FCS EXPRESS 6 RUO EDITION USERS MANUAL

FCS Express 6 RUO Edition

The Future of FCM Analysis

De Novo Software

FCS Express 6 builds on nearly 20 years of cytometry data analysis excellence bringing exceptional speed and ease of use to both flow and image cytometry analysis.

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Chapter

1 Welcome to FCS Express 6

FCS Express is an analysis tool for flow and image cytometry data. FCS Express gives customers the ability to graphically display data generated by any flow cytometer that adheres to the FCS standard. For image data, FCS Express supports a large and growing number of formats .These graphical displays consist of many types of plots, including one-dimensional histograms, and two-dimensional scatter, contour and density plots, and image galleries, among others.

When loading the data, FCS Express reads numerical values for each parameter as well as descriptive information about the parameters, and can display either type of information at any time. In order to display the numerical values, the values can be binned to display in plots with smaller resolutions, or displayed as is. Sub-populations (known as gates) can be defined using graphical means, and used to create further displays.

In addition to the graphical display, FCS Express calculates descriptive statistics based on the original data, as well as any combinations of statistics and operations that the customer creates.

These statistics can be displayed alongside the graphs in tables or embedded in text annotations.

As well as being displayed on the screen, the graphs and statistics can be exported to many types of formats, including text files, Excel, Word, and many image formats, and even custom formats for better integration with customers' existing information storage systems.

This manual applies to FCS Express 6.06.0042.

1.1 About the Manual

The following conventions are used throughout this manual:

In the text, boldface print is used to designate an actual "object" within the program, either text that you will see on your screen, a user interface element such as the **File** tab (note the **File** tab replaces the Applications Button from Version 4) or **Layout Navigation Toolbar**, or an element of FCS Express (e.g., **Display Text** token property). If the text is a hyperlink, it will appear underlined and in blue. Hyperlinked text will not be boldface.

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In the Windows operating system, the designation "left mouse button" refers to the primary mouse button (even if you are left-handed and have switched the buttons so that the primary mouse button is the one under your index finger). By convention, to "right-click" always means to press the secondary mouse button. Throughout the manual, "press the mouse button" refers to your primary button unless the text specifically states to "right-click."

FCS Express uses the Ribbon interface where most menus and toolbars are replaced with the **File** tab as well as **Tabs**, **Groups**, and **Commands**. The drop-down list of commands which appear when selecting the **File** tab or commands with a down-arrow icon are referred to as "galleries" when using the Ribbon interface.

To indicate selecting a particular command, the following convention is often used in this manual "**Tab→Group→Command**". For example, **Home→Pages→New Page** means to select the New Page command from the Pages Group on the Home Tab.

1.2 System Requirements

The minimum system requirements for FCS Express are as follows:

- PC
  - Windows 7/Windows 8/Windows 8.1/Windows 10
  - Intel Core-equivalent or higher
  - 1 GB RAM
  - 400 MB Hard drive space
  - **Recommended system:** 4 GB RAM
- Mac
  - macOS 10.8 (Mountain Lion) or higher.
  - 2 GB ram
  - Intel Core Duo
  - 2 GB Hard drive space
  - **Recommended system:** 8 GB RAM

Notes:

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- FCS Express takes advantage of many features that are available in higher end Intel Chips. For optimum performance, use the newest generation processors. For example, an i7 will significantly outperform an i5 even if the number of cores and clock speed of the processors are equivalent.
- FCS Express can take advantage of all cores in the computer. Thus, while not required, a multi-core computer will be significantly faster than a single core computer for many functions.
- FCS Express (32-bit) (PC Only) will use up to 3 GB of RAM on 32-bit Windows and 4 GB of RAM on 64-bit Windows.
- FCS Express (64-bit) (PC and Mac) will use up to 128 GB on 64-bit machines.

1.2.1 Supported File Formats

FCS Express will read .FCS data files from any instrument and supports a broad range of file formats used for storing flow and imaging cytometry data.

- Supported Flow Cytometry Data Formats 26
- Supported Image Cytometry Data Formats 27
- Other Supported Data Formats 29

1.2.1.1 File formats for Flow Cytometry

FCS Express can read listmode (FCS and LMD) data files from all manufacturers. FCS Express currently supports FCS Version 1 to FCS version 3.1. Simply transfer the files to the computer on which you are running FCS Express (either via the network, flash drive, CD or other method) and FCS Express will be able to load the data 76. FCS Express also supports some specialized data formats native to manufacturers instruments. These specialized formats include Cytek and Sony Spectral data files, BD Accuri C6 and C6 Plus files, and Miltenyi MacsQuant .mqd files.

In addition, FCS Express can also import the analysis/acquisition settings (gates, plots, markers, quadrants etc) from flow cytometers running the BD FACSDiva 868 and the BD Accuri C6 874 software.

	File description	File format
Sample-based files	Listmode files	*.fcs; *.lmd

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	BD Accuri™ C6 / C6 Plus files	*.c6; *.cfl; *.ci;
	Sony Spectral Data 922	*.sraw and *.fcs
	Cytek Spectral Data 922	*.fcs
	Miltenyi MacsQuant files	*.mqd
Plate-based files	BD Accuri™ C6 / C6 Plus Plate files	*.c6; *.ci;
	FCS Plate folders*	FCS Plate Folder (folder directory - see below*)
Importers 868	BD FACSDiva™ Experiment files	*.xml
	BD Accuri™ C6 file	*.c6

* The FCS Plate Folder 1050 format allows users to load individual .FCS files derived from plate based experiments:

- BD instruments with HTS add-on
- Guava
- Coulter Quanta
- Sony
- BD Accuri
- MACSQuant

Plate based data files may be used in conjunction with the High Content Heat Map 1050 add-on.

1.2.1.2 File formats for Imaging Cytometry

FCS Express Image Cytometry allows access to images, numeric analysis results, and image segmentation masks from various instruments and image analysis tools.

In some cases import compatibility with a single image analysis packages enables compatibility with many image formats (e.g. CellProfiler using Bio-Formats). In other cases, direct import of analysis results or links to imaging databases have been developed with specific vendors.

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	Product Name	File format	Images	Numeric Data	Data Base Link
Sample-based files	CellProfiler	*.cpout	Y	Y	NA
	Chemometec	*.acs	Y	Y	NA
	ImageJ files	*.ijout	Y	Y	NA
	Amnis® IDEAS® Software	*.daf and *.fcs	Y	Y	NA
	Fluxion Biosciences - CytoFlux	*.ice	Y	Y	NA
	Phase Holographic Imaging				
	Countstar				
	Molecular Devices® instruments utilizing the MDC Store™ Data Base	*.iso or MDCStore Tab	Y	Y	Y - MDC Store
	Nexcelom Cellometer® Vision	*.nxdat or *.ice	Y	Y	NA
	Perkin Elmer Vectra® / InForm Software	*.tif /*.txt	Y	Y	NA
Plate-based files	Thermo Scientific™ HCS	*.ice	Y	Y	NA

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	Studio™ 2.0 Software				
	Yokogawa™ CQ1	*.ice	Y	Y	NA
	Perkin Elmer® - Opera®, Operetta®, Acapella®, Harmony®, Columbus™	PerkinElmer® Acapella® Folder or Building Blocks Tab.	Y	Y	Y - Columbus™
	Invitrogen™ Tali™	Invitrogen™ Tali™ Data Folder	Y	Y	NA
	Molecular Devices® instruments utilizing the MDC Store™ Data Base	*.iso or MDCStore Tab	Y	Y	Y - MDCStore™

1.2.1.3 Other file formats

FCS Express 6 supports loading data stored as **Comma Delimited files** in the format of (*.csv). Please refer to the Importing External Documents and File Types 868 chapter of this manual for further information.

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1.3 Purchasing and Installing FCS Express

FCS Express can be purchased from our web site at http://www.denovosoftware.com. Simply click the **Purchase** menu on the top of the homepage. You have the choice of either ordering online or downloading an order form. If you download the order form, you can fax the completed form to: +001 (213) 814-1511 or mail it to:

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We accept checks, money orders, wire transfers, purchase orders, Visa™, MasterCard™, and American Express™. In addition to a standalone license, De Novo Software also sells both a network license and site licenses for FCS Express. Please contact us at sales@denovosoftware.com for site license information.

If you purchase the software online, you will be given the option of downloading FCS Express at the time of purchase so that you can begin using it immediately. Whether or not you order online, we will send a CD containing FCS Express to the address you provided. After purchasing the software, the end user will receive a confirmation email containing your configuration file.

FCS Express 6 is available in multiple versions to suit your needs. To learn about the different versions, click on the **Products** menu on the home page.

Information on the different licensing options is available on our web site at Which License is Right for Me?

Complete installation instructions (including information on obtaining configuration files, using dongles and countercodes, and site licenses) are available on the web site at http://www.denovosoftware.com/site/InstallationInstructions.shtml.

1.4 New Features in FCS Express 6

In addition to the new features described in the other sections of the manual, FCS Express has major improvements in the categories below:

- **Mac and PC Compatibility** 31
- **Amnis .DAF file Compatibility** 1040
- **Data Specific Gates** 31
- **Marker to Marker Linking** 36
- **Data Transformations and Cluster Analysis** 32
- **Integrated Spreadsheets** 32

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- Scientific Notation 34
- Standard Operating Procedures 35
- Speed 35
- General Usability 36
- Authorizations 37
- Data File Compatibility 37
- New Plot Types and Enhancements 38
- Custom Grid Options 38

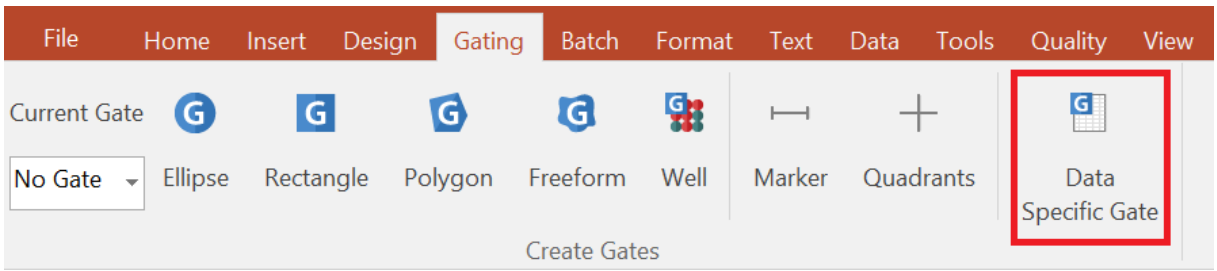
1.4.1 Mac and PC Compatibility

FCS Express is compatible with Mac or PC. Any purchased license of FCS Express may be used seamlessly between either platform, and layouts created on a PC may be opened on a Mac and vice-versa. Users will not have to purchase a separate Mac or PC version. Please visit http://www.denovosoftware.com/site/DownloadLanding.shtml to download the appropriate version for your operating system of choice.

Please see our list of System Requirements 25 for full details on requirements for your Mac or PC operating system.

1.4.2 Data Specific Gates

Data Specific Gates 324 in FCS Express allow you the ability to assign gates to specific data files that have been loaded in the data list. Different gating positions between data files may be stored and automatically used when displaying plots or making calculations when using the **Data Specific Gate** feature, Figure below.



The Data Specific Gate button in the Gating tab is used to enable data specific gates.

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When a gate is converted to a Data Specific Gates for a given file, the shape and position can be modified for the specific file independently from other data files. Thus, when a Data Specific Gates is created, the original gate (here hence referred to as "Master gate") continues to be used for all the remaining files.

Jump to:

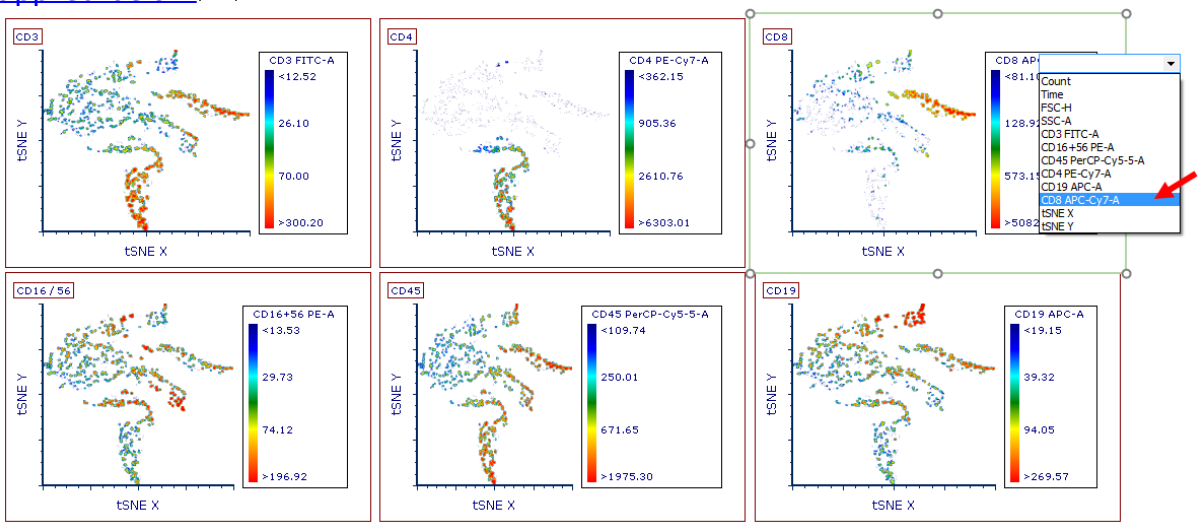
- **Creating Data Specific Gates** 327
- **Deleting/Removing Data Specific Gates** 328
- **Promoting Data Specific Gates to Master Gate** 330
- **Using Data Specific Gate Groups** 331

1.4.3 Data Transformations

FCS Express has new advanced transformation and cluster analysis 932 features that will allow you to perform to following transformation in an easy to use interface.

- Parameter Math 933
- K-Means cluster analysis 958
- Principal Component Analysis (PCA) 946
- R Integration 973 for adding new parameters 982 and cluster transformations 990
- tSNE 1000
- SPADE 1022

Example of tSNE transformed data set with new color based on overlay option applied below 1018.



1.4.4 Integrated Spreadsheets

A new Spreadsheet 414 tool has been added to FCS Express to allow you to easily analyze and organize statistics and information from your experiments.

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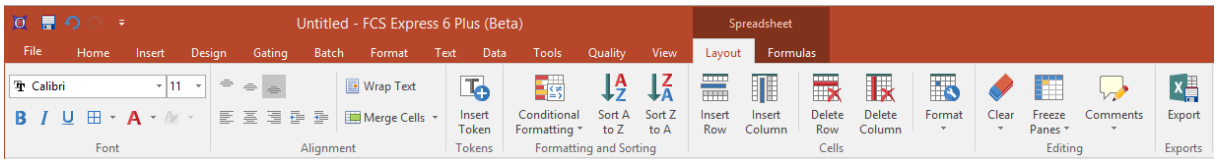
A Spreadsheet may be added to a layout through the **Data** tab→**Insert**→**New Spreadsheet** command.

Once a spreadsheet is inserted you may click on the Spreadsheet to select it, which will make the **Spreadsheet→Layout** and **Spreadsheet→Formula** tabs appear (figures below).

Spreadsheets in FCS Express 6 can be used to perform most of the operations that Microsoft Excel provides. In addition, values in Spreadsheet cells can be derived directly from statistics within the layout, keywords, or from data files in the data list. Inserting statistics for data files in the data list allows additional flexibility in analysis of many statistics for many data files that are not currently open in the layout. Data contained in spreadsheets may be directly exported as .xlsx, .xls, .csv, .html, .xml and .txt files. When exporting as to Excel compatible formats equations and formatting from the FCS Express spreadsheet will be retained.

A number of specialized tutorials are available to guide you through some of the more advanced spreadsheet features:

- HLA Cross matching - Spreadsheet Use Case 1 1620
  - Creating a Spreadsheet for analysis 1624
  - Populating a Spreadsheet with data 1627
  - Working with Custom Tokens and Formula References in spreadsheets 1636
  - Using Conditional Formatting to Classify Data 1646
- Antibody Titration - Spreadsheets Use Case 2 1653
- Calibration via Regression Analysis - Spreadsheets Use Case 3 1679
  - Complete a Spreadsheet and Insert a Scatter with Regression Plot 1680
  - Working with Regression Tokens and Fit Equations 1689
- Working with Pie, Bar, and Scatter Plots - Spreadsheets Use Case 4 1703



The Spreadsheet Layout Tab

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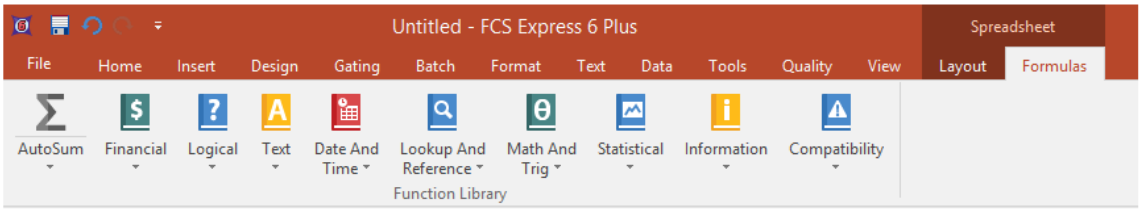
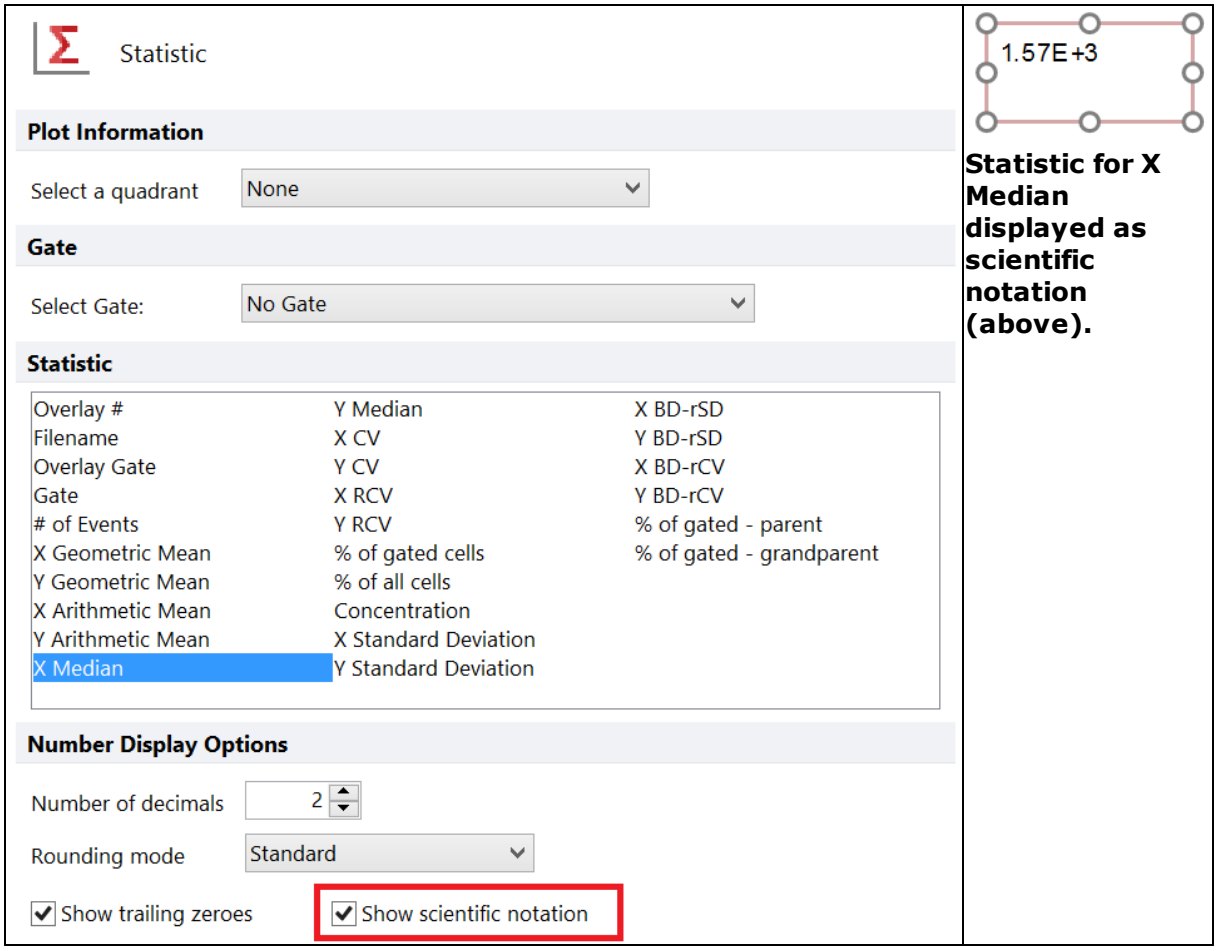


The Spreadsheet Formulas tab.

1.4.5 Scientific Notation

Scientific notation is now available for any statistics in FCS Express and may be accessed via the **Number Display Options** in any **Statistics** dialog.



Statistic for X Median displayed as scientific notation (above).

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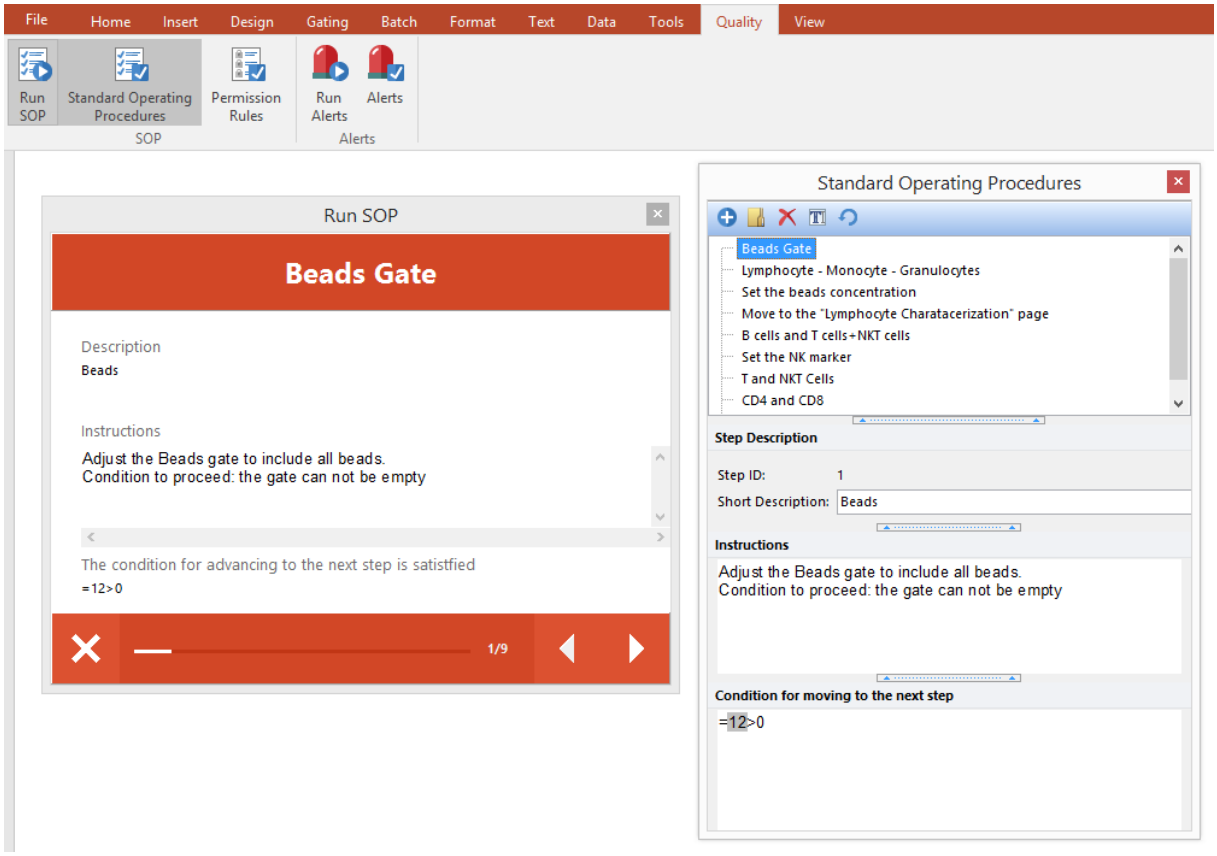
1.4.6 Standard Operating Procedures

Standard Operating Procedures (SOP's) allow you to create a predetermined set of steps in your FCS Express layouts in which each step can be conditionally formatted with tokens so that a requirement must be met before the next step is performed. For instance, a user may not proceed to the next step unless the number of events in a gate is above a certain threshold value or text has been entered in a field.



The Standard Operating Procedure feature in FCS Express.

1.4.7 Speed Improvements

FCS Express has the following new speed enhancements:

Speed:

- Significantly improved speed in the loading of all data files and saving of layouts.

- FCS Express takes advantage of many features that are available in higher end Intel Chips while using all the cores in your computer to dramatically speed up your analysis.
    - For optimum performance, use the newest generation processors. For example, an i7 will significantly outperform an i5 even if the number of cores and clock speed of the processors are equivalent.

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- FCS Express can take advantage of all cores in the computer. While not required, a multi-core computer will be significantly faster than a single core computer for many functions.
- FCS Express (32 bit) (PC Only) will use up to 3 GB of RAM on 32 bit Windows and 4 GB of RAM on 64 bit Windows.
- FCS Express (64 bit) (PC and Mac) will use up to 128 GB on 64 bit machines.
- Heat map loading speed drastically improved.
- Picture plot loading speed drastically improved.
- .ICE file loading speed drastically improved.

1.4.8 General Usability Improvements

FCS Express has the following new improvements to general usability of the software:

General Usability

- New Microsoft office suite style interface 55
- Option to embed or link to images in a layout 146
- Compensation and Spillover Matrices are now color coded 631
- Gate View 345 and Gate Navigator 128 "unified" so any action available in the Gate View 345 is possible within the Gate Navigator 128
- Ability to drag and drop a single cell image from a data grid onto the layout to insert a picture of the cell 685
- Table and text box formatting is retained when copy and pasting from FCS Express to Excel 166
- Heat map statistics grids may be displayed in columns or rows as well as standard tables 1054.
- New Custom Token function for more easily interpolating values from Scatter with Regression Plots 587
- Right click on plot to add text box. 164
- Batch Processing Run window now dockable 846

1.4.9 Marker Linking, Locking, and Visibility options

FCS Express allows users the ability to link any markers together so that moving a marker end point will automatically cause an end point on a different marker to update at the same time. This allows for live updating bifurcation of markers on histograms. Linking markers is accomplished by

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setting the low or high bound for one marker to use a token value for the high or low bound of a separate marker.

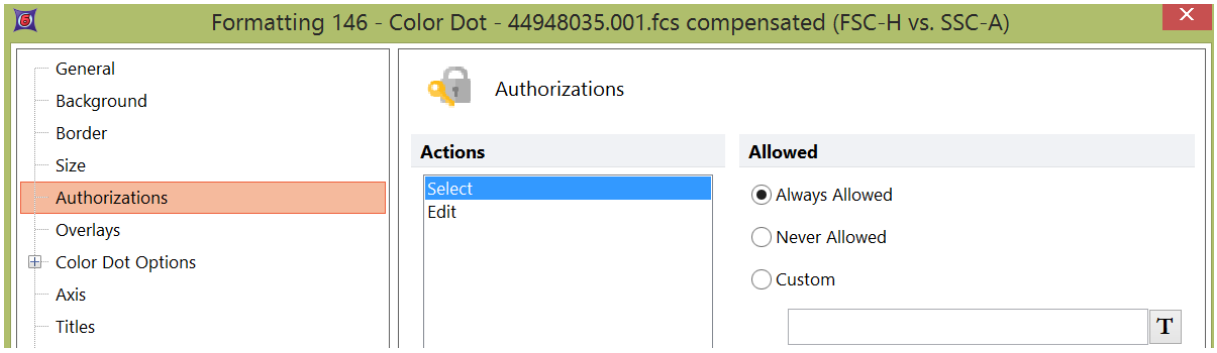
Markers can also be conditionally locked or marked as hidden/visible on any plots

- Markers may be linked together 355
- Markers visibility and ability to move may be set manually or via conditional token values 273
- Marker positions may be locked to a manually entered or token dependent value 273

1.4.10 Authorizations

All objects in FCS Express may now be assigned Authorizations 1136, or rights, which allows or prevents layout users from placing objects in **Select** mode or **Edit** mode or both. **Authorizations** can be set to always allow, never allow, or always allow/do not allow based on the value of a token. For instance, a token value can be set to allow selection of a plot if the current FCS Express user equals a specific person or a plot can remain non-editable unless a particular statistic value is met, ie the number of events for a particular gate is higher than X.

Authorizations allow for an additional layer of control at the layout level for any objects.



The Authorization feature.

1.4.11 Data File Compatibility

FCS Express has the following new data loading and data list features. See our full list of support file formats 26 for additional details.

Data Loading:

- Ability to load Amnis® .DAF files. 1040
- Ability to load new BD Accuri C6 Plus data file (*.ci) format. 874

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- Ability to work with Sony Spectral data files exported in .FCS file format.
- Ability to load very large data files. 35

1.4.12 Plot Types and Enhancements

FCS Express adds the following new plot types and enhancements:

- Heat maps have overlay capabilities 1063
- Heat maps may display any statistic as a radius parameter 1057
- Plots and objects may appear visible or hidden and visibility may be based on token values 240
- Quadrant visibility and ability to move may be set manually or via conditional token values 293
- Tokens may be added to plot titles and footers 250
- Spectrum plots for Cytek and Sony spectral file analysis 922.

1.4.13 Custom Grid Options

FCS Express allows for customization of output format when printing, creating PowerPoint documents, and generating PDF files, from the export and batch processing 784 features by setting **Custom Grid Options** (Figure 4.1). These options allow you to export in a mode where multiple plots or pages are tiled in your resulting document automatically allowing for many plots or pages to be presented on one (or many) page(s) in your final report according to the tiling options you have defined. The **Custom Grid Options** may also be enabled when using the **Multiple Duplicate and Next** and **Multiple Duplicate and Prev**(ious) options available from the **Home** tab→**Clipboard** group→**Duplicate** dropdown menu. The selected plot(s) will be duplicated in a mode where the new plots will be added to same page or other pages of the layout based upon tiling options defined.

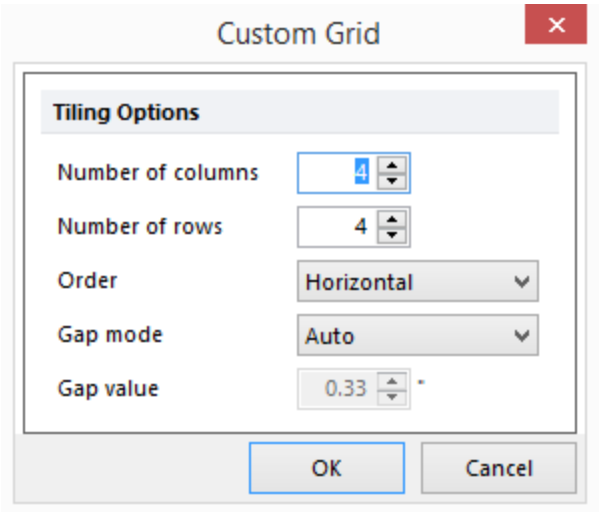

Figure 4.1 Custom Grid Options

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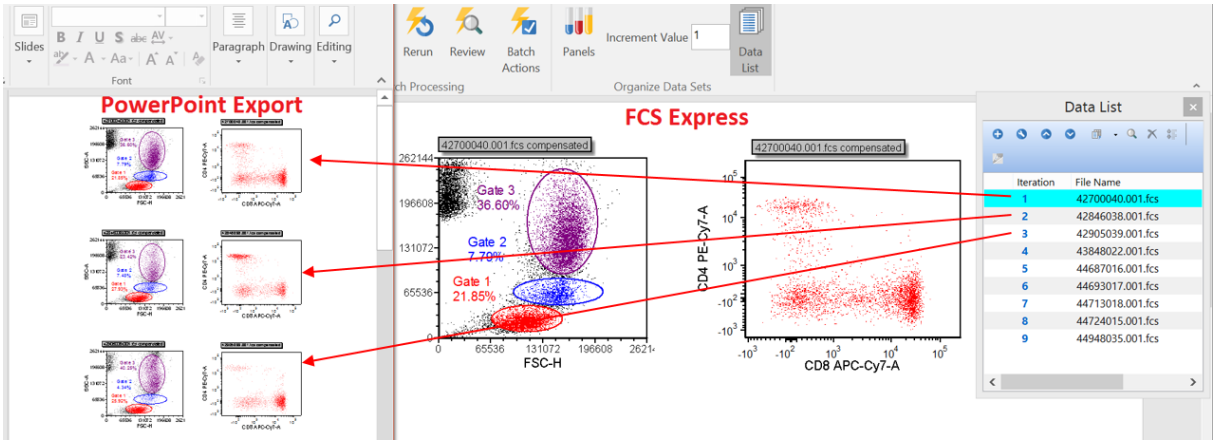
When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear, which will contain the following five settings:

- **Number of columns**: define the number of layout pages displayed across the printed page(s) or exported slide(s) or PDF page(s).
- **Number of rows**: define number of layout pages displayed down the printed page(s) or exported slide(s) or PDF page(s).
- **Order**: arrange the layout pages across (**Horizontal**) or down (**Vertical**) the printed page(s) or exported slide(s) or PDF page(s).
- **Gap mode**: select how to define horizontal distance (**Gap value**) between layout pages.
- **Gap value**: value used for distance of **Gap mode** in inches; will update automatically if **Gap mode** is set to **Auto**; will be **0.02** inches if **Gap mode** is set to **Minimal**; can enter preferred value if **Gap mode** is set to **Manual**.



Example of batching a set of data files from the data list to PowerPoint using the Custom Grid feature. Notice that one set of plots was defined in FCS Express and a series of tiled plots have been exported to PowerPoint in one column in three rows.

1.4.14 Index Sorting Compatibility

FCS Express is now offers the ability to work with index sorting data 1068 from the following instruments to relate individual sorted cells back to the sorted and pre-sort populations.

- **Influx (BD Biosciences)** 1069
- **Summit Software (Beckman Coulter)** 1072
- **Diva (BD Biosciences)** 1076
- **Chorus (BD Biosciences)** 1082

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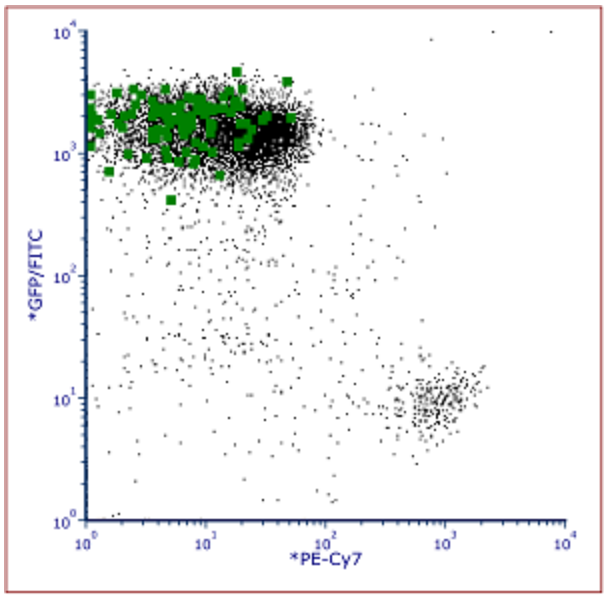
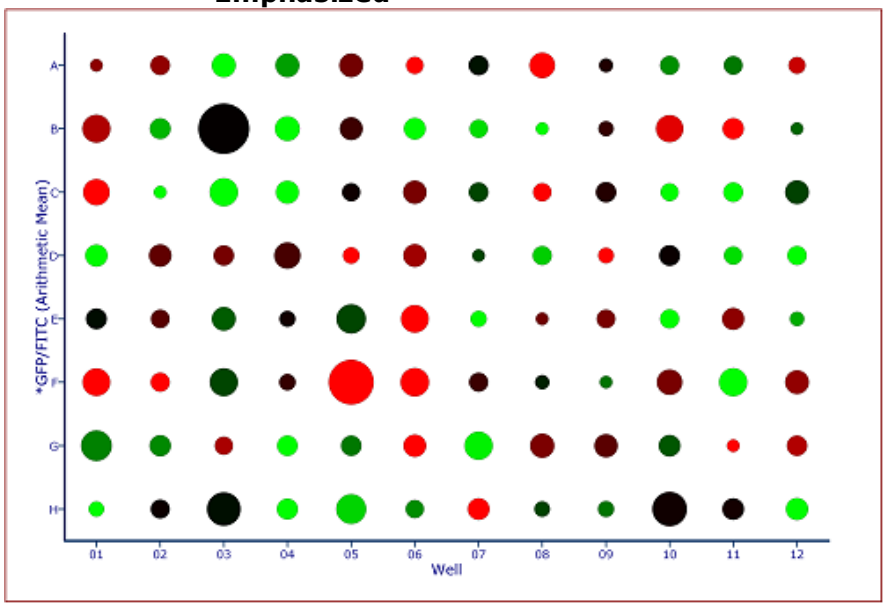


Index Sorting Data with Sorted Events Emphasized



Heat Map of sorted wells with radius size dependent on amount of GFP expression.

1.5 Upgrading From Version 5

FCS Express 6 is the result of extensive customer feedback, giving you the features you need most[30]. FCS Express 6 continues our tradition of innovative, powerful software, designed to let you work in a modern PowerPoint™ style framework. Speed and general usability have been greatly enhanced in Version 6 as highlighted in the New Features in FCS Express 6 chapter[30].

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In addition, Version 6 continues to expand to bring the full power of flow-style analysis to the world of image cytometry. All of the standard FCS Express features to which you have become accustomed are available, plus new innovative abilities, such as gating on cells in your images, gating on events in cell galleries, using heat maps to organize your experiment, and gating on wells.

For more details on upgrading your current FCS Express license to FCS Express 6 please visit our upgrade webpage.

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Chapter

2 Learning How to Use FCS Express

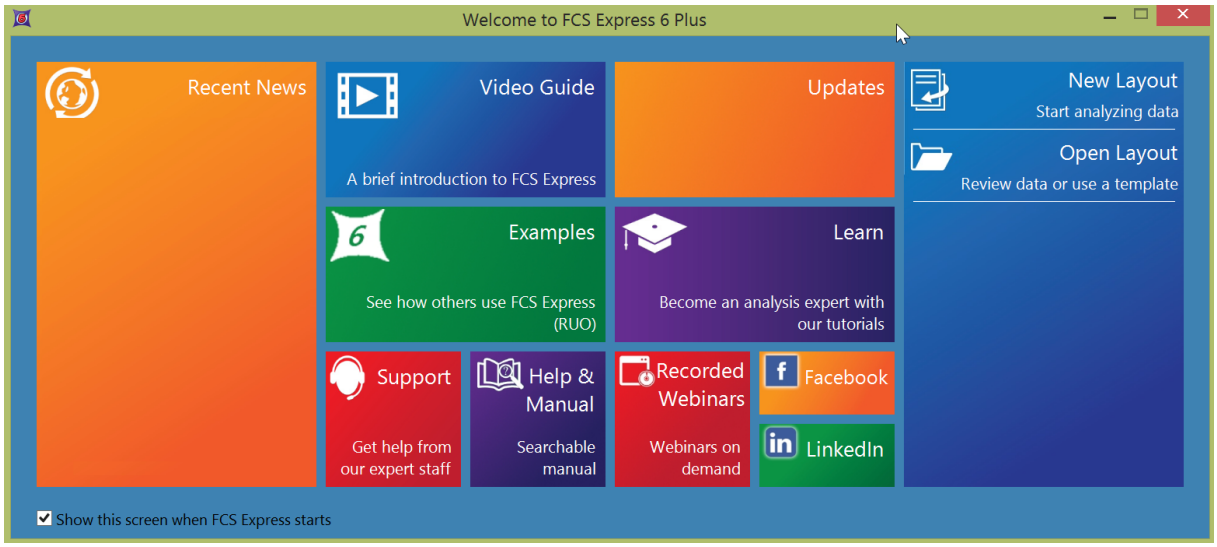
In addition to this manual and the integrated help system, there are a number of other available ways to learn to use FCS Express. These include video 44 and written 45 tutorials, an online forum 46, and email support 47. These resources may also be found in the new Welcome Screen 54 of FCS Express or on the De Novo Software website at www.denovosoftware.com

The expert staff at De Novo Software is also happy to set up short training sessions for free; please write to support@denovosoftware.com to request a session.

Our staff can also be reached for questions by dialing +001 (213) 814-1240 and leaving a message. A member of the De Novo Software team will call you back within 24 hours (usually within the hour).

2.1 Recorded Webinars and Tutorial Videos

A good place to start learning FCS Express is with our online recorded webinars and short instructional videos. These are accessible via the **Recorded Webinars** button on the Start Up Screen 54 and are also available at http://www.denovosoftware.com/site/recordedWebinars.shtml#ShortOverview and on the De Novo Software YouTube channel. These videos provide an overview of software features and a glimpse of the variety of data analysis possibilities in FCS Express.



2.2 Written Tutorials

De Novo Software also provides comprehensive, step-by-step written tutorials, which describe how to use the basic functions of FCS Express. The tutorials[1210] are included in this manual, and are also available offline by selecting the **File** tab→**Help**→**Tutorials**, or at http://www.denovosoftware.com/site/manual/index.html?tutorials.htm. The following tutorials are currently available (check our website for additional tutorials for an updated list).

Sample data is provided so that you can follow along with the examples in the tutorials. The sample data can be downloaded from the De Novo Software web site, from the archive:

https://s3.amazonaws.com/fcsexpressdownloads/TutorialSampleDataV6.zip

- Using Objects[1211]
- Inserting Plots[1225]
- Formatting Plots[1236]
- Gating[1266]
- Text Boxes and Tokens[1298]
- Quadrants[1349]
- Quadrant/Marker to Gate Linking[1362]
- Compensation[1376]
- Snap Shots[1423]
- Batch Processing[1445]
- Creating and Using Panels[1498]
- Docking[1537]
- Data Navigator[1561]
- Gate Navigator[1582]
- Layout Navigator[1597]
- Spreadsheets Use Case 1 - Working with Custom Tokens, Formula References, and Conditional Formatting[1620]
- Spreadsheets Use Case 2 - Creating a regression analysis with an Antibody Titration Series[1653]
- Spreadsheets Use Case 3 - Performing Channel Calibration and Regression Analysis[1679]
- Spreadsheets Use Case 4 - Creating and working with Pie, Bar and Scatter Plots[1703]
- High Content Flow[1739]
- DNA Analysis[1795]
- Proliferation[1833]
- Kinetics[1856]
- Cell Concentration[1885]
- Channel Calibration[1906]

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- CellProfiler Export 1921
- ImageJ Export 2019
- Exporting from IsoCyte 2043
- Picture Plots 2060
-
- Introduction to FCS Express 6 Image Cytometry 2098
- Immunophenotyping 2186
- Changing Common Preferences 2232
- Cluster Analysis 2238

2.3 Integrated Help System

FCS Express comes with a comprehensive integrated help system. The integrated help is available by selecting **File** tab→**Help**→**Contents**. You can use this system to search for specific topics (Figure 2.1). It contains the same information as this manual, in an easy-to-access, cross-referenced manner.

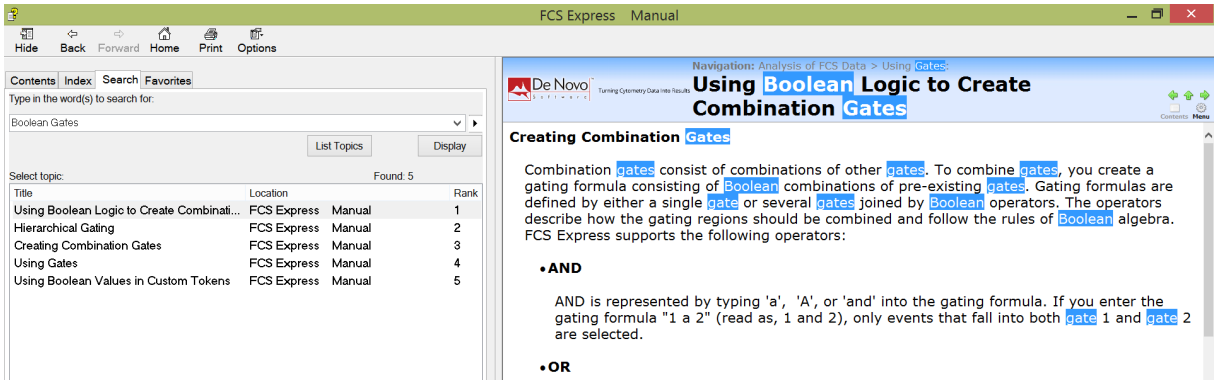

Figure 2.1 The integrated help and manual within FCS Express.

2.4 Comprehensive Manual

The FCS Express manual is a detailed explanation of all of the functions in FCS Express. The manual is available as a .pdf file by selecting the **File** tab→**Help**→**FCS Express Manual**. You can also obtain the latest version of the manual on the De Novo Software website.

2.5 Online Discussion Forum

In addition to the printed materials described above, an online discussion forum is available to discuss any issues pertaining to FCS Express. This list is monitored by the De Novo Software support staff, but has the added advantage of allowing other FCS Express users to respond and learn from your questions and comments. We encourage you to post questions, comments, and suggestions to this forum. You can access the forums by selecting the **File** tab→**Help**→**Online Forum** or at https://forums.denovosoftware.com/.

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2.6 Email Support

If you have any questions pertaining to FCS Express, please do not hesitate to contact the De Novo Software team at support@denovosoftware.com. The De Novo Software team is also available for free live web-based training and support at your request. Please visit http://www.denovosoftware.com/site/support.shtml for additional details.

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Chapter

3 A Quick Tour of FCS Express

The FCS Express 6 interface utilizes the Ribbon 55 system, similar in design to the familiar Microsoft Office suite of programs (Office 2007 and later). The Ribbon 55 helps you quickly find commands that you need to complete a task. The commands are organized into functional groups collected under Tabs. For example, there is a Gating tab 71, a Batch tab 72, an Insert tab 69, etc.

The Applications button found in Version 4 was replaced with the new **File** tab in FCS Express 5 and FCS Express 6, which contains actions previously found in the Applications button.

The **FCS Express User Options** 179 can be found within the **Options** command in the **File** tab. Clicking on the **File** tab→**Options** will launch the **FCS Express User Options** window, which contains the adjustable settings found in Edit Preferences in FCS Express V4.

3.1 Getting Started with FCS Express

When starting FCS Express, the first window to appear is the **User Selection** window (Figure 3.1). In this window, you can select the user profile to be used for the current FCS Express session. To log in with an existing user profile, select either the **Default** user, or a user name that you have created, and click the **Select** button. When a user is selected, the Options previously saved for that user are activated. Therefore, multiple users sharing the same computer can customize their Options independently. To create a new user, click on ***New User...*** and enter a new user name. At the end of the session, the user profile stores the FCS Express User Options 179 for each user. To delete a user, right-click on the user name and select **Delete User** from the pop-up menu. *Note*: *Once a user is deleted, the default Options for that user cannot be recovered*.

The **Import...** button at the bottom left of the **User Selection** window is used for importing FCS Express Version user preference files (.fcg) from the FCS Express V5 installation folder.

For details on changing default user Options, see the section on Customizing Default Options 179. *Note:* An individual user's default Options can be restored to manufacturer default settings, by selecting the **File** tab →**System**→**Restore Options**→**Restore Options to Default Values**.

If only one user will ever be using this copy of FCS Express, you can choose **Remember Me On This Computer** in the **User Selection** window. FCS Express will then automatically log in as that user when the program starts. To stop logging in as the same user, you can log out 59 from the **File**

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tab→**System**→**Current User**→**Logout Current User** or from the right side of the Layout Navigation Toolbar at the bottom of the FCS Express window 56.

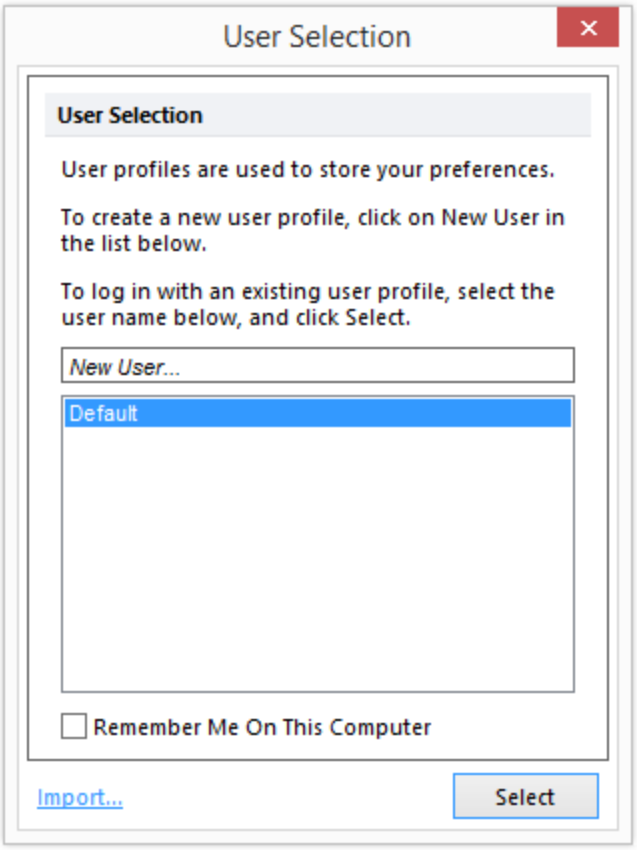

Figure 3.1 User Selection Dialog

Note: If you are running the security system 1142, or are using an Internet Dongle, FCS Express will start up with a login screen prompting you to enter your user name and password.

Once you select a user and press **OK**, FCS Express will start (Figure 3.2) and the Startup Screen 54 will appear.

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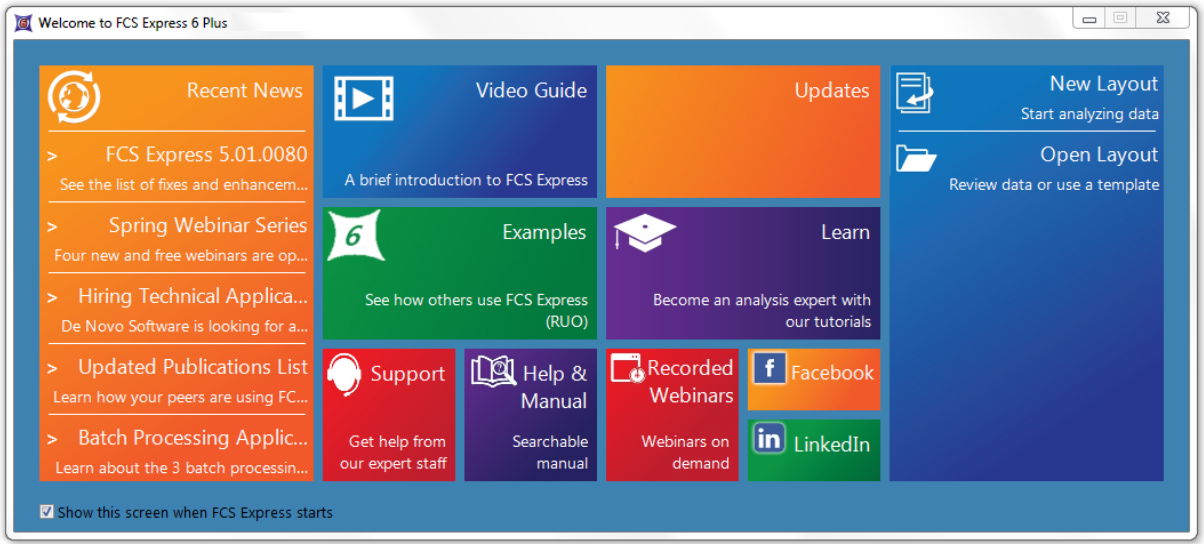

Figure 3.2 Startup Screen

After choosing **New Layout**, a blank document (layout) will be displayed (Figure 3.3). Layouts (.fey files) are the document format of FCS Express.
The default layout is a single page with a white background.

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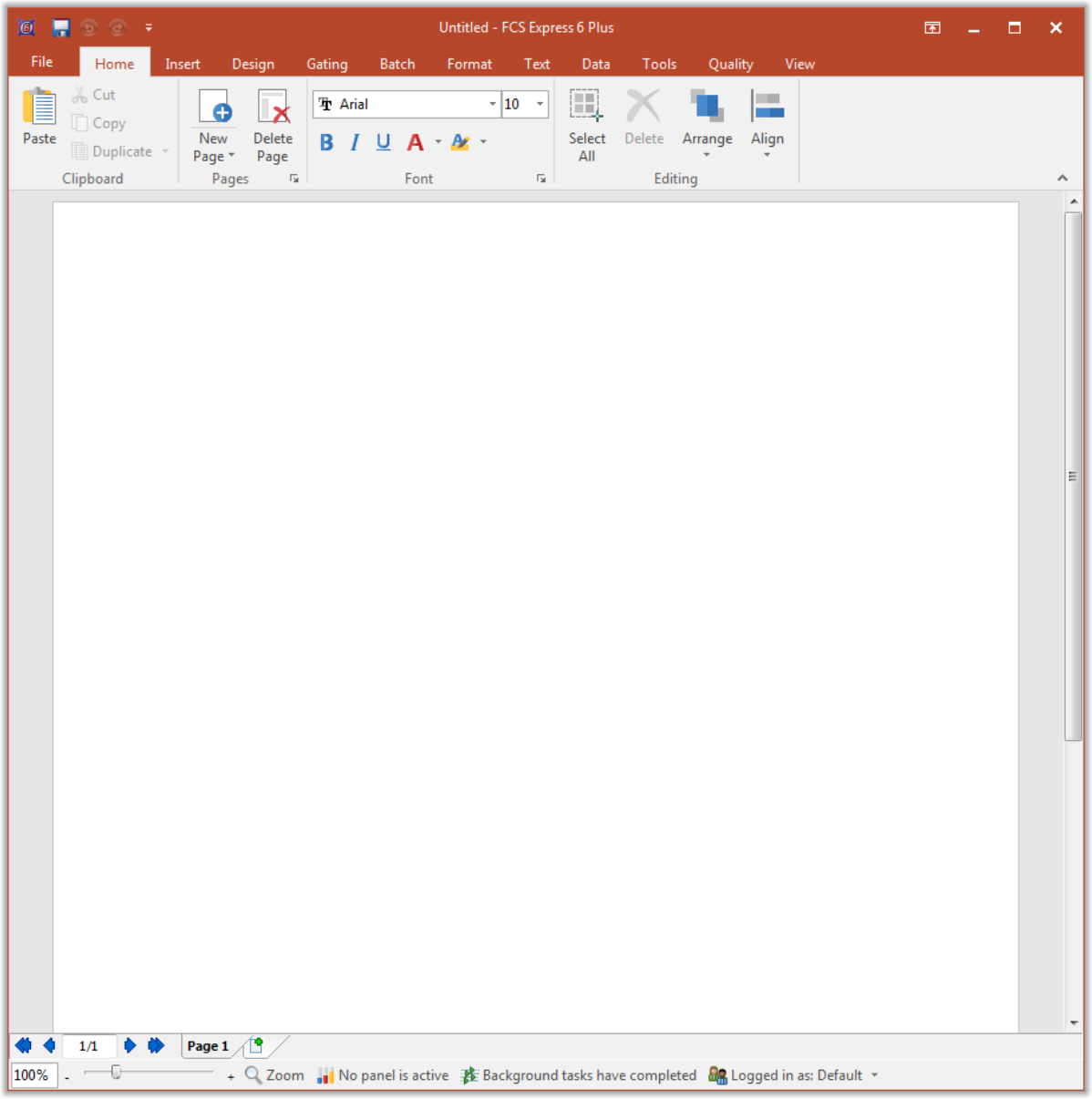

Figure 3.3 Blank layout

You can open an existing layout using the **File** tab→**Open** to bring up a **Load Layout** dialog. You can also create a new layout from a blank document or from a template of an existing layout, discussed in detail in Creating a New Layout 92.

Layouts can contain plots, pictures, text boxes, statistics windows, and data windows. Gating definitions, parameter label sets, and other features of the analysis are also stored directly in the layout file. In addition, .fcs data files can be embedded directly in the layout and saved as part of the layout file.

Any number of layouts can be open at the same time.

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3.2 Startup Screen

The Startup Screen in FCS Express (Figure 3.4) first appears after you choose a User 50 or will open automatically if you have chosen to remember a user on the computer.

The Startup Screen is a dashboard for quickly accessing:

- New layouts
- Saved layouts
- Recently accessed layouts
- FCS Express news and updates
- Video Guides
- Recorded webinars
- Searchable Manual
- Tutorials
- Support
- Applications Examples
- Social media sites for De Novo Software

The Startup Screen will remain open until closed or minimized and can be dismissed for future instances of FCS Express by unchecking the box for "**Show this screen when FCS Express starts up**" in the bottom left of the window.

Once the **"Show this screen when FCS Express starts up**" box has been unchecked, the option to show the startup screen upon startup can be reset in the **File** tab→**Options**→**FCS Express User Options**→**Dialogs**→ **Do Not Show Again** category by checking the box for "**Show startup screen automatically when FCS Express starts up**".

Alternatively, the Startup screen may be opened or closed through the **View** tab→**Welcome**→**Startup Screen** button.

Other options for the Startup screen may be changed in the **File** tab→**Options**→ **FCS Express User Options**→ **Startup** category, such as **Automatically close the startup screen when opening a new or existing layout** and **Reopen the startup screen after closing all layouts**.

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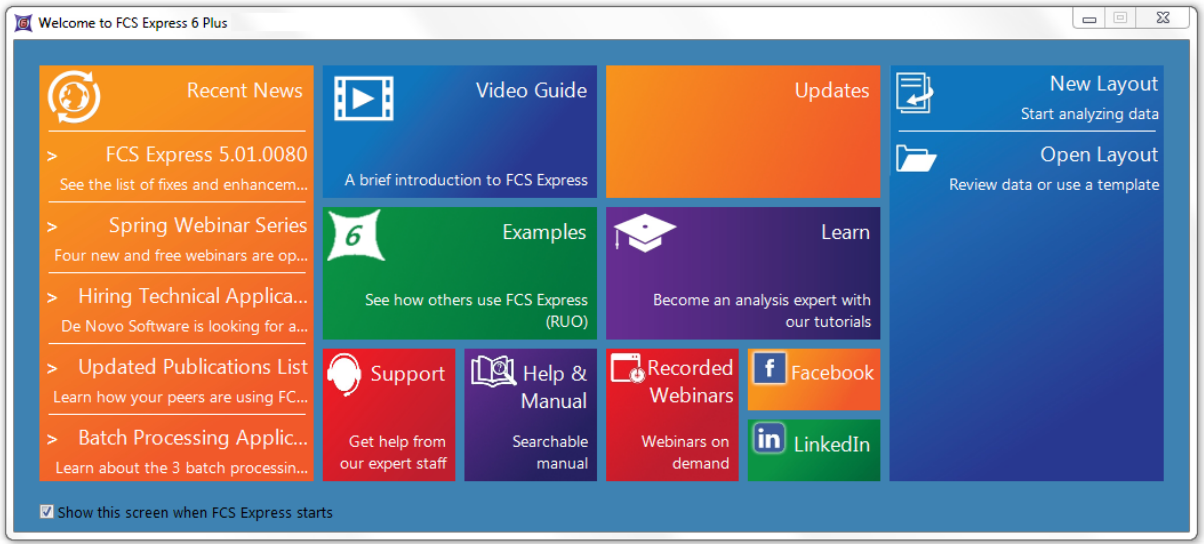

Figure 3.4 The Startup Screen

3.3 A Quick Walk through the Ribbon

The Ribbon (Figure 3.5) is at the top of the page. You can find the File tab[57] and the **Quick Access Toolbar** at the top, left corner of the Ribbon. The **Quick Access Toolbar** is a place to put frequently used functions. You can customize[65] the functions on the **Quick Access Toolbar** (as well as the entire Ribbon itself[67]). Under the **Quick Access Toolbar** are multiple Tabs. Each Tab contains command buttons organized into logical **groups**. Some groups contain a **Dialog Box Launcher Button** with multiple formatting options.

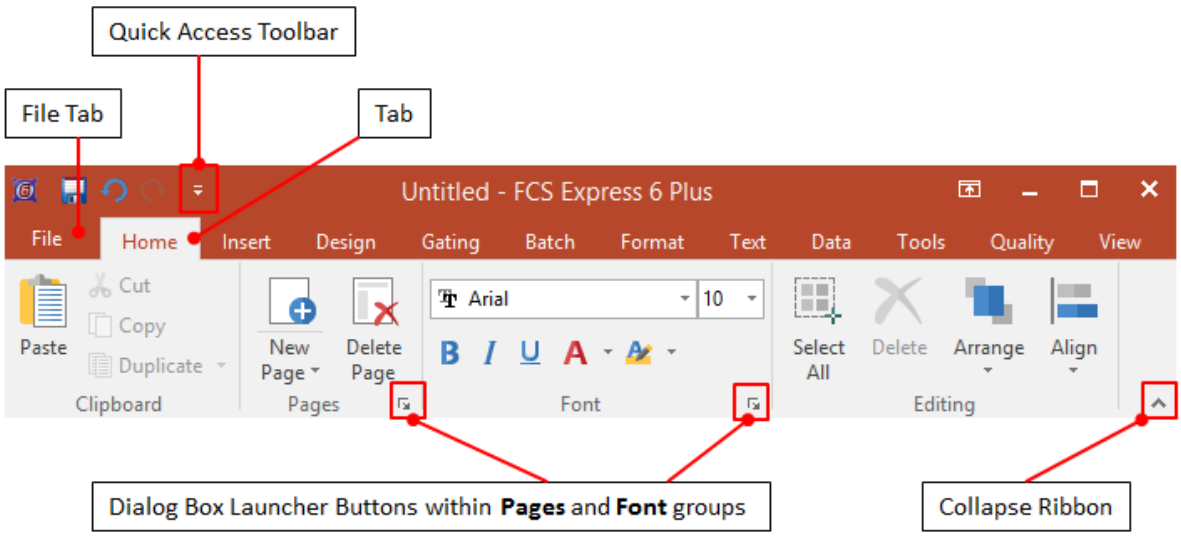

Figure 3.5 The Ribbon

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On the upper right portion of the ribbon you may also choose the ribbon display options (Figure 3.6) or to close, minimize, and/or restore the current layout.

- **Auto-hide Ribbon** will hide the ribbon in a single band until the top band is clicked on to display the ribbon.
- **Show Tabs** will only display tabs until a tab is clicked on, then commands for each tab will be present.
- **Show Tabs and Commands** will show all tabs and all commands at all times.

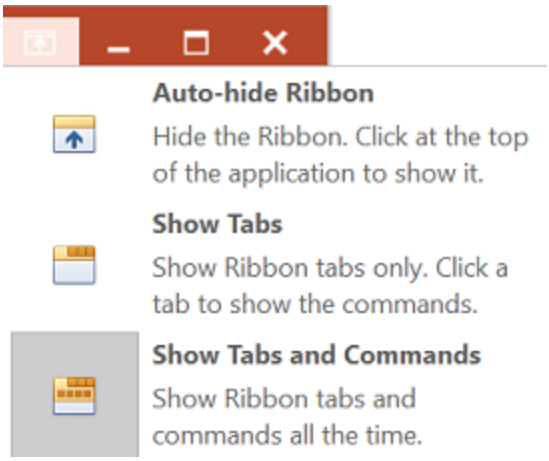

Figure 3.6 Ribbon Display Options

At the very bottom left of the application window is the **Layout Navigation Toolbar** (Figure 3.7) used for navigating through the layout 109. Beneath the **Layout Navigation Toolbar** is the Zoom Toolbar 110. To the right of the **Zoom Toolbar** is a **Status Bar** showing the active panel, described in Working with Panels 860.

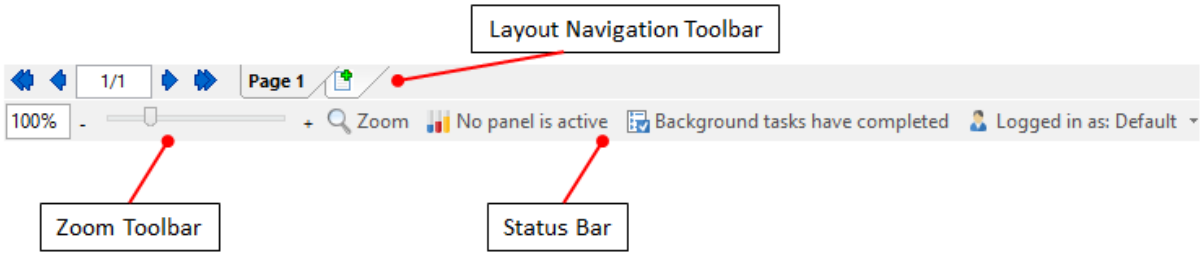

Figure 3.7 Layout Navigation Toolbar with Zoom Toolbar and Status Bar

Individual commands in the **groups** will be enabled or disabled based upon the current status of the layout. For instance, if no items are selected, the **Cut**, **Copy**, and **Duplicate** commands in the **Clipboard** group of the **Home** Tab will be

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disabled. Brief descriptions of the features of the Ribbon are described in the sections below.

Multicycle and **Spreadsheet** tabs are only available when a multicycle plot or spreadsheet has been inserted, respectively.

3.3.1 The File Tab

The **File** tab (Figure 3.8) contains multiple **groups** of items in a list at the left. Clicking on any item on the left will open a new item section on the right specific for that item. The main items and their sub items are as follows:

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Figure 3.8 File tab

- **Save, Save as..., Open, Close, Close All, Open From, Recent, New** (❶)

  These items allow the user to create, open, save, and close layouts. The **Open From** command also allows the user to import BD FACSDiva 868 and BD Accuri C6 874 files.

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- **Export** (❷)

  The **Export** item allows you to export your layout to a PowerPoint presentation 105 or a PDF 107. In addition, you can save the current page or selected items as a Picture 104.

- **Info** (❸)

  This item allows you to:

  - View information about the current layout
  - View the log of all changes that were ever made to the layout (if you have purchased the logging add-on)
  - Create and manage the set of electronic signatures for this layout, and sign or unsign the layout (if you have purchased the security add-on)

- **Print** (❹)

  This item is used to:

  - Select a printer, which pages to print, and other options before printing
  - Choose to print Color or Grayscale

- **Collaborate** (❺)

  This item is for:

  - Publishing your layout 604 using the De Novo Software **Publishing** service
  - Managing the layouts you have published

- **System** (❻)

  With this command, you can:

  - Logout the current user.
  - Edit information for the current user.

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- Restore Options to default values.
- Reset toolbars to default settings.
- Show background tasks to Manage print jobs, downloads and other tasks that are running in the background.
- Logout the current user.
- Change your configuration.

- **Help** (❼)

  With this item, you can:

  - Show the help system's table of contents, and search its contents.
  - View tutorials about using FCS Express.
  - Open the integrated help system and/or the PDF manual.
  - Launch the De Novo Software website in your internet browser.
  - Open the **About** screen to view information about the version of FCS Express that is running.
  - Visit the online forum.

Note: If security is activated, there will be an additional item available labeled "Security". 1142

The **Options** button (❽) brings up the **FCS Express User Options** dialog where you can completely customize the working environment for a selected user by selecting the defaults (Options) for essentially every object category in FCS Express.

The **Exit** button (❾) closes FCS Express. You will be prompted to save your layout if it has changed during your session.

3.3.2 The Home Tab

The **Home** tab (Fig. 3.9) contains the following **groups**:

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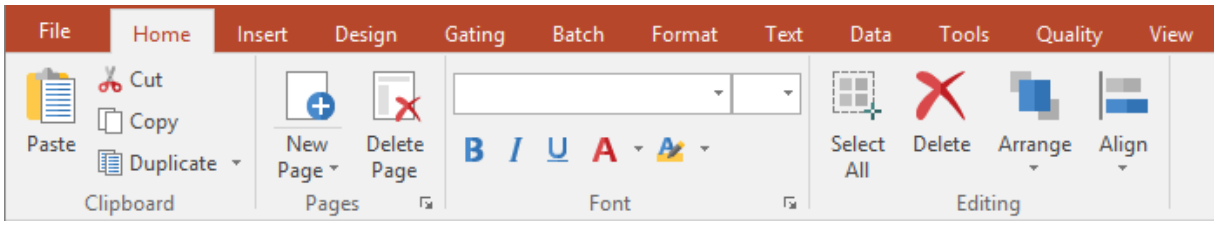

Figure 3.9 Home Tab

- **Clipboard**
  - The **Paste** command allows you to paste something from the clipboard. The label of the **Paste** command will change depending on the type of object(s) in the clipboard.
  - The precise text beside the **Cut** and **Copy** commands will change depending on which object(s) in the layout are currently selected.
  - The **Duplicate** command duplicates the selected object(s). Under the **Duplicate** dropdown menu are five commands: **Duplicate**, **Duplicate and Next**, **Duplicate and Prev**(ious), **Multiple Duplicate and Next**, and **Multiple Duplicate and Prev**(ious). The Next and Prev functions associated with these commands duplicate the selected object(s) and changes them to display the next or previous file(s) in your Data List 785. The Multiple commands allow you to make more than one copy of the selected item, changing successively to the next or previous file(s) in each instance. The positioning of the duplicated objects can be set in the **Duplicate Options** (Fig. 3.10, *top*)or in the default User Options 180:
    - Selecting radio button next to **Place the duplicated objects in a grid** will allow **Custom Grid** settings to be enabled under **Grid Options**. The duplicated objects will displayed on the following new page(s) of the layout.
      - Objects are duplicated at the size appropriate for the page dimensions that allow for the **Number of columns**, **rows**, and **Gap mode** and **value** specified under Custom Grid Tiling Options (Fig. 3.11, *top right*). **Order** specifies whether objects are duplicated across and then down the page ("Horizontal") or down and then across ("Vertical"). Also note that if **Custom Grid** is selected, the file(s) in currently selected object(s) are duplicated as the first object(s) in the Custom Grid. An example of selected objects Multiple Duplicated in a Custom Grid with the options specified below is shown in Fig. 3.12, *bottom*.
    - Selecting the radio button next to **Place the duplicated objects relative to the original** will place the duplicated objects on the same page as the selected object(s) even if pages do not fit on the page. In order to place all duplicated objects on a page, please be

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sure to check the box next to **If duplicated object(s) are off the page, put them on a new page**. Enabling this option will create new page(s) to accommodate all the duplicated objects that do not fit on the same page as the original object(s).

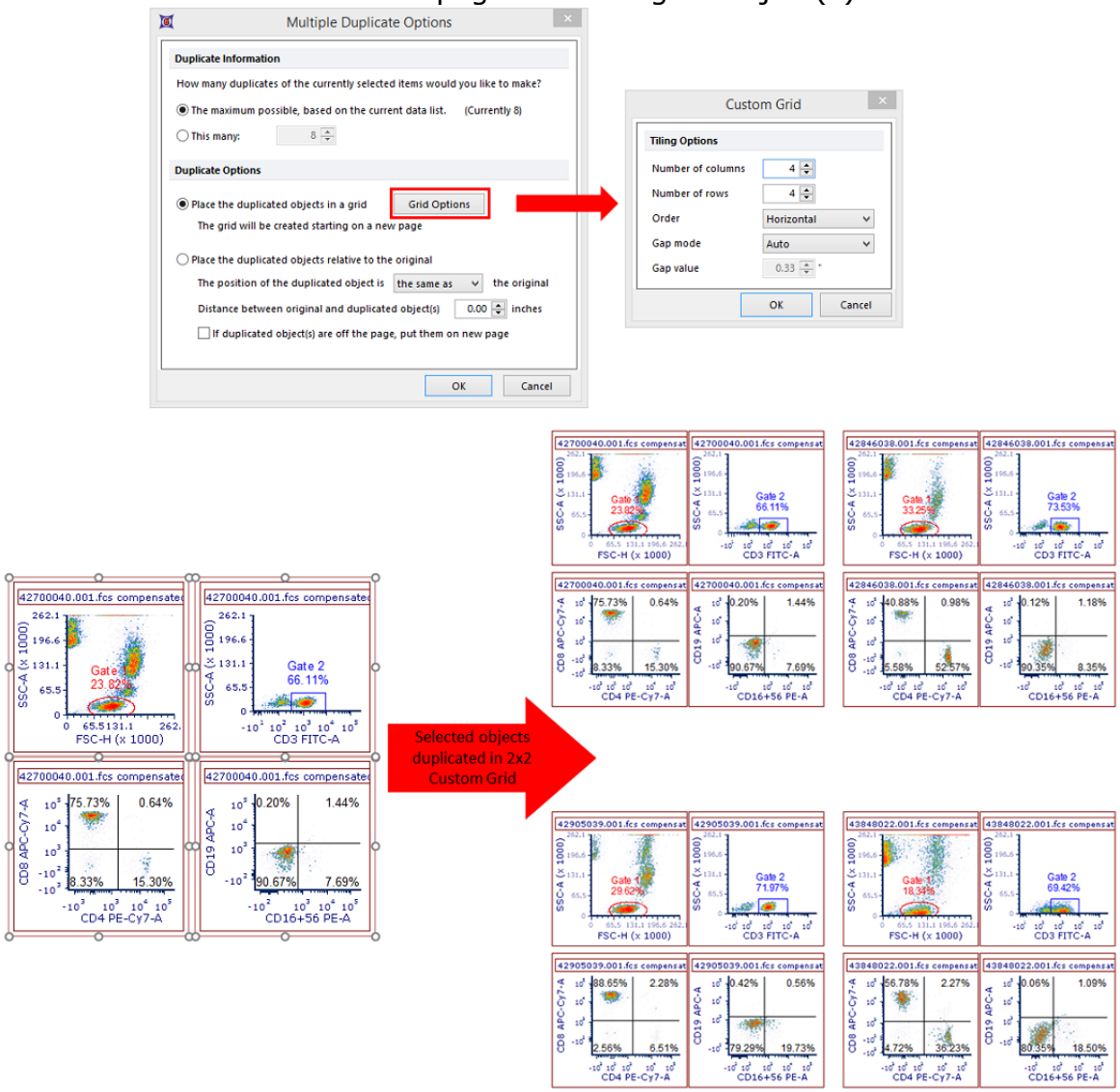

Figure 3.10 Multiple Duplicate Options (top), including Custom Grid Tiling Options (top right), and an example of selected objects Multiple Duplicated with the pictured options (bottom).

- The items in the **Clipboard** group will operate on plot windows, text boxes, statistics windows, and more, depending on which object(s) are currently selected in the layout page. Items (e.g., Text Boxes) can be copied from one layout to another. You can switch between open layouts using the **View** tab→**Windows**→**Switch Windows** command.

- **Pages**

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- The top half of the **New Page** command adds a new page to your layout. The bottom half of the **New Page** command opens a gallery with numerous choices for adding a new page with Place Holders for predefined layouts containing various text boxes, plots, and statistics.

- The **Delete Page** command deletes the current page.

- **Font**

  - Within this group, the **Font Name**, **Font Size**, **Font Color**, and **Font Background Color** can be changed for the selected object(s) with **Bold**, **Italics**, and **Underline** options available. Clicking the expand button will open the **Font Dialog** (Fig. 3.13).

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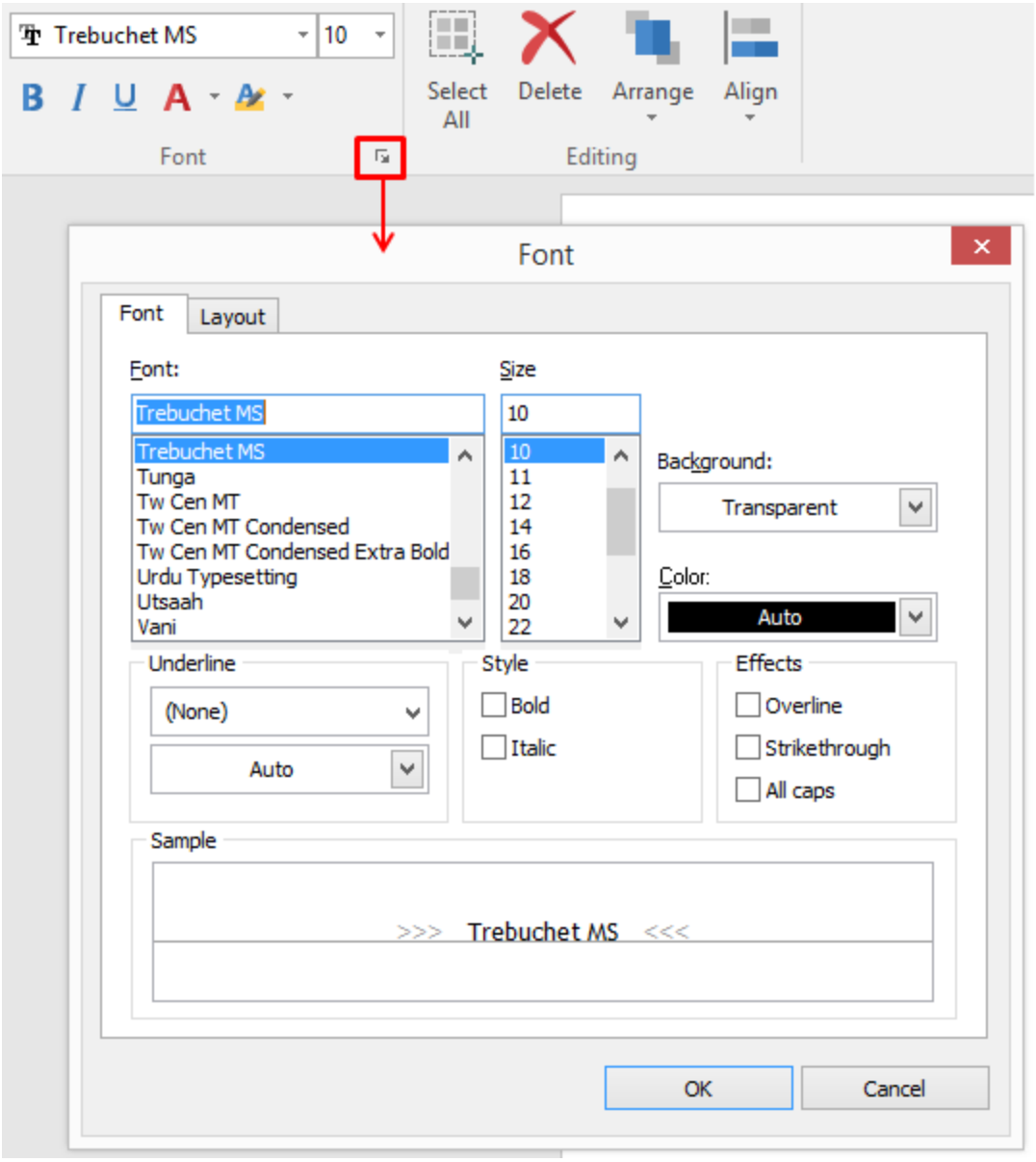

Figure 3.11 - Home Tab Font Group to Font Dialog

- **Editing**
  - The **Select All** command selects all of the object(s) on the current page.
  - The **Delete** command deletes the currently selected object(s).
  - The **Arrange** command expands to allow you to **Order Objects** and **Group Objects**. **Order Objects** allows you to organize which objects are visually on top of other objects. This feature is useful when working with multiple overlapping objects. **Group Objects** allows you to group several

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objects together, so that selecting any one of the objects will automatically select all items in the group.

- The **Align** command expands to allow you to **Horizontally** or **Vertically** align objects and **Position Objects**. These commands are used in aligning the sides, tops, bottoms, centers, heights and/or widths of the selected objects.

3.3.2.1 The Quick Access Toolbar

With the default **Quick Access Toolbar**, there are icons (Figure 3.14) to **Save** the current layout or to **Undo** and **Redo** the last user action. FCS Express remembers every action that you have taken. Therefore, you can undo an unlimited number of actions, allowing you to revert to any earlier state of your layout.

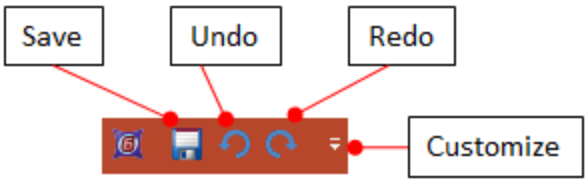

Figure 3.12 Quick Access Toolbar Icons

Clicking the **Customize** command (tooltip "More Buttons") brings up a gallery (Figure 3.15) which allows you to fully customize the interface.

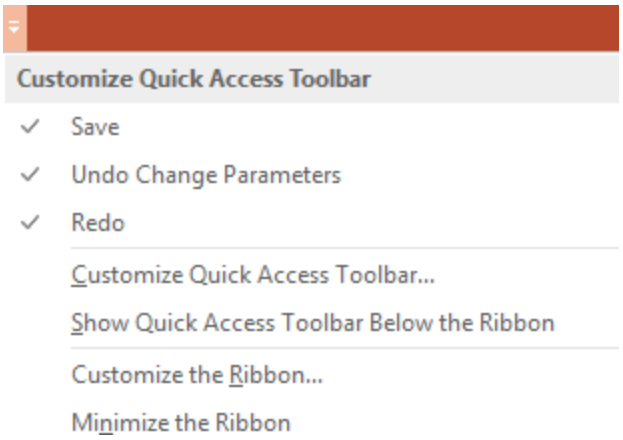

Figure 3.13 Customize Menu

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The **Customize Quick Access Toolbar...** item brings up a **Customize** dialog (Figure 3.16). **Commands** or individual Tabs can be chosen in the left column to be added to or removed from the **Quick Access Toolbar** in the right column, where they can be rearranged.

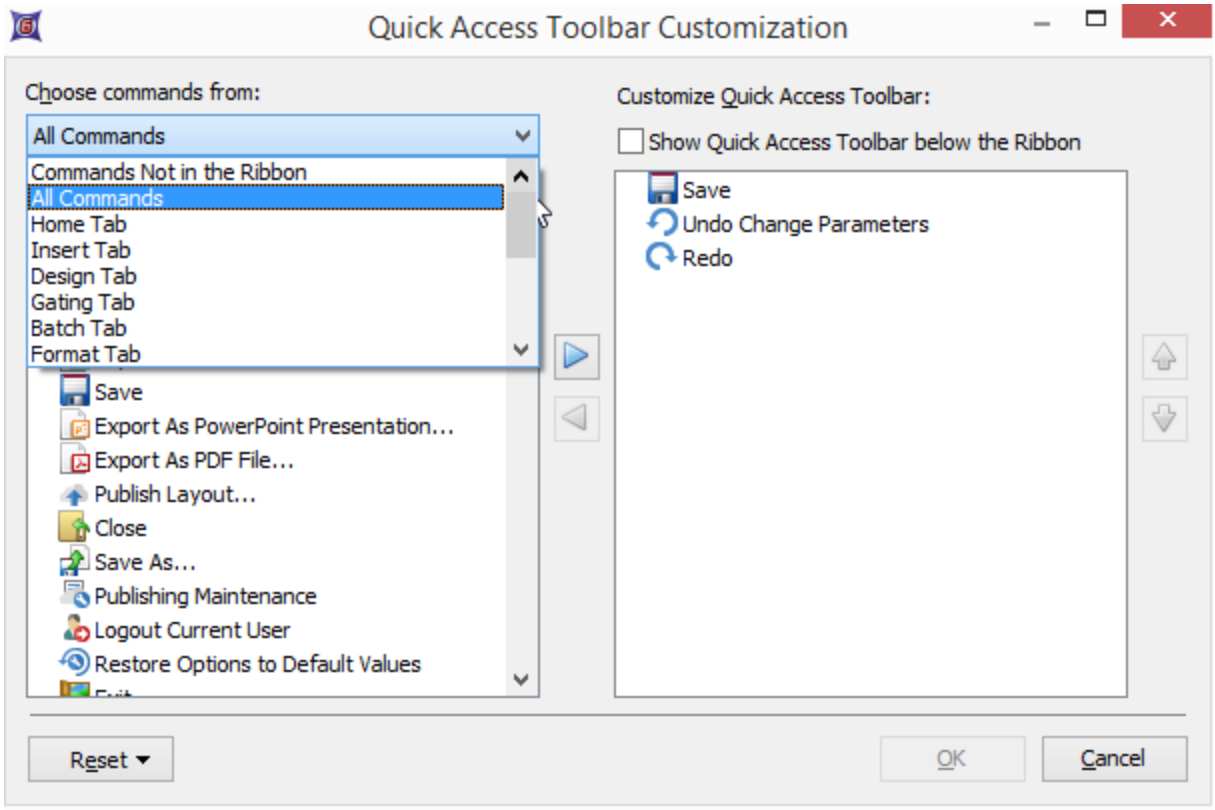

Figure 3.14 Quick Access Toolbar Customization dialog

Under **File** tab→**System**→**Restore Options** drop down, the **Reset Toolbars to Default Settings** command will revert your changes to the manufacturer default (Figure 3.17).

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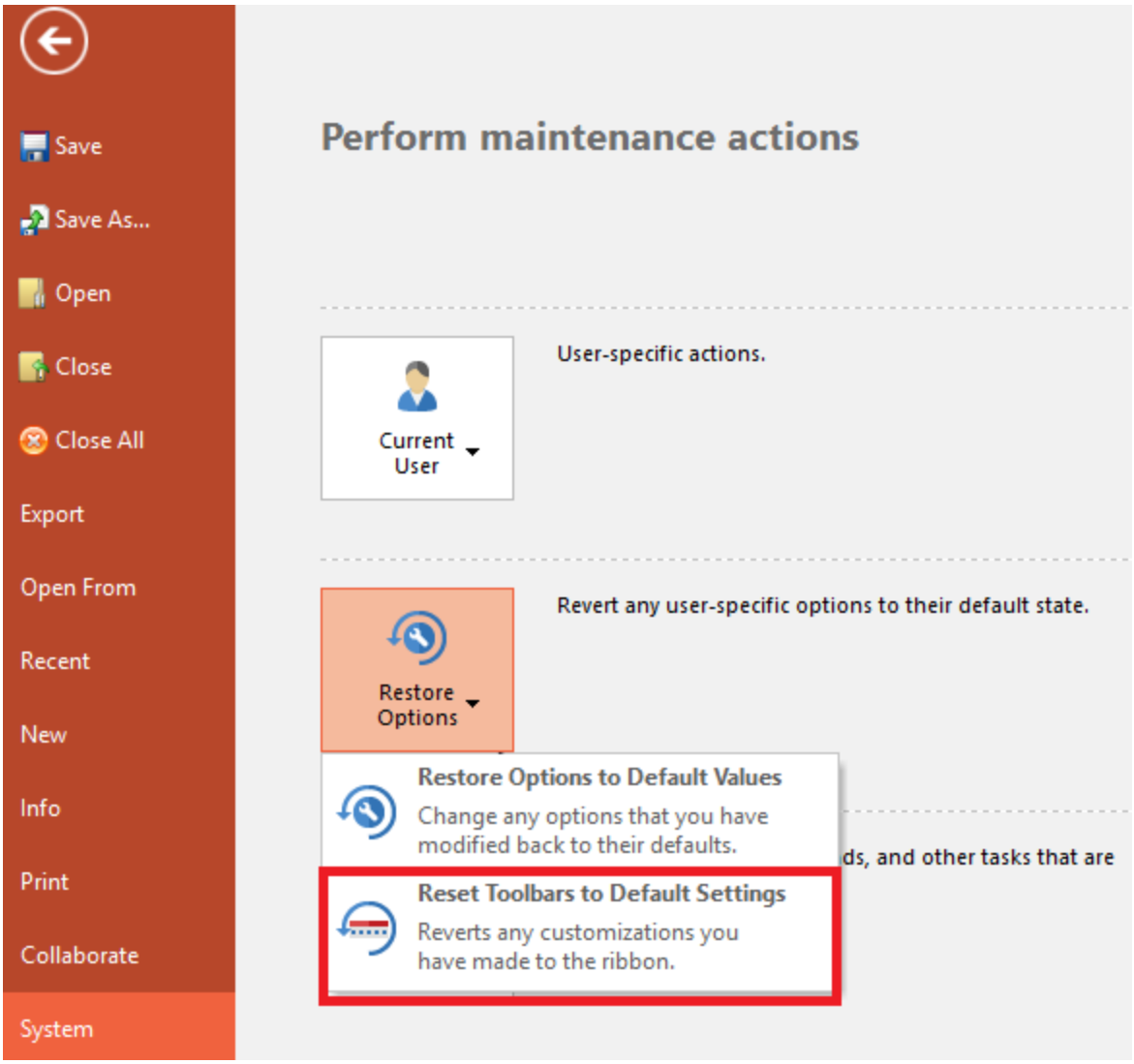

Figure 3.15 - System Restore Options

3.3.2.2 Customizing the Ribbon

In addition to customizing the interface using the customize command to the right of the Quick Access Toolbar 65, right-clicking on an individual command within a group, or right-clicking on the name of a group, brings up a menu (Figure 3.18) which includes the command to **Add to Quick Access Toolbar** (or group of commands) to the Quick Access Toolbar 65.

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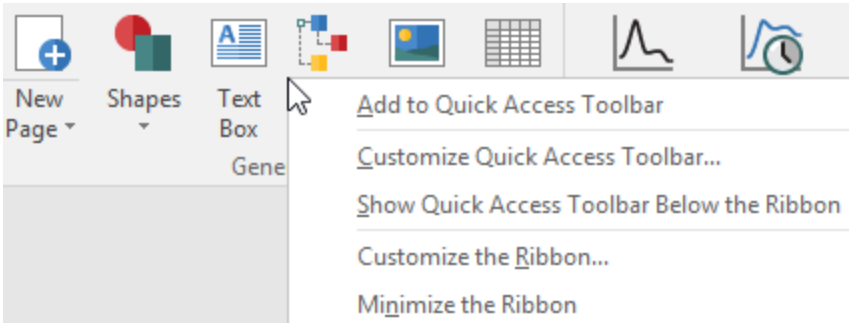

Figure 3.16 Add Command to Quick Access Toolbar

The **Customize the Ribbon...** item brings up a **Ribbon Customization** dialog (Figure 3.19). **Commands** or the **Home**, **Tools**, or **Custom** tabs can be chosen in the left column to be added to or removed from the **Quick Access Toolbar** in the right column, where they can be rearranged or hidden by unchecking the checkboxes.

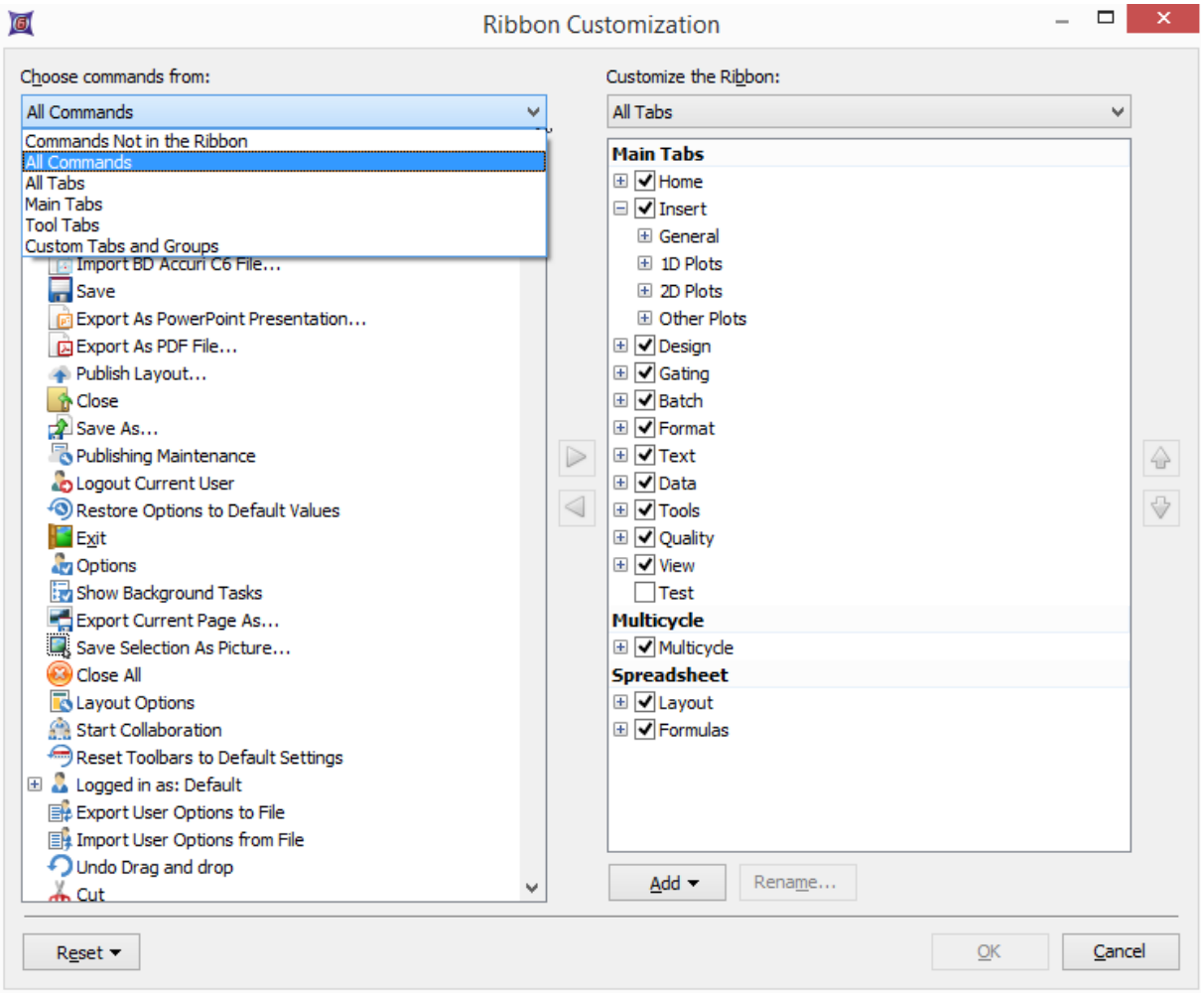

Figure 3.17 - Ribbon Customization dialog

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When the **Customize** dialog is open, you can perform many customizations to the Ribbon 55. To remove items from the Ribbon: click on the item to remove, hold down the mouse button, drag the item to an area within the Customize dialog, and release the mouse. If you drag the item to another part of the Ribbon, it will be moved to the position where you release the mouse. To add commands to the Ribbon, select the **Commands** tab. Select a command from the list and drag it to the position on the Ribbon (or Quick Access Toolbar) where you wish it to appear.

You can reset an individual group (toolbar) to its default state by selecting the toolbar in the **Toolbars** tab and pressing the **Reset** button in the bottom left corner of the window.

Under the **File** tab→**System**→**Restore Options**→**Reset Toolbars to Default Settings** command will restore all toolbars to the default settings.

3.3.3 The Insert Tab

The **Insert** Tab (Figure 3.20) contains four **groups** for inserting objects on a page:

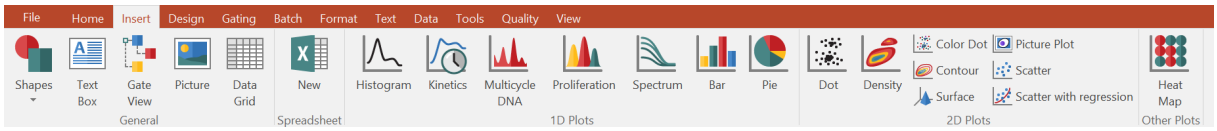

Figure 3.18 Insert Tab

- **General**
  - The top half of the **New Page** command adds a new page to your layout. The bottom half of the **New Page** command opens a gallery with numerous choices for adding a new page with Place Holders for predefined layouts containing various text boxes, plots, and statistics.
  - The **Shapes** command is for inserting a new shape, such as a **Line**, **Arrow**, **Ellipse,** or **Rectangle**.
  - The **Text Box** command inserts a new text box.
  - The **Gate View** command allows the user to insert a Gate View window, from which the properties of all your gates may be viewed and changed.

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- The **Picture** command inserts a picture from a file on your computer.
- The **Data Grid** command allows the user to view data in a table (spreadsheet) format or work with custom data.

- **Spreadsheet**
  - The **New Spreadsheet** command opens up a dialog window for creating a new spreadsheet (Figure 3.21), from which data values and statistical calculations can be plotted in FCS Express. Selecting an inserted spreadsheet will reveal the Spreadsheet Tab group.

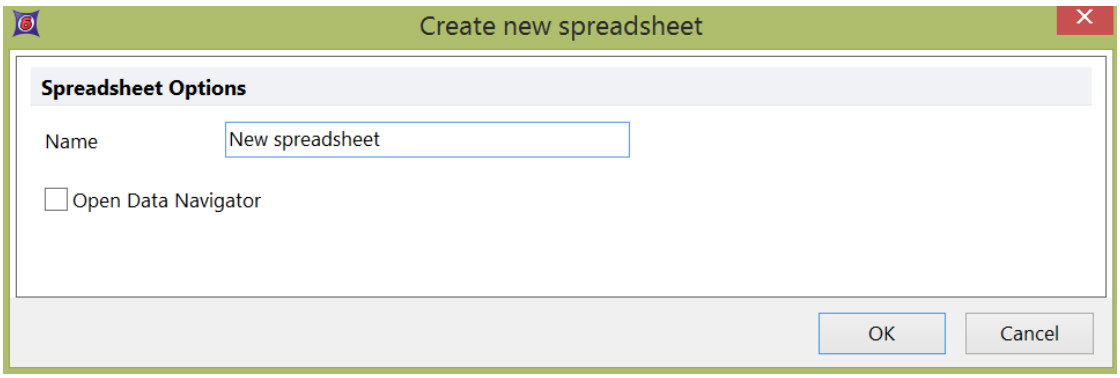

Figure 3.19 - Create New Spreadsheet Dialog

- **1D Plots**
  - The six commands in the 1D Plot group insert Histograms, Kinetics plots, Multicycle DNA histograms, Proliferation histograms, Spectrum, Bar Plots, and Pie Charts.

- **2D Plots**
  - The eight commands in the 2D Plot group insert two-dimensional **Dot** plots, **Density** plots, **Color Dot** plots, **Contour** plots, **Surface** plots, Scatter plots and Scatter with Regression.

- **Other Plots**
  - The Heat Map command inserts a **Heat Map** which is used to view statistical values across an entire microtiter plate.

3.3.4 The Design Tab

The Design Tab (Figure 3.22) contains the **Page Layout** group, which includes the following commands:

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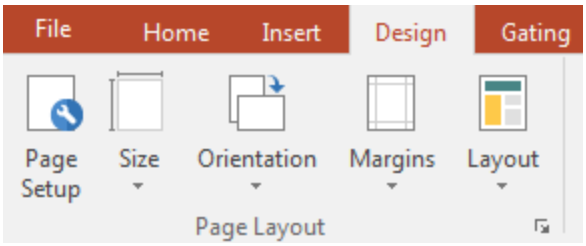

Figure 3.20 Design Tab

- The **Page Setup** command allows you to determine the page Size, Orientation, Margins with any default or custom values as well as to choose to **Maintain the size of all objects on the layout**.
- The **Size** command allows you to pick a page size from a list of default sizes.
- The **Orientation** command allows you to choose to view the layout in a portrait or landscape mode.
- The **Margins** command allows you to choose from four default margin size.
- The **Layout** command opens a gallery for adding Place Holders for predefined text boxes, plots, and statistics to the current page.

3.3.5 The Gating Tab

The **Gating** Tab (Figure 3.23) contains the following commands for working with gates:

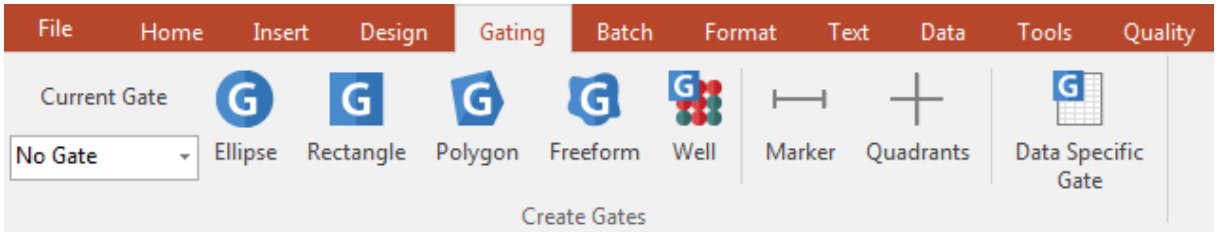

Figure 3.21 Gating Tab

- **Create Gates** is the group under which all of the following fall:
  - commands for creating gates (**Ellipse**, **Rectangle**, **Polygon**, **Freeform**, Well), Markers (**Create Marker**), and **Quadrants** on a plot. It also

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allows you to quickly see which gate is applied to a plot. These functions are described in more detail in the section on Using Gates 316.

- the **Data Specific Gate** command, which permits the assignment of unique gating positions to specific data files. Use of this new feature in FCS Express 6 is described in more detail in the section on Data Specific Gates 324.

Note: adjusting gates on a per-datafile basis during Batch Processing is still possible in FCS Express 6, as in all previous versions of FCS Express.

3.3.6 The Batch Tab

The **Batch** Tab (Figure 3.24) contains two **groups**.

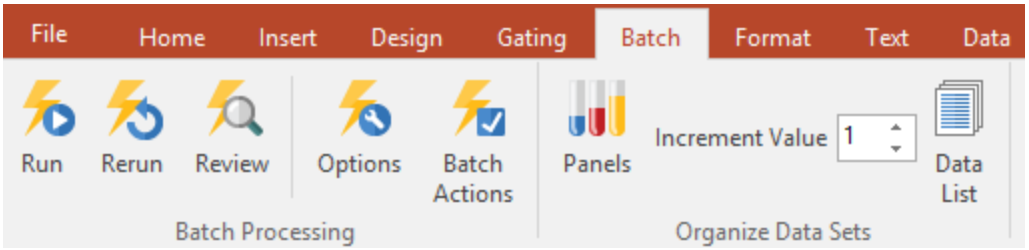

Figure 3.22 Batch tab

All of the commands associated with this Tab are described in detail in the chapter on Batch Processing 784.

- **Batch Processing**
  - Once you have set up and verified all your batch processing options and actions, the **Run** command executes all your actions including printing, saving, and reporting across all samples.
  - Within the **Batch Processing** command you may choose the following options:
    - **Run** - Runs the batch actions with the same gates applied to all samples.
    - **Rerun** - Reruns the previous batch run using any modifications made to the layout when the batch process was run with pauses.

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- **Review** - Runs a series of batch iterations over samples without exporting any actions.
- The **Options** command brings up the **Batch Processing Options** 843 Window where you can:
    - Set the number of iterations to perform.
    - Choose whether or not to run alerts before batch processing.
    - Decide if you want to pause between iterations and which iterations to pause at if iterations are flagged or certain conditions are met.
    - Restore the original files on the layout after batch processing.
- The **Batch Actions** command brings up the **Batch Actions** 803 window where you can set various actions associated with printing, reporting, and saving layouts (to various formats) while batch processing.

- **Organize Data Sets**
    - The **Panels** command brings up the Panel Information dialog for working with Panels 860.
    - In the **Increment Value** field, you can set the increment value, which is the number of files to increment after each iteration in a batch process. An increment value of 1 means to move to the next file.
    - The **Data List** command brings up the dockable Data List 785 window to process the list of current files (or custom data).

3.3.7 The Format Tab

The **Format** Tab (Figure 3.25) contains seven **groups**. The commands allow you to change the properties of one or more selected objects.

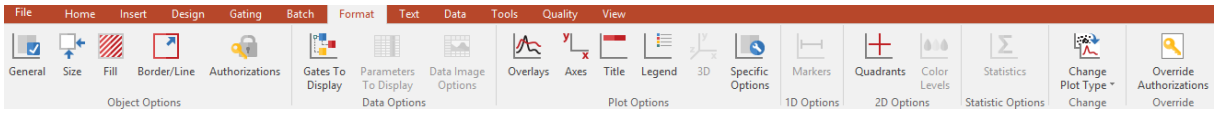

Figure 3.23 Format Tab

- **Object Options** 239: Each command brings up a dialog box to set object properties.
  - The **General** command is for assigning or changing the object name and the object visibility 240.
  - With the **Size** command, you can change or scale the height and width of objects. An option exists to lock the aspect ratio so that the ratio of the height to width are kept constant.
  - The **Fill** command is used for setting the objects background characteristics (color, image), changing the margins of the object, and adjusting the frame.
  - The **Border/Lines** command is used to set the style, color, and width of lines surrounding the border of the selected object(s).
  - The **Authorizations** 1136 command is used to define what select and edit actions are accessible for the object(s).
- **Data Options**
  - The **Gates to Display** command is used for choosing which gates to display in a plot or Data Grid.
  - The **Parameters to Display** command allows you to choose which parameters are shown in a data grid.
  - The **Data Image Options** command controls image placement in the cells of a Data Grid.
- **Plot Options**
  - The **Overlays** command is used to choose the characteristics of an overlay including, x- and y-parameters, gates, compensation, color, and dot size, among other things.
  - The **Axes**, **Title**, and **Legend** commands allow you to format and position the text used in these areas of the object.
  - If you are working with a histogram or surface plot, the **3D** command is used to format the properties of the display, such as zoom, rotation, elevation, offsets, and perspective.
  - The **Specific Options** command allows you to change specific options that are related to the particular plot that is selected, e.g., the resolution of a plot, and to indicate how many cells to plot.

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- **1D Options**

  If marker(s) have been created, the **Markers** command is used for formatting the properties of the markers on single-parameter histograms.

- **2D Options**

  - With the **Quadrants** command, you can format the quadrants of a dual-parameter plot.

  - If you are working with a 2D color plot (e.g., color dot plot, density plot, contour plot) the **Color Levels** command is used to format the properties of the colors displayed.

- **Statistic Options**

  If statistics are displayed in a default statistics window, the **Statistics** command allows you to format the properties of the statistics window.

- **Change**

  The **Change Plot Type** command allows you to change the type of a selected plot. By clicking on the **down arrow** associated with the **Change Plot Type** command, you expand the item to reveal all of the possible plot types.

- **Override**

  The **Override Authorizations** command allows you to override any Authorizations set on objects. See the Overriding Authorizations 1140 topic for additional details.

3.3.8 The Text Tab

The **Text** Tab (Figure 3.26) contains four **groups**.

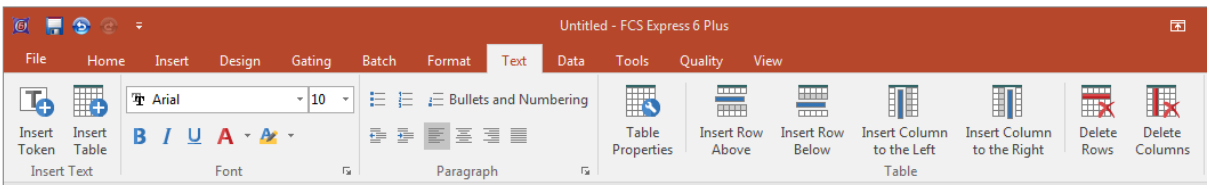

Figure 3.24 Text Tab

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- **Insert Text**

  If a text box is selected, the **Insert Token** command allows you to choose the type of token to insert. The **Insert Table** command is used for inserting a table into a text box and specifying its properties.

- **Font and Paragraph**

  The commands in the **Font** and **Paragraph groups** are used to format the text manually in the current cursor position just as you would in word processing software. If text is selected, the formatting is applied to the selected text. You can display standard **Font** or **Paragraph** dialogs to format multiple properties at the same time by clicking on the Dialog Box Launcher Buttons 55 (little arrow icons at the bottom right) of the **Font** and **Paragraph groups**. While the font and paragraph commands usually apply to text boxes, some paragraph properties can apply to non-text box options. For instance, changing the justification of a data or stats window will change the alignment of the text.

- **Table**

  If a table has been inserted in a text box and the table is selected, the commands in the **Table group** can be used to:

  - Display the table properties.
  - Insert rows above or below the current position.
  - Insert columns to the left or right of the current position.
  - Delete the last row or column of the table.

3.3.9 The Data Tab

The **Data** Tab (Figure 3.27) contains six **groups**:

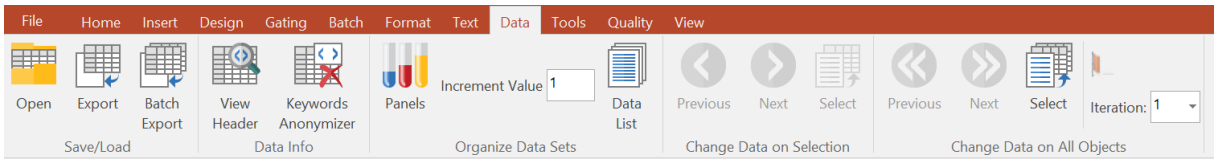

Figure 3.25 - Data Tab

- **Save/Load**

- The **Open** command brings up the **Select a Data File** dialog (Figure 3.28) which allows you to select a data file (e.g., an FCS file). The **Select a Data File** dialog contains a **File Dialog** tab for accessing files anywhere on your computer, a **Data List** tab which allows you to see which files/data sets you are working with in the current experiment, an **Active Files** tab (i.e., the files currently open in the layout), an **MDC Store** tab (accessing files from the Molecular Devices MDC Store (Image Cytometry Version)) and a **Building Blocks** tab (accessing files from PerkinElmer building blocks (Image Cytometry Version)). Using the **Files of type:** drop-down list, you can choose the file extension, e.g., files from different instrument manufacturers.

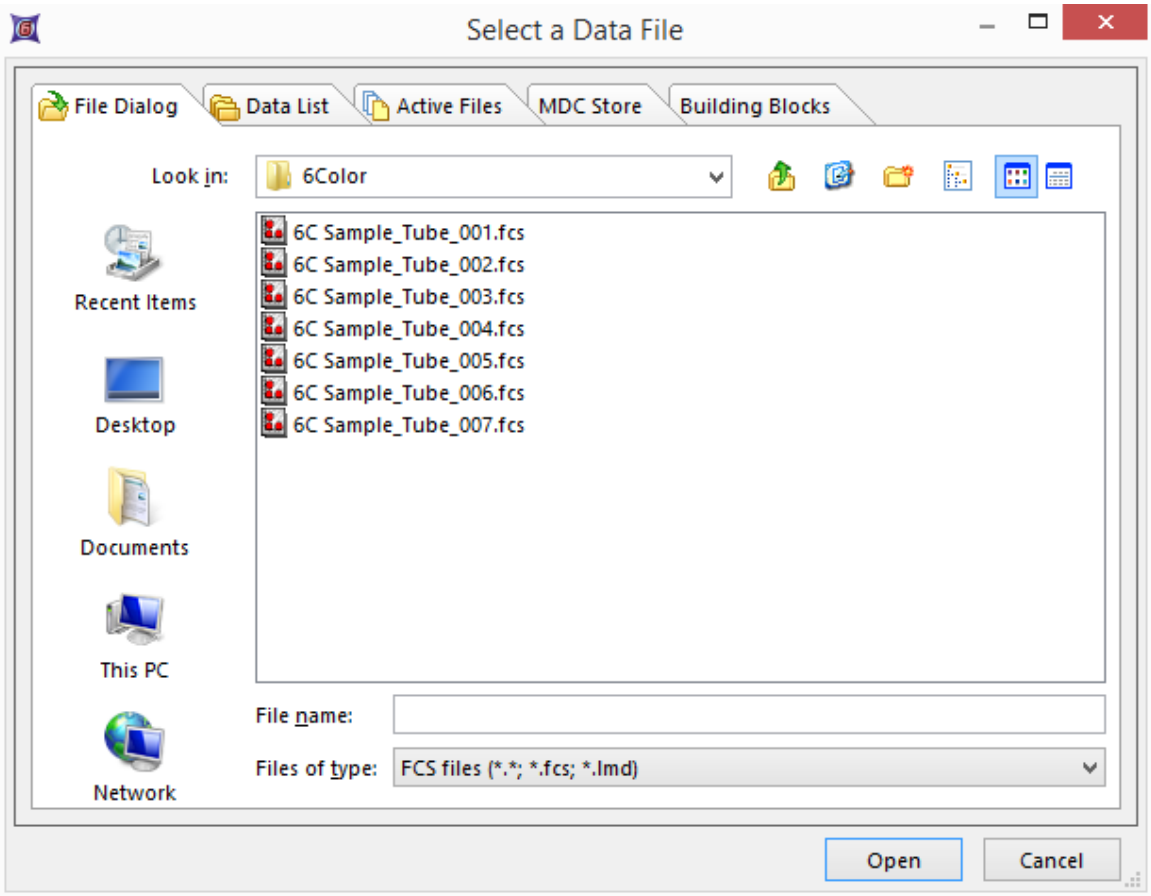

Figure 3.26 - Select a Data File Dialog

Once a data file is opened, choose which type of plot(s) to open from the **Select Plot Types ...** dialog (Figure 3.29).

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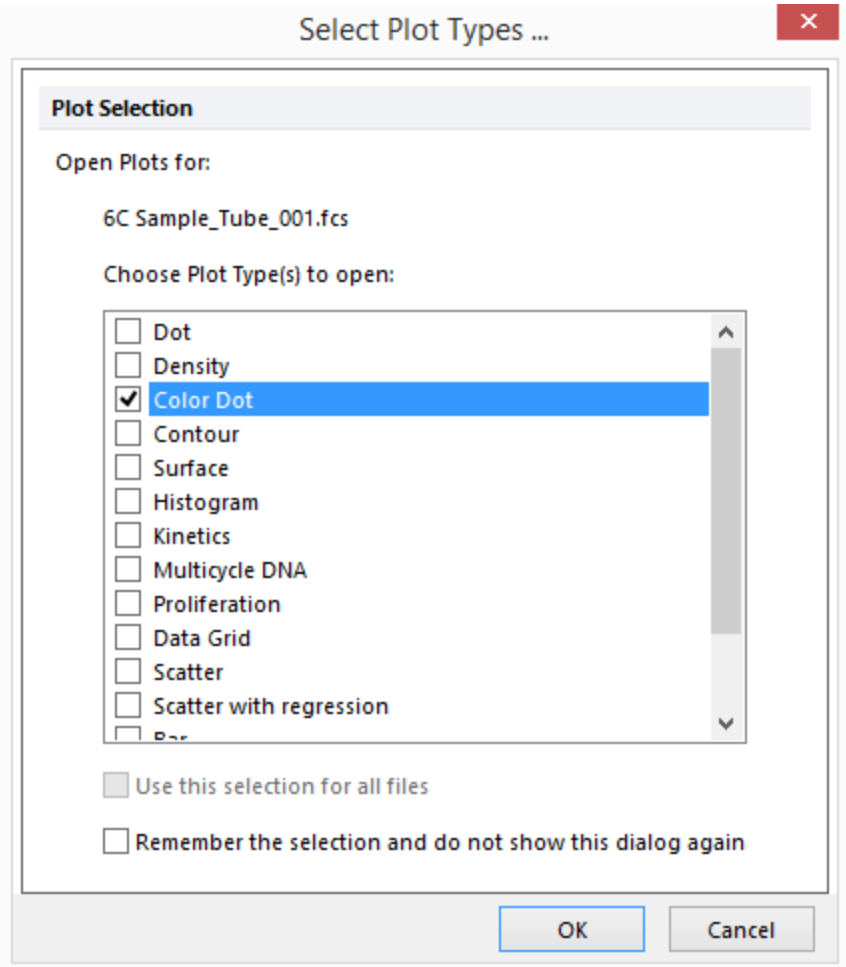

Figure 3.27 - Select Plot Types Dialog

- With the **Export** command, you export the data from a single open file 666.
- You can export data from multiple files consecutively using the 670 **Batch Export** 670 command.

- **Data Info**
  - If a plot is selected, the **View Header** command brings up a dialog box which displays the FCS file header information for that file. If no plot exists on the layout, the **Select a Data File** dialog is displayed, so you can choose which header information to display.

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- The **Keywords Anonymizer** 658 command brings up the **Select Keywords to Anonymize** window, which allows you to remove any or all keywords from a data file.

- **Organize Data Sets**
  - The **Panels** command brings up the Panel Information dialog for working with panels 860.
  - In the **Increment Value** field, you can set the number of files to increment after each iteration in a batch process, or when pressing Next/Prev on Selection or All Objects (below). An increment value of 1 means to move to the next file in the Data List.
  - The **Data List** command brings up the dockable Data List window to process the list of current data 785 files 785.
- **Change Data on Selection**
  - **Previous** changes the .fcs file to the previous file in the **Data List** on the selected plot or plots.
  - **Next** changes the .fcs file to the next file in the **Data List** on the selected plot or plots.
  - **Select** brings up the **Select a Data File** dialog from which you can choose an .fcs file to display in the selected plot or plots.

- **Change Data on All Objects**
  - **Previous** changes the .fcs file to the previous file in the **Data List** on all plots.
  - **Next** changes the .fcs file to the next file in the **Data List** on all plots.
  - **Select** brings up the **Select a Data File** dialog from which you can choose an .fcs file to display in all plots.
  - **Flag this iteration** marks the selected file or iteration to be flagged for review in the Batch Process or Data List.
  - **Iteration** allows you to choose an iteration from a drop down menu that will be displayed in all plots.

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3.3.10 The Tools Tab

The **Tools** tab (Figure 3.30) contains four **groups**:

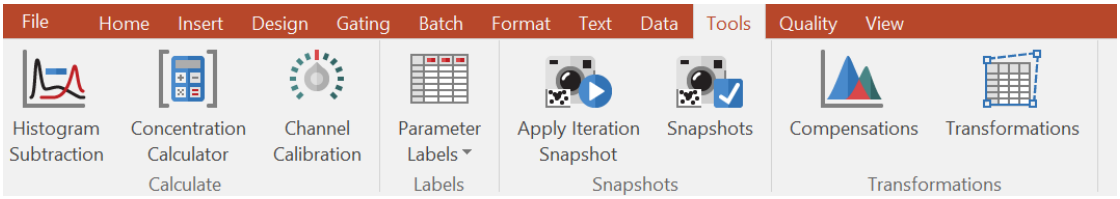

Figure 3.28 - Tools tab

- **Calculate**
  - The **Histogram Subtraction** command opens the **Histogram Subtraction** window where you can apply four mathematical methods of comparing sample and control histograms 598.
  - The **Concentration Calculator** command opens the **Concentration Calculator** window where you can calculate the concentration of events in a sample 592.
  - The **Channel Calibration** command opens the **Channel Calibration** window where you can change the axis fluorescence intensity scales from a linear or logarithmic scale to one indication either the absolute number of antigen molecules or the number of binding sites on the surface of a cell 614.
- **Labels**
  - The **Parameter Labels** opens a gallery containing **Modify Labels**, **Load Labels**, and **Save Labels** commands. These items allow the user to easily create, change, recall, and save alternate parameter labels 410.
- **Snapshots**
  - The **Apply Iteration Snapshot** command allows you to move gates between iterations and have the gates' new positions remembered while navigating using the Next and Previous commands 376.
  - The **Snapshots** command opens the **Snapshot Navigator** window where you can take custom snapshots of your analysis on data files and apply that analysis to other files 374. For example, a snapshot of a gate position may be taken, the gate position changed, and snapshot applied to go back to the original gate position.

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- **Transformations**
  - The **Compensations** command opens the **Compensations Navigator**, which allows you to display the compensation information on the selected file, create new compensations, and load or save compensation information 626.
  - The **Transformations** command opens the **Transformations Navigator**, which allows you access **K means (cluster analysis)** 958, **Principal Component Analysis** 946, Parameter Math 933 (defines a single or sequence of math functions, save the functions, and apply the functions to data files 933), **R integration** 973 to add new parameters or transformations, and **tSNE** 1000.

3.3.11 The Quality Tab

The **Quality** tab (Figure 3.31) contains two **groups**:

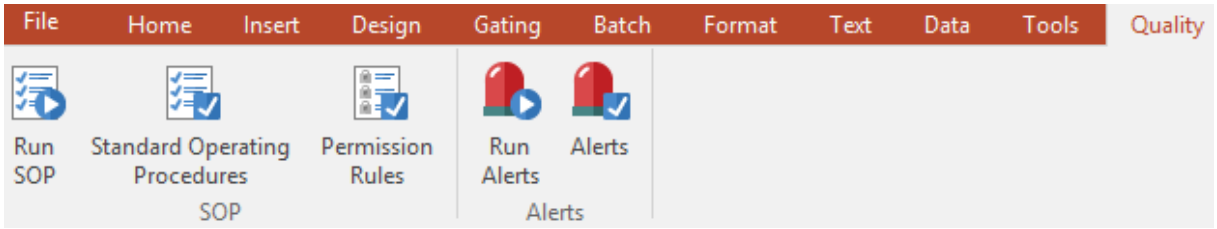

Figure 3.29 - Quality tab

- **SOP**
  - The **Run SOP** command begins the step wise process of running through all of the steps defined in the SOP creation process 1106
  - The **Standard Operating Procedures** command opens the **Standard Operating Procedures** 1105 navigator 1105.
  - The **Permission Rules** command opens the **Permission Rules** Navigator where you can set available permissions 1127 specific to a layout.
- **Alerts**
  - The **Run Alerts** command can be run once at least one alert is created. When clicked, FCS Express will evaluate the formulas written in the **Alerts** Navigator.

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- The **Alerts** command opens the Alerts Navigator (only accessible with FCS Express IVD Version) where you create **True or False** statements for evaluation.

3.3.12 The View Tab

The **View** Tab (Figure 3.32) contains six **groups**:

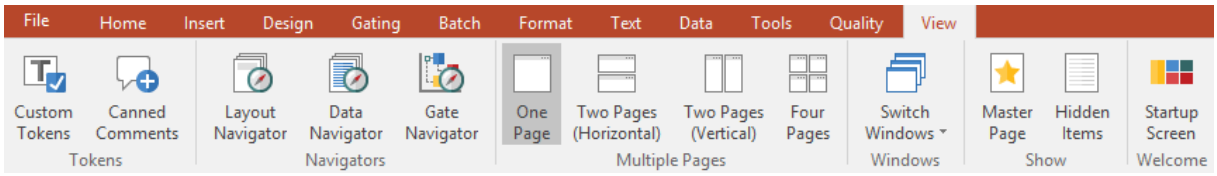

Figure 3.30 View Tab

- **Tokens**
  - The **Custom Tokens** command brings up a dockable **Custom Tokens** window where you can create new custom tokens[728] to generate your own custom statistics.
- **Navigators**
  - The Layout Navigator[123] command brings up a dockable **Layout Navigator** window listing each item in the layout.
  - The Data Navigator[126] command brings up a dockable **Data Navigator** window listing the parameters of each file referenced in the layout.
  - The Gate Navigator[128] command brings up a dockable **Gate Navigator** window describing the gates which have been defined on plots within the layout.
- **Multiple Pages**
  - In the **Multiple Pages** group, there are four commands [**One Page**, **Two Pages (Horizontal)**, **Two Pages (Vertical)**, and **Four Pages**] for displaying the entire layout in different views[109].
- **Windows**

  If there are multiple layouts open, the **Switch Windows** command allows you to choose which layout is active.
- **Show**

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- The **Master Page** command shows the Master Page.
- The **Hidden Pages** command is used to show pages that the user has previously chosen to hide in the layout.

- **Welcome**
  - The **Startup Screen** command allows you to view or hide the Startup Screen.

3.3.13 The Multicycle Tab

The Multicycle Tab[892] allows you to work with the Multicycle AV plugin[884] from Phoenix Flow Systems. To access this tab, a **Multicycle** plot must be inserted. When hovering over a model button, its name will appear (Figure 3.33).

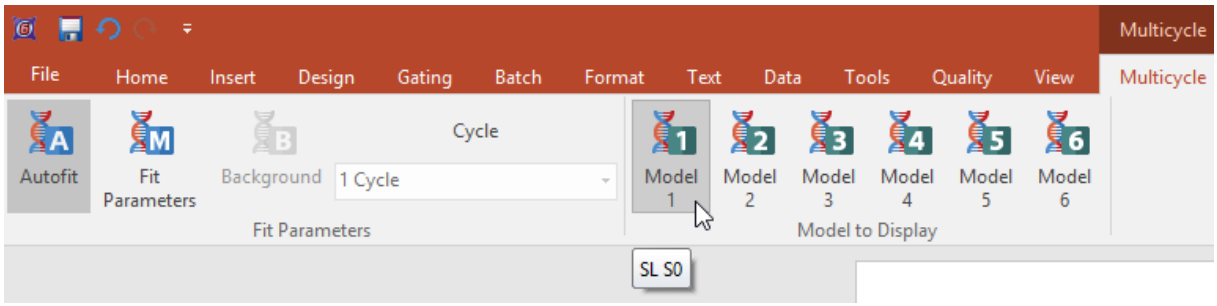

Figure 3.31 Multicycle Tab with Model 1 Name while hovering

It contains two groups:

- **Fit Parameters**

  The **Autofit** command automatically adjusts cycle parameters. In manual mode, the **Fit Parameters** command brings up a dialog to format the Fit Model Properties, and the **Background** command brings up a dialog to format background options. The **Cycle** drop-down list allows you to choose the number and kinds of populations to model.

- **Model to Display**

  The six **Model** commands are used to set the currently displayed model.

3.3.14 The Spreadsheets Tab Group

Once you insert a spread sheet from the data tab and put it in selected mode, the spreadsheets tab group will appear with layout and formulas tab.

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3.3.14.1 The Layout Tab

The **Spreadsheets** 414 **Layout** tab (Figure 3.34) contains seven **groups**:

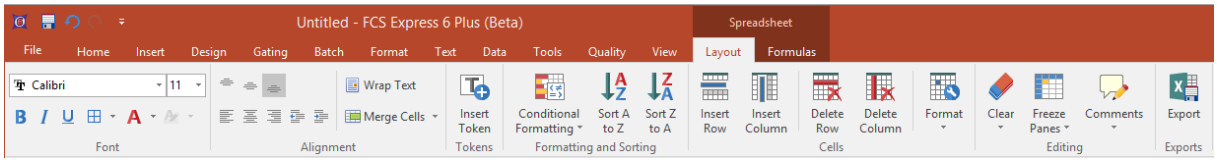

Figure 3.32 - Spreadsheet Layout tab

- **Font**

  The **Font Name**, **Font Size**, **Font Color**, and **Font Background Color** can be changed for the selected object(s) with **Bold**, **Italics**, and **Underline** options available.

- **Alignment**

  - Alignments and indentations can be applied.
  - The **Wrap Text** command sets text to wrap in a cell.
  - The **Merge Cells** drop down allows you to **Merge Cells**, **Merge Across**, **Merge and Center**, or **Unmerge Cells** (Figure 3.35).

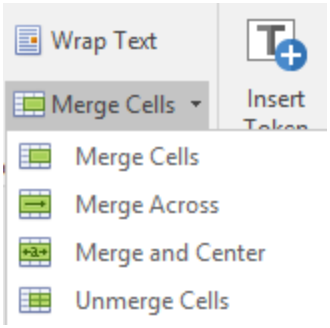

Figure 3.33 - Merge Cells drop down list

- **Tokens**

  The **Insert Tokens** command will open the Insert a Token dialog 692.

- **Formatting and Sorting**

  - The **Conditional Formatting** drop down allows you to access the following:

- **Color Scales**, **Data Bars**, **Icon Sets**, **Top/Bottom Rules** (Figure 3.36),

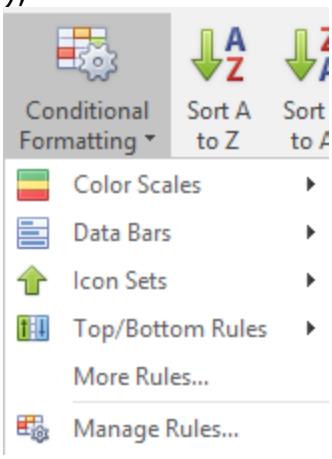

Figure 3.34 - Conditional Formatting drop down list

- **More Rules...**, which opens the **Conditional Formatting Rule** window (Figure 3.37),

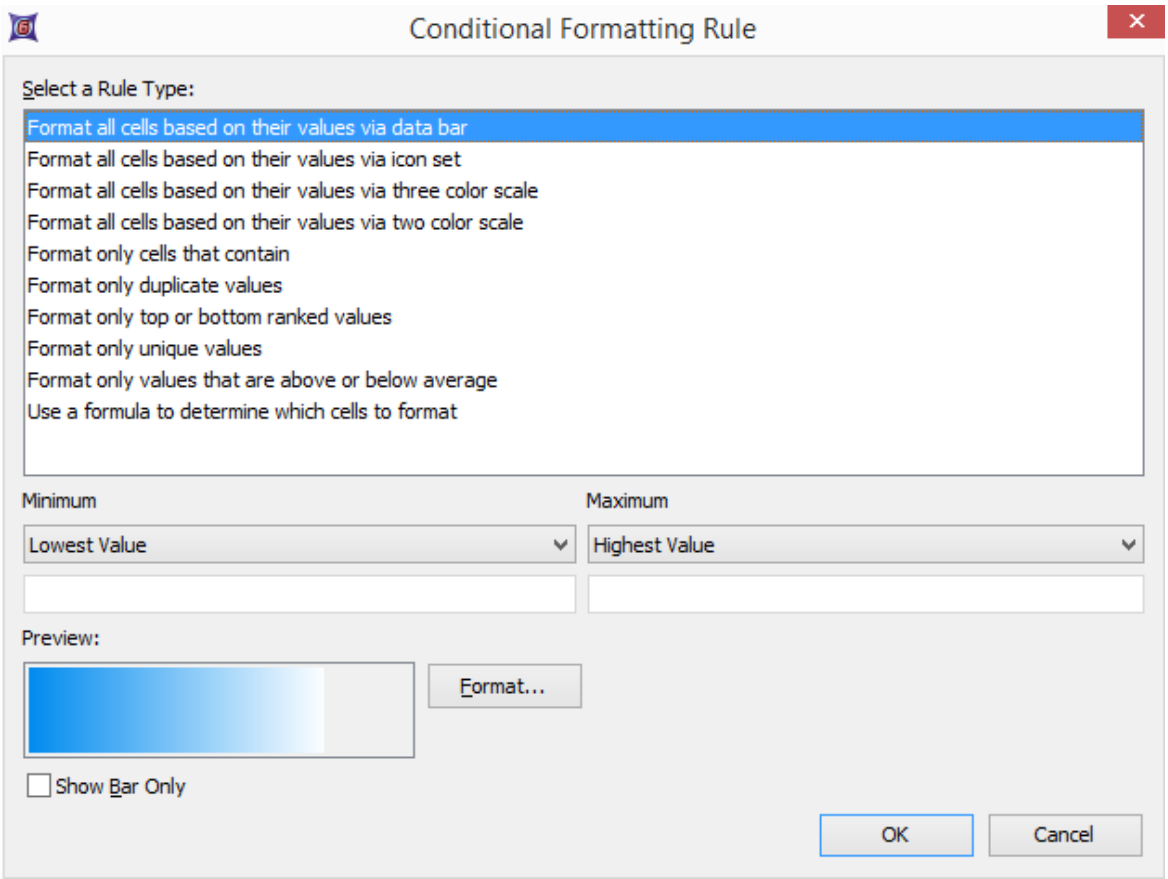

Figure 3.35 - Conditional Formatting Rule window

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- and **Manage Rules...**, which opens the Conditional Formatting Rule Manager (Figure 3.38) to create new rules to be applied to the **Current Selection** or **Sheet**.

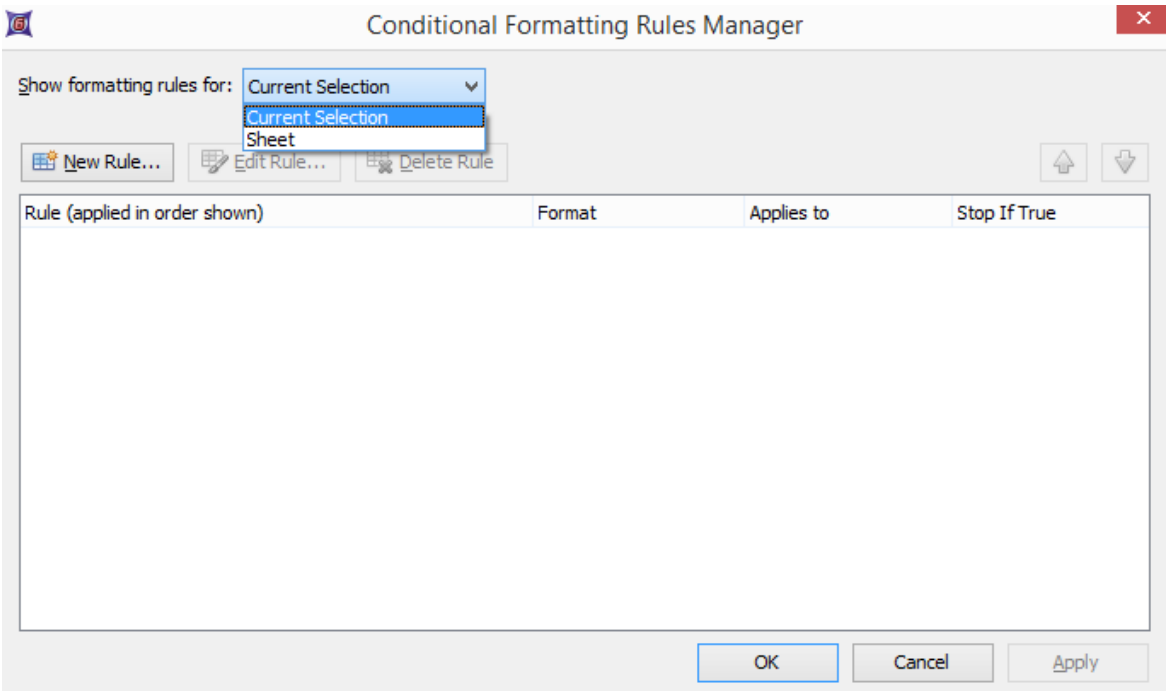

Figure 3.36 - Conditional Formatting Rule Manager

- The **Sort A to Z** command allows you to sort the selected cells in alphabetical order.
- The **Sort Z to A** command allows you to sort the selected cells in reverse alphabetical order.

- **Cells**
  - The **Insert Row** command adds a row above the selected cell(s).
  - The **Insert Column** command adds a column to the left of the selected cell(s).
  - The **Delete Row** command removes a row below the selected cell(s).
  - The **Delete Column** command removes a column to the right of the selected cell(s).
  - The **Formatting** drop down list (Figure 3.39) allows you to perform the following:

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- **AutoFit Row Height**
- **AutoFit Column Width**
- Access **Hide and Unhide** list to **Hide Rows**, **Hide Columns**, **Unhide Rows**, or **Unhide Columns**

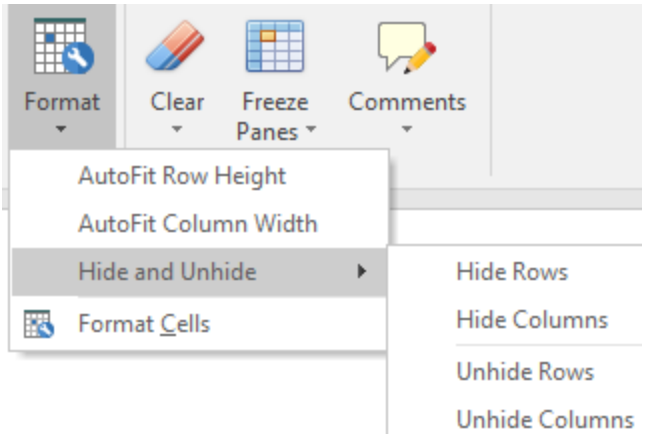

Figure 3.37 - Cells Formatting drop down list

- Open **Format Cells** window (Figure 3.40)

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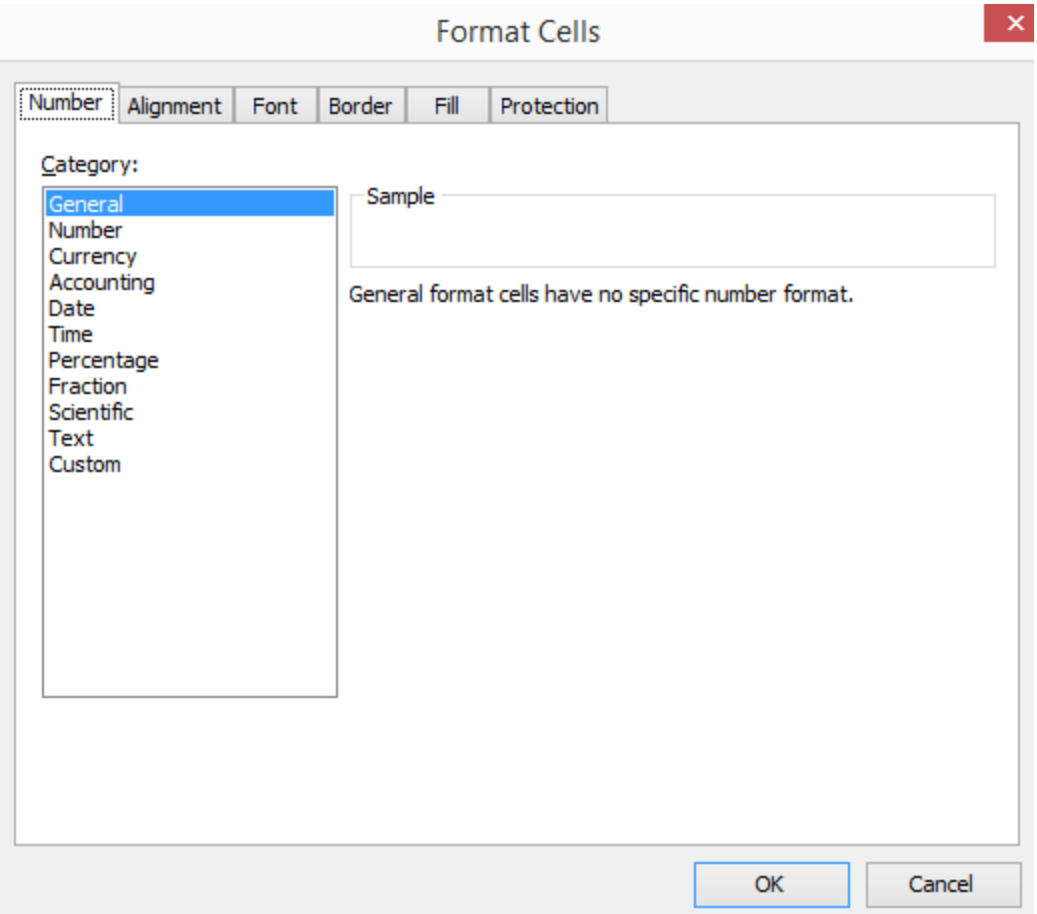

Figure 3.38 - Format Cells window

- **Editing**
  - The **Clear** drop down list allows you to **Clear All**, **Clear Formatting**, and **Clear Contents** of selected cell(s) (Figure 3.41).

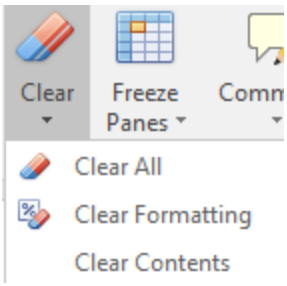

Figure 3.39 - Clear drop down list

  - The **Freeze Panes** drop down list allows you to **Freeze Panes**, **Unfreeze Panes**, **Freeze First Row**, and **Freeze First Column** (Figure 3.42).

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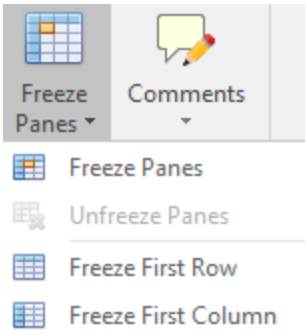

Figure 3.40 - Freeze Panes drop down list

- The **Comments** drop down list allows you to add **New Comment**, **Edit Comment**, **Delete Comments**, move to **Next Comment** or **Previous Comment**, and **Show/Hide Comments** (Figure 3.43).

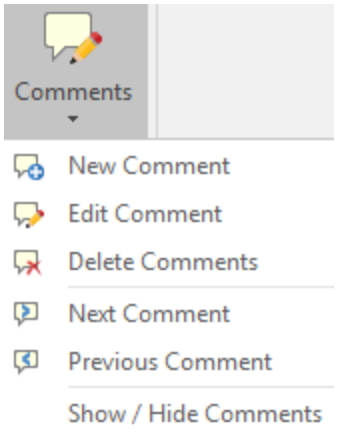

Figure 3.41 - Comments drop down list

- **Exports**
  - The **Export** command allows you to export data contained in the spreadsheet to various formats: .xlsx Excel workbook, .xls 97-2003 Excel Workbook, .html Web Page, .csv Comma Delimited Value files, .xml Data and .txt tab delimited files. When exporting as to Excel compatible formats equations and formatting from the FCS Express spreadsheet will be retained.

3.3.14.2 The Formulas Tab

The **Formulas** tab (Figure 3.44) contains the **Function Library** group with the following drop down lists: **AutoSum, Financial, Logical, Text, Date and Time,**

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Lookup and Reference, Math and Trig, Statistical, Information, and Compatibility 440.

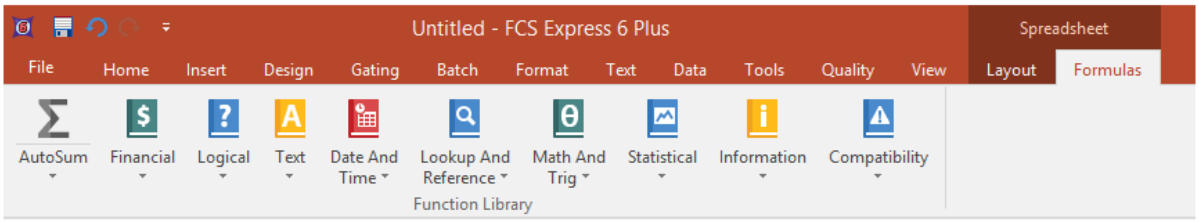

Figure 3.42 - Spreadsheet Formulas tab

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Chapter

4 Working with Layouts

Layouts (.fey files) are the file type of FCS Express. They are usually saved with a link to your .fcs data files, and contain all elements of your analysis including gates, statistics, batch processes, and more.

4.1 Introduction to the Layout

The *layout* is the name for an FCS Express document. The layout contains and organizes plots, regions, quadrants, statistics, text boxes, graphics, printer settings, and more. There is no limit (other than your computer memory) to the number of layouts that can be open at one time. It is possible to copy objects easily from one layout to another. There are many other powerful formatting and graphics features in FCS Express that allow you to display data exactly as you wish.

The layout organization in FCS Express is similar to that in a Microsoft PowerPoint® presentation. Like a presentation containing a series of slides with text and graphics, each FCS Express layout contains a page or series of pages with text boxes, plots, statistics, and other objects. Similar to a slide in PowerPoint, each page in an FCS Express layout can be modified, saved, printed, or exported. The layout itself can be set up with different background colors or images on each page. In addition, a saved layout can be used as a template for batch processing of selected files, or can be linked to specific data files. When a linked layout file is reopened, the data files and plots used to create it are displayed. This feature is useful in documenting clinical cases by providing an archival record of the analysis, or for recreating any kind of repetitive analysis.

Any object inserted into a layout, whether it is a plot, text box, statistics window, or graphic, can be manipulated in many different ways. Again, it is helpful to think of objects on a layout page as if they were objects inserted onto a slide in PowerPoint. Single or multiple objects can be selected, and once selected, the object(s) can be formatted, moved, resized, or aligned with one another.

4.1.1 Creating a New Layout

A new layout can be created by choosing one of the following options:

- Select the **New Layout** button from the Startup Screen 54 (Figure 4.2).
- By selecting the **File** tab→**New**, you can create a new blank layout, or open a previously created, saved layout template. A list of your previously created templates will appear in the **Recent Templates** area of the gallery. Recent templates may be pinned to the list by clicking on the pushpin icon.

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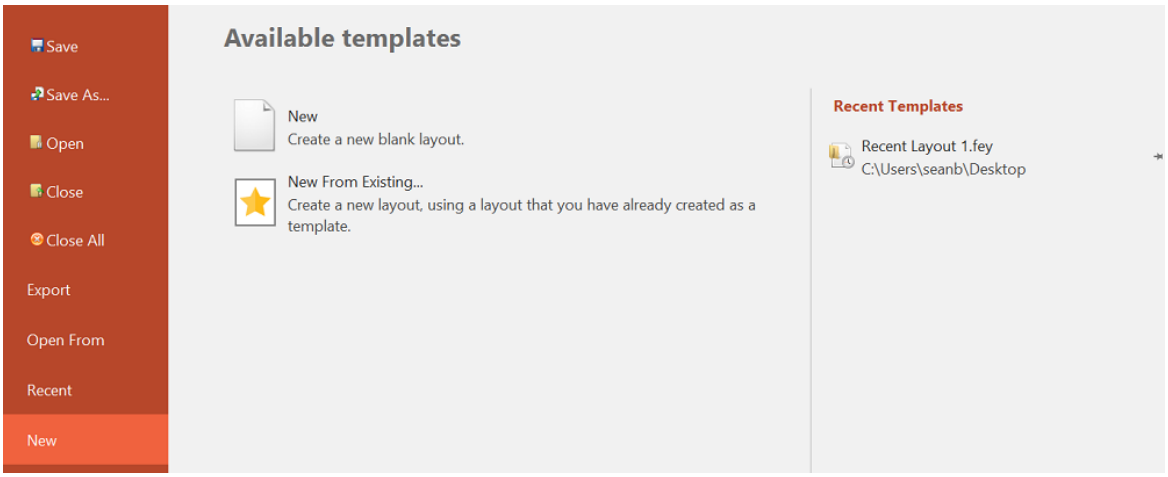

Figure 4.2 Recent Templates

- Templates are very useful for setting up standard document settings, like margins, page orientation, and page size, or analysis-specific layout elements such as plots and statistics. A template is any previously saved layout file that you decide to open as a template. To create a new document from a template, use the **File** tab→**New**→**New From Existing...** command. You can also designate a saved layout file as your default template 208. When a default template is present, FCS Express opens that template file instead of a blank layout when using the **File** tab→**New**→**New** command.

- When FCS Express opens a new layout from a stored template, a new layout identical to the stored template is created. However, the new layout will contain no data files so the plots will be empty. Therefore, when you save the new layout, you will be prompted for a new filename to prevent overwriting the original template.

4.1.2 Saving a Layout

Layouts can be saved in any of the following ways:

- **File** tab→**Save**.
- Click the **Save Current Layout** icon in the Quick Access Toolbar 65.
- Use the **Ctrl+S** keyboard shortcut.

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Using any of these methods will bring up the **Save Layout** dialog (Figure 4.3) where you can name the layout file to be saved and choose the location to save it.

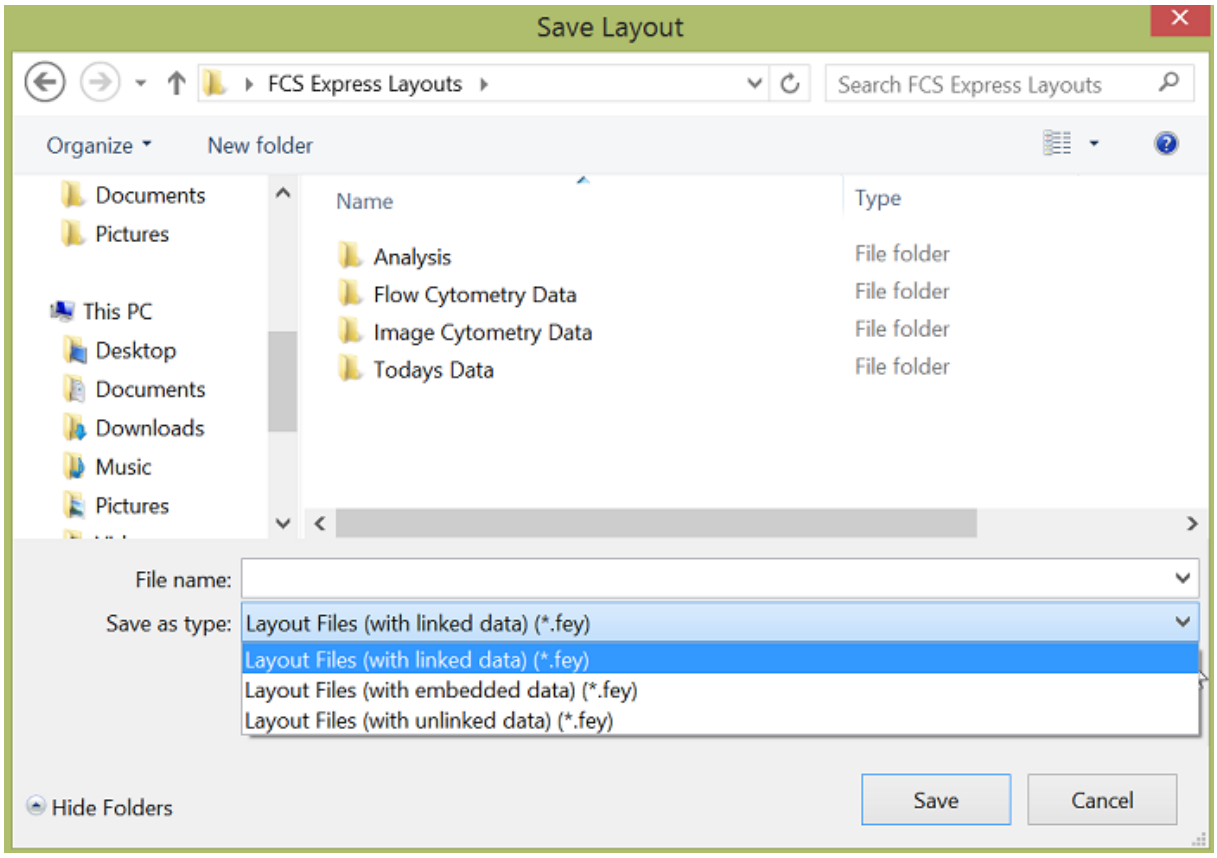

Figure 4.3 Save Layout Dialog

When saving a layout, you have the option to save with a link, save without a link, or embed the data files within the saved layout according to the **Save as type** drop down option you choose.

- **Layout Files (with linked data)(*.fey)**

  The position and formatting of the objects in the layout will be saved. When the file is opened, data will appear if the data files are available or have been copied to the computer where the layout is being opened. However, if the linked layout file is opened on a computer without access to the linked data files, no data will appear in the layout plots or statistics. Linked layout files are smaller than embedded layout files.

- **Layout Files (with embedded data)(*.fey)**

  The position and formatting of the objects will be saved and a copy of the actual data files is saved within the layout file. This allows anyone who opens the layout file to see and analyze the data, without having the original data

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file. Although a layout file with embedded data files can be fairly large, it is actually only about two-thirds of the size of the original data file(s). This makes layouts with embedded files an ideal way to send data to a collaborator.
Note: only .fcs files currently showing on the layout will be saved. Files within the Data List but not currently showing on plots will not be included.

- **Layout Files (with unlinked data)(*.fey)**

  The position and formatting of the objects (plots, statistics boxes, as well as the Data List options, including sorting) will be saved. No data will appear when the unlinked layout is opened. The file sizes of unlinked layout files are smaller than linked or embedded layout files.

4.1.3 Opening a Previously Created Layout

A previously saved layout can be opened by using the **File** tab→**Open** command, the **File** tab→**Open From**→**Open** command. These commands bring up the **Load Layout** dialog. Recently used documents will appear in the **File** tab→**Recent** category (Figure 4.4). To open a recently used document, simply select the document. Note that recent **Places** for file locations may be accessed. **Documents** and **Places** in the **Recent** category may also be pinned using the pushpin icons.

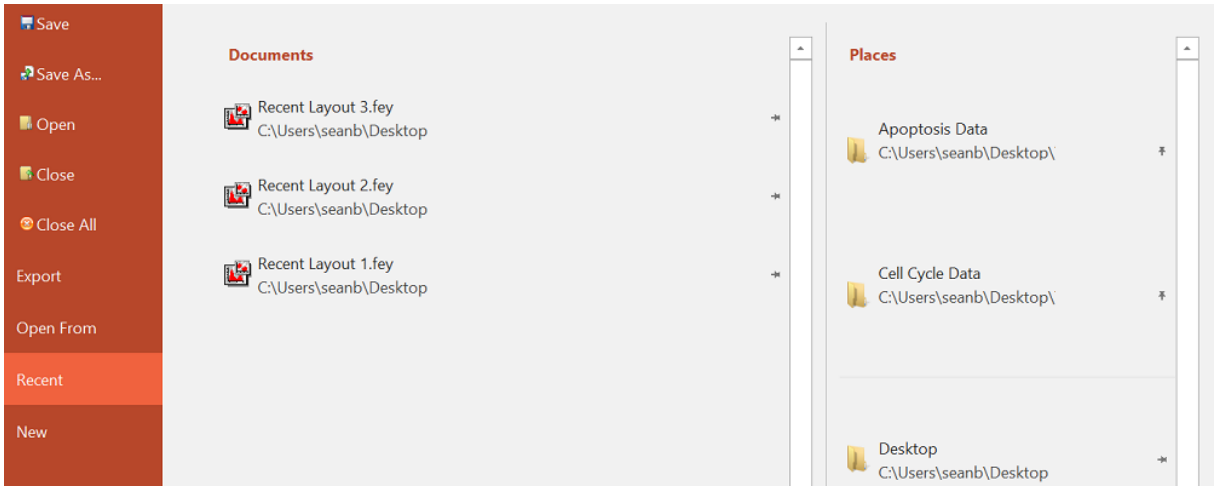

Figure 4.4 Recent Documents Appear on the Right

Navigate to the file you wish to open in the **Load Layout** dialog. Once a layout file has been highlighted, the **Layout Information** at the bottom of the dialog box displays the number of pages, the number of histograms, the number of 2D plots, the Data Link Style 93, and Layout Version of the selected layout file (Figure 4.5).

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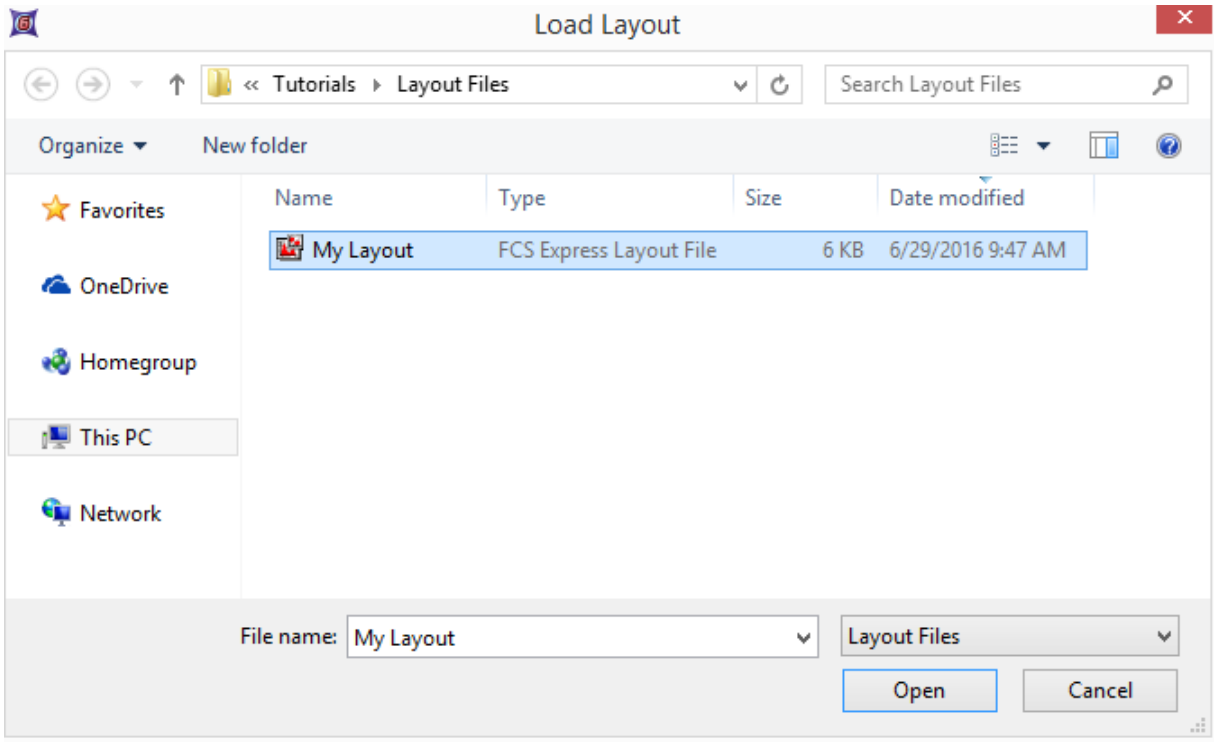

Figure 4.5 Load Layout Dialog

If the layout you are opening has linked data and FCS Express cannot find these .fcs file(s), you will be notified in a **Select the Data Directory** dialog, and will be given the opportunity to load the file(s) from a different folder (Figure 4.6).

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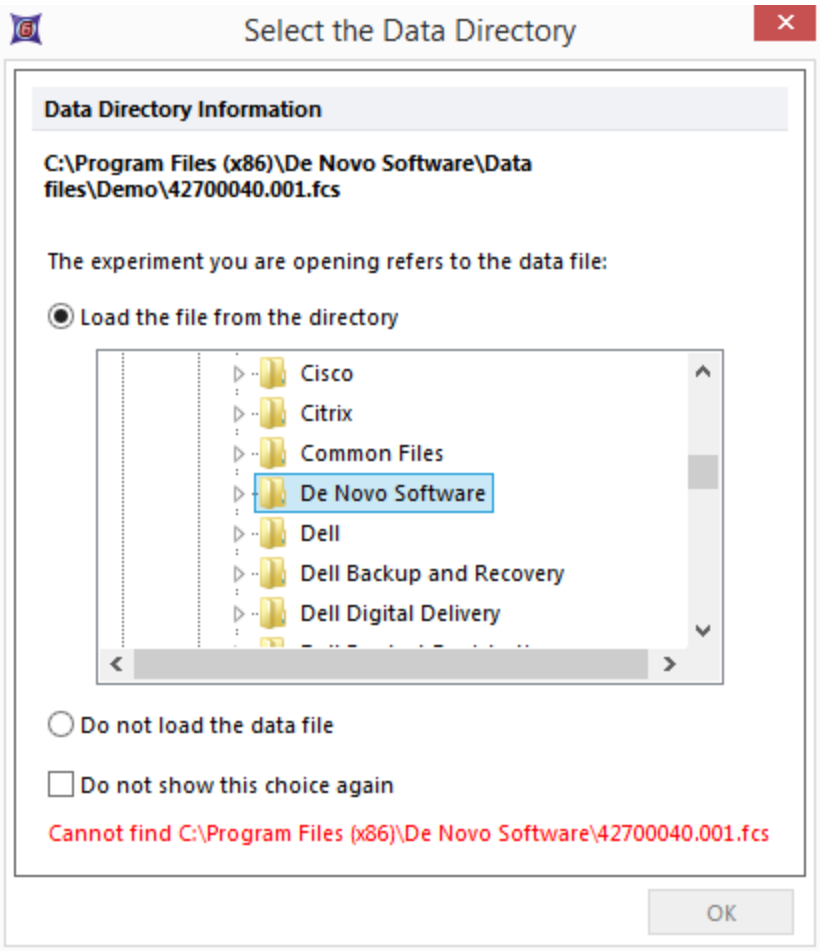

Figure 4.6 Missing Linked Data

4.1.4 Closing Layouts

More than one layout can be open in FCS Express at any time. There are two methods to close the current layout:

- Activate the layout that you want to close (you can use **View→Windows→Switch Windows**, if necessary) and select **File** tab**→Close**. If the only layout that is open is a blank layout which has not been changed, this will close (Exit) FCS Express.

- If more than one layout file is open, you can click on the "X" icon in the upper right of the application window (Figure 4.7).

Note: If more than one layout is open only the current layout will be closed. However, if only one layout is open, clicking on the Close Program "x" will close (Exit) FCS Express.

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Figure 4.7 Close icons

- To close all of the open layout files, select the **File** tab→**Close All**. A new, blank layout will be launched after the others are closed.

If any layout has not been saved when you try to close it, you will be prompted to save it. It is also possible to change your default Save Options so that the layout will close without being prompted to save. In this case, all changes will be discarded without altering the original layout.

4.1.5 Working with Pages

There are several ways to add a new page to a layout. The following three methods will add a new page *after* the current page:

- Select **Home→Pages→New Page** and choose the kind of page you want to insert from the ribbon.
- Right-click on the layout and select **New Page** from the pop-up menu.
- Click on the **Add Page** tab icon (Figure 4.8) of the **Layout Navigation Toolbar** (near the bottom of the FCS Express application window on the left).

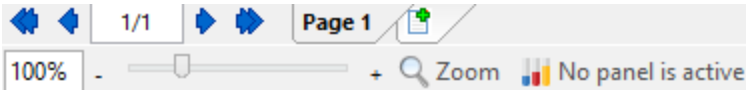

Figure 4.8 Add Page Tab

To insert a page into the middle of an existing layout, right-click on an existing labeled **Page Tab** on the **Layout Navigation Toolbar** (e.g., "**Page 1**") and select **Insert** from the pop-up menu. This will insert a blank page *before* the page you selected. To rearrange pages of a layout, select the tab of a page on the **Layout Navigation Toolbar** and drag the tab left or right to the new location.

You can rename pages. To do so:

1. Right-click anywhere on the page (or on the tab associated with the page) and select **Format** from the pop-up menu, or double-click on the Page Tab.

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2. In the **Formatting Page** dialog that appears, type the new name into the **Name** field (located in the **Name** category).

The visibility of a page can be controlled from the formatting dialog.

1. Right-click anywhere on the page and select **Format** from the pop-up menu.
2. In the **Formatting Page** dialog, click on **Visibility** (Figure 4.9). Using the radio buttons, visibility can be set to **Always visible**, **Never visible** (i.e., hidden), or **Custom**. If you choose **Custom**, you can insert a token that evaluates to a Boolean expression (by clicking on the T button), and the page will only be visible if the token evaluates to True.

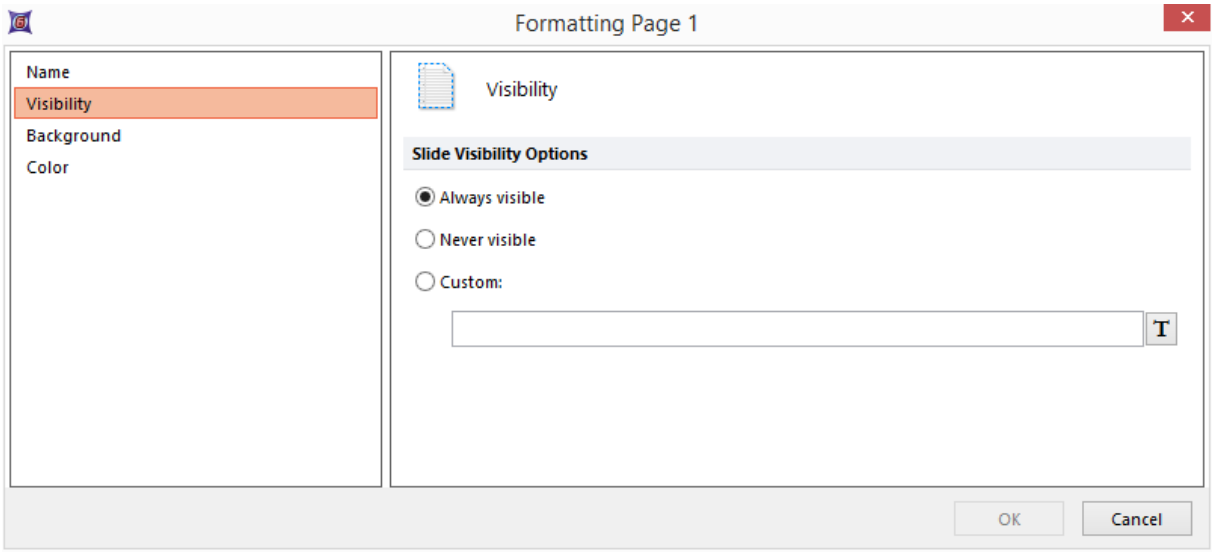

Figure 4.9 Formatting Page Visibility

The default background of a page is the background of the master page. Each page can have a custom background, however. To change the background of an individual page:

1. Right-click anywhere on the page and select **Format** from the pop-up menu.
2. In the **Formatting Page** dialog, navigate to the **Background** category (Figure 4.10).
3. Click on the **Custom** radio button.
4. Choose the **Color/Fill Pattern**, **Gradient**, **Image**, or **Transparent**.

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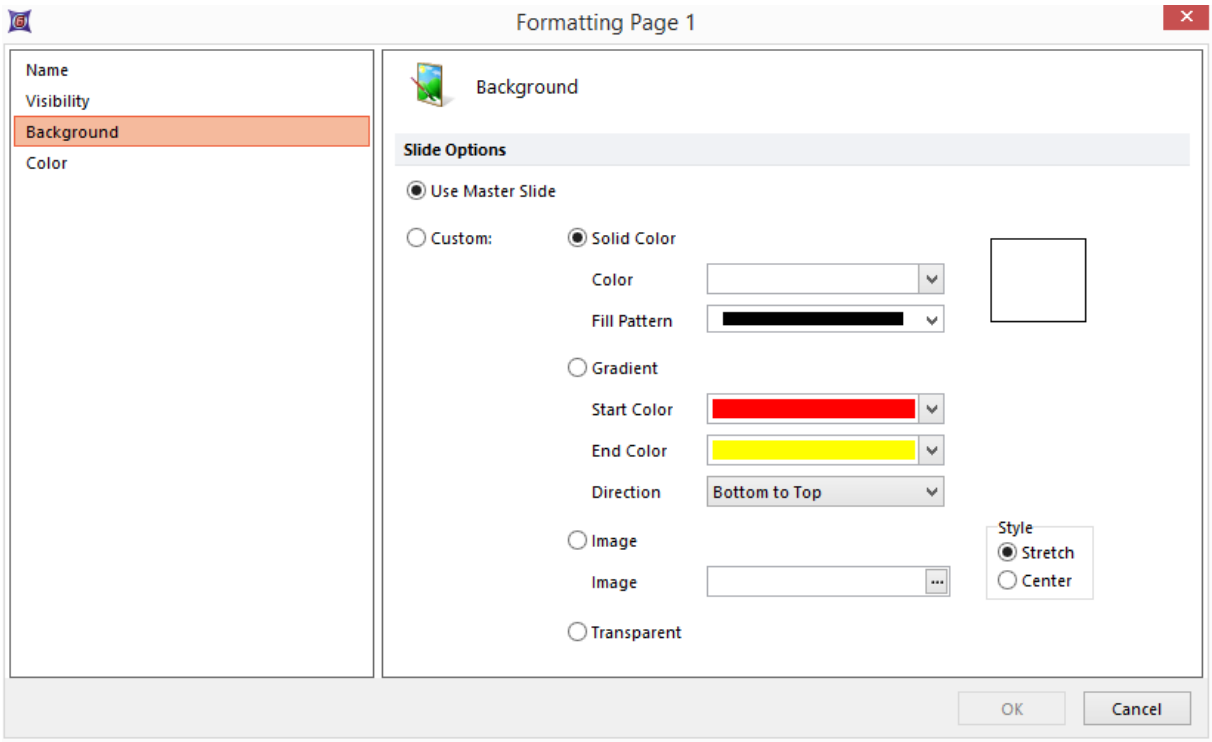

Figure 4.10 Changing an Individual Page Background

The color of a page tab may be controlled from the formatting dialog based on a series of **Color Classifications**.

1. In the **Formatting Page** dialog, click on the **Color** category(Figure 4.11).
2. Using the **Change the color depending on the value of:** dialog you can insert a token that evaluates to a Boolean expression (by clicking on the T button), and the color of the page tab will change if the token evaluates to any of the classifications in the **List of Colors** section.

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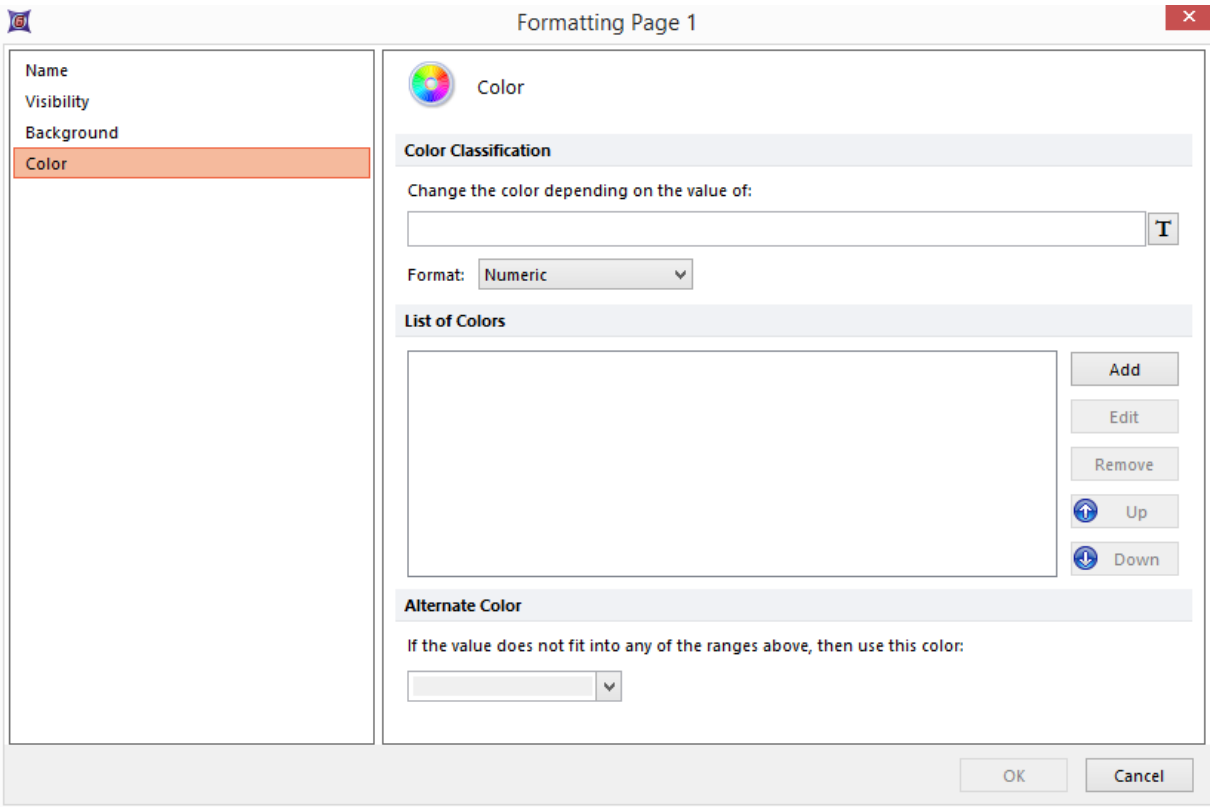

Figure 4.11 Changing the Page tab Color classifiers

3. **List of Colors** allows you to create a series of classifications for colors by clicking the **Add** button in the **List of Colors** dialog.

4. The **Create Classification** dialog will open (Figure 4.12) where you can choose a Color for the page tab if a value, numeric or token based, falls in a range you define.

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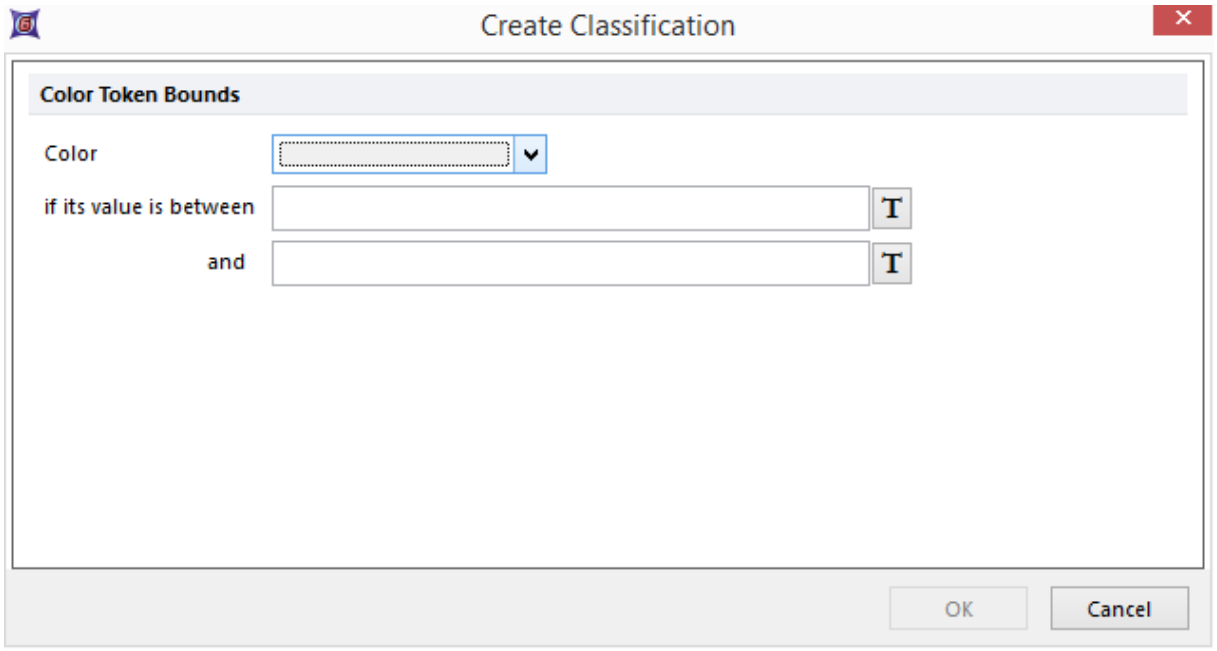

Figure 4.12 Adding a page tab color classifier with Color Token Bounds.

5. The Edit, Remove, Up, and Down commands in the **Formatting→Color** dialog allow you to adjust the **List of Colors** and **Color Token Bounds** as needed.

There are several ways to delete the current page:

- Select **Home→Pages→Delete Page**.
- Right-click on the layout page and select **Delete Page** from the pop-up menu.
- Right-click on a **Page Tab** (e.g., "**Page 2**") on the **Layout Navigation Toolbar** and select **Delete** from the pop-up menu.

4.1.6 Printing Pages from a Layout

To print a page (or range of pages) from the layout, select the **File** tab→**Print** command (Figure 4.13). A standard **Print** gallery will appear and allow you to select what you wish to print. The printed pages will look exactly as they appear within the page borders on the screen.

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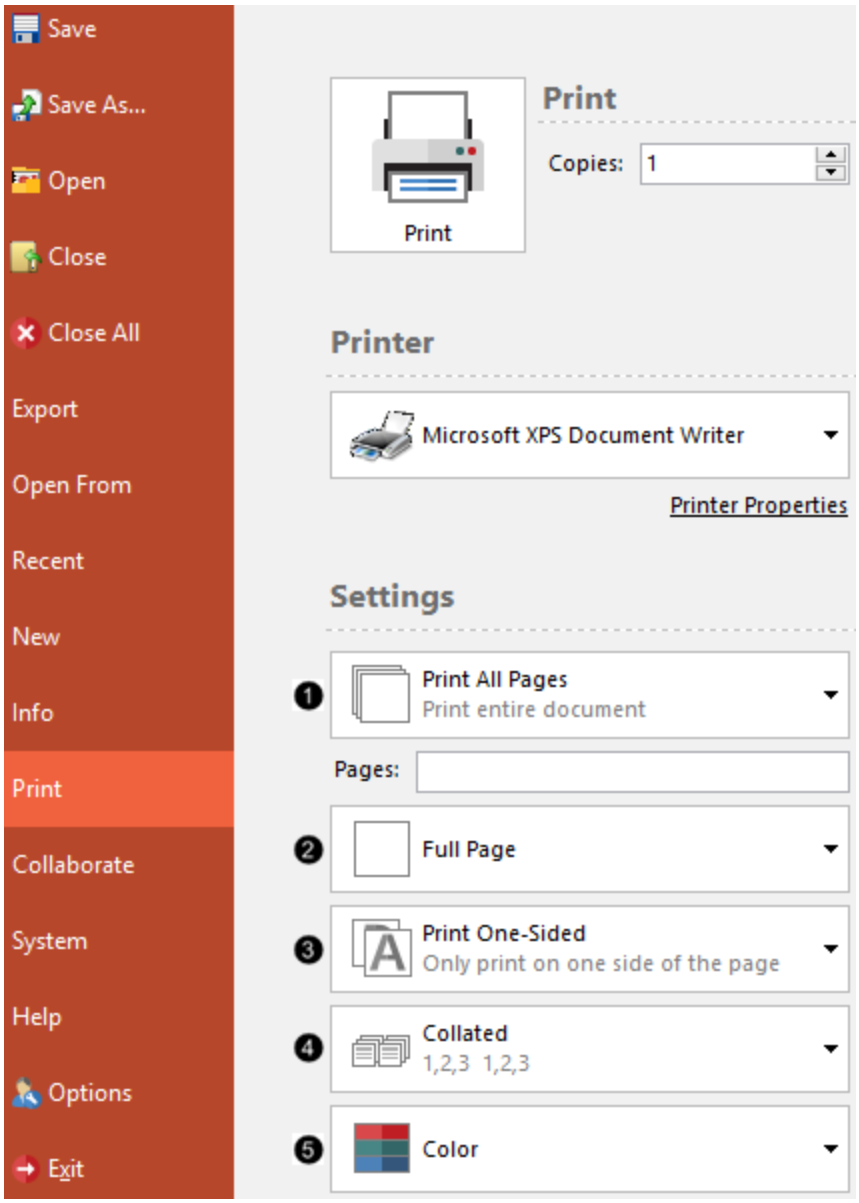

Figure 4.13 Print Setup Dialog

The current **Printer** and **Printer Properties** may be accessed or changed in the **Printer** section.

The sections under **Settings** will allow you to:

1. Choose which layout pages to print (**Print All Pages, Print Current Page,** or **Custom Range**).

2. Choose to print layout pages as single or multiples per printed page(s). With **Full Page** selected, each layout page will be printed as a single page. Multiple layout pages may be printed per page using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options** 38.

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a. To print the layout pages on fewer pages, click the down arrow to the right to expand the dropdown list and select a multiple page option or **Custom Grid Options...** to select a preset multiple page option or customize the tiling, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear (Figure 4.14).

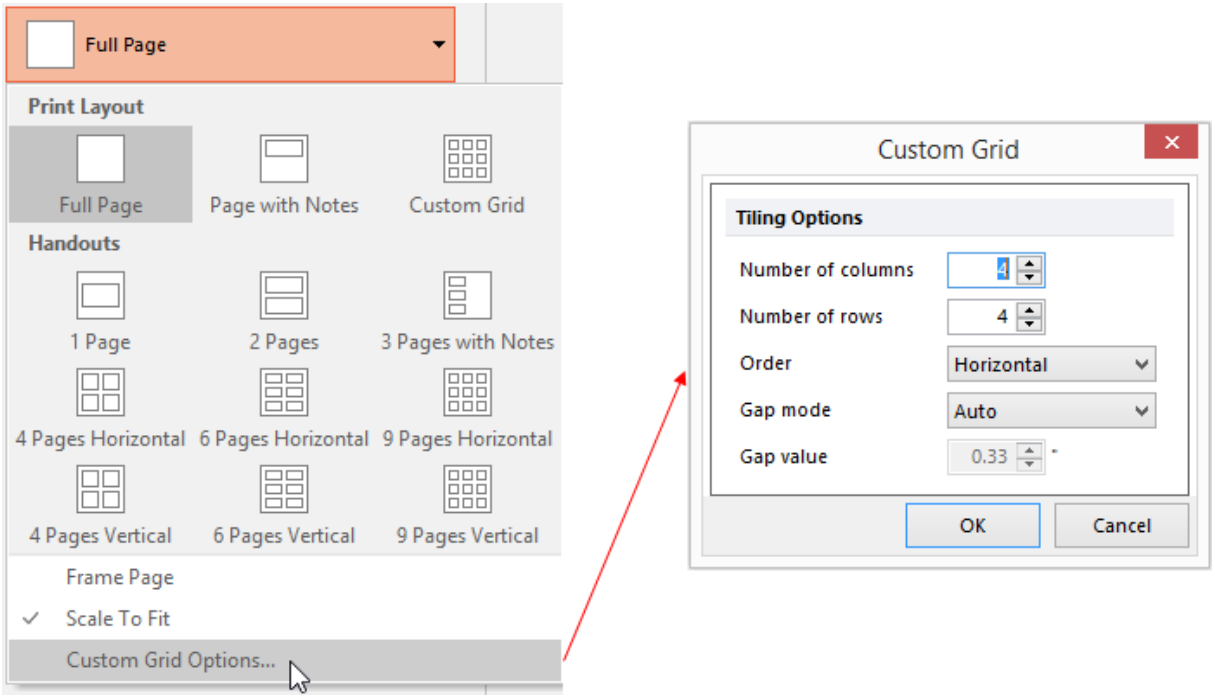

Figure 4.14 Custom Grid Options of Print Setup from File tab

b. **Frame Page**. Check this option to draw a frame around layout pages.

c. **Scale To Fit**. Will be automatically checked. Only uncheck if it is preferred the content from the layout page is not scaled for the paper selected for printing.

3. Choose to print on one side or both sides of the paper.

4. Choose collated or uncollated.

5. Choose to print in color or grayscale.

4.1.7 Exporting a Page as a Picture from a Layout

A page can be exported as a picture by selecting the **File** tab→**Export**→**Export Current Page As...** command.

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The picture can be exported as a Windows Metafile (*.emf), JPEG (*.jpg), Bitmap (*.bmp), Windows Extended Metafile (*.emf), Portable Network Graphics (*.png), GIF (*.gif), or TIFF (*.tif).

4.1.8 Exporting a Layout as a PowerPoint® Presentation

A layout can be directly exported as a Microsoft PowerPoint® presentation. All plots and text boxes are inserted into the PowerPoint presentation as Windows Metafiles and can be moved, resized, and manipulated independently within PowerPoint.

To save as a PowerPoint presentation, select the **File** tab→**Export**→**Export as PowerPoint Presentation...** command.

The **Save as Microsoft PowerPoint Presentation** dialog that appears allows for the options described below:

- **PowerPoint Options**
  - **Name the presentation**. Use this field to select the output folder and the name of the output file.
  - **Include the background (from Master Slide)**. Check this option to to include the background from Master Slide. If you choose to export the background, the objects on the FCS Express Master Page will be placed on the PowerPoint master slide. These background objects will then appear on all of the PowerPoint slides.
  - **Open presentation after saving**. When checked, the PowerPoint presentation will automatically open at the end of the export procedure by launching PowerPoint.
  - **Hide objects that are off the page**. When checked, objects that are not located on the white page in the layout will not be exported to PowerPoint.
  - **If the presentation already exists, append to it**. Check this option to append slides to an existing PowerPoint presentation.
- **Export Options**
  - **Quality**. Set the PowerPoint quality (**High**, **Medium**, **Low**, **On-Screen**) from the dropdown list.

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- **Mode.** This option enables single or multiple layout pages to be exported per slide based on selection. When **Full Page** is selected, each layout page will be exported as a slide. Multiple layout pages may be exported per slide using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options**. To export the layout pages on fewer slides, please select a preset multiple page option or **Custom Grid** by clicking the down arrow to the right to expand the dropdown list and selecting a multiple page option or **Custom Grid Options...**, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear, which will contain following five settings:

- **Frame Page**. Check this option to draw a frame around pages.

- **Crop To Content.** Available when **Mode** is not set to **Full Page**. When selected, empty space of the layout page(s) is cropped during the export.

- **Page Range**

  Choose which layout pages you wish to export.

Comparison of exports to PowerPoint using multiple page preset **4 Pages Horizontal** with or without selecting **Frame Page** and **Crop to Content** (Figure 4.15).

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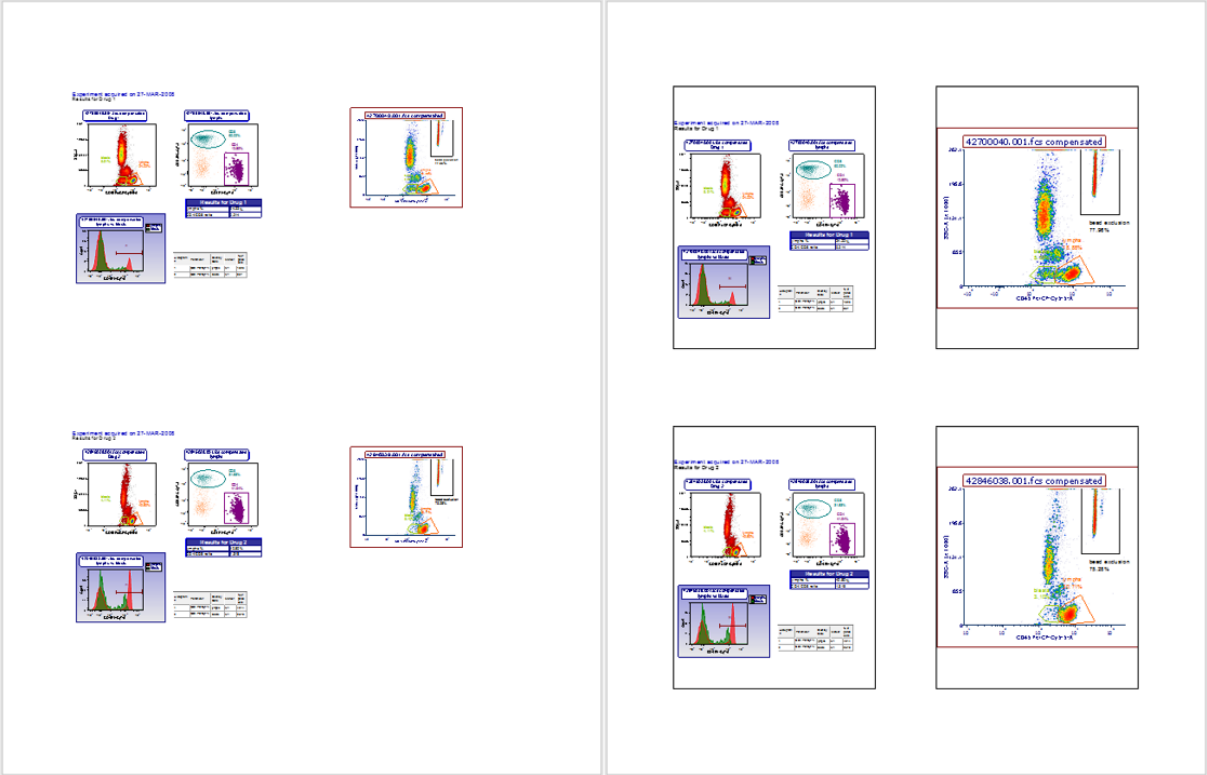

Figure 4.15 Comparison of exports to PowerPoint using preset 4 Pages Horizontal. Left: Frame Page and Crop To Content unchecked. Right: Frame Page and Crop To Content checked

Note about ungrouping objects in PowerPoint for PC versus PowerPoint for Mac

Once exported to PowerPoint, layout objects can be further ungrouped into single components in the PC version of Microsoft PowerPoint. Ungrouping objects saved by FCS Express in PowerPoint for Mac is not supported. However, presentations saved on a Mac may be opened on a PC and ungrouped.

4.1.9 Exporting a Layout as a PDF File

A layout can be directly exported as an Adobe PDF file.

To save as a PDF file, select the **File** tab→**Export**→**Export as PDF File...** command.

The **Save Layout as PDF File** dialog that appears allows for different options:

- **PDF Filename**
  - Use this field to select the output folder and the name of the output file.

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- **Open presentation after saving**. When checked, the PDF file will automatically open at the end of the export procedure.

- **PDF Options**

  Embed fonts (will increase file size). This option ensures that all of the font information used to make your document look the way it does is stored in the PDF file.

- **Export options**

  - **Quality.** Set the PDF quality (**High**, **Medium**, **Low**, **On-Screen**) from the dropdown list. Higher quality documents will have better graphics, but larger file sizes. PDF quality primarily affects the look of statistics windows and gate views.

  - **Mode.** This option enables single or multiple layout pages to be exported per PDF page based on selection. When **Full Page** is selected, each layout page will be exported as a PDF page. Multiple layout pages may be exported per PDF page using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options**38. To export the layout pages on fewer PDF pages, please select a preset multiple page option or **Custom Grid** by clicking the down arrow to the right to expand the dropdown list and selecting a multiple page option or **Custom Grid Options...**, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear.

  - **Frame Page.** Check this option to draw a frame around pages.

  - **Crop To Content.** Available when **Mode** is not set to **Full Page**. When selected, empty space of the layout page(s) is cropped during the export.

- **Page Range**

  Choose which layout pages you wish to export.

Comparison of exports to PDF using default **Custom Grid Options** with or without selecting **Frame Page** and **Crop to Content** (Figure 4.16).

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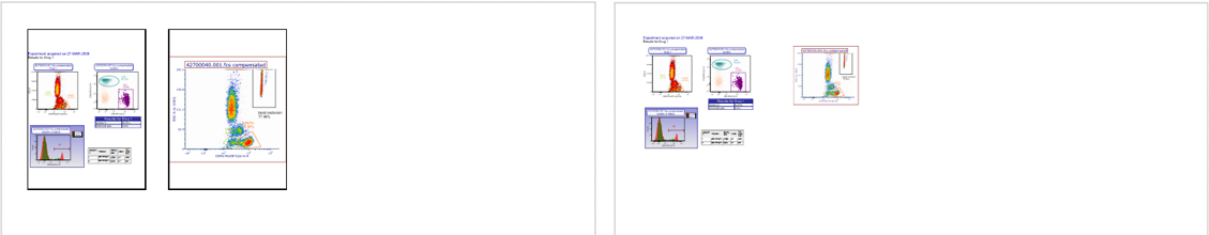

Figure 4.16 Comparison of exports to PDF using default Custom Grid Options (top row only displayed). Left: Frame Page and Crop To Content checked. Right: Frame Page and Crop To Content unchecked

4.1.10 Navigating through the Layout

There are many ways to navigate through the layout:

If the layout is larger than your screen, you can use the vertical scroll bar on the right, or the mouse wheel, to move to different positions on the current page.

- Press the **Page Down** key on your keyboard to move to the next page in the layout.
- Press the **Page Up** key on your keyboard to go to the previous page in the layout.
- Right-click on the layout and choose **Previous Page** or **Next Page** from the pop-up menu.
- Use the Layout Navigation Toolbar 55 (always located at the bottom left corner of the layout). In the **Layout Navigation Toolbar**, you can:
  - Click on the tab of a named page (e.g., "**Page 2**").
  - Type the desired page number directly into the toolbar edit field.
  - Press the arrow buttons located to the left or right of the edit field to go to the next or previous pages. The buttons with single arrows will move in single page increments. The buttons with the double arrows will move to the beginning, or end, of the layout.

4.1.11 Viewing Pages

In the **View→Multiple Pages** group, there are four commands [**One Page**, **Two Pages (Horizontal)**, **Two Pages (Vertical)**, and **Four Pages**] for displaying the entire layout in different views (Figure 4.17).

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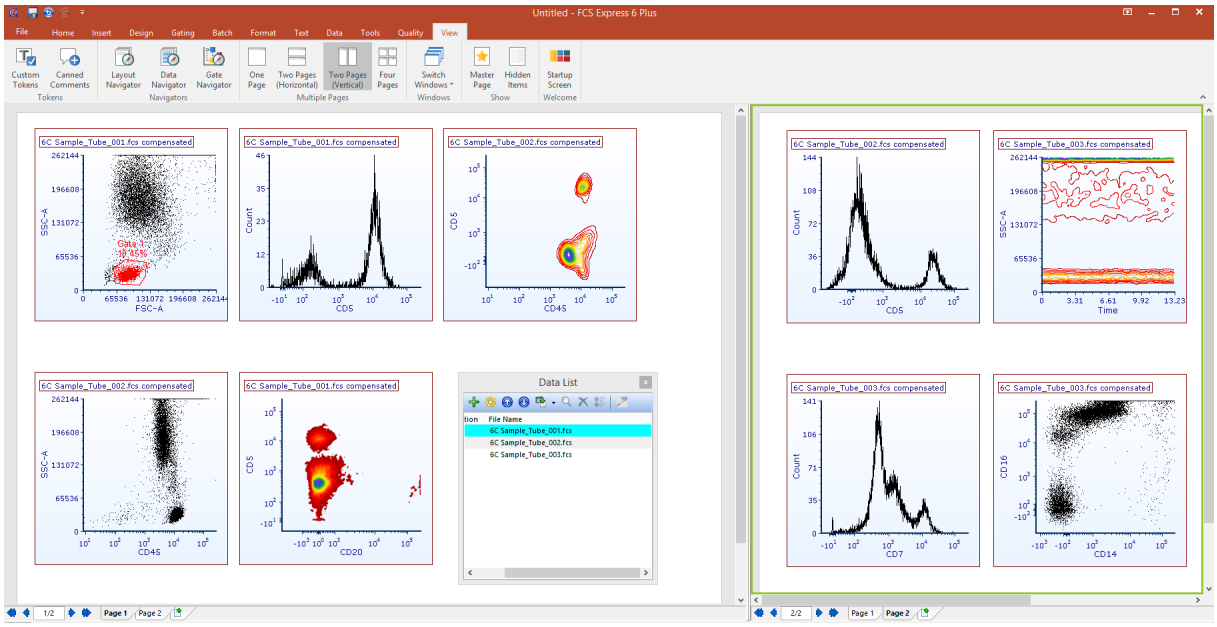

Figure 4.17 Two Pages (Vertical) View

4.1.12 Zooming the Layout

It is possible to change the current zoom factor (in percentages) by using the Zoom Toolbar 55 located below the Layout Navigation Toolbar 109. You can enter the desired zoom percentage directly into the toolbar edit field on the left or press the **Zoom** command (with the magnifying glass icon) to access the **Zoom** window. This window allows you to choose from several predefined zoom factors (or type in a percentage in the edit field).

4.1.13 Auto Recovery Files

FCS Express can automatically create a backup of your layout, known as an Auto Recovery file. By default, an Auto Recovery file will be created every 5 minutes. As a user option, you can turn off this feature, or change the interval between autosaves, by editing the Save page of the User Options 222 dialog. If FCS Express is terminated suddenly, and before the layout could be saved, then the next time you start the program, you will be asked if you want to resume using the AutoSaved file, which will have been given a unique name so as not to overwrite the previous user-saved file.

4.1.14 Working with Multiple Layouts

In FCS Express, multiple layouts can be open at the same time. Each layout opens in its own window. You can drag and drop objects (plots, data files, gates, etc.) from one layout to another.

Use the **View→Windows→Switch Windows** command (and select the appropriate layout from the drop-down list) to change which layout is active.

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4.1.15 Layout and Layout Performance Options

It is possible to access the **Layout Options** and **Layout Performance Options** by selecting the **File** tab→**Info**→**Layout Options**.
If you have security enabled, the **Layout Group Security Access** category will also be available.

The **Layout Options** category (Figure 4.18) displays information about the layout and allows you to assign a password to open the layout.
In addition, it also allows to customize the layout startup via the **Startup Script** tool.

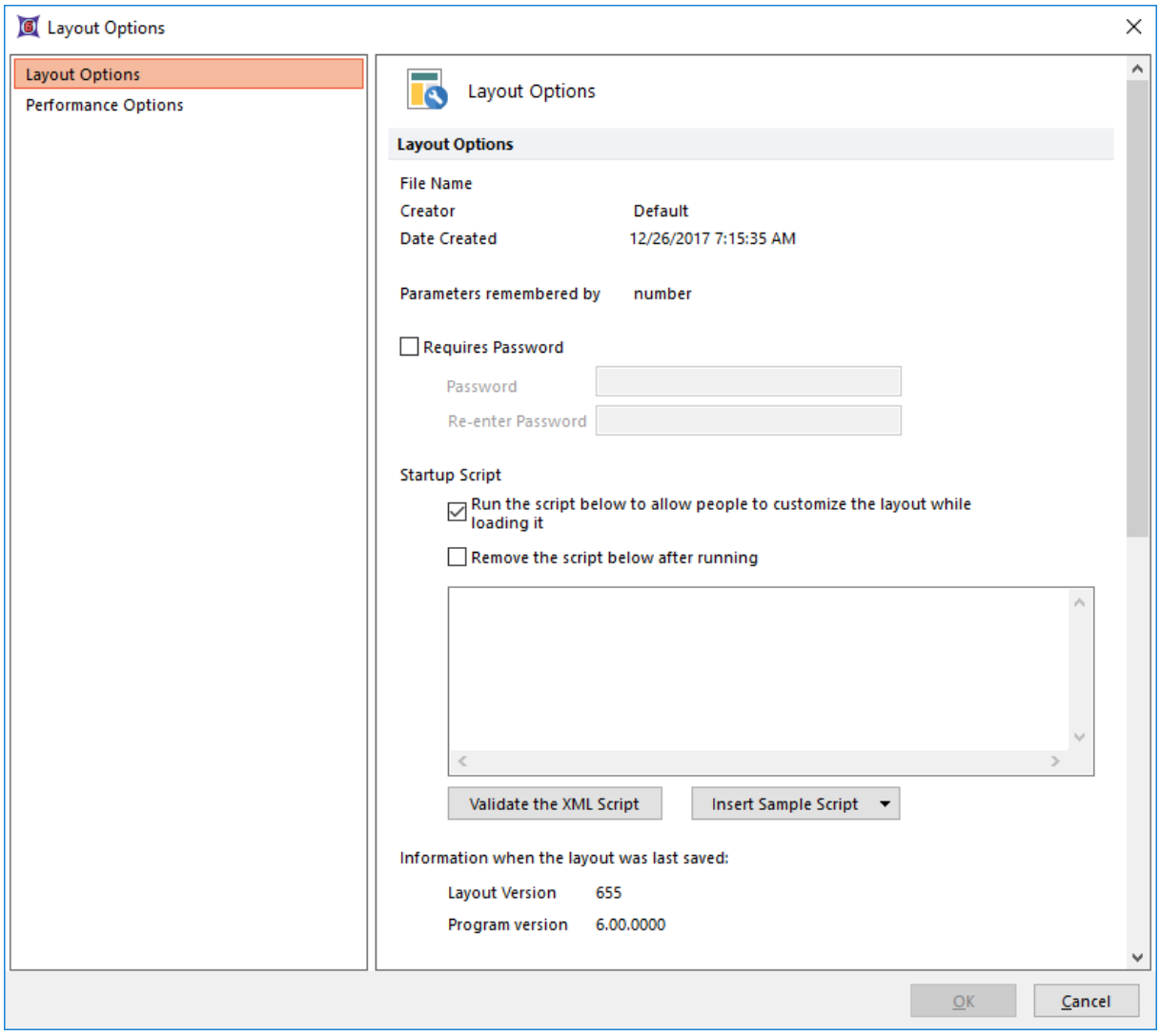

Figure 4.18 Layout Options Dialog

Note: Use caution when deciding to password-protect a layout. If you forget the password, you will not be able to open the layout.

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If you have security enabled, additional properties will be available on the **Layout Options** dialog.

The **Layout Performance Options** category (Figure 4.19) allows you to choose if live updating of gates, quadrants, markers, statistics and tokens is controlled at a global **User Options** level or at the Layout Level.

Choosing the **Specify the value below** radio button will allow you to turn the option to **Update Immediately** on or off for the current open layout via the check box.

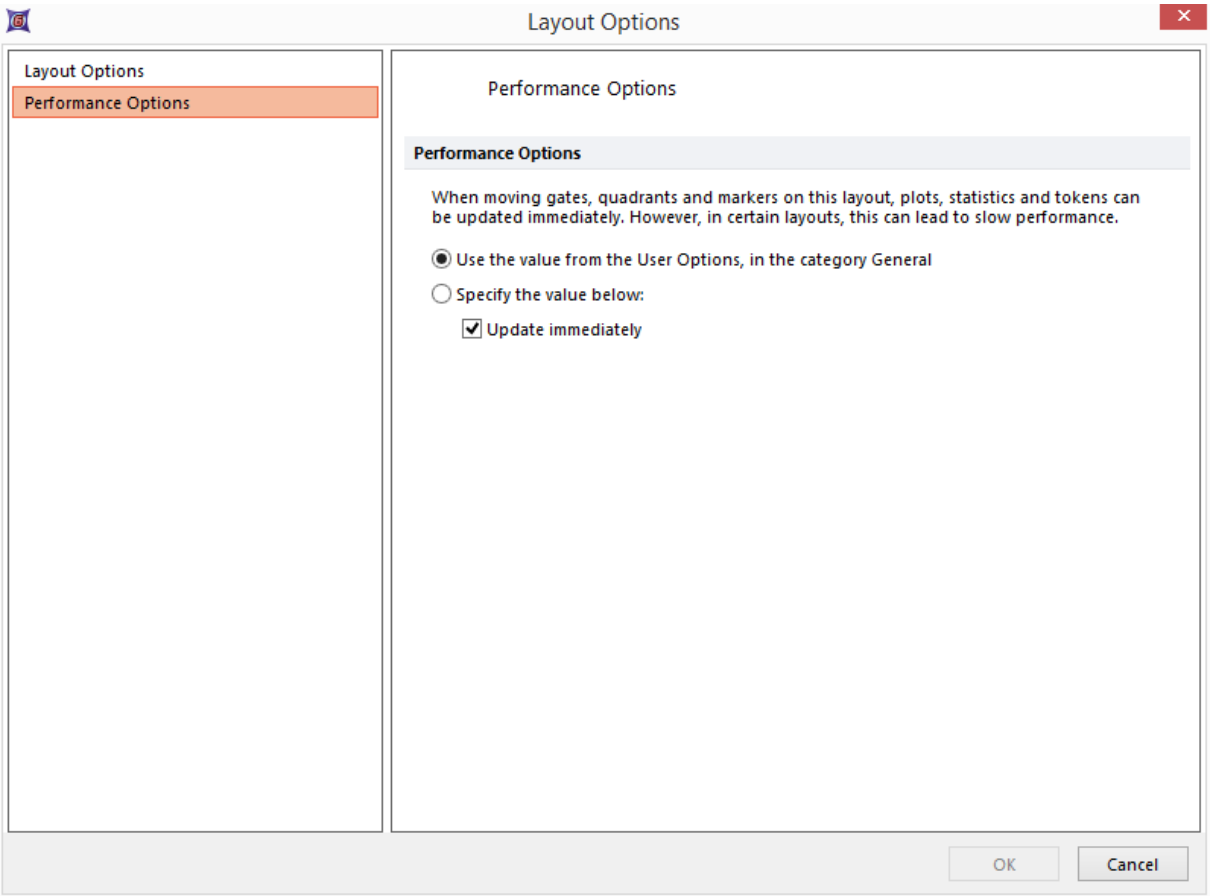

Figure 4.19 Layout Performance Options Dialog

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4.1.15.1 Startup Script

The **Startup Script** tool allows the user to customize the layout every time it is launched and is particularly useful to adapt standardized layouts to data acquired on different instruments with different settings for storing the data files.

Layout customization is performed via a series of questions which appear to the user as messages on the screen when a script is defined. The layout or plots will be customized based on the user's answers to the scripted questions.

Possible scripting customizations are:
- Loading a specific file on specific plots and/or on specific overlays
- Change parameters on specific plots and/or on specific overlays
- Change the parameters used for a specific gate
- Replace or Add parameters to an existing transformation

The questions, and the corresponding actions, are defined via an XML script.

If you are interested in using the scripting features in FCS Express, please contact support@denovosoftware.com for additional information.

4.2 Introduction to Docking

FCS Express 6 has a flexible user interface. Dockable windows give you the ability to customize your analysis workspace to fit your needs.

The following windows are dockable:

- Data List
- Layout Navigator
- Data Navigator
- Gate Navigator
- Compensations
- Snapshots
- Batch Actions
- Batch Run
- Standard Operating Procedures
- Standard Operation Procedures Run
- Standard
- Permission Rules
- Custom Tokens

The remaining sections of this chapter explain how to work with dockable windows.

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4.2.1 Opening a Dockable Window

Dockable windows are opened by clicking on the appropriate Ribbon 55 commands from the Batch Tab 72, the Data Tab 76, or the View Tab 82.

The commands to open the dockable windows are listed in the table below.

Dockable Window	Command
Data List	**Data→Organize Date Sets→Data List** or **Batch→Organize Date Sets→Data List**
Snapshots	**Tools→Snapshots→Snapshots**
Layout Navigator	**View→Navigators→Layout Navigator**
Data Navigator	**View→Navigators→Data Navigator**
Gate Navigator	**View→Navigators→Gate Navigator**
Compensations Navigator	**Tools→Transformations→Compensations**
Transformations Navigator	**Tools→Transformations→Transformations**
Batch Actions	**Batch→Batch Processing→Batch Actions**
Standard Operating Procedures	**View→Tools→Standard Operating Procedures**
Permission Rules	**Quality→SOP→Permission Rules**
Custom Tokens	**View→Tokens→Custom Tokens**

When opened, the window will first appear as a floating window, undocked, in the center of the screen (Figure 4.20). You can leave it as a floating window, or proceed to dock it in the location of your choice 115.

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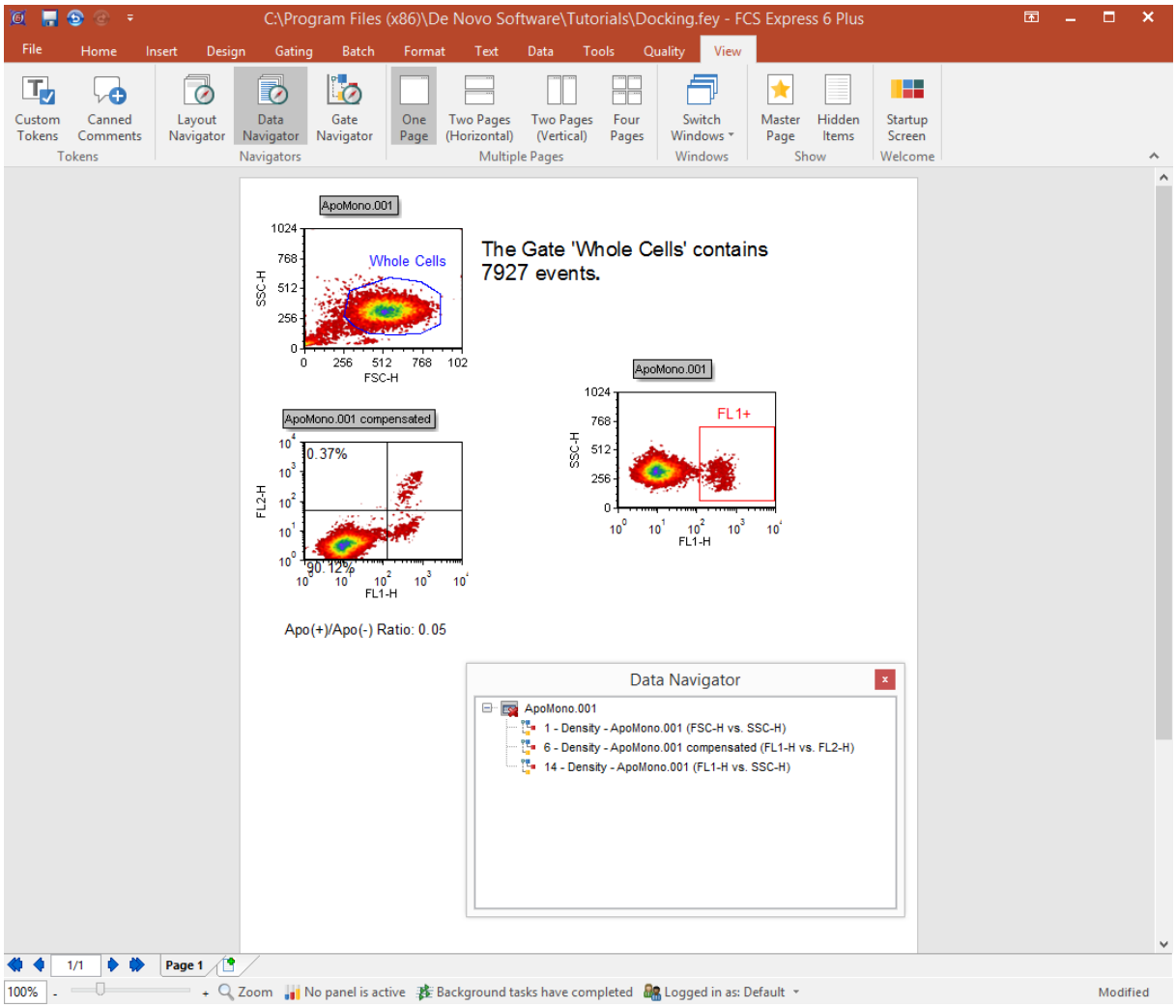

Figure 4.20 Data Navigator, Floating (Undocked)

4.2.2 Docking a Window

To dock a floating window:

- Move the cursor over the title bar of the window (Figure 4.21).

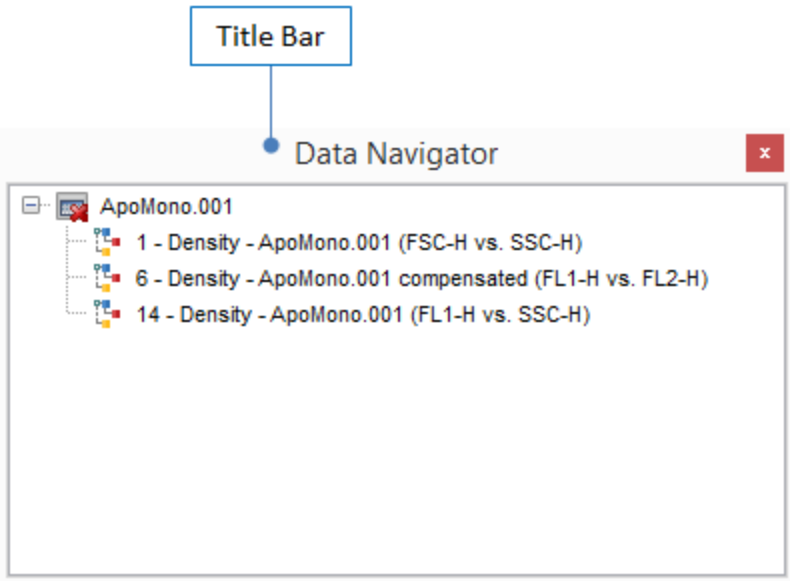

Figure 4.21 Title Bar of Floating Window

- Press and hold the mouse button over the title bar while beginning to drag the window. *Docking Zone Selectors* (a group of rectangles with arrowheads designating the upper, lower, left, and right docking zones, and a central square) will appear on the screen (Figure 4.22).

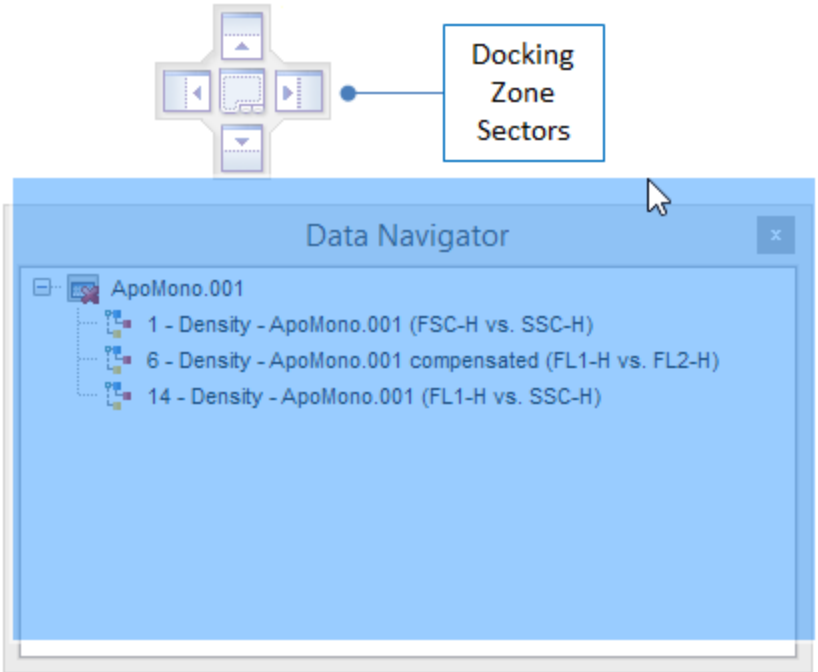

Figure 4.22 Docking Zone Selectors

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- While keeping the mouse button down, drag the cursor over the appropriate zone selector. Not all of the docking zone selectors may be active at all times. When you position the cursor over an active docking zone selector, a blue outline appears over the zone location (and a label confirms the location). In the example (Figure 4.23), the cursor has been positioned over the right docking zone selector. Notice the blue rectangle over the entire right half of the screen indicating where the window will be docked (to better illustrate the label in the lower right corner, it has been slightly repositioned from where it will appear on your screen).

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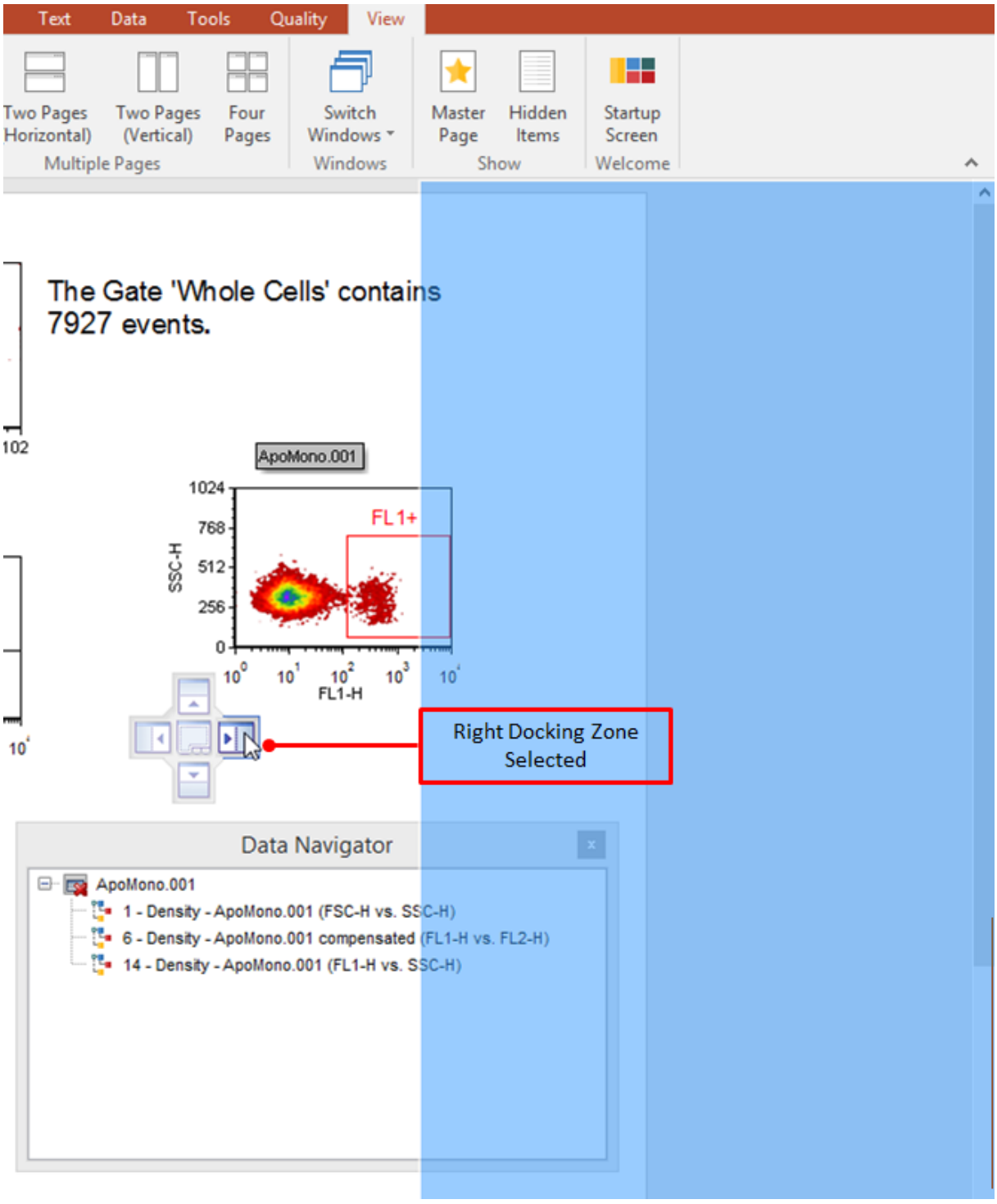

Figure 4.23 Cursor Positioned over Right Docking Zone Selector

- When you release the button with the cursor positioned over a docking selector, the window will dock to the location you selected (Figure 4.24).

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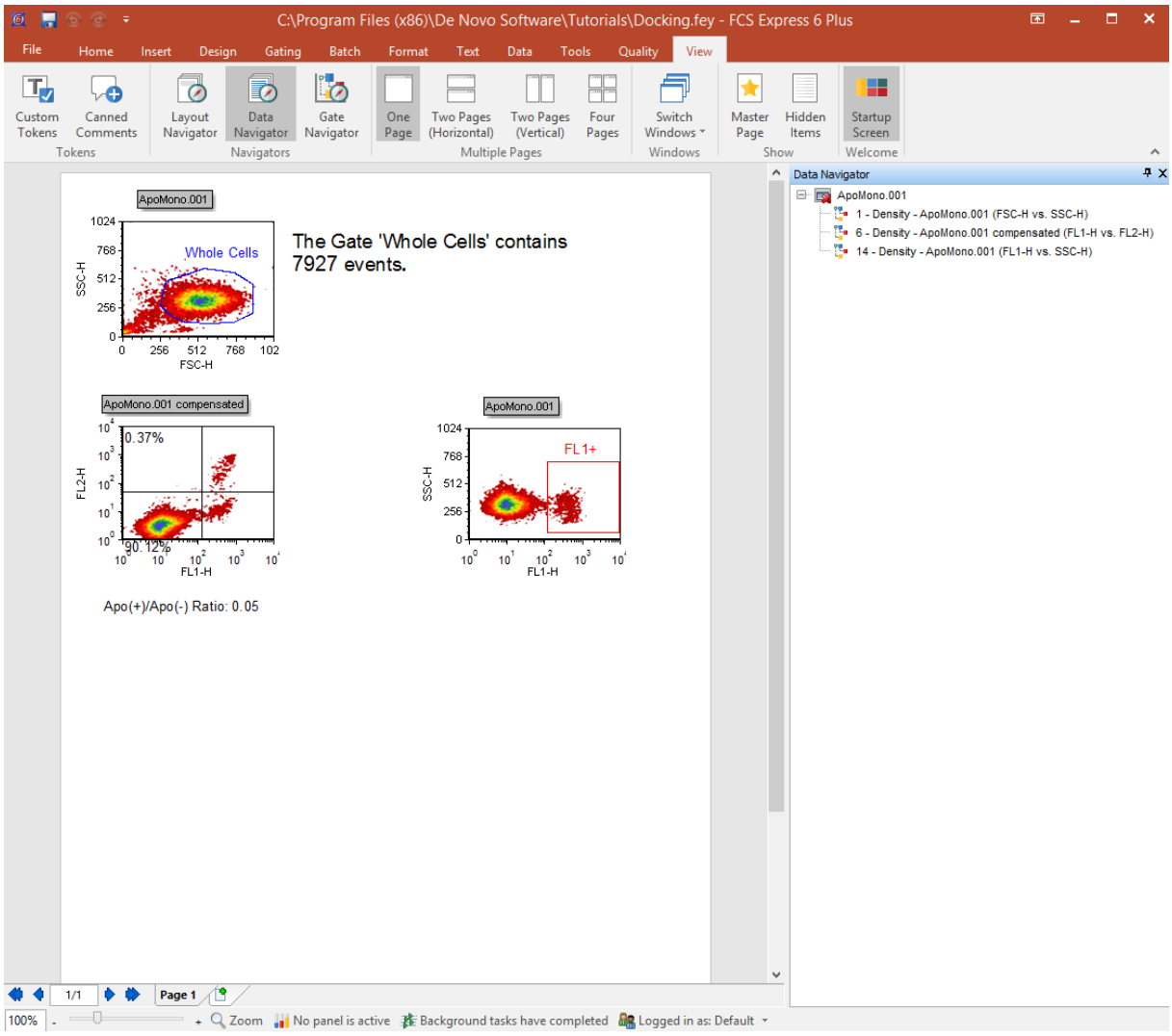

Figure 4.24 Data Navigator Window Docked to the Right

- If a window is already docked, and you are docking a second window, docking zone selectors will appear in the middle of the screen and over the docked window (Figure 4.25). In the example, the **Layout Navigator** is being docked inside the already docked **Data Navigator**. If you dock one window inside another, the two windows will occupy the same location, but will be identified by tabs (Figure 4.26). If you dock two windows in the same location but not inside each other, they will be separated by horizontal or vertical *splitter bars* (Figure 4.27).

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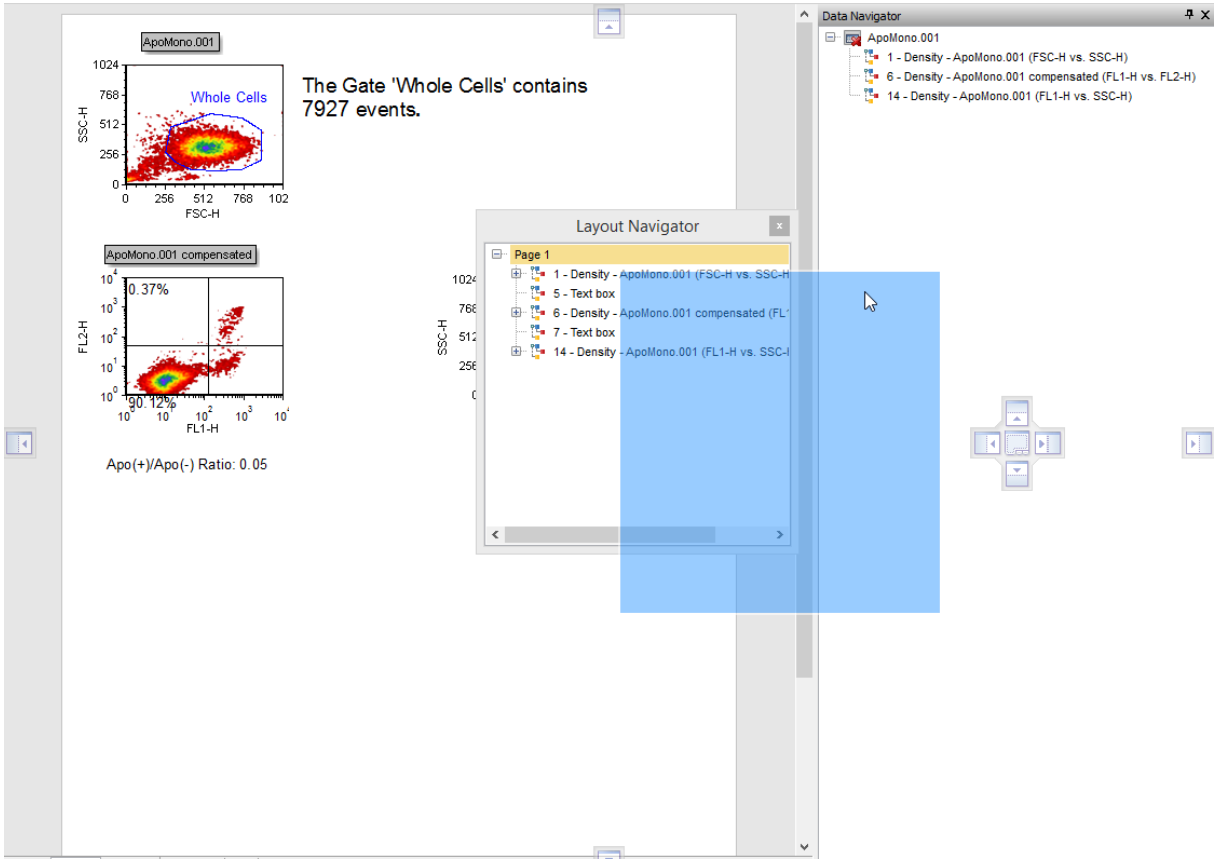

Figure 4.25 Docking the Layout Navigator inside the Data Navigator

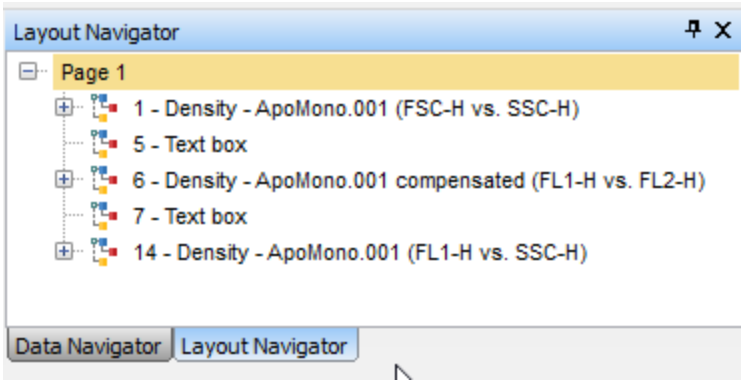

Figure 4.26 Docked Windows Identified by Tabs

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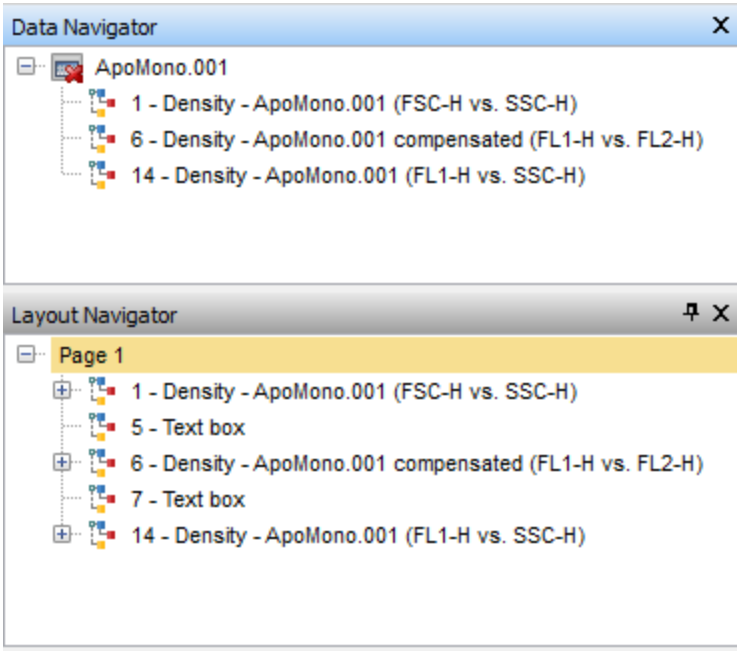

Figure 4.27 Docked Windows Seperated by a Horizontal Splitter Bar

Note: Although docked windows are not part of the layout page and will not be exported to other file formats, their location is saved along with your layout, and they will be present when you re-open the layout.

4.2.3 Pinning a Docked Window

A docked window has a number of icons in the title bar. The pin icon (Figure 4.28) controls the *Auto Hide* feature.

Figure 4.28 Auto Hide Pin Icon in a Docked Window

When the pin is pointing down, it indicates that the docked window is in the standard configuration (unpinned). If you click on the pin icon, the *Auto Hide*

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property will be activated. The pin will turn to the left and a tab with the window name will appear on the edge of the screen (Figure 4.29).

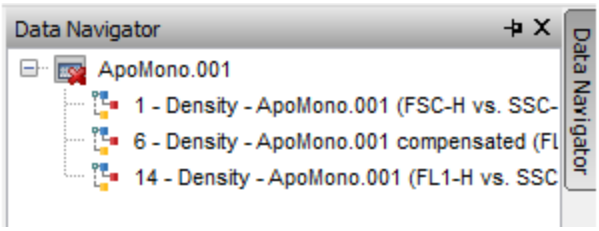

Figure 4.29 Docked Window with Auto Hide Activated

With *Auto Hide* activated, the window will remain open whenever the cursor is over the window. If you move the cursor away (or click on a different part of the screen) the window will slide towards the edge of the screen and only the tab will be visible (Figure 4.30). When a window is pinned, you can also hide it by clicking on the X icon beside the pin. To open a hidden window, hover the cursor over the tab.

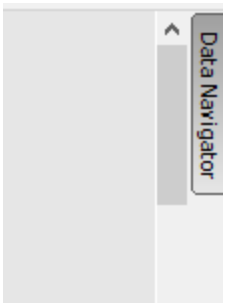

Figure 4.30 Pinned Data Navigator Hidden from View

Note: A window that has been pinned cannot be undocked 122 or closed 123 until it has been unpinned (i.e., *Auto Hide* has been turned off). To unpin the window, make the window visible, and then click on the pin icon.

4.2.4 Undocking a Window

Note: A pinned window cannot be undocked until it is unpinned 121.

To undock an unpinned window:

- Move the cursor over the title bar 115 of the window.

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- While holding down the mouse button on the title bar, drag the window away from its docked position.

4.2.5 Closing a Dockable Window

Note: A pinned window can be hidden on the edge of the screen, but cannot be closed until it has been unpinned 121.

To close an unpinned, dockable window, click on the X icon on the right side of the title bar.

4.3 Navigators

Navigators in FCS Express are dockable windows 113 with drag and drop functionality to help you find and manipulate the objects in your layout 123, the data sources 126 connected to your objects, and the gates 128 on your plots.

4.3.1 Layout Navigator

The **Layout Navigator** shows a hierarchical view of all of the objects in your layout by page. Within each page node, plots and other objects are listed with their data source (Figure 4.31).

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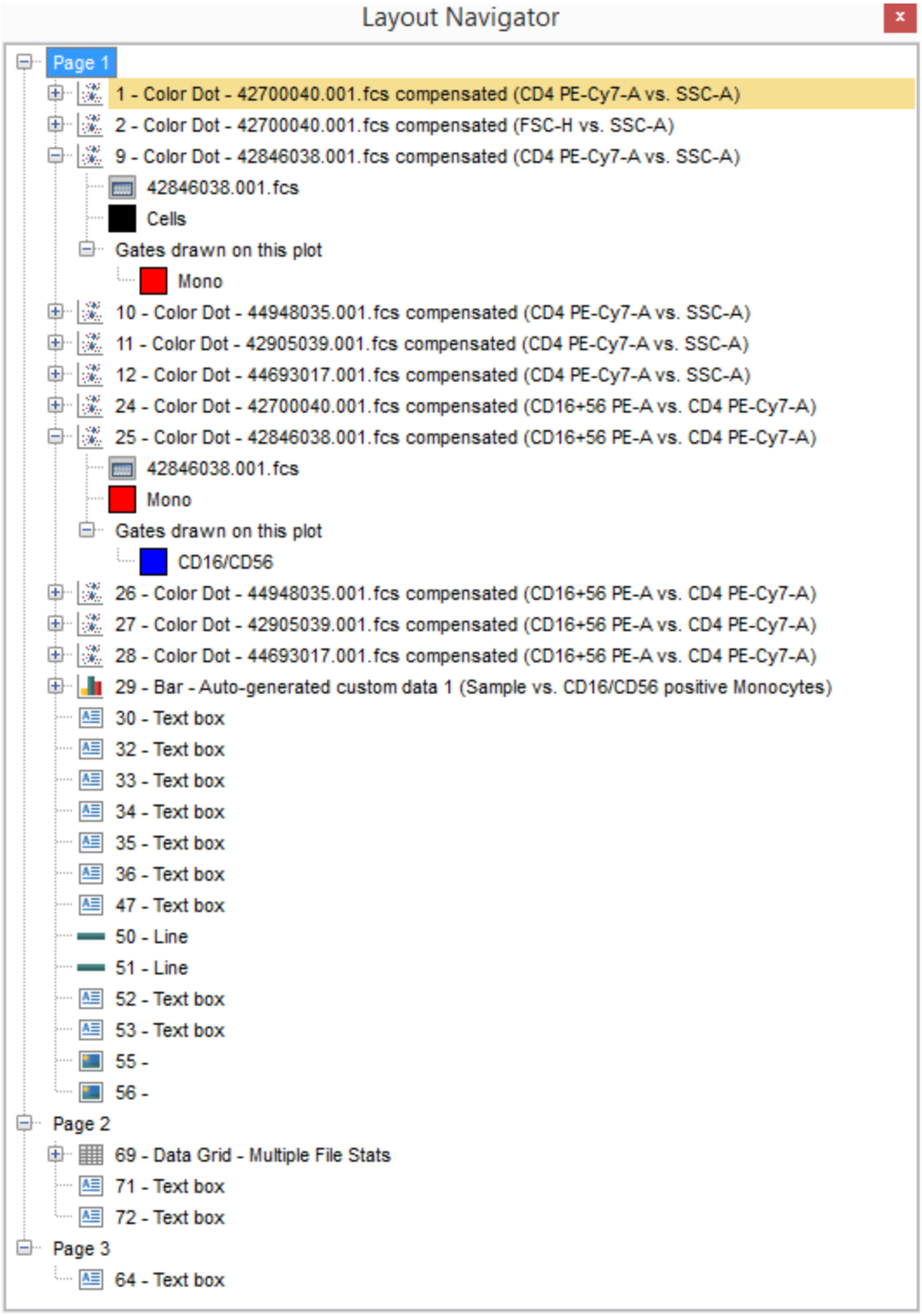

Figure 4.31 Layout Navigator

The first level of the hierarchy in the **Layout Navigator** is the page number. The second level is the objects on your page, such as plots, gate views, text boxes,

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and data grids. If the object contains data (e.g., a plot or data grid), the data set is shown, as well as an indication of whether or not the data is gated.

If you select an object on your layout, the corresponding node in the **Data Navigator** will be highlighted. Hovering the mouse cursor over an object in the docked 113 **Data Navigator** displays a thumbnail view of the object. In a layout with more than one page, clicking on a node in the **Data Navigator** will change the visible layout page to the page where the corresponding object is located.

Click on a node to expand it. While holding the mouse button down, drag an object (e.g., a plot title) stemming from the node to your layout and release the mouse button. The results of drag and drop operations from the Layout Navigator are described in the table below.

Drag and Drop Operation from Layout Navigator	Function
Drag (or Copy & Paste, or Cut & Paste) an object to an empty part of the layout.	Moves the object to the new location. When Copy is used, a copy of the object is created in the new location.
Drag an object onto an existing object in the layout.	Adds the appropriate data to the existing object. You may be prompted to choose special paste format options.
Drag (or Copy & Paste) a data set to an empty part of the layout.	Brings up the Select plot types... 76 dialog to open plot(s) using this data set.
Drag (or Copy & Paste) a data set to an existing plot.	Adds data to the plot. You will be prompted whether to replace the existing data or add it as a new overlay.
Drag a gate to a plot.	Applies the gate to the plot.
Copy a gate and paste it on a plot.	If the plot is an Histogram (i.e. 1D plot), the gate will be applied to all the overlays on that histogram. If the plto is a 2D plot, you will be prompted about creating a new gate on the target plot.
Drag a plot to a text box.	Prompts you to create a keyword or statistics token from that plot in the text box.
Within the navigator, drag (or Copy & Paste, or Cut & Paste) an object onto another Page.	Moves the object to the new location without losing token references. If Cut is used, the original object will be removed. *Note that token references are lost when using cut and paste.*
Within the navigator, drag and drop a page into a new position.	Reorder layout pages.
Within the navigator, copy a page and past it into a new position.	A copy of the page will be created into the new position.
Delete.	This applies to either gates, pages and objects.

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4.3.2 Data Navigator

The **Data Navigator** shows a hierarchical view of all of the data currently showing on your layout. Within each data node, plots and other data grids displaying the data are shown. (Figure 4.32).

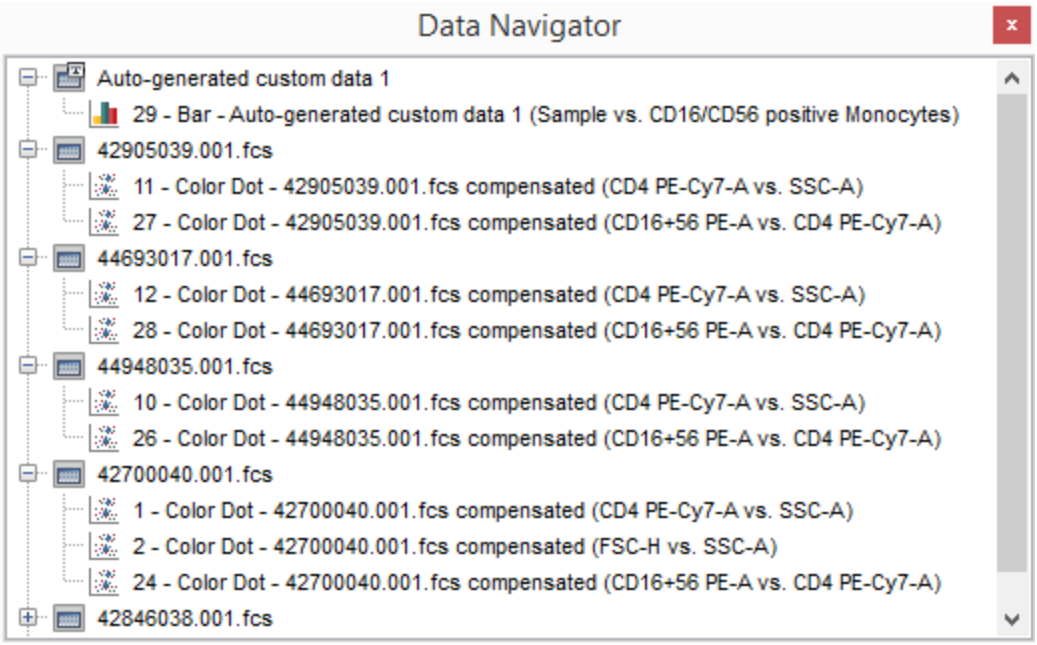

Figure 4.32 Data Navigator

The first level of hierarchy in the **Data Navigator** represents the data set nodes. The second level is the plots and/or data grids associated with the data sets. Hovering the mouse cursor over a plot or data grid in a docked 113 **Data Navigator** window displays a thumbnail view of the object.

Click on a node to expand it. While holding the mouse button down, drag and object stemming from the node to your layout and release the mouse button. The results of drag and drop operations from the Data Navigator are described in the table below.

In FCS Express, plots can be renamed directly in the **Data Navigator** by right-clicking plot title→clicking **Rename** (Figure 4.33). Plot names can still be edited using **Format→General** command, via the ribbon bar, or by right clicking on the plot of interest and updating **Name** field.

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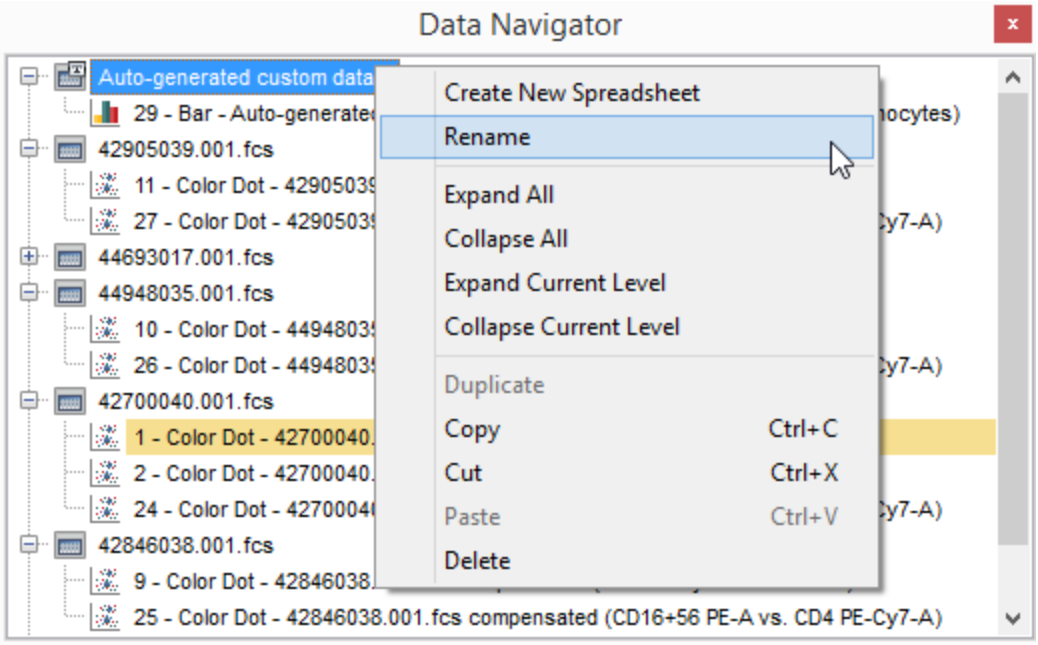

Figure 4.33 - Renaming Plot in Data Navigator

Drag and Drop Operation from Data Navigator	Function
Drag a data set to an existing plot in the layout (or to a plot within the Data Navigator)	Adds data to the plot. You will be prompted whether to replace the existing data or add it as a new overlay. If the target plot does not support overlays, the dragged data set be loaded in the target plot.
Drag a plot to an existing plot in the layout (or to a plot within the Data Navigator)	Adds data to the plot. If the target plot supports overlays, the action brings up a **Paste Special** dialog to insert data or overlays. If the target plot does not support overlays, the data loaded in the dragged plot will be loaded in the target plot.
Drag (or Copy & Paste) a data set to an empty part of the layout	Brings up the Select plot types... 76 dialog to open plot(s) using this data set.
Drag (or Copy & Paste, or Cut & Paste) an object to an empty part of the layout	Opens a copy of the object the object in the new location. When Cut is used, the original object is removed.
Drag an object onto an existing object in the layout	Adds the appropriate data to the existing object. You will be prompted to choose special paste format options.
Cut (or Copy) a plot (from the layout or from the data navigator) and paste it on a data set within the Data Navigator	The data set loaded into the cut (copied) plot will be loaded into all the objects currently loading the target data set. When Cut is used, the cut plot is removed from the Data Navigator.
Drag a plot (from the layout or from the data navigator) and drop it on a data set into the Data Navigator.	The data set loaded into the dragged plot will replace the target data set.

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Drag and Drop Operation from Data Navigator	Function
Cut (or Copy) a plot (from the layout or from the data navigator) and paste it on a plot within the Data Navigator (or on a plot in Edit mode within the layout)	Adds data to the plot. If the target plot supports overlays, the action brings up a **Paste Special** dialog to insert data or overlays. If the target plot does not support overlays, the data loaded in the dragged plot will be loaded in the target plot. When Cut is used, the cut plot is removed from the Data Navigator.
Copy a data set and paste it on a plot within the Data Navigator (or on a plot in Edit mode within the layout)	Adds data to the plot. If the target plot supports overlays, the action brings up a **Paste Special** dialog to insert data or overlays. If the target plot does not support overlays, the data loaded in the dragged plot will be loaded in the target plot.
Copy a data set and paste it on another data set	The copied data set will be loaded into all the objects currently loading the target data set.

4.3.3 Gate Navigator

The **Gate Navigator** shows a hierarchical view of the gates that have been defined in your layout, the plots on which those gates have been applied, and data files the gates or Data Specific Gates are applied to (Figure 4.34). Any action that can be completed in the Gate View 345 (i.e. change hierarchies, edit gate information, and apply gates via drag and drop) can be accomplished with the Gate Navigator except that the Gate Navigator is a separate dockable window rather than an insertable object as is the Gate View. Moreover the Gate Navigator can be used to:

- Work with and view Data Specific Gates 324 and Data Specific Gate Groups 331
- Change plot names (For plots, the name property is the name of the plot to display in lists (**Format→General** 240 options 240) where the user is required to select a plot. The name is also used when displaying objects in different Navigators 123)

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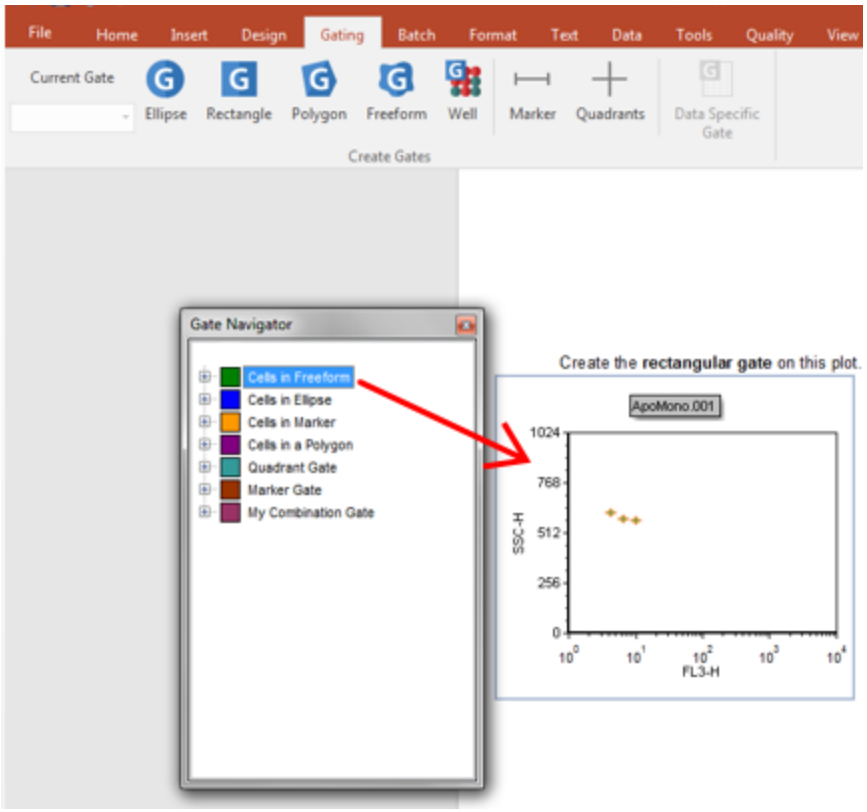

Figure 4.34 Gate Navigator

The first level of hierarchy in the **Gate Navigator** represents the gates defined in your layout. The second level is the plots using those gates. Hovering the mouse cursor over a plot in the docked 113 **Gate Navigator** window displays a thumbnail view of the plot.

Double clicking on a gate node will launch the **Edit Gate dialog** 345 where gate information can be changed or updated.

Click on a node to select it. While holding the mouse button down, drag the node to your layout and release the mouse button. The results of drag and drop operations are described in the table below.

Drag and Drop Operation	Function
Drag a gate to a plot in the layout (or to a plot within the Gate Navigator).	Applies the gate to the plot.
Drag (or Copy & Paste, or Cut & Paste) a plot (in the layout or within the Gate navigator) onto a gate of the Gate Navigator.	The target gate will be made Data Specific for the dragged data. If Cut is used, the cut plot will be removed.

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Drag and Drop Operation	Function
Drag (or Copy & Paste, or Cut & Paste) a plot to an empty part of the layout.	A copy of the plot will be created in the new location. If Cut is used, the cut plot is removed.
Drag a plot onto a plot in the layout (or to a plot within the Gate Navigator)	Adds the appropriate data to the existing object. If the target plot supports overlays, the action brings up a **Paste Special** dialog to insert data or overlays. If the target plot does not support overlays, the data loaded in the dragged plot is loaded in the target plot.
Copy and Paste (or Cut and Paste) a plot from the Gate Navigator onto a plot in Edit mode within the layout	Adds data to the plot. If the target plot supports overlays, the action brings up a **Paste Special** dialog to insert data or overlays. If the target plot does not support overlays, the data loaded in the dragged plot will be loaded in the target plot. When Cut is used, the cut plot is removed from the Data Navigator.
Drag and drop a gate to a different position within the Gate Navigator	The gate is moved into the new position in the hierarchy.

Note: The Gate Navigator is a complementary tool to the Gate View 345. The gate view allows similar functionality in terms of dragging and dropping to/from plots, but allows additional gating functionality.

The Gate navigator window can be formatted in order to display or not display plots on which those gates have been applied as well as data files the Data Specific Gates are applied to.

To format the Gate Navigator:

1. Right click on any empty part of the Gate Navigator window.
2. Select **Format** from the right-click menu. A **Formatting Gate Navigator** window with the following options will open:

- **Create plot navigator items**. Flag this item to display plots on which gates have been applied within the gate navigator window.
- **Show data specific gates**. Flag this item to display data specific gates within the gate navigator window.

4.4 Layout Page Features

Individual objects in FCS Express can be manipulated almost identically to objects in Microsoft PowerPoint®. Not only can individual objects be stretched, resized, and formatted, but any item on the layout can be aligned with respect to any other item. Multiple items can be selected at once and actions can be performed on all the selected items. Every item that can be inserted onto a Layout Page in FCS Express has a formatting menu associated with it. To access the formatting

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dialog for any object, hover the cursor over the object and right-click. When the formatting menu appears, select the desired function.

4.4.1 Inserting Items onto a Page

Use the Insert Tab 69 to insert objects on the current layout page. The objects you can insert are located in five groups:

- **General**

  Shapes (**Line**, **Arrow**, **Ellipse**, **Rectangle**), **Text Box**, **Gate View**, **Picture**, **Data Grid**

- **Spreadsheet** 414

  New spreadsheet

- **1D Plots**

  Histogram, Kinetics, Multicycle DNA, Proliferation, Bar, Pie

- **2D Plots**

  Dot, Density, Color Dot, Contour, Surface, Picture Plot, Scatter, Scatter with Regression

- **Other Plots**

  Heat Map

To insert an object on the layout page in the default size:

1. Click on the command within the group.
2. Position your cursor at the point on the layout page where you would like the upper left corner of the object to be.
3. Click on the layout page and FCS Express will position your object there.

To insert an object on the layout page in a custom size:

1. Click on the command within the group.
2. Drag a sizing rectangle on the layout while holding down the mouse button.
3. Release the mouse. The object will be inserted into the sizing rectangle.

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4.4.2 Selecting Items on a Page

To work effectively with objects/items in FCS Express, you must understand the difference between *edit* mode and *selected* mode.

In order for an object to be manipulated, the object must first be selected. A red border surrounding the object denotes that it is selected. Only selected items can be moved, resized, deleted, etc.

In order to edit items within an object, the object must first be in edit mode. For example, to modify a gate, the plot containing the gate must be in edit mode. When an item is in edit mode, it displays a green border.

Figure 4.35 shows one item in selected mode and one item in edit mode.

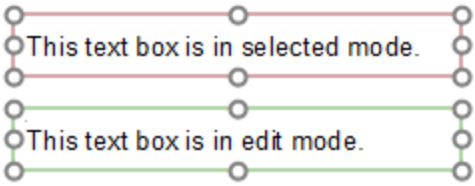

Figure 4.35 Selected and Edit Modes

Many items can be selected at the same time, but only one item can be in edit mode at one time.

To put an object into edit mode, click anywhere within the interior of the object. A green border will appear around the object signifying that it is in edit mode. If an object is in edit mode, but does not contain any items that can be selected, the object will behave as if it is in selected mode. For more information on editing items contained within a plot, refer to the following sections: Adding a Text Box to a Plot 164, Using Gates 316, and Using Markers 352.

To put a single item into selected mode, move the cursor over the border of the item. The cursor will change to a 4-headed arrow . Click the border once. A red border will be displayed around the item indicating that the item has been selected. Alternatively, you can put an item directly into selected mode by holding down the **Shift** key while clicking anywhere on the item.

There are two methods for selecting multiple specific items at the same time. The first method uses a click and drag feature (Figure 4.36). If the items for selection are located near one another on a page, draw a rectangle around all of the items to be selected. Press and hold down the mouse button at the top left of where you want the rectangle to be, then drag the cursor down and to the right. The point that you release the button will be the bottom right corner of the

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rectangle. All items inside this area will now be selected (so that they may be manipulated together).

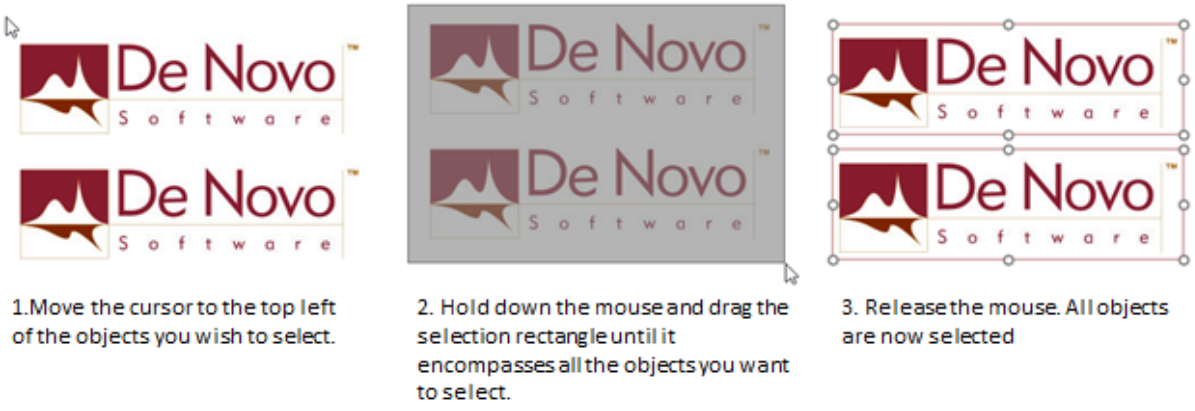

Figure 4.36 Selecting Multiple Objects with Click and Drag

The second method of selecting multiple specific items is useful when the items are not located in close proximity to one another. Once the first item has been selected as a single item, place the cursor anywhere over the next item you wish to select. Press and hold the **Shift** key while clicking. The second item should now have a red border. Continue the **Shift-**Click selection process for each additional item you wish to select. Once multiple objects have been selected, they can be copied, cut, resized, moved, and aligned simultaneously as explained in the sections below.

If you want to select all of the items on the current page, use **Home→Editing→Select All**. Alternately, press **Ctrl+A**.

Clicking outside the selected item(s) will deselect them and put them in a neutral state (no red or green border).

4.4.3 Overlaying Items on a Page

In addition to the *edit* and *selected* modes, FCS Express 6 introduces a third mode called *overlay*. When an object is in Overlay Mode, it is outlined in blue (Figure 4.37).

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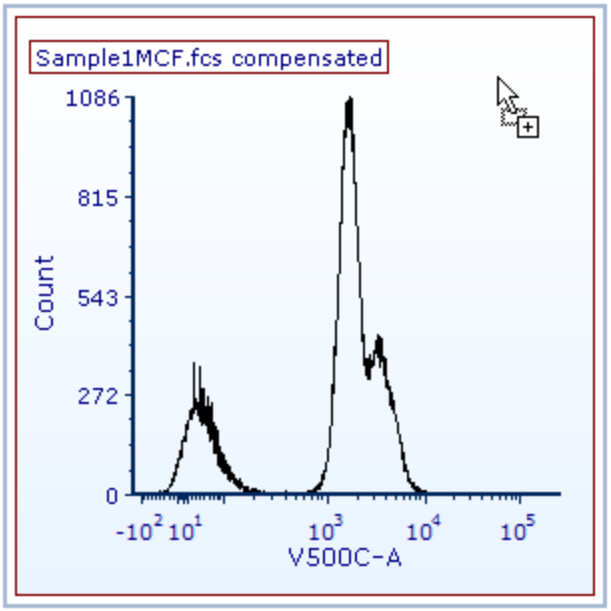

Figure 4.37 - Histogram in Overlay Mode

Dragging and dropping selected data file(s) from the **Data List** onto the following plots, will activate overlay mode and prompt the **Select Drag and Drop Action** window upon mouse release:
Histogram, Kinetics, Multicycle DNA, Proliferation, Scatter, Scatter with Regression, Bar Chart, Pie Chart, Dot, Color Dot, Heat Map

Dragging and dropping selected data file(s) from the **Data List** onto the following plots, will activate overlay mode and replace data upon mouse release:
2D Plots - Contour, Density, Surface

Dragging and dropping **Gates, Markers,** or **Quadrants** onto plots will activate overlay mode and be applied upon mouse release.

Dragging and dropping plots onto **Text boxes** will activate overlay mode and prompt **Paste Special** window to select **Keyword** or **Statistic Token.**

4.4.4 Grouping Items on a Page

Individual objects on a page can be grouped together. After grouping items, all objects in the group will be selected if any one item in the group is selected.

This can be useful, for example, if several objects are in a specific relative location to each other and you want to make sure that you cannot move one without moving all of the others. Since when objects are grouped, selecting any one of them will select all of them, the move operation will be applied to all the selected objects together.

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In order to create a group, select the objects you want to group as described under Selecting Items on a Page 132, then, either:

- Select the **Group** command from the **Home→Editing→Arrange** gallery, or
- Right-click when the cursor is within one of the selected objects and choose **Grouping→Group** from the pop-up menu.

To ungroup objects, use either of the following methods:

- Select the **UnGroup** command from the **Home→Editing→Arrange** gallery, or
- Right-click when the cursor is within one of the selected objects and choose **Grouping→Ungroup** from the pop-up menu.

4.4.5 Cutting, Copying, Deleting, Duplicating, and Pasting Items on Pages

After selecting one or more objects as described under Selecting Items on a Page 132, use the **Home→Clipboard** commands or **Ctrl+X** (cut) **or Ctrl+C** (copy) on your keyboard to perform standard word processing functions (e.g., **Cut**, **Copy**). Objects that have been copied to the clipboard with these functions can be pasted onto different pages or even onto a different layout using the **Home→Clipboard→Paste** command, or **Ctrl+V** on your keyboard.

To delete an object, select it and use the **Home→Editing→Delete** command (or press the **Delete** key on your keyboard).

The **Home→Clipboard→Duplicate** command duplicates the selected item(s). A copy and paste function will be performed at the same time. By clicking on the **down arrow** associated with **Duplicate**, the command expands to reveal five items: **Duplicate**, **Duplicate and Next**, **Duplicate and Prev**(ious), **Multiple Duplicate and Next**, and **Multiple Duplicate and Prev**(ious).

The Next and Prev functions associated with these commands duplicate the selected object(s) and change to the next or previous file in your data list. The Multiple commands allow you to make more than one copy of the selected item(s). The default positioning of the duplicated objects can be set in user options 180.

Note: In FCS Express 6, objects and text can be copied into another application simply using the copy and paste functions, without loss of formatting information. Text boxes, statistics tables, compensations, and other items in tabular format can be copied from FCS Express and pasted into spreadsheet software such as Microsoft Excel in the same format.

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4.4.6 Moving Objects

In order to move an object, or group of objects, they must be in selected mode (as described under Selecting Items on a Page 132).

To move selected object(s), position the cursor on the "moving region" of the selected object, i.e., the red border surrounding the object. *Note*: With some objects, such as pictures, the "moving region" is the entire object. Once the cursor is above the moving region, it changes to a four-headed arrow . When the four-headed arrow appears, hold down the mouse button and move the selected object(s) to the desired location. After the object is in place, release the mouse button.

You can also move selected objects using the keyboard. When the objects are selected, pressing the arrow keys will move items up, down, left, or right. Holding down the **Shift** key when pressing the arrow keys will move the selected objects in finer increments.

4.4.7 Resizing Objects

To resize selected object(s), position the cursor over the one of the corners of a selected object until the cursor becomes a two-headed arrow. When the two-headed arrow cursor is present, hold down the mouse button while moving the cursor to resize the object. Release the mouse button once the item has reached the desired size. If you hold down **SHIFT** while resizing, the aspect ratio (height vs. width) of the selected object(s) will be maintained.

The **Format** tab→ **Size** command opens a dialog window where the desired size can be specified by height X width, or scale (percent).

A convenient way to resize several objects to the same size is using the Align tool 137.

4.4.8 Aligning Objects

The sides, tops, bottoms, centers, heights and/or widths of objects can be easily aligned.

To align multiple items, first select the objects you wish to align, and then go to **Home→Editing→Arrange** or **Align**. This will bring a list of the alignment tools that can be used to align your selected objects. (Figure 4.38).

Clicking on Align, under the Position Objects section, will open the Alignment dialog window, as seen in Figure 4.

Note: The Distribute Equally item in the **Align** dialog ensures that there is an equal distance, horizontally or vertically, between all the selected objects.

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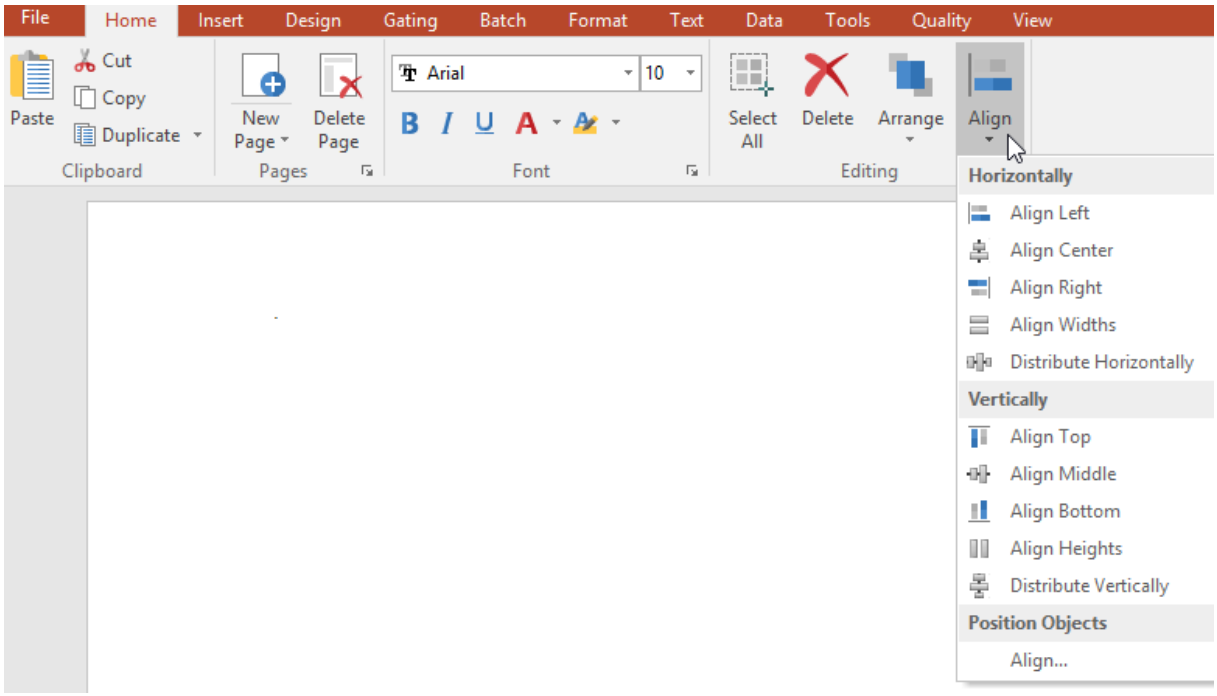

Figure 4.38 Align Menu

Objects are aligned with respect to the first item selected. For example, if you select several items, use the **Home→Editing→Align→Align Top** command, the tops of all the items will be placed in the same position as the first selected item. An example of objects whose horizontal centers were aligned is shown in Figure 4.39.

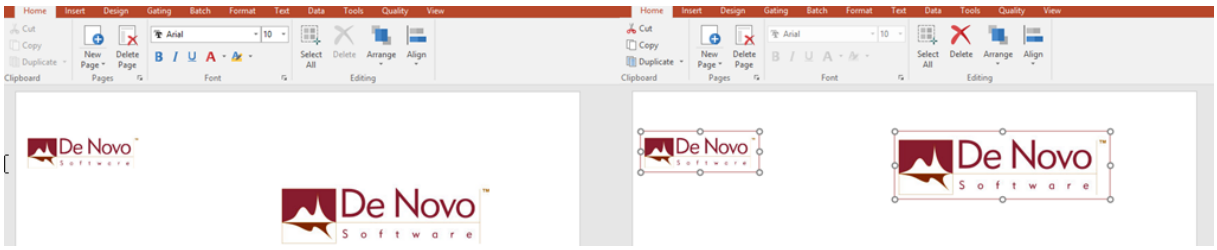

Figure 4.39 - Before Alignment (left); After Alignment of Tops (right)

To make multiple objects the same size:

1. Select the object(s) you wish to resize.
2. **Ctrl + Click** on the objects to be resized.
3. Use both **Home→Editing→Align→Align Widths** and **Home→Editing→Align→Align Heights** commands. All objects will be resized to the dimensions of the first object you selected.

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4.4.9 Ordering Objects

You can also control which object(s) are on top of other objects. This is very useful when objects overlap each other in the layout and you wish to determine which is on top. To change the order of selected object(s), choose **Home→Editing→Arrange** and then select a command from the list in the **Order Objects** portion of the gallery (or right-click on selected object(s) and choose a menu item under **Order**).

The commands function as follows:

- **Bring to Front**

  Brings the selected object(s) to the front of all overlapping objects.

- **Send to Back**

  Sends the selected object(s) all the way to the back of all other overlapping objects.

- **Bring Forward**

  Brings the selected object(s) one level towards the top of all other overlapping objects.

- **Send Backward**

  Sends the selected object one level towards the back of all the other overlapping objects.

Figure 4.40 demonstrates the results when you rearrange the order of the red circle and the green rectangle.

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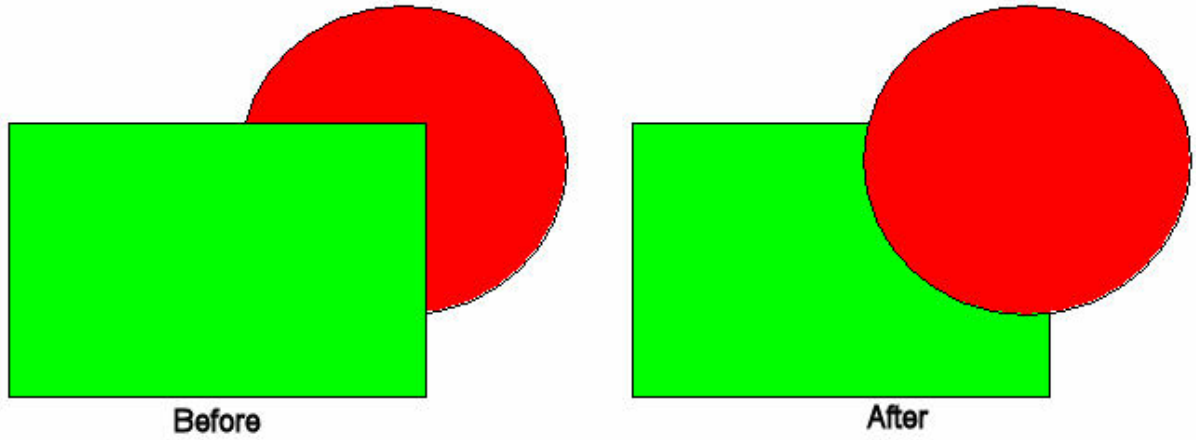

Figure 4.40 Ordering Objects

4.4.10 Undoing and Redoing Changes

FCS Express allows up to 30 operations to be undone or redone by default.

Undo

To undo any changes made to a layout, select (Undo) from the Quick Access Toolbar. The text of the tooltip associated with the Undo icon will indicate which operation will be reversed or undone.

You can also use the **Ctrl+Z** keyboard shortcut to undo the last operation you performed.

Redo

To redo any changes made to a layout, select the (Redo) from the Quick Access Toolbar. The text of the tooltip associated with the Undo icon will indicate which operation will be reversed or undone.

You can also use the **Ctrl+Y** keyboard shortcut to redo the last operation you performed.

4.4.11 Page Layout Using Predefined Placeholders

Using the **Design→Page Layout→Layout** command or **Home→Pages→New Page** command, you can select and apply predefined layouts to the current page (Figure 4.41).

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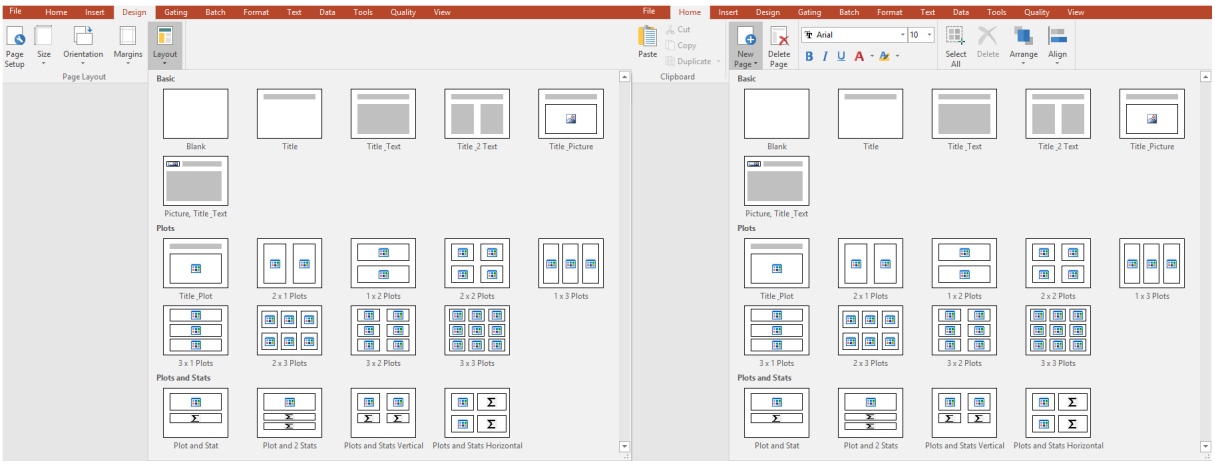

Figure 4.41 Layouts Using Predefined Placeholders from Design tab (left) or Home tab (right)

To most efficiently use this tool, look for a predefined layout containing objects similar to the layout you would like to create. By selecting a predefined layout, the current page is populated with placeholders. Now you can easily insert plots, text boxes, and pictures into the Placeholders with a simple mouse click. The placeholders behave similarly to any other object and can be moved, resized, copied to the clipboard, and aligned. An example **plot placeholder** is shown in Figure 4.42.

Figure 4.42 An Example Plot Placeholder

4.5 Using the Master Page

The Master Page is always present in a layout and therefore does not need to be created. Anything inserted onto the Master Page will appear by default as the background of all pages of a layout. For example, a Text Box containing the current date, time, and page number inserted onto the Master Page will automatically appear on all of the pages in the layout. However, you can choose

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on an individual page basis 98, whether or not the master page appears as the background of that page.

To view the Master Page, select the **View→Show→Master Page** command (Figure 4.43).

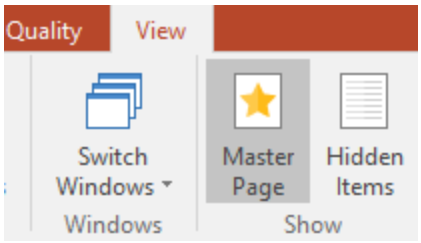

Figure 4.43 - Master Page from Ribbon

The default background that will appear on all the pages can be edited on the Master Page as described in Changing Background Color and Design 141.

4.5.1 Changing Background Color and Design

To change the background of the Master Page, open the Master Page as described in Using the Master Page 140, right-click anywhere on the master page, and choose **Format** from the pop-up menu. When the **Formatting Master Page** dialog appears, choose **Background** from the category at left (Figure 4.44).

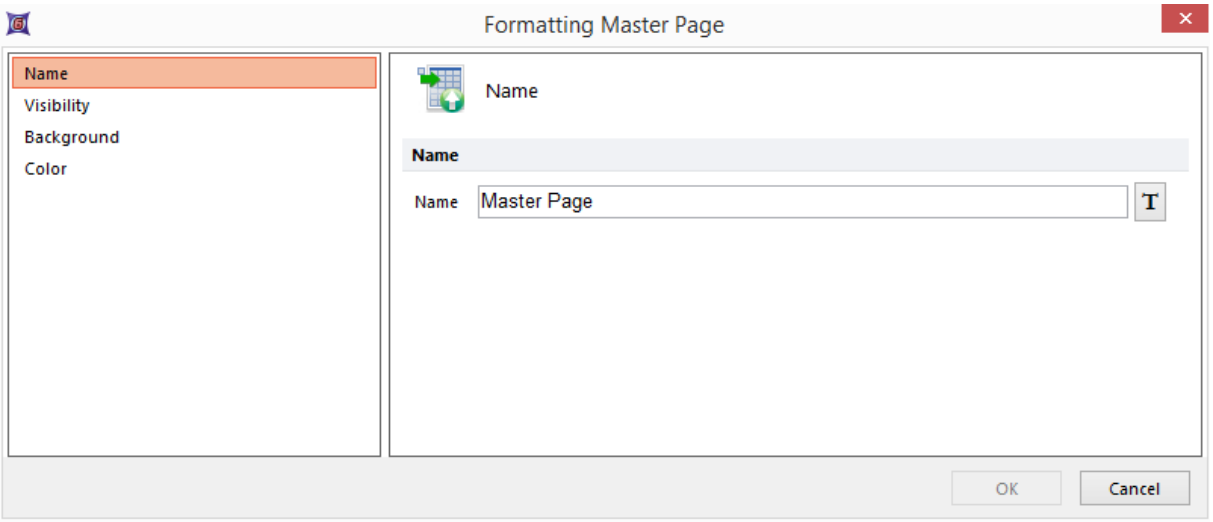

Figure 4.44 Formatting Master Page Background Screen

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The **Master Page** default setting for background is white as a solid color. You may choose another solid color, a color gradient, or an image for the background. If you select an image, you will be able to stretch it over the entire page or center it on the page.

A page with a thin black shadow will always appear around the border of the page, unless you choose the **Transparent** radio button on the **Format Master Page** dialog. Having a transparent page is useful if you simply want to analyze some data and do not care about the specific layout on the page.

4.6 FCS Express Short Cut Keys

FCS Express 6 contains many shortcut keystroke combinations to make navigating and working within the software easier. The table below summarizes the available short cut keys.

Shortcut	Command	Applies To
Ctrl+C	Copy	Objects on layout, tokens, text, items into navigator views
Ctrl+V	Paste	Objects on layout, tokens, text, items into navigator views
Ctrl+X	Cut	Objects on layout, tokens, text, items into navigator views
Shift+Del	Secondary shortcut for Cut	Objects on layout, tokens, text, items into navigator views
Ctrl+Ins	Secondary shortcut for Copy	Objects on layout, tokens, text, items into navigator views
Shift+Ins	Secondary shortcut for Paste	Objects on layout, tokens, text, items into navigator views
Ctrl+Z	Undo	Any action in the layout
Ctrl+Y	Redo	Any action in the layout
Ctrl+B	Bold	Text in text boxes and labels in selected plots
Ctrl+I	Italic	Text in text boxes and labels in selected plots
Ctrl+U	Underline	Text in text boxes and labels in selected plots
F2	Rename	Items in navigator views and batch actions and items
Ctrl+A	Select all	Objects on layout, text, data list, batch actions, custom tokens, canned comment, and alerts navigator
Del	Delete	Selected object on layout, selected item in navigator views, selected text, selected user in non secure login mode

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Ctrl+D	Duplicate	Selected object on layout, selected gate, custom tokens, canned comments and alerts in navigator views
PgDn	Switch to next page in layout	Layouts with multiple pages
PgUp	Switch to previous page in layout	Layouts with multiple pages
Ctrl+N	Open new layout	Any layout
Ctrl+O	Open layout	Any layout
Ctrl+S	Save current layout	Any layout
Ctrl+Shift+S	Save current layout As	Any layout
Ctrl+P	Print current layout	Any layout
Ctrl+Shift+P	Print Setup	Any layout
Ctrl+W	Close current layout	Any layout
F1	Show help	Any layout
Up	Scroll up	Layout or selected object
Down	Scroll down	Layout or selected object
Left	Scroll left	Layout or selected object
Right	Scroll right	Layout or selected object
Esc	Cancel adding or editing	Plots, shapes and gates
Space	Finish creating a polygon gate	Creating polygon gates
Ctrl+Alt+D	Duplicate and Previous	Plots and data grid
Shift+Ctrl+D	Duplicate and Next	Plots and data grid
Alt+Left	Previous on selected objects	Plots and data grid
Shift+Alt+Left	Previous on all objects	Plots and data grid
Alt+Right	Next on selected objects	Plots and data grid
Shift+Alt+Right	Next on all objects	Plots and data grid
Ctrl+L	Change data file for selected plot	Plots and data grid
Shift+Alt+L	Change data file for all plots	Plots and data grid
Ctrl+Alt+E	Multiple duplicate and previous	Plots and data grid
Shift+Ctrl+E	Multiple duplicate and next	Plots and data grid
Ctrl+E	Edit user name	User profiles from User Selection window

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Ctrl+F	Edit format	Objects, Text Boxes, Plots, Data Grids, and Shapes
1-9	Change x parameter for selected plot	Plots
Shift+1-9	Change y parameter for selected plot	Plots
Ctrl+M	Maximize/Restore from the plot's menu	Plots
Double Click on Plot	Opens Format Dialog	Plots
Double Click on Page Tab	Opens Format Dialog	Page tabs
Hold Alt key	Command keys highlight on ribbon - pressing highlighted command keys activates action	Ribbon
Hold Shift key click on object	Put object in selection mode without allowing moving	Objects, Text Boxes, Plots, Data Grids, and Shapes

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Chapter

5 Working with Pictures

In addition to plots and text boxes, FCS Express allows you to insert pictures into layouts. For working with images from Image Cytometry experiments please see the topics on Picture Plots 1044 and Image Galleries 684.

5.1 Inserting Pictures

To insert a picture onto a layout page, use the **Insert→General→Picture** command. Click on the layout where you would like to place the picture and the **Insert Picture** dialog (Figure 5.1) will appear, allowing you to select the picture you want to insert. Once the picture has been selected, click on the arrow located on the **Open** button (to the right of the file name), and choose either **Yes or No** for embedding picture. In both cases, the picture will be placed on the current page.

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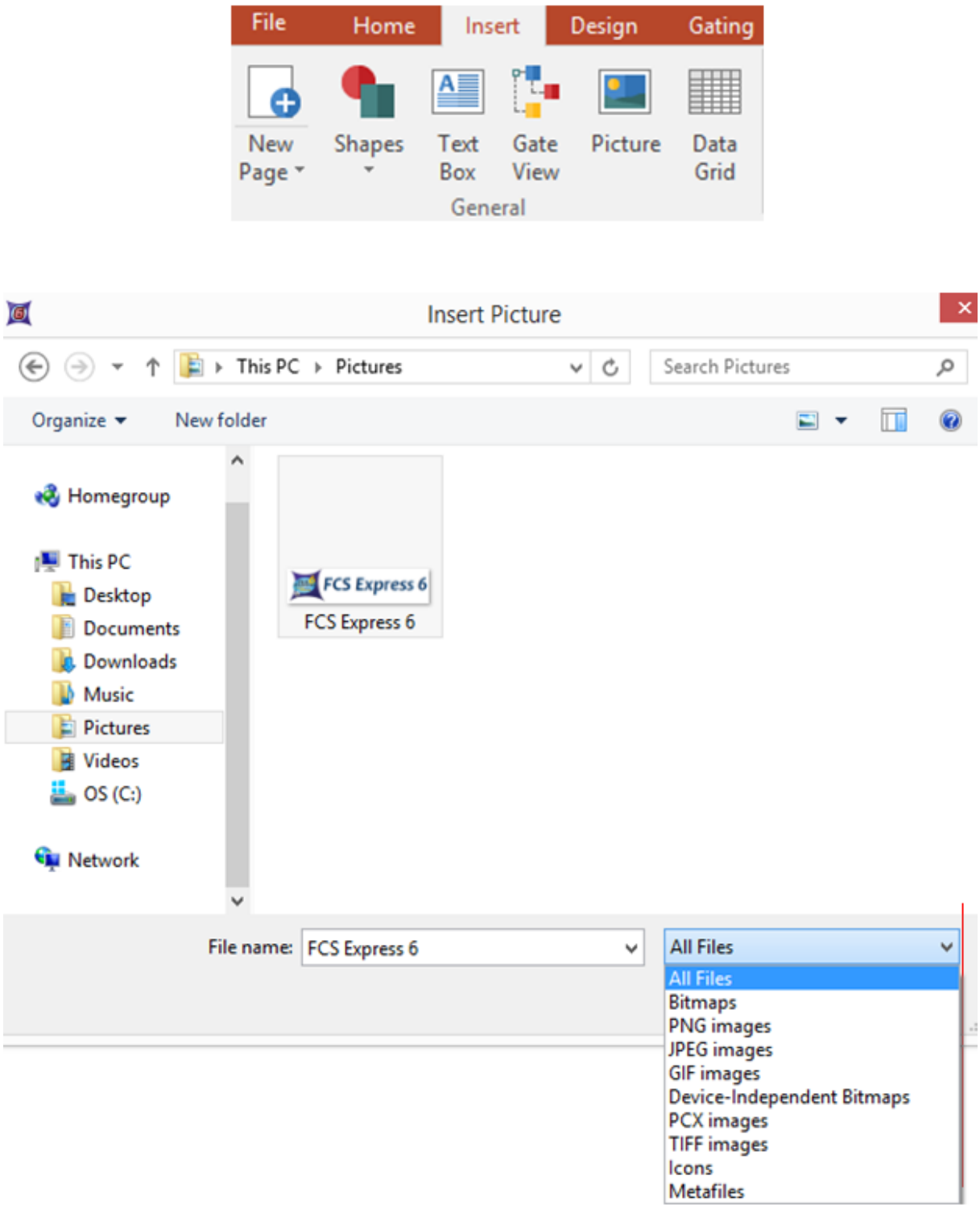

Figure 5.1 - Insert Picture Dialog

The following graphics file types are supported: Windows Bitmaps (*.bmp), Portable Network Graphics (*.png), JPEG files (*.jpg), GIF (*.gif), Device-

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Independent Bitmaps (*.dib), PCX Images (*.pcx), TIFF (*.tif), Icons (*.ico), Windows Metafiles (*.wmf), and Windows Extended Metafiles (*.emf).

Once a picture is selected to be inserted, an Embed Picture Message (Figure 5.2) will appear. If you choose **Yes**, the picture will be saved as part of the layout file. If you choose **No**, the picture will not be saved as part of the layout and the file will be smaller. However, if the layout file is moved to another computer, or the picture is moved to another location, the linked picture will no longer be visible when the layout file is opened.

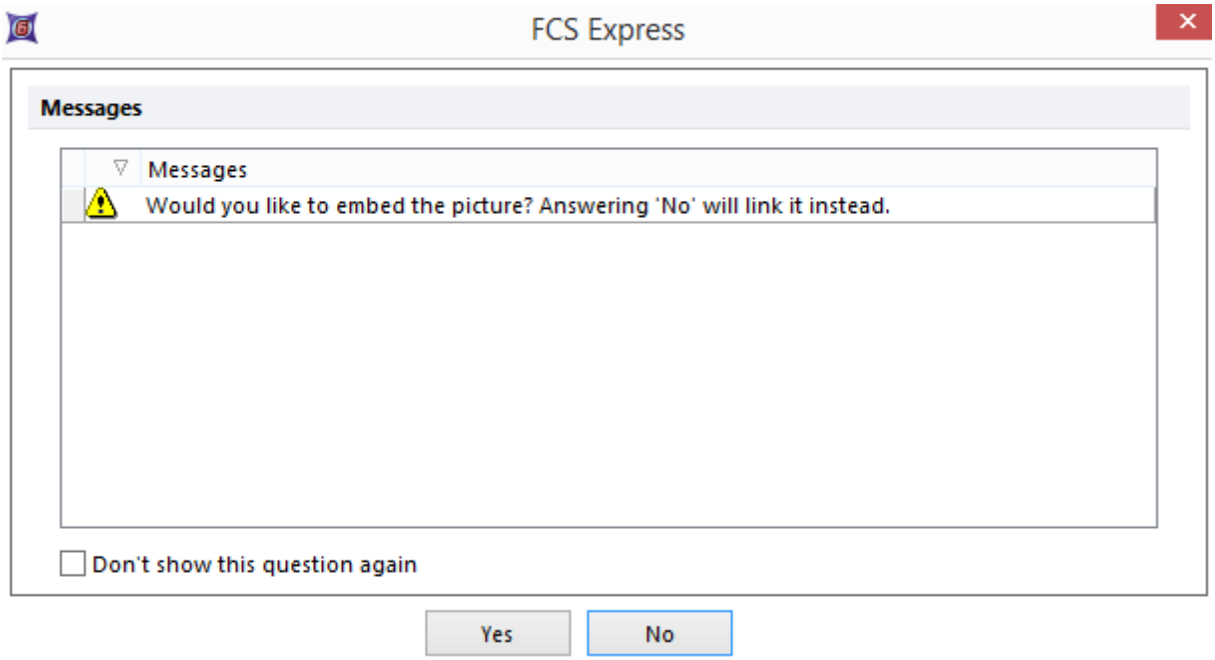

Figure 5.2 - Embed Picture Message

5.2 Formatting Inserted Pictures

To format a picture, select the picture and choose the appropriate command from **Format→Object Options**. Alternatively, right-click on the Picture and select **Format** from the pop-up menu.

The **Formatting Picture** dialog (Figure 5.3)will allow you to edit the border and size 243 of the image as well as general settings and formatting authorizations.

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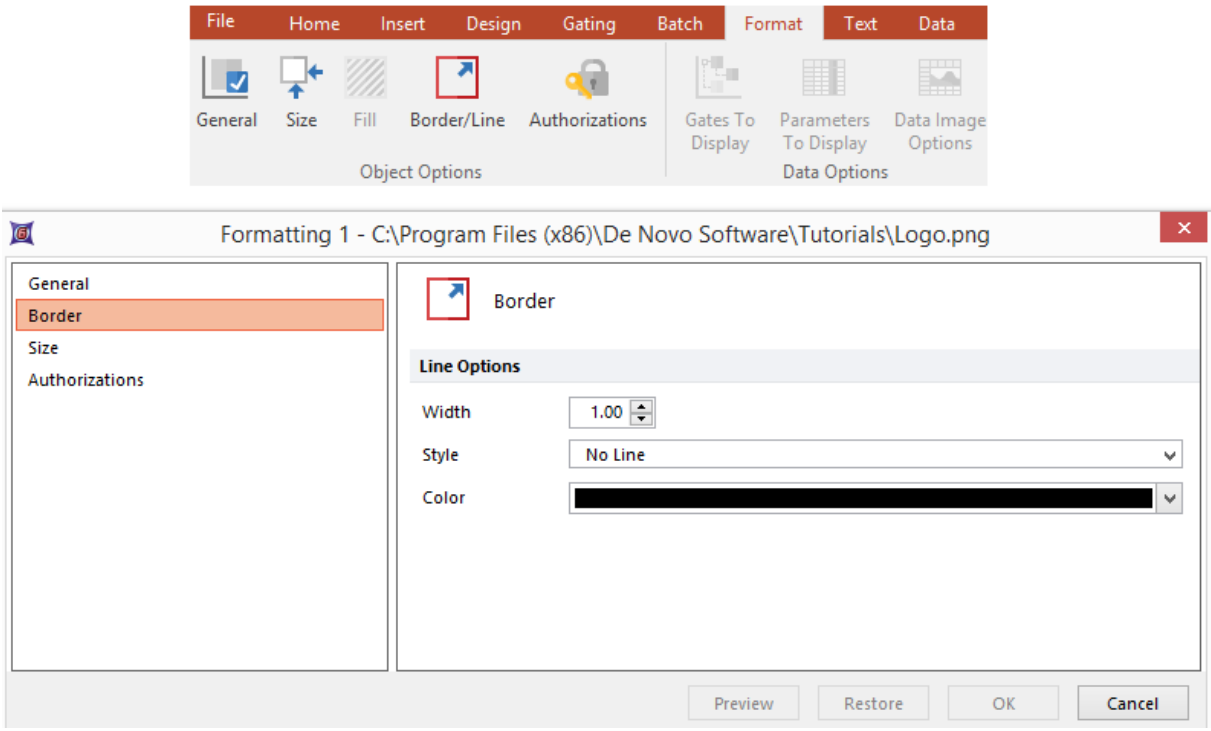

Figure 5.3 - Formatting Picture Dialog

Chapter

6 Working with Text Boxes

Text Boxes are used to insert text anywhere you want on the layout. Text Boxes can be used to display text, statistics, tables, page numbers, as well as entire paragraphs. Text boxes support all the formatting options that are available in modern desktop publishing applications, including bullets, numbering, paragraph alignment, and font properties.

6.1 Inserting Text Boxes onto a Page

To insert a Text Box onto a page, use the **Insert→General→Text Box** command (Figure6.1). Immediately after placing the Text Box on the Page, it will be in edit mode.

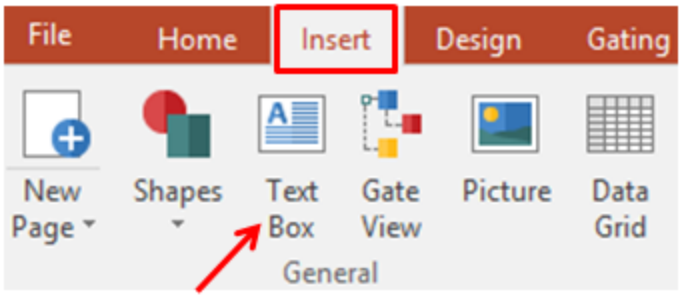

Figure 6.1 - Inserting a Text Box

6.2 Quadrant Label Text Boxes

Plots displaying quadrants have a unique type of text box displayed referred to as a **Label Text Box**. A Label Text Box may be formatted much like a normal text box 153 in FCS Express, but the contents cannot be edited by clicking on and selecting the text box as you would do with other standard text boxes in FCS Express.

Label Text Boxes on plots default to display the quadrant statistic defined by your 2D Plot Option User Options 191.

To change the contents of a Quadrant Label Text Box please follow the steps below.

1. Click on the quadrant Label Text Box to select it.
2. Right click in the selected Label Text Box.
3. Choose **Format**.
4. Choose the **Text Box Contents** category (Figure 6.2).
5. Change the contents in the **Text Box Contents** field to your desired text, symbols, or token 692.

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The Label Text Box on the plot will now reflect the entry in the **Text Box Contents** field.

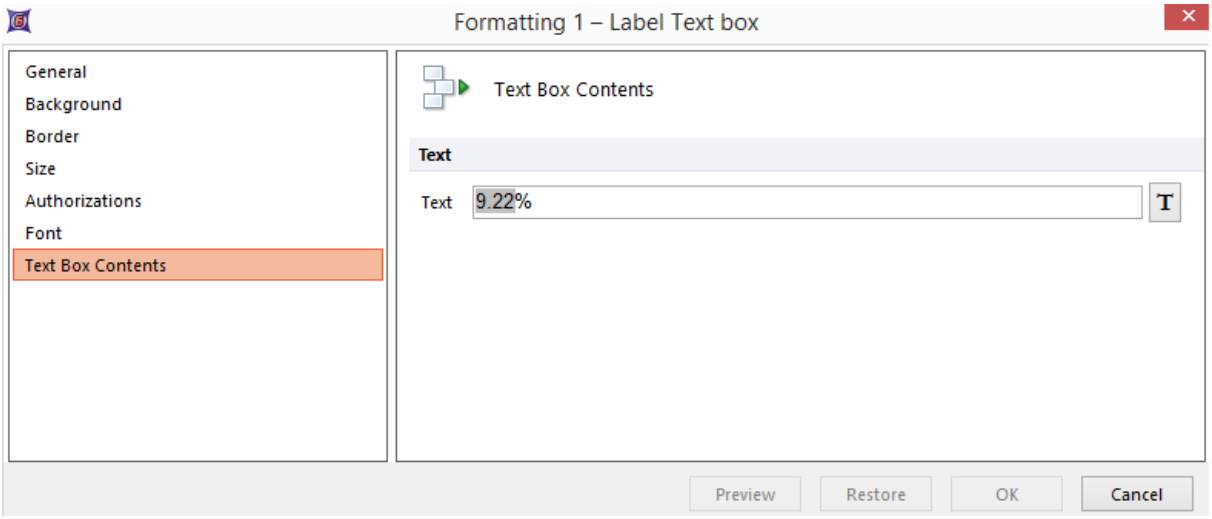

Figure 6.2 - Label Text Box (for quadrant derived text boxes) Dialog

6.3 Entering and Formatting Text in a Text Box

In order to enter text in a Text Box, place it in edit mode 132 by clicking anywhere inside it.

The Text Box can be used just like a word processor meaning you can:

- Cut/Copy/Paste text within the Text Box.
- Format the text (font, alignment, etc.).
- Insert numbering and bullets.
- Paste text from word processing applications.
- Copy text from a Text Box and paste it into a word processor.
- In addition, Tokens, may be placed in text boxes. 692

The most common formatting options are available on the **Text** 75 tab. Detailed text formatting can be performed using the commands of the **Text→Font** and **Text→Paragraph** groups.

If the text box is in edit mode, changing fonts will change the currently selected text. If the text box is in select mode, changes to the font properties will affect all the text in the text box. The Text Box automatically grows when the text being entered reaches the bottom of the Text Box window.

The **Font** and **Paragraph** groups contain dialog box launchers on the lower right, indicated by the button.

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This **Font** dialog (Figure 6.3) allows you to change all font properties. With the **Layout** Tab options, you can change the character spacing and subscript/superscript offset.

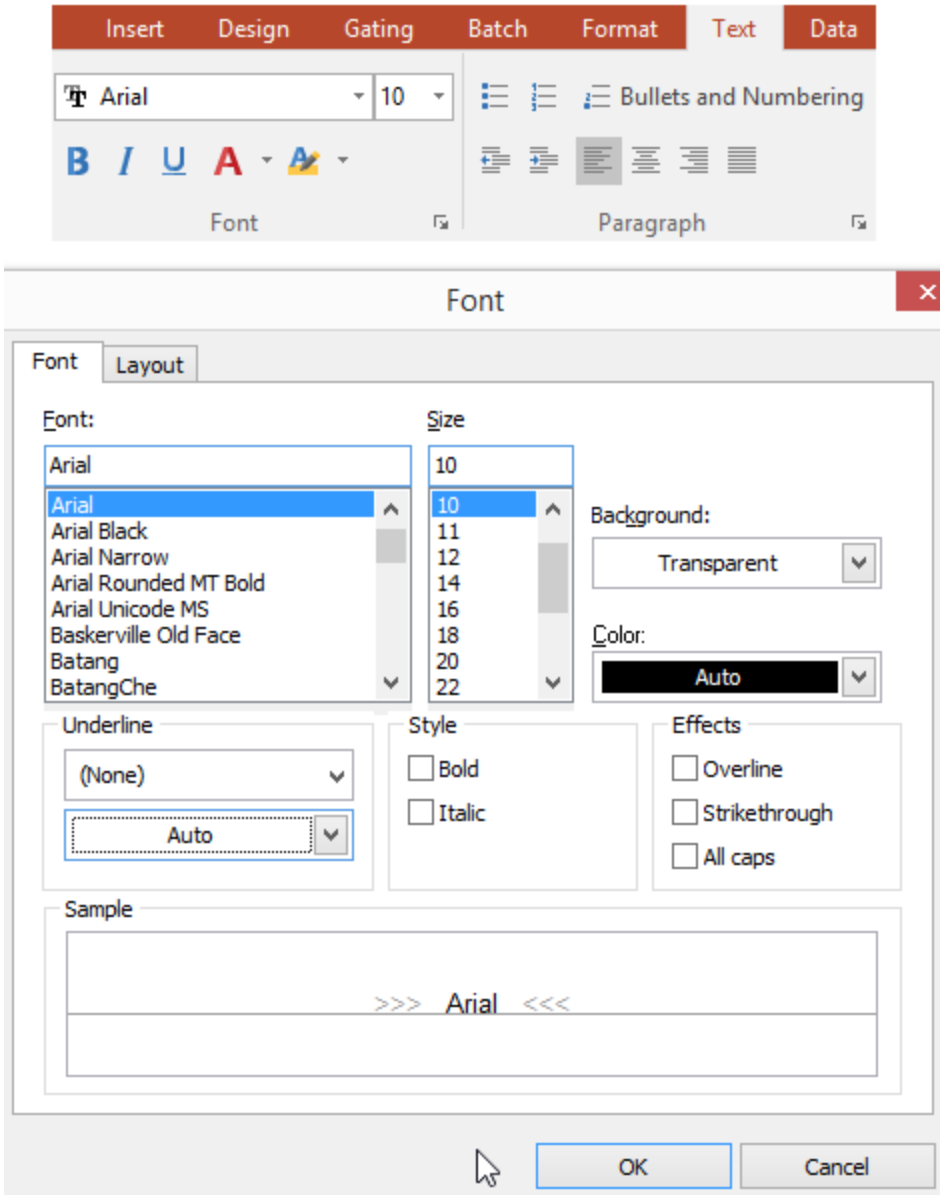

Figure 6.3 - Font Tab and Dialog

To control the detailed aspects of paragraph formatting, use the **Paragraph** dialog box launcher. The **Paragraph** dialog (Figure 6.4) allows you to change paragraph indentation and spacing settings, including text alignment, line spacing, and first-line indentation.

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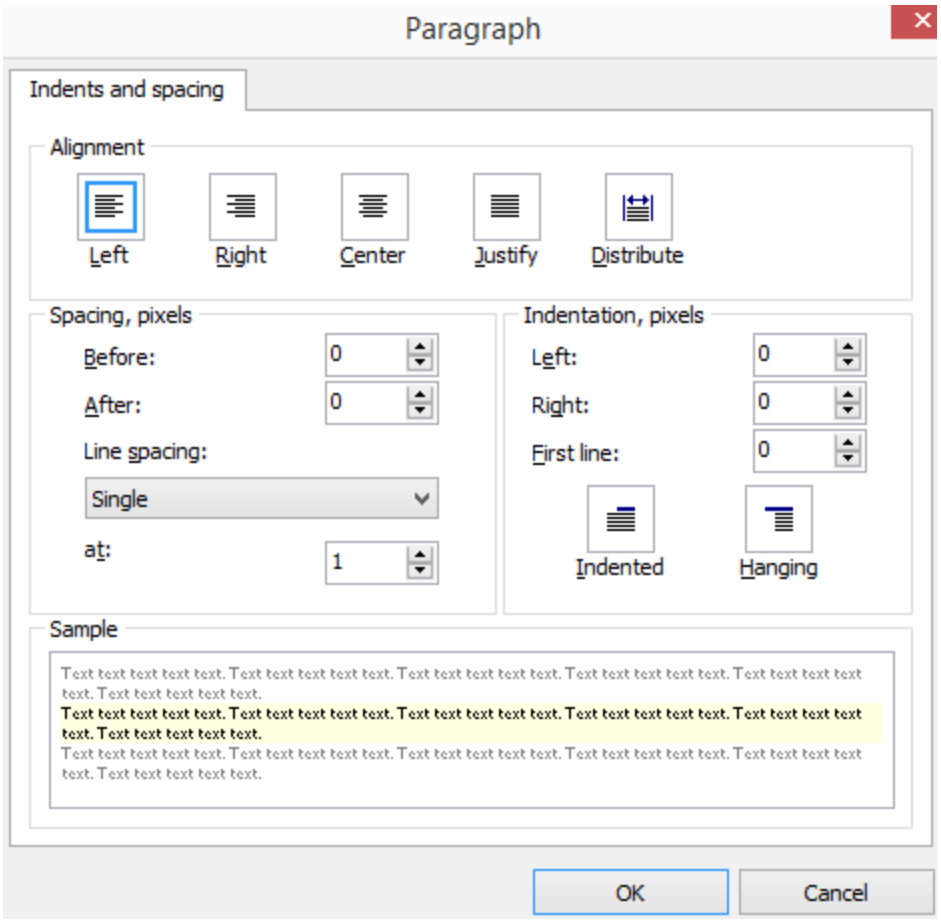

Figure 6.4 - Paragraph Dialog

In order to modify detailed aspects of the bullet and numbering styles, click on the **Bullets and Numbering** button in the paragraph group while a text box is selected. The **Bullets and Numbering** dialog allows you to set bullets and numbering appearance and it provides options for creating new types of bullets (Figure 6.5).

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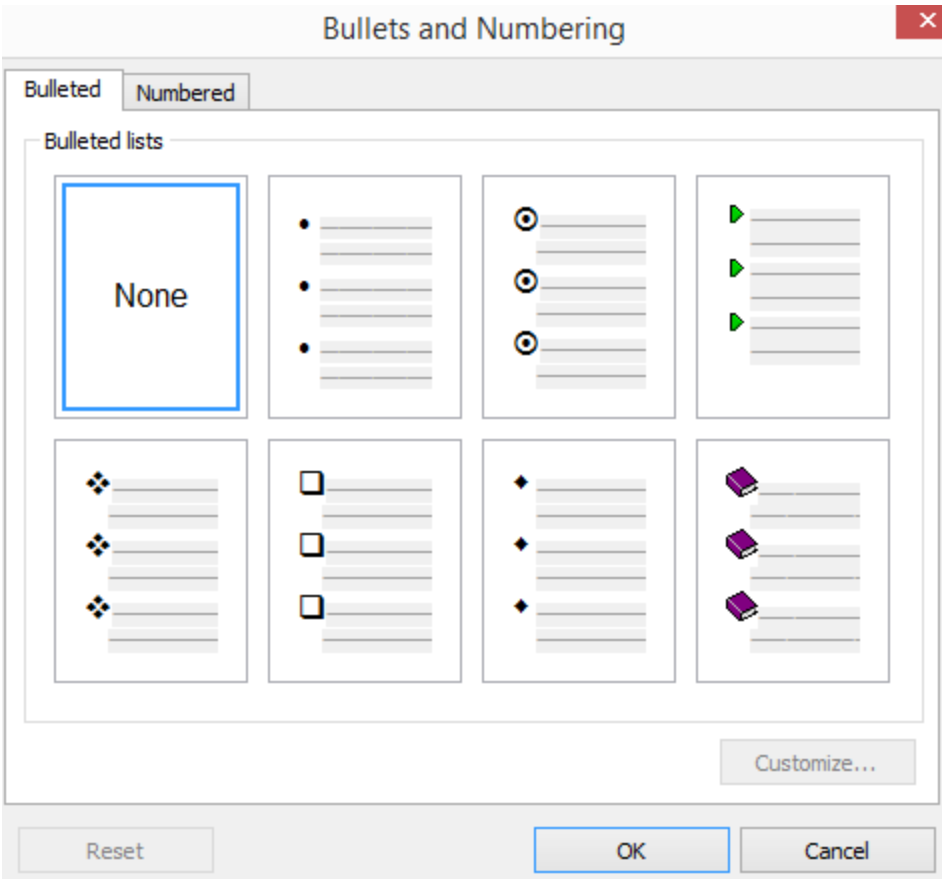

Figure 6.5 - Bullets and Numbering Dialog

6.3.1 Inserting Tables

Tables can be inserted into a Text Box by either of the following methods:

- If the Text box is in edit mode, select the **Text** tab→**Insert Text**→**Insert Table** command.
- Right-click in the Text Box and select **Table**→**Insert Table** from the pop-up menu.

The **Insert Table** dialog will appear (Figure 6.6) where you can choose the number of rows and columns, as well as the size of the table. We recommend keeping the setting at autosize for the best performance. To save these settings for creating future tables, click on the **Remember dimensions for new tables** checkbox.

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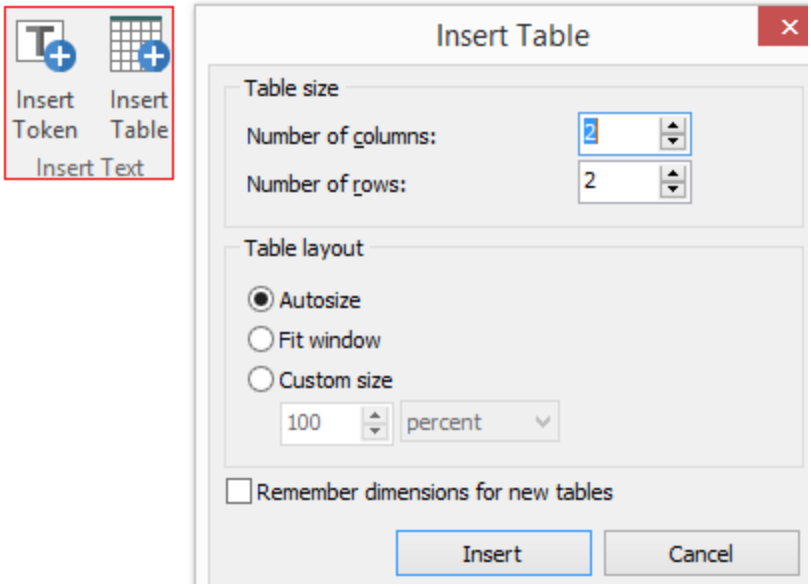

Figure 6.6 - Insert Table Ribbon and Dialog

Tables can also be:

- Pasted from Microsoft Word. Simply copy the table to the clipboard in Word, then select the **Home→Clipboard→Paste** command (or use the **Ctrl+V** keyboard shortcut).
- Pasted from Microsoft Excel. Simply copy the table to the clipboard in Excel, then select the **Home→Clipboard→Paste** command (or use the **Ctrl+V** keyboard shortcut)

6.3.2 Formatting Tables

If you click the cursor in one of the cells of a table (or select a group of cells by dragging the cursor across the cells you want to highlight), you can choose formatting options by one of the following methods:

- Select one of the commands from the **Text→Table** group.
- Right-click while the cursor is in the table, select **Table** from the first pop-up menu, and choose a command from the submenu.

The table below shows the formatting commands that are available.

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Command	Explanation
Insert Row Above	Insert a row above the cell where the cursor resides.
Insert Row Below	Insert a row below the cell where the cursor resides.
Insert Column to the Left	Insert a column to the left of the cell where the cursor resides.
Insert Column to the Right	Insert a column to the right of the cell where the cursor resides.
Delete Rows	Delete the current row or the selected rows.
Delete Columns	Delete the current column or the selected columns.
Table Properties 158	Opens a menu used to set general table properties, as well as set properties for the selected rows and cells.
Merge Cells	The text from all selected cells will be merged into one cell.
Split Cells	The cell(s) will be split into multiple cells.

Note: The **Merge Cells** and **Split Cells** commands are only available through the pop-menu.

6.3.2.1 Table Properties

The Table **Properties** window is accessed by right clicking on a table→**Table**→**Table Properties** and is divided into 3 tabs as outlined below.

- **Table** Tab.

  These settings apply to the whole table (Figure 6.7):

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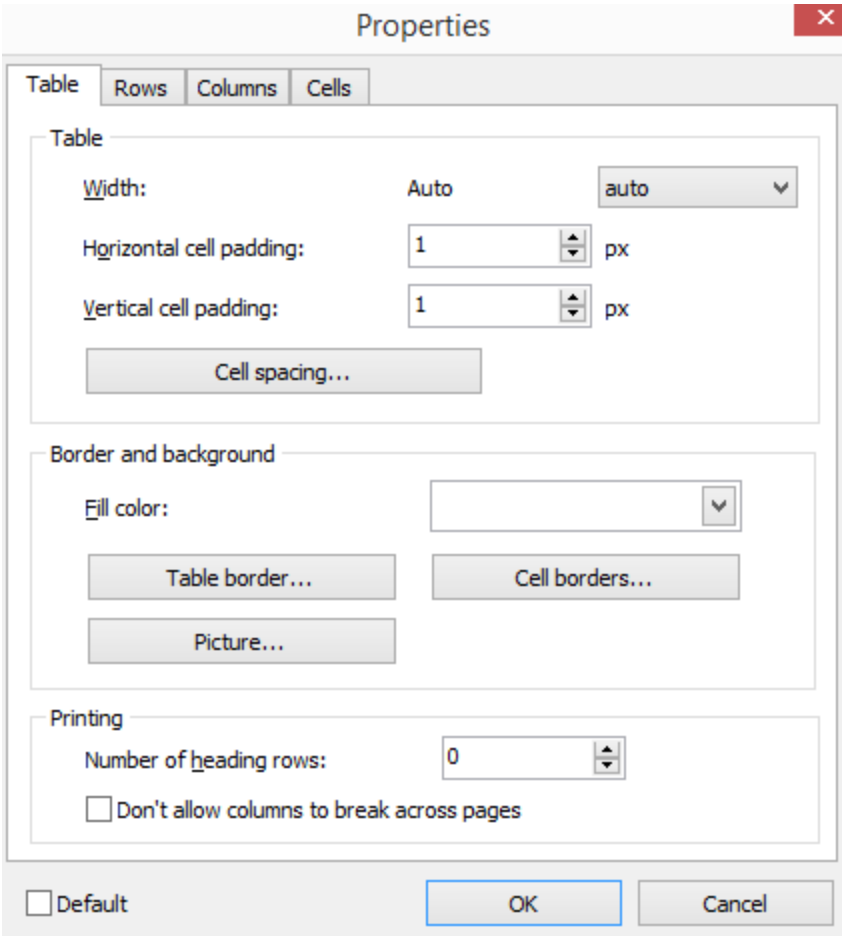

Figure 6.7 - Table Properties - Table Tab

- **Width**

  Set the table width in pixels or as a percentage of Text Box width. (*Note:* Most users find changing the width of individual columns works better than using this option to determine table width.)

- **Fill Color**

  Choose the background color of the table.

- **Cell padding**

  Set the amount of space between the text of a cell and its border.

- **Cell spacing**

  Set the amount of space between cells.

- **Picture**

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Load a picture as the background of the table.

- **Table Border**

  Set the color and width of the table border (the outer frame of the table).

- **Cell Border**

  Set the color and width of the individual cell borders.

- **Printing**

  Allows you to choose to not to allow columns to break across pages during printing.
  Allows you to choose a number of heading rows depending on your needs.

- **Rows** Tab

  These settings apply to the vertical alignment, printing, and cell colors of the selected row(s) (Figure 6.8).

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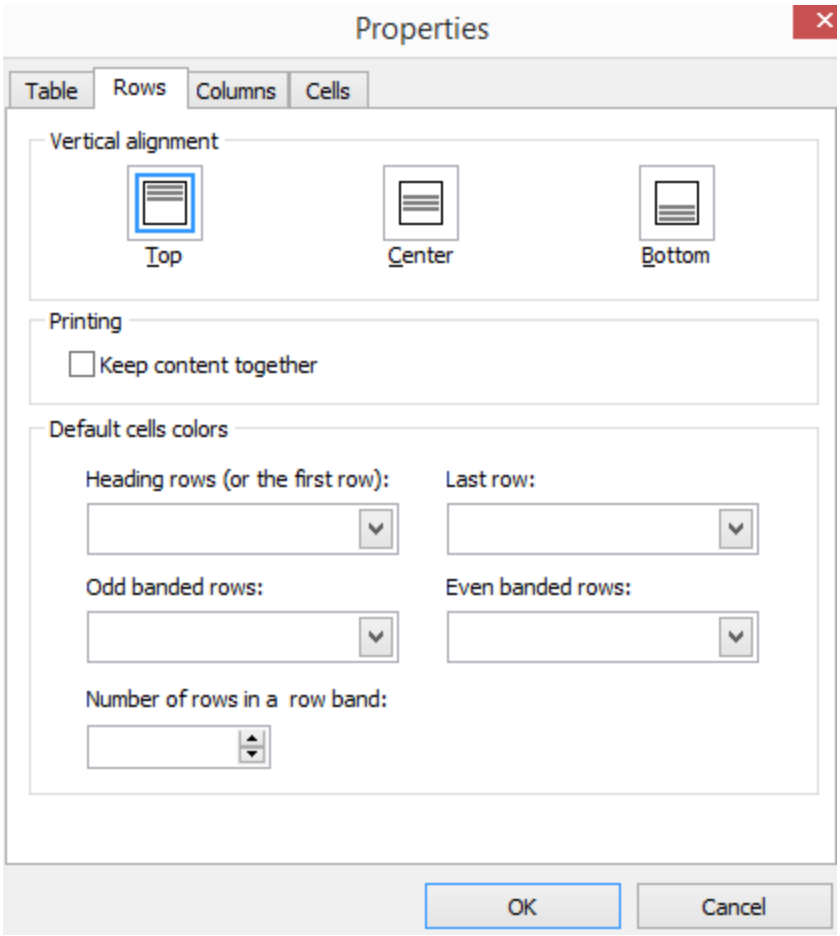

Figure 6.8 - Table Properties - Rows Tab

- **Top**

  Align text to the top of the selected rows.

- **Center**

  Align text to the center of the selected rows.

- **Bottom**

  Align text to the bottom of the selected rows.

- **Columns** Tab

These settings apply to the cell colors and banding of the selected column(s) (Figure 6.9).

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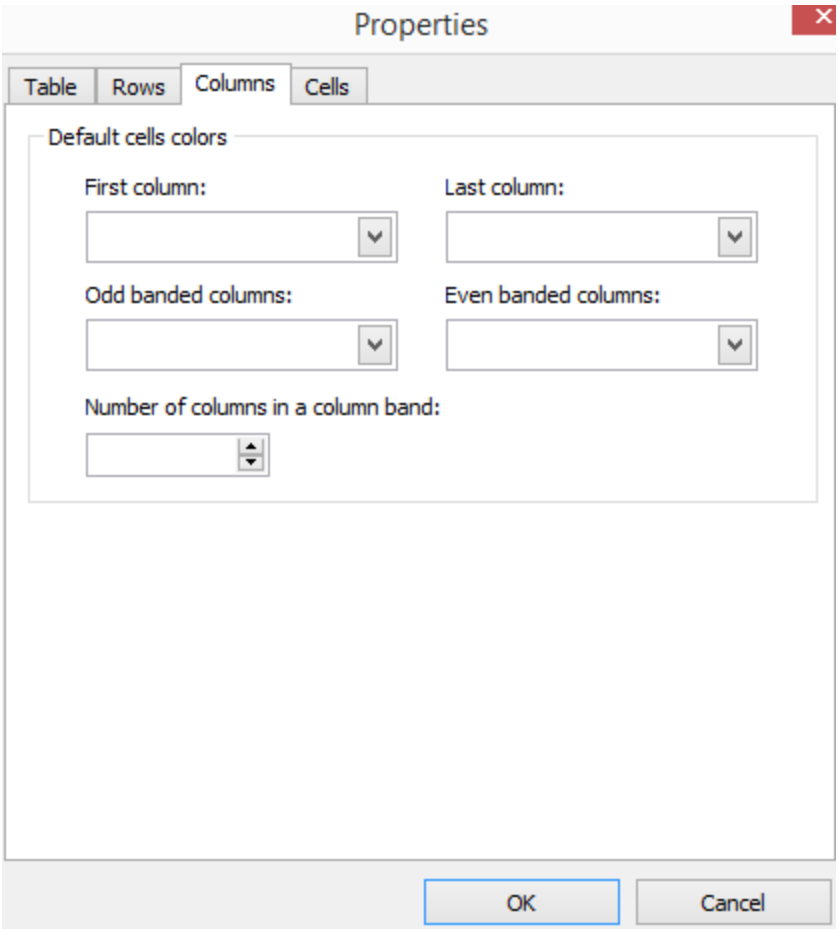

Figure 6.9 - Table Properties - Columns Tab

- **Cells** Tab

  These settings apply to the selected cell(s) (Figure 6.10).

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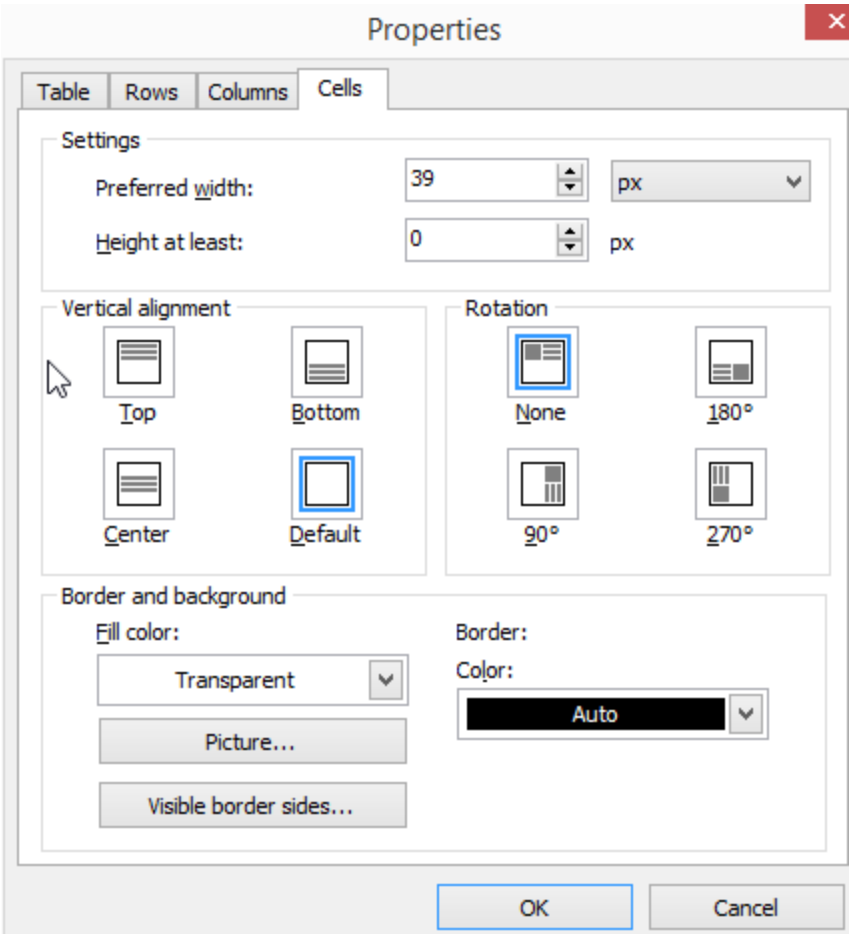

Figure 6.10 - Table Properties - Cells Tab

- **Width**

  Set the width of the current or selected cells.

- **Height**

  Set the height of the current or selected cells.

- **Fill Color**

  Set the background color of the current or selected cells.

- **Picture**

  Load a picture as the background of the current or selected cells.

- **Vertical alignment**

  Set the vertical alignment of the current or selected cells. (*Note: To* align a full Row, use the Table **Properties** - **Rows** Tab.)

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- **Border**

  Set the color of the current or selected cell borders.

6.3.3 Exporting a Table to Excel

A table can be exported to Excel without formatting loss. Please refer to the Exporting a Text Box to Excel 166 to perform this action.

6.4 Resizing Text Boxes

You can change the text box size as described in Formatting Text Box Features 165. Alternately, you can resize text boxes the same way all items are resized in FCS Express 136, with the following exceptions. When a text box contains a token or table, you will not be able to make the text box narrower than the token or table. Furthermore, you will not be able to adjust a text box smaller than what is necessary to display the current text. In either case, attempts to adjust a Text Box too small will cause the text box to automatically resize to the smallest acceptable size. In addition, text boxes will automatically become larger when you add text or insert other items (tables, tokens, etc.) that would not otherwise fit within the current size.

6.5 Adding a Text Box to a Plot

You can also place a text box directly inside a plot using one of the following methods:

- Putting the plot in Edit Mode by clicking anywhere inside it, followed by selecting the **Insert→Text Box** command and then clicking inside the plot.
- Right-click on a plot and select **Add Text Box**. A Text Box will be inserted inside the plot borders.

If you have deleted a marker, quad, or gate text box from your plot, you can recreate them by right-clicking on the plot and choosing the appropriate command (**Recreate Marker Text Boxes**, **Recreate Gate Text Boxes**, or **Recreate Quad Text Boxes**) from the pop-up menu (Figure 6.11).

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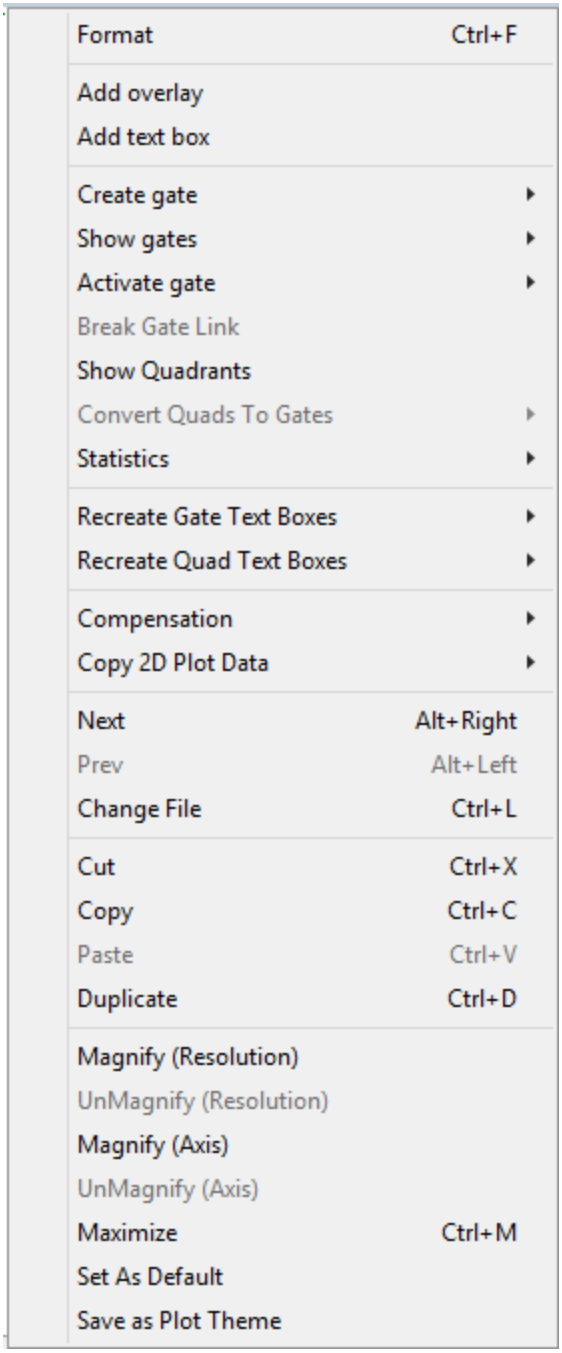

Figure 6.11 - Right-click Pop-up Menu wth Recreate Gate and Quad Text Boxes Commands

6.6 Formatting Text Box Features

You can change the text box border and size, or set a background by:

- Selecting the text box and using commands from the **Format** Tab.

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- Opening the format window. To open the format window, right-click on a text box and select **Format** from the pop-up menu. (*Note:* If the text box is inside a plot make sure that the text box is in edit mode). The format window allows you to change the border, size, and background of the text box. The default background for a text box is transparent.

- Text boxes derived from quadrant statistics are referred to as Label Text Boxes. Please see the section on Quadrant Label Text Boxes for formatting instructions.

6.7 Adding Tokens to Text Boxes

In addition to plain text, you can also insert statistics and other information inside a text box using tokens. The statistics or information in the token text will update as the analysis changes. For more information on tokens, see Using Tokens.

6.8 Exporting a Text Box to Excel

The content of a text box (tables included) can be completely exported to Excel without formatting loss via the following steps:

1. Select the entire text box (please see the Selecting Items on a Page topic for more information on this).
2. Right click on the text box red border and select **Copy** from the right click menu. Alternatively, press **Ctrl+C** on your keyboard.
3. Open Microsoft Excel.
4. Right click on the destination cell and select **Paste** from the right click menu. Alternatively, left click on the destination cells to select it and press **Ctrl+V** on your keyboard.

A part of the text box can be selectively exported to Excel via the following steps:

1. Select the text (or the table) of interest.
2. Right click on the selection select **Copy** from the right click menu. Alternatively, press **Ctrl+C** on your keyboard.
3. Open Microsoft Excel.

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4. Right click on the destination cell and select **Paste** from the right click menu. Alternatively, left click on the destination cells to select it and press **Ctrl+V** on your keyboard.

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Chapter

7 Working with Plots

FCS Express supports many different ways of displaying your data. You can view the raw data in a Data Window 680, or display the data in many different types of plots described in this chapter.

7.1 Data Display Plot Types

FCS Express has 16 different types of plots (grouped into **1D Plots**, **2D Plots**, and **Other Plots** on the **Insert** Tab) to allow you to display your data in many different ways. The different types of plots are:

- **1D Plots -** (one-dimensional) plots allow you to display one parameter of your data. You can also display a cumulative probability distribution of your data.
    - A **Histogram** bins all of your data into a discrete number of bins, and sums all the events that fall into the individual bins. In a **Histogram**, the data is typically displayed as the data value on the X-axis and number of events on the Y-axis.
    - In a Kinetics 914 plot, the X-axis is the Time parameter and all of the Y-value events are averaged for each unit of time.
    - Multicycle DNA histograms 885 are a subset of one-dimensional plots that fit DNA cell cycle statistics.
    - Proliferation histograms 903 are a special subset of one dimensional plots that automatically model proliferation statistics.
    - Spectrum plots 922 are a subset of one-dimensional plots to graphically display spectral data.
    - Bar plots 547 can be used to graphically display statistics from plots, default statistics or text tables, or custom data 414.
    - Pie charts 538 can be used to graphically display statistics from plots, default statistics or text tables, or custom data 414.
- **2D Plots -** (two-dimensional) plots allow you to plot one parameter against another parameter.
    - **Dot** plots represent each cell with an individual dot. Dot plots always display the dots in the overlay color. Dot plots may also be Shaded Based on Density 308.

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- **Density** plots. In a density plot, the color of an area reflect how many events are in that position of the plot. Density plots may also be set to base color on 288 a different parameter and set to shade base on density 308.

- **Color Dot** plots. In a color dot plot, the colors can mean one of two different things. If there is only one overlay on the plot, the colors refer to which cells fall into which gate. If there is more than one overlay on the plot, the color refers to which overlay that dot belongs to. If one dot actually belongs to more than one overly, the color of the dot will be randomly chosen among the overlays. Color Dot plots may also be Shaded Based on Density 308

- **Contour** plots are the same as density plots, with the exception that contour lines represent different numbers of cells, rather than colors. A contour plot looks very similar to a topographical map. Contour plots may also be set to base color on 288 a different parameter

- **Surface** plots. Together with the two parameters on the X- and Y-axes, the Z-axis displays the number of events.

- Picture Plots 1044 are used to display the location of cell (or other) images in two dimensions across an entire area.

- Scatter plots 559 can be used to graphically display custom data 414.

- Scatter with regression plots 579 can be used to graphically display custom data 414 with the regression curve 578 automatically overlaid onto the plot (e.g., in generating a titration curve).

Note: Dot, Density, Contour, and Color Dot plots all bin your data into a discrete number of bins. Therefore, the dot size of these plots is related to the number of bins that the data is being divided into. Scatter plots do not bin your data, but put a symbol at the location of your data value. The symbol size is controlled by the Formatting preferences. The binned plots are a better option when viewing data sets containing large numbers of data points (i.e., >5000). Scatter plots are more useful when displaying smaller numbers of data points, where customization of the point shape and style are more important.

- **Other Plots**

  - Heat Maps 1050 are displays of a microtiter plate which show statistical values (using colors) across all the wells of the plate.

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7.2 Inserting Plots

To insert plots on a layout page in the default size, use either of the following three methods:

- Select the command associated with the plot type you desire from the **1D Plot**, **2D Plot**, or **Other Plot** groups on the **Insert** Tab. Then, click on the layout page at the point where you want the upper left corner of the plot to be (or drag a sizing rectangle on the layout). The file that FCS Express will associate with the plot is explained in the section on The Current Data File 172.

- Drag a file from the Data List 785 and drop it onto an empty spot on your layout. You will be prompted to select which plot type to insert in the **Select Plot Types...** dialog box.

- Drag a gate from an existing plot and drop it onto an empty spot on your layout. A new plot of the same type but with the dragged gate applied will appear.

To change the default size for a plot, see 1D Plots 190 or 2D Plots Preferences 191.

To change the file in a plot see Changing Data Files 173.

7.3 The Current Data File

The first time you add a plot to a layout, the **Select a Data File** dialog (Figure 7.1) will appear. Navigate to the desired file using the **File Dialog**, **File List**, or **Active Files** tabs and select **Open.** After choosing the data file, the plot will be opened and that file will become the current data file. In the future, when you open another plot, you will not be asked to select the data file. Instead, the plot will be created using the current data file.

Every time you select an object displaying a data file, or put that object in edit mode, its data file will become the current data file.

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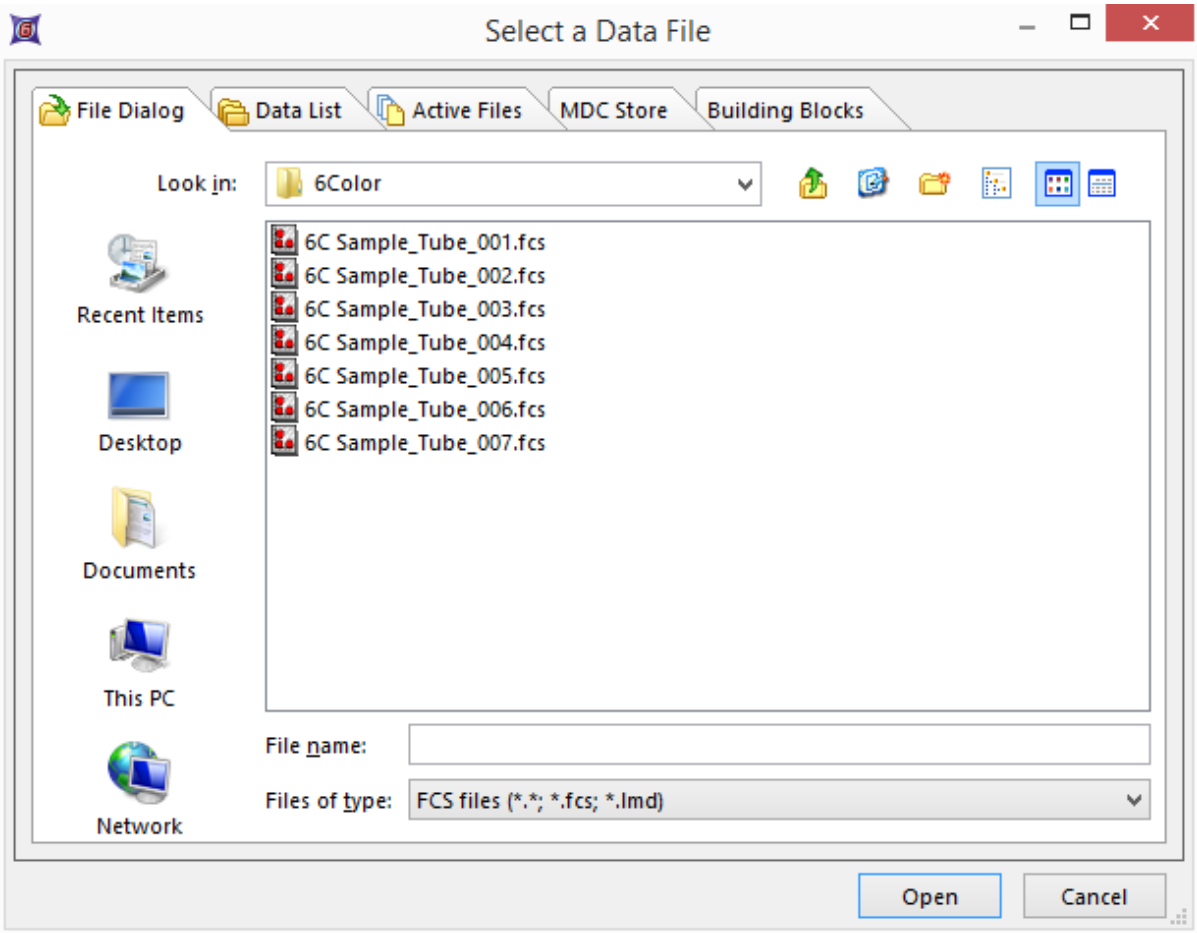

Figure 7.1 - Select a Data File Dialog

You can also configure FCS Express to prompt you for a data file every time you open a plot. To do this, go to **File** tab→**Options**→**Dialogs**. In the **Do Not Show again** category, check the box for **prompt to select plot types to open when dragging and dropping files**. With this configuration, the **Select a Data File** dialog will open every time you create or select a plot.

If a data file has been changed during your analysis (e.g.,, you have performed parameter math to create or modify a parameter), then before that data file is removed from memory upon closing your FCS Express layout, you will be prompted whether or not to save the file. If you do not want this prompt to appear, go to **File** tab→**Options**→**Save** category, and choose the **Don't save modified data files on close** (see Save Options 222) radio button at the bottom.

7.4 Changing Data Files

You can change data files on objects such as plots, data windows, and text boxes in several ways:

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- Dragging and dropping one plot from the layout (or a plot node from a Navigator) onto another plot in the layout. This brings up a Paste Special dialog (Figure 7.2).

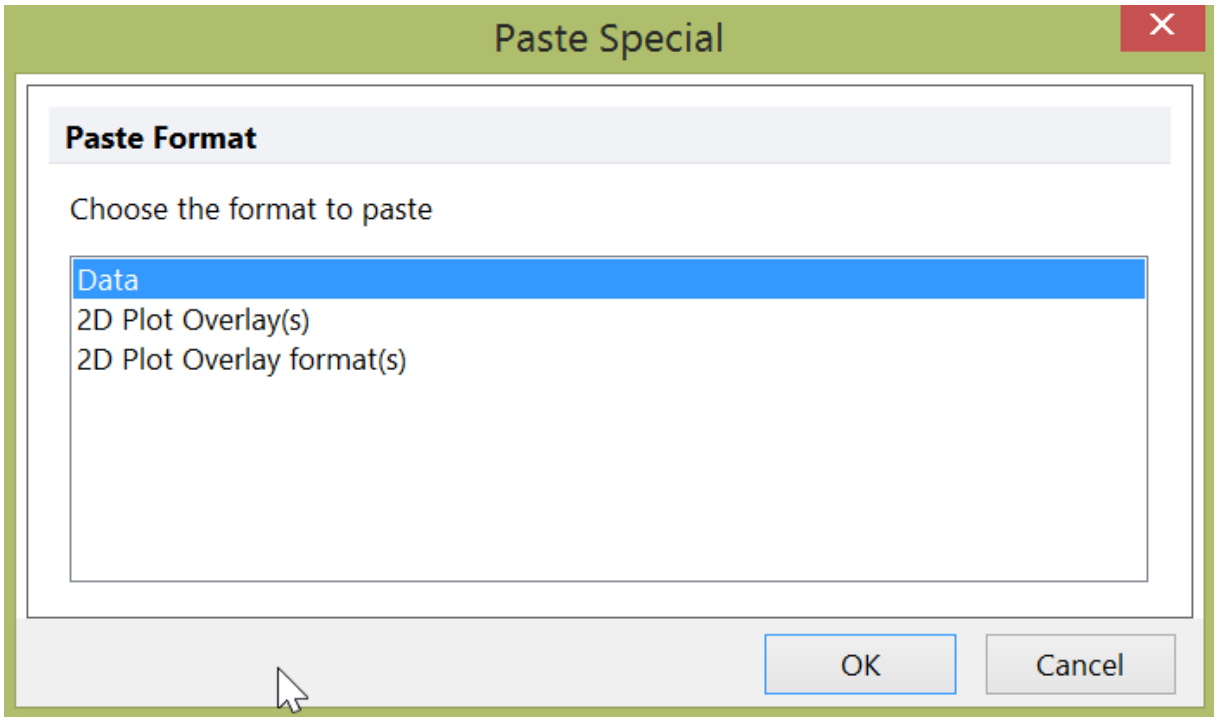

Figure 7.2 - Paste Special Dialog

After choosing **Data** in the **Paste Special** dialog you may see a **Select Drag and Drop Action** dialog (Figure 7.3) where you can choose to **replace the existing overlays on the plot** or **add the files to the plot as new overlays**.

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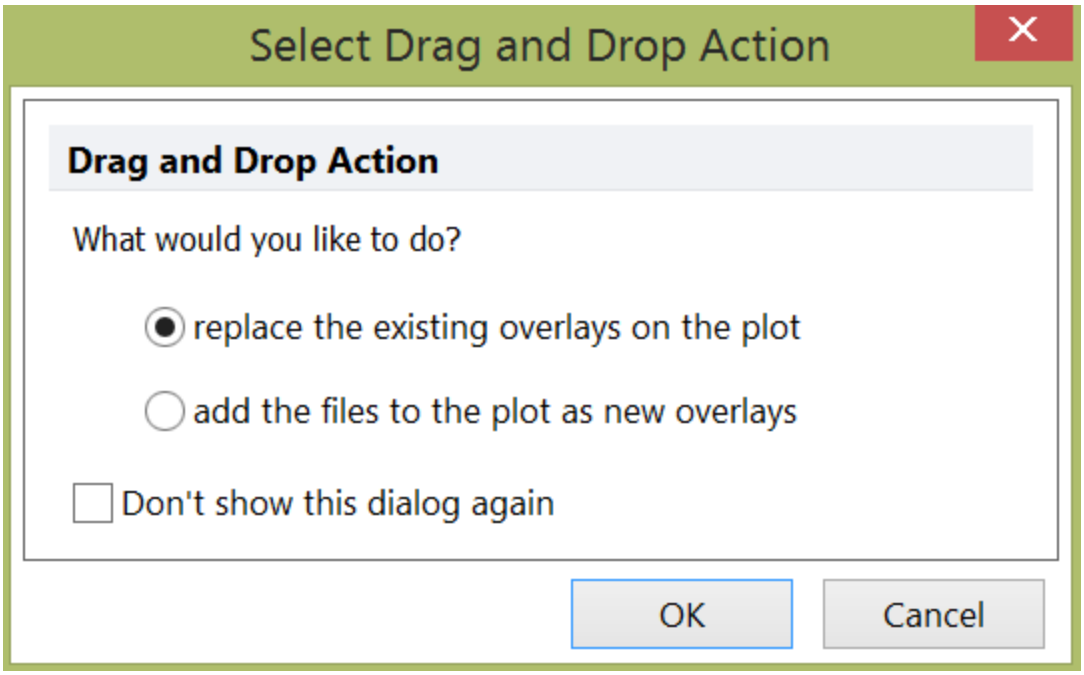

Figure 7.3 - Select Drag and Drop Action Dialog

- Right-click on the plot and select **Next**, **Prev**, or **Change File** from the pop-up menu.
- Use the commands from the **Data→Change Data on All / Selection group** 79 to change the file on the selected object (or on all objects).

In the right click pop-up menu, **Next** and **Prev** will change the data to the next and previous files in the data list (skipping by the increment value), respectively. **Change File** will bring up the **Select a Data File** dialog.

If you have more than just the base overlay (the base overlay is simply a term for the first overlay) on the plot that you are trying to change, use the bottom half of the **Select a Data File** dialog to choose which overlay to change (**Base overlay**, **All overlays**, **Select from the list**).

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Chapter

8 Formatting Objects

FCS Express provides the user with the ability to customize virtually every aspect of each analysis object. Whether editing a plot, a text box or a picture, FCS Express is extremely flexible and offers you many options when it comes to formatting the objects on your layout.

8.1 Overview

All of the objects in FCS Express have many different formatting properties. You will frequently change properties to bring out the important features of your data.

In all cases, you can format the properties on a single object by double clicking on the object or right-clicking on the object and selecting **Format** from the pop-up menu. Properties can also be changed by selecting appropriate commands from the Format tab 73.

All formatting windows work in a similar manner. An example is shown in Figure 8.1. The area on the left, denoted by the ❶, displays a list of categories that can be formatted. Simply click on a category to change it. The properties of the current category will appear on the right of the form, in the area denoted by the ❷.

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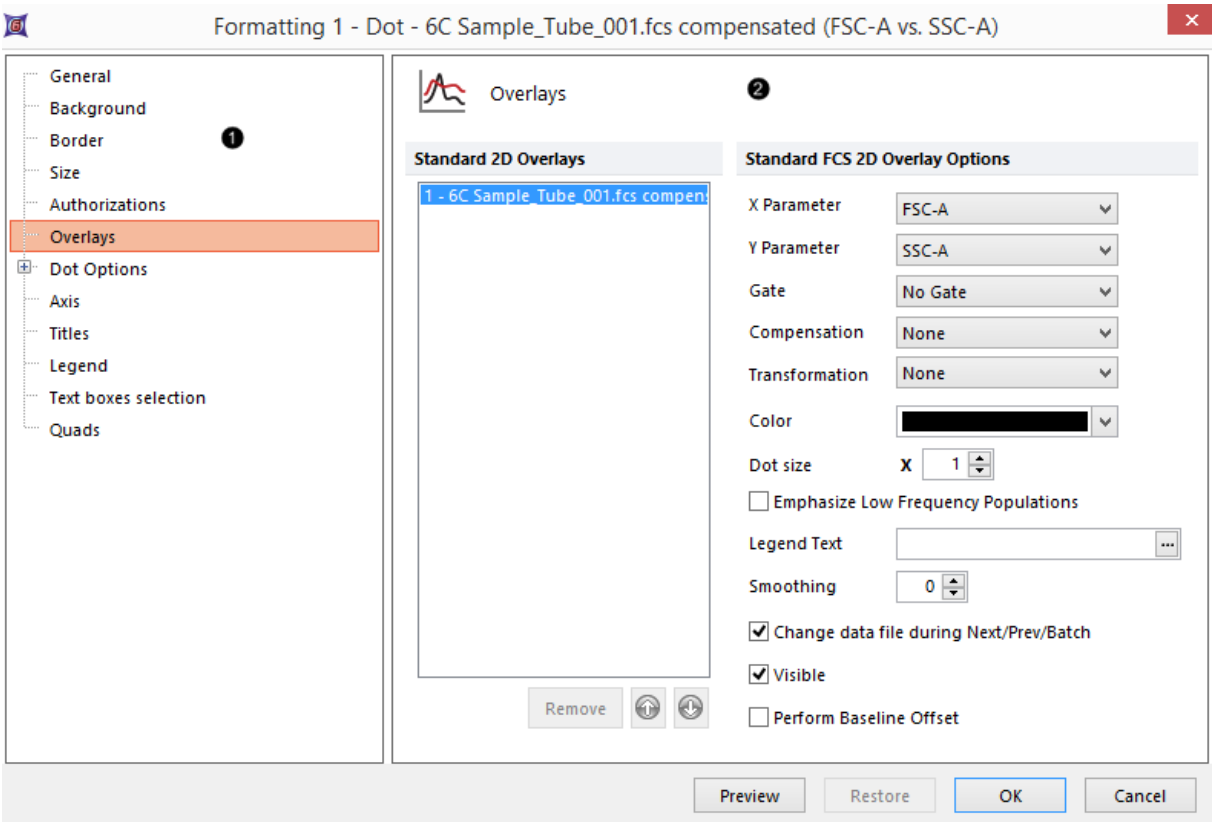

Figure 8.1 Formatting Dialog

8.2 Customizing Default Options

FCS Express contains many options that allow you to completely customize your working environment. You can save time by creating a new user name to save your common options rather than changing the default settings each time you start the program. The settings for these options are called the FCS Express User Options. These user options range from default sizes and formatting options for plots to the way FCS Express copies data to the clipboard.

All FCS Express User Options can be customized and saved under a unique user name. Any changes to the user options during a session will be saved for that user only and will not affect any other user. To customize the Preferences for the current user, select **File** tab→**Options**. The **FCS Express User Options** dialog (Figure 8.2) contains many categories ❶, each of which groups several types of preferences together ❷. The specific categories are discussed in other sections of this chapter.

A tutorial exists with step-by-step instructions for changing commonly edited **FCS Express User Options**.

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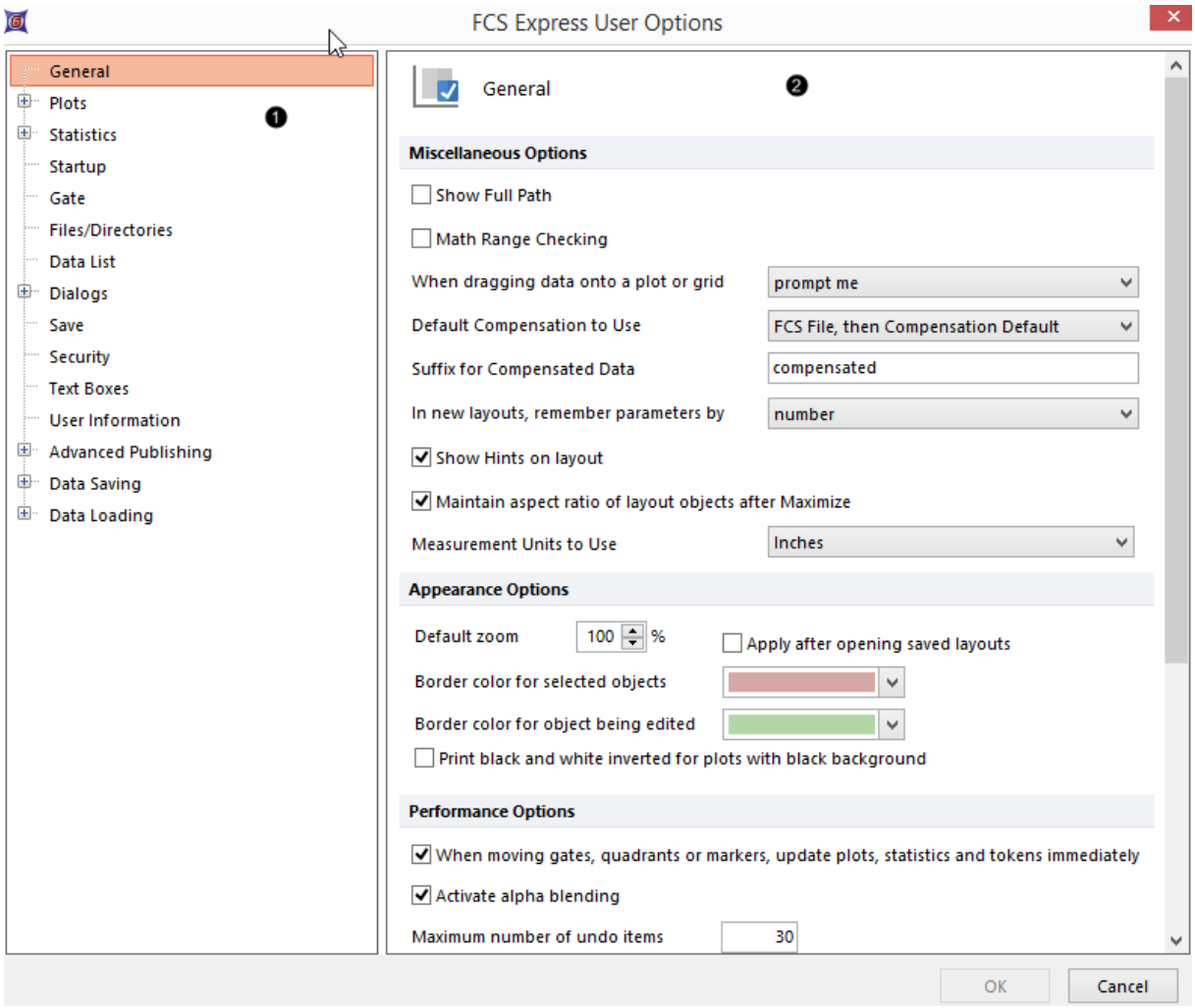

Figure 8.2 FCS Express user options overview

8.2.1 General Options

The **General** category of the **FCS Express User Options** dialog (Figure 8.3) is used to control the overall functioning of FCS Express.

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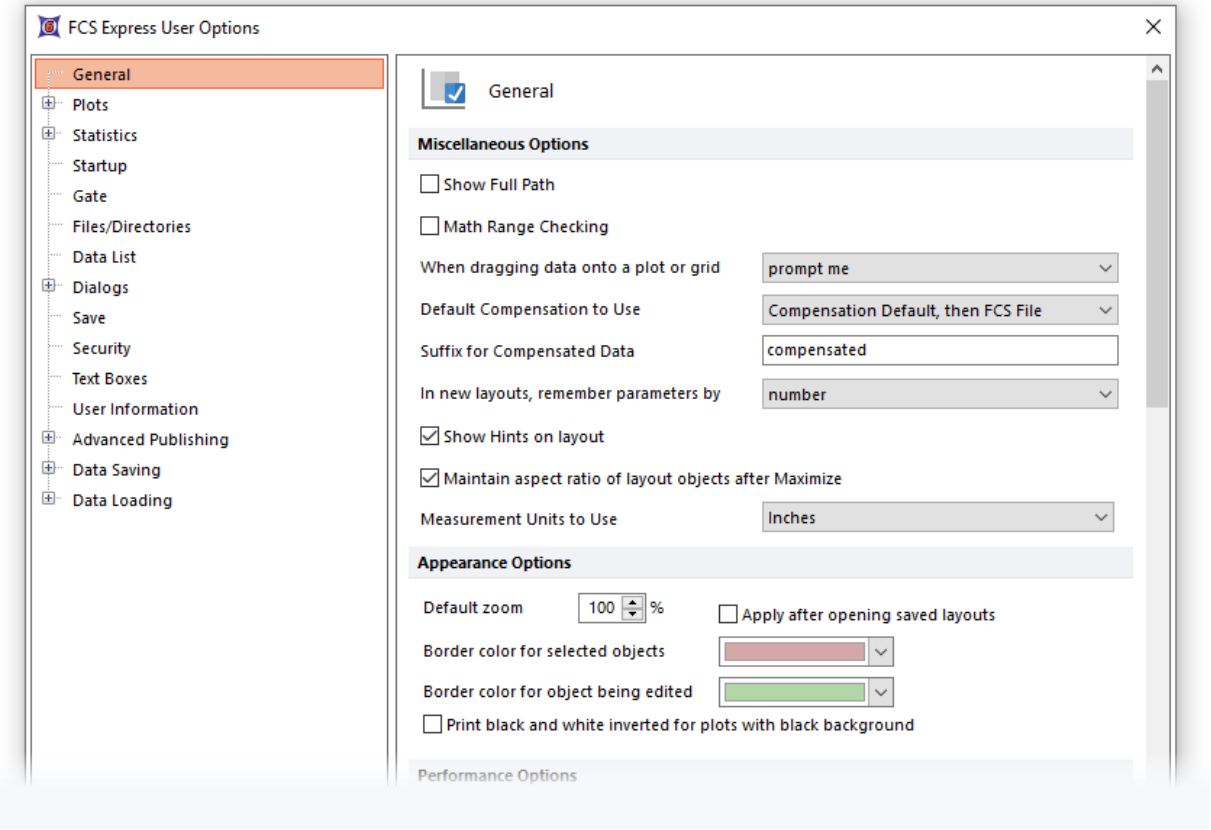

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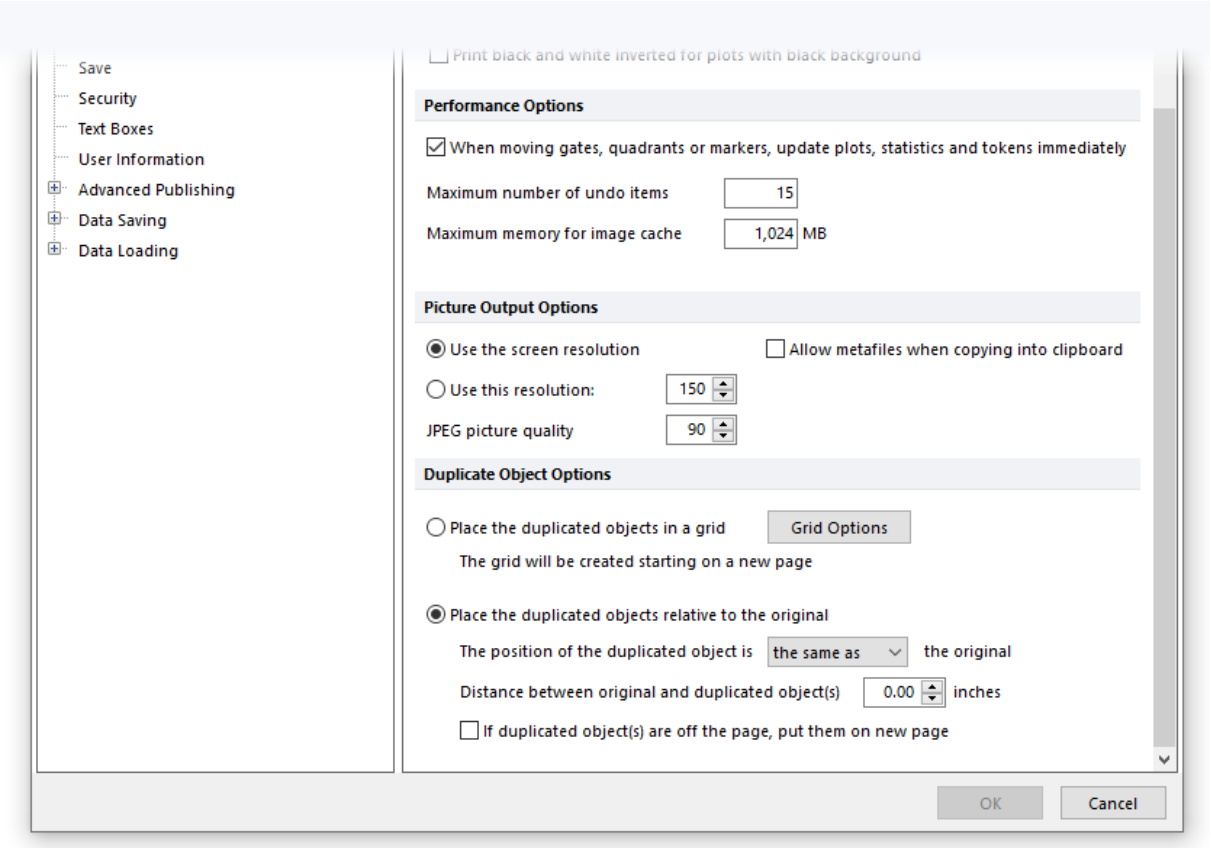

Figure 8.3 General options

The specific options are described in the table below.

Option	Explanation
Show Full Path	Under some circumstances, file names are displayed in FCS Express. This option allows you to choose whether the entire directory path and file name are displayed or only the file name.
Math Range Checking	When performing parameter math, it is possible for the result of a mathematical operation to exceed the allowed range of the file. Storing out-of-range values may cause improper results to be displayed when using data analysis packages other than FCS Express. If **Math Range Checking** is on, values greater than the maximum allowed value will be stored as the maximum allowed value. If **Math Range Checking** is off, the real out-of-range value will be stored.
When dragging a data set onto a plot	When dragging a dataset onto a plot, one of three possible actions will occur: **replace existing overlays** with the file being dragged onto the plot; **add overlays** to append the FCS file to the plot; or, **prompt me** to ask the user what action to take. To set the action, choose it from the drop-down list.
Default Compensation to Use	To set Compensation for new overlays, select one of the options below. See Selecting the Default Compensation 645 for additional details: • **None**. No compensation will be used.

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	• **Compensation Default**. The Compensation Default is selected in the Compensation Info window 627. • **FCS File**. The Compensation used for acquisition will be taken from the FCS file header. • **FCS file, then Compensation Default**. FCS Express will load Compensation from the FCS file. If there is no Compensation stored in the FCS file, the Compensation Default from the Compensation Info window will be used. • **Compensation Default, then FCS file.** FCS Express will load the compensation default from the compensations navigator by default. If a default compensation does not exist, the compensation associated with the FCS file will be used.
Suffix for Compensated Data	When analyzing compensated data, the text typed into this box will be used as a suffix to describe the compensated data. This text can be viewed in the Title 250 of a plot when using the keyword $FCSE_FILENAME or $FCSE_FILENAMENOPATH
In new layouts, remember parameters by	FCS Express can remember the arrangement of parameters in the plots displaying data by their **Parameter Number** (Parameter 1, Parameter 2, etc.) or by their **Parameter Name** (FITC-A, FL2-H, etc.). The **Parameter Name** used by this option is the keyword (or the combination of keywords) specified in the Keyword To Use as Parameter Name 229 option (i.e. Name, Stain, Name and Stain, Stain and Name). This option must be set prior to building the layout.
Show Hints on layout	When this checkbox is enabled, a hint will appear showing the plot ID and names of the files currently displayed when the mouse is hovering over the plot.
Maintain aspect ratio of layout objects after Maximize	When this checkbox is enabled, the aspect ratio (height vs. width) of objects will be maintained.
Measurement Units to Use	Choose **Inches, Centimeters,** or **Millimeters** from the drop-down list.
Appearance Options	These options allow you to chose the border colors for selected objects and objects that are being edited.
Default Zoom	You can customize the default zoom for your layouts. Also, you can choose to **Apply after opening saved layouts** by default.
Apply after opening saved layouts	Applies the chosen default zoom level to layouts that were originally saved with a different zoom level.
Border color for selected objects	Choose the color of the border displayed when an object is selected from the drop-down color pallet.
Border color for object being edited	Choose the color of the border displayed when an object is being edited from the drop-down color pallet.
Print black and white inverted for plots with black background	When this option is selected, when displaying plots with black backgrounds, those plots will print out white, rather than black.
Performance Options	These options allow you to define preferences that may increase the performance speed of FCS Express.
When moving gates, quadrants, and markers, update	When checked, all gated events, backgating, and statistics associated with gates, quadrants, and markers will update immediately ("in real time") when gate, quadrant, or marker positions are changed.

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plots statistics and tokens immediately	
Maximum Number of Undo Items	Sets the maximum number of undo items.
Maximum memory for cell images	Determines how many images stay in memory when picture plots are used. A higher value will increase performance until your computer's memory limit is reached.
Picture output options	These options allow you to choose the format, quality, and resolution of images of a plot copied to the clipboard for future use.
Use the screen resolution	Uses the resolution of the picture on the screen for an exported or copied picture.
Use this resolution	Uses a manually inputted value (in pixels) for the resolution of an exported or copied picture.
JPEG picture quality	Determines the quality of an exported or copied JPEG picture (Max of 100).
Duplicate object options	These options control where duplicated objects are placed after they are duplicated.
The position of the duplicated object is	Determines the position of a duplicated object relative to the original object using the drop-down list to define the position.
Distance between original and duplicated object(s)	Defines the space (in inches) between a duplicated object and the original object after duplication.
If duplicated object(s) are off the page, put them on a new page	If a duplicated object falls off of the page a new page will be created containing the duplicated object.

8.2.2 Plot Options

The **Plots FCS Express User Options** category (Figure 8.4) lets the user customize:

1. **Plot Generic Options**.
2. **Plot Parameter Options**.
3. **Plot Theme**.

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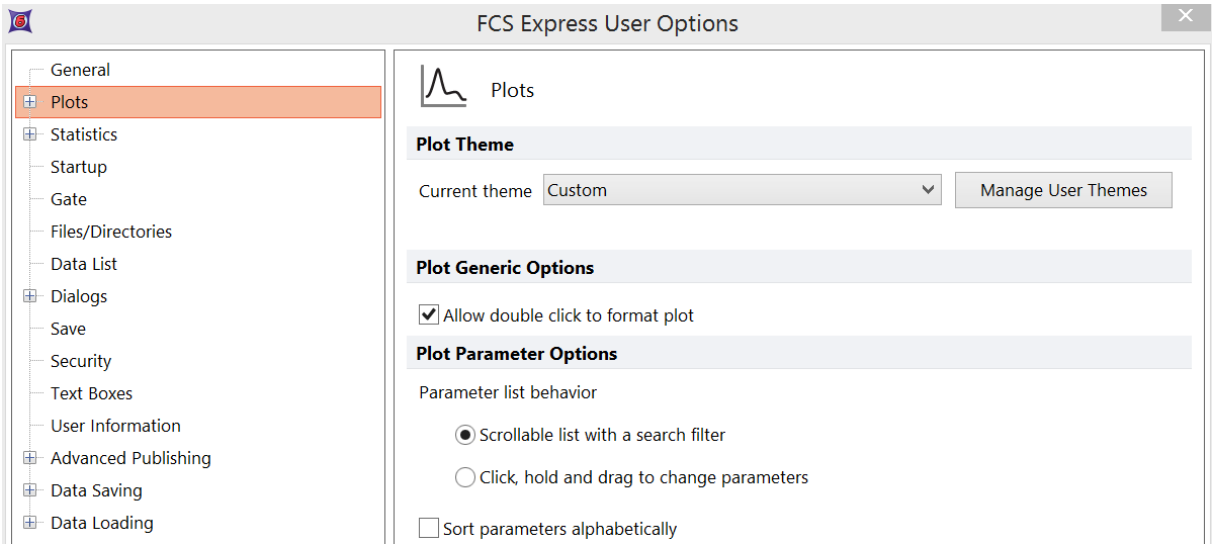

Figure 8.4 - The Plots category in the FCS Express User Options.

1. **Plot Theme**. This option set how plots appear when they are first opened. Almost any option that can be set on a plot can be set as a default value including size, color, font, background, etc.

Alternately, you can either:

- right-click on a plot and select **Set as Default** from the pop-up menu. The plot options selected for that plot will be used as the default options for all plots opened in the future, or
- right-click on a plot and select **Save as Plot Theme** from the pop-up menu, enter a **Theme Name** in the **Add Theme Name** dialog box, and click **OK** (Figure 8.5, *top*). Those plot options can then be set as the default via **Plot Theme** Options (Figure 8.4, *bottom*) and will take effect on subsequently inserted plots. The **Manage User Themes** button enables renaming and deleting of user-defined Themes.

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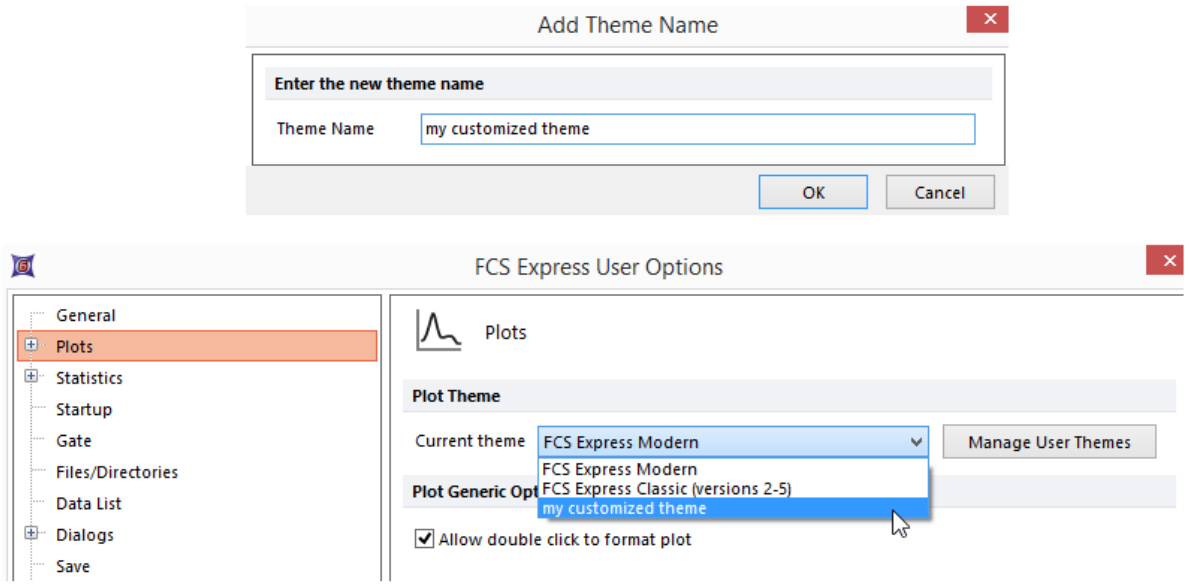

Figure 8.5 Plot Theme options

In most cases, the general FCS Express User Options settings mirror the specific plot FCS Express User Options, as described in Formatting Features Shared by All Plots 246. The option for Fill overlay area is discussed in Overlays 268 (Formatting Single Parameter Histograms). Other discrepancies are described below.

2. **Plot Generic Options**. This option allows to set whether the **Plot Formatting** dialog can be accessed by double click.

3. **Plot Parameter Options**. This options allows to set whether the parameter list accessible on plot axes will appear as **Scrollable list with a search filter** or as a **Multi-column list**. When scrollable list with a search filter is selected you may drop down the parameter list and start typing a parameter name to quickly access the parameter. Additionally, parameter lists can be sorted based on the parameter number (default) or in alphabetical order by choosing the **Sort parameters alphabetically** option.

8.2.2.1 Axis Options

If you commonly work with data files in both the FCS 2.0 and FCS 3.0 standards, you may find that you want to set different default options for each of these file types. For example, fluorescent parameters from FCS 2.0 files will be best viewed in a four-decade log scale, while fluorescent parameters from your FCS 3.0 files might be best viewed in a biexponential scale.

There is a full set of **Axis Options** for each type of data file (Figure 8.6). Therefore, when you open a new plot on your layout, the default settings for each axis will depend on what type of data file it is.

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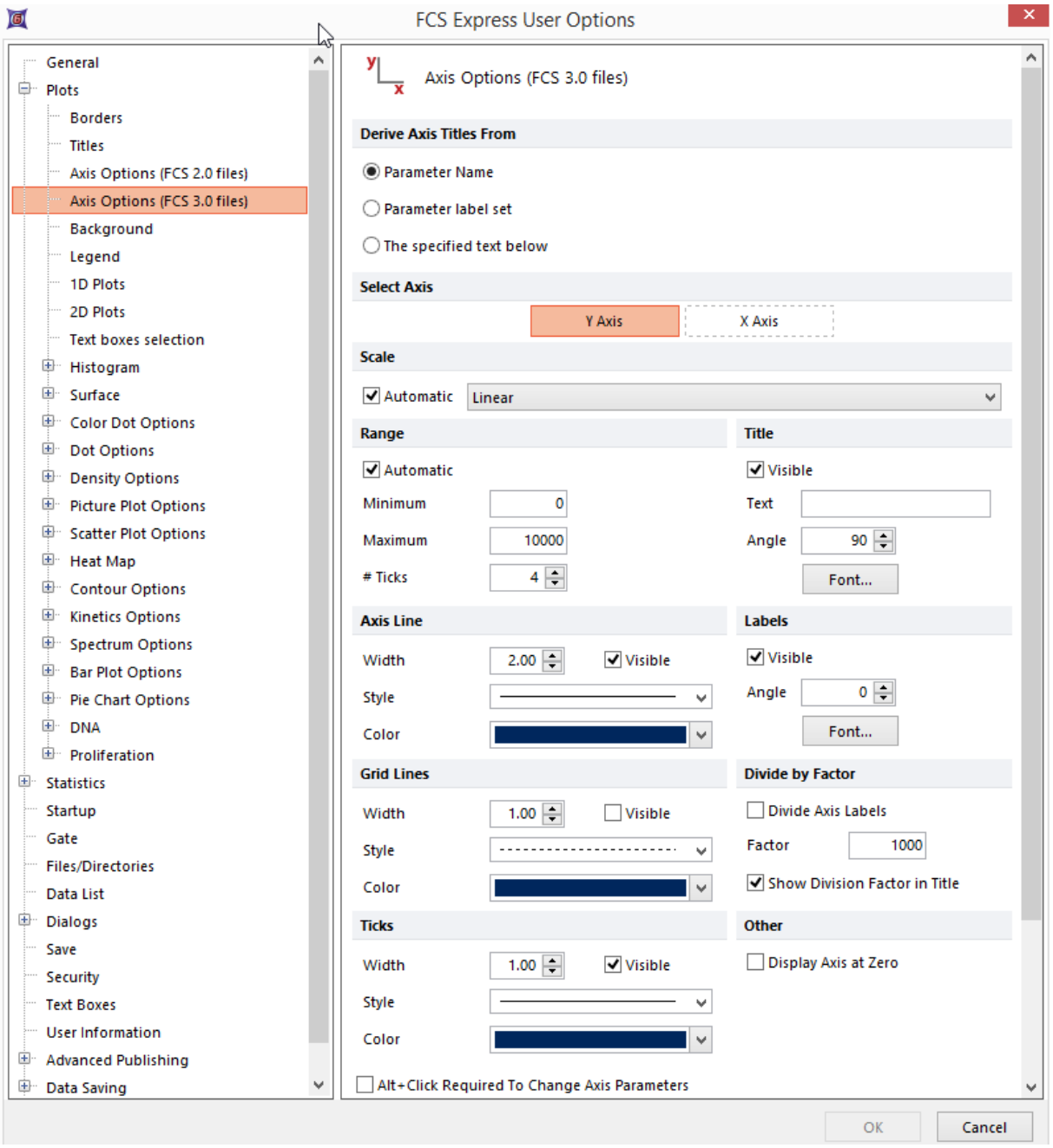

Figure 8.6 Edit Preferences - Axis Options

By default, clicking on the axis label of a plot will allow you to change the parameter for that axis[309]. While this is convenient for users who are designing layouts, parameters may be accidentally changed on occasion. You can force the **Alt** key to be required to change parameters by checking the **Alt+Click Required To Change Axis Parameters** checkbox.

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Technical note: Axis Scale and Statistics

In FCS Express 6, all the statistics are calculated on the raw data, irrespective of the display scaling. However, when Gates and Quadrants are created decisions must be made in cases where an event falls on the line of the gate or quadrant.

In FCS Express 6, Gates and Quadrants are calculated and drawn in a binned space with binning dependent on both the Scaling 254 and the Resolution Minimum/Maximum 247 of the plot. In turn, the inclusion or exclusion of an event on the border line of a gate or in a quadrant is highly dependent on the scaling of the plot, and on the Resolution Minimum/Maximum, of the plot the Gate/Quadrants was drawn on.

When adjustments to the scaling or resolution of a plot are made, an event may then fall to a different side of the border line of the gate/quadrant, and the user may notice very slight changes in the statistics of gated events which are usually quite minimal and make no impact on the interpretation of the results.

Please refer to the Axis Scale and Statistics 256 chapter for more details.

8.2.2.2 Background Options

This category controls the default **background** appearance of plots. The options are explained below and pictured in Figure 8.7.

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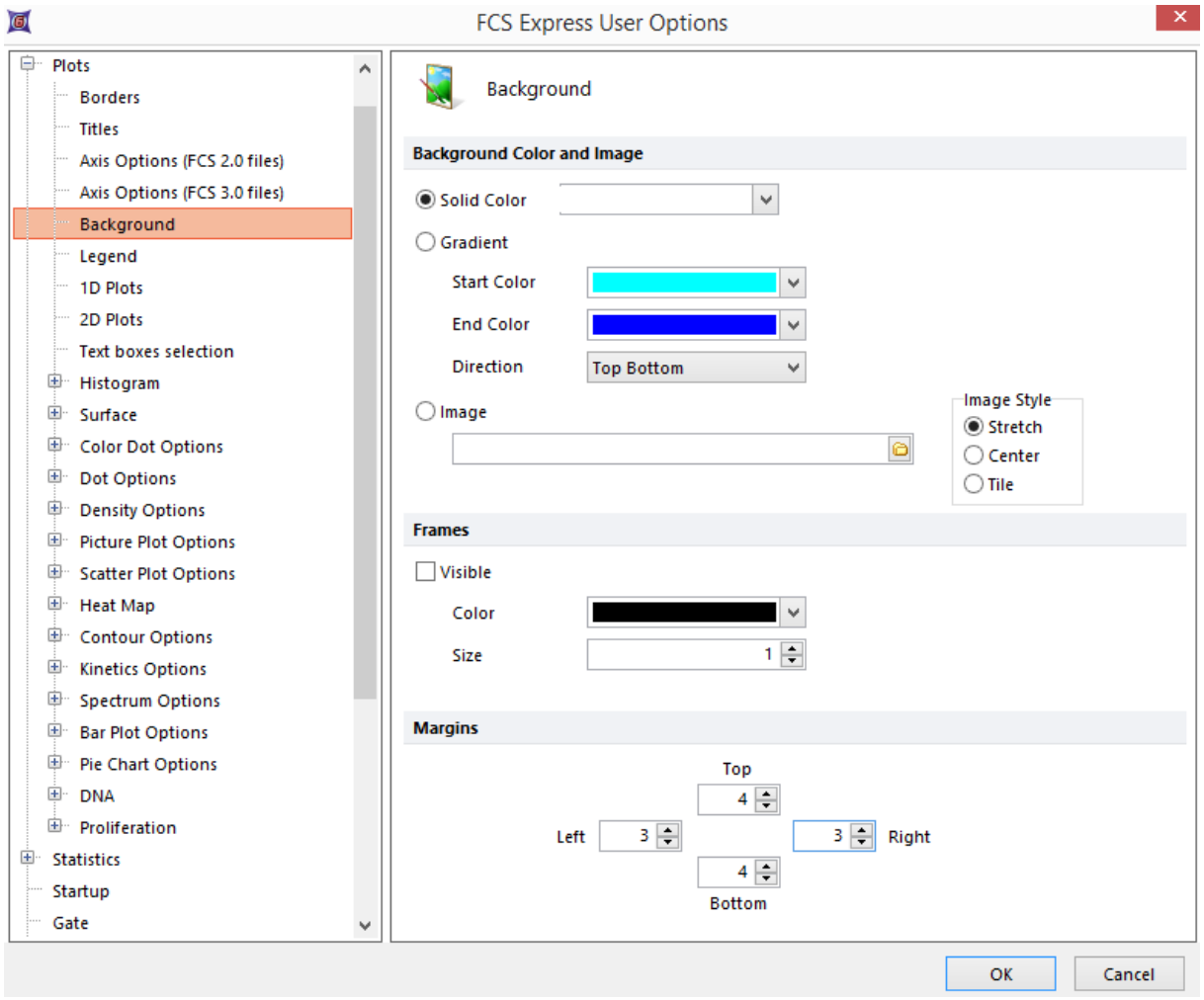

Figure 8.7 Background Options

Option	Explanation
Background color and image	Controls whether plots will appear with a white, other solid color, or gradient background. Images on your computer can also be stretched, centered, or tiled as background.
Frames	Options for adding frames of desired color and thickness appear on the border of the plotted area.
Margins	Add predefined margins of white space around the plot within each object.

8.2.2.3 Legend Options

This category controls the default behavior and appearance of plot **legends**. The options are explained below and pictured in Figure 8.8.

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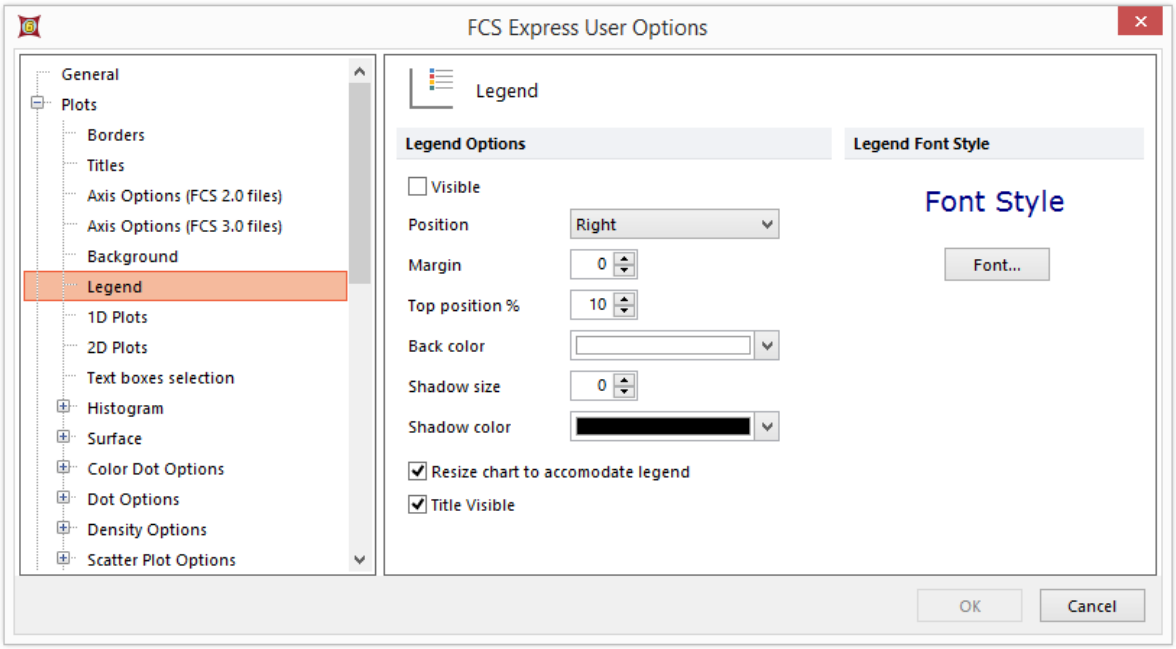

Figure 8.8 Legend Options

Option	Explanation
Legend Options	If **Visible** is checked, the legend will appear by default. If unchecked, it must be added via Formatting options 253 on a per-plot basis. **Position**: This defines the position of the legend within the plot. Options are **Left**, **Right**, **Top**, **Bottom**. **Margin**: This defines how much space is available between the legend and the plotted data. **Top Position**: This defines the distance of the legend from the top of the plot. **Back Color**: This defines that color that is applied to the background of the legend. **Shadow Size**: This defines the degree to which a shadow is applied to a legend. A larger number applies a more intense shadow. A value of zero applies no shadow. **Shadow Color**: This defines the color of the shadow. **Resize chart to accommodate legend**: This defines whether or not the plotted area will resize itself to accommodate the size of the legend. **Title Visible**: Enabling the check box will display the legend title on Density and Contour plots. The legend title is text as well as an interactive drop down menu that allows you to easily change the parameter to use for the **Color based on** 288 option.
Legend Font Style	Defines the default font of legend text.

8.2.2.4 1D Plot Options

The **1D Plot** options are shown in Figure 8.9.

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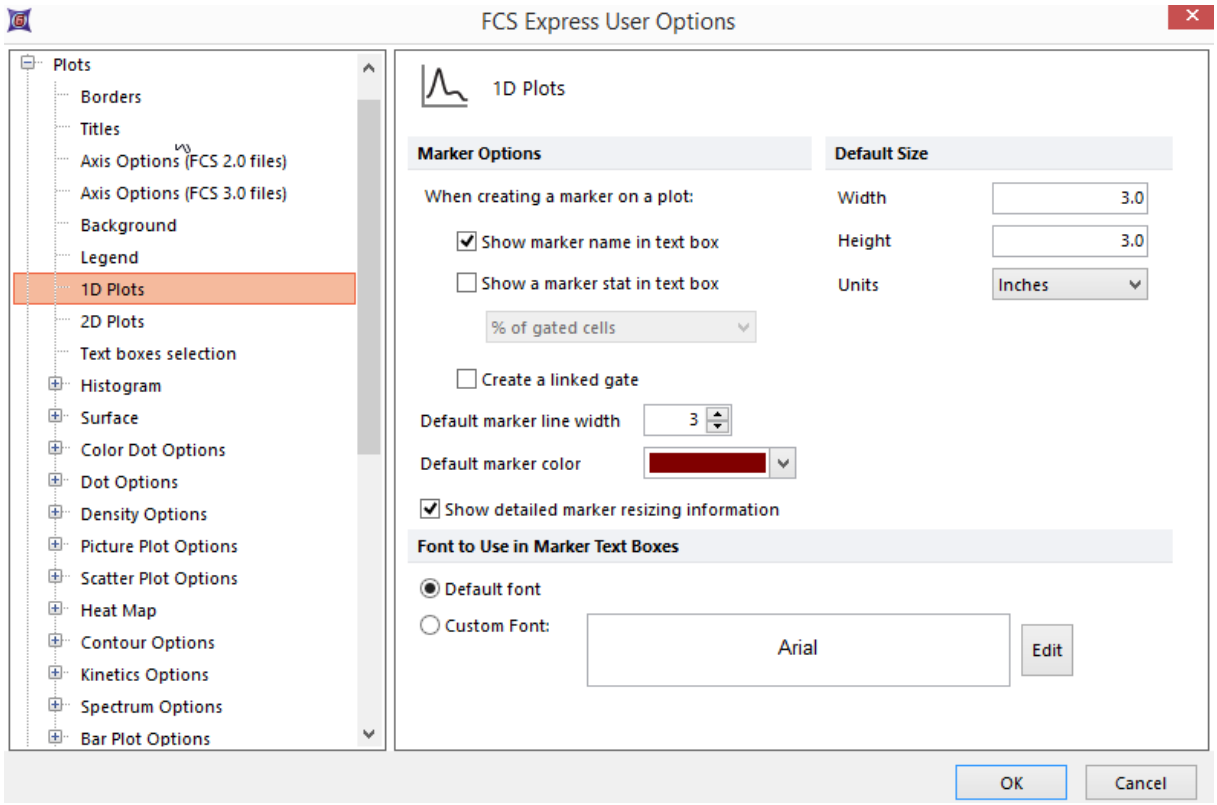

Figure 8.9 1D Plot Options

The options that are specific for 1D plots are described in the table below.

Option	Explanation
When creating a marker on a plot	When a marker is created, you can choose whether or not to show the marker name and/or stat in a text box, and select the default line width and color. If marker stat is enabled, you can choose which statistic to display from the drop-down list. If **Show detailed marker resizing information** is checked, the marker boundaries will appear above the marker and lines will drop to the axes to show where the marker boundaries will be during resizing. If **Create a linked gate** is checked, when new markers are created on this plot, a gate linked to the marker will automatically be created.
Create a linked gate	When checked, any new markers created on the histogram will be automatically converted to linked gates. Any existing markers on the plot will still need to be manually linked to a gate.
Default Size	The default size of 1D Plots.

8.2.2.5 2D Plot Options

The **2D Plot** options category (Figure 8.10) contains items that are specific for all 2D plots.

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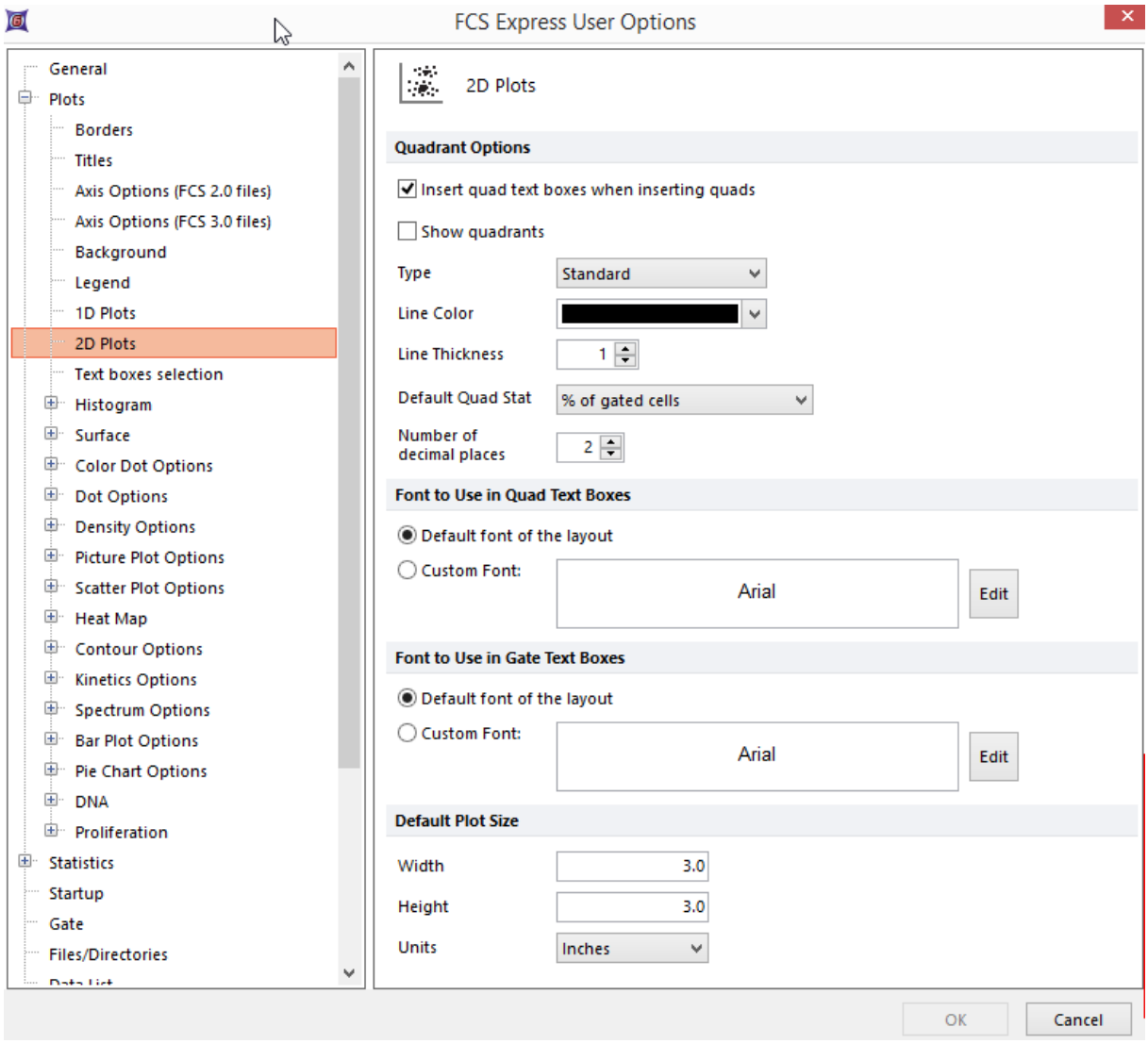

Figure 8.10 2D Plot Options

The 2D plot options are described in the table below.

Option	Explanation
Quadrant Options	The default preferences for the quadrants. The **Default Quad Stat** option allows you to set which statistic appears in a text box in all four quadrants when the quadrants are first inserted.
Font to Use in Quad Text Boxes	The default font to use for Quad Text Boxes on 2D plots showing quadrants.
Default Size	The default size of 2D Plots.

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8.2.2.6 Text Boxes Selection Options

This category (Figure. 8.11) controls whether **text boxes** derived from markers, gates, and quads within plots can be selected[1212] for moving, resizing, deleting, etc. If the checkbox is checked, these text boxes may be selected[1212].

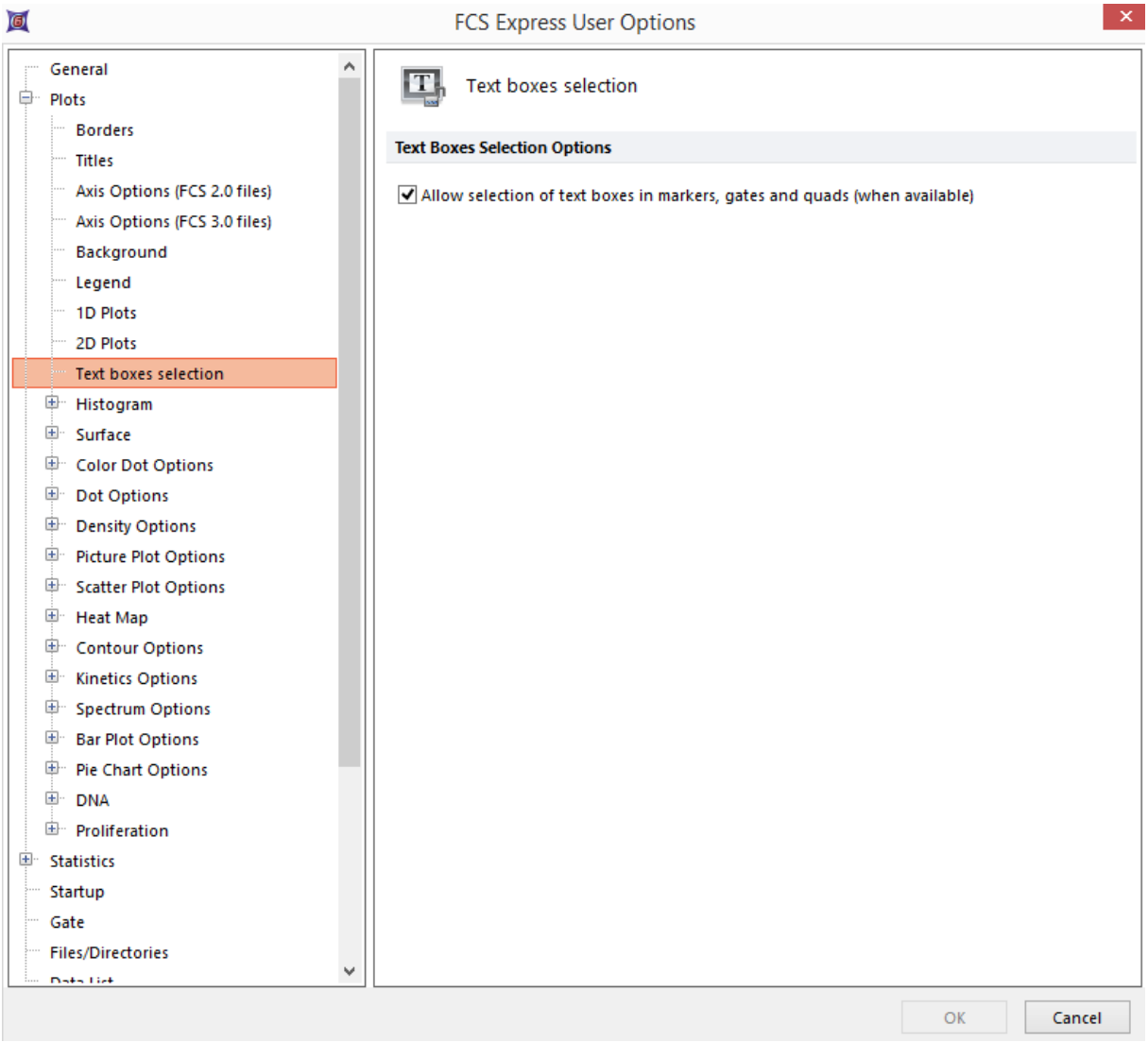

Figure 8.11 Text box selection options

8.2.2.7 DNA Options

The **DNA** options refer to general[193], overlay[194], and position marker options[195] for performing DNA analysis using Multicycle[884].

- **General**

  The General category of DNA Options (Figure 8.12) are miscellaneous properties dealing with the Multicycle fit model and the parameter to plot on DNA histograms.

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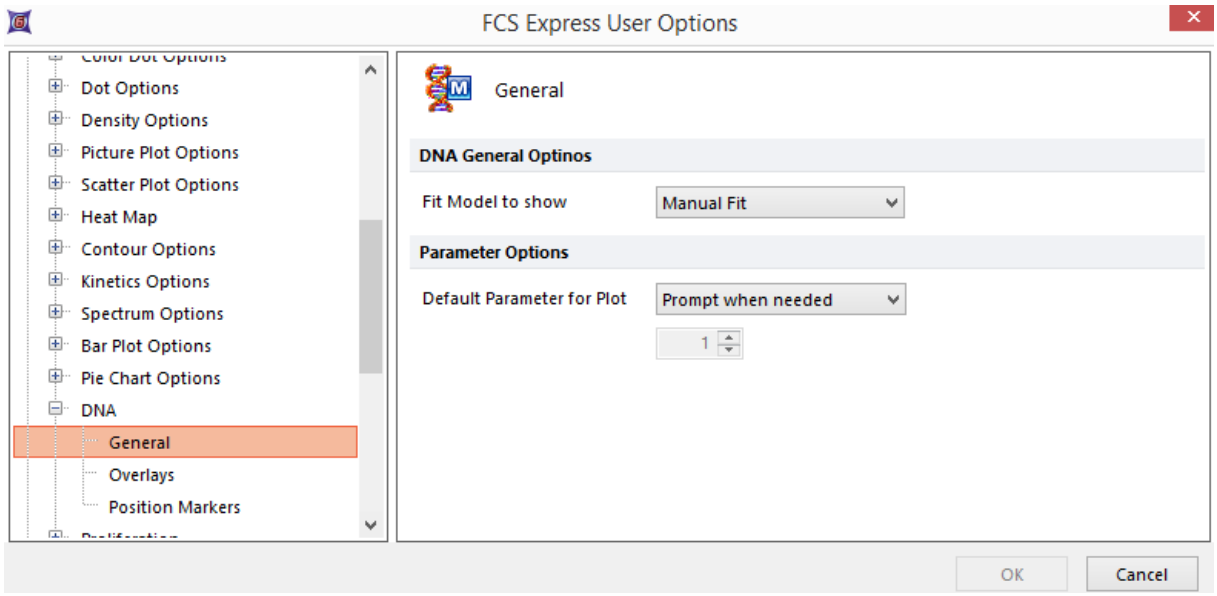

Figure 8.12 DNA General Options

The properties are described in the table below.

Option	Explanation
Fit Model to show	When Multicycle performs a fit, it calculates six different variations on that fit. You can choose which variation to display using this option. The six variations are described in the Multicycle manual.
Parameter Options	Select the parameter to display when changing data files. Use "Prompt when needed" to be prompted for the parameter every time you change a data file. The "Use parameter below" option always displays the parameter you set in the spin control.

- **Overlays**

The Overlays category of DNA preferences (Figure 8.13) allows the user to set up how the calculated overlays look on the DNA histogram.

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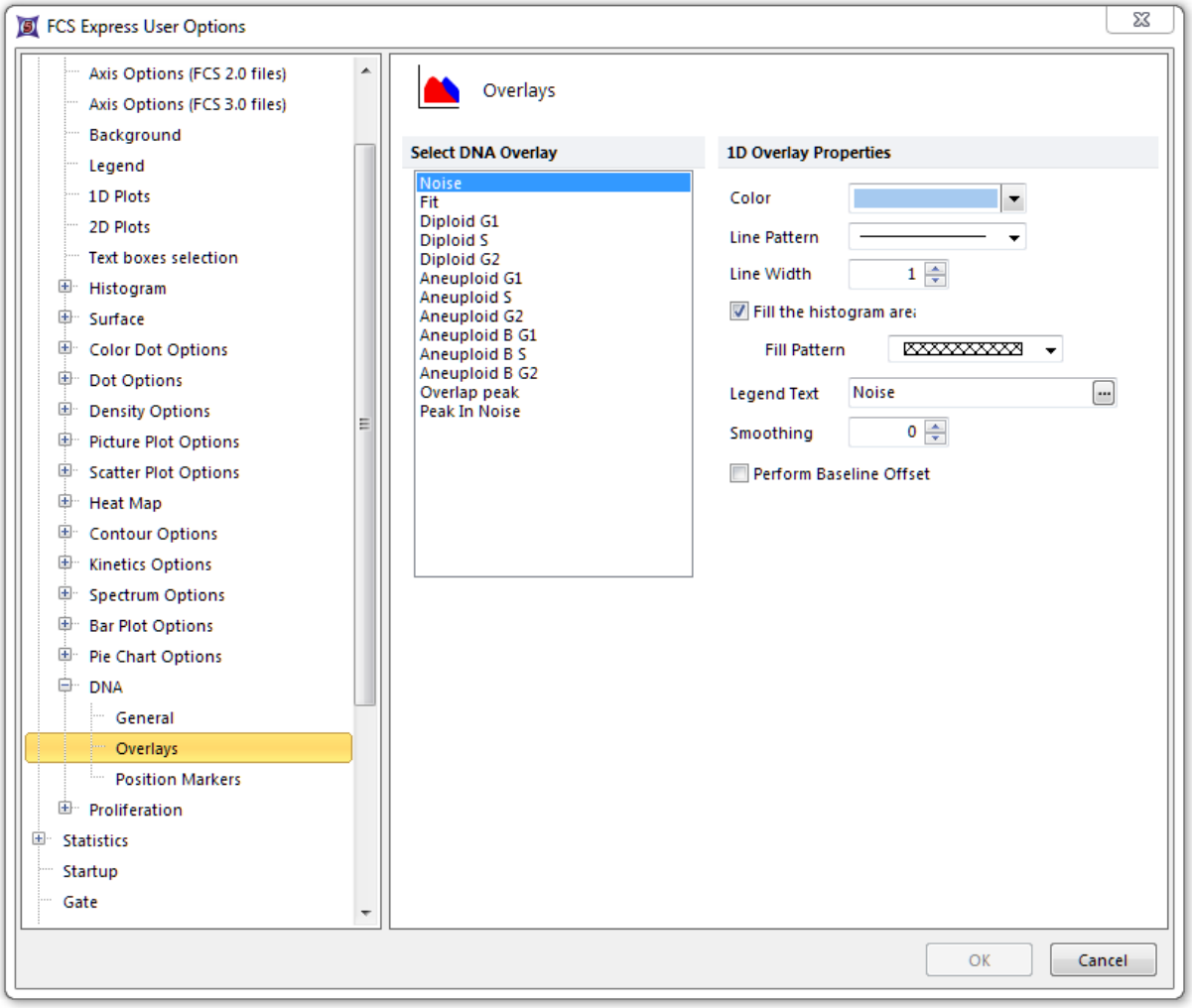

Figure 8.13 DNA Overlays Options

Select one or more overlays from the **Select DNA Overlay** list box on the left of the Overlays page to change the look and feel of that overlay. The visual properties that can be changed are similar to those for regular histogram overlays 268.

- **Position Markers**

  Position markers 889 can be displayed on DNA histograms to mark the position of cell cycle components such as the G1 mean or the background region. The Position Markers category for DNA Options allows the user to set the default properties for these markers.

  Select one or more categories from the list box on the left (Figure 8.14) and choose line options for the markers on the right.

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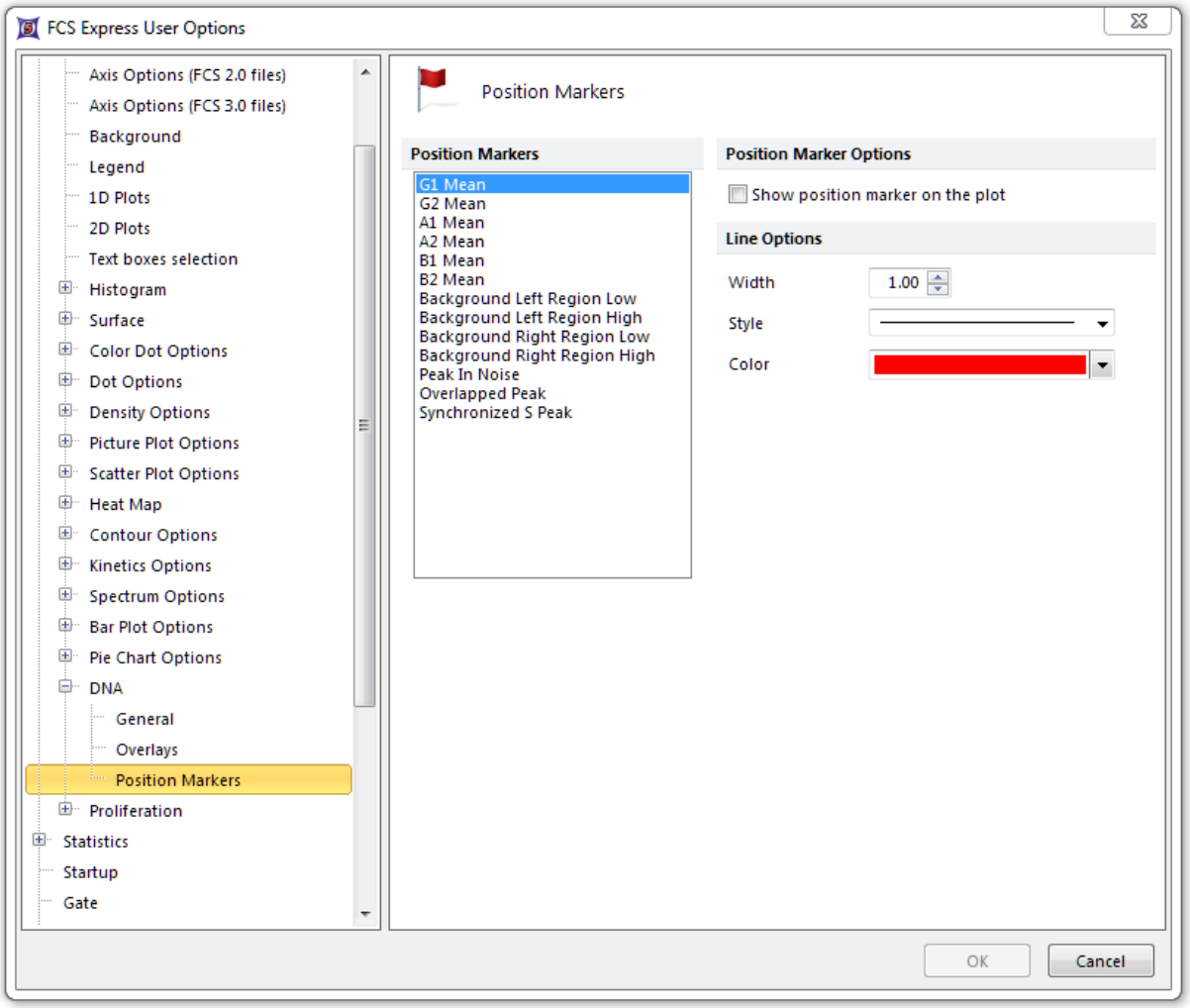

Figure 8.14 DNA Position Markers Options

8.2.3 Statistic Options

The subcategories of the **Statistics** FCS Express User Options category allow the user to select the default format and statistics items to list when statistics windows are opened. There are different options for different types of statistics windows, which are described for each in the following pages.

In addition, the **When copying stats from a statistics grid to clipboard, include column headings** checkbox (Figure 8.15) controls whether or not the description of the statistic is also copied by default.

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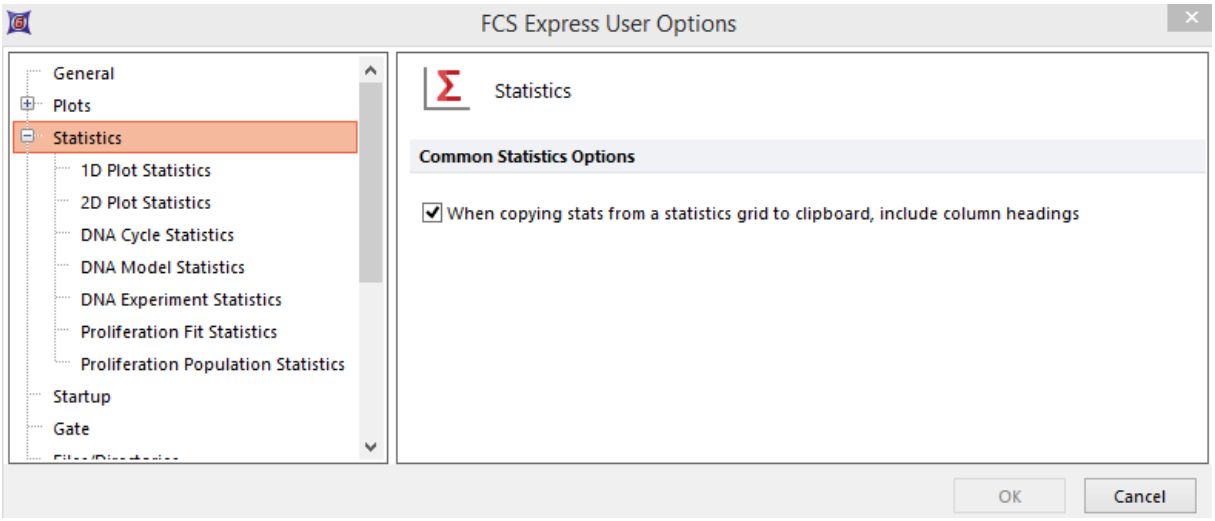

Figure 8.15 Statistics Options

8.2.3.1 1D Plot Statistics

The **1D Plot Statistics** options (Figure 8.16) determine the default visibility and display for 1D plot statistics.

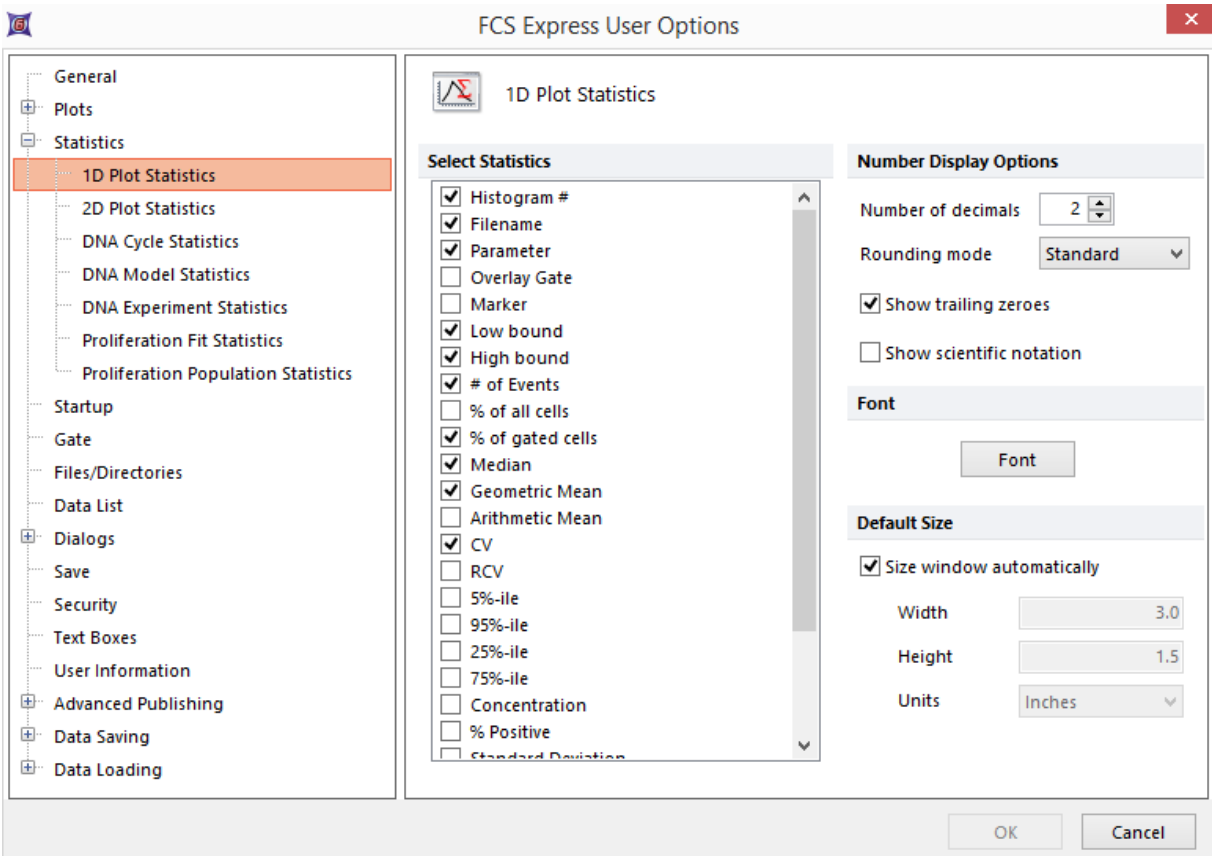

Figure 8.16 1D Plot Statistics Options

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The options are explained in the table below.

Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. The individual statistics items are explained in the statistics section of the chapter on analysis of FCS data 381.
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Options include **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.3.2 2D Plot Statistics

The **2D Plot Statistics** options (Figure 8.17) determine the default visibility and display for 2D plot statistics.

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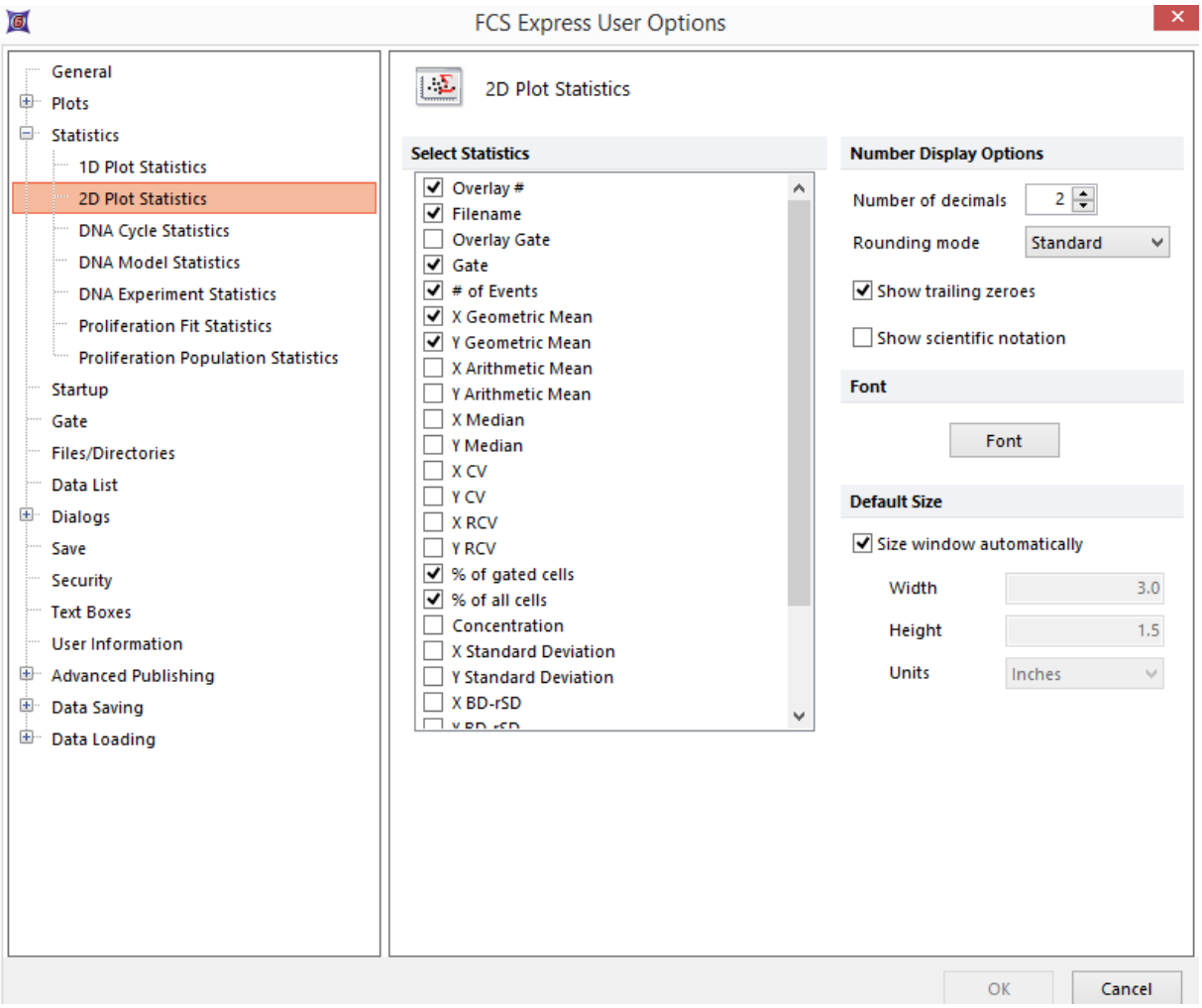

Figure 8.17 2D Plot Statistics Options

The options are explained in the table below.

Option	Explanation
Select Statistics	The check box determines whether or not statistic items are displayed when a statistics window is first opened. The individual statistics items are explained in the statistics section of the chapter on analysis of FCS data 381.
Number Display Options	• **Number of decimals** iindicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **standard form** or **standard index form**: $m \times 10^n$, where m is any real number times ten raised to the n power.
Font	The default font to use when displaying the statistics.

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Option	Explanation
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.3.3 DNA Cycle Statistics

The **DNA Cycle Statistics** FCS Express User Options (Figure 8.18) control which statistics are displayed by default when DNA Cycle Statistics windows are opened.

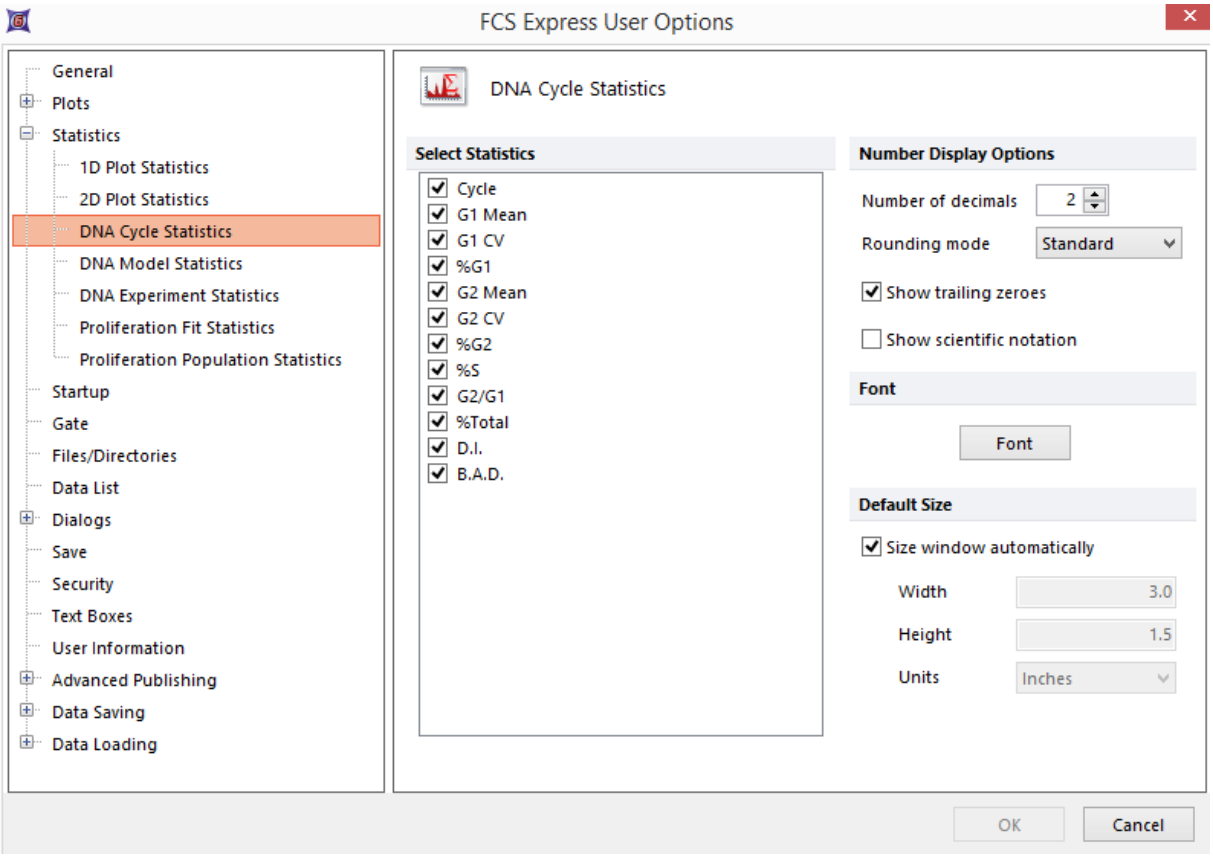

Figure 8.18 DNA Cycle Statistics Options

The options are explained in the table below.

Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. See the FCS Express knowledgebase page on DNA Analysis and Modeling with Multicycle AV for links to information on individual statistics.
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.**

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Option	Explanation
	• **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.3.4 DNA Model Statistics

The **DNA Model Statistics** FCS Express User Options (Figure 8.19) control which statistics are displayed by default when DNA Model Summary Statistics windows are opened.

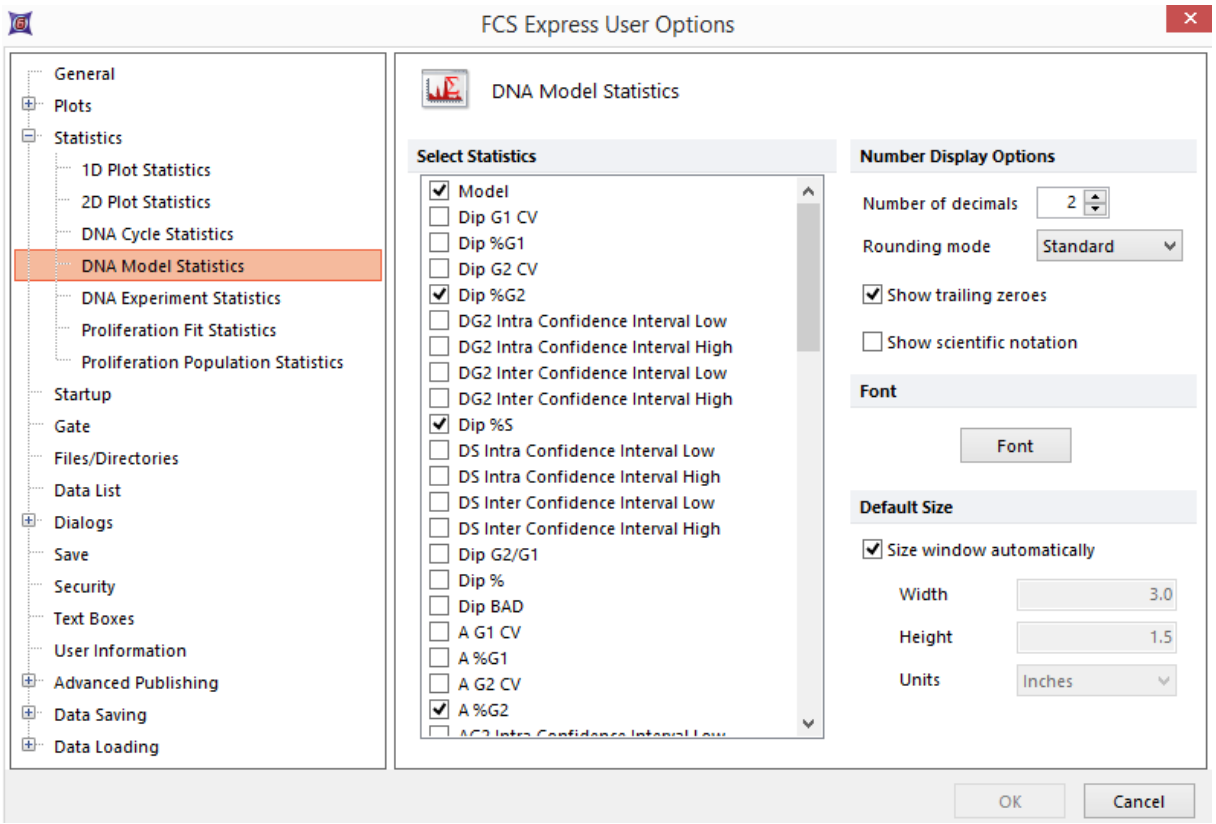

Figure 8.19 DNA Model Statistics Options

The options are explained in the table below.

Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. See the FCS Express knowledgebase page on DNA Analysis and Modeling with Multicycle AV for links to information on individual statistics.

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Option	Explanation
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent".
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.3.5 DNA Experiment Statistics

The **DNA Experiment Statistics** FCS Express User Options (Figure 8.20) control which statistics are displayed by default when DNA Experiment Statistics windows are opened.

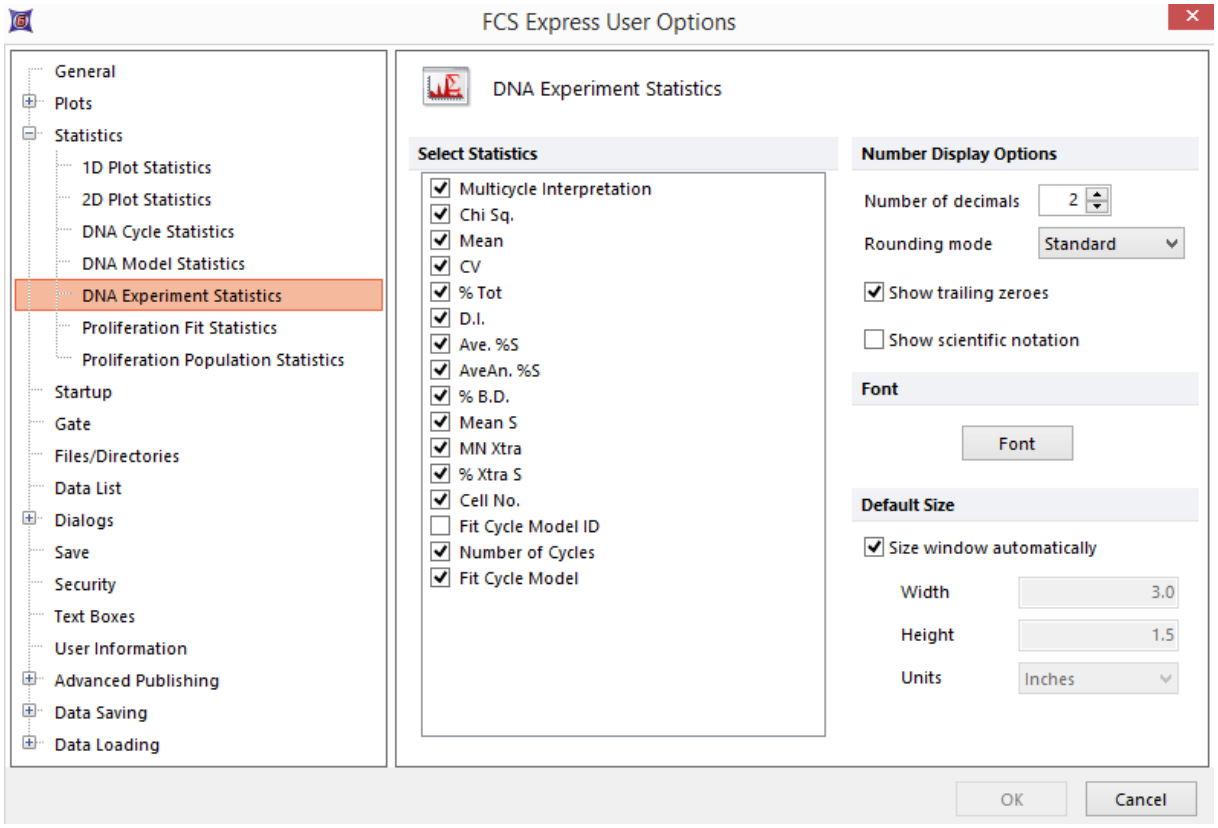

Figure 8.20 DNA Experiment Statistics options

The options are explained in the table below.

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Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. See the FCS Express knowledgebase page on DNA Analysis and Modeling with Multicycle AV for links to information on individual statistics.
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.3.6 Proliferation Fit Statistics

The **Proliferation Fit Statistics** FCS Express User Options (Figure 8.21) control which statistics are displayed by default when Proliferation Fit Statistics windows are opened.

Note: Please refer to the Proliferation Statistics 907 topic for explanations of each statistic and for a list of statistical formulas 908.

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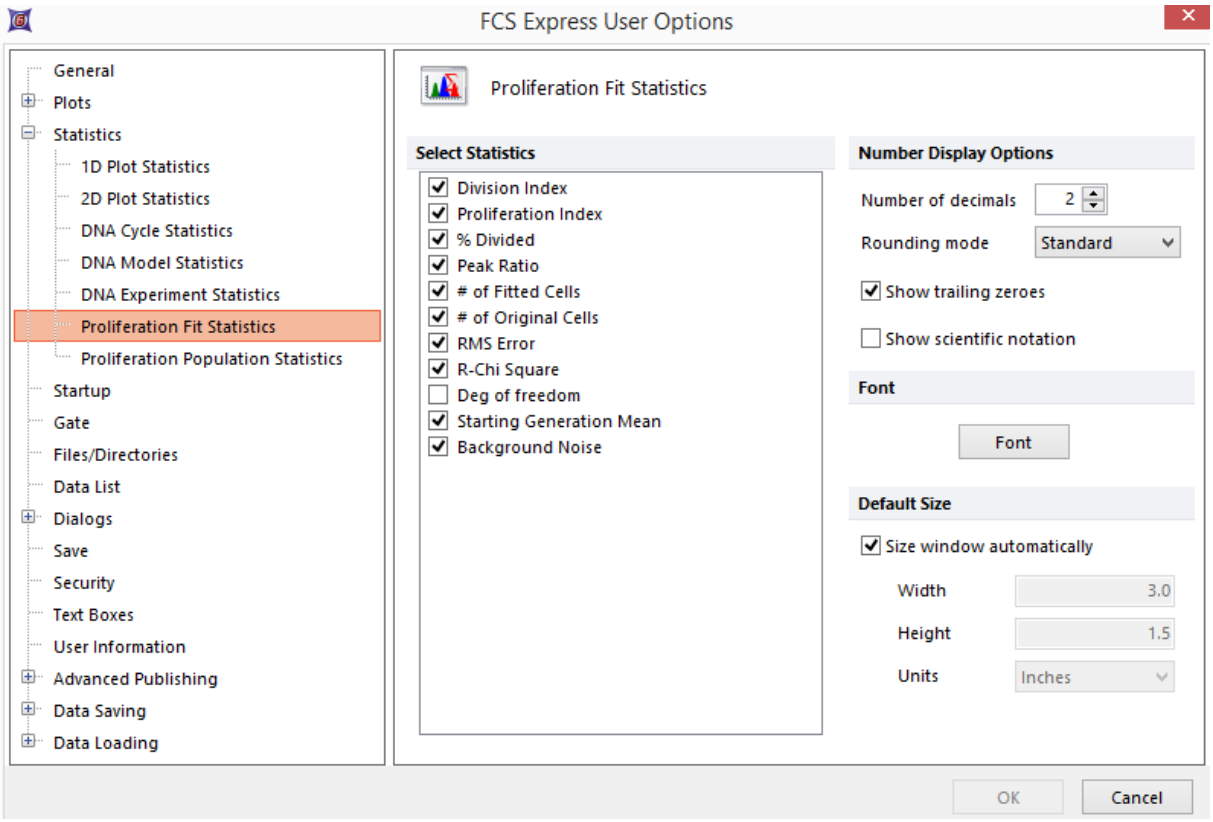

Figure 8.21 Proliferation Fit Statistics options

The options are explained in the table below.

Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. Please refer to the Proliferation Statistics 907 topic for explanations of each statistic and for a list of statistical formulas 908.
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes. • **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

Note: Please refer to the Proliferation Statistics 907 topic for explanations of each statistic and for a list of statistical formulas 908.

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8.2.3.7 Proliferation Population Statistics

The **Proliferation Population Statistics** FCS Express User Options (Figure 8.22) control which population statistics[910] are displayed when the proliferation population statistics window is opened.

Note: Please refer to the Proliferation Statistics[907] topic for explanations of each statistic and for a list of statistical formulas[908].

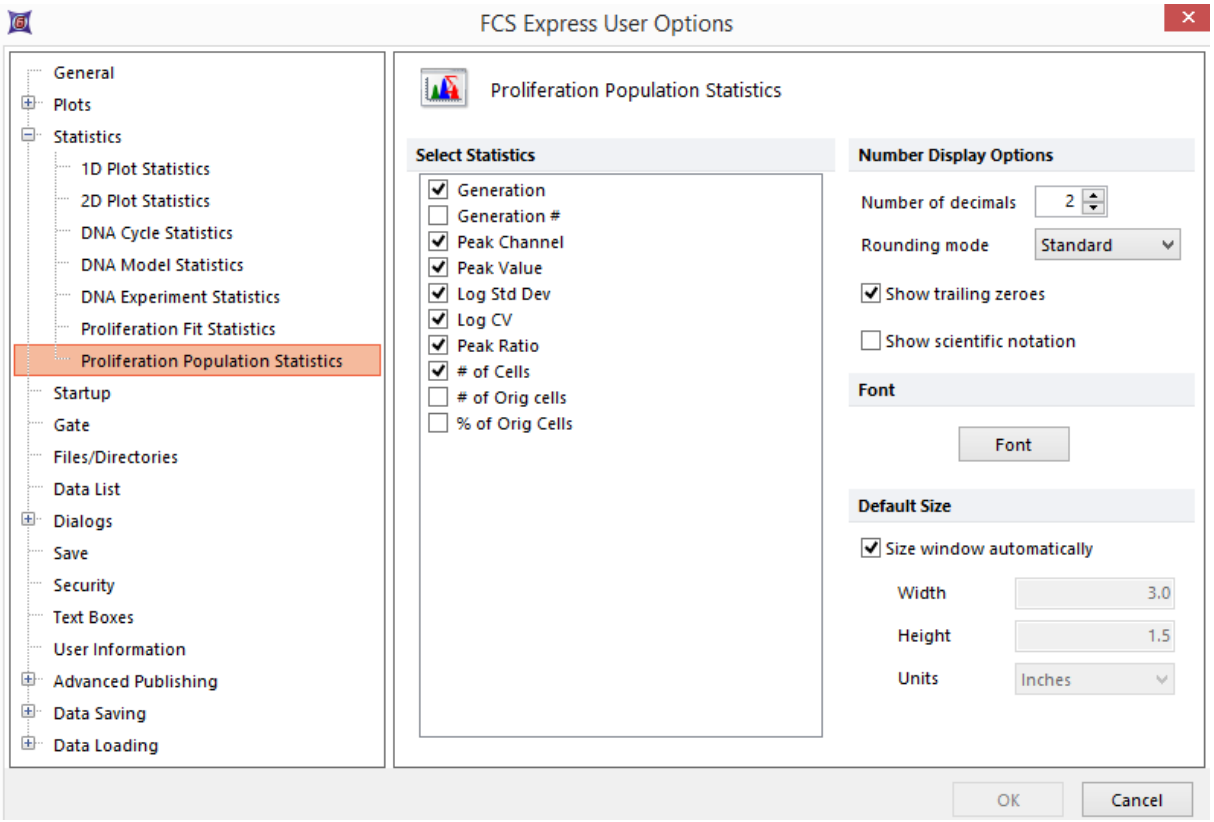

Figure 8.22 Proliferation Population Statistics options

The options are explained in the table below.

Option	Explanation
Select Statistics	The checkbox determines whether or not statistic items are displayed when a statistics window is first opened. Please refer to the Proliferation Statistics[907] topic for explanations of each statistic and for a list of statistical formulas[908].
Number Display Options	• **Number of decimals** indicates the number of decimal places listed to the right of any decimal point. • **Rounding mode** indicates the method for rounding the last digit. Choose between **Standard, Down, Up,** and **Truncate.** • **Show trailing zeroes**, if checked, shows the specified **number of decimals** even if the terminal digits are zeroes.

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Option	Explanation
	• **Show scientific notation**, if checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"
Font	The default font to use when displaying the statistics.
Default Size	Sets the default size of the statistics window. If **Size window automatically** is checked, size will automatically adjust if data is added that extends beyond the window borders.

8.2.4 Startup Options

The **Startup** FCS Express User Options (Figure 8.23) allow the user to control the behavior of FCS Express at startup.

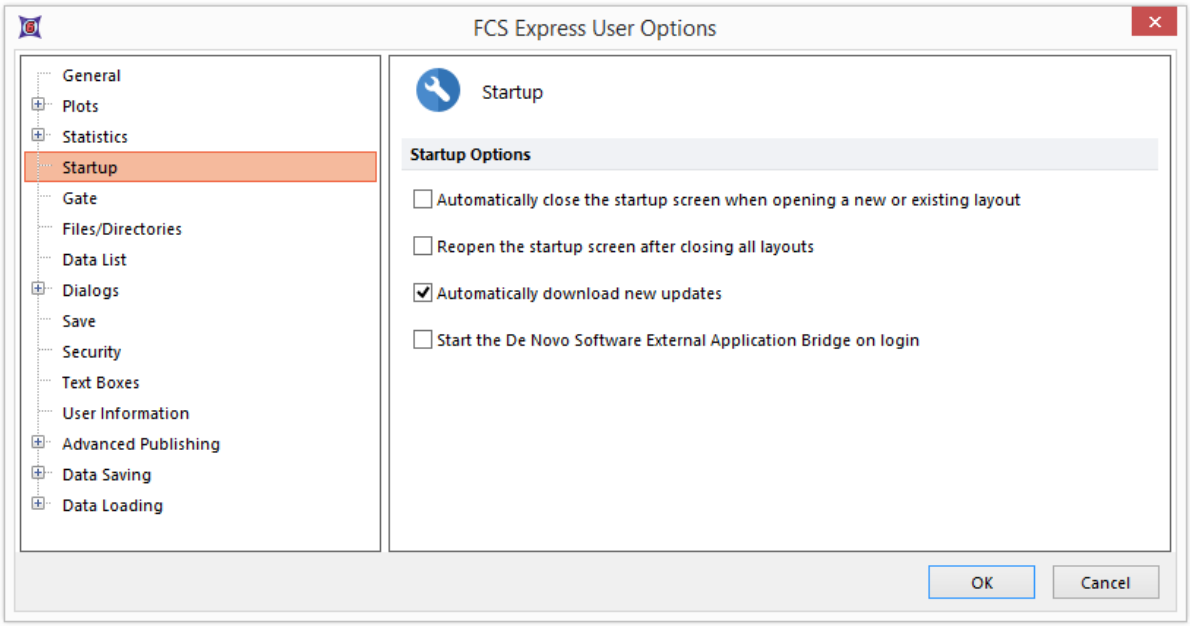

Figure 8.23 Startup screen options

By checking or unchecking the checkbox, you can choose whether or not to:

- Automatically close the Startup Screen 54 when opening a new or existing layout
- Reopen the Startup Screen after closing all layouts
- Automatically download any new updates

  If checked, latest updates to FCS Express will be automatically downloaded when available. They can also be downloaded manually from the De Novo Software website.

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- Start the De Novo Software External Application Bridge on login. The De Novo Software External Application Bridge is required for the R Integration features of FCS Express 973.

8.2.5 Gate Options

The FCS Express User Options **Gate** category (Figure 8.24) lets the user select the default FCS Express User Options for gates.

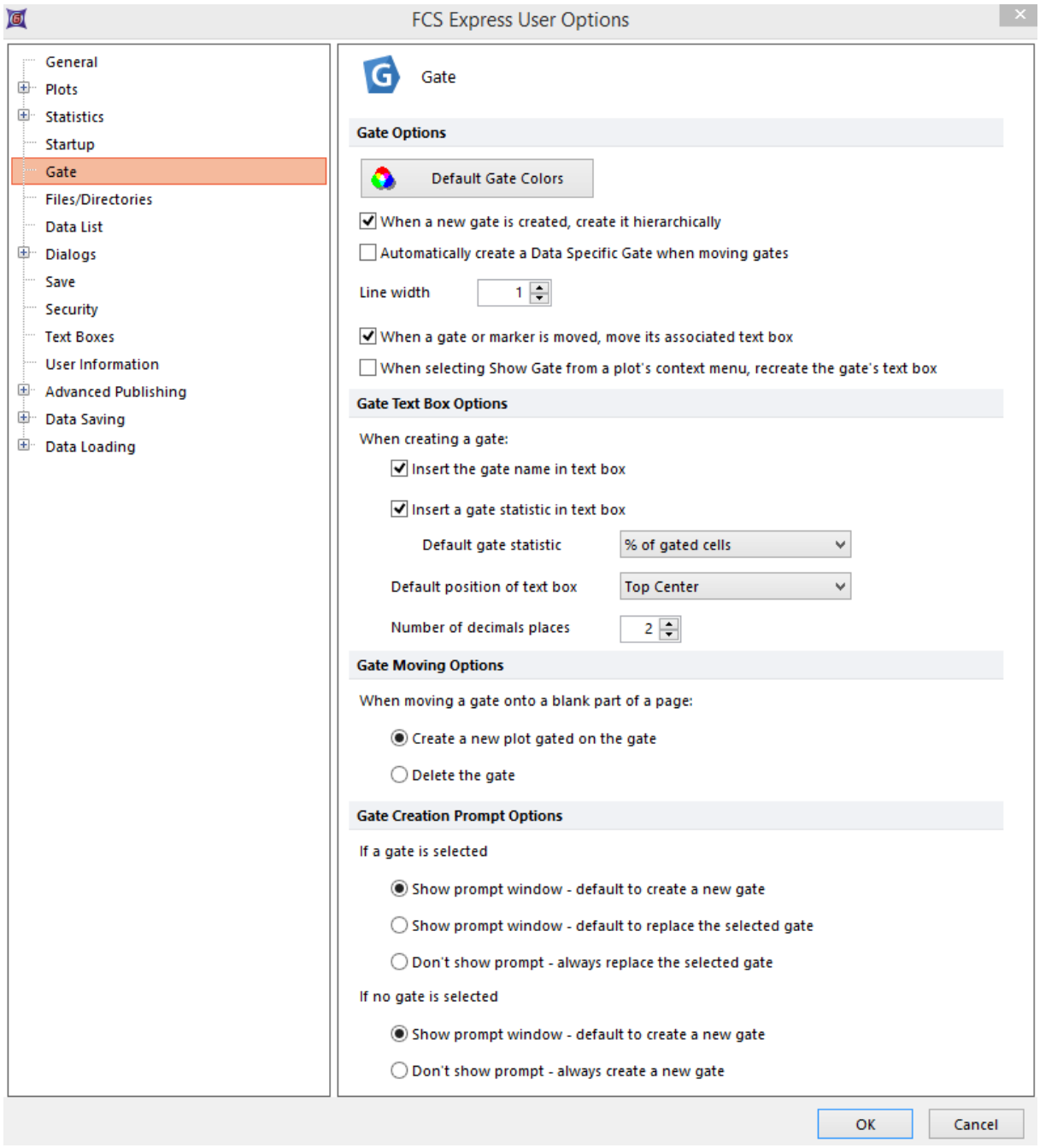

Figure 8.24 Gate options

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These options are described in the table below.

Option	Explanation
Gate options	• Set the default gate colors, which apply to gates according to the order of creation. • Choose to use hierarchical gating 350 as the default logic for new gates. • Choose to create Data Specific Gates 324 when moving. • Set the width of the line used to draw the outline of the gate on a plot. • Move the text box associated with a gate/marker when the gate or marker is moved. • Recreate the gate's text box when selecting **Show Gate** 344 from the plot's context menu.
Gate Text Box options	• Determine whether or not to insert a gate name in the Gate Text Box. • Determine whether to show a gate statistic in the Gate Text Box. From the dropdown menu, choose from several default gate statistics 381. • Define the default position of the Gate Text Box. • Determine number of decimal places.
Gate Moving options	When moving a gate onto a blank part of the page, choose whether to **Create a new plot on the gate** or to **Delete the gate.**
Gate Creation Prompt options	Choose prompt window options that appear when creating a gate 1266, depending on whether an existing gate is selected or not.

8.2.6 Files/Directories

The **Files/Directories** FCS Express User Options category allows the user to select default directories and files for various aspects of FCS Express (Figure 8.25). For example, if you routinely open the same template to create new layouts, the default directory you set here will automatically open your preferred template.

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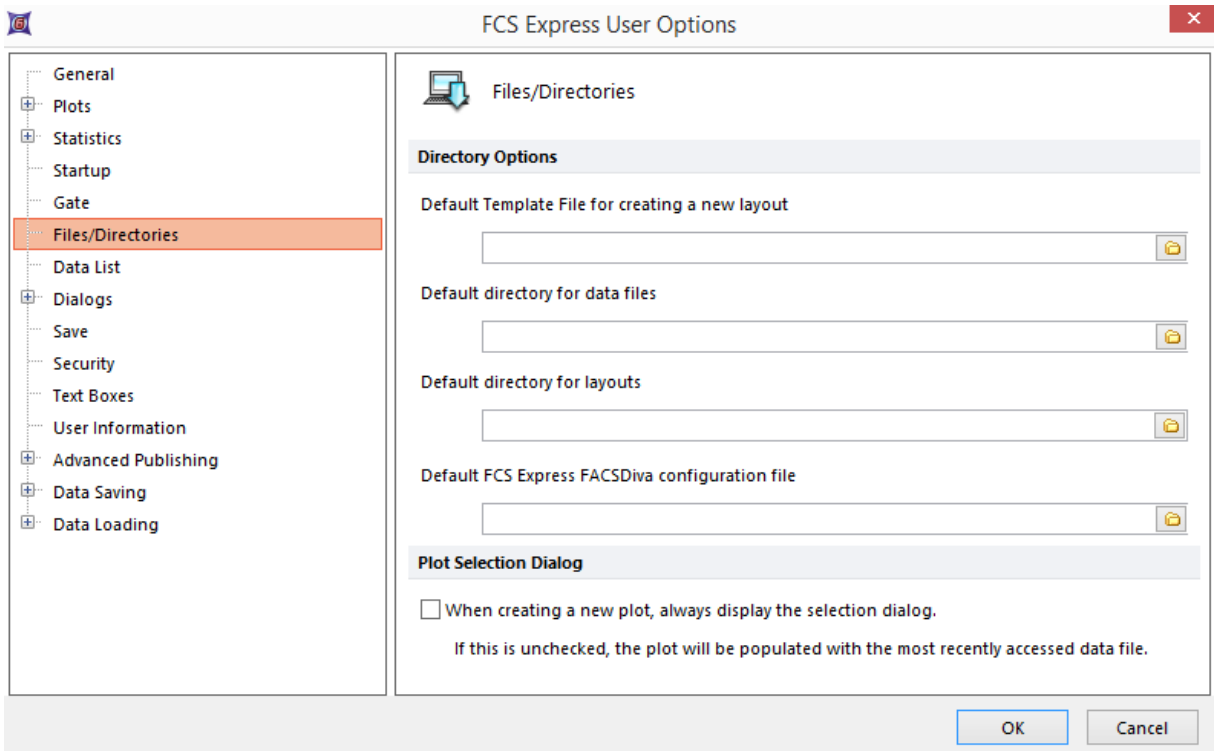

Figure 8.25 Files/Directories options

These options are described in the table below.

Option	Explanation
Default Template File for creating a new layout	If you typically create the same type of layout, this default will automatically open your preferred Template File when creating new layouts. See Using Templates 866 for more information.
Default directory for data files	When an FCS Express layout is first opened and a data file is first required, FCS Express will look in this directory for the data files.
Default directory for layouts	When you first open a layout 95, the Open Layout dialog will first start in this directory.
Default FCS Express FACSDiva configuration file	This is the default FACSDiva configuration file 868.
When creating a new plot, always display the selection dialog	If the checkbox is checked, you will always be prompted to choose the file when creating a new plot. If unchecked, the plot will be loaded with the current data file 172.

8.2.7 Data List

The **Data List** FCS Express User Options category (Figure 8.26) allows the user to control the default display and behavior of the Data List 785.

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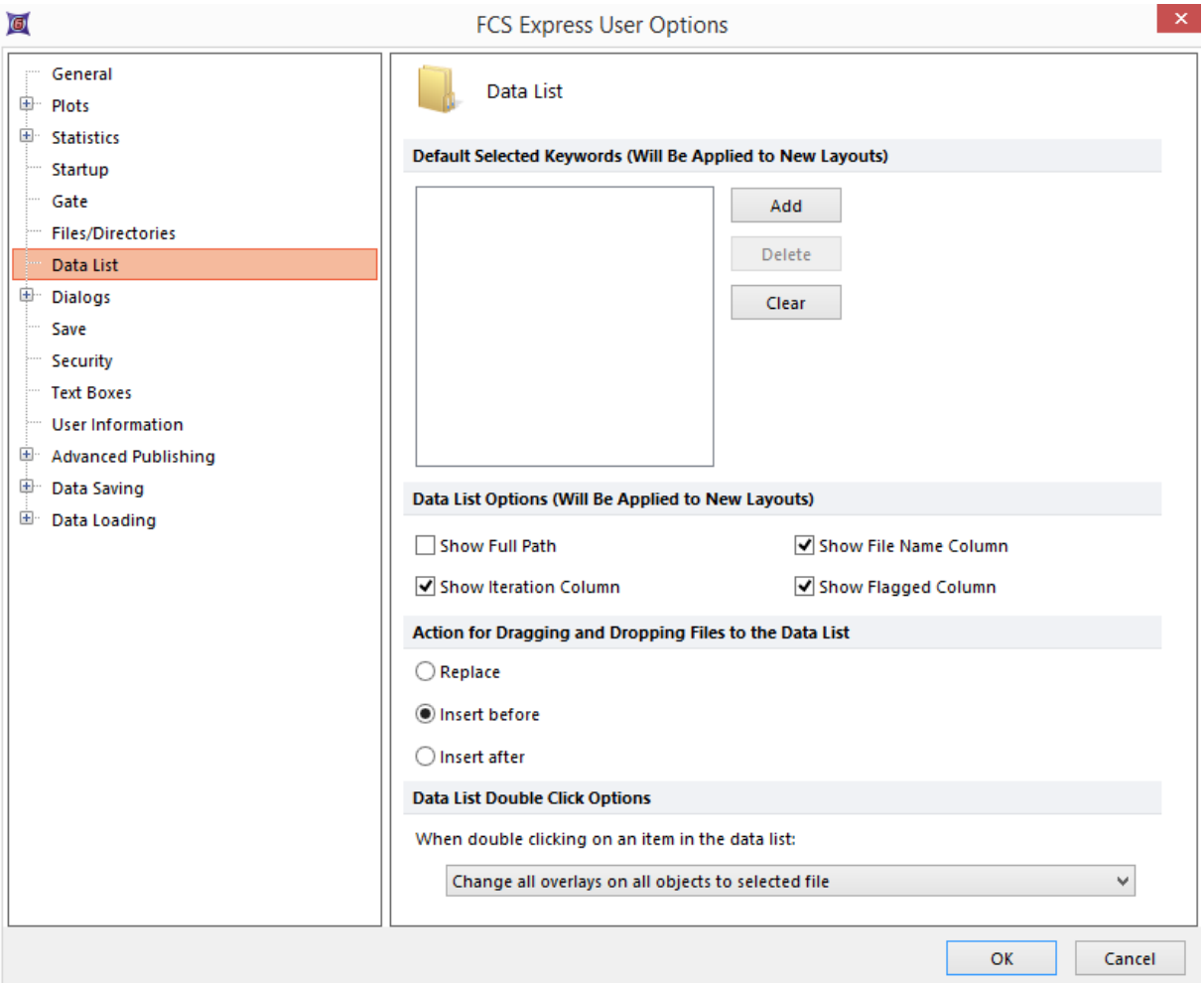

Figure 8.26 Data List options

The options are described in the table below.

Option	Explanation
Selected Keywords	Add or remove keywords by which to identify or sort 791 files.
Data List Options	• Show or hide the Full Path of data files. • Show or hide the Iteration Column. • Show or hide the File Name Column. • Show or hide the Flagged Column.
Action for Drag and Drop	Choose which action to take when dragging and dropping a file from windows explorer on top of an existing file in the Data List. • **Replace** removes the existing file from the data list and replaces it with the newly dropped file. • **Insert before** places the newly dropped file before the existing file in the data list. • **Insert after** - inserts the newly dropped file after the existing file in the data list.
Double-click Options	Choose which of the following actions to take when double-clicking on a file in the Data List. • Change all overlays on all objects to selected file.

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Option	Explanation
	• Change all overlays on selected objects to selected file. • Change base overlay on all objects to selected file. • Change base overlay on selected objects to selected file. • Open new plot with selected file.

8.2.8 Dialogs Options

Some dialog windows appear automatically in FCS Express. Within the **Dialogs** category of user options, there are three subcategories for which you can customize the appearance and behavior of some of these windows: **Keyword Dialog** 211, **Open Data Dialog** 212, and **Do Not Show Again** 220 (Figure 8.27) and are described on the following pages.

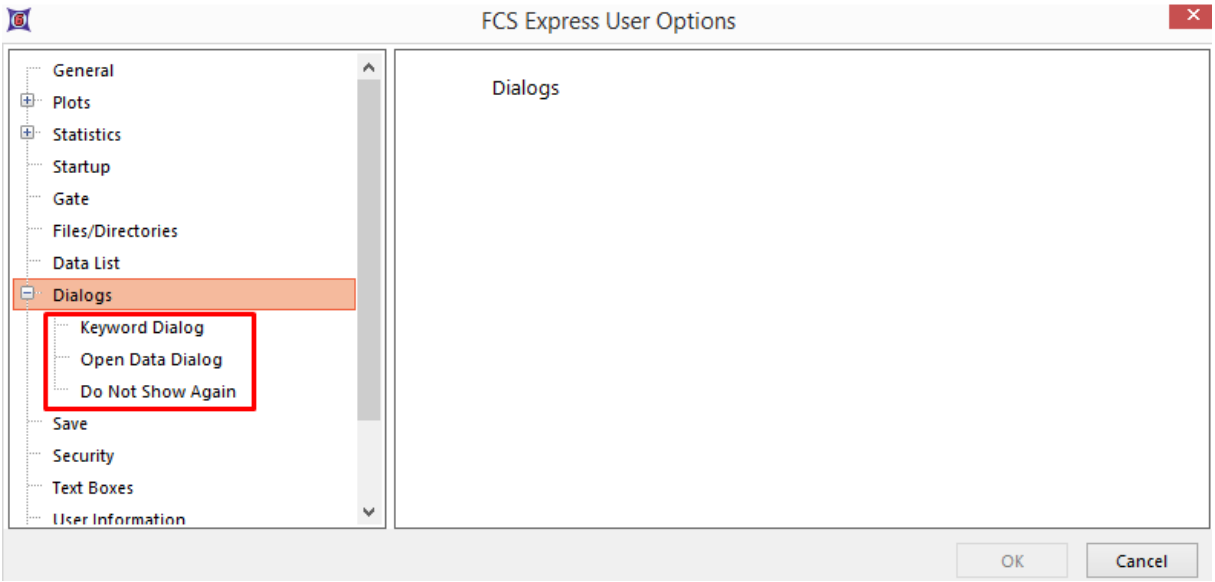

Figure 8.27 - Subcategories for Dialogs outlined in red

8.2.8.1 Keyword Dialog

The **Keyword Dialog** is used throughout FCS Express whenever you are required to select a keyword. For instance, keywords can be selected for insertion in plot titles 250 and in text boxes 152 as keyword tokens 708.

Since many keywords can be present for a data file, the **Dialogs→Keyword Dialog** FCS Express User Options allow you to specify which keywords appear in the keyword dialog (Figure 8.28), providing the user with the opportunity to filter on the keywords that are used the most.

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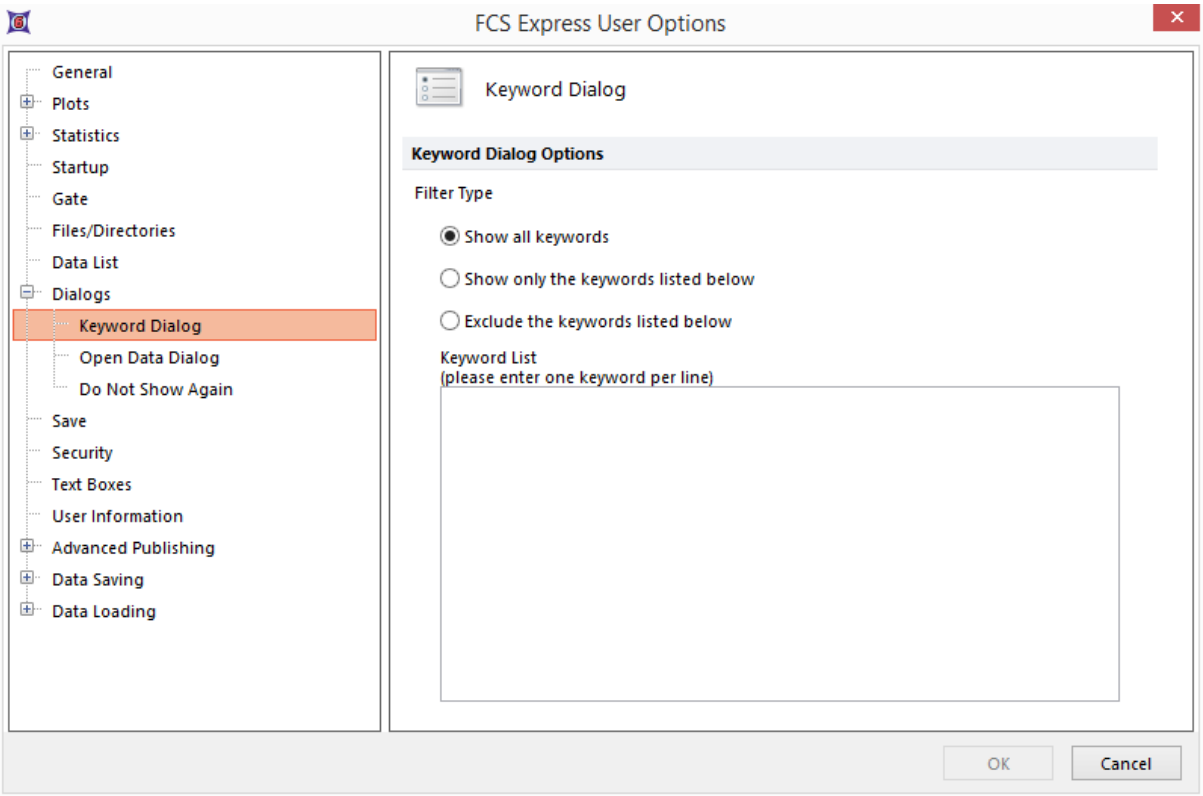

Figure 8.28 Keyword Dialog Preferences

You can choose one of the following:

- Show all keywords.
- Show only keywords in the **Keyword Filter List**.
- Show all keywords except those appearing in the **Keyword Filter List**.

If you do not choose **Show all keywords**, you must enter keywords into the **Keyword Filter List** list box. Enter one keyword per line. Case/capitalization are ignored. You can also use the wild card "*" to denote any characters. For instance, to only display keywords starting with the letter "P", first select the **Show only the keywords listed below** option, and then type in "P*" in the keyword list.

8.2.8.2 Open Data Dialog

The **Open Data Dialog** can be displayed as the **Standard Open Data Dialog** 214 or **Advanced Open Data Dialog** 215 (Figure 8.29).

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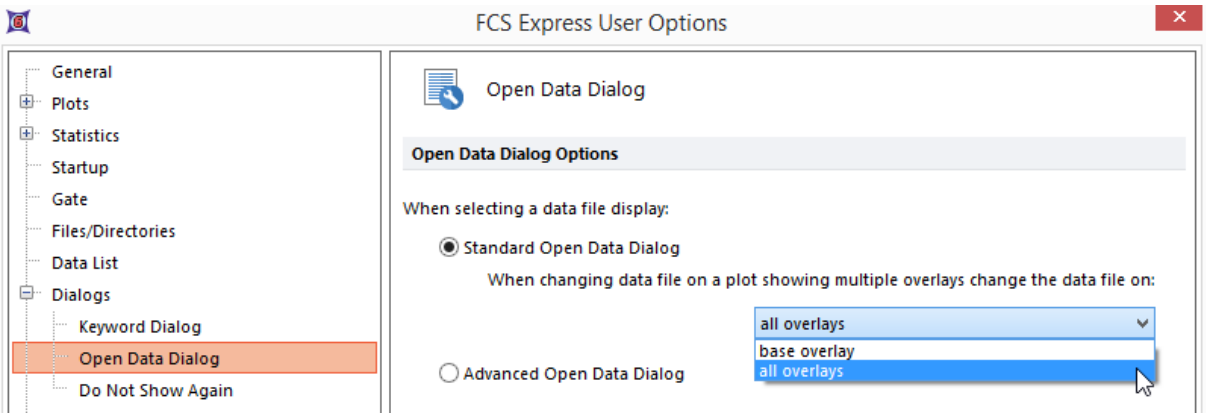

Figure 8.29 - Open Data Dialog with Standard Open Data Dialog Overlay Options Displayed

When the **Standard Open Data Dialog** is selected, the **Select data file** dialog (Figure 8.30) will open when adding files to the **Data List** or changing overlays.

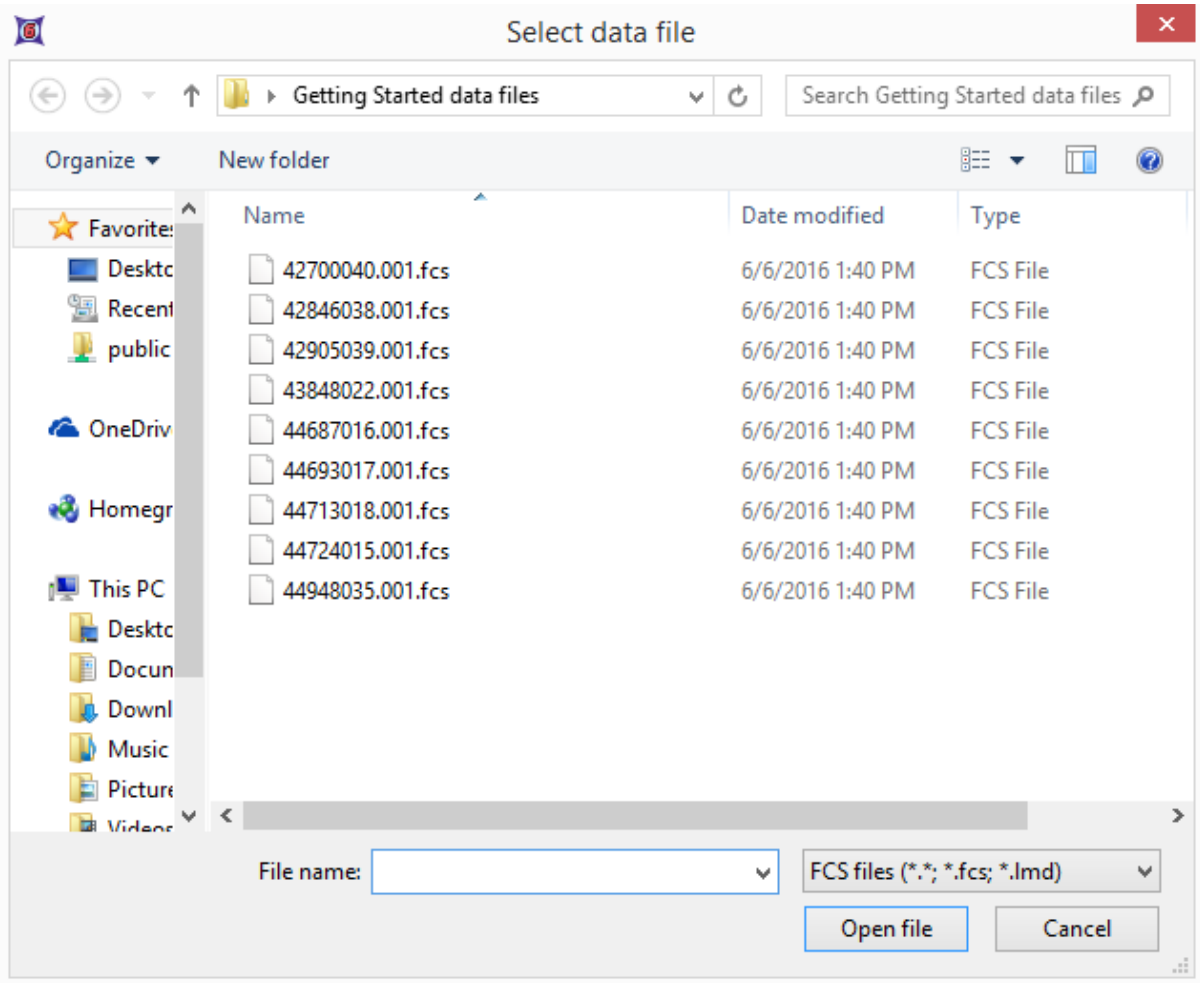

Figure 8.30 - Select data file dialog

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When the **Advanced Open Data Dialog** is selected, the **Open Data File** dialog with five tabs 216 (Figure 8.31) will open when adding files to the **Data List** or changing overlays.

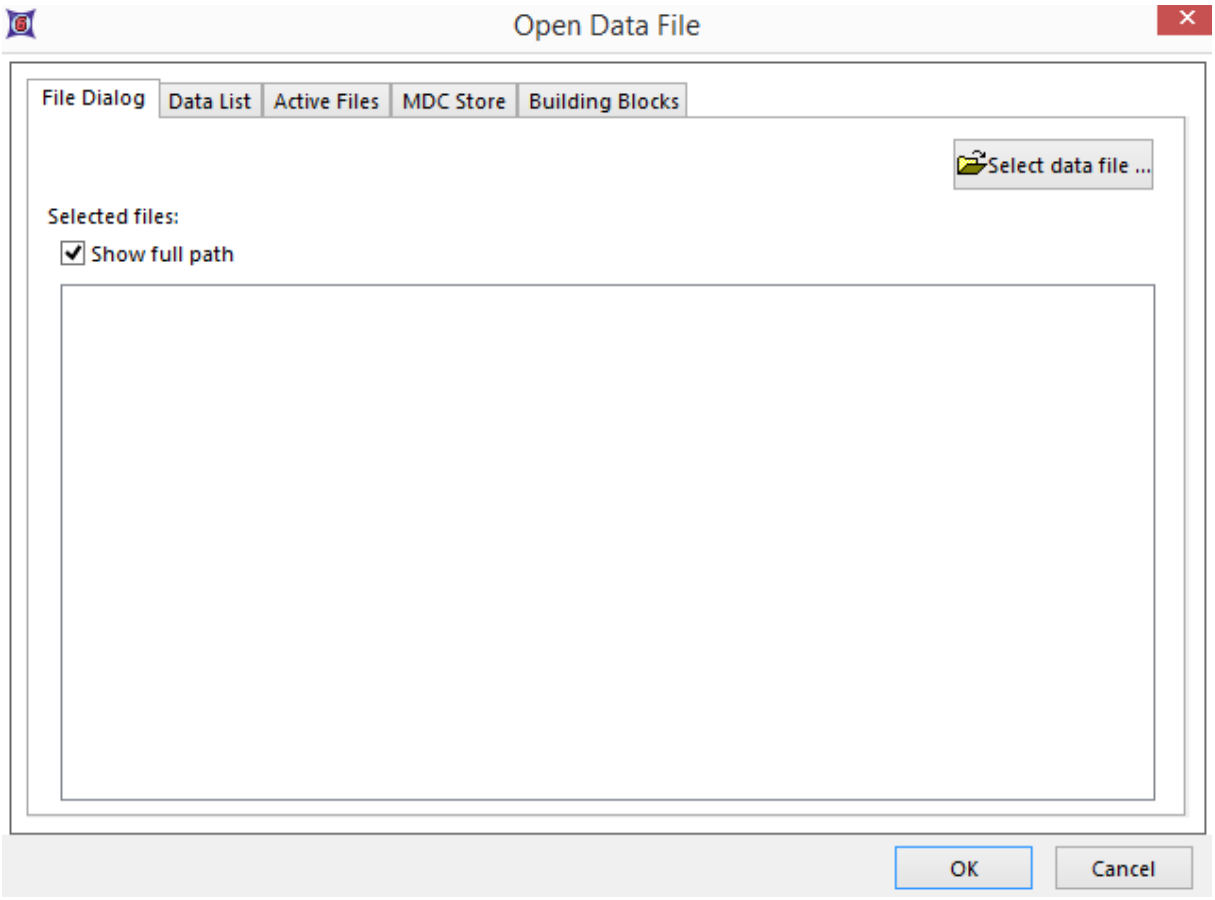

Figure 8.31 - Open Data File dialog

8.2.8.2.1 Accessing the Standard Open Data Dialog

The Standard Open Data Dialog can be accessed when selected in user preferences for **Open Data Dialog** through the following actions:

- creating statistics 699,
- editing statistics 793,
- adding files 788 to the Data List,
- changing overlays on histograms 268, spectrum plots 278, or 2D plots 284,
- changing data by using **Data** tab→**Change Data on Selection** or **Change Data on All Objects** group→**Select** command (Figure 8.32). Please note the **Select** drop down will only appear when the **Standard Open Data Dialog** option has been selected for **All Overlays** in the user preferences.

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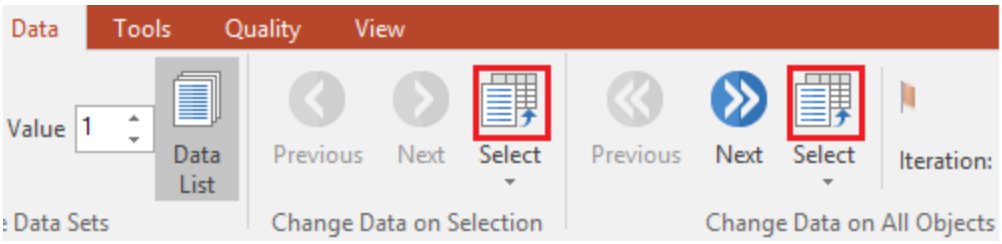

Figure 8.32 - Select command outlined in red from Change Data groups within Data tab on ribbon

8.2.8.2.2 Accessing the Advanced Open Data Dialog

The Advanced Open Data Dialog can be accessed when selected in user preferences for **Open Data Dialog** through the following actions:

- creating statistics 699,
- editing statistics 793,
- adding files 788 to the Data List, or
- changing data by using **Data** tab→**Change Data on Selection** or **Change Data on All Objects** group→**Select** drop down list command (Figure 8.33).

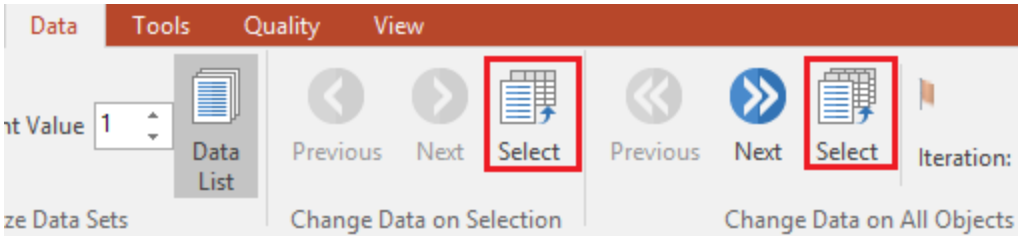

Figure 8.33 - Select command outlined in red from Change Data groups within Data tab on ribbon

and when **Standard Open Data Dialog** for **All Overlays** is set as the preference for **Open Data Dialog**:

- changing data by using **Data** tab→**Change Data on Selection** or **Change Data on All Objects** group→**Select** drop down list command (Figure 8.34).

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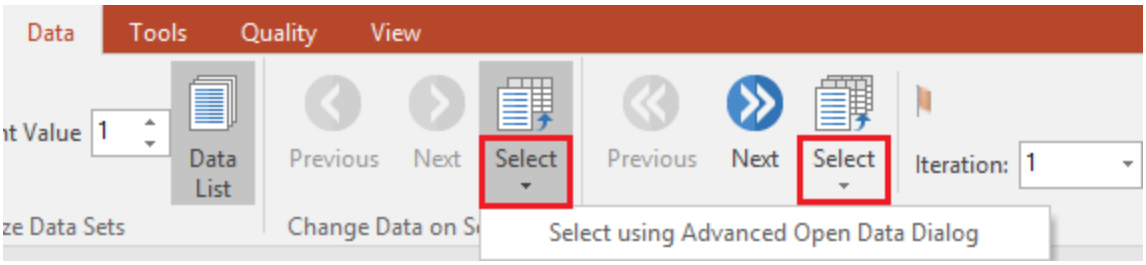

Figure 8.34 - Select dropdown command outlined in red from Change Data groups within Data tab on ribbon

8.2.8.2.3 Advanced Open Data Dialog Tabs

The five tabs for the Advanced Open Data Dialog are listed and explained below. If the checkbox **Show full path** is checked for **Selected Files** under any of the first three tabs, the full pathway will be displayed for each file instead of only the filename.

- **File Dialog** is the first tab and from the user may click **Select data file** button (outlined in red in Figure 8.35 below) to access the **Select data file** window to access file(s) on workstation or network.

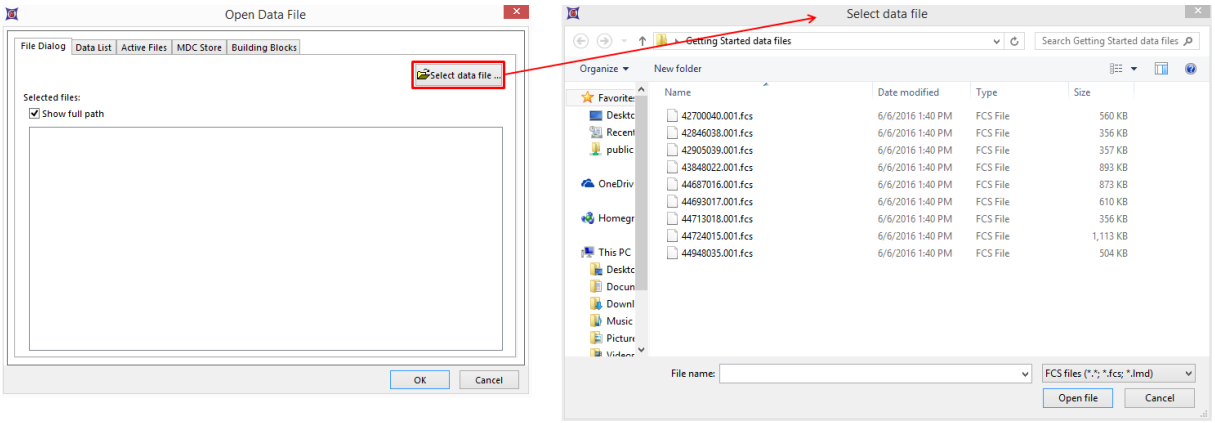

Figure 8.35 - File tab to Select data file dialog

- **Data List** is the second tab from which the user may select file(s) currently added to the Data List for the layout (Figure 8.36).

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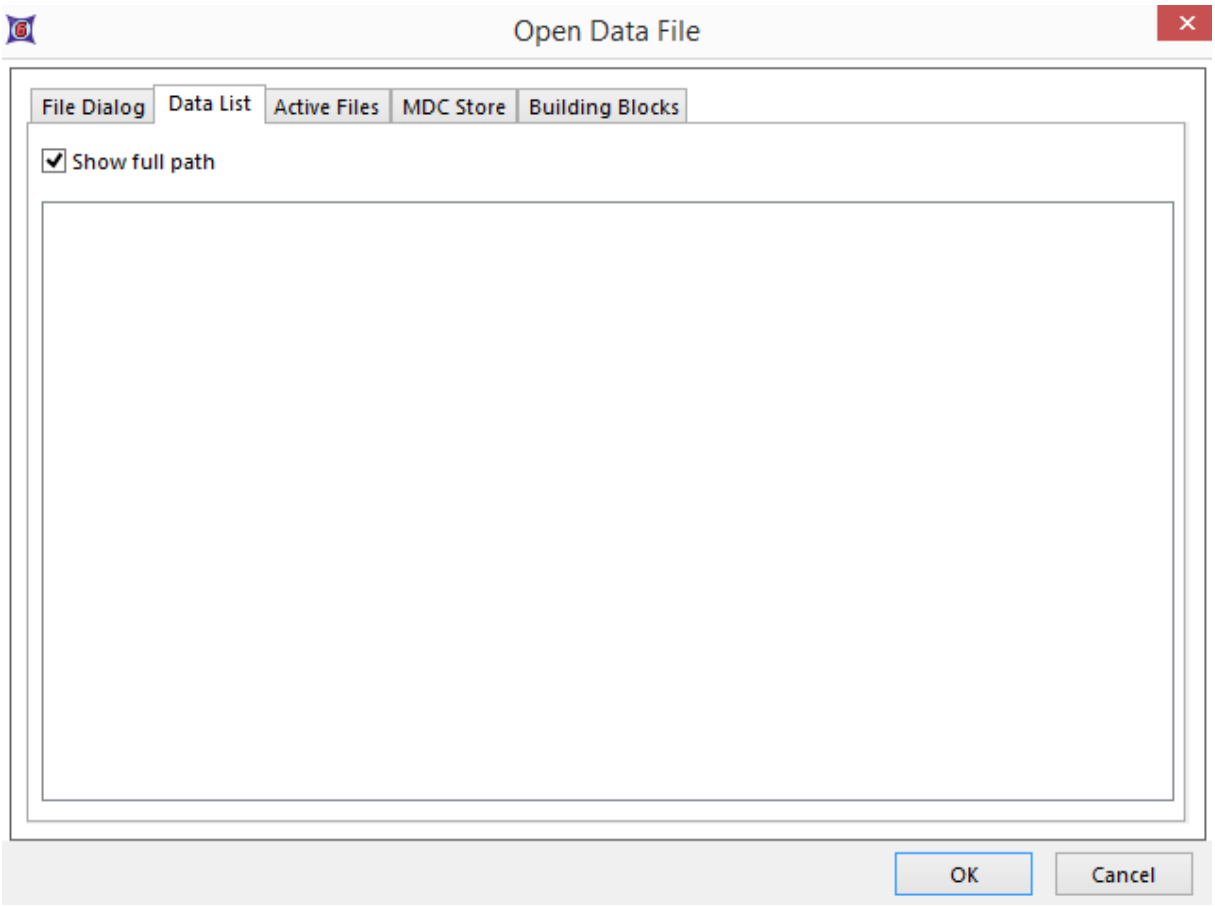

Figure 8.36 - Advanced Open Data Dialog Data List tab

- **Active Files** is the third tab from which the user may select file(s) currently used within the layout (Figure 8.37).

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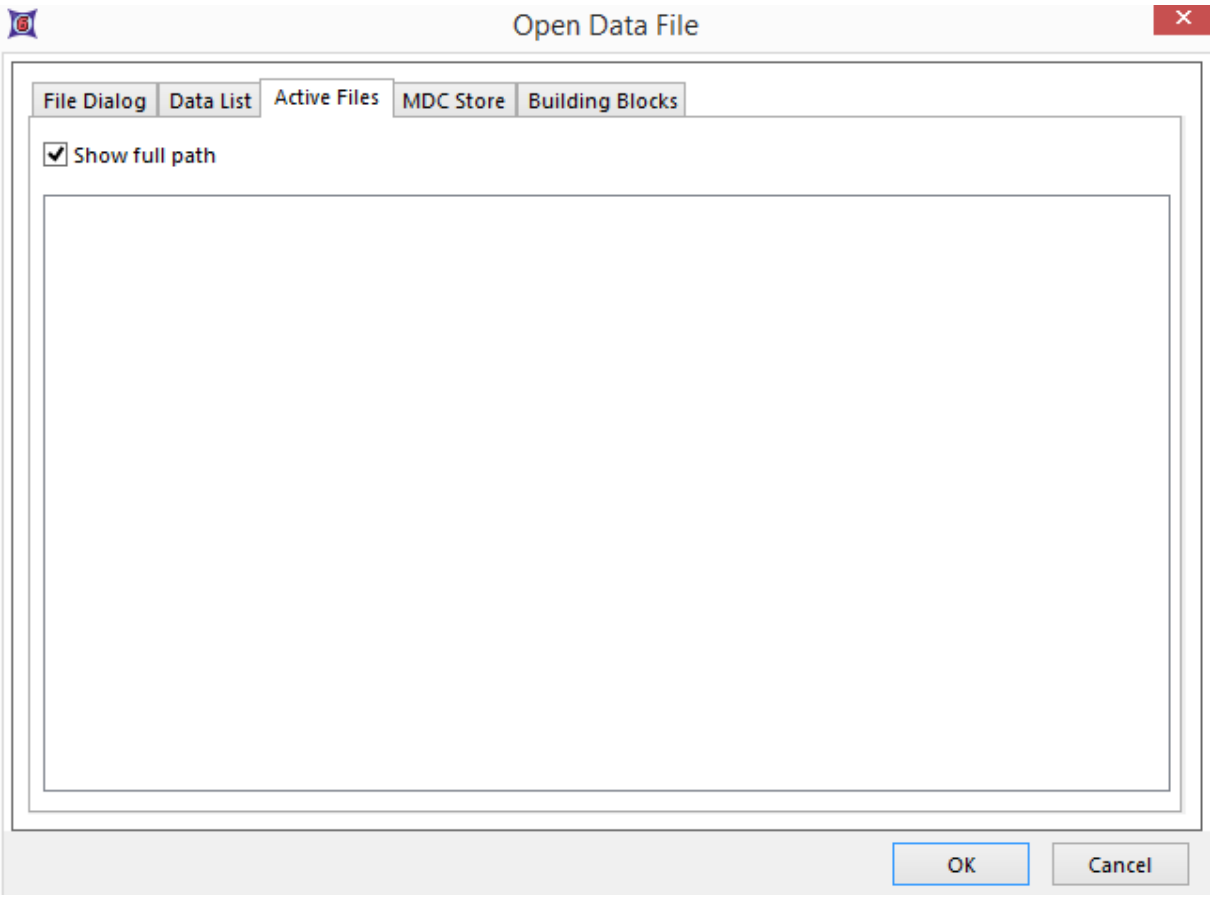

Figure 8.37 - Active Files tab

- **MDC Store** is the fourth tab from which the user may select file(s) if connected to his/her database (Figure 8.38). The **Database Type** drop down list includes **SQL Server 2000**, **SQL Server 2005+**, **Oracle**, and **SQL Server 2000+ OLEDB**. If the checkbox **Remember me on this computer** is checked, the user's credentials for the server will be remembered. The MDC Store tab is only available in FCS Express Image or Plus.

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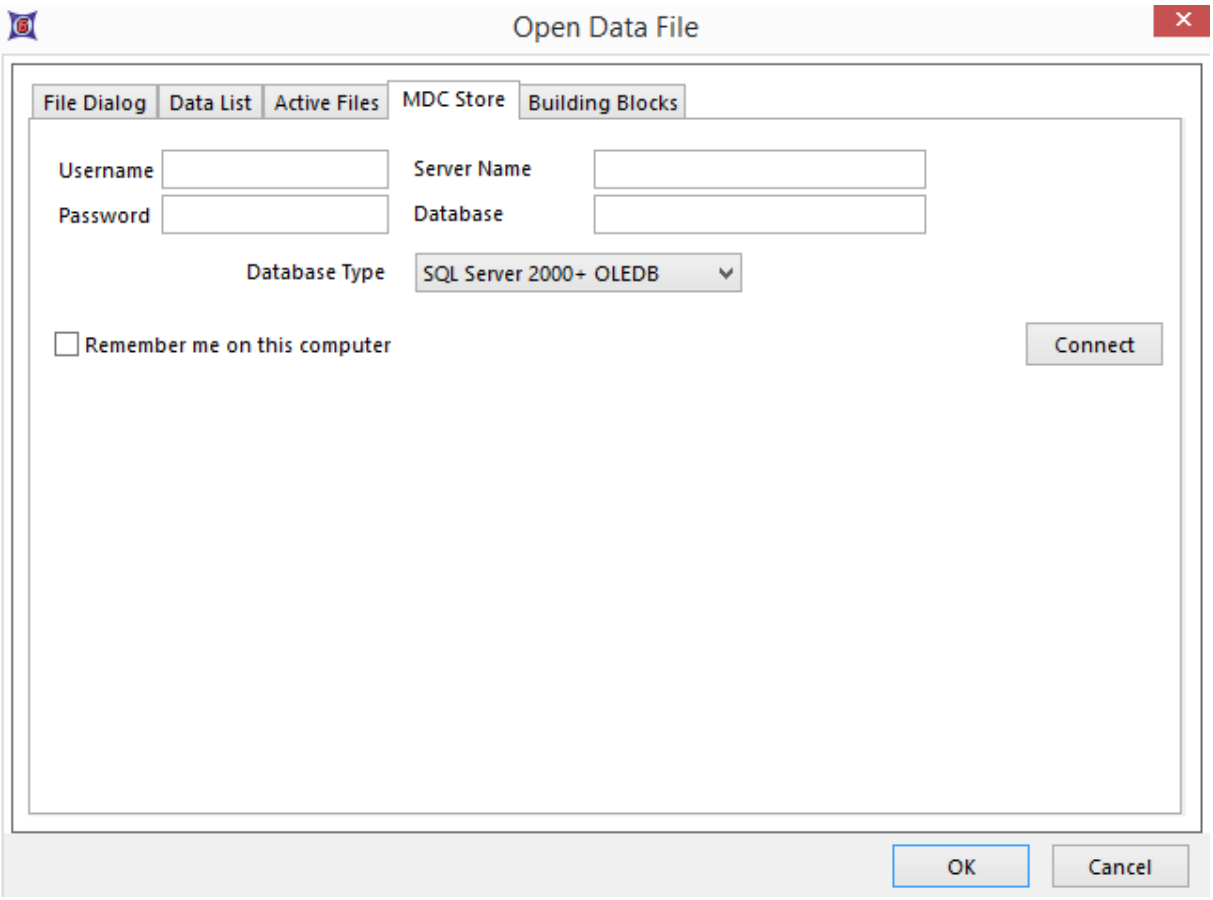

Figure 8.38 - MDC Store tab

- **Building Blocks** is the fifth tab from which the user may select file(s) to **Load cell images**, **Load Background Images**, and **Arrange fields by physical position** (Figure 8.39). Arranging fields by physical position allows you to use the position of the fields to populate regions of a picture plot. You can also define the **default cell width** and **height** as well as the **plane** and **timepoint**. The Building Blocks tab is only available in FCS Express Image or Plus.

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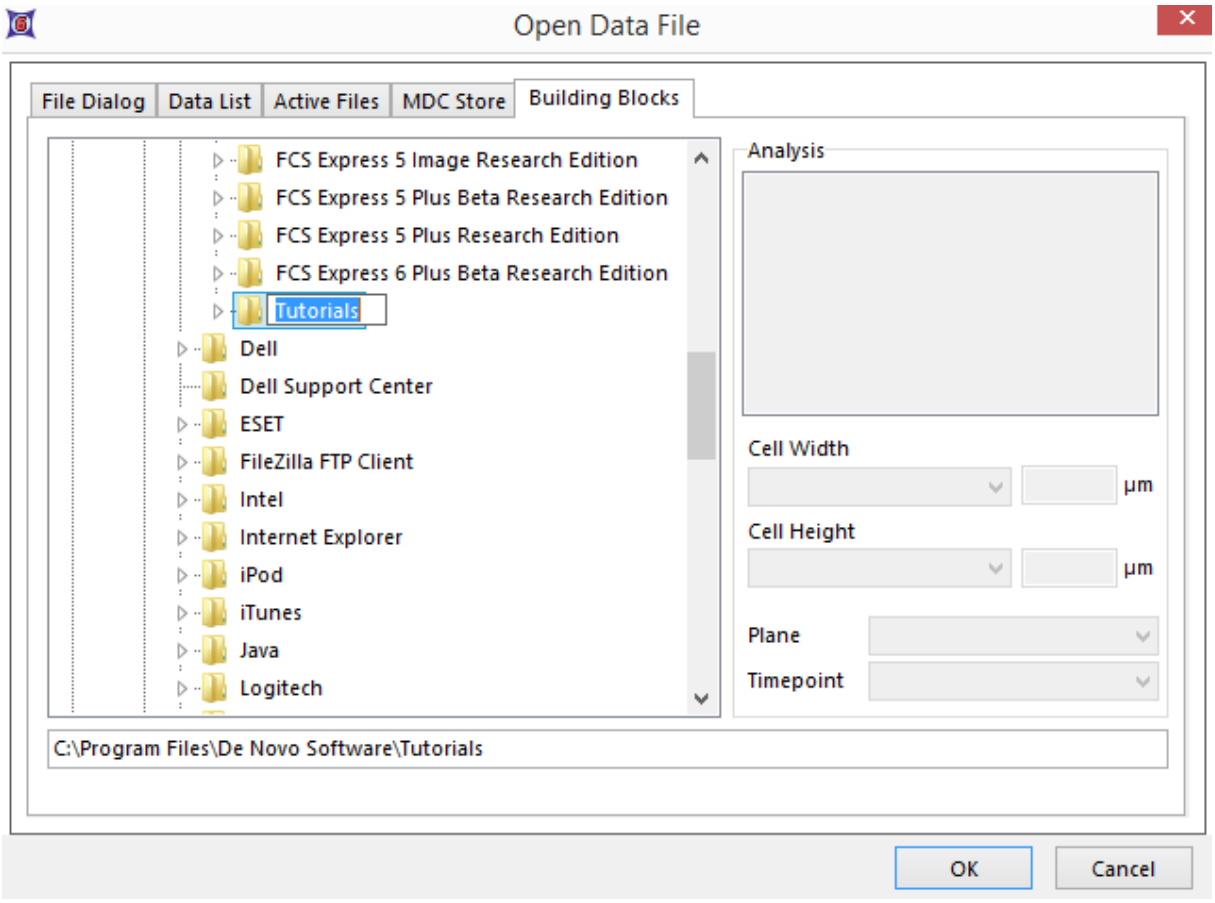

Figure 8.39 - Building Blocks tab

8.2.8.3 Do Not Show Again

Some dialog windows appear automatically in FCS Express. With Do Not Show Again options, you can uncheck those which you wish to not automatically appear as prompts. The dialog windows are listed and explained below (Figure 8.40). The options may be selected or deselected individually or simultaneously using **Select All** or **Deselect All**.

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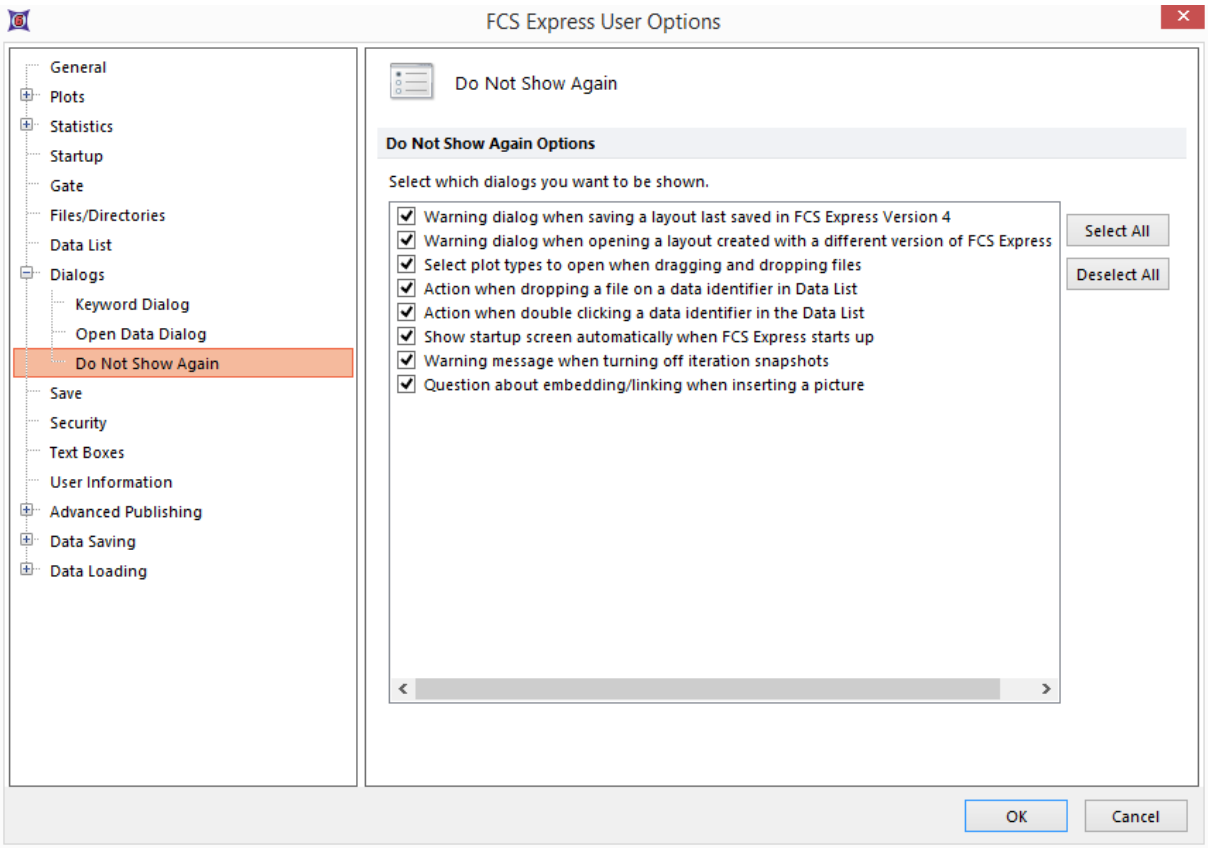

Figure 8.40 - Do Not Show Again options

- **Warning dialog when saving a layout last saved in FCS Express Version 4 or 5**.
  - Saving a legacy layout in a current version of FCS Express will prevent it from opening in the earlier version. Leave this option checked to be warned when saving such a layout.

- **Warning dialog when opening a layout created with a different version of FCS Express**.
  - Leave this option checked to be warned opening a layout created in a different version of FCS Express.

- **Selected plot types to open when dragging and dropping files**.
  - Leave this option checked to have the **Select Plot Types...** dialog appear when dragging and dropping files onto the layout.

- **Action when dropping a file on a data identifier in the Data List**.
  - Leave this option checked to have **Choose Action for Drag and Drop** dialog to appear when dragging and dropping a file from Windows explorer on top of a file in the data list.

- **Action when double clicking a data identifier in the Data List.**

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- Leave this option checked to have the **Select double click action** dialog appear when double clicking on a file in the data list.

- **Show startup screen automatically when FCS Express starts up.**
  - Leave this option checked to show the Startup Screen 54 when FCS Express starts up.

- **Warning message when turning off iteration snapshots.**
  - Leave this option checked to show warning message when turning off iteration snapshots.

- **Question about embedding/linking when inserting a picture.**
  - Leave this option checked to show question when inserting a picture.

8.2.9 Save Preferences

The **Save** category (Figure 8.41) lets the user select how layout files are saved.

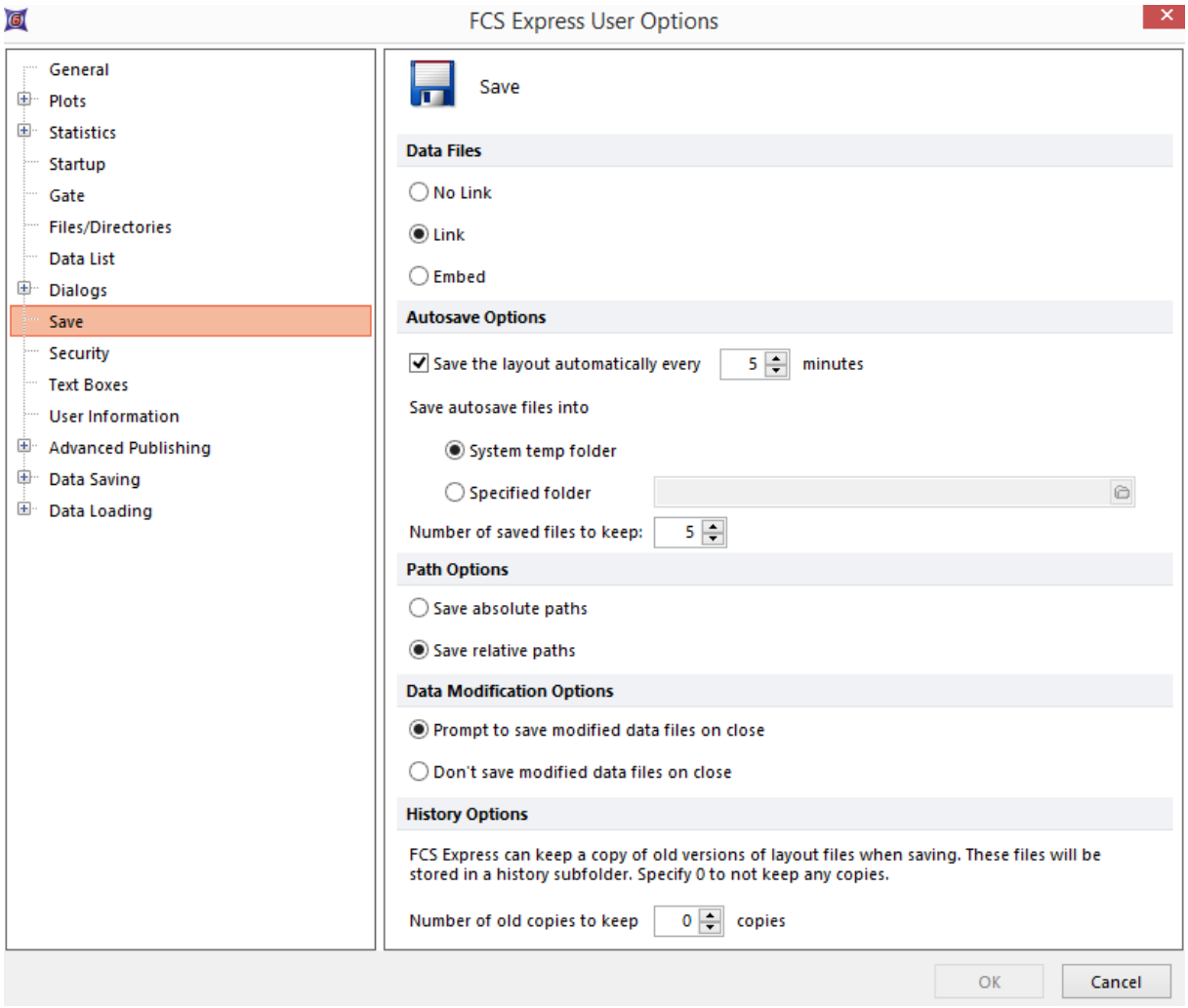

Figure 8.41 Save options

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These FCS Express User Options are described in the table below.

Option	Explanation
Data Files	Layout linking and embedding are described in Saving a Layout 93.
Autosave Options	The layout is automatically saved to the designated location at the specified interval. You can specify the number of saved files to keep. Auto recovery files are described here 110.
Path Options	When layout files are saved with the data files linked, FCS Express has to remember the specific disk location of the data files (see Saving a Layout 93). When the **Save absolute paths** radio button is chosen, the full path of the data files is stored. If the layout is moved, the data files do not need to be moved as well, since the layout will remember their original location. Choosing the **Save relative paths** radio button will store the relative path. As long as the data and the layout file are in the same relative directories on the hard drive or CD, the files will be loaded into the layout. If you check this option, when you move the layout, you should move the data such that the data is in the same relative location to the layout as it was before. *Example:* Assume that your layout and data files were in C:\data. If you saved the layout with **Save Absolute Paths** selected, then the layout will remember to look in C:\data for the data files. If you saved the layout with **Save Relative Paths** selected, the layout will only remember to look for the data files in the same folder as the layout is in, since that is the current relative location of the data files to the layout (remember, they are both in C:\data). If you move the layout to another location (C:\data1), and leave the data files in C:\data, the layout saved with **Save Absolute Paths** will still be able to find the data files, since that layout remembers that the data files were in C:\data. The layout saved with **Save Relative Paths** will not find the data in this case, because it will be looking in C:\data1, since all it remembers is to look for the data in the same folder as the layout is in. **Save Relative Paths** is useful if you are going to be moving the data onto another computer, or external media such as flash drive or CD, because as long as you move the data and the layout together, the layout will be able to find the data when it is opened.

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Option	Explanation
Prompt to save/Don't save modified data files on close	Data file header information can be changed and new parameters can be created 933. These actions create a modified .fcs data file different from the original. Choosing the **Prompt to save modified data files on close** radio button will prompt you with the choice to save the modified files when the layout is closed. If you choose **Don't save modified data files on close**, the modified files will not be saved when the layout is closed and the modifications will be discarded.
History Options	FCS Express can keep a copy of the old versions of FCS Express layout files as a backup when saving. These files will be stored in a "History" subfolder within your original folder. To activate this option, increase the default value of **Number of old copies to keep** from "0".

8.2.10 Security Preferences

The **Security** FCS Express User Options allow the user to set a password to prohibit others from modifying the Options 179 of the current user (Figure 8.42).

Note: If you forget this password, there will be no way to retrieve the password-protected user options.

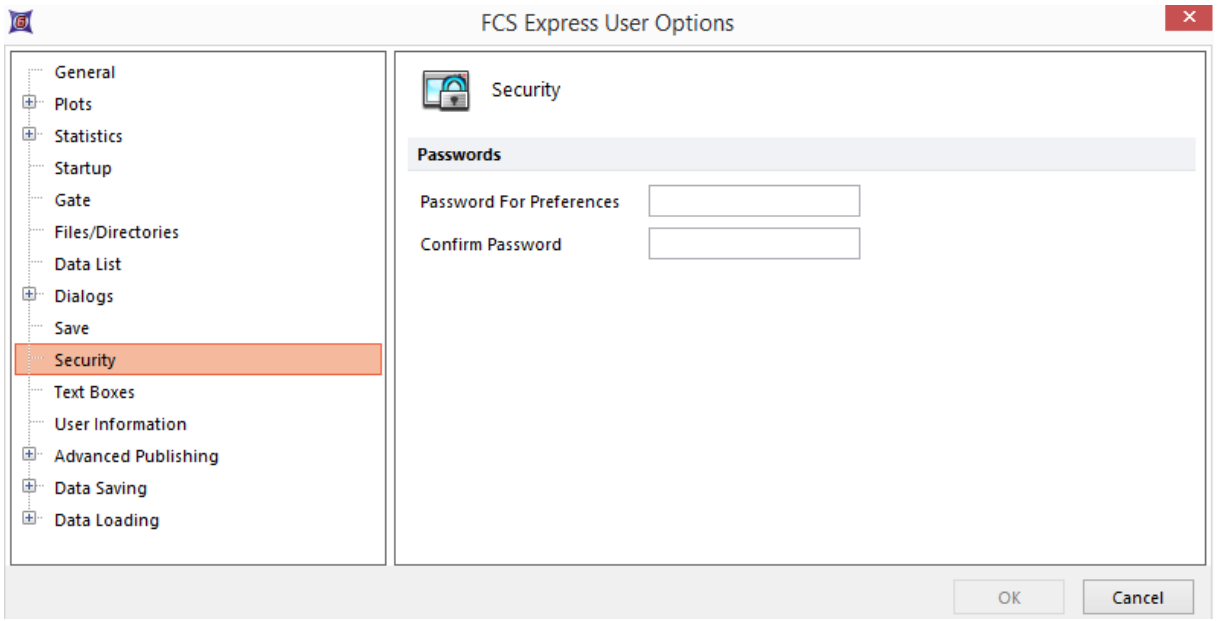

Figure 8.42 Security options

8.2.11 Text Box Preferences

The **Text Box** FCS Express User Options category allows the user to configure the default appearance for text boxes (Figure 8.43).

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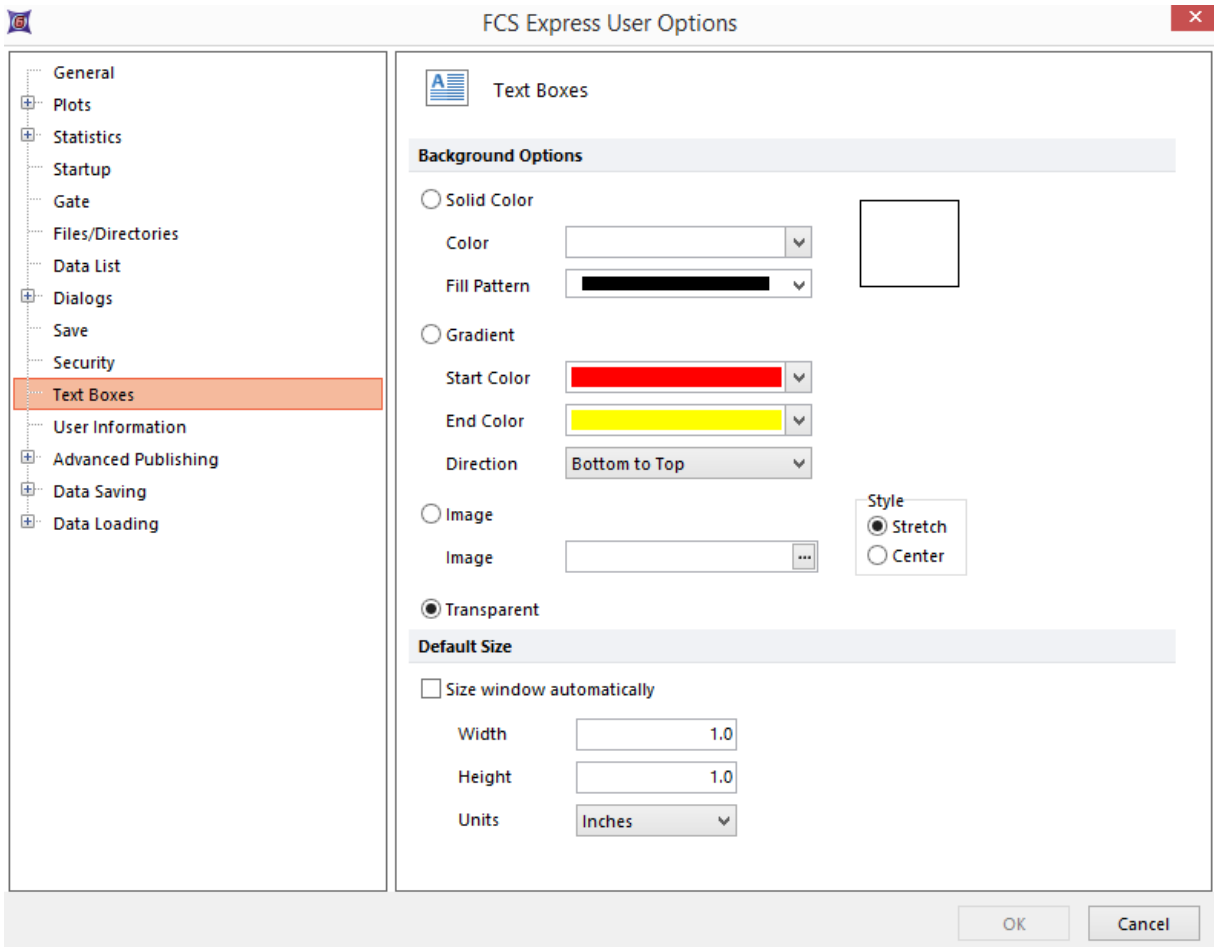

Figure 8.43 Text Boxes options

The default background is **Transparent**. You can choose to fill the background with a color and/or fill pattern, color gradient, or an image. You can also set the default size.

8.2.12 User Information

Under user information (Figure 8.44), you can set an e-mail address, author name, and organization for the user.

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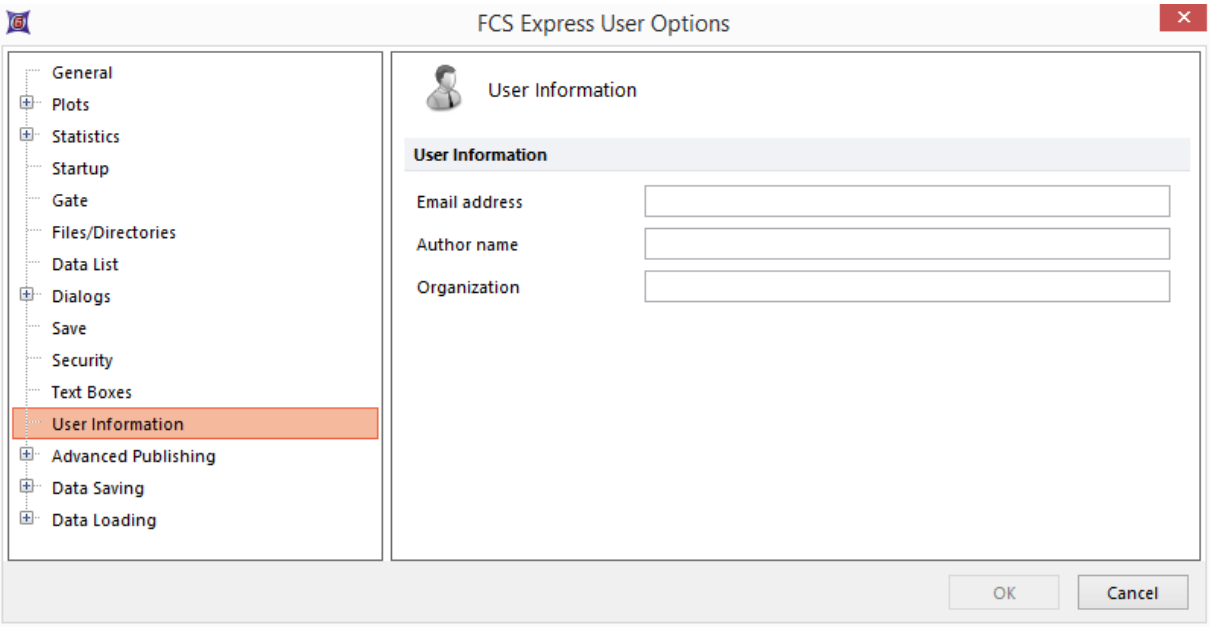

Figure 8.44 User Information options

8.2.13 Advanced Publishing Preferences

There are two subcategories for Advanced Publishing 607 options: **Default Permissions** and **Publishing Options**.

The **Default Permissions** page allows you to limit what others who view your published layout will be able to do in terms of modifying and saving your layout. Checking the box allows the function. The permission to perform almost every feature available in FCS Express can be customized (Figure 8.45).

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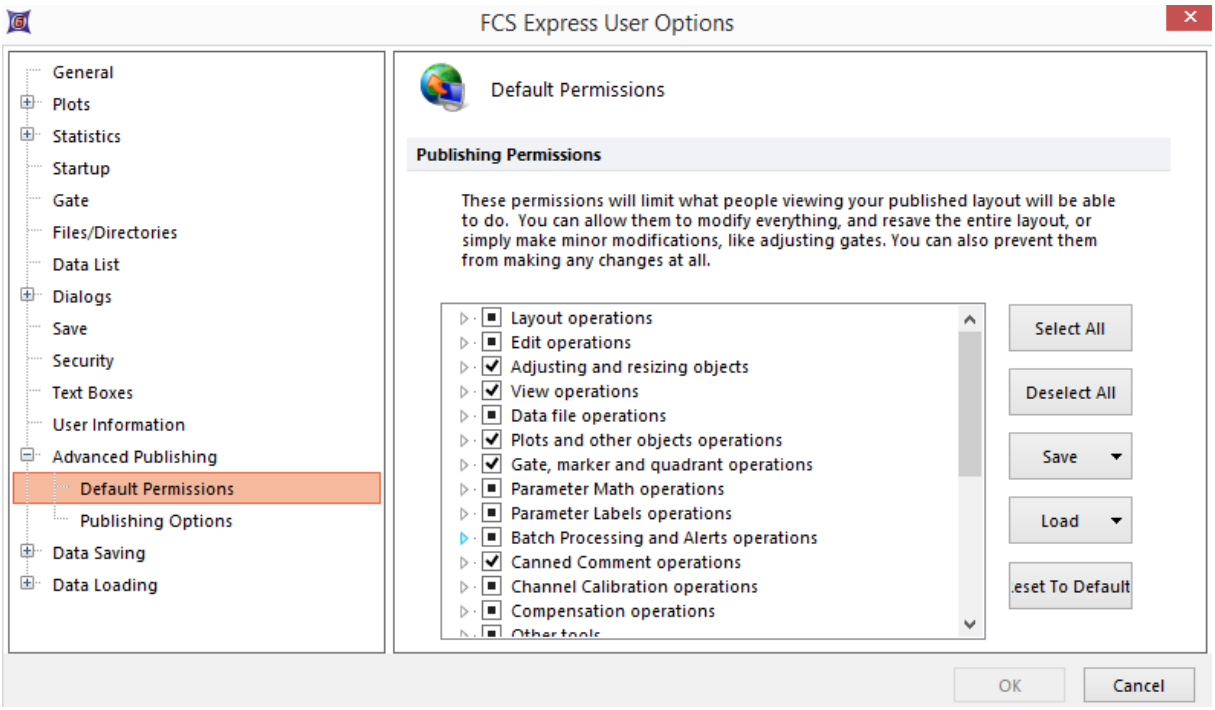

Figure 8.45 Advanced Publishing Default Permissions

The **Publishing Options** page allows you to control whether or not batch processing actions are available in your published layout. In addition, you can decide whether or not to hide objects that are outside of layout pages, and enable or disable token editing in the published layout (Figure 8.46).

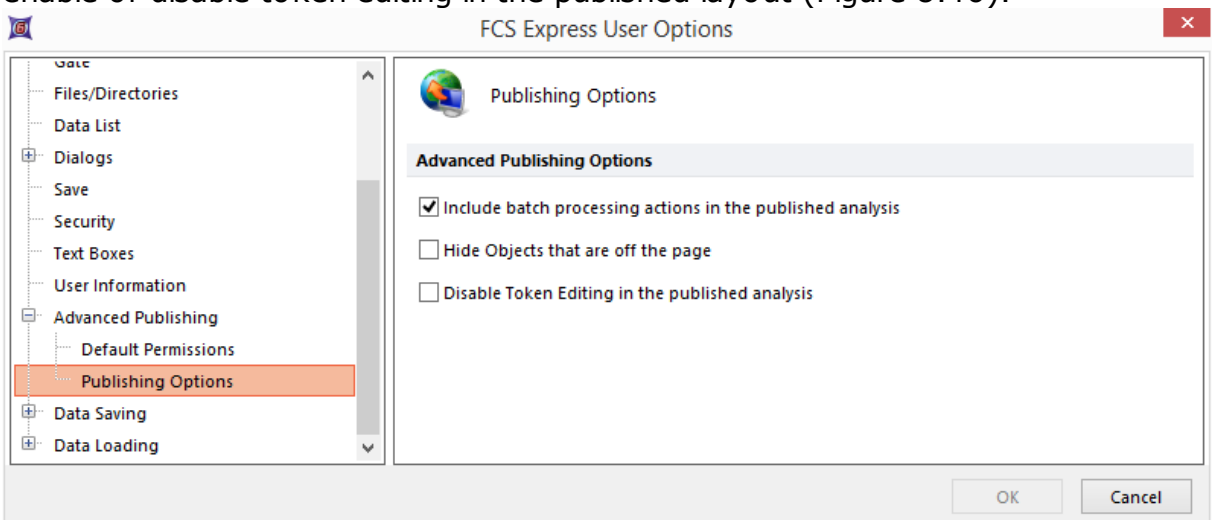

Figure 8.46 Advanced Publishing Options

8.2.14 Data Saving

There are two subcategories of **Data Saving** FCS Express User Options: **Save as Text Format** and **FCS Format**.

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When saving data as a text file, the **Save as Text Format** default options allow you to choose a delimiter ('comma' is the default) and decide whether to **Save Headers** (Figure 8.47).

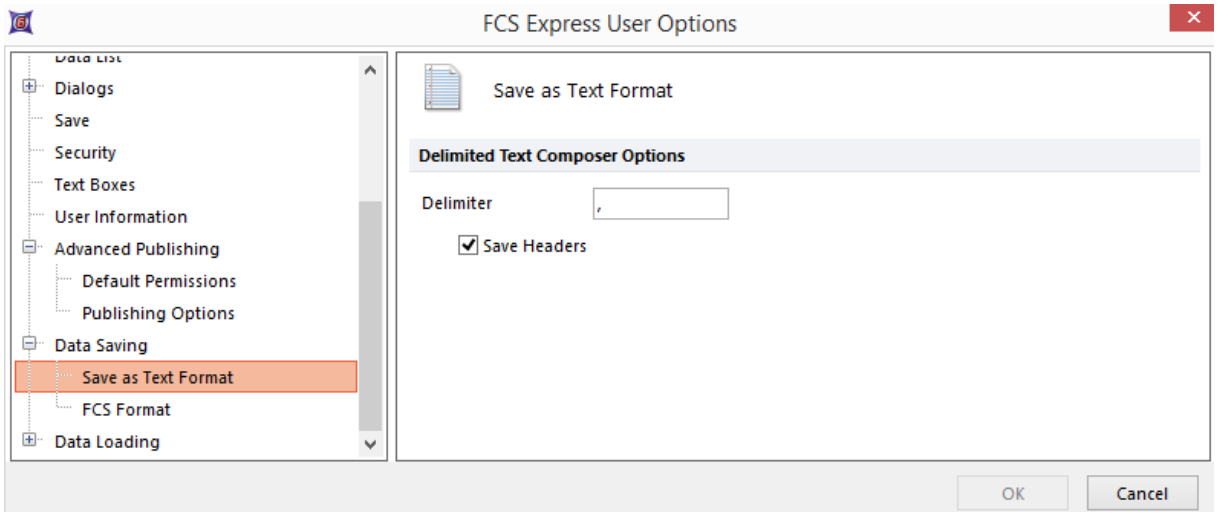

Figure 8.47 Data Saving - Save as Text Format options

You can also set default options for **Data Format**, **Endian Format**, and **FCS Version** when saving files in **FCS Format** (Figure 8.48).

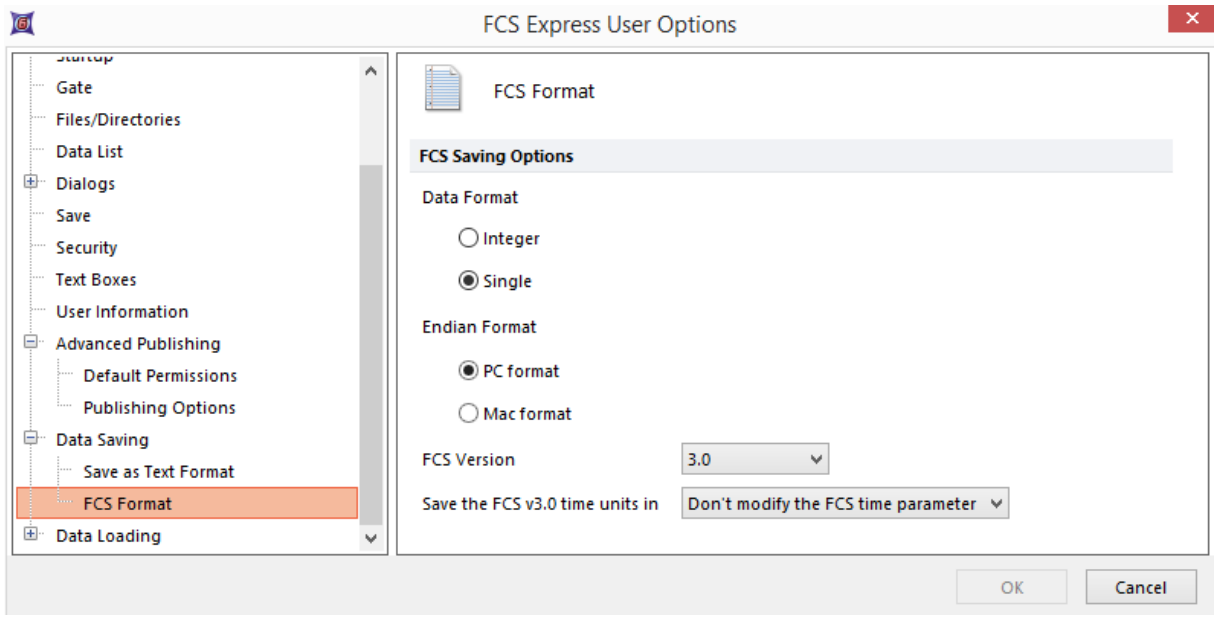

Figure 8.48 Data Saving - FCS Format options

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8.2.15 Data Loading

The **Data Loading** category contains **FCS File Options** ,as well as properties for different instrument data sets including **ICE Format, ImageXPress Velos, CellProfiler, Perkin Elmer Acapella, Building Blocks, Life Technologies Tali, Nexcelom, ImageJ, IDEAS, MDC Store Options, Amnis DAF Reader, and Perkin Elmer Vectra**

Note: Different choices may appear in the data loading options category depending on whether you are using FCS Express Flow, FCS Express Image, or FCS Express Plus.

8.2.15.1 FCS File Options

This option only appears if you are using FCS Express Flow or FCS Express Plus.

The FCS File Options category (Figure 8.49 and 8.50) allows you to select how FCS Files will appear in data selection dialogs (e.g., when changing files 173, adding files to the Data List 788, or inserting new plots 172. The FCS Express User Options are described in the table below.

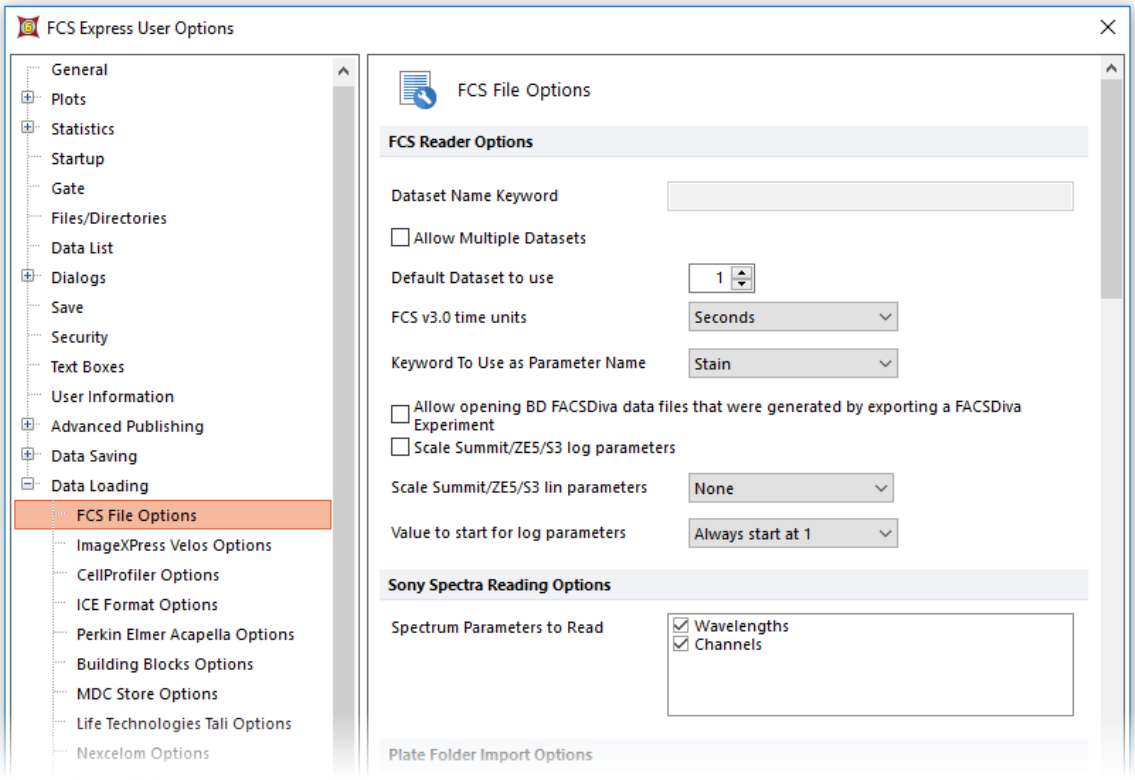

Figure 8.49 FCS File Options dialog (Upper panels)

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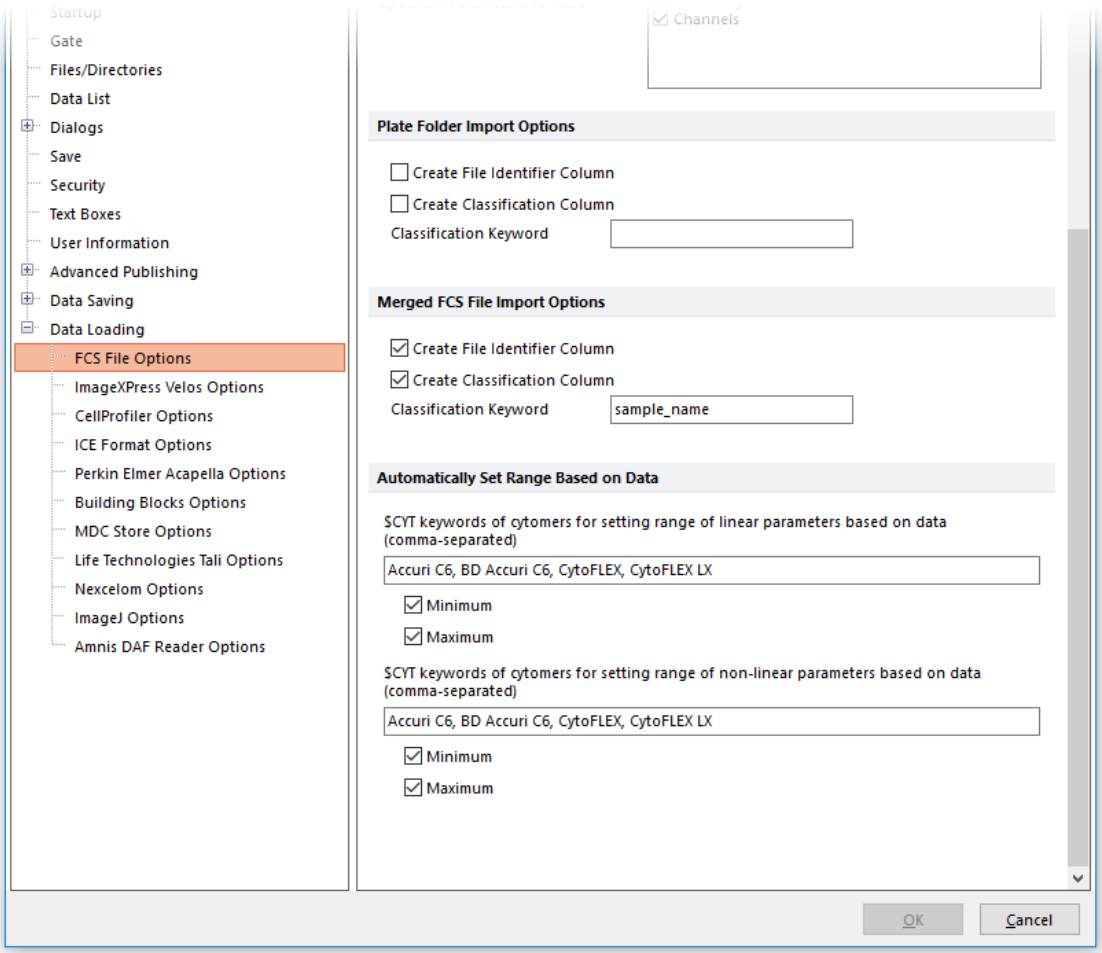

Figure 8.50 FCS File Options dialog (Lower panels)

Option	Explanation
Dataset Name Keyword	Select the keyword to use as the default dataset name when selecting from multiple datasets. Allow Multiple Datasets must be checked.
Allow Multiple Datasets	Some FCS files contain multiple data sets. If this option is checked, you can select one of the embedded datasets. The user option to use **Advanced Open Data Dialog** 216 also must be enabled to be prompted to select which dataset upon loading the data files.
Default Dataset to use	This option specifies which dataset should be loaded by default when **Allow Multiple Datasets** is not checked. In that case, when you select a file in the dialog that has multiple datasets, will load the dataset number corresponding to the value selected instead of displaying a list of

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	datasets. If the dataset does not exist, FCS Express will load the first dataset in the file.
FCS v3.0 $TIMESTEP units	Choose a unit to use when displaying a Time parameter in FCS Express from the drop-down list. The **Default** time option leaves the time parameter at the same raw value as is stored in the FCS file. Choosing a unit from the drop-down list will use the $TIMESTEP keyword to convert the raw value to the specified unit from the list.
Keyword To Use as Parameter Name	Choose a keyword (or combination of keywords) to use as parameter names from the drop-down list.
Allow opening BD FACSDiva data files that were generated by exporting a FACSDiva Experiment	Check this box to allow opening data files that were exported from BD FACSDiva together with the experiment. See http://www.denovosoftware.com/site/kb-invalidfilefromfacsdiva.shtml for a detailed explanation. Re-exporting the files is a better option if you have access to FACSDiva and the experiment data is still available.
Scale Summit/ZE5/S3 log parameters	Check this box to scale log parameters of Summit/ZE5/S3 data in the same manner that they are scaled in Summit/ZE5/S3 software. The scale is applied to newly loaded files.
Scale Summit/ZE5/S3 lin parameters	Chose a value between **None**, **64**, **128**, **256**, **512** and **1024**. Linear parameters of Summit/ZE5/S3 data will be scaled accordingly. The scale is applied to newly loaded files.
Value to start for log parameters	Choose the starting value for parameters displayed in the log scale from the drop-down list.
Sony Spectra Reading Options	This options allows to define which **Spectrum Parameters to Read** when a file from Sony Spectra is loaded.
Plate Folder Import Options	These options allow to automatically create the **File Identifier** (i.e. numeric) and/or the **Classification File Identifier** (i.e. etxt with file names) columns when FCS files are loaded as **FCS Plate Folder** (see the Working with Heat Map 1050 chapter of the manual for more details on the **FCS Plate Folder** format) or **Merge FCS File** (see the Adding Merged Files to the Data List 662 and the Working with Heat Map 1050 chapter of the manual for more details on the **Merge FCS File** format). An additional **Classification** column can also be added using any of the keywords available in the loaded files. If a keyword is specified and multiple files share the same value for that keyword, events from those files will be grouped together in the same well.
Merge FCS File Import Options	

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Automatically Set Range Based on Data	Some flow cytometers save data with a very large dynamic range (stored in the $PnR keywords), but a small actual range, which results in sparse plots. These cytometers include the BD Accuri C6 and Beckman Coulter Cytoflex. These options allow you to automatically adjust plot axes based on the minimum and maximum of the data range, rather than the $PnR keyword. To use them, enter the value of the $CYT keyword of each instrument whose data files you want to affect, separated by commas. Note: the value of the $CYT keyword can be retrieved from the FCS File Header 650. For data from these instruments, the value chosen by the Automatic option in both the Specific Options 305 and Axis Options 186 will be based on the data.

8.2.15.2 Image XPress Velos Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading Image Xpress Velos data, you can choose whether to **Load cell images**, **Load as Plate,** and **Load Background Images.** (Figure 8.51).

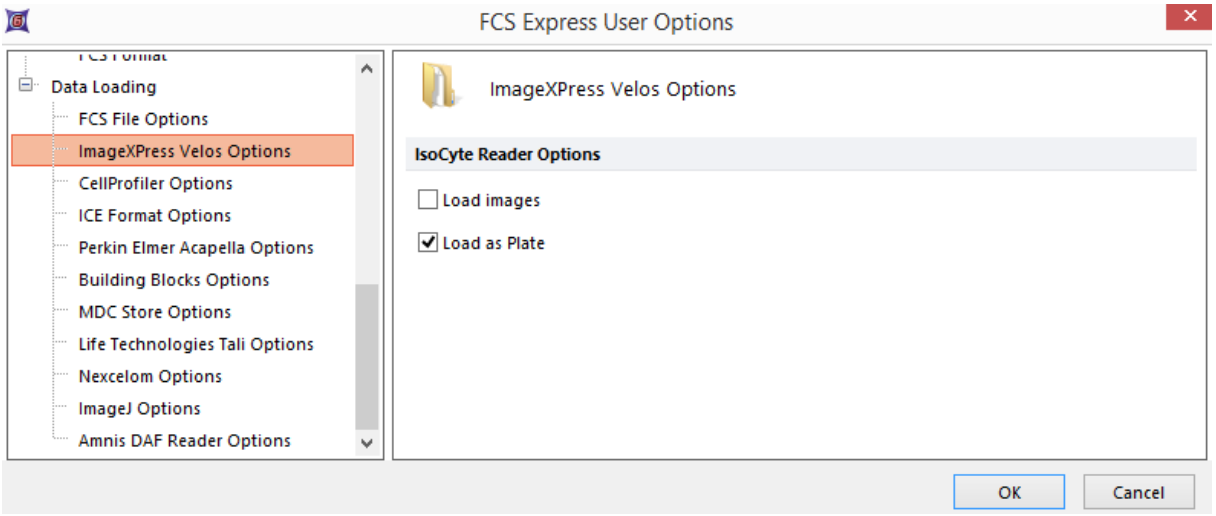

Figure 8.51 IsoCyte Data Loading Options

8.2.15.3 CellProfiler Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading files from CellProfiler, you can choose the format (**File delimiter**), either character separated values (.csv) or tab-delimited files. You can also

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choose whether to **Load Images, Load path names from Cell Profiler table of contents file,** and/or **Load Background Images** (Figure 8.52).

If **Load path names from CellProfiler table of contents file** is checked, then FCS Express will try to load the images from the folder specified in the CellProfiler table of contents file. If it is unchecked, then FCS Express will try to load the images from the same folder as the CellProfiler file.

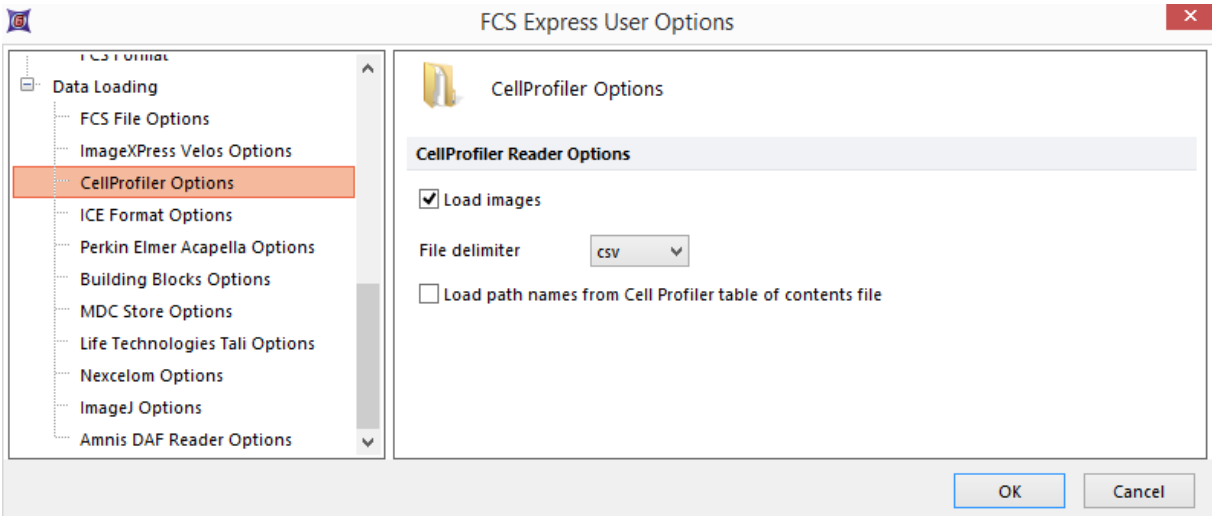

Figure 8.52 Cell Profiler Data Loading Options

8.2.15.4 ICE Format Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading .ICE files you can choose whether to **Load cell images** and **Load Background Images** (Figure 8.53).

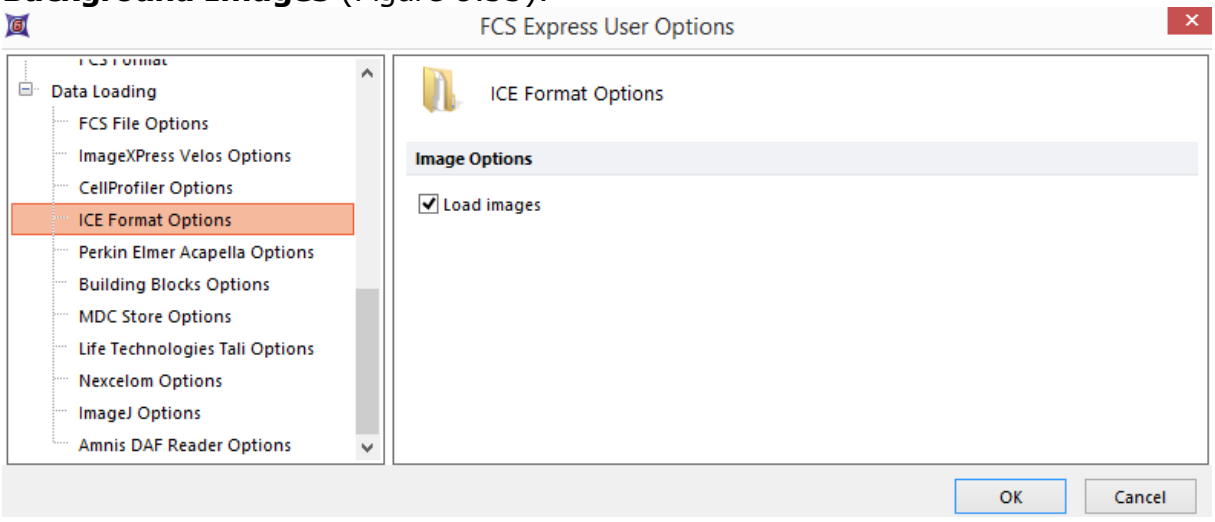

Figure 8.53 ICE Format options

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8.2.15.5 PerkinElmer Acapella Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading Perkin Elmer Acapella Reader files you can choose whether to **Load cell images, Load Background Images,** and **Arrange fields by physical position** (Figure 8.54)**.** Arranging fields by physical position allows you to use the position of the fields to populate regions of a picture plot.

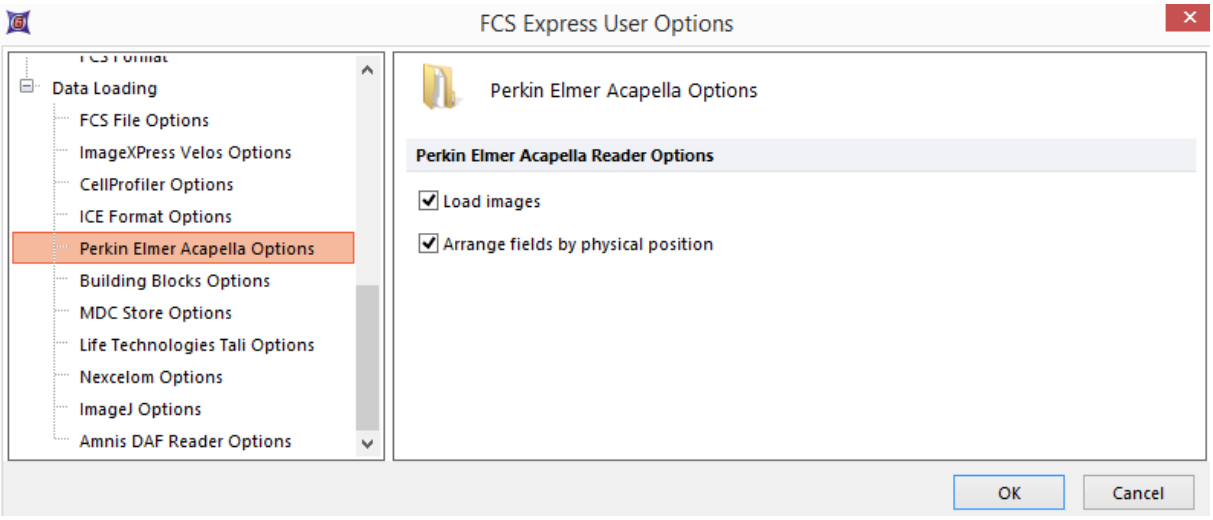

Figure 8.54 Perkin Elmer options

8.2.15.6 Building Blocks Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading Building Blocks files you can choose whether to **Load cell images, Load Background Images,** and **Arrange fields by physical position** (Figure 8.55)**.** Arranging fields by physical position allows you to use the position of the fields to populate regions of a picture plot. You can also define the **default cell width** and **height.**

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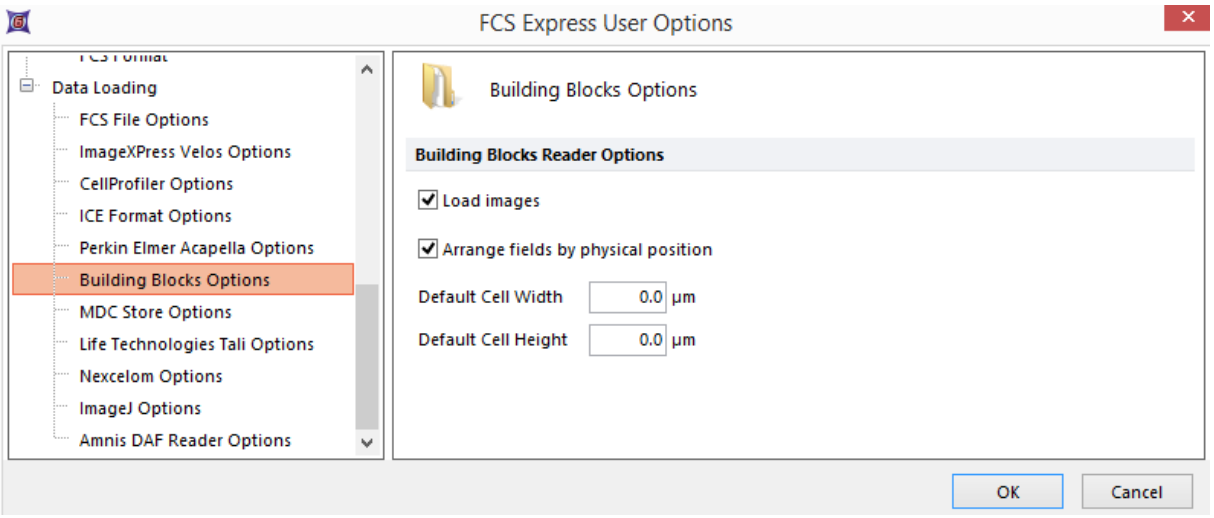

Figure 8.55 Building Blocks options

8.2.15.7 MDC Store Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading data from MDC Store you can choose whether to **Load cell images** and **Load Background Images** (Figure 8.56), and whether to **load as plate**.

Security credentials (**username** and **password**), **ODBC DataSource**, and **Database** name and **type** must be specified in under **MDC Store Options**. The security credentials can be saved with the layout by checking the appropriate box.

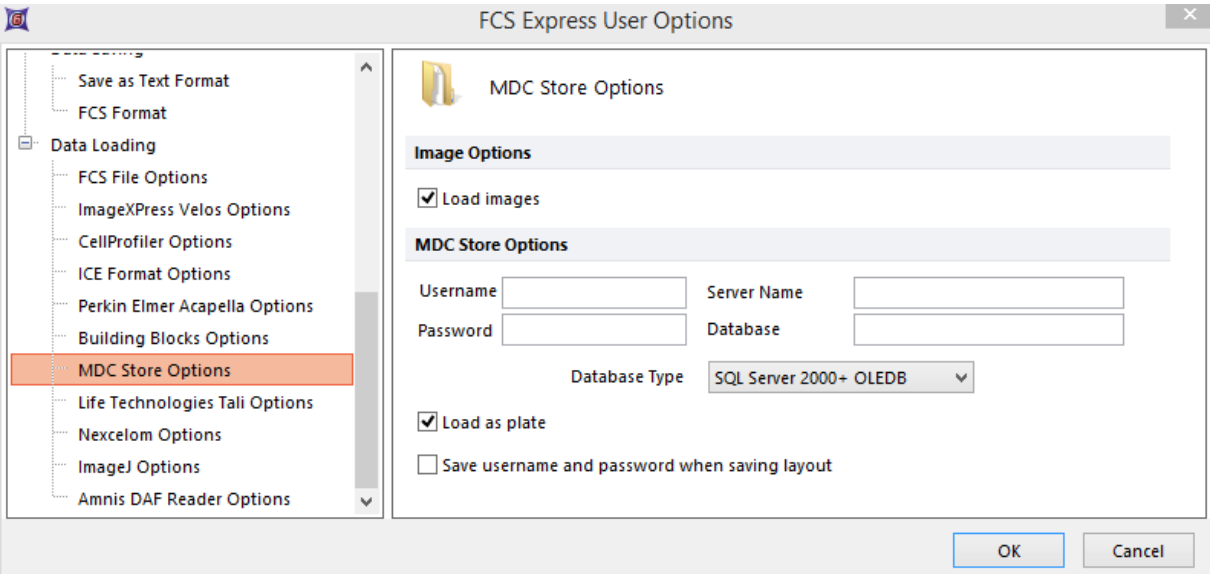

Figure 8.56 MDC Store options

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Please visit the De Novo Software website at http://www.denovosoftware.com/site/kb-loadingdatafromMDCStore.shtml for more details on configuring the MDC Store for use with FCS Express.

8.2.15.8 Life Technologies Tali Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading Life Technologies Tali data files, you can choose whether to **Load cell images** and **Load Background Images** (Figure 8.57). The default **length of the sides of the square around each cell** can also be changed.

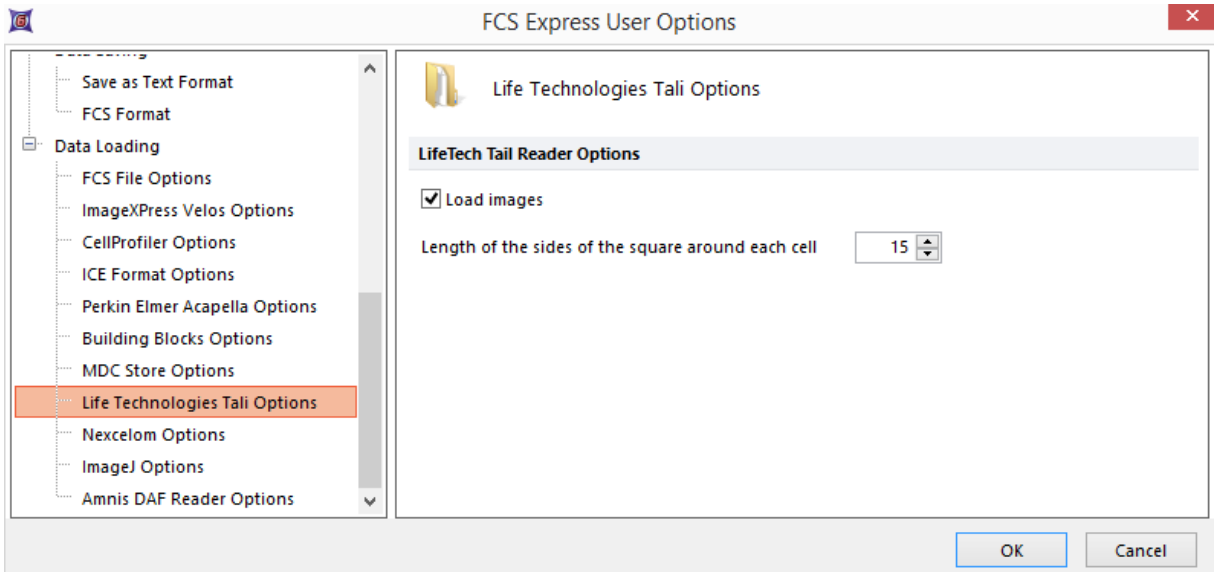

Figure 8.57 LifeTechnologies Tali options

8.2.15.9 Nexcelom Options

This option only appears in FCS Express Flow RUO, FCS Express Image Cytometry and FCS Express Plus.

When loading .nxdat files, you can choose whether the to **import** the original **X** and **Y pixels** (Figure 8.58).

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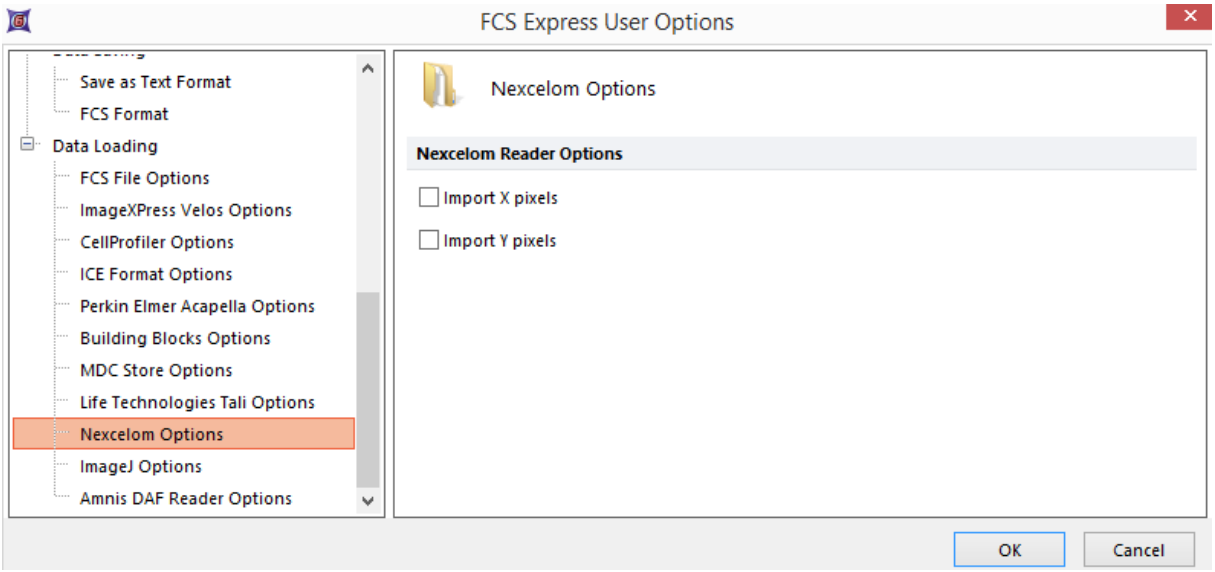

Figure 8.58 Nexcelom options

8.2.15.10 ImageJ Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading .ijout files you can choose whether to **Load cell images, Load Background Images,** and **Load path names from the ImageJ table of contents file** (Figure 8.59).

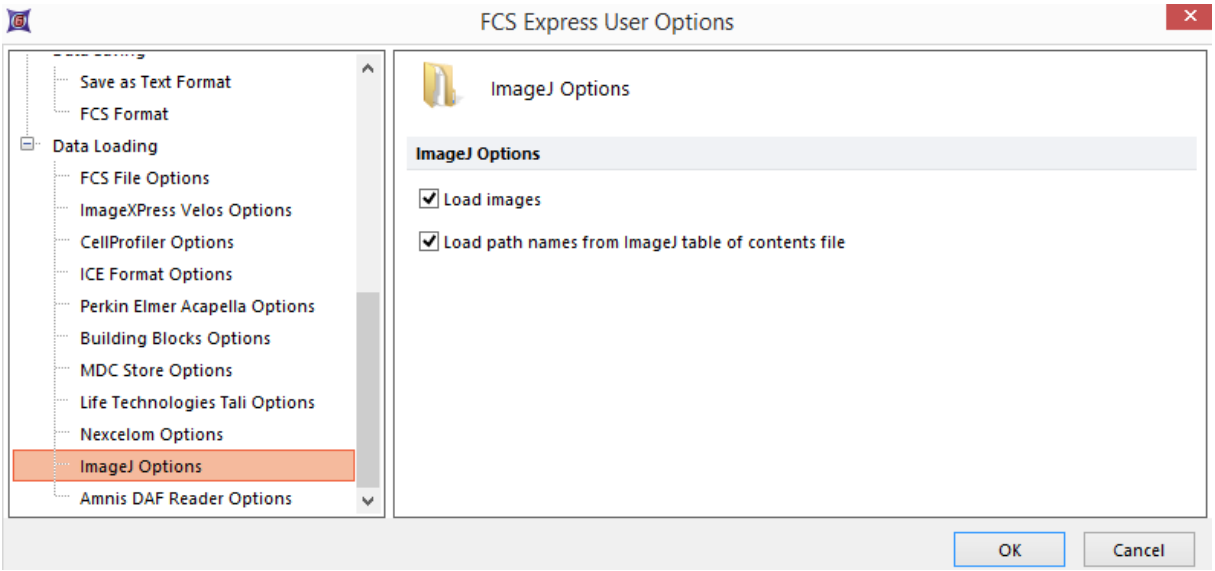

Figure 8.59 ImageJ options

8.2.15.11 Amnis DAF Reader Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

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When loading Amnis *.daf files you have the option to load data with or without images (Figure 8.60). Unchecking the **Load images** box will load *.daf files without the associated images from the relevant *.cif file. Resulting plots will only display number data and data grids will not have columns present for single cell images.

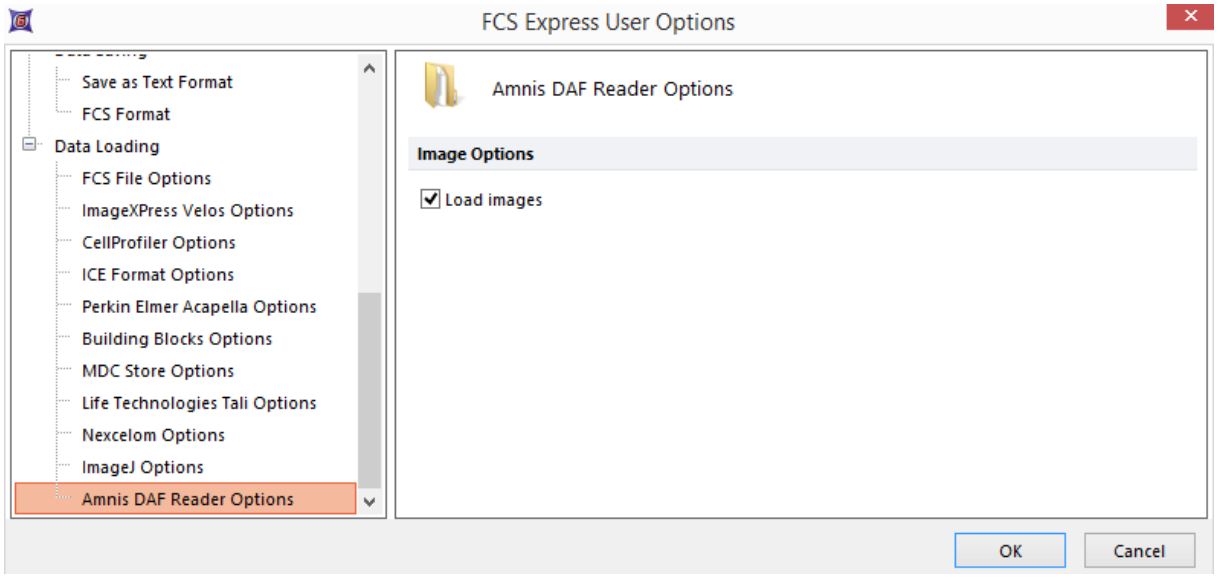

Figure 8.60 IDEAS options

8.2.15.12 Perkin Elmer Vectra Options

This option only appears in FCS Express Image Cytometry and FCS Express Plus.

When loading Vectra files (*.tif, *.txt) you have the option to **Load images**, **Use Default Image Resolution** when loading composite TIFF images, and select which segmentation image/map from the **Segmentation Cell Type** options. (Figure 8.61).

The **Load images** box will be checked by default, and unchecking this box will load Vectra files without any associated images. When unchecked, Picture Plots will not be allowed to be inserted and data grids will not have columns present for single cells images. However, all numeric data may be plotted in any other plot type available.

When the **Use Default Image Resolution** box is checked, the user can set the **Default Image Resolution** for composite TIFF images loaded into FCS Express. Check this box only if you are loading composite TIFF images (*_composite_image.tif), as these images are exported from the Vectra without the image resolution data necessary for tiling the different images into a single

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field of view. For component TIFF images (*_component_data.tif), you may leave this box unchecked.

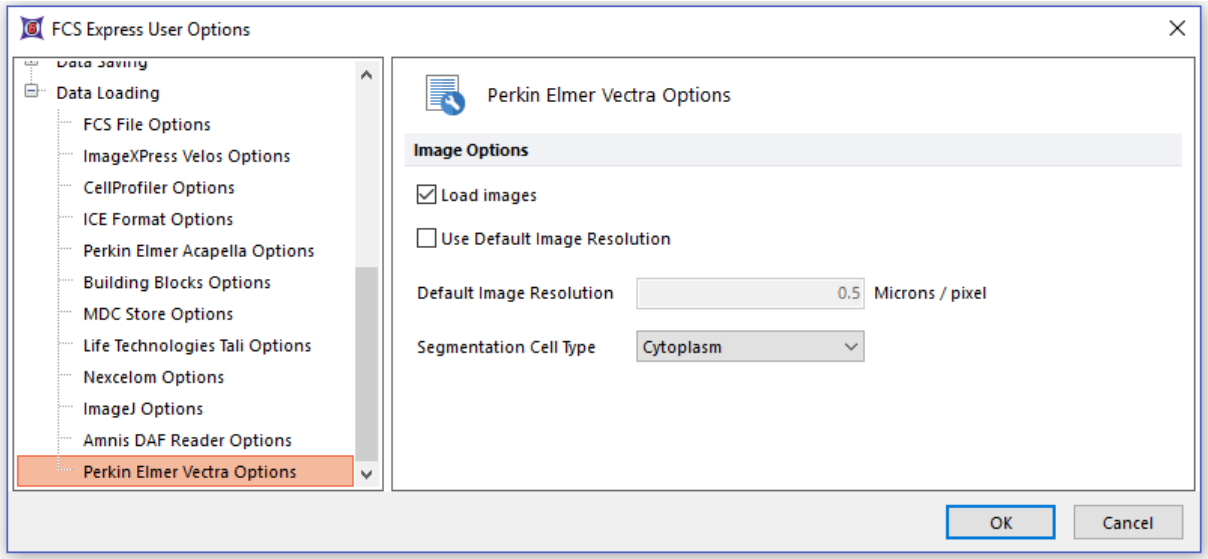

Figure 8.61 Vectra Format options

Please see https://www.denovosoftware.com/site/PerkinElmerApplicationsandImport.shtml for instructions on exporting data from the Vectra / InForm software for use in FCS Express.

8.3 Formatting Options Shared by All Objects

This section discusses the background, border, and size options available to nearly all objects in a layout. Objects can be text boxes, plots, histograms, statistics, pictures, and more. These features can be customized for individual objects, but the customization process is common to all objects.

The options in this section can be set in one of two ways:

- Selecting the object and then using the appropriate commands from the **Format→Object Options** group in the ribbon.
- Right-click on the object, choose **Format** from the pop-up menu, and select the **General, Fill**, **Border**, or **Size** category. With this method, a **Preview** button is available to view changes before actually applying them, as well as a **Restore** button to easily restore the object to its previous state. You can also restore any object to its previous state by using the Undo button in the Quick Access Toolbar 65.

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8.3.1 General

You can edit **General** properties in one of two ways:

- Select the object and use the **Format→Object Options→General** command.
- Right-click on the object, select **Format** from the pop-up menu, and choose the **General** category.

For most objects, the only general formatting options are:
- object's name
- object visibility

For all objects, the name property is the label displayed as tool tip while hovering the mouse cursor over the object. For plots, the name property is the name of the plot to display in lists where the user is required to select a plot. The name is also used when displaying objects in different Navigators 123. The name field includes an option to insert a token as the name by clicking on the T button to the right of the edit field.
Please note that Plots' name can be also changed from the Gate Navigator 128 window.

Object visibility can be set to:

- **Always visible** (default) - The object is always visible in the layout.
- **Never visible -** The object is always hidden unless the **View → Show→ Hidden Items** 82 button of the ribbon bar is selected.
- **Custom -** The option allows you define visibility of the object based on a token or value that evaluates to a conditional expression 747. Any tokens or values may be used to set a conditional custom value and may be inserted 692 via right click in the field, **Insert Token** or by clicking on the **T** token icon. The object will only be visible if the token evaluates to True.

Data Grids have some extra general options (Figure 8.62).

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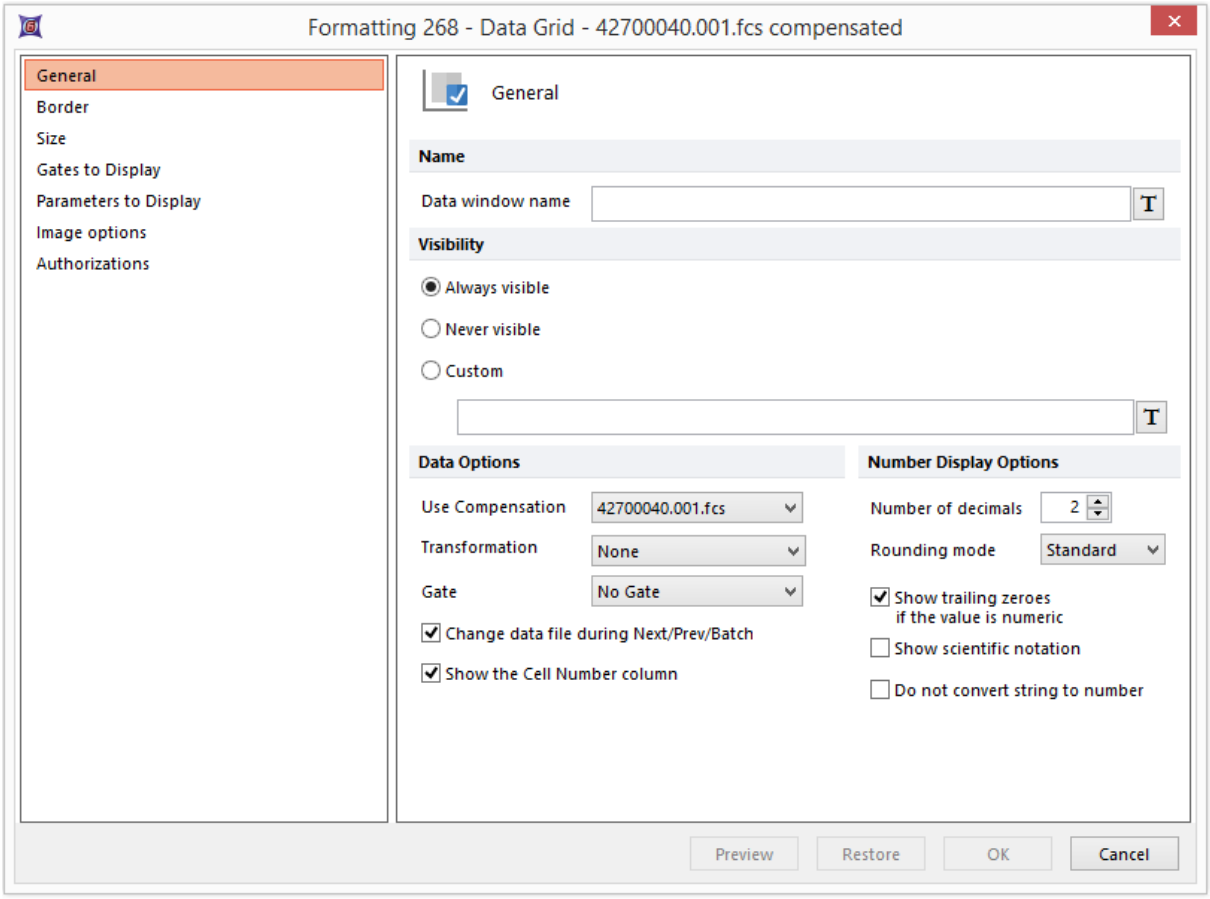

Figure 8.62 Formatting General Dialog for Data Grids

Besides the usual name property (referred to as **Data window name**), you can also choose a compensation 626 definition, a transformation 932, a gate to display data for, how to display the numbers in the grid, and whether or not to change the data in the grid during batch processing 784 or in response to a Next/Previous 79 command.

Lastly, the **Show the Cell Number column** checkbox controls whether the cell or object number (assigned by the cytometer according to the time of sample acquisition) is shown as a column in the Data grid.

8.3.2 Background

The Background options (Figure 8.63) allow you to change the background appearance of an object. To customize the background, click on the object or border. When an object is in edit or selected mode, the background is the area within the red or green outline.

You can format the background in one of two ways:

- Select the object(s) and use the **Format→Object Options→Fill** command.

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- Right-click on the object, select **Format** from the pop-up menu, and choose the **Background** category.

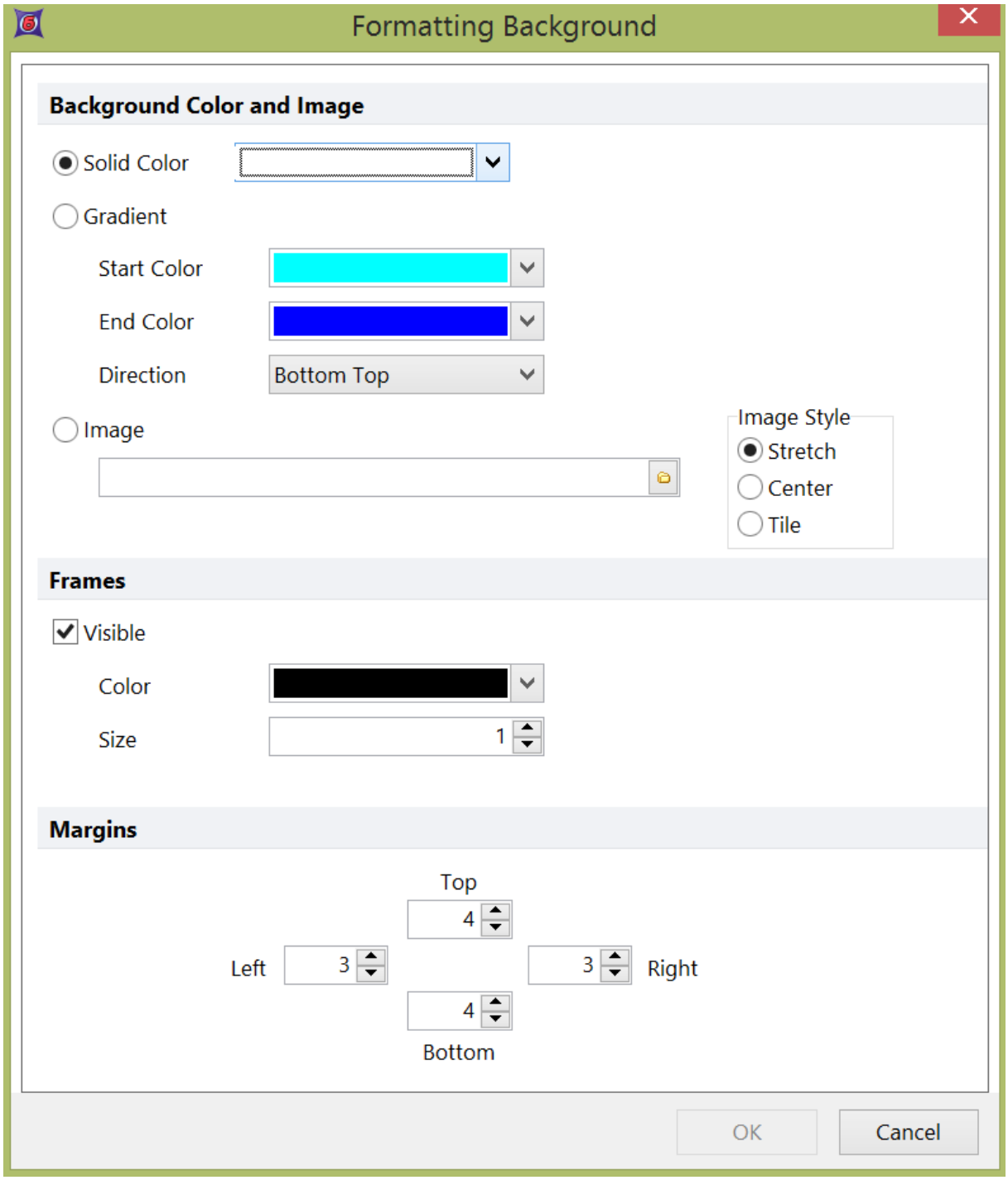

Figure 8.63 Formatting Background dialog for a plot

Once the dialog opens, select a background color (solid or a gradient of colors). Alternatively, an image can be centered, stretched, or tiled in the background.

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With some objects (e.g., text boxes, gate views, or shapes) the dialog title is **Formatting Fill/Background**. With these items, you can also choose a pattern as a fill or make the background transparent. Transparency allows any object which is behind the item to show through. Plots do not support the **Transparent** option.

With the **Margins** options (seen when formatting plots), you can set the space around the margin of the plot. This is most useful when the text on the right-most x-axis label of the plot is being cut off. To correct this, increase the right margin percentage to increase the space between the right margin and the end of the x-axis.

The **Frames** option (for plots) controls whether or not frame lines are visible on top and to the right of the plot. You can also set the color and thickness of the frame lines.

8.3.3 Border

The **Border/Line** options (Figure 8.64) allow you to adjust the border around an object. By default, objects have no border.

When the object is in edit or selected mode, any border will appear inside and adjacent to the red or green outline. You can customize the border/line options in one of two ways:

- Use the **Format→Object Options→Border/Line** command on object(s) in edit or selected mode.
- Right-click on the object, select **Format** from the pop-up menu, and choose the **Border** (or **Line**) category.

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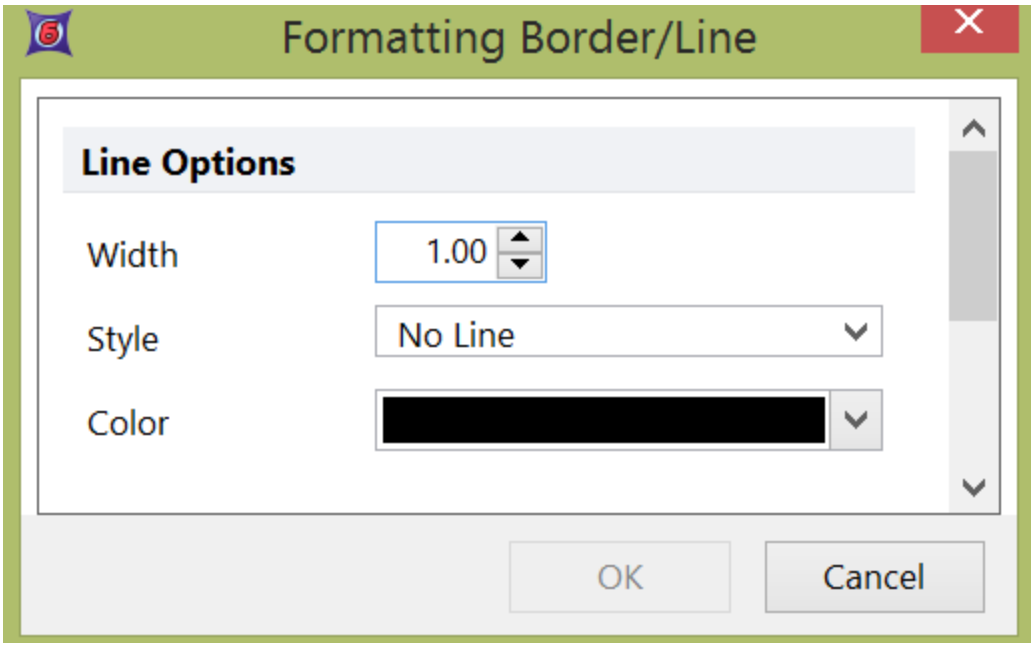

Figure 8.64 Formatting Border/Line dialog

8.3.4 Size

The **Size** category (Figure 8.65) is for adjusting the current size of an object. All sizes are specified in inches.

To change the size of an object, use the **Format→Object Options→Size** command on an object in edit or selected mode, or right-click on the object, select **Format** from the pop-up menu, and choose the **Size** category. You can change the absolute size, or scale the object by a certain percentage. By enabling the **Lock Aspect Ratio** checkbox, you ensure that the width and the height change in the same ratio (thereby keeping the object in the same proportion).

The original size is only meaningful for inserted pictures. For other objects, the original size will always be the same as the current size.

Note: When the object to be resized is a Text Box containing a token, table, or text, you will not be able to make the text box smaller than the minimum size to display the text or the minimum dimensions of the token or table (see Resizing Text Boxes 164).

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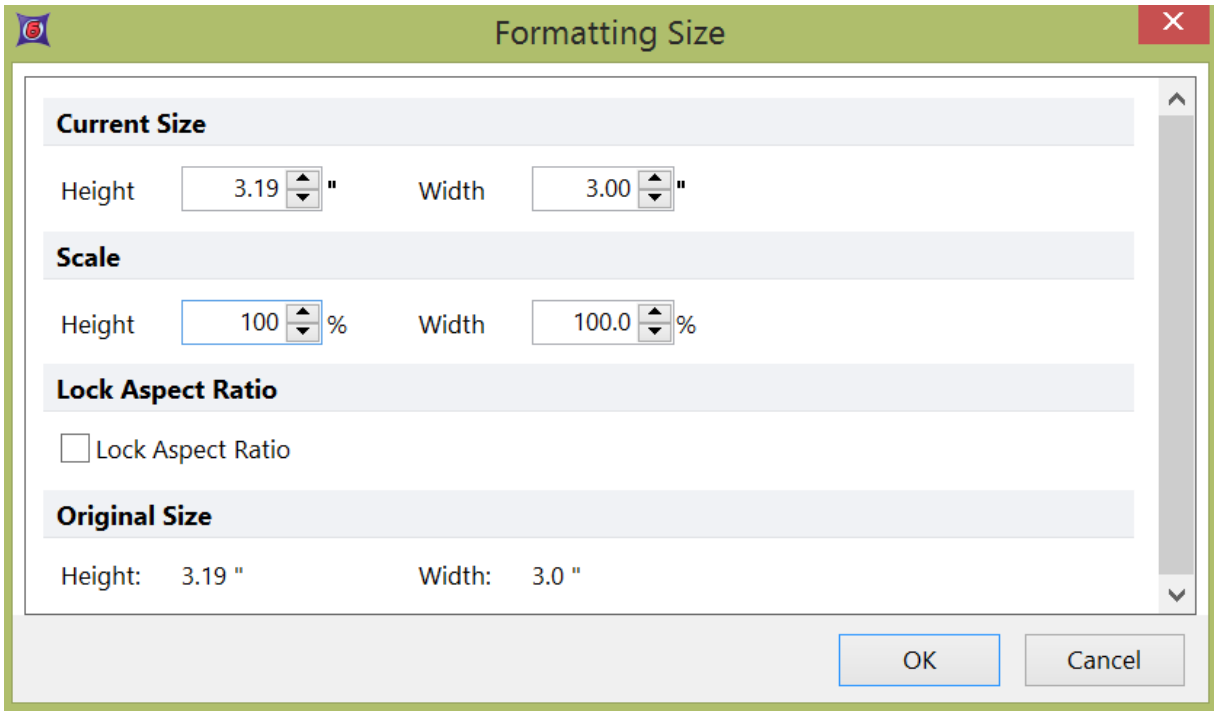

Figure 8.65 Formatting Size dialog

8.3.5 Authorizations

The **Authorizations** category (Figure 8.66) may be adjusted for any object in FCS Express. Authorizations allow user to control when objects are selected or edited based on the chosen settings. Please see Formatting Authorizations 1136 for a detailed explanation of these features.

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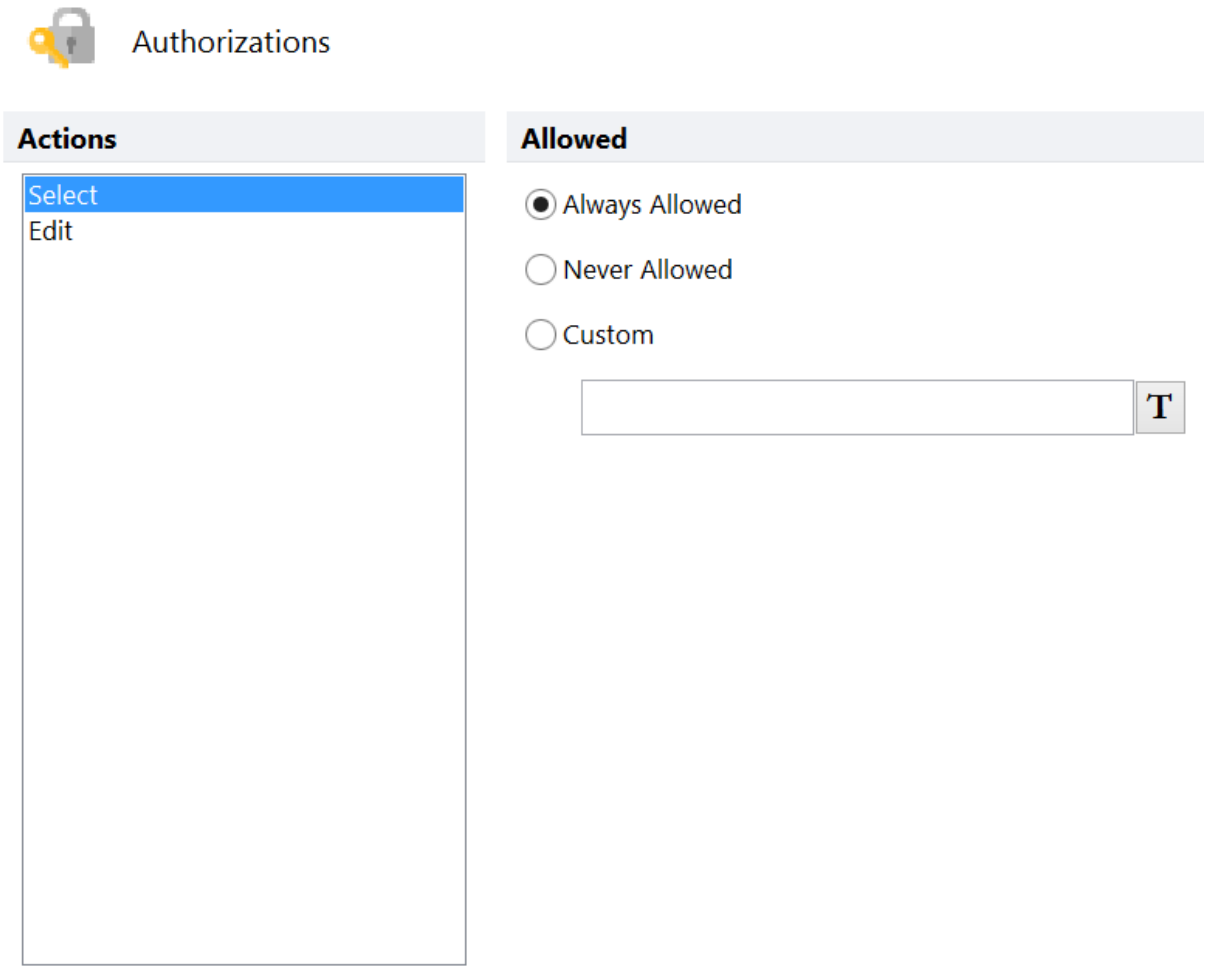

Figure 8.66 - The Formatting Authorizations dialog.

8.4 Formatting Features Shared by All Plots

In addition to the formatting options common to all objects[239], all plots in FCS Express share certain basic features, e.g., title, axes, legend, and 3D properties (note that the latter is available only for Histograms and Surface plots) . These options allow the user to completely customize the look of individual plots. If you open plots with similar properties often, you may want to change the default plot options[184].

You can always format a plot by right-clicking on the plot (or double-clicking on a plot that is already selected), selecting **Format** from the pop-up menu, and choosing a category to format.

After formatting a plot:

- Press **OK** to apply the changes.

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- Press **Cancel** to discard the changes, close the dialog, and return to the original plot layout.

- Press the **Preview** button to see what the changes would look like without actually applying the changes. After **Preview**, press **OK** to apply the changes, or simply continue editing.

- Press **Restore** to discard the changes but continue editing the plot.

You can also format many features by selecting a plot and using the commands on the Format tab of the Ribbon 73. **Preview** and **restore** options are not available using this method, however.

8.4.1 Specific Options

The following default Options can be specified in the Specific Options category for indicated FCS Express User Options plot types:

- Resolution (explained below 247 for Histograms, Kinetics, Dot, Color Dot, Density, Contour, and Surface Plots)
- Normalization 275, Control Histogram for Subtraction Statistics 276, and Stacked vs. Overlaid display 276 (Histograms)
- Cells to Plot 307 (Kinetics, Dot, Color Dot, Density, Contour, and Surface Plots)
- Whether Channel Calibration 614 is applied to axes (Histograms, Kinetics, Dot, Color Dot, Density, Contour, and Surface Plots)
- Dither Color Dot Plots 307 (Dot, Color Dot, and Scatter Plots)
- Shade Based on Density (Dot and Color Dot Plots) 308
- Orientation 1050 (Heat Maps)

The resolution options are located in the **Formatting** dialog in the Histogram→Histogram Specific Options 274 category for histograms 267, and the Dot Options→Binned Dot (or Plot) Specific Options 305 category for 2D plots 284. The properties you set are slightly different for the two kinds of plots, but the basic functionality is similar in both.

An important concept to understand is "What is the resolution of a plot?"

The resolution is the number of *bins* (channels) that parameter data is subdivided into. Before displaying a plot, FCS Express takes the data and compresses it into the number of bins selected by the user. This process is known as *binning*. The values in these bins are displayed in the plots. When the resolution is lower, the same data gets compressed into fewer bins, meaning that the values in the bins will be higher. That is why the peaks in histograms, contour, and density plots increase when the resolution is

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lowered. The way FCS Express puts the data into the bins is determined by the scale 395.

Note: Statistics are always calculated on the full range of data, regardless of how the resolution is set on a particular plot.

In FCS Express, you can set the value of the resolution, and control the range of data (minimum and maximum) that is displayed:

- **Value of the resolution**
  - For histograms (Figure 8.67), you can choose the range specified in the data you are using, or set the resolution to a specific number chosen from the drop-down list.

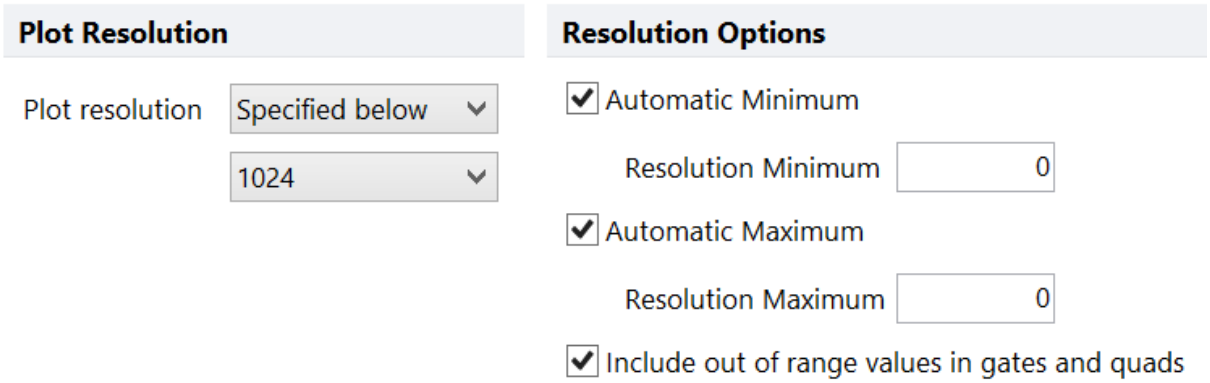

Figure 8.67 Histogram Resolution Options

  - For 2D plots (Figure 8.68), choose from the drop-down list (32 x 32 to 1024 x 1024).

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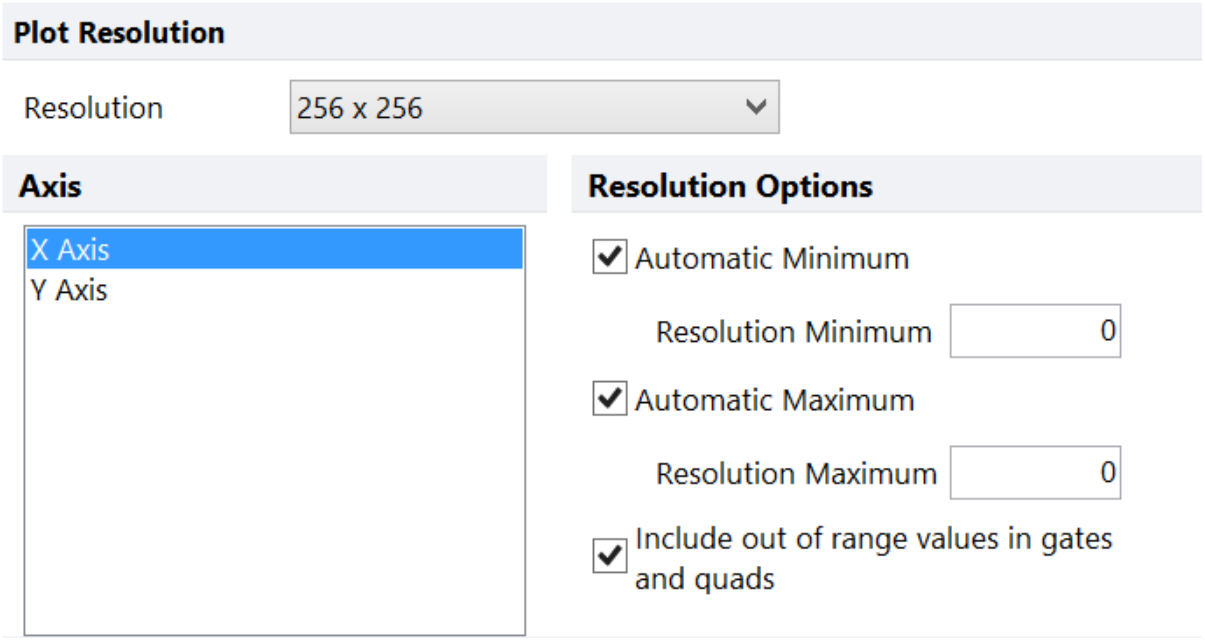

Figure 8.68 2D Plot Resolution Options

- **Range of Data (Minimum and Maximum)**

  FCS Express gives you the option of dividing the full range of data into the resolution (number of bins) you specify, or to "cut out" either the top end or the low end of the data. In most cases, it is preferable to leave the resolution maximums and minimums at the default value, i.e., *automatic*. There are two reasons you might want to set the minimum or maximum. The first is that in certain cases, some instruments may store extreme values in the data. In general, this will just be noise, not significant data. If an extreme data point is included when the plot is calculated, information may be lost in a significant part of the range. In such a case, you can exclude the extreme data point by setting a minimum or maximum value to use for significant data points. The second reason you might want to set the minimum and/or maximum is to provide detailed zooming into a small range of values. You can also decide whether or not to include out of range values in gates and quads.

- **Include Out of Range values in gates and quads**

  If you have not chosen to use an automatic resolution minimum or maximum, there could be data that is outside the visible portion of the plot. You can choose whether or not to include this data in gates or quads drawn on this plot by selecting **include out of range values in gates and quads**. If this option is checked, and the gate touches an extreme edge of the plot, values outside the range of that edge will be included inside the

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gate. Values outside the edge of the plot will also be included in the appropriate quad.

Technical note: Axis Resolution and Statistics

In FCS Express 6, all the statistics are calculated on the raw data, irrespective of the display scaling. However, when Gates and Quadrants are created, decisions must be made in cases when an event falls on the line of the gate or quadrant.

In FCS Express 6, Gates and Quadrants are calculated and drawn in a binned space with binning dependent on both the Scaling 254 and the Resolution Minimum/Maximum 247 of the plot. In turn, the inclusion or exclusion of an event on the border line of a gate or in a quadrant is highly dependent on the scaling of the plot, and on the Resolution Minimum/Maximum, of the plot the Gate/Quadrants was drawn on.

When adjustments to the scaling or resolution of a plot are made, an event may then fall to a different side of the border line of the gate/quadrant, and the user may notice very slight changes in the statistics of gated events which are usually quite minimal and make no impact on the interpretation of the results.

Please refer to the Axis Scale and Statistics 256 chapter for more details.

8.4.2 Title

Title formatting options control the text and appearance of the title (header and/or footer) on each plot.

You can edit the title options in one of two ways:

- Select the plot(s) and use the **Format→Plot Options→Title** command.
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Titles** category (Figure 8.69).

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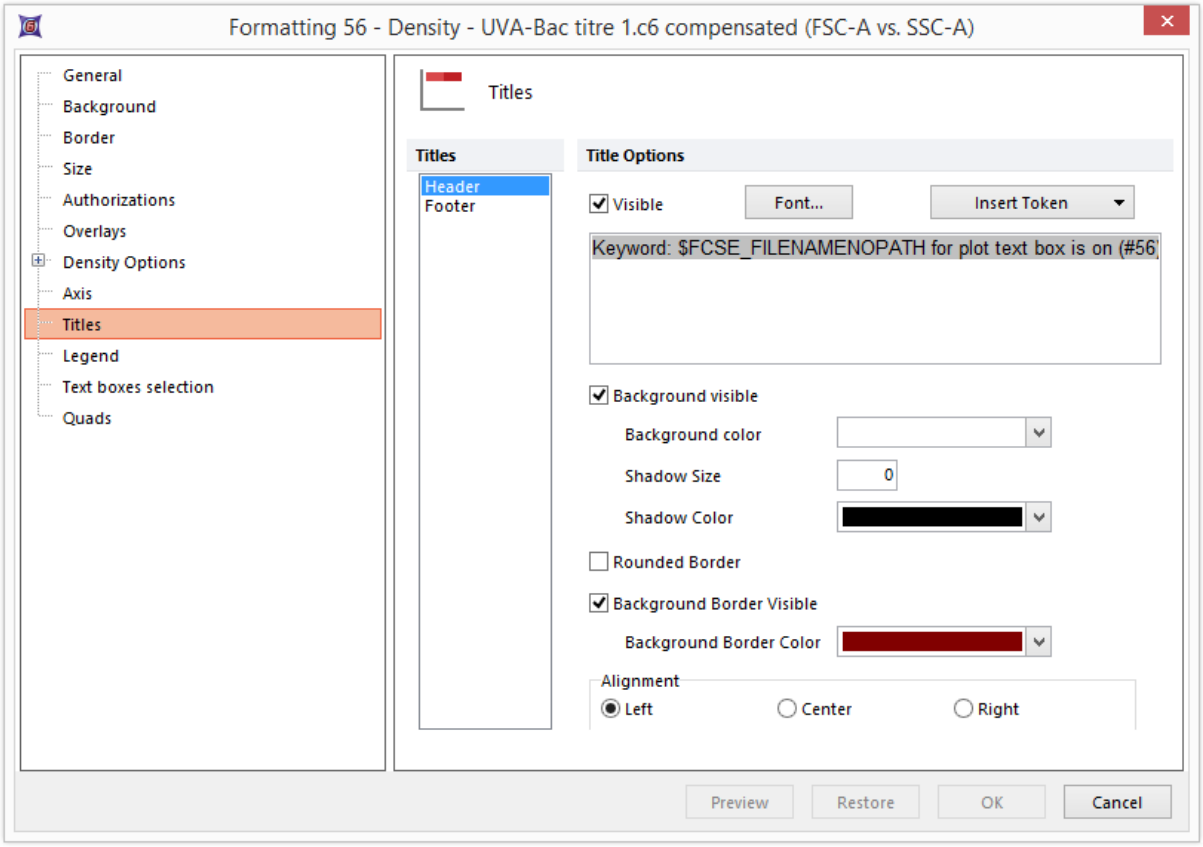

Figure 8.69 Formatting Titles

Visible headers are the default for plot titles, "centered" with a grey background, shadow, and a black border. The header text defaults to the file name keyword code Keyword: **$FCSE_FILENAMENOPATH for plot text box ...** which represents the local file name (no drive\folder) with compensation text that is defined in the **General section of the FCS Express User Options** 180.

The text options for titles are explained in the table below.

Option	Explanation
Header/Footer	Select either a Header or Footer in the upper left corner of the dialog to determine which title location is currently being edited. Headers and footers can co-exist.
Visible	Check or uncheck the checkbox to turn the Header/Footer on or off.
Font	Customize the header/footer font style, size, and text color.
Text Field	Enter text to be displayed in the Header/Footer in this field. Keywords chosen will appear in the text field as codes, but expand to file-specific values in the title.
Insert Keyword	A drop-down a list of special keywords that can be used in plot titles. The following options are available:

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Option	Explanation
	• **Keyword from Data**. Opens the FCS keyword list for the current file. Select keyword(s) from the list (using **Ctrl** to select multiple keywords) to insert in the header/footer. • **File name with Path**. Displays the data file name including the drive and folder. • **File name without path**. Displays the data file name without the drive\folder path. • **File name from Data**. Displays the $FIL keyword (which is part of the FCS standard and is placed into the data file header during sample acquisition). • **Current Gate**. Displays the name of the gate that is applied to the base overlay. • **Plot number**. Displays the Plot Number of the plot (usually visible as part of the plot name in General options 240). • **Other Tokens...**. Let the user insert any of the tokens available in the layout. An Inserting Tokens 692 window will open upon selecting this option.
Alignment	Align the title text left, center, or right.

The appearance of the Header/Footer can be customized by changing the background, shadow size, shadow color, and alignment in relation to the plot. Check the corresponding "visible" checkbox to enable the appearance option. To remove the shadow, set the **Shadow Size** to 0. The **Rounded Border** checkbox controls whether or not to display the rectangle surrounding the header/footer title with rounded edges (Figure 8.70).

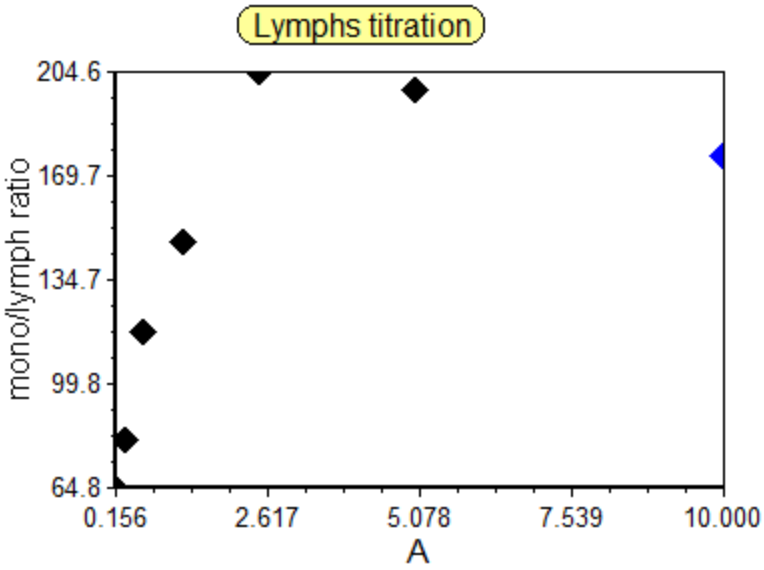

Figure 8.70 Header Title with Rounded Border

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8.4.3 Legend

Legend formatting options control the appearance and position of the legend in relation to the plot.

You can edit the Legend options in one of two ways:

- Select the plot(s) and use the **Format→Plot Options→Legend** command.
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Legend** category (Figure 8.71).

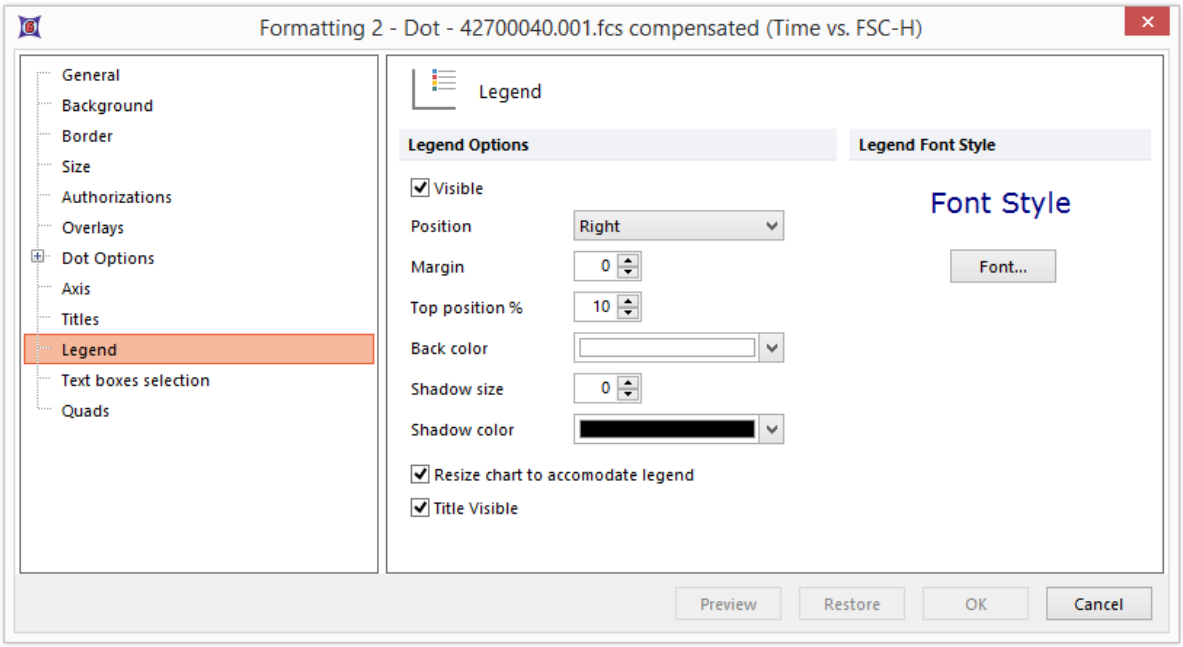

Figure 8.71 Formatting Legend Dialog

The options for legends are explained in the table below.

Option	Explanation
Visible	Check or uncheck the checkbox to turn the legend on or off.
Position	Set the legend to the right, left, top, or bottom of the plot.
Margin	Sets how far the legend is from the plot. Margin = 0 calculates the margin automatically.
Top position %	Sets where the top of the legend is located.
Back color	Sets background color of the legend.
Shadow size and color	The number sets the size of the shadow; use 0 for no shadow. Choose the color from the drop-down list.
Resize chart to accommodate legend	Enabling the checkbox will resize the plot to accommodate the legend. The legend and plot events will overlap if the option is unchecked.

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Option	Explanation
Title Visible	Enabling the check box will display the legend title on Density and Contour plots. The legend title is text as well as an interactive drop down menu that allows you to easily change the parameter to use for the **Color based on** 288 option.
Font...	Customize the Legend font style, size, and text color.

8.4.4 Axes

The **Axis** options control the appearance and text of the plot axes.

You can edit the Axes options in one of two ways:

- Select the plot(s) and use the **Format→Plot Options→Axes** command.
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Axis** category (Figure 8.72).

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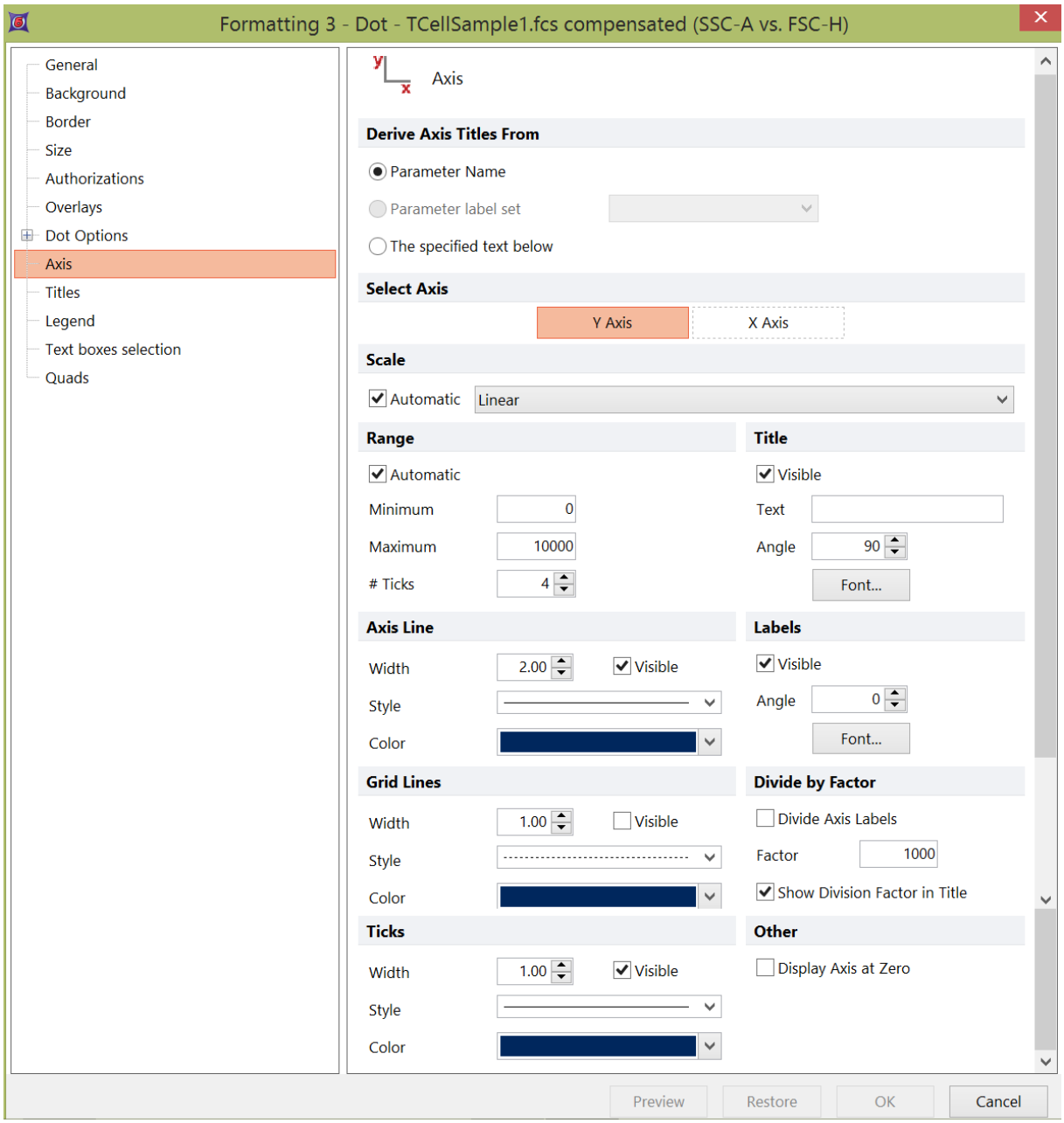

Figure 8.72 Formatting Axes Dialog

The options are explained in the table below. With the **Visible** checkbox, where present, you can determine if the option is displayed or not.

Option	Explanation
Derive Axis Titles From	Choose **Parameter Name**, a Parameter label set 410 (if a label set has been defined), or the text specified in the **Text** field (under **Title**).
Select Axis	Select the **Y Axis** or **X Axis** to determine which axis is currently being edited.

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Option	Explanation
Scale	If **Automatic** scaling is checked, the axis will be shown in the scale chosen automatically by FCS Express for that parameter (from the $PnE keyword). You can override automatic scaling, and choose the axis scaling 395.
Range	For all axes displaying data: • If **Automatic** Range is checked and the resolution options are set to automatic 247, then the range will either start at 0 or the lowest data value (a negative number) and extend to the highest data value. • If **Automatic** Range is turned on, but the resolution options are not automatic, the range will be the minimum to maximum specified in the resolution options. • For the count axis on a histogram, if **Automatic** Range is enabled, the axis range will extend from 0 to the highest event count. • **Minimum**. The minimum value to display on the axis. This option is only meaningful if automatic range is turned off. • **Maximum**. The maximum value to display on the axis. This option is only meaningful if automatic range is turned off. • **# Ticks**. The number of ticks to show along the axis.
Title	Set the visibility, specific text, font style, size, color, and angle for the title of the axis parameter.
Axis Line	Set the Axis Line visibility, style, color, and thickness.
Labels	Set the visibility, font style, size, color, and angle for the numbers which label the scale.
Grid Lines	Set the Grid Lines' visibility, style, color, and thickness.
Divide by Factor	Divide the nominal axis labels by a given factor, and choose whether or not to show the factor in the Axis title.
Ticks	Set the visibility, color, and width for the linear/logarithmic tick markers.
Other	If **Display axis at zero** is checked, the axis currently being edited will be displayed at zero rather than at the plot origin.

8.4.4.1 Axis Scale and Statistics

In FCS Express 6, all the statistics are calculated on the raw data, irrespective of the display scaling. However, when Gates and Quadrants are created decisions must be made in cases where an event falls on the line of the gate or quadrant.

In FCS Express 6, Gates and Quadrants are calculated and drawn in a binned space with binning dependent on both the Scaling 254 and the Resolution Minimum/Maximum 247 of the plot. In turn, the inclusion or exclusion of an event on the border line of a gate or in a quadrant is highly dependent on the scaling of the plot, and on the Resolution Minimum/Maximum, of the plot the Gate/Quadrants was drawn on.

When adjustments to the scaling or resolution of a plot are made, an event may then fall to a different side of the border line of the gate/quadrant, and the user may notice very slight changes in the statistics of gated events which are usually quite minimal and make no impact on the interpretation of the results.

To illustrate the scenario the example below use the exact same Quadrants which have been drawn on different plots using different Scale and/or different Resolution Minimum/Maximum values. In row 1, the three plot are using different

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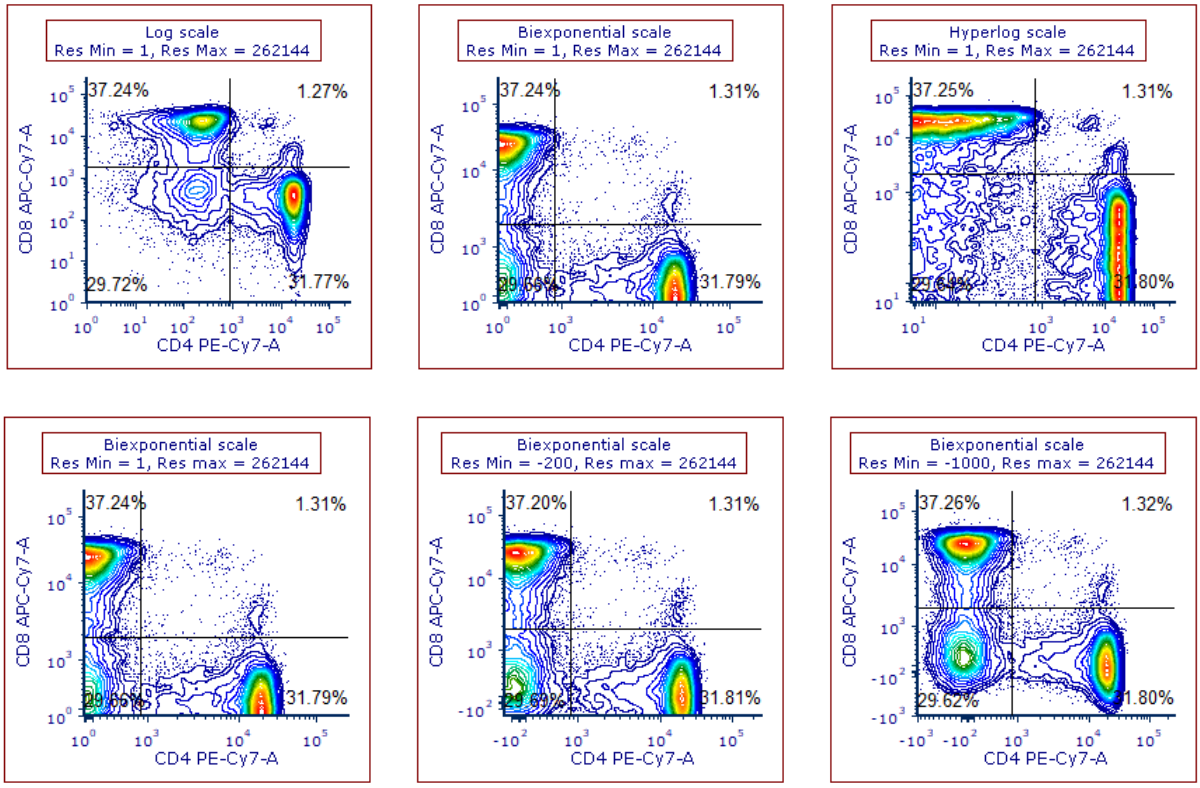
Scales (i.e. Log, Biexponential and Hyperlog respectively) but the same Resolution Minimum/Maximum. In row 2, the three plots are using the same scale (i.e. Biexponential) but different Resolution Minimum/Maximum values. In row 3, plots are using different Scales and different Resolution Minimum/Maximum values.

Each of the plots below is using a different binning, thus, Quadrants drawn on each them, will use a slightly different binning as and very slight differences in percentages may be observed (Figure 8.73).



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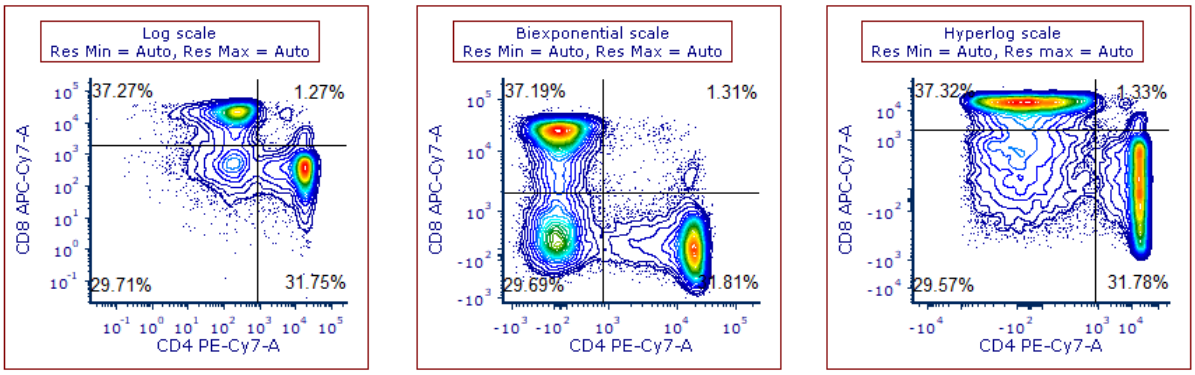

Figure 8.73 The same Quadrants have been displayed on plots using different axis scales and/or different Resolution Minimum/Maximum values. Quadrant statitistics are slightly different (yet statistically insignificant) because of the different binning on the plots and the assignment of events that fall on the borders of the quadrants.

Although small differences exist for both Quadrants and Gates, they are usually only visible for quadrants since they are dependent on the scaling of the plot the quadrant is displayed on. When the axis scale changes, the Quadrant binning on that plot updates as well.

The behavior of Gates is slightly different, as Gates retain the binning of the plot they have been drawn on meaning that a gate can be displayed on plots using different scales but, for consistency, it will always refer to the binning of the plot it has been drawn on.

In the following three scenarios (Figure 8.74), the same Gate has been drawn on a plot using a specific scale (i.e. Log scale in the first row, Biexponential scale in the second row or Hyperlog scale in the third row). The gate has then been displayed on plots using different scales (i.e. Log scale in the first column, Biexponential scale in the second column or Hyperlog scale in the third column). The Resolution Minimum/Maximum on all the plots below is set to Automatic, thus it will vary between the three columns.

The gate statistics are consistent within each row, because in each row the same gate is displayed on different plots. However, small differences are visible between different rows because the gates are using three different binnings.

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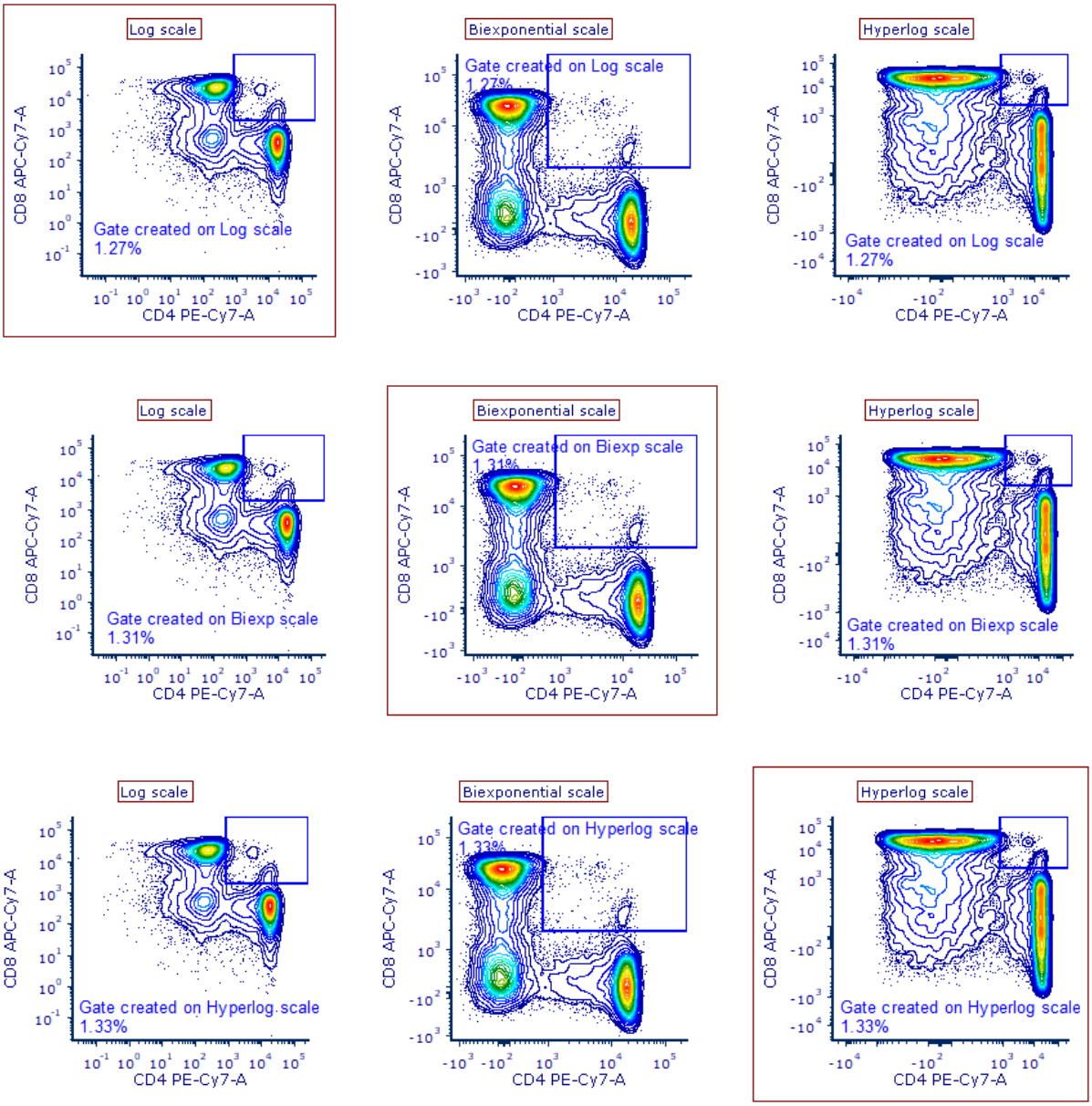

Figure 8.74 The same Gate has been drawn on plots using different axis Scales and different Resolution Minimum/Maximum (Rows 1, 2 and 3) and then displayed on plots using different axis Scales and different Resolution Minimum/Maximum (Columns 1, 2 and 3). Gate statitìstics are slightly different (yet statistically insignificant)change between rows because of the different binning. Gate statitìstics do not change between columns because Gates retain the binning of the plot thay have been drawn on.

8.4.5 3D Display Options

Even though only surface plots have typically been displayed in three dimensions (3D), FCS Express also has the capability of rendering histograms in 3D. The

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appearance of histograms with multiple, filled-in overlays may be enhanced in 3D. 268

The 3D display options control the 3D plot appearance and orientation relative to the user. You can edit the 3D display options for a surface plot or histogram in one of two ways:

- Select the plot(s) and use the **Format** tab→**Plot Options**→**3D** command.
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Surface**→**Plot 3D Options** or **Histogram**→**Plot 3D Options** category, as appropriate (Figure 8.75).

Note: Previewing changes several times without saving is an effective method of becoming familiar with the 3D controls. Right-clicking on the plot and selecting the appropriate 3D Options page has the advantage of bringing up a dialog with Preview and Restore buttons 246.

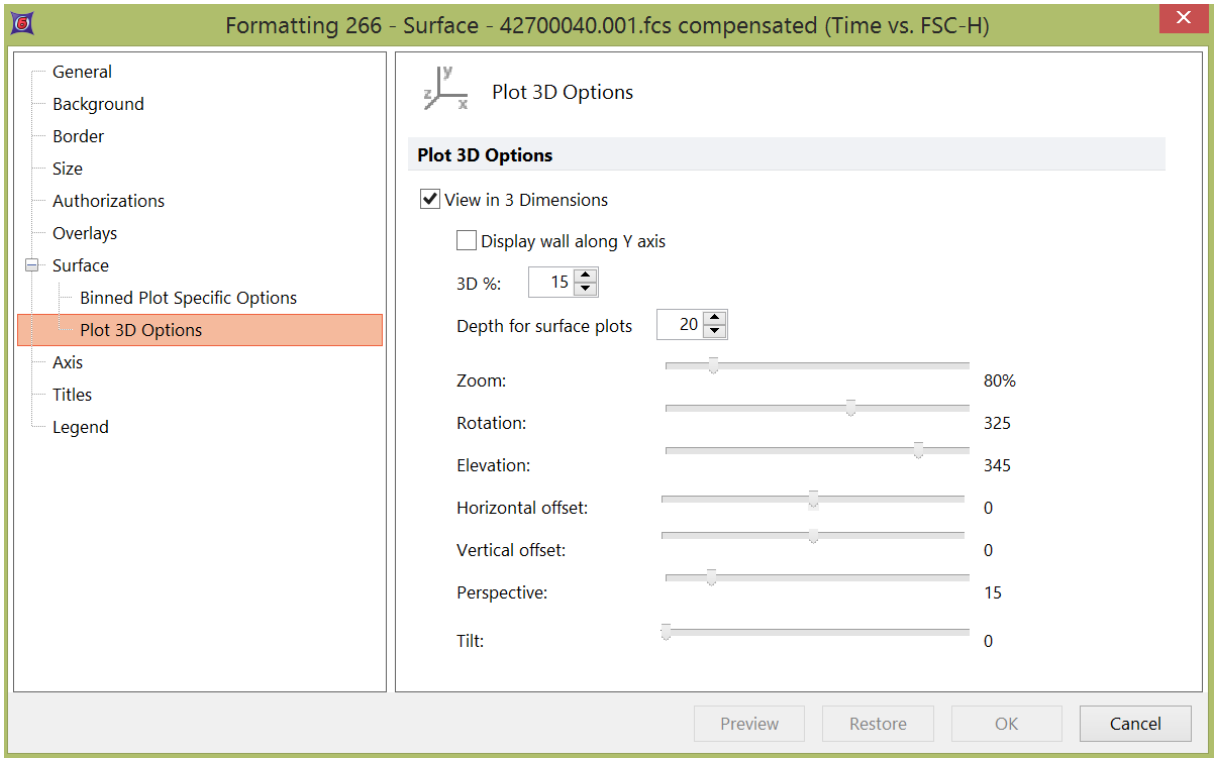

Figure 8.75 Formatting 3D Options for a Surface Plot

Check or uncheck **View in 3 Dimensions** to toggle 3D on or off.

Use the **Display wall along Y-axis** check box to turn on or off a yellow wall along the Y-axis.

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Depth can be controlled (from 0% to 100%) with the **3D%** (for histograms) or the **Depth for surface plots** *spin buttons*. As 3D% increases, the third dimension z-axis widens and the single-plane events progress from dots to short stubble to long lines.

The other options are controlled by sliders:

- **Zoom** resizes the plot within the object border from less than 100% (smaller than current size) to 500%. As the zoom % increases, axes and events may extend beyond the object border until the zoom is decreased again. The zoom is reversible, i.e., no data is lost.
- **Rotation** pivots the plot on the vertical axis from 270 to 360 where 360 is facing the user straight on and 270 rotates the plot clockwise (turns a 90-degree pivot to the left), so the right-hand z-axis is facing the user straight on.
- **Elevation** turns the plot on the horizontal axis from 270 to 360 where 360 is facing the user straight on and 270 tips the top edge of the plot forward (turns a 90-degree pivot down), so the top edge z-axis is facing the user straight on.
- **Horizontal offset** and **Vertical offset** control the position of the 3D plot on the page.
- **Perspective** is a value from 0-100 that controls how far the chart appears to be from the user.
- **Tilt** is a value from 0-360, indicating the number of degrees the plot will be rotated in its 2D-space about an axis perpendicular to the screen.

8.4.6 Magnifying a Plot

It is possible to **Magnify** 261 a region of a histogram 261 or 2D plot 264.

Magnifying Axes is equivalent to setting the minimum and maximum axis range for the plot 254. Magnifying Resolution also resets the range of data displayed on the plot, but also applies your Plot Resolution 247 to the displayed range, rather than to the entire .fcs file.

To magnify a histogram:

- Right-click on the histogram and select either **Magnify (Resolution)** or **Magnify (Axis)** from the pop-up menu.
- Press and hold the mouse button down where you want the lower bound of the magnification to be.

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- Drag the cursor to where you want the upper bound of the magnification to be.
- Release the mouse button.

An example histogram for the Magnification operation is shown in Figure 8.76.

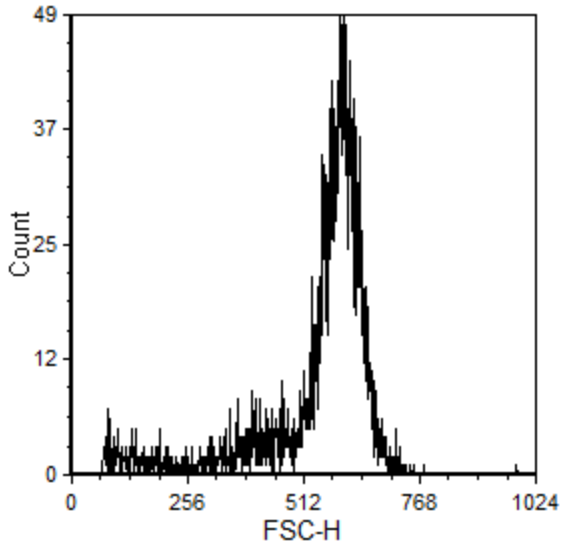

Figure 8.76 An Example Histogram Before Magnification

The result for **Magnify (Resolution)** for the region from channel 445.8 to 776.8 is shown in Figure 8.77.

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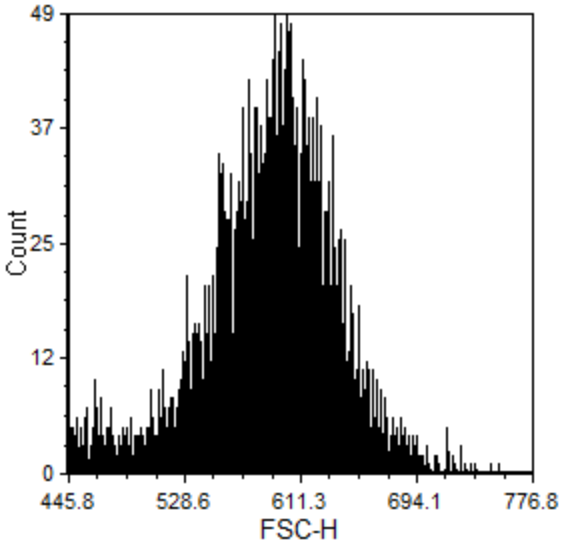

Figure 8.77 Magnify Resolution from Channel 445.8 to 776.8

The result for **Magnify (Axis)** for the region from channel 445.8 to 776.8 is shown in Figure 8.78.

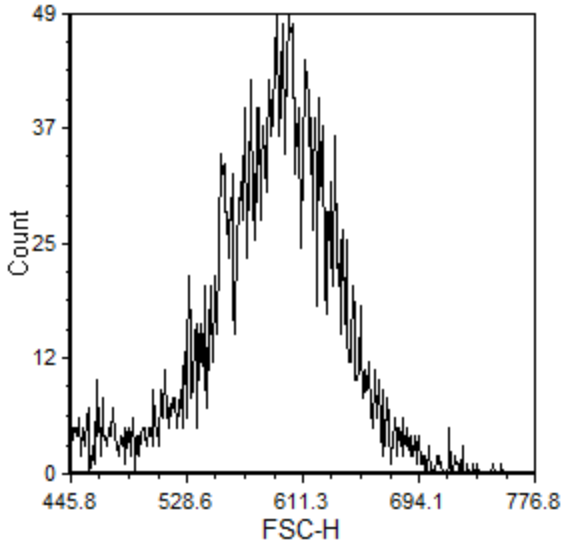

Figure 8.78 Magnify Axis from Channel 445.8 to 776.8

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To release the magnification, right-click on the histogram and select **UnMagnify (Resolution)** or **UnMagnify (Axis)** from the pop-up menu.

Magnifying a 2D plot sets the minimum and maximum values on both the X- and Y-axis scales.

To magnify a 2D plot:

- Right-click on the 2D plot and select either **Magnify (Resolution)** or **Magnify (Axis)** from the pop-up menu.
- Press and hold the mouse button down where you want one end of the magnification "box" to be.
- Drag the cursor to where you want the other end of the magnification "box" to be.
- Release the mouse button.

An example 2D plot for the Magnification operation is shown in Figure 8.79.

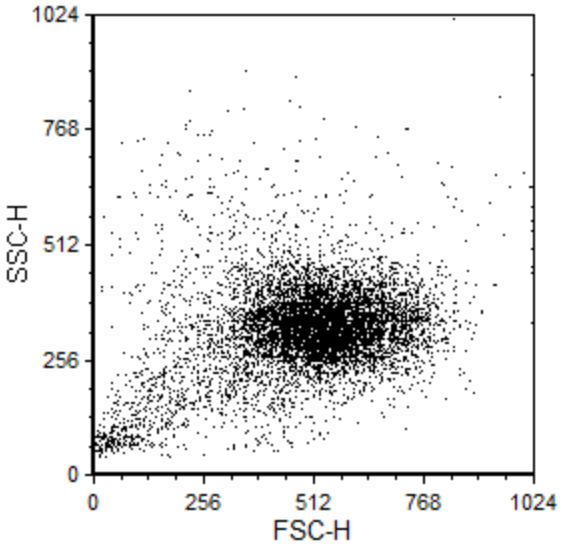

Figure 8.79 An Example 2D Plot Before Magnification

The result for **Magnify (Resolution)** for the region with the majority of cells is shown in Figure 8.80.

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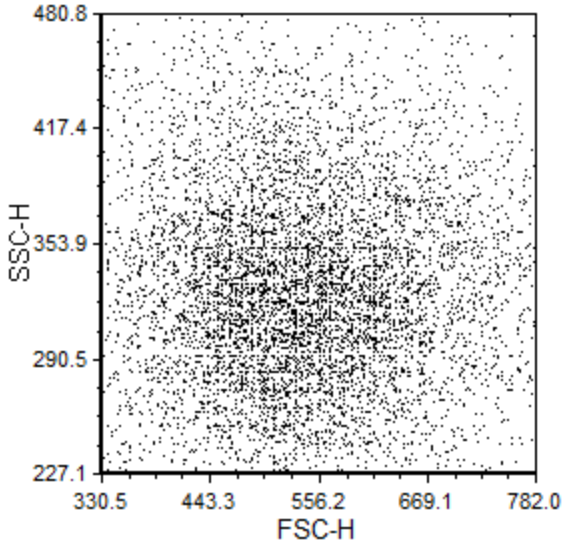

Figure 8.80 Magnify Resolution for a 2D Plot

The result for **Magnify (Axis)** for the region with the majority of cells is shown in Figure 8.81.

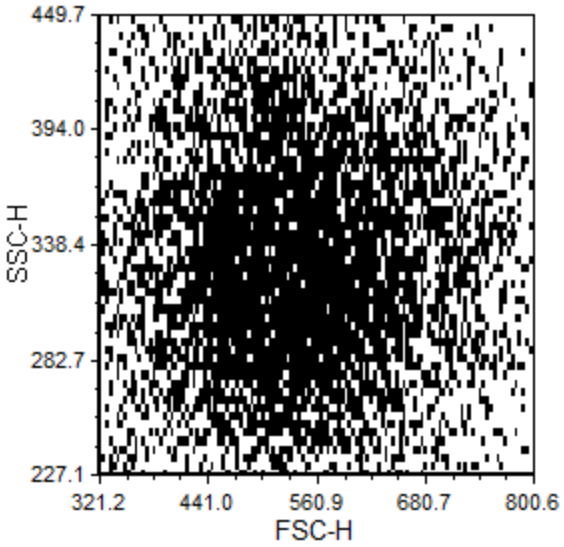

Figure 8.81 Magnify Axis for a 2D Plot

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To release the magnification, right-click on the 2D plot and select **UnMagnify (Resolution)** or **UnMagnify (Axis)** from the pop-up menu.

8.4.7 Changing Plot Type

Existing plot(s) on your layout may be reformatted to a different plot type at any time, by selecting the plot(s) 1216 and using the **Change Plot Type** button on the **Format** tab (Figure 82.)

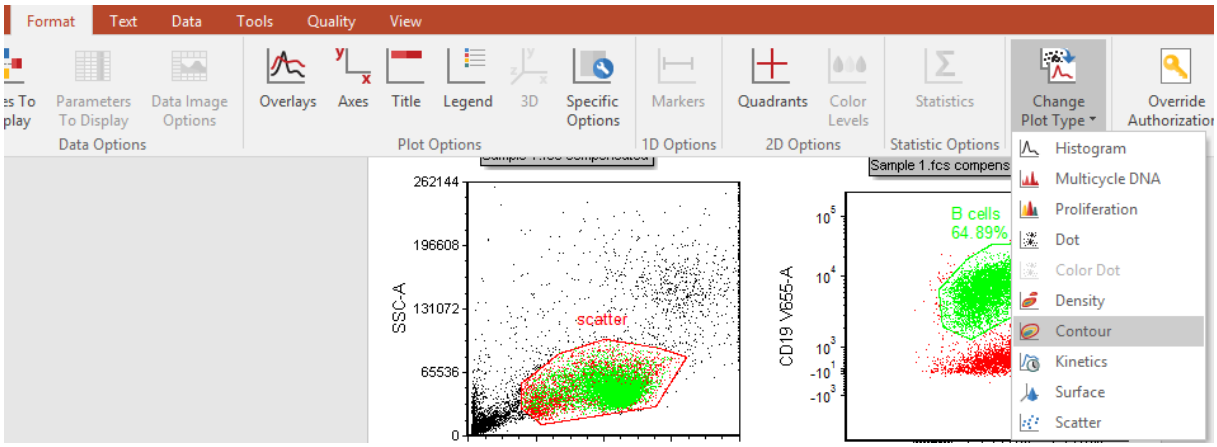

Figure 8.82 Changing the plot type from color dot to contour.

8.4.8 Plot Names

Plot names assigned by default include information about the plot type 170, .fcs file data, displayed parameters, and order of creation in your layout. Plot names appear in hovertext over the plot (Figure 8.83) and in navigators 123, and assist in selecting the data source 699 when inserting a token on your layout or in setting up batch processing actions 803.

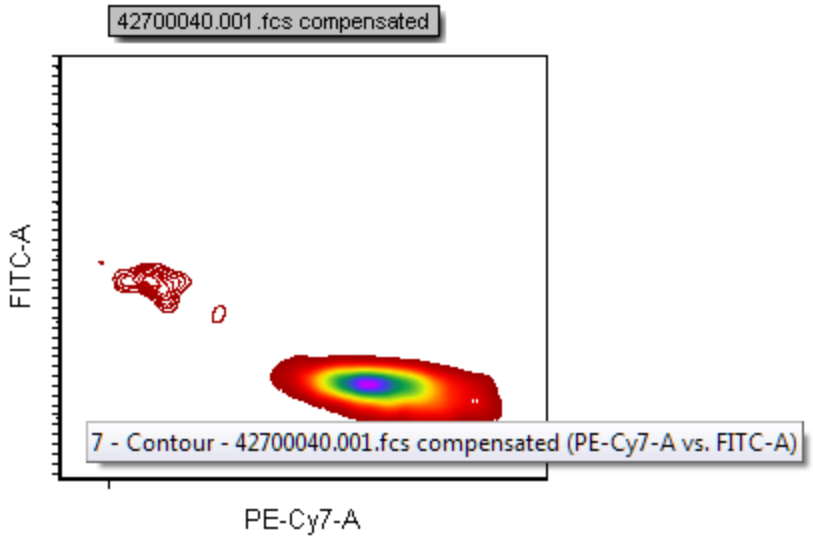

Figure 8.83 Default plot name as shown in plot hovertext.

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Plot names can be customized to contain free text, tokens[692], or a combination thereof. The same plot's hover text is shown below in Figure 8.84 after being changed to a combination of freetext and the *$DATE* keyword token.

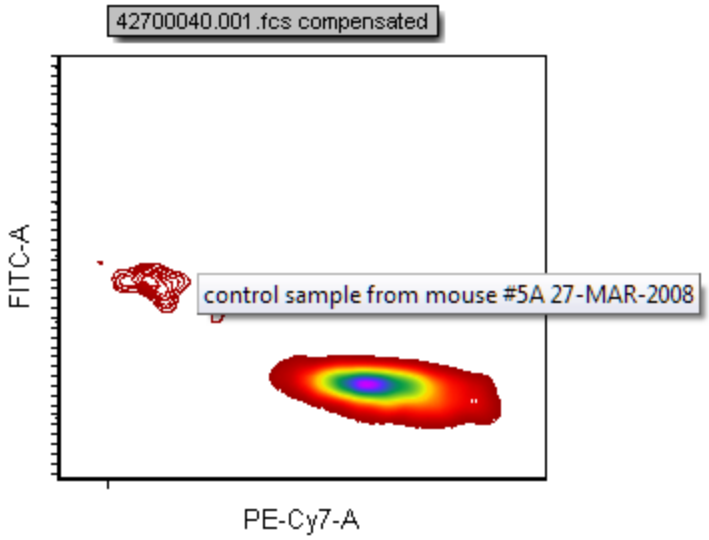

Fig. 8.84 Plot name changed to contain freetext and a keyword token.

To customize the plot name, either:

- Select the plot, use the **Format→Object Options→General** command, and edit the Plot **Name,** or

- Right-click on the plot, select **Format** from the pop-up menu, choose the **General** category, and edit the **Plot name**.
- The **Data, Layout, or Gate Navigator**[123] may also be used to change the **Plot name** by right clicking on the plot within the navigator and choosing Rename.

- Alternatively, a **Suffix for Compensated Data** may be added or removed from the **FCS Express User Options→General→Suffix for Compensated Data Category.**[183]

8.5 Formatting Single Parameter Histograms

In addition to the formatting options common to all Objects[239], and those common to all Plots[246], single parameter histograms have certain properties, such as overlays[268], markers[272], magnification[261], and other specific options[274] which can be formatted.

Overlays[268] are defined in FCS Express as a data set that is displayed on a plot. A data set for a histogram consists of a data file and parameter combination. A histogram must contain one or more overlays. Overlays can be added or deleted

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from a histogram. Furthermore, while any number of data files with parameters different from the first overlay (also known as the *base overlay*) can be added to the same histogram, the axis title and scaling will always be taken from the base overlay. The histogram legend 253 is valuable for displaying and organizing multiple overlays.

Markers 272 are regions defined along a single parameter.

Magnification of a histogram 261 allows you to reset the minimum and maximum axis scale and resolution to restrict your view to a certain region of the histogram.

The histogram specific options 274 include normalization, plot resolution, presentation choices for multiple overlays, and the choice of a control histogram for histogram subtraction statistics.

You can always format a histogram by right-clicking on the plot, selecting **Format** from the pop-up menu, and choosing a category. This method will allow you to use the **Preview** button to see what the changes would look like without actually accepting the changes. Alternatively, once the histogram is selected, you can choose the desired category from the Format tab of the Ribbon 73.

8.5.1 Overlays

The **Overlays** option controls the parameter and appearance of histogram overlays.

All histograms have at least one overlay representing a file and two parameters to display. You can add additional overlays by any of the following methods:

- Right-click on the plot and select **Add overlay** from the pop-up menu (launches a standard browser window called **Select Data File** 214), or
- Right-click on the plot and select **Add Overlay using Advanced Open Data Dialog** (launches the **Open Data File** 215 dialog), or
- Drag-and-drop file(s) from the **Data List** 785 onto the plot you wish to overlay, or
- Drag-and-drop a plot from the layout onto the plot you wish to overlay.

You can edit overlays in one of two ways:

- Select the histogram(s) and use the **Format→Plot Options→Overlays** command.

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- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Overlays** category (Figure 8.85).

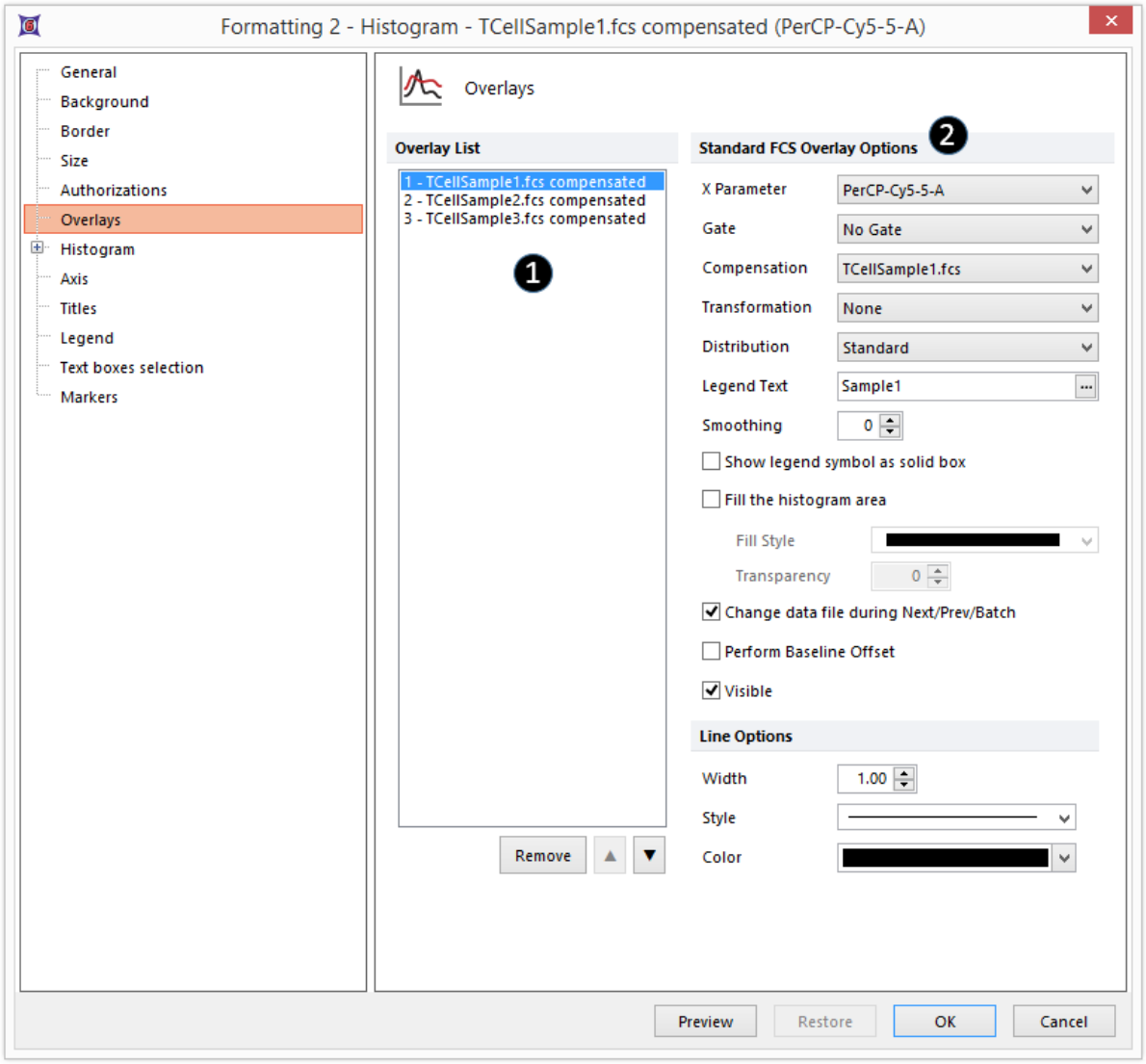

Figure 8.85 Formatting Histogram Overlays Dialog

In the dialog, a list of all the overlays on the histogram will appear in the list box to the left ❶. You can select one or more overlays using standard Microsoft Windows keystroke commands, including **Shift**-Click and **Ctrl**-Click. The properties on the right ❷ pertain to the currently selected overlay(s). If multiple overlays are selected, properties that are not identical in all selected overlays will appear blank or will display a black square in their check box (▣). If the properties are not blank, or they are not displaying a black square in their check box (▣), then they are identical for all selected overlays. Changing an option will

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change that property for all selected overlays. For instance, if you select three overlays and change the Gate, the Gate for all three overlays will be modified.

To remove an overlay from a plot, select the overlay (or multiple overlays) and press the **Remove** button.

To change the order of selected overlay(s) in the legend, use the blue up and down arrows ( ).

For a brief tutorial on how to use overlays, visit us at https://youtu.be/nVqwvDo1RmA.

Overlay properties are explained in the table below.

Option	Explanation
X Parameter	Change the parameter displayed on the histogram.
Gate	If gates are defined, select the gate that will be used to limit the data that is displayed on the histogram.
Compensation	If compensation definitions are set, select the definition to use when compensating the data in the overlay.
Transformation	If transformations are set, select the transformation to use for each overlay.
Distribution	Select the data to be shown. **Standard** displays the data in number of events per channel, and **Cumulative** displays the cumulative distribution frequency (often used for statistical testing). Information about the cumulative distribution frequency can be found in standard statistics textbooks.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. Free text can be inserted. If this field is blank the file name of the overlay will appear. A dropdown menu with additional pre-customized legend content is accessible by pressing the ellipsis button next to the legend text. Possible choices are: ○ **Keyword**. This can be used to insert keywords from the header of the data file. ○ **Gate**. This can be used to display the current gate applied to the overlay. ○ **Tube Number**. This can be used to display the Tube Number of the overlay when a Panel is active. ○ **Tube Name**. This can be used to display the Tube Name of the overlay when a Panel is active. The above mentioned Legend options apply only to **Dot Plots**, **Color Dot Plots**, **Histograms** and **Spectrum Plots**.
Line Options	Choose the overlay line style, color, and/or width.
Smoothing	Set the degree of smoothing for the overlay.
Show legend symbol as solid box	When selected, colored solid boxes will be used in the legend instead of lines. Box colors are defined in the **Line Options > Color** field (see below).

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Option	Explanation
Fill the histogram area	Enable the checkbox to fill in the overlay using the selected Fill Style. Overlays that are filled in may look better on a 3D display 259 (Figure 8.86).
Fill Style	The pattern that will be used to fill the histogram.
Transparency	Transparency of histogram fill, where 0 is opaque and 100 is completely transparent (invisible).
Change data file during Next/Prev/Batch	If this option is checked, the data file for this overlay will change when doing batch processing 784, or selecting Previous File or Next File 72. This option is usually only disabled for a control histogram, where you have multiple overlays, and want the control to stay the same all of the time.
Perform Baseline Offset	If the value of the cell is zero, a random offset will be added so that the dot appears off the axis. This will not affect the statistics.
Visible	You can hide overlays on a histogram without removing them. Select the overlay file from the list and uncheck **Visible**. Check **Visible** to show the overlay again.
Line Options (Width, Style, Color)	Graphical options used for each overlay. These options also applies to the legend unless the "Show legend symbol as solid box" is enabled. In that case, the color only will apply to the legend.

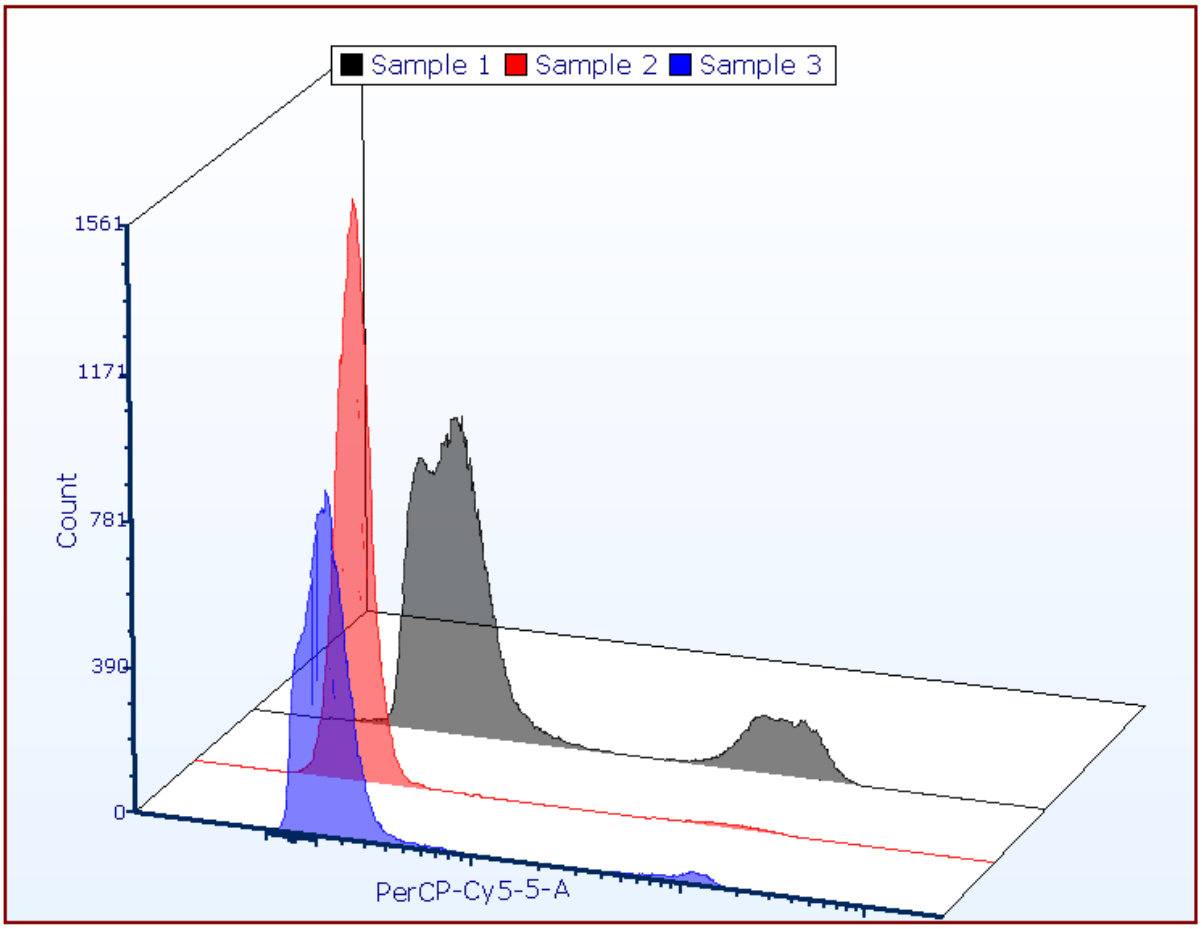

Figure 8.86 Histogram Overlays in 3D

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8.5.2 Histogram Markers

The **Markers** option allows the user to control the formatting of histogram markers.

Please also see the sections on Using Markers 352 for additional details on creating, editing, resizing, linking, and locking markers.

You can edit markers in one of two ways:

- Select the histogram(s) and use the **Format→1D Options→Markers** command.
- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Markers** category (Figure 8.87).

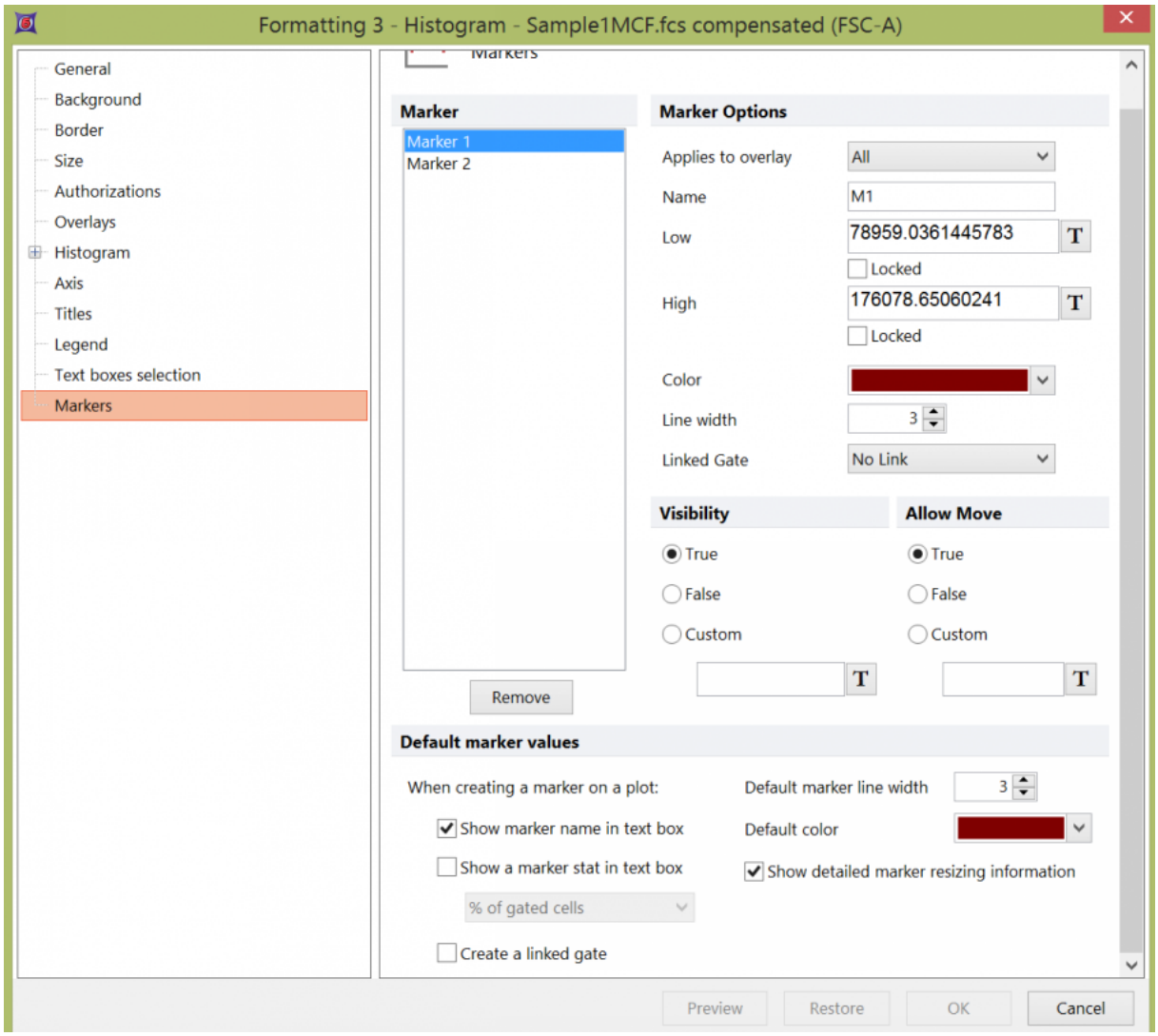

Figure 8.87 Formatting Markers Dialog

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In the dialog, a list of all the markers on the histogram will appear in the **Marker** list box. You can select one or more markers using standard Microsoft Windows techniques, including **Shift**-Click and **Ctrl**-Click. The properties on the right pertain to the currently selected marker(s). If multiple markers are selected, the information will appear blank for those properties that are not identical in all selected markers. If the properties are not blank, then they are identical for all selected markers. Changing an option will change that property for all selected markers. For instance, if you select three markers and change the Color, the Color for all three markers will be modified. To remove a marker, select it and press the **Remove** button.

Marker properties are explained in the table below.

Option	Explanation
Applies to overlay	Select which overlay(s) the marker applies to. Statistics will be calculated for the overlay that the marker applies to.
Name	The name of the marker that appears in text boxes on a plot when the marker name token is selected.
Low	The low channel boundary of the marker. Tokens may be used to define low bound by clicking on the T icon 355. The locked check box locks the low bound position to a manually entered or token defined value. Additionally, A lock may be overridden without accessing the formatting dialog by dragging a new token value onto the marker endpoint.
High	The high channel boundary of the marker.Tokens may be used to define low bound by clicking on the T icon 355. The locked check box locks the high bound position to a manually entered or token defined value. Additionally, A lock may be overridden without accessing the formatting dialog by dragging a new token value onto the marker endpoint.
Color	The color that the marker will be drawn in.
Line width	The line width of the marker.
Linked Gate	The gate that the marker is linked to 366. To break the link between the marker and a gate select **No Link**.
Visibility	Choose to show the marker "**True**", hide a marker without removing it "**False**", or make it visible based on a conditional value "**Custom**". The **Custom** option allows you define visibility of the marker based on a token or value that evaluates to a conditional expression 747. Any tokens or values may be used to set a conditional custom value and may be inserted 692 via right click in the field, **Insert Token** or by clicking on the **T** token icon. The marker will only be visible if the token evaluates to True.
Allow Move	Choose to allow moving the marker "**True**", disallow a marker move "**False**", or make moving the marker based on a conditional value "**Custom**". The **Custom** option allows you define the move status of the marker based on a token or value that evaluates to a conditional expression 747. Any tokens or values may be used to set a conditional custom value and may be inserted 692 via right click in the field, **Insert Token** or by clicking on the **T** token icon. The marker will only be allowed to move if the token evaluates to True.
Default marker values	When a marker is created, you can choose whether or not to show the marker name and/or stat in a text box, and select the default line width and color. If marker stat is enabled, you can choose which statistic to display

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Option	Explanation
	from the drop-down list. If **Show detailed marker resizing information** is checked, the marker boundaries will appear above the marker and lines will drop to the axes to show where the marker boundaries will be during resizing. If **Create a linked gate** is checked, when new markers are created on this plot, a gate linked to the marker 366 will automatically be created.
Show detailed marker resizing information	Enables drop down lines at the end of each marker to show the position on the axis as well as displays marker low and high boundary values.
Create a linked gate	When checked, any new markers created on the histogram will be automatically converted to linked gates. Any existing markers on the plot will still need to be manually linked to a gate 366.

8.5.3 Histogram Specific Options

The Histogram Specific Options category contains a variety of properties that control how data on the histogram is displayed.

You can edit histogram specific options in one of two ways:

- Select the histogram(s) and use the **Format→Plot Options→Specific Options** command.
- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Histogram→Histogram Specific Options** category (Figure 8.88).

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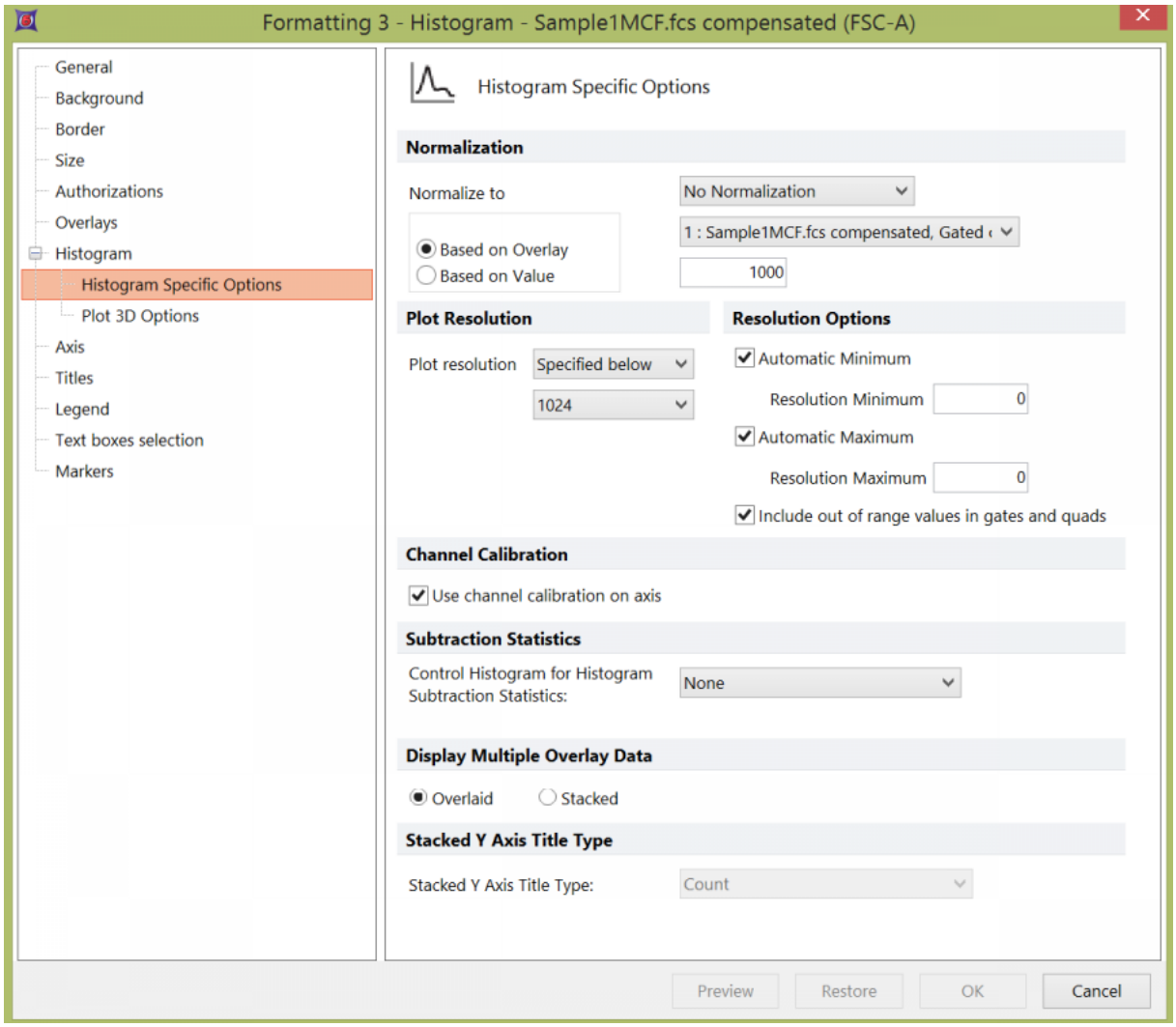

Figure 8.88 Formatting Histogram Specific Options

The options are described in the table below. See Resolution Overview 247 for a further description of resolution options.

Option	Explanation
Normalize to	If different overlays contain different numbers of events in a channel, they may not look alike even if the data comes from an identical population. Overlays can be normalized based on the number of events or based on the peak value. The reference, for both the number of events or the peak value, can be an overlay (select the **Based on Overlay** radio button and select an overlay from the drop-down list. The number of events in other overlays will be normalized to appear to have the same number of events as the selected overlay.) or a custom

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Option	Explanation
	value (select the **Based on Value** radio button and type the custom value in the corresponding field).
Plot resolution	In the first drop-down menu, choose whether to use the resolution specified in the data or select your own resolution from the second drop-down menu. Choosing a resolution lower than the true resolution of the plot is an effective way of smoothing your data.
Automatic Minimum	If this is checked, then FCS Express will start binning the data (dividing it into the number of bins specified by the **Plot resolution**) at either 0 or the lowest data value (if the lowest data value is below 0).
Resolution Minimum	Specify where to start binning the data. If **Automatic Minimum** is checked, then this value is ignored.
Automatic Maximum	If this is checked, then FCS Express will finish binning the data (subdividing it into the number of channels specified by the **Plot resolution**) at the highest value of the true data range.
Resolution Maximum	Specify where to finish binning the data. If **Automatic Maximum** is checked, then this value is ignored.
Include out of range values in gates and quads	If this is checked, data values that are outside the resolution options of the plot will be considered to be part of the lower or upper channels. If a gate or quadrant touches the lower or upper channels, it will include these cells, even though the cells are actually outside the boundary of the gate/quadrant.
Use channel calibration on axis	FCS Express can establish calibrated values for the axes 614. If channel calibrations are defined, toggling this checkbox will determine whether or not to use the calibrated values in place of the generic channel numbers.
Control Histogram for Histogram Subtraction Statistics	FCS Express can automatically perform histogram subtraction 598 to calculate the number of positive cells. Select which overlay is the control.
Display multiple overlay data	Display overlay data in a single histogram (**Overlaid**) or use a split view (**Stacked**). See Figure 8.89.
Stacked Y Axis Title Type	If multiple overlay data is displayed Stacked, choose the Y Axis Title to use.

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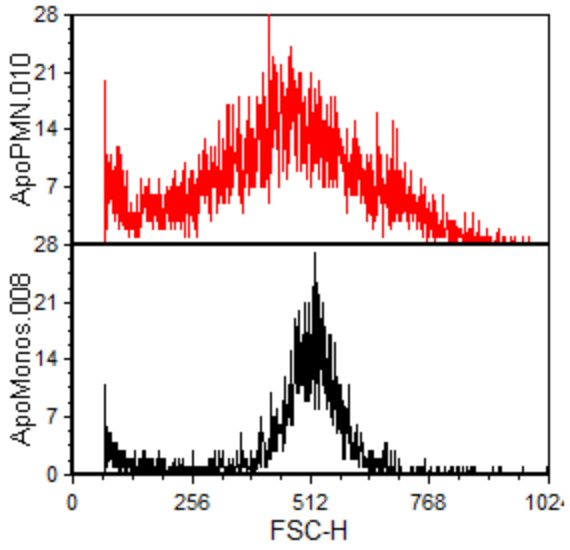

Figure 8.89 A Histogram with Two Overlays in Stacked View

8.6 Formatting 1D Spectrum Plots

In addition to the formatting options common to all Objects 239, and some of those common to all Plots 246, specifically **Axis**, **Titles**, and **Legends**, Spectrum Plots have certain properties, such as overlays 278 and spectrum options 280 which can be formatted.

Overlays 268 are defined in FCS Express as a data set that is displayed on a plot. A data set for a spectrum plot consists the individual spectrum from every event. A Spectrum Plot must contain one or more overlays. Overlays can be added or deleted from a Spectrum Plot, but the first overlay, also known as the *base overlay*, cannot be removed, only reformatted or changed. Furthermore, spectra for all events may be displayed individually or as an average.

Spectrum Options 280 share the same options as Display Gates and Color Levels options. Here, you may automatically match the color of the spectrum line for each event to the color of the gate to which it belongs. These options allow for "backgating" of spectral flow cytometry data files. You may alternatively choose to display the spectral line colors on Density.

You can always format a Spectrum Plot by right-clicking on the plot, selecting **Format** from the pop-up menu, and choosing a category. This method will allow you to use the **Preview** button to see what the changes would look like without actually accepting the changes. Alternatively, once the Spectrum Plot is selected, you can choose the desired category from the Format tab of the Ribbon 73.

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8.6.1 Overlays

The **Overlays** option controls the spectrum and appearance of spectrum overlays. All Spectrum Plots have at least one overlay and a spectrum to display. Additional overlays from the same or different spectral datafiles can be added, and different gates can be applied to each, if desired (e.g., Figure 8.90).

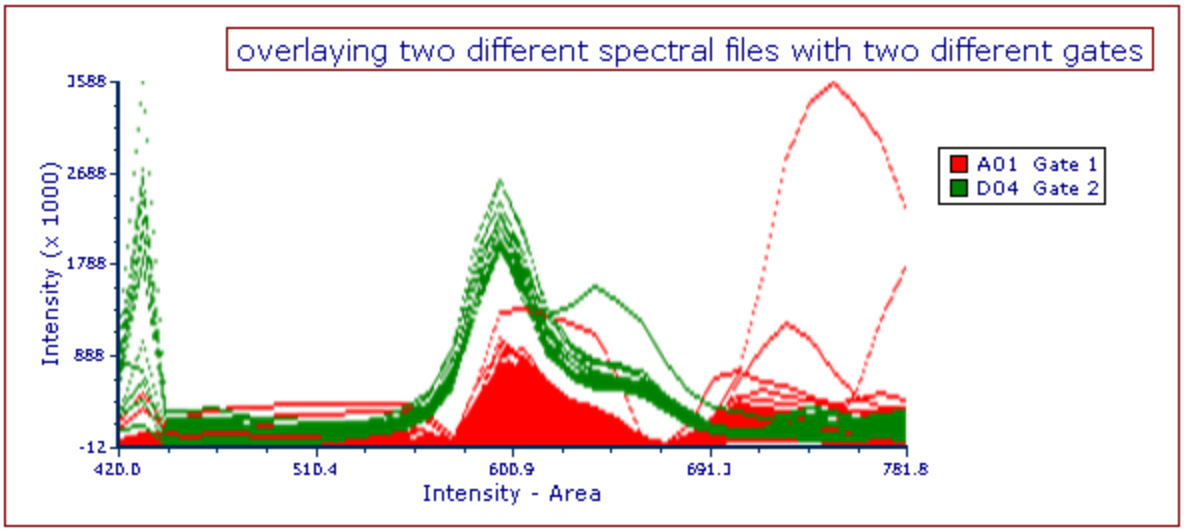

Figure 8.90 - Spectral plot with two overlays from different spectral datafiles, each with a different gate applied.

You can add additional overlays by any of the following methods:

- Right-click on the plot and select **Add overlay** from the pop-up menu (launches a standard browser window called **Select Data File** 214), or
- Right-click on the plot and select **Add Overlay using Advanced Open Data Dialog** (launches the **Open Data File** 215 dialog), or
- Drag-and-drop file(s) from the **Data List** 785 onto the plot you wish to overlay.

To edit overlays: right-click on the Spectrum Plot, select **Format** from the pop-up menu, and choose the **Overlays** category (Figure 8.91).

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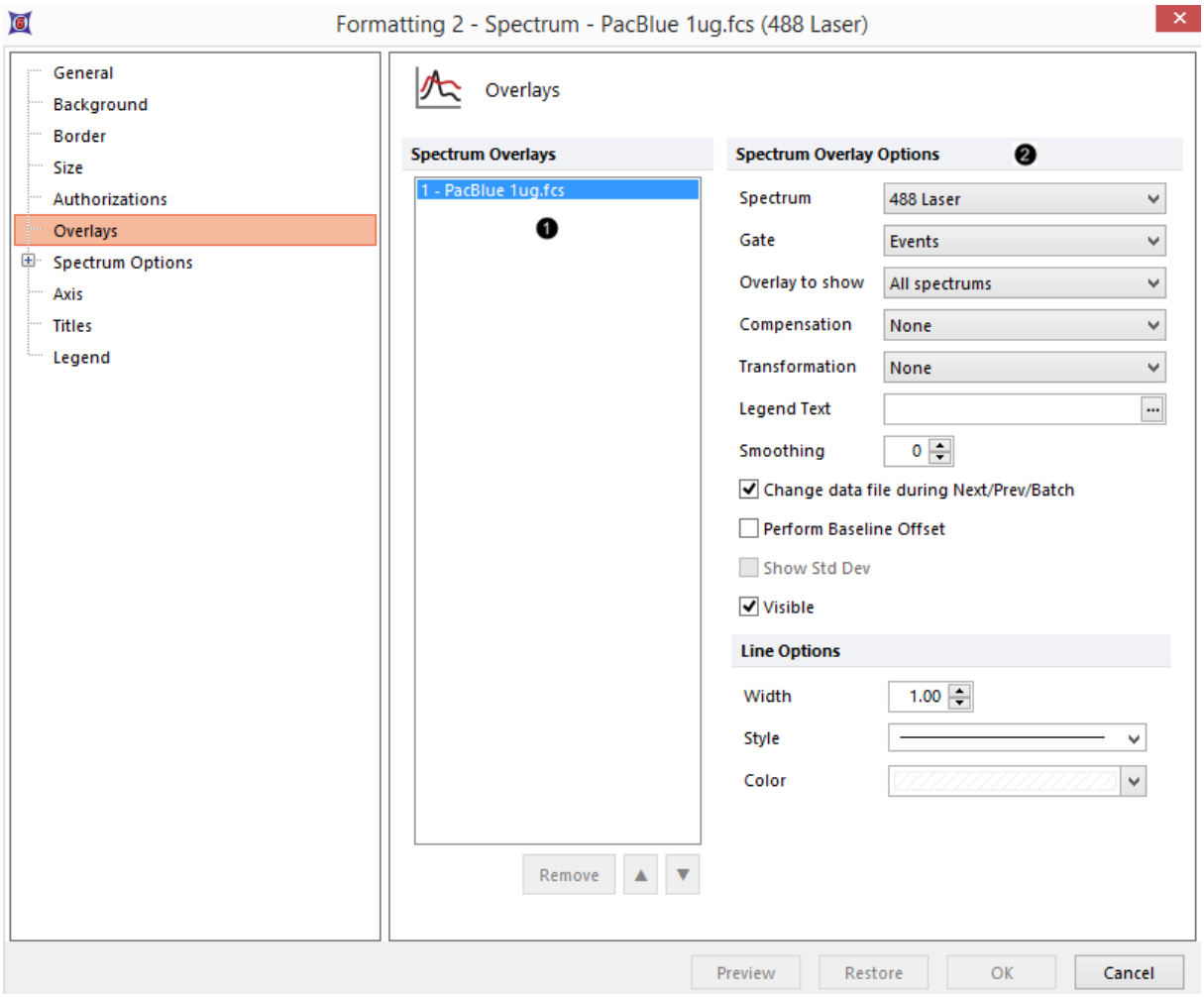

Figure 8.91 - Formatting Spectrum Overlays Dialog

In the dialog, a list of all the overlays on the Spectrum Plot will appear in the list box to the left ❶. You can select one or more overlays using standard Microsoft Windows techniques, including **Shift**-Click and **Ctrl**-Click. The properties on the right ❷ pertain to the currently selected overlay(s). If multiple overlays are selected, the information will appear blank for those properties that are not identical in all selected overlays. If the properties are not blank, then they are identical for all selected overlays. Changing an option will change that property for all selected overlays. For instance, if you select three overlays and change the Gate, the Gate for all three overlays will be modified.

To remove an overlay from a plot, select the overlay(s) and press the **Remove** button.

To change the order of selected overlay(s) in the legend 253, use the blue up and down arrows ( ).

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For a brief tutorial on how to use overlays, visit us at https://youtu.be/nVqwvDo1RmA.

Standard Overlay formatting options are explained in the table below; additional Overlay options specific to Spectrum Plots, which can greatly aid in analysis and report generation, are explained in detail here 924.

Option	Explanation
Spectrum	Change the laser line displayed on the spectrum plot.
Gate	If gates are defined 316, select the gate that will be used to limit the data that is displayed for each Overlay on the spectrum plot.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. Free text can be inserted. If this field is blank the file name of the overlay will appear. A dropdown menu with additional pre-customized legend content is accessible by pressing the ellipsis button next to the legend text. Possible choices are: ○ **Keyword**. This can be used to insert keywords from the header of the data file. ○ **Gate**. This can be used to display the current gate applied to the overlay. ○ **Tube Number**. This can be used to display the Tube Number of the overlay when a Panel 860 is active. ○ **Tube Name**. This can be used to display the Tube Name of the overlay when a Panel 860 is active. The above mentioned Legend options apply only to **Dot Plots**, **Color Dot Plots**, **Histograms** and **Spectrum Plots**.
Smoothing	Set the degree of smoothing for the overlay.
Change data file during Next/Prev/Batch	If this option is checked, the data file for this overlay will change when doing batch processing 784, or selecting Previous File or Next File 72. This option is usually only disabled for a control spectrum, where you have multiple overlays, and want the control to stay the same all of the time.
Visible	You can hide overlays on a spectrum plot without removing them. Select the overlay file from the list and uncheck **Visible**. Check **Visible** to show the overlay again.
Line Options	Choose the overlay line width, style, and/or color.

8.6.2 Spectrum Options

The **Spectrum Options** category contains the **Spectrum Plot Specific Options** 281, **Color Levels** 281, and **Display Gates Options** 282 (Figure 8.92).

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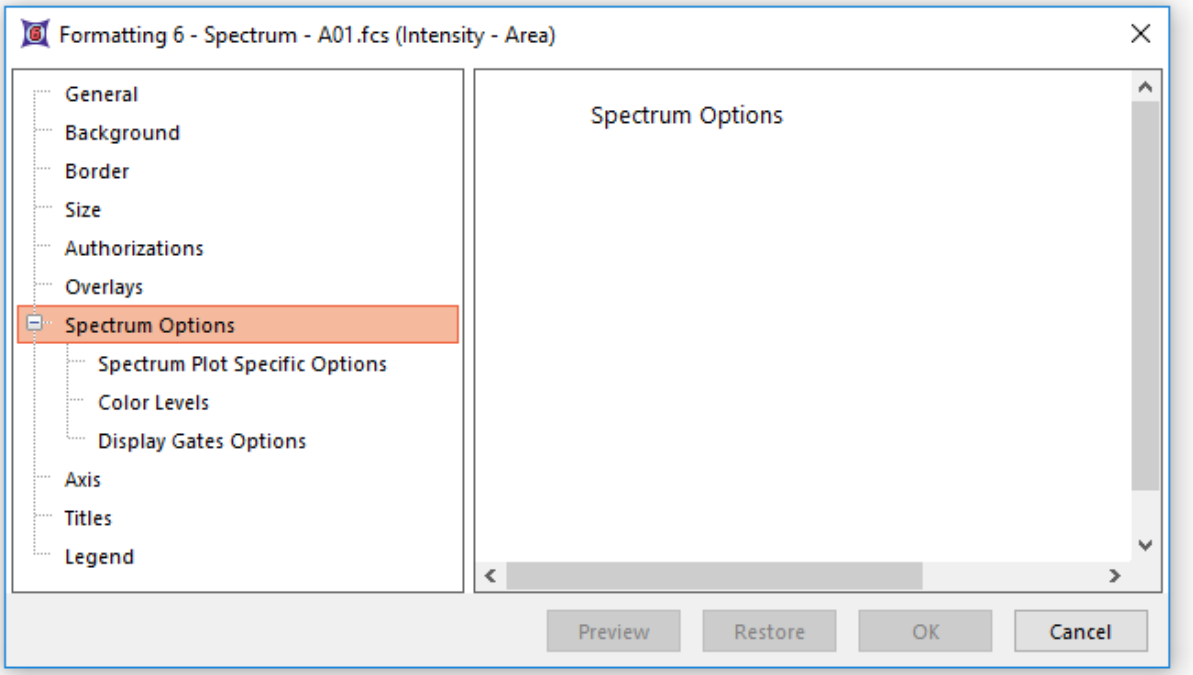

Figure 8.92 - Spectrum Options category

8.6.2.1 Plot Specific Options

Spectrum Plot Specific Options include Y Plot Resolution, Display Mode (Color on Gated/Overlays vs. Color on Density), Shade Based on Density, and Interpolate Values. For details on these options, please see the table in the Formatting a Spectrum Plot 924 topic.

8.6.2.2 Color Levels

Color Level options for Spectrum Plots are enabled in **Color on Density** 925 Display Mode 925 only.

Color Levels for Spectrum Plots can be formatted in the same manner as for 2D-Density Plot Color Levels 295. Below is an example of a plot with customized color levels (Figure 8.93). Please also see the Formatting a Spectrum Plot chapter 926 for additional details and examples.

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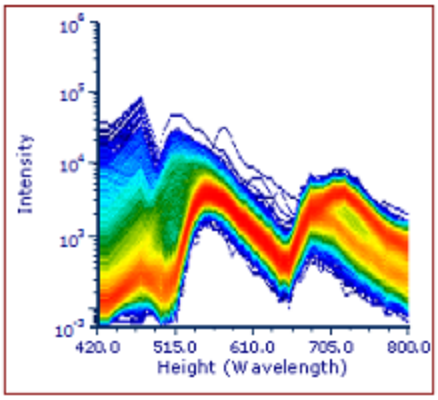

Figure 8.93 - The two Spectrum Plots in the center of the picture have customized Color Levels.

8.6.2.3 Display Gates Options

To edit Display Gates options, either

- Select the Spectrum Plot(s) and use the **Format** tab→ **Data Options→Gates to Display** command, or
- Right-click on the Spectrum Plot, select **Format** from the pop-up menu, and choose the **Spectrum Options→Display Gates Options** category (Figure 8.94).

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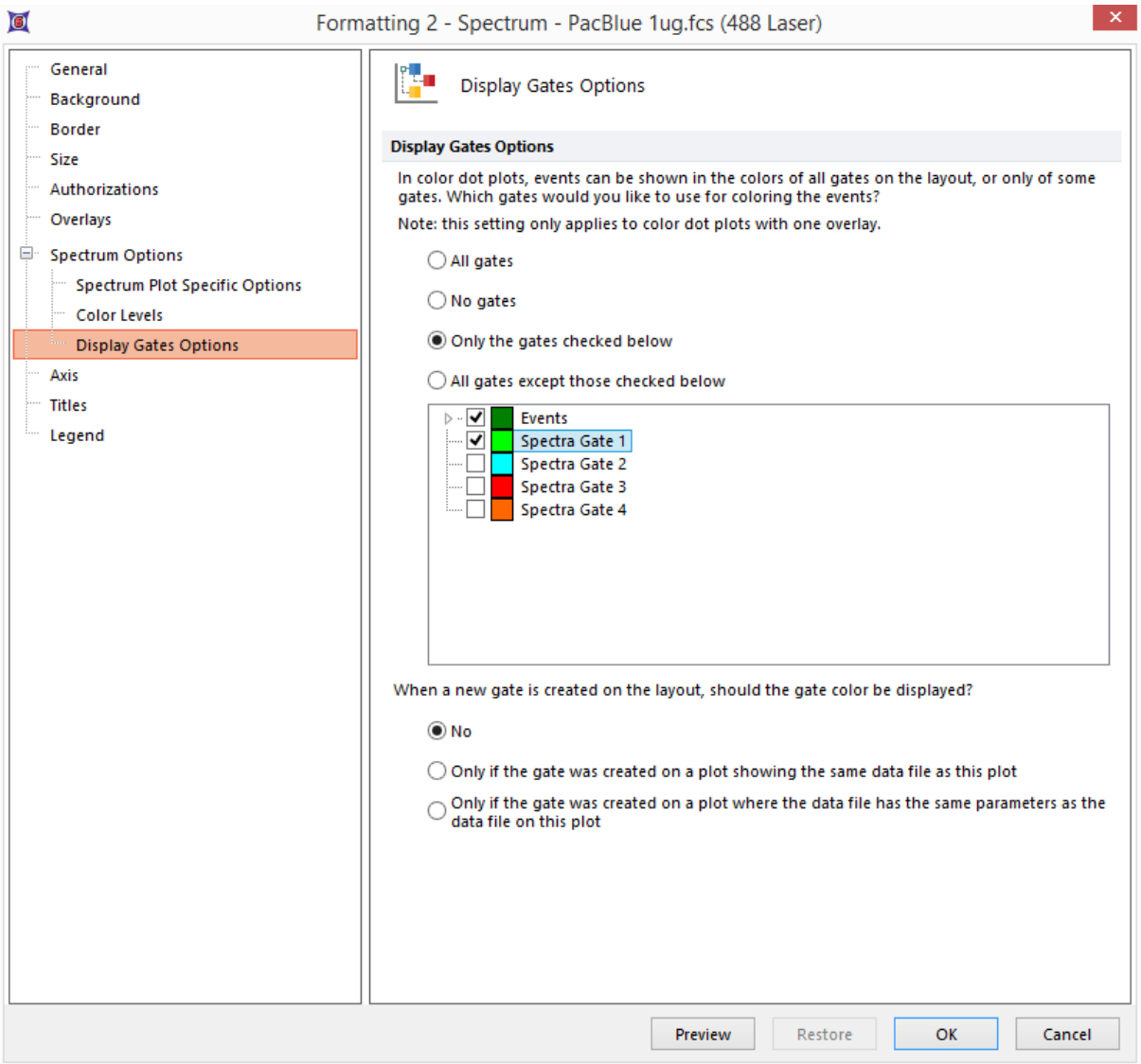

Figure 8.94 - Spectral plot with two overlays from different spectral datafiles, each with a different gate applied.

If you have **Average** set as the **Overlay to display**, the lines are shown as the **Line options** color. If you have **All spectrums** set as the **Overlay to display**, the colors of each line will reflect the color of the gate in which the event falls. If an event falls in more than one of the selected gates, then the line will be drawn in the highest priority gate.

By default, the **All Gates** option is selected. However, you can select **Only the gates checked below** and then select the gates whose colors you wish to display. Alternatively, select **All gates except those checked below** and then choose the gates whose colors you do not wish to display.

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You can also choose how to display the gate color when a new gate is created on the layout. By choosing either the second or third radio buttons in the **When a new gate is created on the layout** category, you can have FCS Express display the gate color on a back-gated plot if the new **gate was created on a plot showing the same data file**, or only **on a plot showing the same parameters** (as the plot where the new gate was created).

8.7 Formatting 2D Plots

In addition to the formatting options common to all objects 239, and those common to all plots 246, 2D plots have certain properties, such as overlays 284, quadrants 291, gates to display 303, color levels 295 for density and contour plots, and other specific options 305 which can be formatted.

You can always format a 2D plot by right-clicking on the plot, selecting **Format** from the pop-up menu, and choosing a category. This method will allow you to use the **Preview** button to see what the changes would look like without actually accepting the changes. Alternatively, once the 2D plot is selected, you can choose the desired category from the Format tab of the Ribbon 73.

8.7.1 Overlays

All 2D plots have at least one overlay representing a file and two parameters to display. With certain 2D plots (e.g., **Dot**, **Color Dot**, and **Scatter**), you can add additional overlays, by any of the following methods:

- Right-click on the plot and select **Add overlay** from the pop-up menu (launches a standard browser window called **Select Data File** 214), or
- Right-click on the plot and select **Add Overlay using Advanced Open Data Dialog** (launches the **Open Data File** 215 dialog), or
- Drag-and-drop file(s) from the **Data List** 785 onto the plot you wish to overlay, or
- Drag-and-drop a plot from the layout onto the plot you wish to overlay.

The **Overlays** option controls the parameters and appearance of 2D overlays.

You can edit overlays in one of two ways:

- Select the 2D plot and use the **Format→Plot Options→Overlays** command (Figure 8.95).

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- Right-click on the 2D plot, select **Format** from the pop-up menu, and choose the **Overlays** category.

Different and specific overlay options are available for the following 2D Plot types:

- Dot Plots and Color Dot plots 286
- Density plots and Contour Plots 287
- Scatter Plots 289

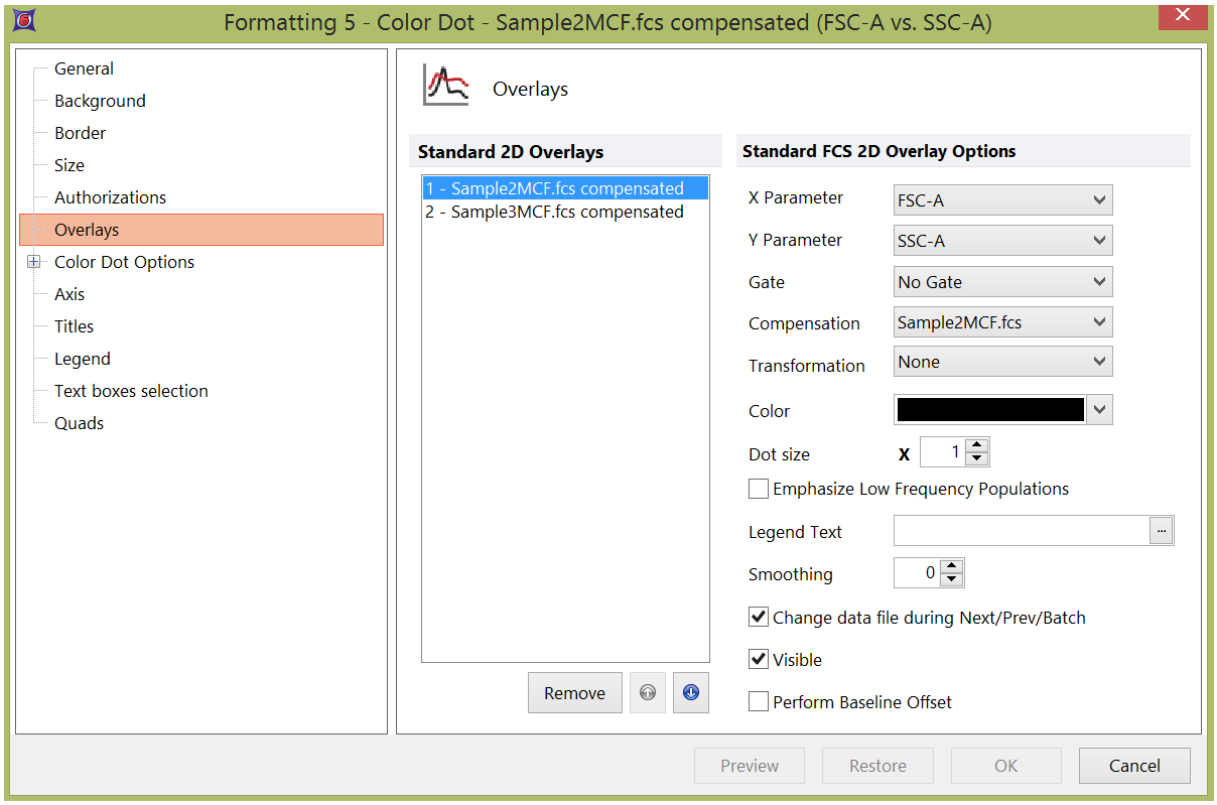

Figure 8.95 Formatting Overlays Dialog for 2D Dot Plots

In the dialog, a list of all the overlays on the plot will appear in the list box to the left. If the plot type permits multiple overlays, you can select one or more overlays using standard Microsoft Windows techniques, including **Shift**-Click and **Ctrl**-Click. The properties on the right pertain to the currently selected overlay(s). If multiple overlays are selected, the information will appear blank for those properties that are not identical in all selected overlays. If the properties are not blank, then they are identical for all selected overlays. Changing an option will change that property for all selected overlays. For instance, if you select three overlays and change the Gate, the Gate for all three overlays will be modified.

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To remove an overlay from a plot, select the overlay (or multiple overlays) and press the **Remove** button.

To change the order of selected overlay(s) in the legend, use the blue up and down arrows ( ).

For a brief tutorial on how to use overlays, visit us at https://youtu.be/nVqwvDo1RmA.

8.7.1.1 Dot and Color Dot Plot Overlays

For Dot plots and Color dot plots the following overlay options are available (Figure 8.96).

Option	Explanation
X Parameter	Change the parameter displayed on the X axis.
Y Parameter	Change the parameter displayed on the Y axis.
Gate	If gates are defined, select the gate that will be used to limit the data that is displayed on the 2D plot.
Compensation	If compensation definitions are set, select the definition to use when compensating the data in the overlay.
Transformation	If transformations are defined, select the transformation to use when displaying the data in the overlay.
Color	The color that the overlay will be drawn in (used for dot plots and color dot plots). The default overlay colors can be set on the 2D plot preferences page of the FCS express User Options Dialog.
Dot Size	FCS Express automatically calculates the dot size based upon the height and width of your plot. By changing this setting, you can force the dot size to be larger or smaller than what FCS Express calculates by the factor chosen in the spin box. *Note:* If you are using several overlays on color dot plots, and you want the overlays to have different dot sizes, then the plot must be set with Dither turned off.
Emphasize Low Frequency Populations	If the % gated for a given gate is < 2%, the dot size for those gated events will increase. Only available for Dot plots displaying gate colors and Color Dot plots.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. Free text can be inserted. If this field is blank the file name of the overlay will appear. A dropdown menu with additional pre-customized legend content is accessible by pressing the ellipsis button next to the legend text. Possible choices are: ○ **Keyword**. This can be used to insert keywords from the header of the data file. ○ **Gate**. This can be used to display the current gate applied to the overlay.

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Option	Explanation
	○ **Tube Number**. This can be used to display the Tube Number of the overlay when a Panel 860 is active. ○ **Tube Name**. This can be used to display the Tube Name of the overlay when a Panel 860 is active. The above mentioned Legend options apply only to **Dot Plots**, **Color Dot Plots**, **Histograms** and **Spectrum Plots**.
Smoothing	Set the degree of smoothing for the presentation of your data.
Change data file during Next/Prev/Batch	If this option is checked, the data file for this overlay will change when doing batch processing 784, or selecting Previous File or Next File 72. This option is usually only disabled for control histograms, where you have multiple overlays, and want the control to stay the same all of the time.
Visible	Controls whether or not to display this overlay on the 2D plot.
Perform Baseline Offset	If the value of the cell is zero, a random offset will be added so that the dot appears off the axis. This will not affect the statistics. This option is typically used when analyzing LMD files from Beckman Coulter.

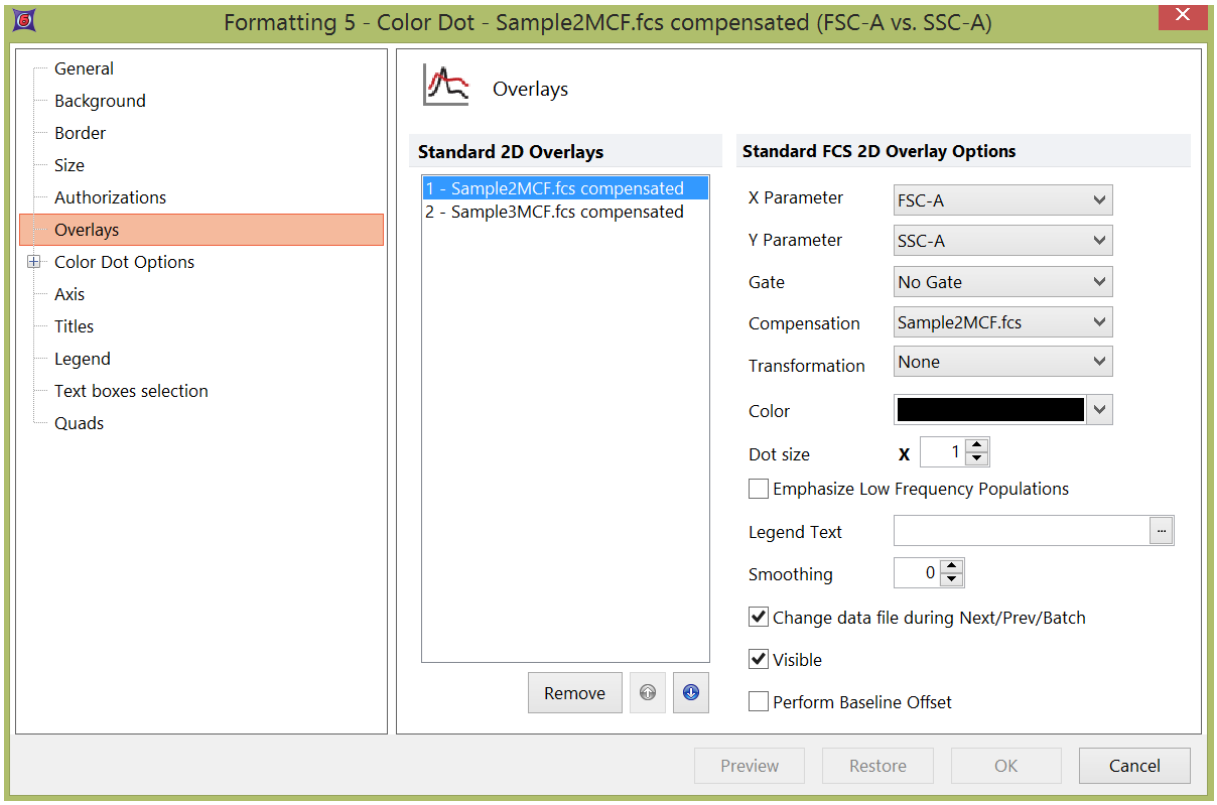

Figure 8.96 Formatting Overlays Dialog for 2D Dot Plots

8.7.1.2 Density and Contour Plot Overlays

For Contour and Density plots, the following overlay options are available (Figure 8.97).

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Option	Explanation
X Parameter	Change the parameter displayed on the X axis.
Y Parameter	Change the parameter displayed on the Y axis.
Color based on	Choose from Count or available parameters to base the density display color palette on. For instance if FSC and SSC are displayed on the X and Y and FL1 is chosen for **Color Based on** then the density display will represent the distribution of FL1 fluorescence on the FSC vs SSC plot. Using Color Based on is a common and useful display type for working with tSNE transformed data sets 1000 where it is useful to view tSNE X vs tSNE Y parameters on separate plots for each measured parameter with the **Color based on** for each plot representing a different parameter. The parameter used by this option, can also be selected via the the legend title 253.
Gate	If gates are defined 316, select the gate that will be used to limit the data that is displayed on the 2D plot.
Compensation	If compensation definitions 626 are set, select the definition to use when compensating the data in the overlay.
Transformation	If transformations are defined 932, select the transformation to use when displaying the data in the overlay.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. If this field is blank the file name of the overlay will appear. Insert keywords from the header of the FCS file by pressing the ellipsis next to the legend text. You can also display the current gate applied to the overlay by inserting "<$FCSE_GATEFORMULA>" into the legend text.
Smoothing	Set the degree of smoothing for the presentation of your data. Smoothing is performed with a box-filter algorithm. The Smoothing parameter controls how many iterations of smoothing are performed.
Outlier Display Mode	Choose from **Do not show**, **Use color below**, **Use lowest color level**. **Do not show** will not display any outliers; **Use color below** will use the **Outlier Dot Color** chosen in the drop down below for the outlier color; **Use lowest color level** will set the color of the outliers to the lowest color level displayed in the density plot.
Outlier Dot Color	If **Use color below** is chosen then the **Outlier Dot Color** selected in the color palette will be used to display outliers.
Outlier Dot size	Choose the size of the dots used to display outliers.
Change data file during Next/Prev/Batch	If this option is checked, the data file for this overlay will change when doing batch processing 784, or selecting Previous File or Next File 72. This option is usually only disabled for control histograms, where you have multiple overlays, and want the control to stay the same all of the time.
Visible	Controls whether or not to display this overlay on the 2D plot.
Perform Baseline Offset	If the value of the cell is zero, a random offset will be added so that the dot appears off the axis. This will not affect the statistics. This option is typically used when analyzing LMD files from Beckman Coulter.

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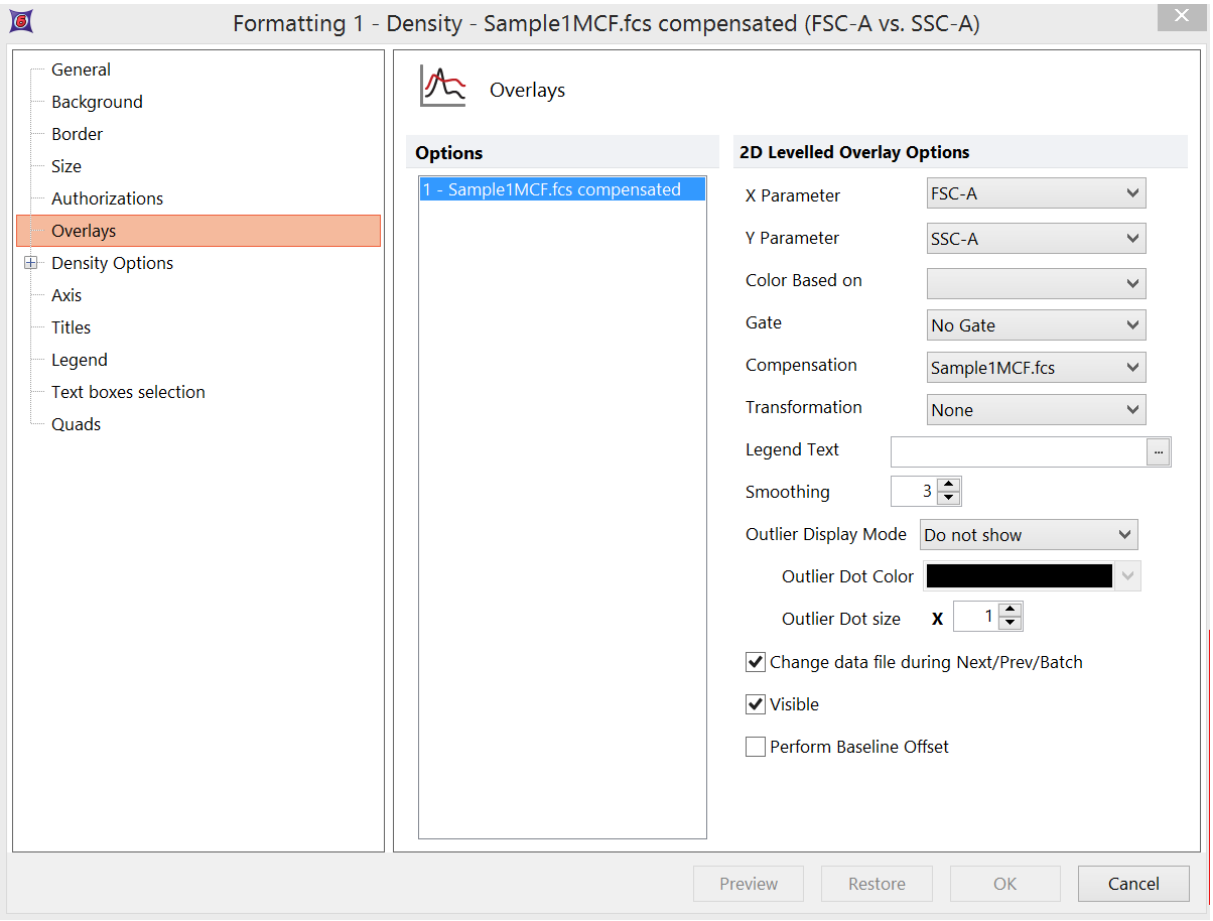

Figure 8.97 Formatting Contour or Density Plots

8.7.1.3 Scatter and Scatter with Regression Plot Overlays

For a full description of working with Scatter Plot specific features such as Error Bars and Highlight parameters please see the Formatting Scatter Plots topic 565 and Scatter Plots Overview topic 559.

Special overlay formatting options for Scatter and Scatter with Regression Plots are indicated by number and referenced in the table and image below (Figure 8.98).

- **Point Color** (❶)
- **Point size** (❷)
- **Point shape** (❸)
- **Highlight Param**(❹)

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- **Highlight Color**(❺)
- **Error Bar Param** (❻)

The formatting options are described in the table below.

Option	Explanation
X Parameter	The parameter (column) from the Spreadsheet 561 displayed on the X axis.
Y Parameter	The parameter (column) from the Spreadsheet 561 displayed on the Y axis.
Error Bar Param. ❻	The parameter (column) from the Spreadsheet displayed as error bars. See below 565 for more information on how to define the error bars.
Highlight Param. ❹	The parameter (column) from the Spreadsheet from which the highlighting rule derives. Points will be highlighted (e.g., the orange point in the plot below 567) in the color defined in ❺ if their value meets the criterion defined by the Highlighting rule. See below 565 for more information on how to define a highlighting rule.
Highlight Color ❺	The display color for highlighted points.
Gate	The gate that is applied to the Scatter Plot.
Point Color ❶	The display color for points.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. Free text can be inserted. If this field is blank the file name of the overlay will appear.
Point size ❷	The size of the points.
Point shape ❸	The shape of the points. Choose between rectangle (default), triangle, circle, cross, star, hexagon, and diamond options.
Connect the points	Whether the points are connected by a line.
Change data file during Next/Prev/Batch	Whether the Scatter Plot changes during batch processing 784 or in response to a Next/Previous 79 command. It is generally recommended to leave this unchecked (default), as the Scatter Plot will update automatically as the data to which it refers is changed.
Visible	Hides overlays without removing them. Select the overlay file from the **2D Unbinned Overlays** list and uncheck **Visible**. Check **Visible** to show the overlay again.
Show marks	Displays the value of the points as text directly above the points.
Line Options for ❼	Toggle between Error Bar Pen, Shape Border, and Connecting Lines editing mode.
Error Bar Pen/Shape Border/Connecting Lines Properties ❽	Width, Style (solid, dotted, and dashed options), and Color of the Error Bar Pen, Shape Border, or Connecting Lines.

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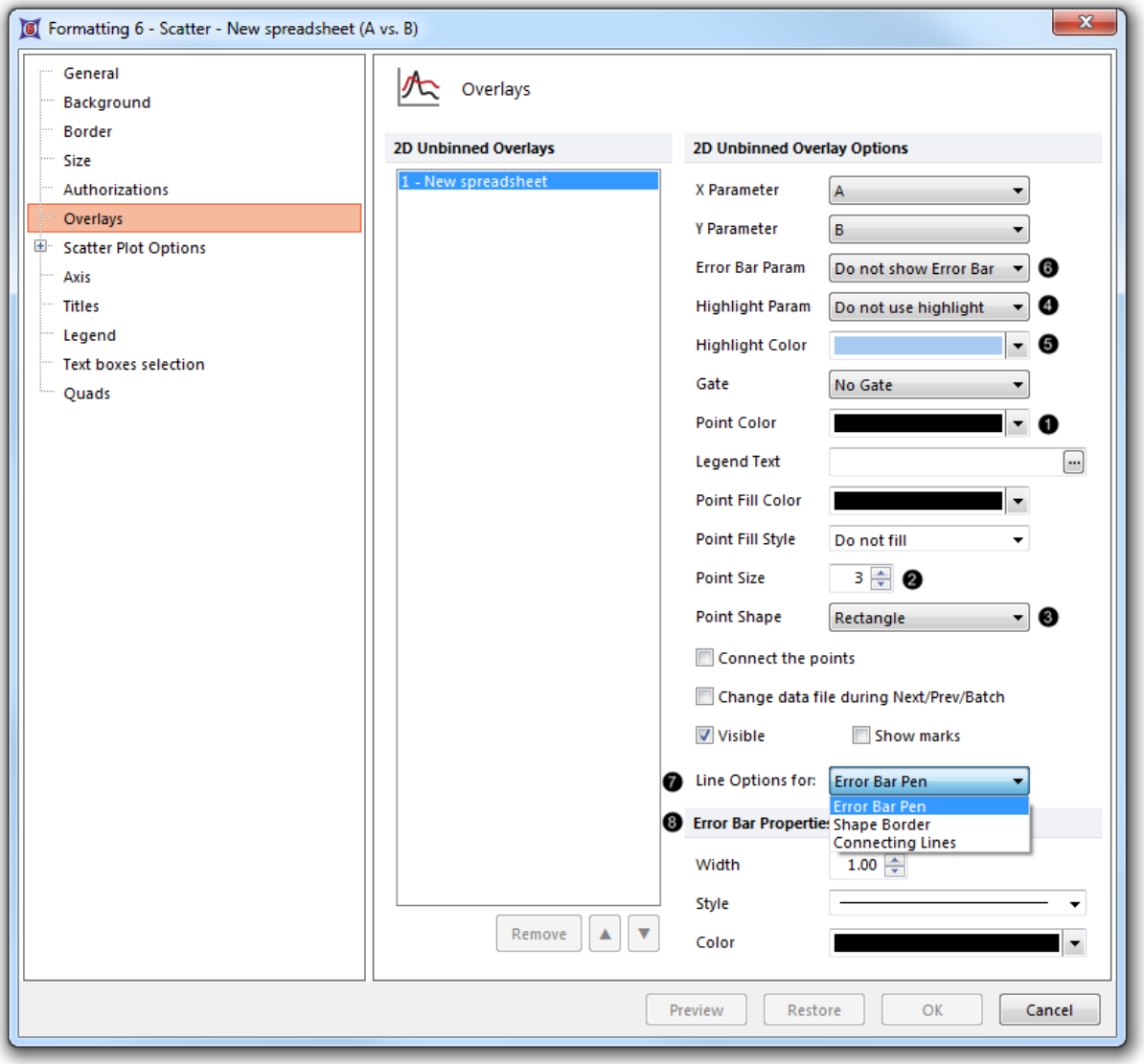

Figure 8.98 Special formatting options for Scatter and Scatter with Regression Plots

8.7.2 Quadrants

The **Quadrants** category allows you to manipulate the quadrants on a 2D plot. Quadrants can also be created and their positions adjusted directly from the plot 368.

You can edit quadrants in one of two ways:

- Select the 2D plot(s) and use the **Format→2D Options→Quadrants** command.

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- Right-click on the 2D plot, select **Format** from the pop-up menu, and choose the **Quads** category (Figure 8.99).

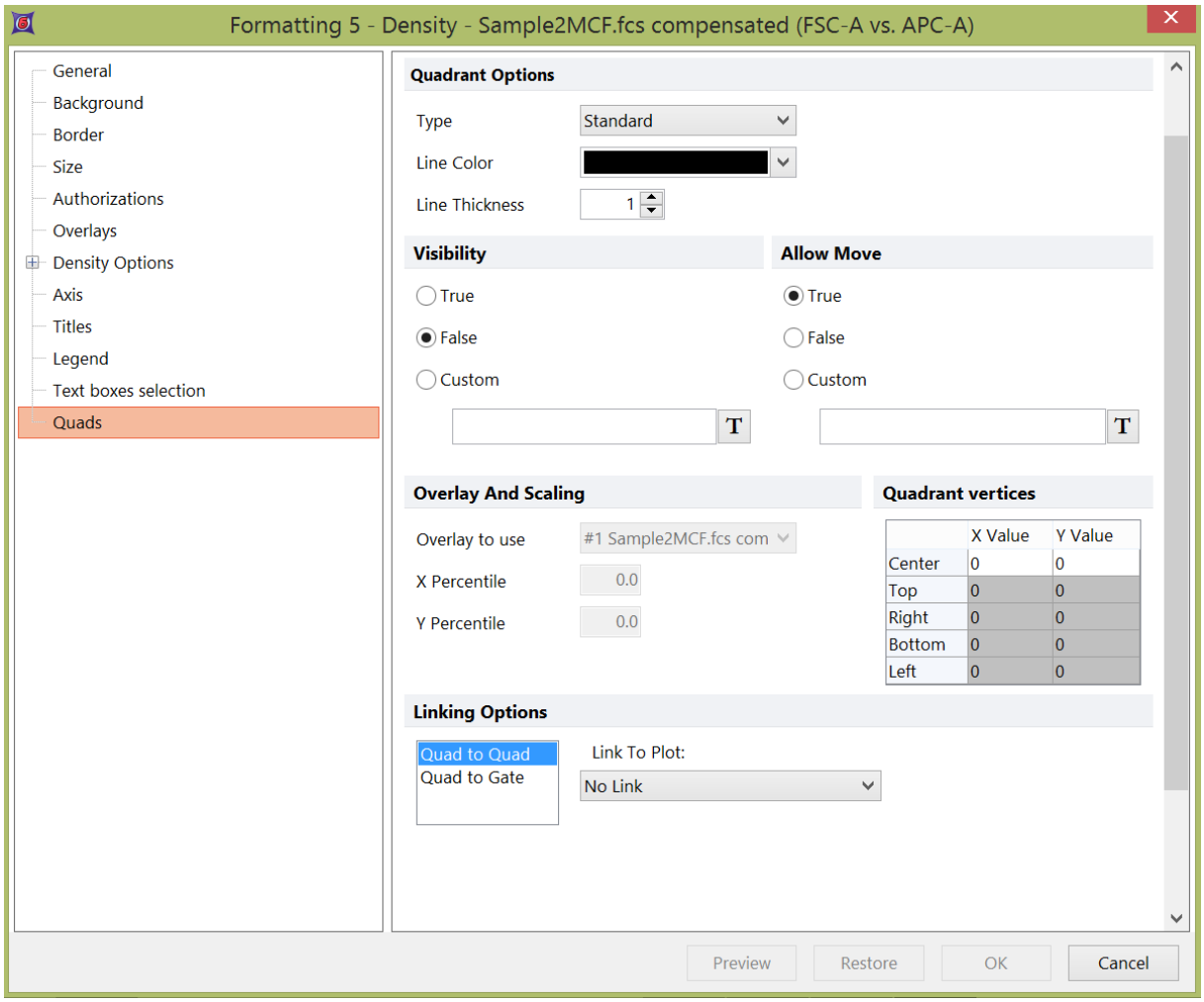

Figure 8.99 Formatting Quadrants dialog

The options on the left side of the dialog are explained in the table below.

Option	Explanation
Type	The type can be one of the following: • **Standard**: All four quadrants are rectangular. • **Floating**: Quadrants can be any shape • **X Percentile**: The quadrants are rectangular, and the X position of the quadrant is determined automatically based on the value in the X Percentile edit field. Please refer to the note at the bottom of this chapter 294 for details about how out of range events are handled by percentile quadrants. • **Y Percentile**: The quadrants are rectangular, and the Y position of the quadrant is determined automatically based on the value in the Y Percentile edit field.Please refer to the note at the bottom

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Option	Explanation
	of this chapter 294 for details about how out of range events are handled by percentile quadrants. • **Both Percentile**: The quadrants are rectangular, and the X and Y positions of the quadrant are determined automatically based on the value in the X Percentile and Y Percentile edit fields, respectively. Please refer to the note at the bottom of this chapter 294 for details about how out of range events are handled by percentile quadrants.
Line color and **thickness**	The color and thickness that the quadrant lines will be drawn in.
Visibility	Choose to show the quad "**True**", hide a quad without removing it "**False**", or make it visible based on a conditional value "**Custom**". The **Custom** option allows you define visibility of the quad based on a token or value that evaluates to a conditional expression 747. Any tokens or values may be used to set a conditional custom value and may be inserted 692 via right click in the field, **Insert Token** or by clicking on the **T** token icon. The quad will only be visible if the token evaluates to True.
Allow Move	Choose to allow moving the quad "**True**", disallow a quad move "**False**", or make moving the quad based on a conditional value "**Custom**". The **Custom** option allows you define the move status of the quad based on a token or value that evaluates to a conditional expression 747. Any tokens or values may be used to set a conditional custom value and may be inserted 692 via right click in the field, **Insert Token** or by clicking on the **T** token icon. The quad will only be allowed to move if the token evaluates to True.
Quadrant vertices	The precise coordinates of the quadrant vertices. These can be entered manually for precise control over quadrant positioning. *Note:* Quadrants are stored in 256x256 resolution; therefore, not all possible values for quad vertices will be available.
Overlay to use	Which overlay to use when calculating the percentiles for the X Percentile and Y Percentile quadrant types.
X Percentile	Which percentile to use for the X Percentile and Both Percentile quadrant types.
Y Percentile	Which percentile to use for the Y Percentile and Both Percentile quadrant types.

Quadrant Linking Options

Quadrants can either be linked to other quadrants 371 or to gates 371. You can change the current links from the **Linking Options** area of the dialog.

To change the quadrant to quadrant link, select **Quad to Quad** and choose the plot you want to link to the current plot. The quadrants on the current plot will become a "slave" to the quadrants on the linked plot, i.e., when the properties of the quadrants on the linked plot change, the quadrants on the current plot will also change. For example, by linking plots to an isotype control plot, the quadrants in the linked plots will automatically adjust if the isotype control quadrants are adjusted.

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If quadrants on the plots have been converted and linked to gates371, these links can be changed or broken in the **Quad to Gate** dialog. The dialog will appear similar to Figure 8.100.

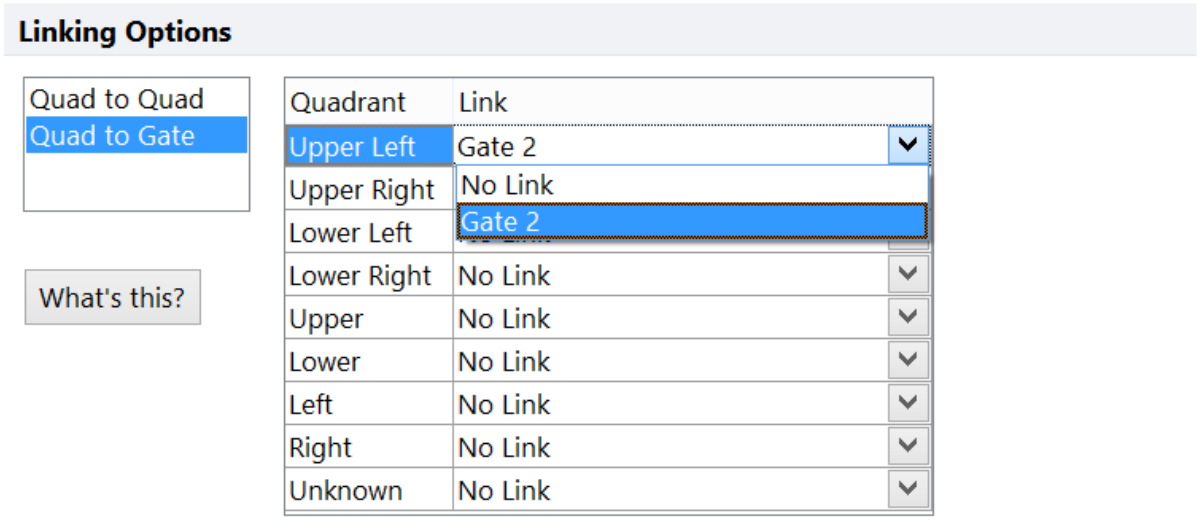

Figure 8.100 Linking Quad to Gate

In order to change or break the link, select the down arrow to the right of the drop-down list. Select **No Link** to break the link between the quadrant and the region. Below the **No Link** item will be a list of gates previously linked to other quadrants on this plot, and now available for linkage to the selected quadrant. A gate can only be linked to one quadrant in a layout. If the desired gate does not appear in the drop-down list, it is already linked to another quad on the current plot or is not derived from these quadrants at all.

Notes regarding percentile quadrants and out of range events

Sometimes a considerable number of events are out of range for a given parameter and thus are assigned to have the maximum value for that parameter. If the top X% of events all equal the maximum value on the plot then the vertex of the last X percentiles will all be set at the maximum value. For an example in which the top 30% of events are all set to the maximum value for a given parameter the vertex of all the percentiles quadrants above the 70th percentile will all be set at the maximum value (Figure 8.101 below. Note the middle two and far right plots.).

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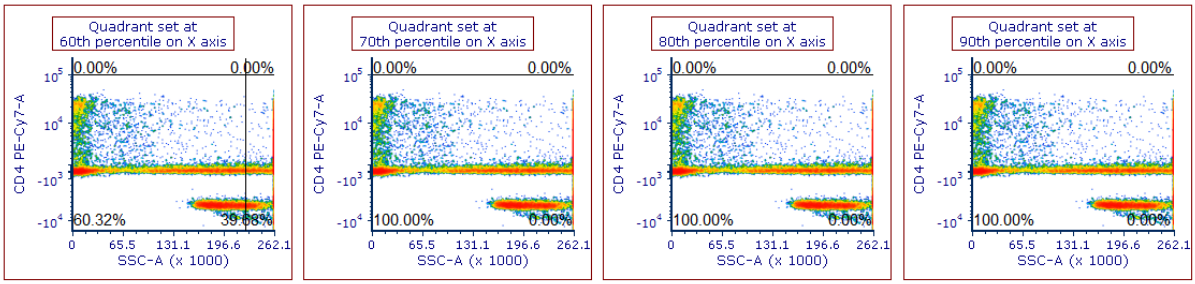

Figure 8.101 In this FCS file more than 30% of the events are set at the maximum value for the SSC parameter. Percentile quadrants have been set at different percentiles of the SSC parameter. Note that the vertex of percentiles from the 70th percentile and above are all at the maximum values for the SSC parameter.

8.7.3 Color Levels

The **Color Levels** options are used to control how **Density** and **Contour** plot are colored. The significance of each color level can be displayed in the plot legend 253. In FCS Express 4, 25 discrete color levels were used to generate gradients in Density and Contour Plots. Since FCS Express 5, Density plots are rendered in 256 discrete color levels, resulting in a smoother color gradient.

You can edit the **Color Levels** for **Density** or **Contour** plots in one of two ways:

- Select **Density** or **Contour** plot(s) and select **Format→2D Options→Color Levels** from the ribbon bar (Figure 8.102).
- Right-click on a **Density** or **Contour** plot, select **Format** from the pop-up menu, and choose **Density Options→Color Levels** or **Contour Options→Color Levels**.

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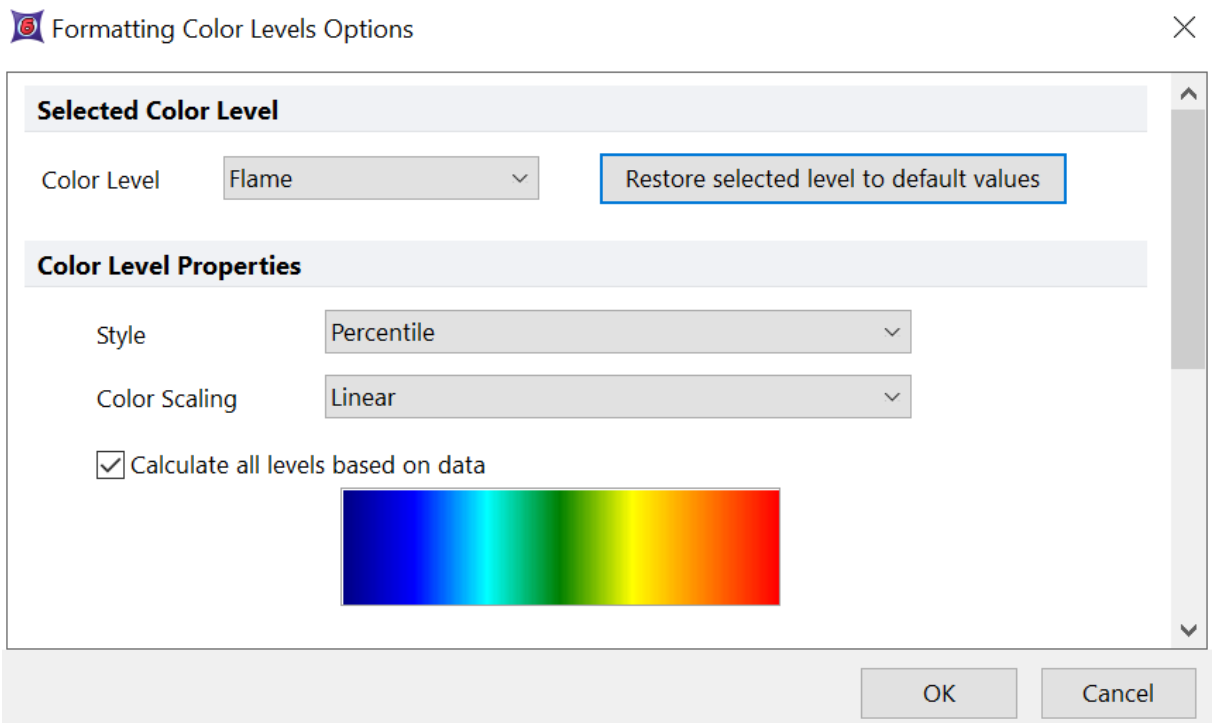

Figure 8.102 Contour Level Options

For **Density** and **Contour** plots, each bin is colored based on the value that bin has for a specific parameter. By default, both Density and Contour plot are colored based on density, thus based on the number of events included in each bin. However, since FCS Express 6, any parameters can also be used to represent a density color shading. The parameter the color is based on is the one shown in the legend[253] and can be changed both through the legend itself and through the Overlay[287] dialog (see the Color based on[288] option).

Note: Colors are calculated taking into consideration both Plot Resolution[305] and Smoothing[287]. The plot legend reports the actual values used to calculate the color.

Three options can be selected to customize the way bins are colored:

1. Color level

Color level options define the color palette (see figures below).

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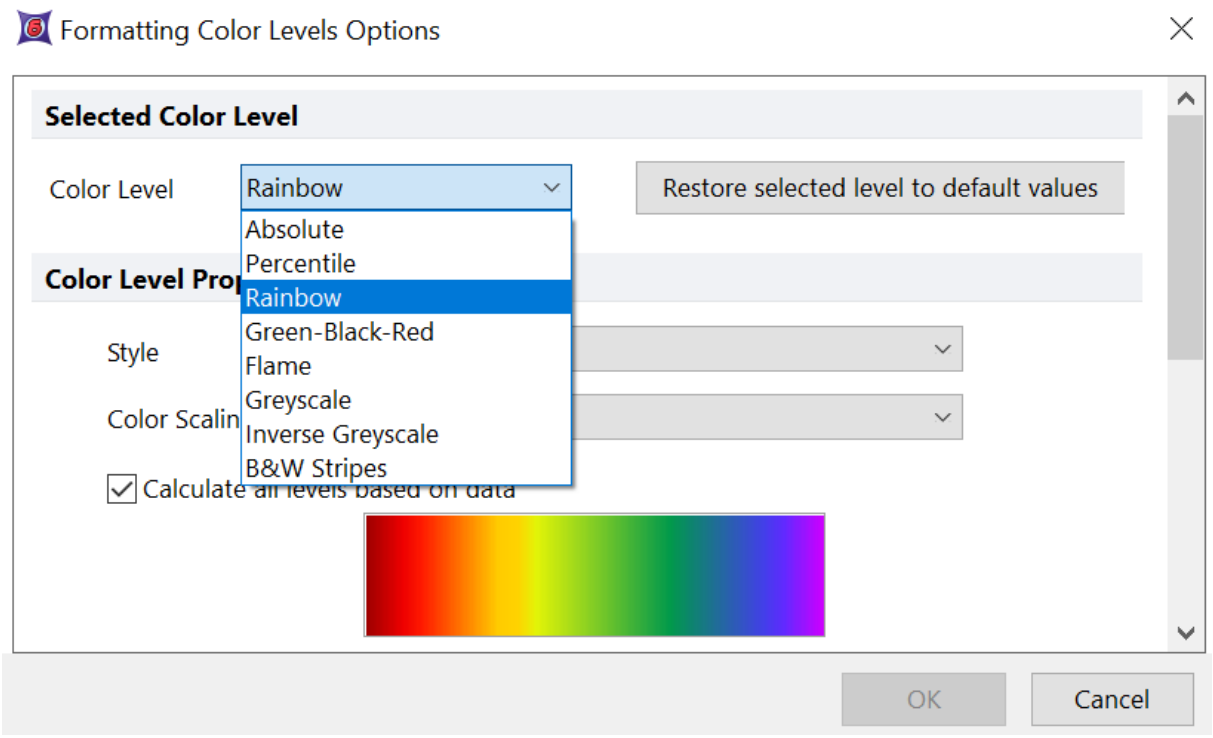
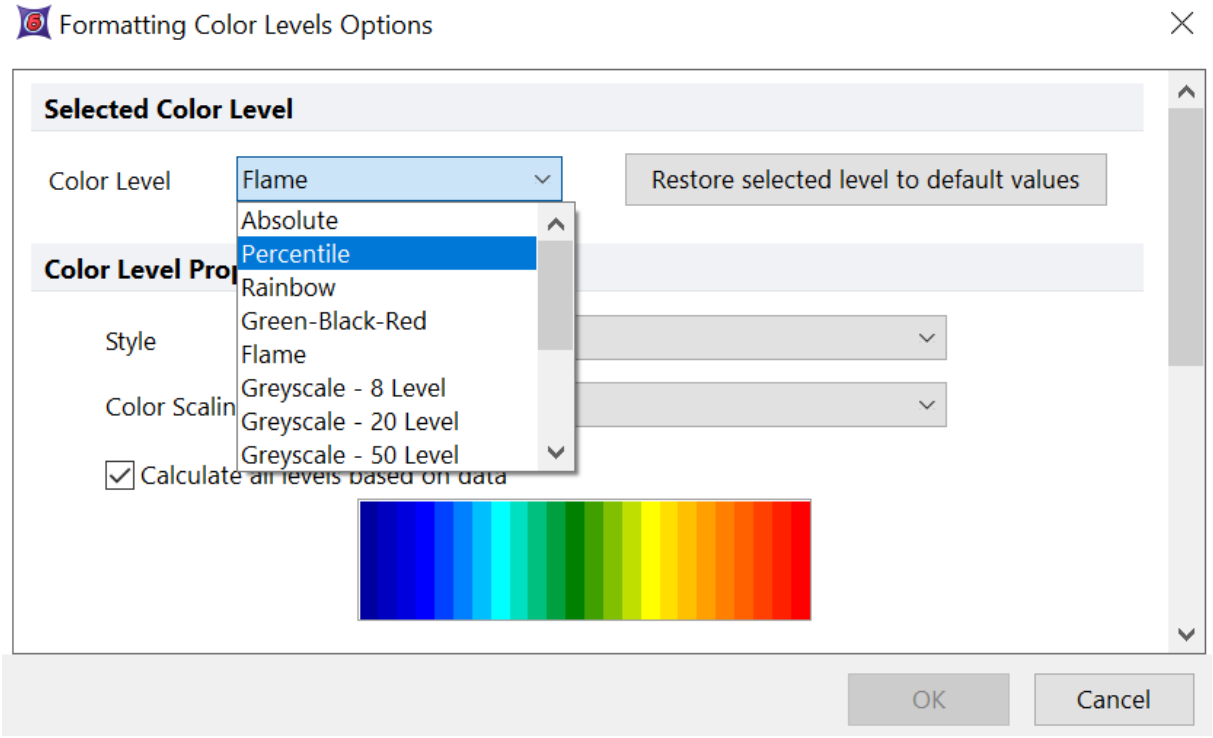


Color Level choices for density plots



Color Level choices for contour plots

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The choices in the list differ for each type of plot:

- Possible choices for **Density plots** are: Absolute or Percentile (the number of levels is the number of colors shown in the list), Rainbow (240 color levels), Green-black-red (250 color levels), Flame (252 color level), Greyscale and Inverse greyscale (255 color levels), B&W stripes (240 color levels).

- Possible choices for **Contour plots** are: Absolute or Percentile (the number of levels is the number of colors shown in the list), Rainbow (24 color levels), Green-black-red (8 color levels), Flame (24 color level). For the remaining choices the number of levels is specified in the drop down menu.

When you select a color level category, the colors in the chosen palette will be displayed in the bottom half of the dialog. In addition, the Preview and Restore buttons 239 in the Format dialog provide a convenient means for evaluating each color scheme with your data. Note that the Preview and Restore buttons 239 are available only when the Format dialog is accessed by right clicking on the plot. They are not available when the Format dialog is accessed via the Ribbon bar.

Most of the Color Level schemes are simply defined by their names, ie, Green-Black-Red uses those colors in the scheme, but two may require further explanation:

- **Absolute**. Color levels may be defined by the user based on absolute values (see below, **Color Level Properties**). If **Calculate Levels based on data** is checked, color levels are instead defined by the software.

- **Percentile**. Color levels may be defined by the user based on percentiles (see below, **Color Level Properties**). If **Calculate Levels based on data** is checked, color levels are instead defined by the software.

The color for an individual level can be changed by selecting the color and clicking the ellipsis inside the color box. A level can be added or removed by selecting the appropriate level and pressing the (Add) or (Remove) buttons. To access these settings for plots displaying Color Levels other than Absolute and Percentile, uncheck Calculate all levels based on data 299.

When selecting grayscale, the shades of gray are automatically displayed for you. You will not be able to edit the shades for an existing level.

You can return to the Rainbow style (or your current default option for 2D Contour or Density plots 191) by clicking on the **Restore selected level to**

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default values button. Clicking the **Default** button will also update any plots created in a Version 4 layout to the newest default color palette for the current version.

2. Style

Style options defines how color levels are used to color the data.

- **Fixed range.** The range of the data in the parameter that was selected for the coloring is divided into evenly spaced color levels. Settings may also be manually defined rather than based on data. 299
- **Percentile.** The range of the data in the parameter that was selected for the coloring is divided into color levels, so that each color level contains the same number of data causing the colors to be centered around the median. Settings may also be manually defined rather than based on data. 299
- **Mean +- SD.** The range of the data in the parameter that was selected for the coloring is divided into color levels, so that the central level is centered around the mean of the data and the color shapes are normally distributed based on the standard deviation specified by the user.
- **Threshold**. The range of the data in the parameter that was selected for the coloring is divided into only two levels regardless of the **Color Level** selected. The two colors can be customized by the user. The color of each bin will be decided based on the threshold specified by the user.

3. Color Scaling

When Logarithmic is selected, the data (i.e. bin values) are log-transformed before dividing the range of the data into color levels.

4. Unchecking Calculate all levels based on data - Manual Settings

For **Fixed range** and **Percentile** styles you may uncheck the **Calculate all levels based on data** check box to manually define your color level settings or fix the low and high levels with two settings.

- **Specify the low and high level; the rest will be calculated automatically**, Figure 8.103: User may enter any value within limits for the low and high bounds of the color levels. Intermediate increments for the color levels will be calculated automatically using the current **Color**

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Level choice. This option is particularly useful when comparing plots with tSNE transformations applied and visualizing different color based on parameters in order to visualize color mappings within the same range/scale.

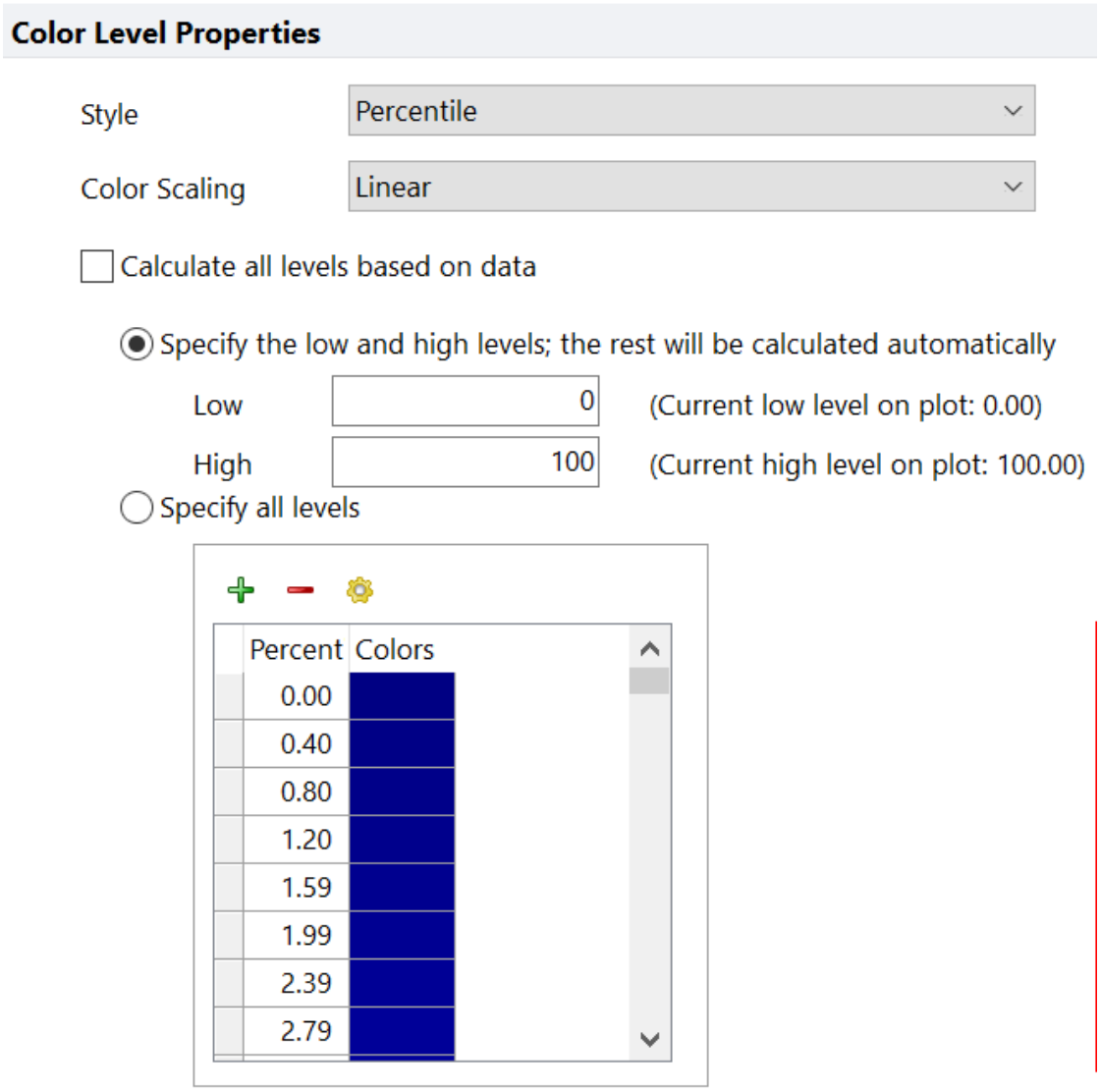

Figure 8.103 - Choosing Specify the low and high levels; the rest will be calculated automatically allows users to set a fixed low and high bound for color scaling.

- **Specify all levels**: The user may specify any levels manually by clicking on the green **+** button to add a value or the red **-** button to remove a color level. Alternatively, you may click on the yellow gear icon to launch the **Increment Information** dialog, Figure 8.104, to more automatically set increment levels based on user input of the lowest and highest color

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level, and how many increments to add in between. The Increment Information dialog will allow you to adjust the following settings in the table below.

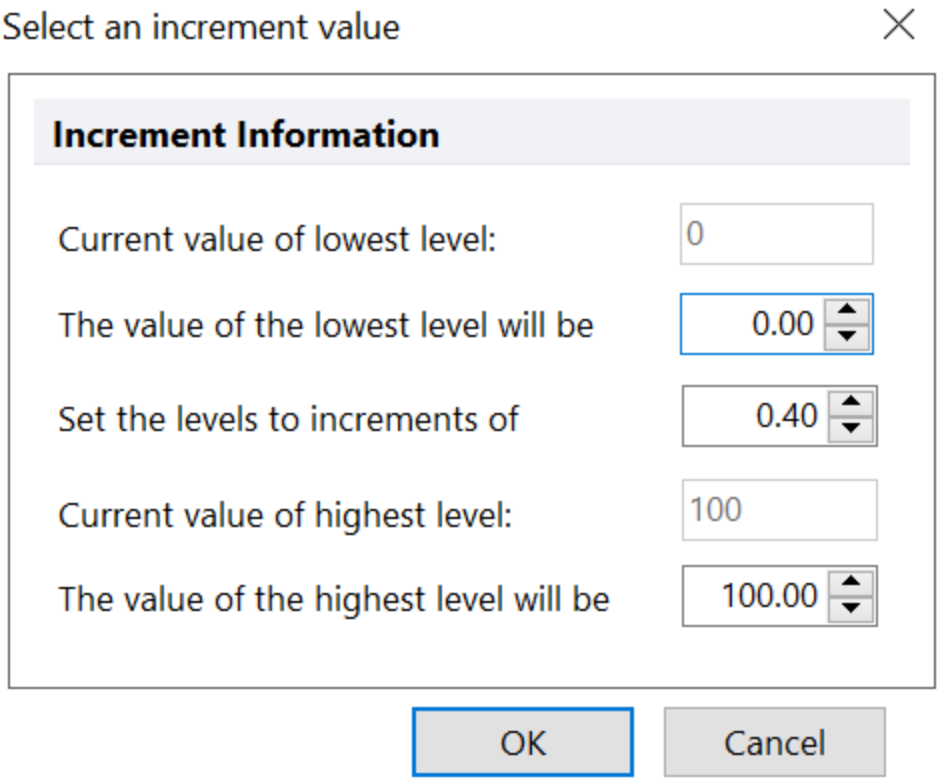

Figure 8.104 - The Select an increment value dialog allows you to automatically set increments for color levels based on the desired low value, high value, and increment value between discreet color levels to use.

Setting	Definition
Current value of lowest level	the value of the lowest color level currently showing on the plot.
The value of the lowest level will be	the new value of the lowest color level you would like to set.
Set the levels to increments of	the incrementation value between discreet color levels that you would like to set.
Current value of highest level	the value of the highest color level currently showing on the plot.
The value of the highest level will be	the new value of the highest color level you would like to set

The color gradient created will be based on the colors of the first and last color in the list, figure 8.105. For instance, if the first color level is purple and the last color level is black you will arrive at a purple to black color gradient. Clicking on ellipsis button (**...**) on the individual color

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levels will reveal the color palette to choose which colors to use for the first and last color level.

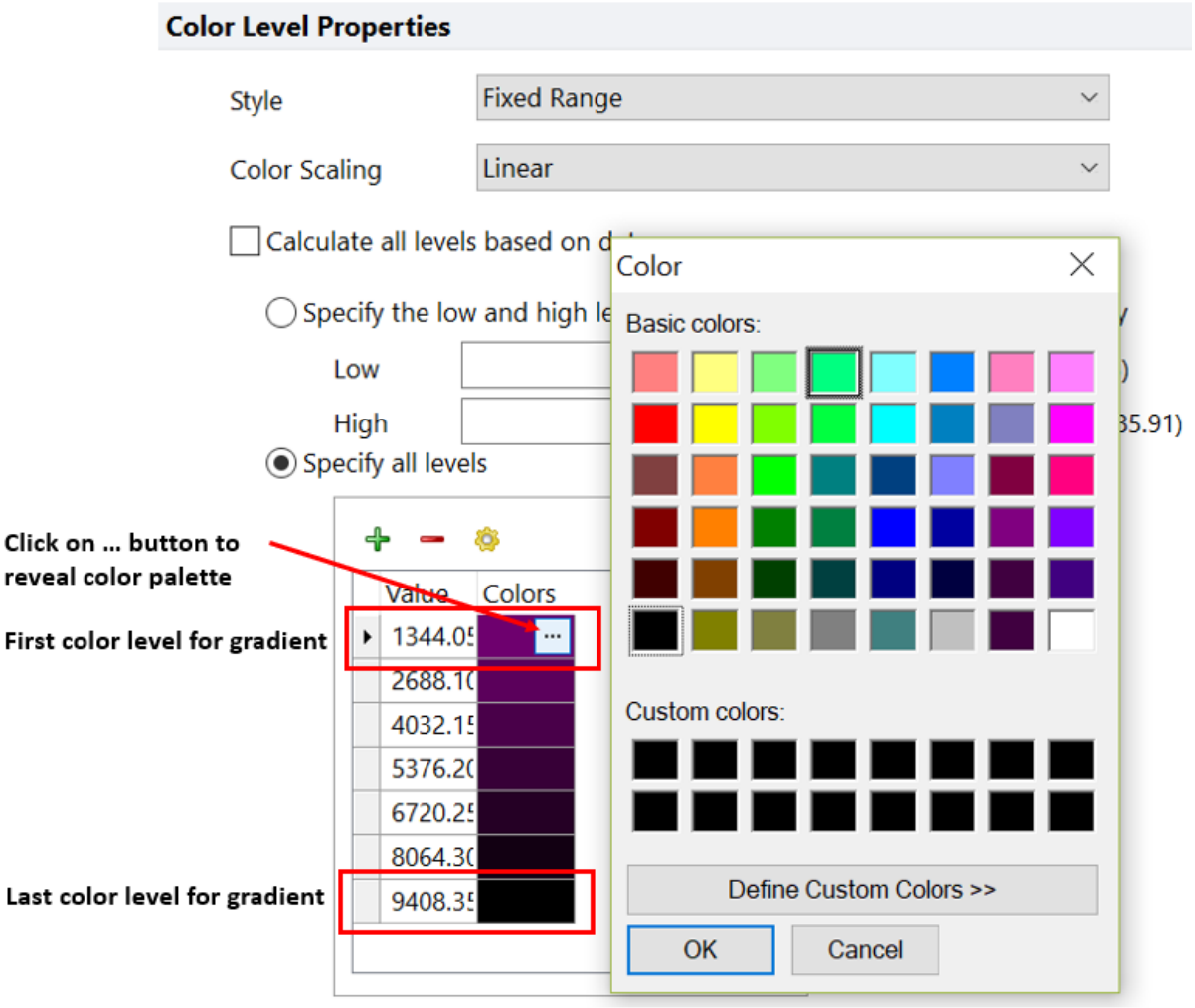

Figure 8.105 - The first and last color levels in the list will determine the beginning and ending colors for the gradient. Clicking on the ... button within the color will allow you to pick a new color for the color palette.

Examples

Figure 8.106 below depicts some examples of plots colored with different combinations of **Color Levels**, **Styles** and **Color Scaling**:

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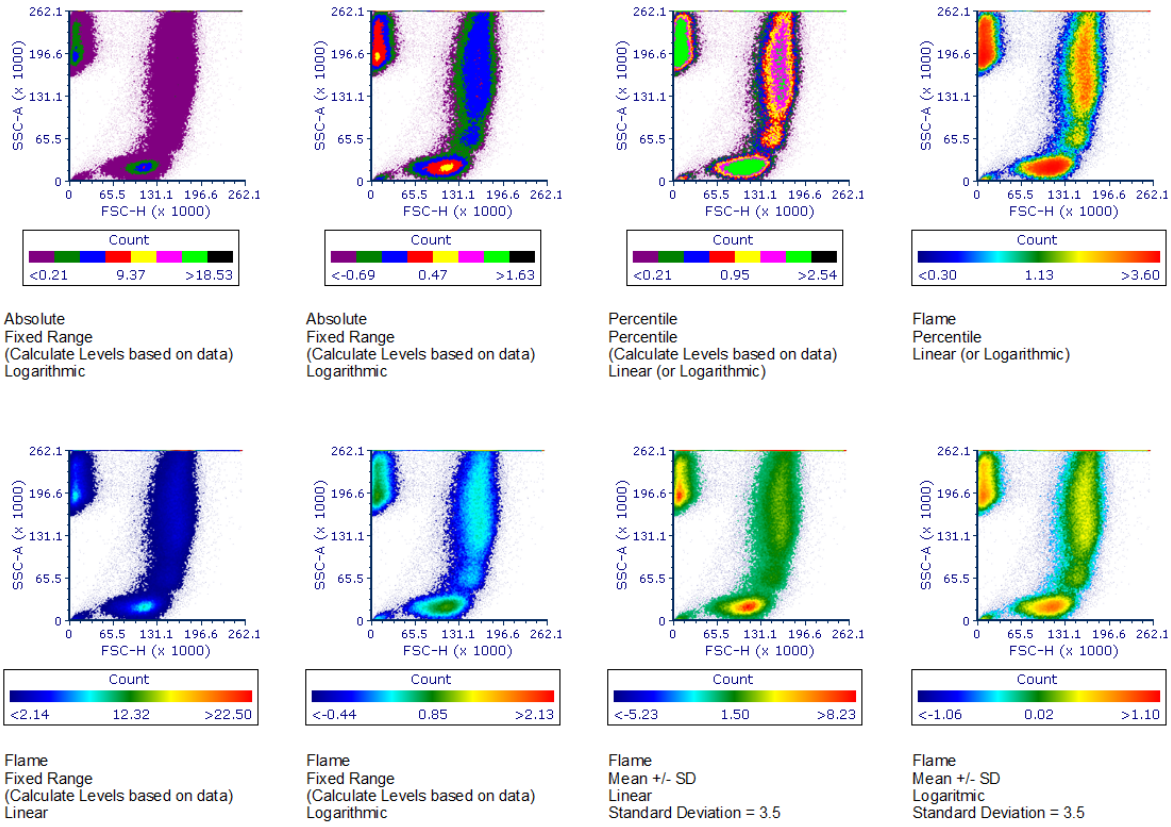

Figure 8.106. Example of different color combinations. The text below each plot reports the Color Levels, the Styles and the Color Scaling used. Additional setting (e.g. "Calculate Levels based on data" and "Standard Deviation") are also reported.

8.7.4 Gate Display

The **Gates to Display** formatting options are for setting which gates to use to color events. Gate display functions are available for dot plots, color dot plots, and scatter plots. You can also choose which gates to display in a data grid 680.

You can edit the Gates to Display in one of two ways:

- Select the plot(s) and use the **Format→Data Options→Gates to Display** command (Figure 8.107).
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Display Gates Options** category (located under the Plot Options category for each specific type of plot).

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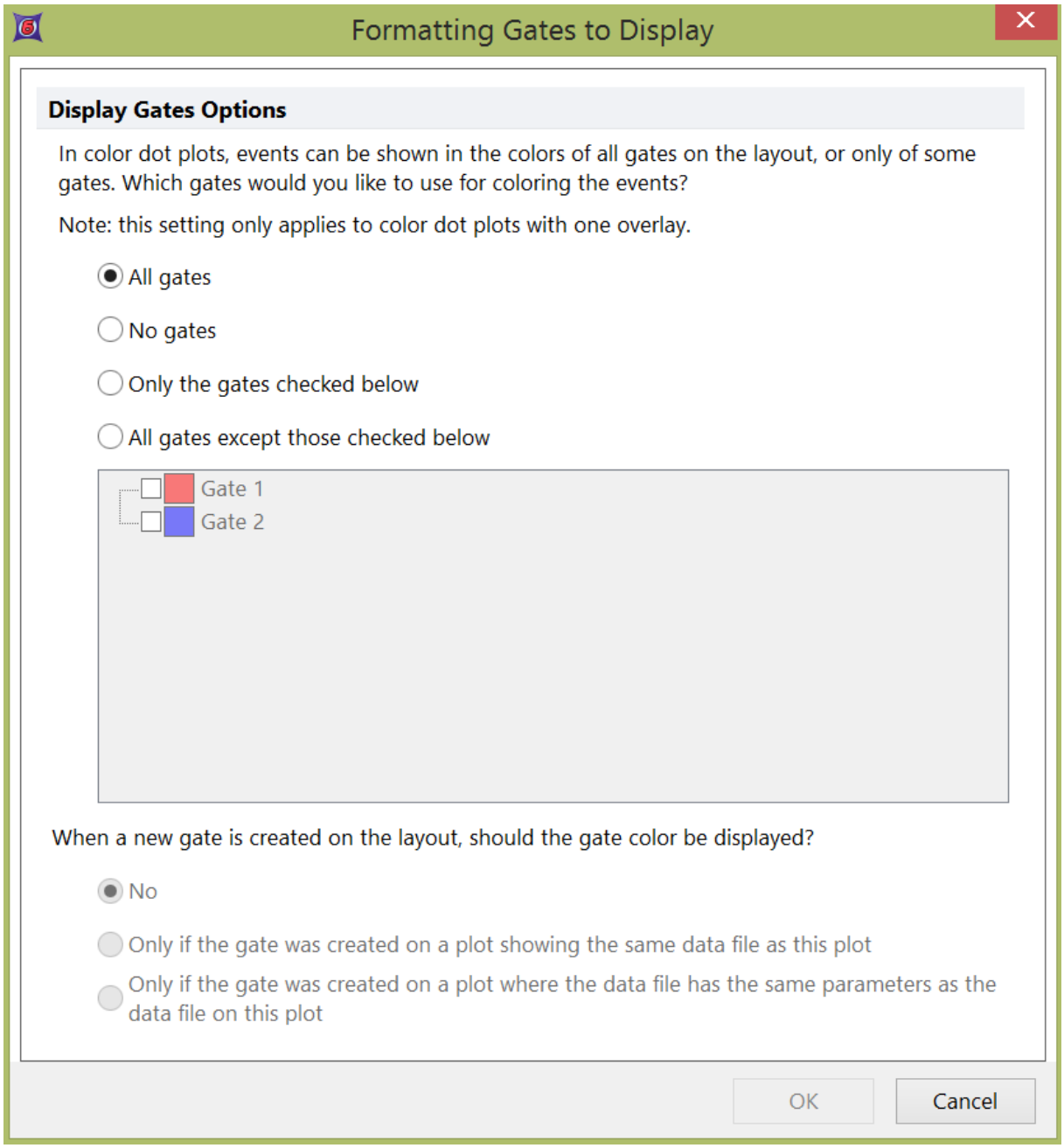

Figure 8.107 Formatting Gates to Display dialog

If you have one overlay on a dot plot, the dots are shown in the gate color. If you have more than one overlay, the colors will reflect the different overlays, which are specified in the Overlays 268 section of the Format window.

By default, the **All Gates** option is selected. However, you can select **Only the gates checked below** and then select the gates whose colors you wish to display. Alternatively, select **All gates except those checked below** and then choose the gates whose colors you do not wish to display.

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You can also choose how to display the gate color when a new gate is created on the layout. By choosing either the second or third radio buttons in the **When a new gate is created on the layout** category, you can have FCS Express display the gate color on a back-gated plot if the new **gate was created on a plot showing the same data file**, or only **on a plot showing the same parameters** (as the plot where the new gate was created).

8.7.5 Specific Options

The **Specific Options** category contains properties to control the way data is displayed on 2D plots.

You can edit 2D plot specific options in one of two ways:

- Select the 2D plot(s) and use the **Format→Plot Options→Specific Options** command. This will bring up the **Formatting Binned Dot Specific Options** dialog for Dot and Color Dot plots (Figure 8.108), or the **Formatting Binned Plot Specific Options** dialog.

- Right-click on the 2D plot, select **Format** from the pop-up menu, click on the triangle next to the specific plot type, and choose the appropriate **Binned** [Dot or Plot] **Specific Options** category.

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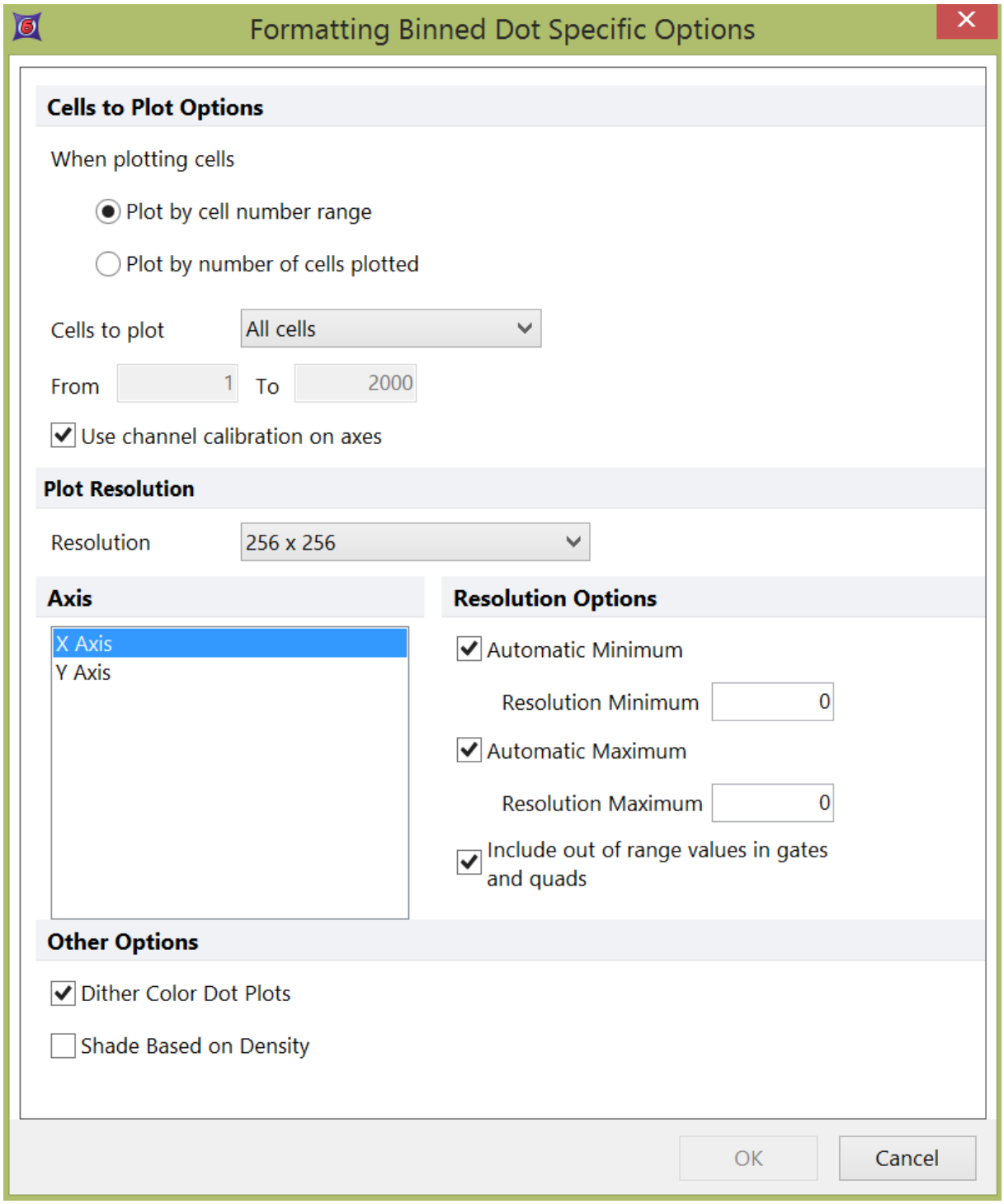

Figure 8.108 Formatting Binned Dot Specific Options Dialog

The options are described in the table below. See Resolution Overview 247 for a further description of resolution options.

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Option	Explanation
Cells to Plot Options	Control how many cells are plotted. If fewer cells are plotted, the plot can draw itself faster. Typically, most of the structure of a plot can be seen if 5000 cells are plotted. With the radio buttons, choose the **cell number range** or **number of cells plotted** option. Then, select a value from the drop-down list or enter in freetext into the **from** and **to** boxes, as appropriate. Statistics are always calculated based upon all of the cells in the data set, irrespective of how many are plotted.
Use channel calibration on axes	FCS Express can establish calibrated values for the axes 614. If channel calibrations are defined, toggling this checkbox will determine whether or not to use the calibrated values in place of the generic channel numbers.
Resolution options	Choose a resolution to display the data from the drop-down list. Lowering the resolution effectively smoothes the data.
Resolution overlays	The resolution options can be changed for the **X Axis/Y Axis**, or together. To edit the options for one of the axes, select that axis in the list, and change the options. The edit the options for both axes simultaneously, select both axes in the list (**Shift**-Click).
Automatic Minimum	If this is checked, then FCS Express will start binning the data (dividing it into the number of bins specified by the **Plot resolution**) at either 0 or the lowest data value (if the lowest data value is below 0).
Resolution Minimum	Specify where to start binning the data. If **Automatic Minimum** is checked, then this value is ignored.
Automatic Maximum	If this is checked, then FCS Express will finish binning the data (subdividing it into the number of channels specified by the **Plot resolution**) at the highest value of the true data range.
Resolution Maximum	Specify where to finish binning the data. If **Automatic Maximum** is checked, then this value is ignored.
Include out of range values in gates and quads	If this is checked, data values that are outside the resolution options of the plot will be considered to be part of the lower or upper channels. If a gate or quadrant touches the lower or upper channels, it will include these cells, even though the cells are actually outside the boundary of the gate/quadrant.
Dither Color Dot Plots (Dot Plots only)	Determine which color to use when two dots of different colors overlap each other (if you have more than one overlay or an event is located in an area where two gates overlap). If dither is on, the color will be chosen randomly from one of the two colors. If dither is off, only the most recent color will be used (i.e., if you have two gates defined, the color for gate 2 will always have priority over the color for gate 1, unless gate 1 is dragged below gate 2 in the Gate View 345.)
Shade Based on Density (Dot, Color Dot, and Density Plots only)(Figure 8.109)	Creates a greyscale shading pattern based on the density of plotted data. When gates are displayed or when checked on a color dot plot the shading pattern will be

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Option	Explanation
	pseudo colored to match the color of the gate displayed. A lighter shade represents a higher density. When using Shade Based on Density with Density plots, the option will further highlight the densest portions of the data set. Note that this option will not apply if multiple overlays are displayed on the plot.

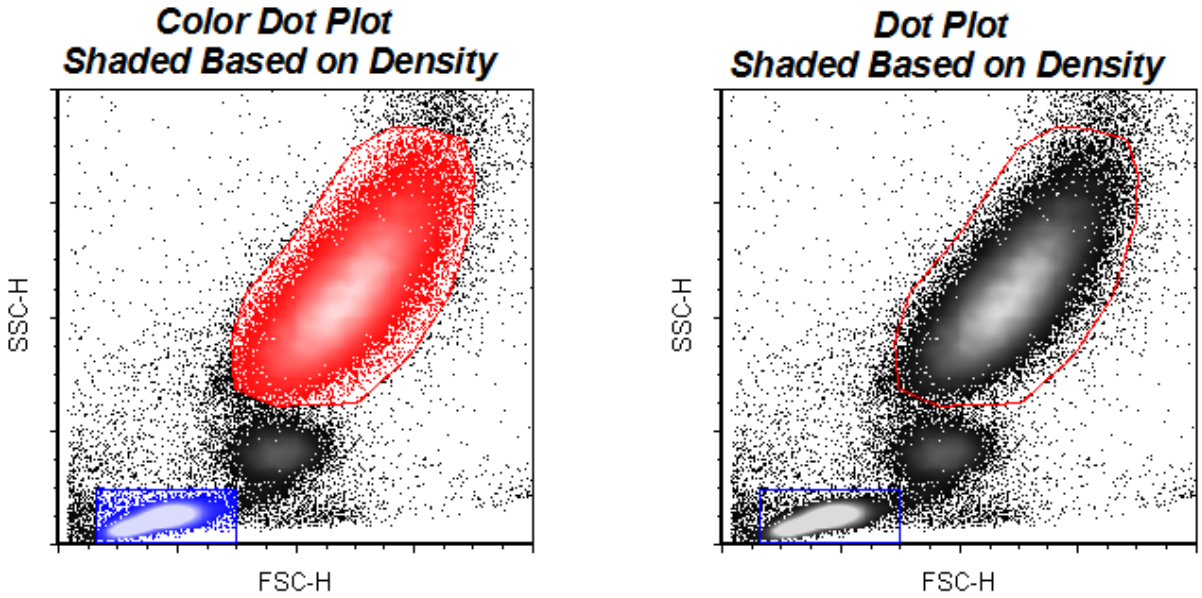

Figure 8.109 An example of color dot and dot plots shaded based on density.

8.8 Formatting Heat Maps

In addition to the formatting options common to all objects 239, and to some of those common to all plots 246, Heat Maps have certain properties, such as Heat Map Overlays 1066, Color Levels 1057 and other specific options 1060 which can be formatted.

You can format an heat Map by right-clicking on it, selecting **Format** from the pop-up menu, and choosing a category. This method will allow you to use the **Preview** button to see what the changes would look like without accepting the changes and allowing you to revert. Alternatively, once the Heat Map is selected, you can choose the desired category from the Format tab of the Ribbon 73.

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8.9 Changing the Parameter on Plot Axes Using Keyboard Shortcuts

The axis parameters can be changed in the formatting dialogs for each plot, as described for histogram overlays 268 and 2D plot overlays 284. You can also change the parameters of the base overlay on each plot using the keyboard or the mouse.

Note: For any of these methods to work, you must make sure that the plot is in edit mode 132.

- **Keyboard**

  To change the parameter displayed on the x-axis with the keyboard, press the number corresponding to the parameter you want to display (e.g., press **3** to view parameter 3). To change the parameter displayed on the y-axis of a 2D plot, simultaneously press the **Ctrl** key and the number corresponding to the parameter to display.

- **Mouse**

  To change the parameter of an axis with the mouse (the x-axis on a histogram, either axis on a 2D plot), move the mouse cursor over the axis label and click on the axis label. A pop-up menu will appear containing the available parameters (if you have set the Alt+Click Required To Change Parameters option 186, you will also have to press the **Alt** key while clicking). From this pop-up menu, you may type in the empty field at the top or scroll through the list using the mouse scroll wheel or arrows in the pop-up menu (if more than 10 parameters are available). If you do not wish to change the parameter after the list has appeared, hit **ESC** on the keyboard or move the cursor anywhere outside the list and click.

8.10 Formatting Data Grids and Custom Data Grids

Data Grids 680 have unique formatting properties that can be accessed by right-clicking on the data grid, selecting **Format** from the pop-up menu, and choosing a category. This method will allow you to use the **Preview** button to see what the changes would look like without actually accepting the changes. Alternatively, once the Data Grid is selected, you can choose the desired category from the Format tab of the Ribbon 73.

Custom Data grids have been deprecated from FCS Express 6 as the functionality has been improved upon with the addition of Spreadsheets 414. Any existing Custom Data grids saved in FCS Express 4 or FCS Express 5 layouts will

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still be available and formattable in FCS Express 6 via the methods in this chapter. Please contact support@denovosoftware.com if you are having any problems working with data grids saved in a previous version of FCS Express in FCS Express 6.

8.10.1 General

In addition to the usual name property (referred to as **Data window name**), Data Grids have some additional **General** options (Figure 8.110).

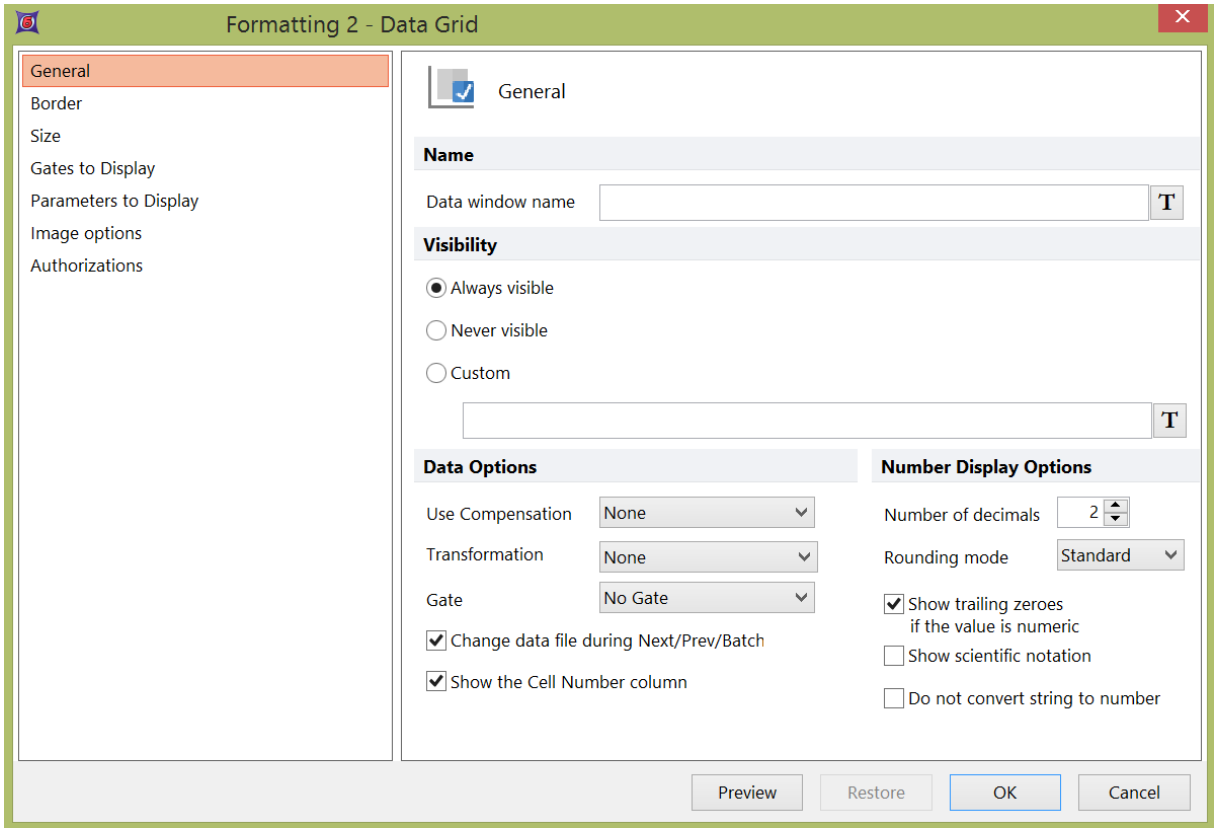

Figure 8.110 Formatting general options for Data Grids and Custom Data Grids

Additional formatting options are described below.

Option	Explanation
Use compensation	Choose which compensation 626 matrix is applied to the data in the grid.
Transformation	Choose which transformation 946 is applied to the data in the grid.
Gate	The gate 352 applied to the data in the grid. Note that a range gate 683 can be created on and applied to Data Grids and Custom Data Grids.
Change data file during Next/Prev/Batch	Whether the data grid changes during batch processing 784 or in response to a Next/Previous 79 command. It is

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Option	Explanation
	generally recommended to leave this unchecked (default), as the data within the grid normally refers to plots and will update automatically as the data in the plots are changed.
Show the cell number column	Whether the cell or object number (assigned by the cytometer according to the time of sample acquisition) is shown as a column in the Data Grid.
Number of decimals	The number of decimal places listed to the right of the decimal point.
Rounding mode	The method for rounding the last digit. Choose between Standard, Down, Up, and Truncate.
Show trailing zeroes	If checked, shows the specified number of decimals even if the terminal digits are zeroes.
Show scientific notation	If checked, shows the number in **E notation**: *m*E*n* which represents m x 10^n, where *m* and *n* are any real number and *E* represents "exponent"

8.10.2 Gate Display

The **Gates to Display** formatting option for Data Grids determines for which gates backgating information is displayed 681. This formatting option does not apply to Custom Data Grids.

You can edit the Gates to Display in one of two ways:

- Select the Data Grid(s) and use the **Format→Data Options→Gates to Display** command.
- Right-click on the Data Grid, select **Format** from the pop-up menu, and choose the **Gates to Display** category (Figure 8.111).

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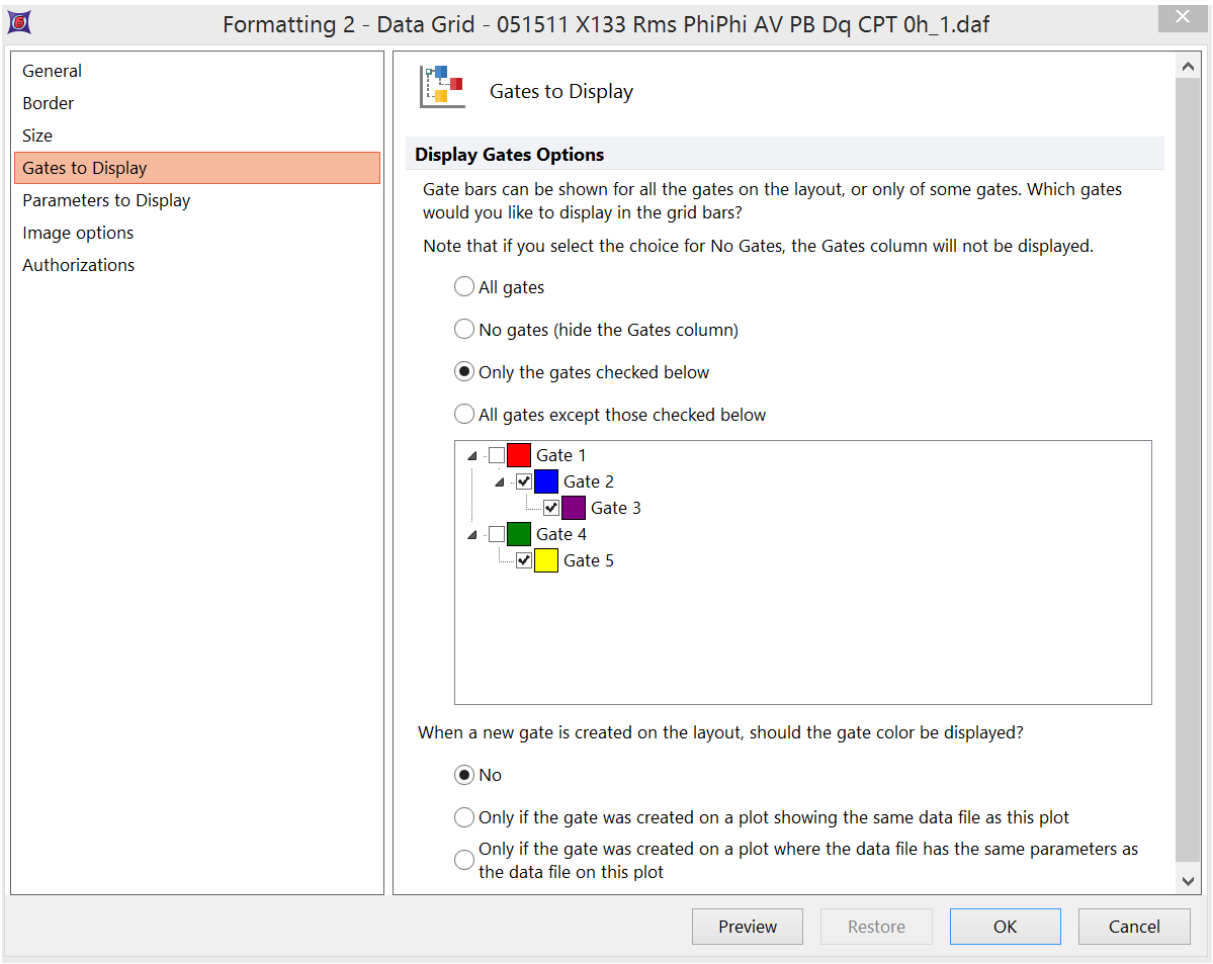

Figure 8.111 Formatting gates to display in a Data Grid

By default, the **No Gates (hide the Gates column)** option is selected. However, you can select **All gates** or **Only the gates checked below** and then select the gates whose colors you wish to display. Alternatively, select **All gates except those checked below** and then choose the gates whose colors you do not wish to display.

You can also choose how to display the gate color when a new gate is created on the layout. By choosing either the second or third radio buttons in the **When a new gate is created on the layout** category, you can have FCS Express display the gate color on a back-gated plot if the new **gate was created on a plot showing the same data file**, or only **on a plot showing the same parameters** (as the plot where the new gate was created).

8.10.3 Parameters to Display

Each column in a Data Grid or Custom Data Grid is a parameter which can be shown or hidden, by accessing the **Parameters to Display** formatting options.

You can edit the Parameters to Display in one of two ways:

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- Select the Data Grid(s) and use the **Format→Data Options→Parameters to Display** command.

- Right-click on the Data Grid, select **Format** from the pop-up menu, and choose the **Gates to Display** category (Figure 8.112).

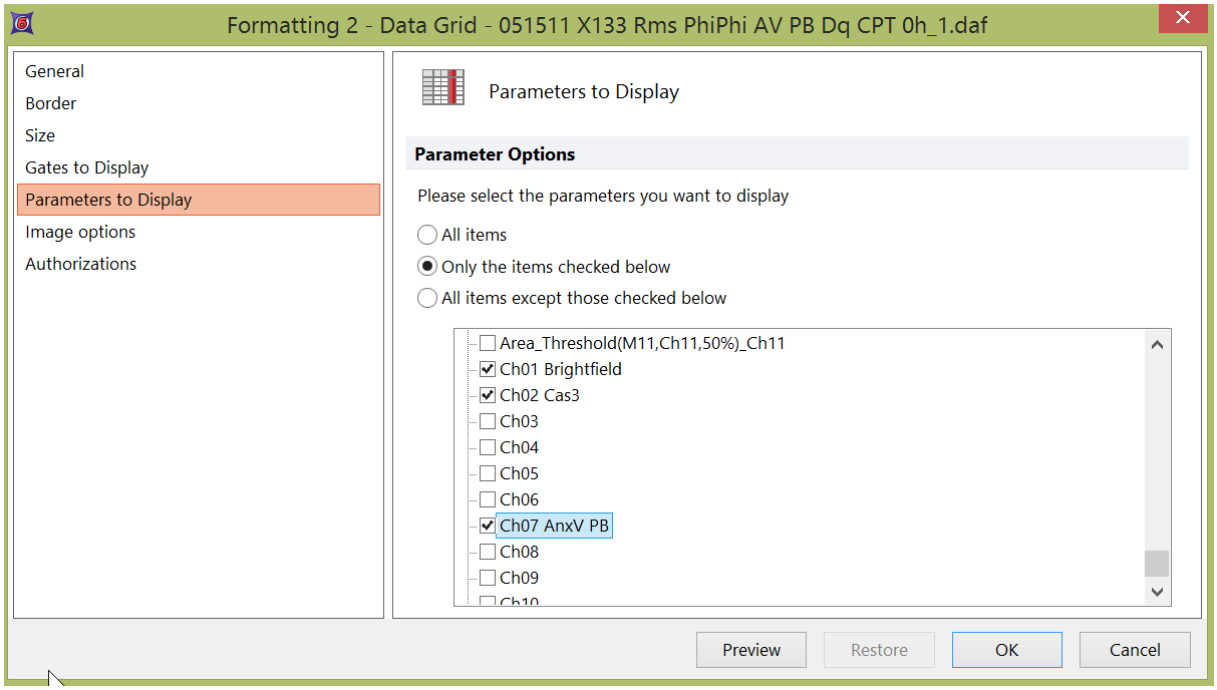

Figure 8.112 Formatting parameters to display in a Data Grid

8.10.4 Image Options

Image Options formatting is available for Data Grids in FCS Express Image or FCS Express Plus only.

If a Data Grid has been formatted to display images for at least one parameter 312, these images will be viewable as an Image Gallery 684 within the Data Grid. These images can fill the grid cell or be centered within it.

You can edit these Image Options in one of two ways:

- Select the Data Grid(s) and use the **Format→Data Options→Data Image Options** command.

- Right-click on the Data Grid, select **Format** from the pop-up menu, and choose the **Image Options** category (Figure 8.113).

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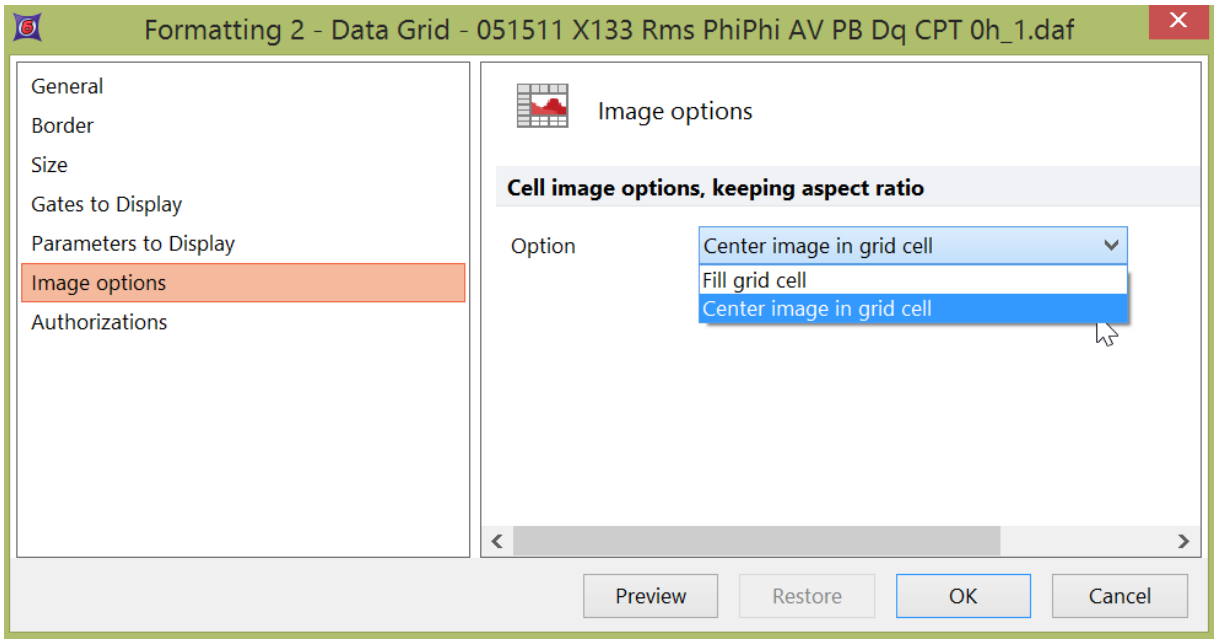

Figure 8.113 Formatting image options in a Data Grid

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Chapter

9 Analysis of Flow and Image Cytometry Data Files

FCS Express provides many tools for flow cytometry analysis, such as gates, markers, quadrants, and statistics. In this chapter, we describe these tools so that you can apply them to your own data.

9.1 Using Gates

Some flow cytometry software packages make a distinction between regions and gates. This distinction does not apply in FCS Express. In FCS Express, you create gates and apply them directly to your data. The term "gate" will be used exclusively throughout this manual.

You can create an unlimited number of individual gates. FCS Express supports seven types of gates.

- Elliptical gates: Gates that are oval, oblong, or circular.
- Rectangular gates: Gates that are square or rectangular.
- Polygonal gates: Gates that are drawn with three or more vertices connected by straight lines.
- Freeform gates: Gates that are drawn with various straight and/or curved sections in an irregular shape.
- 1D gates: Gates that exist only in one dimension, i.e., a one parameter histogram gate or an x-axis plot gate.
- Well gates: Gates that are set on a heat map display showing the wells of a microtiter plate.
- Combination gates: Gates that are Boolean combinations of one or more of the other six types of gates.

Examples of different gate types are shown in Figure 9.1.

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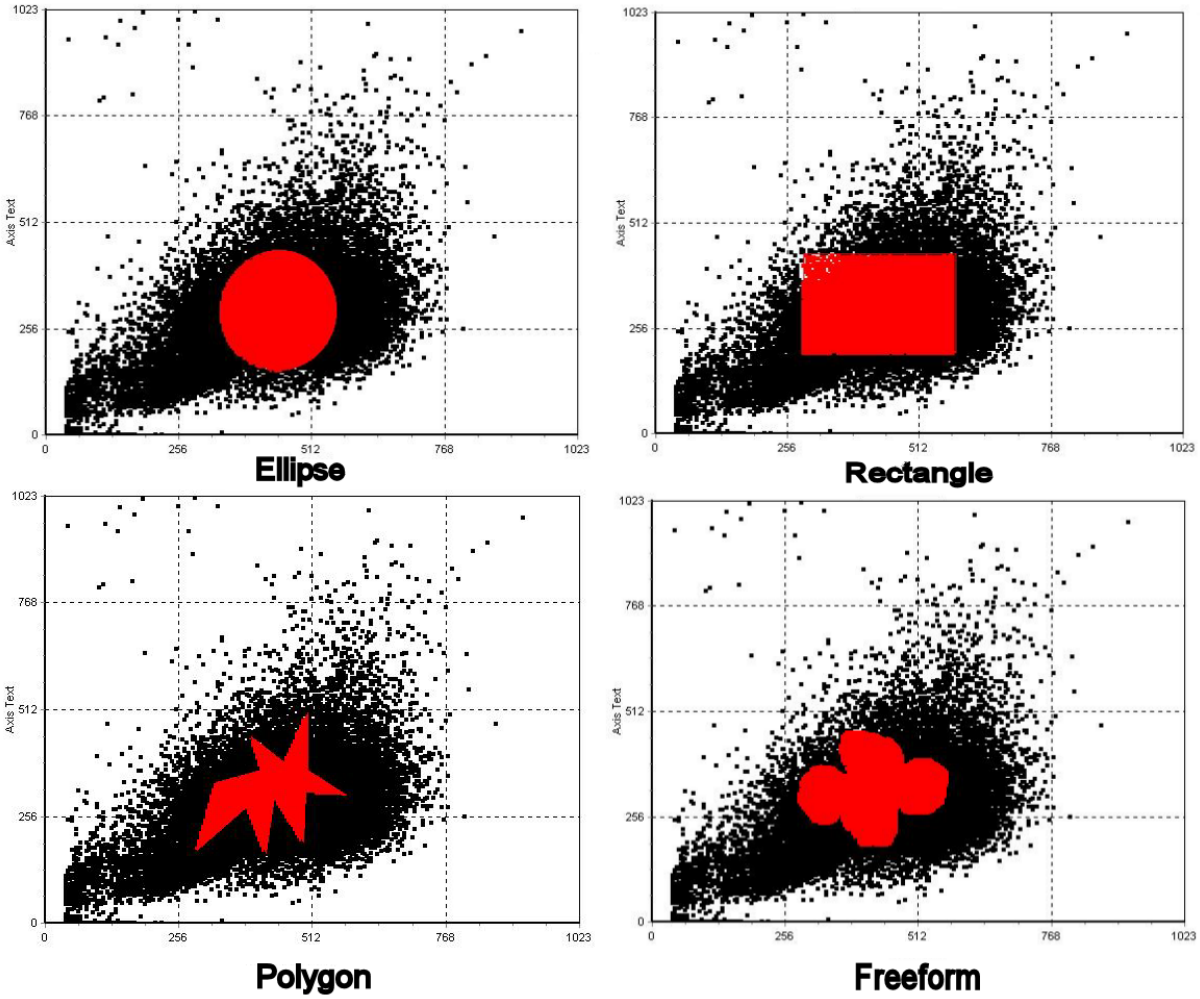

Figure 9.1 Examples of Different Gate Types

9.1.1 Live Updating Gates

FCS Express has the ability to have all gate, quadrant, and marker related changes such as backgating, statistics, and gated events update in real time as the gate is moved.

The option to update immediately, "in real time", is controlled via the **FCS Express User Options** located in the **File** tab→**Options**→**General category**→**Performance Options**→**When moving gates, quadrants and markers, update plots, statistics and tokens immediately.** (Figure 9.2).

The option to enable live updating gating may also be defined at the layout based level via the **Layout Performance Options** 111.

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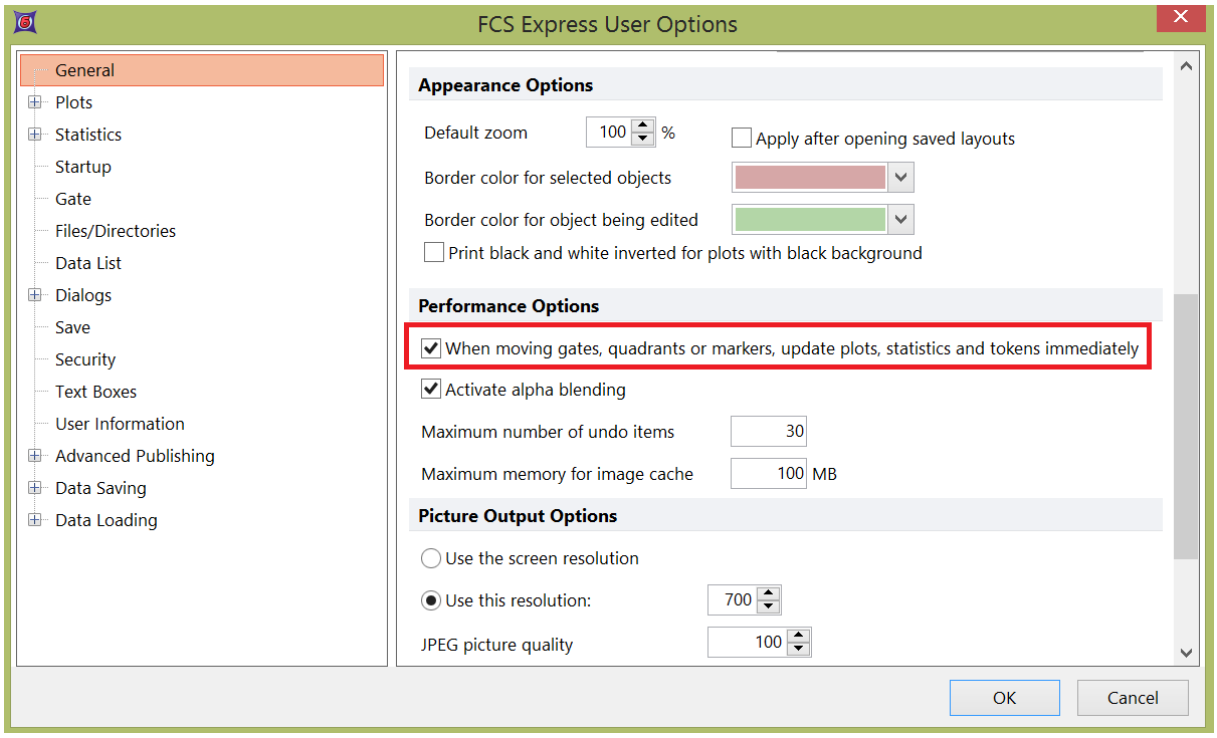

Figure 9.2 The option to enable or disable "real time gating" is controlled by the When moving gates, quadrants and markers, update plots, statistics and tokens immediately check box.

9.1.2 Creating Gates

Gates can be created in several ways. To create a gate efficiently, first select the plot that you would like to create the gate on, and then do one of the following:

- Select a command (e.g., **Ellipse**, **Rectangle**, **Polygon**, **Freeform**) from **Home→Create Gates**.
- Select your desired gate shape from the commands in **Gating→Create Gates**.
- Right-click on the plot and then choose **Create Gate** and a gate type from the pop-up menu.

To cancel the creation of a gate while you are drawing the gate, press the **Esc** key on your keyboard before completing the operation.

- Elliptical or Rectangular Gates

  If you choose the **Ellipse** or **Rectangle** commands, position the cursor on the plot where you want the top left corner of the gate to be. Then, press and hold the mouse button while dragging the cursor down and to the right

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until the gate is the size you want. Release the mouse button to complete the gate. *Note*: You can easily adjust the size later.

- Polygonal Gates

  If you choose the **Polygon** command, position the cursor where you want the first vertex to be. Then, click (and release) the mouse button. Continue repositioning the cursor and clicking at the vertex locations. Straight lines will connect the vertices to create the polygon. To close and complete the polygon gate, click on the square that appeared at the first polygon vertex. Alternatively, you can complete the polygon gate by pressing the space bar. Pressing the space bar will cause the gate to close with a straight line between the first vertex and the last vertex you created. When completed, in addition to the vertices being marked, the polygon will show four outer circles which can be used for proportional resizing.

- Freeform Gates

  If you select the **Freeform** command, position the cursor on the plot where you want to begin drawing the gate. Click and hold the mouse button. While continuing to hold the mouse button down, drag the mouse to draw the shape of the gate. The gate will be drawn along the path of the cursor. To close and complete a **Freeform** gate, release the mouse button. The gate will automatically close with a straight line connecting the first cursor position to the cursor position when the mouse button was released. When completed, the freeform shape will show four outer circles which can be used for proportional resizing.

- Well Gates

  If you choose the **Well** command, you can create a gate on one or more wells of a Heat Map 1061 (microtiter plate).

- 1D Gates

  Create a **Marker** on a histogram as described in Creating Markers 353. Then, right-click on the plot that contains the marker and choose **Convert Marker to Gate**, the **Marker** number, and either **Convert** or **Convert and Link**. If you choose **Convert and Link**, changing the original marker changes the corresponding 1D gate. Otherwise, moving or deleting the original marker will have no effect on the 1D gate.

  To create a 1D Gate on a 2D-plot:

  - Select the **Gating** (or **Home**)→**Create Gates**→**Create Marker** command.

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- Right-click on the plot and select **Create marker** from the pop-up menu.

Then, position the cursor where you want the left marker boundary to be, and press and hold the mouse button down. While holding the mouse button down, move the cursor parallel to the X- or Y-axis. Position the cursor where you want the right marker boundary to be, and release the mouse button.

Once you have finished creating a gate, a dialog will appear (Figure 9.3) asking you to name the gate, choose a color, and decide if a gate should include or exclude the events contained within it.

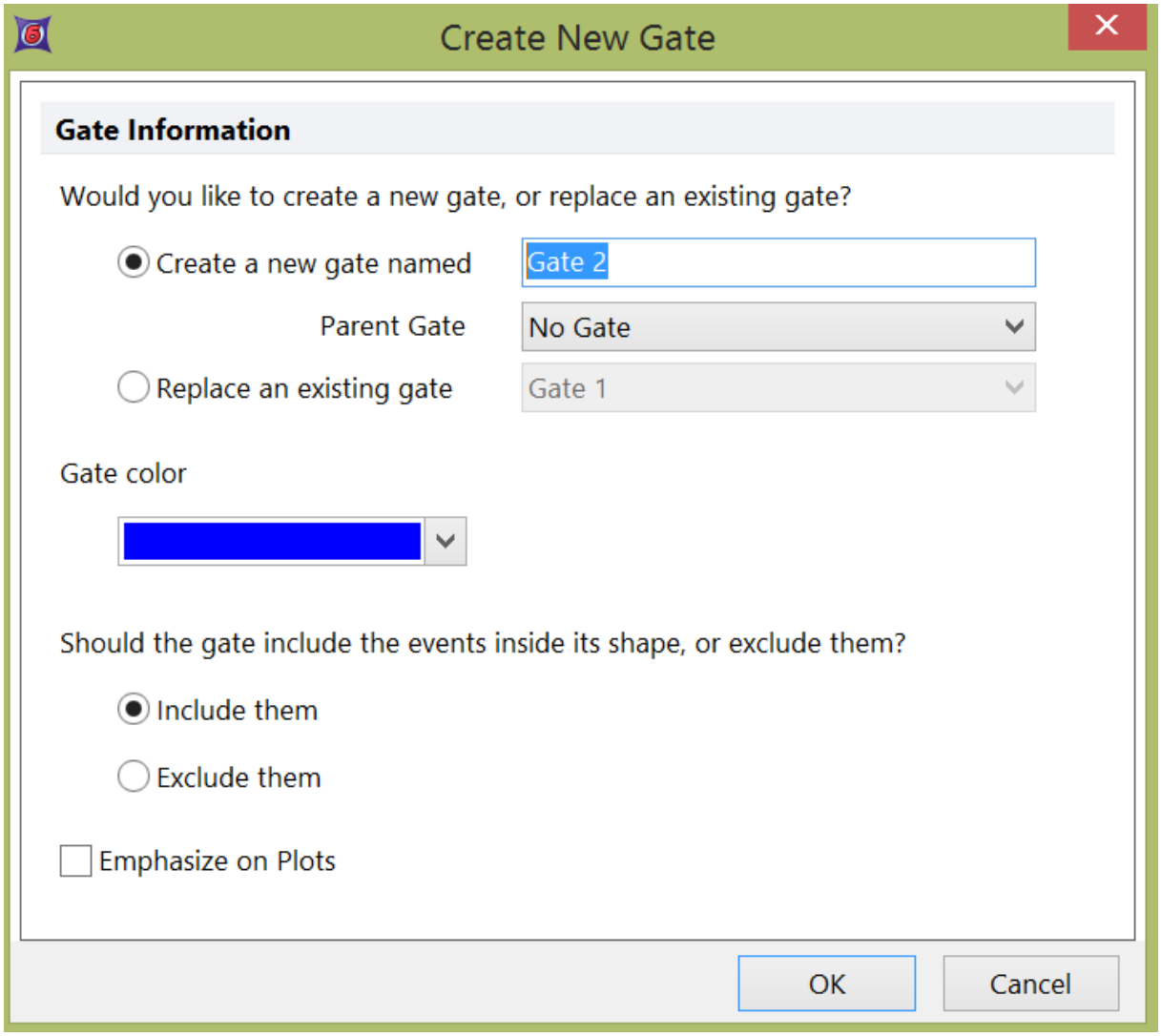

Figure 9.3 Create New Gate Dialog

To replace an existing gate with the newly created gate, select the **Replace an existing gate** radio button and choose the gate you wish to replace from the

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drop-down list. The new gate will replace the old gate without affecting your gating hierarchy 350 at all.

To create the new gate as a child of a specific gate in the hierarchy, choose the parental gate in the hierarchy from the **Parent Gate** drop down. The hierarchy of your gates can be adjusted at any time within the Gate View object 345 as well.

Gates can be copied and pasted from any plot to any other plot. If the recipient plot has different parameters from the original plot you will be prompted to create a new gate that will use the original gating coordinates with the existing parameters of the new plot.

When the **Emphasize on Plots** feature is checked FCS Express will display any events within the gate on a **Color Dot** plot, or dot plots with the gate chosen to display 303, as larger and bolder dots for emphasis (Figure 9.4). Emphasize on plots may also be accessed for specific low frequency populations in overlays on 2D plots through the Formatting 2D Plots dialog 286.

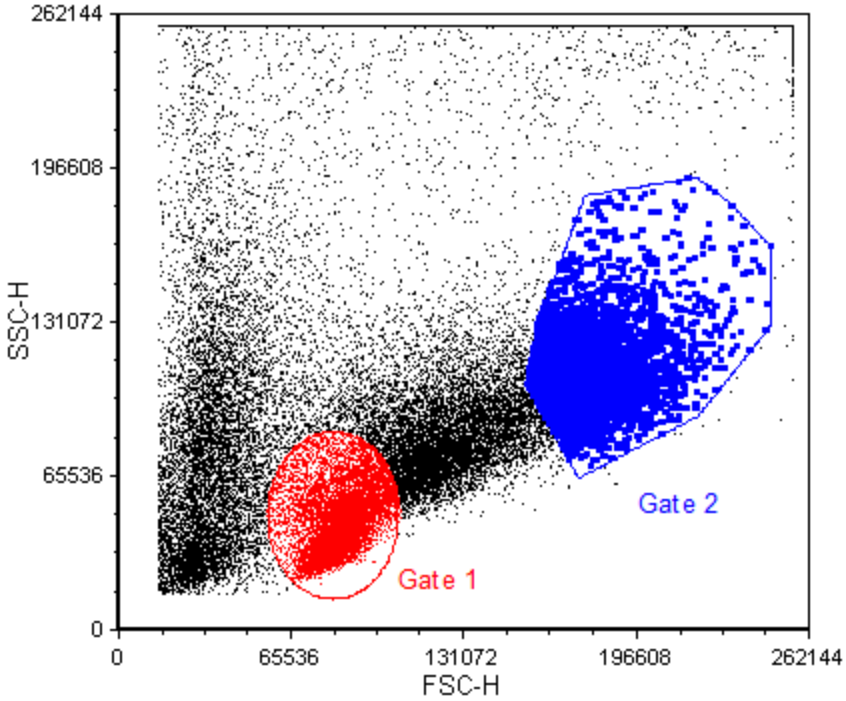

Figure 9.4 - Gate 2 has been created with the Emphasize on Plots feature selected. Notice how dots within Gate 2 appear larger and bolder than Gate 1 which was created without the feature selected.

The **Allow Gate Edit** section within the **Edit Gate** dialog (Figure 9.5) allows you to choose if the gate may be put into edit mode which allows users to move, replace, and delete the gate. Selecting the radio button for **True** allows full

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editing, **False** prevents the gate from being edited, and **Custom** allows the gate to be edited based on the conditions that are set and will only allow editing if the value evaluates to true. You may use the "=" followed by an input of a manual number, token or custom token. "<" or ">" may also be used to specify the condition to be less than or greater than, respectively.

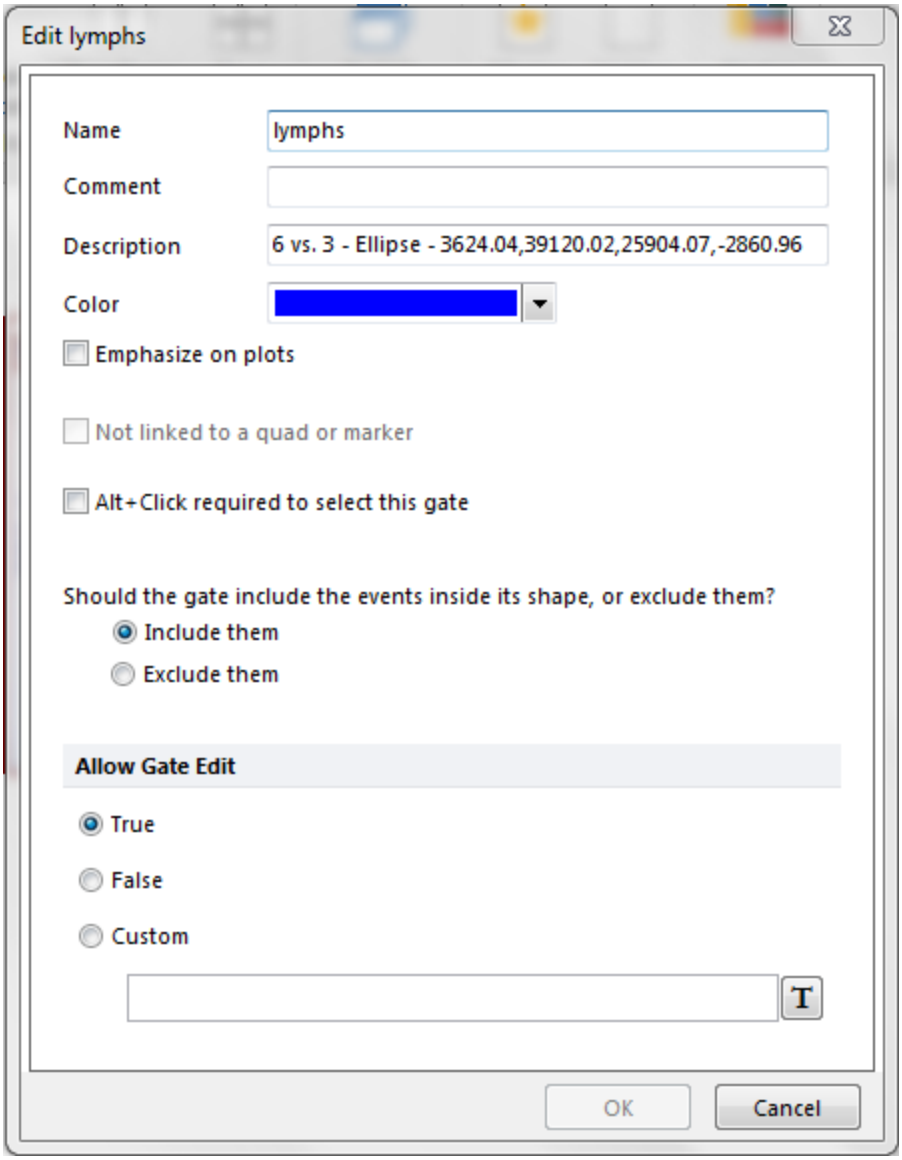

Figure 9.5 Allow gate edit dialog based on radio buttons next to "true", "false", or "custom". The custom setting is based on if the conditions are met.

The **Edit Gate** dialog may be accessed from the **Gate View:**

- Click on **Insert** tab→**General**→**Gate View**.

or from the **Gate Navigator:**

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- Click on **View** tab→**Navigators→Gate Navigator.**

In either dialog double click on the gate to edit to access the **Gate Edit** dialog.

When the radio button for "custom" is selected, the gate may be edited based on whether or not the conditions below are met. You may use the "=" followed by an input of a manual number, token or custom token. "<" or ">" may also be used to specify the condition to be less than or greater than, respectively.

For example, see Figure 9.6 below where a conditional statement was defined in the **Custom** field. The custom field is defined as true since 18.90 is greater than 2 and will allow gate editing if the lymphs gate.

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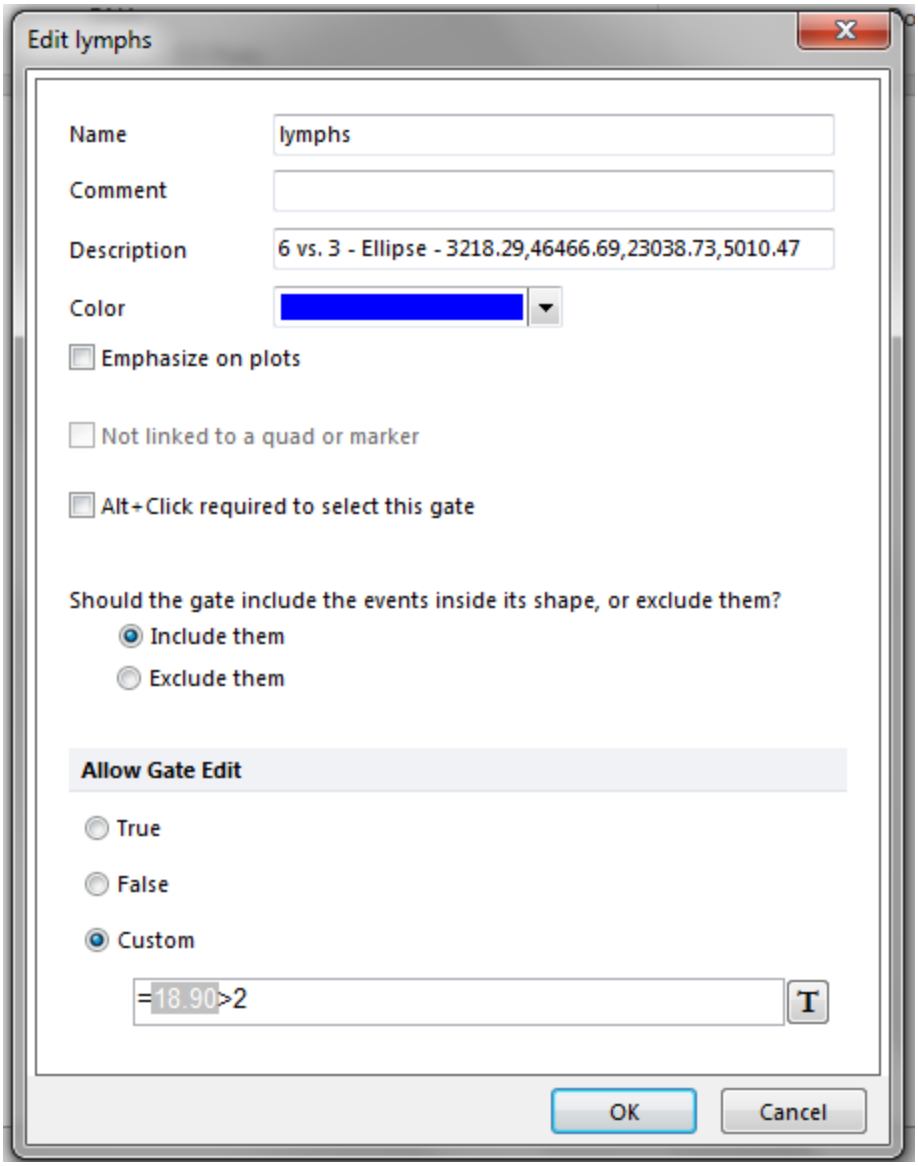

Figure 9.6 Allow Edit Dialog-Defining the Conditions to be met in order to Custom Allow Gate Edit

9.1.3 Data Specific Gates

Data Specific Gates in FCS Express allow you the ability to assign gates to specific data files that have been loaded in the data list. Different gating positions between data files may be stored and automatically used when displaying plots or making calculations when using the **Data Specific Gate** feature, Figure 9.7.

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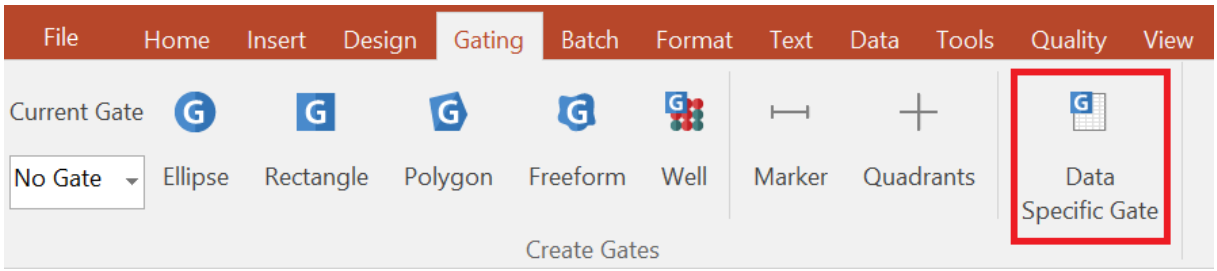

Figure 9.7 The Data Specific Gate button in the Gating tab is used to enable data specific gates.

When a gate is converted to a Data Specific Gates for a given file, the shape and position can be modified for the specific file independently from other data files. Thus, when a Data Specific Gates is created, the original gate (here hence referred to as "Master gate") continues to be used for all the remaining files.

Jump to:

- **Creating Data Specific Gates** 327
- **Deleting/Removing Data Specific Gates** 328
- **Promoting Data Specific Gates to Master Gate** 330
- **Using Data Specific Gate Groups** 331

An example of Data Specific Gates is given in Figure 9.8, where Gate 1 has been converted to be a Data Specific Gates for File 4. Files 1 through 3 continue to use the original position for Gate 1 (i.e. the Master Gate).

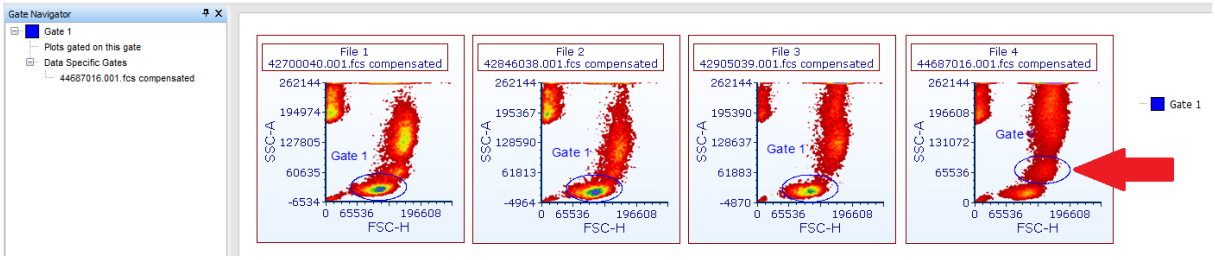

Figure 9.8- Example of Data Specific Gate where Gate 1 has been changed and made specific for File 4.

Data Specific Gates can be easily tracked using the **Gate Navigator** 128 Figure 9.9.

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Figure 9.9 - Using the gate navigator to view which data files are assigned data file specific gates.

Although **Data Specific Gates** may appear to share some similarities with **Iteration Snapshots** 376 and **Manual Snapshots** 374 the features are quite different in term of their applicability:

- **Data Specific Gates** are specific for a data file in the data list, while Snapshot are specific for a given iteration number in the data list. **Data Specific Gates** are suitable for analyzing multiple FCS files simultaneously open in the layout, as well as multiple FCS file using batch process while **Snapshots** are suitable for analyzing multiple FCS files using the batch process.
- **Data Specific Gates** only account for gates and gating positions while **Snapshots** are used to record marker and quadrant positions along with gating positions.

		Data Specific Gates	Snapshots
Record the shape and the position of	**Gates**	**Y**	**Y**
	Markers	**N**	**Y**
	Quadrants	**N**	**Y**
Specific for	**Data Files in Data List**	**Y**	**N**
	Iteration Numbers in Data List	**N**	**Y**
Suitable for analyzing	**Multiple FCS files simultaneously open in the layout**	**Y**	**N**

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	Multiple FCS file using batch process	**Y**	**Y**

9.1.3.1 Using Data Specific Gates

Creating Data Specific Gates

Assigning a gate to be Data Specific is accomplished from the **Gating** tab through the steps below:

1. Click on the gate to convert to a data specific gate (Figure 9.10 - Number 1) on a plot.
2. Click on the **Gating** tab of the Ribbon.
3. Select **Create Gates→Data Specific Gates** (Figure below - Number 2).

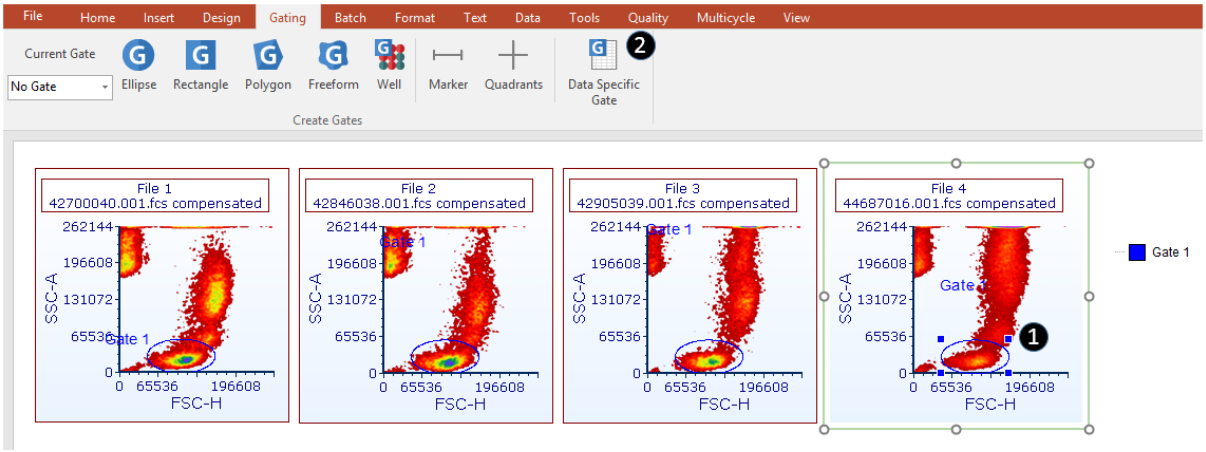

Figure 9.10- Data File Specific gates are created using the Data Specific Gate button from the Gating tab > Create Gates section.

The Gate Navigator will now reflect the presence of a Data Specific Gate for File 4 (ApoMono.004 compensated) (Figure 9.11).

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Figure 9.11 - The gate navigator before and after creating a data file specific gate on File 4 (ApoMono.004).

The shape and position of Gate 1 on File 4 can now be modified independently from the master gate (File 1 through File 3) Figure 9.12.

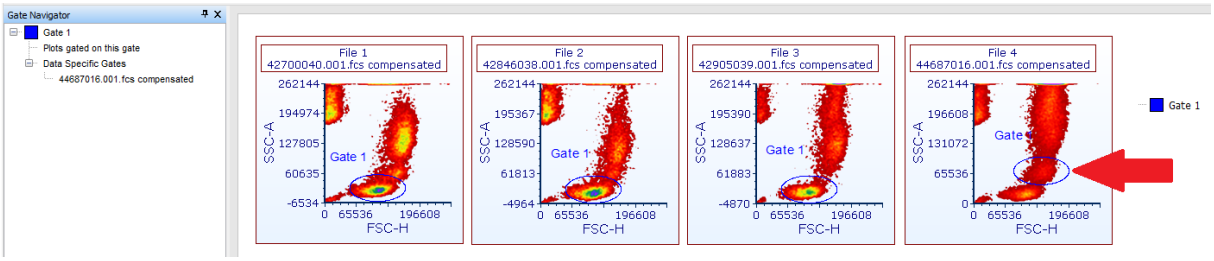

Figure 9.12 - An example of a data file specific gate for Gate 1 that has been made Data File Specific for File 4.

Deleting Data Specific Gates

A Data Specific Gate can be reverted back to resemble the Master gate (i.e. to loose its data specificity) in one of three ways:

Option 1:

1. Click on the gate that need to be reverted to Master gate (Figure 9.13 - Number 1).
2. Click on the **Gating** tab of the Ribbon.

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3. Select **Create Gates→Data Specific Gates** (Figure below - Number 2).

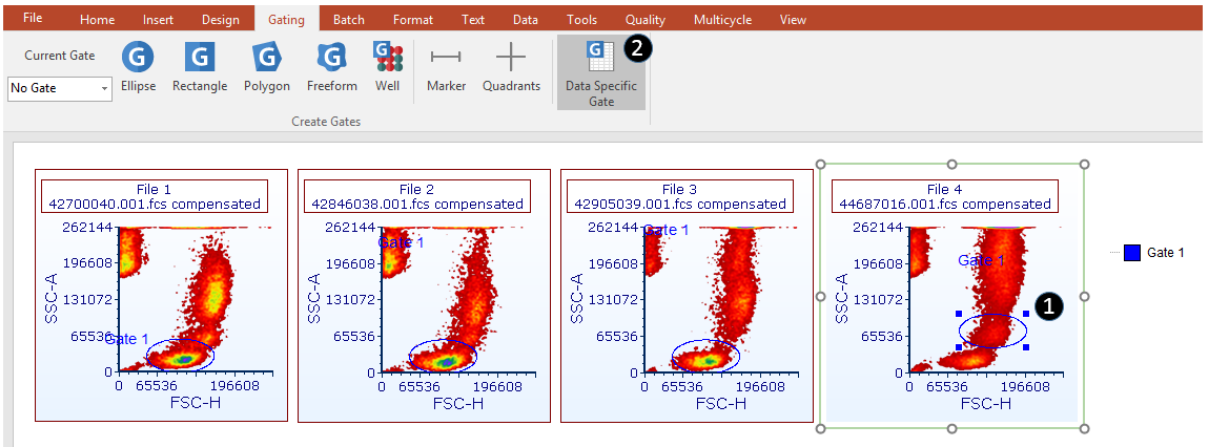

Figure 13 - DSG can be deleted using the Data Specific Gate button from the Gating > Create Gates ribbon bar.

Option 2:

1. Select the **Data Specific Gate** for the File in the **Gate Navigator**.
2. Press **Delete**.

Option 3:

4. Right click on the Data Specific Gate in the Gate Navigator.
5. Select **Delete Data Specific Gate(s)**(Figure 9.14).

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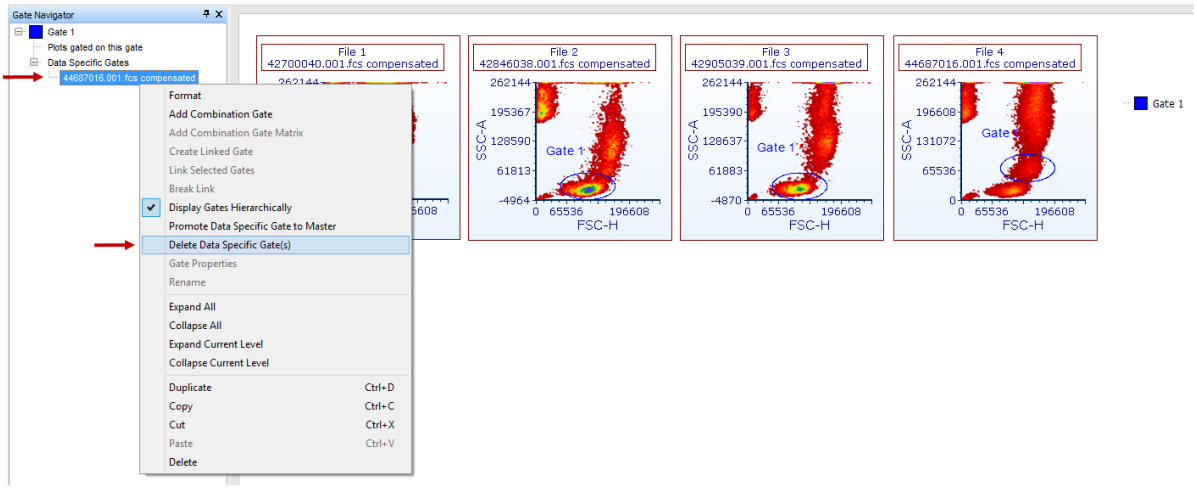


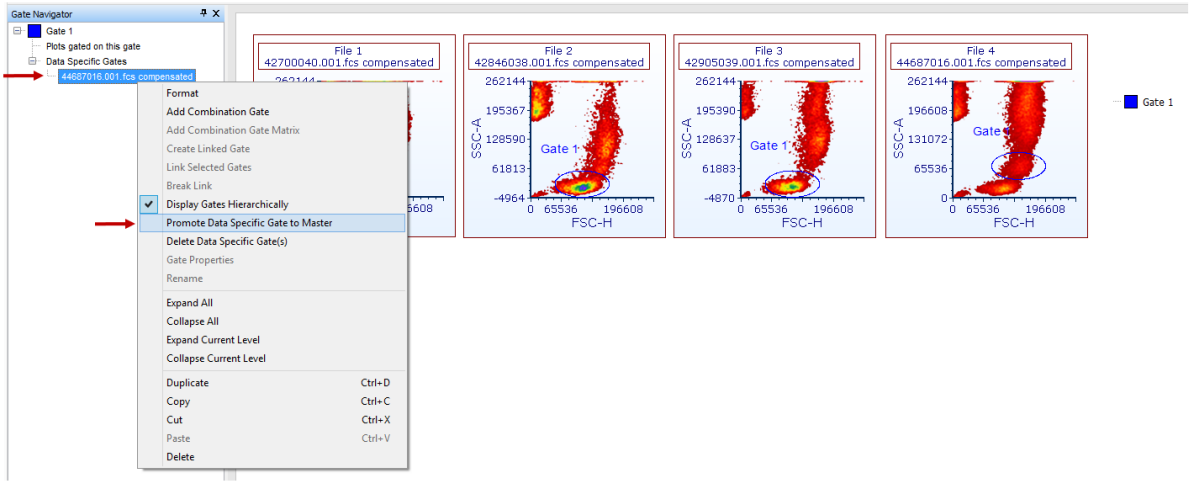
Figure 9.14- A Data File Specific Gate can be removed using the right click menu in the Gate Navigator.

When a Data Specific Gate is deleted the Master Gate will be used on any files that had used the Data File Specific gate.

Promoting a Data Specific Gate to the Master Gate

A Data Specific Gate can be promoted to be the Master gate within the Gate Navigator:

1. Right click on the Data Specific Gate in the Gate Navigator.
2. Select **Promote Data Specific Gate to Master** from the right click menu (Figure 9.15).



(Figure 9.15) - Selecting promote data specific gate to master gate in the gate navigator.

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When promoting a Data Specific Gate to Master Gate:

- All of the files that used the original Master Gate will use the Data Specific Gate.
- The original Data Specific Gate is removed from the Gate Navigator (Figure 9.16) - left).

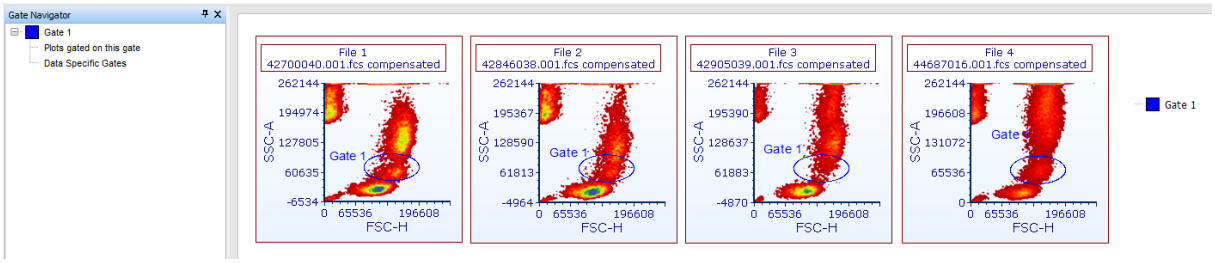

Figure 9.16 - A data specifc gate was promoted to a new master gate. Note the original data specific gate is no longer present.

9.1.3.2 Using Data Specific Gate Groups

Data Specific Gates may be used as gate groups to specify a gating position for multiple data files by creating a Data Specific Gate Group.

Data Specific Gate Groups may be create in three ways:

Multiple Selecting Data Specific Gates 331

Dragging and Dropping within the Gate Navigator 332

Dragging and Dropping from the Data List 332

Data specific gate groups may also be removed via deletion. 336

Multiple Selecting Data Specific Gates to Create a New Group (Figure 9.17)

1. Multiple select the **Data Specific Gates** that will constitute the **Data Specific Gate Group** in the Gate Navigator.

   NOTE: The first Data Specific Gate selected will be used as reference gate for the Data Specific Gate Group.

2. Right click on any of the selected **Data Specific Gate**.
3. Select **Create Data Specific Gate Group** from the right click menu.

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The first Data Specific Gate selected in step 1 will now be used for all other data files specified in the group.

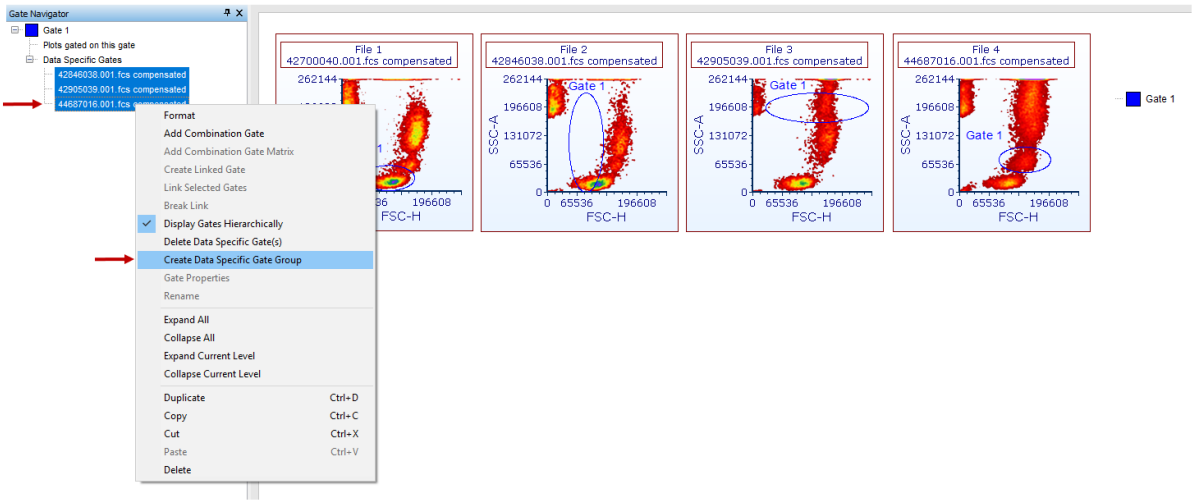

Figure 9.17 - Using multiple select to create a data specific gate group for multiple files.

Dragging and Dropping to Create a Group within the Gate Navigator (Figure 9.18)

1. Select one or more **Data Specific Gates** in the gate navigator.
2. Drag the selected **Data Specific Gates** and release them on top of the **Data Specific Gate** file name that is intended to be used for the gate group master position.

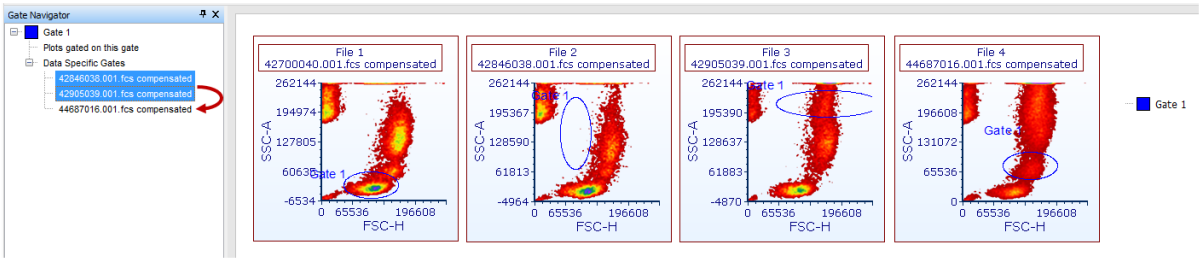

Figure 9.18 - Creating a data specific gate group by dragging and dropping files from in the gate navigator to the data specific gate intended as the new group master gate.

Dragging and Dropping to Create a Group from the Data List (Figure 9.19)

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1. Select one or more **data files** in the **Data List**.
2. Drag the selected **data file(s)** and release them on top of the **Data Specific Gate** in the **Gate Navigator** that is intended to be used for the gate group master position.

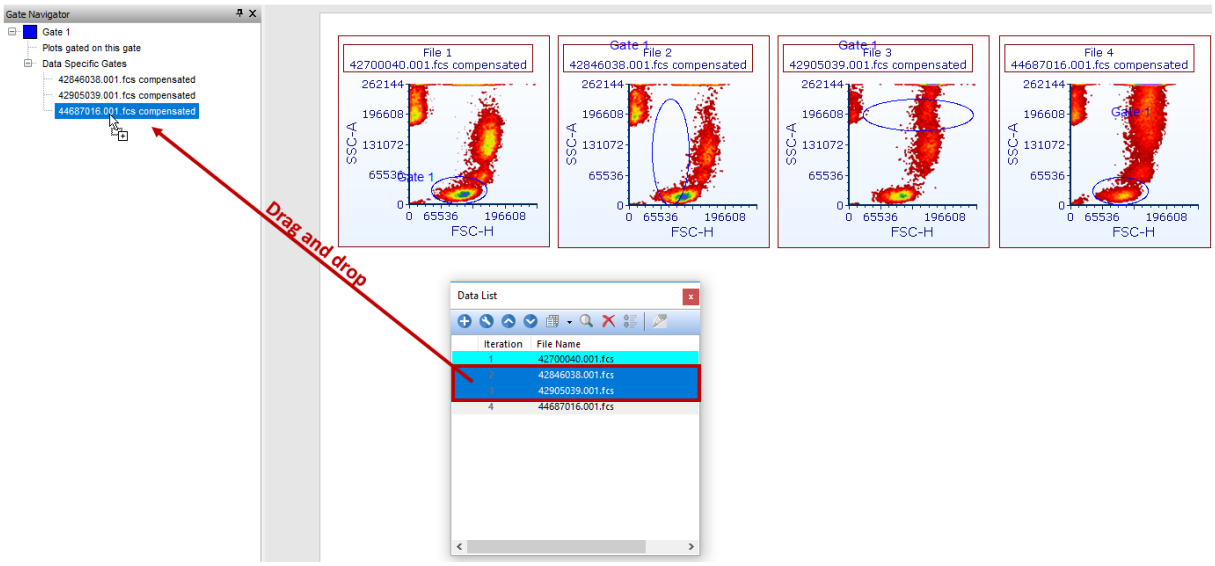

Figure 9.19 - Creating a data specific gate group by dragging and dropping files from the data list to the data specific gate intended as the new group master gate within the gate navigator.

The result for the three above methods for creating a Data Specific Gate Group is that all files within the newly created group will be unified to the Data Specific Gate used for reference. A new Group child will appear under the Data Specific Gates for the gate of choice (Figure 9.20).

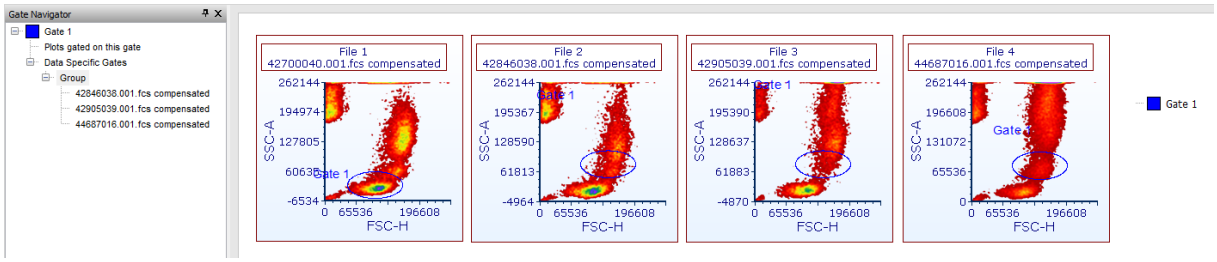

Figure 9.20 - A new data file specific group is created via the multiple select or drag and drop methods for creating groups.

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Note: If the **Data Specific Gate Group** of interest already exists, file(s) can be dragged and dropped on either the **Data Specific Gate Group** entry or on any of the **Data Specific Gates** which are part of it (Figure 9.21).

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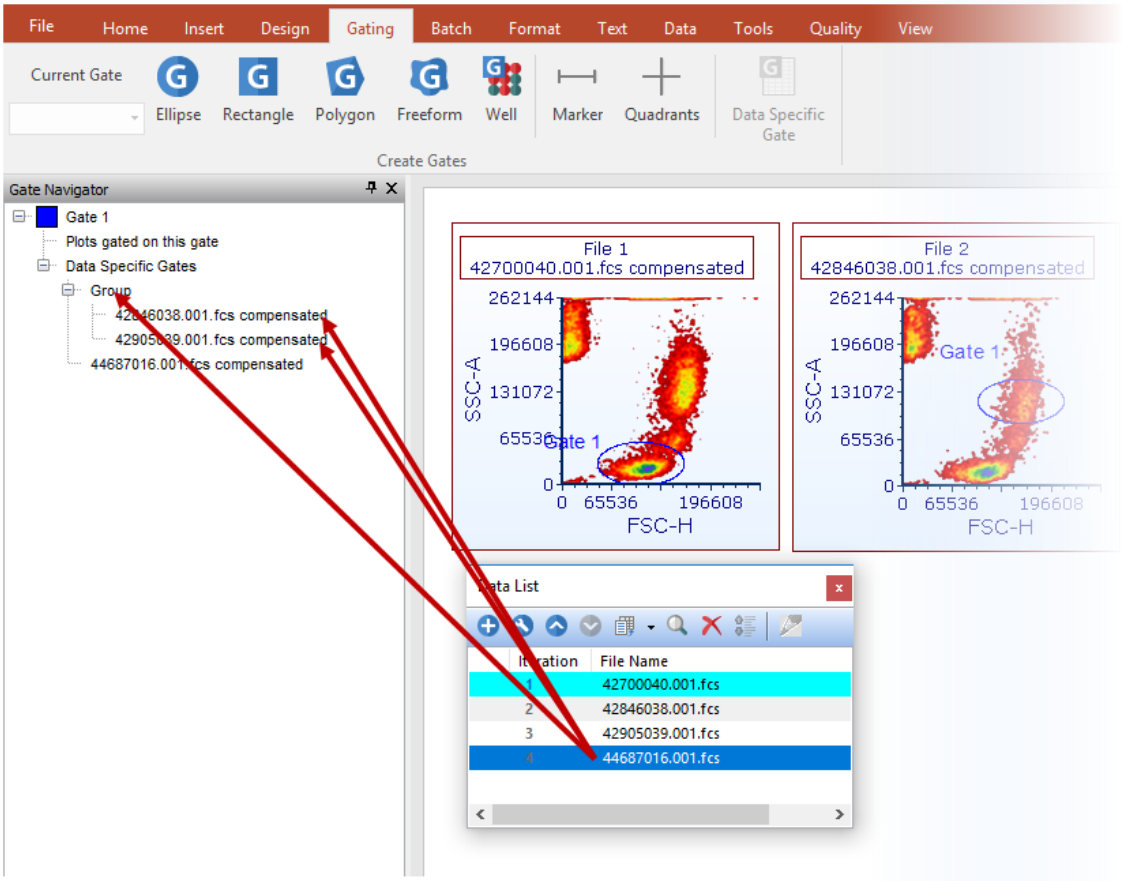

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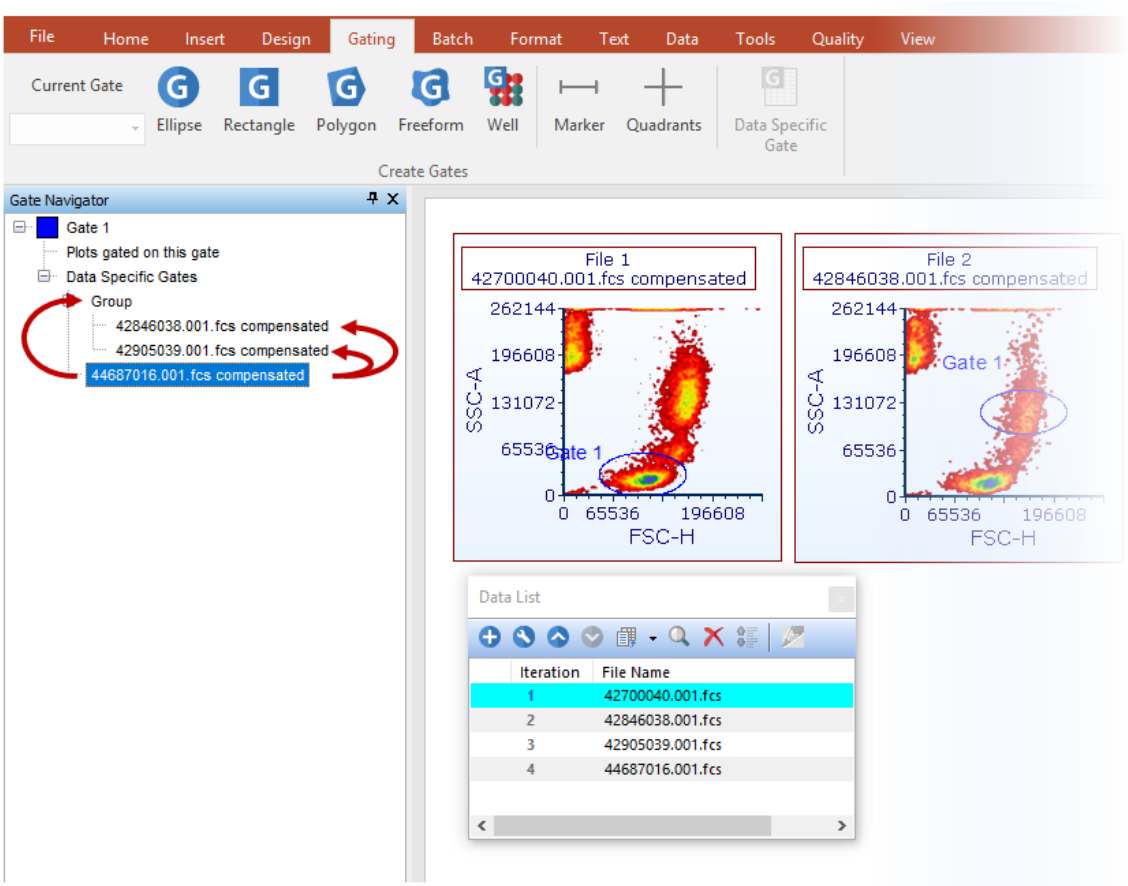

Figure 9.21 - When a DSG group is already present, file(s) can be added to the group by dragging them from either the Data List (upper panel) or the Gate Navigator (lower panel), and realeasing them on top of either the DSG group entry or on top of any DSG already listed in the DSG group of interest.

Removing and Deleting a Data Specific Gate Group

An entire Data Specific Gate group can be deleted by:

1. Selecting the Data Specific Gate Group or multiple selecting the Data Specific Gates composing it in the gate navigator.
2. Press **Delete** on your keyboard.
   a. Alternatively, Right click on the selected Data Specific Gate.
   b. Select "**Delete Data Specific gate(s)**"

One Data Specific Gate can be removed from a Data Specific Gate group by:

1. Selecting the gate within a plot displaying it (Figure 9.22) - Number 1)

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2. Click the Data Specific Gate button in the **Gating** tab→**Create Gates** on the (Figure below - Number 2)

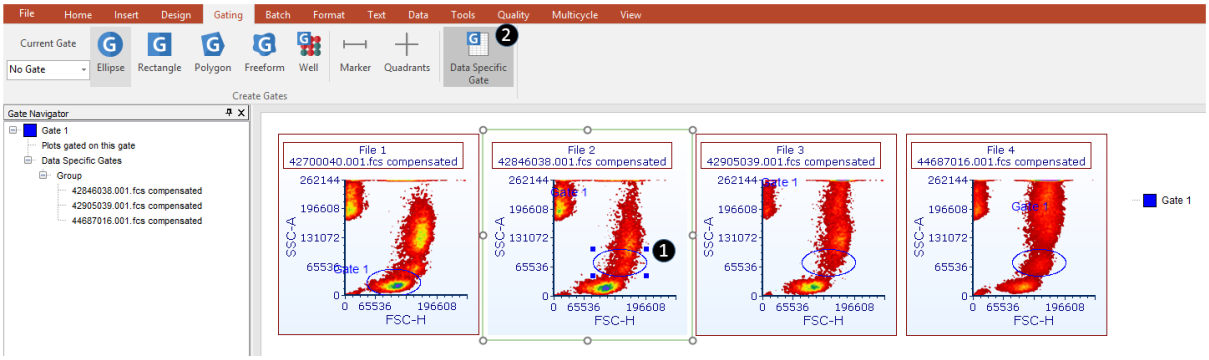

Figure 9.22 - Removing a data specific gate from a group by clicking the Data Specific Gate button twice.

9.1.4 Using Boolean Logic to Create Combination Gates

Creating Combination Gates

Combination gates consist of combinations of other gates. To combine gates, you create a gating formula consisting of Boolean combinations of pre-existing gates. Gating formulas are defined by either a single gate or several gates joined by Boolean operators. The operators describe how the gating regions should be combined and follow the rules of Boolean algebra. FCS Express supports the following operators:

- **AND**

  AND is represented by typing 'a', 'A', or 'and' into the gating formula. If you enter the gating formula "1 a 2" (read as, 1 and 2), only events that fall into both gate 1 and gate 2 are selected.

- **OR**

  OR is represented by typing 'o', 'O', or 'or' into the gating formula. If you enter the gating formula "1 o 2" (read as, 1 or 2), all events that fall into either gate 1 or gate 2 are selected.

- **NOT**

  NOT is represented by typing 'n', 'N', or 'not' into the gating formula. If you enter the gating formula "n1" (read as, not 1), all events except the events in gate 1 are selected.

- **XOR**

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XOR is represented by typing 'x' or 'X' or 'xor' into the gating formula. If you enter the gating formula "1 x 2" (read as, 1 xor 2), only events that fall into gate 1 or gate 2, but not both, are selected. *Note*: XOR stands for exclusive Or. The difference between 'or' and 'xor' is that with 'or' an event that falls into both regions is selected, while with 'xor' an event that falls into both regions is excluded from selection.

Together with the four Boolean operators, Combination Gates can be created using parentheses. The contents of the innermost parentheses are evaluated first, and the remainder of the equation is evaluated from left to right. This allows you to build complex gating formulas quite easily. The following are examples of some valid gating formulas. *Note:* Spaces are not required when inputting the formula into FCS Express some people prefer to use then to make complex formulas more readable.

- "not lymphocytes or monocytes"

  Events that are not in lymphocytes or are in monocytes are selected. The NOT is only applied to the lymphocytes if you do not use parentheses.

- "n (lymphocytes or monocytes)"

  First the "(lymphocytes or monocytes)" is evaluated, then a NOT is applied. Now all events that are not in the lymphocytes gate and not in the monocytes gate are selected. The formula "Not lymphocytes And Not monocytes" would have the same result.

- "(lymphocytes and monocytes) or (granulocytes and blasts)"

  Events that are in both the lymphocytes and monocytes gates, or both the granulocytes and blasts gates are selected. Because 'and' means events must be in both gates to be selected, the only events that will be selected will be those that fall in the overlap between the lymphocytes and monocytes gate OR the overlap between the granulocytes and blasts gates.

Combination gates are created in the following way:

- After inserting a Gate View object 345 onto the layout (**Insert→General→Gate View**), right-click on the **Gate View** and select **Add Combination Gate** from the pop-up menu.

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A dialog box (Figure 9.23) will appear. Select the **Formula** field and enter Boolean operators and the names of preexisting gates. As you type, FCS Express will determine which gate or Boolean operator you are trying to type and will attempt to complete the word for you via autofill. If more than one gate or operator matches the letters you've typed, you can scroll through the available matches by using the arrow keys. Press the **space bar** to accept the choice.

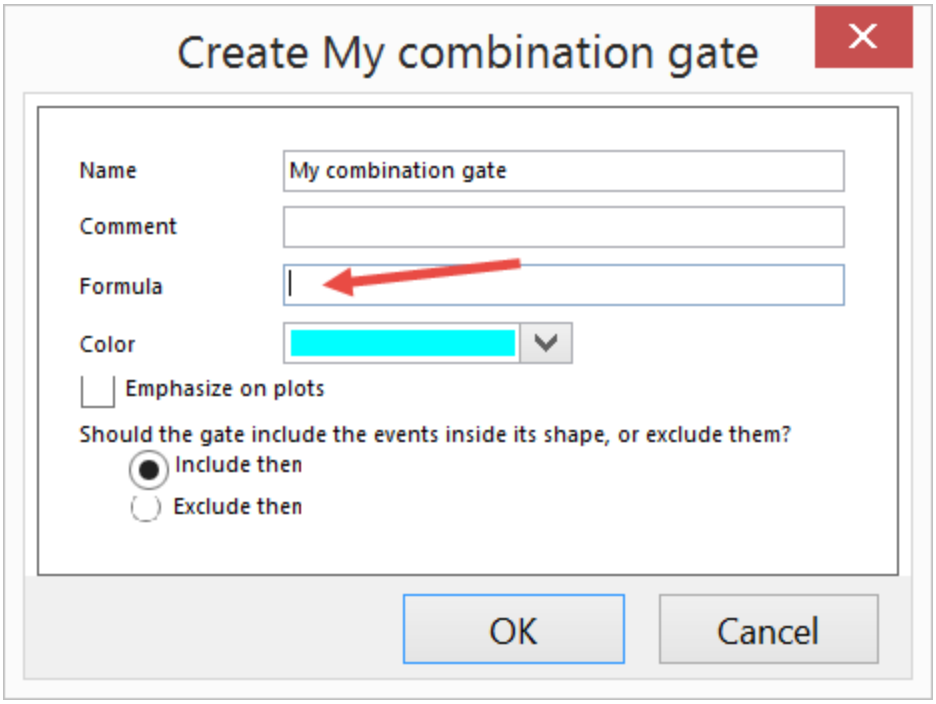

Figure 9.23 Creating a Combination Gate

9.1.5 Adding a Combination Gate Matrix

Adding a Combination Gate Matrix

A combination gate matrix is a set of combination gates, created from one or more previously existing gates. This set of gates contains every possible positive and negative value for the previously existing gates. For example, if we start with a gate labeled "CD4", and another gate labeled "CD25", select both gates, and create a combination gate matrix from them, four new gates will be created (Figure 9.24):

- CD4+/CD25+
- CD4-/CD25+
- CD4+/CD25-
- CD4-/CD25-

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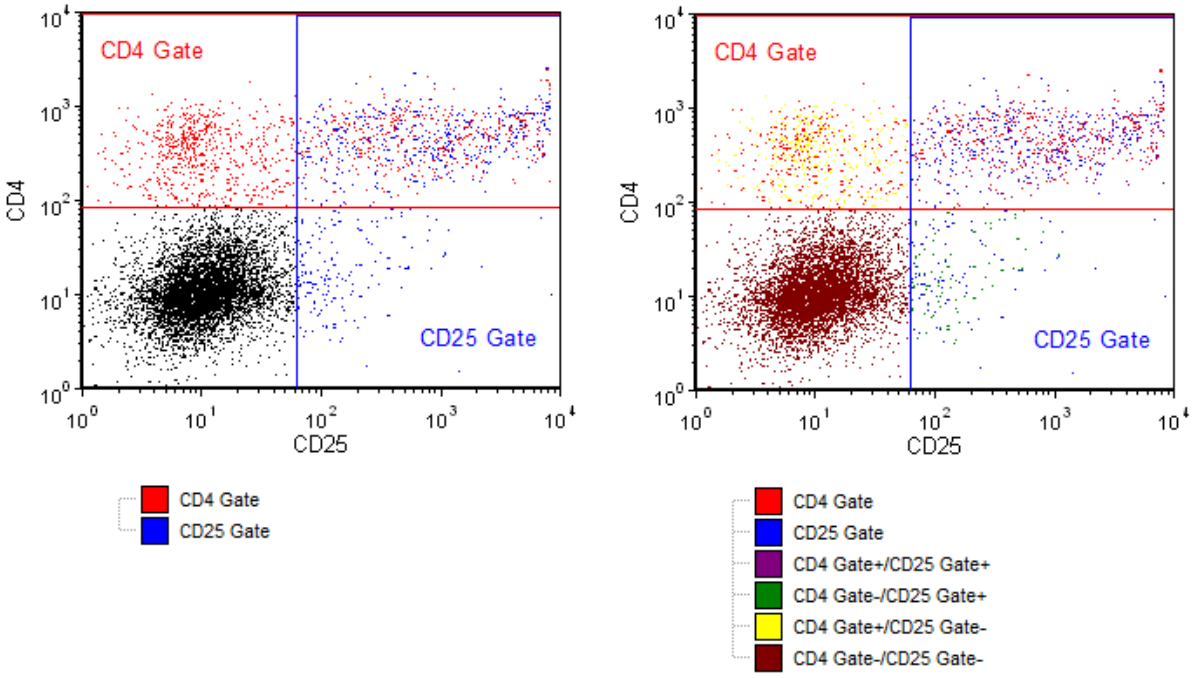

Figure 9.24 Combination Gate Matrix Example. Left: Gates applied to select all CD4+ or CD25+ cells. Right: Combination Gate Matrix applied to create gates for all four combinations.

Once the new gates are created, they are treated as normal gates. Any changes to the original gates (such as renaming) will not cause the new gates to be renamed. The new gates can also be deleted or edited like any other combination gate.

To add the combination gate matrix:

After inserting a Gate View object 345 onto the layout (**Insert→General→Gate View**), select the gates that you want to use via SHIFT-click or **Ctrl**-click, right-click on the **Gate View**, and select **Add Combination Gate Matrix** from the pop-up menu (Figure 9.25).

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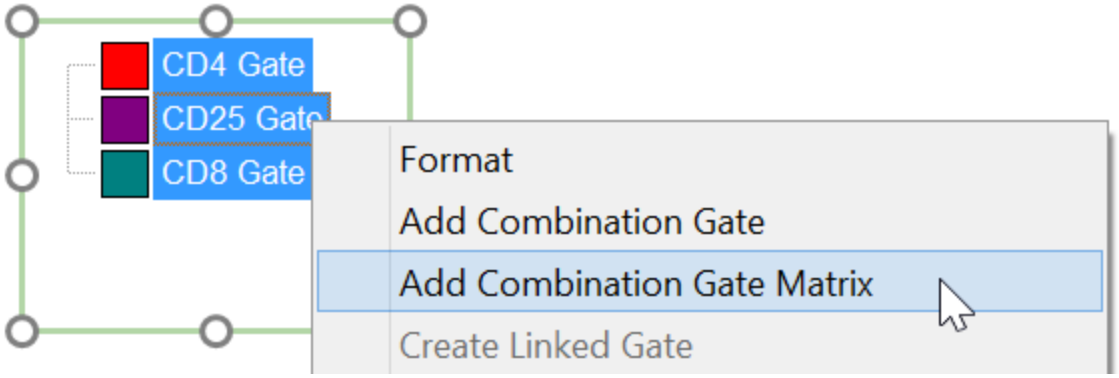

Figure 9.25 Adding a Combination Gate Matrix

If you selected three gates to create the matrix then, after the new matrix has been added, eight new gates will have been created. The gate will now look like Figure 9.26.

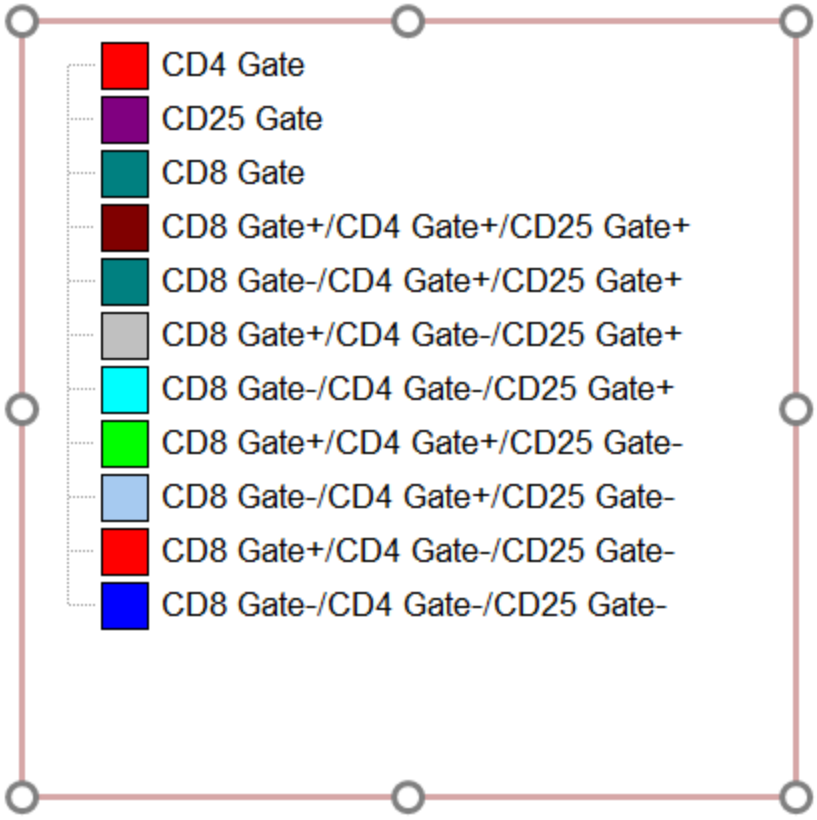

Figure 9.26 After Adding a Combination Gate Matrix

9.1.6 Moving Gates

To move a gate, click the mouse cursor once in the gate that you wish to move. This will activate the gate. The cursor will change to the drag cursor and resizing handles will appear on the vertices of the gate. To move the gate, click and hold

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the mouse button down anywhere inside the gate. Move the cursor while the mouse button is pressed, and the gate will move with the cursor. Release the mouse to drop the gate into the new position.

If one gate falls within the boundaries of a second gate you may right click on the plot→**Activate** gate→select the underlying gate. The position of the underlying gate may now be changed.

To cancel a gate move once you have begun, press and hold the **Esc** key and release the mouse button before you release the **Esc** key.

If you move a gate onto a blank part of a page, you will either:

- Create a new plot gated on the gate, or
- Delete the gate,

according to the options you set in the Gate moving options section on the Gate page of the Options dialog 207.

9.1.7 Resizing Gates

To resize a gate, click the left mouse button when the cursor is in the gate that you wish to resize. This will activate the gate. The cursor will also change to the drag cursor and resizing handles will appear. Move the cursor over one of the resizing handles. You will know you are in the correct position when the cursor changes to the resize cursor, which is a two-headed arrow. With the resize cursor, click and hold down the left mouse button. Move the mouse to move the cursor and the vertex will move with the cursor. Release the mouse button to drop the vertex into the new position.

To cancel a gate resize once you have begun, press the **Esc** key before you release the mouse button.

9.1.8 Deleting Gates

A gate can be deleted in one of two ways:

- Select the gate from a Gate View object 345 or the Gate Navigator 128 and either: Right-click and choose **Delete** from the pop-up menu, as seen in Figure 9.27 or press the **Delete** key on the keyboard.

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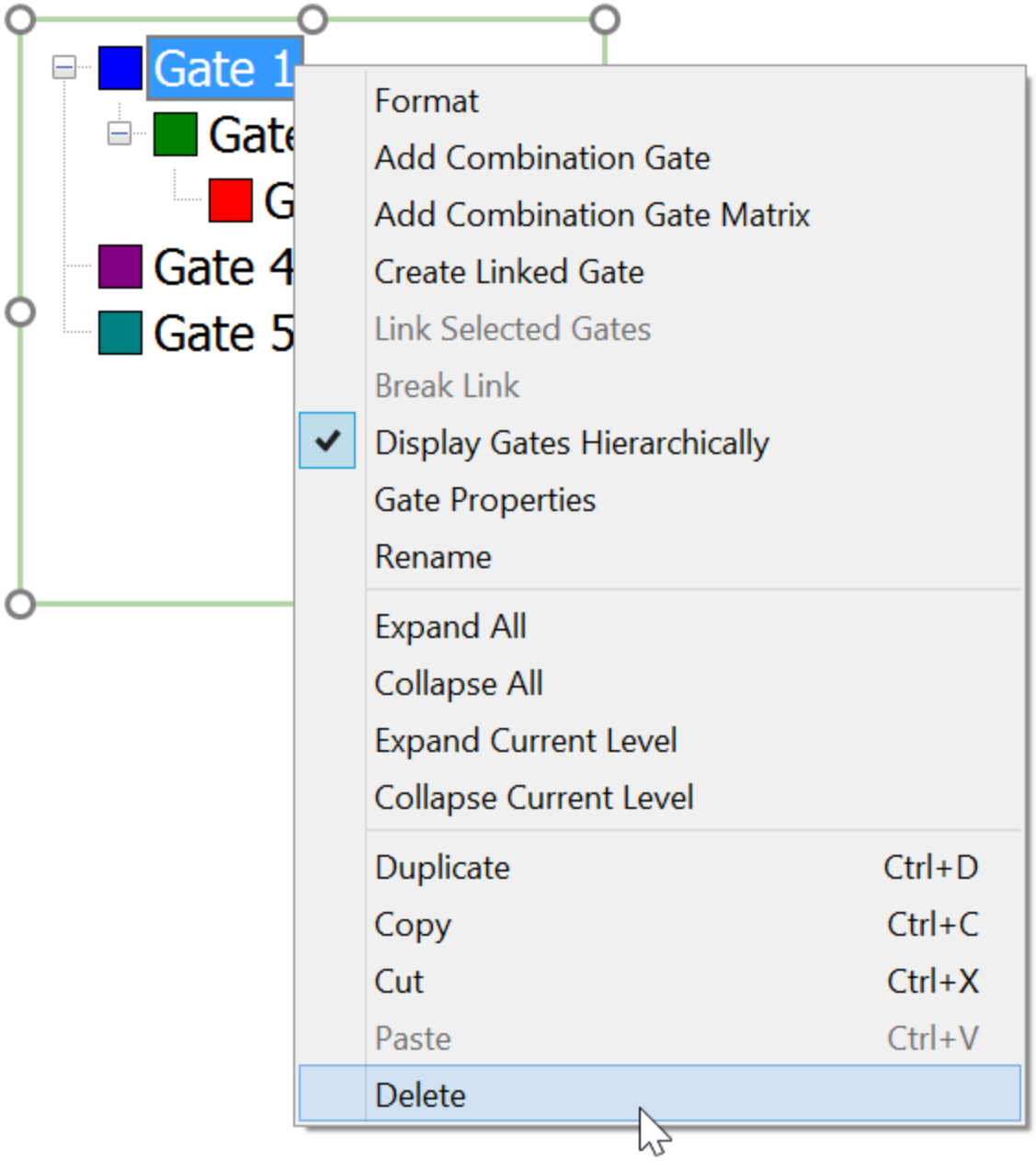

Figure 9.27 - Removing a Gate from the Gate View

- A gate can also be deleted directly from the plot. To delete a gate, click the mouse button when the cursor is in the gate you wish to delete. This will activate the gate. The cursor will then change to the drag cursor and resizing handles will appear on the vertices of the gate. Press the **Delete** key. A dialog box will appear asking whether you want to delete the gate completely, or just hide the gate 344 on that plot, as seen in Figure 9.28.

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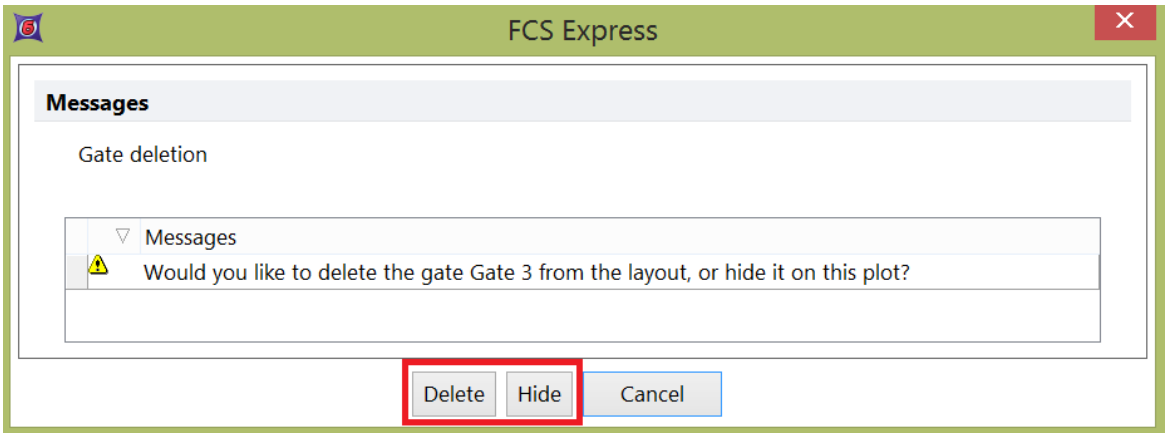

Figure 9.28 - Delete or Hide a Gate

9.1.9 Hiding Gates

To determine whether a gate is visible on a 2D plot or to show or hide a gate, right-click on the plot and select **Show Gates** from the pop-up menu. A submenu will appear listing all currently defined gates. Clicking on the desired gate in the list will toggle between showing or hiding the gate.

You can also hide a visible gate directly from a 2D plot:

1. Activate the gate that you wish to hide, by clicking inside it.
2. Press the **Delete** key.
3. Select the **Hide** button in the dialog box that appears.

Note: A 2D gate can only be displayed if the axes on the plot match the axes that were used when the gate was first created. However, color dot plots can display backgated populations 303 for any gate in your layout.

9.1.10 Using Snapshots with Gates

Snapshots 372 provide a way to save multiple gate, quadrant, or marker positions for quick access in your analysis. An unlimited number of snapshots may be created and saved in a layout. When a snapshot is added to the **Snapshot** navigator using the green + ( ) button, FCS Express will remember the position of a any gates, quadrants, or markers present on open plots. When gates, quadrants, or markers are changed on open plots the saved snapshot may be applied to the layout by dragging and dropping the snapshot from the **Snapshots** navigator to any blank space on the layout. The position of the gates, quadrants, and markers saved in the snapshot will be applied.

In the example below (Figure 9.29) note that the snapshot for Gate Position Number 1 is applied to Sample 1 on the left. When data was changed to Sample 2 on the right, the position of the CD4 and CD25 gates was updated by applying the snapshot for Gate Position Number 2. In this way many positions for gates, quadrants, or markers may be saved and easily accessed to be used as needed.

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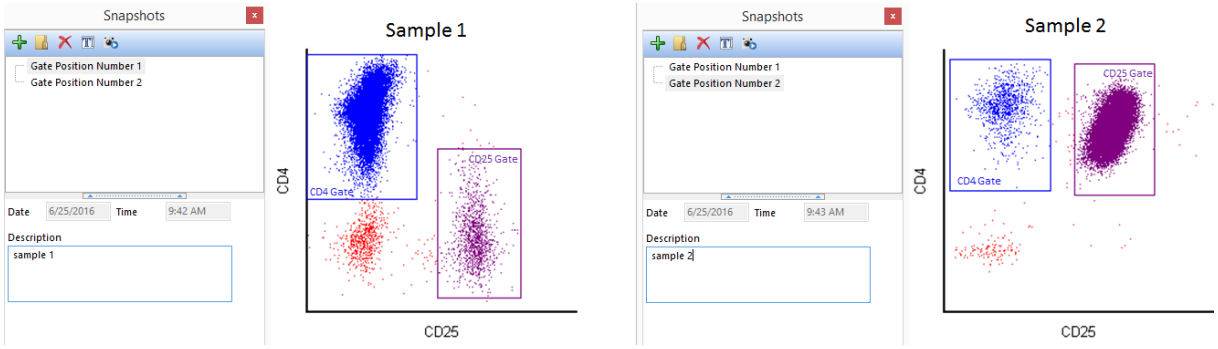

Figure 9.29 - The snapshot for Gate Position Number 1 is applied to Sample 1 on the left. When data was changed to Sample 2 the gate positions were updated by applying the snapshot fo Gate Position Number 2 to the layout.

Please see the **Snapshots** 1498 chapter 1498 for more details on creating **Snapshots**.

Please see the **Snapshots** 852 and Batch Iteration Navigation chapter 852 for more details on using **Snapshots** with a batch process and the Next / Previous commands.

Also see the **Snapshots** 1423 Tutorial 1423 for step by step instructions on creating and using **Snapshots**.

9.1.11 Gate View Object

The **Gate View** object is an interactive legend of all the gates in your layout. As an object, the **Gate View** will be printed and displayed like any other object. For additional object properties, see Formatting Options Shared by All Objects 239. The Gate Navigator 128 may also be used to edit gate information but the Gate Navigator will not be treated as an object and cannot be printed or exported.

To insert a **Gate View**, select the **Insert** tab→**General**→**Gate View** command.

Many of the actions can be performed directly from the **Gate View** as follows:

- To delete a gate from the entire layout, select the gate in the **Gate View** and press the **Delete** key.
- To rename a gate, double click the gate in the **Gate View** and type in the new gate name in the Name field and press **Enter**.
- To edit all of the gate properties, double-click on the gate in the **Gate View** to bring up the **Edit Gate** dialog (Figure 9.30). From the dialog you can change the gate **Name**, add a **Comment**, change the **Description**, change the **Color*** 347, Emphasize the gated events on plots 321, **Include** or **Exclude** the events inside the gates shape, and, if applicable, break the **link** to a quadrant or gate. If you check **Alt+Click required to select this**

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gate, then clicking on the gate will not select the gate unless the **Alt** key is being held. This will prevent accidental changing of the gate vertices.

- Additionally, the ability to edit the gate is controlled through the **Allow Gate Edit** section.
  - Selecting the **True** radio button allows edits to the gate.
  - Selecting the **False** radio button prevents edits to the gate.
  - Selecting the **Custom** radio button allows the user to define a value or condition based on a token 692 or custom token 727 that must be met in order to edit the gate.

- To rearrange the hierarchy of the gates 350, drag and drop the gates within the **Gate View**. For example, to make a gate a child gate of another gate, drag the child gate onto the parent gate. Also note that a gate may be placed in a specific position in the hierarchy at the time of creation using the Parent Gate drop down 320.

- To gate a plot on a gate, drag the gate from the **Gate View** onto that plot.

- The actions above may also be completed through the Gate Navigator 128.

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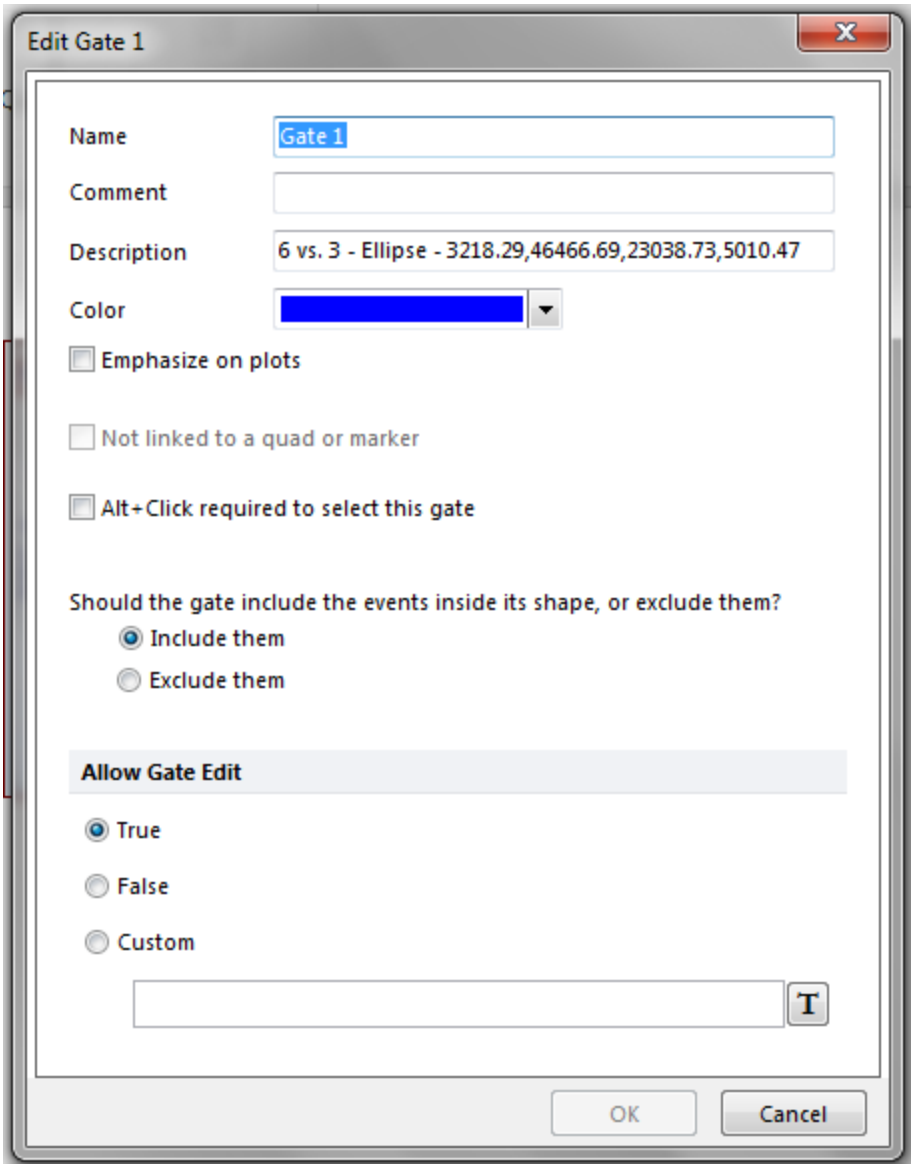

Figure 9.30 Edit Gate Dialog

* Note: If Gate Color has been changed, text color within the Gate Text Box will no longer match. To recreate this text with the current Gate Color, right-click on the plot and select **Recreate Gate Text Boxes** from the pop-up menu.

9.1.12 Gates and the Clipboard

Gates can be copied to and pasted from the clipboard for use within FCS Express. This will create an identical gate with a new name that can be moved independently of the original gate. You can also copy all gates to another layout 348 to reuse your entire gating strategy.

To show a previously created gate on another plot or on a plot on which it is currently hidden, use the Show Gates command 344 instead.

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- **Copying a Gate to the Clipboard**

  Click the mouse button when the cursor is in the gate that you wish to copy. This will activate the gate. Select the **Home→Clipboard→Copy Gate** command or press **Ctrl+C** from the keyboard. Similarly, you can select the gate in the Gate View 345 or Gate Navigator 128 and select **Home→Clipboard→Copy Gate** or press **Ctrl+C** from the keyboard. You can also right click on the Gate and select **Copy Gate** from the right click menu.

- **Cutting a Gate to the Clipboard**

  Click the mouse button when the cursor is in the gate that you wish to cut. This will activate the gate. Select the **Home→Clipboard→Cut Gate** command or press **Ctrl+X** from the keyboard. Similarly, you can select the gate in the Gate View 345 or Gate Navigator 128 and select the **Home→Clipboard→Cut Gate** command or press**Ctrl+X** from the keyboard. If you try to cut a gate from a plot, a dialog will appear asking if you want to delete the gate completely or hide it in that particular plot. You can also right click on the Gate and select **Cut Gate** from the right click menu.

- **Pasting a Gate from the Clipboard**

  Right-click on a plot, the Gate View 345 object, or Gate Navigator 128, and press **Ctrl+V** on the keyboard, or select the **Home→Clipboard→Paste Gate** command. Note that in order to paste a gate as a child of another gate, the prospective parent gate should be selected in the Gate View or Gate Navigator first. Alternatively, you may drag the gate into the prospective parent Gate (see Hierarchical Gating 350 for details) to define it as a child gate.

- **Copying All Gates to Another Layout**

  From the Gate View 345 object, or Gate Navigator 128, press **Ctrl+A** on the keyboard to select all, and **Ctrl+C** to copy. Then, in the new or second layout, open the Gate View or Gate Navigator, and press **Ctrl+V** to paste.

9.1.13 Linking Gates

In FCS Express, you can link two or more gates of the same type together. This will make the gates duplicates of each other. Then, moving or resizing one gate will move all linked gates.

To link two gates:

1. Insert a **Gate View** using the **Insert→General→Gate View** command on a layout with plot(s) containing two or more gates of the same type. *Note:* You can only link gates of the same type, e.g., two rectangular

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gates, or two elliptical gates. You cannot link a rectangular gate to an elliptical gate.

2. Select both gates in the **Gate View** (using **Shift**-Click).

3. Right-click on one of the selected gates and choose **Link Selected Gates** from the pop-up menu (Figure 9.31).

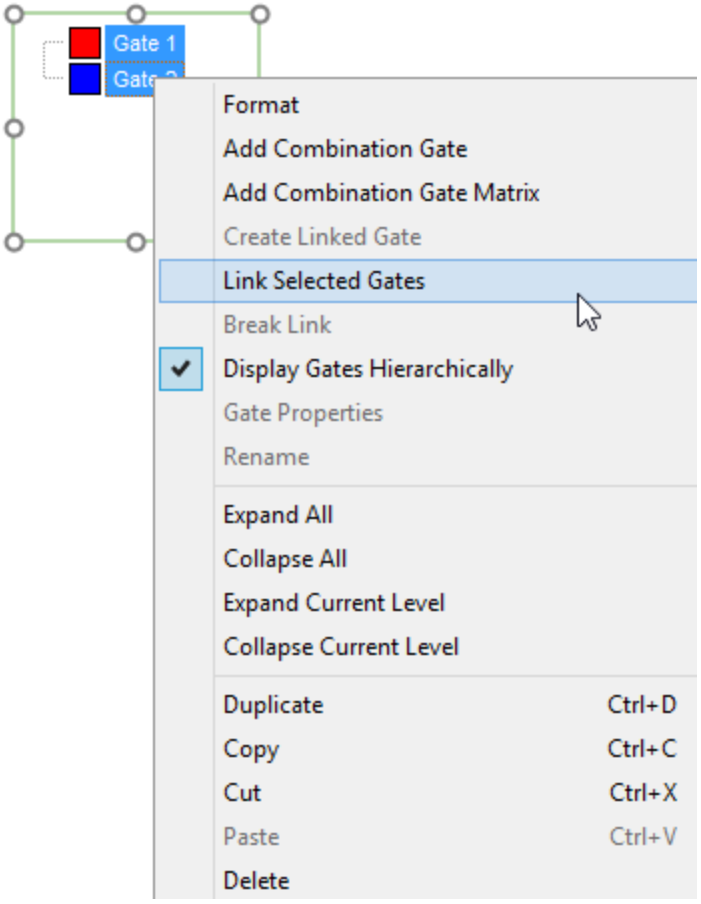

Figure 9.31 Linking Gates

In the **Gate View**, when one of the gates is selected, the icon for the gates will change from a square to a chain to indicate that the gates are linked (Figure 9.32).

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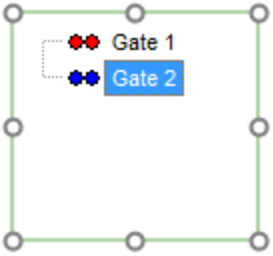

Figure 9.32 Linked Gates with Chain Icon

To break a link:

1. In the **Gate View**, right-click on one of the linked gates and select **Break Link** from the pop-up menu.

You can also clone/duplicate a single gate to create linked gates:

1. In the **Gate View**, right-click on the gate you want to clone and select **Create Linked Gate** from the pop-up menu.
2. In the **Create Linked Gate** window that appears, enter a suffix that you want to use to recognize the linked gate (Figure 9.33).
3. Click **OK**.
4. You will see a new cloned gate added to the Gate View, with the suffix tagged on the end.

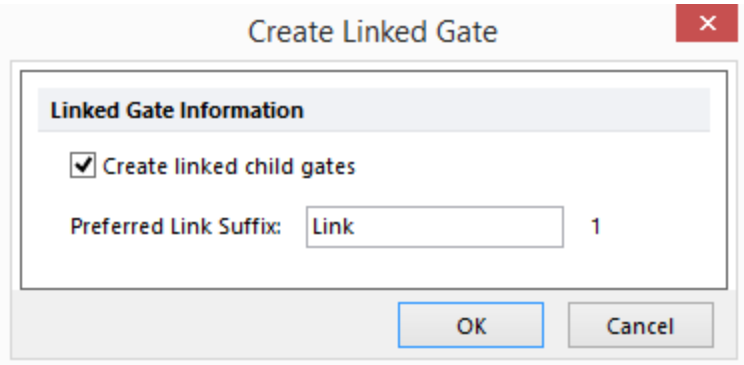

Figure 9.33 - Creating a Linked Gate

9.1.14 Hierarchical Gating

In examining flow cytometry data, a common theme is the analysis and comparison of cellular subsets. In practice, this results in the widespread use of combination gates with the Boolean operator *AND*. For example, you may first specify a live cell subset with the intention of examining lymphocytes,

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monocytes, and granulocytes within the live cells subset. To do so, you first define four gates: *Live cells*, *Lymphocytes*, *Monocytes*, and *Granulocytes*. However, the subsets you wish to examine are combination gates of the type: *Live cells and Lymphocytes*, *Live cells and Monocytes*, and finally, *Live cells and Granulocytes*. Using this Boolean combination gating method, there are now seven gates in the gate list. Adding additional subsets, e.g., *T- and B-lymphocytes*, increases the gate number further.

Hierarchical gating facilitates this type of analysis by introducing the concept of "parent" and "child" gates, thus obviating Boolean operators in a typical gating strategy. A parent gate can contain one or more child gates and the gating criteria of a parent gate are passed on to the child gate. In the example we are discussing, the use of hierarchical gating reduces the number of gates present from seven to four and eliminates the need to manually define combination gate formulas.

The current gating hierarchy for a layout is listed in the Gate View 345 or in the **Gate Navigator** 128.

In Figure 9.34, the hierarchical gating strategy for the gates in our example are displayed in the **Gate View**.

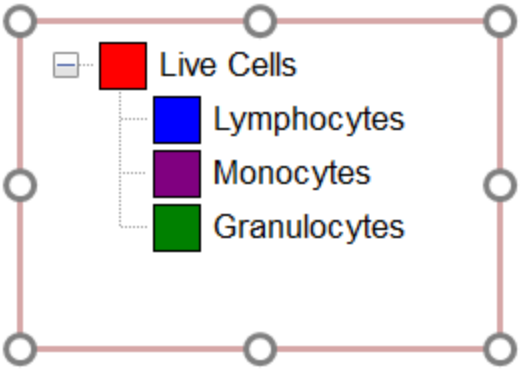

Figure 9.34 Gate View Showing Hierarchy

Note that the **Lymphocytes**, **Granulocytes**, and **Monocytes** gates are children of the parent **Live cells** gate. This means that if you use the **Lymphocytes** gate on a plot, the plot is actually gated on *Live cells AND Lymphocytes*. The same will hold true for the other two child gates.

It is possible to change the gating hierarchy at any time, simply by clicking on the gates in the **Gate View** and dragging the gate to a new hierarchical position.

When creating a new gate you may also choose a specific gate in the hierarchy as the parental gate by selecting the **Parent Gate drop down** 320.

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If you create a new gate on a plot that is already gated, and the **when a new gate is created, create it hierarchically** option is turned on in the gate options 207, the new gate will become a child of the plot's current gate. Note that this option is turned on by default in FCS Express 6.

9.1.15 Applying Gates to Plots

There are several different ways to apply a gate to a plot such that only the events in the applied gate will appear in the plot:

- Create a Gate View 345 from the **Insert→General** group. Press and hold the mouse button on the gate that you want to apply. While holding the mouse button down, drag and drop the gate from the **Gate View** list onto a plot.
- Open the Gate Navigator from the View tab→**Navigators→Gate Navigator**. Press and hold the mouse button on the gate that you want to apply. While holding the mouse button down, drag and drop the gate from the **Gate Navigator** onto a plot.
- Select a gate on a plot. While holding the mouse button down, drag and drop the gate to another plot. If dragged to an empty spot on the layout, a new plot gated on that gate will be created.
- Select a plot or multiple plots 1216 and go to the **Gating→Create Gates** group. Select the gate from the **Current Gates** drop-down list.
- Right-click on a plot and select **Format** from the pop-up menu. Go to the **Overlays** category (see Formatting Single Parameter Histograms 267 or Formatting 2D plots 284) and choose the gate you want to apply from the drop-down list. This is a convenient for applying different gates to overlays on the same plot.

Note: Different overlays on a plot need not have the same gate applied.

9.2 Using Markers

Markers are areas (sometimes known as "regions" in other data analysis software) that you set on a 1D plot (histogram). Markers are accessed by selecting the **Gating** (or **Home**) tab→**Create Gates→Create Marker** command and subsequently drawing a marker on a histogram of interest.

Markers may be:
Created on any histogram 353
Adjusted and moved manually 353
Resized manually 355
Linked to other marker endpoints from the same or different plots 360
Linked to any token value 357

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Locked to a specific position/value 355

9.2.1 Creating Markers

To create a marker on a plot, either:

- Select the **Gating** (or **Home**) tab→**Create Gates**→**Create Marker** command, or
- Right-click on the plot and select **Create marker** from the pop-up menu.

Then, position the cursor where you want the left marker boundary to be, and press and hold the mouse button down. While holding the mouse button down, move the cursor to the right until the desired right marker boundary, and release the mouse button.

Markers can be updated in real time if desired by choosing the FCS Express User Option to update Gates, Markers, and Quadrants immediately. 317

High and Low bounds of markers within one plot or in different plots may be linked so they move together 355 when either marker is adjusted.

9.2.2 Moving Markers

Markers can be moved by clicking and holding on the center point of the marker using the mouse and adjusting the position of the marker.

When the marker is selected by clicking on it, three points will appear: a resizing handle at the left- and right-boundaries, and a point in the center for moving the marker (Figure 9.35). Position the mouse cursor over the central point so it turns into a four-headed arrow. While keeping the mouse button pressed, move the marker to a new position. Release the mouse when the marker is in the new position. To cancel moving the marker mid-way through a move, press the **Esc** key before releasing the mouse button and the marker will return to its original position before a move was attempted.

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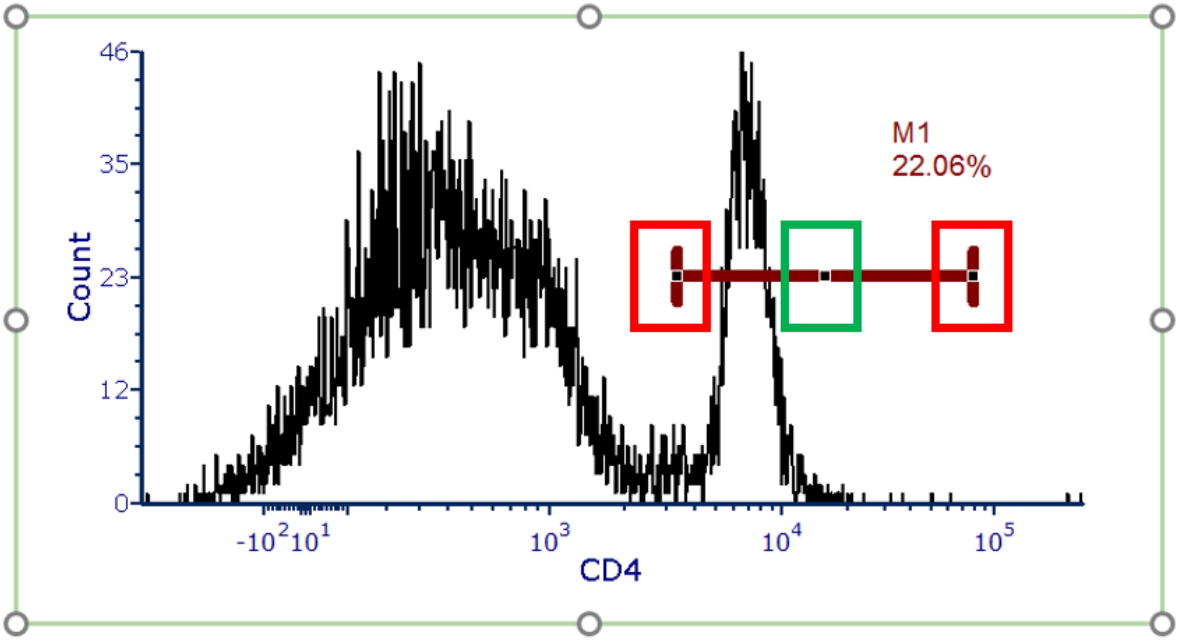

Figure 9.35 - Histogram showing a marker. Red boxes indicate the marker resizing handles and green box indicates the marker center point.

Please refer to the next chapter to learn how to resize a marker with handles or move a marker by defining its boundaries 354.

9.2.3 Resizing Markers

Markers can be resized by either of the following ways:

1. Using the mouse 355.
2. Via the Formatting Marker dialog 355.
3. By dragging and dropping tokens on marker bounds 357.
4. By dragging and dropping a different marker bound to link them 360.
5. By dragging and dropping a gate to choose a statistic to link. 362
6. By dragging and dropping a plot to choose a statistic to link. 363

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9.2.3.1 Resizing marker boundaries using the mouse

Markers can be resized using the mouse in the following way:

- Click anywhere on the marker to select it.
- When the marker is selected, three points will appear: a resizing handle at the left- and right-boundaries, and a point in the center for moving the marker 353 (Figure 9.36).
- Position the mouse cursor over one of the resizing handles so it turns into a two-headed arrow.
- While holding the mouse button down, move that marker boundary to a new position.
- Release the mouse when the marker is the desired size.
- To cancel adjustment of the marker before you have released the mouse button, press the **Esc** key and the marker will return to the original position.

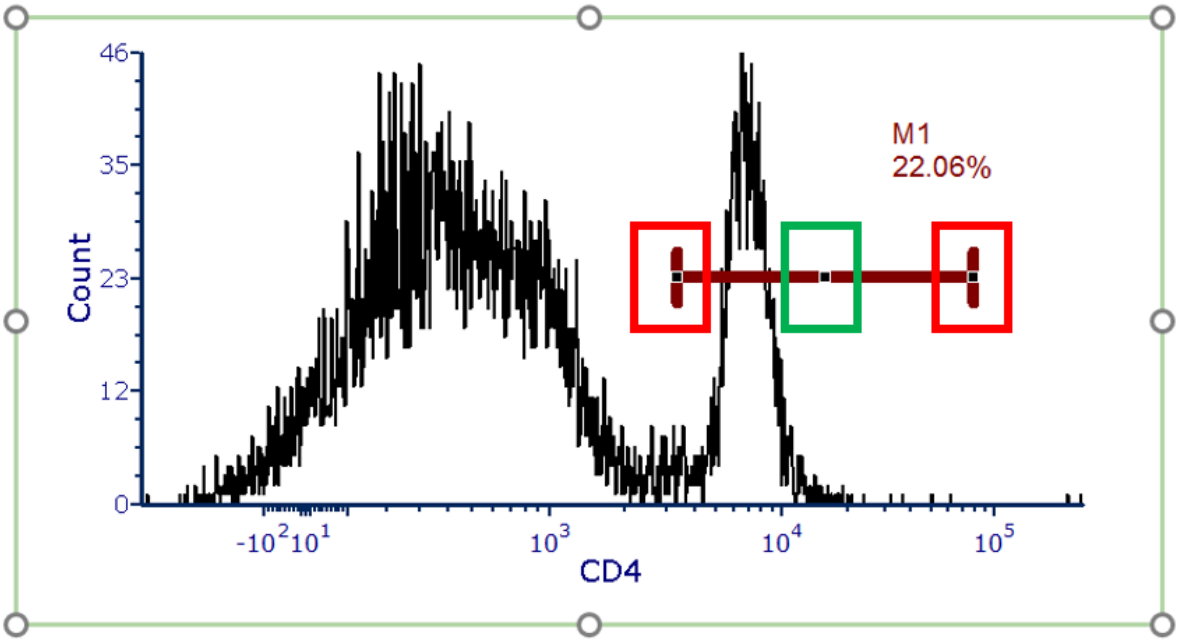

Figure 9.36 - Histogram showing a marker. Red boxes indicate the marker resizing handles and green box indicates the marker center point.

9.2.3.2 Resizing the marker boundaries via the Formatting Marker dialog

Markers end points may be set and/or locked to any number or numeric token via the **Formatting Markers dialog** 272 in the following way:

- With the plot containing the marker you want to resize in selected or edit mode, use the **Format→1D Options→Markers** command from the ribbon bar (alternatively, right click on the plot, choose **Format** and select the **Markers** category) to launch the **Formatting Markers dialog** 272.

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- In the list of **Marker**'s highlight the marker you would like to resize by clicking on it.
- In the **Marker Options** section, **Low** and **High** represent the channel numbers of the left and right marker boundaries, respectively.
- Change the **Low** and/or the **High** value by either typing in numbers (decimal values may also be used) or by clicking on the **T** icon at the right of the bound to select a numeric token. If the **T** icon is selected, then choose **Statistic** from the **Select a Token Type** dialog and select the numeric token of interest.
- Optionally, check the **Locked** check box to lock the marker bound to the specified value. When the marker bound is locked to a token, the marker bound will automatically resize when the token value changes. When a marker bound is locked, the marker bound will not be resizable when attempting to move the marker endpoint with the mouse.
- Clicking **OK** in the **Formatting** dialog will apply the changes.

Please note
*When a marker bound is **not** locked to the specified value, the marker bound will still remain resizable by moving it using the mouse. When this happens, the manually inserted value (or the token value, if a token is used to set the marker bound) will be replaced with the position defined using the mouse. To avoid this, marker bounds can be locked to the given value via the* ***Formatting Markers dialog*** 272.

Although marker end points can be set and locked to any numeric token, a useful application of this feature is setting and locking marker end points together so that moving a marker end point will automatically cause an end point on a different marker to update accordingly while preventing additional independent adjustments of the markers. Markers may be linked within the same plot or between different plots using these features.

Below is a use case examples of linking and locking markers together via the **Formatting Markers dialog** 272.

To link and lock two or more marker end points together:

Linking marker end points is accomplished by setting the **Low** or **High** bound for one marker to use a token value for the **Low** or **High** bound of a separate marker. In the following example, both markers have been created on the same plot and we will link the **High** bound of Marker 1 to the **Low** bound of Marker 2 to create a set of markers that will define positive and negative population.

1. Begin with a layout in which two markers have already been created 353 on one plot.
2. Double click on the plot on which the markers were created to launch the **Formatting** dialog.

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3. Choose the **Markers** category (Figure 9.37 below- Number 1).
4. Select the first marker (Marker 1) from the **Marker** list (Figure below - Number 2).
5. Delete the contents of the field for **High** in the **Marker Options** section (Figure below - Number 3).
6. Click on the **T** icon to choose a token (Figure below - Number 4).
7. Choose **Statistic** from the **Select a Token Type** dialog.
8. Click **Insert**. The **Create Statistic** dialog opens.
9. Choose the **Statistic** category on the left.
10. Select M2 from the **Select a marker** drop down.
11. Choose **Low bound** from the **Statistic** list.
12. Click **OK**.
13. Check the **Locked** box of the **High** bound of Marker 1 in the **Marker Options**.
14. Click **OK** in the **Formatting** dialog.

Since the **Locked** option has been set, when the low bound of Marker 2 is adjusted, the high bound of Marker 1 will now adjust automatically to match. For the same reason, the high bound of Marker 1 cannot be adjusted independently. Please note that dragging a new token value onto the marker will update the value without having to unlock it first.

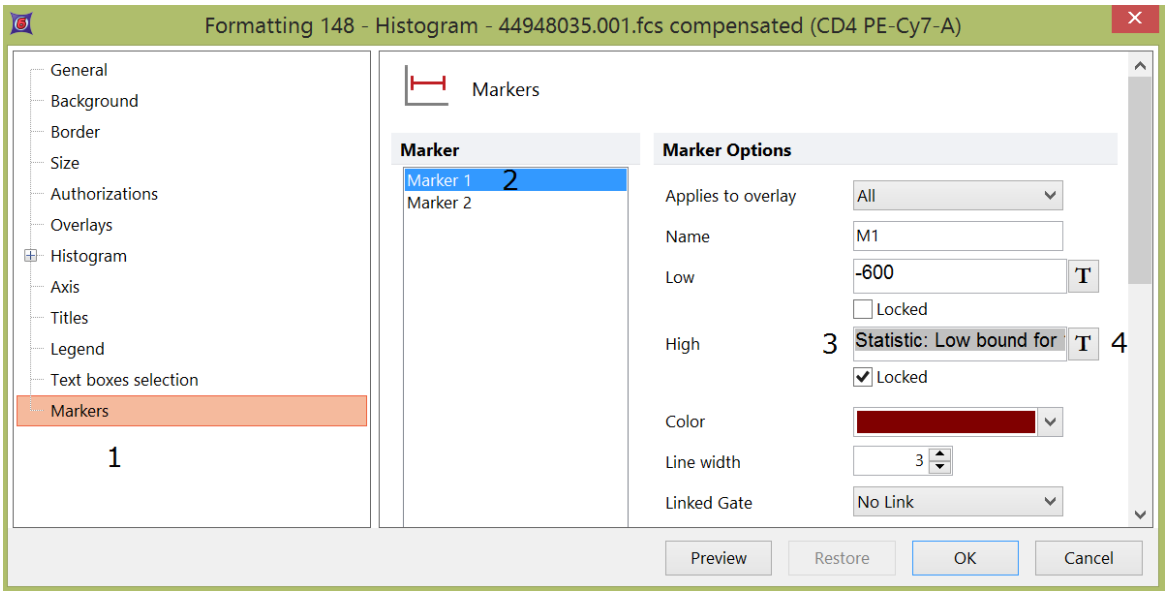

Figure 9.37 - Formatting a marker bound to use the high or low bound of another marker.

9.2.3.3 Resizing marker boundaries by dragging and dropping tokens

A marker endpoint may be set and locked to a token value by dragging and dropping a numeric token onto it. The marker endpoint will be set to the token value and will be automatically resized as soon as the token value will change.

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Please note: The marker bound will automatically be locked to the token, thus the marker bound will not be resizable by using the mouse. The lock can be removed via the ***Formatting Markers dialog*** 272 *and unchecking the* ***Locked*** *check box of the boundary of interest. Additionally, dragging a new token value to the marker end point will override the original lock and lock the newly dragged value to the end point.*

Below are two use case examples of setting and locking marker end point to token using drag and drop:

Using a token in a statistics table to link two markers together.
In this use case the high bound of a marker (M1) is set and locked to the low bound of another marker (M2). The action will be performed via dragging and dropping the token referring to the low bound of marker M2 from a statistics table to the high bound of marker M1 (Figure 9.38). Please note that in this example, the two markers are within the same plot, but the same procedure may be used for markers which reside on different plots as well.

1. Left click the cell of the stat table containing the token of interest to select it (Figure below - Number 1).
2. While pressing the left mouse button drag the cell on top of the right end point of marker M1. Note that the cursor will appear as (Figure below - Number 2).
3. Release the mouse button on top of the right end point of Marker M1. The position of the right end point of Marker 1 now matches the value of the token (Figure below - Number 3).
4. When token value will changes by moving the low bound of marker M2, the right end point of marker M1 will change accordingly (Figure below - Number 4). Please note that the opposite is not true. Since the right end point of marker M1 is automatically locked to the left end point of marker M2, the right end point of marker M1 will not be resizable by using the mouse (Figure below - Number 5, note the absence of the resizing handle at the right-bound). The lock can be removed via the **Formatting Markers dialog** 272 by unchecking the **Locked** check box of the boundary of interest.

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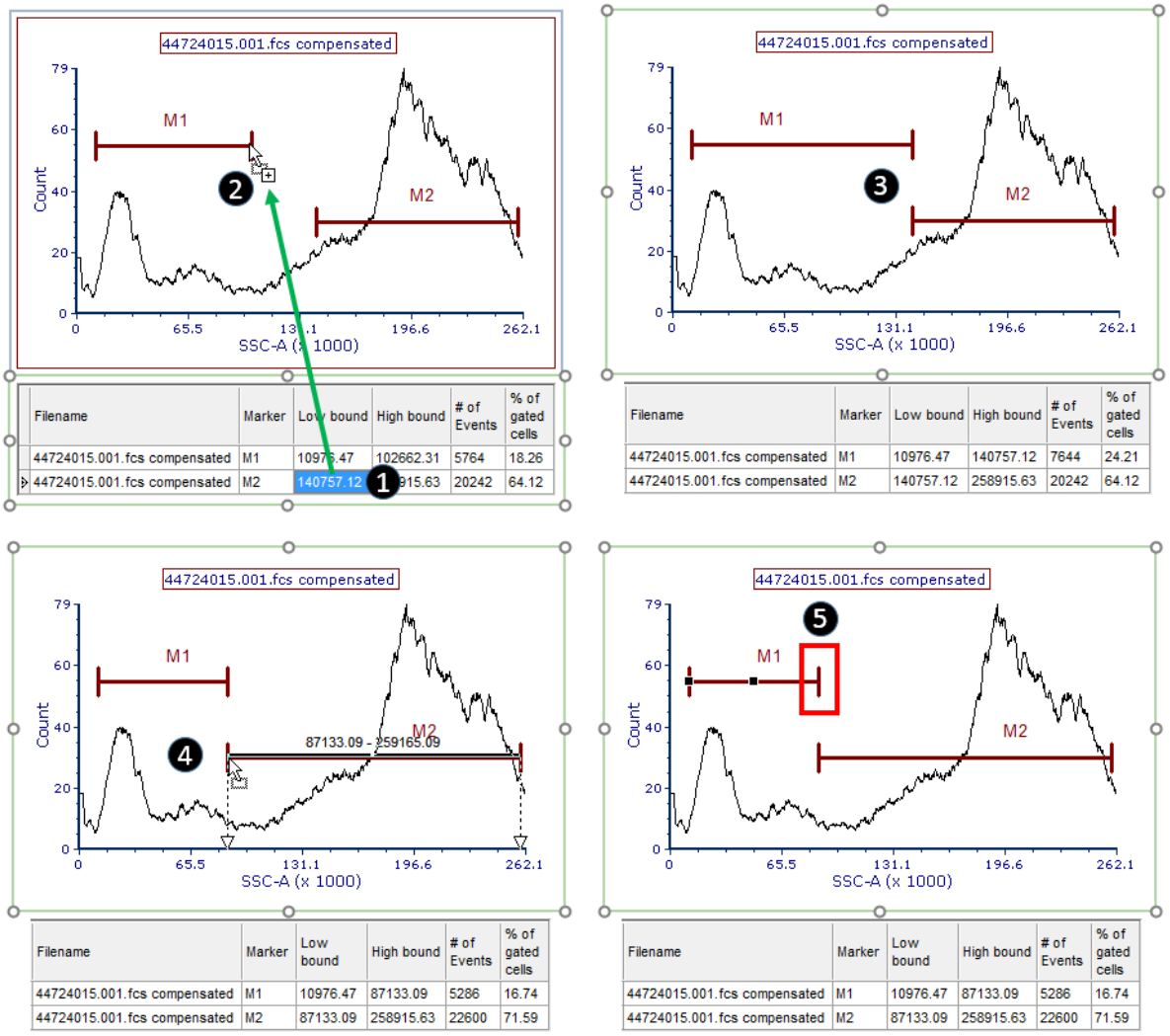

Figure 9.38. The figure shows how to set and lock two marker end points together by dragging and droppging a token contained in a statistics table.

Using a token in a text box to link a marker to it.

In this use case example a statistic token in a text box will be dragged and dropped to the end point of a marker (i.e. Marker M1) to set that end point to be equal to the value of the token (Figure 9.39). Please not that any type of statistical tokens, included custom tokens, can be used when they exist in a text box.

1. Highlight the token of interest (Figure below - Number 1).
2. Left click on the highlighted token and drag it on top of the right end point of Marker 1. Note that the cursor will appear as ⊞ (Figure below - Number 2).
3. Release the mouse button on top of the right end point of Marker M1. The position of the right end point of Marker 1 now matches the value of the token (Figure below - Number 3).

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4. When the token value changes, the right end point of marker M1 will change accordingly. Please note that the opposite is not true. In addition, since the right end point of marker M1 is automatically locked to the token, the right end point of marker M1 will not be resizable by graphically acting on it using the mouse (Figure below - Number 4, note the absence of the resizing handle at the right-bound). The lock can be removed via the **Formatting Markers dialog** 272 by unchecking the **Locked** check box of the boundary of interest.

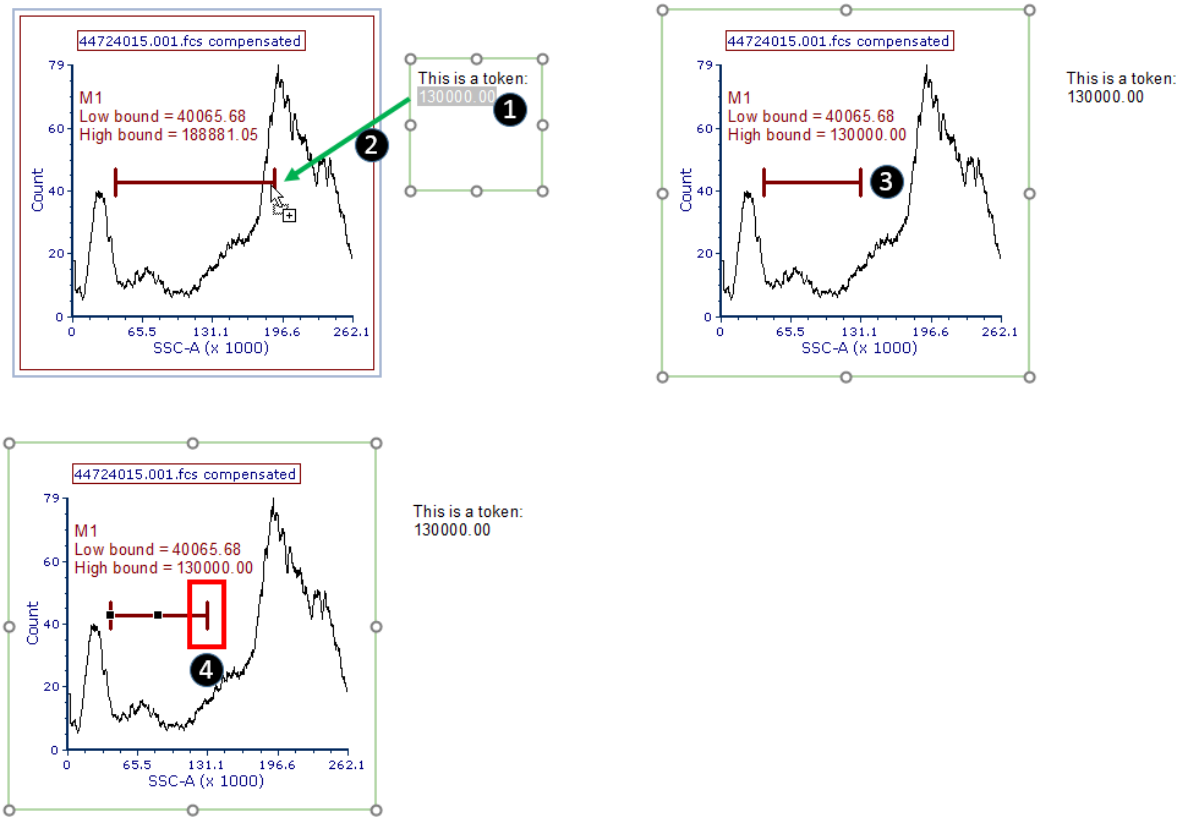

Figure 9.39. The figure shows how to set and lock a marker end point to a token by dragging and dropping the token from a text box.

9.2.3.4 Resizing and linking marker boundaries by dragging and dropping marker bounds

A marker endpoint may be set and locked to an end point of another marker by dragging and dropping one marker endpoint onto another marker endpoint. The dependent marker endpoint will be automatically repositioned as soon as the independent marker endpoint changes.

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Please note: The dependent marker bound will automatically be locked to the independent one, thus the dependent marker bound will not be resizable by using the mouse. The lock can be removed via the ***Formatting Markers dialog*** *by unchecking the* ***Locked*** *check box of the boundary of interest.*

Below is a use case example of linking two marker end points together using drag and drop:

To set and lock two marker end points together using drag and drop.
In this use case the high bound of a marker (M1) is linked to the low bound of another marker (M2). The action will be performed via dragging and dropping the low bound of marker M2 onto the high bound of marker M1 (Figure 9.40). Please note that in this example, the two markers are within the same plot, but the same procedure may be used for markers which reside on different plots as well.

1. Click anywhere on Marker 2 to select it. When the marker is selected, three points will appear: a resizing handle at the left- and right-boundaries, and a point in the center for moving the marker.
2. Position the mouse cursor over the left resizing handles so it turns into a two-headed arrow.
3. Left click to select the left resizing handles.
4. While holding the mouse button down, drag that marker boundary on top of the right end point of marker M1(Figure below - Number 1). Note that the cursor will appear as (Figure below - Number 2).
5. Release the mouse button on top of the right end point of Marker M1. The position of the right end point of Marker 1 now matches the position of the left end point of Marker 2 (Figure below - Number 3).
6. When the left end point of Marker 2 will change, the right end point of marker M1 will change accordingly (Figure below - Number 4). Please note that the opposite is not true. Since the right end point of marker M1 is automatically locked to the left end point of marker M2, the right end point of marker M1 will not be resizable by graphically acting on it using the mouse (Figure below - Number 5, note the absence of the resizing handle at the right-bound). The lock can be removed via the **Formatting Markers dialog** by uncheking the **Locked** check box of the boundary of interest. Additionally, dragging a new token value onto the marker will update the value without having to unlock it first.

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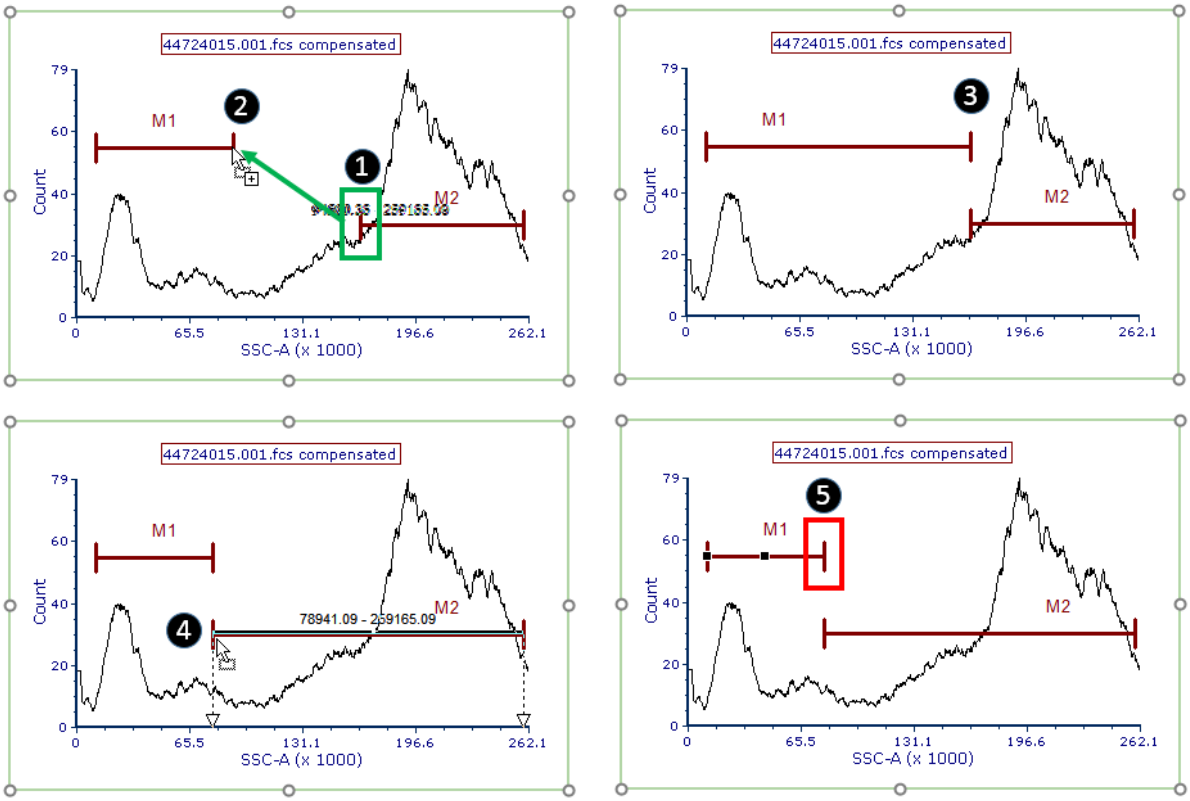

Figure 9.40 The figure shows how to set and lock two marker end points together by dragging and dropping one on top of the other.

9.2.3.5 By dragging and dropping a gate

A marker endpoint may be set and locked to a token value by dragging and dropping a gate onto it. The marker endpoint will be set to the chosen token value and will be automatically resized as when the token value changes.

Please note: The marker bound will automatically be locked to the token, thus the marker bound will not be resizable by using the mouse. The lock can be removed via the ***Formatting Markers dialog*** 272 *and unchecking the* ***Locked*** *check box of the marker bound of interest.*

To Link and lock a marker bound to a gate statistic.
In the example below the low bound of a marker (M1) is linked and locked to the X Median of a gate (Gate 1). The action is be performed by dragging and dropping Gate 1 onto the low bound of marker M1 (Figure 9.41) and selecting the X Median from the statistic list.

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1. Left click and hold anywhere on Gate 1 (Figure below - Number 1) and drag Gate 1 on top of the left end point of marker M1. Note that the cursor will appear as (Figure below - Number 2).
2. Release the mouse button on top of the left end point of Marker M1. An **Edit Statistic** dialog will open.
3. Select **X Median** from the **Statistic** list within the **Edit Statistic** window.
4. Click **OK**.

The position of the lower boundary of marker M1 will now link to the X Median of Gate 1 (Figure below - Number 3).

When Gate 1 is adjusted, the lower boundary of marker M1 will change accordingly (Figure below - Number 4). Please note that the marker endpoint is now locked to the gate based token and cannot be updated by adjusting the marker itself (Figure below - Number 5, note the absence of the resizing handle at the left-bound). The lock can be removed via the **Formatting Markers dialog** 272 by unchecking the **Locked** check box of the boundary of interest.

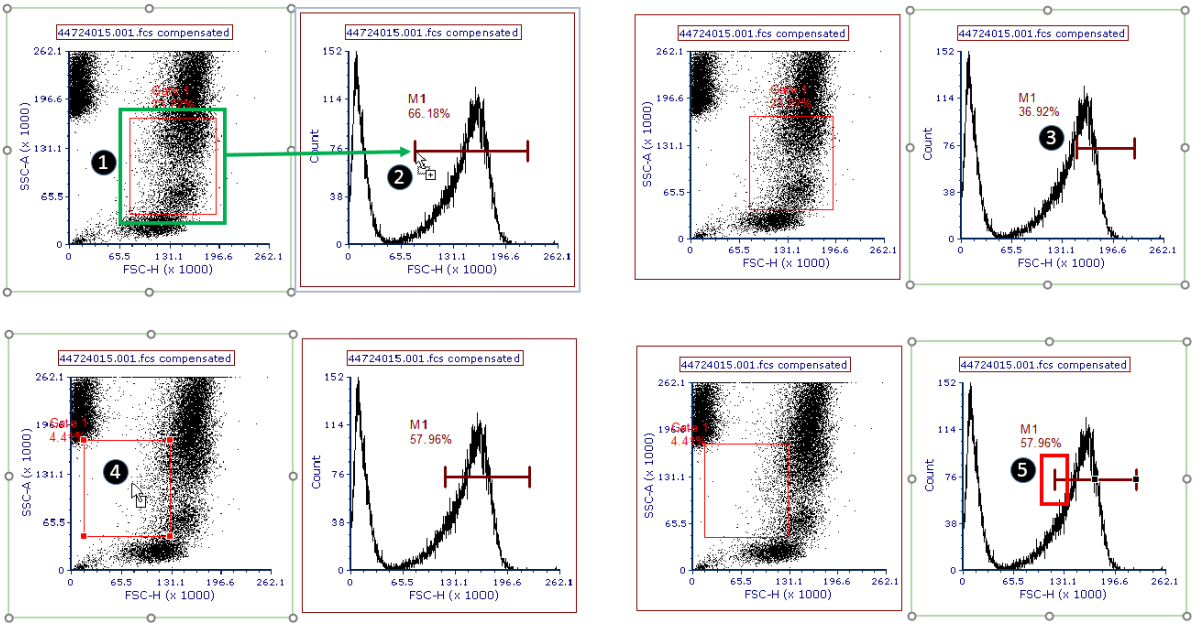

Figure 9.41 Setting and locking a marker end point by dragging and dropping a gate on to the marker boundary.

9.2.3.6 By dragging and dropping a plot

A marker endpoint may be set and locked to a token value by dragging and dropping a plot onto it. The marker endpoint will be set to the chosen token value and will be automatically resized as when the token value changes.

Please note: The marker bound will automatically be locked to the token, thus the marker bound will not be resizable by using the mouse. The lock can be

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*removed via the **Formatting Markers dialog**[272] and unchecking the **Locked** check box of the marker bound of interest.*

To Link and lock a marker bound to a plot statistic.
In the example below the low bound of a marker (M1) is linked and locked to the X Median of a quadrant on another plot. The action is be performed by dragging and dropping the plot where the quadrant is displayed onto the low bound of marker M1 (Figure 9.42) and by then selecting the X Median of the quadrant of interest from the statistic list.

1. Select the plot where quadrant is displayed by left clicking on the plot border (Figure below - Number 1).
2. While holding the mouse button down, drag the plot on top of the left end point of marker M1. Note that the cursor will appear as (Figure below - Number 2).
3. Release the mouse button on top of the left end point of Marker M1. A **Paste Special** dialog will open.
4. Select **Statistic token** from the **Paste Format** list.
5. Click **OK**. An **Edit Statistic** dialog will open.
6. Select the **Lower Right** quadrant from the **Select a quadrant** drop down menu in the **Plot Information** section of the **Edit Statistic** dialog.
7. Select **X Median** from the **Statistic** list within the **Edit Statistic** window.
8. Click **OK**.

The position of the low bound of marker M1 will now link to the X Median of the lower right quadrant (Figure below - Number 3).

When the quadrant is adjusted, the low bound of marker M1 will change accordingly (Figure below - Number 4). Please note that the marker endpoint is now locked to the quadrant/plot based token and cannot be updated by adjusting the marker itself (Figure below - Number 5, note the absence of the resizing handle at the left-bound). The lock can be removed via the **Formatting Markers dialog**[272] by unchecking the **Locked** check box of the boundary of interest.

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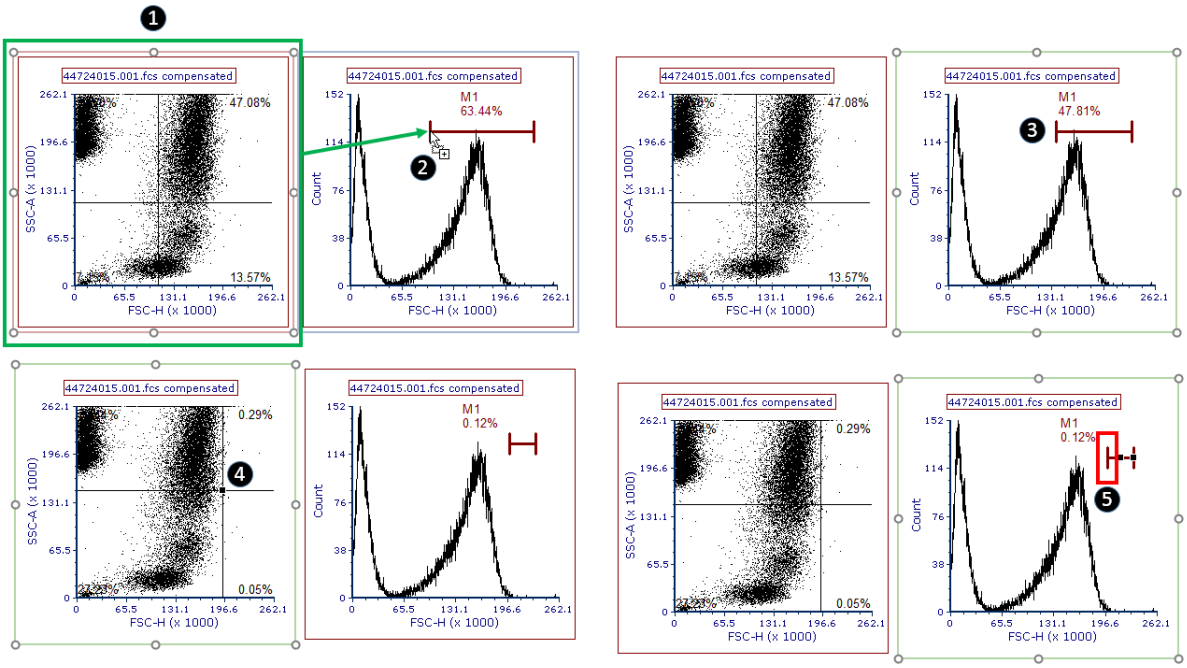

Figure 9.42 Setting and locking a marker end point by dragging and dropping a plot on to the marker boundary.

9.2.4 Deleting Markers

Markers can be deleted in one of the following ways:

- To delete a marker directly from a plot, click anywhere on the marker to select it. When the marker is selected, three points will appear: a resizing handle at the left- and right-boundaries, and a point in the center for moving the marker. Press the **Delete** key on the keyboard or select the **Home→Editing→Delete** command.

- With the plot containing the marker you want to delete in selected or edit mode, use the **Format→1D Options→Markers** command. This brings up a **Formatting Markers** dialog. In the **Marker** list box, highlight the marker(s) you want to delete. Click on the **Remove** button. Click **OK**.

- Right-click on the plot containing the marker and select **Format** from the pop-up menu. Choose the **Markers** category in the formatting dialog. Highlight the marker(s) you want to delete in the list box. Click on the **Remove** button. Click **OK**.

9.2.5 Using Markers and the Clipboard

- Copying/Cutting a marker to the clipboard

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Click anywhere on the marker to select it (three points will appear along the length of the marker). Use the **Home→Clipboard→Cut Marker** (or **→Copy Marker**) command, or standard Windows Cut or Copy keyboard shortcuts (**Ctrl+X** or **Ctrl+C**).

- Pasting a marker

  Select the plot where you want the marker to be pasted. Use the **Home→Clipboard→Paste Marker** command, or standard Windows Paste keyboard shortcut (**Ctrl+V**).

- Markers that have been copy and pasted between different plots can also be linked together 355 to move in union.

9.2.6 Linking Markers to Gates

When a marker is linked to a gate, the gate is automatically resized as the marker is resized or moved. A marker can be linked to any existing 1D gate using the Marker list in the Formatting Markers dialog 272.

You can also create a new gate from a marker and link it automatically. To do so:

- Right-click on the histogram, select the appropriate marker, and then select **Convert Marker to Gate→Convert and Link** from the pop-up menu; note, if you choose **Convert** only, the gate will not update if you move or resize the original marker. If the Show prompt window radio button is set in Gate creation prompt options 207 (Gate category of Options dialog), you will be prompted to enter a name for the new gate.

To break a linked gate, either:

1. Use the **Format→1D Options→Markers** command to bring up the **Formatting Markers** dialog (or right-click on the histogram, select **Format** from the pop-up menu, and choose the **Markers** category).
2. Select the Marker from the list on the left.
3. In the **Linked Gate** drop-down list, change the selection to **No Link**.

or,

1. Access the Edit Gate dialog 345 from the Gate View object.
2. Uncheck the **Linked to...** box (Figure 9.43)

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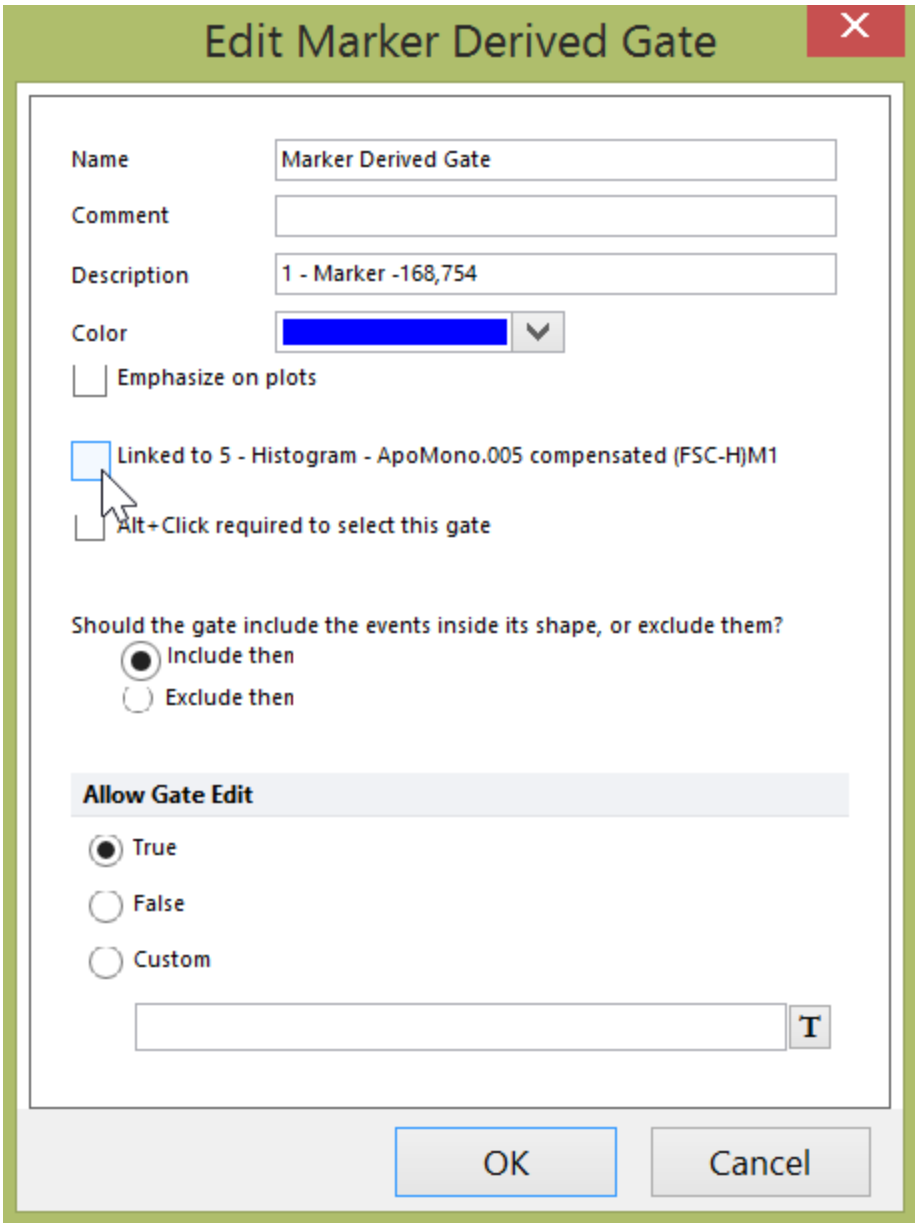

Figure 9.43 Unlinking a gate from a marker in the Edit Gate dialog

A 1D gate can be automatically created every time a marker is created. This option cab be activated:

- On one or more existing histograms by checking the **Create a Linked Gate** check box in the **Histogram Markers** 274 dialog of the histograms of interest.
- By default on every new histogram by by checking the **Create a Linked Gate** check box in the **1D Plot Preferences** 190 user options.

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9.3 Using Quadrants

FCS Express supports three different kinds of Quadrants[291]: Standard, Floating, and Percentile. The four quadrants are defined as follows: Upper Left (UL), Upper Right (UR), Lower Left (LL), and Lower Right (LR).

Standard quadrants are the quadrants found in most flow cytometry software packages. All four quadrants are square or rectangular and are defined by the central vertex. Moving the central vertex of the quadrants will redefine all four quadrants.

Floating quadrants allow the four quadrants to be non-rectangular. When the quadrants are floating, each quadrant can have a different shape. The outer vertices are no longer linked to the central vertex, and each vertex can be manipulated individually (see Floating Quads example, below). Floating quadrants allow the user to quickly and easily get statistics on populations that cannot be demarcated by standard quadrants. This can eliminate the need for compensation when only percentages are needed.

Examples of Standard Quadrants and Floating Quadrants are shown in Figure 9.44.

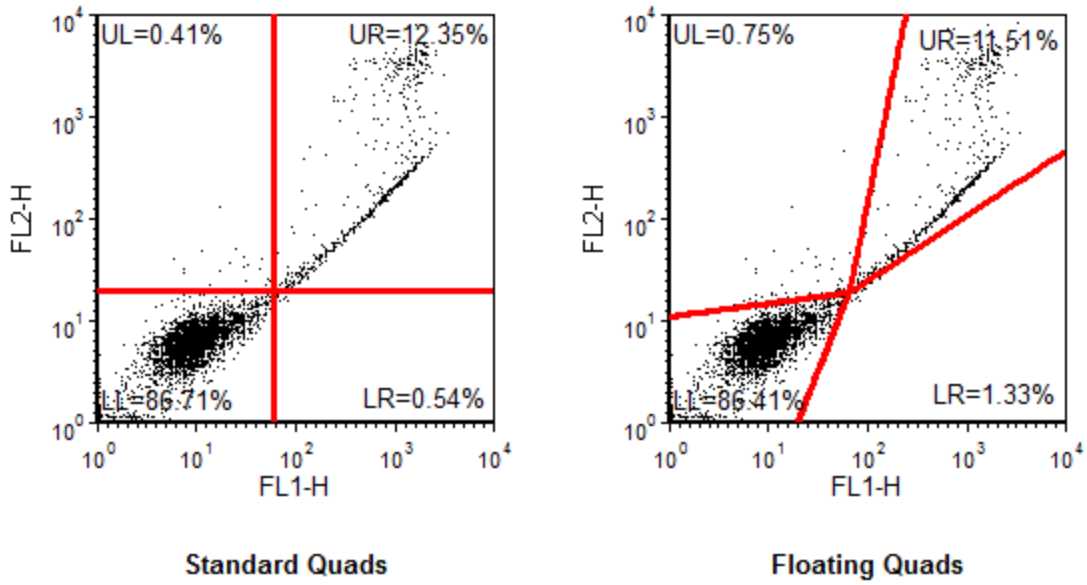

Figure 9.44 Standard vs Floating Quads

Percentile Quadrants are similar to standard quadrants except that the vertex of the quadrant is automatically calculated based on the percentile entered in the

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Quads page of the Format dialog 291. The vertices of the quadrants will automatically update as the data on the plot changes. For instance, if you set the percentile to be 99%, the quads will automatically update so the center quadrant vertex is at the channel where 99% of the events are below it. It can be useful to set Percentile Quadrants on your controls and then link those quadrants to the plots with your sample. Quad linking is described in the section on Linking Quadrants to Quadrants 371.

9.3.1 Showing Quadrants

Only one set of quadrants can exist for a plot. You can control whether quadrants are automatically displayed by checking the **Show quadrants** box in the 2D Plots page of the Options 191 dialog. The default behavior is for the quadrants to be hidden when the plot is created.

You can show the quadrants on a per-plot basis using one of the following methods:

- Right-click on the plot and select **Show Quadrants** from the pop-up menu.
- Select the **Gating→Create Gates→Quadrants** command and click on the plot at the position where you want the central vertex to be located.
- Right-click on the plot and select the **Format** menu item. Choose the **Quads** category. Enable the **Show quadrants** checkbox.

9.3.2 Moving Quadrants

Quadrant vertices can be adjusted either by using the mouse to click and drag the vertex on the plot, or by entering the vertex coordinates on the Quadrants page of a Format window. Quadrants can be updated in real time if desired by choosing the FCS Express User Option to update Gates, Markers, and Quadrants immediately. 317

- **Selecting Quadrants**
  To select the quadrants in order to edit their position, click on the central vertex, where all the lines meet. If a Standard Quadrant is selected, a resizing handle will appear at the central vertex. If Floating Quadrants are selected, resizing handles will appear over all five vertices. Percentile Quadrants cannot be selected, as their position is dictated by the Formatting Quadrants 291 dialog.

- **Moving Standard Quadrants**

  Click on the central vertex of the quadrant you want to move. You will know that you clicked on the correct position when the cursor turns into a four-headed arrow . Press and hold the mouse button while the cursor is a

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four-headed arrow. While holding the mouse button down, move the cursor to the new central vertex position. Release the mouse to move the quadrants to the new position.

- **Moving Floating Quadrants**

  Click on the central vertex of the quadrant you want to move. You will know that you clicked on the correct position when the cursor turns into a four-headed arrow. Press and hold the mouse button while the four-headed cursor is over *any of the five* vertices. While holding the mouse button down, move the cursor to the new vertex position. Release the mouse to move the vertex to the new position. *Note:* Much like the Standard Quadrants, the central vertex can move anywhere within the plot. Outer vertices will only be able to move along their original plot edge.

To enter the vertex coordinates directly on a Format window, see the Quadrants section under Formatting 2D Plots 291. *Note:* Format windows where you can enter vertex coordinates can be reached in one of two ways, either: Right-click a plot and choose **Format** from the pop-up menu; or, select a plot and use the **Format→2D Options→Quadrants** command.

To cancel moving quadrant vertices once you have started, press the **Esc** key at any time. Or, to undo a move, hit **Undo** on the Quick Access Toolbar 65 or **Ctrl+Z** on the keyboard.

Notes:
- If quadrants are linked to the quadrants on another plot 371, the quadrants on the dependent plot will move automatically.
- Percentile quadrants (see the Quadrants section under Formatting 2D Plots 291) cannot be moved manually. Instead, the percentile(s) must be redefined 291.

9.3.3 Hiding Quadrants

Quadrants always exist with a plot. They can never be deleted, but they can be hidden.

There are several ways to hide quadrants:

- Right-click on the plot and deselect the **Show Quadrants** item on the pop-up menu.
- Right-click on the plot and select **Format** from the pop-up menu. Go to the **Quads** page of the format dialog and uncheck the **Show quadrants** box.
- Select the plot and use the **Format** tab→**2D Options→Quadrants** command. Uncheck the **Show quadrants** box.

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- Select the quadrants by clicking on the central vertex. Press the **Delete** key on the keyboard, or select the **Home** tab→**Editing**→**Delete** command.

9.3.4 Linking Quadrants to Quadrants

FCS Express allows you to link quads from one plot to another. This means that when the quads on one plot are moved, the quads on the linked plot will be updated automatically. This is useful in circumstances when you want to create a reference quadrant on your control, from which the quads on your sample data update automatically as the reference control quad is adjusted. To set up linked quadrants, see Quadrant Linking Options 293.

9.3.5 Creating Gates from Quadrants

You can create a gate from a Quadrant. The gate will be created as a polygon gate with the same coordinates as the quadrant. You can create a gate from the **Upper Left**, **Upper Right**, **Lower Left**, **Lower Right**, **Upper** (*Upper Left plus Upper Right*), **Lower** (*Lower Left plus Lower Right*), **Left** (*Upper Left plus Lower Left*), or **Right** (*Upper Right plus Lower Right*) quadrants.

To create a Gate from a Quadrant:

- Right-click on the plot and select the **Convert Quads To Gates** item in the pop-up menu. Select the quad you wish to convert to a gate from the submenu. Select either **Convert** or Convert and Link 371. If you choose **Convert** only, the gate will not update if you move or resize the quad, while **Convert and Link** will allow the gate to be updated as the quad is moved.

The links may be broken at any time, from either the Quadrant Formatting dialog 293 or the Edit Gate dialog 345 from the Gate View object.

9.3.6 Linking Quadrants to Gates

It is also possible to link a quadrant to a gate so that whenever the quadrant is moved, the gate will automatically adjust to the new quadrant coordinates. This allows you to effectively use a quadrant as a gate.

To create a linked gate from a quadrant, either:

- Right-click on a plot, select **Convert Quads to Gates** from the pop-up menu→ choose the quadrant from the submenu→ **Convert and Link**, or

- Right-click on the plot and select **Format** from the pop-up menu. Select the **Quads** category. Choose **Quad to Gate** from the **Linking Options** box. Select a quadrant and an existing polygon or freeform gate from the drop-down list. Further information is available in the Quadrants section under Formatting 2D Plot 291. Alternatively, you can reach the format window by

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selecting a plot and using the **Format→2D Options→Quadrants** command.

The links may be broken at any time, from either the Quadrant Formatting dialog 293 or the Edit Gate dialog 345 from the Gate View object.

9.3.7 Using Quadrants and the Clipboard

It is possible to copy and paste quadrants to and from the clipboard.

- Copying/Cutting a quadrant to the clipboard

  Select the quadrant by clicking on the central vertex. Select the **Home→Clipboard→Copy Quad** (or **→Cut Quad**) command, or standard Windows Cut or Copy keyboard shortcuts (**Ctrl+X** or **Ctrl+C**). *Note*: Cutting does not delete the quadrants, it merely hides them.

- Pasting a quadrant

  Select the plot where you want the quadrant to be pasted. Select the **Home→Clipboard→Paste Quad** command, or right-click on the plot and use the standard Windows Paste keyboard shortcut (**Ctrl+V**).

9.4 Snapshots

Snapshots in FCS Express 6 provide a way to easily save multiple gate, quadrant, or marker positions for quick access across samples in your analysis. Snapshots can be manually defined by working with the Snapshots Navigator 374 374 or automatically each time a gate is moved while advancing through the Data List using the **Data** tab→ **Next/Prev** commands, or during Batch Processing 852. Automatically created Snapshots do not appear in the Snapshots navigator.

The Snapshots navigator can be opened from the **Tools** tab→**Snapshots** category→**Snapshots** (Figure 9.45).

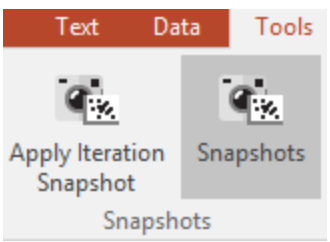

Figure 9.45 - Snapshots Icon from Ribbon

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The Snapshots navigator (Figure 9.46) includes the following options:

- **Create a new Snapshot** - Creates a new Snapshot of the current gate, marker, and quadrant positions.
- **Create a new Folder** - Creates a new folder in the Snapshot navigator to organize Snapshots from the analysis.
- **Delete** - Deletes the selected Snapshot in the navigator.
- **Rename the selected Snapshot** - Renames the selected Snapshot in the navigator.
- **Apply Snapshot** - Applies the selected Snapshot to the layout.
- **Snapshot Date and Time** - Records the Date and Time that a Snapshot of the layout was created.
- **Description** - Allows user to describe selected snapshot

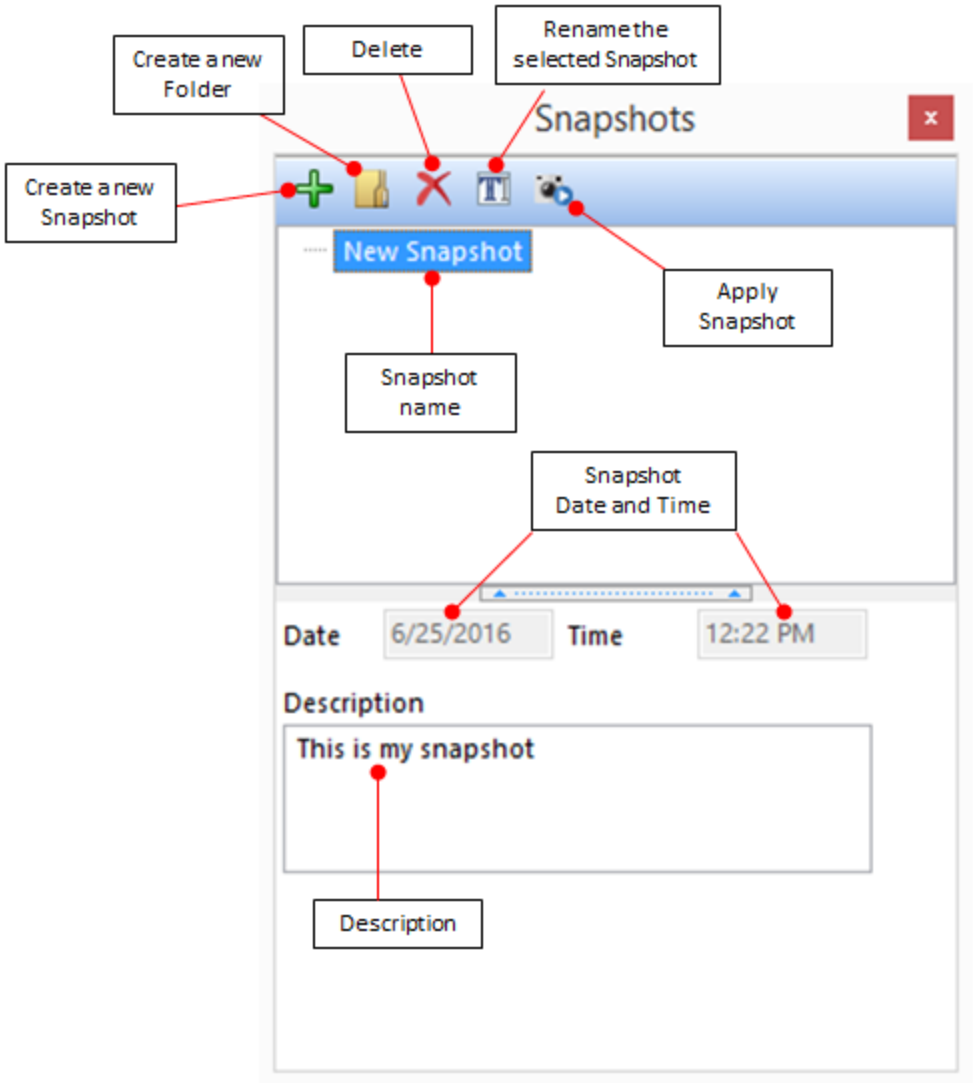

Figure 9.46 The commands available in the Snapshots navigator.

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9.4.1 Creating and Applying Snapshots

Snapshots provide an easy method for remembering multiple gate, marker, and quadrant positions across different samples or analysis runs. Snapshots may be created manual via the steps below or automatically by using Iteration Snapshots 376.

To create a Snapshot on a layout containing plots and data:

1. Open the Snapshots navigator from the **Tool** tab→**Snapshots** category→**Snapshots**.

2. Position any gates, markers, or quadrants as you would like the Snapshot to save.

3. Click on the green plus button to create a new Snapshot.

4. Enter a name for the Snapshot in the Snapshots navigator.

The position of the gates, markers, and quadrants on the layout are now saved within the Snapshot you have created (Snapshot 1 in Figure 9.47 below).

To create multiple Snapshots:

5. Repeat steps 1-4 above and give the new Snapshot(s) a name (Snapshot 1, Snapshot 2, and Snapshot 3 in Figures below).

To apply a Snapshot after the above steps 1-4 have been completed,

6. Move gates, markers, or quadrants to a new position.

7. Highlight the Snapshot in the Snapshots navigator that you intend to apply.

8. Click the camera icon within the navigator (tooltip: "Apply Snapshot")
   a. Alternatively, drag the Snapshot name from the Snapshot navigator to a blank space on the layout and drop it.
   b. Note in Figure 9.48 that "Snapshot 2" has been applied automatically changing the shape of Gate 1, position of Gate 2, the Marker position, and the position and type of Quadrant.

An unlimited number of Snapshots may be created and / or organized using the folder icon (tooltip: "Create a new folder"). Once a folder is created you may drag and drop any existing Snapshots into and out of the folder.

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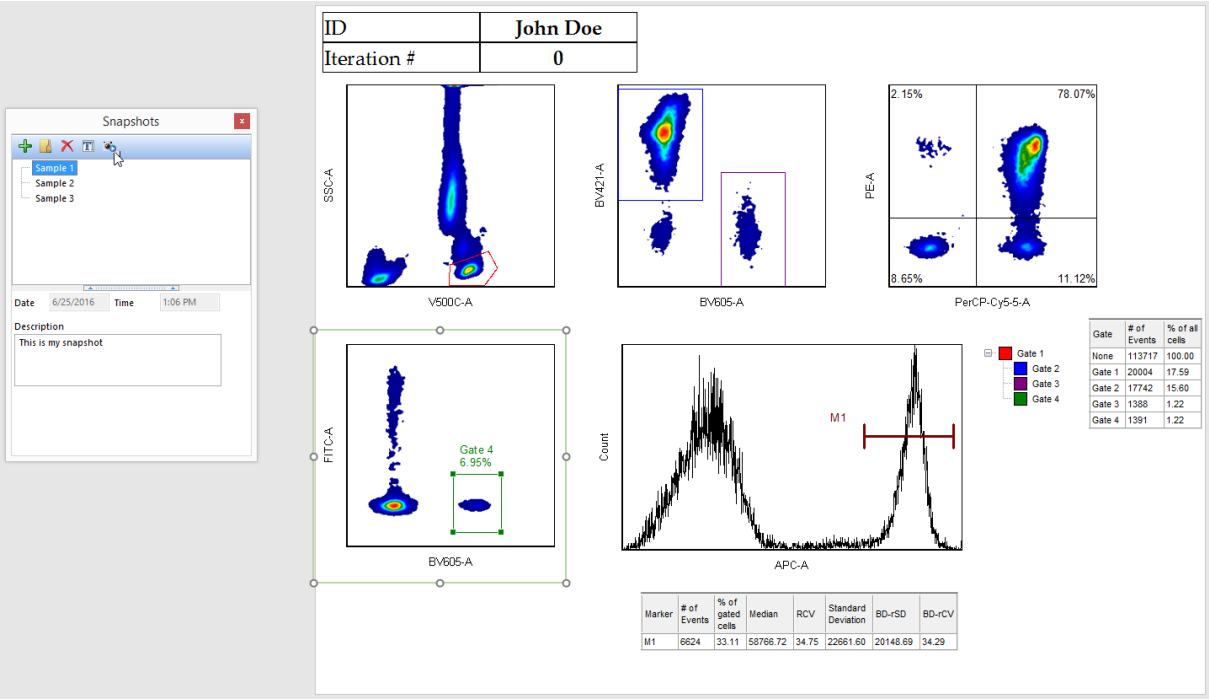
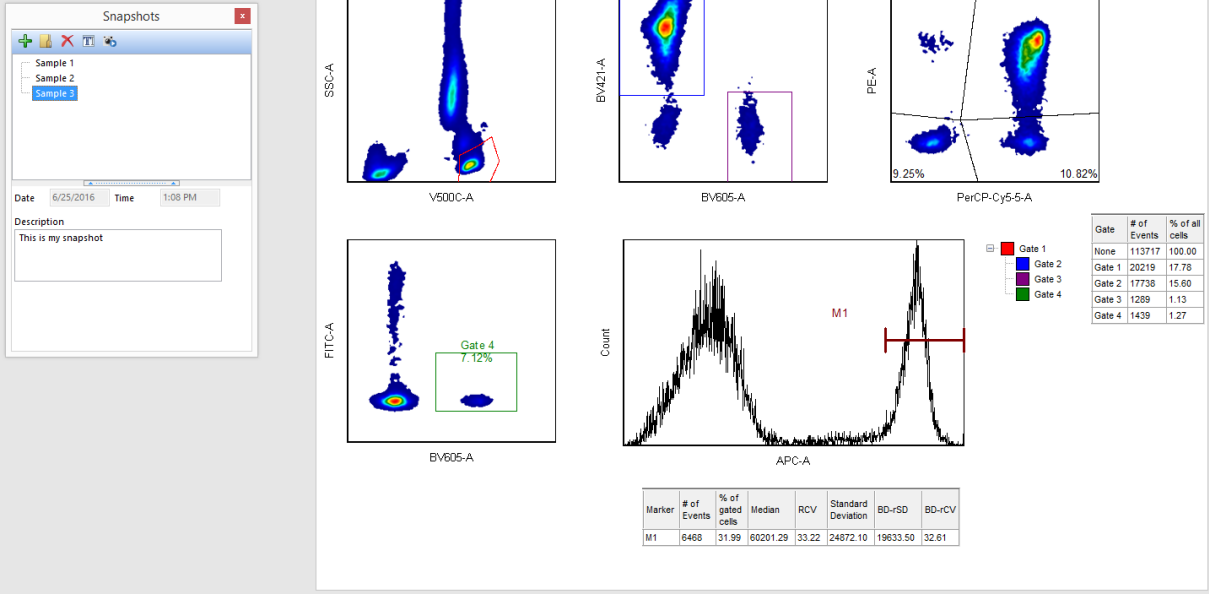


9.47 Snapshot 1 from the Snapshot navigator is applied to the layout. Note the position of Gate 4, the position of M1, and the position and style of the Quadrants.



9.48 Snapshot 2 from the Snapshot navigator has now been applied to the layout. Note the changes from Snapshot 1 to the position of Gate 4, the position of M1, and the position and style of the Quadrants.

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9.4.2 Iteration Snapshots

While analyzing your data, FCS Express automatically takes Snapshots[1498] of your gate, marker, and quadrant positions. Snapshots are recorded automatically when changing between iterations. For instance, by using the Next/Previous buttons[79] to change data on all plots, or by clicking on an Iteration number in the Data List[785] to change between iterations, or automatically while using the Batch Processing[784] functionality. These automatic snapshots are referred to as **Iteration Snapshots**, and do not appear in the Snapshots window[372].

Iteration Snapshots are always recorded but they are only applied in the specific conditions below:

- Never applied: when Batch Processing using the Run[846] command.
- Always applied: when Batch Processing using the Rerun or Review mode[848].
- Applied, or not: depending on whether the **Apply Iteration Snapshot** button is depressed or not, when loading iterations by using the Next/Previous buttons[79] to change data on all plots, or by clicking on an Iteration number in the Data List[785].

Note: Turning off the **Apply Iteration Snapshot** button will continue to record new **Iteration Snapshots** but will stop applying them when changing between iterations by using the Next/Previous buttons[79] to change data on all plots, or by clicking on an Iteration number in the Data List[785]. Importantly, this means that, when changing between iterations, the previously recorded Iteration Snapshots will be overwritten, and thus lost, with the current one (i.e with the current gate, marker, and quadrant positions).

The **Apply Iteration Snapshot** button (Figure 9.49) is found under the **Tools** tab→**Snapshots** category→**Apply Iteration Snapshot** button.

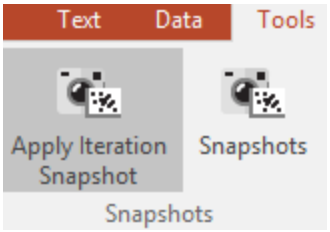

Figure 9.49 - Apply Iteration Snapshot button.

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If the Iteration Snapshot is enabled, FCS Express will take a Snapshot of each iteration when the **Previous/Next** tools are used to manipulate the data in **All** elements simultaneously. The Snapshot will record all the properties of the gating strategy.

The Figures below show how the **Previous/Next** buttons will advance to the next iteration in All objects and record a Snapshot for each iteration. Notice how the Iteration Snapshot button is depressed in figure 9.50 before clicking the Next command. When data is changed to the next iteration in figure Figure 9.51, new gate, quadrant, and marker positions may be created and their positions will be stored within that iteration. Clicking the previous button will revert the layout to the previous file/iteration in the data list along with all of the gate, marker, and quadrant positions remembered for that iteration.

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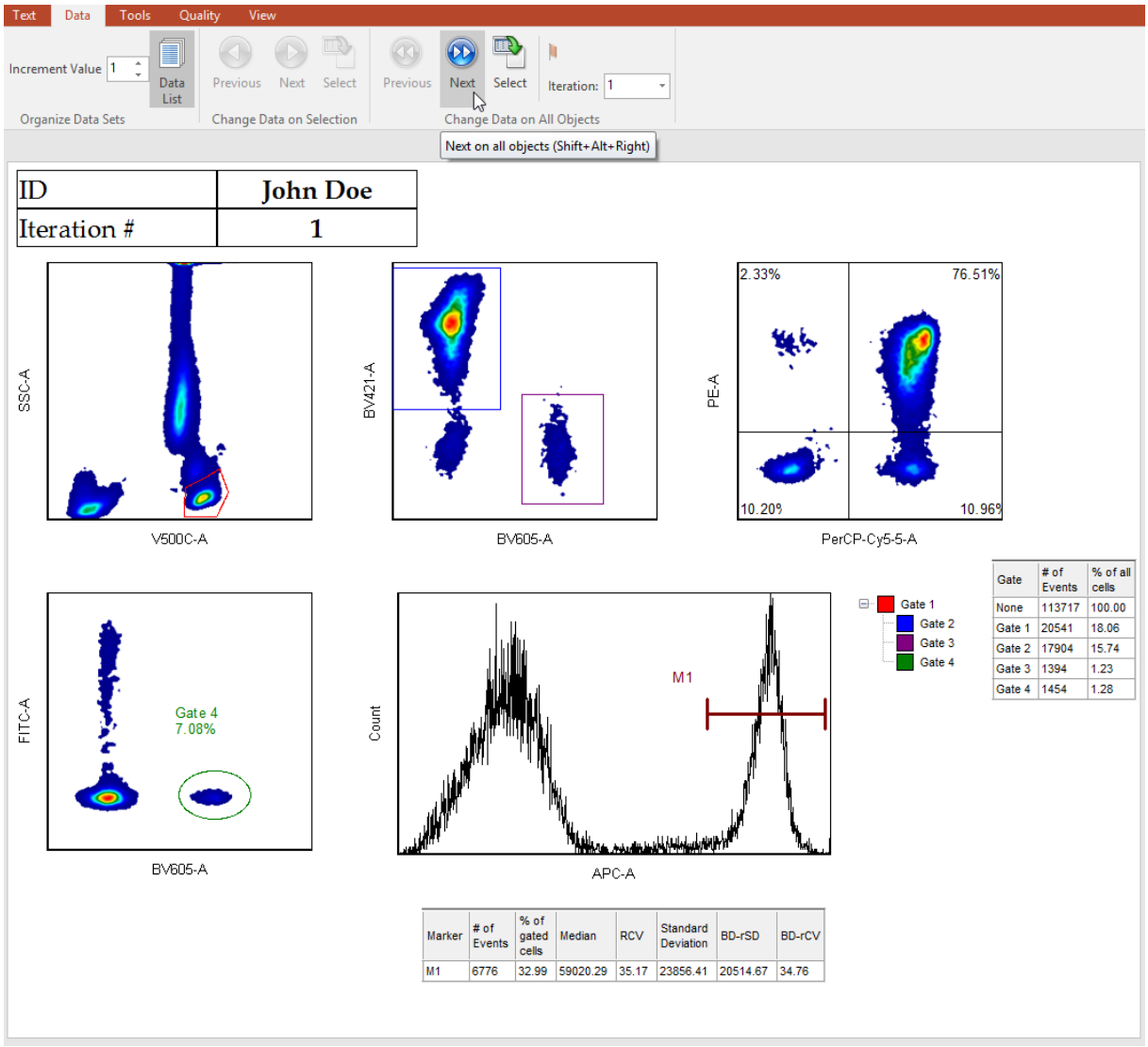

Figure 9.50 Depressing the Snapshot iteration button before clicking the Next button will cause FCS Express to remember the position of any gates, quadrants, or markers before moving to the next file/iteration in the data list.

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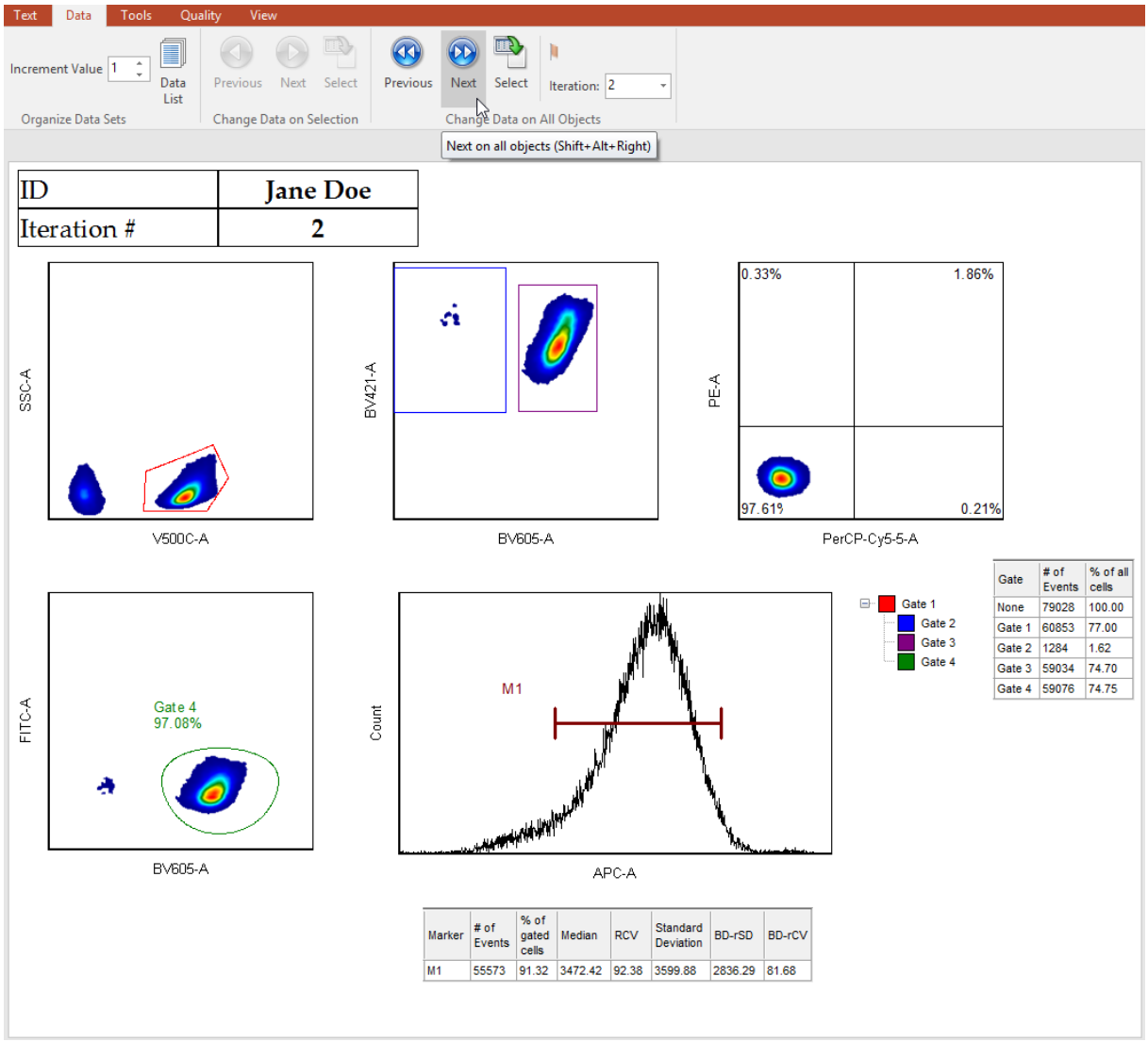

Figure 9.51 Taking Iteration Snapshots Using the Previous/Next Tools

In addition to using the **Previous/Next** button to simultaneously advance through your data and take Snapshots, it is also possible to navigate through your iterations by selecting the iteration number from the dropdown menu that is located in **Data** tab→**Change Data on All** (Figure 9.52).

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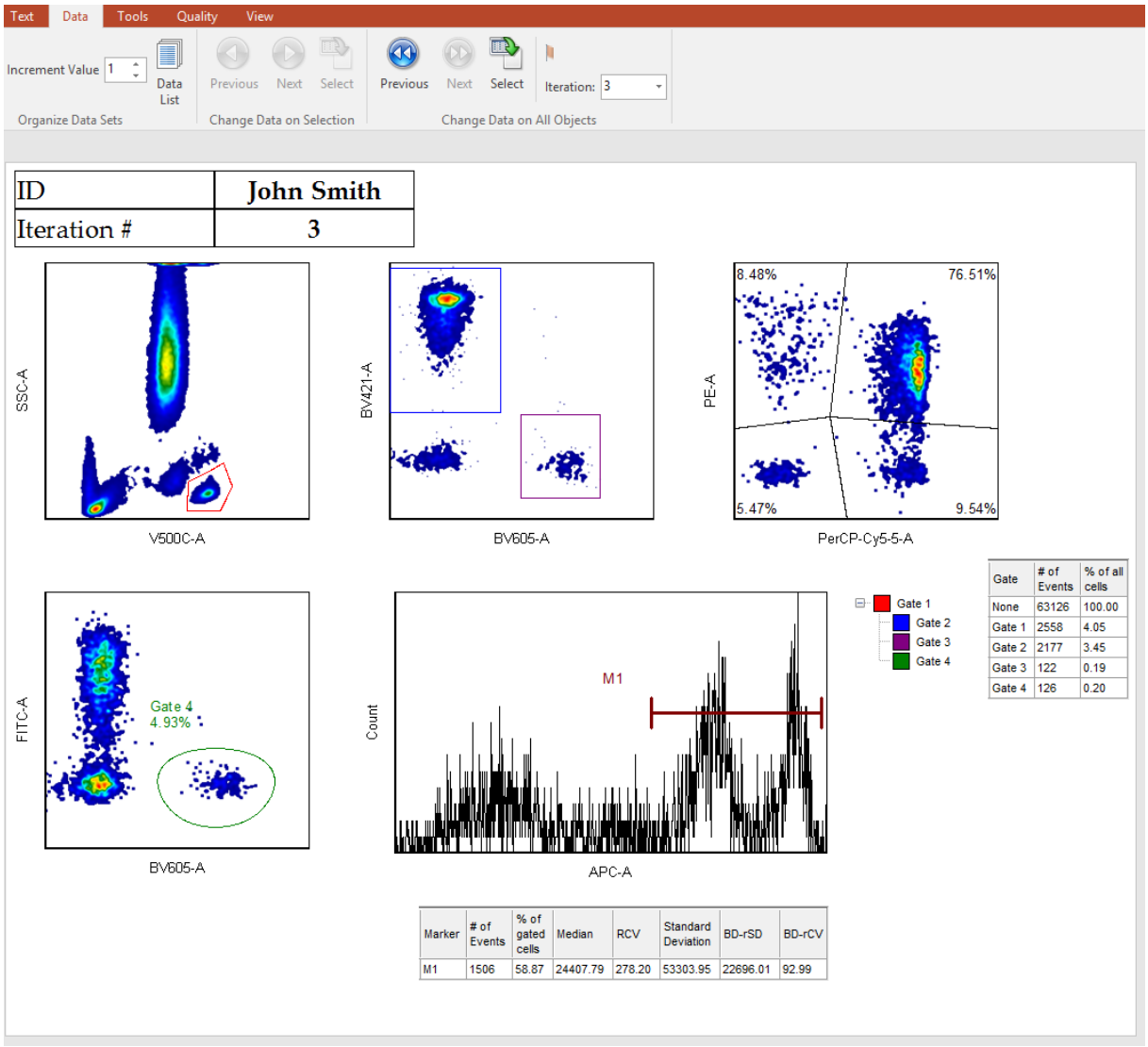

Figure 9.52 Navigating through Data Using the Iteration Number Dropdown Menu

Flagging Iterations

As you analyze your data using the **Previous/Next** tools, it's possible that you might see something about your data that you would like to earmark for further review.

In FCS Express, you can Flag an iteration by selecting the **Flag this iteration** tool. This tool can be found under **Data** tab→**Change Data on All** (Figure 9.53).

You can also flag iterations during Batch Processing 849.

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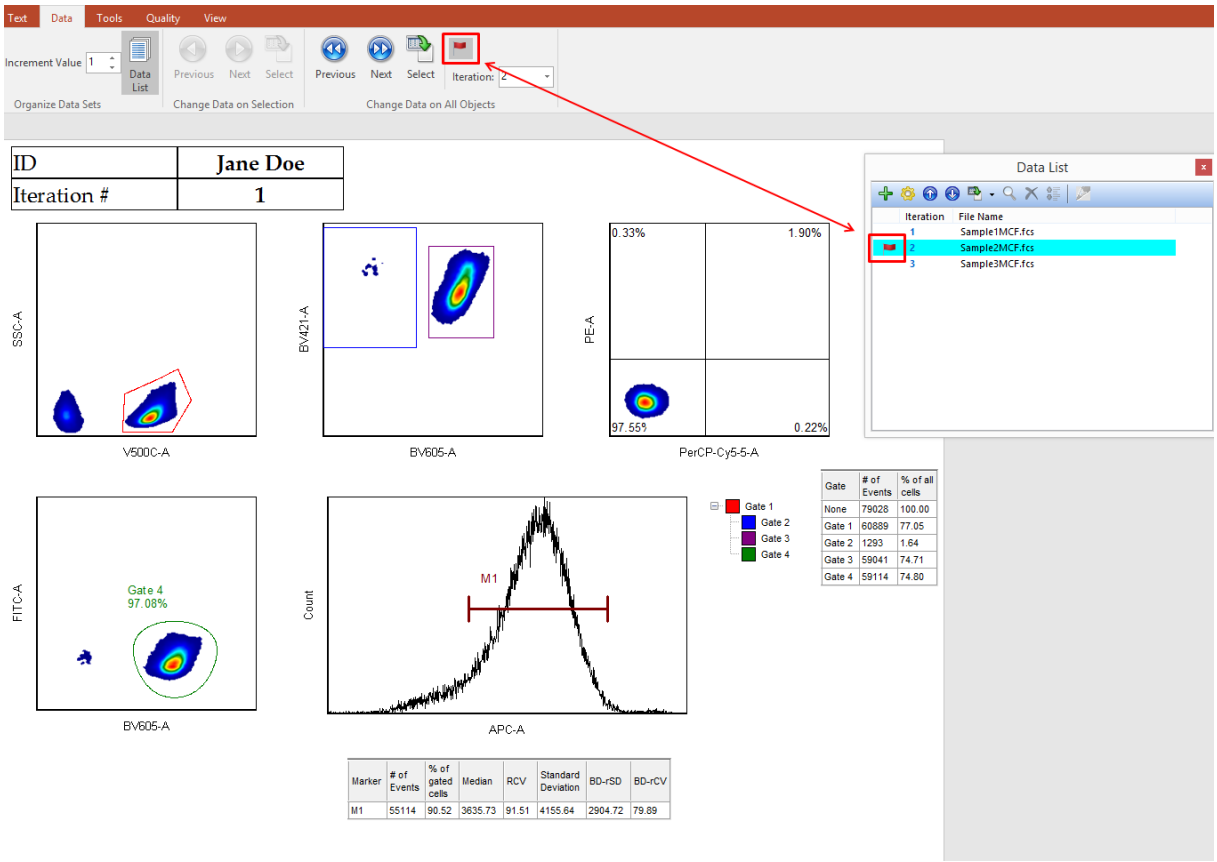

Figure 9.53 Flag This Iteration Tool

Once an iteration is flagged, you can see which iterations are flagged in the Data List 785. Also, you will be able to review this data during Batch Processing by adjusting the Batch Processing Options to 843 **Pause on Flagged Items** 843.

9.5 Statistics

FCS Express can generate any statistic that one would need for analysis of flow cytometry data. Statistics can be calculated either from a 1D or 2D plot. Statistics calculated base on a plot are only calculated for cells currently showing on the plot according to the current gate 1291.

Statistics may be accessed in default statistics windows or tables or represented anywhere within FCS Express as tokens 692. Custom statistics may also be derived from any plot or user based input by accessing custom tokens 696.

FCS Express calculates statistics to be accurate to the IEEE single precision floating point standard, as described in http://ieeexplore.ieee.org/xpl/articleDetails.jsp?arnumber=4610935&contentType=Standards and http://en.wikipedia.org/wiki/Single-precision_floating-point_format. Intermediate calculations may be performed with more precision, if available.

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More information on the default statistics available for 1D- and 2D-plots, as well as using and formatting statistics are in the sections below:

- Histogram statistics 382
- 2D plot statistics 385
- Custom tokens 727 (user defined statistics and more)
- Using statistics and the clipboard 387
- Formatting statistics 389

FCS Express 5 and above includes the following new statistics which are described further in subsequent pages:

- Default Statistics introduced in FCS Express 5 and available in later versions:
  - Percent of Gated - Parent 391
  - Percent of Gated - Grandparent 391
  - BD Robust Standard Deviation (rSD in BD FACSDiva)
  - BD Robust coefficient of variation (rCV in BD FACSDiva)

9.5.1 Histogram Statistics

Statistics will be calculated for every overlay on the histogram. If there are markers, statistics will be calculated for every overlay that the marker applies to (see Creating Markers 353 and Histogram Markers 272).

To view the histogram statistics, right-click on the plot and select **Histogram Statistics** from the pop-up menu (Figure 9.54 below, ❶). Individual statistics may be toggled on and off by right-clicking on the default statistics table (Figure below, ❷), or from the Formatting Statistics 389 menu.

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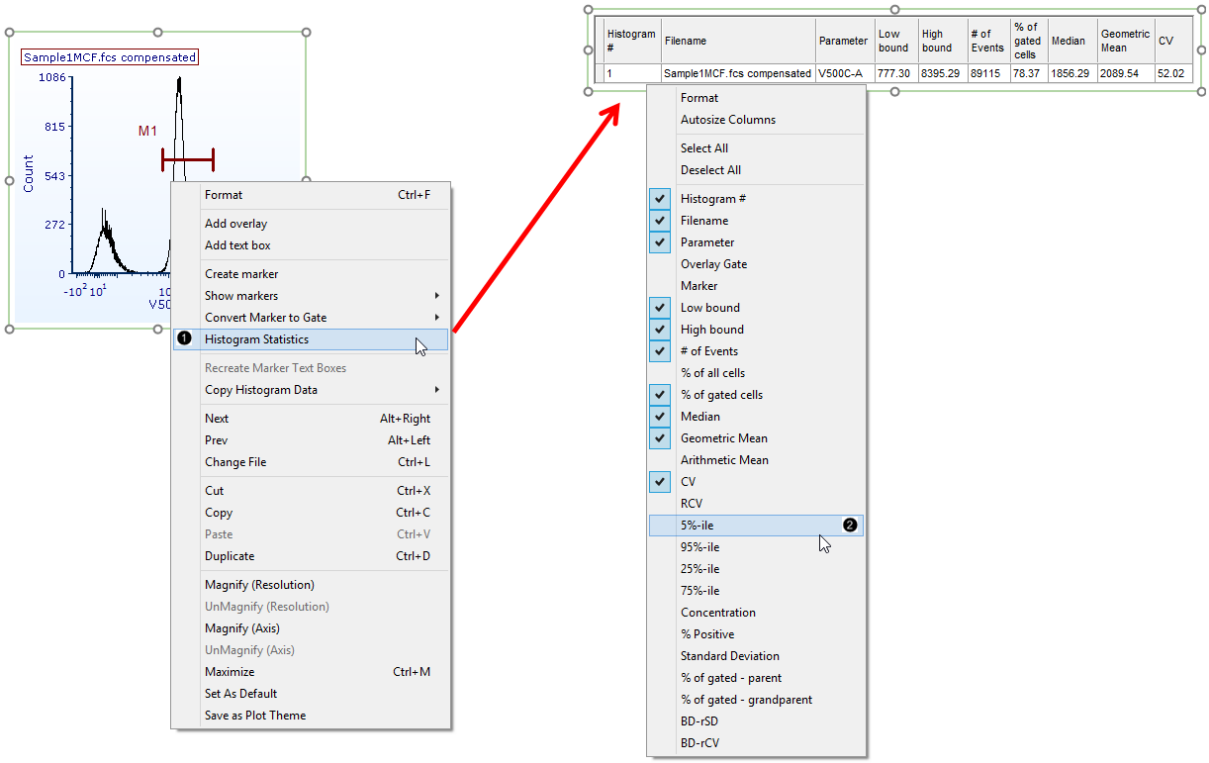

Figure 9.54 Adding a default statistics table from a histogram

The available statistics are shown in the table below.

Statistic	Explanation
Histogram #	If there are multiple overlays visible on a plot, then the statistics will be calculated for every overlay. **Histogram #** displays the overlay number for that row of statistics. The overlay number corresponds to the position in the overlay list on the Overlays 268 page of the Format window.
Filename	The file name of the overlay.
Parameter	The parameter name for the parameter that is being displayed on the overlay. The field that the parameter name is taken from can be set in the **FCS Express User Options** Dialog under Data Loading - FCS File Options 229.
Overlay Gate	The name of the gate that is currently being applied to the overlay.
Marker	If there are several markers visible on a plot, then the statistics will be calculated for each overlay that the marker applies to. **Marker** displays the marker number for that row of statistics. The marker number corresponds to the position in the marker list on the **Markers** page of the **Format** window (see Histogram Markers 272).
Low bound	The channel number of the low boundary of the marker.
High bound	The channel number of the high boundary of the marker.
# of Events	The number of events that are accepted by the gating formula.
% of all cells	The number of events that are accepted by the gating formula and are located within the specified marker divided by the total number of events in the FCS file.

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% of gated cells	The number of events that are accepted by the gating formula and are inside the specified marker divided by the total number of events displayed on the plot.
Median	The median channel value of the events that are accepted by the gating formula that fall within the specified marker.
Geometric Mean	The geometric mean channel number of the events that are accepted by the gating formula that fall within the specified marker. Similar to arithmetic mean except that the numbers are multiplied and the Nth root of the resulting product is taken.
Arithmetic Mean	The arithmetic mean channel number of the events that are accepted by the gating formula that fall within the specified marker.
CV (Coefficient of Variation)	The coefficient of variation of events that are accepted by the gating formula that fall within the specified marker. The CV is a measure of the distribution of your data and is equal to the standard deviation divided by the mean.
RCV (Robust CV)	The Robust Coefficient of Variation of events that are accepted by the gating formula. The RCV may be more applicable than the CV for populations that do not follow a normal Gaussian distribution. The RCV is equal to 0.75 multiplied by the interquartile range divided by the median. The interquartile range is the 75th percentile channel minus the 25th percentile channel.
5%-ile(5th percentile)	The channel corresponding to the 5th percentile of the cells that have been accepted by the gating formula.
95%-ile (95th percentile)	The channel corresponding to the 95th percentile of the cells that have been accepted by the gating formula.
25%-ile (25th percentile)	The channel corresponding to the 25th percentile of the cells that have been accepted by the gating formula.
75%-ile (75th percentile)	The channel corresponding to the 75th percentile of the cells that have been accepted by the gating formula.
Concentration	The concentration of the cells that are accepted by the gating formula. The parameters for the concentration calculation can be set up in the Concentration Calculator 592.
Percent Positive	The percentage of the cells accepted by the gating formula that are positive with respect to the control overlay. The control overlay is set in Histogram Specific Options 274. The Overton Subtraction method statistic will be displayed. 598
Standard Deviation	A measure of the variation from the average for the events. The square root of the variance.
% of gated - parent	The number of events that are accepted by the gating formula and are inside the specified marker divided by the total number of events in the parent gate.
% of gated - grandparent	The number of events that are accepted by the gating formula and are inside the specified marker divided by the total number of events in the grandparent gate.
BD-rSD	The BD Biosciences robust standard deviation. See the full description by BD Biosciences.
BD-rCV	The BD Biosciences robust coefficient of variation of events that are accepted by the gating formula that fall within the specified marker. See the full description by BD Biosciences.

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The right-click→ **Format** pop-up menu→ **Markers→ If there are markers on plot, also show statistics for the base overlay without the marker** checkbox is for displaying statistics for the entire overlay with no marker applied.

9.5.2 2D Plot Statistics

There are two kinds of statistics that can be displayed for a 2D plot (Gate Statistics and Quadrant Statistics). The same statistics are available in either case.

- **Gate Statistics**

  The statistics are calculated for every gate designated in the **Gates to Display** data option for the statistics window, *even if the gate is not being displayed on the plot*. The Gates to Display can be chosen from a dialog accessible from the **Format→Data Options→Gates To Display** command, or in the Formatting Statistics 389 dialog shown by right-clicking on the statistics window and choosing **Format** from the pop-up menu.

- **Quadrant Statistics**

  The statistics are calculated for every quadrant. There will be four rows of statistics for each overlay with each row corresponding to a different quadrant. Quadrant statistics displayed on the plot may be changed by formatting the Label Text Box containing the statistic 152.

In order to open a statistics window, right-click on a 2D Plot and choose **Gate Statistics** (Figure 9.55 below) or **Quadrant Statistics** (if quadrants are not currently shown on the plot, this option is not enabled) from the pop-up menu. Individual statistics may be toggled on and off by right-clicking on the default statistics table (Figure below), or from the Formatting Statistics 389 menu.

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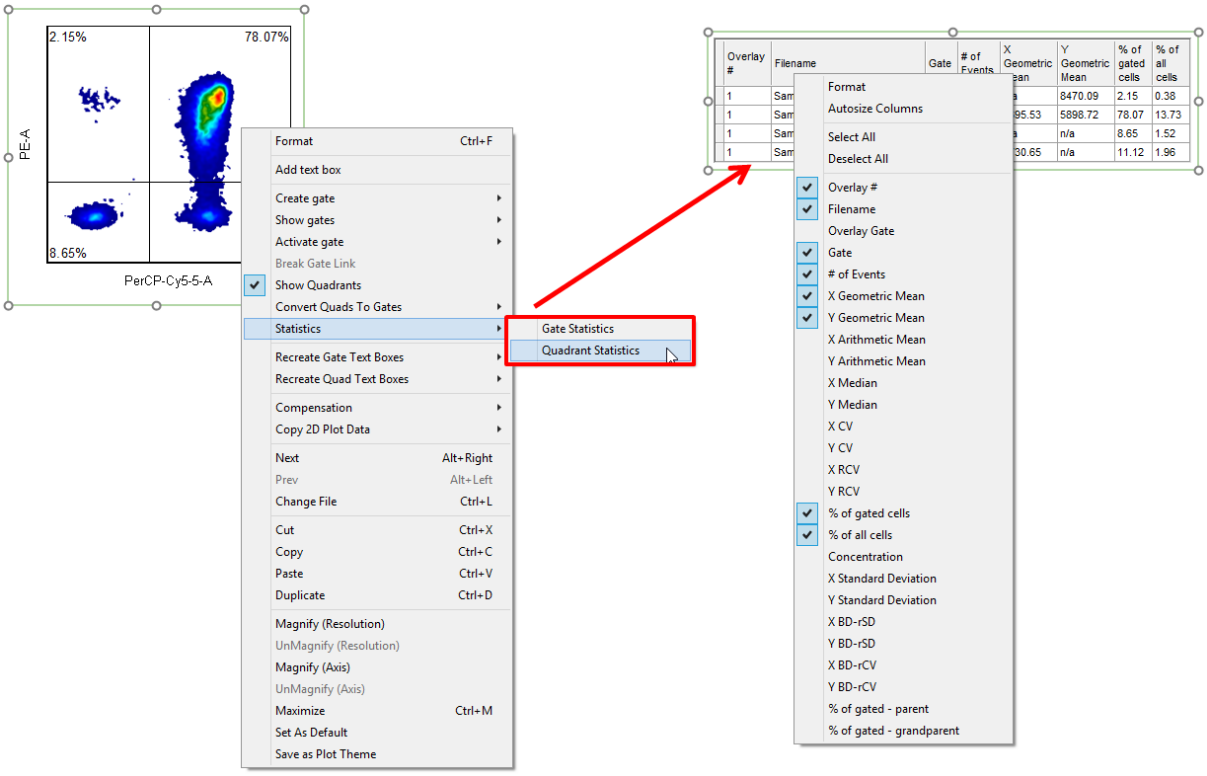

Figure 9.55 Adding a default statistics table from a 2D plot

The available statistics are shown in the table below. A region can refer to a gate or quadrant.

Statistic	Explanation
Overlay #	If there are multiple overlays visible on a plot, the statistics will be calculated for every overlay on the plot. **Overlay #** displays the overlay number for that row of statistics. The overlay number corresponds to the position in the overlay list on the Overlays 284 page of the Format window.
FCS Filename	The file name corresponding to the overlay number.
Overlay Gate	The name of the gate that is currently being applied to the overlay.
Gate	The region for which the statistics apply. This region can be either a gate or quadrant designation.
# of Events	The total number of events in this region accepted by the gating formula.
X Geometric Mean	The geometric mean channel number of the events on the X-axis that are accepted by the gating formula. Similar to arithmetic mean except that the numbers are multiplied and the Nth root of the resulting product is taken.
Y Geometric Mean	The geometric mean channel number of the events on the Y-axis that are accepted by the gating formula. Similar to arithmetic mean except that the numbers are multiplied and the Nth root of the resulting product is taken.
X Arithmetic Mean	The mean X-axis channel value of cells in this region that are accepted by the gating formula.
Y Arithmetic Mean	The mean Y-axis channel value of cells in this region that are accepted by the gating formula.

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Statistic	Explanation
X Median	The median X-axis channel value of cells in this region that are accepted by the gating formula.
Y Median	The median Y-axis channel value of cells in this region that are accepted by the gating formula.
X CV (Coefficient of Variation)	The coefficient of variation of X-axis events that are accepted by the gating formula that fall within the specified marker. The CV is a measure of the distribution of your data and is equal to the standard deviation divided by the mean.
Y CV	The coefficient of variation of Y-axis events that are accepted by the gating formula that fall within the specified marker.
X RCV (Robust CV)	The Robust Coefficient of Variation of X-axis events that are accepted by the gating formula. The RCV may be more applicable than the CV for populations that do not follow a normal Gaussian distribution. The RCV is equal to 0.75 multiplied by the interquartile range divided by the median. The interquartile range is the 75th percentile channel minus the 25th percentile channel.
Y RCV	The Robust Coefficient of Variation of Y-axis events that are accepted by the gating formula.
% of Gated Cells	The total events in the specified region divided by the total number of events on the whole plot accepted by the gating formula.
% of All Cells	The total events in the specified region divided by the total number of events in the FCS file.
Concentration	The concentration of the cells that are accepted by the gating formula. The parameters for the concentration calculation can be set up in the Concentration Calculator 592.
X Standard Deviation	A measure of the variation from the average for the events on the X-axis. The square root of the variance.
Y Standard Deviation	A measure of the variation from the average for the events on the Y-axis. The square root of the variance.
X BD-rSD	The BD Biosciences robust standard deviation for X axis events. See the full description by BD Biosciences.
Y BD-rSD	The BD Biosciences robust standard deviation for Y axis events. See the full description by BD Biosciences.
X BD-rCV	The BD Biosciences robust coefficient of variation of X-axis events that are accepted by the gating formula that fall within the specified marker. See the full description by BD Biosciences.
Y BD-rCV	The BD Biosciences robust coefficient of variation of Y-axis events that are accepted by the gating formula that fall within the specified marker. See the full description by BD Biosciences.
% of gated - Parent	The number of events that are accepted by the gating formula and are inside the specified gate divided by the total number of events of the parent of the gate applied to a plot.
% of gated - Grandparent	The number of events that are accepted by the gating formula and are inside the specified gate divided by the total number of events of the grandparent of the gate applied to a plot.

9.5.3 Using Statistics and the Clipboard

It is possible to copy any single statistic, or a group of contiguous statistics, from the statistics window to the clipboard. First, you must select the cells you wish to copy by clicking. You can then paste the statistics into a text box 1298 that will be

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automatically inserted on your layout, or outside of FCS Express* (e.g, to a text file, or a spreadsheet). Cutting cells from the statistics window is not allowed; instead, toggle 389 the desired statistic off.

To select statistics from a default statistics table:

- Click in a cell to select it. The background of the cell will be highlighted in blue.
- To select a group of contiguous cells, select the first cell of the group to highlight it. Then, while holding the **Shift** key, select the last cell of the group. All of the selected cells will have a blue background.

After you have selected the cells you wish to copy, either:

- use the **Home→Clipboard→Copy Text** command (or **Ctrl+C**) to copy the statistics [i.e., statistic name(s) and selected cell(s)] to the clipboard (Figure 9.56).
- drag the statistic(s) out to an empty spot on your layout to insert them into their own text box.

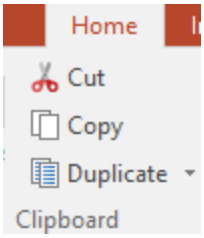

Figure 9.56 - Clipboard from Home tab

* Note: If a large number of statistics from various plots, tables, or from multiple .fcs data files are to be exported, Batch Processing 1455 is a more efficient method.

Copying Histogram Data:

The Bin and Count numbers of histogram data may be copied from any histogram and pasted within FCS Express or any software, such as Excel, for further review. To **Copy Histogram Data (**Figure. 9.57):

1. Right click on a histogram.
2. Choose **Copy Histogram Data**.
3. Choose the **Overlay** to copy data from.
4. Paste the data in FCS Express or any other software with control+V or any other paste command.

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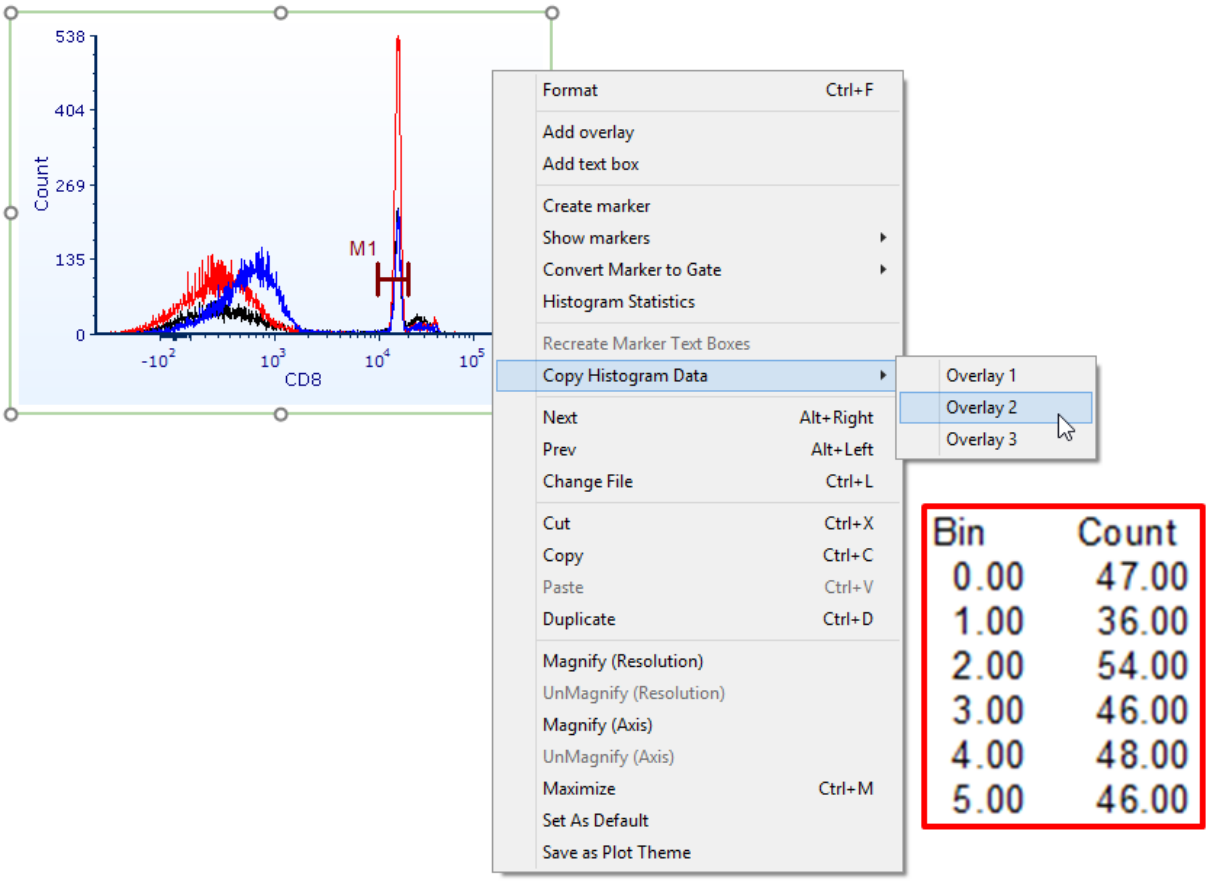

Figure. 9.57 Using the copy histogram data feature. Notice the red highlighted table in which the first 5 bins of data from the histogram are displayed upon a paste command.

9.5.4 Formatting Statistics

There are several options to control which statistics get displayed in any individual statistics window.

You can change the displayed statistics in either of the following ways:

- Right-click on the statistics window. In the pop-up menu, there will be a list of available statistics. You can individually toggle any statistic from this menu. If the statistic is selected, a check mark will appear next to the name (Figure 9.58).

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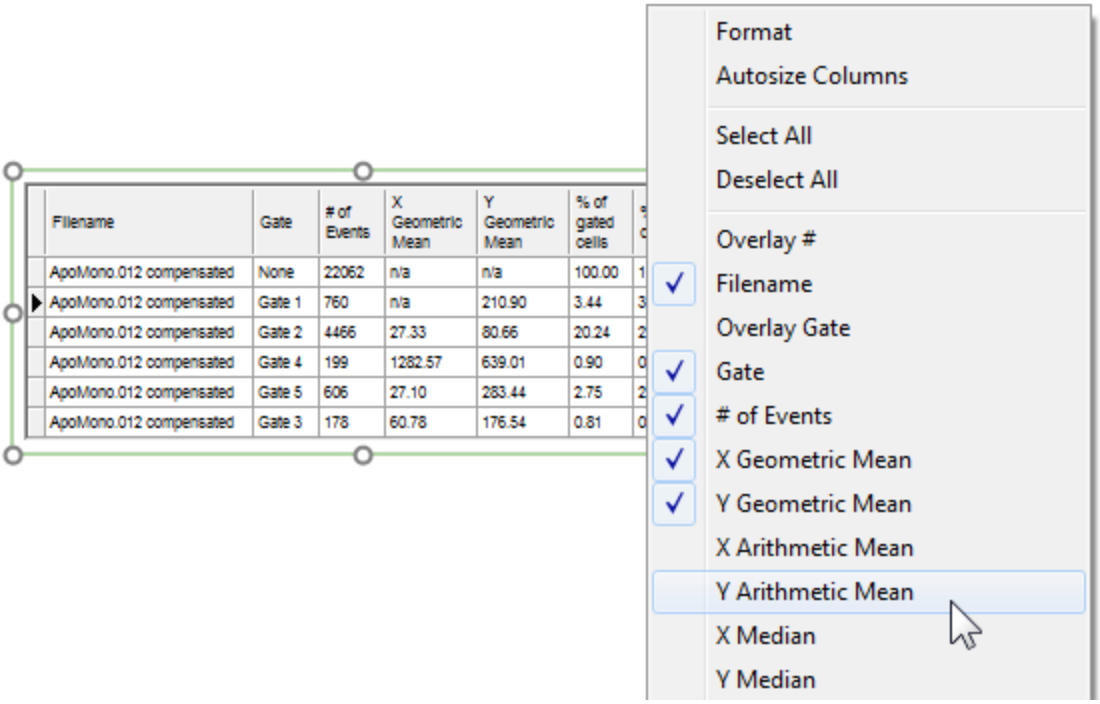

Figure 9.58 Toggling individual statistics on and off via the right-click menu

- Access the Formatting options for the Statistics window by either of these two methods:
    - right-click on the Statistics window and select **Format** from the pop-up menu and the **Stats** category at left, or
    - select the Statistics window and use the **Format** tab→**Statistics Options**→**Statistics** command.

  Then, individually select/deselect each statistic you want to display by checking/unchecking the appropriate checkbox in the **Select Statistics** list box on the **Stats** page. You may also **Select** or **Deselect All** by right clicking in the **Select Statistics** list (Figure 9.59).

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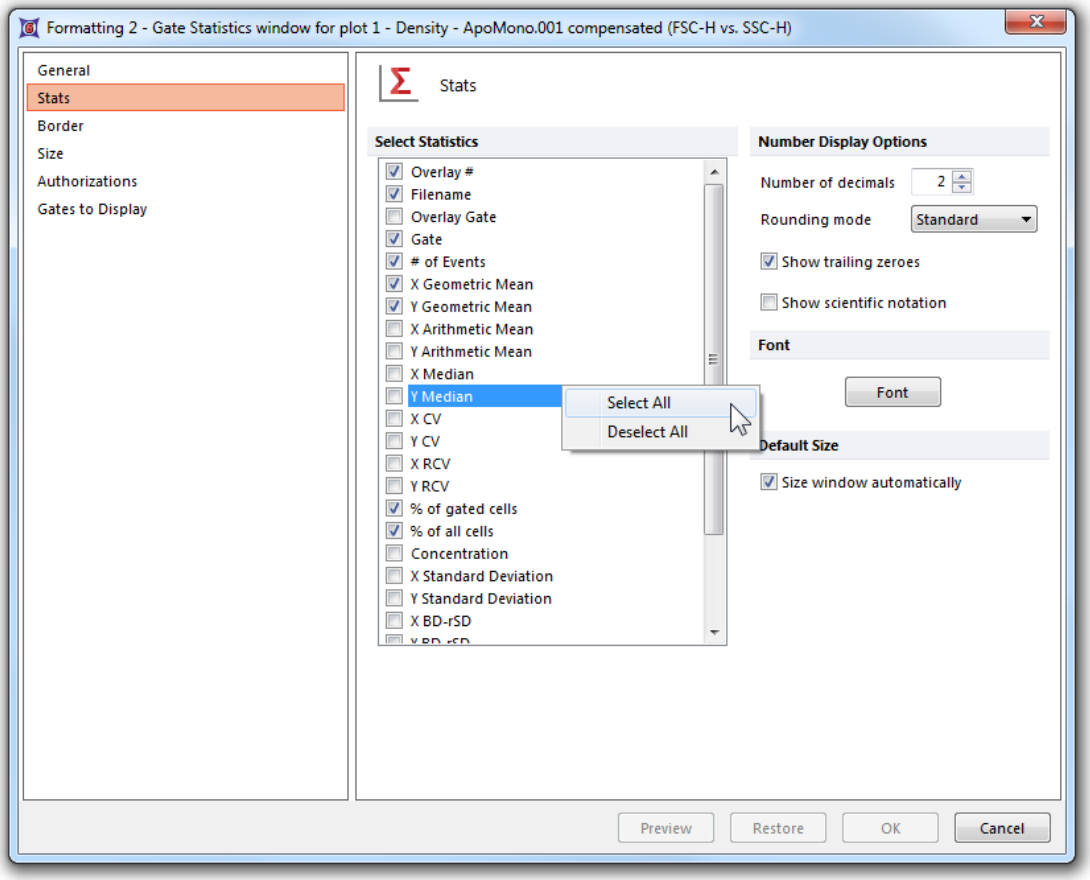

Figure 9.59 Select/Deselect All statistics

To format the statistics window, right-click on it and select **Format** from the pop-up menu. Several categories will be available to set various options, such as the size of the statistics window, border, which statistics are available, and which gate(s) you would like to see statistics for 303.

To set the default statistics that appear when you first open a statistics window, use the 1D Plot Statistics 197 or the 2D Plot Statistics 198 category of the **Options** dialog. This feature is convenient if you routinely prefer to see the same set of statistics during data analysis.

Details on statistic options and number display options may be found in the description for 1D Plot Statistics 197 or the 2D Plot Statistics 198 as they related to FCS Express user options.

9.5.5 Percent Gated - Parent and Percent Gated - Grandparent

The statistics **% Gated - Parent** and **% Gated - Grandparent** are available in FCS Express.

These are defined as follows:

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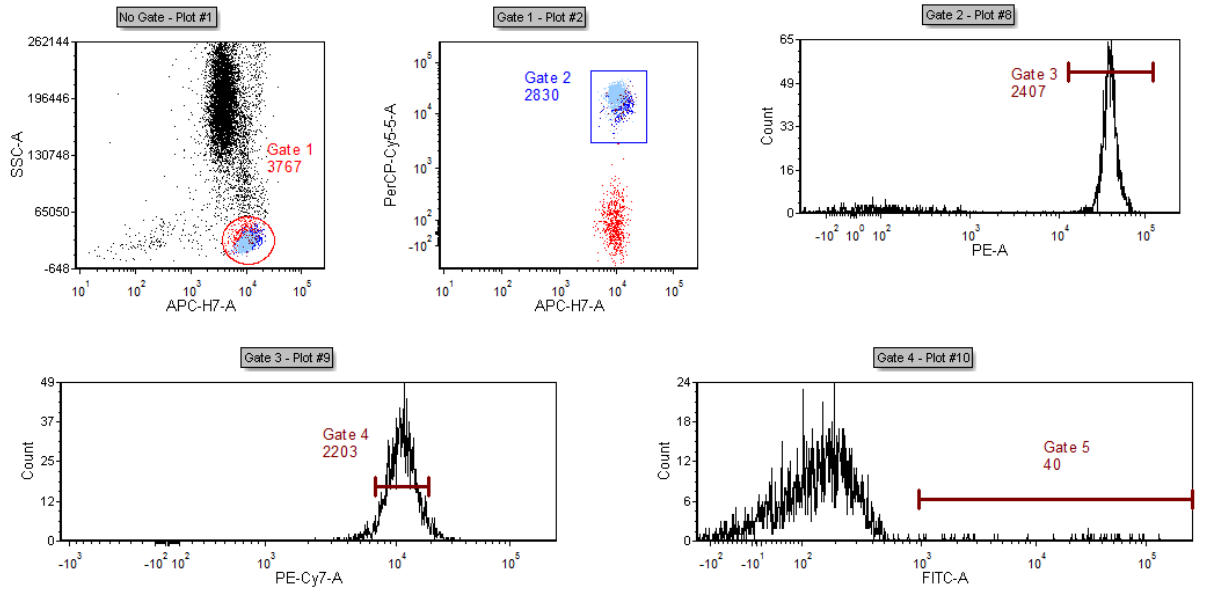
% Gated - Parent	The number of gated events divided by the number of events of the ***parent of the gate that is applied to a plot***, multiplied by 100.
% Gated - Grandparent	The number of gated events divided by the number of events of the ***grandparent of the gate that is applied to a plot***, multiplied by 100.

We will examine these statistics in terms of real data. Figure 9.60 represents an analysis with sequential gating.



Gate	# of Events
None	20000
Gate 1	3767
Gate 2	2830
Gate 3	2407
Gate 4	2203
Gate 5	40

Stats for Gate 5		Written Formula	Numeric Formula
% Gated	1.8157	(# Gate 5 Events/# Gate 4 Events)*100	(40/2203)*100=1.8157
% Total	0.2000	# Gate 5 Events/# of Total Events)*100	(40/20000)*100=0.2000
% Gated - Parent	1.6618	(# Gate 5 Events/# of Gate 3 Events)*100	(40/2407)*100=1.6618
% Gated - Grandparent	1.4134	(# Gate 5 Events/# of Gate 2 Events)*100	(40/2830)*100=1.4134

Figure 9.60 - Analysis with Sequential Gating

When observing Gate 5... In Plot #10 (the lower right 1D histogram), there is a Marker that was converted to Gate 5. Gate 5 contains 40 events.

The gate that is applied to Plot #10 is Gate 4. Therefore, the statistic for Gate 5 **% Gated** is defined as the number of Gate 5 events (40) divided by the number of Gate 4 events (2203) multiplied by 100. In this case, the numerical formula is (40/2203)x100, which equals 1.8157.

The statistic for Gate 5 **% Total** is defined as the number of Gate 5 events (40) divided by the Total Number of events collected (20,000). The numerical formula is (40/20000)x100, which equals 0.2.

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The statistic for Gate 5 **% of Gated - Parent** is defined as the number of gated events (40) divided by the number of events for the parent of the gate applied to Plot #10. As can be seen in Figure 9.61, Gate 4 has been applied to Plot #10. The parent of Gate 4 is Gate 3. So, the statistic for Gate 5 **% of Gated - Parent** is defined as the number of Gate 5 events divided by the number of Gate 3 events multiplied by 100. The numerical formula is (40/2407)x100, which equals 1.6618.

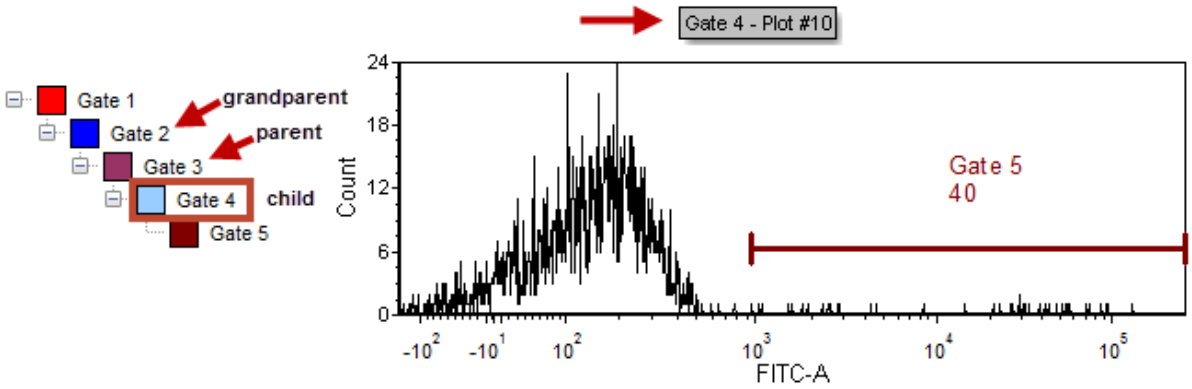

Figure 9.61 - Showing the Parent and Grandparent of Gate 4

The statistic for Gate 5 **% of Gated - Grandparent** is defined as the number of gated events (40) divided by the number of events for the grandparent of the gate applied to Plot #10. As can be seen in Figure 2, Gate 4 has been applied to Plot #10. The parent of Gate 4 is Gate 3. The parent of Gate 3 is Gate 2. So, the statistic for Gate 5 **% of Gated - Grandparent** is defined as the number of Gate 5 events divided by the number of Gate 2 events multiplied by 100. The numerical formula is (40/2830)x100, which equals 1.4134.

9.5.6 Spreadsheet Statistics from Data Files

Spreadsheets may be populated by statistics from the data files available in the Data List 433 using the drag-and-drop feature. The available statistics are shown in the table below.

Statistic	Explanation
Filename	The file name of the file.
Parameter	The name of the parameter selected from the **Parameter** dropdown menu under **Data File Information** within the **Edit Statistic** window.
Gate name	The name of the gate that is currently being applied to the file.
Min value	The number of the minimum value for the selected parameter from the **Parameter** dropdown menu under **Data File Information** within the **Edit Statistic** window for the events in the data file that are accepted by the gating formula.
Max value	The number of the maximum value for the selected parameter from the **Parameter** dropdown menu under **Data File Information** within the **Edit Statistic** window for the events in the data file that are accepted by the gating formula.
# of Events	The number of events that are accepted by the gating formula.

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% of parent	The number of gated events that are accepted by the gating formula and in the specified gate divided by the total number of events in the parent gate of the specified gate.
% of grandparent	The number of gated events that are accepted by the gating formula and are in the specified child gate divided by the total number of events in the grandparent gate of the specified gate.
% of great grandparent	The number of gated events that are accepted by the gating formula and are in the specified gate divided by the total number of events in the great grandparent gate of the specified gate.
% of all cells	The number of gated events that are accepted by the gating formula and are located within the specified gate divided by the total number of events in the FCS file.
Median	The median channel value of the events that are accepted by the gating formula.
Geometric Mean	The geometric mean channel number of the events that are accepted by the gating formula that fall within the specified gate. Similar to arithmetic mean except that the numbers are multiplied and the Nth root of the resulting product is taken.
Arithmetic Mean	The arithmetic mean channel number of the events that are accepted by the gating formula that fall within the specified gate.
CV (Coefficient of Variation)	The coefficient of variation of events that are accepted by the gating formula that fall within the specified gate. The CV is a measure of the distribution of your data and is equal to the standard deviation divided by the mean.
RCV (Robust CV)	The Robust Coefficient of Variation of events that are accepted by the gating formula. The RCV may be more applicable than the CV for populations that do not follow a normal Gaussian distribution. The RCV is equal to 0.75 multiplied by the interquartile range divided by the median. The interquartile range is the 75th percentile channel minus the 25th percentile channel.
5%-ile(5th percentile)	The value corresponding to the 5th percentile of the cells that have been accepted by the gating formula.
95%-ile (95th percentile)	The value corresponding to the 95th percentile of the cells that have been accepted by the gating formula.
25%-ile (25th percentile)	The value corresponding to the 25th percentile of the cells that have been accepted by the gating formula.
75%-ile (75th percentile)	The value corresponding to the 75th percentile of the cells that have been accepted by the gating formula.
Concentration	The concentration of the cells that are accepted by the gating formula. The parameters for the concentration calculation can be set up in the Concentration Calculator 592.
Standard Deviation	A measure of the variation from the average for the events. The square root of the variance.
BD-rSD	The BD Biosciences robust standard deviation. See the full description by BD Biosciences.
BD-rCV	The BD Biosciences robust coefficient of variation of events that are accepted by the gating formula that fall within the specified marker. See the full description by BD Biosciences.

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9.6 Axis Scalings

FCS data has traditionally been acquired on either a linear or logarithmic scale, and the appropriate scaling is stored in the header of the FCS data file. Although FCS Express has supported displaying linear data on a logarithmic scale, the data has traditionally been displayed on the same scale as it was acquired.

The logarithmic scale has the disadvantage that it cannot display values below zero. This is considered a limitation when performing software compensation where it is common for a significant proportion of compensated data to appear in a "negative" channel. When displayed on a log scale, the negative values are placed on the axis, where they can be easily missed, leading you to overcompensate your data.

To overcome this limitation, several additional axis scalings have been developed. These scalings share the general characteristic that they behave linearly close to zero, and behave logarithmically farther away from zero. These scalings also are defined to be able to graph data below zero. Scalings that are a combination of linear and log scalings are called hybrid scalings. More details can be found in the following publications:

- Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parks DR. Interpreting flow cytometry data: a guide for the perplexed. *Nat Immunol* 2006;7:681-685.

- Parks DR, Roederer M, Moore WA. A New "Logicle" Display Method Avoids Deceptive Effects of Logarithmic Scaling for Low Signals and Compensated Data. *Cytometry A* 2006*;*69A:541-545.

- Bagwell CB. Hyperlog – A Flexible Log-like Transform for Negative, Zero, and Positive Valued Data. *Cytometry A* 2005;64A:34-42.

- Battye FL. A Mathematically Simple Alternative to the Logarithmic Transform for Flow Cytometric Fluorescence Data Displays. *Cytometry A* 2006;69A:413-414.

FCS Express supports the following axis scalings:

- Linear – Standard linear scaling

- Log – Standard log scaling

- Log with Negative – This is a modified log scaling.

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$$T(x) = \begin{cases} Log(x), x > 0 \\ -Log(-x), x < 0 \\ 0, x = 0 \end{cases}$$

In the normal log function the zero and negative values are undefined, and if they are obtained during compensation they are commonly represented as events stacked on the axis, as is commonly seen in compensated data. In the log with negative function, if negative values are encountered, the log of its absolute value is calculated and displayed on the negative side of the scale, effectively creating a "negative logarithm" display which is a mirror image of its positive counterpart. Zero values are still kept on axis.

- Biexponential

  The biexponential display was popularized by the BD FACSDiva software. As a result of our close collaboration with BD, FCS Express now supports all of the options that FACSDiva does in terms of displaying biexponential data. In fact, we have implemented FACSDiva's proprietary algorithm for calculating the reflection point, so the results from FCS Express will look identical to the results from the FACSDiva software.

- Hyperlog™ 2 – Described in the Bagwell paper, above.

- Lin/Log – Described in the Battye paper, above.

$$T(x) = \begin{cases} ax, x < t \\ Log(cx), x > t \end{cases}$$

This transform is purely linear below a transition point and purely log above the transition point. The *a* and *c* coefficients are matched so that the function is continuous at the transition point.

- ArcSinh

  ArcSinh is an inverse hyperbolic function most widely used with .fcs files generated by CyTOF Mass Cytometry. ArcSinh values are calculated by applying the ArcSinh equation to the raw intensity value and dividing by a cofactor. The "cofactor" defines the size of the linear region around 0, with smaller cofactors corresponding to a longer linear region.

$$\sinh^{-1} z = \ln\left(z + \sqrt{z^2 + 1}\right)$$

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9.6.1 Data Display

The axis scale for a given plot can be adjusted using the axis formatting options. All data files, regardless of the scale that they were acquired with, can be displayed in any of the scalings supported by FCS Express. The scaling only affects the display of the data, not the statistics 256.

Lin/Log and Hyperlog scalings:
The *Lin/Log* and *Hyperlog* scalings both a have coefficient that can be adjusted. The coefficient can be changed in the **Scale** section of the **Axis** page in the formatting dialog (right-click on the plot, select **Format** from the pop-up menu). The coefficient is called the **Transition Point** because it describes the transition from the predominantly linear to predominantly log portion of the transform. The transition parameter for the *Hyperlog* scaling is identical to the *b* parameter in the Bagwell paper 395.

An example of setting the *Lin/Log* scaling with a Transition Point of 200 is shown in Figure 9.62. Transition values are entered in channel units, and do not have to be scaled as described in the Bagwell paper.

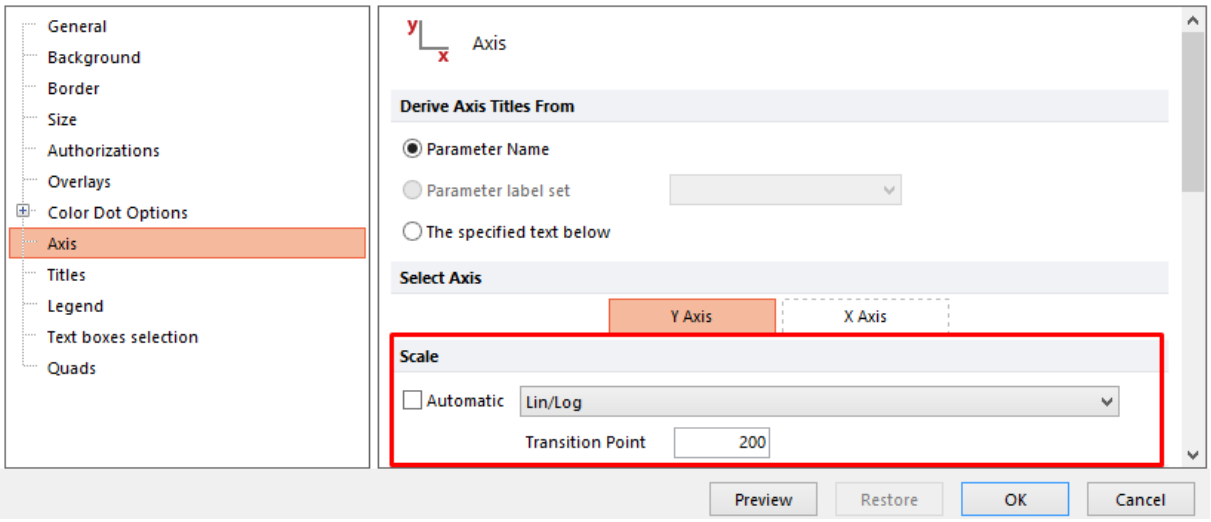

Figure 9.62 Setting the Transition Point for the Y-axis in Lin/Log Scaling

For any of these transforms, smaller transitions values result in plots which more closely resemble the standard log plots. The precise transition value to use will depend for the most part on your data, and generally a value of at least 30 is required.

Biexponential Scaling:
The biexponential display was popularized by the BD FACSDiva software. As a result of our close collaboration with BD, FCS Express now supports all of the

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options that FACSDiva does in terms of displaying biexponential data. In fact, we have implemented FACSDiva's proprietary algorithm for calculating the transition (Below-zero) value, so the results from FCS Express will look identical to the results from the FACSDiva software.

When an FCS file that was acquired with BD FACSDiva is opened in FCS Express you will notice that the automatic scaling in FCS Express applies the Biexponential scaling and chooses the **Below Zero Parameter** (transition point) automatically (Figure 9.63).

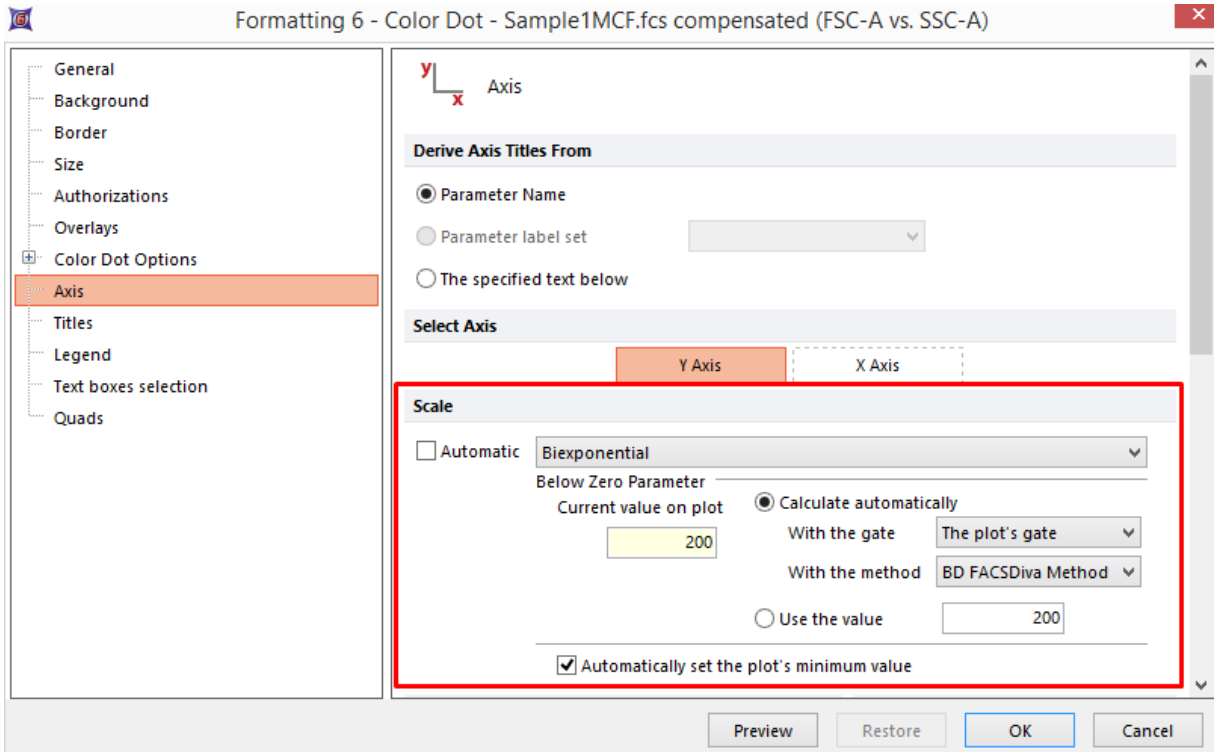

Figure 9.63 Automatic biexponential scaling in FCS Express of a file acquired in BD FACSDiva.

The **Below Zero Parameter** can also be manually adjusted by unchecking the **Automatic** box and selecting the **Use the value** radio button. You may also choose to **Automatically set the plot's minimum value** by checking/unchecking the associated box. When the **Automatically set the plot's minimum value is** checked the axis minimum is set to optimize the display by not displaying outliers. When unchecked the entire range of data, including outliers, will be displayed (Figure 9.64).

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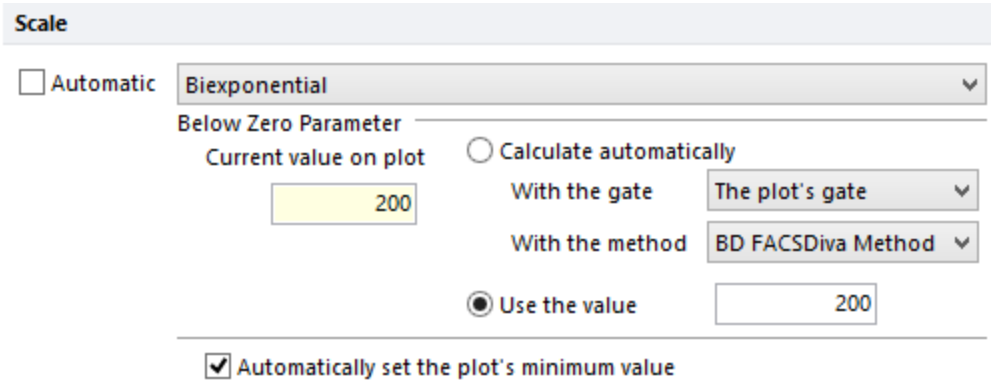

Figure 9.64 Manually entering a Below Zero Parameter value with Biexponential scaling.

The **Calculate automatically** radio button may also be checked if you would like to change the gate for calculating the display (Figure 9.65) or if you would like to change the method for calculation to 5th percentile (Figure 9.66). The Fifth percentile method sets the plots minimum such that the bottom 5% of the data within the selected gate is not visible.

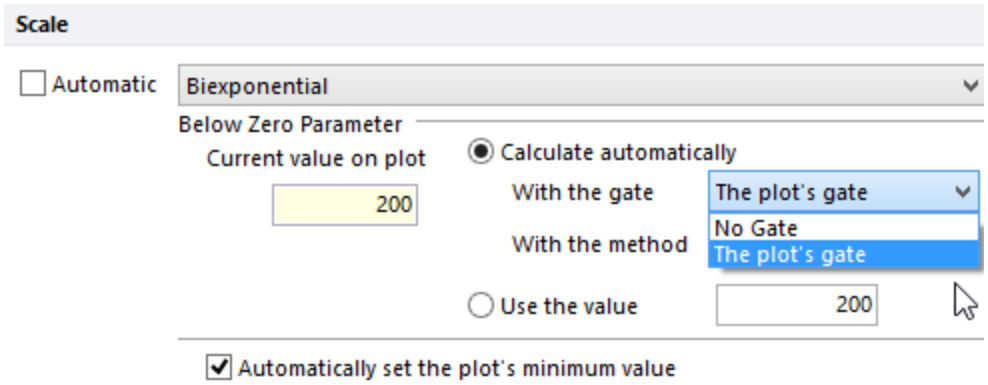

Figure 9.65 Choosing a gate for calculating the biexponential display.

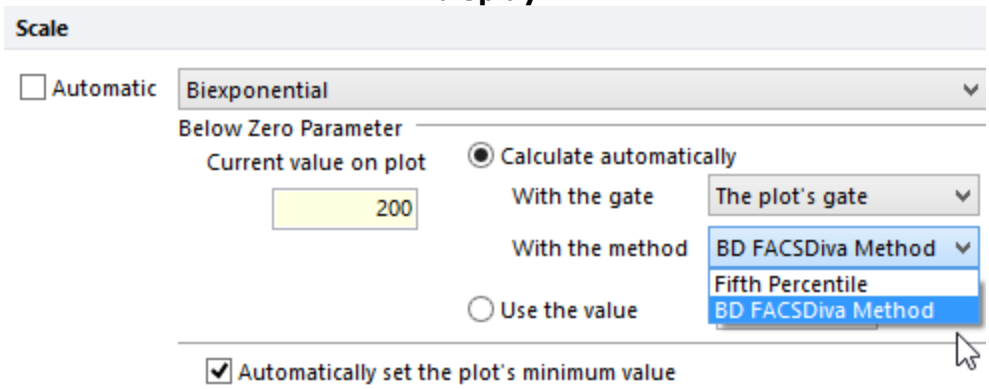

Figure 9.66 Choosing the BD FACSDiva Method or the Fifth Percentile method for display.

Archsinh Scaling:

ArcSinh is an inverse hyperbolic function most widely used with .fcs files generated by CyTOF Mass Cytometry. Unlike standard light based flow

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cytometry, mass cytometry can produce many true zero signal ("intensity") values. An ArcSinh transformation helps display and visualize values at or near zero with a "spread" as we are accustomed to visualizing with standard flow cytometry data. ArcSinh values are calculated by applying the ArcSinh equation to the raw intensity value and dividing by a cofactor. The "cofactor" defines the size of the linear region around 0, with larger cofactors corresponding to a longer linear region. (Figure 9.67).

$$\sinh^{-1} z = \ln\left(z + \sqrt{z^2 + 1}\right)$$

Figure 9.67 - The formula for converting to Archsinh scaling.

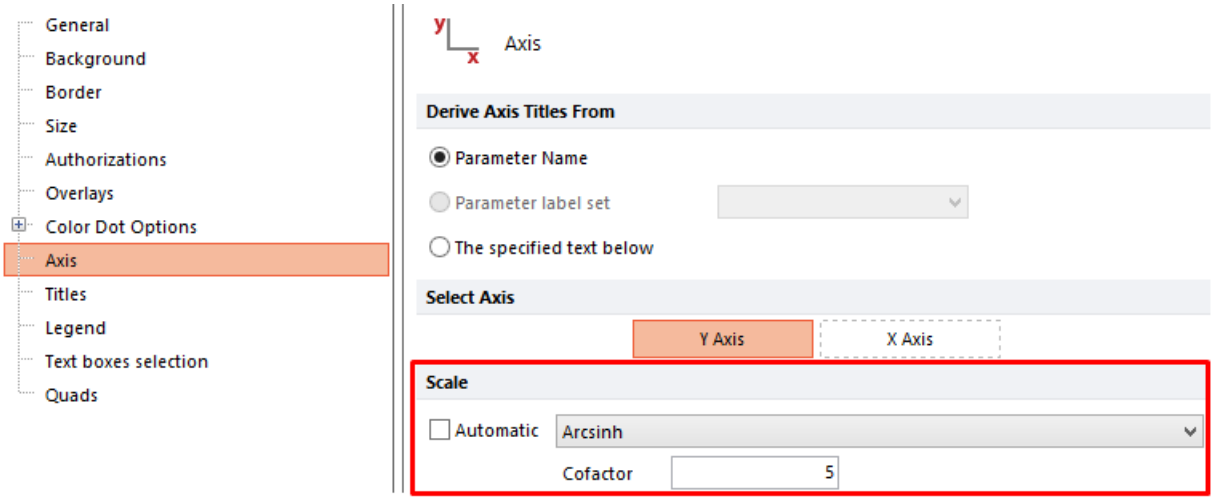

Figure 9.68 - Arcsinh is choosen from the Scale drop down menu. The cofactor value determines the size of the linear region around 0.

9.6.2 Gating and Axis Scalings

When gates are drawn on a plot, the axis scaling of that plot will become the scaling of the gate. This means that if a gate is drawn on a plot using a particular scaling, and then the gate is displayed on a plot with a different scaling, the gate may appear distorted. The gate may also appear distorted if it is displayed on a plot with the same scaling, but a different transition point (e.g., a different "Below zero" parameter for Biexponential scaling). The original scaling that was used when then gate was drawn will be called the native scaling and the alternative scale that it is being displayed will be called the non-native scaling.

Figure 9.68 shows an example of how the gate perimeter can appear distorted when the gate is displayed on a non-native scale. The gate was drawn in linear scale (shown on the left panel) and then displayed on a log scale (shown on the

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right panel). Notice that the straight lines of the gate borders appear curved in the log space. This is correct and is caused by the relative distortion introduced by the log scaling.

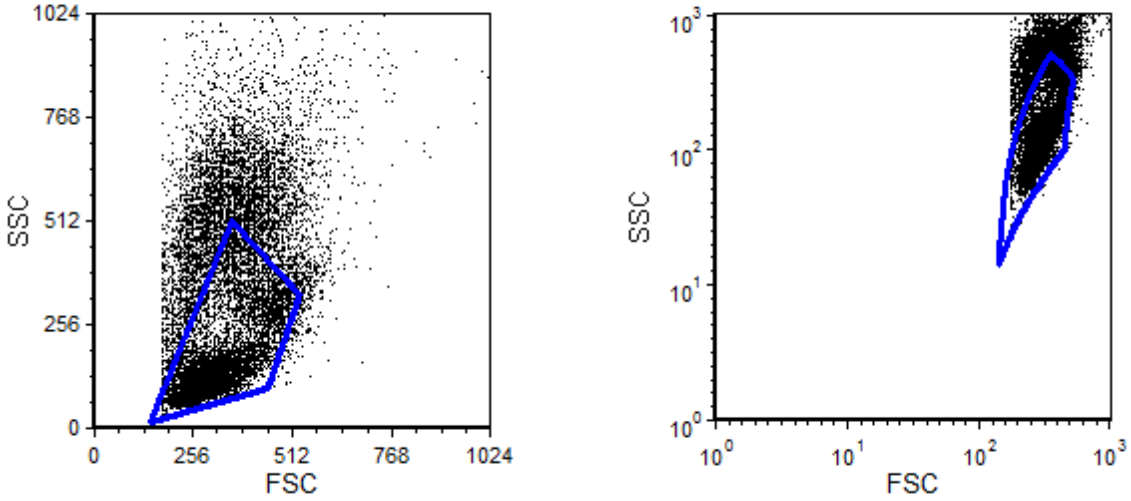

Figure 9.69 Warped Gates in Different Scalings

If you move a gate 341 on a plot displaying a non-native space, the shape of the perimeter of the gate may change as you move the gate, to reflect the mapping of the scales in the different regions of the plot. There are several considerations to take into account when moving a gate on a non-native scale. Since the gate scale is in the native space, the gate may not be able to move to certain positions on the non-native plot. For instance, assume you created a gate on a log scale, which has no negative values, and you are now displaying the gate on a "log with negative" scale, that does have negative values. You will not be able to move the gate to the area of the plot containing negative values, since the native scaling (log) does not support negative values.

A more subtle distortion is depicted in the next figure. The gate shown in the right panel of the figure above (Warped Gates in Different Scalings) was moved to the position shown in Figure 9.69. It is obvious that the gate no longer appears as a four-sided polygon.

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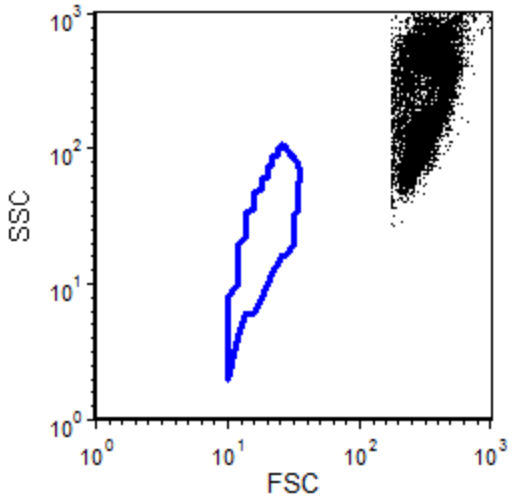

Figure 9.70 Distorted Gate in Non-native Space

The explanation for this can be appreciated by examining the gate in the native space, seen in Figure 9.70. It is evident that the vertices of the gate (located near the origins) comprise a very small area of the data space in the native space. Thus, there is not enough resolution in the native space to accurately draw and calculate a perimeter, and the perimeter appears distorted in the non-native space.

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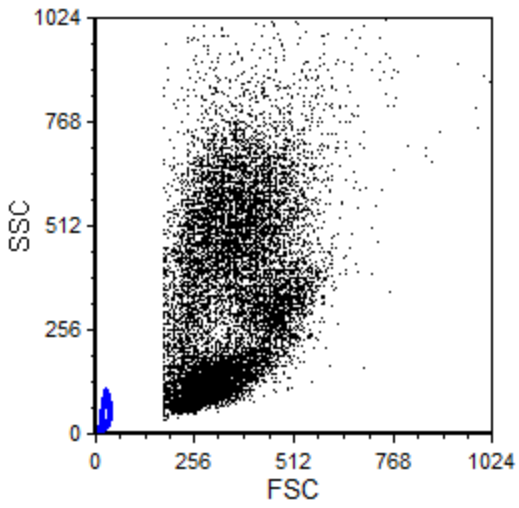

Figure 9.71 Not Enough Resolution in Native Space

If you are displaying a gate on a non-native scale and wish to change it so that scale becomes the native scale, simply draw a new gate on the plot with the non-native scale and replace the existing gate (see Creating Gates 320).

9.6.3 Quadrants and Axis Scaling

Linked quadrants 371 will also appear distorted if they are displayed on plots with different scalings, as shown in Figure 9.71.

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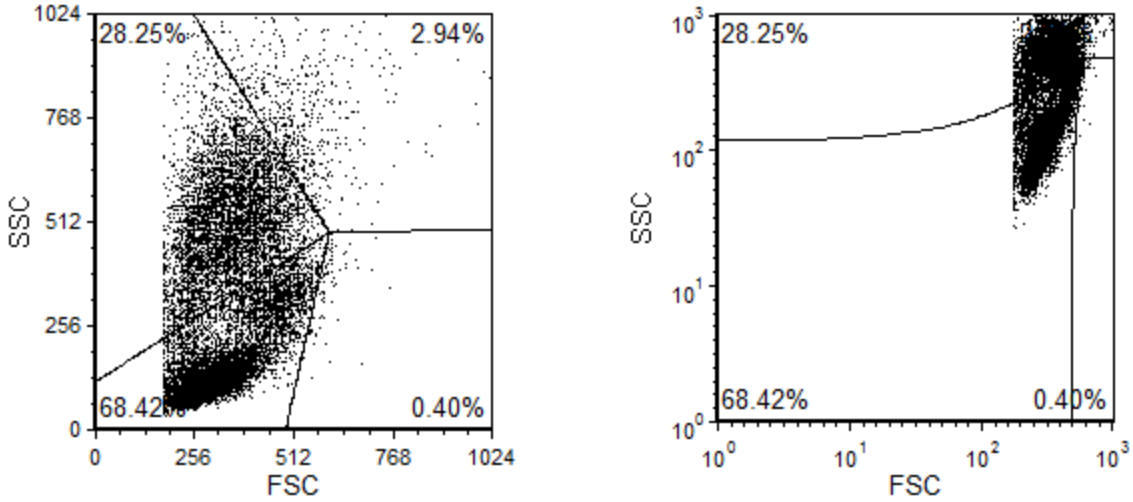

Figure 9.72 Linked Quads Displayed in Non-native Space

9.6.4 Biexponential Scaling Implementation in FCS Express

Biexponential Scale Formula

The biexponential scale formula is defined by the equation[1]:

$$f(x;T,w,m) = Te^{-(m-w)}\left[e^{x-w} - p^2 e^{\frac{-(x-w)}{p}} + p^2 - 1\right], x \geq w$$

Where *x* is the value in scaled space, while *f(x)* is the value in linear space.

In order to generate a formula which is valid for all possible real numbers, we reflect the scale formula about *w* when x < *w* (Equation 2).

$$f(x) = f(w) - f\big(w + (w - x)\big), x < w$$

By definition, $f(w) = 0$, so we can simplify Equation 2.

$$f(x) = -f(2w - x), x < w$$

For this equation,

- *T* represents the range of the data in linear space.
- *m* represents the approximate number of decades of scaled space. For FCS Express, we use the following relation to determine a reasonable value for *m*:

$$m = \left(\frac{4.5 \ln 10}{\ln 262144}\right) \ln T$$

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- *w* is the approximate width of the linear data range in scaled space.
    - The limits of values for *w* are described in the second paper on the scale formula[2].

$$0 \le w \le \frac{m}{2}$$

At *w* = 0, the linear region of the scale formula is 0, while at *w* = *m/2*, the linear region extends for the majority of the data range.

- *p* is defined by its relationship to w:

$$w = \frac{2p \ln p}{p+1}$$

In order to control the width parameter, *w*, the original paper[1] introduced a separate parameter, the "Below zero value". This value should be equal to the most negative values in the data set.

$$|R| = Te^{2w-m}$$

In the original paper, the below zero value, *R*, is always negative. However, in FCS Express, we will display the below zero value as a positive value equal to the absolute value of the paper's below zero value.

In the second paper describing the biexponential scale formula[2], we should pick a value of *w* which is non-negative. Based on this reasoning, we can limit the values used as the below zero value.

$$R \ge Te^{-m}$$

In a standard 18-bit data file, we have *T* = 262144 and *m* = 10.3616. In order to have a non-negative *w* value, *R* ≥ 8.2897.

Invalid below zero values:
The graph of the equation that relates *w* and *p* is shown below.

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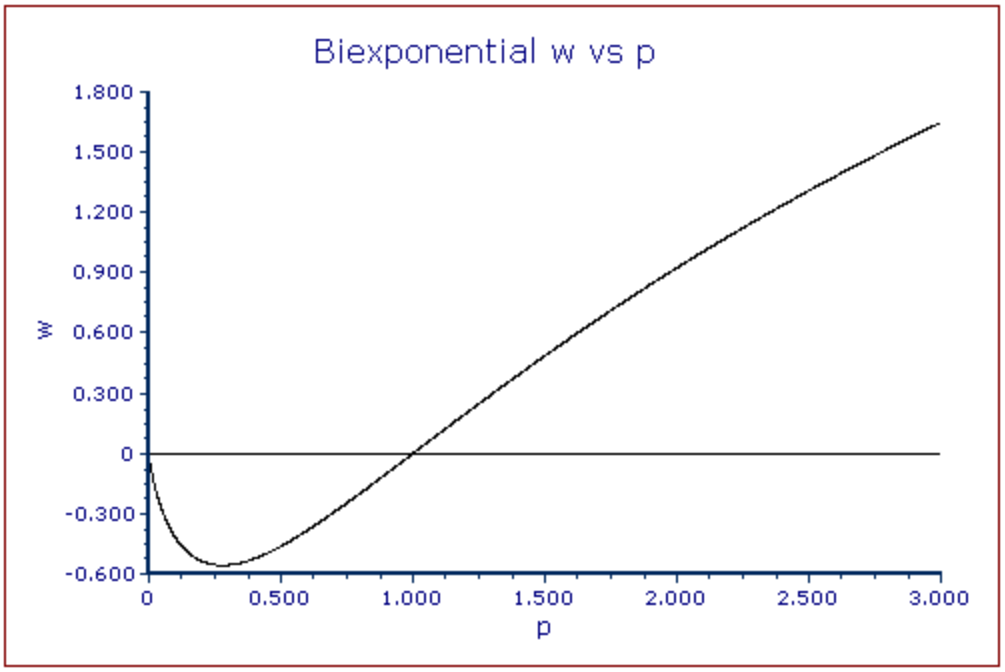

We can see from the graph that if we require *w* to be positive, then the value of *p* will be greater than 1. In this graph, we can also see that if we relax the constraint that w is non-negative we can extend the values for *w* down to a global minimum at *w* = -0.5569290855... = $w_{\min}$

Based on the relationship between *R* and *w,* we can define the minimum value of the below zero value using the following expression.

$$R_{min} = Te^{2w_{\min}-m}$$

For example, in a standard 18-bit data file, *T* = 262144, *m* = 10.3616, and the minimum below zero value will be approximately 2.7214.

A graph of the relation between *T* and R_{min} is shown below.

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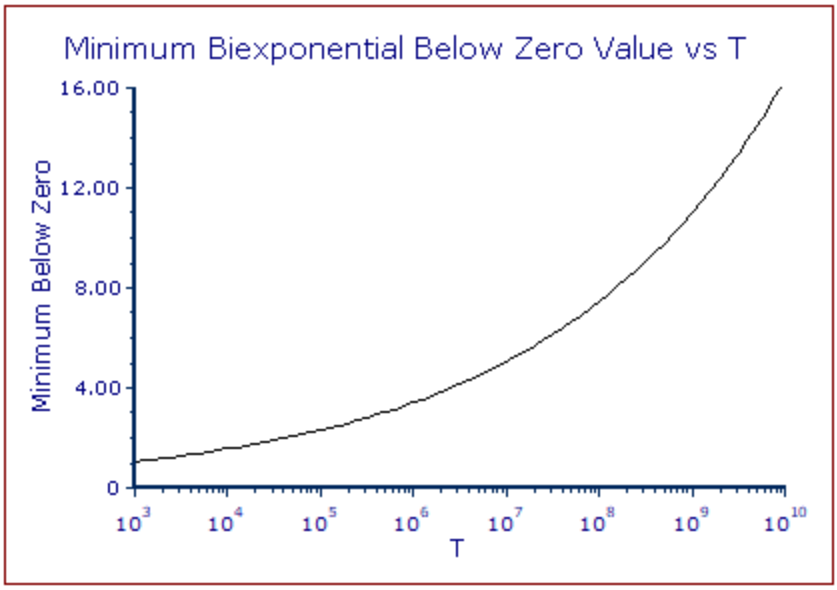

References:

1. Parks, David R.; Roederer, Mario; Moore, Wayne A. A New "Logicle" Display Method Avoids Deceptive Effects of Logarithmic Scaling for Low Signals and Compensated Data. *Cytometry Part A* **2006,** *69A*, 541–551.
2. Moore, Wayne; Parks, David. Update for the Logicle Data Scale Including Operational Code Implementations. *Cytometry A* **2012,** *81(4),* 273-277.

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Chapter

10 Creating and Using Parameter Label Sets

FCS Express allows complete flexibility in labeling the plot axes with whatever information the user desires. Typically, the plot axis labels are taken from the FCS file header or are modified during an analysis session. However, sometimes when acquiring data, the wrong parameter description is entered, or the default parameter description is not modified, and you need to change the axes labels.

FCS Express allows you to create sets of alternate parameter labels associated with the FCS file header parameters. When the new parameter label set is selected during analysis, the alternate parameter label will display on the plot instead of the parameter label listed in the FCS file header. FCS Express allows multiple parameter label sets to be defined, each of which is distinguished by a unique name.

The advantage of a parameter label set is that it allows you to display different axis information without changing the header of the FCS File. By changing the plot axis options to use a parameter label set 254 instead of modifying the data in the FCS File, you can customize the layout the way you want without affecting your data.

To create a new parameter label set:

1. Select the **Tools→Labels→Parameter Labels→Modify Labels** command.
2. Click **...** (ellipsis) button to select or change **Template File** used.
3. Click the **Add Set** button.
4. Enter a unique name for the new parameter set and click **OK**.
5. Click the **Add** button.
6. Select the parameter to modify.
7. Enter the alternate text in the **Label** field.
8. Click **OK**.
9. Continue clicking the **Add** button to modify additional parameters for this parameter label set. You can click on a label and modify it (or use the **Remove** button to delete it).
10. Click **OK** to save the set

Figure 10.1 shows a parameter label set named **Lymphs** that has been created with eight alternate labels.

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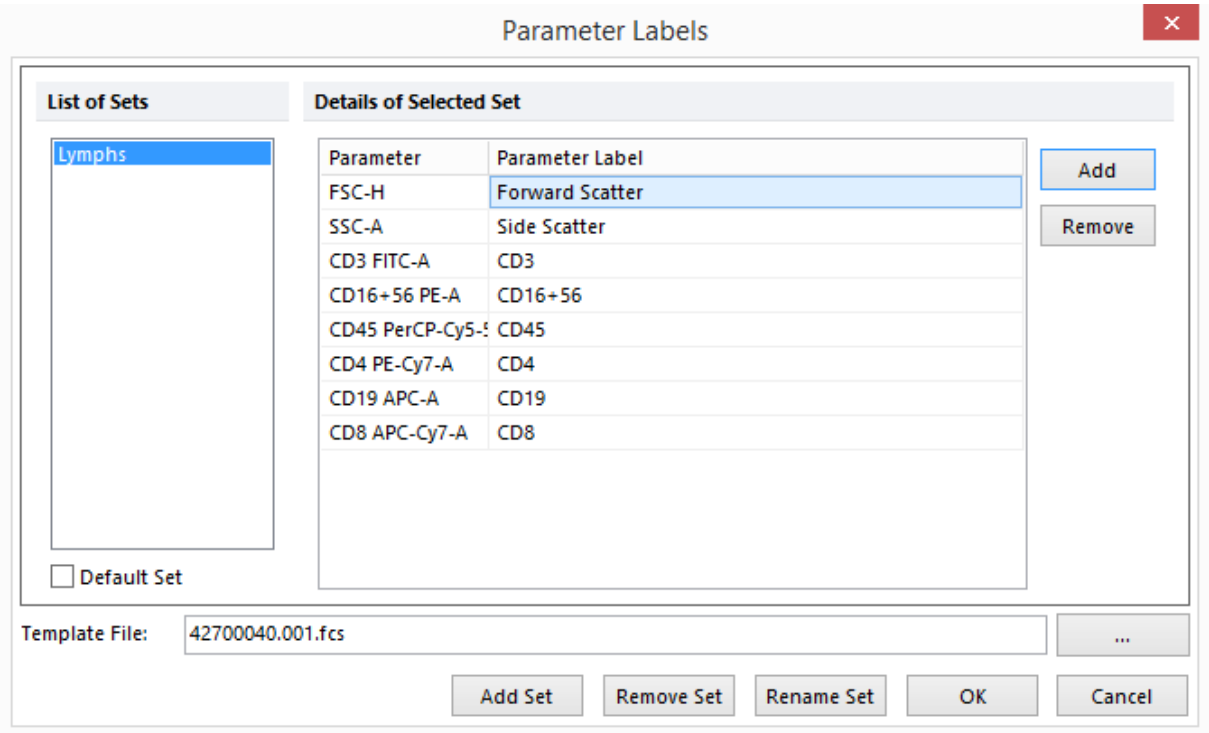

Figure 10.1 Parameter Label Sets

If the axes are set to derive the axis labels from the **Lymphs** set, whenever parameter 3 is shown on the plot, the axis label will be **CD3** instead of the label stored in the header of the FCS file. Selecting a set as the default set means that it will appear by default in the Derive Axis Titles From Parameter label set radio selection drop-down list of the Format Axes dialog 254.

Parameter Label Set Lists can be saved to and loaded from a file by selecting the **Tools→Labels→Parameter Labels→Save Labels** or **Tools→Labels→Parameter Labels→Load Labels** commands, respectively. Parameter labels are automatically saved with the layout file 93.

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Chapter

11 Spreadsheets

Spreadsheets in FCS Express 6 can be used to perform most of the operations that Microsoft Excel provides. In addition, values in Spreadsheet cells can be derived directly from statistics within the layout, keywords, or from data files in the data list. Inserting statistics for data files in the data list allows additional flexibility in analysis of many statistics for many data files that are not currently open in the layout.

A Spreadsheet may be added to a layout through the **Insert** tab→**Spreadsheet**→**New** command.

Once a spread sheet is inserted you may click on the Spreadsheet to select it which will make the **Spreadsheet→Layout** 84 and **Spreadsheet→Formula** 89 tabs appear (figures below).

Once data is tabulated, you can display it graphically, using Pie Charts, Bar Charts and Scatter Plots 537. For example, Spreadsheets can be used to automate the process of generating titration curves.

Spreadsheets created within FCS Express may also be exported 537 to various external file formats such as **.xls**, **.xls**, **.csv**, **.html**, **.xml** and **.txt**. Formulas, formatting, and references will be preserved when selecting to save in the **.xls** or **.xlsx** file format.

Note: Spreadsheets replace **Custom Data** tables from previous versions of FCS Express but have the same capabilities. If you have **Custom Data** tables saved from previous versions FCS Express in your layout then FCS Express 6 will allow you to open them for use. New tables can only be create via the Spreadsheets feature.

Jump to:

Creating a Spreadsheet 415
Working with Spreadsheets 417
Formatting Spreadsheet Cells 423
Using Spreadsheets to access statistics from files in the Data List 433
Spreadsheet Formula Definitions 440
Using Find and Replace 428
Supported Spreadsheet Keystroke Commands 429
List of and description Spreadsheet Statistics available via drag and drop from the data list 393

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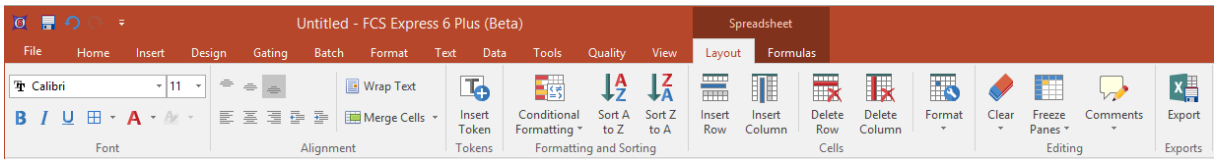
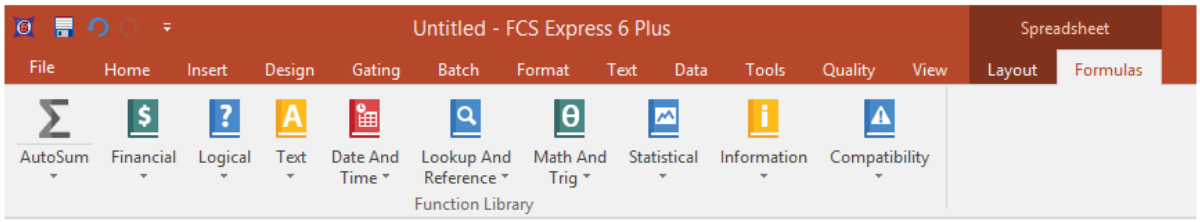


The Spreadsheet Layout Tab



The Spreadsheet Formulas tab.

Additional Resources:

Tutorials:

- HLA Cross matching - Spreadsheet Use Case 1 1620
  - Creating a Spreadsheet for analysis 1624
  - Populating a Spreadsheet with data 1627
  - Working with Custom Tokens and Formula References in spreadsheets 1636
  - Using Conditional Formatting to Classify Data 1646
- Antibody Titration - Spreadsheets Use Case 2 1653
- Calibration via Regression Analysis - Spreadsheets Use Case 3 1679
  - Complete a Spreadsheet and Insert a Scatter with Regression Plot 1680
  - Working with Regression Tokens and Fit Equations 1689
- Working with Pie, Bar, and Scatter Plots - Spreadsheets Use Case 4 1703

11.1 Creating a Spreadsheet

To create a Spreadsheet, use the **Insert** tab→**Spreadsheet**→**New** command to bring up the **Create new spreadsheet** dialog (Figure 11.1).

Alternatively, if a Data Navigator 126 is open, you can right-click on the Data Navigator and choose **Create new spreadsheet** from the pop-up menu.

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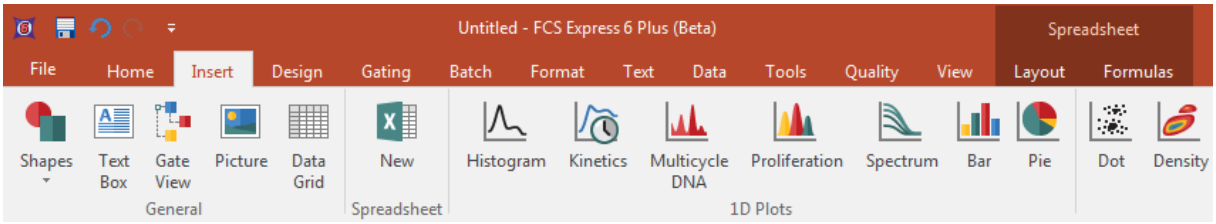

Creating a Spreadsheet from the Insert tab.

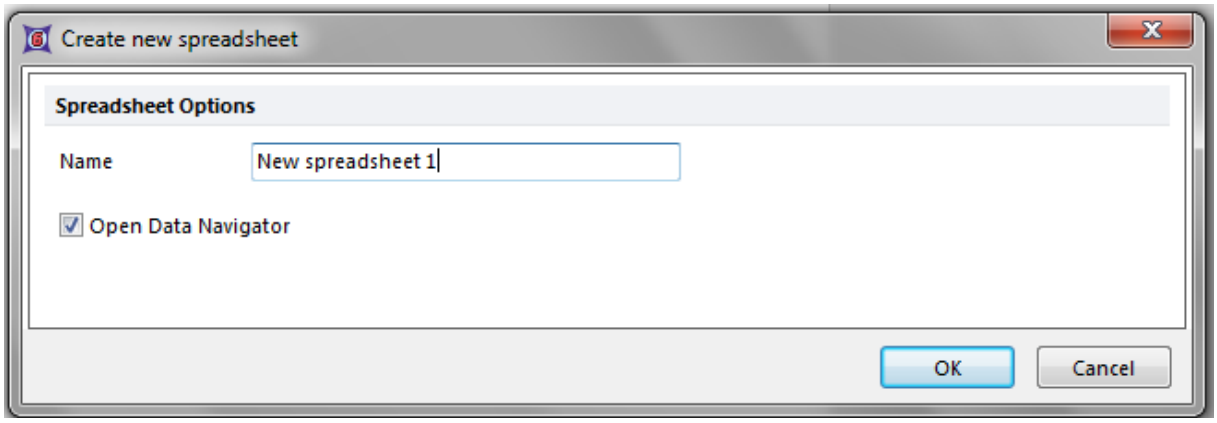

Figure 11.1 Create new spreadsheet Dialog

- The option for **Open Data Grid** is selected by default. You can additionally choose to open the **Data Navigator**. If the Data Navigator is already open then the option will be inaccessible.
- When you are finished, click **OK**. The **Create new spreadsheet** dialog will disappear. A new spreadsheet will appear on your screen, similar to the image below.
- The cells of spreadsheet may be filled with text, numbers or tokens 417.
- Cells of the spreadsheet may reference each other much like Microsoft Excel following the same conventions of Excel.
- Formulas may be created 440 in the cells much like Microsoft Excel.

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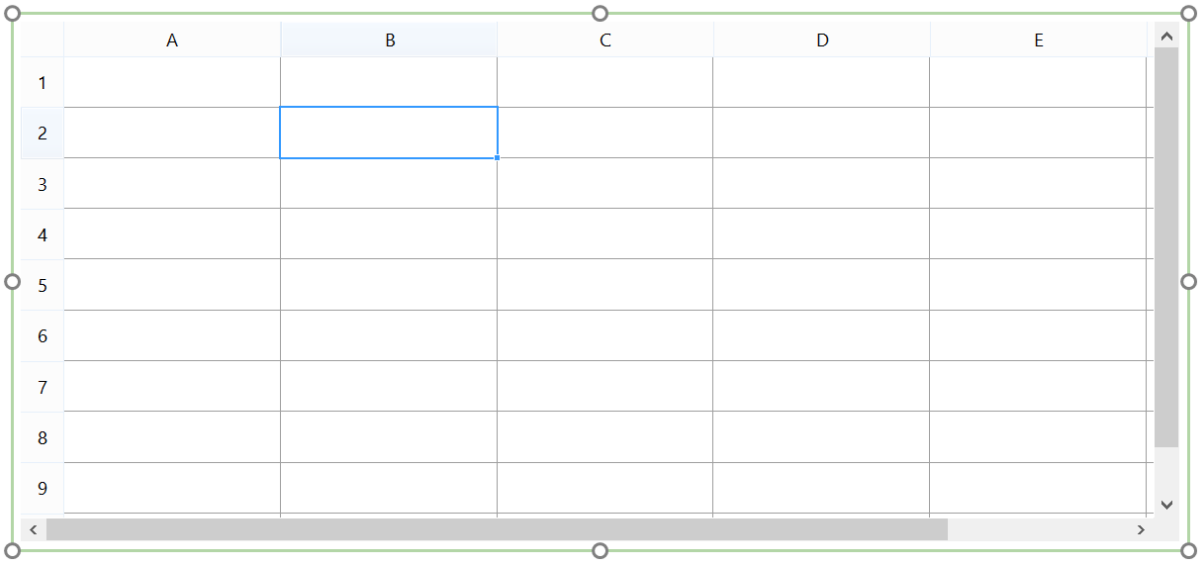

New, Empty Spreadsheet

11.2 Working with Spreadsheets

If you have added a Spreadsheet[415], the Spreadsheet will appear on the layout and a node will appear in the **Data Navigator** with the name you assigned to the spreadsheet (Figure 11.2). Spreadsheets in FCS Express share much of the functionality included in Excel with the added benefit of live updating as statistics or data in your layout changes. A list of some of the common features in Excel that FCS Express will support are:

- **Array Formulas**
- **Appearance Customization and Conditional Formatting**[426]
- **Built in Operators and Functions**[440]
- **Formula Creation**[419] **and AutoSum**[419]
- **Formatting Cells**[423]
- **Find and Replace**[428]
- **Comments**[424]
- **Sorting**[424]

Jump to:

- **Entering text or numeric data in the spreadsheet**[419]
- **Creating a formula in a spreadsheet**[419]
- **Using AutoSum**[419]
- **Referencing cells in a spreadsheet**[420]
- **Inserting a Token or Custom Token in a cell**[420]
- **Inserting Token Tables**[421]
- **Formatting Spreadsheet cells**[423]
- **Spreadsheet Cell Value Tokens**[723]
- **Analyzing Files from the Data List with Spreadsheets**[433]

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- Spreadsheet Statistics Available from Data Files 393

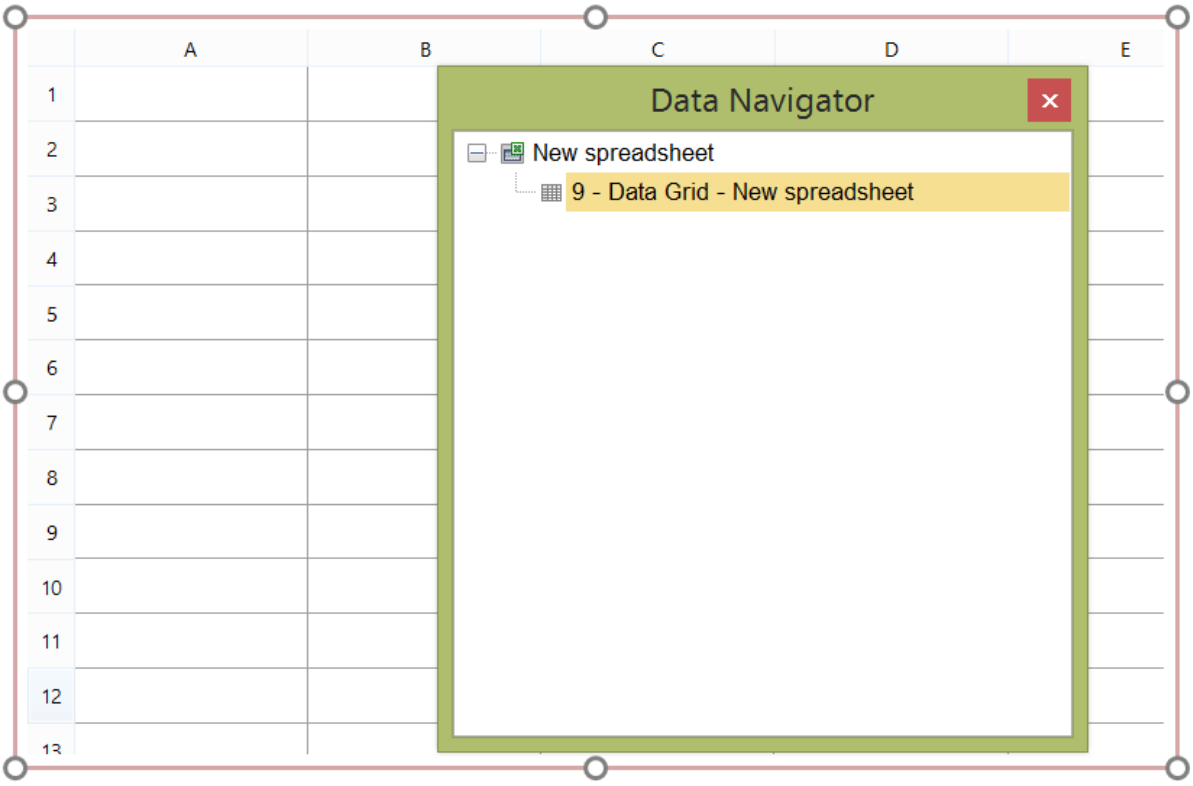

Figure 11.2 An empty spreadsheet open and Data Navigator with New spreadsheet node.

The spreadsheet may be selected, de-selected, moved, re-sized, copied, and pasted just like any object in FCS Express 130.

A spreadsheet that has been copied and pasted will create a duplicate of itself. Any information that is entered in one of the copies will be updated in the additional copies. The copies will be visible in the Data Navigator as separate Data Grids/Spreadsheets under the same node (Figure 11.3) Multiple spreadsheets may exist in the same layout and will appear as separate nodes in the Data Navigator (figure below).

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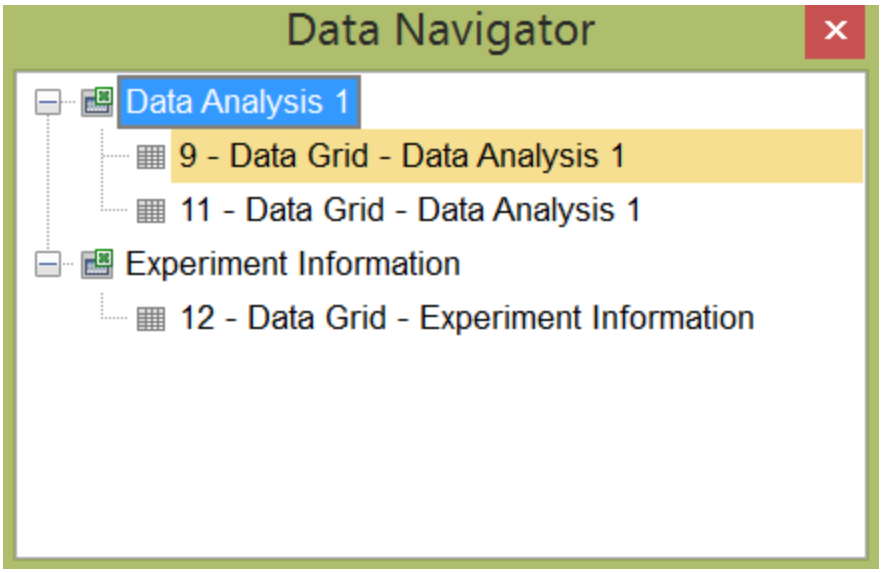

Figure 11.3 - Working with multiple spreadsheets and copies of spreadsheets in the Data Navigator.

Spreadsheets in FCS Express behave very similarly to Microsoft Excel. Just as in Excel there are countless features and options that can be performed. We will cover a few of the basic operations and important points for working with spreadsheets in FCS Express below.

To enter text or numeric data in the spreadsheet:

1. Click on the cell in the spreadsheet.
2. Begin typing in the cell.

To create a formula in a spreadsheet:

1. Click on a cell in spreadsheet.
2. Enter an equals sign "=".
3. Enter a formula, function, or operator 440 to evaluate.
4. The cell will return the value of the information entered.

You may select a cell in the spreadsheet and click on the **Spreadsheet** tab→**Formulas**. Click on the formula or function 440 to use from the menu and it will populate the chosen cell as a blank formula.

AutoSum:
Alternatively, you may select a range of cells and choose a command from the **AutoSum** drop down (Figure 11.4). The **AutoSum** command you have chosen will be calculated across the selected range of data and appear below the final cell in the data range.

Available **AutoSum** functions are:

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Sum	Sums all values within a given range.
Average	Calculates the average for the values within a given range.
Count Numbers	Counts the number of cells containing numbers within a given range. Excludes counting cells that contains text.
Min	Finds the minimum value in a given range.
Max	Find the maximum value in a given range.

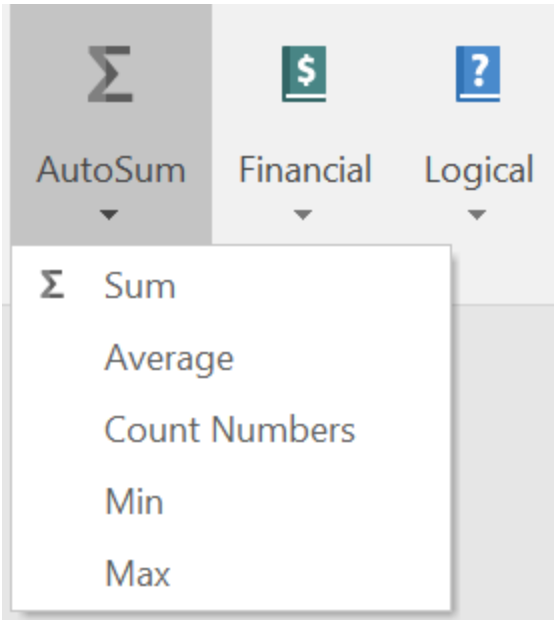

Figure 11.4 - The AutoSum feature is a convenient tool for quickly calculating statistics for a range of values in a spreadsheet.

To reference cells in a spreadsheet:

1. Click on a cell in a spreadsheet.
2. Enter an equals sign "=".
3. Click on a different cell in the spreadsheet that contains data.
4. The original cell will display the data referenced from the second cell.

To insert a Token or Custom Token in a cell:

1. Right click in the cell.
2. Choose **Insert Token.**
3. Select a token to insert in the same manner as inserting a token into a text box 692.

Alternative 1: Drag and drop a statistic, plot, gate, or other FCS Express item from which tokens can be derived into a cell (see more about dragging and dropping tokens and token types 692)

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The token value, or multiple token values, will appear in one or many cells.

Alternative 2:
1. Click on cell in the spreadsheet.
2. Click on the **Spreadsheet** tab→**Layout** tab→Tokens→Insert **Token**.
3. Choose the token of your choice to insert in the cell.

Tokens that are present in cells will update in real time as data updates in plots. Cells that reference other cells or formulas containing token values with also calculate and update in real time. Please note that tokens can also be used elsewhere in FCS Express to reference spreadsheet cells 723.

To insert Token Tables:

Tables in FCS Express from Compensations 626, Signatures 1088, SOPs 1104, Canned Comments, and Heat Maps 1050 may be inserted into a spreadsheet by (Figure 11.5):

1. Right clicking in a cell.
2. Choose **Insert**.
3. Select a token table to insert in the same manner as inserting a token into a text box 692.

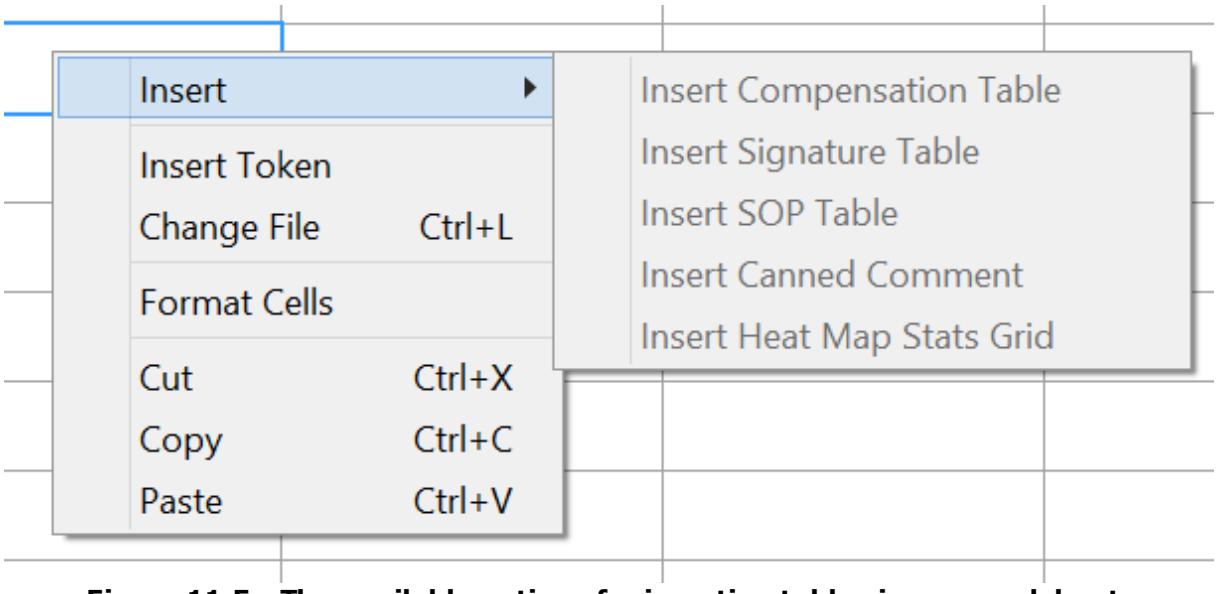

Figure 11.5 - The available options for inserting tables in a spreadsheet.

11.2.1 Formatting

Spreadsheets may be formatted just like any other object in FCS Express 178 and contain many of the same formatting options as other objects. When right

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clicking on a Spreadsheet you will be presented with the option to Format or Format Cells (Figure 11.6). The Format command will allow you to access formatting commands for the Spreadsheet object itself for items like Name, Visibility, Border, Size, Authorizations.

The Format Cells command allows you to adjust formatting options specific to a cell or multiple cells / cell ranges. Please see the next section on Formatting Spreadsheet cells for additional information 423.

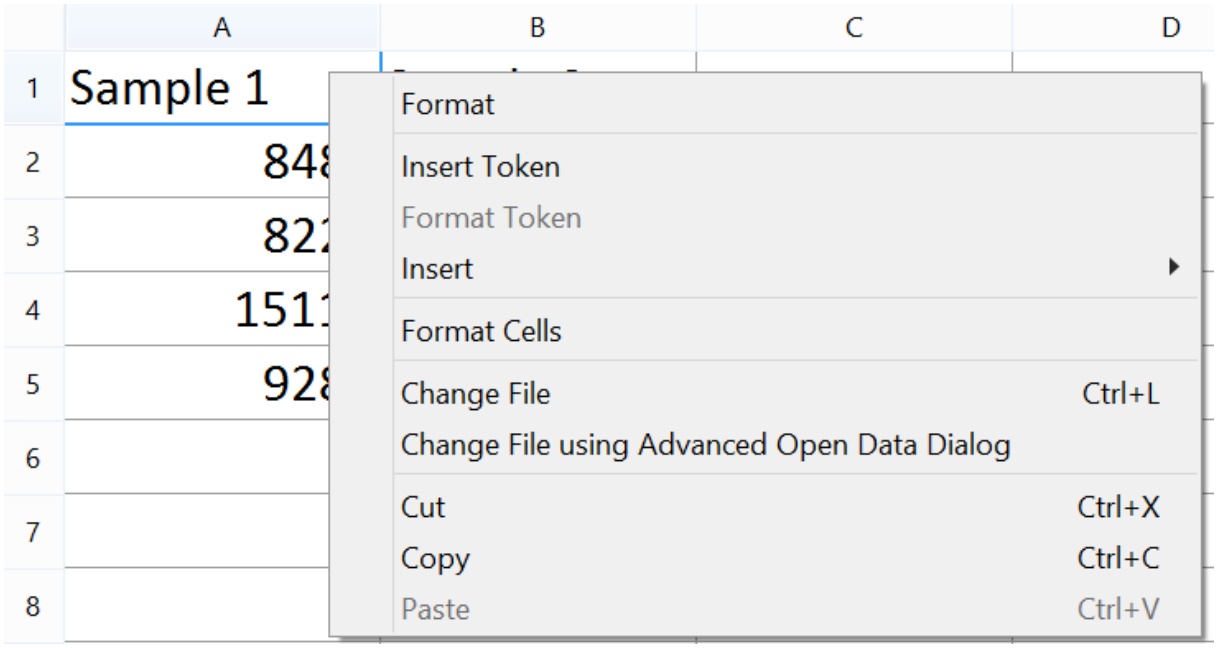

Figure 11.6 - The right click menu available with spreadsheets.

In addition to standard object formatting options 239 spreadsheets have the additional formatting option to **Use values in a first row as labels** (Figure 11.7).

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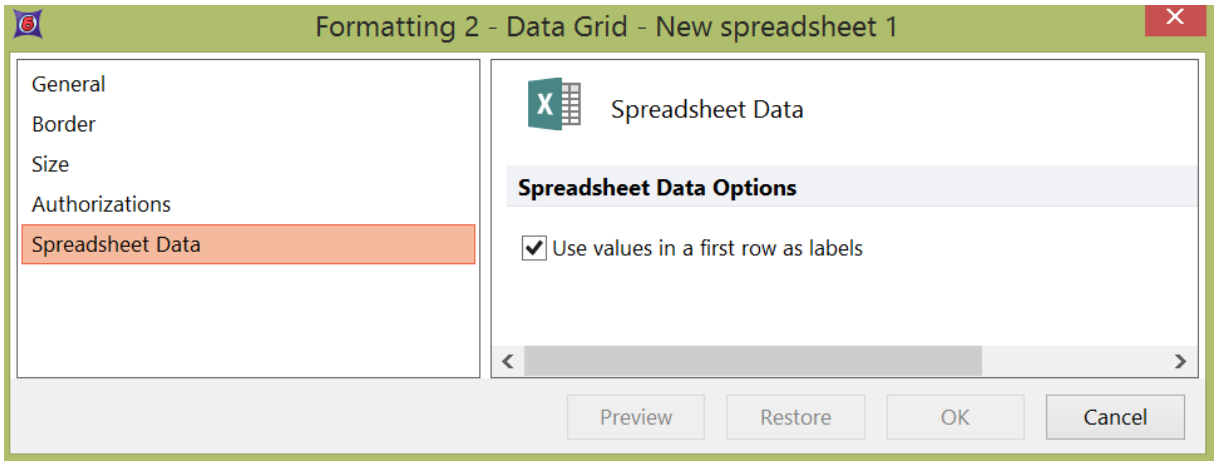

Figure 11.7 - Choosing to Use values in a first row as labels option.

When the option is turned on by checking the box then the first row in the spreadsheet will be used as labels in any additional graph types as in figure 11.8 below.

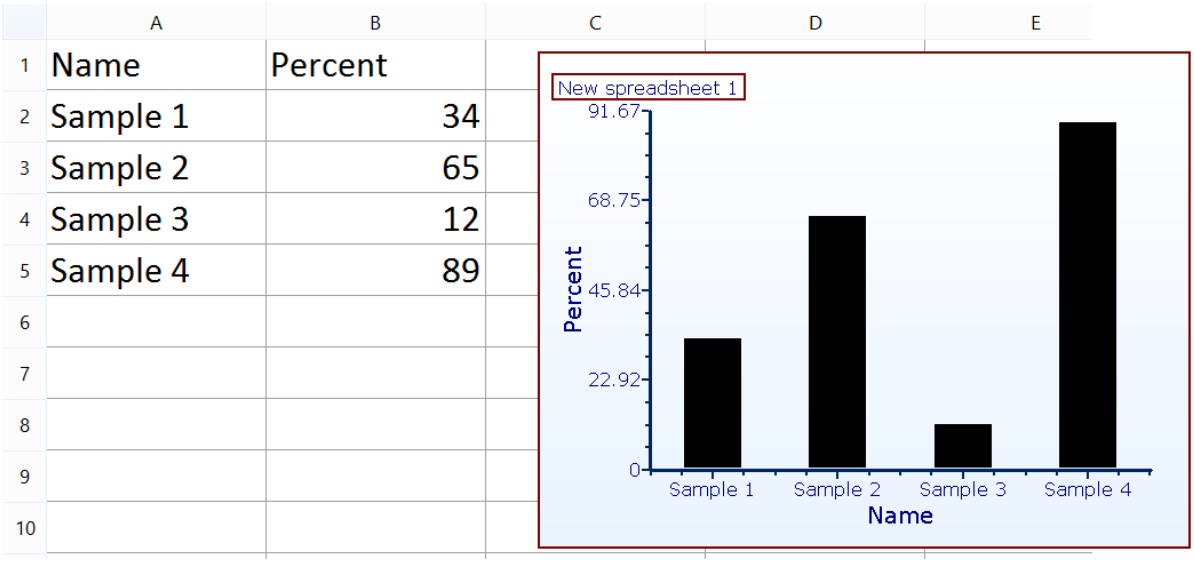

Figure 11.8 - Choosing the Use values in a first row as label option enables bar plots, pie charts, scatter plots, and scatter with regression plots to use the first row in the spreadsheet as a data label.

11.2.1.1 Formatting Spreadsheet Cells

Formatting Spreadsheet cells:

Cells or cell ranges within a spreadsheet in FCS Express may be formatted much as spreadsheets in Microsoft Excel (Figure 11.9).

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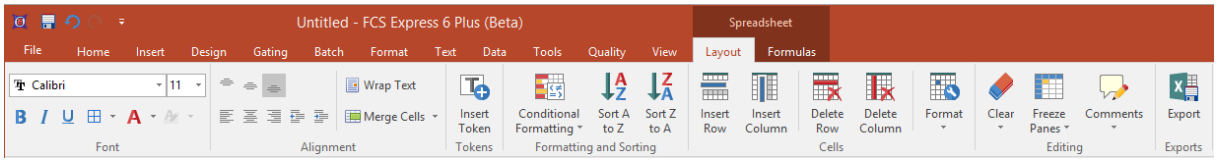

Figure 11.9 - The spreadsheet layout tab provides access to a number of formatting and specialized features for spreadsheets in FCS Express.

The available formatting categories and options from the **Layout** tab are:

Font	Adjust fonts, font sizes, font styles, font color, and cell borders
Alignment	Adjust text alignment for horizontal and vertical placement. Indent text. Wrap text within cells. Merge and unmerge selected cells.
Tokens	Insert any token from FCS Express. 692
Formatting and Sorting	A range of values may be conditionally formatted 426 or sorted.
Cells	Insert or delete rows or columns in the data set. Format cells to autofit contents, hide or unhide contents, and launch the Format Cells dialog.
Editing	Clear the contents formatting or contents of cells. Add or remove freeze panes to the spreadsheet. Create a comment to be associated with the selected cell or cell range.

Additionally, **Number**, **Alignment**, **Font**, **Border**, **Fill** and **Protection** from cells or a range of cells may all be adjusted (Figure 11.10). Cells or cell ranges may be formatted via a right click on the select or via the **Spreadsheet** tab→**Layout** tab commands. To format a cell or range of cells:

1. Select a cell or range of cells to format
2. Right click within the range.
3. Choose **Format Cells**.
4. Choose the formatting feature you would like to adjust from the available tabs just as you would do in Excel.

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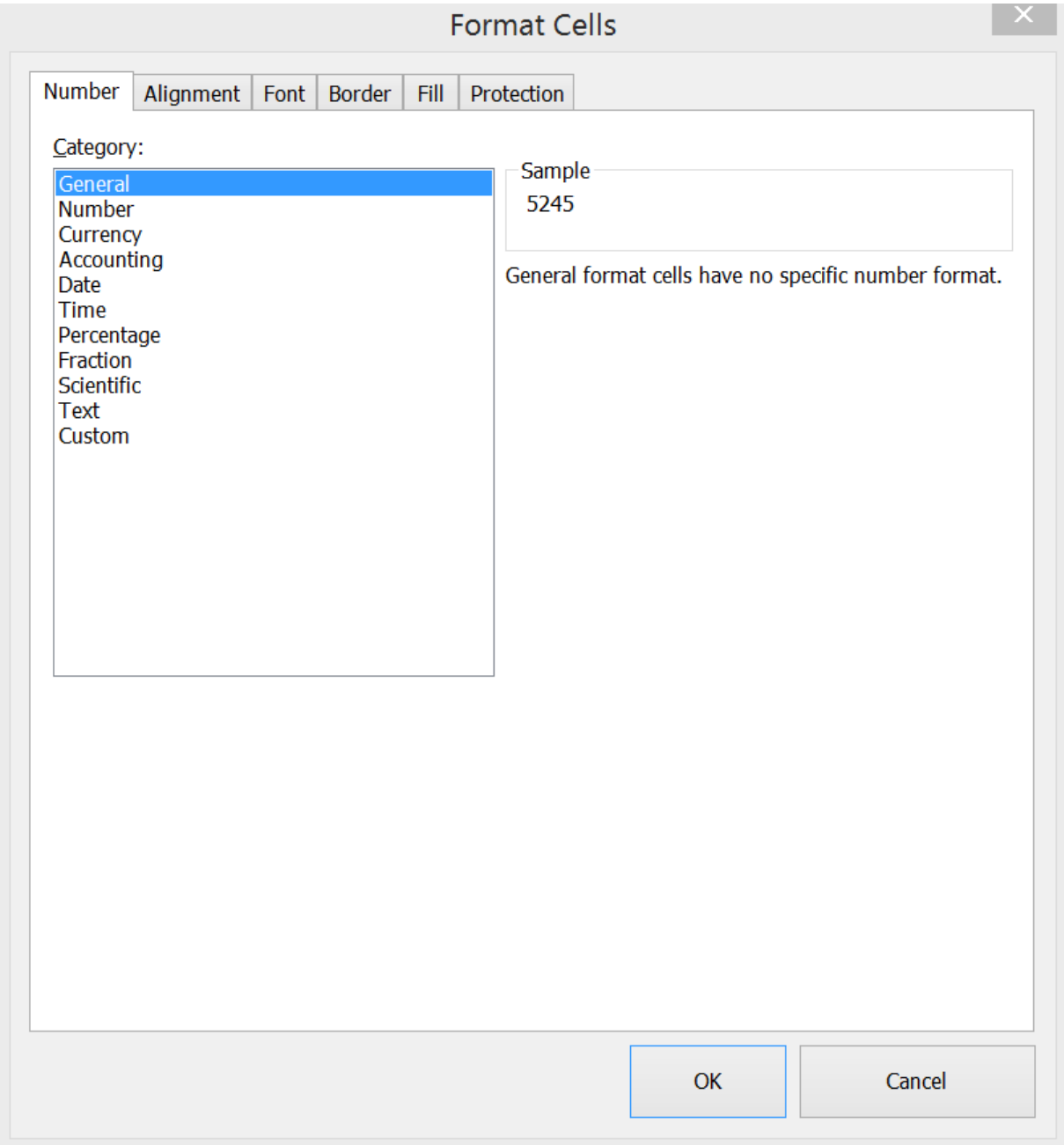

Figure 11.10 - The Format Cell dialog for spreadsheets in FCS Express. The formatting contains all of the familiar features and functionality of Microsoft Excel.

Alternatively:
1. Select a cell or range of cells to format
2. Click on the **Spreadsheet** tab→**Layout** tab.
3. Choose the formatting item you wish to apply to the cells.

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11.2.1.1.1 Conditional Formatting

Conditional formatting is a tool that allows you to apply different displays in the form of Color Scales, Data Bars, and Icon sets to a range of cell values. Conditional formatting may be applied by:
1. Select a range of cells in a Spreadsheet.
2. Click on the **Spreadsheet** tab→**Layout** tab→**Formatting and Sorting**→**Conditional Formatting** dropdown (Figure 11.11).

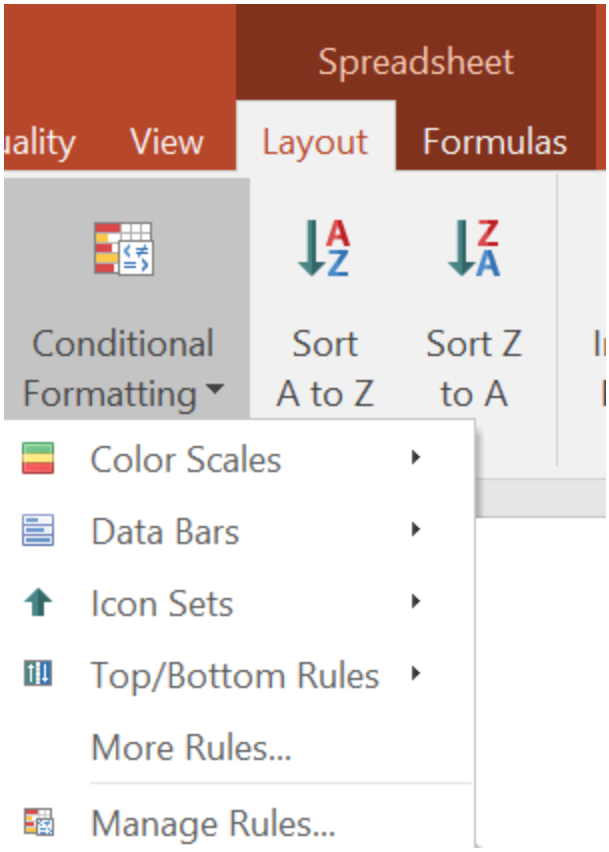

Figure 11.11 - Choosing conditional formatting from the Spreadsheets--> Layout tab.

3. Choose the type of display from **Color Scales**, **Data Bars**, and **Icon Sets**.
4. Choose the sub-type of conditional formatting you wish to apply.

The conditional formatting you choose will be applied to the range of cells. Below is an example of some conditional formatting options applied to a range of data Figure 11.12.

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	A	B	C	D	E	F	G	H
1	Peak	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	V450
2	M1	56.58	44.16	15.18	20.7	187.6	0	313.88
3	M2	1818.84	1718.1	506.46	309.12	1920.8	1250.2	1646.54
4	M3	14735.64	14209.86	4364.94	2644.08	14213.5	9725.8	11231.85
5	M4	41860.92	40173.18	14921.94	10414.86	42119	31798.2	30932.48
6	M5	105084.24	101125.02	54574.86	46011.96	107253.3	88037.6	84377.86
7	M6	202089.95	204531.17	195759.91	198312.21	203804.3	201294.8	201073.39
8								

Figure 11.12 - A variety of conditional formatting options applied to a series of data.

The conditional formatting is based on a series of rules which may be managed by clicking on the **Spreadsheet** tab→**Layout** tab→**Formatting and Sorting**→**Conditional Formatting** dropdown→**Manage Rules** command. From within the **Conditional Formatting Rules Manager** you may select **Edit Rule** to adjust how color scales, bars, and icon sets values are determined.

11.2.2 Spreadsheet Cells

Cells in a spreadsheet allow you to manually enter values, text, formulas or information derived from tokens[417]. Individual cells or cell ranges may be formatted and also contain properties unique to FCS Express in that tokens may exist and be referenced within a cell[420]. Conversely, Spreadsheet Cell Values[723] may be inserted and used as tokens within text boxes and batch processing[723].

Values that currently exist in a cell can be easily moved to a different cell by:

1. Click and hold on the blue square in the lower right portion of the cell to be referenced (Figure 11.13 below).

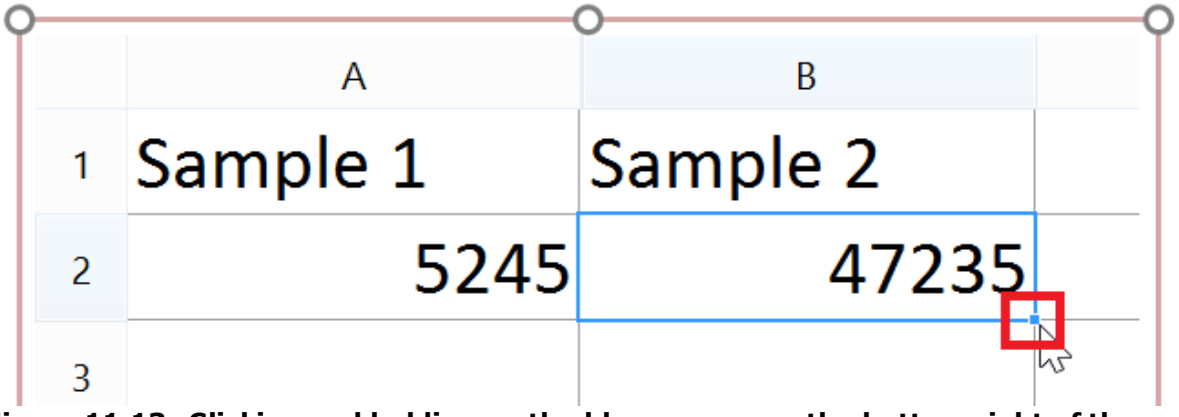

Figure 11.13- Clicking and holding on the blue square on the bottom right of the cell allows you to drag the cell to a new location in the spreadsheet.

2. Drag the cell to the new cell position in the spreadsheet where you would like it to appear.
3. Release the mouse button to place the cell.

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Cell values can be copied down much as you would in Excel to quickly populate a range of cells. To copy a cell down:

1. Select the originating cell by clicking on it.
2. Press **Cntrl+C** to copy.
3. Select the range of cells to copy down including the originating cell.
4. Press **Cntrl+D** to copy down over the range of selected cells.

Right clicking in a Spreadsheet cell reveals options to insert tokens, format cells, and format tokens. If you have already inserted a token in a spreadsheet cell you may format that token by:

1. Right click in the cell that contains the token.
2. Choose **Format Token**.

The token type that is included in the cell may now be edited and changed as desired.

11.2.3 Find and Replace

Spreadsheets in FCS Express support Find and Find and Replace functionality. To access Find and Find and Replace:
1. Click on a spreadsheet or select a range of cells.
2. Press Control+F.

The **Find and Replace** dialog will appear.

In the **Find** tab:
1. Enter a value or text to find within the spreadsheet or selected range.
2. Click **Find All** or **Find Next**.

If matching values or text is found the spreadsheet will highlight the cell in which the information has been found.

The **Options** button allows you to further designate search terms and locations.

In the **Replace** tab:

1. Enter a value or text to find within the spreadsheet or selected range in the **Find what** area.
2. Enter a value or text to replace the found item in the **Replace with** area.
    a. Click **Find All** or **Find Next** to jump to the next found values.
    b. Click **Replace All** or **Replace** to replace the found values with the values indicated in the **Replace with** area.

The **Options** button allows you to further designate search terms and locations.

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11.2.4 Supported Keystrokes

Supported Keystrokes

The Spreadsheets tool in FCS Express provides a familiar Excel-like environment, including multiple corresponding keyboard support. The Spreadsheets tool supports over 70 keys and key combinations available in Microsoft Excel. All currently supported keyboard controls are listed in the following table:

Key/Key Combination	Description
Arrow Keys	Pressing an arrow key produces different effects depending on what object is selected and/or focused (highlighted) in the Spreadsheet control: • If a cell is focused, pressing the Left, Up, Right, or Down key moves focus to the left, top, right, or bottom adjacent cell; • If a cell range is selected, pressing the Left, Up, Right, or Down arrow key clears the selection and shifts focus by one cell in the respective direction; • If the focused cell is located at the left, top, right, or bottom edge of the worksheet area, pressing the Left, Up, Right, or Down key does not move focus;
Del	Pressing the Del key destroys all cell objects within all selected areas.
End	Moves focus to a cell following the rightmost cell object within the same row. If there are no cell objects in this row, the focus is moved to its first (i.e., the leftmost) cell.
Enter / Shift+Enter	Pressing the Enter key or the Shift+Enter key combination produces different effects depending on what object is selected and/or focused in the Spreadsheet control: • If a cell is focused within a worksheet, pressing the Enter key moves focus to the next (i.e., underlying) cell within the same column. If the last cell in a column is focused, the Enter key moves focus to the topmost cell in the next (i.e., the right adjoining) column. If the last cell in the last (i.e., the rightmost) column is focused, pressing the Enter key sets the focus to the top cell in the worksheet's leftmost column. • If a cell range is selected, pressing the Enter key moves cell focus within the range's boundaries in the same manner as described above. In the case of multiple selected cell ranges, the Enter key consecutively moves cell focus through all selected areas in the ascending order of their indexes within the worksheet's collection of selections. Both the Enter key and the Shift+Enter key combination close an in-place cell editor accepting its current contents as a new cell value. The cell focus shifts identically to the cases described above. The

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	Shift+Enter key combination moves focus identically to the Enter key but in the opposite direction.
Esc	If an in-place editor is open, pressing the Esc key closes it and discards its current value.
F2	Opens an in-place editor for the focused cell.
F9	Recalculates all formula expressions within the current spreadsheet document.
Home	Moves cell focus to the leftmost (i.e., the first) cell in the current row.
Page Down	Moves cell focus to the next cell containing data in the column below the current focus. If no data is present, moves cell focus to last row in the spreadsheet.
Page Up	Moves cell focus to the next cell containing data in the column above the current focus. If no data is present, moves cell focus to first row in the spreadsheet.
Tab / Shift+Tab	Pressing the Tab key or the Shift+Tab key combination produces different effects depending on what object is selected and/or focused in the Spreadsheet control: • If a cell is focused within a worksheet, pressing the Tab key moves focus to the next (i.e., the right adjoining) cell within the same row. If the last cell in a row is focused, the Tab key moves focus to the first cell on the next (i.e., the lower adjacent) row. If the last cell in the last row is focused, pressing the Tab key sets the focus to the first cell in the worksheet's topmost row. • If a cell range is selected, pressing the Tab key moves the cell focus within the range's boundaries in the same manner as described above. In the case of multiple selected cell ranges, the Tab key consecutively moves the cell focus through all selected areas in the ascending order of their indexes within the active Table View worksheet's collection of selections. • Both the Tab key and the Shift+Tab key combination close an in-place cell editor, accepting its current contents as a new cell value. The cell focus shifts identically to the cases described above. The Shift+Tab key combination moves focus identically to the Tab key but in the opposite direction.
Alt+Enter	When editing the contents of a cell, starts a new line within the open in-place cell editor.
Ctrl+;	Enters the current date into the focused cell.
Ctrl+`	With a cell in edit mode, switches between displaying formula expressions and their calculated results in the active worksheet.
Ctrl+'	Copies the formula expression to the focused cell from the adjacent cell above.
Ctrl+1	Displays the Format Cells dialog for the current cell selection.

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Ctrl+2 / Ctrl+B	Adds/removes the bold attribute to/from the font used to display the contents of the current cell selections.
Ctrl+3 / Ctrl+I	Adds/removes the italic attribute to/from the font used to display the contents of the current cell selections.
Ctrl+4 / Ctrl+U	Adds/removes the underline attribute to/from the font used to display the contents of the current cell selections.
Ctrl+5	Adds/removes the strikeout attribute to/from the font used to display the contents of the current cell selections.
Ctrl+9	Hides all rows that intersect the current cell selection area.
Ctrl+0	Hides all columns that intersect the current cell selection area.
Ctrl+A	Selects all cells within the active worksheet.
Ctrl+Arrow Keys	Moves focus to the nearest cell object within the current row or column (i.e., the Ctrl+Left, Ctrl+Up, Ctrl+Right, or Ctrl+Down key combination moves focus to the nearest cell object located to the left, above, to the right, and below the currently focused cell). If there are no cell objects on the supposed path of the cell focus, it is moved to the last cell on the respective worksheet's edge (i.e., left, top, right, or bottom, depending on a specific arrow key pressed).
Ctrl+C / Ctrl+Insert	Copies the current cell selection to the clipboard. If there are multiple selections then the full area between the top left cell of those selections and the bottom right cell of the selections will be copied to the clipboard.
Ctrl+D	Populates the currently selected cell range with both the contents and style of its topmost cell (if the range's width does not exceed a single cell) or row.
Ctrl+End	Cancels the current cell selection and moves cell focus to the bottom-right corner of the Dimensions area. Previous cell focus position is irrelevant. If there are no cell objects within the active worksheet, pressing this key combination moves cell focus to the topmost cell in the worksheet's first column.
Ctrl+F	Invokes the Find and Replace dialog with the activated Find tab.
Ctrl+H	Invokes the Find and Replace dialog with the activated Replace tab.
Ctrl+Home	Cancels the current cell selection and moves the cell focus to the upper-left (origin) cell of the active worksheet.
Ctrl+K	Displays the Insert Hyperlink dialog for new hyperlinks or the Edit Hyperlink dialog for a previously created hyperlink. You can add hyperlinks to both the cells and floating containers, including comments.
Ctrl+Page Down	Switches between the currently visible worksheets, consecutively activating their respective tabs from left to right in the caption bar. If the rightmost worksheet tab is already active, pressing this key combination has no effect. The Ctrl+Page Up key combination consecutively activates the currently visible worksheets in reverse.
Ctrl+Page Up	Switches between the currently visible worksheets, consecutively activating their respective tabs from right to left in the caption bar. If the leftmost worksheet tab is already active, pressing this key

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	combination has no effect. The Ctrl+Page Down key combination consecutively activates the currently visible worksheets in reverse.
Ctrl+R	Populates the currently selected cell range with both the contents and style of its leftmost cell (if the range's height does not exceed a single cell) or column.
Ctrl+Shift +7	Applies the outline border to the currently focused cell or selected cell range(s). This key combination is a direct counterpart of the Ctrl+Shift+_ key combination.
Ctrl+Shift +_	Returns the outline border of the currently focused cell or a selected cell range to its original state. This key combination is a direct counterpart of the Ctrl+Shift+& key combination.
Ctrl+Shift +~	Applies the General number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +4	Applies the Currency number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +5	Applies the Percentage number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +6	Applies the Scientific number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +3	Applies the Date number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +2	Applies the Time number format to data within the currently focused cell or selected cell range(s).
Ctrl+Shift +1	Applies the Number format (that has two decimal places, thousands separator, and minus sign (-) for negative values) to the data within the currently focused cell or selected cell range(s).
Ctrl+Shift +8	Selects the current region around the focused cell (the area enclosed by blank rows and columns containing uninitialized cells).
Ctrl+Shift +:	Enters the current time into the focused cell.
Ctrl+Shift +"	Copies the value to the currently focused cell from the adjacent cell above.
Ctrl+Shift +End	Extends the selected cell range to the bottom-right corner of the Dimensions area, i.e., to the cell object with the largest row and column index values.
Ctrl+Shift +Enter	Accepts the changes made in an in-place cell editor and closes it, leaving the current cell focused. If an in-place editor was used to enter a formula expression, this key combination chooses the array formula variant of the function, if applicable.
Ctrl+Shift +Home	Extends the selected cell range to the active worksheet's upper-left cell.
Ctrl+Minus (−)	Displays the Delete dialog for deleting cells, rows, and columns at the currently focused cell or selected cell range.
Ctrl+Shift +O	Selects all cell objects that have an associated comment container in the currently active worksheet.

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Ctrl+Plus(+)	Displays the Insert dialog for inserting cells, rows, and columns at the currently focused cell or selected cell range.
Ctrl+Spacebar	Selects an entire column whose cell object is currently focused. This key combination works even if there are no cell or column objects created within the active worksheet.
Ctrl+V / Shift+Insert	Pastes the current clipboard content into the active worksheet.
Ctrl+X / Shift+Del	Pressing this key combination results in moving (cutting) to the clipboard both the cell data and associated styles from the currently focused cell or a selected cell range.
Shift+Arrow Keys	This key combination expands the currently selected cell range by one cell, row, or column depending on the area's shape and the actual arrow key pressed. If there are several selected cell ranges within the active worksheet, this key combination expands only the last selected cell or cell range.
Shift+Spacebar	Selects the entire row that intersects the cell selection in which the cell focus is currently located.

11.3 Analyzing Files from the Data List with Spreadsheets

Spreadsheets may be used to quickly analyze and compare statistics for many data files from the data list without opening additional plots or creating a batch process.

After data files have been loaded into the data list 785, the data files may individually or multiple selected in the data list, dragged and dropped onto a spreadsheet, and the information or statistics to display will be inserted into the spreadsheet cells. If plots exist on the layout you may choose to derive statistics from gates and as gates are adjusted the values in the spreadsheet will update in real time.

Watch the short video below or review the written steps to learn how to use this feature.

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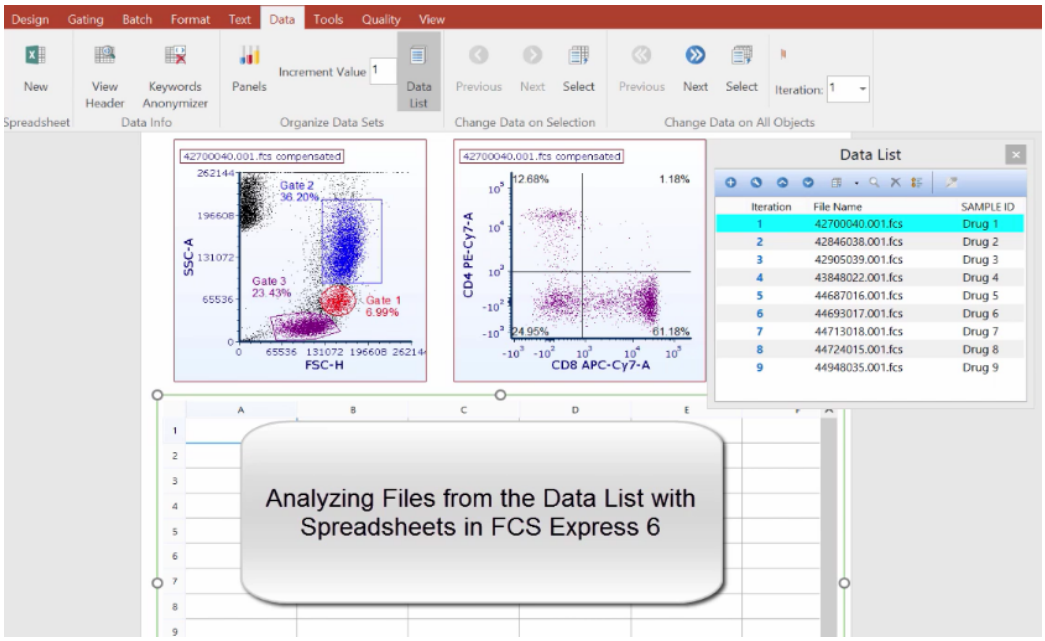

In FCS Express 6 the Spreadsheet feature may be used to quickly analyze and compare statistics for many data files from the data list without opening additional plots or creating a batch process. Watch this short overview to learn about the new feature.

To analyze files from the data list in a spreadsheet:

1. Create a spreadsheet 415 in the layout.
2. Open the data list 785. (Figure 11.14)

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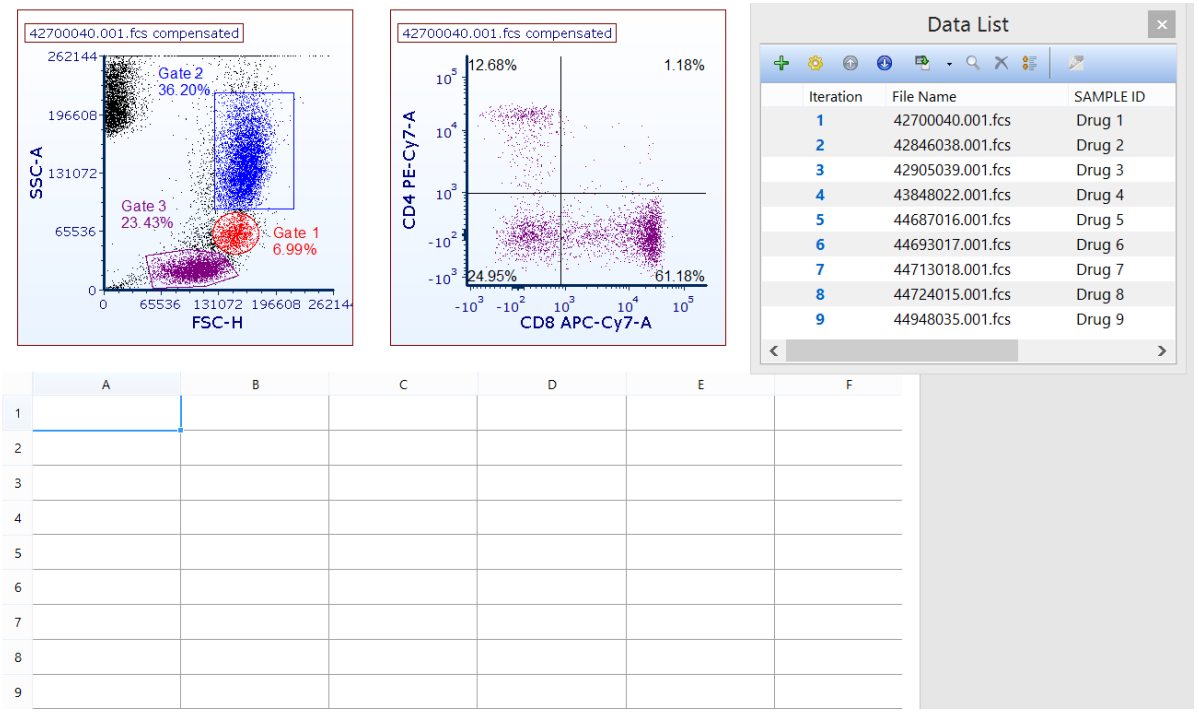

Figure 11.14 - A layout with spreadsheet created and data list opened.

3. Multiple select data files from the data list.
4. Drag and drop data files from the data list into the first cell in the spreadsheet that you would like statistics to appear (Figure 11.15).

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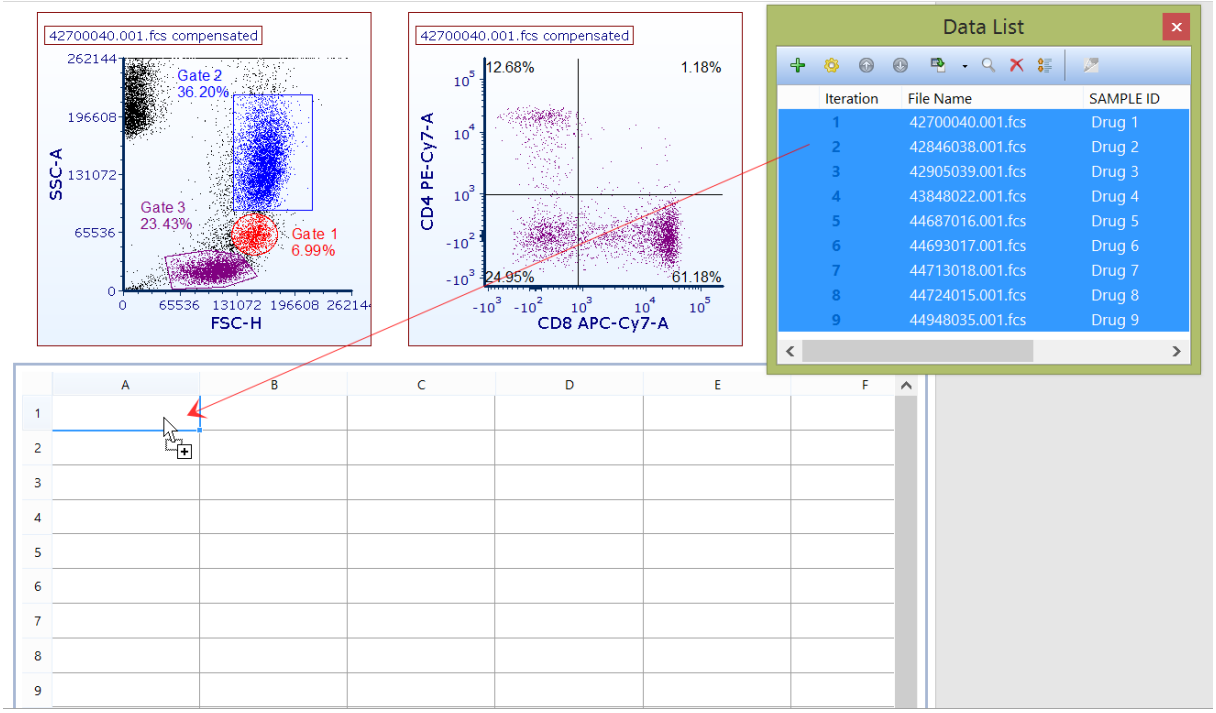

Figure 11.15 - Dragging and dropping data files from the data list into a cell in the spreadsheet.

5. Select **Statistic Token** or **Keyword Token** in the **Paste Special** dialog. In this example, **Statistic Token** has been selected.
6. Select the appropriate parameter for the statistic from the **Parameter** drop down in the **Edit Statistic** dialog (Figure 11.16).

Note - a parameter must be selected regardless of the statistic you choose to insert

7. Select a compensation or choose **Default** or **None** from the **Compensation** drop down.

Note: by setting **Default** from the **Compensation** drop down, the default option for compensation 645 specified in the **User Options** will be used.

8. Select a gate from the **Select Gate** drop down if needed.
9. Select one or multiple statistics from the **Statistic** list (Figure below).

NOTE: The values present in a Spreadsheet for data derived from the data list will reflect the values associated with the data file/iteration and will update in real time. However, in some situations a "fixed" or static value from a particular data file(s)/iteration(s) may be useful to insert in a Spreadsheet. For instance, if you would like to derive the mean from an Isotype control or any other statistic when batch processing. When the having a static or fixed value from a data file or iteration is necessary, the statistics added to the spreadsheet can be made independent from the iteration selected. In other words, the statistics will not change while batch processing or changing through different iterations.

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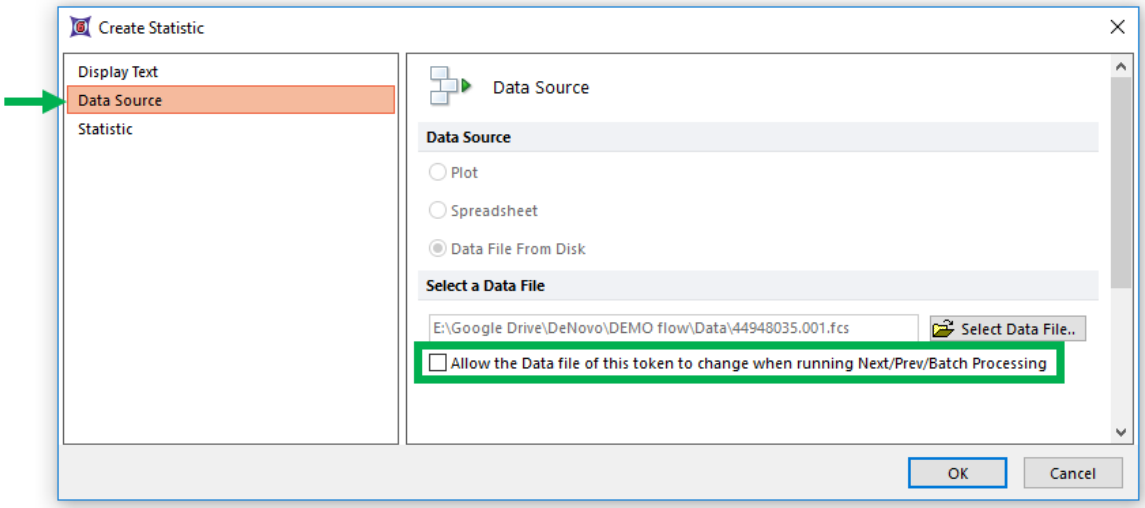
To "fix" a value in the spreadsheet to prevent allowing the value associated with the data file/iteration to change click on the **Data Source** category on the left of the the **Create Statistic** window after dragging and dropping a data file from the data list into the spreadsheet and uncheck the **Allow the Data file of this token**

to change when running Next/Prev/Batch Processing option as seen in the image below. This option is always available for statistics added by dragging and

dropping files from the Data List to the spreadsheet, and can be accessed by right clicking on a spreadsheet cell and by selecting **Format Token** (the **Edit Keyword**

or the **Edit Statistic** dialog will open and the **Data Source** category will be available on the left). However, by unchecking the option during the initial drag and drop action (i.e. before **Step 10** below), the option will apply to all of the data files/iterations at once.



Statistics within the spreadsheet can be made independent from the currently loaded iteration by unchecking the "Allow the Data file of this token to change when running Next/Prev/Batch Processing" option.

10.Click **OK**.

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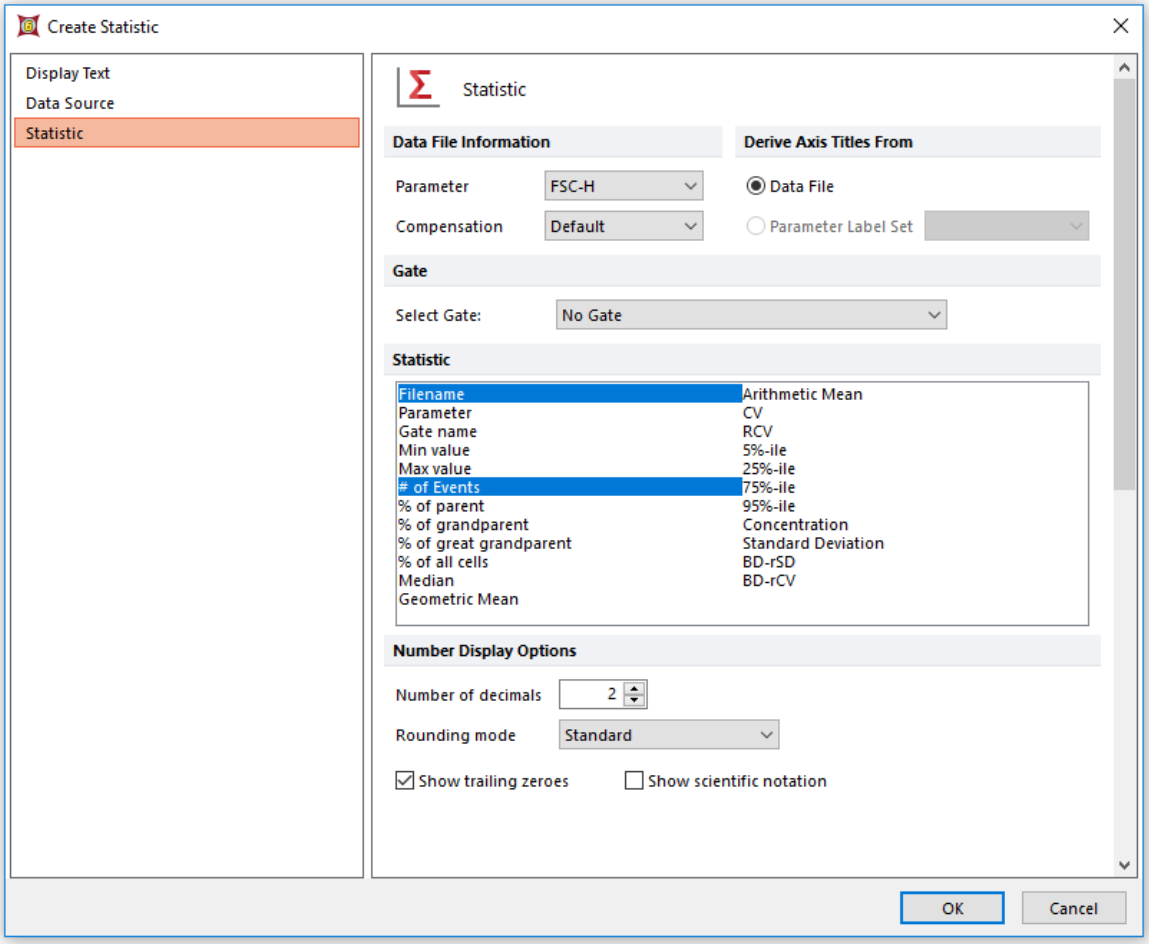

Figure 11.16 - Selecting the parameter, compensation, and statistics to insert in the spreadsheet cells.

The statistics 393 for all of the data files you selected from the data list will appear in the spreadsheet and will update in real-time as gates are adjusted (Figure 11.17).

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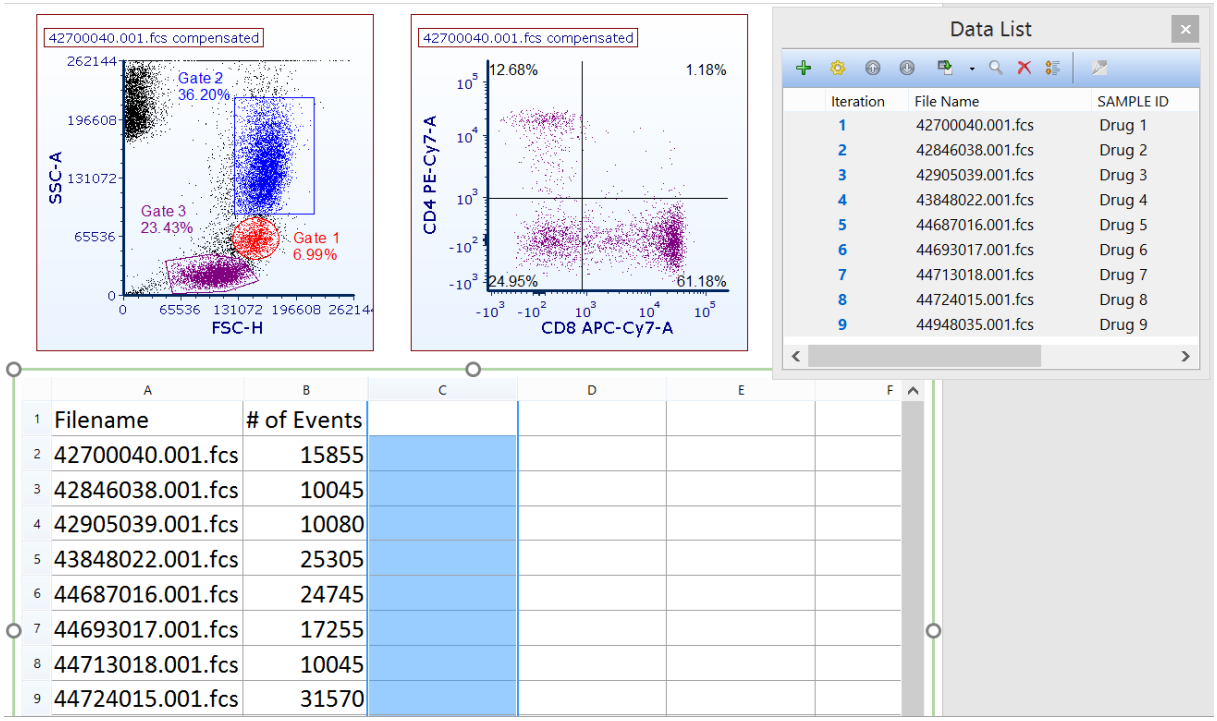

	A	B	C	D	E	F
1	Filename	# of Events				
2	42700040.001.fcs	15855				
3	42846038.001.fcs	10045				
4	42905039.001.fcs	10080				
5	43848022.001.fcs	25305				
6	44687016.001.fcs	24745				
7	44693017.001.fcs	17255				
8	44713018.001.fcs	10045				
9	44724015.001.fcs	31570				

Figure 11.17 - Statistics for all of the data files selected will appear in the spreadsheet.

If additional statistics or statistics from different parameters are needed repeat the steps above. In the example below additional columns were added for gated statistics (Gate 1) and statistics from different parameters (Median CD8) (Figure 11.18).

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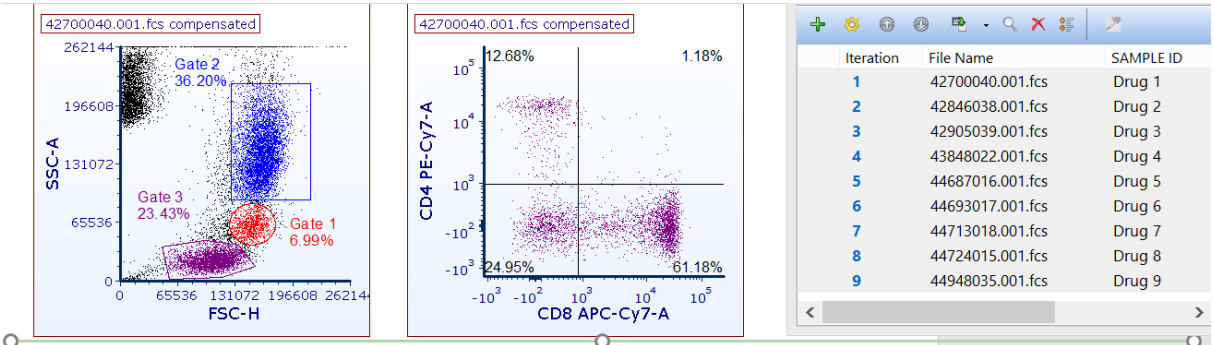

	A	B	C	D	E	F
1	Filename	# of Events	# of Events - Gate 1	% of gated cells - Gate 1	Median CD8	
2	42700040.001.fcs	15855	1108	6.99	6597	
3	42846038.001.fcs	10045	643	6.4	3510	
4	42905039.001.fcs	10080	358	3.55	15180	
5	43848022.001.fcs	25305	399	1.58	3887	
6	44687016.001.fcs	24745	1457	5.89	3985	
7	44693017.001.fcs	17255	414	2.4	3249.5	
8	44713018.001.fcs	10045	675	6.72	3156	
9	44724015.001.fcs	31570	1420	4.5	2299	
10	44948035.001.fcs	14245	568	3.99	3219	

Figure 11.18 - Spreadsheet displaying statistics from 9 data files from different gated populations and parameters. Statistics will update in real time as gates are adjusted.

11.4 Spreadsheet Formula Definitions

Spreadsheets in FCS Express use many of the formulas, functions, and operators with which you are familiar in Excel. Please reference the list of functions and operators below for use in FCS Express. Please also note when implementing a formula below, the delimiter used in list separation may not be a comma based on the computer's regional settings (i.e. replacing the comma with a semi-colon may be necessary to execute the function).

The built-in operators and functions for Spreadsheets can be divided into these groups:

- Compatibility Functions 441
- Arithmetic Operators 453
- Logical Operators 453
- Logical Functions 454
- Lookup and Reference Functions 456
- Arithmetic Functions 467
- Financial Functions 518
- Statistical Functions 478
- Date and Time Functions 524
- Text Functions 529

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- Information Functions 534

Compatibility Functions

Compatibility functions have been replaced with new functions that may provide improved accuracy and whose names better reflect their usage. These functions are still available for compatibility reasons. However, if backward compatibility is not mandatory, you should consider using the new functions from now on, because they offer more accuracy and better functionality.

Mnemonics	Accepted Parameters (Operand Types)	Example	Explanation
BETADIST	Accepts three obligatory parameters: The value at which the **BETADIST** function performs evaluation (*X*) and two parameters of the calculated distribution (*Alpha* and *Beta*). Optionally, you can pass the lower and upper bounds of the interval as the *A* and *B* parameters, respectively.	=BETADIST(X, Alpha, Beta, [A], [B])	Returns the beta cumulative distribution function. If any specified parameter is not numeric, the **BETADIST** function returns the #VALUE! error value. If either the Alpha or Beta parameter is not positive, BETADIST returns the #NUM! error value. If X < A, X > B, or A = B, BETADIST returns the #NUM! error value. If you omit the *A* and *B* parameter values, **BETADIST** uses the standard cumulative beta distribution, so that *A* = **0** and *B* = **1**. **BETADIST** is a deprecated function. For information about its new version, refer to the BETA.DIST function description.
BETAINV	Accepts three obligatory parameters: The value at which the	=BETAINV(Probability, Alpha, Beta, [A],[B])	Returns the inverse of the cumulative distribution function for a specified beta distribution. If any parameter is non numeric, the **BETAINV** function returns the

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	BETAINV function performs evaluation; and two parameters of the calculated distribution (*Alpha* and *Beta*). As with the BETADIST function, you can also pass the lower and upper bounds of the interval as the *A* and *B* parameters, respectively.		#VALUE! error code. If alpha or beta are not positive, BETAINV returns the #NUM! error code. If Probability < 0 or Probability > 1, BETAINV returns the #NUM! error code. If you omit the A and B parameter values, BETAINV uses the standard cumulative beta distribution, so that A = 0 and B = 1. BETAINV is a deprecated function. For information about its new version, refer to the BETA.INV function description.
BINOMDIST	Accepts four obligatory parameters: The number of successes in trials (*NumberS*), the number of independent trials (*Trials*), the probability of success on each trial (*ProbabilityS*), and a logical value that determines the form of the function (*Cumulative*).	=BINOMDIST(NumberS, Trials, ProbabilityS, Cumulative)	Returns the individual term binominal distribution probability. The *NumberS* and *Trials* parameter values are truncated to integers. If the NumberS, Trials, or ProbabilityS parameter value is not numeric, the BINOMDIST function returns the #VALUE! error code. If NumberS < 0 or NumberS > trials, BINOMDIST returns the #NUM! error code. If ProbabilityS < 0 or ProbabilityS > 1, BINOMDIST returns the #NUM! error code. BINOMDIST is a deprecated function. For information about its new version, refer to the BINOM.DIST function description.
CHIDIST	Accepts two obligatory parameters: the value at which you want to evaluate the distribution (*X*)	=CHIDIST(X, DegFreedom)	Returns the one-tailed probability of the chi-squared distribution. If either parameter is not numeric, the **CHIDIST** function returns the #VALUE! error code. If X is negative, CHIDIST returns the #NUM! error code. If the

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	and the number of degrees of freedom (*DegFreedom*).		DegFreedom parameter is not an integer, it is truncated. CHIDIST is a deprecated function. For information about its new versions, refer to the CHISQ.DIST and CHISQ.DIST.RT function descriptions.
CHIINV	Accepts two obligatory parameters: a probability associated with the chi-squared distribution (*Probability*) and the number of degrees of freedom (*DegFreedom*).	=CHIINV(Probability, DegFreedom)	Returns the inverse of the one-tailed probability of the chi-squared distribution. If either parameter is not numeric, the CHIINV function returns the #VALUE! error code. If Probability < 0 or Probability > 1, CHIINV returns the #NUM! error code. If the DegFreedom is not an integer, it is truncated. If DegFreedom < 1, CHIINV returns the #NUM! error value. CHIINV is a deprecated function. For information about its new versions, refer to the CHISQ.INV and CHISQ.INV.RT function descriptions.
COVAR	Accepts two obligatory parameters: the first and second cell ranges of integers (*Array1* and *Array2*).	=COVAR(Array1,Array2)	Returns covariance, the average of the products of deviations for each data point pair in two data sets. The parameters must either be numbers or be names, arrays, or references that contain numbers. If the array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with zero values are taken into account. If Array1 and Array2 have different numbers of data points, COVAR returns the #N/A error value. If either Array1 or Array2 is empty, the COVAR function returns the #DIV/0! error code. COVAR is a deprecated function. For information about its new versions, refer to the

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			COVARIANCE.P and COVARIANCE.S function descriptions.
EXPONDIST	Accepts three obligatory parameters: the value of the function (*X*), the *Lambda* parameter, and the logical value that specifies which form of the exponential function to provide (*Cumulative*).	=EXPONDIST(X, Lambda, Cumulative)	Returns the exponential distribution. If cumulative is TRUE, **EXPONDIST** returns the cumulative distribution function; otherwise – returns the probability density function. If the *X* or *Lambda* parameter is not numeric, the **EXPONDIST function returns the #VALUE! error code.** **If the X parameter is negative, EXPONDIST returns the #NUM! error code.** **If the Lambda parameter is not positive, EXPONDIST also returns the #NUM! error value.** **EXPONDIST is a deprecated function. For information about its new version, refer to the EXPON.DIST function description.**
GAMMADIST	Accepts four obligatory parameters: the value at which you want to evaluate the distribution (*X*), two parameters of the distribution (*Alpha* and *Beta*), and the cumulative logical value (*Cumulative*).	=GAMMADIST(X, Alpha, Beta, Cumulative)	Returns the gamma distribution. You can use this function to study variables that may have a skewed distribution. The gamma distribution is commonly used in queuing analysis. If the *Beta* parameter is **1**, the **GAMMADIST** function returns the standard gamma distribution. If the X, Alpha, or Beta parameter is not numeric, GAMMADIST returns the #VALUE! error code. If the X parameter is negative, GAMMADIST returns the #NUM! error code. If either the Alpha or Beta parameter is not positive, the GAMMADIST function returns the #NUM! error code.

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			GAMMADIST is a deprecated function. For information about its new version, refer to the GAMMA.DIST function description.
GAMMAINV	Accepts three obligatory parameters: the probability associated with the gamma distribution (*Probability*), and two parameters of the distribution (*Alpha* and *Beta*).	=GAMMAINV(Probability, Alpha, Beta)	Returns the inverse of the gamma cumulative distribution. You can use this function to study a variable whose distribution may be skewed. If the *Beta* parameter is **1**, the **GAMMAINV** function returns the standard gamma distribution. If text is passed as any of the parameters, **GAMMAINV** returns the #VALUE! error code. If Probability < 0 or Probability > 1, the GAMMAINV function returns the #NUM! error code. If either the Alpha or Beta parameter is not positive, the GAMMAINV function returns the #NUM! error code. GAMMAINV is a deprecated function. For information about its new version, refer to the GAMMA.INV function description.
HYPGEOMDIST	Accepts four obligatory parameters: the number of successes in the sample (*SampleS*), the size of the sample (*SampleSize*), the number of successes in the population (*PopulationS*), and the population size (*PopulationSize*).	=HYPGEOMDIST(SampleS, SampleSize, PopulationS, PopulationSize)	Returns hypergeometric distribution. The **HYPGEOMDIST** function returns the probability of a given number of sample successes, given the sample size, population successes, and population size. All parameters are truncated to integers. If any parameter is non-numeric, the HYPGEOMDIST function returns the #VALUE! error value. If the SampleS parameter is negative or SampleS is greater than the lesser of SampleSize or PopulationSize, the HYPGEOMDIST function returns the #NUM! error value.

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			If SampleS is less than the larger of 0 or (SampleSize – PopulationSize + PopulationS), the HYPGEOMDIST function returns the #NUM! error value. If the SampleSize parameter is not positive or SampleSize > PopulationSize, the HYPGEOMDIST function returns the #NUM! error value. If PopulationS is not positive or PopulationS > PopulationSize, HYPGEOMDIST returns the #NUM! error value. If the PopulationSize parameter is not positive, HYPGEOMDIST returns the #NUM! error value.
NORMDIST	Four obligatory parameters: the value for which you want the distribution, the arithmetic mean of the distribution, the standard deviation of the distribution, and the logical value that specifies the form of the function. If cumulative = **True**, NORMDIST returns the cumulative distribution function. Otherwise, NORMDIST returns the standard normal distribution, NORMSDIST.	=NORMDIST(X, Mean, StandardDev, Cumulative)	Returns the normal cumulative distribution. This function has a very wide range of applications in statistics, including hypothesis testing. If mean or standard_dev is non-numeric, NORMDIST returns the #VALUE! error value. If standard_dev is not positive, NORMDIST returns the #NUM! error value. If mean = 0, standard_dev = 1, and cumulative = **True**, NORMDIST returns the standard normal distribution, NORMSDIST.

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NORMSDIST	The value for which you want the distribution is passed as the z parameter.	=NORMSDIST(z)	Returns the inverse of the normal cumulative distribution. The distribution has a mean of 0 (zero) and a standard deviation of one. Use this function in place of standard normal curve areas. If z is non-numeric, NORMSDIST returns the #VALUE! error value.
NORMSINV	The probability value corresponding to the normal distribution is passed as the probability parameter.	=NORMSINV(probability)	Returns the inverse of the standard normal cumulative distribution. The distribution has a mean of zero and a standard deviation of one. Given a value for probability, NORMSINV seeks that value z that NORMSDIST(z) = probability. Thus, precision of NORMSINV depends on precision of NORMSDIST. NORMSINV uses an iterative search algorithm. If the search is not converged after 100 iterations, the function returns the #N/A error value. If probability is non-numeric, NORMSINV returns the #VALUE! error value. If probability is not positive or exceeds 1, NORMSINV returns the #NUM! error value.
PERCENTILE	Two obligatory parameters: the array or range of data that defines relative standing and the percentile value in the range 0..1, inclusive.	=PERCENTILE(array, k)	Returns the k-th percentile of values in a range. You can use this function to establish a threshold of acceptance. For instance, you can decide to examine candidates who score above the 90th percentile. If k is non-numeric, PERCENTILE returns the #VALUE! error value. If k < 0 or k > 1, PERCENTILE returns the #NUM! error value. If k is not a multiple of 1/(n – 1), PERCENTILE interpolates to determine the value at the k-th percentile.

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POISSON	Three obligatory parameters: the number of events, the expected numeric value, and the logical value that specifies the form of the probability distribution returned. If cumulative is **True**, POISSON returns the cumulative Poisson probability that the number of random events occurring will be between zero and x inclusive. Otherwise, the POISSON function returns Poisson mass function that the number of events occurring will be exactly x.	=POISSON(x, mean, cumulative)	Returns the Poisson distribution. A common application of the Poisson distribution is predicting the number of events over a specific time, such as the number of cars arriving at a toll plaza in 1 minute. If x is not an integer, it is truncated. If x or mean is non-numeric, POISSON returns the #VALUE! error value. If x is negative, POISSON returns the #NUM! error value. If mean is negative, POISSON returns the #NUM! error value.
QUARTILE	Two obligatory parameters: the array of cell range of numeric values for which you want the quartile value, and the quart value that indicates which value to return. quart = 0 corresponds to	=QUARTILE(array, quart)	Returns the quartile of a data set. Quartiles are often used in sales and survey data to divide populations into groups. For instance, you can use QUARTILE to find the top 25 percent of incomes in a population. If array is empty, QUARTILE returns the #NUM! error value. If quart is not an integer, it is truncated. If quart is negative or if quart > 4, QUARTILE returns the #NUM! error value.`

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	the minimum value; quart = 1 corresponds to the first quartile (25th percentile); quart = 2 corresponds to the median value (50th percentile); quart = 3 corresponds to the third quartile (75th percentile); quart = 4 corresponds to the maximum value.		MIN, MEDIAN, and MAX return the same value as QUARTILE when quart is equal to 0 (zero), 2, and 4, respectively.
RANK	Three parameters: the number whose rank you want to find, an array of, or a reference to, a list of numbers. Non-numeric values in ref are ignored. As an option, you can specify the sorting order as the order parameter.	=RANK(number, ref, [order])	Returns the rank of a number in a list of numbers. The rank of a number is its size relative to other values in a list (i.e. if you were to sort the list, the rank of the number would be its position). If order is 0 (zero) or omitted, the **ExpressSpreadSheet** ranks number as if ref were a list sorted in descending order. If order is any nonzero value, the **ExpressSpreadSheet** ranks number as if ref were a list sorted in ascending order.
STDEV	The one obligatory parameter: the first number corresponding to a sample of a population. As an option, you	=STDEV(number1, [number2], …)	Estimates standard deviation based on a sample. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). STDEV assumes that its parameters are a sample of the population. If your data represents

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	can specify from 2 to 255 parameters corresponding to a sample of a population. It is also possible to use a single array or a reference to an array instead of parameters separated by commas.		the entire population, then compute the standard deviation using STDEVP. STDEV calculates the standard deviation by using the "n – 1" method. Parameters can either be numbers or names, arrays, or references that contain numbers.
STDEVP	The one obligatory parameter: the first number corresponding to a sample of a population. As an option, you can specify from 2 to 255 parameters corresponding to a sample of a population. It is also possible to use a single array or a reference to an array instead of parameters separated by commas.	=STDEVP(number1, [number2], …)	Calculates standard deviation based on the entire population. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). STDEVP assumes that its parameters are the entire population. If your data represents a sample of the population, then compute the standard deviation using STDEV. STDEVP calculates the standard deviation by using the "n" method. Parameters can either be numbers or names, arrays, or references that contain numbers
TDIST	Three obligatory parameters: the numeric value which to evaluate the distribution, the number of degrees of freedom, and the number of	=TDIST(x, deg_freedom, tails)	Returns the Percentage Points (probability) for the Student t-distribution where a numeric value (x) is calculated value of t for which the Percentage Points are to be calculated. The t-distribution is used in the hypothesis testing of small sample data sets. Use this function in place of a table of critical values for the t-distribution.

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	distribution tails to return. If Tails = 1, TDIST returns the one-tailed distribution. If Tails = 2, TDIST returns two-tailed distribution.		If any parameter is non-numeric, TDIST returns the #VALUE! error value. If Deg_freedom < 1, TDIST returns the #NUM! error value. The Deg_freedom and Tails parameters are truncated to integers. If Tails is any value other than 1 or 2, TDIST returns the #NUM! error value. If x < 0, then TDIST returns the #NUM! error value. If Tails = 1, TDIST is calculated as TDIST = P(X > x), where X is a random variable that follows the t-distribution. If Tails = 2, TDIST is calculated as TDIST = P(|X| > x) = P(X > x or X < -x). Since the negative x values are not allowed, to use the TDIST function with x < 0, note that TDIST(-x,df,1) = 1 – TDIST(x,df,1) = P(X > -x) and TDIST(-x,df,2) = TDIST(x,df,2)=P(|X| > x).
TINV	Two obligatory parameters: the probability associated with the two-tailed Student's t-distribution and the number of degrees of freedom with which to characterize the distribution.	=TINV(probability, deg_freedom)	Returns the inverse of the Student's t-distribution. If either parameter is non-numeric, TINV returns the #VALUE! error value. If probability <= 0 or if probability > 1, TINV returns the #NUM! error value. If deg_freedom < 1, TINV returns the #NUM! error value. TINV returns that value t, such that P(|X| > 1) = probability where X is a random variable that follows the t-distribution and P(|X| > t) = P(X < -t or X > t).
VAR	The first number parameter corresponding to a sample of a	=VAR(number1, [number2],…)	Estimates variance based on a sample. VAR assumes that its parameters are a sample of the population. If

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	population is obligatory. Optionally, you can specify from to 2 to 255 number parameters corresponding to a sample of a population.		your data represents the entire population, then calculate the variance by using VARP instead. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values, and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text, or error values in the array or reference are ignored. Parameters that are error values or text cannot be translated into numbers cause errors.
VARP	The first number parameter corresponding to a sample of a population is obligatory. Optionally, you can specify from to 2 to 255 number parameters corresponding to a population.	=VARP(number1 , [number2],…)	Calculates variance based on the entire population. VARP assumes that its parameters are the entire population. If your data represents a sample of the population, then compute the variance by using VAR instead. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values, and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text, or error values in the array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors.
WEIBULL	Four obligatory parameters: the	=WEIBULL(x, alpha, beta,	Returns the Weibull distribution. Use this distribution in reliability

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	value at which to evaluate the function, the alpha and beta parameters to the distribution, and the cumulative logical value that determines the form of the function.	cumulative)	analysis, such as calculating a device's mean time to failure. If x, alpha, or beta is non-numeric, WEIBULL returns the #VALUE! error value. If x is negative, WEIBULL returns the #NUM! error value. If alpha or beta is not positive, WEIBULL returns the #NUM! error value.

Arithmetic Operators

Arithmetic operators allow you to perform basic arithmetic operations (i.e., addition, subtraction, multiplication, division, and exponential functions).

Mnemonics	Operand Type	Example	Explanation
+	Two parameters	= 5 + B1	Adds the first parameter's value to the value of the second parameter.
-	Two parameters	= A5 - B1	Subtracts the second parameter's value from the value of the first one.
*	Two parameters	= A2 * A3	Multiplies the first parameter's value by the value of the second parameter.
/	Two parameters	= A1 / PI()	Divides the first parameter's value by the value of the second parameter.
^	Two parameters	= B1 ^ A2	Raises the first parameter's value to the power determined by the second parameter's value.

Logical operators

Logical operators perform comparison operations and return a logical value (TRUE or FALSE) as a result.

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Mnemonics	Operand Type	Example	Explanation
=	Two parameters of logical types	= A1=A2	The equality operation. Returns TRUE if the first parameter is equal to the second one. Otherwise, returns FALSE.
<>	Two parameters of logical types	= A1<>B2	The inequality operation. Returns TRUE if values within the list are not equal. Otherwise, returns FALSE.
<	Two parameters of logical types	= A1<B1	The "Less than" operation. Returns TRUE if the first parameter is less than the second one. Otherwise, returns FALSE.
>	Two parameters of logical types	= A1>100	The "Greater than" operation. Returns TRUE if the first parameter is greater than the second one. Otherwise, returns FALSE.
<=	Two parameters of logical types	= A2<=C4	The "Less than or equal to" operation. Returns TRUE if the first parameter is less than or equal to the second one. Otherwise, returns FALSE.
>=	Two parameters of logical types	= A2>=1.57	The "Greater than or equal to" operation. Returns TRUE if the first parameter is greater than or equal to the second one. Otherwise, returns FALSE.

Logical functions

Logical functions include Boolean operators, conditional tests and functions.

Mnemonics	Operand Type	Example	Explanation
AND	A list of logical parameters	=AND(1<B4, B4<100)	Logical **AND** operation.

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			Returns TRUE if all values within the list are TRUE; returns FALSE if one or more values within the list evaluates to FALSE. One common use for the **AND** function is to expand the usefulness of other functions that perform logical tests. For example, the IF function performs a logical test and then returns one value if the test evaluates to TRUE and another value if the test evaluates to FALSE.
FALSE	No parameters required	=FALSE()	Returns the logical value FALSE. You can also type the word FALSE directly into a worksheet cell or as a part of a formula expression.
IF	Three parameters: the logical expression, the value to return if the expression succeeds, the value to return if the expression fails.	=IF(A10<=100, "Within budget", "Over budget")	Returns the second parameter if the logical expression evaluates to TRUE and the third parameter otherwise.
IFERROR	Two parameters: the parameter that is checked for an error, and the value displayed in an error occurs.	=IFERROR(value, value_if_error)	Returns the value you specify if a formula evaluates to an error. Otherwise, returns the formula calculation result. The IFERROR function is particularly useful for spreadsheet error handling. The formula evaluates the following error types: #N/A, #VALUE, #REF!, #DIV/0!, #NUM!, #NAME?, and #NULL!.
IFNA	Two parameters: the parameter that is checked for the #N/A error value, and the value returned if the error occurs.	=IFNA(value, value_if_na)	Returns the value you specify if the formula returns the #N/A error value. Otherwise, returns the result of the formula.
NOT	One parameter of logical type	=NOT(1>D4)	Logical **NOT** operation. Reverses the value of its parameter.

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OR	A list of logical parameters	=OR(A1>=10, A1<=−10)	Logical **OR** operation. Returns TRUE if any parameter is TRUE; returns FALSE if all values within the list evaluate to FALSE.
TRUE	No parameters required.	=TRUE()	Returns the logical value TRUE.
XOR	The first parameter is mandatory, subsequent logical values are optional. If the specified range contains no logical values, the **XOR** function returns the #VALUE! error code.	=XOR(logical1, logical2, …)	Returns a logical exclusive OR of all specified parameters. The XOR function allows you to test from 1 to 254 conditions. The result of XOR is TRUE when the number of TRUE inputs is odd and FALSE when the number of TRUE inputs is even.

Lookup and Reference Functions

A set of reference and lookup functions.

Mnemonics	Operand Type	Example	Explanation
ADDRESS	Three parameters: the numeric value that specifies the row number to use in the cell reference, the numeric value that specifies the column number to use in the cell reference. Optionally, you can add a numeric value that specifies the type of reference to return.	=ADDRESS(row_num, column_num, [abs_num], [a1], [sheet_text])	Returns a reference as text to a single cell in a worksheet. You can use the **ADDRESS** function to obtain the address of a cell in a worksheet, given specified row and column numbers. For instance, ADDRESS(2, 3) returns $C$2. As another example, ADDRESS(77, 300) returns $KN$77. You can use other functions, such as the ROW and COLUMN functions, to provide the row and column number parameters to the **ADDRESS** function.

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AREAS	The reference parameter is mandatory. It is a reference to a cell or cell range and can refer to multiple areas. If you want to specify several references as a single parameter, include extra sets of parentheses so the **ExpressSpreadSheet** formula engine will not interpret the comma as a field separator.	=AREAS(reference)	Returns the number of areas in a reference. An area is a range of continuous cells or a single cell. For instance, the AREAS(B2:D4) expression returns **1**. As another example, AREAS((B2:D4,E5,F6:I9)) returns **3**.
CHOOSE	Two mandatory parameters. The index_num parameter specifies which value parameter is selected. The index_num value must be within the range of 1 to 254, or a formula expression or cell reference returning a value within the same range. The value1 parameter is also mandatory while subsequent parameters are optional. You can specify up to 254 value parameters from which the CHOOSE function	=CHOOSE(index_num, value1, [value2], …)	Returns a value from the list of value parameters. You can use the CHOOSE function to select one of the specified values based on the index number. If index_num is 1, CHOOSE returns value1. If the index value is 2, the CHOOSE function returns value 2, and so on. If the index_num value is less than 1 or greater than the number of the last provided value, the CHOOSE function returns the #VALUE! error value. If the index_num parameter value is a fraction, it is truncated to the lowest integer before evaluation. The value parameters can be cell range references as well as single values.

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	selects a value or an action to perform based on the index_num parameter value. The actual parameters can be numbers, cell references, defined names, formulas, functions, or text.		
COLUMN	The reference parameter is optional. If it is omitted or refers to a range of cells, and if the COLUMN function is entered as a horizontal array formula, the COLUMN function returns the column numbers of reference as a horizontal array.	=COLUMN([reference])	Returns the column number of a reference. For instance, the formula =COLUMN(D10) returns 4 since column D is the fourth column. If the reference parameter is a range of cells, and if the COLUMN function is not entered as a horizontal array formula, the COLUMN function returns the number of the leftmost column. If the reference parameter is omitted, it is assumed to be the reference of the cell in which the COLUMN function appears. The reference parameter cannot refer to multiple areas.
COLUMNS	The array parameter is mandatory. It specifies an array or array formula, or a reference to a range of cells for which you want the number of columns.	=COLUMNS(array)	Returns the number of columns in a reference.
FORMULATEXT	The reference parameter is mandatory; it specifies a reference to a cell or range of cells.	=FORMULATEXT(reference)	Returns the formula at the given reference as text. The reference parameter can be to another worksheet or workbook. If the reference parameter is to another workbook that is not open,

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			FORMULATEXT returns the #N/A error value. If the reference parameter is to an entire row or column, or to a range or defined name containing more than one cell, FORMULATEXT returns the value in the upper leftmost cell or the row, column, or range. In the following cases, FORMULATEXT returns the #N/A error value: The cell used as the reference parameter does not contain a formula; The formula in the cell is longer than 8192 characters; The formula cannot be displayed in the worksheet; for example, due to worksheet protection; An external workbook that contains the formula is not currently open. Invalid data types used as inputs will produce a #VALUE error value.
HLOOKUP	Three mandatory parameters: the value to be found in the first row of the table, the table of information in which data is looked up, and the row number in table_array which the matching value will be returned. You can optionally add a logical value that specifies whether you want HLOOKUP to find an exact match or an approximate match.	=HLOOKUP(lookup_value, table_array, row_index_num, [range_lookup])	Looks in the top row of an array and returns the value of the initiated cell. Use HLOOKUP when your comparison values are located in a row across the top of a table of data, and you want to look down a specified number of rows. Use VLOOKUP when your comparison values are located in a column to the left of the data you want to find. If HLOOKUP can't find lookup_value, the range_lookup is TRUE, it uses the largest value that is less than lookup_value. If lookup_value is smaller than the smallest value in the first row of table_array, HLOOKUP returns the #N/A error value. If range_lookup is FALSE and lookup_value is text, you can use the wildcard characters, question mark (?) and asterisk (*), in

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			lookup_value. A question mark matches any single character; an asterisk matches any sequence of characters. If you want to find an actual question mark or asterisk, type a tilde (~) before the character.
INDEX	**Array form:** Two mandatory parameters. The array parameter specifies a cell range or an array constant. If the specified array contains only one row or column, the corresponding row_num or column_num parameter is optional. If the array has more than one row and more than one column, and only the row_num or column_num parameter is provided, the INDEX function returns an array of the entire row or column in array. The mandatory row_num parameter specifies the row in the array from which the INDEX function should return a value. If the row_num parameter is omitted, the	**Array form:** =INDEX(array, row_num, [column_num]) **Reference form:** =INDEX(reference, row_num, [column_num], [area_num])	Returns a value or the reference to a value from within a table or cell range. There are two forms of the INDEX function: the array form and the reference form. **Array form:** Returns the value of an element in a table or an array, selected by the row and column number indexes. Use the array from if the first parameter of the INDEX function is an array constant. If both the row_num and column_num parameters are used, the INDEX function returns the value in the cell at the intersection of row_num and column_num. If you set row_num or column_num to 0 (zero), the INDEX function returns the array of values for the entire column or row, respectively. To use values returned as an array, enter the INDEX function as an array formula in a horizontal range of cells for a row, and in a vertical range of cells for a column. In order to enter the INDEX function as an array formula, press the **Ctrl+Shift+Enter** key combination. The row_num and column_num parameter values must point to a cell within the array. Otherwise, the INDEX function returns the #REF! error value. **Reference form:**

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column_num parameter is required instead.

The optional column_num parameter specifies the column in the array from which the INDEX function should return a value. If the column_num parameter is omitted, the row_num parameter is required instead.

Reference form:

Two mandatory parameters.

The reference parameter specifies a reference to one or more cell ranges. If you are entering a nonadjacent cell range for the reference, enclose the reference in parentheses. If each area in the reference parameter contains only one row or column, the row_num or column_num parameter becomes optional, respectively.

The mandatory row_num

Returns the reference of the cell at the intersection of a particular row and column. If the reference consists of nonadjacent cell ranges, you can pick a particular range.

After the reference and area_num values have been used to select a particular range, the row_num and column_num values are used to select a particular cell. The row_num value set to 1 corresponds to the first row in the range, the column_num value set to 1 corresponds to the first column, and so on. The reference returned by the INDEX function is the intersection of the row and column whose numbers are specified as the row_num and column_num parameters.

If you set the row_num or column_num parameter to 0 (zero), the INDEX function returns the reference for the entire column or row, respectively.

The row_num, column_num, and area_num parameter values must point to a cell within the reference. Otherwise, the INDEX function returns the #REF! error value. If the row_num and column_num parameters are omitted, the INDEX function returns the area in the reference specified by the area_num parameter.

The result of the INDEX function is a reference and can be used as a reference in other formula expressions.

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			parameter specifies the number of the row in the reference from which the INDEX function returns a reference. The optional column_num parameter specifies the number of the column in the reference from which the INDEX function returns a reference. The optional area_num parameter specifies a range in the reference from which the INDEX function returns the intersection of row_num and column_num. The first selected area is numbered 1, the second is 2, and so on. If the area_num parameter is omitted, the INDEX function uses 1 as the area value.
INDIRECT	One mandatory parameter: the reference to a cell that contains an A1-style reference, an R1C1-style reference, a name	=INDIRECT(ref_text, a1)	Returns the reference specified by a text string. References are immediately evaluated to display their contents. Use the INDIRECT function when you need to change the reference to a cell within a formula without changing the formula itself.

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	defined as a reference, or a reference to a cell as a text string. If the a1 parameter is set to TRUE or omitted, the ref_text value is interpreted as an A1-style reference. If the a1 parameter is set to FALSE, the ref_text value is interpreted as an R1C1-style reference.		If the ref_text parameter contains no valid cell reference, the INDIRECT function returns the #REF! error value.
LOOKUP	**Vector form:** Two obligatory parameters: the value that LOOKUP searches for in the first vector, and the range that contains only one row or one column. Additionally, you can specify a range that contains only one row or column (result_vector). **Array form:** Two obligatory parameters: the value that LOOKUP searches for in the array and the range of cells that contains text, numbers, or	**Vector form:** =LOOKUP(lookup_value, lookup_vector, [result_vector]) **Array form:** =LOOKUP(lookup_value, array)	Looks up values in a vector or array. The LOOKUP function returns a value either form a one-row or one-column range or form an array. The LOOKUP function has two syntax forms: the vector form and the array form. **Vector form:** A vector is a range of only one row or one column. LOOKUP looks in a one-row or one-column range (known as a vector) for a value and returns a value from the same position in a second one-row or one-column range. Use the LOOKUP function when you want to specify the range that contains the values that you want to match. If lookup_value is smaller than the smallest value in lookup_vector, LOOKUP returns the #N/A error value. Array form: The array form of LOOKUP looks in the first row or column of an array for the specified value and returns a

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	logical values that you want to compare with lookup_value.		value from the same position in the last row or column of the array. Use this form of LOOKUP when the values that you want to match are in the first row or column of the array. Use the other form of LOOKUP when you want to specify the location of the column or row. Press the **Ctrl+Shift+Enter** key combination to execute the formula. In general, it is best to use the HLOOKUP or VLOOKUP function instead of the array form of LOOKUP. This form of LOOKUP is provided for compatibility with other spreadsheet programs.
MATCH	Two obligatory parameters: the value that you want to match in lookup_array, and the range of cells being searched. Additionally, you can specify the match_type which can be the number –1, 0, or 1. The match_type specifies how the **ExpressSpreadSheet** matches lookup_value with values in lookup_array. The default value for this parameter is 1. If match_type is 1 or omitted, MATCH finds the largest value that is less than or equal to lookup_value.	=MATCH(lookup_value, lookup_array, [match_type])	Looks up values in a reference or array. The MATCH function searches for a specified item in a range of cells, and then returns the relative position of that item in the range. Use MATCH instead of one of the LOOKUP functions when you need the position of an item in a range instead of the item itself. MATCH returns the position of the matched value within lookup_array, not the value itself. For instance, MATCH("b",{"a","b","c"},0) returns 2 which is the relative position of "b" within the array {"a","b","c"}. MATCH does not distinguish between uppercase and lowercase letters when matching text values. If MATCH is unsuccessful in finding a match, it returns the #N/A error value. If match_type is 0, and lookup_value is a text string, you can use the wildcard characters – the question mark (?) and asterisk (*) – in the lookup_value parameter. A question mark matches any single character; an asterisk matches any sequence of

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	If match_type is 0, MATCH finds the first value that is exactly equal to lookup_value. If match_type is –1, MATCH finds the smallest value that is greater than or equal to lookup_value.		characters. If you want to find an actual question mark or asterisk, type a tilde (~) before the character.
OFFSET	Accepts three obligatory parameters: the reference from which you want to base the offset, the number of rows, up or down that you want the upper-left cell to refer to, and the number of columns to the left or right that you want the upper-left cell of the result to refer to. Additionally, you can specify the height and width of the returned reference. Both the height and width must be positive.	=OFFSET(reference, rows, cols, [height], [width])	Returns a reference to a range that is the specified number of rows and columns from a cell or cell range. The returned reference can be a single cell or cell range. You can specify the number of returned rows and/or columns. If the offset reference is outside the worksheet's boundaries, the OFFSET function returns the #REF! error value. If the height or width parameter is omitted, the returned cell range has the same height or width as the specified reference. The OFFSET function does not actually move any cell or change the selection while returning a reference. You can use OFFSET with any function expecting a reference parameter. For example, the formula expression SUM(OFFSET(C2,1,2,3,1)) calculates the total value of a range that is three rows in height and one column width that is 1 row below and 2 columns to the right of the C2 cell.
ROW	The reference parameter is optional. If reference is omitted, it is assumed to be the	=ROW([reference])	Returns the row number of a reference.

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	reference of the cell in which the ROW function appears. If reference is a range of cells, and if ROW is entered as a vertical array, ROW returns the row numbers of reference as a vertical array. Reference cannot refer to multiple areas.		
ROWS	The array parameter is mandatory. An array, an array formula, or a reference to a range of cells for which you want the number of rows.	=ROWS(array)	Returns the number of rows in a reference or array.
TRANSPOSE	One obligatory parameter: an array or range of cells within a worksheet that you want to transpose. The transposition of an array is created by using the first column of the new array, the second row of the array as the second column of the new array, and so on.	=TRANSPOSE(array)	Returns a vertical range of cells as a horizontal range, and vice versa. You must enter the TRANSPOSE function as an array formula in a range that has the same number of rows and columns, respectively, as the source range has columns and rows. Use TRANSPOSE to swap the vertical and horizontal orientation of an array or range within a worksheet. The steps are as follows: 1. Highlight the new area for the transposed cells. 2. Select TRANSPOSE and highlight the source cells so they appear within the parentheses of the formula.

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3. Press **Ctrl+Shift+Enter** key combination to execute the formula; the new area will now contain the transposed data.

VLOOKUP	Three obligatory parameters: the value to search in the first column of the table or range, the range of cells that contains the data, and the column number in the table_array parameter from which the matching value must be returned. Additionally, you can specify the range_lookup that switches the VLOOKUP finding between an exact match and an approximate match.	=VLOOKUP(lookup_value, table_array, col_index_num, [range_lookup])	Looks in the first column of an array and moves across the row to return the value of a cell. You can use the VLOOKUP function to search the first column of a range of cells, and then return a value from any cell on the same row of the range. The V in VLOOKUP stands for vertical. Use VLOOKUP instead of HLOOKUP when your comparison values are located in columns to the left of the data that you want to find. When searching number or date values, ensure that the data in the first column of table_array is not stored as text values. In this case, VLOOKUP might return an incorrect or unexpected value. If range_lookup is FALSE and lookup_value is text, you can use the wildcard characters – the question mark (?) and asterisk (*) – in lookup_value. A question mark matches any single character; an asterisk matches any sequence of characters. If you want to find an actual question mark or asterisk, type a tilde (~) preceding the character.

Arithmetic functions

A set of mathematic and trigonometric functions, including basic arithmetic, conditional sums and products, and trigonometric ratios. Mathematicians may also find the Statistical Functions 478 useful.

Mnemonics	Operand Type	Example	Explanation

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ABS	One parameter of double type.	=ABS(B6)	Returns the absolute value.
ACOS	One parameter of double type.	=ACOS(B16)	Returns the arccosine.
ACOSH	One parameter of double type.	=ACOSH(A1)	Returns the inverse hyperbolic cosine.
ACOT	One parameter of double type.	=ACOT(A1)	Returns the principal value of the arccotangent, or inverse cotangent, of a number. The returned angle is given in radians in the range 0 (zero) to pi.
ACOTH	One parameter of double type. The absolute value of the parameter must be greater than 1.	=ACOTH(A1)	Returns the inverse hyperbolic cotangent of a number. If the absolute value of the parameter is less than 1, the ACOT function returns the #VALUE! error value.
ASIN	One parameter of double type.	=ASIN(B6)	Returns the arcsine.
ASINH	One parameter of double type.	=ASINH(C2)	Returns the inverse hyperbolic sine of the parameter.
ATAN	One parameter of double type.	=ATAN(C5)	Returns the arctangent.
ATAN2	Two parameters of double type.	=ATAN2(A1, A2)	Returns the arctangent using x- and y- coordinates.
ATANH	One parameter of double type.	=ATANH(D4)	Returns the inverse hyperbolic tangent.
BASE	Two parameters of integer type: the first is greater than 0 and less than 2^53; the second (radix) must be greater than or equal to 2 and less than or equal to 36. Third parameter is optional, which	=BASE(A1, Radix[Min_length])	Converts a number into a text representation with the given radix (base). If A1, Radix, or Min_length are outside the minimum or maximum constraints, BASE returns the #NUM! error value. If A1 is a non-numeric value, BASE returns the #VALUE! error value. Any non-integer number used as an parameter is truncated to an integer.

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	must be greater or equal to 0.		If the Min_length parameter is included, leading zeros are added to the result if the result would otherwise be shorter than the minimum length specified. For example, BASE(16,2) returns 10000 but BASE(16,2,8) returns 00010000.
CEILING	Two parameter of double type	=CEILING(2.5, 1)	Rounds the first parameter up to the nearest multiple based on the significance specified by the second parameter.
CEILING.MATH	Three parameters. The first parameter must be less than 9.99E+307 and greater than −2.229E-308; the second parameter is the multiple to which the number is rounded; the optional Mode parameter.	=CEILING.MATH(A1, [significance], [mode])	Rounds a number up to the nearest integer or to the nearest multitude of significance. By default, significance is +1 for positive numbers and −1 for negative numbers. By default, both positive and negative numbers with decimal portions are rounded up to the nearest integer. If you specify the Significance and Mode parameters, you can change the direction of rounding for negative numbers. For example, rounding −6.3 to a significance of 1 with a mode of 1 rounds away from 0, to −7. There are many combinations of Significance and Mode values that affect rounding of negative numbers in different ways. The Mode parameter does not affect positive numbers.
CEILING.PRECISE	Two parameters. The first parameter is a number of double type. The Significance parameter is optional.	=CEILING.PRECISE(A1, [significance])	Returns a number that is rounded up to the nearest integer or to the nearest multiple of significance. Regardless of the sign of the number, the number is rounded up. However, if the number or the significance is zero, **CEILING.PRECISE** returns zero. If the Significance parameter is omitted, its default value is 1.

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CONCATENATE	At least one text item is mandatory; you can add more text items, up to a maximum of 255 items. Commas must separate the specified items.	=CONCATENATE(text1, [text2], …)	Joins several text items into a single text item. The **CONCATENATE** function joins up to 255 text strings into one text string. The joined items can be text, numbers, cell references, or a combination of those items. Note that you can also use the ampersand (&) calculation operator instead of the **CONCATENATE** function to join text items. For instance, =A1 & B1 returns the same value as =CONCATENATE(A1, B1).
COS	One parameter of double type	=COS(A1)	Returns the cosine.
COSH	One parameter of double type	=COSH(D7)	Returns the hyperbolic cosine.
COMBIN	Two parameters of integer type. Non-integer numeric parameters are truncated to integers.	=COMBIN(number, number_chosen)	Returns the number of combinations for a given number of items. Use **COMBIN** to determine the total possible number of groups for a given number of items. If either parameter is non-numeric, COMBIN returns the #VALUE! error value. If either parameter is negative, COMBIN returns the #NUM! error value.
COMBINA	Two parameters of integer type. Non-integer numeric parameters are truncated to integers.	=COMBINA(number, number_chosen)	Returns the number of combinations (with repetitions) for a given number of items. If the value of either parameter is outside of its constraints, COMBINA returns the #NUM! error. If either parameter is a non-numeric value, COMBINA returns the #VALUE! error value.
COT	One parameter of double type, which should be more than 0 and less than 2^27.	=COT(number)	Returns the cotangent of an angle specified in radians. If the specified parameter is outside its constraints, the COT function returns the #NUM! error value.

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			If the specified parameter is not a numeric value, the COT function returns the #VALUE! error value. The COT(0) expression returns the #DIV/0! error value.
COTH	One parameter of double type, which should be less than 2^27.	=COTH(number)	Returns the hyperbolic cotangent of a hyperbolic angle. If the specified parameter is outside its constraints, the **COTH** function returns the #NUM! error value. If the specified parameter is a non-numeric value, the COTH function returns the #VALUE! error value.
CSC	One parameter of double type, which should be less than 2^27.	=CSC(number)	Returns the cosecant of an angle specified in radians. If the specified parameter is outside its constraints, the CSC function returns the #NUM! If the specified parameter is a non-numeric value, the CSC function returns the #VALUE! error value. CSC(n) equals 1/SIN(n).
CSCH	One parameter of double type, which should be less than 2^27.	=CSCH(number)	Returns the hyperbolic cosecant of an angle specified in radians. If the specified parameter is outside its constraints, the CSCH function returns the #NUM! error value. If the specified parameter is a non-numeric value, the CSCH function returns the #VALUE! error value.
DECIMAL	One parameter is a text string that must be less or equal to 255 characters, other parameter is an integer that must be greater or equal to 2 and less than or equal to 36.	=DECIMAL(text, radix)	Converts a text representation of a number in a given base into a decimal number. A radix greater than 10 uses the numeric values 0 – 9 and the letters A-Z as needed. For example, base 16 (hexadecimal) uses 0 – 9 and A – F, and base 36 uses 0 – 9 and A – Z. If either parameter is outside its constraints, DECIMAL may return the #NUM! or #VALUE! error value.
DEGREES	One parameter of double type	=DEGREES(PI()/2)	Converts radians to degrees.

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	that specifies angle in radians		
EVEN	One parameter of double type	=EVEN(-1)	Rounds the parameter up to the nearest even integer.
EXP	One parameter of double type	=EXP(C1)	Returns the exponent value of the parameter.
FACT	One non-negative parameter. If a number is not an integer, it is truncated.	=FACT(2)	Returns the factorial of the parameter.
FACTDOUBLE	One non-negative parameter. If a number is not an integer, it is truncated.	=FACTDOUBLE(number)	Returns the double factorial of a number. If the number is non-numeric, the FACTDOUBLE function returns the #VALUE! error value. If the number is negative, the FACTDOUBLE function returns the #NUM! error value.
FLOOR	Two parameters of double type	=FLOOR(-2.5, -2)	Rounds the first parameter down, towards zero, to the nearest multiple of the significance specified by the second parameter.
FLOOR.MATH	Three parameters: first is the number to be rounded down, second is the multiple to which you want to round, and third is the direction (either towards or away from 0) to round negative numbers.	=FLOOR.MATH(number, significance, mode)	By default, both positive and negative numbers are rounded down to the nearest integer. By using 0 or a negative number as the Mode parameter, you can change the direction of the rounding for negative numbers. The Significance parameter rounds the number down to the nearest integer that is a multiple of the significance specified. The exception is where the number to be rounded is an integer. For example, for a Significance of 3, the number is rounded down to the next integer that is a multiple of 3. If Number is divided by a Significance of 2 or greater results in a remainder, the result is rounded.

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FLOOR.PRECISE	Two parameters. First is the number to be rounded down, second is the multiple to which the number is to be rounded. The Significance parameter is optional.	=FLOOR.PRECISE(number, [significance])	Returns a number that is rounded down to the nearest integer or to the nearest multiple of significance. Regardless of the sign of the number, the number is rounded down. However, if the number of the significance is zero, zero is returned. If the significance is omitted, its default value is 1. The absolute value of the multiple is used, so that the **FLOOR.PRECISE** function returns the mathematical floor irrespective of the signs of the number and significance.
INT	Parameter of type double	=INT(E4)	Rounds the parameter down to the nearest integer.
ISO.CEILING	Two parameters. First is the value to be rounded; second is the optional multiple to which the number is to be rounded.	=ISO.CEILING(number, [significance])	Returns a number that is rounded up to the nearest integer or to the nearest multiple of significance. Regardless of the sign of the number, the number is rounded up. However, if the number or the significance is zero, zero is returned. If the significance is omitted, its default value is 1.
LN	One parameter of double type	=LN(C1)	Returns the natural logarithm.
LOG	Two parameters: the number and the base	=LOG(100, 10)	Returns the logarithm of a number to the specified base.
LOG10	One parameter of double type	=LOG10(1000)	Returns the base-10 logarithm of the parameter.
MMULT	Two obligatory parameters: the arrays you want to multiply. The number of columns in array1 must be the same as the number of rows	=MMULT(array1, array2)	Returns the matrix product of two arrays. The result is an array with the same number of rows as array1 and the same number of columns as array2. You must enter formulas that return arrays only as array formulas. The MMULT function returns #VALUE! error when any of the

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	in array2, and both arrays must contain only numeric values. Both arrays can be specified as cell ranges, array constants, or references.		specified cells are empty or contain non-numeric values. If the number of columns in array1 is different from the number of rows in array2, MMULT also returns the #VALUE! error.
MOD	Two parameters: a number and divisor	=MOD(4,3)	Returns the remainder after the number is divided by the divisor.
MROUND	Two parameters: the value to be rounded, and the multiple to which you want to round the value.	=MROUND(number, multiple)	Returns a number rounded to the desired multiple. **MROUND** rounds up, away from zero, if the remainder of dividing the number by the multiple is greater than or equal to half the value of the multiple.
ODD	One parameter of double type	=ODD(3.5)	Rounds the parameter up to the nearest odd integer.
PI	No parameters required	=PI()	Returns the value of Pi.
POWER	Two parameters of double type: the number and the power	=POWER(A1, 4)	Raises the number to the base.
PRODUCT	The first parameter is the number or range that you want to multiply. Additional optional parameters that specify additional numbers or ranges that you want to multiply, up to a maximum of 255 parameters.	=PRODUCT(number1, [number2], …)	The **PRODUCT** multiplies all the numbers given as parameters and returns the product. For example, if cells A1 and A2 contain numbers, you can use the formula =PRODUCT(A1, A2) to multiply those two numbers together. You can also perform the same operation by using the multiply (*) mathematical operator; for example, =A1 * A2. The **PRODUCT** function is useful when you need to multiply multiple cells or cell ranges together. For example, the formula

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			=PRODUCT(A1:A3, C1:C3) is equivalent to = A1 * A2 * A3 * C1 * C2 * C3.
QUOTIENT	Two parameters: the dividend and the divisor.	=QUOTIENT(numerator, denominator)	Returns the integer portion of a division. Use this function when you want to discard the remainder of a division.
RADIANS	One parameter of type double that specifies angle in degrees.	=RADIANS(180)	Converts degrees to radians.
RAND	No parameters required	=RAND()	Returns a random number between 0 and 1.
RANDBETWEEN	Two parameters of integer type: bottom that specifies the smallest returned value, and top that specifies the largest returned value.	=RANDBETWEEN(bottom, top)	Returns a random integer between the numbers you specify. A new random integer is returned every time the worksheet is calculated.
ROUND	Two parameters: the number of double type and the number of digits	=ROUND(20.57, 1)	Rounds the first parameter to the specified number of digits.
ROUNDDOWN	Two parameters of double type	=ROUNDDOWN(2.75,0)	Rounds the parameter toward zero. The first parameter specifies a number to round up. The second parameter defines the number of digits to which you want to round the first parameter.
ROUNDUP	Two parameters of double type	=ROUNDUP(-3.42,1)	Rounds the parameter toward infinity. The first parameter specifies a number to round up. The second parameter defines the number of digits to which you want to round the first parameter.
SEC	One parameter of double type.	=SEC(number)	Returns the secant of an angle.

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	Number is the angle in radians for which you want to calculate the secant.		The absolute value of the parameter must be less than 2^27. If the parameter is outside of its constraints, SEC returns the #NUM! error value. If the parameter is a non-numeric value, SEC returns the #VALUE! error value.
SECH	One parameter of double type. Number is the angle in radians for which you want to calculate the hyperbolic secant.	=SECH(number)	Returns the hyperbolic secant of an angle. The absolute value of the parameter must be less than 2^27. If the parameter is outside of its constraints, SECH returns the #NUM! error value. If the parameter is a non-numeric value, SECH returns the #VALUE! error value.
SIGN	One parameter of double type	=SIGN(A2)	Returns the sign.
SIN	One parameter of double type	=SIN(A1)	Returns the sine.
SINH	One parameter of double type	=SINH(1)	Returns the hyperbolic sine.
SQRT	One parameter of double type	=SQRT(B5)	Returns the square root.
SQRTPI	One parameter of double type.	=SQRTPI(number)	Returns the square root of (number * pi). If the parameter is negative, SQRTPI returns the #NUM! error value.
SUBTOTAL	The first parameter is a number from 1 to 11 (includes hidden values) or from 101 to 111 (ignores hidden values) that specifies which function to use in calculating	=SUBTOTAL(function_num, ref1, [ref2], …)	Returns a subtotal in a list or database. If there are other subtotals within ref1, ref2, … (or nested subtotals), these nested subtotals are ignored to avoid double counting. The SUBTOTAL function ignores any rows that are not included in the result of a filter, no matter which function_num value you use. The SUBTOTAL function is designed for columns of data, or vertical

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	subtotals within a list. The second parameter is the first named range or reference for which you want the subtotal. Subsequent parameters are optional and specify named ranges or references 2 to 254 for which you want the subtotal.		ranges. It is not designed for rows of data, or horizontal ranges. For example, when you subtotal a horizontal range using a function_num of 101 or greater, such as SUBTOTAL(109,B2:G2), hiding a column does not affect the subtotal. However, hiding a row in a subtotal of a vertical range does affect the subtotal. If any of the references are 3-D references, SUBTOTAL returns the #VALUE! error value.
SUM	A list of parameters	=SUM(A1:C12) =SUM(A1, 3.14, 1.57)	Sums all the values in the list.
SUMIF	Three parameters. The range of cells that you want to evaluate by criteria. Blank and text values are ignored. The criteria in the form of a number, expression, a cell reference, text, or a function that defines which cells will be added. The optional sum_range parameter that allows you to add more cells to those	=SUMIF(range, criteria, [sum_range])	The **SUMIF** function is designed to sum the values in a range that meet a criteria you specify. For example, suppose that in a column that contains numbers, you want to sum only the values that are larger than 5. You can use the following formula: =SUMIF(B2:B25, ">5") In this example, the criteria is applied the same values that are being summed. If you want, you can apply the criteria to one range and sum the corresponding values in a different range. For example, the formula =SUMIF(B2:B5, "John", C2:C5) sums only the values in the range C2:C5, where the corresponding cells in the range B2:B5 equal "John". Any text criteria or any criteria that includes logical or mathematical symbols must be enclosed in double

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	specified ion the range parameter.		quotation marks ("). If the criteria is numeric, double quotation marks are not necessary. You can use both the question mark (?) and asterisk (*) – as the criteria parameter. A question mark matches any single character; an asterisk matches any sequence of characters. If you need to find an actual question mark or asterisk, type a tilde (~) preceding the character.
SUMSQ	A list of parameters	=SUMSQ(B1:D1) =SUM(1, 2, 3, 4)	Sums the square of values in the list
TAN	One parameter of double type	=TAN(C3)	Returns the tangent.
TANH	One parameter of double type	=TANH(0)	Returns the hyperbolic tangent.
TRUNC	One parameter of double type	=TRUNC(PI())	Returns the integer part. Compare this with the INT function, which returns a double

Statistical functions

The **ExpressSpreadSheet** control provides a large selection of statistical functions, performing most of the common statistical calculations, from basic mean, median and mode calculations to more complex statistical distribution and probability tests.

Mnemonics	Operand Type	Example	Explanation
AVEDEV	Number1 is required, subsequent parameters are optional. You can specify from 1 to 255 numbers for	=AVEDEV(number1, [number2], …)	Returns the average of the absolute deviations of data points from their mean. AVEDEV is a measure of the variability in a data set. AVEDEV is influenced by the unit of measurement in the input data.

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	which you want the average of the absolute deviations. Alternatively, you can use a single array or a reference to an array instead of parameters separated by commas.		Parameters must either be numbers or be names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included.
AVERAGE	A list of parameters	=AVERAGE(A1:A5) =AVERAGE(10.3, 9.1)	Calculates the average value of values within the list.
AVERAGEA	A list of parameters	=AVERAGEA(A1:A5)	Calculates the average value of the non-empty cells referenced. AVERAGEA(range) equals SUM(range)/COUNTA(range).
AVERAGEIF	Two mandatory parameters: the range which specifies one or more cells to average, including numbers or names, arrays or references that contain numbers; the criteria in the form of a number, expression, cell reference, or text that defines which cells are averaged. Additionally, you can specify the actual set	=AVERAGEIF(range, criteria, [average_range])	Returns the average (arithmetic mean) of all the cells in a range that meet a given criteria. Cells that contain TRUE or FALSE are ignored. If a cell in average_range is an empty cell, AVERAGEIF ignores it. If range is a blank or text value, AVERAGEIF returns the #DIV/0! error value. If a cell in criteria is empty, AVERAGEIF treats it as a 0 value. If no cells in the range meet the criteria, AVERAGEIF returns the #DIV/0! error value. You can use the wildcard characters, question mark (?) and asterisk (*), in criteria. A question mark matches any single character; an asterisk matches any sequence of characters. If you want to find an actual question mark or asterisk,

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	of cells to average. If omitted, the range parameter is used.		type a tilde (~) before the character. The average_range parameter does not have to be the same size and shape as range. The actual cells that are averaged are determined by using the top, left cell in average_range as the beginning cell, and then including cells that correspond in size and shape to range.
BETA.DIST	Six parameters, the first four of which are mandatory: X is the value between A and B at which the function should be evaluated; Alpha and Beta are parameters of the distribution; Cumulative is a logical value that determines the form of the function; A and B are lower and higher bounds to the interval of X, respectively.	=BETA.DIST(x, alpha, beta, cumulative, [A], [B])	Returns the beta distribution. The beta distribution is commonly used to study variation in the percentage of something across samples, such as fraction of the day people spend watching television. If any parameter is non-numeric, BETA.DIST returns the #VALUE! error value. If either alpha or beta is not positive, BETA.DIST returns the #NUM! error value. If x < A, x > B, or A = B, BETA.DIST returns the #NUM! error value. If you omit values for A and B, BETA.DIST uses the standard cumulative beta distribution, so that A = 0 and B = 1. If cumulative is TRUE, BETA.DIST returns the cumulative distribution function; if FALSE, it returns the probability density function. If you omit values for A and B, BETA.DIST uses the standard cumulative beta distribution, so that A = 0, and B = 1.
BETA.INV	Five parameters, the first three of which are mandatory: Probability is the probability associated with	=BETA.INV(probability, alpha, beta, [A], [B])	Returns the inverse of the beta cumulative probability density function (BETA.DIST). If probability = BETA.DIST(x, … TRUE), then BETA.INV(probability, …) = x. The beta distribution can be used in project planning to model probable completion times given an

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	the beta distribution; Alpha and Beta are parameters of the distribution; A and B are lower and higher bounds to the interval of X, respectively.		expected completion time and variability. If any parameter is non-numeric, BETA.INV returns the #VALUE! error value. If either alpha or beta is not positive, BETA.INV returns the #NUM! error value. If probability is either below zero or higher than 1, BETA.INV returns the #NUM! error value. If you omit values for A and B, BETA.INV uses the standard cumulative beta distribution, so that A = 0 and B = 1. Given a value for probability, BETA.INV seeks that value x such that BETA.DIST(x, alpha, beta, TRUE, A, B) = probability. Thus, the precision of BETA.INV depends on the precision of BETA.DIST.
BINOM.DIST	Four obligatory parameters: the number of successes in trials, the number of independent trials, the probability of success on each trial, and the logical value that determines the form of the function. If cumulative is TRUE, then BINOM.DIST returns the cumulative distribution function, which is the probability that	=BINOM.DIST (number_s, trials, probability_s, cumulative)	Returns the individual term binominal distribution probability. Use BINOM.DIST in problems with a fixed number of tests or trials, when the outcome of any trial are only success or failure, when trials are independent, and when the probability of success is constant throughout the experiment. For example, BINOM.DIST can calculate the probability that two of the next three babies born are male. The number_s and trials parameters are truncated to integers. If number_s, trials, or probability_s is non-numeric, BINOM.DIST returns the #VALUE! error value. If number_s < 0 or number_s > trials, BINOM.DIST returns the #NUM! error value. If probability_s < 0 or probability_s > 1, BINOM.DIST returns the #NUM! error value.

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	there are at most number_s successes; if FALSE, it returns the probability mass function, which is the probability that there are number_s successes.		
BINOM.DIST.RANGE	Three obligatory parameters: the number of independent trials, the probability of success in each trial, and the number of successes in trials. Additionally, you can provide an additional range bound for the number of successes in trials.	=BINOM.DIST.RANGE(trials, probability_s, number_s, [number_s2])	Returns the probability of a trial result using a binomial distribution. If any parameters are outside of their constraints, BINOM.DIST.RANGE returns the #NUM! error value: • The trials parameter must be greater than or equal to zero. • The probability_s parameter must be greater than or equal to zero and less than or equal to 1. • The number_s parameter must be greater than or equal to zero and less than or equal to trials. • The number_s2 parameter, if provided, must be greater or equal to number_s, and less than or equal to trials. If any parameters are non-numeric values, BINOM.DIST.RANGE returns the #VALUE! error value. Numeric parameters are truncated to integers.
CHISQ.DIST	Three obligatory parameters: the value at which you want to evaluate the distribution, the number of degrees of freedom, and	=CHISQ.DIST(x, deg_freedom, cumulative)	Returns the cumulative beta probability density functions. The chi-squared distribution is commonly used to study variation in the percentage of something across samples, such as the fraction of the day people spend watching television.

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	the logical value that determines the form of the function. If cumulative is TRUE, CHISQ.DIST returns the cumulative distribution function; otherwise, it returns the probability density function.		If any parameter is non-numeric, CHISQ.DIST returns the #VALUE! error value. If x is negative, CHISQ.DIST returns the #NUM! error value. If deg_freedom is not an integer, it is truncated. If deg_freedom < 1 or deg_freedom > 10^10, CHISQ.DIST returns the #NUM! error value.
CHISQ.DIST.RT	Two obligatory parameters: the value at which you want to evaluate the distribution and the number of degrees of freedom.	=CHISQ.DIST.RT(x, deg_freedom)	Returns the one-tailed probability of the chi-squared distribution. The chi-squared distribution is associated with a chi-squared test. Use the chi-squared test to compare observed and expected values. For instance, a genetic experiment might hypothesize that the next generation of plants will exhibit a certain set of colors. By comparing the observed results with the expected ones, you can decide whether your original hypothesis is valid. If either parameter is non-numeric, CHISQ.DIST.RT function returns the #VALUE! error value. If deg_freedom is not an integer, it is truncated. If deg_freedom < 1 or deg_freedom > 10^10, CHISQ.DIST.RT returns the #NUM! error value.
CHISQ.INV	Two obligatory parameters: the probability associated with the chi-squared distribution and	=CHISQ.INV(probability, deg_freedom)	Returns the inverse of the left-tailed probability of the chi-squared distribution. The chi-squared distribution is commonly used to study variation in the percentage of something across samples, such as

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	the number of degrees of freedom.		the fraction of the day people spend watching television. If parameter is non-numeric, CHISQ.INV returns the #VALUE! error value. If probability < 0 or probability > 1, CHISQ.INV returns the #NUM! error value. If deg_freedom is not an integer, it is truncated. If deg_freedom < 1 or deg_freedom > 10^10, CHISQ.INV returns the #NUM! error value.
CHISQ.INV.RT	Two obligatory parameters: the probability associated with the chi-squared distribution and the number of degrees of freedom.	=CHISQ.INV.RT(probability, deg_freedom)	Returns the inverse of the right-tailed probability of the chi-squared distribution. If probability = CHISQ.DIST.RT(x,…), then CHISQ.INV.RT(probability,…) = x. Use this function to compare observed results with expected ones in order to decide whether your original hypothesis is valid. If either parameter is non-numeric, CHISQ.INV.RT returns the #VALUE! error value. If probability < 0 or probability > 1, CHISQ.INV.RT returns the #NUM! error value. If deg_freedom is not an integer, it is truncated. If deg_freedom < 1, CHISQ.INV.RT returns the #NUM! error value. Given a value for probability, CHISQ.INV.RT seeks that value x such that CHISQ.DIST.RT(x, deg_freedom) = probability. Thus, precision of CHISQ.INV.RT depends on precision of CHISQ.DIST.RT. CHISQ.INV.RT uses an iterative search technique. If the search has not converged after 64 iterations, the function returns the #N/A error value.

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CHISQ.Test	Two obligatory parameters: array1 specifies a cell range of values and array2 specifies a second range of values.	=CHISQ.Test(array1, array2)	Returns the test for independence. CHISQ.TEST returns the value from the chi-squared (χ^2) distribution for the statistic and the appropriate degrees of freedom. The first parameter is an array of observed frequencies, the second parameter is an array of estimated (theoretical) frequencies.
CORREL	Two obligatory parameters: array1 specifies a cell range of values and array2 specifies a second range of values.	=CORREL(array1, array2)	Returns the correlation coefficient of the array1 and array2 cell ranges. Use the correlation coefficient to determine the relationship between two properties. For example, you can examine the relationship between a location's average temperature and the use of air conditioners. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however cells with the zero value are included. If array1 and array2 have a different number of data points, CORREL returns the #N/A error value. If either array1 or array2 is empty, or if s (the standard deviation) of their values equals zero, CORREL returns the #DIV/0! error value.
COUNT	A list of parameters	=COUNT(A1:A4) =COUNT(1, 5, 8)	Returns the number of cells in a given range.
COUNTA	A list of parameters	=COUNTA(A1:A4)	Counts the number of non-empty cells in a given range.
COUNTBLANK	A list of parameters	=COUNTBLANK(A1:E1)	Counts the number of empty cells.
COUNTIF	Two parameters: the range of cells and the	=COUNTIF(A1:A7,">2")	Counts the number of nonblank cells within a range, which meet a given condition.

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	condition of type string.		
COVARIANCE.P	Two obligatory parameters: array1 specifies the first cell range of integers and array2 specifies the second range of integers.	=COVARIANCE.P(array1, array2)	Returns population covariance, the average of the products of deviations for each data point pair in two data sets. Use covariance to determine the relationship between two data sets. For example, you can examine whether greater income accompanies greater levels of education. The parameters must either be numbers or be names, arrays, or references that contain numbers. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. If array1 and array2 have different numbers of data points, COVARIANCE.P returns the #N/A error value. If either array1 or array2 is empty, COVARIANCE.P returns the #DIV/0! error value.
COVARIANCE.S	Two obligatory parameters: array1 specifies the first cell range of integers and array2 specifies the second range of integers.	=COVARIANCE.S(array1, array2)	Returns the sample covariance, the average of the products of deviations for each data point pair in two data sets. The parameters must either be numbers or be names, arrays, or references that contain numbers. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. If array1 and array2 have different numbers of data points, COVARIANCE.S returns the #N/A error value. If either array1 or array2 is empty or contains only 1 data point each,

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			COVARIANCE.S returns the #DIV/0! error value.
DEVSQ	The first parameter is required, subsequent numbers are optional. You can specify from 1 to 255 parameters for which you want to calculate the sum of squared deviations. You can also use a single array or a reference to an array instead of parameters separated by commas.	=DEVSQ(number1, [number2], …)	Returns the sum of squares of deviations of data points from their sample mean. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. Parameters that are error values or text that cannot be translated into numbers cause errors.
EXPON.DIST	Three obligatory parameters: the value of the function, the parameter value, and the logical value that indicates which form of the exponential function to provide. If cumulative is TRUE, EXPON.DIST returns the cumulative distribution function; otherwise, it returns the probability	=EXPON.DIST(x, lambda, cumulative)	Returns the exponential distribution. Use EXPON.DIST to model the time between events, such as how long an automated bank teller takes to deliver cash. For example, you can use EXPON.DIST to determine the probability that the process takes at most 1 minute. If x or lambda is non-numeric, EXPON.DIST returns the #VALUE! error value. If x < 0, EXPON.DIST returns the #NUM! error value. If lambda is not positive, EXPON.DIST returns the #NUM! error value.

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	density function.		
FORECAST	Three obligatory parameters: the data point for which you want to predict a value, the dependent array or range of data, and the independent array or range of data.	=FORECAST(x, known_y's, known_x's)	Calculates, or predicts, a future value by using existing values. The predicted value is a y-value for a given x-value. The known values are existing x-values and y-values, and the new value is predicted by using linear regression. You can use this function to predict future sales, inventory requirements, or consumer trends. If x is non-numeric, FORECAST returns the #VALUE! error value. If known_y's and known_x's are empty or contain different number of data points, FORECAST returns the #N/A error value. If the variance of known_x's equals zero, then FORECAST returns the #DIV/0! error value.
F.Test	Two obligatory parameters: array1 specifies a cell range of values and array2 specifies a second range of values.	=F.Test(array1,array2)	Returns the result of F-Test, the two-tailed probability that the variances in array 1 and array2 are not significantly different. The parameters are the first and second datasets.
GAMMA	The number parameter is mandatory.	=GAMMA(number)	Returns the gamma function value. If number is not positive, GAMMA returns the #NUM! error value. If number contains characters that are not valid, GAMMA returns the #VALUE! error value.
GAMMA.DIST	Four mandatory parameters: X – the value at which you want to evaluate the distribution; Alpha and Beta are parameters	=GAMMA.DIST(x, alpha, beta, cumulative)	Returns the gamma distribution. You can use this function to study variables that may have a skewed distribution. The gamma distribution is commonly used in queuing analysis. If x, alpha, or beta is non-numeric, GAMMA.DIST returns the #VALUE! error value.

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	to the distribution; Cumulative is a logical value that determines the form of the function.		If x is negative, GAMMA.DIST returns the #NUM! error value. If either alpha or beta is not positive, GAMMA.DIST returns the #NUM! error value. If beta = 1, GAMMA.DIST returns the standard gamma distribution. If cumulative is TRUE, GAMMA.DIST returns the cumulative distribution function; if FALSE, it returns the probability density function.
GAMMA.INV	Three mandatory parameters: Probability is the probability associated with the gamma distribution; Alpha and beta are parameters to the distribution.	=GAMMA.INV (probability, alpha, beta)	Returns the inverse of the gamma cumulative distribution. If p = GAMMA.DIST(x, …), then GAMMA.INV(p, …) = x. You can use this function to study a variable whose distribution may be skewed. If beta = 1, GAMMA.INV returns the standard gamma distribution. If any parameter is a text string, GAMMA.INV returns the #VALUE! error value. If the probability is either negative or higher than 1, GAMMA.INV returns the #NUM! error value. If either alpha or beta is not positive, GAMMA.INV returns the #NUM! error value. Given a value for the probability, GAMMA.INV seeks that value x such that GAMMA.DIST(x, alpha, beta, TRUE) = probability. Thus, the precision of GAMMA.INV depends on precision of GAMMA.DIST.
GAMMALN	A single mandatory parameter of double type, which is the value for which you want to calculate GAMMALN.	=GAMMALN(X)	Returns the natural logarithm of the gamma function, $\Gamma(x)$. If x is non-numeric, GAMMALN returns the #VALUE! error value. If x is not positive, GAMMALN returns the #NUM! error value. The number e raised to the GAMMALN(i) power, where i is an integer, returns the same result as (I – 1)!.

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GAMMALN.PRECISE	A single mandatory parameter of double type, which is the value for which you want to calculate the natural logarithm of the gamma function.	=GAMMALN.PRECISE(x)	Returns the natural logarithm of the gamma function, $\Gamma(x)$. If x is non-numeric, GAMMALN.PRECISE returns the #VALUE! error value. If x is not positive, GAMMALN.PRECISE returns the #NUM! error value. The number e raised to the GAMMALN.PRECISE(i) power, where i is an integer, returns the same result as (I – 1)!.
GAUSS	The z parameter is mandatory and specifies a number.	=GAUSS(z)	Calculates the probability that a member of a standard normal population will fall between the mean and z standard deviations from the mean. If z is not a valid number, GAUSS returns the #NUM! error value. If z is not a valid data type, GAUSS returns the #VALUE! error value. Because NORM.S.DIST(0, True) always returns 0.5, GAUSS(z) will always be 0.5 less than NORM.S.DIST(z, True).
GEOMEAN	Up to 255 parameters, for which you calculate the mean. Only the first parameter is mandatory. You can also use a single array instead of number of parameters.	=GEOMEAN(number1, [number2], …)	Returns the geometric mean of an array or range of positive data. For example, you can use GEOMEAN to calculate average growth rate given compound interest with variable rates. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included.

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			Error values or text strings that cannot be translated into numbers cause errors, if you use them as parameters. If any data point is not positive, GEOMEAN returns the #NUM! error value.
Grubbs.Test	One obligatory parameter: array1 specifies a cell range of data.	=GRUBBS.TEST(array1)	Performs the Grubbs' test for outliers. Test is used to detect outliers in a univariate dataset. It is based on the assumption of normality. That is, you should first verify your data can be reasonably approximated by a normal distribution before applying the Grubbs' test. The parameter is the dataset.
HYPGEOM.DIST	Five obligatory parameters: the number of successes in the sample, the size of the sample, the number of successes in the population, the population size, and the logical value that determines the form of the function. If cumulative is TRUE, then HYPGEOM.DIST returns the cumulative distribution function; otherwise, it returns the probability mass function.	=HYPGEOM.DIST(sample_s, number_sample, population_s, number_pop, cumulative)	Returns the hypergeometric distribution. HYPGEOM.DIST returns the probability of a given number of sample successes, given the sample size, population successes, and population size. Use HYPGEOM.DIST for problems with a finite population, where each observation is either a success or a failure, and where each subset of a given size is chosen with equal likelihood. All parameters are truncated to integers. If any parameter is non-numeric, HYPGEOM.DIST returns the #VALUE! error value. If sample_s < 0 or sample_s is greater than the lesser of number_sample or population_s, HYPGEOM.DIST returns the #NUM! error value. If number_sample is not positive or number_sample > number_population, HYPGEOM.DIST returns the #NUM! error value. If population_s is not positive or population_s > number_population,

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			HYPGEOM.DIST returns the #NUM! error value. If number_pop is not positive, HYPGEOM.DIST returns the #NUM! error value.
INTERCEPT	Two obligatory parameters: the dependent and independent sets of observations or data.	=INTERCEPT(known_y's, known_x's)	Calculates the point at which a line will intersect the y-axis by using existing x-values and y-values. The intercept point is based on a best-fit regression line plotted through the known x-values and known y-values. Use the INTERCEPT function when you want to determine the value of the dependent variable when the independent variable is 0 (zero). For example, you can use the INTERCEPT function to predict a metal's electrical resistance at a certain temperature when your data points were taken at room temperature and higher. The parameters should be either numbers or names, arrays, or references that contain numbers. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. If known_y's and known_x's contain a different number of data points or contain no data points, INTERCEPT returns the #N/A error value.
KS.Test	Two obligatory parameters: array1 specifies a cell or cell range of values and array2 specifies a second cell or cell range of values. Two optional	=KS.TEST(array1, array2) or =KS.TEST(array1, array2, 0|1|2, Alpha)	Performs two-sample Kolmogorov-Smirnov goodness of fit test on independent random samples Data1 and Data2. Test determines if Data1 and Data2 samples are drawn from the same continuous population. The mandatory parameters are the first and second datasets. The optional parameters are tailing and alpha (significance)

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	parameters: Tailing and alpha (significance). For tailing, a value of 0=two tailed, 1=right tailed, 2=left tailed. Alpha may be an significance level.		
LARGE	Two parameters: the array or range of data for which you want to determine the k-th largest value. K is the position (from the largest) in the array or cell range of data to return.	=LARGE(array, k)	Returns the k-th largest value in a data set. You can use this function to select a value based on its relative standing. For example, you can use LARGE to return the highest, runner-up, or third-place score. If array is empty, LARGE returns the #NUM! error value. If k is not positive or if k is greater than the number of data points, LARGE returns the #NUM! error value. If n is the number of data points in a range, then LARGE(array, 1) returns the largest value, and LARGE(array, n) returns the smallest value.
Lilliefors.Test	One obligatory parameter: array1 specifies a cell range of vales.	=LILLIEFORS.TEST(array1)	Performs the Lilliefors goodness of fit test to a normal distribution with unknown parameters mu and sigma. The parameter is the dataset. A minimum of 4 values must be present to calculate.
MannWhitney.Test	Five obligatory parameters: array1 specifies a cell or range of cells for dataset 1, array2 specifies a cell or range of cells for dataset 2, the	=MANNWHITNEY.TEST(array1, array2,hypothesis type, alpha,approximation)	Performs the non-parametric test of the null hypothesis, that two samples come from populations of equal means, against an alternative hypothesis, especially that a particular population has a larger mean than the other one. It operates under the assumption that all the observations from both groups are independent of each

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	hypothesis type, the significance level, and the approximation.		other. Will evaluate to **TRUE** when failing to reject the null hypothesis or **FALSE** when rejecting the null hypothesis. (Credit: Wikipedia) The variables for the function are as follows: **[Array1…]** - cell range of values for first dataset **[Array2…]** - cell range of values for second dataset **Hypothesis Type - 0** for two-tailed, **1** for right-tailed, or **2** for left-tailed **Alpha** - the significance level for which to test (i.e. **0.05**) **Use Normal Approximation - True** (or **1**)/**False** (or **0**)
MAX	A list of parameters	=MAX(A1:D1) =MAX(A1, 100, C2)	Returns the largest parameter value.
MAXA	From 1 to 255 parameters. Value1 is the mandatory parameter for which you want to find the largest value; the following values from 2 to 255 are optional parameters for which you want to find the largest value.	=MAXA(value 1, [value2], …)	Returns the largest value in a list of parameters. Parameters can be the following: numbers; names, arrays, or references that contain numbers; text representations of numbers; logical values, such as TRUE and FALSE, in a reference. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only values in that array or reference are used. Empty cells and text values in the array or reference are ignored. If the parameters contain no values, MAXA returns 0 (zero). If you do not want to include logical values and text representations of numbers in a reference s part of the

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			calculation, use the MAX function instead.
MEDIAN	From 1 to 255 parameters. Only the number1 is required, subsequent numbers are optional.	=MEDIAN(number1, [number2], …)	Returns the median of the given numbers. The median is the number in the middle of a set of numbers. Note that the MEDIAN function measures central tendency, which is the location of the center of a group of numbers in a statistical distribution.
MIN	A list of parameters	=MIN(A1:D1) =MIN(0, C2)	Returns the smallest parameter value.
MINA	From 1 to 255 parameters. Only the value1 is required, subsequent values are optional.	=MINA(number1, [number2], …)	Returns the smallest value in a list of parameters, including members, text, and logical values. Parameters can be the following: numbers; names, arrays, or references that contain numbers; text representations of numbers; logical values, such as TRUE and FALSE, in a reference. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only values in that array or reference are used. Empty cells and text values in the array or reference are ignored. If the parameters contain no values, MINA returns 0 (zero). If you do not want to include logical values and text representations of numbers in a reference s part of the calculation, use the MIN function instead.
NORM.DIST	Four obligatory parameters: the value for which you want the distribution, the arithmetic mean of the	=NORM.DIST(x, mean, standard_dev, cumulative)	Returns the normal distribution for the specified mean and standard deviation. This function has a very wide range of applications in statistics, including hypothesis testing.

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	distribution, the standard deviation of the distribution, and the logical value that determines the form of the function. If cumulative is TRUE, NORM.DIST returns the cumulative distribution function; otherwise, it returns the probability mass function.		If mean or standard_dev is non-numeric, NORM.DIST returns the #VALUE! error value. If standard_dev is not positive, NORM.DIST returns the #NUM! error value. If mean = 0, standard_dev = 1, and cumulative = TRUE, NORM.DIST returns the standard normal distribution, NORM.S.DIST.
NORM.INV	Three mandatory parameters: the probability corresponding to the normal distribution, the arithmetic mean of the distribution, and the standard deviation of the distribution.	=NORM.INV(probability, mean, standard_dev)	Returns the inverse of the normal cumulative distribution for the specified mean and standard deviation. If any parameter is non-numeric, NORM.INV returns the #VALUE! error value. If probability is not positive or if probability > 1, NORM.INV returns the #NUM! error value. If standard_dev is not positive, NORM.INV returns the #NUM! error value. If mean = 0 and standard_dev = 1, NORM.INV uses the standard normal distribution. Given a value for probability, NORM.INV seeks that value x such that NORM.DIST(x, mean, standard_dev, TRUE) = probability. Thus, precision of NORM.INV depends on precision of NORM.DIST.
NORM.S.DIST	Two mandatory parameters: the value for	=NORM.S.DIST(z, cumulative)	Returns the standard normal distribution (has a mean of zero and a standard deviation of one).

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	which you want the distribution, and the cumulative logical value that determines the form of the function. If cumulative is TRUE, NORM.S.DIST returns the cumulative distribution function; otherwise, it returns the probability mass function.		Use this function in place of a table of standard normal curve areas. If z is non-numeric, NORM.S.DIST returns the #VALUE! error value.
NORM.S.INV	The probability parameter is mandatory.	=NORM.S.INV (probability)	Returns the inverse of the standard normal cumulative distribution. The distribution has a mean of zero and a standard deviation of one. If probability is non-numeric, NORM.S.INV returns the #VALUE! error value. If probability is not positive or if probability >= 1, NORM.S.INV returns the #NUM! error value. Given a value for probability, NORM.S.INV seeks that value z that NORM.S.DIST(z, TRUE) = probability. Thus, precision of NORM.S.INV depends on precision of NORM.S.DIST. NORM.S.INV uses an iterative search technique.
PEARSON	Two mandatory parameters: array1 and array2 which represent two sets of independent and dependent	=PEARSON(array1, array2)	Returns the Pearson product moment correlation coefficient, r, a dimensionless index that ranges from −1.0 to 1.0 inclusive and reflects the extent of a linear relationship between two data sets. The parameters must be either numbers or names, array

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	values, respectively.		constants, or references that contain numbers. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. If array1 and array2 are empty or have a different number of data points, PEARSON returns the #N/A error value.
PERCENTILE.INC	Two mandatory parameters: the array or range of data that defines relative standing, and the percentile value in the range 0..1, inclusive.	=PERCENTILE.INC(array, k)	Returns the k-th percentile of values in a range. You can use this function to establish a threshold of acceptance. For example, you can decide to examine candidates who score above 90th percentile. If array is empty, PERCENTILE.INC returns the #NUM! error value. If k is non-numeric, PERCENTILE.INC returns the #VALUE! error value. If k is < 0 or if k > 1, PERCENTILE.INC returns the #NUM! error value. If k is not a multiple of 1/(n – 1), PERCENTILE.INC interpolates to determine the value at the k-th percentile.
PERCENTILE.EXC	Two mandatory parameters: the array or range of data that defines relative standing, and the percentile value in the range 0..1, exclusive.	=PERCENTILE.EXC(array, k)	Returns the k-th percentile of values in a range, where k is in the range 0..1, exclusive. If array is empty, PERCENTILE.EXC returns the #NUM! error value. If k is non-numeric, PERCENTILE.EXC returns the #VALUE! error value. If k is not positive or if k is equal to or exceeds 1, PERCENTILE.EXC returns the #NUM! error value. If k is not a multiple of 1/(n – 1), PERCENTILE.EXC interpolates to determine the value at the k-th percentile.

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			PERCENTILE.EXC will interpolate when the value for the specified percentile lies between two values in the array. If it cannot interpolate for the percentile, specified by the k parameter, the **ExpressSpreadSheet** returns #NUM! error.
PERMUT	Two mandatory parameters: integer values that describe the number of objects and the number of objects in each permutation.	=PERMUT(number, number_chosen)	Returns the number of permutations for a given number of objects that can be selected from number objects. A permutation is any set or subset of objects or events where internal order is significant. Permutations are different from combinations, for which the internal order is not significant. Use this function for lottery-style probability calculations. Both of the accepted parameters are truncated to integers. If number or number_chosen is non-numeric, PERMUT returns the #VALUE! error value. If number is not positive or if number_chosen is negative, PERMUT returns the #NUM! error value. If number < number_chosen, PERMUT returns the #NUM! error value.
POISSON.DIST	Three mandatory parameters: the number of events, the expected numeric value, and the cumulative logical value that determines the form of the probability	=POISSON.DIST(x, mean, cumulative)	Returns the Poisson distribution. A common application of the Poisson distribution is predicting the number of events over a specific time, such as the number of cars arriving at a toll plaza in 1 minute. If x is not an integer, it is truncated. If x or mean is non-numeric, POISSON.DIST returns the #VALUE! error value. If x < 0, POISSON.DIST returns the #NUM! error value.

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		distribution returned. If cumulative is TRUE, POISSON.DIST returns the cumulative Poisson probability that the number of random events occurring will fall between zero and x inclusive; otherwise, it returns the Poisson probability mass function that the number of events occurring will be exactly x.	If mean < 0, POISSON.DIST returns the #NUM! error value.
QUARTILE.INC	Two mandatory parameters: the array or cell range of numeric values for which you want the quartile value, and the quart that indicates which value to return. If quart = 0, QUARTILE.INC returns the minimum value. If quart = 1, QUARTILE.INC returns the first quartile (25th percentile).	=QUARTILE.INC(array, quart)	Returns the quartile of a data set, based on percentile values from 0..1, inclusive. Quartiles often are used in sales and survey data to divide populations into groups. For example, you can use QUARTILE.INC to find the top 25 percent of incomes in a population. If array is empty, QUARTILE.INC returns the #NUM! error value. If quart is not an integer, it is truncated. If quart < 0 or if quart > 4, QUARTILE.INC returns the #NUM! error value. MIN, MEDIAN, and MAX return the same value as QUARTILE.INC when quart is equal to 0 (zero), 2, and 4, respectively.

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			If quart = 2, QUARTILE.INC returns the median value (50th percentile). If quart = 3, QUARTILE.INC returns the third quartile (75th percentile). If quart = 4, QUARTILE.INC returns the maximum value.
QUARTILE.EXC	Two mandatory parameters: the array or cell range of numeric values for which you want the quartile value, and the quart that indicates which value to return.	=QUARTILE.EXC(array, quart)	Returns the quartile of the data set, based on percentile values from 0..1, exclusive. If array is empty, QUARTILE.EXC returns the #NUM! error value. If quart is not an integer, it is truncated. If quart is not positive or if quart equals to or exceeds 4, QUARTILE.EXC returns the #NUM! error value. MIN, MEDIAN, and MAX return the same value as QUARTILE.EXC when quart is equal to 0 (zero), 2, and 4, respectively.
RANK.AVG	Two mandatory parameters: the number whose rank you want to find, and the ref parameter specifies an array of, or a reference to, a list of numbers. Additionally, you can set the	=RANK.AVG(number, ref, [order])	Returns the rank of a number in a list of numbers: its size relative to other values in the list. If more than one value has the same rank, the average rank is returned. If order is 0 (zero) or omitted, the **ExpressSpreadSheet** ranks number as if ref were a list sorted in descending order. If order is any non-zero value, the **ExpressSpreadSheet** ranks

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	way of ranking the number as the order parameter.		number as if ref were a list sorted in ascending order.
RANK.EQ	Two mandatory parameters: the number whose rank you want to find, and the ref parameter specifies an array of, or a reference to, a list of numbers. Additionally, you can set the way of ranking the number as the order parameter.	=RANK.EQ(number, ref, [order])	Returns the rank of a number in a list of numbers. Its size is relative to other values in the list; if more than one value has the same rank, the top rank of that set of values is returned. If you were to sort the list, the rank of the number would be its position. If order is 0 (zero) or omitted, the **ExpressSpreadSheet** ranks number as if ref were a list sorted in descending order. If order is any non-zero value, the **ExpressSpreadSheet** ranks number as if ref were a list sorted in ascending order. RANK.EQ gives duplicate numbers the same rank. However, the presence of duplicate numbers affects the ranks of subsequent numbers. For example, in a list of integers sorted in ascending order, if the number 10 appears twice and has a rank of 5, then 11 would have a rank of 7 (no number would have a rank of 6). For some purposes one might want to use a definition of rank that takes ties into account. In previous example, you would want a revised rank of 5.5 for the number 10. This can be done by adding the following correction factor to the value returned by RANK.EQ. This correction factor is appropriate oth for the case where rank is calculated in descending order (order = 0 or omitted) or ascending order (order = non-zero value).

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			Correction factor for tied ranks=[COUNT(ref) + 1 – RANK.EQ(number, ref, 1)]/2.
REGRESSIONPLOTXTOY	Three mandatory parameters: the Plot number of a regression plot (numeric), the overlay number of a regression fit (numeric) and the X known value (numeric).	=REGRESSIONPLOTXTOY(plot_number, overlay_number,x_known_value)	Returns the Y value of the regression function given the X input value. Only a single X value is allowed as input.
REGRESSIONPLOTYTOX	Three mandatory parameters: the Plot number of a regression plot (numeric), the overlay number of a regression fit (numeric) and the Y known value (numeric).	=REGRESSIONPLOTYTOX(plot_number, overlay_number,y_known_value)	Returns the X value of the regression function given the Y input value. Only a single Y value is allowed as input.
RSQ	Two mandatory parameters are two arrays or ranges of data points.	=RSQ(known_y's, known_x's)	Returns the square of the Pearson product moment correlation coefficient through data points in known_y's and known_x's. For more information, refer to the description of the PEARSON function. The r-squared value can be interpreted as the proportion of the variance in y attributable to the variance in x. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that

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			you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. Parameters that are error values or text that cannot be translated into numbers cause errors. If known_y's and known_x's are empty or have a different number of data points, RSQ returns the #N/A error value. If known_y's and known_x's contain only 1 data point, RSQ returns the #DIV/0! error value.
SKEW	The SKEW function accepts from one to 255 parameters for which you want to calculate skewness. You can also use a single array or a reference to an array instead of parameters separated by commas. Note that you must specify at least one parameter.	=SKEW(number1, [number2], ...)	Returns the skewness of a distribution. Skewness characterizes the degree of asymmetry of a distribution around its mean. Positive skewness indicates a distribution with an asymmetric tail extending toward more positive values. Negative skewness indicates a distribution with an asymmetric tail extending towards more negative values. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. Parameters that are error values or text that cannot be translated into numbers cause errors. If there are fewer than three data points, or the sample standard

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			deviation is zero, SKEW returns the #DIV/0! error value.
SKEW.P	The SKEW function accepts from one to 255 parameters for which you want to calculate skewness. You can also use a single array or a reference to an array instead of parameters separated by commas. Note that you must specify at least one parameter.	=SKEW.P(number1, [number2], …)	Returns the skewness of a distribution based on a population: a characterization of the degree of asymmetry of a distribution around its mean. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. SKEW.P uses the standard deviation of an entire population, not a sample. If parameters are values that are not valid, SKEW.P returns the #NUM! error value. If parameters use data types that are not valid, SKEW.P returns the #VALUE! error value. If there are fewer than three data points, or the sample standard deviation is zero, SKEW.P returns the #DIV/0! error value.
SLOPE	Two mandatory parameters: an array or cell range of numeric dependent data points and the set of independent data points.	=SLOPE(known_y's, known_x's)	Returns the slope of the linear regression line through data points in known_y's and known_x's. The slope is the vertical distance divided by the horizontal distance between any two points on the line, which is the rate of change along the regression line. The parameters must be either numbers or names, arrays, or references that contain numbers.

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			If an array or reference parameter contains text, logical values, or empty cells, those values are ignored; however, cells with the zero value are included. If known_y's and known_x's are empty or have a different number of data points, SLOPE returns the #N/A value.
SMALL	Two mandatory parameters: an array or range of numerical data for which you want to determine the k-th smallest value and the position (from the smallest) in the array or range of data to return. , k)	=SMALL(array	Returns the k-th smallest value in a data set. Use this function to return values with a particular relative standing in a data set. If array is empty, SMALL returns the #NUM! error value. If k is not positive or if k exceeds the number of data points, SMALL returns the #NUM! error value. If n is the number of data points in array, SMALL(array, 1) equals the smallest value, and SMALL(array, n) equals the largest value.
SSMD	Six mandatory parameters: the mean of the positive control, the mean of the negative control, the sample size of the positive control, the sample size of the negative control, the standard deviation of the positive control, and the standard deviation of the negative control.	=SSMD(mean max,mean min,n max,n min,SDmax,SDmin)	Short for Strictly Standardized Mean Difference. Has been used as a ranking metric for hit selection in high throughput screening assays.

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StainIndex	Three mandatory parameters: mean or median of positive population, mean or median of negative population, and standard deviation of negative population	=STAININDEX (mean or median of positive population,mean or median of negative population,standard deviation of negative population)	Performs a stain index calculation using the means/medians or positive and negative populations and the negative population's standard deviation based on the Stain Index formula of (Median of Positive - Median of Negative) / (SD of Negative *2).
STDEV.S	This function accepts from 1 to 255 parameters. Only the number1 is required, subsequent values are optional. You can also use a single array or a reference to an array instead of parameters separated by commas.	=STDEV.S(number1, [number2], …)	Estimates the standard deviation based on a sample. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). STDEV.S assumes that its parameters are a sample of the population. If your data represents the entire population, then calculate the standard deviation using the STDEV.P function instead. The standard deviation is calculated using the "n-1" method. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text, or error values in the array or reference are ignored. Parameters that are error values or text that cannot be converted into numbers cause errors.

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			If you want to include logical values and text representations of numbers in a reference as part of the calculation, use the STDEVA function instead.
STDEV.P	From 1 to 255 parameters. Only the number1 is required, subsequent values are optional. You can also use a single array or a reference to an array instead of parameters separated by commas.	=STDEV.P(number1, [number2], …)	Estimates the standard deviation based on the entire population. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). STDEV.P assumes that its parameters are the entire population. If you data represents a sample of the population, then calculate the standard deviation using STDEV. For large sample sizes, STDEV.S and STDEV.P return approximately equal values. The standard deviation is calculated using the "n" method. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values, and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text, or error values in the array or reference are ignored. Parameters that are error values or text that cannot be converted into numbers cause errors. If you want to include logical values and text representations of numbers in a reference part of the calculation, use the STDEVPA function instead.
STDEVA	From 1 to 255 parameters. Only the value1	=STDEVA(value1, [value2],	Estimates the standard deviation based on a sample. The standard deviation is a measure of how

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	is required, subsequent values are optional. You can also use a single array or a reference to an array instead of parameters separated by commas.	…)	widely values are dispersed from the average value (the mean). STDEVA assumes that its parameters are a sample of the population. If your data represents the entire population, you must calculate the standard deviation using STDEVPA instead. The standard deviation is calculated using the "n−1" method. Parameters can be the following: numbers, names, arrays, or references that contain numbers; text representations of numbers, or logical values, such as TRUE and FALSE, in a reference. Parameters that contain TRUE evaluate as 1; parameters that contain text or FALSE evaluate as 0 (zero). If an parameter is an array or reference, only values in that array or reference are used. Empty cells and text values in that array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors. If you do not want to include logical values and text representations of numbers in a reference as part of the calculation, use the STDEV function instead.
STDEVPA	From 1 to 255 parameters. Only the value1 is required, subsequent values are optional. You can also use a single array or a reference to an array instead of	=STDEVPA(value1, [value2], …)	Calculates the standard deviation based on the entire population, including numbers, text, and logical values. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). STDEVPA assumes that its parameters are the entire population. If your data represents a sample of the population, you must calculate the standard

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	parameters separated by commas.		deviation by using the STDEVA function. The standard deviation is calculated using the "n" method. Parameters can be the following: numbers, names, arrays, or references that contain numbers; text representations of numbers, or logical values, such as TRUE and FALSE, in a reference. Text representations of numbers that you type directly into the list of parameters are counted. Parameters that contain TRUE evaluate as 1; parameters that contain text or FALSE evaluate as 0 (zero). If an parameter is an array or reference, only values in that array or reference are used. Empty cells and text values in the array or reference are ignored. Parameters that are error values or text that cannot be converted into numbers cause errors. If you do not want to include logical values and text representations of numbers in a reference as part of the calculation, use the STDEVP function instead.
STEYX	Two mandatory parameters: the two arrays or ranges of dependent and independent data points, respectively.	=STEYX(known_y's, known_x's)	Returns the standard error of the predicted y-value for each x in the regression. The standard error is a measure of the amount of error in the prediction of y for an individual x. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values and text representations of numbers that you type directly into the list of parameters are counted. In an array or reference parameter contains text, logical values, or

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			empty cells, those values are ignored; however, cells with the zero value are included. Parameters that are error values or text that cannot be converted into numbers cause errors. If known_y's and known_x's have a different number of data points, STEYX returns the #N/A error value. If known_y's and known_x's are empty or have less than three data points, STEYX returns the #DIV/0! error value.
SUM	A list of parameters	=SUM(A1:C12) =SUM(A1, 3.14, 1.57)	Sums values within the list.
SUMSQ	A list of parameters	=SUMSQ(B1:D1) =SUMSQ(1, 2, 3, 4)	Sums squares of values within the list.
T.DIST	Three obligatory parameters: the numeric value at which to evaluate the distribution, the integer value indicating the number of degrees of freedom, and the cumulative logical value that determines the form of the function. If cumulative is TRUE, T.DIST returns the cumulative distribution function;	=T.DIST(x, deg_freedom, cumulative)	Returns the Student's left-tailed t-distribution. The t-distribution is used in the hypothesis testing of small sample data sets. Use this function in place of a table of critical values for the t-distribution. If any parameter is non-numeric, T.DIST returns the #VALUE! error value. If deg_freedom < 1, T.DIST returns an error value. The deg_freedom parameter should be at no less than 1.

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	otherwise, it returns the probability density function.		
T.DIST.2T	Two obligatory parameters: the numeric value at which to evaluate the distribution, and the integer value that indicates the number of degrees of freedom.	=T.DIST.2T(x, deg_freedom)	Returns the two-tailed Student's t-distribution. The Student's t-distribution is used in the hypothesis testing of small sample data sets. Use this function in place of a table of critical values for the t-distribution. If any parameter is non-numeric, T.DIST.2T returns the #VALUE! error value. If deg_freedom < 1, T.DIST.2T returns the #NUM! error value. If x < 0, then T.DIST.2T returns the #NUM! error value.
T.DIST.RT	Two obligatory parameters: the numeric value at which to evaluate the distribution, and the integer value that indicates the number of degrees of freedom.	=T.DIST.RT(x, deg_freedom)	Returns the right-tailed Student's t-distribution. The t-distribution is used in the hypothesis testing of small data sets. Use this function in place of a table of critical values for the t-distribution. If any parameter is non-numeric, T.DIST.RT returns the #VALUE! error value. If deg_freedom < 1, T.DIST.RT returns the #NUM! error value.
T.INV	Two obligatory parameters: the probability associated with the Student's t-distribution and the number of degrees of freedom with which to characterize the distribution.	=T.INV(probability, deg_freedom)	Returns the left-tailed inverse of the student's t-distribution. If either parameter is non-numeric, T.INV returns the #VALUE! error value. If probability <= 0 or if probability > 1, T.INV returns the #NUM! error value. If deg_freedom is not an integer, it is truncated. If deg_freedom < 1, T.INV returns the #NUM! error value.

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T.INV.2T	Two obligatory parameters: the probability associated with the Student's t-distribution and the number of degrees of freedom with which to characterize the distribution.	=T.INV.2T(probability, deg_freedom)	Returns the two-tailed inverse of the Student's t-distribution. If either parameter is non-numeric, T.INV.2T returns the #VALUE! error value. If probability <= 0 or if probability > 1, T.INV.2T returns the #NUM! error value. If deg_freedom < 1, T.INV.2T returns the #NUM! error value. T.INV.2T returns that value t, such that P(|X| > t) = probability where X is a random variable that follows the t-distribution and P(|X| > t) = P(X < -t or X > t). A one-tailed t-value can be returned by replacing probability with 2*probability. For a probability of 0.05 and degrees of freedom of 10, the two-tailed value is calculated with T.INV.2T(0.05, 10), which returns 2.28139. The one-tailed value of the same probability and degrees of freedom can be calculated with T.INV.2T(2 * 0.05, 10), which returns 1.812462. Given a value for probability, T.INV.2T seeks that value x such that T.DIST.2T(x, deg_freedom, 2) = probability. Thus, precision of T.INV.2T depends on precision of T.DIST.2T.
T.Test	Four mandatory parameters: array1 specifies a cell or range of cells for dataset 1, array2 specifies a cell or range of cells for dataset2, the hypothesis type, and the	=T.TEST(array1, array2,hypothesis type or tails,type)	It compares Array1 mean value with Array2 mean value. The assumption is Number1 and Number2 variances are equal but unknown. The variables for the function are as follows: **[Array1…]** - cell range of values for first dataset **[Array2…]** - cell range of values for second dataset

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	last to define paired or unpaired		**Hypothesis Type - 1** for one-tailed (left-tailed) or **2** for two-tailed **Type** - **1** for paired data with equal variance (homoscedastic) (data set size should be equal), **2** for unpaired data with equal variance (homoscedastic) (data set size do not have to be equal).
VAR.P	From 1 to 255 parameters. Only the number1 is required, subsequent values are optional.	=VAR.P(number1, [number2], …)	Calculates variance based on the entire population (ignores logical values and text in the population). VAR.P assumes that its parameters are the entire population. If your data represents a sample of the population, than calculate the variance by using the VAR.S function instead. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values, and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text or error values in the array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors. If you want to include logical values and text representations of numbers in a reference as part of the calculation, use the VARPA function instead.
VAR.S	From 1 to 255 parameters. Only the number1 is required, subsequent	=VAR.S(number1, [number2], …)	Estimates variance based on a sample (ignores logical values and text in the sample). VAR.S assumes that its parameters are a sample of the population. If your data represents the entire population, then calculate the

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	values are optional.		variance by using the VAR.P function instead. Parameters can either be numbers or names, arrays, or references that contain numbers. Logical values, and text representations of numbers that you type directly into the list of parameters are counted. If an parameter is an array or reference, only numbers in that array or reference are counted. Empty cells, logical values, text, or error values in the array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors. If you want to include logical values or text representations of numbers in a reference as part of the calculation, use the VARA function instead.
VARA	From 1 to 255 parameters. Only the value1 is required, subsequent values are optional.	=VARA(value 1, [value2], …)	Estimates variance based on a sample. VARA assumes that its parameters are a sample of the population. If your data represents the entire population, you must calculate the variance by using the VARPA function instead. Parameters can be the following: numbers, names, arrays, or references that contain numbers; text representations of numbers, or logical values, such as TRUE and FALSE, in a reference. Logical values and text representations of numbers that you type directly into the list of parameters are counted. Parameters that contain TRUE evaluate as 1; parameters that contain text or FALSE evaluate as 0 (zero).

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			If an parameter is an array or reference, only values in that array or reference are used. Empty cells and text values in the array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors. If you do not want to include logical values and text representations of numbers in a reference as part of the calculation, use the VAR function instead.
VARPA	From 1 to 255 parameters. Only the value1 is required, subsequent values are optional.	=VARPA(value 1, [value2], …)	Calculates variance based on the entire population. VARPA assumes that its parameters are the entire population. If your data represents a sample of the population, you must calculate the variance by using the VARA function instead. Parameters can be the following: numbers, names, arrays, or references that contain numbers; text representations of numbers, or logical values, such as TRUE and FALSE, in a reference. Logical values and text representations of numbers that you type directly into the list of parameters are counted. Parameters that contain TRUE evaluate as 1; parameters that contain text or FALSE, evaluate as 0 (zero). If an parameter is an array or reference, only values are used in that array or reference. Empty cells and text values in the array or reference are ignored. Parameters that are error values or text that cannot be translated into numbers cause errors. If you do not want to include logical values or text representations of

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			numbers in a reference as part of the calculation, use the VARP function instead.
WEIBULL.DIST	Four obligatory parameters: the value at which to evaluate the function, the alpha and beta parameters to the distribution, and the cumulative parameter that determines the form of the function.	=WEIBULL.DIST(x, alpha, beta, cumulative)	Returns the Weibull distribution. Use this distribution in reliability analysis, such as calculating a device's mean time to failure. If x, alpha, or beta is non-numeric, WEIBULL.DIST returns the #VALUE! error value. If x is negative, the WEIBULL.DIST function returns the #NUM! error value. If either alpha or beta is not positive, WEIBULL.DIST returns the #NUM! error value.
Wilcoxon	Four mandatory parameters: array1 specifies a cell or range of cells for dataset 1, array2 specifies a cell or range of cells for dataset 2, hypothesis type, and the significance level	=Wilcoxon(array1, array2,hypothesis type,alpha)	Performs a non-parametric test that compares two paired groups. The test calculates the difference between each set of pairs and analyzes these differences. The Wilcoxon Rank Sum test can be used to test the null hypothesis that two populations have the same continuous distribution. Will evaluate to **TRUE** when failing to reject the null hypothesis or **FALSE** when rejecting the null hypothesis. (Credit: Investopedia) The variables for the function are as follows: **[Array1…]** - cell range of values for first dataset **[Array2…]** - cell range of values for second dataset **Hypothesis Type - 0** for two-tailed, **1** for right-tailed, or **2** for left-tailed **Alpha** - the significance level for which to test (i.e. **0.05**)

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ZPrime	Four mandatory parameters: the mean of the negative control, the standard deviation of the negative control, the mean of the positive control, and the standard deviation of the positive control	=ZPRIME(mean negative,SD negative,mean positive,SD positive)	Calculates the Z Factor, which is an attempt to quantify the suitability of a particular assay for use in a full-scale, high-throughput screen by comparing the Mean and Standard Deviation of a negative control to the Mean and Standard Deviation of a positive control within the experiment.
Z.Test	Three mandatory parameters: array1 specifies a cell or range of cell values, X specifies the expected mean, and sigma specifies the expected standard deviation	=Z.TEST(array1,X,sigma)	Compares the normally distributed data elements, mean value with known standard deviation, **expected SD**, to a mean value **expected mean**. **array1** is the dataset.

Financial functions

Financial functions are designed for financial calculations and analysis. Use them to perform many of the commonly used financial calculations, such as the calculation of yield, interest rates, investment valuations, internal rate of return, payments and asset depreciation.

Mnemonics	Operand Type	Example	Explanation
FV	Three mandatory parameters: the	=FV(rate, nper, pmt, [pv], [type])	Returns the future value of an investment.

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	interest rate per period, the total number of payment periods in an annuity, and the payment made each period; it cannot change over the life of the annuity. Typically, pmt contains principal and interest but no other fees or taxes. If pmt is omitted, you must specify the pv parameter instead. Additionally, you can specify the lump-sum amount that a series of future payments is worth right now. If pv is omitted, it is assumed to be 0 (zero), and you must include the pmt parameter. The second optional parameter is type that indicates when payments are due. If type is omitted, it is assumed to be 0.		Make sure that you are consistent about the units you use for specifying rate and nper. If you make monthly payments on a four-year loan at 12 percent annual interest, use 12%/12 for rate and 4*12 for nper. If you make annual payments on the same loan, use 12% for rate and 4 for nper. For all parameters, cash you pay out, such as deposits to savings, is represented by negative numbers; cash you receive, such as dividend checks, is represented by positive numbers.
IPMT	Four mandatory parameters include: the	=IPMT(rate, per, nper, pv, [fv], [type])	Returns the interest payment for an investment for a given period.

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	interest rate per period, the period for which you want to find the interest and must be in the range 1 to nper, the total number of payment periods in an annuity, and the lump-sum that a series of future payments is worth right now. Additionally, you can specify two more parameters that include: the future value, or a cash balance you want to attain after the last payment is made (if this parameter is omitted, it is assumed to be 0), and the type that indicates when payments are due (if type is omitted, it is assumed to be 0).		Make sure that you are consistent about the units you use for specifying rate and nper. If you make monthly payments on a four-year loan at 12 percent annual interest, use 12%/12 for rate and 4*12 for nper. If you make annual payments on the same loan, use 12% for rate and 4 for nper. For all parameters, cash you pay out, such as deposits to savings, is represented by negative numbers; cash you receive, such as dividend checks, is represented by positive numbers.
NPER	Three mandatory parameters include: the interest rate per period, the payment made each period which cannot change over the	=NPER(rate, pmt, pv, [fv], [type])	Returns the number of periods for an investment based on periodic, constant payments and a constant interest rate.

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	life of annuity, and the lump-sum amount that a series of future payments is worth right now. Additionally, you can specify the cash balance you want to attain after the last payment is made (if this parameter is omitted, it is assumed to be 0), and the type that indicates when payments are due.		
NPV	Two mandatory parameters include the rate of discount over the length of one period and the value1 parameter specifies the payments and income. You can specify up to 254 value parameters.	=NPV(rate, value1, [value2], …)	Calculates the net present value of an investment by using a discount rate and a series of future payments (negative values) and income (positive values). The NPV investment begins one period before the date of the value1 cash flow and ends with the last cash flow in the list. The NPV calculation is based on future cash flows. If your first cash flow occurs at the beginning of the first period, the first value must be added to the NPV result, not included in the value parameters. NPV is similar to the PV function (present value). The primary difference between PV and NPV is that PV allows cash flows to begin either at the end or at the beginning of the period. Unlike the variable NPV cash flow values, PV cash flows must be constant throughout the investment. For information about annuities and financial functions,

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			refer to the description of the PV function.
PMT	Three mandatory parameters include: the interest rate per period, the payment made each period which cannot change over the life of annuity, and the lump-sum amount that a series of future payments is worth right now. Additionally, you can specify the cash balance you want to attain after the last payment is made (if this parameter is omitted, it is assumed to be 0), and the type that indicates when payments are due.	=PMT(rate, nper, pv, [fv], [type])	Calculates the payment for a loan based on constant payments and a constant interest rate. The payment returned by PMT includes principal and interest but no taxes, reserve payments, or fees sometimes associated with loans. Make sure that you are consistent about the units you use for specifying rate and nper. If you make monthly payments on a four-year loan at an annual interest rate of 12 percent, use 12%/12 for rate and 4 * 12 for nper. If you make annual payments on the same loan, use 12 percent for rate and 4 for nper.
PPMT	Four mandatory parameters include: the interest rate per period, the period for which you want to find the interest and must be in the range 1 to nper, the total number of payment periods in an	=PPMT(rate, per, nper, pv, [fv], [type])	Returns the payment on the principal for a given period for an investment based on periodic, constant payments and a constant interest rate. Make sure that you are consistent about the units you use for specifying rate and nper. If you make monthly payments on a four-year loan at 12 percent annual interest, use 12%/12 for rate and 4*12 for nper. If you make annual

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	annuity, and the lump-sum that a series of future payments is worth right now. Additionally, you can specify two more parameters that include: the future value, or a cash balance you want to attain after the last payment is made (if this parameter is omitted, it is assumed to be 0), and the type that indicates when payments are due (if type is omitted, it is assumed to be 0).		payments on the same loan, use 12% for rate and 4 for nper.
PV	Three mandatory parameters: the interest rate per period, the total number of payment periods in an annuity, and the payment made each period; it cannot change over the life of the annuity. Typically, pmt contains principal and interest but no other fees or taxes. If pmt is omitted, you	=PV(rate, nper, pmt, [fv], [type])	Returns the present value of an investment. The present value is the total amount that a series of future payments is worth now. For example, when you borrow money, the loan amount is the present value to the lender. Make sure that you are consistent about the units you use for specifying rate and nper. If you make monthly payments on a four-year loan at 12 percent annual interest, use 12%/12 for rate and 4*12 for nper. If you make annual payments on the same loan, use 12% for rate and 4 for nper. An annuity is a series of constant cash payments made over a continuous period. For example, a car loan or a mortgage is an

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must specify the pv parameter instead.	annuity. For more information, refer to the description of each annuity function.
Additionally, you can specify the lump-sum amount that a series of future payments is worth right now. If pv is omitted, it is assumed to be 0 (zero), and you must include the pmt parameter.	In annuity functions, cash you pay out, such as a deposit to savings, is represented by a negative number; cash you receive, such as a dividend check, is represented by a positive number. For example, a $1000 deposit to the bank would be represented by the parameter –1000 if you are the depositor and by the parameter 1000 if you are the bank.
The second optional parameter is type that indicates when payments are due. If type is omitted, it is assumed to be 0.	

Date and Time functions

Use this set of functions to identify the current date, extract a part of the date, convert the **DateTime** values to other data types, and perform calculations with the dates.

Mnemonics	Operand Type	Example	Explanation
DATE	Three operands defining the year, month and day.	=DATE(1900,1,1)	Calculates the serial number that represents a specified date.
DATEVALUE	The date_text parameter specifies a date in the Excel date format, or a reference to a cell	=DATEVALUE(date_text)	The DATEVALUE function converts a date that is stored as text to a serial number that the **ExpressSpreadSheet** recognizes as a date.

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	that contains text that represents a date in the Excel date format. For example, "1/30/2008" or "30-Jan-2008" are text strings within quotation marks that specify dates.		Like Excel, the **ExpressSpreadSheet** stores dates as sequential serial numbers so that they can be used in calculations. By default, January 1, 1900 is a serial number 1, and January 1, 2008 is serial number 39448 because it is 39447 days after January 1, 1900. Most functions automatically convert date values to serial numbers. The DATEVALUE function returns the #VALUE! error value if the value of the date_text parameter falls outside the range of dates between January 1, 1900 and December 31, 9999.
DAY	One parameter defining the serial number of the required date.	=DAY(TODAY())	Returns the day portion of a given date.
DAYS	Two mandatory parameters: start_date and end_date are two dates between which you want to know the number of days.	=DAYS(end_date, start_date)	Returns the number of days between two dates. If both date parameters are numbers, DAYS uses EndDate-StartDate to calculate the number of days in between both dates. If either one of the date parameters is text, that parameter is treated as DATEVALUE(date_text) and returns an integer value instead of a time component. If date parameters are numeric values that fall outside the range of valid dates, DAYS returns the #NUM! error value. If date parameters are strings that cannot be parsed as valid dates, DAYS returns the #VALUE! error value.
DAYS360	Two mandatory parameters:	=DAYS360(start_date,	The DAYS360 function returns the number of days between two

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	start_date and end_date are two dates between which you want to know the number of days. If start_date occurs after end_date, the DAYS360 returns a negative number. Additionally, you can specify if the **ExpressSpreadSheet** should use the European method instead of the U.S. method in the calculation.	end_date, [method])	dates based on a 360-day year (twelve 30-day months), which is used in some accounting calculations. Use this function to help calculate payments if your accounting system is based on twelve 30-day months. Like Excel, the **ExpressSpreadSheet** stores dates as sequential serial numbers so that they can be used in calculations. By default, January 1, 1900 is a serial number 1, and January 1, 2008 is serial number 39448 because it is 39447 days after January 1, 1900.
EDATE	Two mandatory parameters: start_date specifies the start date, and months specifies the number of months before or after start_date. A positive value for months yields a future date; a negative value yields a past date.	=EDATE(start_date, months)	Returns the serial number of the date that is the indicated number of months before or after a specified date (the start_date). Use EDATE to calculate maturity dates or due dates that fall on the same day of the month as the date of issue. Like Excel, the **ExpressSpreadSheet** stores dates as sequential serial numbers so that they can be used in calculations. By default, January 1, 1900 is a serial number 1, and January 1, 2008 is serial number 39448 because it is 39447 days after January 1, 1900.
EOMONTH	Two mandatory parameters: start_date (specifies the start date), and months (specifies the number of months before or after start_date). A positive value for	=EOMONTH(start_date, months)	Returns the serial number of the last day of the month that is the indicated number of months before or after start_date. Use EOMONTH to calculate maturity dates or due dates that fall on the last day of the month.

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	months yields a future date; a negative value yields a past date.		
HOUR	One parameter defining the serial number of the required date/time value.	=HOUR(NOW())	Returns the hour portion of a given date/time value.
ISOWEEKNUM	The date parameter is the date-time code used by the **ExpressSpreadSheet** for date and time calculations.	=ISOWEEKNUM(date)	Returns the number of ISO week number of the year for a given date.
MONTH	One parameter defining the serial number of the required date.	=MONTH(TODAY())	Returns the month portion of a given date.
MINUTE	One parameter defining the serial number of the required date/time value.	=MINUTE(NOW())	Returns the minutes portion of a given date/time value.
NOW	No parameters required.	=NOW()	Returns the current time in general format. You can apply further formatting to the result of the function.
SECOND	One parameter defining the serial number of the required date/time value.	=SECOND(NOW())	Returns the seconds portion of a given date/time value.
TIME	Three parameters defining hour, minute and second parts of a time value.	=TIME(16, 48, 10)	Returns a decimal number for a specified time.
TIMEVALUE	The time_text parameter is a text string specifies a time in any one of	=TIMEVALUE(time_text)	Returns the decimal number of the time represented by a text string. The decimal number is a value ranging from 0 (zero) to

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	the Microsoft Excel® time formats; for example, "6:45 PM" and "18:45" text strings within quotation marks that specify time.		0.99988426, representing the times from 0:00:00 (12:00:00 AM) to 23:59:59 (11:59:59 PM). Date information in time_text is ignored. Time values are a portion of a date value and represented by a decimal number (for example, 12:00 PM is represented as 0.5 because it is half of a day).
TODAY	No parameters required.	=TODAY()	Returns the serial number of the current date.
WEEKDAY	Two parameters: the serial number of the required date, weekday base.	=WEEKDAY(TODAY())	Returns the day of the week corresponding to the specified date.
		=WEEKDAY(DATE(2002, 12, 1),1) returns 1(Sunday)	The weekday base identifies the first day of the week and determines the return value type: 1 or omitted: the first day of the week is Sunday. The function returns 1 for Sunday, 2 for Monday, etc. 2: the first day of the week is Monday. The function returns 1 for Monday, 2 for Tuesday, etc. 3: the first day of the week is Monday. The function returns 0 for Monday, 1 for Tuesday, etc.
WEEKNUM	The serial_number parameter specifies a date within the week. Dates should be entered by using the DATE function, or as results of other formulas or functions. Additionally, you can specify the return_type parameter that determines on which day the	=WEEKNUM(serial_number, [return_type])	Returns the week number of a specific date. For example, the week containing January 1 is the first week of the year, and is numbered week 1.

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	week begins (the default value is 1).		
YEAR	One parameter defining the serial number of the required date.	=YEAR(TODAY())	Returns the year portion of a given date.
YEARFRAC	Two mandatory parameters include start_date and end_date that represent start date and end date, respectively. Additionally, you can specify which type of the day count basis to use.	=YEARFRAC(start_date, end_date, [basis])	Calculates the fraction of the year represented by the number of whole days between two dates (the start_date and end_date parameters). Use the YEARFRAC worksheet function to identify the proportion of a whole year's benefits or obligations to assign to a specific term.

Text functions

The text functions allow you to work with text strings.

Mnemonics	Operand Type	Example	Explanation
& (ampersand)	Two parameters of type string	= "Yellow "&"river"	Concatenates specified strings.
CHAR	The number parameter allows you to specify a number between 1 and 255 representing the required character. The character is from the character set used by your computer.	=CHAR(number)	Returns the character specified y a number. Use CHAR to translate code page numbers you might get from files on other types of computers into characters.
CLEAN	The text parameter	=CLEAN(text)	Removes all non-printable characters from text. Use CLEAN on text imported from other

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	specifies any worksheet information from which you want to remove non-printable characters.		applications that contains characters that may not print with your operating system. For example, you can use CLEAN to remove some low-level computer code that is frequently at the beginning and end of data files and cannot be printed. The CLEAN function was designed to remove the first 32 nonprinting characters in the 7-bit ASCII code (values 0 through 31) from text. In the Unicode character set, there are additional nonprinting characters (values 127, 129, 141, 143, 144, and 157). By itself, the CLEAN function does not remove these additional nonprinting characters.
CODE	The text parameter specifies the text for which you want the code of the first character.	=CODE(text)	Returns a numeric code for the first character in a text string. The returned code corresponds to the character set used by your computer.
CONCATENATE	A list of strings	=CONCATENATE("Developer", " Express")	Joins several text strings in one text string. An alternative to "&".
DOLLAR	Two parameters defining the value and the number of digits to the right of the decimal point in the output string.	=DOLLAR(957.344, 2)	Converts the number to text using currency format $#,##0.00_); ($#,##0.00), with the decimals rounded to the specified number of places.
EXACT	The text1 and text2 parameters specify the first and second text strings.	=EXACT(text1, text2)	Compares two text strings and returns TRUE if they are exactly the same, FALSE otherwise. EXACT is case-sensitive but ignores formatting differences. Use EXACT to test text being entered into a document.
FIND	The find_text parameter specifies the text you want to find,	=FIND(find_text, within_text, [start_num])	FIND locates one text string within a second text string, and returns the number of the starting position of the first text string from the first

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	the within_text parameter specifies the source text for the text fragment you want to find. Additionally, you can specify the character at which to start the search as the start_num parameter. The first character in within_text is character number 1. If you omit start_num, it is assumed to be 1.		character of the second text string. FIND always counts each character, whether single-byte or double-byte, as 1, no matter what the default language setting is.
FIXED	The first parameter of type double is required. The second parameter of type integer is optional (the default value is 2). The third parameter of type Boolean is optional.	=FIXED(1234.567, 1)	Rounds the first parameter to the number of decimals determined by the second parameter and returns it as a string. The third parameter specifies whether to omit commas in the output string.
LEFT	The first parameter of type string is required. The second parameter of type integer is optional.	=LEFT(A1) =LEFT(A1, 3)	Returns the first character or characters in a text string. The second parameter defines the number of characters to extract. The default value is 1.
LEN	One parameter of type string.	=LEN("ABC")	Returns the length of a given string.
LOWER	One parameter of type string.	=LOWER(A1)	Converts a string to lowercase.
MID	The first parameter is of type string, the second and the third parameters are of type integer.	=MID("ABC",3,1)	Returns the substring of a given text string. The position of the substring is defined by the second parameter. The third parameter specifies the number of characters to extract.

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PROPER	The text parameter of the string type is mandatory. Text enclosed in quotation marks, a formula that returns text, or a reference to a cell containing the text you want to partially capitalize.	=PROPER(text)	Capitalizes the first letter in a text string and any other letters in text that follow any character other than a letter. Converts all other letters to lowercase letters.
REPLACE	Four mandatory parameters: the text in which you want to replace some characters, the position of the character in old_text that you want to replace with new_text, the number of characters in old_text that you want REPLACE to replace with new_text, and the text that will replace a specified number of characters in old_text.	=REPLACE(old_text, start_num, num_chars, new_text)	Replaces part of a text string, based on the number of characters you specify, with a different text string. REPLACE always counts each character, whether single-byte or double-byte, as 1, no matter what the default language setting is.
REPT	Two obligatory parameters: the text you want to repeat, and the positive number of times to repeat text.	=REPT(text, number_times)	Repeats text a given number of times. Use REPT to fill a cell with a number of instances of a text string. If number_times is 0 (zero), REPT returns "" (empty string). If number_times is not an integer, it is truncated. The result of the REPT function cannot be longer than 32767 characters, otherwise REPT returns the #VALUE! error value.

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RIGHT	The first parameter of type string is required. The second parameter of type integer is optional.	=RIGHT(B2, 3)	Returns the last character or characters in a text string. The second parameter defines the number of characters to extract. The default value is 1.
SEARCH	Two obligatory parameters of string type.	=SEARCH("n", "printer")	The SEARCH function locates one text string with a second text string, and returns the number of the starting position of the first text string from the first character of the second text string. For example, to find the position of the letter "n" in the word "printer", you can use this function as =SEARCH("n", "printer").
SUBSTITUTE	Three obligatory parameters of string type plus an optional instance_num parameter that allows you to specify what instance of the old text should be replaced.	=SUBSTITUTE (text, old_text, new_text, [instance_num])	Substitutes new_text for old_text in a text string. Use SUBSTITUTE when you want to replace specific text in a text string; use REPLACE when you want to replace any text that occurs in a specific location in a text string.
T	One obligatory parameter of any type.	=T(value)	Returns the text referred by value.
TEXT	Two obligatory parameters: first is a numeric value or a reference to a cell containing a numeric value; second is a numeric format as a text string enclosed in quotation marks, for example "m/d/yyyy" or "#,##0.00".	=TEXT(A1, "$0.00")	The TEXT function converts a numeric value to text and allows you to specify the display formatting by using special format strings. This function is useful in situations where you want to display numbers in a more readable format, or you want to combine numbers with text or symbols. For example, suppose cell A1 contains the number 23.5. To format the number as a dollar amount, you can use the following formula: =TEXT(A1, "$0.00")

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TRIM	One parameter of string type	=TRIM(" Express Spread Sheet ")	Removes all spaces from text except for single spaces between words.
TRUNC	Two parameters: the number that is truncated, and the optional num_digits parameter that specifies the precision of the trunctaion. The default value for num_digits is 0 (zero).	=TRUNC(number, [num_digits])	Truncates a number to an integer by removing the fractional part of the number.
UPPER	One parameter of type string.	=UPPER(A1&A2)	Converts a string to uppercase.
VALUE	One obligatory parameter of string type.	=VALUE(text)	Converts a text string that represents a number to a member. Text can be in any of the constant number, date, or time formats recognized by the **ExpressSpreadSheet**. If text is not in one of these formats, VALUE returns the #VALUE! error value. You do not generally need to use the VALUE function in a formula because the **ExpressSpreadSheet** automatically converts text to numbers as necessary. This function is provided for compatibility with other spreadsheet applications.

Information functions

Functions which test the type of a value and return a Boolean result.

Mnemonics	Operand Type	Example	Explanation

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ISBLANK	A value of any type.	=ISBLANK(A1)	Returns TRUE if cell is empty.
ISERR	A value of any type.	=ISERR(H1)	Returns TRUE if the cell contains any error value except #N/A.
ISEVEN	A value of any type.	=ISEVEN(B1)	Returns TRUE if the number is even
ISERROR	A value of any type.	=ISERROR(A7)	Returns TRUE if the cell contains any error value (#N/A, #VALUE!, #REF!, #DIV/0!, #NUM!, #NAME?, or #NULL!).
ISLOGICAL	A value of any type.	=ISLOGICAL(C3)	Returns TRUE if the specified value refers to a logical value.
ISNA	A value of any type.	=ISNA(E5)	Returns TRUE if the cell contains #N/A (value not available) error value.
ISNONTEXT	A value of any type.	=ISNONTEXT(D4)	Returns TRUE if the cell does not contain text. Returns TRUE for blank cells.
ISNUMBER	A value of any type.	=ISNUMBER(A2)	Returns TRUE if the cell contains a number.
ISTEXT	A value of any type.	=ISTEXT(D1)	Returns TRUE if the specified cell contains text.
ISODD	A value of any type.	=ISODD(A2)	Returns TRUE if the number is odd.
ISTEXT	A value of any type.	=ISTEXT(A1)	Returns TRUE if the value is a text.
N	A value of any type.	=N(F7)	Returns a value converted to a number.
NA	A value of any type.	=NA(B2)	Returns the error value #N/A.

11.5 Renaming Spreadsheets

To rename your **Spreadsheet**:

1. Open the Data Navigator 126.
2. Right click on the spreadsheet to rename.
3. Select **Rename** from the list.(Figure 11.19)
4. Enter the new name in the edit field and press **Enter**.

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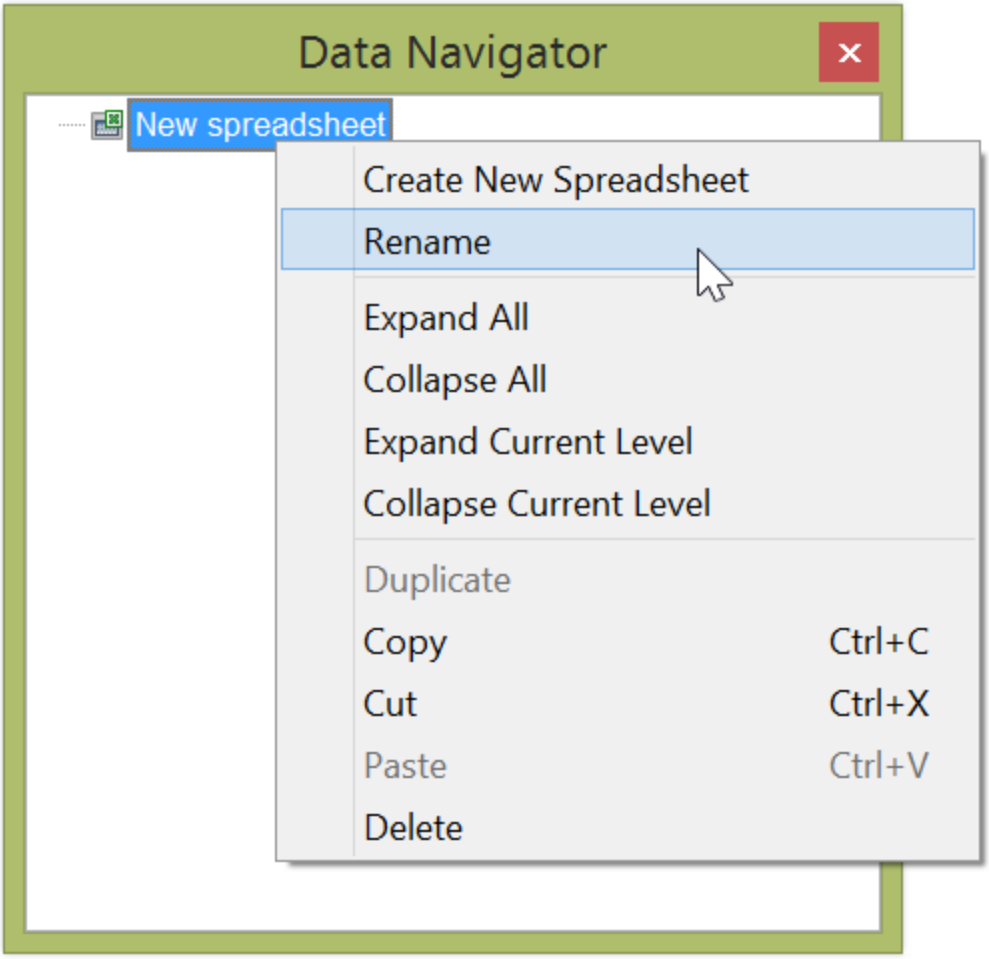

Figure 11.19 Rename a spreadsheet.

11.6 Deleting Spreadsheets

To delete a **Spreadsheet**

1. Select the spreadsheet in the layout.
2. Press **Delete**. If you want to immediately restore a spreadsheet that was deleted by mistake, use the Undo command in the Quick Access Toolbar 65 .

Please note that the steps above will only delete the spreadsheet object from the page but it will still remain associated with the layout in the Data Navigator 126. To completely remove a spreadsheet from the layout right-click on the spreadsheet in the **Data Navigator** and choose **Delete** from the pop-up menu.

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11.7 Exporting Data in Spreadsheets

Spreadsheets created within FCS Express may be exported to various external file formats such as **.xls**, **.xls**, **.csv**, **.html**, **.xml** and **.txt**.
Formulas, formatting, and references will be preserved when selecting to save in the **.xls** or **.xlsx** file format.

To export a spreadsheet created within FCS Express:
1. Left click within the desired spreadsheet to select it. The spreadsheet will enter into Edit mode (green borders) and the Spreadsheet category will appear in the Ribbon bar (Figure 11.20, Step 1).
2. Select **Spreadsheet→Layout→Export→Export** (Figure below, Step 2) in the Ribbon.
3. Navigate to the desired file path location in the **Export Spreadsheet To** dialog (Figure below, Step 3).
4. Enter the file name to be used.
5. Select the desired file format (Figure below, Step 4).
6. Click **Save**.

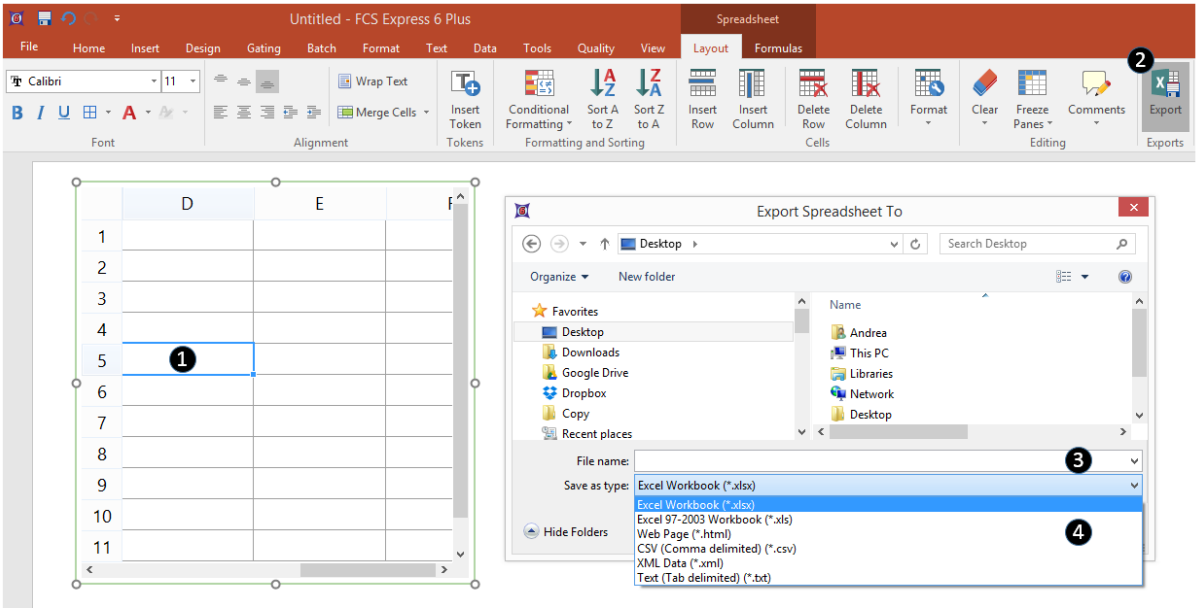

Figure 11.20 How to Export a Spreadsheet created whitin FCS Express.

11.8 Graphing Data with Pie Charts, Bar Plots, and Scatter with Regression Plots

Data in a spreadsheet can be graphed in FCS Express as Pie Charts 538, Bar Plots 547, Scatter Plots 559, and Scatter with Regression Plots 579. Graphing data from spreadsheets in FCS Express allows you to easily visualize trends in your data as plots update in real time in response to gate, marker, and quadrant adjustments or as data files update via Next/Previous 79 or Batch Processing 784.

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Data may also be graphed from data files values inserted in a spreadsheet from the data list 433.

- Graphing Bar Plots 547
- Graphing Pie Charts 538
- Graphing Scatter Plots 559
- Scatter with Regression Plots 579.

Please note that in all graph types the first row of a corresponding spreadsheet may be enable for use as the data label source. Please see the Formatting Spreadsheet section for additional details 422.

11.8.1 Pie Charts

Pie charts are a convenient means for visualizing how several statistics relate to one another and combine to form a conceptual whole (e.g., Figure 11.21).

Pie Charts can be inserted anywhere on your layout 539, and can be populated via drag-and-drop 540 or from Spreadsheets 542 containing statistical tokens or freetext. They are also fully formattable 544.

The appearance of Pie Charts in FCS Express is customizable via special formatting options 544.

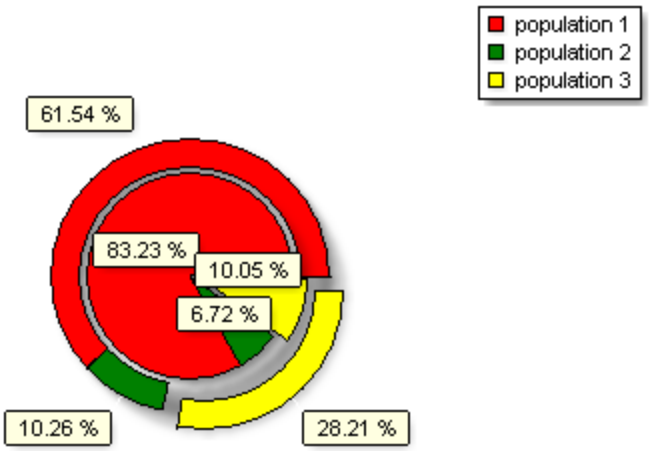

Figure 11.21 Pie Chart in FCS Express

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11.8.1.1 Inserting Pie Charts

Pie Charts are inserted like any other plot in FCS Express (see below). In FCS Express, Pie Charts may be created easily by drag and drop 540, or from an existing spreadsheet 542.

To insert a blank Pie Chart to populate by drag and drop 540:

1. Click the **Insert** tab→**1D Plots**→**Pie** command.
2. Click on a blank space of the layout to insert the Pie Chart.

An empty Pie Chart will appear, to which you can drag and drop any statistic in FCS Express to populate the pie 540. Note: if a spreadsheet exists in your layout, the inserted Pie Chart will be populated with its data. Simply insert another Pie Chart to create an empty Pie Chart for receiving the dragged and dropped items. The first Pie Chart may be deleted, if desired.

To insert a Pie Chart from a spreadsheet:

1. Select a spreadsheet in the Layout 415.
2. Click the **Insert** tab→**1D Plots**→**Pie** command (Figure 11.22).
3. Click on a blank space of the layout to insert the Pie Chart.

Note: The spreadsheet should be formatted so the first column (A) contains the name of the series and the second column (B) contains the values to be plotted. If the data exists in different rows the Pie Chart may be formatted to select the appropriate columns of data. 544

In both cases, a new Pie Chart will appear on the layout that is populated with the data from the spreadsheet. The Pie Chart may now be populated with the desired parameters 542 and formatted as you wish 544.

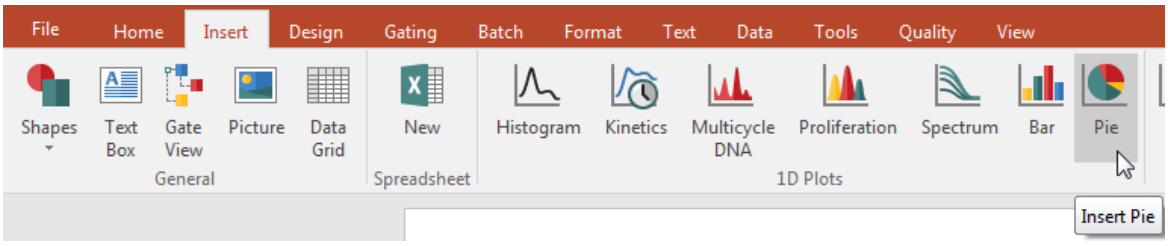

Figure 11.22 Inserting a Pie Chart

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11.8.1.1.1 Populating Pie Charts by Drag and Drop

Pie Charts are easy to populate through the drag-and-drop functionality in FCS Express. You can drag plots or gates[541], statistical tokens[541], or free text to create or replace sectors (slices)[542] on a blank Pie Chart that has already been inserted on your layout[539].

When populating a previously blank Pie Chart by drag-and-drop, the following actions are available:

- Insert a new statistic or value to be plotted as a slice in the pie chart.[541]
- Replace the value of an existing slice in pie chart with a new value.[542]

See the figures and steps below to learn how to populate Pie Charts by drag-and-drop.

After inserting a blank Pie Chart[539], dragging and dropping gates onto it will cause the **Edit Statistic** dialog to appear. The specific statistic relating to the gate can be chosen for plotting (e.g., "% of Gated Cells" in Fig. 11.23).

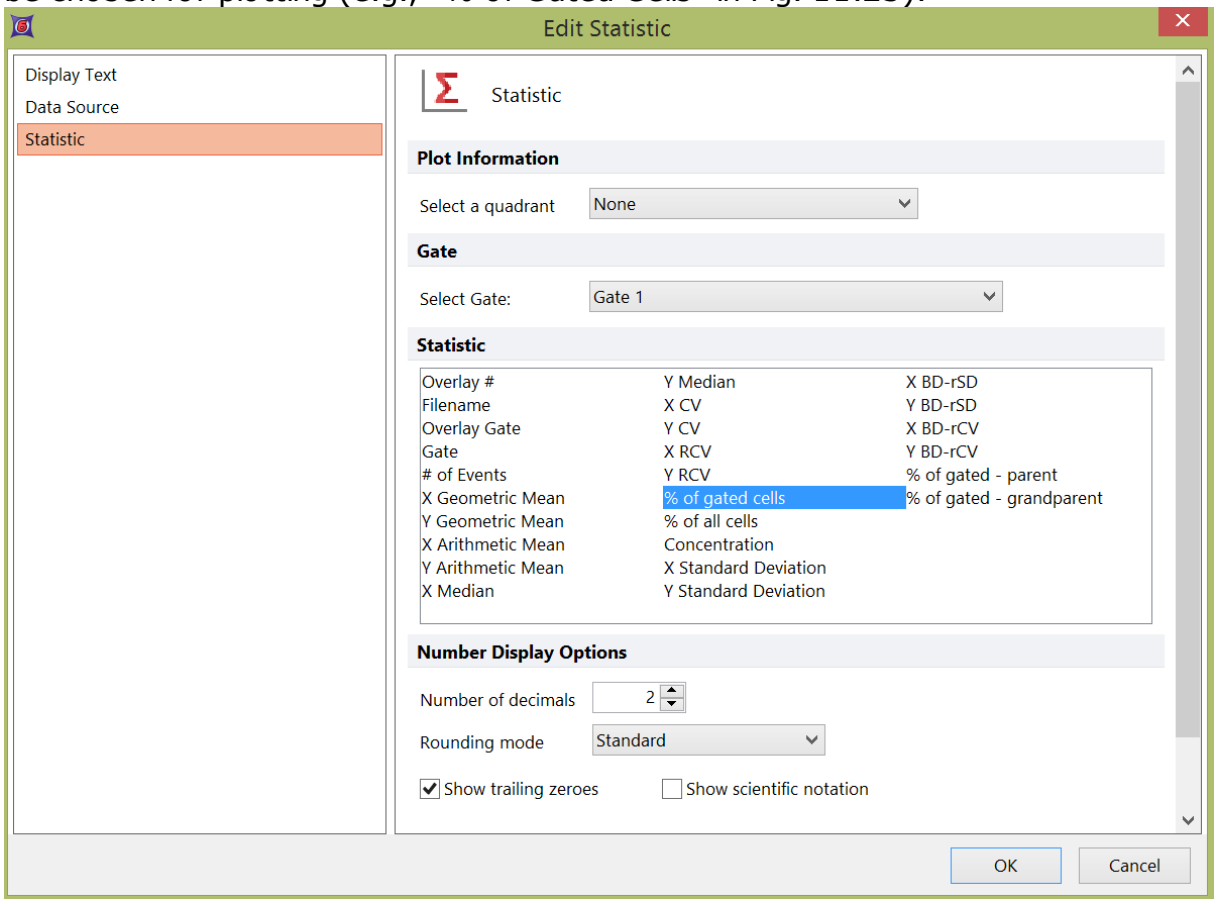

Figure 11.23 Edit Statistic dialog appearing after dragging a gate to a Pie Chart

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The result of dragging and dropping a gate to a Pie Chart and choosing the desired statistic in the Edit Statistic 540 dialog is shown in Fig. 11.24 below.

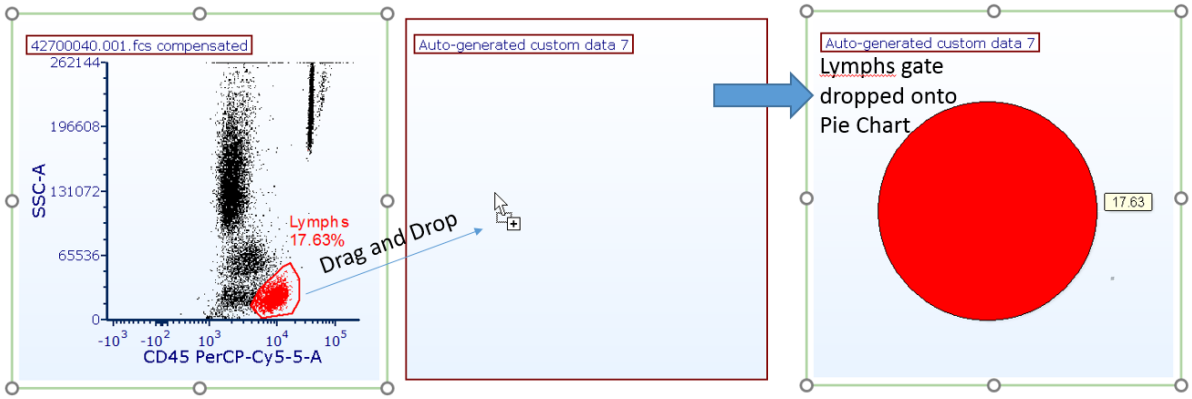

Figure 11.24 Dragging a gate to a Pie Chart to add a sector (slice)

Statistical tokens or freetext from default statistics windows or text boxes on your layout can also be dragged directly to Pie Chart. The data value must be dropped on the plot outside of the existing pie area (if dropped on the pie area the current value representing the slice will be replaced with the dropped value 542). In this case, the **Edit Statistic** dialog will not appear. In the example below, the selected "Gate 1, % of gated cells" statistic is dragged to an empty space on the Pie Chart, in order to add a new sector (slice) (Fig. 11.25).

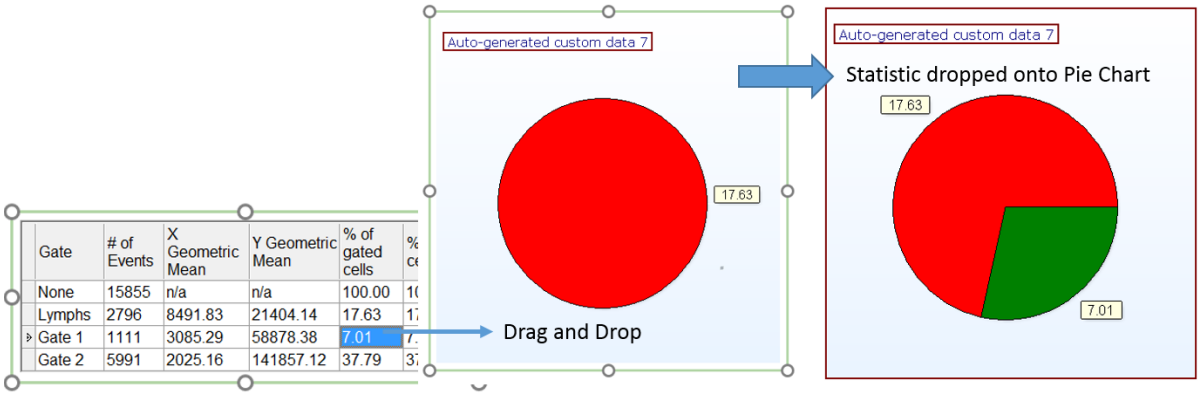

Figure 11.25 Dragging and dropping a statistic token to the Pie Chart

Dragging a plot, gate, or statistic onto an existing sector (slice) will replace it with the desired statistic, as shown below (Fig. 11.26).

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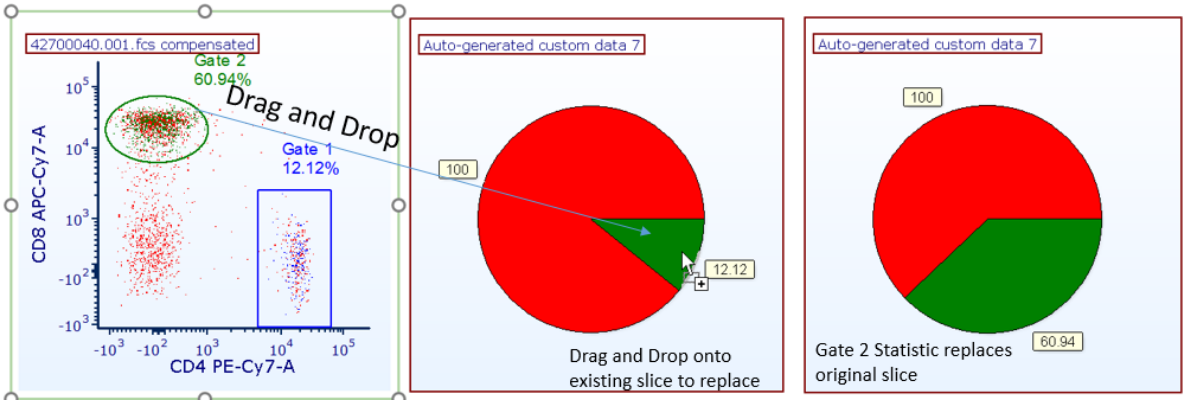

Figure 11.26 Dragging and dropping a gate to replace an existing sector (slice) on a Pie Chart

11.8.1.1.2 Populating Pie Charts from Spreadsheets

An alternative to populating Pie Charts by dragging and dropping values is to populate a pie chart from an existing Spreadsheet 414.

If a Spreadsheet exists on the layout, an inserted Pie Chart 539 will be automatically populated with the data from Columns A and Column B within the Pie. Column A will be the series name and column B the value used in the Pie Chart. If more than one spreadsheet exists, select 1212 that which is intended as the data source prior to inserting the Pie Chart 539.

By default, the first two columns of the Custom Data Grid for the Name- and Value-parameters of each sector (slice) will be plotted on the Pie Chart, respectively.

To populate the Pie Chart with parameters other than these first two columns of the Custom Data Grid,

1. Select the **Format** tab→**Plot Options**→**Overlays** command (Figure 11.27)

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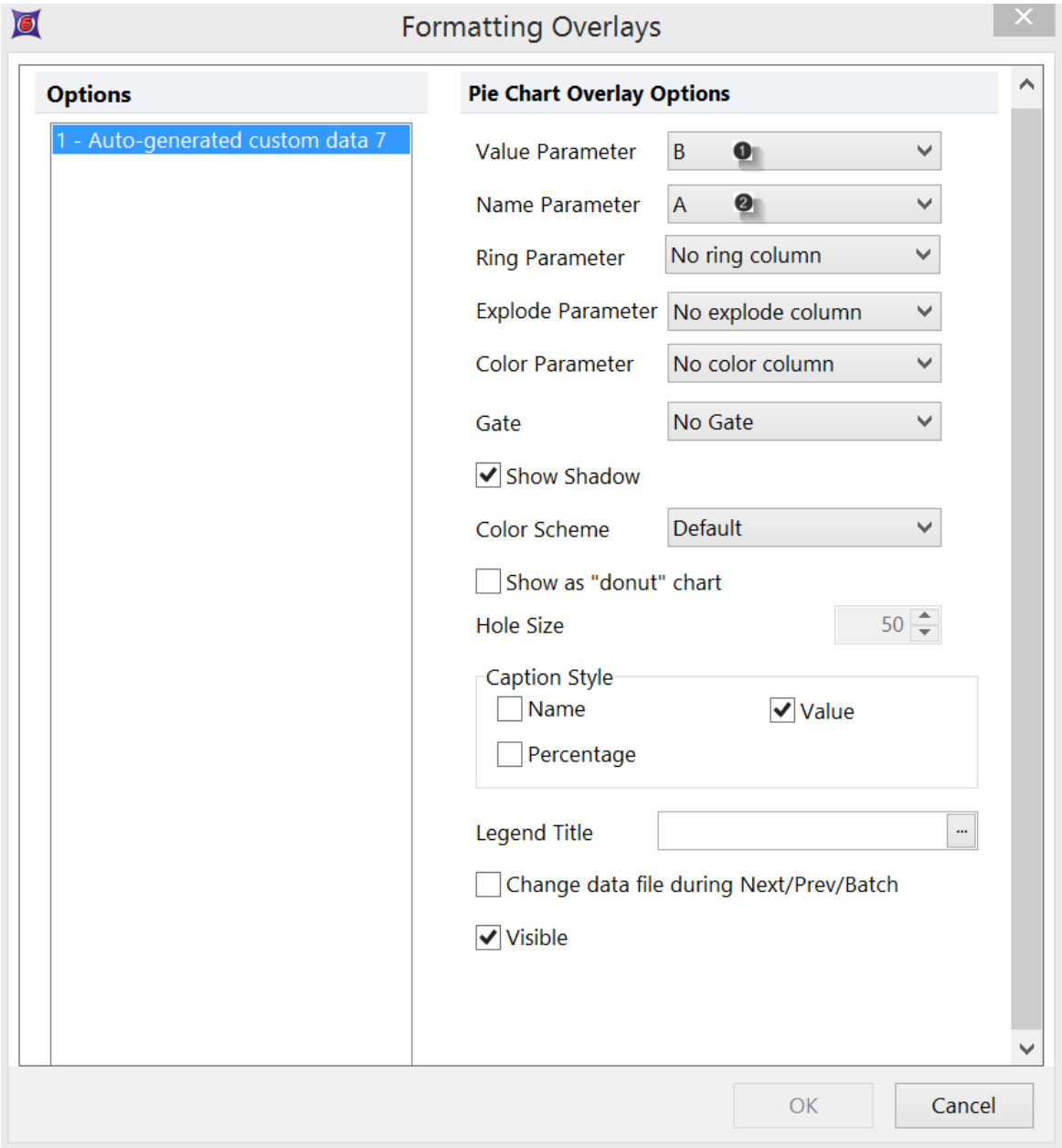

Figure 11.27 Choosing parameters for Pie Chart

2. Choose the Spreadsheet column intended for the value of the sector (slice), from the **Value Parameter** drop-down menu (❶)
3. Choose the Spreadsheet column intended for the name of the sector (slice), from the **Name Parameter** drop-down menu (❷)

See Formatting Pie Charts 544 for details on how to customize colors, legends, rings, and more.

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11.8.1.2 Formatting Pie Charts

Pie Charts in FCS Express are fully formattable. In addition to many of the formatting options shared by all plots 246 (border, size, background, titles, and legend), special formatting options are accessible from the **Overlays** category. To access these special options:

- Select the Pie Chart and use the **Format** tab→**Plot Options**→**Overlays** command (Figure 11.28), or
- Right-click on the Pie Chart, select **Format** from the pop-up menu, and choose the **Overlays** category.

Special formatting options for Pie Charts are pictured below and include:

- Plotting additional data as a **Ring Parameter** (❶)
- **Explode Parameter** for exploding individual sectors (slices) for emphasis (❷)
- **Color Parameter** for sector (slice) colors (❸, ❹)
- **Shadow** (❺)
- Displaying of Pie Chart as filled pie (default) or "**donut**" (❻)
- **Caption Style** (❼)

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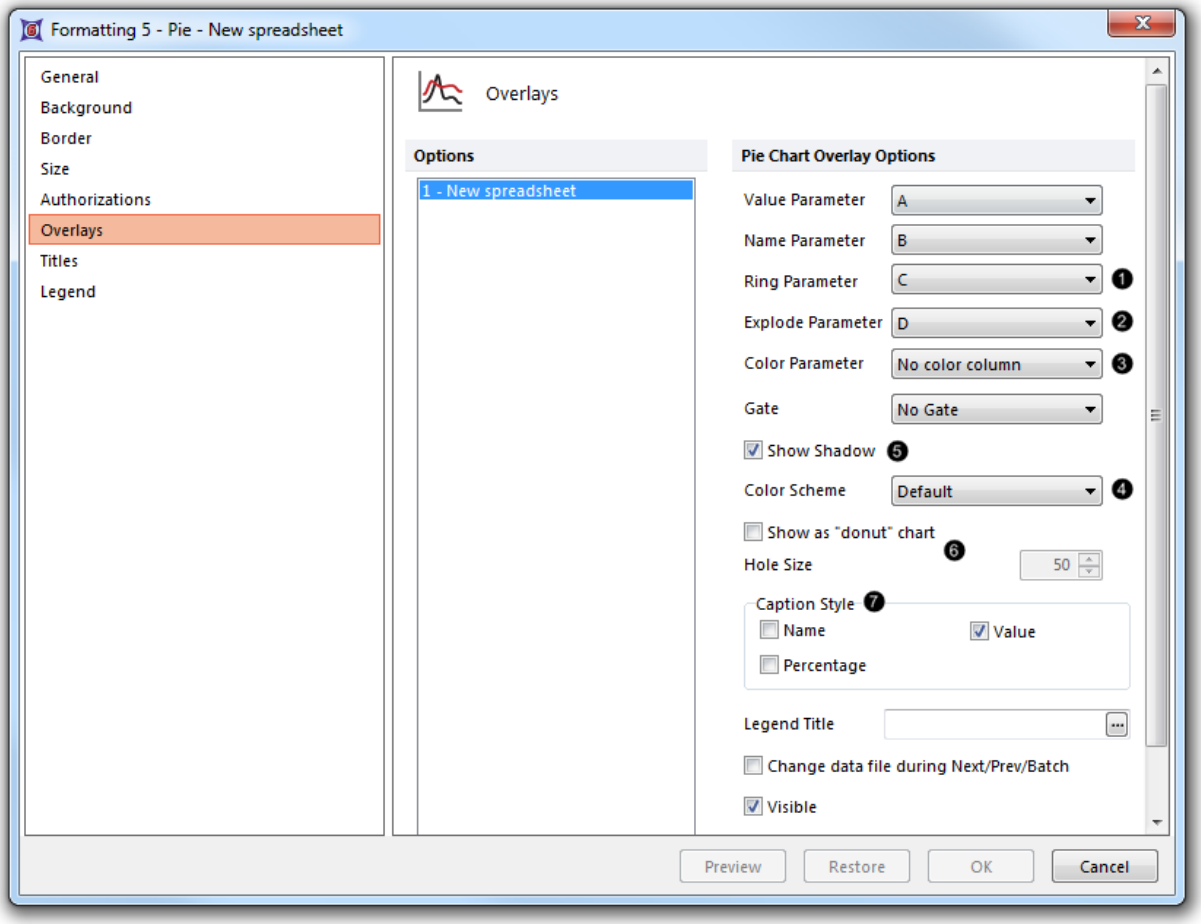

Figure 11.28 Special formatting options for Pie Charts

The formatting options are described in the table below.

Option	Explanation
Value Parameter	The parameter (column) from the Spreadsheet displayed as value of the sector (slice).
Name Parameter	The parameter (column) from the Spreadsheet displayed as name of the sector (slice).
Ring Parameter ❶	The parameter (column) from the Spreadsheet displayed as sector values of the Ring. See below for more information on displaying additional data as a Ring on Pie Charts.
Explode Parameter ❷	The parameter (column) from the Spreadsheet from which the Explode rule derives. A sector (slice) will explode away from the pie for emphasis (e.g., the yellow sector shown below) if its value meets the criterion defined by the Explode rule. See below for more information on how to define am Explode rule.
Color Parameter ❸	The parameter (column) from the Spreadsheet defining the display color the sector (slice). See the Pie Charts tutorial for details.
Show Shadow ❺	Whether a shadow behind the Pie Chart is shown.

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Option	Explanation
Color Scheme ❹	Any of twenty preset coloring schemes which can be applied to the Pie Chart.
Show as "donut" chart ❻	Whether the Pie Chart is shown with a hole in the center.
Hole size ❻	If the Pie Chart is shown as "donut" (❻), the size of the donut hole.
Caption Style ❼	The Name, Value, percentage of the pie (note: "percentage of the pie" differs from "percent gated") or any combination thereof can be shown as text directly beside each sector of the pie or ring.
Legend Title	Text title for the Name parameters listed in the Legend.
Change data file during Next/Prev/Batch	Whether the Pie Chart changes during batch processing 784 or in response to a Next/Previous 79 command. It is generally recommended to leave this unchecked (default), as the Pie Chart will update automatically as the data to which it refers is changed.
Visible	Whether the Pie Chart is visible within the object.

To add Ring values as additional data at the periphery of the pie 546 or Exploding for emphasis 546 to a Pie Chart, these values must be entered into designated columns in the Spreadsheet.

To define values for the Ring:

1. Go to an empty column of the Spreadsheet 414.
2. Enter the value 419 of the Ring for each row (each row corresponds to an individual slice, or sector, of the pie). This value may be freetext or a custom token 727.
3. Choose the appropriate Spreadsheet column for the Ring Parameter (Figure 11 above, ❶)

An example of a Pie Chart with additional Ring values (plotted at the periphery of the Pie) and its associated Spreadsheet is shown in Figure 11.29 below.

To define an Explode rule:

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the rule for each row (each row corresponds to an individual slice, or sector, of the pie). The rule must be a formula evaluating to "TRUE" or "FALSE"; if "TRUE," the sector (slice) will explode away from the pie. An example of an Explode rule referring to a cell within the Spreadsheet is shown in Figure 11 below.
3. Choose the appropriate Spreadsheet column for the Explode Parameter (Figure 11 above, ❷)

An example of a Pie Chart with the Explode option and its associated Spreadsheet is shown in Figure 11 below.

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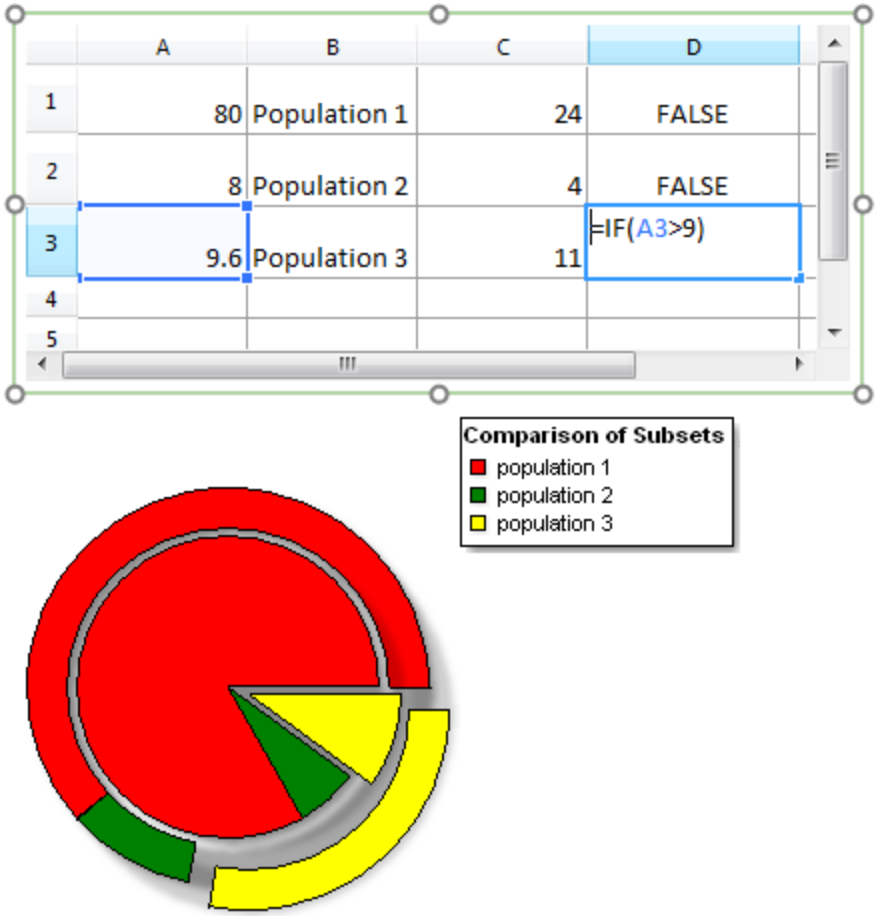

Figure 11.29 Pie Chart and associated Spreadsheet with Ring and Explode formatting options

11.8.2 Bar Plots

Bar Plots are a convenient visualization tool for comparing several statistics at a glance, whether these derive from different gates, plots, data files, ratios, or complex statistical formulae. Bar plots are unitless along the X-axis, being labeled with user-defined, nominal categories instead (e.g., Figure 11.30). Thus bar plots are not truly bivariate, unlike scatter plots.

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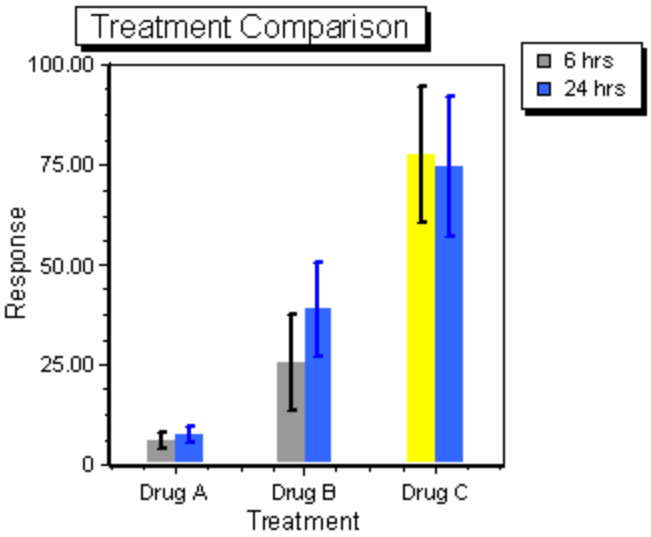

Figure 11.30. Bar Plot in FCS Express

Bar Plots can be inserted anywhere on your layout[548], and can be populated from Spreadsheets[553] or by dragging and dropping gates, plots, or statistical tokens[549]. They are also fully formattable[556].

11.8.2.1 Inserting Bar Plots

Bar Plots are inserted like any other plot in FCS Express (see below). In FCS Express 6, Bar Plots may be created easily by drag and drop[548], or from a Spreadsheet[548].

To insert a blank Bar Plot to populate by drag and drop[549]:

1. Click the **Insert** tab→**1D Plots**→**Bar** command (Figure 11.31).
2. Click on a blank space of the layout to insert the Bar Chart.

An empty Bar Plot will appear, to which you can drag and drop any statistic in FCS Express to populate the bars[549]. Note: if a Custom Data Grid exists in your layout, the inserted Bar Plot will be populated with its data. Simply insert another Bar Plot to create an empty Bar Plot for receiving the dragged and dropped items. The first Bar Plot may be deleted, if desired.

To insert a Bar Plot from an existing Spreadsheet:

1. Select a Spreadsheet in the Layout[414].
2. Click the **Insert** tab→**1D Plots**→**Bar** command.
3. Click on a blank space of the layout to insert the Bar Plot.

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Alternatively:
1. Drag a Spreadsheet from the Data Navigator 126 to an empty part of the page.
2. Release the mouse button.
3. Select **Bar** from the **Select Plot Types...** dialog.

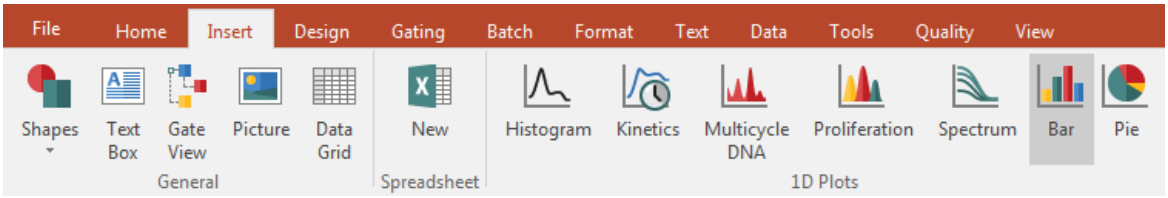

Figure 11.31 Inserting a Bar Plot

See the following sections to learn how to add data to Bar Plots:
- **Populating Bar Plots by Drag-and-Drop** 549
- **Populating Bar Plots from Spreadsheets** 553

11.8.2.1.1 Populating Bar Plots by Drag-and-Drop

Bar Plots are easy to populate through the drag-and-drop functionality in FCS Express 6. You can drag gates 550, statistical tokens 551, or freetext to create bars on a Bar Plot that has been inserted on your layout 548.

When populating a Bar Plot by drag-and-drop, the following attributes can be controlled:

- The specific statistic 549 relating to the gate to be plotted.
- The position of the new bar 551 relative to existing bars.
- The addition of new overlays 552 to the Bar Plot.
- The X-axis label 553 for each bar.

See the figures and steps below to learn how to populate Bar Plots by drag-and-drop.

After inserting a Bar Plot 548, dragging and dropping gates onto it will cause the **Edit Statistic** dialog to appear. The specific statistic relating to the gate can be chosen for plotting (e.g., "% of Gated Cells" in Fig. 11.32).

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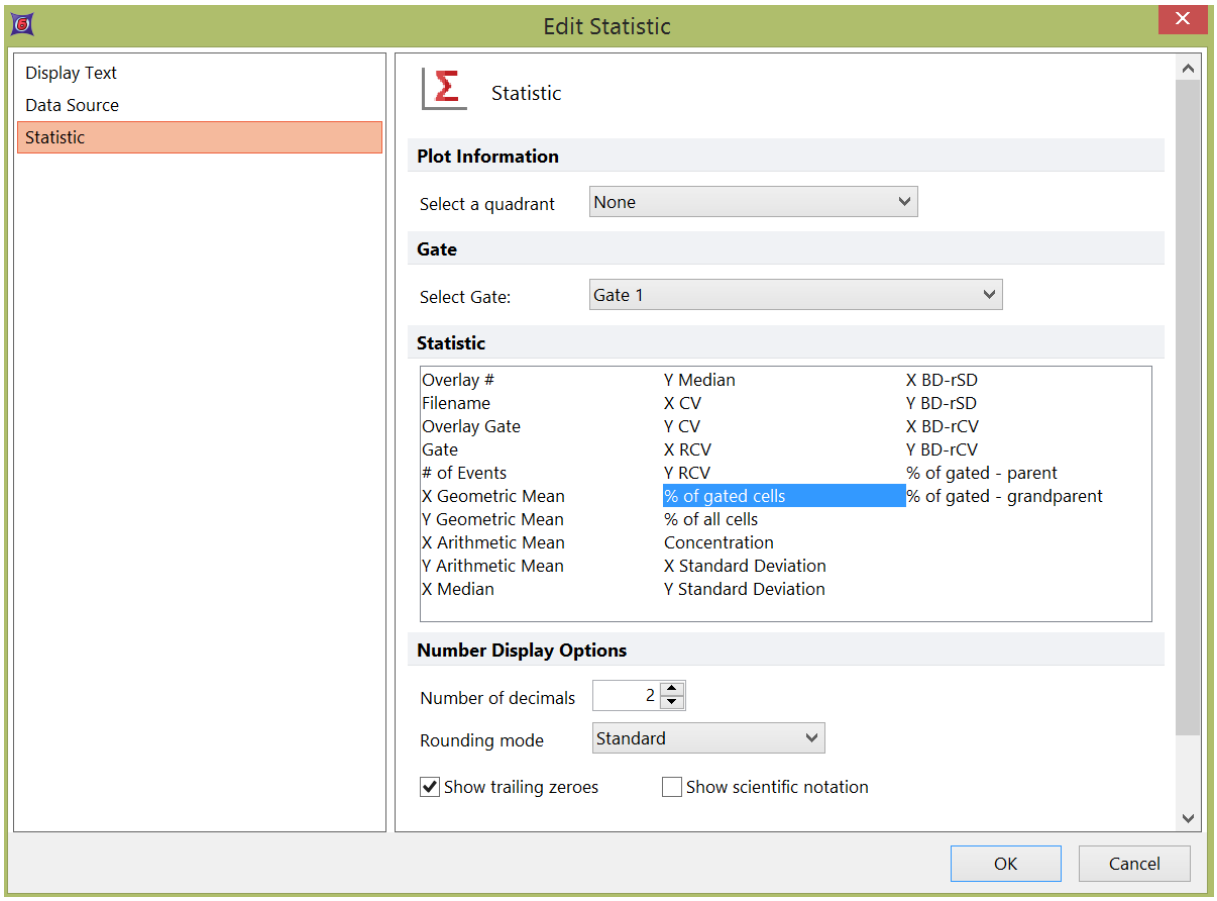

Figure 11.32 Edit Statistic dialog appearing after dragging a gate to a Bar Plot

The result of dragging and dropping a gate to a bar plot and choosing the desired statistic in the Edit Statistic 549 dialog is shown in Fig. 11.33.

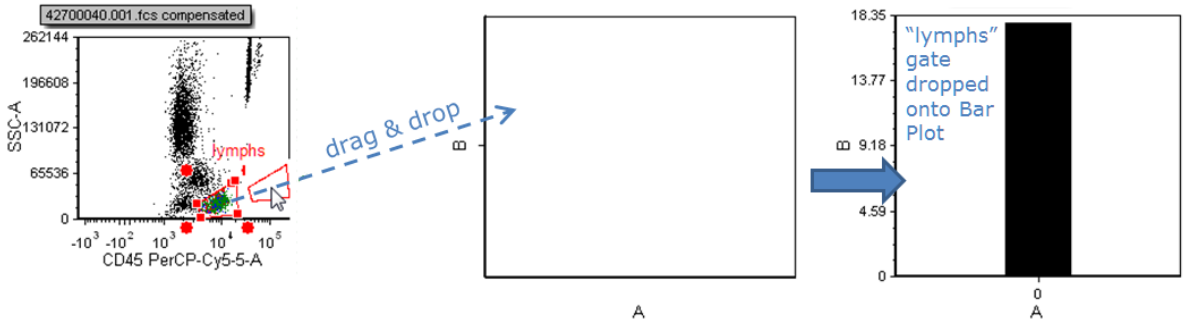

Figure 11.33 Dragging and dropping a gate onto a Bar Plot

A second gate can be dragged to the Bar Plot to open the Edit Statistic 549 dialog, from which a statistic for the second bar is chosen (Fig. 11.34).

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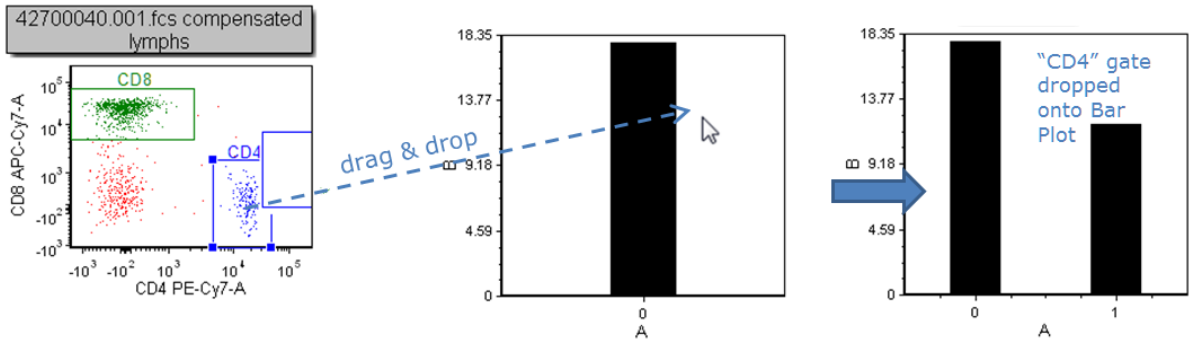

Figure 11.34 Dragging and dropping a second gate onto a Bar Plot

Statistical tokens or freetext from default statistics windows or text boxes on your layout can also be dragged directly to Bar Plots. In this case, the **Edit Statistic** dialog will not appear. In the example below, the selected "CD8, % of gated cells" statistic is dragged to the second position, to form a new bar between the existing two (Fig. 11.35).

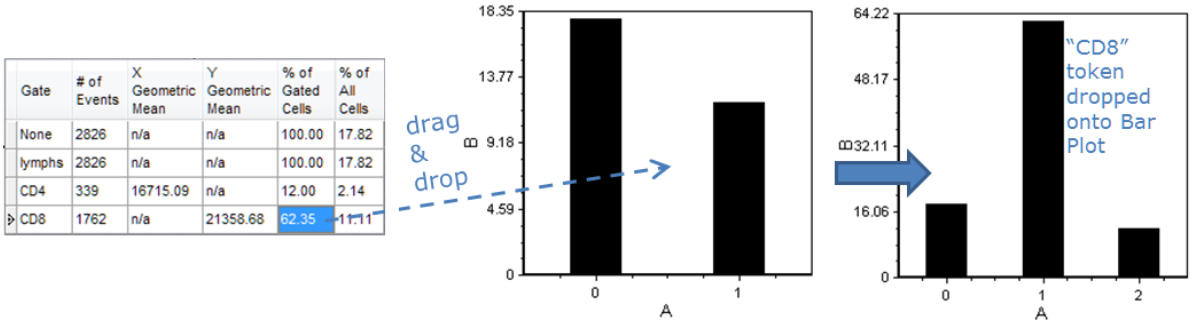

Figure 11.35 Dragging and dropping a statistic token to the middle position in a Bar Plot

To add an overlay, drag the data source toward the Bar Plot and drop it below the axis (Fig. 11.36, ❶); the overlay will be overlaid onto the first bar.

To add subsequent bars, drag the data source toward the Bar Plot and drop it to the right of the intended bar of the first overlay (Fig. 11 (below) ❷).

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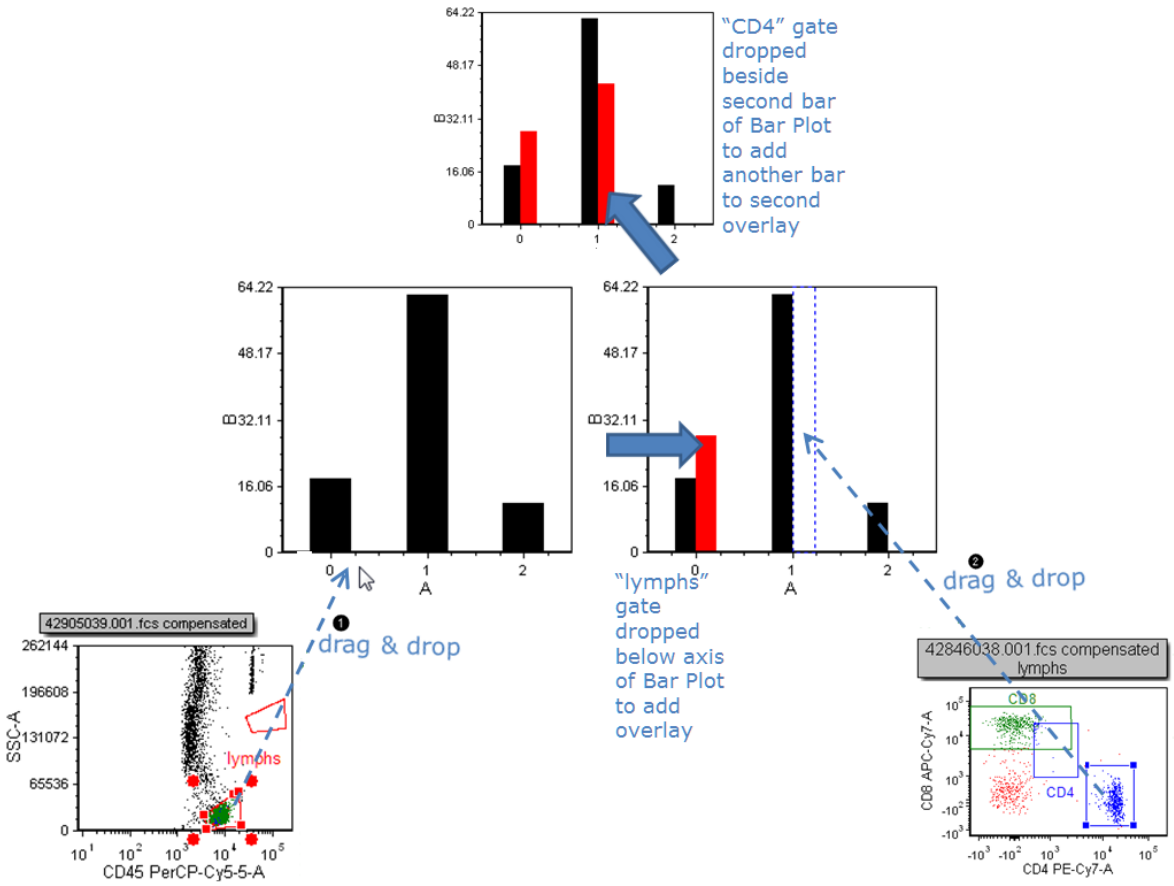

Figure 11.36 Dragging and dropping an overlay to a Bar Plot

To add a descriptive X-axis label for each bar:

1. Click on the label
2. Enter freetext or tokens[692] (using the T button) into the label editor bar. Fig. 11.37.
3. Click the green check mark in the label editor bar or hit **Enter** on the keyboard to accept the change. In the example below, the label for the second bar has been edited to "population 2".

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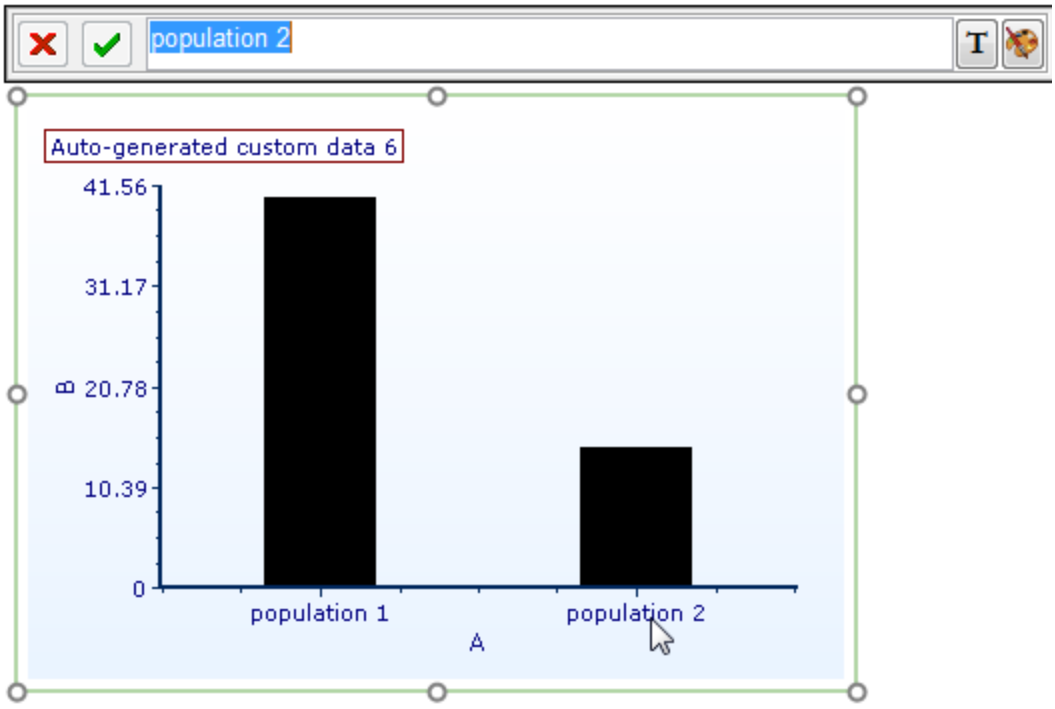

Figure 11.37 Clicking on a Bar Plot axis label to edit it

See the Formatting Bar Plots section[556] for details on how to customize colors, bar style, error bars, and more.

11.8.2.1.2 Populating Bar Plots from Spreadsheets

It is generally more convenient to populate Bar Plots via drag-and-drop[549], but the following situations require use of a Spreadsheet[414]:

- Bars derived from:
  - Custom Tokens[727] that cannot serve as a drag source during drag-and-drop (e.g., those not visible within a text box[692])
  - The results of Parameter Math or array formulas[748] calculated within a Spreadsheet[417], and

- Error bars or highlighting parameters[556].
  Note: Bar Plots requiring these formatting elements can still be populated with bars via drag-and-drop[549], but accessing the Spreadsheet is required to add them.

If a Spreadsheet exists on the layout, an inserted Bar Plot[548] will be automatically populated with the data within the Spreadsheet. If more than one Spreadsheet exists, select[1212] that which is intended as the data source prior to inserting the Bar Plot[548].

By default, the Bar Plot will plot the first two columns of the Spreadsheet for the X- and Y-parameters, respectively.

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To populate the Bar Plot with parameters other than the first two columns of the Spreadsheet,

1. Select the **Format** tab→**Plot Options**→**Overlays** command (Figure 11.38).

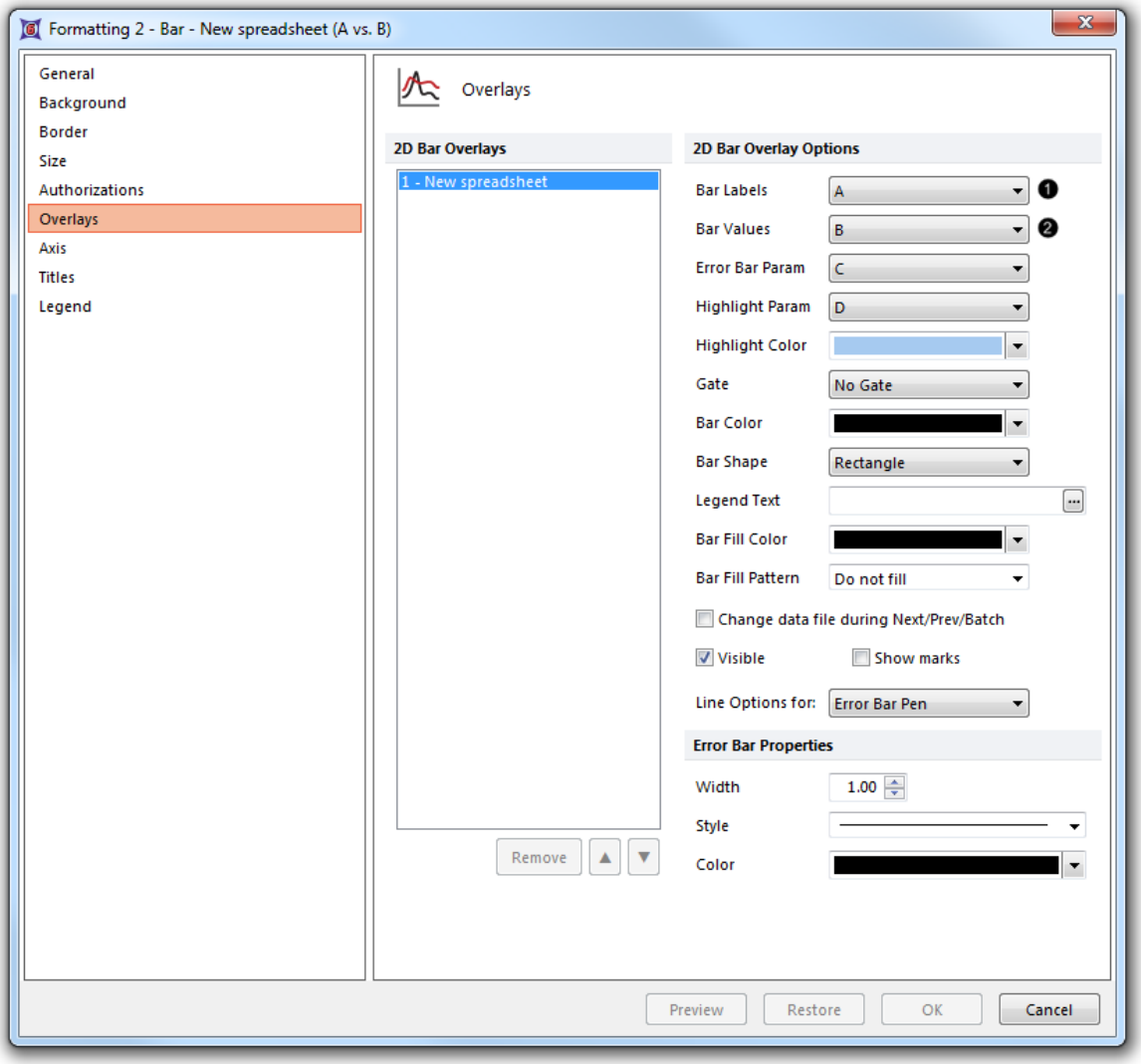

Fig. 11.38 Populating a Bar Plot from Spreadsheet parameters

2. Choose the parameter (i.e., Spreadsheet column) intended for the X-axis, from the **Bar Labels** drop-down menu (❶).

3. Choose the parameter (i.e., Spreadsheet column) intended for the Y-axis, from the **Bar Values** drop-down menu (❷).

To add an overlay to an existing Bar Plot, either

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- Drag and drop a Spreadsheet onto the Bar Plot, or
- Drag and drop another Bar Plot onto the recipient Bar Plot, or
- Right-click on the Bar Plot

1. Select **Add Overlay using Advanced Open Data Dialog** from the pop-up menu.
2. Select the data source in the **Active Files** tab of the **Open Data File** dialog (Figure 11.39).
3. Click **OK.**

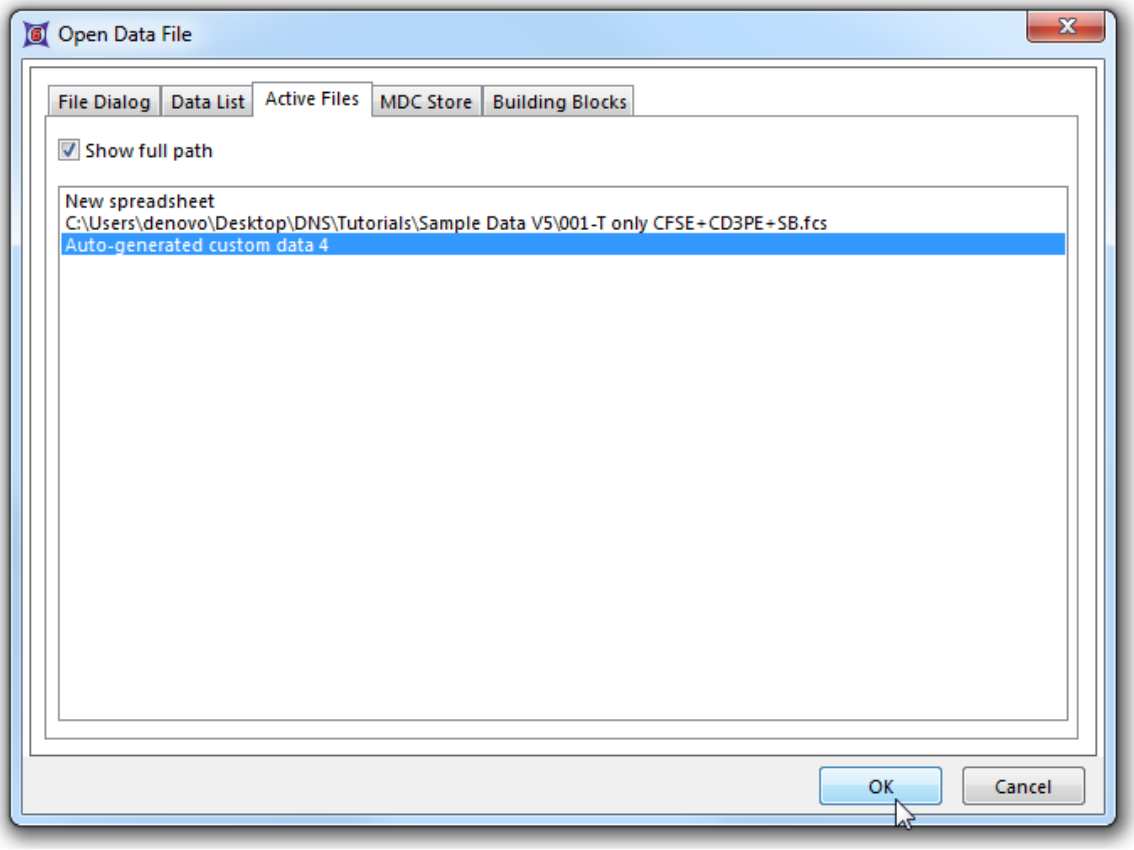

Figure 11.39 Selecting a Spreadsheet to overlay onto an existing Bar Plot

4. Select the parameters for the new Overlay in the **Add overlays** dialog.
5. Click **OK** (Figure 11.40).

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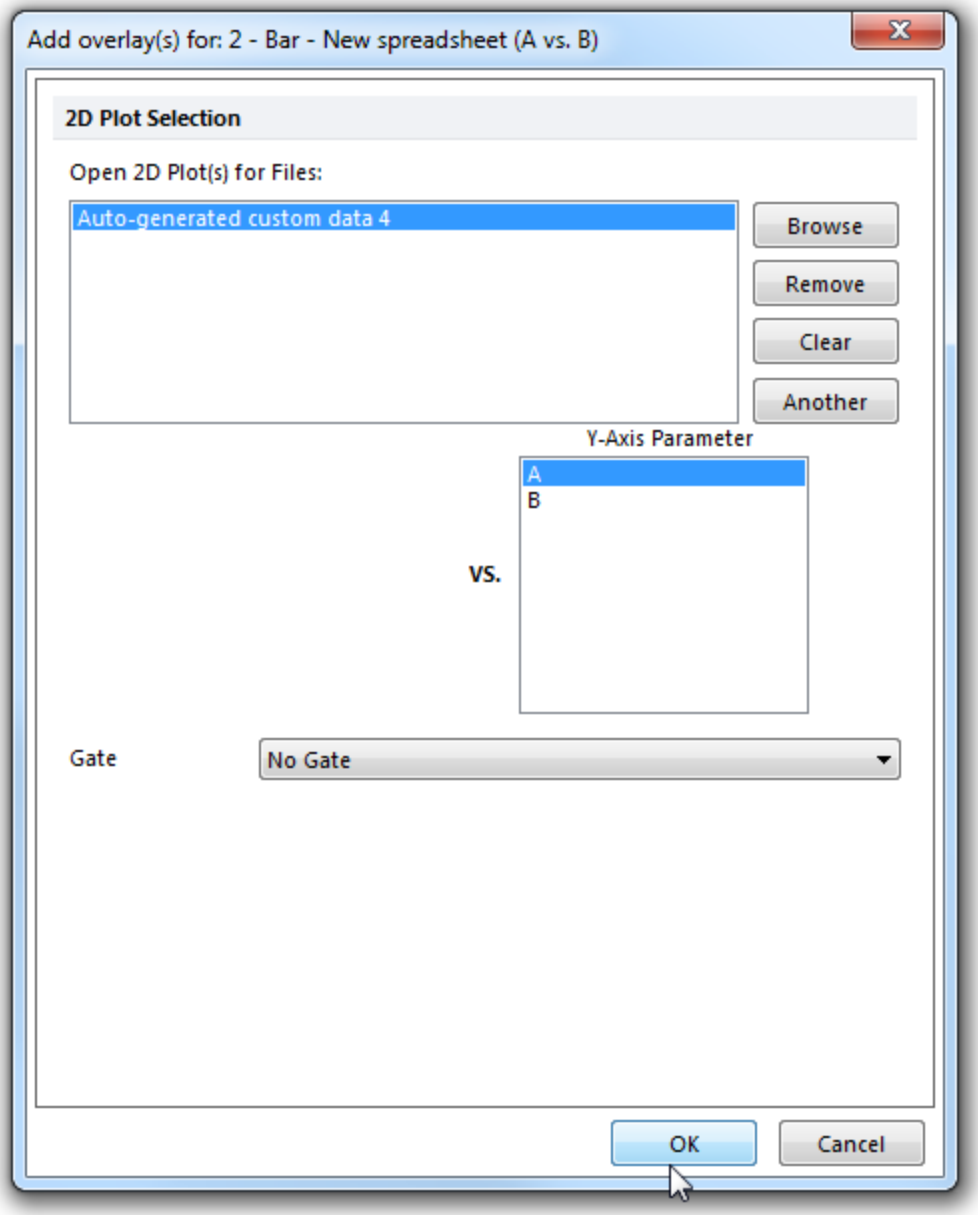

Figure 11.40 Selecting parameters to overlay onto an existing Bar Plot

See the Formatting Bar Plots section 556 for details on how to customize colors, bar style, error bars, and more.

11.8.2.2 Formatting Bar Plots

Bar Plots in FCS Express are fully formattable. In addition to the formatting options shared by all plots 246 (e.g., border, size, background, axis 559, titles, and legend), special formatting options are accessible from the Overlays category. To access these special options, either

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- Select the Bar Plot and use the **Format** tab→**Plot Options**→**Overlays** command, or

- Right-click on the Bar Plot, select **Format** from the pop-up menu, and choose the **Overlays** category.

Special formatting options for Bar Plots are indicated by number below (Figure 11.41) and include:

- Bar color (❶), shape (❷), fill (❸, ❹), and border (❻).
- Highlighting (❼, ❽).
- Error bars (❾, ❿).

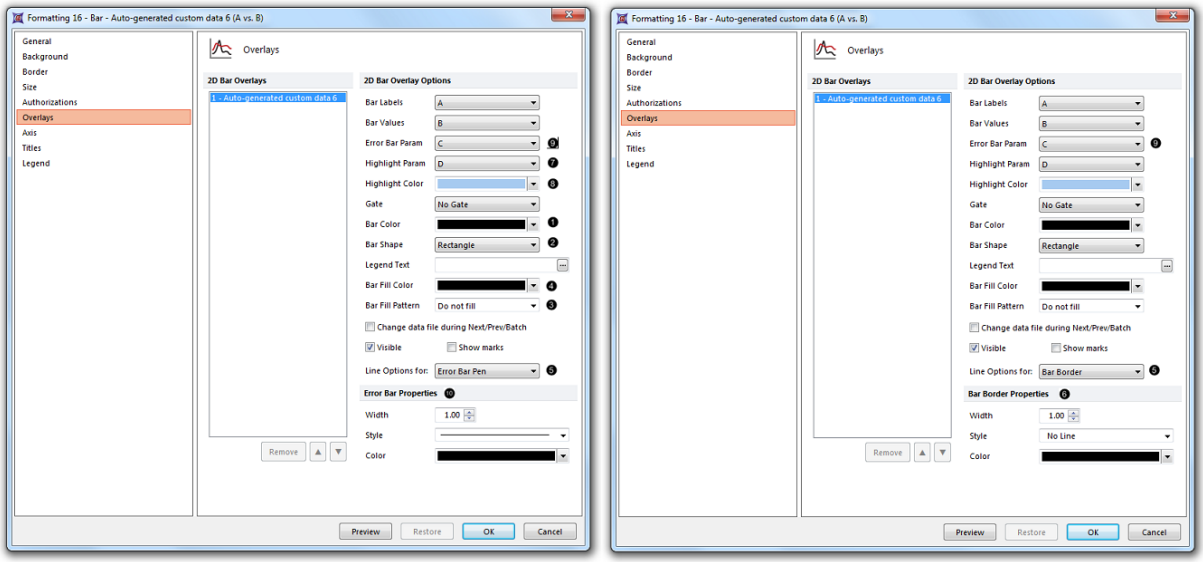

Figure 11.41 Special formatting options for Bar Plots

The formatting options are described in the table below.

Option	Explanation
Bar Labels	The parameter (column) from Spreadsheets 554 displayed on the X axis.
Bar Values	The parameter (column) from Spreadsheets 554 displayed on the Y axis.
Error Bar Parameter ❾	The parameter (column) from the Spreadsheet displayed as error bars. See below 558 for more information on how to define the error bars.
Highlight Param. ❼	The parameter (column) from the Spreadsheet from which the highlighting rule derives. Bars will be highlighted (e.g., the yellow bar shown in this example 547) in the color defined in ❽ if their value meets the criterion defined by the Highlighting rule. See below 558 for more information on how to define a highlighting rule.
Highlight Color ❽	The display color for highlighted bars.

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Option	Explanation
Gate	The gate that is applied to the Bar Plot.
Bar Color ❶	The display color for bars.
Bar Shape ❷	The shape of the bar. Choose between rectangle (default), pyramid, cylinder, ellipse, arrow, cone, bevel, cube, and diamond options.
Legend Text	The text that will appear in the plot legend beside the symbol for the overlay.
Bar Fill Color ❹	The stripe fill color for bars with fill patterns.
Bar Fill Pattern ❸	The fill pattern of the bar. Choose between none (default), cross, diagonal, horizontal, and vertical stripe options.
Change data file during Next/Prev/Batch	Whether the Bar Plot changes during batch processing 784 or in response to a Next/Previous 79 command. It is generally recommended to leave this unchecked (default), as the Bar Plot will update automatically as the data to which it refers is changed.
Visible	Hides overlays without removing them. Select the overlay file from the **2D bar Overlays** list and uncheck **Visible**. Check **Visible** to show the overlay again.
Show marks	Displays the value of the bars as text directly above the bars.
Line Options for ❺	Toggle between Bar Border and Error Bar Pen editing mode.
Bar Border/Error Bar Properties ❻/❿	Width, Style (solid, dotted, and dashed options), and Color of the Bar Border or Error Bars.

To add error bars 558 or highlighting 558 to a Bar Plot, these values must be entered into designated columns in the Spreadsheet.

To define values for the Error Bars,

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the error for each row (each row corresponds to an individual bar). This value may be freetext or a custom token 727.
3. Choose the appropriate Spreadsheet column for the Error Bar Parameter (Figure 11 (above), ❾).

An example of a Bar Plot with Error Bars and its associated Spreadsheet is shown in Figure 11.42 below.

To define a Highlighting rule,

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the rule for each row (each row corresponds to an individual bar). The rule must be a formula evaluating to "TRUE" or "FALSE"; if "TRUE," the bar will be highlighted with the color defined in the **Formatting Overlays** window, ❽, in the chart shown above. An example of a Highlighting rule referring to a cell within the Spreadsheet is shown below in Figure 11 below.
3. Choose the appropriate Spreadsheet column for the Highlight Param. (as shown in the **Formatting Overlays** window, ❼, in the Special formatting options figure above the chart).

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An example of a Bar Plot with Highlighting and its associated Spreadsheet is shown below.

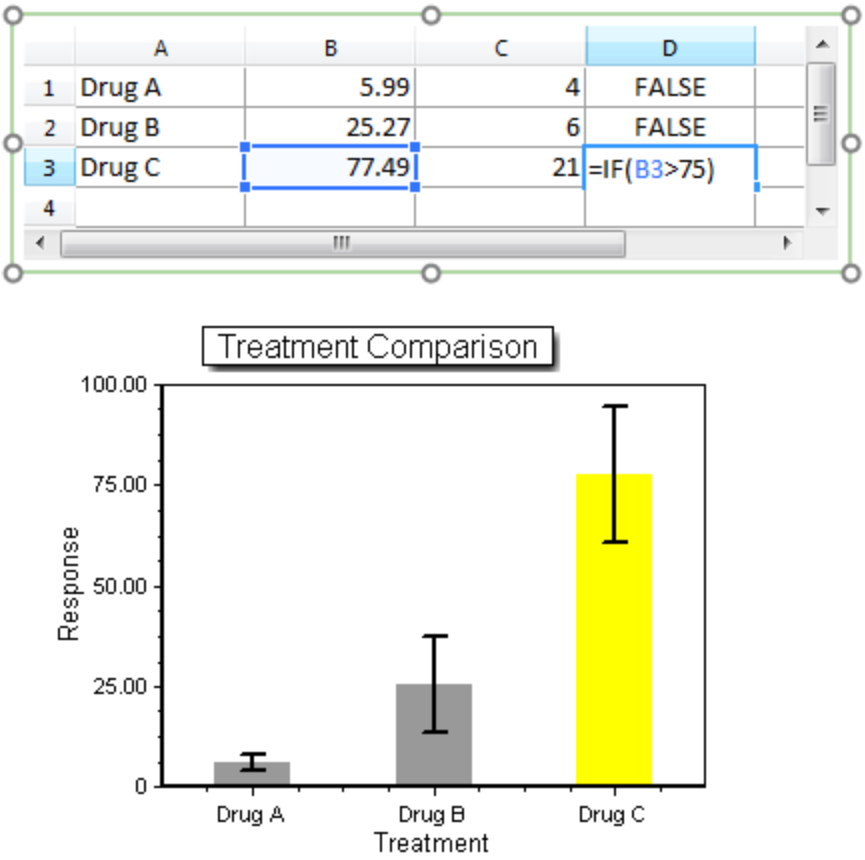

Figure 11.42 Bar Plot and Spreadsheet with Error Bars and Highlighting.

Note: Bar Plot **Axis titles** may be defined in the Axes 254 category of Formatting options.

11.8.3 Scatter Plots

Scatter plots facilitate the observation of relationships between two variables*. These variables may be native to the experiment (e.g., *time* and *fluorescence* .fcs file parameters), simple statistical tokens 692 derived from different plots or populations, or complex statistical formulae defined in Custom Tokens 727. Unlike bar plots and pie charts, scatter plots are true 2D plots.

Scatter Plots (e.g., Figure 11.43)can be inserted anywhere on your layout 560, and are populated from Spreadsheets 560 containing statistical tokens or freetext. They are also fully formattable 563.

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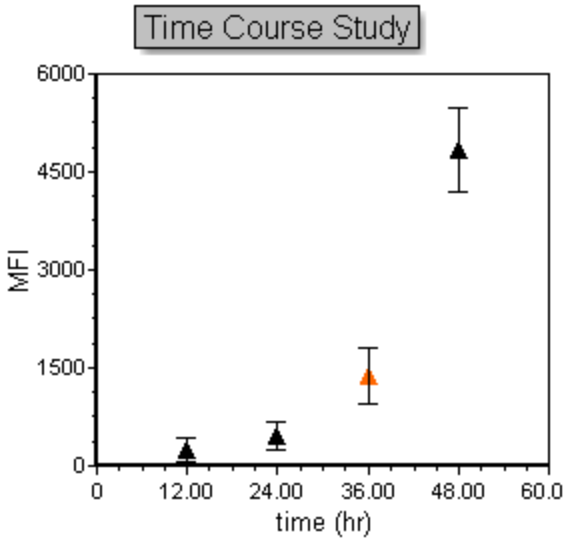

Figure 11.43. Scatter Plot in FCS Express

* Note: If you plan on performing a regression analysis (best-fit curve) of your data, it is strongly recommended to insert a Scatter with Regression Plot[579] instead. Scatter with Regression Plots facilitate automatic overlay of the regression curve onto your raw data.

11.8.3.1 Inserting Scatter Plots

To insert a Scatter Plot:

- Use the **Insert** tab→**1D Plots→Scatter** command (Figure 11.44) and click on a blank space of the layout

Learn more about Populating Scatter Plots from Spreadsheets[560]

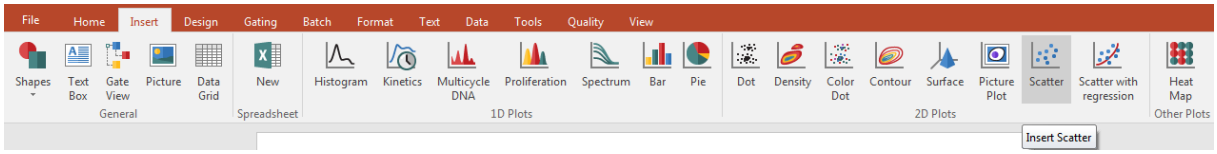

Figure 11.44 Inserting a Scatter Plot

11.8.3.1.1 Populating Scatter Plots from Custom Data Grids

Scatter Plots are populated from Spreadsheets[414].

If a Spreadsheet exists on the layout, an inserted Scatter Plot[560] will be automatically populated with the data from the Spreadsheet.

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If more than one Spreadsheet exists on the layout, select the Spreadsheet 1212 that which is intended as the data source prior to inserting the Scatter Plot 560.

By default, the Scatter Plot will plot the first two columns of the Spreadsheet for the X- and Y-parameters, respectively.

To populate the Scatter Plot with parameters other than the first two columns of the Spreadsheet,

1. Select the **Format** tab→**Plot Options**→**Overlays** command (Figure 11.45)

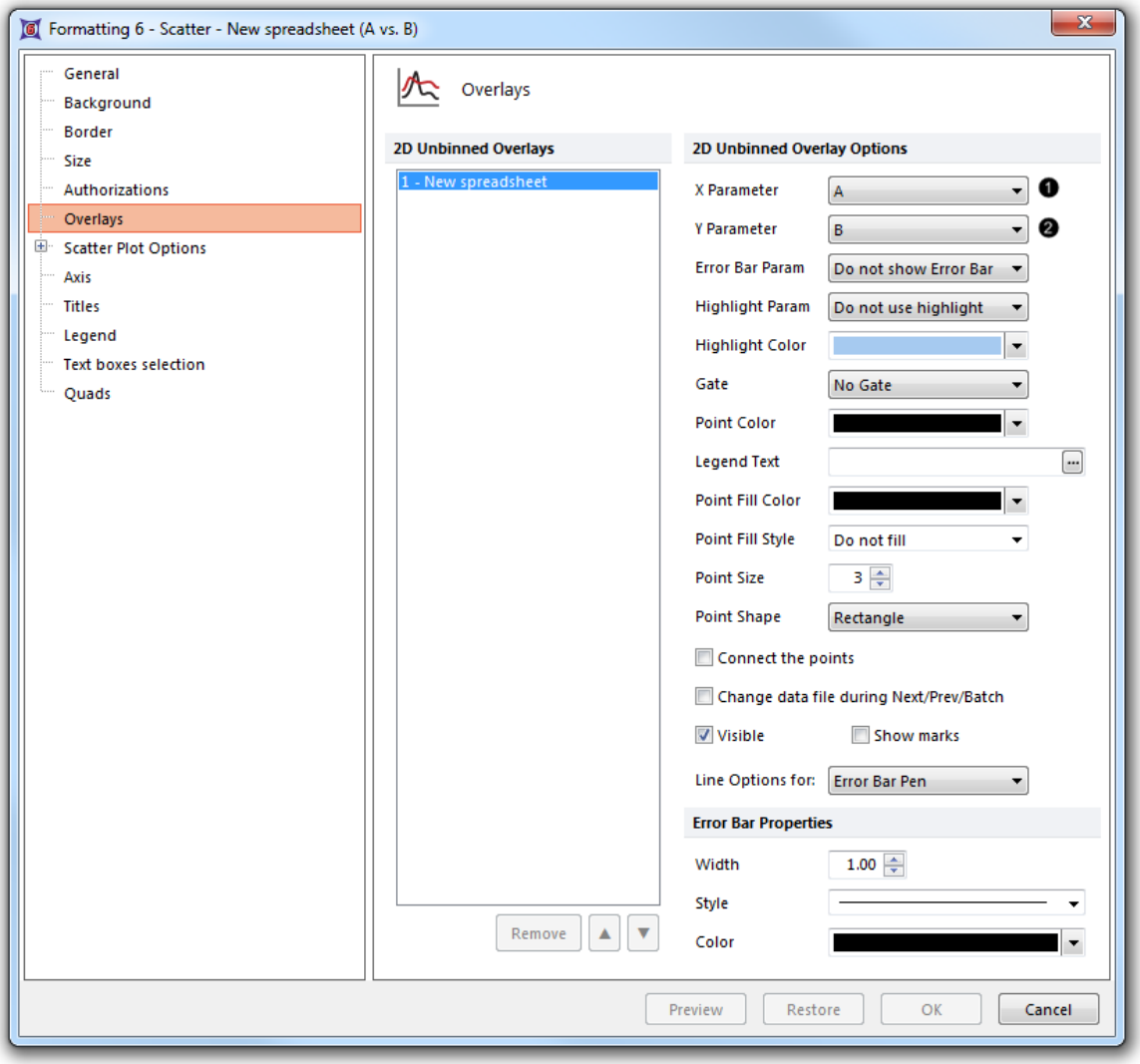

Fig. 11.45 Populating a Scatter Plot from Spreadsheet parameters

2. Choose the parameter (i.e., Spreadsheet column) intended for the X-axis, from the **X Parameter** drop-down menu (❶)

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3. Choose the parameter (i.e., Spreadsheet column) intended for the Y-axis, from the **Y Parameter** drop-down menu (❷)

Note: Alternatively, click and hold on the axis parameter name and select the intended parameter from the list of available parameters.

To add an overlay to an existing Scatter Plot, either

- Drag and drop a Spreadsheet onto the Scatter Plot.
- Drag and drop another Scatter Plot onto the recipient Scatter Plot.
- Right-click on the Scatter Plot.
    1. Select **Add Overlay using Advanced Open Data Dialog** from the pop-up menu.
    2. Select the data source in the **Active Files** tab of the **Open Data File** dialog (Figure 11.46).

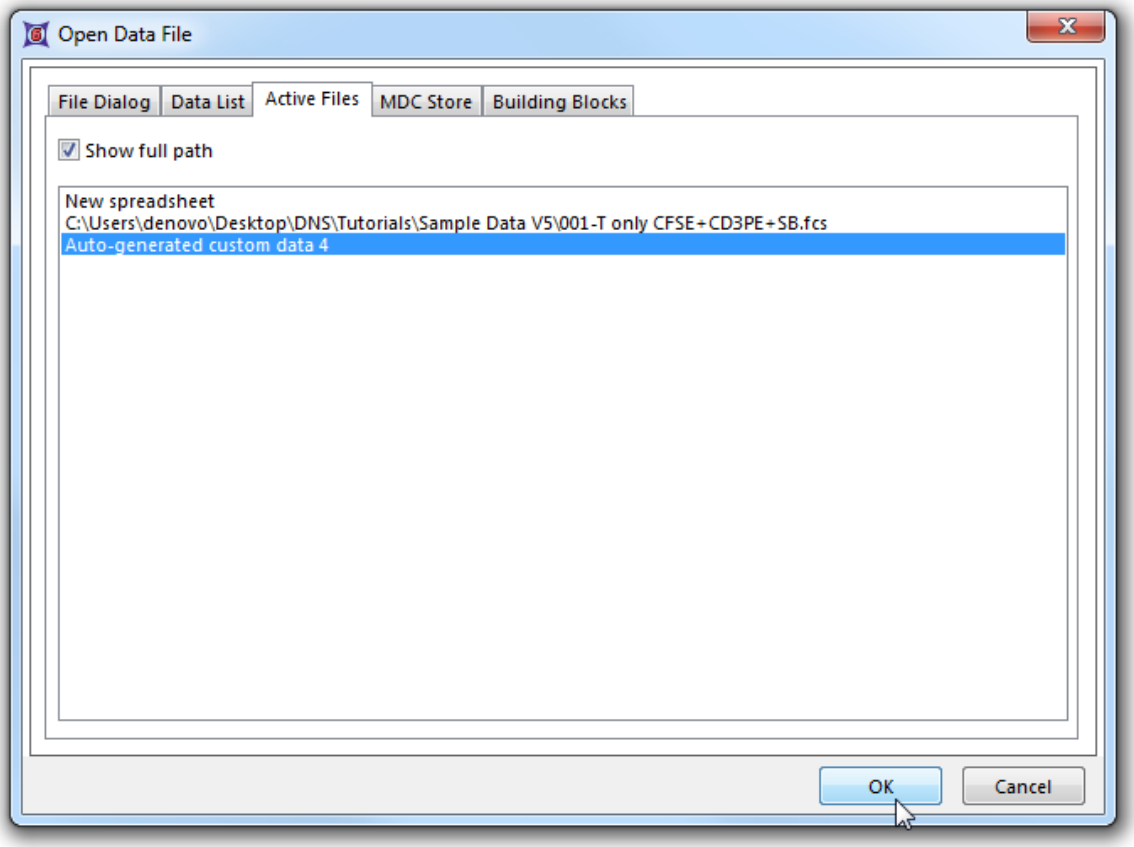

Figure 11.46 Selecting a Spreadsheet to overlay onto an existing Scatter Plot

3. Click **OK.**
4. Select the parameters for the new Overlay in the **Add overlays** dialog.
5. Click **OK** (Figure 11.47).

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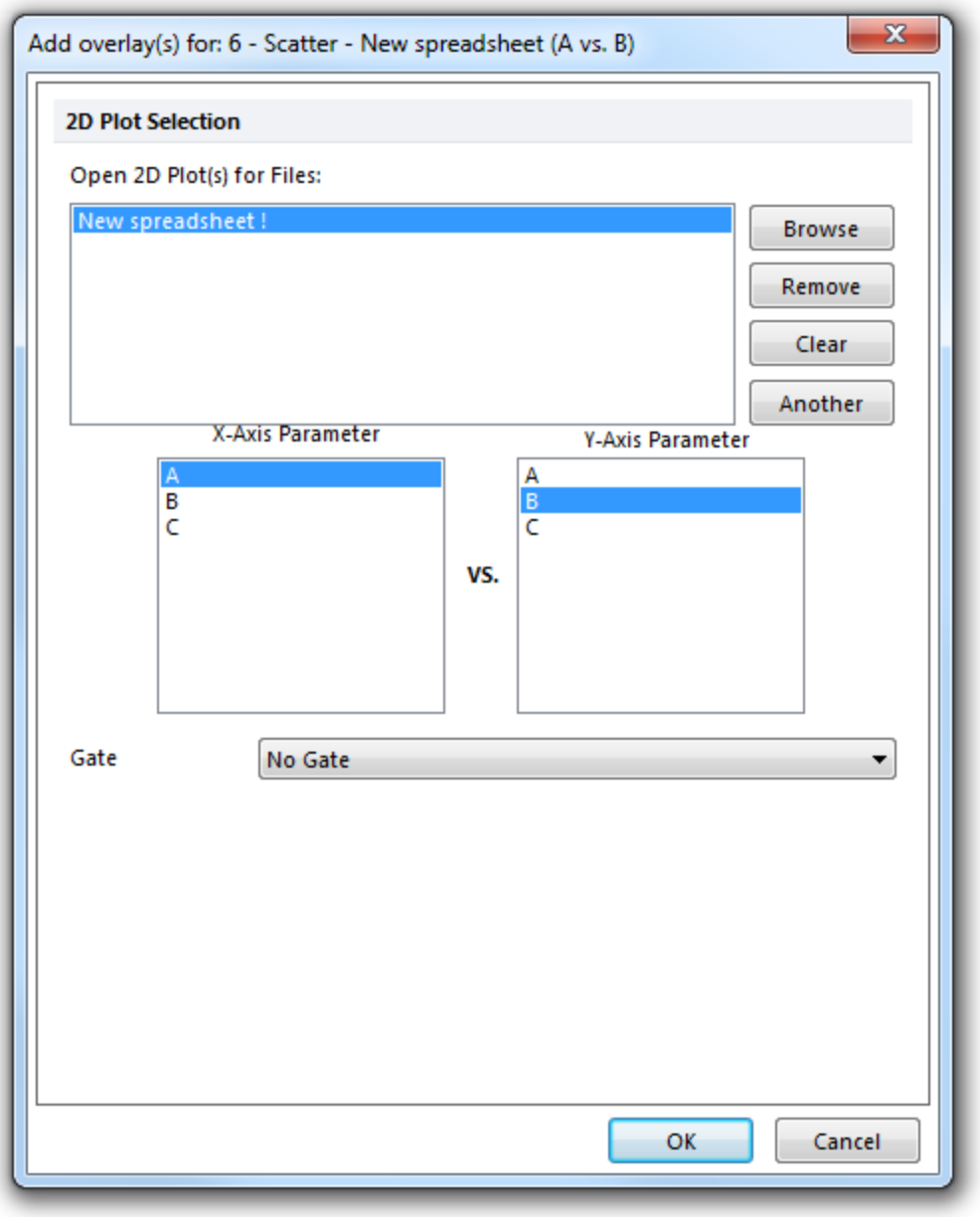

Figure 11.47 Selecting parameters to overlay onto an existing Scatter Plot

11.8.3.2 Formatting Scatter Plots

Scatter Plots in FCS Express are fully formattable. In addition to the formatting options shared by all plots 246 (e.g., border, size, background, axis 567, titles, and legend), special formatting options are accessible from the Overlays category. To access these special options, either

- Select the Scatter Plot and use the **Format** tab→**Plot Options**→**Overlays** command, or

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- Right-click on the Scatter Plot, select **Format** from the pop-up menu, and choose the **Overlays** category.

Special formatting options for Scatter Plots are indicated by number below (Figure 11.48) and include:

- **Point Color** (❶)
- **Point size** (❷)
- **Point shape** (❸)
- **Highlight Param**(❹)
- **Highlight Color**(❺)
- **Error Bar Param** (❻)

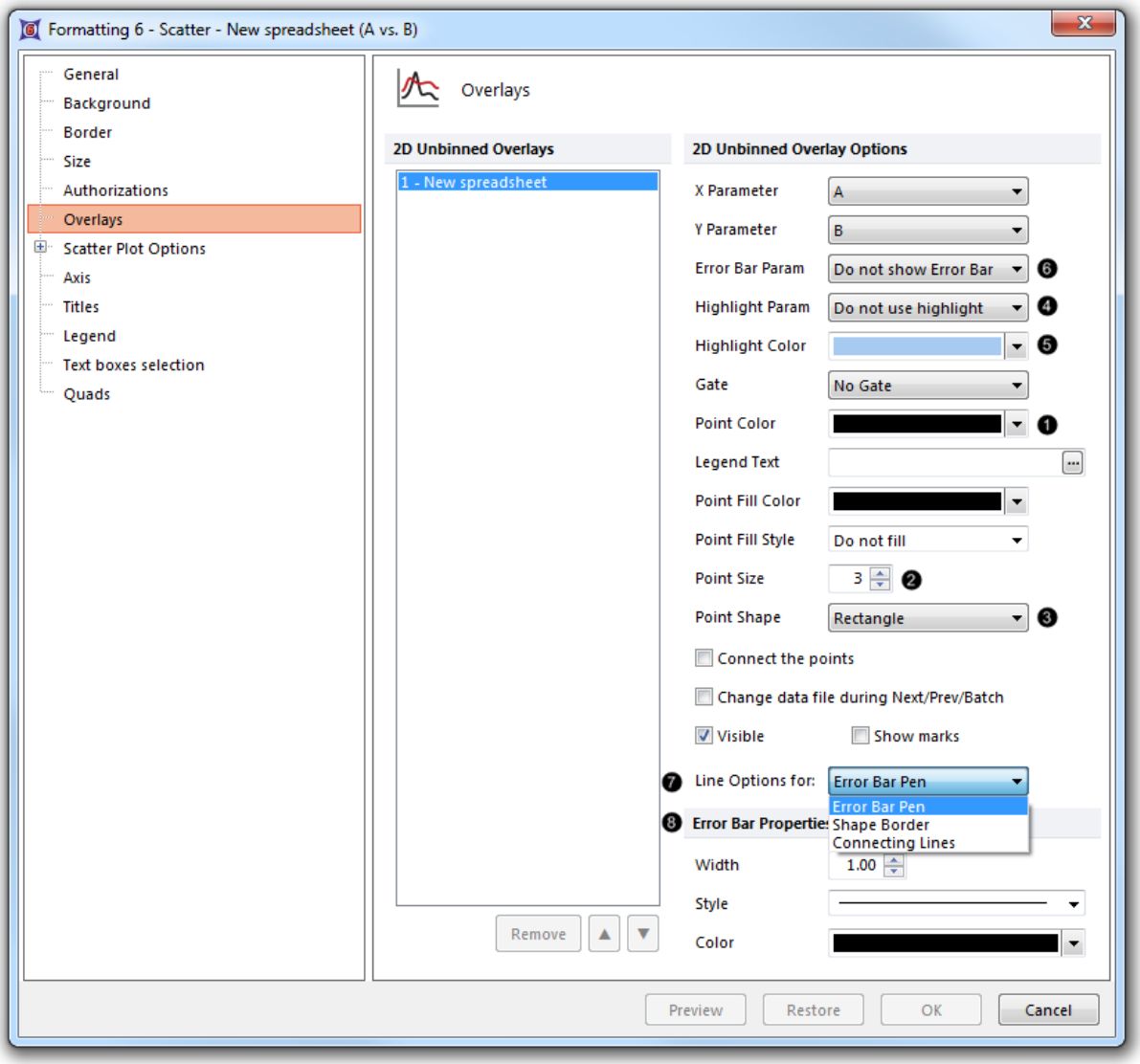

Figure 11.48 Special formatting options for Scatter Plots

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The formatting options are described in the table below.

Option	Explanation
X Parameter	The parameter (column) from the Spreadsheet displayed on the X axis.
Y Parameter	The parameter (column) from the Spreadsheet displayed on the Y axis.
Error Bar Param. ❻	The parameter (column) from the Spreadsheet displayed as error bars. See below for more information on how to define the error bars.
Highlight Param. ❹	The parameter (column) from the Spreadsheet from which the highlighting rule derives. Points will be highlighted (e.g., the orange point in the plot below) in the color defined in ❺ if their value meets the criterion defined by the Highlighting rule. See below for more information on how to define a highlighting rule.
Highlight Color ❺	The display color for highlighted points.
Gate	The gate that is applied to the Scatter Plot.
Point Color ❶	The display color for points.
Legend Text	The text that will appear in the plot legend beside the symbol for the overlay.
Point Fill Color	The color used to fill points.
Point Fill Style	The style used to fill points.
Point size ❷	The size of the points.
Point shape ❸	The shape of the points. Choose between rectangle (default), triangle, circle, cross, star, hexagon, and diamond options.
Connect the points	Whether the points are connected by a line.
Change data file during Next/Prev/Batch	Whether the Scatter Plot changes during batch processing or in response to a Next/Previous command. It is generally recommended to leave this unchecked (default), as the Scatter Plot will update automatically as the data to which it refers is changed.
Visible	Hides overlays without removing them. Select the overlay file from the **2D Unbinned Overlays** list and uncheck **Visible**. Check **Visible** to show the overlay again.
Show marks	Displays the value of the points as text directly above the points.
Line Options for ❼	Toggle between Error Bar Pen, Shape Border, and Connecting Lines editing mode.
Error Bar Pen/Shape Border/Connecting Lines Properties ❽	Width, Style (solid, dotted, and dashed options), and Color of the Error Bar Pen, Shape Border, or Connecting Lines.

To add error bars or highlighting to a Scatter Plot, these values must be entered into columns in the Spreadsheet.

To define values for the Error Bars,

1. Go to an empty column of the Spreadsheet

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2. Enter the value 419 of the error for each row (each row corresponds to an individual bar). This value may be freetext or a custom token 727.
3. Choose the appropriate Spreadsheet column for the Error Bar Parameter (Figure 11 above, ❻).

An example of a Scatter Plot with Error Bars and its associated Spreadsheet is shown in the figure below.

To define a Highlighting rule,

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the rule for each row (each row corresponds to an individual point). The rule must be a formula evaluating to "TRUE" or "FALSE"; if "TRUE," the point will be highlighted with the color defined in Figure 11 above, ❺. An example of a Highlighting rule referring to a cell within the Spreadsheet is shown below in Figure 11.49.
3. Choose the appropriate Spreadsheet column for the Highlight Param. (Figure 11 above, ❹).

An example of a Scatter Plot with Highlighting and its associated Spreadsheet is shown in Figure 11 below.

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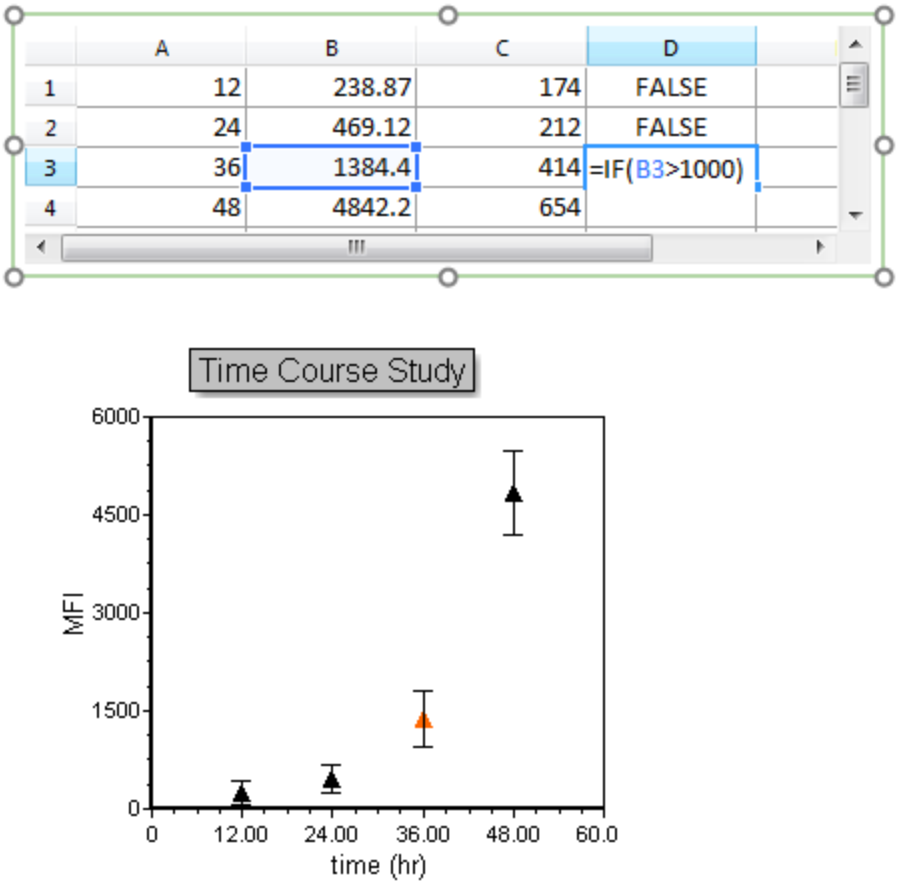

Figure 11.49 Scatter Plot and associated Spreadsheet with Error Bars and Highlighting

Note: Scatter Plot **Axis titles** may be defined:

- directly in the Spreadsheet by using the column header (this require to check the **Use values in a first row as labels** 422 option in the Spreadsheet Formatting dialog 421).
- in the Axes 254 category of Formatting options.

11.8.3.3 Regression Analysis (Manually fitting data)

FCS Express has the ability to perform regression analysis on your data through manual formulas and custom data grids or at a click through Scatter with Regression plots 579.

FCS Express provides the user with many mathematical functions to create custom tokens 727 and custom data 414 sets. These functions operate similar to functions within Microsoft Excel™. When it comes to regression analysis there are four basic functions that apply to all of the available equations that a regression can be performed on. In the table below the functions are listed with

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the red italic, *xxx,* representing the FCS Express Function name for a particular equation (The FCS Express Function names can be found in the Available Equations.). A description of the function argument is given in the right side of the table.

Regression Function Type	Description	Arguments	Result
Perform*xxx* Fit	Performs a fit of the specified X-Y values to a particular equation.	([X Values],[Y Values],[SD],[Fixed Parameters]) Where **X Values** is a comma delimited list of X values, generally specified by a series of tokens. Where **Y Values** is a comma delimited list of Y values, generally specified by a series of tokens. **Fixed Parameters** allows you to specify variables that should not be fit, but will be forced to use the value that you specify. The format of the Fixed parameters is a comma delimited list of *var=value* where *var* is one of the available variables for that equation and *value* is the value to fix it to. **SD,** or Standard Deviation, allows you to specify a standard deviation for each list of the Y values, generally specified by a series of tokens. The SD value assigns a "weight" to each associated X and Y value with a lower SD value resulting in a higher weight and a higher SD value resulting in a lower weight. If the SD is not specified, an SD of zero is assumed for each Y value.	Comma delimited list of all of the fit variables and their fit values
Extract*xxx* Results	Extracts a particular variable from the result of a Perform*xxx*Fit custom token.	Extract*xxx*Results([modelParameters], "*Variable 1*" | "*Variable 2*" | "*Variable 3*") Where *xxx* represents an equation (ie. Linear, Sigmoidal, Exponential, etc...). Where **modelParameters** represents a comma delimited list of all of the fit variables and their values, generally specified by a token result of the Perform*xxx*Fit function. Where *Variable* represents a variable defined by the Perform*xxx*Fit function and "|" represents "or". Each Perform*xxx*Fit function will define a unique set of variables that will replace the example of "*Variable 1, Variable 2, Variable 3*" when the function is used: ie... "m" | "b" | "r2" or "A" | "B" | "C"| "r2" for Linear and Sigmoidal equations	The value of the specified variable.

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		respectively. Unwanted variables must be deleted to return a result. For example: ExtractLinearResults([modelParameters], "m") will return the fit value result of the "m" variable associated with the equation.	
*xxx*XtoY	Uses the X values of a given data set and the "Model Parameters" or "Fit" of a regression to return an unknown Y Value.	*xxx*XToY(X, [modelParameters]) Where *xxx* represents an equation 578 (ie. Linear, Sigmoidal, Exponential, etc...). Where **X** is a comma delimited list of X values, generally specified by a token 692. Where **modelParameters** represents a comma delimited list of all of the fit variables and their fit values. Generally specified by a token 692 result of the Perform*xxx*Fit function.	Comma delimited list of all of the Y values calculated from the associated equation, X value, and fit variables.
*xxx*YtoX	Uses the Y values of a given data set and the "Model Parameters" or "Fit" of a regression to return an unknown X Value.	*xxx*YToX(Y, [modelParameters]) Where *xxx* represents an equation 578 (ie. Linear, Sigmoidal, Exponential, etc...) Where **Y** is a comma delimited list of Y values, generally specified by a token 692. Where **modelParameters** represents a comma delimited list of all of the fit variables and their fit values. Generally specified by a token 692 result of the Perform*xxx*Fit function.	Comma delimited list of all of the X values calculated from the associated equation, Y value, and fit variables.

To view a list of all possible equations please see the topic of Available Equations 578. To see an example of using these equations please see the topic of Regression Analysis Example 569 or visit the Linear Regression Analysis 1679 tutorial.

11.8.3.3.1 Manual Regression Analysis Example

In order to perform a manual regression analysis, you will use a custom data set. To demonstrate the use of this feature, we will fill a new data set with fixed values. Normally, you would use Tokens 692, representing some statistic from your analysis. Defining regressions manually is easy in FCS Express 6 with Scatter with Regression plots 579. We highly recommend you visit the Scatter with Regression plots 579 chapter before proceeding with a manual fit.

In Figure 11.50, we have a Spreadsheet 415, with values typed into the cells. Please note that these are freetext values, but in practice many of these will be tokens 692 derived from plots on your layout.

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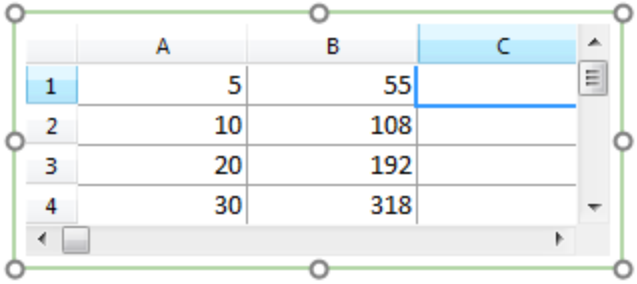

	A	B	C
1	5	55	
2	10	108	
3	20	192	
4	30	318	

Figure 11.50 Spreadsheet Data for Regression Analysis

With this data, a linear regression should yield a slope of approximately 10 and a Y-intercept of approximately 1.

Regression analysis is performed using Custom Tokens 727.

1. First, create a new custom token and name it **Linear Regression**.

If you right-click in the formula area of the **Custom Token** window and choose **Insert Function**, you will find a list of available regressions, each beginning with the word **Perform** (Figure 11.51).

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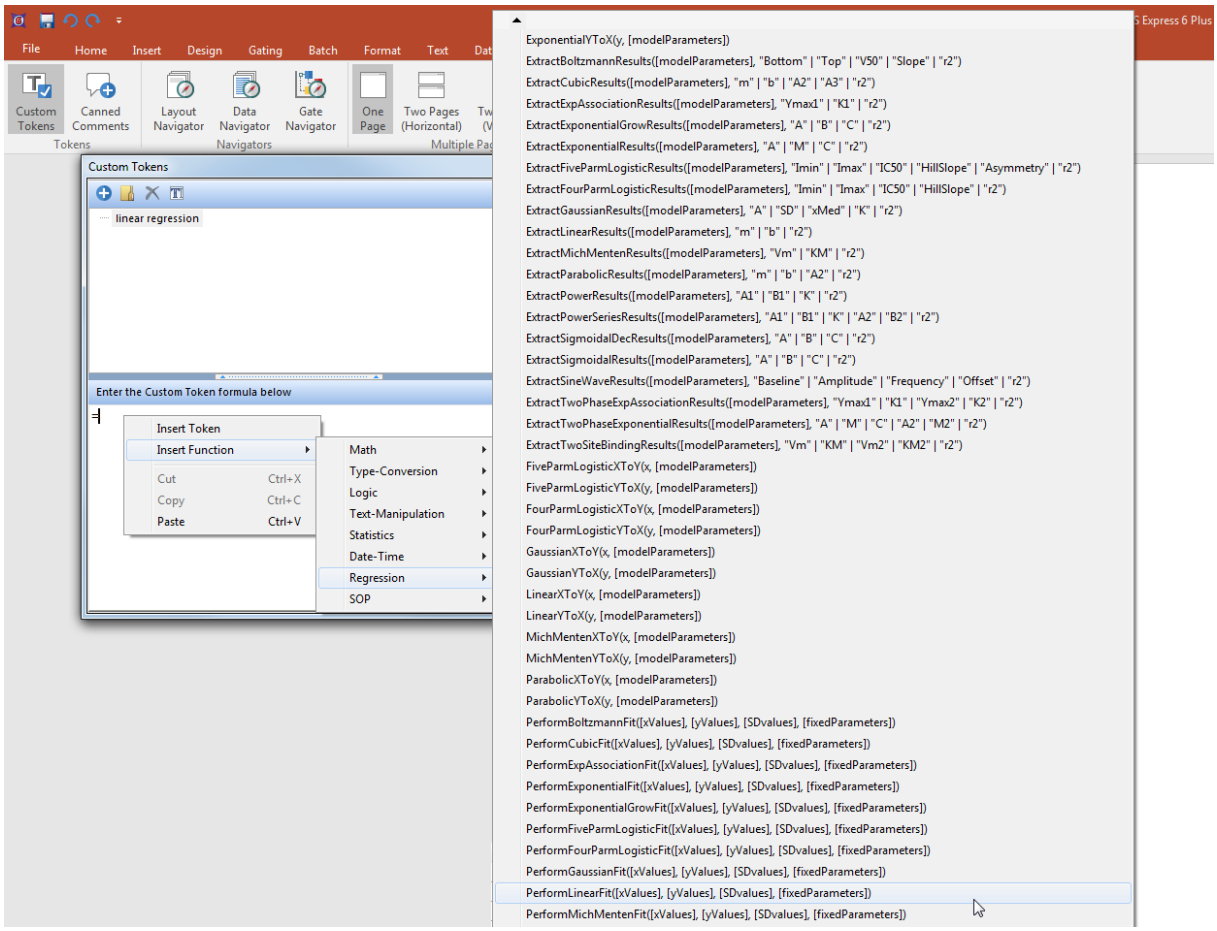

Figure 11.51 Regression Functions

2. Start a formula with an equals sign (**=**), right-click to bring up the pop-up menu, and choose **Insert Function→PerformLinearFit([xValues, [yValues], [SDvalues], [fixedParameters])** (Figure 11.52).

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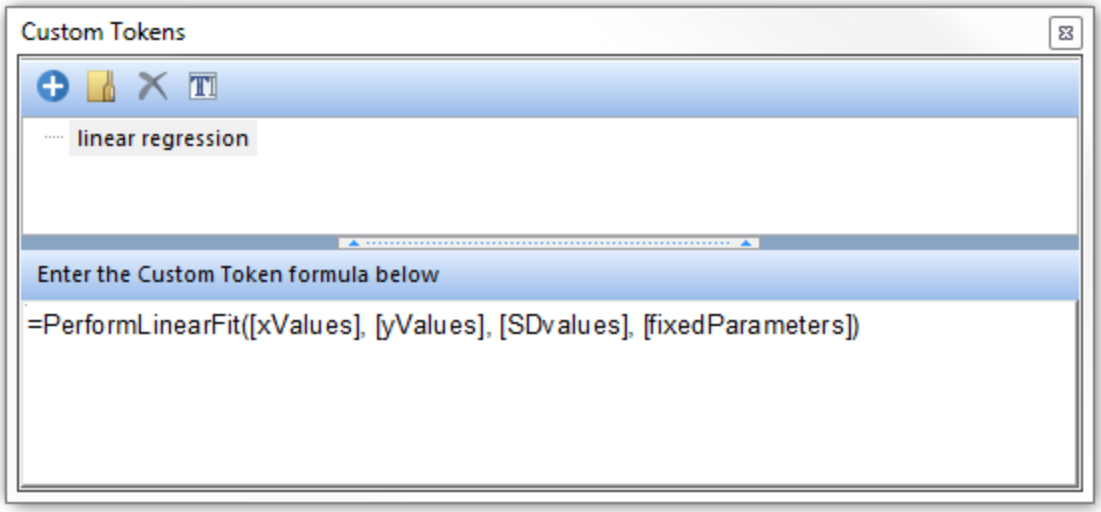

Figure 11.52 Linear Regression Formula

3. In the formula, highlight **xValues**, right-click to bring up the pop-up menu, and choose **Insert Token** (Figure 11.53).

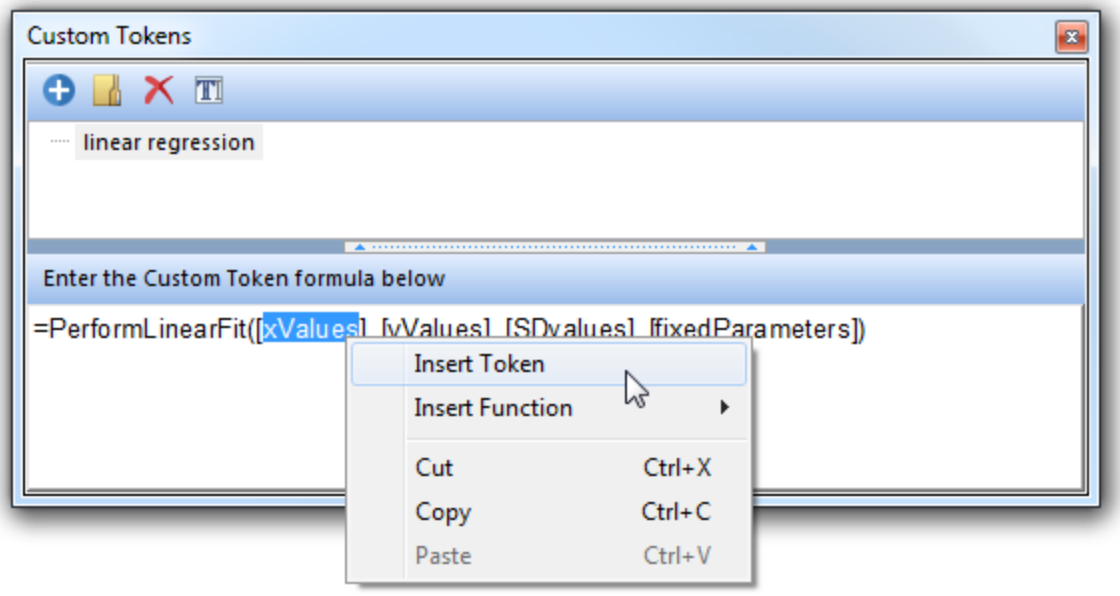

Figure 11.53 Inserting a Token into a Linear Regression Formula

An **Insert a Token** dialog will appear that contains a **Data Parameter** token (Figure 11.54).

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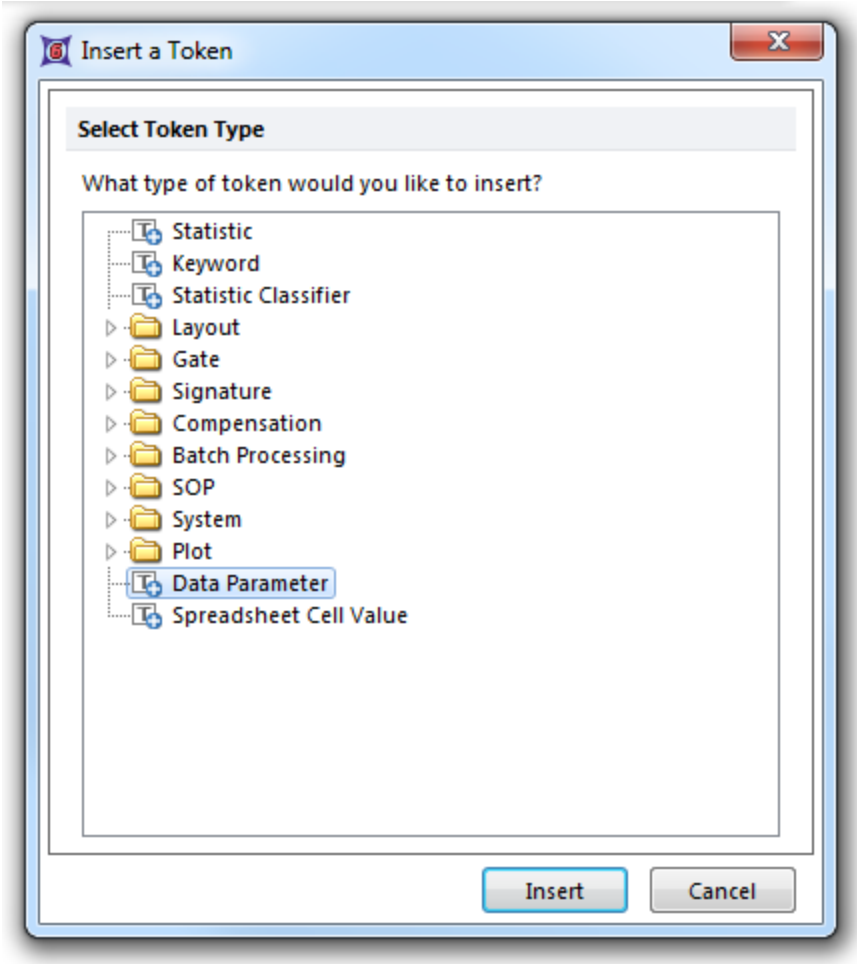

Figure 11.54 Data Parameter Token

4. Select the **Data Parameter** token and click **Insert**.

5. In the **Create Data Parameter** dialog which appears, choose the **Spreadsheet** radio button as a **Data Source**, and select **New Spreadsheet** (Figure 11.55).

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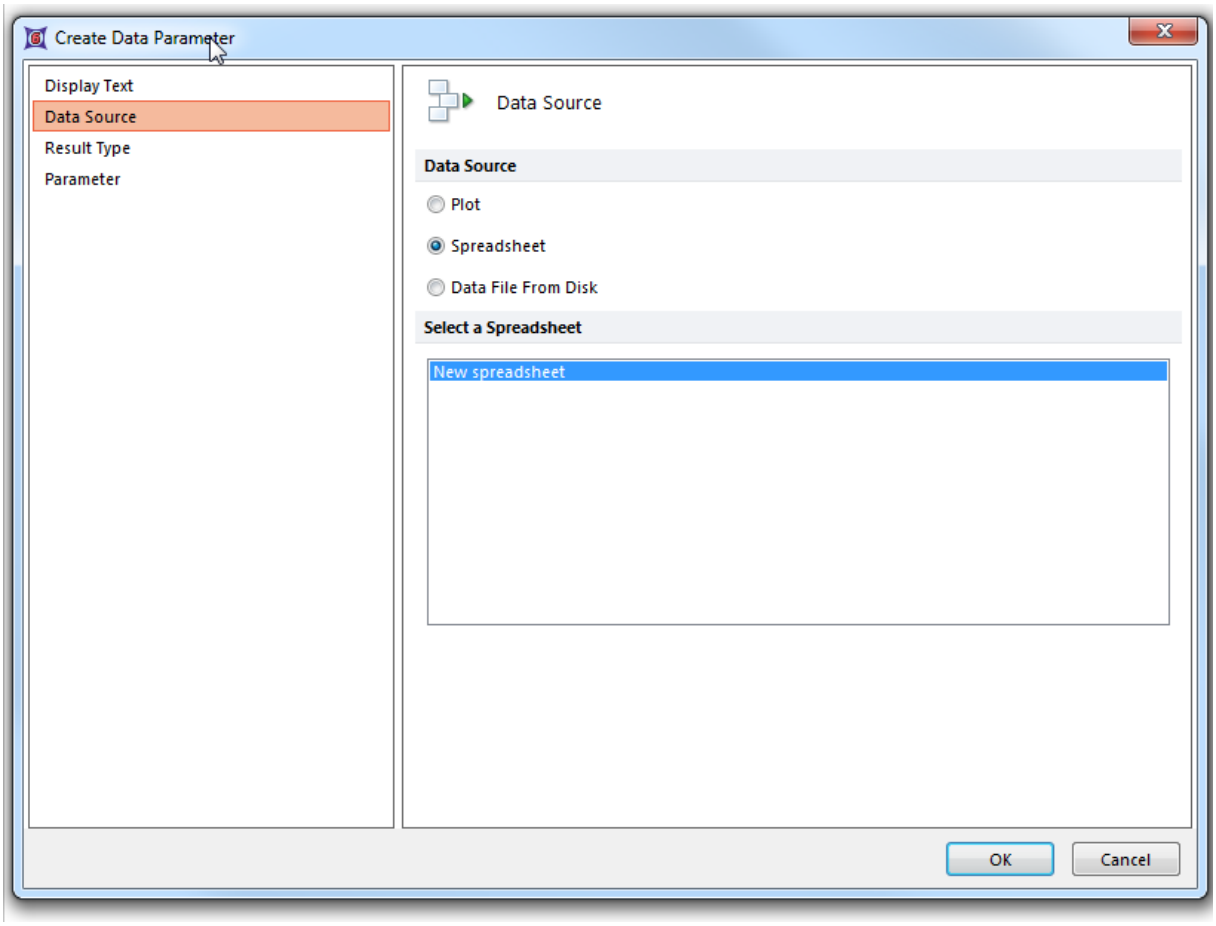

Figure 11.55 Create Data Parameter -- Data Source

6. Then, go to the **Parameter** category and from the **Parameter** drop-down list, choose the data in column **A** (Figure 11.56). Leave **Macro** for the **Output As** drop-down selection. Choosing **Data Values** converts all of the data to string values which is not efficient when there is a large amount of data.

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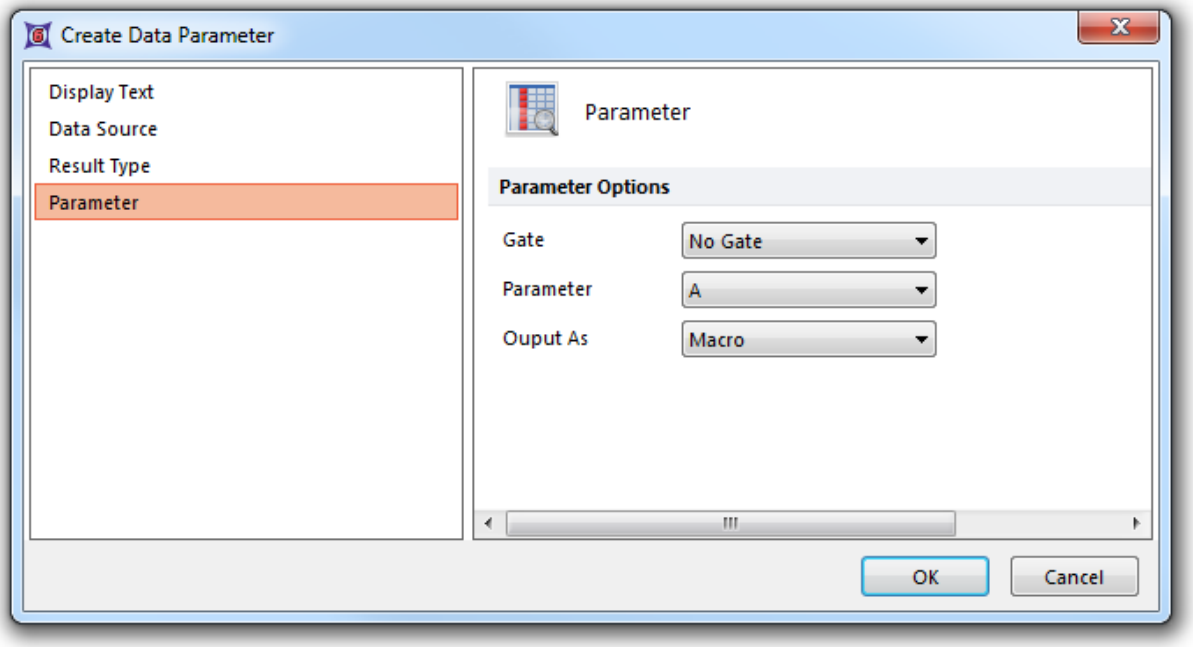

Figure 11.56 Create Data Parameter -- Parameter Page

7. Repeat steps 3-6 using **yValues** in the formula and choosing Parameter **B**.

8. Highlight **SDvalues** and press the Del key (leaving the area between the brackets blank. Do the same for **fixedParameters** (Figure 11.57).

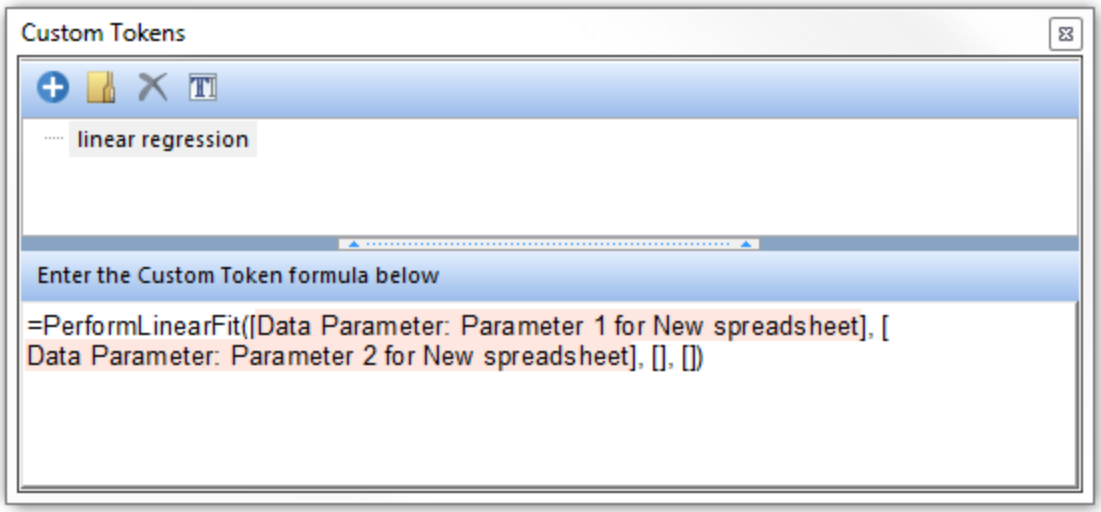

Figure 11.57 Completed Linear Regression Formula

If the measurement in your B column was made from multiple data points and you know the standard deviation of each of the data points, you can enter a list of comma-delimited values in the **SDvalues** parameter of the linear regression

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formula. The model will weigh the points with the lower standard deviation more strongly than the points with higher SD values.

The fixed Parameters for a linear regression are m (slope) and b (Y-intercept). By leaving the parameter blank, the model will fit both of them. If you wanted to force a Y-intercept of 0, for example, you could enter **b=0** in **fixedParameters**, and the model will fix that parameter, rather than letting it float.

9. Convert both Data Parameter values in the formula to display a numeric result type 746 (they will be highlighted in blue instead of pink, as shown below in Figure 11.58).

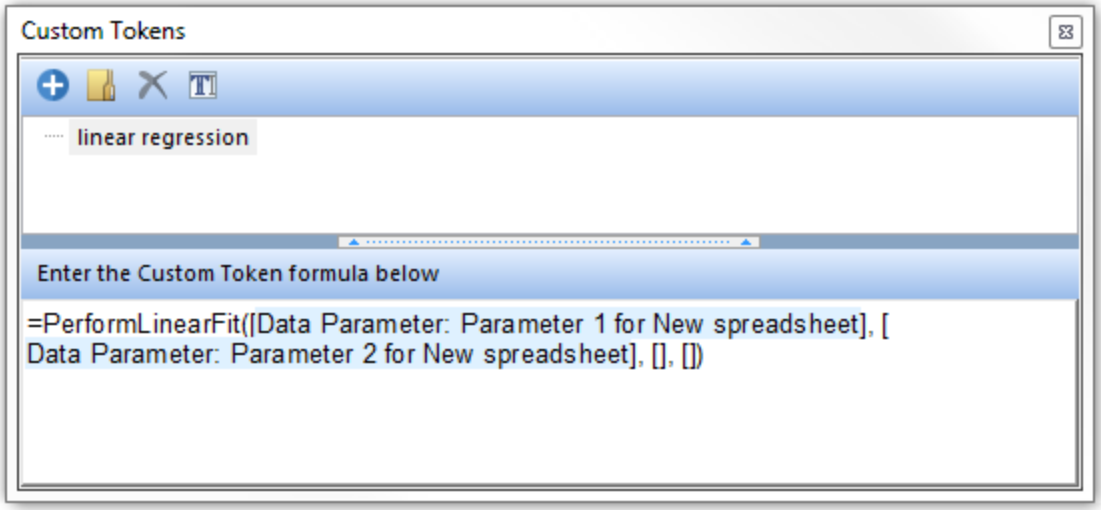

Figure 11.58 Completed Linear Regression Formula with result type converted to Numeric

10. Insert a Text Box 152 onto your layout.

11. Drag the custom token **Linear Regression** into the text box. The m, b, and r2 (r-squared) values will be displayed (Figure 11.59).

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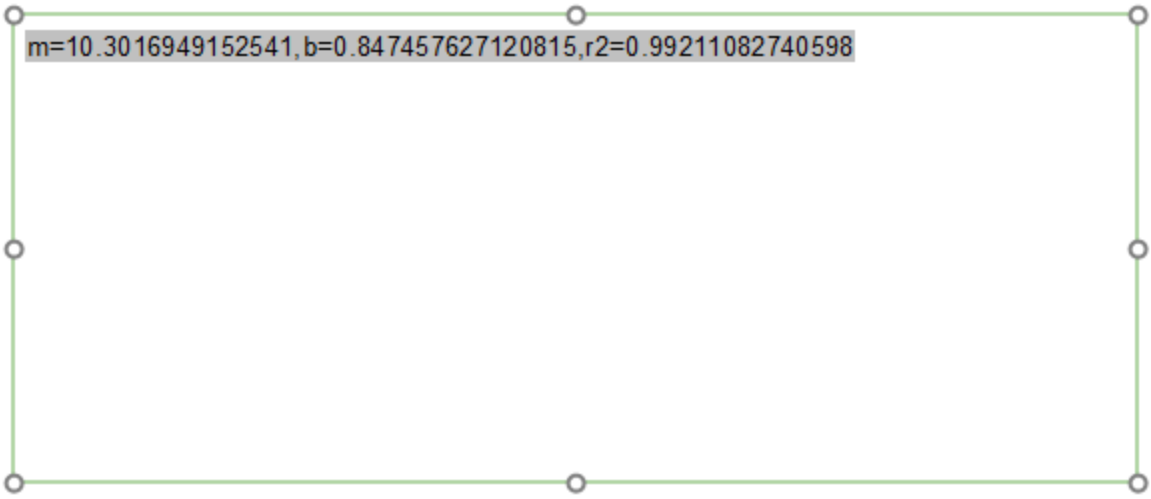

Figure 11.59 Linear Regression Token inserted into a Text Box

The result is a comma-delimited list of every parameter that the linear regression fit.

If you want to display just the slope of the linear regression in a text box, you create a new custom token, and:

12. Use the function **ExtractLinearResults([modelParameters], "m" | "b" | "r2")** (Figure 11.60) as in step 2.

13. Then, select **modelParameters** and insert the **Linear Regression** token, as in step 4.

14. Delete the "b" and "r2" parameters.

15. Create a text box and insert the text: "The slope is: ".

16. Drag the Slope token into the text box (Figure 11 below).

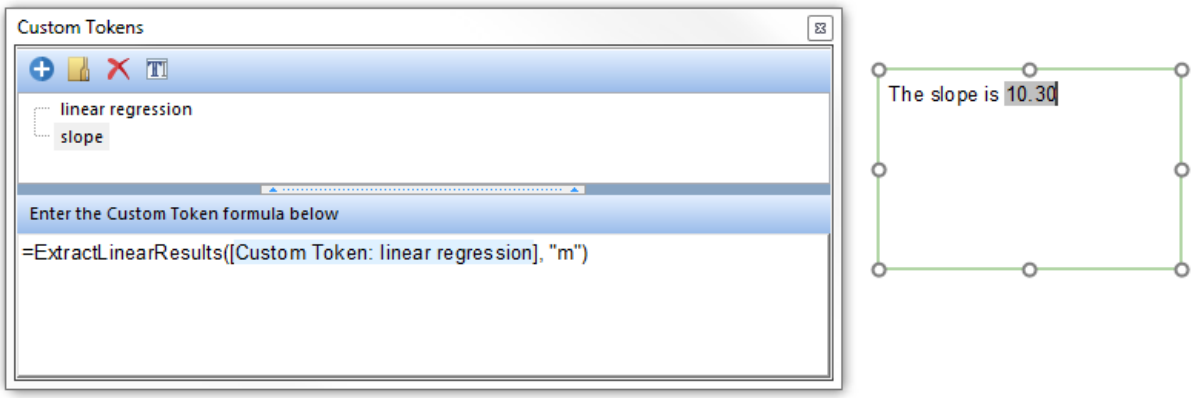

Figure 11.60 Slope Parameter of Linear Regression Analysis Inserted into a Text Box as a Token

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In summary, it is a two-step process. You make one custom token for the fit, and a second custom token for the parameter that you want to use from that fit.

11.8.3.3.2 Available Equations

FCS Express has the ability to fit your data to many different types of equations.

Below you will find a table indicating the equation, the formula associated with it, and the parameters defined in FCS Express when a fit is performed.

Equation Name	FCS Express Function Name	Equation	Variables
Michaelis Menten	MichMenten	$Y = \frac{Vmax \cdot x}{Km + x}$	Vmax, Km
TwoSiteBinding	TwoSiteBinding	$Y = \frac{Vmax \cdot x}{Km + x} + \frac{Vmax_2 \cdot x}{Km_2 + x}$	Vmax, KM, Vmax2, KM2
Four Parameter Logistic (4PL)	FourParmLogistic	$y = Imax + \frac{(Imin - Imax)}{1 + (x / IC50)^{Hill}}$	Imax, Imin, IC50, Hill
Five Parameter Logistic (5PL)	FiveParmLogistic	$y = Imax + \frac{(Imin - Imax)}{(1 + (x / IC50)^{Hill})^{Asym}}$	Imax, Imin, IC50, Hill, Asym
Boltzmann	Boltzmann	$y = Bottom + \frac{(Top - Bottom)}{1 + e^{\frac{(V50 - x)}{Slope}}}$	Bottom, Top, V50, Slope
Exponential	Exponential	$y = A \cdot e^{-\left(\frac{x}{M}\right)} + C$	A, M, C
Two Phase Exponential	TwoPhaseExponential	$y = A \cdot e^{-\left(\frac{x}{M}\right)} + A_2 \cdot e^{-\left(\frac{x}{M_2}\right)} + C$	A, M, A2, M2, C
Exponential Association	ExpAssociation	$y = Ymax1 \cdot (1 - e^{-K1 \cdot x})$	Ymax1, K1
Two Phase Exponential Association	TwoPhaseExpAssociation	$y = Ymax_1 \cdot (1 - e^{-K_1 \cdot x}) + Ymax_2 \cdot (1 - e^{-K_2 \cdot x})$	Ymax1, K1, Ymax2, K2
Exponential Grow	ExponentialGrow	$y = A \cdot e^{Mx} + C$	A, M, C
Power	Power	$y = A_1 \cdot x^{B_1} + K$	A1, B1, K

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Power Series	PowerSeries	$y = A_1 \cdot x^{B_1} + A_2 \cdot x^{B_2} + K$	A1, B1, A2, B2, K
Linear	Linear	$y = mx + b$	m, b
Parabolic	Parabolic	$y = mx + b + A_2 x^2$	m, b, A2
Cubic	Cubic	$y = mx + b + A_2 x^2 + A_3 x^3$	m, B, A2, A3
Sine Wave	SineWave	$y = B + A \sin(Fx + \varphi)$	Baseline, Amplitude, Frequency, Offset
Gaussian	Gaussian	$y = \frac{A}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2} + K$	A, SD, xMed, K
Sigmoidal	Sigmoidal	$y = \frac{C}{1 + A \cdot e^{-Bx}}$	A, B, C
Decreasing Sigmoidal	SigmoidalDec	$y = \frac{C}{1 + A \cdot e^{Bx}}$	A, B, C

11.8.4 Scatter With Regression

FCS Express can display data from a Spreadsheet 414 or any file as a Scatter with Regression Plot 580 which automatically overlays one or multiple best-fit regression curves of your choice 578 onto your data. The resulting regression parameters 567 can be extracted and added as statistical tokens 585 anywhere on your layout. The regression curve will update as you adjust your gates or advance through your dataset, as shown in Figure 11.61 below:

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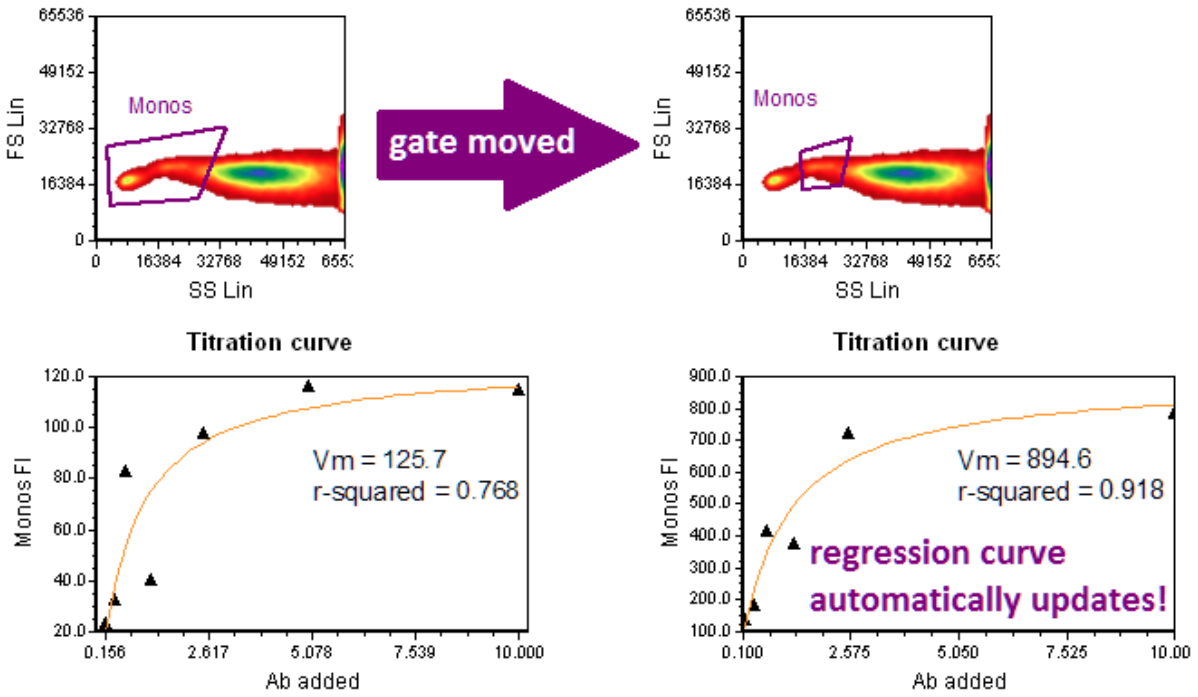

Figure 11.61 Scatter with Regression Plot updating as gate is moved.

11.8.4.1 Scatter With Regression Plots

To insert a **Scatter with Regression** plot,

1. Select the **Insert→2D Plots→Scatter with Regression** command (Figure 11.62).

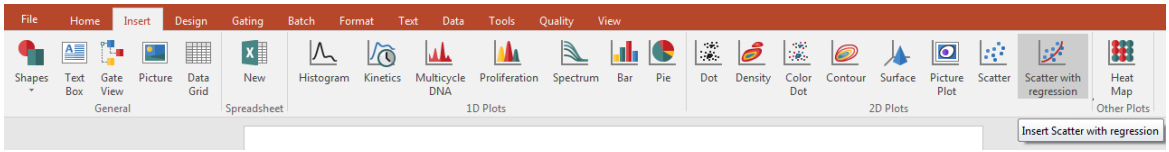

Fig. 11.62 Inserting a scatter with regression plot.

2. Click (or click-drag) on your layout where you want the plot to appear.

   Note: The plot will appear showing data from the top Spreadsheet 414, i.e., the Spreadsheet which was most recently selected or edited. The top Spreadsheet is the one most recently accessed.

3. Change the X- and Y-axes 254 to the desired parameters.

To Format a Scatter with Regression Plot:

1. Right click on the plot.
2. Choose **Format** to access plot formatting options 284.
3. Choose the **Regression fit** category.

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In the regression fit category you may choose the **Regression Type** (Figure 11.63) from the dropdown list of available regression curve equations[578] to best fit your data.

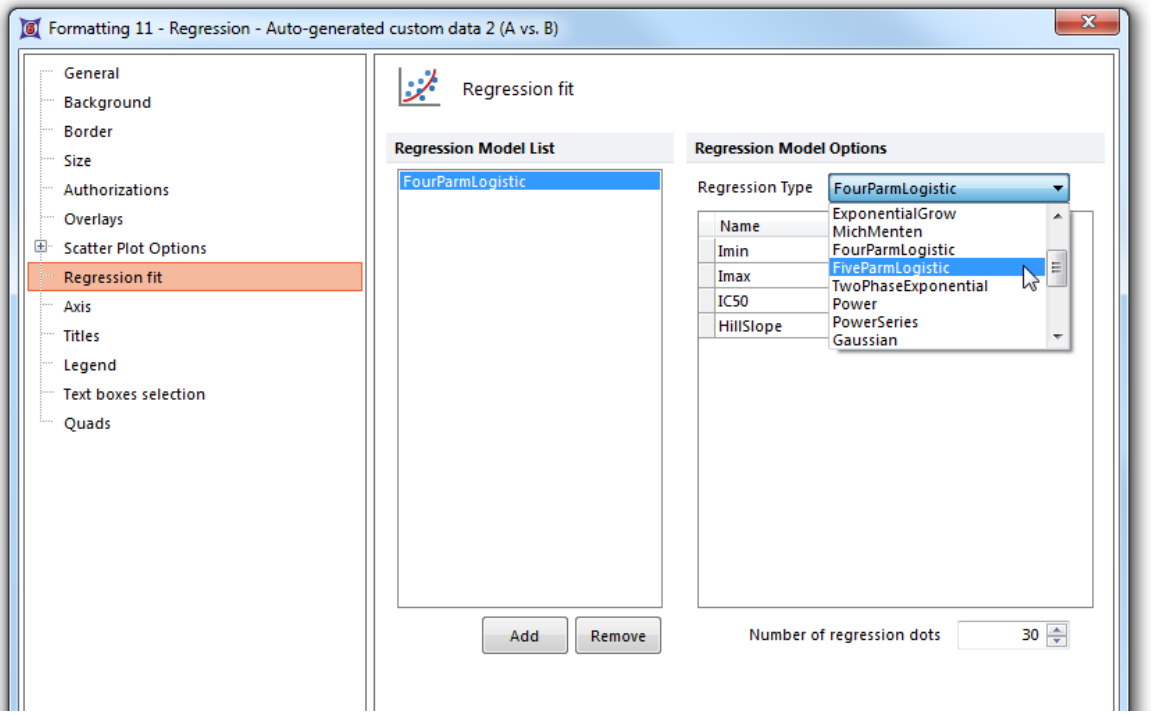

Fig. 11.63 Formatting a scatter with regression plot.

Note that multiple fits may be added or removed by using the **Add** and **Remove** buttons under the **Regression Model List**. The **Regression Model Options** also allow you to see the model parameters and enter a fixed parameter where applicable (Figure 11.64). The model parameters may later be added to a layout as live-updating tokens via Scatter with Regression Tokens[585].

You may also increase the **Number of regression dots** to give the curve a smoother appearance, though the default of "30" is sufficient in most cases.

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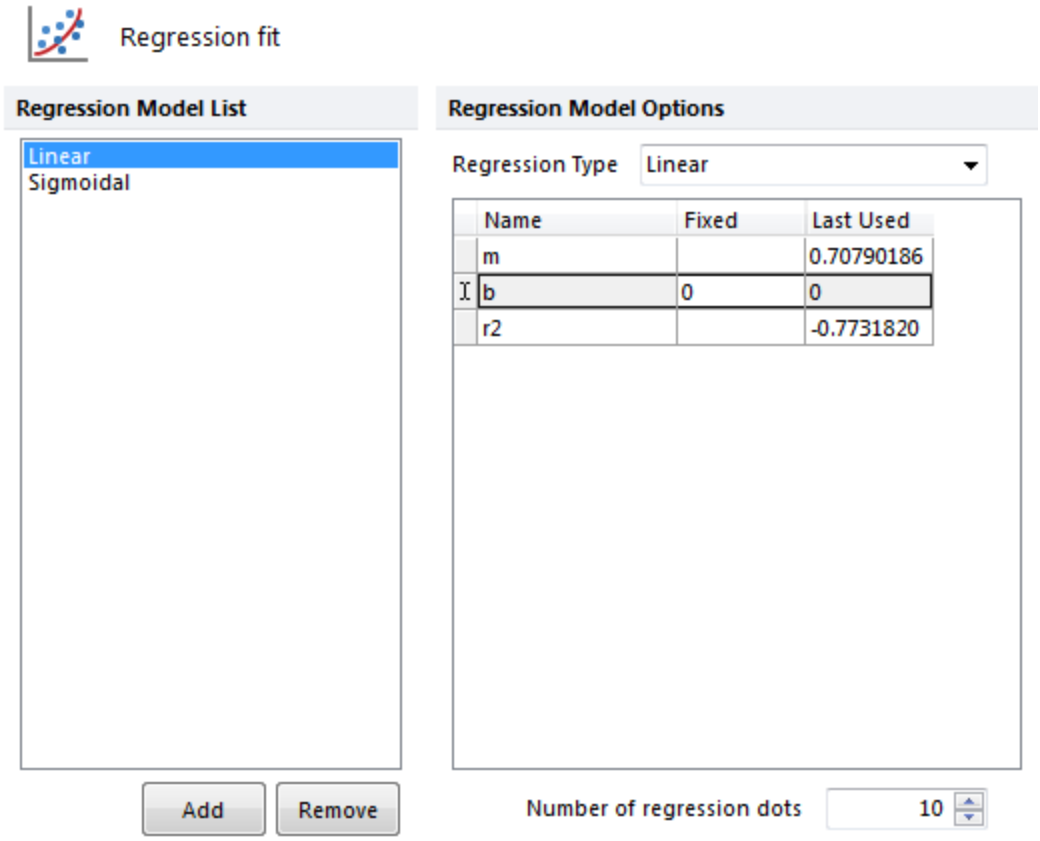

Fig. 11.64 Regression Model Options with a Linear and Sigmoidal fit added to the Regression Model List for comparison. Parameters for the regression model are displayed on the right and a fixed parameter may be typed in the Fixed column. Note in the example that the line was forced through zero by adding a fixed value of "0" to the column.

11.8.4.2 Formatting Scatter With Regression Plots

Scatter with Regression plots in FCS Express are fully formattable. In addition to the formatting options shared by all plots 246 (e.g., border, size, background, axis 585, titles, and legend), special formatting options are accessible from the Overlays category. To access these special options, either

- Select the Scatter Plot with Regression and use the **Format** tab→**Plot Options→Overlays** command, or
- Right-click on the Scatter Plot with Regression, select **Format** from the pop-up menu, and choose the **Overlays** category.

Special formatting options for Scatter with Regression plots allow to customize the appearance of both the data (i.e., Overlay 1, ❶ in Figure 11.65) and the regression (i.e., Overlay 2, ❷ in the figure below).

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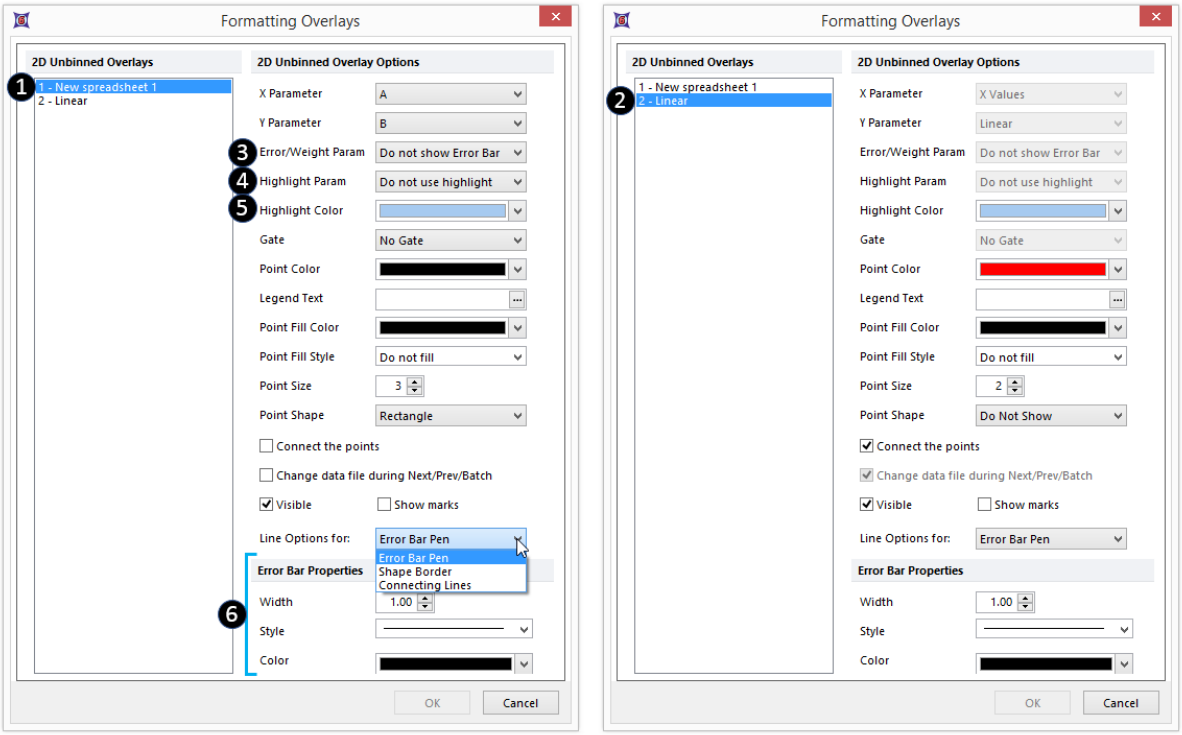

Figure 11.65 Special formatting options for Scatter with Regression Plots

The formatting options are described in the table below.

Option	Explanation
X Parameter	The parameter (column) from the Spreadsheet 561 displayed on the X axis.
Y Parameter	The parameter (column) from the Spreadsheet 561 displayed on the Y axis.
Error/Weights Bar Param. ❸	The parameter (column) from the Spreadsheet displayed as error bars and used by the regression to calculate the weights. Note, if an Error/Weights parameter is selected, a weighted regression will be performed. Weights will be calculated as the inverse of the Error/Weights Bar Parameter. For the sake of simplicity, if the Error/Weights Bar Parameter contains the following values "a, b, c, d,..." the weights that will be used to perform the weighted regression will be "1/a, 1/b, 1/c, 1/d,...". See below 584 for more information on how to define the error bars.
Highlight Param. ❹	The parameter (column) from the Spreadsheet from which the highlighting rule derives. Points will be highlighted (e.g., the orange point in the plot below 585) in the color defined in the **Highlight Color** drop down menu if their value meets the criterion defined by the Highlighting rule. See below 584 for more information on how to define a highlighting rule.
Highlight Color ❺	The display color for highlighted points.
Gate	The gate that is applied to the Scatter with Regression Plot.
Point Color	The display color for points.

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Option	Explanation
Legend Text	The text that will appear in the plot legend beside the symbol for the overlay.
Point Fill Color	The color used to fill points.
Point Fill Style	The style used to fill points.
Point size	The size of the points.
Point shape	The shape of the points. Choose between rectangle (default), triangle, circle, cross, star, hexagon, and diamond options.
Connect the points	Whether the points are connected by a line.
Change data file during Next/Prev/Batch	Whether the Scatter with Regression Plot changes during batch processing 784 or in response to a Next/Previous 79 command. It is generally recommended to leave this unchecked (default), as the Scatter with Regression Plot will update automatically as the data to which it refers is changed.
Visible	Hides overlays without removing them. Select the overlay file from the **2D Unbinned Overlays** list and uncheck **Visible**. Check **Visible** to show the overlay again.
Show marks	Displays the value of the points as text directly above the points.
Line Options for	Toggle between Error Bar Pen, Shape Border, and Connecting Lines editing mode.
Error Bar Pen/Shape Border/Connecting Lines Properties ❻	Width, Style (solid, dotted, and dashed options), and Color of the Error Bar Pen, Shape Border, or Connecting Lines.

To add error bars 565 or highlighting 566 to a Scatter Plot, these values must be entered into columns in the Spreadsheet.

To define values for the Error Bars,

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the error for each row (each row corresponds to an individual bar). This value may be freetext or a custom token 727.
3. Choose the appropriate Spreadsheet column for the Error Bar Parameter (Figure 11 above, ❸).

An example of a Scatter Plot with Error Bars and its associated Spreadsheet is shown in the figure below.

To define a Highlighting rule,

1. Go to an empty column of the Spreadsheet 414
2. Enter the value 419 of the rule for each row (each row corresponds to an individual point). The rule must be a formula evaluating to "TRUE" or "FALSE"; if "TRUE," the point will be highlighted with the color defined in Figure 11 above, ❺. An example of a Highlighting rule referring to a cell within the Spreadsheet is shown below in Figure 11.66.
3. Choose the appropriate Spreadsheet column for the Highlight Param. (Figure 11 above, ❹).

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An example of a Scatter with Regression Plot with Highlighting and its associated Spreadsheet is shown in Figure 11 below.

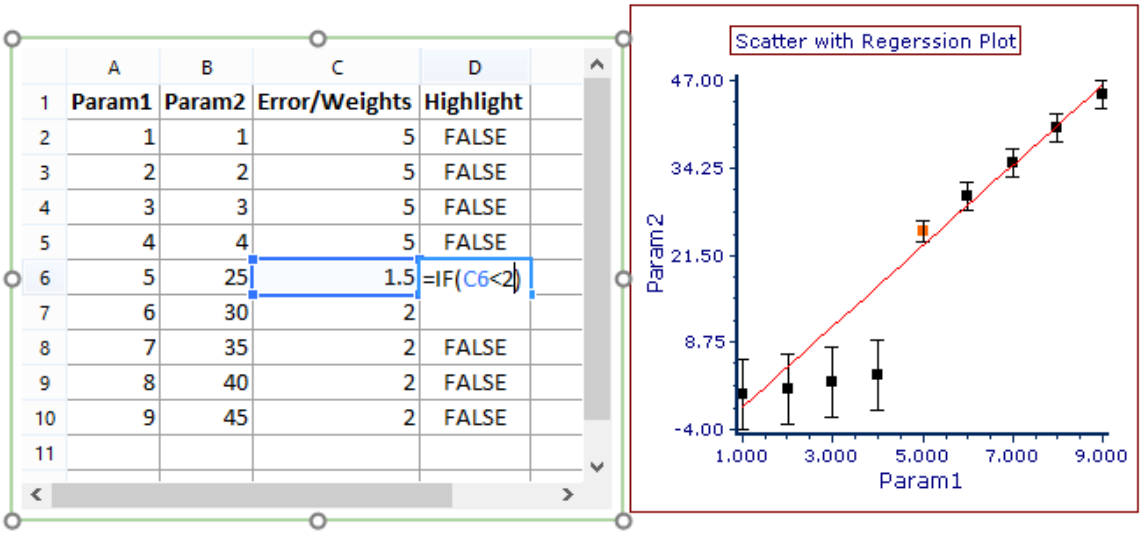

Figure 11.66 Scatter with Regression Plot and associated Spreadsheet with Error Bars and Highlighting

Note: Scatter with Regression Plot **Axis titles** may be defined:

- directly in the Spreadsheet by using the column header (this require to check the **Use values in a first row as labels** 422 option in the Spreadsheet Formatting dialog 421).
- in the Axes 254 category of Formatting options.

11.8.4.3 Scatter with Regression Tokens

Scatter with Regression parameters (**Regression Param Statistics**) can be extracted and used as tokens 692 or displayed in a default Regression Parameter Statistics window 586. These tokens can be inserted into text boxes 692, used in Custom Token 727 or any formulas, and exported to reports during batch processing 835. Regression Parameter Statistics will update in real time as regression curves change just like any other token in FCS Express.

To insert a **Regression Parameter Statistics** token into a text box:

1. Insert a text box using the **Insert** tab→**General**→**Text Box** command.
2. Right-click on the text box and select **Insert Token**.
3. Choose **Statistic** and click on the **Insert** button to bring up the **Create Statistic** dialog.
4. Choose a Scatter with Regression 580 plot as a **Data Source** from the **Select a Plot** box.
5. Open the **Statistic** category.

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6. Choose **Regression Parameter Statistics** from the **Select a Statistic** dropdown list (Figure 11.67).
7. Choose a statistic from the **Statistic** list box.
8. Click **OK**.

The statistic or **Model name** will now be displayed in the text box and will update in real time as the regression overlay updates.

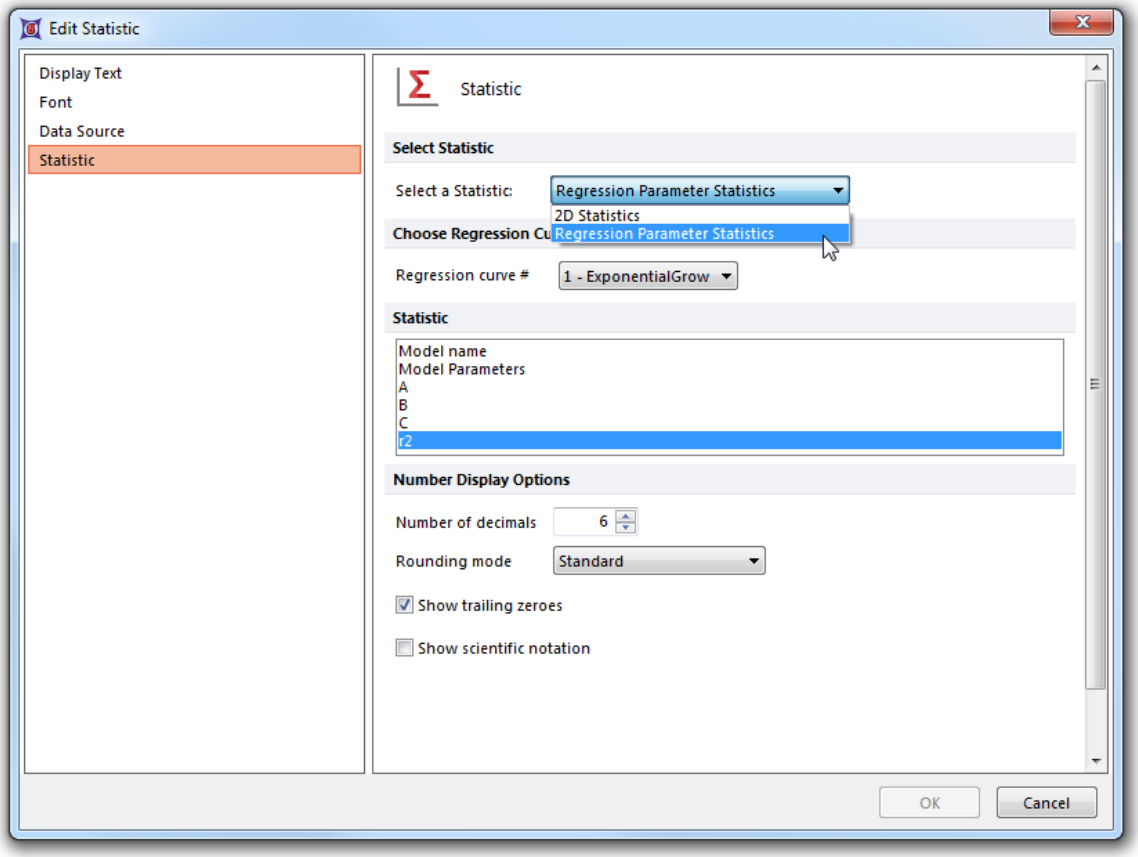

Fig. 11.67 Choosing Regression Parameter Statistics

Scatter with Regression parameters can also be displayed in a Regression Parameter Statistics window, by right-clicking on the plot, and choosing **Statistics**> **Regression Parameter Statistics** from the pop-up menu (Figure 11.68). The tokens therein may also be dragged and dropped elsewhere in your layout, which will result in a copy-paste operation.

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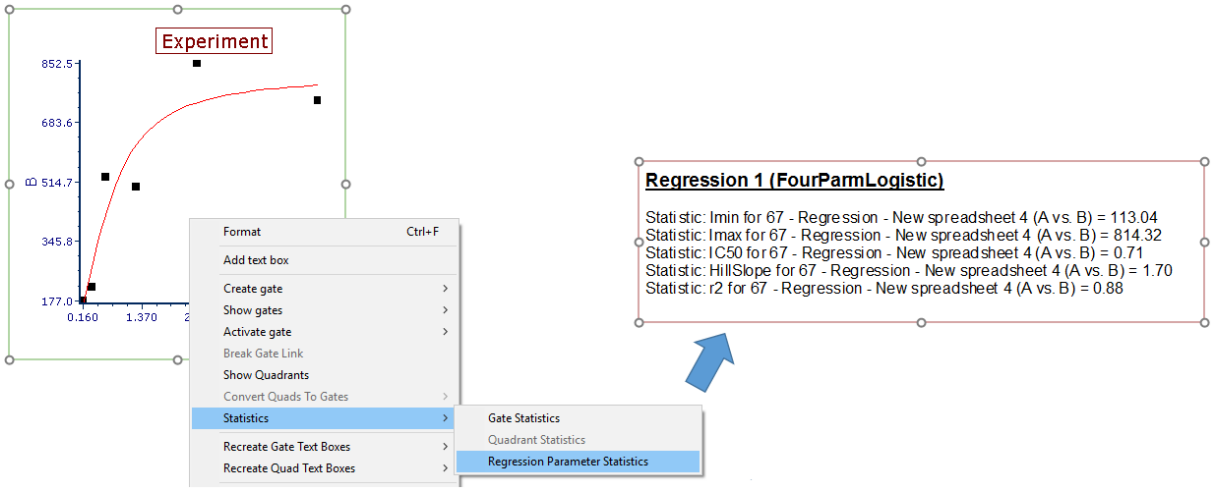

Fig. 11.68 Displaying the default Regresion Parameter Statistics window from the Scatter with Regression Plot

11.8.4.4 Interpolating values from Scatter with Regression Plots

X- or Y-values can be interpolated from Scatter with Regression Plots using the "RegressionPlotYToX" and "RegressionPlotXtoY" functions, via the Custom Tokens 728 window (Figure 11.69) or via a formula in the Spreadsheet 588. See below or this Use Case Tutorial section 1675 for details.

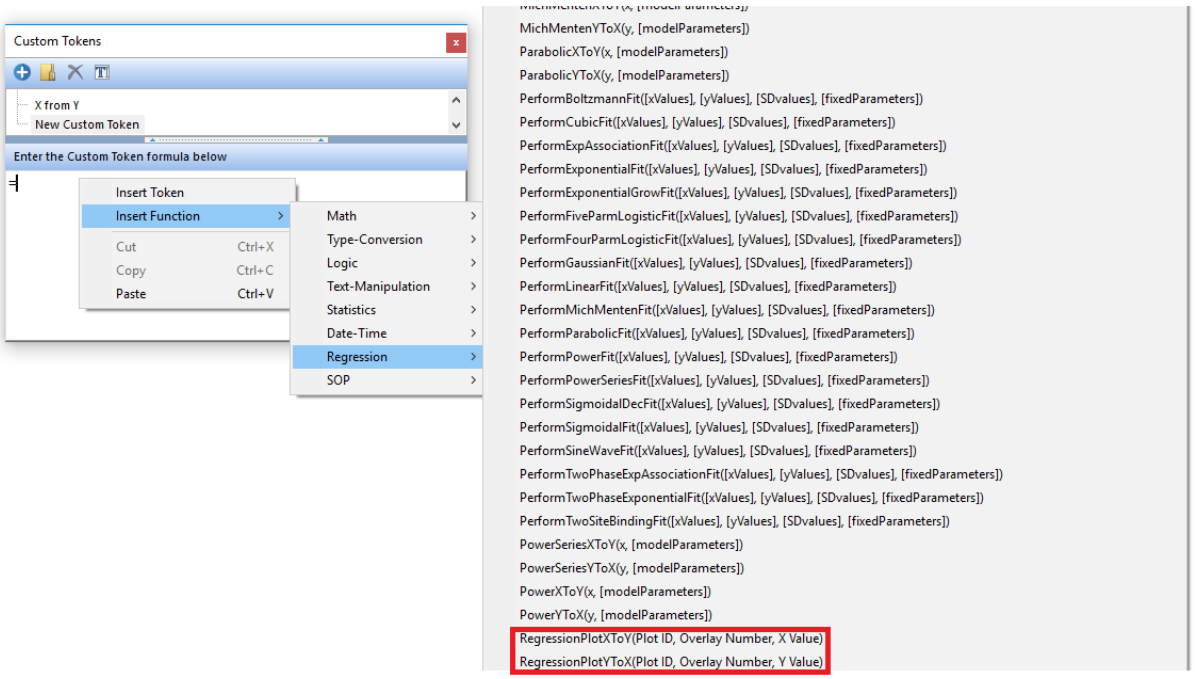

Fig. 11.69 Regression Plot Interpolation Custom Token Functions

The components of the Custom Token are Plot ID (i.e., the Plot Number token 719), Overlay Number for the Regression Overlay (note: the overlays are 1-based, so the raw data is Overlay #1, and the first regression is Overlay #2), and the X- or Y-value from which the interpolated value will be derived. An example of an

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unfilled and filled Custom Token formula is shown below in Figure 11.70, *top* and *bottom*, respectively.

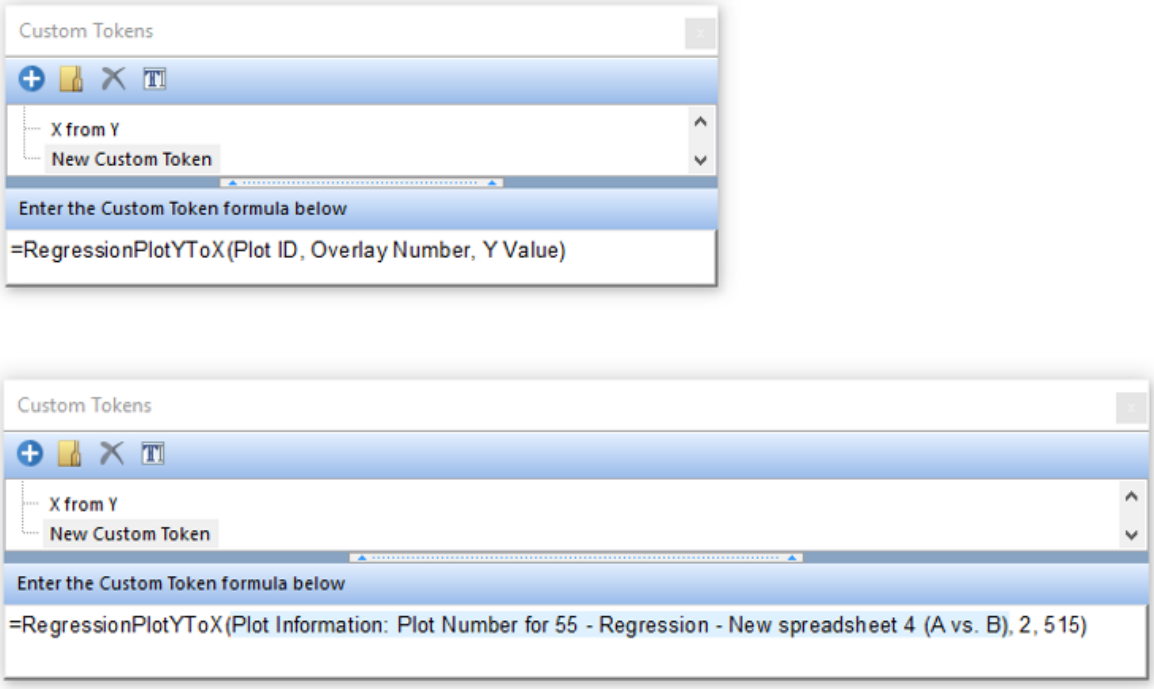

Fig. 11.70 Interpolation Custom Token formula components unfilled (top) and filled (bottom)

Interpolation formulas can also be entered into a Spreadsheet 414 from the **Spreadsheet** tab→**Formulas**→**Statistical** group of functions, as shown in Figure 11.71.

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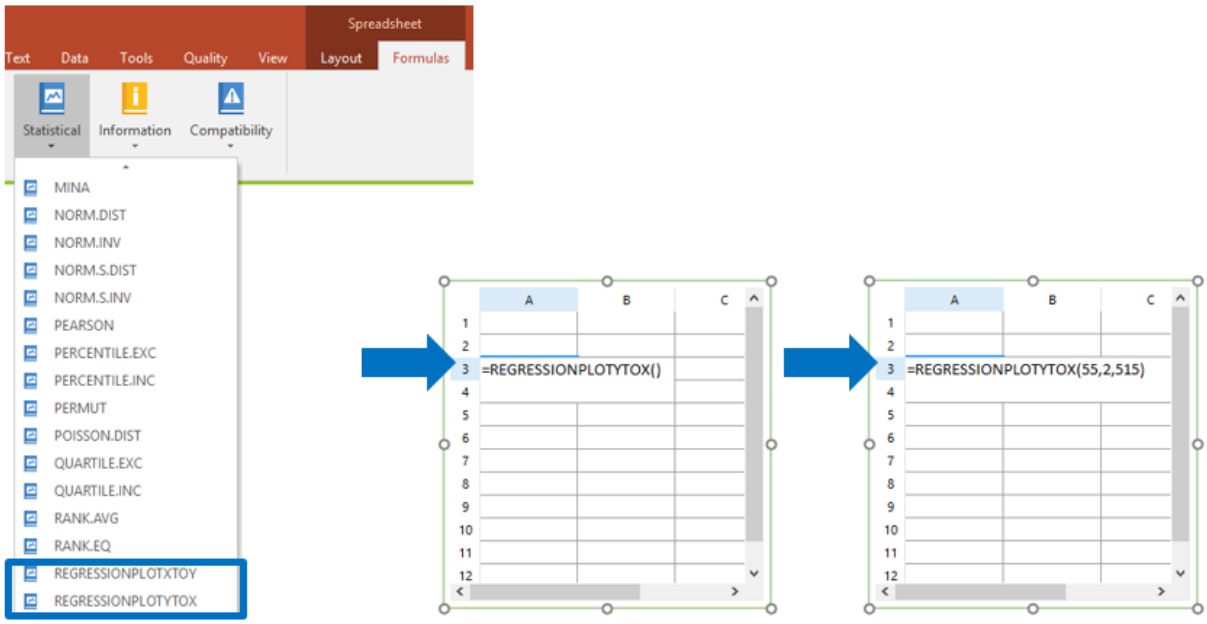

Fig. 11.71 Regression Plot Interpolation Formula (left) inserted in Spreadsheet (center) and with components filled (right).

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Chapter

12 The Concentration Calculator

Sometimes, you need to know the absolute concentration of the cells in your sample. For example, you may be determining absolute lymphocyte counts or counting bacterial populations. While most instruments allow for the accurate determination of counts, they often do not report the volume of the sample that was measured to obtain those counts.

FCS Express can calculate the sample volume from the flow rate and the sample acquisition time, or from counts of beads added to a sample at a known concentration. It can also read the $VOL in FCS 3.1 files or the equivalent value from Amnis DAF files.

FCS Express allows you to:

- Calculate individual concentrations.
- Have the concentration calculated automatically and appear directly on the statistics window.

12.1 Calculating a Single Concentration

To set up the proper parameters for the concentration calculator, select the **Tools** tab→**Calculate**→**Concentration Calculator** command.

To calculate a single concentration:

- Go to the **Individual** tab of the **Concentration Calculator** dialog (Figure 12.1).

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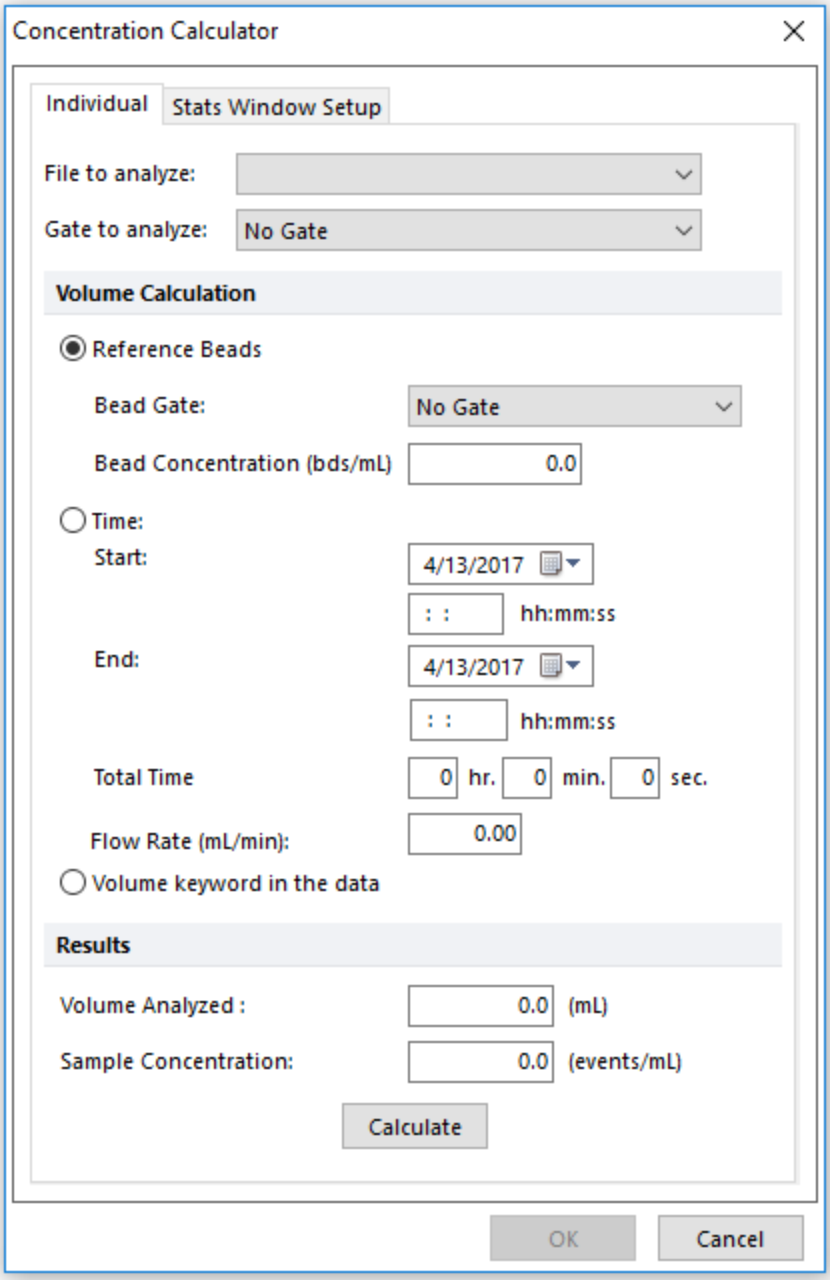

Figure 12.1 Concentration Calculator - Individual Tab

- Use the **File to analyze** drop-down list box to select the FCS file on which you want to calculate the concentration. You can choose to calculate the concentration for only a subset of cells by selecting a gate in the **Gate to analyze** list box.

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- Select a method to calculate volume using **Reference beads**, acquisition **Time** or **Volume keyword in the data** (see below).

- Press **Calculate** to calculate the actual concentration. The sample volume will be displayed in the **Volume Analyzed** field and the concentration will be displayed in the **Sample Concentration** field.

- **Calculating Volume from Reference Beads**

  A common way to calculate the volume of a sample is to add a known concentration of beads to your sample. By counting how many beads were acquired in the time it took to acquire the sample, it is possible to calculate the volume of the sample.

  - Before opening the **Concentration Calculator** dialog, draw a gate around your population of beads on a 2D Plot.
  - Select the **Reference Beads** radio button.
  - Select the gate created around beads from the **Bead Gate** drop-down menu.
  - Enter the concentration of the beads in the **Bead Concentration** field. The concentration must be entered in units of beads/ml. You can enter the concentration in scientific notation (e.g., 5 x 10^6/ml is entered as **5e6**).

- **Calculating Volume by Time**

  Some instrument sheath fluid flows at a well-defined rate. If you know the flow rate, and the acquisition time, you can calculate the volume acquired.

  - Enter the flow rate in the **Flow Rate** field in ml/min.
  - The **Start Time** and **End Time** fields are automatically filled in from data in the topmost FCS file header. If those times are not correct, you can enter the start time and end time directly. The **Total Time** is calculated as the difference between the start time and end time. You can also enter data into the total time field directly.

- **Calculating Volume by Keyword**

  A standardized keyword for sample volume run during acquisition ($VOL) was introduced with the FCS 3.1 file format. The keyword value is a floating point number, which represents sample volume in nanoliters. FCS Express can read the keyword from FCS files and the equivalent from Amnis .daf files to be used for calculating concentrations.

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- Before opening the **Concentration Calculator** dialog, insert a plot. If preferred, draw a gate around your population of interest.
- Select the **Volume keyword in the data** radio button.

12.2 Calculating Concentrations on the Statistics Page

You can also have the concentration calculated automatically on the statistics window of a plot. In that case, you must tell FCS Express how the volume is to be analyzed for that data file. The concentration will be calculated as the number of cells in that statistics row divided by the volume. To set up statistics concentration calculations, go to the **Stats Window Setup** tab of the **Concentration Calculator** dialog (Figure 12.2) and enter the volume parameters (by **Reference Beads**, **Time**, or **Volume keyword in the data**) in a similar manner as for a single concentration 592.

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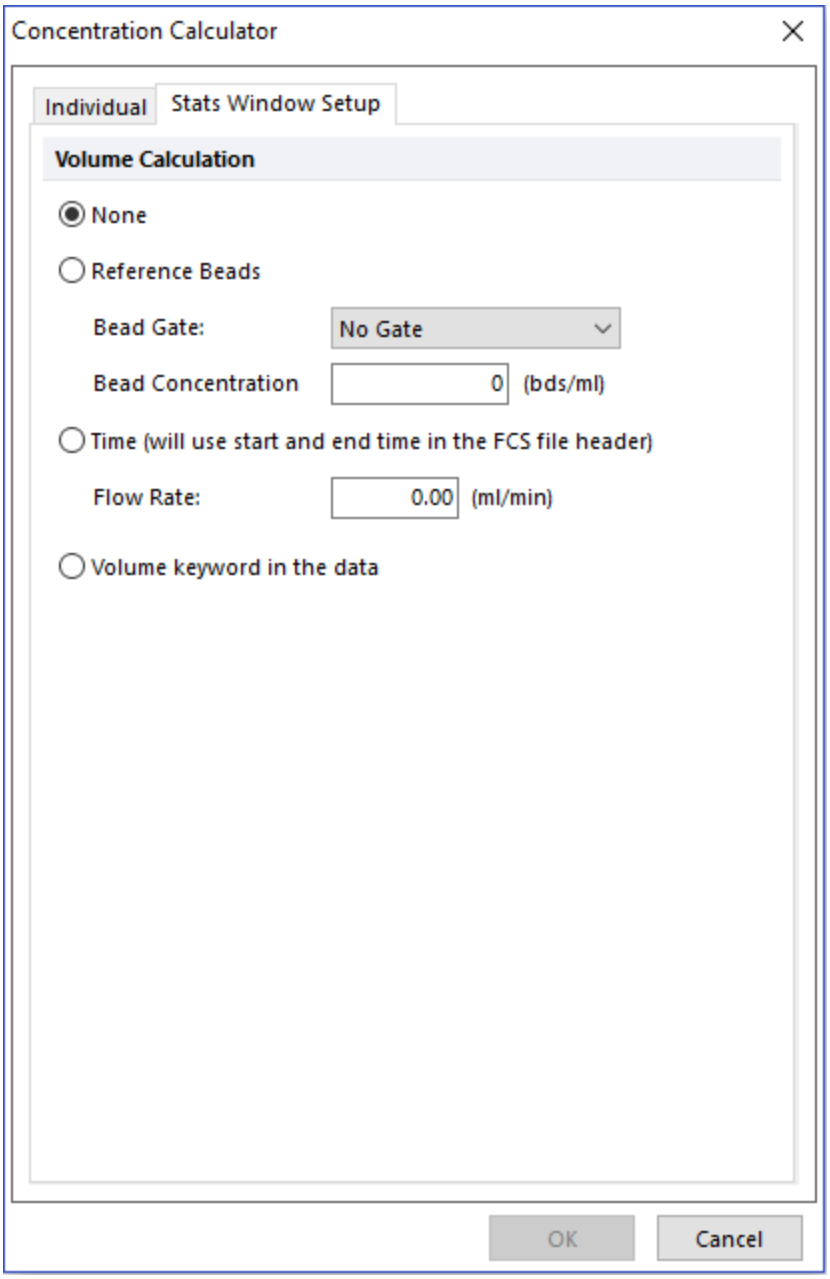

Figure 12.2 Concentration Calculator Dialog - Stats Window Setup

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Chapter

13 The Histogram Subtraction Tool

Histogram subtraction allows you to determine the percent positive on a histogram by subtracting a negative control histogram from a sample histogram. Histogram subtraction can be

1. Set up to be automatically calculated and appear on the Histogram Statistic 382 table. In this case, the **Overton Subtraction** method is used.
2. Performed on an individual basis using the **Histogram Subtraction** tool. In this case, the subtraction can be performed using different method.

To have the histogram subtraction automatically performed and displayed in a histogram statistics window:

- Make sure the percent positive column is visible for the statistics window 389.
- Use the **Format→Plot Options→Specific Options** command (or right-click on the histogram, choose **Format**, and select the **Histogram→Histogram Specific Options** category). Set the **Control Histogram for Histogram Subtraction Statistics** field to the appropriate negative control (Figure 13.1). Statistics histogram subtraction always uses the *Overton Subtraction* method, described below.

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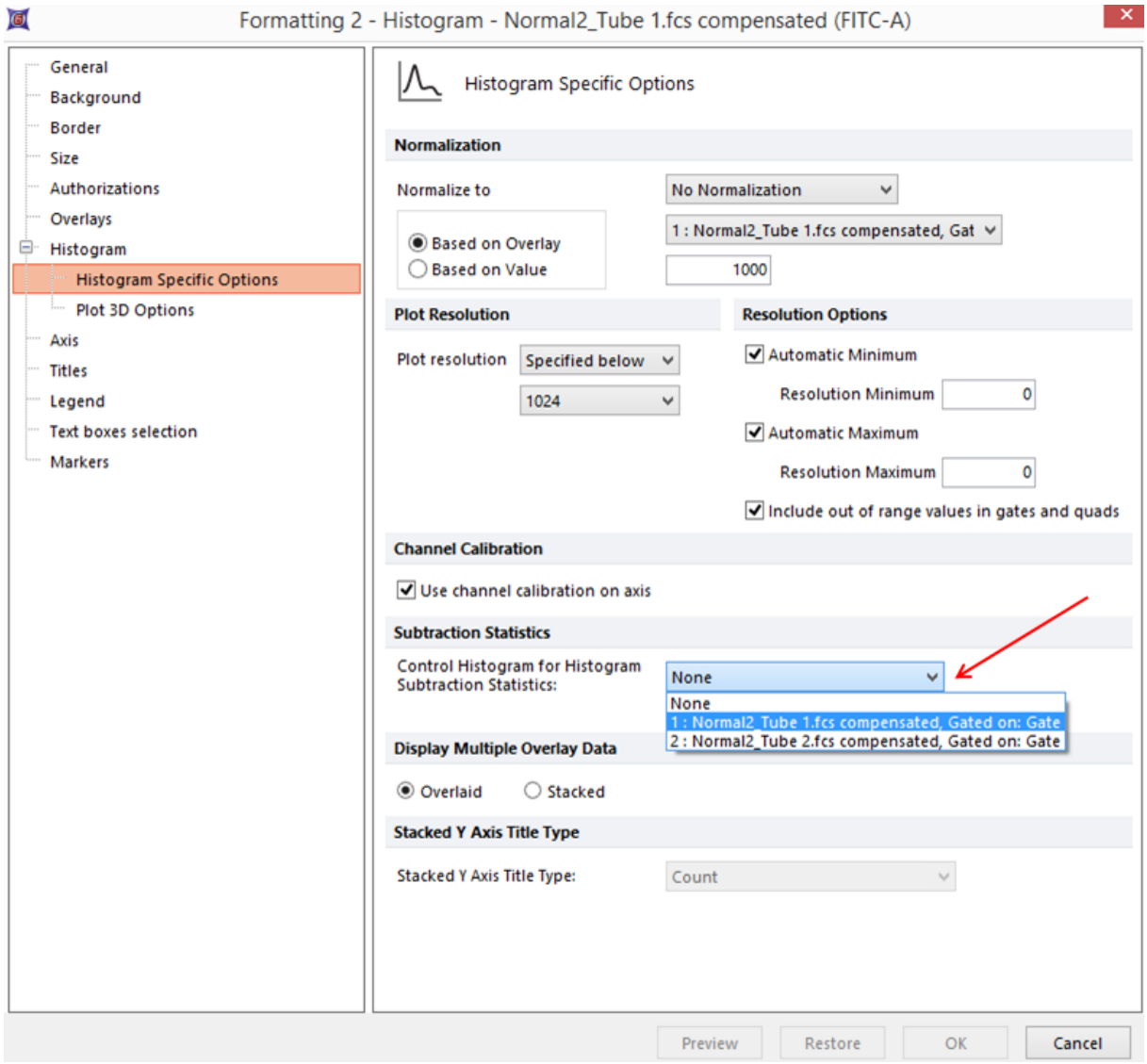

Figure 13.1 Setting the Control Histogram in the Formatting Histogram Specific Options Dialog

To perform an individual histogram subtraction, select the **Tools** tab→**Calculate** category→click **Histogram Subtraction** command on the ribbon (Figure 13.2).

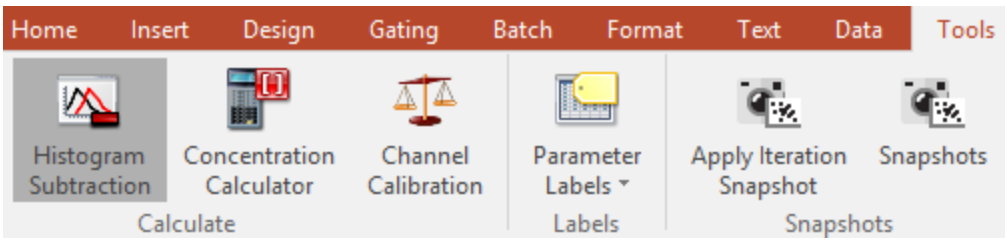

Figure 13. 2 - Histogram Subtraction command on ribbon

The **Histogram Subtraction** window (Figure 13.3).

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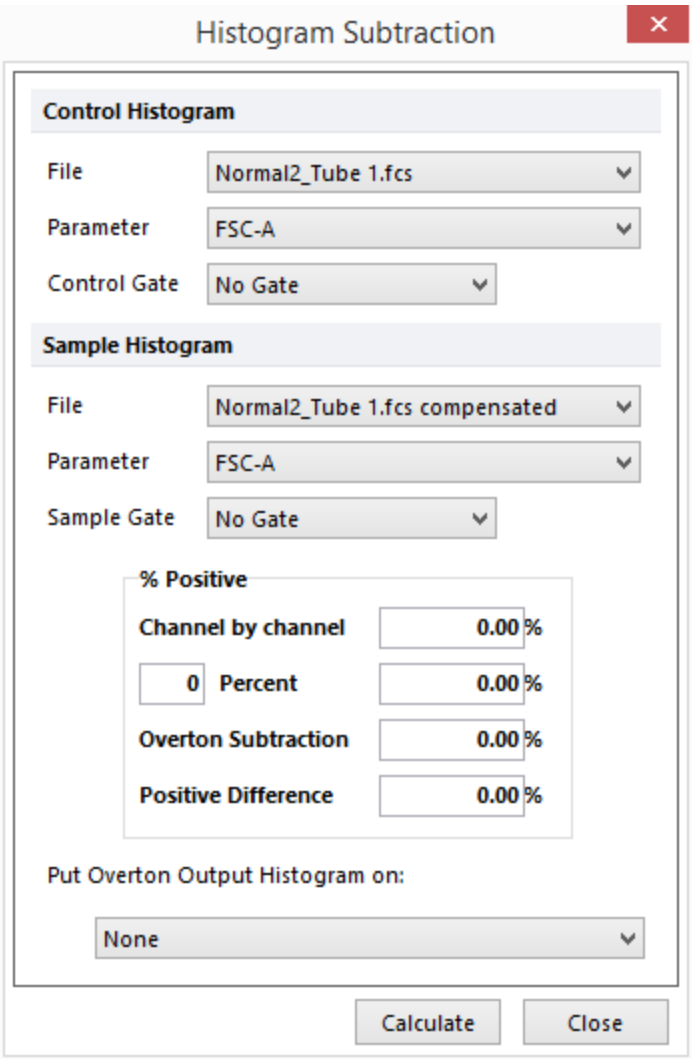

Figure 13.3 Histogram Subtraction Window

A histogram subtraction can be performed for any FCS file that is currently open. Select the **File**, **Parameter**, and **Control** or **Sample Gate** for the **Control Histogram** and **Sample Histogram** in the appropriate places. Press the **Calculate** button to perform the histogram subtraction. The percent positive is calculated in four different ways, of which the Overton Subtraction and Positive Difference methods are usually the most accurate.

The methods used to calculate **% Positive** are:

- **Channel by channel**

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The control histogram is subtracted from the sample histogram on a channel by channel basis. In other words, the number of cells in channel N of the control is subtracted from the number of events in channel N of the sample. All of the positive results (i.e., where there are more cells in the sample channel N than in the control channel N) are summed and are then divided by the total number of cells to yield a positive result.

- X **Percent**

  In this method, you set a cutoff channel value at the top of the control population and consider all cells above this channel to be positive. To determine the cutoff value, enter a value in the edit field to the left of the **Percent** label. If you enter the value **2**, the cutoff channel will be below the highest two percent of the control cells or the 98th percentile. Any cells in the sample histogram that are above this channel are considered positive.

- **Overton Subtraction**

  This technique is similar to the channel by channel subtraction method except that negative differences (where the number of cells in control channel N is greater than the number of cells in sample channel N) are added to the positive differences in lower channels. After all channels have been analyzed, residual negative results are set to zero, and the sum of the positive results is divided by the total number of cells to yield a percent positive.

- **Positive Difference**

  The percent of cells between channel N and channel N-1 are calculated for the sample and control histogram and the difference between these percentages is determined. This difference is determined as N moves from channel 1 to the last channel. The largest difference found becomes the percent positive.

The Overton Subtraction results in a histogram overlay that contains all the positive cells. This overlay can be placed on a histogram by choosing an existing histogram in the **Put Overton Output Histogram On** field. Statistics can be calculated on this overlay as if it were a regular overlay on the histogram.

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Chapter

14 Publishing Layout Tools

FCS Express has the unique capability to share your data and analysis with other scientists and researchers. You can share your layouts by publishing them. Once a layout is published, it can be placed on a web site and can be read by anyone in the world using the FCS Express Reader 611, which is available free at http://www.denovosoftware.com/site/reader.shtml.

A published layout is a specialized FCS Express layout with embedded data and certain restrictions about what can be changed. FCS Express Reader will allow users to perform a complete analysis using your data (including moving gates and creating new plots) subject to certain limitations 611.

De Novo Software also provides a free service to publish your layouts 604. These layouts will be hosted on our web site and can be accessed by anyone on the internet. You do not have to worry about uploading your published files to a web site and exposing it. That is all handled behind the scenes by FCS Express.

Every FCS Express customer has 10 free megabytes on our web site that they can use to publish their layouts. If more space is required, you can purchase additional hosting space 612. In addition, you can publish the layout to your own web site 607.

14.1 Publishing a Layout to the De Novo Software Publishing Site

To publish a layout to the De Novo Software web site, use the **File** tab→**Collaborate**→**Publish Layout...** command. The **Publishing Wizard** will appear to guide you through the publishing process.

You enter demographic information about your layout (**Title**, **Author**, **Organization**) in the first page after the welcome screen (Figure 14.1).

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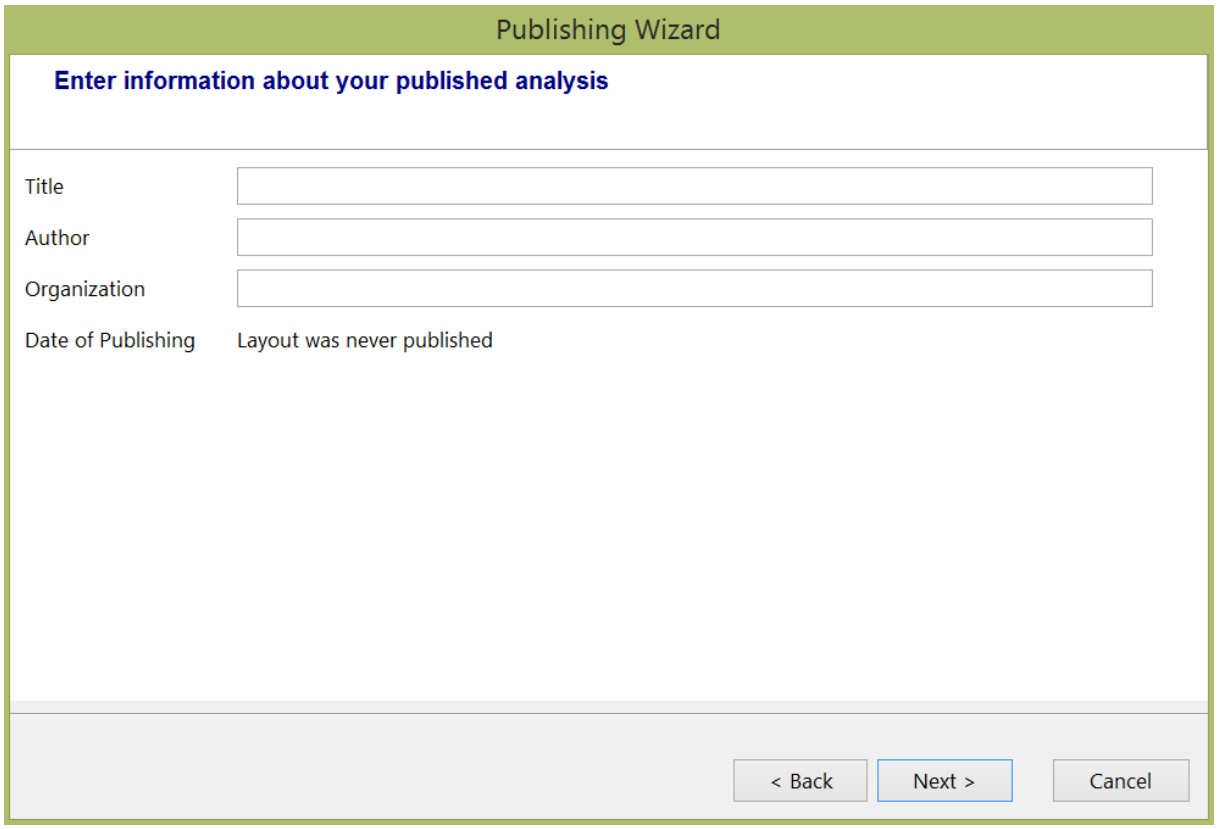

Figure 14.1 Publishing Information

This information is optional and is only used for descriptive purposes. Press **Next** when you are done entering the demographic information.

The next page (Figure 14.2) allows you to choose the location to publish your layout. Everyone can publish on the De Novo Software web site. To publish to your local hard drive, please see the section on Advanced Publishing 607. Press **Next** to publish your layout on the De Novo Software web site.

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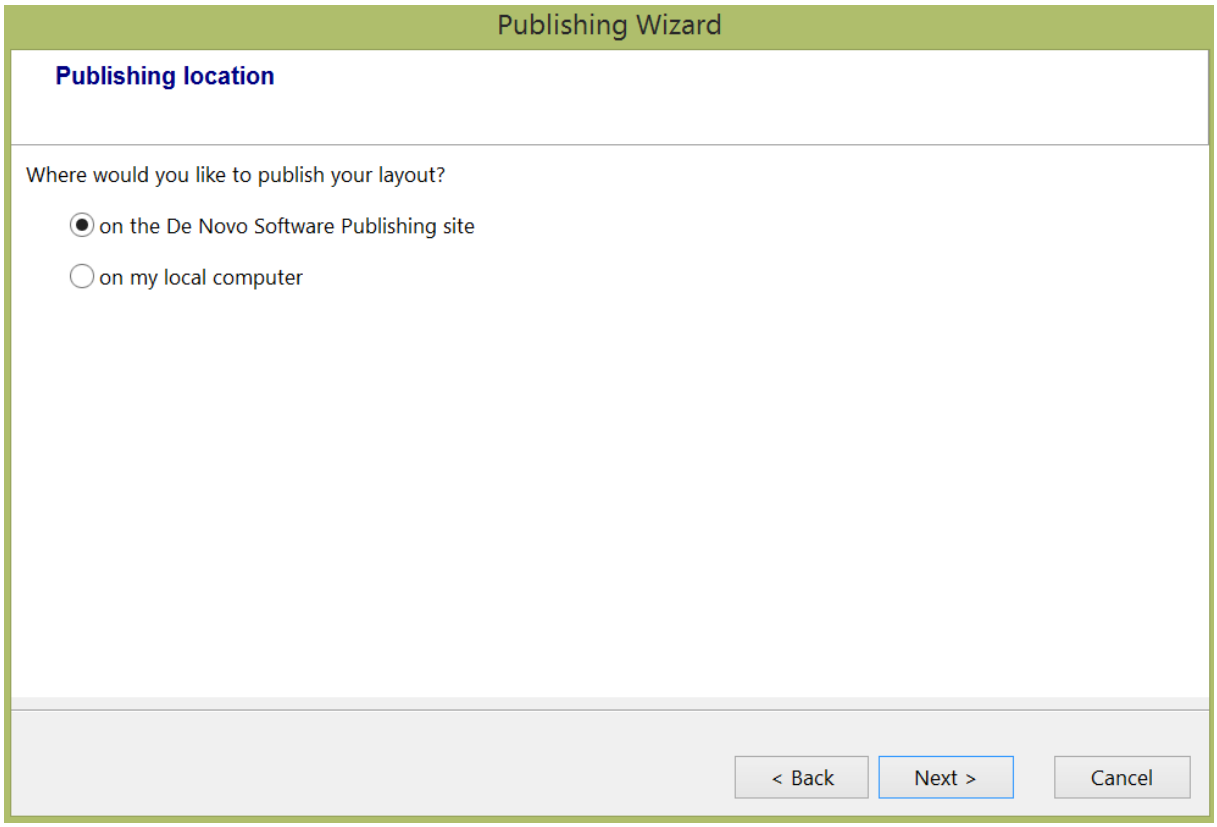

Figure 14.2 Publishing Location

When using the Advanced Publisher you will be presented with two additional dialogs that are discussed in the Advanced Publishing section 607.

The **Publishing Wizard** will ask you to log in using your email address and password (Figure 14.3). This must be the same email address and password that you used when registering for an account on the De Novo Software web site.

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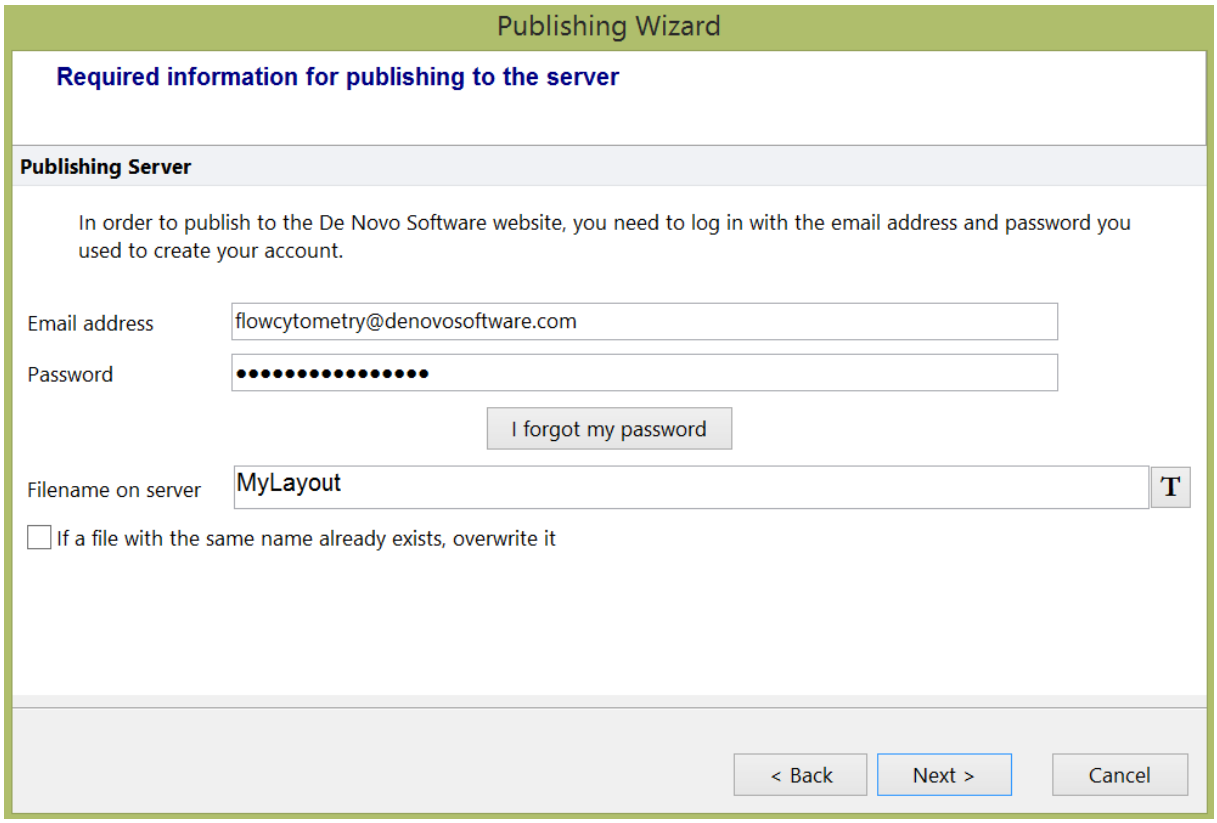

Figure 14.3 Logging in to the De Novo Software Server

You can also provide a filename that will be used when publishing. This will be the name of the layout that will appear in the maintenance list 609.

Press **Next** after entering the required information. The final page of the wizard shows you the URL that you distribute to have other people view your published layout. Press **Copy** to copy the URL to the clipboard to paste it into an e-mail. Pasting the URL into a browser will allow users with the FCS Express Reader 611 to view your data files.

14.2 Advanced Publishing

The advanced publishing module allows you to publish your layout to any location, as well as giving you several more options for controlling how your published layouts are used by others. Publishing permissions may be saved just like regular permissions and loaded from a previously saved permissions file 1125.

If you are using the advanced publisher, the **on my local computer** option will be available on the Location page of the Publishing Wizard 604. If you select this radio button and press **Next**, the **Set permissions for the published layout** page (Figure 14.4) will appear.

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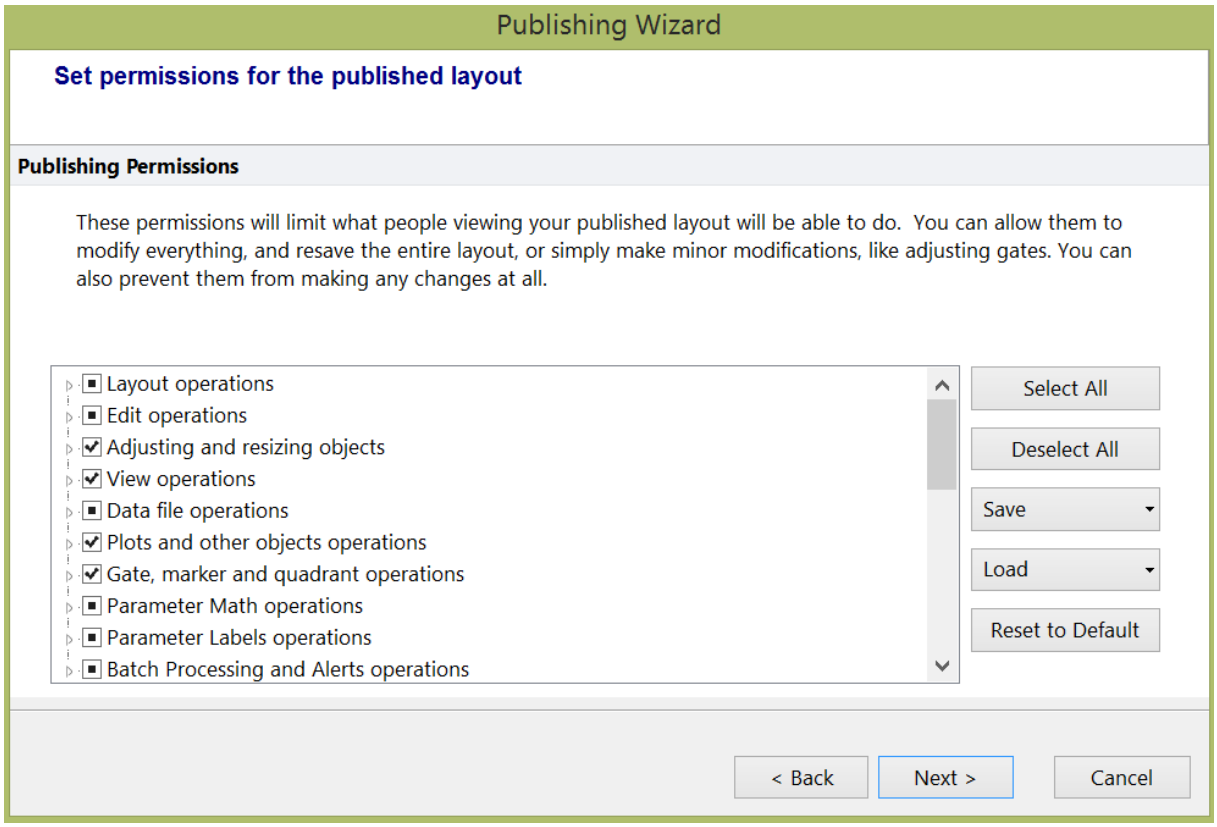

Figure 14.4 Publishing Security Permissions

On this page, you can select which actions viewers of your layout will be allowed to perform. For example, if you wish to prevent users from printing your layout, you could disable the Print permission (located under **Layout operations**). Almost every action in FCS Express has an associated permission code that you can choose to allow or not to allow. These permissions will be respected if the published layout is loaded both in FCS Express and FCS Express Reader. Press **Next** after you have selected your permissions.

The next page (**Advanced publishing options**) allows you to control additional features, including whether to include batch processing actions and/or disable token editing in the published document.

The next page (**Information for keywords anonymizing**) allows you to use the features associated with the Keyword Anonymizer 658 to remove any or all keywords from the data in the published layout.

When publishing to your local computer, the next page requires you to select the file name. Your published file will have the same extension as a standard FCS Express layout (.fey) and the data will automatically be embedded.

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When publishing to the De Novo Software website, the next page requires you to enter the email address and password associated with you De Novo Software account as well as a file name for the layout on the server.

Any published layout can be opened in the FCS Express Reader.

14.3 Default Publishing Options

The **Default Permissions** and **Publishing Options** can be set in the Advanced Publishing category of the FCS Express User Options dialog 226.

14.4 Managing Your Published Layouts

FCS Express allows you to delete and rename the layouts that you currently have published on the De Novo Software web site.

Select the **File** tab→**Collaborate**→**Publishing Maintenance** command to open the **Manage your Published Layouts** dialog (Figure 14.5).

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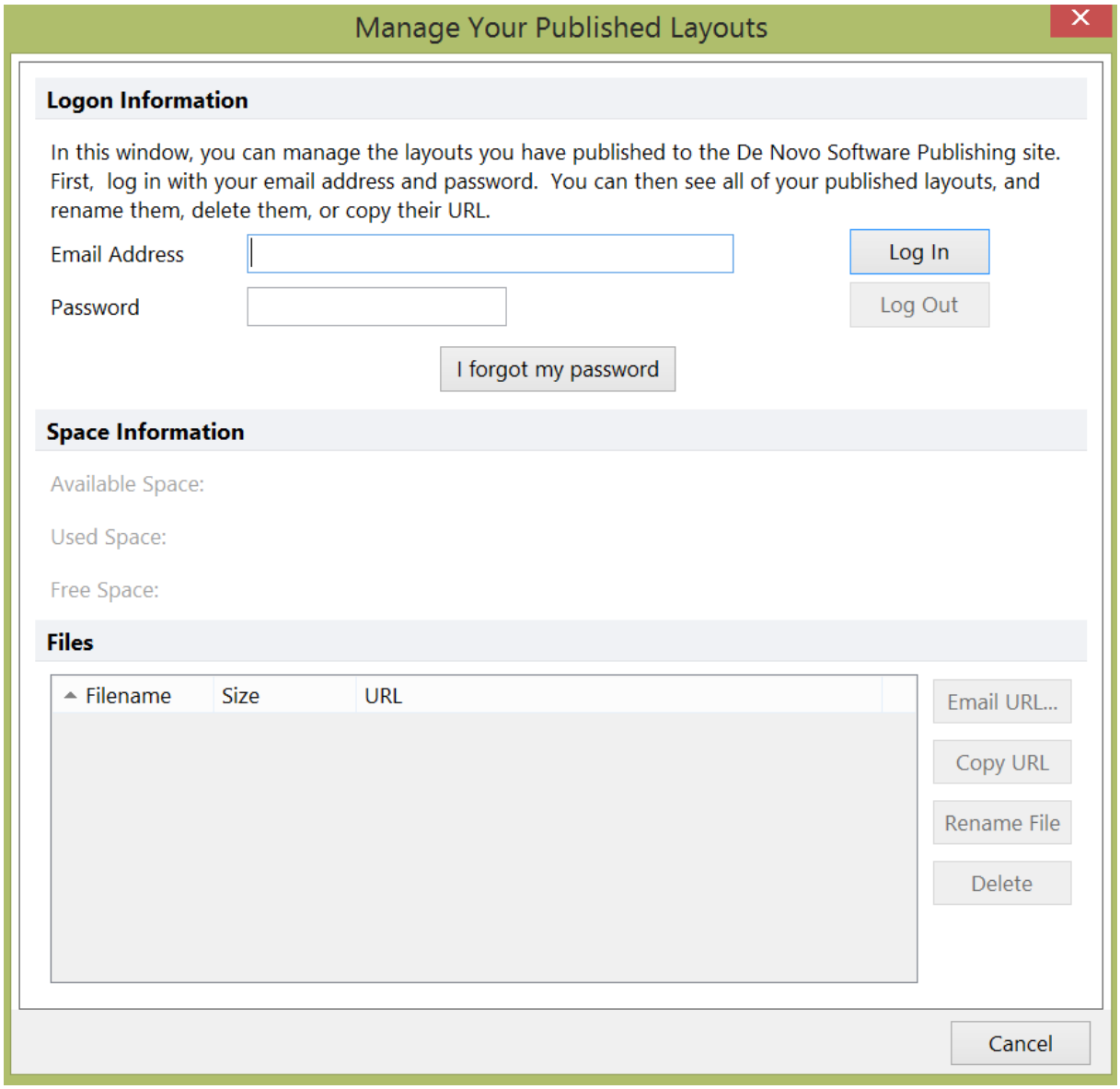

Figure 14.5 Managing Published Layouts

Enter your **Email Address** and **Password** and press the **Log In** button. You must use the same email address and password that you used when registering for an account on the De Novo Software web site. When you log in, a summary of all your published layouts on the De Novo Software web site will appear in the bottom portion of the window. You can **Delete** or **Rename** files, as well as obtain the URL for any of your published layouts. If you need more than 10 MB of space for your layouts, you can purchase a larger hosting plan 612.

14.5 Sharing Published Layouts with Others

To share your published layouts with others, they must have either FCS Express or FCS Express Reader installed on their computer.

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Simply distribute the URL that was generated by FCS Express 604 (or that you chose when publishing to your own site). Paste the URL into any web browser (Figure 14.6) and press **Enter**.

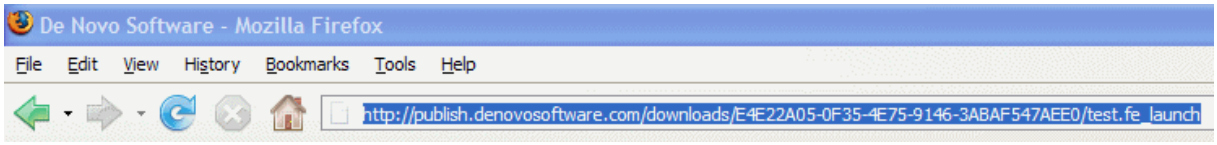

Figure 14.6 Pasting the URL of a Published Layout into a Web Browser

14.6 FCS Express Reader

With FCS Express Reader, you can:

- See published analysis from the web.
- Perform all actions that the publisher of the layout has given you access to.
- Re-analyze the data. All analysis capabilities of the FCS Express Professional version are available.
- Find templates and demos on the web from reagent suppliers to help get started with using their products.
- Upgrade to the full demo, or the purchased version, at any time.

14.6.1 Obtaining the FCS Express Reader

FCS Express Reader is available free at http://www.denovosoftware.com/site/reader.shtml.

14.6.2 FCS Express Reader Limitations

FCS Express Reader allows you to perform almost all of the analysis tasks of the regular version of FCS Express. For example, you can add new plots, manipulate gates, get statistics, and print. However, FCS Express Reader does have the following limitations:

- You can only use the data files that are embedded in the published layout. You cannot change data files, load new data files, or run batch processing on the layout.

- There is restricted saving capability. A published layout that was saved from FCS Express Reader can only be opened on the same computer on which it was saved, or the computer from which it was originally published. This allows teachers to create layouts which students can manipulate and save. The changed layouts can then be returned to the teacher for subsequent grading (as long as the grading occurs on the same computer which created the published layouts).

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14.6.3 Upgrading from FCS Reader to FCS Express

To upgrade from the FCS Express Reader to FCS Express, purchase the appropriate version from http://www.denovosoftware.com. Once you have made your purchase, you can upgrade your FCS Express Reader by selecting the **File** tab →**System**→**Change your configuration.** (Figure 14.7) and follow the steps in the configuration wizard.

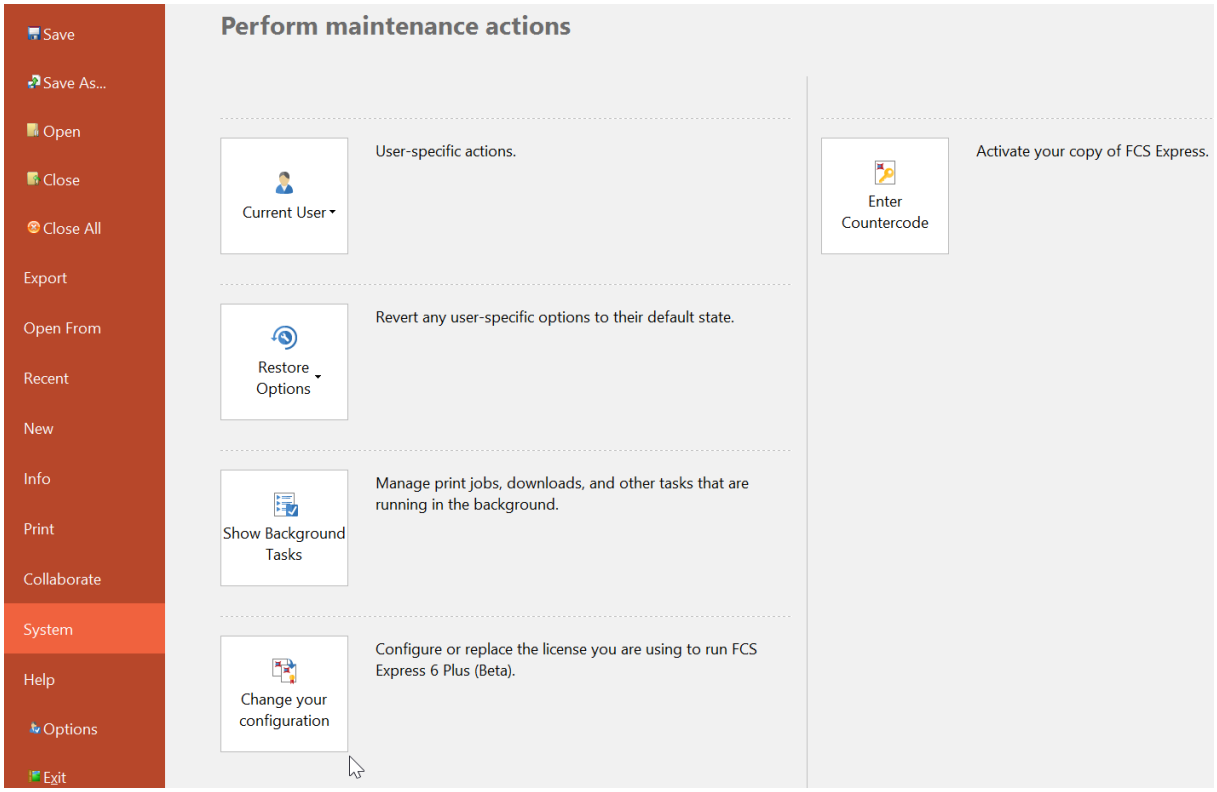

Figure 14.7 Upgrade FCS Express Reader

14.7 Extra Hosting Plans

If you want to publish more than 10 MB worth of layouts on the De Novo Software publishing site, you can purchase extra hosting plans. These allow you additional space on our servers. Several different hosting plans are available. Details can be found at http://www.denovosoftware.com.

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Chapter

15 The Channel Calibration Tool

Quantitative flow cytometry is used to determine the actual number of fluorescent ligands (fluorochromes or fluorochrome-protein conjugate molecules) labeling each particle. The technique requires calibration of the fluorescence channel number with standards of known fluorochrome content. The standards define the relationship between the observed fluorescence level and the number of bound fluorochrome molecules.

Several companies have specially designed beads of different intensities where the precise number of dye molecules per bead is known. By establishing a standard curve correlating the channel number to the number of dye molecules per bead, it is possible to determine the absolute number of molecules per cell of antigen, antibody, and other useful markers.

This type of calibration has traditionally been a very labor intensive process, requiring you to use your flow cytometry software to obtain a set of statistics from the beads and the sample, then entering the statistics into a spreadsheet, creating a calibration curve, determining the standard channel values, and finally performing more calculations using the spreadsheet to convert the statistics to the number of binding sites per cell.

FCS Express automates the entire process for you. Once you have set up a channel calibration, all plots will automatically display the calibrated axes, and the statistics will be displayed as calibrated values. Tokens may be used to monitor if a calibration has been applied to an axis 620 or what the last channel calibration loaded in the layout 621 was. To set up a channel calibration 614, you must have a set of beads for which the number of fluorochrome molecules is precisely known. In addition, you must know the effective fluorescence-protein ratio to determine the number of dye molecules attached to each marker on the cells.

15.1 Creating a New Channel Calibration

To create a new channel calibration:

- Display the bead data in a histogram and create a marker 353 bounding each bead as shown in Figure 15.1.

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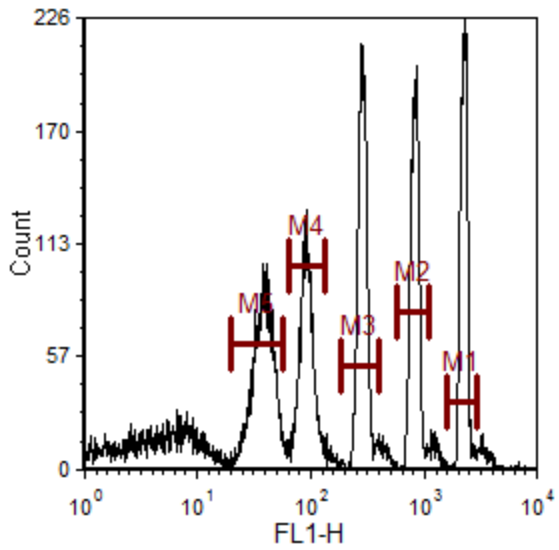

Figure 15.1 Setting Up Markers for Channel Calibration

- Ensure that the plot being used as the raw data source for the calibration does not itself have calibration applied by following Steps 11-15 of the Channel Calibration tutorial 1908.

- Select the **Tools** tab→click **Channel Calibration** command (Figure 15.2).

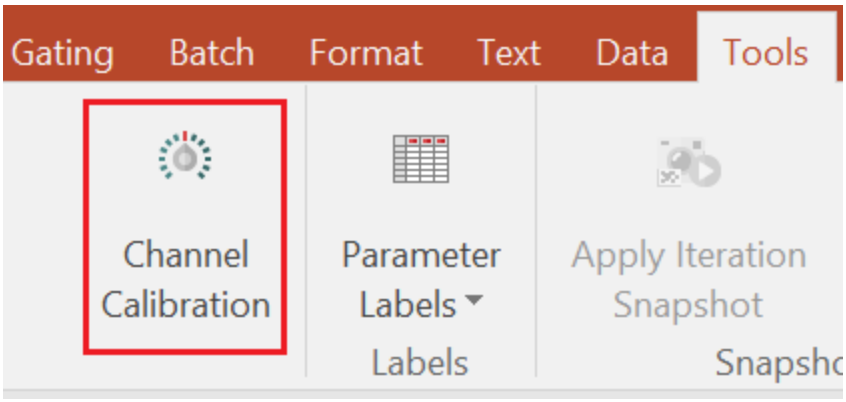

Figure 15.2 - Channel Calibration Ribbon

- The Channel Calibration window 622 will appear (Figure 15.3).

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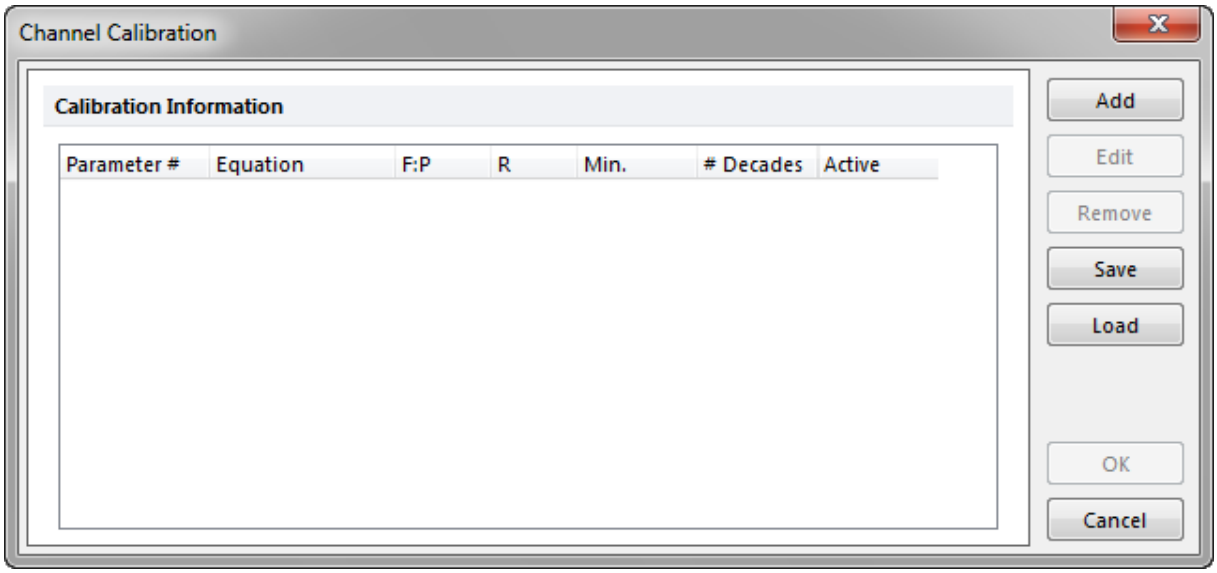

Figure 15.3 Channel Calibration Window

- Click **Add** to create a new calibration. The **New Calibration** dialog will appear (Figure 15.4).

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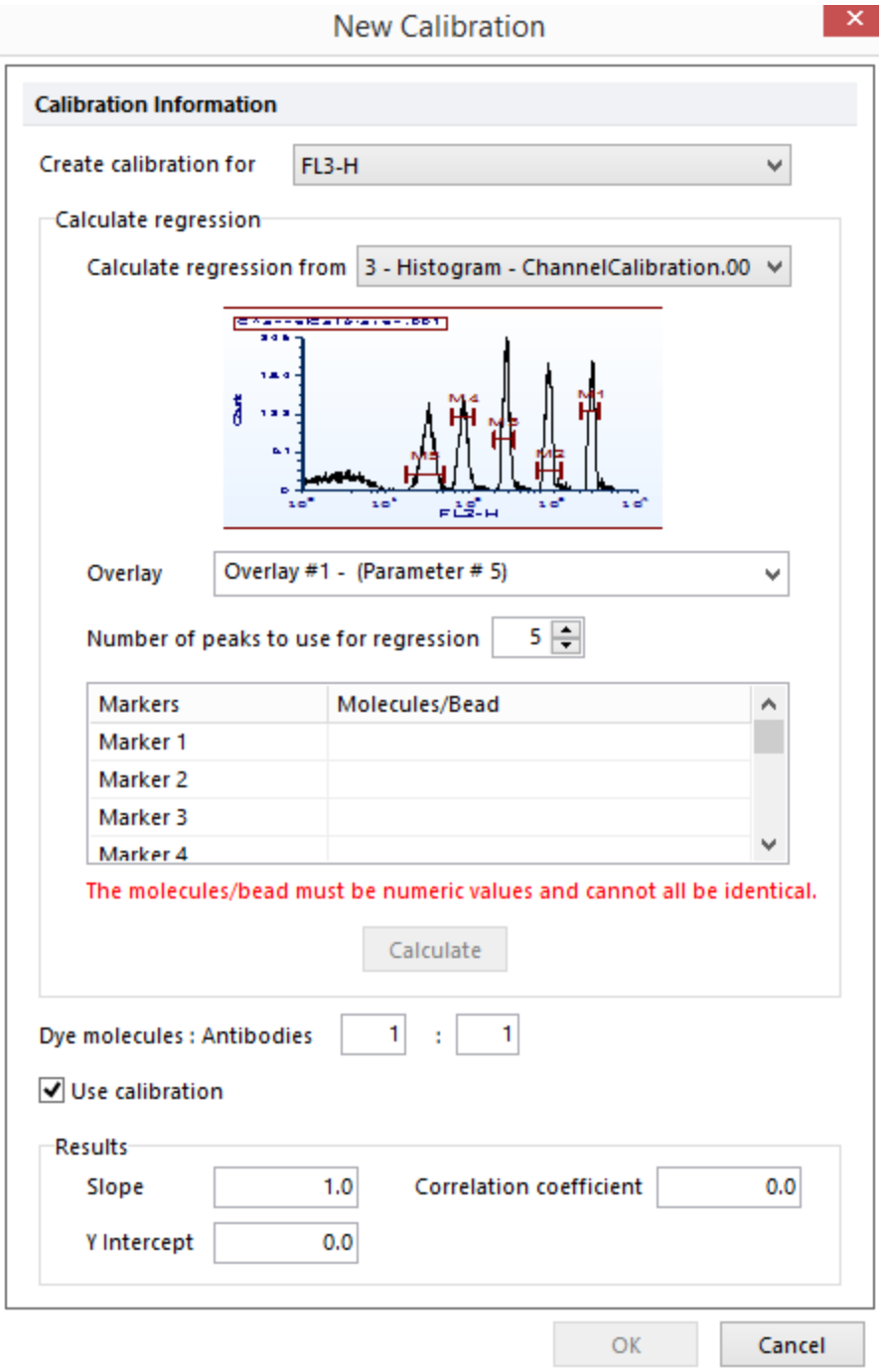

Figure 15.4 New Calibration Dialog

- Select the parameter you wish to create the calibration for in the **Create calibration for** drop-down list.
- Choose the histogram which will serve as a data source for the calibration from the **Calculate regression from** dropdown list.

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- Select the correct overlay from the histogram in the **Overlay** drop-down list. When the histogram is selected, the marker box will automatically create an entry for every marker that exists on that histogram. You can choose to use fewer markers by changing the value in the **Number of peaks to use for regression** field. You must use a minimum of three markers to calculate the regression.

- Enter the number of molecules per bead next to the marker for that peak. This information is supplied by the manufacturer of the beads.

- Click **Calculate** to calculate the new channel calibration. The equation for the new calibration (**Slope**, **Y Intercept**, **Correlation coefficient**) will appear in the **Results** box.

- The **Dye molecules:Antibodies** (also known as the F:P) ratio is typically 1:1, but you can change it to another value. The F:P ratio is supplied by the manufacturer of the antibodies you are using in your experiment.

- Click **OK** to accept the new calibration.

The channel calibration may now be saved, edited, or reloaded from the Channel Calibration dialog 618.

15.2 Using a Channel Calibration

FCS Express offers two levels of control in determining whether a parameter will have a calibration applied to it: A global control, and an individual plot-by-plot control.

If you do not want the calibration to be used at all, you can select the **Tools** tab→click **Channel Calibration** command and uncheck the **Active** checkbox beside that calibration (Figure 15.5).

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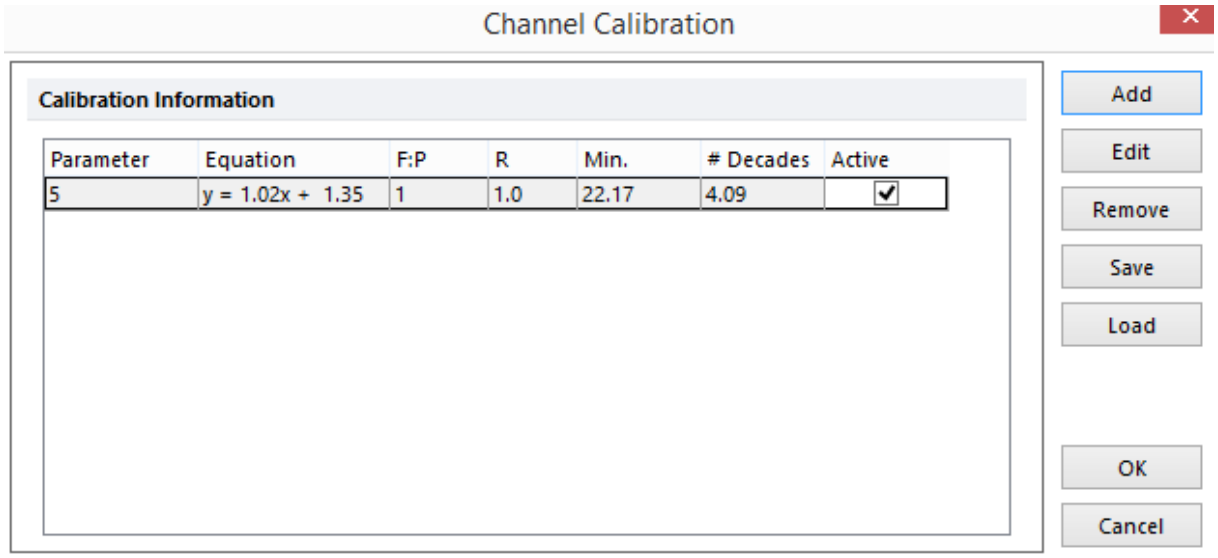

Figure15.5 - Channel Calibration Window with Active Checkbox

You can also choose which individual plots use the calibrations. The **Specific Options** page of the **Format** window for both histograms 274 and 2D Plots 305 contains a **Use channel calibrated on axis** checkbox. If this checkbox is set, calibrations will be used for any parameter that has a calibration defined. If the **Use channel calibrated on axis** checkbox is not set, the calibration will not be used, irrespective of whether or not that calibration is active (Figure 15.6).

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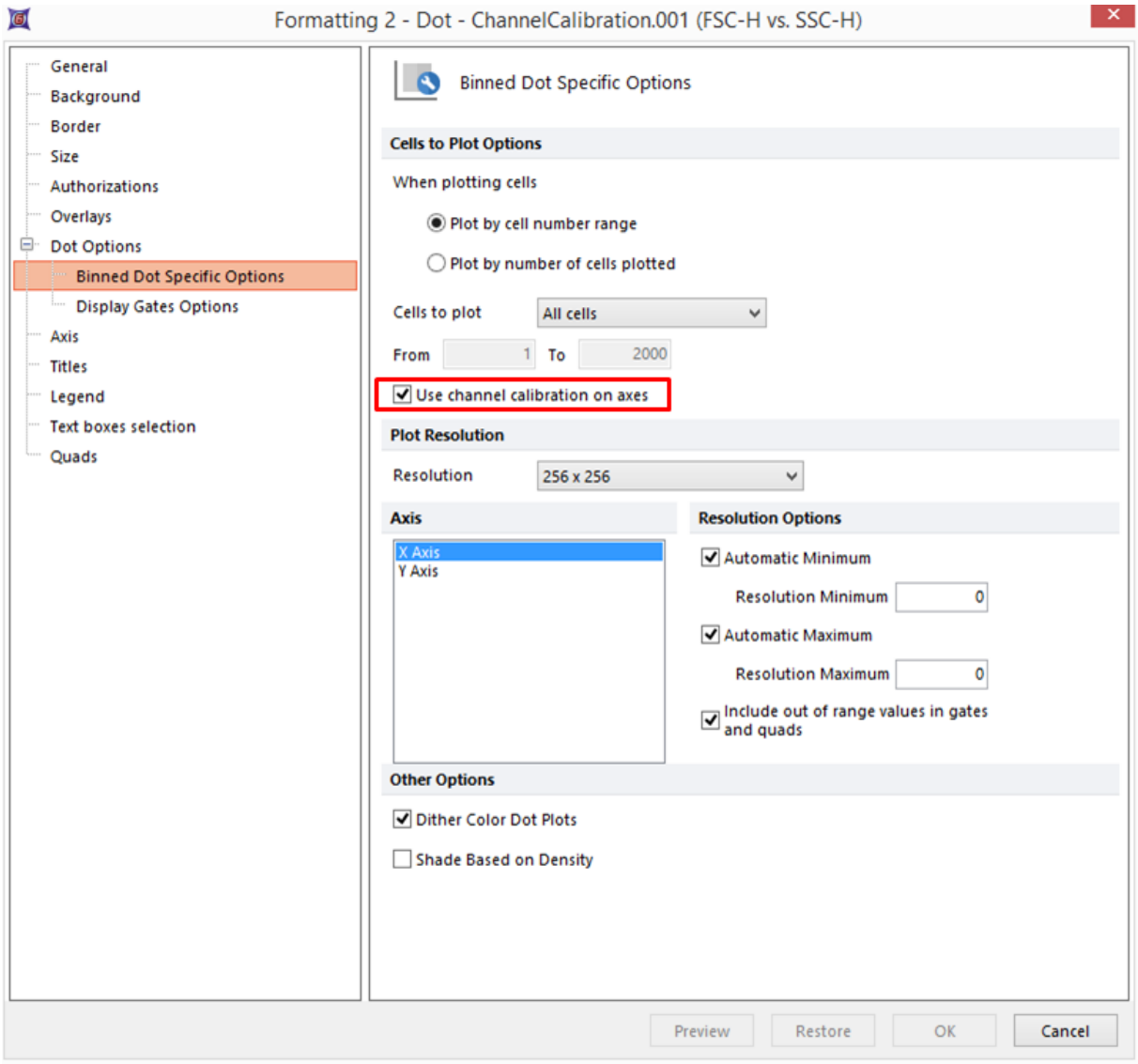

Figure 15.6 - Channel Calibration on Axes in Formatting Dialog

Tokens[692] in FCS Express also allow you to monitor if channel calibrations are applied to any axis on your plots[620] and to monitor the last loaded calibration file in the layout[621].

To monitor channel calibrations on either axis of a plot using the "**Is channel Calibration applied on the axis?**" token type:

1. Right click on a text box.
2. Choose **Insert Token**.
3. Choose **Plot→Plot Information**.
4. Choose "**Is channel Calibration applied on the X/Y axis?**" in the **Plot Elements** category.
5. Click **OK**.
6. Click **Insert**.

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A new token will now be displayed in the text box indicating the calibration status of the axis you chose for the indicated plot (Figure 15.7).

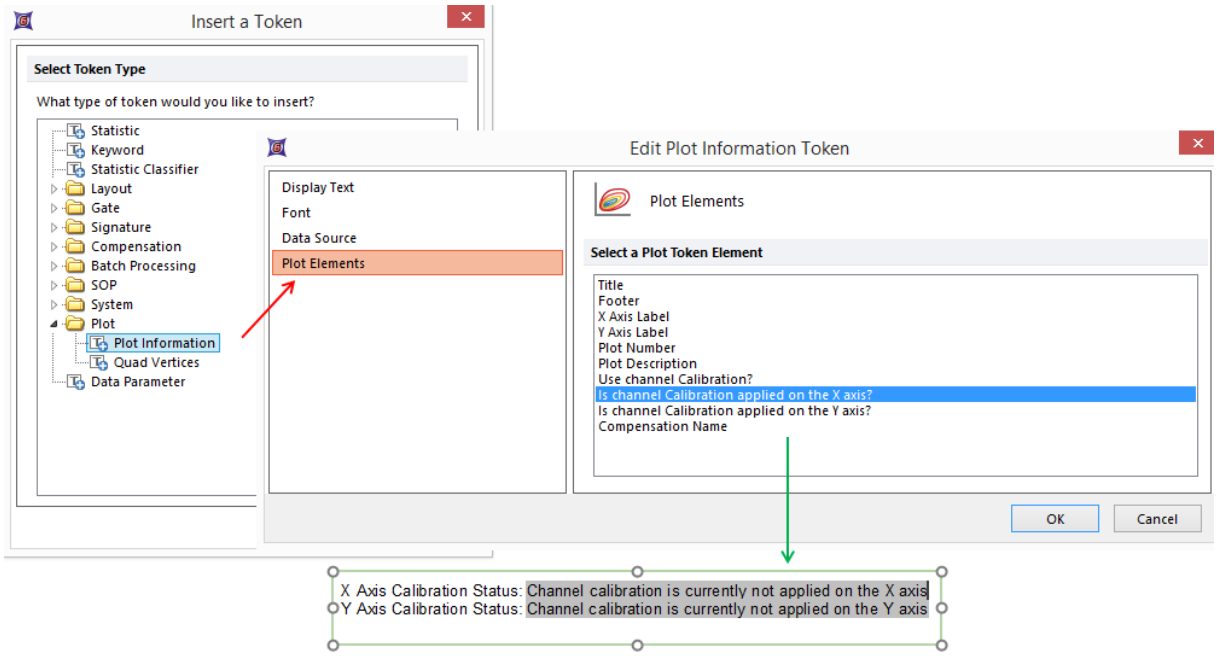

Figure 15.7 Using the "Is channel Calibration applied on the axis?" token type. The token can be access through Plot Information>Plot Elements (red arrow). When the token is inserted it will indicate if a channel calibration is applied on the X or Y axis of a plot (green arrow).

To monitor the **Last Loaded Channel Calibration File** token type:

1. Right click on a text box.
2. Choose **Insert Token**.
3. Choose **Layout→Last Loaded Channel Calibration File.**
4. Click **Insert**.

A new token will now be displayed in the text box indicating the last calibration file that was loaded into the layout (Figure 15.8).

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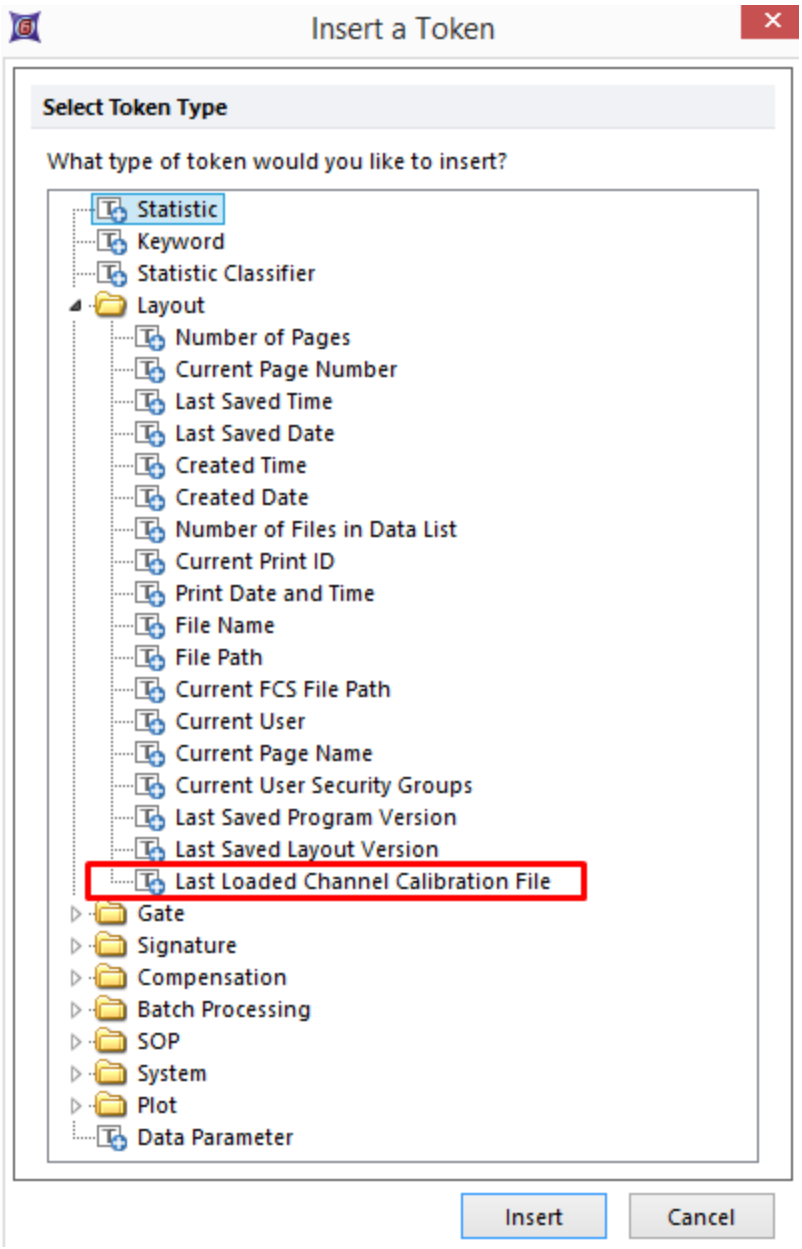

Figure 15.8 - Last Loaded Channel Calibration File Token Type

15.3 The Channel Calibration Window

The Channel Calibration window (Figure 15.9) shows all the current calibrations. It is possible to have different calibrations defined for different parameters. FCS Express will automatically use the appropriate calibration depending on which parameters are displayed on a plot.

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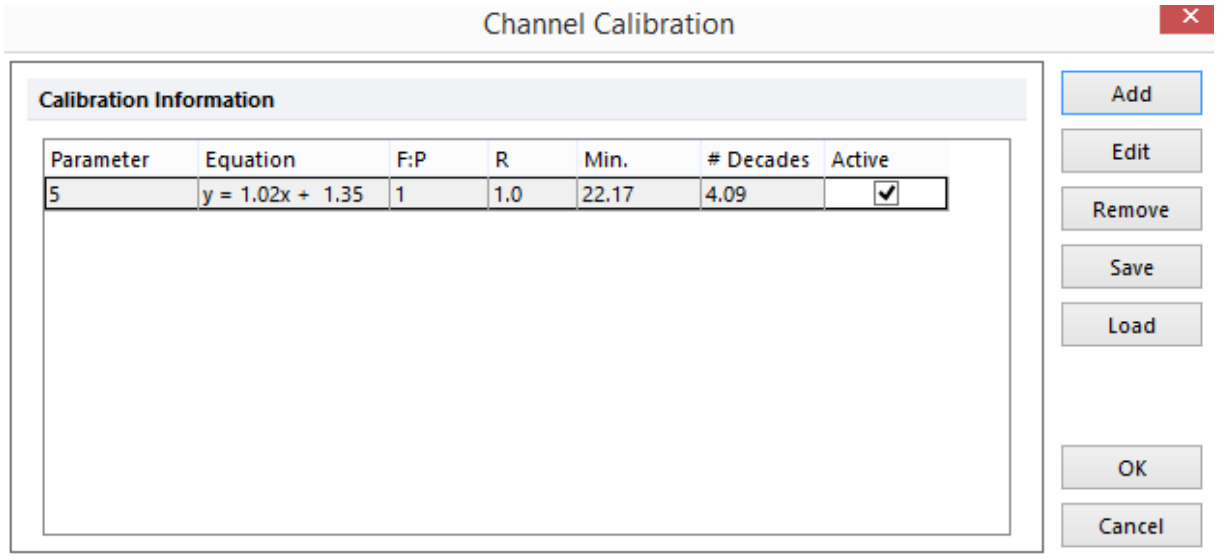

Figure 15.9 Channel Calibration Window

In the **Channel Calibration** window:

- **Parameter #** indicates which parameter the calibration applies to.
- **Equation** is the linear regression formula that will be used to calibrate the channels.
- **F:P** refers to the dye molecules:antibody ratio that you selected.
- **R** tells you how closely your equation fit the actual data, with a value of 1 being a perfect fit.
- **Min** is the minimum number of fluorochromes you are able to resolve.
- **# Decades** represents the number of decades of dynamic range your instrument is capable of measuring.
- **Active** indicates whether this calibration will be applied for the selected parameter.

To edit an existing calibration, click on a row (e.g., Parameter #2 above) to highlight/select it and press **Edit**. This will bring up the **New Calibration** dialog for that data, where you can change the values.

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Chapter

16 The Compensation Tool

Ideally, when one uses a dye in an experiment, its emission spectrum will be narrow enough that fluorescence from that dye is only detected by a single detector in the instrument. In practice, however, because of the broad emission spectra of available fluorochromes, the dye you are using will likely emit significant amounts of fluorescence in several different detectors. In other words, the light reaching a given detector consists of the signals from multiple fluorochromes.

Compensation is the process of transforming the data such that the values from a single detector come from an individual dye. In order to do this, a percentage of the overlapping emission is subtracted from the target emission. Traditionally, this compensation was performed by the instrument during acquisition. However, modern instruments are capable of storing the data in uncompensated form and compensation can be applied by the analysis software.

Example of the calculation: Compensated Parameter 2 Fluorescence = Observed Parameter 2 Fluorescence minus 5% of Observed Parameter 1 Fluorescence.

Compensation involves creating two matrices. The spillover matrix represents the percentage of the signal from a given channel that spills into adjacent channels. The compensation matrix, used to correct for the spillover, is the inverse of the spillover matrix.

FCS Express uses the following terminology when dealing with compensation. The target parameter is the parameter that is detecting signal (potentially from multiple sources). The source parameter is the primary parameter that you want the signal to be in, but that dye is also bleeding (potentially) into multiple targets. In other words, we are subtracting the percentage of the source that is "bleeding" into the target.

In the example given above, Parameter 2 is the target and Parameter 1 is the source. A family of sources and targets is called a *compensation definition*. A compensation definition describes all the ways that fluorescence from different channels affect each other under a given set of conditions and is equivalent to a single compensation matrix. Typically, the instrument user will set the gains for all the channels at the beginning of the experiment and use these settings for the duration of the experiment. Thus, the compensation definition would apply for the entire experiment.

FCS Express can store multiple compensation definitions in a single layout. This is useful when one wants to analyze data acquired on different days in the same layout. It is likely that the gains will be different on different days and will require

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a different compensation definition for each day. Each overlay on a plot can use a different compensation definition.

In addition, the compensation definition can be assigned to a data file. This means that when the .fcs file is loaded into a plot, it will automatically use the proper compensation. In this way, you do not have to remember the appropriate compensation definition for each of your data files.

16.1 Software Compensation Caveats

Software compensation is not a panacea for all compensation problems. Software compensation works best on high-resolution (> 1024 channel) linear data. Compensation must be performed on linear data. Therefore, data that is stored in logarithmic format (as is sometimes the case with FCS 2.0 data files) must be converted to linear prior to compensation. This conversion from logarithmic to linear can induce rounding errors. In addition, data that is stored as less than 1024 channels is rarely compensated accurately with software. However, the majority of data stored as 1024 channels or greater can be software compensated.

Additionally, certain file formats supported by FCS Express do not support storing compensation matrices separately from the data file and the these data sets will only display and utilize the raw data values, compensated values, from the time of acquisition. Examples include .csv files 29 and .MQD (MacsQuant) files 26.

For a detailed description of how software compensation is implemented in FCS Express, please see our **Frequently Asked Questions** at http://www.denovosoftware.com/site/compensationdetails.shtml.

16.2 Setting Up Compensation Definitions

In order to view all the current compensation definitions in the layout, select the **Tools** tab→**Transformations**→**Compensations** command. A dockable window titled **Compensations** will open. The **Compensations** window will list all of the compensation definitions that are available in the layout within the **Loaded automatically with data files** or the **Created manually** folders. (Figure 16.1). The **Loaded automatically with data files** folder shows compensation definitions that were automatically loaded with a data file presently showing on the layout (e.g., one defined during acquisition and saved as metadata with a modern FCS 3.0 or greater format .fcs data file) and the **Created manually** folder shows compensation definitions that have been created manually within FCS Express.

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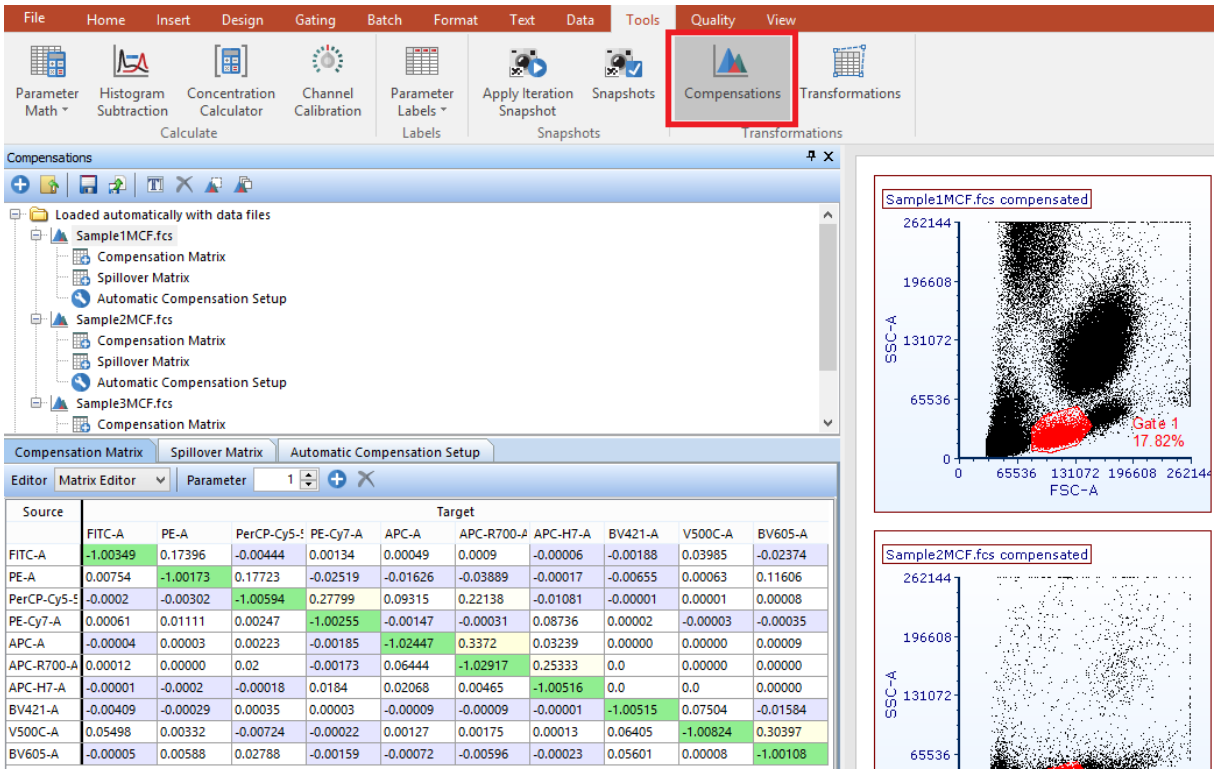

Figure 16.1 Compensations Navigator showing compensations available in the layout.

The **Compensations** window (Figure 16.2) is dockable and pinnable[113] like many other windows in FCS Express. There are many actions you can perform from the **Compensations** window tool bar. You can perform the action either by pressing the appropriate button (hover the mouse over the button for tooltip) or by right-clicking on a compensation folder or sub-item. The actions are summarized in the table below:

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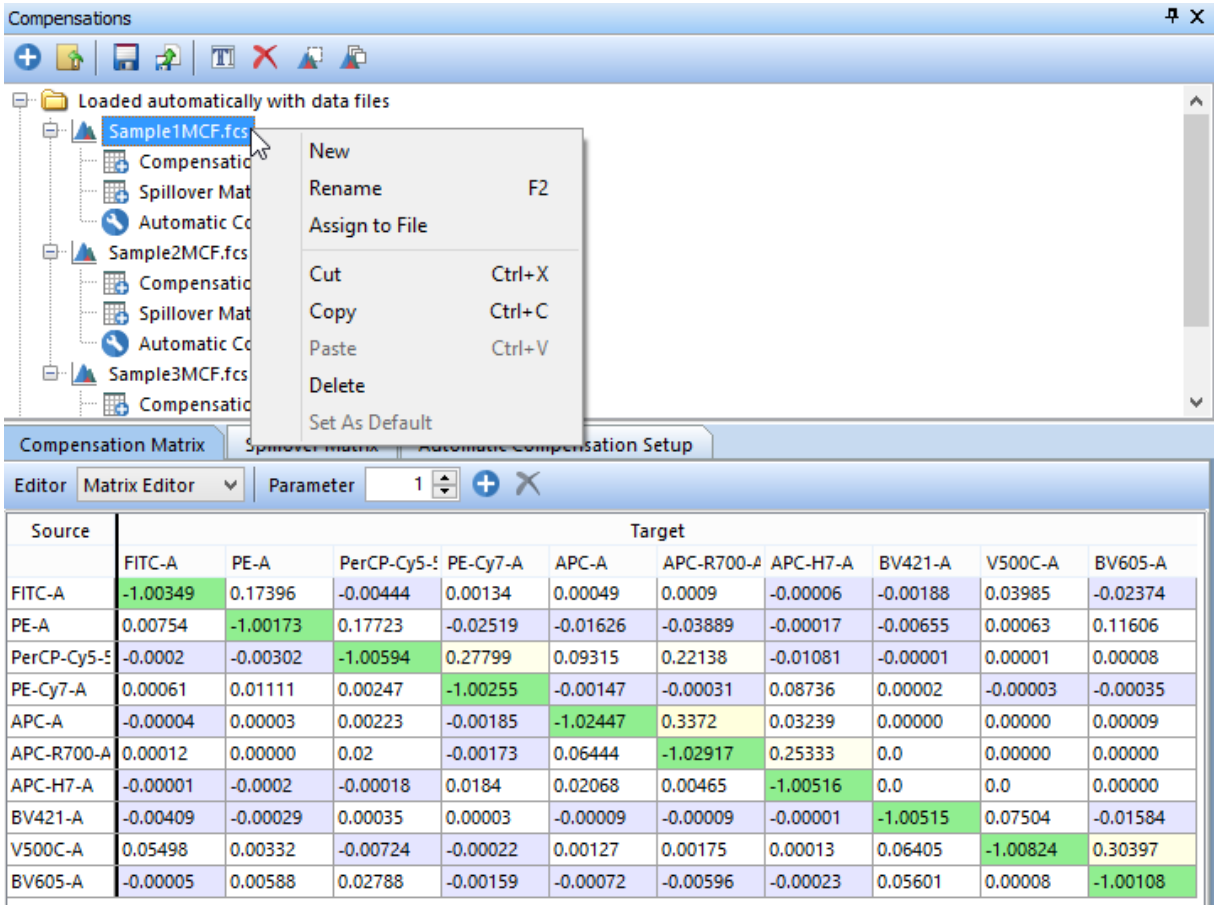

Figure 16.2 Compensations Navigator showing available commands and actions.

Action	Command	Explanation
Create a new compensation	or right-click on Folder or Compensation	Add a new compensation definition.
Import a compensation from a file		Import a previously saved compensation (*.compensation) file.
Export the selected compensation		Export a compensation (*.compensation) file for use in other layouts.
Assign this compensation to a data file	or right-click on Folder or Compensation→ **Assign to file**	Assign 647 a compensation definition to a file or series of files.
Rename the selected compensation	or right-click on Folder or Compensation **→ Rename**	Renames the selected compensation definition.

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Delete	or right-click on Folder or Compensation→ **Delete**	Deletes a compensation definition from the compensation window and the plots to which it was applied.
Cut Object	Right-click on compensation→ **Cut Object**	Performs a cut command on the compensation definition. If performed on the Compensation or Spillover Matrix, the Matrix can be pasted into a Text Box or other document.
Copy Object	Right-click on compensation→ **Copy Object**	Performs a copy command on the compensation definition. If performed on the Compensation or Spillover Matrix, the Matrix can be pasted into a Text Box or other document.
Set as Default	Right-click on compensation→ **Set as Default**	Sets the selected compensation definition as the default compensation. The default compensation is the one that will be used when adding new plots. Only **Created manually** compensation definitions may be **Set as Default**; however, compensation definitions **Loaded automatically with data files** may be copied into the **Created manually** category for this purpose.

The **Compensations** window also has tabs devoted to the **Compensation Matrix**, **Spillover Matrix**, and Automatic Compensation Setup 634 (Figure 16.3). The **Compensation Matrix** and **Spillover Matrix** tabs allow you to view and Edit a Compensation Definition 631 by utilizing the compensation or spillover matrix. You can choose to view the **Matrix Editor** or **List Editor** from the **Editor** drop-down (Figure 18 below) to view a matrix of **Source** vs. **Target** (Figure 16 below and *left*) or to view a list of **Target - % Source** (Figure 16 below and *right*). Please see the **Automatic Compensation Setup** 634 section for details on this tab.

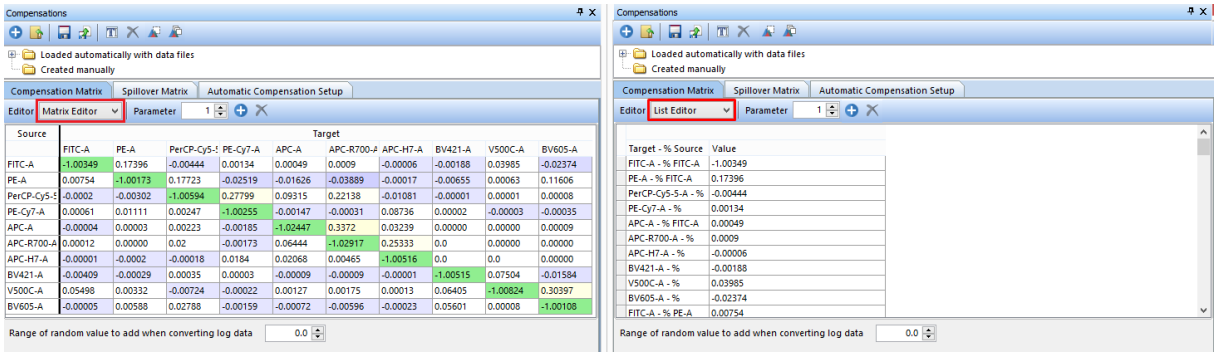

Figure 16.3 Compensations Navigator showing available tabs. Matrix Editor View (left) and List Editor View (right).

Once a **Compensation** or **Spillover Matrix** is defined, you can drag and drop either matrix directly onto a layout, into a text box, or even directly into Microsoft Excel to display the raw matrix data (Figure 16.4).

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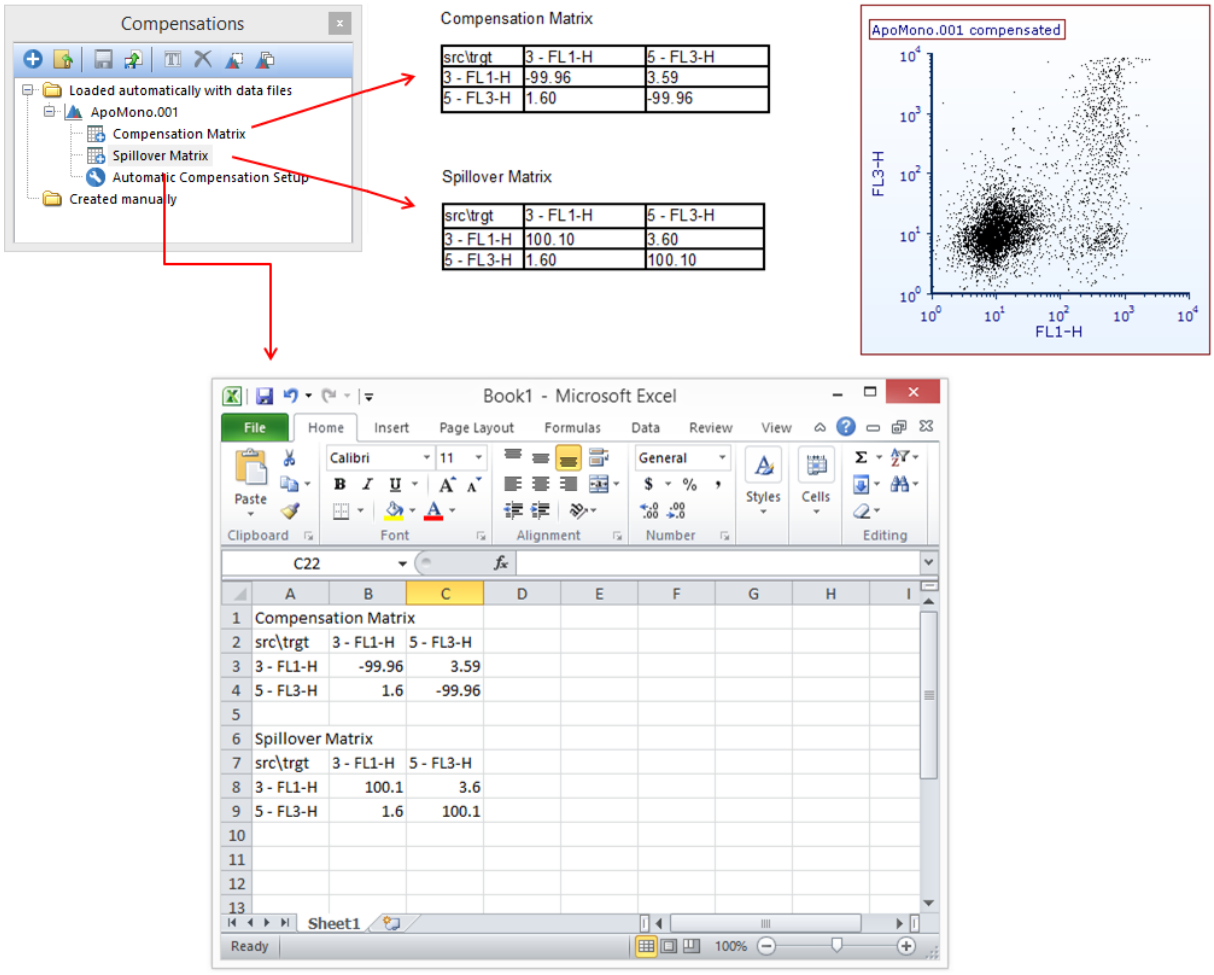

Figure 16.4- Drag and drop a compensation matrix from the Compensations navigator onto a layout, into a text box, or directly into Microsoft Excel to display the values.

16.3 Editing a Compensation Definition

Selecting a file within the **Compensations** navigator displays the compensation definition in the tabs below. Expanding the compensation file node will reveal the **Compensation Matrix**, **Spillover Matrix**, and **Automatic Compensation Setup** 634 items. Clicking on items in the expanded view is just like choosing the different tabs in the lower pane of the window (Figure 16.5).

FCS Express has color-coding for values in compensation and spillover matrices. The color heat map allows users quick identification of same parameter pairings (green) and potentially high (shades of yellow to red) or low (shades of purple to blue) values.

You can choose to view the **Matrix Editor** or **List Editor** from the **Editor** drop-down to view a matrix of **Source vs. Target** or to view a list of **Target - % Source**, respectively.

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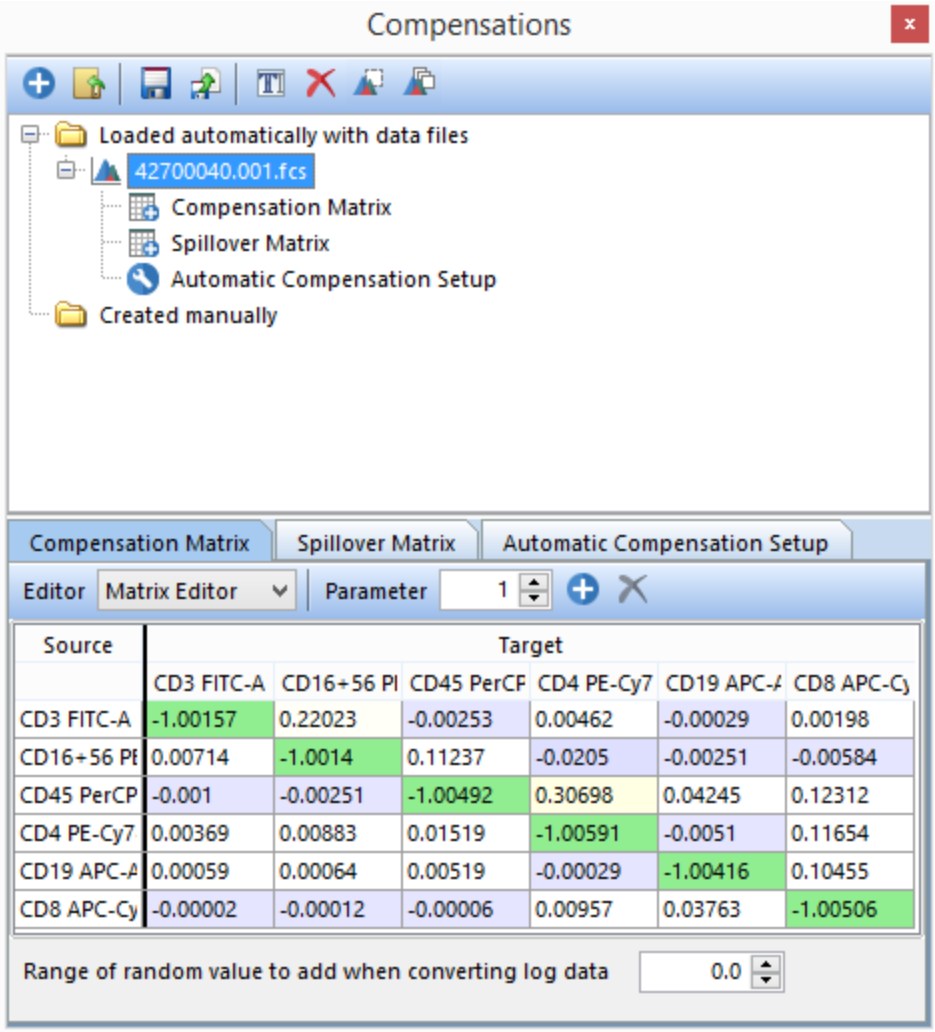

Figure 16.5 The Compensations Navigator displaying the Compensation Matrix in Matrix Editor View.

Compensations can be edited directly from the **Compensation Matrix** tab and **Spillover Matrix** tab by clicking on the **Target** (**Matrix Editor**) (Figure 16.6, *left*) or **Value** (**List Editor**) (Figure 16 below and *right*) and using the up and down arrows (outlined in red below) or entering a value to adjust the compensation.

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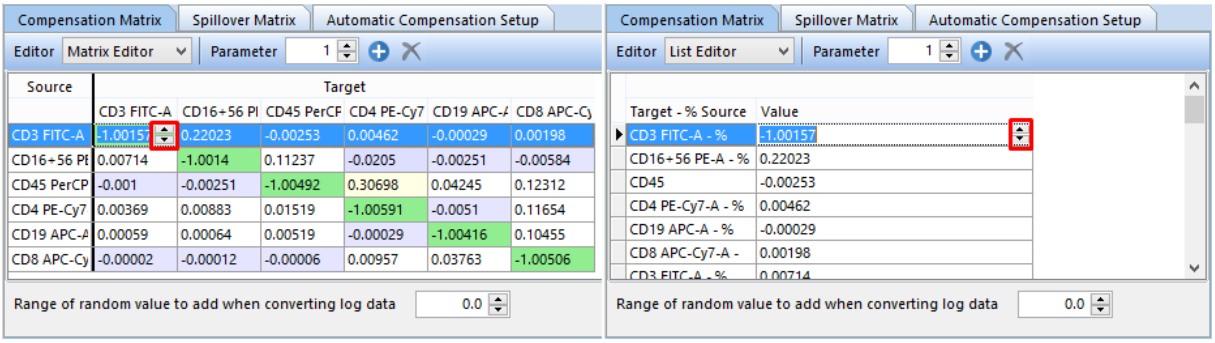

Figure 16.6 Adjusting compensation values using the compensation Matrix (left) and List (right) Editors. Use the up and down arrows or manually enter a value to adjust the compensation.

Every compensation must be given a unique name. The name of your compensation may be changed at any time by selecting the compensation and clicking the **Rename selected compensation** button or by right-clicking on a compensation and choosing **Rename.** You may find it helpful to enter the date or experiment number as the compensation name.

The **Parameter** field allows you to add or remove parameters to/from the matrix by clicking the or buttons (Figure 16.7).

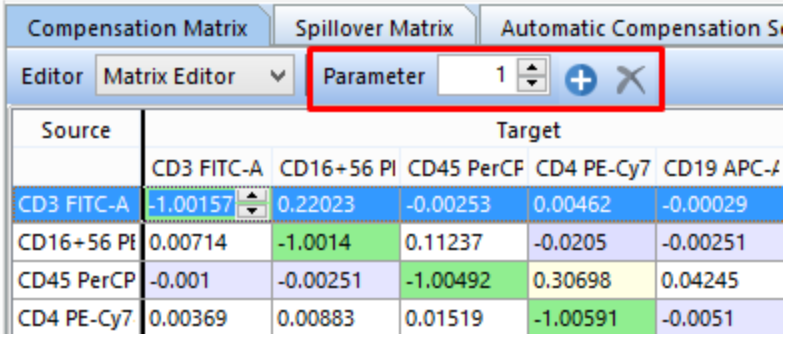

Figure 16.7 The Parameter field allows you to add or remove parameters to your compensation matrix.

Compensation must be performed on linear data. If you have stored data on a logarithmic scale in your FCS file (common with FCS 2.0 data files), this data has to be converted to a linear scale before compensation is applied. It is then converted back to log for display. Depending on the resolution of your original file, this can result in digitization errors, which can cause large numbers of events to group in a single channel. The conversion to linear and reconversion back to log occurs automatically during the software compensation process. However, if you notice digitization errors, you can enter a value to spread the grouped population out using the **Range of random value to add when converting log data** field (Figure 16.8). Typically, this value should be less than one. This will add a small random value to the linear value prior to it being

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reconverted back to log. It is recommended to leave this value at zero unless these digitization errors become apparent.

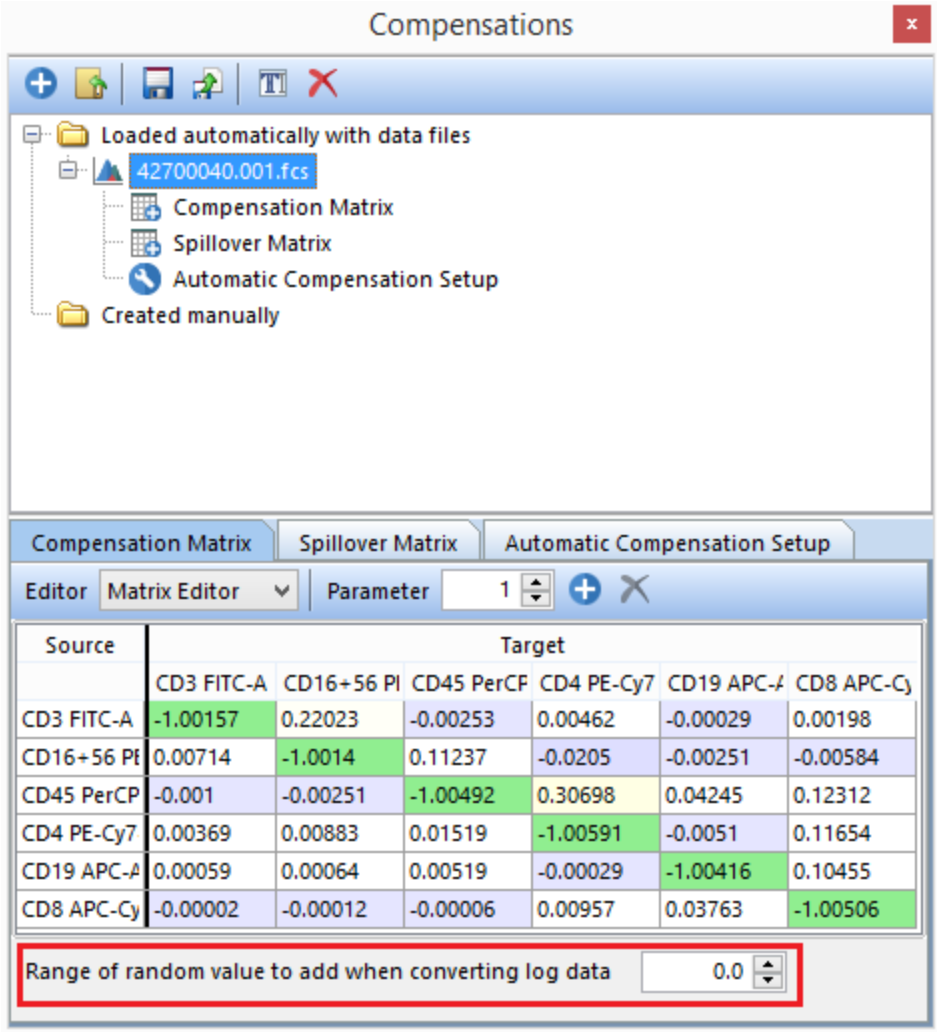

Figure 16.8 The Range of random value to add when converting log data field allows you to easily correct for digitization errors.

16.4 Automatically Calculating Compensation Definitions

Instead of manually entering a value to subtract for every target-source pair, or guessing the compensation by eye using old-fashioned sliders, you can have FCS Express calculate the entire compensation definition for you. For step-by-step instructions on using the Automatic Compensation Setup please see our **Automatic Compensation Tutorial**. To get started, use the **Tools** tab→**Transformations**→**Compensations** command, open the **Compensations** navigator, and add a new compensation with the **Add a new compensation button** or you can then click on the **Automatic Compensation Setup** command (outlines in red below) tab to access the automatic settings (Figure 16.9).

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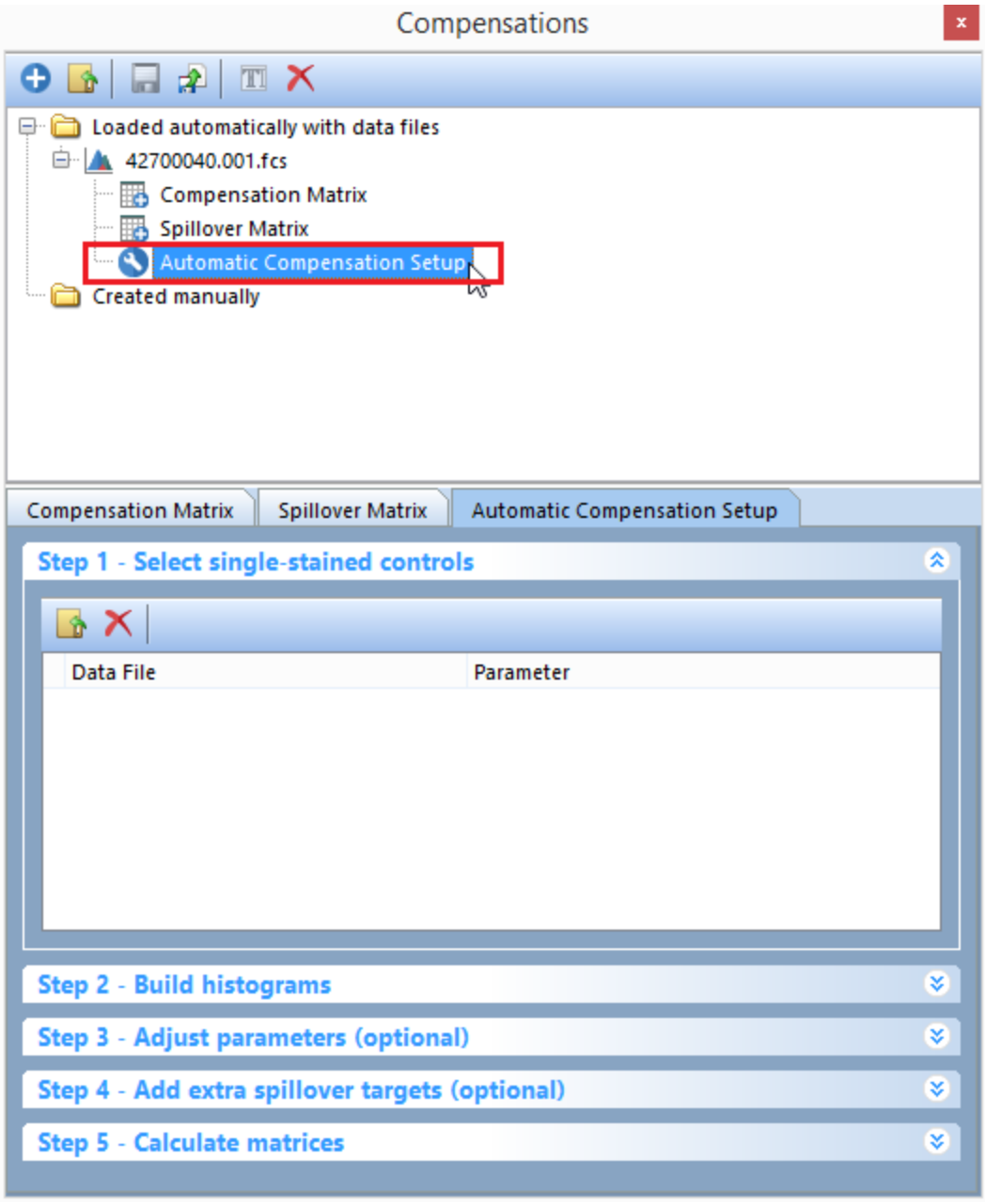

Figure 16.9 The Automatic Compensation Setup tab.

The **Automatic Compensation Setup** tab contains 5 steps to calculate your compensation. Step 3 and step 4 are optional and do not have to be completed.

- **Step 1 - Select single-stained controls** (Figure 16.10)

In order to automatically calculate the compensation definition, you will need an .fcs file with a single stain control for each parameter you wish to compensate and an unstained control. The unstained control can be a universal negative, a negative population defined by a gate, or the negative population with the tube

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of the positive control. You will also have the option of using an unstained control for each parameter you wish to compensate or to use populations defined in user-created gates in **Step 3: Adjust parameters**.

1. Click the **Add data for auto compensation** button, , to add files that will serve as compensation controls.
2. Choose the parameter that was stained for each control sample and the universal negative using the **Parameter** dropdown menu.

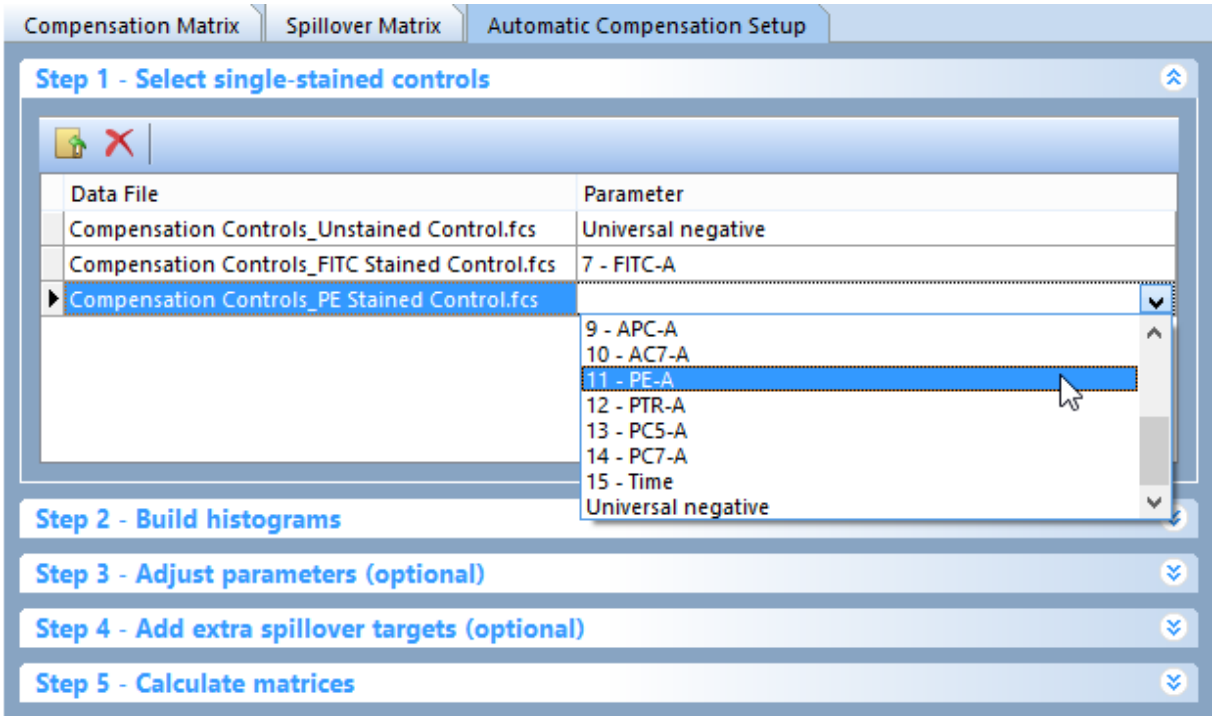

Figure 16.10 Step 1 - Select single-stained controls. Add compensation control data files and choose their respective channels from the Parameter drop down list.

- **Step 2: Build Histograms** (Figure 16.11)

1. Click the **Build Histograms** button. If there was something wrong with the files or parameters you selected in the previous step, an error message will appear here to let you know what the problem was, and the **Build Histogram** button will be disabled until the error is corrected.

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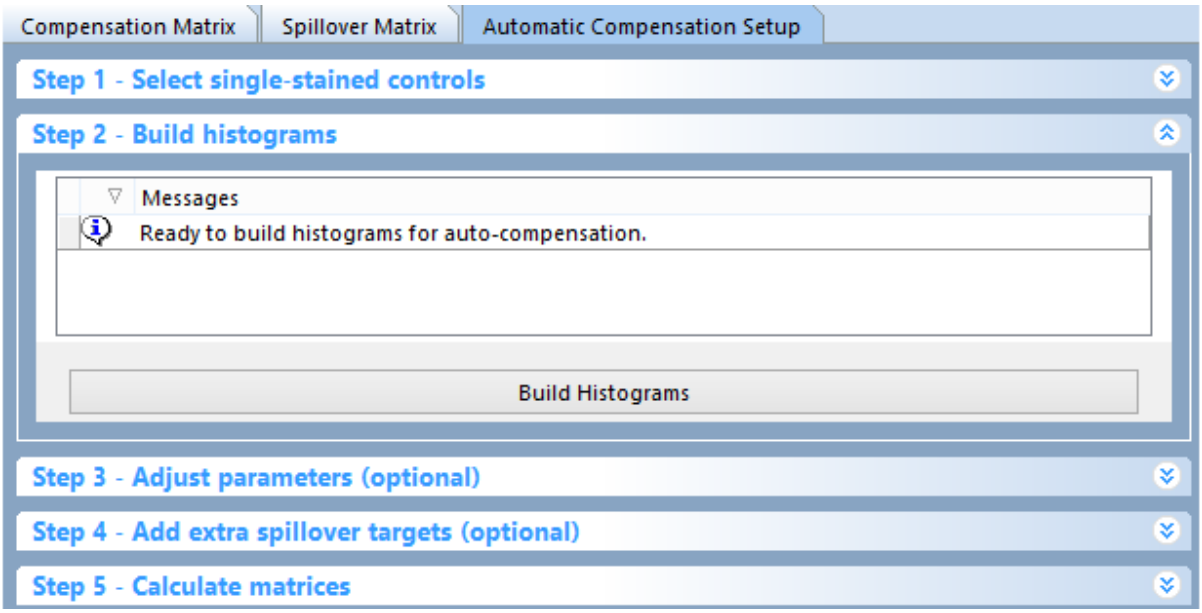

Figure 16.11 Step 2: Build Histograms

If there were no errors, the **Ready to build histograms for auto-compensation** message appears in the **Build histograms** dialog. **The Build histograms** step will automatically create the following items (Figure 16.12):

- Scatter Plot Layout Page: A page will be created with a plot displaying SSC vs FSC and a **Scatter Gate**. The **Scatter Gate** is automatically created and used to define the "live" cells or cells of interest in your compensation controls. All of the fluorescence gates are children of the **Scatter Gate,** which means adjusting the **Scatter Gate** will cause all other gates to update in real time. If the **Scatter Gate** does not appear on the right parameters (e.g., SSC-H vs FSC-H), change the gate using the replace gate feature 320. To replace the **Scatter Gate** with a new gate:

    . Change the X and Y axes to the correct parameters.
    . Draw a gate around the population of interest.
    . Choose the **Replace an existing gate** radio button in the **Create New Gate** dialog.
    . Select **Scatter Gate** from the drop down menu.
    . Click **OK**.

    (Note: These steps are also described in the Automatic Compensation Setup 1381 tutorial.)

- Single Stained Control Layout Pages: A new page will be created for every parameter you are compensating (e.g., "FITC-A control", "PE-A control" in the image below). The page will display one histogram with the stained population marked and one histogram with the unstained population

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marked. Markers will be placed based on FCS Express auto recognition of peaks, but can be adjusted 354.

Note: the unstained population histogram will only appear if a universal negative is chosen. If it was not, only a single plot with two markers (for positive and negative populations) will appear.

- Gates: New gates will be created for the **Scatter Gate** and each stained and unstained set up markers will be converted and linked to gates. After the initial "auto-gating" all the gates and markers created in the **Build histograms** can be manually adjusted 354. *(Note: A Gate View 345 will not automatically be inserted.)*

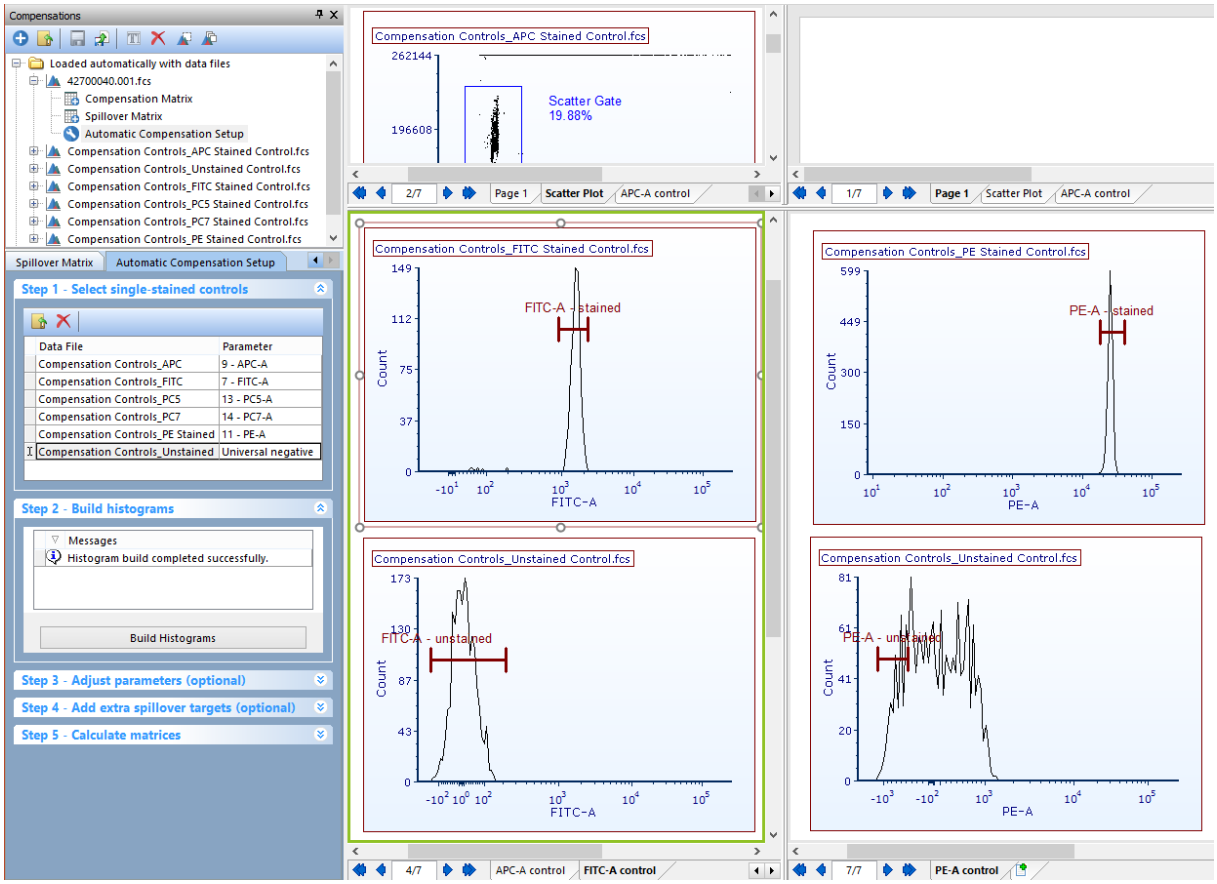

Figure 16.12 Layout after Step 2 of Autocompensation setup. Note the Scatter Plot, FITC control, and PE control pages. Also notice that a plot for SSC vs FSC has been created with a Scatter Gate automatically applied. Histograms with markers for the stained and unstained populations have also been automatically created.

- **Step 3: Adjust Parameters (optional)** (Figure 16.13)

This step is rarely used, but can be helpful with certain kinds of data (e.g., where the Stained or Unstained population must be defined on a different plot). Once you have determined your initial compensation parameters via Step 1 and Step 2

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(or completed your compensation matrix in step 5) you can add **Adjust parameters** in Step 3.

Step 3 - Adjust parameters (optional) is useful when you would like to refine the populations that are used for calculating compensation. Dragging and dropping new plots, files, or gates in this section will allow you to override the automatic setup and use gates and plots of your choice. For instance, if the **Automatic Compensation Setup** does not make a good estimate of the population or if you would like to use a population from another file, gate, or plot, **Step 3** would be useful. Parameters may be adjusted or changed altogether by dragging and dropping a plot or gate onto the parameter for the **Stained** and **Unstained** columns. If a gate is dropped, the compensation definition will be based on that gate, including its hierarchical position 350 visible in the Gate View object. If a plot is dropped, the compensation definition will be based on the entire population on the plot. If you have adjusted a parameter after the matrix has been calculated you must click on the **Calculate matrices** button in **Step 5** again to apply the change.

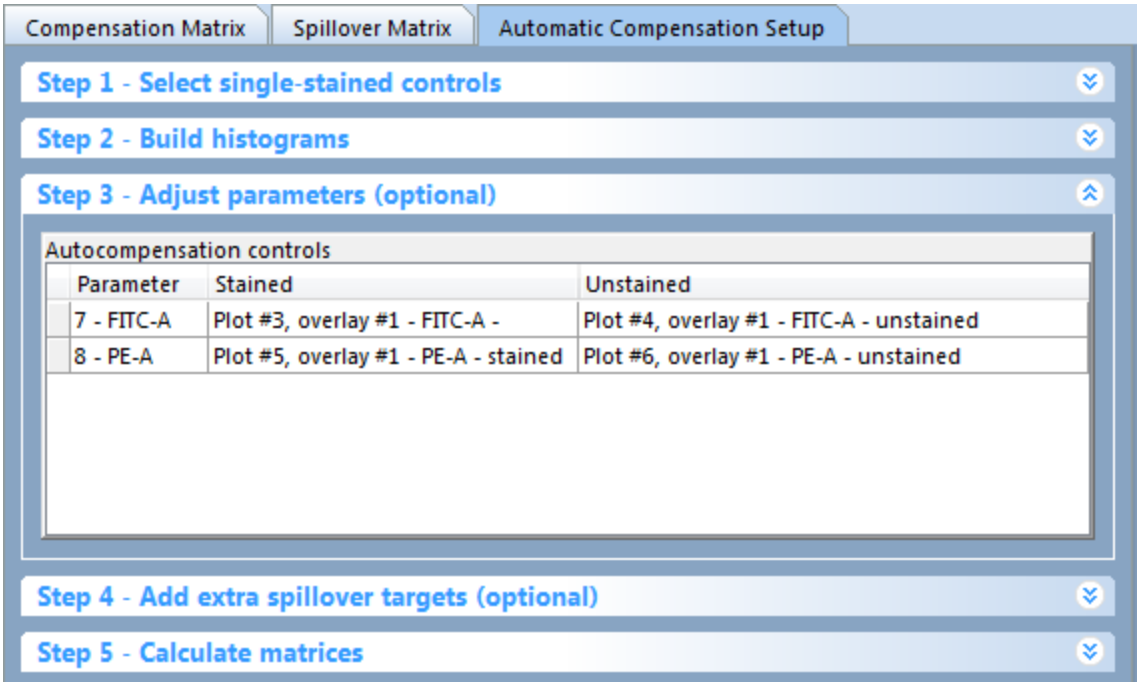

Figure 16.13 The Adjust parameters dialog displaying the currently defined parameters, plots, and gates.

Instructions for adjusting parameters (Figure 16.14):

1. Click and hold on a gate or plot.
2. Drag the gate or plot to appropriate cell in the **Autocompensation controls** table (red arrow).
3. Release the mouse button.

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The new gate and or plot will appear by name in the appropriate cell in the **Autocompensation controls** table of the **Adjust parameters** pane (Figure 16 below, green arrow).

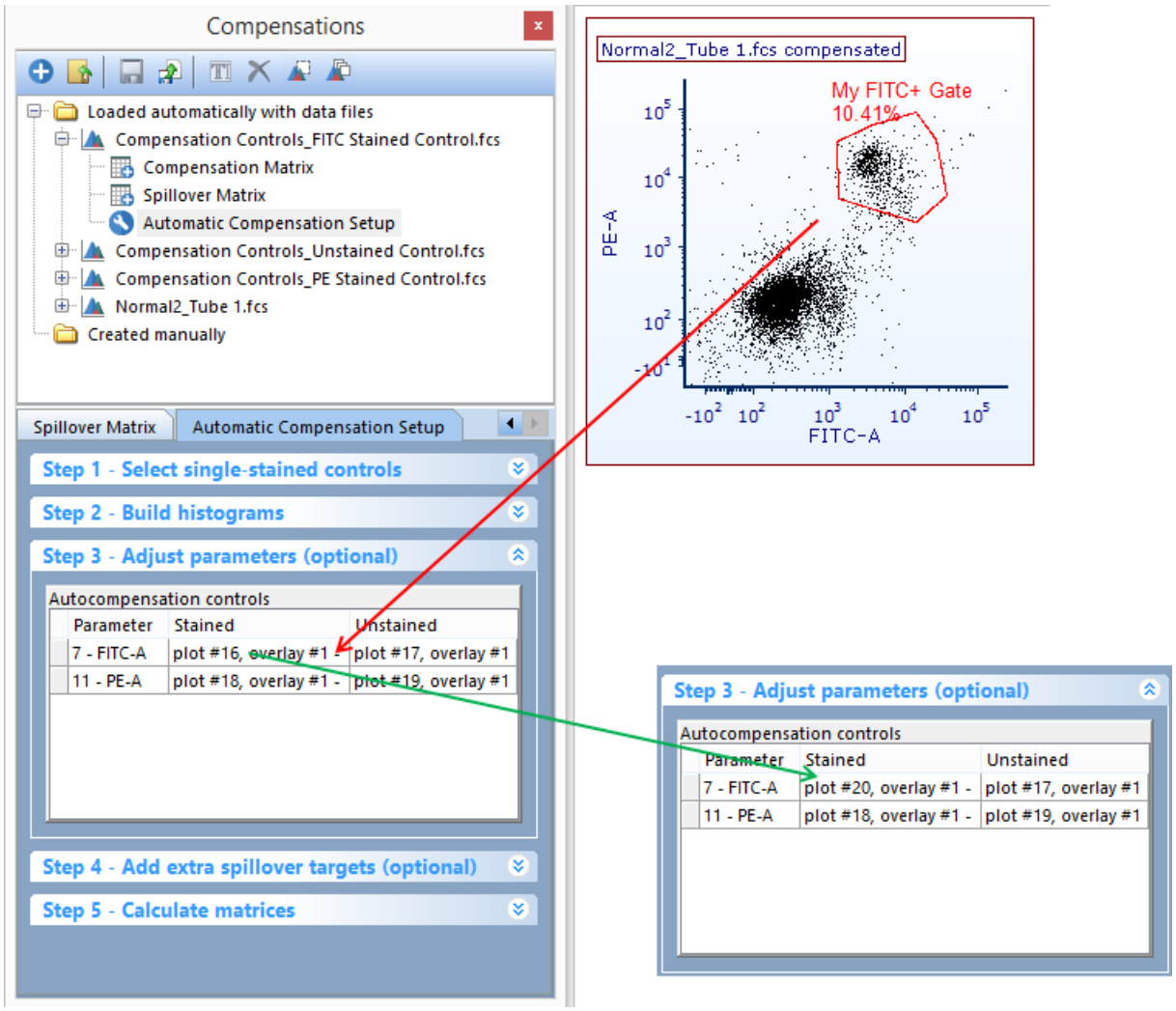

Figure 16.14 To change a parameter plot or gate drag the gate or plot onto the plot you wish to change. The plot information will then update to the newly choosen gate or plot.

• **Step 4: Add extra spillover targets (optional)** (Figure 16.15)
Adding extra spillover targets allows you to have other targets defined without defining a source. This is useful if you would like to have an idea of what spillover would look like if an extra parameter was added in future experiments. To add an extra spillover target simply check the box for the extra target you would like to add. Note: extra spillover targets will only appear for parameters that were acquired with the data.

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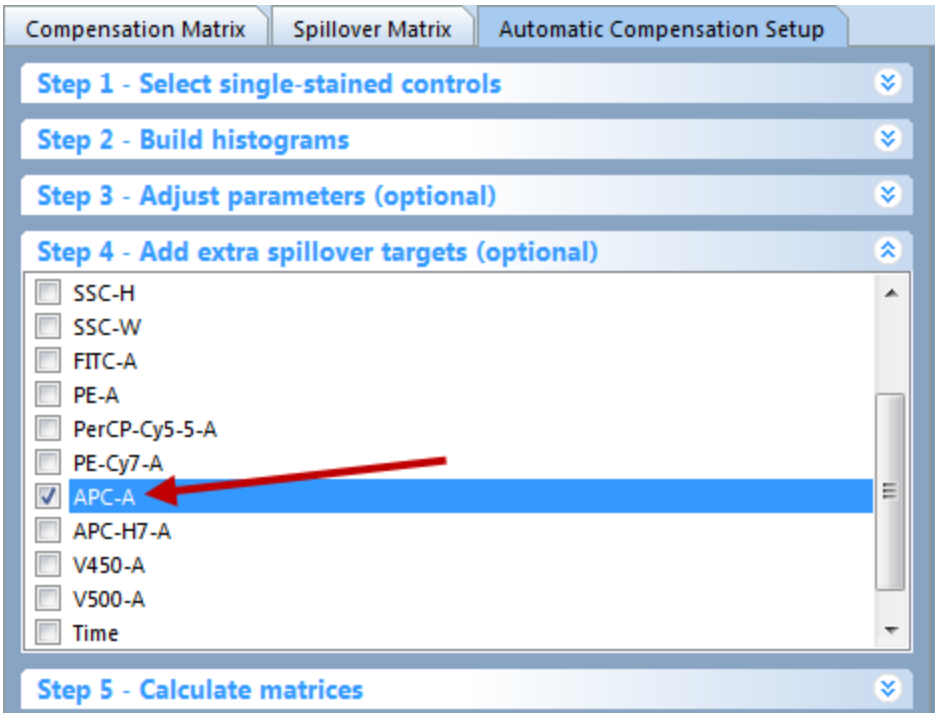

Figure 16.15 The Add extra spillover targets (optional) dialog. Checking a box will calculate the spillover for targets that have no source.

• **Step 5: Calculate matrices** (Figure 16.16)
In this final step you can choose to **Calculate Matrices** based on the **Mean** or **Median** depending on your preference for your data and experiment. Click on the radio button for either choice to apply your preference.

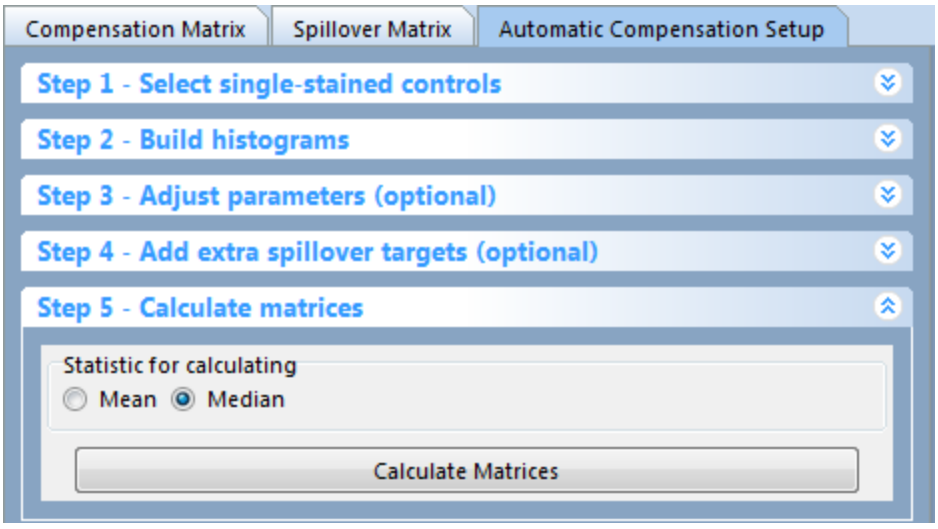

Figure 16.16 Step 5: Calculate Matrices button and choosing Mean or Median.

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Your **Spillover Matrix** will then appear in the **Compensations** navigator (Figure 16.17)and you may edit any of the source-target pairs 631 through the **Compensation Matrix**, **Spillover Matrix**, or make new adjustments in the **Automatic Compensation Setup**.

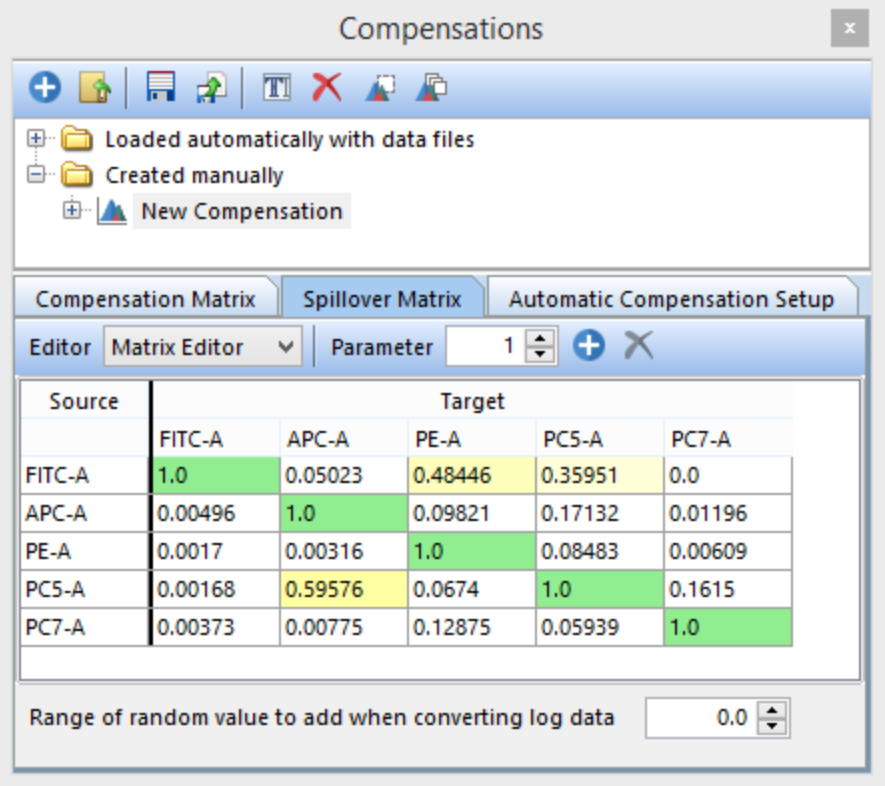

Figure 16.17 The Compensation Matrix after the Automatic Compensation Setup is complete.

The automatic compensation setup settings are saved with the layout 648. To create another compensation matrix from a different set of data, simply add a new compensation with the **Create a new compensation button** and begin the Autocompensation setup process again.

We also suggest that you use the compensation determined in the **Automatic Compensation Setup** and apply it to different layouts and files 642 rather than re-creating the same compensation definition many times.

A step-by-step description of the **Automatic Compensation Setup** feature is provided in our tutorials at **Compensation Tutorial** 1376.

16.5 Applying Compensation Definitions

Every plot, overlay on a plot, and every file loaded as a plate, can have a different compensation definition. To change the compensation for an overlay,

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right-click on the plot and select **Format** from the pop-up menu. Go to the **Overlays** category, select the overlay(s) of interest, and select the compensation definition you want to use. Alternatively, you can use the **Format** tab→**Plot Options**→**Overlays** command to edit individual or multiple plots.

There are also many drag and drop commands for compensations. You can drag and drop compensations from the **Compensations** navigator to any plot, any layout, and from one matrix to another to apply and edit compensations (Figure 16.18).

To apply a compensation definition by dragging and dropping from the **Compensations** navigator, follow the steps below.

1. Left click and hold on a compensation.
2. Drag the compensation to the intended plot (red arrow).
3. Drop the compensation by releasing the left mouse button.
   The compensation will then be applied to the plot (green arrow).

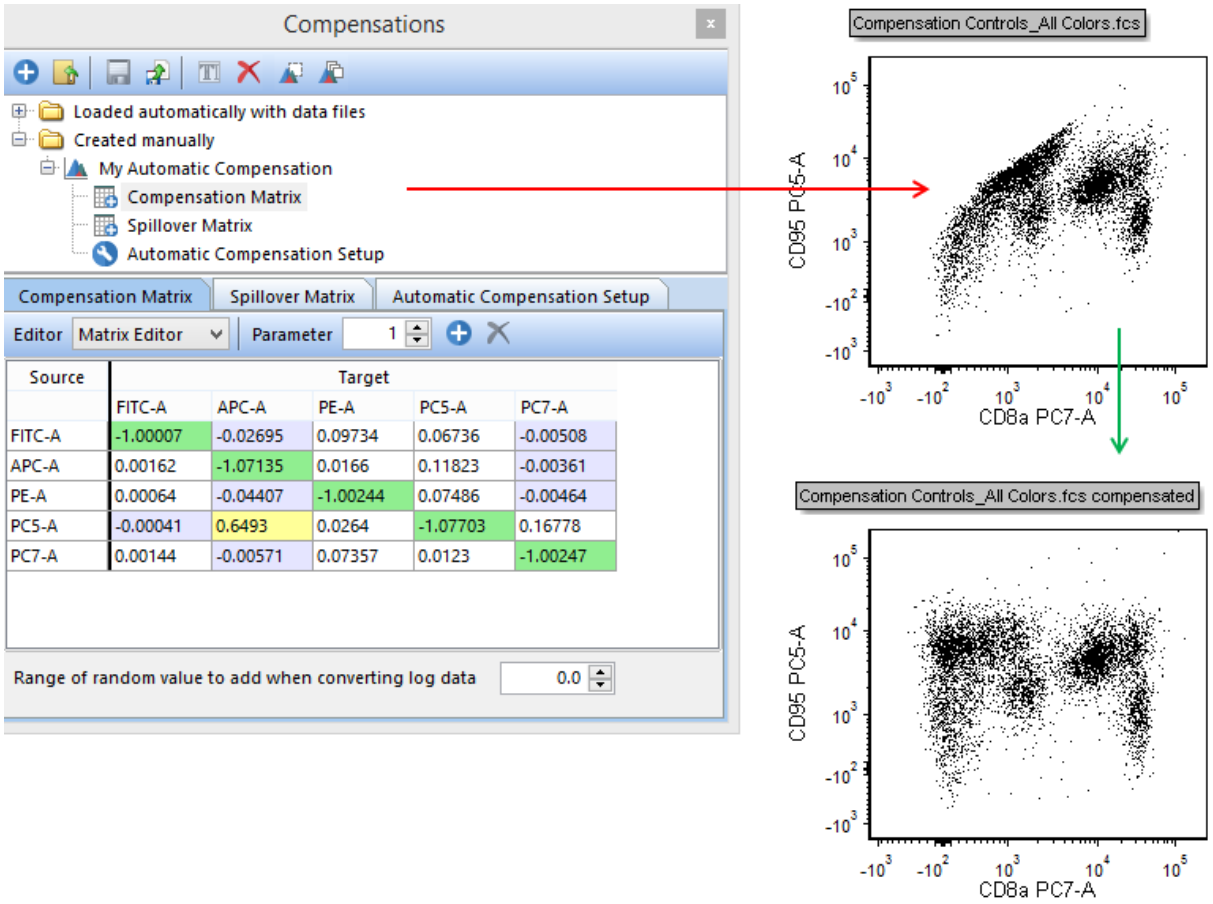

Figure 16.18 Dragging and dropping a compensation from the Compensations navigator onto a plot to apply the compensation.

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Compensations may be dragged and dropped between different layouts by dragging the compensation definition from the **Compensations** navigator in one layout to the **Compensations** navigator or on a plot in another layout much like Steps 1 - 3 above. This will allow you to easily apply compensations between different layouts and experiments.

A compensation definition may also be dragged and dropped onto another compensation definition. This allows you to easily copy matrices from one compensation definition to another.

You can set a compensation as default for the layout, so it will automatically apply to all experimental files in your Data List as you click Next/Prev or perform a Batch Process. See the Automatic Compensation and Batch Processing section 1414 of the relevant tutorial.

You can save compensation definitions to folders 647, and have them automatically apply to the FCS files in the folder.

Compensations may be applied to individual or multiple plots by performing either option in the **Compensations** navigator below. Note if user option **Default Compensation to Use** is set to any option other than **None**, the compensation applied will revert to the preference (i.e. **Compensation Default** or **FCS file**) when changing the data displayed on the plot or during batch processing.

- Left-click to select compensation name from **Loaded automatically with data files** folder or **Created manually** folder and click **Apply to selected plots** or **Apply to all plots** button in top menu bar (Figure 16.19)

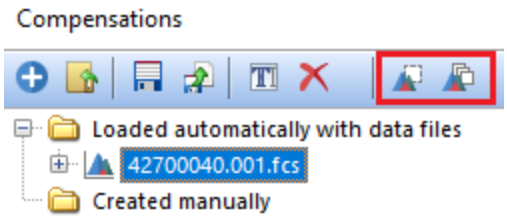

Figure 16.19 -Compensation navigator menu to access options to apply selected compensation to selected or all plots (outlined in red)

- Right-click compensation and click **Apply to selected plot** or **Apply to all plots** button in popup menu (Figure 16.20)

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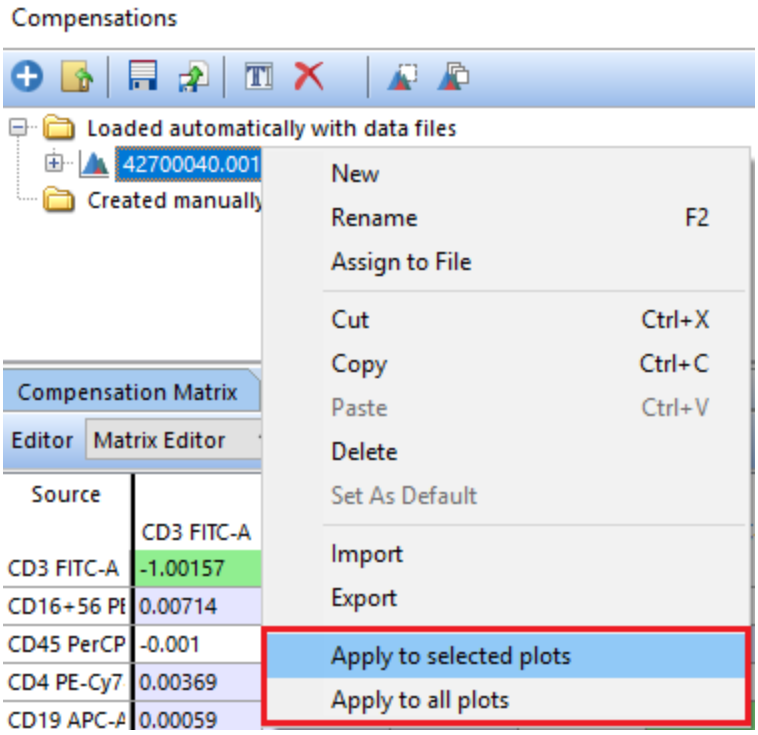

Figure 16.20 - Right-click popup menu to access options to apply selected compensation to selected or all plots (outlined in red)

Additionally, standard copy and paste commands 142 may be used to move matrices from one compensation definition to another within the **Compensations** navigator.

Note that various other functions in FCS Express allow you to apply a compensation definition. For instance, when performing Parameter Math you can choose whether a compensation definition is applied prior to performing the math.

16.6 Selecting the Default Compensation

FCS Express has many default options for compensation definitions when opening files or adding overlays to a plot. These options are found on the **General** page of the **Options** dialog (**File** tab→**Options**) in the **Default Compensation to Use** drop-down list. The options are described in the table below.

Option	Explanation
None	When adding a new plot, no compensation will be used. When changing a file on an existing plot, the compensation that was applied to the plot 642 before the change will continue to be used.
Compensation Default	The compensation definition that was manually Set as Default will be used. To set the definition, see Setting Up Compensation Definitions 630.

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Option	Explanation
FCS File	The compensation definition stored with the file is automatically used when the data file is displayed on a plot. This is commonly the matrix defined at the time of acquisition (for FCS 3.0 or later format files), or it may be a compensation that was Assigned to an .fcs file by FCS Express 630.
FCS File, then Compensation Default	See FCS File, above. However, if there is no definition, the default compensation definition will be applied (see Setting Up Compensation Definitions 630).
Compensation Default, then FCS File	See compensation default above. However, if there is no Compensation Default, the compensation stored with the file will be automatically loaded as described in FCS File above.

16.7 Adjusting Compensation Definitions from a 2D Plot

It is also possible to adjust the compensation definition from a 2D plot itself. Right-click on the plot and select **Compensation** from the pop-up menu. The compensation item contains one submenu for every compensation definition that is used on the plot (maximum one per overlay). For step-by-step instructions on using the this feature please see our **Manual Compensation Tutorial** 1377.

The first item in the **Compensation** submenu is **Create New**, which allows you to create a new compensation definition. If you select **Create New**, a dialog will appear asking you to enter a name for the new compensation as well as some additional information (Figure 16.21). To edit an existing compensation definition, select the appropriate submenu item.

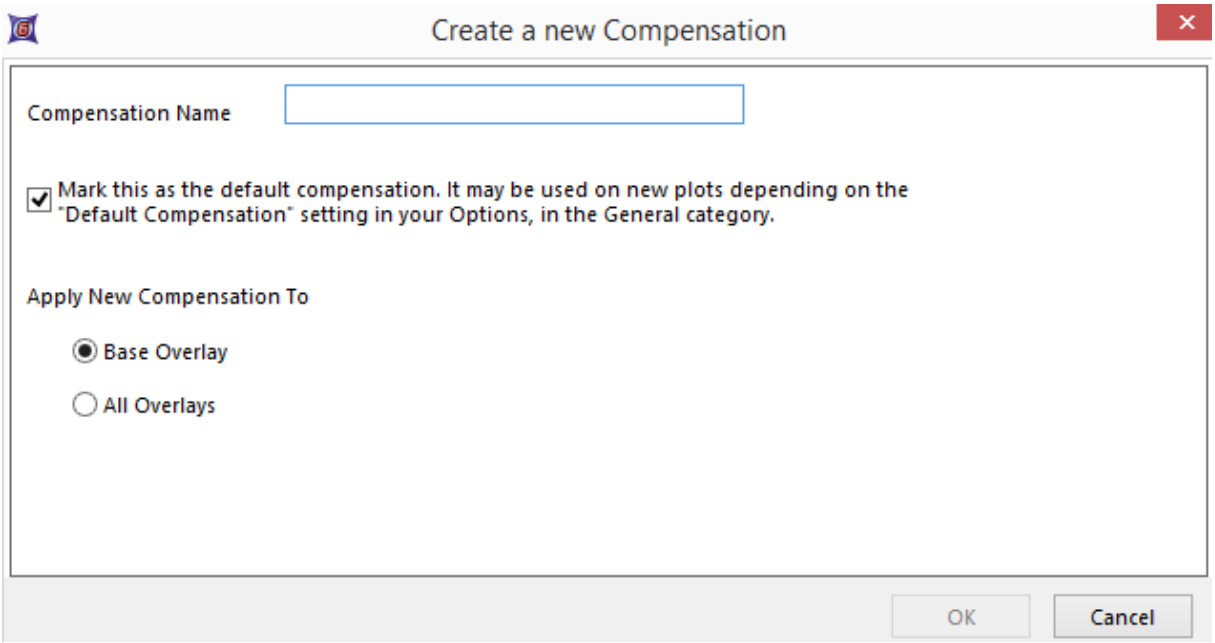

Figure 16.21 Creating a New Compensation from a 2D Plot

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After naming a new compensation and clicking **OK**, or selecting a compensation definition to edit, the **Edit Compensation** dialog will appear (Figure 16.22). Use this dialog to compensate the two parameters against each other on the plot. Move the slider to either increase or decrease the amount of compensation. If you check **Automatic Preview**, the plot will update with the new compensation settings as you edit the compensation in real-time. If the **Compensations** window is open and displaying the compensation currently being edited, the Compensation Matrix and Spillover Matrix 631 values will update in real time, as will plots to which the compensation has been applied.

Click **Cancel** to reject the compensation changes and exit the dialog, or **Restore** to discard the changes but continue editing the plot. Once you press **OK**, the compensation settings for any plot on your layout to which this compensation was applied will be changed to the new values.

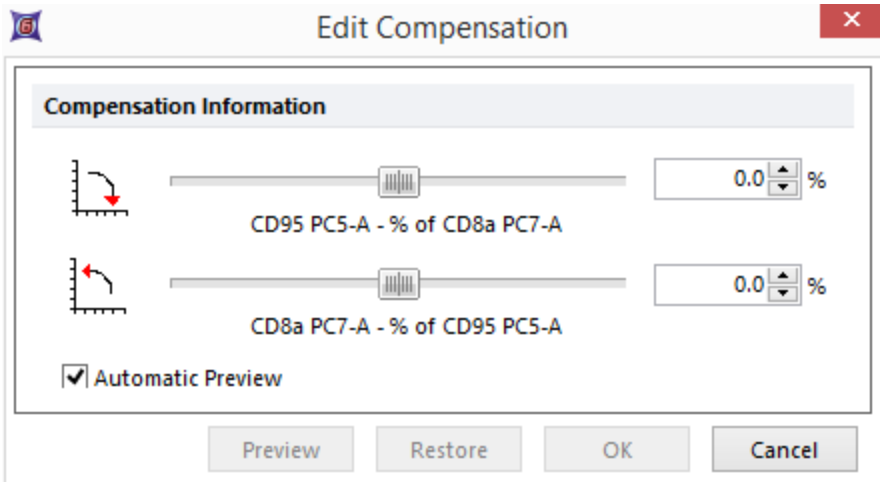

Figure 16.22 Edit Compensation Dialog

16.8 Working with Individual Compensation Files

The extension for an individual compensation matrix in FCS Express is *.compensation*. This is the extension that is applied in an **Assign this compensation to a data file or Export the selected compensation command from the Compensations** 630 command.

If **FCS File** or **FCS File, then Compensation Default** is specified as the default Option for loading compensations 645, when loading a list mode file, FCS express will look in the folder where the file is being loaded for a file with the same name, but ending in *.compensation*. For instance, if you are loading *sample1.fcs* then FCS Express will look for *sample1.fcs.compensation*. If it finds this file, it will load that matrix as if it was specified in the .fcs file itself.

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If it does not find the specific compensation file, it will look for a file of the type **.allcompensation* in the same folder as the data file. The individual matrix in the **.allcompensation* file will be applied to all of the files in that folder.

*Note: If there are two or more *.allcompensation files in the same folder, e.g., type1.allcompensation and type2.allcompensation, the results cannot be predicted. In such a case, FCS Express will pick one randomly.*

16.9 Saving Compensation Definitions

All compensation definitions are automatically saved with the layout file when the layout is saved. The Automatic Compensation Setup 634 is also saved with each compensation.

Save an individual compensation definition (**.compensation*) by selecting the **Data** tab→**Data Info**→**Compensations** command, select a compensation definition by clicking on it, and click the **Export the selected compensation** icon .

16.10 Loading Compensation Definitions

The compensation definitions that were saved with the layout are automatically loaded when the layout is loaded. You may also choose to open a saved compensation file (**.compensation*) by choosing the **Import a compensation from a file** command, then navigating to the compensation file, and selecting it to be opened.

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Chapter

17 Working with FCS Data Files

FCS Express gives you the power to modify .fcs data files directly. You will be able to edit existing 650 or add new keywords 652, and export the raw data 666 as modified .fcs files or other generic formats. You may also choose to merge several FCS files into one file "on the fly" or export the merged data as a new file.

17.1 Viewing and Changing FCS File Headers

To view the header details of the current FCS file 172, use the **Data** tab→**Data Info**→**View Header** command. The View Header window will appear (Figure 17.1). If you do not have any open .fcs files, you will be presented with a dialog to select the desired file.

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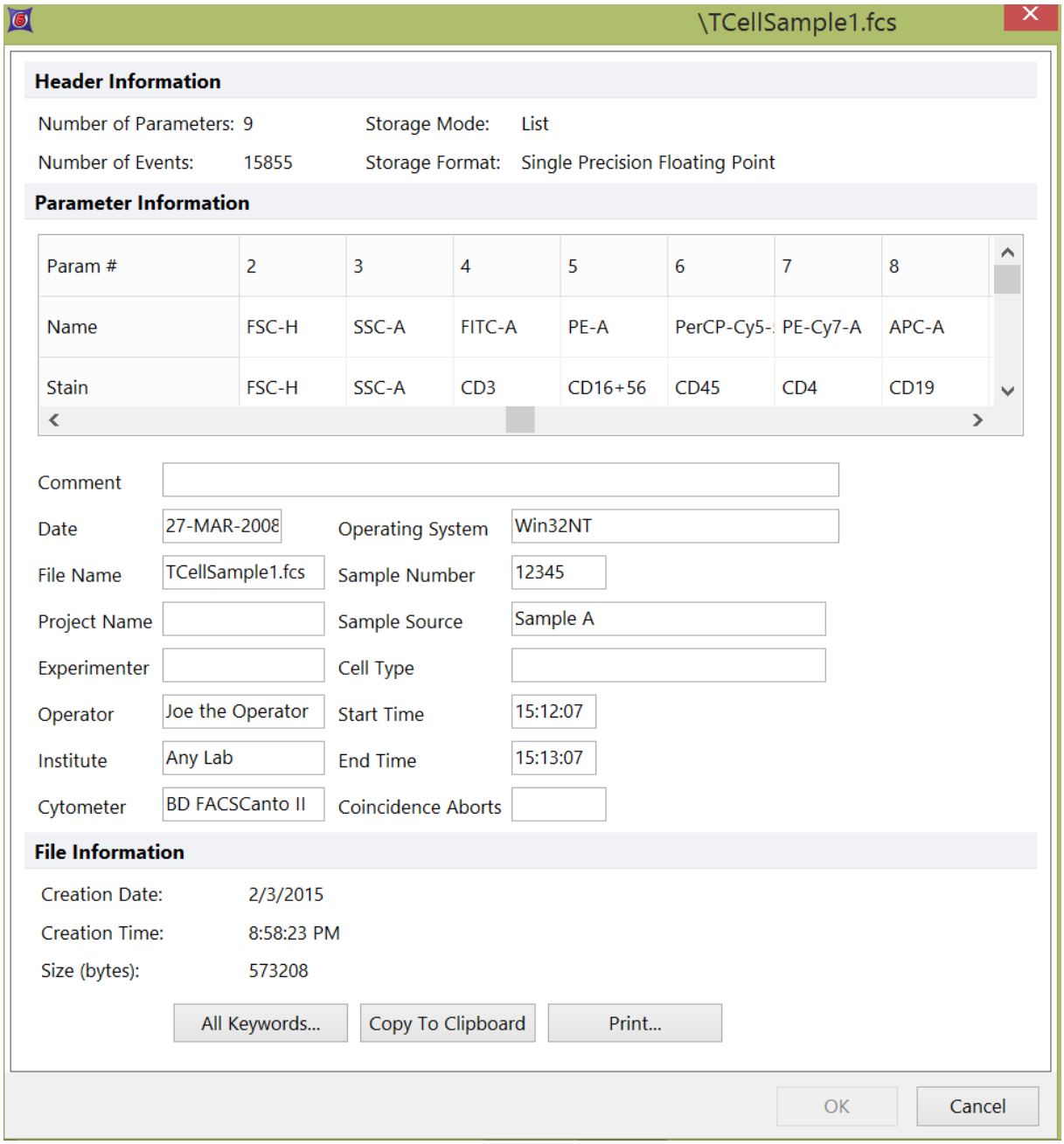

Figure 17.1 View Header window

Some of the more common keywords associated with each parameter are displayed in the **Parameter Information** field. You can view the entire list of keywords by pressing the **All Keywords...** button. A dialog listing all the keywords in the file will appear (Figure 17.2).

To copy all of the keywords to the clipboard in the $Keyword=Value format (for pasting into a text document or a text box in FCS Express), press the **Copy to Clipboard** button.

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To print the keywords in the $Keyword=Value format, press the **Print...** button.

You can modify keywords by clicking the **All Keywords...** button near the bottom of the window and entering in the new **Value** in the designated column (Figure 17.2); note that not all values can be modified. You can also modify any of the values in the editable freetext fields in the middle of the window.

To add keywords, click the **Add Keyword** button and a dialog will appear asking you to fill in the new keyword label and value. When you press **OK**, your new keyword will be added to the bottom of the list. To efficiently add multiple keywords to a data file or keywords to multiple files 652, please see instructions on the following page 652.

To remove a keyword, press the **Remove Keyword** button. If the keyword is required, there will be another dialog informing you that the keyword cannot be removed. If the keyword is not required, you will see a confirmation dialog after the change has been made.

When you have finished making your changes in the **View keywords - edit mode** dialog, press **OK** and the changes will be saved in the FCS file header.

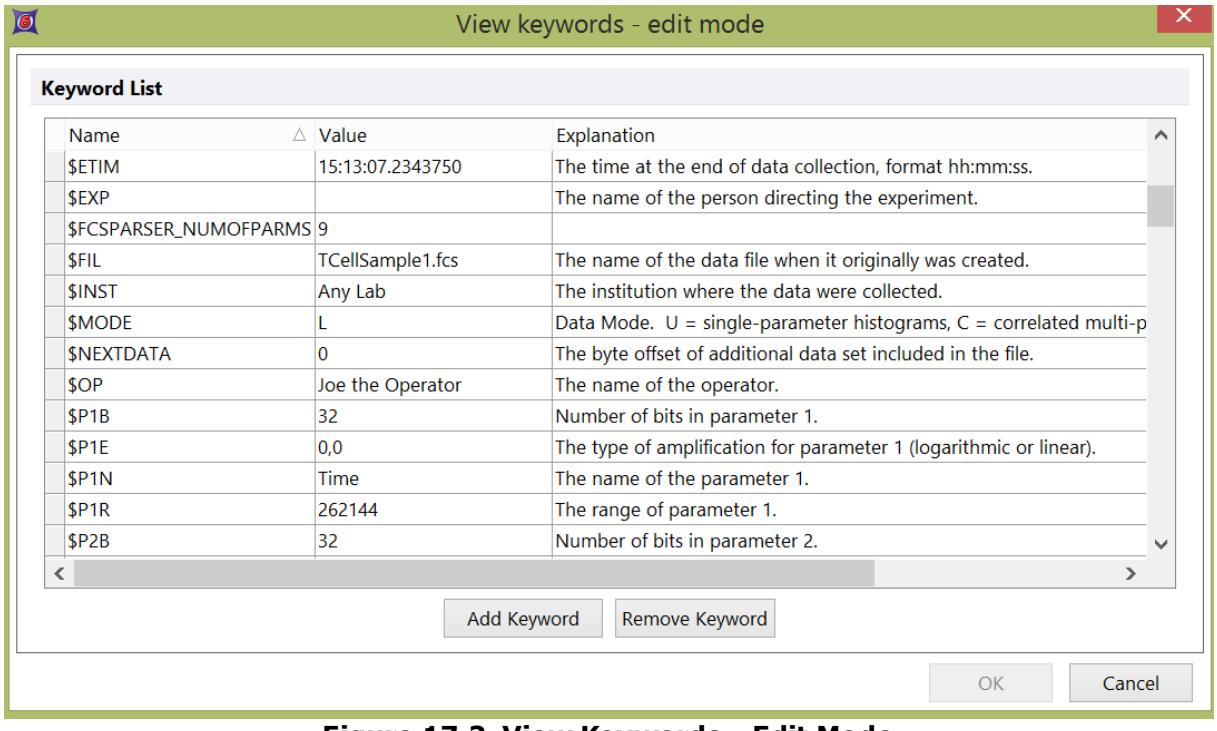

Figure 17.2 View Keywords - Edit Mode

17.2 Extra Keywords

FCS Express allows you to add keywords contained in a text file (with the form **Keyword=Value**) to an existing data file. This is useful for adding descriptors to

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your data files that were not saved as metadata at the time of sample acquisition, or for loading data as multiwell plate Heat Maps 658 in FCS Express.

To add extra descriptive keywords to your .fcs files, use any of the following methods:

- Add a keyword text file with the name **<filename>.extrakeywords.txt** to the same folder as a data file with the name **<filename>**. FCS Express will display the extra keyword(s) 692 with the data file.

- Add a keyword text file with the name **<descriptor>.allextrakeywords.txt** to the same folder containing your data files. FCS Express will display the extra keyword(s) 692 with all data files in the folder.

- Add a .csv file with the name **ExtraKeywordsTable.csv** containing columns for filename and extra keyword(s) to the same folder containing your data files. FCS Express will display the extra keyword(s) 654 with the appropriate data file on a per-row basis.
  The format of the .csv file should be as follows and as seen in Figure 17.3 below:

    - Column A contains a header named "filename"
      - Additional rows contain the file name of the file to add the keyword value to within the folder. Make sure to include the file extension as part of the name of the file.

    - Additional columns contain headers named with the name of the keyword you would like to add.
      - Additional rows contain under each header contain the information for the keyword associated with each file in text or numeric form.

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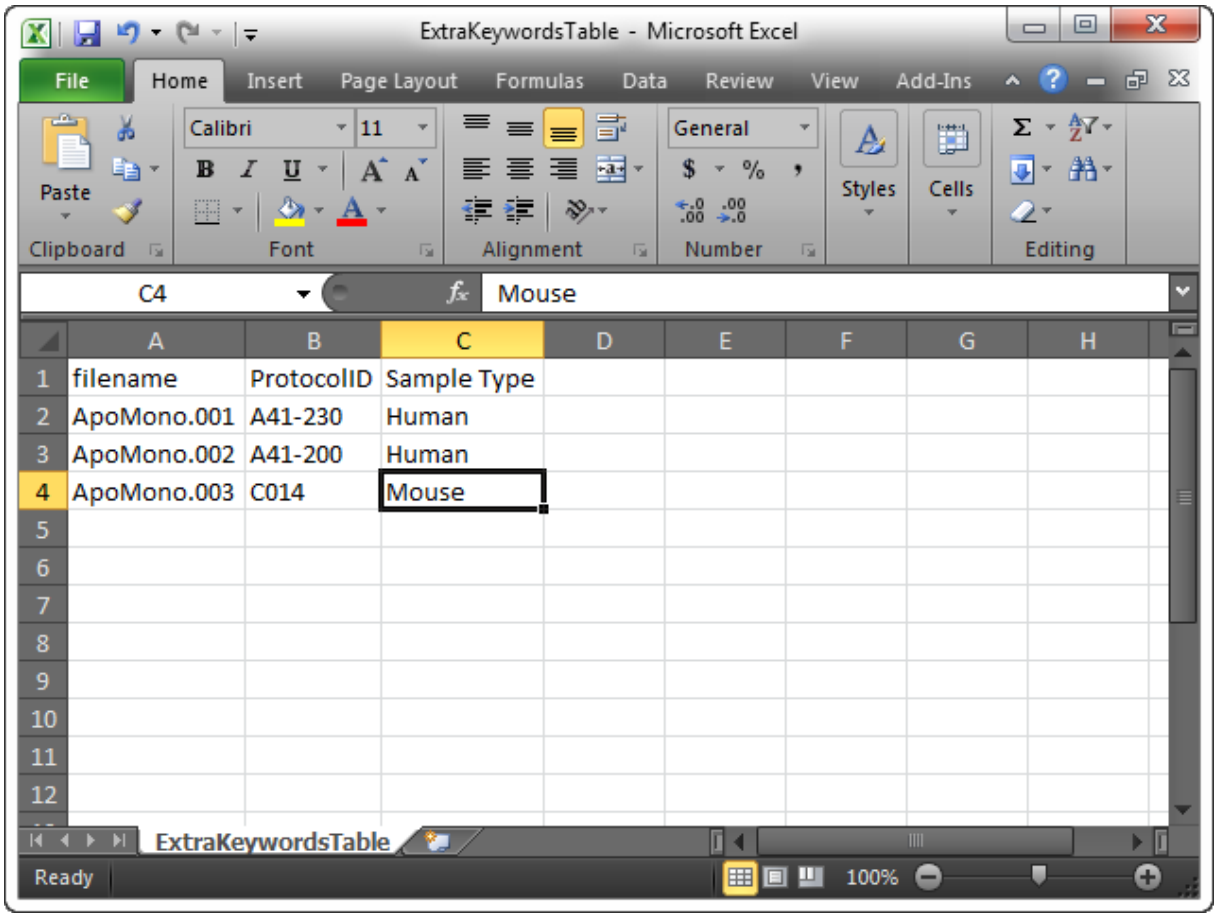

Figure 17.3 Extra keywords .csv file

Option	When you would use this option?	Why would you use this option?	Considerations
<filename>.extrakeywords.txt	When you want to annotate a specific file with a single keyword or a list of keywords.	If you have an individual file or a series of files where impor	The <filename> must match the name of the file to which the

		tant information was not entered prior to the acquisition of data, such as keywords that pertain to sample concentration and you would like to display or otherwise use the different keywords or values for each file in FCS Express.	keyword(s) will be written.

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		For example, a series of samples where different concentration values are added for each file.	
<descriptor>.allextrakeywords.txt	When you want to annotate an entire folder of files.	When you want to add keywords that pertain to all the files in the folder. For example, experiment name, protocol information, and plate infor	The <descriptor> can be any word that describes the type of keywords that are being added to the data files, like "Plate". This option will assign

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		matio n that is releva nt acros s an entire series of sampl es.	all keyw ords value s within the .txt file to all files within the folder .
ExtraKeywordsTable.csv	When you have many files that you want to annotate at once.	When you want to annot ate multip le files, each with differe nt keyw ords and differe nt value s. Much like using an .extra keyw ords.t xt file, but allowi ng you	The file must be name d Extra Keyw ordsT able.c sv and it must reside in the same folder as the data files to be analy zed for the file to be recog nized by FCS

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		the ability to quickly add and edit many files and keyword values in one .csv document.	Express.

Special Extra Keywords:

Plate Based Data:

When loading data as a multiwell plate Heat Map, the size of the plate must be specified in an extra keywords file residing in the same directory folder as your data file(s). The keywords file must contain the keywords $PlateRows and $PlateColumns. In a typical 96 well plate, that file would contain the following text:

$PlateRows=8
$PlateColumns=12

Extra keyword .txt files for 6-, 12-, 24-, 48-, 96, and 384-well plates can be downloaded on the De Novo Software website.

17.3 Keyword Anonymizer

The Keywords Anonymizer allows you to remove sensitive keyword information from your data files.

Keywords are written into the file header of data files. Many of these keywords contain the identification of the sample or patient from which these files came. To maintain the privacy of these files, you can use the Keywords Anonymizer to strip any keyword information that contains an identifiable feature.

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To use the Keyword Anonymizer, go to **Data** tab→**Data Info→Keywords Anonymizer.**

In the open **Select Keywords to Anonymize** window, you can:

- Select the **Files to anonymize ❶:**
  - **All active files**, which will anonymize all the files currently active and displayed within the layout, or
  - **Only the ones selected below**, which allow selection of the files to be anonymized ❷.

- **Remove** keywords ❸:
  - From this list, you can choose to remove:
    - **all keywords**
    - **only the keywords listed below**
    - **all keywords except the ones listed below**
      - To add specific keywords to the list, click on the **Get keywords** ❹ button to open the **Please select one or more keywords** dialog, or you can type the keyword into the list ❺**.**
      - You can select multiple keywords using the **Ctrl** or **Shift** key.

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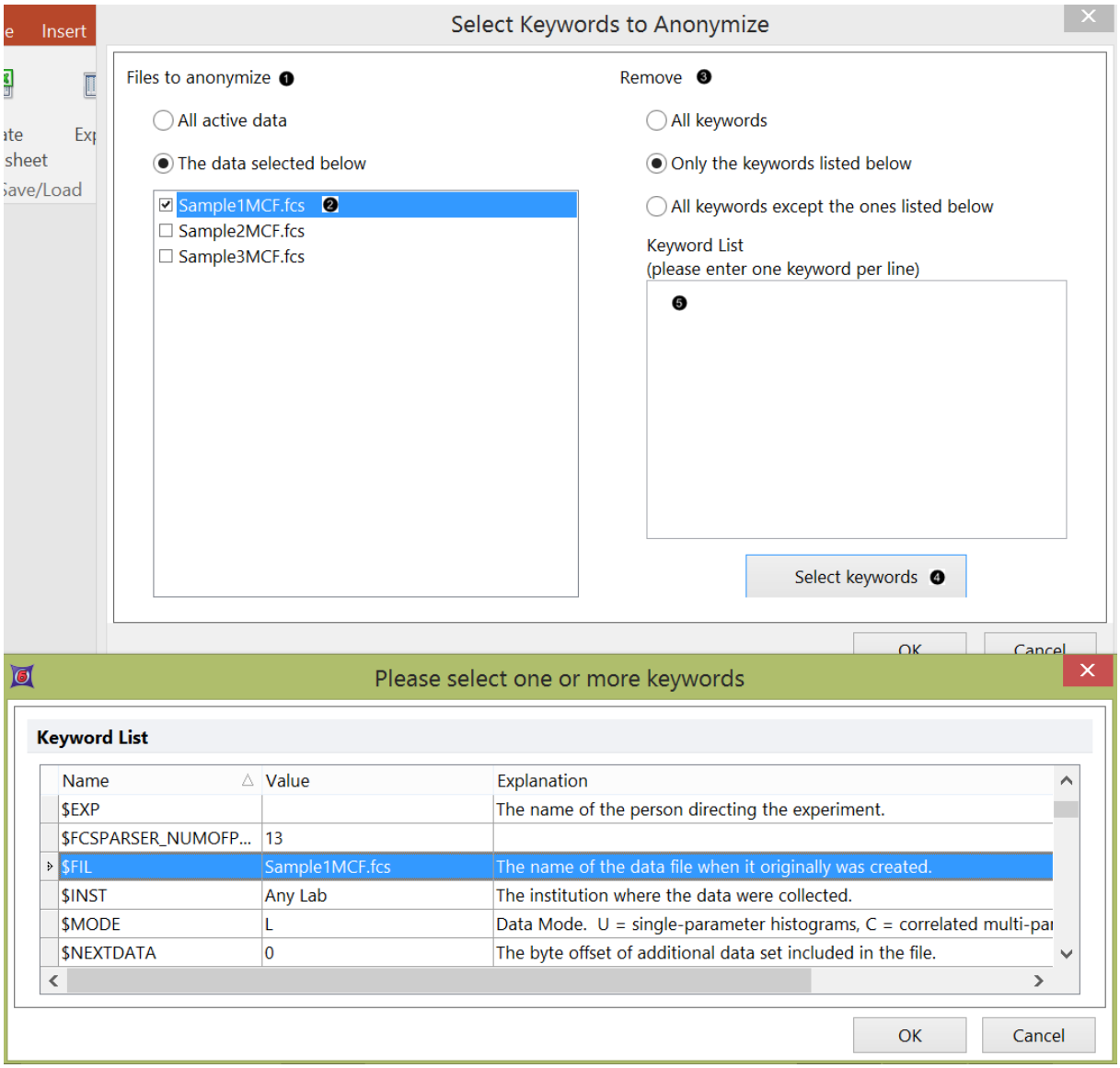

Figure 16 - The Keywords Anonymizer

Options to use the Keyword Anonymizer are also available when publishing your layout using the advanced publisher add-on 607.

17.4 Merging / Concatenating Multiple Files

FCS Express allows users to merge / concatenate multiple data files. This is particularly useful every-time it is necessary to combined the data points across multiple samples (e.g. different experimental conditions, different experimental groups, different replicates,...).

Some examples for using file merging are as follows:

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- To visualize changes across multiple files in one plot (see Example A in Figure 16.23 below, depicting a titration experiment);
- To easily display multiple samples not acquired in plate format using Heat Map plot (i.e. to present them in a multiwell "virtual" plate) (see Example B in the figure below where 8 files are depicted in a multiwell plate format);
- To perform data transformations on multiple files simultaneously without ending up with different transformation results for each file (see the Example C below where tSNE is calculated on 9 samples).

Data file merging can be accomplished in two ways in FCS Express.

1. Virtually Merging Files "on the fly" via the Merge FCS Files 662 option when loading data files in the Data List ().

2. Merging and exporting data files as a new .FCS or file type. 670

Note that the above mentioned strategies are not super-imposable. The Virtual Merge is faster but less flexible. For example, since the compensation matrix of the first file is applied to all the subsequent files, the Virtual merge it is not recommended if you want to use different compensation matrix for different files.

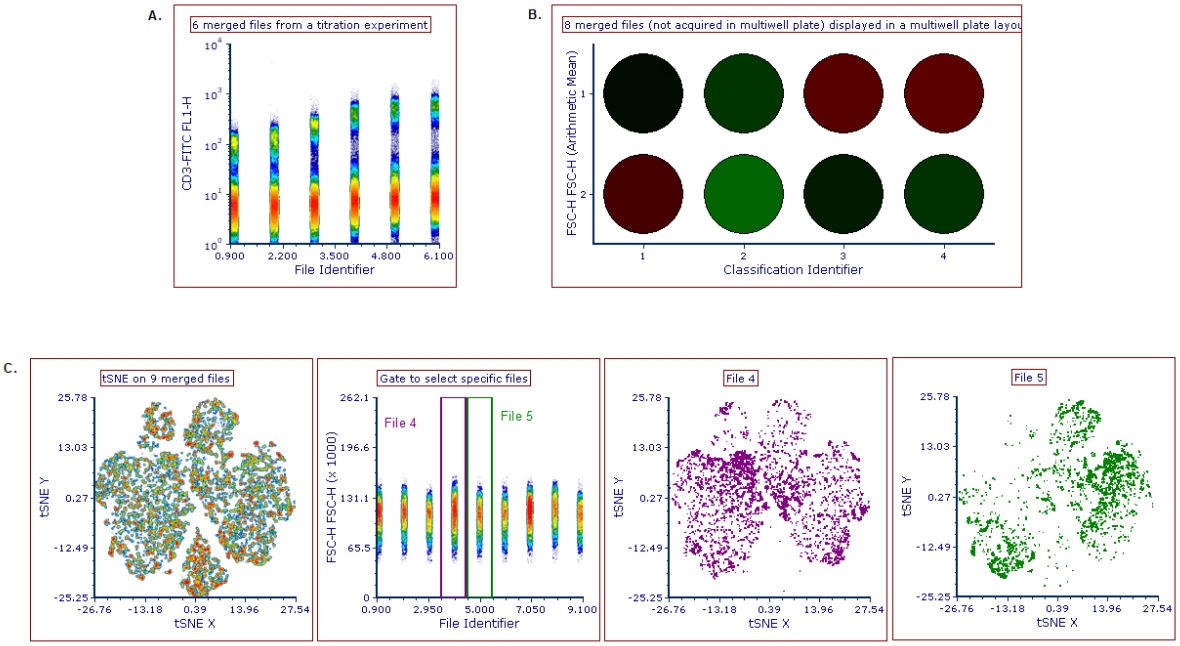

Figure 16.23 - Use cases examples of file merging.

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17.4.1 Adding Merged Files to the Data List

Usually, Flow and Image cytometry files are added as separate data files in the data list. However, multiple files may be merged together and loaded as a new file using the **Merge FCS Files** file format from the file of type drop down. Once a merged data file is added to the data list it may be used in any of the plots and analysis your perform in FCS Express.

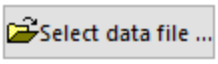
Merging data files using the **Merge FCS Files** file of type is a convenient way to load and visualize multiple files on one plot, for instance, with an antibody titration or drug treatment series, and may prove particularly useful when performing transformations 932 where an algorithm must be run on a file that is representative of all samples in the experiment. Merged files may also be represented in a heat map even if they were not acquired on a plate 1050. When using the **Merge FCS Files** option there is no need to export as a new data file as the files that are selected will be "virtually" merge on the fly. Virtually merged files can be used as any other .FCS File would be used. They can be displayed in any plot types 172 and statistics 381 can be derived from them as well.

Please also see the chapter on Exporting FCS File Data from Multiple Files 670 if you would like to merge multiple data files and save the resulting merged data file as a new .FCS file.

To begin merging data files using the **Merge FCS Files** option:

1. Open the Data list 785.
2. Click on the button.

The **Standard Open Data Dialog** will appear as in Figure 16.24 below.
Note: based on your User Options 212, the Advanced Open Data Dialog 216 may appear. If the **Advanced Open Data** dialog appears, please select the



button to access the **Standard Open Data Dialog**.

3. Select **Merge FCS Files (*.fcs; *.lmd; *.*)** from the files of type drop-down menu (Step 1 figure below).
4. Select multiple files from your computer (Step 2 in figure picture below).
5. Click **Open File**.

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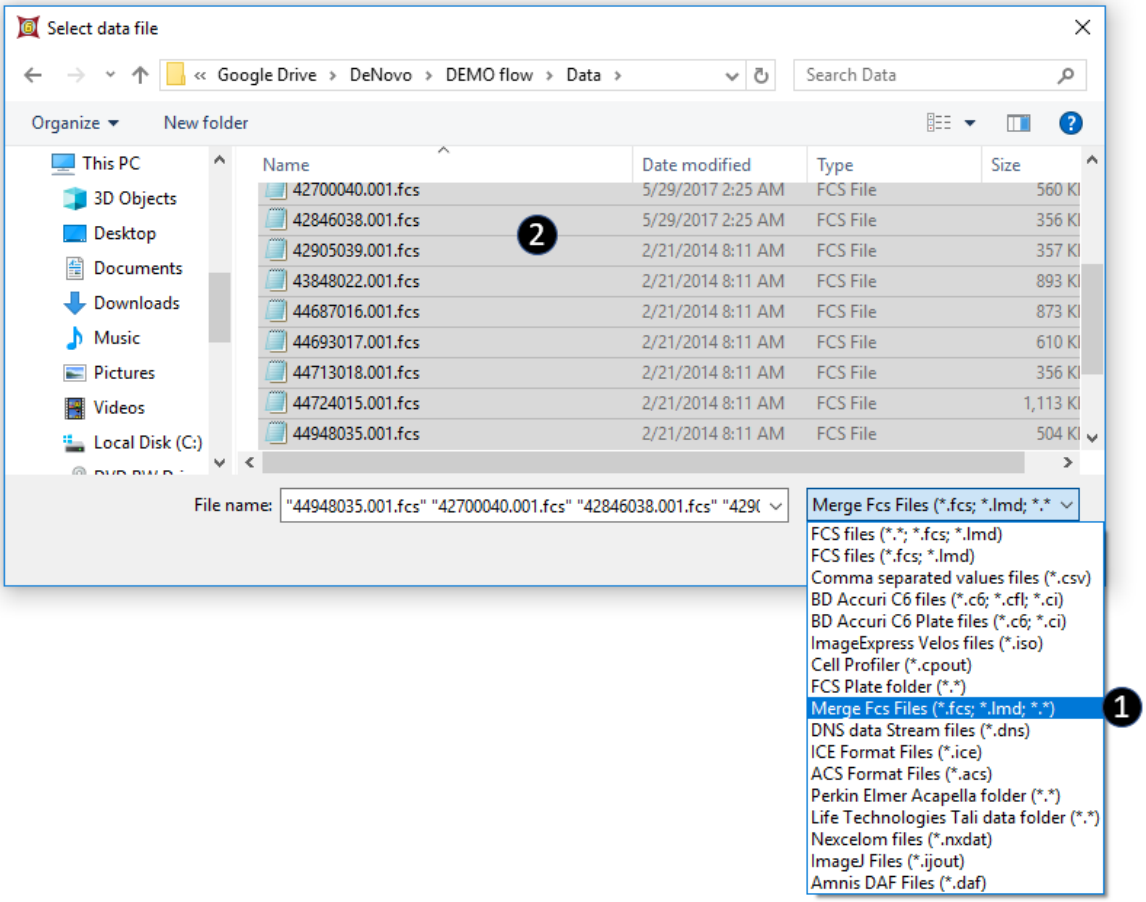

Figure 16.24 - The Standard Open Data dialog

The selected files will be virtually merged / concatenated in alphabetical order and a single file will be listed for them in the Data List (Figure 16.25 below).

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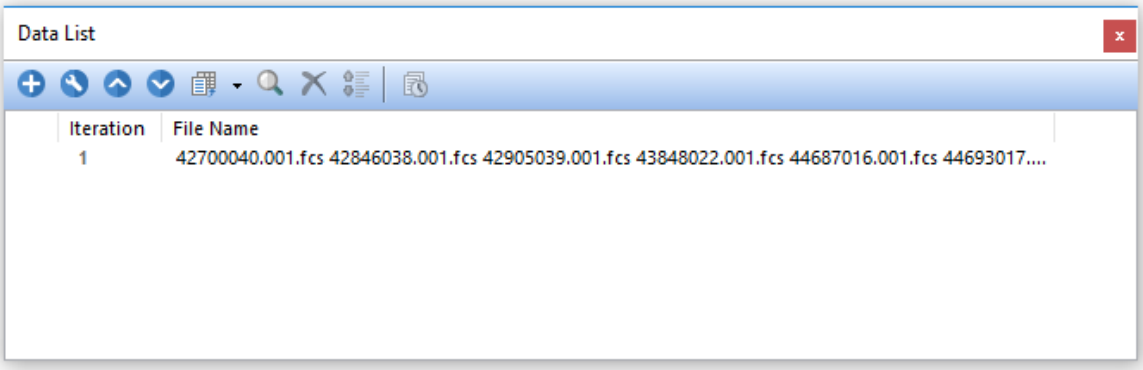

Figure 16.25 below. The Data List with the virtually merged file loaded.

The virtually merged file is concatenated as follow:

- Files are concatenated in alphabetical order.
- The compensation matrix of the first file is applied to all the following files (the compensation matrices of the following files are not used).
- A File Identifier 671 and a Classification File Identifier 672 are added. The first one can be used to discriminate the merged files using 1D and 2D plots afterwards. The second one can be used to discriminate merged files using an Heat Map 1050 plot afterwards.

An example of virtually merged file is depicted below. The **File Identifier** parameter can be displayed on 1D and 2D plots. Single files can be selected by Converting and Linking Markers 366 on 1D plots, or by using 1D or regular gates 316 on 2D plots (Figure 16.26, upper panes). The **Classification File Identifier** parameter can be displayed in Heat Map 1050 plots. With Heat Maps, single files can be selected by by using Well Gates 1061 (Figure below, lower pane).

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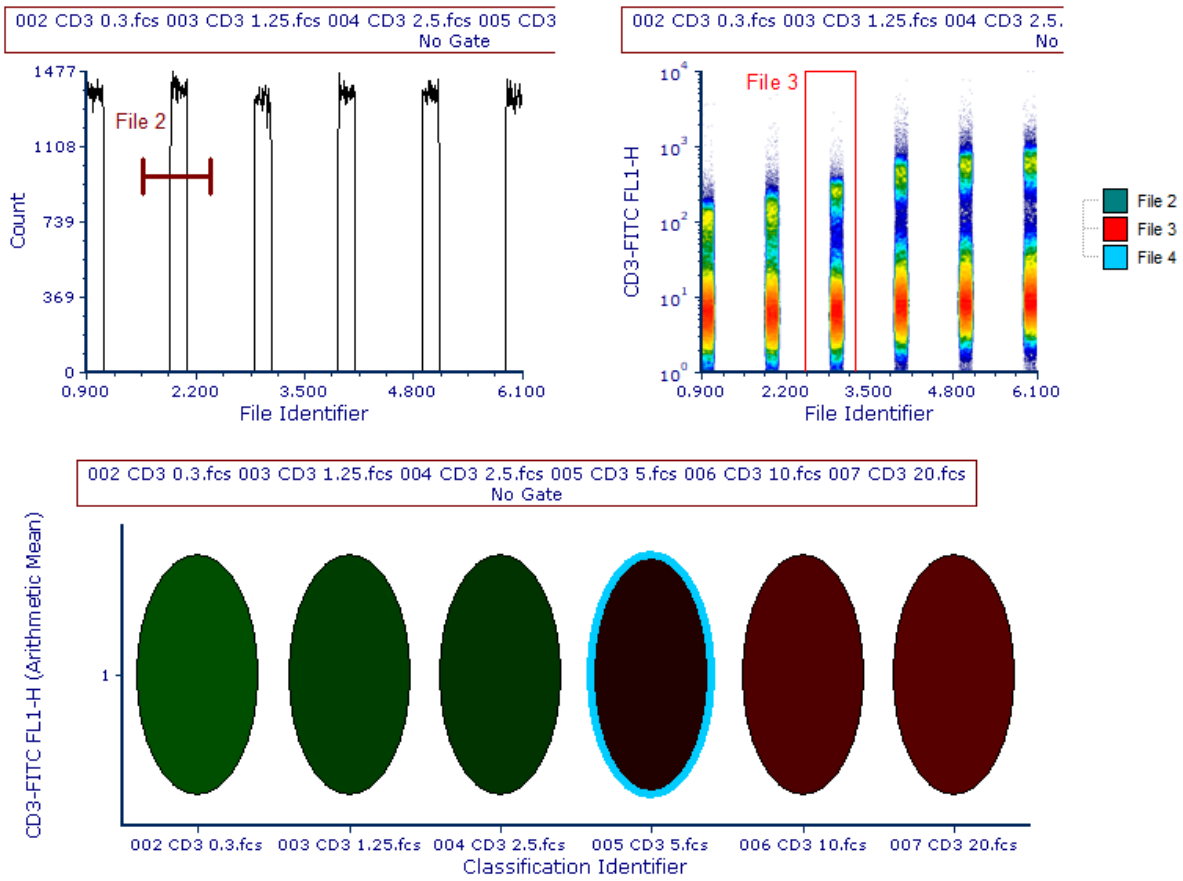

Figure 16.26. The virtually merged file is loaded into different plots displayin either the File Identifier or the Classificiation File Identifier paranmeter. Single files are also selected by Markers (Converted and Linked to gates), 1D Gates or Well Gates.

Heat Map plots displaying the virtually merged files can be customized in term of both Color Scheme 1057 and well layout 1060. The example below shows the same Heat Map plot above, with both the color scheme and the well layouts customized (Figure 16.27).

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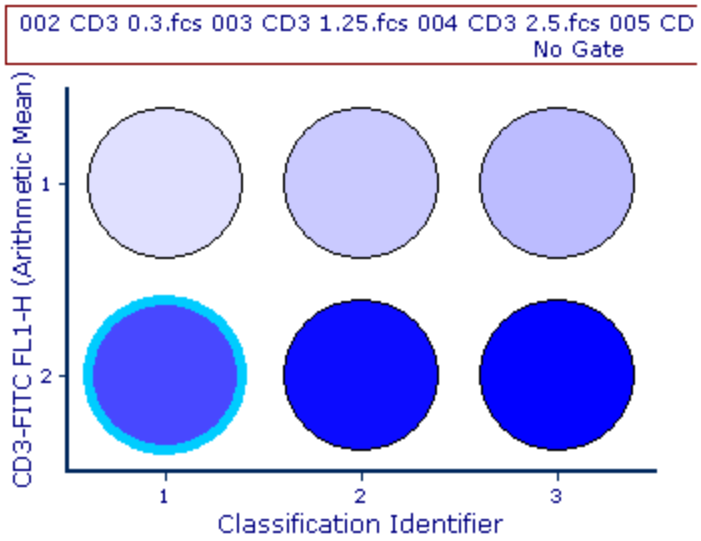

Figure 16.27 The same Heat Map plot of the figure above, customized both in term of color scheme and well layouts.

Note: the virtually merged file can be exported as a merged file by using the Single File Export tool 666 and by selecting the **DNS data stream file (*.dns)** file type.

17.5 Exporting FCS File Data

FCS Express gives you two ways to export (i.e., save) the data from an FCS file in different formats. You can export data from a single file 666 or you can "batch export" data from several files 670 at one time. Files may also be **Merged/Concatenated** 670 to create one new file that can display and compare parameters in a single plot for multiple data files.

17.5.1 Exporting FCS File Data from a Single File

To export the data from an open FCS file:

- Select a plot displaying the file to be exported.

Note: the compensation applied to the selected plot will also be applied to the exported data.

- Use the **Data** tab→**Save/Load**→**Export** command to bring up the **Export Data** dialog (Figure 17.4).

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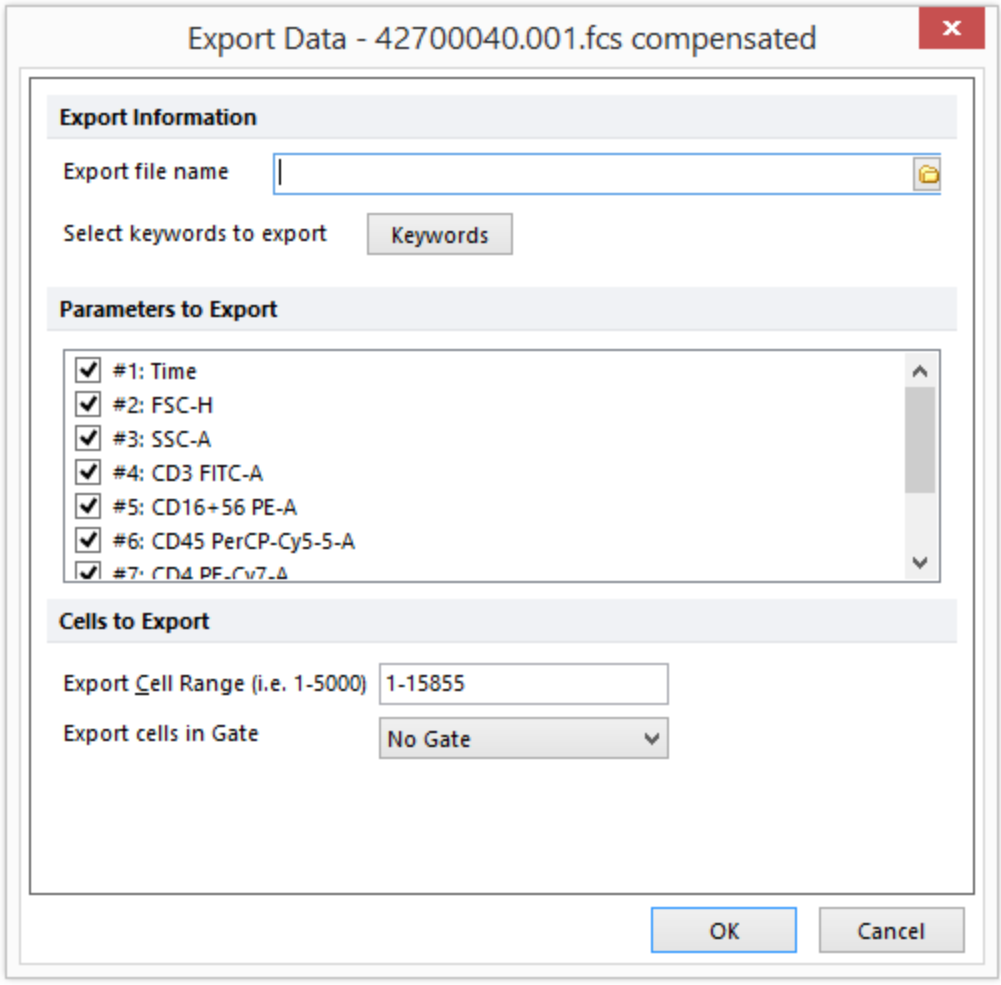

Figure 17.4 Export Data dialog

You must assign a name and file type for the data you are exporting. It is recommended to click the folder icon to the right of the **Export file name** field. This will bring up a **Save As** dialog (Figure 17.5) where you can choose the folder to save the data in, enter a file name, and choose the file type from a drop down menu.

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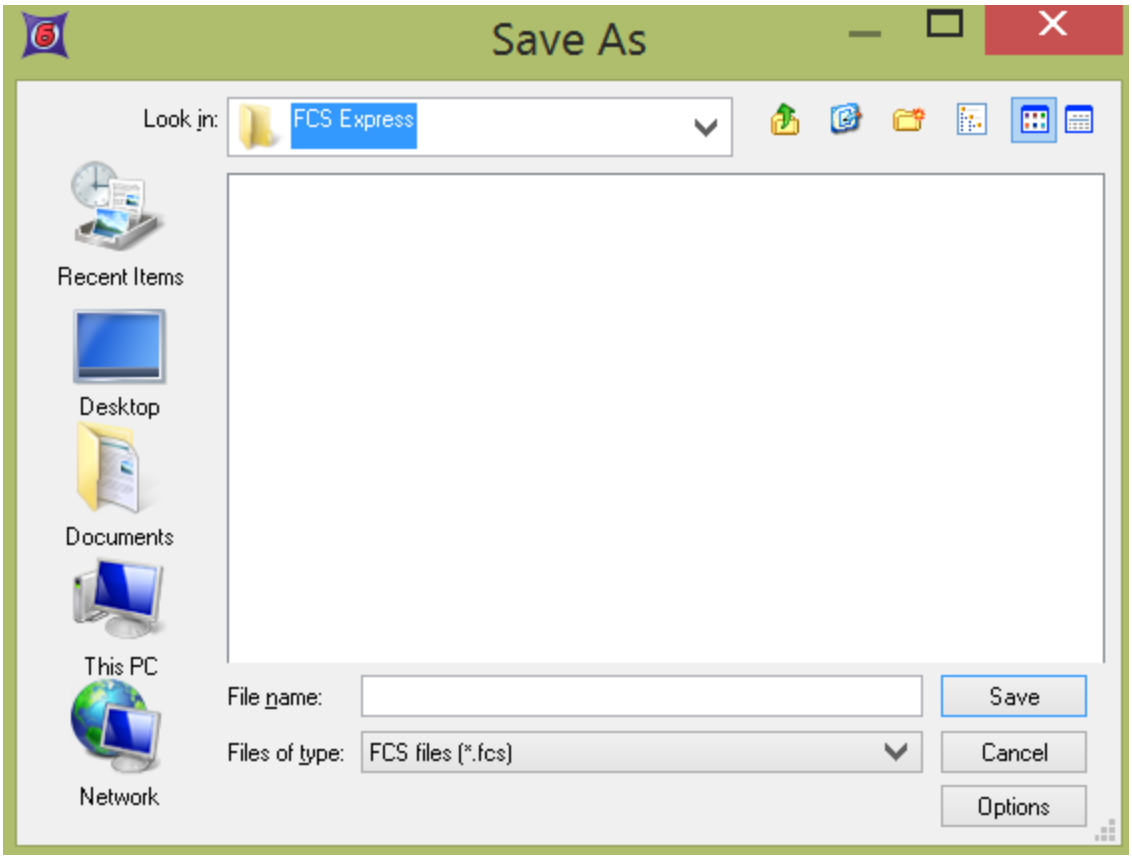

Figure 17.5 - The file export Save As dialog. Note the files of type dropdown list and Options button.

The **Options** button in the **Save As** dialog will allow you to set the specific options for the selected data format. Figure 17.6 depicts the Saving Options when **FCS files (*.fcs)** is selected as file type.

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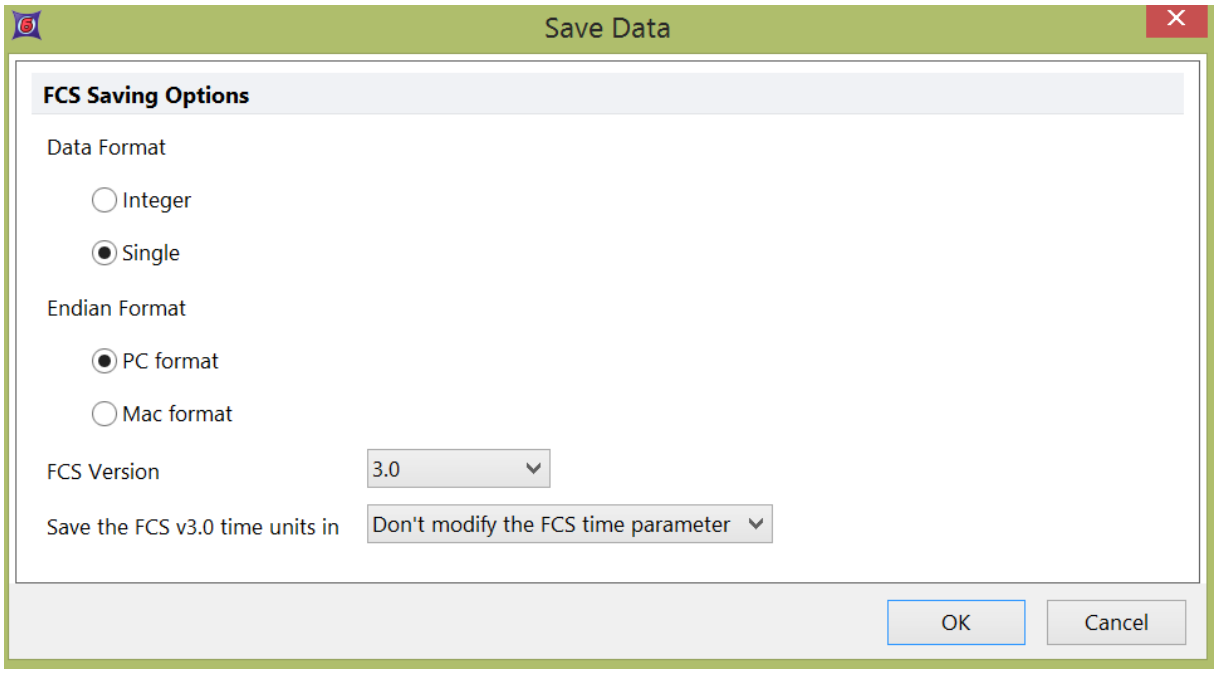

Figure 17.6 - The Save Data dialog allows you to choose the format of the data file saved.

The following table lists the options available when exporting data as FCS files.

Option	Explanation
Data Format	Select the data format to use: Integer or Single Precision Floating Point. The default option is to export the data with Single Precision Floating Point.
Endian Format	Select the endian format to use: PC format (little-endian) or Mac Format (big-endian). The default option is to export the data in PC format.
FCS Version	Select the Flow Cytometry Standard (FCS) format version: 2.0 or 3.0. The default option is to export the data as FCS 3.0.
Save the FCS v3.0 time units in	Select the units to use for the Time Parameter (it only applies if 3.0 is selected in the FCS Version in the option above). Possible choices: Don't modify the FCS time parameter, milliseconds, seconds, minutes or hours. The default option is Don't modify the FCS time parameter.

In addition to the **FCS file (*.fcs)** format, you can export the data as a **text file (*.txt)**, **comma separated values file (*.csv)**, **tab separated values file**

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(*.tab), **space separated values file (*.prn)**, **ICE file (*.ice)**, or a **DNS data stream file (*.dns)**. After assigning the file name and type, click **Save**.

The **Export Data** dialog allows to select also additional options:

- **Select keywords to export**. Keywords can be selectively exported using the **Keywords to export** button.
- **Parameters to Export.** Select which parameters to export in the **Parameters to Export** list box.
- **Export Cell Range.** Choose which cells to export, by cell number in the **Export Cell Range** field. The format is [first cell number-last cell number]. By default, the numbers represent the first and last cells in the file.
- **Export cells in Gate.** Use the **Export cells in Gate** drop-down list, to export cells only within the specified gate.

When you are ready to export, press **OK**.

17.5.2 Exporting FCS File Data from Multiple Files: Batch Export / Merging/ Concatenation

You can export data from multiple files consecutively, or merged multiple files into a single file, using batch export. The process is also sometimes referred to as concatenation.

1. Select the **Data** tab→**Save/Load**→**Batch Export** command to launch the **Batch Export** dialog (Figure 17.7).
2. Click **Add** to select the files to export. You can remove files from the export list by clicking the **Remove** button (or clear all files with the **Clear** button).
3. Use the **Export as Individual Files** radio button to export all files separately and **Export as Merged File** to export all files merged into a single file. When **Export as Merged File** is selected, you may check the **Create File Identifier column** 671 box and/or the **Create Classification File Identifier Column** 672 box, which both allow each event in the merged file to be traced back to the original file (see details below 671).

The options, with respect to file types, and the choices for data to export, are similar to those when exporting data from a single file 666.

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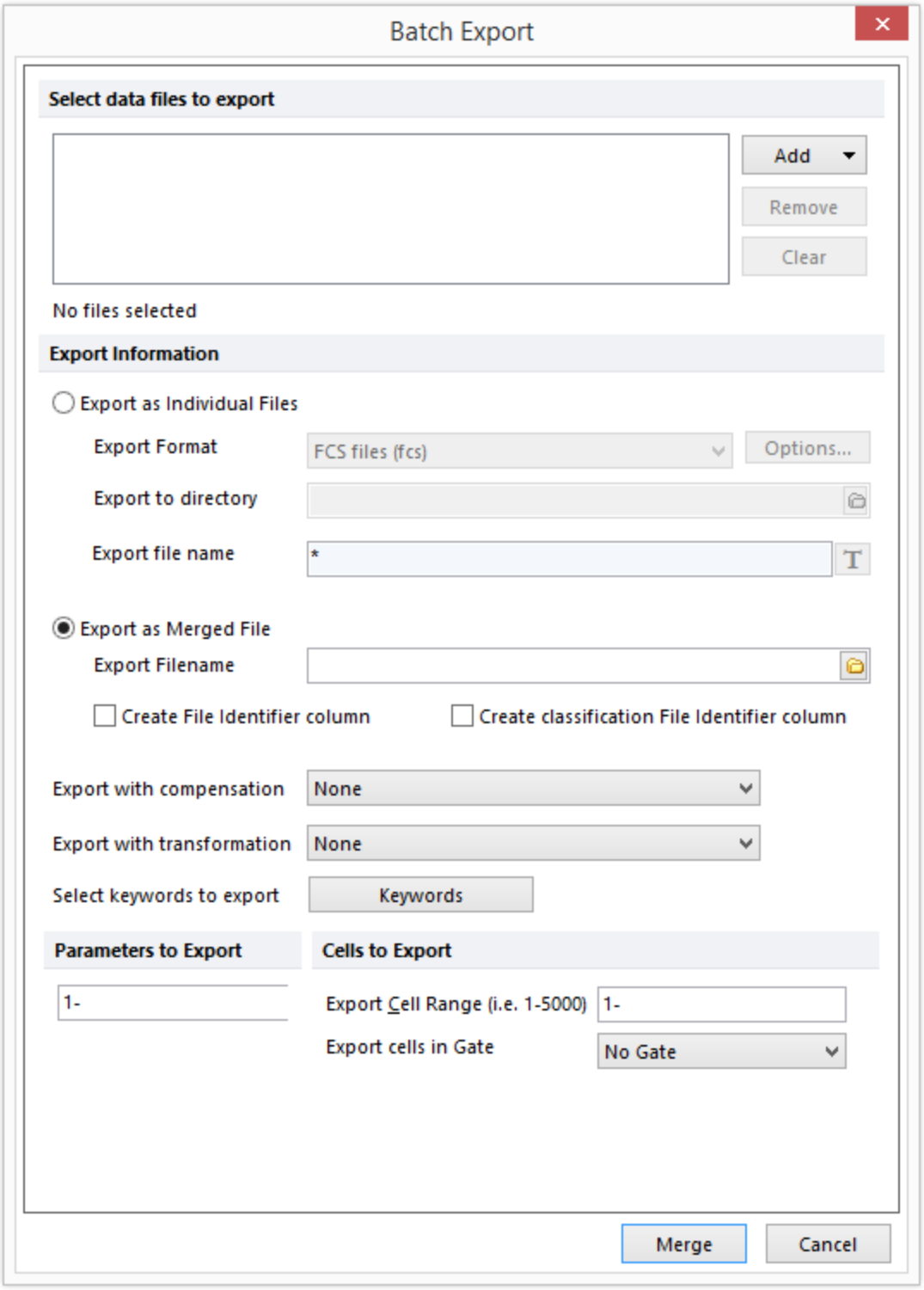

Figure 17.7 Batch Export Dialog

Create File Identifier column adds a new column (parameter) to the merged output file. The values for the File Identifier parameter are incremented for each

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file so that events in the merged file may be traced back to and gated on the original files. In the example below (Figure 17.8), a merged output file has been created from a dataset containing four original files. Three separate gates have been drawn on events originating from files 1, 2, and 3 to allow for separate analysis of these populations.

Note: In order for the File Identifier parameter scaling to display correctly, please ensure that the default FCS Saving Option for Data Format 227 is set to Single (default).

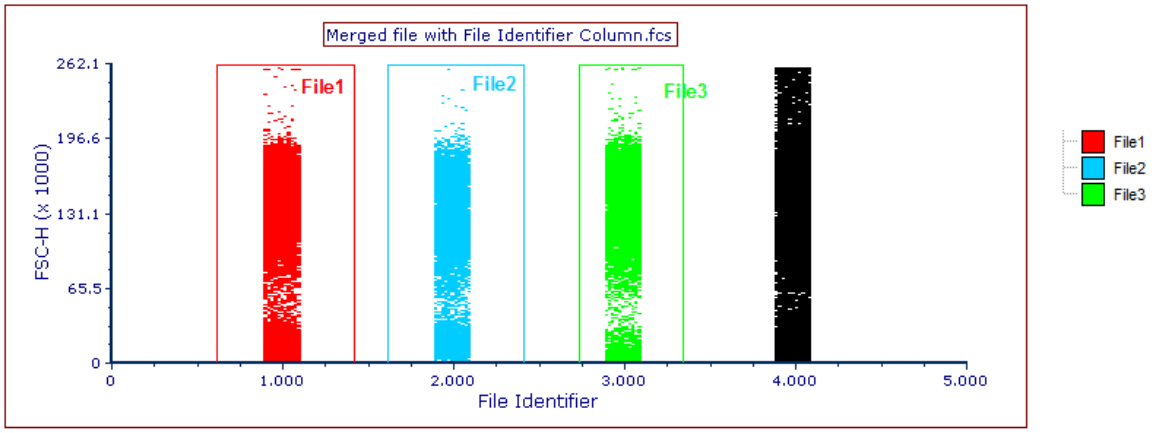

Figure 17.8 Merged file with File Identifier Column, with separate gates drawn on events from original files

Create Classification File Identifier Column adds a new column (parameter) to the merged output file. The values for the Classification File Identifier parameter are text labels matching the file name to which each event belongs to allowing each event in the merged file to be traced back to and gated per file. The difference between the **File Identifier parameter** and **Classification File Identifier** is that the **Classification File Identifier** parameter is compatible with Heat Map plots 1050. Thus, each original file may be represented as a well of an Heat Map plot. Moreover, the **Classification File Identifier parameter** reports the actual file name instead of a number as is the case with the **File Identifier parameter**.

Please note that to create the **Classification File Identifier** parameter, the **DNS data Stream files (*.dns)** format must be selected as the file of type for the output merged file (see the Merging / Concatenating FCS Files - Use Case Example 674 chapter for more information on how to use this feature).

In the example below (Figure 17.9), a merged output file has been created from a dataset containing four files. Three separate well gates have been drawn on the wells of the Heat Map plot representing files 1, 2, and 3, to allow for separate analysis of these populations.

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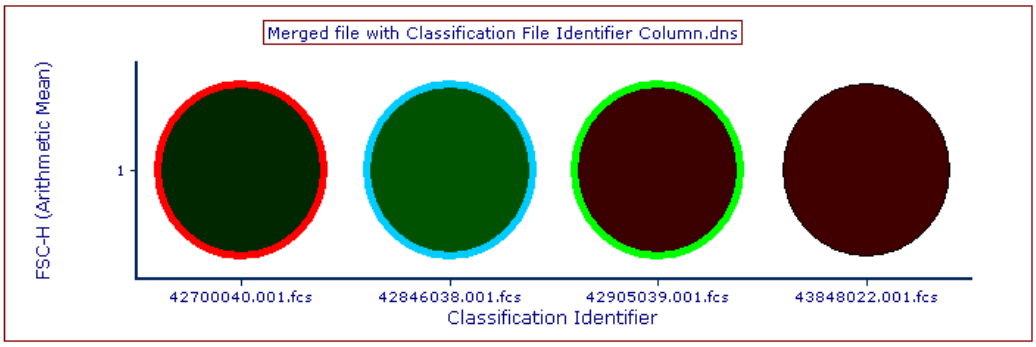

Figure 17.9 Merged file with Classification File Identifier Column, with separate well gates drawn on the Heat Map plot to specifically select events from original files

See an example of the Multiple File Merging feature in the Merging / Concatenating FCS Files - Use Case Example 674.

The **Batch Export** dialog also allows you to set the following options:

- **Export with compensation** allows you to select the compensation that will be applied to the data before exporting (default is **None**).
  Possible choice are:
  - **None** (non-compensated data will be exported)
  - **Default** (the default option for compensation 645 specified in the **User Options** will be used)
  - Any of the compensation definitions that are currently loaded into the layout.

  If more than one compensation has been applied to the files for export, a single compensation must be selected from the **Export with Compensation** option or no compensation will be applied to the merged output file.

  When merging multiple data files, if a Data Specific Gate was used to select the **Cells to Export**, the compensation applied to the merged output file must be the same compensation applied to the Data Specific Gate. In this case, it is recommend to select Default in the **Export with Compensation** option and set the compensation used to create the Data Specific Gate as the Default for the layout (see Applying Compensation Definitions 642 for more information on how to set a compensation as Default for a layout).

- **Export with transformation** allows to select the Transformation 932 that will be applied to the data before exporting. Default is **None**.

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- **Select keywords to export** allows to select which keywords will be maintained or removed after the export. Default is to export all keywords.

- **Parameters to Export** allows to select the parameters to export. Default is all parameters.

- **Cells to Export** allows to select a subset of events to export.
  Possible choices are:
  - **Export Cell Range**. Only the events in the specified range will be exported. Default is all cells.
  - **Export cells in gate**. Only the events belonging to the selected gate will be exported. Default is **No gate**.

17.5.2.1 Merging / Concatenating FCS Files Via Batch Export - Use Case Example

Here we will present a use case example for **Merging/Concatenating Data Files** 670 . In the example, files from a titration experiment will be concatenated. Briefly, each sample has been stained with a different dilution of the antibody of interest (e.g. CD3 FITC). The file resulting from file merging will then be loaded into FCS Express for further analysis. Both the **File Identifier Column** and the **Classification File Identifier Column** will be created in this example. Of course, the user can decide to create either one or both the above-mentioned Identifier Columns.

1. Select **Data** tab→**Save/Load**→**Batch Export** from the Ribbon. The **Batch Export** dialog will open.
2. Click on **Add** from the **Select Data File to Export** section of the dialog→ **Add using Standard Open Data Dialog** or **Add using Advanced Open Data Dialog** (Figure 17.10). *Note: The Advanced Data Dialog allows to select files from the Data List.*

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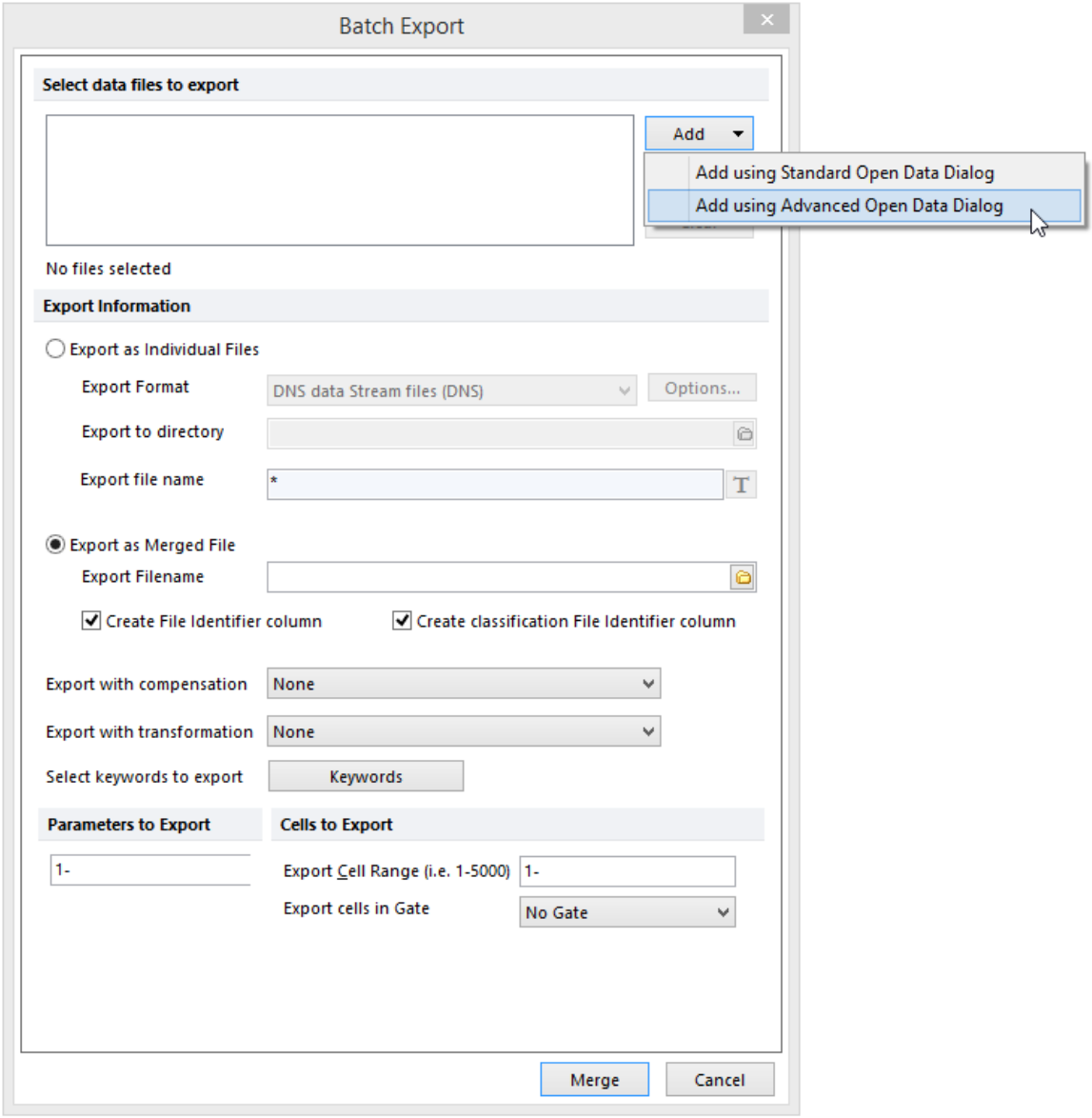

Figure 17.10 The Batch Export Dialog displaying the different Add options.

3. Browse and multiple select the files to merge.
4. Check the **Export as Merged File** radio button.
5. Browse to a location in the **Export Filename** field for the output file →select an output file name (❶ and ❷ in Figure 17.11).

a. Select **DNS data Stream files** as **File of type** (❸ in the figure below). Note that this file extension is required to create the **Classification File Identifier**

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Column. If only the **File Identifier Column** has to be created, **FCS files (*.fcs)** can be selected as **File of type**.

b. Note: When the **File Identifier Column** is created, in order for the File Identifier parameter scaling to display correctly, please ensure that the default FCS Saving Option for Data Format 227 is set to Single (default). Note that this option is only available when **FCS files (*.fcs)** is selected as **File of type**. When working with DNS files, the plot axis displaying the File Identifier parameter may be manually customized with regards to the **Resolution Options** via the 1D plot Specific Options 274 or the 2D plot Specific Options 305.

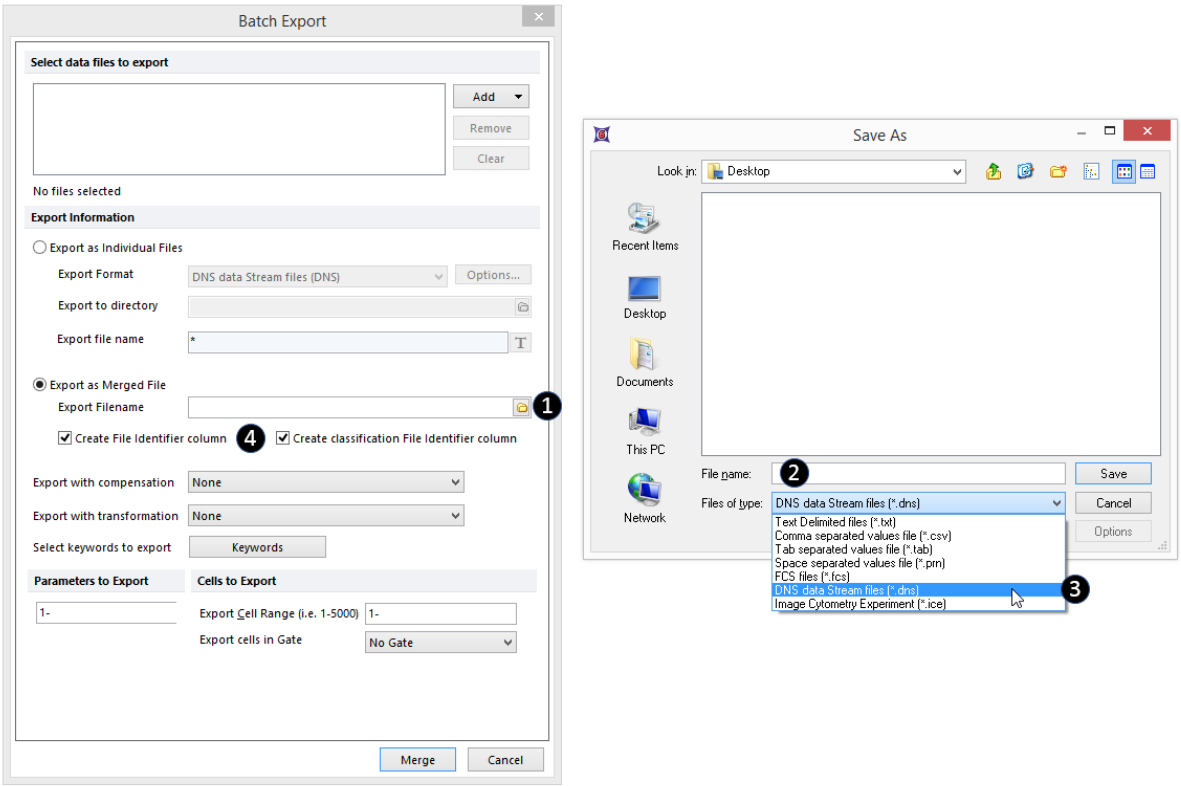

Figure 17.11 The Save As dialog allows to specify the location, the name and the type of the oputput file.

6. Check the **Create File Identifier Column** and the **Create classification File Identifier Column** check boxes (❹ in the Figure above).
7. Click **OK**. The selected file will be merged/concatenated and the corresponding merged file will be saved in the location specified in **Step 5**.

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Note that files are merged/concatenated in the same order in which they are listed in the **Select data files to export field** (**Step 2**).

The merged file can be now loaded into the layout using 1D plots, 2D plots, Heat Maps and Data grids.

Below is an example of a Density plot, Contour plot and Heat Map with the newly merged file loaded. Note that the **File Identifier Column** and **Classification File Identifier Column** created in **Step 6** above can now be used to create gates and well gates and thus to virtually separate the different merged files (Figure 17.12).

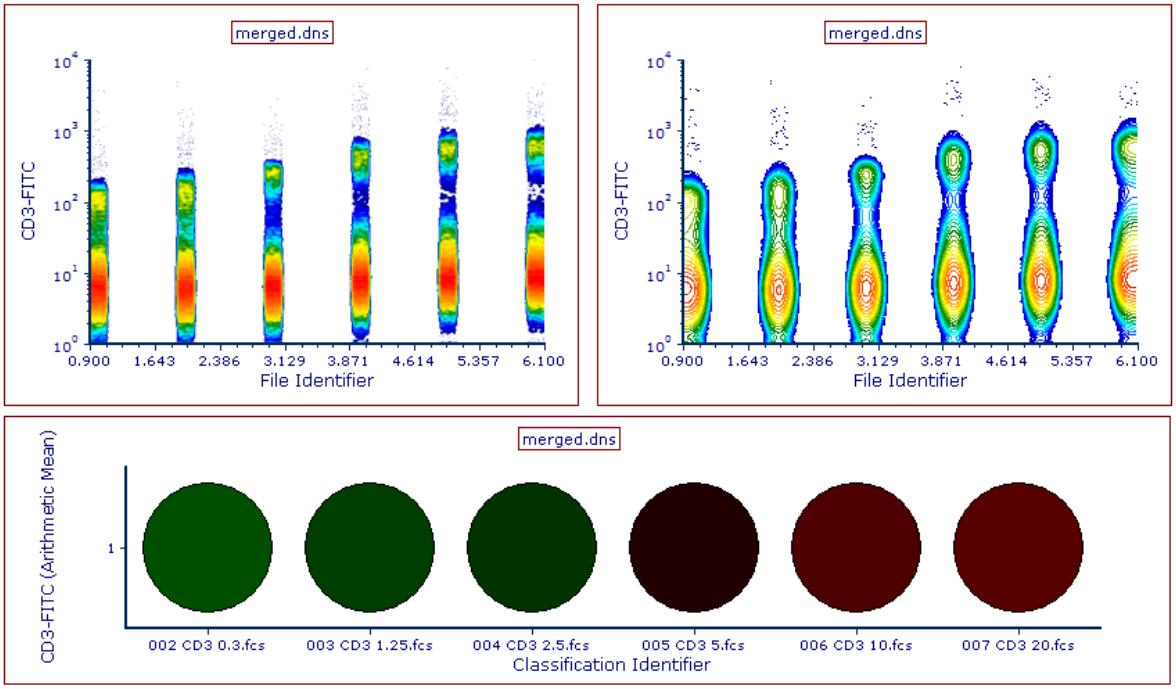

Figure 17.12 An example of Density (left) and Contour (right) plots of the file resulting from the merge.

Plots displaying the File Identifier Column and file-specific gates can be both integrated in any of your layout as they are compatible will all functionality of FCS Express (Figure 17.13).

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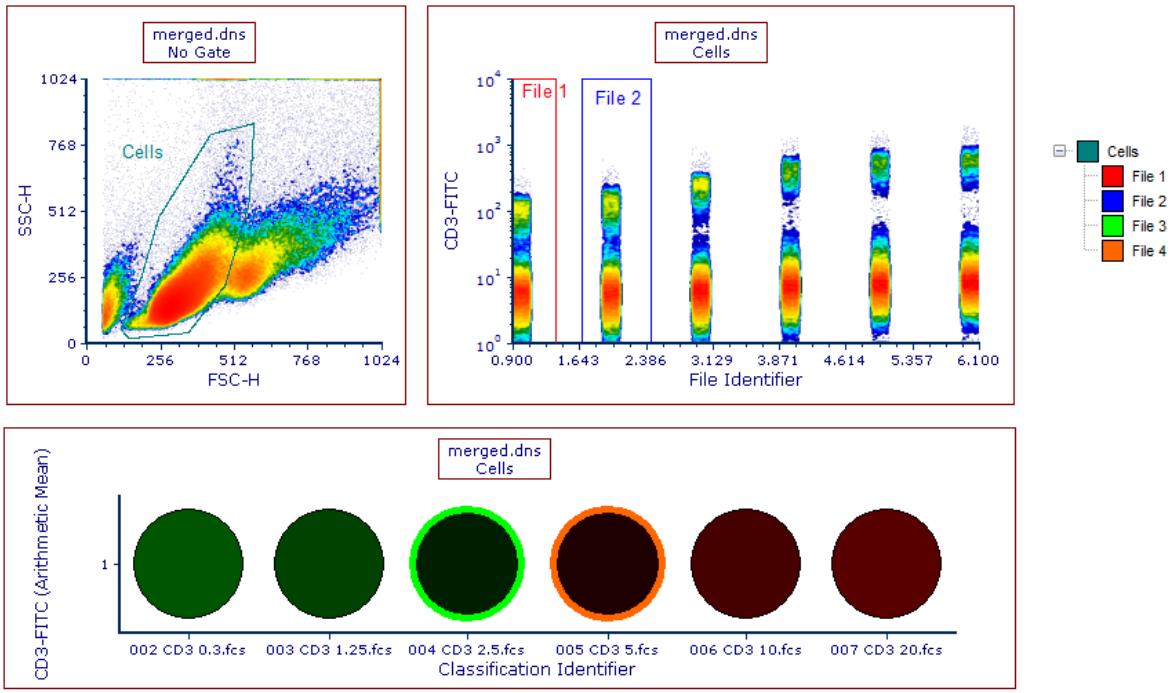

Figure 17.13 An example of file-specific gates drawn on the file resulting from the merge.

Please see also the Antibody Titration Spreadsheet use Cases 2 to see how to use spreadsheet for this type of assay.

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Chapter

18 Working with Data Grids

FCS Express allows you to view the raw data acquired for every event or "cell" in a data set, including cell images if applicable 684.

Select the **Insert→General→Data Grid** command to insert a **Data Grid** in the layout to show the data from the top data set (Figure 18.1). If no data set is open, you will be prompted to select a file.

Cell #	FSC-Height	SSC-Height	FL1-H	FL2-H	FL3-H	Time (20.48
1	509.00	351.00	403.15	395.96	626.43	3.00
2	571.00	516.00	198.10	254.83	421.70	3.00
3	482.00	564.00	518.61	264.16	319.08	3.00
4	428.00	355.00	1321.58	1321.58	2090.80	3.00
5	396.00	376.00	770.40	1134.19	1963.22	3.00
6	337.00	317.00	973.38	1274.88	2246.79	3.00
7	247.00	291.00	1055.45	474.00	582.94	3.00
8	397.00	408.00	716.92	151.25	23.08	3.00
9	693.00	631.00	930.57	182.69	20.35	3.00
10	256.00	446.00	9.65	11.04	7.30	3.00
11	498.00	621.00	1084.32	421.70	449.10	3.00
12	717.00	423.00	342.89	74.32	15.12	3.00
13	435.00	360.00	557.31	649.38	1175.74	3.00
14	434.00	350.00	8.51	4.14	4.14	3.00
15	525.00	345.00	661.17	138.24	21.48	3.00
16	572.00	452.00	433.23	93.06	18.94	5.00
17	529.00	525.00	316.23	433.23	835.36	5.00
18	496.00	511.00	330.77	271.39	414.18	5.00
19	430.00	396.00	421.70	86.60	14.59	5.00
20	335.00	318.00	1036.63	964.66	1582.04	5.00
21	380.00	294.00	399.54	82.05	10.18	5.00

Figure 18.1 Data Grid

To copy the raw data to the clipboard, select the cells to copy, and then use the **Home→Clipboard→Copy Text** command. To select an entire column, click on the column header for the parameter. To select an entire row, click in the small grey box to the left of the cell number.

To export multiple parameters to a text file, see Exporting FCS File Data 666.

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18.1 Formatting Data Grids to Display Only Images

In order to format a **Data Grid** to only display images, right-click on the **Data Grid** and select **Format** from the pop-up menu. Choose the parameters to show as columns in the grid by using the **Parameters to Display** category (Figure 18.2). To show selected columns, choose **Only the items checked below** or **All items except those checked below**, and then check the appropriate items.

For all other formatting options please see Formatting Data Grids and Custom Data Grids 309.

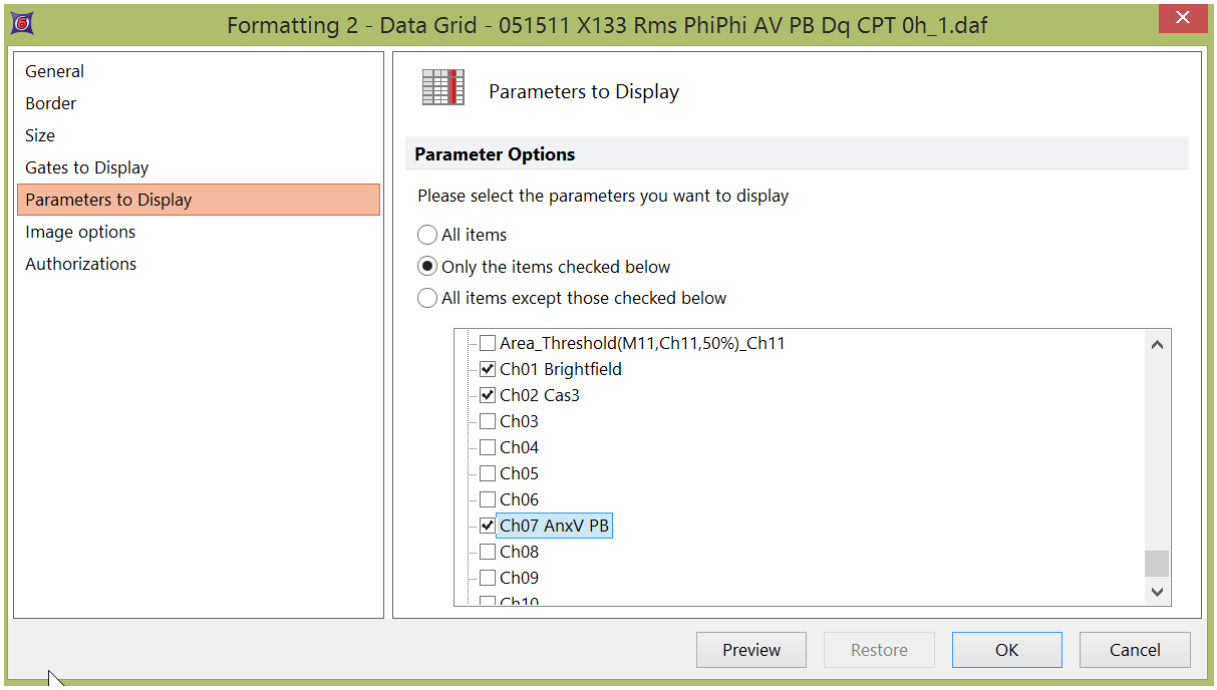

Figure 18.2 Showing and Hiding Columns in a Data Grid to only display images.

18.2 Applying Gates to a Data Grid

Gates can be applied to data grids to show just the data within the gate. To do so, use the **Gating→Create Gates** group and choose a gate from the **Current Gate** drop-down list.

Gates may also be applied to a Data Grid by dragging the gate and dropping it on top of the Data Grid. Please see the section on Using Gates 316 for additional details and options.

18.3 Backgating on a Data Grid

A **Data Grid** can also be backgated 316 using the formatting options:

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1. Right-click on the grid and select **Format** from the pop-up menu.
2. Choose the **Gates to Display** category.
3. Select a gating scheme using the radio buttons and check the gates appropriate to your scheme (Figure 18.3).

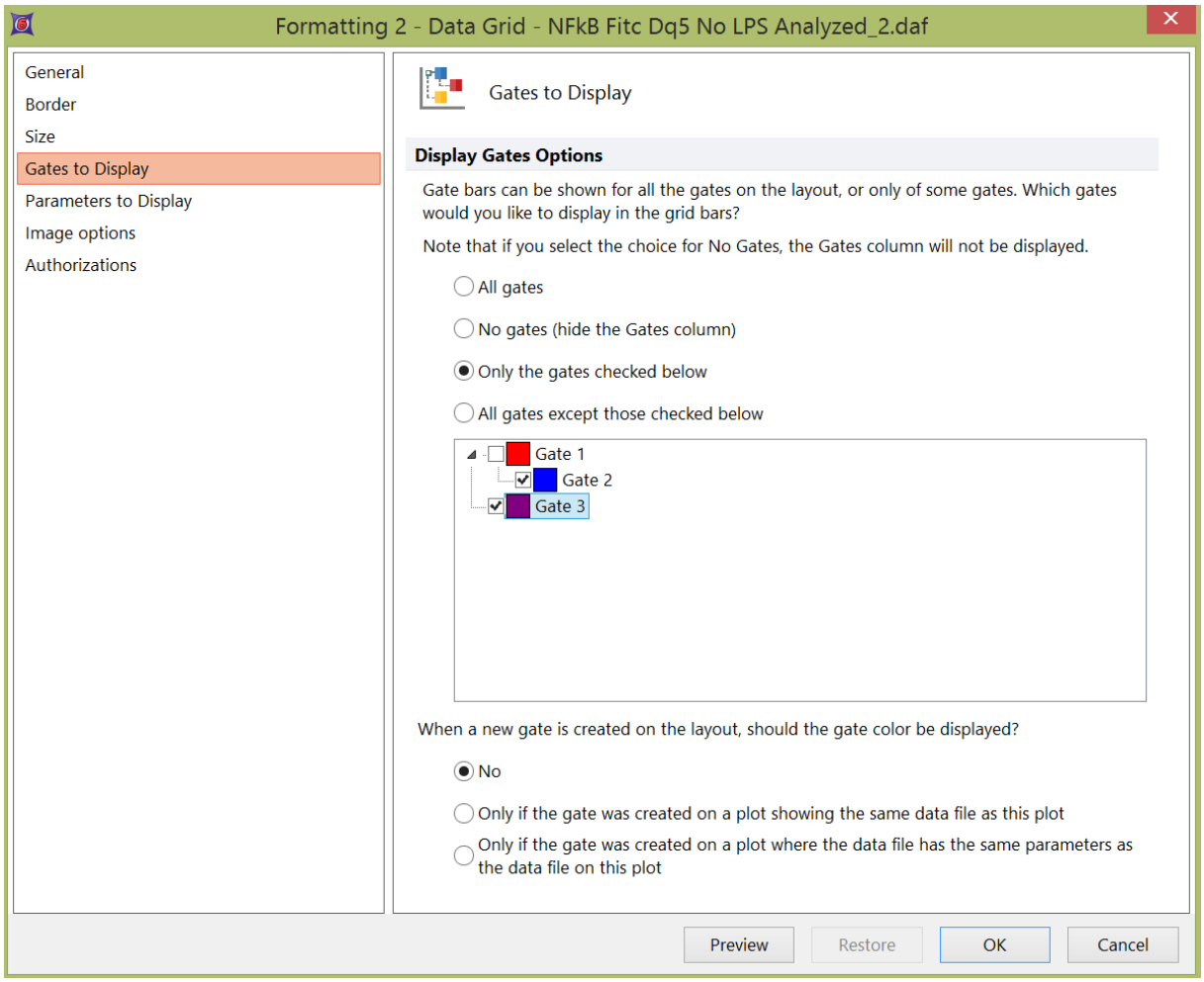

Figure 18.3 Formatting a Data Grid - Gates to Display

When gates are displayed in a **Data Grid**, a **Gates** column is present. If an event falls in one or more gates, colored bars (according to the gate colors) will be shown in the **Gates** column (Figure 18.4).

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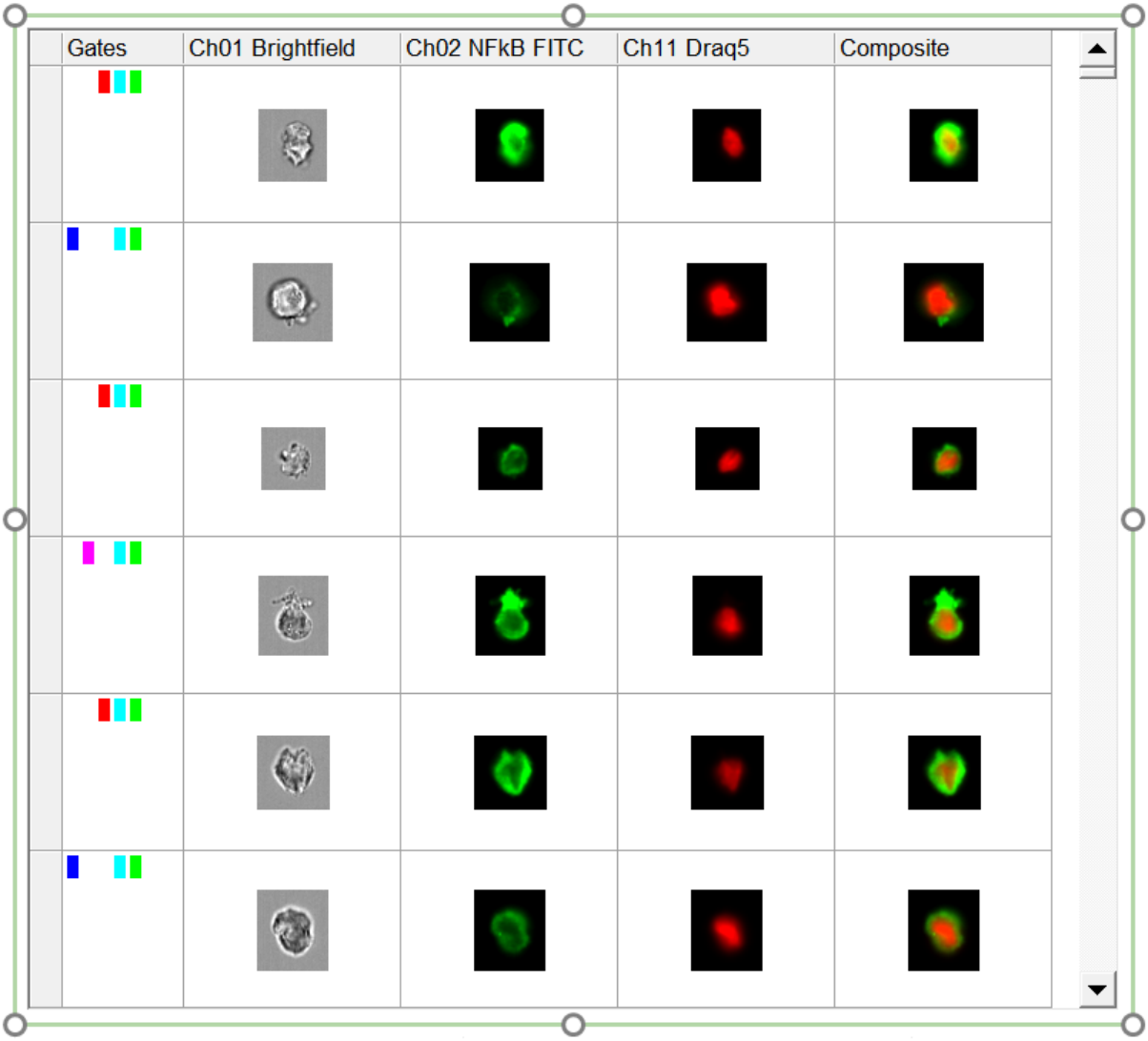

Figure 18.4 Backgated Data Grid. Bars in the Gates column of some events show in which gate(s) the cells fall.

18.4 Creating Cell Number Gates on a Data Grid

To create a gate on a **Data Grid**, right-click on the grid and select **Create Gate** from the pop-up menu. A new column will appear in the grid (Figure 18.5).

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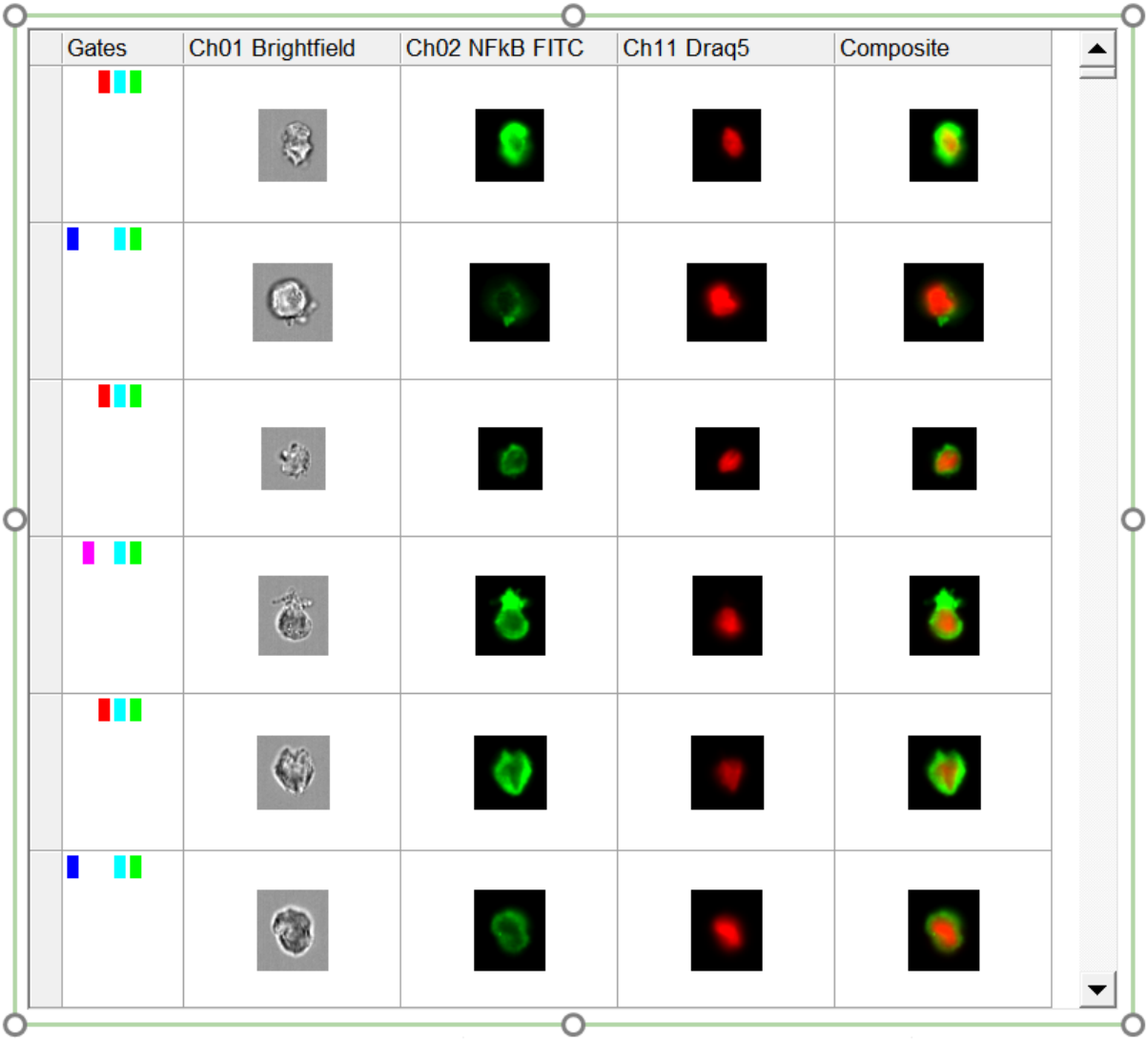

Figure 18.5 Creating a Gate on a Data Grid

Check the events in the **New Gate** column that you want to include in your gate (so-called "Cell Number gates") and click **OK** to bring up a Create New Gate dialog where you can assign a name to the gate and choose the color 318.

You can also create a gate containing a **Selection** or **Range** using the appropriate drop-menu items.

18.5 Using Data Grids as Image Galleries

If you are using FCS Express Image Cytometry or FCS Express Plus, it is possible to use the Data Grid as an Image Gallery. Raw images associated with your Image Cytometry experiment will be located in the last columns in the far right of

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the data grid. You may also choose to select only to display the parameters with the image data from the Data Grid format screen 681 Figure 18.6. Multiple data grids may be inserted in the layout 680 for the same or different data files to compare results.

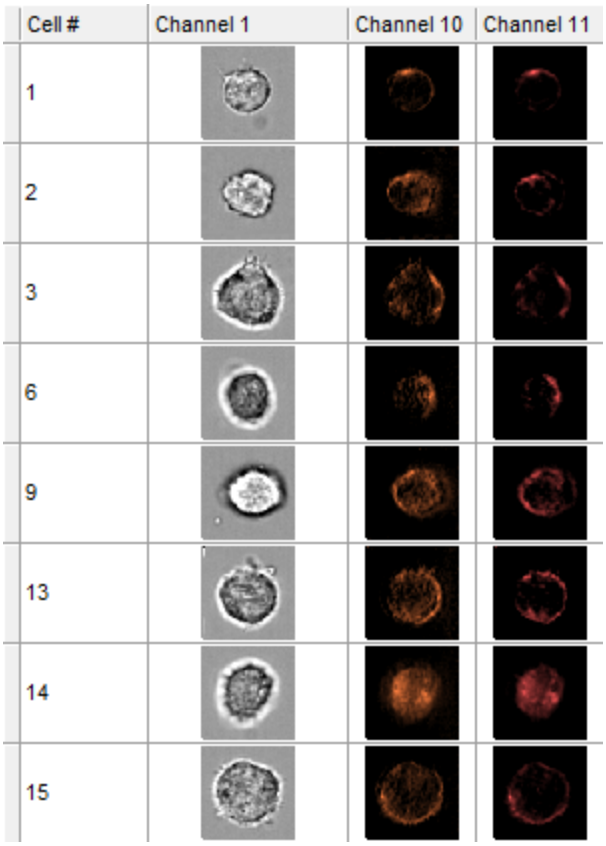

Figure 18. 6 An image gallery displaying only the image parameters.

To view images of only the cells within a particular gate apply a gate 681 to the Data Grid.

Single cell images may also be inserted in the layout via the following steps.

1. Left click and hold on the image in the Data Grid.
2. Drag the image to an empty space in the layout.
3. Release the mouse button.

The image from the data grid that was dragged onto the layout will now be inserted as a new picture object on the page.

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18.6 Viewing Compensated and Transformed Values in Data Grids

Data grids may be formatted to display compensated and transformed values. Please note only one compensation and one transformation may be applied at a time for each plot or data grid selected. Also, the transformation will have its own column(s) displayed in the data grid, the number of which are dependent upon the transformation selected.

Compensations may be applied to data grids by any of the following commands:

- Drag and drop compensation from Compensations navigator (Figure 18.7).

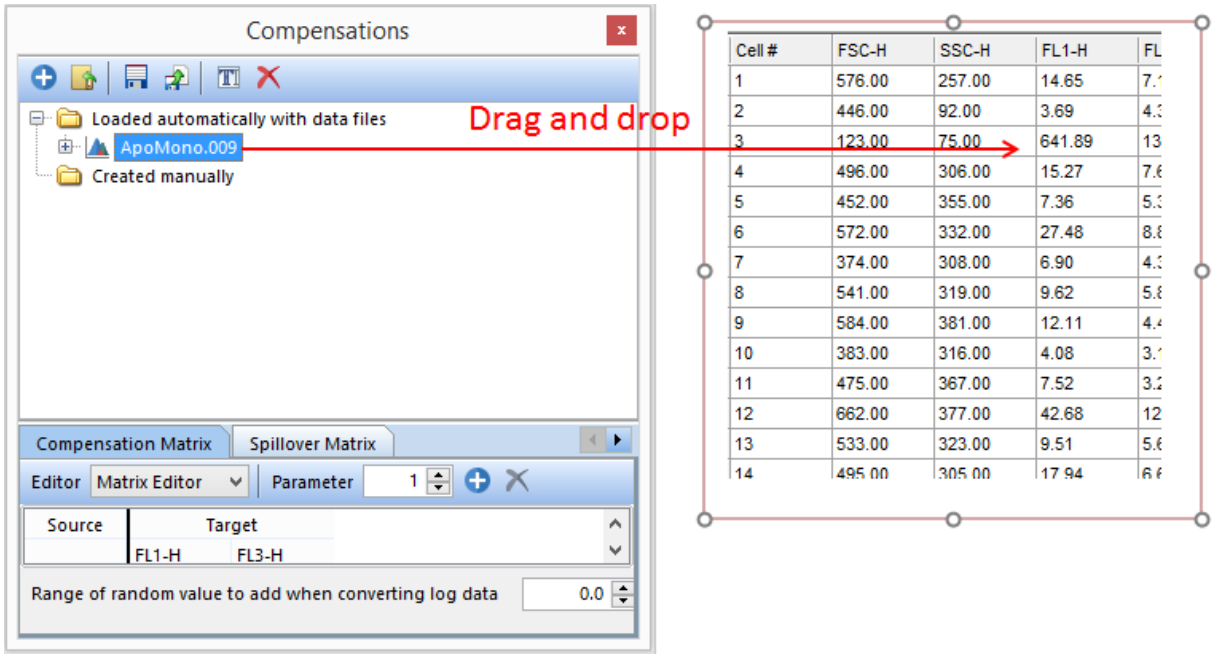

Figure 18.7 - Drag compensation from Compensations navigator and drop onto data grid (red arrow).

- Right-click data grid→**Show Compensated Values**→selecting **None** or one of listed options (Figure 18.8).

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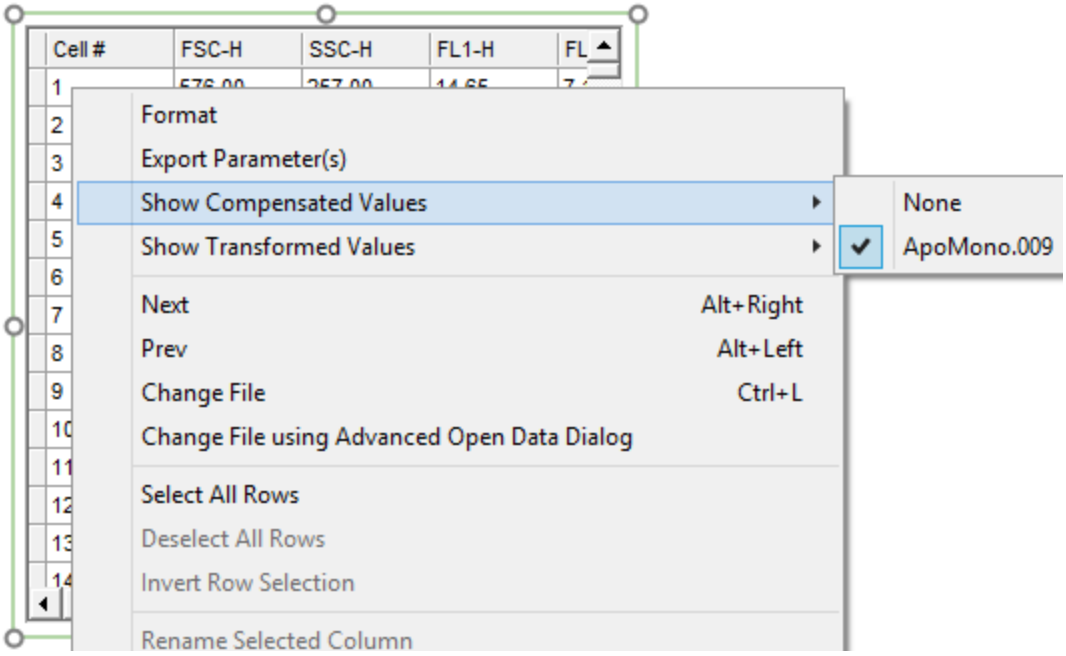

Figure 18.8 - Right-click data grid to Show Compensated Values menu.

- Access Formatting dialog for general options 310 →**Data Options**→select compensation to use from **Use Compensation** drop down list→**OK** (Figure 18.9).

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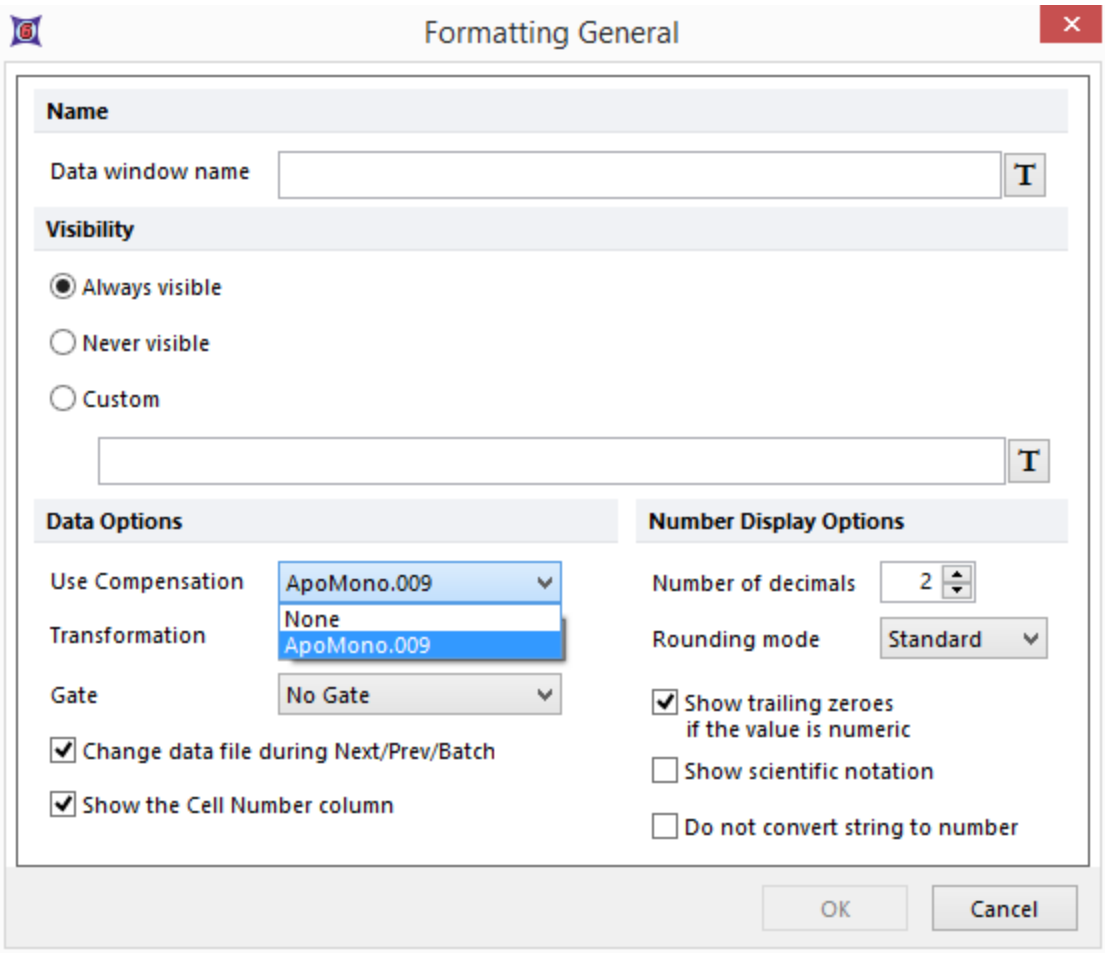

Figure 18.9 - Using Formatting dialog to select from Use Compensation drop down list under Data Options.

Transformations may be applied to data grids by any of the following methods:

- Drag and drop from the Transformations navigator to the intended data grid (Figure 18.10).

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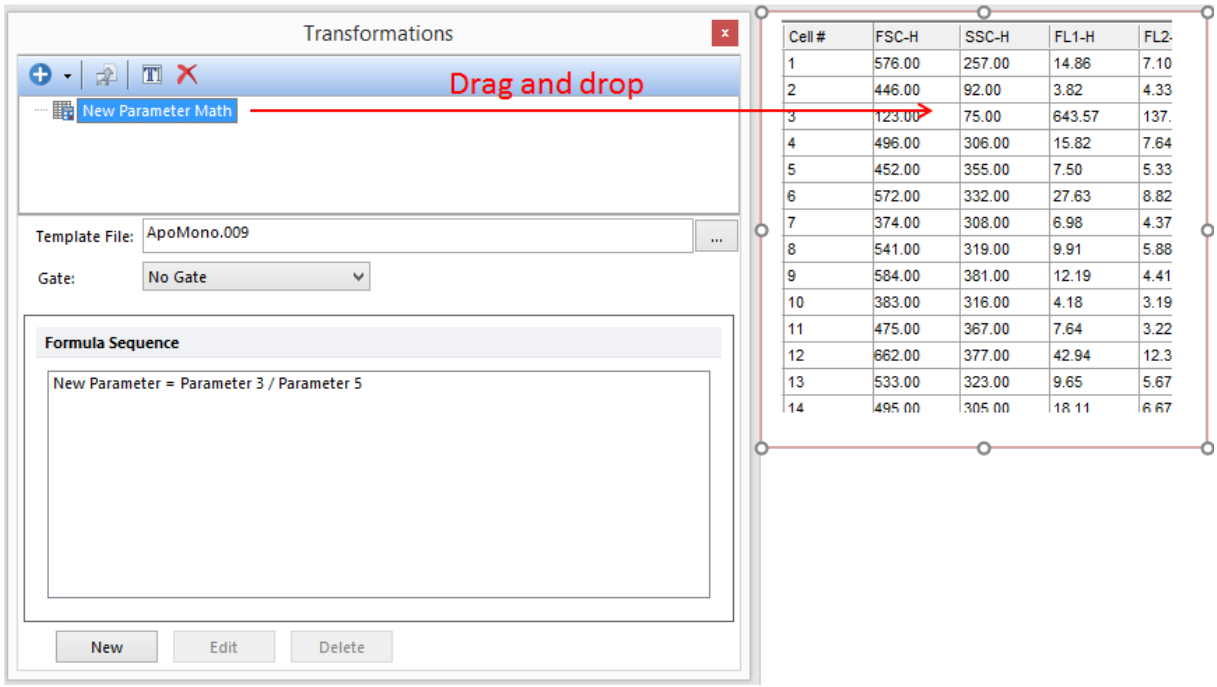

Figure 18.10 - Applying parameter math transformation to a plot by dragging from the Transformations navigator to the data grid (red arrow).

- Right-click data grid→**Show Transformed Values**→select appropriate transformation from menu.(Figure 18.11).

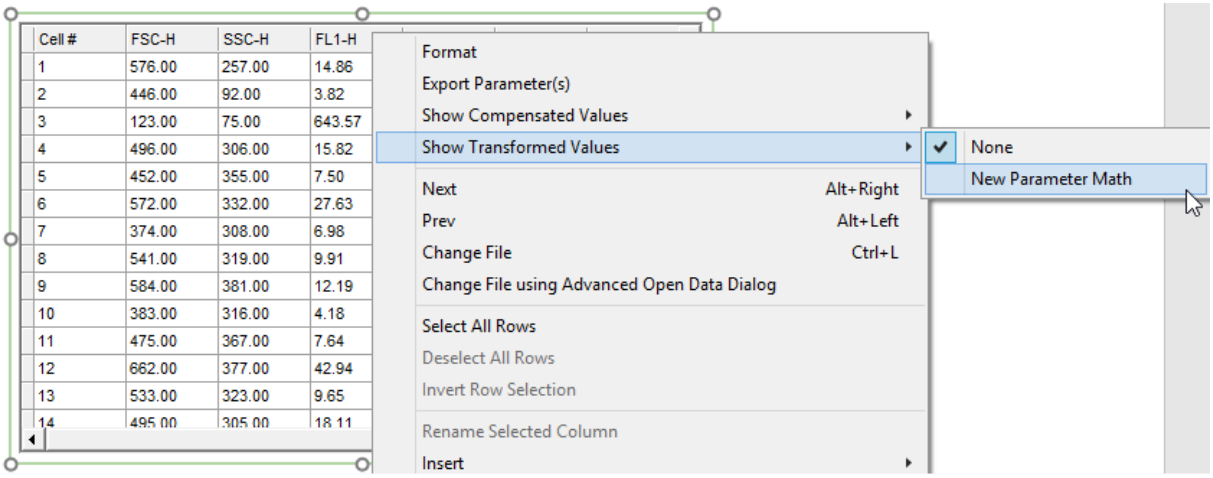

Figure 18.11 - Accessing parameter math transformation by right-clicking data grid and navigating to Show Transformed Values menu.

- Access Formatting dialog for general options 310→**Data Options**→select parameter math to use from **Transformation** drop down list→**OK**. (Figure 18.12).

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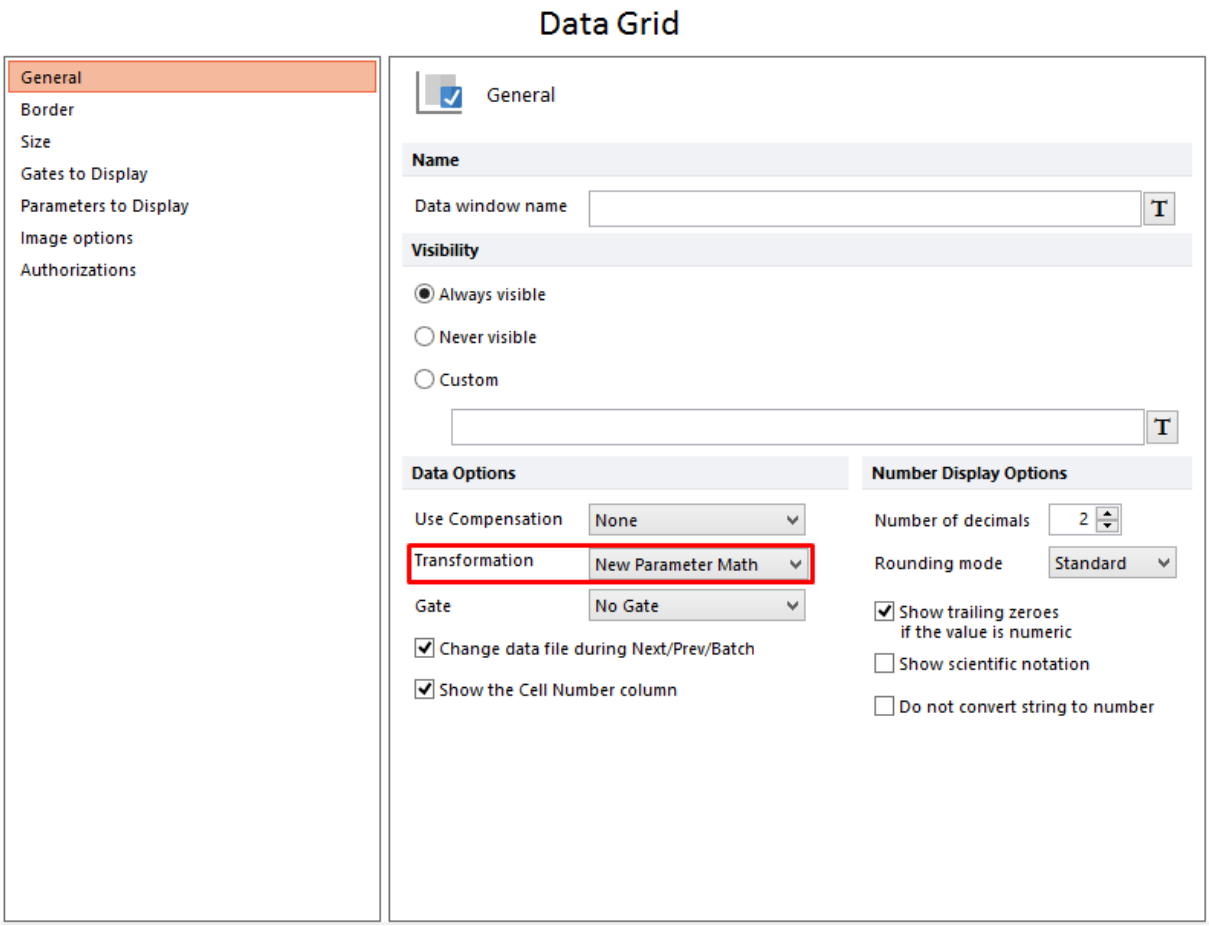

Figure 18.12 - Applying parameter math transformation to a data grid by selecting the parameter math in the Transformation drop down from the Formatting Overlays dialog.

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Chapter

19 Using Tokens

FCS Express allows you to insert special items, called Tokens, into a Text Box. Tokens appear as text, but are dynamically evaluated and can display information such as plot statistics, FCS file keywords, layout properties, and system information. For example, tokens can be used to display the number of pages in a layout, the percentage of events in a quadrant, and the instrument used to acquire data.

Once a token is placed in a Text Box, the displayed information acts like any other text and can be formatted like normal text. Simply select the token text and format it using the appropriate commands in the **Text** Tab, or, right-click on the selected token text and choose the **Format Token** option from the pop-up menu.

Tokens are evaluated automatically, which means that whenever the underlying information changes, the text that the token displays will change accordingly.

19.1 Inserting Tokens

To insert a Token in a Text Box (or any other edit field that accepts tokens), use either of the following methods:

- Right-click in the body of the text box and select **Insert Token** from the pop-up menu (or use the **Text→Insert Text→Insert Token** command). The **Insert a Token** dialog will appear (Fig. 19.1):

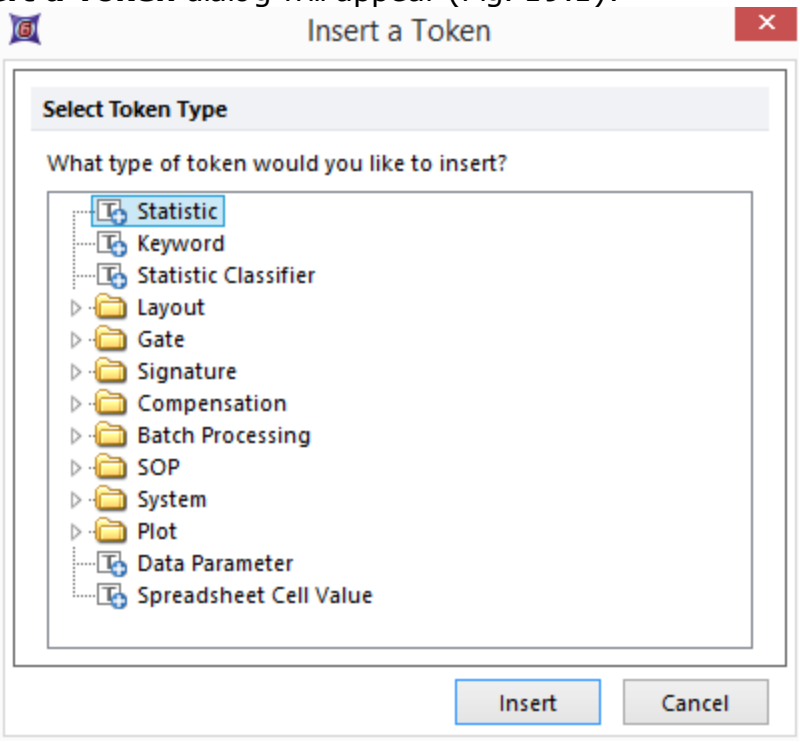

Figure 19.1 - Insert a Token dialog

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- Drag and drop a plot into the text box. The **Paste Special** dialog will appear. Choose **Statistic token** or **Keyword token** (Fig. 19.2). The **Edit Statistic** or **Edit Keyword** dialog box will appear, from which token(s) can be selected for insertion. Multiple statistics may be chosen and inserted at the same time by multiple selecting statistics from the Statistic category.
    - Multiple plots may also be selected and dropped into a text box. You will be prompted by the Paste Special dialog separately for each plot you have selected.

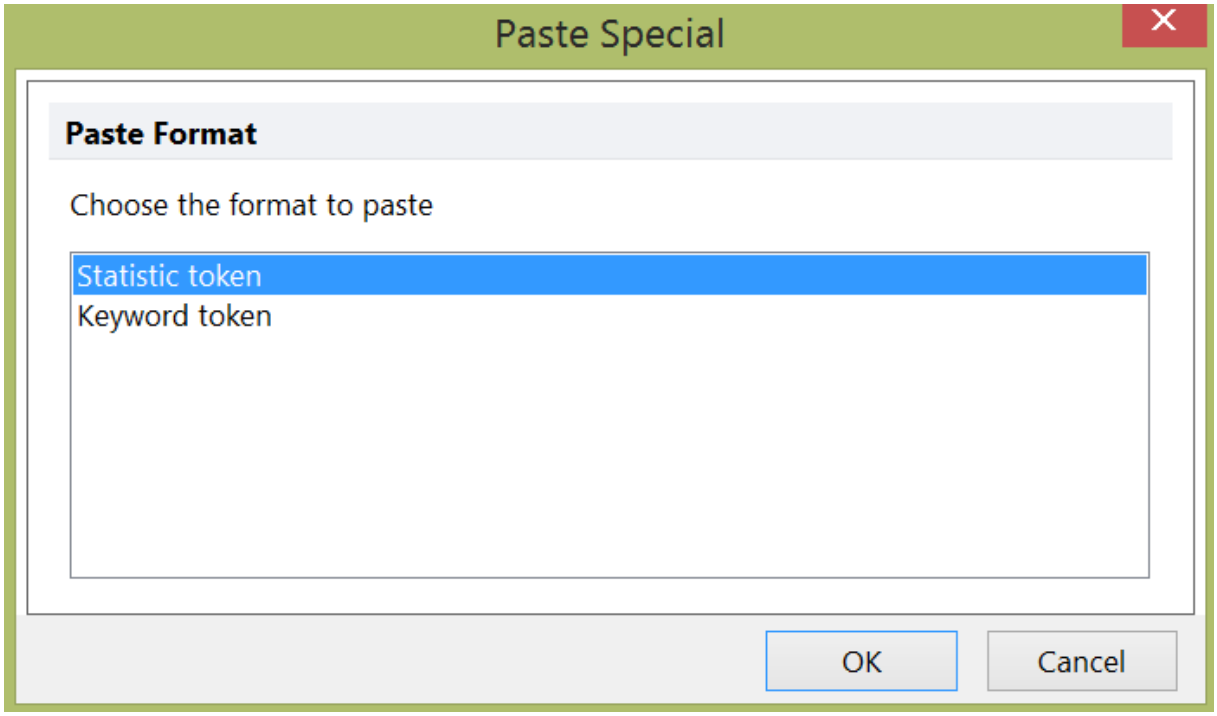

Figure 19.2 - Paste Special dialog

- Drag and drop a gate into the text box. The **Edit Statistic** dialog box will appear, from which token(s) can be selected for insertion. To select multiple items, press **Ctrl** or SHIFT on the keyboard.

- Drag and drop a default statistics into the text box. With a default gate or histogram statistics window open you can drag and drop individual statistics or hold down shift while clicking on different statistics to multiple select them. One or many statistics may be inserted into a new text box in this way.
    - When inserting a token into a text box embedded within a plot, you may select the plot on which the token is on or from a different plot listed for the **Data Source** category (Figure 19.3 below).

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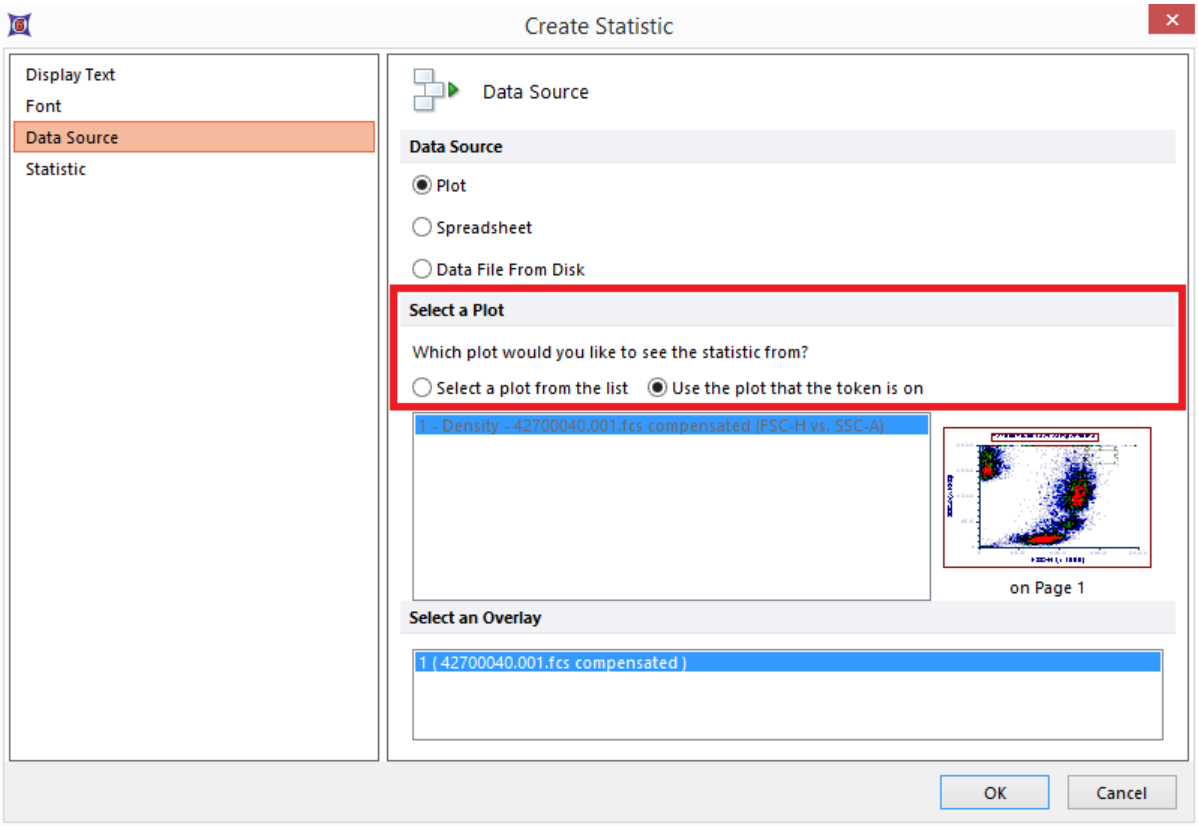

Figure 19.3 - Create Statistic dialog displaying options outline in red for selecting plot as source for token to be inserted.

There are many kinds of tokens that can be inserted:

- **Statistic** 703

  Displays plot, quadrant, and gate statistics. The value of a statistic token updates automatically, whenever a change pertaining to that statistic is made.

- **Keyword** 708

  Displays the information which is stored in the FCS file header. Each keyword contains a different piece of information, i.e., acquisition date, number of events, etc.

- **Statistic Classifier**

  Allows you to classify a statistic in terms of text, i.e., positive or negative cells (see Statistic Classifier Tokens).

- **Layout** 709

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Displays information related to the layout, including the number of pages, the layout creation date, compensation settings, batch processing, and gate information.

- **Gate** 712

Displays information related to gate information, such as the gate name, comments, link description, gate points, gate wells, combination formula or gate color.

- **Signature** 715

Displays information related to signatures such as Name, Description, Status, Data, Time, Signer, and Stamping Notes

- **Compensation** 715

Displays information for the Spill Over or Compensation Matrices.

- **Batch Processing** 715

Displays information related to batch processing including the Current Iteration, Number of Iterations, and Start time of the current batch process.

- **SOP (Standard Operating Procedure)** 716

Displays information related to SOP steps.

- **System** 717

Displays information related to the current user and other system-related data.

- **Plot** 719

Displays text related to the Plot Information, such as:

1. Title
2. Footer
3. X axis label
4. Y axis label
5. Plot Number
6. Plot Description
7. Whether the plot is using channel calibration?
8. Is channel calibration applied on the X and/or Y axis?

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This token can also display information as it relates to the *Center, Top, Right, Bottom*, or *Left* Quad Vertices in the plot.

- **Data Parameter** 720

Provides a shortcut to a column of data in a data set. Normally used for evaluating numeric data from a parameter in a custom token.

- **Spreadsheet Cell Value** 723

Displays information from the specified cell address in the user-selected spreadsheet on the layout.

- **Custom Tokens** 727

These are tokens, which can contain user-defined formulas, that are created from the Custom Token window 728. A custom token formula can contain arithmetic operations, such as division and multiplication, and conditional operations, such as IF … THEN … ELSE. See Custom Tokens 727 for a more detailed explanation. *Note: Since a custom token must be created before it can be inserted into a text box, the Custom node will not appear in the Insert a Token dialog until one or more custom tokens have been created.*

To insert a token, select the kind of token you want and then click **Insert**. Alternatively, you can double-click on the token you want to insert. After you **Insert**, a dialog box may appear asking you to set the properties for that particular token. The token properties are described under Common Token Properties 696.

19.2 Common Token Properties

The **Display Text** token property is shared by all Tokens, whereas the **Data Source** token property is only common to **Statistic** and **Keyword** tokens. These two properties will be described in the following sections.

19.2.1 Display Text Token Property

When you insert or edit a Token in a text box, you have the option to select what text will be displayed by changing the **Display Text** property. All tokens can display one of the following three items:

- **Token Value**

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The token value is the default setting when a token is inserted into a text box. The token will display the current evaluated information for the selected statistic, keyword, etc. For example:

- The "number of pages on the layout" token would display the number "2" if the layout consisted of 2 pages. If a page was added, the number displayed would change to "3".
- The token value representing the number of events on a particular plot would display "3043" in the text box if there were 3043 events in that plot.
- The token value for the data collection date (e.g., for an .fcs file on the layout) would display "January 1, 2005" if the data was collected on the first day of 2005.

- **Token Description**

  Displays the description of the token rather than the value. This is the default setting when a token is used as part of a formula, for instance when creating custom token 727. For example:

  - The "number of pages on the layout" token would display the description "Number of Pages".
  - The "number of events" token for the first overlay on the second plot would display the description "Statistic: # of Events for Plot #2, overlay #1".

- **Text**

  Displays user-defined text for the token. For example:

  - If you enter the text "apple" then the token will display "apple" in the text box.
  - If you enter the text "My first draft" then the token will display "My first draft" in the text box.

The following example will demonstrate how to change the **Display Text** property:

1. Follow steps 1 – 5 from the example given in Inserting a Page Layout Token 710.
2. Double-click on the highlighted "1", which is the token value for the number of pages token you inserted. The **Edit Number of Pages** dialog will appear (Figure 19.4). Note that the three options are listed with the **Token Value** selected by default.

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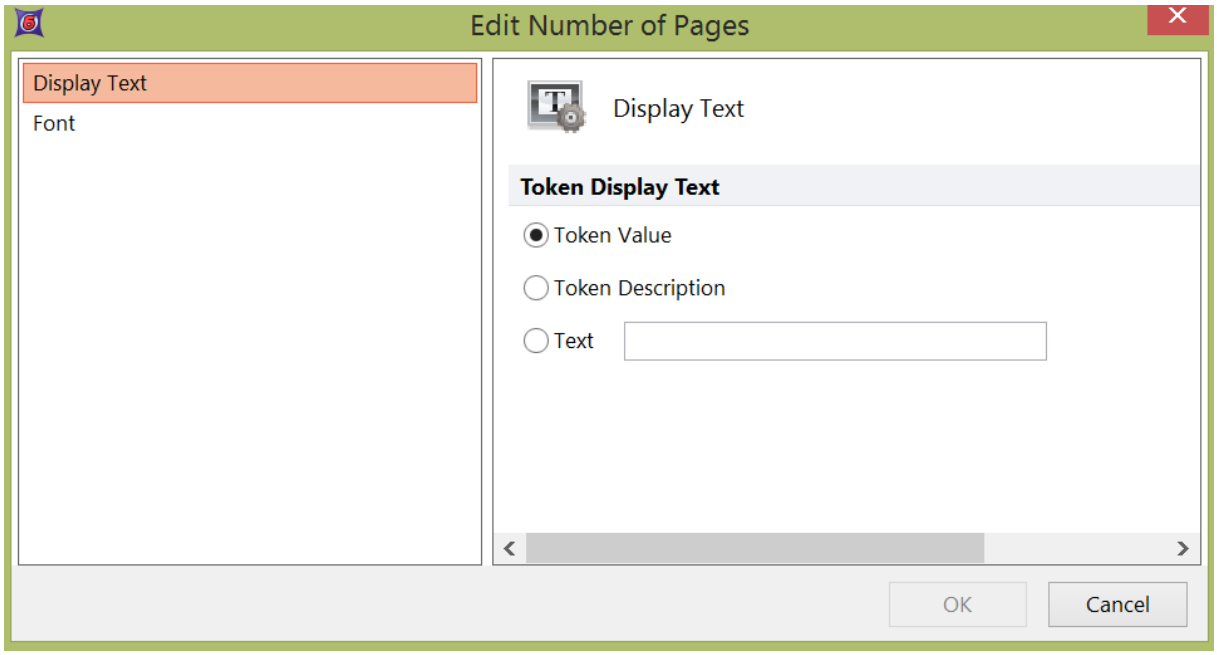

Figure 19.4 Edit Number of Pages Dialog

3. Choose the **Token Description** radio button and then click **OK**. The token now displays the token description, "Number of Pages" as shown in Figure 19.5 rather than the token value "1".

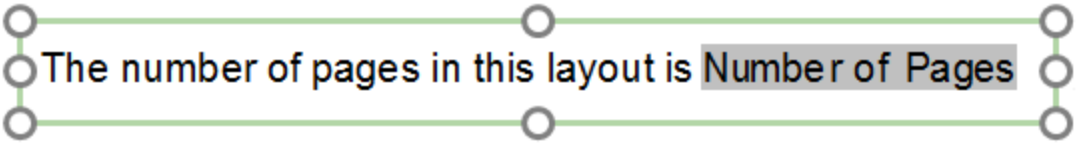

Figure 19.5 Display Text - Token Description

4. Double-click on the highlighted "Number of Pages" token to return to the **Edit Number of Pages** dialog.
5. Click on the **Text** radio button, and then enter "# of Pages on my Layout" in the edit field.
6. Click **OK**.

The token now displays the text just entered on the previous screen (Figure 19.6).

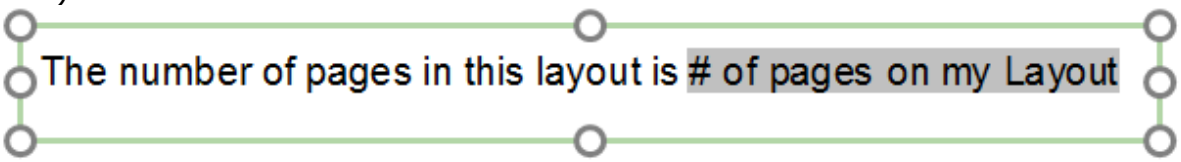

Figure 19.6 Display Text - User Defined Text

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The Display Text option can also be changed by right-clicking on the text box token and changing the Display Text option from the pop-up menu (Figure 19.7). The user-defined **Text** option is primarily useful when creating custom tokens 727.

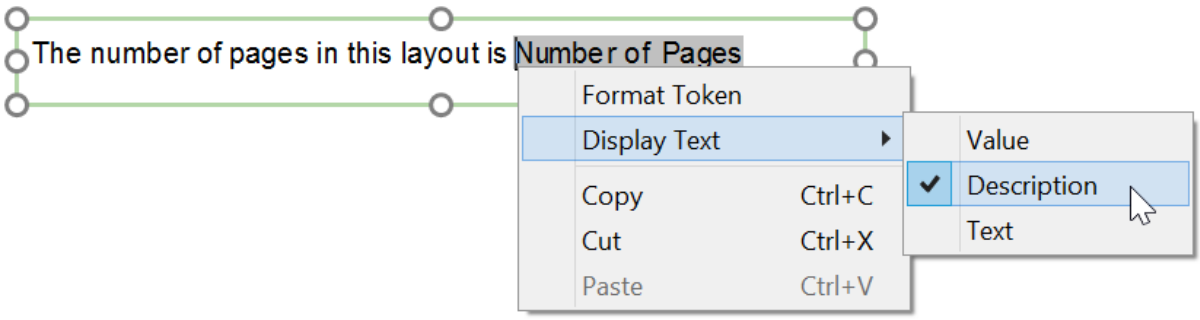

Figure 19.7 Display Text Pop-up Menu

19.2.2 Data Source Token Property

The **Data Source** token property is available only for **Statistic** and **Keyword** tokens. Many of these tokens are used to obtain information from an .fcs data file or from a plot on the layout. As the data changes in a layout, the token information will update as well.

Keyword tokens display .fcs file keyword information and **Statistic** tokens display statistics calculated from the data. Each .fcs file contains multiple keywords which can also be viewed from the View Header window 650.

The **Data Source** token property is used to define where a token gets its information. A token can obtain information directly from an .fcs file or from the data that is being displayed on a plot. If the data source is a plot, then the token will update whenever the data on that plot changes.

To configure a **Data Source** token property for a **Statistic** token using an .fcs data file:

1. Create a new layout 92.
2. Insert a Text Box 152 on the page and type in "The number of events in my data file are: " (with a <space> after colon). Make sure that the flashing cursor is located after the <space>.
3. Right-click near the flashing cursor in the text box, and select **Insert Token** from the pop-up menu.
4. Choose the **Statistic** token and then click the **Insert** button. The **Create Statistic** dialog will appear. If there are no plots on the layout (as in this example), choose the **Data File from Disk** radio button (Figure 19.8).

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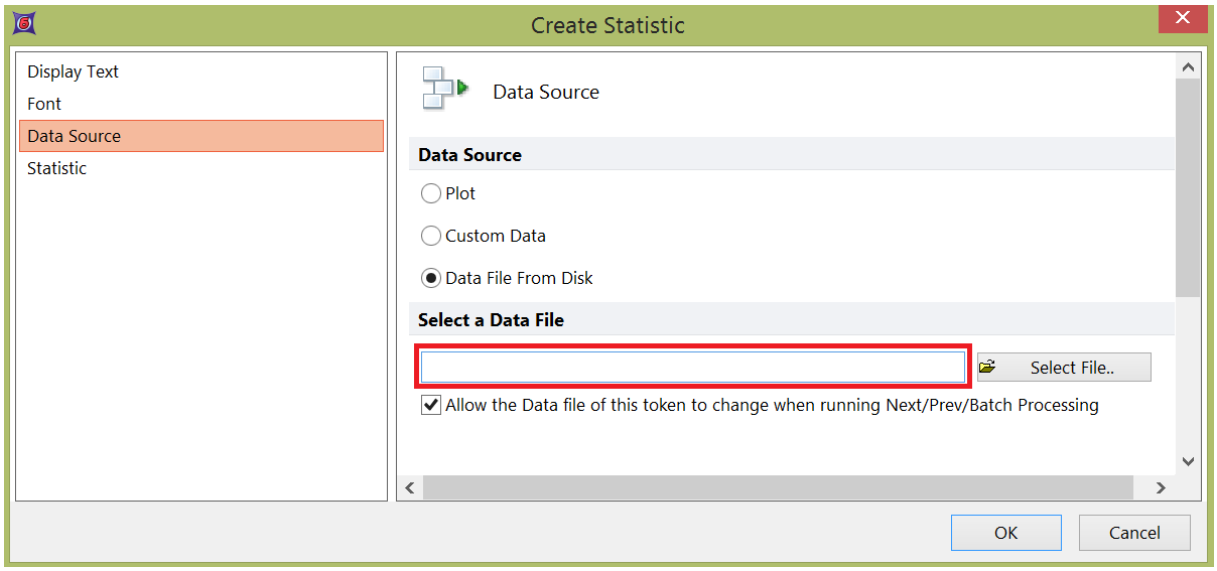

Figure 19.8 Create Statistic Dialog - Data Source - Data File from Disk

5. Click on the **Select Data File** button to locate an .fcs file, which will cause the **Select Data File** 214 dialog to appear if **Standard Open Data Dialog** is chosen under user options or **Open Data File** 215 dialog to appear if **Advanced Open Data Dialog** is chosen under user options. When you have chosen an .fcs file, the filename will appear in the **Select a Data File** field (outlined in red in above figure.)
6. Leave the default option: **Change data file during Next/Prev/Batch** enabled, so that the token information will update when the **Data Source** changes.

Note: Instead of performing **Steps 3 to 6**, you can also drag a file from the Data List 785 and drop it near the flashing cursor in the text box then directly select **Statistics** from the **Paste Special** dialog. By clicking **OK** you will be directly prompted with Step 7 below. You may still go to the **Data Source** section to change any options if you choose.

7. Click on **Statistic** from the list of token properties on the top-left of the **Create Statistic** dialog.
8. Select a **Parameter** from the drop down for the statistic.
9. Select a compensation or choose **Default** or **None** from the **Compensation** drop down.

Note: by setting **Default** from the **Compensation** drop down, the default option for compensation 645 specified in the **User Options** will be used.

10. Choose a gate from the drop-down list if any gates exist on the plot. The default is **No Gate** which will use all events on the plot.
11. Select one, or multiple select, a Statistic option, such as **# of Events** (Figure 19.9).

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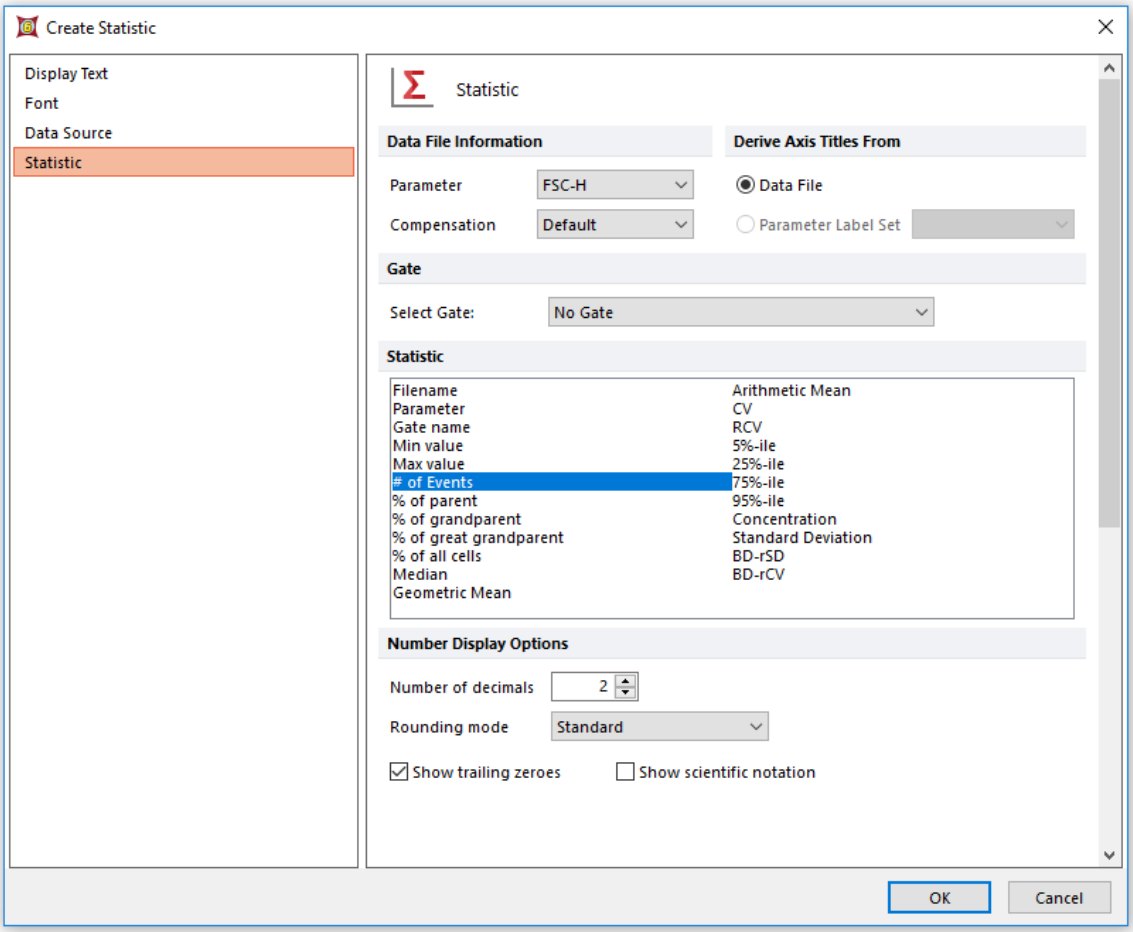

Figure 19.9 Create Statistic Dialog - Statistic Page

9. Click **OK**. The text box will display the text entered in step 2 followed by the statistical data from step 7 for the .fcs file selected in step 5.

To configure a **Data Source** token property for a **Statistic** token using a plot:

1. Open an existing layout 95, or create a new one 92.
2. Insert a 2D Dot Plot 172 on the page if there are none.
3. Insert a Text Box 152 on the page.
4. Right-click on the text box, and select **Insert Token** from the pop-up menu.
5. Select **Statistic**, and then click the **Insert** button. The **Data Source** page of the **Create Statistic** dialog will appear with **Plot** selected as the default **Data Source** type (Figure 19.10).

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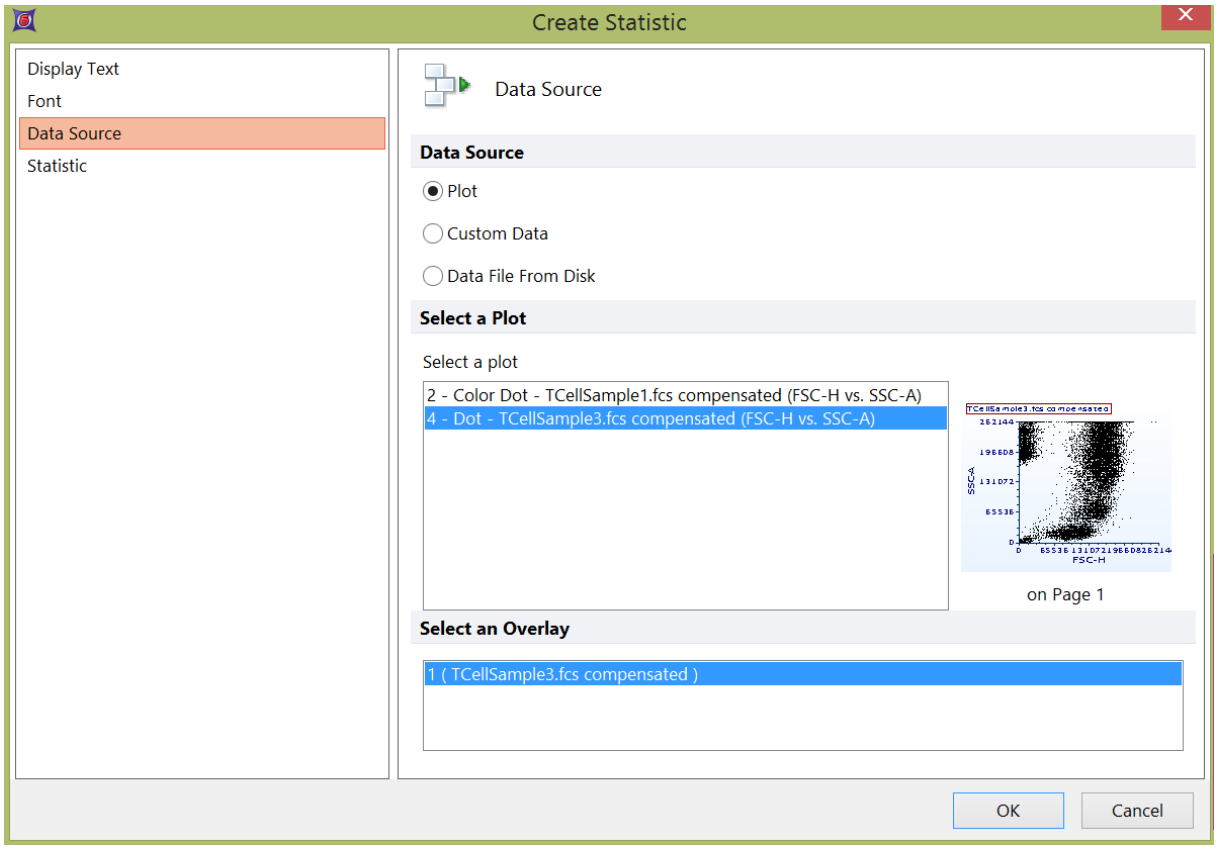

Figure 19.10 Create Statistic Dialog - Data Source Page

6. Select a plot from the **Select a plot** field, which lists the plots on the layout.
   Note: If multiple overlays exist on the selected plot, the desired overlay must be selected from the **Select an Overlay** field appearing below the Select a plot field.
7. Click on the **Statistic** category on the left.
8. Select a Statistic from the **Statistic** list, such as **# of Events**.
9. Click **OK**.

The **Create Statistic** dialog closes, and you will see that the text box now displays the number of events in the plot.

Keyword tokens work in the same way as **Statistics** tokens, with the following exceptions:

- To insert a keyword token, select **Keyword** after selecting **Insert Token** in Step 4 above.

- In the **Create Keyword** dialog, there will be a **Keyword** category on the left side of the window. After you select that category, you will be able to

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type in a keyword, or select one from the list of default keywords (see Editing Keyword Tokens 708).

19.3 Token Descriptions and Editing Tokens

Each of the different categories of tokens contains a number of properties which can be edited.

Jump to:

- Editing Statistic Tokens 703
- 2D Plot Statistic Token Options 703
- Histogram Statistic Token Options 706
- Editing Keyword Tokens 708
- Statistic Classifier Tokens
- Layout Tokens 709
- Inserting a Page Layout Token 710
- Gate Tokens 712
- Signature Tokens 715
- Compensation Tokens 715
- Batch Processing Tokens 715
- SOP Tokens 716
- System Tokens 717
- Plot Tokens 719
- Data Parameter Tokens 720
- Spreadsheet Cell Value Tokens 723

19.3.1 Editing Statistic Tokens

To insert or edit a **Statistic** token, the following properties need to be set:

- Display Text 696 to select the token value, description, or user-defined text.
- Data Source 699 to select the plot or FCS file as a source of data for the token.

Note: The **Statistic** page of the **Create Statistic** dialog is used to choose the statistic to display in a statistic token. Because the **Statistic** property options differ depending on the **Data Source**, you must select the **Data Source** first. The **Data Source** page is the default page shown when you select **Statistic** from the **Insert a Token** dialog.

19.3.1.1 2D Plot Statistic Token Options

If a 2D Plot is the Plot type chosen as a **Data Source**, the following properties can be set (Figure 19.11).

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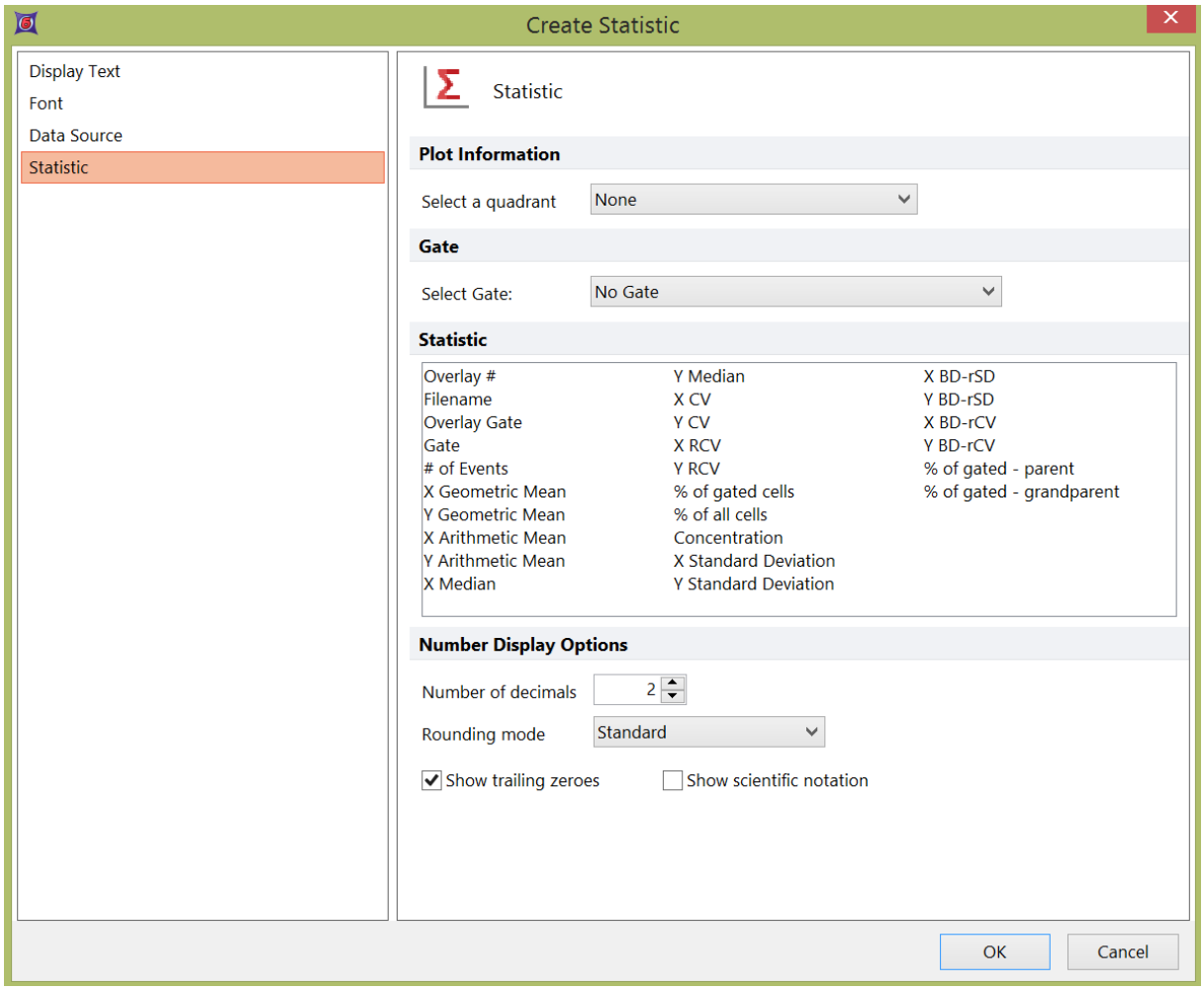

Figure 19.11 Statistic Options - 2D Plot as Data Source

- **Plot Information**

  Select a quadrant from the drop-down list to use as a basis for your statistic. The default selection is **None** which will use all events on the plot.

- **Gate**

  Choose a gate from the drop-down list if any gates exist on the plot. The default is **No Gate** which will use all events on the plot.

- **Statistic**

  Select the statistic to display. Multiple statistics may be selected to inserted together. See 2D Plot Statistics 385 for descriptions of each.

- **Number Display Options**

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Select the number of decimals to be displayed for this statistic and the rounding mode for this statistic. Rounding mode options include **Standard**, **Down**, **Up**, and **Truncate**. The default value is 2 decimal places. You can also choose whether or not to display trailing zeroes.

- Show scientific notation allows you to display the statistic in the form of **X.XXE+Y**

It is also possible to insert or edit a **Statistic** token with an .fcs file as a **Data Source**, though if the desired file is showing on your layout, it is preferable to use a Plot as the Data Source as described above. The following properties need to be set when an .fcs file is the Data Source (Figure 19.12):

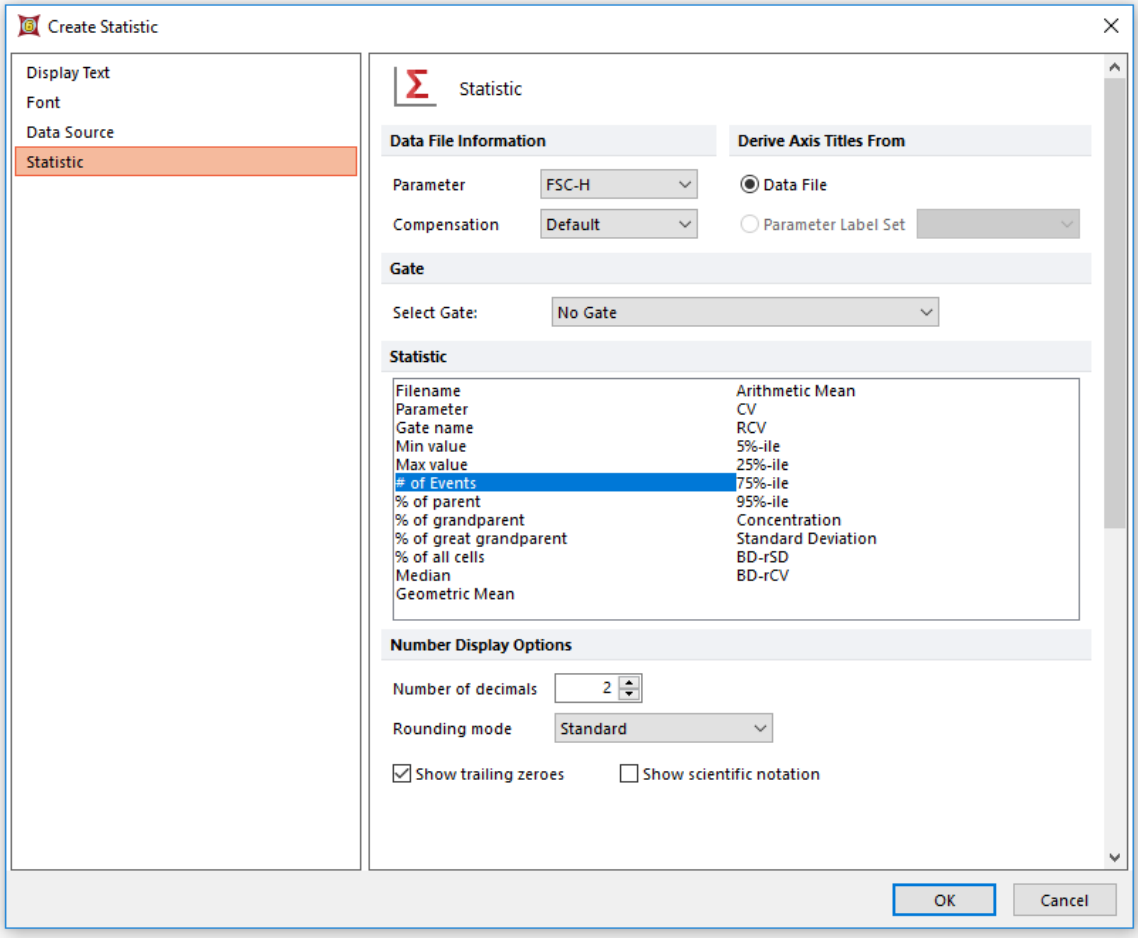

Figure 19.12 Statistic Options - FCS File as a Data Source

- **Data File information**

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- **Parameter**

  Select the parameter data to use from the drop-down list.

- **Compensation**

  Select a compensation definition 627 for the data or choose **Default** or **None** from the **Compensation** drop down.
  Note: By setting **Default** from the **Compensation** drop down, the default option for compensation 645 specified in the **User Options** will be used.

- **Derive Axis Titles From**

  Choose from the **Data File** axis titles, or axis titles from a predefined Parameter Label Set 410.

- **Gate**

  Choose a gate from the drop-down list for calculating the statistics on gated events.

- **Statistic**

  Select the statistic to evaluate from the list box.

- **Number Display Options**

  Select the number of decimals to be displayed for this statistic, the Rounding Mode and whether or not to show trailing zeroes. The options for the Rounding Mode include **Standard**, **Down**, **Up**, and **Truncate**. The default is 2 decimal places.

  - Show scientific notation allows you to display the statistic in the form of **X.XXE+Y**

19.3.1.2 Histogram Statistic Token Options

If a histogram is the Plot type chosen for a Data Source of a Statistic Token, the following properties can be set (Figure 19.13).

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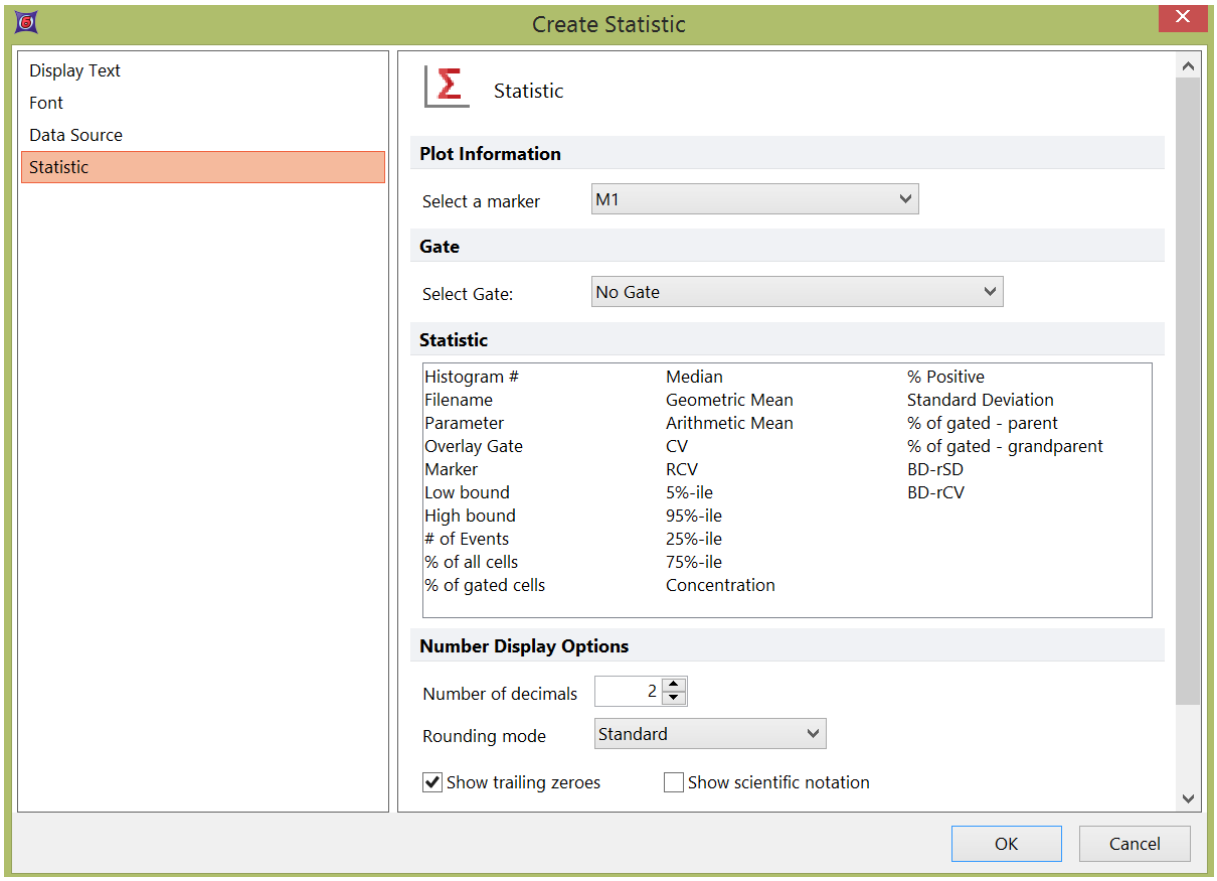

Figure 19.13 Statistic Options - Histogram as Data Source

- **Plot Information**

  Select a marker from the drop-down list to use as a basis for your statistic. The default selection is **None** which will use all events on the plot.

- **Gate**

  Choose a gate from the drop-down list (if the histogram data is based on a gate from a 2D plot). The default is **No Gate**.

- **Statistic**

  Select the statistic to display. See Histogram Statistics 382 for descriptions of each.

- **Number Display Options**

  Select the number of decimals to be displayed for this statistic and the rounding mode to be used for this statistic. The options include **Standard**, **Down**, **Up**, and **Truncate**. The default value is 2 decimal places. You can also choose whether or not to display trailing zeroes.

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- Show scientific notation allows you to display the statistic in the form of **X.XXE+Y**

19.3.2 Editing Keyword Tokens

When you insert or edit a **Keyword** token, the following properties need to be set:

- Use the Display Text 696 category of the **Create Keyword** dialog to choose a token value (default), description, or user-defined text to display in the token.
- Use the Data Source 699 category of the **Create Keyword** dialog to select the plot or FCS file as a source of data for the token.
- Use the **Keyword** page of the **Create Keyword** dialog to edit the keyword that will be displayed (Figure 19.14). *Note:* Because the Keyword options differ depending on the **Data Source**, you must select the **Data Source** before you can navigate to the **Keyword** page.

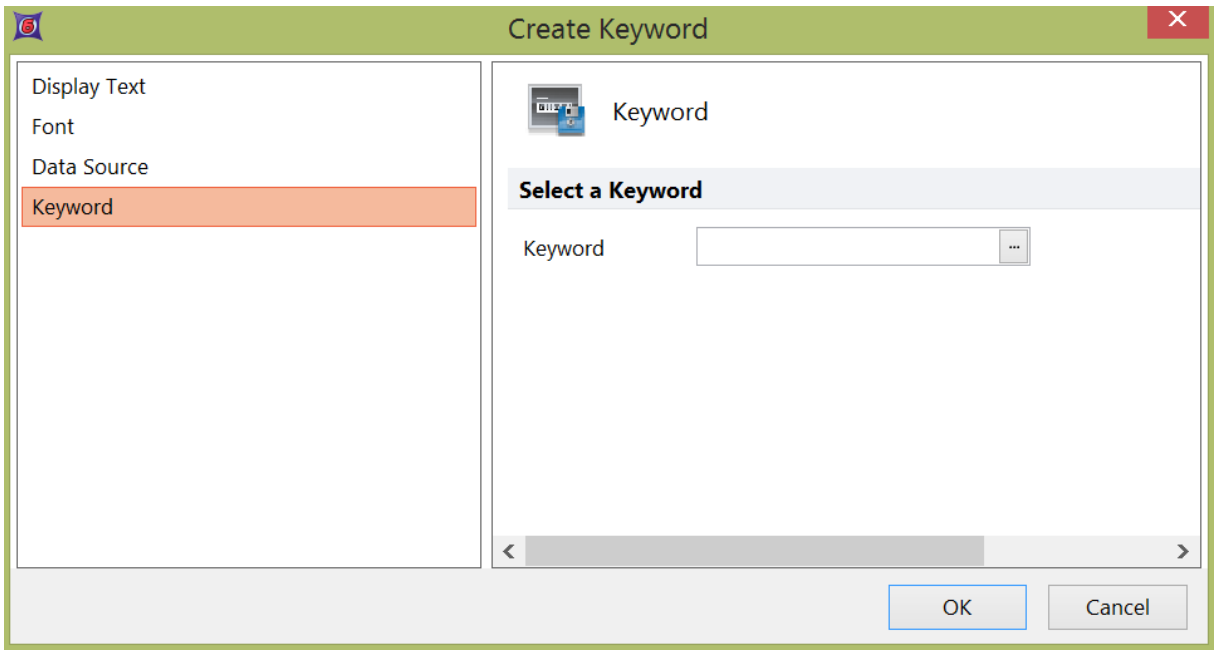

Figure 19.14 Keyword Properties

- Select a keyword. Click on the ellipsis to display the scrollable **Please select a keyword** dialog (Figure 19.<%19F++++%>). Click on the **Keyword**

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you want to display, and then click **OK** to return to the **Create Keyword** dialog. Click **OK** to complete the Keyword edit or insert procedure.

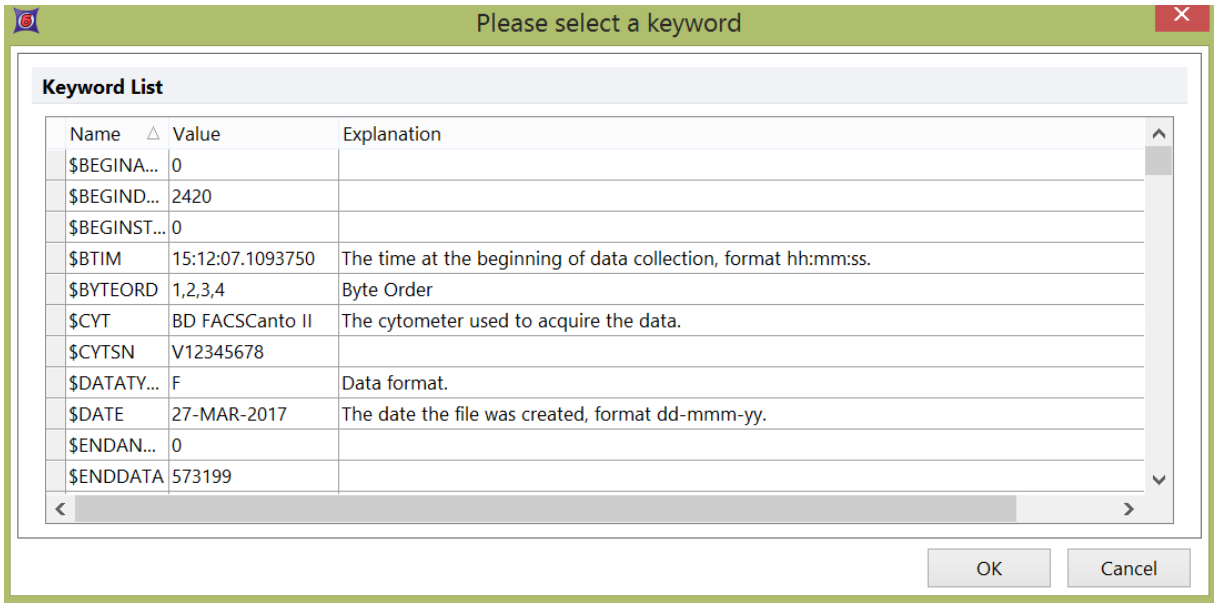

Figure 19.<%19C++++%> Select a Keyword

For more information regarding Keywords, please watch our YouTube video that goes into greater detail about what Keywords are and how to use them within your analysis layout.

19.3.3 Layout Tokens

Layout tokens display information related to the layout, e.g., the number of pages on the layout or the date the layout was created.

To insert Layout tokens directly into a Text Box:

1. Right-click on the Text Box.
2. Select **Insert Token** from the pop-up menu.
3. Select the desired **Layout** token.
4. Click the **Insert** button.

To edit the text format or display properties, right-click on the Layout token and select **Format Token** or one of the Display Text options from the pop-up menu. Or, double-click the Layout token to access the Edit dialog.

Layout Token	Definition
Number of Pages	The overall number of pages in the current layout.
Current Page Number	The page number of the layout page currently in use for the token.
Last Saved Time	The time at which the layout was last saved.

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Last Saved Date	The date on which the layout was last saved.
Created Time	The time at which the layout was created.
Created Date	The date on which the layout was created.
Number of Files in the File List	The total current number of data files in the Data List.
Current Print ID	Identification number used for the print job.
Print Date and Time	The date and time when the layout was last printed.
File Name	The file name of the current layout.
File Path	The file path specific for the current layout.
Current FCS File Path	The file pathway for the file which was last used in the layout.
Current User	The user name of who is currently logged into FCS Express.
Current Page Name	The name of the page that is currently being viewed.
Current User Security Groups	For users of the Security option, this token displays the name of the security group to which the user belongs.
Last Saved Program Version	The last version number of FCS Express used when saving the layout.
Last Saved Layout Version	The last layout version number used when saving the layout.
Last Loaded Channel Calibration File	The last file that was used when performing Channel Calibration 621.

Also see the Inserting a Page Layout Token example 710.

19.3.3.1 Inserting a Page Layout Token

The following is an example on inserting a Page Layout Token:

1. Create a new layout 92, or open an existing one.
2. Insert a Text Box 152 on the page and type in "The number of pages on this layout is " (with the <space> after the word "is").
3. Right-click near the flashing cursor in the text box, and select **Insert Token** from the pop-up menu. The **Insert a Token** dialog will appear (Figure 19.15).

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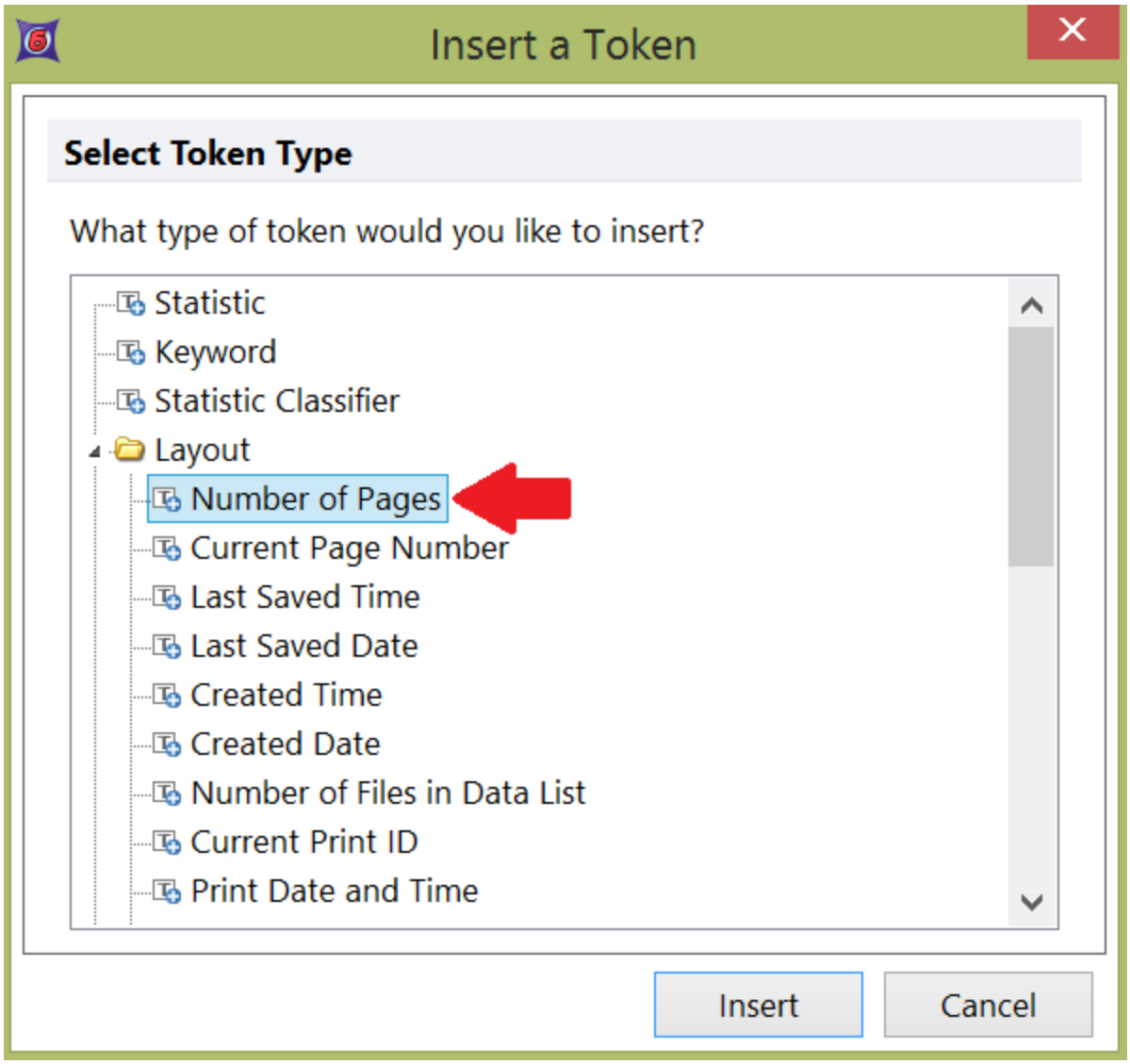

Figure 19.15 Insert a Token Dialog

4. Select the **Number of Pages** token (located under **Layout**) and then click the **Insert** button.
5. The text box will then look like Figure 19.16. Note how the background of the number 1 is gray. When the Text Box is in edit mode, this signifies that the text is actually a token rather than freetext.

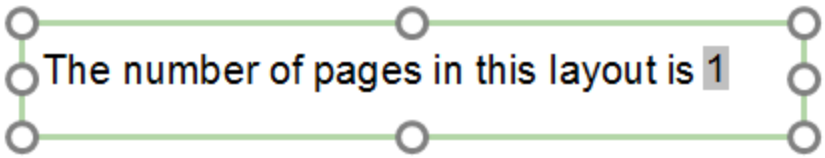

Figure 19.16 Number of Pages Token in a Text Box

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6. Insert a new page on the layout by using the **Insert** tab→**General**→**New Page**→**Blank** command.
7. The FCS Express window will display the new, empty page. Return to the page with the Text Box and Token by clicking on the **Page 1** tab, located at the bottom left of the layout screen. Since the text displayed in the token changes as the information it is displaying changes, the token text now displays "2" indicating the new number of pages (Figure 19.17).

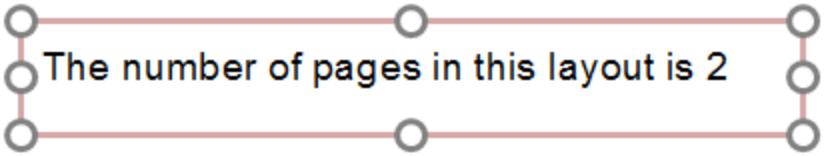

Figure 19.17 Token Automatically Updates

19.3.4 Gate Tokens

Gate tokens display information related to the gates on the Layout. To see the list of available gate tokens:

1. Right-click on a Text Box.
2. Select **Insert Token** from the pop-up menu.
3. Click on the triangle next to **Gate** on the **Insert a Token** dialog (Figure 19.18).

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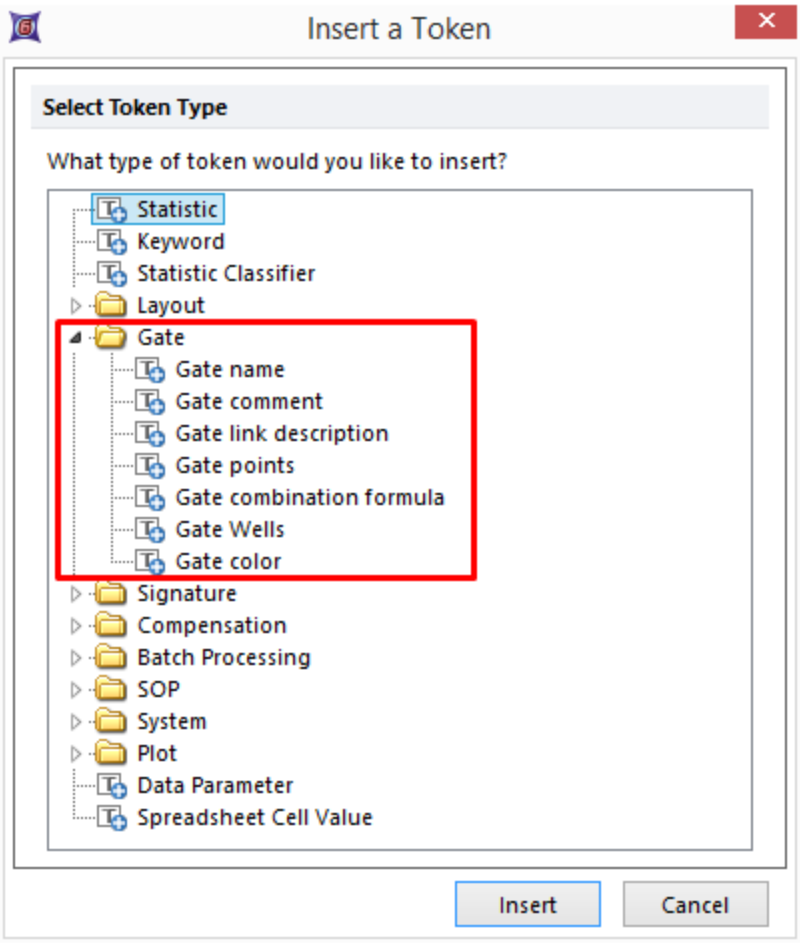

Figure 19.18 - Choose a Gate Token

4. Select a **Gate** token and click the **Insert** button. Or, double-click the **Gate** token.

When you insert or edit a **Gate** token, the **Create Gate Token** dialog appears.

Use the Display Text 696 page of the **Create Gate Token** dialog to choose a token value (default), description, or user-defined text to display in the token.

Use the **Gate information** page (Figure 19.19) to:

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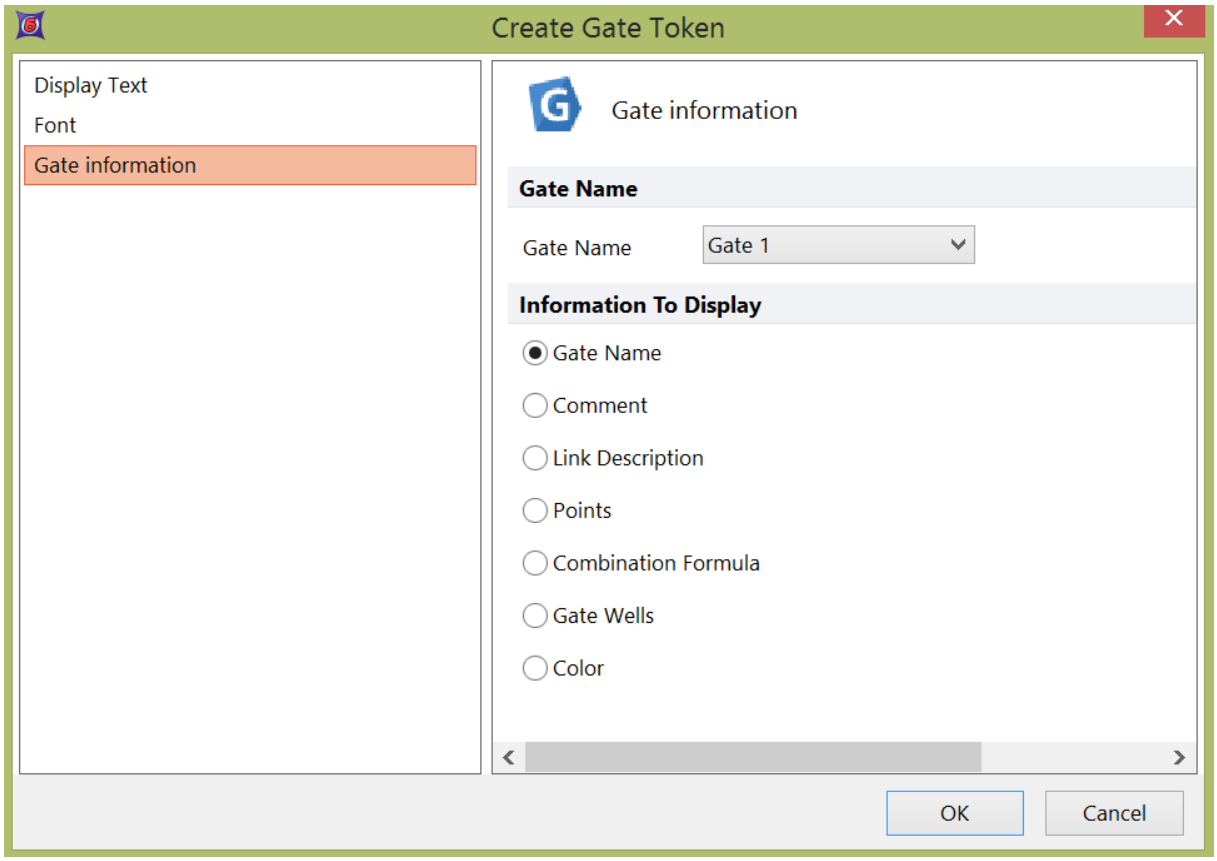

Figure 19.19 Gate Token Options

- Select the **Gate Name** (on which to base the token data) from the drop-down list.
- Choose one of the following options for which **Information to Display**:

Gate Token	Definition
Gate Name	This is the name that you specified when the gate was created. This name that is used to reference the gate throughout FCS Express.
Comment	This is text that you can specify when you are editing the gate from the Gate View 345.
Link Description	If the gate is linked to a quadrant or a marker, this is the information that describes that link.
Points	If the gate is one of the basic shapes (elliptical, rectangular, polygonal, or freeform) and not a combination gate, then this is the set of points that define it on the plot.
Combination Formula	If the gate is a combination gate, this is the formula that you used to create it.

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Gate Wells	If the Gate is a Well Gate, this information tells you which well, or wells, makes up the Gate.
Color	This token will describe the color used for the Gate.

19.3.5 Signature Tokens

Signature tokens are described in detail in the Displaying Signature Information 1093 chapter.

19.3.6 Compensation Tokens

Compensation tokens allow you to insert information pertaining to the current compensation 626 settings. You can choose different source and target parameters and display either the crossover or spillover matrix values (Figure 19.20).

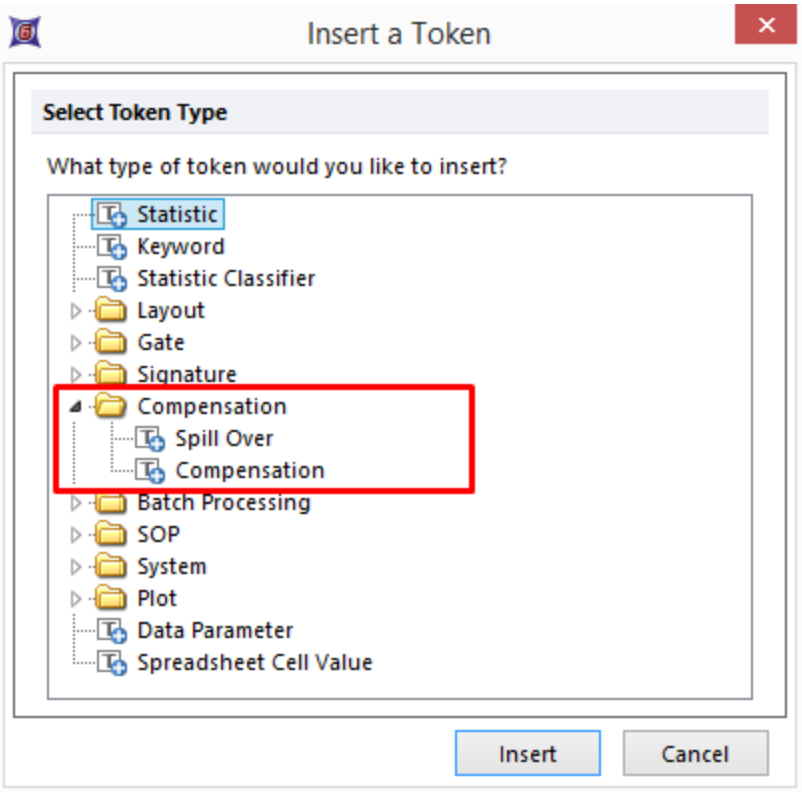

Figure 19.20 - Compensation Tokens

19.3.7 Batch Processing Tokens

Batch processing tokens allow you to insert information relating to the current state of the batch process 784 (Figure 19.21. These tokens are used to create dynamic file names that change as a batch process proceeds thru a collection of files (see Batch Processing - Save Layout 806).

The options include:

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Gate Token	Definition
Current Iteration	This is the iteration number of the current iteration that is being displayed within your plots during batch processing, as defined within the Data List.
Number of Iterations	This is the total number of iteration numbers, as defined within the Data List.
Start Time of the Current Batch Process	This will list the time at which batch processing commences. It will be listed in a hh:mm:ss AM/PM format.

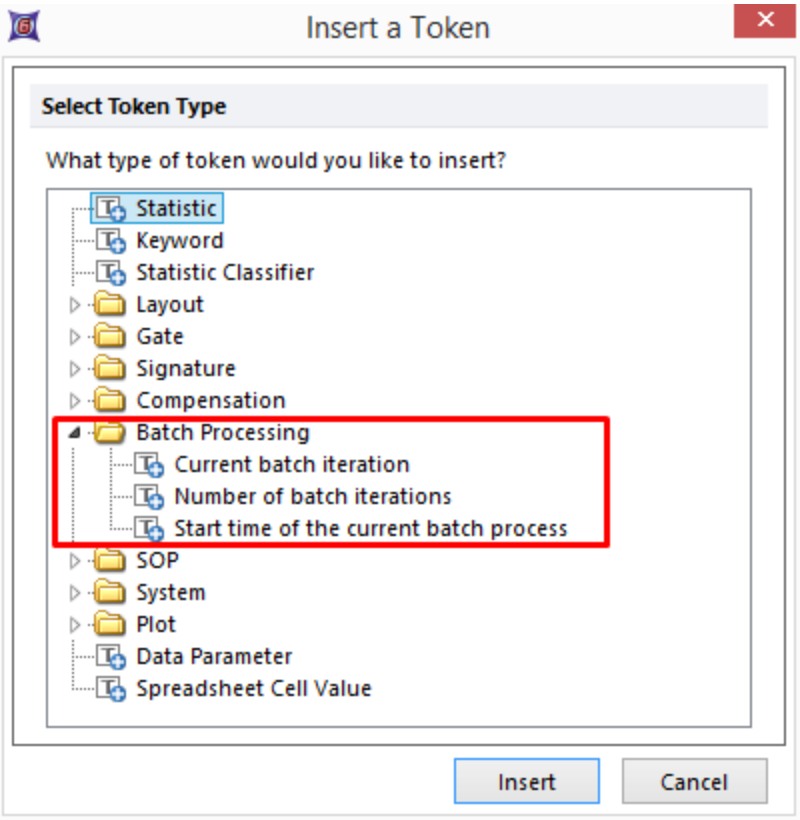

Figure 19.21 - Batch Processing Tokens

19.3.8 SOP Tokens

Standard Operating Procedure tokens are described in detail in the Displaying SOP Information 1113 chapter. See token list below (Figure 19.22).

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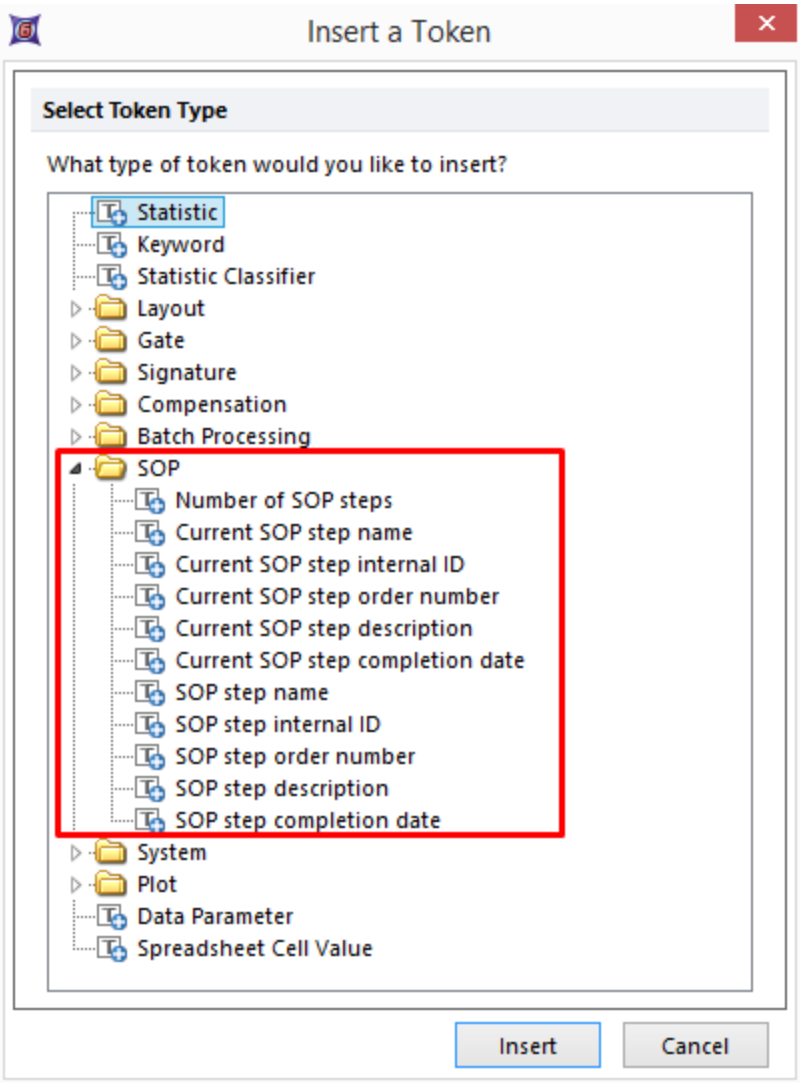

Figure 19.22 - SOP Token List

19.3.9 System Tokens

System tokens allow you to insert information pertaining to the current computer session. The current system tokens are listed in the table below.

To insert a **System** token:

1. Right-click on a Text Box.
2. Select **Insert Token** from the pop-up menu. Scroll the list box until you see the **System** category, if necessary.
3. Click on the triangle next to **System** (Figure 19.23).

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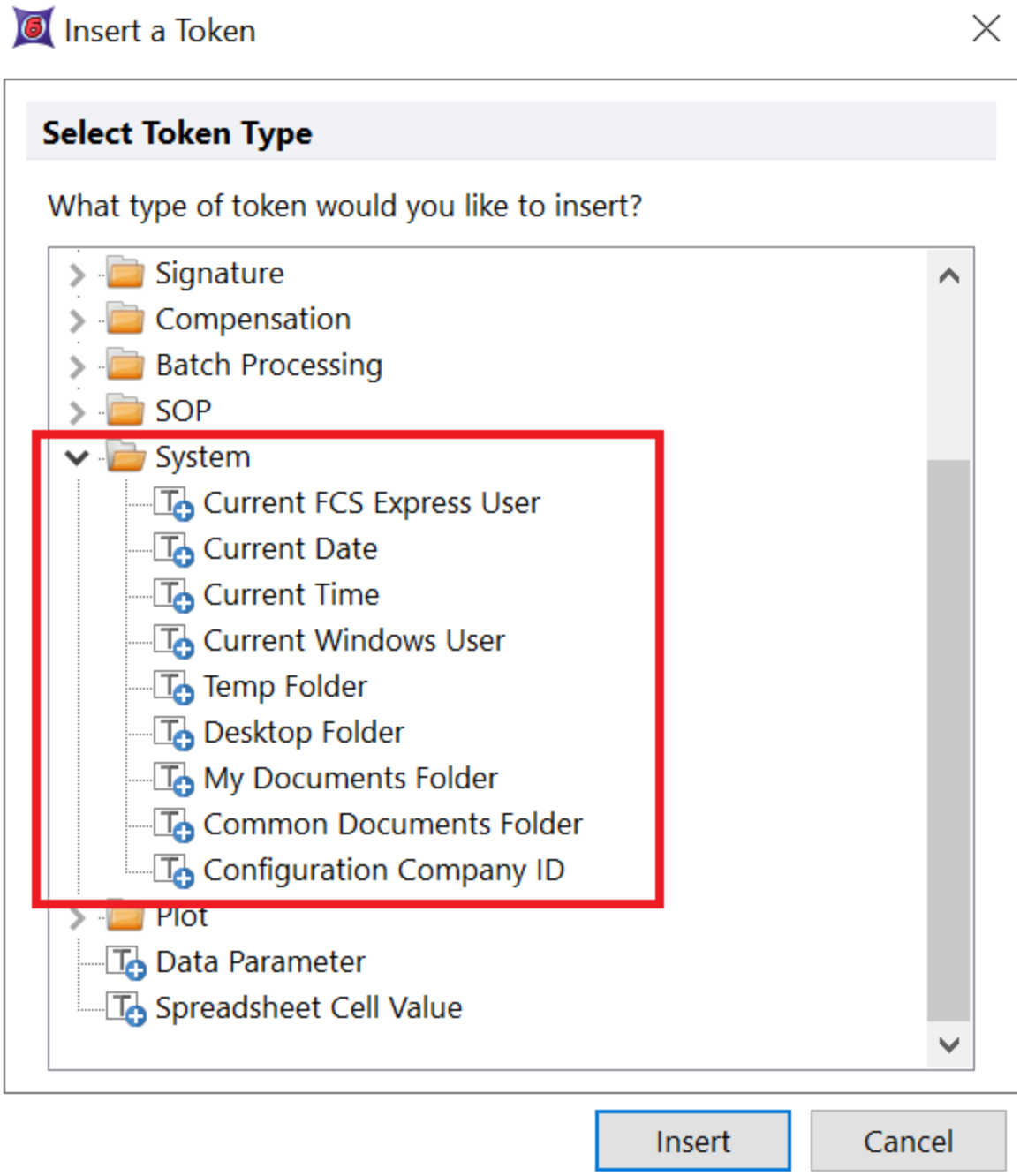

Figure 19.23 - System Tokens

4. Choose one of the **System** tokens and click the **Insert** button.

System Token	Definition
Current FCS Express User	The name of the user currently logged into FCS Express.

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Current Date	The current date as shown on the workstation.
Current Time	The current time as shown on the workstation.
Current Windows User	The user name for whoever is currently logged into Windows.
Temp Folder	The destination pathway for the Temporary folder.
Desktop Folder	The destination pathway for desktop folder of the user currently logged into FCS Express.
My Documents Folder	The destination pathway for the user's **Documents** folder.
Common Documents Folder	The destination pathway for the **Public Documents** folder.
Configuration Company ID	Identifier associated with license configuration file.

19.3.10 Plot Tokens

Plot Tokens allow you to insert information pertaining to a plot selected as Data Source 699. To insert a **Plot token**:

1. Right-click on a Text Box.
2. Select **Insert Token** from the pop-up menu.
3. Click on the triangle next to **Plot** (Figure 19.24).

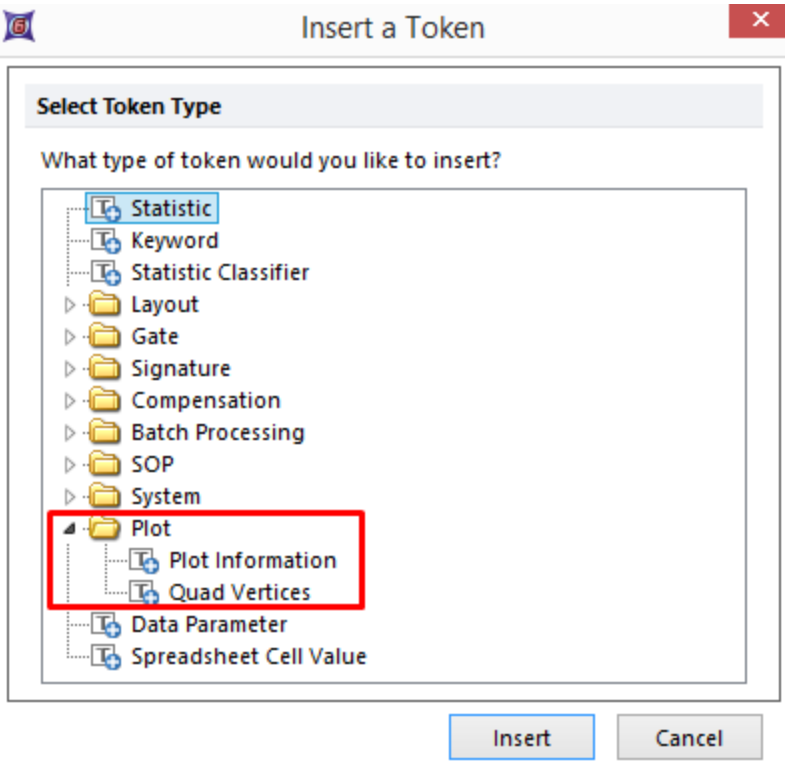

Figure 19.24 - Selecting Plot Token from the Select Token Type dialog

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4. Double-click on **Plot Information** or **Quad Vertices**.
5. Choose the desired plot from the **Select a Plot** list from the **Data Source** category of the **Edit Plot Information Token** dialog
6. Choose the desired token from the list in the Plot Elements 720 or Quad Vertices 720 category, and click **OK**.

Plot Elements

The available Plot Elements tokens are described below.

Plot Element	Definition
Title	The text within the plot title 250.
Footer	The text contained with the plot footer.
X Axis Label	The label used for the X axis within the plot.
Y Axis Label	The label used for the Y axis within the plot.
Plot Number	The number given to the plot by FCS Express as it is created.
Plot Description	An overall description of the plot, showing the Plot Number, Plot Type, the data file name being displayed within the plot, and the X and Y axis parameters for the selected plot.
Use Channel Calibration?	Has the Use Channel Calibration on Axes option been selected for that plot? The output is Off or On 620.
Is Channel Calibration Applied on X axis?	A description of whether or not channel calibration has been applied to the X axis.
Is Channel Calibration Applied on Y axis?	A description of whether or not channel calibration has been applied to the Y axis.

Quad Vertices

This token will display the X- and Y- coordinates for a selected vertex of a Quadrant 291.

The options are **Center**, **Top**, **Right**, **Bottom**, **Left**.

19.3.11 Data Parameter Tokens

Data Parameter tokens provide a shortcut to a column of data in a data set. **Data Parameter** tokens are normally used for evaluating numeric data from a parameter in a custom token, for instance, the mean value of a particular parameter (Figure 19.25).

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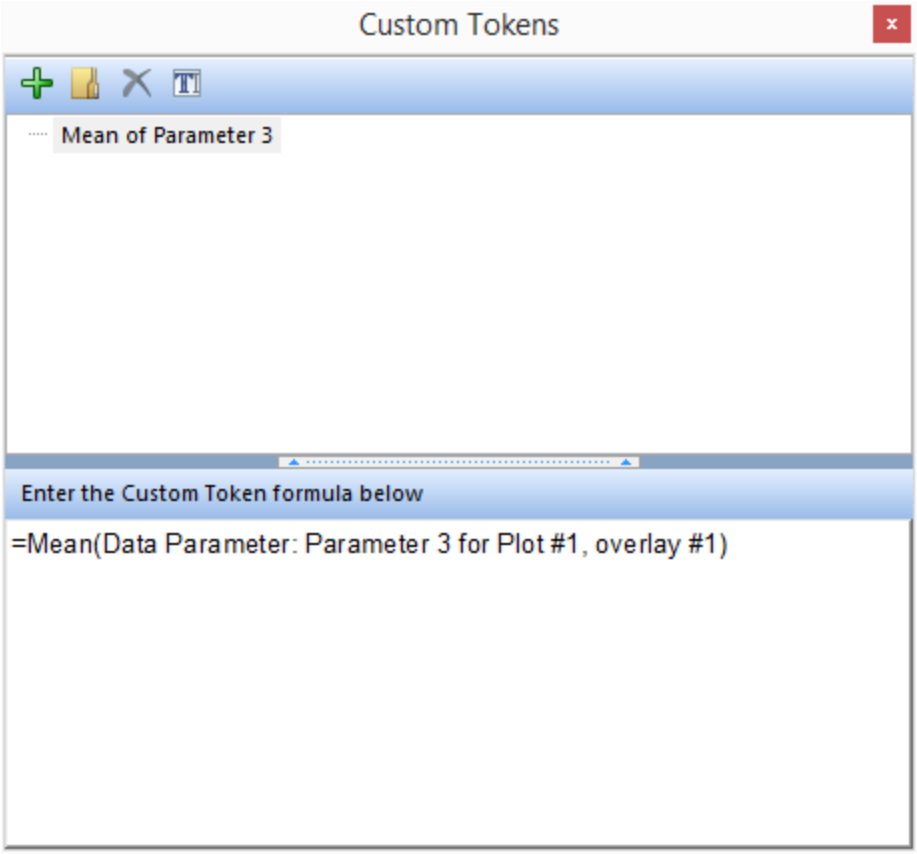

Figure 19.25 A Data Parameter token for Parameter 3 of plot 1 being used in a custom token formula to calculate the Mean of Parameter 3.

To insert a **Data Parameter** token:

1. Right-click on a Text Box.
2. Select **Insert Token** from the pop-up menu. Scroll the list box until you see the **Data Parameter** token type, if necessary (Figure 19.26).
3. Choose the **Data Parameter** token and click the **Insert** button.

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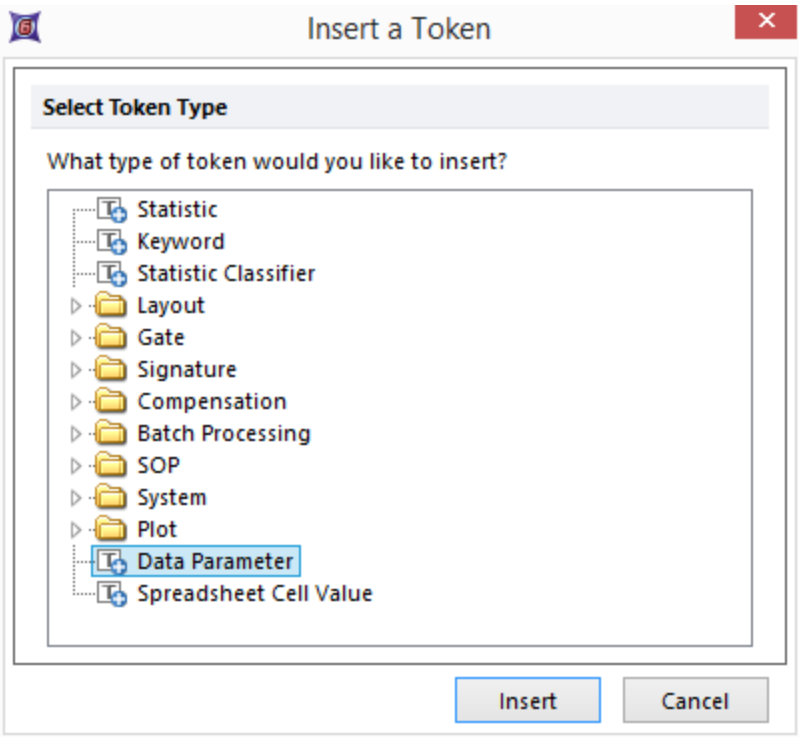

Figure 19.26 - Data Parameter Tokens

Upon choosing to insert a **Data Parameter** token, the **Create Data Parameter** dialog will open (Figure 19.27). After selecting the Data Source[699], choose which **Gate** and **Parameter** to derive the column of data from in the **Parameter** category.

- Choosing **Macro** from the **Output As** dropdown will retrieve all values and input them in the function without using any text derived values.
- Choosing **Data Values** from the **Output As** dropdown will retrieve all values and input them in the function as text derived value (Not recommended for large data sets)

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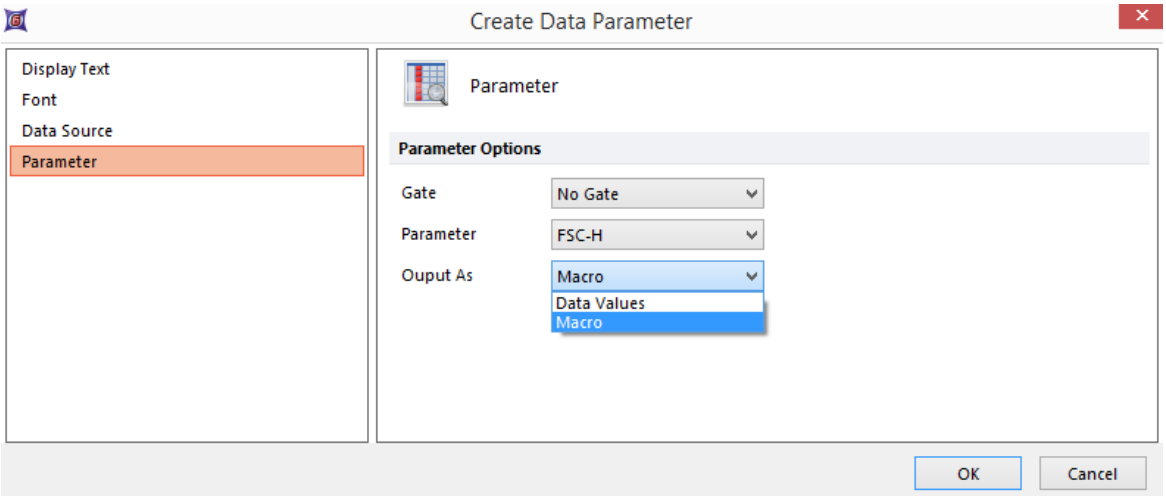

Figure 19.27 Create Data Parameter token dialog.

19.3.12 Spreadsheet Cell Value Tokens

Spreadsheet Cell Value tokens provide a shortcut to data within a cell of a spreadsheet[414]. The data may be derived directly from statistics within the layout, keywords, or from data files in the data list. Inserting statistics for data files in the data list allows additional flexibility in analysis of many statistics for many data files that are not currently open in the layout. Spreadsheet Cell Value tokens may be used in text boxes or in the batch actions.

To insert a **Spreadsheet Cell Value** token in a text box or layout:

Drag and Drop method:

1. Click and hold on the blue square in the lower right portion of the cell to be referenced (Figure 19.28 below).

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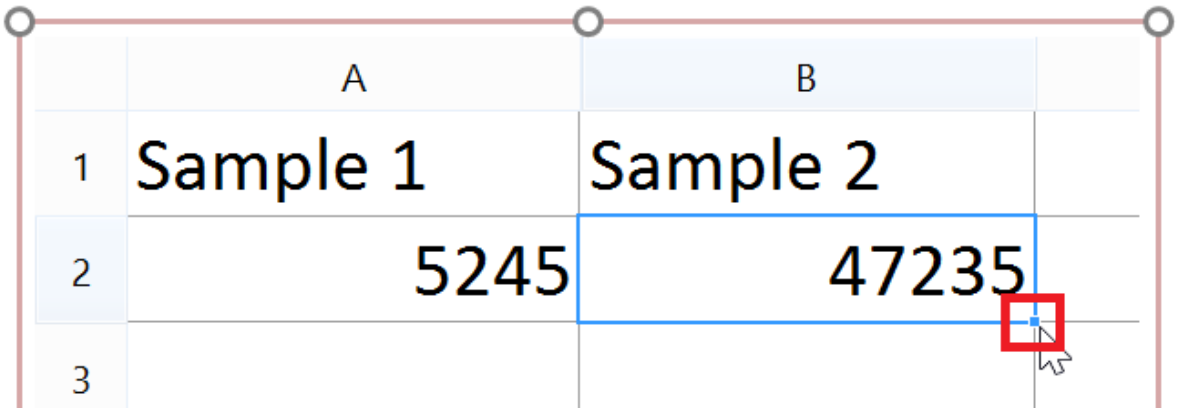

Figure 19.28- Clicking and holding on the blue square on the bottom right of the cell allows you to drag the cell value out of the spreadsheet to insert it as a Cell Value Token in a text box or batch action.

2. Drag the cursor onto a blank section of the layout. A new text box will be created that contains the **Spreadsheet Cell Value** token.
3. Alternatively, Drag the cursor into an existing text box. The **Spreadsheet Cell Value** token will be inserted in the text box.

The drag and drop method may also be used to quickly populate **Batch Actions** as in Figure 19.29 below.

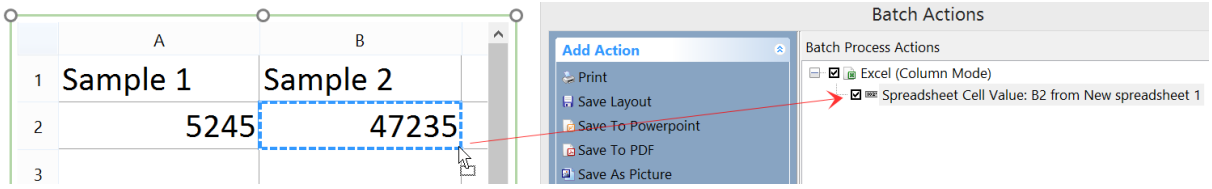

Figure 19.29 - Dragging and dropping a Spreadsheet Cell Value token to an Excel (Column Mode) action.

Right click method:

1. Right-click on a Text Box.
2. Select **Insert Token** from the pop-up menu. Scroll the list box until you see the **Spreadsheet Cell Value** token type, if necessary Figure 19.30.
3. Choose the **Spreadsheet Cell Value** token and click the **Insert** button.

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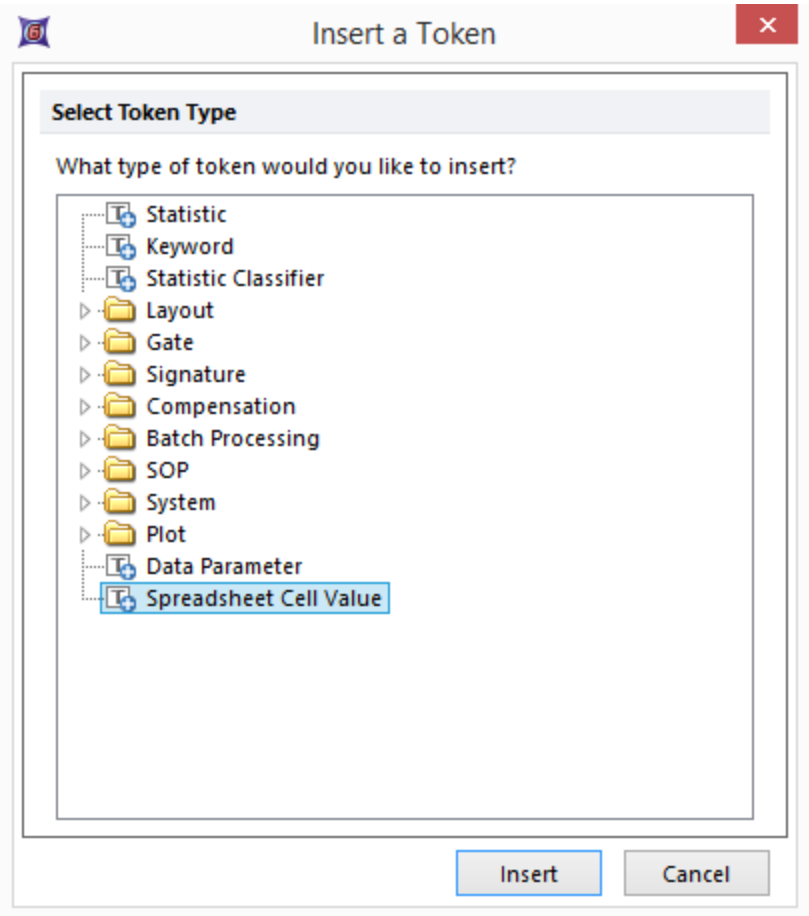

Figure 19.30 - Insert Spreadsheet Cell Value Token

Upon choosing to insert a **Spreadsheet Cell Value** token, the **Create Spreadsheet Cell Value** dialog will open (Figure 19.31). After selecting a spreadsheet as the Data Source 699, choose which **Cell Address** to derive the value inserted.

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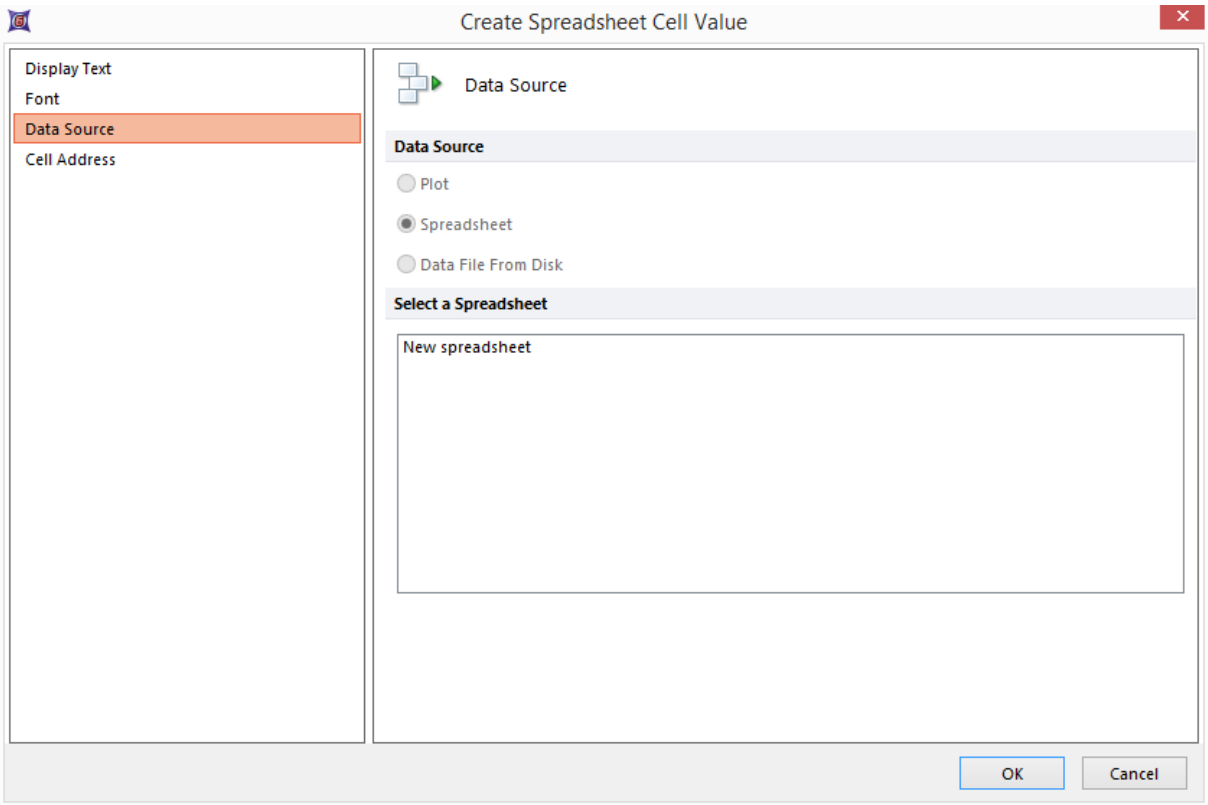

Figure 19.31 - Create Spreadsheet Cell Value dialog

If a spreadsheet is not selected first as the **Data Source** before proceeding to **Cell Address**, a warning message will appear (Figure 19.32) prompting you **Continue** or **Correct**.

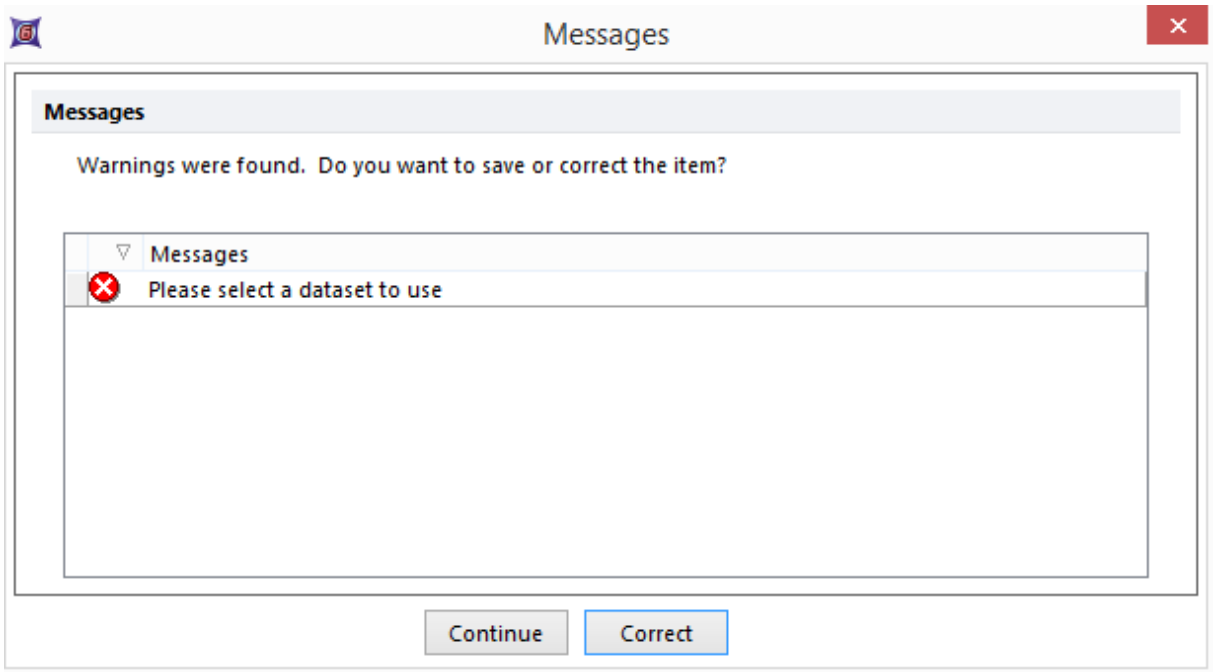

Figure 19.32 - Spreadsheet Cell Value without dataset Message

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If **OK** is clicked before selecting **Cell Address**, a warning message will appear (Figure 19.33) prompting you **Continue** or **Correct**.

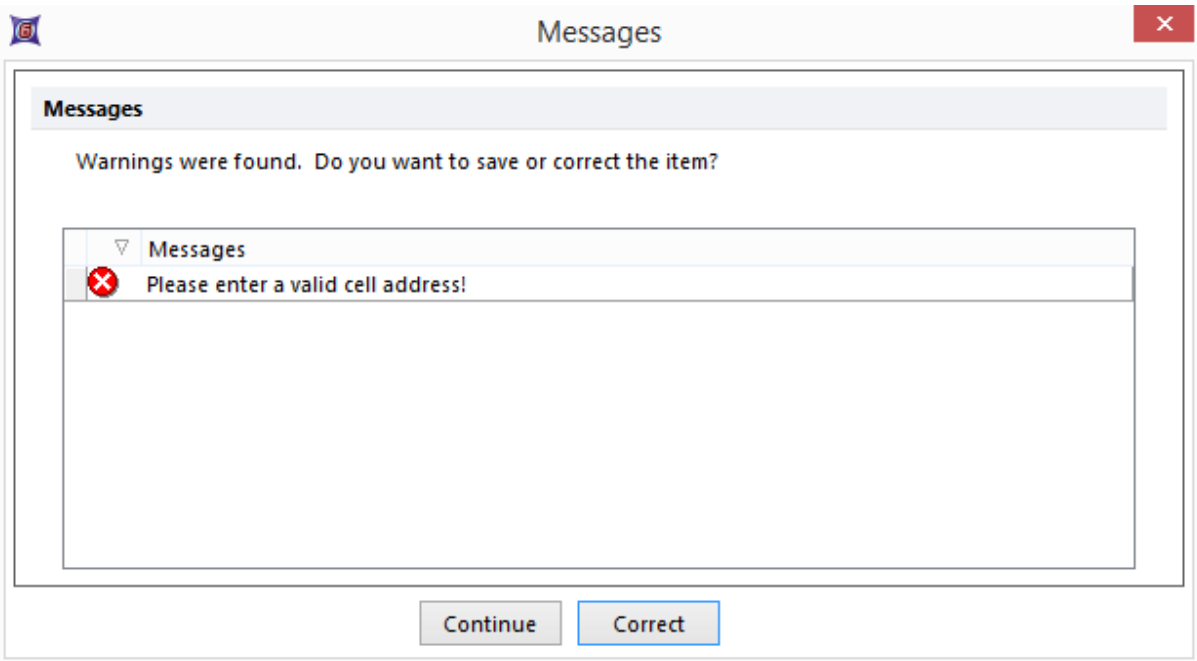

Figure 19.33 - Spreadsheet Cell Value without Cell Address Message

19.4 Custom Tokens

Custom Tokens contain user-defined combinations of tokens and text to create custom statistics. The custom combinations are created using mathematical calculations and/or functional operators to define the custom token formula. These formulas can contain any token including **Statistic** tokens, **Keyword** tokens, or even other **Custom** tokens.

Once defined, custom tokens are inserted into text boxes the same way other tokens are inserted. During data analysis, the custom token formula is evaluated by FCS Express and the result is displayed in the text box containing the custom token. With custom token formulas you can:

- Create mathematical expressions to combine tokens and/or text
- Create conditional statements using IF..THEN..ELSE qualifiers
- Convert a string of numeric values into a text result such as *Positive* or *Negative*

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The following sections will help you understand the power of **Custom Tokens**. A comprehensive list of all Custom Token formula operators and functions[769] is also provided.

Note: FCS Express 6 uses Excel-style formula evaluation. Therefore, formulas must start with "=".

19.4.1 Creating Custom Tokens

To create a **Custom Token**:

1. Click on the **View** tab→**Tokens**→**Custom Tokens** command to bring up the dockable **Custom Tokens** window.
2. As with many of the functional windows in FCS Express, the **Custom Tokens** window can be docked[115] to the sides of the screen. This helps keep the window within reach, while not getting in the way. Once docked, the window can be pinned to enable the Auto Hide feature.
3. The two areas of the **Custom Tokens** window, the Custom Token List (upper pane) and the Custom Token Formula Editor (lower pane), are separated by a splitter which can be moved to re-size the areas. (Figure 19.34)

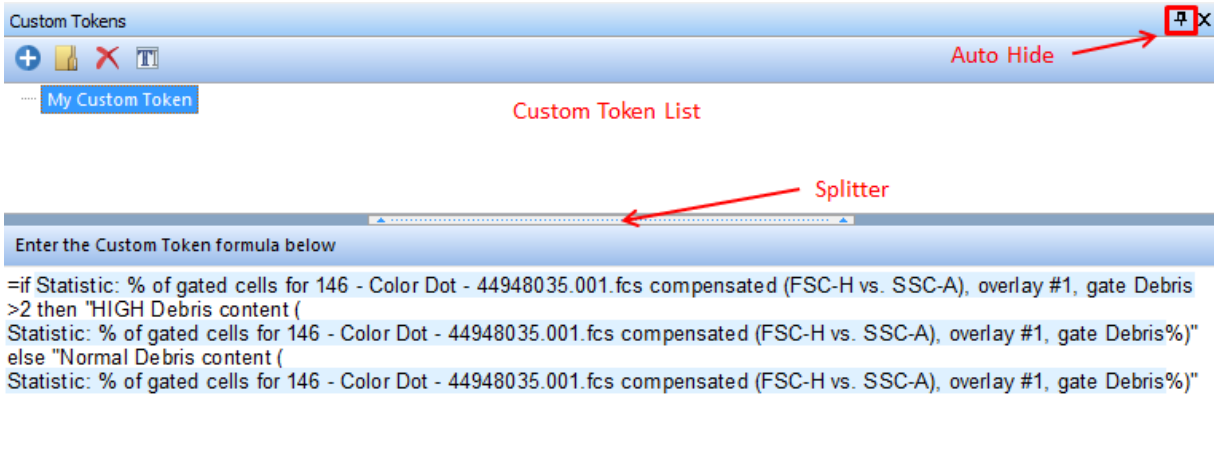

Figure 19.34 Docked Custom Tokens Window

3. Click on the (Add) icon.
4. Give the **New Custom Token** a unique name.
5. Click on the Token name in the list so that it is highlighted.
6. Enter or edit the formula:

   - Right-click in the lower pane of the Custom Tokens window, select **Insert Token** from the pop-up menu, and choose a token from the **Insert a Token** dialog; alternatively, drag and drop a gate or plot from

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your layout which serves as Data Source 699 into the lower pane of the Custom Tokens window to access the Edit Statistic dialog.
- Modify the formula. You can use any of the operators and functions that are available for custom tokens 769.

The icon buttons in the **Custom Tokens** window are explained in the table below.

Icon Button	Explanation
	Add a new Custom Token.
	Create (and name) a new folder to manage Custom Tokens. Drag & Drop Custom Tokens into the folder once it has been created.
	Delete the selected item. *Note: You can undo a delete by immediately clicking Undo in the Quick Access Toolbar 65.*
	Rename the currently selected item.

To understand how **Custom Token** formulas operate, and how to use them, a number of examples are shown in the next sections of this chapter.

19.4.2 Custom Token Example 1: Mathematical Calculation

In the first example, you will create a Custom Token with a simple formula, and then insert it into a Text Box.

1. Click on (Add) in a docked Custom Token window 728.
2. Edit **New Custom Token** to read *Mathematical Calculation*.
3. In the Formula area below the splitter 728, enter the following: *=100 * 30.*
4. Click on **Mathematical Calculation** to highlight/select it. Your **Custom Token** window should look like Figure 19.35.

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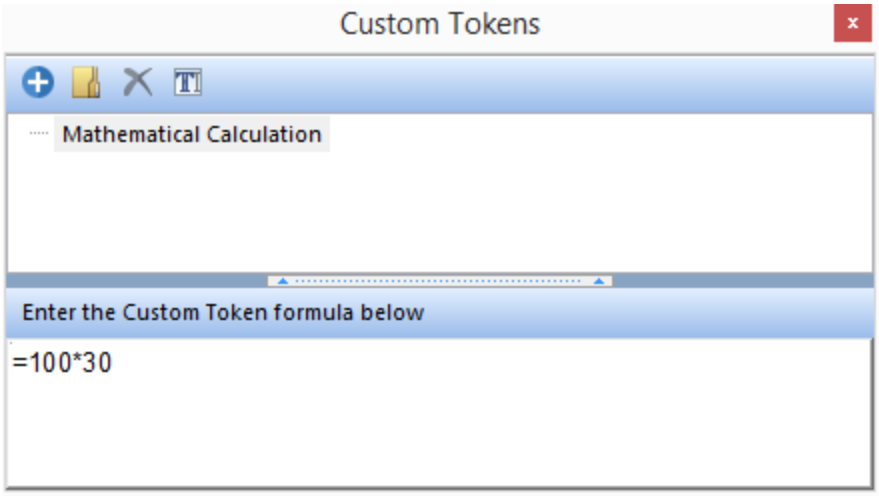

Figure 19.35 Custom Token Example 1

5. Create a Text Box on a layout page.
6. In the Text Box, enter the phrase: *The number of stars in the sky is: <space>*
7. Right-click at the end of the text, and select **Insert Token** from the pop-up menu.
8. At the bottom of the **Insert a Token** dialog (Figure 19.36), make sure that the **Custom** category is open (click on the triangle next to **Custom**, if necessary), and select **Mathematical Calculation.**

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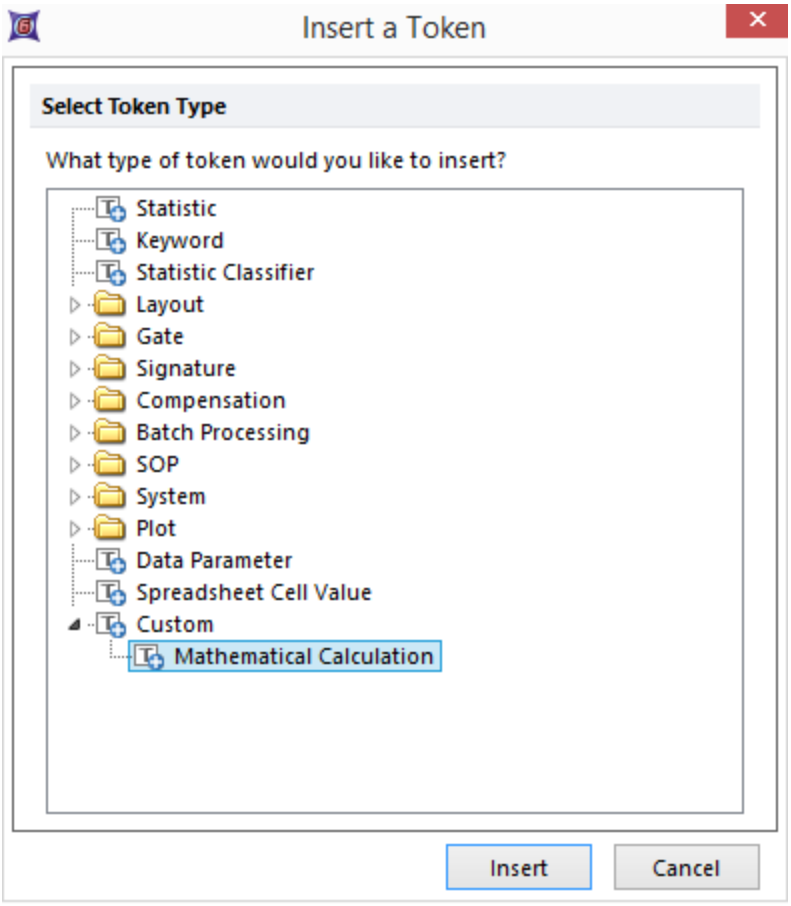

Figure 19.36 Insert a Token Dialog

9. With **Mathematical Calculation** highlighted, Click the **Insert** button. The Text Box should now read: "The number of stars in the sky is: 3000.00". (The default numeric output for a numeric result is two decimal places. This can be changed by right-clicking on the token in the text box, selecting **Format Token** from the pop-up menu, and changing the value on the **Output Format** page with the spin control). Once the Text Box is no longer in edit mode, the *Mathematical Calculation* token will display its result without a gray background.

This example demonstrates that when a **Custom Token** is inserted into a Text Box, its formula is calculated and the result is displayed.

19.4.3 Custom Token Example 2: Mathematical Calculation with a Token

The second example demonstrates how to insert tokens in your formulas to create more complex custom tokens.

1. Insert a plot 172 and a text box 152 on a layout page. Open the Custom Token window 728 and dock 115 it.
2. Click on (Add) in the docked Custom Token window.

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3. Edit **New Custom Token** to read "Math Calc with Token".
4. Enter = in the Formula area below the splitter[728]. *Note: FCS Express 6 uses Excel-style formula evaluation. Therefore, formulas must start with "=".*
5. Choose the Data Source[699] of the token by either of the following methods:
   - right-click in the Formula area (lower pane of Custom Tokens window) and choose **Insert Token** from the pop-up menu. Select **Statistic** and click on the **Insert** button, and choose a plot from the **Data Source** category
   - drag and drop the desired gate or plot from your layout into the lower pane of the Custom Tokens window, and double-click **Statistic token**.
6. From the **Statistic** category, highlight **# of Events** as the statistic. Click **OK**.
7. In the Formula area, enter "/2" to the right of the token.
8. Click on **Mathematical Calculation with a Token** to highlight/select it. Your **Custom Token** formula should look similar to Figure 19.37.

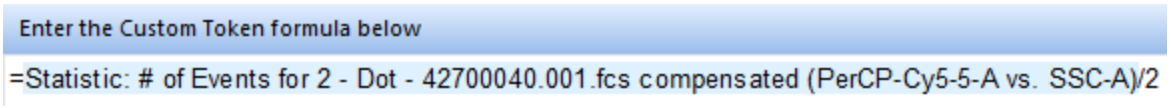

Figure 19.37 Custom Token Example 2

9. Insert the Custom Token you have just created into the Text Box by either of the following methods:
   - right-click on the Text Box[1298], choose **Insert Token** from the pop-up menu, select **Math Calc with Token** from **Custom** (clicking on the triangle next to Custom to expand the list, necessary), and click the **Insert** button, or
   - drag and drop **Math Calc with Token** from the Custom Token List (upper pane of the Custom Tokens window) to an empty spot on your layout or to an existing Text Box.

The Text Box should now display the number of events in your dataset divided by 2.

19.4.4 Custom Token Example 3: Appending Text

Up until now, you have seen how formulas calculate and return numeric results. However, formulas can also be used to display text. The following example demonstrates how to create a formula that appends text with Keyword tokens to return information:

1. Insert a plot[172] and a text box[152] on a layout page. Open the Custom Token window[728] and dock[115] it.
2. Click on (Add) in the docked Custom Token window.
3. Edit **New Custom Token** to read "Appending Text".

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4. Choose the Data Source 699 of the token by either of the following methods:
   - right-click in the Formula area (lower pane of Custom Tokens window), choose **Insert Token** from the pop-up menu, double-click **Keyword**, and choose a plot from the **Data Source** category
   - drag and drop the desired plot from your layout into the lower pane of the Custom Tokens window, and double-click **Keyword token**
5. Select the **Keyword** category of the **Create Keyword** dialog, and click the ellipsis to bring up the **Please select a keyword** dialog.
6. Select **$FIL** and click **OK** to exit the **Please select a keyword** dialog and then OK again to exit the **Create Keyword** dialog.
7. Enter the following after the keyword token: *<space> was created on <space>*
8. Add the **$DATE** keyword (similar to steps 4–6). The Formula field should resemble Figure 19.38.

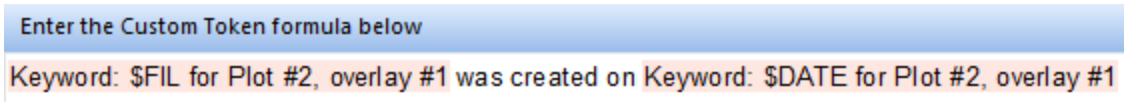

Figure 19.38 Custom Token Example 3 Formula

9. Insert the Custom Token you have just created into the Text Box by either of the following methods:
   - right-click on the Text Box 1298, choose **Insert Token** from the pop-up menu, select **Appending Text** from **Custom** (clicking on the triangle next to Custom to expand the list, necessary), and click the **Insert** button, or
   - drag and drop **Appending Text** from the Custom Token List (upper pane of the Custom Tokens window) to an empty spot on your layout or to an existing Text Box.

The Text Box should now resemble Figure 19.39.

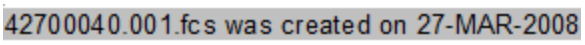

Figure 19.39 Custom Token Example 3

19.4.5 Custom Token Example 4: Conditional Statements

This example demonstrates using conditional IF..THEN..ELSE statements in a Custom Token formula.

1. Insert a plot 172 and a text box 152 on a layout page. Create a gate 318 around your cells of interest.
2. Open the Custom Token window 728 and dock 115 it.
3. Click on (Add) in the docked Custom Token window.
4. Edit **New Custom Token** to read "Conditional Statement".

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5. In the Formula field enter: *=IF <space>*
6. Choose the Data Source[699] of the token by either of the following methods:
   - right-click in the Formula area (lower pane of Custom Tokens window) and choose **Insert Token** from the pop-up menu, and double-click **Statistic.** Choose the plot from Step 1 from the **Data Source** category
   - drag and drop the plot from Step 1 from your layout page into the lower pane of the Custom Tokens window, and double-click **Statistic token**.
7. From the **Statistic** category, select the gate that you created in Step 1 from the drop-down list in the Select Gate field. Highlight **# of Events** as the statistic. Click **OK**.
8. Type the following in the formula area: *>2000 THEN "There are a lot of cells." ELSE "There are very few cells"*. The Formula field should resemble Figure 19.40.

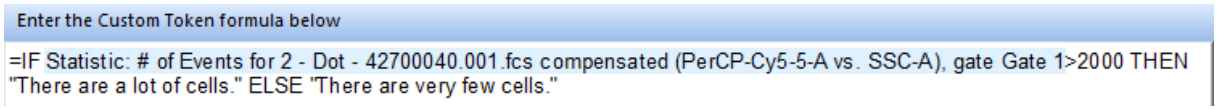

Figure 19.40 Custom Token Example 4 Formula

9. Insert the Custom Token you have just created into the Text Box by either of the following methods:
   - right-click on the Text Box[1298], choose **Insert Token** from the pop-up menu, select **Conditional Statement** from **Custom** (clicking on the triangle next to Custom to expand the list, necessary), and click the **Insert** button, or
   - drag and drop **Conditional Statement** from the Custom Token List (upper pane of the Custom Tokens window) to an empty spot on your layout or to an existing Text Box.

The token will display ***There are a lot of cells.*** if the number of events in the Plot #2 is greater than 2000 and ***There are very few cells.*** if the number of events is less than or equal to 2000. If you move the gate, the Custom Token formula is recalculated. Therefore, the token will display a different result if the number of events within the gate changes from less than 2000 to greater than 2000 (or vice versa).

19.4.6 Custom Token Example 5: Hiding Signature Tokens

Once you have created and set up your signatures[1089] in FCS Express, you may only want to display their signing status on your layout pages if the layout is signed and forgo signatures labeled as "unsigned" or "never signed".

Creating a new custom token based on your signature token[692] will accomplish the hiding effect.

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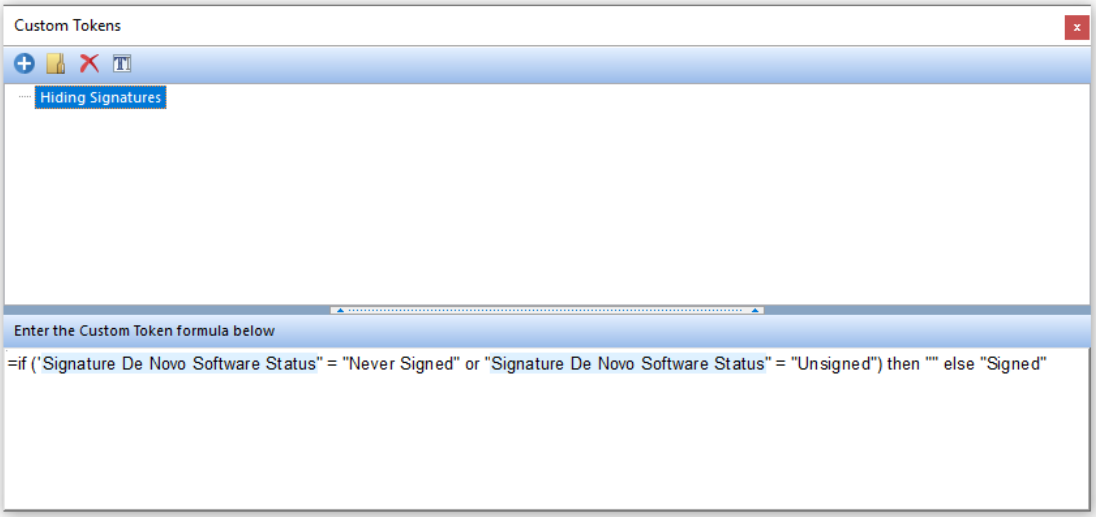
The custom token will take the form of a conditional statement 733 (IF, THEN, ELSE statement) as follows where <signature XXXXX status> represents your signature status 1098 token. Use the following custom token equation to create the token:

=if ("<signature XXXXX status>" = "Never Signed" or "<signature XXXXX status>" = "Unsigned") then "" else "Signed"

Please note that:

1. The custom token is case-sensitive so that **Never Signed**, **Unsigned**, and **Signed** strings must be entered with capital letter when the match with the **Signature Token** is evaluated (see example above).

2. If the **Signature Status** token is compared to a text string as in this example, the **Signature Status** token must be formatted to be a text string by adding quotes around it (as in the example above and the screenshot below).



Once the new custom token has been defined you may drag and drop or insert it anywhere in the layout and the token will only be displayed when the signing status is "Signed".

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19.4.7 More Complex Custom Token Examples

The following two examples demonstrate more complex custom token formulas:

- Custom Token Example 5 demonstrates the use of parentheses to control the order in which mathematical operations are applied. The part of the formula inside the parentheses is performed first. The Custom Token formula in Figure 19.41 calculates the average number of events across three plots.

```
=(Statistic: # of Events for Plot #1, overlay #1 +
Statistic: # of Events for Plot #4, overlay #1 +
Statistic: # of Events for Plot #5, overlay #1) / 3
```

Figure 19.41 Custom Token Example 5 Formula

- Custom Token Example 6 demonstrates the use of nested IF..THEN..ELSE statements. The custom token formula shown in Figure 19.42 categorizes the fluorescence of a histogram based on the percentage of events within marker regions (Figure 19.43). If 80% of the events exist in the right marker region (M1), then the formula returns *Bright*. If the middle marker region (M2) contains 80% of the events, then *Medium* is returned. If 80% of the events exist in the left marker region (M3), then *Dim* is returned. If none of the markers contain more than 80% of the events, then *Unknown* is returned.

```
=IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 1>80 THEN "Bright"
ELSE IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 2>80 THEN "Medium"
ELSE IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 3>80 THEN "Dim"
ELSE "Unknown"
```

Figure 19.42 Custom Token Example 6 Formula

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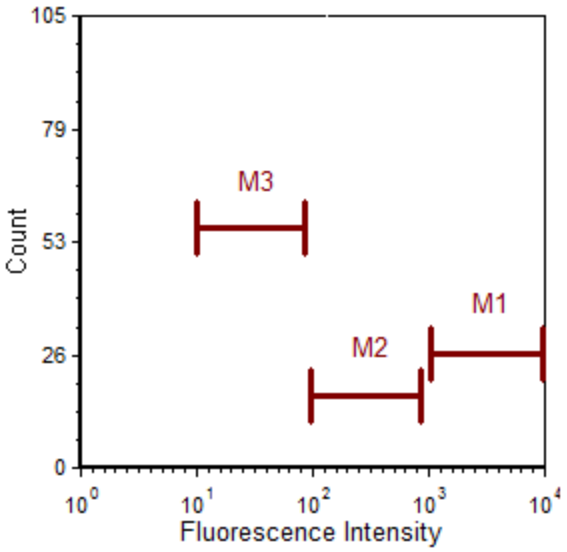

Figure 19.43 Fluorescence Intensity Marker Example

Additional conditions could be applied in Example 6. For example, if the sum of two markers contains more than 80% of the events, then *Bimodal* could be returned.

19.4.8 Inserting Functions into Formulas

A number of mathematical functions[769] can be inserted into custom token formulas. To insert a function, right-click in the formula area at the point you want the function to appear, choose **Insert Function** from the pop-up menu, and select the appropriate function (Figure 19.44).

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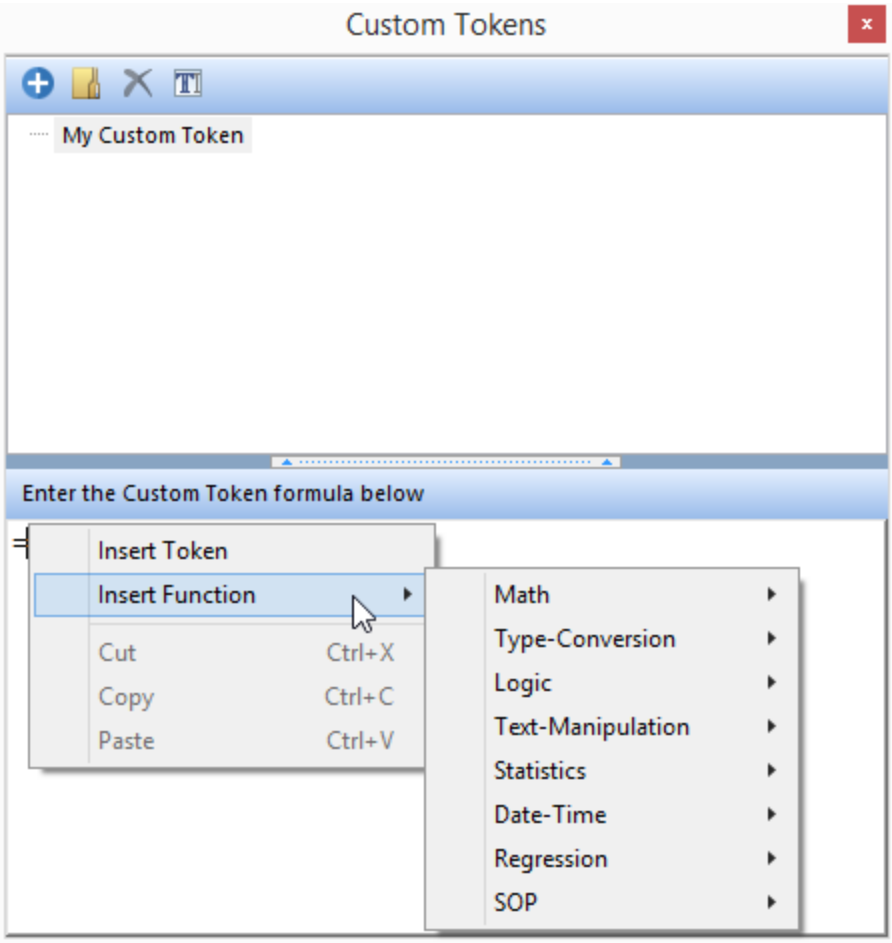

Figure 19.44 Inserting a Function into a Formula

The available functions in each group are pictured below.

Math

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```
Abs(number)
ArcCos(number)
ArcCosh(number)
ArcSin(number)
ArcSinh(number)
ArcTan(number)
ArcTanh(number)
ConvertLinToLog(lin_value,#_of_decades,output_range,offset)
ConvertLogToLin(log_value,#_of_decades,output_range,offset)
Cos(number)
Cosh(number)
CoTan(number)
Ln(number)
Log(logBase,number)
Max(number1,number2)
Maximum([number1,number2,...])
Mean([number1,number2,...])
Min(number1,number2)
Minimum([number1,number2,...])
Range([number1,number2,...])
Sin(number)
Sinh(number)
Sqr(number)
Sqrt(number)
StdDev([number1,number2,...])
Tan(number)
```

Type-Conversion

```
NumericToString(number)
StringToNumeric("text")
```

Logic

```
Between(number1,number2,number3)
FalseValue
IsFalse(number)
IsSameText("text1,text2,...,textn",CaseSensitive)
IsTrue(number)
TrueValue
```

Text Manipulation

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```
AreValuesInRange([number1,number2,...],tolerance,["name1", "name2",...])
Copy("text",number1,number2)
ExtractFilename("file_path")
ExtractFolderFromFilename("file_path")
FormatFloat("format string", number)
FragmentCount("text1", "text2")
GetContainingFolder("file_path")
GetFragment(n, "text1", "text2")
IncludeTrailingPathDelimiter("file_path")
Pos("text1", "text2")
PositionInText("text1", "text2", startingPosition, n)
RemoveSpaces("text")
RemoveText("text1", "text2", RemoveAll)
ReplaceText("text1", "text2", "text3", ReplaceAll)
Trim("text")
```

Statistics

```
ChiSquareTest([number1,..],[number1,... ])
FTest([number1,...],[number1,...])
GrubbsTest([number1,...])
KSTest([number1,...],[numer1,...])
KSTest2([number1,...],[numer1,...], tails, signif)
LillieforsTest([number1,...])
MannWhitney([number1,...],[number1,...],hypothesis type,alpha, use normal approximation)
SSMD(mean max, mean min, n max, n min, SD max, SD min)
StainIndex(positive population mean/median, negative population mean/median, negative population standard deviation)
TTest1S([number1,...],expected mean)
TTest2S([number1,...],[number1,...],tails, paired)
Wilcoxon([number1,...],[number1,...],hypothesis type,alpha)
ZPrime(mean negative, SD negative, mean positive, sd positive)
ZTest([number1,...],expected mean, expected sd)
```

Date Time

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```
DateKWToDate("DateKeyword", number)
DateTimeToStrFMT(time, format)
DaysBetween(start date, end date)
FormatDateTime("format","Date and/or time")
HoursBetween(start time, end time)
MillisecondsBetween(start time, end time)
MinutesBetween(start time, end time)
SecondsBetween(start time, end time)
StrToDateFMT(text, format, delimiter)
StrToTimeFMT(text, time delimiter, milisecond delimiter)
TimeKWToTime("TimeKeyword")
YearsBetween(start date, end date)
```

Regression

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BoltzmannXToY(x, [modelParameters])
BoltzmannYToX(y, [modelParameters])
CubicXToY(x, [modelParameters])
CubicYToX(y, [modelParameters])
ExpAssociationXToY(x, [modelParameters])
ExpAssociationYToX(y, [modelParameters])
ExponentialGrowXToY(x, [modelParameters])
ExponentialGrowYToX(y, [modelParameters])
ExponentialXToY(x, [modelParameters])
ExponentialYToX(y, [modelParameters])
ExtractBoltzmannResults([modelParameters], "Bottom" | "Top" | "V50" | "Slope" | "r2")
ExtractCubicResults([modelParameters], "m" | "b" | "A2" | "A3" | "r2")
ExtractExpAssociationResults([modelParameters], "Ymax1" | "K1" | "r2")
ExtractExponentialGrowResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractExponentialResults([modelParameters], "A" | "M" | "C" | "r2")
ExtractFiveParmLogisticResults([modelParameters], "Imin" | "Imax" | "IC50" | "HillSlope" | "Asymmetry" | "r2")
ExtractFourParmLogisticResults([modelParameters], "Imin" | "Imax" | "IC50" | "HillSlope" | "r2")
ExtractGaussianResults([modelParameters], "A" | "SD" | "xMed" | "K" | "r2")
ExtractLinearResults([modelParameters], "m" | "b" | "r2")
ExtractMichMentenResults([modelParameters], "Vm" | "KM" | "r2")
ExtractParabolicResults([modelParameters], "m" | "b" | "A2" | "r2")
ExtractPowerResults([modelParameters], "A1" | "B1" | "K" | "r2")
ExtractPowerSeriesResults([modelParameters], "A1" | "B1" | "K" | "A2" | "B2" | "r2")
ExtractSigmoidalDecResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractSigmoidalResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractSineWaveResults([modelParameters], "Baseline" | "Amplitude" | "Frequency" | "Offset" | "r2")
ExtractTwoPhaseExpAssociationResults([modelParameters], "Ymax1" | "K1" | "Ymax2" | "K2" | "r2")
ExtractTwoPhaseExponentialResults([modelParameters], "A" | "M" | "C" | "A2" | "M2" | "r2")
ExtractTwoSiteBindingResults([modelParameters], "Vm" | "KM" | "Vm2" | "KM2" | "r2")
FiveParmLogisticXToY(x, [modelParameters])
FiveParmLogisticYToX(y, [modelParameters])
FourParmLogisticXToY(x, [modelParameters])
FourParmLogisticYToX(y, [modelParameters])
GaussianXToY(x, [modelParameters])
GaussianYToX(y, [modelParameters])

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LinearXToY(x, [modelParameters])
LinearYToX(y, [modelParameters])
MichMentenXToY(x, [modelParameters])
MichMentenYToX(y, [modelParameters])
ParabolicXToY(x, [modelParameters])
ParabolicYToX(y, [modelParameters])
PerformBoltzmannFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformCubicFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExpAssociationFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExponentialFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExponentialGrowFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformFiveParmLogisticFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformFourParmLogisticFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformGaussianFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformLinearFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformMichMentenFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformParabolicFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformPowerFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformPowerSeriesFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSigmoidalDecFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSigmoidalFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSineWaveFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoPhaseExpAssociationFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoPhaseExponentialFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoSiteBindingFit([xValues], [yValues], [SDvalues], [fixedParameters])
PowerSeriesXToY(x, [modelParameters])
PowerSeriesYToX(y, [modelParameters])
PowerXToY(x, [modelParameters])
PowerYToX(y, [modelParameters])
SigmoidalDecXToY(x, [modelParameters])
SigmoidalDecYToX(y, [modelParameters])
SigmoidalXToY(x, [modelParameters])
SigmoidalYToX(y, [modelParameters])
SineWaveXToY(x, [modelParameters])
SineWaveYToX(y, [modelParameters])
TwoPhaseExpAssociationXToY(x, [modelParameters])
TwoPhaseExpAssociationYToX(y, [modelParameters])
TwoPhaseExponentialXToY(x, [modelParameters])
TwoPhaseExponentialYToX(y, [modelParameters])
TwoSiteBindingXToY(x, [modelParameters])
TwoSiteBindingYToX(y, [modelParameters])

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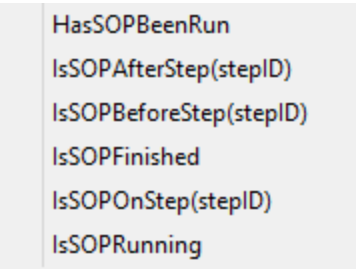
SOP



For in-depth definitions for each non-Regression Function, please visit our Custom Token Formula Reference 769.

For in-depth definitions for each Regression Function, please visit our Regression Analysis section 567.

For a table indicating the equation, the formula associated with it, and the parameters defined in FCS Express when a fit is performed, please visit our section on the Available Equations 578.

19.4.9 Editing Custom Tokens

You can edit the formula of a Custom Token directly in the Custom Tokens window 728 by simply clicking on an available Custom Token from the list.

If you have already inserted a Custom Token into a text box, you can open an **Edit** dialog (Figure 19.45) by either of the following methods:

- right-click on the Custom Token in the Text Box and choose **Format Token** from the pop-up menu, or
- double-click on the token in the text box.

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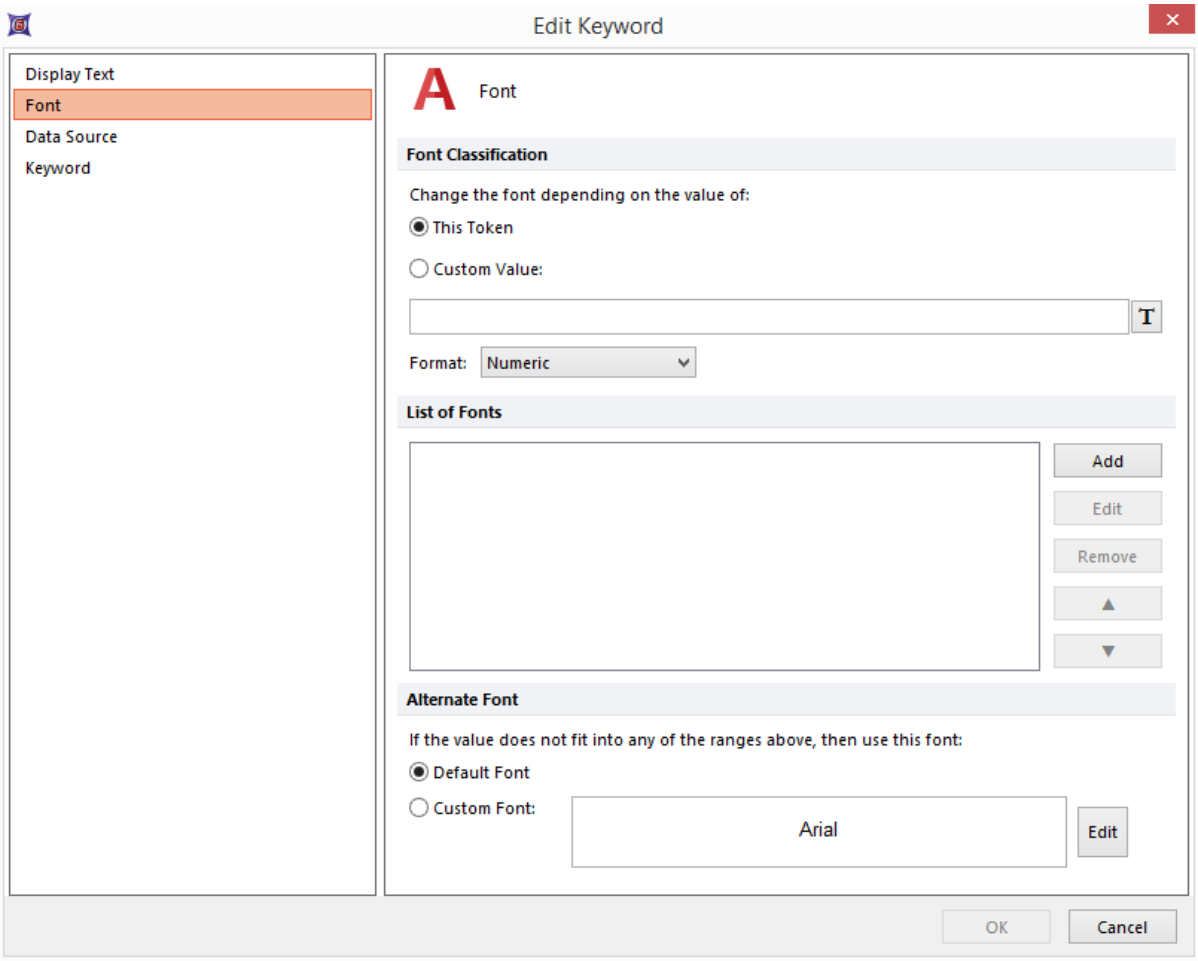

Figure 19.45 Editing a Custom Token -- Font Page

By default, Custom Tokens will contain 2 decimal places. You can edit the number of decimal places under the **Number Format** page of the dialog. On this page of dialog, you can also edit the Rounding Mode (from either **Standard**, **Down**, **Up**, or **Truncate**) and whether or not the value shows trailing zeroes.

On the **Font** page of the dialog, you can modify which font to use for the token depending on the value of the token or a custom value. Click **Add** to bring up a **Create Classification** dialog (Figure 19.46). To change the font style or color for a range of values, select the **Custom Font** radio button and click the **Edit** button. Then set the range and click **OK**. Repeat for other ranges.

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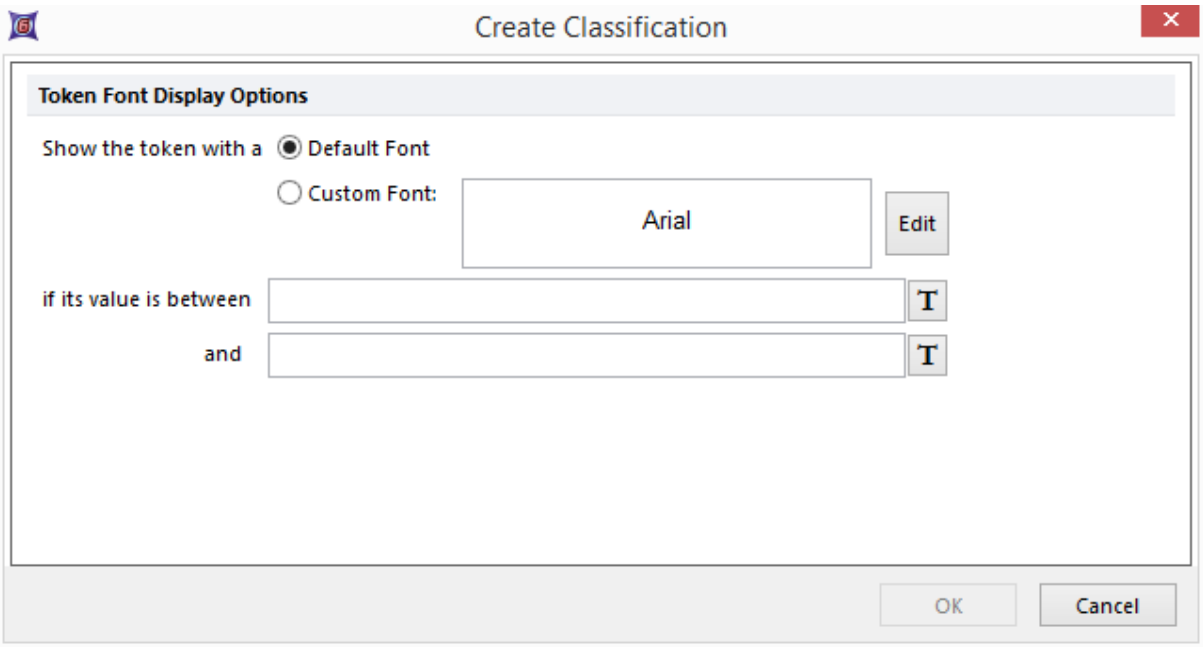

Figure 19.46 Create Classification Dialog

If you edit the formula of a Custom Token in a text box (as opposed to editing it in the Custom Token window), you will be prompted whether you want to modify the existing token (throughout the layout), or create a new token that only applies to that text box. If you choose **Create New**, a new token with a default name will be created. To modify the default name, right-click on the new token in the **Custom Token** window, select **Rename** from the pop-up menu, and edit the name.

19.4.10 Custom Token Formatting

FCS Express custom token formulas must follow standard rules for mathematical expressions in order to generate the desired token output. In addition to standard mathematical operations, FCS Express can also perform text manipulation, i.e., joining two text phrases together. The following table shows examples of correct and incorrect formulas for generating text results.

Desired Output	Correct Formula	Incorrect Formula
There are 5000 cells	"There are" + "5000 cells"	"There are" + 5000 cells
4	2+2	"2"+"2"
22	"2"+"2"	2+2

When tokens are inserted into formulas, they are considered to be one of two types: numeric result tokens or text result tokens. Numeric tokens have a blue background and text tokens have a peach background when a formula is being

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edited. It is possible to change the result type of a token by right-clicking on the token and selecting the alternate result type from the drop-down menu (Figure 19.47).

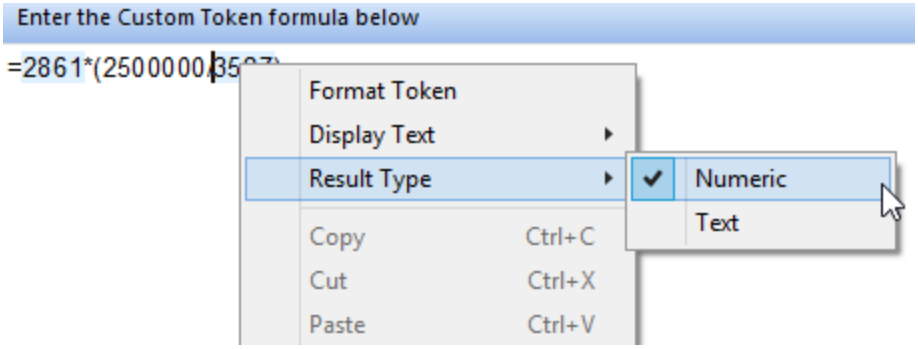

Figure 19.47 Changing the Result Type in a Custom Token Formula

When a new token is inserted into a formula, FCS Express will try to determine the correct result type. However, some tokens can make sense either as a number or text, depending on the context. It is up to you to ensure that the correct type is selected. The result type is important when FCS Express is trying to evaluate the result of a formula. Consider the case shown in the next table where there are 5000 cells on plot 2.

Desired Output	Correct Formula	Incorrect Formula
There are 5000 cells on plot 2	"There are " + # of Events for Plot #2 + " cells on plot 2"	"There are " + # of Events for Plot #2 + " cells on plot 2"
10000	# of Events for Plot #2 * 2	# of Events for Plot #2 * 2

In the first example, the incorrect formula tried to link a numeric result token (blue) to text phrases. The correct formula contains a text result token (peach).

In the second example, the incorrect formula tried to apply a mathematical operation to a text result token (peach). The correct formula contains a numeric result token (blue).

An incorrect formula will display **##ERROR##** if the token is placed in a text box.

19.4.11 Using Boolean Values in Custom Tokens

It is possible to create a custom token that returns a Boolean value. For example, the statistic chosen in the custom token, **Condition1**, shown in Figure 19.48, was set on a gate drawn around the lymphocyte population. **Condition1**

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will evaluate to TRUE or FALSE, depending on the number of cells in the lymphocyte gate.

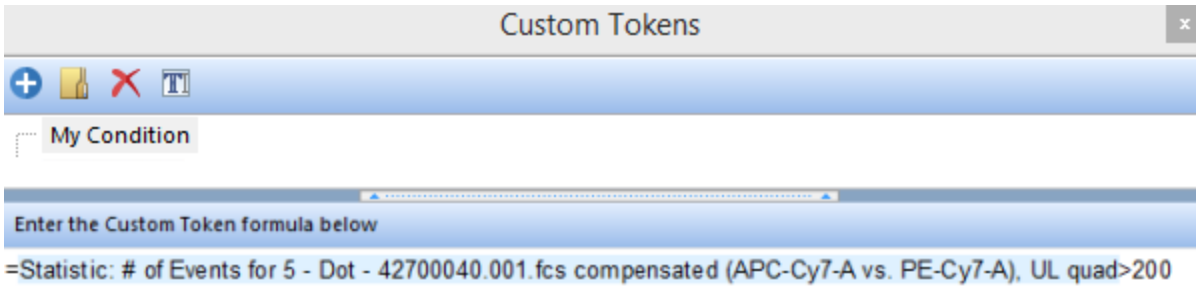

Figure 19.48 Token that Evaluates to TRUE or FALSE

You can use the **My Condition** token inside another token. To do so, set the result type of the token to Numeric and use the IsTrue or IsFalse function, as shown in Figure 19.49.

```
=IF IsTrue(Custom Token: My Condition) THEN "There are at least 200 events
in my quadrant." ELSE "There are less than 200 events in my quadrant."
```

Figure 19.49 Using a Boolean token

19.4.12 Using Array Formulas in Custom Tokens

Several mathematical functions described in the Custom Token Formula Reference, such as Mean, StdDev, Minimum, Maximum, and Range, work on arrays. The arrays can contain an unlimited number of elements enclosed in square brackets and separated by commas. These elements can be constants or tokens.

For example:

- Using the formula *=Minimum([54,36,75,89,39])* returns *36* when the custom token is inserted in a text box.
- The formula in Figure 19.50 will return the mean of the Y Median values of the Upper Left quadrant across four dot plots.

```
=Mean([Statistic: Y Median for 5 - Dot - 42700040.001.fcs compensated (APC-Cy7-A vs. PE-Cy7-A), UL quad,
Statistic: Y Median for 8 - Dot - 42846038.001.fcs compensated (APC-Cy7-A vs. PE-Cy7-A), UL quad,
Statistic: Y Median for 9 - Dot - 42905039.001.fcs compensated (APC-Cy7-A vs. PE-Cy7-A), UL quad,
Statistic: Y Median for 10 - Dot - 43848022.001.fcs compensated (APC-Cy7-A vs. PE-Cy7-A), UL quad])
```

Figure 19.50 Using Tokens in an Array Formula

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19.4.12. Custom Token Example 3: Appending Text 2 1

Up until now, you have seen how formulas calculate and return numeric results. However, formulas can also be used to display text. The following example demonstrates how to create a formula that appends text with Keyword tokens to return information:

1. Insert a plot 172 and a text box 152 on a layout page. Open the Custom Token window 728 and dock 115 it.
2. Click on (Add) in the docked Custom Token window.
3. Edit **New Custom Token** to read "Appending Text".
4. Choose the Data Source 699 of the token by either of the following methods:
   - right-click in the Formula area (lower pane of Custom Tokens window), choose **Insert Token** from the pop-up menu, double-click **Keyword**, and choose a plot from the **Data Source** category
   - drag and drop the desired plot from your layout into the lower pane of the Custom Tokens window, and double-click **Keyword token**
5. Select the **Keyword** category of the **Create Keyword** dialog, and click the ellipsis to bring up the **Please select a keyword** dialog.
6. Select **$FIL** and click **OK** to exit the **Please select a keyword** dialog and then OK again to exit the **Create Keyword** dialog.
7. Enter the following after the keyword token: *<space> was created on <space>*
8. Add the **$DATE** keyword (similar to steps 4–6). The Formula field should resemble Figure 19.51.

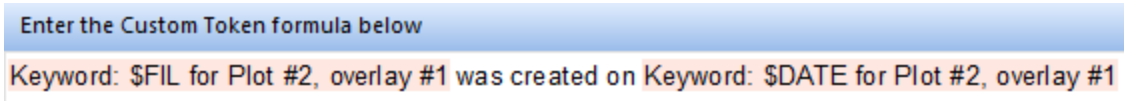

Figure 19.51 Custom Token Example 3 Formula

9. Insert the Custom Token you have just created into the Text Box by either of the following methods:
   - right-click on the Text Box 1298, choose **Insert Token** from the pop-up menu, select **Appending Text** from **Custom** (clicking on the triangle next to Custom to expand the list, necessary), and click the **Insert** button, or
   - drag and drop **Appending Text** from the Custom Token List (upper pane of the Custom Tokens window) to an empty spot on your layout or to an existing Text Box.

The Text Box should now resemble Figure 19.52.

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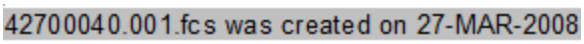

Figure 19.52 Custom Token Example 3

19.4.12. Custom Token Example 4: Conditional Statements 2 2

This example demonstrates using conditional IF..THEN..ELSE statements in a Custom Token formula.

1. Insert a plot[172] and a text box[152] on a layout page. Create a gate[318] around your cells of interest.
2. Open the Custom Token window[728] and dock[115] it.
3. Click on (Add) in the docked Custom Token window.
4. Edit **New Custom Token** to read "Conditional Statement".
5. In the Formula field enter: *=IF <space>*
6. Choose the Data Source[699] of the token by either of the following methods:
   - right-click in the Formula area (lower pane of Custom Tokens window) and choose **Insert Token** from the pop-up menu, and double-click **Statistic.** Choose the plot from Step 1 from the **Data Source** category
   - drag and drop the plot from Step 1 from your layout page into the lower pane of the Custom Tokens window, and double-click **Statistic token**.
7. From the **Statistic** category, select the gate that you created in Step 1 from the drop-down list in the Select Gate field. Highlight **# of Events** as the statistic. Click **OK**.
8. Type the following in the formula area: *>2000 THEN "There are a lot of cells." ELSE "There are very few cells"*. The Formula field should resemble Figure 19.53.

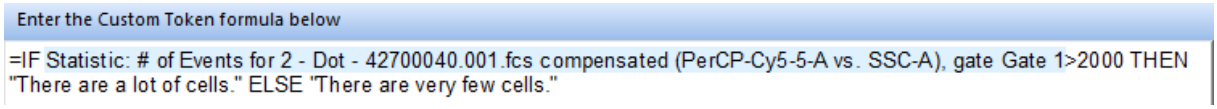

Figure 19.53 Custom Token Example 4 Formula

9. Insert the Custom Token you have just created into the Text Box by either of the following methods:
   - right-click on the Text Box[1298], choose **Insert Token** from the pop-up menu, select **Conditional Statement** from **Custom** (clicking on the triangle next to Custom to expand the list, necessary), and click the **Insert** button, or
   - drag and drop **Conditional Statement** from the Custom Token List (upper pane of the Custom Tokens window) to an empty spot on your layout or to an existing Text Box.

The token will display ***There are a lot of cells.*** if the number of events in the Plot #2 is greater than 2000 and ***There are very few cells.*** if the number of

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events is less than or equal to 2000. If you move the gate, the Custom Token formula is recalculated. Therefore, the token will display a different result if the number of events within the gate changes from less than 2000 to greater than 2000 (or vice versa).

19.4.12. Custom Token Example 5: Hiding Signature Tokens 2 3

Once you have created and set up your signatures 1089 in FCS Express, you may only want to display their signing status on your layout pages if the layout is signed and forgo signatures labeled as "unsigned" or "never signed".

Creating a new custom token based on your signature token 692 will accomplish the hiding effect.

The custom token will take the form of a conditional statement 733 (IF, THEN, ELSE statement) as follows where <signature XXXXX status> represents your signature status 1098 token. Use the following custom token equation to create the token:

=if <signature XXXXX status> = "Never Signed" or <signature XXXXX status> = "Unsigned" then "" else "signed"

Please note that the custom token is case-sensitive so that Never Signed, Unsigned, and signed must be entered exactly as above.
Once the new custom token has been defined you may drag and drop or insert it anywhere in the layout and the token will only be displayed when the signing status is "Signed".

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19.4.12. More Complex Custom Token Examples 24

The following two examples demonstrate more complex custom token formulas:

- Custom Token Example 5 demonstrates the use of parentheses to control the order in which mathematical operations are applied. The part of the formula inside the parentheses is performed first. The Custom Token formula in Figure 19.54 calculates the average number of events across three plots.

```
=(Statistic: # of Events for Plot #1, overlay #1 +
Statistic: # of Events for Plot #4, overlay #1 +
Statistic: # of Events for Plot #5, overlay #1) / 3
```

Figure 19.54 Custom Token Example 5 Formula

- Custom Token Example 6 demonstrates the use of nested IF..THEN..ELSE statements. The custom token formula shown in Figure 19.55 categorizes the fluorescence of a histogram based on the percentage of events within marker regions (Figure 19.56). If 80% of the events exist in the right marker region (M1), then the formula returns *Bright*. If the middle marker region (M2) contains 80% of the events, then *Medium* is returned. If 80% of the events exist in the left marker region (M3), then *Dim* is returned. If none of the markers contain more than 80% of the events, then *Unknown* is returned.

```
=IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 1>80 THEN "Bright"
ELSE IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 2>80 THEN "Medium"
ELSE IF Statistic: % of gated cells for Plot #6, overlay #1,in marker 3>80 THEN "Dim"
ELSE "Unknown"
```

Figure 19.55 Custom Ton Example 6 Formula

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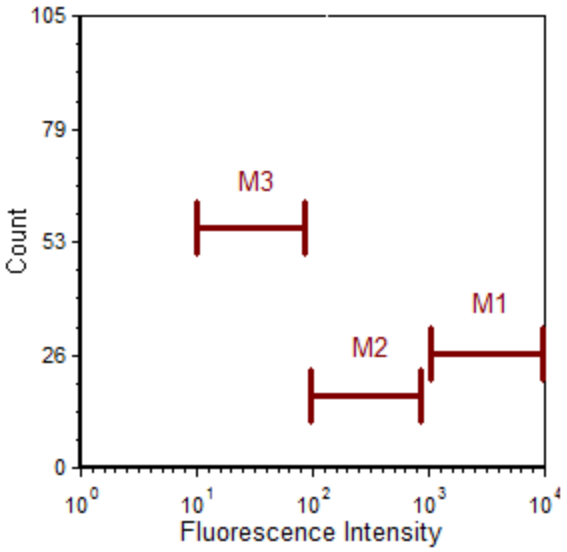

Figure 19.56 Fluorescence Intensity Marker Example

Additional conditions could be applied in Example 6. For example, if the sum of two markers contains more than 80% of the events, then *Bimodal* could be returned.

19.4.12. Inserting Functions into Formulas 25

A number of mathematical functions[769] can be inserted into custom token formulas. To insert a function, right-click in the formula area at the point you want the function to appear, choose **Insert Function** from the pop-up menu, and select the appropriate function (Figure 19.57).

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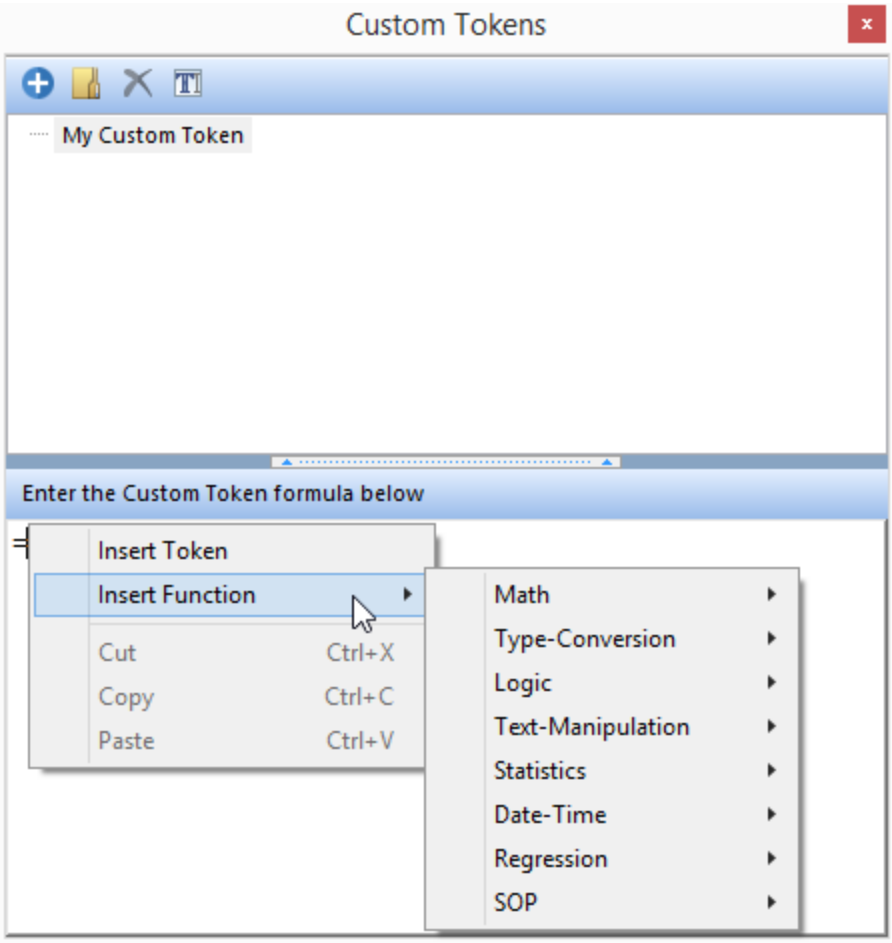

Figure 19.57 Inserting a Function into a Formula

The available functions in each group are pictured below.

Math

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```
Abs(number)
ArcCos(number)
ArcCosh(number)
ArcSin(number)
ArcSinh(number)
ArcTan(number)
ArcTanh(number)
ConvertLinToLog(lin_value,#_of_decades,output_range,offset)
ConvertLogToLin(log_value,#_of_decades,output_range,offset)
Cos(number)
Cosh(number)
CoTan(number)
Ln(number)
Log(logBase,number)
Max(number1,number2)
Maximum([number1,number2,...])
Mean([number1,number2,...])
Min(number1,number2)
Minimum([number1,number2,...])
Range([number1,number2,...])
Sin(number)
Sinh(number)
Sqr(number)
Sqrt(number)
StdDev([number1,number2,...])
Tan(number)
```

Type-Conversion

```
NumericToString(number)
StringToNumeric("text")
```

Logic

```
Between(number1,number2,number3)
FalseValue
IsFalse(number)
IsSameText("text1,text2,...,textn",CaseSensitive)
IsTrue(number)
TrueValue
```

Text Manipulation

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```
AreValuesInRange([number1,number2,...],tolerance,["name1", "name2",...])
Copy("text",number1,number2)
ExtractFilename("file_path")
ExtractFolderFromFilename("file_path")
FormatFloat("format string", number)
FragmentCount("text1", "text2")
GetContainingFolder("file_path")
GetFragment(n, "text1", "text2")
IncludeTrailingPathDelimiter("file_path")
Pos("text1", "text2")
PositionInText("text1", "text2", startingPosition, n)
RemoveSpaces("text")
RemoveText("text1", "text2", RemoveAll)
ReplaceText("text1", "text2", "text3", ReplaceAll)
Trim("text")
```

Statistics

```
ChiSquareTest([number1,..],[number1,... ])
FTest([number1,...],[number1,...])
GrubbsTest([number1,...])
KSTest([number1,...],[numer1,...])
KSTest2([number1,...],[numer1,...], tails, signif)
LillieforsTest([number1,...])
MannWhitney([number1,...],[number1,...],hypothesis type,alpha, use normal approximation)
SSMD(mean max, mean min, n max, n min, SD max, SD min)
StainIndex(positive population mean/median, negative population mean/median, negative population standard deviation)
TTest1S([number1,...],expected mean)
TTest2S([number1,...],[number1,...],tails, paired)
Wilcoxon([number1,...],[number1,...],hypothesis type,alpha)
ZPrime(mean negative, SD negative, mean positive, sd positive)
ZTest([number1,...],expected mean, expected sd)
```

Date Time

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```
DateKWToDate("DateKeyword", number)
DateTimeToStrFMT(time, format)
DaysBetween(start date, end date)
FormatDateTime("format","Date and/or time")
HoursBetween(start time, end time)
MillisecondsBetween(start time, end time)
MinutesBetween(start time, end time)
SecondsBetween(start time, end time)
StrToDateFMT(text, format, delimiter)
StrToTimeFMT(text, time delimiter, milisecond delimiter)
TimeKWToTime("TimeKeyword")
YearsBetween(start date, end date)
```

Regression

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BoltzmannXToY(x, [modelParameters])
BoltzmannYToX(y, [modelParameters])
CubicXToY(x, [modelParameters])
CubicYToX(y, [modelParameters])
ExpAssociationXToY(x, [modelParameters])
ExpAssociationYToX(y, [modelParameters])
ExponentialGrowXToY(x, [modelParameters])
ExponentialGrowYToX(y, [modelParameters])
ExponentialXToY(x, [modelParameters])
ExponentialYToX(y, [modelParameters])
ExtractBoltzmannResults([modelParameters], "Bottom" | "Top" | "V50" | "Slope" | "r2")
ExtractCubicResults([modelParameters], "m" | "b" | "A2" | "A3" | "r2")
ExtractExpAssociationResults([modelParameters], "Ymax1" | "K1" | "r2")
ExtractExponentialGrowResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractExponentialResults([modelParameters], "A" | "M" | "C" | "r2")
ExtractFiveParmLogisticResults([modelParameters], "Imin" | "Imax" | "IC50" | "HillSlope" | "Asymmetry" | "r2")
ExtractFourParmLogisticResults([modelParameters], "Imin" | "Imax" | "IC50" | "HillSlope" | "r2")
ExtractGaussianResults([modelParameters], "A" | "SD" | "xMed" | "K" | "r2")
ExtractLinearResults([modelParameters], "m" | "b" | "r2")
ExtractMichMentenResults([modelParameters], "Vm" | "KM" | "r2")
ExtractParabolicResults([modelParameters], "m" | "b" | "A2" | "r2")
ExtractPowerResults([modelParameters], "A1" | "B1" | "K" | "r2")
ExtractPowerSeriesResults([modelParameters], "A1" | "B1" | "K" | "A2" | "B2" | "r2")
ExtractSigmoidalDecResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractSigmoidalResults([modelParameters], "A" | "B" | "C" | "r2")
ExtractSineWaveResults([modelParameters], "Baseline" | "Amplitude" | "Frequency" | "Offset" | "r2")
ExtractTwoPhaseExpAssociationResults([modelParameters], "Ymax1" | "K1" | "Ymax2" | "K2" | "r2")
ExtractTwoPhaseExponentialResults([modelParameters], "A" | "M" | "C" | "A2" | "M2" | "r2")
ExtractTwoSiteBindingResults([modelParameters], "Vm" | "KM" | "Vm2" | "KM2" | "r2")
FiveParmLogisticXToY(x, [modelParameters])
FiveParmLogisticYToX(y, [modelParameters])
FourParmLogisticXToY(x, [modelParameters])
FourParmLogisticYToX(y, [modelParameters])
GaussianXToY(x, [modelParameters])
GaussianYToX(y, [modelParameters])

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LinearXToY(x, [modelParameters])
LinearYToX(y, [modelParameters])
MichMentenXToY(x, [modelParameters])
MichMentenYToX(y, [modelParameters])
ParabolicXToY(x, [modelParameters])
ParabolicYToX(y, [modelParameters])
PerformBoltzmannFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformCubicFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExpAssociationFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExponentialFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformExponentialGrowFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformFiveParmLogisticFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformFourParmLogisticFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformGaussianFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformLinearFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformMichMentenFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformParabolicFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformPowerFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformPowerSeriesFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSigmoidalDecFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSigmoidalFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformSineWaveFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoPhaseExpAssociationFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoPhaseExponentialFit([xValues], [yValues], [SDvalues], [fixedParameters])
PerformTwoSiteBindingFit([xValues], [yValues], [SDvalues], [fixedParameters])
PowerSeriesXToY(x, [modelParameters])
PowerSeriesYToX(y, [modelParameters])
PowerXToY(x, [modelParameters])
PowerYToX(y, [modelParameters])
SigmoidalDecXToY(x, [modelParameters])
SigmoidalDecYToX(y, [modelParameters])
SigmoidalXToY(x, [modelParameters])
SigmoidalYToX(y, [modelParameters])
SineWaveXToY(x, [modelParameters])
SineWaveYToX(y, [modelParameters])
TwoPhaseExpAssociationXToY(x, [modelParameters])
TwoPhaseExpAssociationYToX(y, [modelParameters])
TwoPhaseExponentialXToY(x, [modelParameters])
TwoPhaseExponentialYToX(y, [modelParameters])
TwoSiteBindingXToY(x, [modelParameters])
TwoSiteBindingYToX(y, [modelParameters])

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For in-depth definitions for each non-Regression Function, please visit our Custom Token Formula Reference 769.

For in-depth definitions for each Regression Function, please visit our Regression Analysis section 567.

For a table indicating the equation, the formula associated with it, and the parameters defined in FCS Express when a fit is performed, please visit our section on the Available Equations 578.

19.4.12. Using Boolean Values in Custom Tokens 26

It is possible to create a custom token that returns a Boolean value. For example, the statistic chosen in the custom token, **Condition1**, shown in Figure 19.58, was set on a gate drawn around the lymphocyte population. **Condition1** will evaluate to TRUE or FALSE, depending on the number of cells in the lymphocyte gate.

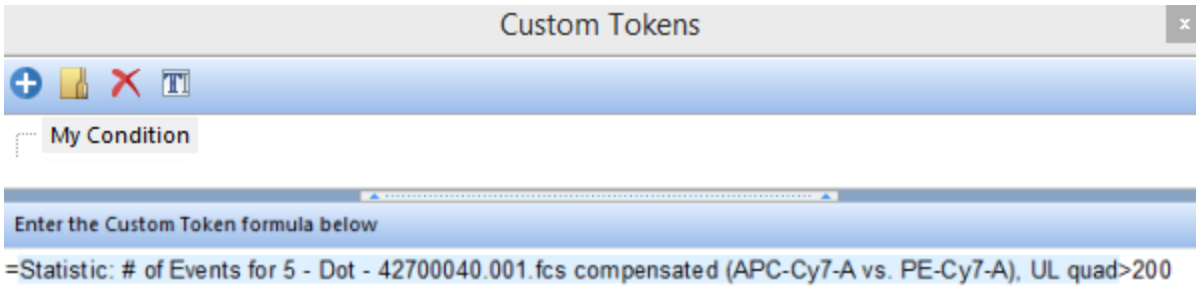

Figure 19.58 Token that Evaluates to TRUE or FALSE

You can use the **My Condition** token inside another token. To do so, set the result type of the token to Numeric and use the IsTrue or IsFalse function 769, as shown in Figure 19.59.

```
=IF IsTrue(Custom Token: My Condition) THEN "There are at least 200 events
in my quadrant." ELSE "There are less than 200 events in my quadrant."
```

Figure 19.59 Using a Boolean token

19.4.13 Deleting Custom Tokens

To delete a custom token, right-click on the token in the Custom Token window list 728 and select **Delete** from the pop-up menu. If the deleted token is used in the formula of another Custom Token, the remaining token will display **##ERROR##** if it is used in a text box.

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Also, a custom token can be deleted by selecting the token in the Custom Token window List, and by clicking the Delete button in the Custom Token window toolbar.

19.4.14 Custom Token Display Types

FCS Express 6 has new custom token **Display types** which allow you to insert an interactive **Edit box**, **Check box**, or **Dropdown list** directly in a layout (Figure 19.60). The new Display type feature can be accessed by right-clicking on any custom token displayed on the layout and choosing **Format Token** (alternatively, double click on the custom token to open the **Edit** custom token dialog). Within the **Edit** custom token dialog click on the **Display type** dropdown and choose:

- **Edit box** 762 **-** creates an editable box directly in the layout where you can change custom token values which will update the original custom token and subsequently any other instance of the custom token
- **Check box** 764 **-** creates a check box directly in the layout that displays the name of the custom token. Checking the box returns a "TrueValue" entry for the custom token while unchecking the box returns a "FalseValue" entry for the custom token.
- **Dropdown list** 767 **-** creates a dropdown list of comma separated choices entered in the **Choices** section of the **Edit** custom token dialog **Display** category**.**

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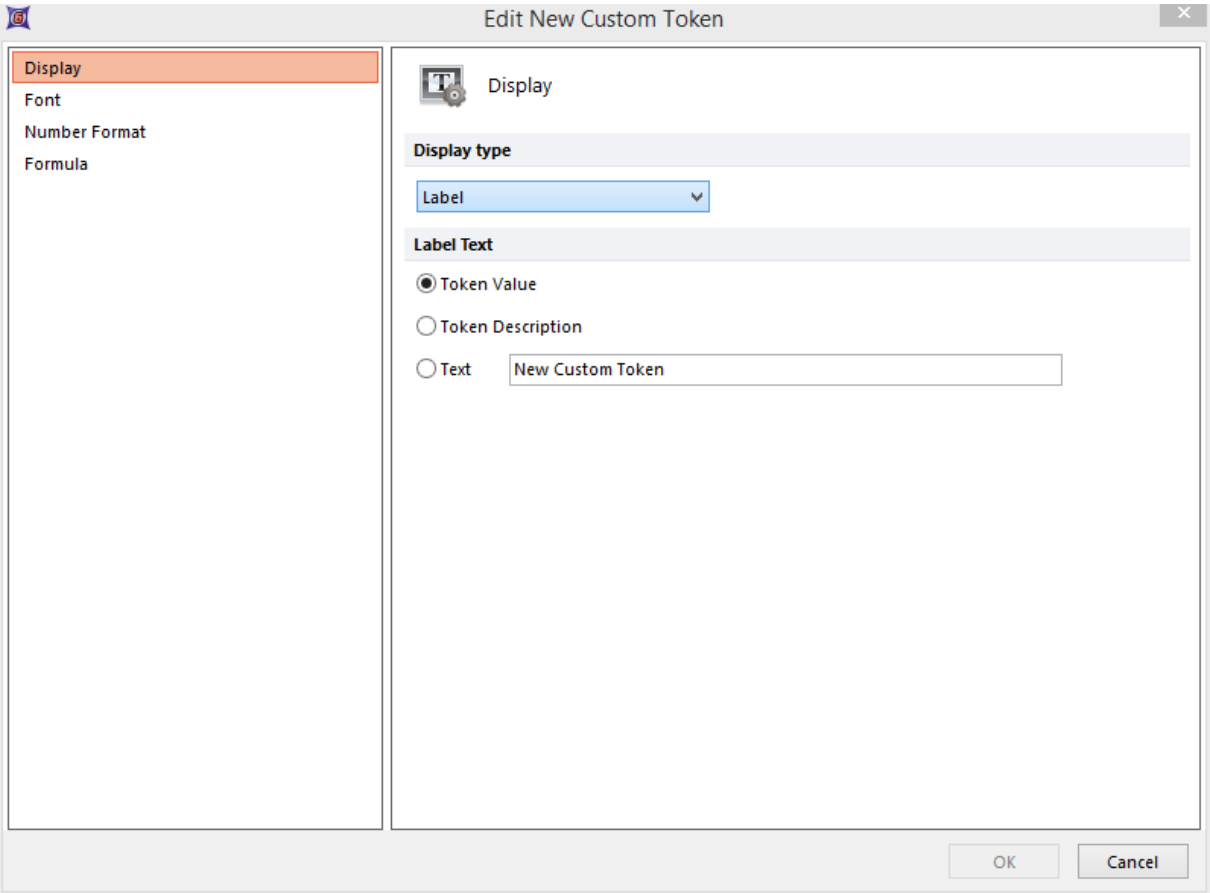

Figure 19.60 The Display type feature in FCS Express allows you to create editable text fields, check boxes, and dropdown lists directly in your layout.

19.4.14. Edit Box Custom Tokens
1

The **Edit box** Display type allows you to create an editable box directly in the layout where you can change Custom Token values which will update the original Custom Token and subsequently any other instance of the Custom Token. To create an Edit Box Custom Token:

1. Create a new Custom Token with or without text.
2. Drag and drop the Custom Token from the **Custom Token** window onto the layout to insert it.
3. Double-click the Custom Token in the layout to open the **Edit** custom token dialog.
4. Change the **Display** type to **Edit box** (Figure 19.61).

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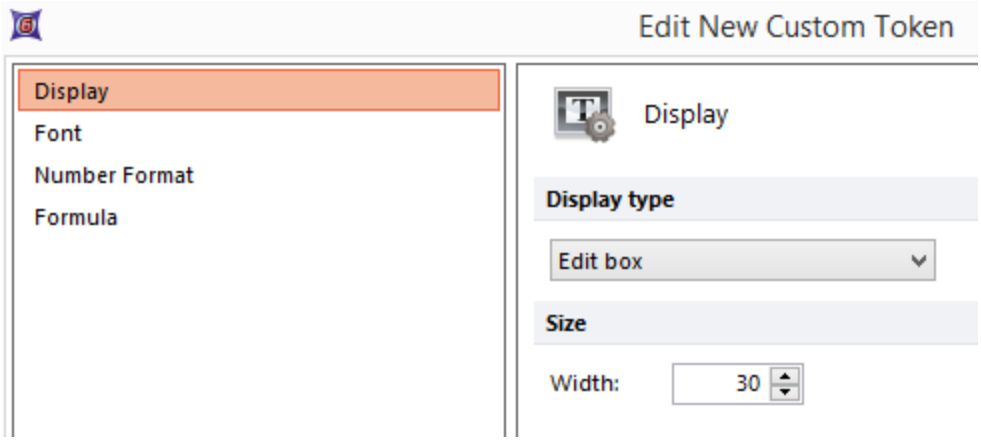

Figure 19.61 The custom token display type is set to edit box.

5. Click **OK**.

An **Edit box** will appear within the text box in the layout where the token was inserted (Figure 19.62).

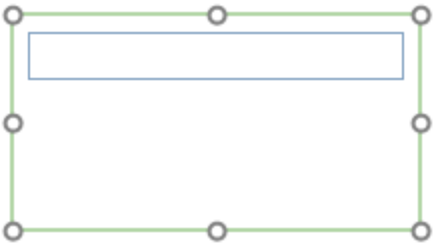

Figure 19.62 When the Custom Token Display type is set to Edit box, an editable box will appear in the layout. This one appears empty as the original Custom Token formula was not defined (i.e., left empty)

6. Enter or change any text in the **Edit box**.

Once a value has been entered, the custom token value in the **Custom Tokens** navigator will reflect the value entered in the **Edit box**. The Custom Token may then be dragged and dropped from the **Custom Tokens** window to any other location in the layout. When dragging and dropping the token from the **Custom Tokens** window, the default display type of "label" will be used thus displaying only the value entered in the **Edit box**. When the value is updated in the **Edit box,** the Custom Token will update wherever displayed. (Figure 19.63)

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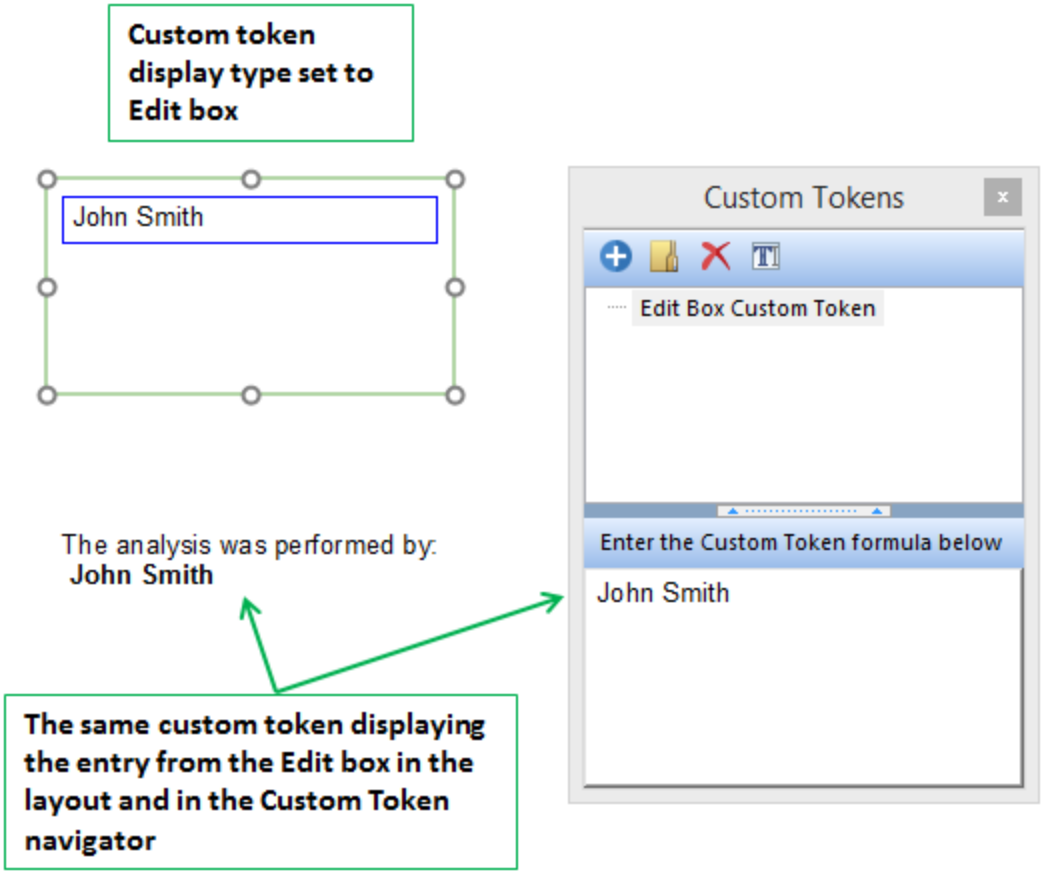

Figure 19.63 The text of "John Smith" was entered in the Edit box. The value was updated in the Custom Token window and also in the secondary text box containing the token.

19.4.14. Check Box Custom Tokens 2

The **Check box** Display type allows you to create a check box directly in the layout that displays the name of the Custom Token. Checking the box returns a "TrueValue" entry for the Custom Token while unchecking the box returns a "FalseValue" entry for the custom token. To create a Check box Custom Token:

1. Create a new Custom Token with or without text.
2. Drag and drop the Custom Token from the **Custom Token** window onto the layout to insert it.
3. Double-click the Custom Token in the layout to open the **Edit** custom token dialog.
4. Change the **Display type** to **Check box** (Figure 19.64).

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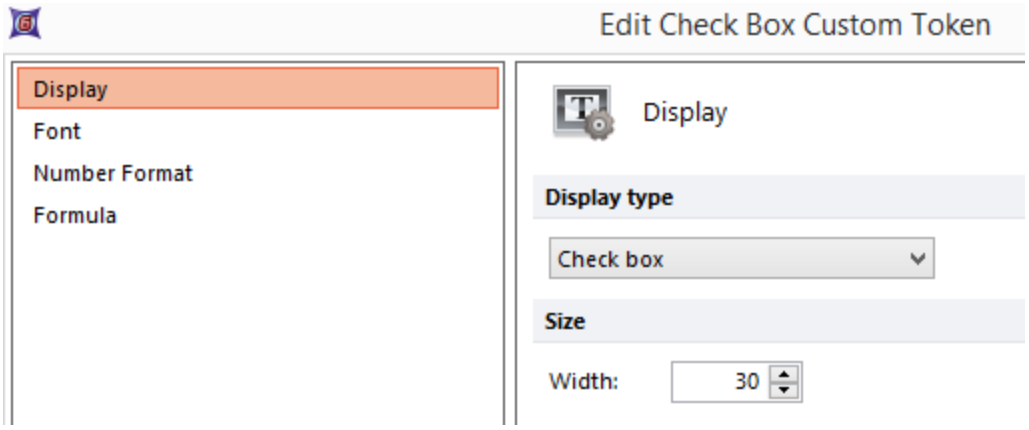

Figure 19.64 The custom token display type is set to Check box.

5. Click **OK**.

A **Check box** will appear within the text box in the layout where the token was inserted (Figure 19.65).

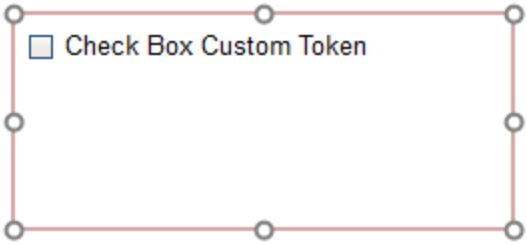

Figure 19.65 When the custom token display type is set to Check box, a check box will appear in the layout displaying the name of the custom token.

6. Check or uncheck the box next to the Custom Token name in the layout.

Once the box has been checked, the Custom Token returns a "TrueValue" entry for the Custom Token. Unchecking the box returns a "FalseValue" entry for the Custom Token (Figure 19.66). The token value, "TrueValue" or "FalseValue" may then be used in any location within FCS Express where a True or False evaluation is made via tokens such as displaying pages or IF, THEN, ELSE statements.

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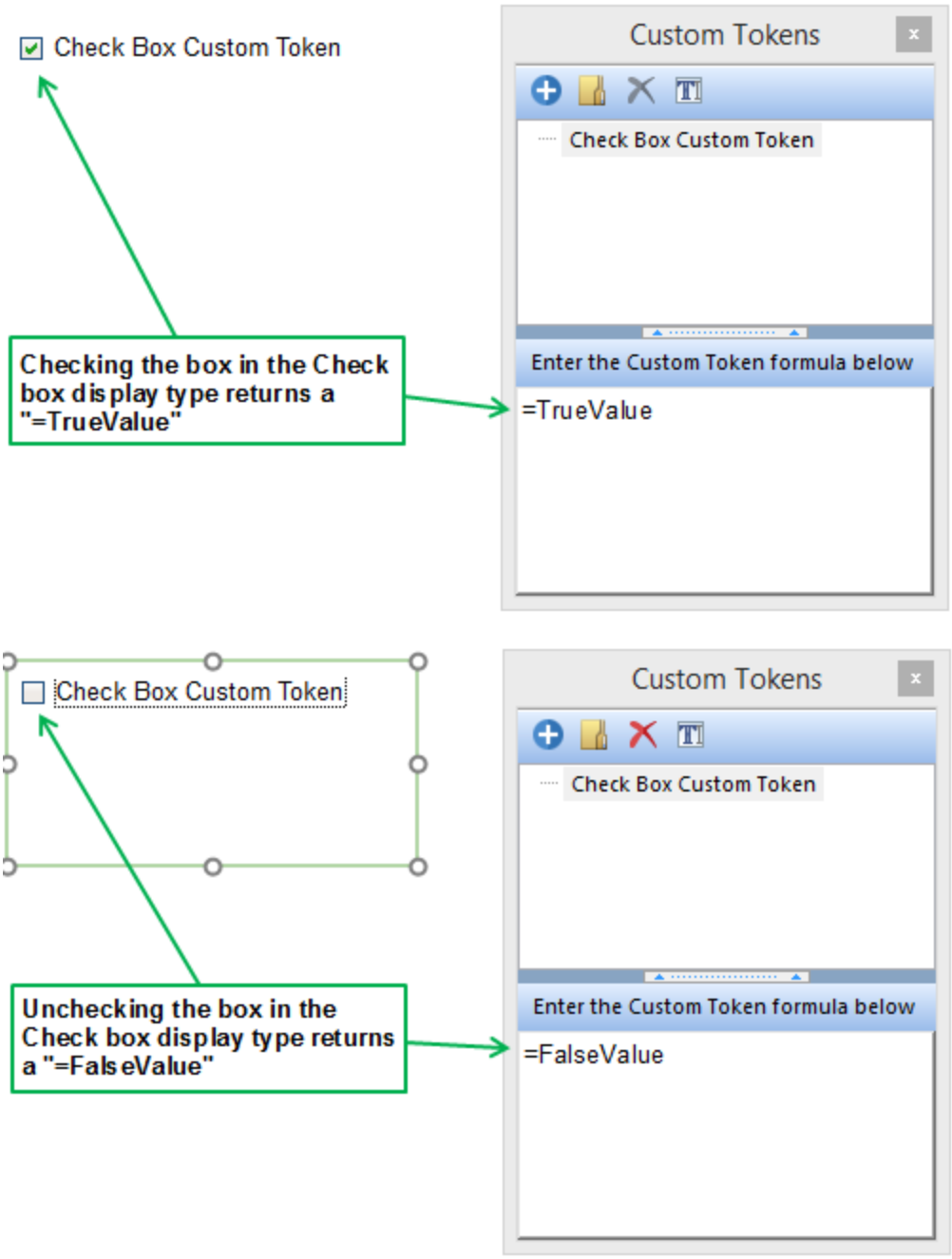

Figure 19.66) Once the box has been checked, the Custom Token returns a "TrueValue" entry for the Custom Token. Unchecking the box returns a "FalseValue" entry for the Custom Token.

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19.4.14. Dropdown Custom Tokens 3

The **Dropdown list** Display type allows you to create a list of comma-separated values to appear in a dropdown list within the layout. To create a **Dropdown list**:

1. Create a new custom token with or without text.
2. Drag and drop the custom token from the **Custom Token** window onto the layout to insert it.
3. Double-click the custom token in the layout to open the **Edit** custom token dialog.
4. Change the **Display** type to **Dropdown list**.
5. Enter a list of comma-separated choices for the list in the text field (alternatively, choose to use any token values by clicking on the T button) (Figure 19.67).

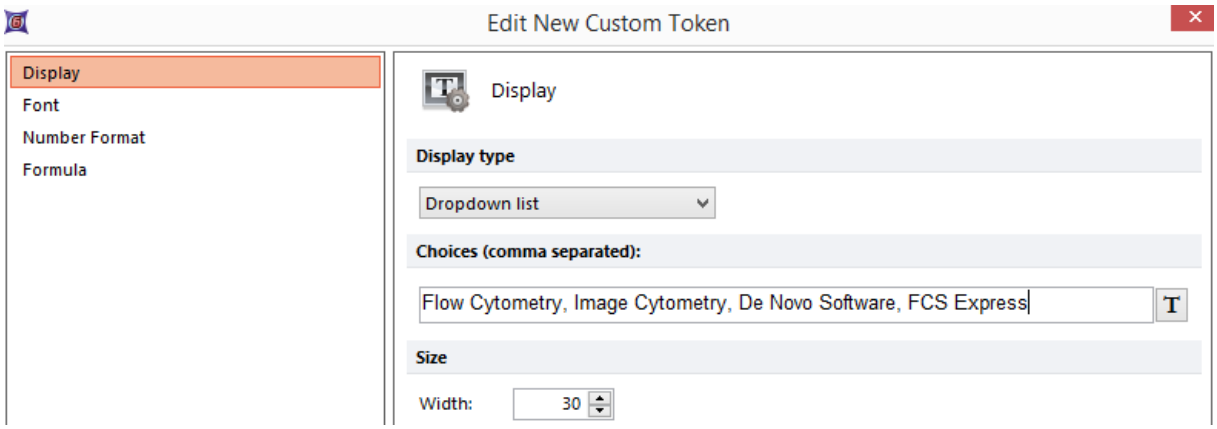

Figure 19.67 Enter a list of choices separated by a comma to be displayed in the dropdown list.

6. Click **OK**.

An empty box will appear within the text box in the layout where the token was inserted (Figure 19.68).

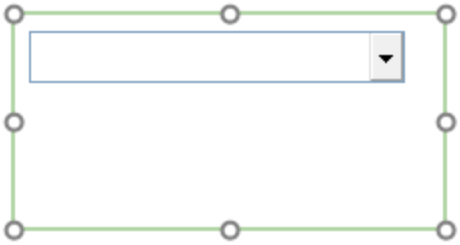

Figure 19.68 An empty dropdown list box appears in the layout where the token was originally inserted.

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7. Click on the dropdown arrow to reveal the contents of the Dropdown list (Figure 19.69).

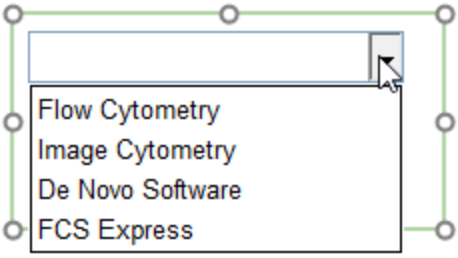

Figure 19.69 Once the dropdown arrow is pressed a list of the comma separated choice that were entered will appear.

8. Click on any of the choices in the Dropdown list to fill the box (Figure 19.70).

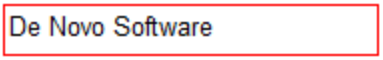

Figure 19.70 The dropdown list after the "De Novo Software" choice was selected.

Once a choice has been selected, the Custom Token value in the **Custom Tokens** window will reflect the choice made in the Dropdown list. The Custom Token may then be dragged and dropped from the **Custom Tokens** window to any other location in the layout. When dragging and dropping the token from the **Custom Tokens** window, the default display type of "label" will be used, thus displaying only the value chosen in the Dropdown list. When the value is updated via the Dropdown list the custom token will update wherever displayed. (Figure 19.71)

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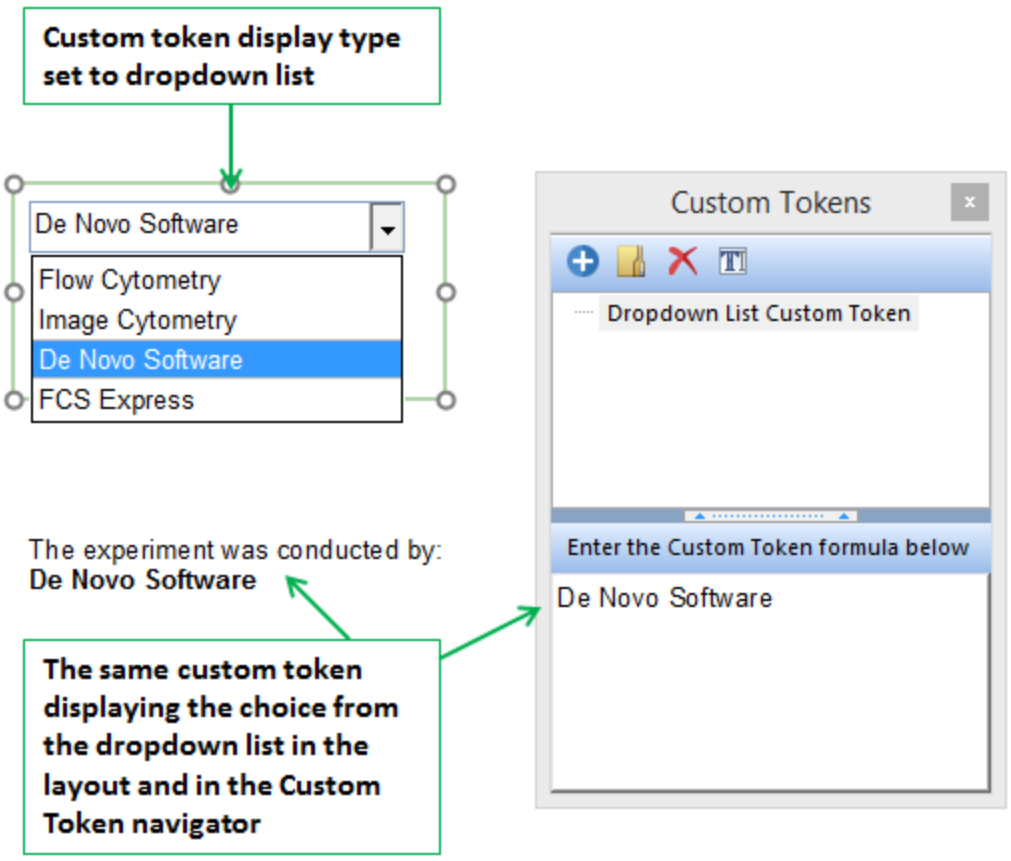

Figure 19.71 The value of "De Novo Software" was chosen from the dropdown list. The value was updated in the Custom Token navigator and also in the secondary text box containing the token.

19.5 Custom Token Formula Reference

The following tables are a reference of all operators and functions available for creating custom tokens in the Custom Token Editor 728 and with Parameter Math 933.

Arithmetic Operators

Operator	Description
x+y	Add x and y
x-y	Subtract y from x
x*y	Multiply x and y
x/y	Divide x by y
x^y	x to the y power

Conditional/Comparison Operators. Use these with IF..THEN..ELSE statements.

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Operator	Description
x>y	x is greater than y
x<y	x is less than y
x=y	x is equal to y
x>=y	x is greater than or equal to y
x<=y	x is less than or equal to y
x<>y	x is not equal to y

Logical Operators. These are used in IF..THEN..ELSE statements or to combine two equations.

Operator	Description
x OR y	x OR y
x AND y	x AND y
NOT (x AND y)	Inverse of the value of (x AND y)
Example: if (x>20 and y>20) then.....else If combining multiple logical operators, parenthesis must surround each operator. Example: if ((x>20 and y>20) and (a<1 or b>10)) then.....else	

Mathematical Functions

Function	Description
Abs(x)	Absolute value of x
ArcCos(x)	Arc Cosine of x
ArcCosh(x)	Hyperbolic Arc Cosine of x
ArcSin(x)	Arcsine of x
ArcSinh(x)	Hyperbolic Arcsine of x
ArcTan(x)	Arctangent of x
ArcTanh(x)	Hyperbolic Arctangent of x
ConvertLinToLog(I,J,K,L)	Converts a linearly scaled value into a logarithmically scaled value, using the following parameters: I = Lin value, J = # of decades, K = output range (with values of 0, 256, 512, or 1024), L = offset This will convert a value from 1-10,000 to 0-1024.
ConvertLogToLin(I,J,K,L)	Converts a Logarithmically scaled value into a Linearly scaled value, using the following parameters: I = Log value, J = # of decades, K = input range, L = offset This will convert a value from 0-1024 to 1-10,000.
Cos(x)	Cosine of x
Cosh(x)	Hyperbolic Cosine of x
CoTan(x)	Cotangent of x
Ln(x)	Natural logarithm of x
Log(x,y)	Logarithm of y in base x
Max(x,y)	Returns the higher value of x and y.
Maximum([x,y,z...])	Returns the maximum value in the array ([x,y,z,...]). The array can hold an unlimited number of elements.

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Function	Description
Mean([x,y,z...])	Returns the mean of the values in the array [x,y,z,…]. The array can hold an unlimited number of elements.
Min(x,y)	Returns the lower value of x and y.
Minimum([x,y,z...])	Returns the minimum value in the array ([x,y,z,…]). The array can hold an unlimited number of elements.
Range([x,y,z...])	Returns the result of (Maximum([x,y,z,…]) – Minimum([x,y,z,…])).
Sin(x)	Sine of x
Sinh(x)	Hyperbolic Sine of x
Sqr(x)	x squared
Sqrt(x)	Square root of x
StdDev([x,y,z...])	Returns the standard deviation of the values in the array [x,y,z,…]. The array can hold an unlimited number of elements.
Tan(x)	Tangent of x

Type Conversion Functions. These are used in more advanced formulas, to convert a number to text and vice versa. They are used when the value returned by a particular token is not the type that you need, and you want to convert it to a type that you can include in your formula. You could also change the result type as described in Custom Token Formatting 746.

Function	Description
NumericToString(x)	Converts the number x to text (also known as a string).
StringToNumeric(x)	Converts the string x to a number.

Logic (True/False) Functions. These functions return the truth values of applying an operation to the incoming parameters. Some of the functions return a numerical representation of True (-1) or of False (0).

Function	Description
Between(x,y,z)	Returns True if x is between y and z (including being equal to y or z), False otherwise.
FalseValue	Returns 0.
IsFalse(x)	Returns True if x has a value of 0.
IsSameText([Text1, Text2],CaseSensitive)	Returns True if Text1 and Text2 are the same. If CaseSensitive is true, the words must match in case, in addition to its content.
IsTrue(x)	Returns True if x has a value of -1.
TrueValue	Returns -1.

Text Manipulation Functions. These functions can be used to manipulate or get information out of existing text values.

Function	Description
AreValuesInRange([x,y,z...],tolerance,	If the values in the array [x,y,z...] are all within tolerance % of each other, it returns True. If a pair of values in the array are not

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<table>
<tr><th>Function</th><th>Description</th></tr>
<tr><td>[xName,yName,zName ...])</td><td>within tolerance % of each other, it returns text indicating which ones are not.</td></tr>
<tr><td>Copy(x,y,z)</td><td>X is the full text. Y is the position to start copying text. Z is the number of letters (also known as characters) to copy from the full text. Returns a subset of the text x, starting at position y, copying z characters in total.</td></tr>
<tr><td>ExtractFilename("file_path")</td><td>Accepts a file name and will return the text of the data file name. For example, if a file path is C:\Data Files\Today\FlowData1.fcs, ExtractFilename("file_path")will return FlowData1.fcs.
Keyword tokens using the keywords $FIL, $FCSE_FILENAME or $FCSE_FILENAMENOPATH can be used for this function.</td></tr>
<tr><td>ExtractFolderFromFilename("file_path")</td><td>Accepts a file name and will return the text of the file path of the folder in which the data is contained.
For example, if a file path is C:\Data Files\Today\FlowData1.fcs, ExtractFolderFromFilename("file_path") will return C:\Data Files\Today.
Keyword tokens using the keyword $FCSE_FILENAME can be used for this function.</td></tr>
<tr><td>FormatFloat("format string",number)</td><td>FormatFloat formats the floating-point value given by Number using the format string given by Format String. The following format specifiers are supported in the format string:

Specifier — Represents
0 — Digit placeholder. If the value being formatted has a digit in the position where '0' appears in the format string, then that digit is copied to the output string. Otherwise, a '0' is stored in that position in the output string.
— Digit placeholder. If the value being formatted has a digit in the position where '#' appears in the format string, then that digit is copied to the output string. Otherwise, nothing is stored in that position in the output string.
. — Decimal point. The first '.' character in the format string determines the location of the decimal separator in the formatted value; any additional '.' characters are ignored. The actual character used as a decimal separator in the output string is determined by the DecimalSeparator global variable or its equivalent.
, — Thousand separator. If the format string contains one or more ',' characters, the output will have thousand separators inserted between each group of three digits to the left of the decimal point. The placement and number of ',' characters in the format string does not affect the output, except to indicate that thousand separators are wanted. The actual character used as a thousand separator in the output is determined by the ThousandSeparator global variable or its equivalent.
E+ — Scientific notation. If any of the strings 'E+', 'E-', 'e+', or 'e-' are contained in the format string, the number is</td></tr>
</table>

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Function	Description
	formatted using scientific notation. A group of up to four '0' characters can immediately follow the 'E+', 'E-', 'e+', or 'e-' to determine the minimum number of digits in the exponent. The 'E+' and 'e+' formats cause a plus sign to be output for positive exponents and a minus sign to be output for negative exponents. The 'E-' and 'e-' formats output a sign character only for negative exponents. Learn more about working with Scientific Notation. 'xx'/"xx" Characters enclosed in single or double quotation marks are output as such and do not affect formatting. ; Separates sections for positive, negative, and zero numbers in the format string. The locations of the leftmost '0' before the decimal point in the format string and the rightmost '0' after the decimal point in the format string determine the range of digits that are always present in the output string. The number being formatted is always rounded to as many decimal places as there are digit placeholders ('0' or '#') to the right of the decimal point. If the format string contains no decimal point, the value being formatted is rounded to the nearest whole number. If the number being formatted has more digits to the left of the decimal separator than there are digit placeholders to the left of the '.' character in the format string, the extra digits are output before the first digit placeholder. To allow different formats for positive, negative, and zero values, the format string can contain between one and three sections separated by semicolons, as follows: * One section: The format string applies to all values. * Two sections: The first section applies to positive values and zeros, and the second section applies to negative values. * Three sections: The first section applies to positive values, the second applies to negative values, and the third applies to zeros. If the section for negative values or the section for zero values is empty, that is if there is nothing between the semicolons that delimit the section, the section for positive values is used instead. If the section for positive values is empty or if the entire format string is empty, the value is formatted using general floating-point formatting with 15 significant digits. General floating-point formatting is also used if the value has more than 18 digits to the left of the decimal point and the format string does not specify scientific notation.
FragmentCount("text1","text2")	Returns the number of string fragments in text2 separated by text1. Text1 will equal a delimiter, such as "\", for example. Text 2 will be a pathway.

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Function	Description
	For example, **FragmentCount("\","C:\Documents\Data\Sample.fcs")** will return **4**, since there are 4 fragments that are delimited by "\". Keywords that return a pathway can be used for Text2.
GetContainingFolder("file_path")	Returns the name of the folder containing the file named in file_path. Example, if file **Sample.fcs** was located in the directory *C:\Program Files\De Novo Software\FCS Express 6 Plus Research Edition/Data*, the output for this function would be **Data**.
GetFragment(n, "text1", "text2")	Returns the **N**th string fragment in **Text2**, delimited by **Text1**. **N** = the nth occurrence, **Text1** = a delimiter, such as "\", **Text2** = a string that uses **Text1** as a delimiter. Some examples of **Text2** could be: ▪ a pathway that uses "\" as a delimiter, such as **C:\Documents\Data\Sample.fcs.** ▪ a name of a file where several identifiers have been concatenated together and delimited by "_", such as **FirstnameLastname_ID1_ID2_ID3_Date**. For example, **GetFragment(1, "\", "C:\Documents\Data\Sample.fcs")** will return **C:**. **GetFragment(2, "\", "C:\Documents\Data\Sample.fcs")** will return **Documents**. If **n** is a negative number, the function will start evaluating the text string backwards. For example, **GetFragment(-2,"_","SheldonCooper_ABC_DEF_GHI")** will return **DEF.**
IncludeTrailingPathDelimiter("file_path")	Accepts the value of "File_path", while including the delimiter (\, for example) at the end of the string.
Pos(x,y)	X is the text that you are looking for. Y is the text that you are searching in. Returns the position of x inside y. If x cannot be found in y, the return value is 0.
PositionInText("text1","text2",StartingPosition,N)	**PositionInText** returns the position of a string within another string. **Text1** = the text for which you are searching **Text2** = the text in which you are searching **StartingPoint** = the position at which to start searching (1,2,3...etc.) **N** = the Nth occurrence for the string for which you are searching (1 for 1st occurrence, 2 for 2nd occurrence, etc.)

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Function	Description
	For example **=PositionInText("d","abcdefgabcdefg",1,1)** would return 4 because the first occurrence (N=1) of "d" occurs at position 4. **=PositionInText("d","abcdefgabcdefg",1,2)** would return 11 because the second occurrence (N=2) of "d" occurs at position 11. This function would return a "0" if no occurrence can be found.
RemoveSpaces(x)	Removes the spaces from the text x, and returns the resulting text.
RemoveText(x,y,RemoveAll)	This function will remove the string **x** from **y**. RemoveAll will equal True or False depending on whether only the first occurrence is to be removed. For example, **=RemoveText("def","abcdefg",True)** will return **abcg.**
ReplaceText(x,y,z,RemoveAll)	This function will replace the string **x** with the string **y** located within the string **z**. RemoveAll will equal True or False depending on whether only the first occurrence is to be removed. For example, **=ReplaceText("def","xyz","abcdefg",True)** will return **abcxyzg**.
x+y	If x and y are both text (i.e., strings), returns the concatenation of x with y.
Trim	Removes spaces before and after a string of text. For example, =**Trim(" abcd efgh ")** will return **abcd efgh**

Statistical Functions

Operator	Description
ChiSquareTest([number1,...], [number2,...])	Performs the Chi-squared goodness of fit test to test if data is coming from specific distribution. The first parameter is an array of observed frequencies, the second parameter is an array of estimated (theoretical) frequencies.
FTest([number1,...],[number2,...])	Performs F-Test by comparing number1 and number2 standard deviations. The parameters are the first and the second dataset.
GrubbsTest([number1,...])	Performs the Grubbs test for outliers. Test is used to detect outliers in a univariate data set. It is based on the assumption of normality. That is, you should first verify that your data can be reasonably approximated by a normal distribution before applying the Grubbs' test. The parameter is the dataset.
KSTest([number1,...],[number2,...])	Performs two-sample Kolmogorov-Smirnov goodness of fit test of independent random samples Data1 and Data2. Test determines if Data1 and Data2 samples are drawn from the same continuous population. The parameters are the first and the second dataset.

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Operator	Description
KSTest2([number1,...],[number1,...], tails, significance)	Performs two-sample Kolmogorov-Smirnov goodness of fit test of independent random samples Data1 and Data2. Test determines if Data1 and Data2 samples are drawn from the same continuous population. The parameters are the first and the second dataset, the hypothesis type ("**TWO_TAILED**", "**LEFT_TAILED**" or "**RIGHT_TAILED**" (not case sensitive and requires quotes)), and significant level for which to test. For example, 0.05.
LillieforsTest([number1,...])	Performs the Lilliefors goodness of fit test to a normal distribution with unknown parameters mu and sigma. The parameter is the dataset.
MannWhitney([number1,...], [number1,...],hypothesis type,alpha, use normal approximation)	The Mann Whitney test is is a nonparametric test of the null hypothesis that two samples come from populations of equal means, against an alternative hypothesis, especially that a particular population has a larger mean than the other one. It operates under the assumption that all the observations from both groups are independent of each other. (Credit: Wikipedia) The variables for the functions are as follows: **[Number1.....]** - Comma separated values from the 1st dataset. **[Number2.....]** - comma separated values from the 2nd dataset. **Hypothesis Type** - **TWO_TAILED**, **LEFT_TAILED** or **RIGHT_TAILED** (not case sensitive) **Alpha** - the significance level for which to test. For example, **0.05**. **Use Normal Approximation** - **True/False**
SSMD(mean max,mean min,n max,n min,SDmax,SDmin)	Short for Strictly Standardized Mean Difference. Has been used as a ranking metric for hit selection in high throughput screening assays.
StainIndex(positive population mean/median, negative population mean/median, negative population standard deviation).	Performs a stain index calculation using the means/medians of positive and negative populations and the negative population standard deviation based on the Stain Index formula of (Median of Positive - Median of Negative) / (SD of Negative * 2).
TTest1S([number1,...],expected mean)	Performs the one sample T-test. It compares Data mean value with the expected mean. The first parameter is the dataset.
TTest2S([number1,...], [number2,...],tails,paired)	It compares Number1 mean value with Number2 mean value. The assumption is Number1 and Number2 variances are equal, but unknown. The first 2 parameters are the first and the second datasets. Tails parameter can be **1** or **2**. It is

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Operator	Description
	used to define the type of null hypothesis, a value of 1 means left tailed, a value of 2 means two tailed. Paired can be **True** or **False**. If **True**, it will perform Two-Sample Paired t-Test. If **False**, it will perform Two-Sample Pooled (unpaired) t-Test.
Wilcoxon([number1,...], [number1,...],hypothesis type,alpha)	The Wilcoxon test is a nonparametric test that compares two paired groups. The test calculates the difference between each set of pairs and analyzes these differences. The Wilcoxon Rank Sum test can be used to test the null hypothesis that two populations have the same continuous distribution. (credit: Investopedia) The variables for the functions are as follows: **[Number1.....]** - Comma separated values from the 1st dataset. **[Number2.....]** - comma separated values from the 2nd dataset. **Hypothesis Type** - **TWO_TAILED**, **LEFT_TAILED** or **RIGHT_TAILED** (not case sensitive) **Alpha** - the significance level for which to test, for example, **0.05**.
ZPrime(mean negative,SD negative,mean positive,SD positive)	The ZPrime function is used to calculate the Z Factor. The Z-factor is an attempt to quantify the suitability of a particular assay for use in a full-scale, high-throughput screen by comparing the Mean and Standard Deviation of a negative control to the Mean and Standard Deviation of a positive control within the experiment.
ZTest([number1,...],expected mean,expected SD)	Compares the normally distributed Data elements mean value with known standard deviation **expected SD**, to a mean value **expected mean**. **([Number1,...])** is the dataset.

Date and Time Manipulation Functions

Function	Description
DateKWToDate($DATE keyword,n)	Takes the standardized format of the $DATE keyword, which is in either dd-mmm-yy or dd-mmm-yyyy format, and returns mm/dd/yyyy. If $DATE has the year listed as yy, n will be placed in front of yy to produce yyyy. A keyword token will be used to insert the value of $DATE. For example if the $DATE keyword equals 03-Nov-05, **DateKWToDate($DATE,20)** will return 11/03/2005.

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Function	Description
	If the year is already formatted as yyyy, the function will still return mm/dd/yyyy. If the year is already formatted as yyyy, n must still be accounted for in the function. For example, if $DATE keyword equals 04-Jul-2008, **DateKWToDate($DATE,20)** will return 07/04/2008. Also, **DateKWToDate($DATE,0)** will return 07/04/2008.
DaysBetween(StartDate,EndDate)	Returns the number of days between the values entered for the Start Date and End Date. Dates in anything other than a mm/dd/yyyy format will need to be run through **DateKWToDate** and/or **FormatDateTime** prior to being used within this function.
FormatDateTime("format","Date or Time")	FormatDateTime formats the Time or Date value given by using the format given. Example: =FormatDateTime("yyyy-mm-dd",(<Current Date token>)) will return the Current Date in a format showing Year-Month-Day, with 2 digits for the month and day, and 4 digits for the year. =FormatDateTime("hh:mm:ss",(<Current Time token>)) will return the Current Time in a format showing Hour:Minute:Second, with 2 digits for Hour, Minute and Second. *Please note that if using the <Current Date> token, no quotation marks are needed, as the **Result Type** for this token is **Text**. Formats can be as follows below: **Specifier Displays** : c Displays the date using the format given by the ShortDateFormat global variable, followed by the time using the format given by the LongTimeFormat global variable. The time is not displayed if the date-time value indicates midnight precisely. d Displays the day as a number without a leading zero (1-31). dd Displays the day as a number with a leading zero (01-31). ddd Displays the day as an abbreviation (Sun-Sat) using the strings given by the ShortDayNames global variable. dddd Displays the day as a full name (Sunday-Saturday) using the strings given by the LongDayNames global variable. ddddd Displays the date using the format given by the ShortDateFormat global variable. dddddd

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Function	Description
	Displays the date using the format given by the LongDateFormat global variable. e (Windows only) Displays the year in the current period/era as a number without a leading zero (Japanese, Korean, and Taiwanese locales only). ee (Windows only) Displays the year in the current period/era as a number with a leading zero (Japanese, Korean, and Taiwanese locales only). g (Windows only) Displays the period/era as an abbreviation (Japanese and Taiwanese locales only). gg (Windows only) Displays the period/era as a full name (Japanese and Taiwanese locales only). m Displays the month as a number without a leading zero (1-12). If the m specifier immediately follows an h or hh specifier, the minute rather than the month is displayed. mm Displays the month as a number with a leading zero (01-12). If the mm specifier immediately follows an h or hh specifier, the minute rather than the month is displayed. mmm Displays the month as an abbreviation (Jan-Dec) using the strings given by the ShortMonthNames global variable. mmmm Displays the month as a full name (January-December) using the strings given by the LongMonthNames global variable. yy Displays the year as a two-digit number (00-99). yyyy Displays the year as a four-digit number (0000-9999). h Displays the hour without a leading zero (0-23). hh Displays the hour with a leading zero (00-23). n Displays the minute without a leading zero (0-59). nn Displays the minute with a leading zero (00-59). s Displays the second without a leading zero (0-59). ss Displays the second with a leading zero (00-59). z Displays the millisecond without a leading zero (0-999). zzz Displays the millisecond with a leading zero (000-999). t

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Function	Description
	Displays the time using the format given by the ShortTimeFormat global variable. tt Displays the time using the format given by the LongTimeFormat global variable. am/pm Uses the 12-hour clock for the preceding h or hh specifier, and displays 'am' for any hour before noon, and 'pm' for any hour after noon. The am/pm specifier can use lower, upper, or mixed case, and the result is displayed accordingly. a/p Uses the 12-hour clock for the preceding h or hh specifier, and displays 'a' for any hour before noon, and 'p' for any hour after noon. The a/p specifier can use lower, upper, or mixed case, and the result is displayed accordingly. ampm Uses the 12-hour clock for the preceding h or hh specifier, and displays the contents of the TimeAMString global variable for any hour before noon, and the contents of the TimePMString global variable for any hour after noon. / Displays the date separator character given by the DateSeparator global variable. : Displays the time separator character given by the TimeSeparator global variable. 'xx'/"xx" Characters enclosed in single or double quotation marks are displayed as such, and do not affect formatting.
HoursBetween(start time,end time)	Counts the number of hours between the start time and the end time. The keywords $BTIM or $ETIM can be used for the start and end times. However, user-defined times would need to be fed through the StrToTimeFMT function prior to use in this function to establish the proper formatting.
MillisecondsBetween(start time,end time)	Counts the number of milliseconds between the start time and the end time. The keywords $BTIM or $ETIM can be used for the start and end times. However, user-defined times would need to be fed through the StrToTimeFMT function prior to use in this function to establish the proper formatting.
MinutesBetween(start time,end time)	Counts the number of minutes between the start time and the end time. The keywords $BTIM or $ETIM can be used for the start and end times. However, user-defined times would need to be fed through the StrToTimeFMT function prior to use in this function to establish the proper formatting.
SecondsBetween(start time,end time)	Counts the number of seconds between the start time and the end time. The keywords $BTIM or $ETIM can be used for the start and end times. However, user-defined times would need to be fed through the StrToTimeFMT function prior to use in this function to establish the proper formatting.

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Function	Description
StrToDateFMT(text, "format", "delimiter")	Returns a date string in the format as defined by the user, using the specifier displays as seen in FormatDateTime("format","Date or Time") of ddmmyyyy using the delimiter designated, such as "/".
StrToTimeFMT(text, "time delimiter", "millisecond delimiter")	Returns a time string using a time delimiter and a millisecond delimiter.
YearsBetween(Start Date,End Date)	Returns the number of years between the values entered for the Start Date and End Date. Dates in anything other than a mm/dd/yyyy format will need to be run through **DateKWToDate** and/or **FormatDateTime** prior to being used within this function.

Regression. These function allow to fit several regression functions and to calculate the corresponding results. Two of those functions allow to retrieve the X (or the Y) value for a given Y (or a given X) from a Scatter with Regression plot 580 fit.

Function	Description
RegressionPlotXToY(Plot ID, Overlay Number, X Value)	The three mandatory parameters are the Plot number of a regression plot (numeric), the overlay number of a regression fit (numeric) and the X known value (numeric). The function returns the Y value of the regression function given the X input value. Only a single input X value is allowed.
RegressionPlotYToX(Plot ID, Overlay Number, Y Value)	The three mandatory parameters are the Plot number of a regression plot (numeric), the overlay number of a regression fit (numeric) and the Y known value (numeric). The function returns the X value of the regression function given the Y input value. Only a single input Y value is allowed.

SOP (Standard Operating Procedure) Functions. These functions can be used to derive information from SOP's 1104.

Function	Description
HasSOPBeenRun	Returns true or false in the form of -1 (True) or 0 (False) to determine if any SOP has been previously run in the layout.
IsSOPAfterStep(StepID)	Returns true or false in the form of -1 (True) or 0 (False) to determine the the current SOP step is after a specific SOP Internal Step ID.
IsSOPBeforeStep(StepID)	Returns true or false in the form of -1 (True) or 0 (False) to determine the the current SOP step is before a specific SOP Internal Step ID.
IsSOPFinished	Returns true or false in the form of -1 (True) or 0 (False) to determine if the SOP has finished running in the layout.
IsSOPOnStep(StepID)	Returns true or false in the form of -1 (True) or 0 (False) to determine if the running SOP is currently on a specific SOP Internal Step ID.

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Function	Description
IsSOPRunning	Returns true or false in the form of -1 (True) or 0 (False) to determine if an SOP is currently running.

Parameter Math Variables. These functions can be used to manipulate or get information out of existing parameter values.

Function	Description
Row	The variable 'row' describes the current value in a particular row, or cell number, of the data file. 'Row' may be used to perform sequential calculations on a series of values in a file. For example, 'row' may be used to create a time parameter. By using the 'row' variable, you use the cell number as a parameter and can plot fluorescence against cell number to check for uniform flow over time. Or, if the acquisition took 5 minutes and you have 5000 cells you could use the following formula to get the actual time (in minutes) where that cell was acquired. Formula: =(row-1)*(5/5000)
ConvertLinToLog(I,J,K,L)	Same as above, except that variable I will use the Parameter number that is being converted, for example P4 or P14.
ConvertLogToLin(I,J,K,L)	Same as above, except that variable I will use the Parameter number that is being converted, for example P4 or P14.

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Chapter

20 Batch Processing

Batch Processing allows you to analyze many data files consecutively in a single layout without having to set up new plots for each file. Batch processing also gives you the ability to print, save, or export your layout and statistics in many different formats, including a PowerPoint presentation, a Word document, an Excel spreadsheet, a PDF file, or a text file.

Batch processing applies the analysis on the layout to every item in the **Data List**. During batch processing, FCS Express automatically performs a Data→Change File on All Objects→Next File command 793 to systematically increment all the data files in your layout. The items will be incremented in their order in the **Data List** by the increment value specified in the batch processing options 843, or the currently active panel 860.

Changing the files during a batch process is called iteration. During iteration, the FCS files on every plot will be incremented and tokens will be evaluated.

You can choose not to increment a particular overlay by disabling the **Change data file during Next/Prev/Batch** option for histograms 268 , spectrum plots 278 , or 2D plots 284. For example, you may choose not to increment a control file in one overlay, while allowing iteration in the other overlays. In addition, you can choose not to increment a particular token function by disabling the Change data file during Next/Prev/Batch function for token Data Sources 699.

FCS Express allows you to define batch processes that can be performed during iteration. These processes are divided into *Action* or *Report* processes:

- Action Processes 803

  These processes allow you to print and/or save the layout in various formats. The following lists all print/save processes:

  - Print
  - Save Layout
  - Save to PowerPoint
  - Save as PDF
  - Save as Picture

- Report Processes 822

  These processes allow you to export information from your layout to:

  - Microsoft Excel
  - Microsoft Word

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- A PDF file
- A text file
- De Novo Software Data Services (DDS)

The information you can export includes plots (as pictures), statistics (as columns, cells, or text), and tokens (i.e., the token information displayed in the text box). Once you have chosen where to export the data by adding a Report, you assign items to transfer to that Report.

There are four distinct steps to using **Batch Processing** properly after you have set up your analysis layout:

1. Add the data sets you wish to analyze, in the correct order, to the Data List 788.
2. Add Batch Action and Report processes to the Batch Processing Action Screen 800.
3. Specify the Batch Processing Options 843.
4. Run the Batch Process 846.

20.1 Working with the Data List

Once you have created a layout to analyze your initial data, you can begin to review data or build a **Batch Process** to sequentially analyze the remainder of your items. The first step in setting up batch processing is to add items to the **Data List**. The order in which items appear in the **Data List** is an important consideration. Items are batch processed in the order that they appear in the list, and the **Next** and **Prev** commands change items in the order that they appear in the list, as well.

To access the **Data List**, use the **Batch** (or **Data**)→**Organize Data Sets**→**Data List** command to bring up the **Data List** window (Figure 20.1). The **Data List** is a dockable window 113.

The Data List window will show the File Name, the Tube number and the Iteration number.

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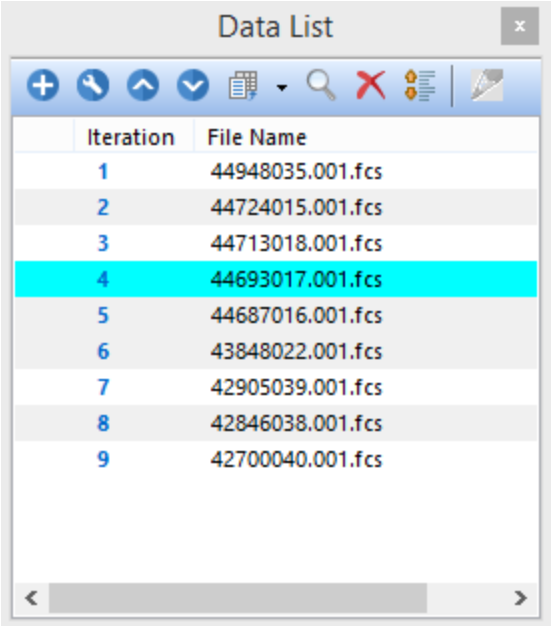

Figure 20.1 Data List Window

The icon buttons in the **Data List** window are explained in the table below.

Icon Button	Explanation
	Add File. Brings up the Select a Data File Dialog 214 or Advanced Open Data Dialog 215
	Format. Brings up the **Format Data List** dialog where you can add keywords to be used for sorting, show or hide file paths, or show the Iteration Column. You can also hide file paths by default by adjusting your FCS Express User Options 2233.
	Move selected file(s) up.
	Move selected file(s) down.
	Change file drop down list to access **Change Data on Selected Plots** command or **Change Data on All Plots** command.
	Search for files using Find Data Files 788 dialog.
	Delete the selected file(s).
	Sort on All Keywords. The sort is performed based on the priority order of the keywords in the "Selected Keywords" dialog, or on the order of the extra keywords columns (the default order of the columns is the same order as the selected keywords). You can change the order by dragging the columns around. Note, if you drag the keyword columns around, or if you click on another column header, it turns OFF the "sort by multiple keywords" option and you have to click it again.
	Defer layout changes 798. When this button is selected and used in conjunction with Panels 860, FCS Express will not refresh the data within the

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Icon Button	Explanation
	layout until all changes have been made and the button has been de-selected.

20.1.1 Data List Actions

In FCS Express, you can use the Data List to manipulate your data within the layout.

For example, if you wanted to change the base overlay in all of the plots in your layout, you can double-click on the data file within the Data List, and choose the appropriate action.

By double-clicking on a data file in the Data List, you will be prompted with the **Select double click action** window, as seen below (Figure 20.2).

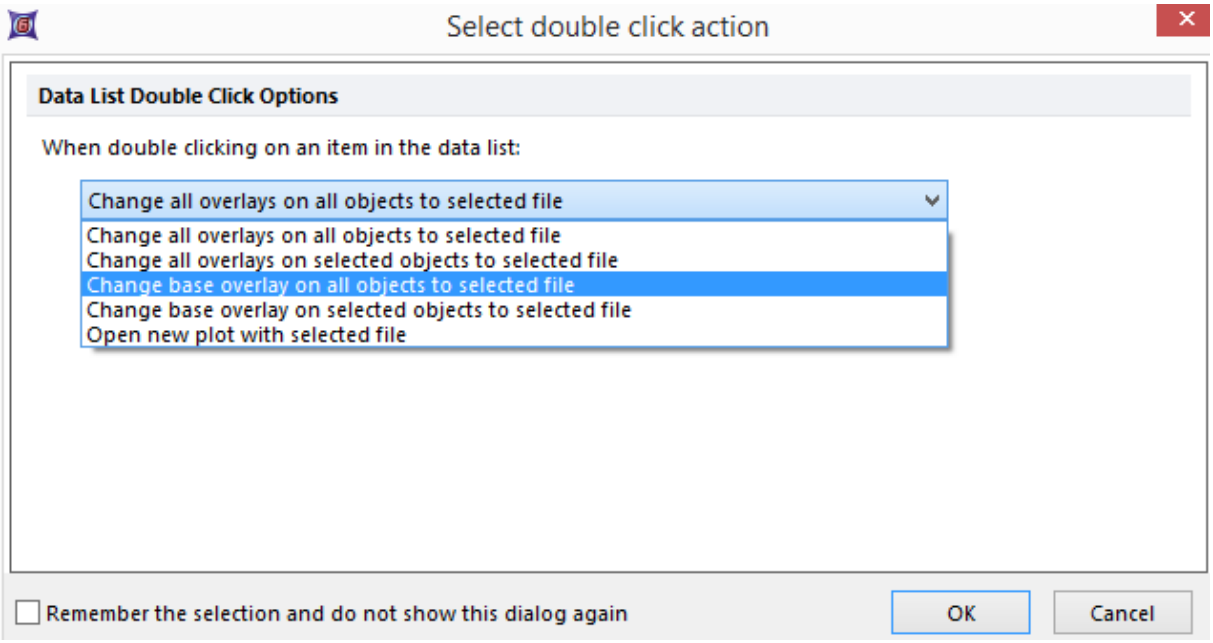

Figure 20.2 Select double click action dialog window

Once a selection has been made, FCS Express can remember your selection and not prompt you for an action.

As your data update, any tokens that are included in the layout will automatically update to reflect the new data.

Also, the user can navigate to an iteration within the Data List by clicking on the iteration number within the Iteration Column (Figure 20.3).

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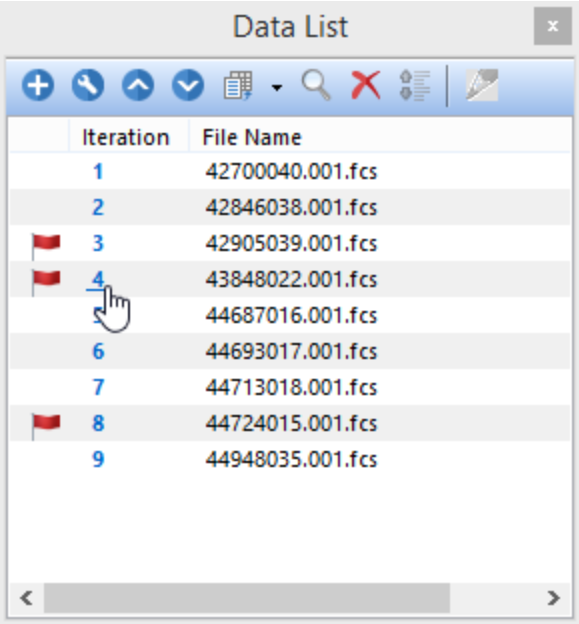

Figure 20.3 Click on the Iteration Number to view that Iteration

20.1.2 Adding Files to the Data List

Two methods can be used to add data sets to the **Data List**:

- **Add files directly without searching**.

  1. Access the Data List using the Batch (or Data)→Organize Data Sets→Data List command 785.
  2. Click on the button to bring up the **Select Data File** 214 dialog if **Standard Open Data Dialog** is chosen under user options or **Open Data File** 215 dialog if **Advanced Open Data Dialog** is chosen under user options.
  3. Select the appropriate files and click on the **Open** button to add the files to the **Data List**. This dialog allows you to add multiple files at once using standard Windows techniques (e.g., **Shift-**Click or **Ctrl-**Click to select multiple files at the same time).

  Alternatively, you may drag and drop data files into the **Data List** from any folder on your computer.

  Note: If you have set an active Panel 860, you can simply choose the first file/tube in your Panel, and FCS Express will ask if you want to automatically load the rest of the files/tubes in the Panel.

- **Search for files or conditionally select files**.

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If you do not know where your data files are located, or if you wish to select files based on one or more conditions, you can perform a search.

Note: Since FCS Express Version 4, FCS Express is no longer limited to opening only FCS files. Therefore, to properly utilize functions which are related to Keywords, you must highlight the appropriate data set/file (so that FCS Express knows which type of Keywords to display) before doing a keyword search.

1. Access the Data List using the Batch (or Data)→Organize Data Sets→Data List command 785.
2. Click on the Search button 785 to bring up the **Find Data Files** dialog (Figure 20.4).

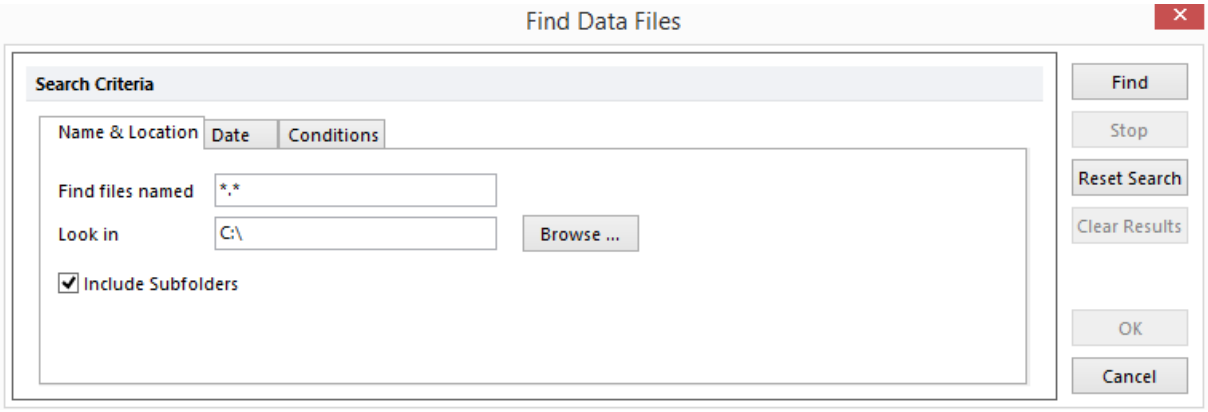

Figure 20.4 Find Data Files Dialog

Select one or more of the three tabs at the top of the **Find Data Files** dialog to set search criteria for finding the files you want to add to the **Data List**. All the search criteria work together, so you can select any combination before clicking the **Find** button.

- On the **Name & Location** tab, click the **Browse** button, navigate to the directory to search, then press the **Find** button. *Note*: Setting an appropriate directory on the **Name & Location** tab first will make searches faster even if you are using the date or keyword conditions below.

- To search for files based on their date, click on the **Date** tab and enter the search criteria.

- To search for files based on the value of a keyword, select the **Conditions** tab. (Recall that you must highlight/select a data set/file in the **Data List** for Keyword searches to work properly.) By default, there are no conditions set, so click the **Add Condition** button. A new line

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appears in the table, and the first column will be unavailable. Click on the button in the **Keyword** column and select a keyword (e.g., **$TOT** for total number of events) from the **Please select a keyword** dialog. You can also type in the keyword within the field. In the **Relation** and **Value** columns, select the keyword range to search. For example, to search for files with more than 20000 events, select **>** (for less than) in the **Relation** column, then enter 20000 in the **Value** column.

- To search for files based on more than one keyword, click on the **Add Condition** button again. This time, select the Operator from the first column. The Operator default is **AND** to specify both keyword conditions must be met. Otherwise, select the operator **OR** to specify either keyword condition may be met. After selecting the operator, enter the **Keyword**, **Relation** and **Value** options as before.

When you are ready to run the search, click on the **Find** button. Remember that all of the conditions work together, so you can select a starting folder, date criteria, and keyword conditions before starting the search.

While FCS Express is finding the files that match your search requirements, the window will expand to include the **Search Results** field. As files are found, they will be added to that field. To halt the search before it is complete, click on the **Stop** button. Figure 20.5 shows a search for all files within a specified directory with greater than 20,000 cells acquired.

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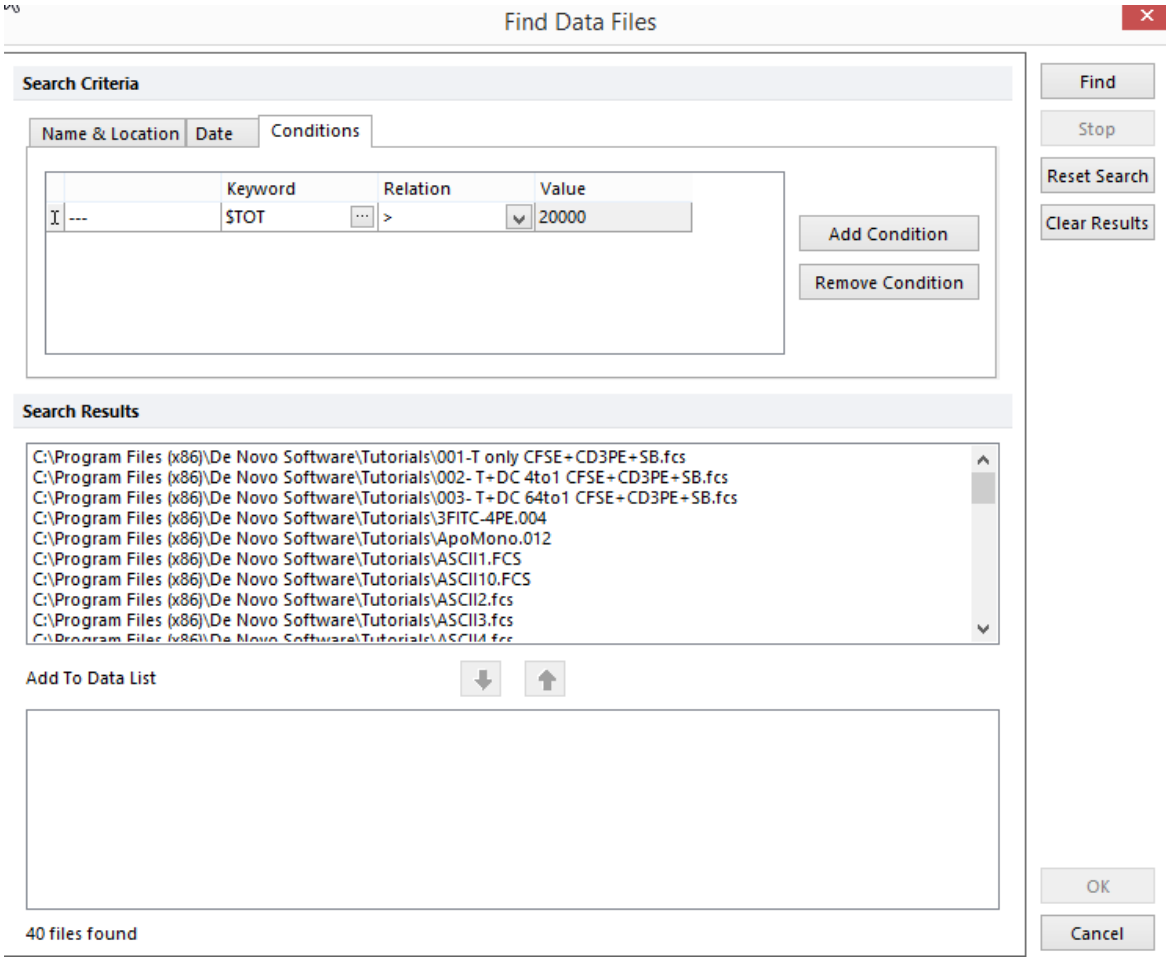

Figure 20.5 Search Files by Keyword

Once all the files have been found, add the files you want to use in the **Data List**. To add files individually, click on a file name to select it. To select more files, press **Ctrl** (or **Shift**) while you click on a file name, then press **OK**. Once the appropriate files have been highlighted, press the **Down Arrow** button. To remove files from entry into the **Data List**, select the files to be removed and click the **Up Arrow** button. When you have finished choosing files for the **Data List**, press **OK**.

If you wish to perform a second search, first add the files from the current search, then perform subsequent searches. When files are added to the **Data List** and an active Panel 860 is set, each group of files (representing the tubes of the Panel) will appear in alternating colors. This makes it easier to distinguish between the different files of a Panel.

20.1.3 Sorting in the Data List

The **Data List** window allows you to look at the values of certain keywords, and to sort the list based on these keywords.

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To select keywords to view:

1. Access the Data List using the Batch (or Data)→Organize Data Sets→Data List command 785.
2. Click on an appropriate entry in the **Data List** to let FCS Express know which keywords to display.
3. Click on the (Format) button to bring up the **Format File List** dialog.
4. Click on **Add**. The **Please select one or more keywords** dialog will appear.
5. Click on a keyword to select it. To select more keywords, press **Ctrl** while you are clicking the mouse button.
6. Once you have selected all the keywords you would like to view, press **OK**.

The **Data List** will have a new column for each of the keywords that you selected (Figure 20.6).

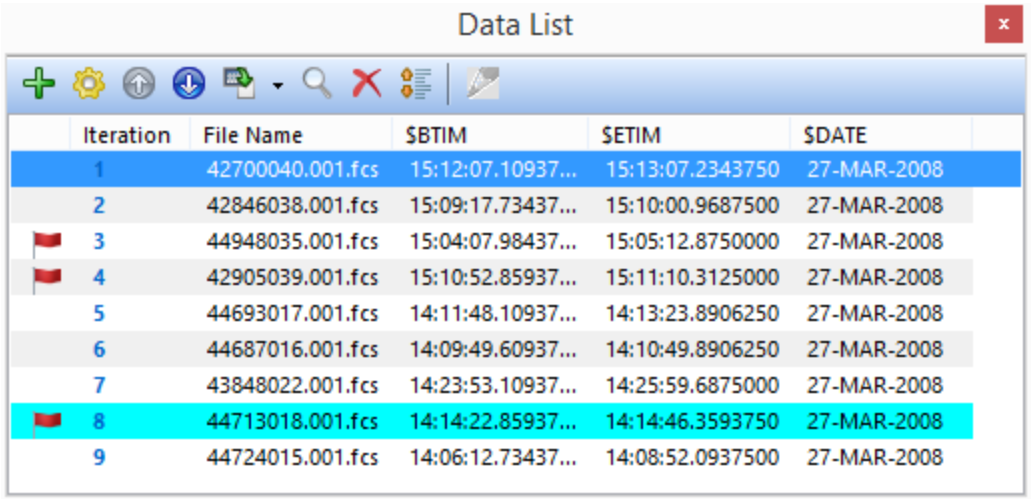

Figure 20.6 Data List with Keyword Columns

By default, the **Data List** is sorted in alphabetical order based on data set name. To sort the data sets by other criteria, click on any column title. The list will be sorted based on the values in that column. Clicking on the title once will sort in ascending order; clicking on the title a second time will sort in descending order; clicking on the title a third time will return the list to the previous sort criteria.

You can also sort individual files (or groups of files) without clicking on the column titles. Click on a row in the list to select it, then press the (Up) button to move that file up in the list, or the (Down) button to move it down in the list. You can move several file rows at the same time by selecting them together

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using standard Windows techniques for selecting multiple objects (**Shift**-Click, **Ctrl**-Click), then pressing the or buttons.

20.1.4 Using the Data List to Change the Data Set in a Plot or Token

Batch Processing is an effective tool to use for processing multiple sets of data with a common layout and exporting your data into various formats.

Once a **Data List** has been created, you can also use the list to manipulate the data set in plots 787.

Also, when editing Tokens, you can edit the Data Source to include data files that are included in the Data List by selecting the option for **Data File From Disk**, and then choosing data from the **Select Data File** 214 or **Open Data File** 215 dialog (Figure 20.7).

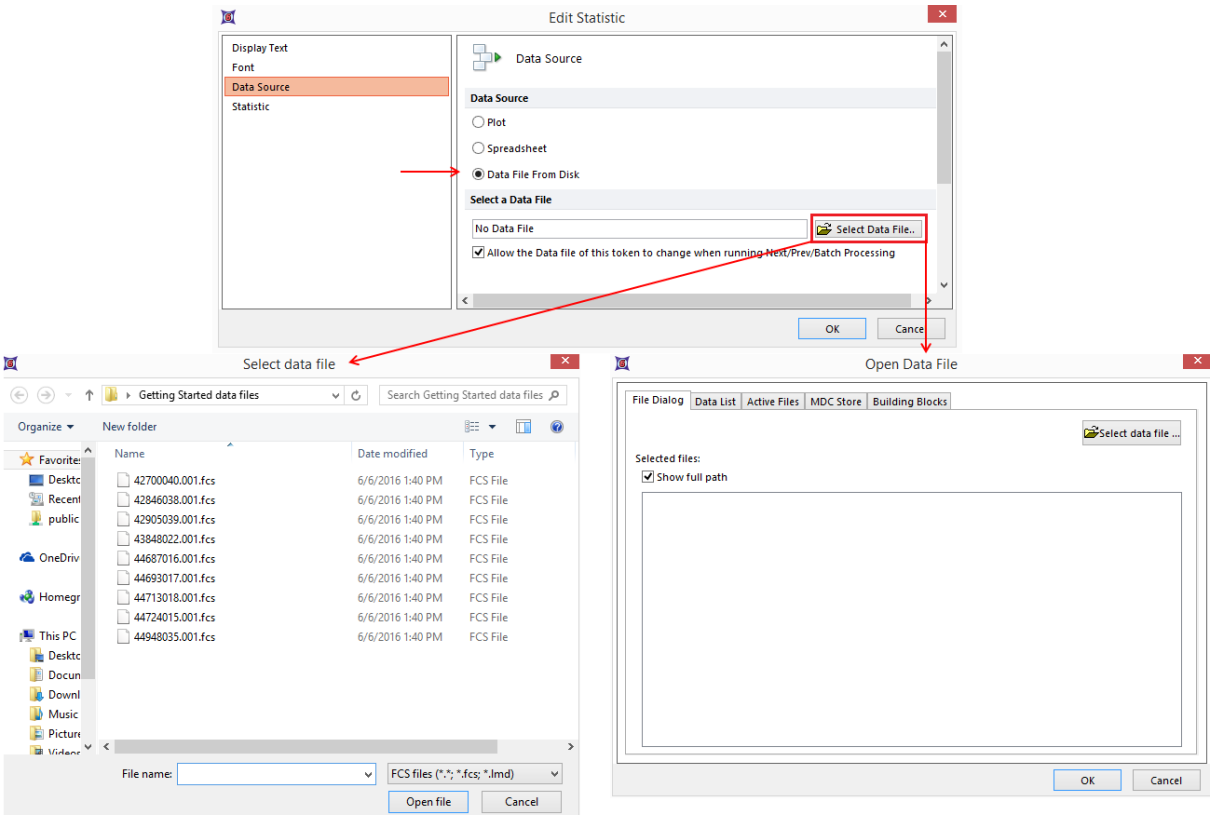

Figure 20.7 - Editing the Data Source of a Token through the Data List to Select Data File (lower left) or Open Data File (lower right) dialog options.

20.1.4.1 Next/Previous

The Next and Previous functions will change the dataset on the following objects:

- Plots
- Data Windows
- Tokens in Text Boxes

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- Custom Tokens
- Batch Processing Actions

In order to use the Next/Previous functions to change datasets, each of the following conditions must be met:

- A **Data List** has been defined;
- The object(s) to change (e.g., a plot) are selected or in edit mode, if datasets are to be changed on only certain objects;
- The selected object(s) display or are linked to a dataset in the list, and finally;
- The selected object(s), e.g., a histogram 268, 2D plot 284, and/or Token 699, must have the **Change data file during Next/Prev/Batch** option enabled (Figure 20.8).

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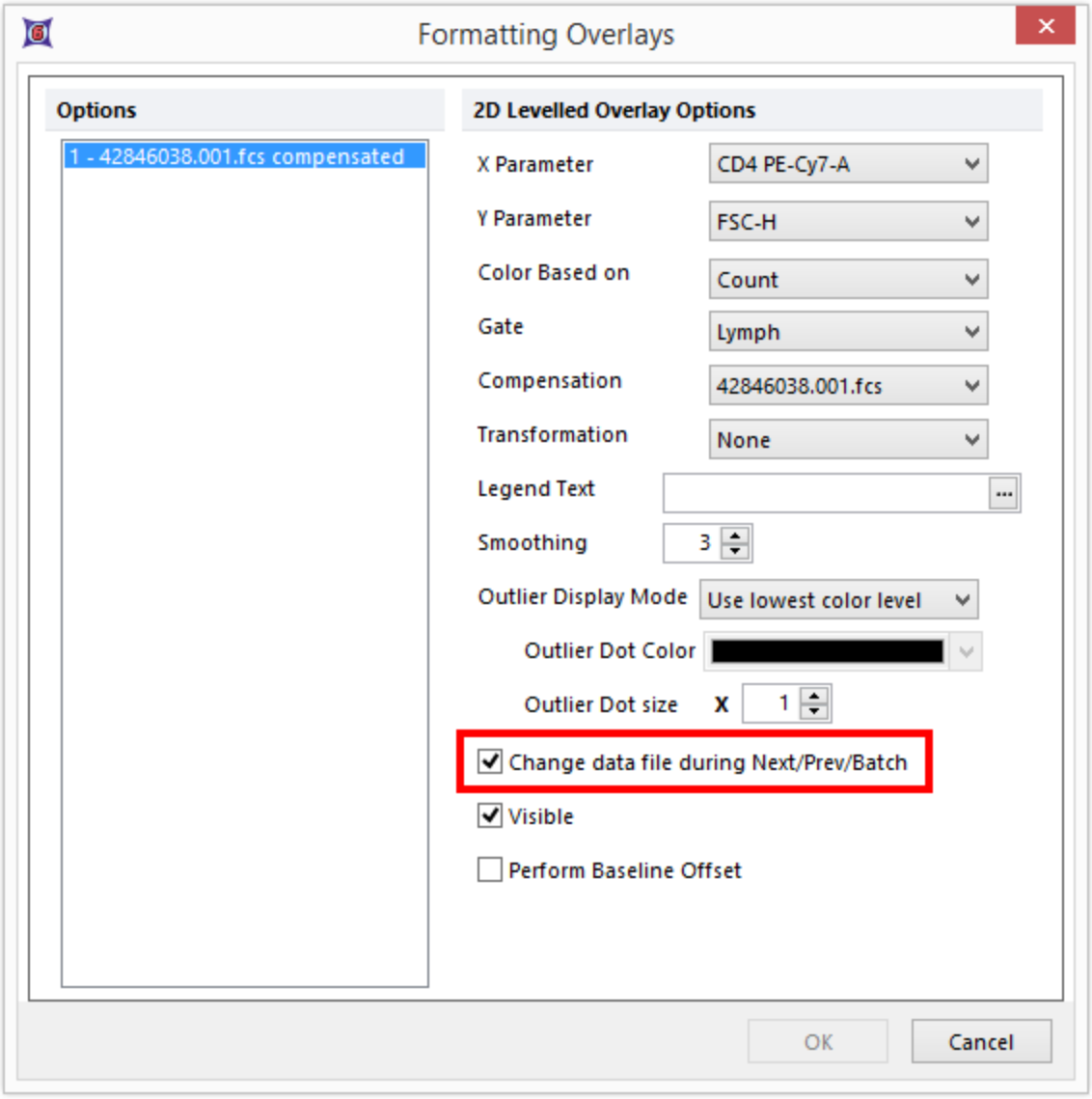

Figure 20.8 - Example of Formatting dialog for 2D plots in which the Change data file during Next/Prev/Batch option is selected. The same option is available for histograms and Tokens.

To change the dataset on one or more selected objects to the next or previous dataset:

1. Select the plots you wish to change. If you wish to change all plots, you need not select them and the current selection is irrelevant.
2. Use one of the three following methods:
   - Use the **Data→Change Data on Selection/Change Data on All Objects→Next** (or **Previous**) command.
   - Use the keyboard shortcuts **Alt+Right arrow** for Next or **Alt+Left arrow** for Previous.

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- Right-click on the selected object and choose **Next** or **Prev** from the pop-up menu.
- In the Data List, click on the file to which you would like to change and select the appropriate choice from the Data List Actions 787 window.
- Use the **Data→Change Data on Selection/Change Data on All Objects→Select.** This command will open the Select a Data File window, which will allow you to select another data file, either in the Data List or from another location.

3. Please note that *new Snapshots will not be recorded while changing the data on Selected object(s), and previous Iteration (automatic) Snapshots will be discarded.*
4. The user can also change the data within all objects by selecting an iteration number from the **Iteration** drop down menu.

20.1.4.2 Dragging and Dropping

Like other dockable windows 113 in FCS Express 6, the **Data List** has drag and drop functionality. You can drag a data set directly from the **Data List** to your layout to either change the data in an existing plot, to add the data as an overlay in an existing plot, or to create a new plot.

The **When dragging a data set onto a plot** drop-down list in the General category of the FCS Express User Options dialog 180 controls the functionality when you drag a data set onto an existing plot. The drag and drop operation will either:

- Replace the current FCS file on the plot;
- Create a new overlay with the dragged file, or;
- Open a dialog to select one of the first two options.

If you drag a file from the **Data List** directly onto a blank area of the layout, a **Select Plot Types...** dialog will appear asking which object(s) you wish to open (Figure 20.9).

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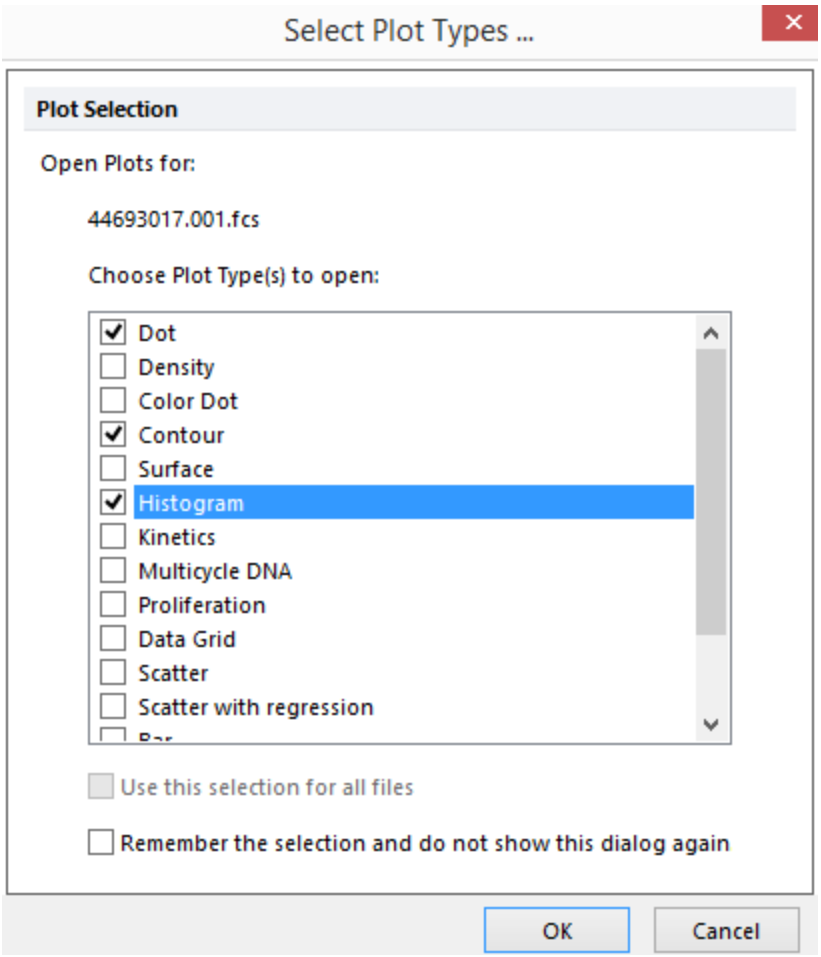

Figure 20.9 Select Plot Types... Dialog

One or more objects can be chosen by checking the appropriate box(es). Once you have chosen the object type(s), the new object(s) will open showing the data from the data set that you dragged from the **Data List**.

20.1.4.3 The Change File Button

To use the **Data List** to change the files/data sets on your plots (Figure 20.10):

1. Click on a dataset in the Data List to highlight it.
2. Click on the icon to **Change Data On Selected Plots** or **Change Data on All Plots** from the drop down list.

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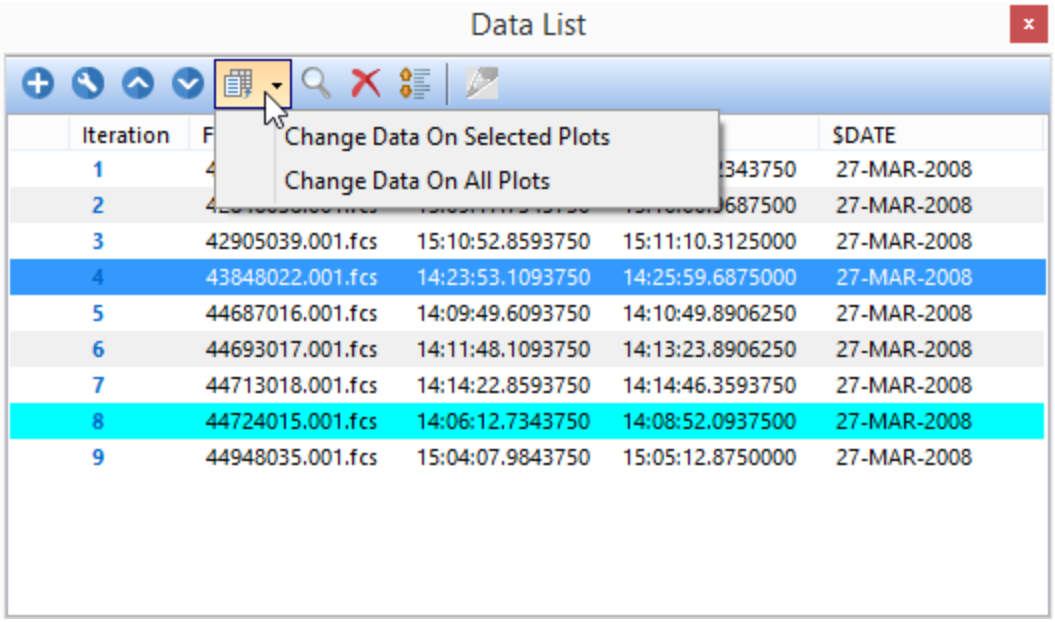

Figure 20. 10 - Available commands from Change File drop down list

20.1.4.4 Defer Layout Changes

When using the Panels feature in FCS Express, a user needs to load data into the Data List as it pertains to the panel.

FCS Express will automatically load the data into the plots to which the files were assigned. If a panel set of data files were placed in the wrong order, you can easily re-arrange the order of the tubes by selecting the file, then, clicking the button or the button to place the files in the correct sequence.

However, if the data files are large in size and they needed to be moved Up or Down in sequence, the amount of time needed for FCS Express to correctly display the data in their proper plots and to recalculate statistics and tokens can be significant.

Users can make changes to the sequence of tubes within their panel and have FCS Express defer those changes until all the changes have been made rather than recalculate each time a file is moved in the Data List.

In the example illustrated below, we can see how this tool is utilized by using a 3-tube panel dataset.

1. Open the Data List.
2. Load the data specific for the panel.
3. Select the Defer Layout Changes icon.
4. Once the icon is selected, a grey play button icon and a red "X" will appear next to the Defer Layout Changes icon .

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5. Move a tube within the sequence Up or Down using the arrows within the Data List toolbar (Figure 20.11).

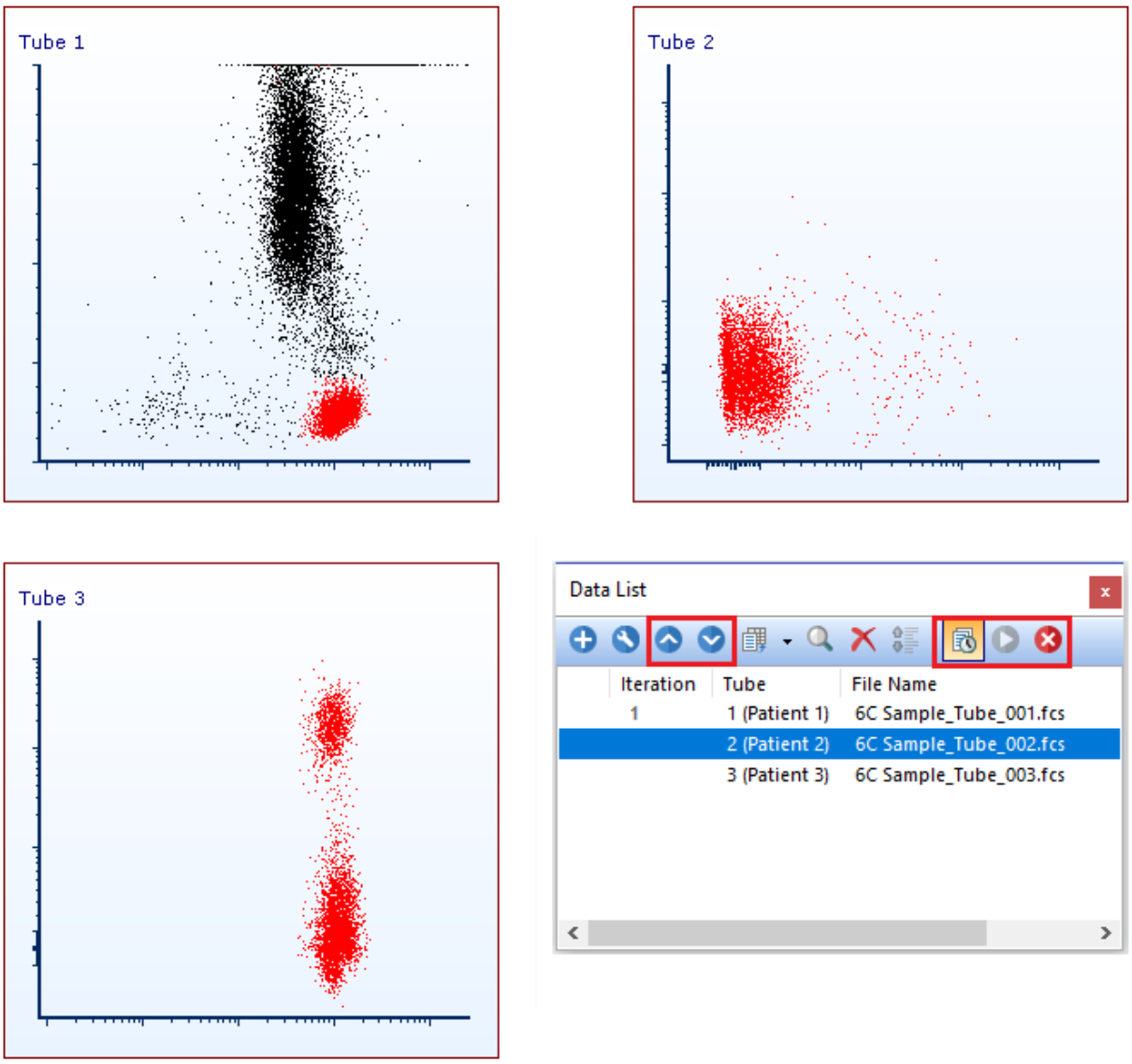

Figure 20.11 - Panel Data Before Deferring Layout Changes

6. Once the tube is moved to the correct sequence, click the blue play button icon to accept the changes .
7. FCS Express will now display the data in the appropriate plots (Figure 20.12).

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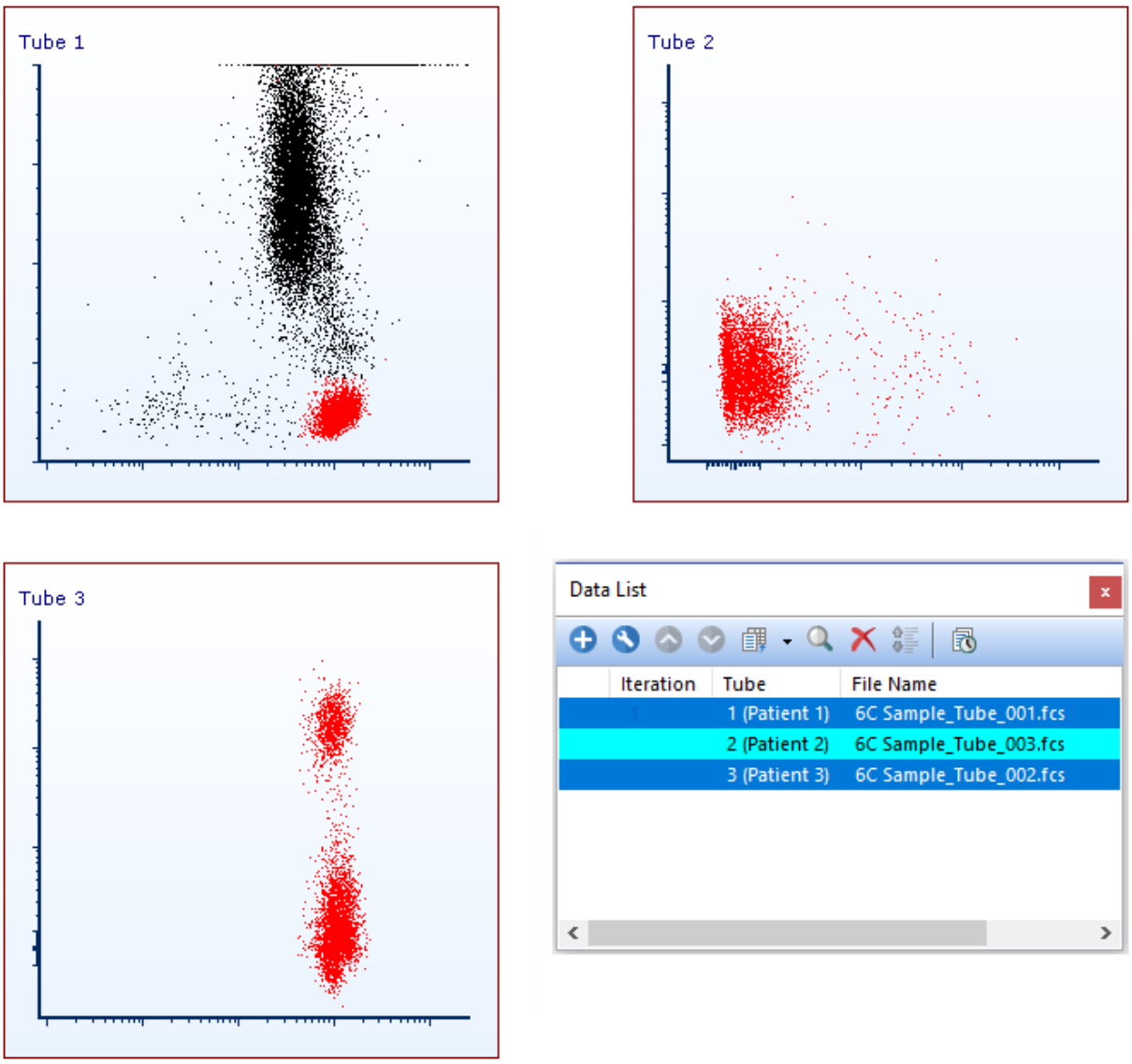

Figure 20.12 - Panel Data After Deferring Layout Changes

20.2 Adding Batch Processes

After creating the **Data List**, the second step in Batch Processing is to define which processes to perform. FCS Express allows you to perform actions and reports during the batch process 784.

To add actions to the batch process, use the **Batch→Batch Processing→Batch Actions** command to bring up the dockable **Batch Actions** window (Figure 20.13).

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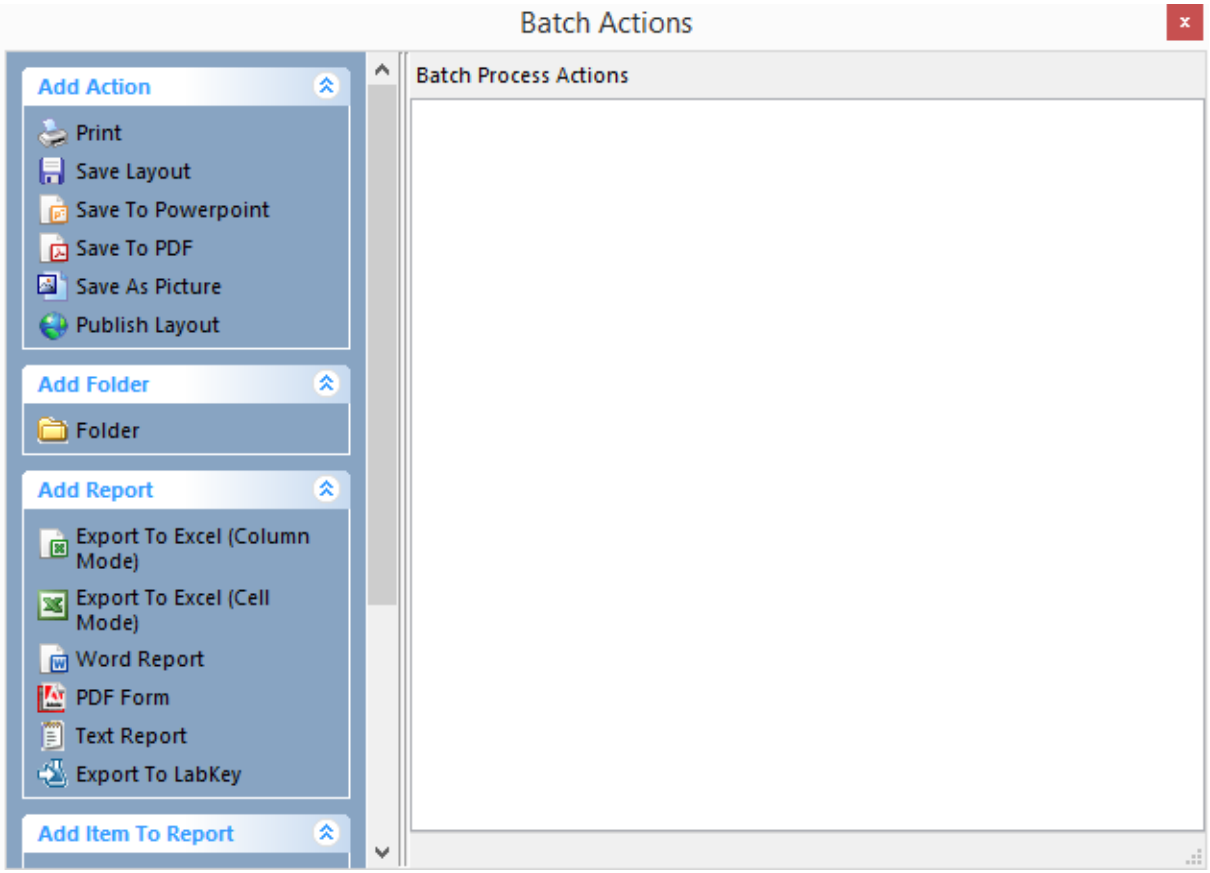

Figure 20.13 Batch Actions Window

To add a new process to the **Batch Process Actions** list, click on the process from the list of available items on the left of the **Batch Actions** window. An appropriate dialog box will appear to further specify the action for each process. For example, to add a Print 803 action, you must specify the printer, the number of pages, and the number of copies to print.

Once a process has been added to the **Batch Process Actions** list, it is automatically set as active and will execute when running the Batch (the box to the left of the process in the **Batch Process Actions** list will be checked). To deactivate a process so that it will not execute in the Batch, uncheck the checkbox to the left of the process name.

Multiple actions of the same type can be added to the **Batch Process Actions** list. For example, two print processes can be active at once. The first print process could print pages 1-5 on printer #1 and the second print process could print pages 6-10 on printer #2.

Because both of the actions in the previous example will be listed as "Print" in the list, it could be helpful to change the process names in a complex batch list. To rename an action in the list:

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1. Select the process name and press **F2** (or right-click on the action and choose **Rename** from the pop-up menu).
2. Type in the new process name.
3. Press **Enter**.

Figure 20.14 shows an example of a Batch Process with several actions defined. The two active print actions have been renamed with descriptive text. The **Save Picture of Plot** action has been deactivated, indicating that it will not be used the next time the Batch Process is run.

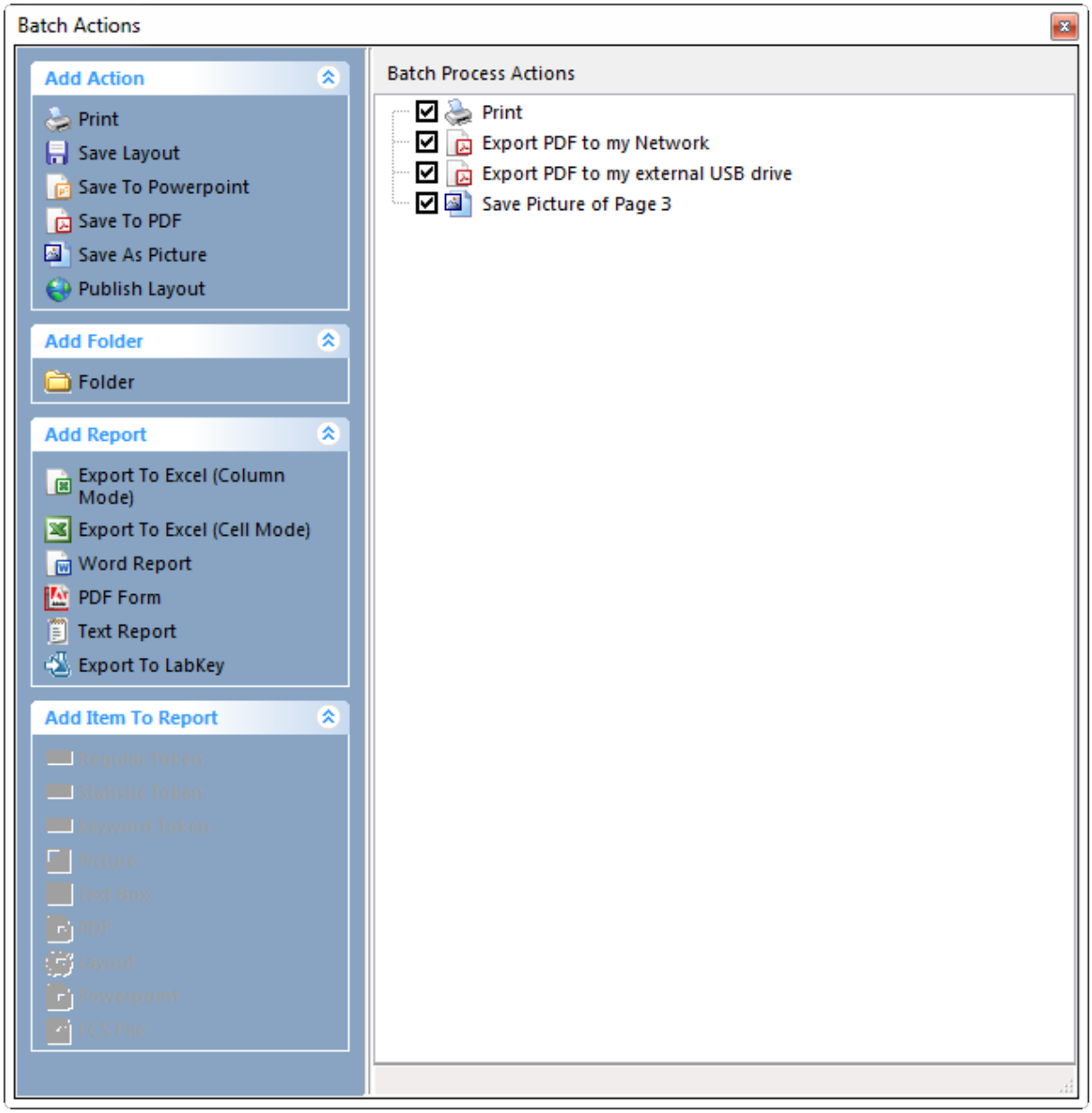

Figure 20.14 Batch Process Actions List with Actions

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Right-clicking on a process in the **Batch Process Actions** list brings up a pop-up menu with the following options:

- **Cut**/**Copy**/**Paste**: Allows copying and pasting processes. This facilitates moving tokens from one report to another.
- **Delete**: Delete the selected action.
- **Rename**: Enter a meaningful name for the selected action.
- **Add Items**: For Reports, you can add Items that can be found in the **Add Item To Report** section.
- **Properties**: Display the dialog box for the selected action to allow changes to the action properties.

20.2.1 Batch Processing Actions

The Batch Processing Actions include printing a layout 803, saving a layout 806, saving to PowerPoint 810, saving to PDF 815, and saving as a picture 820. You can also group your actions using a folder (action container) 821.

20.2.1.1 Print

A Print action will allow you to automatically print a range of pages during a batch process.

To create a Print Action, click on **Print** (in the **Add Action** section of the **Batch Actions** window). The **Create Print Action** dialog (Figure 20.15) is used to set up how the layout will be printed with each iteration of the batch process.

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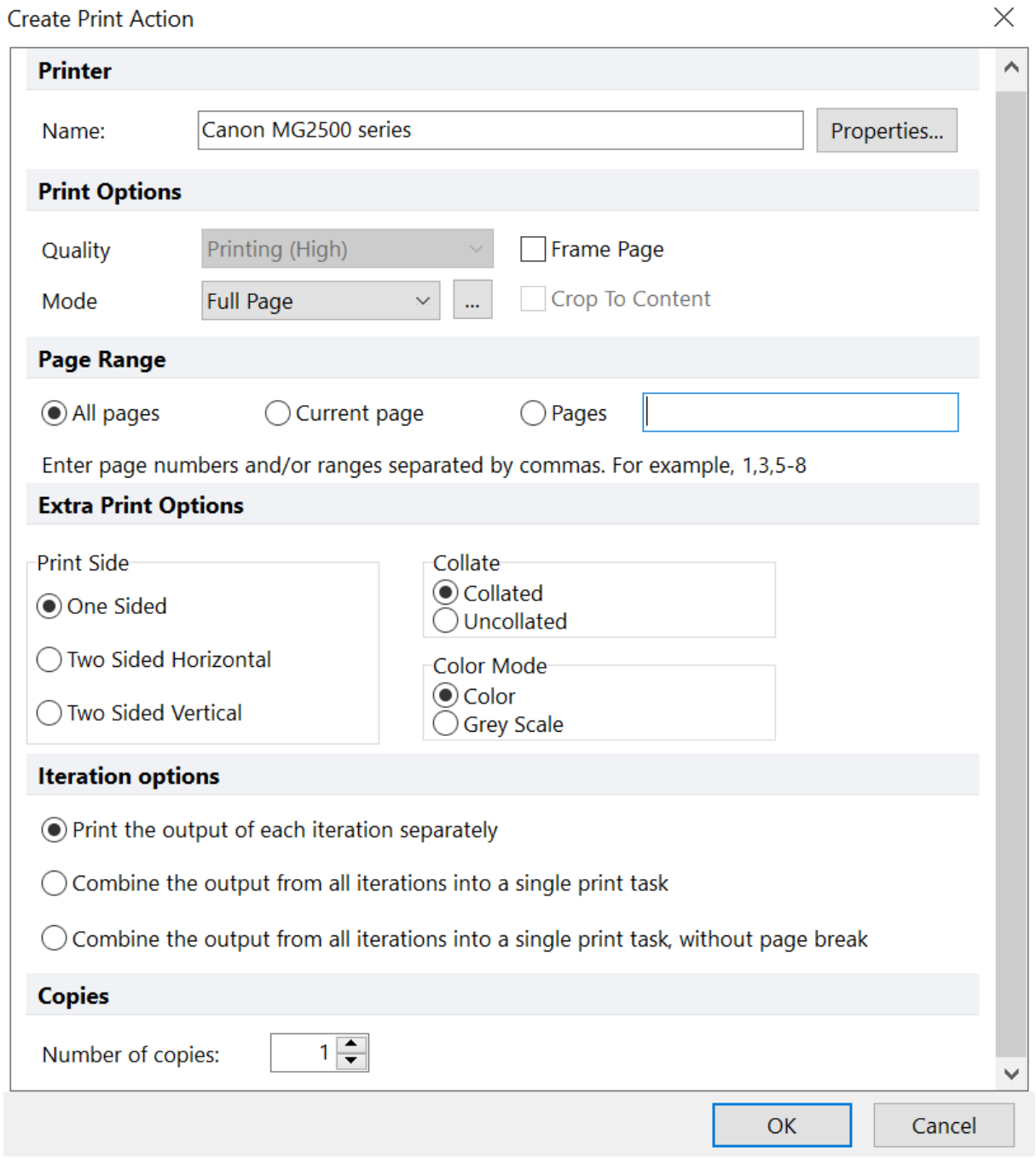

Figure 20.15 Create Print Action Dialog

The properties in the **Create Print Action** dialog are divided into five sections:

- **Printer**

  Select which printer to use to print the layout.

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- **Print Options**
  - **Quality.** Will be set to **On-screen**.
  - **Mode.** Choose to print layout pages as single or multiples per printed page(s). With **Full Page** selected, the layout page(s) for each iteration 785 will be printed as individual page(s). Multiple layout pages may be printed per page using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options** 38. To print the layout pages on fewer pages, please select a preset multiple page option or **Custom Grid**. To select a preset multiple page option or customize the tiling, click the down arrow to the right to expand the dropdown list and select a multiple page option or **Custom Grid Options...**, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear.
  - **Frame Page.** Flag this option to draw a frame around layout page(s).
  - **Crop To Content.** Available for selection when **Mode** is not set to **Full Page**. When selected, empty space of the layout page(s) is cropped during the export.

- **Extra Print Options**
  - **Print Side.** Choose to print **One Sided**, **Two Sided Horizontal**, or **Two Sided Vertical**.
    - Select **One Sided** for single-sided printing.
    - Select **Two Sided Horizontal** for long-edge page turning.
    - Select **Two Sided Vertical** for short-edge page turning.
  - **Collate.** May be set to **Collated** for which all pages are printed in ordered sets or **Uncollated** for which all copies of the first page followed by copies of subsequent page(s).
  - **Color Mode.** May be set to **Color** or **Grey Scale**.

- **Page Range**

  Choose which page(s) of the layout to print.

- **Iteration options**

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Choose whether to save the output of each iteration to a separate document or combine all the iterations into a single document. **Combine the output of all iterations into a single print task, without page break** must be selected when **Mode** is not set to **Full Page**.

- **Print the output of each iteration separately.**
  - Each iteration will be printed separately.
- **Combine all the output from all iterations into a single print task.**
  - All iterations will be combined into one print task for printing.
- **Combine the output of all iterations into a single print task, without page break**
  - All iterations will be combined into one print task. Depending on your **Export Options→Mode** settings many iterations may be combined into one page for printing.

- **Copies**

  Designate how many copies of the layout to print.

Click **OK** when you have finished. At this point, the Printer action will be added to the list of batch actions.

20.2.1.2 Save Layout

To create a Save action, click on **Save Layout** (in the **Add Action** section of the **Batch Actions** window).

The **Create Save Layout Action** dialog (Figure 20.16) is used to set up how the layout will be saved after each iteration.

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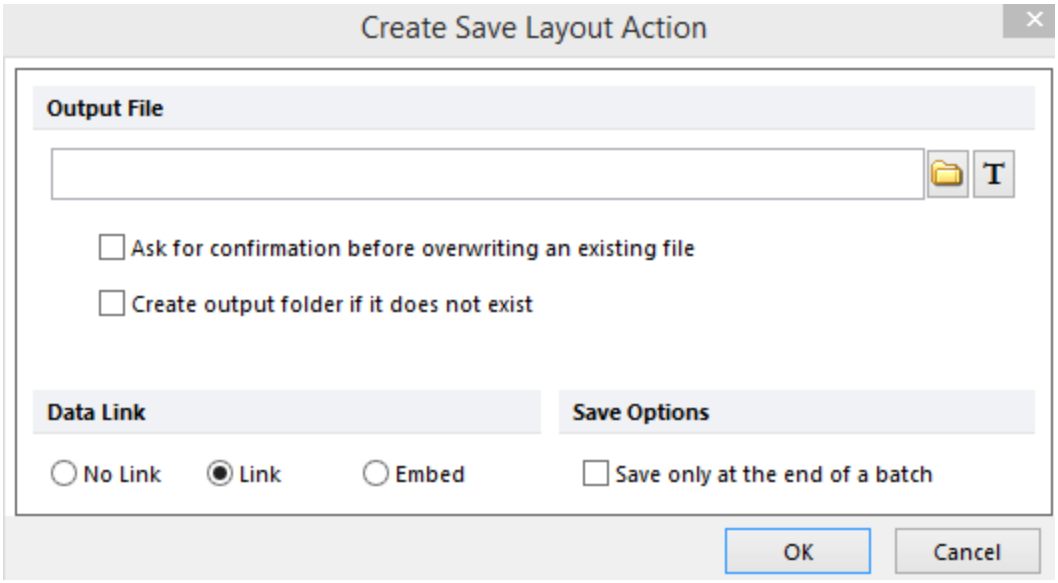

Figure 20.16 Create Save Layout Action Dialog

The properties are divided into three sections:

- **Output File**

  The **Output file** field determines the name and location of each saved layout. Since you will need different names for each file that is saved in each iteration, the filename you enter must contain dynamic information that changes for every iteration. In order to create a dynamic filename, you have to insert a token 692 as part of the name. The most common token used in conjunction with a dynamic filename in Batch Processing is the *Current iteration* token.

  Before you insert the token into the **Output file** field, make sure you have entered a filename in the field, and then position the cursor where you want the token text to appear. To insert a Current Iteration token:

  1. Click on the T button to bring up the **Insert a Token** dialog.
  2. Click on the triangle next to **Batch Processing**.
  3. Select **Current iteration** (Figure 20.17).
  4. Click **Insert**.

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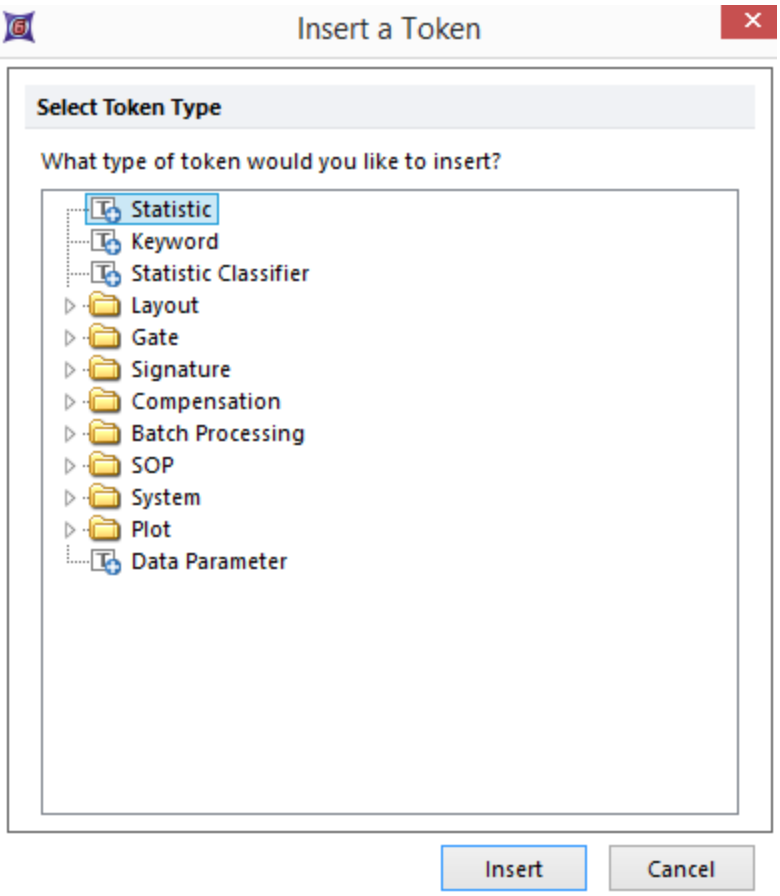

Figure 20.17 Insert a Current Iteration Token

Figure 20.18 shows an example of Output file, which will create a dynamic filename using the Current iteration token.

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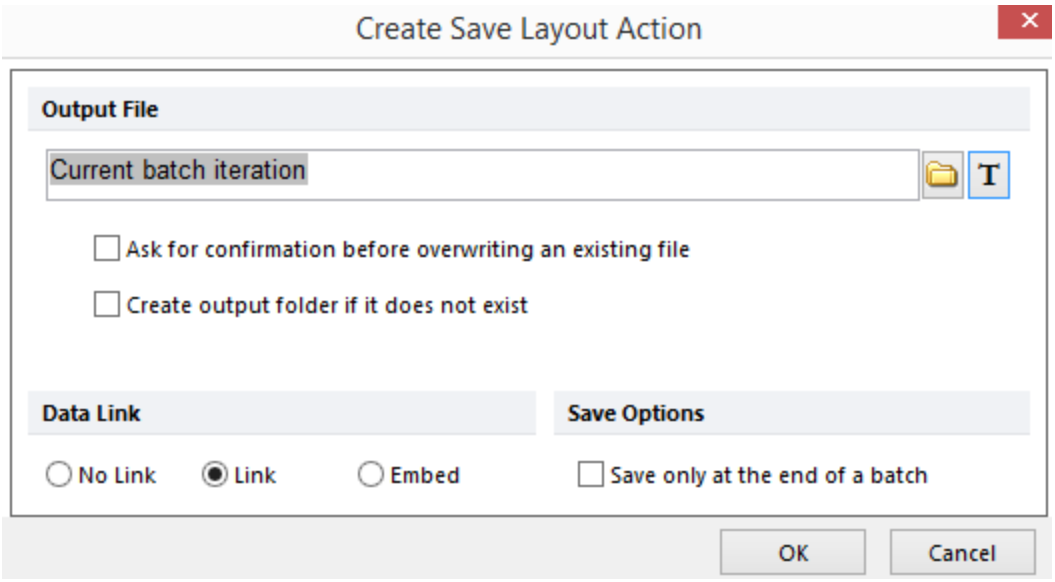

Figure 20.18 Example Output File with Current Iteration Token

Running the Batch with this output file would generate files such as *C:\Temp\Layout-1.fey*, *C:\Temp\Layout-2.fey*, etc. If **Ask for confirmation before overwriting an existing file** is checked, you will be prompted to approve overwriting already existing filenames when the batch process is run.

You do not have to use the *Current iteration* token. You can use any token whose information changes with every increment. For example, Patient ID, Sample ID, or some calculated value could all be used to generate unique dynamic file names.

- **Data Link**

  The options are the same as in the Save Layout dialog 93.

- **Save Options**

  By checking this box, you can tell FCS Express to hold the process of saving the layouts until the end of the batch process.

Click **OK** to finish editing the Save Layout properties. At this point, FCS Express verifies that the Output file has been filled in properly. If you have left the Output file field empty, or if the path name contains characters that are not allowed in a filename, then a **Messages** with descriptions of the errors will appear (Figure 20.19).

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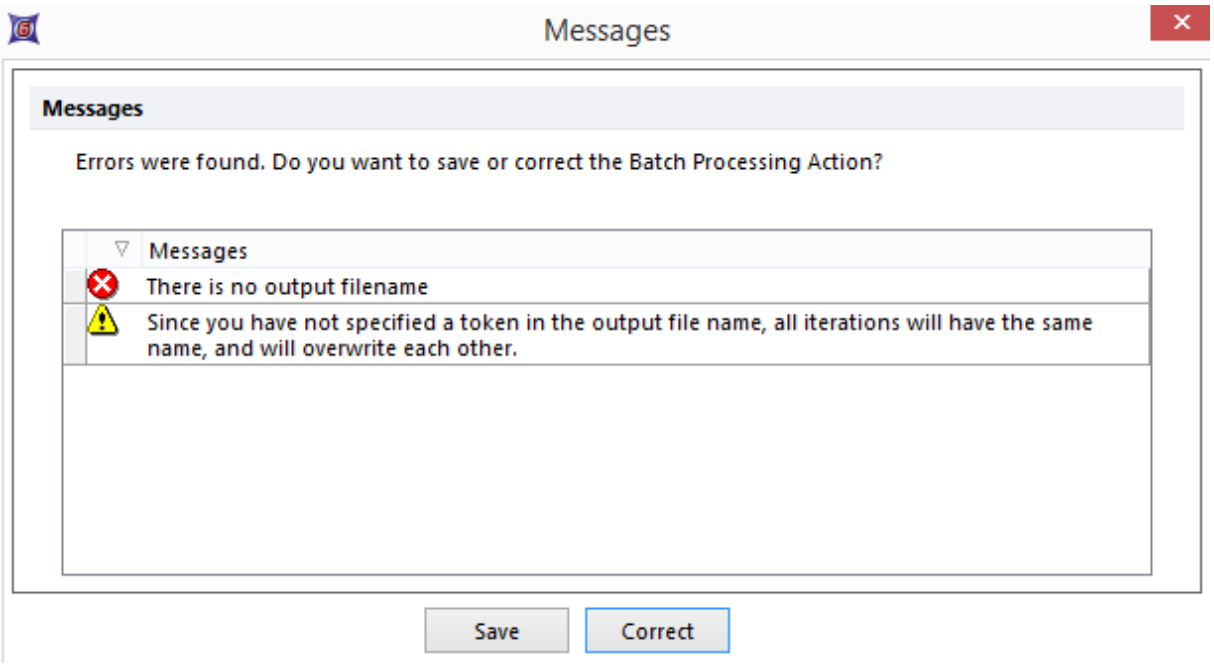

Figure 20.19 Batch Processing Error Messages

This dialog allows you to **Save** the action with the error, or to **Correct** it. If you choose to save the action without correcting the error, this action will not be executed in the Batch.

20.2.1.3 Save To PowerPoint

To create a Save To PowerPoint action, click on **Save To PowerPoint** (in the **Add Action** section of the **Batch Actions** window).

The **Create Export to Powerpoint Action** dialog (Figure 20.20) is used to set up how each iteration of the layout will be saved as a PowerPoint presentation file.

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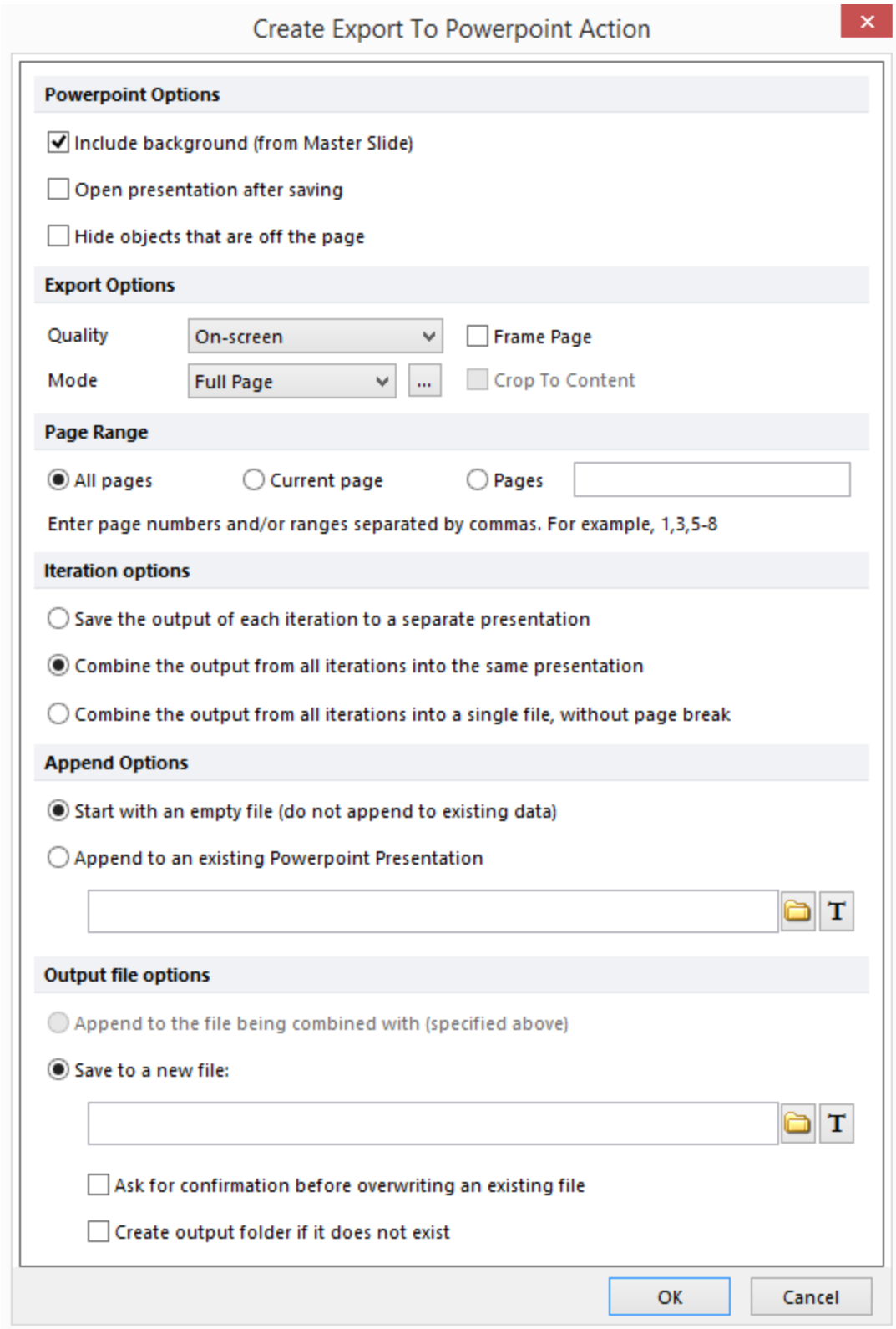

Figure 20.20 Create Export to Powerpoint Action Dialog

The properties are divided into six sections:

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- **Powerpoint Options**
  - **Include background (from Master Slide).** Flag this option to to include the background from Master Slide. If you choose to export the background, the objects on the FCS Express Master Page will be placed on the PowerPoint master slide. These background objects will then appear on all of the PowerPoint slides.
  - **Open presentation after saving.** When checked, the PowerPoint presentation will be opened after the batch process has ended.
  - **Hide Objects that are off the page.** When checked, objects that are not located on the white page in the layout will not be exported to PowerPoint.

- **Page Range**

  Choose which layout pages to export.

- **Export Options**
  - **Quality.** Set the PowerPoint quality (**High**, **Medium**, **Low**, **On-Screen**) from the dropdown list.
  - **Mode.** This option enables single or multiple layout pages per slide based on selection. With **Full Page** selected, the layout page(s) for each iteration 785 will be exported as individual slide(s). Multiple layout pages may be printed per slide using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options** 38. To export the layout pages on fewer slides, please select a preset multiple page option or **Custom Grid** by clicking the down arrow to the right to expand the dropdown list and selecting a multiple page option or **Custom Grid Options...**, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear. See an example of the output below in (Figure 20.21).
  - **Frame Page.** Flag this option to draw a frame around layout page(s).
  - **Crop To Content.** Available for selection when **Mode** is not set to **Full Page**. When selected, empty space of the layout page(s) is cropped during the export.

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- **Iteration options**
  - **Save the output of each iteration to a separate presentation.**
    - Each iteration will be saved as a new PowerPoint presentation.
  - **Combine all the output from all iterations into the same presentation.**
    - All iterations will be combined into one new PowerPoint presentation.
  - **Combine the output of all iterations into a single file, without page break.**
    - All iterations will be combined into one new PowerPoint presentation. Depending on your **Export Options→Mode** settings many iterations may be combined into one PowerPoint slide as seen in the figure below.

- **Append Options**

  Choose whether to export to a new file or append the output to an existing PowerPoint presentation.

- **Output file options**

  Click on the folder icon to choose a location on the computer or server and create the filename for the exported PowerPoint file(s). See Save Layout - File Options for a description of batch generated filenames 806.

Click **OK** when you are finished editing the properties.

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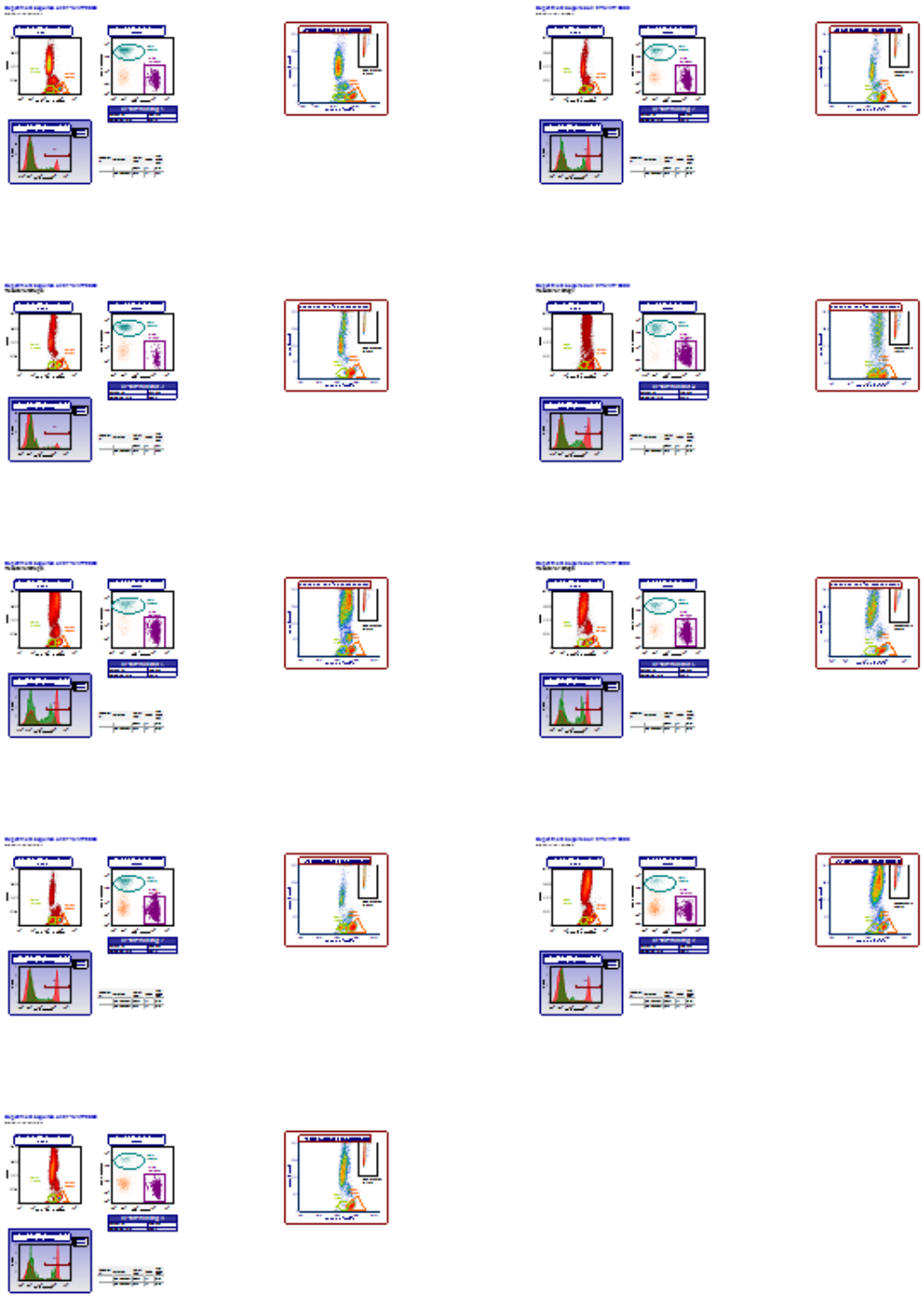

Figure 20.21 Custom Grid Options enabled for batch processing 9 data files for two layout pages exported to a single PowerPoint slide.

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Note about ungrouping objects in PowerPoint for PC versus PowerPoint for Mac

Once exported to PowerPoint, layout objects can be further ungrouped into single components in the PC version of Microsoft PowerPoint. Ungrouping objects saved by FCS Express in PowerPoint for Mac is not supported. However, presentations saved on a Mac may be opened on a PC and ungrouped.

20.2.1.4 Save To PDF

To create a Save To PDF action, click on **Save To PDF** (in the **Add Action** section of the **Batch Actions** window).

The **Create Export to PDF Action** dialog (Figure 20.22) is used to set up how each iteration of the layout will be saved as a PDF file.

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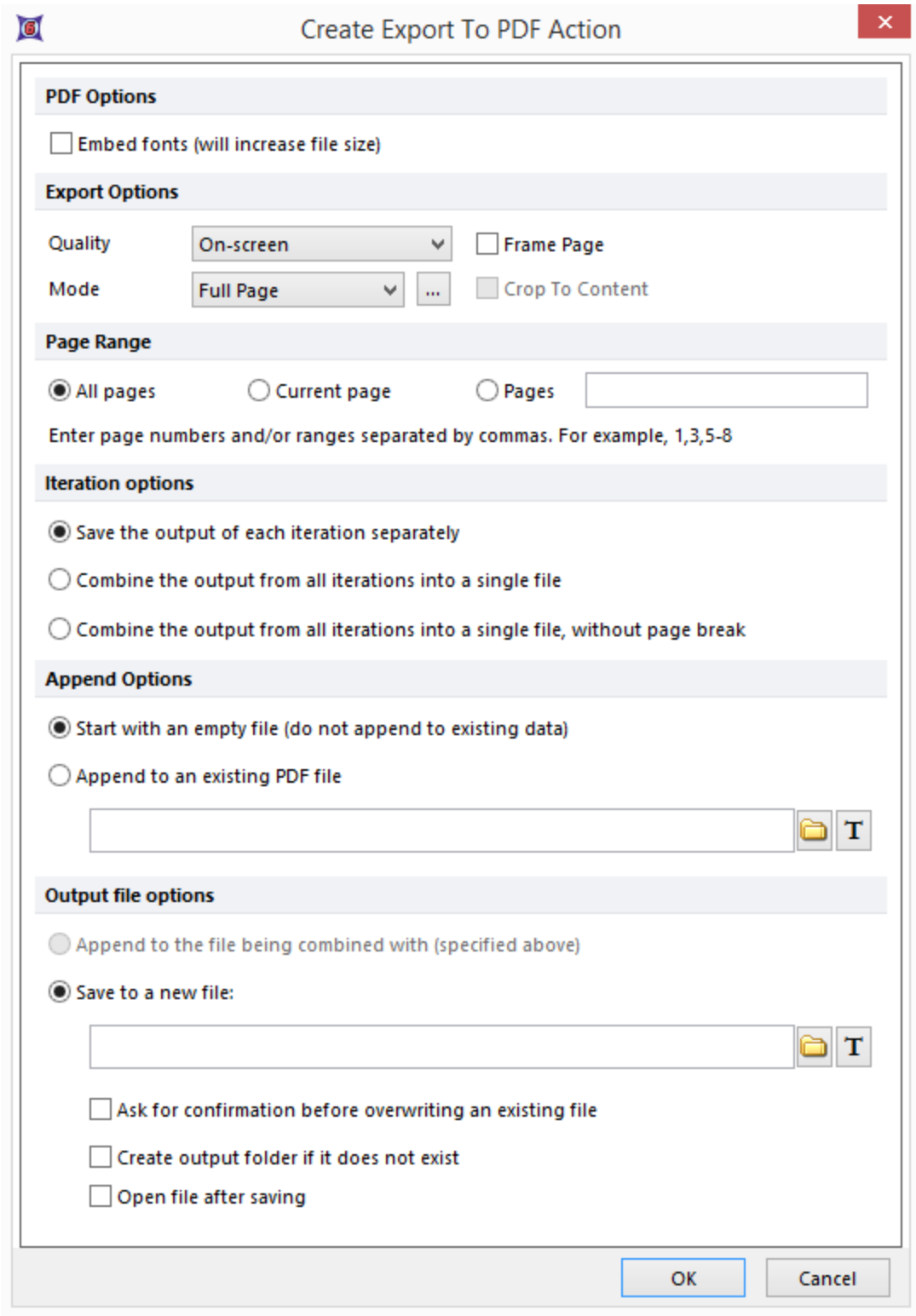

Figure 20.22 Create Export to PDF Action Dialog

You can choose to Open the PDF file after saving. The properties are divided into the sections below:

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- **PDF Options**

  Embed fonts (will increase file size). This option ensures that all of the font information used to make your document look the way it does is stored in the PDF file.

- **Export options**

  - **Quality.** Set the PDF quality (**High**, **Medium**, **Low**, **On-Screen**) from the dropdown list.

  - **Mode.** This option enables single or multiple layout pages per page based on selection. With **Full Page** selected, the layout page(s) for each iteration 785 will be exported as individual slide(s). Multiple layout pages may be printed per slide using a preset multiple page option (i.e. **3 Pages with Notes** or **9 Pages Vertical**) or **Custom Grid Options** 38. To export the layout pages on fewer pages, please select a preset multiple page option or **Custom Grid** by clicking the down arrow to the right to expand the dropdown list and selecting a multiple page option or **Custom Grid Options...**, respectively. When selecting **Custom Grid Options**, the **Custom Grid** popup window will appear, which will contain following five settings:

  - **Frame Page.** Check this option to draw a frame around layout page(s).

  - **Crop To Content.** Available for selection when **Mode** is not set to **Full Page**. When selected, empty space of the layout page(s) is cropped during the export.

    See an example of the output in Figure 20.23 below.

- **Page Range**

  Choose which layout pages to export.

- **Iteration options**

  - **Save the output of each iteration separately.**
    - Each iteration will be saved as a new file.
  - **Combine all the output from all iterations into a single file.**
    - All iterations will be combined into one new file.
  - **Combine the output of all iterations into a single file, without page break.**

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- All iterations will be combined into one new file. Depending on your **Export Options→Mode** settings many iterations may be combined into one page in the file as seen in the figure below.

- **Append Options**

  Choose whether to start with a new file or append the output to an existing PDF file.

- **Output file options**

  - **Append to the file being combined with (specified above)**. This option is only available when both the **Combine the output from all iterations into a single file** and the **Append to an existing PDF file** are selected.

  - **Save to a new file**. Use this option to generate a new PDF file and enter the filename for the generated PDF file. See Save Layout - File Options for a description of batch generated filenames 806.

  - **Ask for confirmation before overwriting an existing file**. Chose this option to be prompted about the existence of a file with the same name before overwriting it.

  - **Create output folder if it does not exist.** Chose this option to automatically create the output folder if it does not exist.

  - **Open the PDF file after saving.** When checked, the PDF file will open at the end of each iteration.

Click **OK** when you are finished editing the properties.

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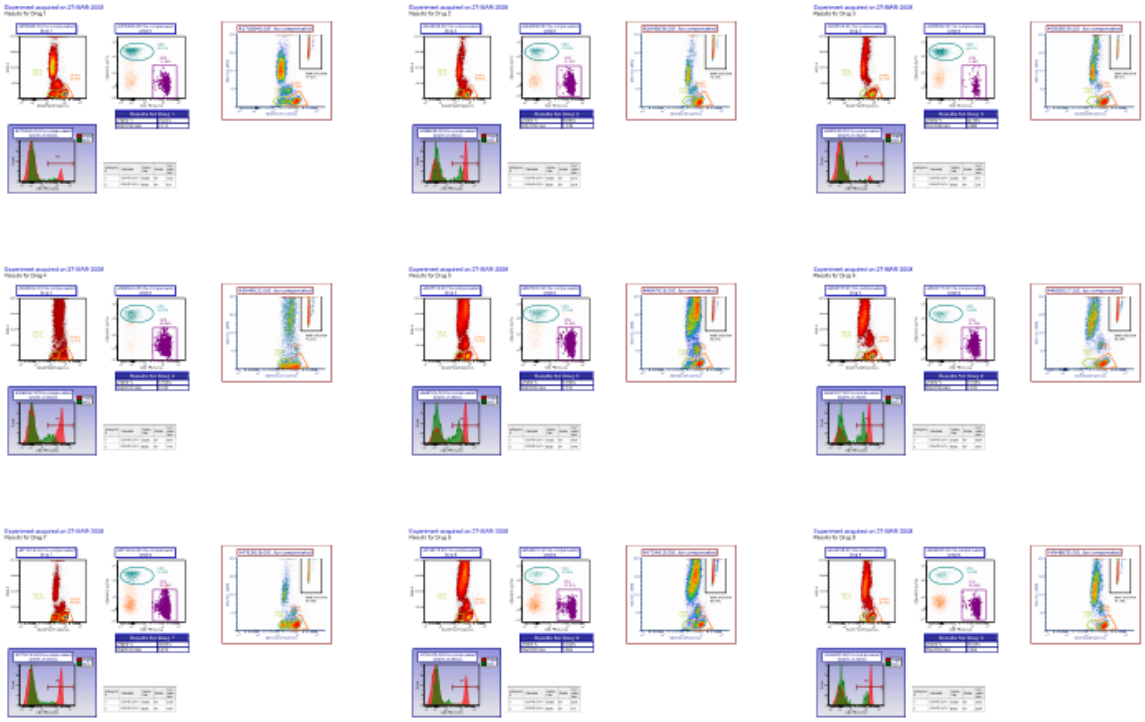

Figure 20.23 Custom Grid Options enabled for batch processing 9 data files fortwo layout pages exported to a single PDF page.

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20.2.1.5 Save As Picture

To create a Save As Picture action, click on **Save As Picture** (in the **Add Action** section of the **Batch Actions** window).

The **Create Save Picture of Plot Action** dialog (Figure 20.24) is used to set up how, in each iteration, plots or layout pages will be saved as picture files.

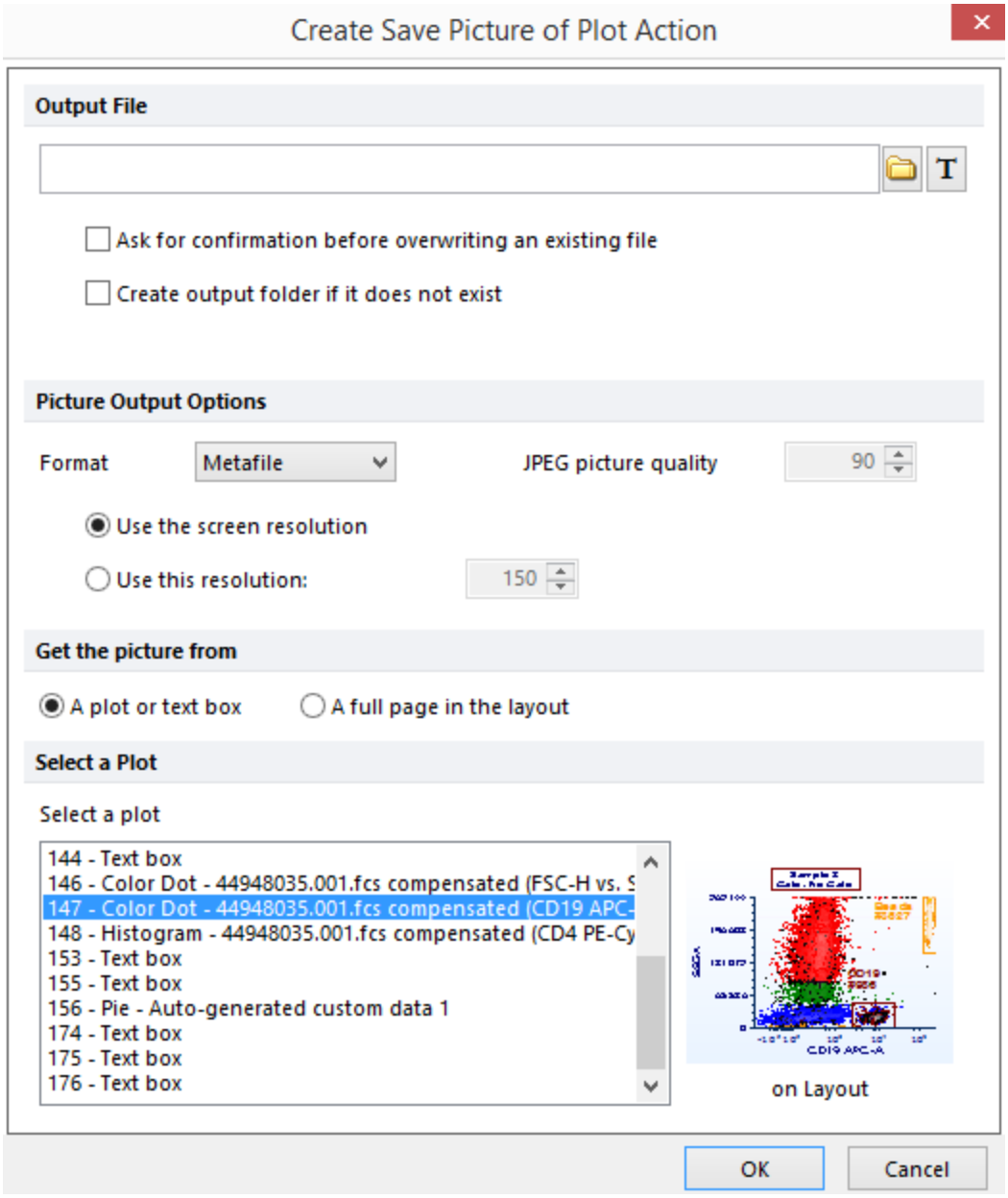

Figure 20.24 Create Save Picture of Plot Action Dialog

The properties are divided into three sections:

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- **File Options**

  Enter the filename for the exported file. See Save Layout - File Options for a description of batch generated filenames 806.

- **Picture output options**

  Choose the file type (**JPEG**, **Bitmap**, **Metafile**, **GIF**, **PNG**, **TIF**) from the drop-down list. For JPEG files, choose a picture quality using the spin control. Set the resolution using the radio buttons. For Metafiles, choose whether or not to embed the plot data as a bitmap.

- **Get a picture from**

  Choose to save a particular plot/text box or a full page in the layout using the radio buttons. Select the plot or layout page from the list box.

Click **OK** when you are finished editing the properties.

20.2.1.6 Folders (Action Containers)

Folders (Action Containers) are used for organizational purposes. They do not generate output by themselves. Think of them simply as a container for a group of related actions.

The advantage of folders is that by unselecting the folder, the actions within the folder will not be executed during the batch.

In Figure 20.25, two folders are defined. The actions in the *Main Actions* folder will all be run, since the *Main Actions* folder is checked. However, the actions in the *Supplemental Actions* folder will not be executed (even though the individual actions are checked), because the *Supplemental Actions* folder is not checked.

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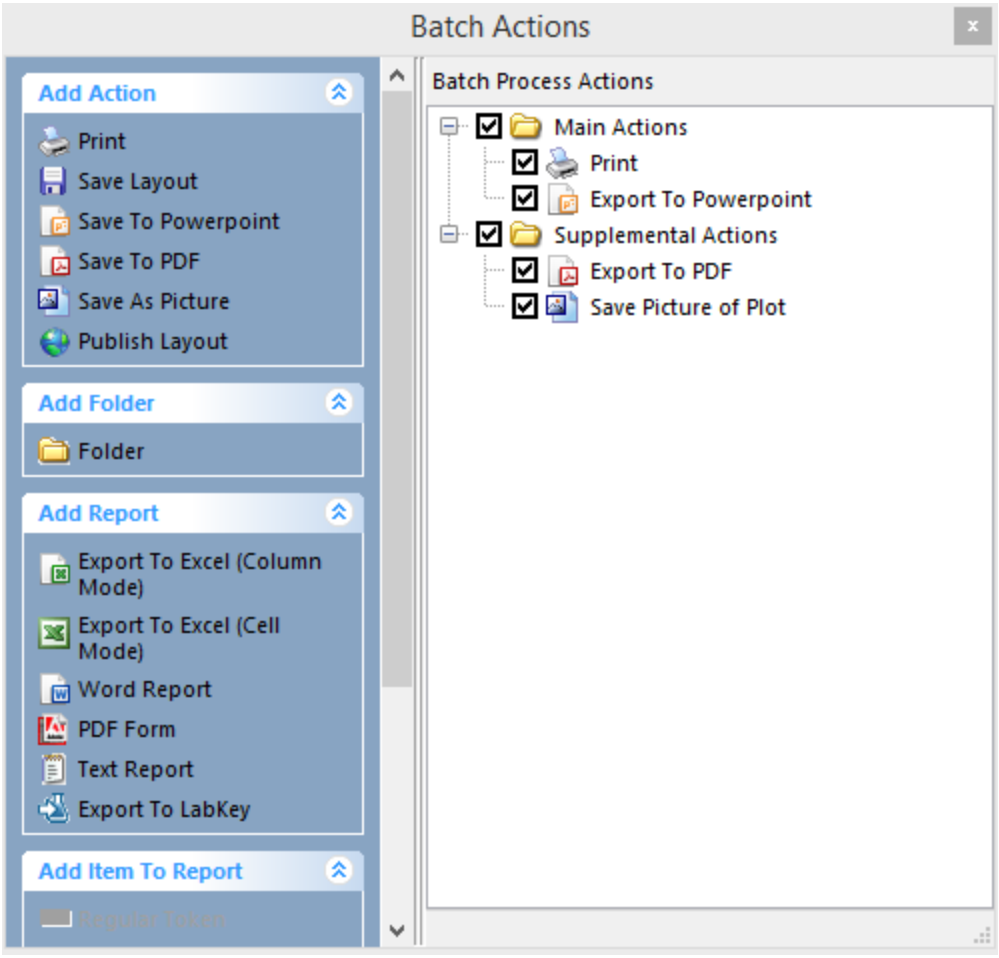

Figure 20.25 Folders (Action Containers)

If a folder is deleted, all its contained actions will be deleted. You can drag and drop a folder like any other action, and all the contained actions will move with it. If you copy a folder to the clipboard, all the contained actions will be copied.

20.2.2 Batch Processing Reports

Reports are used to export items (e.g., tokens, pictures) into Excel, Word, as Text/PDF files, or to LabKey Server 1483. All reports can contain tokens, and Word/Excel reports can also contain images. Exporting items using Reports is a two-step process:

1. Add the Report type to the Batch Actions list by selecting it from the **Add Report** section of the **Batch Actions** window.
2. Add the item(s) to be exported to the appropriate Report process.

There are seven types of reports:

- Excel - Column Mode 825 (text)

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- Excel - Cell Mode 828 (text and images)
- Word Report 830 (text and images)
- PDF Form 832 (text)
- Text Report 833 (text)
- LabKey Server 833 (tokens, images, PDF files, FCS files, FCS Express Layouts)

The items which can be exported to a report are determined by the report type. Depending on the report type, the following items may be available:

- Regular Token 692 (all available tokens, including Custom tokens 727)
- Statistic Token 703
- Keyword Token 708
- Picture 839 (a plot in the layout, or an entire page of the layout, only used in Word, Excel-Cell Mode, and DDS reports)
- Text Box 152 (text in a text box (e.g., canned comments) can be exported to a column in an Excel worksheet or other location)
- PDF 107
- Layout 92
- PowerPoint 1445
- FCS File

An example of the Batch Process Actions for an Excel (Cell Mode) report is shown in Figure 20.26.

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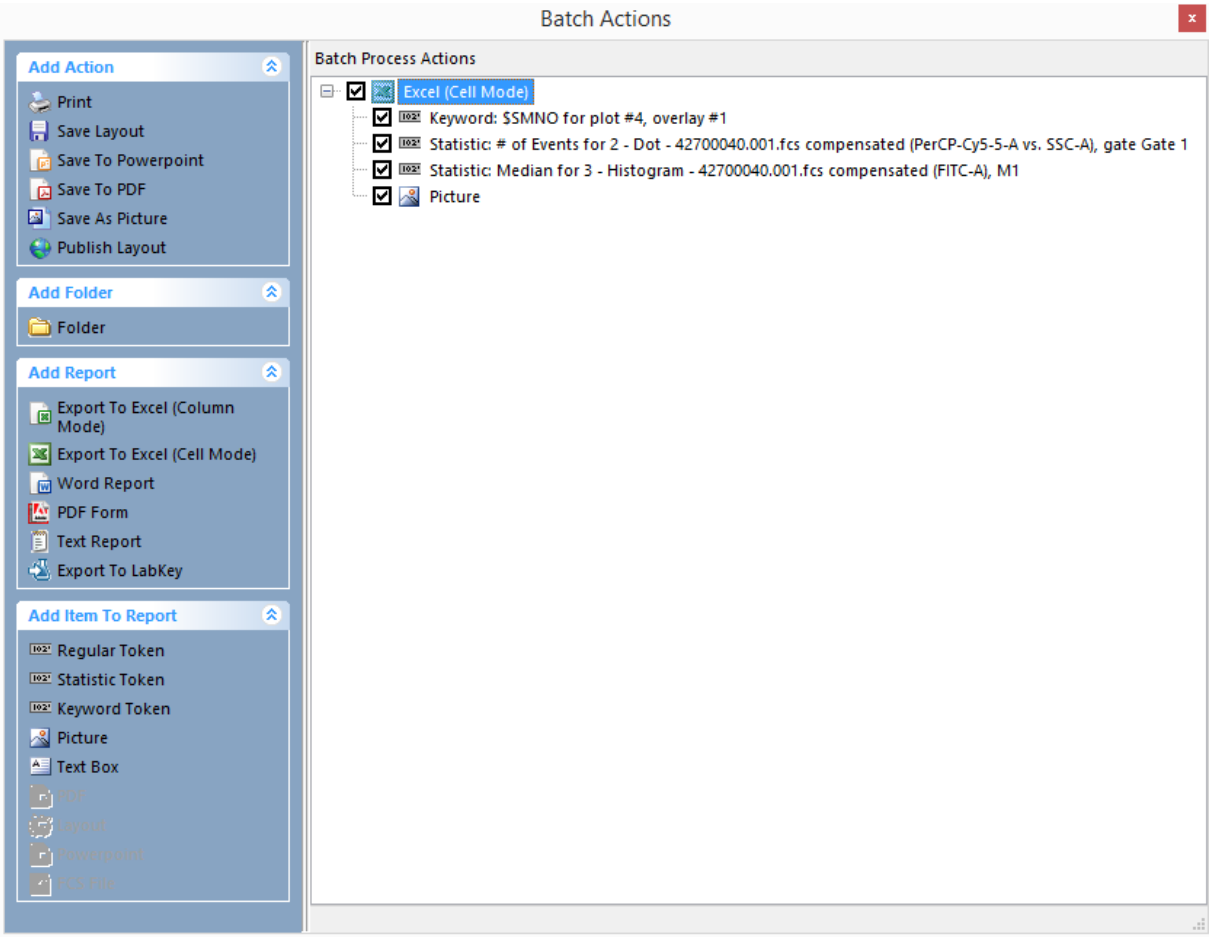

Figure 20.26 Batch Process Actions for Excel Report

This report will export two statistics and a picture of a specified plot for each iteration of the batch process. Only items that are checked will be exported.

Using the check boxes for each of the Batch Process Actions, an item can be enabled or disabled to run with the Batch Process. If you double click on the Batch Process Action (or right-click and choose **Properties** from the pop-up menu), you can navigate to the **Running Options** page of the **Options** dialog to control the activation state of an action (Figure 20.27). If you choose the **Set the checkbox from the value of the expression below** radio button, you can input a formula (using standard or custom tokens) to control the action.

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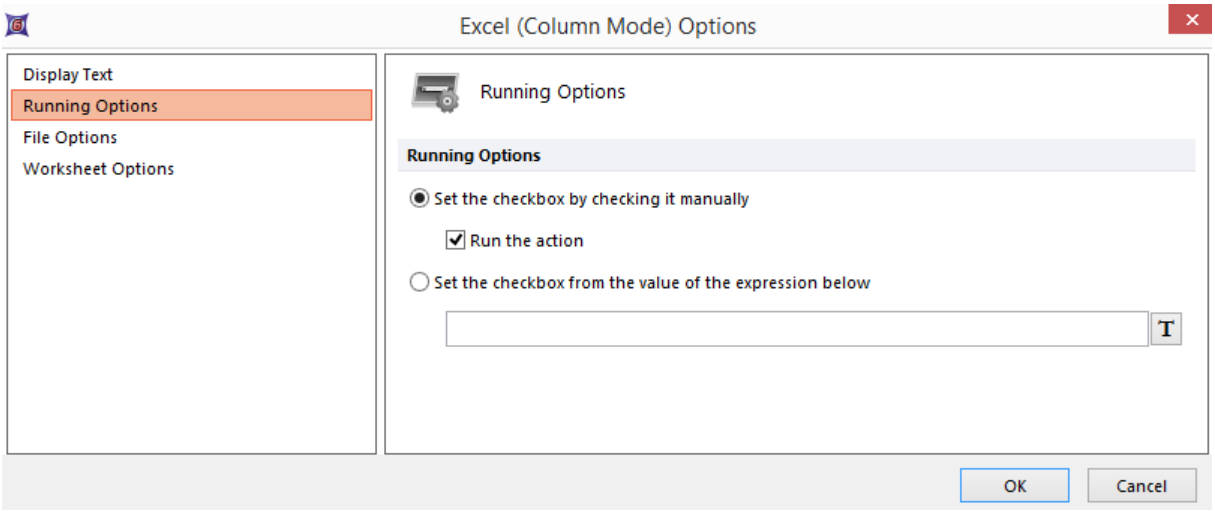

Figure 20.27 Running Options

20.2.2.1 Working with Reports

To create a new report:

1. Use the **Batch→Batch Processing→Batch Actions** command.
2. Select the report type from the **Add Report** section of the **Batch Actions** window. A properties dialog for that report type will appear. Once the properties are set, the report will be added to the list of **Batch Process Actions**.

There are seven different types of reports:

- Excel (Column Mode) 825
- Excel (Cell Mode) 828
- Word 830
- PDF form 832
- Text 833
- LabKey Server 833

- **Excel (Column Mode)**

  All tokens contained in this report will be written to a Microsoft Excel file. Pictures cannot be added to this report. Each token will be reported in its own column. The values for each iteration are written in the same column, but in new rows. An example of the output of an Excel (Column Mode) report is shown in Figure 20.28.

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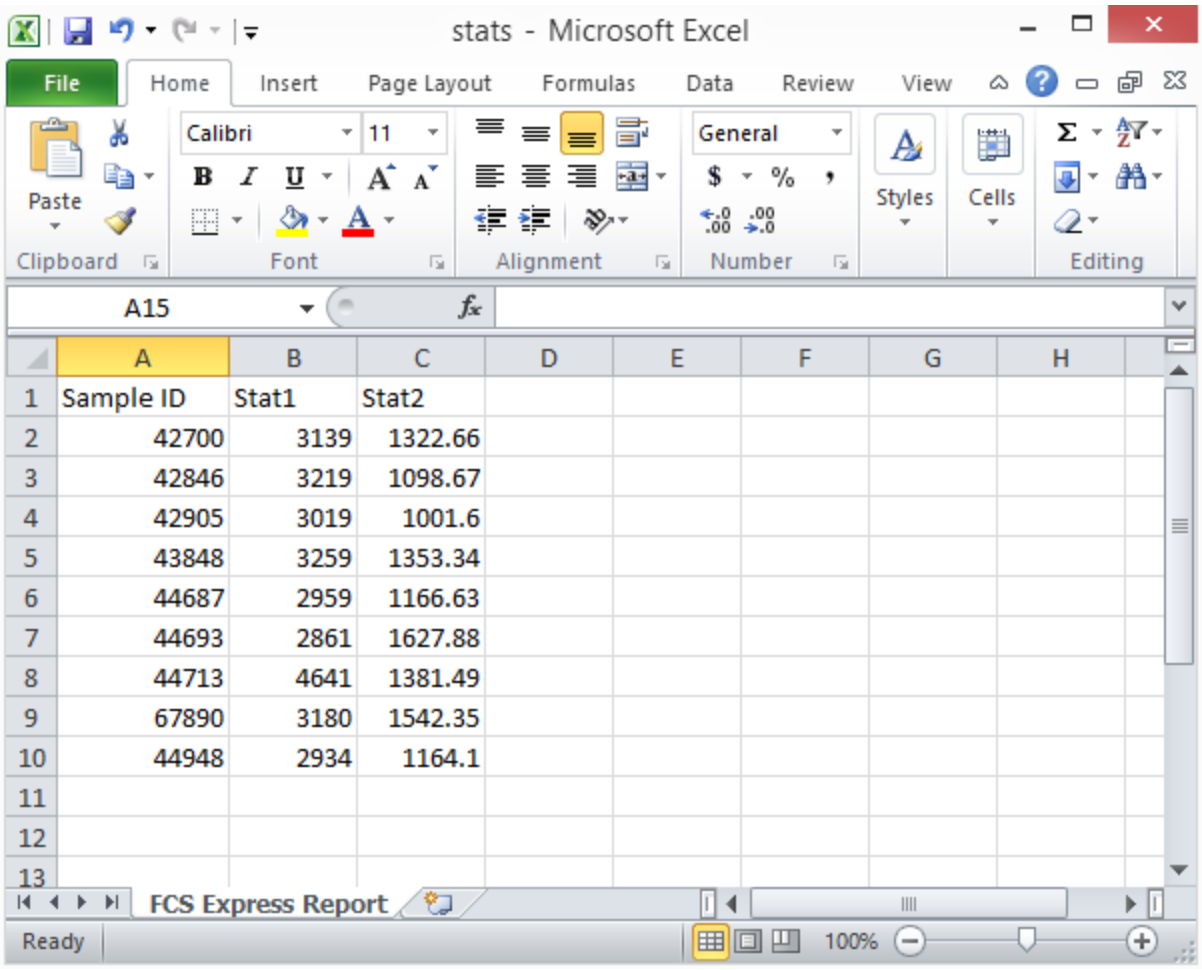

Figure 20.28 Excel (Column Mode) Sample Report

The **File Options** page of the **Excel (Column Mode) Options** dialog (Figure 20.29) has two sections:

- **Append Options**

  With the radio buttons, you can choose to **Start with an empty file** or **Combine the output with the data in an existing file**. If you combine data from an existing file, FCS Express will join the Batch Processing output with the data that exists in the Excel file (.xls) entered in the text box. The final output (new iterations plus existing data) will be written to another worksheet.

- **Output file options**

  When combining data with an existing file, the destination file can be a new file (.xls) or the existing Excel worksheet.

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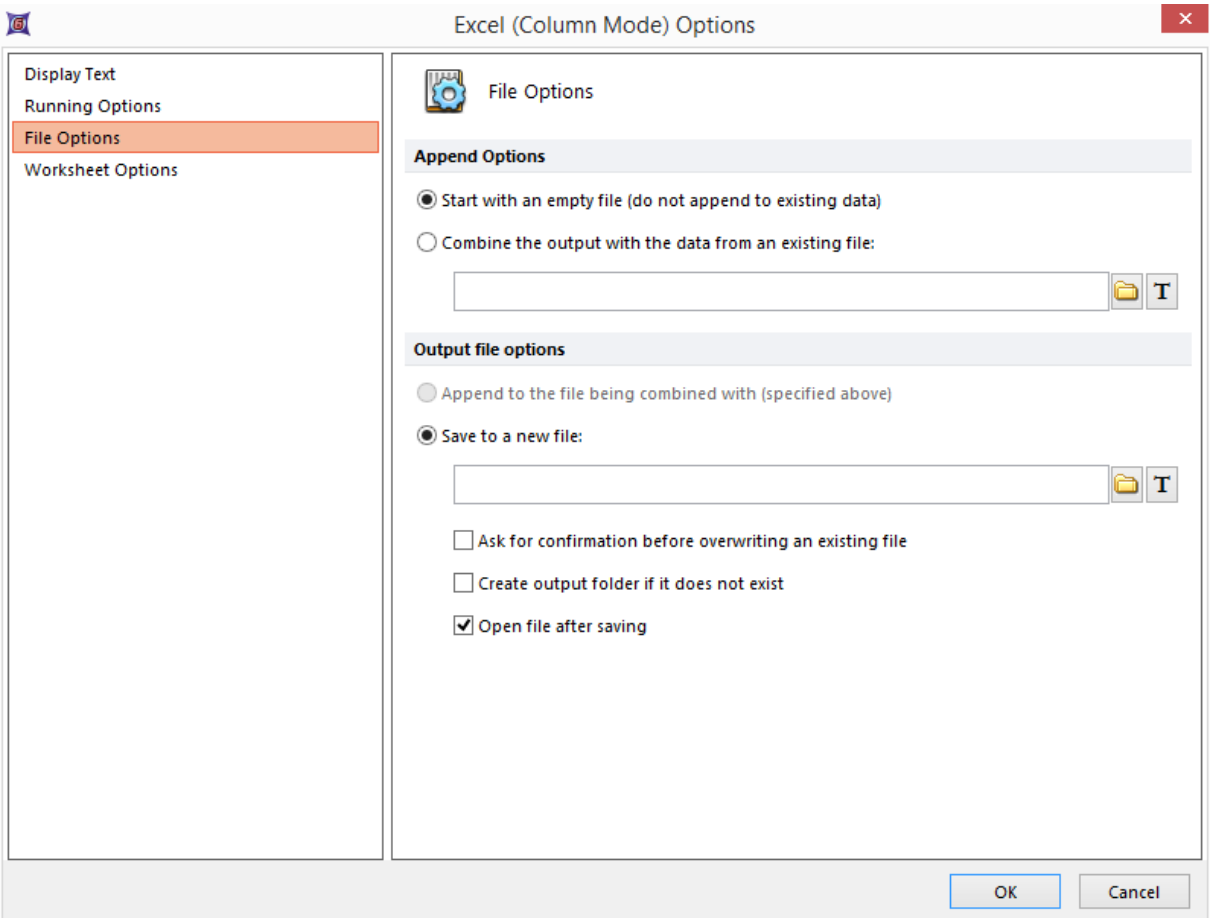

Figure 20.29 Excel (Column Mode) File Options

The **Worksheet Options** (Figure 20.30) page of the **Excel (Column Mode) Options** dialog allows you to assign the name of the worksheet (optionally using a token). You can use either **a new worksheet** or **an existing worksheet**. If you use an existing worksheet, you can designate **Row Options** and **Column Options** (e.g., start at the first available row or column or start at a particular row or column).

The **Row/Column Arrangement** radio button controls whether iterations are exported as new columns or new rows within a document.

The **Header Options** allow FCS Express to automatically write the Field Label 837 to the result Excel document. By default, this option is enabled.

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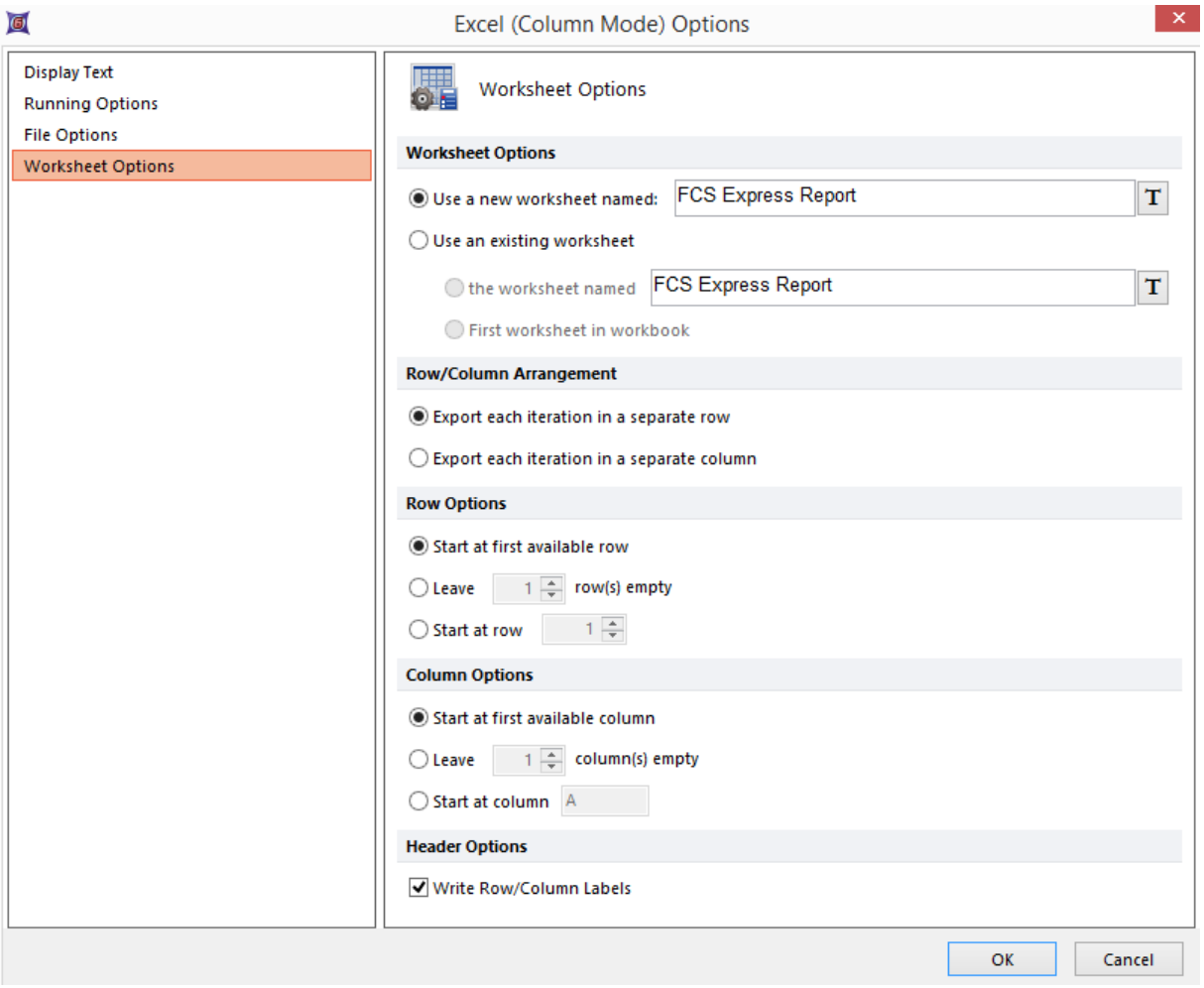

Figure 20.30 Worksheet Options

- **Excel (Cell Mode)**

  The Excel (Cell Mode) report allows you to merge you data with a preexisting spreadsheet. You select a Template Document (spreadsheet) containing headings, fonts, and colors, and then specify the exact cell destination for each output item, including pictures. An example is shown in Figure 20.31.

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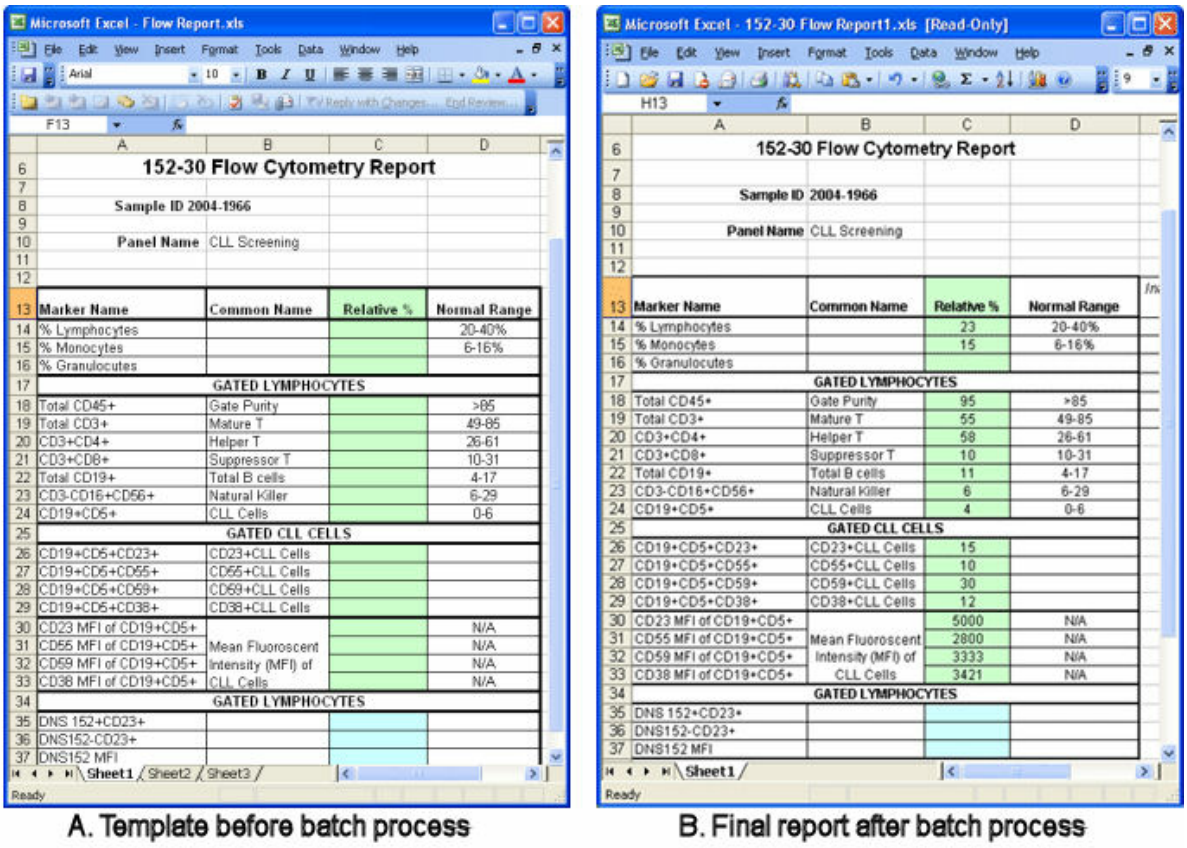

Figure 20.31 An Example Excel (Cell Mode) Report

The **File Options** page of the **Excel (Cell Mode) Options** dialog (Figure 20.32) has four sections:

- **Template Document**

  Enter the filename (and path) to your preformatted Excel workbook. The first worksheet in the workbook will be used as the template.

- **Iteration options**

  Output each Batch iteration to a separate file (the default), or combine the output from all Batch iterations to a single file. In the latter case, each iteration is written to a separate worksheet within a single Excel workbook.

- The **Append Options** and **Output file options** are identical to those for the Column Mode described above.

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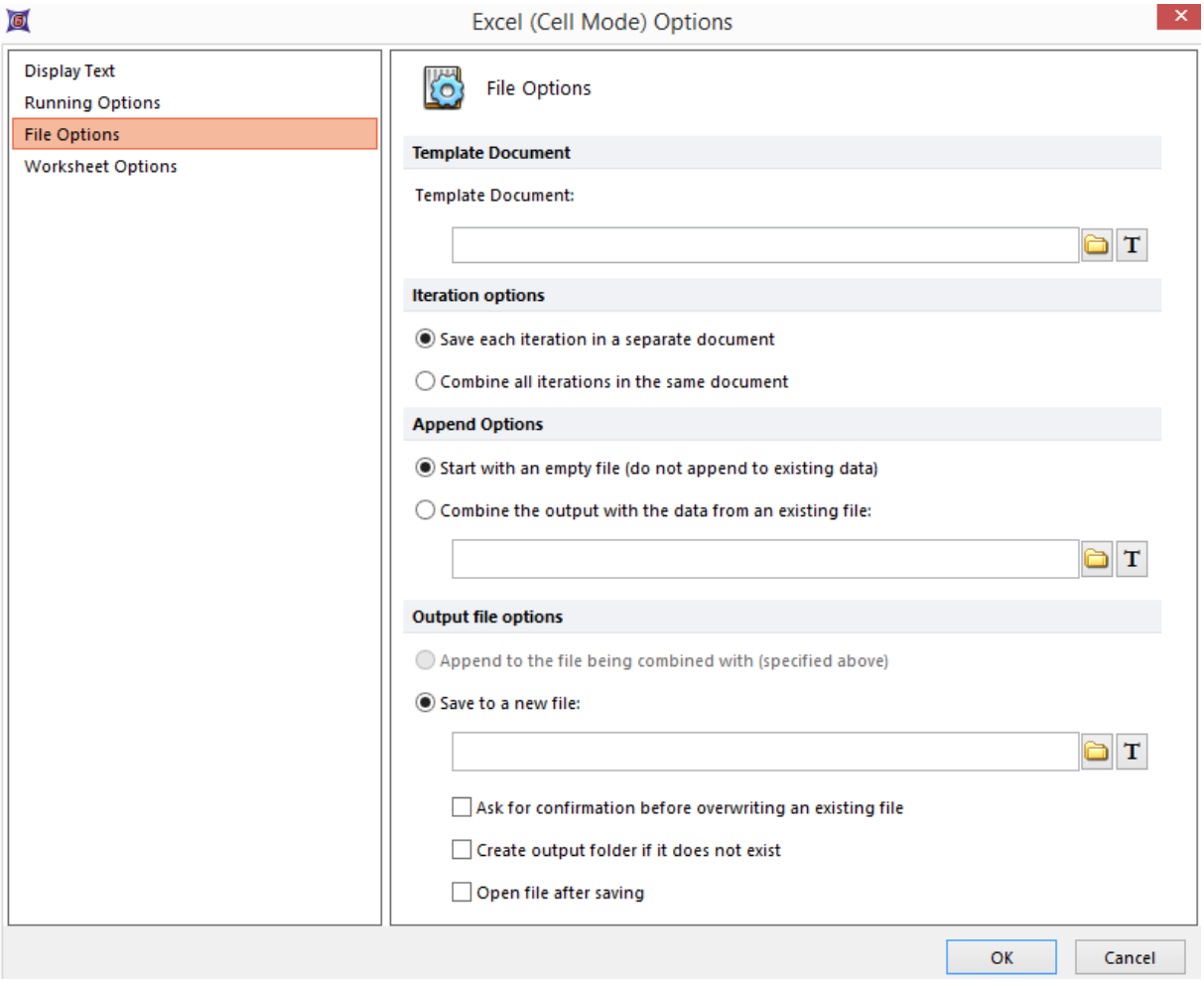

Figure 20.32 Excel (Cell Mode) File Options

- **Word Report**

  With this report type you can create Word files resembling Figure 20.33. A Word Template Document contains all the headings and text formatting. When combined with the output of the values from Batch Processing, you can create professional quality reports. All tokens contained in a Word Report, including pictures, will be written to a Microsoft Word file during each iteration using the mail merge features of Word. Once each token has been configured with a Field Name837, the token value will be merged with the Word Merge Field. Details on setting up the Word report with mail merge fields are described under Creating Templates for Reports834.

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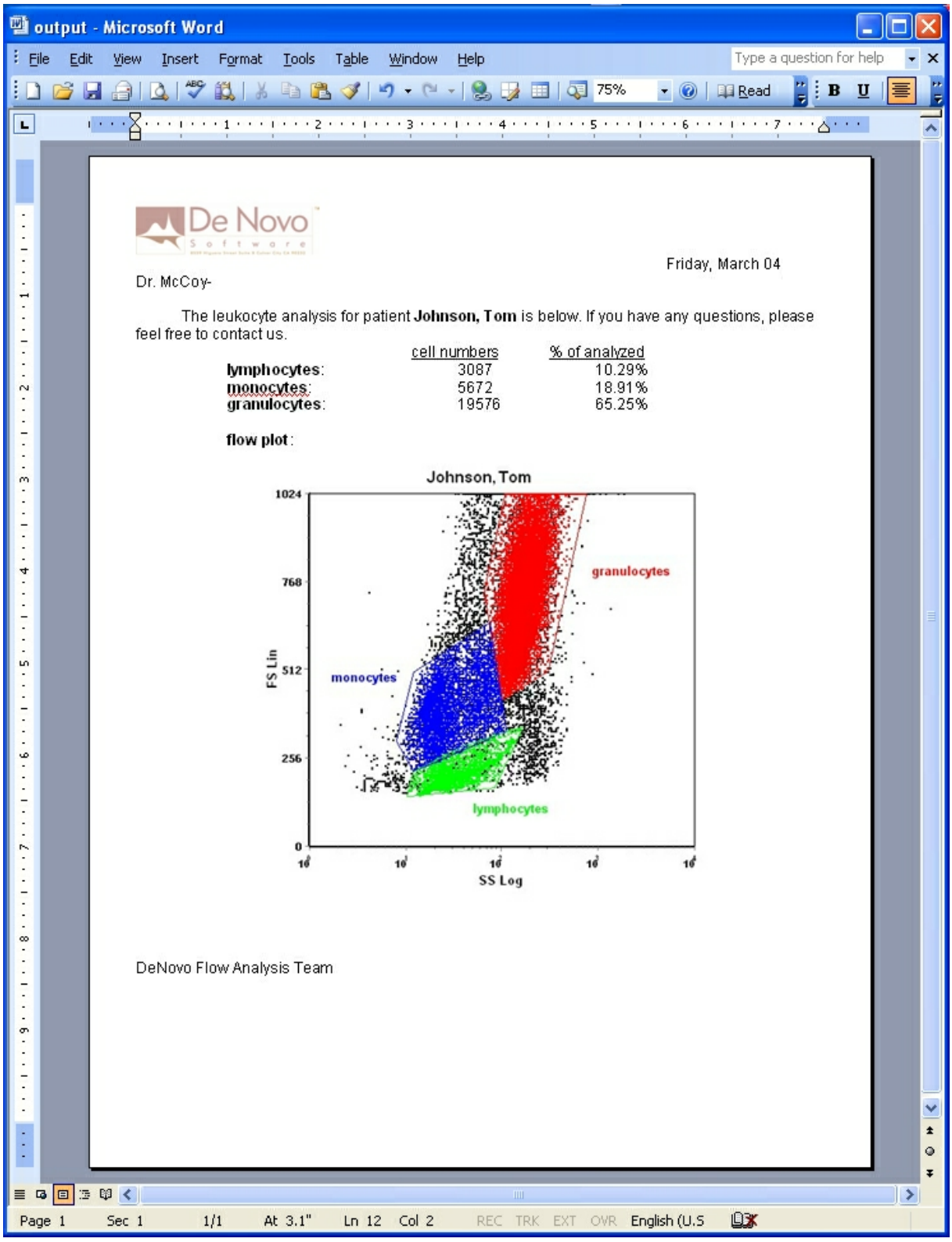

Figure 20.33 Example Word Report

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The **File Options** page of the **Word Report Options** dialog has four sections similar to those described for Excel (Cell Mode) reports:

- **Template Document**

  Enter the filename (and path) to your preformatted Word document.

- **Iteration options**

  Output each Batch iteration to a separate file (the default), or combine the output from all Batch iterations to a single file.

- The **Append Options** and **Output file options** are identical to those for the Column Mode described above.

- **PDF Form Report**

In addition to exporting your layout directly as a standard PDF file 107, FCS Express can merge your statistics with PDF Form files. PDF forms are special PDF files that allow you to enter information in specific fields. FCS Express will export all tokens contained in the report to the specific fields in the PDF file. Once each token has been configured with a Field Name 837, the token values will be placed into the report fields designated in a preformatted PDF file.

The **File Options** page of the **Export to PDF Options** dialog has four sections similar to those described for Excel (Cell Mode) reports:

- **Template Document**

  Enter the filename (and path) of the preformatted PDF template document.

- **Iteration options**

  Output each Batch iteration to a separate file (the default), or combine the output from all Batch iterations to a single file. In the latter case, each iteration is written to a new page in the PDF document.

- The **Append Options** and **Output file options** are identical to those for the Column Mode described above.

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- **Text Report**

  All tokens in a Text Report will be written to a text file, with custom separator (see below the **Encoding Options**). The first row of the text file will contain the column header of each exported item (see below the **Header Options**). A new row of outputted values will be created for each iteration. *Note:* Pictures cannot be exported to this type of report.

  The **File Options** page of the **Text File Report Options** dialog is similar to the Excel (Column Mode) format (described above) with two sections:

  - **Append Options**

    With the radio buttons, you can choose to **Start with an empty file** or **Combine the output with the data in an existing file**. If you combine data from an existing file, FCS Express will join the Batch Processing output with the data that exists in the text file entered in the text box. The final output (new iterations plus existing data) will be written to another text file.

  - **Output file options**

    This option goes along with the previous one.
    When **Start with an empty file** is selected in the **Append Options**, a file name must be specified. Available file extensions are **.tab**, **.prn** and **.csv**. To generate a Txt file, the **.txt** extension needs to be manually specified in the file name. When combining data with an existing file, the destination file can be a new file or the existing text file.

  Two additional sections are also present:

  - **Encoding Options**

    Two drop-down menus allow to select the desired Separator (Tab, Space or Comma) and the Encoding (ANSI or Unicode (UTF8)).

  - **Header Options**

    This check box defines whether the first exported raw will contain the column header of each exported item.

- **LabKey Server Report**

  LabKey Server is free, open source software that supports automated quality control, centralized data management, and web-based data

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sharing. You may use the batch processing function of FCS Express to export your data to LabKey Server.

After registering with LabKey Server, downloading their free server, and preparing LabKey Server to receive your exported data, simply click on the **Export to LabKey** icon under "**Add Report**" from the Batch Actions window. The **Export to LabKey Options** dialog window appears (Figure 20.34). Select **Web Service** in the left pane of the window, and enter in your **LabKey Server URL**, **Protocol ID** (must be an integer), **Name** of the report, and, if desired, a **Comment**, into the fillable fields. Note that tokens may be entered into the **Name** and **Comments** fields. Enter in the Username and Password you used to register with LabKey Server, and click OK.

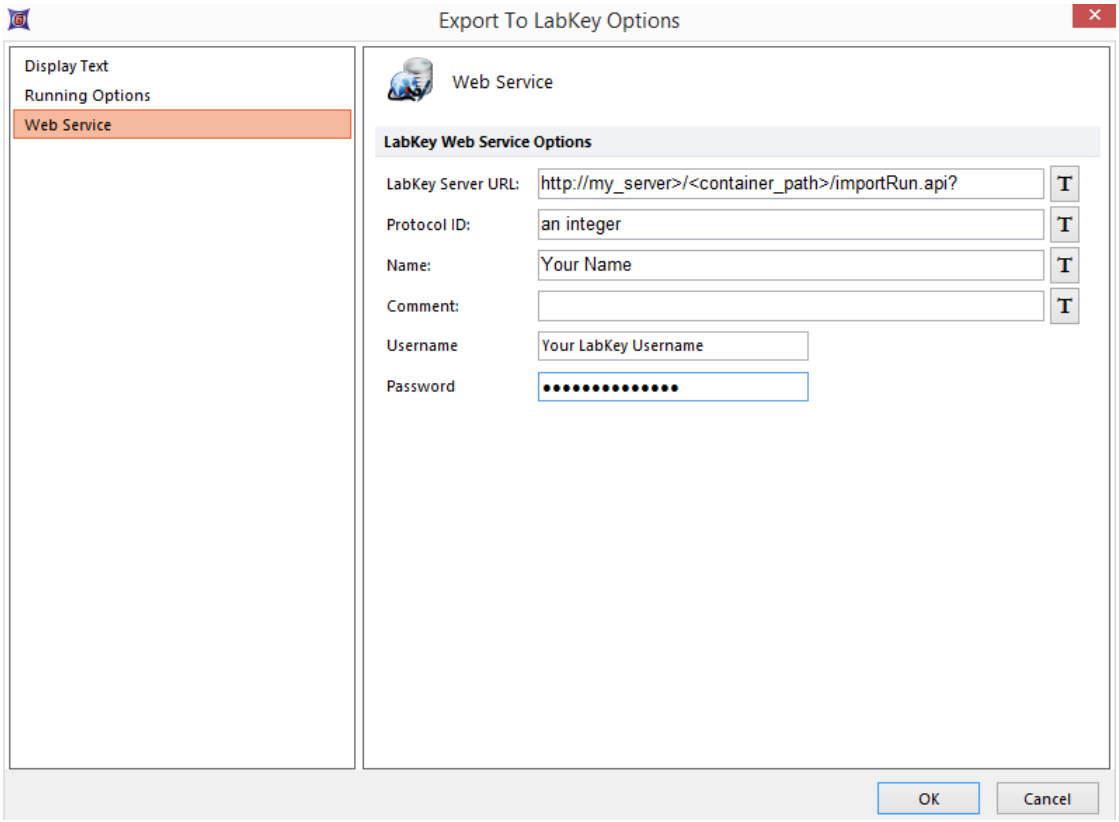

Figure 20.34 Export to LabKey Options

20.2.2.2 Creating Templates for Reports

You can create templates for Excel (Cell Mode) reports, as well as PDF and Word reports.

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- *Creating Excel Templates*

  Excel templates are simply Excel spreadsheets. You can use any Excel functionality in the spreadsheets that are used as templates. Simply leave blank cells where you want FCS Express to put the data or pictures. Enter those cells as the **Cell Address** on the **Field** page of the options dialog corresponding to the exported item.

- *Creating PDF Form Templates*

  When creating a PDF template, the PDF file must contain text fields (which must be uniquely named) where FCS Express will place the data from your layouts. PDF files containing text fields can be created with numerous PDF creation programs, such as Adobe Acrobat®. Please refer to your PDF creation program for instructions on how to create PDF files with text fields.

- *Creating Word Templates*

  In order to create a report using Word, you must use the Word mail merge functions. A brief overview of how to create mail merge templates is given below. For more details, please consult the documentation for your version of Microsoft Word®.

  When creating a Word report template, you must enter *fields* into your Word document where FCS Express will place the exported data from the layout during batch processing. Enter those fields as the **Mail Merge field name** on the **Field** page of the options dialog corresponding to the exported item.

  Please refer to the help files in your version of Word for detailed instructions on how to create Word documents with mail merge fields.

20.2.2.3 Adding Items to Reports

An *item* is something that is exported from FCS Express to a particular report. Reports usually contain multiple items. Whenever you add an item to a report, it is added to the bottom of the list of existing items in the report. To see all the items contained in a report, expand the report by clicking on the **+** to the left of the report name. You can reorder the items in the report 841 by selecting item(s) and dragging them to a new order position. You can also drag item(s) from one report to another.

Items can only be added to the **Batch Process Actions** list after a Report has been added because the item must be added directly to a report. There are four methods to add items to reports:

- Adding items from a Text Box or a Statistics window.

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If you have a layout with an open statistics window or with tokens in a text box, you can add some or all of these items to an existing report. Perform the steps as follows:

1. Open the Batch Actions window by using the **Batch→Batch Processing→Batch Actions** command.
2. Make sure you have a report in the **Batch Process Actions** list.
3. Dock the Batch Actions window (or position it such that you can view the statistics window or Text Box in the layout).
4. To add statistics, highlight the cell(s) in the statistics grid and drag them onto the report in the **Batch Process Actions** area. When the report is highlighted, release the mouse button.
5. To add token(s) from a Text Box, highlight one or more tokens and drag them onto the report in the **Batch Process Actions** area. Once the report is highlighted, release the mouse button.

- Adding items from a plot or gate.

  Perform the steps as follows:

  1. Open the Batch Actions window by using the **Batch→Batch Processing→Batch Actions** command.
  2. Make sure you have a report in the **Batch Process Actions** list.
  3. Click on the page of the layout containing the plot(s) from which the items will be added.
  4. Drag and drop the plot or gate onto the report in the **Batch Process Actions** area. When the report is highlighted, release the mouse button.
  5. Double-click **Statistic token** or **Keyword token** from the **Paste Special** dialog.
  6. Select the item(s) from the **Statistic** list box or the **Please select one or more keywords** dialog.

  Note: Multiple items may be selected and added while holding down Ctrl on the keyboard.

- Adding items from the Add Item to Report list.

  When you first open the **Batch Actions** window, the items in the **Add Item to Report** section will be disabled. After adding a report to the Batch Process Actions list, items appropriate to that report type will be enabled. To add an item to a report:

  1. Open the Batch Actions window by using the **Batch→Batch Processing→Batch Actions** command.
  2. Click on the report that will contain the item.
  3. Click on an enabled item in the **Add Item to Report** section.

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4. Once an item has been added to a report, you will need to configure the item's "report-specific" property 837. This property tells FCS Express where to insert the item in the final report.

- Adding items by right clicking on the Action (Figure 20.35).

1. Open the Batch Actions window by using the **Batch→Batch Processing→Batch Actions** command.
2. Right click on the report that will contain the item.
3. Mouse over **Add Item.**
4. Choose an item from the list of available items in the dropdown.

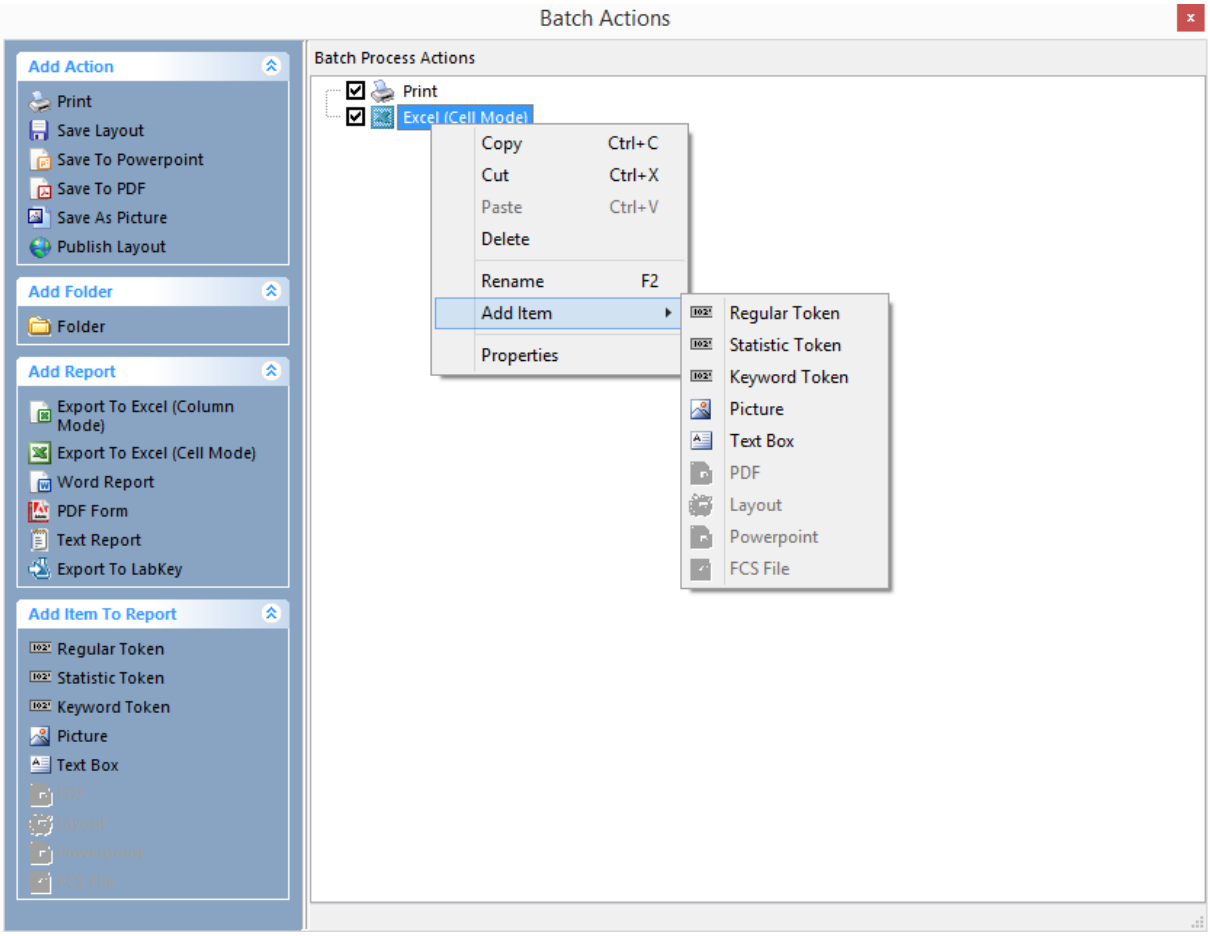

Figure 20.35) Adding items to a batch action by right clicking on the action.

20.2.2.3.1 Configuring a Report Item's Report Specific Property

When adding items to reports, there is a report-specific property that needs to be set to tell FCS Express where to insert the item in the final report. This property differs depending on report type. It is usually found on the **Field** page of the report item options dialog. For example, when you add a **Regular Token** from the **Add Item to Report** section of the **Batch Actions** window for either

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of these two report types, after the specific token has been chosen a dialog box will open to the **Field** page and the appropriate property can be entered in the corresponding edit field. You can also reach the **Field** page by right-clicking on the item in the **Batch Process Actions** list and selecting **Properties** from the pop-up menu.

- *Excel (Column Mode) and Text Reports*

  The **Column Name** specifies the text that will appear in the report at the top of each column of data. For example, if the token you are exporting displays the number of events for a plot, a useful Column Name could be *Number of Events*. In FCS Express V5 and later versions, the report-specific property of Excel (Column Mode) and Text Reports defaults to the name of the item in your layout (e.g., the token description in Fig. 20.36). To change this default behavior so that the field is left blank, see the Header Options 827.

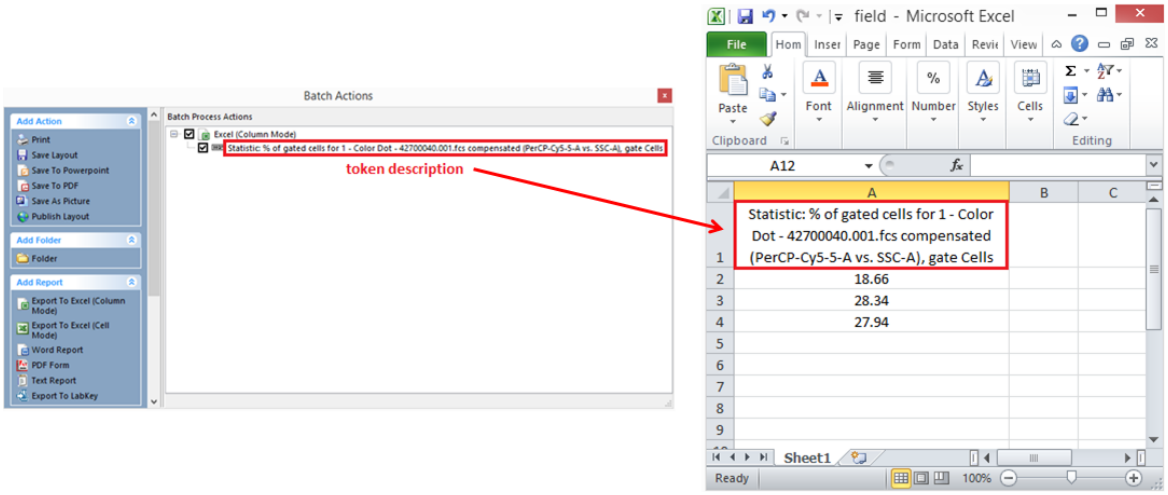

Figure 20.36 Token description automatically exported to Excel as Column Name in FCS Express V5

If you wish to manually define the report-specific property in lieu of the default, enter it in **Column Name** field of the **Field** category (Figure 20.37).

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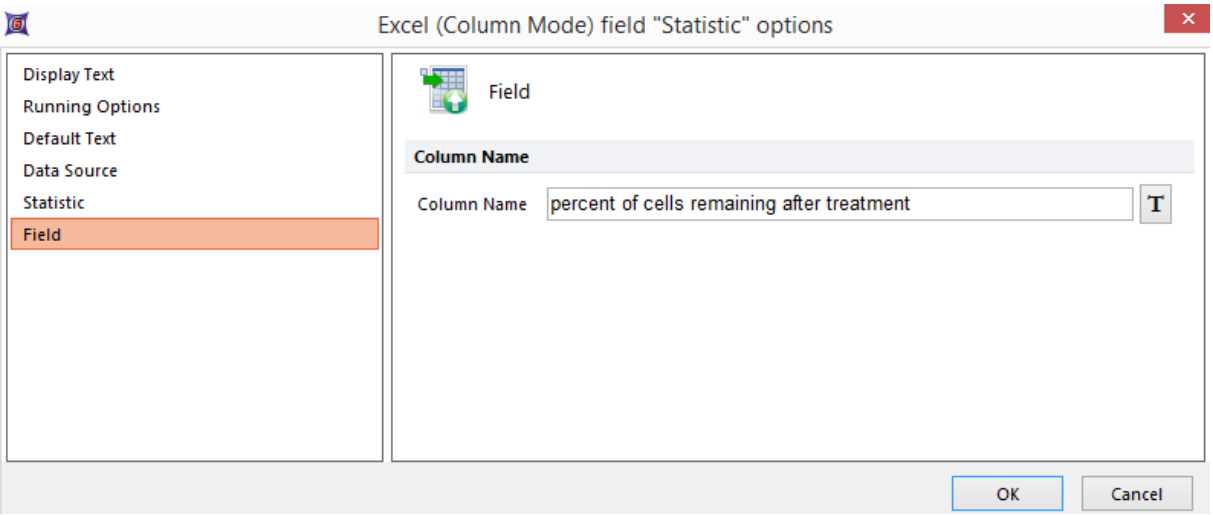

Figure 20.37 Manually entering the Column Name property

- *Excel (Cell Mode)*

  The report-specific property for Excel (Cell Mode) reports is set in the **Cell Address** field. The Cell Address specifies the Excel cell to write the data.

- *Word*

  The specific property for Word reports is set in the **Mail Merge field name** which specifies the mail merge field in your Word template 834 where the layout item will be placed. The field name must exactly match the name in the Word document.

- *PDF Form*

  For PDF reports, the specific property is set by editing the **Field Name**. This specifies the text field in your PDF template 834 where the layout item will be placed. The field name must match the corresponding field in the PDF.

- *LabKey Server*

  For LabKey Server reports, the specific property is set by editing the **XML Node**. This specifies the Data Field in LabKey Server to which the layout item will be sent. The **XML Node** must match the corresponding Data Field name in LabKey Server.

20.2.2.4 Adding Pictures to a Report

A picture of a Text Box, plot, or an entire page of the Layout can be exported to Excel (Cell Mode), Word, and LabKey Server reports. To add a picture to a report:

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1. Click on the appropriate report type in the Add Report section of the Batch Actions window 822.
2. Highlight the appropriate report type and click on **Picture** in the **Add Item to Report** section of the **Batch Actions** window.

- *Excel (Cell Mode)*

  The **Picture** page of the **Excel (Cell Mode) field "Picture" options** dialog will appear (Figure 20.38). This page has two sections:

  - **Picture output options**

    Choose the file format and resolution 820. *Note:* JPEG picture quality is directly related to the size of the image; a higher quality image will be less compressed and larger in size.

  - **Get the picture from**

    Choose whether to export an object from the layout (a plot or text box) or to export an entire page from the layout.

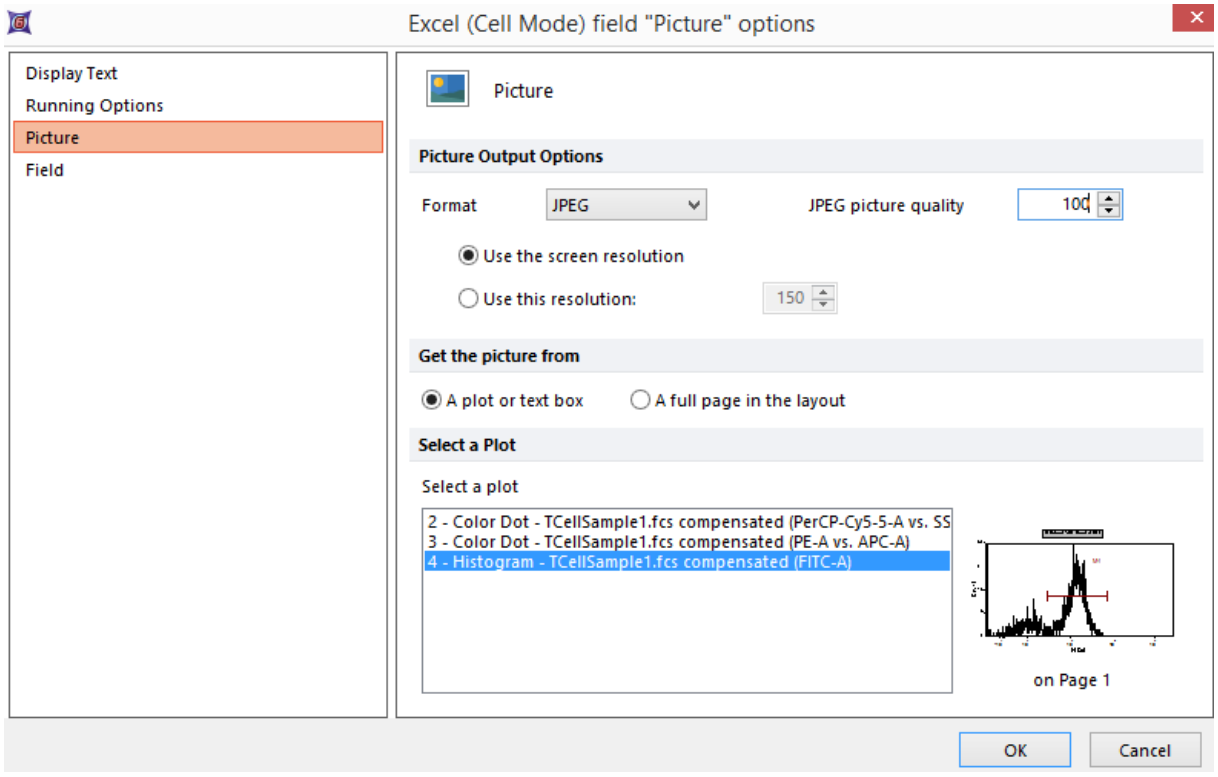

Figure 20.38 Excell (Cell Mode) Picture Options

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- *Word*

  The **Picture** page of the **Word Report field "Picture" options** dialog is similar to the Excel (Cell Mode) dialog described above.

  The Mail Merge field Name edit field on the **Field** page of the **Word Report field "Picture" options** dialog is where you identify the field in the Word template file where the picture will be inserted. The specified field name must exactly match the corresponding field in the Word template file. See the section on report-specific properties 837 for a description of the Field Name property.

- *LabKey Server*

  The **Picture** page of the **Export to LabKey field "Picture" options** dialog is similar to the Excel (Cell Mode) dialog described above. See the LabKey Server Export Tutorial 1483 to learn how to prepare pictures and other items for export to LabKey Server.

20.2.2.5 Reordering and Copying Items in Reports

In Excel (Column Mode) and Text File reports, the token order in the **Batch Actions** list is significant since the values will be written to columns in the order that they are listed within the report.

To reposition an item (e.g., a token) within a report, click on the item and drag it to the new location in the list. As you drag the item over other items in the list, you will see a blue line appear above the other items (Figure 20.39). Release the mouse button to place the item in the position designated by the line. If you want to reposition the item at the end of the list, drag it onto the report name at the top of the list. You can also select multiple items and drag them all at once.

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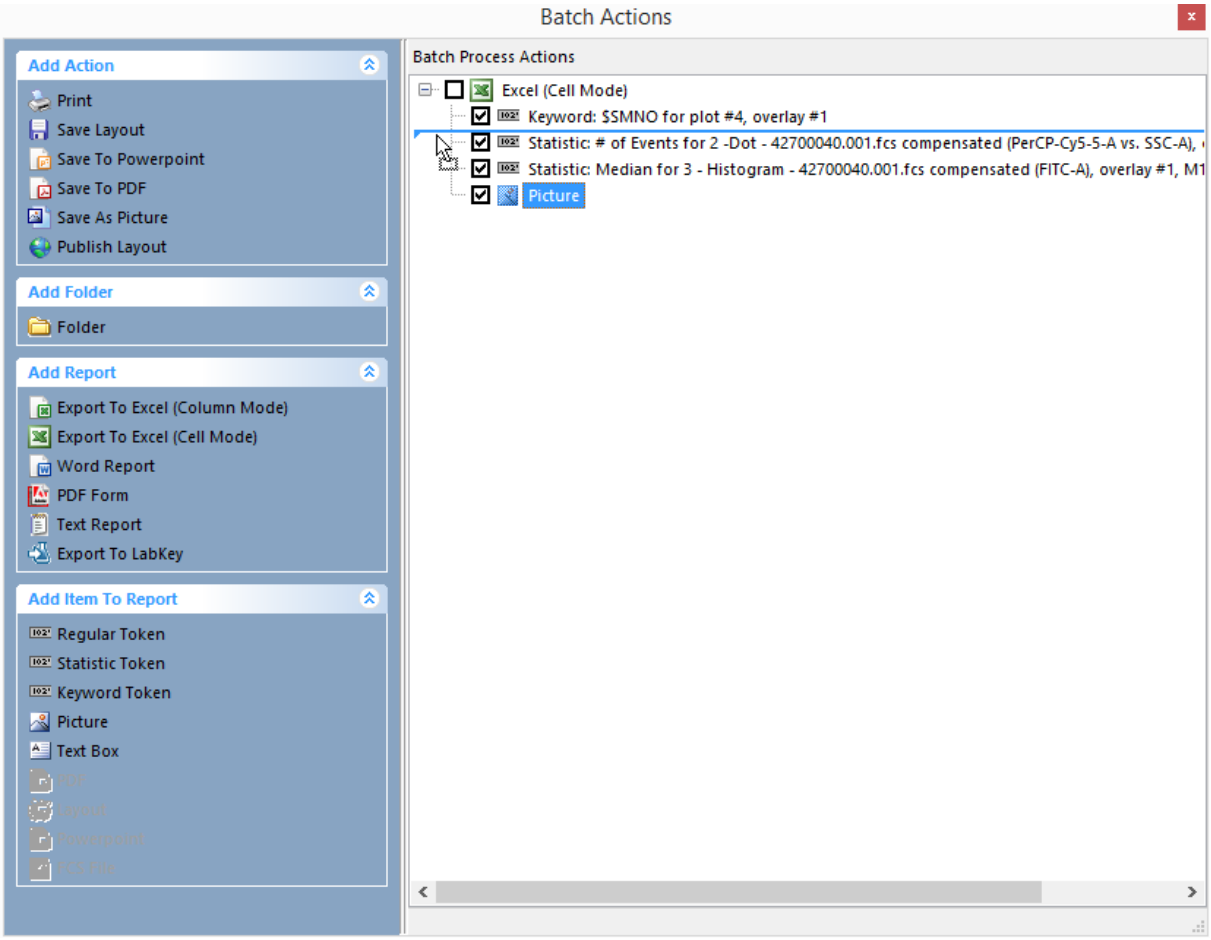

Figure 20.39 Repositioning a Token in a Report

To move an item to a new report, drag the item from its current report to the title of another report. This removes the item from the current report and adds it to the end of the destination report. Alternately, right-click on the item and select **Cut BP Actions** from the pop-up menu. Click on the destination report, right-click, and select **Paste BP Actions** from the pop-up menu.

To copy an item to a new report, click on an item to select it, then right-click and choose **Copy BP Actions** from the pop-up menu. Click on the destination report, right-click, and select **Paste BP Actions** from the pop-up menu. Alternatively, you can copy items by dragging them from one report to another while holding down the **Ctrl** key.

Note: After you have moved or copied an item from one report to another, it is important to ensure that the report-specific properties 837 in the destination report are configured correctly. FCS Express attempts to set the new properties, but it is impossible to set them correctly in all cases programmatically. For example, FCS Express has no way of tracking which new report cell to use for a new token.

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20.3 Batch Processing Options

To set batch processing options, use the **Batch→Batch Processing→Options** command to bring up the **Batch Processing Options** dialog (Figure 20.40). With the Batch Processing Options, you can control how many iterations are to be performed during batch processing, whether or not to Pause during batch processing (either conditionally or unconditionally), and how FCS Express is to prepare the layout for the analysis of the next iteration.

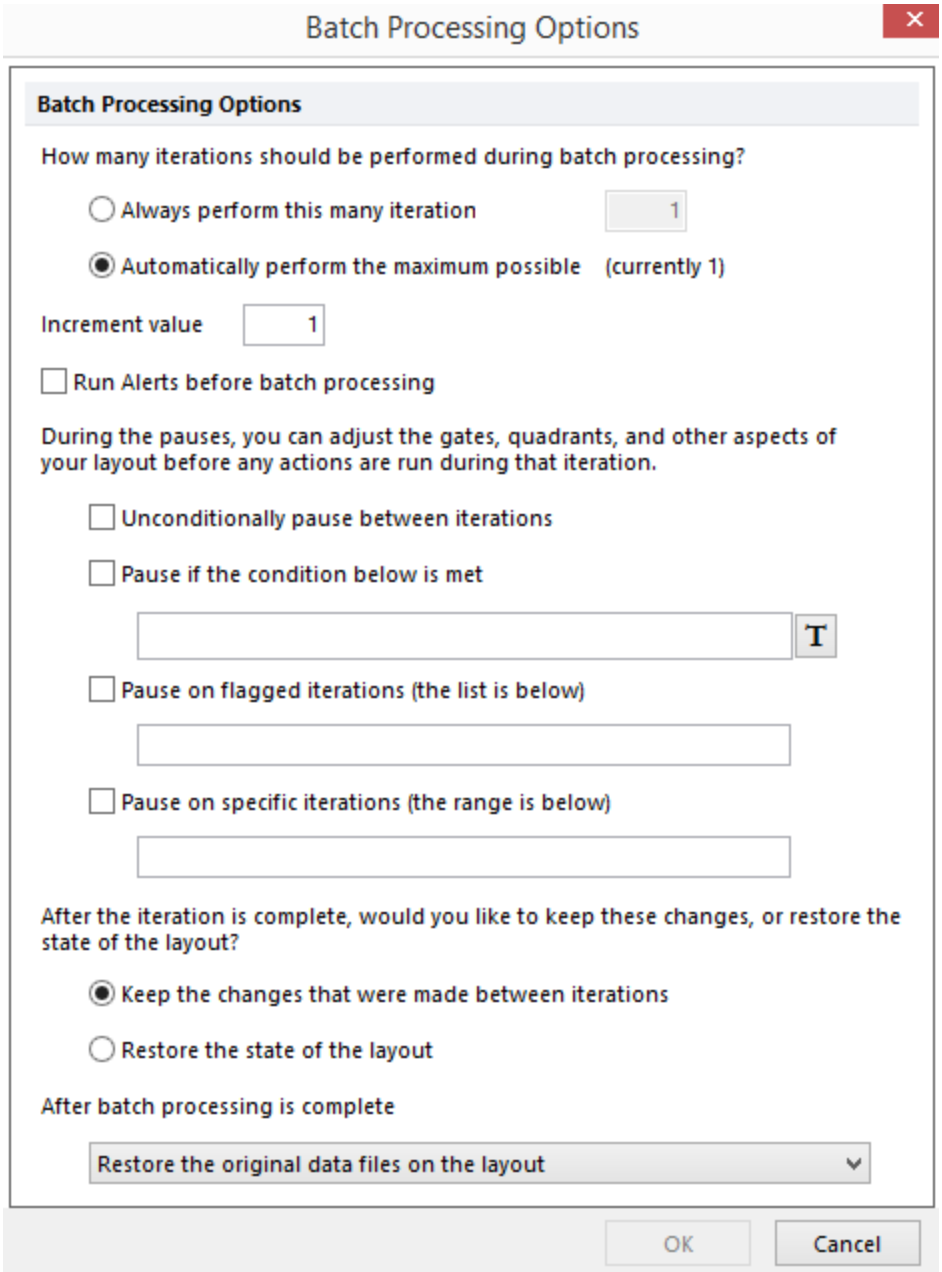

Figure 20.40 Batch Processing Options Dialog

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The properties which can be set are as follows:

- **How many iterations should be performed during batch processing?**

  Specify the number of iterations that the Batch will perform. Normally, you will want to have as many iterations as can be accommodated by the number of data sets in the Data List. If you choose **Automatically perform the maximum possible**, FCS Express will determine the number of iterations to perform based on the number of data sets in the Data List, the position of files in the layout, and the increment value. You can override this by selecting **Always perform this many iterations**, and entering a value in the text field.

- **Increment value**

  The increment value is the number of data sets to increment after each iteration of the batch process. An increment value of 1 means to move to the next data set. An increment value of 2 means to skip over the next data set and use the third data set. For example, if the increment value is 1 and an open plot contains the first FCS file in the Data List, the open plot will contain the second FCS file in the list after the first iteration. If the increment value is 2, then the plot will contain the third FCS file after the first iteration, and the fifth file after the second iteration. If a panel 860 is applied, the Batch increment value will be ignored and the iteration value from the panel will be used to move through the data sets.

- **Iteration start count**

  The iteration start count is the starting value for the number in the Current Iteration token (commonly used in dynamic filenames). See Save Layout - File Options for a description of batch generated filenames 806 for more information about the Current Iteration token. The default value is 1. All filenames generated in subsequent iterations will be incremented by 1.

- **Run alerts before batch processing**

  If this property is checked, any alerts that you have set will be run before each iteration is processed. If an alert does not pass the defined criteria the user will be notified and will have the ability to continue or abort the batch process.

- **Pause between iterations**

  There are several options available in which FCS Express can pause during batch processing to give the user an opportunity to finalize the analysis for

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each iteration. These settings allow the user to adjust gates and quadrants as needed before the next iteration. If any of these options are selected, a window will appear during the batching process, prompting the user to click **Continue, Run to end, or Cancel** 846. The options to Pause the batch process are:

Unconditionally pause between iterations	If this option is enabled, you will be prompted to click the **Continue** button in the Batch Run progress window at the beginning of each iteration unconditionally. In other words, FCS Express will pause between iterations, whether or not the iteration has been manually flagged or conditionally flagged, as determined by the conditions set up by the user. The Batch will run uninterrupted if this property is unchecked. Using the radio buttons, you can choose to **Keep the changes that were made between iterations** or **Restore the state of the layout** for the next iteration. Also, if the iteration was not flagged previously, you can flag the iteration during the process.
Pause if the condition below is met	With the option, the user can create conditions through the use of tokens. If the condition is True, FCS Express will pause during batch processing at the iteration which fulfills the condition.
Pause on flagged iterations (the list is below)	If any iterations were Flagged, FCS Express will list the iteration numbers which were flagged. FCS Express will also pause batch processing when the data from the flagged iterations is currently being viewed.
Pause on specific iterations (the range is below)	With this option, the user can designate at which iterations FCS Express will pause during batch processing. The user can enter the iteration number, an iteration range (for example, 1-9) or specific iterations (for example, 1,3,5).

- **Keep the changes that were made between iterations** or **Restore the original FCS files on the layout after completing batch processing**

  When one of these options are selected, all objects on the layout will display their original data sets at the end of the Batch Process. If unchecked, then at the end of the batch process, the Data List and corresponding objects will point to the last data set.

- **After batch processing is complete -** After FCS Express has completed batch processing, how would you like FCS Express to treat the layout? The options are:

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Do nothing	FCS Express will do nothing and leave the layout, as is, after the completion of batch processing.
Restore the original data files in the layout	After the completion of batch processing, FCS Express will reload the original data files that were being viewed within the layout.
Clear the data files	After the completion of batch processing, FCS Express will clear the Data List of all the data files were present within it.

Click **OK** to save your settings.

20.4 Running the Batch Process

Once you have set up all your actions, the Batch Processing options, and verified that all files you want included in the Batch are listed in the Data List, then you are ready to run the Batch. Running the Batch executes all actions, including the Print, Save, and Report actions.

To run the Batch, use the **Batch→Batch Processing→Run** command. The Batch Processing Run, Rerun, and Review windows are now dockable 115, so that you may perform other actions while the Batch Process runs or modify Batch Actions, as well as keep the window open at all times if you wish.

While the Batch Process is running, a progress window will appear showing how many iterations have passed and how many are left. If you chose to pause between iterations, then the progress window will have 2 options from which you can select:

1. **Keep the changes -** this option will keep the changes that were made to the layout during the analysis and keep the changes for the next iteration.
2. **Restore the state of the layout before the next iteration -** this option will restore the layout to its original state, ignoring any changes that were made to the layout during the previous batch processing iteration.

Once a selection has been made, the user can choose to Continue running the batch process, while pausing for each iteration, or by choosing Run to end, where the batch process will continue to its end, without pausing (Figure 20.41).

If the Batch Process aborts for any reason, FCS Express will now save all of the reports up until the abort (Figure 20.42).

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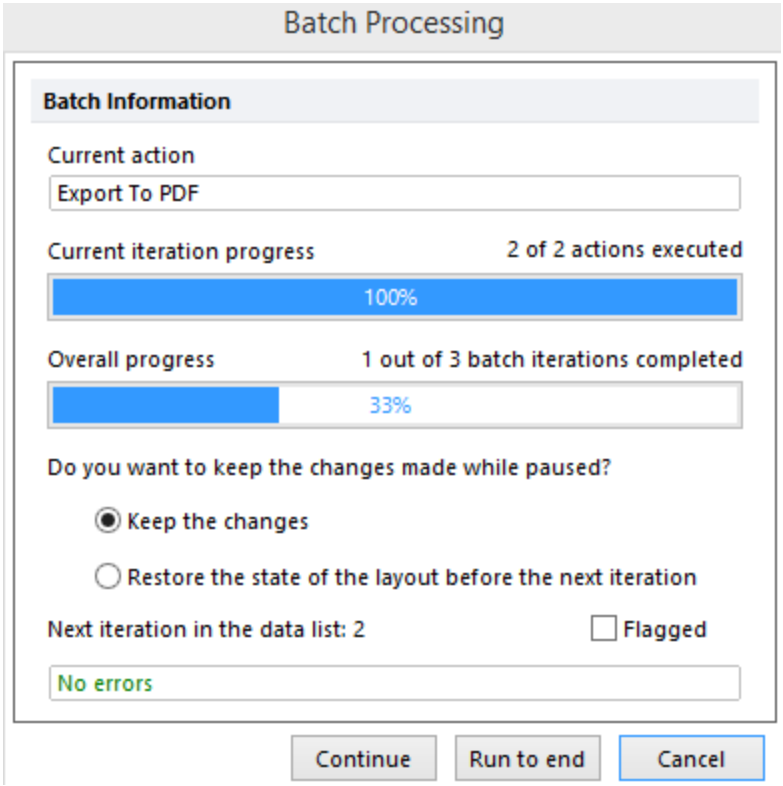

Figure 20.41 Batch Processing Progress Window showing the options that are available between iterations.

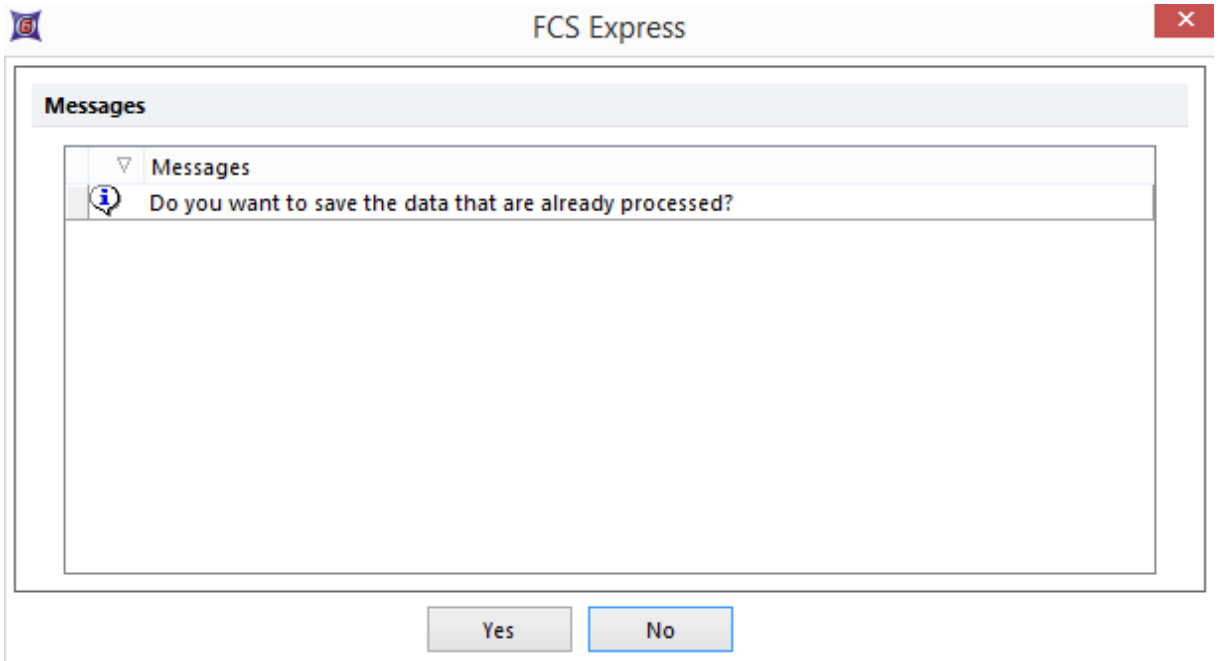

Figure 20.42 If the Batch Process is cancelled you will be prompted to save the data that was already processed.

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20.5 Rerunning and Reviewing the Batch Process

After the Batch Process has been Run 846, the user now has the option to:

- **Rerun -** Reruns the previous batch process including all changes to gates, markers, and quadrants applied during a pause of the batch by using Iteration Snapshots 852.
- **Review -** Reruns the previous batch process including all changes to gates, markers, and quadrants applied during a pause of the batch by using Iteration Snapshots 852 *without completing* the batch actions.

To rerun the Batch, use the **Batch→Batch Processing→Rerun** command (Figure 20.43). To review the Batch, use the **Batch→Batch Processing→Review** command (Figure below).

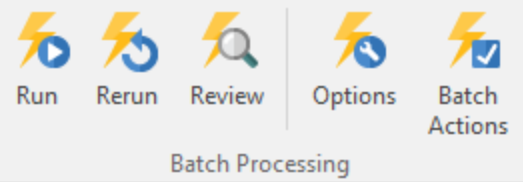

Figure 20.43 Rerun or Review Batch Processing

Rerun and **Review** will be dependent on the pause options that available in the Batch Processing Options 852 and Flags 849. If there are no pause options selected when running in **Review** mode, FCS Express will display a message, prompting the user whether they would like to choose a pause option (Figure 20.44). Clicking **Yes** will open the **Batch Processing Options** window.

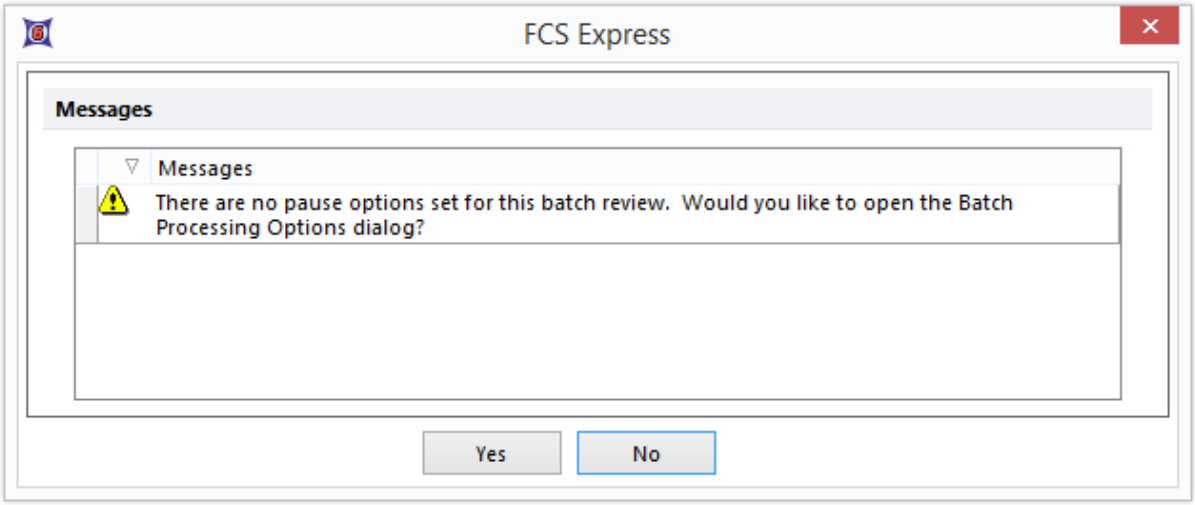

Figure 20.44 No pause options were set for Batch Review prompting the message to open the options dialog.

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20.5.1 Using Flags

During analysis, you will most likely encounter data that needs to be reviewed or re-analyzed. In FCS Express, you can designate iterations for review by Flagging them.

You can find the **Flag This Iteration** button at **Data** tab→**Change Data on All Objects** (Figure 20.45).

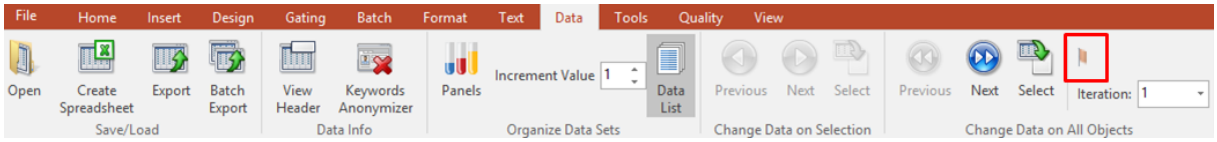

Figure 20.45 The Flag Iteration Tool Showing a Flagged Iteration.

If any iterations are flagged, as seen in the above image, it is possible to review those items during Batch Processing.

During Batch Processing, it will be possible to flag iterations from the **Batch Processing** progress window (Figure 20.46).

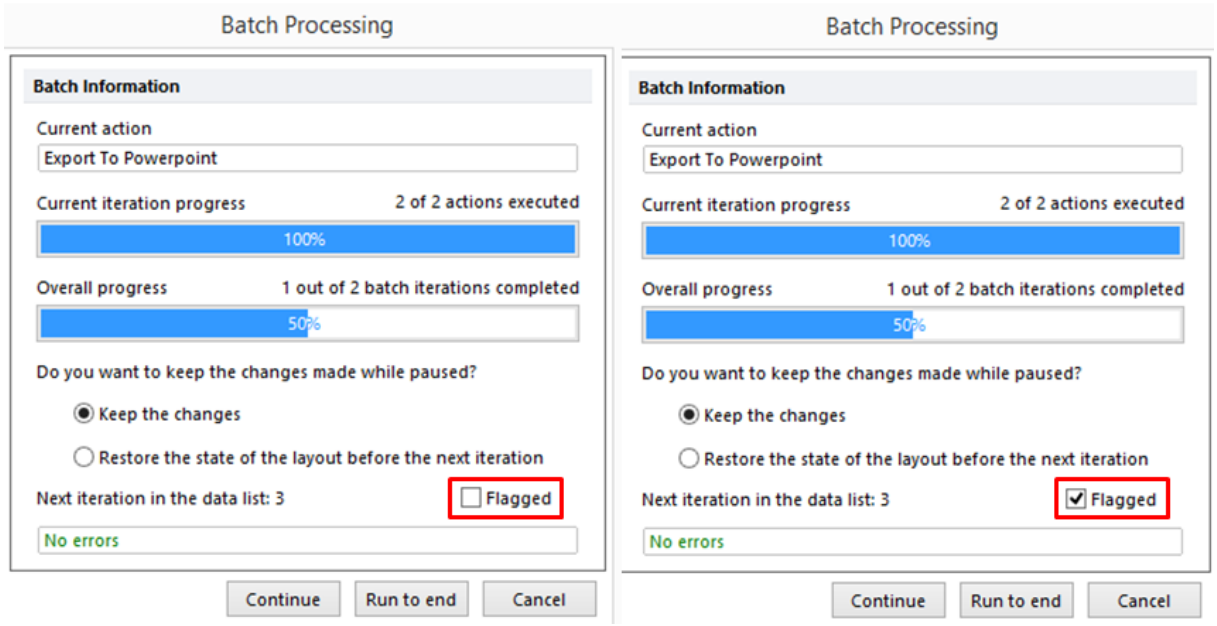

Figure 20.46 An Iteration during Batch Processing that is Unflagged (Left) and Flagged (Right).

By adjusting the Batch Processing Options 843 to **Pause on flagged iterations**, Batch Processing can be **Run**, **Rerun** or **Reviewed** for those selected items (Figure 20.47).

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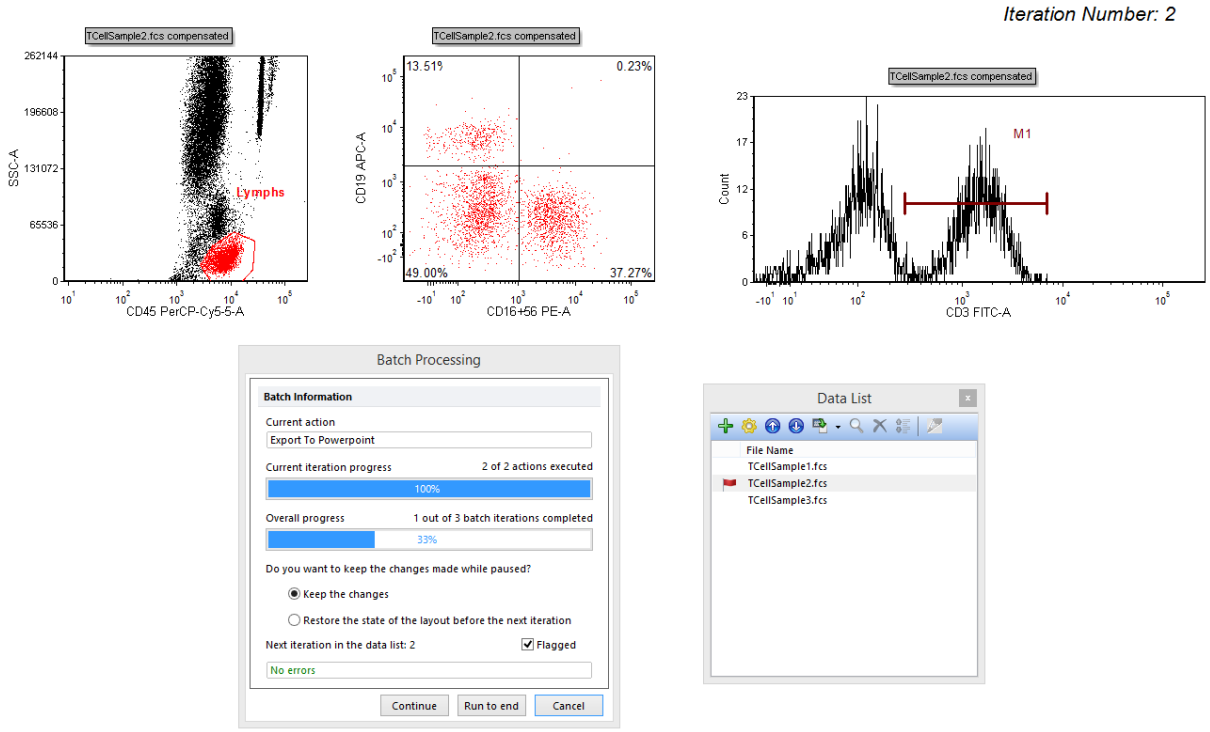

Figure 20.47 Batch Processing Paused on Flagged Iteration for further review.

Flagged iterations will be visible within the Data List if the option for **Show Iteration Column** is checked within the Data List options. The Data List can be formatted to show this column by clicking on the **Format** button.

After clicking on the **Format** button, select **Show Iteration Column** (Figure 20.48).

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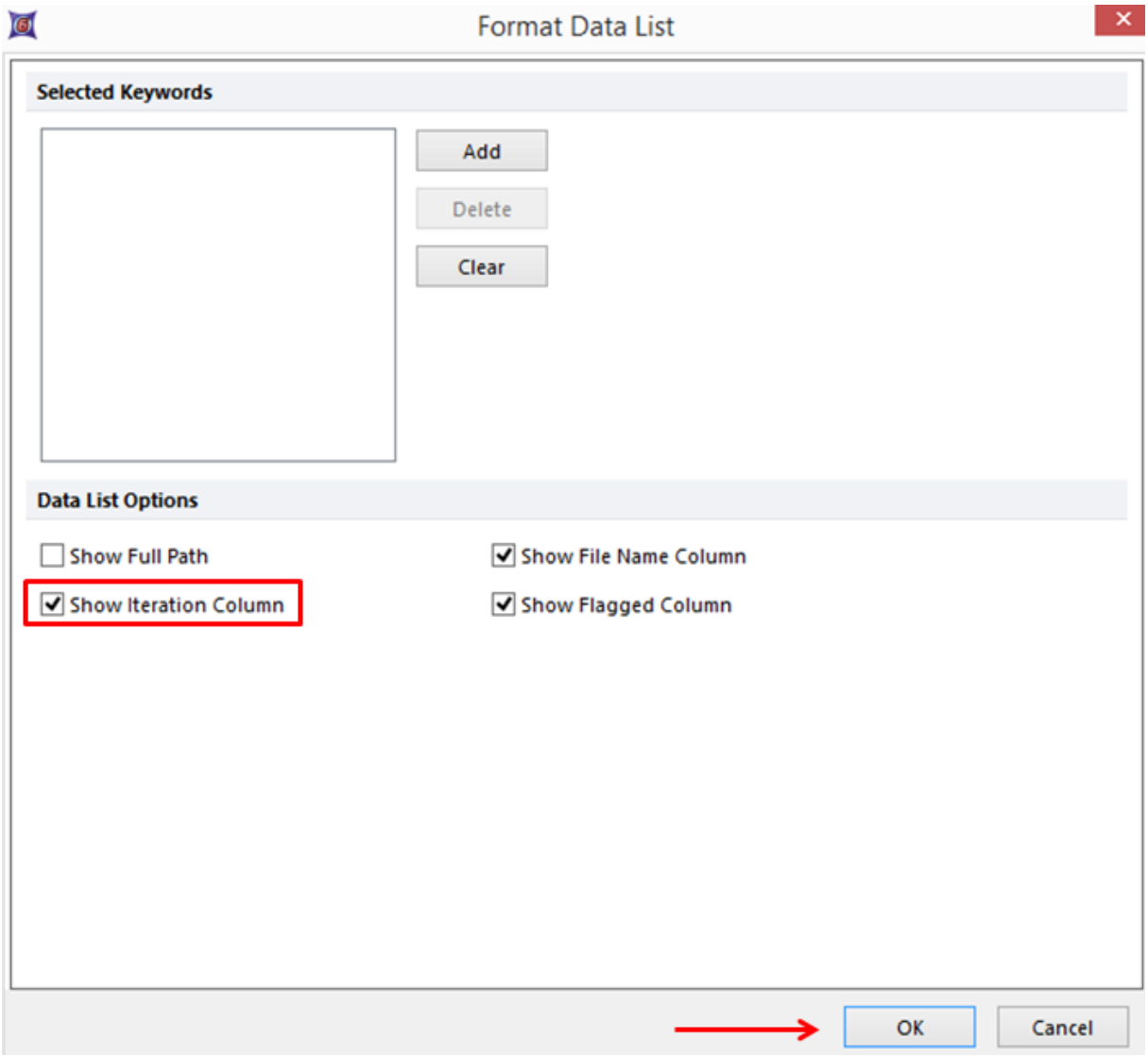

Figure 20.48 Choosing to Show the Iteration Column within the Data List.

After selecting the **Show Iteration Column** option, a **Flag** will appear in front of the iteration that was flagged, either during Batch Processing, or from the **Data** tab (Figure 20.49).

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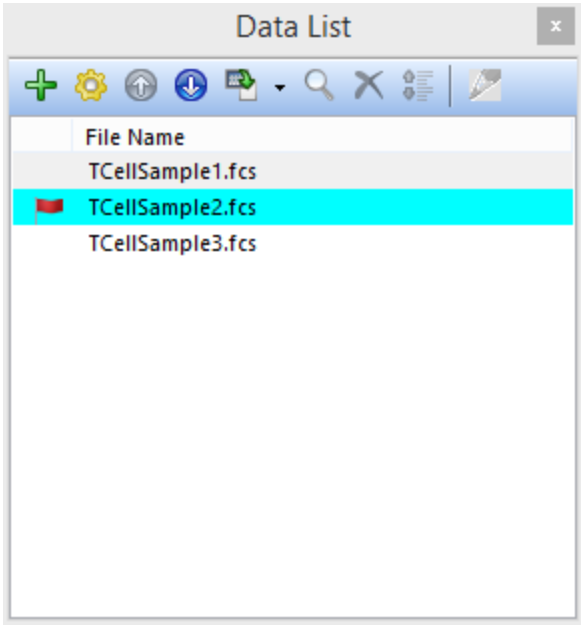

Figure 20.49 The Data List displaying Flagged Iterations

20.5.2 Snapshots and Batch Iteration Navigation

FCS Express now has the ability to automatically take Iteration Snapshots[376] of your analysis for each iteration during Batch Processing.

During Batch Processing[784], as gates, markers and quadrants are adjusted, an Iteration Snapshot[376] is automatically captured for each iteration to store:

- all gates and their coordinates.
- all markers and their coordinates.
- all quadrants and their coordinates.

When a Pause[843] is enabled in the **Batch Processing Options** (Figure 20.50) the user can make adjustments of all gates, markers and quadrants and record those elements for each iteration as part of Batch Processing, through the use of automatic Iteration Snapshots[376].

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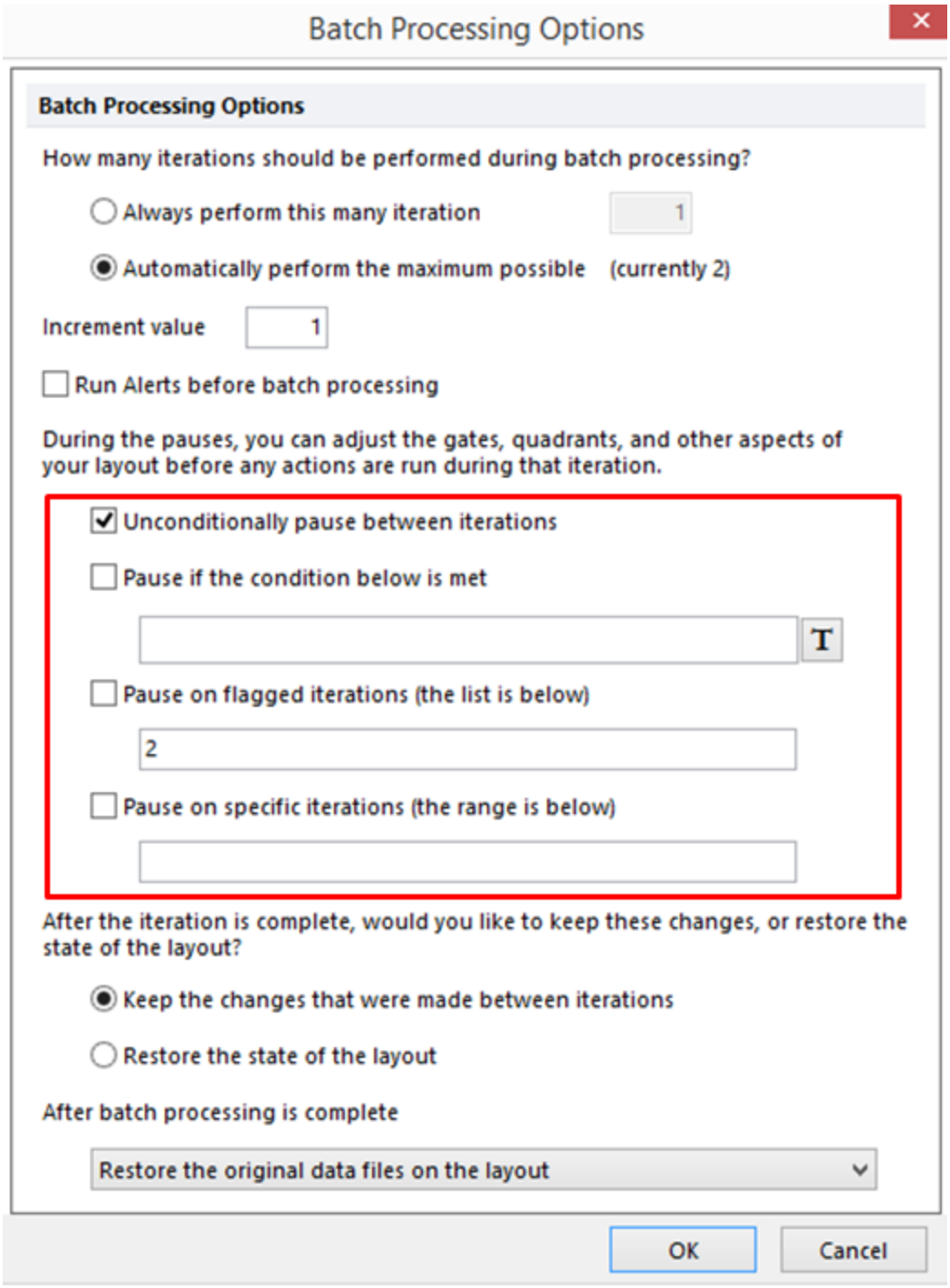

Figure 20.50 Enabling Batch Processing Pause Options.

As the analysis progresses through batch processing and pauses between iterations, gates can be adjusted manually or by applying Snapshots[1498] that were taken with the Snapshots list. If any iteration needs to be reviewed, a flag[849] can be applied to that iteration.

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If the **Batch Processing Options** for **Pause if the condition below is met** is selected, a condition, in the form of a Token, can entered into the field. If the condition is True for an iteration, batch processing will pause and the user will have the opportunity to alter the gating or flag the iteration for further review.

If the **Batch Processing Options** for **Pause on flagged iterations** 849 is selected (Figure 20.51), batch processing will only stop at those iterations that are flagged. The iterations that have been flagged will be listed in the field below the option.

If the Batch Processing Option for **Pause on specific iterations** is selected, the user can manually list the iteration or iterations during at which batch processing should pause.

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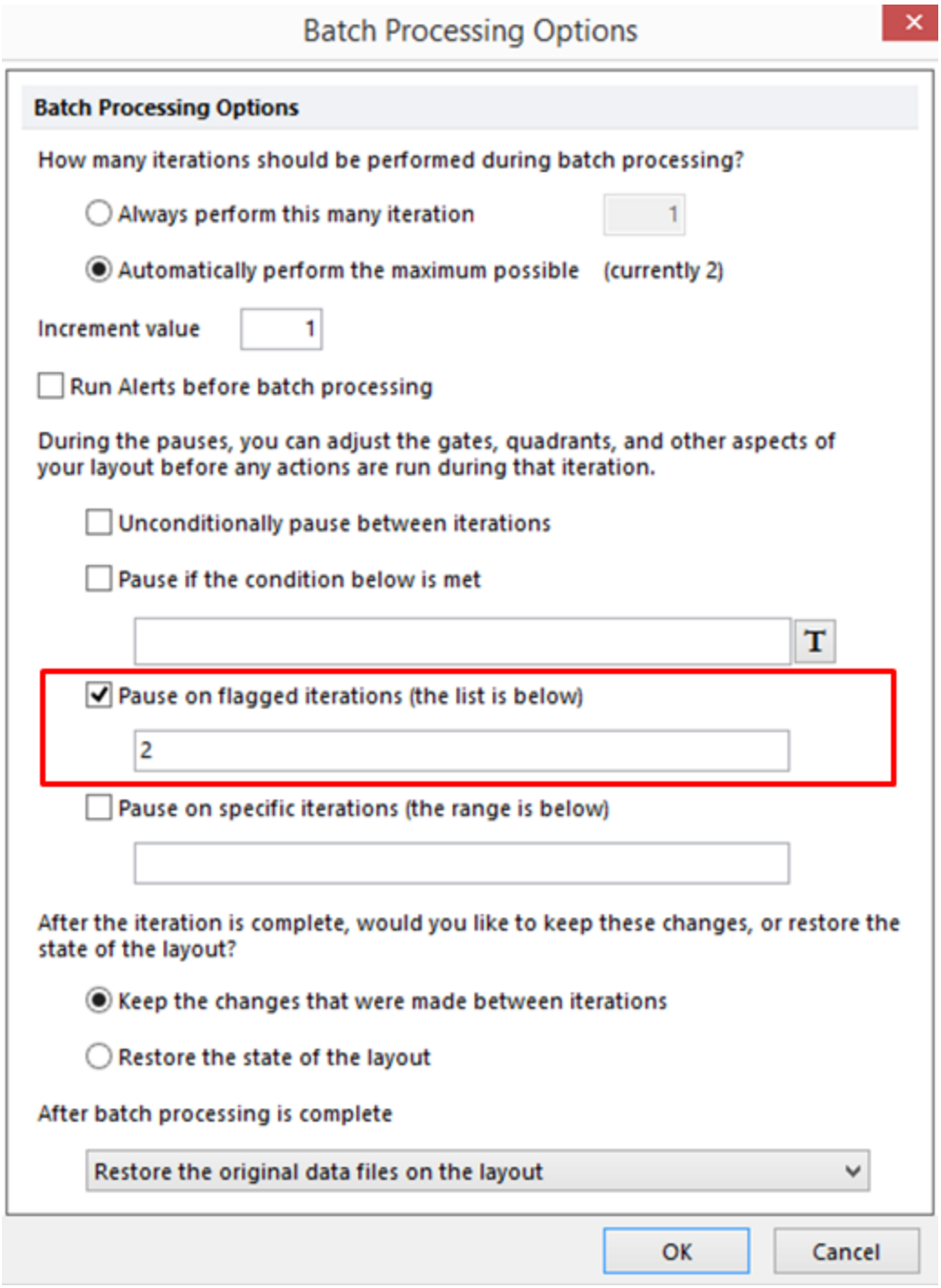

Figure 20.51 Batch Processing Options Showing Pause for Flagged Iterations.

Once the gates have been adjusted to their correct positions for an iteration, click on **Continue** or **Run to end** (Figure 20.52). FCS Express will automatically store a Snapshot of that iteration, move to the next iteration, or to complete the sequence of iterations.

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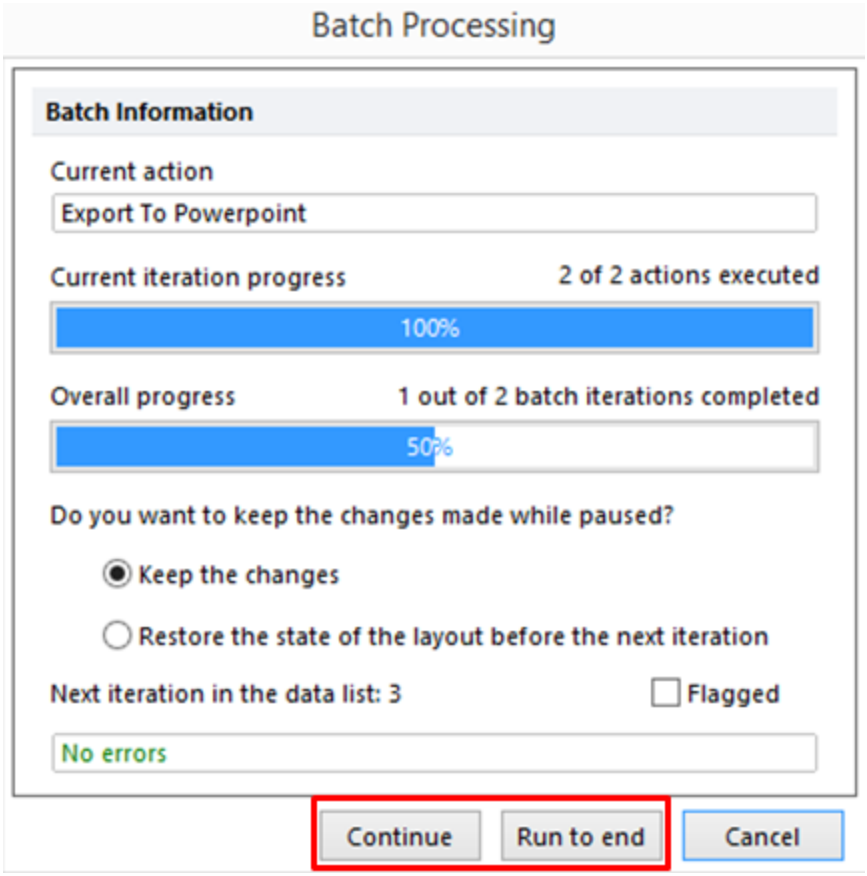

Figure 20.52 Choosing Continue, Run to End or Cancel.

If **Cancel** is selected, FCS Express will prompt you whether you want to save the data that has already been processed (Figure 20.53).

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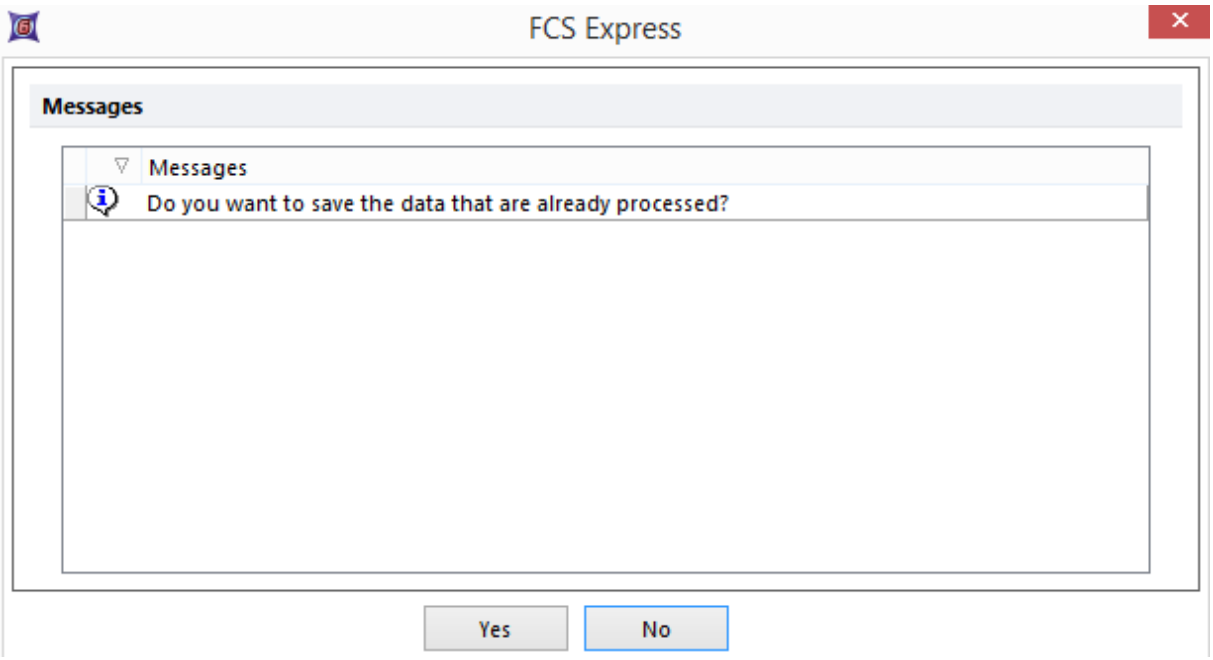

Figure 20.53 Cancelling a batch process will prompt you with "Do You Want To Save the Data That Has Already Been Processed?"

Once Batch Processing is complete, you can review the gating changes that were made to an iteration during the batch by navigating to the iteration in the Data List. You may also use the Batch Rerun or Review options 848 from the **Batch** tab→**Batch Processing**→**Run** dropdown to run the batch and review your previous changes. The gating changes that were made will be recorded and shown with the data (Figure 20.54 and Figure 20.55).

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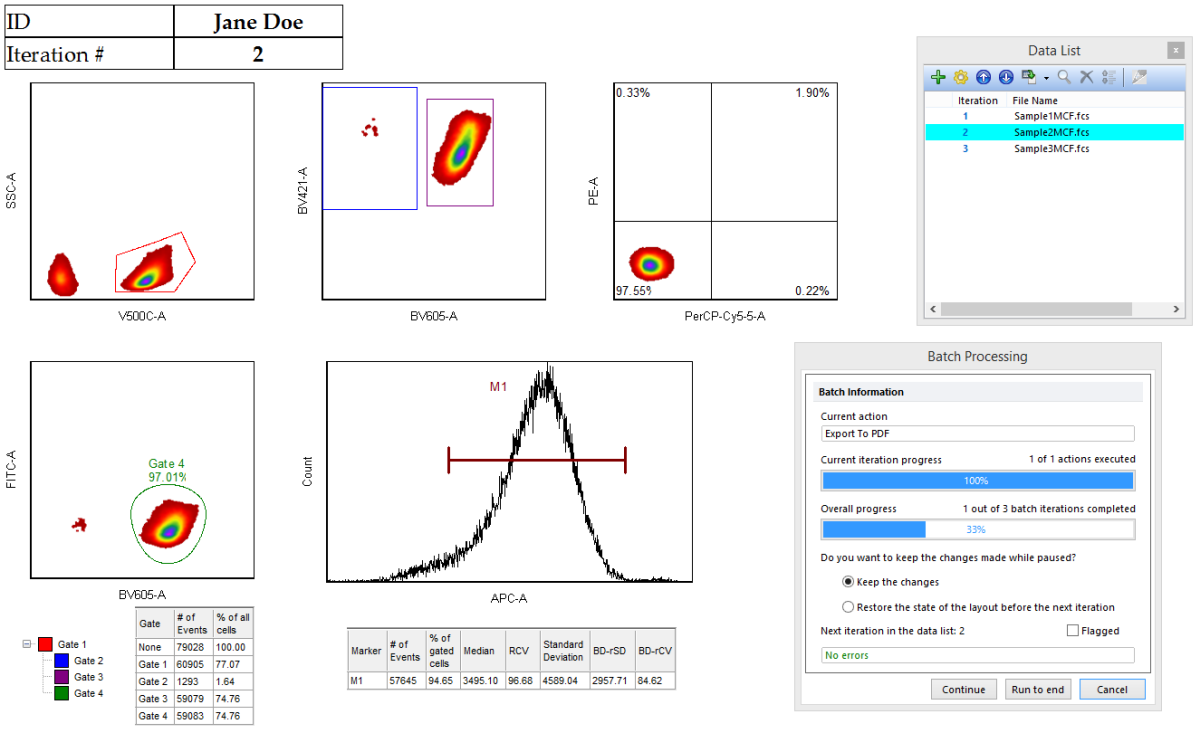

Figure 20.54 Snapshot showing gating changes made during the batch process for Iteration #2.

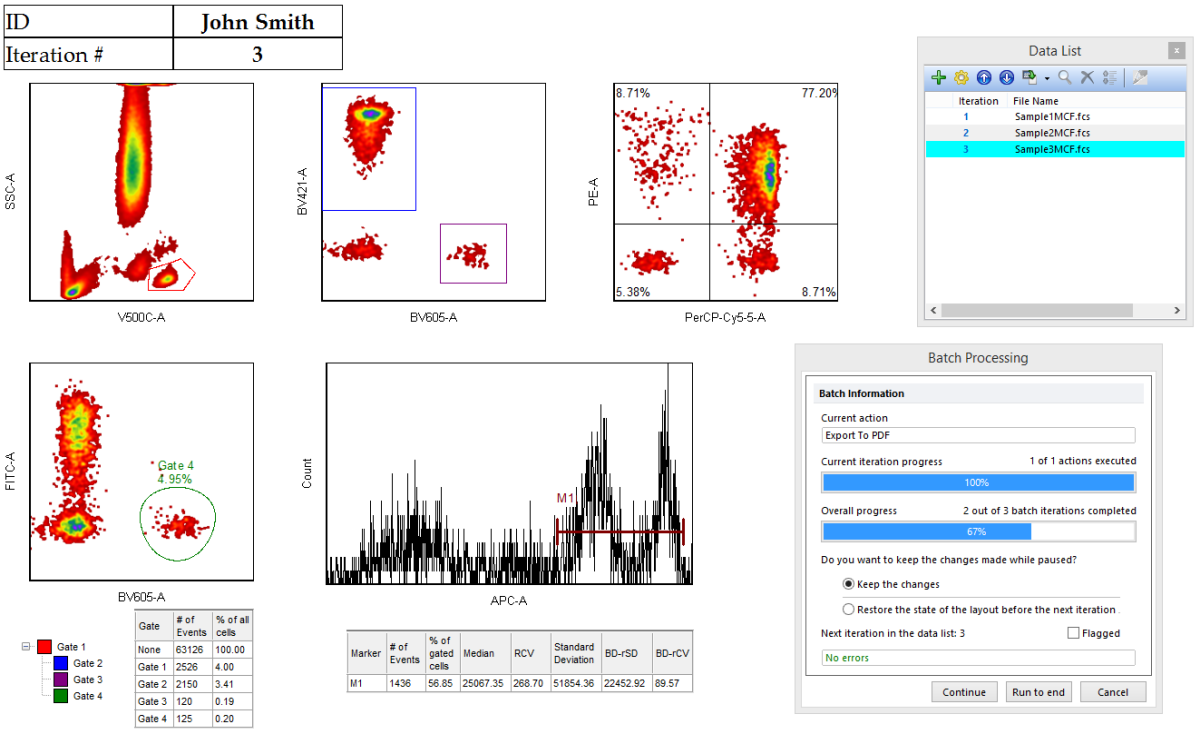

Figure 20.55 Snapshot showing gating changes made during the batch process for Iteration #3.

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Chapter

21 Using Panels

In a typical experiment, you can repeat the same set of staining protocols many different times, with different samples, but using the same number and sequence of tubes for your experiment. This type of staining protocol is often referred to as a **Panel**. You can specify the number of tubes in the panel, as well as the name of each tube. Once you have designated an active panel, and added files to the Data List, FCS Express will automatically determine which data file corresponds to which tube in your panel. You can change this association from the **Data List**. With an active panel, when you create plots with files from the **Data List**, FCS Express will know which plots will display the proper file. When you change the **Data List**, the plots will automatically update with the correct file for that tube.

Panels can be added, deleted, edited, saved, and loaded from the **Panel Information** dialog which can be opened by using the **Batch→Organize Data Sets→Panels** command, or by clicking on the Panel status bar button (with the test tubes icon) at the bottom of the layout.

21.1 Working with Panels

The primary benefit of a panel is that it allows you to change the data on your layout simply by changing the **Data List**. For example, on Day 1 you analyze your tubes using FCS Express, creating a plot for each tube. On Day 2, you need to load new data into the same plots that you used on Day 1. Once you set up the **Panel** and the **Data List** correctly, the layout from Day 1 can be modified to show the data from Day 2, simply by loading the Day 2 data into the **Data List**.

The following example will guide you through creating a **Panel** and taking advantage of it using the **Data List**.

1. Select the **Data→Organize Data Sets→Panels** command. The **Panel Information** dialog, shown in Figure 21.1, will appear.

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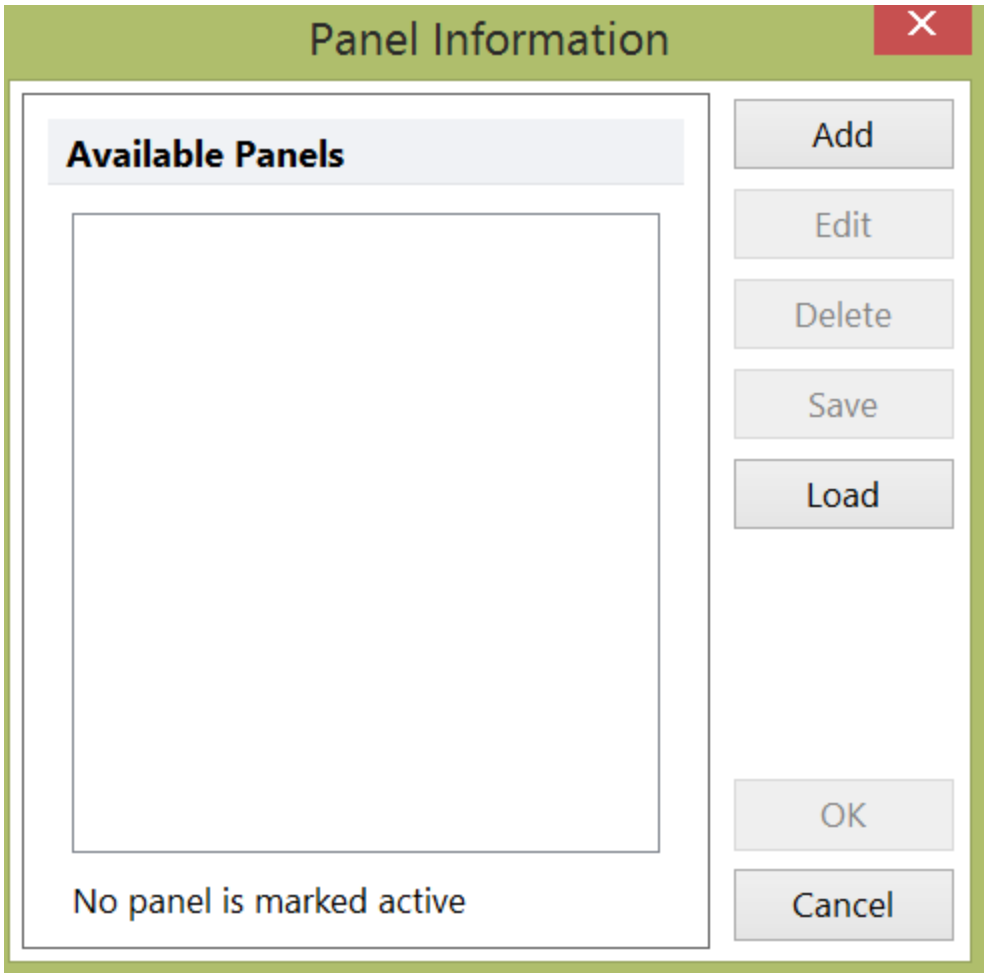

Figure 21.1 Panel Information Dialog

2. Click on the **Add** button to create a blank Panel. This will bring up the Add Panel dialog (Figure 21.2).

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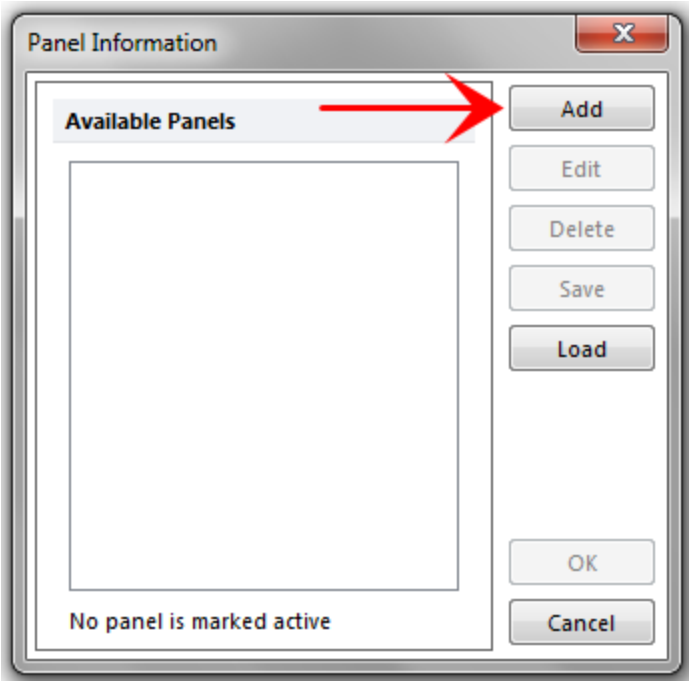

Figure 21.2 Add Panel Window

3. Enter a name in the **Panel Name** field, and then select the number of tubes in the panel by clicking on the arrows to the right of **Number of Tubes**. For each tube added, a blank line appears in the table.

4. Give each tube a name (optional), by typing the name into the name column.

5. Click **OK** when you have finished setting up the panel. You will be returned to the **Panel Information** dialog, where you will see the newly created panel. Panel information is saved with the layout. However, you can save your panel for use in other layouts. The **Panel Information** dialog allows you to **Edit**, **Delete**, **Save**, and **Load** panels.

6. Since each layout can have only one active panel, verify that the panel that you want to use is marked active (the box to the left will be checked).

7. Click **OK** to continue. Now create plots on your layout and populate them with data. The **Data List** will automatically be populated with the data files in the current directory.

8. Use the **Data→Organize Data Sets→Data List** command to bring up the **Data List** window (Figure 21.3). The first column shows the tubes from the Panel and the second column shows each tube's associated data file. Notice how the data files are grouped into the panel automatically. If a tube is associated with the wrong data file, you can reorder the files using the Up and Down buttons.

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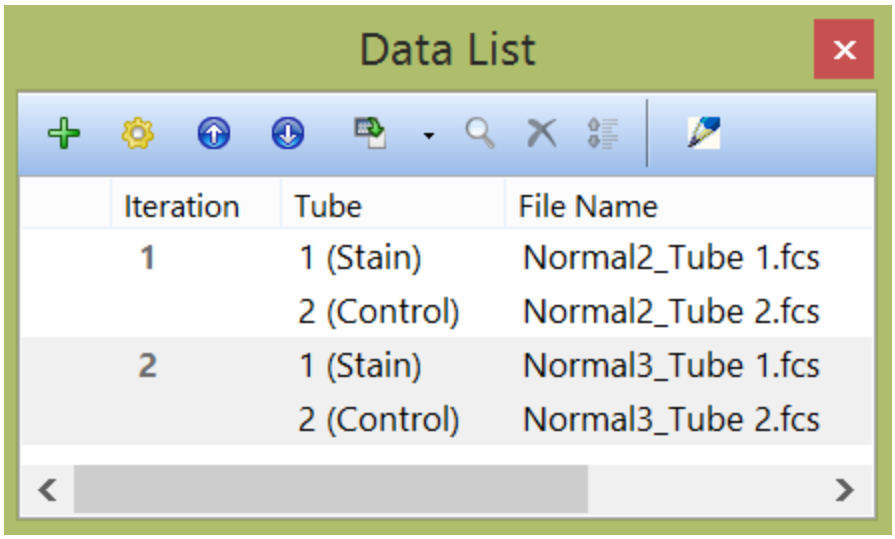

Figure 21.3 Data List with 2-tube Example Panel

To use the **Data List** in conjunction with the panel to control the data files in the plots in your layout, do the following:

1. Remove the data files from the **Data List** by selecting all the files and clicking **Remove**.

2. Click **OK**. The plots which previously displayed data are now empty.

3. Return to the **Data List**, and add a new set of data files. To do this, click on **Add** and select the data files to be added.

4. Click **OK**. The previously empty plots are now displaying data from their associated data files.

If you have a Panel applied, and are adding files to the file list, you do not have to select all the files that you want to add. You can select the first file that you wish to add, and FCS Express will attempt to determine how many additional files to include (Figure 21.4).

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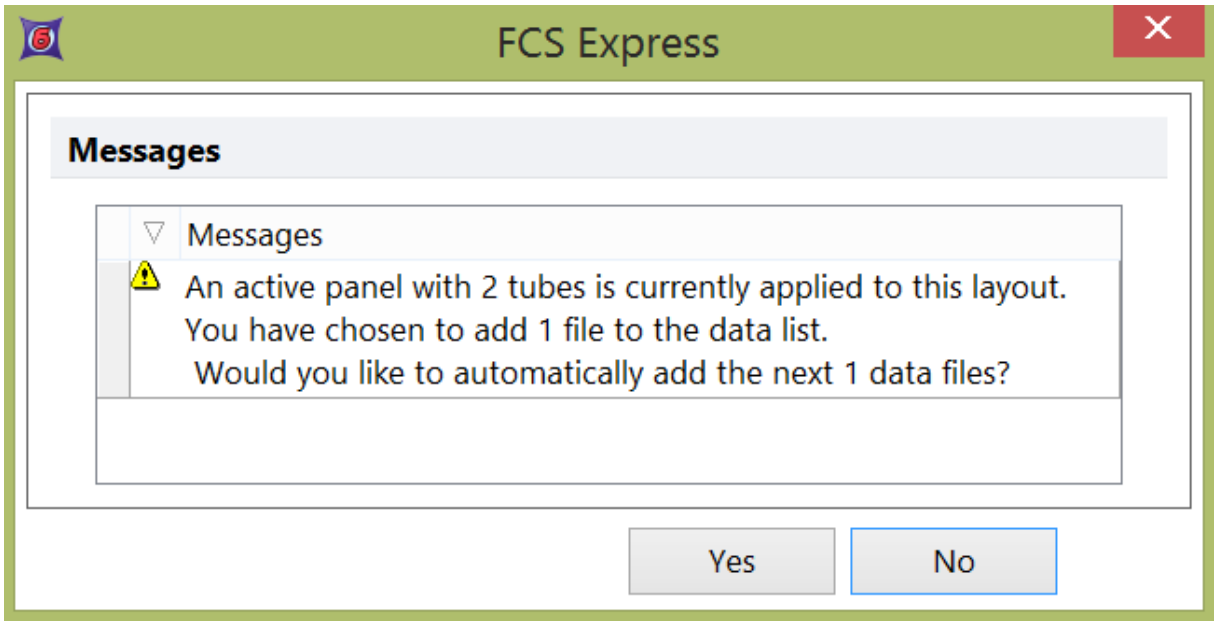

Figure 21.4 Automatically Populating a Panel

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Chapter

22 Using Templates

Templates allow you to set up a default look and feel for your experiment. For instance, if you like a certain paper size, or certain margins, or wish to include your company logo on every layout, you can do so by creating a template.

A template is nothing more than an FCS Express layout that is set up the way that you want, providing the user with the opportunity to use a previously created layout as a starting point for a new analysis layout. Otherwise, it is just like any other layout. FCS Express remembers the layout as a template when you select it using the **File** Tab→**New Layout**→**New From Existing** command/gallery. Clicking on **New From Existing** will bring up a **Create New Layout from Template** dialog where you can choose any existing layout. If you have previously created one or more templates, you can also choose one from the Recent Documents or Places area of the gallery 92.

When the new layout is opened, it will look identical to the template layout. However it will "forget" that it came from the template, and when you save the new layout for the first time, FCS Express will prompt you to select a new file name.

You can also set a "default template" for use when any new layout is opened. If a default template has been set, selecting the **File Tab→New Layout** command will bring up the default template instead of a blank layout. Setting the default template is described in the section on Default Files/Directories 208.

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Chapter

23 Importing External Documents and File Types

FCS Express supports importing two external layout types:

- BD FACSDiva™ experiments.
- BD Accuri™ C6 experiments.

FCS Express also supports importing / loading of comma delimited files stored in the format of (*.csv)

23.1 BD FACSDiva Files

The basic version of FCS Express can load FCS files from BD FACSDiva™ software, similar to loading data files from any other instrument. However, the FACSDiva Importer add-on allows you to import your entire experiment (FACSDiva version 4 and above) as well.

A FACSDiva experiment file, which has the extension *.xml*, is created when you export your data using the FACSDiva software (see "Exporting Experiments" in the FACSDiva Software Reference Manual for information). The FACSDiva experiment file contains information on plots, gates, and compensation settings.

The following FACSDiva experiment information will be maintained when the file is imported into FCS Express:

- All acquisition templates and analysis worksheets.
- The native, high resolution, floating point FCS data.
- All gates with the proper gating hierarchy.
- All compensation settings.
- All markers and quadrants.

Because the FACSDiva experiment file does not contain information about the pages its plots should appear on, FCS Express needs additional data to import the file properly. In particular, FCS Express needs to know how you want the plots distributed across your layout pages. For example, a given FACSDiva experiment file has three plots laid out horizontally. This file could be imported into FCS Express such that each plot exists on its own layout page, or all three plots could be placed on a single layout page. If you leave the size of the FCS layout page at the standard 8.5 x 11 inches, then importing the three plots may cause the size of the plots to shrink, in order to fit them on a single page. However, while importing, you can configure the size of the FCS Express layout page to contain more plots of standard size.

Once you have set up the page size for the FACSDiva experiment file import, along with the distribution of the plots on the layout page, you can save the

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configuration for use again with other FACSDiva experiment files. The configuration is saved to an FCS Express FACSDiva configuration file 869.

23.1.1 How to Import FACSDiva Experiment Files

To import a FACSDiva experiment file:

1. Use the **File** tab→**Open From**→**Import BD FACSDiva File...** command.

2. Browse your directories to select the FACSDiva experiment file (.xml) to import. Click **Open** when you have located it.

3. The **Import BD FACSDiva File Wizard** appears, beginning with the **Import Options** page (Figure 24.1). The worksheets available in the FACSDiva experiment file are displayed, including their type (either **Analysis worksheet** or **Acquisition template**). In the **Import** column, check the box for the Worksheet(s) you want to load. You will need to select at least one Worksheet to continue.

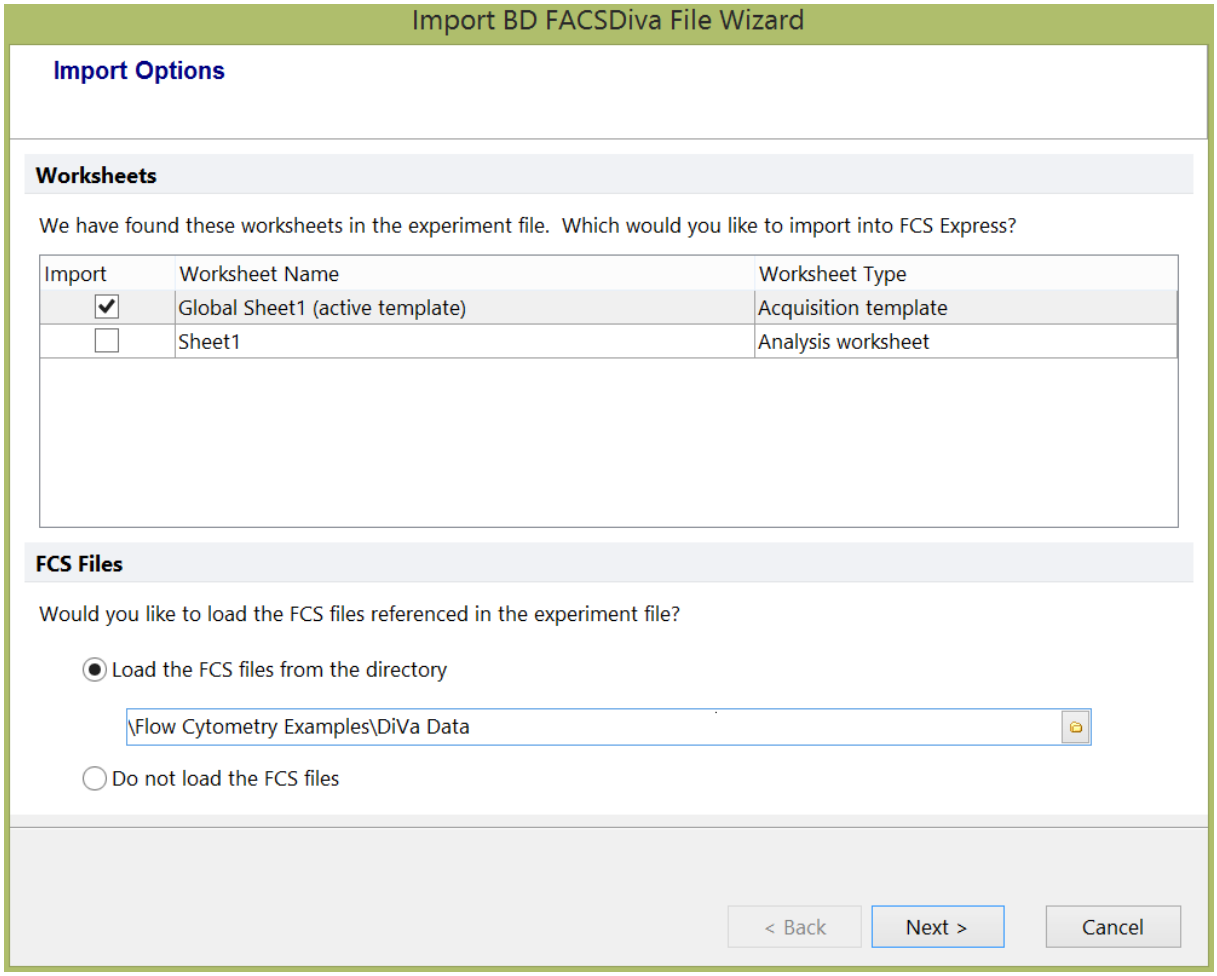

Figure 24.1 Import BD FACSDiva File Wizard - Import Options

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4. Towards the bottom of the window, choose whether you want to load the FCS files corresponding to the experiment, and navigate to the folder containing the files (if different from the .xml-containing folder). If you do not want to load the FCS files, choose the **Do not load the FCS files** radio button. Click **Next** to continue.

5. The **FCS Express FACSDiva configuration file** page appears (Figure 24.2). You can choose to load an existing configuration file, or create a new one. If you choose to load an existing configuration file, select the **Use an existing configuration file** radio button, select the file, click **Next**, *and proceed to Step 8*. If you choose to create a new configuration file, select the **Create a new configuration** radio button, and click **Next**.

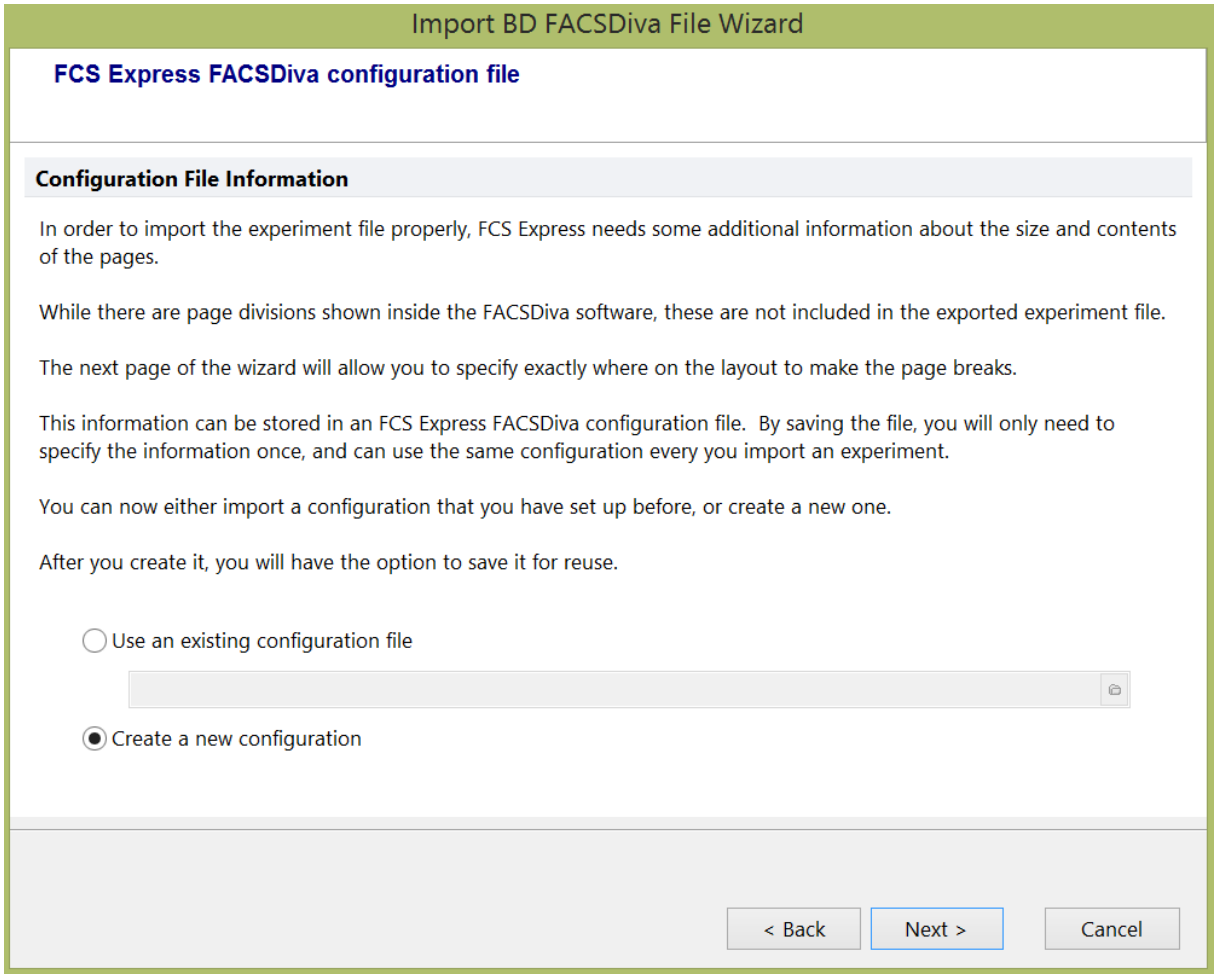

Figure 24.2 Import BD FACSDiva File Wizard - Configuration File

6. If you decided to create a new configuration, the **Setting up the page size for the layout in FCS Express** (Figure 24.3) will appear. Here, you

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set up how the plots from the FACSDiva experiment will be imported into the FCS Express layout.

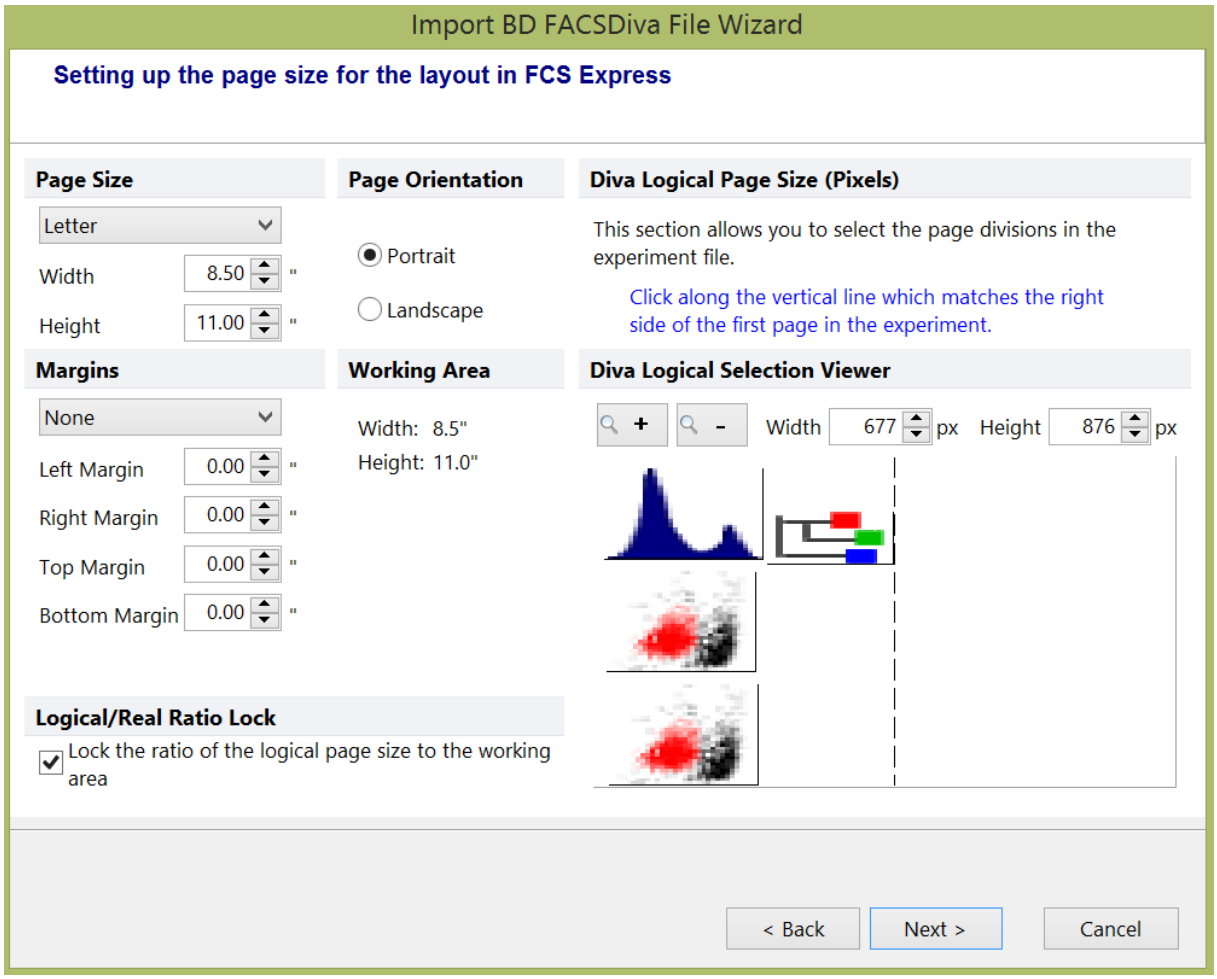

Figure 24.3 Import BD FACSDiva File Wizard - Setting Page Size

The window is divided into two sections:

- **Real Page Size (inches)**

  This section allows you to configure the page size of the layout. The default is 8.5 x 11 inches. You can also set the margin widths.

- **Logical Page Size (pixels)**

  This section allows you to configure how the FACSDiva experiment file will be divided when it is imported into FCS Express. It displays all the dot plots and histograms from the FACSDiva experiment, using mock diagrams to represent the appropriate plot. By clicking in the

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representation of the FACSDiva experiment file page, you can divide the experiment into multiple pages. After the initial click, you will see a dotted vertical line which represents the right side of each layout page, and a horizontal dotted line, which represents the bottom of the layout page. If one of these lines is not visible, zoom in or out using the magnifying glass buttons. If a plot extends across more than one page in the diagram, it will be placed on the layout page where its top-left corner exists in the diagram. By default, the ratio of the page is locked, which means that as the width of the page increases, so does the height.

When you have completed setting up how the FACSDiva experiment's plots will be imported, click **Next**.

7. The next page of the wizard, **Saving the configuration**, allows you to choose whether or not to save the settings from the previous screen to a file. Saved configuration files can be used to import other FACSDiva experiments (in Step 5). Click **Next** to continue.

8. If, in Step 3, you chose to load an *Analysis worksheet*, then FCS Express will display the experiment. If you loaded an *Acquisition template*, along with the FCS data files, then the **Select the Specimen and Tube** dialog will appear (Figure 24.4). Expand the name headings, as appropriate, and select one FCS file from the list. After highlighting an FCS file name, click **OK**. If FCS Express cannot find the file, you will be prompted to locate it.

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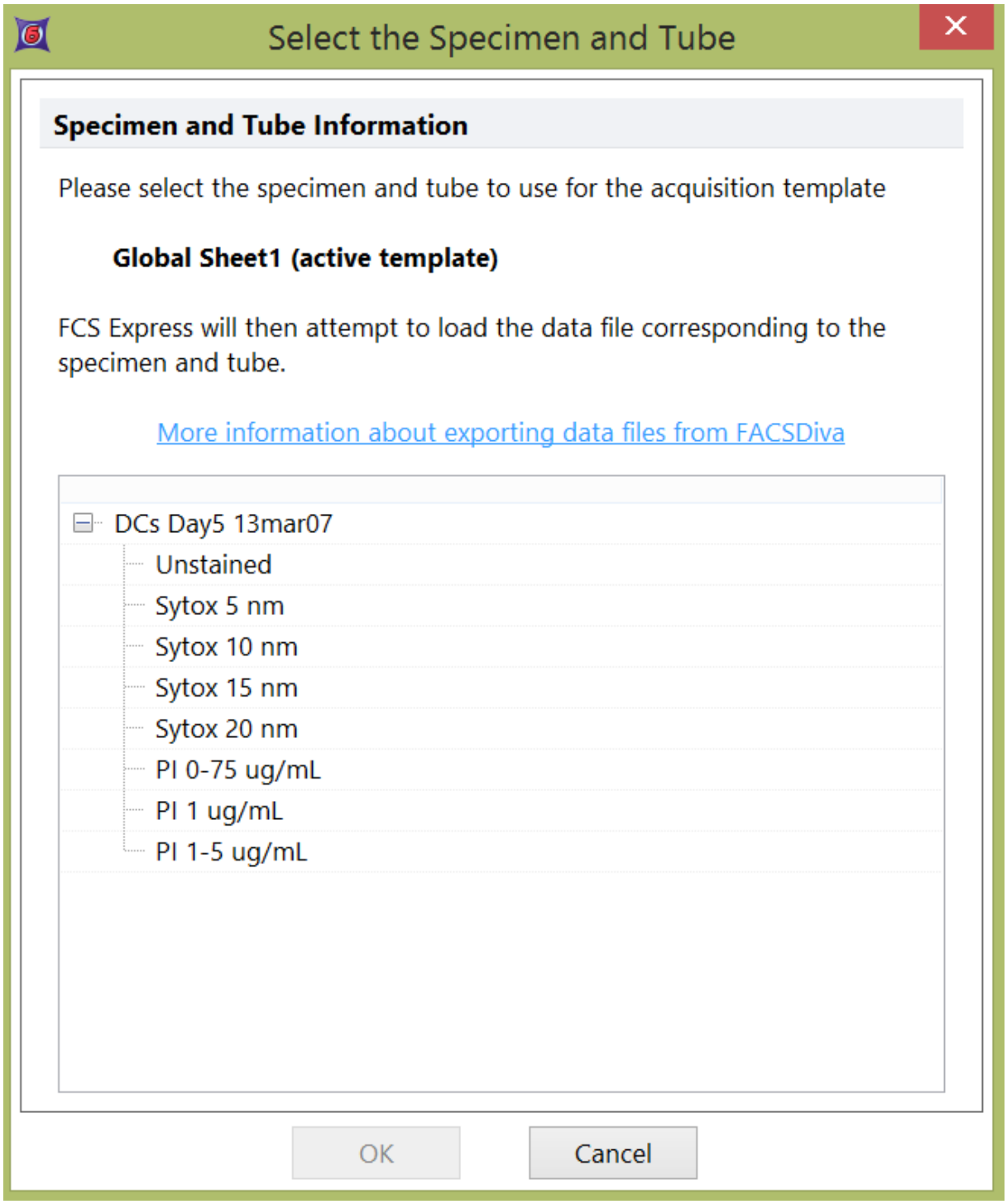

Figure 24.4 Selecting FCS File Data for an Imported FACSDiva Experiment

9. For each worksheet with an acquisition template you selected in Step 3, you will see the above dialog to load FCS data file.

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FCS Express will then display the imported FACSDiva experiment, which can be treated like any other layout.

23.1.2 Tips for Importing FACSDiva Experiments

The following tips are valuable when considering importing FACSDiva experiment files into FCS Express.

When importing FACSDiva experiments into FCS Express, all plots in the experiment will be recreated on your layout. Although each plot can be reformatted using standard FCS Express techniques 246, it is useful to remember that the default format (i.e., fonts, axes labels, plot resolution, overlay settings, etc.) can be defined before a plot is created, in FCS Express User Options 179. To ensure that imported plots are displayed in the manner that you prefer, it is recommended that you take advantage of the default options before you import FACSDiva experiments. For convenience, you can create a user 50 (e.g., "FACSDiva Import"), under whom the specific default options may be defined without affecting your default settings for working with other types of data files.

Among the plot settings that you will most likely want to configure are:

- Legend visibility

  Displaying the legend takes away viewable space from the plot. Therefore, you should consider hiding the legend 189 if your FACSDiva experiment displays many plots on a page.

- Histogram resolution

  Typically, the histogram resolution is set to be derived from the FCS File. In high resolution data with lots of channels, there are likely to be very few events in each channel. Lowering the resolution to 1024 channels may improve the appearance of the histograms.

- Scaling the Axes

  When you import your experiment into FCS Express, your plots are displayed with the axes scale settings that are stored in the experiment file. You are not obligated to keep these axes scales, however. For example, if an imported plot is displayed with five decades, you may decide that it is better displayed with only four decades. You can also change the scale between linear, logarithmic, and biexponential 395.

23.2 BD Accuri CFlow and C6 Files

The BD Accuri™ C6 and C6 Plus are bench-top systems that deliver performance and features similar to those of the market-leading flow cytometers, yet the

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instruments and the C6 software that controls the C6 are easy to use and allow even a novice to quickly set up an experiment and acquire data.

FCS Express is pleased to provide the BD Accuri C6 importer, which allows you to apply the sophisticated analysis features of FCS Express to the data acquired using C6. FCS Express will import all of the experiment information from the C6 file, including:

- All plots on the Collect and Analyze tab.
- The native, high resolution, 24-bit FCS data.
- All gates with the proper gating hierarchy.
- All compensation settings.
- All markers and quadrants.
- All plot formatting settings.

Once your experiment is loaded into FCS Express, you can treat your plots just like a plot created in FCS Express.

If you have purchased the Heat Map add-on with FCS Express you may load any .C6 file in FCS Express as a 96 well heat map regardless if it was collect from a plate. Simply insert a heat map and choose **BD Accuri C6 Plate files (*.c6, *.ci)** from the files of type dropdown list.

23.2.1 How to Import CFlow Experiment Files

To import a CFlow experiment file:

1. Use the **File** tab→**Open From**→**Import BD Accuri C6 File...** command.

2. Browse your directories to select the C6 experiment file (*.c6; *.ci)to import. Click **Open** when you have located it.

3. The **Import BD Accuri C6 File Wizard** appears, beginning with the **Import Selections** page (Figure 24.5). On this page, you can choose to import plots from the C6 Collect and/or Analyze tabs.

4. If you choose to import plots from the Collect tab, select the data file to use from the drop-down list.

5. Click **Next** to continue.

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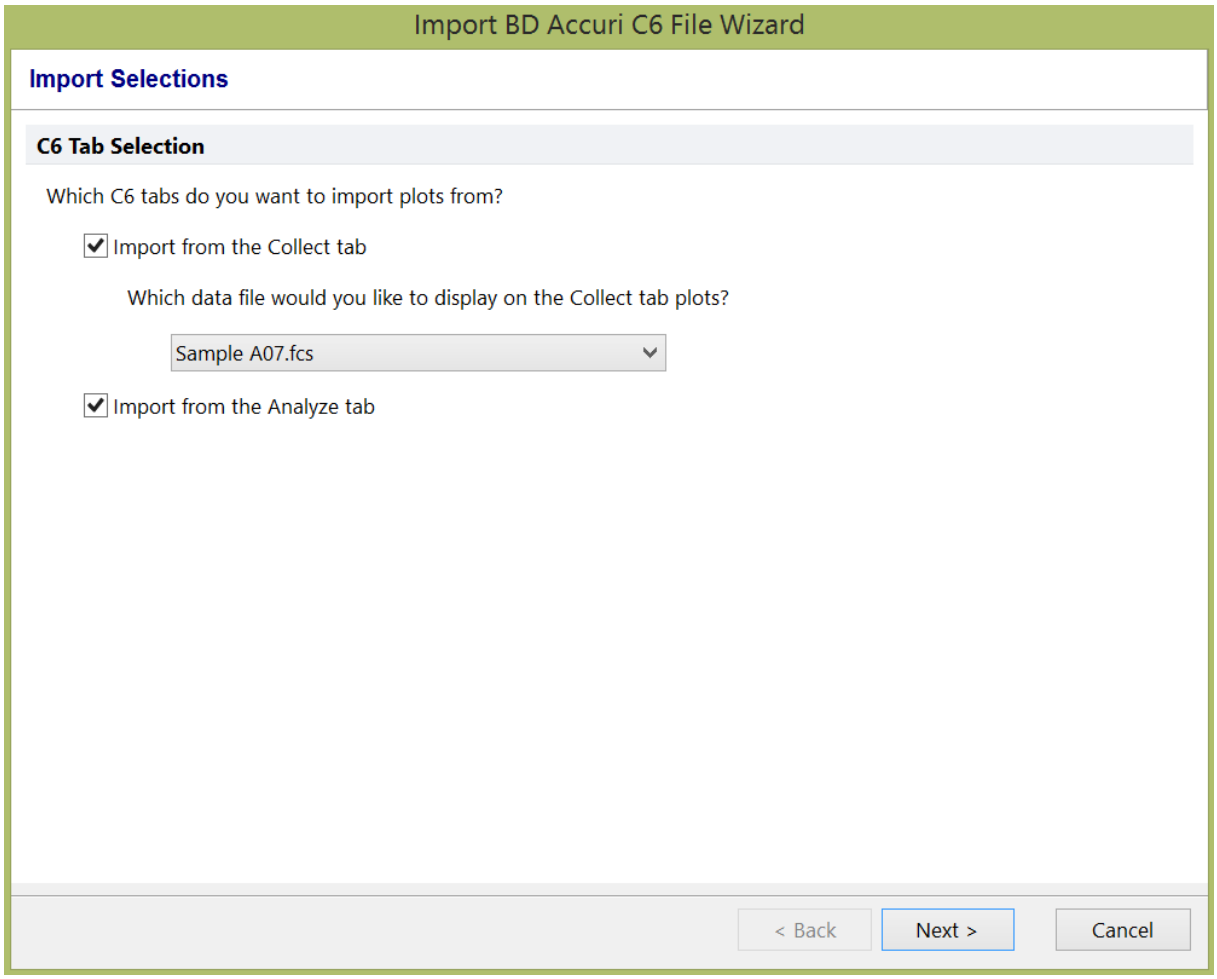

Figure 24.5 Import BD Accuri CFlow File Wizard - Import Selections

The **Page Layout Options** page (Figure 24.6) will appear. Here, you configure how the plots from the C6 experiment will be imported into FCS Express.

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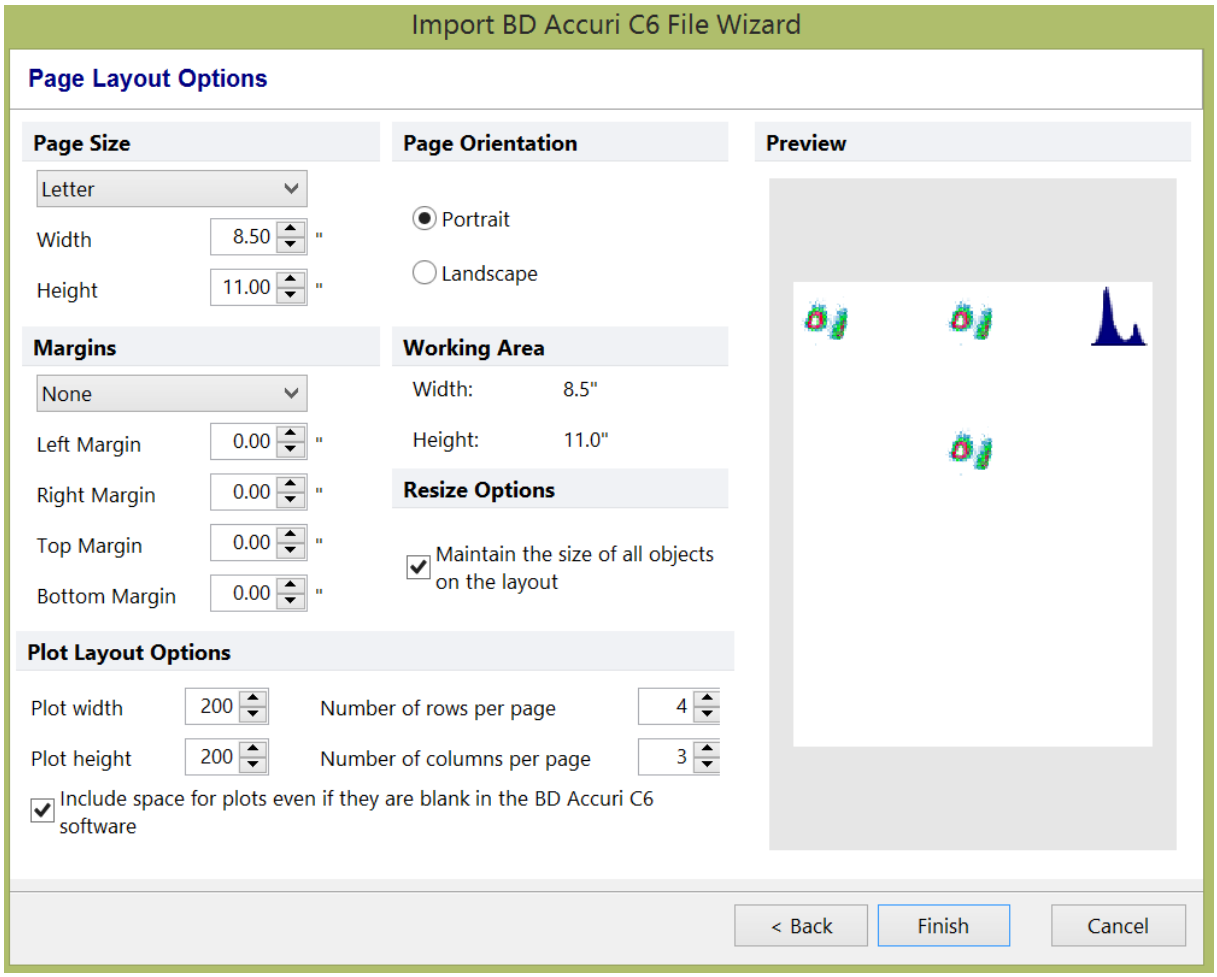

Figure 24.6 Import BD Accuri CFlow File Wizard - Page Layout Options

6. Under the **Page Size** section, you can select a standardized size from the drop down menu, or you can manually enter a Width or Height. You are also able to adjust the **Margins**, **Page Orientation** and whether or not FCS Express is to *Maintain the size of all objects on the layout*. Underneath the **Plot Layout Options** section, you can set the *Plot width*, *Plot height*, *Number of rows per page*, and *Number of columns per page*. A preview of your layout is shown on the right. Click **Finish** when you are ready. FCS Express will now create an exact copy of the C6 workspace displayed as an FCS Express layout. The plots will all be scaled and zoomed the same way in FCS Express as in C6. All regions, markers, and gating are retained. Parameter names and compensation settings are also applied.

23.2.2 Tips for Importing CFlow Experiments

The following tips and instructions will aid in importing and using Accuri CFlow experiments:

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- Recommended settings in FCS Express to help improve performance when importing BD Accuri C6 experiments.

  1. Open the **Options** dialog using the File tab→Options 179 command.

  2. Select the **Data Loading→FCS File Options** category. Make sure that **Allow Multiple Datasets** is unchecked. Although this setting is not necessary for loading a C6 data file, it will allow you to add files to the data list, which is necessary for batch processing.

- Loading BD Accuri C6 software data into FCS Express plots.

  1. Insert a plot 172 from the Insert Tab. When the Select a Data File dialog opens, change the file type to **C6 files (*.c6; *.cfl; *.ci)**.

  2. Navigate to the BD Accuri C6 software file that you want to open, and double-click on it. The dialog will then display all of the fcs files contained inside the C6 file. Select the one that you are looking for.

  3. Press **Open**.

23.3 Comma Separated Value / Delimited (.csv) Files

FCS Express supports loading data stored as Comma Delimited files in the format of (*.csv).

Data files stored in the .csv format must contain a header row for each parameter that contains a parameter descriptor as well as a comma-separated list of values for each parameter created as seen in Figure 24.7.

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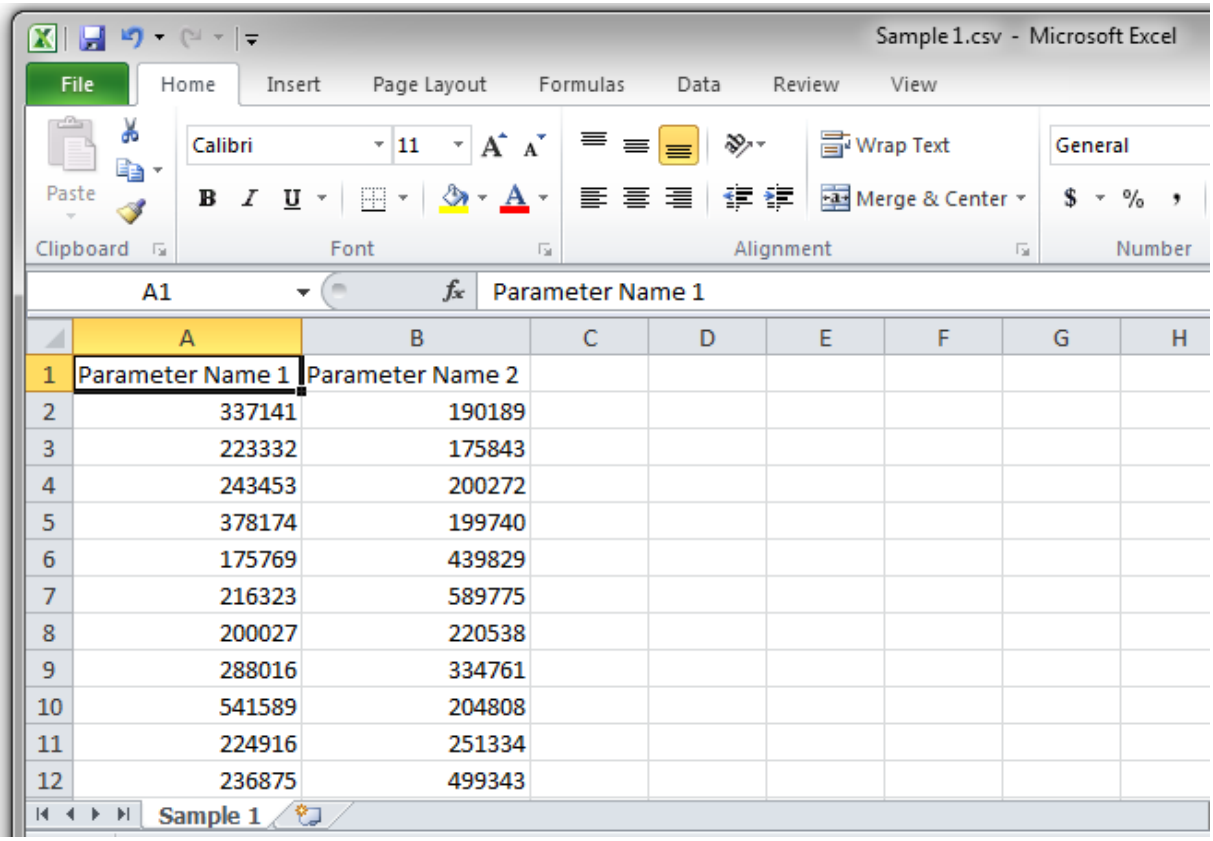

	A	B	C	D	E	F	G	H
1	Parameter Name 1	Parameter Name 2						
2	337141	190189						
3	223332	175843						
4	243453	200272						
5	378174	199740						
6	175769	439829						
7	216323	589775						
8	200027	220538						
9	288016	334761						
10	541589	204808						
11	224916	251334						
12	236875	499343						

Figure 24.7 - A comma-separated value (.csv) file created in the proper format for import to FCS Express Version 5.

Comma Delimited files may be opened and used in FCS Express by choosing **Comma Delimited files (*.csv)** from the **Files of type** dropdown list in any **Select a Data File dialog** (Figure 24.8.).

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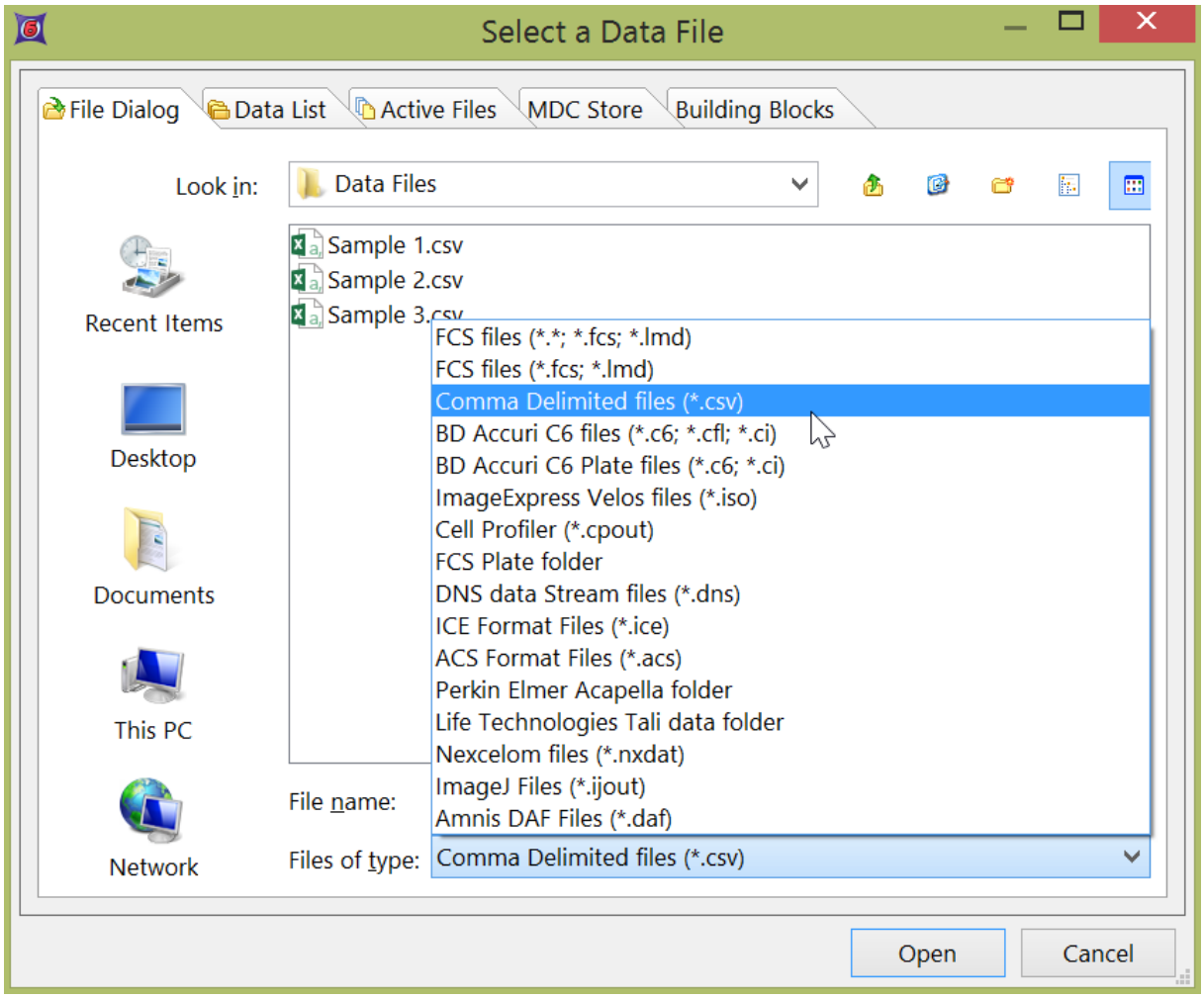

Figure 24.8 - Choosing the Comma Delimited files (*.csv) file type in the select a data file dialog.

Once a file is selected it may be inserted as a plot in FCS Express for analysis (Figure 24.9).

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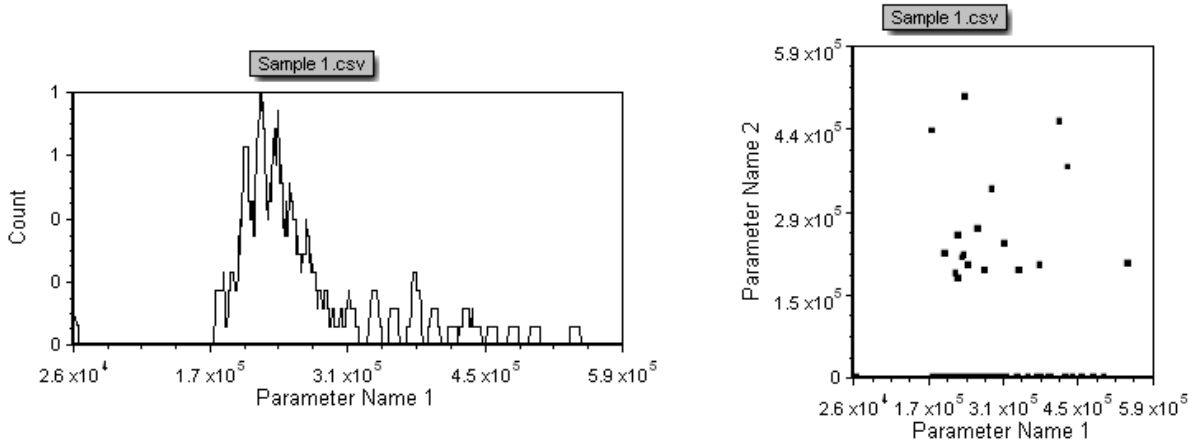

Figure 24.9 A comma delimited value file opened in a histogram and dot plot.

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Chapter

24 DNA Analysis

FCS Express provides a complete DNA analysis solution based upon the widely used MultiCycle AV™ DNA analysis package written by Dr. Peter Rabinovitch at the University of Washington, and distributed by Phoenix Flow Systems (http://www.phoenixflow.com).

From the Multicycle manual:

> *"DNA content histograms require mathematical analysis in order to extract the underlying G1, S, and G2 phase distributions...The most flexible and accurate methods of cell cycle analysis are based upon building a mathematical model of the DNA content distribution, and then fitting this model to the data using curve-fitting methods." Multicycle contains "the most well established models," like those "proposed by Dean and Jett, based upon the prediction that the cell cycle histogram is a result of the Gaussian broadening of the theoretically perfect distribution," and Fox, whose "model provides a more flexible S phase shape, but still retains the smoothness of the S phase that is characteristic of the Dean and Jett model." In addition, "careful attention to fitting of the background aggregates and debris is also" a feature of Multicycle "in order to maximize the reliability of cell cycle analysis of complex histograms."*

A free, limited version of Multicycle is included with FCS Express. This free version only reports the data on a basic 1-cycle Autofit with no background correction. When using the free version, there will be a warning message if more than one cycle is detected in your .fcs data files, and/or if a background correction would improve the fit. Both of these conditions require the full version of Multicycle for accurate analysis.

The full version of Multicycle is available for purchase as an add-on. The complete version allows 1-, 2-, and 3-cycle fits with the ability to change parameters manually and perform background correction.

24.1 Multicycle

FCS Express comes with a free version of Multicycle capable of performing a 1-cycle fit with no background correction. The full version of Multicycle may be purchased as an add-on for FCS Express from De Novo Software, or as standalone software from Phoenix Flow Systems (http://www.phoenixflow.com).

The Multicycle model has been described extensively in the following publications:

- Rabinovitch, P.S. DNA content histogram and cell-cycle analysis. *Methods in Cell Biology*. 41:263-296, 1994.

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- Rabinovitch, P.S. Practical considerations for DNA content and cell cycle analysis. In: *Clinical Flow Cytometry: Principles and Applications* (Bauer, K.D., Duque, R.E., and Shankey, T.V., eds.) Williams and Wilkins, Baltimore, pp. 117-142, 1992.

- Rabinovitch, P.S. DNA Histogram Analysis. In: *Cell Growth and Division*, 2nd Edition. (G. Studinski, ed.). Oxford University Press, Oxford, 1996.

- Kallioniemi, O-P., Visakorpi, T., Holli, K., Isola, J.J., and Rabinovitch, P.S. Automated peak detection and cell cycle analysis of flow cytometric histograms. *Cytometry* 16:250-255, 1994.

24.2 Inserting a DNA Histogram

DNA Histograms are inserted onto a layout page just like other histograms 172.

Use the **Insert** tab→**1D Plots**→**Multicycle DNA** command and either click on the layout page to insert a standard size histogram, or click-drag to insert a plot of the desired size. A file dialog will then appear allowing you to select your DNA data file. A fitted Multicycle DNA histogram will appear. A legend 253 can be displayed when the standard histogram features are formatted (Figure 25.1).

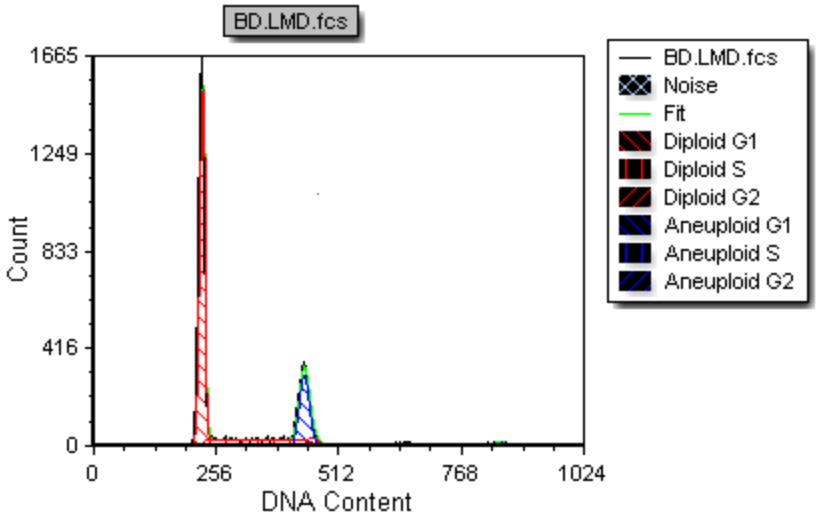

Figure 25.1 Multicycle DNA Histogram with Legend

FCS Express can fit DNA curves from files generated from any cytometer (both standard FCS list mode data files as well as uncorrelated FCS histogram files).

24.3 Formatting a DNA Histogram

To format a Multicycle DNA histogram,

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- Right-click on the histogram, select **Format** from the pop-up menu, expand the **DNA** node, and choose one of the four DNA-specific categories: General 886, Cycle 887, Background 888, or Position Markers 889

The other categories which can be formatted are described in formatting options for standard histograms 267. Note that overlays cannot be added to Multicycle DNA histograms.

24.3.1 General Options

The **General** category of DNA formatting options contains properties that control how the data on the DNA histogram is presented (Figure 25.2).

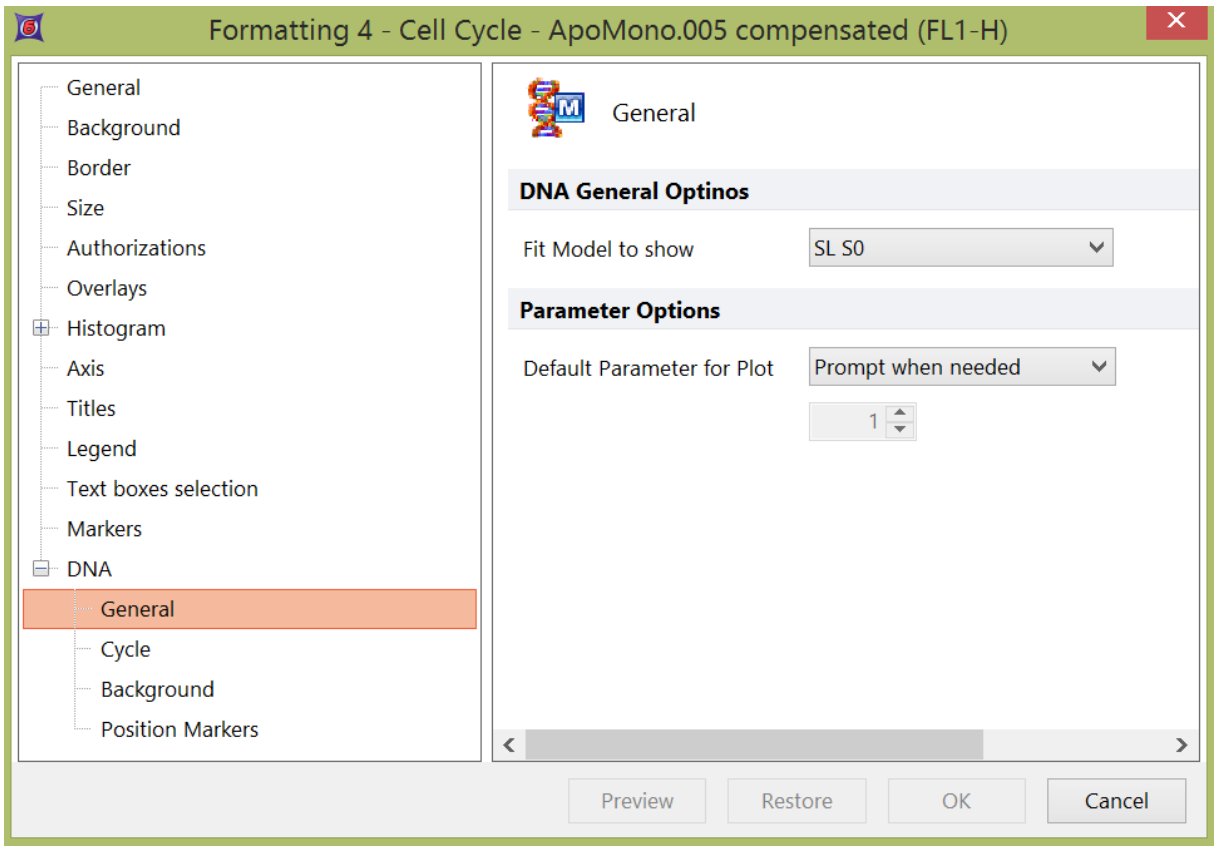

Figure 25.2 Formatting a DNA histogram - general options

The options are described in the table below.

Property	Explanation
Fit Model to show	Every time Multicycle performs a fit, it calculates six different variations on that fit. You can choose which variation to display by changing this option. The six variations are described in detail in the Multicycle manual.

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Property	Explanation
Default Parameter For Plot	Select which parameter to display when changing data files. "Prompt when needed" will prompt you to select a parameter every time you change a data file. "Use parameter below" allows you to always display the parameter selected from the spin control.

You can find a summary of the Cycles and Models used in Multicycle here 898.

24.3.2 Cycle Options

The **Cycle** category contains options describing different parameters of the DNA fit. This category allows you to choose what kind of fit you wish to apply, as well as set specific parameters for the fit.

FCS Express defaults to selecting the fit type automatically (the **Detect fit type automatically** checkbox is checked). When detecting the fit type automatically, all other options are disabled because Multicycle will calculate all the fit parameters.

To set the fitting values yourself, uncheck the **Detect fit type automatically** checkbox. The other options will then be enabled (Figure 25.3).

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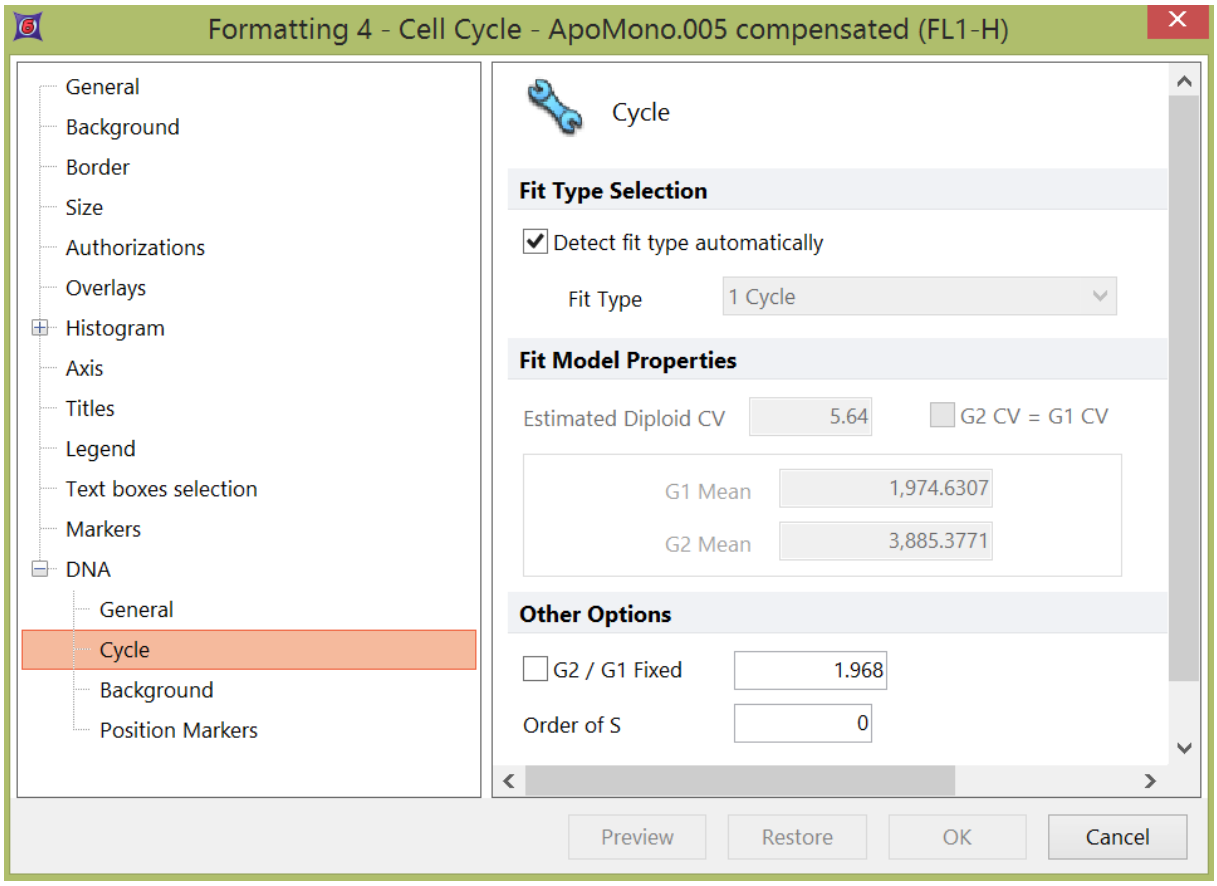

Figure 25.3 Formatting a DNA histogram - Cycle options

FCS Express will show different fitting options depending on which fit type is selected from the **Fit Type** drop-down list. The example shows a **2 Cycle** fit. Links to a complete listing of all the supported fit types and the various parameters that apply for those fits can be found on the FCS Express - Multicycle knowledgebase page.

After changing the parameters for a fit, you can apply those parameters by pressing the **OK** or **Preview** buttons. Multicycle will use the input parameters as the starting point for the fit, but may vary the parameters while calculating the best fit. To see the final fit values that Multicycle calculated you can open a DNA statistics window 895 or create Text Boxes with DNA-specific tokens 897.

24.3.3 Background Options

The **Background** category (Figure 25.4) contains options which control how Multicycle calculates the background (in order to remove debris from the sample and calculate an accurate fit).

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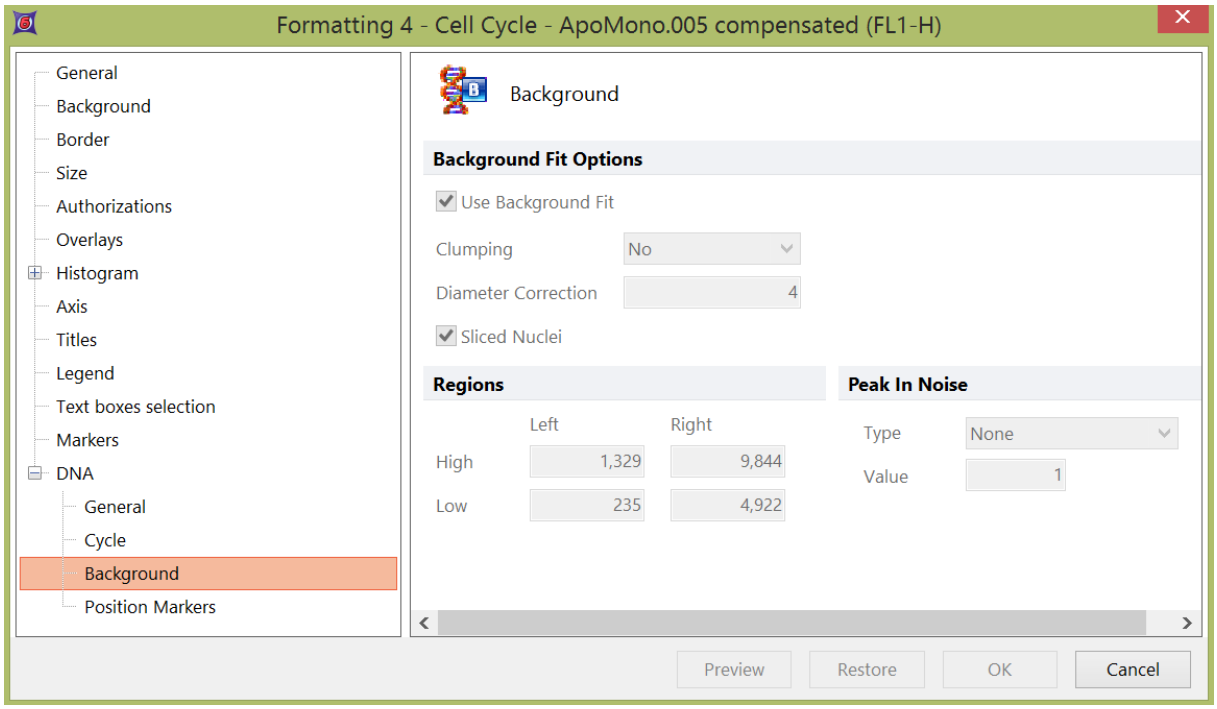

Figure 25.4 Formatting a DNA histogram - background options

The **Use Background Fit** checkbox controls whether or not Multicycle will attempt to calculate a background and remove it from the fit data. The other options control the specific background fitting parameters and are explained in detail in the Multicycle manual. Please see the FCS Express - Multicycle knowledgebase page for additional resources.

24.3.4 DNA Position Markers

The position of cell cycle parameters (e.g., the G1 mean or the background regions) can be displayed with the use of **DNA Position Markers** (Figure 25.5).

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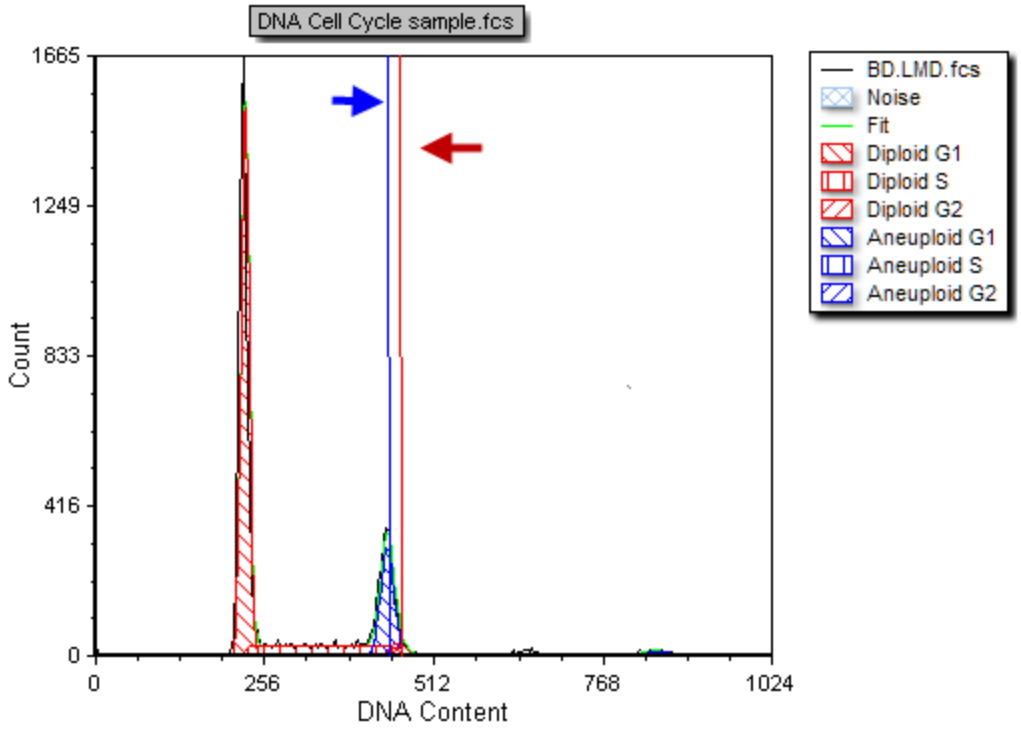

Figure 25.5 DNA Histogram showing Diploid G2 Position Marker (Red) and Aneuploid G1 Position Marker (Blue)

You can show or hide a position marker by either:

- Right-clicking on the DNA Histogram, selecting **DNA Position Markers** from the pop-up menu, and toggling the appropriate position marker on or off from the submenu (Figure 25.6), or
- Right-clicking on the histogram, selecting **Format** from the pop-up menu, expanding the **DNA** category node, and choose the **Position Markers** category

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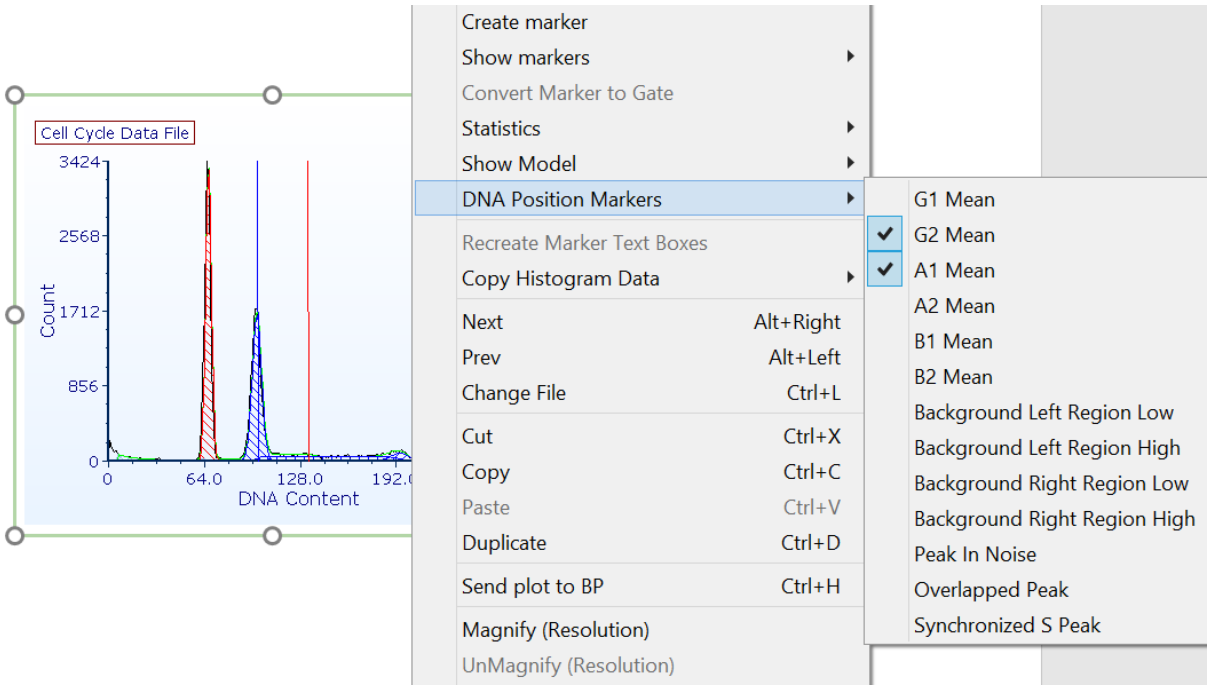

Figure 25.6 Showing or Hiding DNA Position Markers

To adjust the visibility, style, color, and/or width of a marker, right-click on the DNA Histogram, select **Format** from the pop-up menu, expand the **DNA** node, and choose the **Position Markers** category (Figure 25.7). Select the marker from the list on the left (use **Ctrl+Click** to select multiple markers). The **Show position marker on the plot** checkbox controls the visibility of the selected marker.

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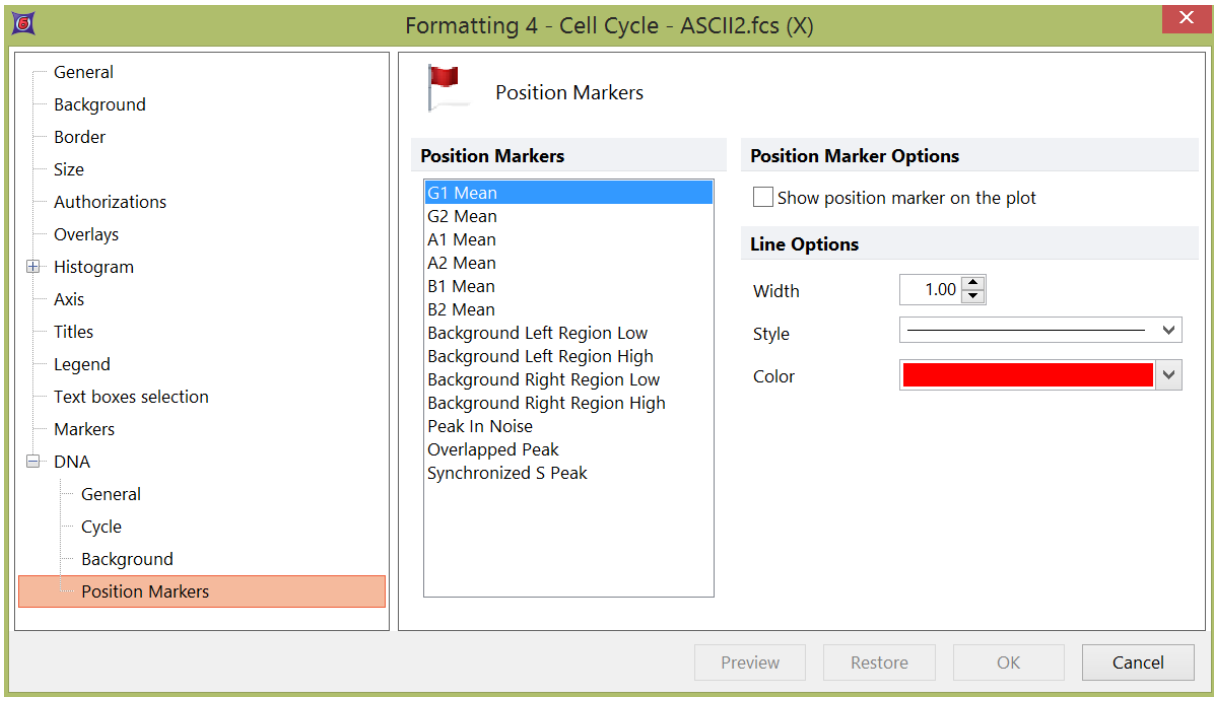

Figure 25.7 Formatting a DNA histogram - Position Markers

24.4 Setting Default DNA Options

The default DNA options[193] including general options, overlay options, and position marker preferences for performing DNA analysis using Multicycle[884] are described in the appropriate section of the chapter on formatting objects[193].

24.5 The Multicycle Tab

MultiCycle can automatically fit and analyze cell cycle histograms with multiple models. You can control the number and type of populations to fit, the currently displayed model, and the background options using the MultiCycle Tab (Figure 25.8). The Multicycle tab will only appear when a Multicycle DNA Analysis plot has been selected in the layout.

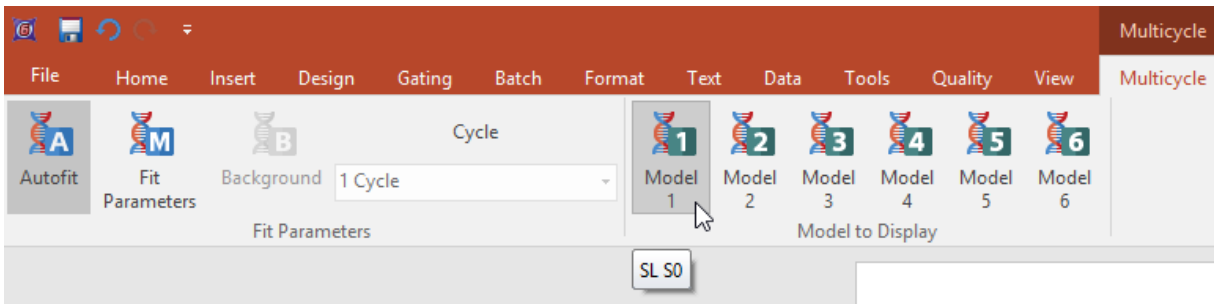

Figure 25.8 The Multicycle Tab

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To analyze a DNA histogram automatically:

1. Insert a DNA Histogram onto your layout using the **Insert→1D Plots→MultiCycle DNA** command.
2. Select the DNA Histogram and verify that the **MultiCycle→Fit Parameters→Autofit** command is highlighted. With Autofit, MultiCycle will choose the Cycle parameter for you.
3. Right-click on the DNA Histogram and select **Statistics→DNA Cycle Statistics** from the pop-up menu. Adjust the position of the statistics window.
4. Right-click on the DNA Histogram and select **Statistics→DNA Model Summary Statistics** from the pop-up menu. Adjust the position of the statistics window.

The current model being displayed in the DNA Cycle Statistics is highlighted in the **MultiCycle→Model to Display** group. To change the model, click on one of the other five **Model** commands within the **Model to Display** group.

To change to a manual fit, click on **MultiCycle→Fit Parameters→Autofit** and verify that the command/button is not highlighted. With a manual fit:

- To change the number of populations for the fit, or the parameters for a 1 Cycle fit, use the **MultiCycle→Fit Parameters→Cycle** drop-down list.

- To change the Fit Model Properties, use the **MultiCycle→Fit Parameters→Fit Parameters** command to bring up the **Formatting Fit Model Options** dialog (Figure 25.9). You can alter the means and CVs for populations manually. The properties that can be adjusted will change according to the **Fit Type** chosen in the drop-down list.

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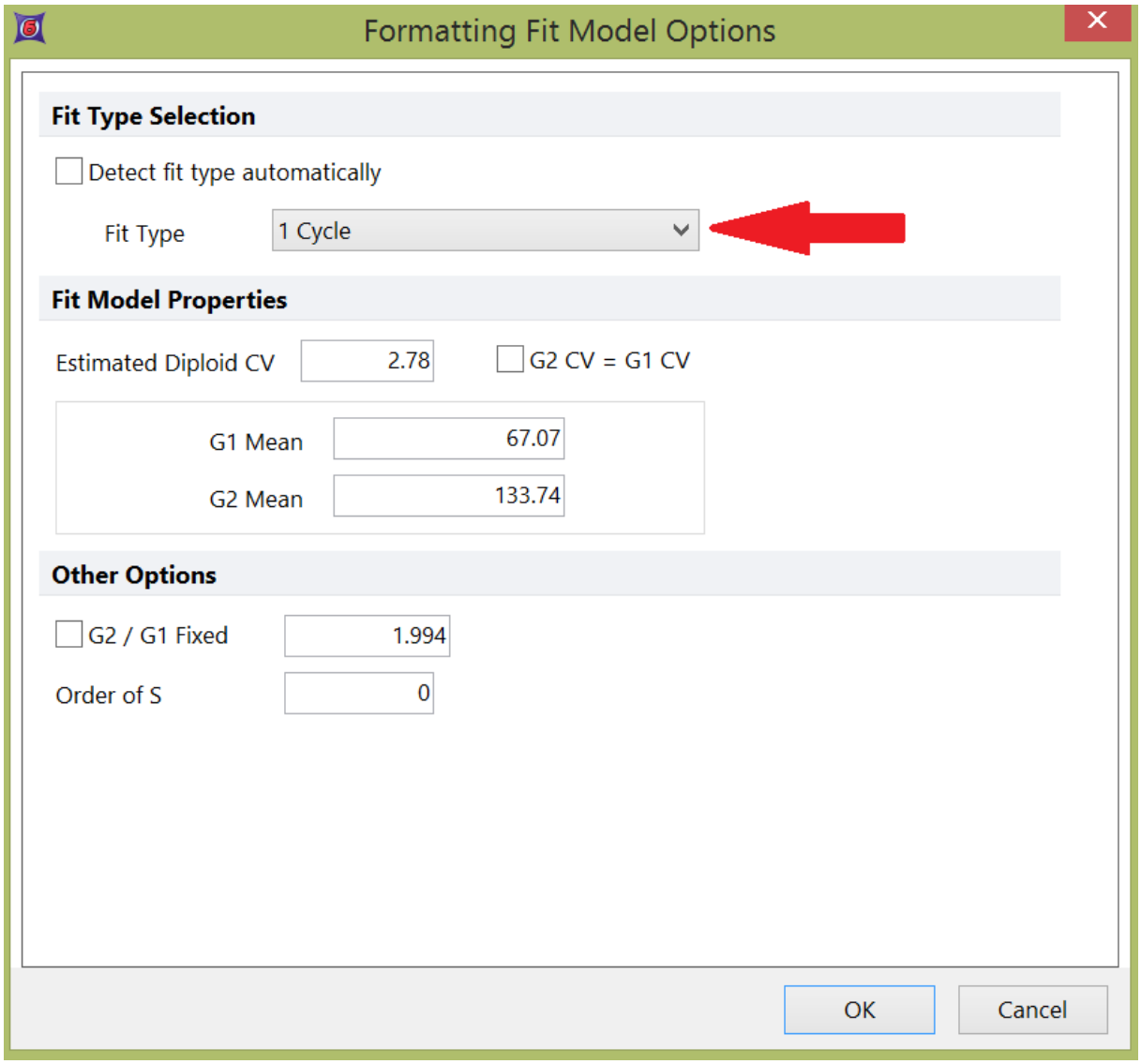

Figure 25.9 Formatting Fit Model Options Dialog

- To change background parameters, select the **MultiCycle→Fit Parameters→Background** command to bring up the **Formatting Background Options** dialog (Figure 25.10). The parameters are described in the MultiCycle manual.

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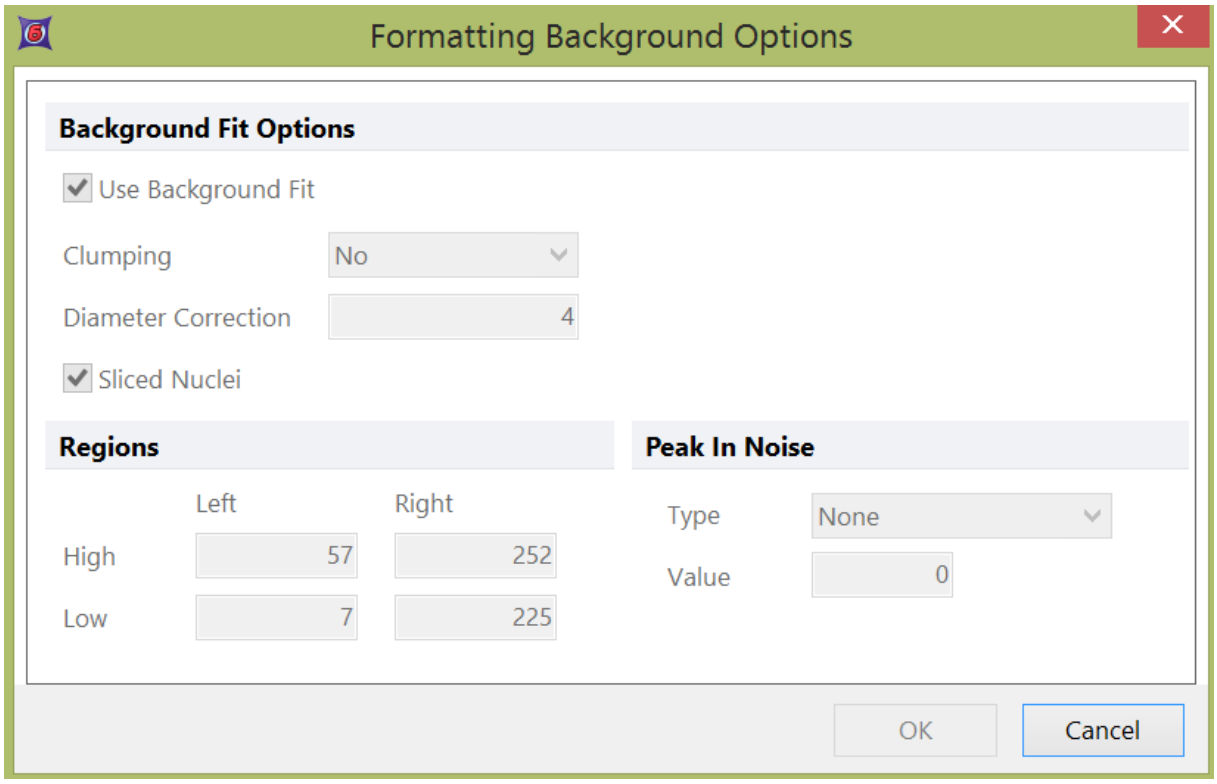

Figure 25.10 Formatting Background Options Dialog

24.6 DNA Statistics

To open a DNA statistics window, right-click on the DNA histogram, move the cursor over **Statistics** in the pop-up menu, and select the desired type from the submenu (Figure 25.11).

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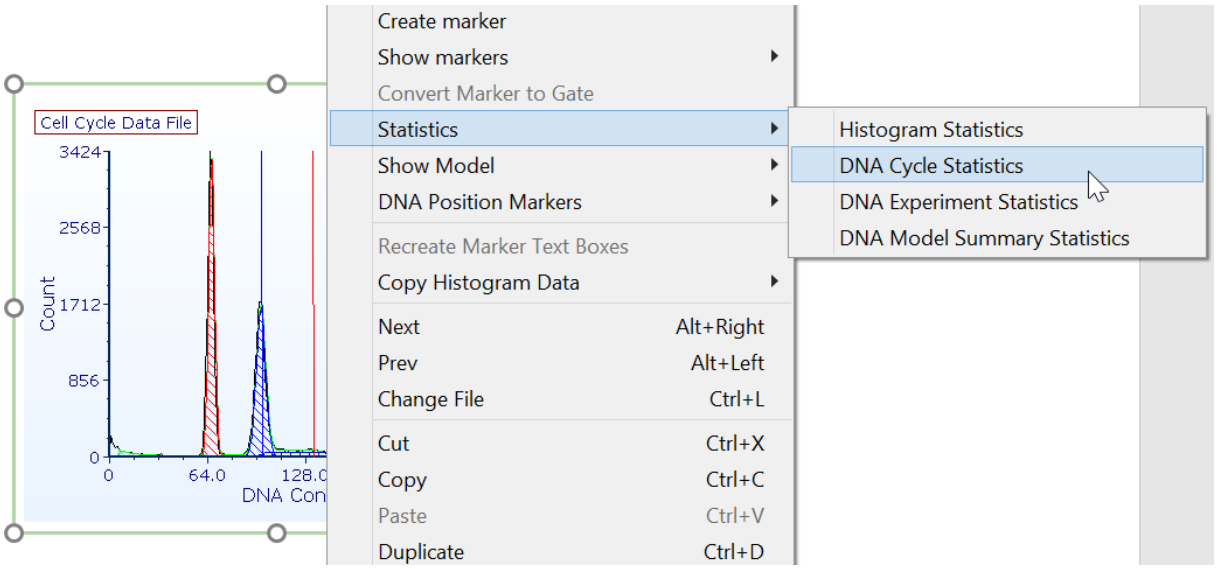

Figure 25.11 Choosing DNA Statistics

The first choice, **Standard Histogram Statistics**, represents the statistics available for non-DNA histograms 382.

DNA Cycle Statistics describe each of the populations within your sample. The three possible populations are Diploid, Aneuploid A (or Aneuploid 1), and Aneuploid B (or Aneuploid 2). Each population will appear in a different row depending on the currently applied fit. Figure 25.12 shows an example of DNA cycle statistics for a two cycle fit. A detailed description of the different cycle statistics can be found in the Multicycle manual.

Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	226.96	2.55	75.42	460.25	2.55	2.65	21.94	2.03	76.56	n/a	0.14
Aneuploid A	442.96	2.22	79.73	855.00	2.22	6.51	13.76	1.93	23.44	1.95	0.10

Figure 25.12 DNA Cycle Statistics for a Two Cycle Fit

The **DNA Model Summary Statistics** pertain to the six different models that Multicycle calculates each time a DNA analysis is performed. Each model will appear in a different row. A detailed description of the different DNA model statistics can be found in the Multicycle manual.

The **DNA Experiment Statistics** display several additional statistics as well as an interpretation of the experiment as a whole. The interpretation, designed to give you an estimate of the goodness of the fit, is described in more detail in the Multicycle manual (Figure 25.13).

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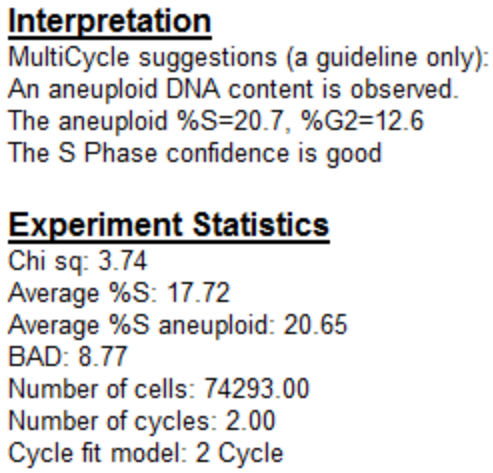

Figure 25.13 DNA Interpretation and Experiment Statistics

Please see the FCS Express - Multicycle knowledgebase page for additional resources.

24.7 DNA-Specific Tokens

All of the DNA statistics can be used as tokens[692]. DNA tokens can be inserted into text boxes[692], used in Custom Token[727] or Alert formulas, and exported to reports during batch processing[835].

To insert a DNA token into a text box:

1. Insert a text box using the **Insert** tab→**General**→**Text Box** command.
2. Right-click on the text box and select **Insert Token**.
3. Double-click **Statistic** to bring up the **Create Statistic** dialog.
4. Choose a DNA Histogram as a **Data Source**.
5. Open the **Statistic** category.
6. Choose a DNA statistic[895] from the **Select a Statistic** drop-down list. If you choose DNA Cycle Statistics, the dialog will look like Figure 25.14. If you choose a different DNA statistic (DNA Model Summary Statistics or DNA Experiment Statistics), the options and the specific items in the list box will change.
7. Click **OK**.

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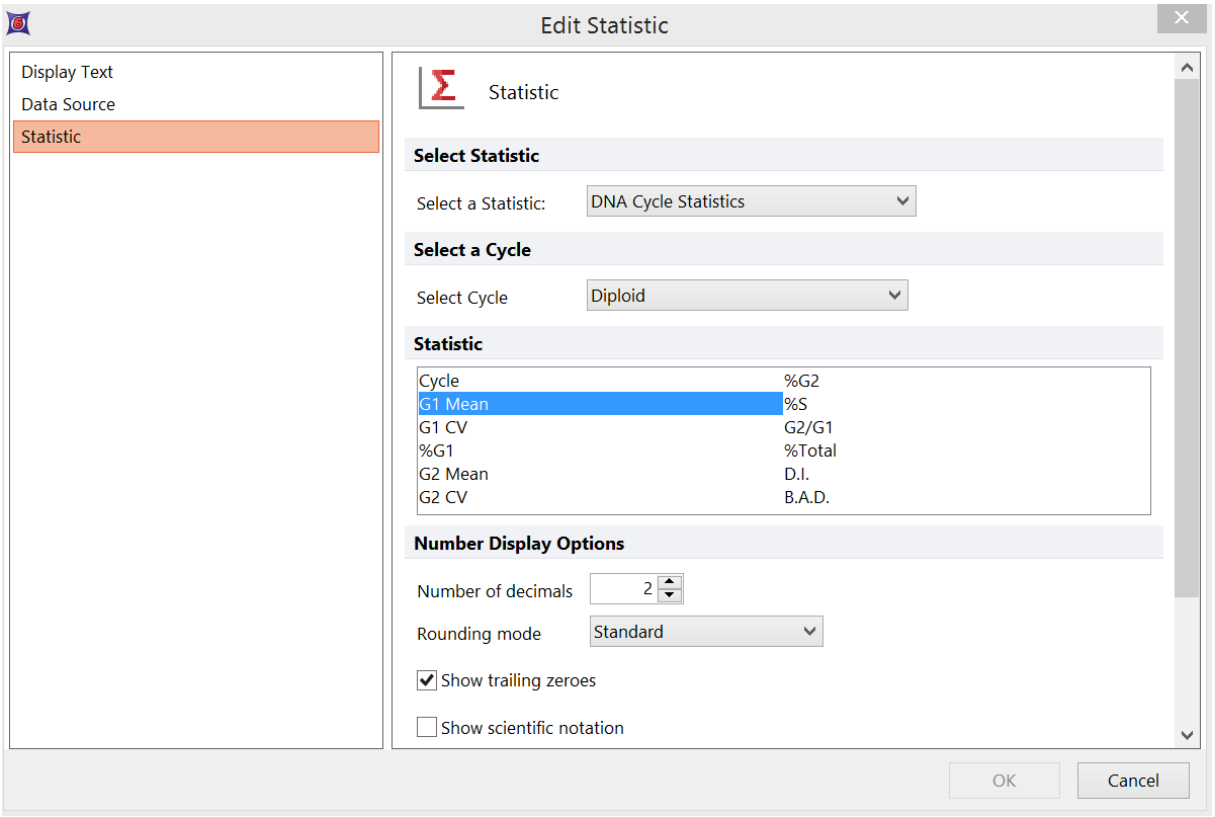

Figure 25.14 Create Statistics Dialog - DNA Cycle Statistics

24.8 DNA Cycle and Model Summary Reference

MultiCycle AV for FCS Express is capable of autofitting the appropriate Cycle to suit your data. However, users have the ability to override the autofit and choose a Cycle to fit to their data.

Here is a summary of the various Cycles that are available in MultiCycle AV.

Cycles	
1 Cycle	The most common and frequently used. Used when there is 1 full cell cycle present.
1 Cycle Standard Overlay Peak	Similar to 1 Cycle. Ideal when using a standardization tool like chicken erythrocytes.
1 Cycle Apoptotic Overlay Peak	This cycle is performed when it is assumed that there is one extra peak to the left of the G0/G1 peak.
1 Cycle Aneuploid Overlay Peak	This cycle is performed when there is an Aneuploid peak present that does not contain a full cell cycle.
1 Cycle Diploid	This cycle is performed when there is a Diploid peak that does not contain a full cell cycle.

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Overlay Peak	
2 Cycle	This cycle is used when there are 2 full cell cycles present.
3 Cycle	This cycle is used when there are 3 full cell cycles present.
Synchronous S Phase	This cycle should be used when performing an experiment where S phase is arrested, leading to a peak in S peak, rather than a smooth gradual curve.

MultiCycle AV for FCS Express applies six different models to your data at the same time.

In FCS Express, you can create a DNA Model Statistics Summary box that shows your statistics as it applies to each of the six different models (Figure 25.15).

Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL CV S0	2.65	21.94	6.51	13.76	1.85
SL CL S0	2.87	21.92	6.17	7.82	1.38
+G2/G1 Fixed	2.44	21.98	0.50	17.59	2.25
+Aggregates	0.00	20.70	4.34	8.30	1.60
+S Order = 1	2.44	21.78	6.64	13.47	1.82
+CVs Fixed	0.06	22.67	6.43	12.51	1.87

Figure 25.15 - DNA Model Statistics

Here is a summary of the various Cycles that are available in MultiCycle AV.

Models	
SL S0	Most common model used. Sliced nuclei background modeling with zero order S phase, but without other options. Used when debris curve looks like a ski slope.
SL CL S0	Sliced nuclei background modeling with clumping compensation with zero order S phase. Used if debris curve is flat and cuts into G0/G1.
+G2/G1 Fixed	Sliced nuclei background modeling, plus the constraint of the G2/G1 ratio. For 1 cycle fitting models, the G2/G1 ratio is the current default stored within Multicycle AV. Please note, for 2 Cycle models, the G2/G1 ratio of the diploid cycle is constrained to be equal to the aneuploid G2/G1 ratio that resulted from the user-chosen fitting model. For 3 Cycle models, the G2/G1 ratio of all 3 cell cycles is constrained to be equal to the average of the 2 aneuploid G2/G1 ratios that resulted from the user-chosen fitting model.

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+Aggregates	Sliced nuclei modeling plus aggregation modeling and addition of G2/G1 constraint, as detailed above. Basically, this is a software version of doublet discrimination. This model looks at all possible combinations of G1 and G2 stuck together and figures out the % of cells that are stuck together.
+S Order = 1	A 1st order S phase polynomial is used with sliced nuclei background modeling. This model is to be used when the S phase is more in the shape of a broadened trapezoid. S order = 0 (broadened rectangle) S order = 1 (broadened trapezoid) S order = 2 (higher on the sides, lower in the middle)
+CVs Fixed	Sliced nuclei modeling with 1st order S phase, plus the CVs of all peaks are constrained. This model can be used when the G2/M peak is buried in the S phase.

References for this information can be found at our Multicycle Knowledgebase Page.

In cases where the data is suboptimal or the cell cycle peaks are not well defined, users can get Intra-model or Inter-model errors. You can find the definition for these errors at http://www.denovosoftware.com/site/kb-DNAMulticycleInfo.shtml.

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Chapter

25 Proliferation Analysis

The introduction of fluorescent cell staining and division tracking dyes has made it possible to monitor the number of cell divisions during proliferation, and to examine the relationship between proliferation and differentiation.

Fluorescent tracking dyes can be categorized into two major groups:

- *General protein labeling*

  General protein labeling dyes such as carboxyfluorescein diacetate succinimidyl ester (CFSE) act by forming random covalent bonds with amino groups on cellular proteins resulting in a highly fluorescent membrane impermeable marker. CFSE is highly fluorescent and when excited with a 488 nm laser it emits in the "green" channel at 515 nm.

- *General membrane labeling dyes*

  General membrane labeling dyes such as PKH and Cellvue are lipophilic compounds that stably but non-covalently insert into the plasma membrane. General membrane labeling dyes vary in fluorescent intensity and can be obtained in many colors, making them useful for multi-color experiments.

With each cell division, the dye is divided roughly equally between the two daughter cells. Therefore, the dye fluorescence intensity is a measure of the number of divisions any given cell has undergone.

After staining, the cell population is analyzed at regular time intervals by flow cytometry. A histogram of the number of cells as a function of dye fluorescence intensity is generated. Typically, cell proliferation dyes can be used to track up to 8 rounds of division before fluorescence intensity is reduced to the autofluorescence of unstained cells.

The FCS Express Proliferation Add-on provides curve fitting algorithms to estimate the proliferation fraction.

For more information on proliferation analysis, please see http://www.denovosoftware.com/site/ProliferationAnalysis.shtml.

25.1 Obtaining the Proliferation Add-on

The Proliferation add-on module in FCS Express performs sophisticated curve fitting in order to quantify the proliferation characteristics of your population. This

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allows for easy determination of the fraction of proliferating cells, as well as other derived statistics.

The Cell Proliferation Analysis add-on module is included with any Professional license of FCS Express 6 Flow Cytometry.

25.2 Inserting a Proliferation Histogram

Proliferation histograms are a special subset of one dimensional plots that automatically model proliferation statistics according to the fit parameters you set.

Note: The axis scale 254 of Proliferation histograms should be set to Log.

Prior to inserting a Proliferation histogram for curve fitting analysis, you should gate your cell population on the desired cells. See the proliferation tutorial 1833 for further information.

To insert a Proliferation histogram, use the **Insert→1D Plots→Proliferation** command. If necessary, change the x-axis parameter 309 to reflect the appropriate parameter for the proliferation dye used in the experiment, such as CFSE or CellTrace Violet, for example.

An example of a Proliferation histogram is shown in Figure 26.1.

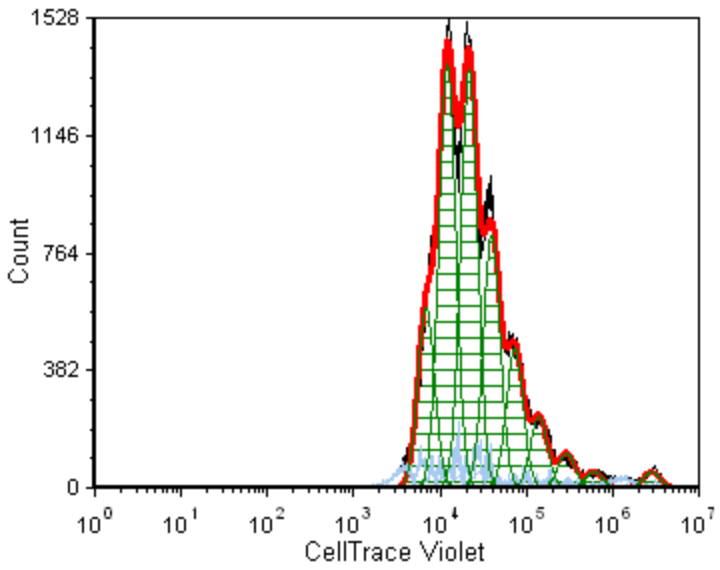

Figure 26.1 Proliferation Histogram

The default colors are explained in the table below.

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Color	Explanation
Black	Overall histogram contour. This includes all the data and does not represent an output of the fitting algorithm.
Red	Overall contour of the fitted data. This excludes what the algorithm determines to be noise.
Light Blue	This is the data that the algorithm determines to be noise, and is not included in the statistics.
Green	Modeled populations of proliferating cells. These are the actual curves fitted to the data, representing the parent, undivided population, and all the proliferating generations.

25.3 Formatting a Proliferation Histogram

In addition to the options common to all histograms 267, Proliferation Histograms have two additional property categories which can be formatted: Fit Parameters 904 and Proliferation Position Markers 906.

25.3.1 The Fit Parameters Option

If the algorithm finds a significant population of events that it determines to be noise (shown in Light Blue on the Proliferation histogram), you can try to modify the **Proliferation Fit Parameters** in order to attempt to improve the model's fit.

To format the fit parameters of a Proliferation histogram:

1. Right-click on the Proliferation histogram and choose **Format** from the pop-up menu.

2. Click on the triangle next to **Proliferation** to expand the category.

3. To change the fit model, select the **Fit Parameters** category (Figure 26.2).

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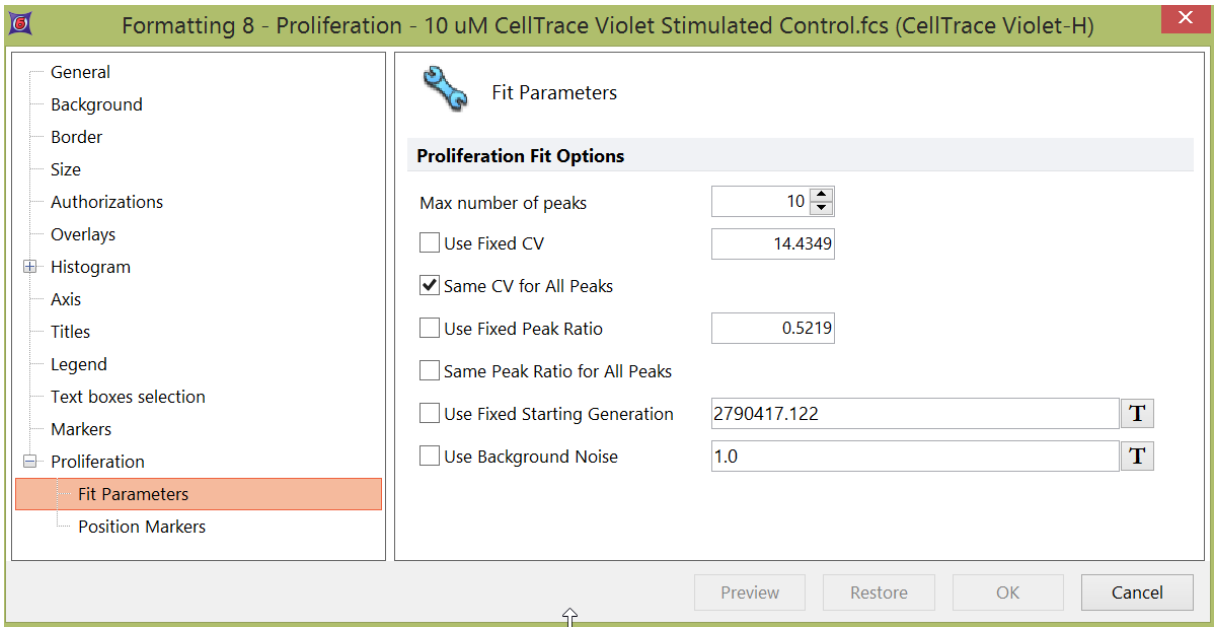

Figure 26.2 Formatting Proliferation Fit Parameters

On the **Fit Parameters** page, you can modify a number of properties related to the fit algorithm, including:

- **Maximum number of peaks** to model.
- **Fixed CV**. Decide whether or not to use a fixed CV (defined by the user) for all peaks. Note that, the value defined in the **Fixed CV** field is actually interpreted by the software as the Standard Deviation of the peaks, calculated, on log-transformed values, after binning.
- **Same CV for All Peaks**. When this option is selected, the CV, calculated on linear-scaled data (i.e. raw data), is made the same between peaks. Note that the CV reported in the Proliferation Statistics table, is calculated on the log-scaled data instead.
- **Fixed Peak Ratio** and/or **Same Peak Ratio for All Peaks**.
- **Fixed Starting Generation**
- **Background Noise**. The values defined in this field is subtracted from the channel values prior to doing any fitting.

The following is a brief description of how the fitting algorithm works, which will help you to set the properties:

- The fitting process starts by determining where the population of cells that have not divided is located. The fitting process attempts to find this peak automatically, although you can specify it manually. In some cases, you have to specify it manually since there are no cells in the population that have not divided.

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- Once the undivided population is found, the model attempts to find populations that have decreased in fluorescence by 50% from each other. Since not all experiments work according to theory, the model starts looking at the 50% point but can move left or right to find the true peak. You can control this by using the **Same Peak Ratio for All Peaks** option.

- In order to find a peak, the model makes certain assumptions about the width of each peak compared to the undivided control. You can also control these settings using the **Same CV for All Peaks** option. When this options is selected, the CV, calculated on linear-scaled data (i.e. row data), is made the same between peaks.

- The model can also subtract background fluorescence from all the calculations, if you provide a value for the background fluorescence.

- Since the algorithm uses the actual histogram contour to model the fit, the histogram data should not be smoothed. Only in special cases, when the original data is very noisy, should a little smoothing be applied to allow the model to better fit the data.

- These options exist in order to accommodate the wide range of data sources and biological behaviors that the model has to encompass. Thus, in order to obtain the best fit, it is important that the correct initial settings be selected. In practice, this is usually performed by trial and error until the settings providing the best fit are found.

- The two ways to assess the goodness of the fit are visual inspection, which gives a rough measure, and the proliferation fit statistics 907.

25.3.2 Proliferation Position Markers

Position Markers can be displayed on Proliferation histograms to mark the position of the undivided cells peak and/or the background region.

To format proliferation position markers:

1. Right-click on the Proliferation histogram.

2. Choose **Format** from the pop-up menu.

3. Click on the triangle next to **Proliferation** to expand the category.

4. Select the **Position Markers** category.

5. To make the position marker for a specific peak, select the peak and check the box for **Show position marker on the plot**.

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Select the **Undivided cells peak** and/or **Background** properties from the list box on the left (Figure 26.3) and choose line options for the selected markers on the right.

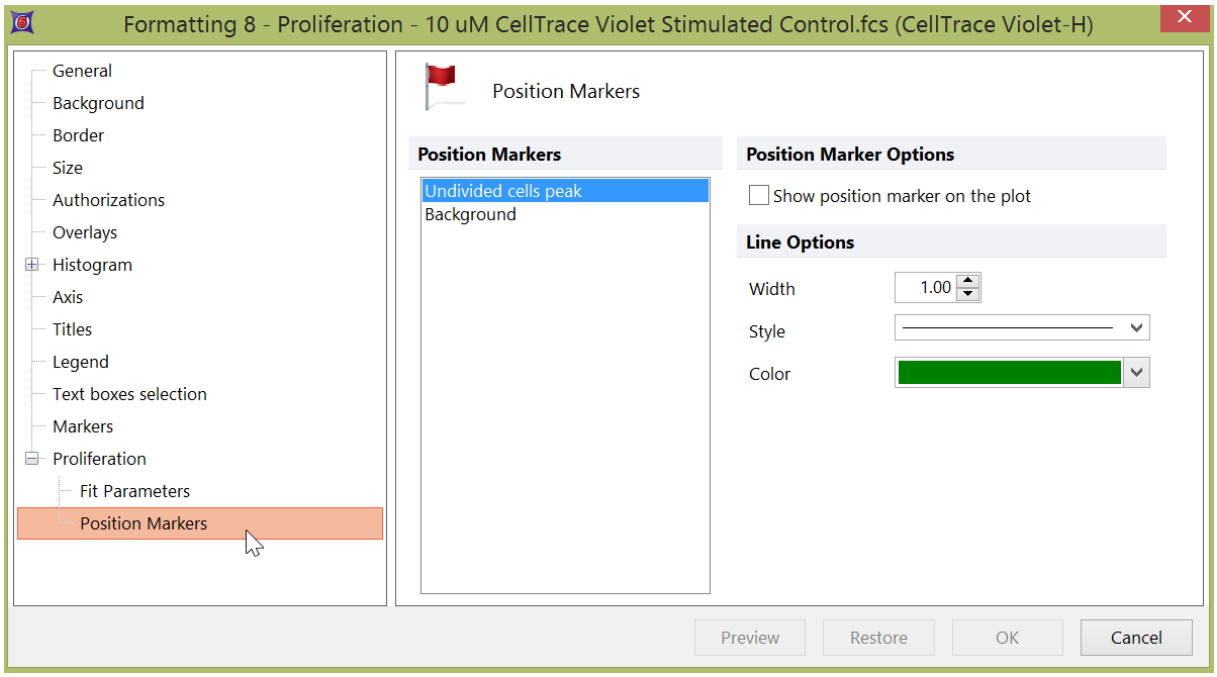

Figure 26.3 Formatting Proliferation Position Markers

25.4 Proliferation Statistics

When performing a Proliferation Analysis, FCS Express automatically calculates several proliferation fit 908 and proliferation population 910 statistics from your data. To change user preferences for which of these statistics are displayed, refer to the Proliferation Fit Statistics preferences 203 and Proliferation Population Statistics preferences 205 sections of the Statistic Preferences 196 sub chapter of Formatting Objects 178.

To open a Proliferation statistics window, right-click on the Proliferation histogram, move the cursor over **Statistics** in the pop-up menu, and select the desired type from the submenu (Figure 26.4).

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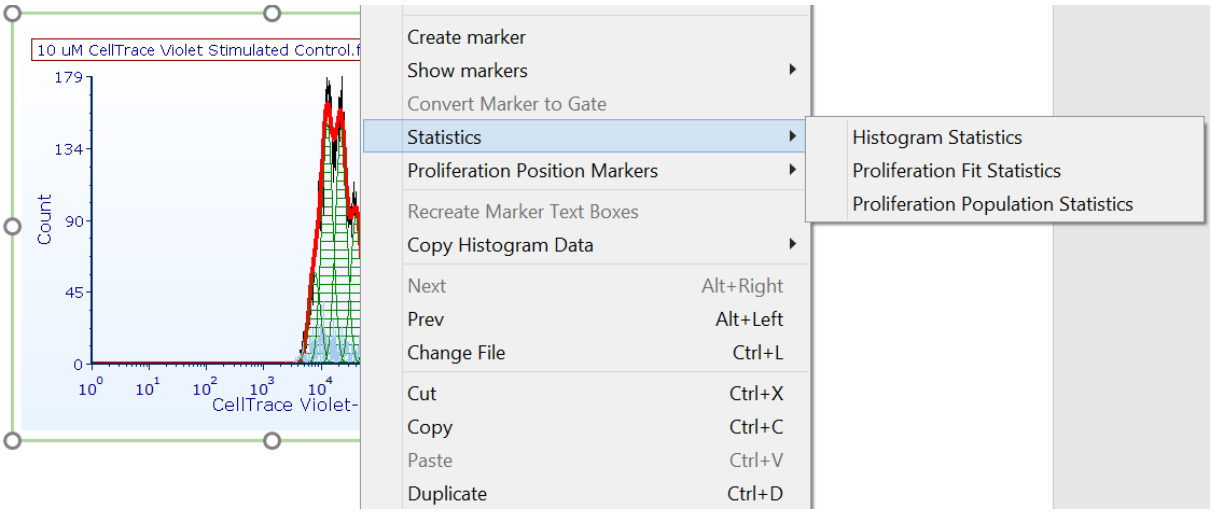

Figure 26.4 Choosing Proliferation Statistics

The first choice, **Standard Histogram Statistics**, represents the statistics available for non-Proliferation histograms 382.

Proliferation Fit Statistics 203 describe the fitted data. Figure 26.5 shows an example of proliferation fit statistics. The statistic equations that are calculated are shown in the table below.

Division Index	Proliferation Index	% Divided	Peak Ratio	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square	Starting Generation Mean	Background Noise
58.39	37.14	62.97	0.51	182173	4899.26	32.87	48.42	2734437.20	0.00

Figure 26.5 Proliferation Fit Statistics

The statistics shown in this window are explained in the table below

P is the total number of peaks found (where *P0* is the undivided generation).
N is the number of cells in a generation.
μ is the mean of a population.

All statistics below assume that no cell death occurred.

Statistic	Equation	Description
Proliferation Index	$\frac{\sum_{i=0}^{P-1} N_i}{\sum_{i=0}^{P-1} \frac{N_i}{2^i}}$	The average number of cells that an initial cell became.

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Division Index	$\frac{\sum_{i=1}^{P-1} N_i}{\sum_{i=1}^{P-1} \frac{N_i}{2^i}}$	The average number of cells that a dividing cell became.
# of Original Cells	$\sum_{i=0}^{P-1} \frac{N_i}{2^i}$	The number of cells that existed prior to any division.
% Divided	$\frac{\sum_{i=1}^{P-1} \frac{N_i}{2^i}}{\text{\# of Original Cells}} \times 100$	The percentage of the original cells that divided.
Peak Ratio	$\frac{\sum_{i=1}^{P-1} \left(\frac{\mu_i}{\mu_i - 1}\right)}{P-1}$	The average ratio of all the peak positions.
# of Fitted Cells	$\sum_{i=0}^{P-1} N_i$	The total number of cells calculated by the fitting.
Degrees of Freedom	N/A	The number of channels minus the number of fit parameters minus 1.
R-Chi Square	$\frac{\sum_{i=0}^{R-1} \left(\frac{O_{(i)} - F_{(i)}}{\sigma(i)}\right)^2}{\text{Degrees of Freedom}}$	An estimate of the "goodness of fit" of the model. Values under 5 indicate a good fit. R is the range of the data. O(i) is the data value at i. F(i) is the fit value at i. σ(i) is the measurement error at i. Measurement error is estimated by comparing a smoothed version (7 point smoothing).

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RMS Error	$\sqrt{\frac{\sum_{i=0}^{R-1}\left(O_{(i)}-F_{(i)}\right)^2}{\text{Degrees of Freedom}}}$	An estimate of the "goodness of fit" of the model. R is the range of the data. O(i) is the data value at i. F(i) is the fit value at i.

Proliferation Population Statistics 205 pertain to the multiple generations that have proliferated during the experiment (Figure 26.6). The statistic equations that are calculated are shown in the table below.

Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells	# of Orig cells	% of Orig Cells
Undivided Cells	3223.90	0.00	0.00	0.00	0.00	0	0.00	0.00
Generation 1	1175.74	4.00	18.07	2.28	0.38	147	73.50	8.78
Generation 2	542.47	4.00	18.07	2.55	0.48	162	40.50	9.67
Generation 3	273.84	8.00	18.07	2.87	0.49	336	42.00	20.06
Generation 4	139.49	9.00	18.07	3.26	0.50	383	23.94	22.87
Generation 5	72.34	9.00	18.07	3.74	0.52	390	12.19	23.28
Generation 6	40.68	5.00	18.07	4.28	0.56	215	3.36	12.84
Generation 7	21.87	1.00	18.07	5.00	0.56	39	0.30	2.33

Figure 26.6 Proliferation Population Statistics

An explanation of the shown in this window is in the table below. Note that all statistics are generation-specific.

Statistic	Description
Peak Channel	The x-value of the peak.
Peak Value	The y-value of the peak.
Log Std. Dev.	The Standard Deviation (Std. Dev.) of the log-scaled data. The Std. Dev. is a measure of the variation from the average for the events, or the square root of the variance. Log values are used due to histogram data being binned in log space.
Log CV	The coefficient of variation (CV) of the log-scaled data. The CV is a measure of the distribution of your data and is equal to the

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	standard deviation divided by the mean. Log values are used due to histogram data being binned in log space.
Peak Ratio	The ratio of the fluorescence to the previous generation's fluorescence. The ideal is 0.5.
# of Cells	The number of cells calculated by the fitting.
# of Orig Cells	The number of original cells (i.e., cells prior to any cell division) giving rise to the cells calculated by the fitting.
% of Orig Cells	The number of original cells expressed as a percent of the total number of original cells.

25.5 Proliferation-Specific Tokens

All of the Proliferation statistics can be used as tokens 692. Proliferation tokens can be inserted into text boxes 692, used in Custom Token 727 or Alert formulas, and exported to reports during batch processing 835.

To insert a Proliferation token into a text box:

1. Insert a text box using the **Insert→General→Text Box** command.
2. Right-click on the text box and select **Insert Token**.
3. Choose **Statistic** and click on the **Insert** button to bring up the **Create Statistic** dialog.
4. Choose a Proliferation Histogram as a **Data Source**.
5. Open the **Statistic** category.
6. Choose either **Proliferation Population Statistics** or **Proliferation Fit Statistics** from the **Select a Statistic** drop-down list. If you choose **Proliferation Population Statistics**, the dialog will look like Figure 26.7. Choose a population from the **Select Population** drop-down list, and one or multiple statistics from the list box, and click **OK**.

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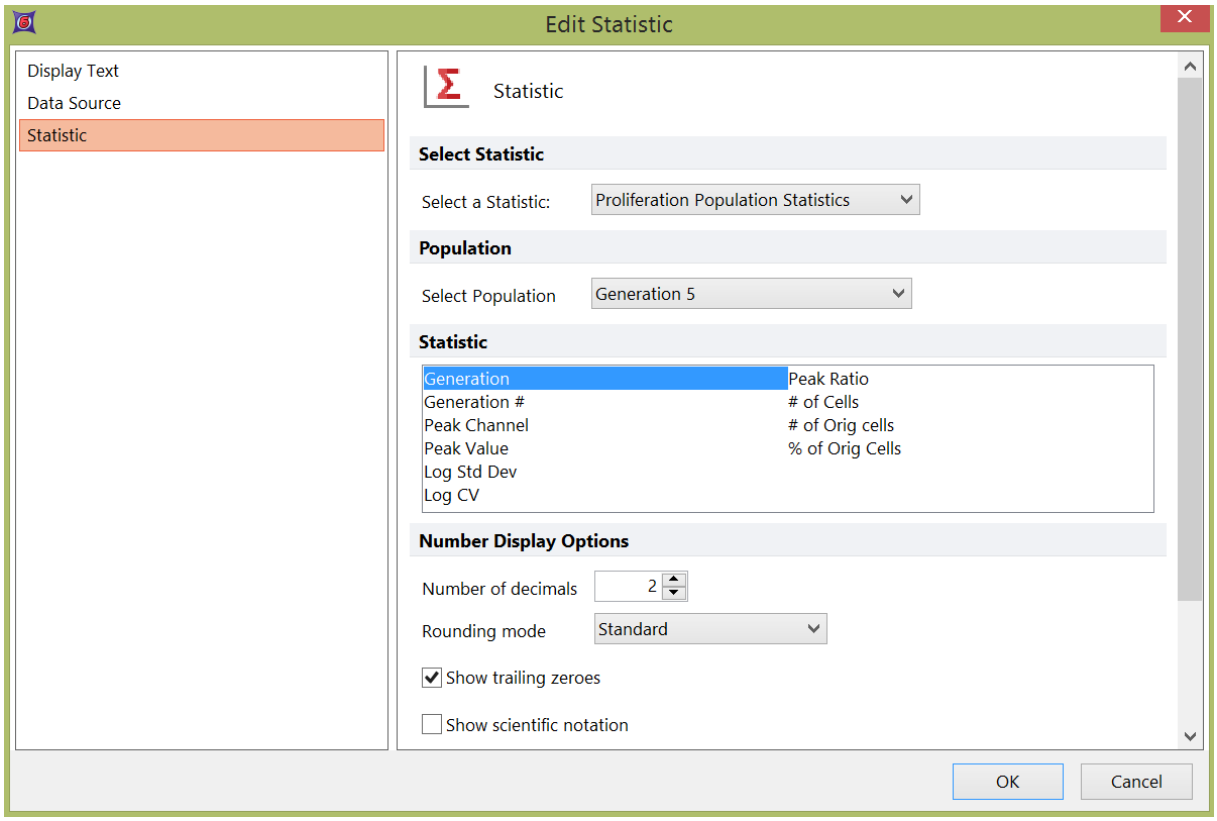

Figure 26.7 Choosing a Proliferation Population Statistic Token

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Chapter

26 Kinetics Analysis

Kinetics experiments are particularly useful for examining changes in DNA content, antibody binding, and calcium flux over time. For example, calcium ions play an important role in cell signaling. The intracellular concentration of calcium ions often show transient changes in response to external stimuli. Several fluorescent dyes can be used to measure bound Ca^{++} by flow cytometry, including Indo-1 (which requires a UV laser) and a combination such as Fluo-3 and Fura Red, which can be used in instruments limited to excitation at 488 nm. Fluo-3 (with maximal fluorescence at 530 nm) increases its fluorescence with increasing Ca^{++} concentration. Fura Red (maximal fluorescence at 650 nm) decreases its fluorescence with increasing Ca^{++} concentration. The ratio of Fluo-3 to Fura Red fluorescence can be used to follow changes in Ca^{++} concentration over time.

26.1 Inserting a Kinetics Plot

In Kinetics analysis, the X-axis value is Time. Cells are usually stimulated by adding one or more drugs after a baseline is established at the beginning of the experiment.

Kinetics plots are a special subset of one dimensional plot that automatically *average* all of the Y-axis values per unit of Time and displays one dot (chosen from Mean, Geometric Mean, Median or % Above Threshold) for each X-axis value (along with the option of displaying the standard deviation of the Y-axis value in color).

To insert a Kinetics plot, use the **Insert→1D Plots→Kinetics** command. If necessary, change the X-axis parameter to Time and format the X-axis range or resolution to suit your experiment (right-click on the histogram, choose **Format** from the pop-up menu, and select the **Axis** page to adjust the range or the **Kinetics Options→Binned Plot Specific Options** category to set the resolution).

An example of a Kinetics plot is shown in Figure 27.1.

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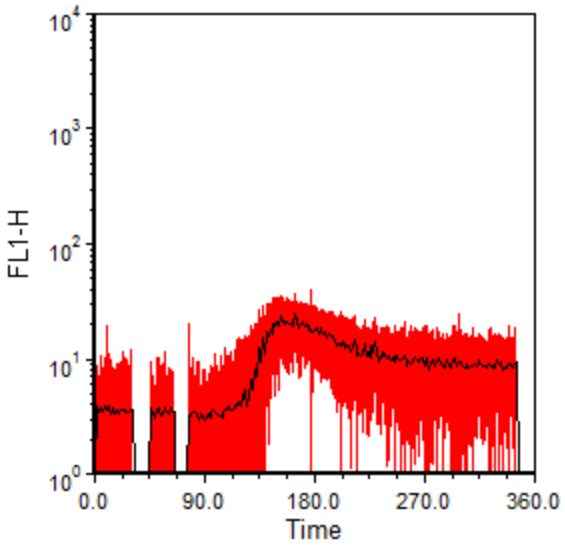

Figure 27.1 Kinetics Histogram (Median FL1-H vs Time). Standard Deviation shown in red.

26.2 Formatting a Kinetics Plot

When formatting Kinetics plots, the Overlays category has important properties which control their appearance. You can format these properties in one of two ways:

- Select the Kinetics plot and use the **Format→Plot Options→Overlays** command.
- Right-click on the Kinetics plot, select **Format** from the pop-up menu, and choose the **Overlays** page (Figure 27.2).

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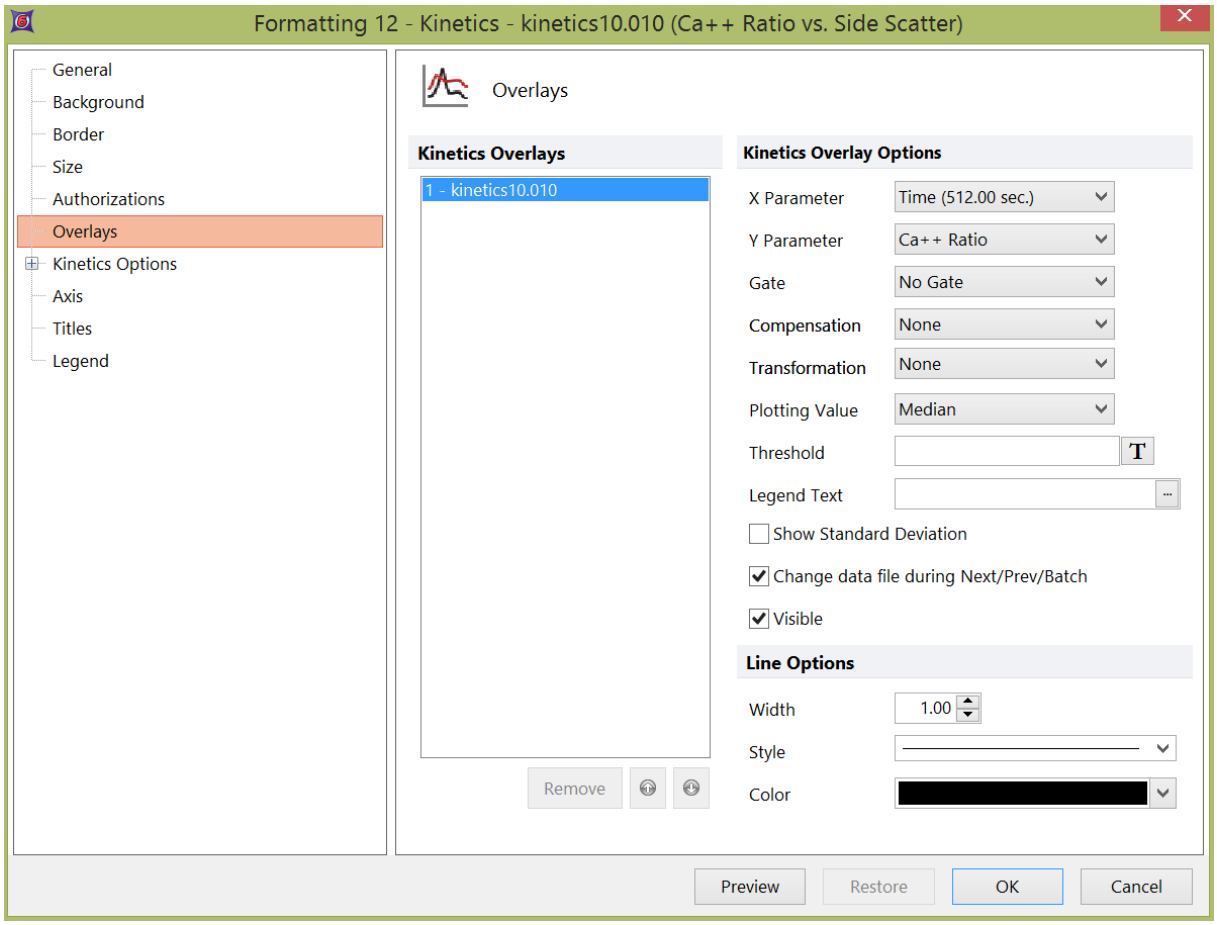

Figure 27.2 Formatting Kinetics Overlays

Choose a **Plotting Value**, which represents either the method used to average the Y-axis values (**Mean**, **Geometric Mean**, **Median, Count**) or the **% Above Threshold**. Count represents events versus Time (i.e., event rate). If there are no problems running the sample, Count should be relatively constant.

Using **% Above Threshold** as a plotting value (Figure 27.3) is useful to determine what percent of the cells responded in the kinetics analysis. A threshold value (set in the **Threshold** field) can either be set manually after determining a cut-off value from the Y-axis or by inserting one or more statistic token(s) into a formula 706.

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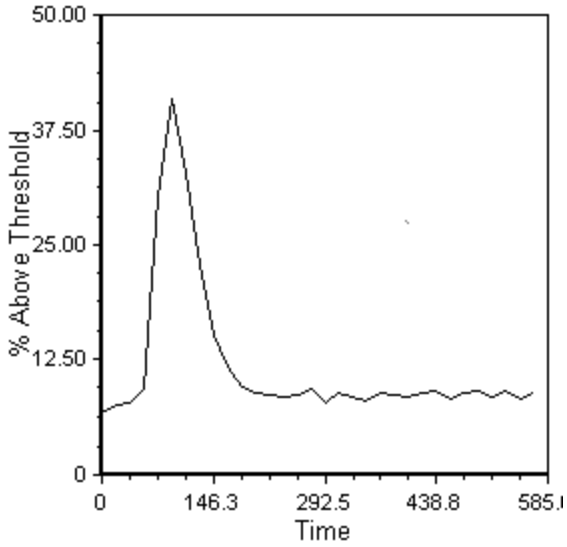

Figure 27.3 Plotting the % Above Threshold Value

The **Show Standard Deviation** check box controls whether or not one standard deviation of the Y-axis value is shown (in red) around the plotted value.

26.3 Kinetics Statistics

To display kinetics statistics in a default statistics table(Figure 27.4), right-click on the Kinetics plot and select **Kinetics Statistics** from the pop-up menu. Kinetics statistics can also be inserted as tokens 918 anywhere on your layout.

Overlay Number	Overlay Legend	Gate	Start Time	End Time	Elapsed Time	Peak Value	Peak Time	Start to Peak Elapsed Time	Start to Peak Slope	Start to End Slope	Rising Phase baseline	Start Rising Phase	Rise to Peak Elapsed Time	Rise to Peak Slope	Area under the curve
1	kinetics10.010	Marker Gate 2	9.14	475.31	466.17	0.64	91.41	82.27	0.01	0.00	n/a	n/a	n/a	n/a	13.92

Figure 27.4 Kinetics Statistics

The default kinetics statistics are explained in the table below. See Formatting Statistics 389 to choose which statistics to display.

Statistic	Explanation
Overlay Number	If there are multiple overlays visible on a plot, the statistics will be calculated for every overlay on the plot. **Overlay #** displays the overlay number for that row of statistics. The overlay number corresponds to the position in the overlay list on the Overlays page of the Format window.
Overlay Legend	The default legend is the file name. The legend can be changed in the Legend Text field of the Overlays page of the Format window.
Gate	The gate for which the statistics apply. This region is the 1D gate on the time parameter.

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Statistic	Explanation
Start Time	The time at the left marker boundary, or the left boundary of the plot if there is no gate defined.
End Time	The time at the right marker boundary, or the right boundary of the plot if there is no gate defined..
Elapsed Time	End Time minus Start Time.
Peak Value	The Y-axis value at the peak.
Peak Time	The X-axis value (Time) at the peak.
Start to Peak Elapsed time	Peak Time minus Start Time.
Start to Peak Slope	The slope from the left marker boundary to the peak, or from the left boundary of the plot to the peak.
Start to End Slope	The slope from the left marker boundary to the right marker boundary.
Rising Phase Baseline	What the algorithm determined was the baseline prior to the rising phase starting.
Start Rising Phase	The time at which the algorithm determined the rising phase started.
Rise to Peak Elapsed Time	Peak time minus Start Rising Phase.
Rise to Peak Slope	The slope from Start Rising Phase to Peak Time.
Area Under the Curve	Area under the curve in units of the Y-axis.

Kinetics statistics can be calculated and displayed for the entire plot, or for gated events. Drawing gates on kinetics plots is advisable when the left boundary of the plot is not a reliable indicator of the baseline (e.g., when the signal is unsteady at the beginning of acquisition, or when a drug is added and the return to baseline is delayed). In such a case, the user may wish to define the baseline by creating a 1D gate 1277 on the *Time* parameter of the Kinetics plot. The left boundary of the 1D gate will define the **Start Time** statistic and the baseline, and the right boundary the **End Time** statistic.

The algorithm is designed to automatically detect the beginning of the rising phase and the peak.

26.4 Kinetics-Specific Tokens

All of the Kinetics statistics can be used as tokens 692. Kinetics tokens can be inserted into text boxes 692, used in Custom Token 727 or Alert formulas, and exported to reports during batch processing 835.

To insert a Kinetics statistics token into a text box:

1. Insert a text box using the **Insert→General→Text Box** command.
2. Right-click on the text box and select **Insert Token**.
3. Choose **Statistic** and click on the **Insert** button to bring up the **Create Statistic** dialog.
4. Choose a Kinetics plot as a **Data Source**.
5. Choose the **Statistic** category.

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6. Choose **Kinetics Statistics** from the **Select a Statistic** drop down.
7. Select the **Gate**, if any, (Figure 27.5). Choose one or multiple statistics from the list box, then click **OK**.

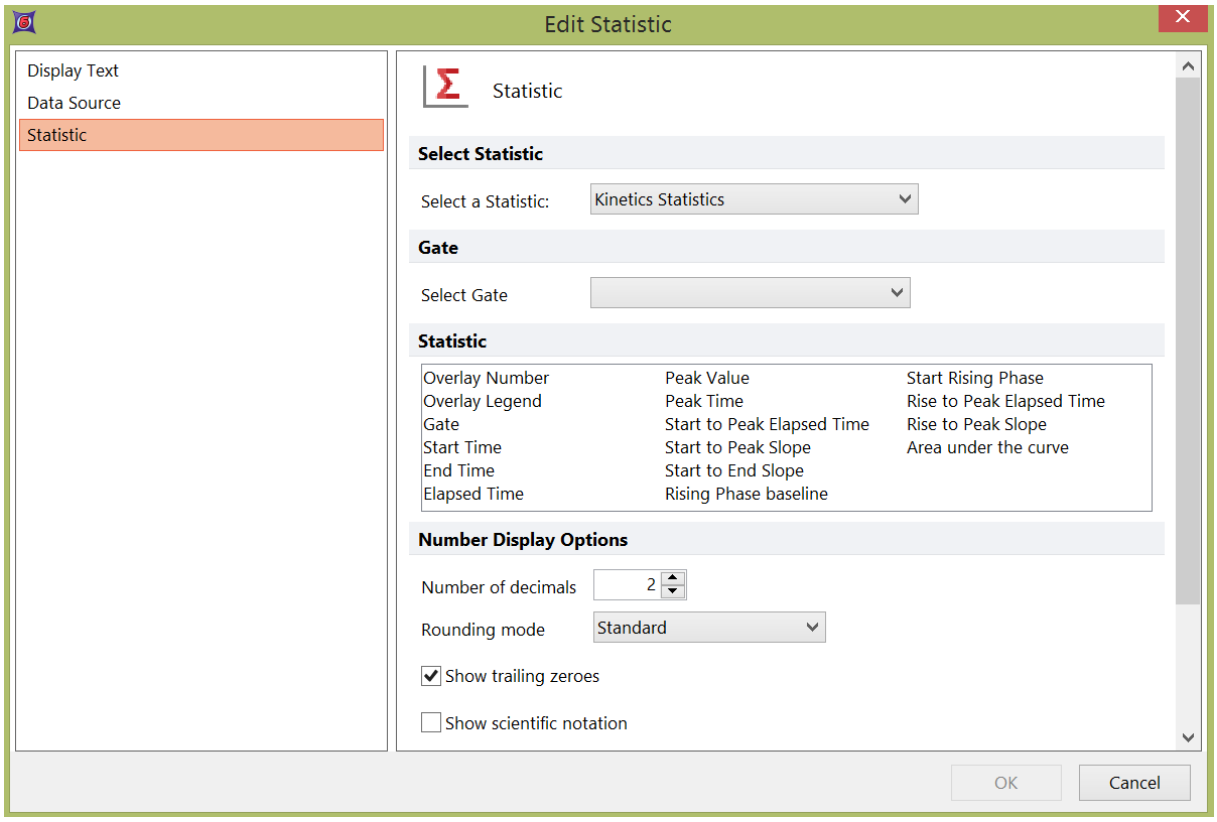

Figure 27.5 Creating a Kinetics Statistics Token

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Chapter

27 Spectrum Analysis

Spectral flow cytometry is used to sample more parameters per single cell or particle than traditional flow cytometry. In contrast to conventional flow cytometers, spectral flow analyzers and sorters substitute mirrors, optical filters, and photomultiplier tubes (PMTs) with a spectrograph and multichannel detector to use fluorescence or Raman spectroscopy.

FCS Express has the capability to read and display raw spectral data from Cytek and Sony instruments 26 through **Spectrum Plots** 922. Spectral data plotted in Spectrum plots links directly the standard flow cytometry single cell measurements to allow seamless transitions between standard flow cytometry analysis and spectral cytometry analysis.

Supported Data Formats:

Sony Instruments:

- Spectral data is stored with the **.SRAW** file format with both an .SRAW and .FCS file being produced. To analyze data from Sony instruments in spectrum plots the .sraw and .fcs files produced by the instrument must be placed in the same source folder. The names of the .SRAW and .FCS files must match.

- **Cytek Instruments:** Spectral data is stored with the standard **.FCS** file format however two files will be produced, an "unmixed" data file and a "mixed" data file. The unmixed data file will have the same name as the mixed data file except it will use a suffix of "Unmixed" in the data file name. For instance:

  sample1.fcs and ***sample1_Unmixed.fcs***

  - The file containing the suffix of "Unmixed" contains the FCS file data that was unmixed via the Cytek system while the file with the standard file name remains mixed.
  - To view spectrum plots for the unmixed data set both data files most be placed in the same source folder with the names matching except for the "Unmixed" suffix.
  - To view spectrum plots for the mixed data set, only the original file without the "Unmixed" suffix must be present.

27.1 Inserting a Spectrum Plot

Spectrum Plots are a special subset of the one-dimensional plot type that displays spectra for all events within a gating strategy as individual lines or an

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average of all events within a gating strategy as one line. Spectral lines for cells or particles are displayed as **Counts** (Y-axis) vs. **wavelength** from 500 nm to 800 nm (X-axis) for the specified laser line. Compensations 626 and/or transformations 932 may be applied to spectra displayed.

Data can be presented in the spectrum plot with one spectrum line per event (**Format→Plot Options→Overlays→Overlays to show→All Spectrums** command) or one line as an average of events (**Format→Plot Options→Overlays→Overlays to show→Average** command). The color of each line will reflect the color of the gate in which the event falls if **Overlay to show** is set to **All Spectrums**. If set to **Average**, the color will be derived from **Line options** 278 color. See Formatting a Spectrum Plot 924 for more information on these and other formatting options.

To insert a Spectrum plot, first ensure that the data files meet the requirements for the data format 922. Then, use the **Insert→1D Plots→Spectrum** command (Figure 28.1).

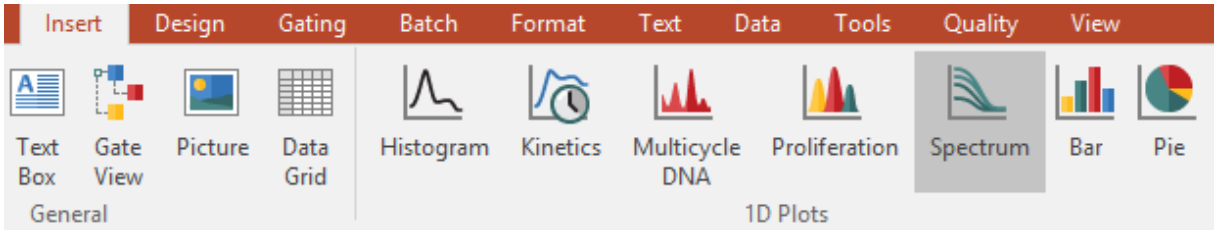

Figure 28.1 - Insert Spectrum Plot command

An example of a Spectrum Plot is shown below in Figure 28.2.

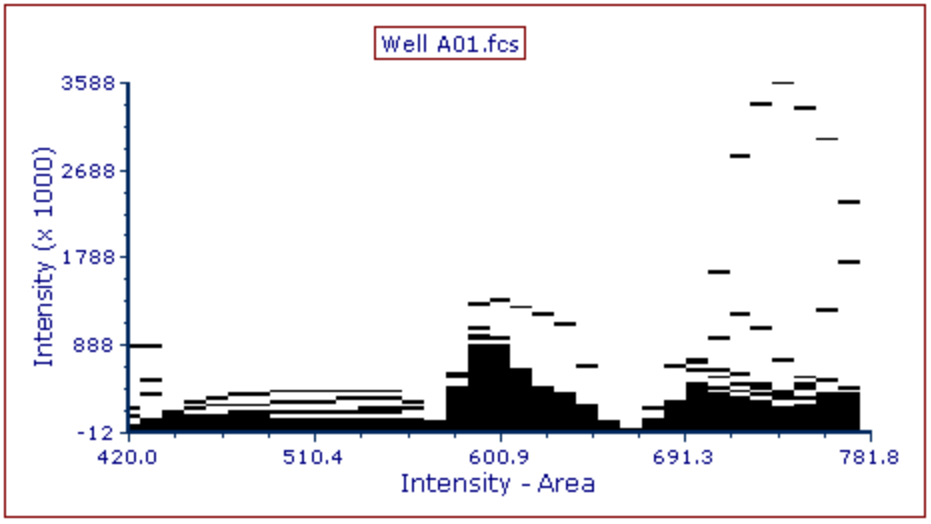

Figure 28.2 - Inserted Spectrum Plot

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Spectrum Plots can be formatted 924 to show interpolated values, gate colors, averages, population density, and more.

27.2 Formatting a Spectrum Plot

Formatting options specific to Spectrum Plots are pictured below and include:

- Plotting **All spectrums** or **Average** (❶), or **Average Showing Standard Deviation** (❷)
- Setting Display Mode to **Color on Gated/Overlays** or **Density** (❸)
- **Interpolating values** with desired number of **Interpolation points** (❹)

To access these formatting options, either

- Select 1216 the Spectrum plot(s) and use the **Format** tab→**Plot Options**→**Overlays** or **Specific Options** command, or

- Right-click on the Spectrum plot, select **Format** from the pop-up menu, and choose the **Overlays** or **Spectrum Plot Specific Options** category (Figure 28.3).

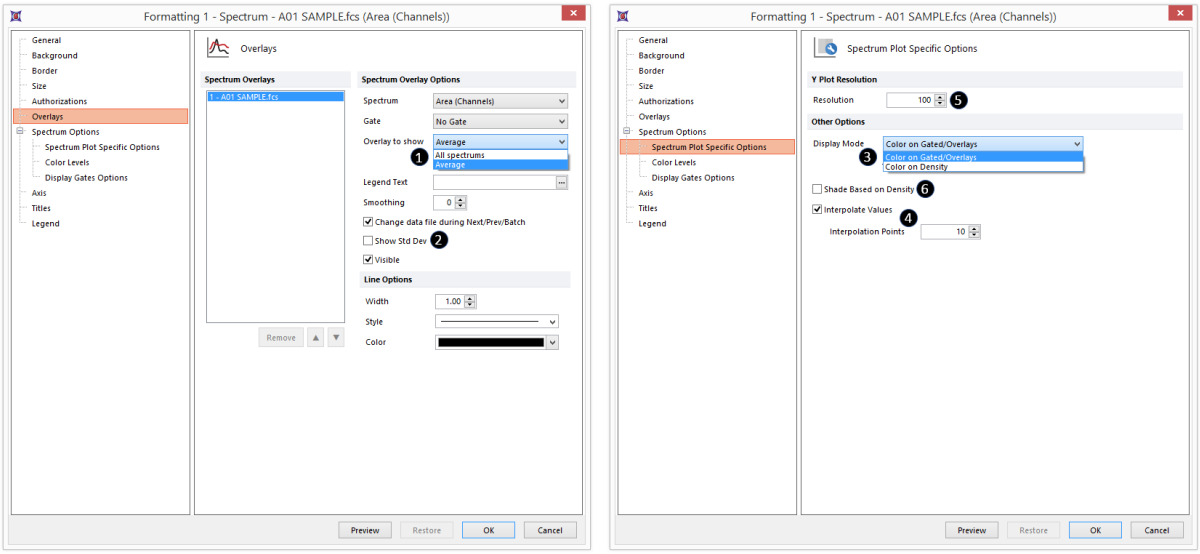

Figure 28.3 - Formatting options specific to Spectrum Plots.

These formatting options specific to Spectrum Plots are detailed in the table below. Standard formatting options for Spectrum Plot overlays are listed here 280 .

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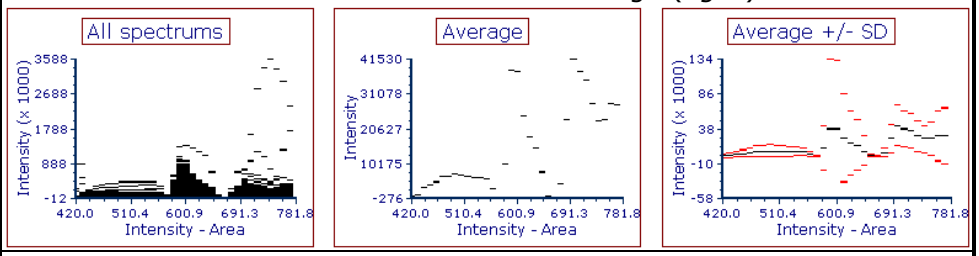
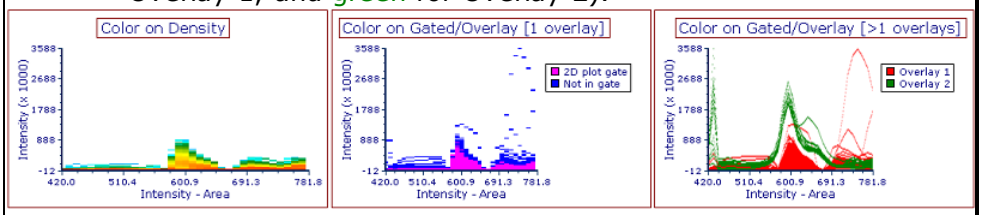
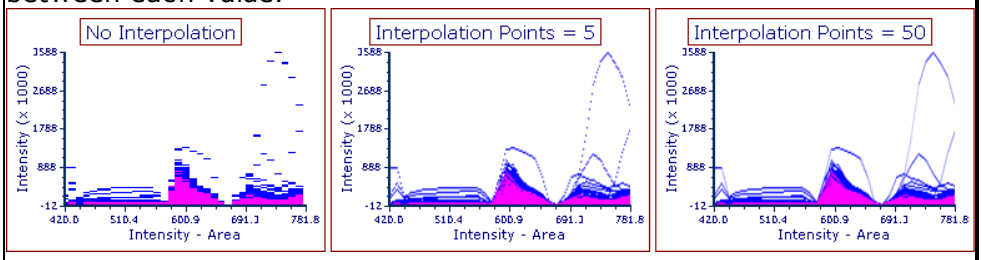
Option	Explanation
Overlay to show ❶	**All spectrums** shows each event as an individual line or dot (*left*); **Average** shows the average of all events as one line or dot (*center*); Average with **Show Std Dev** ❷ additionally plots the Standard Deviation as a red dot or line above and below the Average (*right*). 
Display Mode ❸	The colors in **Color on Density** mode (*left*) reflect population density of events; the colors in **Color on Gated/Overlays** mode (*center* and *right*) reflect the following, depending on the number of overlays 278: • for plots with 1 overlay (*center*), the gate to which each event belongs (only for gates selected in the Display Gates Option 282) (e.g., pink for events in the "2D plot gate" below and the base color of the overlay (e.g., blue, below) for cells not in one of the selected gates). • for plots with >1 overlay (*right*), the overlay (e.g., red for Overlay 1, and green for Overlay 2). 
Interpolate values ❹	FCS Express can **interpolate values** to impart a smoother look to your data, so that each event is depicted as a continuous line rather than an array of discrete values. Below are examples of plots with no interpolation (*left*), and 5 (*center*) and 50 (*right*) **Interpolation Points** between each value.  **Interpolate values** is also applicable to Spectrum Plots with the Color on Density Display Mode:

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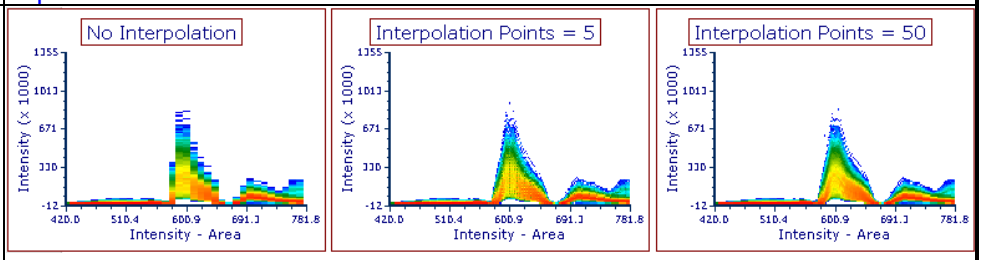
Option	Explanation
	 Please note that interpolation does impact the speed of displaying the plots. Thus, it is recommended to use the lowest interpolation value that provides the desired plot appearance.
Resolution ❺	Increase or decrease the display **resolution,** or number of bins, of the Y-dimension of the plot.
Shade Based on Density ❻	Superimposes a grayscale shading pattern based on the density of plotted data. When gates are displayed on a Spectrum Plot in the Color on Gated/Overlay Display Mode, the shading pattern will be pseudocolored to match the color of the gate displayed. A lighter shade represents a higher density. Note that this option will not apply if multiple overlays are displayed on the Spectrum Plot.

NOTE: In addition, the formatting options shared by all objects 239 and plots 246 exist for Spectrum Plots.

Below are some example of Spectrum plot customizations.

Figure 28.4 displays two Spectrum Plots with the **Display Mode** option set to **Color on Density** (Option ❸ in the table above) and a Spectrum plot showing two Overlays as **Average** (Option ❶ in the table above). Please note that a legend 253 is also displayed in the latter.

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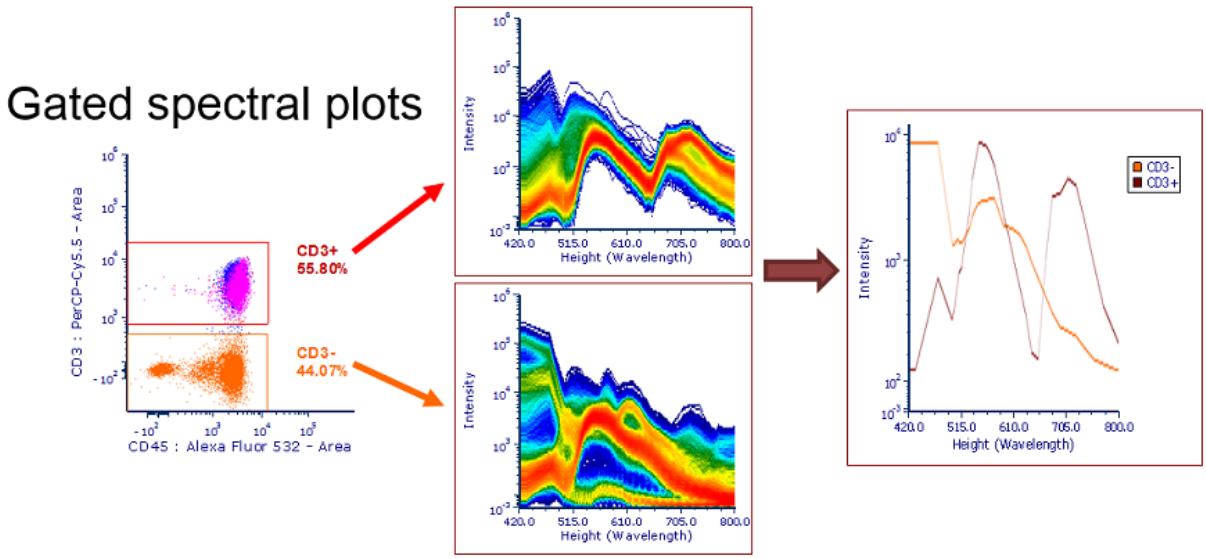
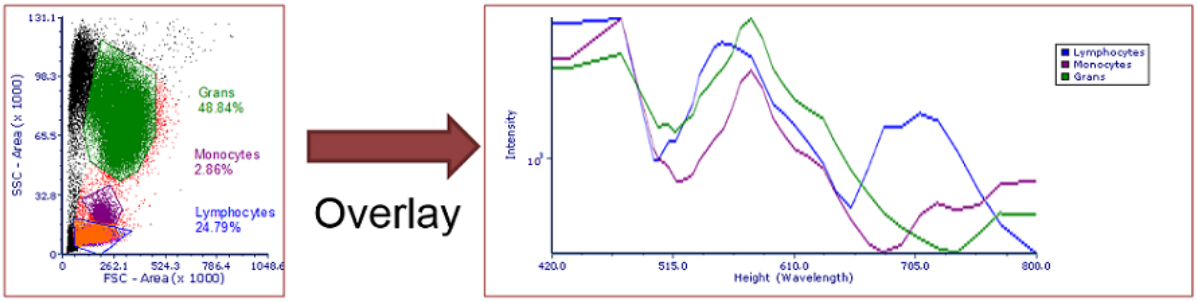


28.4 Example of Spectrum plot customizations.

Figure 28.5 shows a Spectrum plot with three Overlays as an **Average** (Option ❶ in the table above). Please note that a legend[253] is also displayed.



28.5 Example of Spectrum plot customizations.

27.3 Gating and Backgating on Spectrum Plots

Gates may be created on[318] and applied to[352] Spectrum Plots, much like any other plot type in FCS Express.

Gating and backgating each work bidirectionally between Spectrum Plots and other plot types. In other words, gates can be:

- created on Spectrum Plots and applied to[352] / backgated onto[303] other plot types (Figure 28.6)
- created on other plot types and applied to[352] / backgated onto[303] Spectrum Plots (Figure 28.7)

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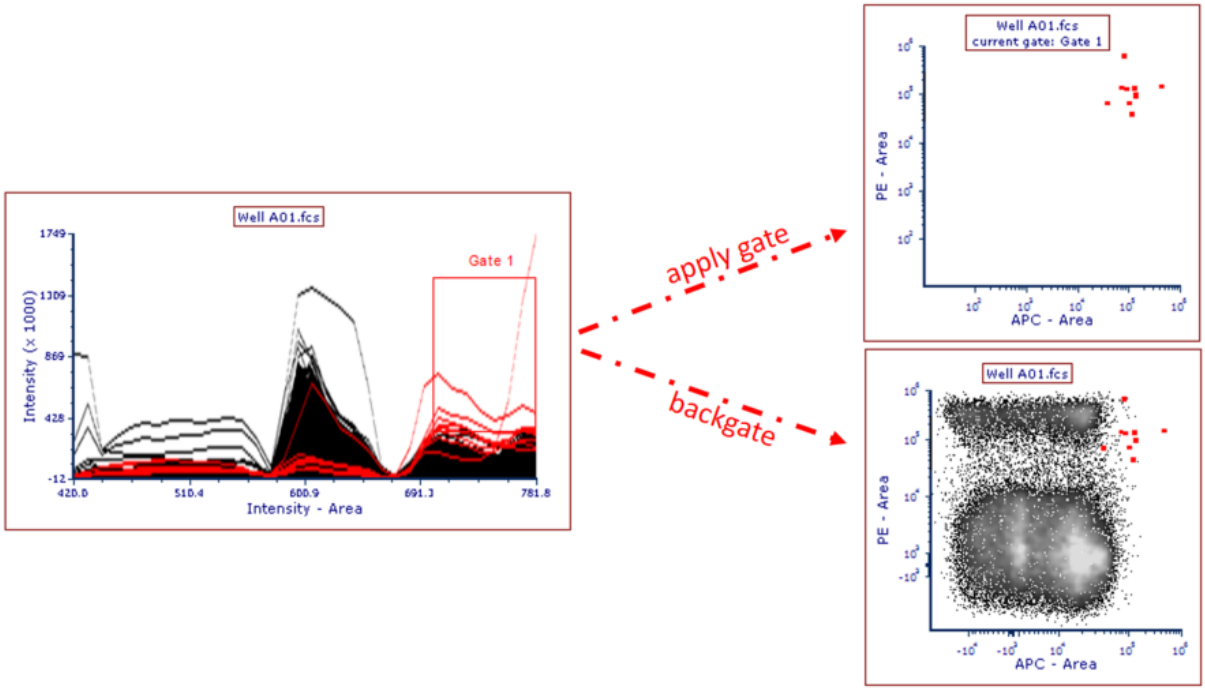

Figure 28.6 - Gate created on Spectrum Plot and applied or backgated onto a Color Dot Plot.

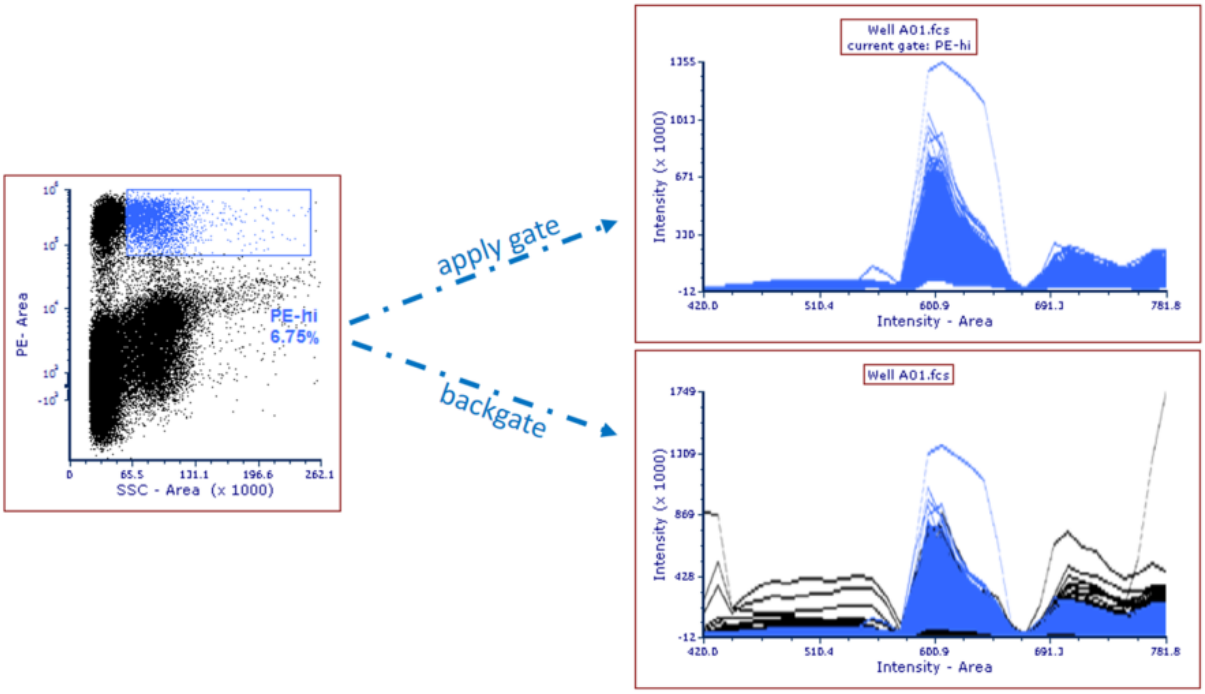

Figure 28.7 - Gate created on Color Dot Plot and applied or backgated onto a Spectrum Plot.

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27.4 Overlaying Spectrum Plots

Spectrum Plots can be overlaid upon one another (e.g., Figure 28.8), in the same way that overlays are added to histograms and other plots in FCS Express. For details on creating and formatting these overlays, see the Overlays subtopic of Formatting 1D Spectrum Plots.

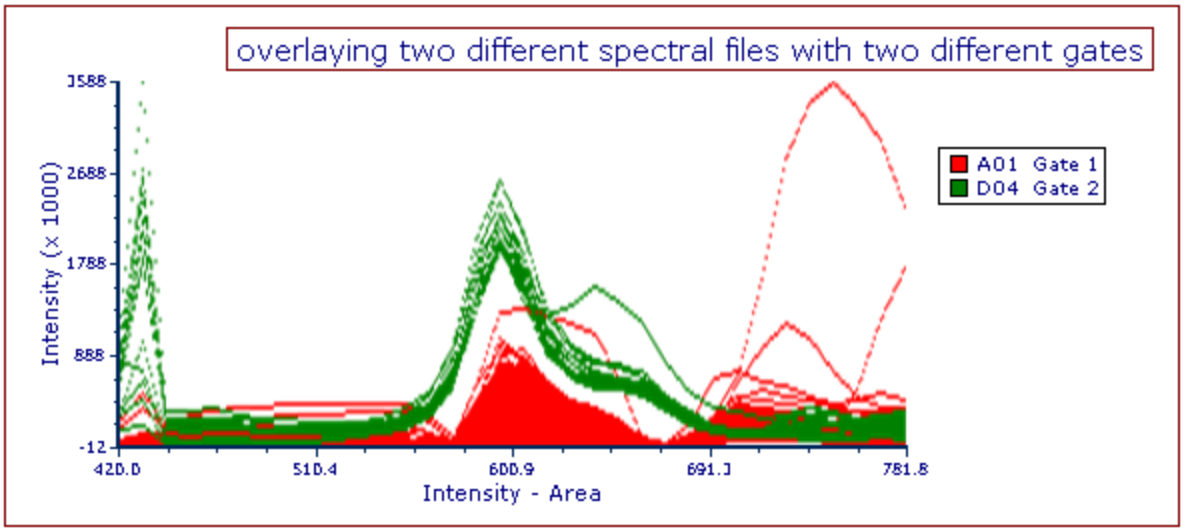

Figure 28.8 - Spectral plot with two overlays from different spectral datafiles, each with a different gate applied.

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Chapter

28 Transformations

The Transformations navigator is access via the **Tools** tab→**Transformations**→**Transformations** button. The transformations navigator (Figure 29.1) will launch and new data transformations may be added by clicking on the down arrow next to the blue plus button. The following transformations are currently available in FCS Express:

- Parameter Math 933
- Principal Component Analysis (PCA) 946
- K-Means cluster analysis 958
- R Integration 973 for adding new parameters 982 and cluster transformations 990
- tSNE transformation 1000
- SPADE 1022
- Please also note our sections on Merging / Concatenating files 660 which is often used for tSNE and SPADE data sets.

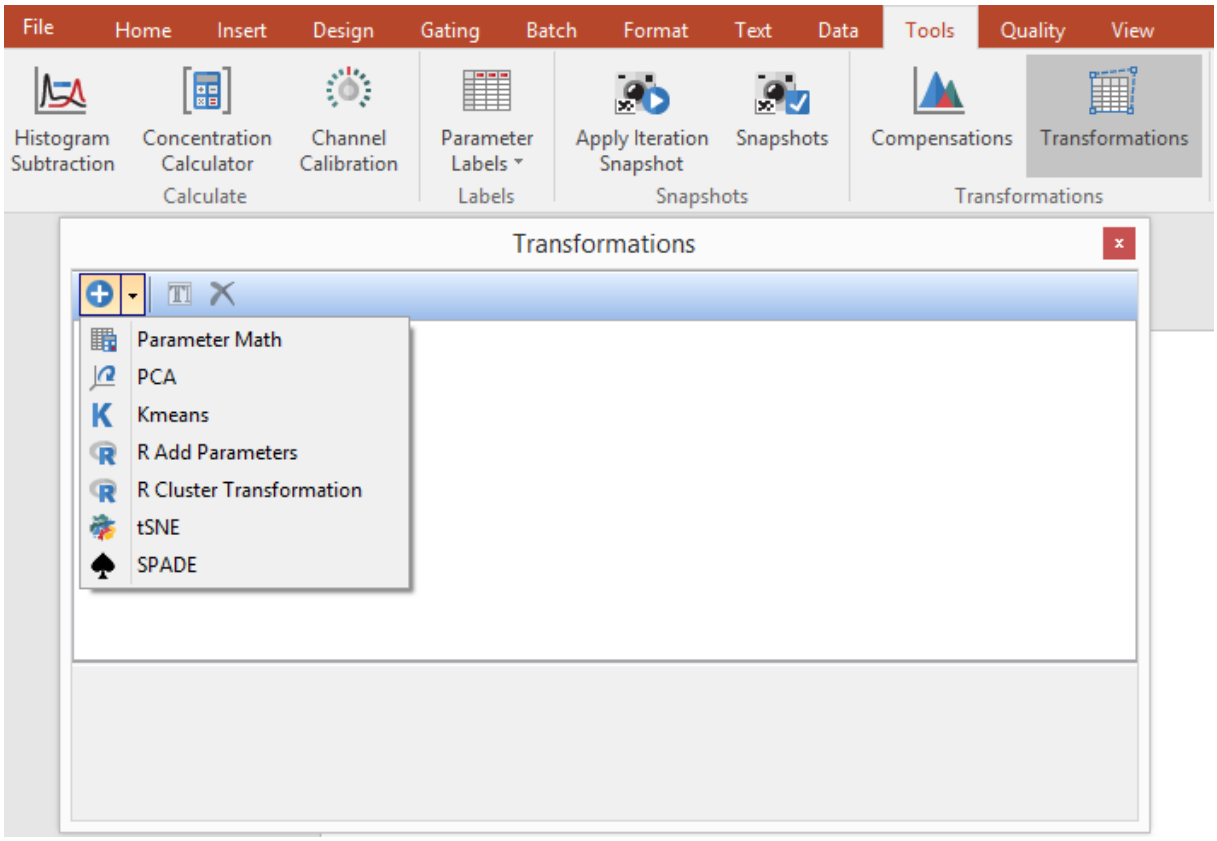

Figure 29.1 - The transformations navigator in FCS Express. Clicking on the down arrow next to the blue plus button allows access to a variety of transformations.

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28.1 Parameter Math

Parameter Math is a tool that allows you to create new parameters or change current parameters, based on mathematical combinations, or formula sequences, of existing parameters for a data file.

Continue to:

- **Defining Parameter Math** 933
- **Applying Parameter Math** 941

28.1.1 Defining Parameter Math

Formula sequences may be single calculations or a series of calculations. There may be situations where you want to perform a series of parameter math calculations. Although it is possible to perform many calculations in a single formula 933, sometimes it is necessary to store and plot the intermediate results of a calculation, which makes parameter math sequences very useful.

To create new parameter math, complete the following commands:

1. Select the **Tools→Transformations→Transformations** command to open the Transformations dialog.
2. In the **Transformations** dialog, click the down arrow to the right of the blue plus to open the drop down menu and select **Parameter Math** (Figure 29.2).

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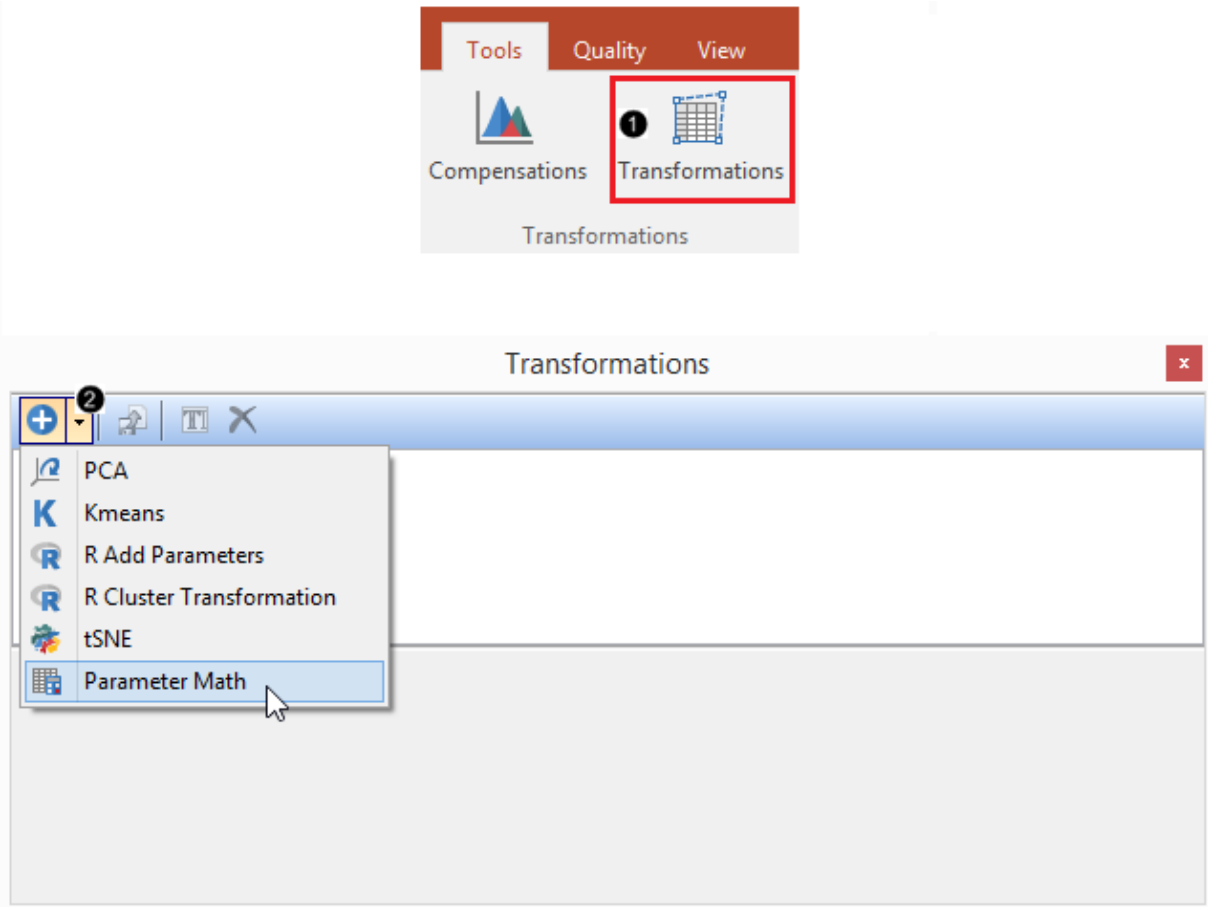

Figure 29.2 - Transformation command on ribbon and Transformation Dialog with Add New drop down menu displayed

If no plots are inserted into the layout when adding new parameter math, a **Template File** will need to be selected by clicking the ... button (outlined in red in Figure 29.3 below) to open the **Select data file** dialog. This button will also need to be used if plots are inserted and a different file is preferred to be used rather than the selected file. If preferred, a gate may also be selected in this dialog by selecting the gate of interest from the **Gate** drop down list. Parameter math will only be performed on cells that are accepted by the gating formula listed. The calculation will be performed on all cells in the file if **No Gate** is selected from the drop down list.

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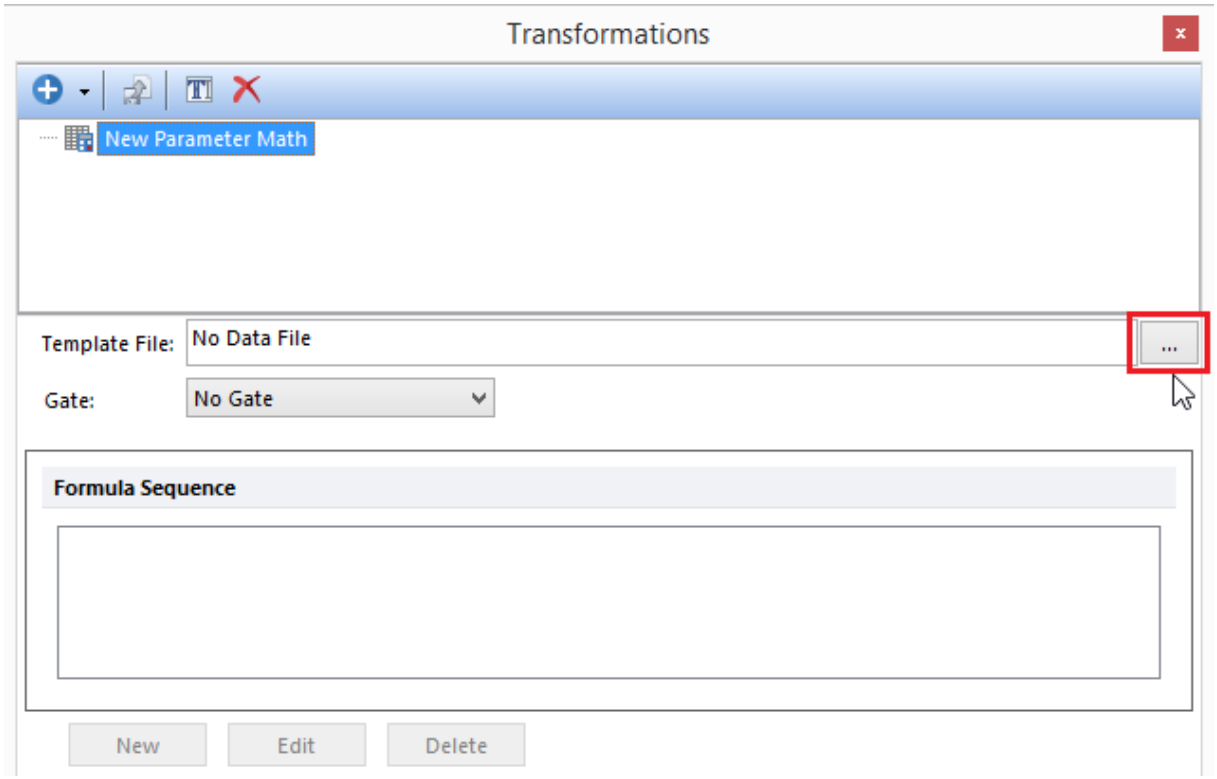

Figure 29.3 - Select Template File from Transformations dialog

3. Once the **Template File** and **Gate** have been selected, click **New** to access **Create New Sequence Item** dialog (Figure 29.4).

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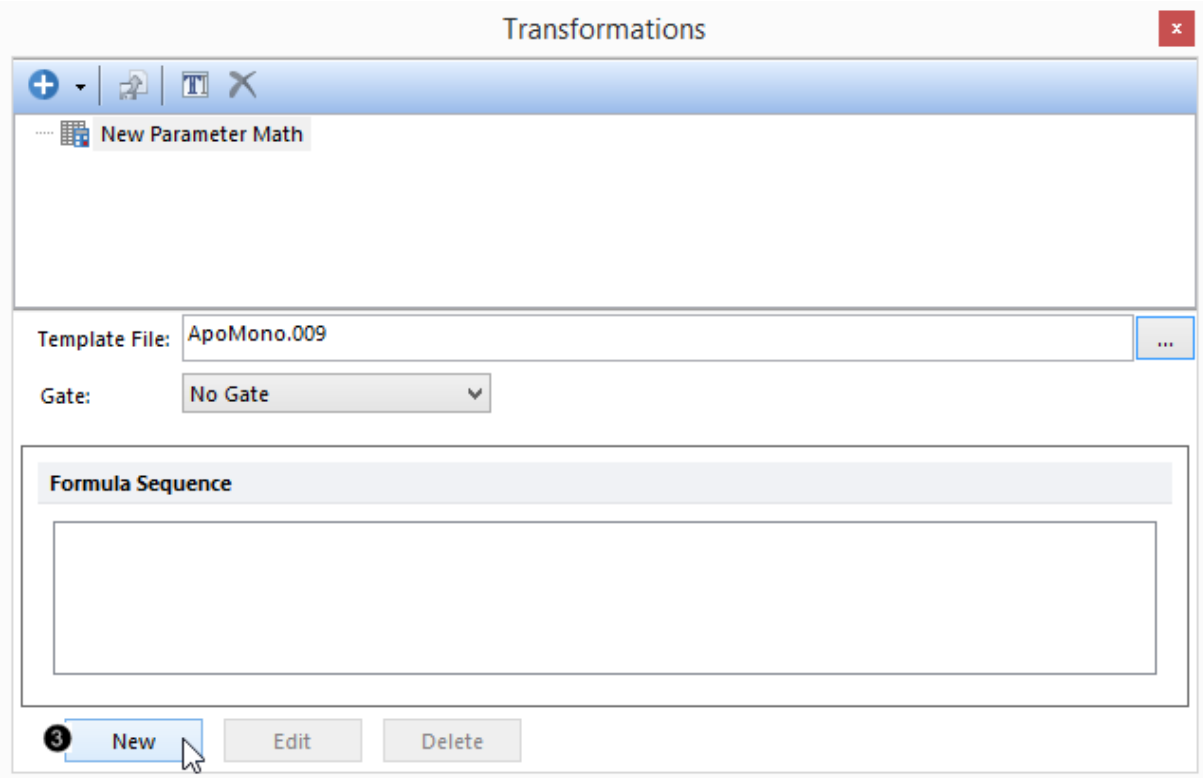

Figure 29.4 - Transformations Dialog with Template File Selected

The **Output Parameter** field controls where the result of the calculation will be stored. It can either be **New**, in which case a new parameter will be created, or you can select a currently existing parameter, which will overwrite that parameter (Figure 29.5 below). If you are creating a new parameter, you can enter the name of the new parameter in the **Output Parameter Name** field.

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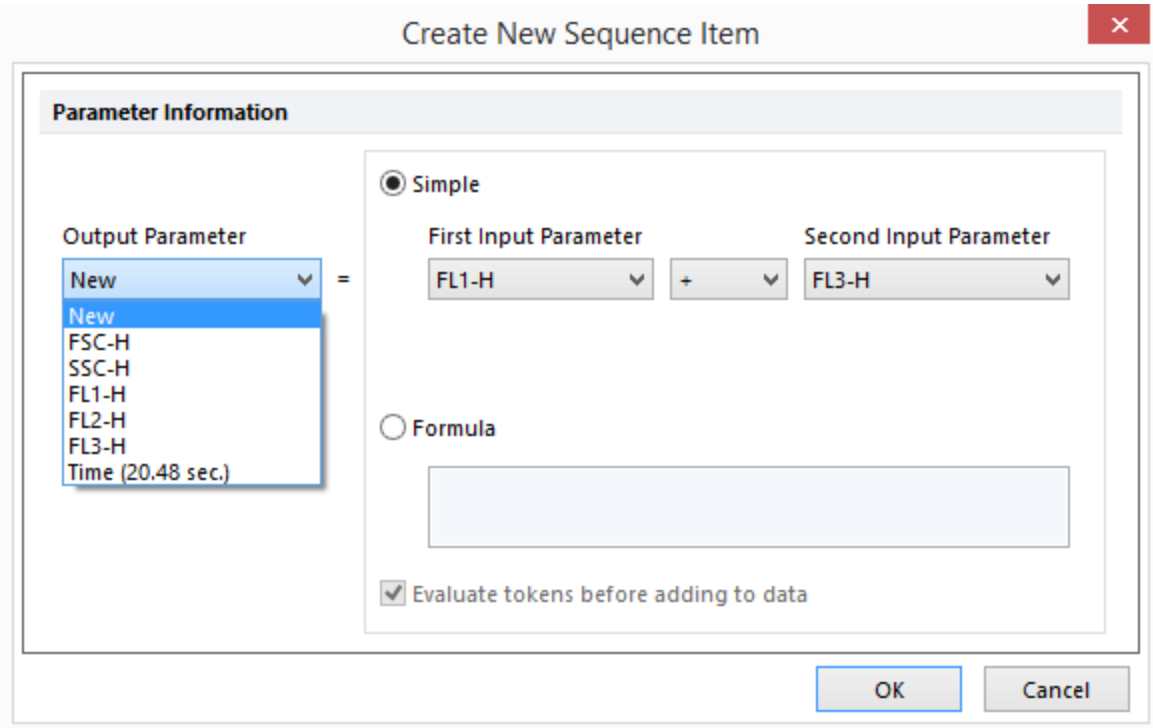

Figure 29.5 - Output Parameter drop down list in Create New Sequence Item dialog

There are two ways to set up a Parameter Math calculation: **Single** or **Formula**. Click on the appropriate radio button to select either **Simple** or **Formula** for parameter math entry.

For typical calculations, the Simple approach is usually easiest. Select the **First Input Parameter**, followed by the mathematical operation [only addition (**+**), subtraction (**–**), multiplication (*****), and division (**/**) are supported], and then the **Second Input Parameter**. The **Second Input Parameter** can also be a constant, in which case an entry field will appear below the **Second Input Parameter** allowing you to enter the constant value.

A more complicated but flexible way to set up a Parameter Math calculation is to enter a **Formula** directly. The formula has the advantage that you can combine multiple parameters, including regular and custom tokens 692, along with different calculations, in a single formula. Formulas are entered as mathematical equations. Each parameter is represented by Pn, where "n" is the parameter number. Some valid formulas are:

- P1+P2
- (P1+P2)/(P4-100)
- sin(P3)/cos(P4)

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Functions can also be inserted in the Formula. To enter a token or a function into your equation, right-click on the **Formula** field and choose **Insert Token** or **Insert Function** from the pop-up menu (Figure 29.6).

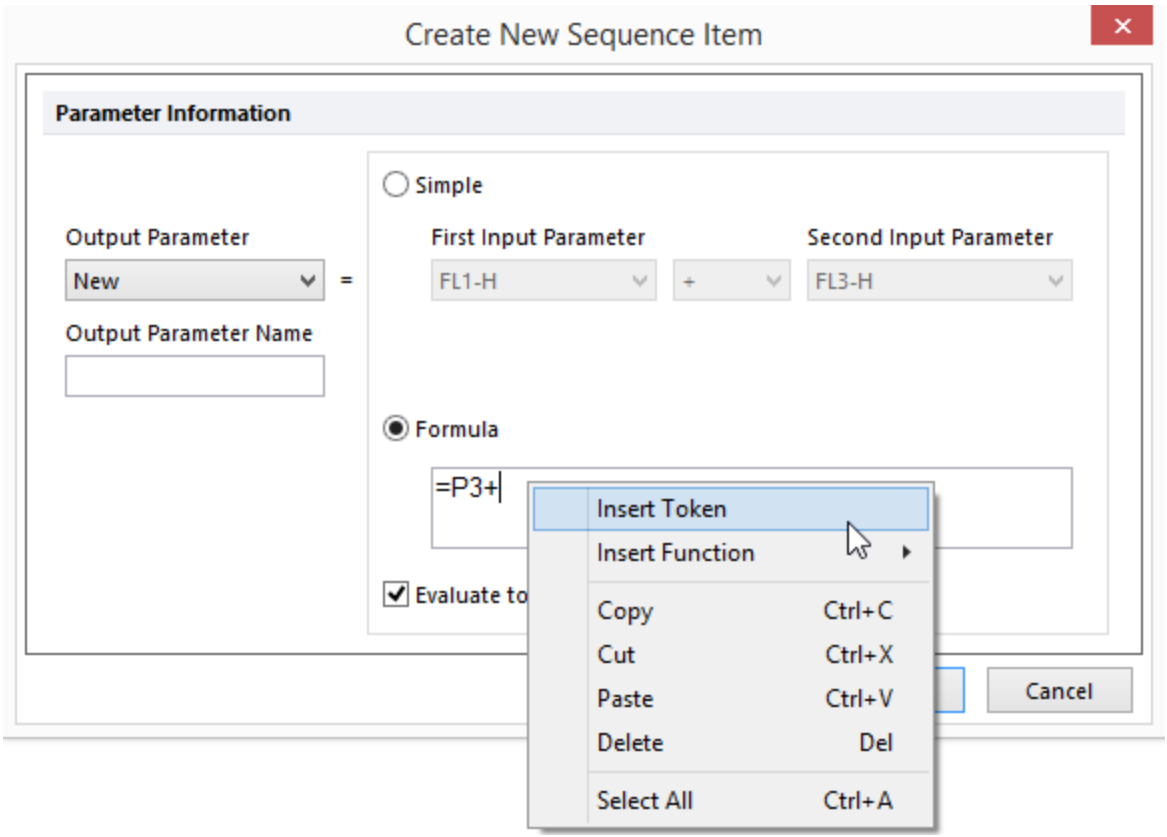

Figure 29.6 Inserting a Token into a Parameter Math Formula

In addition to inserting tokens, you can use the word *Row* as a special keyword in Parameter Math formulas. Row refers to the cell number column in a data grid[680]. For example, the formula *P1+Row* would add 1 to the P1 parameter for the first cell, 2 for the second cell, and so on. The *Row* keyword is also useful for creating parameters with increasing values, such as a time parameter.

The **Evaluate tokens before adding to data** checkbox controls whether or not tokens added to the formula will remain as tokens in the resulting data parameter. If that box is checked (the default), you will only see a single, calculated result in the new parameter column in a data grid. If it is unchecked, the value of the individual components of the formula (e.g., tokens or special keywords) will appear separately within the data grid cell, so that you can see the contribution of each component.

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A comprehensive set of all formula operators and functions 769 is available. This available set is the same for Custom Tokens.

If the parameter math calculation uses a simple calculation, or if the calculation uses a formula and the checkbox **Evaluate tokens before adding to data** is checked, then the results of the operation will be stored as floating point numerical values. If the parameter math calculation uses a formula, and the checkbox is unchecked, then the results will be stored as a formula.

Any final results that are above the maximum channel value in the FCS file are either truncated to the maximum value or stored without truncation, depending on the setting of the Math Range Checking option on the General page of the FCS Express User Options dialog 180.

4. To create the new sequence item, click the **OK** (Figure 29.7).

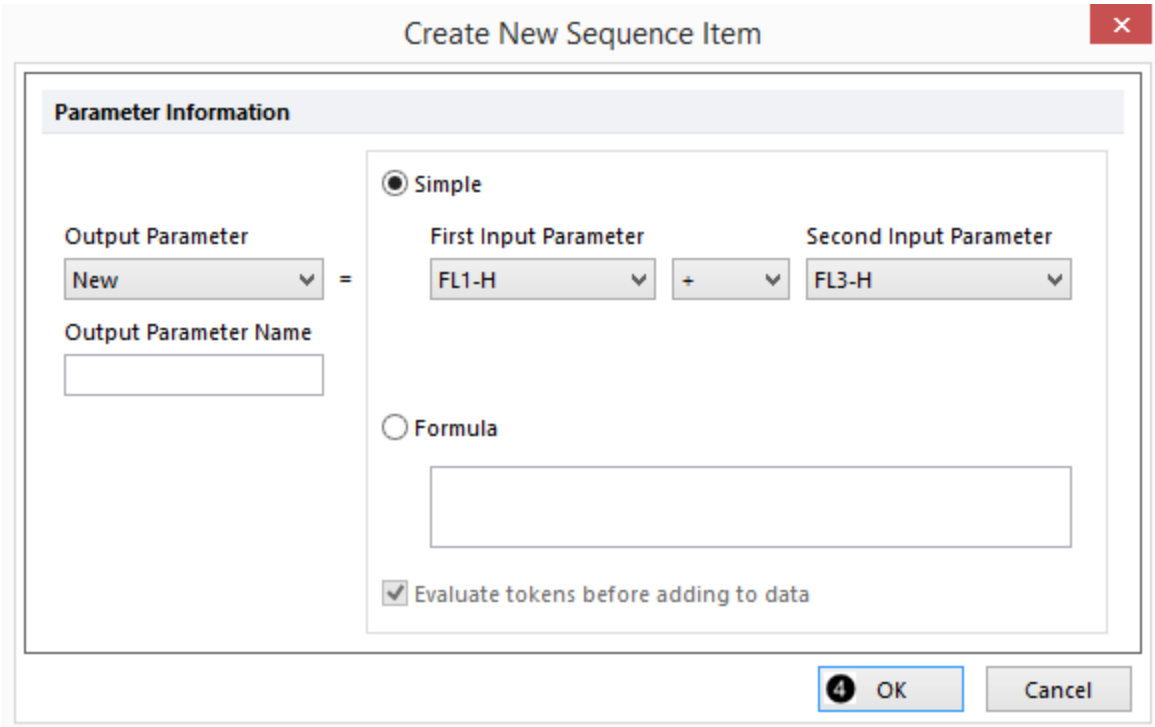

Figure 29.7 - Finish calculation creation in Create New Sequence Item dialog

It will now appear in the **Formula Sequence** box (outlined in red in Figure 29.8 below) of the **Transformations** dialog.

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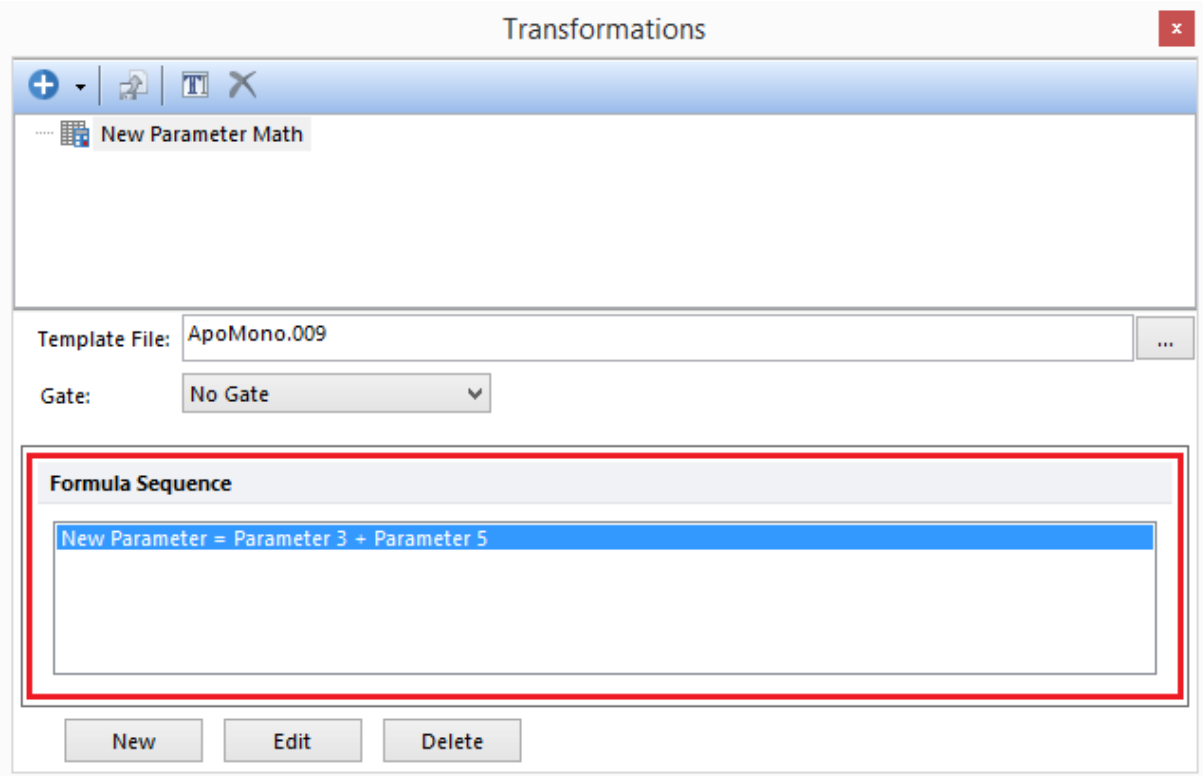

Figure 29.8 - Newly created calculation added to Formula Sequence box of Transformations dialog.

You can delete a sequence: select it in the Formula Sequence box and click the **Delete** button. To edit the sequence, select it and click the **Edit** button. These commands may be accessed by right-clicking within the Formula Sequence box as well. You may also save or load a sequence by right-clicking within the Formula Sequence box. See right-click menu below in Figure 29.9.

After adding or selecting **Parameter Math** in the **Transformations** dialog, the right-click in **Formula Sequence** box→**Save Sequence to File** command brings up a standard dialog to save your Parameter Math Sequence (*.fcf).

After adding or selecting **Parameter Math** in the **Transformations** dialog, the right-click in **Formula Sequence** box→**Load Sequence from File** command brings up a standard dialog to load a saved sequence.

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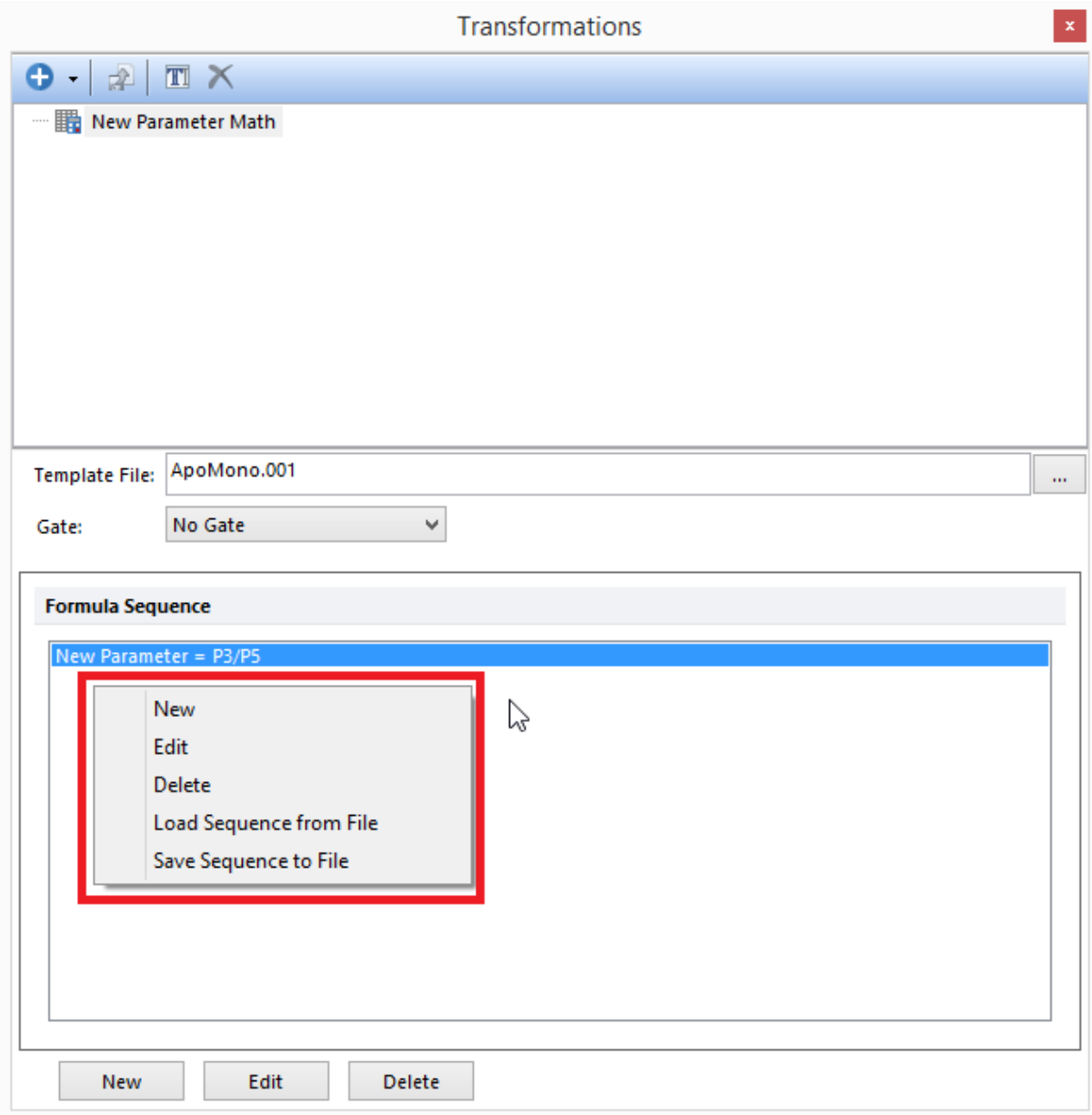

Figure 29. 9 - Right-click menu for Formula Sequence box of Parameter Math in Transformations dialog

28.1.2 Applying Parameter Math

The new parameters derived by Parameter Math can now be applied to any plot or data grid in FCS Express, and they can be chosen from the list of available parameters (Figure 29.10 below). Please note only one transformation may be applied at a time for each plot or data grid selected.

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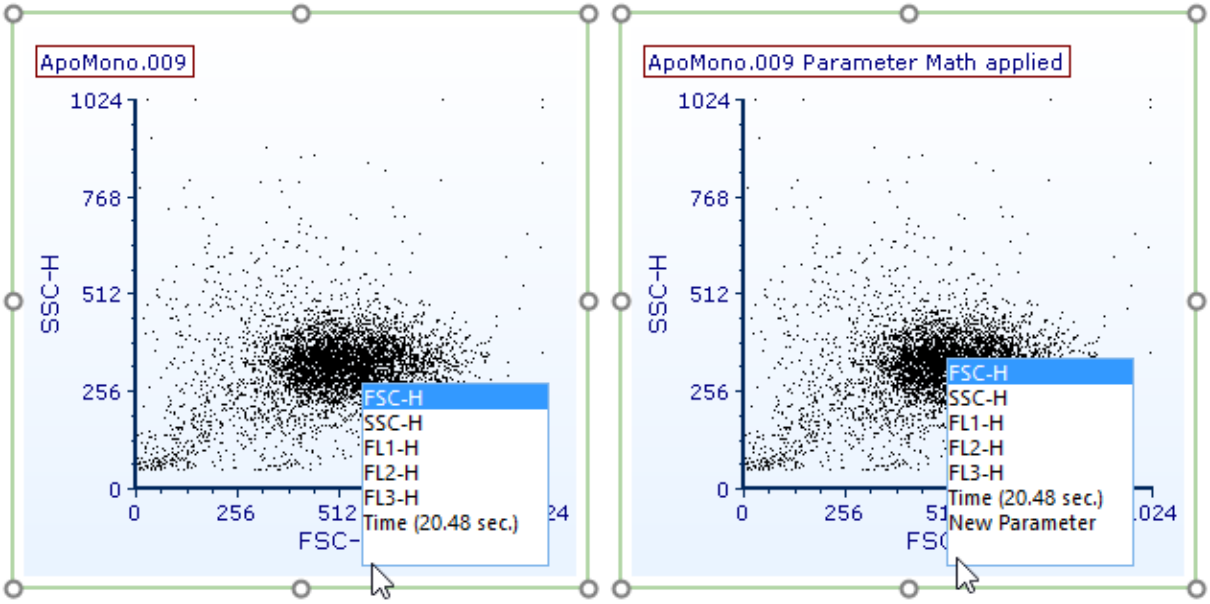

Figure 29.10 - Parameters created in Parameter Math can be accessed as standard parameters

Once parameter math has been used to define new parameter(s), it may be applied to any plot and data grid in FCS Express. Standard gating and statistics may be used to study the result of the parameter math.

Note: When Parameter math has been applied, the text "**Parameter Math applied**" will appear in the plot title.

Parameter math may be applied to 1D and 2D plots by either of the following methods:

- Drag and drop a parameter math from the Transformations navigator to the intended plot (Figure 29.11).

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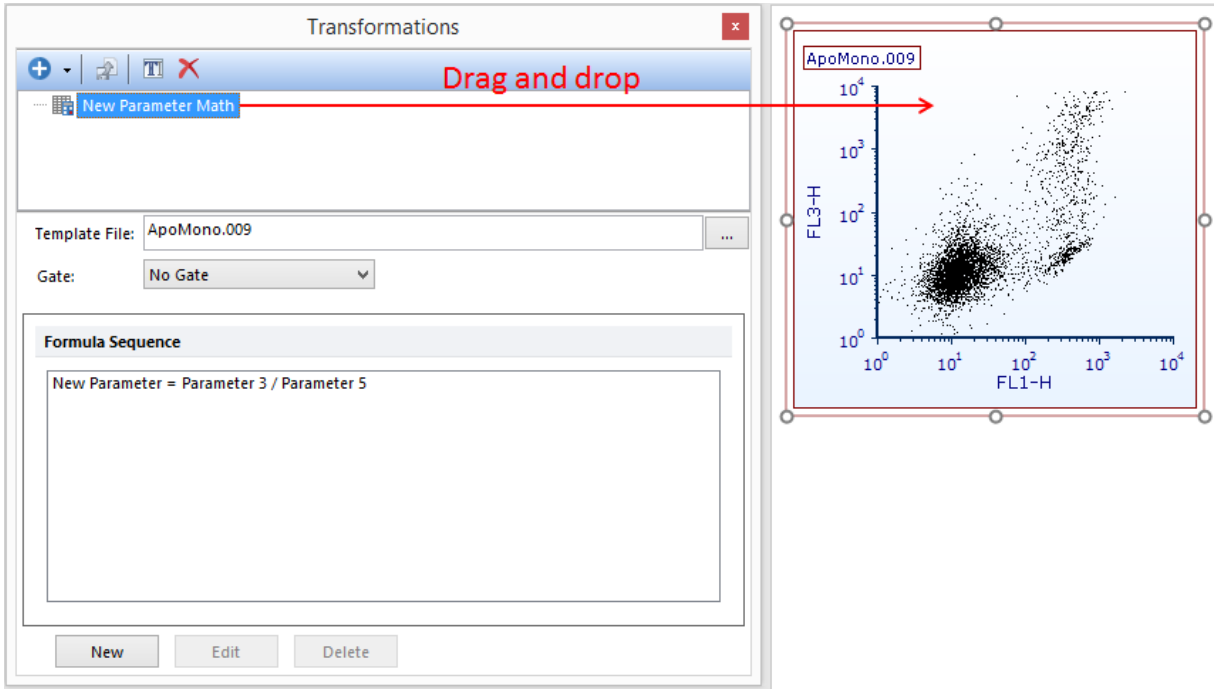

Figure 29.11 - Applying parameter math to a plot by dragging from the Transformations navigator to the plot (red arrow).

- Access the Formatting Overlays dialog 284 and choose the parameter math to apply from the **Transformation** drop down list (Figure 29.12).

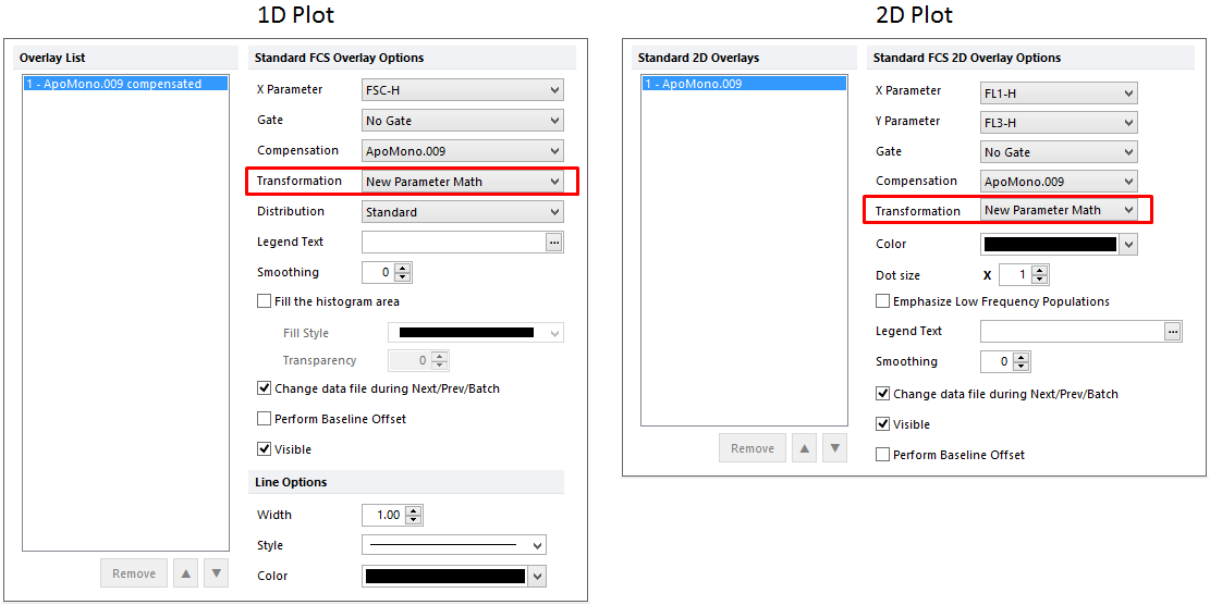

Figure 29.12 - Applying parameter math to 1D (left) or 2D (right) plots by selecting the parameter math in the Transformation drop down from the Formatting Overlays dialog.

Parameter math may be applied to data grids by any of the following methods:

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- Drag and drop a parameter math from the Transformations navigator to the intended data grid (Figure 29.13).

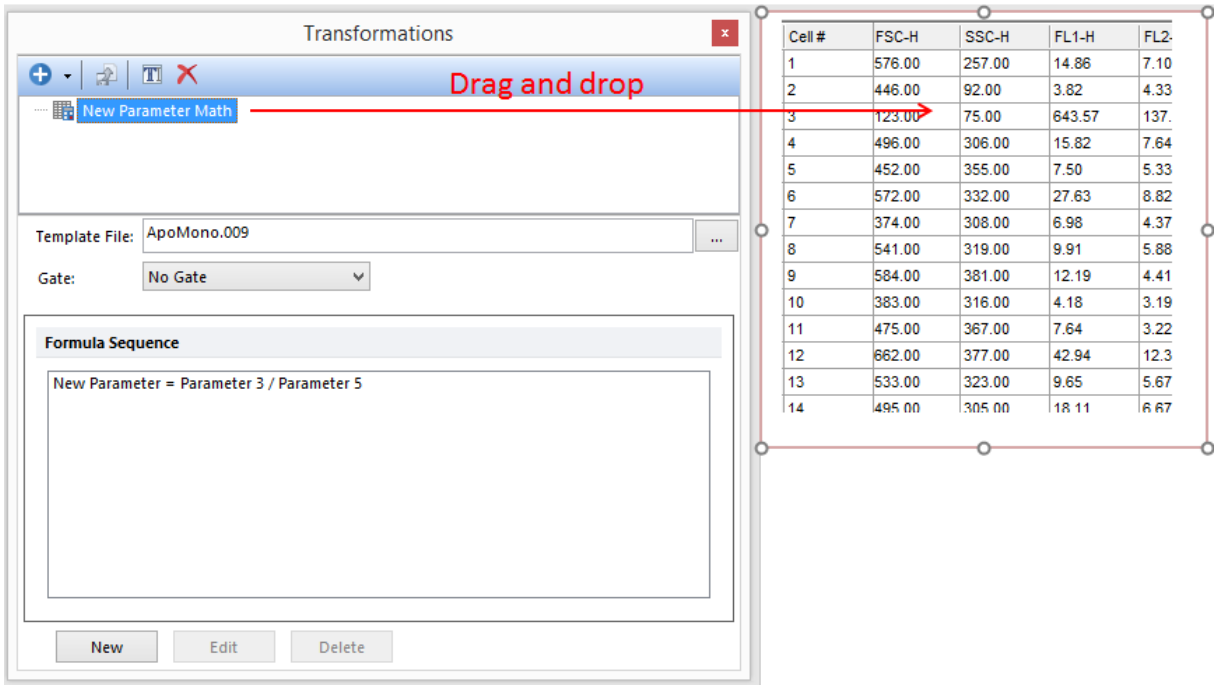

Figure 29.13 - Applying parameter math to a plot by dragging from the Transformations navigator to the data grid (red arrow).

- Access Formatting dialog for general options 310. **Data Options**→select parameter math to use from **Transformation** drop down list→**OK**. (Figure 29.14).

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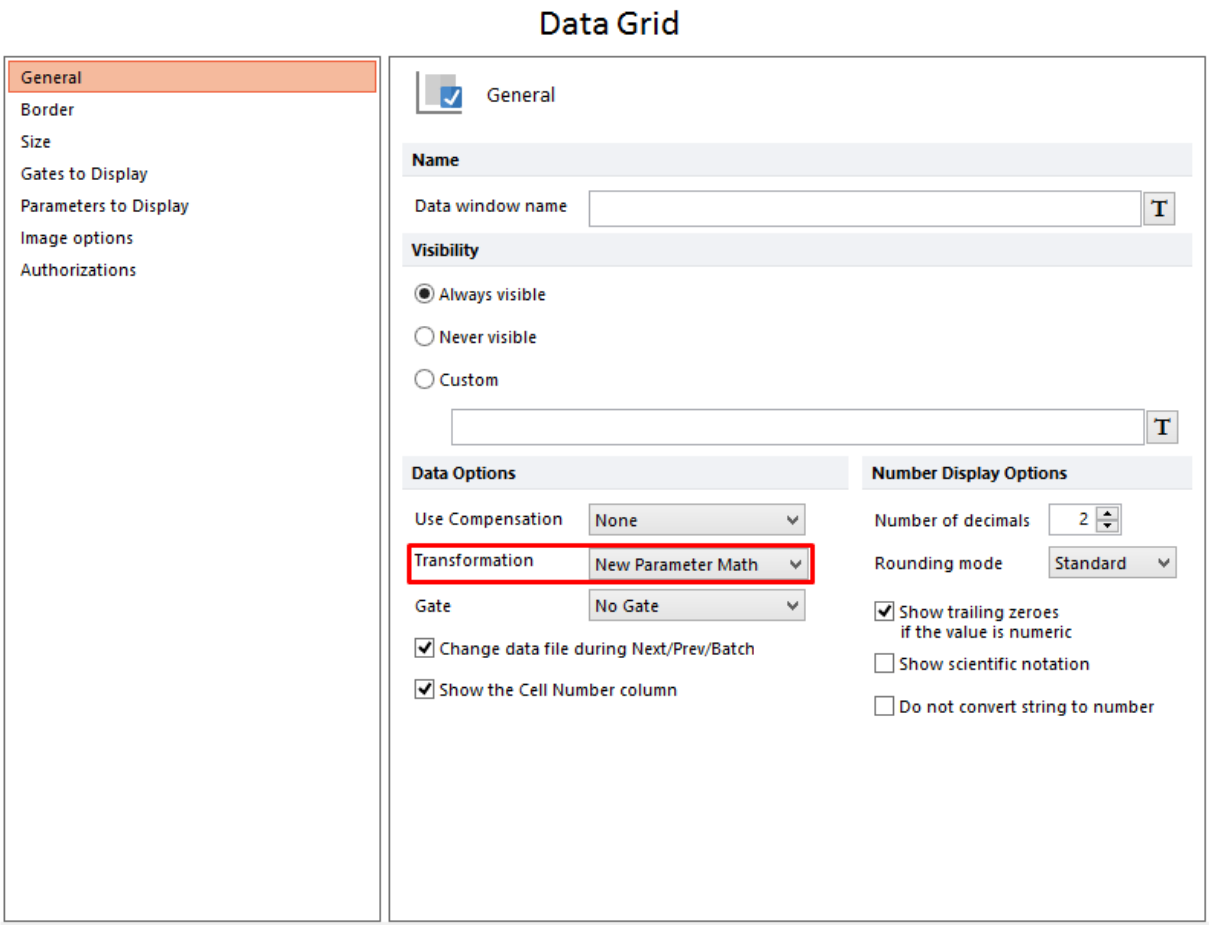

Figure 29.14 - Applying parameter math to a data grid by selecting the parameter math in the Transformation drop down from the Formatting Overlays dialog.

- Right-click data grid→**Show Transformed Values**→select appropriate parameter math from menu.(Figure 29.15).

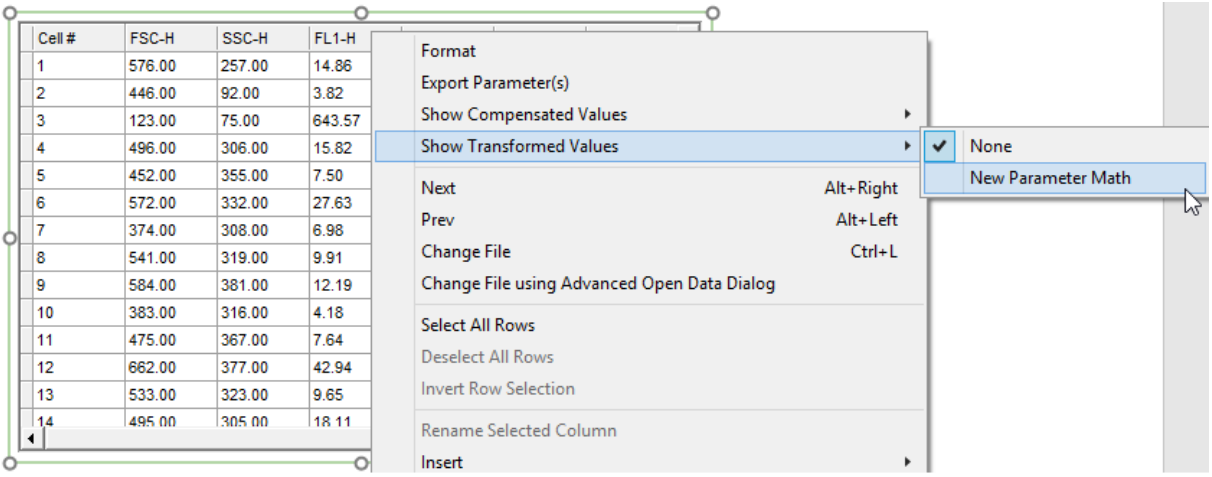

Figure 29.15 - Accessing parameter math by right-clicking data grid and navigating to Show Transformed Values menu.

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28.2 Principal Component Analysis

Principal Component Analysis (PCA) is a statistical procedure that uses an orthogonal transformation to convert a set of observations, in this case cytometry based events, into new variables called principal components. The transformation is defined in such a way that the first two principal components generally define the maximum variance while each succeeding component maximizes the variance at a 90 degree rotation. Principal components defined and applied to a plot in FCS Express are accessible for analysis as new parameters on plots.

To begin working with PCA analysis in FCS Express please see the topics on:

- **Defining a PCA Transformation** 946
- **Applying a PCA Transformation** 956

28.2.1 Defining a PCA Transformation

Principal Component Analysis is performed in FCS Express through the **Transformation** window.
The steps below outline the process of creating a new PCA transformation on your data:

1. Open the Transformation window by selecting the **Tools** tab → **Transformations** → **Transformations** command. The **Transformations** window will open (Figure 29.16, Step 1).
2. Click on the dropdown arrow adjacent to the blue plus button (Figure below, Step 2).
3. Click on **PCA** in the dropdown list (Figure below, Step 3).

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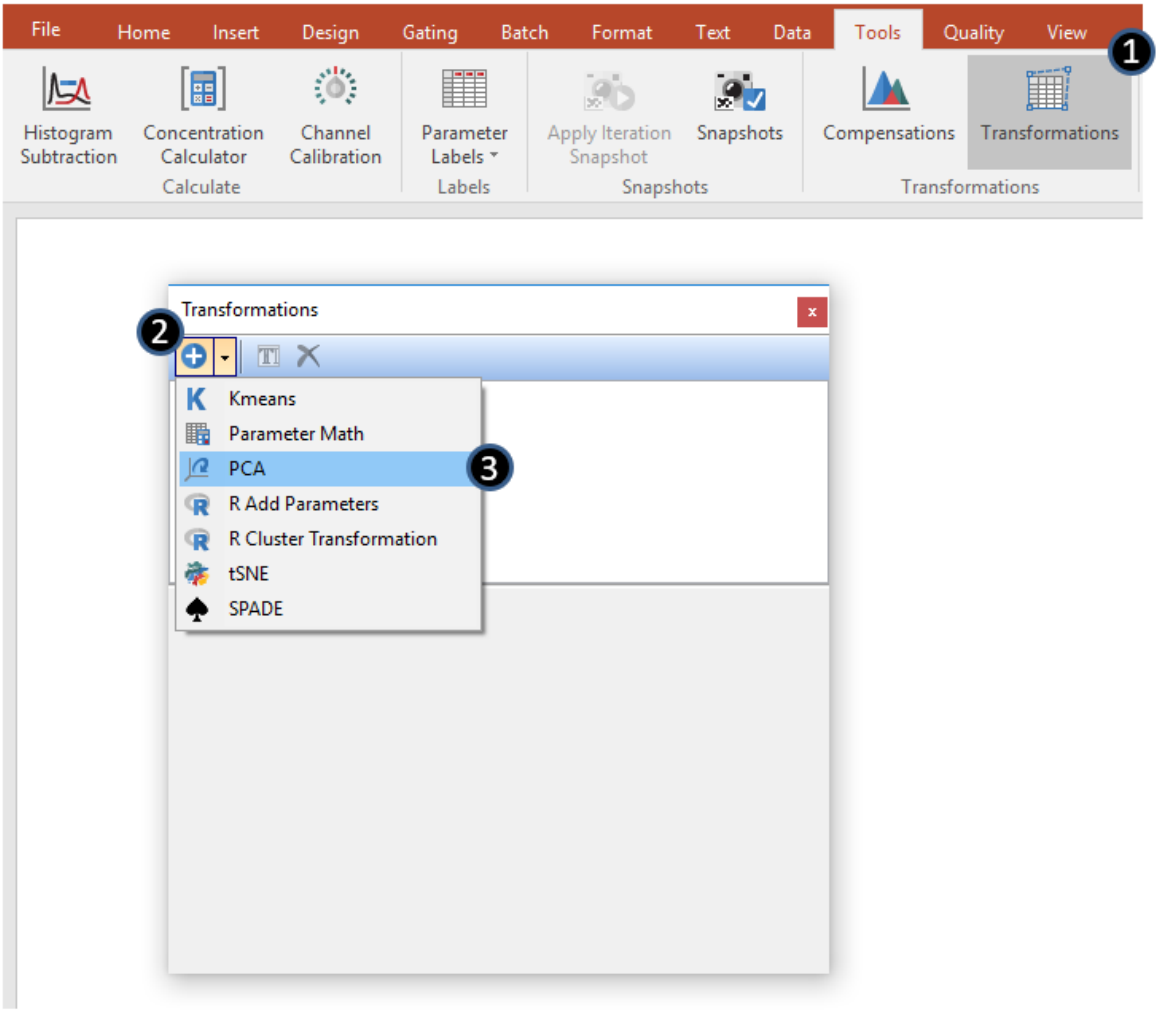

Figure 29.16 Initial steps to create a new PCA transformation.

The PCA dialog will now appear in the **Transformation** window as seen in Figure 29.17.

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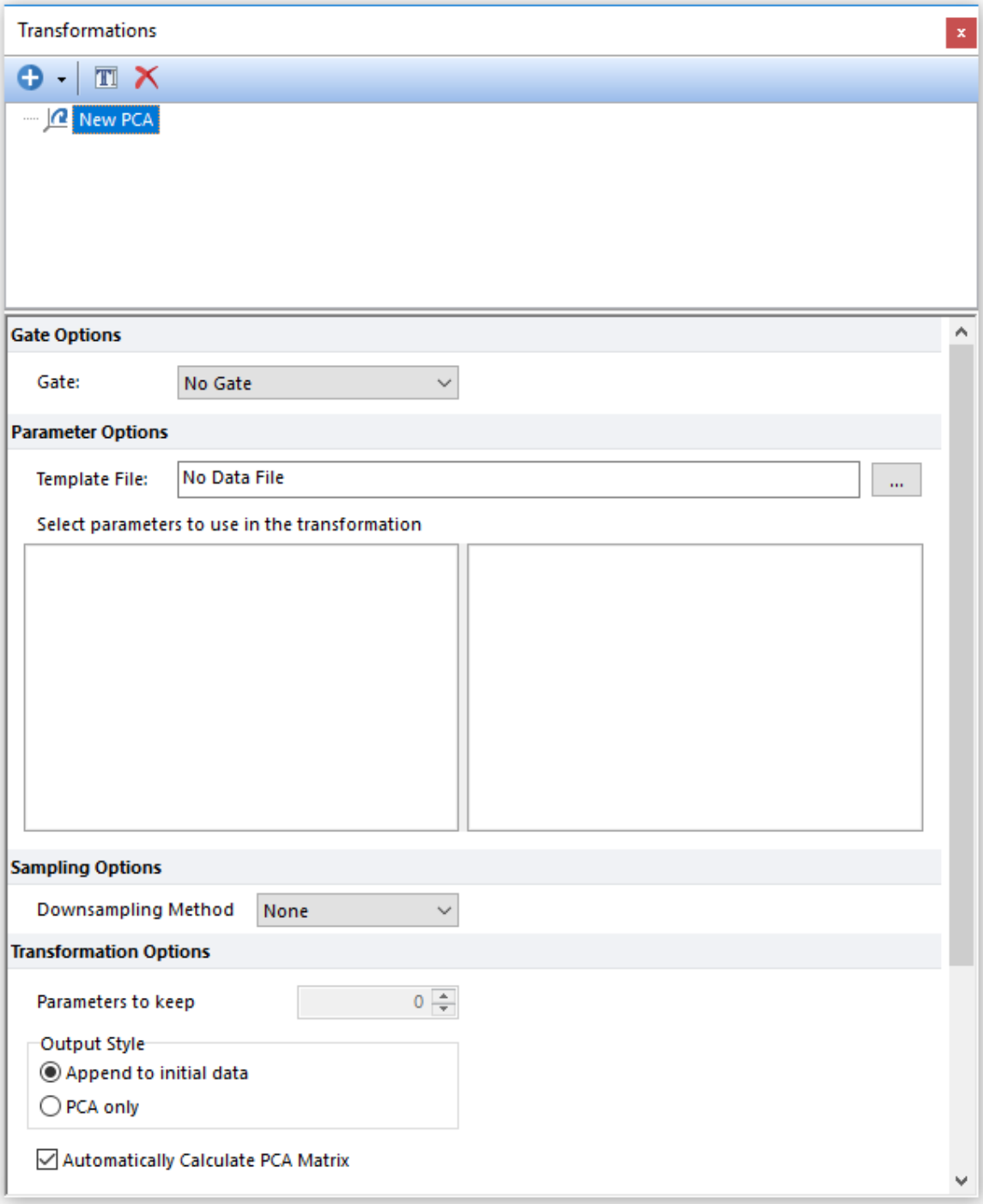

Figure 29.17 The PCA dialog in the Transformations window

The new PCA transformation is named as **New PCA** by default but it can be easily renamed by either of the following methods:

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- Right click on the PCA transformation that needs to be renamed→Select **Rename**.
- Select the PCA transformation that need to be renamed via left click and press **F2** on your keyboard.
- Select the PCA transformation that need to be renamed via left click and click on the button in the **Transformations** tool bar.

4. Choose a **Template File** for the PCA transformation by clicking on the ellipsis to the right of the **Template File** text box (Figure 29.18).

The PCA dialog will be populated with a list of available parameters to perform a PCA transformation based on available parameters from the template file (Figure below).

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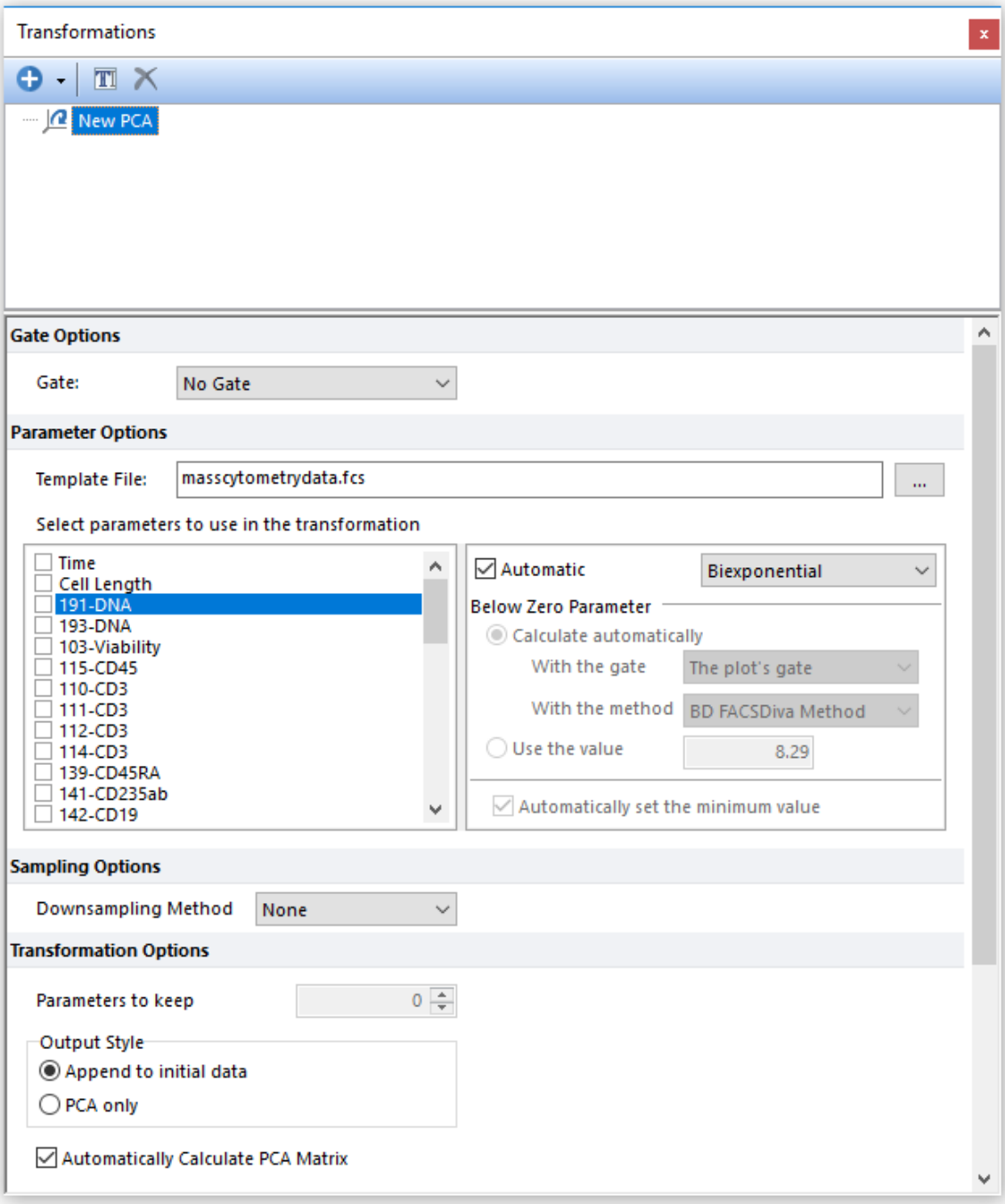

Figure 29.18 A template file has been chosen which subsequently populates the list of possible parameters to perform a PCA transformation.

5. Choose the parameters to define the transformation by checking the boxes next to the parameter names (Figure 29.19, Step 1).
6. Choose or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below, Step 2).

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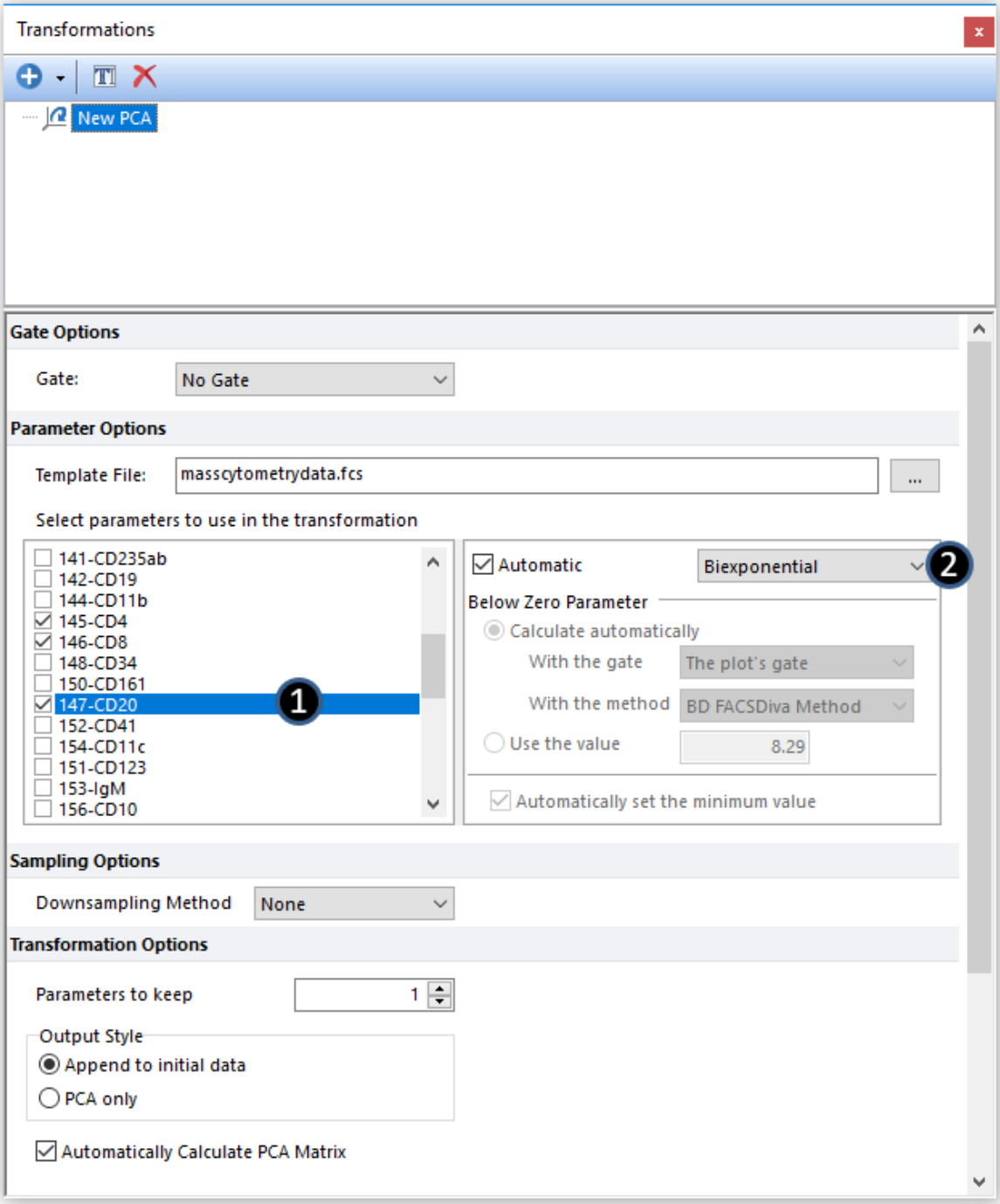

Figure 29.19 Parameters for the PCA transformation are selected by checking the boxes next to the parameter names.

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The PCA transformation has now been defined and automatically calculated by default. The PCA transformation can now be applied to any plot in FCS Express (see, Applying a PCA Transformation 956).

Note the following options:

- A gate may be selected to only evaluate the matrix for events falling within the gate from **Gate** dropdown menu.
- **Sampling Options** (optional). Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default **Downsampling Method** for PCA is **None**, thus all available events (i.e. the ones defined with the **Gate** drop-down menu) will be used.
- The **Parameters to keep** option allows you to define how many principal components are defined for the data set (the figure below depicts an example in which 3 parameters have been kept).
- The **Output Style** choices are:
  - **Append to initial data** adds the newly created principal component (PC) parameters to the end of the parameter list in the data file when the PCA is applied (Figure 29.20, *left*).
  - **PCA only** only displays the PC parameters on a plot when the PCA is applied (Figure below and *right*).

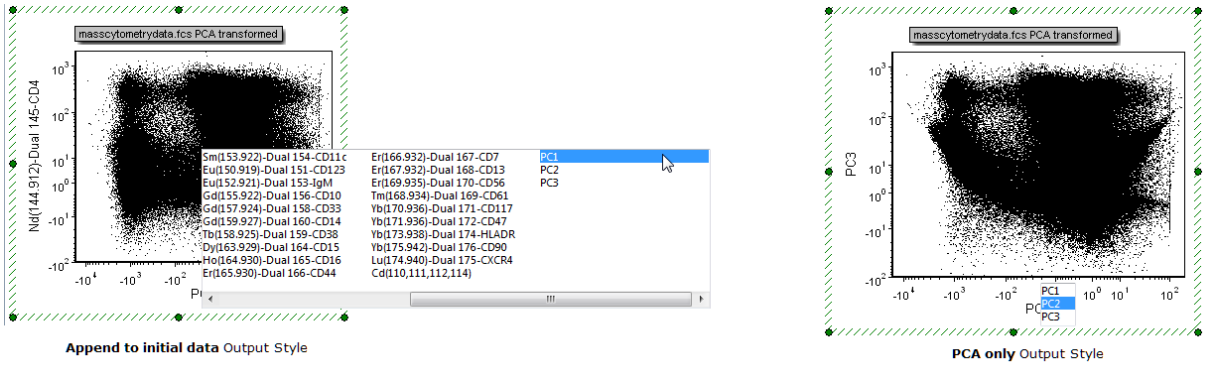

Figure 29.20 PCA transformation Output Styles

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To defer calculating the PCA matrix, uncheck the box for **Automatically Calculate PCA Matrix**. Doing so will present you with the button to **Calculate the PCA Matrix** (Figure 29.21).

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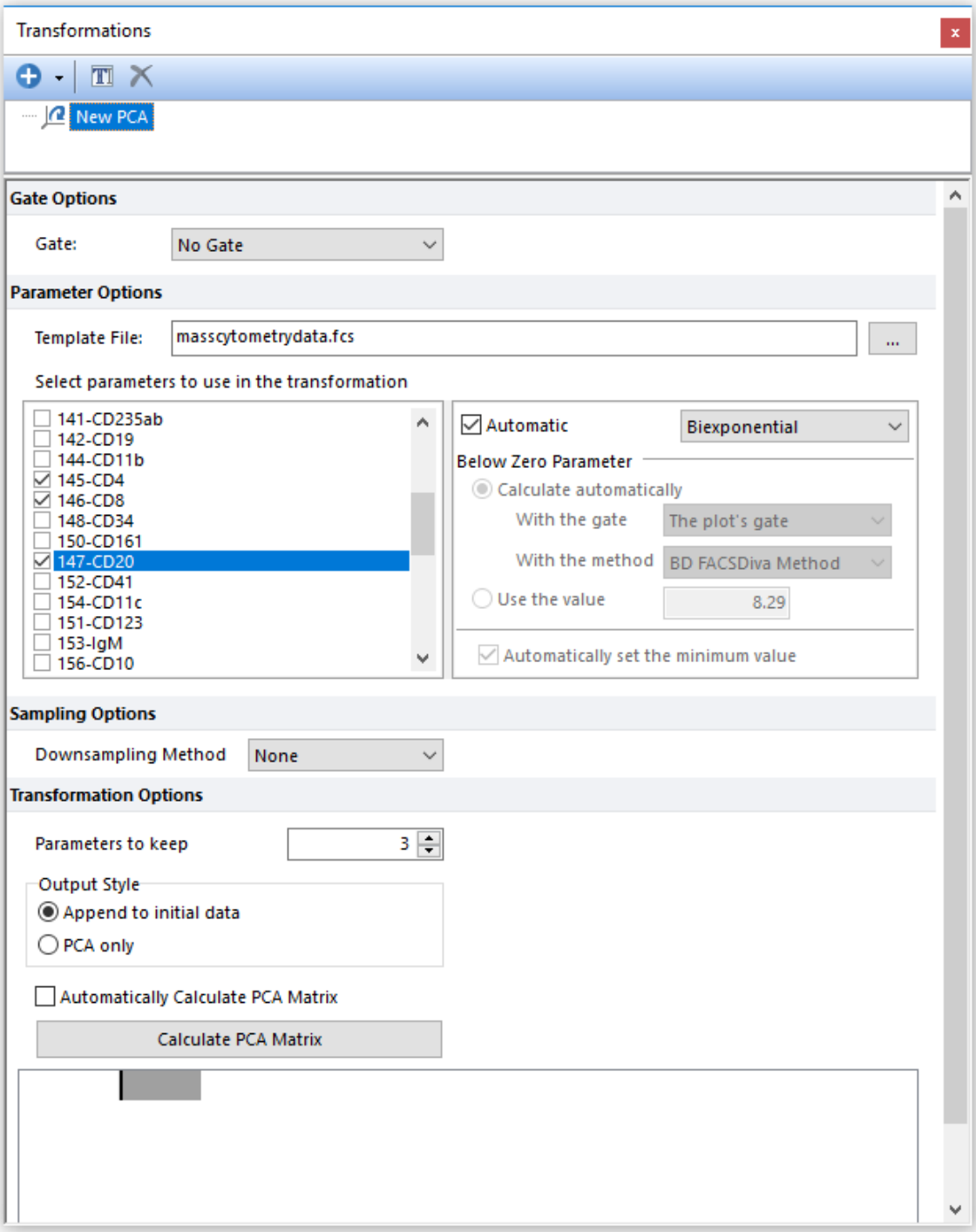

Figure 29.21 Unchecking the Automatically Calculate PCA Matrix box will defer calculating the PCA matrix.

After pressing the button to **Calculate the PCA Matrix** the matrix will be displayed in the bottom pane of the window (Figure 29.22).

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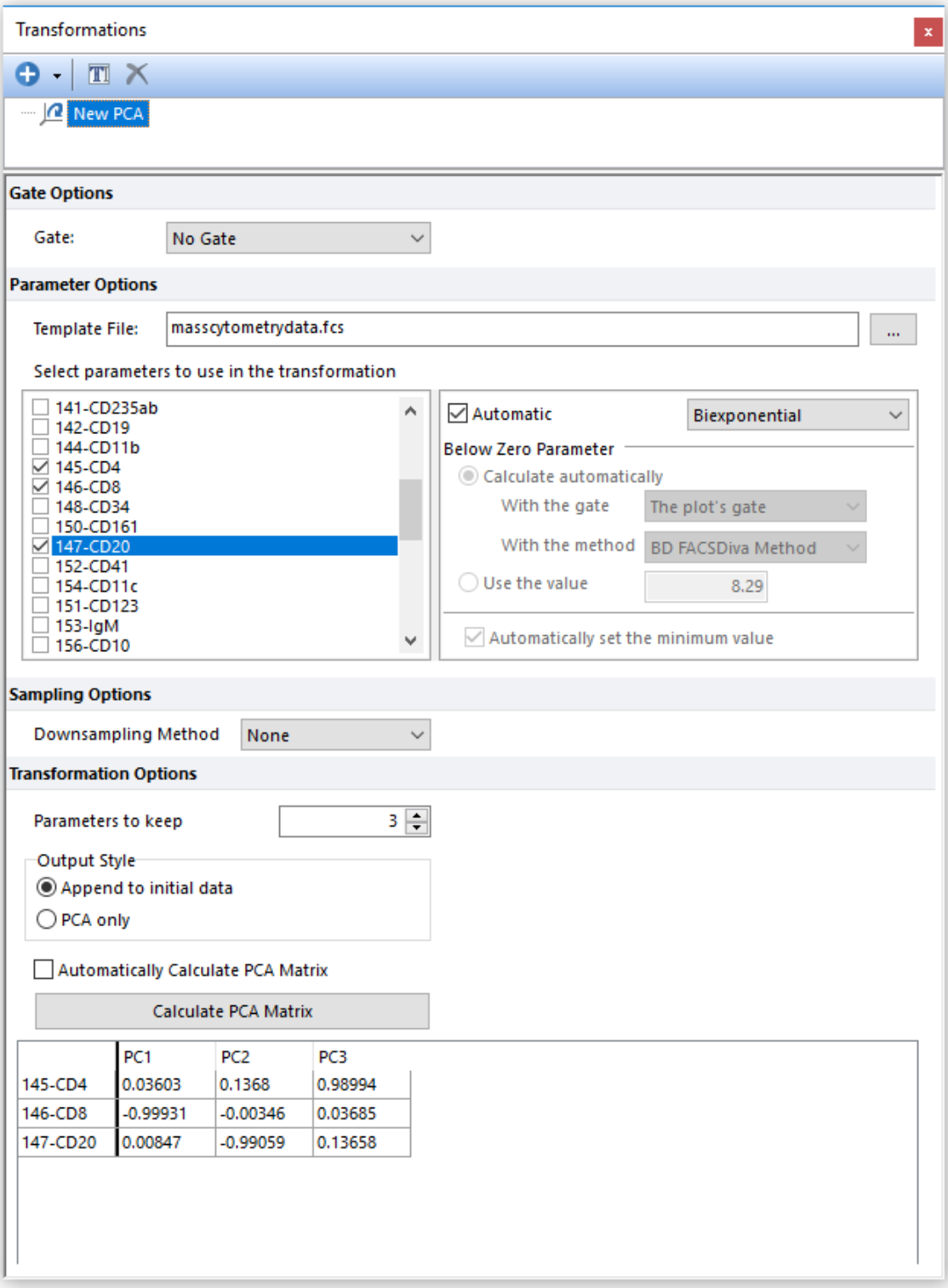

Figure 29.22 Once the Calculate PCA Matrix button is pressed the matrix will be calculated and shown in the dialog.

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28.2.2 Applying a PCA Transformation

Once a PCA transformation has been defined946, it may be applied to any plot in FCS Express. Once a PCA is defined and applied to a plot, standard gating and statistics may be used to evaluate the new principal component parameters.

Note: When a PCA transformation has been applied, the text "**PCA transformed**" will appear in the plot title.

PCA transformations may be applied to 1D and 2D plots by either of the following methods:

- Drag and drop a PCA transformation from the **Transformation** window to the intended plot (Figure 29.23).

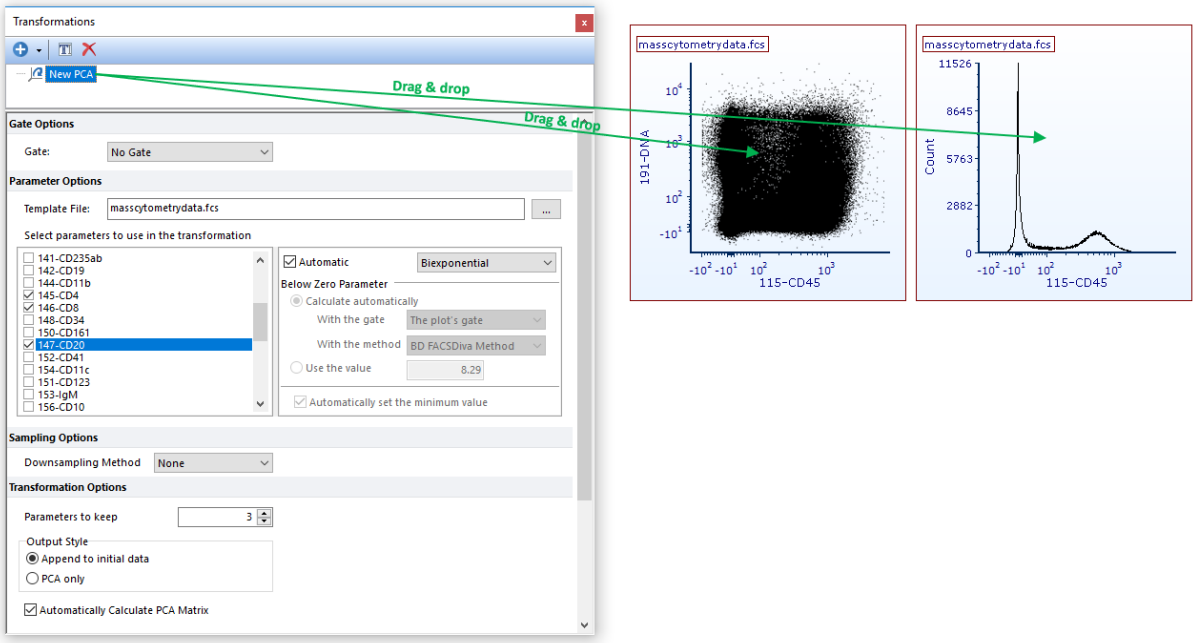

Figure 29.23 Applying a PCA transformation to a plot by dragging the PCA transformation from the Transformations navigator to the plot (green arrow).

- Access the Formatting Overlays dialog284 and choose the PCA to apply from the **Transformation** dropdown menu (Figure 29.24).

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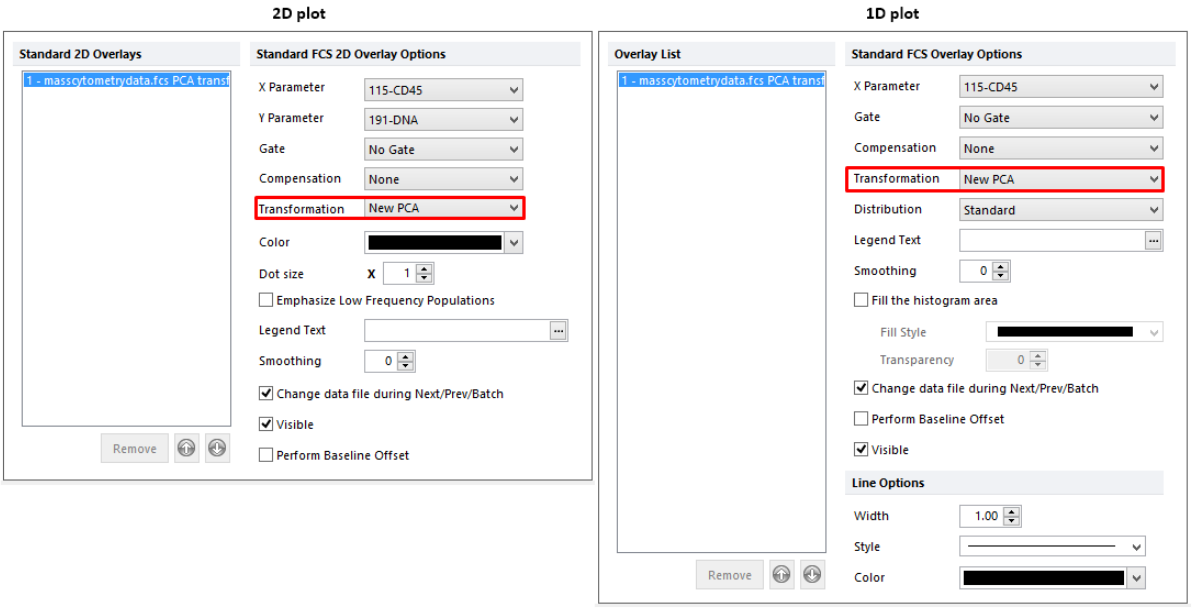

Figure 29.24. Applying a PCA transformation to a plot by selecting the PCA transformation from the Transformation dropdown in the Formatting Overlays dialog.

The result of both method will be the following (Figure 29.25). Please note the suffix "**PCA transformed**" in the plot title.

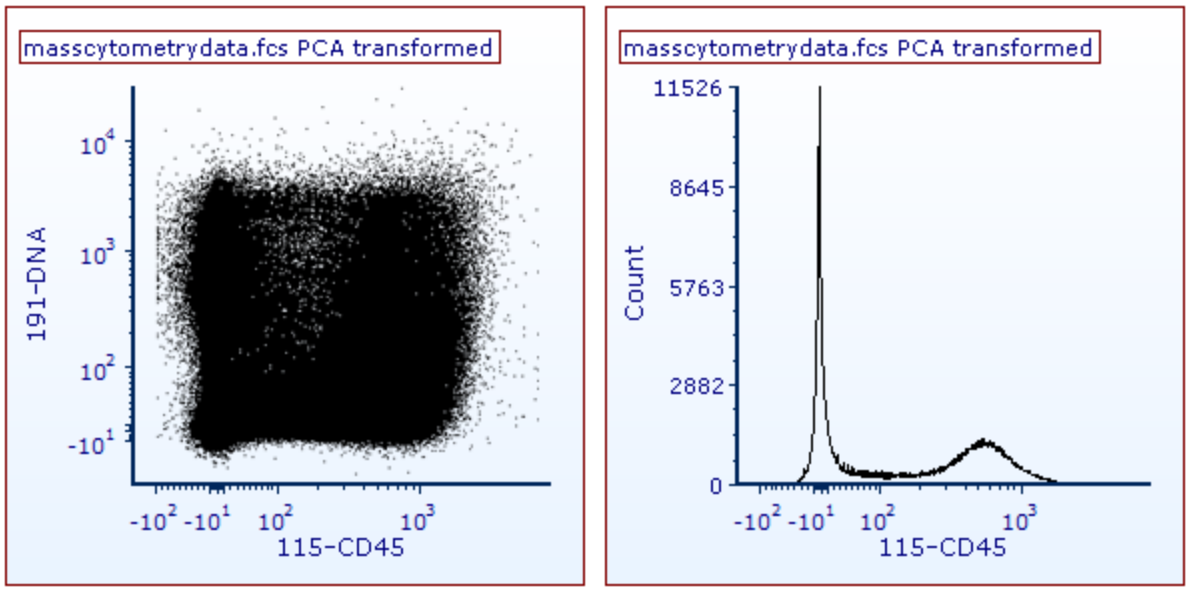

Figure 29.25. 1D and 2D plot with PCA transformation applied. Note the "PCA Transofrmed" suffix in the plot title.

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Principal Component will be accessible as additional or as unique plot parameters based on the selected output style (see the previous section 946 for more info about this) (Figure 29.26).

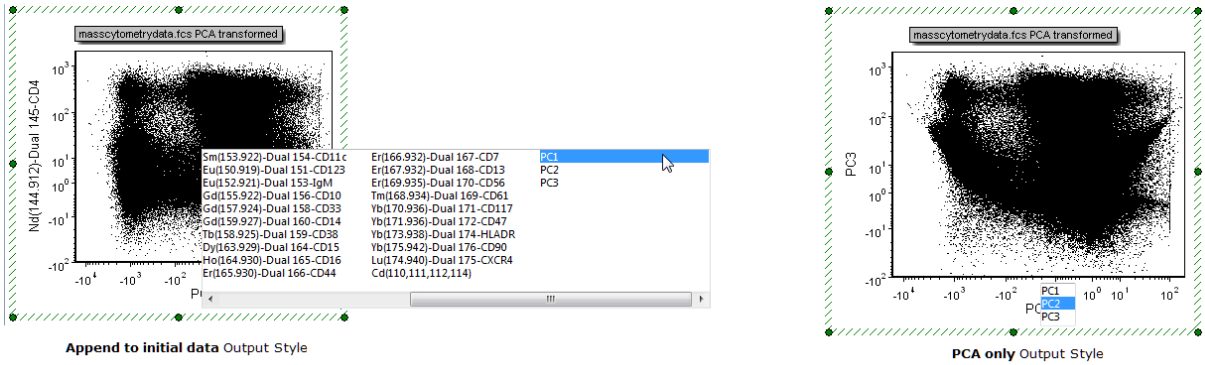

Figure 29.26 - PCA Transformation output styles.

28.3 K means (Cluster Analysis)

FCS Express can perform cluster analysis using *k*-means methodology.

Cluster analysis aims to group a set of objects/events in such a way that objects/events in the same group (i.e. a cluster) are more similar (in some sense or another) to each other than to those in other groups (*i.e.* the other clusters).

k-means is a partitioning-based clustering algorithm. *k*-means method for clustering is an iterative process in which an initial partition of given *k* clusters is then improved by applying a search algorithm to the data. Simplifying, given a pre-defined number (*k*) of clusters, the algorithm:

- begins with an initial set of *k* cluster centers (*i.e.* the centroids)
- (re)assigns objects to the closest centroids
- recalculates centroids according to new memberships of the data points.
- repeats the last two steps until a consistent result is found or until the maximum number of iterations is reached.

The basic *k*-means clustering is based on a non-deterministic algorithm. This means that running the algorithm several times on the same data, could give different results. However, to ensure consistent results, FCS Express performs *k*-means clustering using a deterministic method.

To begin working with cluster analysis in FCS Express please see the topics on:
Defining a k-means Clustering Analysis 959
Applying a k-means Clustering Analysis 968
Working with k-means parameters 970

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28.3.1 Defining a k-means cluster analysis

k-means cluster analysis is performed in FCS Express through the **Transformation** button within the **Tools** tab→ **Transformations.** The steps below outline the process of creating a new *k*-means cluster analysis on your data.

1. Open the **Transformation** window by selecting the **Tools** tab→**Transformations** →**Transformations** command (Figure below, Step 1).
2. Click on the drop down arrow adjacent to the blue plus button (Figure below, Step 2).
3. Click on **Kmeans** in the drop down list (Figure 29.27, Step 3).

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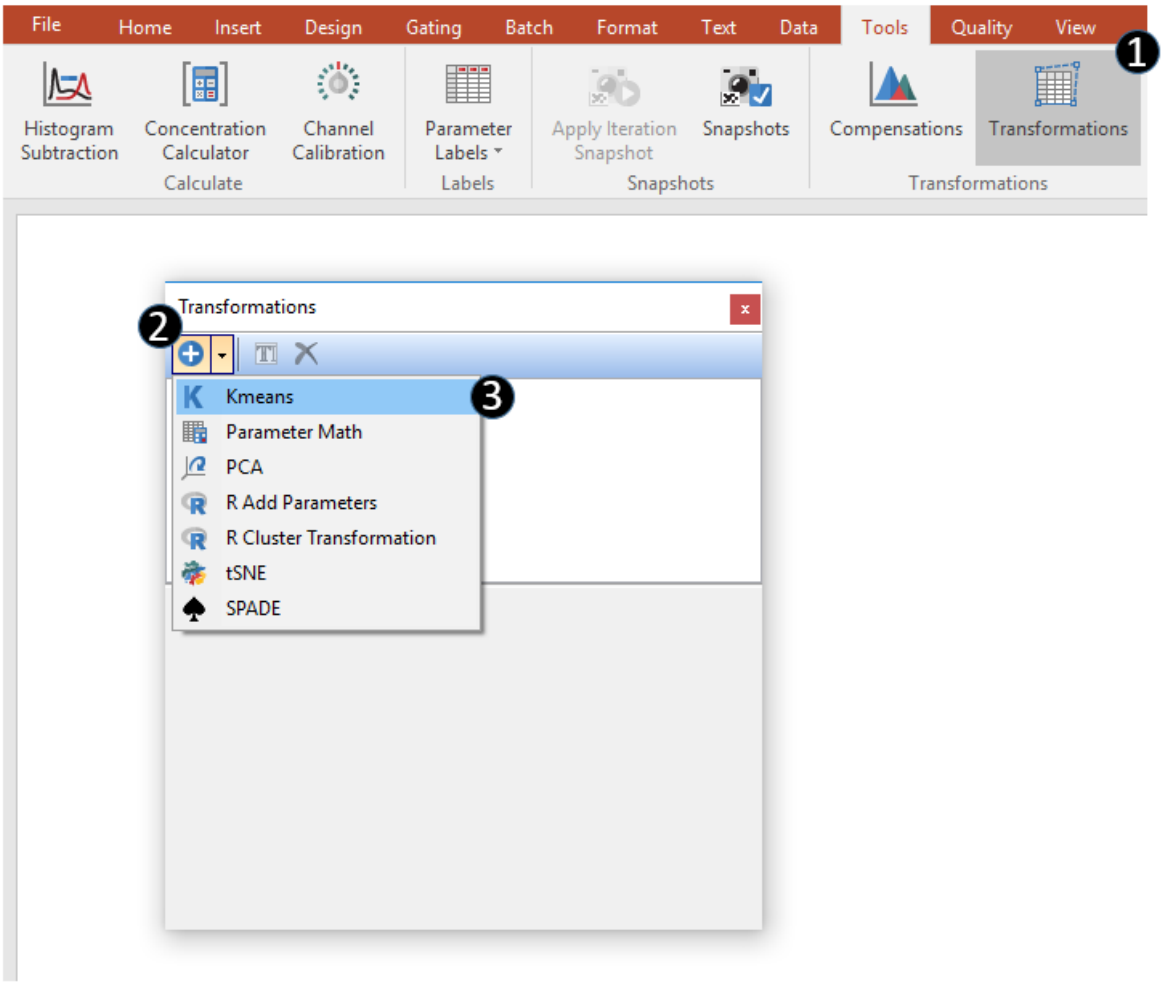

(Figure 29.27) - A Kmeans transformation can be created via the Transformations navigator.

The **Kmeans** dialog will now appear in the **Transformation** window as seen in Figure 29.28.

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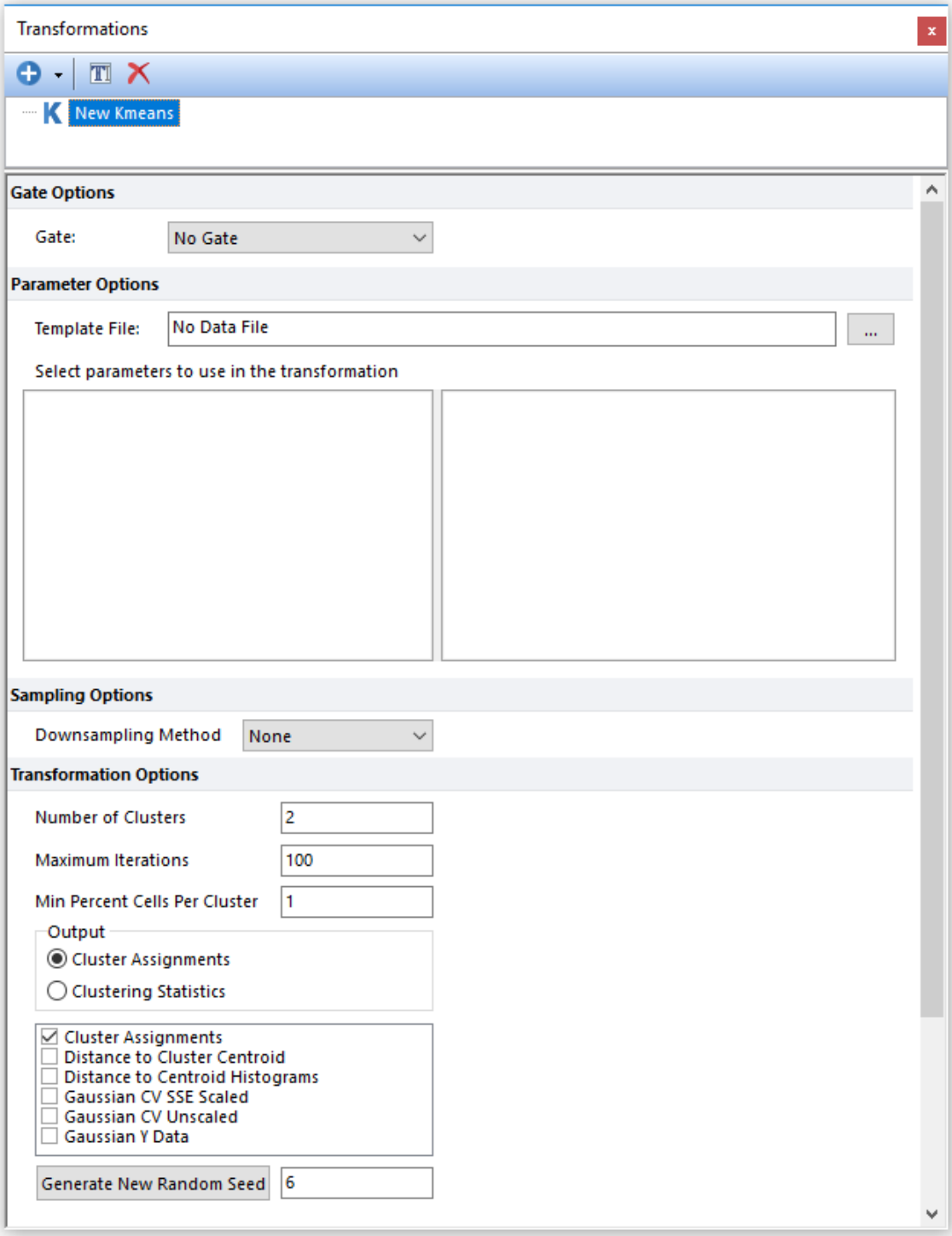

Figure 29.28 - The Kmeans dialog opens when a new k-means transformation is created.

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The new Kmeans transformation is named as **New Kmeans** by default and it can be easily renamed by either of the following methods:

- Right click on the Kmeans transformation that needs to be renamed→Select **Rename**.
- Select the Kmeans transformation that need to be renamed via left click and press **F2** on your keyboard.
- Select the Kmeans transformation that need to be renamed via left click and click on the button in the **Transformations** tool bar.

4. Choose a **Template File** for the k-means clustering by clicking on the ellipsis to the right of the **Template File** edit box.

Once the file of interest has been selected, the dialog will be populated with a list of available parameters to perform a *k*-means clustering based on available parameters from the template file (Figure 29.29).

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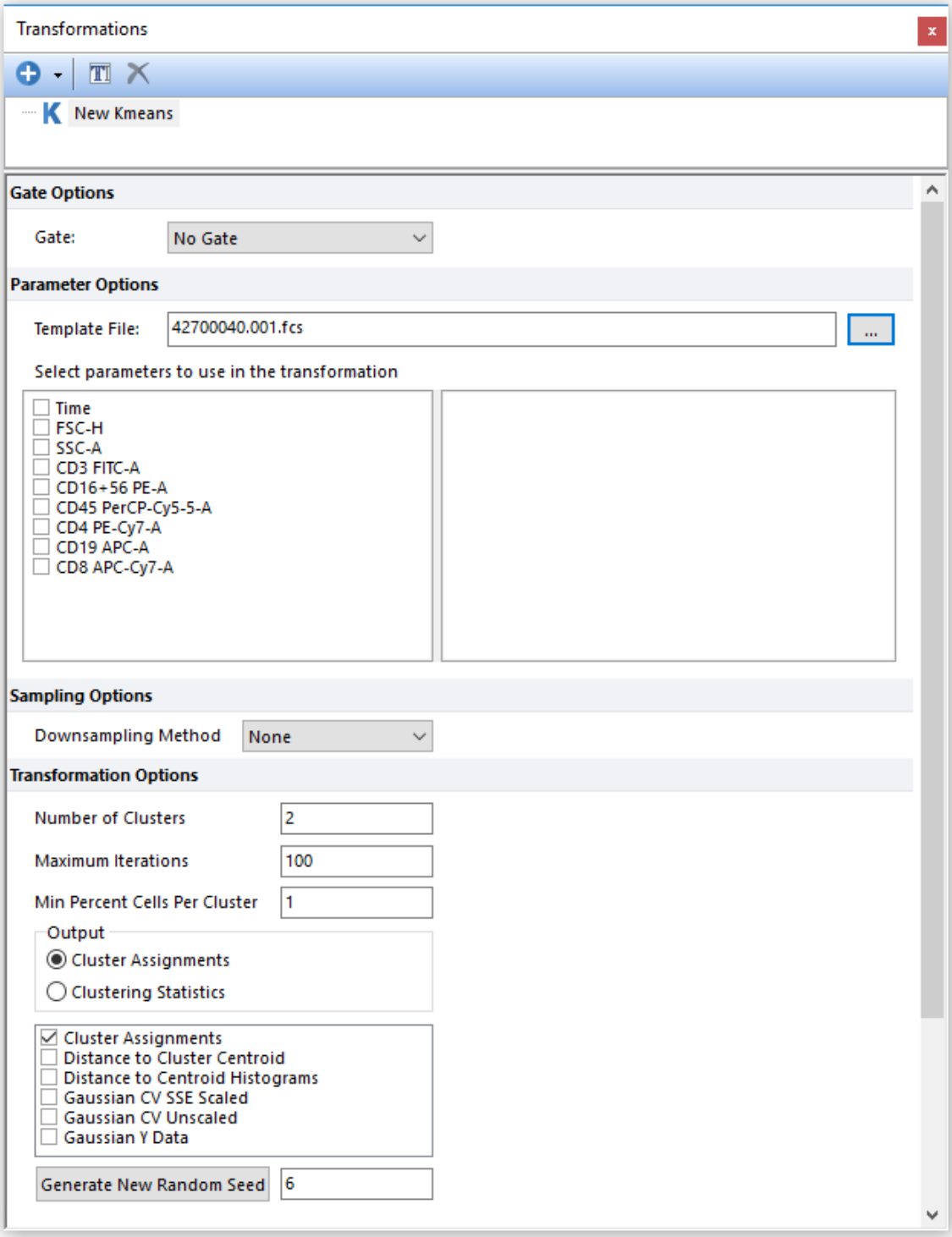

Figure 29.29 - A template file has been loaded into the New Kmeans dialog.

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5. Choose the parameters to define the Kmeans clustering by checking the boxes next to the parameter names (Figure 29.30, Step 1).
6. Choose or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below, Step 2).

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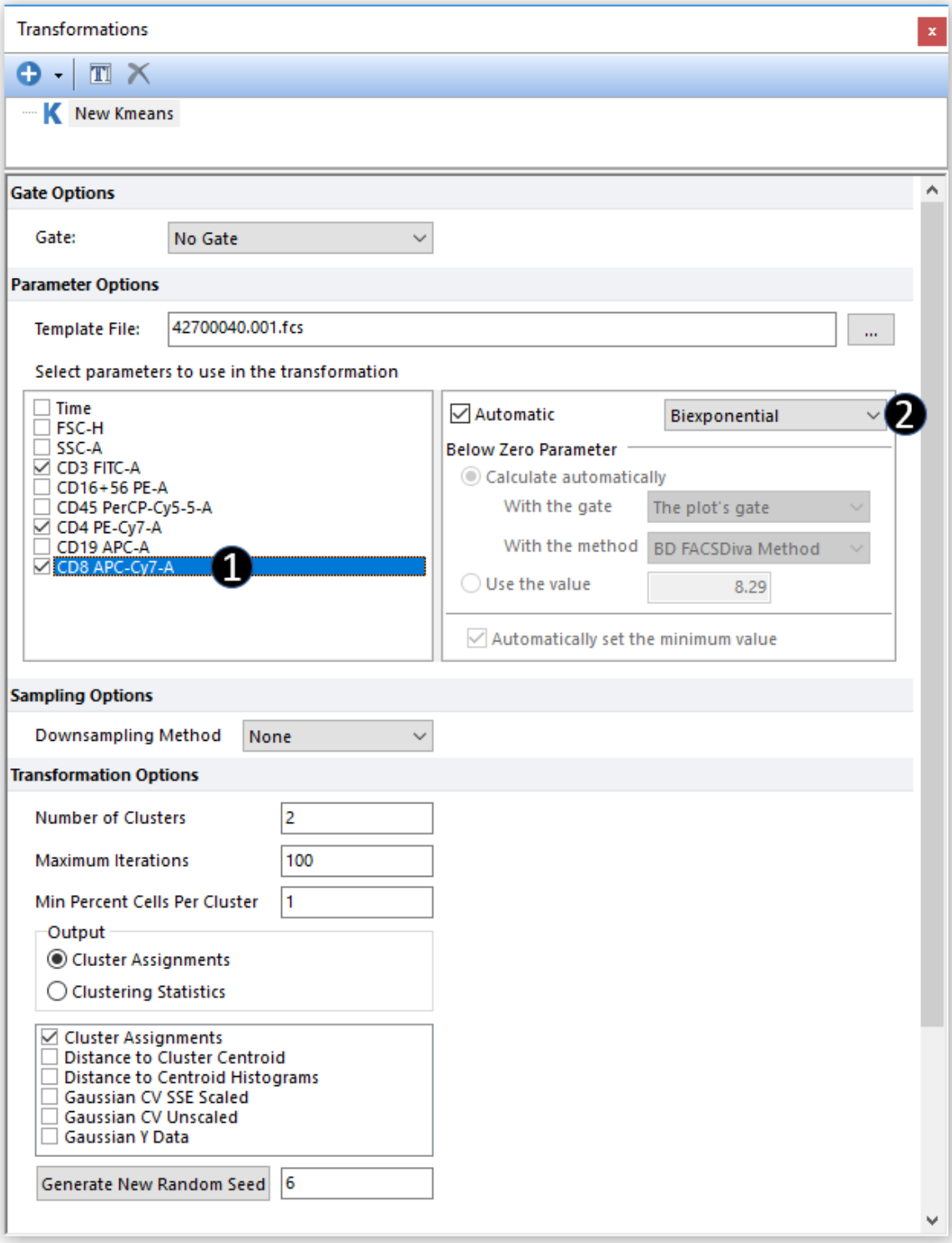

Figure 29.30 - Choosing the parameters to perform k-means cluster analysis.

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A number of options can be set at this point:

6. (Optional) Select a gate from the **Gate** drop down if you are interested in performing the clustering only for the events belonging to it.
7. **Sampling Options** (optional). Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default **Downsampling Method** for k-Means is **None**, thus all available events (i.e. the ones defined with the **Gate** drop-down menu in **Step 6**) will be used.
8. Set the **Number of Clusters** the events should be clustered by. It's equivalent to the number of centroids.
9. Set the number of **Maximum Iterations**. This value indicate the maximum number of iterations that FCS Express should perform before quitting the clustering process.
10. Set the **Minimum Percent of Cells Per Cluster**. This value indicates the minimum size, in term of percentage, of clusters.
10. Set **Output** Style for Kmeans clustering.

The available choices are **Clustering Assignments** and **Clustering Statistics**. Please see the detailed list below explaining each option.

- **Clustering Assignments:** By selecting this output style, the following statistics can be assigned to each event the cluster has been performed on:
  - **Cluster Assignment:** An internal label indicating the cluster membership is automatically assigned to each event. This label is internally used by FCS Express for heatmap representation of clusters but it is also accessible through a Data grid 680.
  - **Distance to Cluster Centroid:** The distance of a given event to the centroid of its cluster. Values are plotted as unique histogram.
  - **Distance to centroid Histograms:** The same as the Distance to Cluster Centroid but in this case values are grouped, and thus plotted,

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separately for each cluster. Clusters are numbered from 1 to **Number of Clusters**.

- **Gaussian CV SSE Scaled:** In this representation each event is plotted based on its cluster membership, thus clusters appear as separated histograms. Given that cluster membership is an integer value that goes from 1 to **Number of Clusters**, an artificial noise is introduced to spread out the data. **Gaussian SSE scaled** means that the standard deviation of the Gaussian (normal distribution) used to represent each cluster is proportional to the Sum of Squared Error of the cluster.
- **Gaussian CV Unscaled:** In this representation each event is plotted based on its cluster membership, thus clusters appear as separated histograms. Given that cluster membership is an integer value that goes from 1 to **Number of Clusters**, an artificial noise is introduced to spread out the data. **Gaussian CV unscaled** means that the standard deviation of the Gaussian (normal distribution) used to represent each cluster is the same among clusters.
- **Gaussian Y Data:** Events are plotted following a Gaussian (normal distribution).

- **Clustering Statistic**: By selecting this output style, the following statistics will be accessible through plots and Data grid 680:
  - **Kmeans Cluster ID:** An integer value that goes from 0 to **Number of Clusters-1**.
  - **Centroid coordinates:** The coordinates of each centroid are given for each of the parameters considered for the clustering.
  - **Sum Of Square (WCSS):** The Within-Cluster Sum of Squares is reported for each cluster.

The following table lists the objects suitable for displaying each output style:

	Can be displayed as		
	Heatmap	**1D or 2D plots**	**Data grid**

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Clustering Assignments	**Cluster Assignment**	Y		Y
	Distance to Cluster Centroid		Y	Y
	Distance to Centroid Histograms		Y	Y
	Gaussian CV SSe Scaled		Y	Y
	Gaussian CV Unscaled		Y	Y
	Gaussian Y Data		Y	Y
Clustering Statistics			Y	Y

11. The *k*-means clustering has now been defined and automatically calculated. *k*-means clustering can now be applied to plots for display and analysis. (see Applying a k-means Cluster Analysis 968).

28.3.2 Applying a k-means cluster analysis

Once *k*-means clustering has been performed, results can be accessed as 1D plots, 2D plots 172, Heatmaps 1050 and Data Grid 680 depending on the selected output style (see the previous section 959). The Cluster Assignments chosen to be calculated 966 may be accessed on plots on which the transformation has been applied as new parameters 970.

Although clustering results are automatically displayed when inserting a Data grid, to access clustering results as parameters in 1D plots, 2D plots and/or Heatmaps, the k-means transformation must be applied to the plots.

k-means clustering may be applied to 1D plots, 2D plots and heatmaps by either of the following methods:

Drag and Drop (Figure 29.31)
1. Left click and hold on *k*-means clustering from the **Transformation** window.
2. Drag the Kmeans to the intended plot.
3. Release the mouse button.

The Kmeans will be applied to the plot and the title of the plot will now include the text "Kmeans clustered" 970 to indicate the transformation has been applied.

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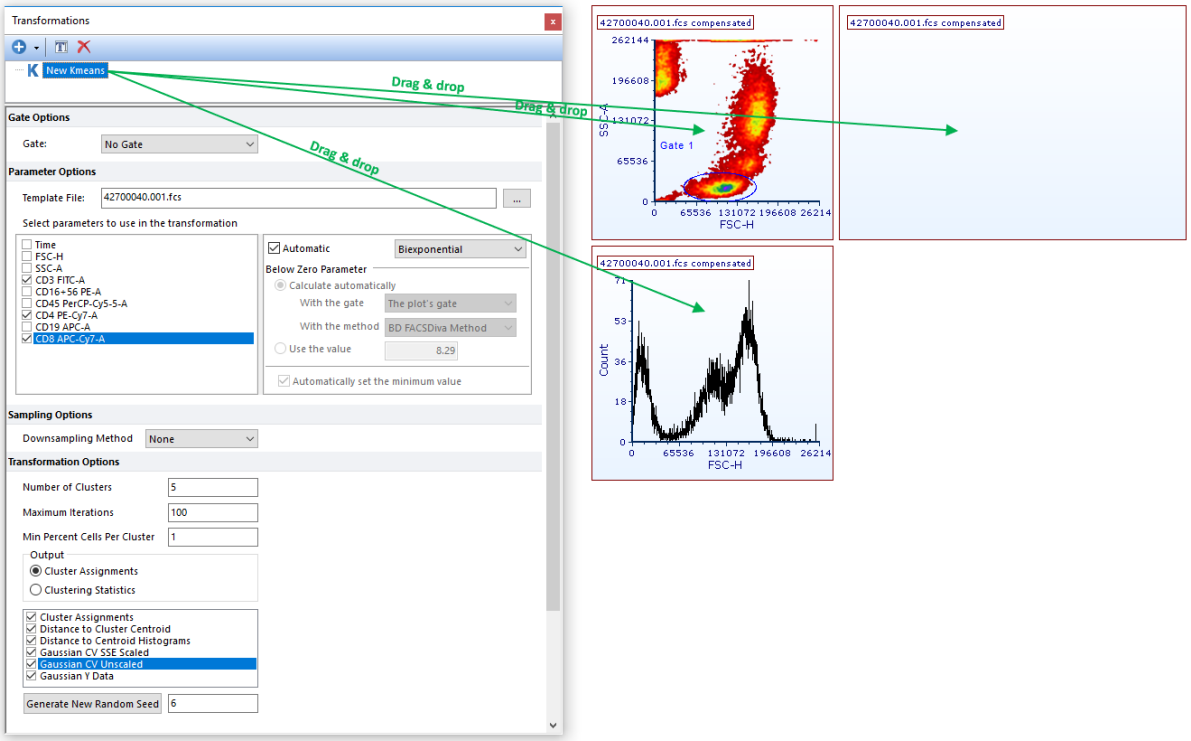

Figure 29.31 Applying a k-means transformation to a plot by dragging the k-means transformation from the Transformation navigator to the plot (green arrows).

Using the Formatting Dialog (Figure 29.32)

1. Access the **Formatting→Overlays dialog** 284 (for 1D plots, 2D plots and Heat map) or the **Formatting→General dialog** 310 (for Data Grids).
2. Choose the Kmeans to apply from the **Transformation** drop down.
3. Click **OK**.

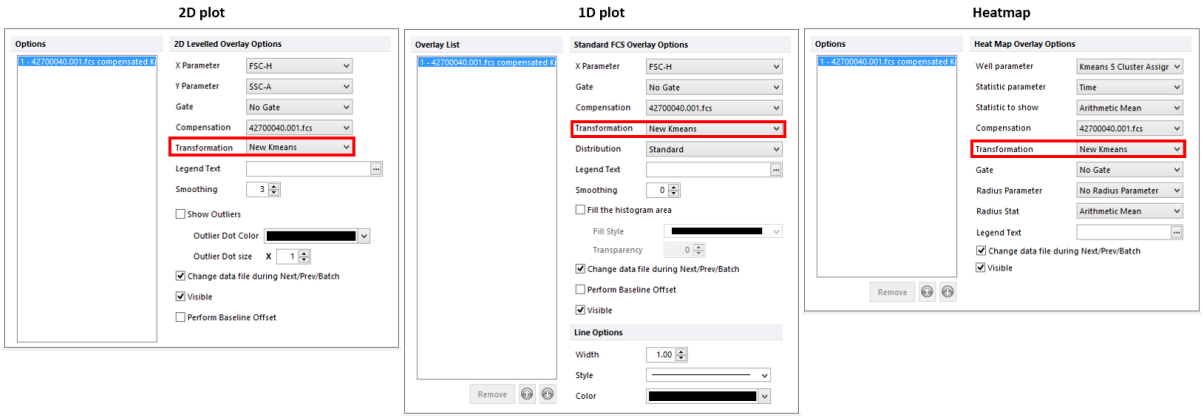

Figure 29.32 Applying a k-means transformation to a plot by selecting the k-means transformation from the Formatting Overlays dialog in the Transformation dropdown.

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The result of both the previous methods is represented in Figure 29.33 below. When a *k*-means clustering has been applied to a plot or to an heatmap, the suffix "**Kmeans clustered**" will appear in the object title.

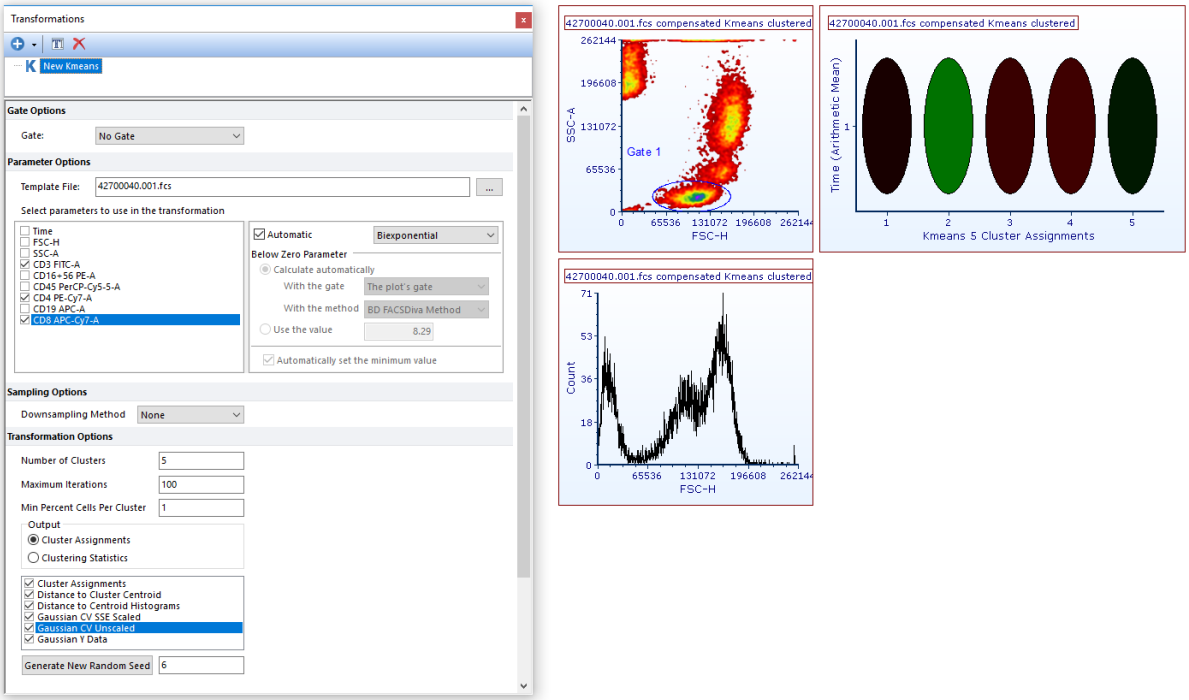

Figure 29.33 The plot title contains "Kmeans clustered" siffix when a Kmeans transformation is applied.

In the next section we will see how to work with clustering parameters 970.

28.3.3 Working with k-means parameters

Once a *k*-means transformation is applied to a plot, the newly created **Cluster Assignment** parameters can be accessed by clicking on the axis label of the plot Figure 29.34. Please note that heatmaps can only display **Cluster Assignments**, which is automatically displayed when a k-means transformation is applied.

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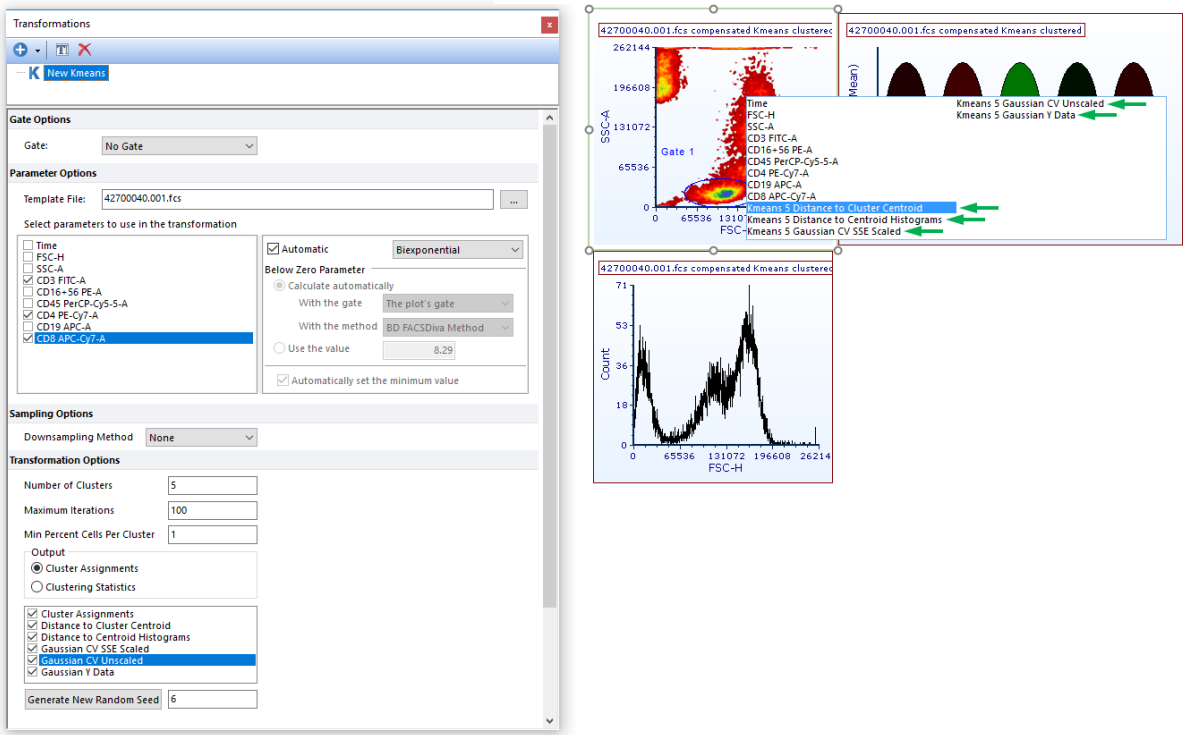

Figure 29.34 Newly created pareameters can be accessed by clicking on the axis label of the plot of interest.

Gates[316] can now be created on the following **Cluster Assignment** parameters to specifically select the events within one or more clusters.:

- **Cluster Assignments** (heatmap only),
- **Distance to centroid Histograms**(1D/2D plot only),
- **Gaussian CV SSE Scaled** (1D/2D plot only),
- **Gaussian CV Unscaled** (1D/2D plot only),

These gates can then be used to back gate[303] clustered events on non-clustering parameters.

Here are some examples of cluster selection using the above-mentioned parameters:

1D plots

Markers can be created on 1D plot displaying clustering parameters as one would on non-clustering parameters. After converting the marker to be a gate[366], that marker can be used to back gate clustered events on non-clustering parameters (Figure 29.35).

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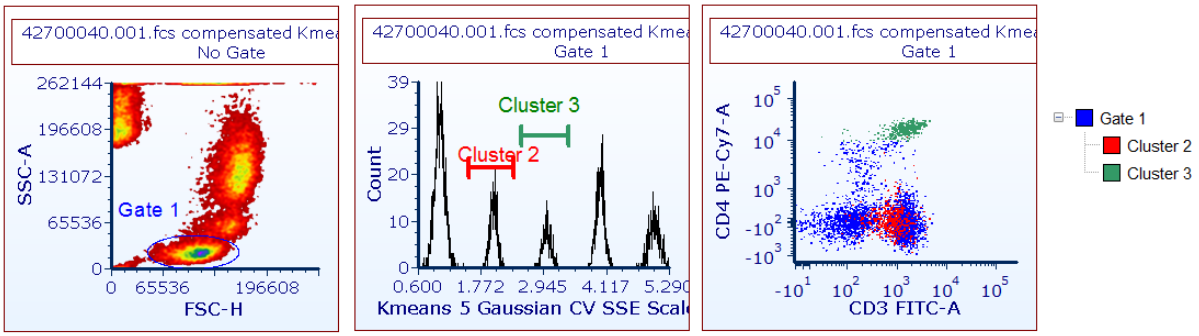

Figure 29.35. Markers can be used to select cluster on 1D plot displaying a clustering parameter. Gate 1 was defined on a density plot and events within it have been clustered. Gate 1 has also been then applied to the k-means transformed histogram. Markers from the histogram were converted to gates and back gated onto the CD4 vs CD3 dot plot.

2D plots

Gates can be created on 2D plots displaying clustering parameters as one would on 2D plots displaying non-clustering parameters. In the example below gates have been created on a 2D plot displaying clustering parameters on both X and Y axis but please consider that a clustering parameter can be displayed together with a non-clustering parameter as well (Figure 29.36).

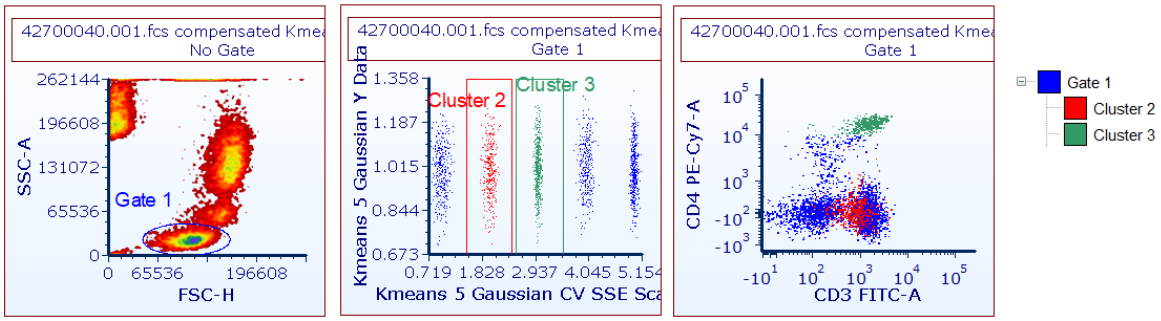

Figure 29.36. Gates can be used to select clusters on 2D plot displaying one or more clustering parameters. Gate 1 was defined on a density plot and events within it have been clustered. Gate 1 has also been then applied to the k-means transformed plots. In this example 1D Gates (i.e. Markers) have been used to select Cluster 2 and Cluster 3. Events in those gates have been back gated onto the CD4 vs CD3 dot plot.

Heatmaps

Clustering Assignments are automatically displayed on heatmaps when a transformation is applied to them. Well gates[1061] can then be used to select one or more clusters (i.e. wells) and then used for downstream analysis. In the following example (Figure 29.37) two well gates have been created on cluster 2 and 3 (well gate 2 is red highlighted on the heatmap).

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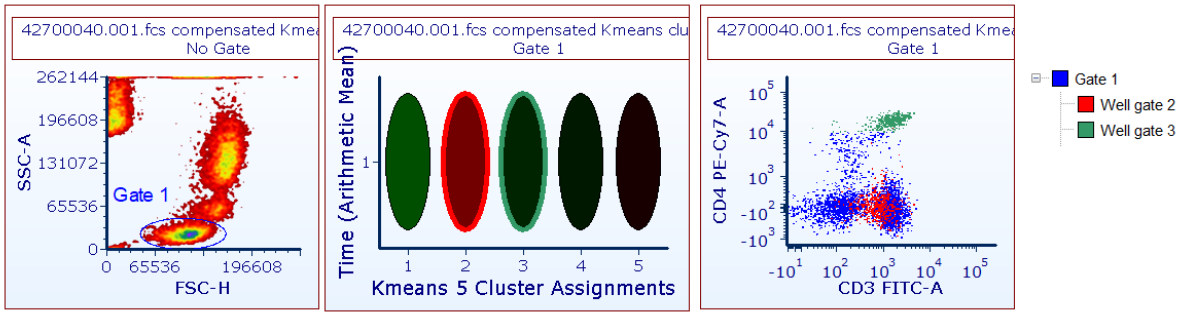

Figure 29.37. Well gates can be used to select clusters on heatmaps. Gate 1 was defined on a density plot and events within it have been clustered. Gate 1 has also been then applied to the k-means transformed plots. In this example Well Gates have been used to select Cluster 2 and Cluster 3. Events in those gates have been back gated onto the CD4 vs CD3 dot plot.

28.4 R Integration

FCS Express can integrate R scripts via the new R integration tools in the **Tools tab→Transformations→Transformations** 932 button which will launch the **Transformations** navigator. Please refer to www.r-project.org to learn more about R. The goal of R integration and implementation in FCS Express is to allow users to run their own R scripts and work on the resulting output directly within FCS Express as new parameters and cluster transformations. If you have any questions about R integration with FCS Express after reading the topic information below do not hesitate to contact support@denovosoftware.com.

Note: On the PC version of FCS Express, R and any R packages required to run R scripts, need to be installed in order to take advantage of the R integration tools. The MAC version of FCS Express does not require R to be installed on the machine. However, any R package required to run the user's R script, needs to be installed in order to take advantage of the R integration tools. Please refer the **Installing R packages on the MAC version of FCS Express 6** 976 chapter to learn more on how to install R packages on the MAC version of FCS Express.

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28.4.1 Enabling R integration in FCS Express

FCS Express integrates R via an **External Application Bridge** that can be enabled in the Startup User Options 206 by going to **File→Options→Startup** and checking the box for **Start the De Novo Software External Application Bridge on login** (29.38).

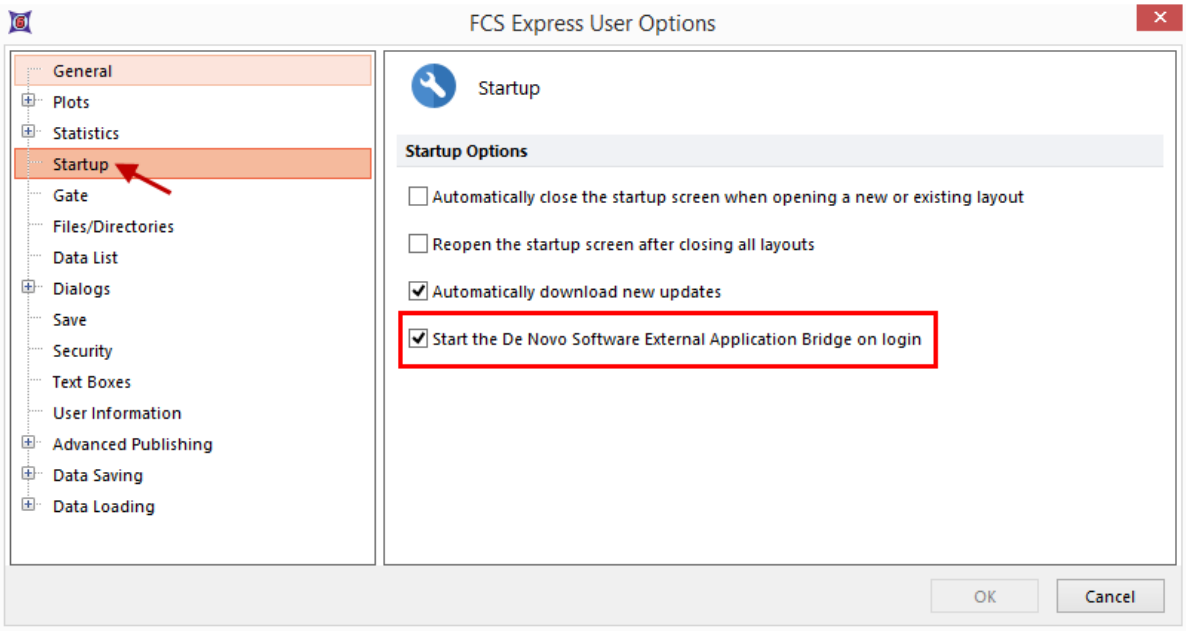

Figure 29.38 Enabling the External Application Bridge at the FCS Express startup to integrate R.

FCS Express must then be closed and reopened, including the startup screen. The next time that FCS Express is launched, a **Windows Security Alert** message by **Windows Firewall** may pop up under Windows environment. If this happen, please check both options in order to allow DNSEABridge to communicate on both Private and Public Network, and the click **Allow access** (Figure 29.39).

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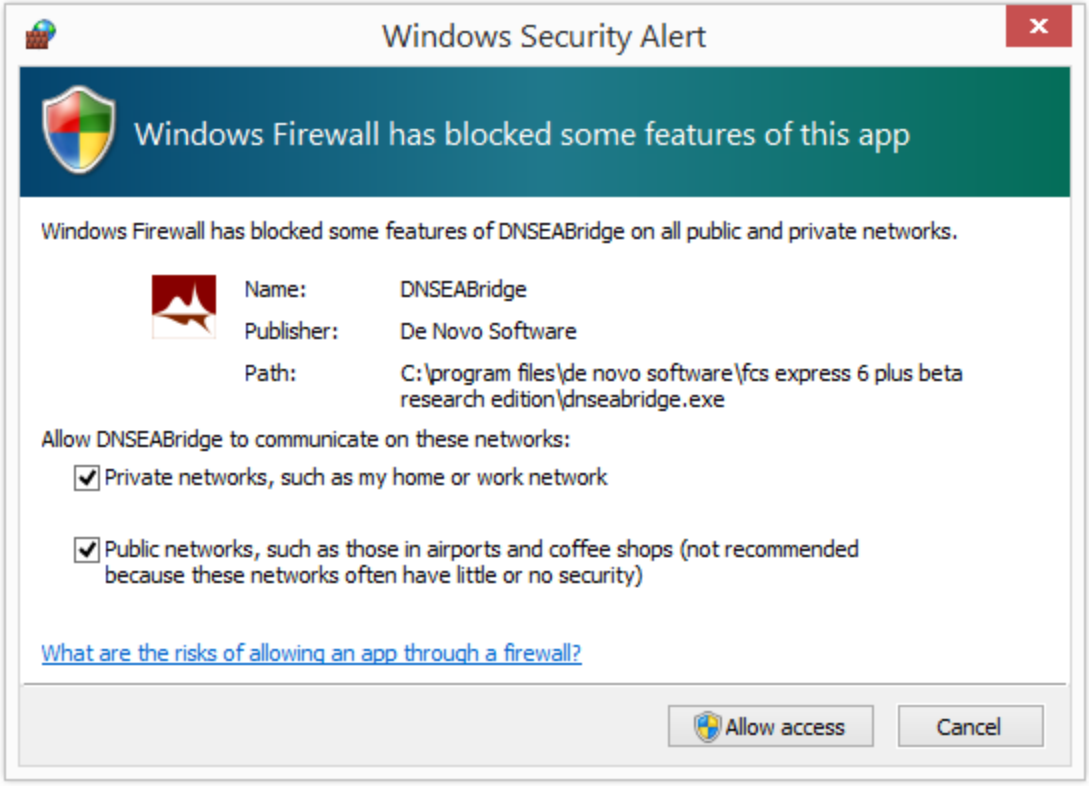

FIgure 29.39 The Windows Security Alert by Windows Firewall that may pop up under Windows environment.

Once FCS Express will be launched, an icon referring to the External Application Bridge will appear in your tray confirming that the External Application Bridge is active (Figure 29.40 and Figure 29.41).

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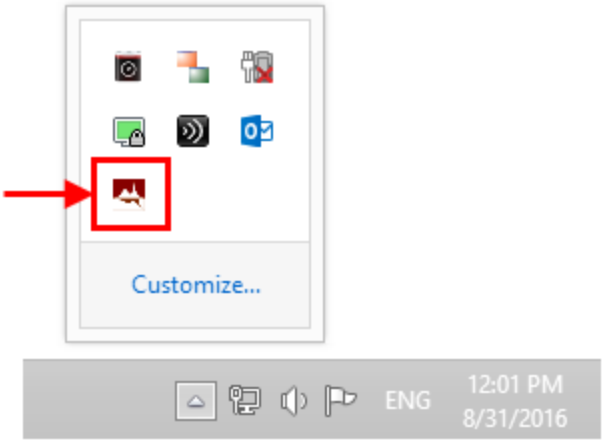

29.40 The External Application Bridge icon as it appears in the Windows tray.

29.41 The External Application Bridge icon as it appears in the macOS tray.

28.4.2 Installing R packages on the MAC version of FCS Express

MAC users running FCS Express can install R packages by running one of the following two scripts directly within FCS Express via either the **R Add Parameters** or the **R Cluster Transformation** module. The scripts download and install the packages of interest and create a text log report. The log report can be used to check that the installation worked properly and that the packages of interest can be loaded. The name of the log report file will be in the form "FCSExpress installation report YYYY-MM-DD.txt", where YYYY-MM-DD is the current date. The output directory can be defined by the user (see below). Two scripts are available base on the repository (CRAN or Bioconductor) the package is on. Please, chose the script suitable for you.

##Bioconductor Packages Installation for MAC users

```
Execute <- function(mat)
```

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```
{
  FCSE6_log_file <- file(paste0("A/FCSExpress installation report
",Sys.Date(),".txt"), open="wt") ##creating the file and opening it
for writing
  sink(FCSE6_log_file, type="message") ##diverting R messages to
the file

  ##Installing the required packages
  source("https://bioconductor.org/biocLite.R")
  biocLite("B",suppressUpdates=TRUE)

  ##Checking that the installed package can be properly loaded
  library("B")

  sink(type = "message") ## reset sink
  close(FCSE6_log_file) ##close the file
}
```

##CRAN Packages Installation for MAC users

```
Execute <- function(mat)
{
  FCSE6_log_file <- file(paste0("A/FCSExpress installation report
",Sys.Date(),".txt"), open="wt") ##creating the file and opening it
for writing
  sink(FCSE6_log_file, type="message") ##diverting R messages to
the file

  ##Installing the required packages
  install.packages("B")

  ##Checking that the installed package can be properly loaded
  library("B")

  sink(type = "message") ## reset sink
  close(FCSE6_log_file) ##close the file
}
```

Where ***A*** and ***B*** are defined by the user as follow:
A: is the path where the text log report will be saved
(e.g. /Users/*YOURNAME*/Desktop).
B: is the name of the package that need to be installed.

Please do the following before proceeding with the installation:

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1. Open a text editor (e.g. TextEdit / NotePad).
2. Copy and past one of the scripts above into a blank page (either the **Bioconductor Packages Installation for MAC users** or the **CRAN Packages Installation for MAC users**).
3. Complete the script by inserting ***A*** and ***B***.
4. Save the script using ".R" (without quotes) as the file extension.

Now that the R script is ready, you can run it in FCS Express via the R Integration tools by following the next steps:

1. Be sure that the R Integration is enabled (if not, please see the **Enabling R integration in FCS Express** 974 section of this chapter)
2. Open the **Transformation** window by selecting the **Tools** tab→**Transformations** →**Transformations** command from the ribbon bar.
3. Click on the drop down arrow adjacent to the blue plus button.
4. Select either the **R Add Parameters** or the **R Cluster Transformation**.
5. Click on the ellipsis at the right of the **Template File** field to browse for a template *.FCS file. The Transformation window will be populated with the parameters contained in the selected *.FCS file.
6. Select any of the available parameters.
7. Click on the ellipsis at the right of the **R Script File** field to browse for the R script created few steps ago.
8. Apply the transformation to any plot of your layout. This can be done via either **Drag and Drop** 987 or via the **Formatting dialog** 988.
9. A text log report will be generated in the ***A*** location.

In order to check that the installed packages can be properly loaded, you can simply open the text log report and check the output messages (if any) generated in response to the `library("B")` command.
Please, do not hesitate to contact us at support@denovosoftware.com for any question you may have.

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28.4.3 Introduction to R integration in FCS Express

Although any R function can be run by FCS Express via the R integration tool, the user should consider the following specifications for creating the R script containing the function of interest:

1. The user's functions need to be included in a general function named Execute and defined as follow:

```
Execute <- function(mat) { }
```

2. The input of the Execute function, mat, is a matrix of fluorescence values. FCS Express allows the user to select which parameters and which events will be included the matrix, as well as the matrix orientation, thus to keep events in rows and parameter in columns (Figure 29.42, on the left) or vice-versa (Figure below, on the right).
Please note that the name of the parameters included in the mat matrix will be accessible in R by using the `rownames()` or the `colnames()` functions (when parameter are in rows and in columns respectively).

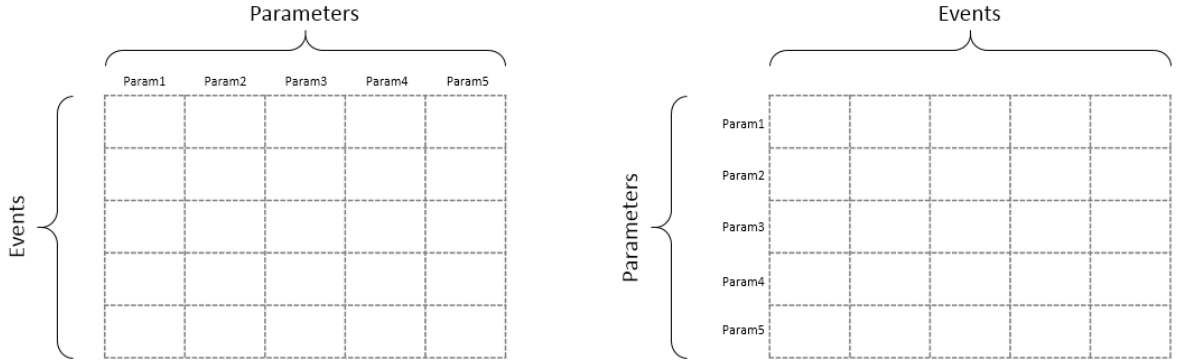

Figure 29.42. The mat matrix used as input by the Execute function.

3. The aim of the above-mentioned Execute function is to place the user defined data and meta data together in an S4 object suitable to be used by FCS Express.
4. The output of the Execute function is an S4 object containing the user defined data and the metadata. The output represents the new input for FCS Express.

The workflow of how R integration works in FCS Express is given in the figure below (Figure 29.43).

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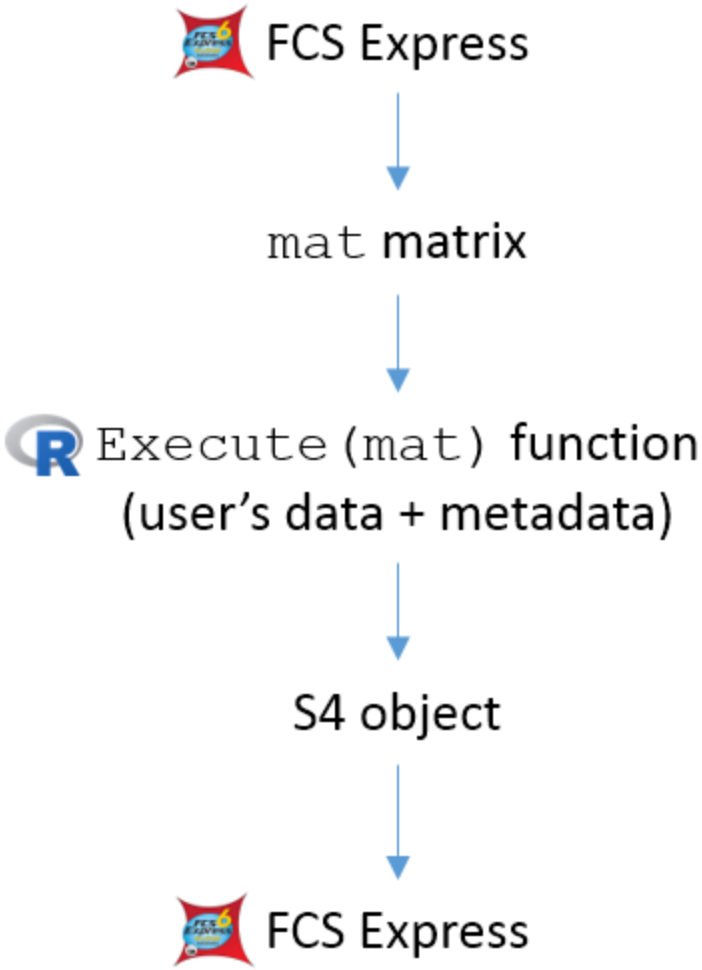

Figure 29.43. Simplified schematic of R integration with FCS Express.

FCS Express currently supports two types of S4 objects as input:

1. **R Add Parameter** 982 **-** The method is suitable for adding new columns to the `mat` matrix. Columns must contain numeric values and can be named.
2. **R Cluster Transformation** 990 -The method is suitable for cluster based analysis. A column containing the cluster membership of each event is added and clusters may be labeled.

Both input methods can be accessed by selecting the **Tools** tab→**Transformations** →**Transformations** command to launch the

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Transformations navigator. Clicking on the drop down arrow adjacent to the blue plus button (Figure 29.44) will reveal the **R Add parameters** and **R Cluster Transformation** modules described above and presented in further detail in the next sections:

Jump to:

- **R Add parameters**
- **R Cluster Transformation**

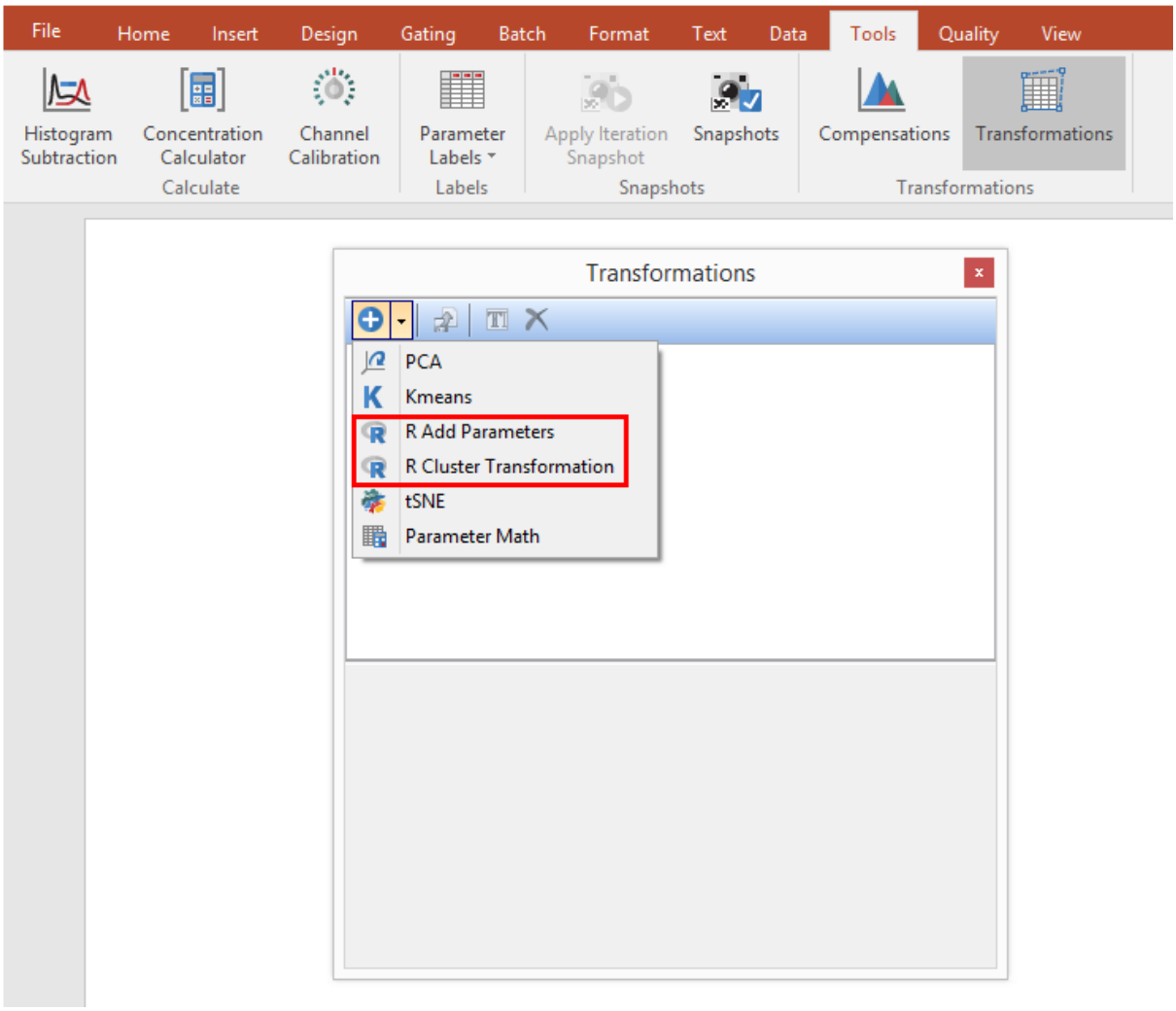

Figure 29.44. Selecting the input method for R integration scripts.

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28.4.4 R Add Parameters

New parameters can be added to a file of interest using the **R Add Parameters** input method.

To be compatible with the R Add Parameters method, the `Execute` function defined in the R script should result in an S4 object containing the following slots:

1. **numberOfNewParams** : integer of the number of new parameters;
2. **newParamData** : numeric matrix with the data for the new parameters. It has to be oriented with events in columns and parameters in rows, irrespective of the orientation set for the `mat` matrix;
3. **newParamNames** : character vector containing the names of new parameters.

A general example of an R script compatible with the **R Add Parameters** input method follows:

```
Execute <- function(mat)
{
  setClass("TransformationResult",
           representation(
               newParamData="matrix",
               numberOfNewParams="integer",
               newParamNames="character"
               ),
           prototype(
               newParamData=c(),
               numberOfNewParams=as.integer(0),
               newParamNames=""
               )
  )

  result <- new("TransformationResult")

  result@numberOfNewParams <- as.integer(A)
  result@newParamData <- B
  result@newParamNames <- C

  return(result)
}
```

Where ***A***, ***B*** and ***C*** values are defined by the user. As stated above:

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- ***A*** can be any integer number. Note that the `as.integer()` function is required since numbers are otherwise stored as object of class numeric.
- ***B*** can be the result of any user-defined transformation. ***B*** must be an object of class matrix with events in columns and parameters in rows. The number of events (i.e. the number of columns) must match the number of events in the `mat` matrix and the number of parameters (i.e. the number of rows) must match the value defined in ***A***.
- ***C*** is a vector of class character containing the names of the new parameters. Multiple names can be specified using the `c()` function (e.g. `c("name1","name2","name3")`). Please also note that the name of the parameters included in the `mat` matrix are accessible in R by using the `rownames()` or the `colnames()` functions (when parameter are in rows and in columns respectively). Thus parameter names can be derived and used as part of generating new parameter names.

An R script structured as above can be integrated into FCS Express using the **R Add Parameters** tools via the following steps:

1. Open the Transformation window by selecting the **Tools** tab→**Transformations** →**Transformations** command.
2. Click on the drop down arrow adjacent to the blue plus button.
3. Select **R Add Parameters**. The Transformation window will appear as in the picture below (Figure 29.45).
4. Click on the ellipsis at the right of the **Template File** field to browse for a template *.FCS file (Figure below - Step 1).

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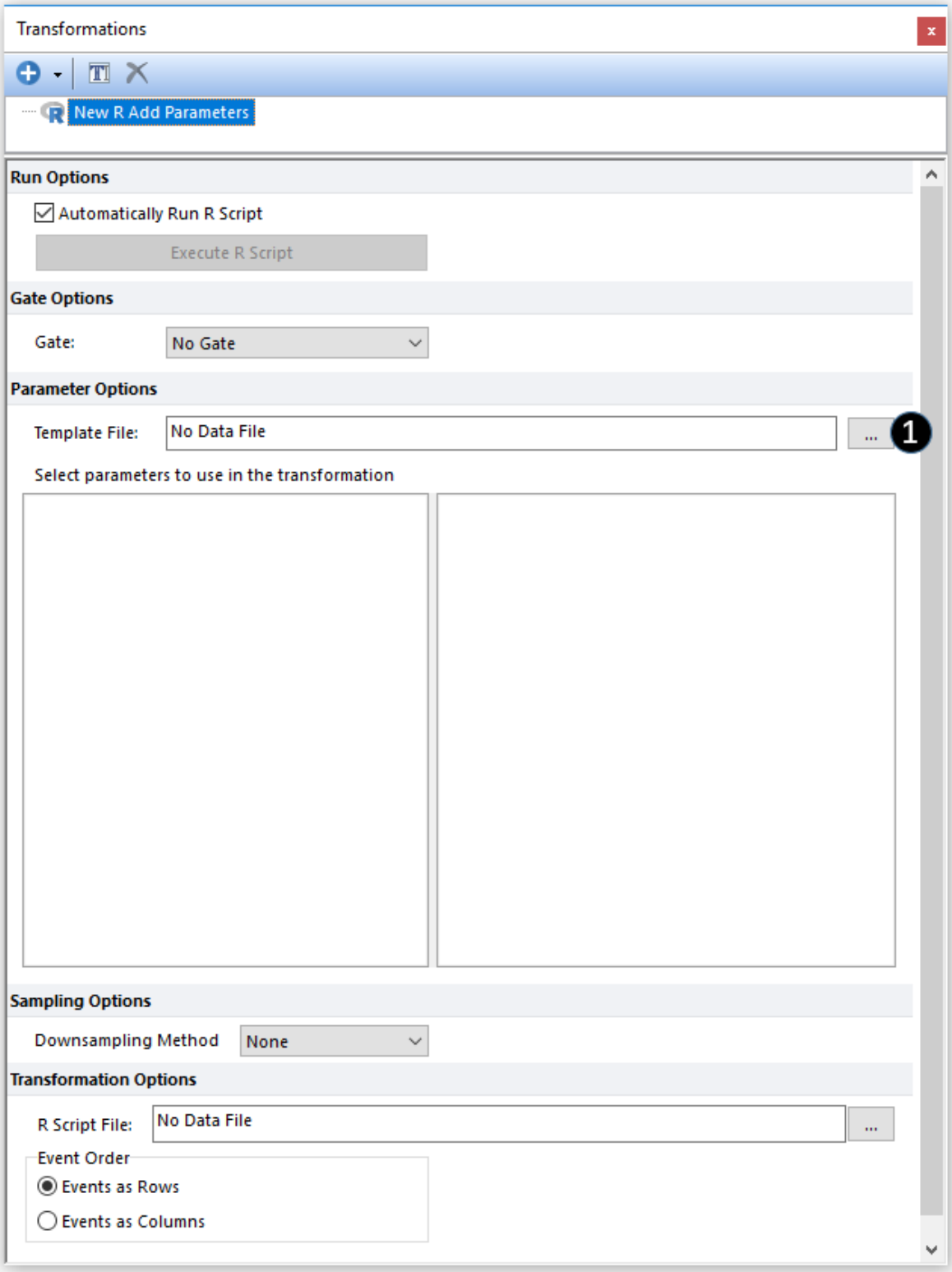

Figure 29.45. The transformation windows populated with a New R Add Parameter transformation.

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The Transformation window will be populated with the parameters contained in the selected *.FCS file (Figure 29.46).

5. Select the parameters that will be included in the `mat` matrix (Figure below - Step 2).
6. Select or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below - Step 3).
7. (Optional) Select a gate from the **Gate** drop down menu to only include the events within that gate in the `mat` matrix (Figure below - Step 4).
8. (Optional) Select a **Sampling Option** (Figure below - Step 5). Although multiple Sampling Methods are available (please refer to the Sampling Options chapter for more details), the default **Downsampling Method** for an **R Add Parameters** transformation is **None**, thus all available events (i.e. the ones defined with the **Gate** drop-down menu) will be used.
9. Click on the ellipsis at the right of the **R Script File** field to browse for an R script file (Figure below - Step 6).
10. Select the **Event Order**. This option defines the orientation of the `mat` matrix. If **Events as Row** is selected, the `mat` object will have events in rows and parameters in columns. If **Events as Columns** is selected, the `mat` object will have events as column and parameters as rows.(Figure below - Step 7).

The result of the previous steps can be seen in the Figure below:

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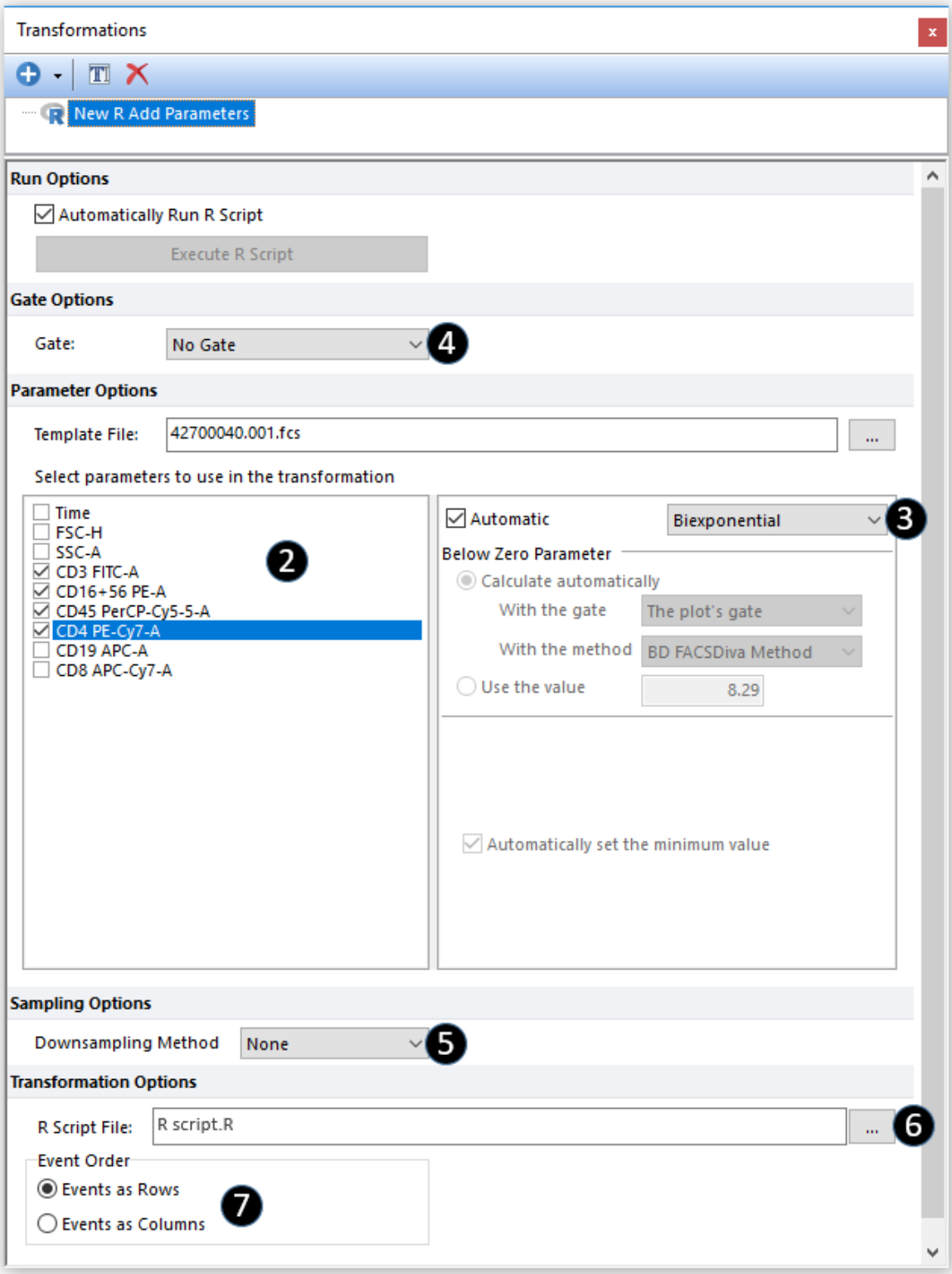

Figure 29.46. The transformation window with a fully set New R Add Parameter transformation.

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The custom **R Add Parameters** transformation is now ready to be applied to plots and Data Grids.

The R Script will be automatically run as soon as it will be applied on a plot or a data grid, or as soon as one of the option in the Transformation dialog are modified. To defer running the R Script, uncheck the box for **Automatically Run R Script**. Doing so will present you with the button to **Execute R Script** (Figure 29.47) which can be pressed to calculate on demand.

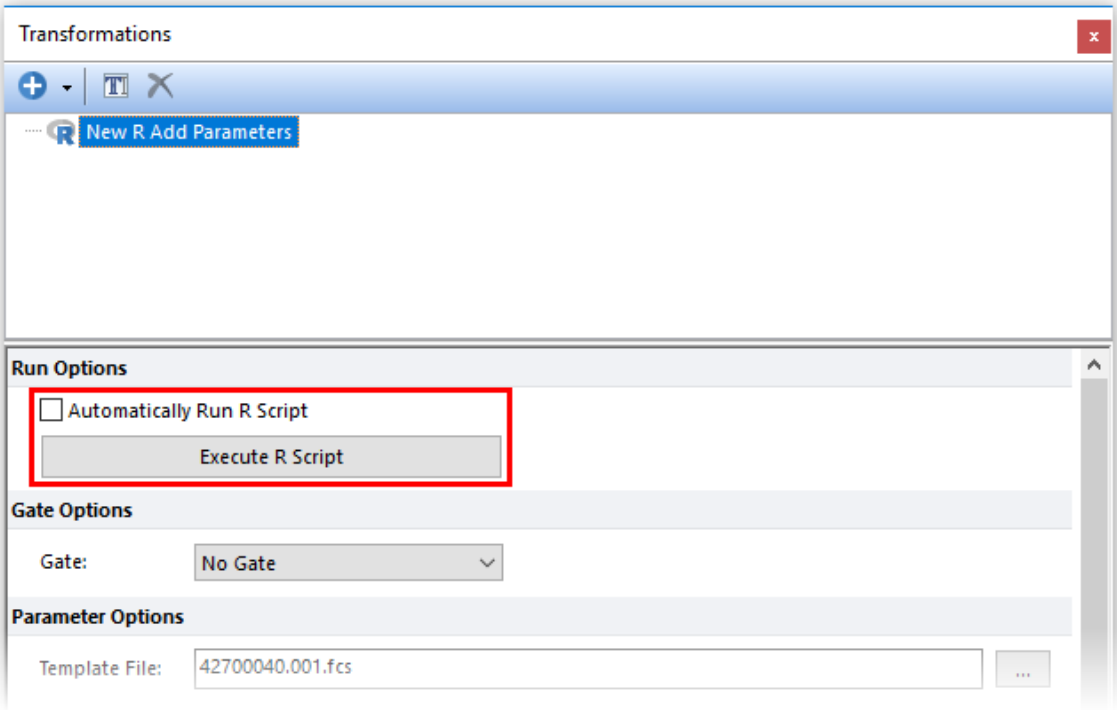

Figure 29.47 Unchecking the Automatically Run R Script box will allow the user to run the R script manually only when required.

Once an **R Add Parameters** transformation has been created, results can be accessed as 1D plots, 2D plots 172 and Data Grid 680 as new parameters. In order to do so, the R transformation must be applied to the object of interest.

R Transformations may be applied to 1D plots, 2D plots and data grids by either of the following methods:

Drag and Drop (Figure 29.48)

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1. Left click and hold on **New R Add Parameters** from the **Transformation** window.
2. Drag the transformation to the intended object (1D plots, 2D plots or a data grid).
3. Release the mouse button.

The R transformation will be applied to the object. For 1D and 2D plots the title of the plot will now include text "**R add parameters added**" indicating the transformation has been applied.

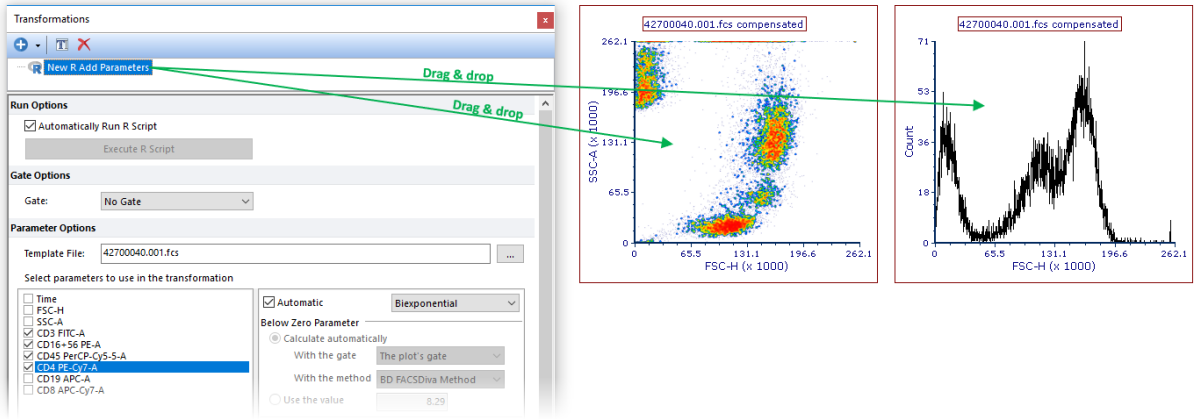

Figure 29.48 Applying an R Add Parameters transformation to a plot by dragging the transformation from the Transformation navigator to the plot (green arrows).

Using the Formatting Dialog (Figure 29.49)

1. Access the **Formatting→Overlays dialog** 284 (for 1D and 2D plots) or the **Formatting→General dialog** 310 (for Data Grids).
2. Choose the **R Add Parameters** to apply from the **Transformation** drop down.
3. Click **OK**.

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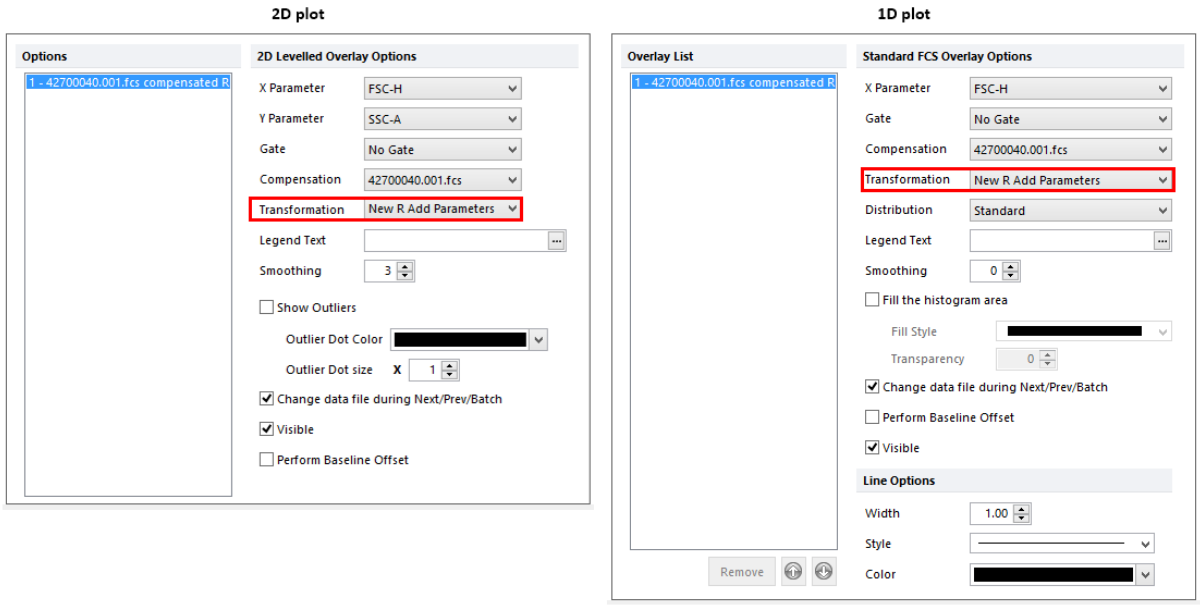

Figure 29.49. Applying an R Add Parameters transformation to a plot by selecting the R Add Parameters transformation from the Transformation dropdown of the Formatting Overlays dialog.

The result of the previous methods for 1D and 2 Plots is represented in Figure 29.50 below. When an **R Add Parameters** has been applied to a plot, the suffix "**R Add Parameters Added**" has appear in the object title.

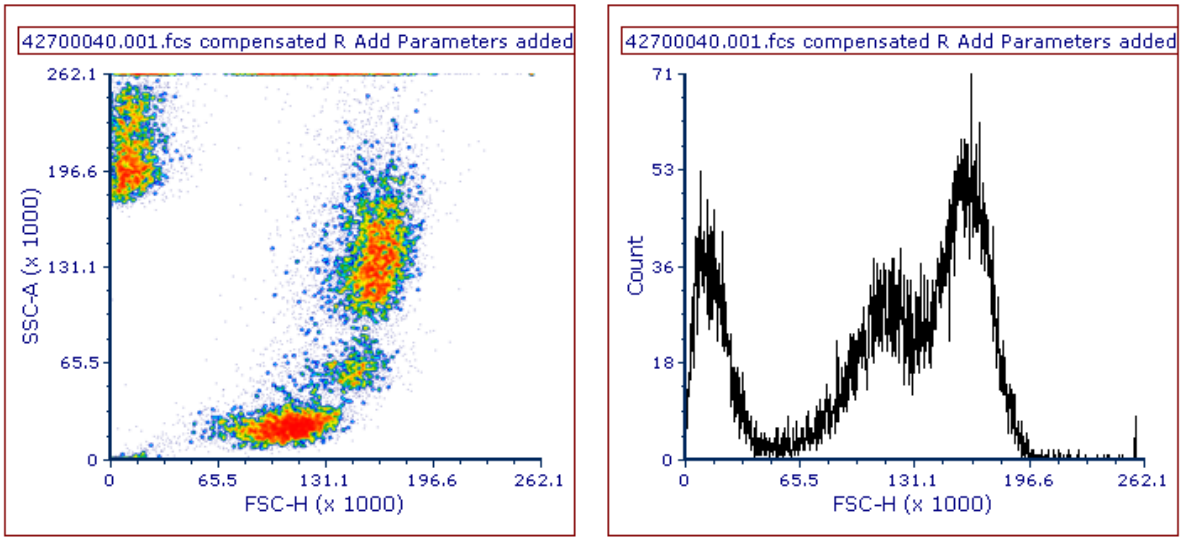

Figure 29.50. The plot title contains the "R Add Parameters added" suffix when an R Add parameters transformation is applied.

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Once the transformation is applied, transformation results can be accessed as new parameters (Figure 29.51).

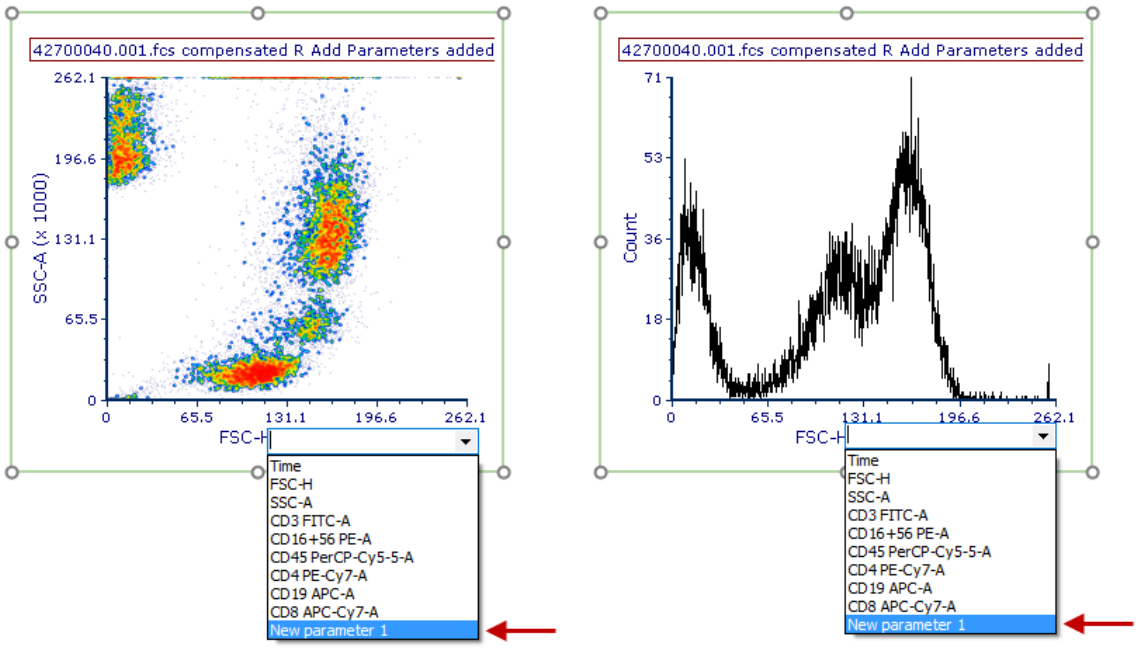

Figure 29.51 When an R Add Parameter transformation is applied to a plot, transformation results can be accessed as new parameters.

Next Topic: R Cluster Transformations 990

28.4.5 R Cluster Transformation

Customized cluster analysis can be applied to a file of interest using the **R Cluster Transformation** input method.

To be compatible with the method, the `Execute` function defined in the R script should result in an S4 object containing the following slots:

1. **clusterAssignments** : integer vector of cluster assignments;
2. **numberOfClusters** : integer of the number of new clusters;
3. **clusterNames** : character vector containing the names of new clusters.

A general example of an R script compatible with the **R Cluster Transformation** input method follows:

```
Execute <- function(mat)
{
  setClass("ClusteringResult",
           representation(
```

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```
                clusterAssignments="integer",
                numberOfClusters="integer",
                clusterNames="character"
                ),
            prototype(
                clusterAssignments=c(),
                numberOfClusters=integer(0),
                clusterNames=""
                )
  )

  result <- new("ClusteringResult")

  result@numberOfClusters <- as.integer(A)
  result@clusterAssignments <- as.integer(B)
  result@clusterNames <- C

  return(result)
}
```

Where ***A***, ***B*** and ***C*** values are defined by the user. As stated above:

- ***A*** can be any integer number. Note that the `as.integer()` function is required since numbers are otherwise stored as object of class numeric;
- ***B*** can be the membership resulting from any user-defined clustering. It must be a vector of integer values of length equal to the number of events in the `mat` matrix. The number of unique integer values must be equal to ***A***. The numbering of clusters must begin at 0.
- ***C*** is a vector of class character containing the names of the clusters. Multiple names can be specified using the `c()` function (e.g. `c("name1","name2","name3")`). The length has to be equal to ***A***.

An R script as structured above can be integrated into FCS Express using the **R Cluster Transformation** tools via the following steps:

1. Open the transformation window by selecting the **Tools** tab→**Transformations** →**Transformations** command.
2. Click on the drop down arrow adjacent to the blue plus button.
3. Select **R Cluster Transformation**. The Transformation window will appear as in the following Figure 29.52:
4. Click on the ellipsis at the right of the **Template File** field to browse for a template *.FCS file (Figure below - Step 1).

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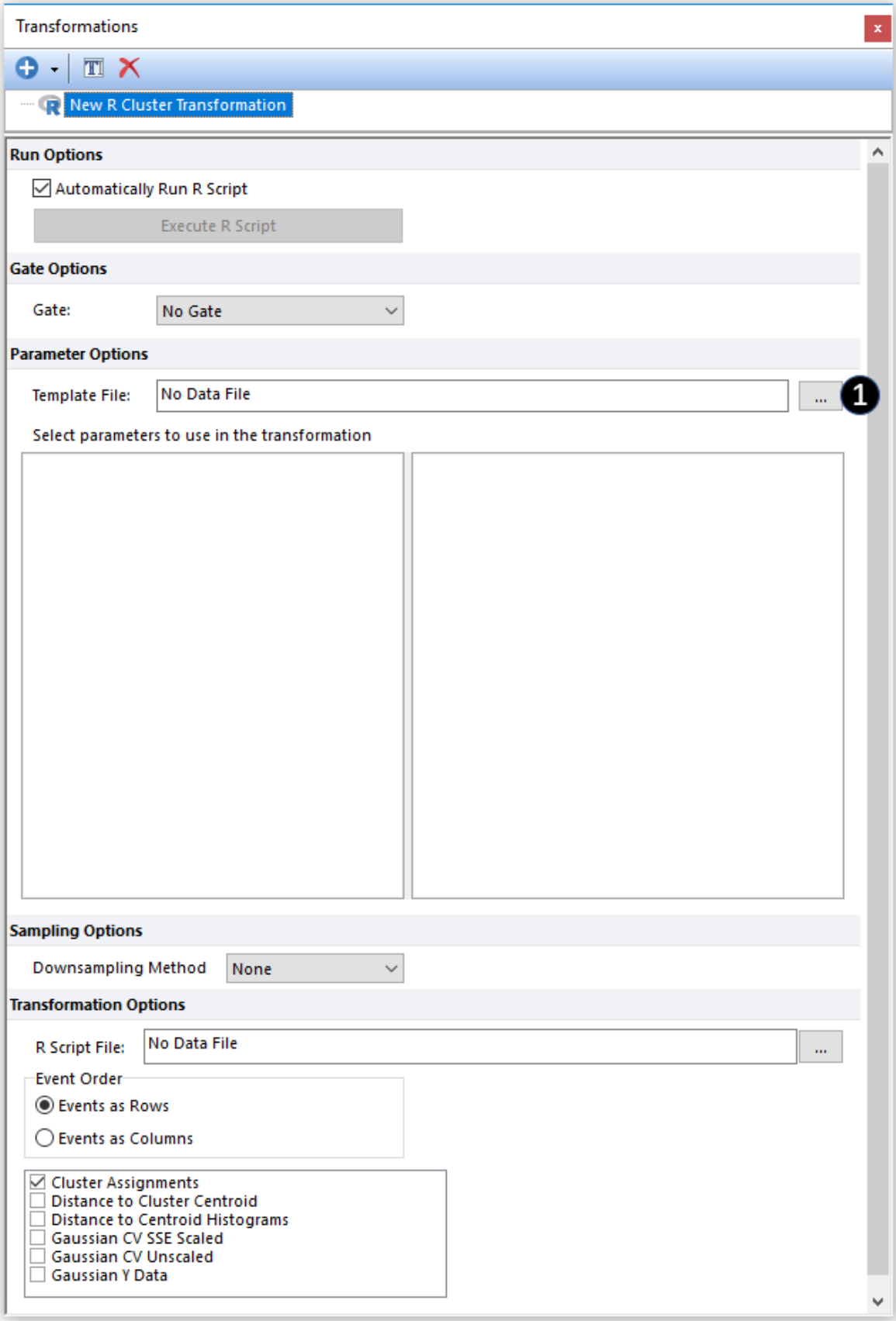

Figure 29.52. The transformation windows populated with a New R Cluster Transformation.

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The Transformation window will be populated with the parameters contained in the selected *.FCS file.

5. Select the parameters that will be included in the `mat` matrix (Figure below - Step 2).
6. Select or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below - Step 3).
7. (Optional) Select a gate from the **Gate** drop down menu to only include the events within that gate in the `mat` matrix (Figure below - Step 4).
8. (Optional) Select a **Sampling Option** (Figure below - Step 5). Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default **Downsampling Method** for an **R Cluster Transformation** transformation is **None**, thus all available events (i.e. the ones defined with the **Gate** drop-down menu) will be used.
9. Click on the ellipsis at the right of the **R Script File** field to browse for an R script file (Figure below - Step 6).
10. Select the **Event Order**. This option define the orientation of the mat matrix. If **Events as Row** is selected, the `mat` object will have events in rows and parameters in columns. If **Events as Columns** is selected, the `mat` object will have events as column and parameters as rows (Figure below - Step 7).
11. Set the output parameters for the clustering (Figure below - Step 8). Here are reported the available cluster output format.

    - **Cluster Assignment:** An internal label indicating the cluster membership assigned to each event. This label is internally used by FCS Express for heatmap representation of clusters but it is also accessible through a Data grid 680.
    - **Distance to Cluster Centroid:** The distance of a given event to the centroid of its cluster. Values are plotted as unique histogram.
    - **Distance to centroid Histograms:** The same as the Distance to Cluster Centroid but in this case values are grouped, and thus plotted, separately for each cluster. Clusters are numbered from 1 to **Number of Clusters**.
    - **Gaussian CV SSE Scaled:** In this representation each event is plotted based on its cluster membership, thus clusters appear as separated histograms. Given that cluster membership is an integer value that goes

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from 0 to **Number of Clusters-1**, an artificial noise is introduced to spread out the data. **Gaussian SSE scaled** means that the standard deviation of the Gaussian (normal distribution) used to represent each cluster is proportional to the Sum of Squared Error of the cluster.

- **Gaussian CV Unscaled:** In this representation each event is plotted based on its cluster membership, thus clusters appear as separated histograms. Given that cluster membership is an integer value that goes from 0 to **Number of Clusters-1**, an artificial noise is introduced to spread out the data. **Gaussian CV unscaled** means that the standard deviation of the Gaussian (normal distribution) used to represent each cluster is the same among clusters.
- **Gaussian Y Data:** Events are plotted following a Gaussian (normal distribution).

The result of the steps above will be the following (Figure 29.53):

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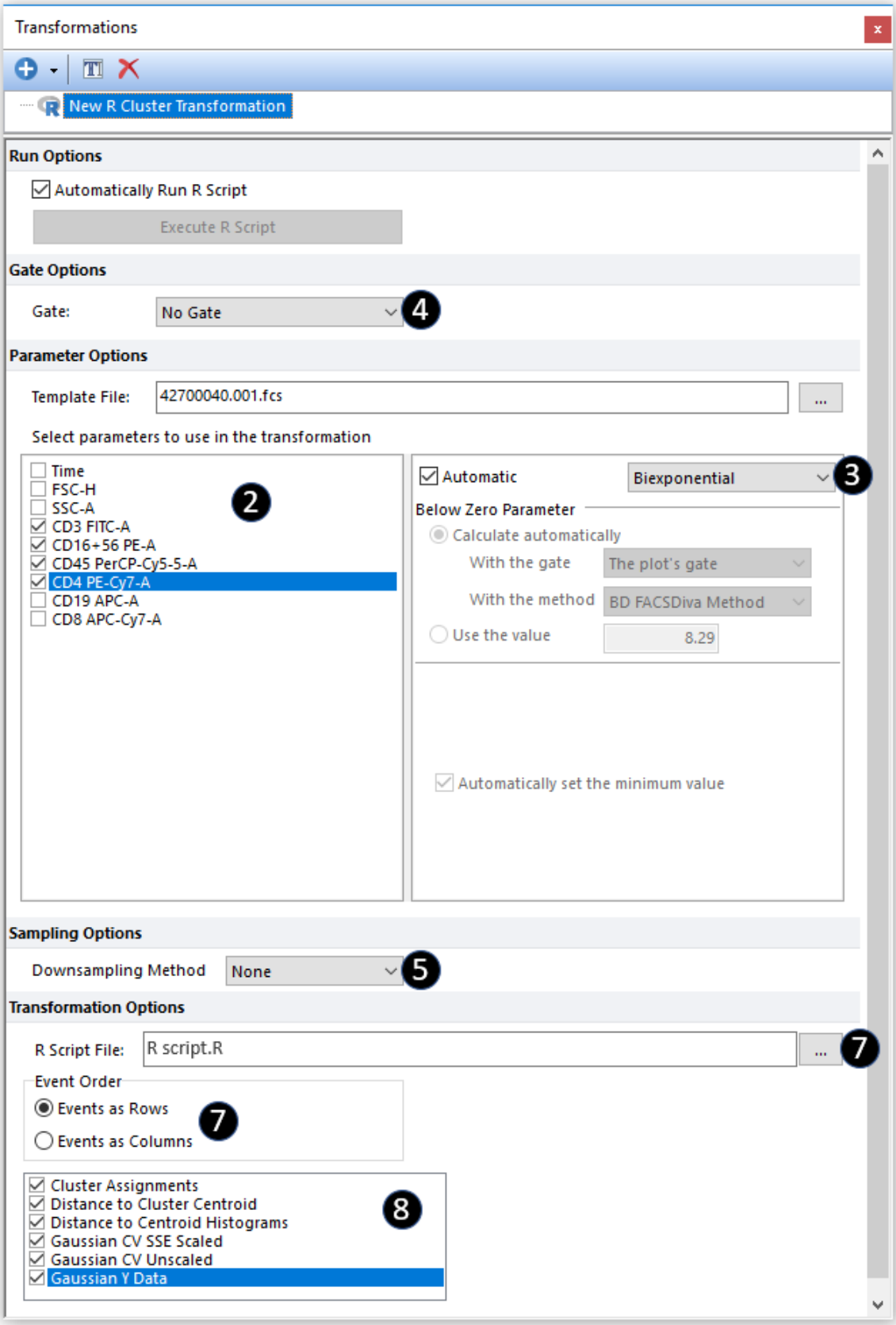

Figure 29.53. The transformation window with a fully set New R Cluster Transformation.

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The custom **R Cluster Transformation** transformation is now ready to be applied to plots.

The R Script will be automatically run when it is applied to a Heat Map or a data grid, or when one of the option in the Transformation dialog are modified. To defer running the R Script, uncheck the box for **Automatically Run R Script**. Doing so will present you with the button to **Execute R Script** (Figure 29.54) which can be pressed to calculate on demand.

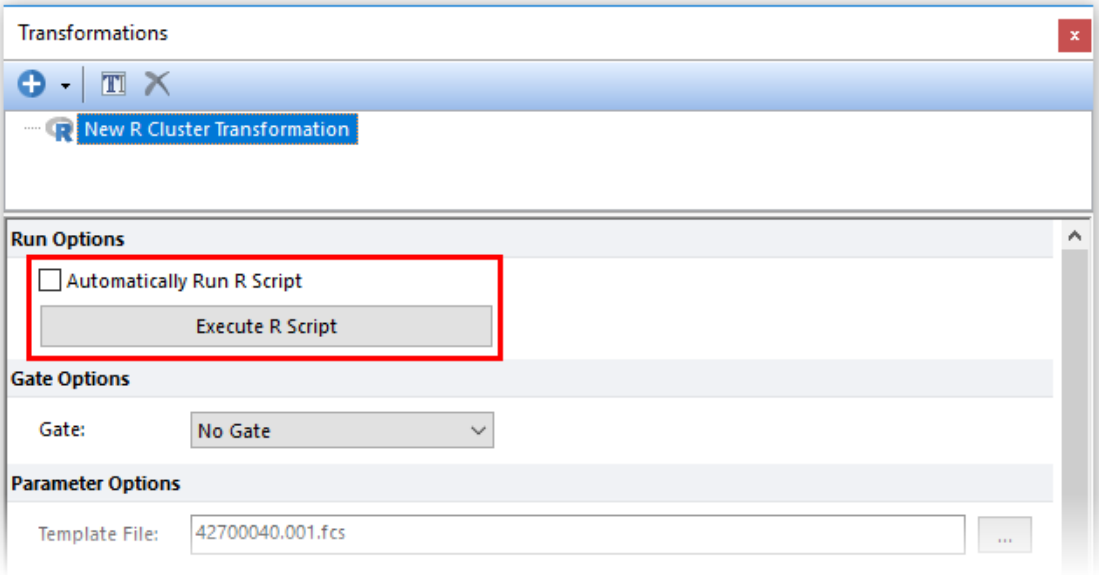

Figure 29.54 Unchecking the Automatically Run R Script box will allow the user to run the R script manually only when required.

Once an **R Cluster Transformation** transformation has been created, results can be accessed through Heat Maps 1050 and Data Grids 680. The R transformation must be applied to to the plots.

R Transformation may be applied to Heat Maps and Data grids by either of the following methods:

Drag and Drop (Figure 29.55)

1. Left click and hold on **New R Cluster Transformation** from the **Transformation** window.
2. Drag the transformation to the intended object (Heat Map or Data Grid).
3. Release the mouse button.

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The R transformation will be applied to the object. If the object is an Heat Map plot, the title of the plot will now include the text "**R Cluster Transformation clustered**" to indicate the transformation has been applied.

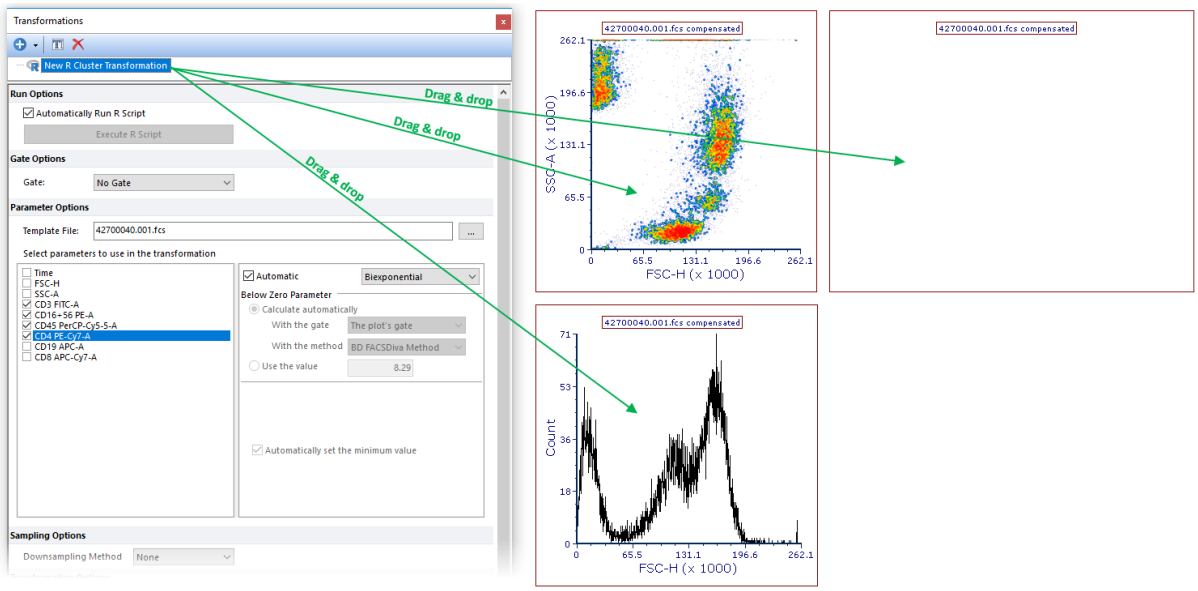

Figure 29.55. Applying an R Cluster Transformation to a plot by dragging the transformation from the Transformation navigator to the plot (green arrow).

Using the Formatting Dialog (Figure 29.56)

1. Access the **Formatting→Overlays dialog** 284 (for 1D plots, 2D plots and Heat map) or the **Formatting→General dialog** 310 (for Data Grids).
2. Choose the **R Cluster Transformation** to apply from the **Transformation** drop down.
3. Click **OK**.

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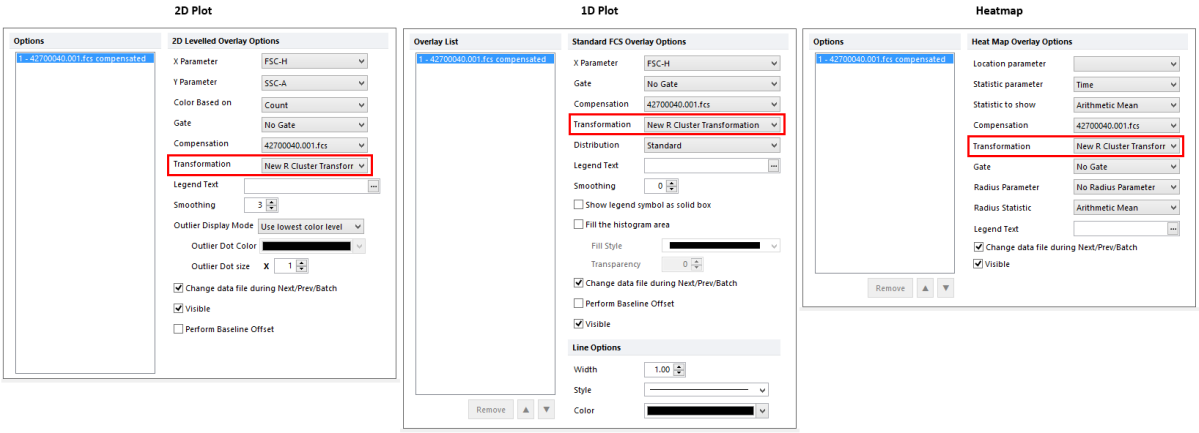

Figure 29.56. Applying an R Cluster Transformation to a plot by selecting the R Cluster transformation from the Transformation dropdown of the Formatting Overlays dialog.

The result of the previous methods for 2D plot, Heat Map and 1D plot is represented in Figure 29.57. When an R Cluster Transformation has been applied to a 2D plot, Heat Map or 1D plot, the suffix "**R Cluster Transformation clustered**" will appear in the plot title.

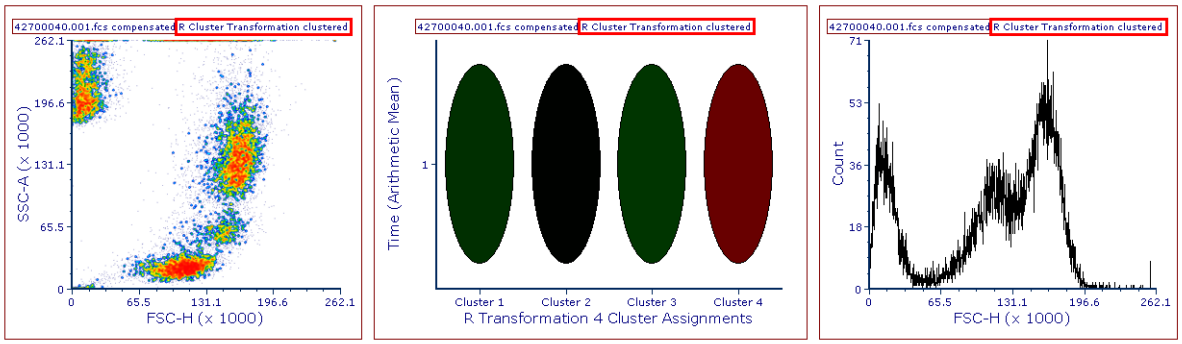

Figure 29.57. The heatmap title contains the "R Cluster Transformation clustered" siffix when an R Cluster Transformation is applied.

2D plots, 1D plots and heatmap can now be used to create gates, markers and well gates suitable for downstream analysis (please refer to the **Heat Map** section of the **Working with k-means parameter** 970 chapter for more info on how to create well gates) (Figure 29.58).

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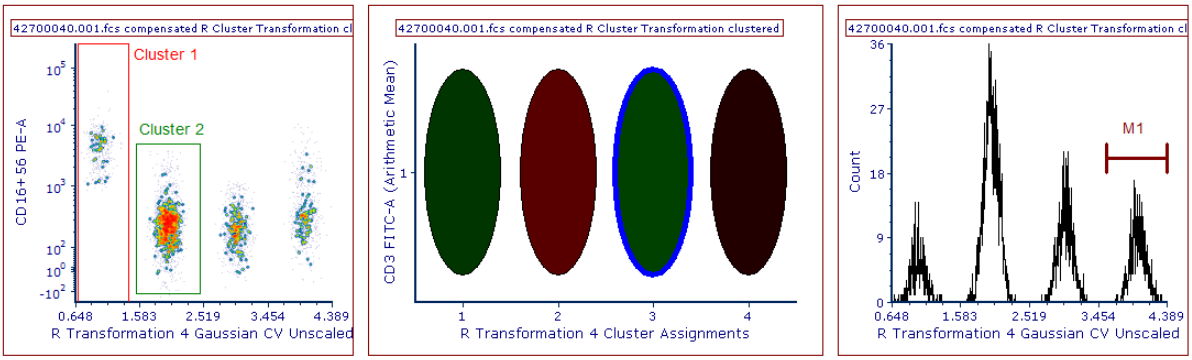

Figure 29.58 Examples of 1D Gate ("Cluster 1" gate on the left), rectangle 2D Gate ("Cluster 2" gate on the left), Well Gate (blue well gate in the Heat map) and Marker (M1 marker on the right).

28.5 tSNE (viSNE)

High-dimensional single-cell technologies, such as multicolor flow cytometry, mass cytometry, and image cytometry, can measure dozens of parameters at the single-cell level. FCS Express integrates t-Distributed Stochastic Neighbor Embedding, otherwise known as **t-SNE**, which is a tool that allows you to map high-dimensional cytometry data onto a two dimension plot while conserving the original high-dimensional structure to help you visualize and analyze high-dimensional data.

viSNE, which uses the Barnes-Hut implementation of the t-SNE algorithm, was developed by El-ad David Amir and colleagues in 2013 and is based on the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm developed by Laurens van der Maaten and Geoffrey Hinton in 2008.

Briefly, the optimization algorithm included in viSNE searches for the best low-dimensional (e.g. two or three dimensions) representation of the high-dimensional events, so that the pairwise distances between events are best conserved between the high- and the low-dimensional space.

The final result of the algorithms in FCS Express is a 2D plot in which the positions of cells reflect their proximity in their original high-dimensional space. Plots can further be colored with density or heat mapping of each parameter allowing for easy visualization of populations.

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Given that t-SNE is an highly demanding algorithm, in 2014 Laurens van der Maaten developed a faster version of the tSNE algorithm exploiting the Barnes-Hut algorithm. The Barnes-Hut method gives an approximate result of tSNE but it is faster than the exact tSNE. FCS Express allows the user to use both the exact tSNE algorithm and the Barnes-Hut approximation. Moreover, the developers team at De Novo Software worked hard to make both the exact algorithm and the Barnes-Hut approximation even faster. You can find a performance test in the next chapter 1001.

Although t-SNE is extremely useful for visualizing high-dimensional data, t-SNE plots can be mysterious and esoteric. For a more comprehensive understanding of t-SNE, please click here.

More details on viSNE/tSNE can be found in the following publications:

- Van der Maaten, L. Accelerating t-SNE using Tree-Based Algorithms. J. Mach. Learn. Res. 15, 3221-3245 (2014).
- Amir el-AD et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat. Biotechnol.* 31, 545–552 (2013).
- Van der Maaten, L. & Hinton, G. Visualizing data using t-SNE. *J. Mach. Learn. Res.* 9, 2579–2605 (2008).

Additional resources:

- Github by Laurens van der Maaten, co-author of original implementation: https://lvdmaaten.github.io/tsne/
- Distill article discussing the effective use of t-SNE: http://distill.pub/2016/misread-tsne/

To begin working with viSNE in FCS Express please see the topics on:

- **t-SNE performance in FCS Express V6** 1001
- **Defining a tSNe transformation** 1003
- **Applying a tSNe transformation** 1014
- **Working with tSNE transformations** 1016
- **Saving a tSNE transformation** 1021
- **t-SNE FAQ**

28.5.1 tSNE performance in FCS Express

Given that t-SNE is an highly demanding algorithm, the development team at De Novo Software made a great effort to improve the speed of running the algorithm with FCS Express.

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To give you an idea of how t-SNE is performing within FCS Express, a performance test has been run comparing FCS Express and the independent R package in running both the exact tSNE algorithm and the Barnes-Hut approximation.

Here is a brief description of how the test was run:

File	Machine	FCS Express	R
• FCS format version: 3.0 • Total number of events: 500397 (different sample sizes were used in the performance test) • Total number of parameters: 40 (20 parameters have been selected in the performance test)	• CPU: Intel Core i5-4200M CPU 2.5GHz • Memory: 8Gb • System type: Windows 8.1 64 bit	• Version: 6.00.0045 (64 bit)	R version 3.3.1 (2016-06-21) R Studio Version 0.99.903

The graph below (**Figure 29.59**) show the elapsed time (in minutes) for t-SNE calculation (using both the **exact t-SNE** and the **Burnes-Hut Approximation (Amount of Approximation = 0.5)**) in both FCS Express and R. A fixed number of parameters (i.e. n=20) and different numbers of events have been used. t-SNE was not estimated for unsampled events. Please refer to the Defining a tSNE transformation 1003 chapter for more information about all the above mentioned options.

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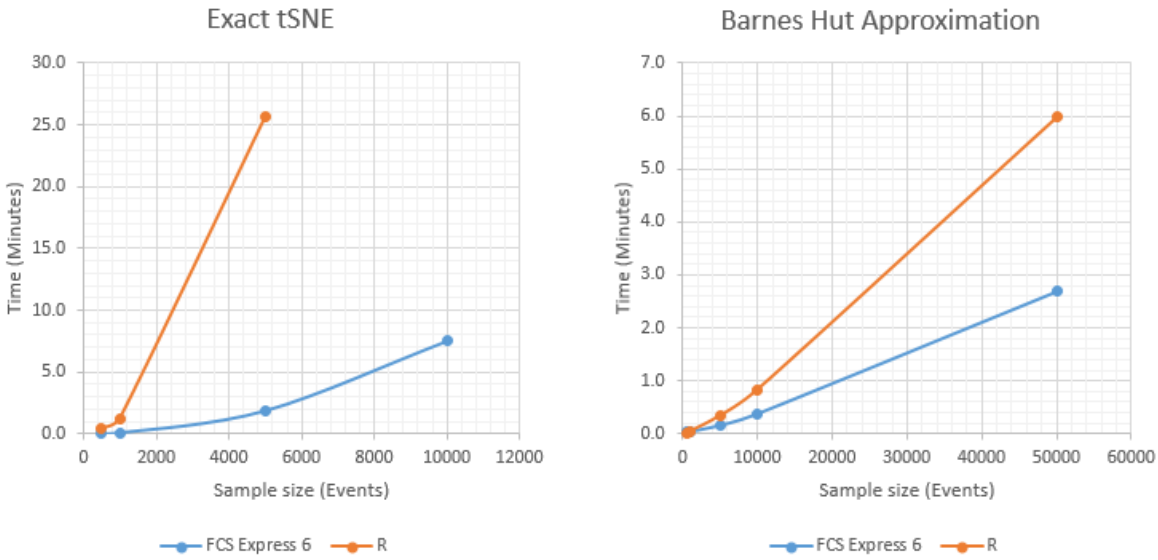

Figure 29.59 - Performance comparison between FCS Express and R in running both the exact tSNE and the Barnes-Hut Approximation.

28.5.2 Defining a tSNE transformation

tSNE transformations are performed in FCS Express through the **Transformations** navigator.
The steps below outline the process of creating a new tSNE transformation for your data:

1. Open the Transformation window by selecting the **Tools** tab → **Transformations** → **Transformations** command (Figure 29.60, Step 1). The **Transformations** window will open as in the figure below.
2. Click on the dropdown arrow adjacent to the blue plus button (Figure below, Step 2).
3. Click on **tSNE** in the dropdown list (Figure below, Step 3).

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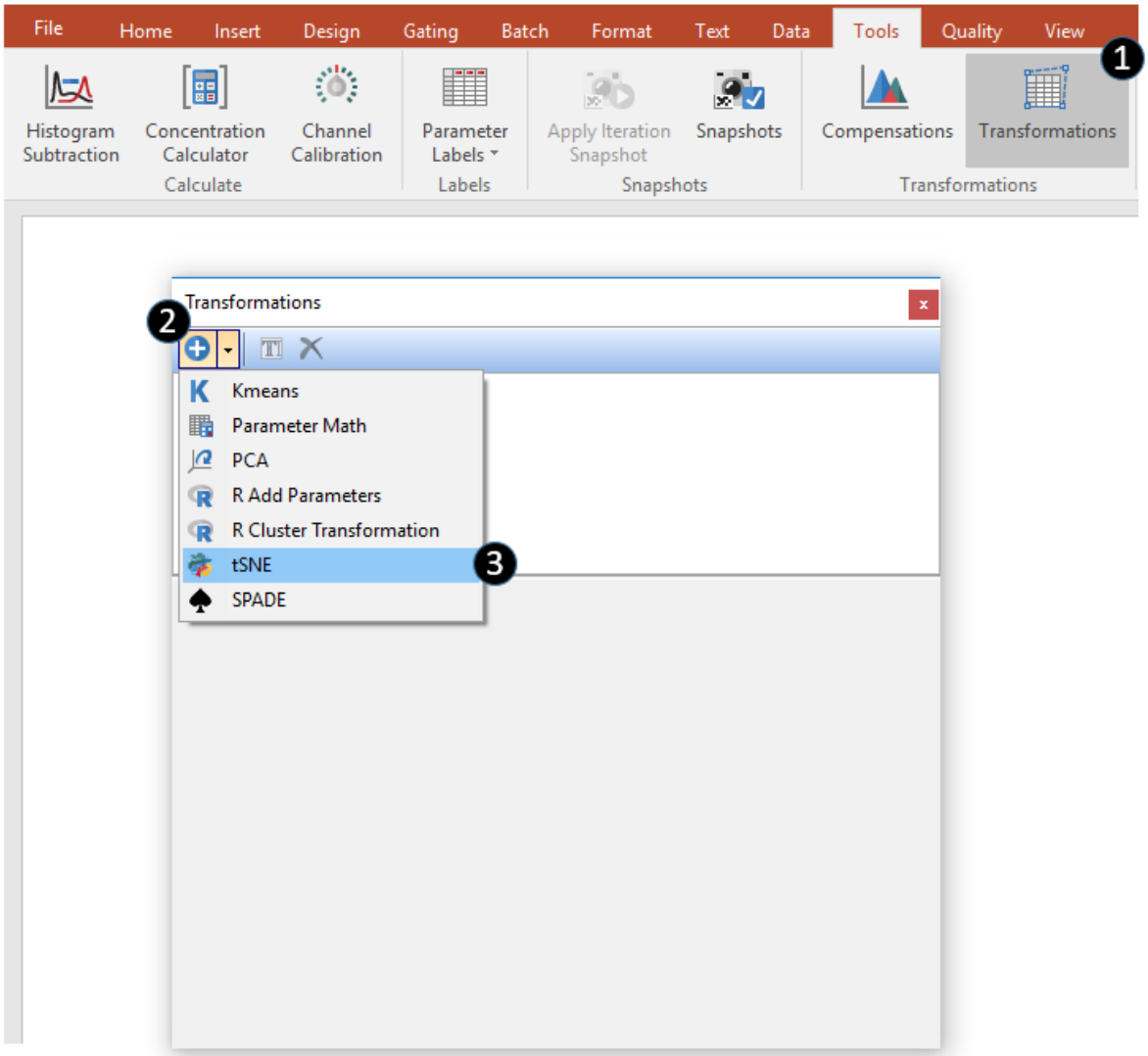

Figure 29.60 Initial steps to create a tSNE transformation.

The **tSNE** dialog will now appear in the **Transformation** window as seen in Figure 29.61.

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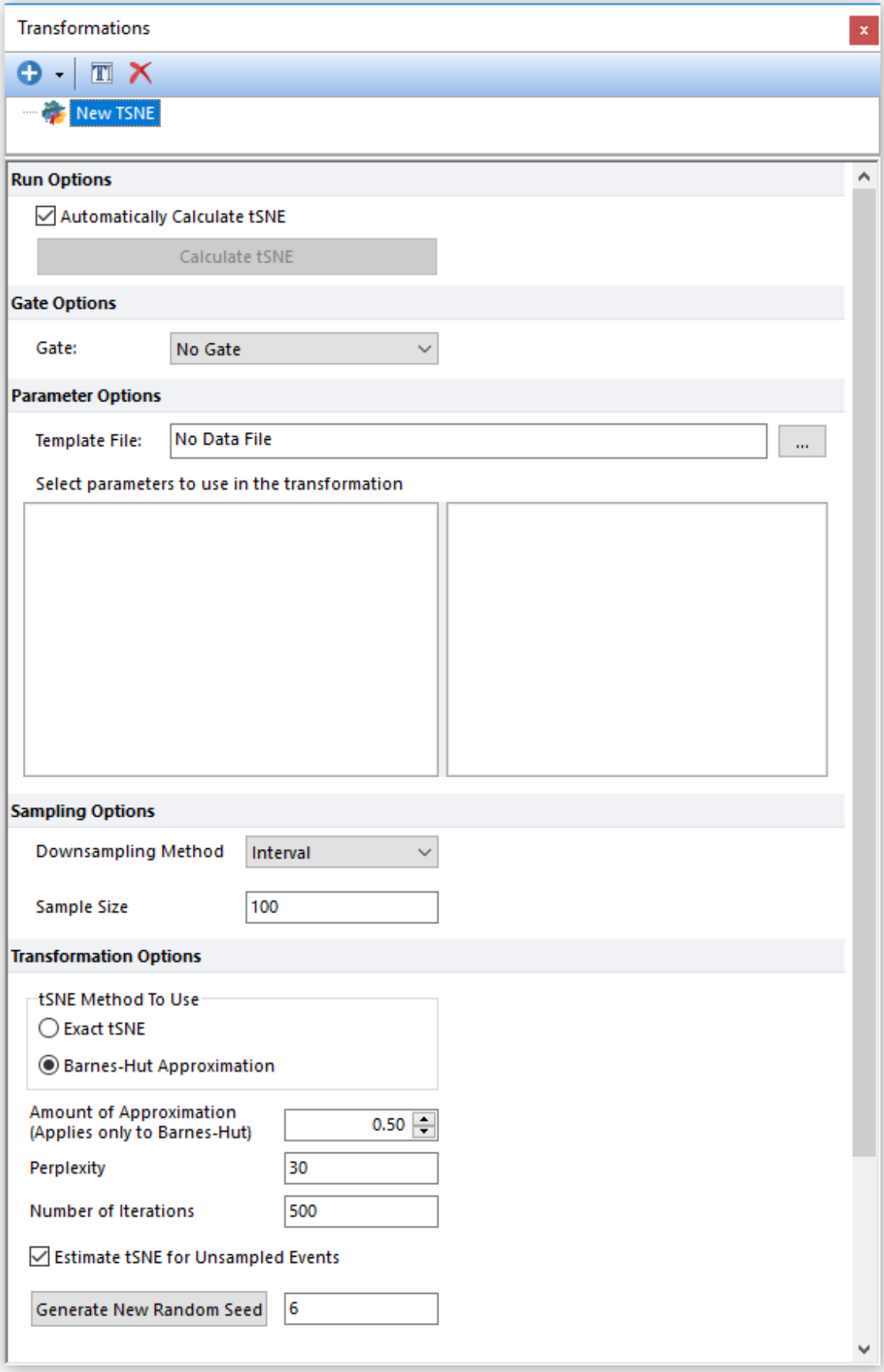

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Figure 29.61 - The tSNE dialog opens when a new tSNE transformation is created.

The new tSNE transformation is named as **New TSNE** by default but it can be easily renamed by either of the following methods:

- Right click on the tSNE transformation that needs to be renamed→Select **Rename**.
- Select the tSNE transformation that need to be renamed via left click and press **F2** on your keyboard.
- Select the tSNE transformation that need to be renamed via left click and click on the button in the **Transformations** tool bar.

4. Choose a **Template File** for the tSNE transformation by clicking on the ellipsis to the right of the **Template File** text box.

   Note: if you wish to define the tSNE transformation on your entire dataset rather than an individual datafile, you can use a merged datafile with a File Identifier Column 671 as the Template File. The transformation can then be applied 1014 to the merged datafile, and events therein can be related back to the original datafiles with the File Identifier Column 671.

The tSNE dialog will be populated with a list of available parameters to perform a tSNE transformation based on available parameters from the template file (Figure 29.62).

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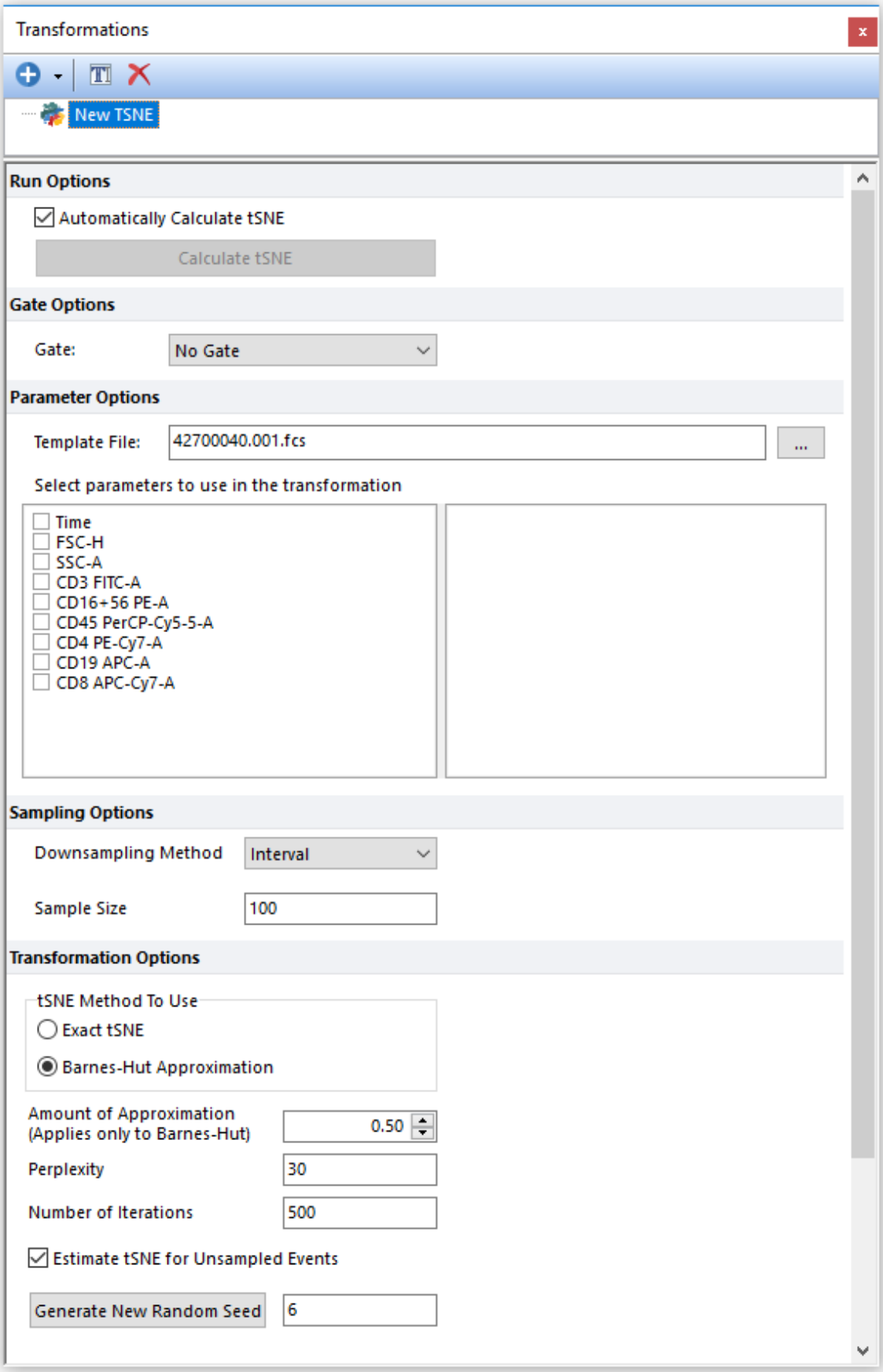

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Figure 29.62 When a template file is selected, the possible parameters to perform a tSNE transformation are automatically listed in the tSNE dialog.

5. Choose the parameters to be used in the tSNE transformation by checking the boxes next to the parameter names (Figure 29.63 below, Point 1).
6. Select or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below, Point 2).

Options can now be set to customize the transformation.

Options	Description
Gate	Allows the user to run tSNE only on the events within a specific gate.
tSNE Method to Use	Allows the user to set the method to use for running tSNE. Possible choices are **Exact tSNE** and **Barnes-Hut Approximation**. The Exact tSNE is the original implementation of the algorithm and is only practical for sample sizes less than 5000 due to speed and memory constraints of this approach. The Barnes-Hut method gives an approximate result of tSNE and is faster than the exact tSNE for larger data sets but there will be little difference in calculation times for smaller data sets. The amount of approximation for the Barnes-Hut method can be specified in the **Amount of Approximation (Applies only to Barnes-Hut)** field (see next option). In FCS Express 6, the default Method for tSNE is the **Barnes-Hut Approximation.**
Amount of Approximation (Applies Only to Barnes-Hut)	Allow the user to set the **Amount of Approximation** for the Barnes-Hut method. The Amount of Approximation is a trade-off between speed and accuracy of the approximation. The value ranges from 0 to 1, where higher values indicate a faster computation time but a less accurate approximation and lower values results in a slower computation time but a more accurate approximation. If the approximation value is less than 0.2, the exact tSNE method may be used as well as the Barnes Hut approximation may be slower than the exact tSNE method. In FCS Express 6, the default **Amount of Approximation** is 0.50.

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Perplexity	**Perplexity** is a measure for information. In tSNE, the perplexity is used to sets the number of nearest neighbors considered. In tSNE, typical values for the perplexity range between 5 and 50. In FCS Express 6, the default value for **Perplexity** is 30.
Number of Iterations	tSNE in an iterative process. This option allows you to set the number of iteration that will be performed by the algorithm. In FCS Express 6, the default **Number of Iterations** is 500.
Sampling Options	tSNE can be performed on a subsample of the data with the rest of the data subsequently mapped to the transformed subsampled data after the initial run (see the **Estimate tSNE for Unsampled Events** option). Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default setting for a tSNE transformation are: **Downsampling Method**. Default setting is **Interval**. **Sample Size**. This parameter indicates how many cells to subsample and perform tSNE on. If the specified **Sample Size** is higher than the number of available events, tSNE will be run on all available events. Note that the number of available events is dictated by the **Gate** option. In FCS Express 6, the default **Sample Size** is 100.
Estimate tSNE for Unsampled Events	This option determines whether events that did not participate in the tSNE calculation (if you chose less than the total number of events in the **Sample Size** option) will be mapped to the nearest point that did participate in the tSNE mapping. If this option is unselected, events that did not participate in the tSNE calculation will be have undefined values in the resulting tSNE parameters and will not be plotted, participate in statistical calculations of the tSNE parameters or able to be gated based on the tSNE parameters. In FCS Express 6, tSNE is Estiamted by default for Unsampled Events.

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Change Random Seed	The Random Seed is a number which is used by the random number generator to generate the N random values which are used as input data points for the tSNE calculation. The random number generator will use the Random Seed value and perform a series of math operations on it which result in N random values. Since tSNE is a stochastic algorithm, a seed can be set to make results reproducible over the time when tSNE is applied to the same data. Alternatively, the seed can be changed when a different tSNE map is desired. The seed can be changed by either clicking on the **Change Random Seed** button or by manually inserting a seed in the field. In FCS Express 6, the default **Random Seed** is 6.
Automatically Calculate tSNE	By default tSNE transformation is recalculated as soon as an option in the tSNE dialog is changed. Given that tSNE is an highly demanding algorithm that can take several seconds to be calculate, FCS Express allows the user to disable the automatic calculation of tSNE and to manually calculate tSNE only when required. To defer calculating the tSNE algorithm, uncheck the box for **Automatically Calculate tSNE**. Doing so will present you with the button to **Calculate tSNE** which can be pressed to calculate tSNE on demand. Please note that this option only regulates the autoupdate upon changes made within the tSNE dialog. If tSNE is calculated on a gate and that gate is moved or resized, tSNE will be automatically recalculated regardless this option. If you want to avoid tSNE autoupdate upon gate moving/resizing, please refer to the Live Updating Gate 317 chapter of this manual.

7. (Optional) Select a gate from the **Gate** drop down menu to only run tSNE transformation on the events within that gate (Figure below, Point 3).

8. (Optional) Select a **Downsampling Method** (Figure below, Point 4). Although multiple Sampling Methods are available (please refer to the Sampling

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Options 1036 chapter for more details), the default **Downsampling Method** for a **tSNE** transformation is **Interval**.

9. Enter a value in the **Sample Size** field by typing the number of events on which tSNE will be performed (Figure below, Point 5).

10. Select which method to use for tSNE transformation. **Exact tSNE** or the **Barnes-Hut Approximation** (Figure below, Point 6).

11. If the Barnes-Hut approach is selected, set the **Amount of Approximation** for the Barnes-Hut method in the **Amount of Approximation (Applies only to Barnes-Hut)** field (Figure below, Point 7).

12. (Optional) Change the **Perplexity** by manually inserting a value in the field (Figure below, Point 8).

13. (Optional) Change the **Number of Iterations** that will be performed by manually inserting a value in the field (Figure below, Point 9).

14. (Optional) Select whether to **Estimate tSNE for Unsampled events** (Figure below, Point 10).

15. (Optional) Change the the random seed used for tSNE calculation by either clicking on the **Change Random Seed** button or by manually inserting a seed in the field (Figure below, Point 11).

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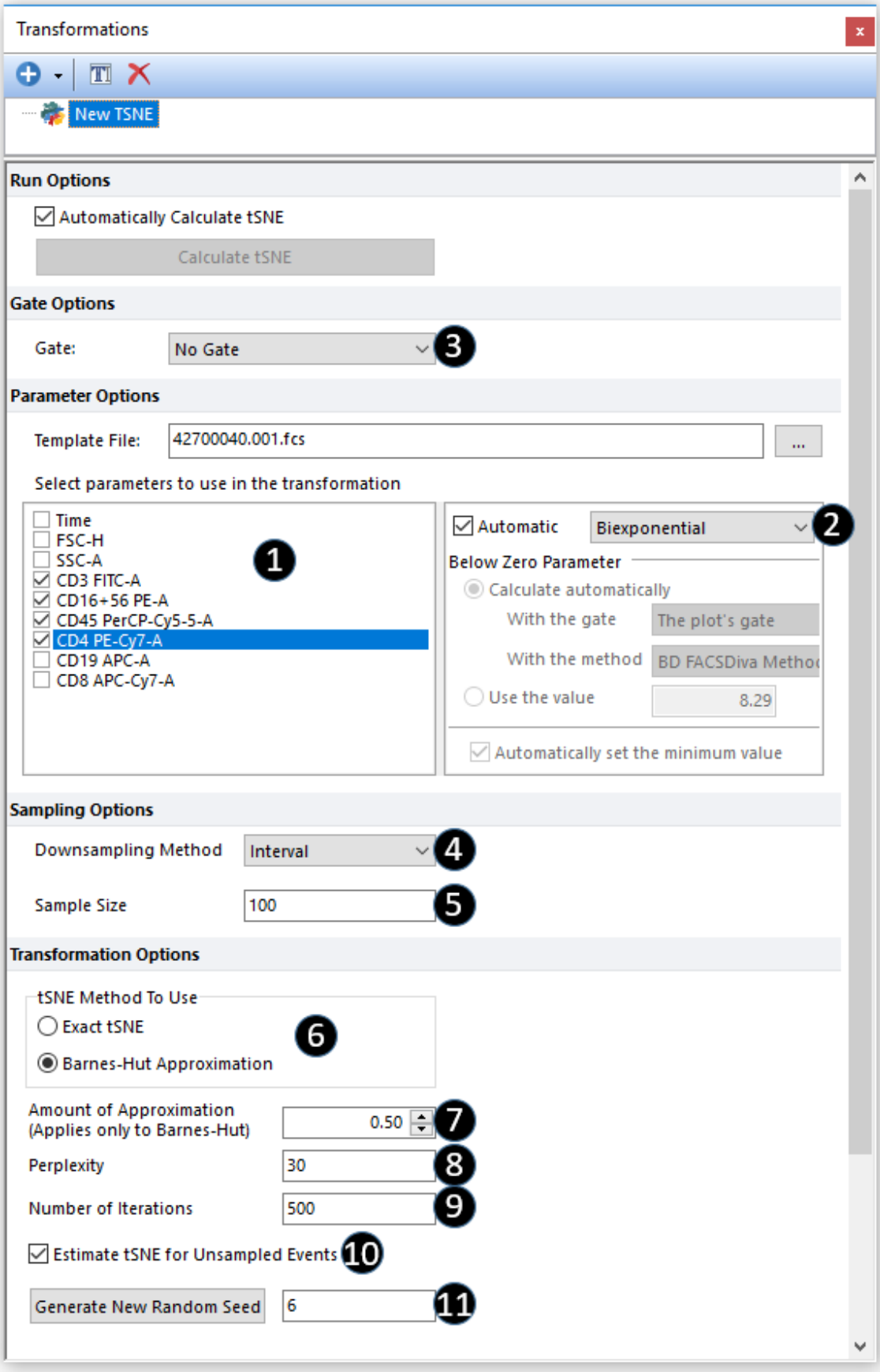

Figure 29.63 Steps to complete the tSNE dialog.

The tSNE transformation has now been defined and will be automatically calculated as soon as it will be applied to a plot[1014]. In addition, tSNE transformation will be automatically recalculated as soon as an option in the tSNE dialog will be modified.

Given that tSNE is an highly demanding algorithm that can take several seconds to be calculate, FCS Express allows the user to disable the automatic calculation of tSNE upon options changing and to manually calculate tSNE only when required. To defer calculating the tSNE algorithm, uncheck the box for **Automatically Calculate tSNE**. Doing so will present you with the button to **Calculate tSNE** (Figure 29.64) which can be pressed to calculate on demand. Please note that this option only regulates the auto update of tSNE upon changes made within the tSNE dialog. If tSNE is calculated on a gate and that gate is moved or resized, tSNE will be automatically recalculated regardless this option. If you want to avoid tSNE autoupdate upon gate moving/resizing, please refer to the Live Updating Gate[317] chapter of this manual.

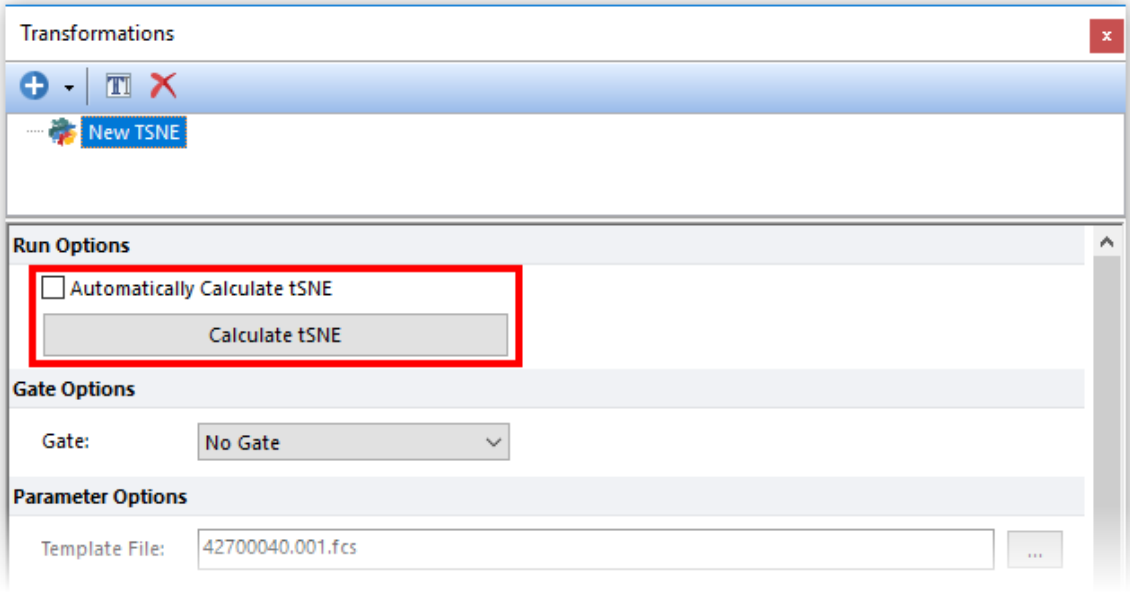

Figure 29.64 Unchecking the Automatically Calculate tSNE box will allow the user to calculate tSNE manually only when required.

The tSNE transformation can now be applied to any plot in FCS Express and the new tSNE X and tSNE Y parameters chosen from the list of available parameters

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(Figure 29.65 below). Please also see the Applying a tSNE Transformation 1014 section for additional details on applying tSNE mapped transformations to plots.

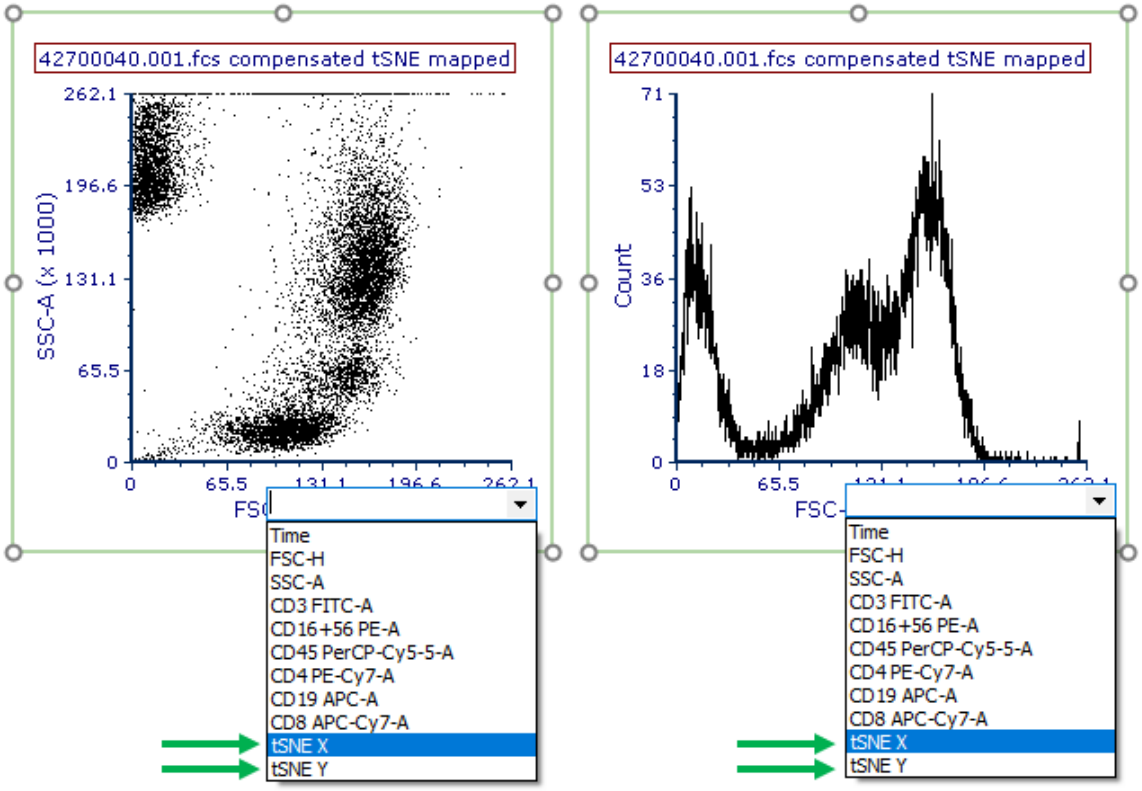

Figure 29.65 tSNE parameters can be accessed as standard parameters.

28.5.3 Applying a tSNE transformation

Once a tSNE transformation has been defined 1003, it may be applied to any plot in FCS Express. Once a tSNE transformation is applied to a plot, standard gating and statistics may be used to study the result of the tSNE transformation.

Note: When a tSNE transformation has been applied, the text "**tSNE mapped**" will appear in the plot title.

tSNE transformations may be applied to 1D and 2D plots by either of the following methods:

- Drag and drop the tSNE from the **Transformation** window to the intended plot or data grid (Figure 29.66).

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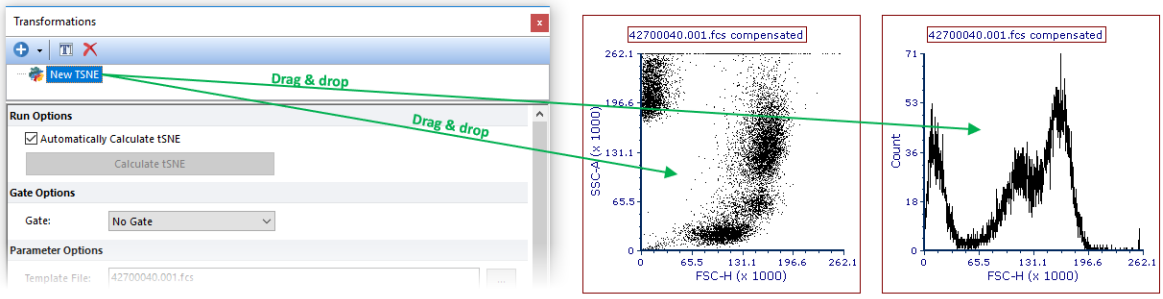

Figure 29.66 Applying a tSNE transformation to a plot by dragging the tSNE transformation from the Trasnformations navigator to the plot (green arrow).

- Access the Formatting Overlays dialog 284 and choose the tSNE transformation to apply from the **Transformation** drop down menu (Figure 29.67).

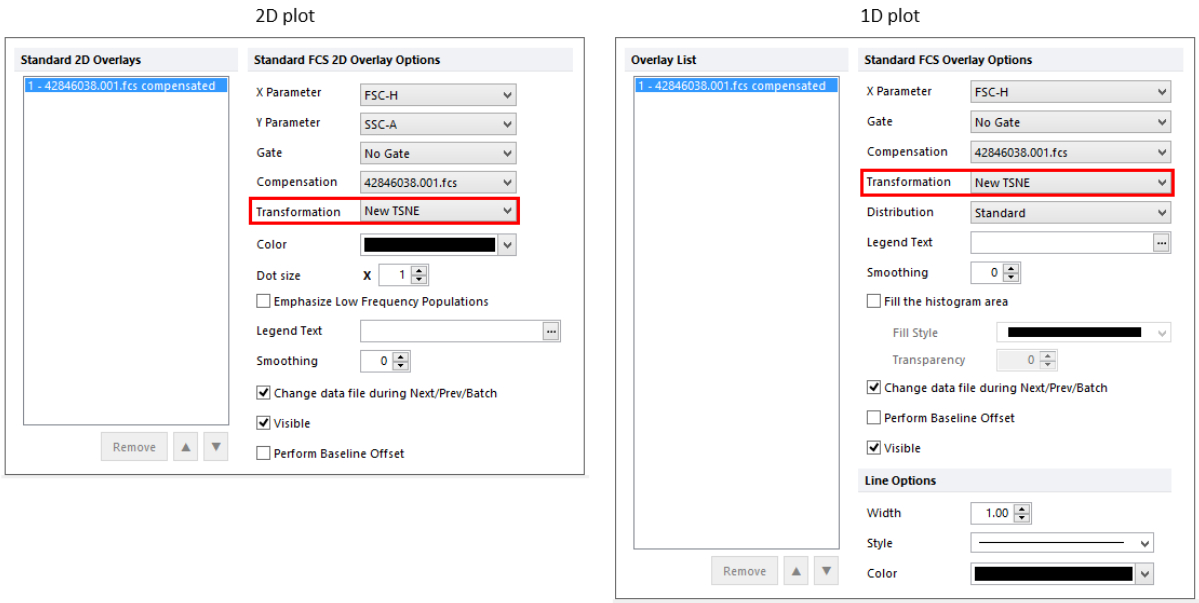

Figure 29.67. Applying a tSNE transformation to a plot by selecting the tSNE transformation from the Formatting Overlays dialog in the Transformation dropdown.

The result of both methods will be plots that denote they are **tSNE mapped** in the plot title (Figure 29.68). To access the tSNE parameters click and hold on the axis of the plot and choose the appropriate tSNE X or Y parameters 1016.

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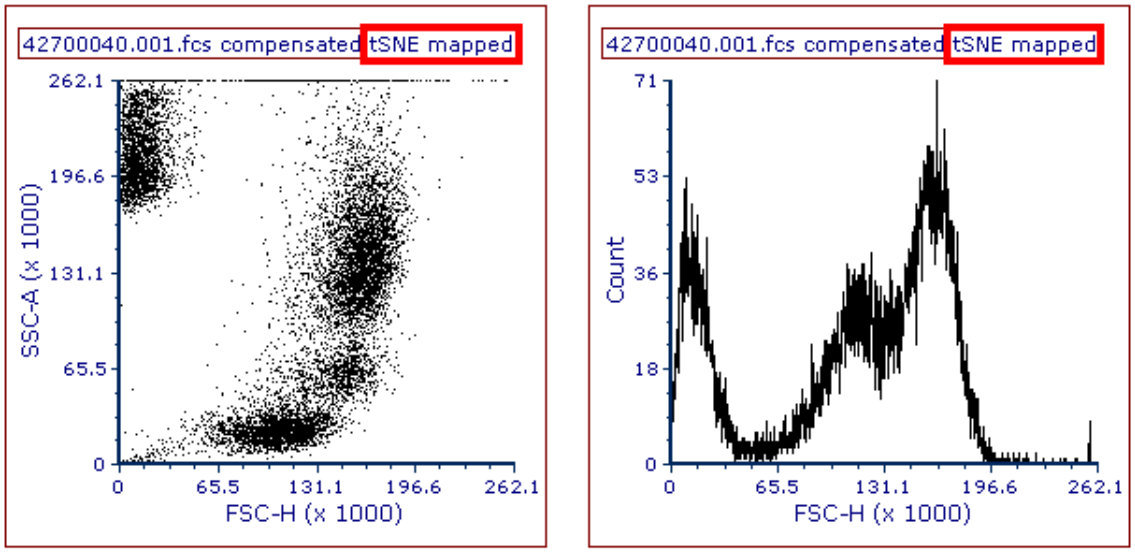

Figure 29.68. 2D and 1D plot with tSNE transformation applied. Note the "tSNE mapped" suffix in the plot title.

In the next section, we will see how to work with tSNE parameters[1016].

28.5.4 Working with tSNE transformations

Once a tSNE transformation is applied to a plot[1014], tSNE parameters can be accessed by clicking on the axis label of the plot Figure 29.69. Gates[316] may be created on any combination of standard or tSNE parameters on any plot type to specifically select the events within one or more tSNE clusters. *Jump to Gating tSNE transformed data.*[1020]

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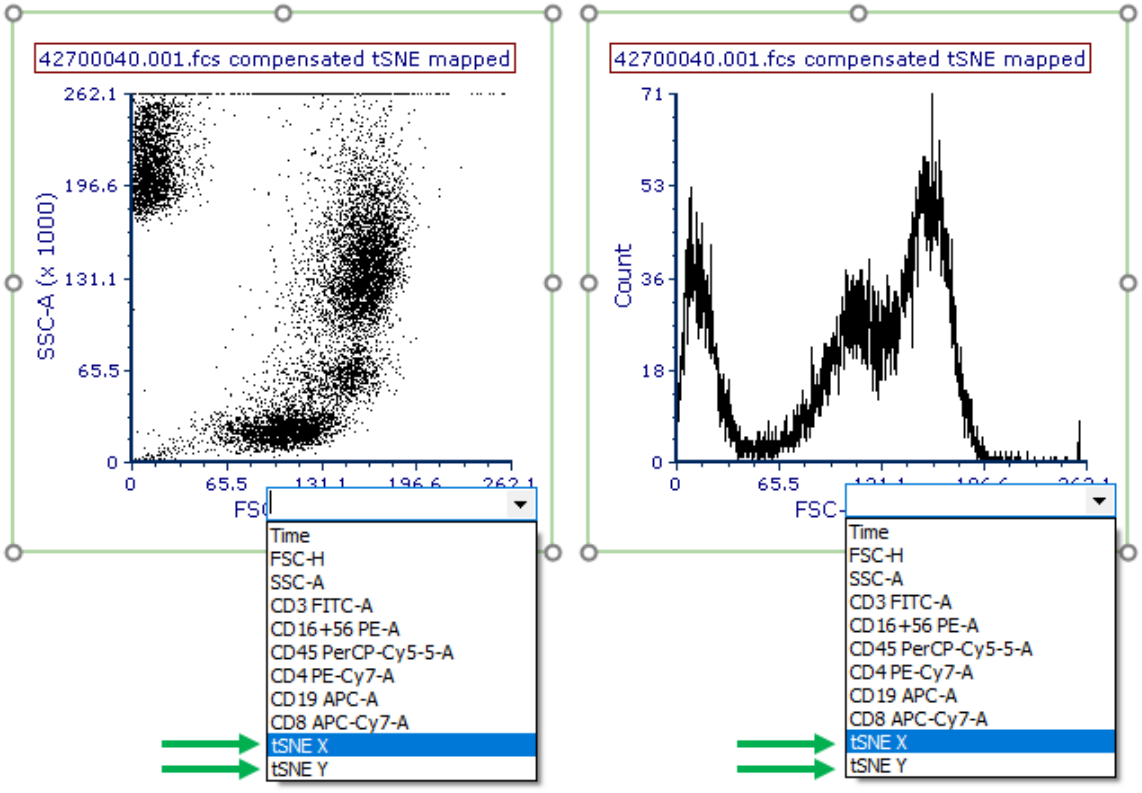

Figure 29.69 tSNE parameters can be accesses as standard parameters.

While any plot type can display tSNE based parameters, Density plots and Contour plots have a unique overlay option called **Color Based on** 288 (Figure 29.70) that is helpful for visualizing and understanding tSNE transformed data sets.

The **Color Based on** option is access via either:

- The Formatting→Overlays category 284 of Density and Contour plots. The **Color Based on** dropdown menu will allow you to choose from **Count** or any available parameter for the data set to display as the Density or Contour map on the plot.

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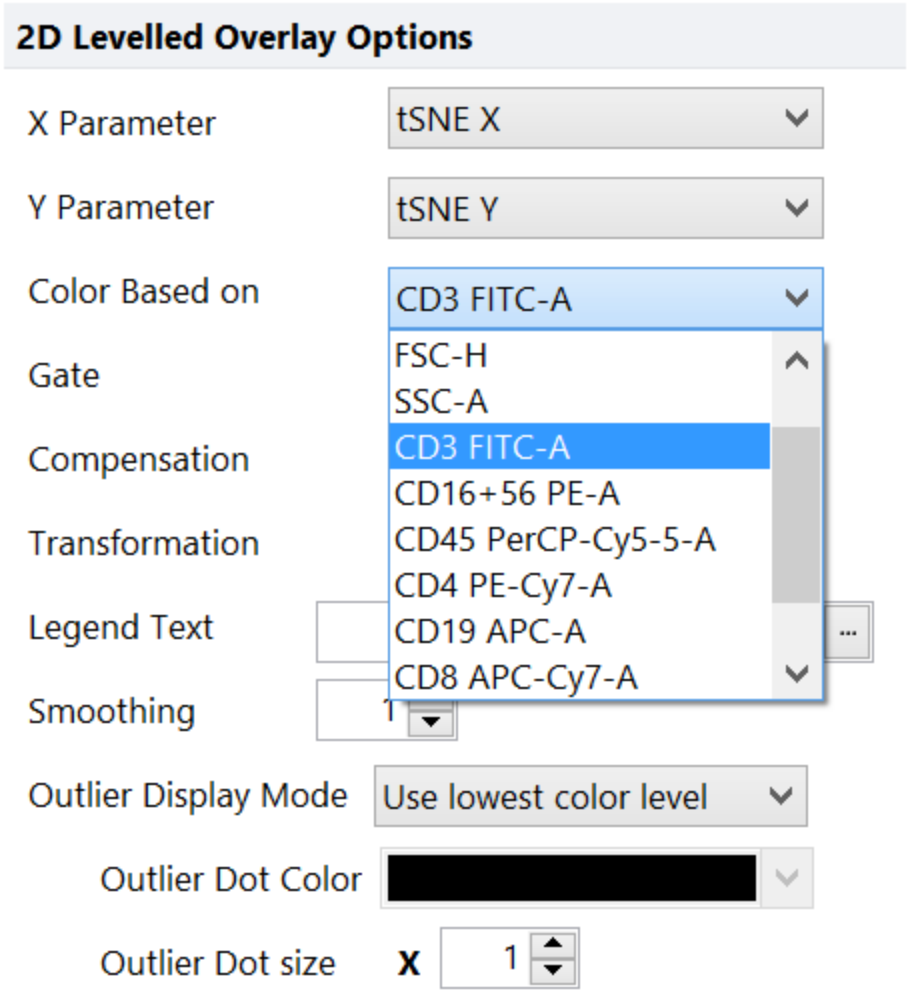

Figure 29.70 - The Color based on option in the formatting>overlays category of density and contour plots allows you to choose a parameter that will be used to display a density or contour map on tSNE X and tSNE Y transformed parameters.

- The Legend title 253, when the plot legend is visible (see the red arrow in the pictures below).

For tSNE transformed data sets it is useful to display tSNE X and tSNE Y parameters on their respective X Parameter and Y Parameter axis and visualize each additional target parameter as a **Color Based on** selection. In the examples below the same tSNE transformation for an analysis is shown in three different display types with each plot using the **Color Based on** parameter indicated in the upper left of the plot. The top example shows Density plots with the **Flame Color Level**, the middle example shows Contour plots with outliers enabled,

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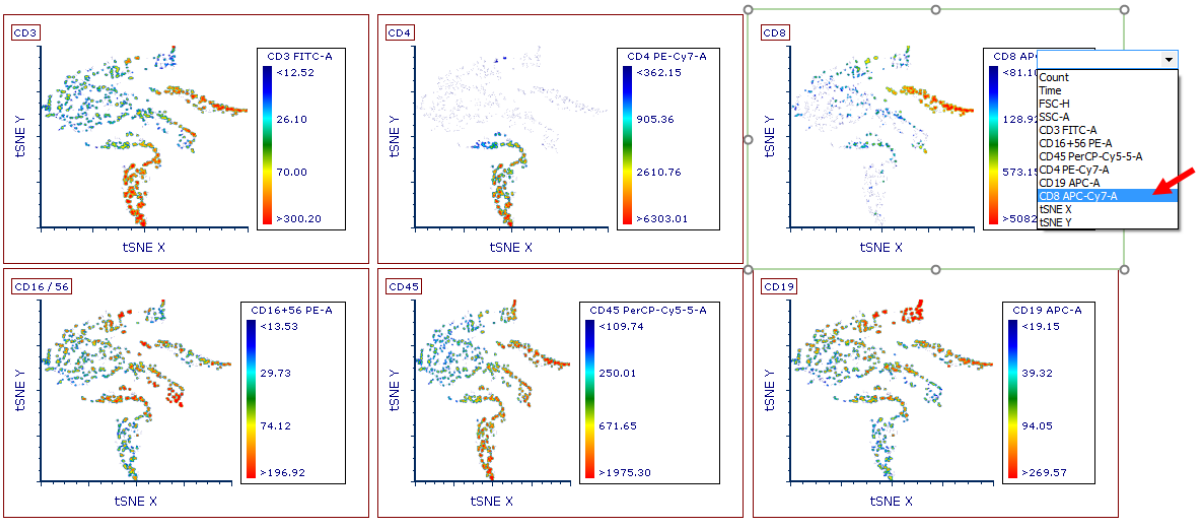
while the bottom plot shows Density plots withe **Green-Black-Red Color Level** with a **Style** of **Mean +/- SD** and **Standard Deviations** set to **2.00**.

Note that there are a number of options in FCS Express that may be changed to display tSNE transformed data in unique ways and seemingly unlimited combinations. The following list of formatting options may be useful for reviewing and preparing data visualizations:

Please note that reducing Smoothing 288 to 0 or 1 on plot overlays or reducing the resolution 305 may significantly enhance your plot visualizations.

- Overlays 287 - To choose the Color Based on 288 parameter and to show or hide outliers and outlier coloring.
- Color Levels 295 - To choose the overall color scheme and color level properties for Density and Contour Plots.
- Specific Options 305 - To choose the resolution to display plots at and the select **Shade Based on Density** 308 if desired.



Color Based on parameter indicated in the upper left of the plot. Example above shows Density plots with the Flame Color Level.

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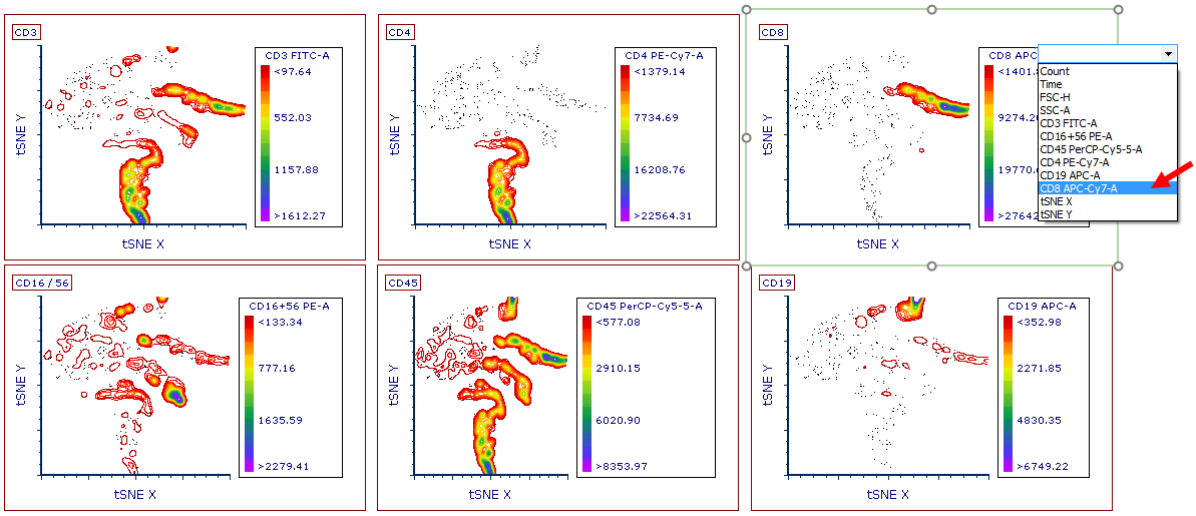
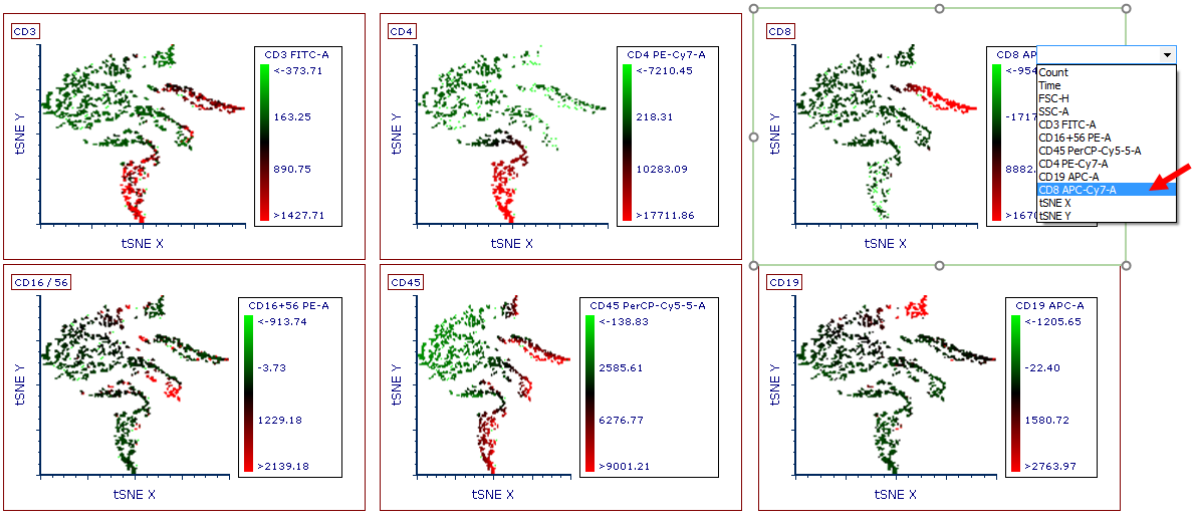


Color Based on parameter indicated in the upper left of the plot. Example above shows Contour plots with outliers enabled.



Color Based on parameter indicated in the upper left of the plot. Example above shows Density plots with Green-Black-Red Color Level with a Style of Mean +/- SD and Standard Deviations set to 2.00.

Gates 316 may be created on any combination of standard or tSNE parameters on any plot type to specifically select the events within one or more tSNE clusters. Gated populations may also be used to gate or back gate 303 events on any tSNE transformed plot.

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An example of gates drawn, backgated, and gated on tSNE parameters is shown in the Figure below (Figure 29.71).

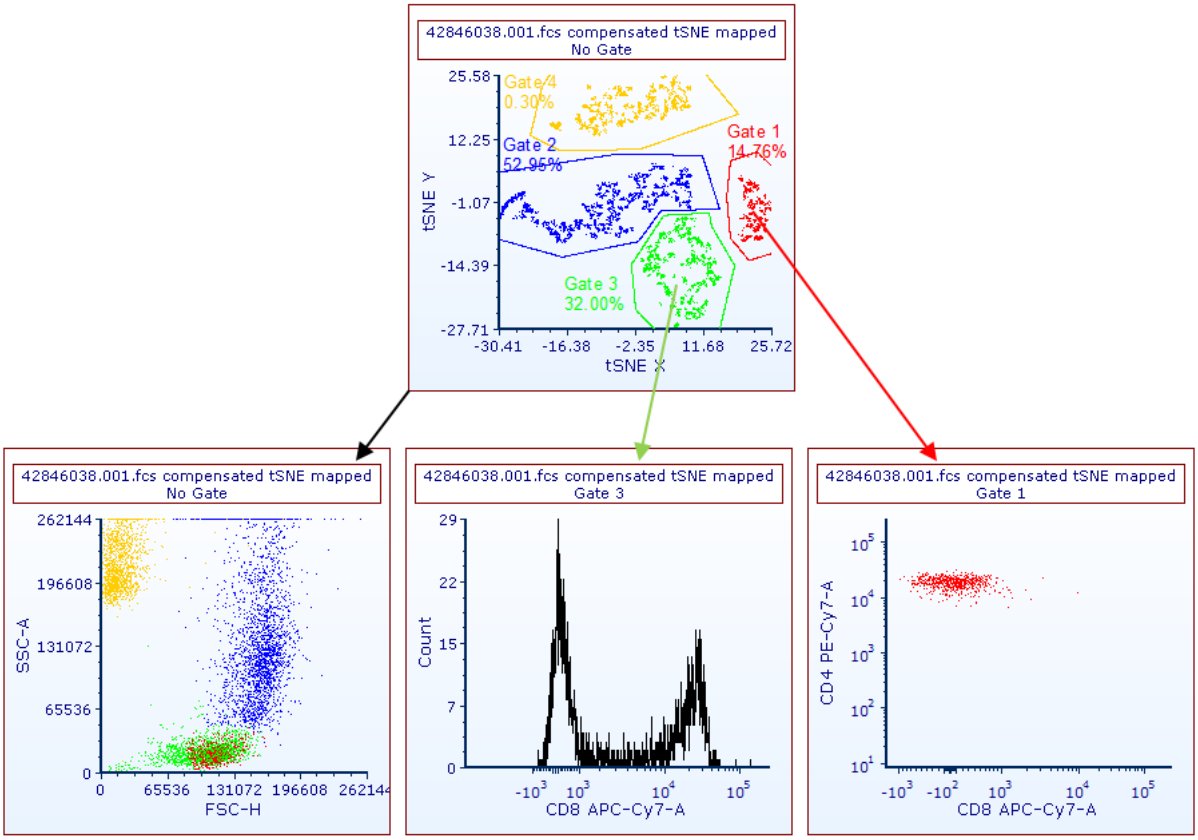

Figure 29.71. Gates have been drawn on tSNE parameters (upper panel) and have been displayed (lower left plot) or applied (lower central and lower right plots) to other plots.

28.5.5 Saving tSNE results with an FCS File

Newly calculated tSNE X and tSNE Y parameters are not saved within the layout or data file but are instead calculated automatically (or manually) and applied to plots/files when the layout is reopened. When working with large data sets or computationally intensive transformations the team at De Novo Software recommends saving/exporting a new .FCS file that contains the newly calculated tSNE X and tSNE Y parameters so you may more easily work with the data set without having to recalculate the tSNE results.

Exporting/Saving the newly create tSNE X and Y Parameters as part of a new .FCS file is easily accomplished by:

1. Apply the tSNE transformation of interest to a given plot.
2. Click on the plot to select it.
3. Click on the **Data** tab→**Save/Load**→**Export** from the ribbon to launch the **Export Data** dialog.

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4. Select the additional tSNE X and tSNE Y parameters from the list of **Parameters to Export**.
5. Perform the export normally according to the full instructions at **Exporting FCS File Data from a Single File** 666.

It is also recommend that the layout in which the transformation was create is saved so you may review the settings used to create the tSNE transformation at anytime in the future.

28.6 SPADE

FCS Express can perform the Spanning-tree Progression Analysis of Density-normalized Events (SPADE) algorithm. The transformation allows for direct analysis and visualization for highly multiparametric single-cell flow, image, and mass cytometry data.

The final result of the algorithm in FCS Express is a heat map in which the positions of cells in related nodes are positioned within a hierarchical minimum spanning tree. Heat maps can be modified easily for improved visualization of populations while Gates 1061 may be created to identify node(s) of interest.

SPADE was developed at Stanford University by members of the Plevritis and Nolan laboratories.

More details on SPADE can be found in the following publications:

- Qiu, P., et al. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. Nat. Biotech. 29, 886-891 (2011).
- Qiu, P. Inferring phenotypic properties from single-cell characteristics. PLoS One. 5, Web (2012).

To begin working with SPADE in FCS Express, please see the topics on:
Defining SPADE 1022
Working with SPADE 1031

28.6.1 Defining a SPADE transformation

SPADE is performed in FCS Express through the **Transformations** navigator. The steps below outline the process of creating a new SPADE transformation for your data:

1. Open the Transformation navigator by selecting the **Tools** tab → **Transformations** → **Transformations** command (Figure 29.72, Step 1). The **Transformations** navigator will open as in the figure below.

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2. Click on the dropdown arrow adjacent to the blue plus button (Figure below, Step 2).

3. Click on **SPADE** in the dropdown list (Figure below, Step 3).

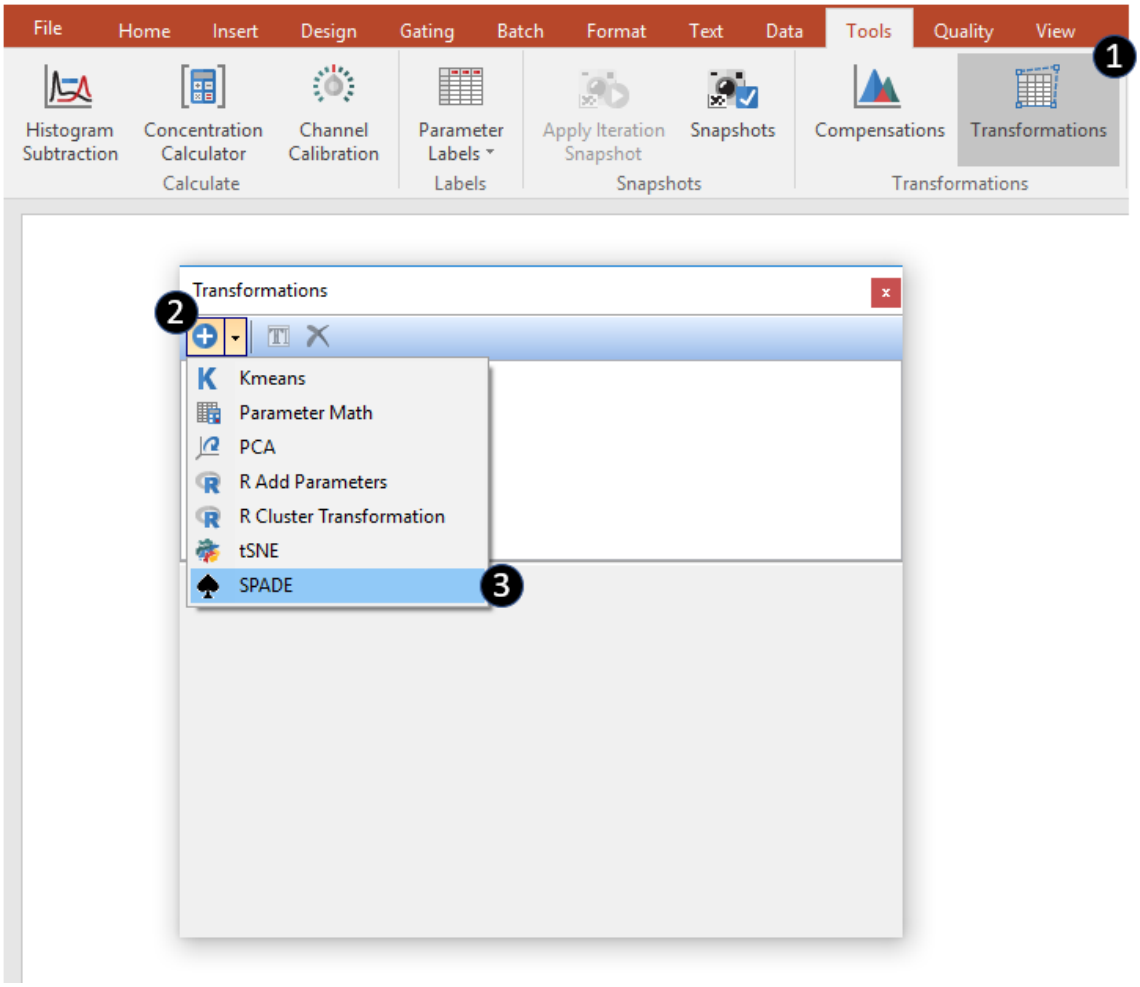

Figure 29.72 Initial steps to create a SPADE transformation.

The SPADE dialog will now appear in the **Transformation** navigator as seen in Figure 29.73.

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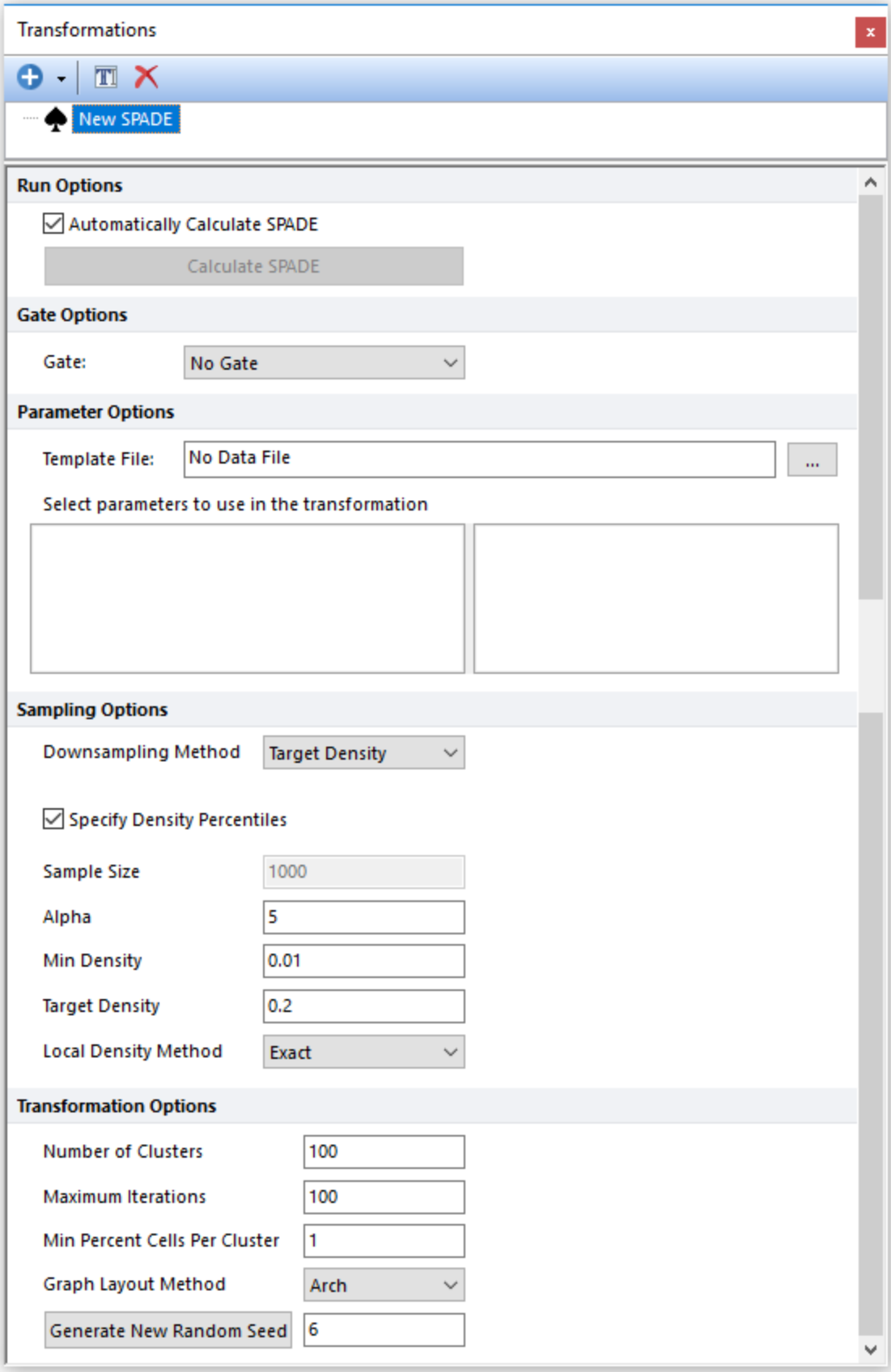

Figure 29.73 The SPADE dialog in the Transformations navigator.

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The new SPADE transformation is named as **New SPADE** by default, but it can be easily renamed by any of the following methods:

- Right click on the SPADE transformation that needs to be renamed→select **Rename**.
- Select the SPADE transformation that need to be renamed via left click and press **F2** on your keyboard.
- Select the SPADE transformation that need to be renamed via left click and click on the button in the **Transformations** tool bar.

1. Choose a **Template File** for the SPADE transformation by clicking on the ellipsis to the right of the **Template File** text box (Figure 29.74).

The SPADE dialog will be populated with a list of available parameters to perform SPADE based on available parameters from the template file (Figure below).

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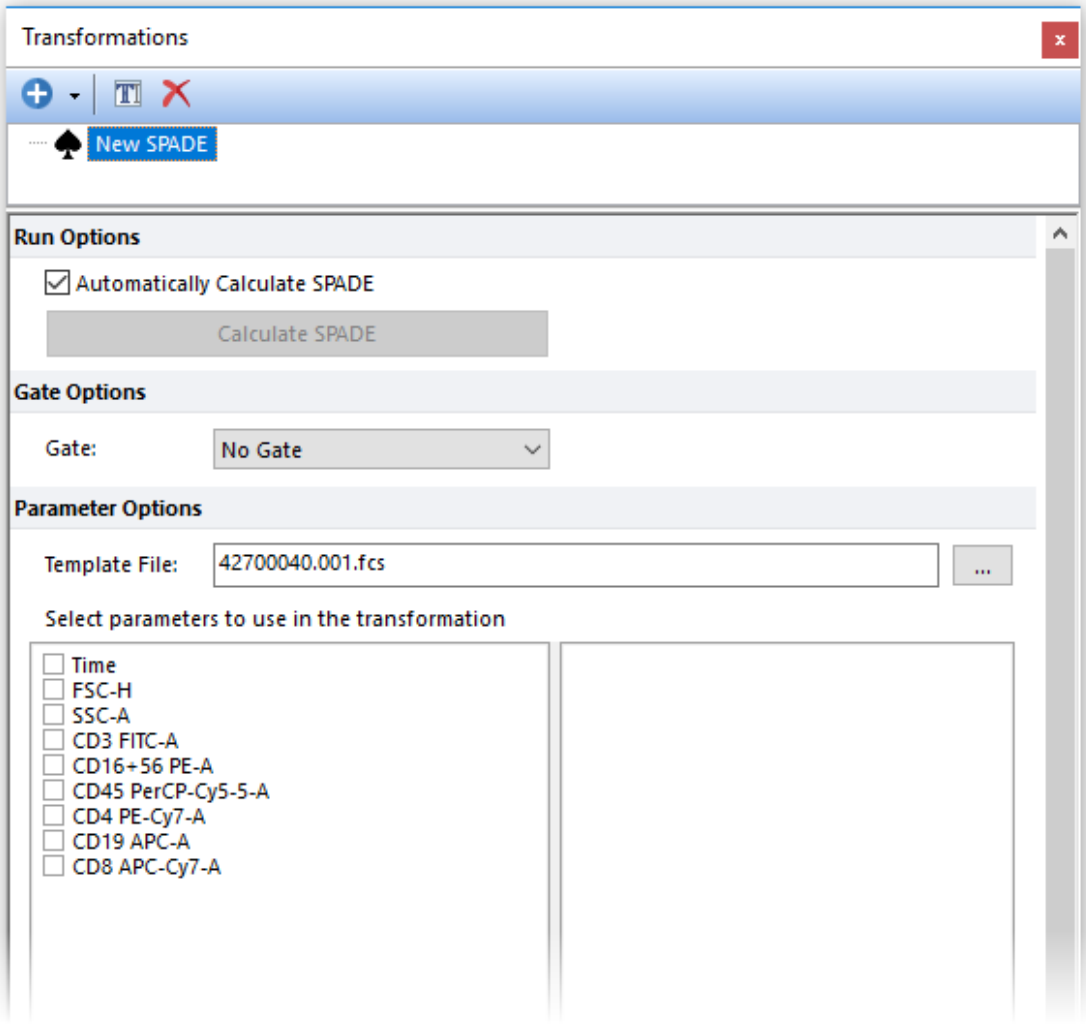

Figure 29.74 When a template file is selected, the possible parameters to perform a SPADE transformation are automatically listed in the SPADE dialog.

2. Choose the parameters to be used in the SPADE transformation by checking the boxes next to the parameter names (Figure 29.75, Point 1).
3. Select or change to the appropriate **Parameter Scaling Options** for each of the selected parameters if required (Figure below, Point 2).

Options can now be set to customize the transformation.

Options	Description
Gate	Allows the user to run SPADE only on the events within a specific gate.

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Number of Clusters	Indicates the number of nodes for the SPADE tree.
Maximum Iterations	Sets the maximum number of iterations that FCS Express should perform before quitting the clustering process.
Min Percent of Cells Per Cluster	Defines the minimum size, by percentage, of clusters and should be a value between 0 and 100.
Sampling Options	Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default setting for a SPADE transformation are: **Downsampling Method**. Default setting is **Target Density**. **Specify Density Percentiles.** By default the option is checked, and the user can define how the density-dependent downsampling is performed by eentering **Min Density** and **Target Density** values. If the box is unchecked, **Sample Size** may be defined instead of these two values. **Alpha**. Default value is **5**. **Min Density**. Values range from 0 to 1. Default value is **0.01**. **Target Density**. Values range from 0 to 1. Default value is **0.20**. **Local Density method**. Default setting is **Exact**. **Sample Size**. Since **Specify Density Percentiles** is checked by default, the **Sample Size** field is not available by default. This option becomes available when the **Specify Density Percentiles** check-box is unchecked, . If using this option, the **Min Density** and **Target Density** will be automatically estimated to select cells. If the specified **Sample Size** is higher than the number of available events, SPADE will be run on all available events. Note that the number of available events is dictated by the **Gate** option. The minimum value that can be entered is 10.
Graph Layout Method	Defines the layout style used for the SPADE tree. Possible choices are **Arch** (default) or **Radio**. This option determines the display style used to plot the tree, it does not affect results.
Change Random Seed	The Random Seed is a number which is used by the random number generator to generate the N random values which are used as input data points for the SPADE calculation. The random number generator will use the Random Seed value

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	and perform a series of math operations on it which result in N random values. Since SPADE relies on k-Means clustering, which is a stochastic algorithm, a seed can be set to make results reproducible over the time when SPADE is applied to the same data. The seed can be changed by either clicking on the **Change Random Seed** button or by manually inserting a seed in the field. In FCS Express 6, the default **Random Seed** is 6.
Output	The available choices are **Clustering Assignments** and **Clustering Statistics**. Please refer to the Defining a k-Means cluster analysis 966 chapter for more details on Clustering Statistics.
Automatically Calculate SPADE	By default SPADE is recalculated as soon as an option in the SPADE dialog is changed. FCS Express allows the user to disable the automatic calculation of SPADE and to manually calculate SPADE only when required. To defer calculating the SPADE algorithm, uncheck the box for **Automatically Calculate SPADE**. Doing so will present you with the button to **Calculate SPADE**, which can be pressed to calculate SPADE on demand. Please note that this option only regulates the auto-update upon changes made within the SPADE dialog. If SPADE is calculated on a gate and that gate is moved or resized, SPADE will be automatically recalculated regardless this option. If you want to avoid SPADE auto-updating upon gate moving/resizing, please refer to the Live Updating Gate 317 chapter of this manual.

4. (Optional) Select a gate from the **Gate** drop down menu to only run SPADE on the events within that gate (Figure below, Point 3).

5. (Optional) Select a **Downsampling Method** (Figure below, Point 4). Although multiple Sampling Methods are available (please refer to the Sampling Options 1036 chapter for more details), the default **Downsampling Method** for a **SPADE** transformation is **Target Density** (see above for more details and default settings).

6. Enter value for **Number of Clusters** (Figure below, Point 5).

7. Enter value for **Maximum Iterations** (Figure below, Point 6).

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8. (Optional) Change the value for **Min Percent of Cells Per Cluster** (Figure below, Point 7).

9. Select the **Graph Layout Method** to be used for the SPADE tree (Figure below, Point 8). Possible choices are **Arch** (default) or **Radio**.

10. (Optional) Change the the **Random Seed** used for SPADE calculation by either clicking on the **Change Random Seed** button or by manually inserting a seed in the field (Figure below, Point 9).

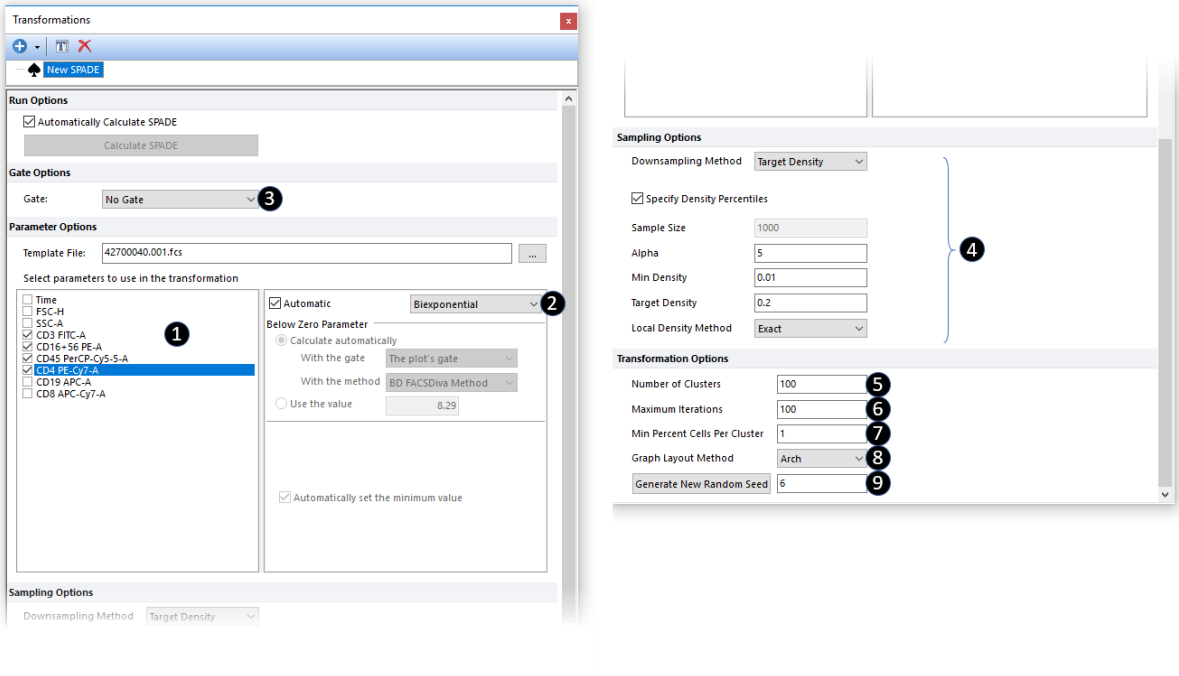

Figure 29.75 Steps to complete the SPADE dialog.

The SPADE transformation has now been defined and will be automatically calculated as soon as it will be applied to a heat map 1031. In addition, SPADE will be automatically recalculated as soon as an option in the SPADE dialog will be modified.

Given that SPADE is a demanding algorithm that can take several seconds to be calculate, FCS Express allows the user to disable the automatic calculation of SPADE upon options changing and to manually calculate SPADE only when required. To defer calculating the SPADE algorithm, uncheck the box for **Automatically Calculate SPADE**. Doing so will present you with the button to **Calculate SPADE** (Figure 29.76) which can be pressed to calculate on demand.

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Please note that this option only regulates the auto update of SPADE upon changes made within the SPADE dialog. If SPADE is calculated on a gate and that gate is moved or resized, SAPDE will be automatically recalculated regardless this option. If you want to avoid SPADE autoupdate upon gate moving/resizing, please refer to the Live Updating Gate 317 chapter of this manual.

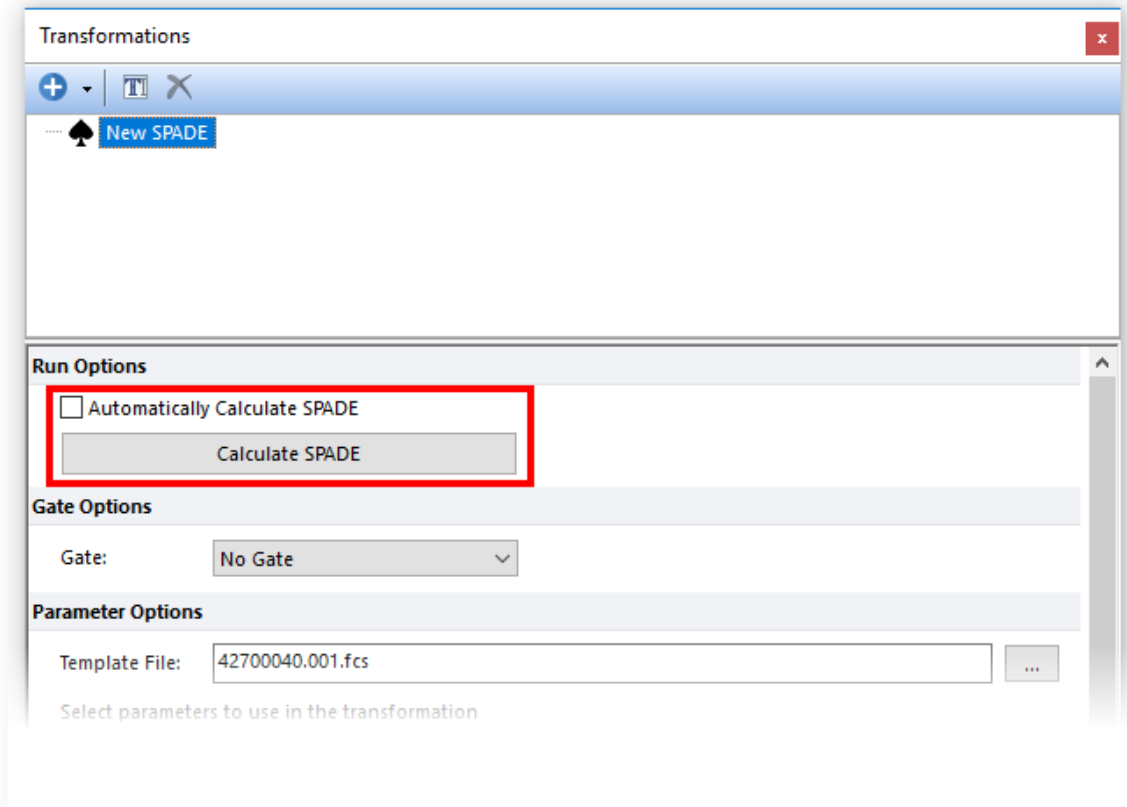

Figure 29.76 Unchecking the Automatically Calculate SPADE box will allow the user to calculate SPADE manually only when required.

For visualization of the SPADE transformation, a **Heat Map** 1050 may be inserted and modified (Figure 29.77 below). Please also see the Working with SPADE transformations 1031 section for additional details on applying SPADE transformations to plots.

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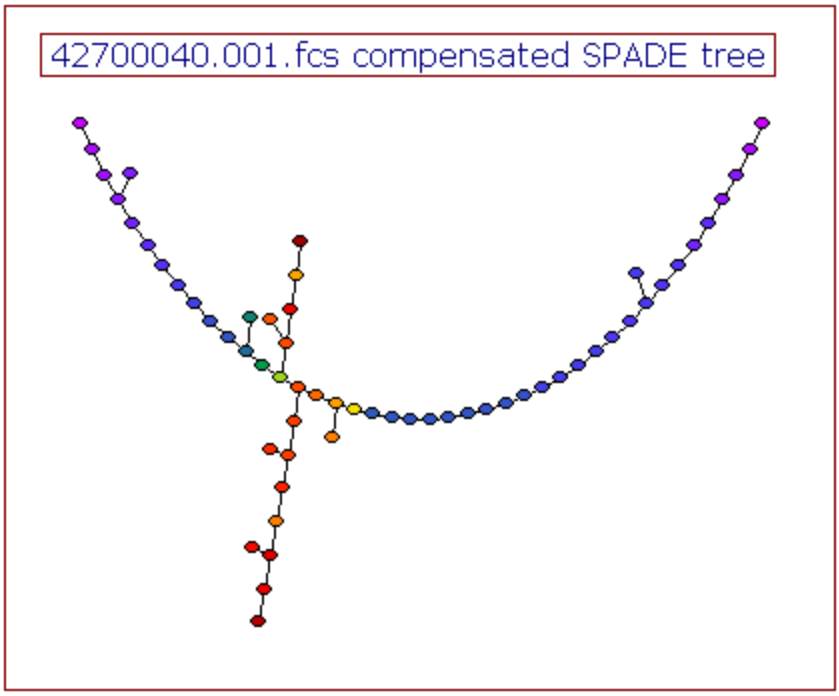

Figure 29.77 Heat Map Displaying SPADE transformation.

28.6.2 Working with SPADE transformations

Once SPADE has been defined[1022], it may be applied to empty Heat Maps[1050] in FCS Express. Please note when inserting a Heat Map[1050] for non-multiwell plate data, a warning message will appear (Figure29.78).

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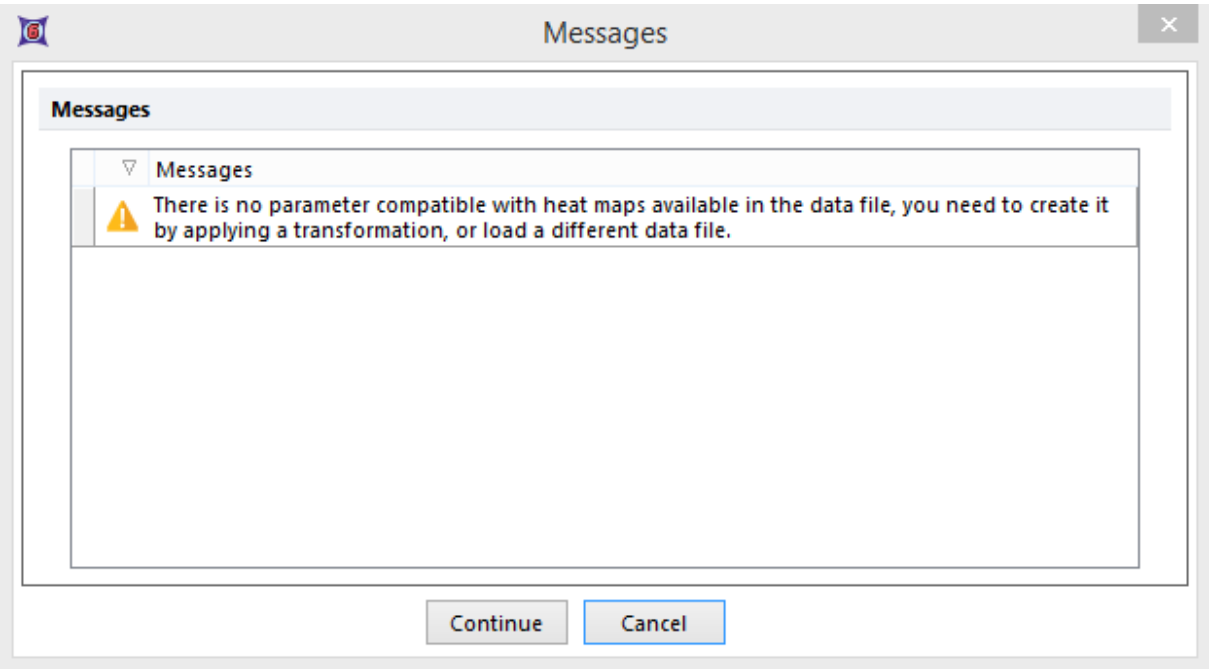

Figure 29.78 Warning message when inserting a heat map for data sets not derived from a plate based data file.

Click **Continue** and proceed to apply the SPADE transformation by either of the methods below:

- Drag and drop SPADE from the **Transformation** navigator on to the intended plot (Figure 29.79). Note that the SPADE tree can only be displayed on Heat Map plots. However, applying the SPADE transformation on 1D and/or 2D plots is required for downstream analysis of SPADE results (e.g. back-gating of SPADE nodes on 1D and 2D plots).

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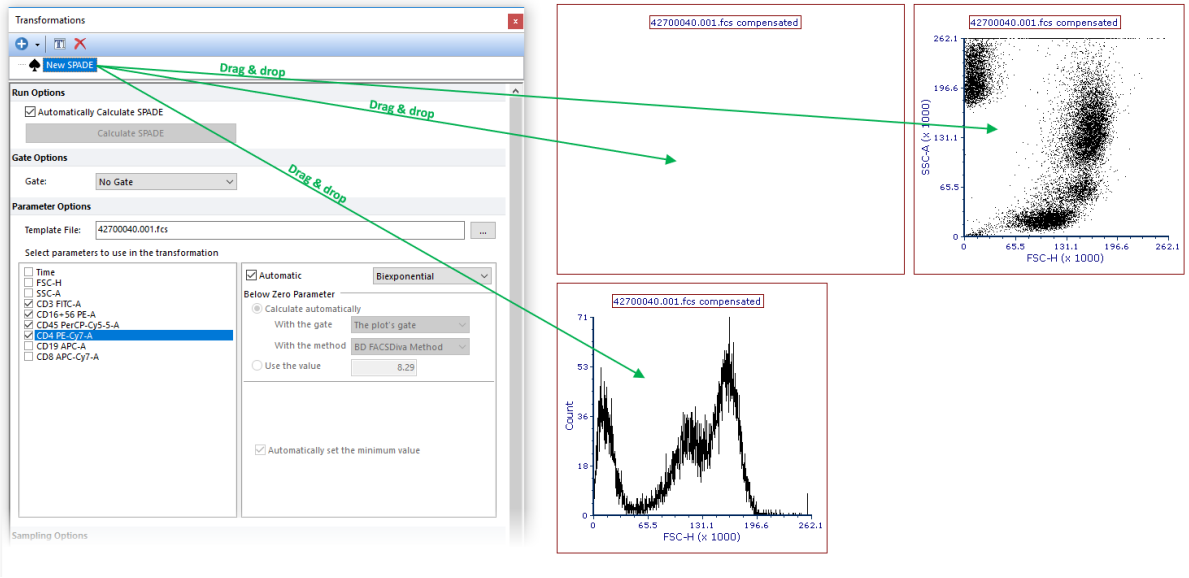
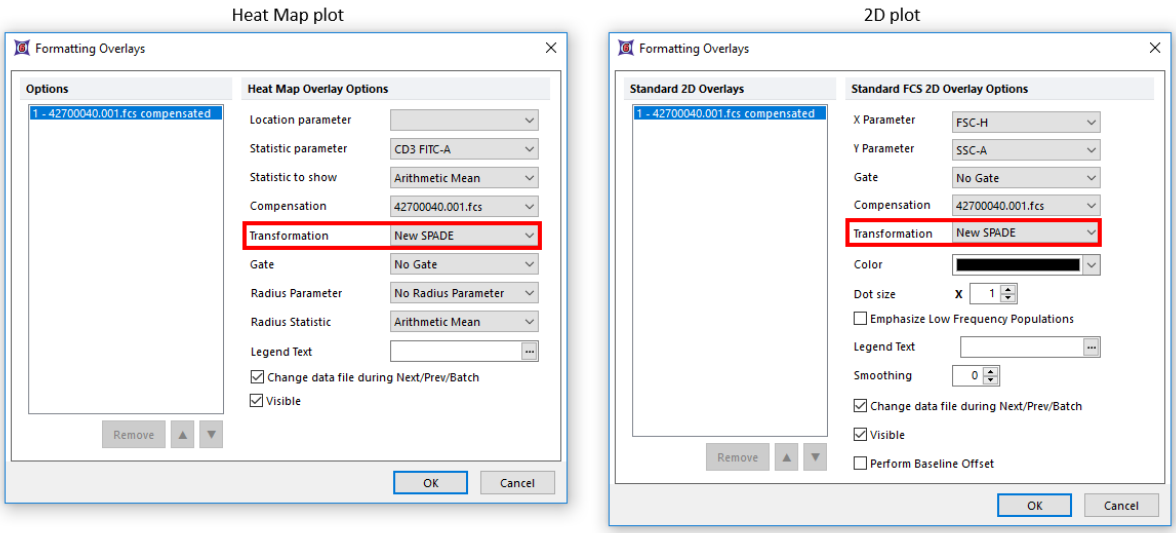
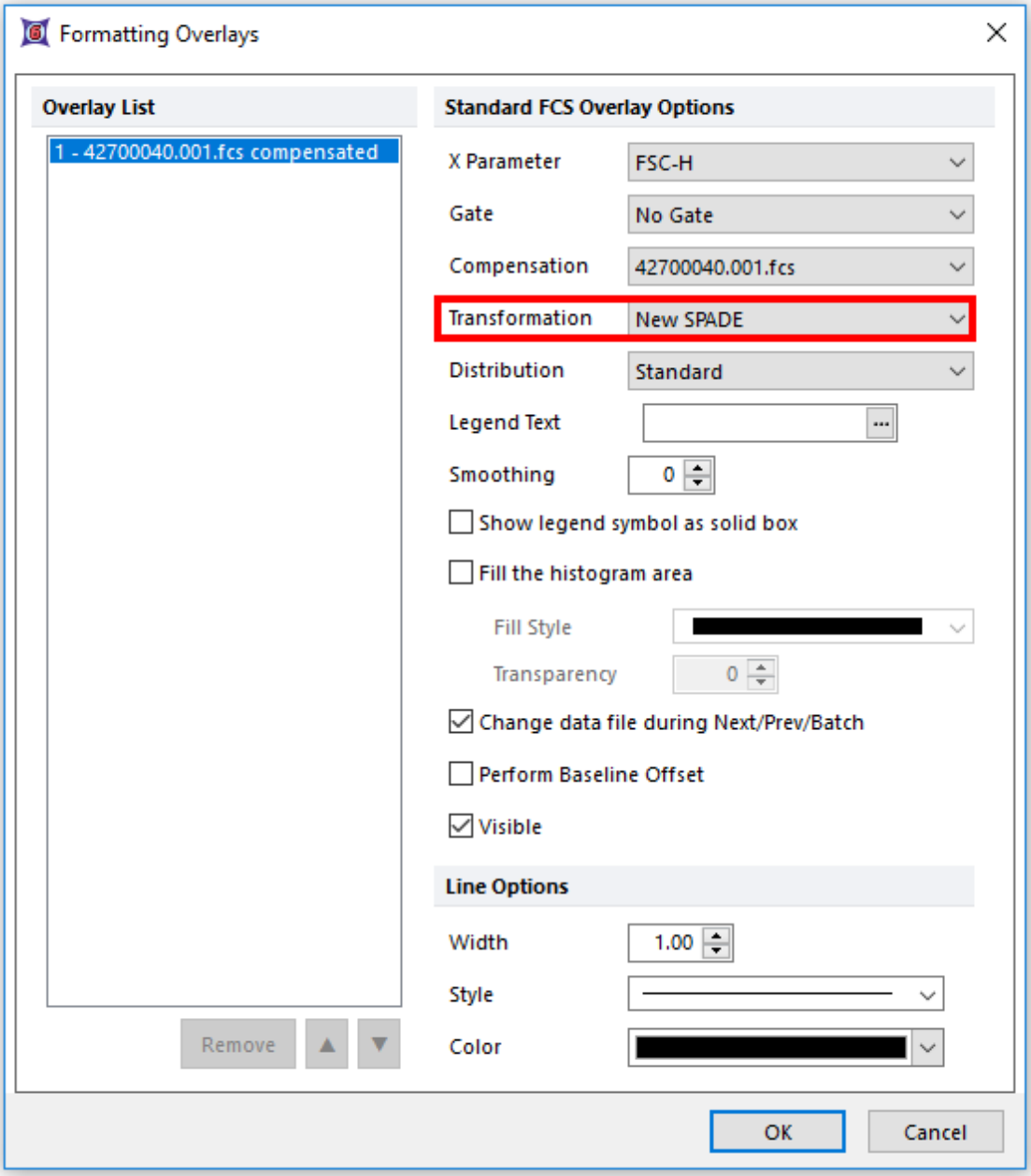


Figure 29.79 Drag and drop SPADE from Transformations navigator to an empty heat map. When the transformation has been applied the heat map will populate with SPADE nodes.

- Access the Formatting Overlays dialog 284 and choose the SPADE transformation to apply from the **Transformation** dropdown list (Figure 29.80).



1D plot



The result of both methods will be a plot that denotes **SPADE tree** in the title, outlined in red below (Figure 29.81).

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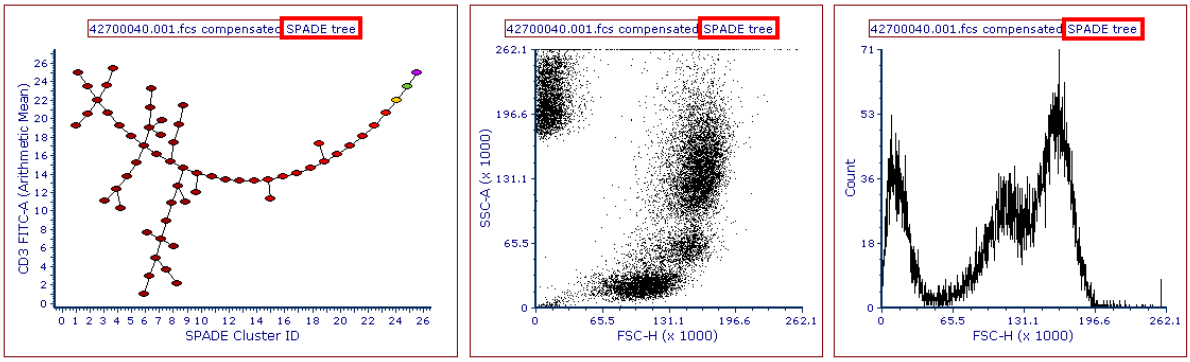

Figure 29.80 Heat Map with title appended with SPADE tree, outlined in red.

Once SPADE is applied, the Heat Map may be formatted to:

- Have well size dependent on a a statistic such as number of events in the node 1057
- Set the Parameter and Statistic for display 1054
- Change the Color Level and Color Scheme 1057
- Create gate(s) to identify node(s) of interest, and edit other features 1061

28.6.3 Saving SPADE results with a DNS file

Results from a SPADE transformation are not saved within the layout or data file but are instead calculated automatically (or manually) 1022 and applied to plots/files 1031 when the layout is reopened. When working with large data sets or computationally intensive transformations the team at De Novo Software recommends saving/exporting SPADE results in a new file so you may more easily work with the data set without having to recalculate the SPADE transformation. SPADE result are not compatible with the Flow Cytometry Standard (i.e. FCS) file format and need to be saved as De Novo Software data Stream (i.e. DNS) file format.

Exporting/Saving SPADE results as part of a new .DNS file is easily accomplished by:

1. Apply the SPADE transformation 1031 of interest to a given plot.
2. Click on the plot to select it.
3. Click on the **Data** tab→**Save/Load**→**Export** from the ribbon to launch the **Export Data** dialog.
4. Select the additional **SPADE Cluster ID** parameter from the list of **Parameters to Export**.
5. Perform the export normally according to the full instructions at **Exporting FCS File Data from a Single File** 666, being sure to select **DNS data Stream file** as file type for the output file.

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It is also recommend that the layout in which the transformation was create is saved so you may review the settings used to create the SPADE transformation at anytime in the future.

28.7 Sampling Options

Sampling is a method used to reduce the sample size of a data set prior calculating a statistics of interest. Different sampling methods are available in FCS Express and can be used in conjunction with several Transformation tools 932.

Available sampling methods are:

- **None**. Sampling is not performed. All events selected in the **Gate** drop-down menu of the **Transformation** dialog are used.

- **Interval**. A defined number of cells (i.e. the Sample Size) is sampled from the main population (i.e. the one selected in the **Gate** drop-down menu of the **Transformation** dialog) according to a fixed periodic interval. This interval, called the sampling interval, is calculated by dividing the population size by the desired **Sample Size**. For example, if the main population counts 1000 events and the Sample Size is 100, every 10th event will be selected (e.g. 1st, 11th, 21st, 31st...). If the specified **Sample Size** is higher than the number of available events, all available events will be considered.

Target Density and **Weighted Density** are examples of Density Based sampling. This means the probability that an event will be sampled (i.e. included in the dataset for analysis) is based on the local density around that event. The local density of an event x is defined as the number of other events within a certain radius around event x. The radius used is arbitrary, and properties to control it are defined below.
Density based sampling involves two stages. The first stage is to calculate the local density around each cell. The second stage is to decide whether or not to sample the cell based on the local density.

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Local Density Calculation

The local density is calculated identically for both the Target Density and Weighted Density options. In order to calculate the local density, a radius must be determined. In order to determine the radius, 2000 events are randomly selected and the distance matrix of these events are calculated. The median distance is then multiplied by the **Alpha** value that can be entered in the properties to determine the radius for the local density.

Once the radius is determined as described above, the number of events within the radius is calculated based on the **Local Density Method** whose options are:

- **Exact -** the exact number of events within the radius is determined
- **Kernel** - an approximation of the number of events within the radius is determined. Generally, the kernel method is much faster than the exact method and provides comparable results.

Sampling Calculation

After the local density has been calculated, the cells are sampled based on the density using two different approaches which the user can select: Target Density 1037 and Weighted Density 1038.

28.7.1 Target Density Downsampling

Target Density downsampling is a method to discard events based on their local density. Please read our introduction to sampling 1036 if you have not done so already to learn about your options for calculating local density.

Target density downsampling is implemented as originally described in Qui, P et. al. "Extracting a Cellular Hierarchy from High-dimensional Cytometry Data with SPADE", *Nature Biotechnology*, 29(10):886-891, 2011. The goal is to ensure that the final density of the sampled population falls within a certain density range. Events with local densities below the desired density range are discarded. Events with the local densities within the desired range are kept. Events with local densities above the desired range with are discarded with a probability that depends on how much the local density is greater than the desired range. The higher the local density is compared to the desired range, the more likely it is to be discarded.

Target Density Downsampling has several options

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Specify Density Percentiles	By default the option is checked, and the user can define how the density-dependent downsampling is performed by entering **Min Density** and **Target Density** values. If the box is unchecked, **Sample Size** may be defined instead of these two values.
Min Density	If the local density of a cell is less than the Min Density value, it will be excluded from sampling. Values range from 0 to 1. Default is **0.01**.
Target Density	If the local density of a cell is greater than the **Min Density** percentile and less than the **Target Density**, it will be sampled. When the **Local density** of a cell is greater than the **Target Density**, it will be sampled randomly with a probability equal to the ratio of **Target Density/Local density**. Values range from 0 to 1. Default is **0.20**.
Sample Size	Indicates how many cells to subsample when performing the transformation. This option becomes available when the **Specify Density Percentiles** check-box is unchecked, . If using this option, the **Min Density** and **Target Density** will be automatically estimated to select cells. If the specified **Sample Size** is higher than the number of available events, SPADE will be run on all available events. Note that the number of available events is dictated by the **Gate** option. The minimum value that can be entered is 10.

28.7.2 Weighted Density Sampling

Weighted Density sampling is a method to discard events based on their local density. Please read our introduction to sampling 1036 if you have not done so already to learn about your options for calculating local density.

Weighted density sampling is implemented as originally described in Kollios, G et. al "Efficient Biased Sampling for Approximate Clustering and Outlier Detection in Large Data Sets", IEEE Transactions on Knowledge and Data Engineering, 15(5):1170-1187, 2003.
There are several options for Weighted density sampling:

Sample Size	Indicates how many events to sample.
Weight	The **Weight** parameter controls the probability with which an events is sampled based on the local density. Positive **weights** mean that events from higher density regions will be preferentially sampled. Negative **weights** mean that events from low density regions will be preferentially sampled. A **weight** of 0 means random sampling will be performed, with no bias for density. In general values from -3 to 3 are appropriate. In order to mimic Target Density sampling, positive values between 0 - 1 are generally appropriate.

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Chapter

29 Working with Amnis .DAF files

FCS Express 6 Image Cytometry and Plus are directly compatible with the Amnis *.daf file format to allow loading of native .daf files and the images associated with them (in .cif format) in FCS Express.

Amnis .daf files behave just like any other data file in FCS Express for inserting plots 172, creating, gates, and deriving statistics 316. Single cell images may be accessed via a Data Grid 684 which serves as an Image Gallery just as with any other image cytometry data file formats in FCS Express.

Please note that .DAF files can be loaded with images or without image (numeric data only) via the FCS Express User Options 237 depending on the needs of your analysis. By default, if an Amnis .cif file with the same name as the corresponding Amnis .daf file are located in the same folder, images will be loaded. If no Amnis .cif file was created, or if the .daf files have been moved to a different folder location than the .cif files, images will not be loaded.

Please see the following topics for working with data in FCS Express:

Analysis of Flow and Image Cytometry Data Files 316
Inserting plots 172
Using Data Grids as Image Galleries 684
Applying Gates to a Data Grid (Image Gallery) 681
Creating Gates on a Data Grid (Image Gallery) 683
Backgating on a Data Grid (Image Gallery) 681

To open an Amnis .DAF file in FCS Express:

1. Choose to insert a plot 172 or add a data file to the data list 788.
2. Click on the **Files of type drop down** in the **Select a Data File** dialog (Figure 30.1)
3. Choose **Amnis DAF File (*.daf)** from the list.
4. Select the Amnis .daf file you wish to use.
5. Press **OK**.

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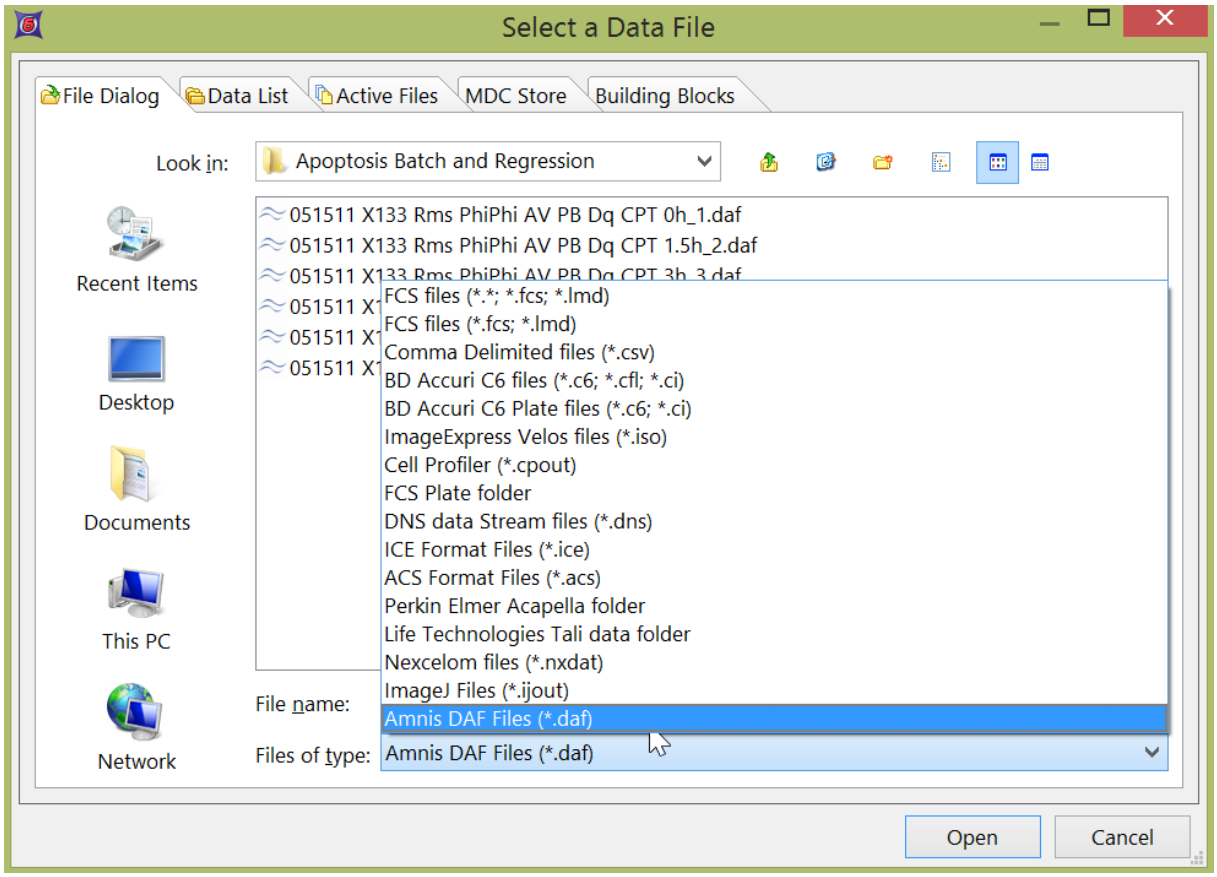

Figure 30.1 - Selecing the Amnis DAF File of type from the Select a Data File dialog.

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Chapter

30 Picture Plots

Picture Plots are two-dimensional displays of the morphology of cells (or other objects). Think of them as gated collections of cell images. The location of individual cells is shown along the x- and y-axes of the plot. Gates can be set on individual cells or groups of cells.

30.1 Working with Picture Plots

To insert a **Picture Plot** on your layout, use the **Insert** tab→**2D Plots**→**Picture Plot** command.

(Also see File Formats for Imaging Cytometry 27)

To format the plot, right-click on the plot, select **Format** from the pop-up menu, and choose the **Overlays** category. Set the **Image to display** and, if desired, choose an appropriate **Gate** (e.g., a gate defined on a 1D- or 2D-plot 316, or a single well gate that you set on the corresponding Heat Map 1061) using the drop-down lists (Figure 31.1). Alternatively, you can select the **Picture Plot** and use the **Format** tab→**Plot Options**→**Overlays** command.

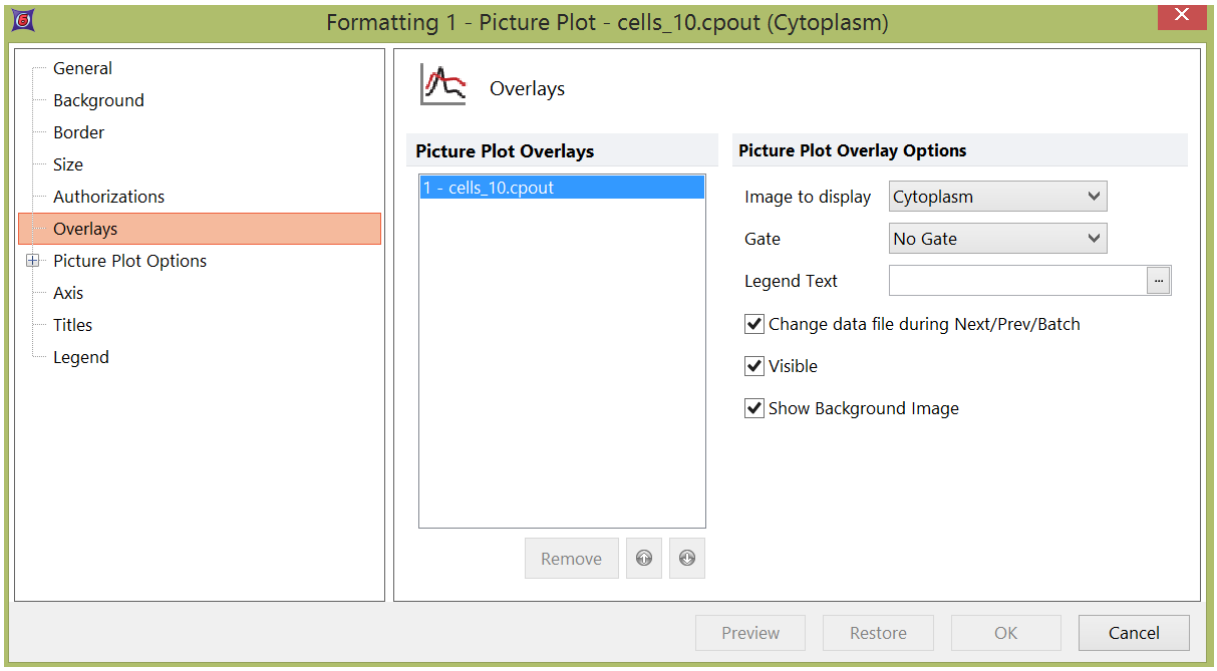

Figure 31.1 Formatting a Picture Plot - Overlays Category

The **Picture Plot** will display the images of your cells with their location in the field (e.g., a single well that was chosen in a well gate) that was imaged (Figure 31.2). The axes are in pixels with the origin (0,0) located in the upper left corner

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of the field. You can change the parameter being displayed by clicking on the x-axis label and choosing a new parameter from the pop-up list.

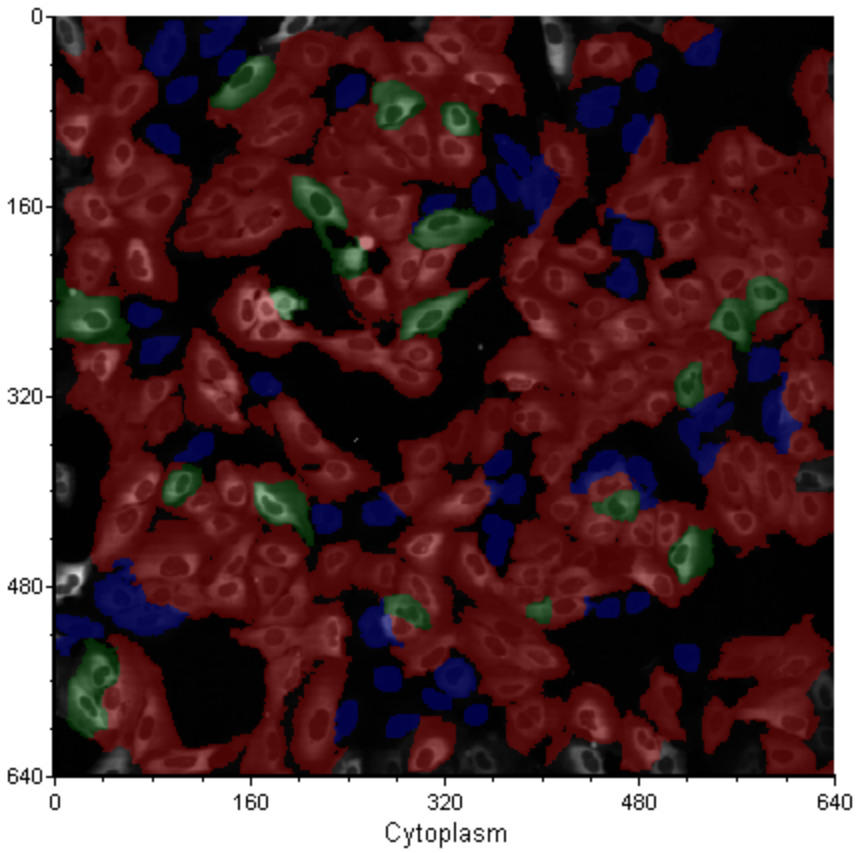

Figure 31.2 A Picture Plot

To display the images from a different well, modify the single well gate by right-clicking on the Heat Map, selecting Edit Gate, choosing the appropriate gate from the submenu, and then selecting a different well[1062]. Alternatively, you may create additional Well Gates[1061] and apply them to the Picture Plot using any of the methods for applying gates[352] in FCS Express.

Picture Plots can be backgated just like any other color dot plot. In other words, the events (in this case, the images of cells) can be drawn in the color of the gate that they belong to.

To implement backgating:

1. Right-click on the **Picture Plot**.
2. Select **Format** from the pop-up menu.
3. Expand the **Picture Plot Options** node.
4. Choose the **Display Gates Options** category.
5. Set the gates to display according to your criteria (Figure 31.3).

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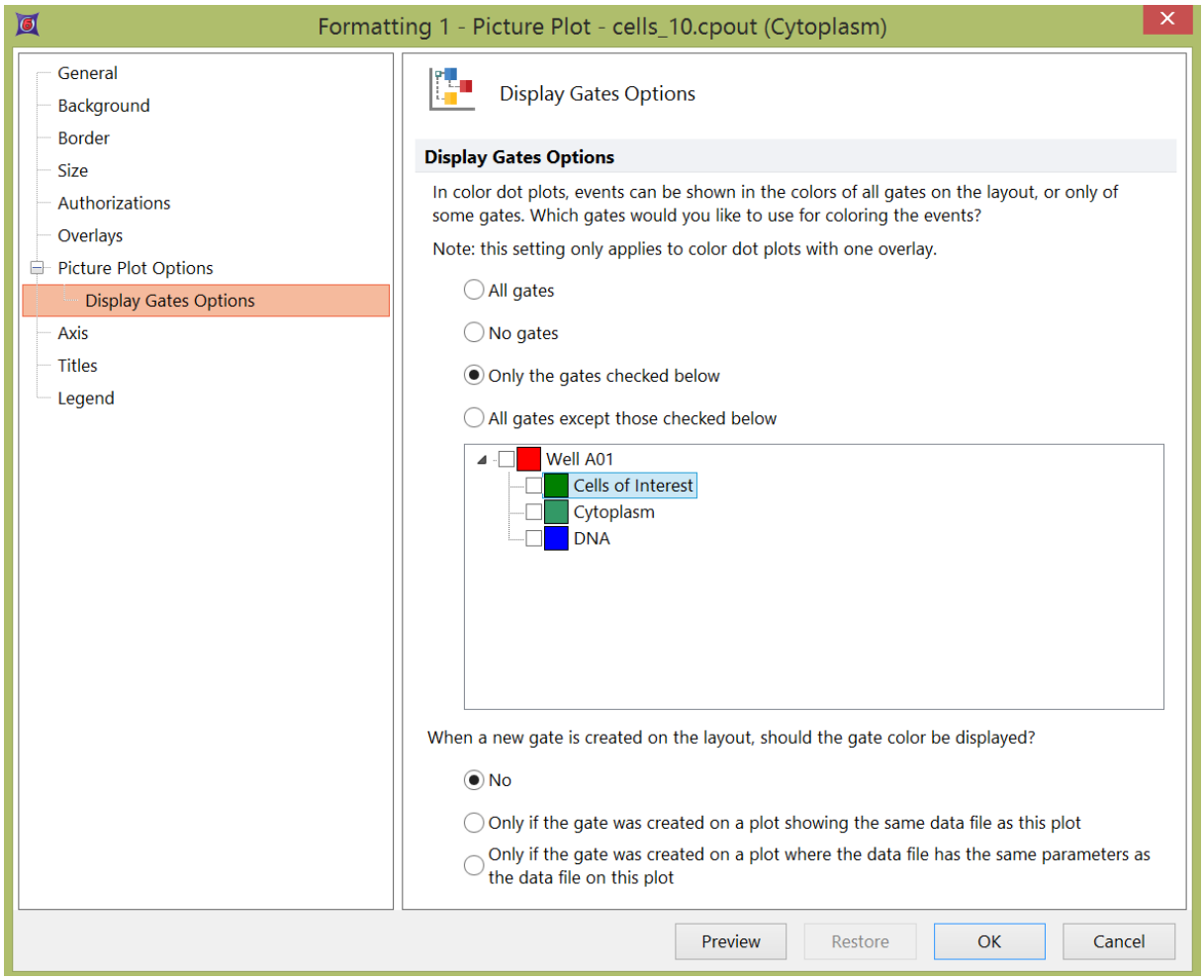

Figure 31.3 Picture Plot - Display Gates Options

In the example, Cells of Interest (pink) has been chosen as the sole backgating color. When you click **OK**, the objects in Cells of Interest will be colored pink (Figure 31.4).

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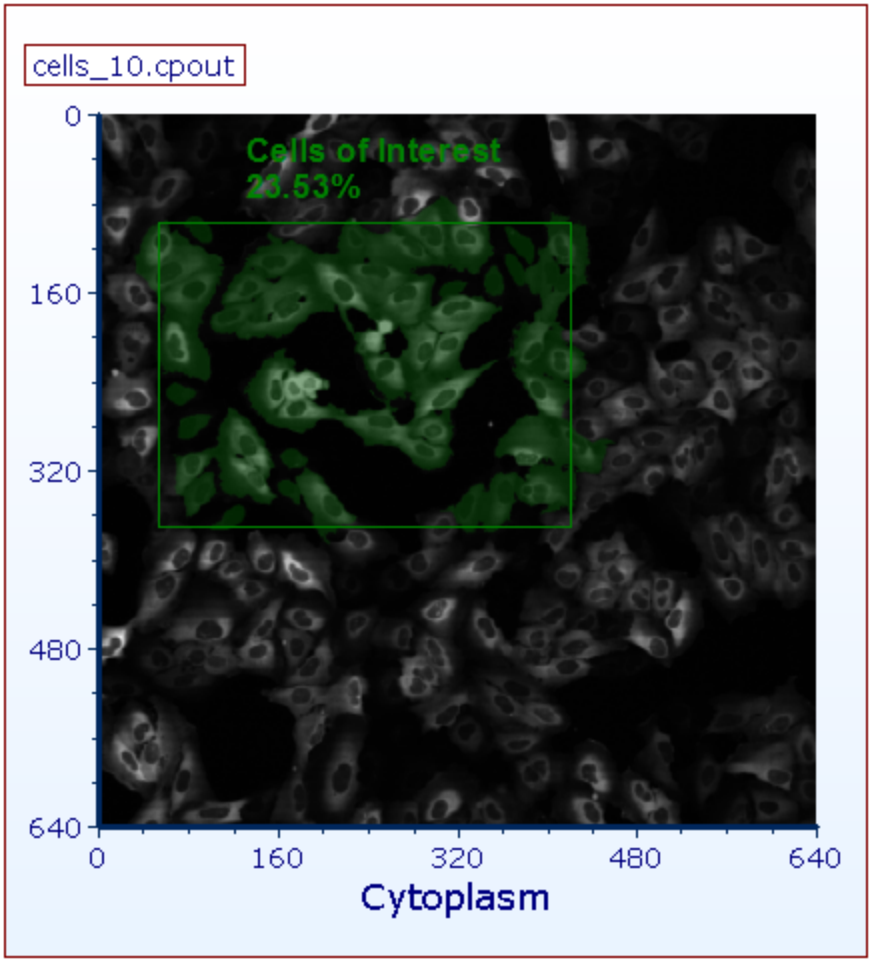

Figure 31.4 Back-Gated Picture Plot. The Cells of Interest display the Pink color of their gate.

You can also gate on a single cell or group of cells on a **Picture Plot**. Choose a gate shape from the **Gating** tab→**Create Gates** group and draw the gate on the plot.

In Figure 31.5, a single cell gate (light blue) has been drawn on the **Picture Plot**. You can use that gate just like any other gate in FCS Express. For example, you can backgate a Data Grid 681 to show your single cell gate, or you can drag the gate to a Data Grid or other plot to re-gate either one.

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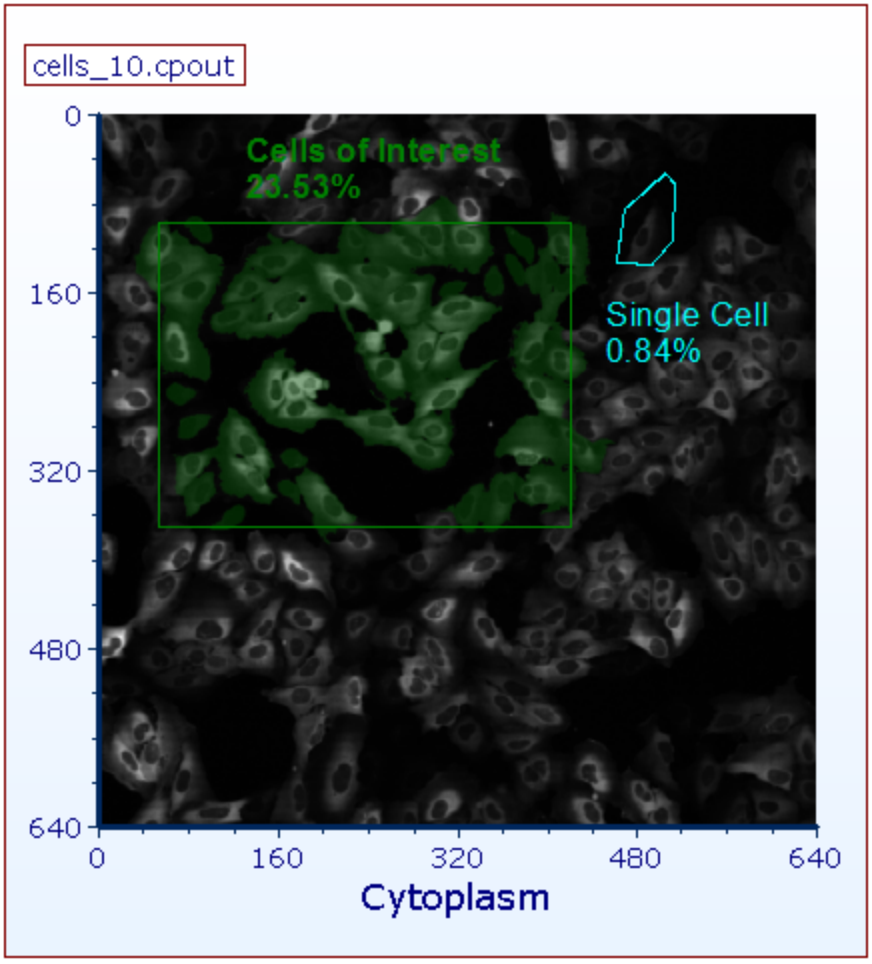

Figure 31.5 Single Cell Gate Drawn on a Picture Plot

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Chapter

31 Heat Maps

In high-throughput laboratories, flow cytometry and cell imaging are often done in multiwell microtiter plates. The data from a 96-well microtiter plate, for example, normally contains 96 separate data files. FCS Express can automatically merge the files from any size plate into one dataset and associate each "cell" with the particular well that it was located in. Even if samples were acquired in tubes FCS Express can merge the individual files so they may be represented in a heat map or "virtual plate".

A **Heat Map** is a graphical representation of your data in a plate-shaped grid with each well of the plate color-coded based on the statistic being displayed. This enables rapid visualization of wells and outliers by comparing the statistical data in an individual well to the statistical data of the entire microtiter plate.

Heat Maps also provide a visualization when **SPADE** is calculated with the **Transformations** navigator and applied. Please see the Working with SPADE Transformations section to learn how to use Heat Maps in conjunction with SPADE transformations.

By choosing an appropriate color scheme and statistic to display, you can get an overview of all of the wells in your experiment or node(s) within the **SPADE tree**. You can also create Well Gates to conveniently pool your data or identify node(s) of interest within the **SPADE tree**.

31.1 Working with Heat Maps

To display a **Heat Map**:

1. Select the **Insert→Other Plots→Heat Map** command.
2. Click (or click-drag) on your layout where you want the heat map to appear.
3. Browse to the data file you wish to insert:
    i. If you are loading a list of data files collected together in a microtiter plate (e.g., data files derived from a BD HTS instrument), each file will likely already contain a **WellID keyword** with the well position for that sample. To open those files as a plate, choose **FCS Plate folder** from the **Files of type** drop-down list in the Select a Data File dialog, open the folder that contains the files, choose any individual well's file in that folder, and click **Open File**.

Note: The folder containing the files should also contain an extra keywords file to indicate the size of the plate, if this information is

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not already present as keywords[708] in the data files. Ready-to-use extra keywords files indicating the size of the plate can be downloaded from the De Novo Software web site.

ii. If you are loading a single file representing all the wells in a plate (e.g., an Accuri .C6 or .CI file), choose the appropriate file type from the **Files of type** drop-down list in the Select a Data File[76] dialog (e.g., "BD Accuri .c6 Plate files"), and click **Open File**. No additional extra keyword files will be required since both the WellID and plate size are likely already contained in the data set.

iii. If you would like to display a heat map for a series of files that have not been acquired in a microtiter plate (i.e. files that contain do not contain a WellID keyword or the plate size), you may do so in the following ways:

- If you would like to control the well which the file will be associated with, the folder containing the files should also contain an extra keywords file[658] to indicate the size of the plate and extrakeywordstable.csv file[652] indicating the WellID (or $WellID) column with the well position for each file.
- If you do not need to control which well the file will be associated with, you can use the Merge FCS Files loader[662] which will load samples into the plate map in the order they were selected during the file merge. Setting the **number of columns to display**[1060] will determine the plate format.

iv. If you are visualizing SPADE tree please insert a Heat Map as described in step 1 above and follow the instructions for Working with SPADE transformations[1031].

v. If you are visualizing result of a k-Means clustering please insert a Heat Map as described in step 1 above and follow the instructions for Applying a k-Means cluster analysis[968].

The data in a **Heat Map** is displayed as a two-dimensional grid of the wells on a microtiter plate. For example, for a 96-well plate, the heat map contains eight rows (labeled A through H) and 12 columns (labeled 01 through 12). The parameter being displayed[1054] is shown as the title of the y-axis. Each well displays a color according to the chosen color scheme[1057], and the well's radius can be dependent upon a chosen parameter[1054] (Figure 32.1).

You can now create Well Gates[1061] to pool your data from multiple wells, and display the parameters of each event on a per-well basis in a Data Grid[1053].

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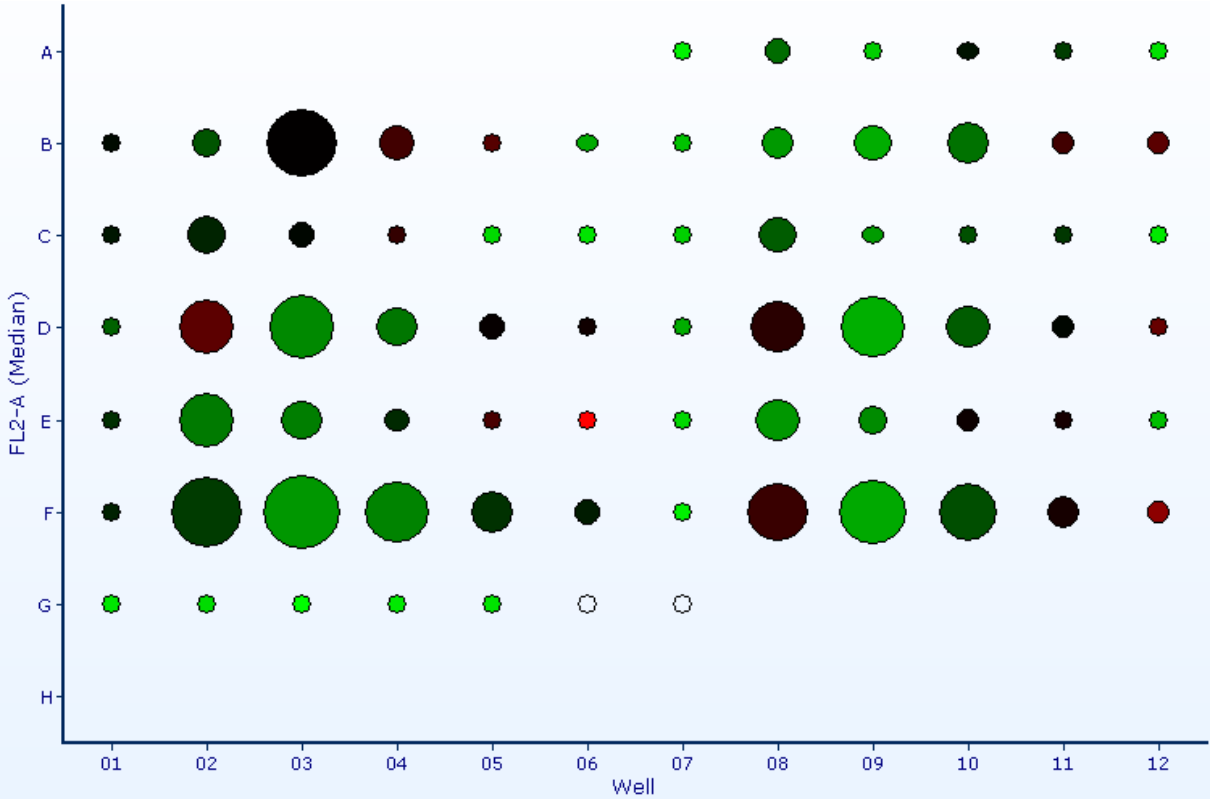

Figure 32.1 Heat Map

When a **Data Grid** is displayed for the **Heat Map**, a **Well** column will be present showing the well where each cell or event is located. The example below is from an image cytometry experiment, where the Data Grid has been formatted as an image gallery 684 (Figure 32.2).

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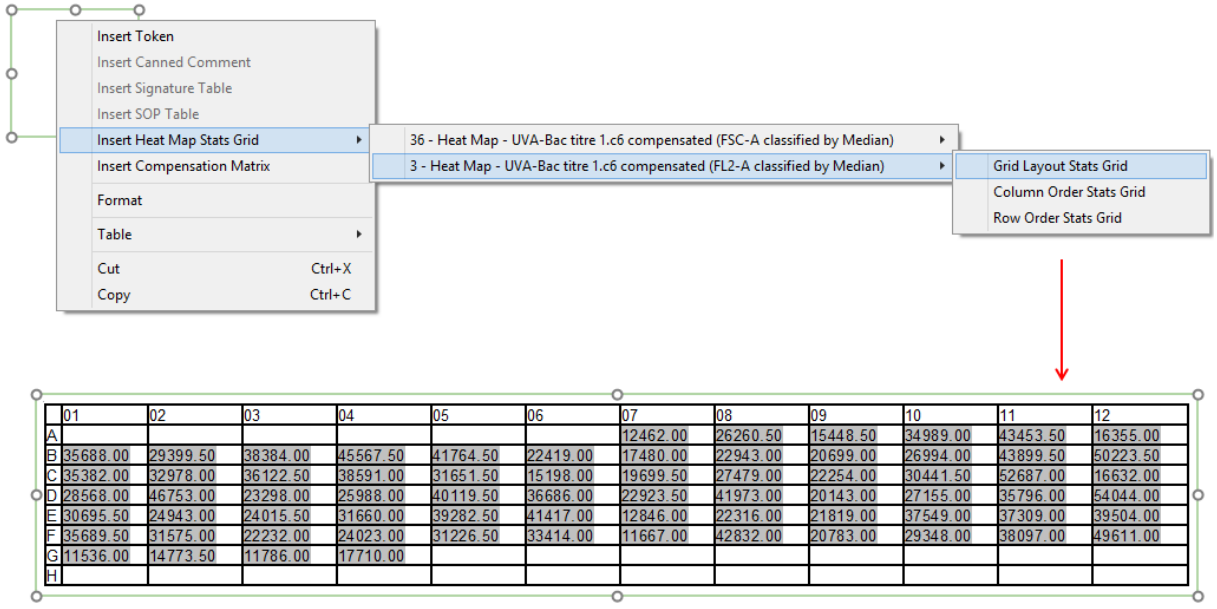
Cell #	Well	AreaShape_Area	Cytoplasm	DNA
1529	A07	1063.00		
1530	A07	1377.00		
1531	A07	374.00		
1532	A07	740.00		
1533	A07	515.00		
1534	A08	984.00		
1535	A08	473.00		
1536	A08	1472.00		
1537	A08	1217.00		
1538	A08	902.00		
1539	A08	1117.00		

Figure 32.2 Data Grid with Well Column

You can insert a table displaying the actual values from each well of your Heat Map into a Text Box[1298]. For a 96-well microtiter plate, this will insert an 8 x 12 data grid. To do so, right-click on the Text Box, choose Insert Heat Map Stats Grid, and select the Heat Map to use as a data source from the menu. (Figure 32.3). Tables can be displayed in Grid Layout Stats Grid, Column Order Stats Grid, or Row Order Stats Grid[1054].



	01	02	03	04	05	06	07	08	09	10	11	12
A							12462.00	26260.50	15448.50	34989.00	43453.50	16355.00
B	35688.00	29399.50	38384.00	45567.50	41764.50	22419.00	17480.00	22943.00	20699.00	26994.00	43899.50	50223.50
C	35382.00	32978.00	36122.50	38591.00	31651.50	15198.00	19699.50	27479.00	22254.00	30441.50	52687.00	16632.00
D	28568.00	46753.00	23298.00	25988.00	40119.50	36686.00	22923.50	41973.00	20143.00	27155.00	35796.00	54044.00
E	30695.50	24943.00	24015.50	31660.00	39282.50	41417.00	12846.00	22316.00	21819.00	37549.00	37309.00	39504.00
F	35689.50	31575.00	22232.00	24023.00	31226.50	33414.00	11667.00	42832.00	20783.00	29348.00	38097.00	49611.00
G	11536.00	14773.50	11786.00	17710.00								
H												

Figure 32.3 - Insert Heat Map Stats Grid into a Text Box

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Grid Layout Stats Grid - displays the heat map well statistics in a table similar to the heat map (Figure above).
Column Order Stats Grid - displays the heat map well statistics according to the plate column order, ie, A01, B01, C01.... (Figure 32.4)
Row Order Stats Grid - displays the heat map well statistics according to the plate row order, ie, A01, A02, A03.... (Figure below)

Column Order Stats Grid

Well	Value
A01	866.44
B01	1101.46
C01	1217.30
D01	1175.83
E01	659.48
F01	878.73
G01	611.47
H01	573.77
A02	1038.41
B02	1195.64

Row Order Stats Grid

Well	Value
A01	866.44
A02	1038.41
A03	1013.46
A04	1138.54
A05	1088.19
A06	973.10
A07	932.78
A08	794.70
A09	789.34
A10	850.15

Figure 32.4 - Heat map stats grids displayed in Column and Row order.

All stats grids may be copied and pasted into Excel or the table dragged into a batch process export action for easy reporting.

31.1.1 Choosing Parameters and Statistics

To select the parameter and statistic to display on the **Heat Map**, use one of the following methods:

- Select the **Heat Map** and use the **Format** tab→**Plot Options**→**Overlays** command (Figure 32.5), and choose from the drop-down menus under **Heat Map Overlay Options**.

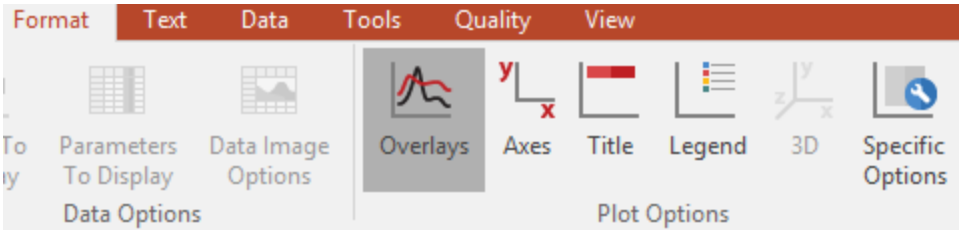

Figure 32.5 - Heat Map Overlays Command from Ribbon

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- Right-click on the **Heat Map**, select **Format** from the pop-up menu, choose the **Overlays** category, and choose from the dropdown menus under **Heat Map Overlay Options** (Figure 32.6).

- Click and hold down the mouse button over the y-axis label and choose an available parameter. Set the statistic to show using one of the above two methods.

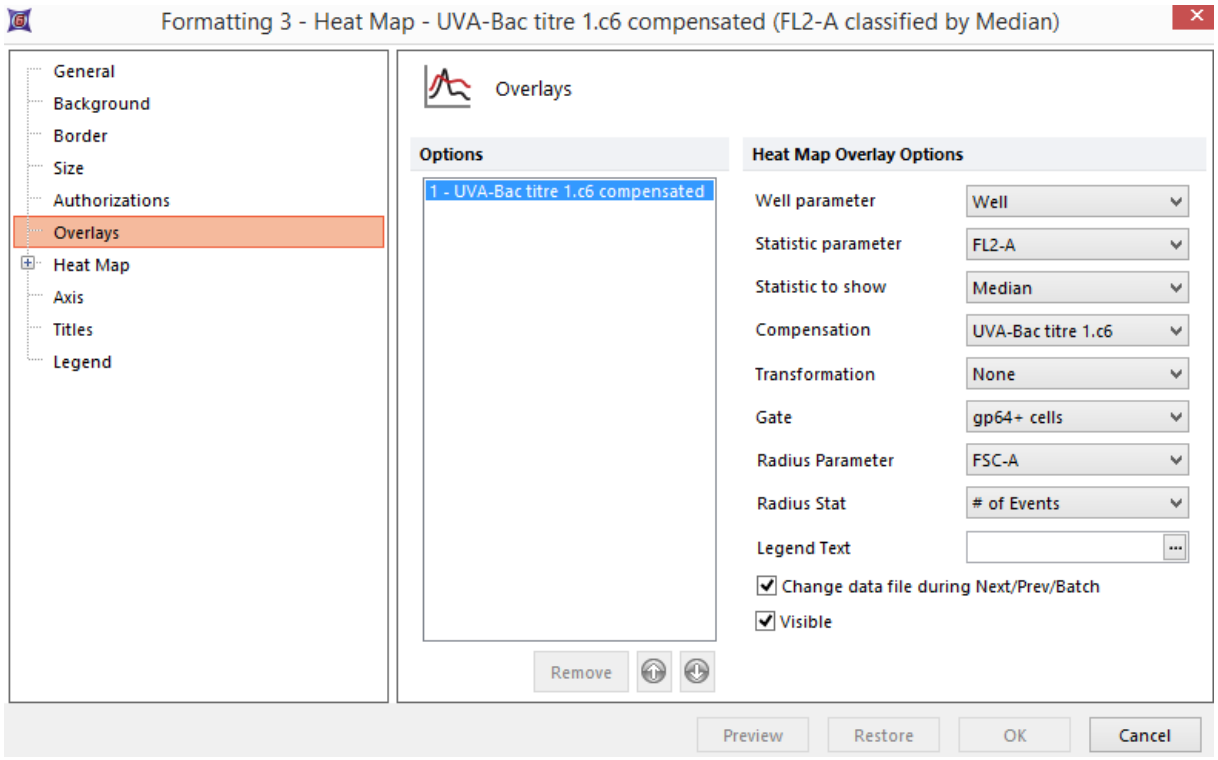

Figure 32.6 Heat Map - Setting the Statistic Parameter and Statistic to Show

The data that is displayed is a combination of the **Statistic parameter** and the **Statistic to show** (e.g., Mean, Standard Deviation, CV, Median, # of events, 75%-ile) properties. FCS Express calculates the average of the statistic chosen across all of the wells in the microtiter plate. Then, a color is displayed for each well according to the color scheme property 1057. The usual procedure is to assign the color by comparing the value of the statistic in each well to either:

- The mean ± SD of the entire plate.

- A predetermined threshold.

Choose the parameter from the **Statistic parameter** drop-down list. The list will normally be populated by a wide range of parameters that were measured or

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calculated by your instrument. Select the **Statistic to show** from the predefined statistics in the corresponding drop-down list.

Available Statistics to Show:

Min (or Max) value	The Min (or Max) value is calculated for each well
# of Event	The number of events is calculated for each well
% of parent	For a given overlay, the % of parent for a given well is calculated as the number of events in that well (for that overlay), divided by the number of events displayed on the entire Heatmap (for that overlay), multiplied by 100. E.g. If Gate 1 is applied to a given overlay of the Heatmap plot, the % of parent for a given well of that overlay is calculated as the number of events in that well (for that overlay), divided by the number of events of Gate 1 (i.e. the total number of events for that overlay), multiplied by 100.
% of grandparent	not available
% of great grandparent	not available
% of all cells	Same as **% of parent** but the total number of events in the entire data file is used as reference.
Median (or Geometric Mean, or Arithmetic Mean)	The Median (or Geometric Mean, or Arithmetic Mean) for the parameter selected in **Parameter Statistics** drop-dow menu is calculated for each well.
CV (or rCV)	The Coefficient of Variation (or the robust Coefficient of Variation) for the parameter selected in **Parameter Statistics** drop-dow menu is calculated for each well.
5%-ile (or, 25%-ile, 75%-ile, 95%-ile)	The selected 5th (or 25th, 75th, 95th) percentile for the parameter selected in **Parameter Statistics** drop-dow menu is calculated for each well.
Concentration	The concentration in each well is calculated based on the Concentration 595 tool.
Standard Deviation	The Standard Deviation for the parameter selected in **Parameter Statistics** drop-dow menu is calculated for each well.
BD-rSD (or BD-rCV)	The BD robust Standard Deviation(or the BD robust Coefficient of Variation) for the parameter selected in **Parameter Statistics** drop-dow menu is calculated for each well.

Please note: *If a **Statistic to Show** for a well cannot be calculated, for instance, the Geometric Mean of a negative value, the well will appear "empty" with vertical and horizontal hatch marks as in the screen shot to the right. Choosing a different **Statistic to Show** may be appropriate.*

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A **Heat Map** can have a **Gate** set similar to any other plot in FCS Express. For example, you can draw a gate on a dot plot displaying the data and then drag the gate onto the heat map. You can also set the gate using the drop-down list in the Formatting window pictured above.

Radius Parameter and Statistic
FCS Express allows a statistic of a parameter be assigned to represent the well's radius (Figure 32.7). The radius on the heat map can change based on the statistic selected. Choose the parameter from the **Radius Parameter** drop-down list. Then, select from the **Radius Stat** drop-down list.

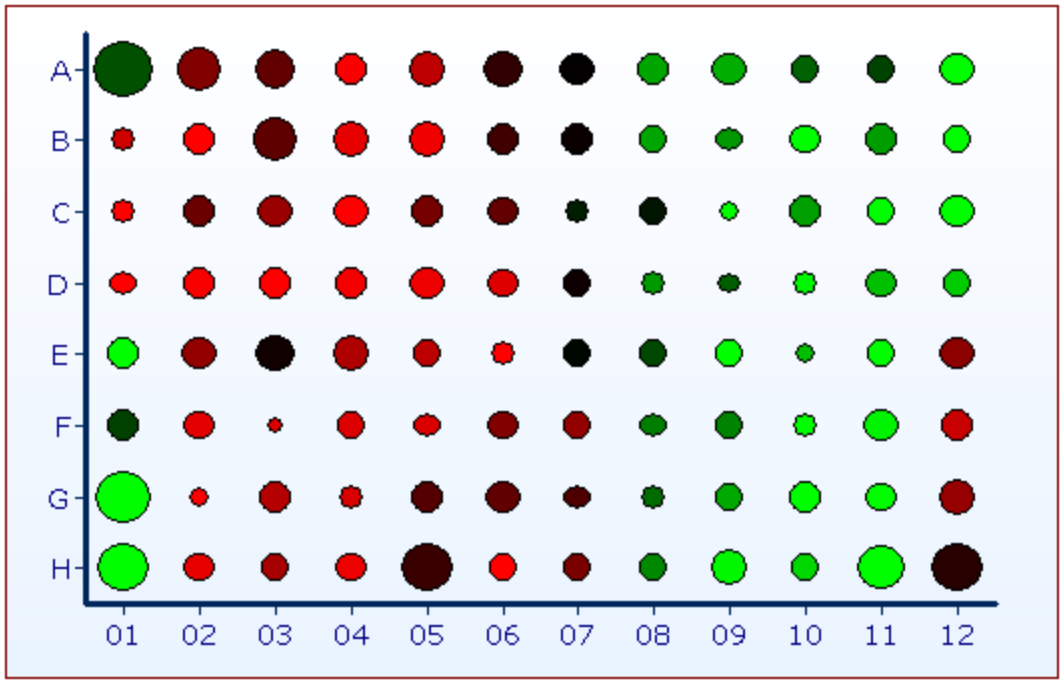

Figure 32.7 - A heat map using the number of events per well as the radius statistic and the mean intensity as the heat statistic. Larger size wells represent wells with more events present in the well.

31.1.2 Choosing a Color Scheme

To choose the color scheme on a heat map, select the Heat Map, and either:

- Select the **Format** tab→**2D Options**→**Color Levels** command (Figure 32.8), or
- Right-click on the **Heat Map**, select **Format** from the pop-up menu, then choose **Heat Map**→**Color Levels**.

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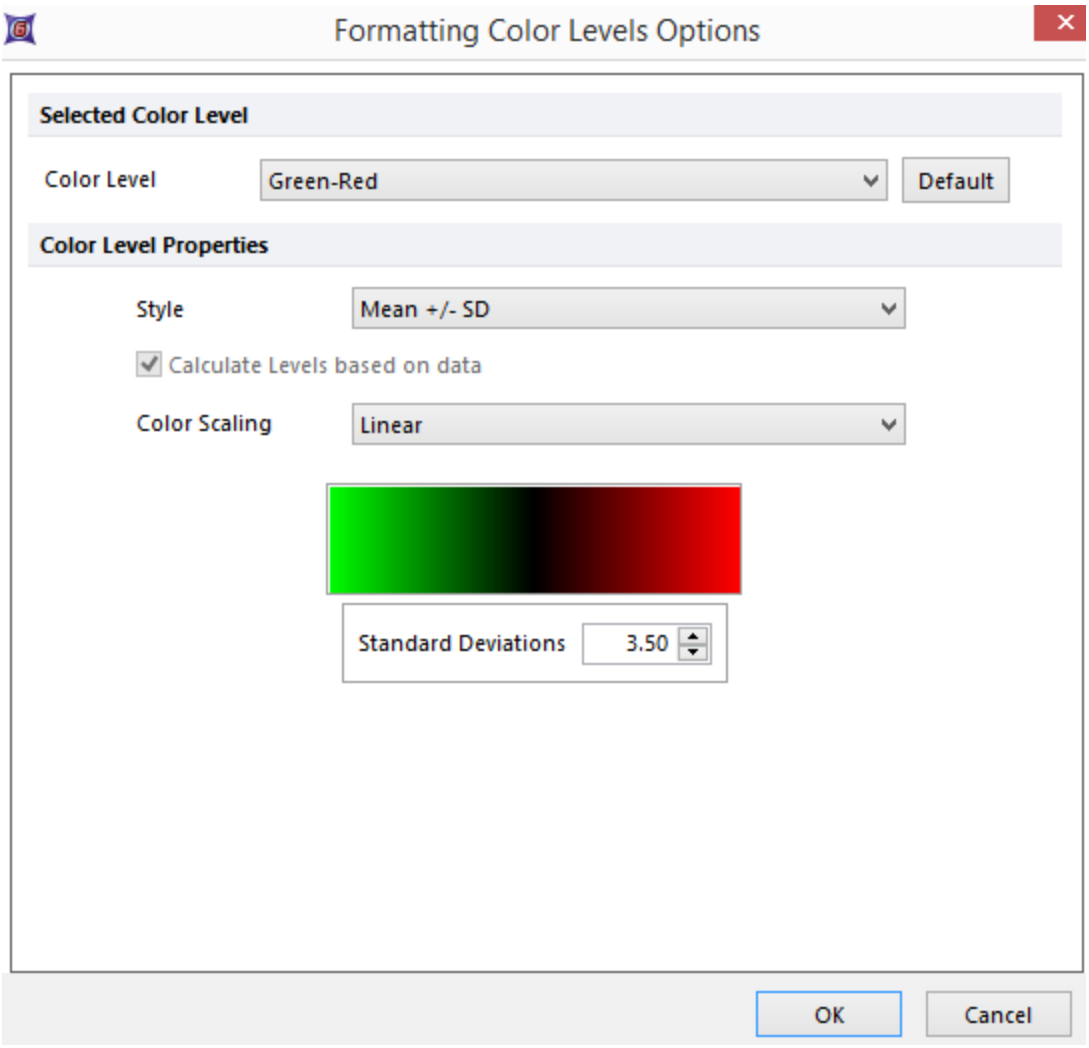

Figure 32.8 Formatting Color Level Options

The default settings are a **Green-Red** color scheme, a **Style** of **Mean ± SD**, and **3.5** standard deviations on either side of the scale. This means that wells close to the average for the statistic being displayed are colored black, wells that are +3.5 SD from the average are pure red, and wells that are -3.5 SD are pure green.

You can select another color scheme using the **Selected Color Level** drop-down list.

Available color schemes are:

- Rainbow
- Threshold
- Green-Red
- Green-Blue

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- Greyscale
- Inverse Greyscale

The **Threshold** scheme has specific **Color Level Properties** related to the threshold value (Figure 32.9).

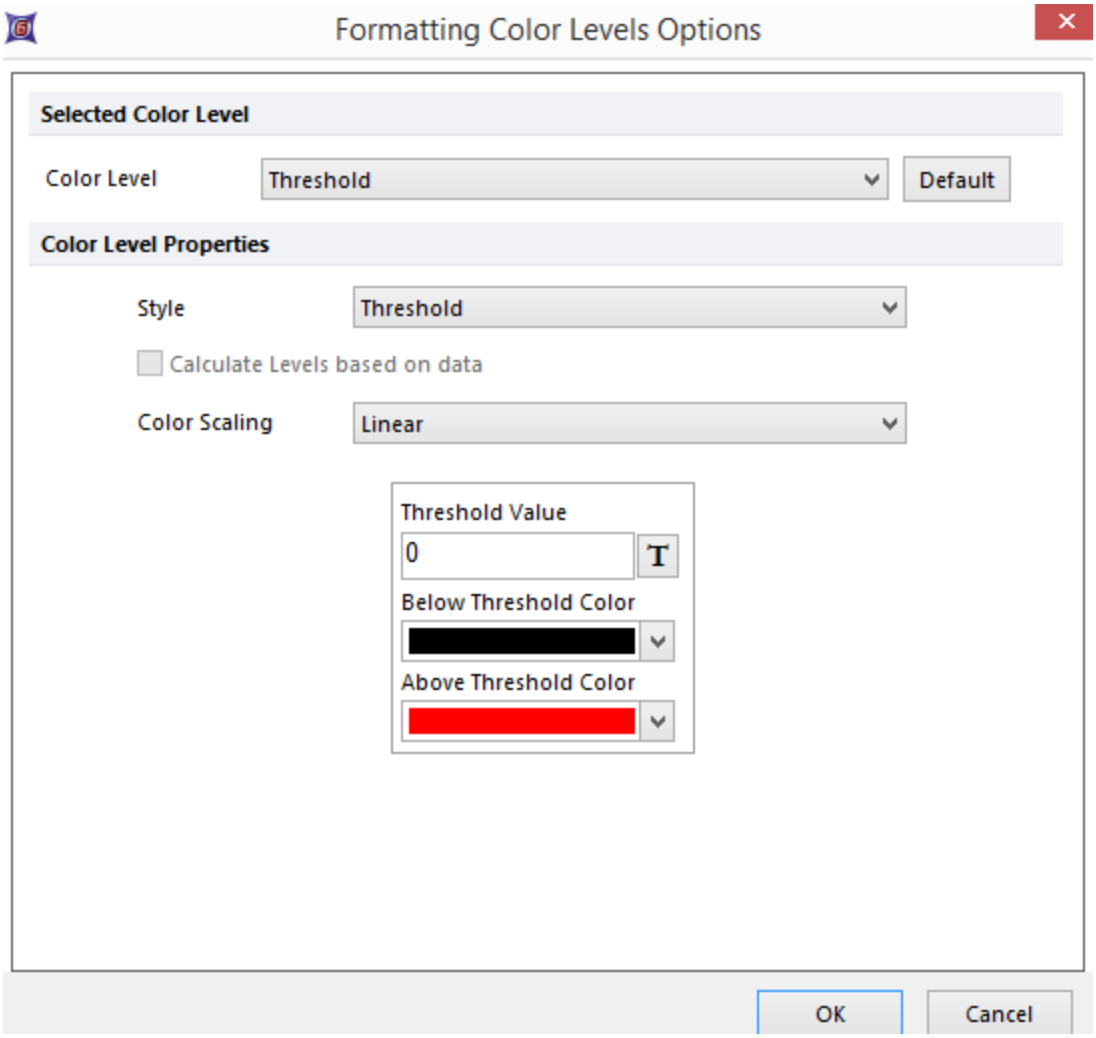

Figure 32.9 Threshold Color Scheme

When the **Selected Color Level** or **Style** are set to **Threshold**, you can choose a token (e.g., a statistic based on a control well) to represent the **Threshold Value** by clicking on the T button, or simply type in an arbitrary freetext value. Then, set the **Below Threshold** and **Above Threshold** colors using the drop-down lists. Wells whose value is below the threshold value will be displayed in the first color, and wells with a value above the threshold value will be displayed in the other color.

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31.1.3 Heat Map Specific Options

Heat Map Specific Options may be accessed by selecting the Heatmap, and either:

- Select the **Format** tab→**Plot Options**→**Specific Options** command from the ribbon bar (Figure 32.10), or
- Right-click on the **Heat Map**, select **Format** from the pop-up menu, then choose **Heat Map**→**Heat Map Specific Options**.

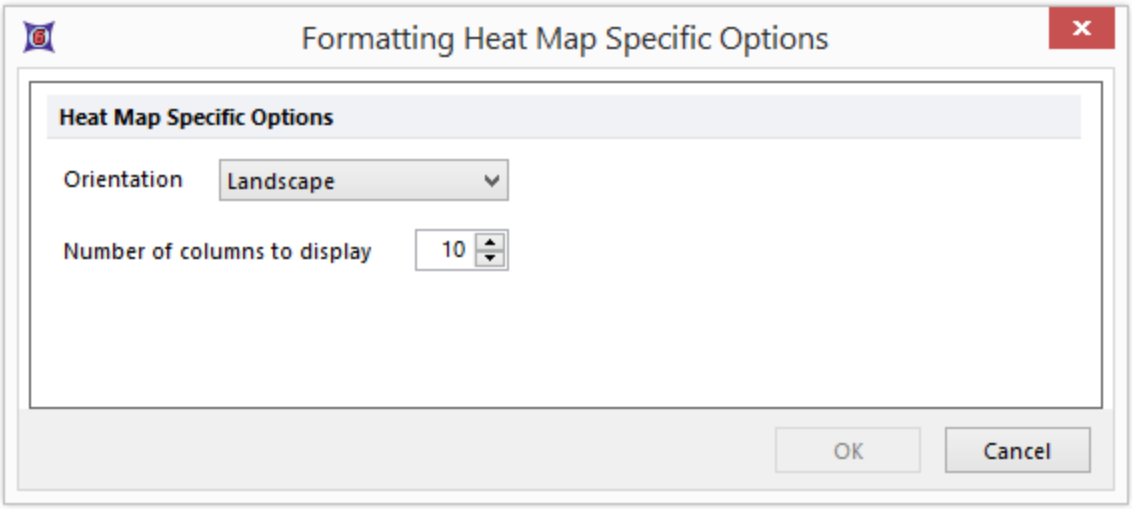

Figure 32.10 The Heat Map Specific Options dialog when accessed via the ribbon bar.

Heat Map Specific Options allow to modify two Heat Map properties:

- **Orientation**. This option allow to display the Heat Map with Landscape or Portrait orientation. For Heat Maps displaying **SPADE**, do not change this setting.
- **Number of columns to display**. This option applies to Heat Maps that display Cluster Assignments from a k-Means transformation 958 and for heat maps that are displaying merged files 662. It allows you to specify how many columns to display for Clustering Assignment rather than choosing a default number of well columns to display. For merged data files it determines how many columns wide the heat map should represent before "wrapping" to the next row. For instance, if 96 samples are merged together that represent a plate map, setting the value to 12 would result in a heat map with 12 columns and 8 rows.

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31.1.4 Well Gates

You can set a gate on one or more wells in a heat map. The wells need not be contiguous. To create a **Well** gate:

1. Select the **Gating** tab→**Create Gates**→**Well** command. (Alternatively, right-click on the heat map and choose **Create gate**→**Well**).
2. Click anywhere on the heat map. An **Editing New Well Gate** dialog (with instructions) for the well gate will appear above the heat map (Figure 32.11).

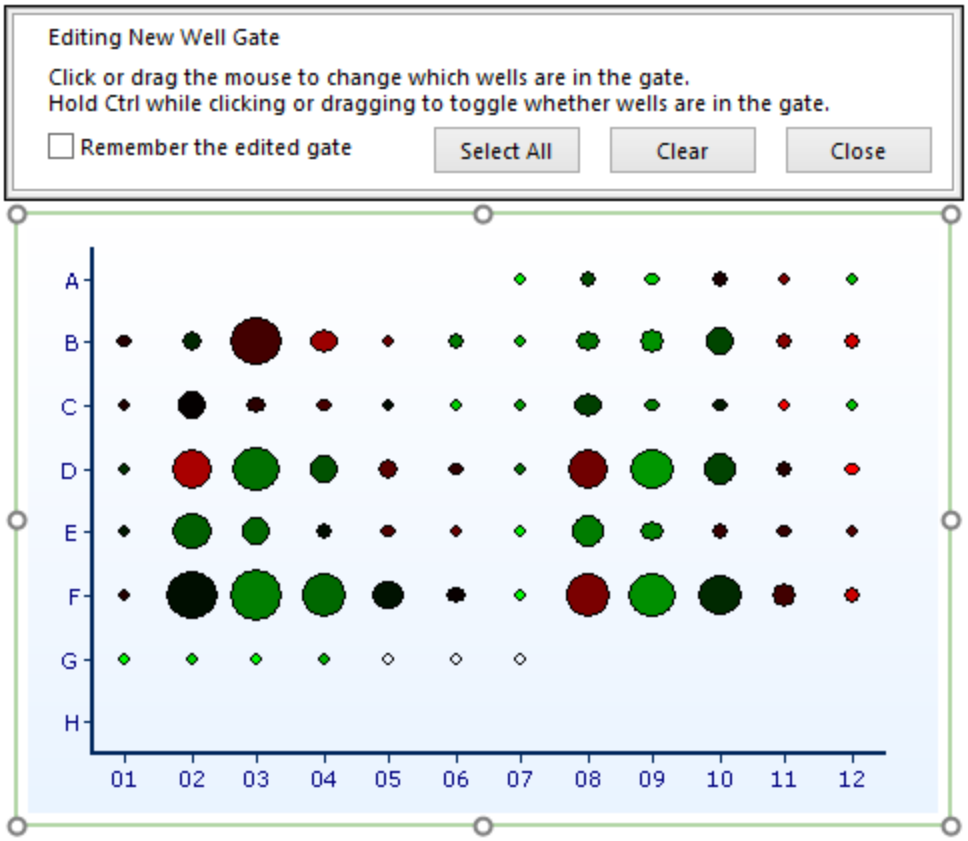

Figure 32.11 Setting a Well Gate

To set the specific gate do one or more of the following:

- Click on an individual well to select it as part of the gate.
- While holding the **Ctrl** key, click on multiple wells.
- Click and drag a rectangle across multiple wells to select all wells within the rectangle as part of the gate.

The **Create New Gate** dialog will appear. Choose a name and color, and click on **OK**.

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The well(s) in the gate will be outlined by a circle that is the same as the gate color (e.g., the wells outlined in light blue in Figure 32.12).

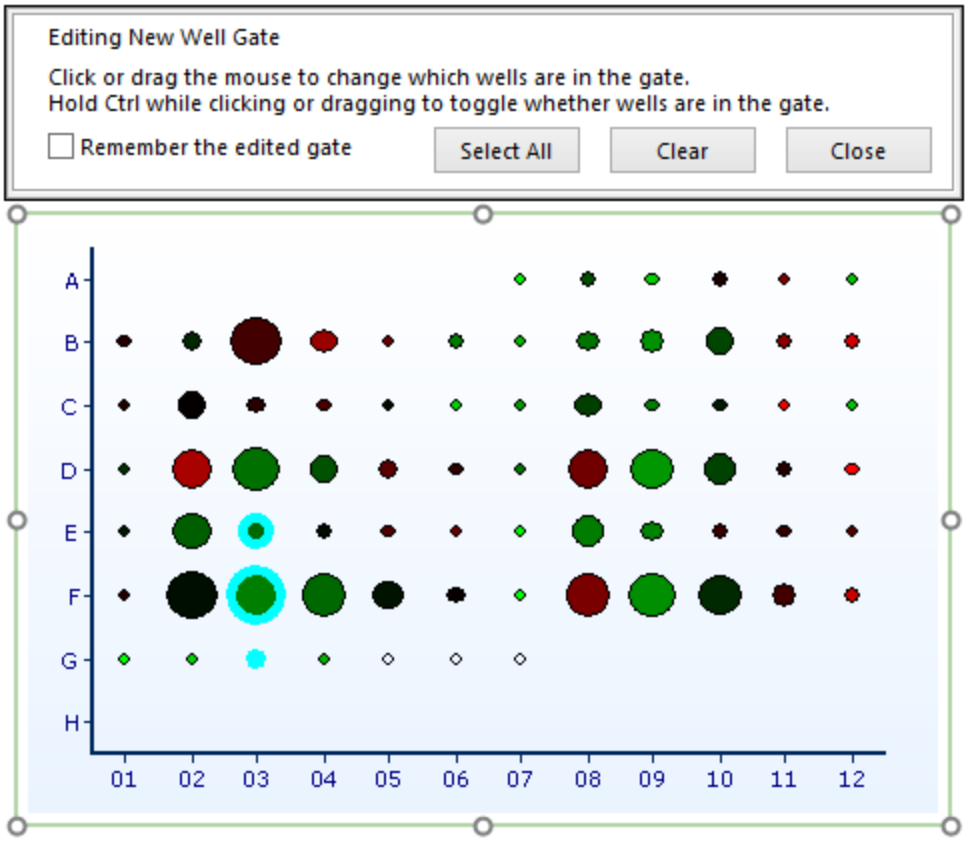

Figure 32.12 - Well Gate. Three wells are outlined in blue (rows E-G, column 03)

To change the gate you just created, click or click-drag on new well(s). The original well(s) will no longer be part of the gate.

To extend the gate you just created to additional wells, hold down the **Ctrl** key while clicking on individual wells (or while dragging a rectangle across a group of cells). The new well(s) will be added to the original well(s) in the gate. Notice a non-contiguous region was selected (Figure 32.13).

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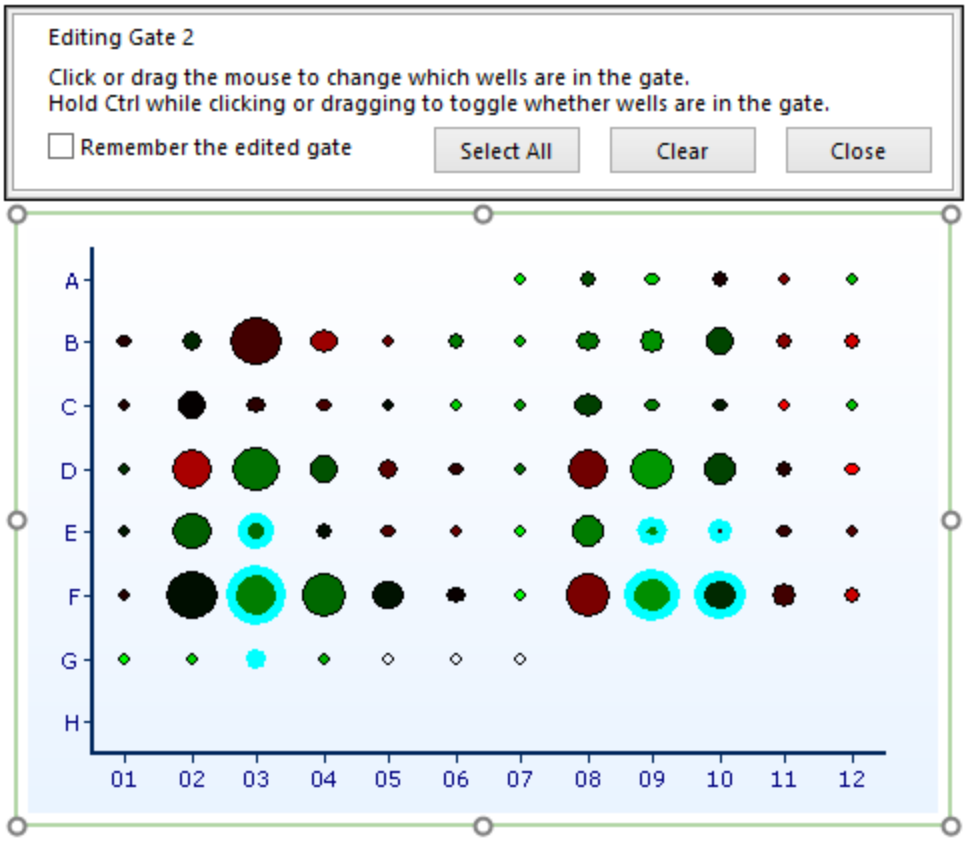

Figure 32.13 Well Gates. Four new wells (rows E-F, columns 09-10) have been added to the previous gate by holding down the Ctrl key when dragging across the new wells.

Click **Close** when you are finished editing.

To activate (and modify) an existing **Well** gate, right-click on the **Heat Map**, select **Edit gate**, and choose the gate to activate from the submenu.

31.1.5 Heat Map Overlays

FCS Express allows users to view multiple statistics and parameters from a high content experiment in a single heat map by adding each statistic as a new overlay. Each well is represented by a circle with each overlay represented as a slice similar to a pie chart. Different overlays may represent different parameters and statistics from the same data set or multiple data sets. One **Color Level** will be applied globally to all overlays of a **Heat Map**.

To create a heat map overlay (Figure 32.14):

1.) Right-click on the **Heat Map**.
2.) Click **Add overlay** from pop-up menu.

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3.) Select data files(s) to use for the overlay from **Select a Data File** dialog (note that the existing data file may be chosen in the **Data List** tab or new files accessed in the **File Dialog** tab).
4.) Click **Open**.

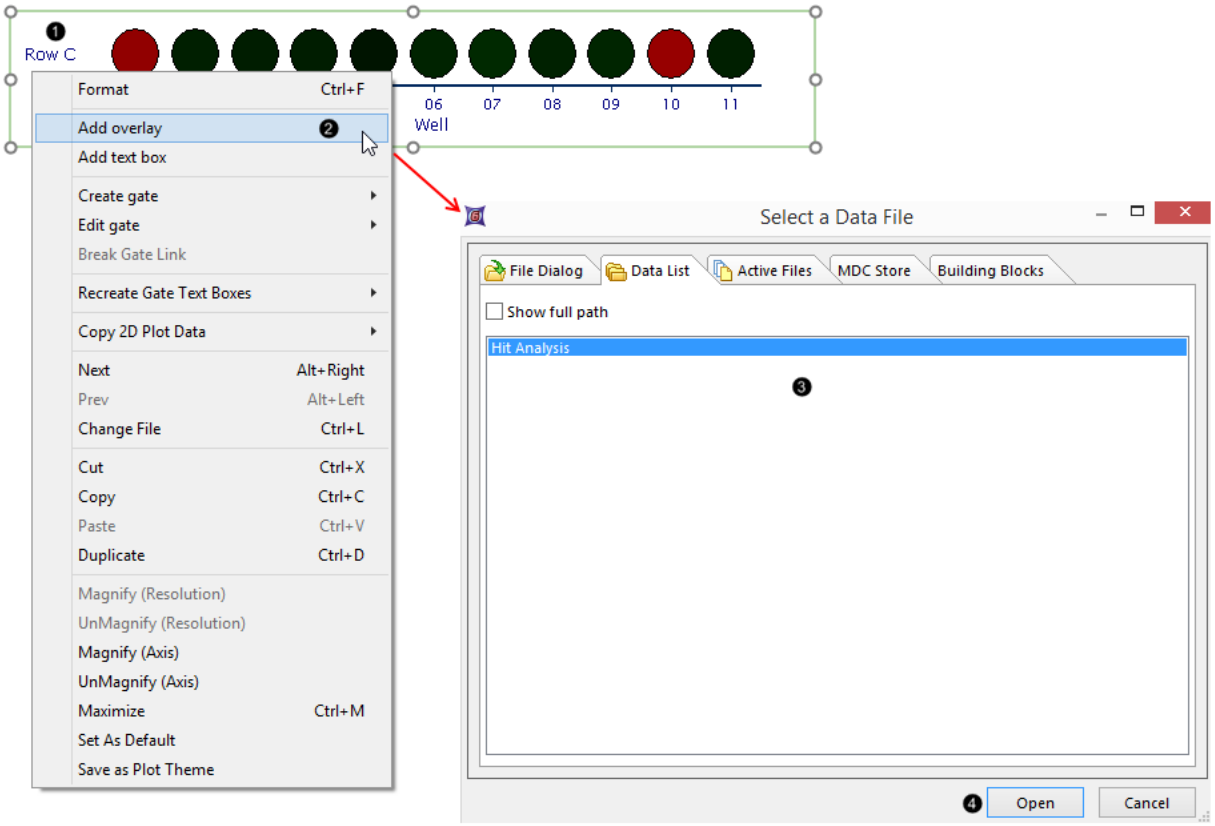

Figure 32.14 - Adding Overlays to a Heat Map, Steps 1-4.

5.) Choose 2D Plot(s) under **2D Plot Selection**.
6.) Select the parameter to use for the overlay from the **Y-Axis Parameter** list.
7.) Click **OK**.

The new overlay will be added to the heat map as in Figure 32.15.

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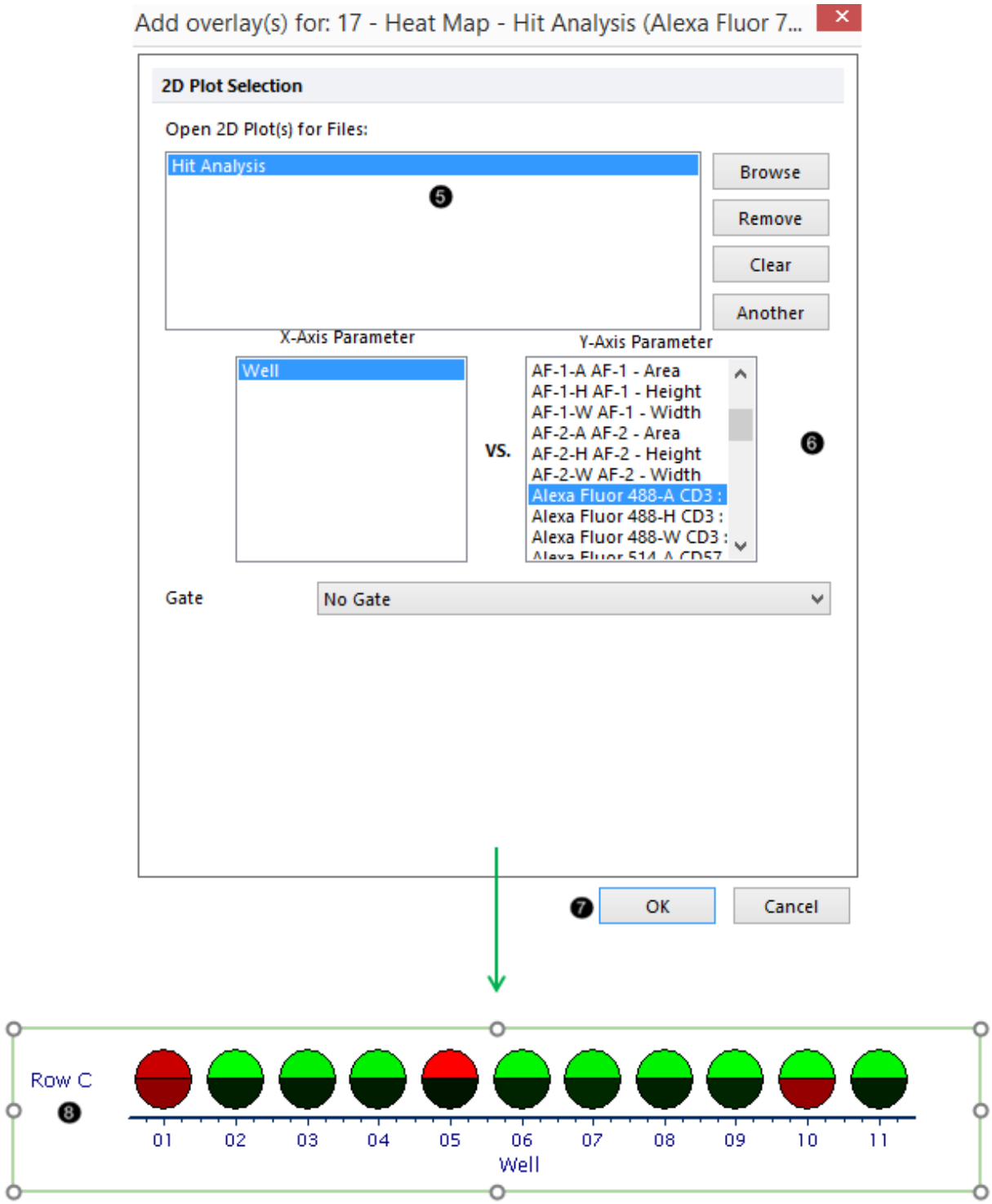

Figure 32.15 - Adding Overlay to Heat Map, Steps 5-7.

The first overlay occupies the lower-right sector of the well, while new overlays are added in a clockwise fashion (Figure 32.16).

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Figure 32.16 - New overlay sectors added in clockwise fashion to Heat Map, starting from lower-right sector.

Please note when visualizing SPADE, only one SPADE tree may be displayed on a Heat Map.

31.1.6 Formatting Heat Map Overlays

Once overlays have been added to a **Heat Map**, they can be edited similarly to other overlays much like **Histogram Overlays** 268 and **2D Plot Overlays** 284.

The **Overlays** option controls the parameter, statistics to display, compensations, transformations, gates, and appearance of heat map overlays.

All heat maps have at least one overlay representing a file and well parameter to display. You can add additional overlays by any of the following methods:

- Right-click on the plot and select **Add overlay** from the pop-up menu, or
- Drag-and-drop file(s) from the **Data List** 785 onto the plot you wish to overlay, or
- Drag-and-drop a plot from the layout onto the plot you wish to overlay.

You can edit overlays in one of two ways:

- Select the heat map and use the **Format→Plot Options→Overlays** command.
- Right-click on the map, select **Format** from the pop-up menu, and choose the **Overlays** category (Figure 32.17).

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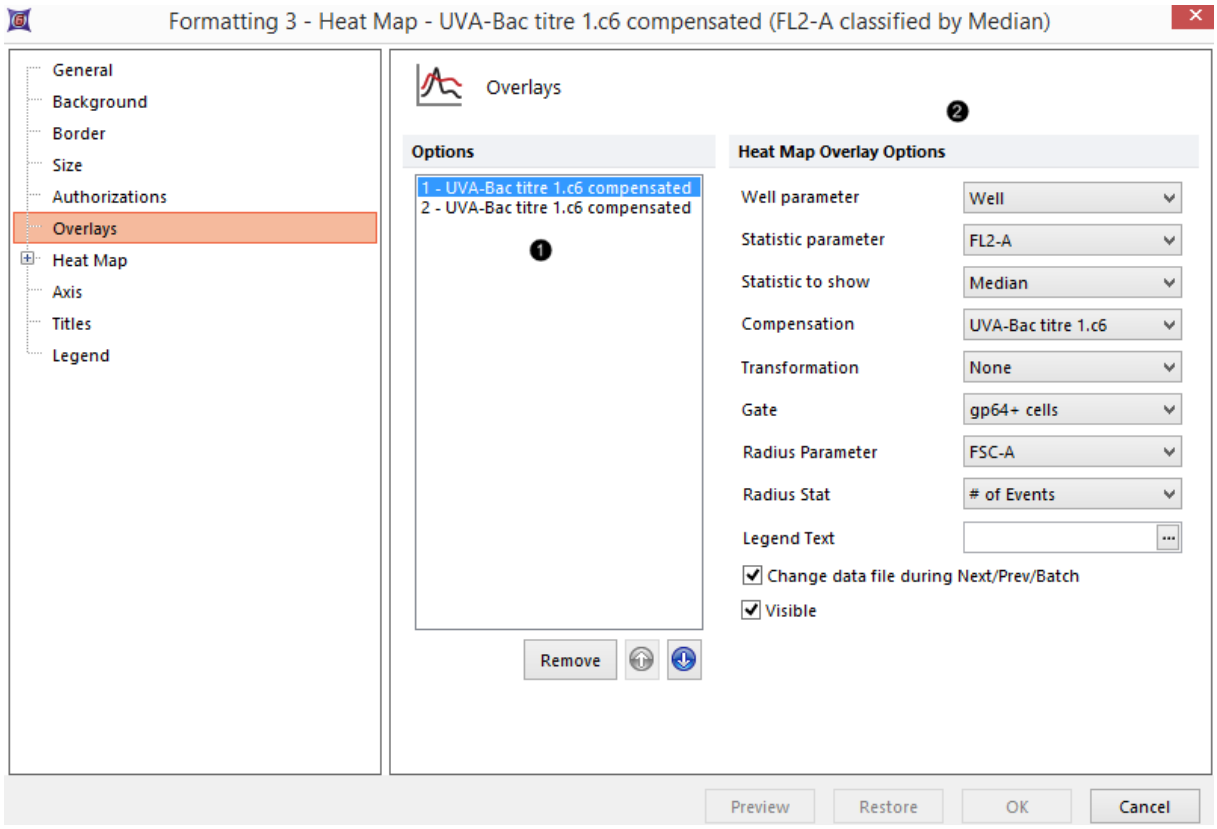

Figure 32.17 - Editing a Heat Map Overlay

In the dialog, a list of all the overlays on the heat map will appear in the list box to the left ❶. You can select one or more overlays using standard Microsoft Windows techniques, including **Shift**-Click and **Ctrl**-Click. The properties on the right ❷ pertain to the currently selected overlay(s). If multiple overlays are selected, the information will appear blank for those properties that are not identical in all selected overlays. If the properties are not blank, then they are identical for all selected overlays. Changing an option will change that property for all selected overlays. For instance, if you select three overlays and change the Gate, the Gate for all three overlays will be modified.

To remove an overlay from a plot, select the overlay (or multiple overlays) and press the **Remove** button.
To change the order of selected overlay(s) in the legend, use the blue up and down arrows ( ).

Heat Map Overlay Options are explained in the table below.

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Option	Explanation
Well parameter	Is the X parameter (well parameter) displayed on the heat map.
Statistic parameter	A drop-down menu to select the parameter from which a statistic will be chosen to be displayed. Please refer to the Setting the Parameter to Display 1054 chapter for more details.
Statistic to show	A drop-down menu to select the statistic 381 to show as an overlay on the heat map. Please refer to the Setting the Parameter to Display 1054 chapter for more details.
Compensation	If compensation definitions 626 are set, select the definition to use when compensating the data in the overlay.
Transformation	If transformations 932 are applied to the data set, select the transformation to use when transforming the data in the overlay.
Gate	If gates are defined 316, select the gate that will be used to limit the data that is displayed on the histogram.
Radius Parameter	*Optional:* A drop-down menu to select the parameter from which a **Radius statistic** will be chosen. A **Radius parameter** must be chosen to use a **Radius statistic**.
Radius Statistic	*Optional:* A drop-down menu to select the statistic to adjust the radius of each well.
Legend Text	The text that will appear in the legend beside the symbol for the overlay. If this field is blank the file name of the overlay will appear. Insert keywords from the header of the data file by pressing the ellipsis next to the legend text. You can also display the current gate applied to the overlay by typing "<$FCSE_GATEFORMULA>" into the legend text.
Change data file during Next/Prev/Batch	If this option is checked, the data file for this overlay will change when doing batch processing 784, or selecting Previous File or Next File 72. This option is usually only disabled for a control histogram, where you have multiple overlays, and want the control to stay the same all of the time.
Visible	You can hide overlays on a heat map without removing them. Select the overlay file from the list and uncheck Visible. Check Visible to show the overlay again.

31.2 Working with Index Sort Data in Heat Maps

Index sorting allows researchers to review the complete phenotype of every single event sorted into a plate and associate that event with all sorted and non sorted events. FCS Express allows for analysis and reporting of index sorting data using **Heat Maps** along with the standard plot offerings 170. For index sort data, Heat maps will display the number of events (usually one) and parameter based phenotypic data for all sorted events. Wells may be gated on individually, or as groups, with the well gates backgated onto other plots to view the

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individually sorted cells in context of the entire acquired/sorted samples. All heat map functionality is available for use with index sorting data.

FCS Express currently supports index sorting data files from the following instruments:

- **Influx (BD Biosciences)** 1069
- **Summit Software (Beckman Coulter)** 1072
- **Diva (BD Biosciences)** 1076
- **Chorus (BD Biosciences)** 1082

31.2.1 Influx Index Sorting Data Files

Influx Index Sorting Data Files contain fluorescence information on both the sorted and unsorted events. To isolate the sorted events:

1. Insert a heat map 1050 for the index sort data file.
2. Select the parameter of interest on the y-axis (heat maps will contain only Index Sorted Data) (Figure 32.18).

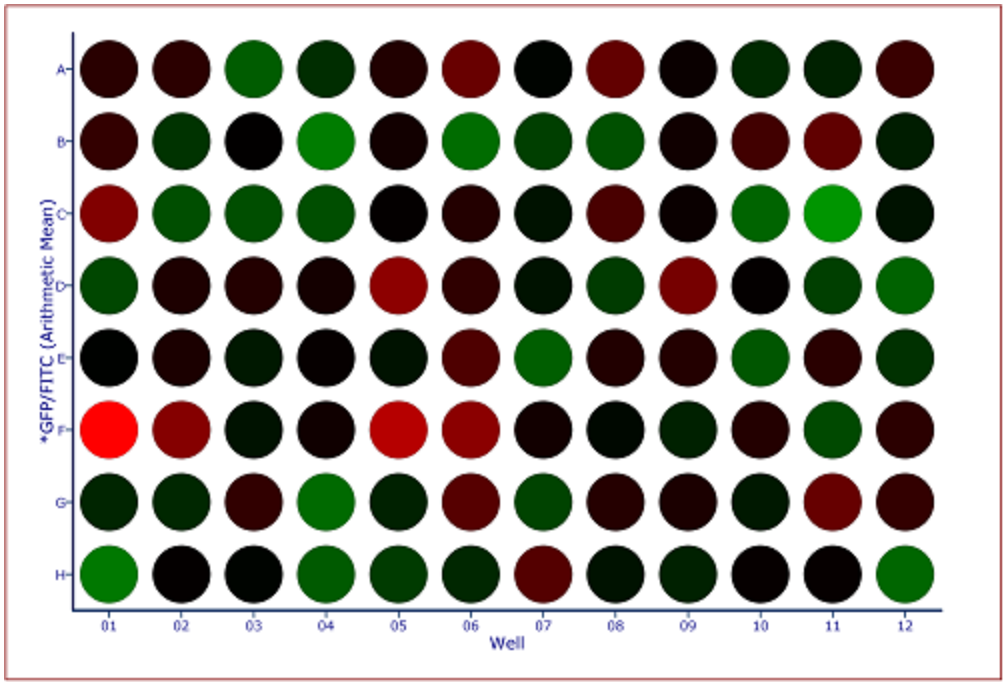

Figure 32.18 Default Heat Map with GFP Arithmetic Mean Y Parameter

3. Insert a Color Dot Plot 170.
4. Change the axes on the color dot plot to the parameters of interest.
5. Create a Well Gate 1061 on all wells.

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Notes:

- *In addition to creating a gate on all wells a gate may be created on one, or any number of wells, to visualize the individually sorted cells.*
- *Since Influx Index Sorting Data Files also contain data for the unsorted events, we can use the [Emphasize on Plots]321 feature of gates to differentiate the sorted from non sorted events* (Figure 32.19).

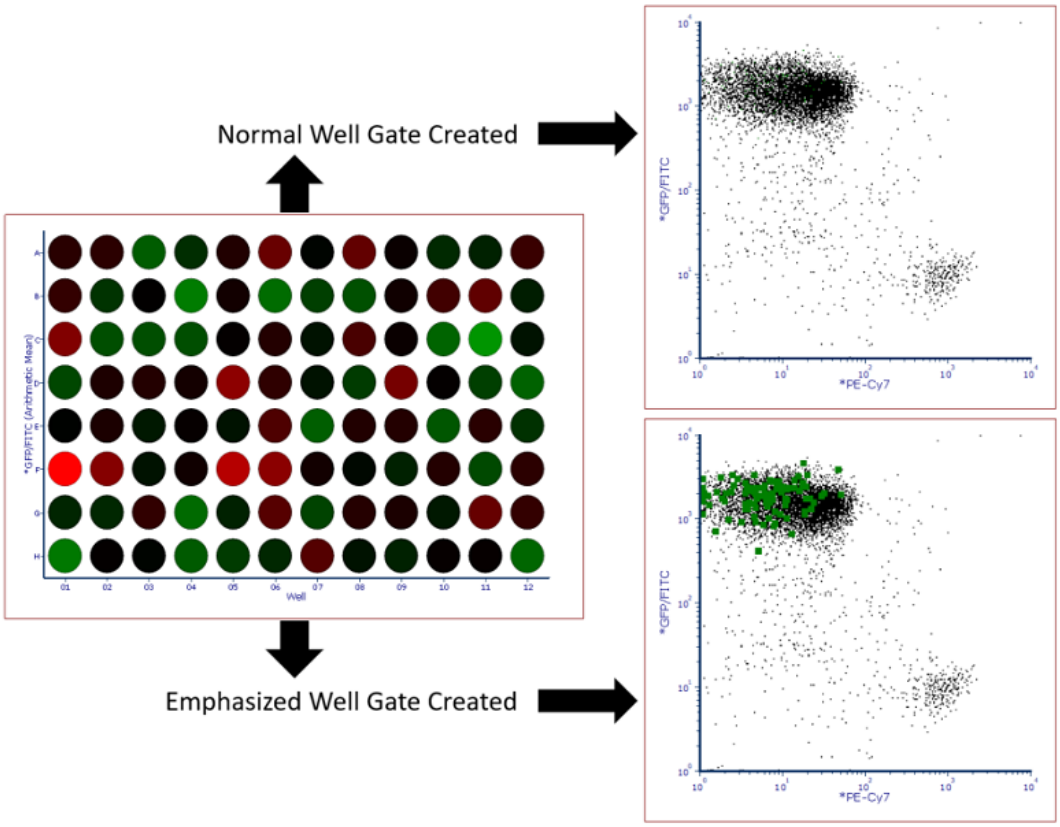

Figure 32.19 Heat map with Well Gate created on all wells. Standard gate is shown above while a gate with the Emphasize on Plots check box checked is shown below. Note that events highlighted in green fall within the well gate and in this case represent all events that were sorted into the plate.

6. **Optional:** Apply the Well Gate created in step 3 to the color dot plot to only view Index Sorted Events (Figure 32.20).

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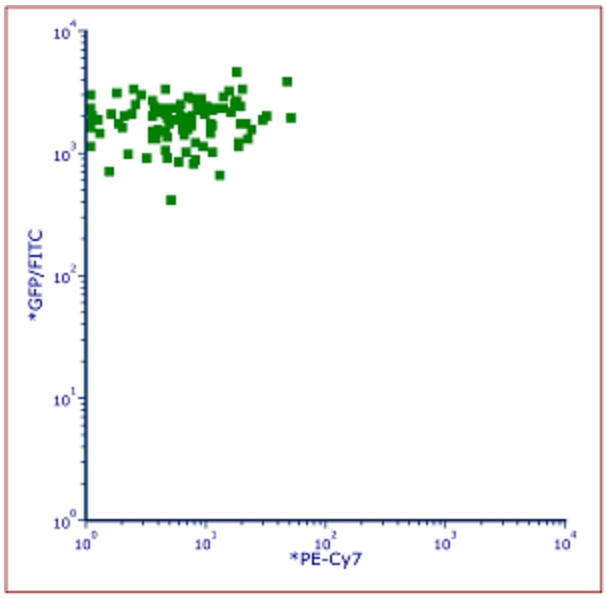

Figure 32.20 Color Dot Plot gated on Well Gate with events emphasized

It is possible to insert a heat map statistics grid in a text box to display statistics for each well in the heat map as done in Figure 32.21.

	01	02	03	04	05	06	07	08	09	10	11	12
A	2358.08	2367.71	1003.24	1479.26	2279.87	3007.11	1889.32	2939.01	2035.71	1523.14	1591.63	2529.03
B	2455.48	1410.83	1952.76	668.83	2123.67	822.66	1293.65	1127.68	2099.34	2606.98	2919.25	1645.31
C	3261.12	1130.22	1142.35	1142.35	1987.09	2294.66	1738.01	2685.07	2045.46	912.95	410.12	1752.72
D	1205.87	2226.05	2289.50	2152.82	3388.20	2406.96	1738.01	1337.65	3144.11	1972.34	1293.65	922.76
E	1908.81	2201.78	1674.47	1996.61	1738.01	2733.81	976.39	2269.96	2294.66	1056.93	2358.08	1435.23
F	4608.66	3290.59	1738.01	2099.34	3807.92	3348.90	2113.85	1845.50	1601.28	2294.66	1174.09	2367.71
G	1567.22	1542.74	2441.03	844.57	1591.63	2821.63	1240.07	2309.21	2206.73	1689.12	2987.73	2460.66
H	707.91	1977.06	1903.98	1005.64	1323.07	1528.07	2802.27	1747.80	1601.28	1991.84	2001.67	891.03

Figure 32.21 Heat Map Statistics Grid displaying statistics for the Y parameter of the Heat Map

Another useful feature for index sorting data is the Heat Map Overlay function. Heat Map Overlays allow a user to view multiple statistics and parameters on a single heat map by adding each statistic as a new overlay. For instance, in Figure 32.22 left, the upper slice of the well represents PE-CY7 arithmetic mean while the lower slice represents GFP arithmetic mean so single and double positive events can be more easily visualized.

Additionally, users may add a **Radius Statistic**, which changes the size of the well to reflect the selected parameter. For instance, in Figure 32 below (right), the heat/color for each well is dependent on the GFP arithmetic mean while the size of the well radius is dependent on the PE-Cy7 arithmetic mean.

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Please see the Heat Map Overlay 1063 section of the manual for full instructions on how to create a heat map overlays of different parameters and types as well as setting the radius parameter statistic.

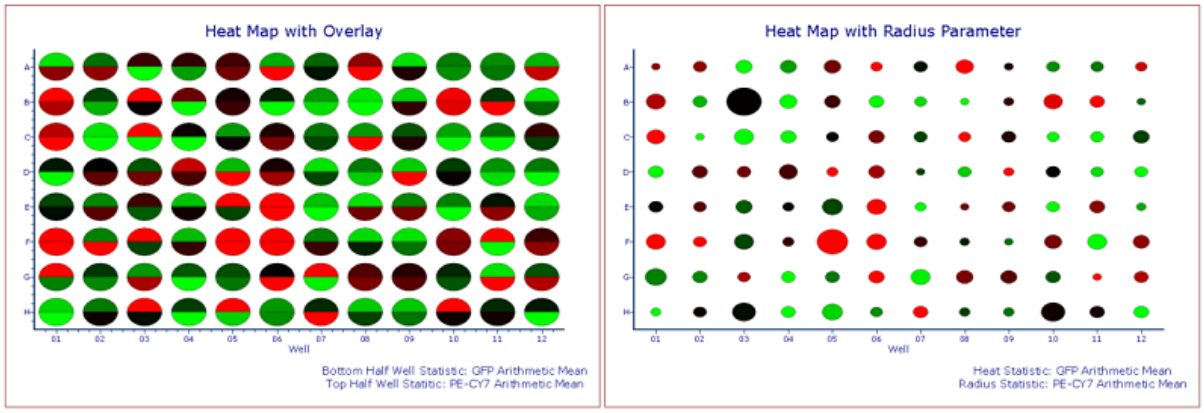

Figure 32.22 Custom Heat Maps with Overlay (left) and Radius Parameter (right). Standard Deviations of Color Levels was changed to 1.

31.2.2 Summit Index Sorting Data Files

Summit Index Sorting Data Files contain fluorescence information on both the sorted and unsorted events. To isolate the sorted events:

1. Insert a heat map 1050 for the index sort data file.
2. Select the parameter of interest on the y-axis (heat maps will contain only Index Sorted Data) (Figure 32.23).

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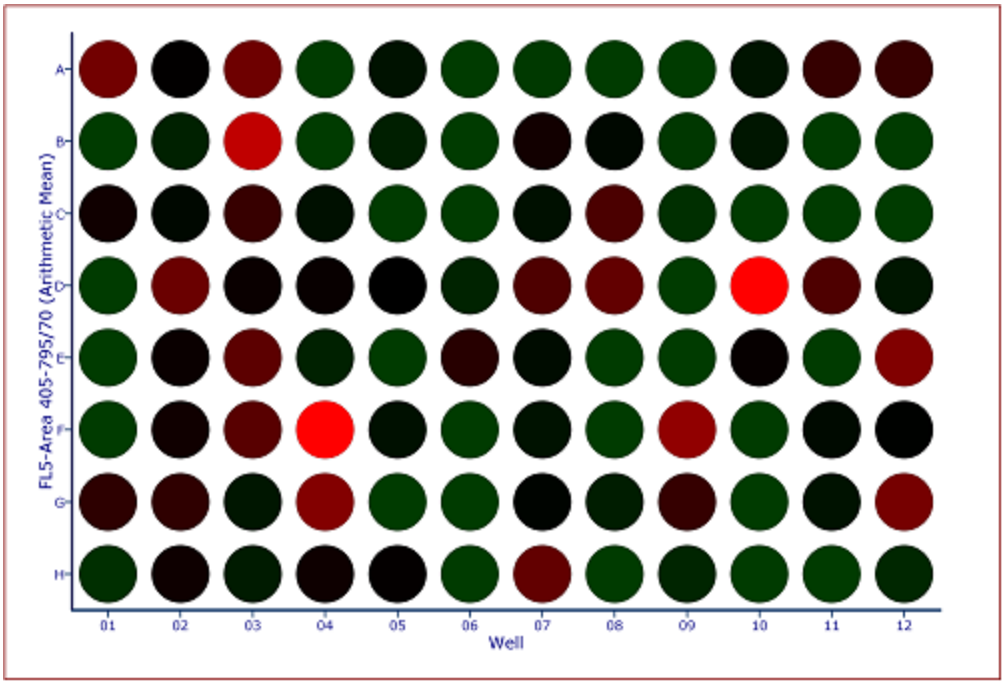

Figure 32.23 Default Heat Map with FL5 Arithmetic Mean Y Parameter

3. Insert a Color Dot Plot 170.
4. Change the axes on the color dot plot to the parameters of interest.
5. Create a Well Gate 1061 on all wells.
   Notes:
   - *In addition to creating a gate on all wells a gate may be created on one, or any number of wells, to visualize the individually sorted cells.*
   - *Since Summit Index Sorting Data Files also contain data for the unsorted events, we can use the Emphasize on Plots 321 feature of gates to differentiate the sorted from non sorted events* (Figure 32.24).

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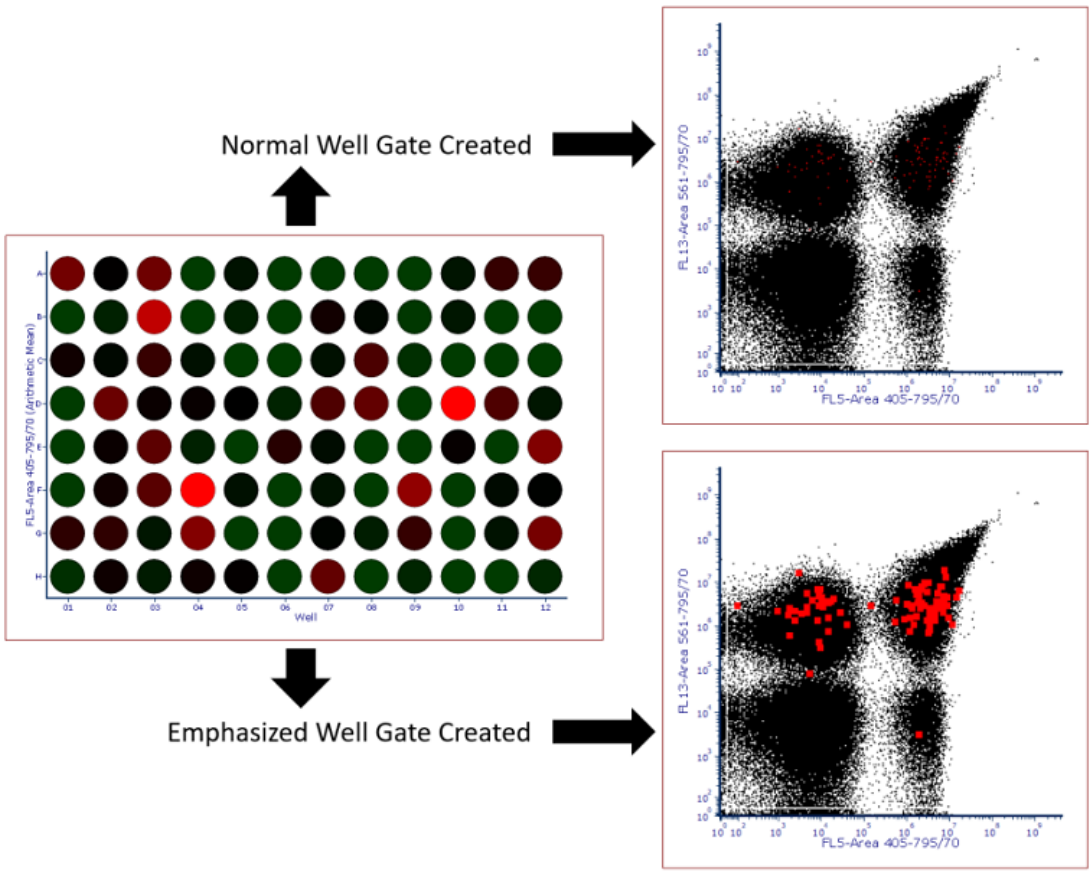

Figure 32.24 Heat map with Well Gate created on all wells. Standard gate is shown above while a gate with the Emphasize on Plots check box checked is shown below. Note that events highlighted in red fall within the well gate and in this case represent all events that were sorted into the plate.

6. **Optional:** Apply the Well Gate created in step 3 to the color dot plot to only view Index Sorted Events (Figure 32.25).

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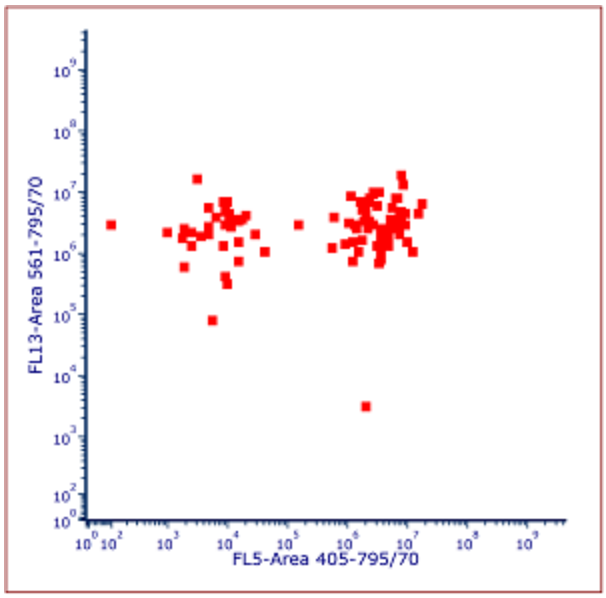

Figure 32.25 Color Dot Plot gated on Well Gate with events emphasized

It is possible to insert a heat map statistics grid 1054 in a text box to display statistics for each well in the heat map as done in Figure 32.26.

	01	02	03	04	05	06	07	08	09	10	11	12
A	8159549.00	2875538.00	8047695.00	9742.00	1902273.00	8243.00	41609.00	4743.00	2559.00	1812175.00	5289792.00	5384204.00
B	15529.00	1198036.00	11862044.00	9195.00	1295829.00	4667.00	3696243.00	2383005.00	150978.00	1680673.00	14899.00	1855.00
C	3566573.00	2396483.00	5376771.00	2034729.00	5529.00	8415.00	2004673.00	6407628.00	592883.00	10637.00	9411.00	1703.00
D	20011.00	7798970.00	3229392.00	3175210.00	2784627.00	1170102.00	6431277.00	7401585.00	6374.00	15022325.00	6443666.00	1717777.00
E	2544.00	3297874.00	7162933.00	1250490.00	9081.00	4629905.00	2170118.00	4693.00	17433.00	3061206.00	95.00	8846847.00
F	999.00	3602360.00	6966886.00	17093972.00	1989512.00	1869.00	1913365.00	3717.00	9608234.00	10477.00	2278094.00	2721665.00
G	4901985.00	5005901.00	1702622.00	8955595.00	9974.00	9231.00	2638343.00	1443168.00	5267456.00	15469.00	1946119.00	8382049.00
H	565156.00	3478054.00	1499686.00	3470526.00	2969502.00	11018.00	7472195.00	3213.00	1089478.00	28029.00	14657.00	947443.00

Figure 32.26 Heat Map Statistics Grid displaying statistics for the Y parameter of the Heat Map

Another useful feature for index sorting data is the Heat Map Overlay 1063 function. Heat Map Overlays allow a user to view multiple statistics and parameters on a single heat map by adding each statistic as a new overlay. For instance, in Figure 32.27 left, the upper slice of the well represents FL13 arithmetic mean while the lower slice represents FL5 arithmetic mean so single and double positive events can be more easily visualized.

Additionally, users may add a **Radius Statistic** 1063, which changes the size of the well to reflect the selected parameter. For instance, in Figure 32 below (right), the heat/color for each well is dependent on the FL5 arithmetic mean while the size of the well radius is dependent on the FL13 arithmetic mean.

Please see the Heat Map Overlay 1063 section of the manual for full instructions on how to create a heat map overlays of different parameters and types as well as setting the radius parameter statistic.

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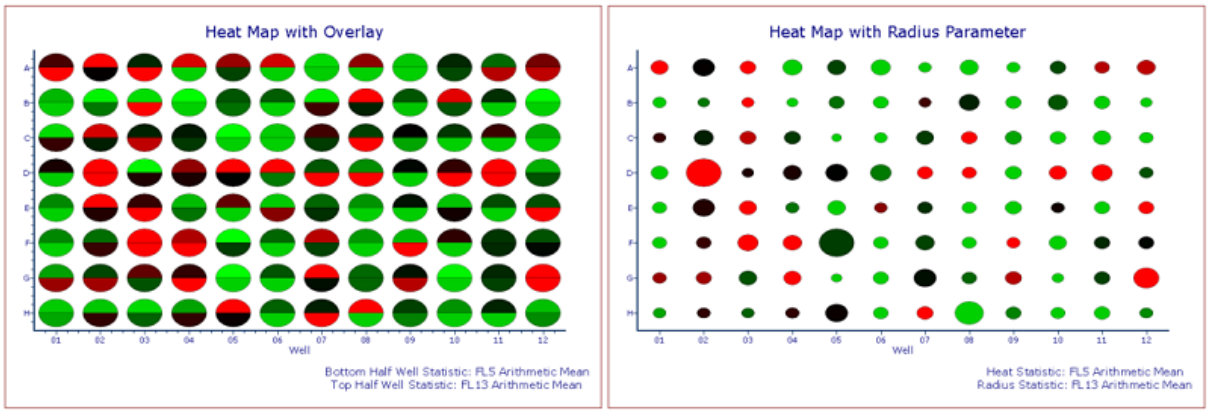

Figure 32.27 Custom Heat Maps with Overlay (left) and Radius Parameter (right). Standard Deviations of Color Levels was changed to 1.

31.2.3 Diva Index Sorting Data Files

Diva Index Sorting Data Files contain fluorescence information only on the indexed sort events. A larger "master" .fcs file contains information on all of the events collected during a sort while a single, or number of, smaller .fcs files contain only the sorted events. See Figure 32.28 below where the master file is 354 kB in size while the file containing only sorted events is 7 kB in size. These two files may be used individually or in conjunction via plot overlays to help visualize index sorting data acquired with Diva.

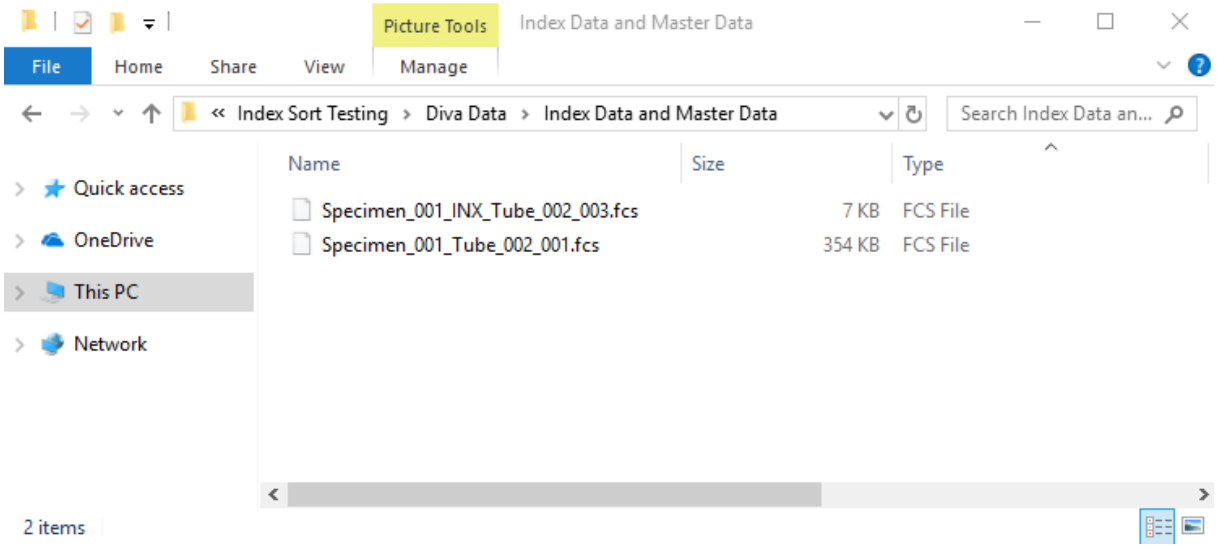

Figure 32.28 Diva Index Sorting Data Files. Note the larger sized "master" file that contains all events collected and the smaller size file that only contains sorted events.

To analyze Index Sort Data:

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1. Insert a heat map 1050 for the index sort data file.
2. Select the parameter of interest on the y-axis (Figure 32.29).

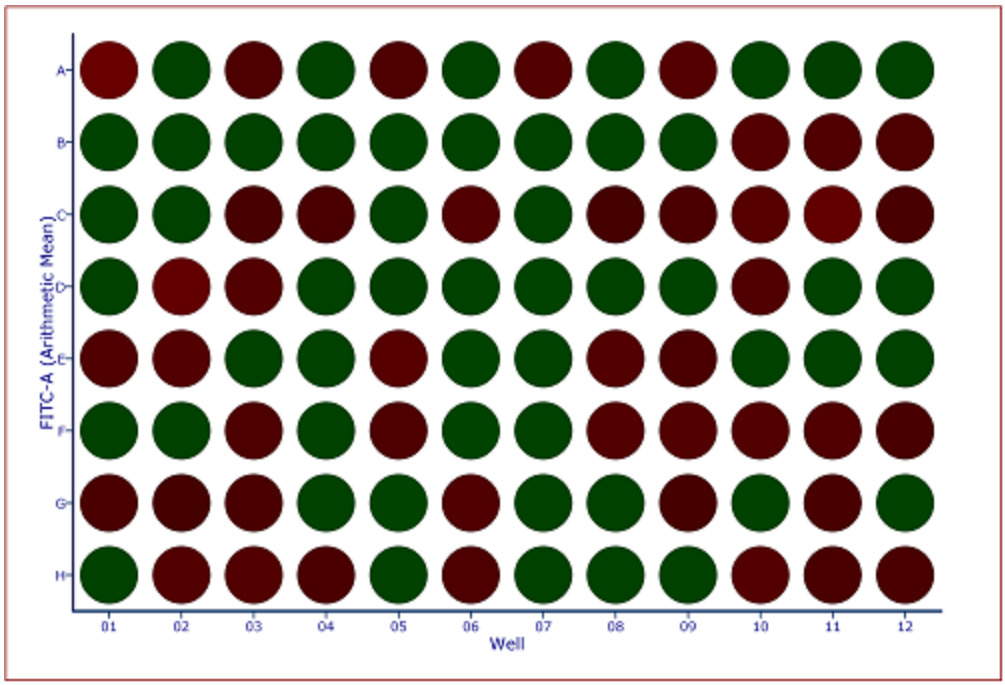

Figure 32.29 Default Heat Map with FITC Arithmetic Mean Y Parameter

3. Insert a Color Dot Plot 170.
4. Change the axes on the color dot plot to the parameters of interest.
5. Create a Well Gate 1061 on all wells.

Notes:

- *In addition to creating a gate on all wells a gate may be created on one, or any number of wells, to visualize the individually sorted cells.*
- *Since Diva Index Sorting Data Files only contain data for the unsorted events, we can use the Emphasize on Plots 321 feature of gates to emphasize the events that fall within the gate* (Figure 32.30).

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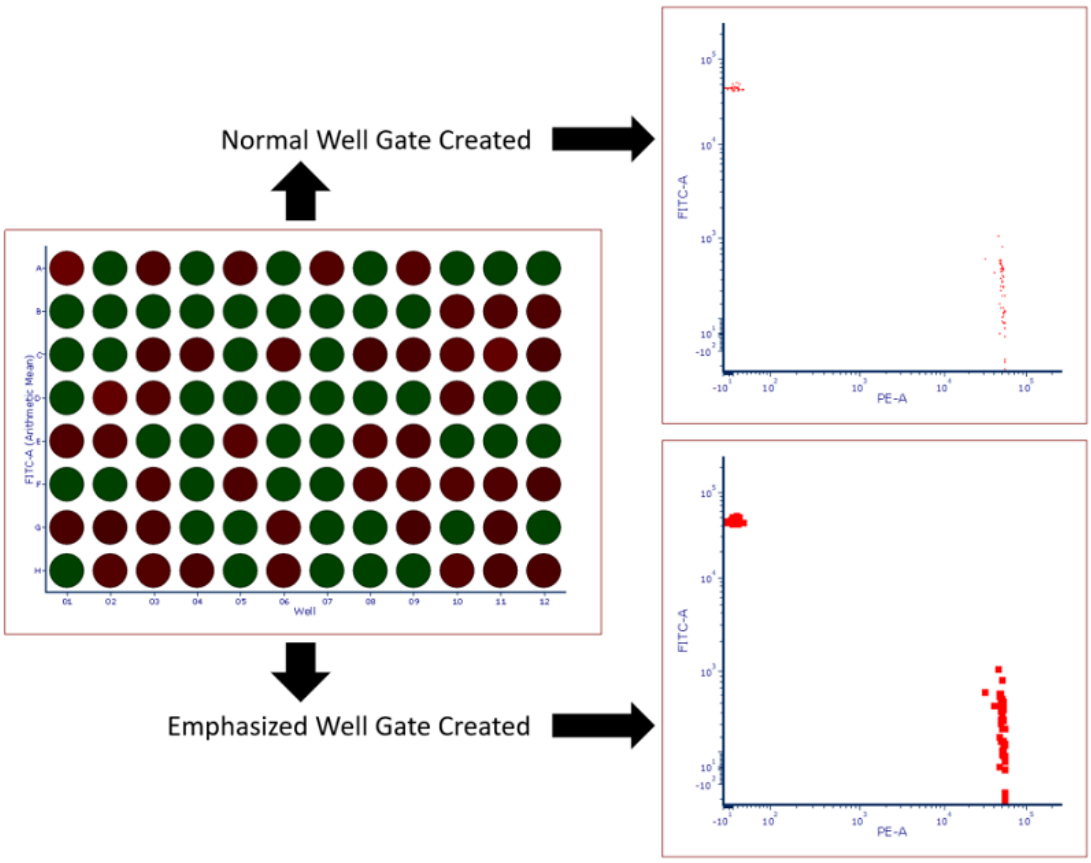

Figure 32.30 Heat map with Well Gate created on all wells. Standard gate is shown above while a gate with the Emphasize on Plots check box checked is shown below. Note that events highlighted in red fall within the well gate and in this case represent all events that were sorted into the plate making them easier to visualize.

6. **Optional:** Create additional Well Gates to isolate an individual well (blue event) or a group of wells (purple events) (Figure 32.31).

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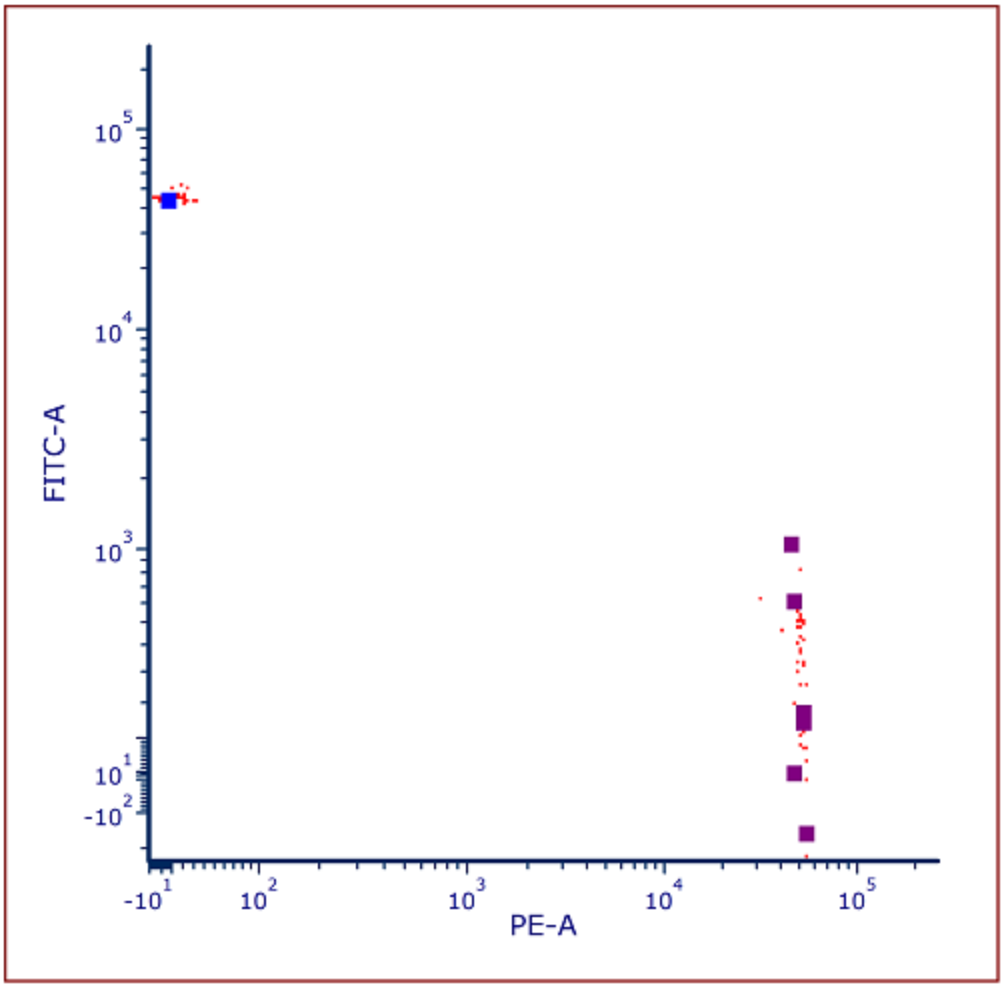

Figure 32.31 Color Dot Plot gated on Well Gate with Children Gates Applied and Emphasized

In the event that you would like to compare the master file to the Index Sort File, you can create a Color Dot Plot Overlay[286] with increased dot size for the Index Sort Data. To do so:

1. Insert a **Color Dot Plot** for the larger "master" file.
2. Change axes to reflect parameters of interest (Figure 32.32).

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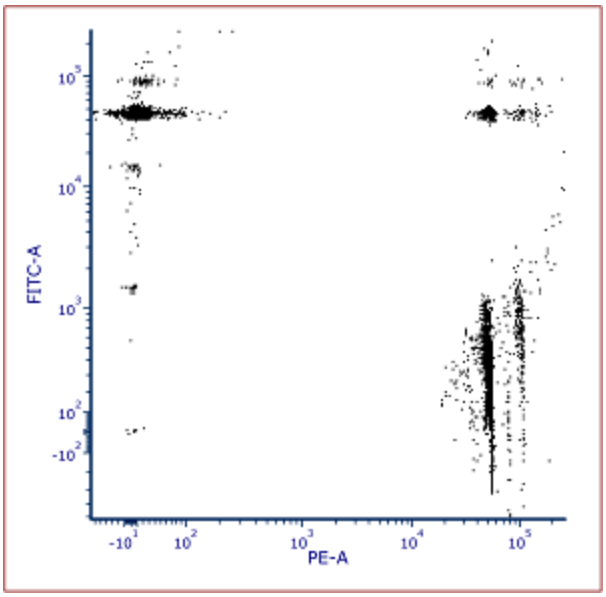

Figure 32.32Color dot plot of the "master" file (largest file in the data directory).

3. Drag and drop index sort data file (smaller data file in the data directory) from the data list onto the Color Dot Plot.
4. Select **add the files to the plot as new overlays** radio button (Figure 32.33).

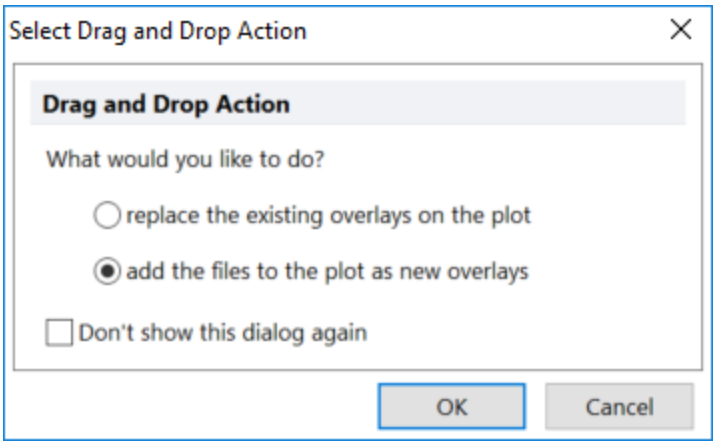

Figure 32.33 Select Drag and Drop Action Window to add Overlays to Color Dot Plot

5. Click **OK.**
6. Right click on the color dot plot.
7. Select **Format**.
8. Chose the **Overlays** category.
9. Select the second overlay.
10. Increase the **Dot Size** to 5.
11. Click **OK** (Figure 32.34).

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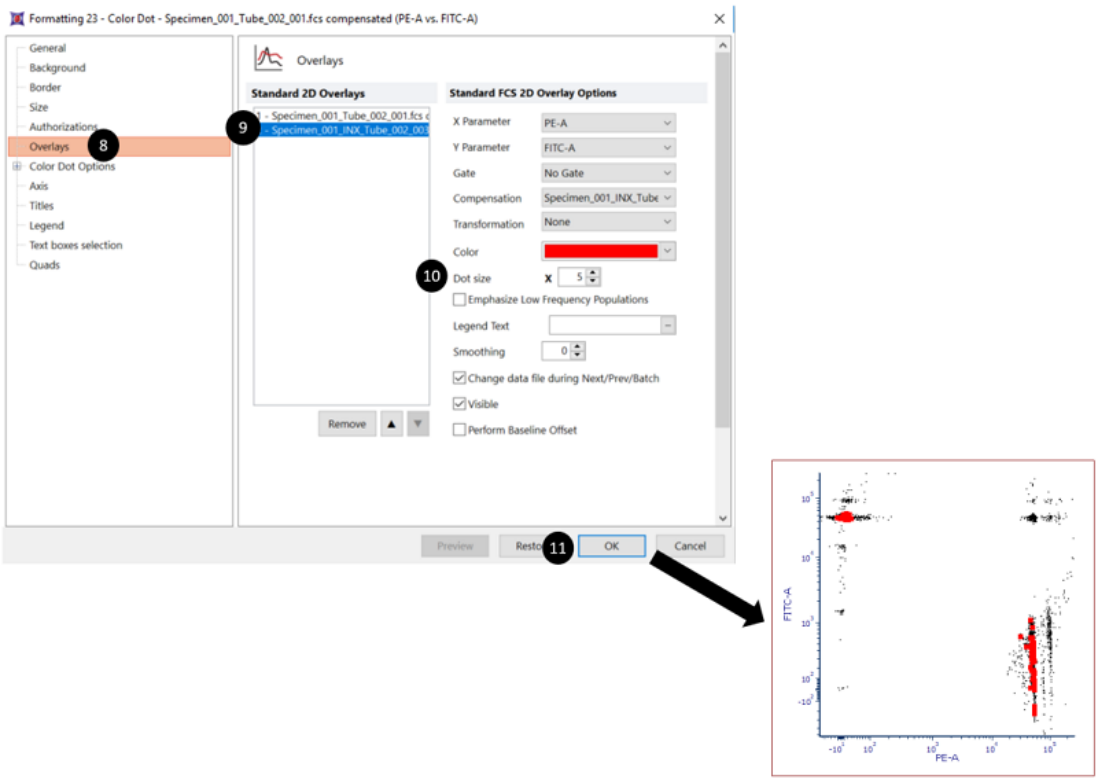

Figure 32.34 Adding an Overlay to Color Dot Plot with increased dot size for the index sorted data file.

It is possible to insert a heat map statistics grid[1054] in a text box to display statistics for each well in the heat map as done in Figure 32.35.

	01	02	03	04	05	06	07	08	09	10	11	12
A	51070.32	131.99	42779.79	253.30	43210.86	109.18	43993.89	559.94	44725.13	370.98	810.36	491.17
B	-187.80	494.11	160.88	443.84	32.70	328.08	602.94	68.05	557.71	44604.73	43686.14	42734.34
C	321.76	413.55	41848.21	42210.68	492.88	44209.70	124.31	40457.31	42279.23	45361.39	49029.46	41659.32
D	398.29	48795.23	44011.23	595.23	1017.23	131.80	-163.93	165.36	0.74	43326.69	459.12	522.65
E	43716.32	44326.72	246.96	99.87	45177.70	-18.32	356.48	44379.16	42553.08	466.18	142.70	425.26
F	79.11	564.44	42764.92	312.21	43208.97	64.93	106.53	44056.00	43896.30	44122.50	43371.80	41976.24
G	42570.93	40721.00	42326.46	514.70	-239.13	43530.55	163.43	473.49	41200.99	284.63	41470.43	428.03
H	121.80	43923.55	44172.72	42505.20	416.42	43116.39	192.88	541.70	590.59	45099.99	42428.33	42321.62

Figure 32.35 Heat Map Statistics Grid displaying statistics for the Y parameter of the Heat Map

Another useful feature for index sorting data is the Heat Map Overlay[1063] function. Heat Map Overlays allow a user to view multiple statistics and parameters on a single heat map by adding each statistic as a new overlay. For instance, in Figure 32.36 left, the upper slice of the well represents PE arithmetic mean while the lower slice represents FITC arithmetic mean so single and double positive events can be more easily visualized.

Additionally, users may add a **Radius Statistic**[1063], which changes the size of the well to reflect the selected parameter. For instance, in Figure 32 below (right),

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the heat/color for each well is dependent on the FITC arithmetic mean while the size of the well radius is dependent on the PE arithmetic mean.

Please see the Heat Map Overlay 1063 section of the manual for full instructions on how to create a heat map overlays of different parameters and types as well as setting the radius parameter statistic.

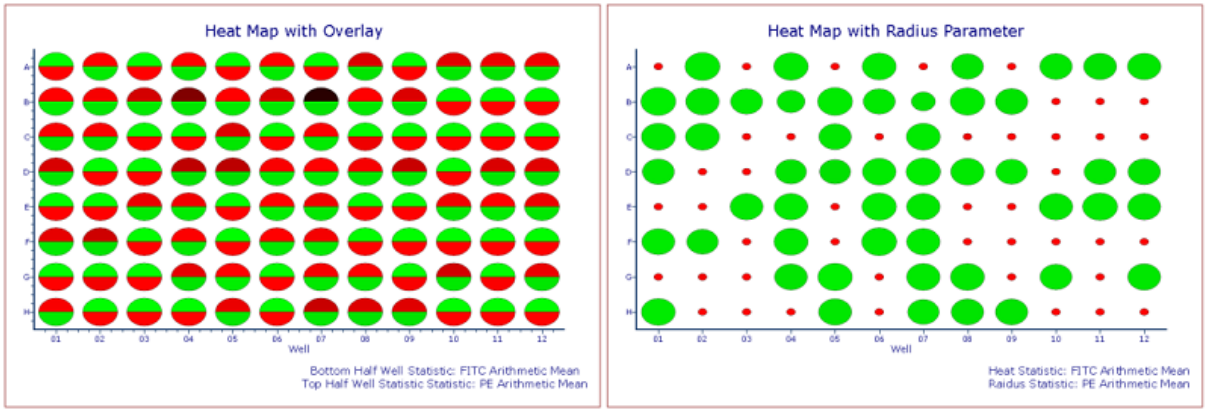

Figure 32.36 Custom Heat Maps with Overlay (left) and Radius Parameter (right). Standard Deviations of Color Levels was changed to 1.

31.2.4 Chorus Index Sorting Data Files

Chorus Index Sorting Data Files only allow for analysis on indexed sort events.

To analyze Index Sort Data:

1. Insert a heat map 1050 for the index sort data file.
2. Select the parameter of interest on the y-axis (Figure 32.37).

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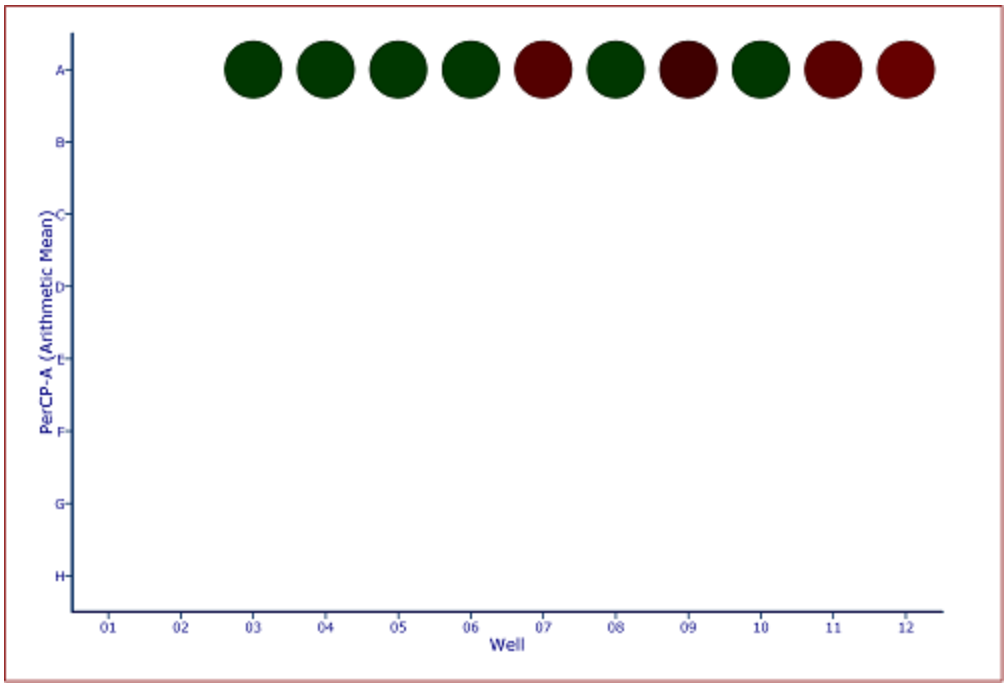

Figure 32.37 Default Heat Map with PerCP Arithmetic Mean Y Parameter

3. Insert a Color Dot Plot 170.
4. Change the axes on the color dot plot to the parameters of interest.
5. Create a Well Gate 1061 on all wells.

   Note:

   - *In addition to creating a gate on all wells a gate may be created on one, or any number of wells, to visualize the individually sorted cells.*
   - *Since Chorus Index Sorting Data Files only contain data for the unsorted events, we can use the Emphasize on Plots 321 feature of gates to emphasize the events that fall within the gate* (Figure 32.38).

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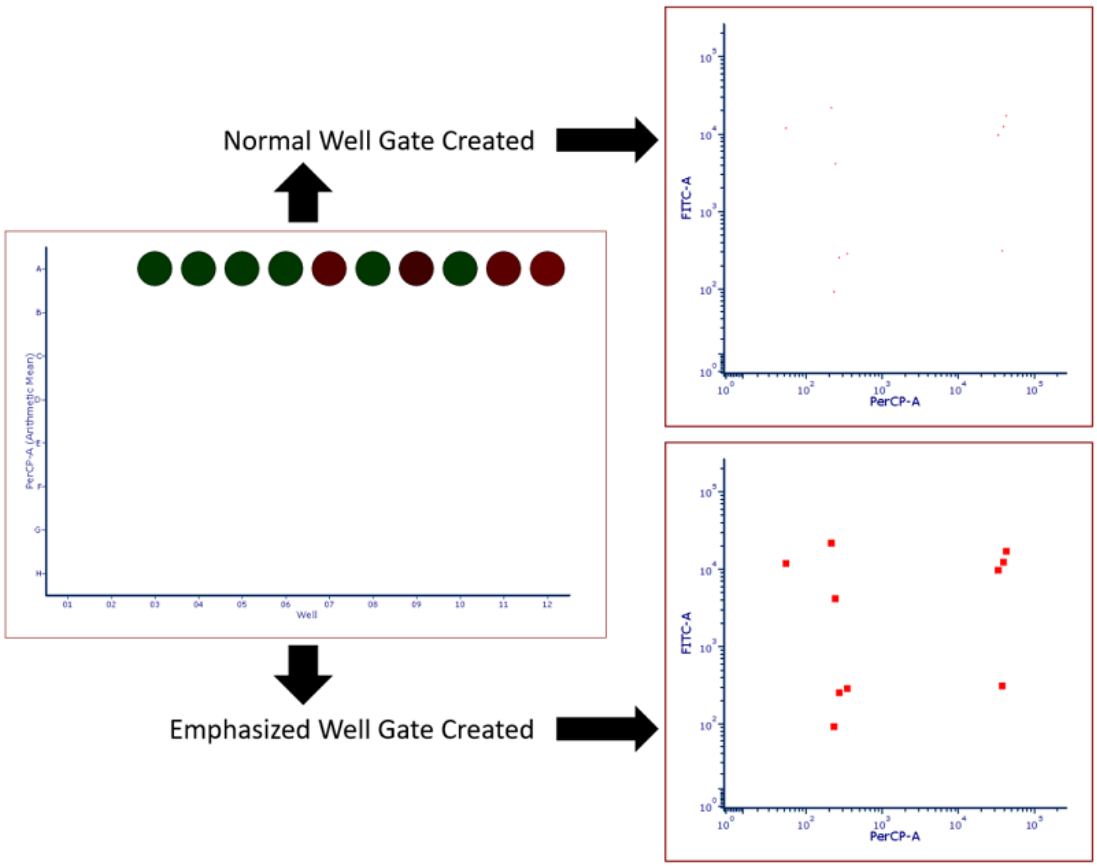

Figure 32.38 Heat map with Well Gate created on all wells. Standard gate is shown above while a gate with the Emphasize on Plots check box checked is shown below. Note that events highlighted in red fall within the well gate and in this case represent all events that were sorted into the plate making them easier to visualize.

6. Select the **Insert** tab→**General**→**Gate View** command to insert a Gate View 345.
7. Drag and Drop the Well Gate from the Gate View to the Color Dot Plot to apply the Well Gate (Figure 32.39).

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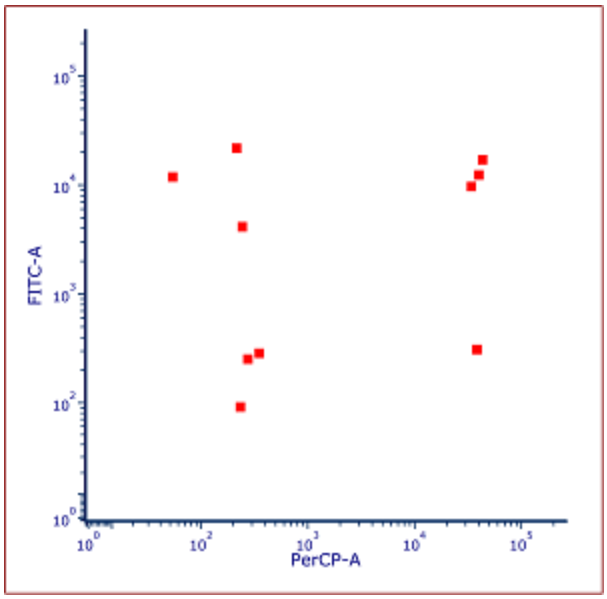

Figure 32.39 Color Dot Plot gated on Well Gate with events emphasized

8. **Optional:** Create additional Well Gates to isolate any number of individual wells (blue events below) or a group of wells (purple events below) (Figure 32.40).

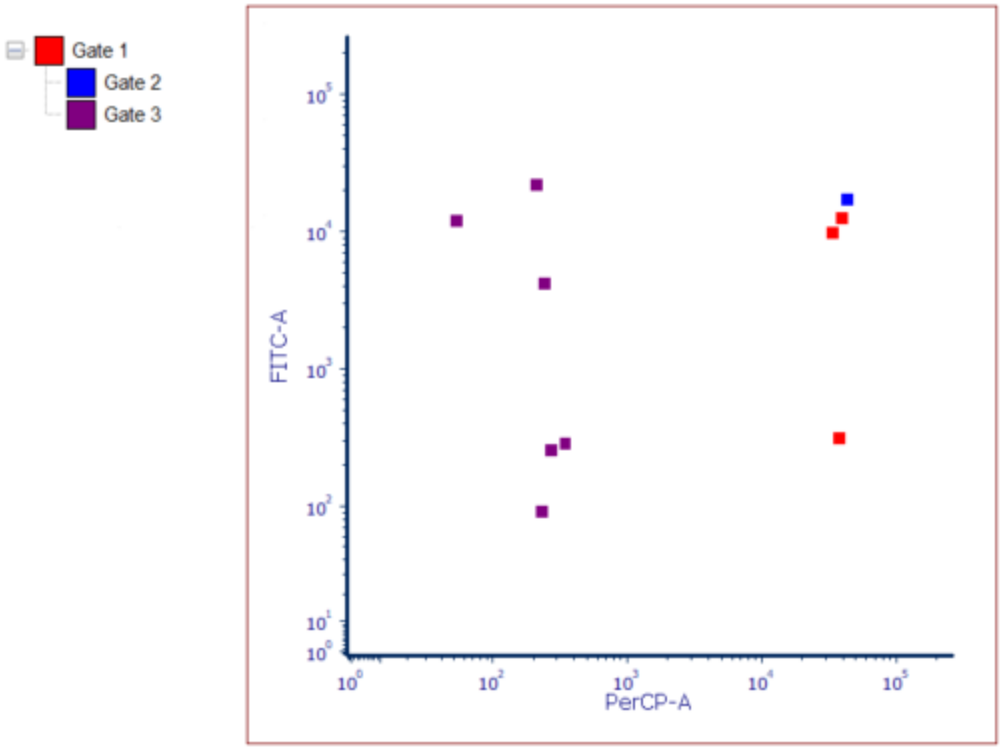

Figure 32.40 Color Dot Plot gated on Well Gate with Children Gates Applied and Emphasized

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It is possible to insert a heat map statistics grid 1054 in a text box to display statistics for each well in the heat map as done in Figure 32.41.

	01	02	03	04	05	06	07	08	09	10	11	12
A			210.06	263.95	51.77	220.24	37129.55	232.13	32027.44	343.31	39066.33	42403.09
B												
C												
D												
E												
F												
G												
H												

Figure 32.41 Heat Map Statistics Grid displaying statistics for the Y parameter of the Heat Map

Another useful feature for index sorting data is the Heat Map Overlay 1063 function. Heat Map Overlays allow a user to view multiple statistics and parameters on a single heat map by adding each statistic as a new overlay. For instance, in Figure 32.42 left, the upper slice of the well represents FITC arithmetic mean while the lower slice represents PerCP arithmetic mean so single and double positive events can be more easily visualized.

Additionally, users may add a **Radius Statistic** 1063, which changes the size of the well to reflect the selected parameter. For instance, in Figure 32 below (right), the heat/color for each well is dependent on the PerCP arithmetic mean while the size of the well radius is dependent on the FITC arithmetic mean.

Please see the Heat Map Overlay 1063 section of the manual for full instructions on how to create a heat map overlays of different parameters and types as well as setting the radius parameter statistic.

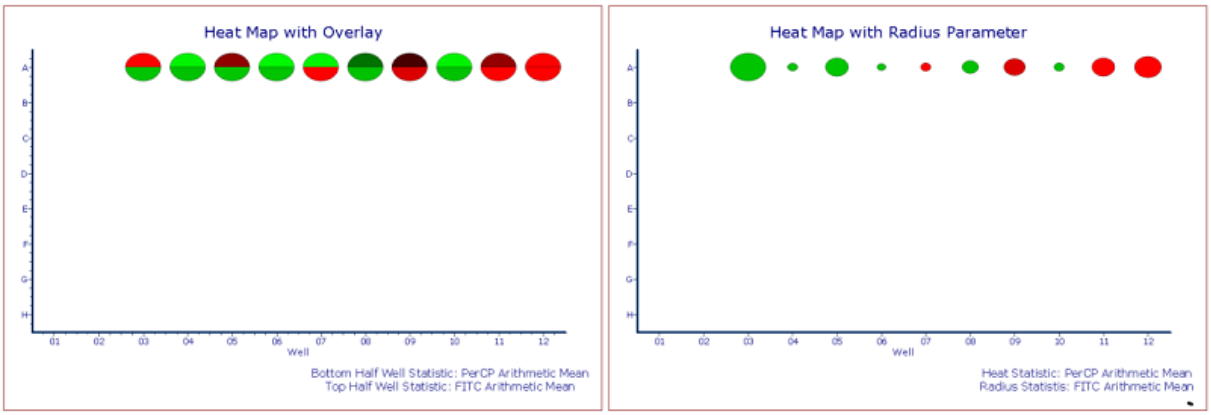

Figure 32.42 Custom Heat Maps with Overlay (left) and Radius Parameter (right). Standard Deviations of Color Levels was changed to 1.

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Chapter

32 Electronic Signatures

FCS Express supports a comprehensive set of features to allow you to manage and record electronic signatures.

Electronic signatures generally work in conjunction with the security features. With the security features activated, a new **Electronic Signatures** gallery will be present when you select **File** tab→**Info**→**Electronic Signatures** (Figure 33.1).

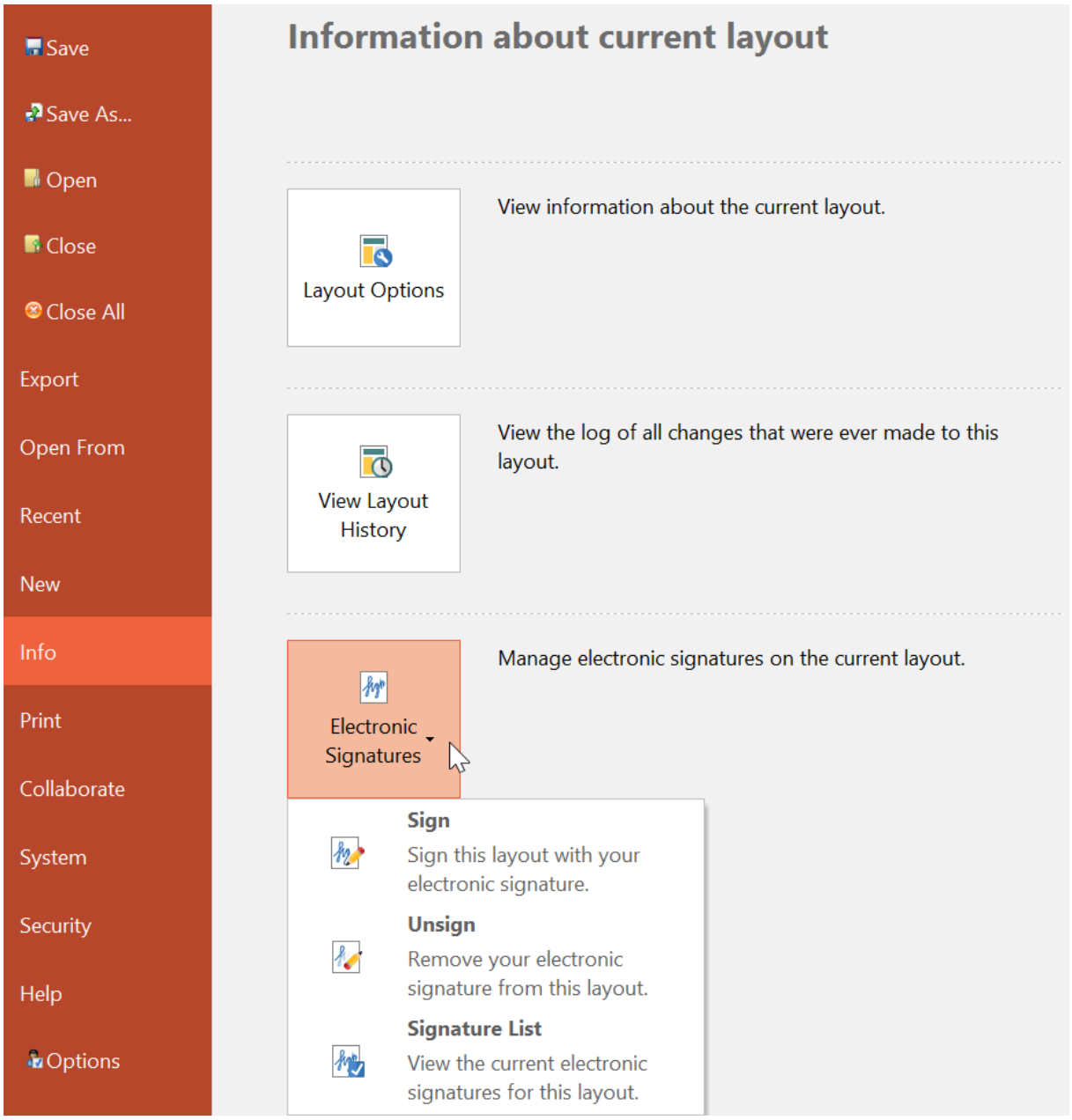

Figure 33.1 Electronic Signatures Gallery

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Click on the black triangle to **Sign, Unsign or to view the Signature List**[1098]. The number and types of signatures can be completely defined by the user. If the logging features are activated, FCS Express will keep a record of all signing and unsigning events.

32.1 Maintaining Signatures

Signatures can be maintained from the **Signature List** dialog (Figure 33.2) by selecting the **File** tab→**Info**→**Electronic Signatures**→**Signature List** command.

Figure 33.2 Signature List Dialog

When you are finished adding[1089], modifying[1090], or deleting[1091] signatures, press **OK** to save your changes or **Cancel** to discard them.

32.1.1 Adding Signatures

To add a new signature:

1. Use the **File** tab→**Info**→**Electronic Signatures**→**Signature List** command to bring up the **Signature List** dialog.
2. Press the **Add** button. A new signature will appear in the list box on the left (Figure 33.3).
3. Type a name for the signature into the **Signature Name** field.
4. Under the **Allow signature for** section, select the User Group[1174] for which the signature is to be enabled.

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Figure 33.3 Adding a New Signature

The signature properties are described in the section on Modifying Signatures 1090.

32.1.2 Modifying Signatures

To modify an existing signature:

1. Use the **File** tab→**Info**→**Digital Signatures**→**Signature List** command to bring up the **Signature List** dialog (if it isn't already visible).
2. Select the signature to modify in the list box on the left. The properties for that signature will appear in the **Signature Properties** portion of the window (Figure 33.4).

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Figure 33.4 Modifying a Signature

Signature properties are defined in the table below.

Property	Explanation
Signature Name	The name of the signature as it will appear in a token in the layout. It usually corresponds to the specific state of the layout (e.g., under review, approved, etc.).
Allow signature for	Only users who are members of the security groups 1173 that are checked will be allowed to sign under this signature.
Description	A short description of the signature. This is used for reference purposes only.
Notes	Notes associated with the signature

32.1.3 Deleting Signatures

To delete a signature:

1. Use the **File** tab→**Info**→**Electronic Signatures**→**Signature List** command to bring up the **Signature List** dialog.
2. Select the signature from the list on the left.
3. Press the **Delete** button.

32.2 Signing Layouts

To sign a layout, use the **File** tab→**Info**→**Electronic Signatures**→**Sign** command to bring up the **Sign** dialog (Figure 33.5).

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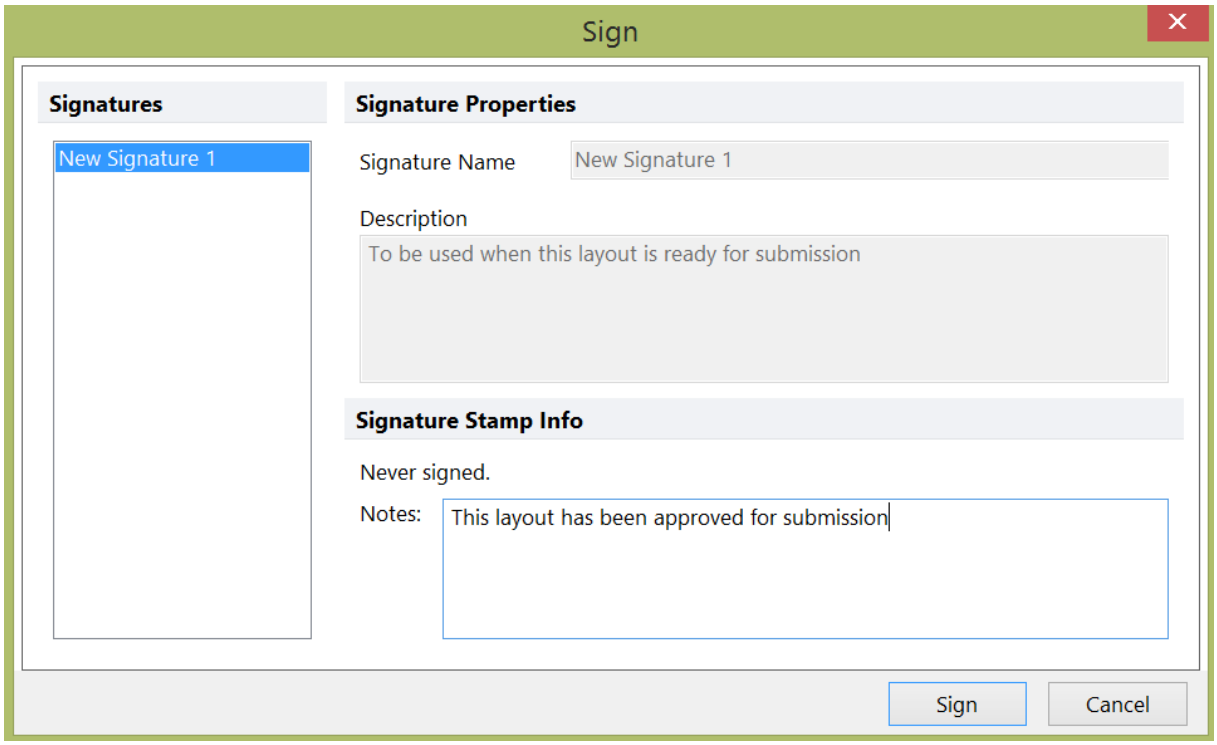

Figure 33.5 Signing a Layout

The list on the left will show signatures that are allowed, based upon the current user's security group.

Press the **Sign** button to sign the layout. An optional note or comment can be entered in the **Notes** field. After pressing **Sign**, the user will be required to confirm their password.

32.3 Unsigning Layouts

To unsign an already signed layout, use the **File** tab→**Info**→**Electronic Signatures**→**Unsign** command to bring up the **Unsign** dialog (Figure 33.6).

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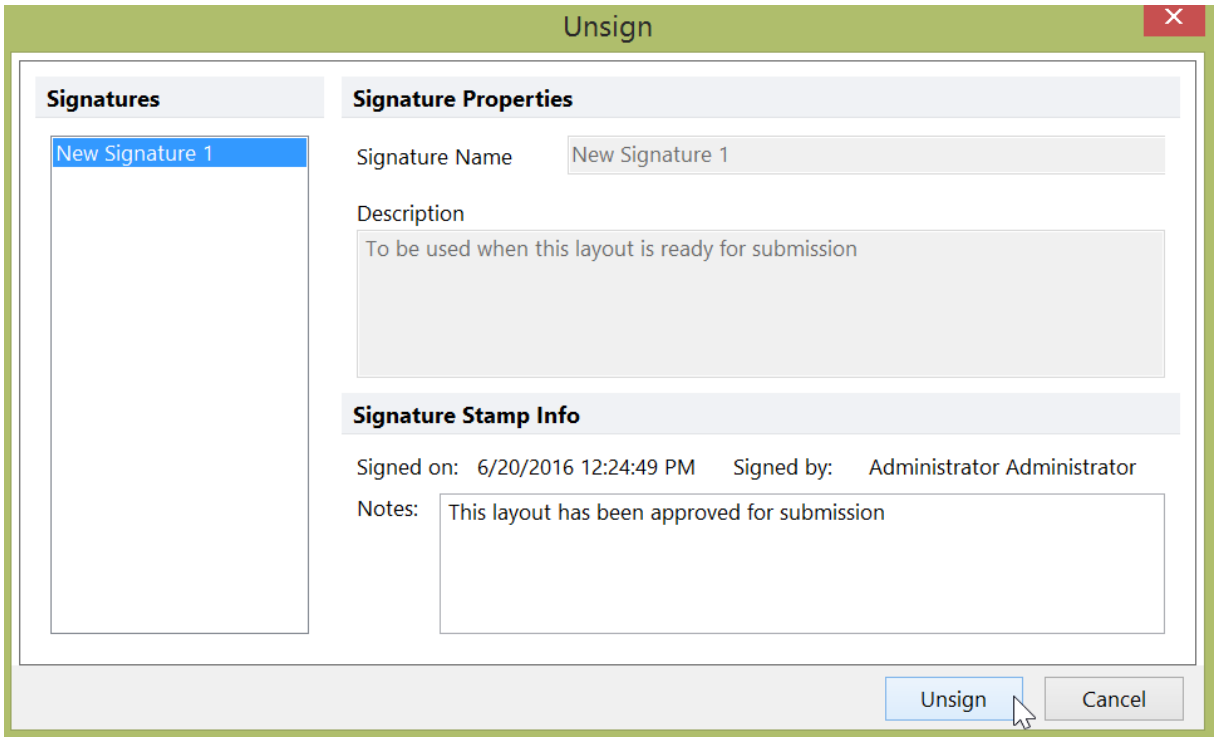

Figure 33.6 Unsigning a Layout

The list on the left will contain the current user's signatures for the present layout. Select the signature you wish to remove and press the **Unsign** button. An optional note can be entered in the **Notes** field. After pressing the **Unsign** button, the user will be required to confirm their password.

32.4 Displaying Signature Information

Once you have created and set up your signatures[1089] in FCS Express, you may want to display their details and signing status[1098] on your layout pages.

By default, the signature[1089] details and status do not appear on a layout page. However, you can easily display the information about the signatures[1089] using signature tokens[692].

A signature token can display the following kinds of information (Figure 33.7):

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1. **Signature Name**
2. **Signature Description**
3. **Signature Status**
   Never Signed*|*Signed*|*Unsigned
4. **Signature Date**
5. **Signature Time**
6. **Signature Signer**
   Never Signed *or Name*
7. **Signature Stamping Notes**

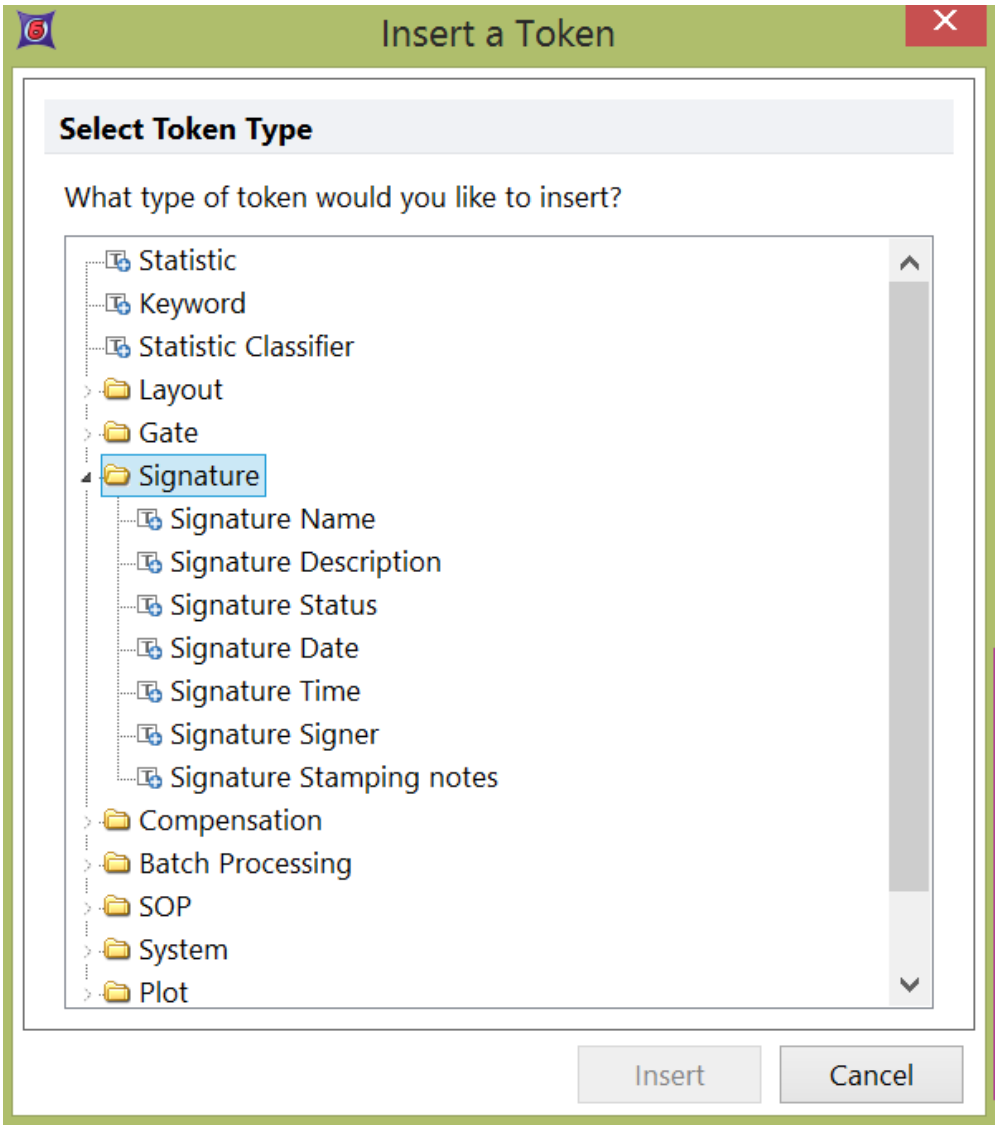

Figure 33.7 Signature tokens available in FCS Express.

To display the signature tokens, you need to first create a text box. Once you have the text box, there are two ways to insert signature tokens into the text box:

1. Using the signature table

Right-click in the text box, and select **Insert Signature Table**. A table will be created inside the text box, as shown below (Figure 33.8).

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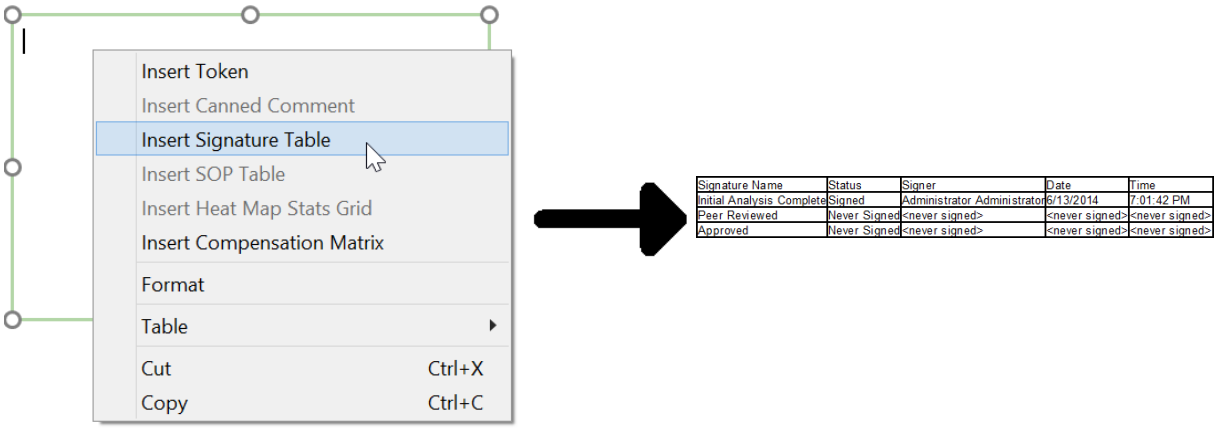

Figure 33.8 Inserting a signature table.

2. Using individual signature tokens

a. Right-click in the text box, and select **Insert Token** 692. The **Insert a Token** 692 window will appear (Figure 33.9).

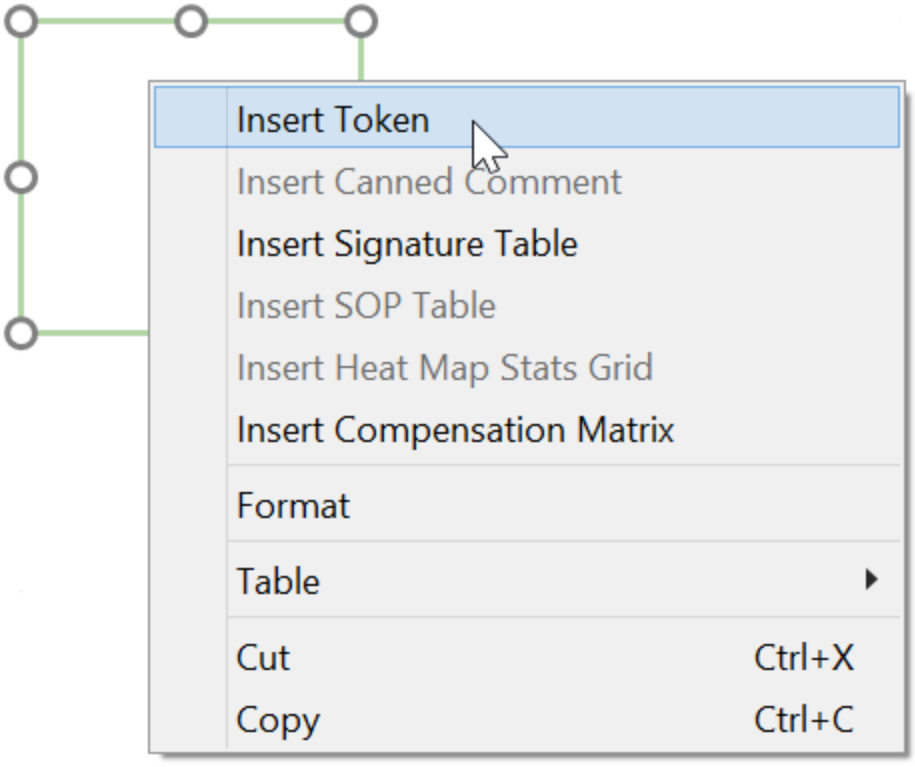

Figure 33.9 The insert token window.

b. In the **Insert a Token** 692 window, expand the **Signature** 1089 node then select the type of signature 1089 information you want to display, and press **Insert**

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(in this example we will choose **Signature Name**) (Figure 33.10). The token editor will appear.

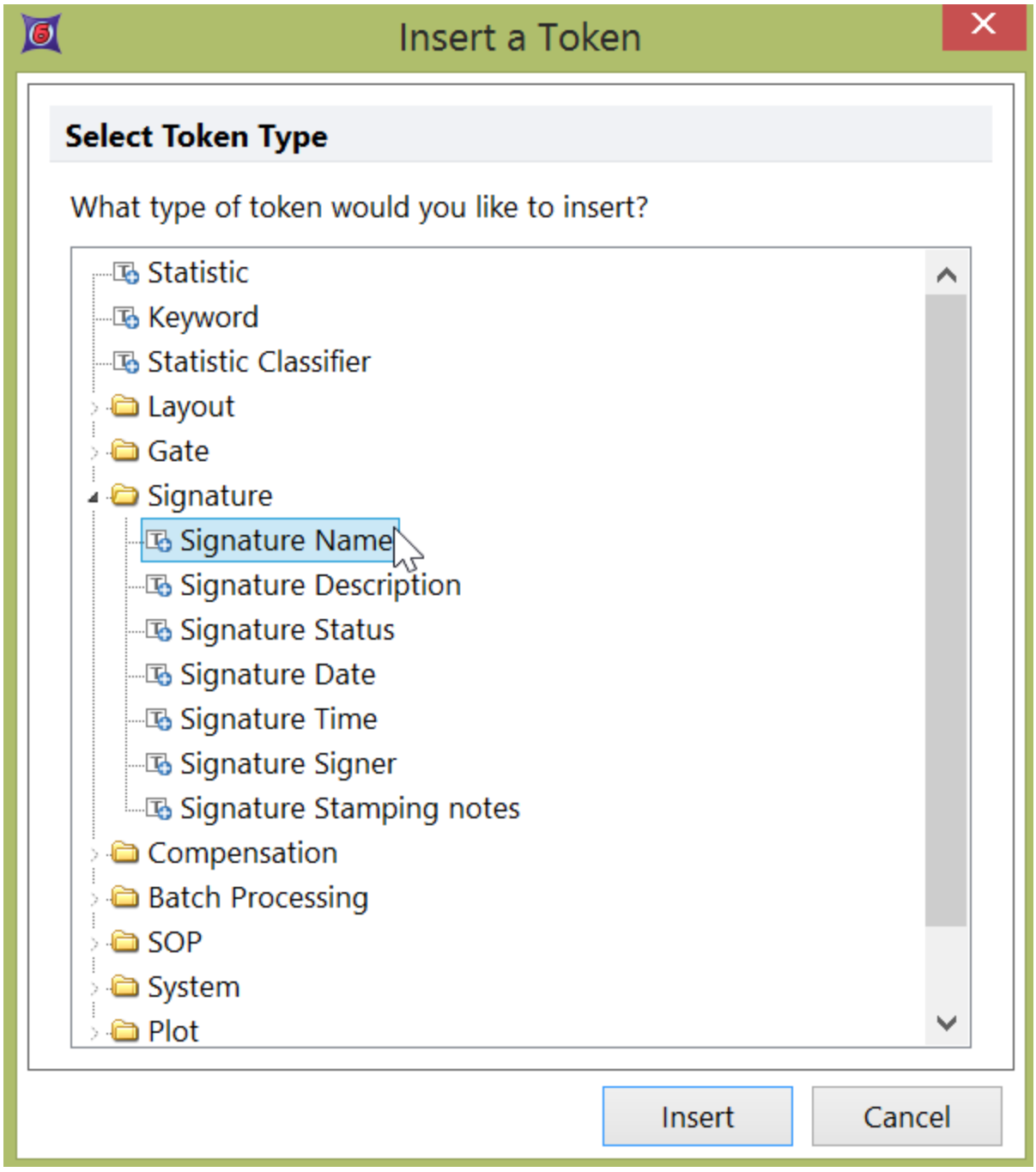

Figure 33.10 Inserting a token.

c. In the token editor click on the **Signature information** category to confirm your choice (Figure 33.11).

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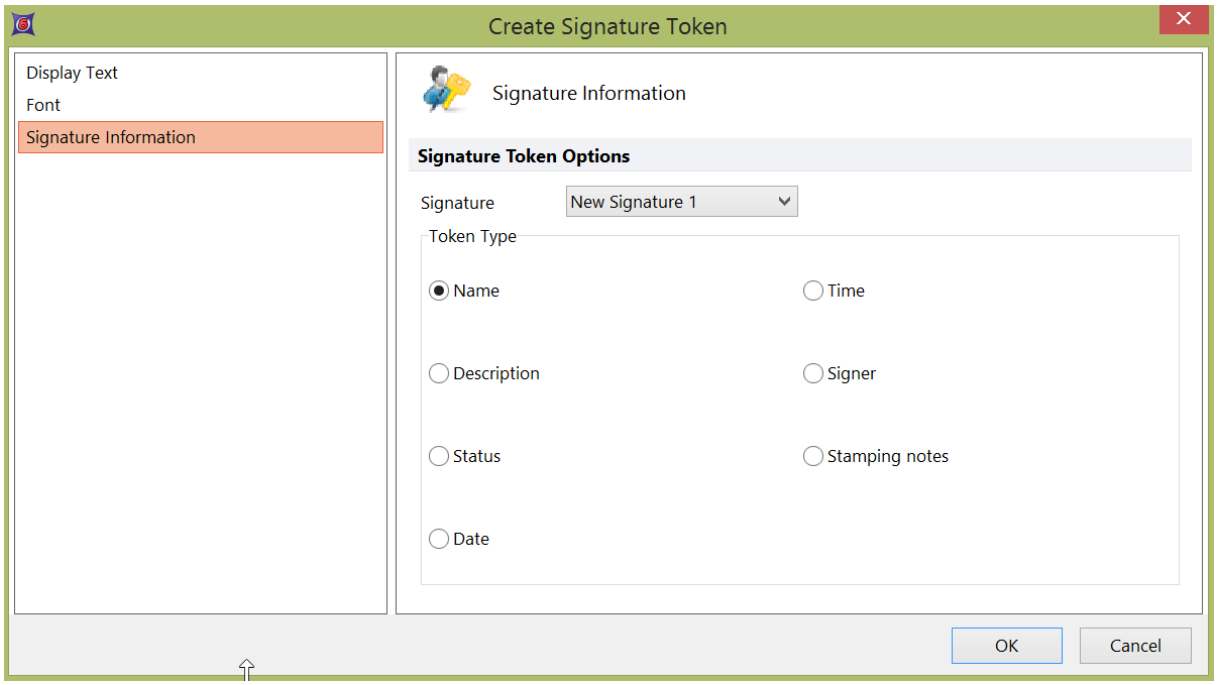

Figure 33.11 Signature information in the Create Signature Token window.

Note: You will notice in the **Signature information** category you have the option to choose any defined signature from the **Signature** drop down. You can choose which signature token you would like to insert from the **Token Type** radio buttons. This is useful if you would like to edit or change the token in the layout without deleting and inserting a new token.

d. Click **OK** to insert the token.

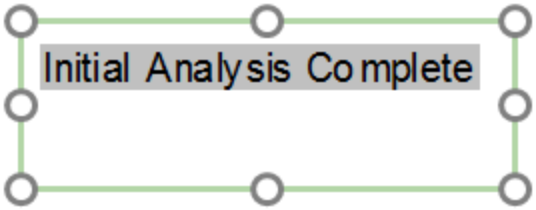

Figure 33.12 The signature token inserted in a text box.

The new signature token will appear in the text box as in the image above (Figure 33.12).

As with other tokens, you can change the formatting of individual signature tokens, for instance to mimic a typical signature section on a printed report.

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32.4.1 Signature Status

Once you have created and set up your signatures[1089] in FCS Express, you may want to display their signing status on your layout pages.

Signature Status can be designated as:

- ***Never Signed -*** Indicates the signature has never been signed.
- ***Signed -*** Indicates the signature is currently signed.
- ***Unsigned -*** Indicates that the signature has been signed at least once and is currently unsigned.

You may also create a custom signing status using custom tokens[727] to hide the signature status if the layout has never been signed or has been unsigned. A custom token[727] of this type will therefore only display the signature status when the layout has been signed. Instructions for creating this type of custom token[727] can be found in the hiding signature tokens[734] section of the custom tokens[727] chapter.

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Chapter

33 Layout History

FCS Express tracks every action performed by a user on a layout. To view the history, use the **File** tab→**Info**→**View Layout History** command to bring up the **Layout History Viewer** window (Figure 34.1).

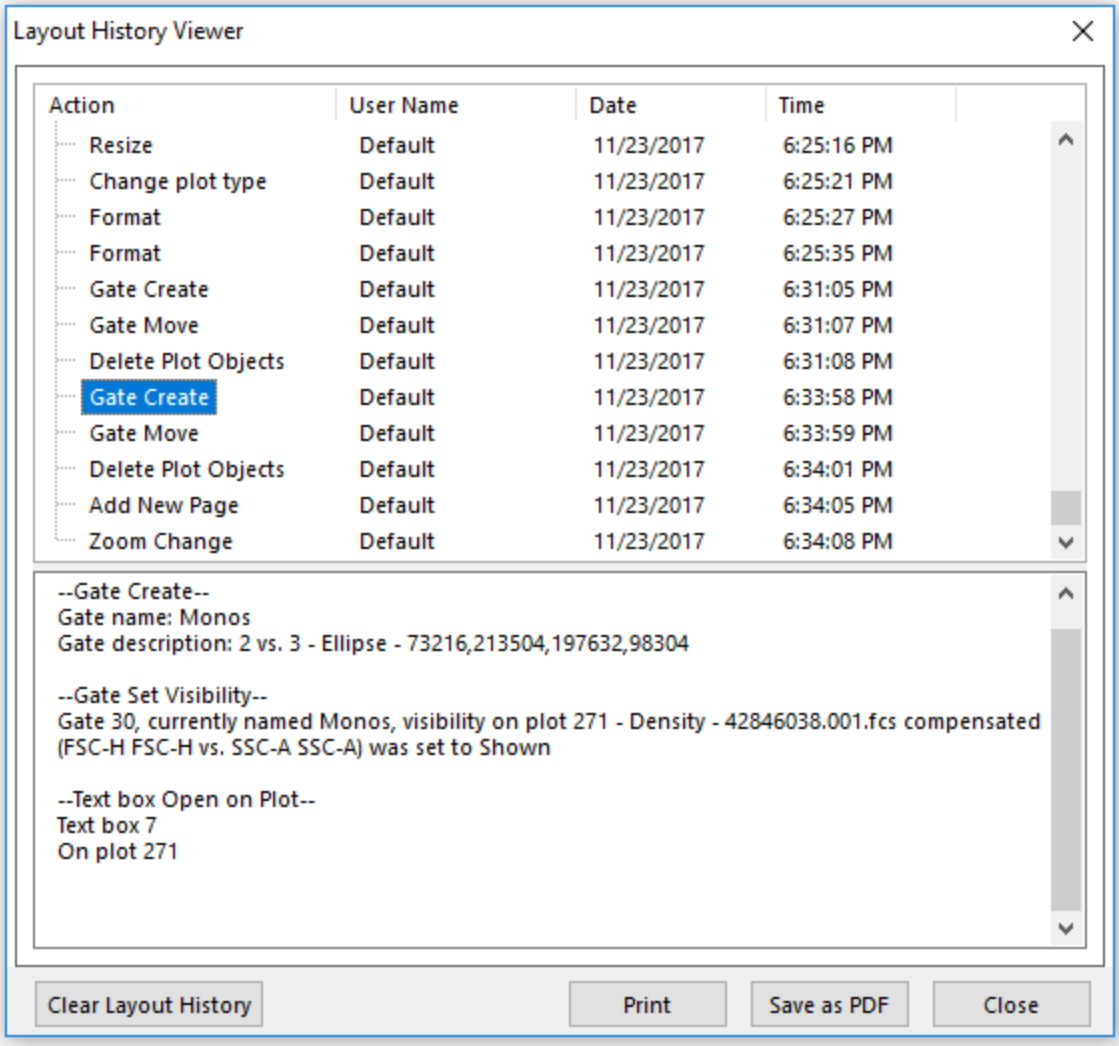

Figure 34.1 Layout History Viewer

Every action performed contains the following information:

- In the **Action** column, there is a description of the action performed.
- If security is enabled, the **User Name** column lists the name of the user who performed the action.
- The **Date** column shows the date when the action was performed.

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- The **Time** column contains the time the action was performed.

Some actions also cause other actions to be performed. For example, when creating a gate, a text box is also automatically created. All of the associated actions are grouped under a single entry. In the figure, the **Open Dot** entry at the top of the list box contains two history sub-entries, **Compensation Create** and **Open 2D Plot**. To view all history entries for an action, click on the **+** to the left of the text of the action. The lower text region of the **Layout History Viewer** contains more details about the currently selected action.

You can **Print** the layout history or **Save it as a PDF**.

The **Layout History** may also be cleared:

- Select **Clear Layout History** at the bottom of the **Layout History Viewer** dialog (Figure 34.2 Step 1).
- Enter a Note and press **OK** (Figure Below, Step 2) in the **Clear Layout History** dialog.
- The Layout History will be cleared and a single action referring to the clearance will be listed in it (Figure Below, Step 3).

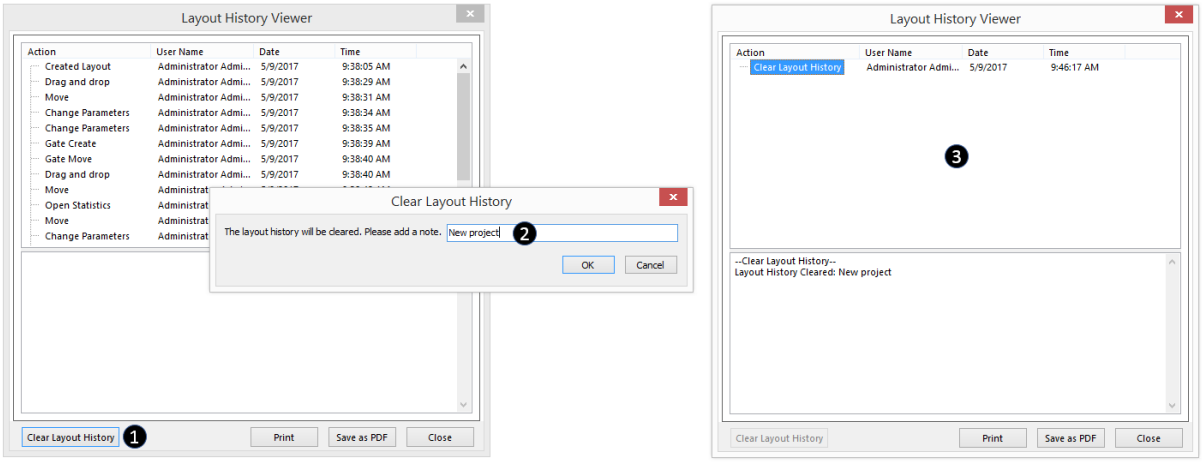

Figure 34.2 Layout History can be cleared via the Clear Layout History button.

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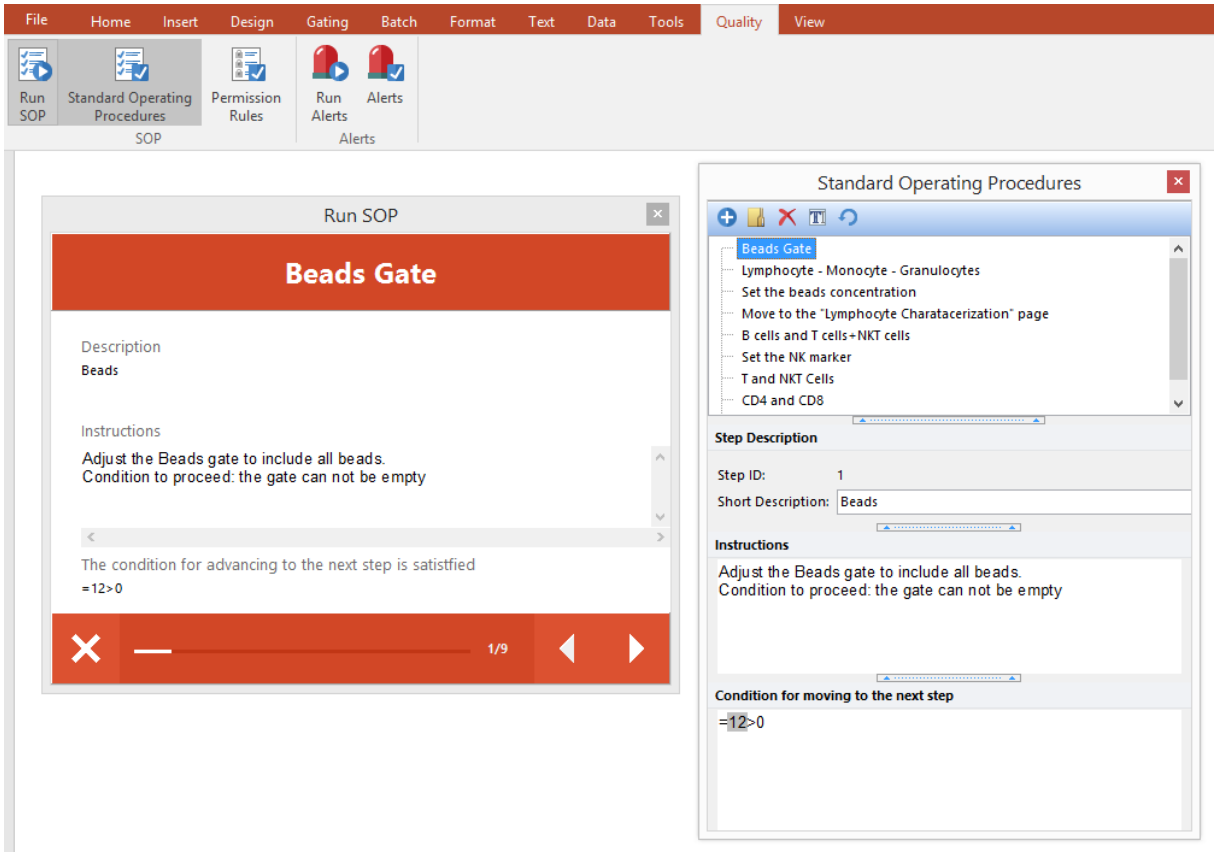
Chapter

34 Standard Operating Procedures

To help maximize efficiency and quality of routine analysis workflows, FCS Express allows user to create **Standard Operating Procedures** (SOP's), which are sets of step-by-step instructions and actions to perform in FCS Express. These steps allow you to create a predetermined procedure in your FCS Express layouts in which each step can be conditionally formatted with tokens so that a requirement must be met before the next step is performed. For instance, a user may not proceed to the next step unless the number of events in a gate is above a certain threshold value or text has been entered in a field.

Jump to:

[Accessing and Navigating SOP's](1105)
[Creating SOP's](1106)
[Reordering SOP Steps](1109)
[Deleting SOP Steps](1109)
[Using SOP Permission Rules](1110)
[Running SOP's](1110)
[Displaying SOP information and SOP tokens](1113)



The Standard Operating Procedure feature in FCS Express

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34.1 Accessing and Docking SOP Navigator

To access the **Standard Operating Procedures** navigator, click on the **Quality** tab→**SOP**→ **Standard Operating Procedures** command (Figure 35.1).

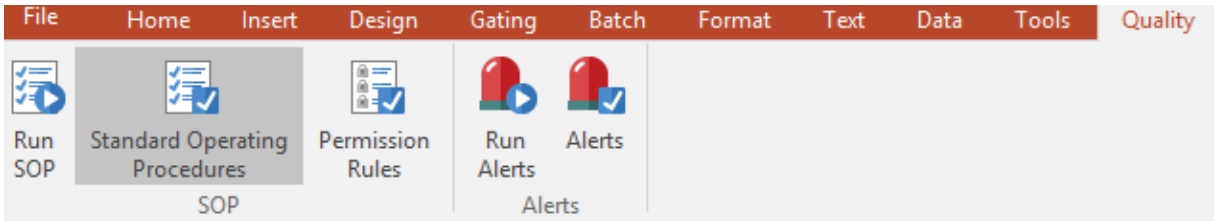

Figure 35.1 Accessing SOP navigator

To dock the **SOP** navigator, click and drag the window's title bar to an edge of the layout to activate docking zone sectors. Select the area by releasing mouse on docking zone sector of choice (Figure 35.2). See docking a window 115 for more details.

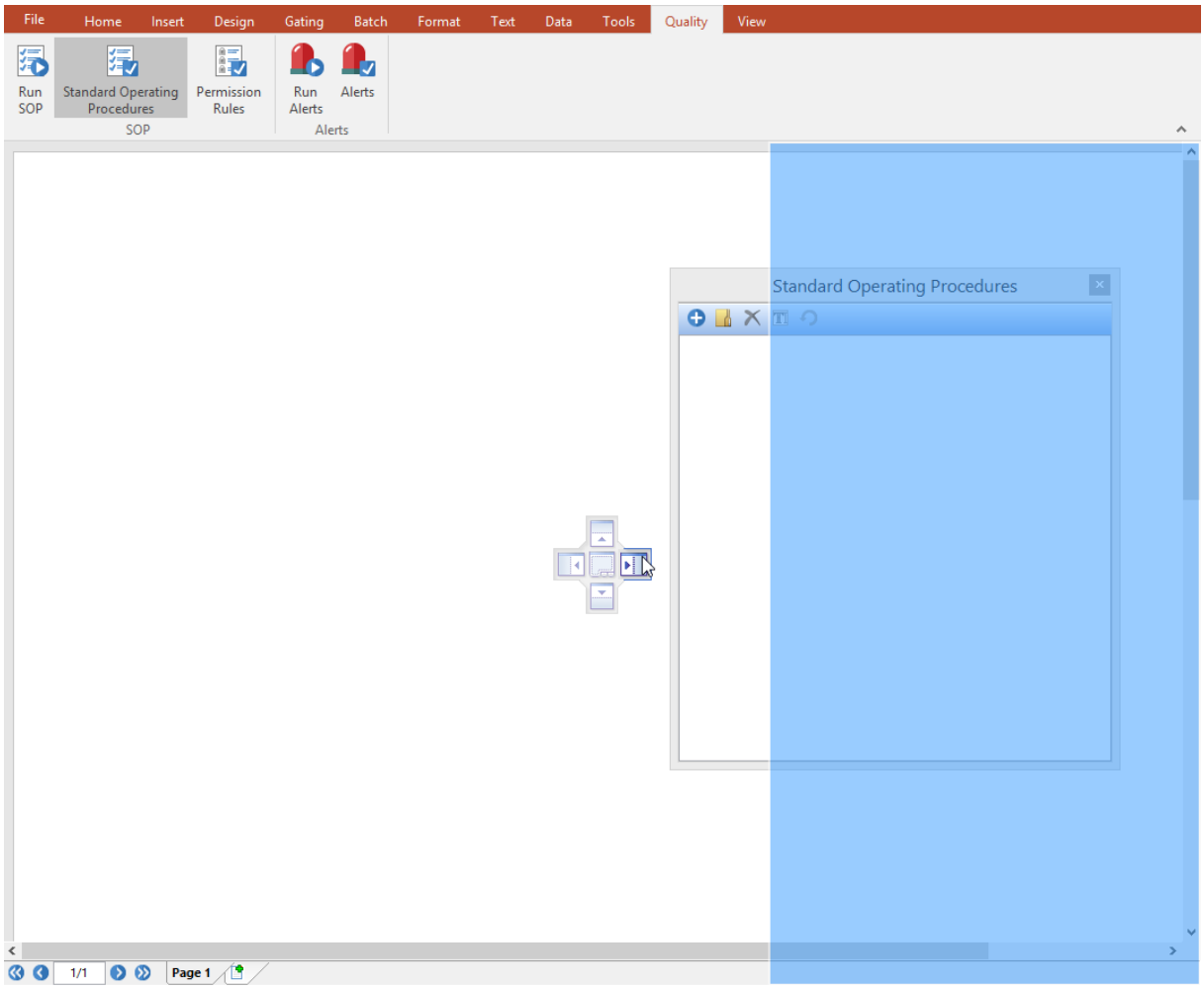

Figure 35.2 Docking SOP navigator

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34.2 Creating the SOP

Standard Operating Procedures are created in a step-wise manner. Steps may simply be instructions to follow in the **Instructions** field or may include conditions that must be met before the next step in the SOP may be accessed. Conditions, can be specified in the **Condition for moving to the next step** field of the SOP dialog.
For instance, in the example below, Figure 35.3, a formula was defined in the **Condition for moving to the next step** field for the **Beads Gate** step (Step 1). When the SOP is run, the step may not be advanced until the token value meets the criteria of being above the reference value (0). In this case, the step is meant to check to make sure events are present in the gate before proceeding to the next step. Any tokens may be used to set a conditional custom value and may be inserted 692 via right click in the **Condition for moving to the next step** field, **Insert Token**.

Any manually entered value or token in FCS Express 692 may be used in the **Condition for moving to the next step**. Tokens derived from the SOP itself may also be used 1113 and there are a number of specialized SOP functions available within the custom token functions list 781.

Values must be evaluated to "true" to allow advancing to the next step. Please see the examples below 1108 showing how a condition may be evaluated to allow moving to the next step.

Providing detailed instructions in the **Instructions** field is highly recommended, so users may easily follow the necessary steps to advance when Running the SOP 1110.

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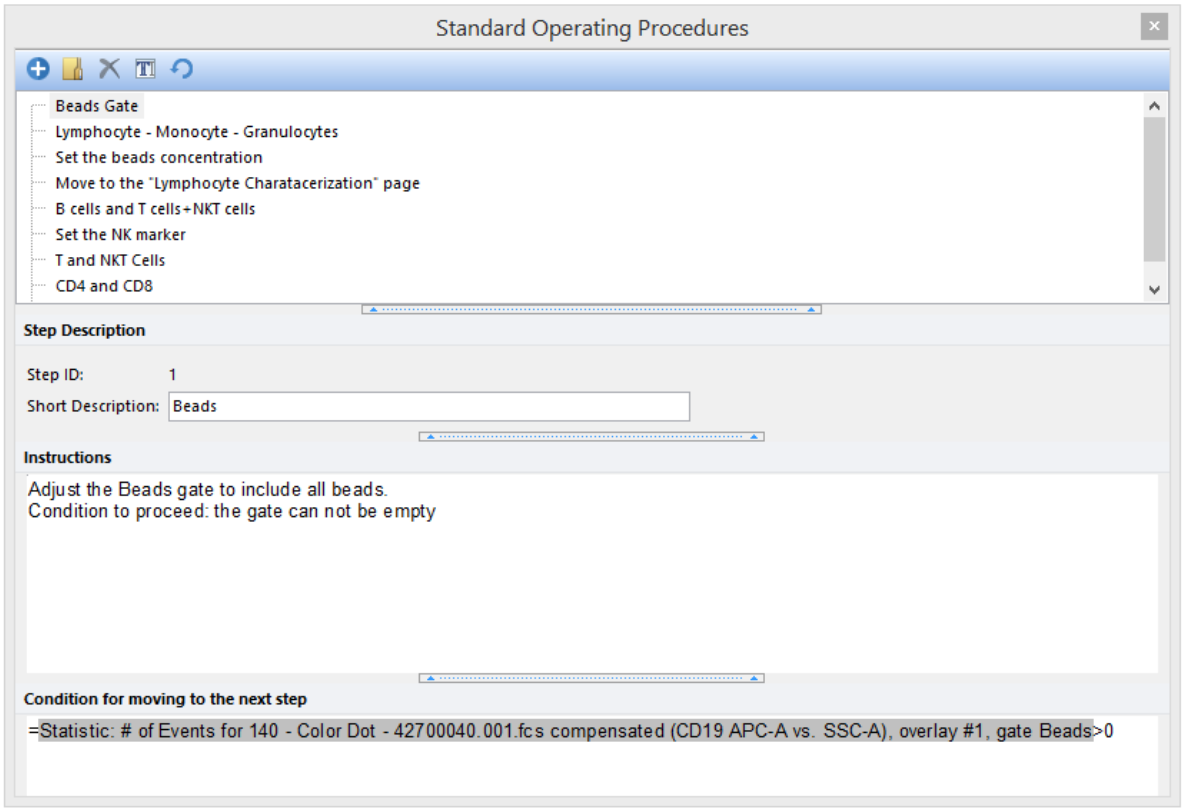

Figure 35.3 - An SOP has been defined where the first step is dependent on the Condition for moving to the next step. In this example, the condition requires that the number of events in the beads gate must be greater than 0.

To create a series of **Standard Operating Procedure** steps, add steps using **SOP** navigator (Figure 35.4):

1.) Click to add new step.

2.) Rename the **New SOP Step** by clicking the icon. Alternatively, right-click on the step and select **Rename**, or highlight the step and press **F2**.

3.) Enter information into **Short Description** field. The text in the **Short Description** field will appear in the **Description** area when running the SOP.

4.) Populate **Instructions** field. The text in the **Instructions** field will appear in the **Instructions** area when running the SOP.

Optional: Create a token expression to control advancing to next step in SOP if by entering an "**=**" sign under **Condition for moving to the next step.**

5.) Right-click next to "**=**" sign in the field.

6.) Choose **Insert Token**.

7.) Choose a token to evaluate.

8.) Repeat steps 1-8 to add more steps until complete.

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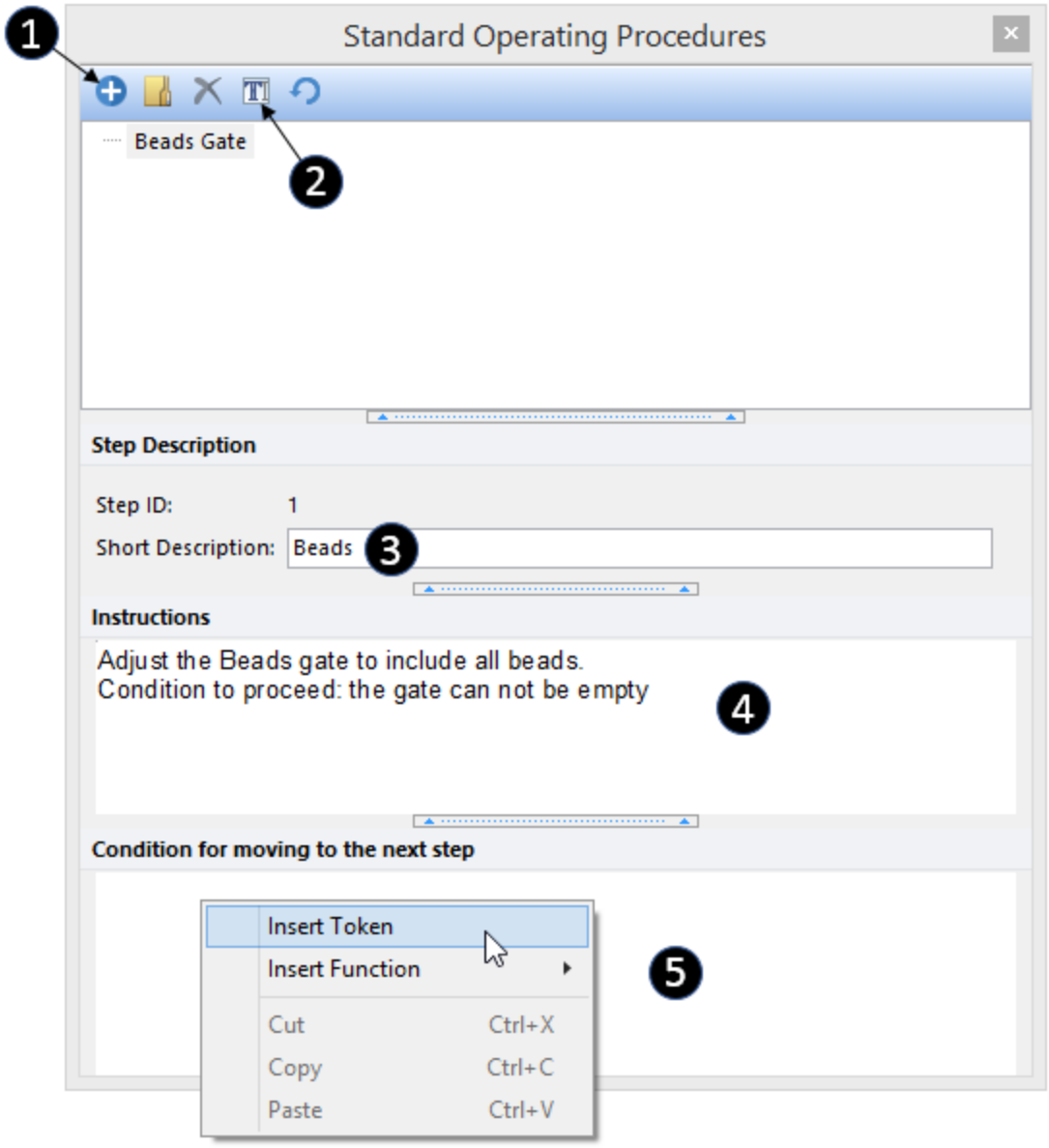

Figure 35.4 Adding Steps to SOP

Use case examples of formulas to evaluate a result for moving to the next step

Allow moving to the next SOP step only if...	Condition for moving to the next step formula	Equivalent formula
Token1 is higher then a given value (i.e. 50)	=Token1>50	
Token1 is lower then Token2	=Token1<Token2	

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Checkbox1 is checked	=CheckBox1=-1	=IsTrue(CheckBox1)
Token1 and Token2 are both higher than zero	=(Token1>0)and(Token2>0)	

34.3 Changing Order of SOP Steps

The order of steps in an **SOP** may be rearranged easily by using drag and drop.

1. Click on **SOP** step to highlight it.
2. Hold the left mouse button.
3. Drag the step into the new position.
4. Release the mouse when blue line is positioned where desired (Figure 35.5).

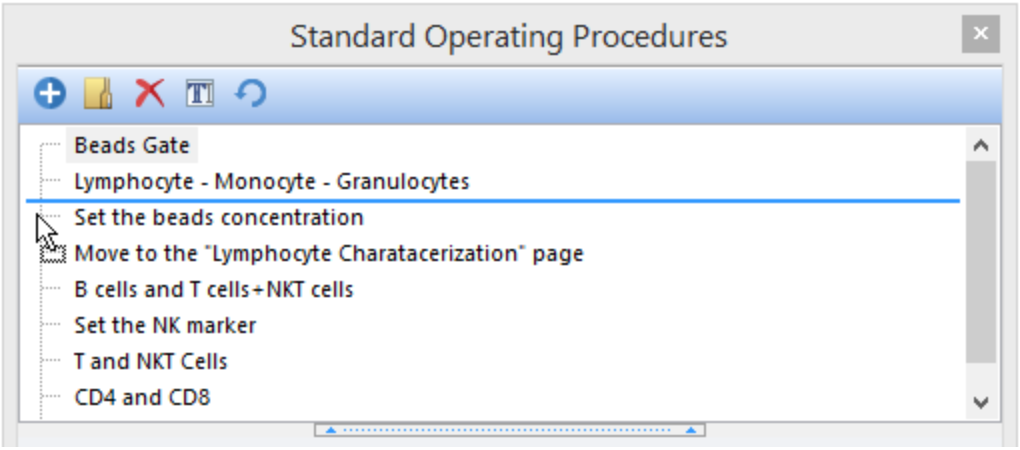

Figure 35.5 Changing Order of Steps in SOP by clicking and dragging

The order of steps will update accordingly and may also be displayed in the layout 1113.

34.4 Deleting SOP Step

Using the **SOP** navigator, steps may be deleted by:
1. Click on the step to highlight it.
2. Click on the (Figure 35.6 left). Alternatively, right-click on the step and choose **Delete** (Figure below - right).

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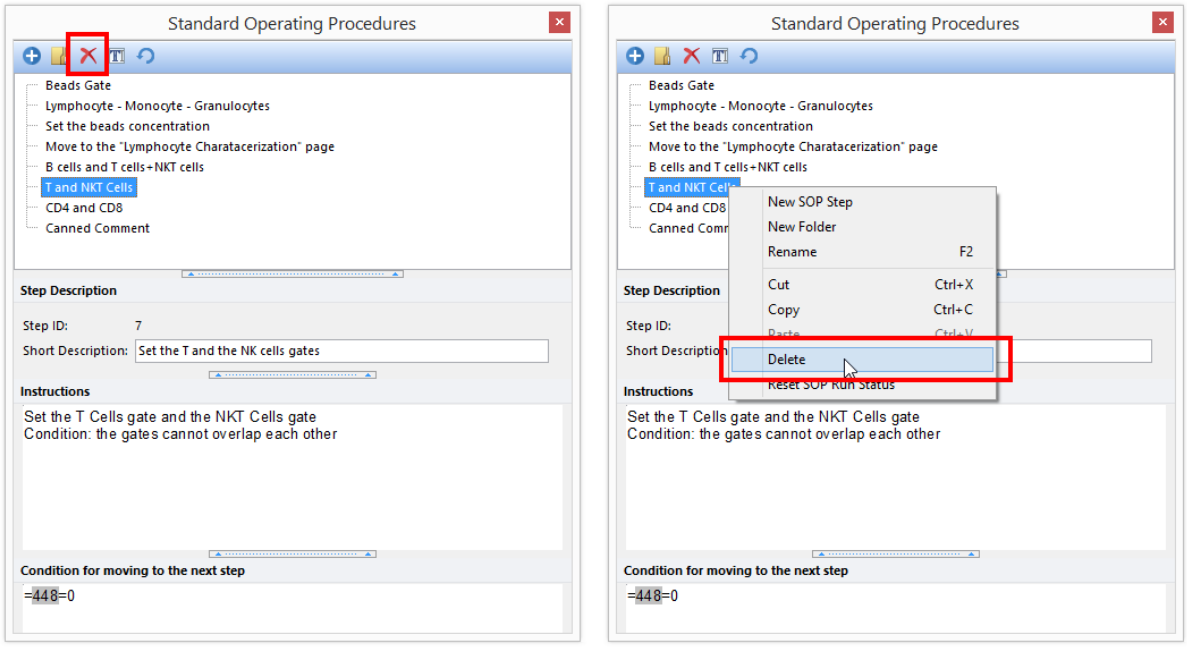

Figure 35.6 Deleting SOP steps.

The order of steps will update accordingly due to the deleted step and may also be displayed in the layout[1113].

34.5 SOP Permission Rules and Authorizations

Permission Rules[1120] and **Authorizations**[1136] may be used in conjunction with SOP's to allow or deny users of the layout to perform specific actions or to Edit/Select objects.

Please see the Permissions Rules[1120] section of the manual to learn more about locking down specific actions that may be performed in the layout.

Please see the Authorizations[1136] section of the manual to learn more about allowing or never allowing selection or editing of plots. Authorizations may also be conditionally formatted[1138] to allow or never allow editing or selecting of objects.

34.6 Running SOP

The **Run SOP** command begins the step wise process of running through all of the steps defined in the SOP creation process[1106]. When the **Run SOP** command is selected from the **Quality** tab→**SOP** the **Standard Operating Procedure** dialog will appear (Figure 35.7) and will display the first step defined. The **Description** area will display the information entered in the **Short Description**[1107] field, the **Instructions** area will display the information entered in the Instructions field[1107]. The current result of the condition (satisfied or not satisfied), if any, is also reported in the SOP dialog.

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The left and right arrows in the bottom right of the dialog (figure below) will allow the user to advance or go back through the **Standard Operating Procedure** steps. If an **Condition for moving to the next step** 1107 conditional statement was defined, the user will not be able to advance until the condition is met.

To run an SOP (Figure below):
1.) Click on the **Quality** tab→**SOP**→**Run SOP** command to launch the **Standard Operating Procedure** dialog.
2.) Perform the instructions indicated in the **Instructions** area.
3.) Click on the right arrowheads in lower right of SOP dialog to advanced through the SOP steps. Note that if an Condition for moving to the next step 1107 was defined the condition must be met before you are allowed to advance.
4.) ***Optional:*** Click on the left arrowhead to go back to the previous SOP step.

Note: The SOP Runner window is now dockable 115, so that you may perform other actions while the SOP runs.

Once all SOP steps have been completed the **Standard Operating Procedures** dialog will close.

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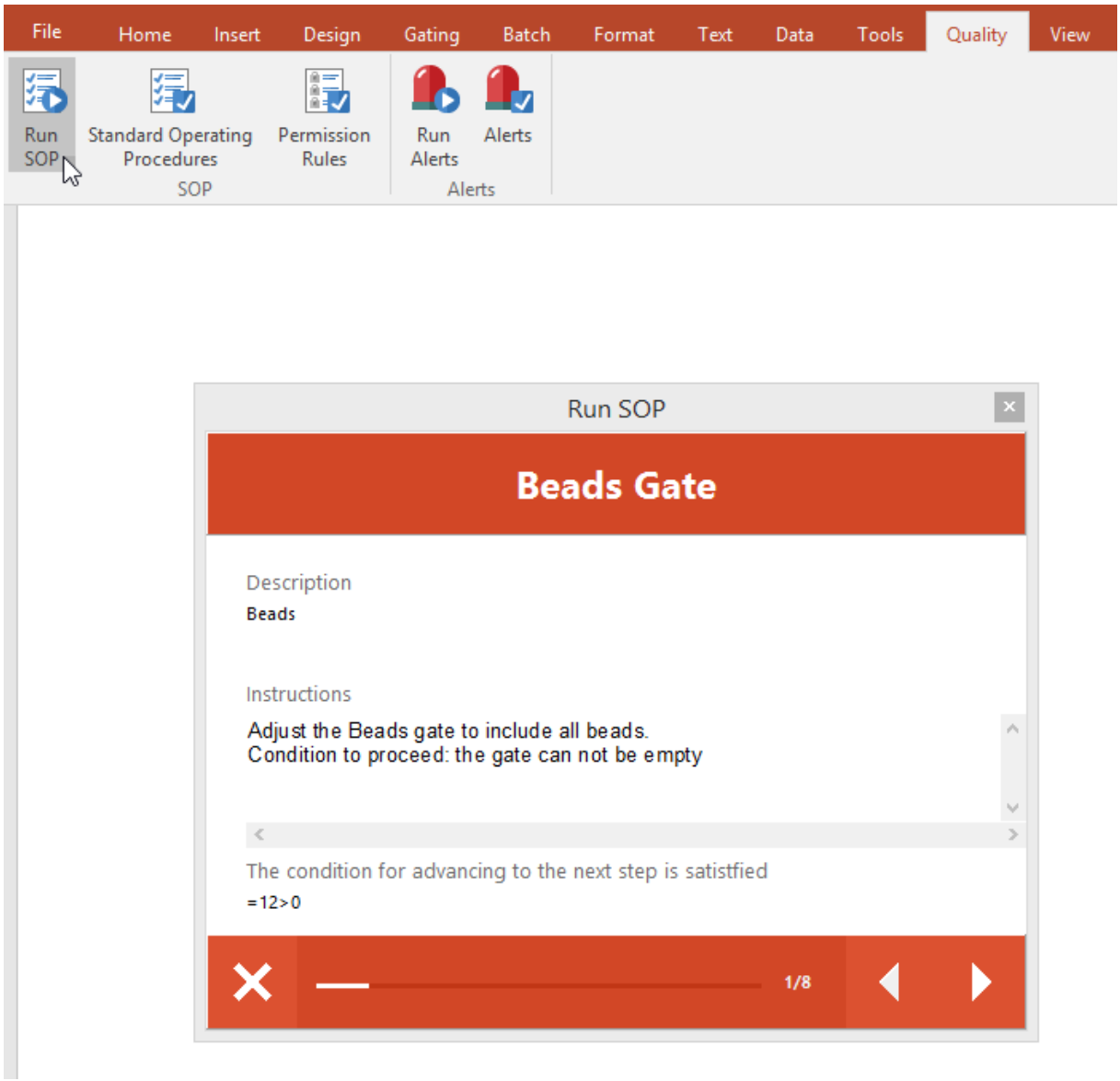

Figure 35.7 Opening SOP Window from ribbon

If the **Standard Operating Procedure** was run to completion previously, a message will appear and ask if you wish to restart the SOP (Figure 35.8).

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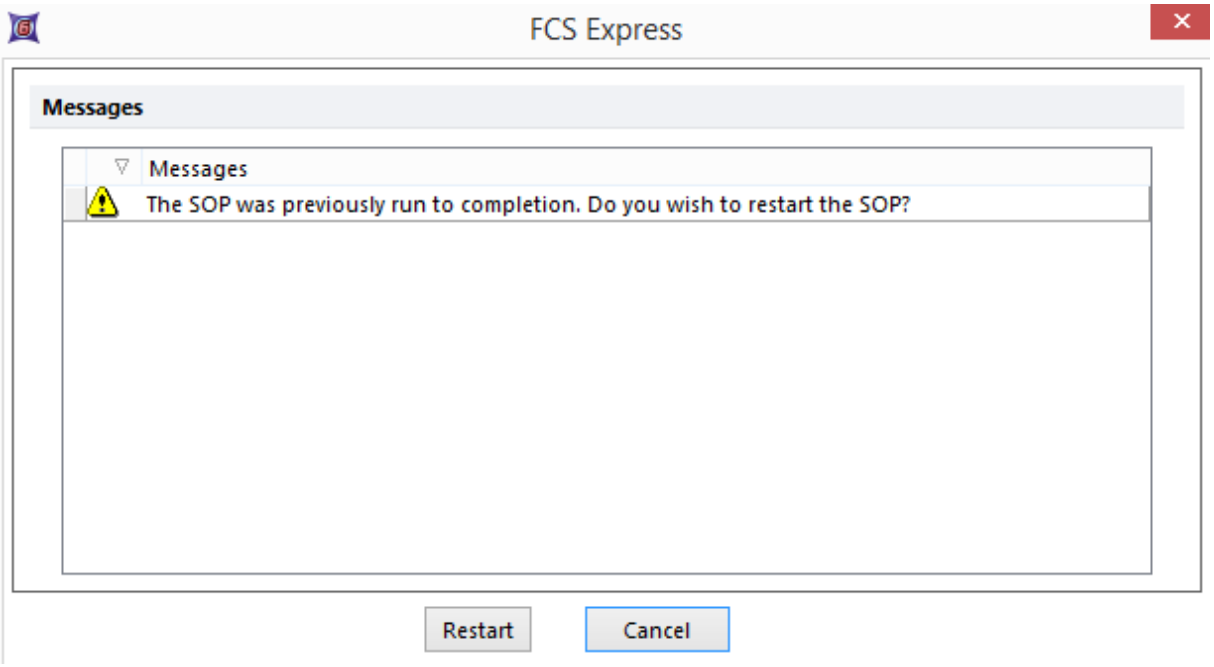

Figure 35.8 Run SOP Message

34.7 Displaying SOP Information and SOP tokens

Once you have created an SOP in FCS Express, you may want to display the details on your layout pages.

SOP's details and status information can easily be displayed using the **Insert SOP table** when right clicking in any text box 152 or by using tokens 692.

An SOP token can display the following kinds of information:

SOP Token Type	Explanation
Number of SOP steps	The total number of steps in an SOP
Current SOP step name	The name of the currently active step when running SOP as defined by the user
Current SOP step internal ID	The internal ID of the currently active step when running SOP
Current SOP step order number	The step order number when running SOP
Current SOP step description	The step description when running SOP
Current SOP step completion date	The last date the current SOP step was completed on.
SOP step name	The name of the selected step from the **Create SOP Token** dialog
SOP step internal ID	The internal ID of the selected step from the **Create SOP Token** dialog

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SOP step order number	The step order number of the selected step from the **Create SOP Token** dialog
SOP step description	The step description of the selected step from the **Create SOP Token** dialog
SOP step completion date	The last date the selected step from the **Create SOP Token** dialog was completed on

To display the SOP tokens, you need to first create a text box. There are two ways to insert SOP tokens into the text box:

Using the SOP table

1. Right-click in the text box.
2. Select **Insert SOP Table**.

A table will be created inside the text box, as shown below (Figure 35.9).

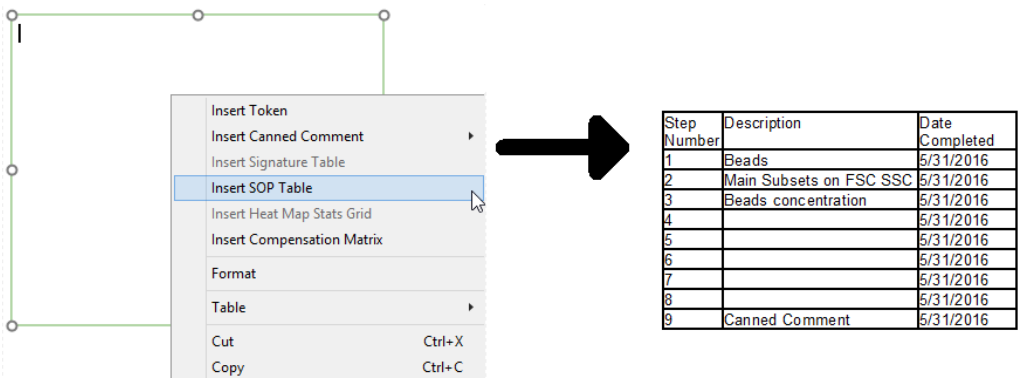

Figure 35.9 Inserting the SOP Table

Using individual SOP tokens

1. Right-click in a text box.
2. Select **Insert Token**. The **Insert a Token** window will appear (Figure 35.10).

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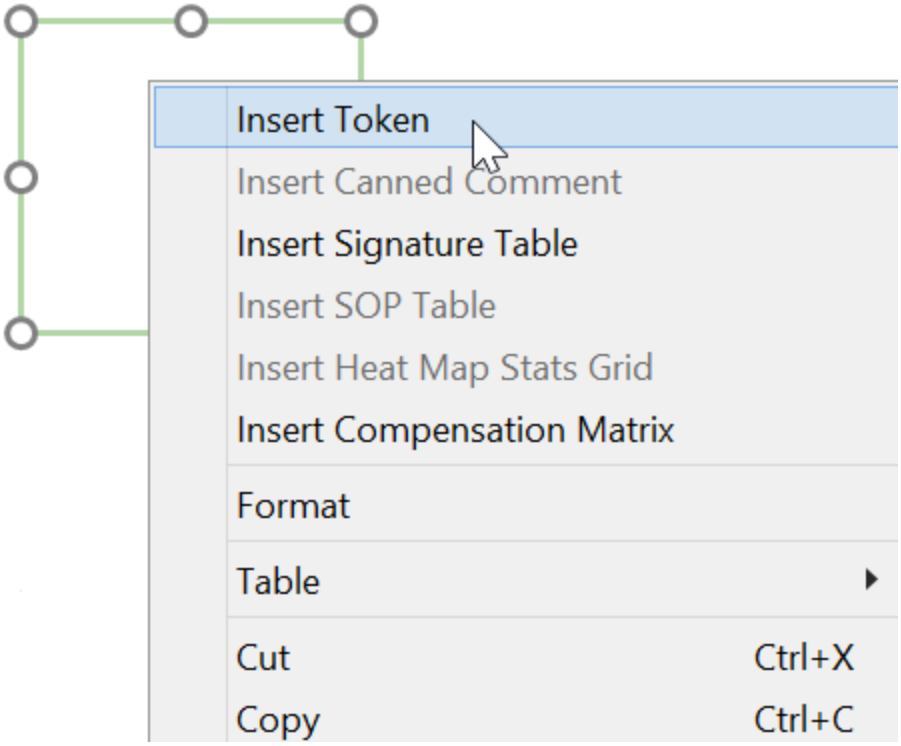

Figure 35.10 Insert Individual SOP tokens

3. Click on the **SOP** node.
4. Select the type of **SOP** information you want to display.
5. Press **Insert** (in this example, we will choose **SOP step name**) (Figure 35.11). The **Create SOP token** dialog will appear. Note: if the SOP is not running when any of the follow tokens are inserted, **"SOP is not running"** will be the token value's display text in the text box: **Current SOP step name**, **Current SOP step internal ID**, **Current SOP step order number**, **Current SOP step description**, and **Current SOP step completion date**.

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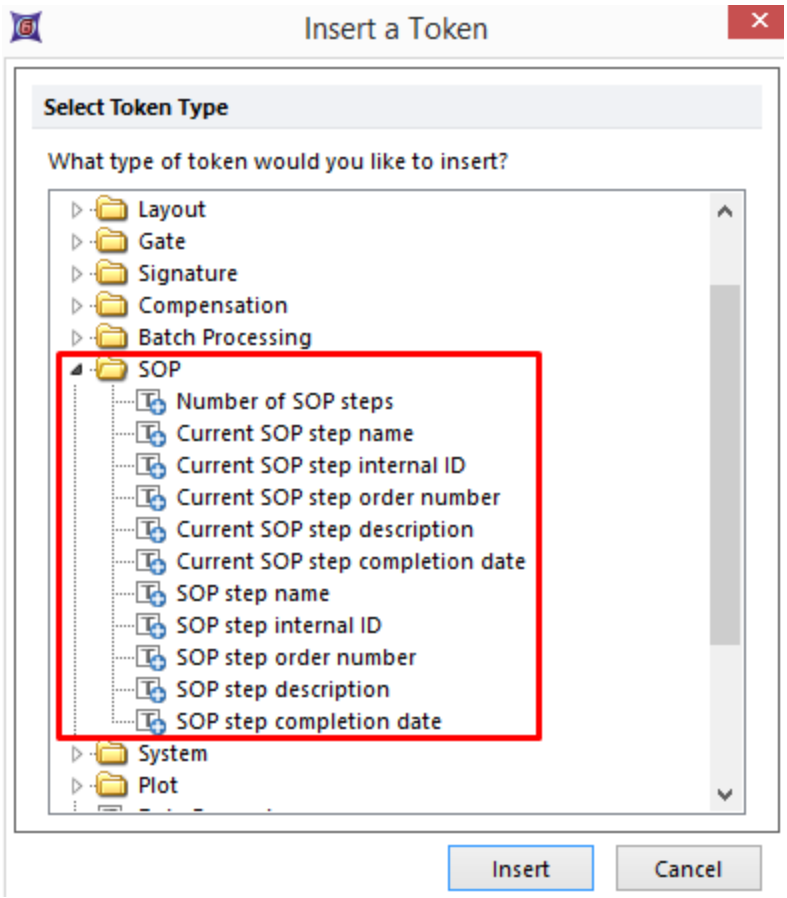

Figure 35.11 SOP Tokens

6. Choose the **SOP Information** category in the **Create SOP Token** dialog.
7. Select the **Linked SOP step name** from **Token to Display** drop down list.
8. Choose the step of interest from the **Sop Step** drop down list under **Linked SOP Step** (Figure 35.12).

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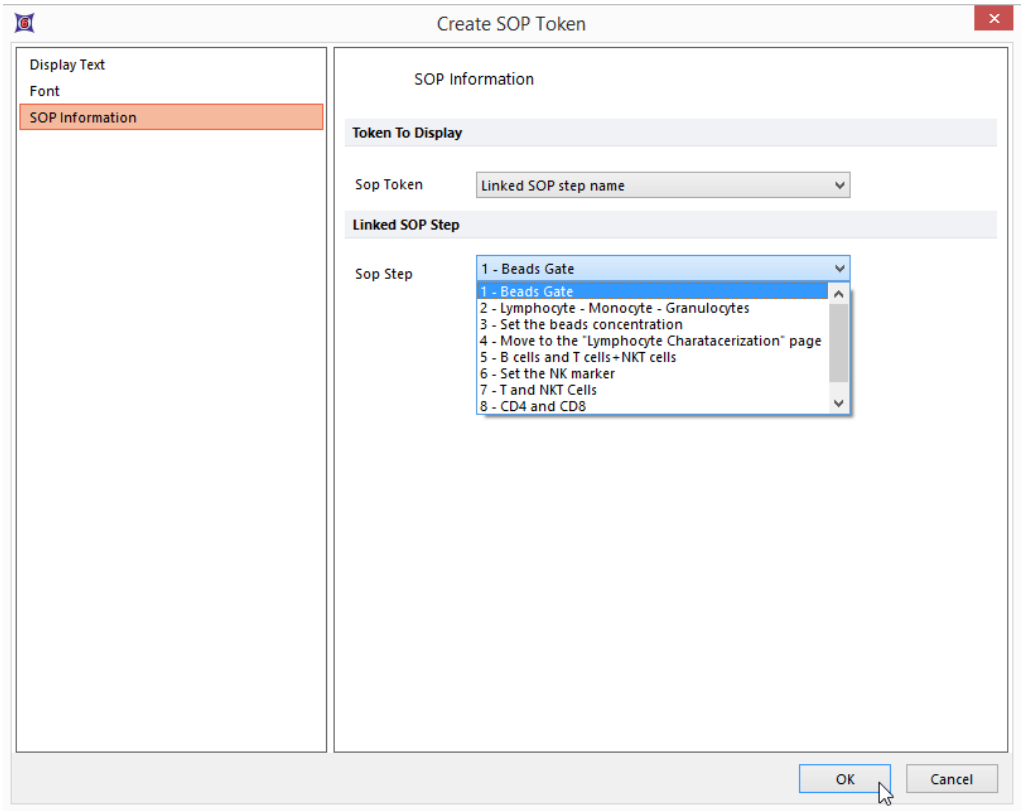

Figure 35.12 SOP Token Editor

9. Click **OK.** The token will appear in the text box (Figure 35.13).

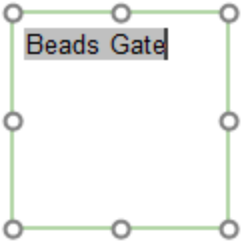

Figure 35.13 - Text box with Inserted Token

As with other tokens, you can change the Common Token Properties 696 of **Display Text** and **Font** of individual SOP tokens in the SOP Token Editor.

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Chapter

35 Permission Rules

Permission Rules allow you to set available permissions[1127] specific to a layout. They may be activated or disabled manually or via formula statements[1124] in the **Permission Rules** navigator. Please note that layout based[1179] and user based[1173] security group permissions will always take precedence over permission rules.

Permission Rules can be accessed via the **Quality** tab→**SOP**→**Permission Rules** button.(Figure 36.1).

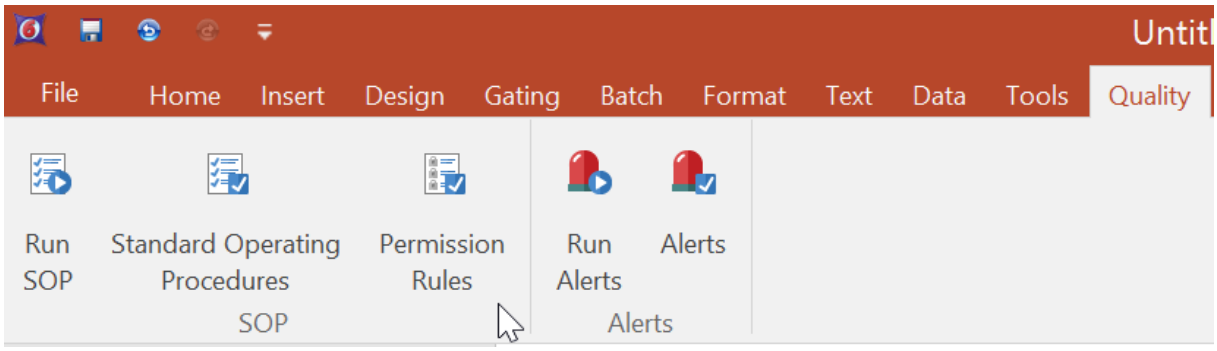

Figure 36.1 - Accessing the Permission Rules navigator.

The **Permission Rules** navigator will appear (Figure 36.2) allowing you to create and edit Permission Rules.

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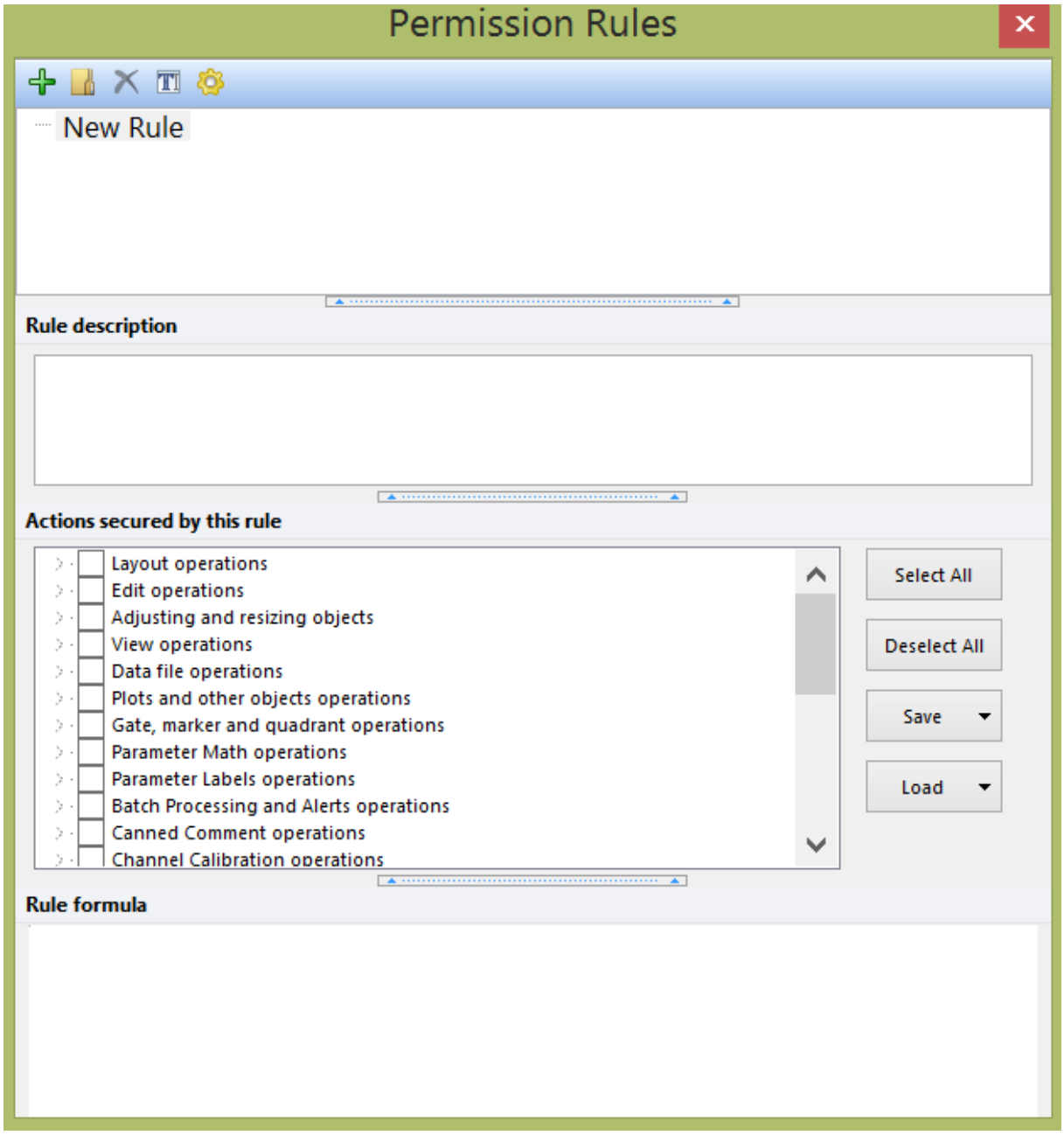

36.2 - The Permission Rules navigator.

The top menu bar of the **Permission Rules** navigator allows you to (Figure 36.3) :

1. Create a new rule.
2. Create a new permission rule folder.
3. Delete one or more permission rules.
4. Rename the selected rule.

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5. Access the permission rule option.

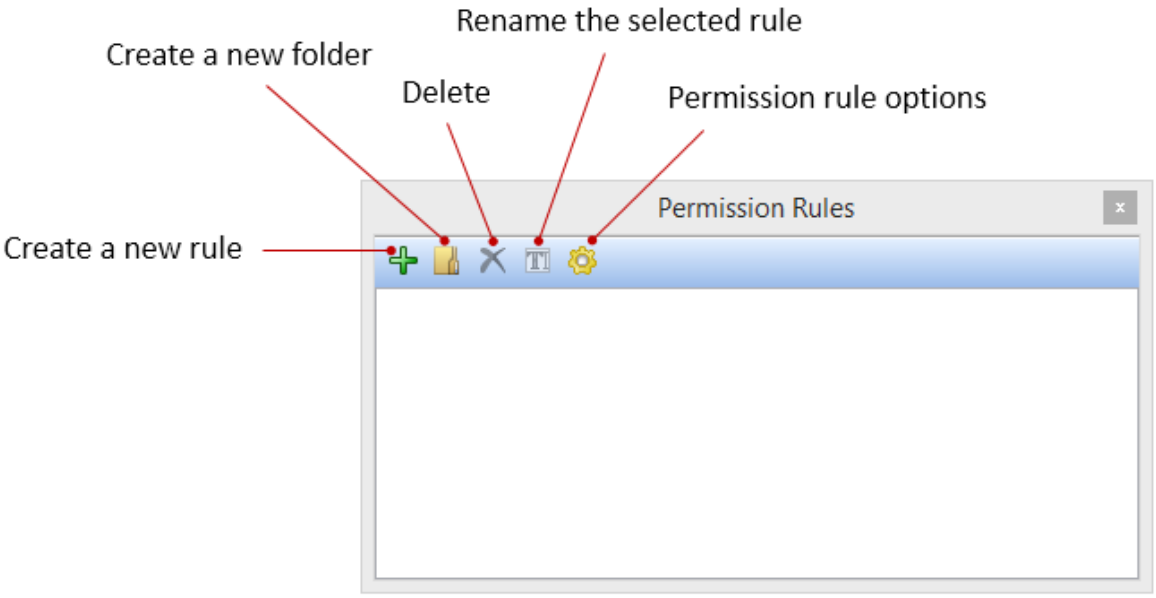

Figure 36.3 - The Permission Rules navigator menu bar.

35.1 Creating a Permission Rule

To create a new **Permission Rule** first open the **Permission Rules** navigator using the **Quality** tab→**SOP**→**Permission Rules** button. The **Permission Rules** navigator will appear.

Click on the button to create a new **Permission Rule** (Figure 36.4). A **New Rule** will populate the Permission Rule window.

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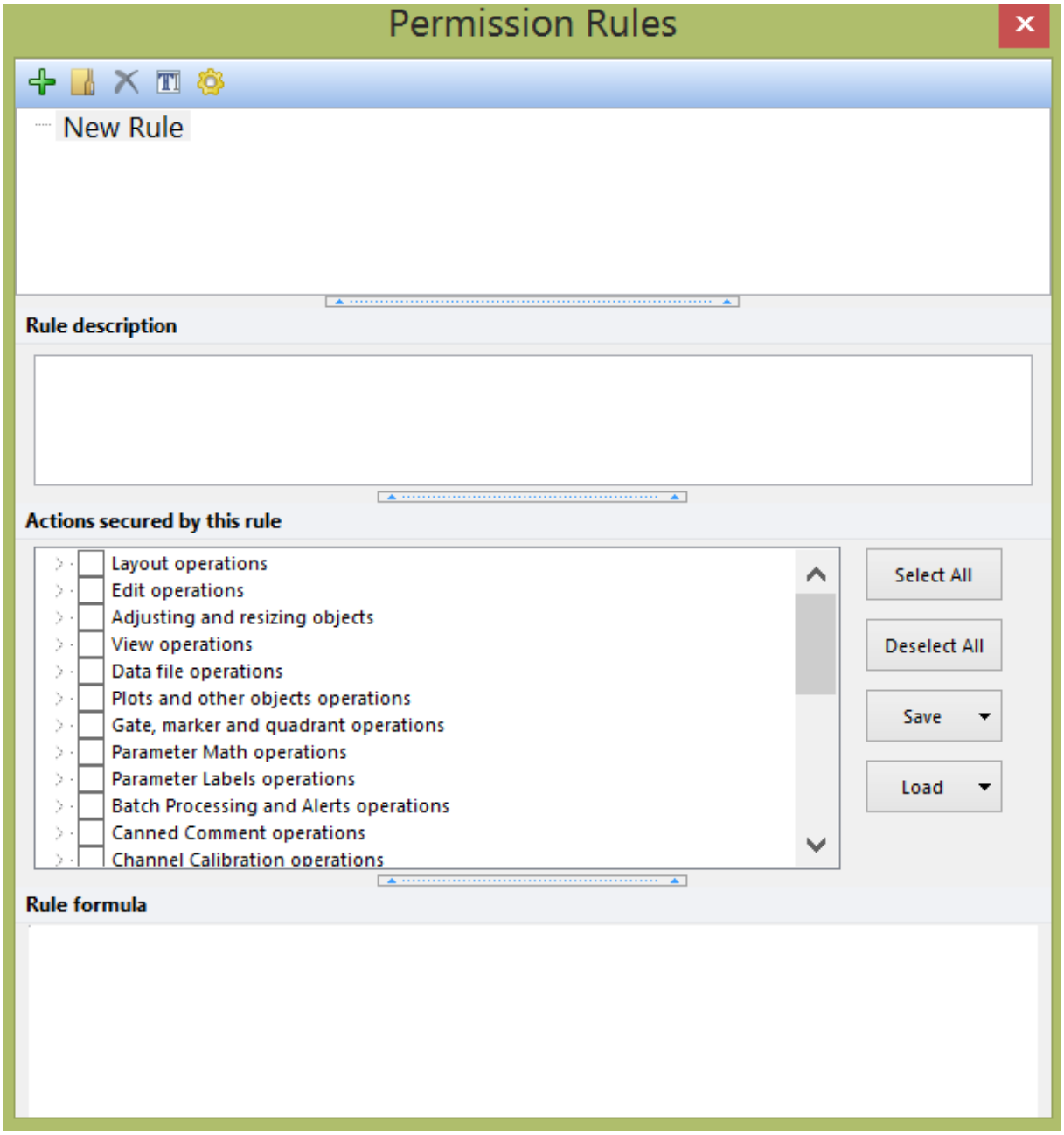

36.4 - The Permission Rules navigator.

The new **Permission Rule** is named as **New Rule** by default but it can be easily renamed by either of the following methods:

- Right click on the **Permission Rule** that needs to be renamed and select **Rename** from the right click menu.

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- Select the **Permission Rule** that needs to be renamed via left click and press **F2** on your keyboard.
- Select the **Permission Rule** that needs to be renamed via left click and click on the button in the **Permission Rule** window.

A free-text description of the Permission Rule can also be added in the **Rule Description** field of the Permission Rule window.

The **Actions secured by this rule** section of the **Permission Rule** window contains the list of action that can be locked down via the permission rule. A complete list of actions that can be secured via a Permission Rule can be found in the Available Permission Rules section 1127.

The following buttons are available in the **Actions secured by this rule** section of the **Permission Rule** window:

- **Select All**: to select all the available actions.
- **Deselect All**: to deselect all the available actions.
- **Save→Save Permissions to File**: to export the list of secured actions as text file (please see the Save and Load Permission Rules Action List 1125 topic).
- **Load→Save Permissions to File**: to export the list of secured actions as text file (please see the Save and Load Permission Rules Action List 1125 topic).

A permission rule can be turned on or off via a conditional formula. The conditional formula a **Permission Rule** will be subjected to can be inserted in the **Rule Formula** section of the **Permission Rule** window. The formula can contain any of the operators and functions available for creating custom tokens (please refer to the Custom Token Formula Reference 769 topic for the complete list of available operators and functions). However, given that the formula has to be conditional, one of the following operator need to be present:

- Conditional/Comparison operators.
- Logical Operators.

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- Logic (True/False) functions.

NOTE: The results of Permission Rules are dependent on the Permission Rules Options 1127.

35.2 Organizing Permission Rules in folders

Permission Rules can be organized in folders directly within the Permission Rules window by either of the following methods:

- Right click on any empty area of the upper section of the **Permission Rules** window and select **New Folder** from the Right Click menu.
- Right click on any Permission Rule and select **New Folder** from the Right Click menu.
- Click on the button in the **Permission Rule** window.

A folder can be renamed by either of the following methods:

- Right click on the folder that needs to be renamed and select **Rename** from the Right Click menu.
- Select the folder that needs to be renamed via left click and press **F2** on your keyboard.
- Select the folder that needs to be renamed via left click and click on the button in the **Permission Rule** window.

35.3 Save and Load Permission Rules Action List

The list of actions secured by a Permission Rule 1127 can be exported as text file in order to be reloaded when needed.

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To export the list of actions secured by a Permission Rule:

1. Click on the **Save** button of the **Permission Rules** window and select **Save Permission to File**.
2. A **Select a file for saving permissions** window opens.
3. Select the file destination and enter a file name in the **Select a file for saving permissions** window and click **Save**.

To Load a previously exported list of action secured by a Permission Rule:

1. Click on the **Load** button of the **Permission Rules** window and select **Load Permission to File**.
2. A **Select a file for loading permissions** window opens.
3. Select a previously exported lust of action in the **Select a file for loading permissions** window and click **Open**.
4. The list of actions is now loaded and displayed in the **Actions secure by this rule** section of the **Permission Rules** window.

35.4 Editing a Permission Rule

To edit a Permission Rule first open the Permission Rules dialog using the **Quality** tab→**SOP**→**Permission Rules** command. The **Permission Rules** dialog will appear and will list the available Permission Rules.

Select the Permission Rule that need to be edited. The **Rule Description**, the list **Actions secured by this rule** and the **Rule Formula** can now be edited.

35.5 Deleting a Permission Rule

To edit a Permission Rule first open the Permission Rules dialog using the **Quality** tab→**SOP**→**Permission Rules** command. The **Permission Rules** dialog will appear and will list the available Permission Rules.

A Permission Rule can be deleted by either of the following methods:

- Right click on the Permission Rule that need to be deleted and select **Delete** from the Right Click menu.

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- Select the Permission Rule that need to be deleted via left click and press **Delete** on your keyboard.
- Select the Permission Rule that need to be deleted via left click and click on the button in the **Permission Rule** window.

35.6 Permission Rules Options

Permission Rules Options can be accessed by either of the following methods:

- Click on the button of the **Permission Rules** window.
- Right click on any empty area of the upper section of the **Permission Rules** window and select **Options** from the Right Click menu.

Permission rules options defines whether the **Action that are not included in any rule will be:**

- **Allowed**: all the actions not selected as permission rules will be allowed by default (permissive approach).
- **Not Allowed**: all the action not selected as permission rules will be denied (restrictive approach).

35.7 Available Permission Rules

Permission Rules allow the user to lock down one or more of the following actions:

- ❖ **Layout operations**
  - Open layout
    - Open
  - Navigators and Other Dockable Windows
    - View Batch Actions
    - View Data List
    - View Alerts
    - View Custom Tokens
    - View Canned Comments
    - View Compensations

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    - View Snapshots
    - View Standard Operating Procedures
    - View Permission Rules
  - Save layout
  - Save layout as
  - Print layout
  - Change Page Setup
  - Save layout to PowerPoint
  - Save PDF
  - Publish Layout
- **Edit operations**
  - Copy FCS data to the clipboard
  - View layout properties
  - Change layout properties
  - Insert new page
  - Rearrange pages
  - Delete page
  - View page options
  - Change page options
  - Align objects
  - Change Object Order
- **Adjusting and resizing objects**
  - Move objects
  - Resize objects
  - Group objects
  - Ungroup objects
- **View operations**
  - View the layout history
  - View the master page
  - Change the number of visible pages
  - View hidden pages
- **Data file operations**
  - Open a Data file
  - Modify the contents of a data file
  - Add a column to a data file
  - Delete a column from a data file
  - Insert a row into a data file
  - Delete a row from a data file
  - Export a Data file
  - Load an Data file that is not already loaded
  - Create a custom data set
  - Add to data list
  - Search the data list
  - Remove from the data list
  - Format the Data List Options

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- Move a file Up in the data list
- Move a file Down in the data list
- Increment/Decrement file on selected/all objects
- Change file on selected/all objects
- View file header information
- Modify file header information
- Change the increment value

❖ **Plots and other objects operations**

- Insert stats window
- Insert plots and other objects
  - Insert 2D Plots
  - Insert Histogram
  - Insert Kinetics
  - Insert MultiCycle DNA
  - Insert Proliferation
  - Insert Text Box
  - Insert Gate View
  - Insert Picture
  - Insert Data Grid
  - Insert Scatter
  - Insert Bar
  - Insert Pie
  - Insert Heat Map
  - Insert AutoShapes
- Remove 2D plots
- Remove histograms
- Remove DNA histograms
- Remove Proliferation histograms
- Remove text boxes
- Remove picture
- Remove data
- Remove Gate View
- Remove AutoShape
- Remove stats window
- Change parameters on a histogram
- Change parameters on a 2D plot
- Add overlay to a histogram
- Add overlay to a 2D plot
- Remove overlay from a histogram
- Remove overlay from a 2D plot
- Reorder overlay in a histogram
- Reorder overlay in a 2D plot
- Edit the text on a text box
- View the format of a 2D plot
- View the format of a 1D plot

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- View the format of an AutoShape
- View the format of a text box
- View the format of a picture
- View the format of a data object
- View the format of a statistics window
- Change the format of a 2D plot
- Change the format of a 1D plot
- Change the format of an AutoShape
- Change the format of a text box
- Change the format of a picture
- Change the format of a data object
- Change the format of a statistics window
- Change the visibility of statistics
- Autosize statistics window
- Delete Table Cells
- Insert Table
- Insert Table Cells

❖ **Gate, marker and quadrant operations**

- Create a new gate
- Modify a gate
- Remove a gate
- Modify gate information
- Add a combination gate
- Change Gate Properties
- Create a new marker
- Modify a marker
- Remove a marker
- Gate fellowship Link
- Break Gate fellowship
- Show quadrants
- Hide quadrants
- Modify quadrants

❖ **Parameter Math operations**

- Perform single calculation
- View the current sequence
- Modify the current sequence
- Perform the current sequence on the top FCS file
- Perform the current sequence on the all FCS files
- Load a sequence
- Save the current sequence

❖ **Parameter Labels operations**

- View parameter label sets
- Modify parameter label sets
- Load a set
- Save the list of sets

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- **Batch Processing and Alerts operations**
  - Alerts
    - Run alerts
    - Add a new alert
    - Delete an alert
    - Edit the text in an alert
    - Add token to alert formula
    - View token properties for the alert formula
    - Edit alert formula token properties
  - Batch Processing
    - Flag an iteration
    - View batch processing options
    - Change batch processing options
    - Run batch processing
    - Replay batch processing
    - Add save batch actions
      - Add Save Layout batch action
      - Add Save PDF batch action
      - Add Save Picture batch action
      - Add Save PowerPoint batch action
      - Add Print batch action
      - Add Publish Layout batch action
    - Add batch folders
      - Add Folder
    - Add report batch actions
      - add Export To Excel (Column Mode)
      - add Export To Excel (Cell Mode)
      - add Word Report
      - add PDF Form
      - add Text Report
      - add Export To LabKey
    - Add items to reports
      - add Regular Token
      - add Statistic Token
      - add Keyword Token
      - add Picture
      - add Text Box
      - add PDF
      - add Layout
      - add PowerPoint
      - add FCS File
    - Rename batch actions
    - Paste batch actions
    - Delete batch actions
    - View batch action details
    - Change batch action details
- **Canned Comment operations**

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- Add a new Canned Comment
- Delete a Canned Comment
- Edit the text in a canned comment
- Add a token to a canned comment
- View canned comment token properties
- Edit canned comment formula token properties

❖ **Channel Calibration operations**

- View Channel Calibration settings
- Add a Channel Calibration
- Delete a Channel Calibration
- Load Channel Calibration settings
- Save Channel Calibration settings
- Modify settings
- Calculate Channel Calibration
- Edit a Channel Calibration

❖ **Compensation operations**

- Modify compensation
- Import compensation
- Export compensation
- Create compensation
- Delete compensation
- Assign compensation
- Run Automatic Compensation

❖ **Other tools**

- Modify Concentration calculator settings
- Concentration calculator calculate
- Histogram subtraction
- Batch export
- Diva Import
- CFlow Import

❖ **Security Operations**

- Electronic Signature operations
  - View signature list
  - Change signature list
  - Sign
  - Unsign

❖ **Snapshot operations**

- Add snapshot
- Edit snapshot
- Delete snapshot
- Apply snapshot

❖ **SOP operations**

- Add SOP Step
- Delete SOP Step
- Edit SOP Step

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- Run SOP

❖ **Help operations**

- View help
- View tutorials
- View manual
- View online forum
- Go to the De Novo Software web site
- Enter countercode
- View About screen

❖ **Panel operations**

- View the panel information
- Add a panel
- Edit a panel
- Remove a panel
- Load panels from a file
- Save panels to a file
- Change the panel information
- Change the name of a panel
- Change the number of tubes in a panel

❖ **Token operations**

- Custom Tokens
  - Add custom token
  - Delete custom token
  - Add token to custom token formula
  - View custom formula token properties
  - Edit custom formula token properties
  - Edit the text in a custom formula
- Change Font Classification
- Add Font Classification
- Edit Font Classification
- Reorder Font Classification
- Remove Font Classification
- Token Bounds Change
- Add Statistic Classification
- Edit Statistic Classification
- Reorder Statistic Classification
- Remove Statistic Classification
- Add Keyword
- Remove Keyword
- Add token to a text box
- View text box token properties
- Edit text box token properties

❖ **Formatting Operations**

- Bold
- Bullets

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- Font
- Font Background
- Font Color
- Font Name
- Font Size
- Gate Formula Change
- Indent Text
- Italics
- Justify Text
- Format 3D Options
- Format Axes Options
- Format Color Levels
- Format Fill Options
- Format General Options
- Format Legend Options
- Format Line Options
- Format Overlays Options
- Format Size Options
- Format Stats Options
- Format Title Options
- Underline

❖ **Keywords Anonymizer**

❖ **Page Tab Operations**

- Change Page Tab Color Classification
- Add Page Tab Color Classification
- Edit Page Tab Color Classification
- Reorder Page Tab Color Classification
- Remove Page Tab Color Classification

❖ **Override Authorizations**

❖ **Format Authorizations**

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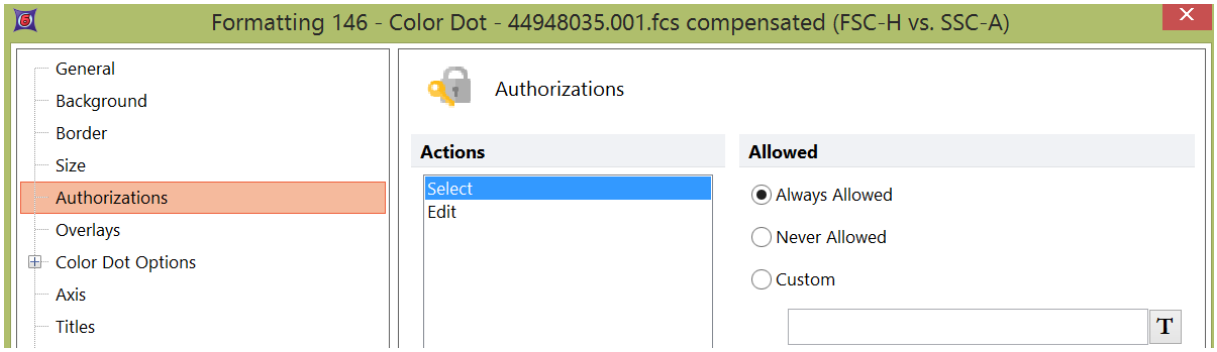
Chapter

36 Formatting Authorizations

FCS Express allows user to control when objects are selected or edited based on settings in **Formatting Authorizations**.

All objects in FCS Express may be assigned Authorizations[1136], or rights, which allows or prevents layout users from placing objects in **Select** mode or **Edit** mode or both. **Authorizations** can be set to always allow, never allow, or always allow/do not allow based on the value of a token. For instance, a token value can be set to allow selection of a plot if the current FCS Express user equals a specific person or a plot can remain non-editable unless a particular statistic value is met, e.g., the number of events for a particular gate is higher than an arbitrary value. See the Working with Authorizations[1138] section in the manual for additional details on setting up these conditions.

Authorizations allow for an additional layer of control at the layout level for any object.



The Authorization feature.

36.1 Accessing and Docking Formatting Authorizations Navigator

Users can access the **Formatting Authorizations** navigator by using the ribbon after selecting object(s) or right-clicking selected object(s) and choosing **Format**.

To access **Authorizations** using the ribbon:
1. Click on an object to select it.
2. Click **Format** tab→**Object Options**→**Authorizations** command (Figure 37.1).

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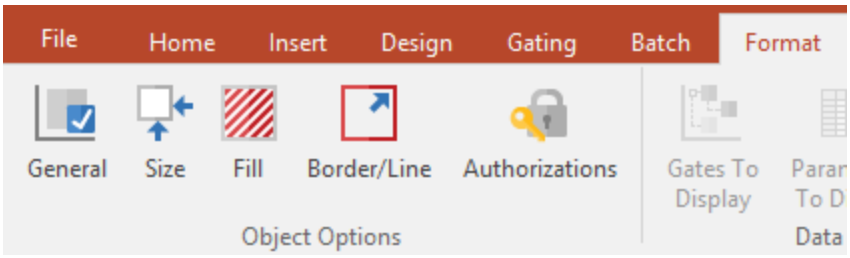

Figure 37.1 Accessing Formatting Authorizations for selected object(s)

The **Formatting Authorizations** navigator will appear allowing actions to be formatted when allowed as seen in Figure 37.2 below.

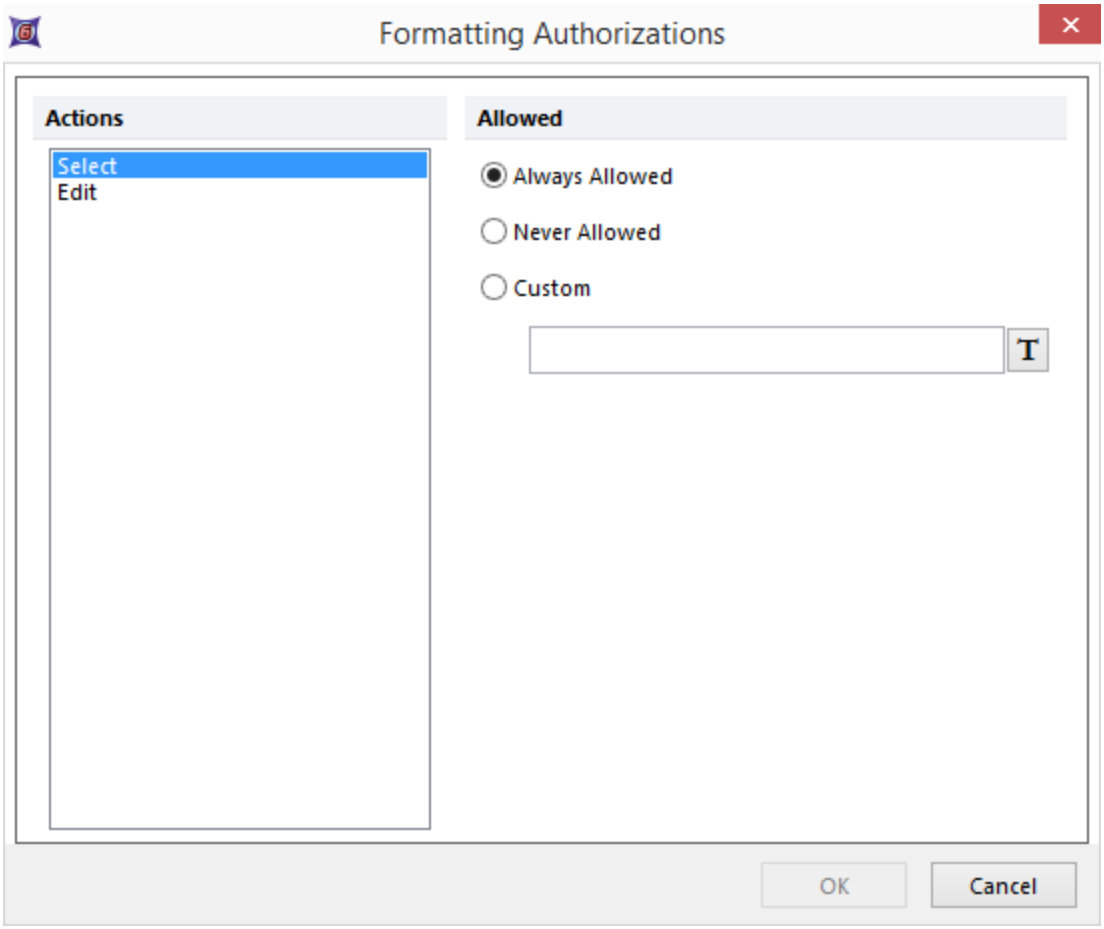

Figure 37.2 Formatting Authorizations navigator via ribbon

To access Formatting Authorizations via an object:
1. Right-click on a selected object(s).
2. Choose **Format**.
3. Select the **Authorizations** category (Figure 37.3).

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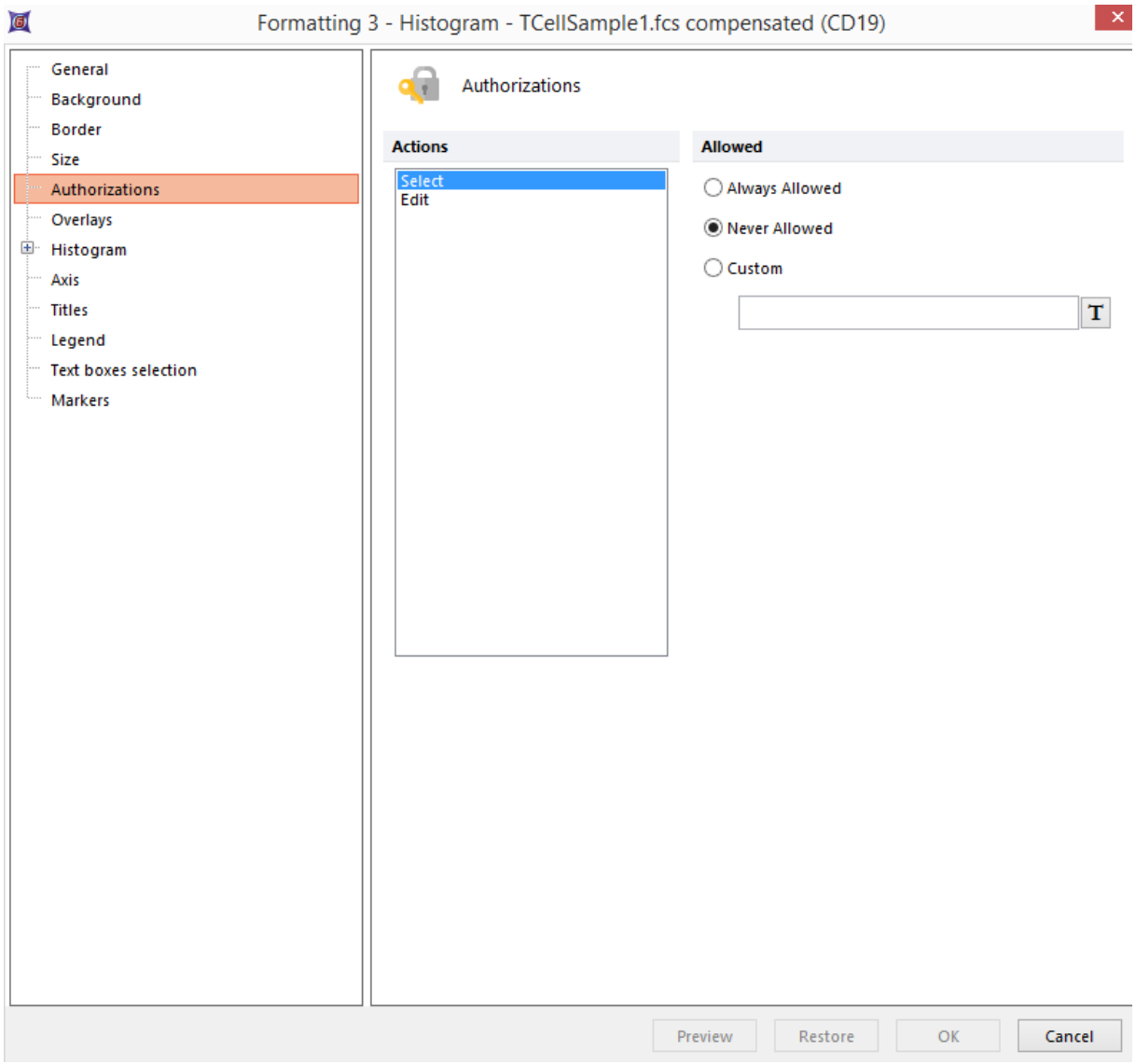

Figure 37.3 Formatting Authorizations navigator via right-clicking selected object(s)

To dock the **Formatting Authorizations** navigator, click and drag the window's title bar to an edge of the layout to activate docking zone sectors; select area by releasing mouse on docking zone sector of choice. See docking a window 115 for more details.

36.2 Working with Authorizations

The Authorizations options may be accessed by formatting plots as described in the previous section 1136.

Authorizations may be set for two **Actions**, **Select** and **Edit**. Either option may be chosen individually in the **Actions** list or both **Actions** may be multiple

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selected to change them together. Setting an Authorization for either or both **Actions** will control if an object may be placed into Select mode or Edit mode 132 thus limiting the specific actions that may be taken with a plot. For instance, if **Never Allowed** is chosen for the **Select** action then a plot may not be placed into selection mode and moved or resized on the page. If **Never Allowed** is chosen for the **Edit** action then the plot cannot be formatted, gates cannot be created, or nor can any actions available via the right click menu. If **Never Allowed** is chosen for both the **Select** and **Edit** actions then the plot cannot be formatted or moved.

The default status of **Actions** on all objects is **Always Allowed**. Selecting the **Never Allowed** radio button will disallow access to the **Select** or **Edit Action**. Choosing the **Custom** radio button will allow you to define a condition that must be met to Allow or Never Allow the Action. For instance, in the example below, Figure 37.4, a formula was defined in the **Custom** field. The chosen plot will not be allowed to be in **Select** mode if the token value (highlighted in grey - 79.05)) is above the reference value (70). Any tokens may be used to set a conditional Custom value and may be inserted 692 via right-click in the field, **Insert Token** or by clicking on the **T** token icon.

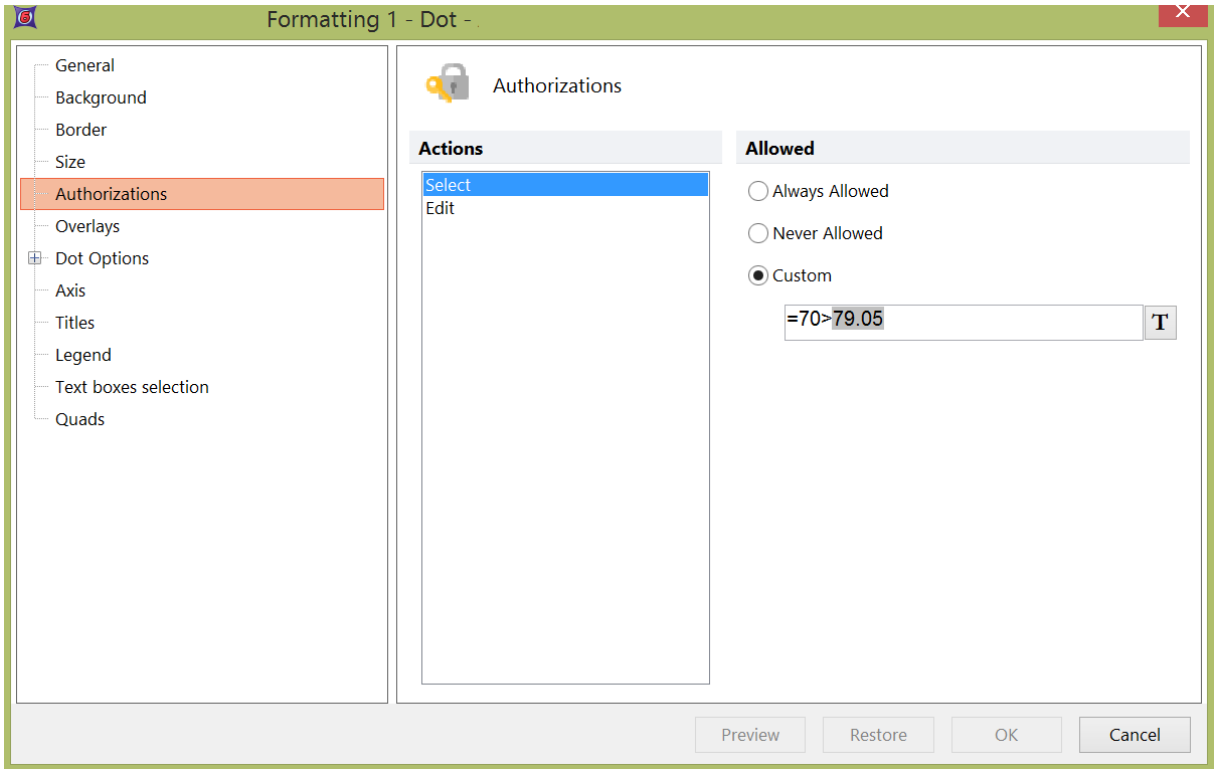

Figure 37.4 - The Authorizations formatting dialog. In the example a Select action was set to be Custom and is only allowed if the formula returns a true result. In this case the plot will remain not selectable because the token value (highlighted in grey) is higher than the reference value of 70.

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Values must be returned as "true" to be allowed / "false" to never allow. Please note that if are evaluating a token whose result type is text, then the standard value must exist in quotes (e.g. the "FACScan" in the figure below).

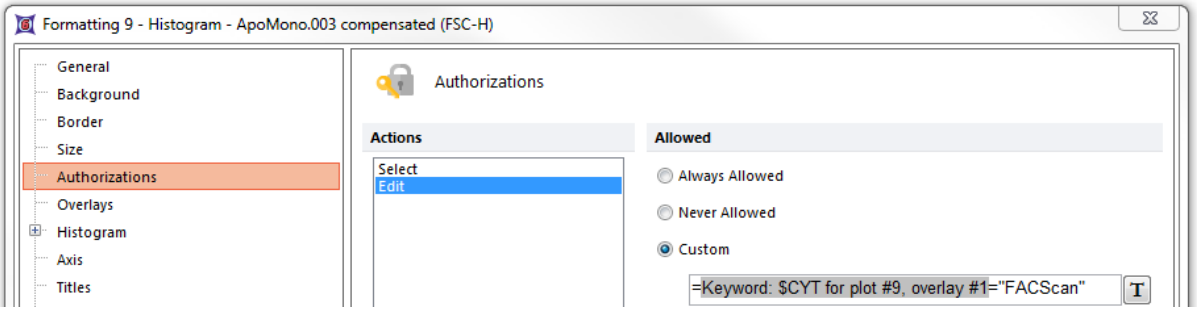

Figure 37.5Authorizations window showing proper syntax for text-type tokens to return a true/false value.

Authorizations may also be overridden. Overriding Authorizations[1140] is particularly useful if the **Edit Action** is set to not be allowed but an edit or change to the plot or Authorization is needed.

36.3 Overriding Authorizations

To override authorization(s), click to select object(s) and click **Format** tab→**Override**→click **Override Authorizations** (Figure 37.5).

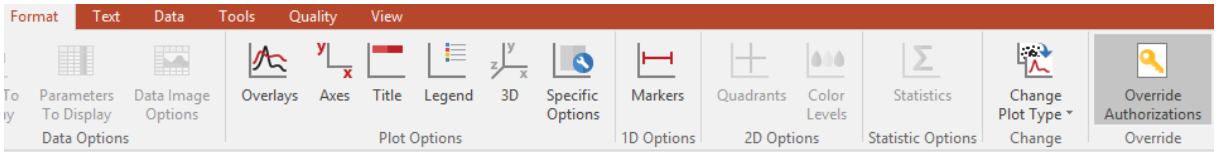

Figure 37. 6 Override Authorizations under Format tab

Please note, for users not using the security add-on[1142], any user can override authorizations. Users using the security add-on can allow or prohibit access to **Formatting Authorizations**[1136] or Override Authorizations at the User[1142], Layout[1143], or Security Group[1173] level.

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Chapter

37 Security

FCS Express has a comprehensive security system that allows you to restrict access to various functions to particular users. This can be important for ensuring the integrity of your data analysis and to conform with certain government regulations such as 21 CFR Part 11. FCS Express offers multiple layers of security, described in the remaining sections of this chapter.

37.1 User-Based Security

The first level of security is based on restricting functionality based on a particular user. When security is activated, each user must log in to FCS Express with a user name and password (Figure 38.1).

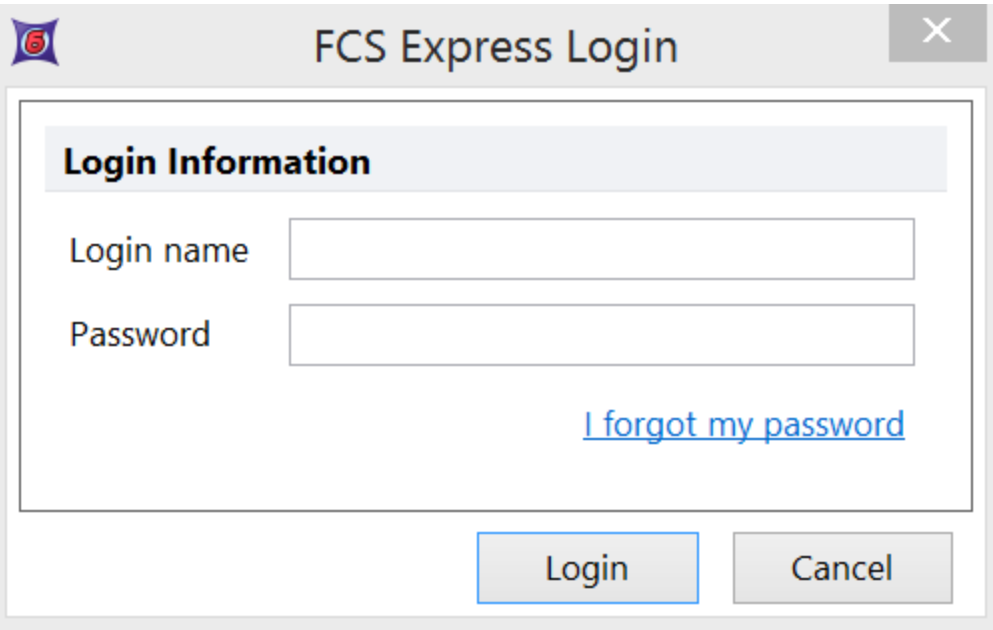

Figure 38.1 Security Login

Users are created and managed from the **File** tab→**Security** gallery within FCS Express (Figure 38.2).

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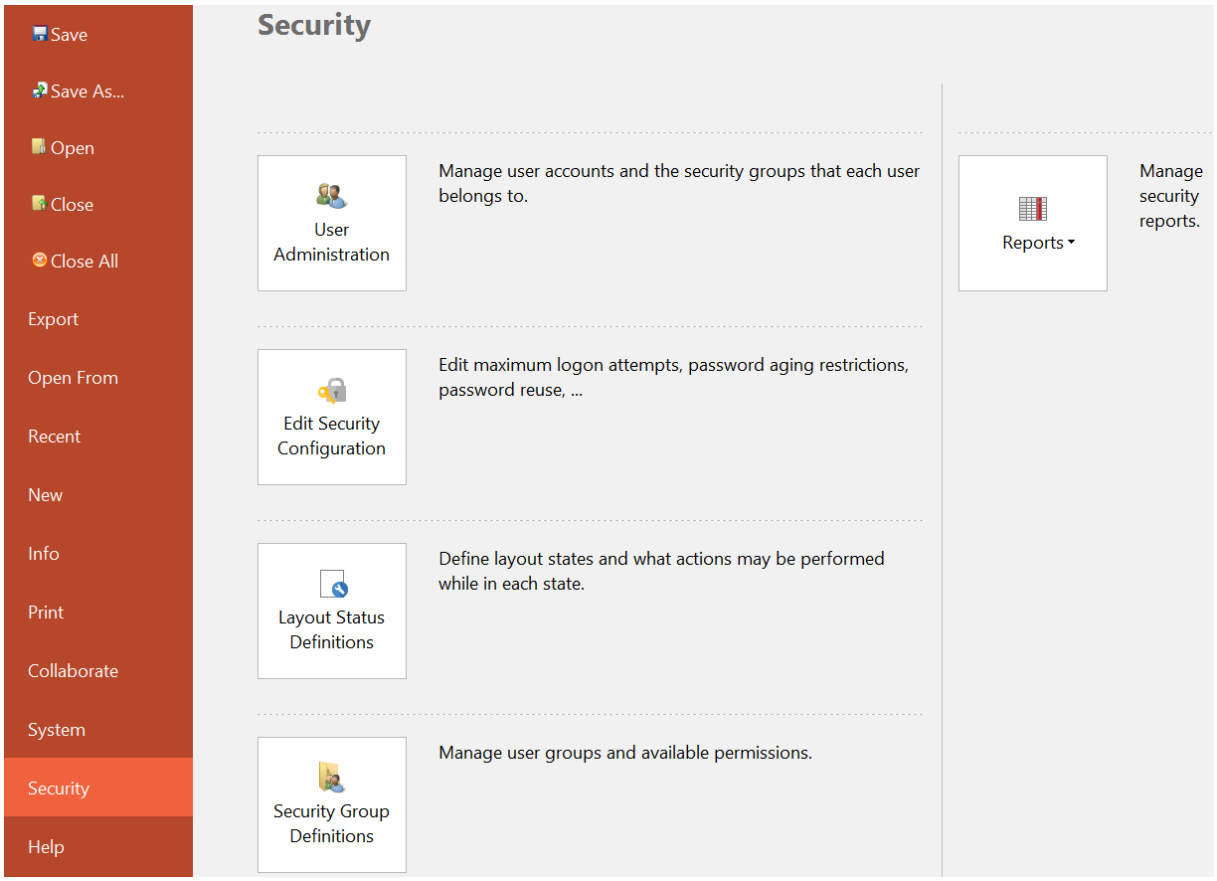

Figure 38.2 Security Gallery

Each user is also assigned to a User Security Group and can only perform functions that are allowed by that group. Hence, if Group A does not allow its members to print layouts, then any user who is a member of Group A will not be allowed to print layouts.

37.2 Layout-Based Security

The second level of security is based on restricting functionality for particular layouts. Every layout has a Layout Security Group 1179 assigned to it. Only the functions allowed by the Layout Security Group can be performed on that layout. These functions are identical to the ones that can be assigned to a User Security Group 1181. For example, if the layout security was set to a Layout Security Group that did not allow printing, then any user (regardless of their User Security Group permissions) would not be able to print that layout.

In addition to having security permissions for each action on the layout, a password can be specified for the layout. You will not be able to open the layout without entering the password. The password is stored in an encrypted format. If

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the password is lost, the only way to open the layout is to send it to De Novo Software.

37.3 Working with User-Based Security

FCS Express comes with one default user, the Administrator. The default **Login name** for the Administrator is *admin*. The default Password for the Administrator is *password*.

Note: This password should be changed immediately after using FCS Express the first time.

Changing a user's password is described in Editing a User 1152. The Administrator is part of the Administrator Security Group. For more information on Security Groups, see Working with Security Groups 1173. By definition, the Administrator Security Group can perform every action and cannot be deleted.

Users may be sorted by **Name** or **Last Name** by clicking on the Up or Down arrows in the column headers.

To manage users, use the **File** tab→**Security**→**User Administration** command. This will bring up the User Administration dialog (Figure 38.3). Users are listed in alphabetical order by last name.

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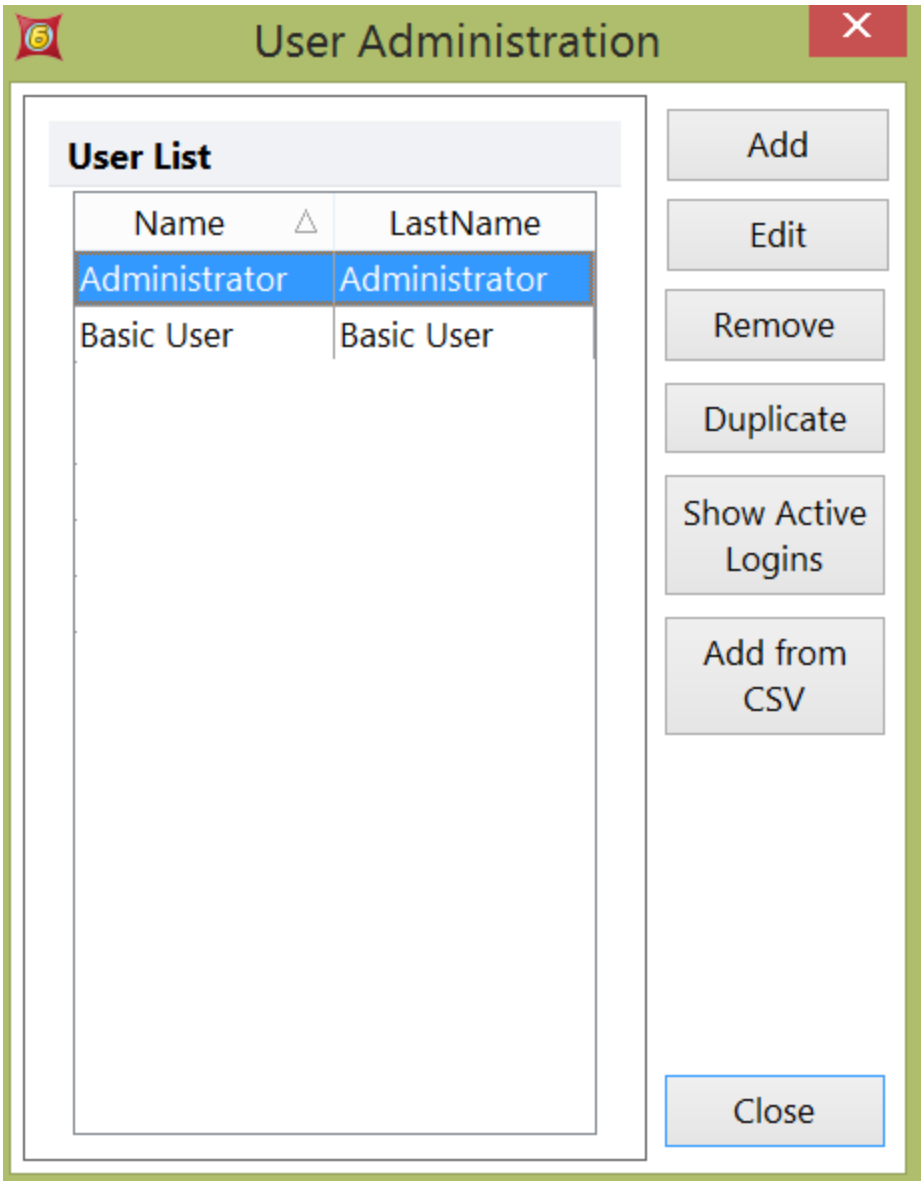

Figure 38.3 User Administration Dialog

Clicking on the **Show Active Logins** button will bring up the **Active Logins** dialog (Figure 38.4) which will display all of the currently active users. To logout a user, highlight the user and press the **Logout** button.

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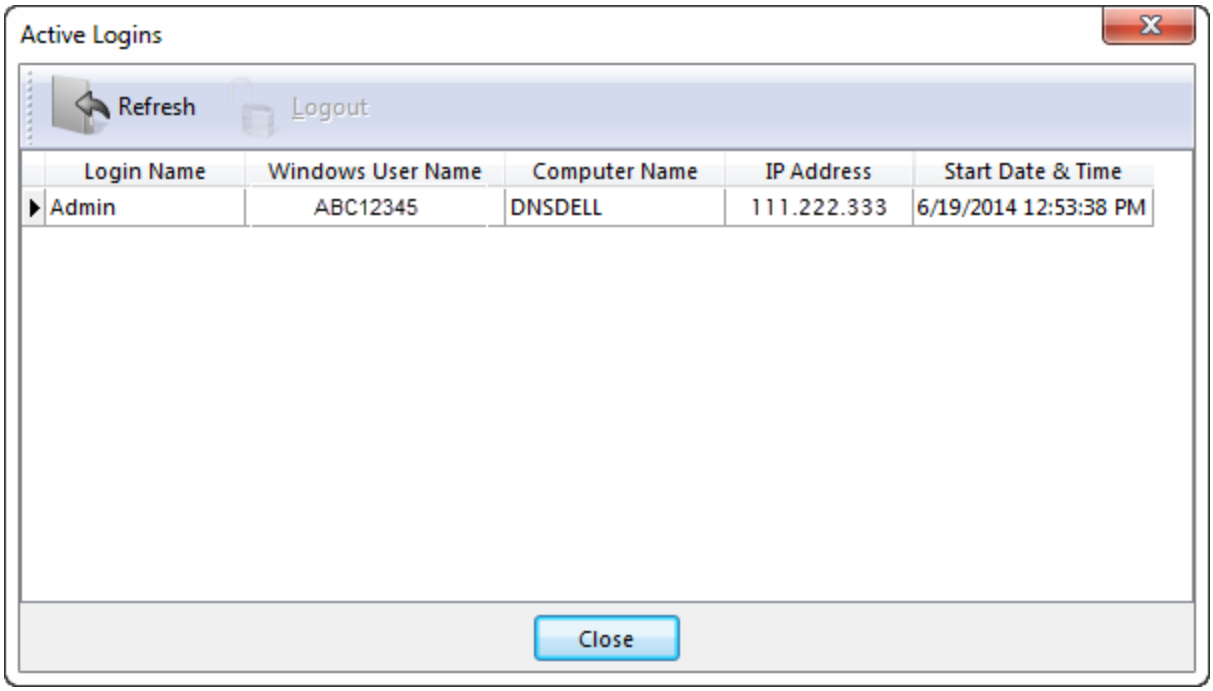

Figure 38.4 Active Logins Dialog

Note: If a user is logged out while they are currently using the software, all functionality will be disabled and they will only be able to Save or Exit.

Please see the Internet Dongle and Site License KnowledgeBase page for more information on working with user-based security for these license types.

37.3.1 Creating a User

To create a new user:

1. Use the **File** tab→**Security**→**User Administration** command.
2. Click on the **Add** button in the **User Administration** dialog (Figure 38.5).

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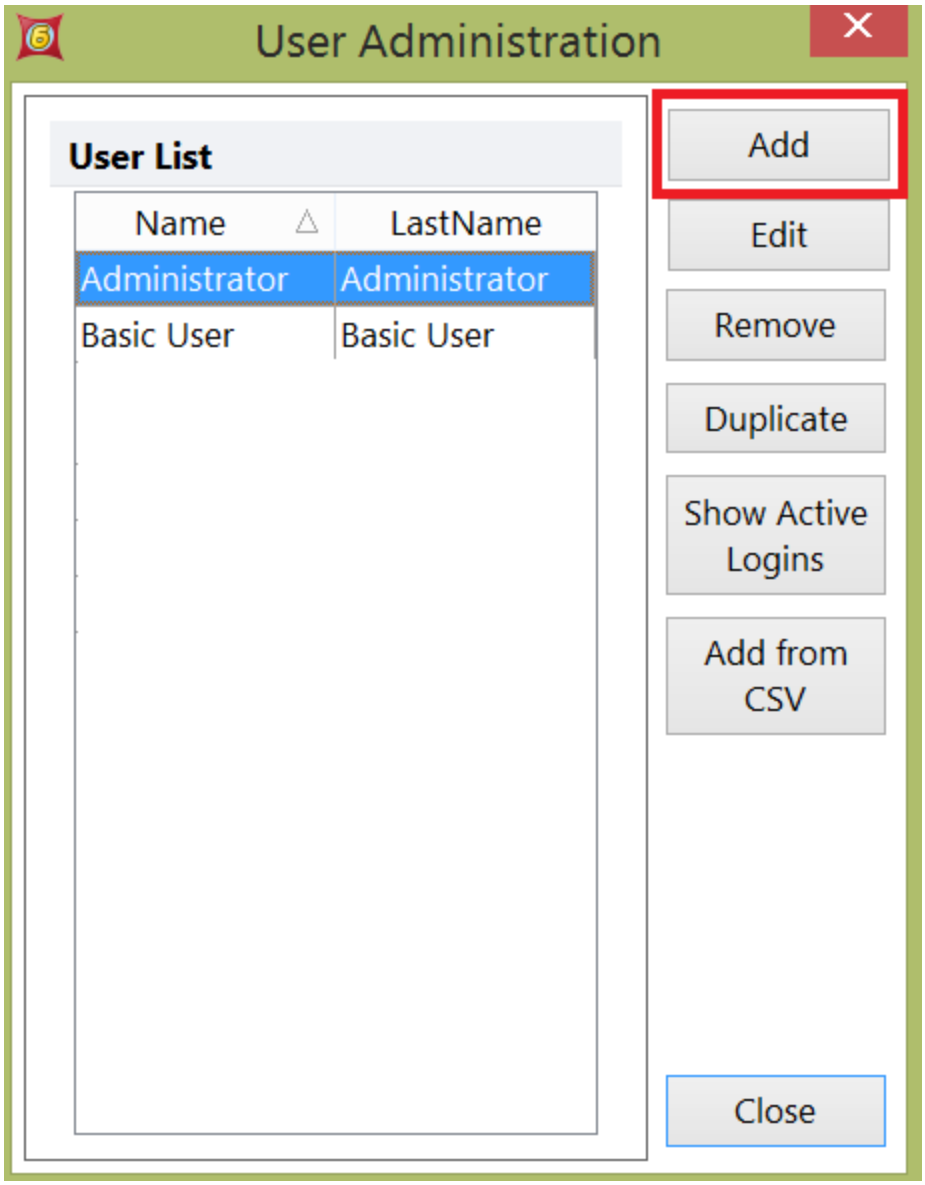

Figure 38.5 The Add button in the User Administration dialog.

The **Edit User Information** dialog will appear (Figure 38.6).

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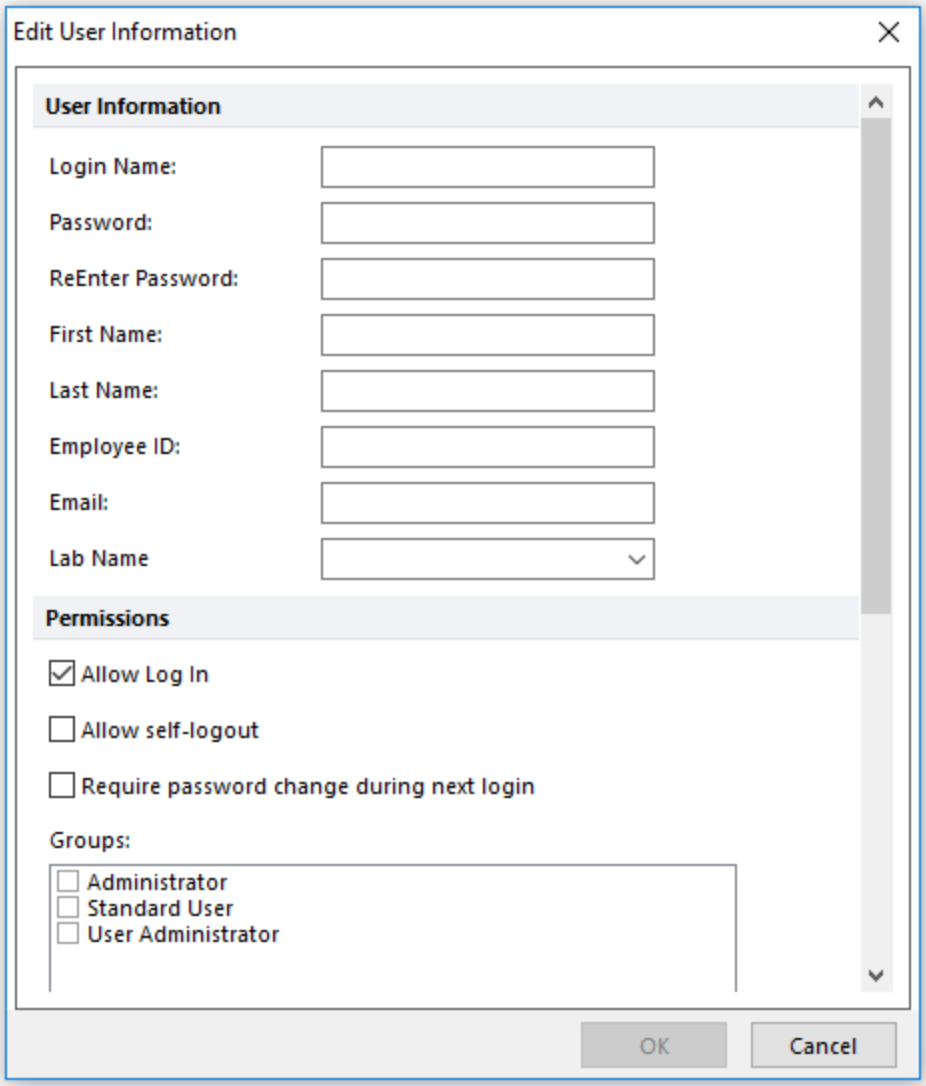

Figure 38.6 Edit User Information Dialog

All of the fields except for **Employee ID** and **Lab Name** are mandatory. The **Login Name** field must be unique (no two users in FCS Express can have the same Login Name). The **Lab Name** field allow to assign the user to a specific lab. Previously inserted Lab Names will be available in the **Lab Name** dropdown menu. The **Lab Name** is used to organize users in the Billing Report 1199.

The **Allow Log In** checkbox determines whether that user can log in. The **Allow self-logout** checkbox determines if a user can log themselves out from a

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remote location. The **Require password change during next login** checkbox will prompt the user to change his or her password during the next login.

At least one option must be selected in the **Groups** box for the user. Customers who hold the Security add-on, can manage Security Groups via the **Security Group Definitions** tools (see Working with Security Groups) 1173. Internet Dongle and Site License customers have the ability to assign the user to one of the following pre-defined user groups:

- **Administrator:** User assigned to this group will be able to use the software to analyze data, to add, edit and delete users, to view Security Reports 1193 and to edit the Security Configuration 1154.
- **Standard user:**User assigned to this group will only be able to analyze data.
- **User Administrator:** User assigned to this group will only be able to to add, edit and delete users, to view Security Reports 1193 and to edit the Security Configuration 1154.

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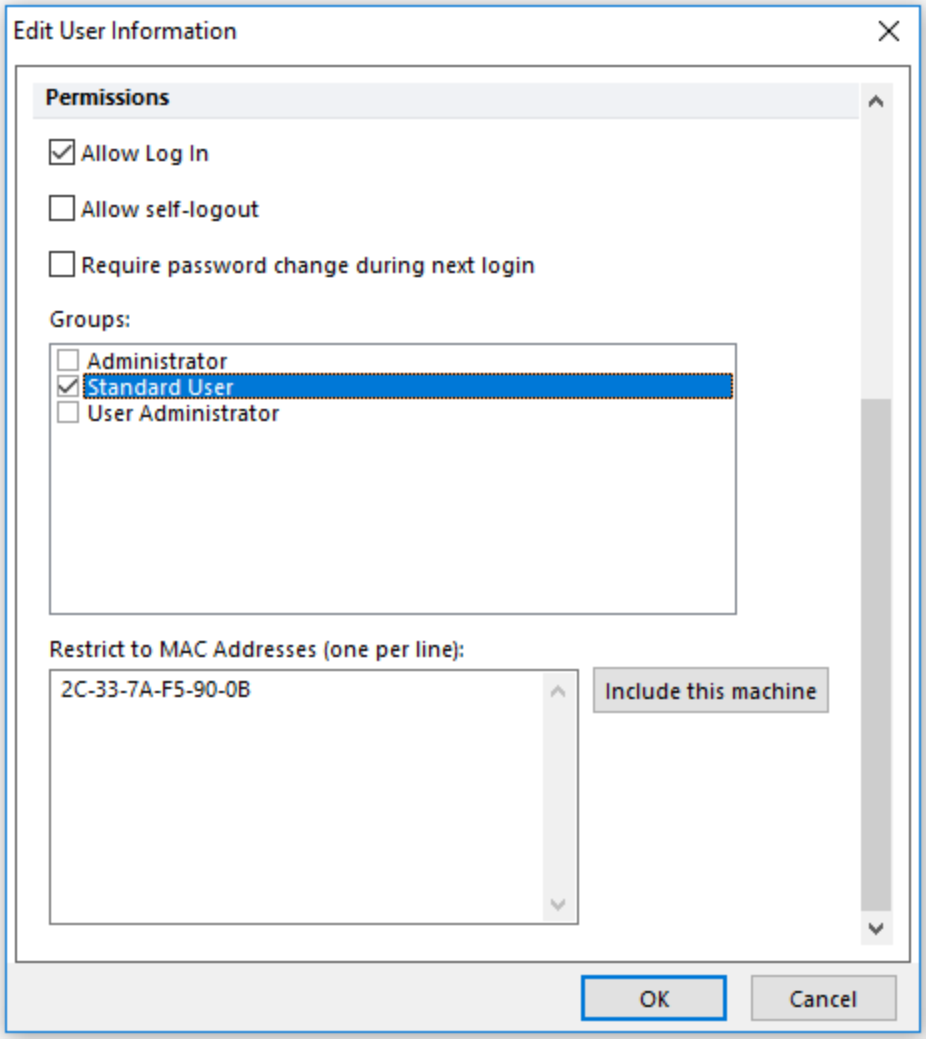

Figure 38.7 Restrict User to Defined MAC Address(es) in User Information Dialog

The user may be restricted to defined MAC address(es) as well. Clicking **Include this machine** button will populate the **Restrict to MAC Addresses (one per line):** box with the MAC address from the computer used to create the user (Figure 38.7).

Multiple user can be automatically added to the database via the **Add from CSV button**. The CSV file can be either a comma-delimited or a semicolon-delimited file and has to contain the following columns:

Column Header	Specific requirements	Example
LoginName	None	JohnSmith
Password	None	password123

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FirstName	None	John
LastName	None	Smith
Email	None	John.Smith@DNS.com
EmployeeID	None	123456
LabName	None	Flow Lab
CanLogin	Enter: T or F or True or False	True
CanLogMyselfOut	Enter: T or F or True or False	F
ForcePasswordExpired	Enter: T or F or True or False	T
SecurityGroups	For internet dongle user types: **Administrator**, **Standard User or User Administrator** (see above for additional details 1149). User group names can be inserted as comma-delimited list. For comma-delimited CSV files, said list has to be surrounded by quotes. If a group name include comma in it, that name has to be included in double quotes for both comma-delimited and semicolon-delimited CSV files. Note: If MS Excel is used to edit the CSV files, user group names can be simply listed as comma-delimited list with a single quote character around any group names that have commas in them.	Standard User or Comma delimited: "Group 1, Group 2, Group 3" or "Group 1, ""Group,2"", Group 3" Semicolon-delimited CSV file: Group 1, Group 2, Group 3 or Group 1, ""Group, 2"", Group 3
RestrictToMACAddresses	N/A	N/A

Example of .CSV import in Excel below. A template for the the .CSV file import may also be downloaded for use.

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	A	B	C	D	E	F	G	H	I	J
1	LoginName	Password	FirstName	LastName	Email	SecurityGroups	EmployeeID	CanLogin	CanLogMyselfOut	ForcePasswordExpired
2	DeNovoSoftware	iLoveFlow	John	Smith	John.Smith@DNS.com	Standard User	338477	T	T	T
3	Adminstrator	FCSExpress	Joe	Flow	joe.flow@dns.com	Administrator	837390	TRUE	FALSE	TRUE

37.3.2 Editing a User

To edit an existing user:

1. Use the **File** tab→**Security**→**User Administration** command.
2. Either double-click on the user, or select the user and click on the **Edit** button in the **User Administration** dialog.
3. Edit the information and Click **OK**.

The currently logged in user can change their own password by selecting the **File** tab→**System**→**Edit Information for Current User** command. The **Edit User Information** dialog will appear, but only the password field will be enabled (Figure 38.8).

Please note that for the User to be able to change their password, the User must have the permission for **Security Options**→**Current User Operations** enabled for the User Group to which they belong.

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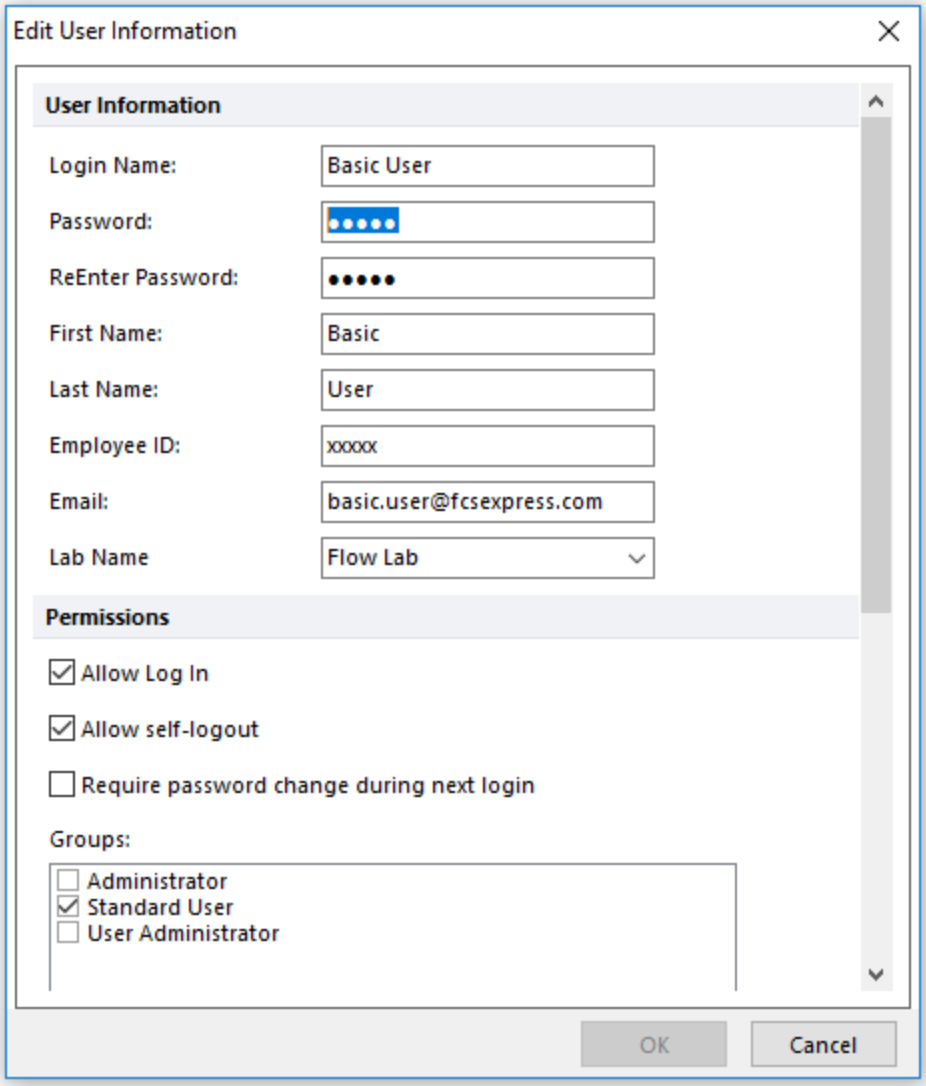

Figure 38.8 Change Current User Password

37.3.3 Deleting a User

To delete a user:

1. Use the **File** tab→**Security**→**User Administration** command.
2. Select one or multiple users, and click on the **Remove** button in the **User Administration** dialog (Figure 38.9).
3. A message will ask for confirmation for the deletion. Click **Yes** to confirm or **No** to avoid deleting the selected user(s).

FCS Express will not allow you to delete the last administrator who can log in.

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Note: You cannot undelete a user, so please use this feature with caution.

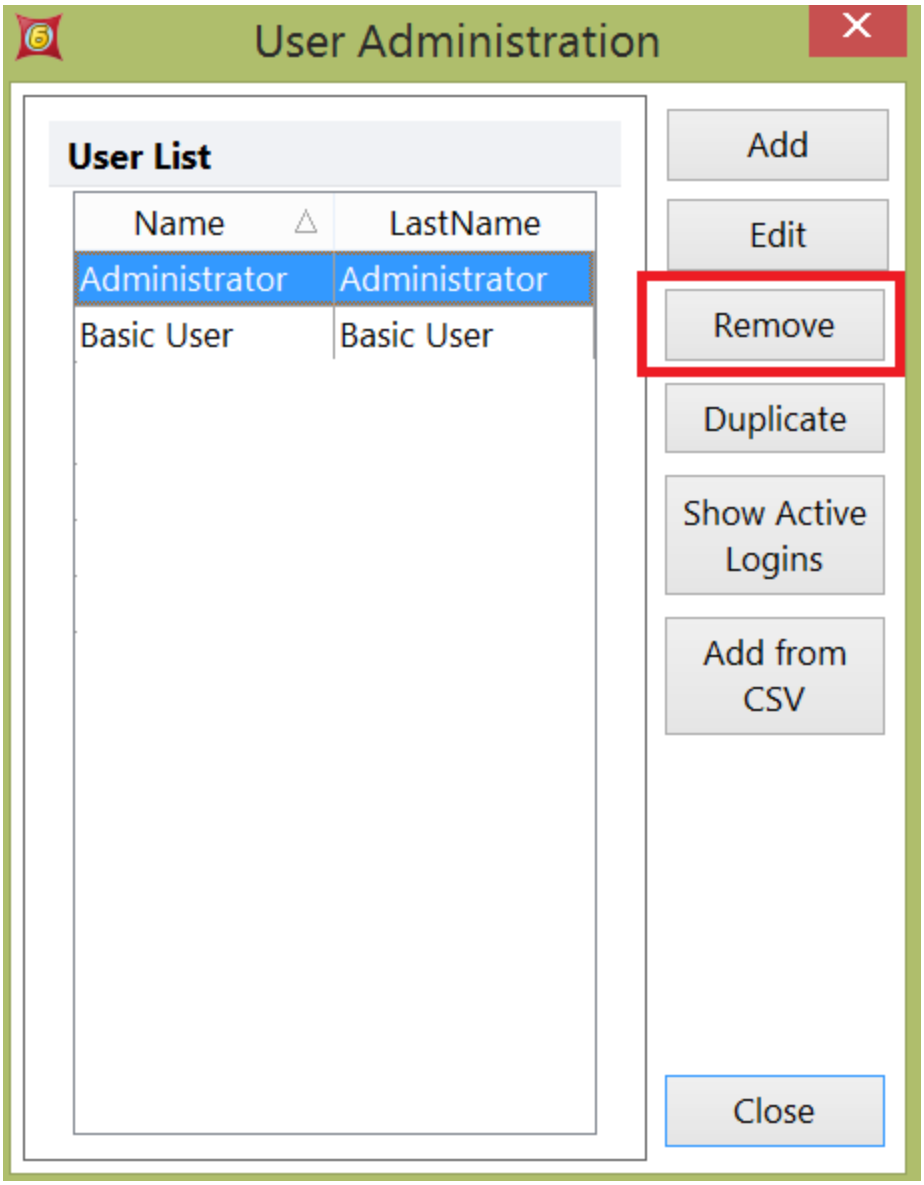

Figure 38.9 Removing a Security User

37.3.4 Editing Security Configurations

Under **File** tab→**Security**→**Edit Security Configuration**, users can find the **Edit Security Configuration** dialog (Figure 38.10) which is used to manage Password Policies[1155], SMTP Servers[1158], E-Mail Notifications[1156], and Online Registration (site license customers only)[1159].

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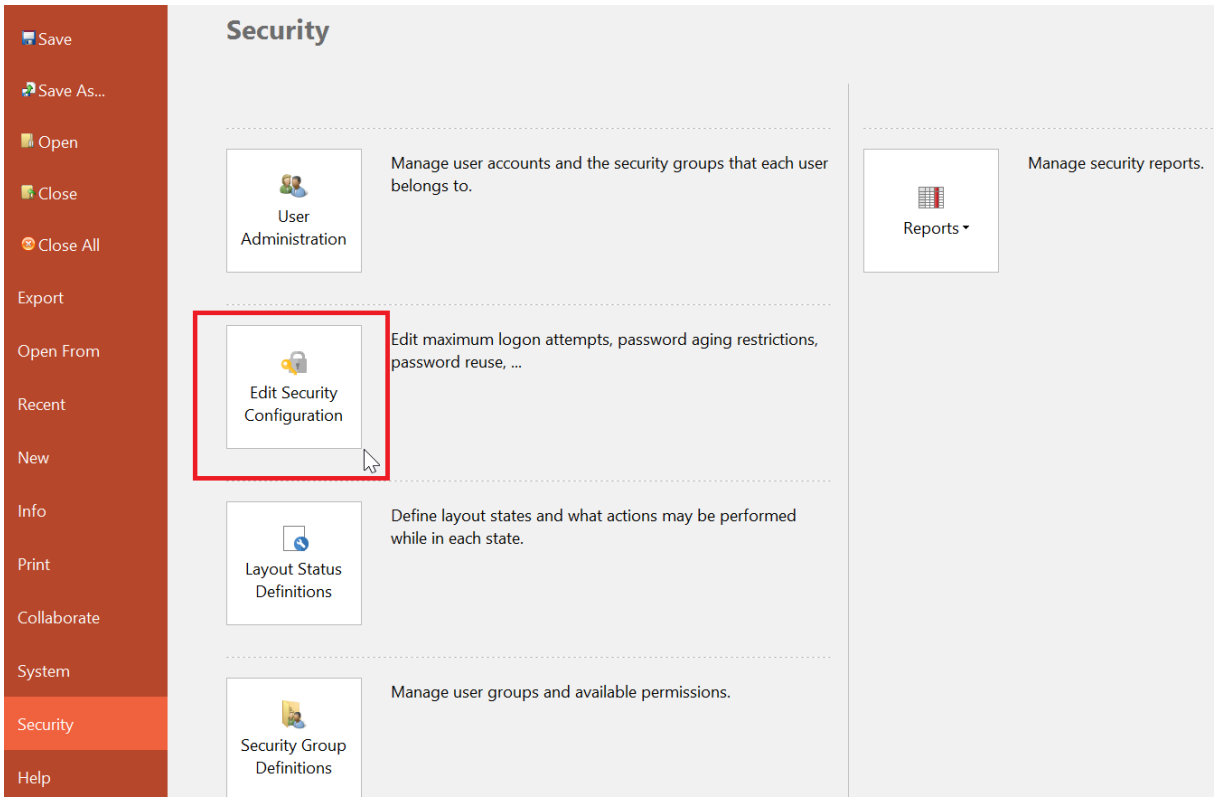

Figure 38.10 Navigating to the Edit Security Configuration dialog

37.3.4.1 Managing Password Configurations

Note: In previous releases FCS Express used an XML file to manage password configurations. Upon updating and running the latest release of FCS Express for the first time the values from your XML file will automatically be imported and the original XML file will then be ignored.

To manage your **Password Policies** navigate to the **File** tab→**Security**→**Edit Security Configuration**.

The **Edit Security Configuration** dialog will appear as in Figure 38.11 below.

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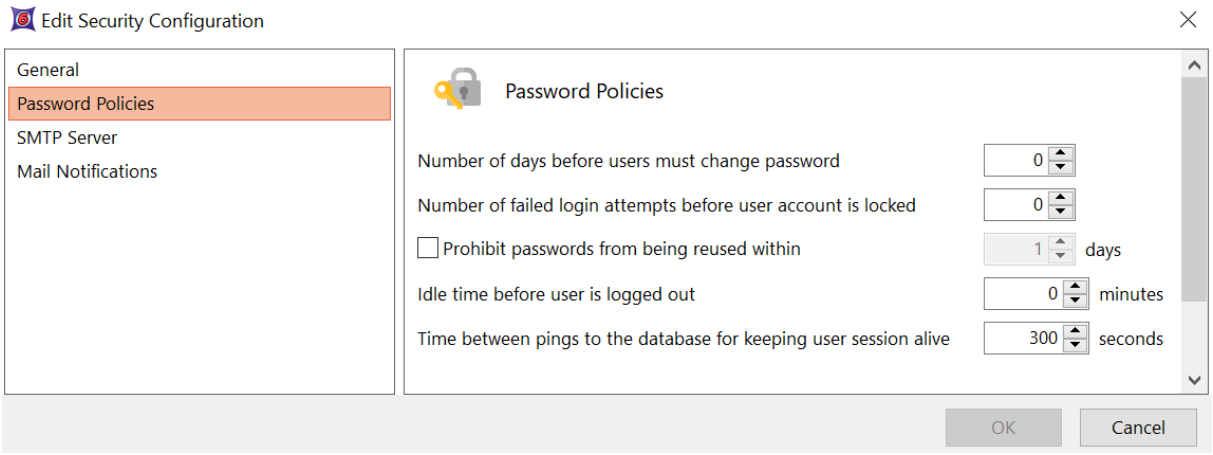

Figure 38.11 The Password Policies Dialog

The options for password policies allow you to define:

Password Policy Option	Notes
Number of days before users must change password	A value of 0 (zero) means the user never has to change their password.
Number of failed login attempts before user account is locked	A value of 0 (zero) indicates the account will never lock upon any number of failed attempts
Prohibiting passwords from being reused	Checking this option will determine the number of days before a password may be reused. A value of 0 (zero) means that the user can reuse their password at any time.
Idle time in seconds before user is logged out	A value of 0 (zero) indicates the user will never be logged out upon software idling
The time between pings to the database for keeping user session alive	Determines how often an FCS Express session communicates back to the server that it is still active. When a new user logs in, the software checks for any sessions that have not communicated back within this period and cancels that session. Smaller values indicate that a session can be reused more quickly in the event of an unexpected program halt (such as a power failure or crash). The maximum value is 300 seconds.

Once your **Password Policies** are defined click **OK** to apply them or continue to setup the SMTP Server 1158 or Mail Notifications 1156.

37.3.4.2 Mail Notifications

The **Mail Notification** dialog (Figure 38.12) can be accessed through the **File** tab→**Security**→**Edit Security Configuration**→**Mail Notifications**.

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Mail Notifications allow notifications to be sent to a specific email address when certain events happen within FCS Express. A list of available notifications can be found in the table below.

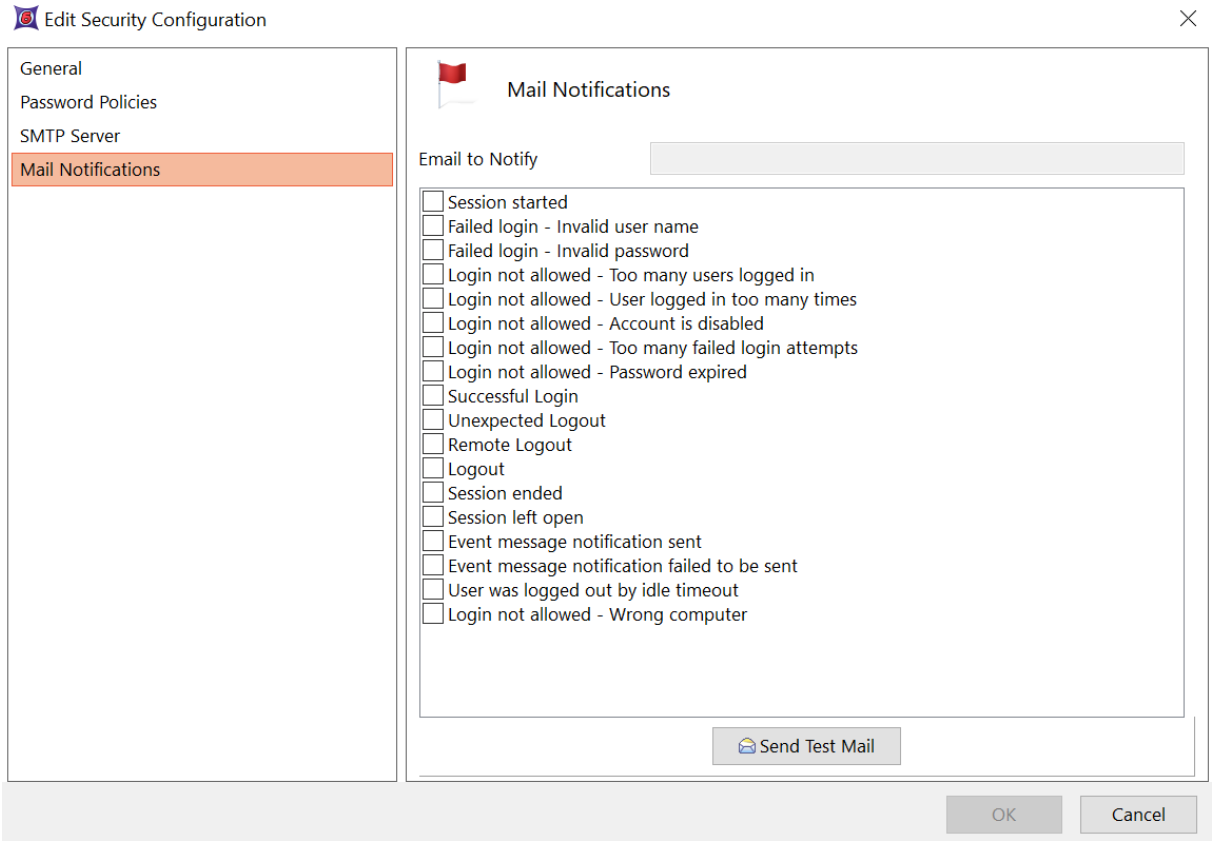

Figure 38.12 Mail Notifications Dialog

To setup a mail notification:

1. Configure your SMTP Server 1158.
2. Check boxes for each notification trigger you would like to be notified about.
3. Enter the email address to receive the notification in the **Email to Notify** box.
4. Click on **Send Test Mail** to confirm your selection.
5. Click **OK**.

When a notification is triggered you will receive an email at the specified email address.

Available Notifications:

Mail Notification	Notes
FCSE session started	Notification when the software is started

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Invalid UserName	Notification when an invalid User Name is entered
Invalid Password	Notification when an invalid Password is entered
Too many users	Notification when a user tries to log in and there were too many other users already logged in
Too many users in the same account	Notification when a user tries to log in and there are too many users are logged into the same account
User account is disabled	Notification if a user account is disabled because of too many invalid login attempts
Too many failed login attempts	Notification if the number of failed login attempts allowed is exceeded
Password expired	Notification if a password is determined to be expired
Successful Login	Notification of a successful login
Unexpected Logout	Notification of an unexpected logout. This occurs if the program shuts down unexpectedly and the sessions times out.
Remote Logout	Notification if a user is logged out remotely by another user
Logout	Notification of a regular logout
FCSE session ended	Notification if FCSE session is ended
FCSE session left open	Notification if FCSE session is left open
Event message notification sent	Confirmation notification of message sent
Event message notification failed to deliver	Notification when a message fails to deliver
User was logged out by idle timeout	Notification if user was automatically logged out by idle timeout

37.3.4.3 SMTP Server

The **SMTP Server** dialog (Figure 38.13) can be accessed through the **File** tab→**Security**→**Edit Security Configuration**→**SMTP Server**.

Enter the SMTP Server information for your preferred email provider in this category to allow Mail Notifications 1156 to be sent out.

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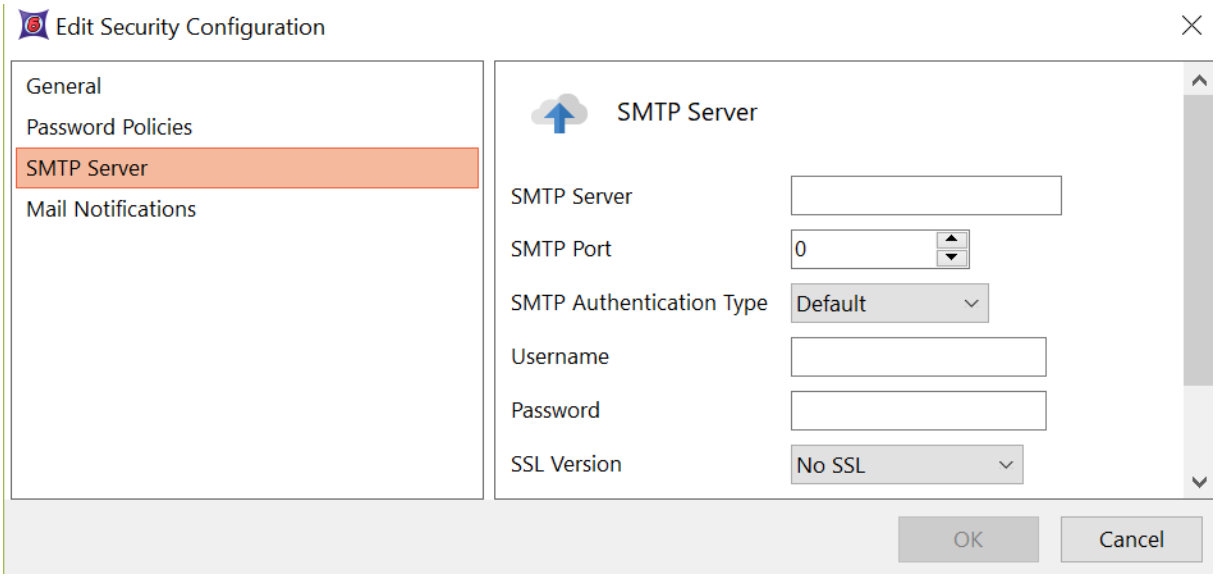

Figure 38.13 The SMTP Server Dialog

Note that if you configure your SMTP server to use SSL, then you will need to make sure that there are SSL libraries in your computer's system Path, or in the same folder as FCS Express.exe. You can download and extract the OpenSSL package for this purpose. Links to download it are available from https://www.openssl.org/ . The only two libraries that are required from OpenSSL are libeay32.dll and libeay32.dll.

37.3.4.4 Online Registration

The **Online Registration** dialog (Figure 38.14) can be accessed through the **File** tab→**Security**→**Edit Security Configuration**→**Online Registration**.

The Online Registration dialog will only be available to you if you are an FCS Express Internet Dongle or Site License customer and have Administrator privileges assigned 1149 to your user in FCS Express.

Online registration will allow you to set up a unique webpage customized to your institutions needs for allowing FCS Express end user registration. Registration can be set up to allow users to register with or without approval. Multiple levels of approval may be assigned and custom registration email messages may be created directly within FCS Express.

To enable Online Registration for your users:

1. Click on the **General** category in the **Edit Security Configuration** dialog.
2. Enter the name of your institution. The name entered here will appear on your custom registration web page as in the image below where "**Your Institution Name Here**" was entered.

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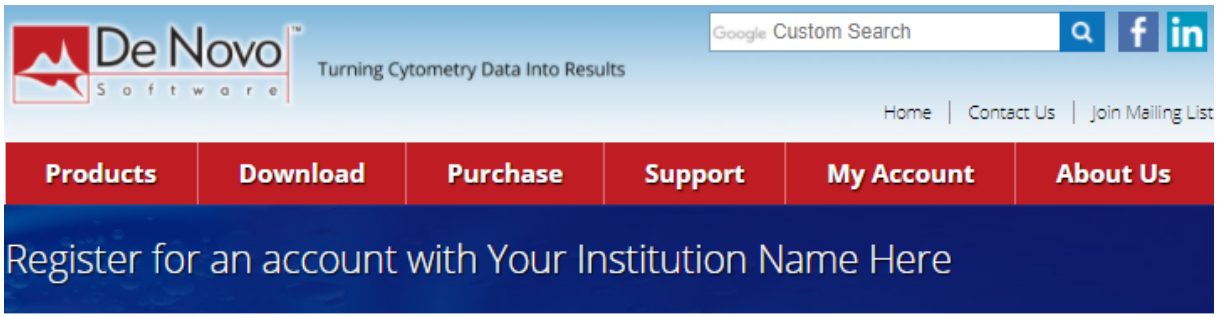

3. Check the box for **Allow new users to be registered through the De Novo Software website**.
4. Click on or copy the web address provided to access the web page (please note it will not be active until you click **OK** in the next step).
5. Click **OK** to save your changes.

The web link provided is unique to your license. You can distribute the web link to any prospective users of FCS Express to allow them to register as an end user of your license. There are a number of customizations including access controls and custom messages that are outlined in the table below.

All emails sent to users, approvers, and admins will originate from denovosoftware@denovosoftware.com. Please ensure your institution and users can receive messages from that email address. The inbox for denovosoftware@denovosoftware.com is not monitored, please do not reply directly to this email address but use support@denovosoftware.com.

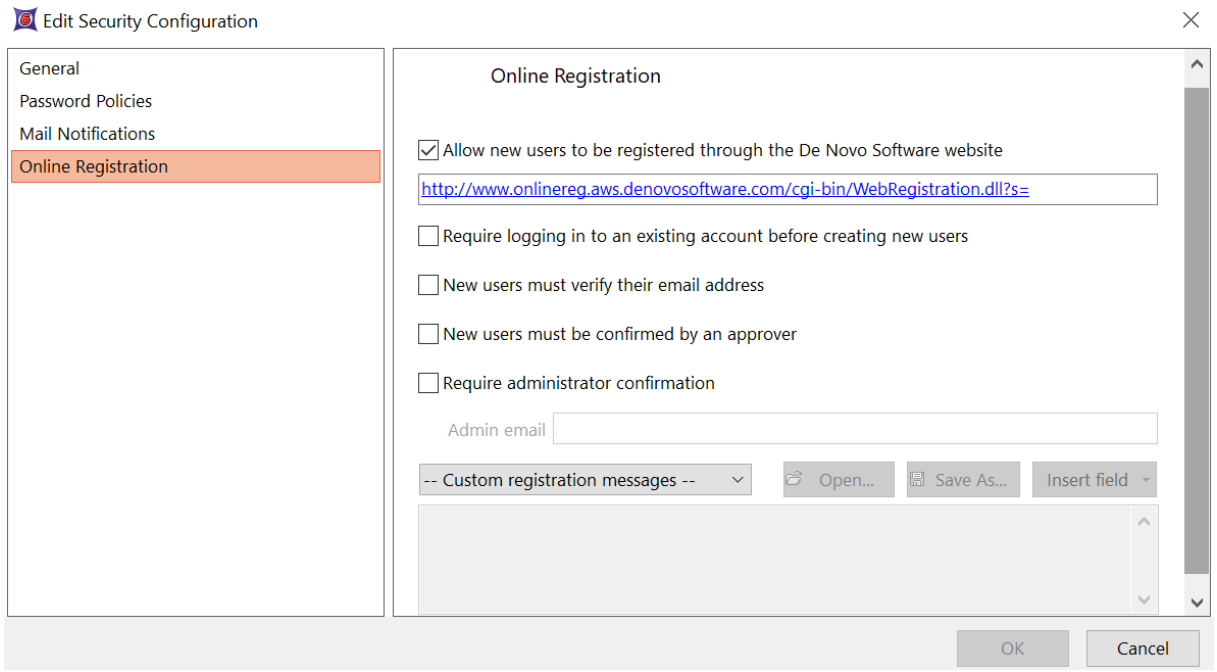

Figure 38.14 - The Online Registration dialog in FCS Express is available to site license adminstrators.

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Option	Action when checked
Allow new users to be registered through the De Novo Software website	Makes a website active that is unique to and associated with your license so end users can self-register. The link provided in the dialog may be provided to end users to self register.
Require Logging in to an existing account before creating new users	When attempting to register, a log in screen will first be presented. A license user with Administrator or User Administrator rights 1149 must enter their credentials to access the registration form. The option is useful if you would like to have only an administrator be able to create end users on the license without having to access FCS Express directly.
New users must verify their email address	When new users register for a license user they are first sent an email to the email address they provided in the form with a link to validate that the email address is active. User must click on the verification link before their user may be approved.
New users must be confirmed by an approver	When new users register a field will be present on the registration form for "**Approval email address**". The email address associated with the approval email address field will be sent an email message to approve the user via a web link. If Require administrator confirmation (below) is not checked, the end user will be approved immediately. If Require administrator confirmation is checked, a subsequent approval request will be send to the license admin email.
Require administrator confirmation	When checked the email address entered in the **Admin email** field will be sent an email message to approve the user via a web link.

Website Registration Form and Customizations

The website form for your institution (Figure 38.15 - left) will minimally contain the following fields that when filled out will map to the Edit User Information dialog 1147 and create a license user on your license. Fields for Employee ID and Lab name are optional.

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Note that the Approval email address* field will be displayed only when choosing the option (figure 38 below and right) for "**New users must be confirmed by an approver**" in the Online Registration options. The Approval email address should be the email address of the person who will be providing approval of the user. If only "**New users must be confirmed by an approver**" is checked, they will be the final line of approval for user creation. If "**Require administrator confirmation**" is also checked then the **Approval email address** will first be messaged for approval before sending a subsequent message to the **Admin email** for final approval.

- Login Name
- Email Address
- Password
- Re-enter password
- First Name
- Last Name
- Employee ID
- Lab Name
- Approval email address*

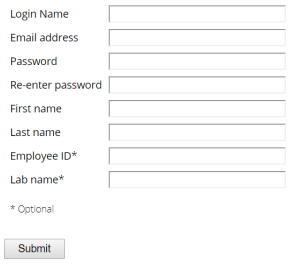

Figure 38.15 - The website form in a web browser. No additional options for confirmation applied.

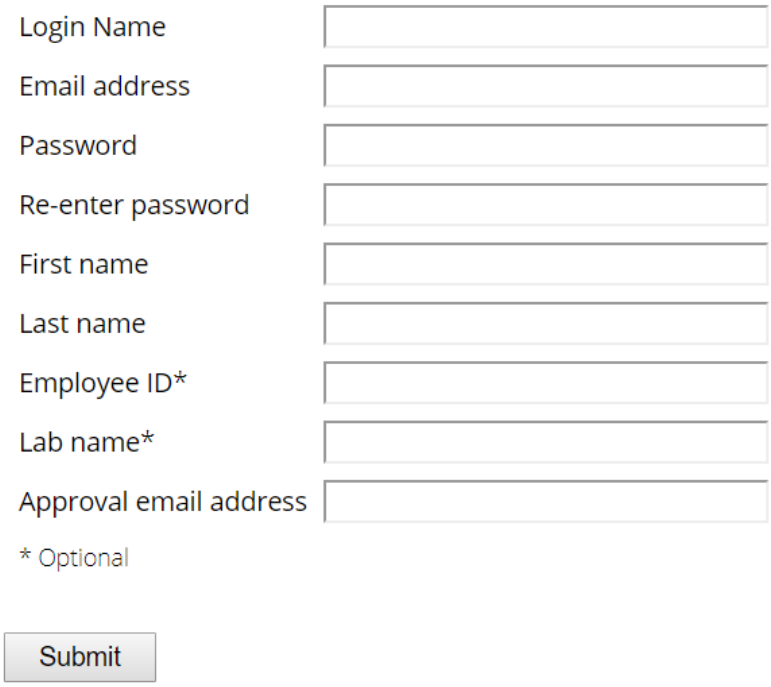

Figure 38 - The website form in a web browser with Approval email address displayed after checking option for "New users must be confirmed by an approver" in the Online Registration options.

If **Require Logging in to an existing account before creating new users** has been checked in the **Registration Options** the web page will first display the log in screen as seen in figure 38.16. A license user with Administrator or User Administrator rights 1149 must enter their credentials to access the registration

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form. The option is useful if you would like to have only an administrator be able to create end users on the license without having to access FCS Express directly.

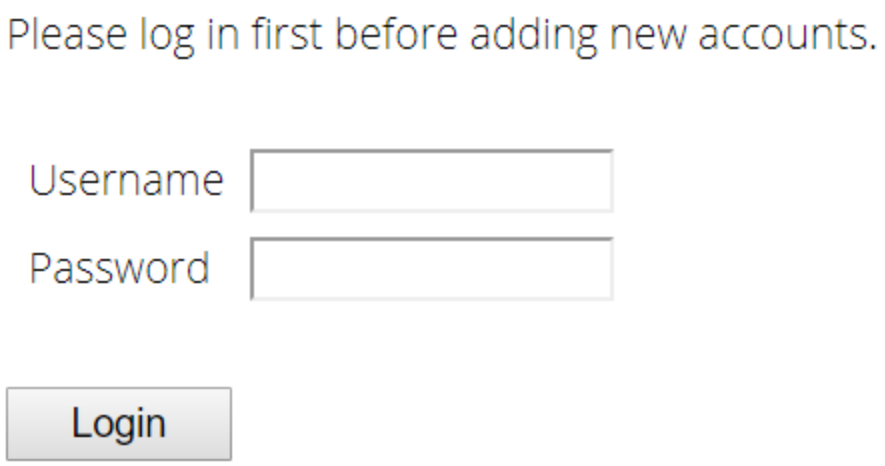

Figure 38.16 - The log in screen presented on the web page if "Require Logging in to an existing account before creating new users" is checked.

Custom Registration Messages

Custom registration messages can be defined for the web form and emails sent at various points in the registration process. Custom registration messages may be set by clicking on the **-- Custom registration messages --** drop down as seen in figure 38.17 below.

- The **Open** button will allow you to import a message as a text file.
- The **Save As** button will allow you to save a message as a text file.
- The **Insert Field** drop down will allow you to automatically populate the message via a field from the User's information entered in the form (figure below-right). For instance, if users have questions regarding their account you might consider including the Administrator email address in the User registration complete message. Or if an approver needs to easily access the Lab name or requesting users email address it may be included in the Approver and Administrator confirmation emails.

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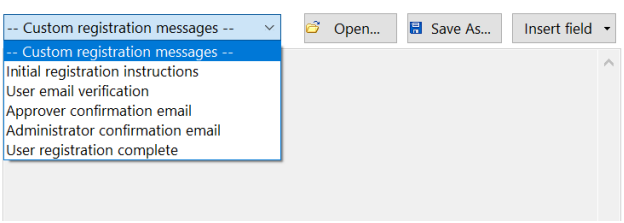
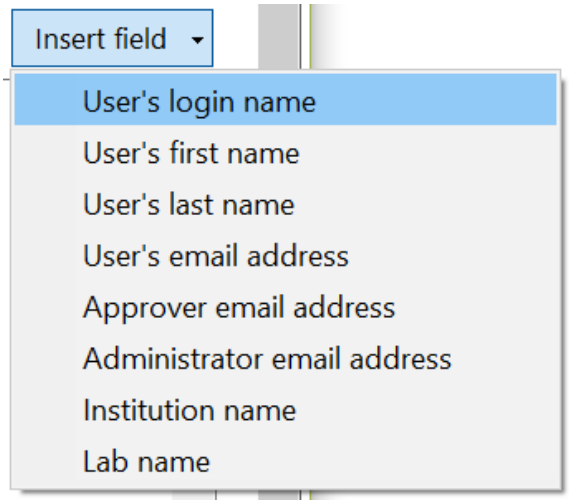
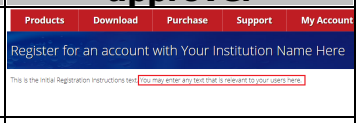


38.17 - The custom registration message dialog.



Custom Registration Message Option	Dialog box and text in FCS Express	Form on custom web page or email delivered to end user / approver / admin approver
Initial registration instructions	Initial registration instructions Open... Save As... Insert field This is the Initial Registration Instructions text. You may enter any text that is relevant to your users here.	
User email verification	User email verification Open... Save As... Insert field Please verify you email address.	**Email sent to user/approver (yellow indicates location of custom text)** First Name Last Name, Thank you for registering for an FCS Express account at Your Institution Name Here. **Please verify you email address.** Please confirm your email address by clicking this link:
Approver confirmation email	Approver confirmation email Open... Save As... Insert field Enter text for your Approver confirmation email.	**Email sent to user/approver (yellow indicates location of custom text)** First Name Last Name wants to register a new FCS Express

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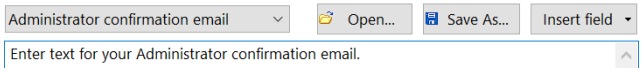
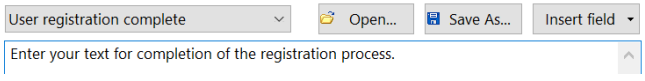
Custom Registration Message Option	Dialog box and text in FCS Express	Form on custom web page or email delivered to end user / approver / admin approver
		account, with you as their reference. Account name: CellDye Email address: email address here Billing code: Enter text for your Approver confirmation email. To approve this registration, please click this link:
Administrator confirmation email		**Email sent to user/approver (yellow indicates location of custom text)** First Name Last Name wants to register a new FCS Express account. Account name: CellDye Email address: email address here Billing code: Approved by: email address here Enter text for your Administrator confirmation email. To approve this registration, please click this link:
User registration complete		**Email sent to user/approver (yellow indicates**

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Custom Registration Message Option	Dialog box and text in FCS Express	Form on custom web page or email delivered to end user / approver / admin approver
		location of custom text) First Name Last Name, Your FCS Express account "CellDye" is now active. You can log in at any time with the password you created during registration. Enter your text for completion of the registration process.

37.3.4.5 LDAP and Single Sign On

Lightweight Directory Access Protocol (LDAP) (also known as Active Directory or Directory Server) with Single Sign On capability is currently available in FCS Express. To request access and pricing for LDAP integration please contact support@denovosoftware.com. LDAP in FCS Express is only supported for Simple Bind configurations.

The **LDAP** dialog (Figure 38.18) can be accessed through the **File** tab→**Security**→**Edit Security Configuration**→**LDAP** tab.

If you are attempting to edit the security configuration for LDAP you must be connected to the LDAP server.

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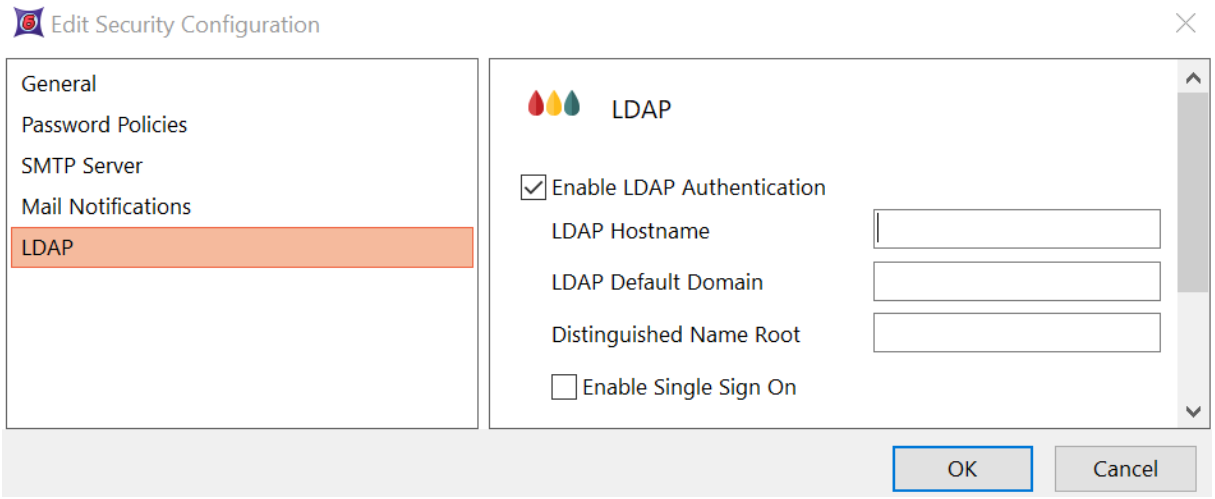

Figure 38.18 The LDAP Dialog

LDAP Authentication may be configured via the LDAP dialog via the options listed in the dialog and as follows:

- When the check box for **Enable LDAP Authentication** is checked FCS Express will attempt to auto fill the **LDAP Default Domain** and **Distinguished Name Root**. If a different **LDAP Default Domain** and **Distinguished Name Root** are required you may enter them manually.

- The **LDAP Hostname** must be entered by the end user setting up LDAP.

- The **Enable Single Sign On** check box will control if Single Sign On should be used by FCS Express when opening the software.

    - If **Enable Single Sign On** is **unchecked**, then the user will be prompted via a log in dialog to enter their credentials.

        - The user name may be entered as the domain user name or with the domain plus user name. For instance, JQFlow or FlowCytometry\JQFlow where JQFlow is the user name and the domain is FlowCytometry.

    - If **Enable Single Sign On** is **checked**, and the end user of FCS Express is assigned as a user of FCS Express, the end user will not be prompted to log into FCS Express as long as they are already signed on to the system.

        - If a user would like to force a sign in prompt, for instance, to switch to a different domain user to access FCS Express, you may hold down the **Control**, or **Alt**, or **Shift** key immediately after running FCS Express until the log in dialog appears.

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- Users may also force a sign in prompt if they switch to another user by logging out of FCS Express. To logout, click on the **Logged in as:** drop down in the bottom bar of FCS Express (Figure 38.19) or from the **File** tab→**System**→**Current User**→**Logout Current User.**

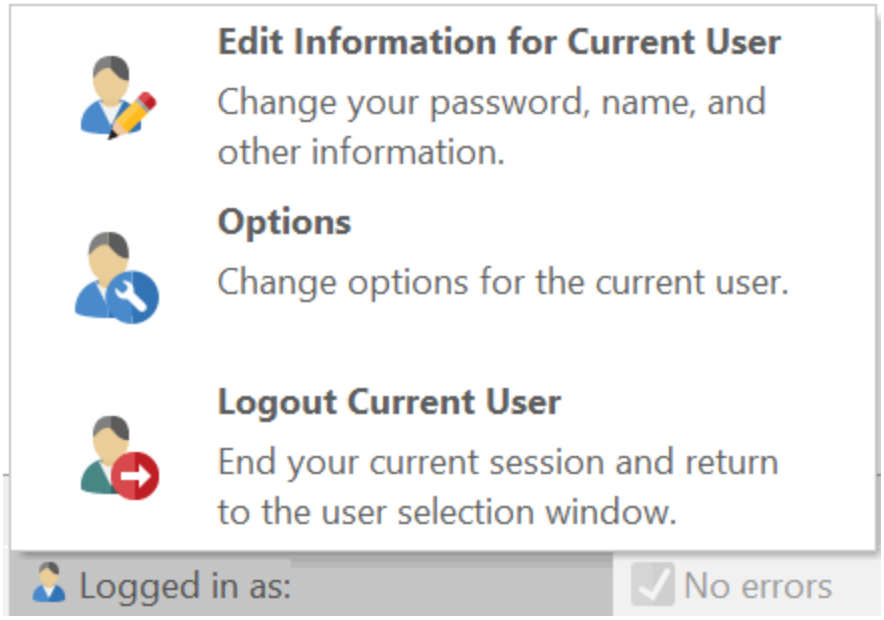

Figure 38.19 Logging out via the logout current user drop down in the bottom bar of FCS Express.

See the next chapter for **Creating and Administering LDAP Users**[1168].

37.3.4.5.1 Creating and Administering LDAP Users

FCS Express users may be added from the LDAP server from the **File** tab→**Security**→**User Administration**→**Add from LDAP** button (Figure 38.20).

Users added via LDAP may utilize all of the security group[1173] and electronic signature[1088] features available in FCS Express.

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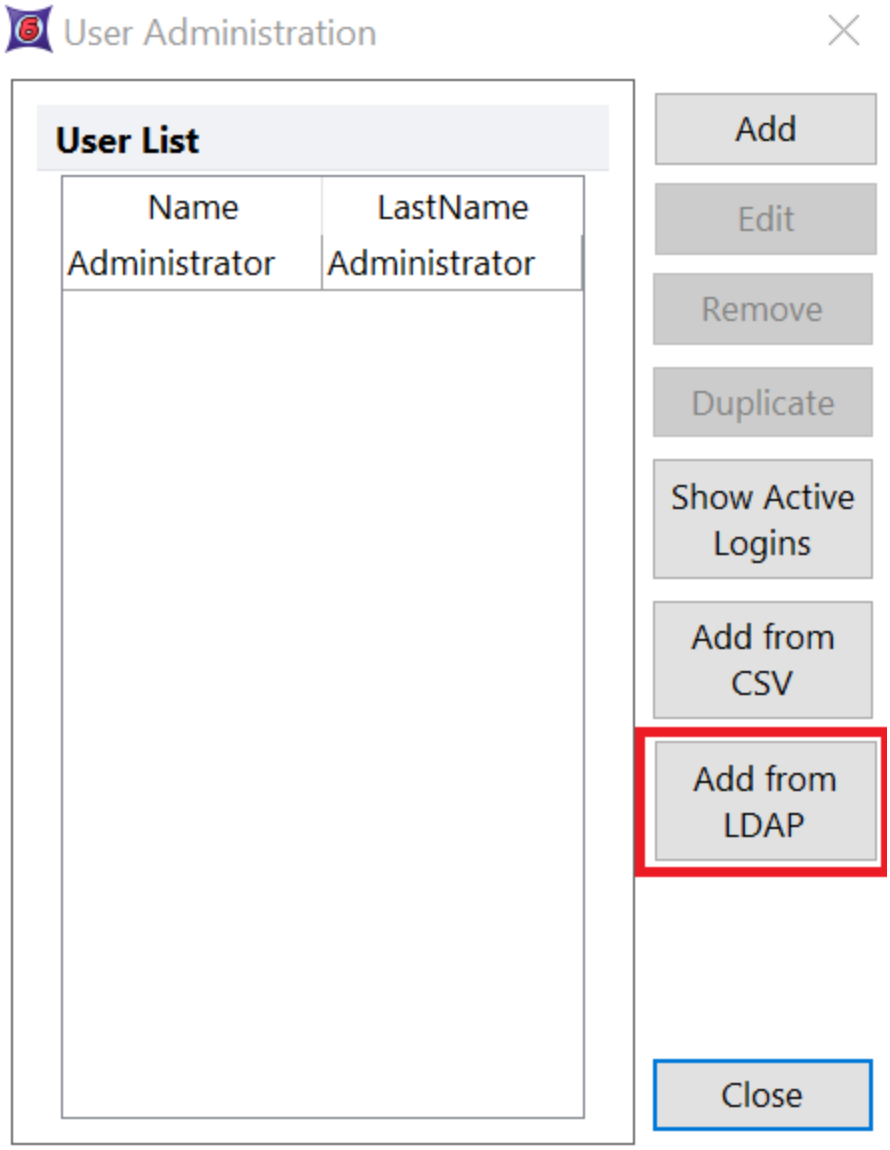

Figure 38.20The User Administration Dialog with Add from LDAP highlighted.

Click on the **Add from LDAP** button to launch the **Select Users** dialog (Figure 38.21).

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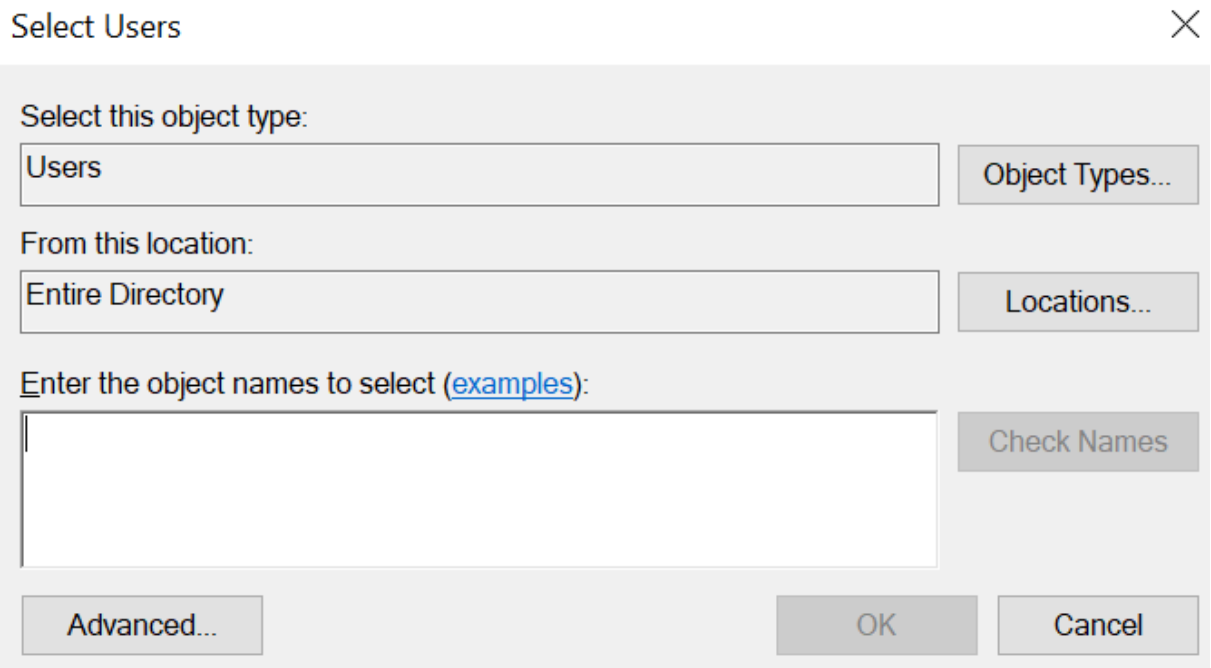

Figure 38.21The Select Users dialog for LDAP user selection in FCS Express.

The **Select Users** dialog will all you to add users by entering the user names in the **Enter the object names to select (examples):** field.
The field is searchable by clicking on the **Check Names** button after entering text. For instance, if a user on the system existed as joeflow@cytometry.com you could simply type *Joe* and click **Check Names** to find the user. If multiple users appeared in the search a **Multiple Names Found** dialog will appear allowing you to select one or multiple users.

If multiple users need to be added to the system at the same time you may separate user names or search terms with a semi-colon. For instance, *Joe;Jane* will search for any users in the system by those names. If users are found, the full user name will appear separated by a semi colon in the dialog. If multiple users are selected in the **Multiple Names Found** dialog they will appear in the **Enter the object names to select (examples):** dialog separated by a semi-colon.

An Advanced user selection dialog (Figure 38.22) may be launched by clicking on the **Advanced...** button which will allow you to use additional tools to search for and select users.

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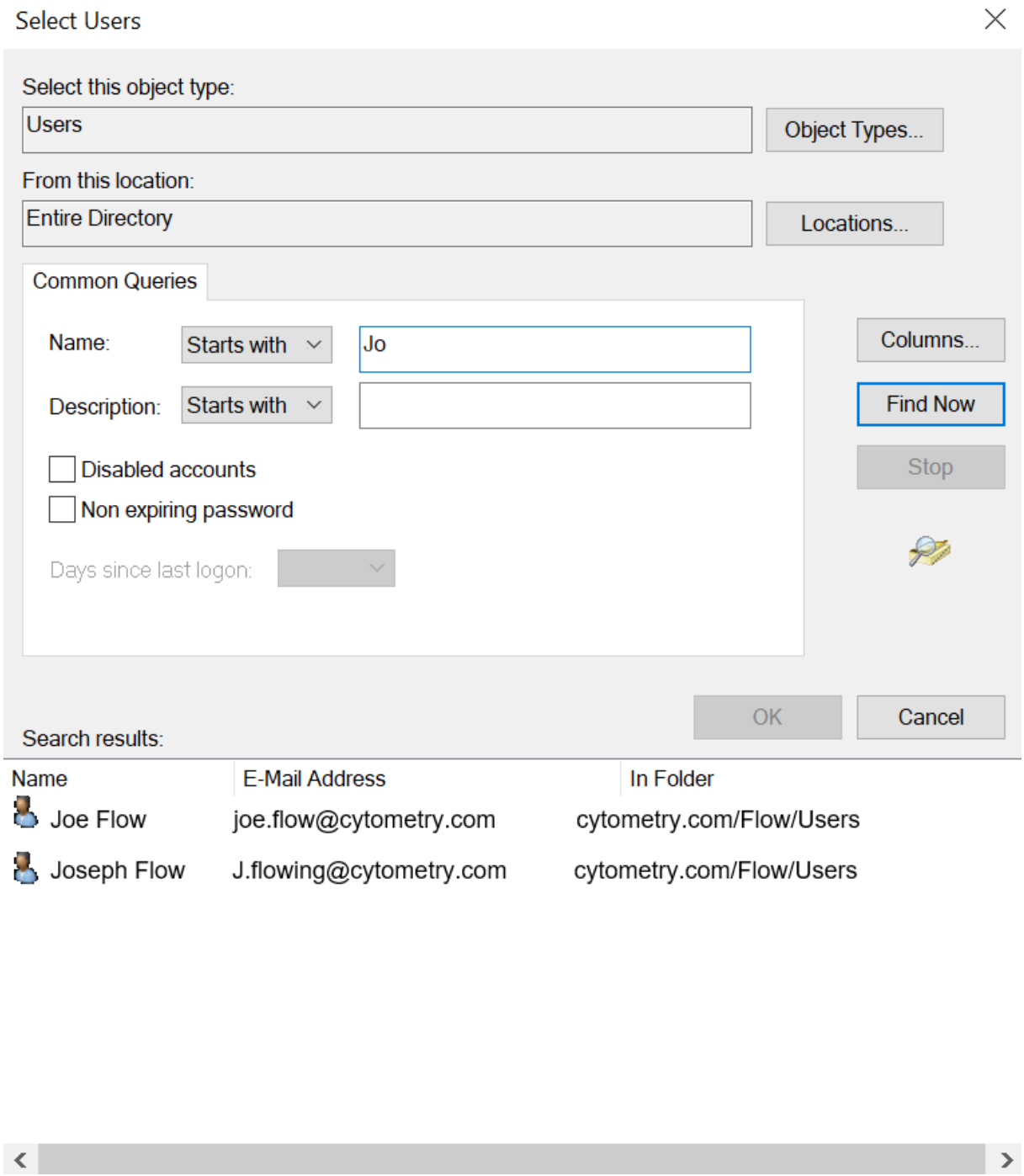

Figure 38.22The advanced Select Users dialog.

Once a user is selected and added all fields except **Employee ID** and **Lab Name** will be grayed out since they are being populated by LDAP (Figure 38.23). If a change is made to the user via LDAP, for instance, the password has changed, FCS Express will only update after the user logs out and logs back into FCS Express.

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Please note that **Permissions** and **Groups** for each user added to FCS Express via LDAP must be updated manually when adding a single user, or by selecting the user from the **User Administration→User List** and clicking on the **Edit** button when adding multiple users.

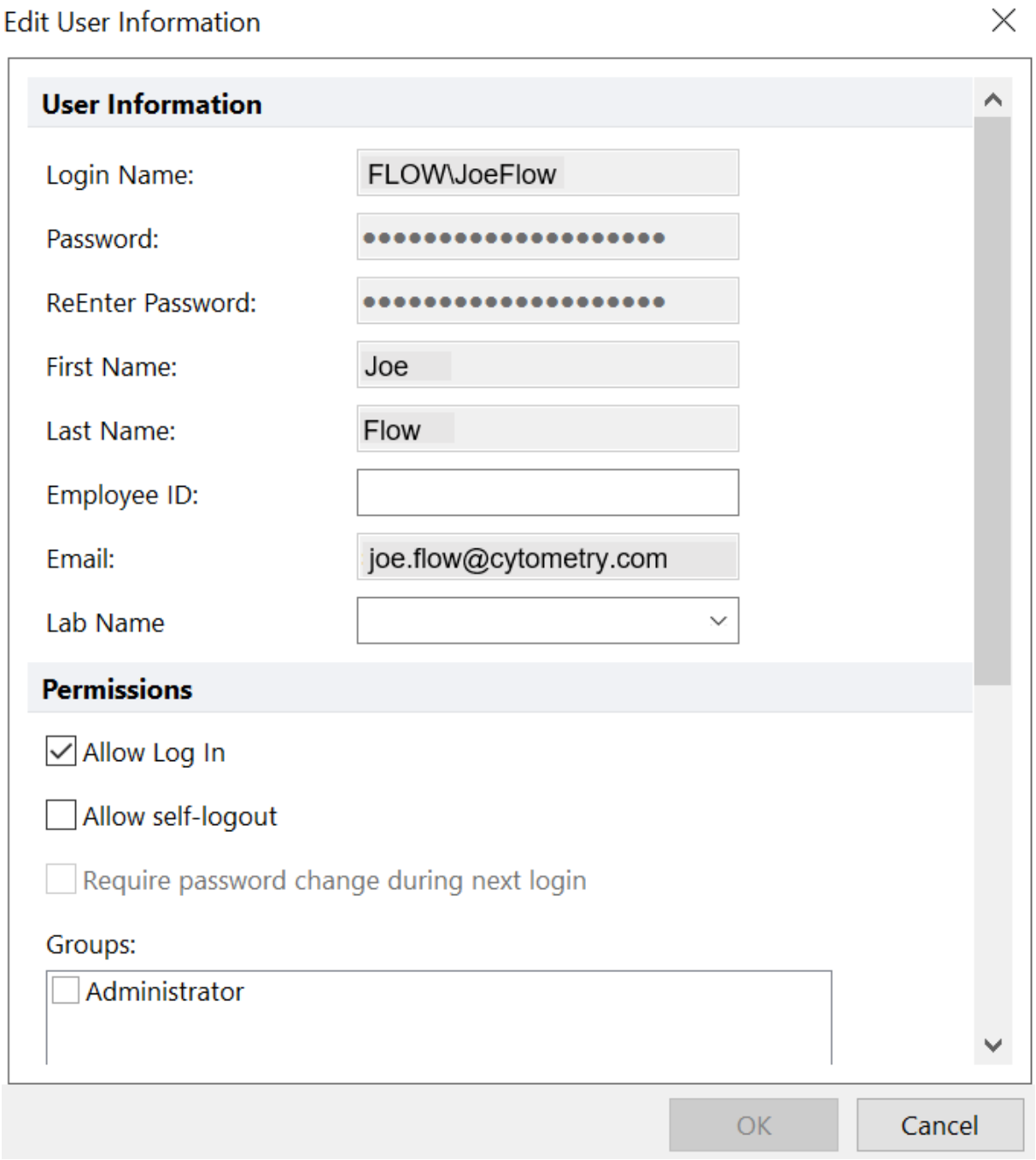

Figure 38.23The Edit User Information dialog after a single user has been added via LDAP.

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37.4 Working with Security Groups

Every action in FCS Express has a corresponding Permission. Security Groups contain lists of Permissions that are allowed to be performed. The Permissions are grouped in logical categories, as shown in Figure 38.24.

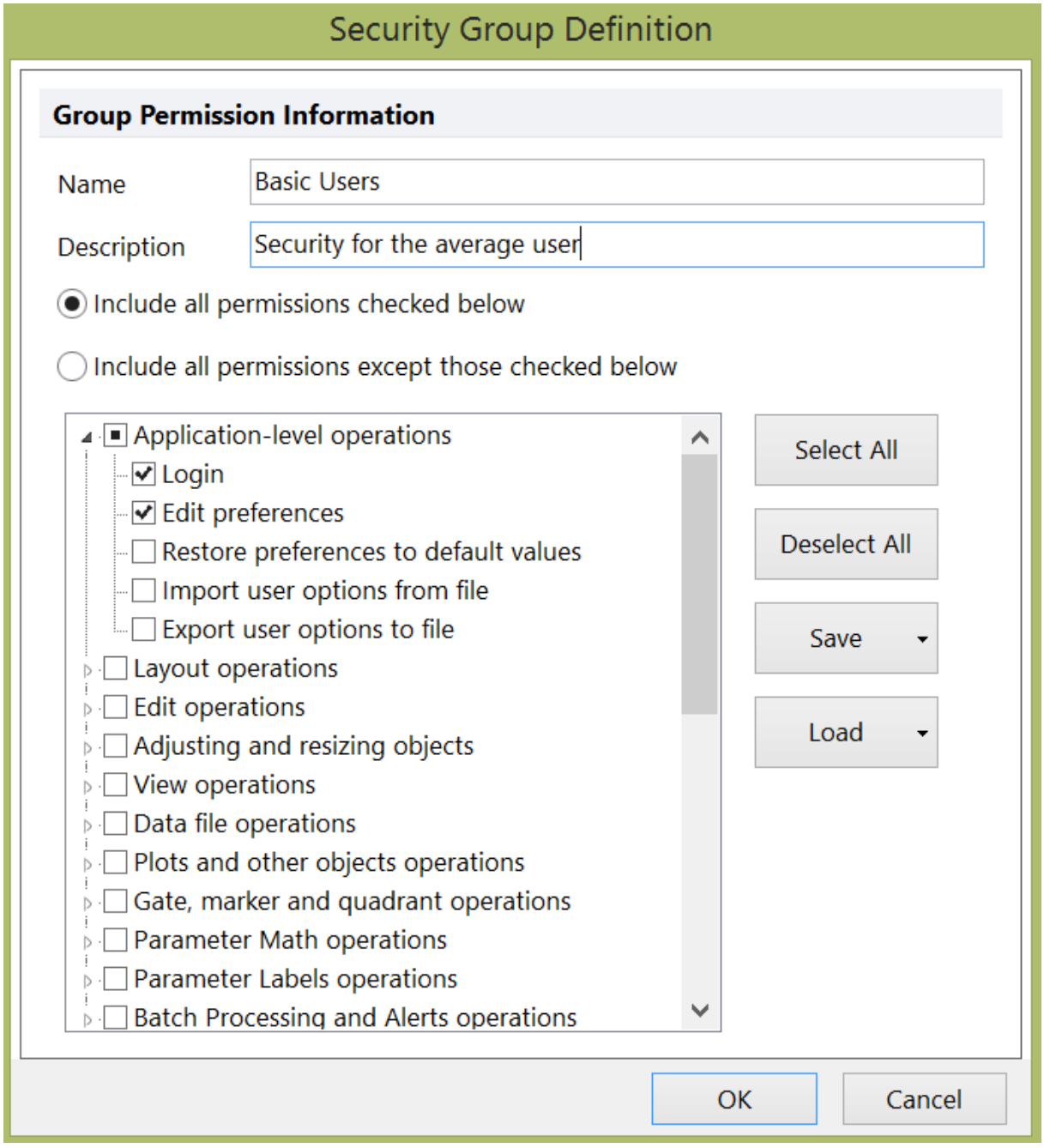

Figure 38.24 Security Group Definition Dialog

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You can choose to include all checked permissions, or, if you only want to exclude a few permissions, include all permissions except those checked in the list. Once a Security Group is created, it can be assigned to a user 1152. The user will then only be allowed to perform the actions for which they have permission.

FCS Express comes with the Administrator group, which permits all actions, and cannot be deleted.

The following example illustrates how permissions behave when a user logs in:

A user is assigned to a Group which can perform all actions, except for viewing the security group list. The user logs in to FCS Express. They are able to load a layout, and make changes to it. However, when the user attempts to change the security group list, they will see the message shown in Figure 38.25. This notifies the user that they are unable to perform the action.

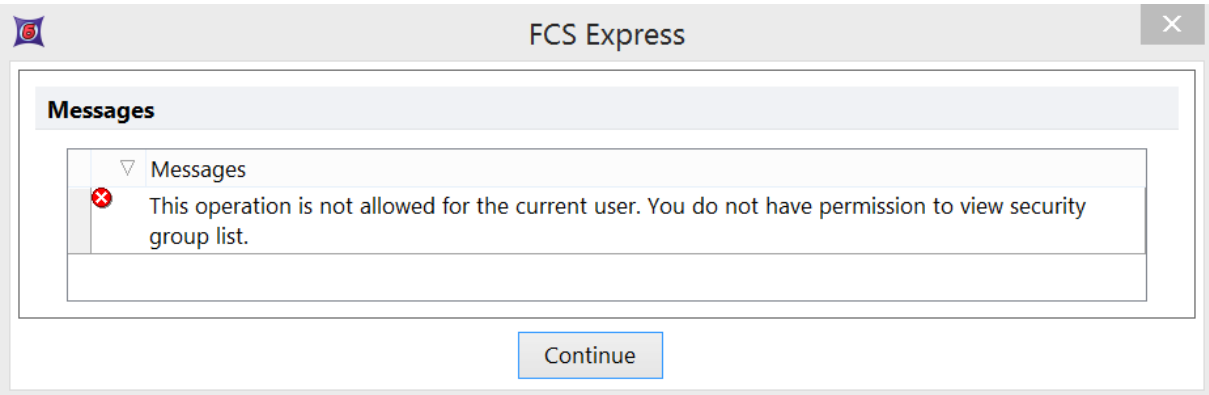

Figure 38.25 Operation Not Allowed

37.4.1 Creating a User Security Group

To create a new Security Group, use the **File** tab→**Security**→**Security Group Definitions** command. The **Security Group Definitions** dialog will appear (Figure 38.26).

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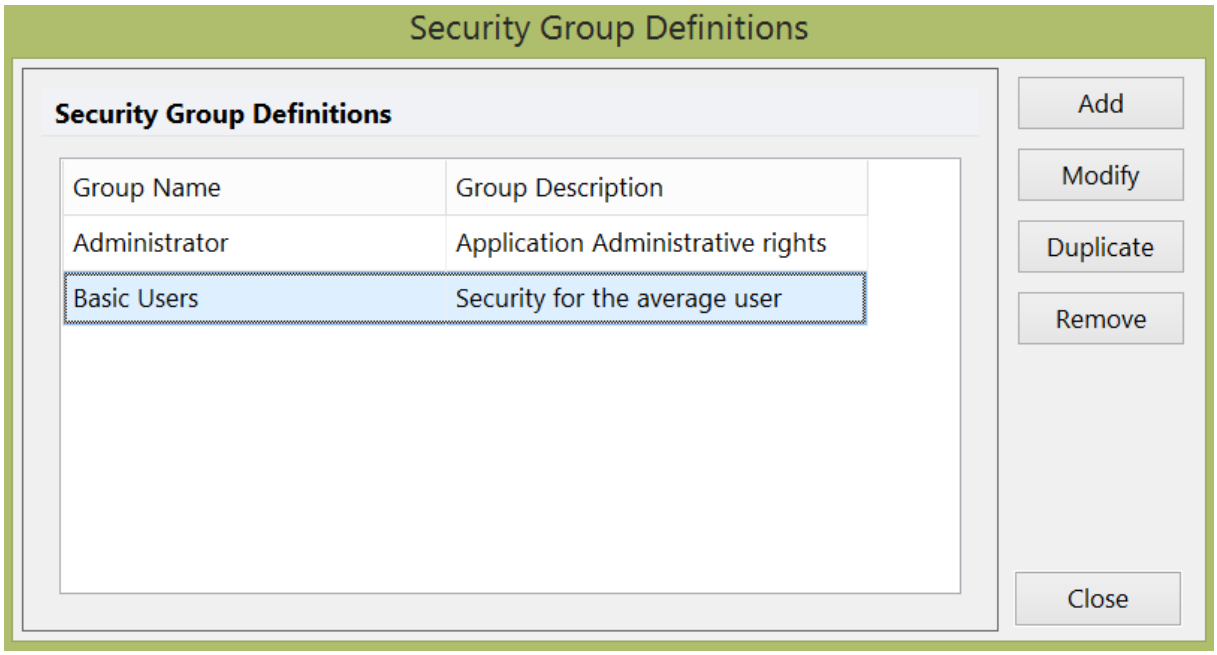

Figure 38.26 Security Group Definitions Dialog

The security groups are listed in alphabetical order. Click on the **Add** button to create a new group and bring up the **Security Group Definition** dialog (Figure 38.27).

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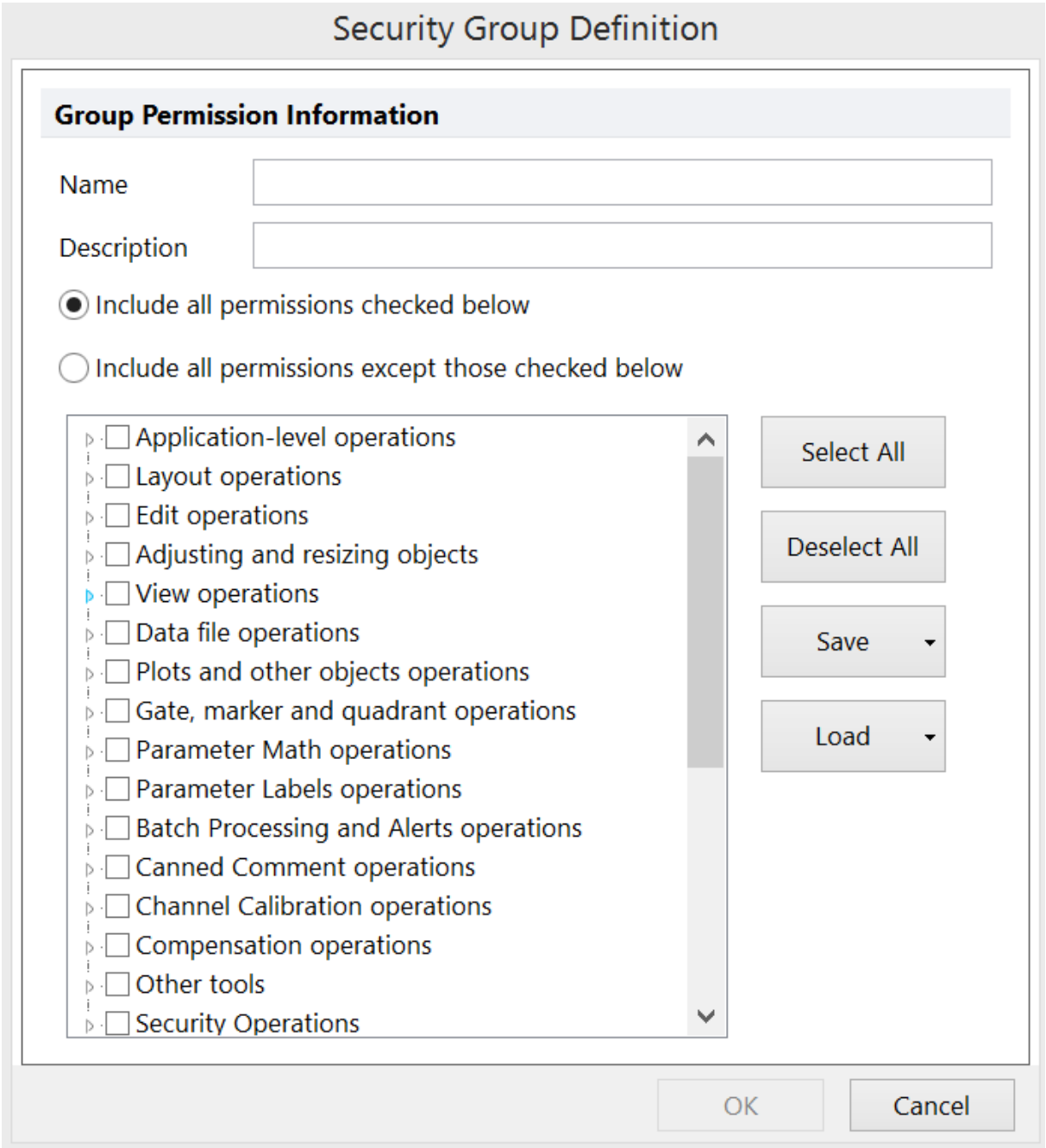

Figure 38.27 Adding a New Security Group

Assign a **Name** and optional **Description** to the Group. To select all permissions associated within a category, click on the checkbox next to the title. To view all associated permissions within a category, click on the triangle next to the checkbox. To select an individual permission, check the checkbox to the left of the permission. Using the radio buttons, you can choose to either include all checked permissions, or include all permissions except those that are checked. When you are finished defining the group permissions, click **OK**.

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To view a list of Available Security Group Permissions in FCS Express 6, click here 1181.

37.4.2 Editing a Security Group

To edit a Security Group, use the **File** tab→**Security**→**Security Group Definitions** command, and either double-click on the group, or select the group and click the **Modify** button. That will bring up the Security Group Definition dialog 1173, where you can then modify the groups permissions.

Permissions are only applied when the user logs in. If a user's permissions change while they are logged in, the new permissions will only be effective after the user logs out and in again.

Please note that the **Administrator** security group can not be modified.

37.4.3 Duplicating a Security Group

Duplicating a group allows you to easily create similar Security Groups. To duplicate a group, use the **File** tab→**Security**→**Security Group Definitions** command. Select the group to be copied, and click the **Duplicate** button. A new group will be created which has the same name as the original group, but with the "(copy)" added to it.

You can then select the new group, click the **Modify** button, rename it, describe it, and set the exact permissions you need.

37.4.4 Deleting a Security Group

To delete a group, use the **File** tab→**Security**→**Security Group Definitions** command. Select the group to be deleted and click the **Remove** button.

FCS Express will not delete a group which still has users assigned to it. Once all users who are assigned to this Security Group are assigned to another group, FCS Express will allow the group to be deleted.

37.4.5 Loading and Saving Security Group and Layout Status

Security Permissions, Groups, and Layout Status Definitions may be saved to a .txt file for quick access and loading or saved as a convenient text report.

To save security Permissions, Groups, or Layout Status Definitions to a file or report:

1. Choose the **File** tab→**Security**.
2. Choose **Security Group Definitions** (Figure 38.28, left) or **Layout Status Definitions** (Figure below, right).
3. Click on **Add** or **Modify**.
4. Click on the **Save** dropdown.

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5. Choose **Save Permissions To File**, **Save Group To File**, or **Save Group as Report** from the dropdown.
6. Browse to a location to save the file.
7. Enter a file name for the .txt file.
8. Click **Save**.

The text file will then be saved in the location specified. Permissions or Group Files may be loaded in the following steps listed below 1178 or Group Reports may be opened for review. Note that the permission files created in the steps below may also be used when creating Permission Rules for the Standard Operating Procedure features 1110.

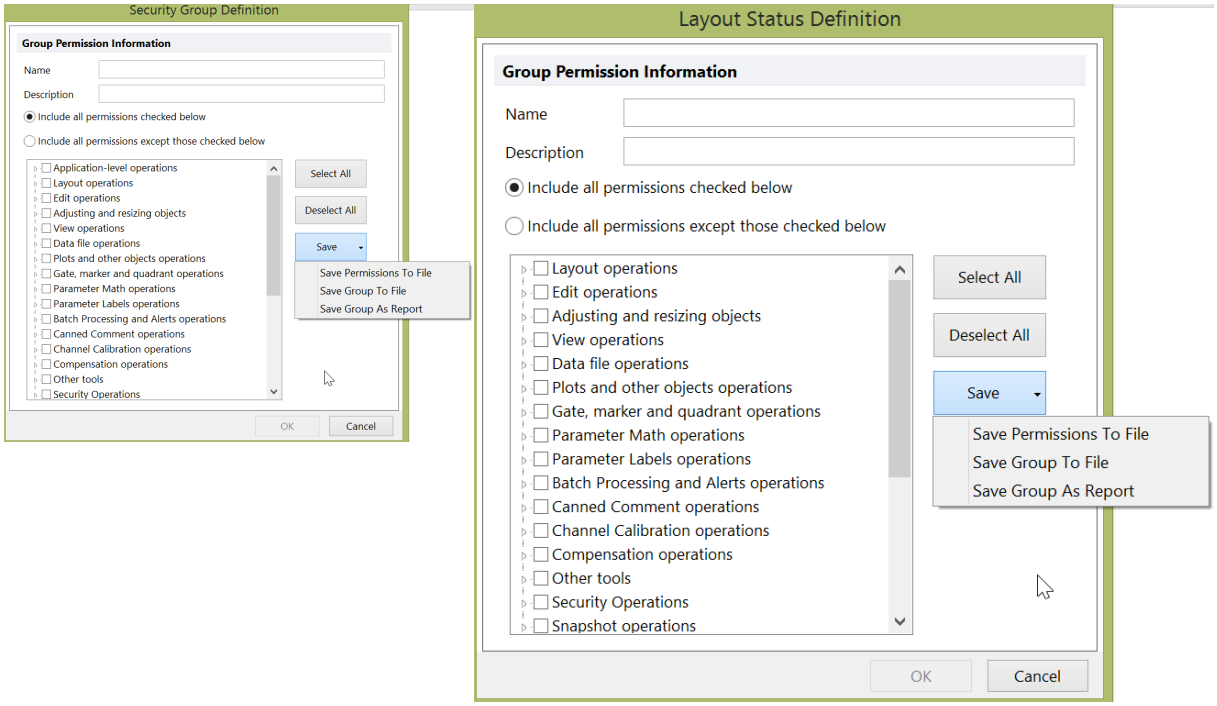

Figure 38.28 - Choosing to Save Security Group Permissions, Groups, and Reports (left) and Choosing to Save Layout Status Definition Permissions, Groups, and Reports (right).

To load security Permissions, Groups, or Layout Status Definitions files:

1. Choose the **File** tab→**Security**.
2. Choose **Security Group Definitions** (Figure 38.29, left) or **Layout Status Definitions** (Figure below, right).
3. Click on **Add** or **Modify**.
4. Click on the **Load** dropdown.
5. Choose **Load Permissions From File** or **Load Group From File** from the dropdown.
6. Browse to the location of the saved file.
7. Enter a file name for the .txt file.

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8. Click **Open**.
9. Click **OK**.

The previously saved security permission or group will now be loaded.

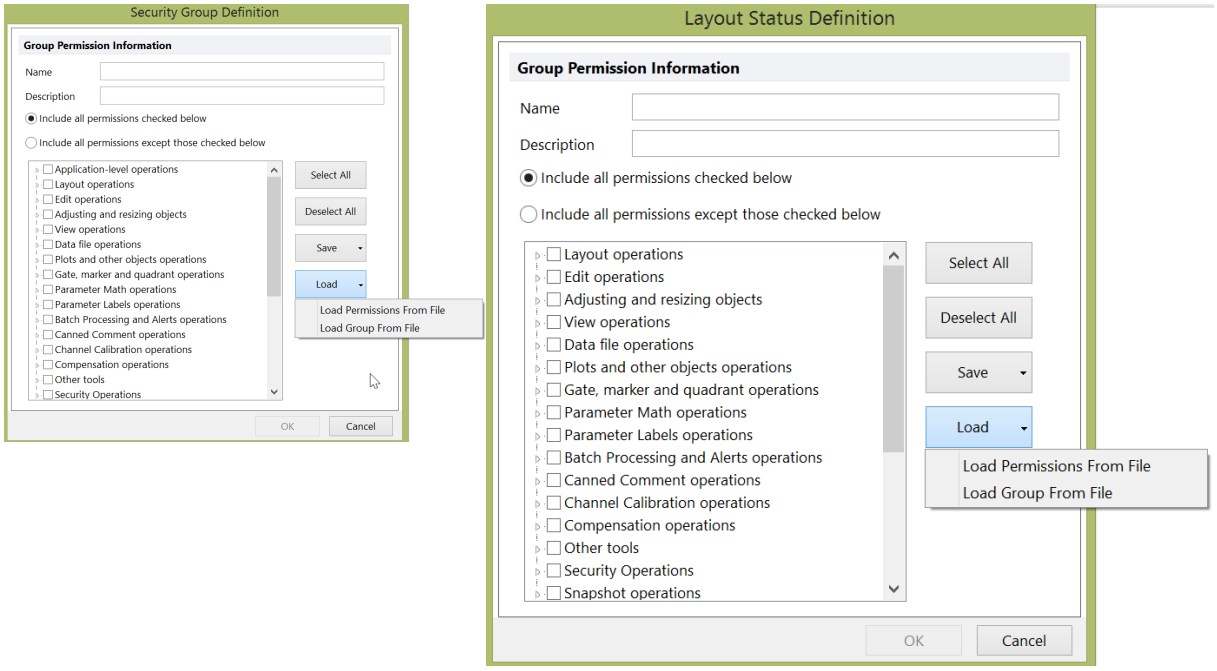

Figure 38.29 - Choosing to Load Security Group Permissions or Groups (left) and Choosing to Load Layout Status Definition Permissions or Groups (right).

37.5 Working with Layout Security Groups

A layout security group behaves in the same way as a user security group. However, the permissions associated with the layout security group apply to a particular layout file (as opposed to a user). Layout security groups are managed in a manner identical to user security groups.

In order to manage a layout security group, use the **File** tab→**Security**→**Layout Status Definitions** command to bring up the **Layout Status Definitions** dialog (Figure 38.30).

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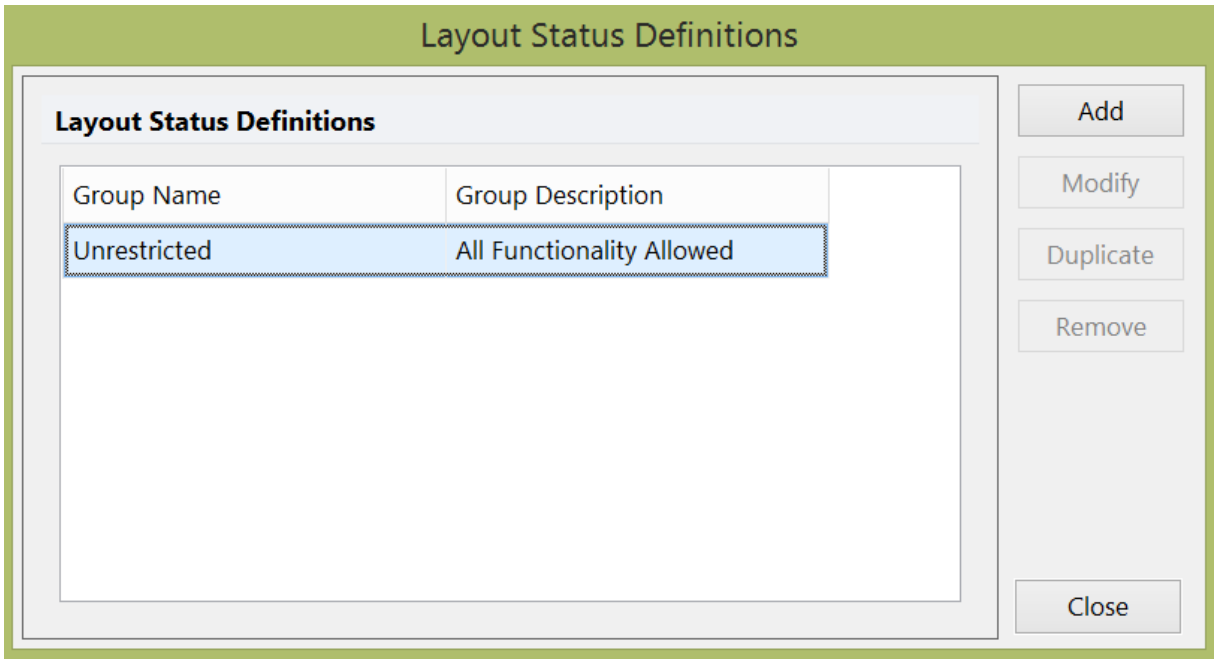

Figure 38.30 Layout Status Definitions Dialog

Layout security groups are modified, added, and deleted in the same manner as user security groups. Layout security groups may also be saved and loaded.

In the event that the current layout belongs to a layout security group that restricts a certain action, but the current user is part of a user security group that has permission to perform that action, that action will not be allowed. Therefore, both user permissions and layout permissions must be allowed for a particular action to be performed.

To associate a layout with a particular security group, use the **File** tab→**Info**→**Layout Options**→**Layout Group Security Access** command (Figure 38.31). The default group is **Unrestricted**. This means that all actions can be performed on this layout. To change the group, select a different one from the list box.

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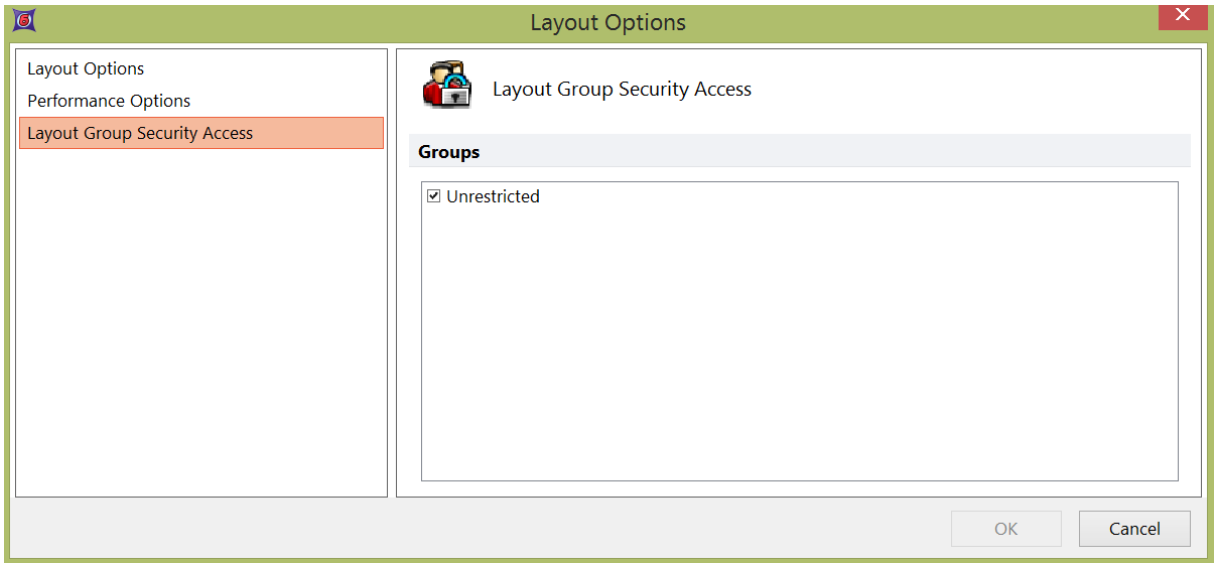

Figure 38.31 Assigning a Layout to a Security Group

The **Layout Options** window shows other pertinent information related to the layout. The file name, the name of the user who created the layout, and the date it was created is shown. Password protection can be enabled on the layout here, as well. If password protection is enabled, when the layout file is loaded, the user will be prompted to enter the password. If the password is not correct, the file will not open.

37.6 Managing the Databases

Regular maintenance of the database is not required. However, in the rare occurrence that the database becomes corrupt, or is accidentally deleted, there are tools to help maintain the databases. Please contact De Novo Software at support@denovosoftware.com prior to performing maintenance to ensure that this is required in your case.

37.7 Available Security Group Permissions

- **Application Level operations**
  - Login
  - Edit preferences
  - Restore preferences to default values
  - Import user options from file
  - Export user options to file
- **Layout operations**
  - Create New Layout
  - Open layout
    - Open
  - Navigators and Other Dockable Windows
    - Run Batch Processing

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    - Rerun Batch Processing
    - Review Batch Processing
    - View Batch Actions
    - View Data List
    - View Alerts
    - View Custom Tokens
    - View Canned Comments
    - View Compensations
    - View Transformations
    - View Run SOP
    - View Snapshots
    - View Standard Operating Procedures
    - View Permission Rules
  - Save layout
  - Save layout as
  - Print layout
  - Clear Layout History
  - Change Page Setup
  - Save layout to PowerPoint
  - Save PDF
  - Publish Layout
  - Perform Publishing Maintenance
- **Edit operations**
  - Copy FCS data to the clipboard
  - View layout options
  - Change layout options
  - Insert new page
  - Rearrange pages
  - Delete page
  - View page options
  - Change page options
  - Align objects
  - Change Object Order
- **Adjusting and resizing objects**
  - Move objects
  - Resize objects
  - Group objects
  - Ungroup objects
- **View operations**
  - View the layout history
  - View the master page
  - Change the number of visible pages
  - View hidden pages
- **Data file operations**
  - Open a Data file

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- Modify the contents of a data file
- Add a column to a data file
- Delete a column from a data file
- Insert a row into a data file
- Delete a row from a data file
- Export a Data file
- Load data that is not already loaded
- Create a new spreadsheet
- Add to data list
- Search the data list
- Remove from the data list
- Format the Data List Options
- Move a file Up in the data list
- Move a file Down in the data list
- Increment/decrement file on selected/all objects
- Change file on selected/all objects
- View file header information
- Modify file header information
- View files in memory
- Change the increment value

❖ **Plots and other objects operations**

- Insert stats window
- Insert plots and other objects
  - Insert 2D Plots
  - Insert Histogram
  - Insert Kinetics
  - Insert MultiCycle DNA
  - Insert Proliferation
  - Insert Text Box
  - Insert Gate View
  - Insert Picture
  - Insert Spectrum
  - Insert Data Grid
  - Insert Scatter
  - Insert Bar
  - Insert Pie
  - Insert Heat Map
  - Insert AutoShapes
- Remove 2D plots
- Remove histograms
- Remove DNA histograms
- Remove Proliferation histograms
- Remove text boxes
- Remove picture
- Remove data

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- Remove Gate View
- Remove AutoShape
- Remove stats window
- Change parameters on a histogram
- Change parameters on a 2D plot
- Add overlay to a histogram
- Add overlay to a 2D plot
- Remove overlay from a histogram
- Remove overlay from a 2D plot
- Reorder overlay in a histogram
- Reorder overlay in a 2D plot
- Edit the text on a text box
- View the format of a 2D plot
- View the format of a 1D plot
- View the format of an AutoShape
- View the format of a text box
- View the format of a picture
- View the format of a data object
- View the format of a statistics window
- Change the format of a 2D plot
- Change the format of a 1D plot
- Change the format of an AutoShape
- Change the format of a text box
- Change the format of a picture
- Change the format of a data object
- Change the format of a statistics window
- Change the visibility of statistics
- Autosize statistics window
- Delete Table Cells
- Insert Table
- Insert Table Cells

❖ **Gate, marker and quadrant operations**

- Create a new gate
- Modify a gate
- Remove a gate
- Modify gate information
- Add a combination gate
- Change Gate Properties
- Create a new marker
- Modify a marker
- Remove a marker
- Gate fellowship Link
- Break Gate fellowship
- Show quadrants
- Hide quadrants

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- Modify quadrants

❖ **Parameter Labels operations**

- View parameter label sets
- Modify parameter label sets
- Load a set
- Save the list of sets

❖ **Batch Processing and Alerts operations**

- Alerts
  - Run alerts
  - Add a new alert
  - Delete an alert
  - Edit the text in an alert
  - Add token to alert formula
  - View token properties for the alert formula
  - Edit alert formula token properties
- Batch Processing
  - Flag an iteration
  - View batch processing options
  - Change batch processing options
  - Add save batch actions
    - Add Save Layout batch action
    - Add Save PDF batch action
    - Add Save Picture batch action
    - Add Save PowerPoint batch action
    - Add Print batch action
    - Add Publish Layout batch action
  - Add batch folders
    - Add Folder
  - Add report batch actions
    - add Export To Excel (Column Mode)
    - add Export To Excel (Cell Mode)
    - add Word Report
    - add PDF Form
    - add Text Report
    - add Export To LabKey
  - Add items to reports
    - add Regular Token
    - add Statistic Token
    - add Keyword Token
    - add Picture
    - add Text Box
    - add PDF
    - add Layout
    - add PowerPoint
    - add FCS File
  - Rename batch actions
  - Paste batch actions

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  - Delete batch actions
  - View batch action details
  - Change batch action details

❖ **Canned Comment operations**

- Add a new Canned Comment
- Delete a Canned Comment
- Edit the text in a canned comment
- Add a token to a canned comment
- View canned comment token properties
- Edit canned comment formula token properties

❖ **Channel Calibration operations**

- View Channel Calibration settings
- Add a Channel Calibration
- Delete a Channel Calibration
- Load Channel Calibration settings
- Save Channel Calibration settings
- Modify settings
- Calculate Channel Calibration
- Edit a Channel Calibration

❖ **Compensation operations**

- Modify compensation
- Import compensation
- Export compensation
- Create compensation
- Delete compensation
- Assign compensation
- Run Automatic Compensation
- Apply to all plots
- Apply to selected plots

❖ **Transformation operations**

- Create Parameter Math transformation
- Create PCA transformation
- Create Kmeans transformation
- Create R Add Parameters transformation
- Create R Add Clustering transformation
- Create tSNE transformation
- Create SPADE transformation
- Modify transformation
- Load Parameter Math Sequence from file
- Save Parameter Math Sequence to file
- Delete transformation

❖ **Other tools**

- Modify Concentration calculator settings
- Concentration calculator calculate
- Histogram subtraction

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- Batch export
- Diva Import
- CFlow Import

❖ **Security Operations**

- Current user operations
  - View information for the current user
  - Change information for the current user
- Security System operations
  - View Security System configuration
  - Change Security System configuration
  - Security System configuration - SMTP
- All user operations
  - View information for all users
  - Add a user
  - Modify a user
  - Duplicate a user
  - Remove a user
  - Can Log Any User Out
- Reports
  - Report All Events
  - Report Failed Logins
  - Report Full Sessions
  - Report Remote Logouts
- Layout Status Definitions
  - View Layout Status List
  - Add new Layout Status
  - View and modify a Layout Status
  - Duplicate a Layout Status
  - Remove a Layout Status
- Security Group Definitions
  - View Security Group List
  - Add new Security Group
  - View and modify a Security Group
  - Duplicate a Security Group
  - Remove a Security Group
- Electronic Signature operations
  - View signature list
  - Change signature list
  - Sign
  - Unsign

❖ **Snapshot operations**

- Add snapshot
- Edit snapshot
- Delete snapshot
- Apply snapshot

❖ **SOP operations**

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- Add SOP Step
- Delete SOP Step
- Edit SOP Step
- Reset SOP Run Status
- Rerun SOP

❖ **Permission Rule operations**

- Add Permission Rule
- Delete Permission Rule
- Edit Permission Rule

❖ **Help operations**

- View help
- View tutorials
- View manual
- View online forum
- Go to the De Novo Software web site
- Enter countercode
- View About screen

❖ **Panel operations**

- View the panel information
- Add a panel
- Edit a panel
- Remove a panel
- Load panels from a file
- Save panels to a file
- Change the panel information
- Change the name of a panel
- Change the number of tubes in a panel

❖ **Token operations**

- Custom Tokens
  - Add custom token
  - Delete custom token
  - Add token to custom token formula
  - View custom formula token properties
  - Edit custom formula token properties
  - Edit the text in a custom formula
- Change Font Classification
- Add Font Classification
- Edit Font Classification
- Reorder Font Classification
- Remove Font Classification
- Token Bounds Change
- Add Statistic Classification
- Edit Statistic Classification
- Reorder Statistic Classification
- Remove Statistic Classification

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- Add Keyword
- Remove Keyword
- Add token to a text box
- View text box token properties
- Edit text box token properties

❖ **Formatting Operations**

- Bold
- Bullets
- Font
- Font Background
- Font Color
- Font Name
- Font Size
- Gate Formula Change
- Indent Text
- Italics
- Justify Text
- Format 3D Options
- Format Axes Options
- Format Color Levels
- Format Fill Options
- Format General Options
- Format Legend Options
- Format Line Options
- Format Overlays Options
- Format Size Options
- Format Stats Options
- Format Title Options
- Underline

❖ **Keywords Anonymizer**

❖ **Page Tab Operations**

- Change Page Tab Color Classification
- Add Page Tab Color Classification
- Edit Page Tab Color Classification
- Reorder Page Tab Color Classification
- Remove Page Tab Color Classification

❖ **Override Authorizations**

❖ **Format Authorizations**

37.8 Security Database Options

If you purchase FCS Express with the Security add-on, you have the choice of two database options

- Microsoft SQL Server
- Elevate DB Local Server

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Click here for more information about compatibility requirements for Microsoft SQL Server.

We strongly recommend Microsoft SQL Server for your production environment.

Please note that before or after you purchase the security add-on, you must inform sales@denovosoftware which database option you would like. We can then customize the configuration file for your license.

Microsoft SQL Server

Microsoft SQL Server is the most robust database option available for FCS Express. This option is ideal if your organization already has an SQL Server installation and has experience with it. It is also ideal if you will be running several network installations of FCS Express, or many standalone licenses, or any combination of network and standalone licenses. Each of those installations will be able to use the same configuration 1190, and share the same security system. Note that to use FCS Express with SQL Server, you must purchase your own license for SQL Server.

Elevate DB local server

The Elevate DB local server is a database that does not require a database server to be installed on any computer. The database files reside in a shared folder, and each FCS Express client accesses the database files directly. Multiple instances of FCS Express can access the database at the same time. This is the simplest option as it requires no extra installation of an additional database server. It does suffer from performance issues inherent to non-client server architectures, however, the security databases are quite simple and this may not have a practical impact in your organization. This option can be provided for free with purchase of your security system.

Because Elevate DB is not a client-server database, it is **critical** that you perform regular backup and restore tests in the event of data corruption. This is one of the reasons we recommend SQL Server, since it is much less prone to data corruption. Instructions regarding backing up your databases can be found here 1192.

37.9 Configuring MS SQL Server to host Security Databases

Microsoft SQL Server is one of several database options supported by FCS Express for hosting your security system. This option is ideal if your organization already has an SQL Server installation and has experience with it. To configure FCS Express to use SQL Server, please follow the following instructions.

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1. Create a database in the SQL server instance. This will be the database that FCS Express will use.

2. Set up a file containing the connection information to the database, in the same folder as the FCS Express configuration file. This file can either be stored in plain text, or encrypted.
   a. If you want to store the database details and login credentials in a plain text file, create a text file called FCSEDB.ini and start editing it in notepad.
      i. In the first line, type [MSSQLConnection]
      ii. In the second line, type ConnectionString=
      iii. The connection string itself will be placed immediately at the end of the second line (without any spaces following the = sign)
   b. If you want to encrypt the database details and login credentials, run the program FCSEDBIniFileEditor.exe. You will find this program in the same folder as FCS Express.exe
      i. A window will appear with fields for entering the connection string, and the password.

3. Create a connection string, and enter it into the appropriate place in either the text file or the field in FCSEDBIniFileEditor. The connection string includes information about the type of connection to create, the server where the database is located, the name of the database, and user id / password details.

Sample connection string if you will be using an ADO connection:

Provider=SQLOLEDB.1;Integrated Security=SSPI;Persist Security Info=False;Initial Catalog=<database>;Data Source=<database host>\<database instance>

Modify the text above so that it uses your database information. <Database> should be replaced with the name of database you created in step #1 above. <Database host> should be replaced with the IP address or path to your SQL Server instance. <Database instance> will be the instance on the database host, and may not be required, depending on your database installation. Note that this connection string assumes integrated (ie Windows login) security. If you would prefer to set it up with a named user, update the text above so that it includes the user name and password, and removes the Integrated Security value. For example, it might look like:

Provider=SQLOLEDB.1;Initial Catalog=<database>;Data Source=<Database Host>\<Database Instance>;User ID=<user name>;Password=<password>

Sample connection string if you will be using an ODBC connection

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Provider=MSDASQL.1;Persist Security Info=False;User ID=<user name>;Password=<password>;Data Source=<Data Source Name created with ODBC Manager>;Initial Catalog=<database>

Modify the text above so that it uses your database information. <User name> should be replaced with the username, <password> replaced with the password. <Data Source Name created with ODBC Manager> should be replaced with the ODBC connection name and <database> should be replaced with the name of the database you created in step #1 above.

4. After you have entered the connection string, save the file.
   a. Select the menu item File->Save in either notepad or the FCSEDBIniFileEditor. Then navigate to the same folder where your configuration file is stored, and enter a file name.
   b. If you are using a plain text file, the file name must be FCSEDB.ini. If you are using the FCSEDBIniFileEditor, the file name must be FCSEDB.cini .

Please note: when creating the connection string in the .INI file, the "<>" are not needed and should not be included. They are being used in this text to denote a placeholder for a custom value that needs to be entered by the user or administrator.

When FCS Express starts, it will read the information in the file, connect to your database, and automatically create all the required tables and indices. FCS Express will not create a database. A database needs to be created ahead of time for this process to occur.

37.10 Backing up the Databases

We recommend that you perform regular backups of your database, as well as regular tests where you restore from backups.

SQL Server

There are several ways to back up and restore SQL Server databases, and we do not recommend any particular ones. Please consult your SQL Server administrator for the best choice for your environment.

Elevate DB Local Server

Elevate DB Local Server stores data in a set of files located in a subfolder of the program folder. The subfolder is called ElevateDBData. In order to back it up, please perform the following steps:

1. Make sure everyone is logged out of FCS Express, and that the program is shut down on every computer.

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2. Copy the folder ElevateDBData to your backup storage location.

To restore, simply copy the backed up folder back to the original location.

37.11 Preparing for Database Updates

For certain releases, FCS Express will update the structure of the security database. After the database is updated, previous releases of FCS Express will no longer be able to use it. Because of this, we recommend the following:

1. Before installing a version of FCS Express which will update the database, perform a backup of the existing database. Please see the topic Backing up the Databases 1192 for details. To determine whether a specific release will update the database, please check the release notes on the download page.

2. If you plan on testing the new FCS Express release before switching to using it, then perform the following steps

   a. Create a new database for testing, and initialize it with the contents of the backup from step 1.

   b. Install the new release to a new folder

   c. Copy the file **FCSEdb.ini** from your previous installation to the new folder, and **edit it so that it refers to the database you created in step 1**.

   d. Run FCS Express, and perform your testing. The user names and permissions will be the same as in your original databases. Note that since you are now using a new database, any changes you make will not be propagated back to the previous release.

3. Install the new release to the same folder as the previous release, and run.

37.12 Security Reports

FCS Express allows you to view and manage reports for user activity. Reports can be accessed from the **File** tab→**Security**→**Reports** gallery (Figure 38.32).

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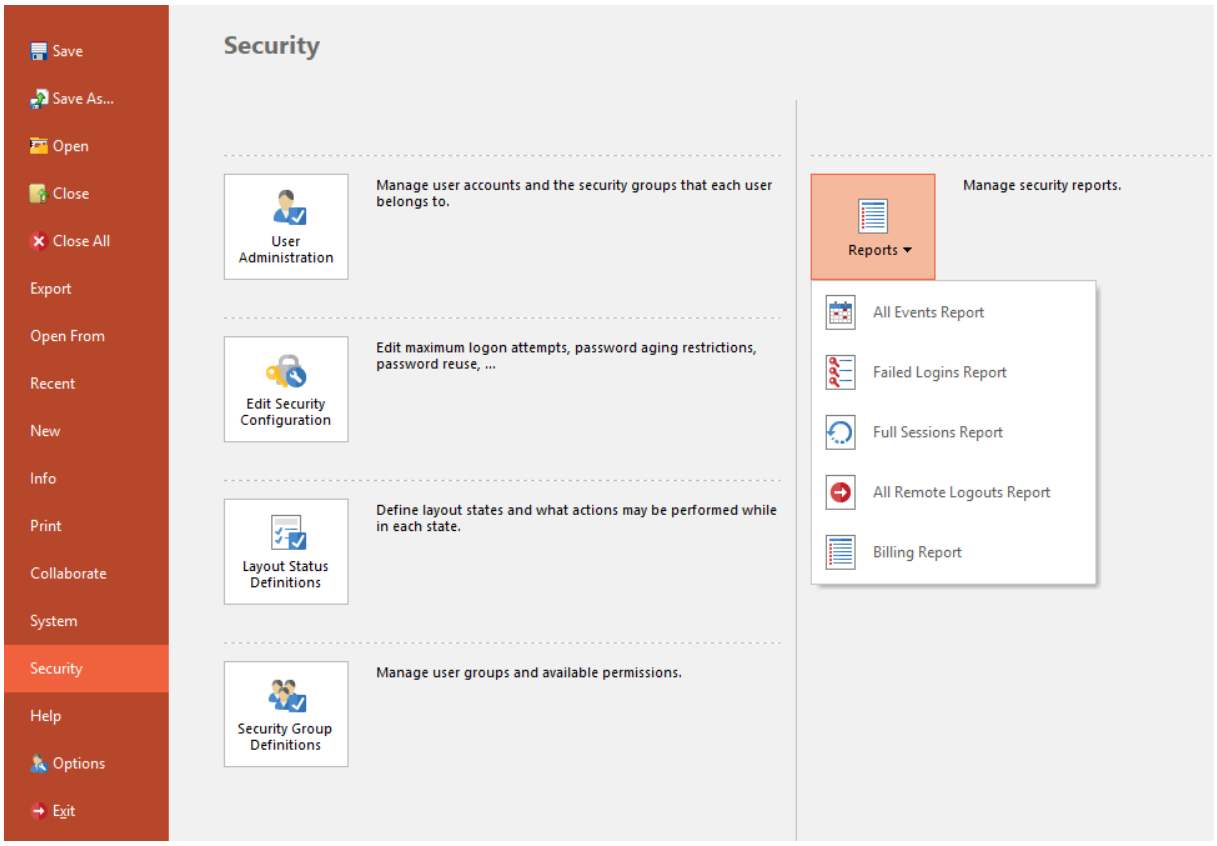

Figure 38.32 Navigating to the Security Reports Gallery

Four types of reports are available (click on each link for more details):

- **All Events Report** 1195 **-** Lists a history of all logging events
- **Failed Logins Report** 1196 **-** Reports a list of failed logins and the reason for failure
- **Full Sessions Report** 1197 **-** Reports a list of all full sessions in FCS Express
- **All Remote Logouts Report** 1197 **-** Reports a list of all logouts from a remote location
- **Billing Report** 1199 - Reports a list of Unique Users that accessed the software and the corresponding Quarters Billed. Users are grouped based on the Lab they belong to.

All security reports contain a list of columns that can be arranged 1200 or grouped 1200 as well as the option to define Filter Criteria 1200 and **Refresh the**

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Report for the new filter (Figure 38.33). You also have the option to export the report[1205] in the following formats:

- **TXT**
- **HTML**
- **XML + XSL**
- **XLS**

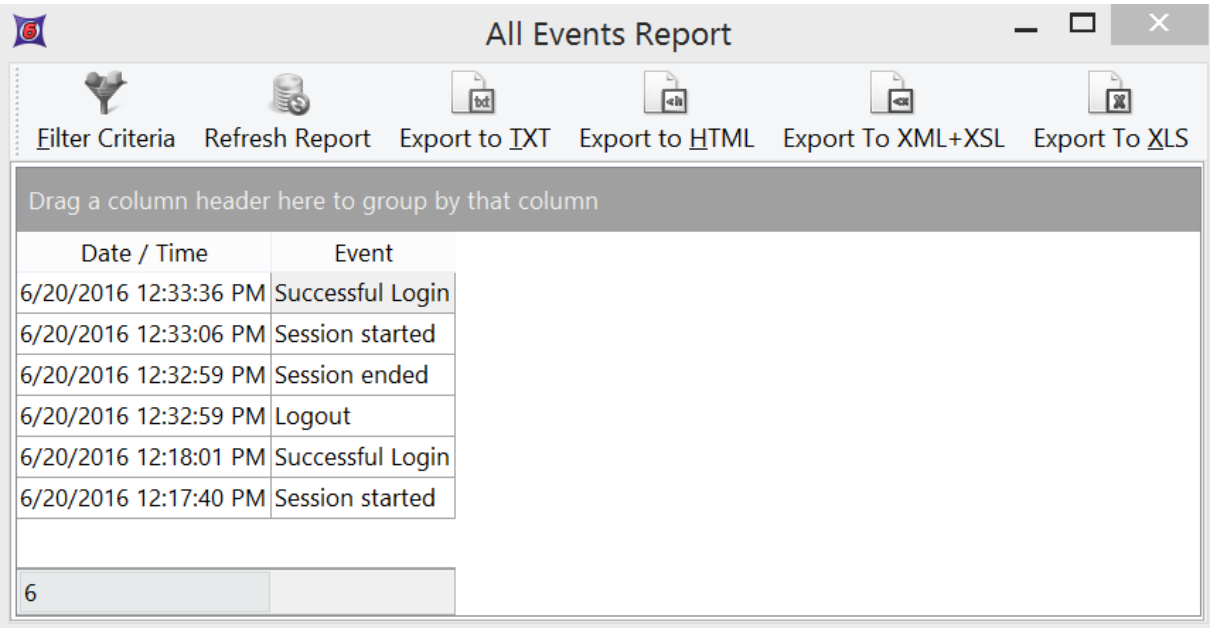

Figure 38.33 - Example of Security Report dialog.

37.12.1 All Events Report

All Events Report can be accessed from the **File** tab→**Security**→**Reports**→**All Events Report**.

Choosing an **All Events Report** (Figure 38.34) will allow you to see the **date and time** of all session actions and user logging **event** activity.

The report can be arranged, grouped, and filtered[1200] according to your needs.

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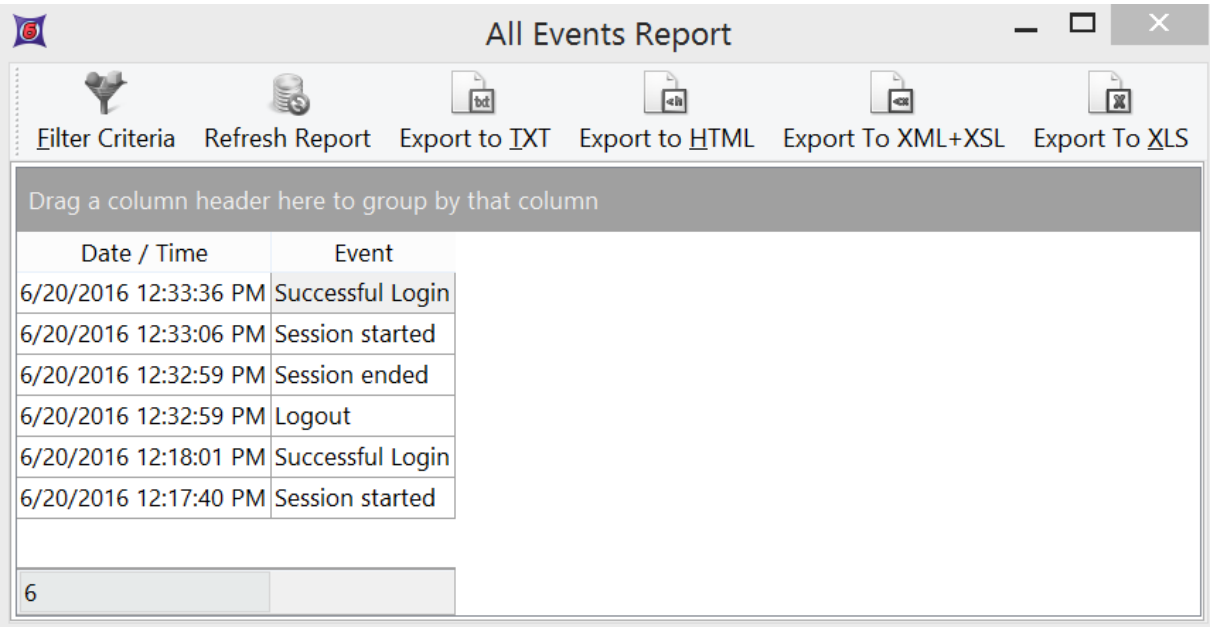

Figure 38.34 The All Events Report Dialog

37.12.2 Failed Logins Report

Failed Logins Report can be accessed from the **File** tab→**Security**→**Reports**→**Failed Logins Report**.

Being able to track failed logins in a requirement of the 21 CFR Part 11.

Choosing a **Failed Logins Report** (Figure 38.35) will allow you to view:

- **Date and Time** of failed login
- **Reason** for failure
- **User Name** when failure occurred
- **Login Name** when failure occurred
- **Windows User Name** when failure occurred
- **Computer name** on which failure occurred
- **IP Address** used to access server at time of failure
- **First Name** of user associated with failure
- **Last Name** of user associated with failure

The report can be arranged, grouped, and filtered according to your needs.

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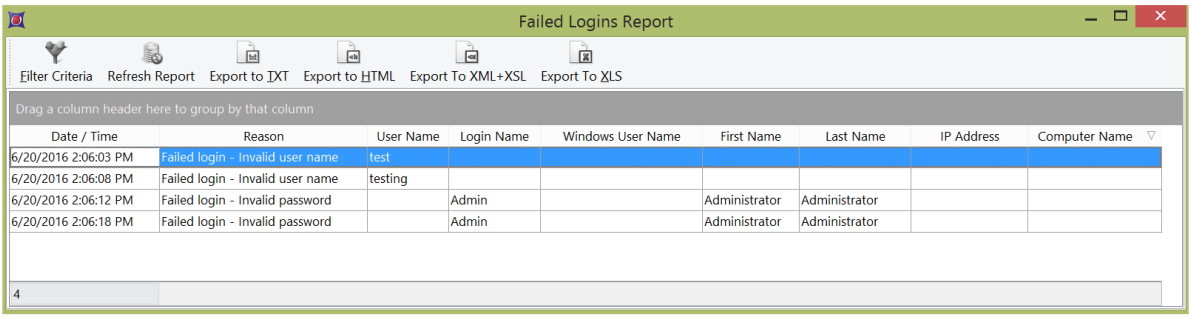

Figure 38.35 The Failed Logins Report Dialog

37.12.3 Full Sessions Report

Full Sessions Report can be accessed from the **File** tab→**Security**→**Reports**→**Full Sessions Report**.

Choosing a **Full Sessions Report** (Figure 38.36) will allow you to view:

- Date and Time of **Connection**
- **Windows User Name** during sessions
- **Computer name** on which session occurred
- **IP Address** used to access server for session
- Session **Login** time
- Session **Login Name**
- **First Name** of user associated with session
- **Last Name** of user associated with session
- **Logout** date and time
- **Logout Reason** status
- Time of **disconnection**
- **Disconnection reason** status
- **Session Time** (in hours) of each session

The report can be arranged, grouped, and filtered 1200 according to your needs.

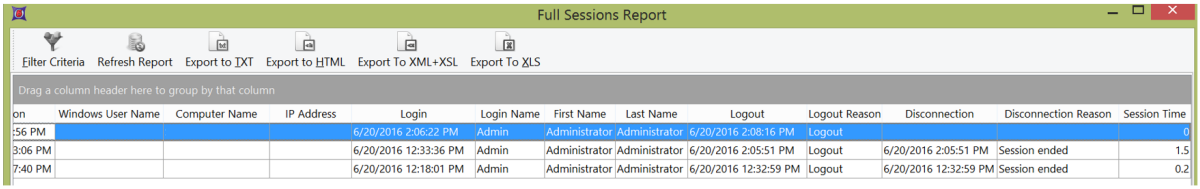

Figure 38.36 The Full Session Report Dialog

37.12.4 All Remote Logouts Report

All Remote Logouts Report can be accessed from the **File** tab→**Security**→**Reports**→**All Remote Logouts Report**.

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Choosing a **All Remote Logouts Report** (Figure 38.37) will allow you to view:

- For the **User who was logged out**
  - **Logout Time**
  - **Login Time**
  - **Login Name** of the user
  - **First Name** of user associated with session
  - **Last Name** of user associated with session
  - **Windows User Name** for the session
  - **Computer Name** for the session
  - **IP Address** used to access server for session

- For the **User who requested the logout**
  - **Login Name** of the user
  - **First Name** of user associated with session
  - **Last Name** of user associated with session
  - **Windows User Name** for the logout
  - **Computer Name** for the logout
  - **IP Address** used to access server for the logout

The report can be arranged, grouped, and filtered 1200 according to your needs.

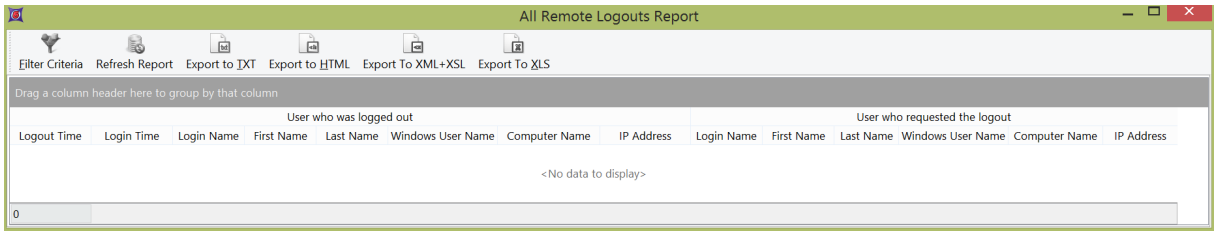

Figure 38.37 The Remote Logouts Report Dialog

37.12.5 Report Unique Users

The total number of **Unique Users** can be viewed in the footer of the Full Sessions Report (Figure 38.38, red arrow), which is accessible from the **File** tab→**Security**→**Reports**→**Full Sessions Report**. Note that filter criteria 1204 may be used to return the results for a specific date range.

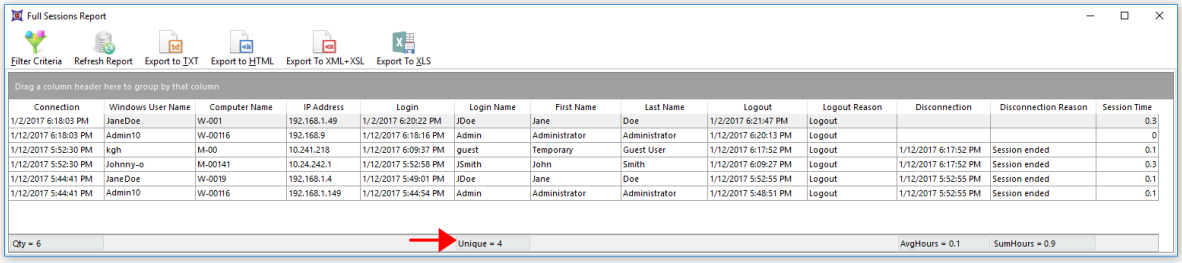

Figure 38.38 Number of Unique Users displayed in footer of Full Sessions report

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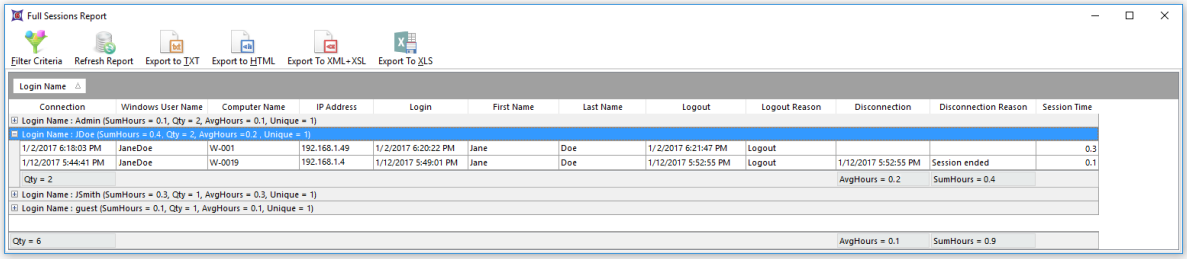
By dragging the Login Name column header to the shaded gray area at the top of the report[1201], the activity per user (by Login Name), can be reported. This includes the sum of all hours logged in (SumHours), the number of logins (Qty), and average length of session (AvgHours) (Figure 38.39).



37.12.6 Billing Report

Billing Report can be accessed from the **File** tab→**Security**→**Reports**→**Billing Report**.

Choosing a **Billing Report** (Figure 38.40) will allow you to see the number of Unique Users and the total of Quarters Billed for each Lab group.

The report can be arranged, grouped, and filtered[1200] according to your needs.

An example of Billing report is depicted in the figure below. Three unique users have been created under the Joe Flow group, and another three under the Jane Cytometry group. Only one unique user from the Joe Flow name group accessed the software during the quarter, while all three users from the Jane Cytometry group accessed the software during the quarter. In total four active accessed the software during the quarter each, thus billing for four quarters would be assessed.

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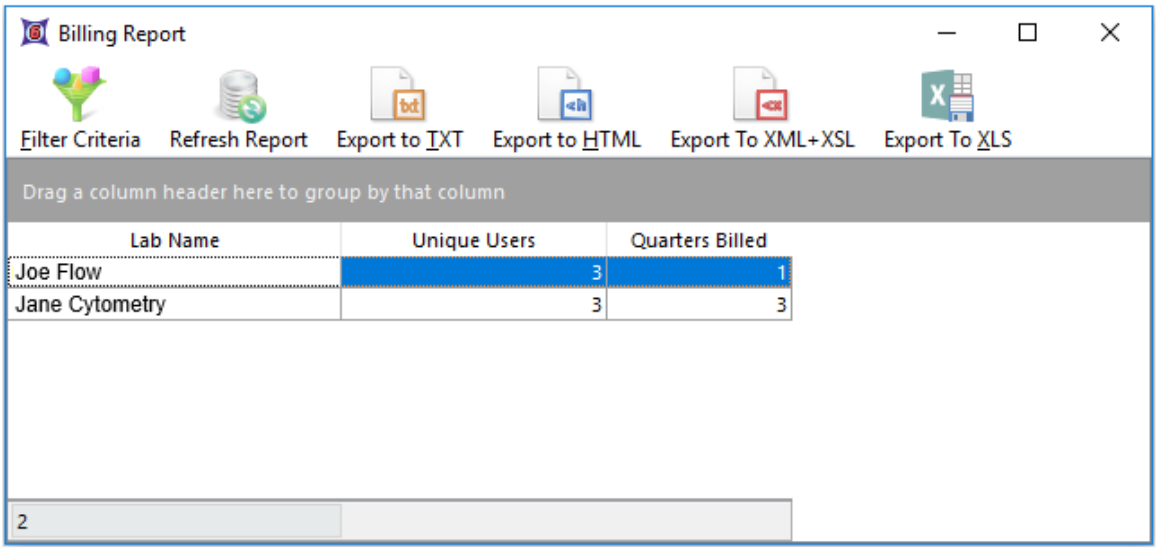

Figure 38.39 The Billing Report Dialog

37.12.7 Arranging, Grouping, and Filtering Reports

All security reports contain a list of columns that can be arranged[1200] or grouped[1201] as well as the option to define Filter Criteria[1204] (for a date range) and **Refresh the Report** for the new filter.

Please see the following topics for details:
- Arranging Columns[1200]
- Grouping Reports[1201]
- Filtering on a Date Range[1204]

37.12.7. Arranging Columns in Reports 1

Columns for any report can easily be rearranged.

To rearrange columns:

1. Click on the column header to select it.
2. Click and hold on the column header.
3. Drag the column header to a new location (green arrows will indicate where the column will be placed) (Figure 38.41).
   - In the example below the column header for **Windows User Name** was dragged between **IP Address** and **Login**.
4. Drop the column header by releasing the mouse button (Figure 38.42).

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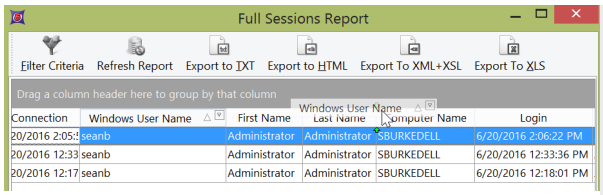

Figure 38.40 Dragging a column to a new location.

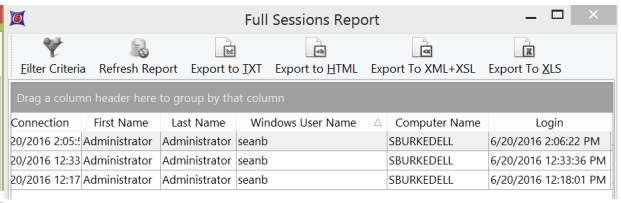

Figure 38.41 The report after the column has been moved.

Date range filtering may also be done on any column, group, or custom filter using the Filter Criteria 1204 button.

37.12.7. Grouping and Filtering Reports 2

Reports can be arranged into groups based on column headers.

To create a grouped report:

1. Click on a column header to select it.
2. Click and hold on the column header.
3. Drag the column header to the grey area just above the header (green arrows and the text "**Drag a column header here to group by that column**" will indicate where the column header will be placed) (Figure 38.43).
   - In the example below the column header for **Connection** was dragged to the grouping area.

4. Drop the column header by releasing the mouse button (Figure 38.44).

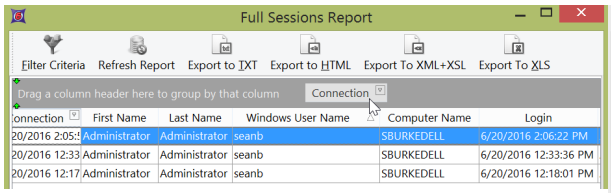

Figure 38.42 Dragging a column header into the grouping area

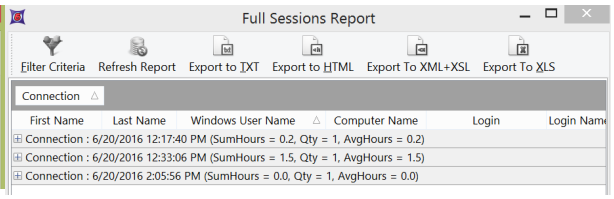

Figure 38.43 A report after being grouped by Connection

Subgrouping of the report is also possible.

To create subgroups:

1. Click on a column header to select it.
2. Click and hold on the column header.

3. Drag the column header to the grey area just above the header (green arrows and the text "**Drag a column header here to group by that column**" will indicate where the column header will be placed) (Figure 38.45).
   - In the example below the column header for Computer Name and IP Address were subsequently was dragged to the grouping area.

4. Drop the column header by releasing the mouse button.

The hierarchy of subgroups can also be changed by dragging and dropping a sub-grouped item before or after another grouped or sub-grouped item.

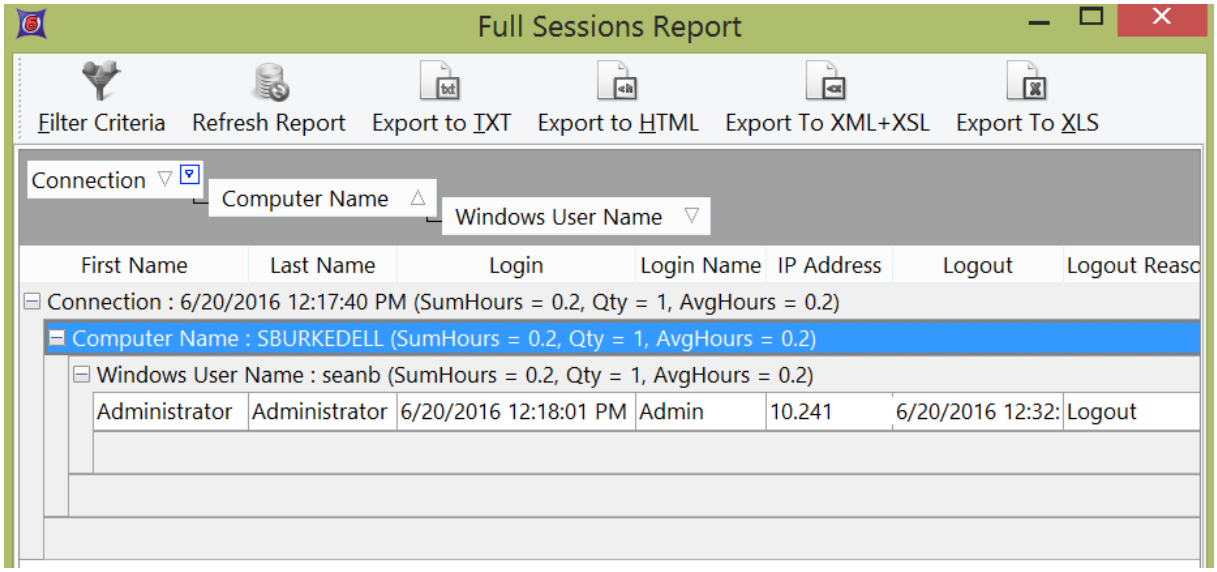

Figure 38.44 Using the grouping feature to create sub-groups

Grouped items can also be further filtered by clicking on the group or sub-grouped heading and checking the box for the sub-group classification (Figure 38.46).

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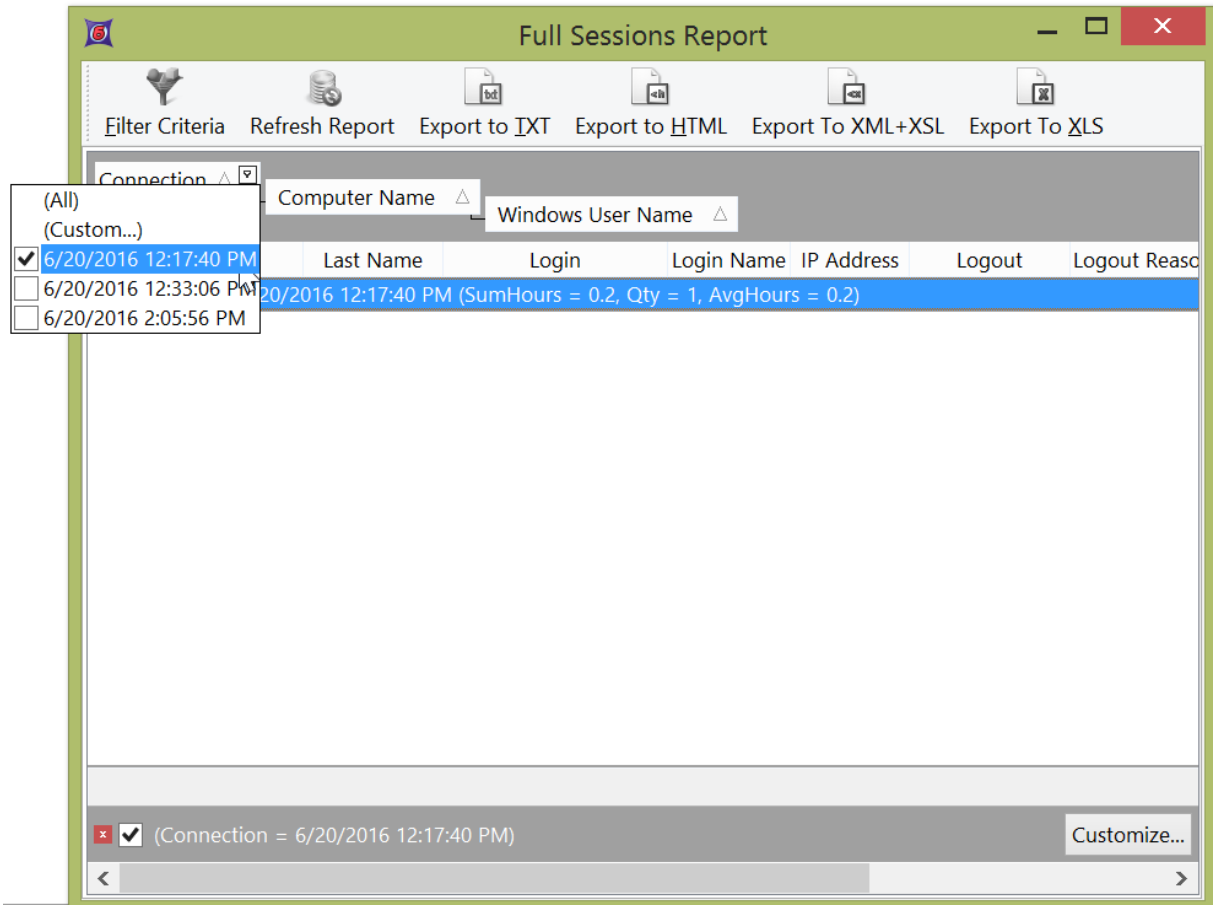

Figure 38.45 Using a check box to further classify a group or sub-group

If the **(Custom...)** category is chosen you will be prompted with the Custom Filter dialog in which you can define a custom classification for your reports grouping (Figure 38.47 and Figure 38.48 below).

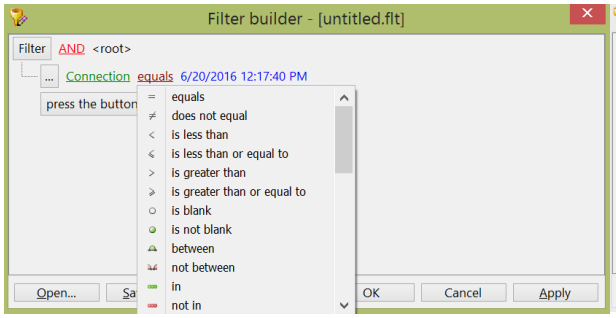

Figure 38.46 Choosing a custom classification for reports

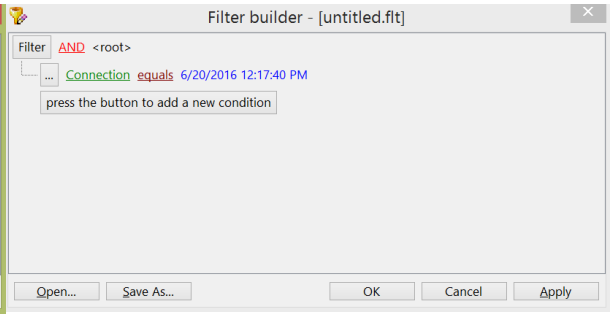

Figure 38.47 Using a custom classification for reports

Date range filtering may also be done on any column, group, or custom filter using the Filter Criteria 1204 button.

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Filters may also be saved by clicking on the **Save As...** button (Figure 38.49 below). The **Save the active filter to file dialog** will appear where you may save your **Filters (*.flt)** file to be used again at a later date by clicking **Save**.

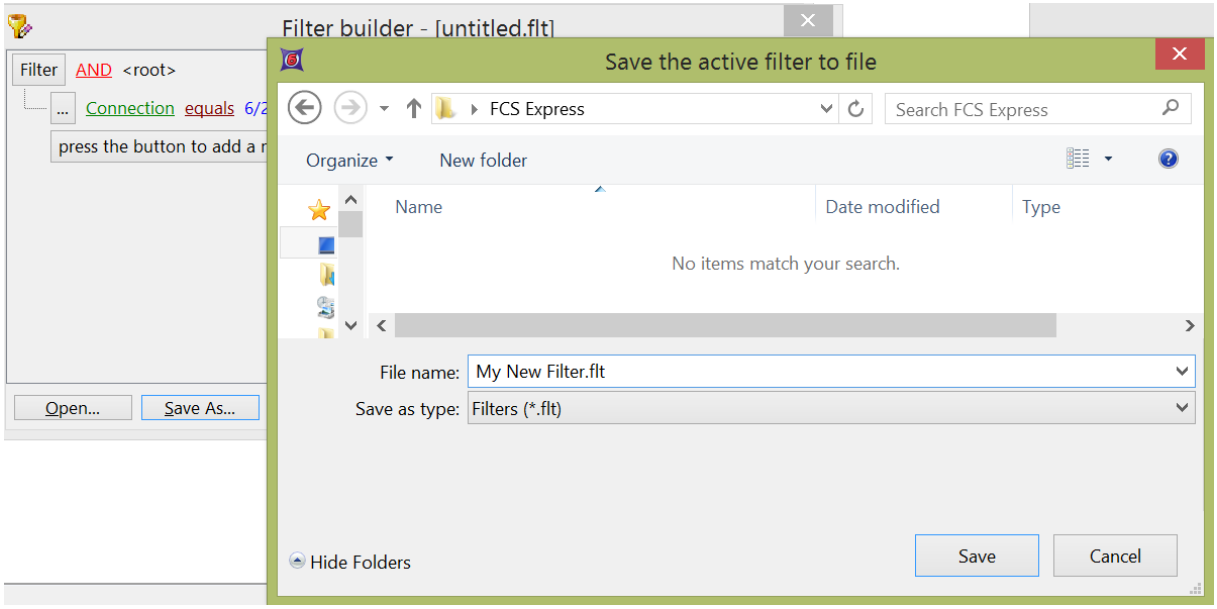

Figure 38.48 Saving a customized filter.

37.12.7. Filtering on a Date Range 3

All reports can be further classified by using **Filter Criteria** to choose a date and time range.

To define the **Filter Criteria**:

1. Click on the **Filter Criteria** button.
2. Enter a date and time range in the **Time Interval Filter** dialog (Figure 38.50).
3. Click **OK**.

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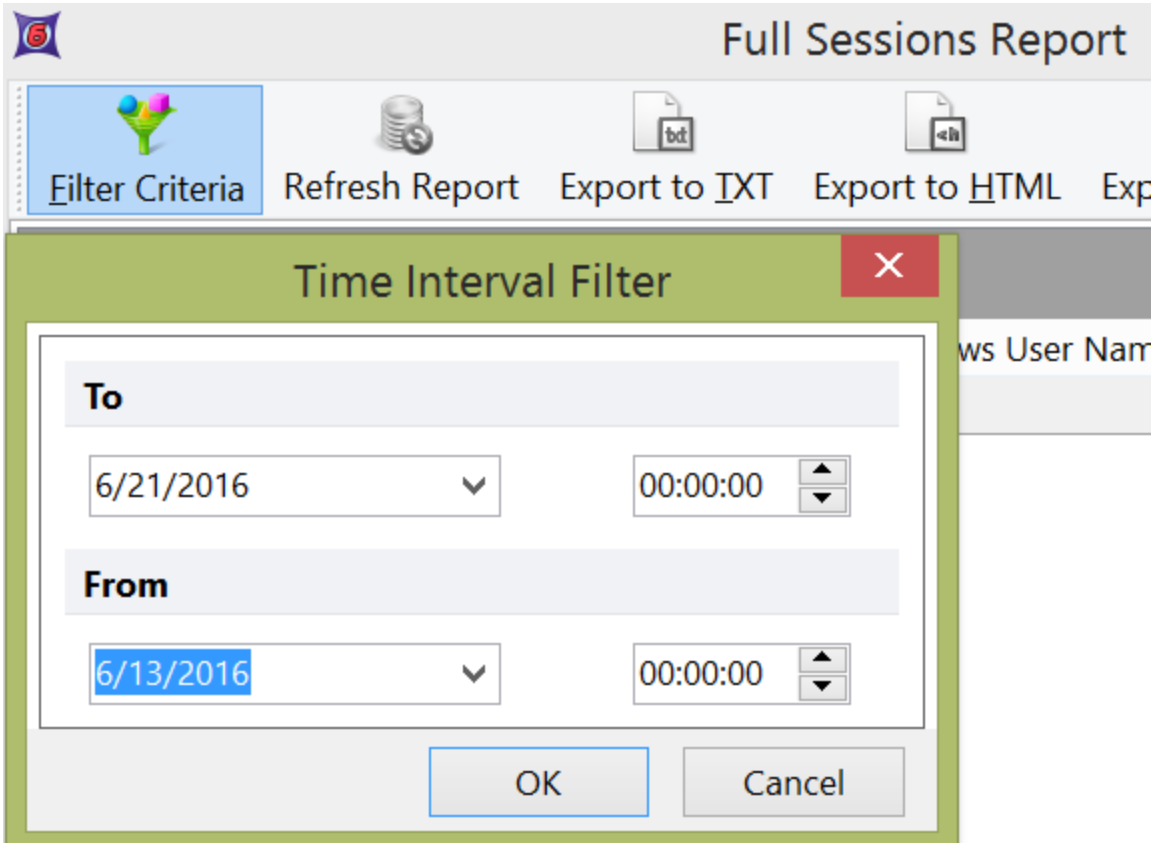

Figure 38.49 Choosing the date and time Filter Criteria

4. Click on the **Refresh Report** button to apply the new **Filter Criteria** to the report (Figure 38.51).

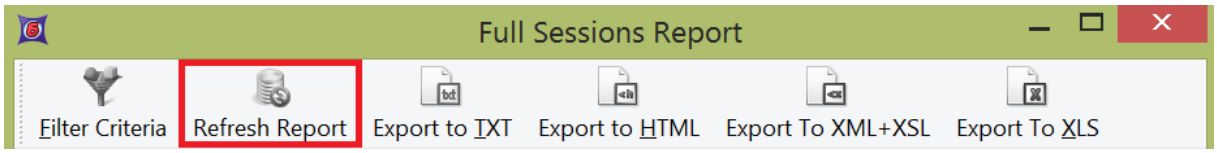

Figure 38.50 Choosing to refresh the report to the new Filter Criteria

37.12.8 Exporting Reports

You have the option to export any report in the following formats:

- **TXT**
- **HTML**
- **XML + XSL**

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- **XLS**

To export a report:

1. Click on the button for the export type you want (**Export to HTML** in this examples) (Figure 38.52).

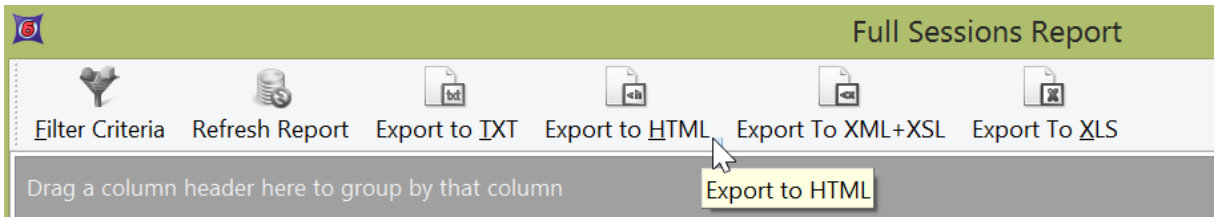

Figure 38.51 Choosing to export the report as an HTML file

2. Choose a location and name for the file on your drive (Figure 38.53).

3. Click **Save**.

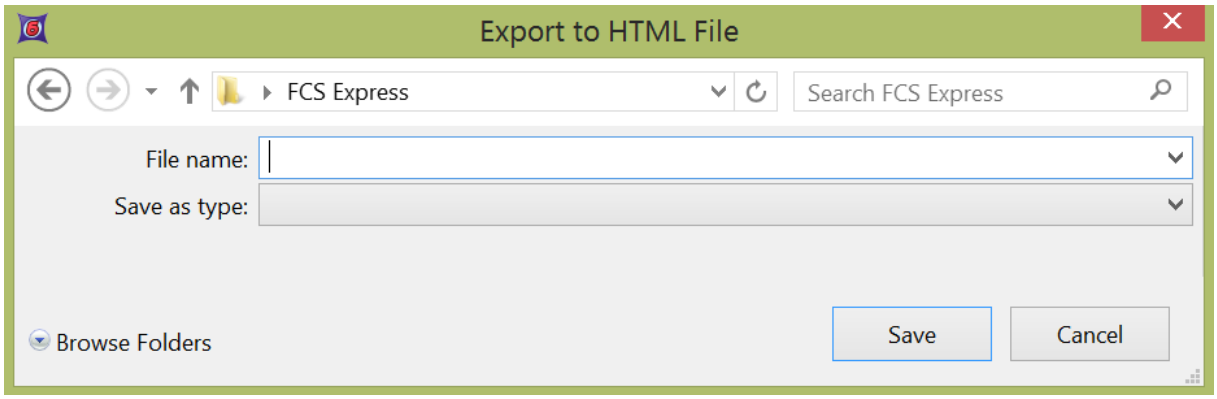

Figure 38.52 The export to HTML file dialog

37.13 Layout and Data Security

To help you fulfill your requirements under 21 CFR Part 11, 11.10(c), FCS Express provides Record Protection. This is implemented in two forms:

1. Your layouts are stored in an encrypted format, and require the correct encryption key to open. This holds true whether or not any data has been embedded in the layout. If the layout has been tampered with, or corrupted in any way, FCS Express will not be able to decrypt it, and will inform you that the layout cannot be opened.

Note that we encourage you to retain secure backups of your layouts if the above situation were to ever occur, so that you would be able to retrieve your analysis.

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2. When data is loaded in a layout, FCS Express makes a digital signature of the data, and stores it in the layout. After the analysis is completed, if in the future someone attempts to open the layout to look at your analysis, then the layout will only display the data if the digital signature is still the same. In other words, if the data has changed, the person will not be able to see that data in your analysis, and all plots will appear blank. This holds true whether the data was saved embedded in the layout, or is stored separately on disk.

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Chapter

38 Tutorials

This chapter contains detailed tutorials of the functions of FCS Express Version 6 to make learning commonly used features as easy as possible.

Sample data is provided so that you can follow along with the examples in the tutorials. The sample data can be downloaded from the De Novo Software web site, from the archive:

https://s3.amazonaws.com/fcsexpressdownloads/TutorialSampleDataV6.zip

After downloading the archive, you may extract the contents anywhere you like, for example, in My Documents.

These tutorials are currently available:

- Using Objects 1211
- Inserting Plots 1225
- Formatting Plots 1236
- Gating 1266
- Text Boxes and Tokens 1298
- Quadrants 1349
- Quadrant/Marker to Gate Linking 1362
- Compensation 1376
- Snap Shots 1423
- Batch Processing 1445
- Creating and Using Panels 1498
- Docking 1537
- Data Navigator 1561
- Gate Navigator 1582
- Layout Navigator 1597
- Spreadsheets Use Case 1 - Working with Custom Tokens, Formula References, and Conditional Formatting 1620
- Spreadsheets Use Case 2 - Creating a regression analysis with an Antibody Titration Series 1653
- Spreadsheets Use Case 3 - Performing Channel Calibration and Regression Analysis 1679
- Spreadsheets Use Case 4 - Creating and working with Pie, Bar and Scatter Plots 1703
- High Content Flow 1739
- DNA Analysis 1795
- Proliferation 1833
- Kinetics 1856
- Cell Concentration 1885
- Channel Calibration 1906

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- CellProfiler Export 1921
- ImageJ Export 2019
- Exporting from IsoCyte 2043
- Picture Plots 2060
- Introduction to FCS Express 6 Image Cytometry 2098
- Immunophenotyping 2186
- Changing Common Preferences 2232
- Cluster Analysis 2238

38.1 Using Objects

Objects (e.g., plots, text boxes, statistic tables, and pictures) are items that are placed onto an FCS Express document (called a layout). Objects in FCS Express can be manipulated similarly to objects in Microsoft PowerPoint, which means they can be moved, resized, deleted, grouped, aligned, cut, copied, and duplicated. In order for an object to be manipulated, the object must first be **selected**. A red border surrounding the object denotes a **selected** object. An object can also be in **Edit** mode, which is denoted by a green border. **Edit** mode will be described in detail later.

The first section of this tutorial will describe Inserting an Object 1211.

38.1.1 Inserting an Object

To insert an object on a page, simply choose the object from the **Insert** tab on the Ribbon. In the following example, we will insert a picture.

To insert a Picture:

1. Select **Insert→General→Picture** (Figure T1.1).
2. Click the mouse where you want the top-left corner of the picture to appear on the page. A file selection dialog will appear to allow you to choose the picture file you wish to insert.

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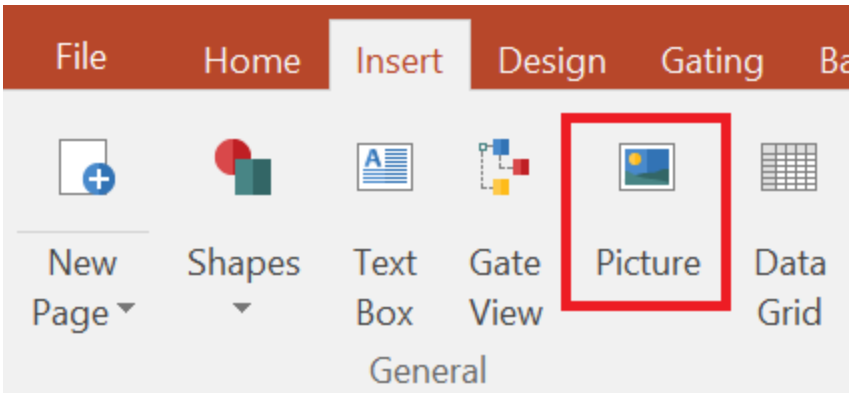

Figure T1.1 Insert Picture

3. Select the file *Logo.png* as shown in Figure T1.2 (we will use the De Novo Software Logo as an example for the rest of the tutorial). If *Logo.png* is not available, you can use any picture file.

Figure T1.2 De Novo Software Logo

The red border around the object signifies that the object is selected. For more information on selected and edit modes, refer to the *Layout Page Features* section in the FCS Express manual.

In the next section, we will practice selecting and deselecting objects 1212.

38.1.2 Selecting and Deselecting Objects

Clicking anywhere outside the object on the layout background will deselect the object. To select the object again:

1. Move the mouse until it is on the border of the object.

2. Click on the border. You will know the cursor is in the right place because the cursor will change to a four-headed arrow.

Now, we will resize an object 1213.

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38.1.3 Resizing an Object

Once an object is selected, you can resize it by moving the cursor to one of the **resizing handles** (red circles, Figure T1.3) along the border of the object.

The example below shows how to resize the object both horizontally and vertically at the same time:

1. Move the cursor above the resized handle on the bottom right hand corner of the object until the arrow cursor changes to a two-headed diagonal arrow cursor.
2. Press and hold down the left mouse button while moving the cursor to an appropriate position. A transparent preview image of the object will appear showing the new size as you move the mouse. You can maintain the aspect ratio of an object by holding down the **Shift** key while you resize the object.
3. Release the mouse button and the object will resize to the size of the preview image.

Figure T1.3 Resizing an Object

We will now learn to move an object.

38.1.4 Moving an Object

To move an object (Figure T1.4):

1. Move the cursor along the border until a four-headed arrow cursor appears.
2. Press and hold down the left mouse button.

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3. Move the mouse while holding down the button. A transparent preview image showing the new position of the object will appear as you move the mouse.

4. Continue moving the mouse until the preview image is in the desired location.

5. Release the mouse button. The object will now move to the new location.

Figure 1.4 Moving an Object

Alternatively, you can move the object by selecting it and pressing the arrow keys on the keyboard (up, down, left, right). To move the object in smaller increments using the keyboard, hold down the **Shift** key while pressing the arrow keys.

The next topic is deleting an object 1214.

38.1.5 Deleting an Object

To delete an object:

1. Select the object as described above 1212.

2. Press the **Delete** key on the keyboard

OR

3. Select the **Home→Editing→Delete** command (Figure T1.5).

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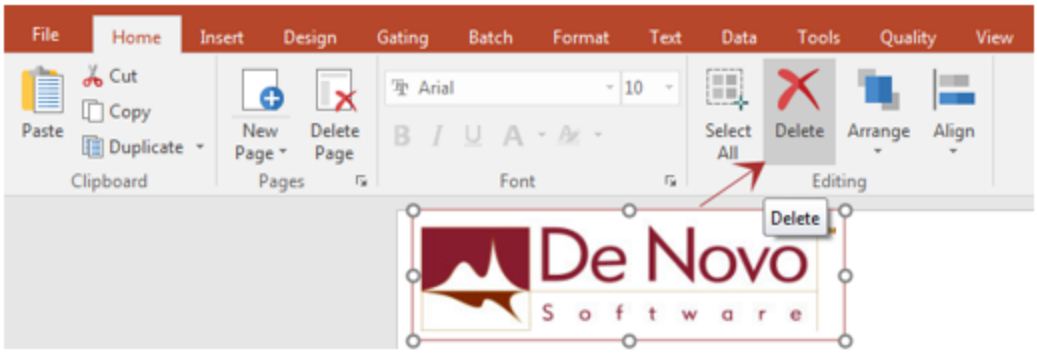

Figure T1.5 Deleting Objects

If you decide that you didn't want to delete the object, you can undo or redo your changes in the next section.

38.1.6 Undoing and Redoing Changes

FCS Express 6 allows for any number of previous operations to be undone and redone.

To undo or redo any changes:

1. Click the **Undo** button in the **Quick Access Toolbar** (Figure T1.6). The text on the **Undo** menu item will change to inform you which operation can be undone. For example, to undo the delete of the picture in the step above, click the **Undo** button. You can also press **Ctrl+Z** to undo changes.

2. Click the **Redo** button in the **Quick Access Toolbar**. The text on the Redo menu item will change to inform you which operation can be undone. You can also press **Ctrl+Y** to redo changes.

The next topic is duplicating objects.

38.1.7 Duplicating Objects

To duplicate an object:

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1. Select the object as described above 1212.
2. Select the **Home→Clipboard→Duplicate** command. The new object will appear offset slightly lower and to the right of the original object (Figure T1.7).

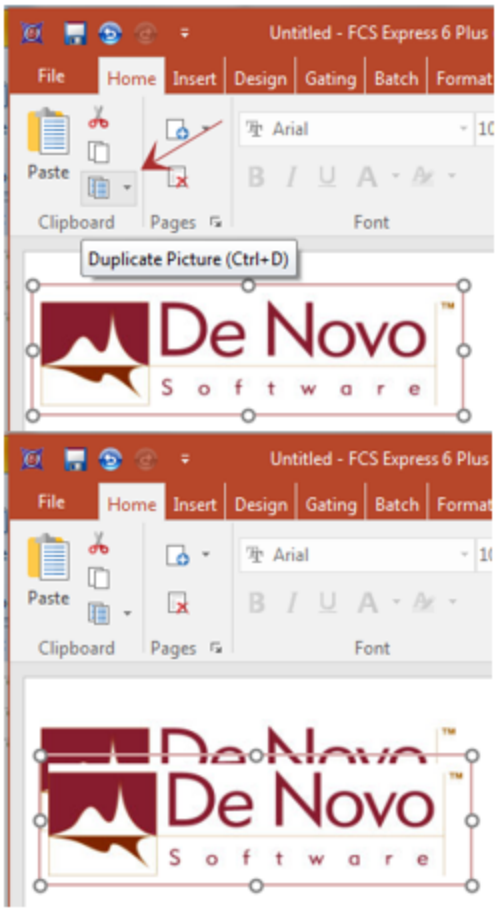

Figure T1.7 Duplicating Objects

In the next section, we will select multiple objects 1216.

38.1.8 Selecting Multiple Objects

To select multiple objects using the mouse (Figure T1.8):

1. Place the cursor outside the border region near a corner of one of the objects you would like to select.

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2. Press and hold the left mouse button.
3. Move the cursor. A gray highlighted box will appear as you move the cursor around the layout.
4. Size the gray highlighted box until all the objects to be selected are contained within the box.
5. Release the left mouse button.

All of the objects will now be selected as seen by the presence of a red border around each one.

Figure T1.8 Selecting Multiple Objects

To select multiple objects using the keyboard:

1. Hold down the **Shift** key on the keyboard.
2. Click inside or on the border of an object that you want to select. When the object is selected the border will change to red.
3. Continue holding down **Shift** and move the cursor to another object you want to select.
4. Click inside or on the border of this object. You may also deselect during this multiple selection process by shift-clicking an already selected object.
5. Continue following steps 3 & 4 until all the objects that you want to select are selected.
6. Release the **Shift** key. All the objects you have selected will have a red border.

The next section is copying objects 1218.

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38.1.9 Copying Objects

FCS Express allows you to copy one or more objects to the clipboard and paste them onto the same or a different layout page.

To Copy objects:

1. Select the object or objects to be copied as described above 1212.
2. Select the **Copy Picture** command (Figure T1.9), from the **Clipboard** Group on the **Home** tab. The text of the **Copy** menu item will inform you which object will be copied. Alternatively, you can use the **Ctrl+C** shortcut.

Figure T1.9 Copying Objects

We will now proceed to pasting objects 1218.

38.1.10 Pasting Objects

To paste objects that are on the clipboard:

1. Select the **Paste Bitmap** command (Figure T1.10) from the **Clipboard** group on the **Home** tab. The text of the **Paste** menu item will inform you which object will be pasted. Alternatively, you can use the **Ctrl+V** shortcut.

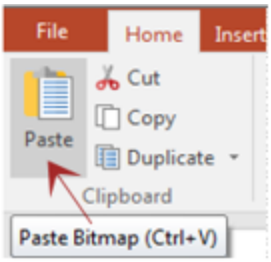

Figure T1.10 Pasting Objects

In the next step, we will learn to group objects 1219.

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38.1.11 Grouping Objects

Individual objects on a page can be *grouped* together. This means that as soon as one object in the group is selected, all of the objects in the group will become selected. This is useful, for example, if several objects are in a specific relative location to each other and you want to make sure that you cannot move one without moving all of the others.

To group several objects together:

1. Select the objects that you want to group, as described above 1212.
2. Select **Group** from the **Home→Editing→Arrange** command drop-down list (Figure T1.11).

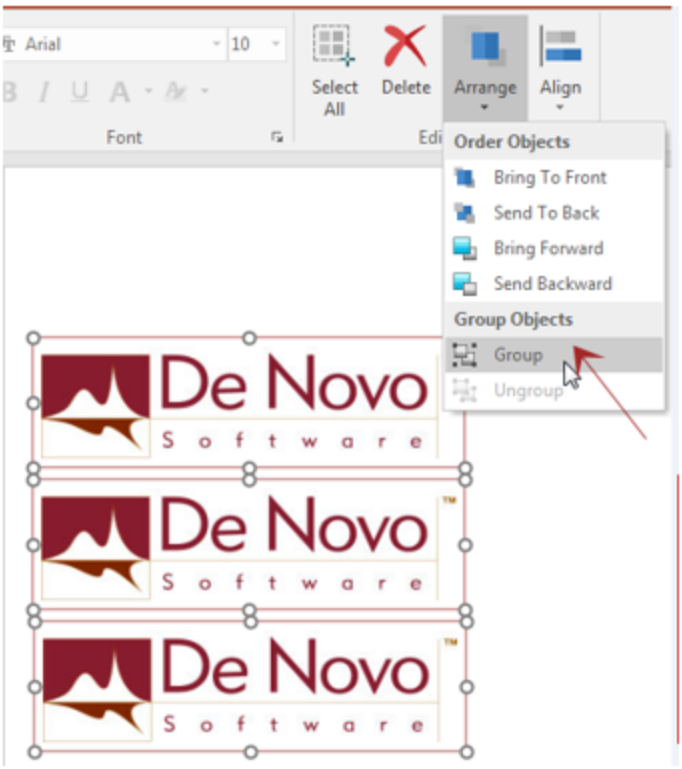

Figure T1.11 Grouping Objects

3. Click on a blank area of the layout to unselect all the objects.
4. Click on the border of one of the grouped objects. They should all become selected.

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To ungroup grouped objects:

1. Select the group of objects you want to ungroup, as described above.
2. Select **Ungroup** from the **Home→Editing→Arrange** command drop-down list.

In the next section, we will learn to align objects 1220.

38.1.12 Aligning Objects

FCS Express allows both horizontal and vertical alignment of objects by their sides, tops, bottoms or centers. This alignment feature also allows you to resize an object's height or width with respect to another object's size. Objects are always aligned with respect to the first object selected.

To horizontally align multiple objects:

1. Select 1212 all of the objects you want to align.
2. Select **Align** from the **Home→Editing→Arrange** command drop-down list (Figure T1.12). An **Alignment** drop-down list will appear.
3. Select **Centers** from the horizontal alignment list (Figure T1.12).

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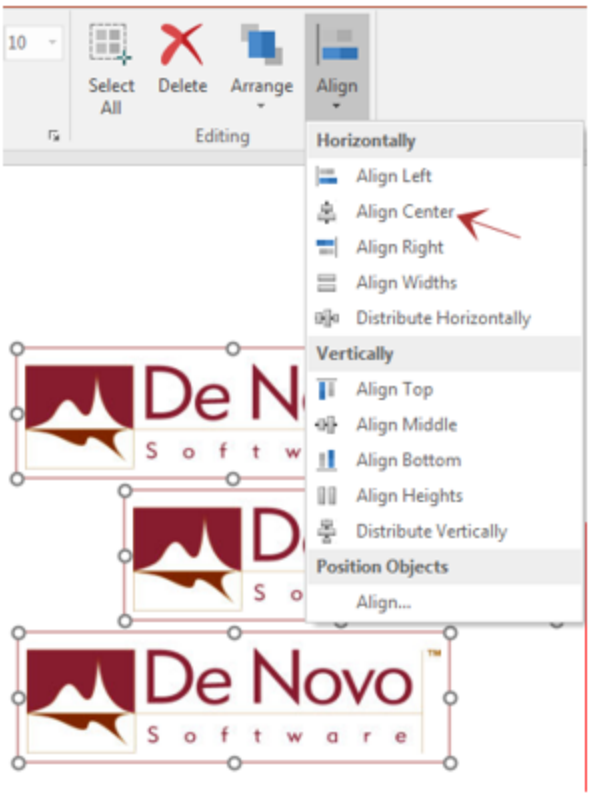

Figure T1.12 Aligning Objects (Step 2)

The selected objects are now horizontally aligned (Figure T1.13).

Figure T1.13 The objects before and after the alignment.

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To make several objects have the same height and width:

1. Select the object that is the size you wish all the other objects to be.
2. Select the objects you wish to resize.
3. Select **Align** from the **Home→Editing→Arrange** drop-down list (Figure T1.14a).
4. An Alignment dialog box will appear in the center of the layout (Figure T1.14b).
5. Select **Widths** from the horizontal alignment list.
6. Select **Heights** from the vertical alignment list.
7. Press the **OK** button.

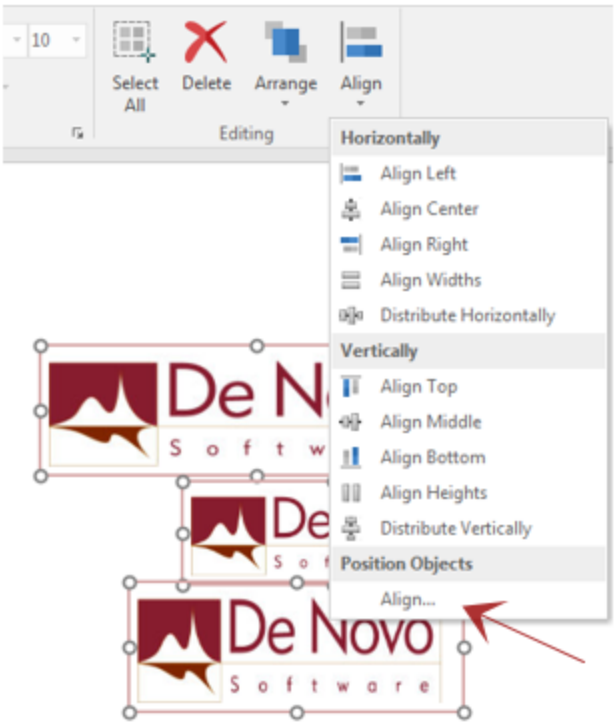

Figure 1.14a Equalize Height and Width

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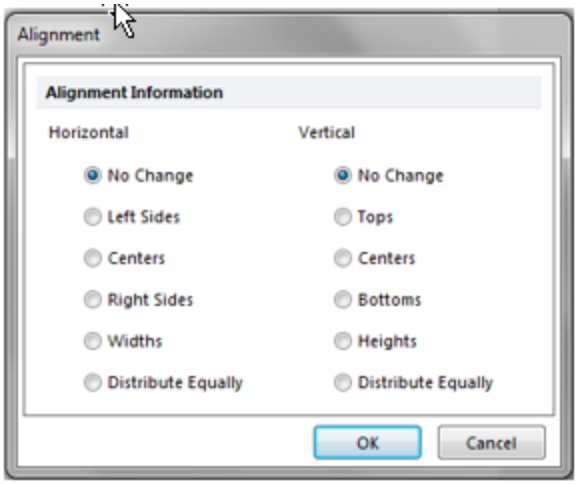

Figure T1.14b Alignment Dialog Box

All of the selected objects are now the same size as the first object selected (Figure T1.15). Alternatively, the height and width could have been aligned separately.

Figure T1.15 Aligning Height and Width

In the last section of this tutorial, we will set the order of different objects 1224.

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38.1.13 Ordering Objects

FCS Express allows for explicit placement of objects in front or behind other objects.

To move an object in front of another one:

1. Select the object that you want to move in front, as described above.
2. Select **Bring to Front** from the **Home→Editing→Arrange** command drop-down list (Figure T1.16).

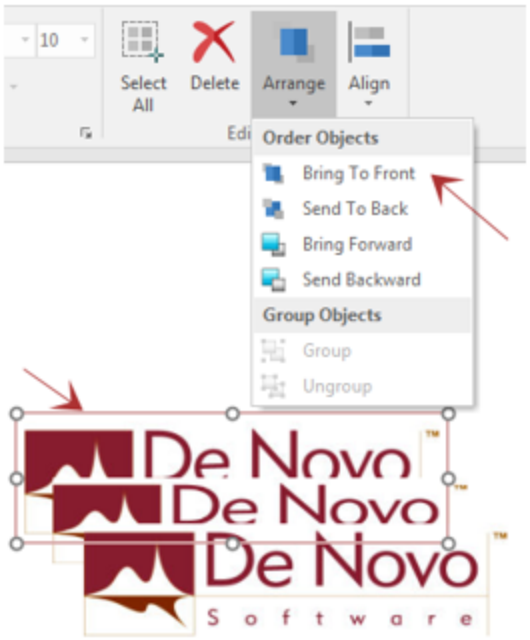

Figure T1.16 Ordering Objects (Step 2)

The selected object will be brought to the front (Figure T1.17).

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Figure T1.17 Ordering Objects (Final Result)

38.2 Inserting Plots

In the course of this example, we will:

- Learn how to insert plots on a layout.

The basic way to insert a plot is to use commands from the **Insert→1D Plots**, **→2D Plots**, or **→Other Plots** groups.

Although any data files of your own can be used to follow along, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive 1210. The files **GFPcontrol.001** and **GFPsample.002** are used here.

We will start by inserting a 2D plot 1225.

38.2.1 Inserting a 2D Plot

To insert a 2D plot:

1. Select the **Insert→2D Plots→Dot** command (Figure T2.1).

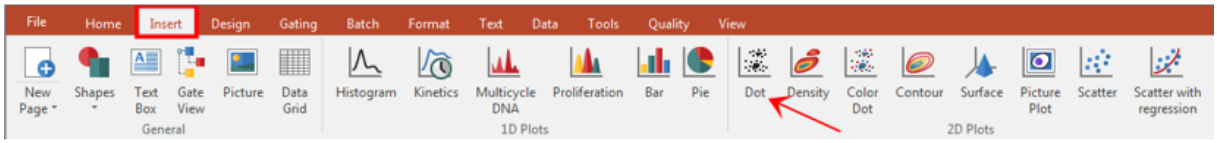

Figure T2.1 Inserting a Dot Plot

2. Move the mouse to a place on the layout where you would like the top-left corner of the plot to be located.

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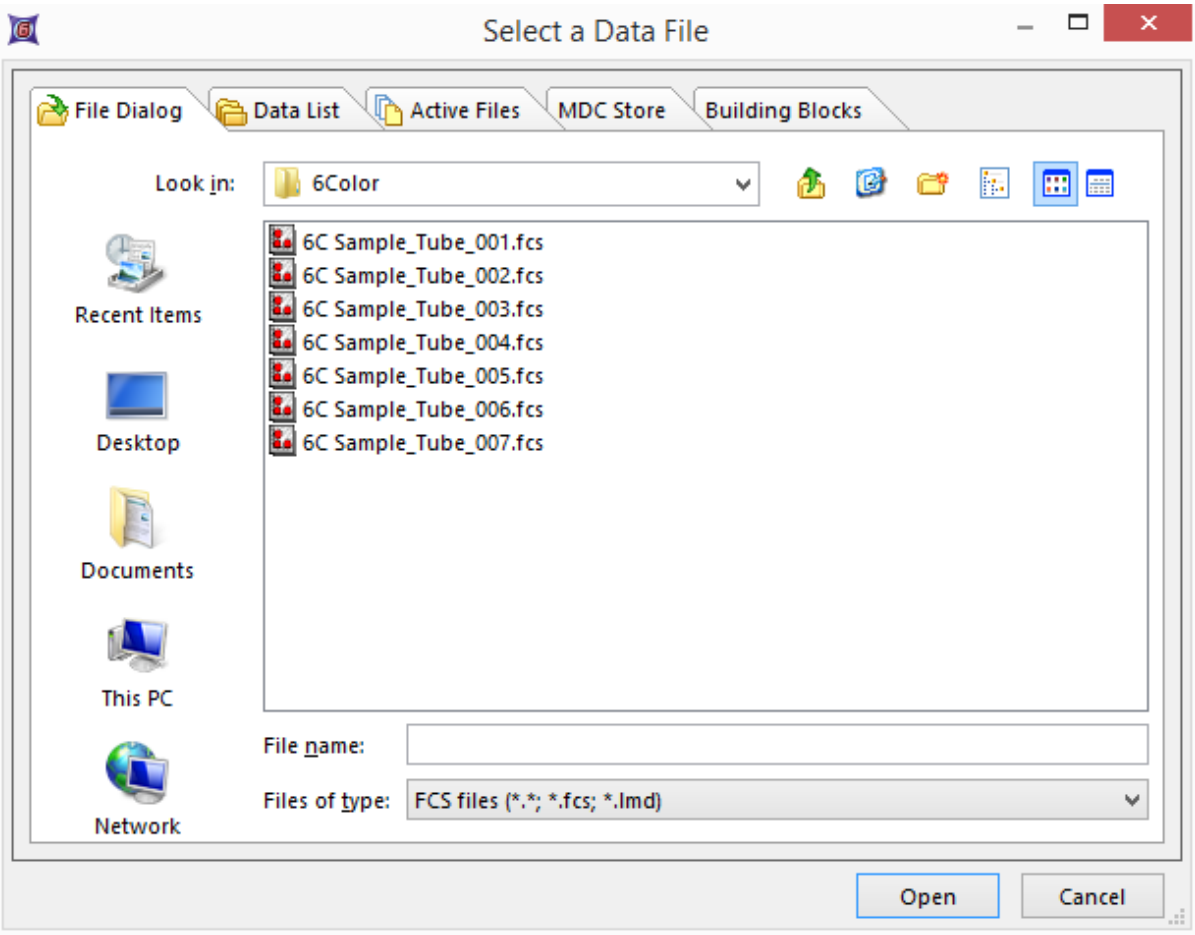
3. Click the mouse on the page where you want the top-left corner of the plot to appear.

A **Select a Data File** dialog box will appear (Figure T2.2).

Notice that all the data files in the dialog box appear with an FCS file icon, even though their extension is not ".fcs". This is because FCS Express knows that many FCS files are not saved with a ".fcs" extension, and it performs special checking (and appropriately sets the icon to make sure you can easily recognize your FCS data files).



4. Select the **GFPcontrol.001** data file from the FCS Express **Sample Data** directory (Figure T2.2).

5. Press the **Open** button**.**

The newly inserted 2D dot plot will now appear in the layout with a default size (Figure T2.3). You can change the default size by placing the cursor over

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any of the red handles on the selected plot and adjusting as necessary. Refer to the tutorial on using objects for more information on resizing objects 1213.

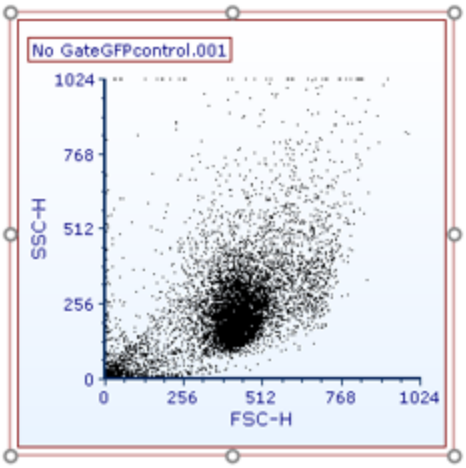

Figure T2.3 Inserting a 2D Plot

Next, we will insert a histogram 1227.

38.2.2 Inserting a Histogram

To demonstrate inserting a 1D plot in a custom size, we will add one to the layout made in the previous section.

1. Select **Insert→1D Plots→Histogram** (Figure T2.4).

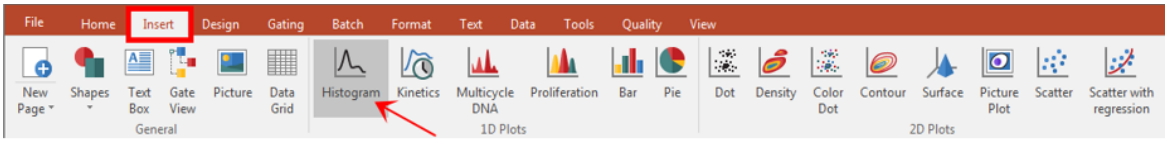

Figure T2.4 Insert Histogram Command

2. Move the mouse to a place on the layout where you would like the top left corner of the plot to be located.

3. Press and hold the left mouse button down.

4. Move the cursor, while holding the mouse button down, until the sizing rectangle is the size you want the histogram to be (Figure T2.5).

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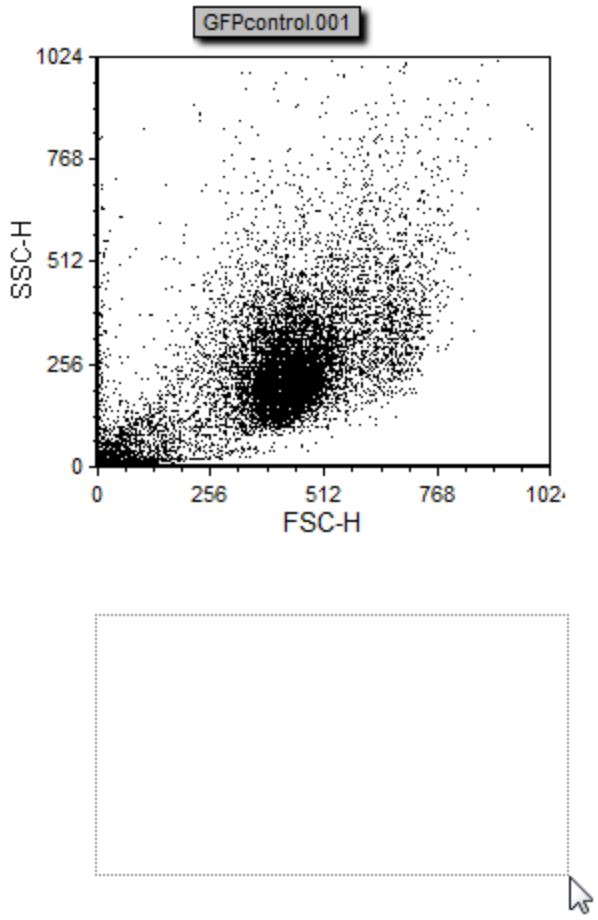

Figure T2.5 Insert Histogram (Step 4)

5. Release the mouse button and the histogram (shown with the red border) will be inserted in the place of the sized rectangle (Figure T2.6).

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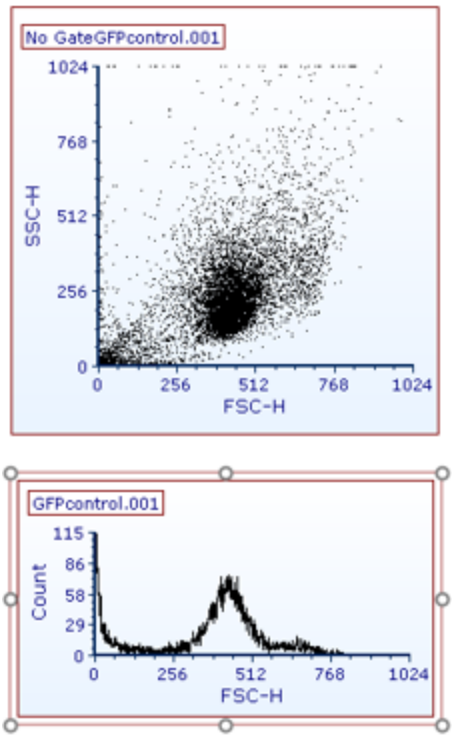

Figure T2.6 Insert Histogram (Step 5)

Note that when inserting the histogram, you were not prompted to select a file. This is because FCS Express uses the most recent data file when adding a new plot. You are only prompted to select a data file if no data files have been opened, or you select the **When creating a new plot, always display the selection dialog** preference from the **FCS Express User Options** (described below 1231).

Plots can also be inserted using the Data→Save/Load→Open command 1229.

38.2.3 Inserting from the Data Tab with the Open Command

To insert a plot:

1. Select the **Data→Save/Load→Open** command (Figure T2.7).

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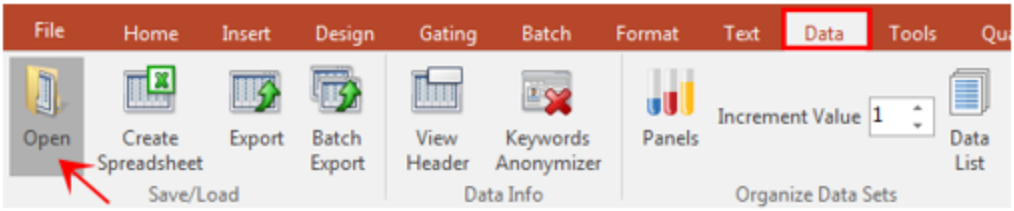

Figure T2.7 Open Command

A **Select a Data File** dialog will open as previously shown in Figure T2.2 1225.

2. Select the **GFPcontrol.001** data file from the *FCS Express Sample Data* directory.

3. Select **Open**.

A **Select Plot Types...** dialog will appear (Figure T2.8).

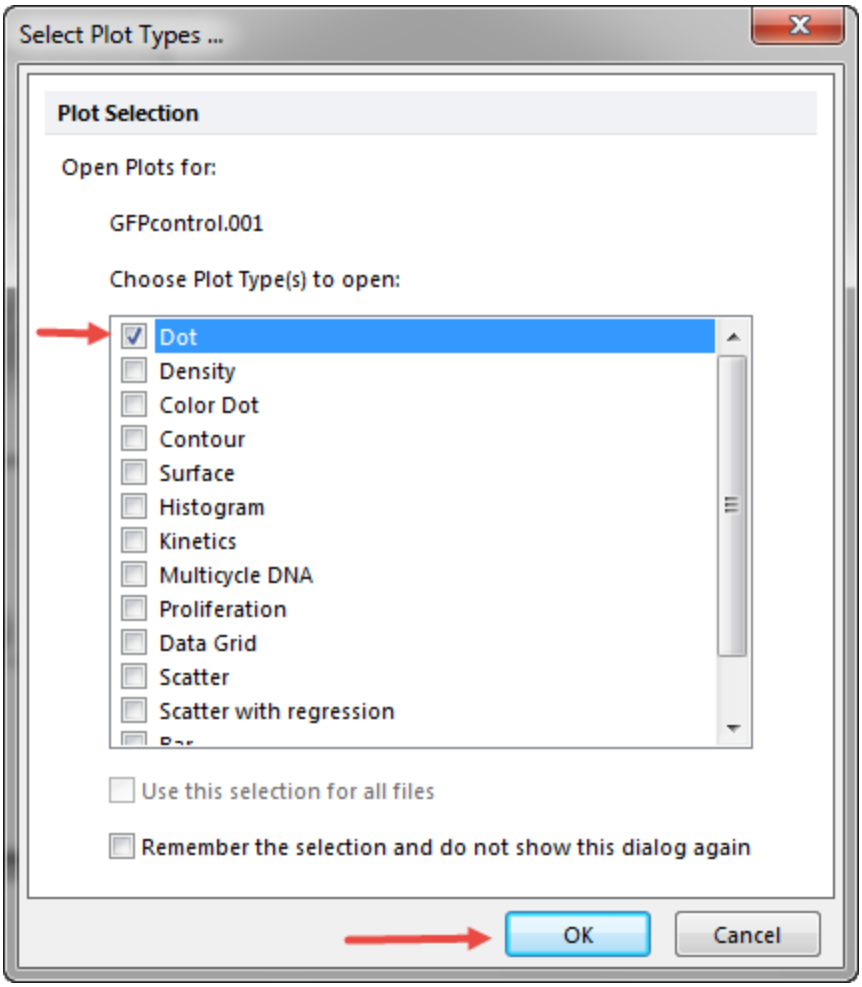

Figure T2.8 Select Plot Types... Dialog

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4. Select **Dot** as the Plot Type to open.
5. Select **OK**.

A 2D dot plot will now appear on the layout (with a red border to indicate it is in selected mode) as previously shown in Figure T2.3 1225.

You can force the display of the selection dialog 1231 using by setting a user preference.

38.2.4 Always Display Selection Dialog

When the default preferences are used, inserting a plot will insert the same data file as previously selected. To select alternate files, the **When creating a new plot, always display the selection dialog** preference can be set in **Options**.

1. Select the **File** tab (circled in red in the upper left-hand corner of Figure T2.9).
2. Select **Options** (shown in the lower left of Figure T2.9).

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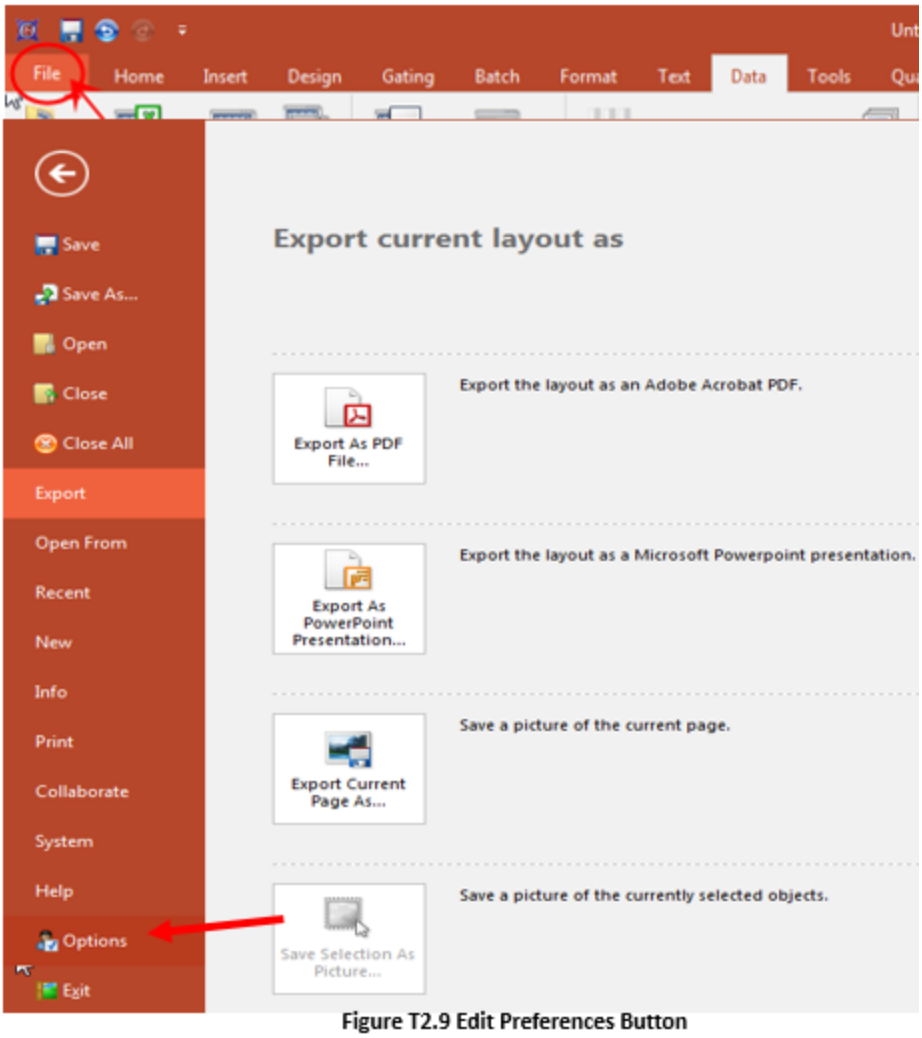

Figure T2.9 Edit Preferences Button

The **FCS Express User Options** dialog will appear (Figure T2.10).

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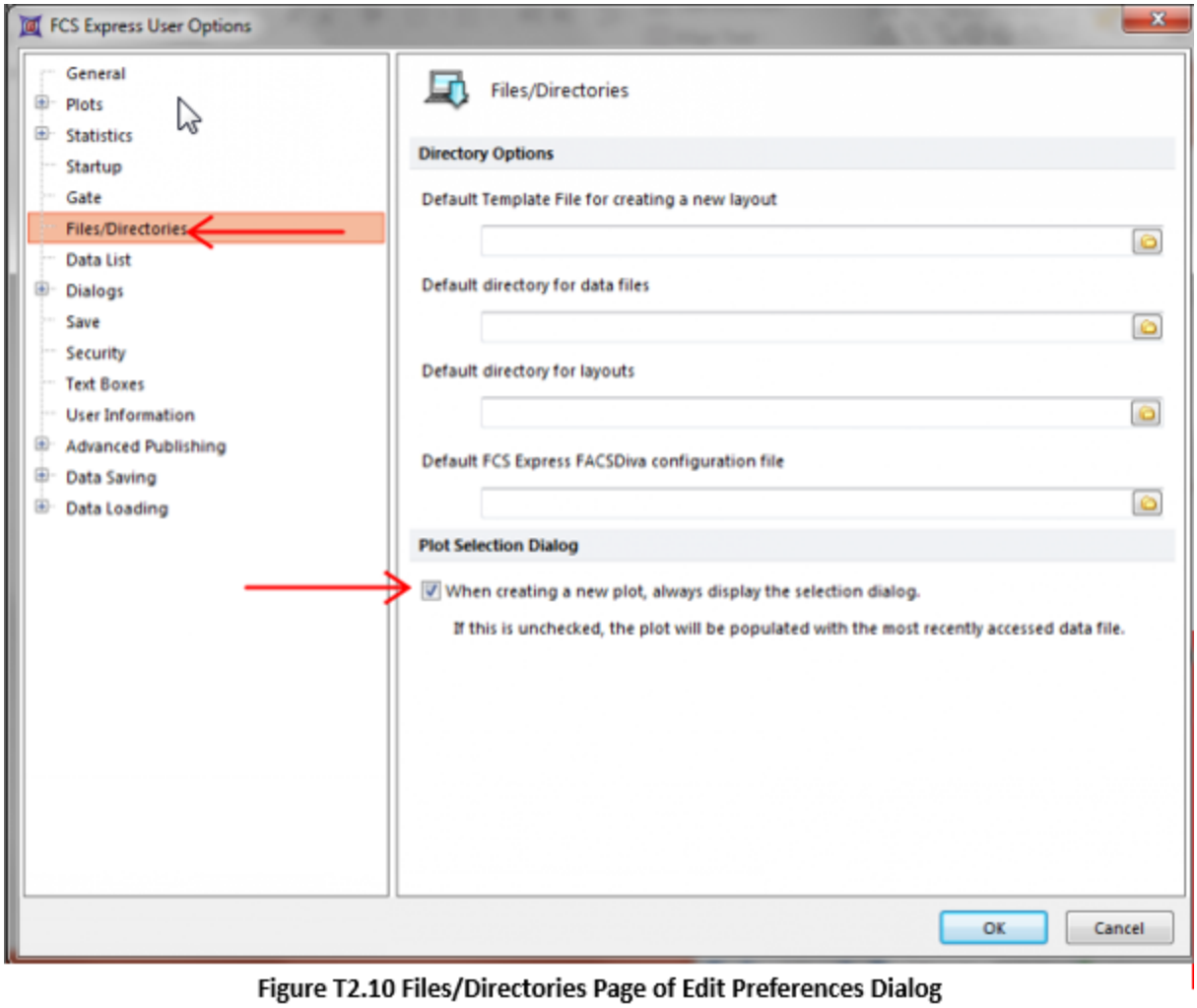

Figure T2.10 Files/Directories Page of Edit Preferences Dialog

3. Select the **Files/Directories** category from the left column.
4. Select the checkbox for **When creating a new plot, always display the selection dialog** (Figure T2.10).
5. Click **OK**.

Now we will open a data file and insert a 2D plot.

6. Select the **Insert→2D Plots→Dot** command.

The **Select a Data File** dialog box will appear, as previously shown in Figure T2.2 1225.

7. Select the file **GFPsample.002** from the **Select a Data File** dialog box.
8. Select **Open**.

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An **Open 2D Plots** dialog box for file GFPsample.002 appears (Figure T2.11). This is where you can select the desired X and Y parameters for the 2D plot.

9. Select **OK** to select the default parameters on the **Open 2D Plots** dialog box.

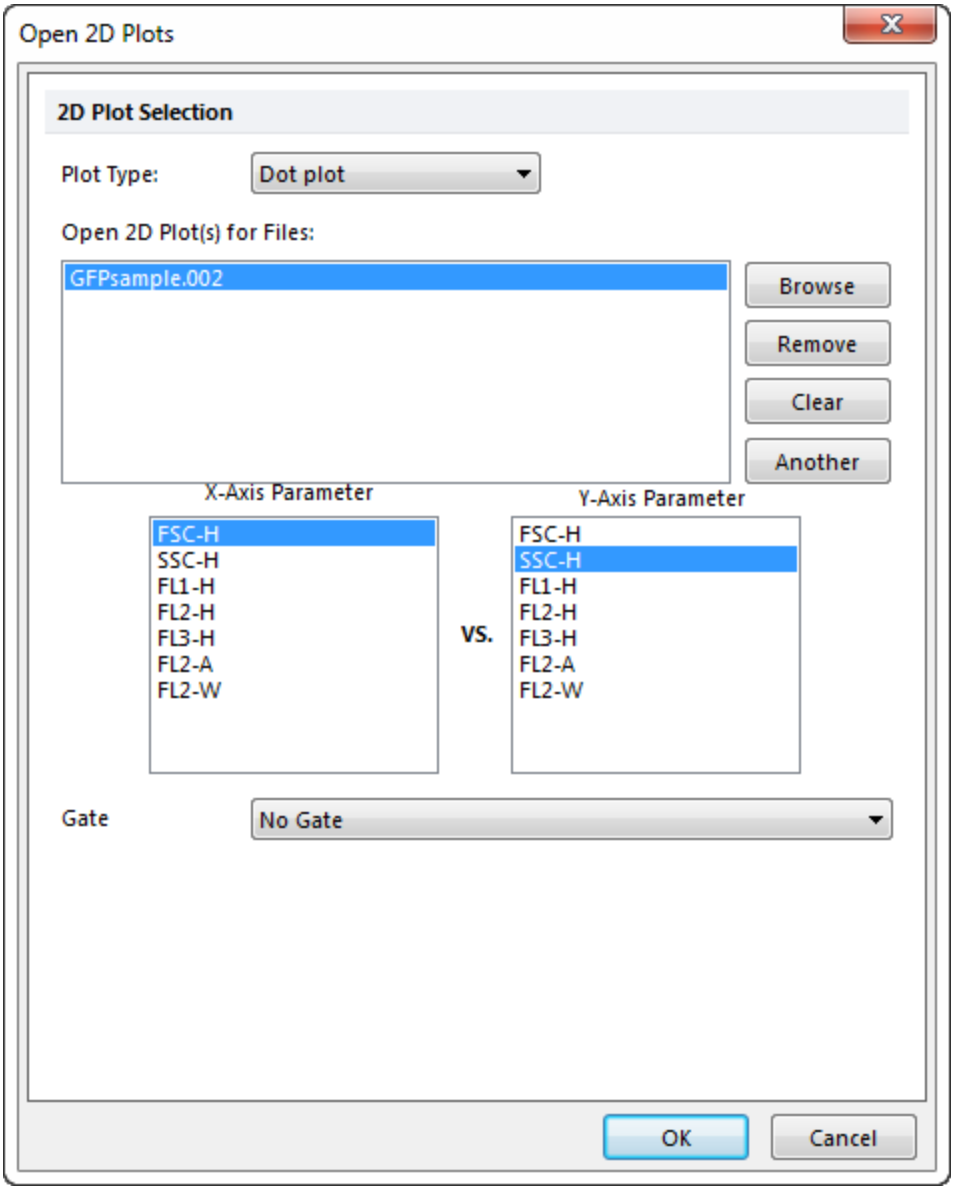

Figure T2.11 Open 2D Plots Dialog

The 2D dot plot of GFPsample.002 will now appear on the layout as shown in Figure T2.12.

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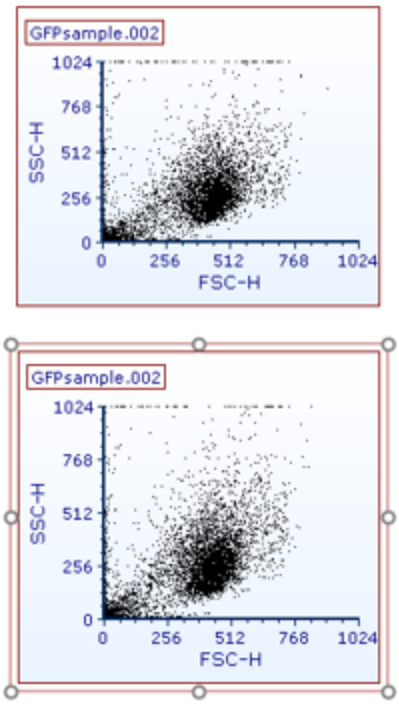

Figure T2.12 Inserted 2D Plot

Finally, we will insert plots from Windows Explorer.

38.2.5 Inserting Plots from Windows Explorer

FCS Express provides a variety of ways to open and select data files for plots. These options allow you to choose your preferred method of opening a plot from a file. In addition to the methods described above, you can select files directly from Windows Explorer and then plot them with FCS Express.

Opening a new plot with a file from Windows Explorer:

1. Open Windows Explorer.
2. Navigate to the *FCS Express Sample Data* directory using Windows Explorer**.**
3. Select the file **GFPSample.002** in Windows Explorer.

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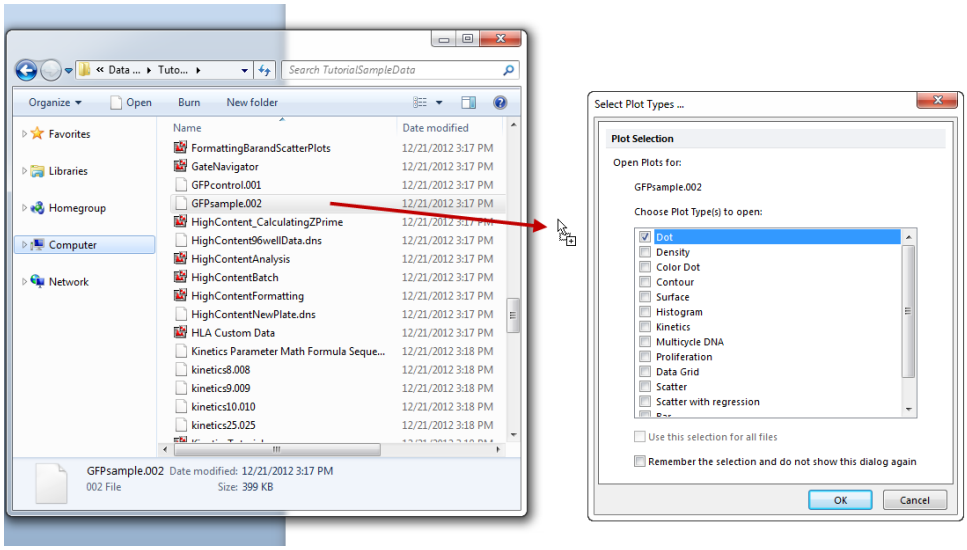
4. Press and hold down the left mouse button and drag the file **GFPSample.002** onto the layout in FCS Express.

The **Select Plot Types...** dialog appears as previously shown in Figure T2.8 1229.

5. Select **Dot** from the **Choose Plot Type(s) to open** window on the **Select Plot Types...** dialog.

6. Click **OK.**

A 2D Dot Plot of **GFPSample.002** will now appear on the layout using default settings.



Dragging and dropping data from Windows Explorer onto the Layout

38.3 Formatting Plots

All plot types share basic formatting options that include altering the titles, axes, background, legend, and 3D presentation. These options allow you to completely customize the look of your plots.

Although any data files of your own can be used to follow along, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive 1210. The layout **section4.fey** and the **ApoMono.001**, **ApoMono.002** data files will be used here.

We will start by using the pop-up menu to format plots 1237.

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38.3.1 Formatting Plots Using the Pop-up Menu

In the course of this example, we will:

- Open a Layout file.
- Insert data into a plot.
- Format a plot using the pop-up menu.

1. Select **File** tab→**Open** (Figure T3.1).

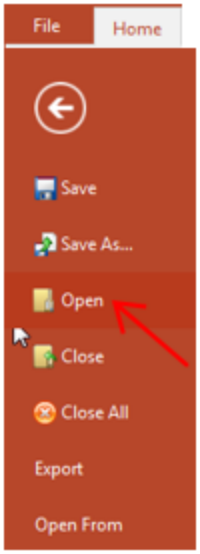

Figure T3.1 Open Layout

2. Select the **section4.fey** layout file located in the Tutorial Sample Data archive from the **Load Layout** dialog (Figure T3.2).

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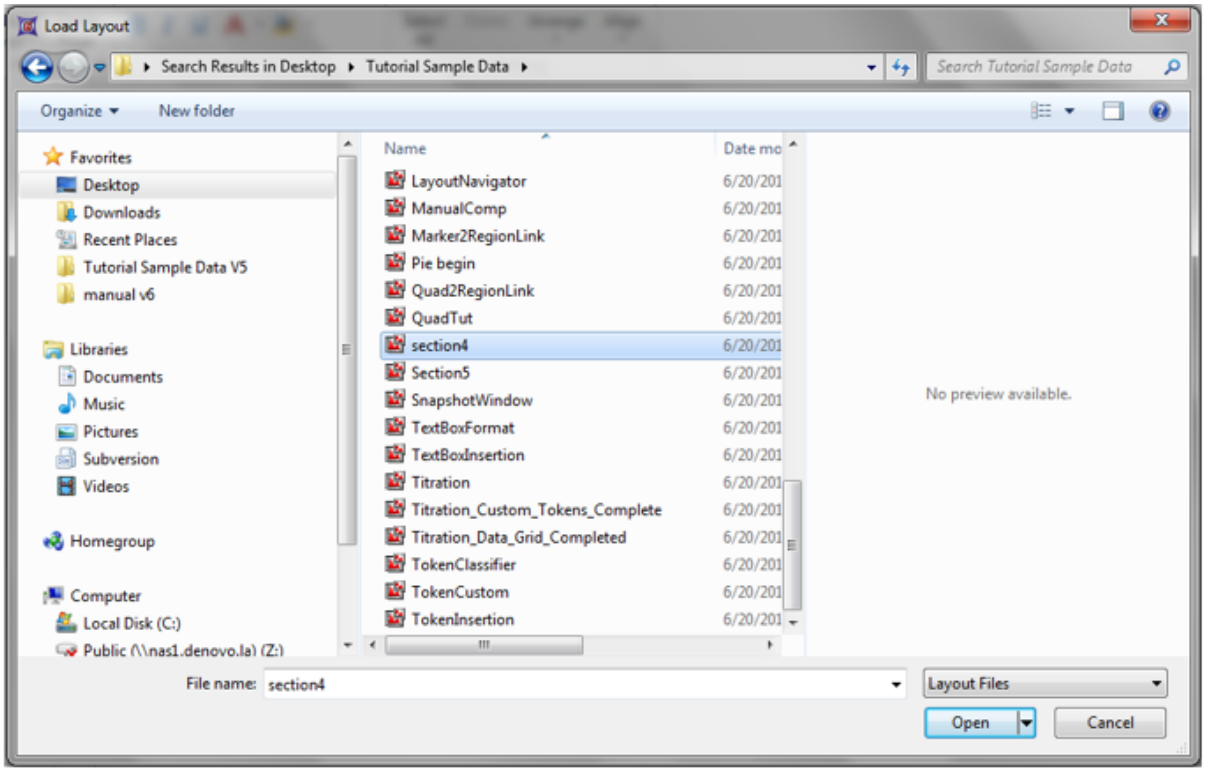

Figure T3.2 Load Layout Dialog

The Layout should have a single blank plot (Figure T3.3).

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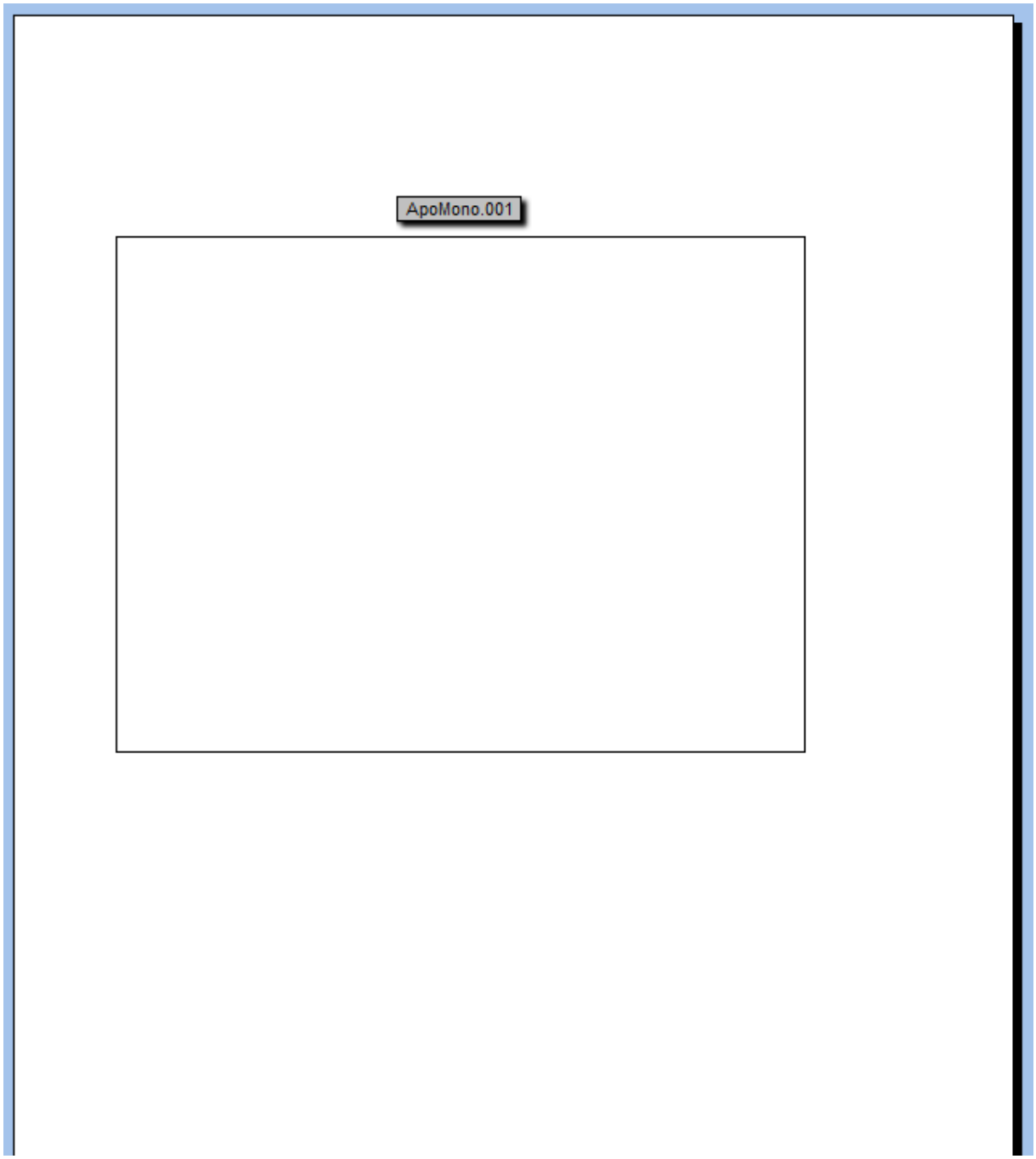

Figure T3.3 Section 4 Layout

3. Select the **Data→Change Data on All Objects→Select** command to insert a data file into the plot (Figure T3.4).

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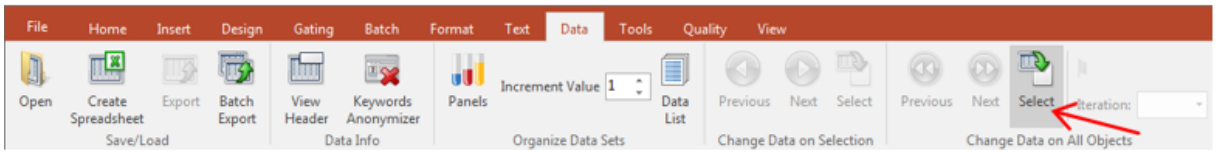

Figure T3.4 Select Command (Change Data on All Objects)

4. Select the **ApoMono.001** FCS file located in the Tutorial Sample Data archive.

5. Click **Open.**

6. Right-click on the plot so the pop-up menu appears (Figure T3.5).

7. Select **Format** from the pop-up menu.

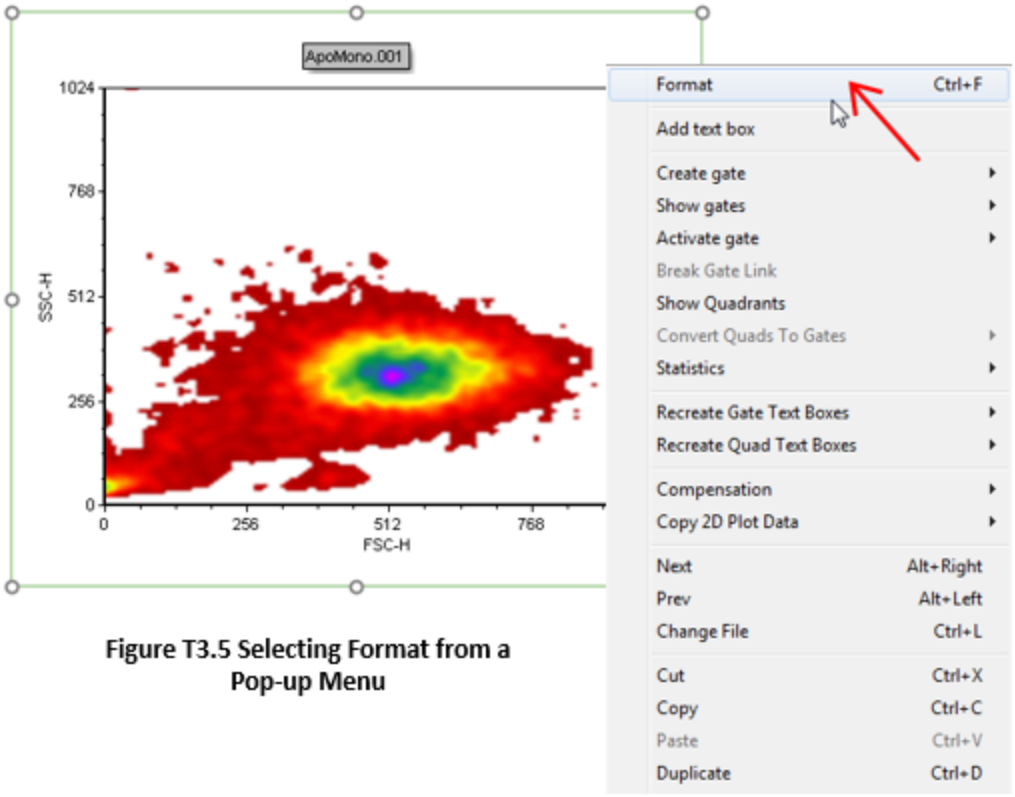

Figure T3.5 Selecting Format from a Pop-up Menu

Even though we have shown this example using a 2D plot, all objects in FCS Express have an associated pop-up menu and are formatted in a similar manner. Simply right-click on the object and select **Format** from the pop-up menu.

The **Formatting** dialog for 2D plots is shown in Figure T3.6.

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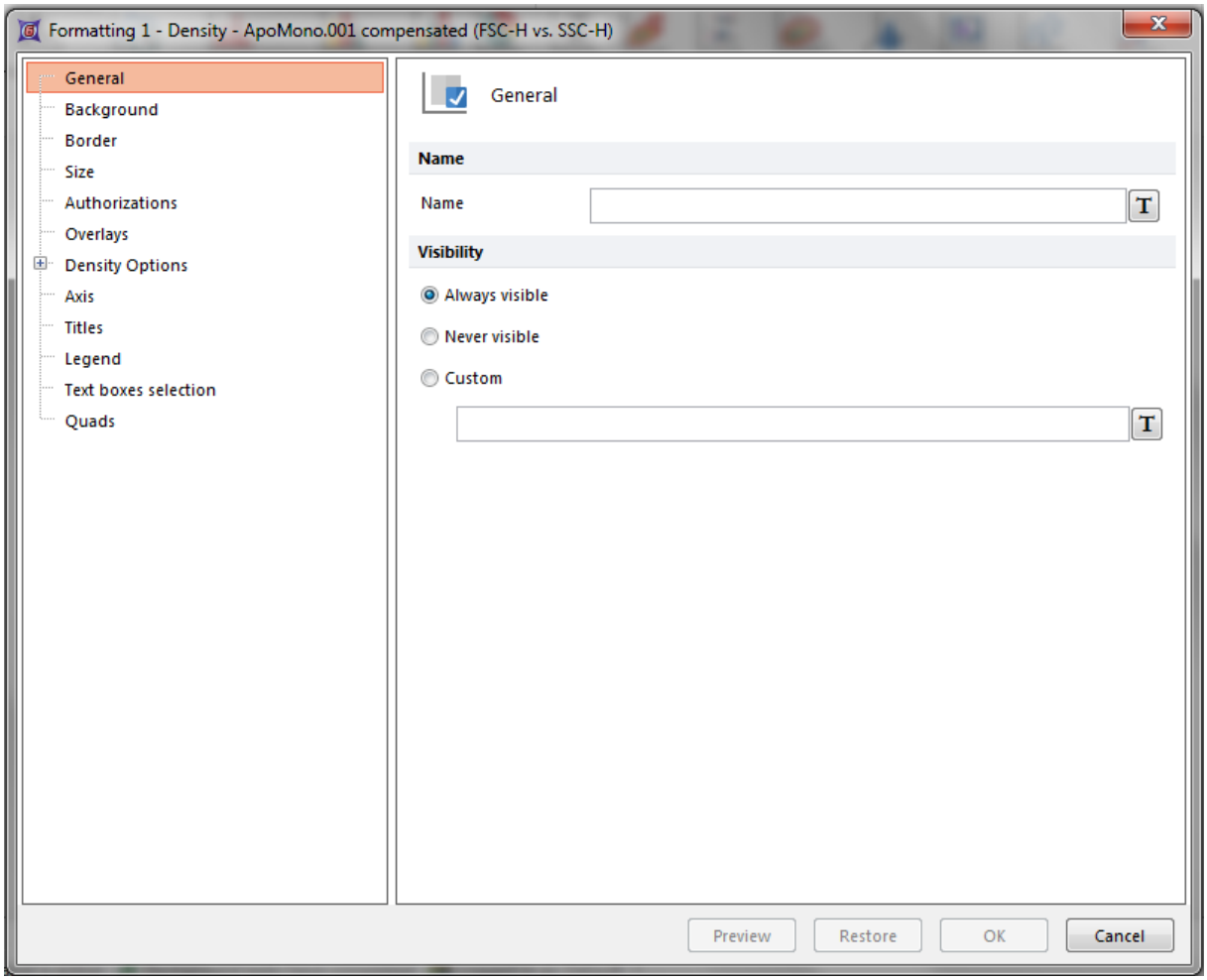

Figure T3.6 Formatting Dialog for 2D Plots

All formatting dialogs in FCS Express share several common features. The section on the left is called the *Category List*. This list organizes all of the different options for a particular object. The specific categories in the list change depending on which object is being formatted. When you click on a category in the category list, the right hand side of the dialog will change to show the specific options that pertain to that category.

The **OK** button of the format dialog will become enabled when you change the value of an option. You can also **Preview** and **Restore** the options without closing the formatting dialog. Remember, even if you press **OK** to change your options, and then decide that you don't like them, you can always undo your changes by selecting the **Undo** command from the Quick Access Toolbar 65.

In the next section, we will format multiple plots at the same time 1242.

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38.3.1.1 Formatting Multiple Plots

In the course of this example, we will:

- Duplicate a plot using the pop-up menu.
- Use the **Format** tab from the ribbon to format multiple plots simultaneously.

1. Right-click on the plot to bring up the pop-up menu.
2. Select **Duplicate** from the pop-up menu (Figure T3.7).

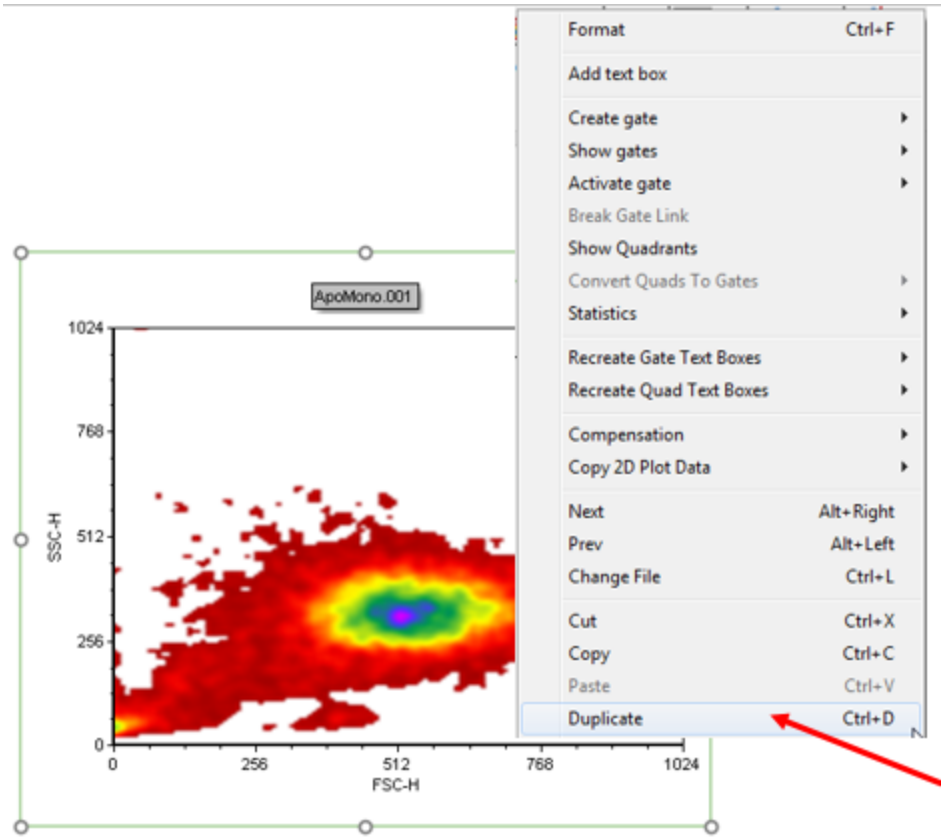

Figure T3.7 Duplicating a Plot

A duplicate copy of the plot should appear on top of the original plot (Figure T3.8).

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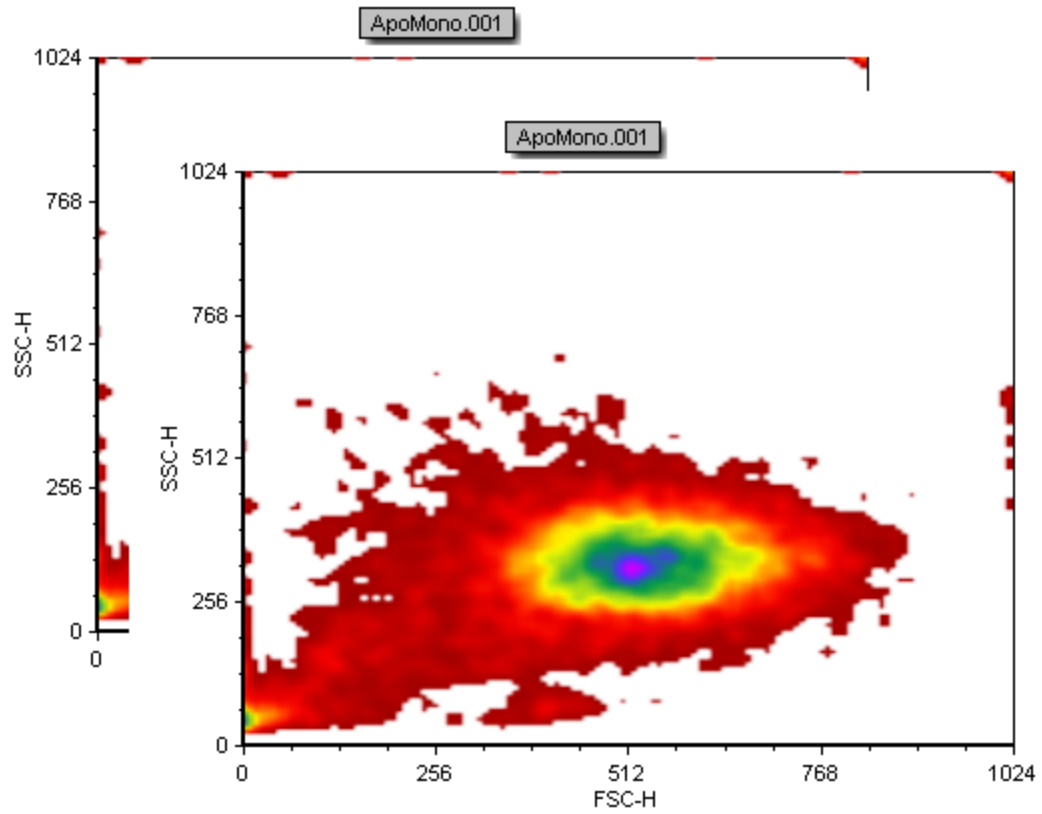

Figure T3.8 Duplicated Plot

3. Move the mouse over the top border of the duplicate plot, the cursor should change to a four-headed arrow.
4. Hold down the left mouse button while moving the duplicate plot down the page.
5. Release the mouse button when the duplicate plot is at a desired location.

There should now be two identical plots on the page as shown in Figure T3.9.

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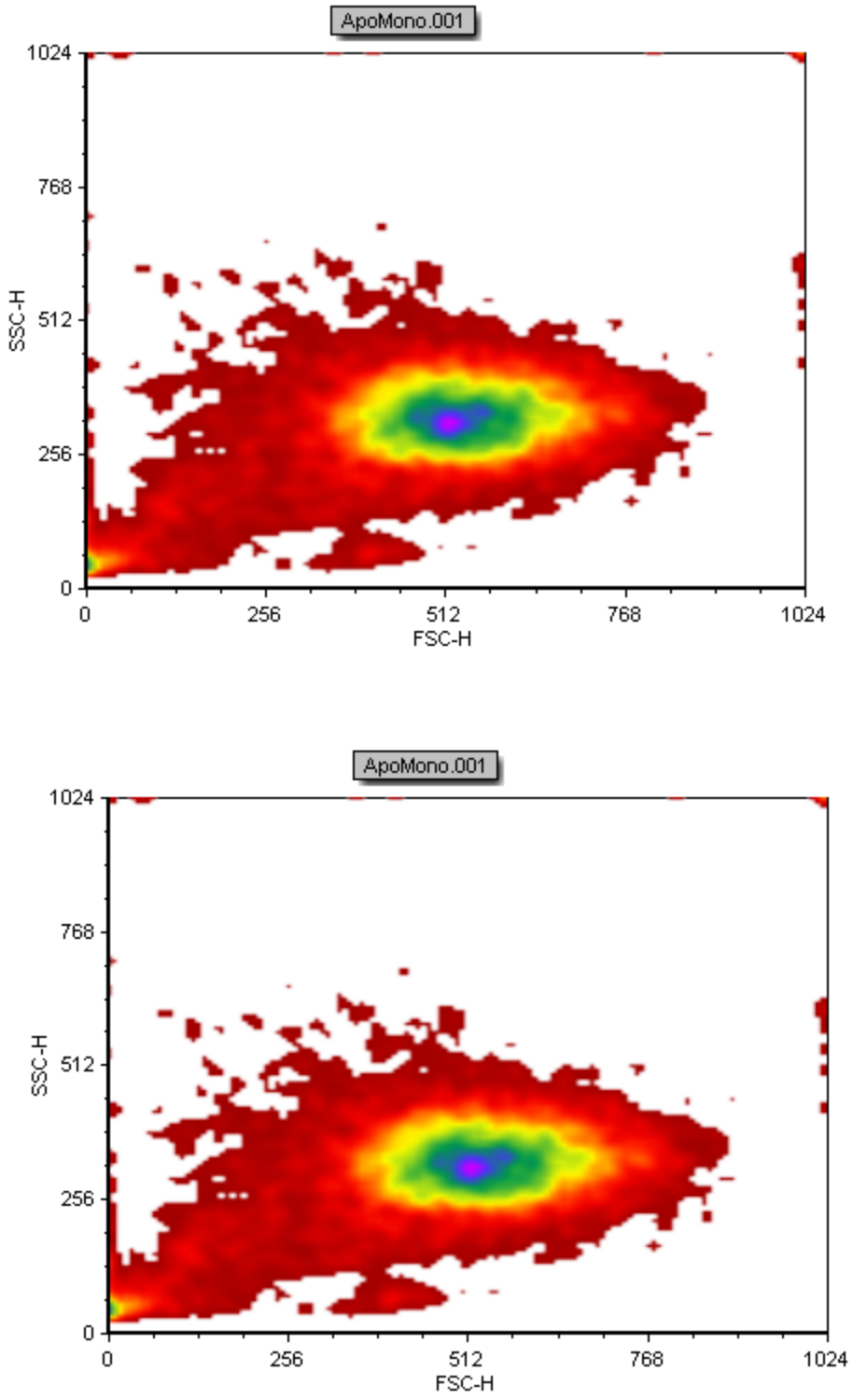

Figure T3.9 Duplicated Plots on a Layout Page

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6. Select both plots on the layout (see the tutorial on selecting multiple objects 1216).
7. Select **Format→Object Options→Border/Line** (Figure T3.10).

We will format the border/line of both plots at the same time.

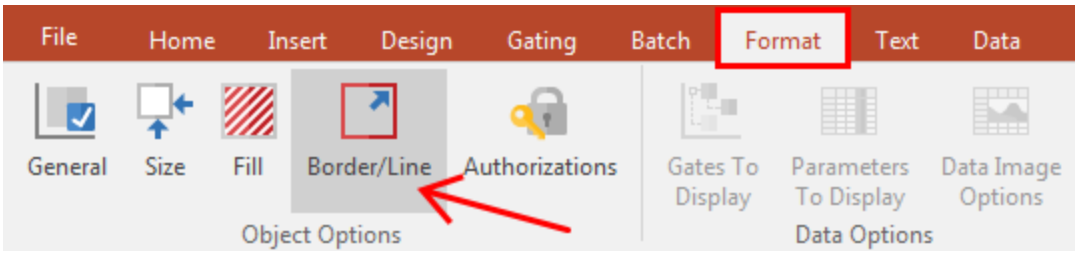

Figure T3.10 Format Border/Line Command

8. Select Line from the **Formatting Border/Line** dialog.
9. Select a solid line is the **Style**.
10. Select Blue as the Color (Figure T3.11).
11. Click OK.

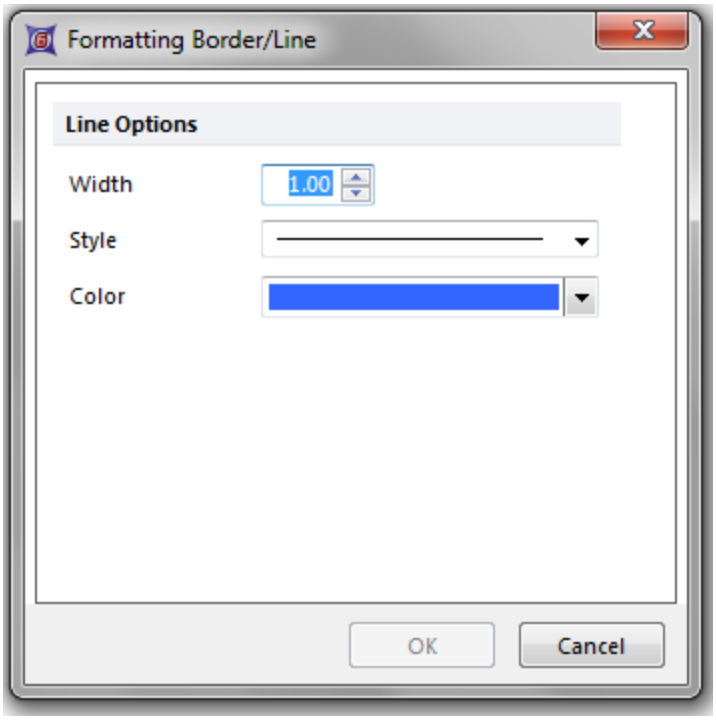

Figure T3.11 Formatting Border/Line Dialog

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Both plots will now have a blue border, as shown in Figure T3.12.

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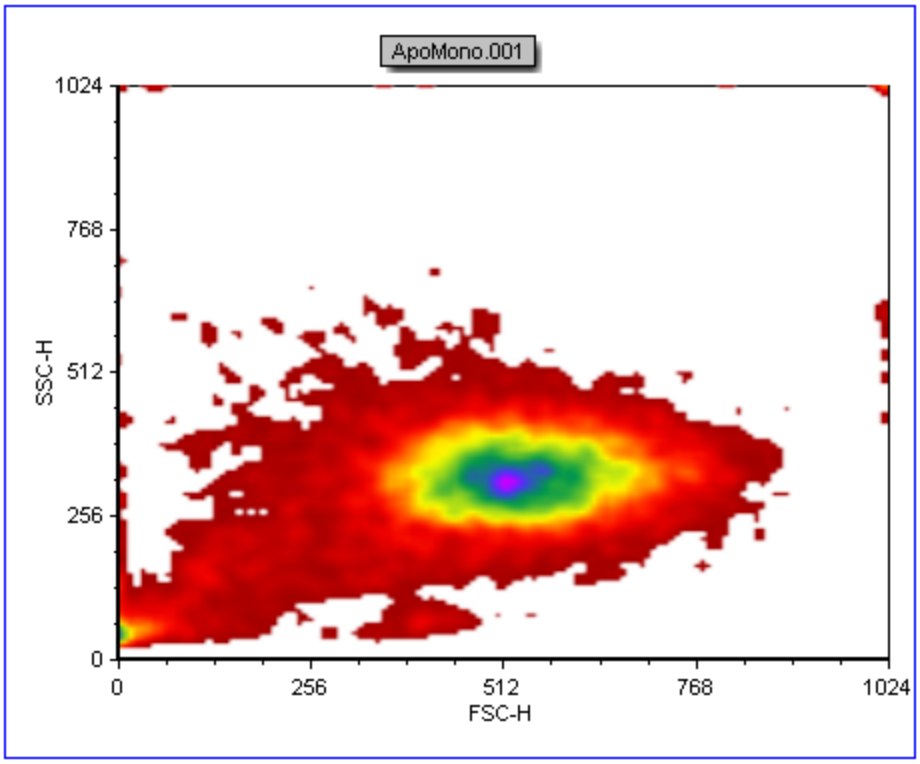

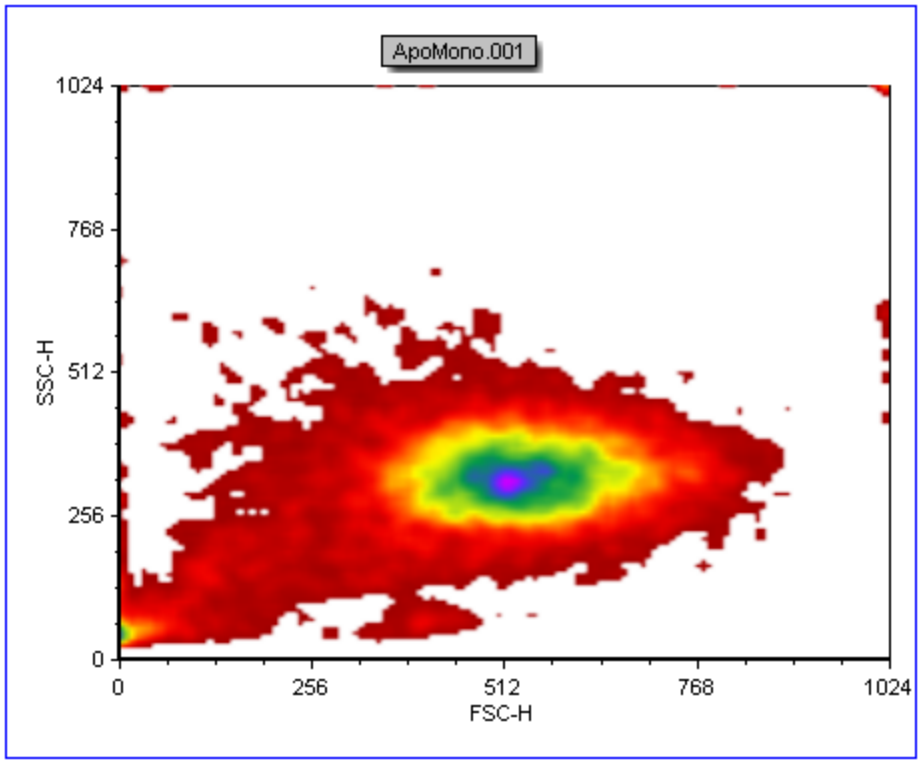

Figure T3.12 Plots with a Blue Border

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12. Select **Undo** from the Quick Access Toolbar 65 to undo the formatting changes to the plots (Figure T3.13).

Figure T3.13 Undo

The plots will now be restored to their original formatting with no border.

There are many options available for formatting objects using the **Format** tab (see Figure T3.14). If you hover your mouse over a command on the **Format** tab, a brief description of the command's actions appears, as shown for the **Fill** command in Figure T3.14.

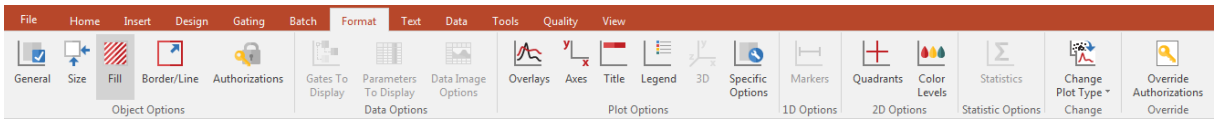

Figure T3.14 Format Tab

In the next section, we will format plot titles 1248.

38.3.2 Formatting Plot Titles

In the course of this example, we will:

- Acquire an overview of formatting plot titles.
- Change the title text.
- Change the title font.
- Change the shadow size.
- Change the alignment of the title.
- Change the footer properties.
- Insert keywords.

To change the plot title (numbers refer to Figure T3.15):

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1. Right-click on the top plot to open the pop-up menu.
2. Select **Format** from the pop-up menu (Figure T3.5 1237).
3. Click on the **Titles** category from the **Plot Formatting** dialog (Figure T3.6 1237).

The Formatting Titles category options shown in Figure 35.44 will appear.

4. Select **Header** from the Header/Footer list box ❶.
5. Press the **Font** button ❷ and increase the font size to **12** pt.
6. Press **OK** on the **Font** dialog when you have changed the font size.
7. Replace the existing title with the text "Light Scatter" ❸. This is the actual text that will appear as the title of the plot.
8. Change the **Shadow Size** to **0** to remove the shadow from the background of the header. ❹
9. Select **Right** from the **Alignment** list to right align the title. ❺

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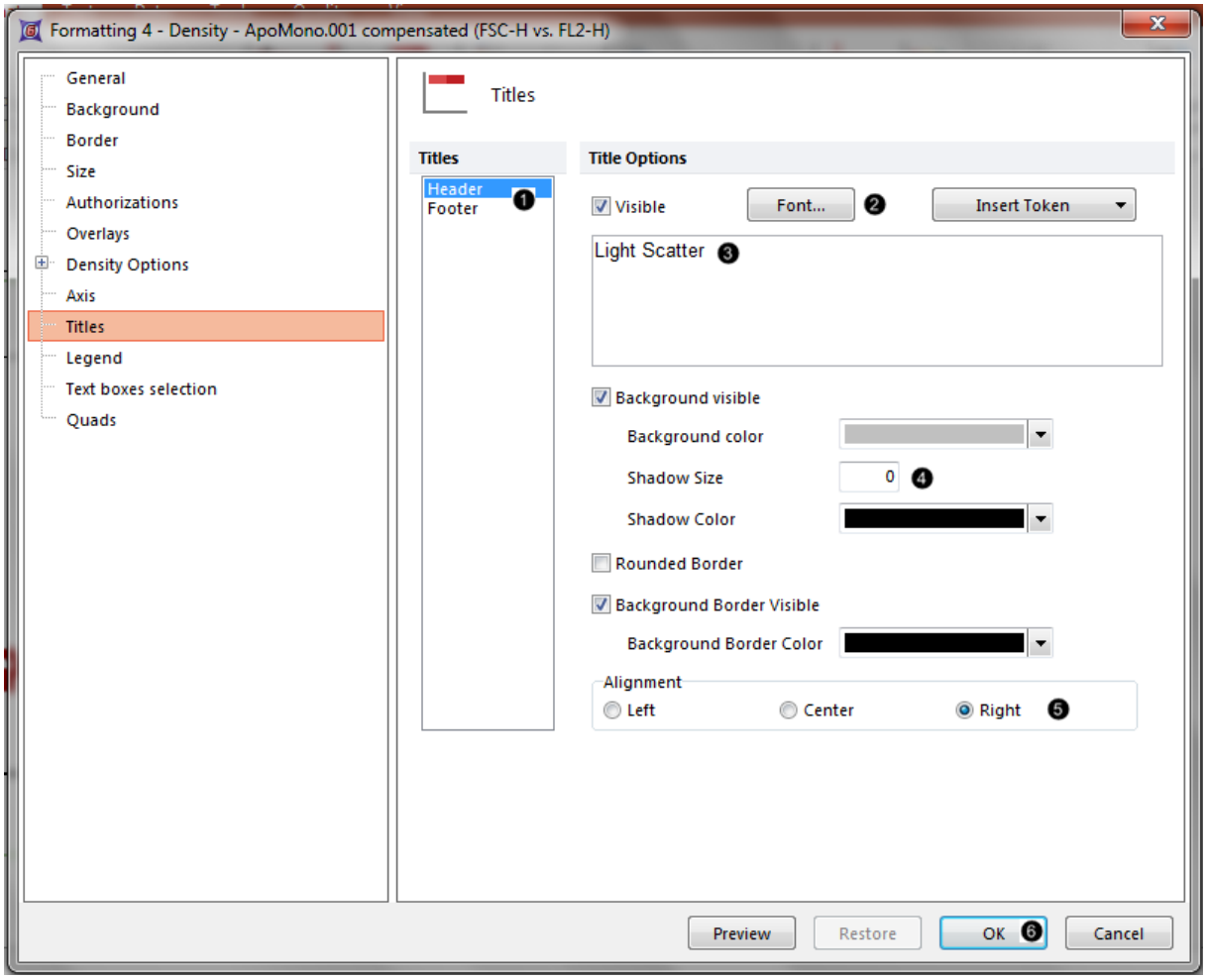

Figure T3.15 Formatting Titles Dialog

10. Press **OK** to apply the changes. ❻

The plot should now look like the plot in Figure T3.16B.

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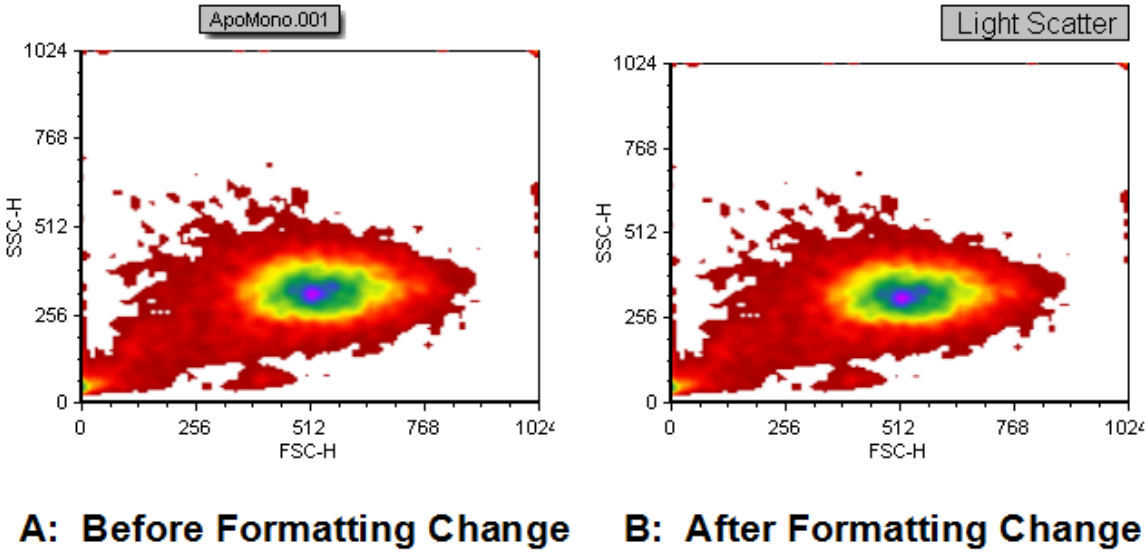

Figure T3.16 Changing the Header Properties

Now, we will change the footer properties (numbers refer to Figure T3.18):

1. Right-click on the plot and select **Format** from the pop-up menu.
2. Click on the **Titles** category.
3. Select **Footer** from the Header/Footer list box ❶.
4. Check the **Visible** checkbox ❷ to enable the visibility of the footer.
5. Delete the **$FIL** keyword from the header text.
6. Press the **Insert Token** dropdown menu ❸ and select **Keyword from Data** from the dropdown list. The Keyword Dialog will appear (Figure T3.17). This allows you to insert any keyword that is located in the header of the FCS File as the footer for the plot. As the value of the keyword changes, (i.e., because you change the data file) the plot will automatically update with the correct value.

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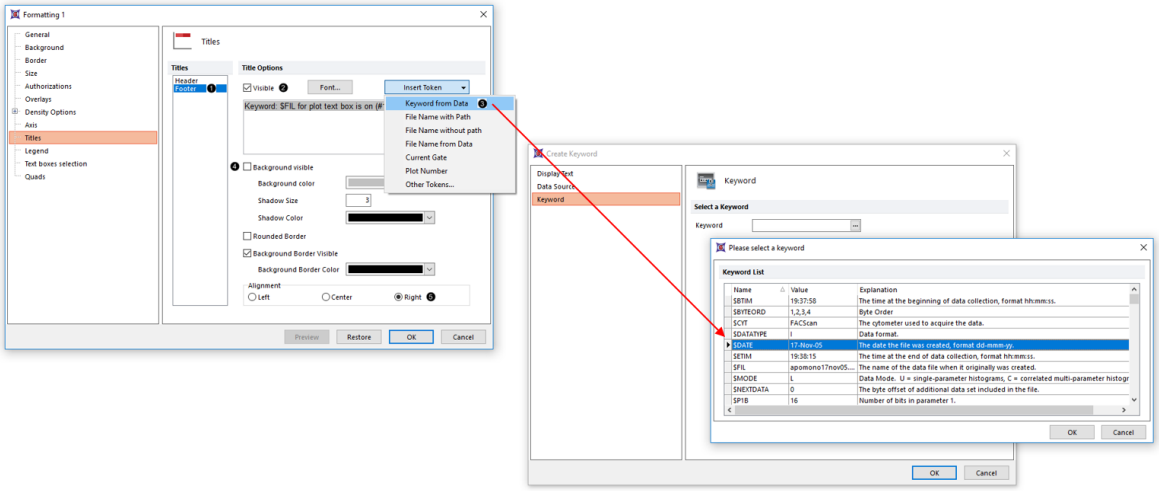

Figure T3.17 Select FCS Keyword

7. Select the **$Date** keyword (red arrow). This will insert the acquisition date.

8. Press **OK**.

9. Uncheck the **Background visible** checkbox ❹.

10. Select **Right** from **Alignment** to right-align the footer ❺.

The screen should look like Figure T3.18.

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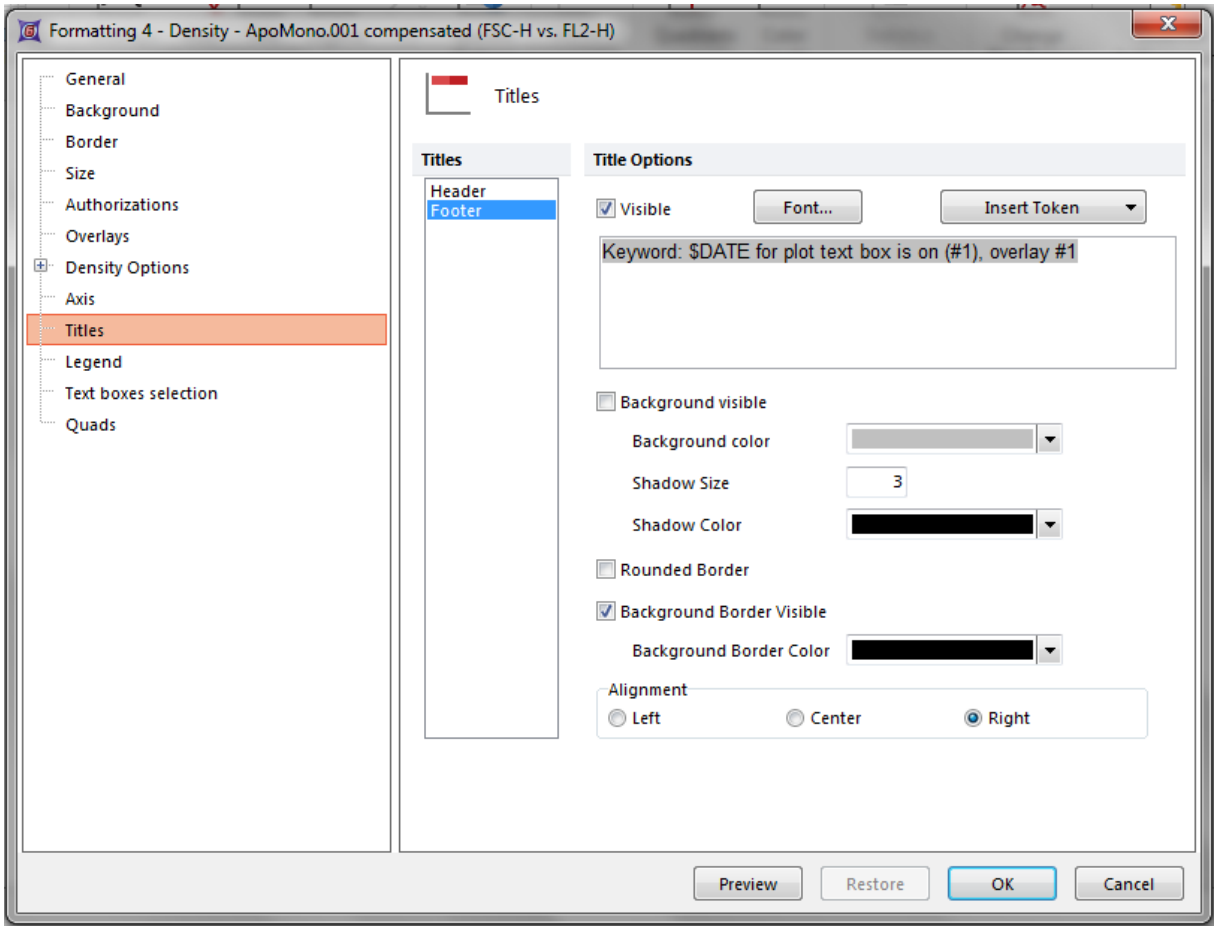

Figure T3.18 Formatting Titles Category (Footer)

11. Press **OK** to apply the changes.

The plot should now look like Figure T3.19B.

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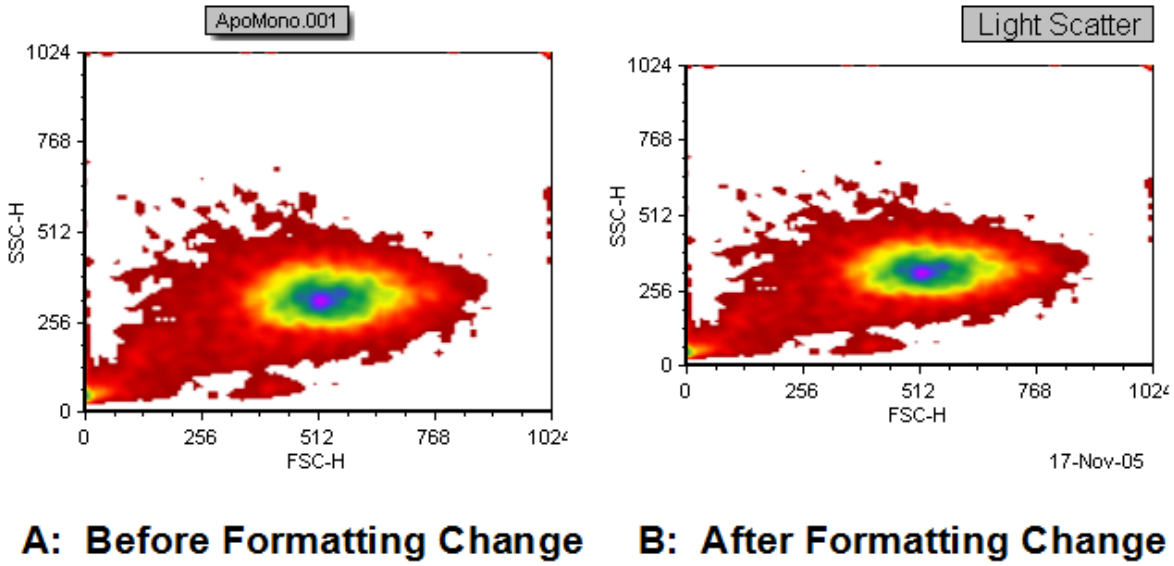

Figure T3.19 Changing the Footer Properties

Next, we will format the plot axes.

38.3.3 Formatting Plot Axes

FCS Express has many axis formatting options which allow you to customize the appearance of your axes any way you like. You have full control over the axis text, font, colors, and many other aspects of its appearance.

In order to demonstrate modifying the axis properties, we will continue with the layout we were using above.

In the course of this example, we will:

- Acquire an overview of formatting axes.
- Modify the axis font properties.
- Add grid lines to a plot.
- Change the angle of the axis labels.

To format the axes (numbers refer to Figure T3.20):

1. Right-click on the plot and select **Format** from the pop-up menu.
2. Click on the **Axis** category ❶.

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3. Click on the **Y Axis** ❷ to set the properties for the Y axis.
4. Click on the **Font** button ❸ and change the font to **Bold**.
5. Press **OK** on the **Font** dialog to accept the font changes.
6. Click the **Show grid lines** checkbox ❹ to turn it on.
7. Click on the **X Axis** ❺ to set the properties for the X axis.
8. Click on the **Font** button ❸ and change the font to **Bold**.
9. Press **OK** on the **Font** dialog to accept the font changes.
10. Change the **Angle** ❻ to 10.

Press **OK** to accept the changes.

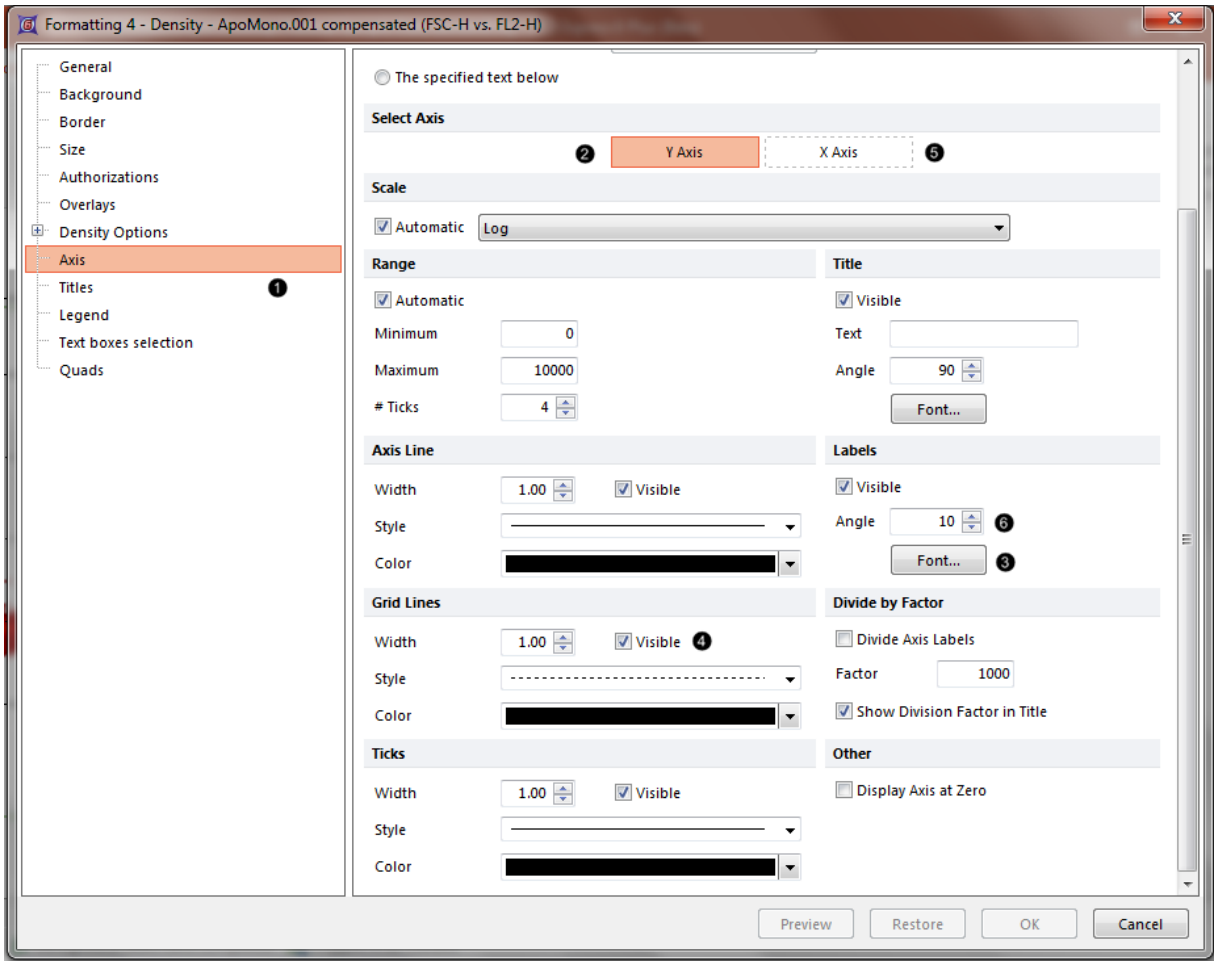

Figure T3.20 Axis Formatting

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The axes of the plot have now been modified (see Figure T3.21).

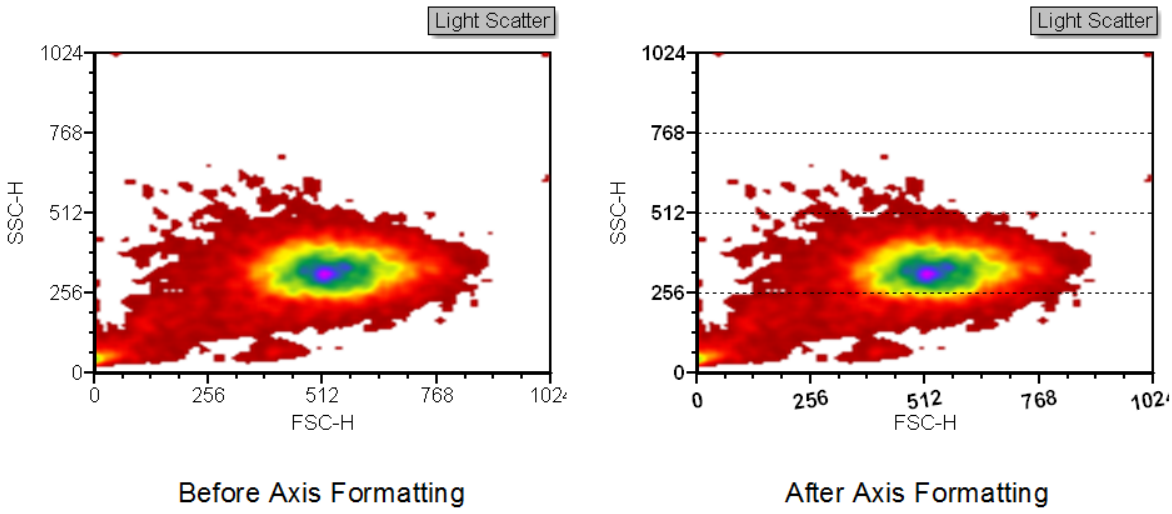

Figure T3.21 Formatting Axis Example

In addition to changing the parameters displayed on the axis or axes of a plot via the Format window, you may use these keyboard or mouse commands 309.

In the next section, we will format the plot background 1256.

38.3.4 Formatting Plot Background

FCS Express also allows complete flexibility in formatting the background of your plots. Almost any object in FCS Express has the same background options as the ones we describe below. The background of any object can display any solid color, a gradient of colors, or an image. Many objects can also be set to be transparent. The plot background options are unique in that they also allow you to adjust the plot margins.

In order to demonstrate modifying the background properties, we will continue with the layout we were using above. In the course of this example, we will:

- Acquire an overview of formatting the background.
- Modify the background gradient.
- Modify the plot margins.

To format the background (numbers refer to Figure T3.22):

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1. Right-click on the plot and select **Format** from the pop-up menu.
2. Click on the **Background** category ❶.
3. Click the **Gradient** radio button ❷.
4. Change the **Right Margin** ❸ to 8%. This will move the right plot margin, allowing you to see the last axis label that is cut off in Figure T3.21 1254.
5. Press **OK** to accept the changes.

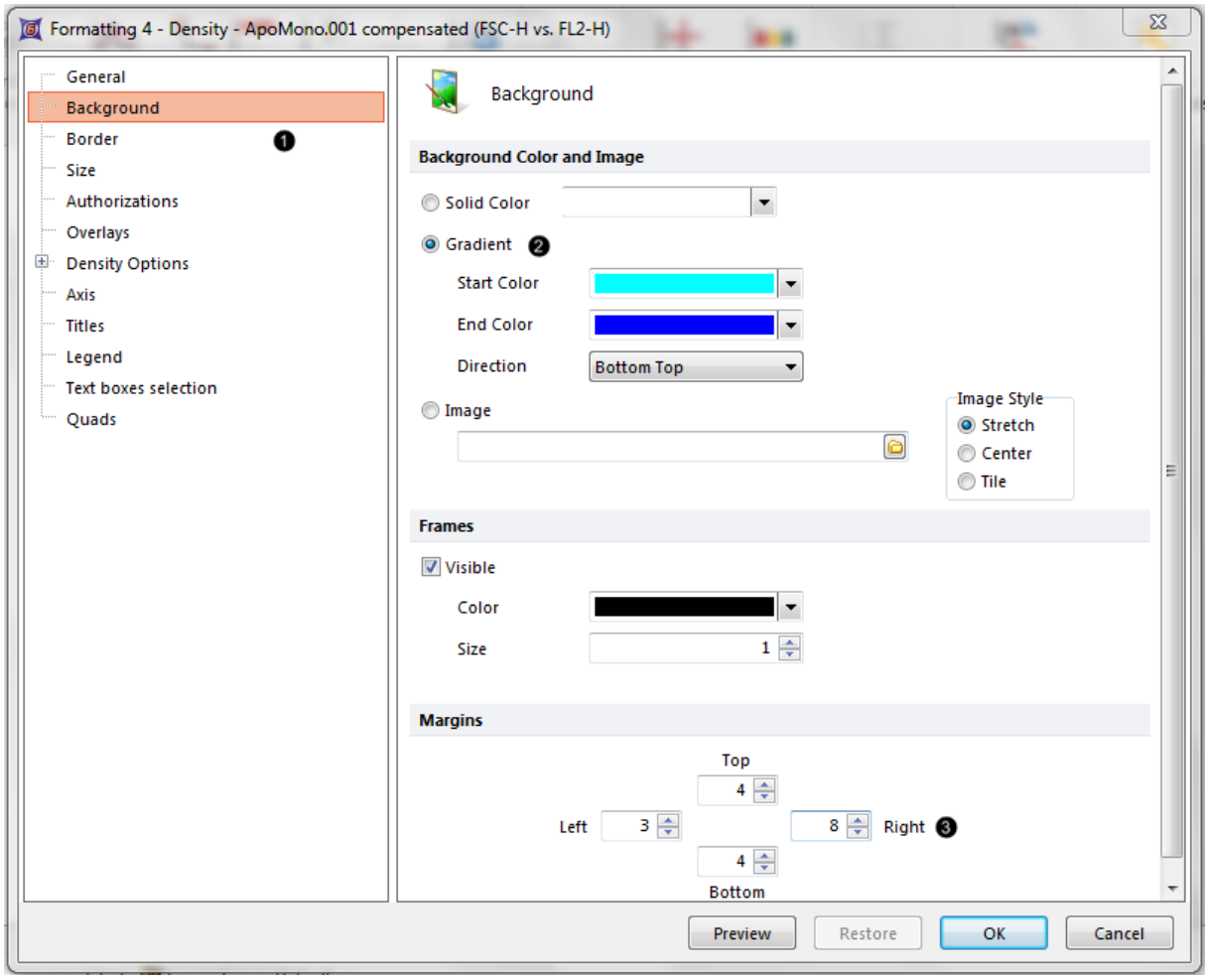

Figure T3.22 Background Formatting

The plot background has now been modified to display a gradient background as shown in Figure T3.23.

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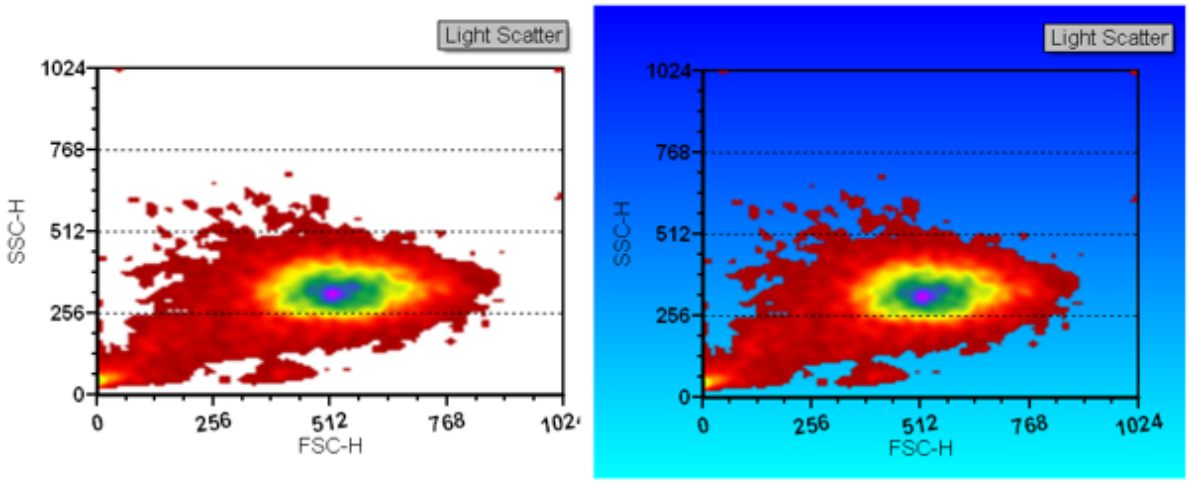

BEFORE BACKGROUND FORMATTING **AFTER BACKGROUND FORMATTING**

Figure T3.23 Formatting Background

Next, we will format the legend 1258.

38.3.5 Formatting the Legend

Legends are useful to describe the data presented in your plots and to help distinguish between several data files that are overlaid onto a single plot.

In the course of this example, we will:

- Learn how to add an overlay to a histogram.
- Acquire an overview of legend formatting.
- Change the legend font.
- Change the legend position.

In order to format the legend, we will first create a histogram with an overlay.

1. Insert a histogram using the data file **ApoMono.001**. If you do not know how to insert a histogram, see the Inserting Plots 1225 chapter of this tutorial.
2. Right-click on the histogram.
3. Select **Add Overlay** from the pop-up menu.

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4. Choose the **ApoMono.002** data file. Leave the rest of the properties on the add overlay window alone.

5. Press **OK** to add the overlay.

The histogram should look like Figure T3.24. *If the legend is already on your plot, your preferences may be set to automatically add legends.*

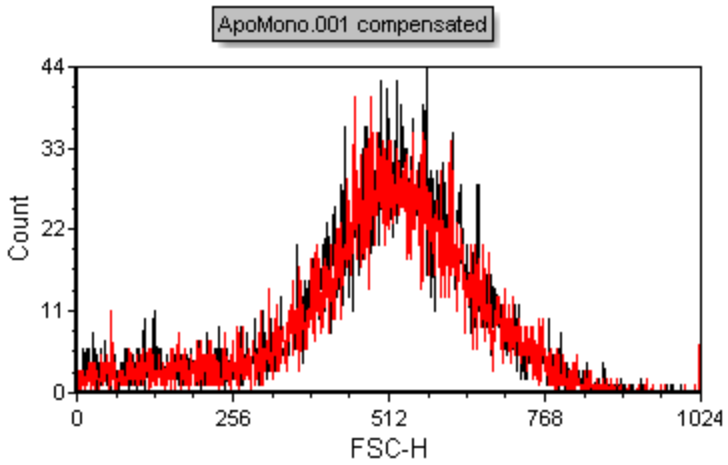

Figure T3.24 Histogram with Two Overlays

To format the legend:

1. Right-click on the histogram and select **Format** from the pop-up menu. The **Formatting** window appears (Figure T3.25)

2. Select the **Legend** category on the left (❶).

3. Check the **Visible** checkbox (❷) (if it is not already checked).

4. Press the **Font** button (❸) and change the font to **Times New Roman**, **10** pt, **Bold**.

5. Press **OK** on the font dialog to accept the changes.

6. Change the **Position (❹)** to "Top".

7. Press **OK** to accept your changes.

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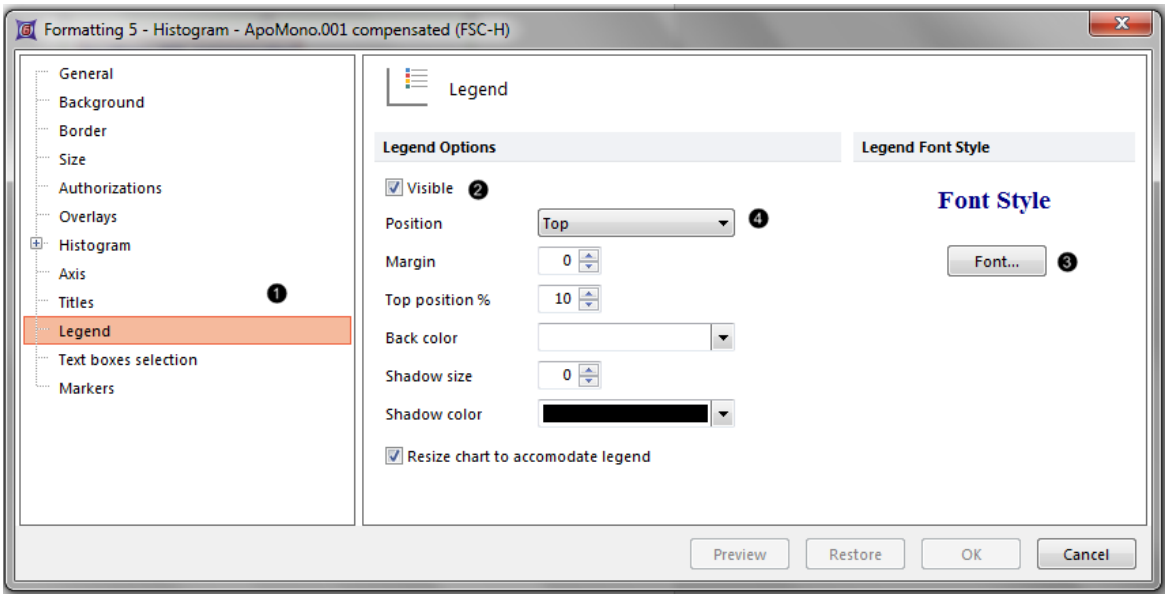

Figure T3.25 Formatting - Legend Category

The plot should now look like Figure T3.26.

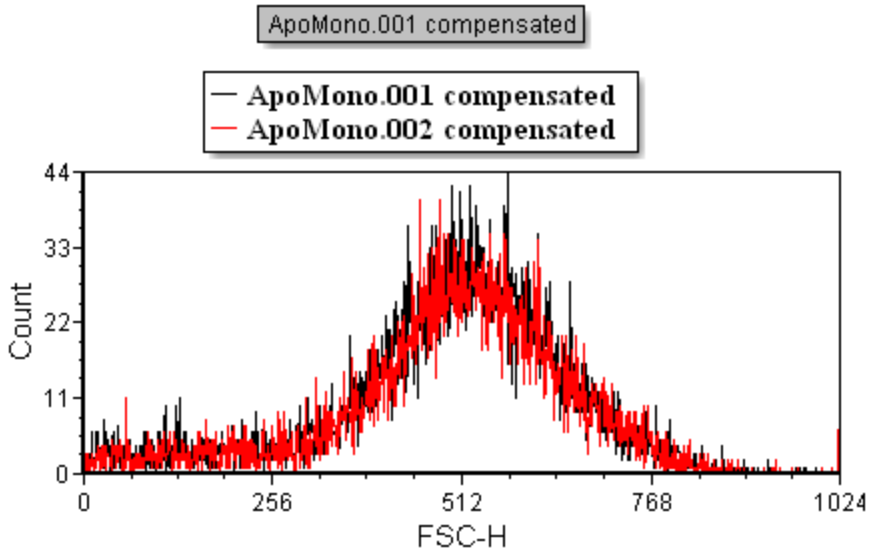

Figure T3.26 Histogram with Formatted Legend

Overlays can be formatted either as stacked or overlaid.

38.3.6 Changing Histogram Overlays

In the course of this example, we will learn how to change histogram overlays to stacked:

For convenience, we are going to start with the same layout as before. The images contained within this section will continue with the layout from the previous section.

1. Select the histogram (see selecting objects 1212).
2. Select the **Format→Plot Options→Specific Options** command (Figure T3.27).
   Alternatively you can Right-click on the histogram and select **Format→Histogram→Histogram Specific Options**.

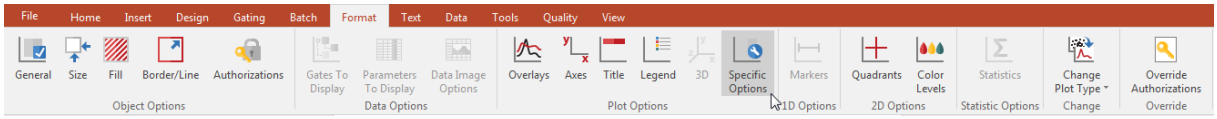

Figure T3.27 Format Specific Options Command

A **Formatting Histogram Specific Options** dialog will appear (Figure T3.28).

3. Check **Stacked** from the **Display multiple overlay data** section (❶).
4. Click **OK**.

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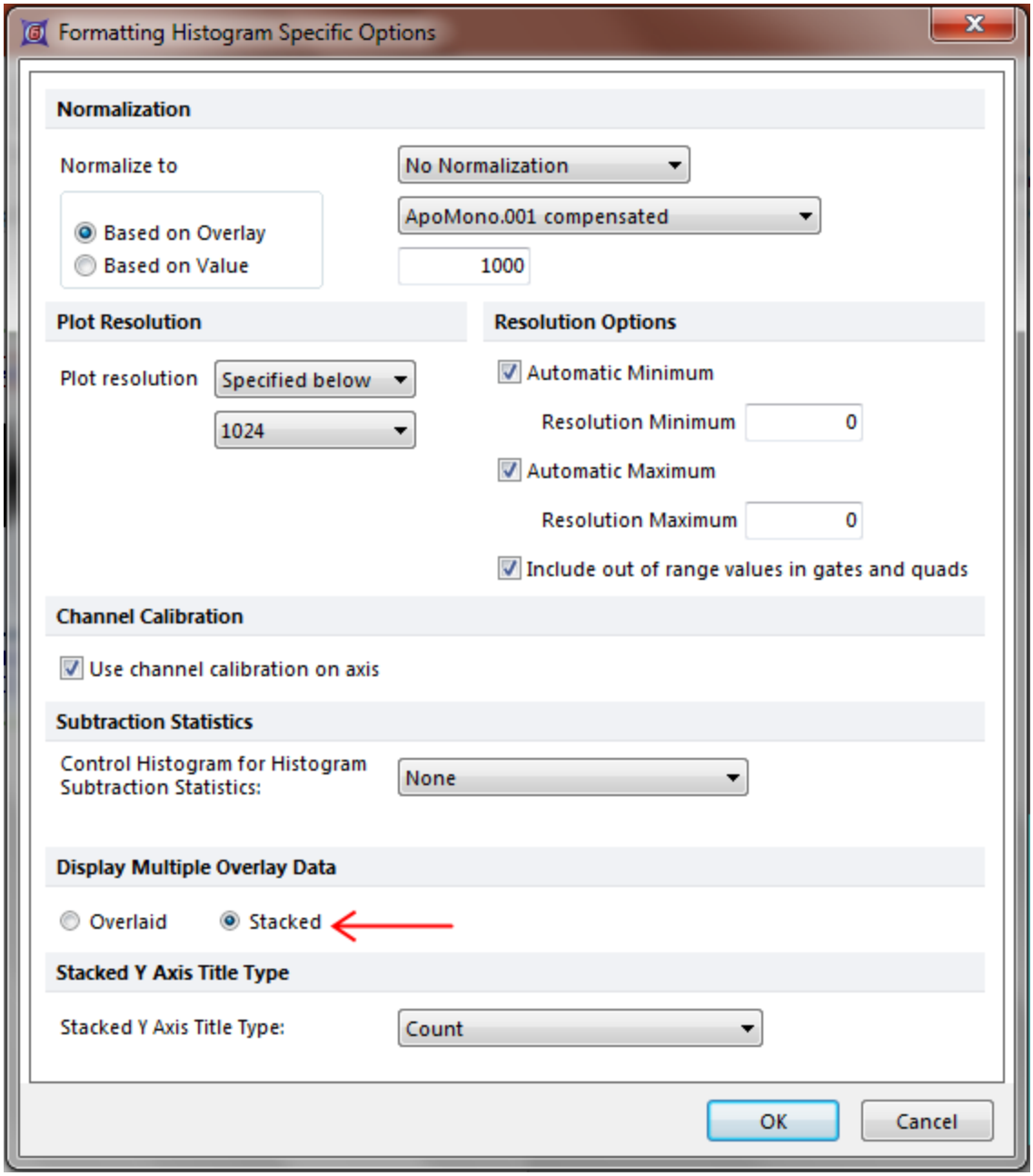

Figure T3.28 Format Histogram (Stacked Overlays)

The histogram will change to stacked (Figure T3.29).

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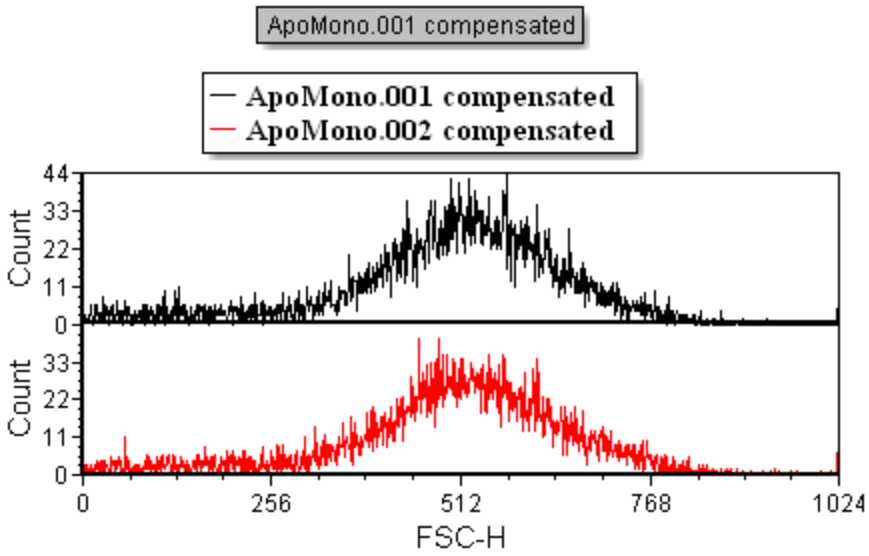

Figure T3.29 Stacked Histograms

To change the histogram overlays back to overlaid press **Ctrl+Z** on your keyboard.

Alternatively repeat steps 1-4 selecting **Overlaid** from the **Display multiple overlay data** section (❶).

The histogram will change back to the overlaid style (Figure T3.26 1258).

Certain plots can be formatted in 3D 1264.

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38.3.7 Formatting 3D Options

FCS Express also allows you to view your histogram overlays in 3D.

In the course of this example we will:

- Acquire an overview of the 3D options.
- Turn 3D on.
- Change the 3D percent.

We will use the histogram shown in Figure T3.26 1258.

1. Right-click on the histogram and select **Format**. The Formatting window appears (Figure T3.31).
2. Click on the **Plot 3D Options** category under the **Histogram** sub-menu (❶).
3. Check **View in 3 Dimensions** (❷).
4. Change the **3D %** to 100% (❸).
5. Adjust the **Zoom** slider to 70% (❹).
6. Adjust the **Vertical offset** slider to approximately 10% (❺).

Press **OK** to accept the changes.

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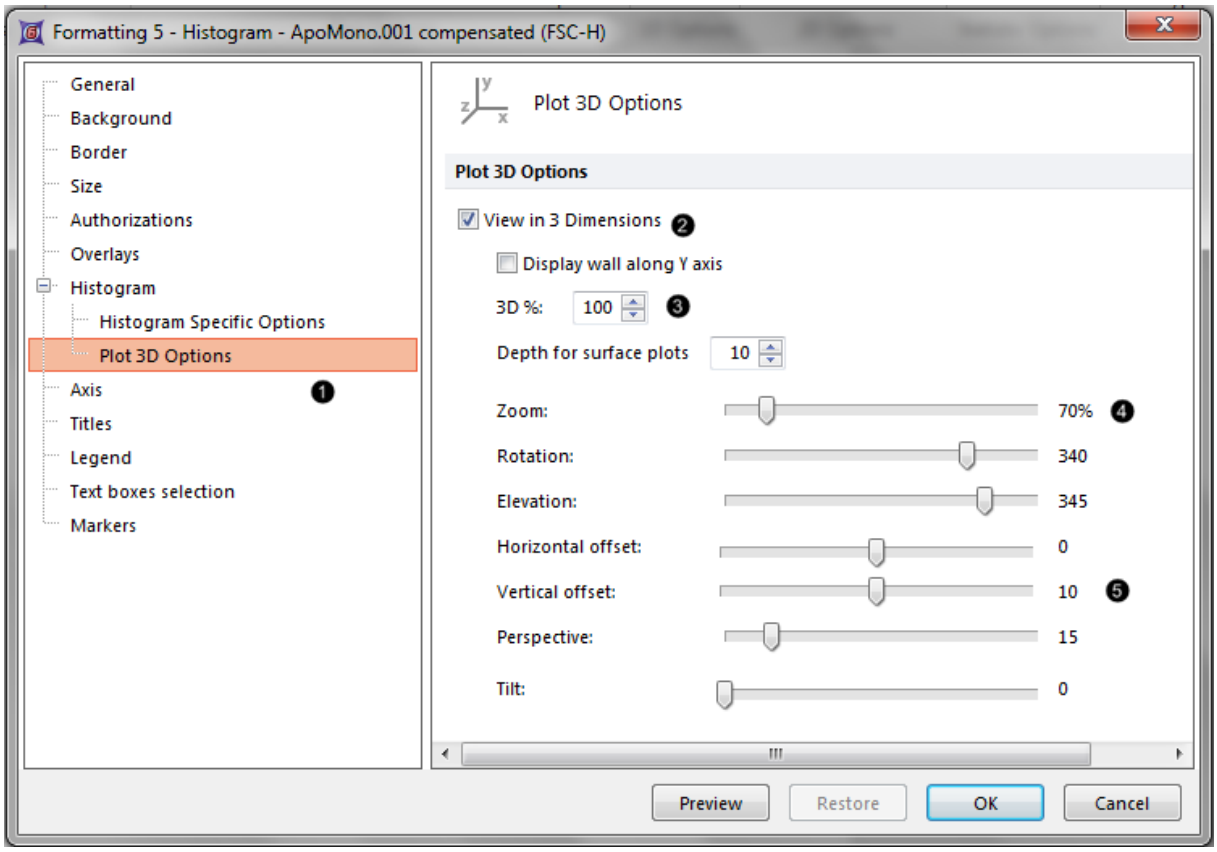

Figure T3.31 Histogram 3D Options

The plot should now look similar to Figure T3.32.

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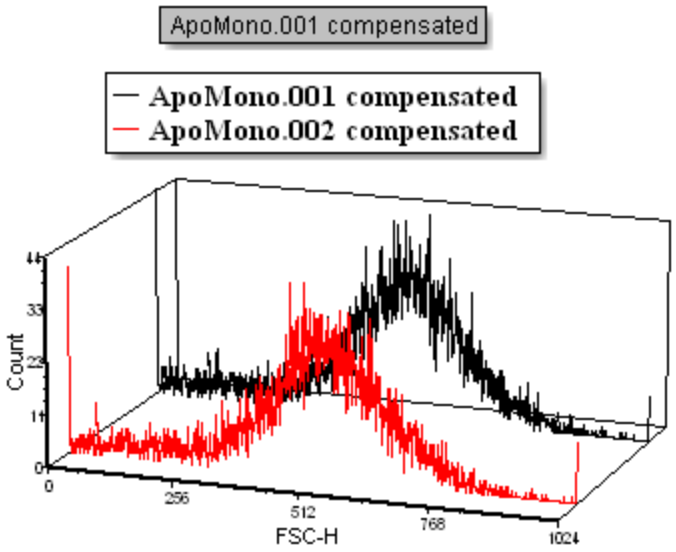

Figure T3.32 Histogram with 3D Overlays

38.4 Gating

In FCS Express 6, you can create six types of gates as tools for flow cytometry analysis. These gates include rectangular, elliptical, polygonal, freeform, markers (1D), and combination gates. This tutorial will demonstrate how to create, manipulate, and apply gates. More information on gates can be found in the Analysis of FCS Data chapter of the manual 316.

The first section, will introduce creating gates 1266.

38.4.1 Creating Gates

In the course of this example, we will:

- Open a previously created layout.
- Create rectangular, elliptical, polygonal, and freeform gates.
- Change a data file.
- Create a 1D gate on a 2D plot.
- Create a 1D gate from a marker.
- Convert a quadrant to a gate.

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Although any data files of your own can be used to follow along in this tutorial, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive. The **section5.fey** layout and the **ApoMono.001** and **ApoMono.002** data files will be used here.

To Open a Layout:

1. Select **File** tab→**Open** (Figure T3.1).
2. Select the **section5.fey** layout file located in the Tutorial Sample Data archive from the **Load Layout** dialog.
3. Select the **Data→Change Data on All Objects→Select** command to insert a data file into the plot (Figure T3.4).
4. Select the **ApoMono.001** FCS file located in the Tutorial Sample Data archive.
5. Click **Open.**

The layout should now look like Figure T4.0.

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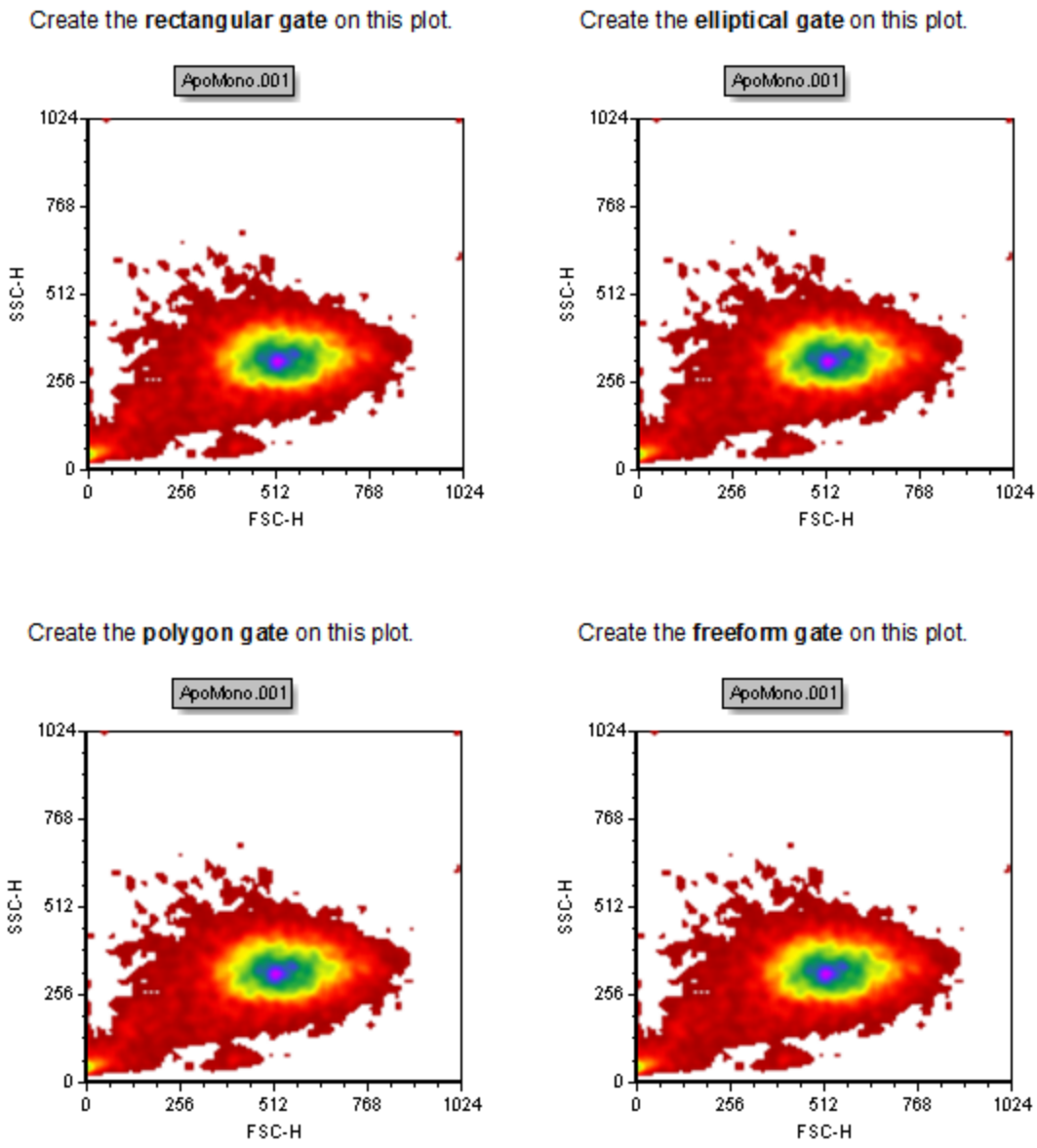

Figure T4.0 Section5 layout after file loading

We are going to insert four different kinds of gates on the different plots on the layout.

Using the commands from the **Create Gates** groups on the **Gating** tab (Figure T4.1) is the most convenient way to create gates, but all gates except markers (1D) can also be created using **Create Gate** from the 2D plot pop-up menu (right-clicking on a plot brings up the pop-up menu). The Gating commands are shown in Figure T4.1 and described in the table below.

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Note: Gating of data obtained from multiwell plates will be discussed in a subsequent tutorial (see Analyzing Data in Heat Maps 1758 section).

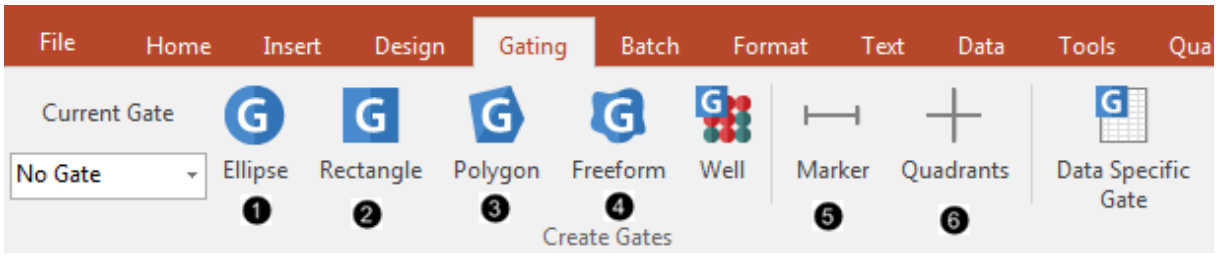

Figure T4.1 Gating Commands

Symbol	Description
❶	Insert an elliptical gate
❷	Insert a rectangular gate
❸	Insert a polygonal gate
❹	Insert a freeform gate
❺	Insert a marker
❻	Insert a quadrant

If you hover your mouse briefly over any of the commands in the **Create Gates** group, a hint describing the command is shown.

To see the gating command hint (Figure T4.2):

5. Move the mouse over the **Rectangle** gate button.
6. Pause for half of a second.

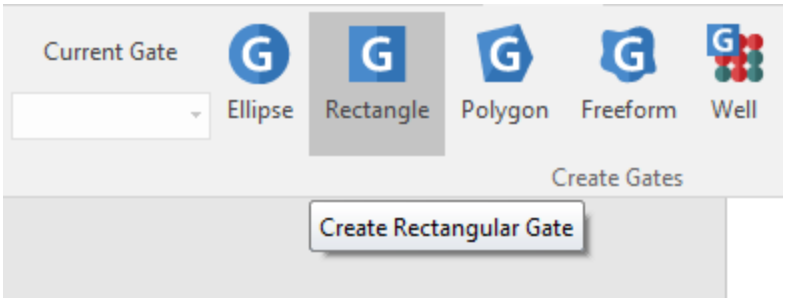

Figure T4.2 Gating Command Hint

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To create a Rectangular Gate:

7. Select the **Gating→Create Gates→Rectangle** command (Figure T4.1 ❷).

You will know it is pressed because it will have a different color background

.

We will create the rectangular gate on the upper left density plot (referred to as "Create the **rectangular gate** on this plot") (Figure T4.3) .

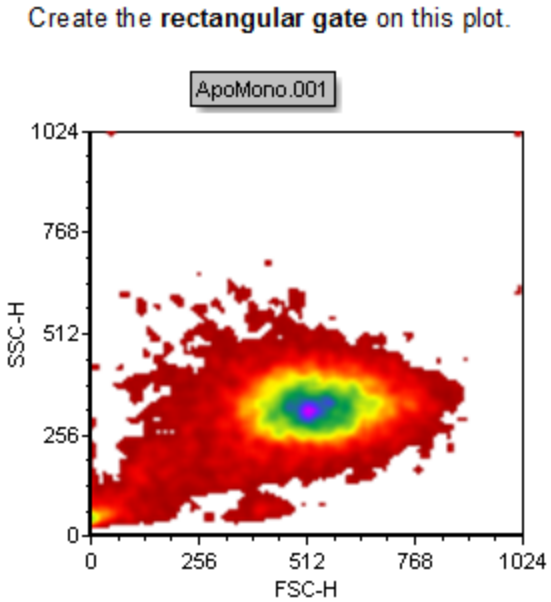

Figure T4.3 Density Plot for First Gating Excercise

8. Move the cursor on the plot wherever you would like the gate to begin.
9. Press and hold the left mouse button.
10. Move the cursor on the plot wherever you would like the gate to stop. (Figure T4.4).
11. Release the mouse button.

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Create the **rectangular gate** on this plot.

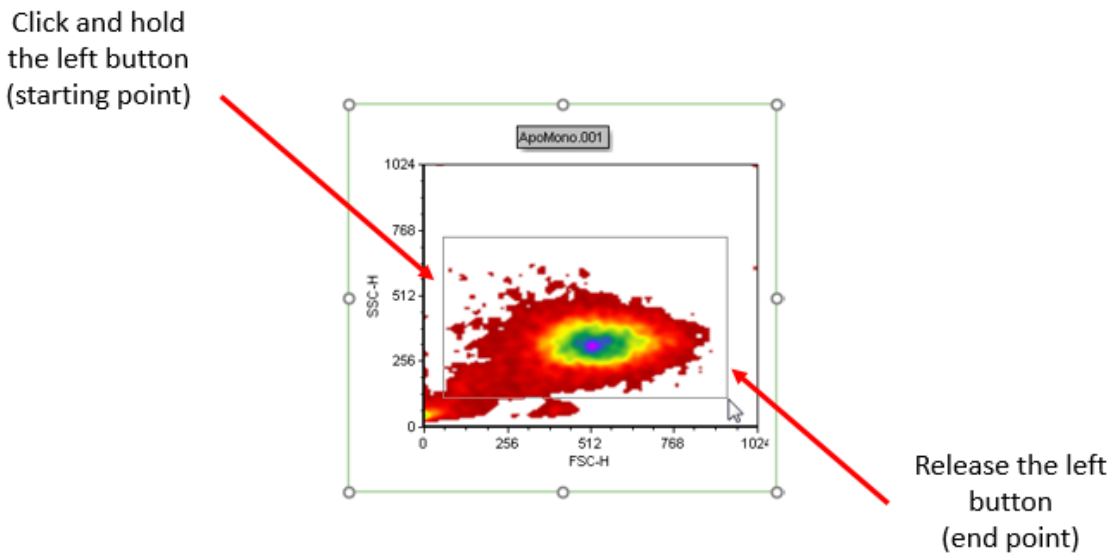

Figure T4.4 Creating a Rectangular Gate

The **Create New Gate** dialog will appear (Figure T4.5). We can now give the gate a name.

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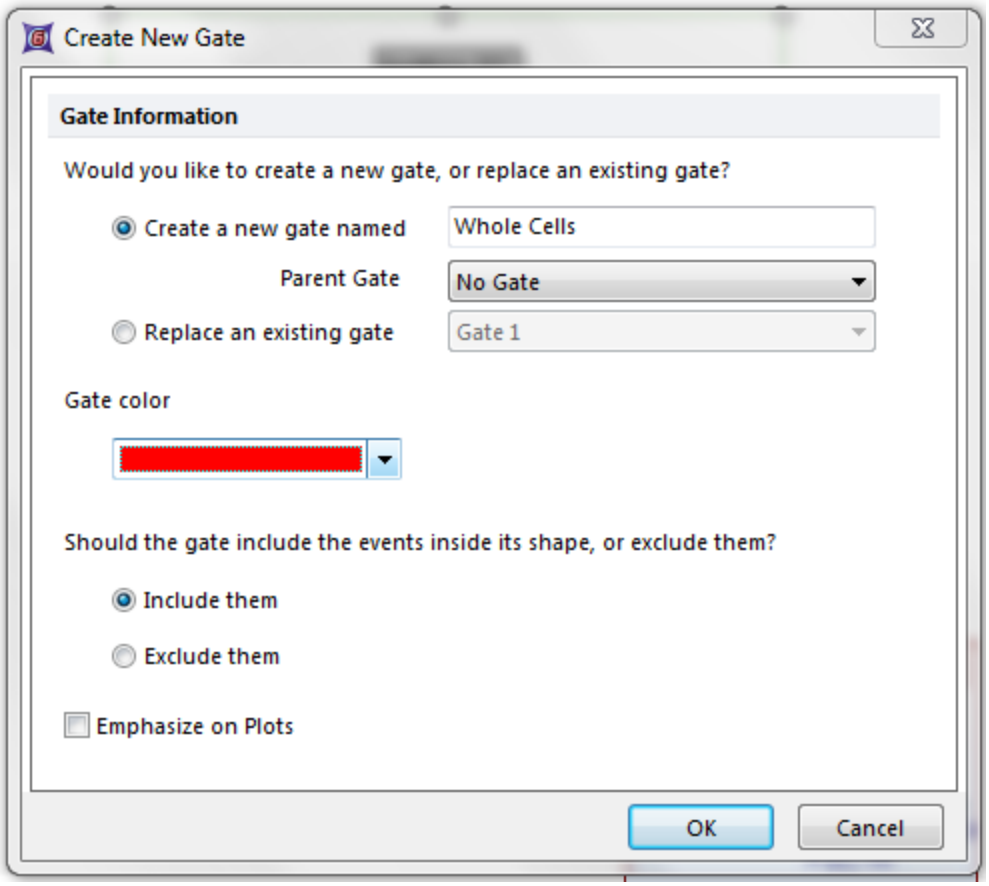

Figure T4.5 Create New Gate Dialog

12. Enter "Whole Cells" in the **Create a new gate named** edit field.
13. Click **OK** to accept the name.

The gate and a text box displaying the gate name and the % of gated cells will appear in the plot (Figure T4.6).

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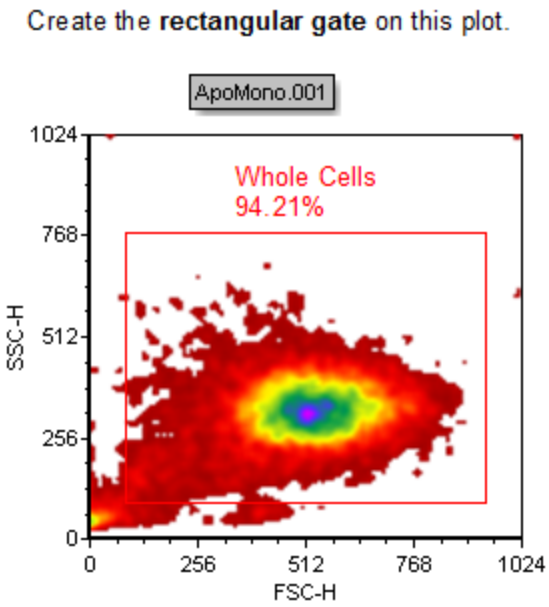


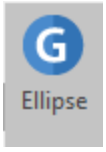
Figure T4.6 Newly Created Rectangular Gate

Note: You can cancel the creation of gates anytime during step 7-12 by pressing ESC on the keyboard. This technique applies to all types of gates.

To create an Elliptical Gate:

14. Select the **Gating→Create Gates→Ellipse** command (Figure T4.1 ❶).

You will know it is pressed because it will have a different color background



.

We will create the elliptical gate on the upper right density plot (referred to as "Create the **elliptical gate** on this plot").

15. Follow steps 8-11 above to create the elliptical gate.

16. Enter "Cells in ellipse" in the **Create a new gate named** edit field.

17. Press **OK** to accept the name.

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The gate and a text box displaying the gate name and the % of gated cells will appear in the plot (Figure T4.6b).

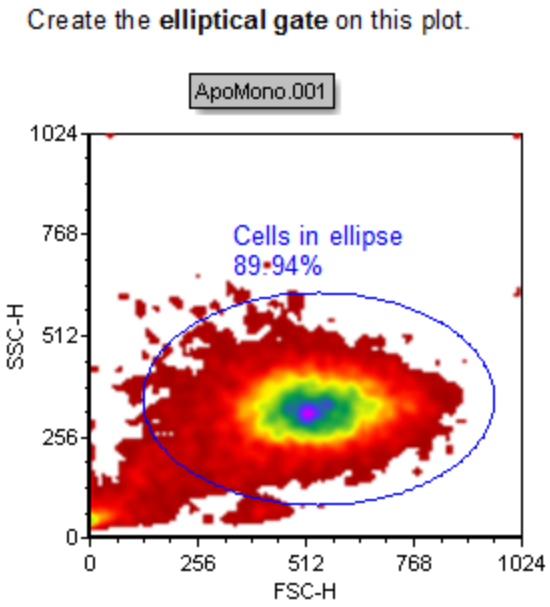

Figure T4.6b Newly Created Elliptical Gate

To create a Polygon Gate:

We will create the polygon gate on the lower left density plot (referred to as "Create the **polygon gate** on this plot").

18. Select the **Gating→Create Gates→Polygon** command (Figure T4.1 ❸**)**.

    You will know it is pressed because it will have a different color background

.

19. Move the cursor on the plot wherever you would like the add the first point of your polygon gate.

20. Press the left mouse button. The first point of your gate is created

21. Move the cursor on the plot wherever you would like the add the second point of your polygon gate.

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22. Press the left mouse button. The second point of your gate is created
23. Repeat steps 19-22 until you want to close the polygon.
24. Close the polygon gate by clicking on the first point created in step 20. Alternatively, you can press the space bar to close the polygon.

A **Create New Gate** dialog will appear, similar to Figure T4.5. We can now give the gate a name:

25. Enter "Cells in a polygon" in the **Create a new gate named** edit field.
26. Click **OK** to accept the name.

The gate and a text box displaying the gate name and the % of gated cells will appear in the plot (Figure T4.7).

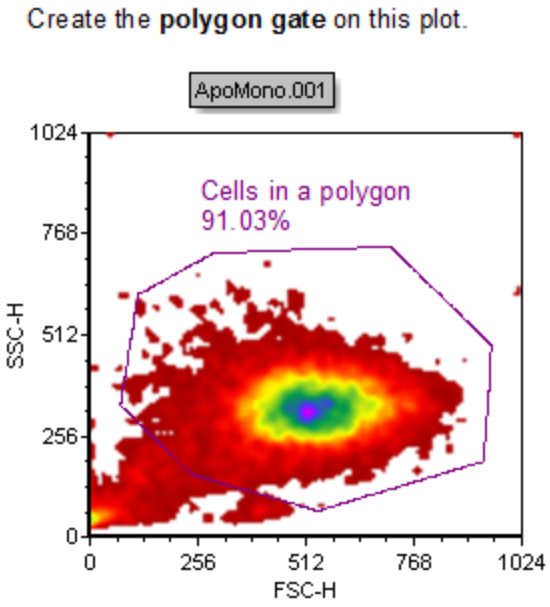

Figure T4.7 Polygon Gate

To create a Freeform Gate:

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We will create the freeform gate on the lower right density plot (referred to as "Create the **freeform gate** on this plot").

27. Select the **Gating→Create Gates→Freeform** command (Figure T4.1 ❹).

You will know it is pressed because it will have a different color background

.

28. Move the cursor to the place where you want to start the gate.
29. Press and hold down the left mouse button.
30. Move the cursor while holding the mouse button down to create the shape of the gate. A line will follow the cursor showing where the gate will appear.
31. Release the mouse to finish creating the gate (Figure T4.8).

A **Create New Gate** dialog will appear, again similar to Figure T4.5. We can now give the gate a name:

32. Enter "Cells in freeform" in the **Create a new gate named** edit field.
33. Click **OK** to accept the name.

The gate and a text box displaying the gate name and the % of gated cells will appear in the plot (Figure T4.6b).

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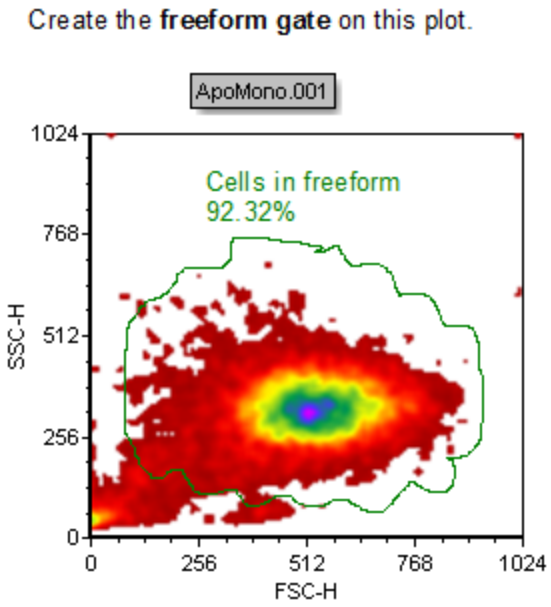

Figure T4.8 Freeform Gate

In the next section, we will create a 1D gate on a 2D plot.

38.4.2 Creating a 1D Gate on a 2D Plot

1D gates can be applied to 2D plots as well as single parameter histograms (where they are called *Markers*). 1D gates are unique in the respect that they only gate on data in a single dimension. We will continue using the same layout we were using above in the previous section.

We will now create a 1D gate on the 2D plot underneath where it says "Create the **elliptical gate** on this plot" (Figure T4.6b).

To create a 1D Gate on a 2D plot:

1. Select the **Gating** tab→**Create Gates**→**Create Marker** command, or right-click on the plot and select **Create gate**→ **Marker** from the pop-up menu.

You will know the **Create Marker** command is selected because it will have a different color background

.

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2. Place the cursor on the 2D plot at the location you want to start the gate.
3. Press and hold down the left mouse button.
4. While holding the mouse button down, move the cursor from the left to the right to create a 1D gate in the horizontal direction.

Note: Moving the cursor from the right to left, or left to right, will create a 1D gate along the x-axis. Moving the cursor in the vertical direction, either up or down, will create a 1D gate along the y-axis. In this example, we will create a gate along the x-axis.

5. Release the mouse button when at the desired location (Figure T4.9).

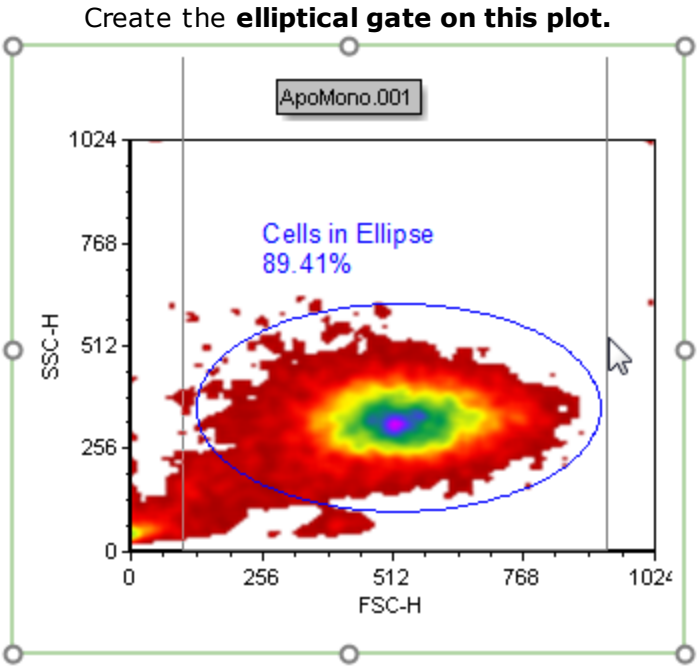

Figure T4.9 Creating a 1D Gate on a 2D Plot

A **Create a new gate** dialog will appear, as previously shown.

6. Enter "Cells in marker" in the **Create a new gate named** edit field.
7. Press **OK** to accept the name.
8.

A 1D gate now appears on the 2D plot (Figure T4.10).

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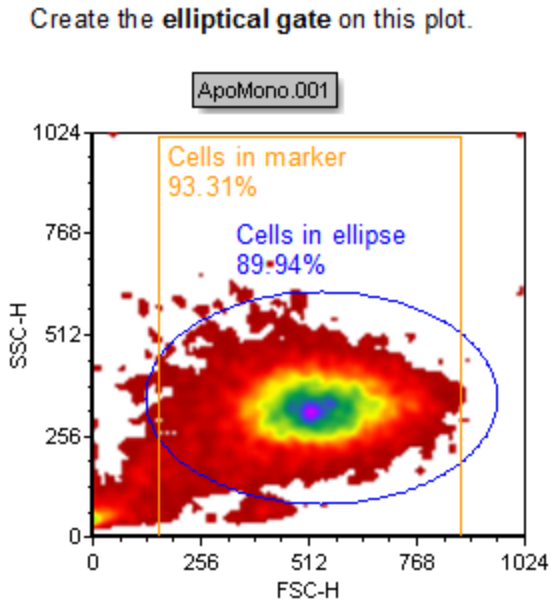

Figure T4.10 1D Gate on a 2D Plot

Next, we will create a gate from a marker.

38.4.3 Creating a Gate from a Marker

We will continue using the same layout we were using above in the previous section.

To create a Gate from a Marker on a 1D plot:

1. Press the **Page 2** tab from the **Layout Navigation Toolbar** (lower left of the window). You will see a blank histogram and dot plot on that page.
2. Select both the histogram and the 2D plot contained in this page by pressing **Ctrl+A** on your keyboard
3. Select the **Data→Change Data on Selection→Select** command to insert a data file into the plot (Figure T3.4).
4. Select the **ApoMono.002** FCS file located in the Tutorial Sample Data archive.
5. Click **Open.**

The histogram should look like Figure T4.11.

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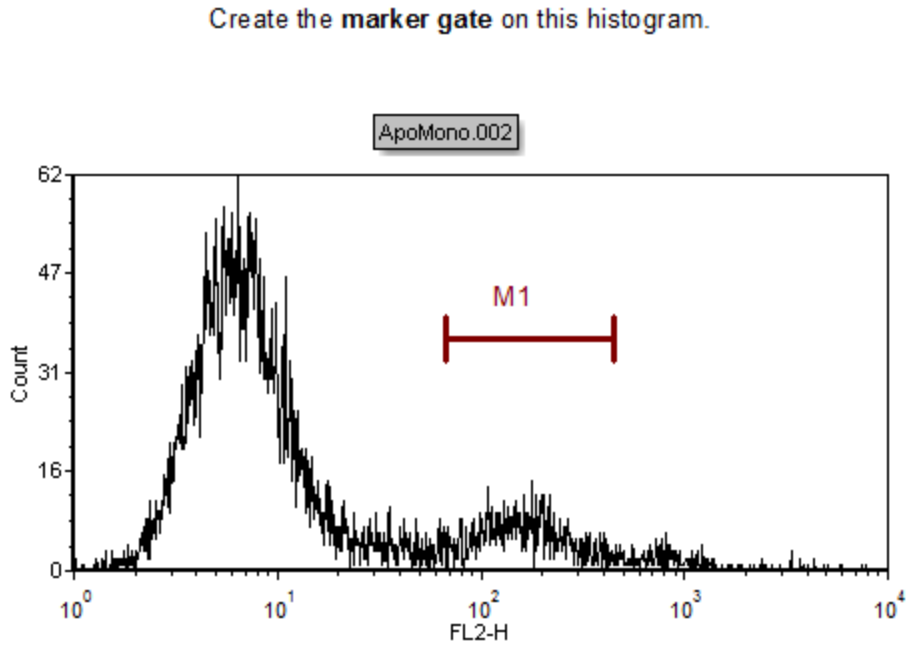

Figure T4.11 Histogram with Marker

6. Right-click on the histogram to bring up the pop-up menu.
7. Select **Convert Marker to Gate→Marker #1 (M1) →Convert and Link** from the pop-up menu (Figure T4.12).

Create the **marker gate** on this histogram.

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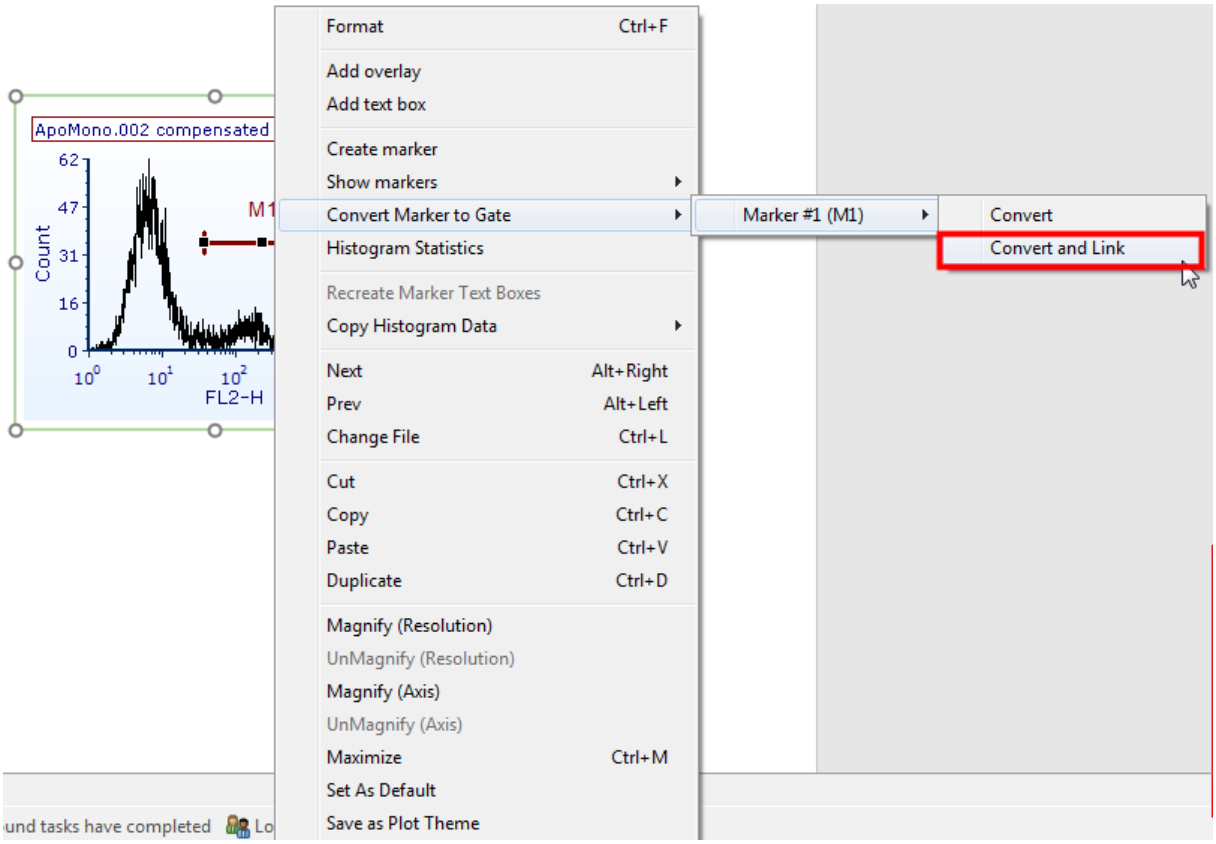

Figure T4.12 Convert Marker to Gate

A dialog will appear asking you to name the gate.

8. Enter "Marker Gate" in the **Create a new gate named** edit field.

9. Press **OK** to accept the name.

The gate is now linked to the marker because we selected **Convert and link** above. This means that whenever we move the marker, the gate will also move to reflect the new marker position.

Note you can track gate creation and hierarchy through the **Gate View**.:

To open a **Gate View** object:

10. Select the **Gate View** button in the **Insert** bar (Figure T4.12)

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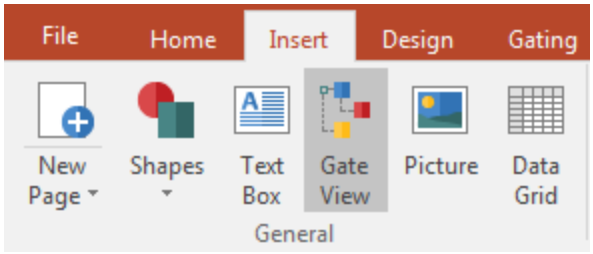

Figure T4.12 Inserting a Gate View Object

11. Click the left mouse button on the layout wherever you want to insert the Gate View object

10. The Gate View appear (Note the presence of the new "Marker gate" gate)

Figure T4.13 Example of Gate View

In the next section, we will create a gate from a quadrant 1282.

38.4.4 Creating a Gate from a Quadrant

It is also possible to create a gate from a quadrant. It does not matter if the quadrant is standard, floating, or percentile (for an in-depth description of the different types of quadrants, see the section on Using Quadrants 368). Creating a quadrant gate is very similar to creating a marker gate.

We will continue using the same layout we were using above in the previous section.

On the bottom of **Page 2** of the layout, you should see a dot plot with quadrants.

1. Right-click on the dot plot to bring up the pop-up menu.

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2. Select **Convert Quads to Gate→Lower Right→Convert and Link** from the pop-up menu (Figure T4.13).

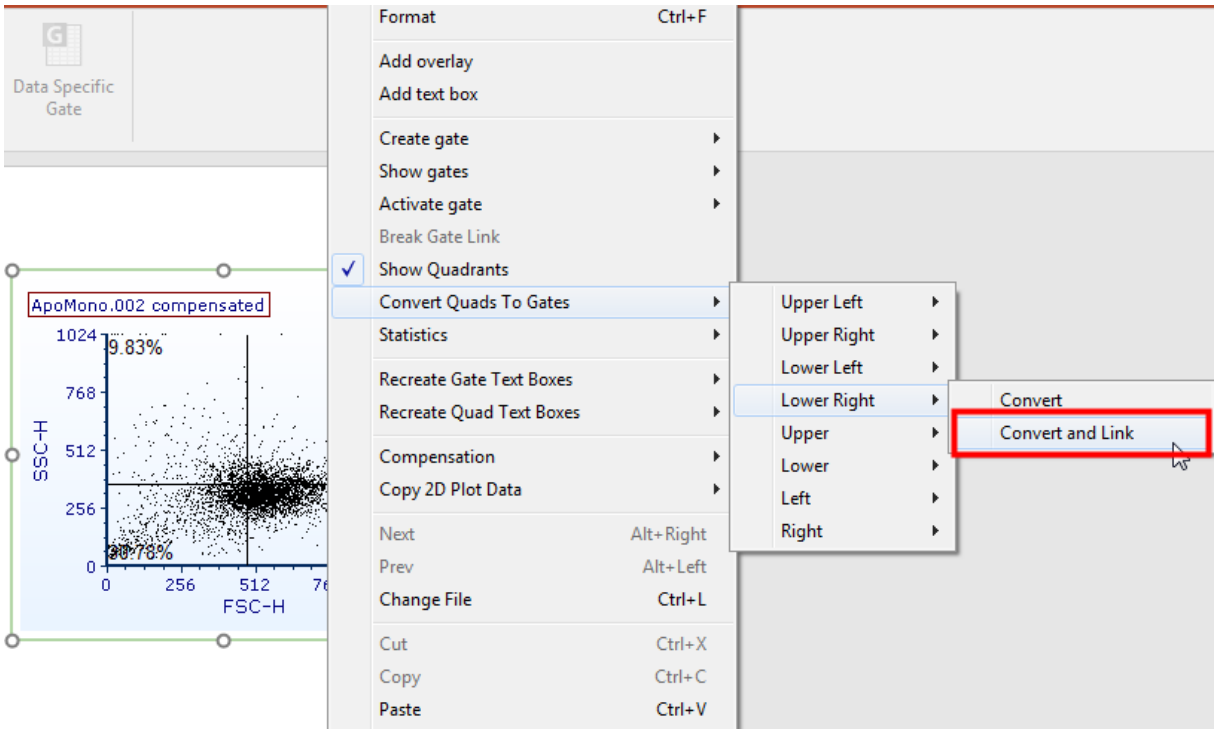

Figure T4.13 Converting a Quadrant to a Gate

A dialog will appear asking you to name the gate.

3. Enter "Quadrant Gate" in the **Create a new gate named** edit field.

4. Press **OK** to accept the name.

The gate is now linked to the lower right quadrant because we selected **Convert and link** above. This means that whenever we move the quadrants, the gate will also move to reflect the new quadrant position.

It is also possible to create combination gates.

38.4.5 Creating Combination Gates

Combination gates consist of combinations of other gates. To combine gates, you create a gating formula using Boolean combinations of pre-existing gates. Gating formulas are defined by either a single gate, or gate numbers joined by Boolean operators. The Operators describe how the gates should be combined and follow the rules of Boolean algebra.

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We will continue using the same layout as we were using above.

As a first step we will insert a gate view object to keep track of the new gates we are going to create.

To insert a Gate View:

1. Select the **Gate View** button in the **Insert** bar (Figure T4.12 1279).
2. Click the left mouse button on the layout wherever you want to insert the Gate View object
3. The **Gate View** (Figure T4.14) now appears on the layout.

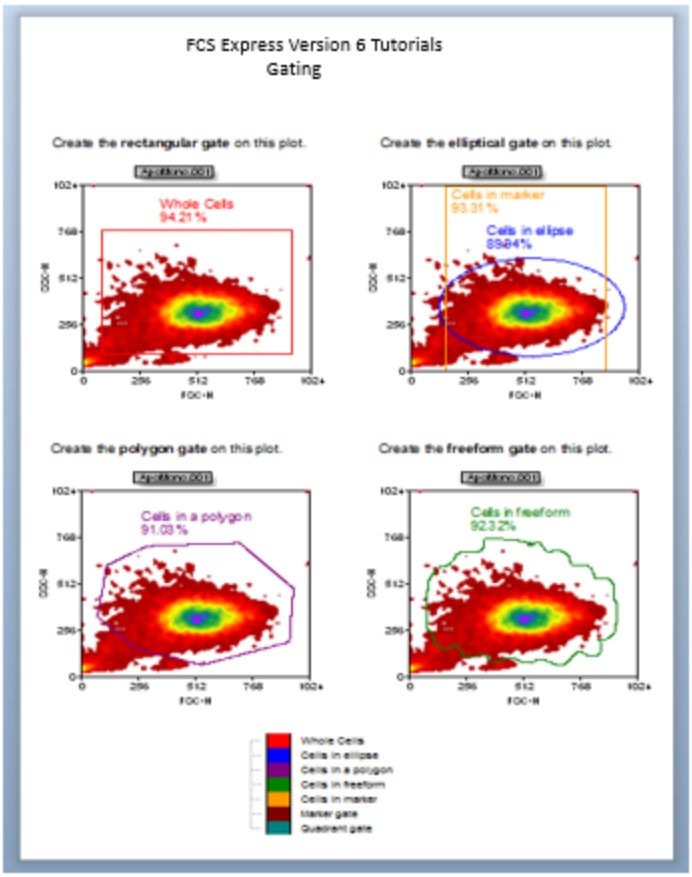

Figure T4.14 Gate View on a Layout Page

To create a Combination Gate:

1. Right-click on the **Gate View** to bring up the pop-up menu.

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2. Select **Add Combination Gate** from the menu (Figure T4.15).

Create the **polygon gate** on this plot. Create a **freeform gate** on this plot.

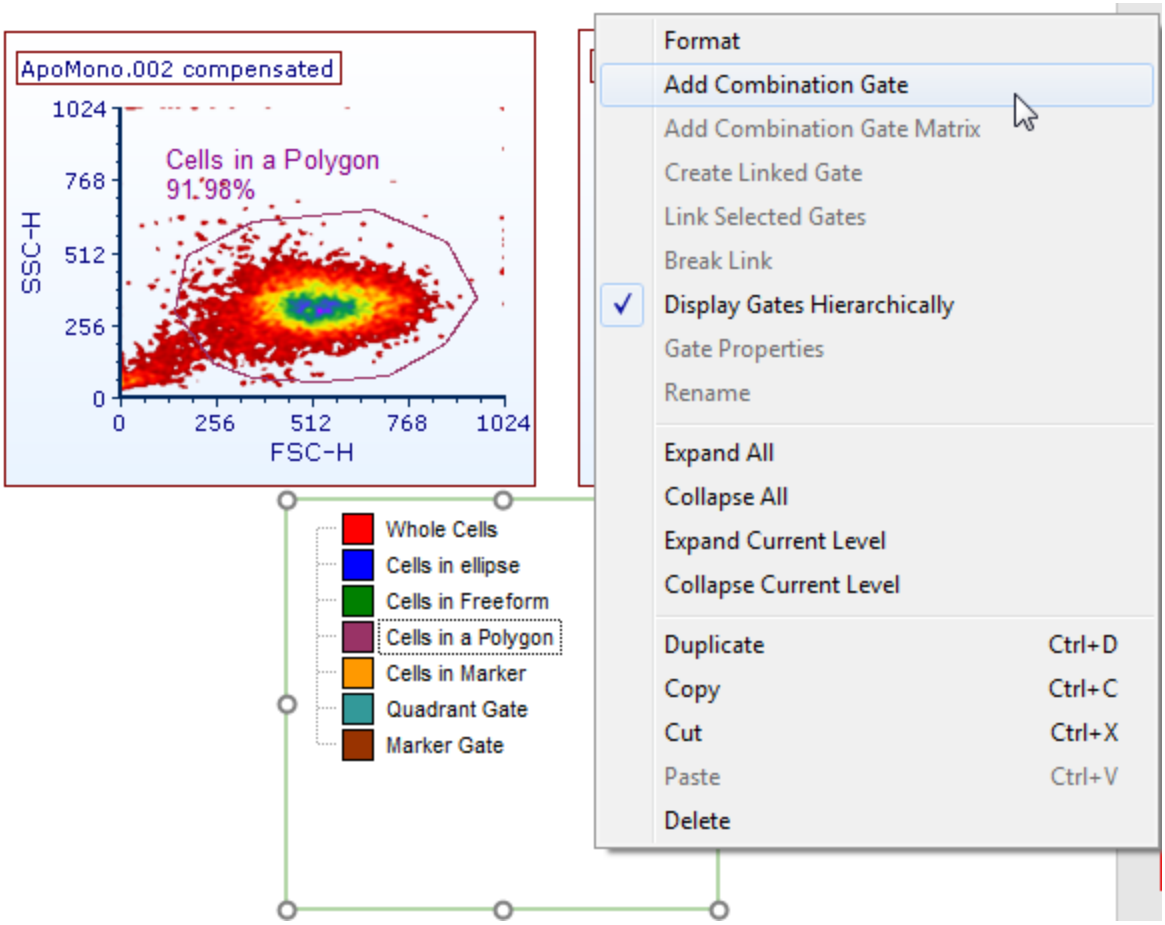

Figure T4.15 Add a Combination Gate

3. The **Create Gate** editor will appear (Figure T4.16).
4. Type "My Combination Gate" in the **Name** field (❶).
5. Click in the formula field (❷).
6. Type "m".
7. Notice that the text "marker Gate" is filled in for you. That is because FCS Express knows that is the only gate that starts with the letter "m".
8. Type <SPACE> to accept the "marker gate" choice.
9. Type "o".

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10. Type <SPACE> to accept the "or".
11. Type "q".
12. Type <SPACE> to accept the "quadrant Gate" choice.
13. Change the color to "Plum" (❸).

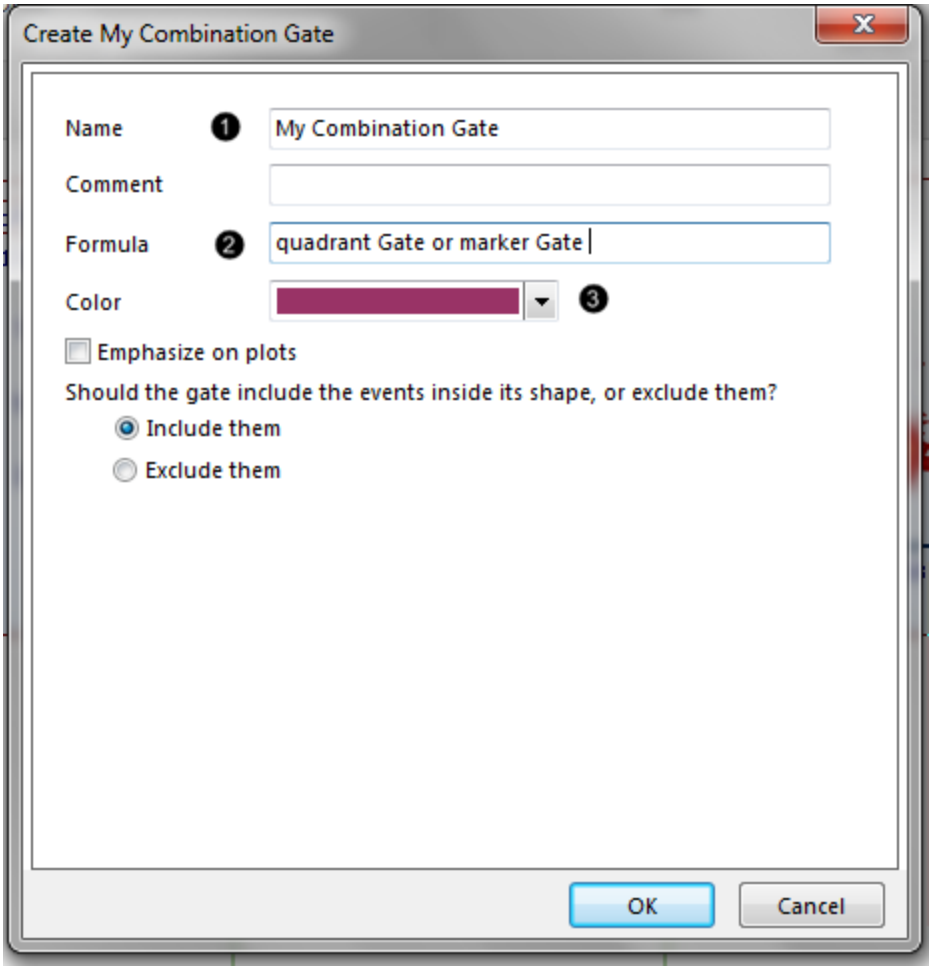

Figure T4.16 Create Combination Gate Dialog

14. Press the **OK** button to create the combination gate.

More information about creating combination gates and all the different possible operators can be found in the section on Creating Gates 318 in the main part of the manual.

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Next, we will learn how to move a gate 1287.

38.4.6 Moving Gates

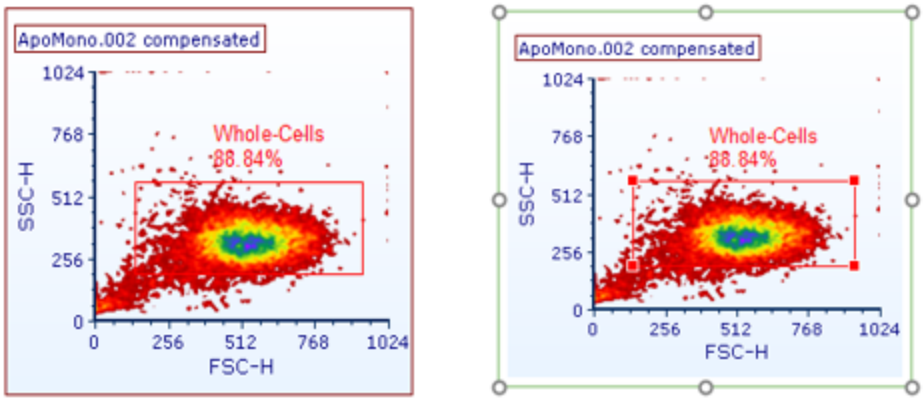
In the course of this example we will:

- Activate a gate
- Move a gate

We will continue using the same layout as we were using above.

1. Click anywhere inside the "Whole-Cells" gate. This will activate the gate. You know the gate is activated when there are resizing handles (red squares) on the corners (Figure T4.17).

Create the **rectangular gate** on this plot. Create the **rectangular gate** on this plot



Before **After**

Figure T4.17 Gate activation by clicking on it

2. Press and hold down the left mouse button anywhere inside the gate.
3. Move the mouse while holding down the mouse button. An outline of the gate will move as you move the mouse.
4. Release the mouse button when the gate is in the new, desired position.

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Note: You can press **Esc** on the keyboard while moving a gate (Steps 1-3) to cancel the action.

The next topic is resizing a gate.

38.4.7 Resizing Gates

In the course of this example we will:

- Re-size a gate.
- Re-size a gate with multiple vertices proportionally.
- Re-size a vertex of a gate with multiple vertices independently.

We will continue using the same layout as we were using above.

1. Activate the "Whole-Cells" gate by clicking inside the gate (Figure T4.17).
2. Move the mouse to the top right resizing handle of the gate. You will know you are in the correct place when the mouse cursor changes to a two-headed arrow. Press and hold down the left mouse button.
3. Move the mouse while holding down the mouse button. An outline of the gate will move as you move the mouse.
4. Release the mouse button when the gate is in the new, desired position.

Note: You can press **Esc** on the keyboard while resizing a gate (Steps 1-3) to cancel the action.

Polygon and freeform gates can be resized proportionally by accessing the outer set of resizing vertices.

Proportional Resizing of Polygon Gates

1. Activate the polygon gate by clicking inside the gate.

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2. Move the mouse to the top right large outer resizing handle of the gate (Figure T4.18). You will know you are in the correct place when the mouse cursor changes to a two-headed arrow.

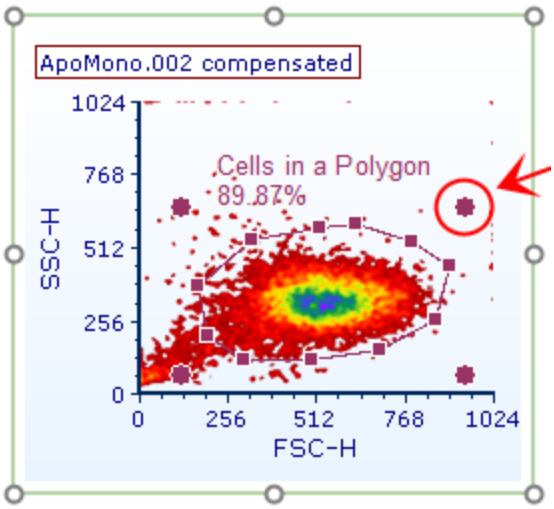

Figure T4.18 Proportional Resizing of a Polygon Gate

3. Press and hold down the left mouse button.

4. Move the mouse while holding down the mouse button. An outline of the gate will move as you move the mouse. The gate will change height as you move the mouse up or down, width, as you move the mouse right or left, and overall size as you move the mouse in a diagonal direction.

5. Release the mouse button when the gate is sized appropriately.

Resizing Polygon Gate Vertices Independently

1. Activate the Polygon Gate by clicking inside the gate.

2. Move the cursor over the upper left vertex of the polygon gate (Figure T4.19). You will know you are in the correct place when the mouse cursor changes to a two-headed arrow.

3. Press and hold down the left mouse button.

4. Move the mouse while holding down the left mouse button. As you move the mouse, the one vertex will move and all the other vertices of the polygon gate will remain fixed.

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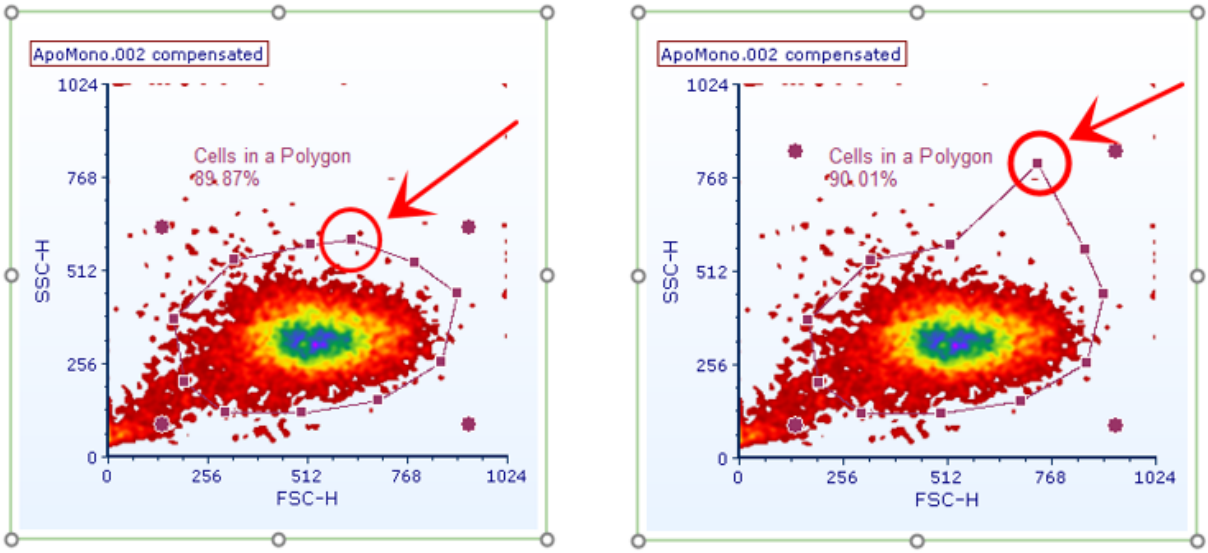
5. Release the mouse button when the vertex of the gate is in the appropriate location (Figure T4.19).

Create the **polygon gate** on this plot. Create the **polygon gate** on this plot.



Before

After

Figure T4.19 Re-size a Polygon Vertex Independently

In the next example, we will delete a gate.

38.4.8 Deleting Gates

In the course of this example we will:

- Delete a gate

We will continue using the same layout as we were using above.

1. Activate the "Whole Cells" gate by clicking in the gate (Figure T4.17).
2. Press the **Delete** button on the keyboard.

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A dialog will appear asking if you want to delete or hide the "Whole Cells" gate (Figure T4.20).

3. Press **Delete** to delete the "Whole Cells" gate.

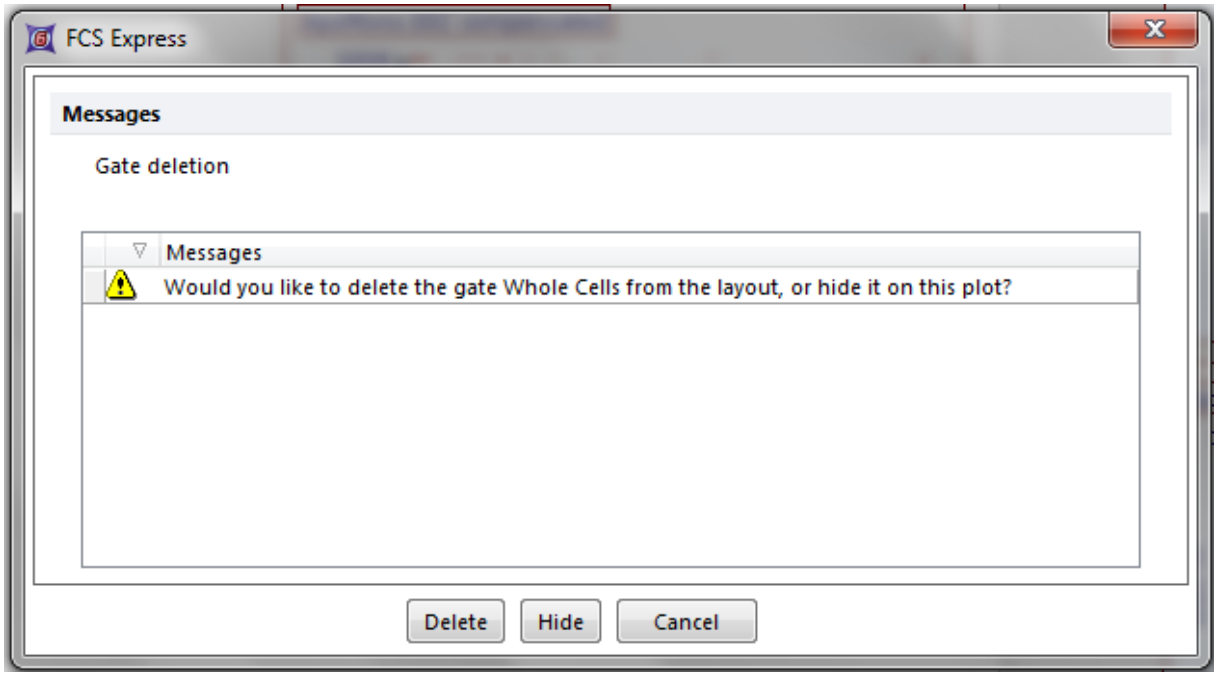

Figure T4.20 Deleting a Gate

The "Whole Cells" gate has now been deleted from the layout and thus disappears from the plot.

Once gates are created, they can be applied to a plot 1291.

38.4.9 Applying Gates

In the examples above, we created several gates. We will now learn how to apply them to a plot.

In the course of this example we will:

- Apply a gate from the format screen.

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- Apply a gate from the Gating tab.
- Apply a gate from the Gate Navigator.

We will continue using the layout we have been using until now. Numbers refer to Figure T4.21.

1. Right-click on the plot that used to have the "Whole Cells" gate on it.
2. Select **Format** from the pop-up menu.
3. Select the **Overlays** Category ❶.
4. Change the **Gate** ❷ to "Cells in ellipse".

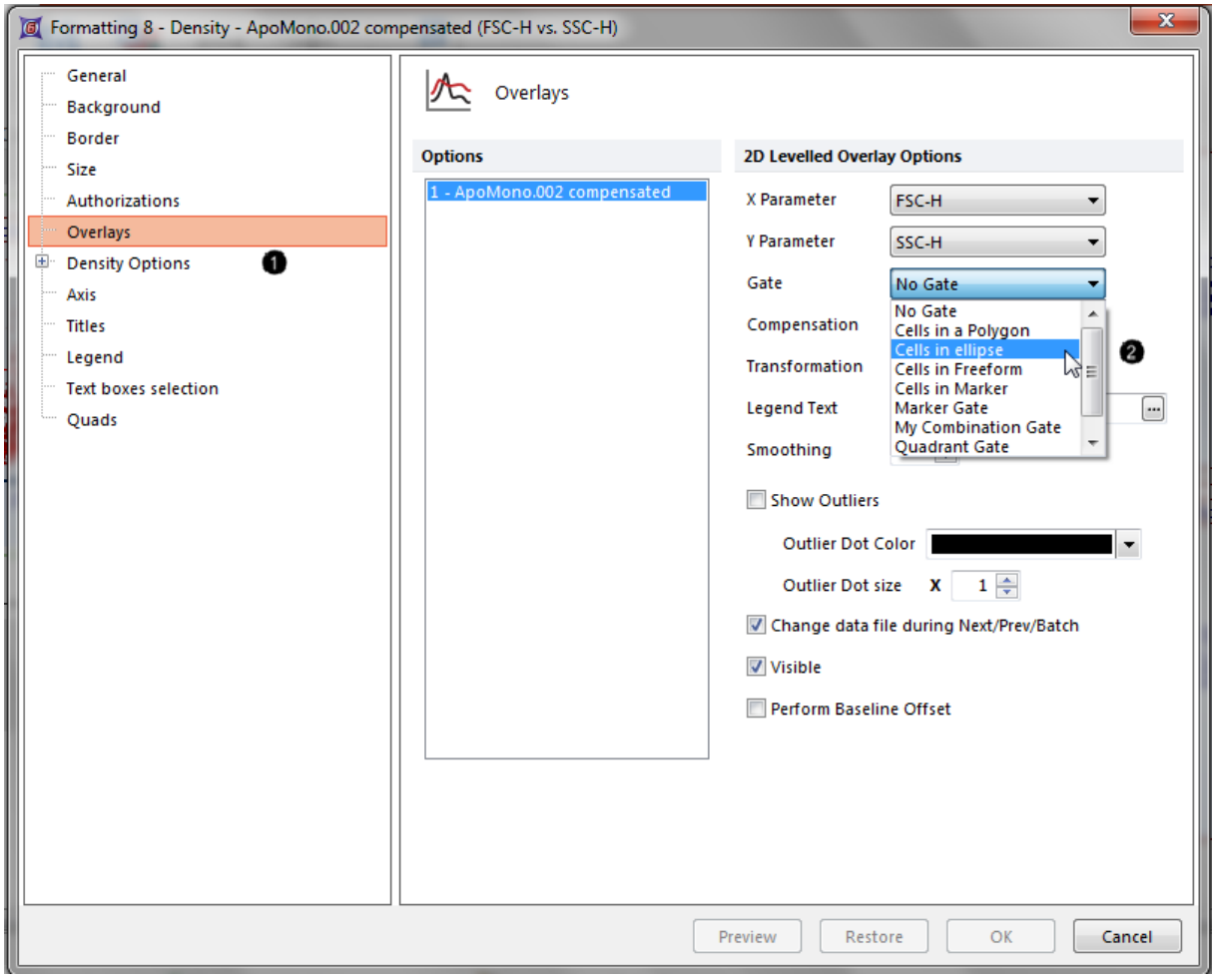

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Figure T4.21 Formatting 2D Plots (Overlays Category)

5. Press the **OK** button to accept the change.

The plot should now look similar to the right panel of Figure T4.22.

Note: The plot may not look exactly like Figure T4.22 because you may have placed your ellipse gate in a slightly different position.

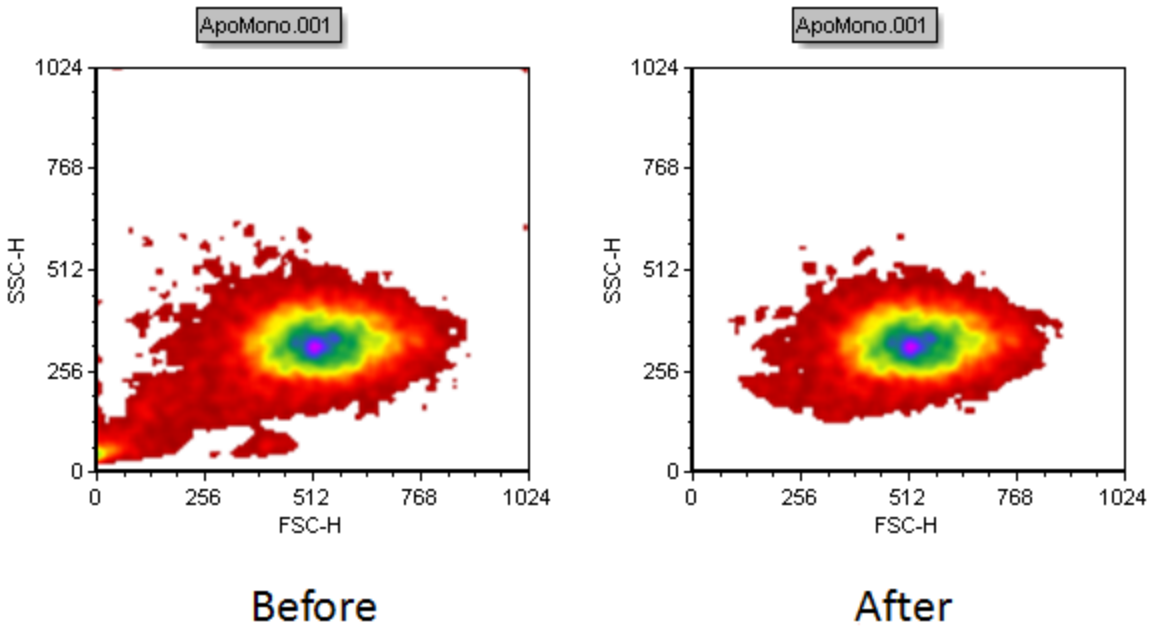

Figure T4.22 Plot Ungated (left) become gated on "Cells in Ellipse" (right)

6. Move the ellipse gate. You will see the plot that is gated on "Cells in ellipse" update as the "Cells in ellipse" gate is moved.

7. Click on the plot that you have gated so that it is in **edit mode**. You will know it is in edit mode because there will be a green border around the plot.

The **Current Gate** field on the left side of the **Create Gates** group of the **Gating** tab will display "Cells in ellipse" (see Figure T4.23), indicating that the current plot is gated on "Cells in ellipse".

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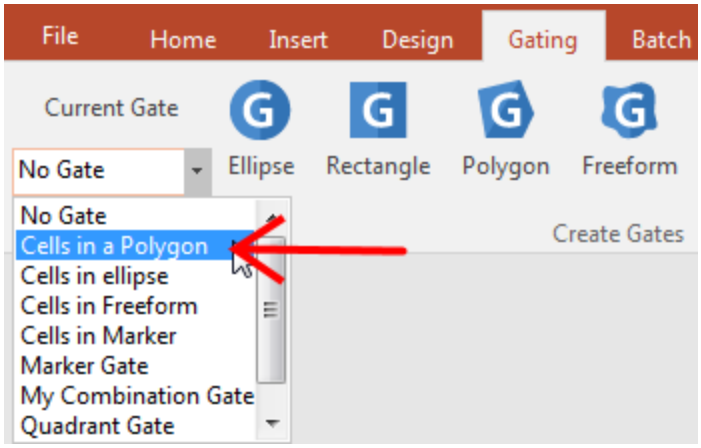

Figure T4.23 Selecting the "Cells in a Polygon" Gate

8. Click on the arrow next to "Cells in ellipse" to drop down the list of available gates.

9. Select the "Cells in a polygon" gate (Figure T4.23).

Your plot should only display cells that are in the "Cells in polygon" gate, as shown in Figure T4.24.

Note: The plot may not look exactly like Figure T4.22 because you may have placed your ellipse gate in a slightly different position.

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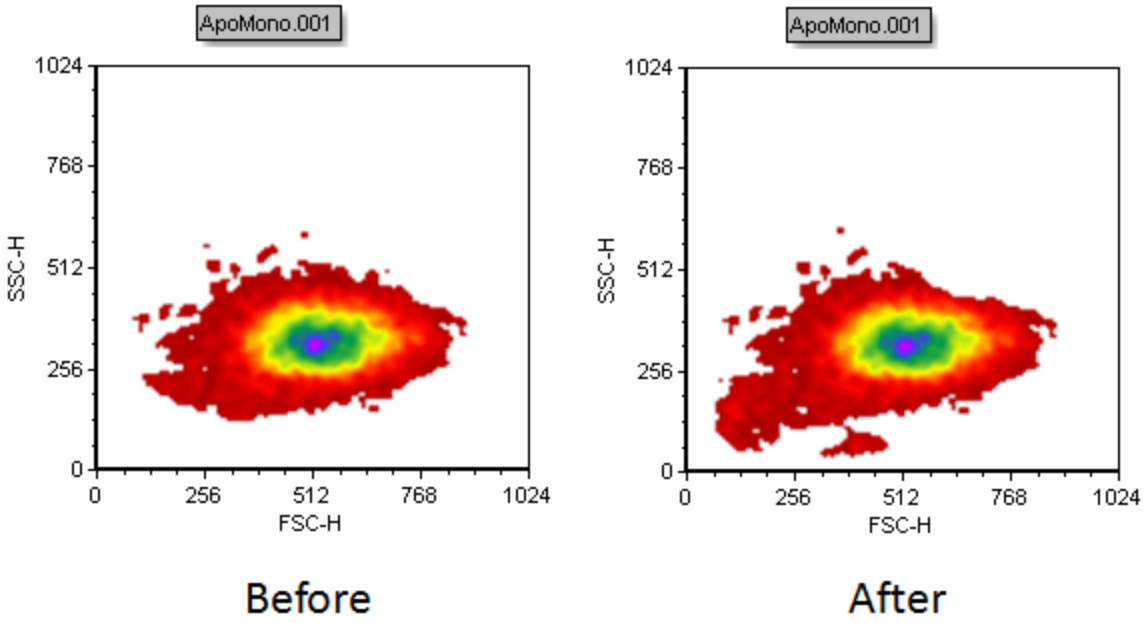

Figure T4.24 Plot Gated on "Cells in Ellipse" (left) become gated on "Cells in Polygon" (right)

10. Select the **View→Navigators→Gate Navigator** command.

11. The **Gate Navigator** will appear (Figure T4.25). The **Gate Navigator** displays all the current gates and indicates which gates are being applied to a plot.

12. Move the **Gate Navigator** to the side of the layout in order to have a complete view of your plots.

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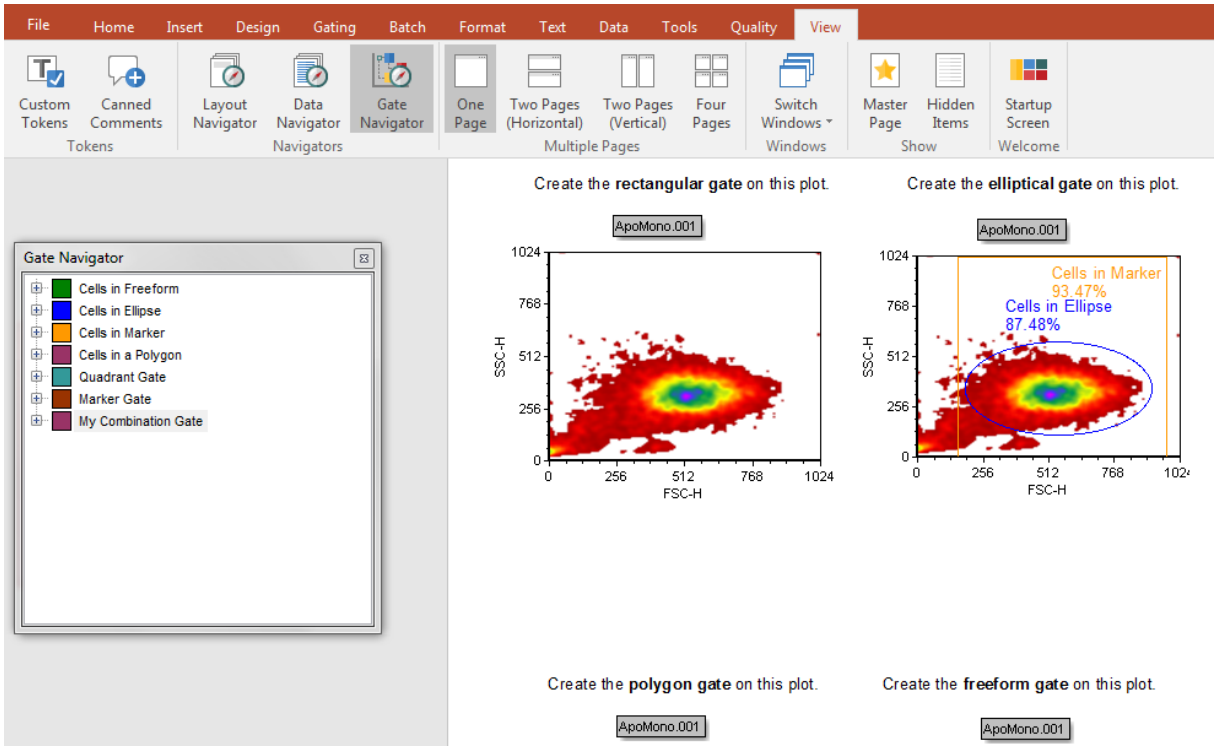

Figure T4.25 Opening the Gate Navigator Window

13. Drag and drop the "Cells in freeform" gate onto the 2D plot labeled "Create the **rectangular gate** on this plot". As soon as the mouse will be onto the plot, the latter will become blue highlighted (Figure T4.26)

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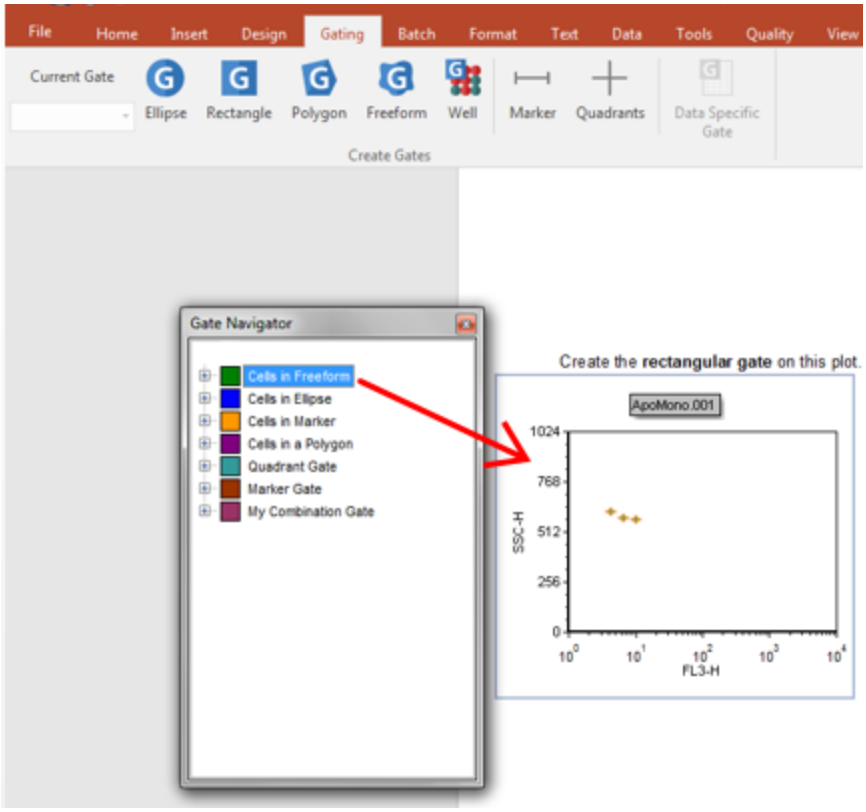

Figure T4.26 Drag&Drop a Gate from the Gate Navigator window onto a plot

14. Release the mouse button to apply the gate.

The "Cells in freeform" gate is now applied to the plot as indicated on the updated **Gate Navigator** (Figure T4.27).

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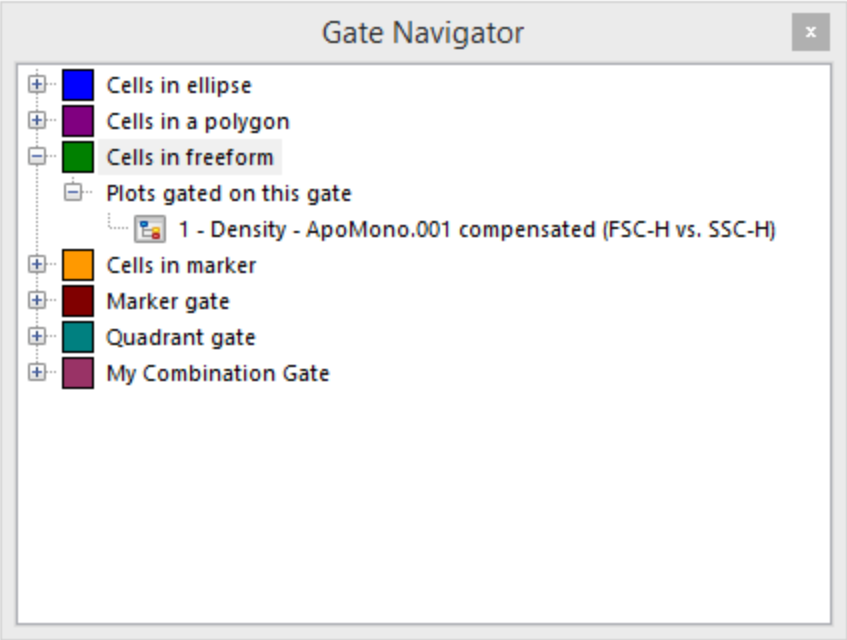

Figure T4.27 Gate Navigator Updated

Note: you can also drag and drop a gate from the **Gate View** (Figure T4.13 1279) onto any plot, exactly in the same way we did here from the **Gate Navigator** window.

38.5 Text Boxes and Tokens

Text boxes allow you to enter any text you like in your layout. They support many of the features found in modern word processing software, i.e., text formatting, tables, bullets, numbering, and many other advanced features. In addition to standard word processing features, FCS Express also supports adding tokens to a text box. Tokens 692 are dynamic pieces of information that update as you change your data. For instance, if you insert a statistics token in your text box, the value of the token will constantly be changing as you change the data.

In the first section, we will cover inserting text boxes 1298.

38.5.1 Inserting Text Boxes

There are several ways to insert a text box, but the two most common methods are: inserting a default-sized text box, or a custom-sized text box. You

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can also add a text box directly into a 2D plot or histogram so that when you move the plot, the text box will also move.

In the course of this example, we will:

- Insert a text box.
- Insert a custom-sized text box.
- Insert a text box into a plot.

To insert a default-sized text box:

1. Select **File** tab→**New**→**New** to create a new layout.
2. Select the **Insert**→**General**→**Text Box** command (Figure T5.1).
3. Click anywhere on the layout to place the text box.

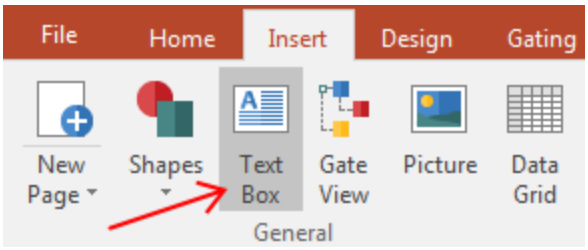

Figure T5.1 Selecting the Insert Text Box Command

By default, a text box of 1 inch by 1 inch will appear where you place and click your left mouse button on the layout. Note: To change this default size setting, select **File** tab→**Options**→**Text Boxes**.

The text box will appear in edit mode (green border) with a blinking mouse cursor (Figure T5.2). This means that you can now enter text into the text box. Entering text is similar to typing in a text box in Microsoft PowerPoint™.

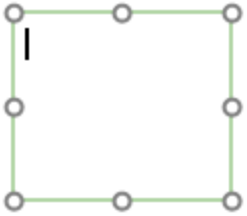

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Figure T5.2 Empty Text Box

To insert a custom-sized text box:

1. Select the **Insert→General→Text Box** command.
2. Place the mouse on the layout where you would like the top left corner of the text box to begin.
3. Press and hold down the left mouse button.
4. Move the mouse while holding down the mouse button. A dashed outline showing the dimensions of the object will appear as you move the mouse (Figure T5.3).
5. Release the mouse button once you have enlarged the dashed outline to the desired size. An empty text box will appear in edit mode (green border) at that position.

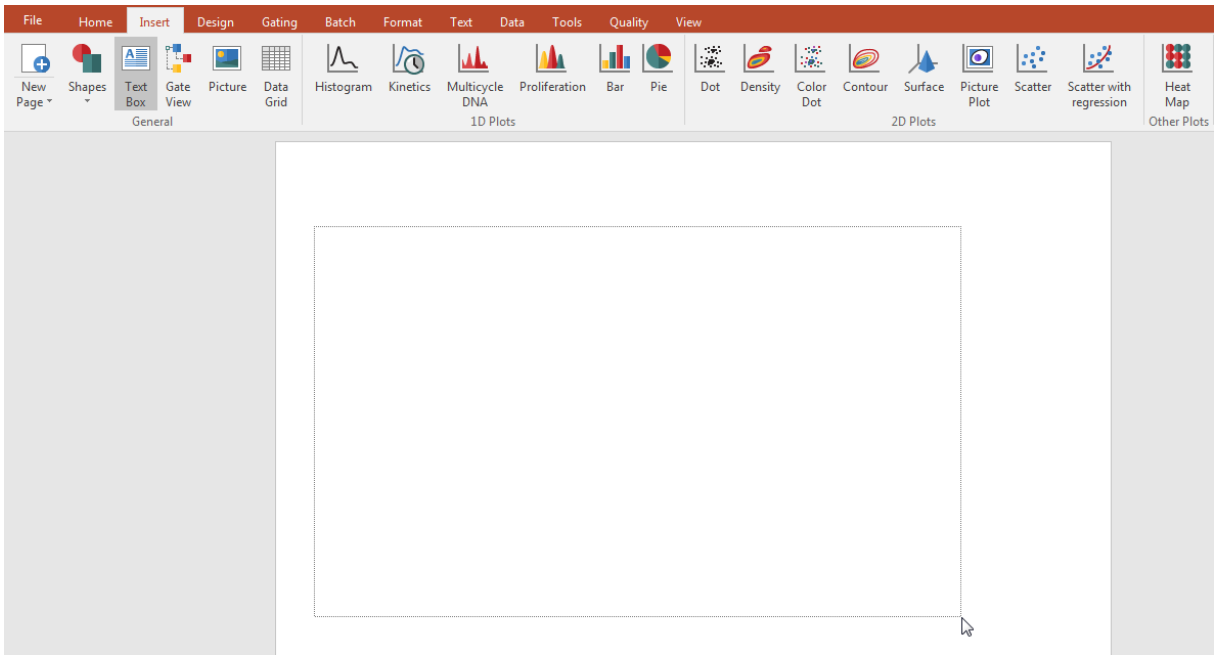

Figure T5.3 Inserting a Custom-sized Text Box

To insert a text box into a 2D plot or histogram:

1. Select **File→Close** to close the layout we were just working on.
2. Select **File→Open**.

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3. Open the layout **TextBoxInsertion.fey** found in the *FCS Express Sample Data* folder.

This layout contains a density plot of APC-A vs. PE-A.

4. Right-click on the plot. A green border will appear indicating that the plot is selected and the pop-up menu will appear.

5. Select **Add Text Box** (Figure T5.4) from the pop-up menu.

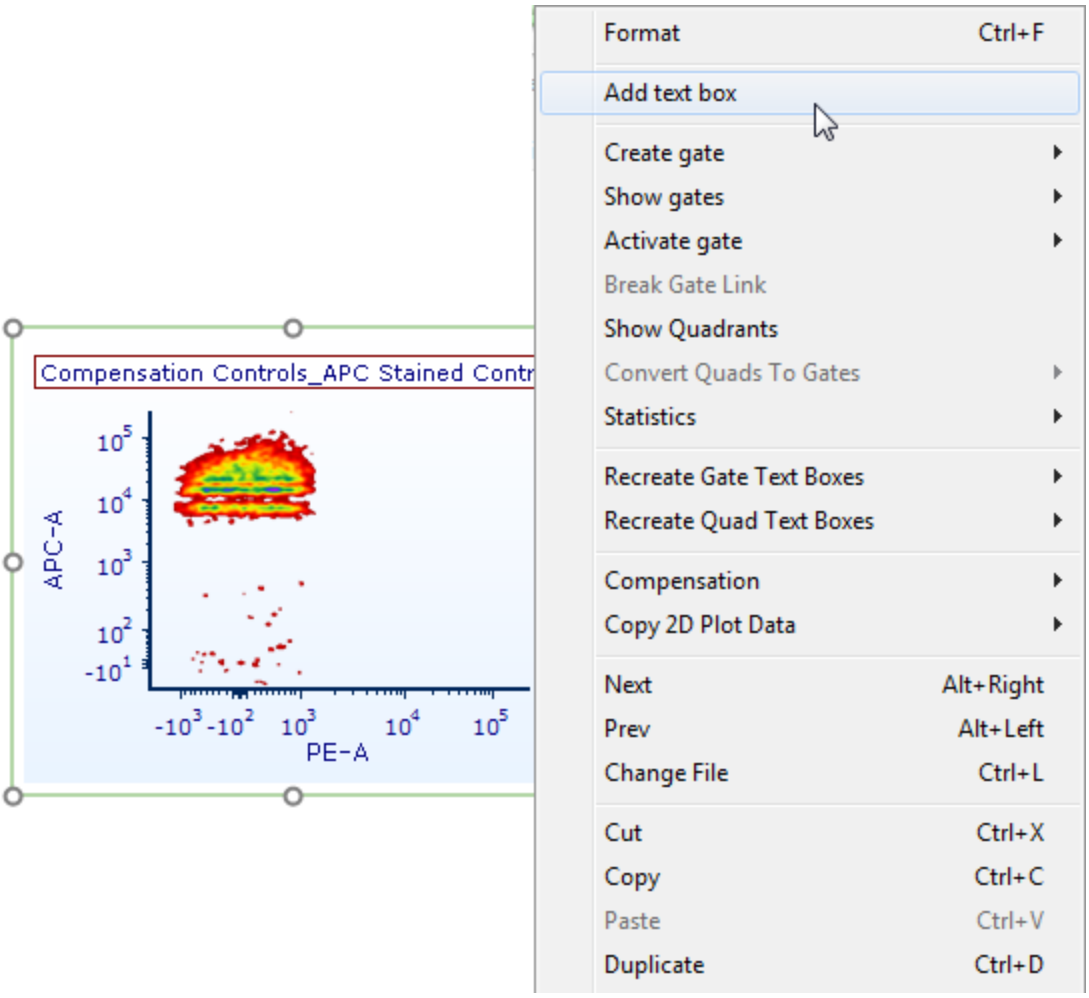

Figure T5.4 Adding a Text Box to a Plot

A small text box will appear in the upper left-hand corner of the plot (Figure T5.5). You can now begin entering text.

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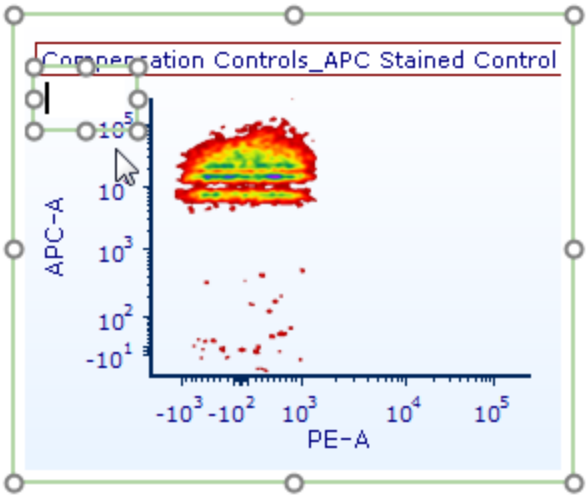

Figure T5.5 Text Box Added to a Plot

The text box is now embedded within the plot. Moving the plot you will also move the text box. You can also move the text box anywhere within the plot. For help with moving objects, please see the tutorial on Using Objects.

6. Select **File→Close** to close the layout we were just working on.

In the next section, we will format the text in a text box.

38.5.2 Formatting Text in a Text Box

The text within a text box can be formatted similar to formatting text in Microsoft Word™.

In the course of this example, we will:

- Change the font.
- Change the font size.
- Change the paragraph alignment.

1. Select **File** tab→**Open** (Figure T3.1).
2. Open the layout **TextBoxFormat.fey** found in the Tutorial Sample Data archive.

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This layout contains a single text box in the center of the page with the text "This is a text box" (Figure T5.6). The default settings for text are Arial, 10 pt., and left alignment.

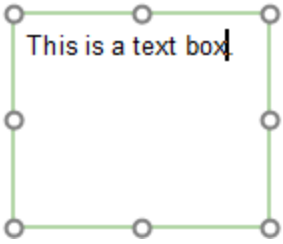

Figure T5.6 Text Box

You can change the text by selecting the **Text** tab on the ribbon (Figure T5.7). The Text editing commands are very similar to those in Microsoft Word™.

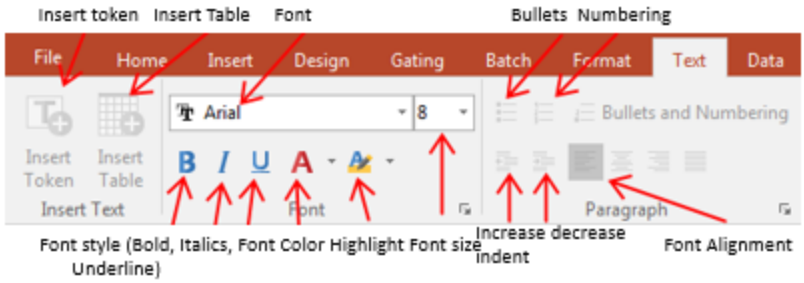

Figure T5.7 Text Formatting Commands

3. Highlight the text in the text box.
4. Change the **Font** to Courier Bold.
5. Increase the **Font Size** to 16pt.
6. Change the **Font Alignment** to Right.

The text should now look like Figure T5.8 (right panel).

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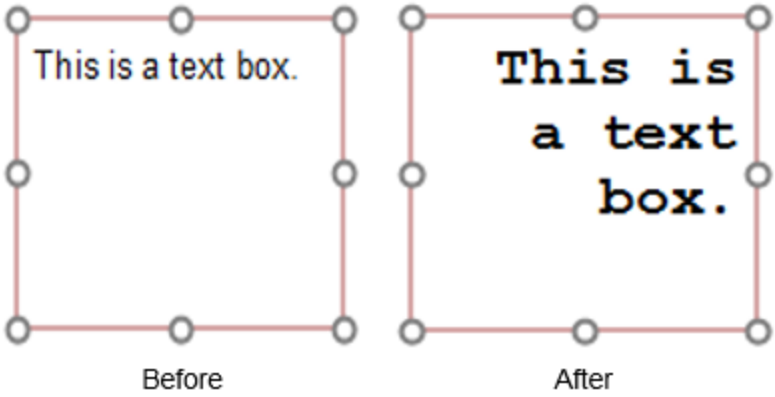

Figure T5.8 Formatting a Text Box

Next, we will change the text box properties 1304.

38.5.3 Formatting Text Box Properties

In addition to formatting the text within a text box, the properties of the text box itself (e.g., size, background color, and border style) can also be formatted.

In the course of this example, we will:

- Change the border.
- Change the size.
- Change the background.

We will continue using the same layout we were using above in the previous section.

To format the Text Box properties:

1. Right-click in the text box from Figure T5.6 1302.
2. Select **Format** from the pop-up menu (Figure T5.9).

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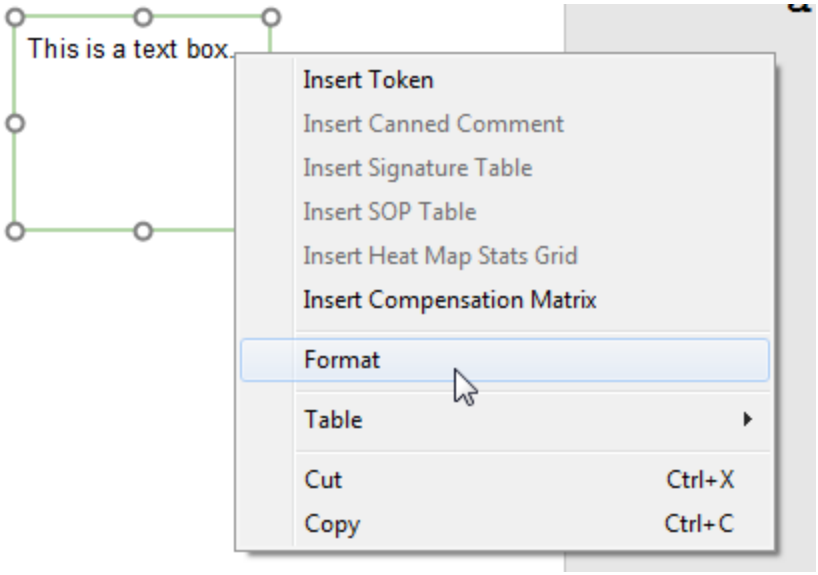

Figure T5.9 Selecting Format from the Pop-up Menu

The **Formatting Text box** dialog (Figure T5.10) will appear with five categories on the left:

A. General – To change the name of a text box.

B. Border – To change or add a border to a text box.

C. Size – To change the size of a text box.

D. Background – To change the background of the text box.

E. Authorizations – To control when objects may be selected or edited.

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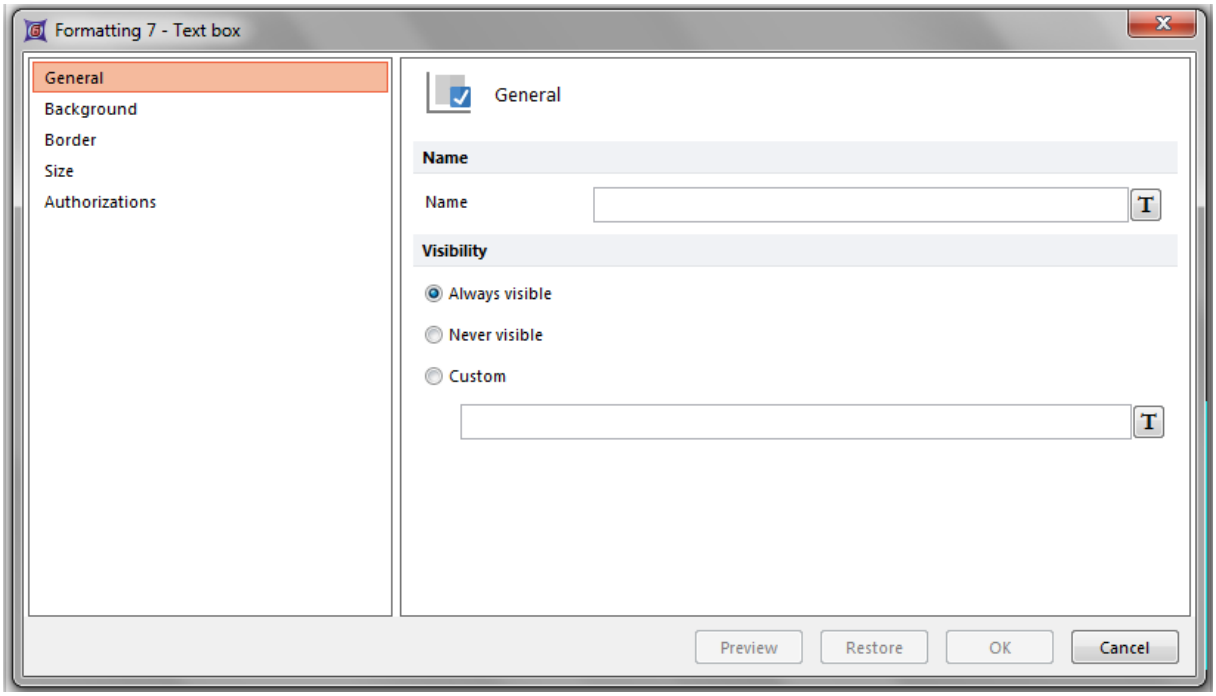

Figure T5.10 Formatting Text Box Dialog

To Modify the Border:

1. Click on the **Border** category in the **Formatting Text box** dialog (Figure T5.11).

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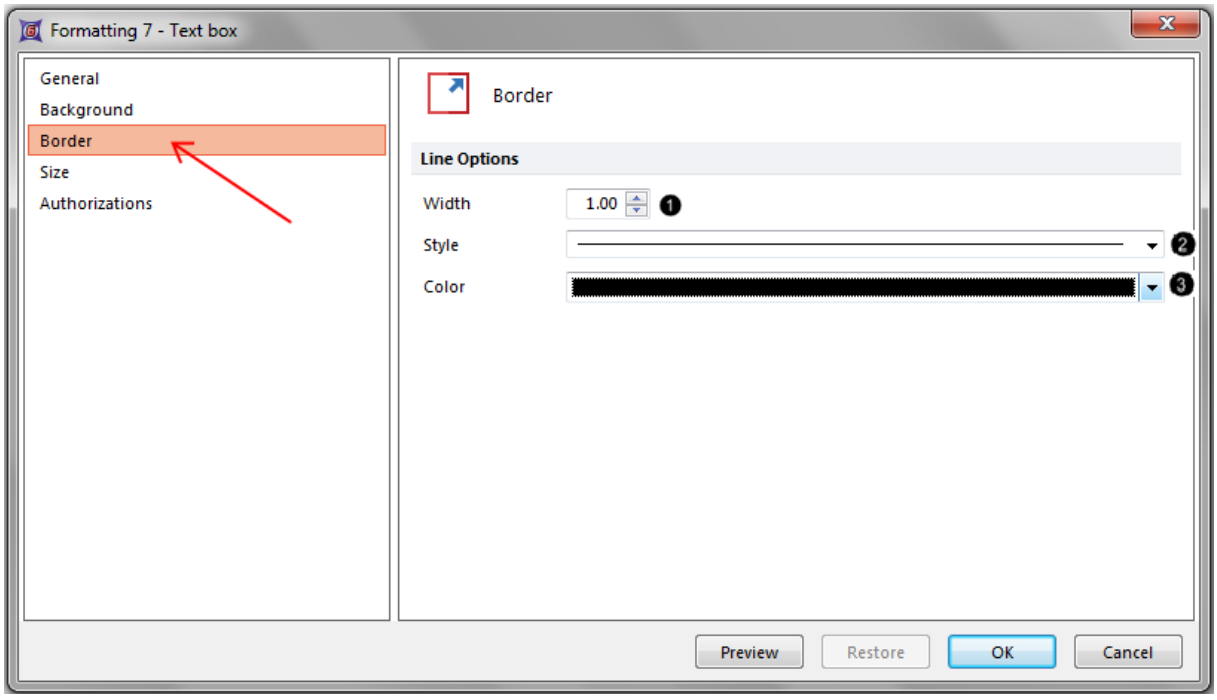

Figure T5.11 Formatting Text Box Dialog--Border Category

From the category you can change the line style, color, and width. The default setting is no line.

2. Select **1.00** points from the **Width** spin control (Figure T5.11 ❶).
3. Select **Solid Line** from the **Style** drop-down list (Figure T5.11 ❷).
4. Select **Black** from the **Color** drop-down list (Figure T5.11 ❸).
5. Select **OK**.
6. Click on the layout to remove the selection highlighting from the text box.

The text box should now look like Figure T5.12 (Right panels).

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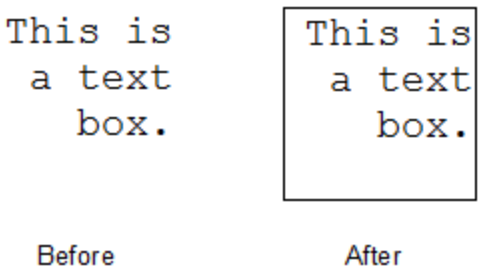

Figure T5.12 Text Box with Border

To Modify the Size:

1. Right-click in the text box..
2. Select **Format** from the pop-up menu..
3. Click on the **Size** category in the **Formatting Text box** dialog (Figure T5.13).

From the category you can change you can change the dimensions of the text box.

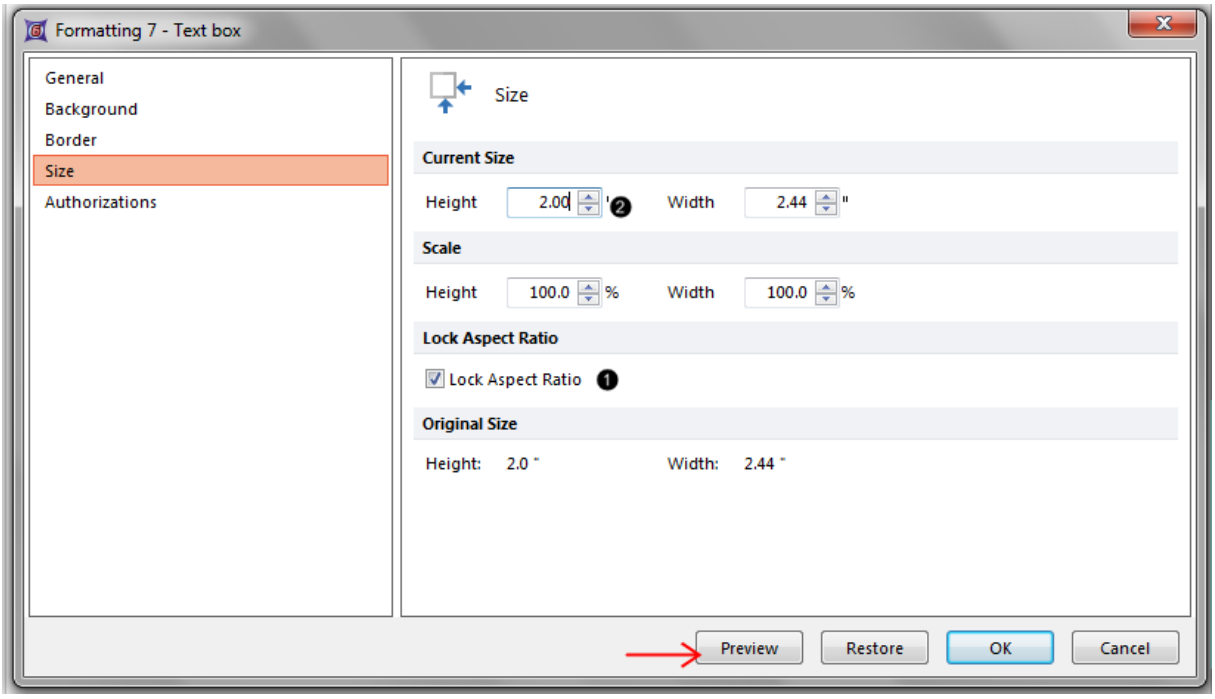

Figure T5.13 Formatting Text Box Dialog--Size Category

Click on **Lock Aspect Ratio** (Figure T5.13 ❶). This will maintain the ratio of the text box size relative to the original text box.

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4. Change the **Height** to **2.00 inches** (Figure T5.13 ❷).
5. Select **Preview**. By changing the height to 2.00 inches, the width has automatically change to 2.00 (because Lock Aspect Ratio is checked in Figure T5.13).
6. Select **OK**.
7. Click on the layout to remove the selection highlighting from the text box.

The text box should now look like the 'After' image on the right of Figure T5.14.

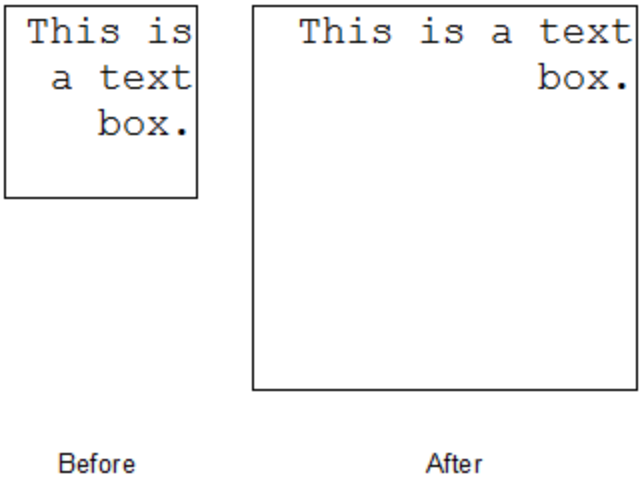

Figure T5.14 Modified Text Box Size

To Modify the Background of a text box:

1. Click on the **Background** category of the **Formatting Text box** dialog (Figure T5.15).

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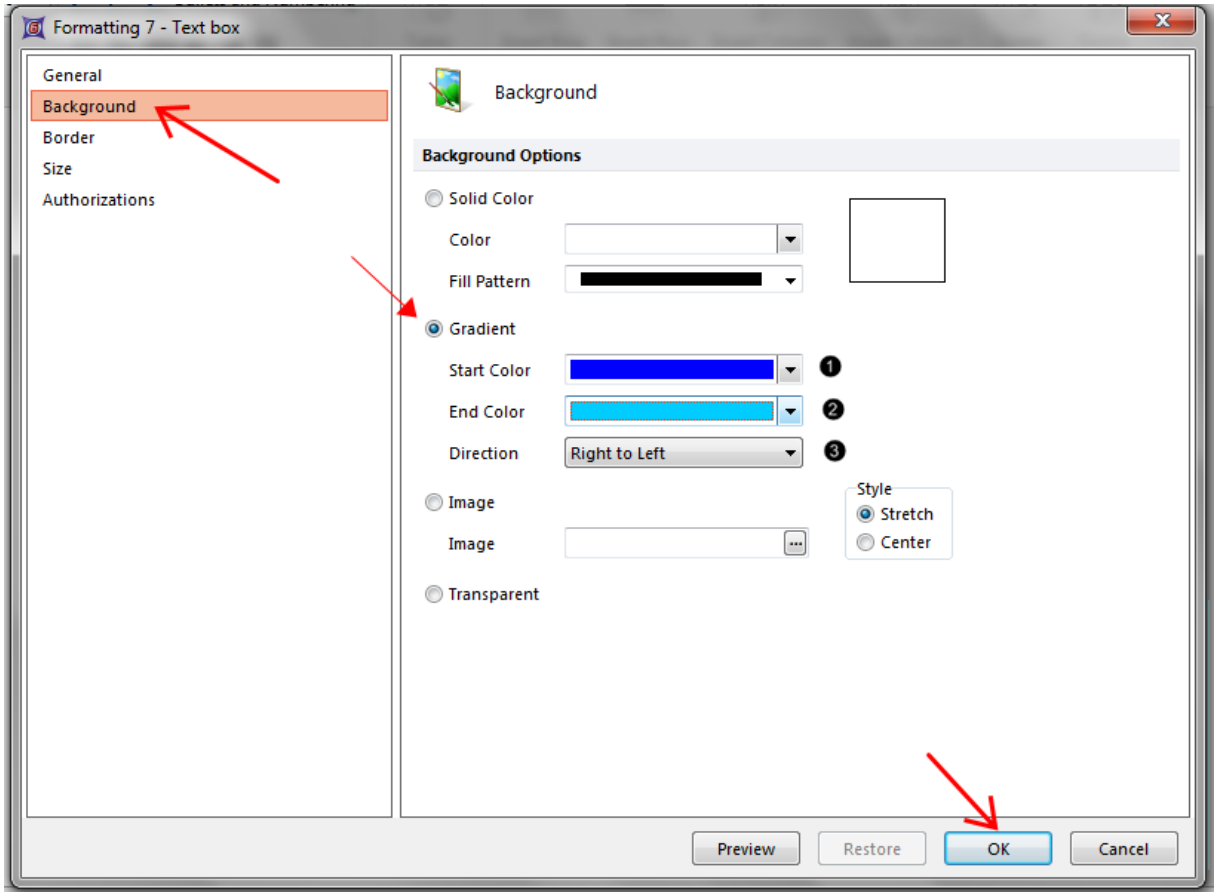

Figure T5.15 Formatting Text Box Dialog--Background Category

The **Background** category page will appear (Figure T5.15). Here, you can change the background of the text box. The default is a solid white background.

2. Select **Gradient** for the **background**.
3. Change the **Start Color** to **Blue** (Figure T5.15 ❶).
4. Change the **End Color** to **Pale Blue** (Figure T5.15 ❷).
5. Change the **Direction** to **Right to Left** (Figure T5.15 ❸).
6. Select **OK**.

The resulting image should now look like the image on the right of Figure T5.16.

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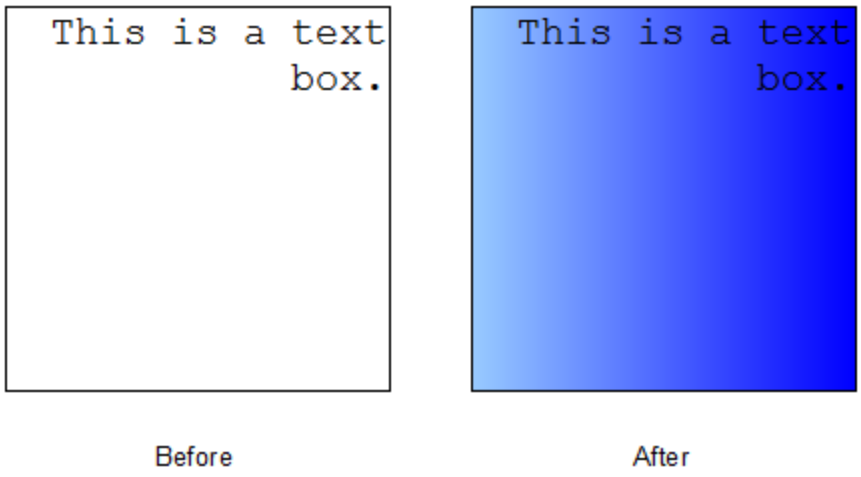

Figure T5.16 Text Box with New Background

Select **File** tab→**Close** to close the layout we were just working on.

Next, we will insert and modify a table 1311.

38.5.4 Inserting and Modifying a Table

Tables are another way to insert and organize data in a layout. FCS Express supports a wide variety of formatting options for tables.

In the course of this example, we will:

- Insert a table into a text box.
- Modify table properties.

To insert a table:

1. Select **File** tab→**New Layout** to open a new layout.
2. Insert a text box and have it open in edit mode (green border). For help with inserting a text box, see the previous section of this tutorial, "Inserting Text Boxes" 1298.

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3. Select the **Text→Insert Text→Insert Table** command (Figure T5.17). (You can also right-click within a text box and select **Table→Insert Table** from the pop-up menu.)

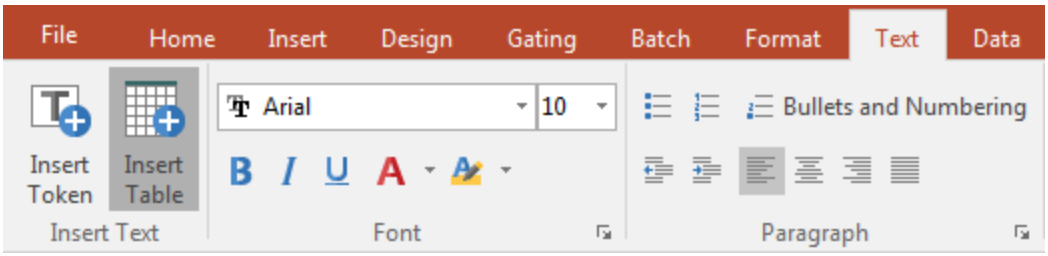

Figure T5.17 Insert Table Command

An **Insert Table** dialog will now appear (Figure T5.18).

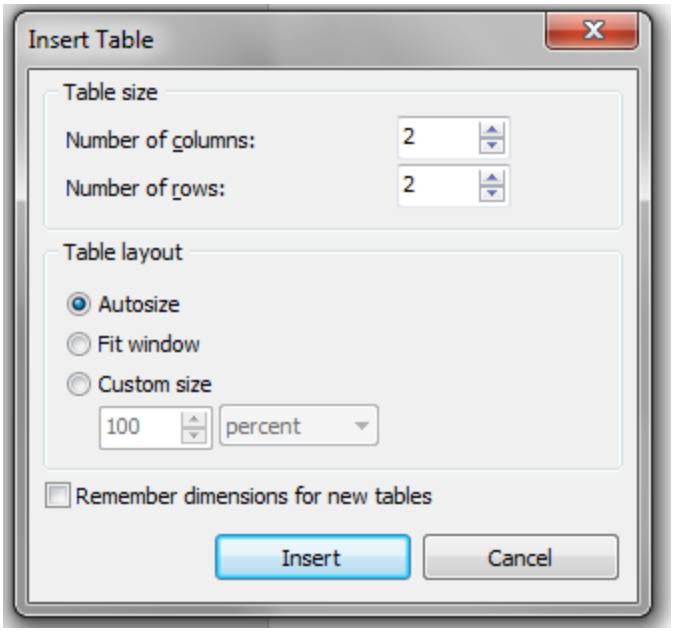

Figure T5.18 Insert Table Dialog

Here you can select the properties of the new table. The default settings are two columns and two rows.

4. Press **OK** to accept the default properties.

A new table will appear in the text box (Figure T5.19).

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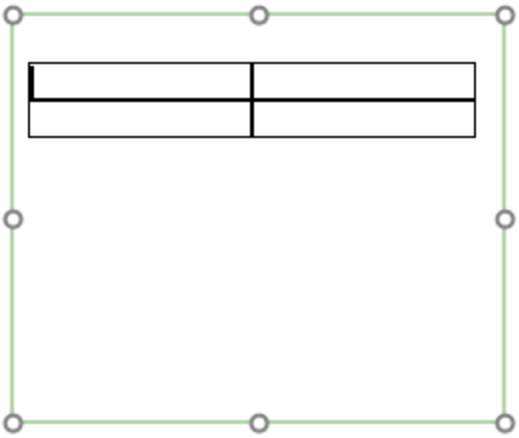

Figure T5.19 Table Inserting in a Text Box

To Modify a Table:

1. Click inside the table to select it.
2. Select the **Text→Table→Table Properties** command (Figure T5.20) or right-click on the table and select **Table→Table Properties** from the pop-up menu.

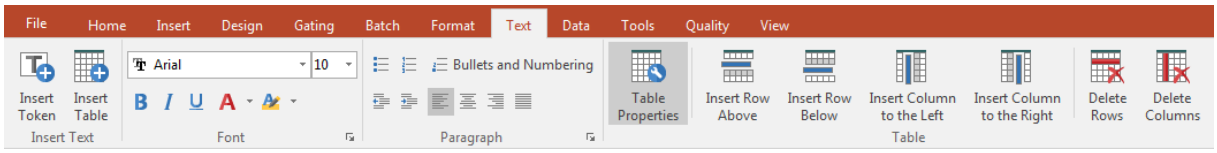

Figure T5.20 Table Properties Command

A Table **Properties** dialog will appear (left side of Figure T5.21).

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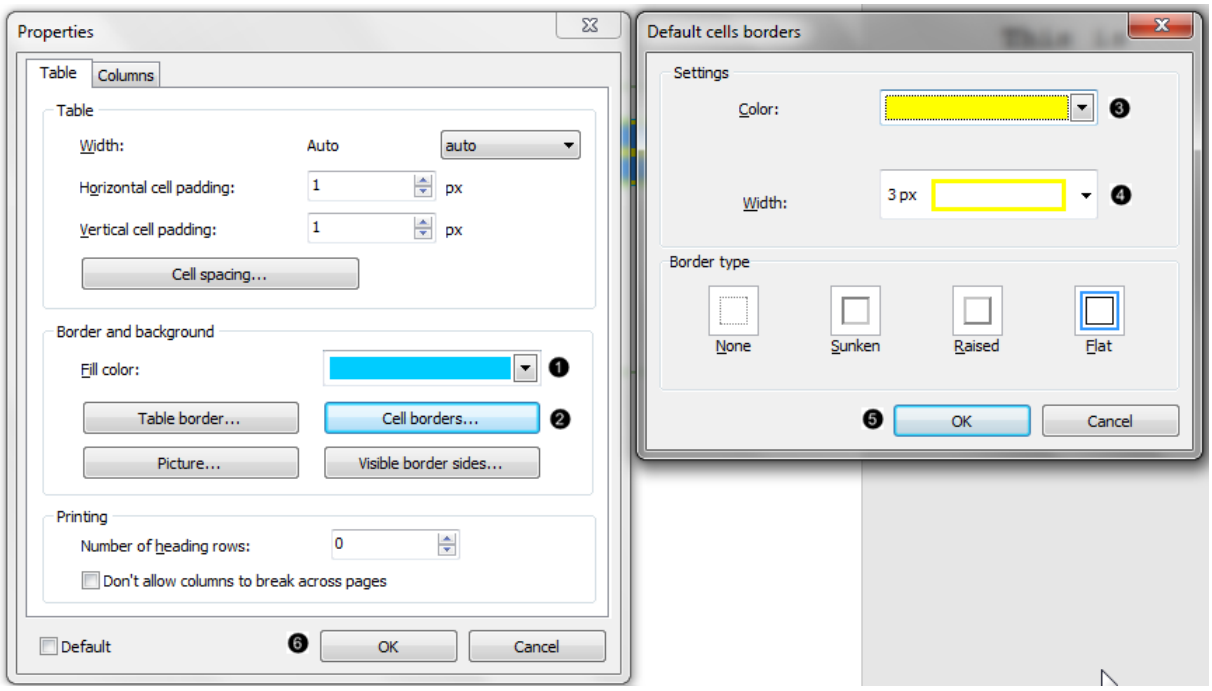

Figure T5.21 Table Properties and Default Cells Border Dialogs

3. Select the color **Sky Blue** from the **Fill Color** drop-down list (Figure T5.21 ❶).
4. Click on the **Cell Borders... button** (Figure T5.21 ❷). The **Default Cells Borders** dialog will appear (Figure T5.21 right panel).
5. Change the cell border **Color** to **Yellow** (Figure T5.21 ❸).
6. Change **Width** to 3 (Figure T5.21 ❹).
7. Press the **OK** button on the **Default Cells Borders** dialog (Figure T5.21 ❺).
8. Press the **OK** button on the Table **Properties** dialog to accept the changes (Figure T5.21 ❻).

The resulting table should now look like the one on the right of Figure T5.22.

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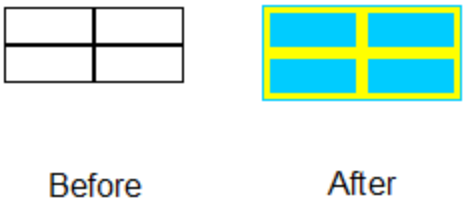

Figure T5.22 Formatted Table

Select **File** tab→**Close Layout** to close the layout we were just working on.

We will now proceed to inserting and modifying a token.

38.5.5 Inserting and Modifying a Token

Tokens can represent items of many types including: statistics, classifiers, file information, or customized values. You can insert tokens in text boxes. For the following three sections on tokens, the file we will be using is **ApoMono.002** found in the Tutorial Sample Data archive.

In the course of this example, we will:

- Insert a token into a text box.
- Modify the formatting of a token.

To insert a token:

1. Select **File** tab→**Open** (Figure T3.1).
2. Open the layout **TokenInsertion.fey** found in the Tutorial Sample Data archive.
3. Left-click after the word "contains" in the text box containing the incomplete sentence "The Gate 'Whole Cells' contains". The text box should change to have a green border, indicating it is in edit mode, and the cursor should be flashing after the word *contains*.

We will be completing this sentence with the statistic # **of Cells** in Gate 'Whole Cells'.

4. Right-click inside the selected text box.

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5. Select **Insert Token** from the pop-up menu (Figure T5.23).

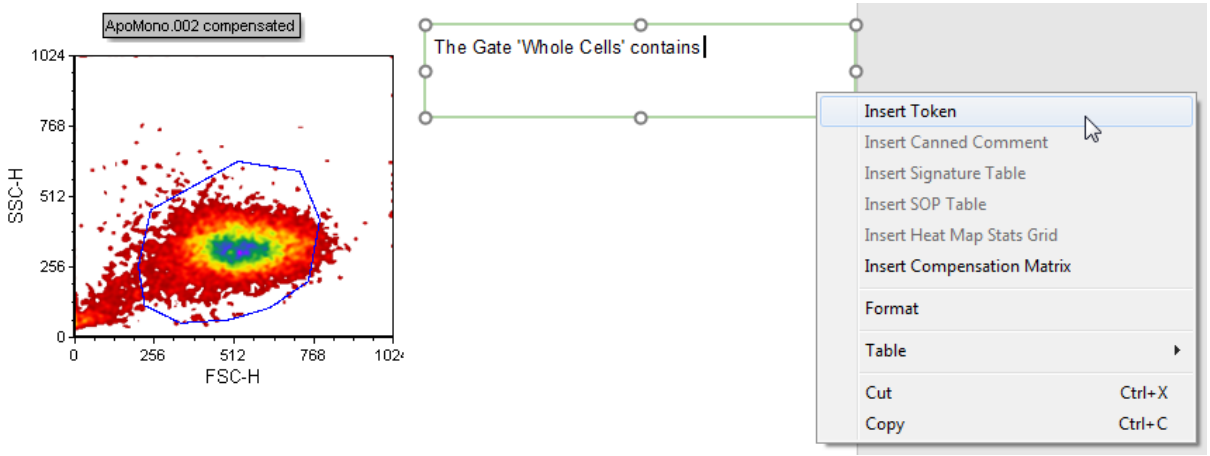

Figure T5.23 Inserting a Token

You can also insert a token using the **Text→Insert Text→Insert Token** command from the Ribbon Bar.

An **Insert a Token** dialog will appear (Figure T5.24).

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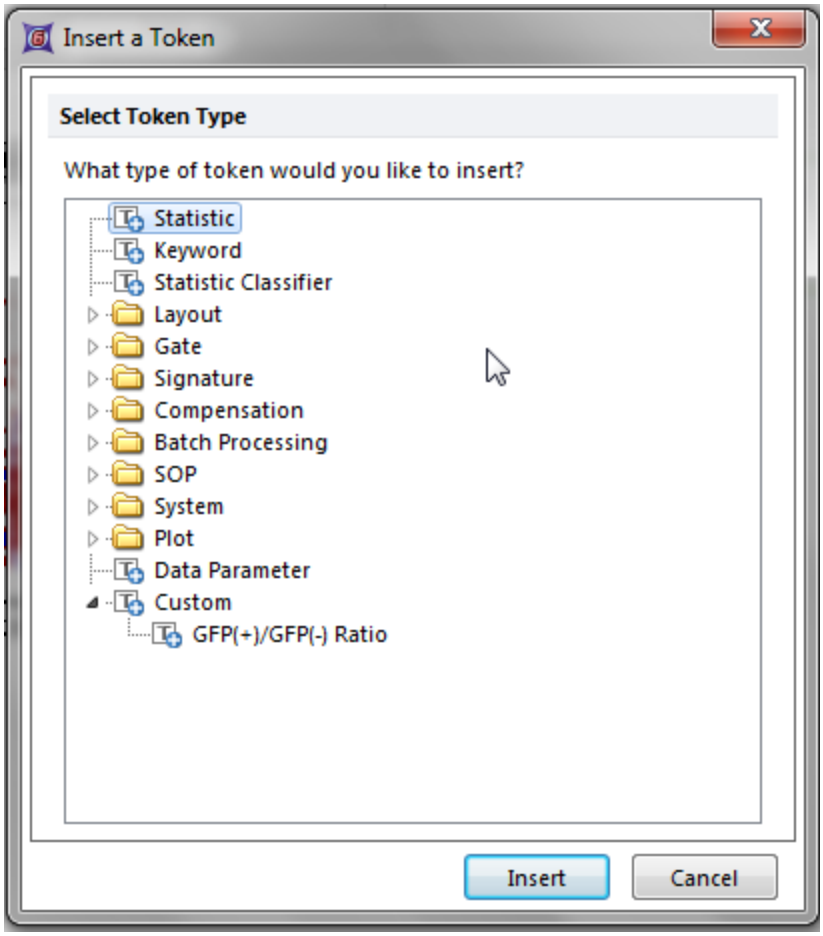

Figure T5.24 Insert a Token Dialog

6. Click on **Statistic**.
7. Click **Insert** at the bottom of the dialog.

A **Create Statistic** dialog will appear with the **Data Source** category open (Figure T5.25). There is no need to change the data source, since it is already showing the source as the FSC vs. SSC plot of ApoMono.002.

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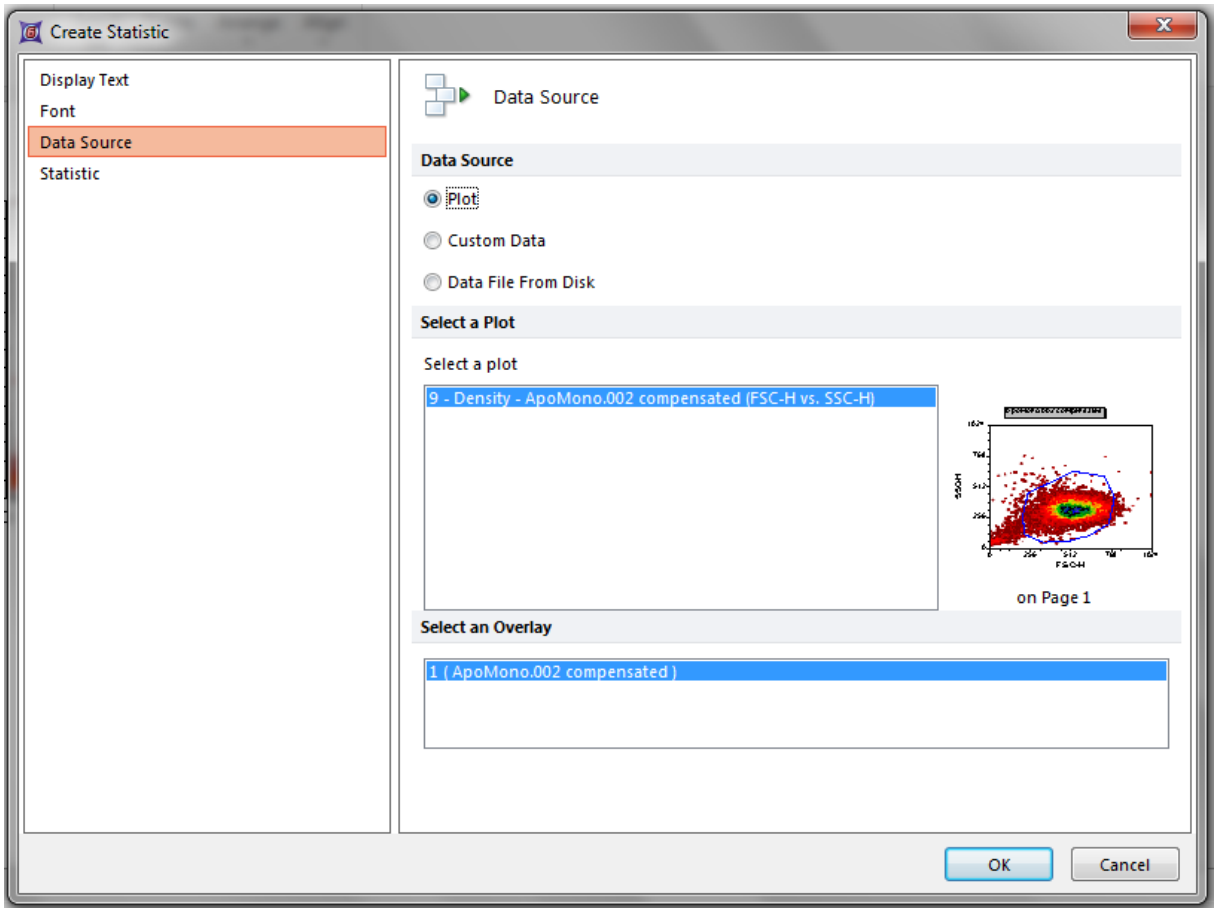

Figure T5.25 Create Statistic Dialog--Data Source Category

8. Click on the **Statistic** Category of the **Create Statistic** dialog (Figure T5.26).

The **Statistic** page of the **Create Statistic** dialog will now appear.

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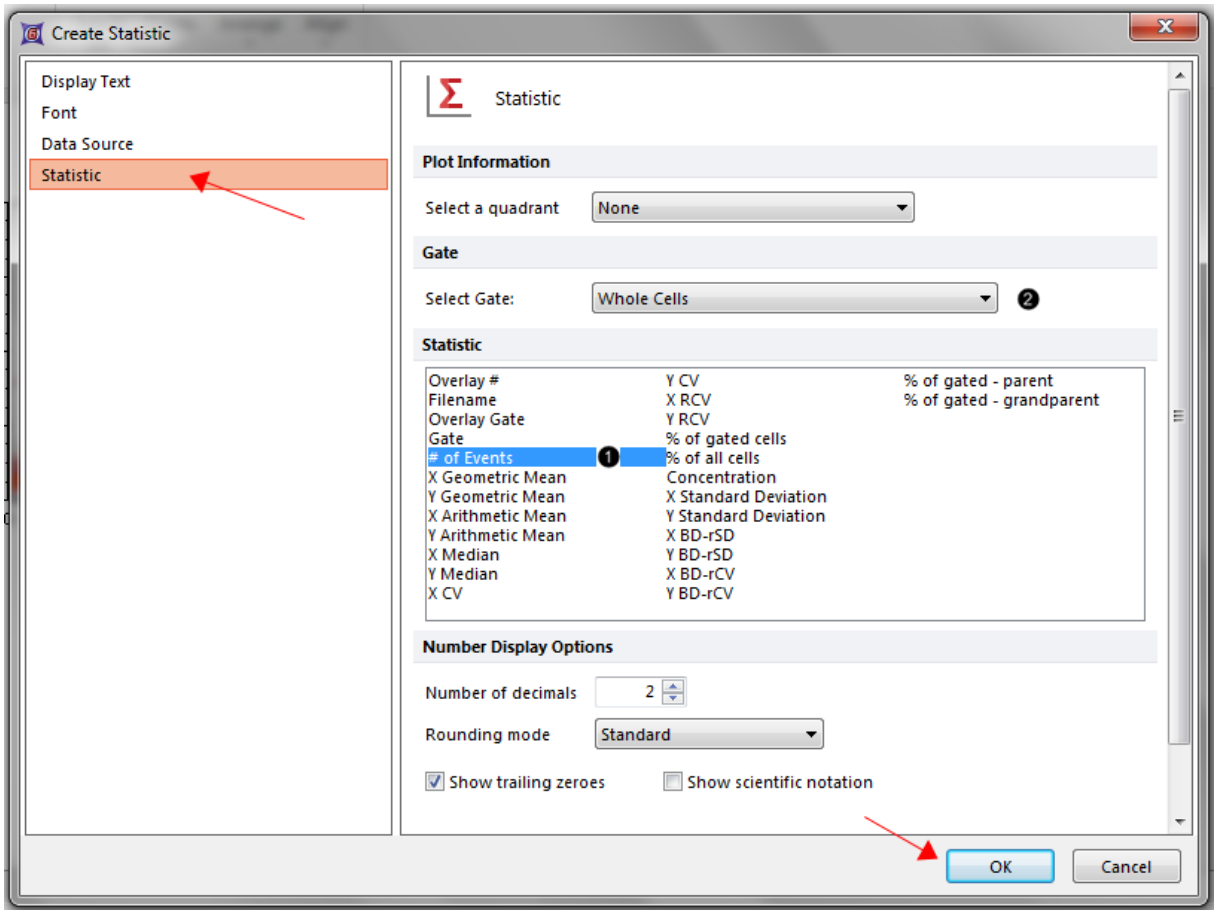

Figure T5.26 Create Statistic Dialog--Statistic Category

We will select the statistic we want the token to display (Figure T5.26).

9. Select # **of Events** in the **Statistic** list box (Figure T5.26 ❶).
10. Select **Whole Cells** from the **Gate** drop-down list (Figure T5.26 ❷).
11. Select **OK**.

The Statistic (# of events) will now appear in the text box after the text 'The Gate Whole Cells contains' and will be highlighted gray whenever the text box is in edit mode (green border). The grey background is to show that the text is a token, and cannot be directly edited.

12. Push the "space" bar key and type 'events.' in the text box after the token to complete the sentence.

The text box should now look like Figure T5.27. The token will appear with the same text settings as the text already in the box.

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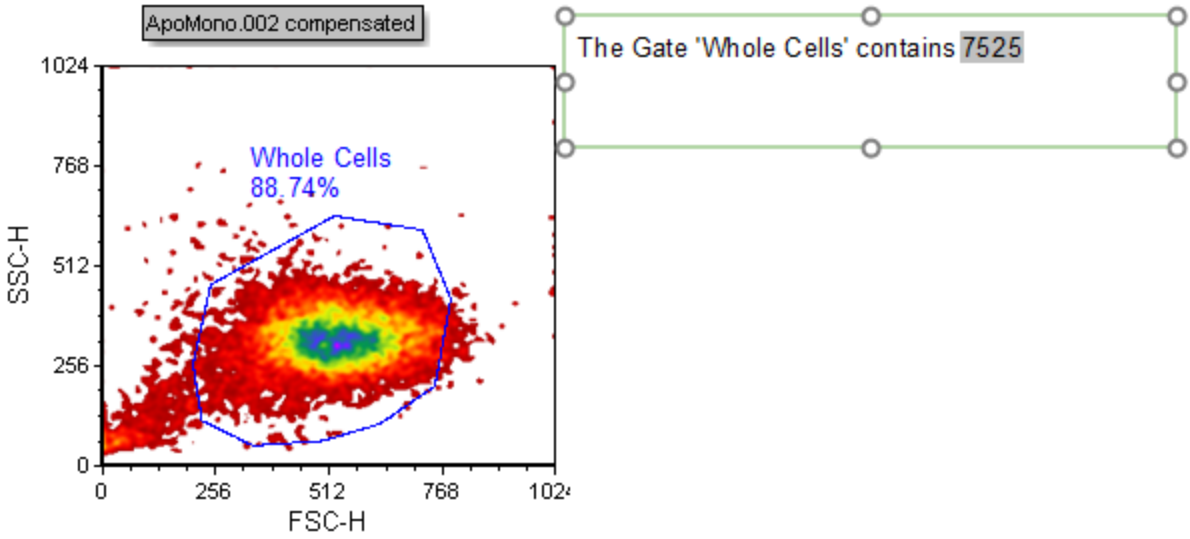

Figure T5.27 Text Box with Token

Note: Tokens can also be inserted into tables.

You can format the font of a token identically to the way you format the font for normal text, described above. Figure T5.28 shows the same text box as Figure T5.27 except the font was changed to Courier New, 15pt, and Bold. For more help on changing text in text boxes, see the section on Formatting Text in a Text Box 1302.

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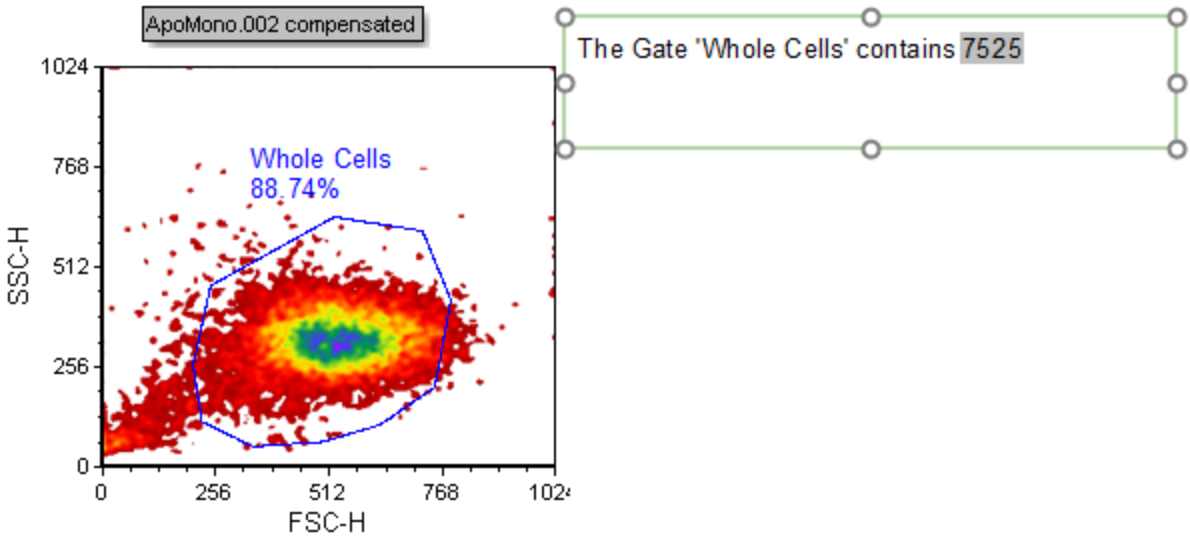

Figure T5.28 Formatted Token Font

Tokens can also be inserted by dragging and dropping the plot onto the text box.

To insert a Token by Drag and Drop:

First, we need to delete the token in the text box.

13. Highlight "**7525**" events in the text box.
14. Hit the **Delete** key to delete the token.
15. Left-click on the border of the **SSC-H v FSC-H ApoMono.002** plot. The border of the plot should turn green.
16. Left-click on the green border. The border of the plot should turn red to indicate it is in selected mode.
17. While holding down the left mouse button, drag the cursor (Figure T5.29) to the space after the word "**contains**" in the text box.
18. Release the mouse button to drop the plot into the text box.

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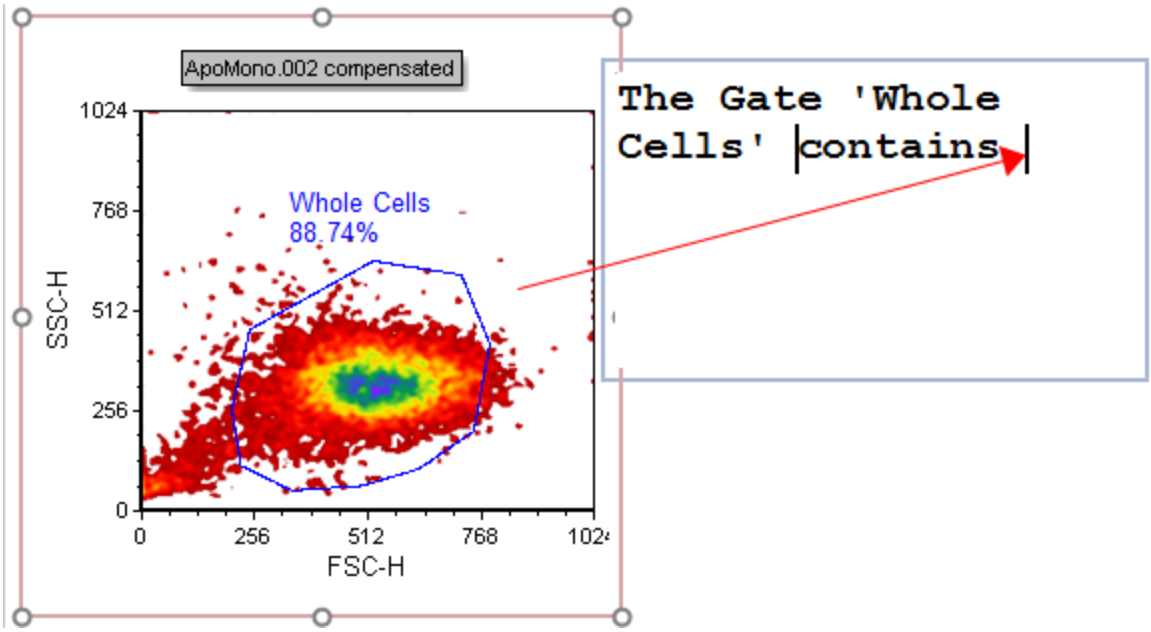

Figure T5.29 Dragging Token from Plot onto Text Box

The **Paste Special** dialog will appear (Figure T5.30).

19. Select **Statistic Token** from the **Paste Special** dialog (Figure T5.30).

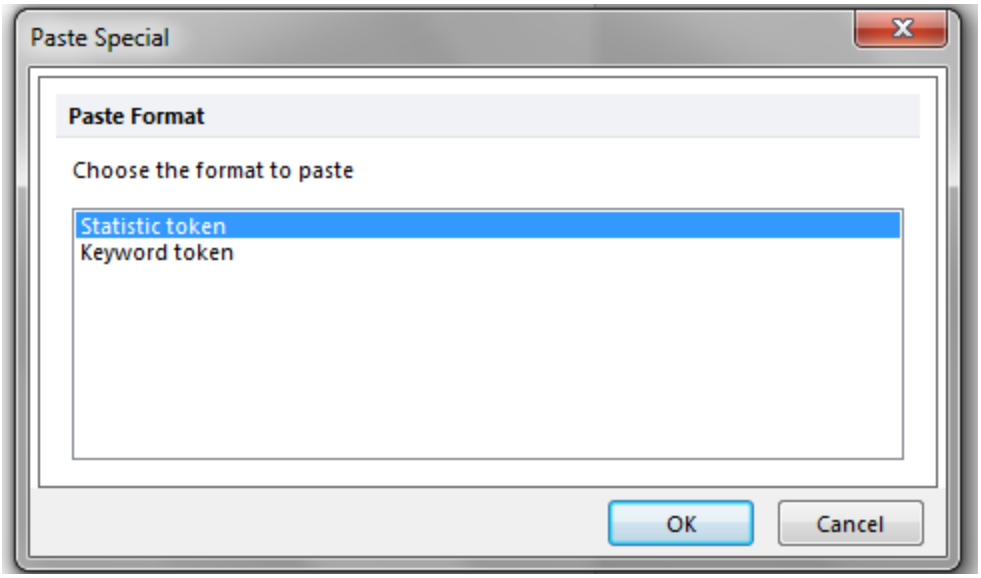

Figure T5.30 Paste Special Dialog

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20. Click **OK**

The **Create Statistic** dialog will now appear with the **Statistic** category open as previously seen in step 8 (Figure T5.26)

21. Select **Whole Cells** in the **Gate** field (Figure T5.26 ❶).
22. Select # **of Events** from the **Statistic** box (Figure T5.26 ❷).
23. Select **OK**.

The number of events will now be inserted as a token, highlighted in grey, after the word "**contains**" in the text box (Figure T5.29).

24. Click after the number of events token (highlighted in grey) in the text box.
25. Push the "space" bar key and type "events." to complete the sentence in the text box.

The text box will now appear as in Figure T5.31.

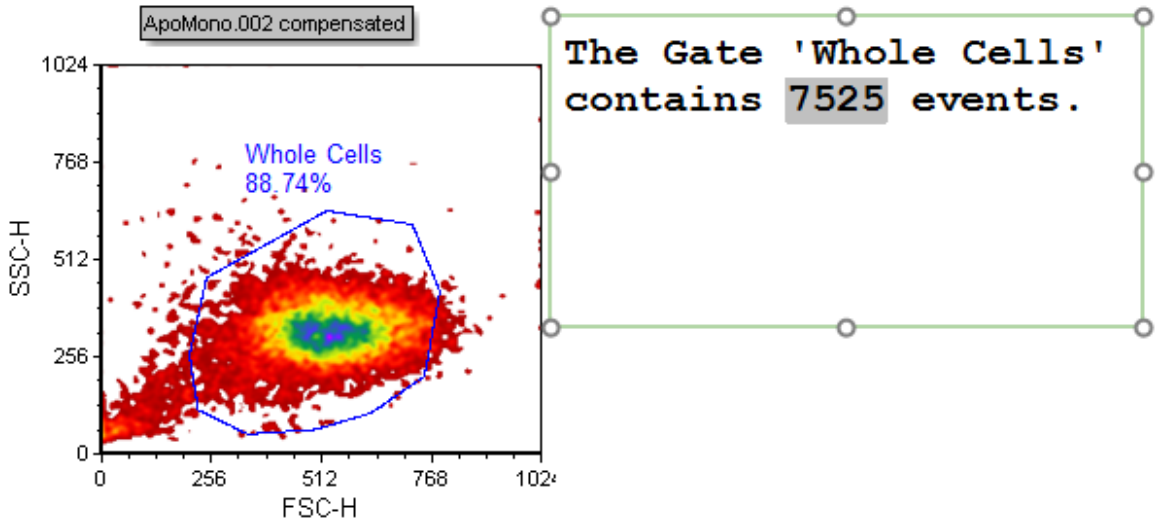

Figure T5.31 The Layout as it will be appear at the end of this section

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Select **File** tab→**Close** to close the layout we were just working on.

In the next example, we will creating a custom token 1324.

38.5.6 Creating a Custom Token

Tokens can also be customized to create any kind of statistic that you like. In this example, we will calculate the Apo(+)/Apo(-) ratio in the positive sample file **ApoMono.002**. This is calculated by taking the # of positive cells and dividing them by the # of negative cells.

To insert a Custom Token:

1. Select **File** tab→**Open.**
2. Open the layout **TokenCustom.fey** found in the Tutorial Sample Data archive 1210.

The layout, shown in Figure T5.43, contains two 2D plots a text box containing the text 'Apo(+)/Apo(-) ratio:'.

We will be inserting a custom token statistic here (Figure T5.43).

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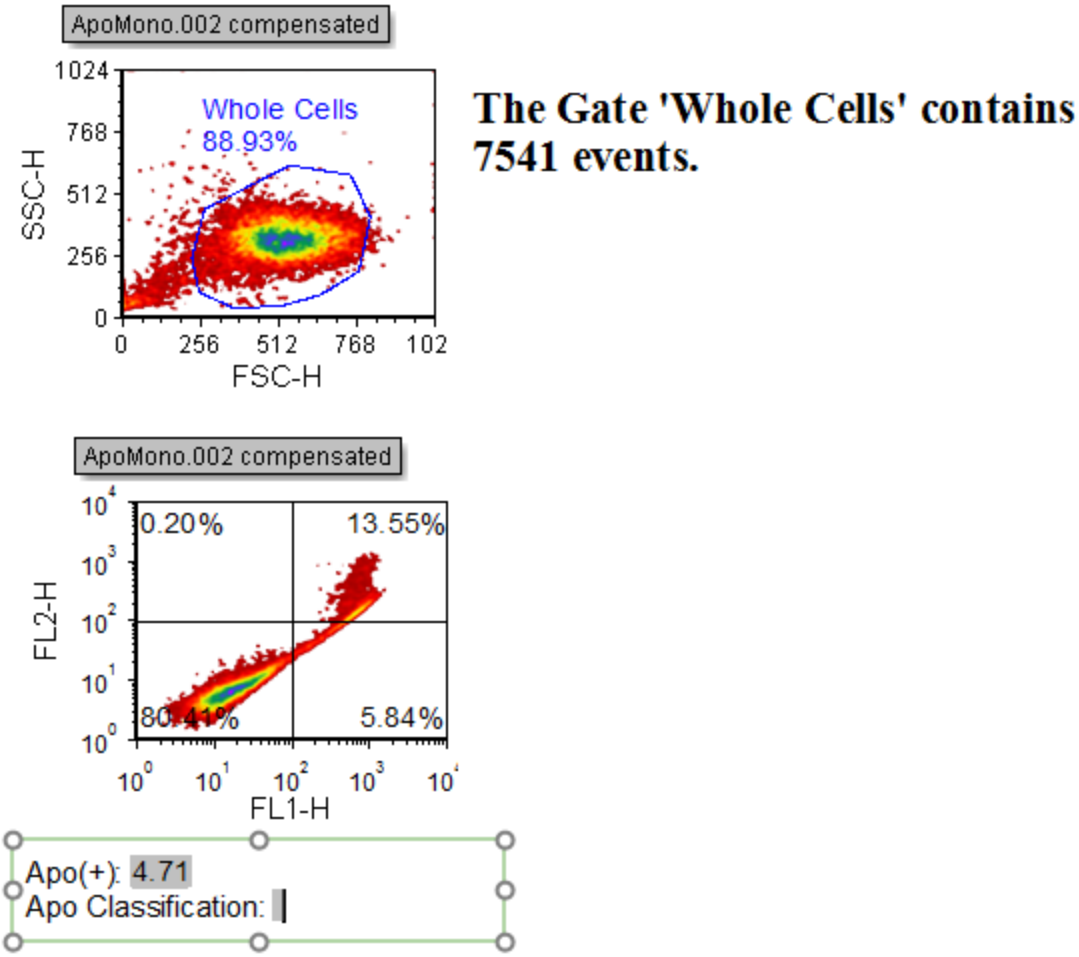

Figure T5.43 Custom Token Layout (TokenCustom.fey)

3. Click in the text box at the end of the incomplete text '**Apo(+)/Apo(-) Ratio:**'.

4. Select the **View→Tokens→Custom Tokens** command (Figure T5.44).

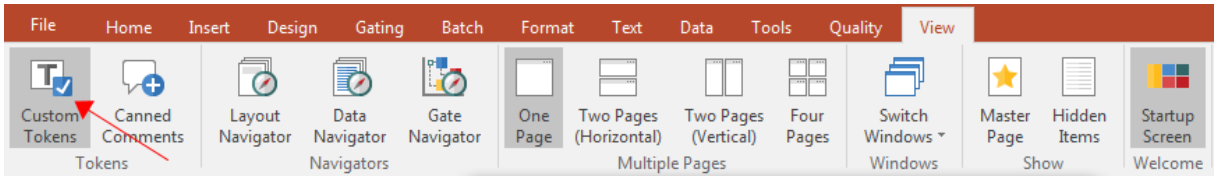

Figure T5.44 Selecting the Custom Tokens Command

The **Custom Tokens** window should now appear (Figure T5.45).

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5. Right-click inside the upper half of the **Custom Tokens** window and select **New Custom Token** from the pop-up menu (Figure T5.45).

Alternatively click on the button within the **Custom Tokens** window.

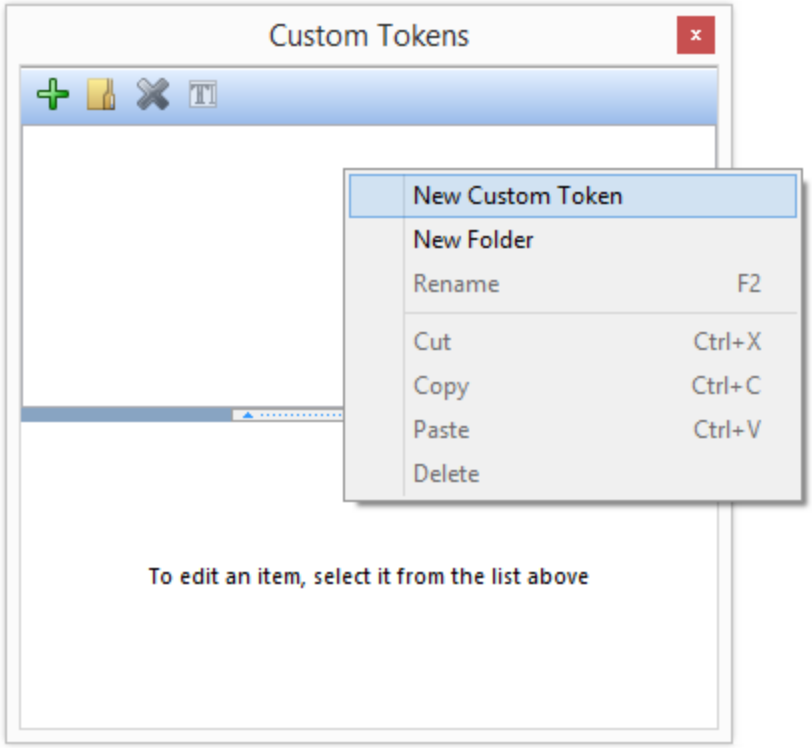

Figure T5.45 Creating a New Custom Token

7. Type "**Apo(+)/Apo(-) Ratio:** " into the Name field of the New Custom Token (Figure T5.46).
8. Press **Enter** to accept the new name.
9. The Custom Token "Apo(+)/Apo(-) Ratio:" should now be highlighted (Figure T5.46).

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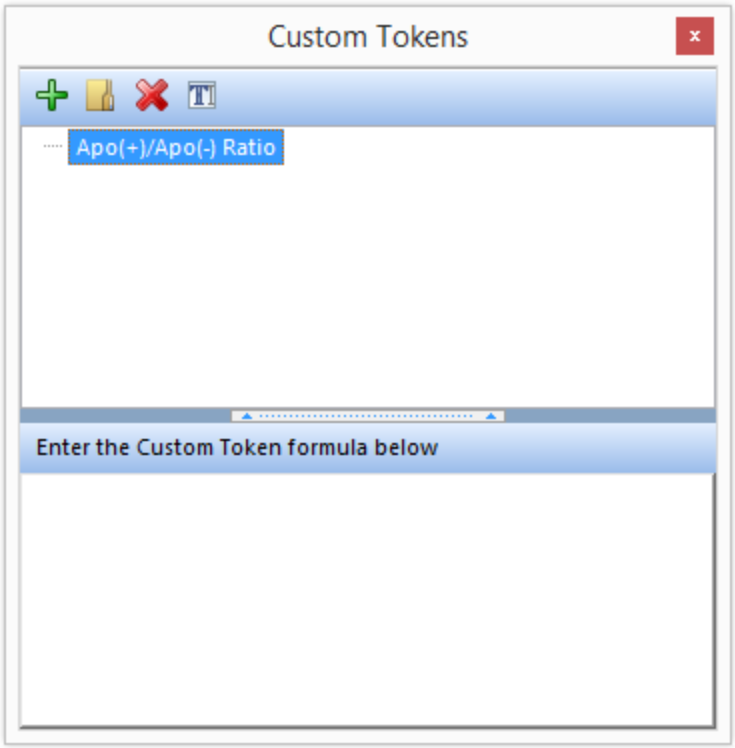

Figure T5.46 Custom Tokens Editor

To calculate the ratio for this example, we will take the number of events in the positive population (upper right quadrant) and divide it by the number of events in the negative population (lower quadrants).

9. Left-click in the lower half of the Custom Tokens window and enter an equals sign **"="** (Figure T5.47).
10. Right-click just after the "=" sign to bring up the associated pop-up menu.
11. Select **Insert Token** from the pop-up menu (Figure T5.47).

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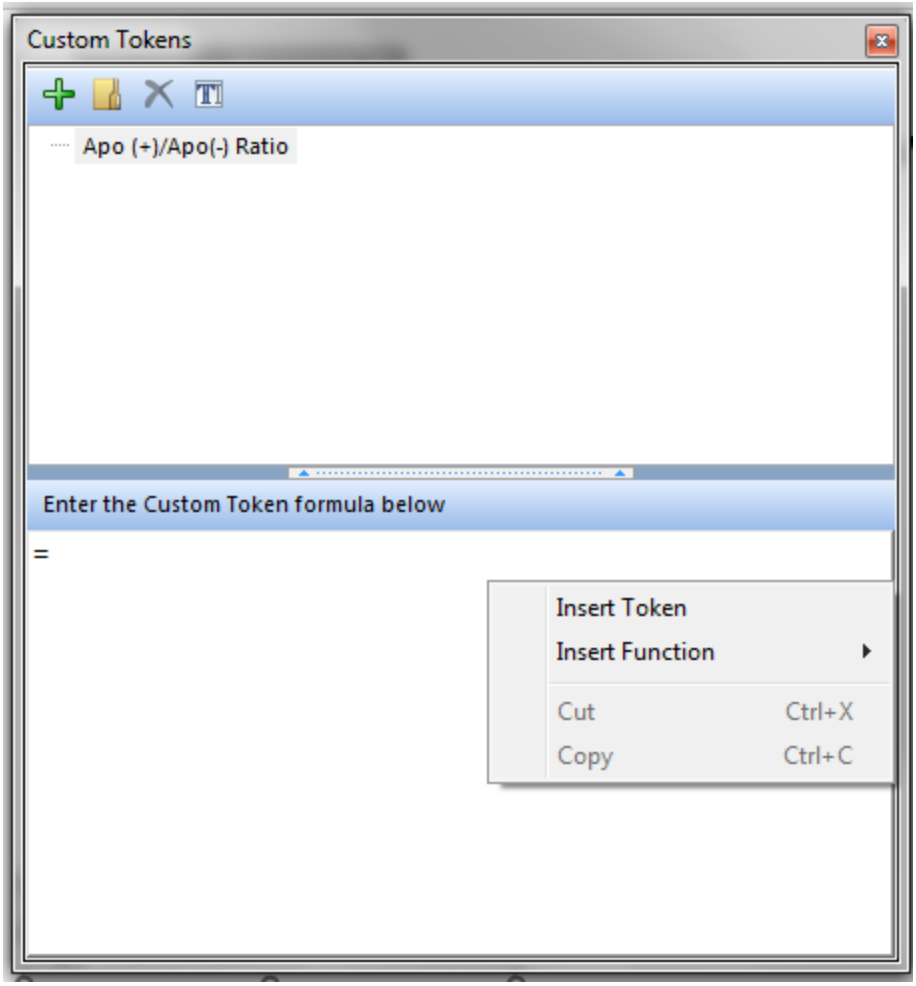

Figure T5.47 Entering a Custom Token Formula

An **Insert a Token** dialog will appear as in Figure T5.24 1315.

12. Click on **Statistic**.
13. Click on **Insert**.

A **Create Statistic** dialog will appear with the **Data Source** Category open as in Figure T5.36.

14. Select **2D plot - ApoMono.002 (FL1 vs. FL2)** as the data source.
15. Click now on the **Statistic** Category (on the left).

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The **Statistic** Category of the **Create Statistic** Window will appear as in Figure T5.37. We will now select the statistic that will define the numerator of our custom token.

14. Select **Upper Right** in the **Select a quadrant** drop-down list (Figure T5.37 ❶).
15. Select **Whole Cells** in the **Gate:** drop-down list (Figure T5.37 ❷).
16. Select **% of Gated Cells** from the **Statistic:** list box (Figure T5.37 ❸).
17. Press **OK**.

The **Custom Tokens Editor** (Figure T5.48), will show the statistic in the formula field highlighted in light blue. This is the numerator for our custom token. We will now define the operator and the denominator of our custom token.

20. Enter the division symbol "/" just after the statistic we created in the last steps (Figure T5.48).
21. Right-click after the "/" symbol to bring up the pop-up menu.
22. Select **Insert Token** from the pop-up menu (Figure T5.48).

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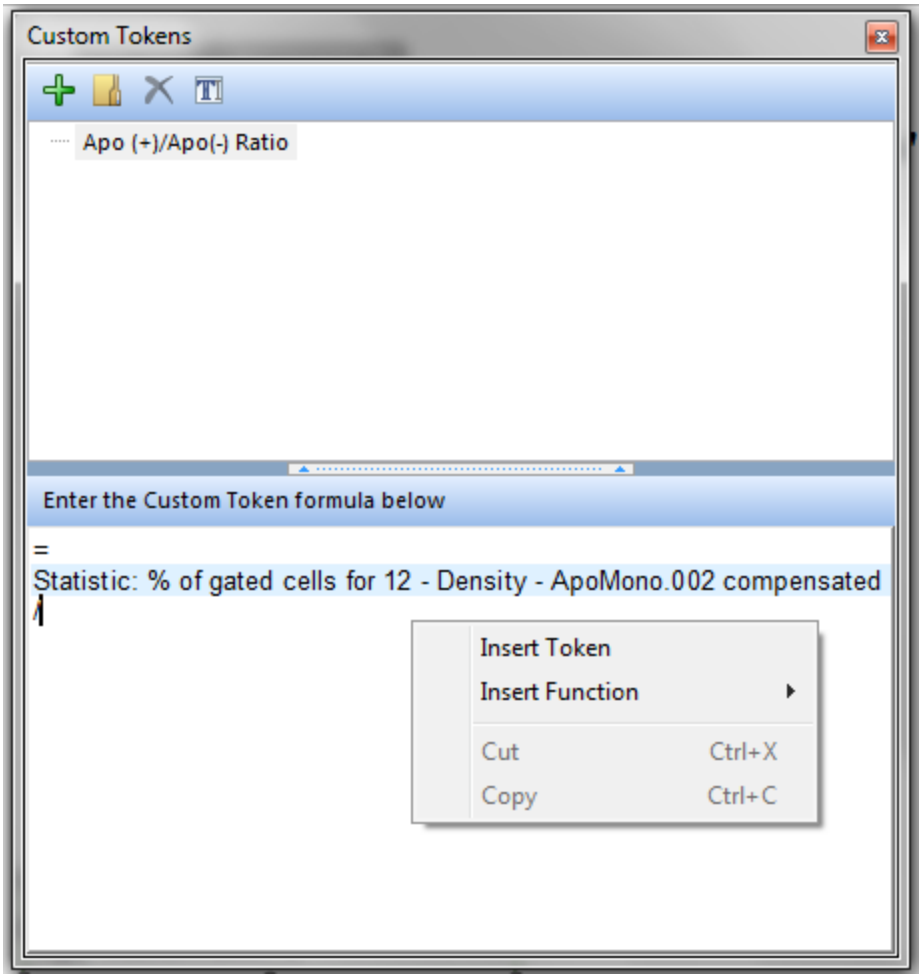

Figure T5.48 Numerator Definition for Custom Token

An **Insert a Token** dialog box will appear (Figure T5.24 1315).

23. Click **Statistic**

24. Click **Insert**

A **Create Statistic** window will appear with the **Data Source** Category open . We will now define the denominator of our custom statistic token.

25. Select **2D plot - ApoMono.002 (FL1 vs. FL2)** as the data source .

26. Click on the **Statistic** Category (Figure T5.37 ❶).

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The **Statistic** Category of the **Create Statistic** Window will appear as in Figure T5.37. We will now select the statistic that will define the denominator of our custom token.

27. Select **Lower** in the **Select a quadrant** drop-down list (Figure T5.37 ❷).
28. Select **% of Gated Cells** from the **Statistic:** list box (Figure T5.37 ❸).
29. Select **Whole Cells** in the **Gate:** drop-down list (Figure T5.37 ❹).
30. Press **OK**.

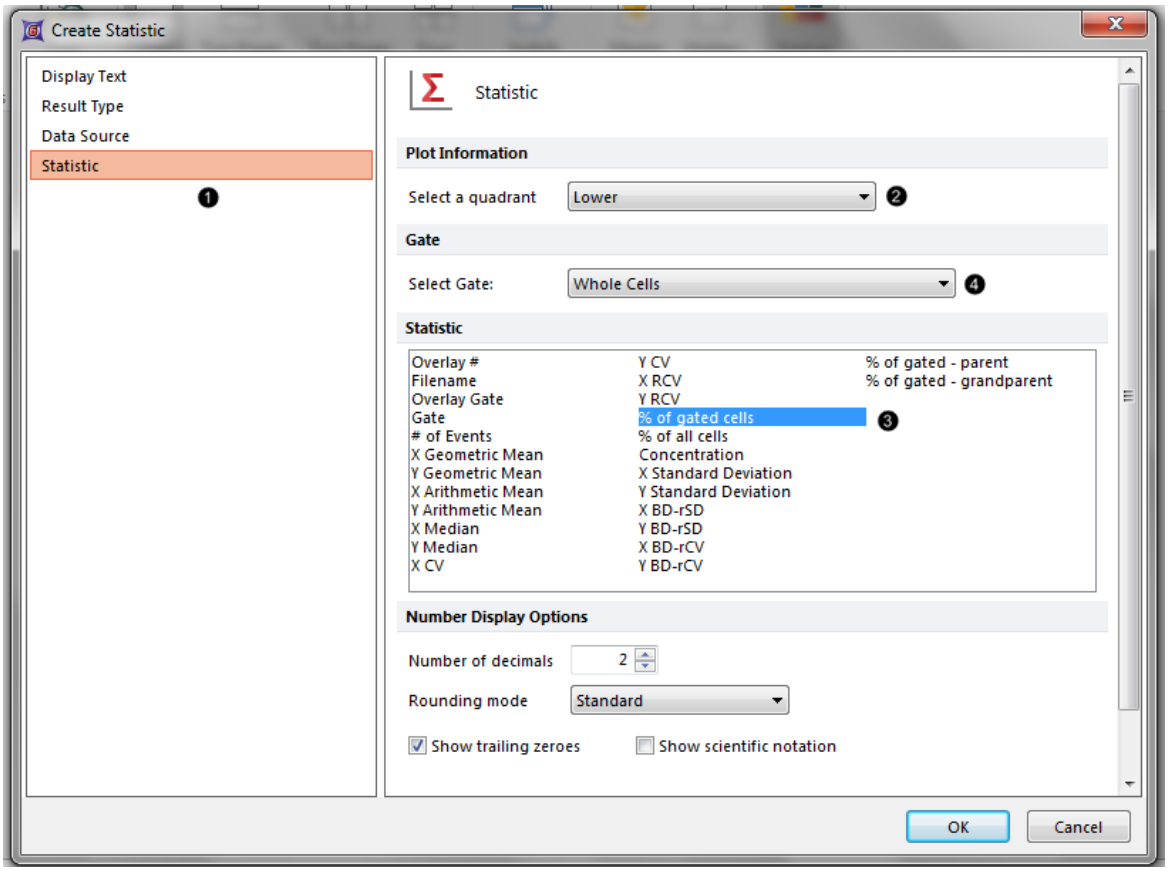

Figure T5.49 Defining the Denominator for the Custom Token

30. Press **OK**.

The **Custom Tokens** window (Figure T5.50) will show the upper right quadrant statistic divided by the lower quadrants statistics in the formula field. The tokens

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are highlighted in blue to indicate that they are being treated as numbers, as opposed to text.

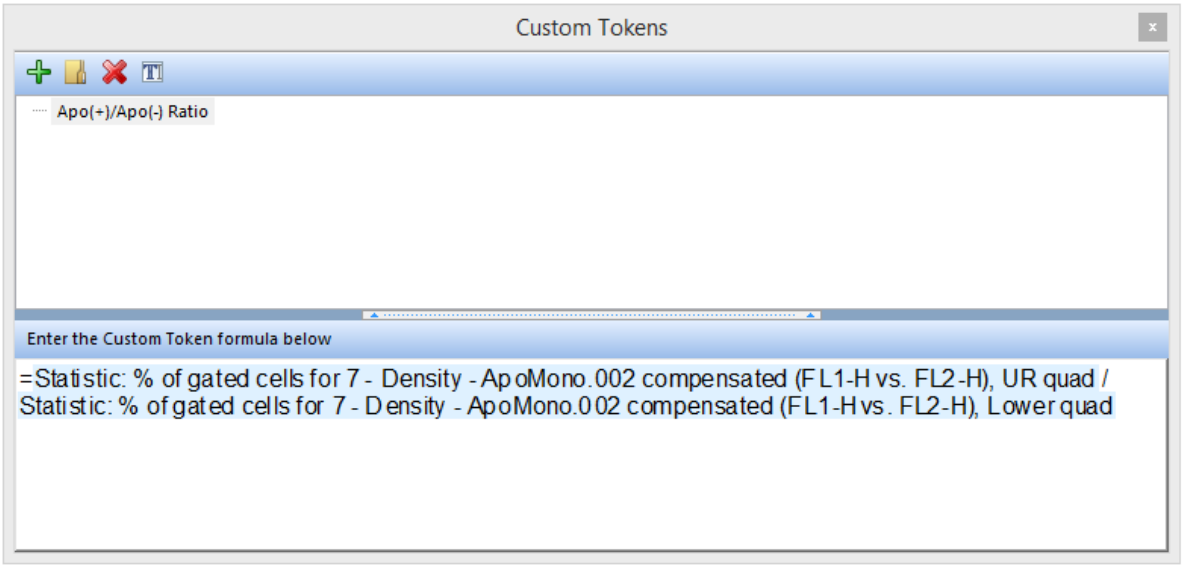

Figure T5.50 Final Custom Token Formula

Now that we have created the custom token, we must insert it into the text box.

31. Position the cursor to the end of the text "Apo(+)/Apo(-) Ratio:" within the text box (Figure T5.51).

32. Select **Text→Insert Text→Insert Token** from the ribbon (Figure T5.51).

    Alternatively, right-click after the "**Apo(+)/Apo(-) Ratio:** " text to bring up the pop-up menu and select **insert token**).

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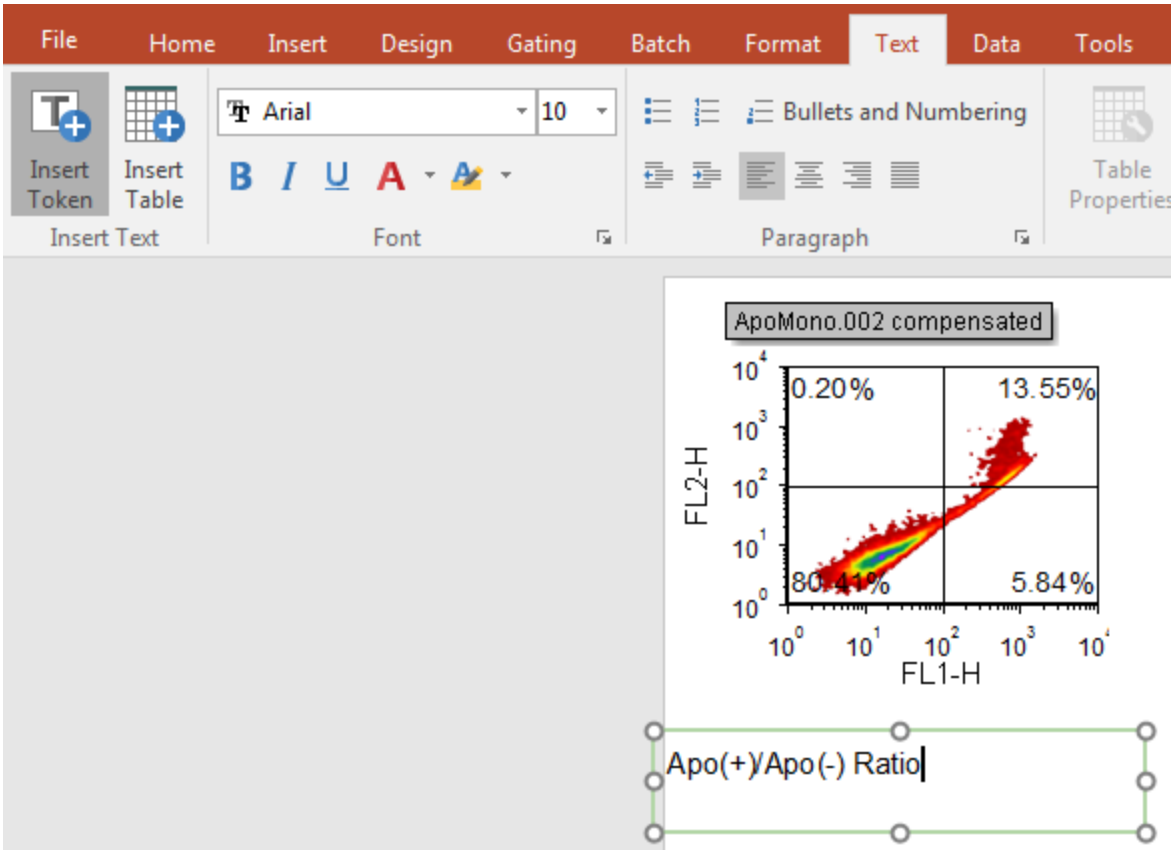

Figure T5.51 Insert Token Command

The **Insert a Token** dialog will appear with the new custom token **Apo(+)/Apo(-) Ratio:** listed (Figure T5.52).

33. Click **Custom→Apo(+)/Apo(-) Ratio:** as the token to be inserted.

34. Click **Insert** to insert the token into the text box.

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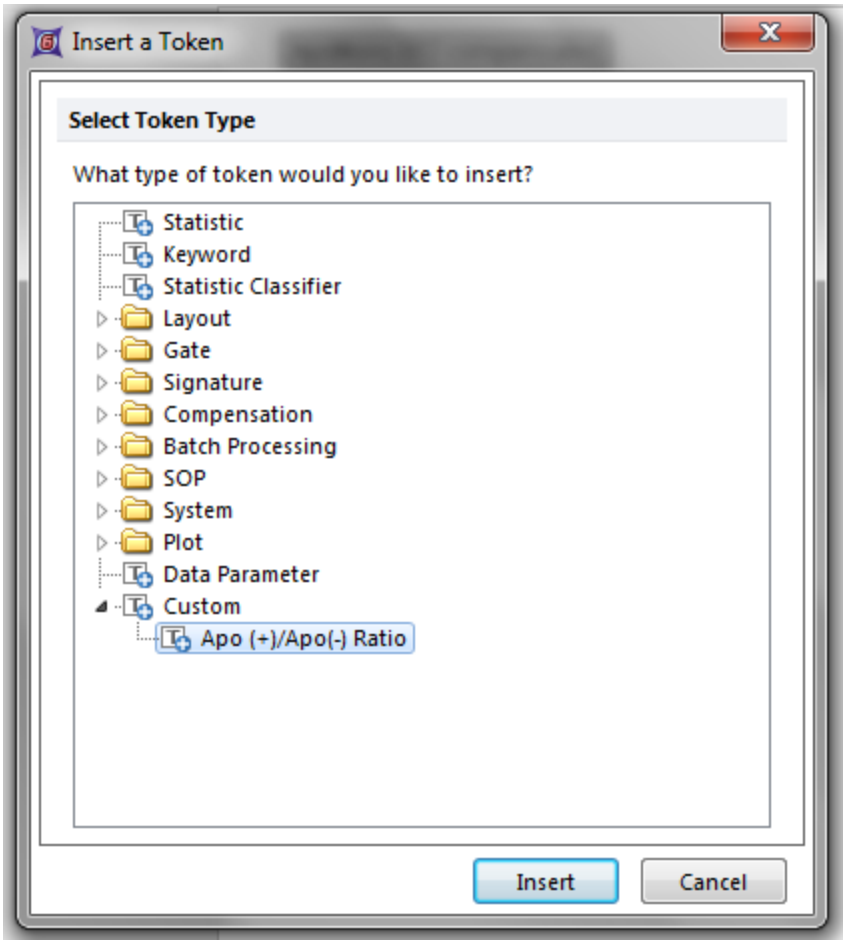

3.Figure T5.52 Selecting the Custom Token

The Apo(+)/Apo(-) Ratio: value of '3.89' will appear highlighted in grey in the text box (Figure T5.53).

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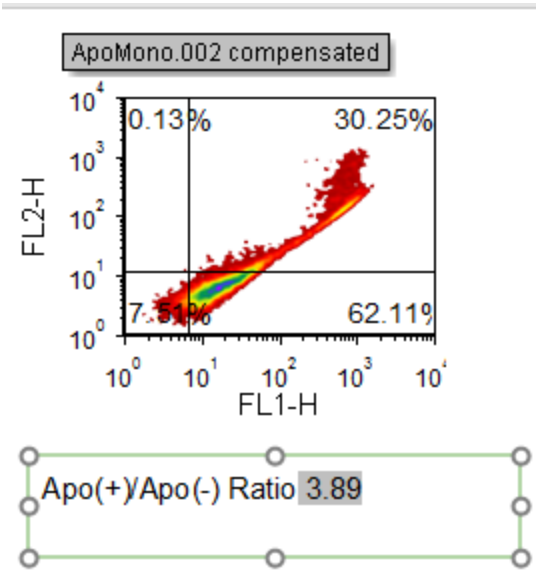

Figure T5.52 Displaying the Custom Token in the Text Box

We will now delete the Custom Token and then insert it by dragging it from the **Custom Tokens** window.

35. Select the custom token we just insert after the "**Apo(+)/Apo(-) Ratio:**" text (you can easily recognize it since it is grey highlighted) (Figure T5.53).

36. Press the **Delete** on you keyboard to remove the custom token.

The **Custom Tokens** window should still be on the layout as shown in Figure T5.54.

37. Press and hold the left mouse button down on the **Apo(+)/Apo(-) Ratio** custom token ❶ highlighted in blue in the **Custom Tokens** window (Figure T5.54).
38. Drag the mouse, while holding the left mouse button down, to the spot after the **Apo(+)/Apo(-) Ratio:** text ❷ in the text box on the layout page.

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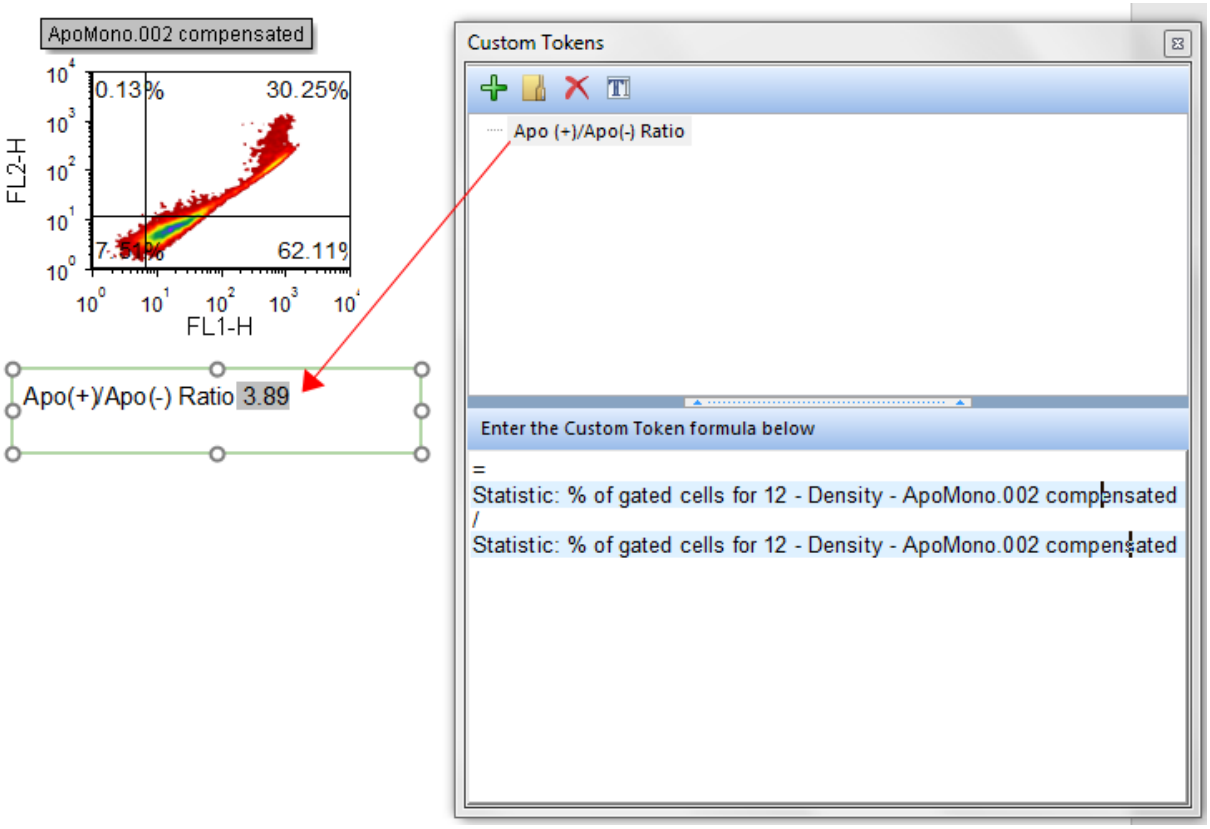

Figure T5.54 Dragging Custom Token onto Text Box

39. Release the mouse button to drop the custom token into the text box.

The custom token is now reinserted in the text box as shown in Figure T5.53.

38.5.7 Using Custom Token Display Types

FCS Express has **Custom Token display types** 761 which allow you to insert an interactive **Edit box** 762, **Check box** 764, or **Dropdown list** 767 directly in a layout. These new display types provide increased flexibility for Custom Token appearance and functionality.

In the course of this tutorial you will learn how to:

- Create and use an Edit Box Custom Token. 1337
- Create an use a Check Box Custom Token. 1341
- Create and use a Dropdown List Custom Token. 1345

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38.5.7.1 Creating an Edit Box Custom Token

Edit Box 762 Custom Token display types are used to create an editable box directly in the layout where you can change Custom Token values, which will update the original custom token and subsequently any other instance of the custom token. Values can be numeric or text. In the steps below we will describe how to view a token as an Edit box, how to modify the token value, and how to adjust the width of the Edit Box.

1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open**.
2. Open the layout **EditBoxTutorial.fey**.

The layout contains a table at the top that contains the *# of Lymphs* token from the "Lymphs" gate, a user-defined division factor, and a Custom Token value for the *# of Lymphs* divided by the division factor (Figure T5.44). The values are all dependent on Custom Tokens 727 that were previously created for use in this tutorial. In the following steps we will adjust the division factor token to be an Edit Box that will dynamically update the token value in other tokens.

# of Lymphs	Division Factor	Lymphs / 5
16.05	5.00	3.21

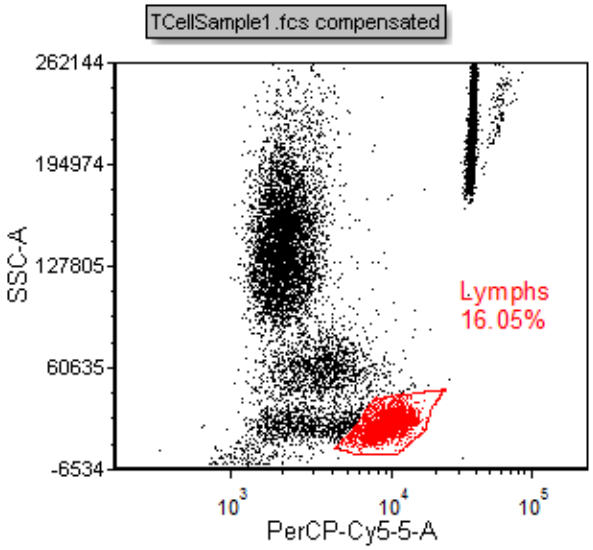

Figure T5.44 - The EditBoxTutorial.fey layout. The Division factor will be adjusted to be an editable box within the layout in the tutorial.

3. Double click the token value "5.00" in the Division Factor column.
4. Choose **Edit Box** from the **Display** category→**Display type** dropdown menu (Figure T5.45).
5. Click **OK**.

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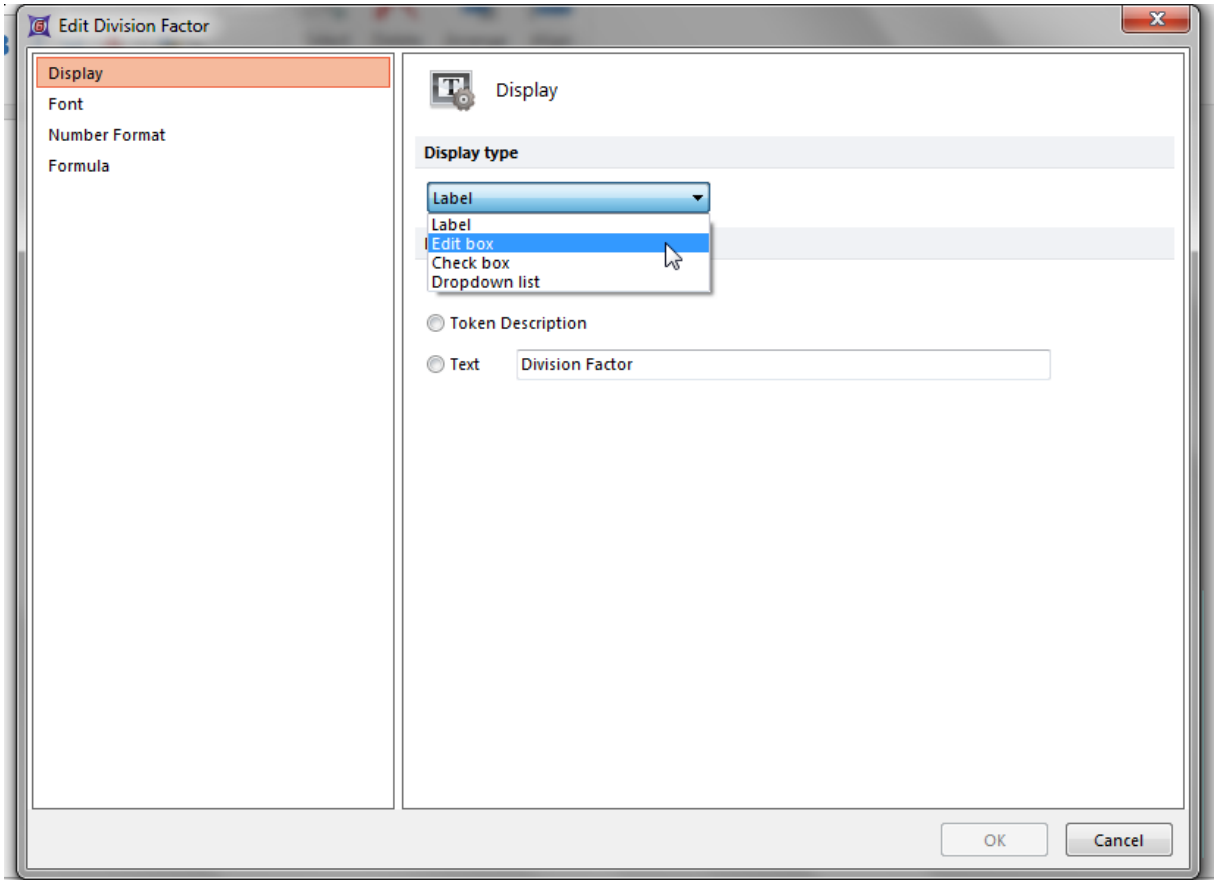

Figure T5.45 - Choosing the Edit box custom token display type.

6. Click on the text box in the layout to select it.

Notice that the Division Factor value is now surrounded by a blue box (Figure T5.46). The blue box represents an editable field for the token value.

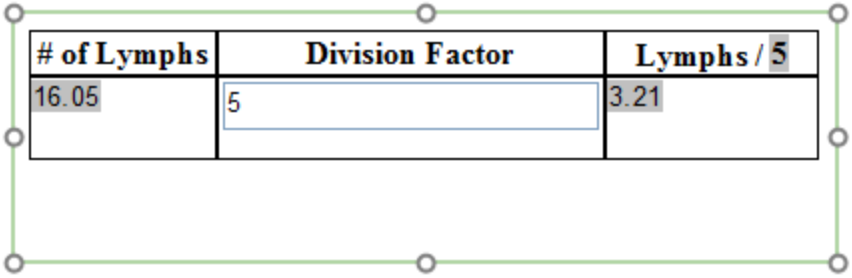

Figure T5.46- The token display type for the division factor is now an editable box denoted by the blue box surrounding the token value.

7. Delete the value "5" from the edit box.

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8. Enter the value "10" in the edit box.

Notice that the token value in the "Lymphs / " column header has changed as well as the token value in the cell underneath the header. Any tokens that are dependent on the token in the Edit Box will update in real time as the value is adjusted.

You may also notice that the division factor Edit Box is quite large for the table size. We will now format the size of the edit box to more appropriately fit the table (Figure T5.47).

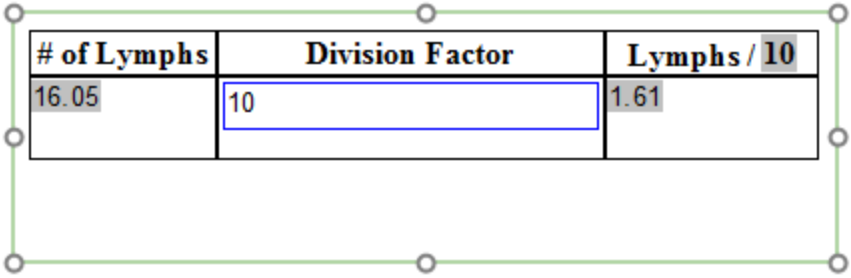

Figure T5.47 - The value in the Edit box has been changed to 10 thus modifying the original Custom Token value and updating any other token or displayed location in the layout.

9. Right click on the edit box in the table.

10. Choose **Format token**.

11. Enter "10" in the **Width** dialog under the **Display** category (Figure T5.48).

12. Click **OK**.

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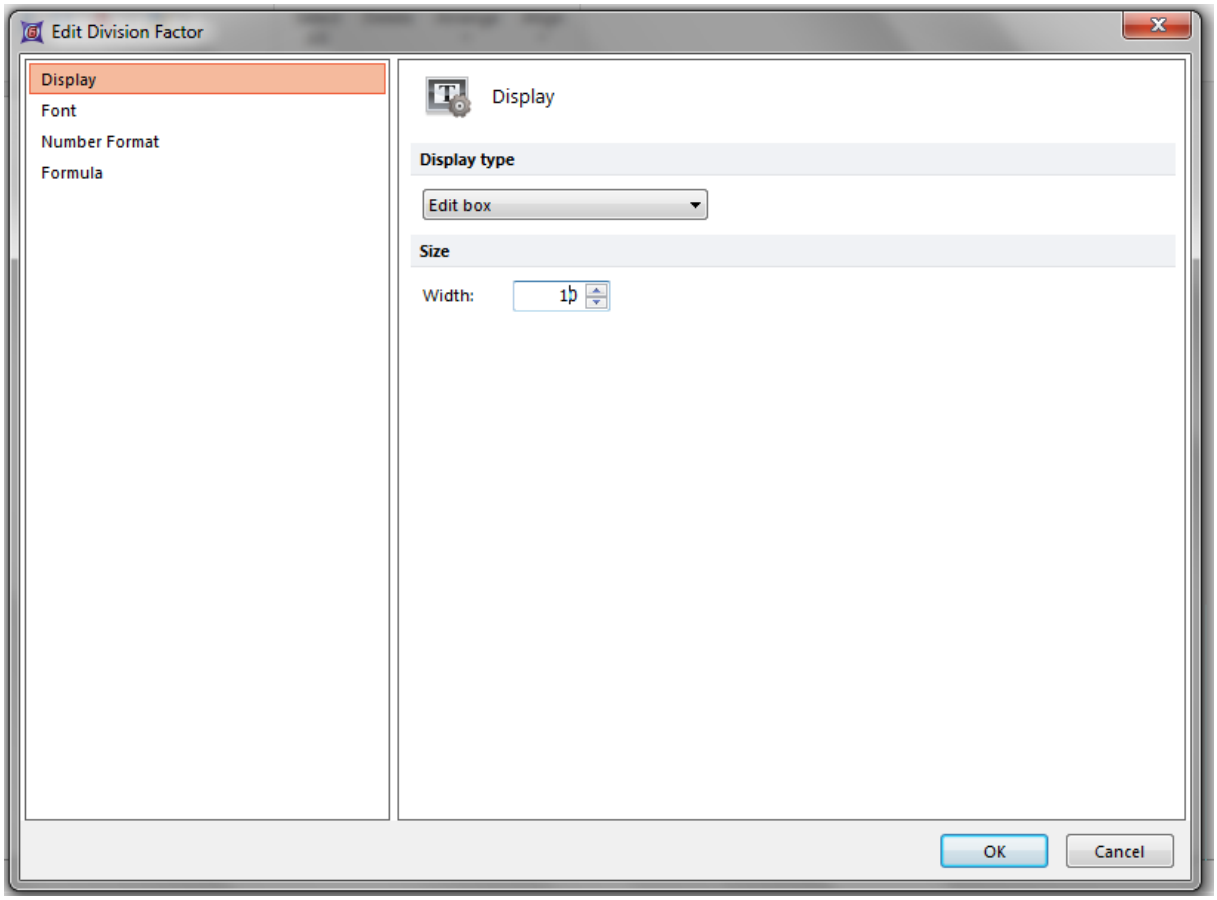

Figure T5.48 -Adjusting the width of the Edit box.

13. Click on the text box to select it.

Notice that the edit box size has decreased to a width of 10, thus fitting the table cell correctly (Figure T5.49).

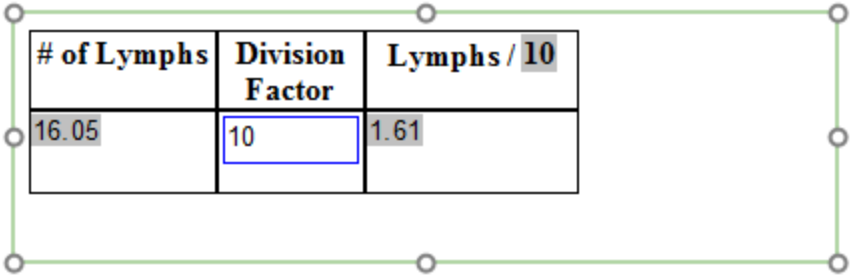

Figure T5.49 - The Edit box has been adjusted to a width of 10 to fit within the table cell.

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In the next section we will create a Check Box Custom Token display type.

38.5.7.2 Creating a Check Box Custom Token

Check box Custom Token display types create a check box directly in the layout that displays the name of the Custom Token. Checking the box returns a "TrueValue", also equivalent to -1, entry for the Custom Token while unchecking the box returns a "FalseValue", also equivalent to 0, entry for the Custom Token. The True or False value of the token can then be used to control any number of true or false dependent actions such as displaying pages. In the course of this tutorial we will create a check box Custom Token and use it to display

1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open**.
2. Open the layout **CheckBoxCustomToken.fey**.

The layout only contains a Custom Tokens window. We will use the predefined Custom Tokens in the window to work with the Check box feature.

3. Drag and drop the Check Box Custom Token from the **Custom Token** window onto the layout to insert it.

A text box containing the text "This will be our check box custom token" will appear as in Figure T5.50 below:

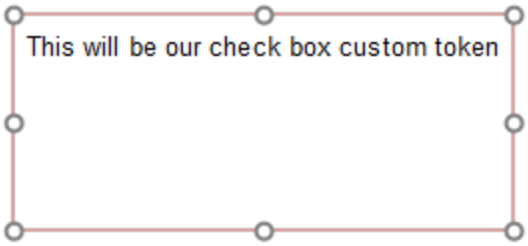

Figure T5.50 - The Check box custom token inserted in the layout.

4. Double click on the token text of "This will be our check box custom token" to open the **Edit Token** dialog.
5. Choose **Check box** from the **Display type** dropdown menu within the **Display** category (Figure T5.51).

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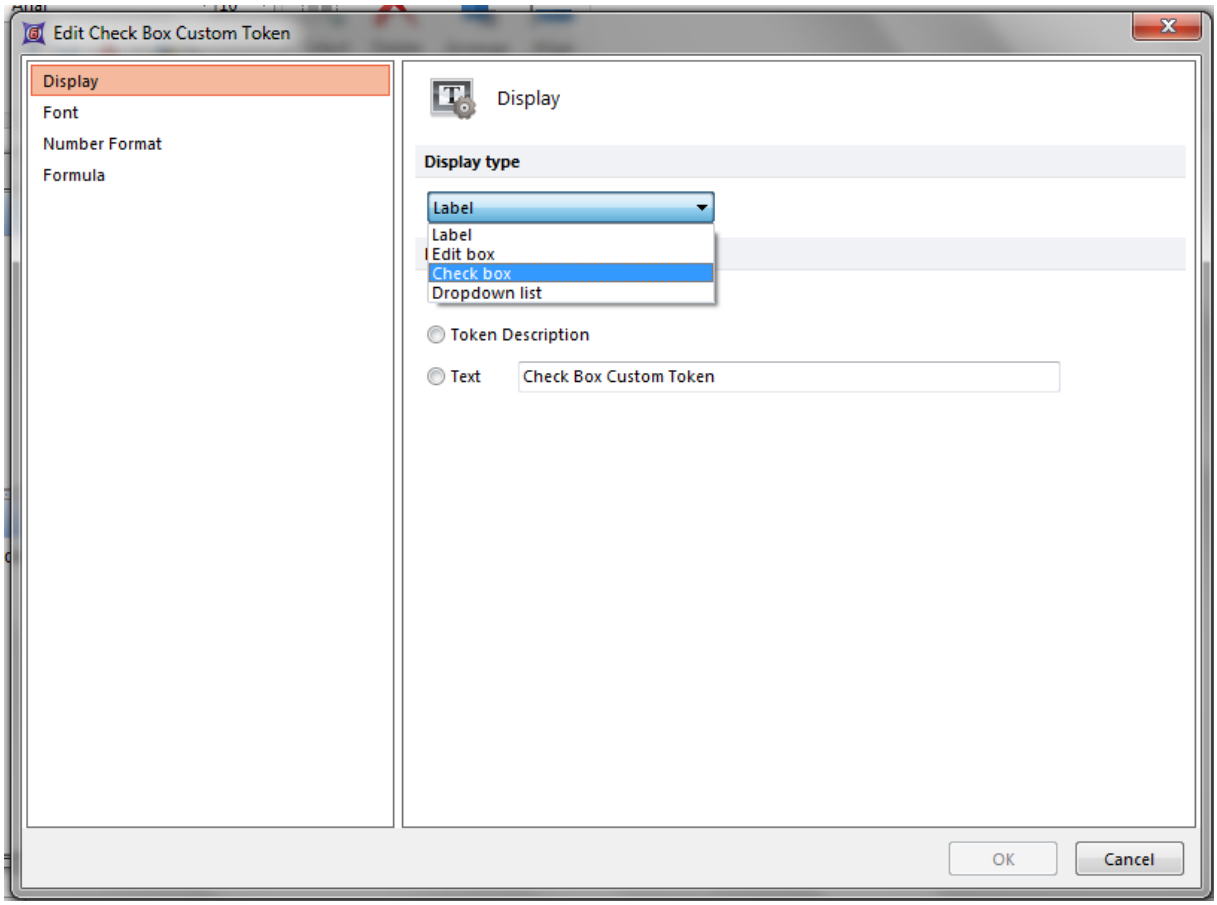

Figure T5.51 - Choosing the Check box display type from the drop down list.

6. Click **OK**.

A **Check box** will appear within the text box in the layout where the token was inserted that displays the name of the Custom Token (Figure T5.52).

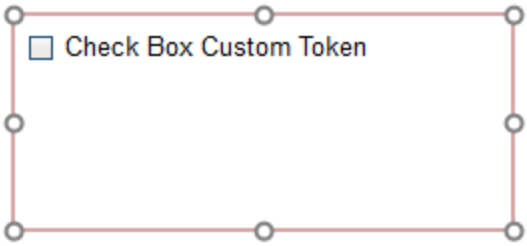

Figure T5.52 - When the Custom Token display type is set to Check Box, a check box will appear in the layout displaying the name of the Custom Token.

7. Check the check box in the text box.

Notice that the value of the token in the **Custom Tokens** window now changed to "=TrueValue"

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8. Uncheck the check box in the text box.

Notice that the value of the token in the **Custom Tokens** window now changed to "=FalseValue"

The *True* and *False* values returned through using the Custom Token display type of check box can be used elsewhere in the layout. Before we continue using the *True* and *False* values, we will rename the token which will in turn rename the value in the check box.

9. Click on the Check Box Custom Token name within the Custom Tokens window.
10. Press F2, which is the short-cut for the **Rename** command . Alternatively, right-click on the token and choose **Rename.**
11. Enter the text "A New Check Box"
12. Press Enter.

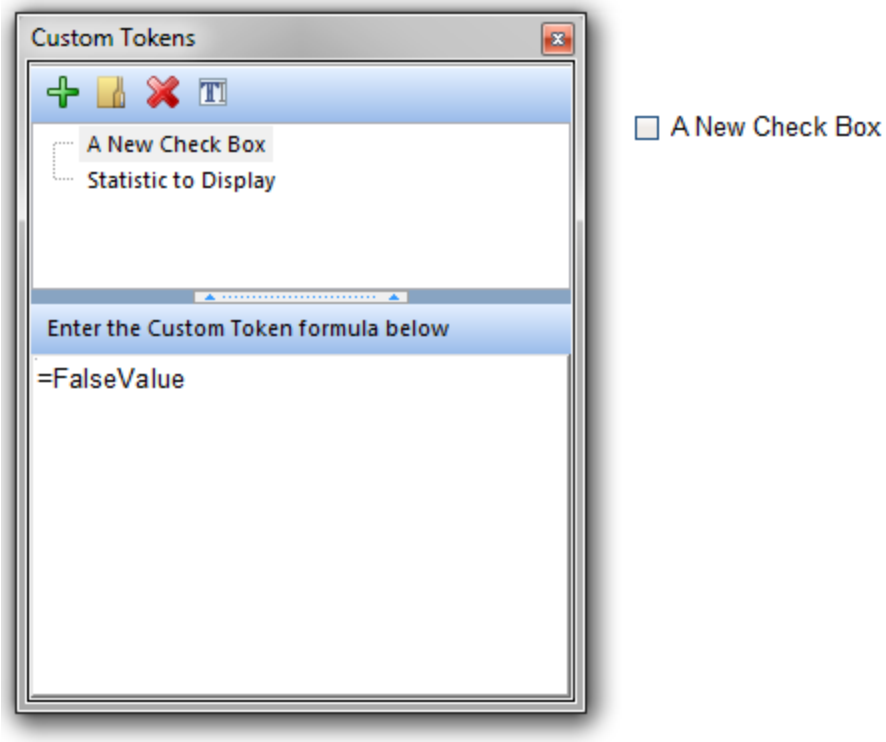

Figure T5.53 - Renaming the Check box token to rename the text in the Check box display.

Once renamed, the token name will appear in the Custom Tokens window with the new name, and the check box text within the text box will also be renamed (Figure T5.53).

We will now utilize the Check box token to display a message when checked / unchecked.

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13. Drag and drop the Message to Display Custom Token from the **Custom Token** window onto the layout to insert it.
14. Check the check box in the first text box containing the Check Box Custom Token, which was inserted onto the Layout in Step 3.

Notice that the message updates as the Check box is changed. The action occurs because the Message to Display Custom Token uses an *IF Then Else* Custom Token format that evaluates the status of the Check Box Custom Token (Figure T5.54). If the value equals "-1" (the numeric representation of "TrueValue"), then "The Check Box Has Been Checked" is returned in the field. If the value equals "0" (the numeric representation of "FalseValue"), then "The Check Box Has NOT Been Checked" is returned in the field. The messages between quotations can be any custom text or can be replaced with any token or Custom Token values from your layout and analysis.

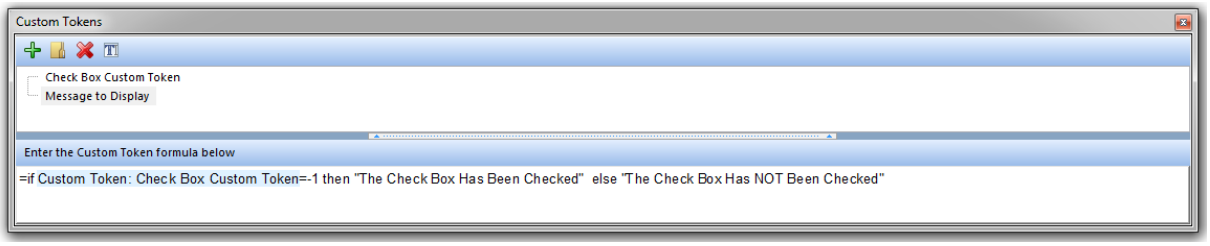

Figure T5.54 - The message to display custom token uses an IF THEN ELSE Custom Token format to return values based on the status of the Check box Custom Token.

We will now utilize the Check box token to display a page when checked / unchecked.

15. Right click on the Page 2 tab.
16. Choose **Format**.
17. Choose the **Visibility** category.
18. Click on the **Custom** radio button.
19. Enter an '=' sign in the text field.
20. Click on the "T" button to the right to insert a token.
21. Choose the **A New Check Box Custom Token** from the **Insert a Token→Custom** list.
22. Enter the text "=truevalue" after the token. The Visibility formatting category should look like Figure T5.55.

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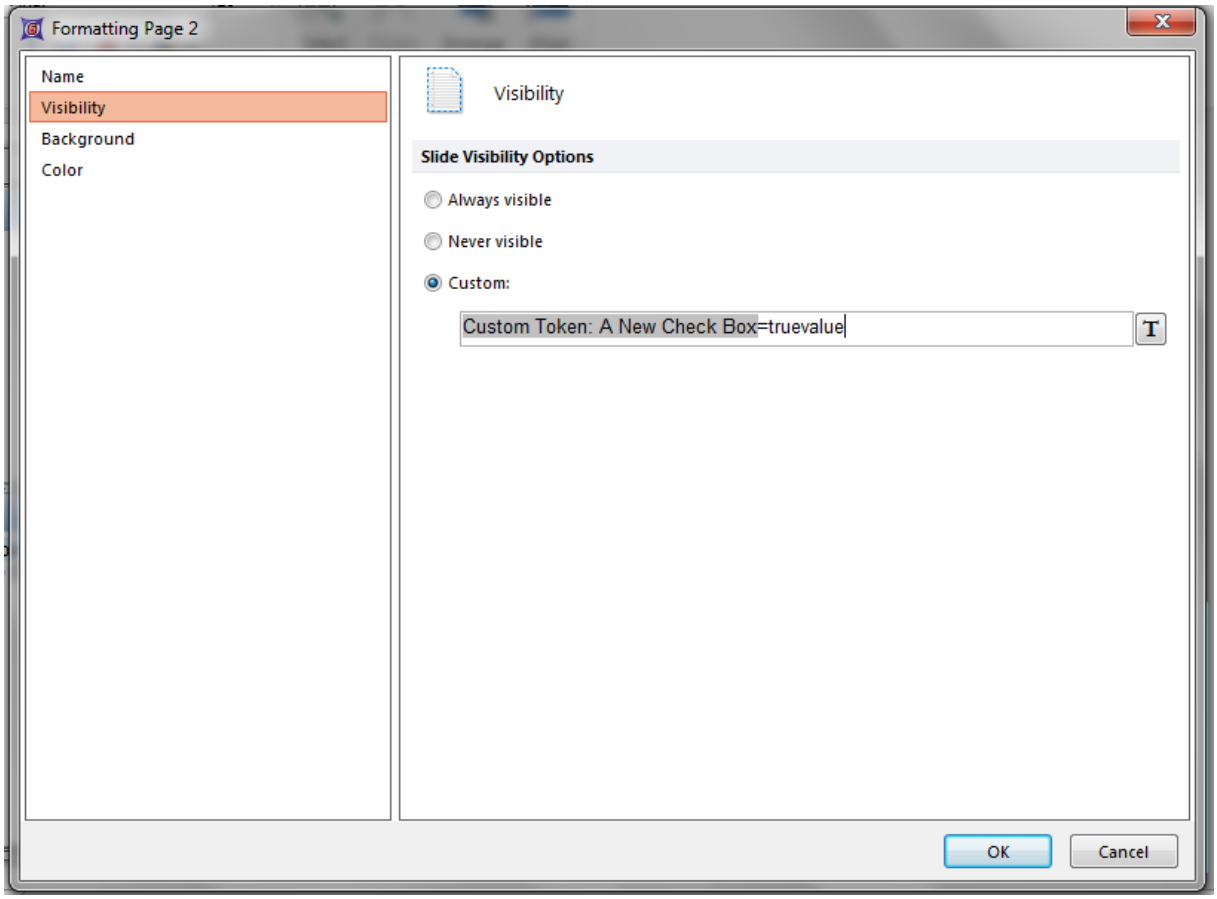

Figure T5.55 Entering a custom page visibility formula to view or hide a page based on the status of a check box being checked or unchecked.

23. Click **OK**.
24. Check or Uncheck the check box in the text box containing the Check Box Custom Token (on Page 1 of the Layout).

Notice in the page tabs that Page 2 appears and is hidden depending on the status of the Check box. In this example we have used the "truevalue" text to evaluate the token rather than the numeric "-1" and "0" values used in step 14. Checking the box returns true value in turn displaying the page. In contrast, if the text "=falsevalue" was entered the page would display when the Check box is unchecked.

In the next section we will create a drop down list Custom Token display type. 1345

38.5.7.3 Creating a Dropdown list Custom Token

Dropdown list 767 Custom Token display types create a dropdown list of comma separated choices entered in the **Choices** section of the **Edit** custom token dialog **Display** category. The dropdown list is useful to choose and display a value from a list of predefined entries. In the steps below we will describe how to create a dropdown list for use in a layout.

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1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open**.
2. Open the layout **Dropdownlist Custom Token.fey**.

The layout only contains a Custom Tokens window. We will use the predefined Custom Tokens in the window to work with the Dropdown feature.

3. Drag and drop the Drop Down List Custom Token from the **Custom Token** window onto the layout to insert it.

A text box containing the text "This will be our Dropdown List Custom Token" will appear as in Figure T5.56 below:

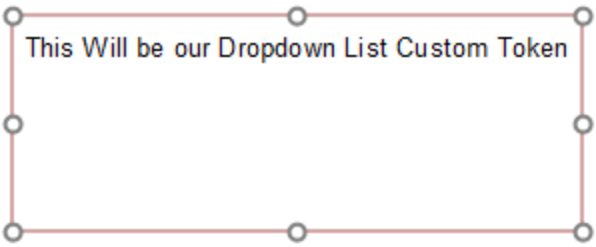

Figure T5.56 -The Dropdown list Custom Token inserted in the layout.

4. Double click on the token text of "This will be our Dropdown List Custom Token" to open the **Edit Token** dialog.
5. Choose **Dropdown list** from the **Display type** dropdown menu within the **Display** category (Figure T5.57).

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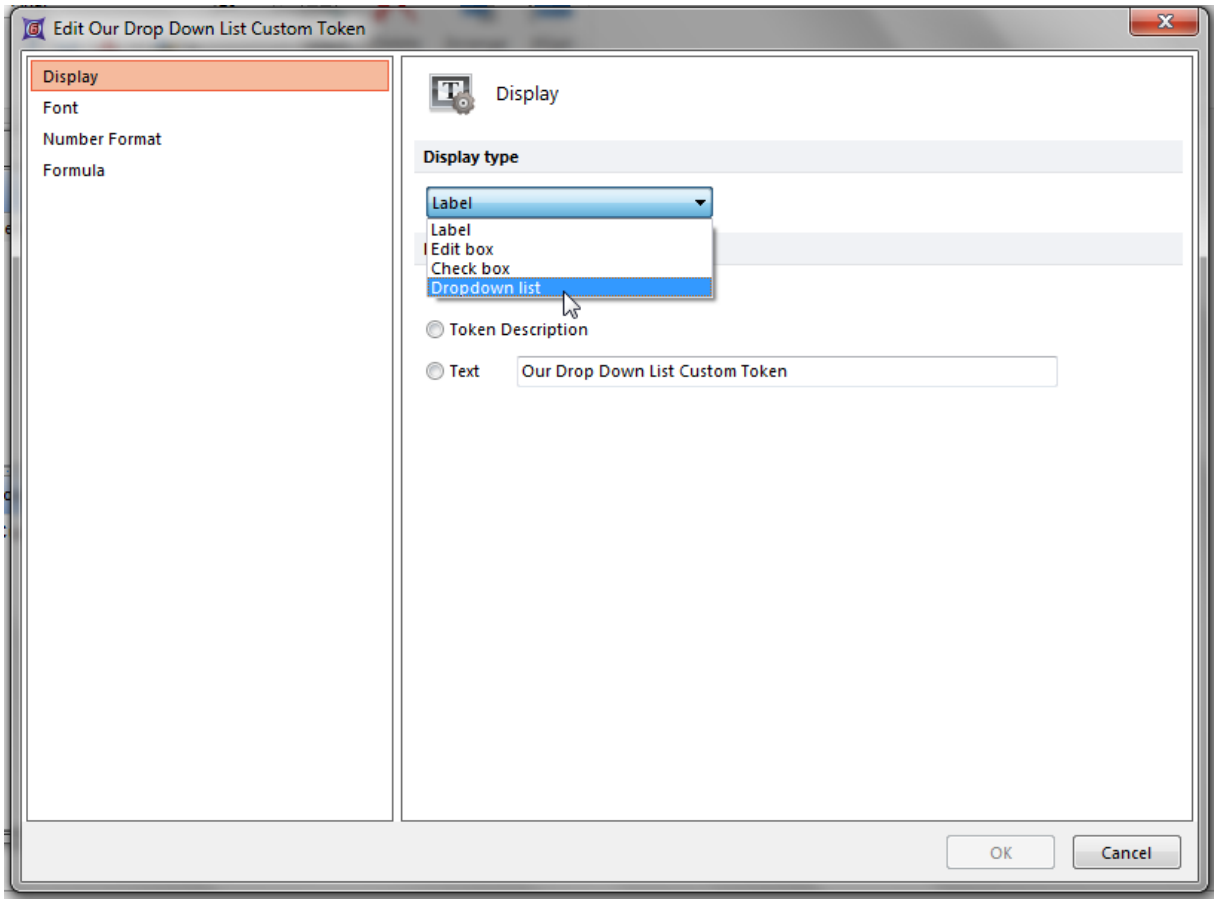

Figure T5.57 -Choosing the Dropdown list display type from the drop down list.

6. Type "Option 1, Option 2, Option 3" in the **Choices (comma separated):** field (Figure T5.58).

The Edit Drop Down List Custom Token dialog should look like figure 3. Notice that our choices for "Option 1 - Option 3" are separated by commas. Any text, tokens, or Custom Tokens may be entered in the dialog as dropdown choices as long as they are separated by commas.

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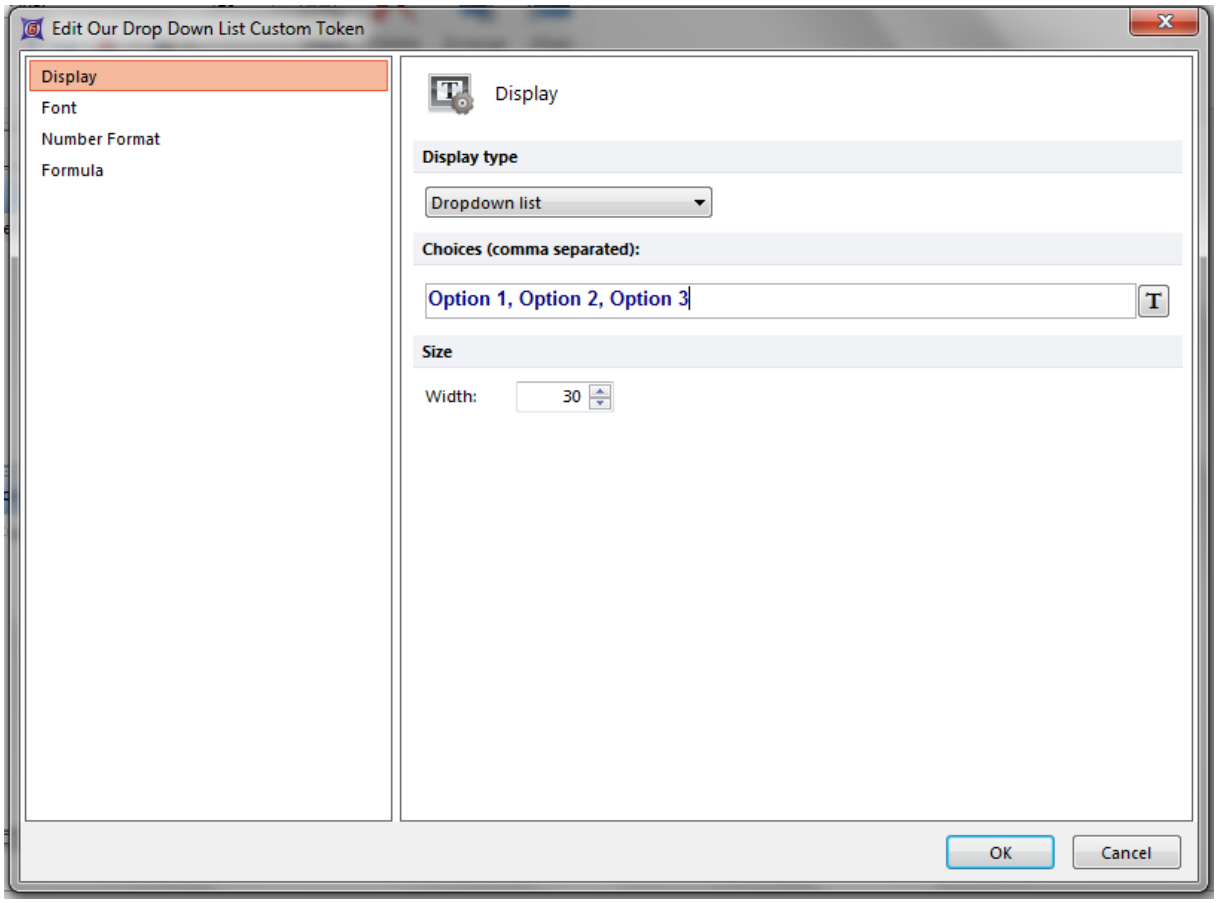

Figure T5.58 - The Edit Drop Down List Custom Token dialog after entering a comma separated list of options to display in the Dropdown list.

7. Click **OK**.
8. Click on the dropdown arrow within the text box to reveal the Dropdown choices.

We will now place the Custom Token elsewhere on the layout and observe how making a choice from the Dropdown list updates the token value.

9. Drag and drop the Drop Down List Custom Token from the **Custom Token** window onto the layout to insert it.
10. Click on the dropdown arrow within the text box containing the Dropdown list to reveal the Dropdown choices.
11. Choose Option 2.
12. Choose Option 3.

Notice that the newly inserted text box containing the token from Step 9 now reflects the value selected from the Dropdown list Custom Token display type (Figure T5.59).

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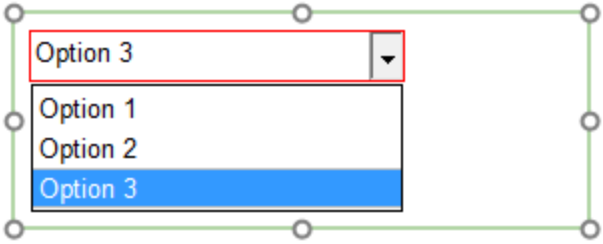

Figure T5.59 - The text "Option 3" appear underneath the selected Dropdown list Custom Token display type because "Option 3" is selected from the list.

38.6 Quadrants

FCS Express has powerful quadrant features. You can set "standard" rectangular quadrants, as well as floating quadrants and percentile quadrants, among other options. This allows you to analyze your data with the flexibility that you need.

In the first section, we will set up rectangular and floating quadrants 1349.

38.6.1 Rectangular and Floating Quadrants

In the course of this example, we will:

- Learn how to create rectangular quadrants and move them.
- Learn how to create floating quadrants and move them.

To place a rectangular quadrant and move it:

1. Select **File** tab→**Open Layout**.
2. Open the layout **QuadTut.fey** found in the Tutorial Sample Data archive 1210 .

Two density dot plots now appear on the layout.

3. Select the **Gating→Create Gates→Quadrants** command (Figure T6.1).

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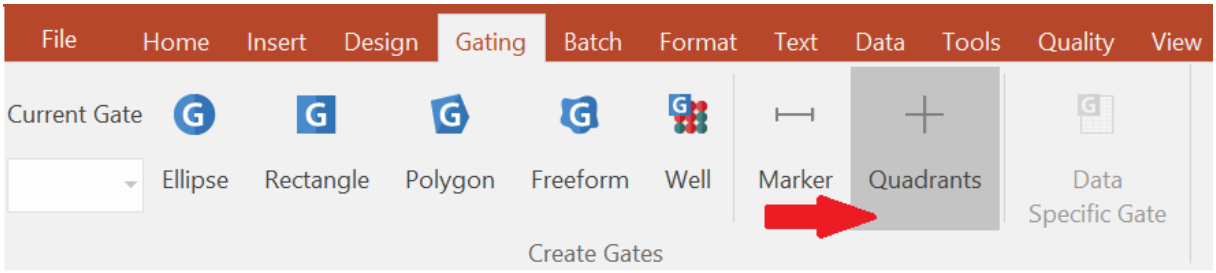

Figure T6.1 Selecting the Quadrants Command on the Gating Tab

4. Click in the middle of the left density dot plot.

A rectangular or "standard" quadrant now appears on the plot with the center of the quadrant located where you clicked in the plot. Note that the quadrant is selected, as indicated by the rectangular symbol in the center of the quadrant where the lines cross (the central node), indicated by the cursor in Figure T6.2. Also note the quadrant's percentages are automatically placed in the corners of the plot (Figure T6.2).

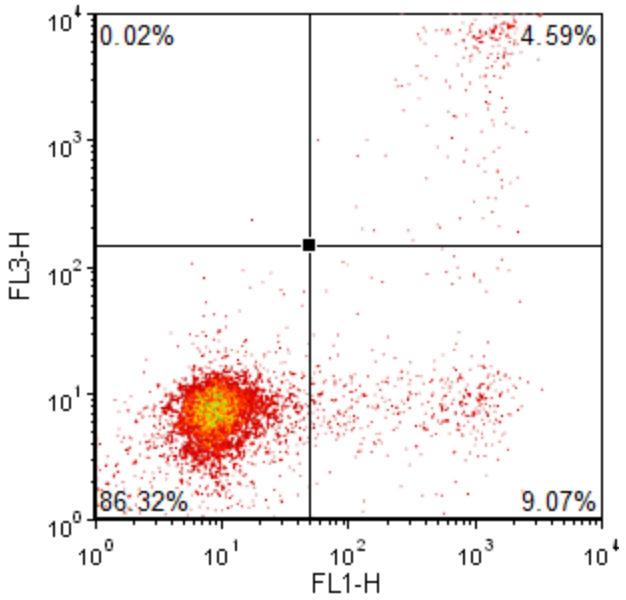

Figure T6.2 Plot with Quadrants

If the quadrant is currently selected then skip to step 6.

5. Click in the middle of the quadrant at the central node to select the quadrant (Figure T6.2).

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6. Hover the mouse over the central node of the quadrant; the cursor should change to a four-headed arrow.

We will now move the position of the central node of the quadrant to redefine the quadrant.

7. Press the left mouse button when the cursor is a four-headed arrow.
8. Continue to hold the left mouse button and move the central node to the desired location.
9. Release the mouse when the quadrants properly define the three populations in the plot.

Notice that when you move the central node, the percentages of each quadrant change accordingly. The final plot should look similar to Figure T6.3.

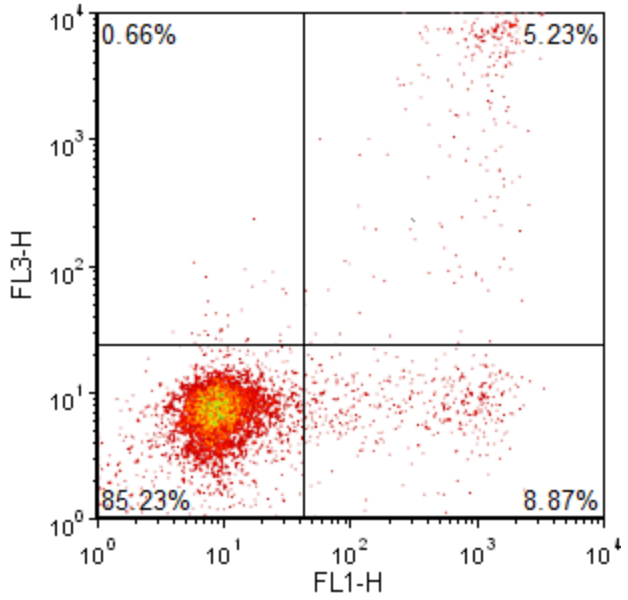

Figure T6.3 Redefined Quadrants

We will now turn to the plot on the right side of the layout. In this case, if we try to use a standard rectangular quadrant we will not be able to clearly separate the three populations in the plot, as demonstrated in Figure T6.4.

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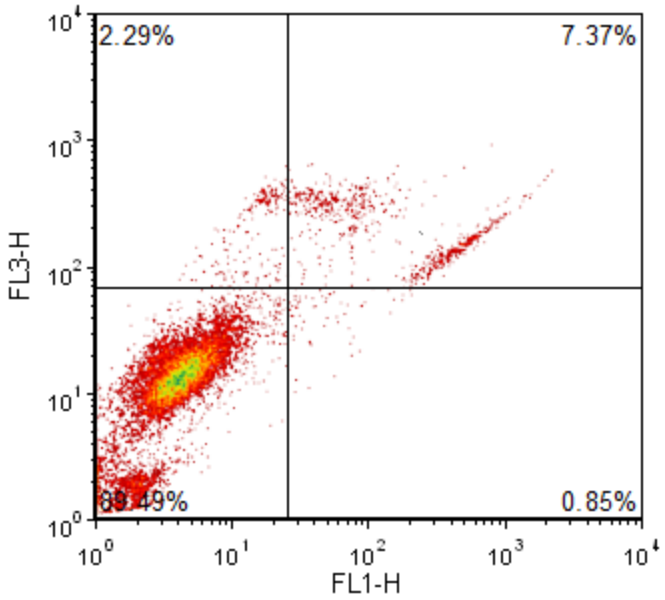

Figure T6.4 Improperly Defined Regions Using Standard Quadrants

To surmount this problem we will create a floating quadrant on this plot.

1. Select the **Gating→Create Gates→Quadrants** command (Figure T6.1).
2. Click in the middle of the right plot.

A standard quadrant will appear similar to Figure T6.4. If the quadrant in right plot is not already selected then select it.

3. Select the **Format→2D Options→Quadrants** command (Figure T6.5).

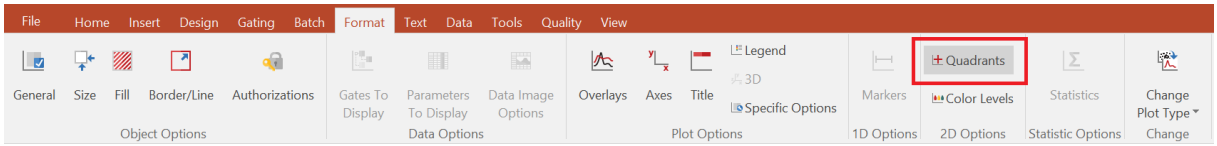

Figure T6.5 Selecting the Format Quadrants Command

The **Formatting Quadrants** dialog will now appear (Figure T6.6).

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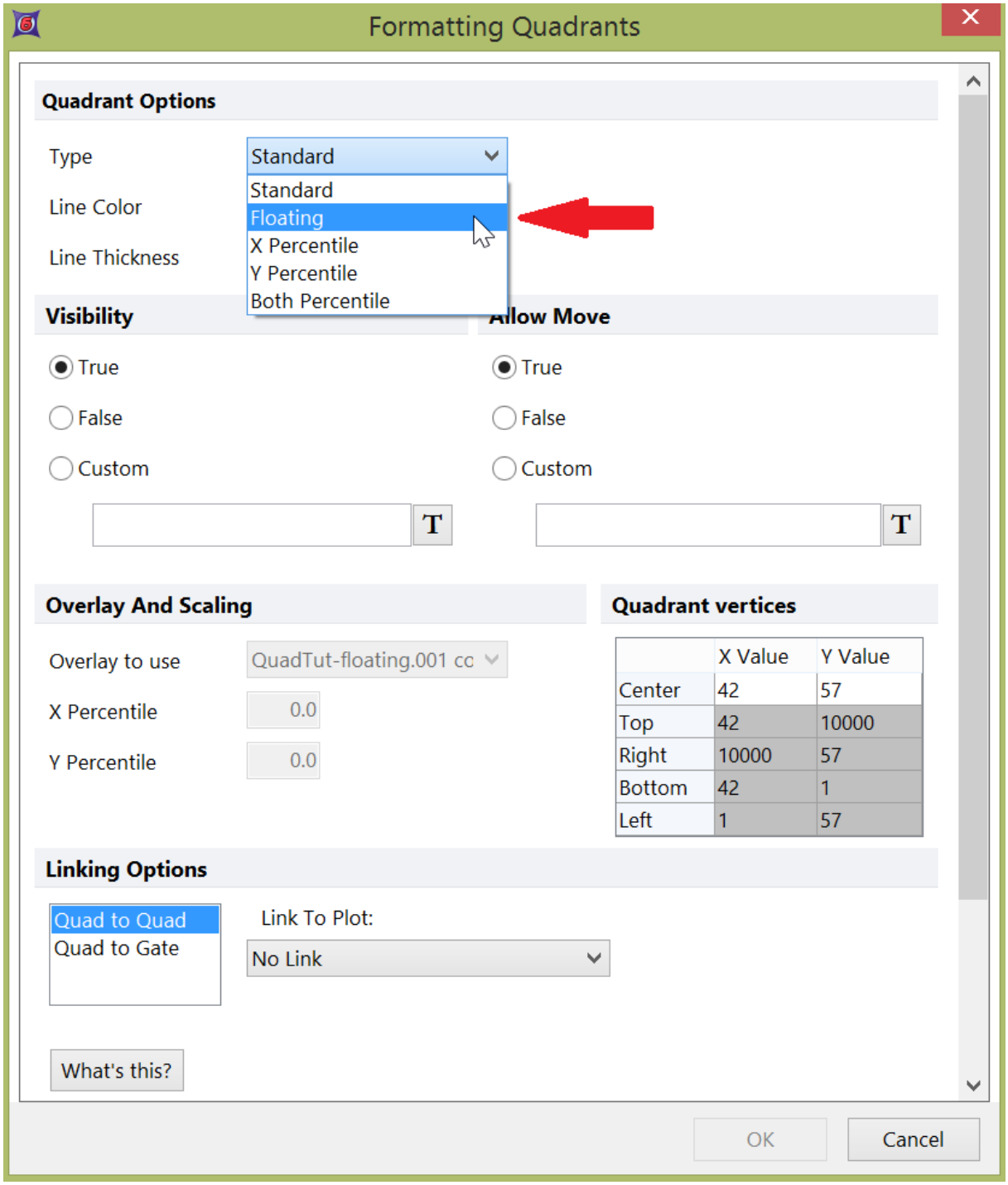

Figure T6.6 Formatting Quadrants Dialog

4. Change the **Type** of the quadrant from **Standard** to **Floating** from the drop-down list, shown by the cursor in Figure T6.6.

5. Click **OK**.

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Alternatively, you can format the quadrants using the plot associated pop-up menu.

- Right-click on the right plot to bring up the associated pop-up menu.
- Select **Format** from the pop-up menu.

The **Format** dialog appears, similar to Figure T6.7.

- Select the **Quads** category on the left side of the **Format** dialog (Figure T6.7).
- Change the **Type** of the quadrant from **Standard** to **Floating** from the drop-down list, shown by the cursor in Figure T6.7.
- Click **OK**.

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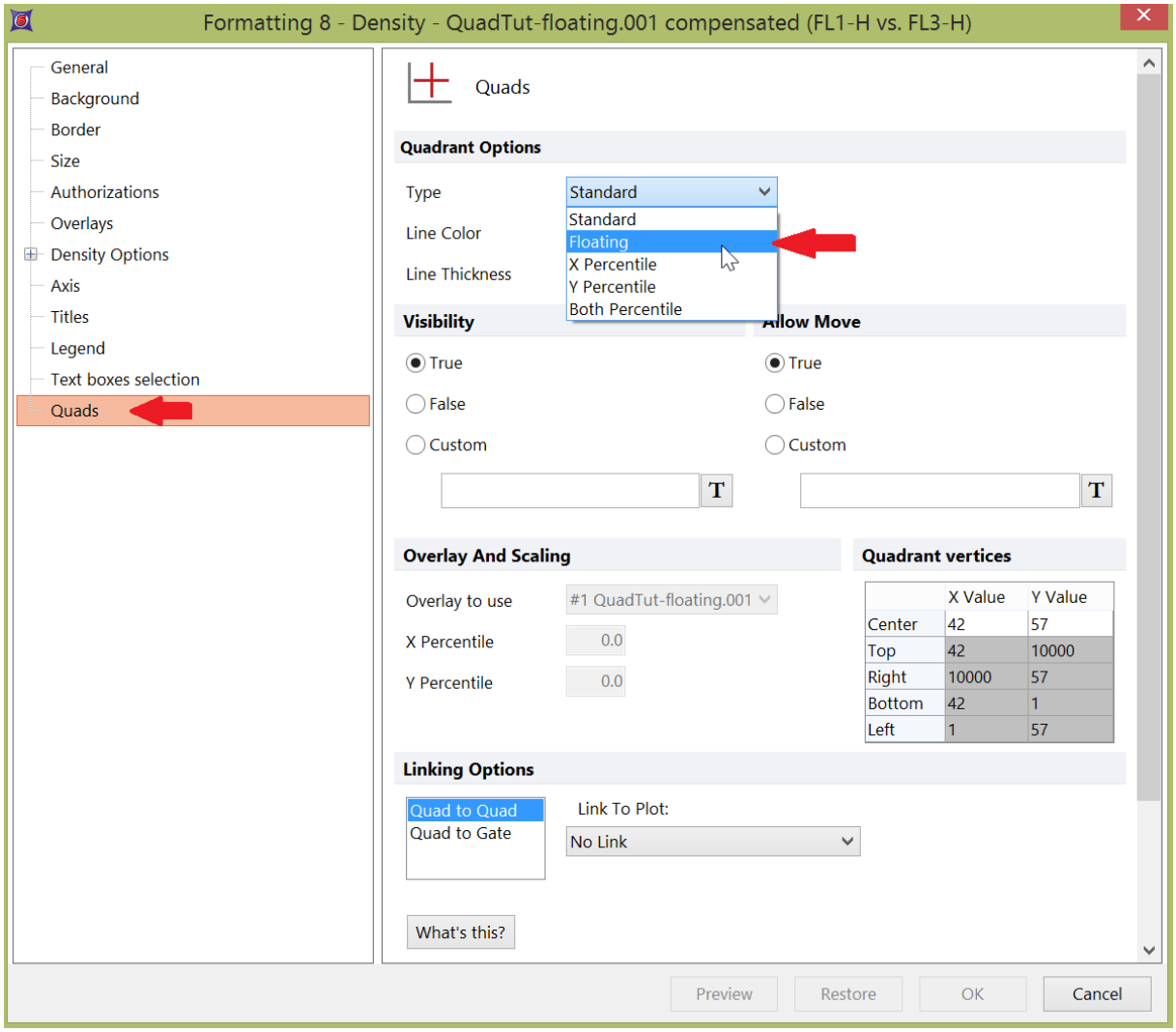

Figure T6.7 Quads Category of the Formatting Dialog

6. Click on the right plot in the middle of the quadrant, on the central node, to activate the quadrant.

Notice that not only does the central node show a small square, but also the ends of the quadrant lines have small squares as well, indicated by the red circles on Figure T6.8. The ends of the quadrant lines are now movable nodes, along with the central node.

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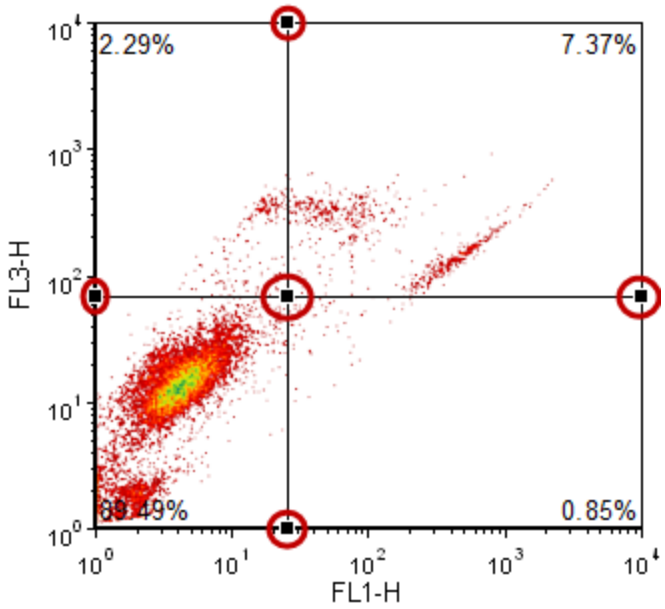

Figure T6.8 Floating Quadrant Selected for Editing

7. Place the cursor over the small square on the central node; the cursor should change to a four-headed arrow.

8. Hold down the left mouse button and move the central node into the middle of the three populations on the plot.

9. Release the mouse button when the central node is in the desired location.

10. Place the cursor over the small square at the end of one of the quadrant lines; again, the cursor should change to a four-headed arrow.

11. Hold down the left mouse button and move the square node at the end of the quadrant line along the axes to separate the three populations.

12. Release the mouse button when the quadrant line node is in the desired location.

Repeat steps 10 through 12 until all of the nodes on the quadrant lines have been moved. The final result should look similar to Figure T6.9.

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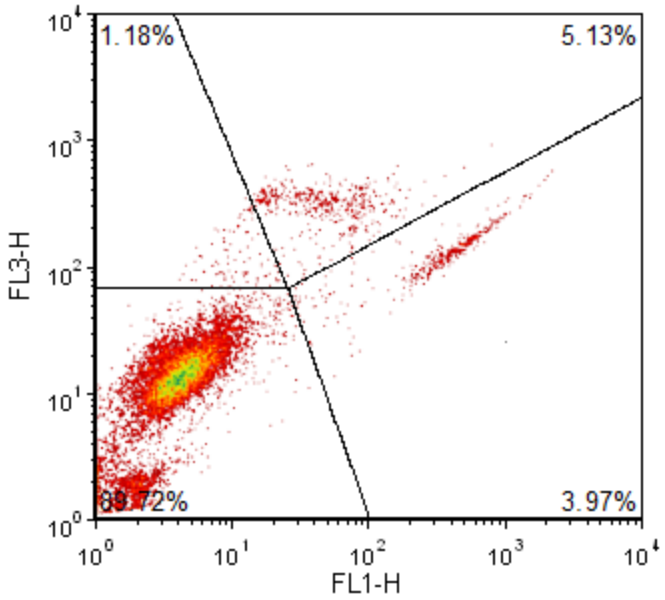

Figure T6.9 Floating Quadrants

Next, we will set up percentile quadrants.

38.6.2 Percentile Quadrants

FCS Express allows you to set quadrants according to percentages instead of manual positioning.

In the course of this example, we will:

- Learn how to set rectangular quadrants using percentiles.

For convenience, we are going to start with the same layout as before. If you have the previous layout still open, please jump to Step 3.

1. Select **File** tab→**Open Layout**.
2. Open the layout **QuadTut.fey** found in the Tutorial Sample Data archive.
3. Select the **Gating→Create Gates→Quadrants** command (Figure T6.1).
4. Click in the middle of the left density dot plot.

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Two density dot plots now appear on the layout with quadrants appearing on the left plot.

To set a quadrant with percentages:

3. Click once on the left plot to select it.
4. Select the **Format→2D Options→Quadrants** command (Figure T6.5 1349).

The **Formatting Quadrants** dialog will appear, similar to Figure T6.10.

5. Change the **Type** of the quadrants listed in the drop-down menu from **Standard** to **Both Percentile**, indicated by the cursor in Figure T6.10.

Notice that the **X Percentile** and **Y Percentile** options on the Formatting Quadrants dialog are now enabled (Figure T6.10).

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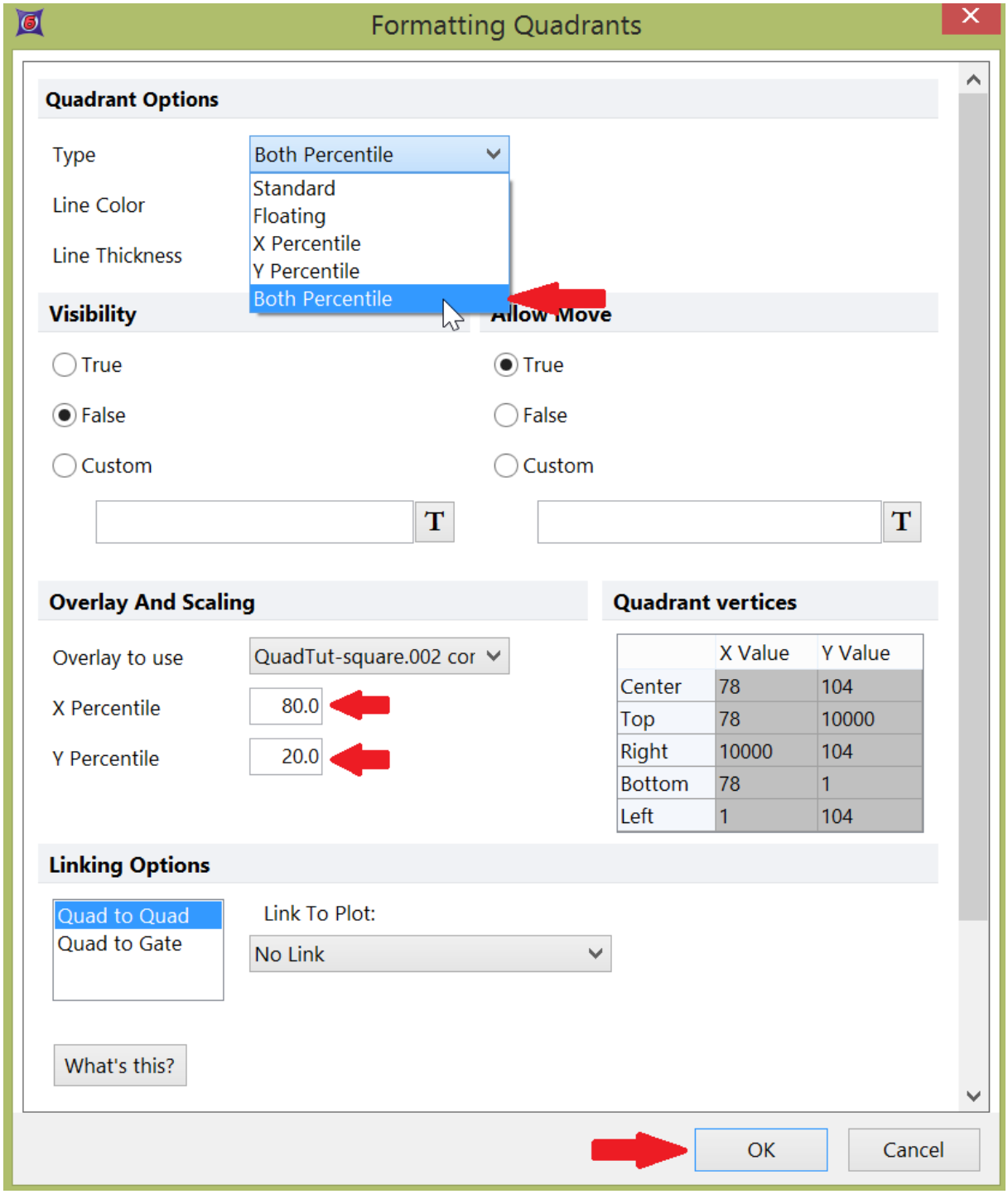

Figure T6.10 Formatting Quadrants Dialog -- Setting Type to Both Percentile

6. Enter "**80**" in **X Percentile** field (Figure T6.10).
7. Enter "20" in the **Y Percentile** field (Figure T6.10).

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8. Click **OK**.

The quadrants have adjusted automatically and appear similar to Figure T6.11. The sum of the Upper Left and Lower Left quadrants is approximately 80% (± rounding errors), and the sum of the Upper Right and Lower Right quadrants is approximately 20% (± rounding errors).

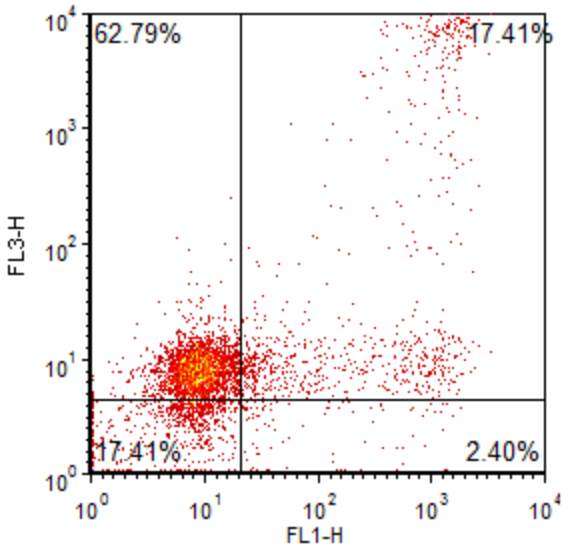

Figure T6.11 Percentile Quadrants of 80% and 20%

We will now use the plot pop-up menu to format the quadrant and change the X and Y percentile values.

9. Right-click on the left plot to bring up the associated pop-up menu.

10. Select **Format** from the pop-up menu.

11. Select the **Quads** category from the left of the **Formatting** dialog (Figure T6.12).

12. Change the **X and Y Percentiles** to **82 and 8** respectively (Figure T6.12).

13. Click **Preview**, indicated by the cursor in Figure T6.12.

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Using **Preview** allows you to quickly manipulate the percentiles until you reach your desired result.

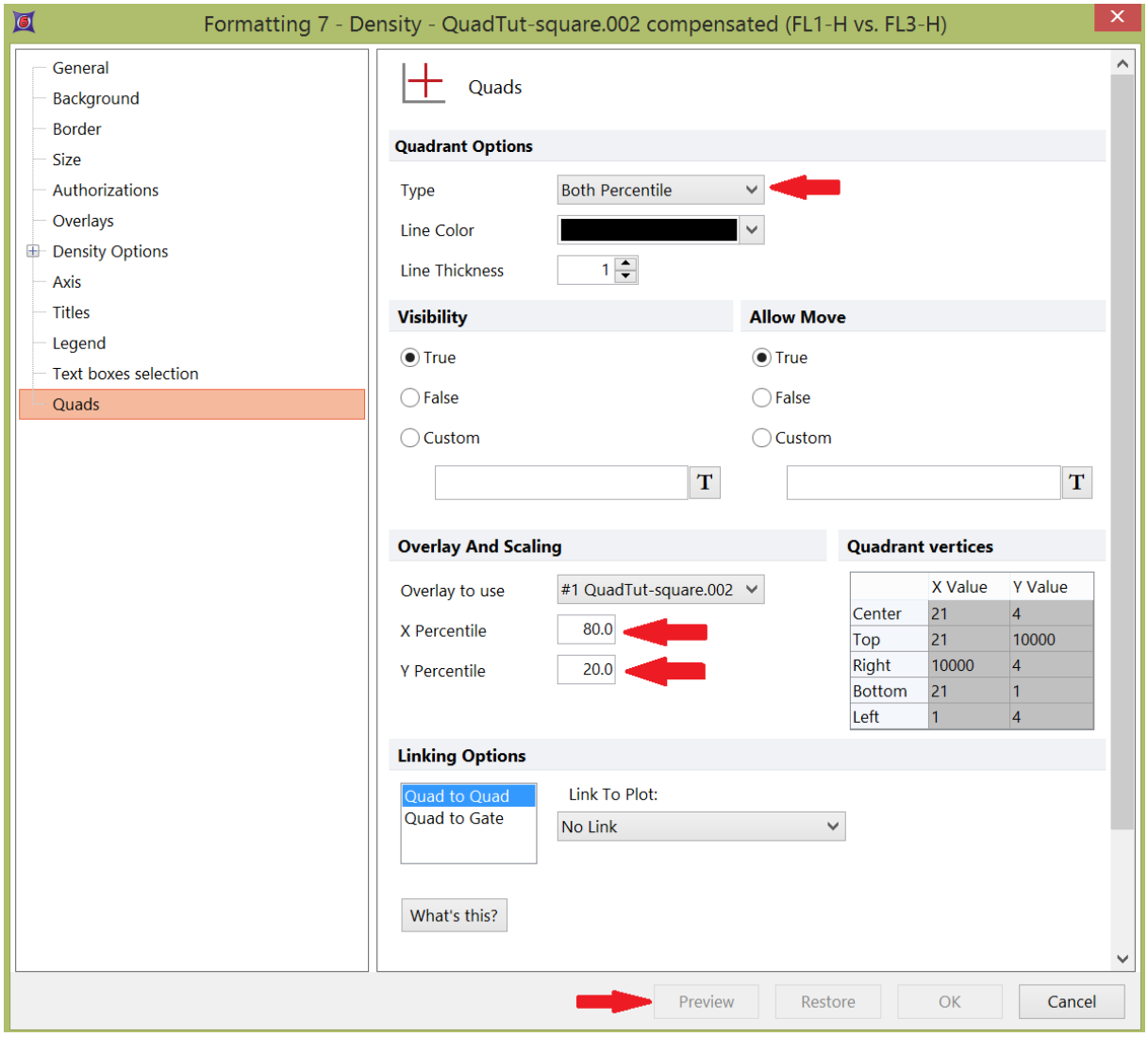

Figure T6.12 Using Preview while Setting Percentile Quadrant Values

Percentile quadrants are very useful when you have a plot that has a control population. You can have the quadrants automatically adjust to ensure that a specific fraction of the cells are in the lower left quadrant. You can then link the quadrants on the control plot to the quadrants on the sample plots, and all of the quadrants will update appropriately.

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38.7 Quadrant/Marker to Gate Linking

One of the many time saving features that FCS Express offers is the ability to use a quadrant to define a gate. This is useful if you want to use your quadrants to define a negative (or positive) population and then have your plots show only cells in the gated population. Markers can also be used to define a gate.

In the first exercise, we will link a quadrant to a gate 1362.

38.7.1 Quadrant to Gate Linking

In the course of this example, we will:

- Link a quadrant to a gate.
- Move a quadrant with a linked gate and watch the gate automatically update.

To link a region to a quadrant:

1. Select **File** tab→**Open Layout**.
2. Open the layout **Quad2RegionLink.fey** found in the Tutorial Sample Data archive 1210.

This layout contains three 2D Color Dots of the file **TCellSample1.fcs** (Figure T7.1). The left plot is of SSC-A vs. PerCP-Cy5.5-A with a gate on **Lymphs,** which are colored Red. The upper right plot is of FITC-A vs. APC-A gated on **Lymphs** with quadrants. The lower plot is a 2D Color Dot Plot looking at PE-Cy7 vs APC and gated on **Lymphs**.

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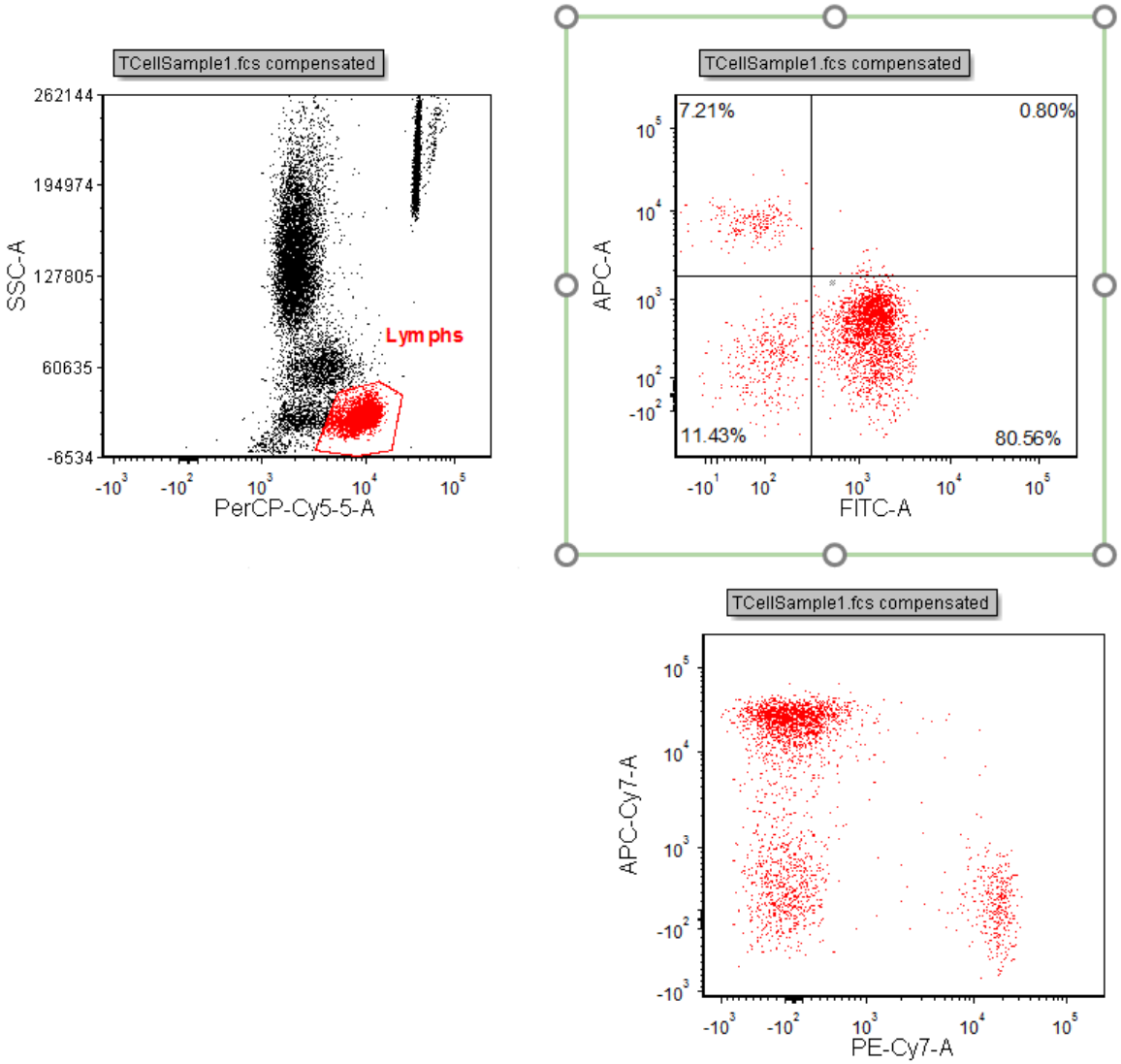

Figure T7.1 Quad2RegionLink.fey Layout

3. Right-click on the upper right 2D plot of FITC-A vs. APC-A with quadrants, shown with the green border in Figure T7.1.

This will bring up the associated pop-up menu (Figure T7.2).

4. Select **Convert Quads to Gates→Lower Right→Convert and Link** from the pop-up menu (Figure T7.2).

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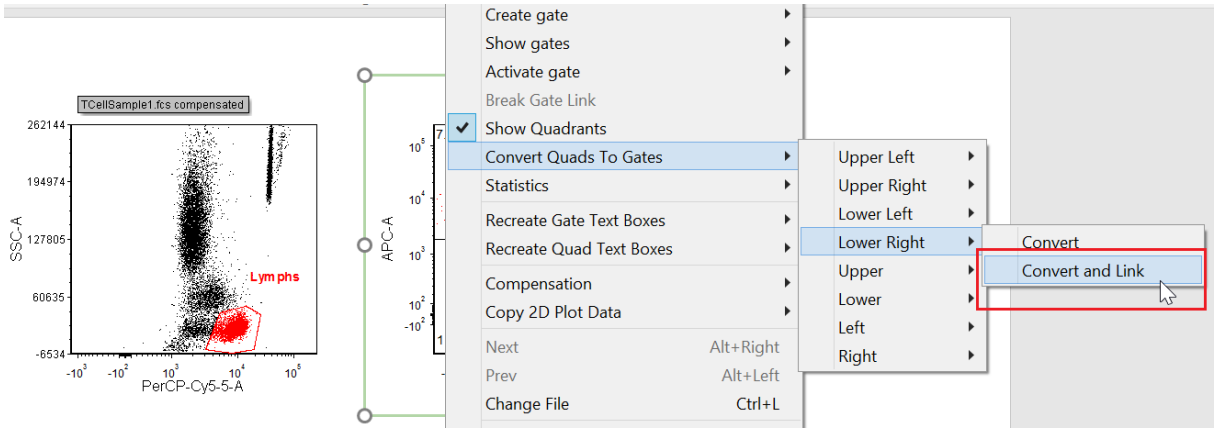

Figure T7.2 Converting and Linking the Lower Right Quadrant to a Gate

A **Create New Gate** dialog will appear (Figure T7.3). Here, we will name the new gate that will be linked to the lower right quadrant.

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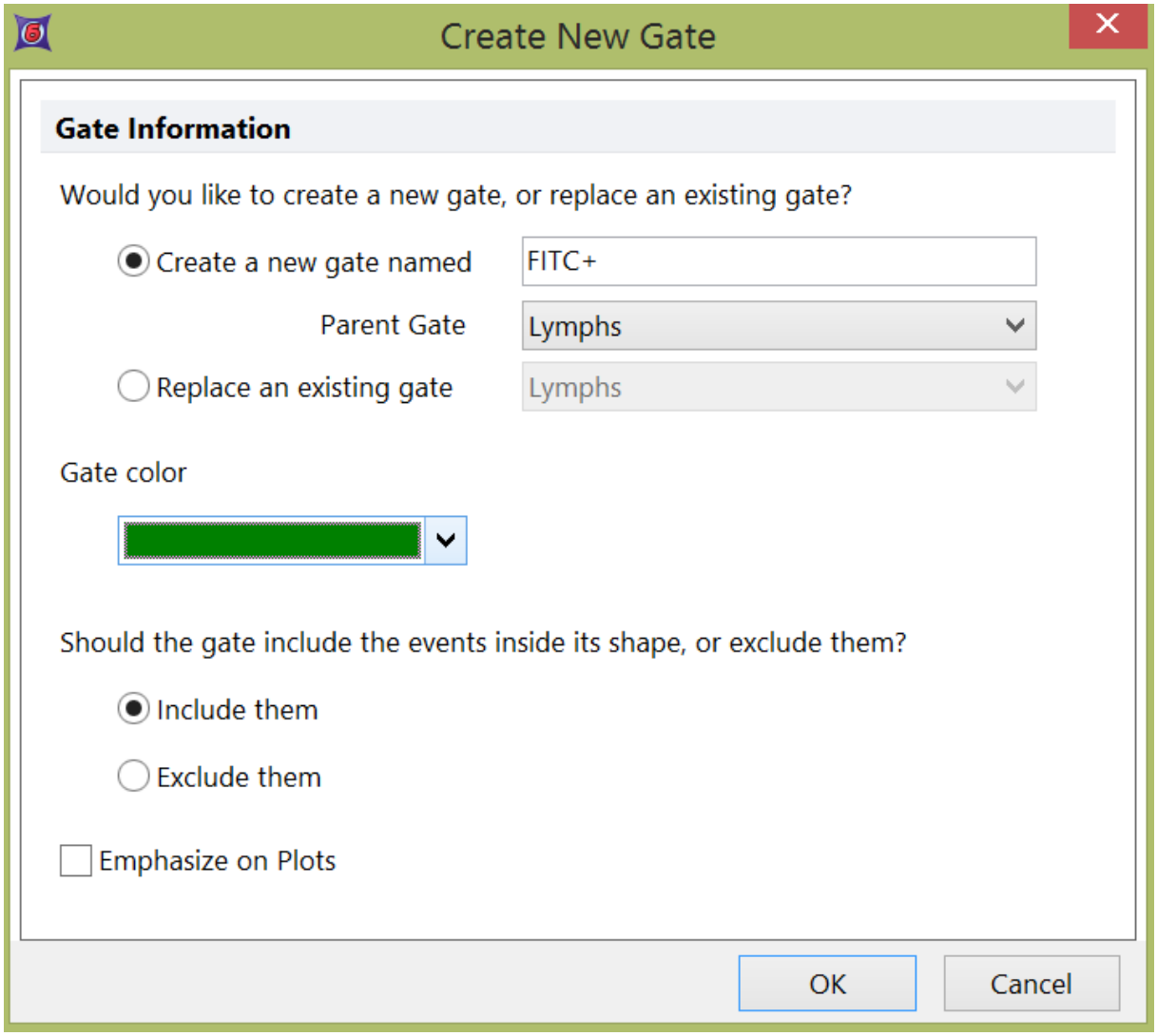

Figure T7.3 Create New Gate Dialog

5. Enter **FITC+** in the **Create a new gate named** field.
6. Change the Gate Color to **Green**.
7. Click **OK**.

We will now apply the newly created **FITC+** gate, that is linked to the lower right quadrant, to the lower right 2D plot.

7. Click inside the 2D plot of PE-Cy7-A vs APC-Cy7-A on the lower right without the quadrants. The border will appear green (Figure T7.4).
8. Select the **Gating** tab (Figure T7.4).

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9. Select **FITC+** from the **Current Gate** drop-down list (Figure T7.4).

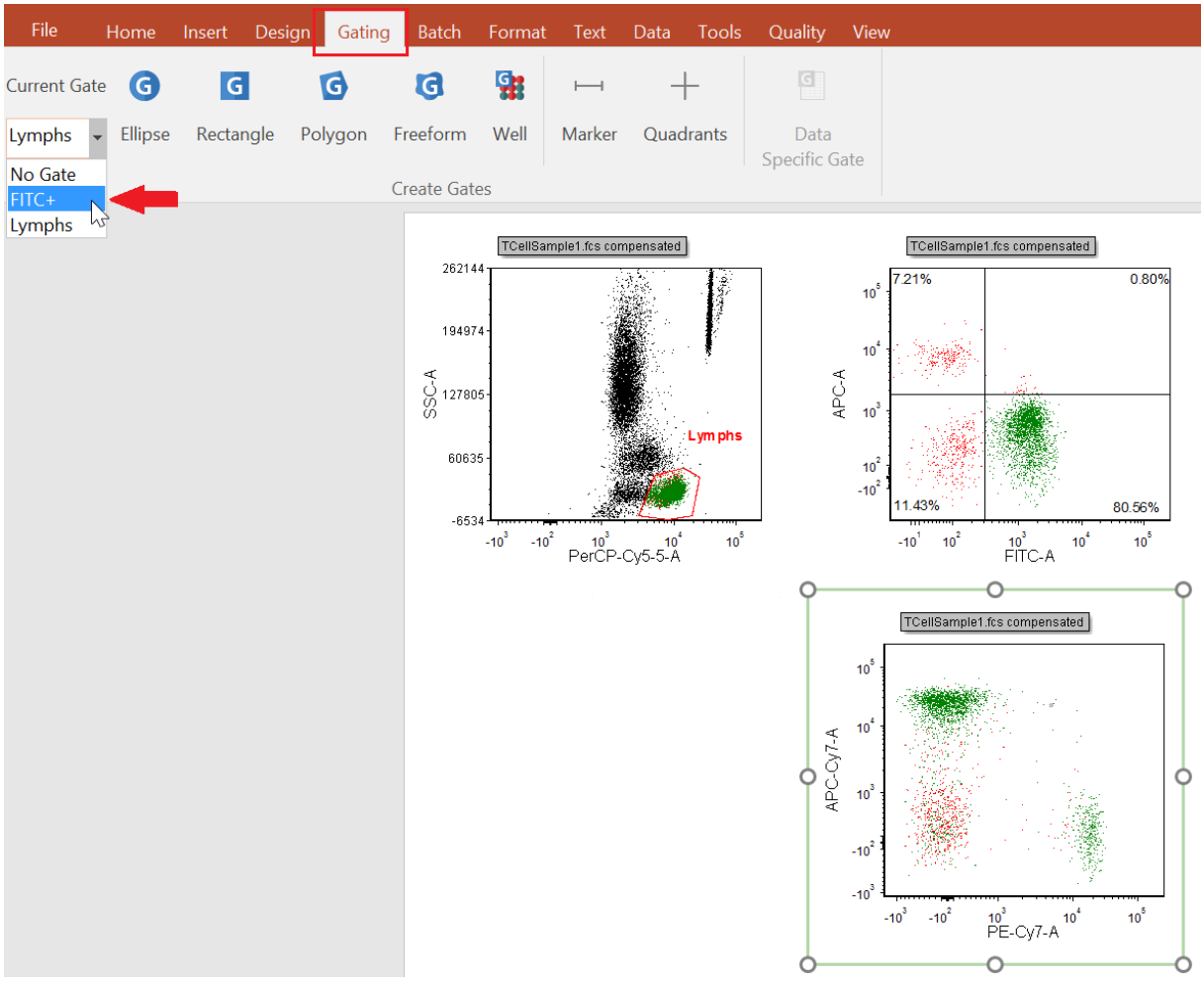

Figure T7.4 Selecting the FITC+ Gate for the Lower Right 2D Plot

The 2D plot on the lower right will now appear with only the events found in the lower right quadrant (Figure T7.5).

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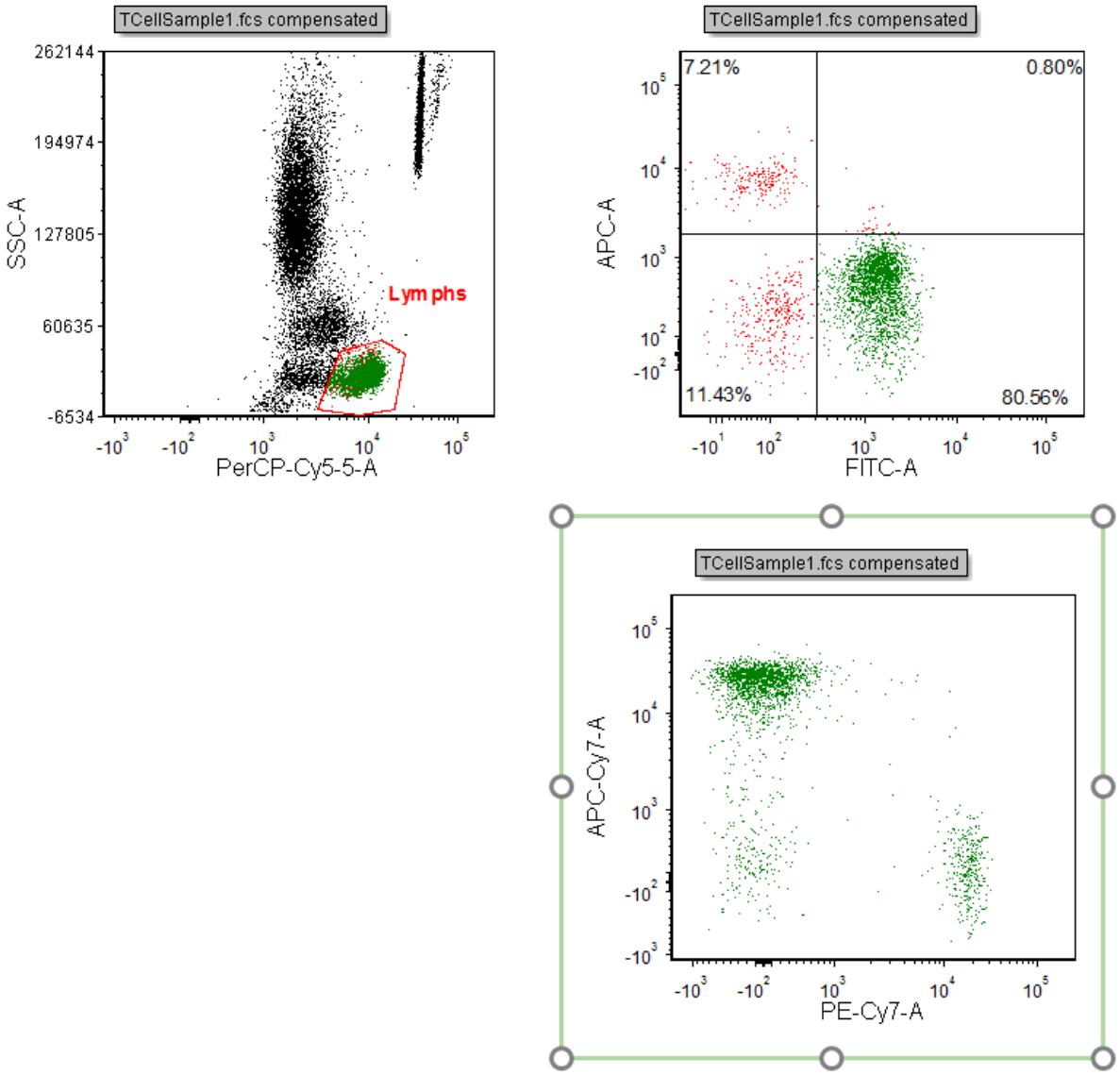

Figure T7.5 Lower Right Plot Gated on GFP(+)

Because the quadrants are linked to the gate, changing the quadrant position will also change the gate **GFP(+)**. We will now move a quadrant to demonstrate how the **GFP(+)** gate linked to the quadrant will also change.

To move a quadrant:

10. Click inside the 2D plot of FL1 vs. FL2 with the quadrants on the upper right of the layout. The border will appear green (Figure T7.6).

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11. Click on the vertex of the quadrant. You will know you have selected the quadrant for editing because the mouse will change to a four-headed arrow.

12. Press and hold the down the left mouse button on the vertex.

13. Move the mouse to the right while continuing to hold down the mouse button.

14. Release the mouse button when the lower right quadrant definition is smaller (Figure T7.6).

The **Lower 2D plot of FL1 vs. FL2** will update to reflect the changes made to the lower right quadrant (Figure T7.6).

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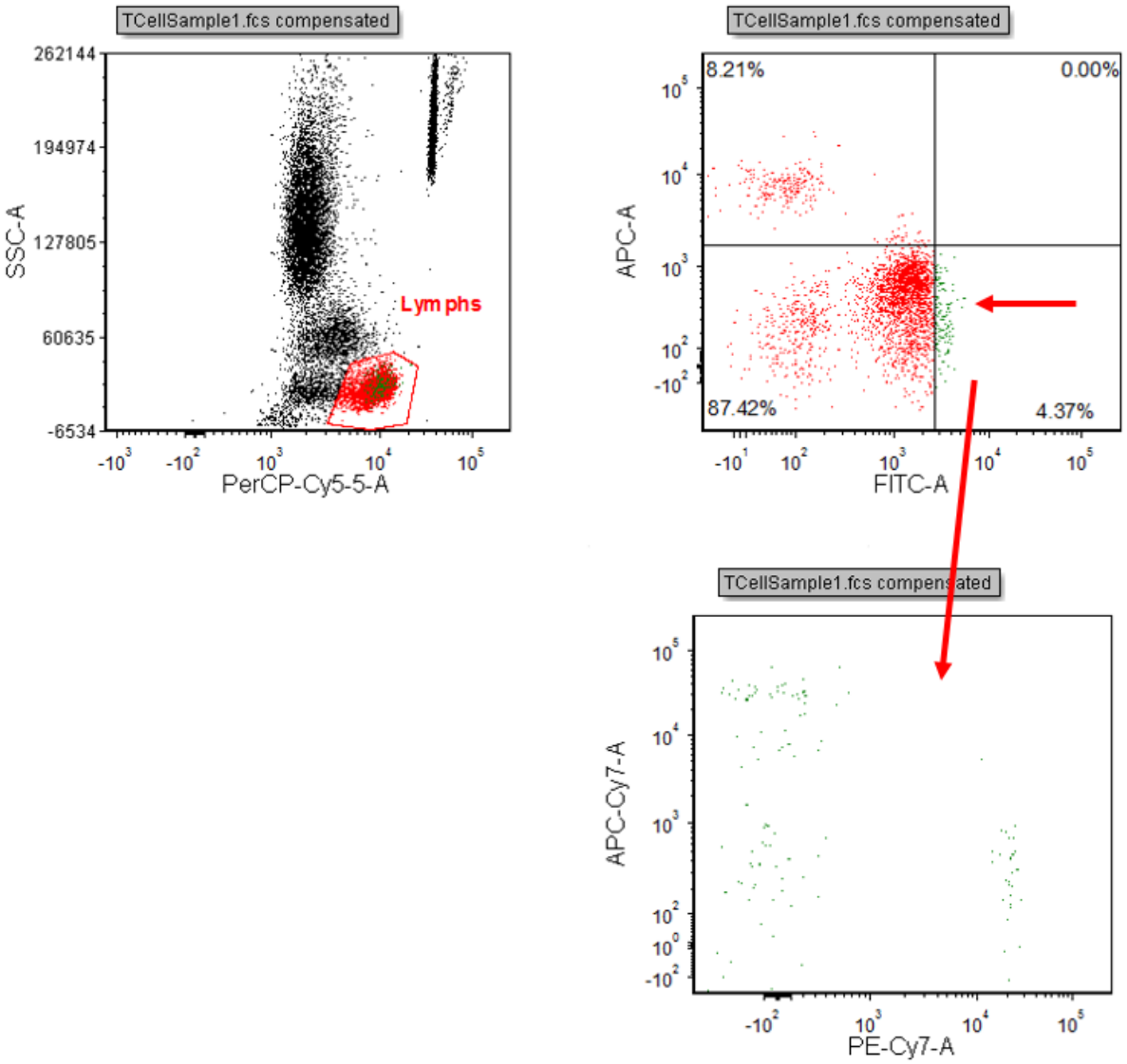

Figure T7.6 GFP(+) Gate Updated after Quadrant Move

15. Select **File** tab→**Close Layout**.

In the next section, we will link a marker to a gate 1369.

38.7.2 Marker to Gate Linking

In the course of this example, we will:

- Link a marker to a gate.

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- Move a marker with a linked gate and watch the gate automatically adjust.

A marker can also be created directly on a 2D plot by selecting the **Gating→Create Gates→Create Marker** command. For more information on creating a 1D gate on a 2D plot, please refer to the appropriate section in the tutorial on Using Gates.

1. Select **File** tab→**Open Layout**.
2. Open the layout **Marker2RegionLink.fey** found in the Tutorial Sample Data archive.

This layout contains one 2D plot and two histograms of the file **GFPsample.002** (Figure T7.7). The upper left plot is of FSC vs. SSC with a gate on **Whole Cells**. The upper histogram is of FL1 gated on **Whole Cells** with a marker **M1**. The lower histogram is also a histogram of FL1 but without a marker.

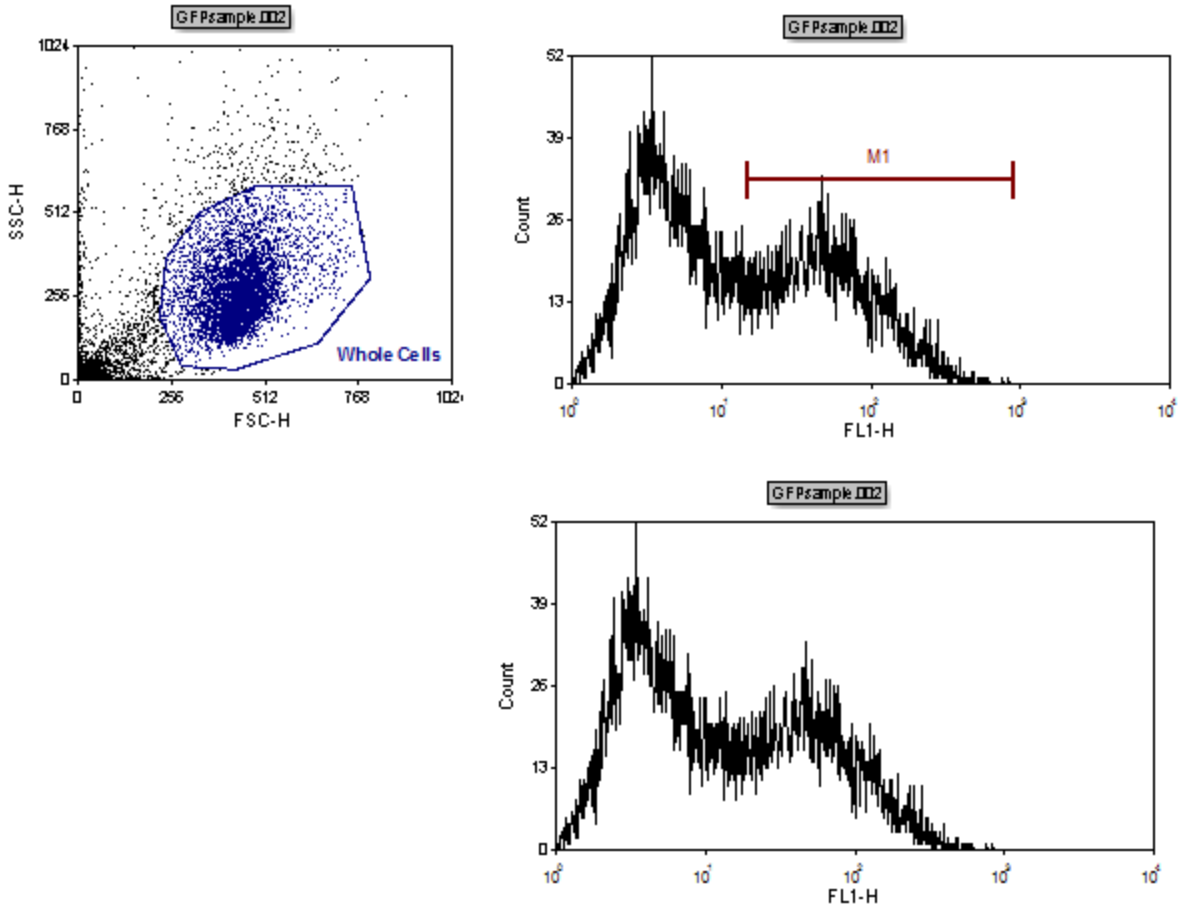

Figure T7.7 Marker2RegionLink.fey Layout

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3. Right-click on the upper right histogram of FL1 with the **M1** marker.

This will bring up the associated pop-up menu.

4. Select **Convert Marker to Gate→Marker #1 (M1)→Convert and Link** from the pop-up menu (Figure T7.8).

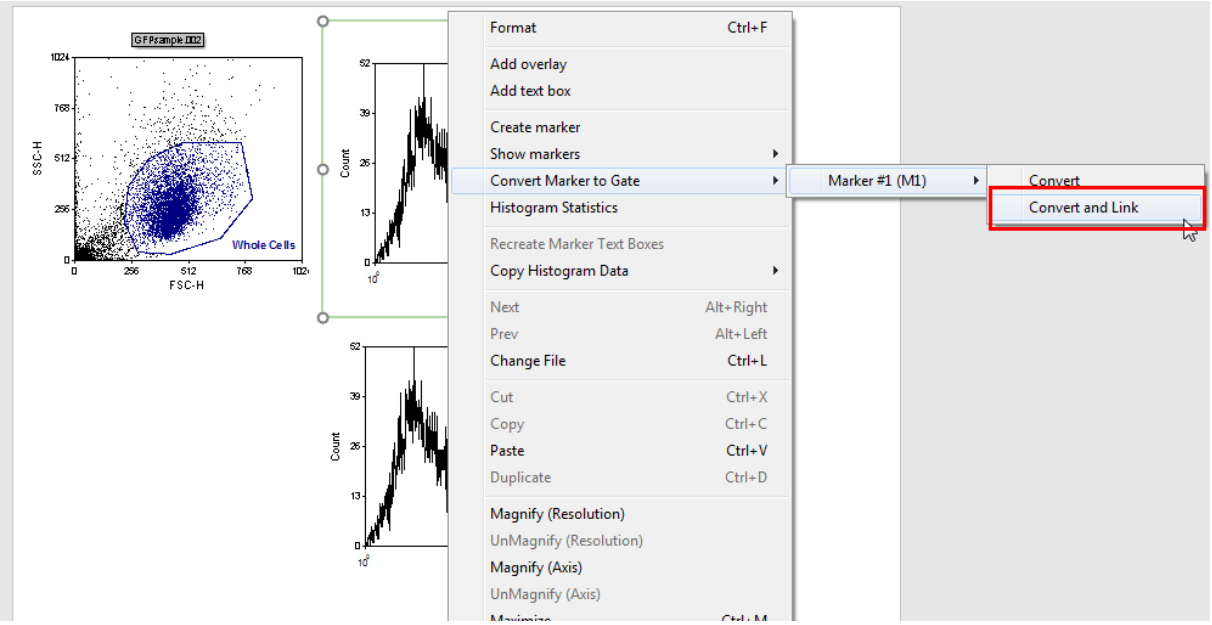

Figure T7.8 Converting the Marker M1 to a Gate

A **Create New Gate** dialog will appear as shown in Figure T7.3 1362. Here, we will name the new gate that is linked to the marker.

5. Enter "**GFP(+)**" in the **Create a new gate named** field.
6. Click **OK** (Figure T7.3 1362).
7. Click inside the histogram of FL1 on the lower right, without the marker. The border will appear green (Figure T7.9).
8. Select the **Gating** tab as previously shown in Figure T7.4 1362.
    - Alternatively, select the **Home** tab (Figure T7.9).
9. Select **GFP(+)** from the **Current Gate** drop-down list (Figure T7.9).

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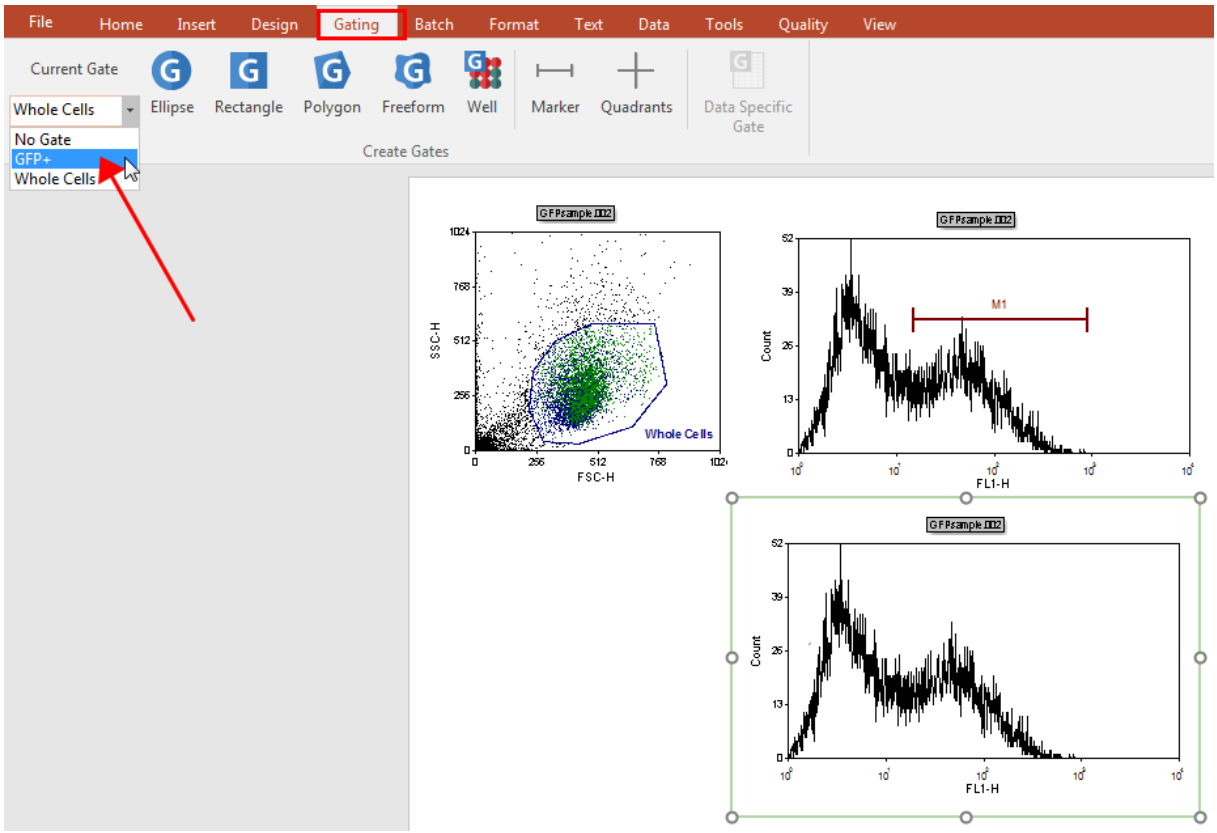

Figure T7.9 Applying the GFP(+) Gate to the Lower Right Histogram

The histogram on the lower right will now appear with only the events found in the marker **M1** (Figure T7.10).

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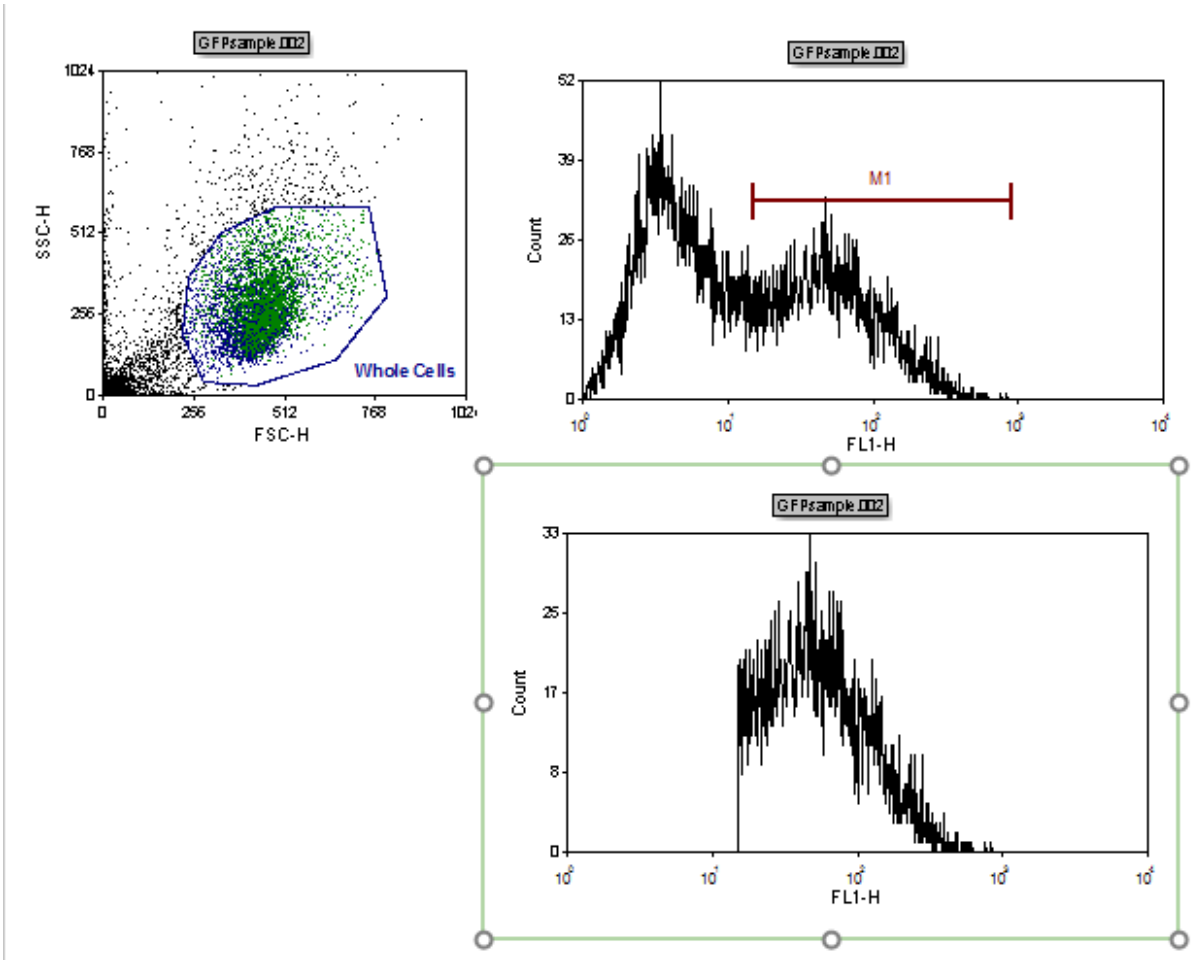

Figure T7.10 Lower Right Histogram Gated on GFP(+)

Because the marker **M1** is linked to the gate, changing the marker position will also change the gate **GFP(+)**.

10. Click inside the histogram of FL1 with the marker **M1**, located in the upper right of the layout. The border will appear green.

11. Click the left mouse button anywhere on the marker **M1** to select it. The marker will have three resizing rectangles when it has been selected for editing (Figure T7.11).

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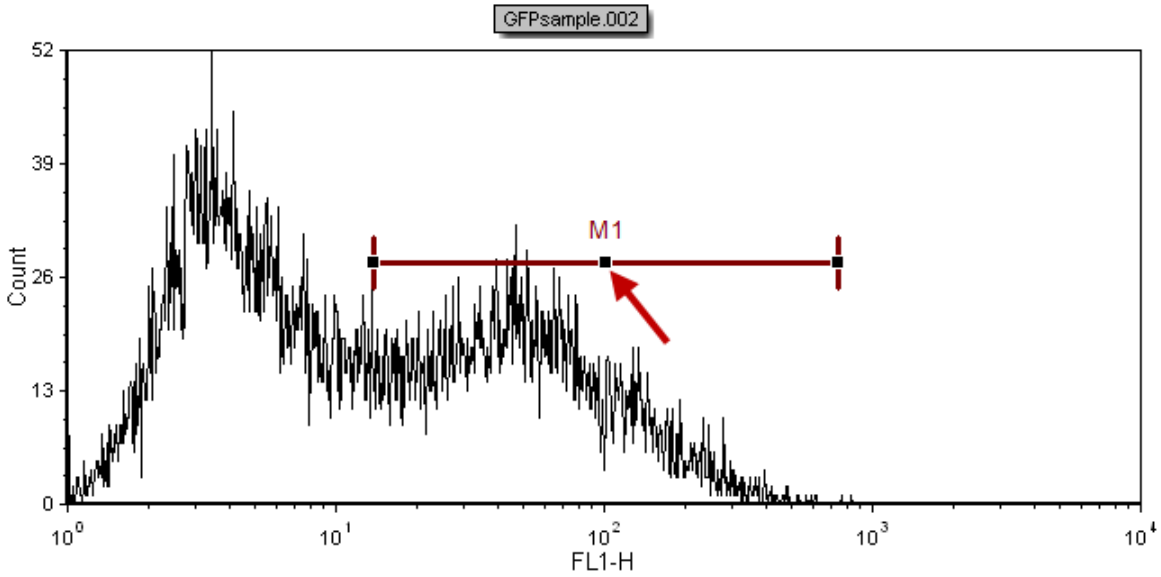

Figure T7.11 Active Marker M1 with Resizing Rectangles

12. Move the mouse over the central resizing rectangle. You will know you are in the correct place because the cursor will change to a four-headed arrow.

We will now move the entire marker **M1** to a new location on the histogram. Alternatively we could also resize the marker by selecting one end of the marker with the cursor and then moving it.

13. Press and hold down the left mouse button.

14. Move the mouse to the right while holding down the mouse button (Figure T7.12).

Arrows pointing down will appear indicating the location of the ends of the marker on the x-axis. On top of the marker will be the channel numbers indicating the end boundaries of the marker (Figure T7.12).

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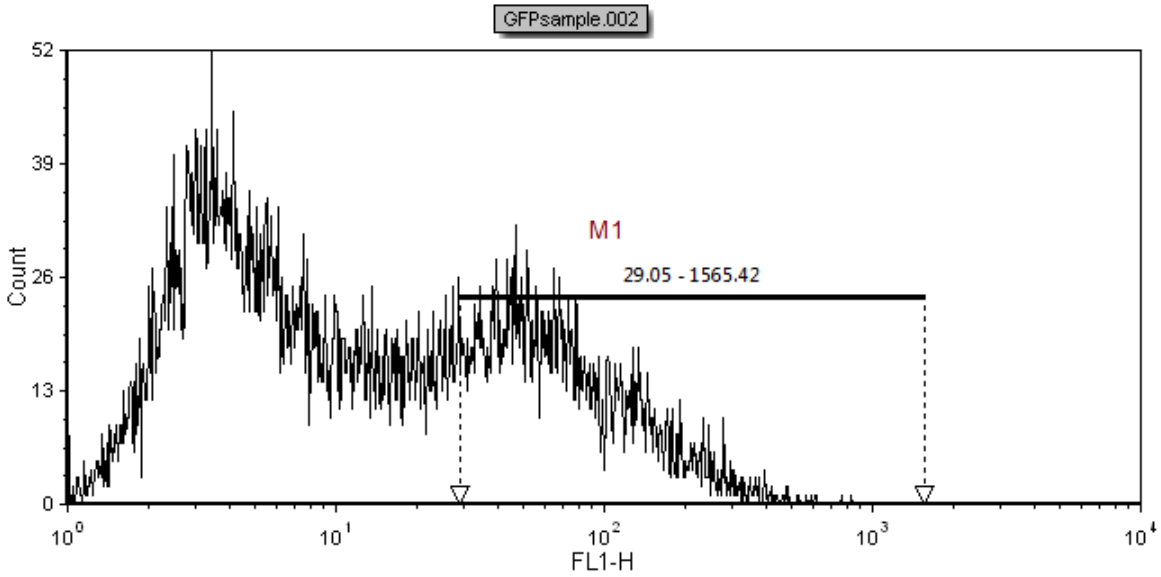

Figure T7.12 Moving Marker M1

15. Release the mouse button when the marker is in the desired position (Figure T7.12).

The histogram of FL1 located on the lower right will update to reflect the changes made to the marker **M1** (Figure T7.13).

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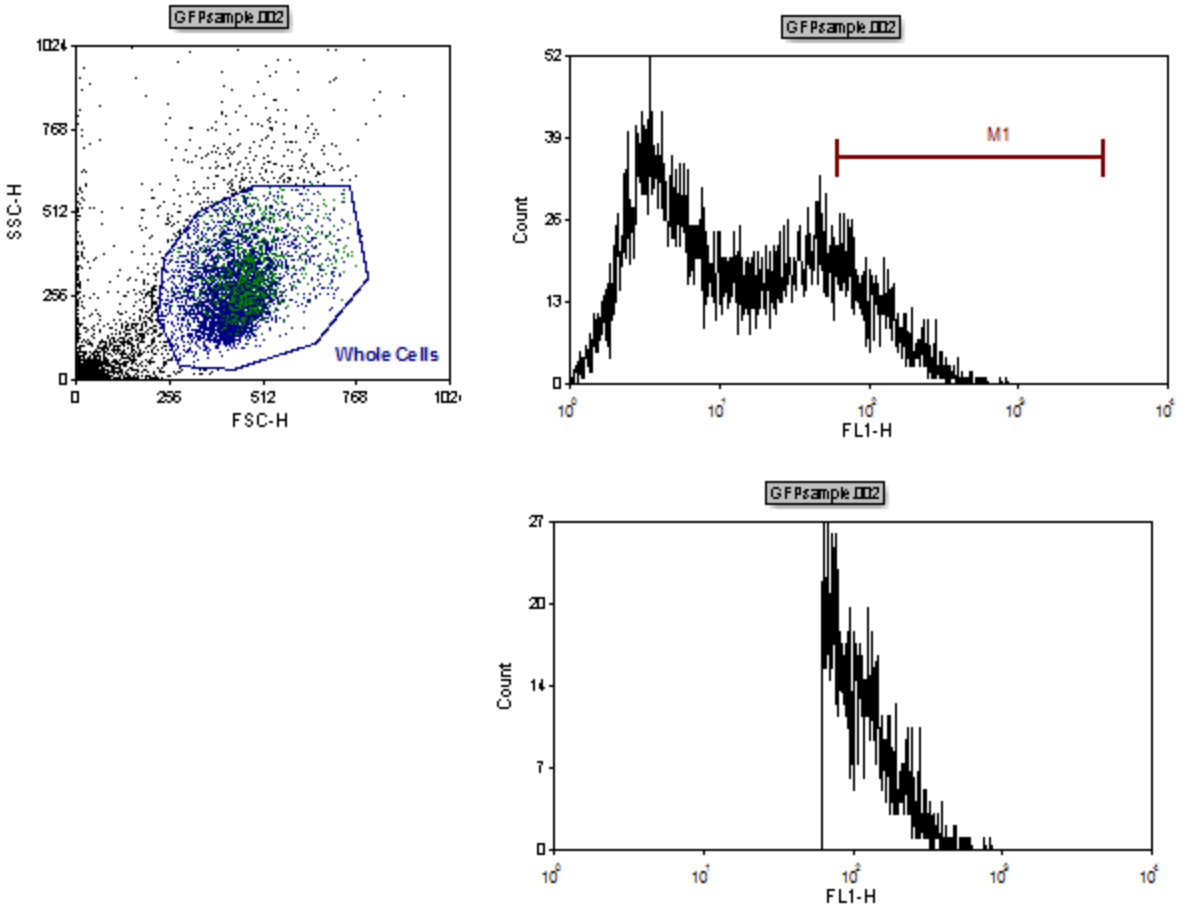

Figure T7.13 Histogram Gated on GFP(+) Updated to Reflect Changes made to Marker M1 Position

38.8 Compensation

Normally, FCS Express imports the compensation matrix that was used when acquiring the sample. But sometimes you want to make your own compensation matrix.
There are two ways to create your own compensation definition in FCS Express:

- The Manual 1377 method allows you to adjust the compensation levels using sliders that allow you to adjust the amount of compensation.
- The Automatic 1381 method allows you to take single stained controls and automatically calculate the appropriate compensation values.

FCS Express allows you to quickly and easily set up either compensation method. Sometimes compensation may also introduce digitization errors which will be discussed in the Compensation Digitization Error 1421 tutorial. In the following examples, we will be opening two layouts in FCS Express which already have a gate defined on the compensation population of interest.

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The first example will show Manual Compensation 1377.

38.8.1 Manual Compensation

To adjust the compensation manually:

1. Select **File** tab→**Open Layout**.
2. Open the layout **ManualComp.fey** found in the Tutorial Sample Data archive 1210.

This layout contains two plots. The dot plot of FSC-H vs. SSC-H is showing a gate based on size (gate 1). The right plot of CD95 PC5 (PE CY5) vs. CD8a PC7 (PE CY7) is gated on gate 1 (Figure T8.1).

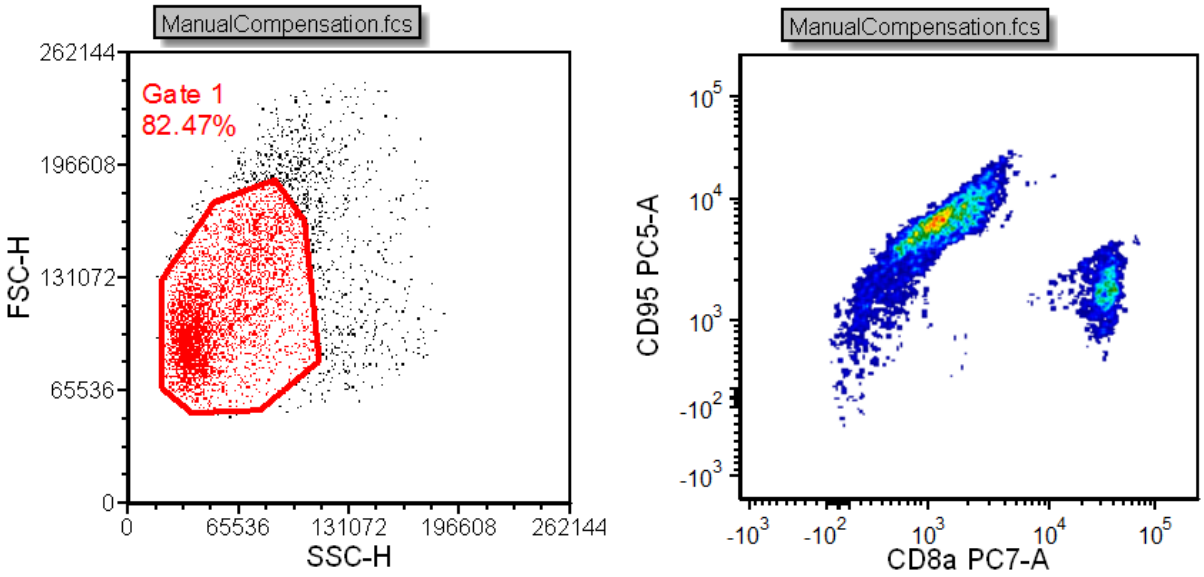

Figure T8.1 Uncompensated Plots

3. Right-click on the right density plot of CD95 PC5-A vs. CD8a-PC7-A to bring up the associated pop-up menu.
4. Select **Compensation→Create New** from the pop-up menu (Figure T8.2).

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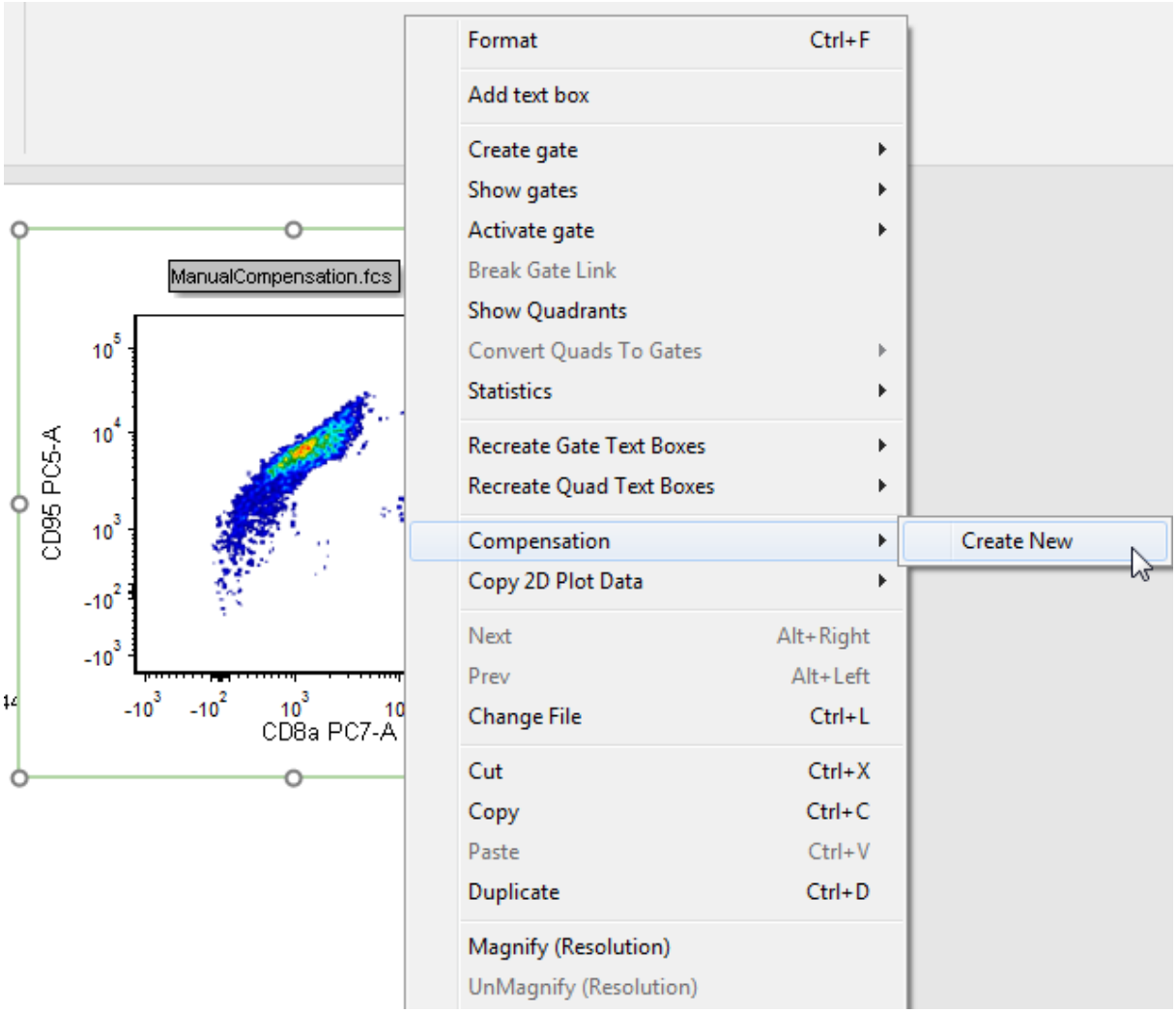

Figure T8.2 Selecting Create New Compensation

A **Create a new Compensation** dialog will appear (Figure T8.3).

5. Type "**ManualComp**" in the **Enter a name for the new Compensation** field.
6. Deselect the checkbox for **Use this compensation as default on new plots**.
7. Click **OK**.

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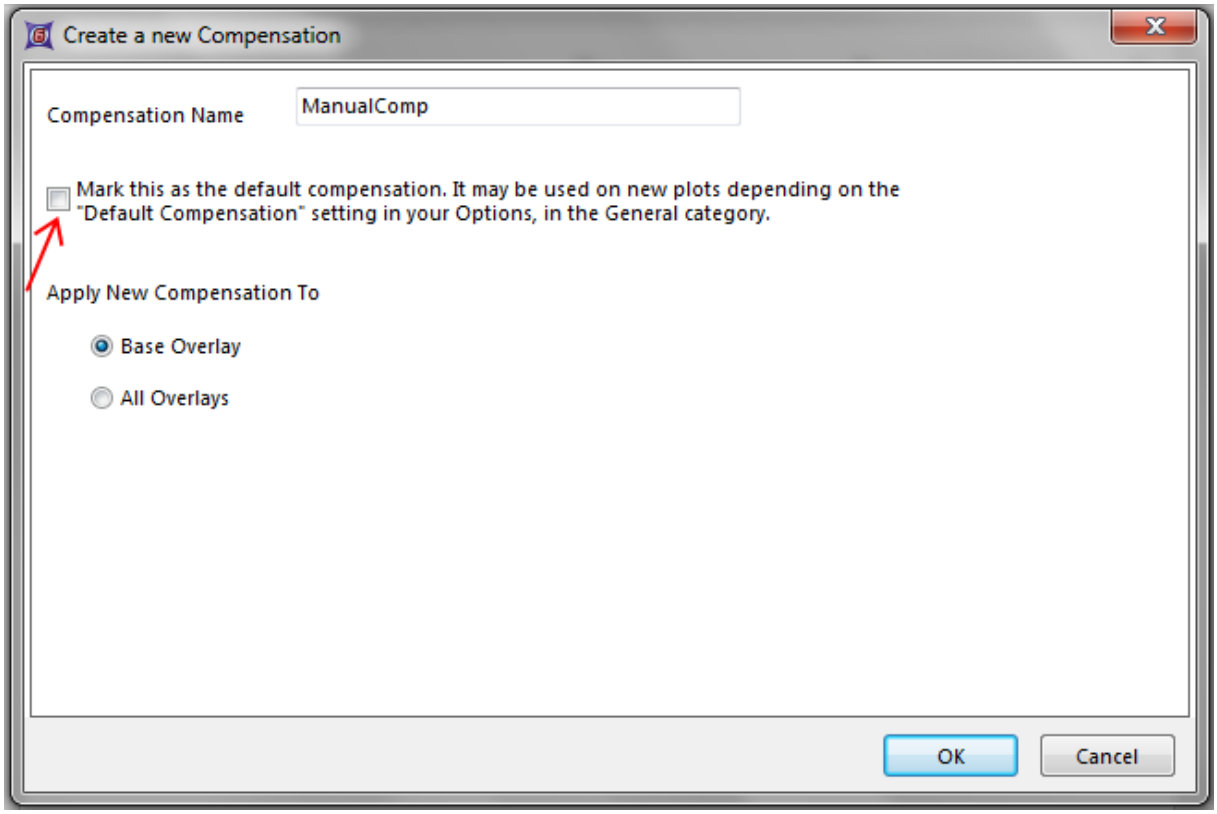

Figure T8.3 Create a new Compensation Dialog

An **Edit Compensation** dialog will now appear (Figure T8.4).

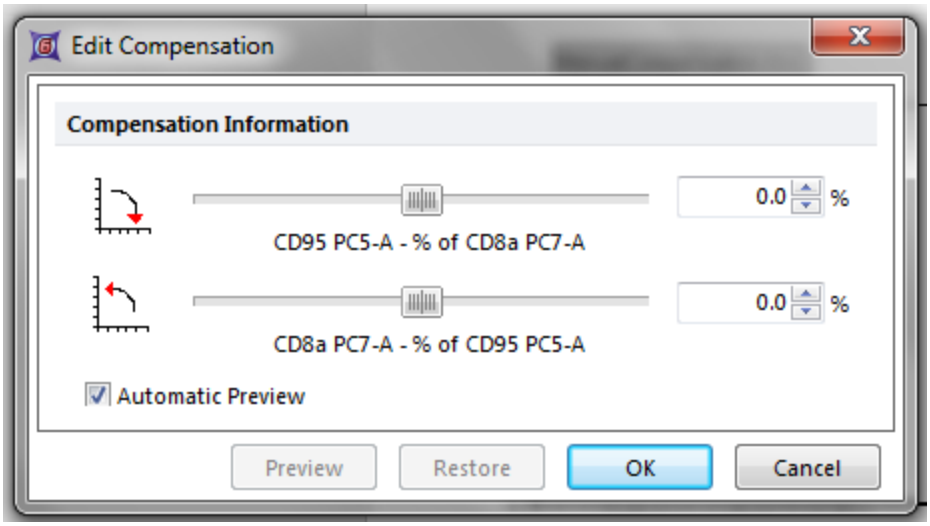

Figure T8.4 Edit Compensation Dialog

8. Adjust the sliders shown in Figure T8.4 until the 2D plot looks like Figure T8.5. Note that moving the slider to the right causes the population to move in the direction of the arrow in the image to the left of the slider.

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Be careful not to overcompensate! Proper compensation will require around 6.0% of CD95 PC5-A - % of CD8a PC7-A and 18.0% of CD8a PC7-A - % of CD95 PC5-A.

9. Click **OK** to select the manual compensation settings.

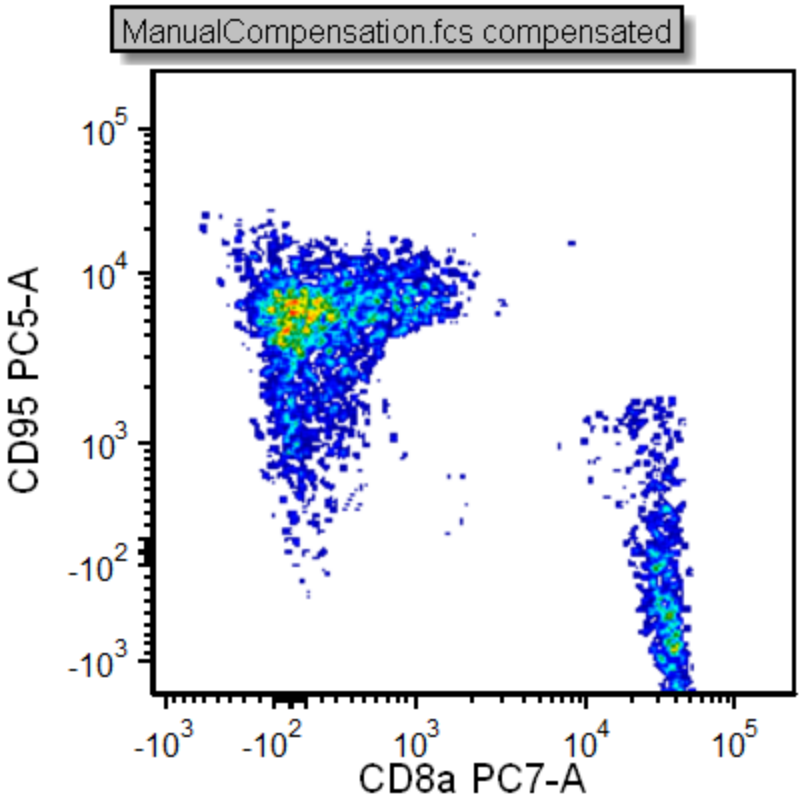

Figure T8.5 Manual Compensation using Edit Compensation Sliders

Manual compensation of the file is now complete.

Note: *Sometimes when working with your own data digitization errors may occur as in Figure T8.21 below. If you wish to correct for the digitization errors please see the brief* Compensation Digitization Errors 1421 *tutorial.*

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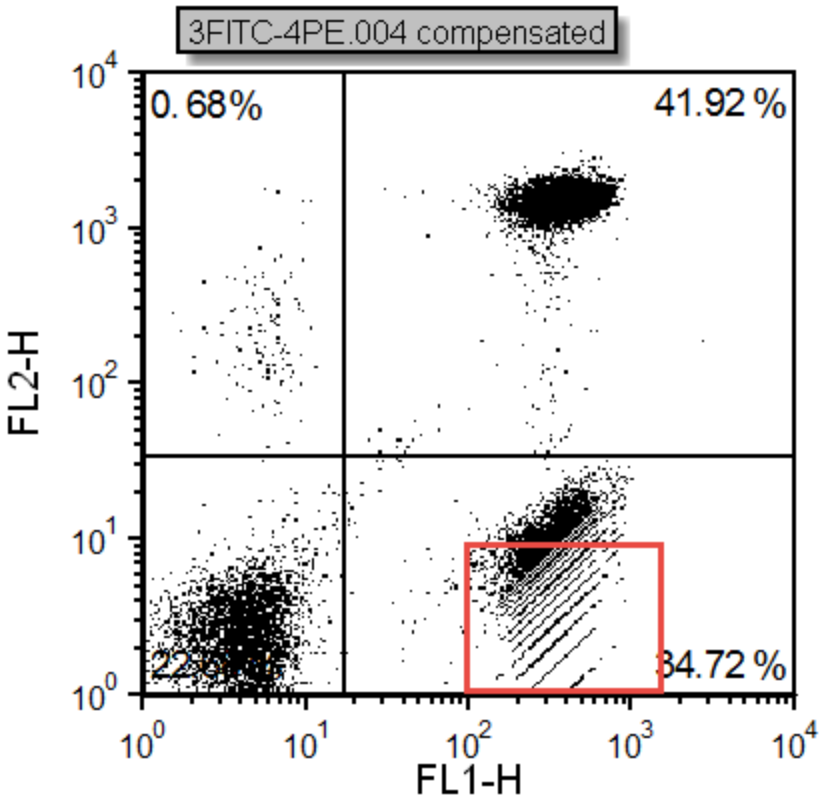

Figure T8.21 - Digitization errors after compensation can be seen in the red box. Please refer to the Compensation Digitization Errors tutorial to correct this type of error.

Note: *Existing compensations may also be adjusted on a 2D plot. Please see the Adjusting Compensation Definitions 646 section of the manual for more details.*

In the next section, we will use Automatic Compensation 1381.

38.8.2 Automatic Compensation

To create a Compensation Definition automatically from single stained controls:

1. Select **File** tab→**Open** (Figure T3.1 1237).
2. Open the layout **AutoComp.fey** found in the Tutorial Sample Data archive 1210.

This is the layout that we are going to use to illustrate how to set up automatic compensation (Figure T8.6). Typically you would start with a blank layout but we have inserted plots so you can more easily see your data for the purposes of

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this tutorial. The top-left most plot is of FSC vs. SSC data with a gate based on a bead population. In order to use the automatic compensation, you need a single-stained control for every parameter that you wish to compensate as well as an unstained sample.

(Note: The unstained control can be a universal negative, a negative population defined by a gate, or the negative population with the tube of the positive control. In this case we will use an unstained sample)

The following data set contains an Unstained Control, All Color Control, and five single stain controls for the following parameters: FITC, PE, PC5 (PE Cy5), PC7 (PE Cy7), and APC. All of the histograms are gated on the Beads gate and display the data file and parameter for each control. For example, the top-right most histogram is the *Compensation Controls_FITC Stained Control.fcs* file and the FITC-A parameter is being displayed on the X-axis.

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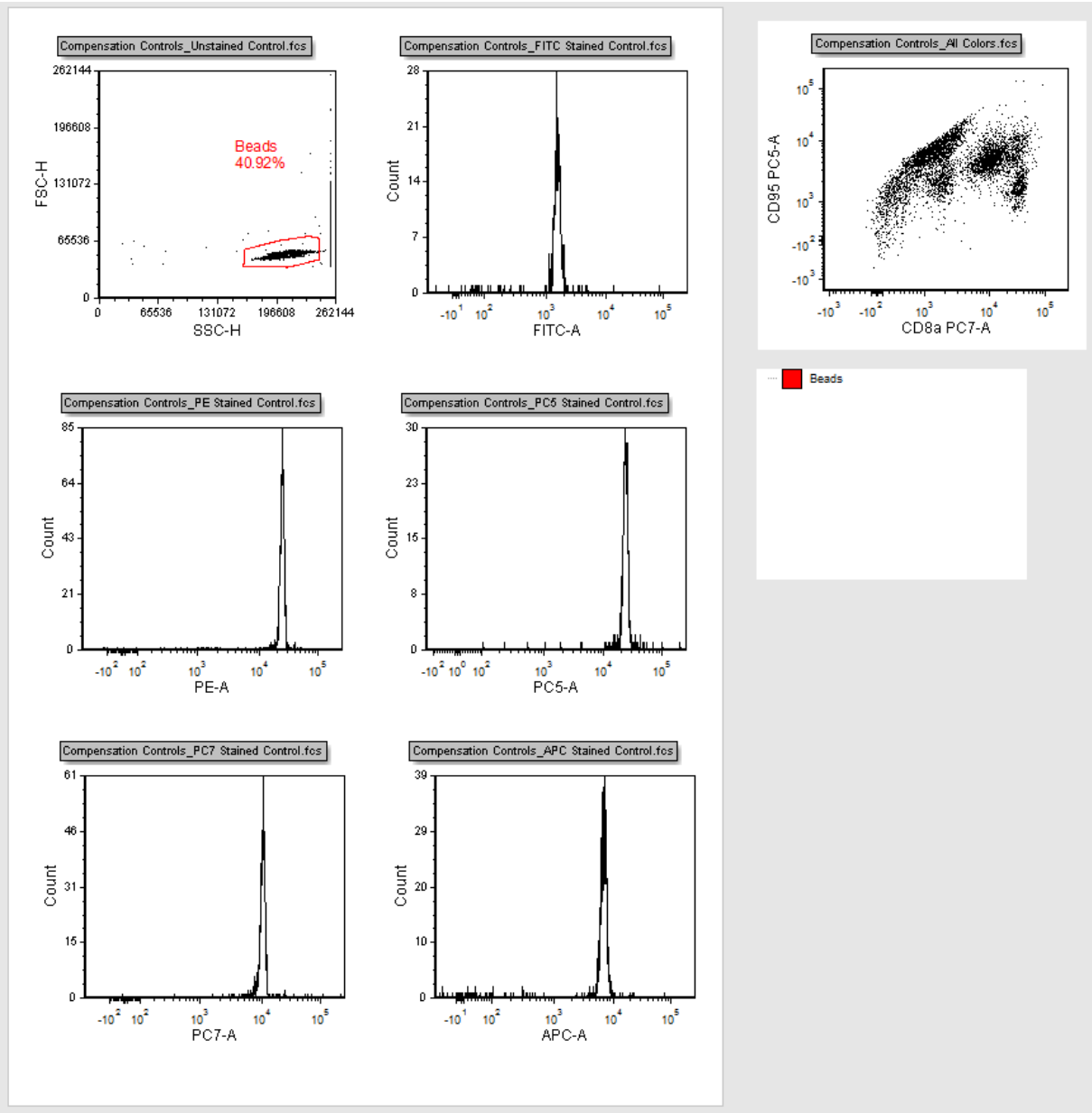

Figure T8.6 Autocomp.fey Layout

In this example, we are setting compensation for all five single stained controls/colors. However, you can set compensation for as many channels as you like, provided you have a single-stained control for each particular channel.

3. Click on the **Tools** tab→**Transformations**→**Compensations** command to open the **Compensations** navigator (Figure T8.7).

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The **Compensations** navigator can be moved anywhere on the screen and it is dockable and pinnable[113]. Please feel free to move it around the screen as needed while working on this tutorial.

4. Click on the blue plus button, , to **Create a new compensation** (Figure T8.7).

A text box highlighted in blue will appear with the text **New Compensation** (Figure T8.7).

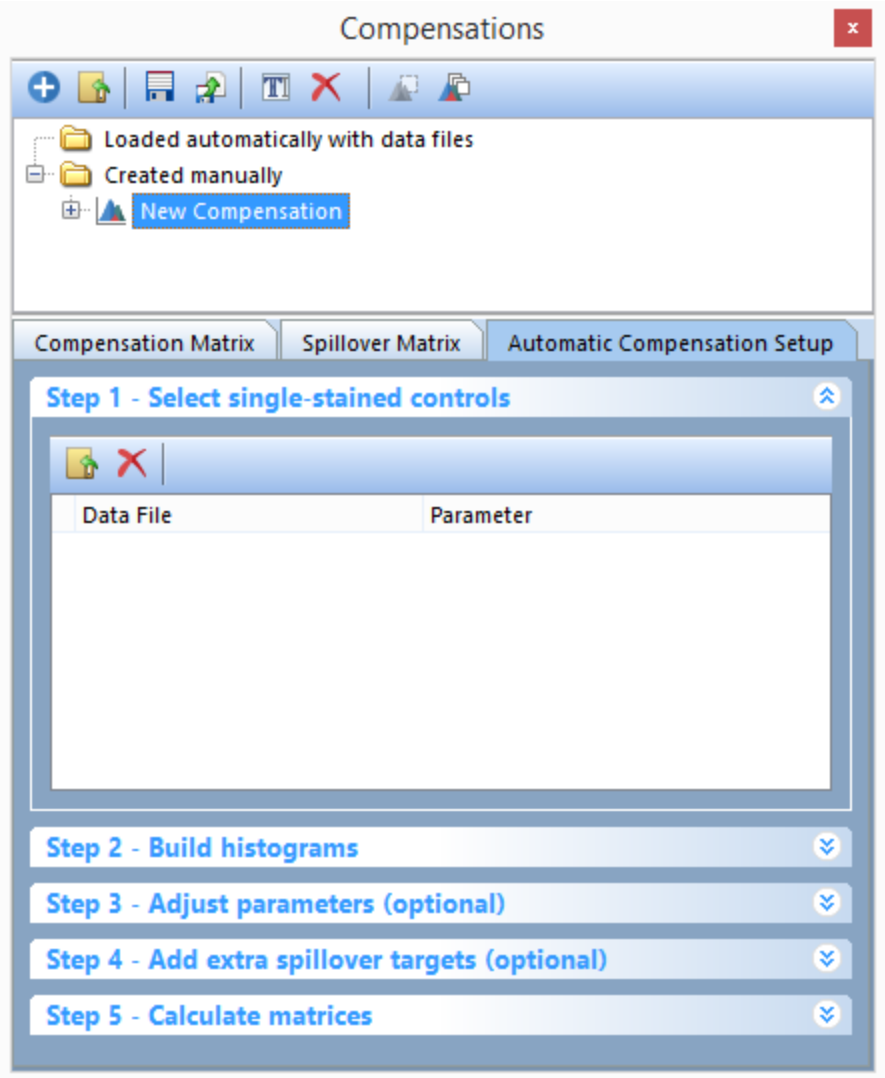

Figure T8.7 - The Compensations navigator. Use the navigator to add new compensations, edit definitions, and to create new Automatic Compensation Setups.

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5. Click on the Add data for compensation button, , in the **Automatic Compensation Setup→Step 1 - Select single-stained controls** window (Figure T8.7).

6. The **Standard Open Data Dialog** will appear (Figure T8.8).

Note: based on your User Options 212, the Advanced Open Data Dialog 216 may appear. If the **Advanced Open Data dialog** appears, please select the Select data file ... button to access the **Standard Open Data Dialog**.

. Select **FCS Files (*.*; *.fcs; *.lmd)** from the files of type drop-down menu (Step 1, Figure T8.8).

. Select multiple files from your computer (Step 2, Figure T8.8).

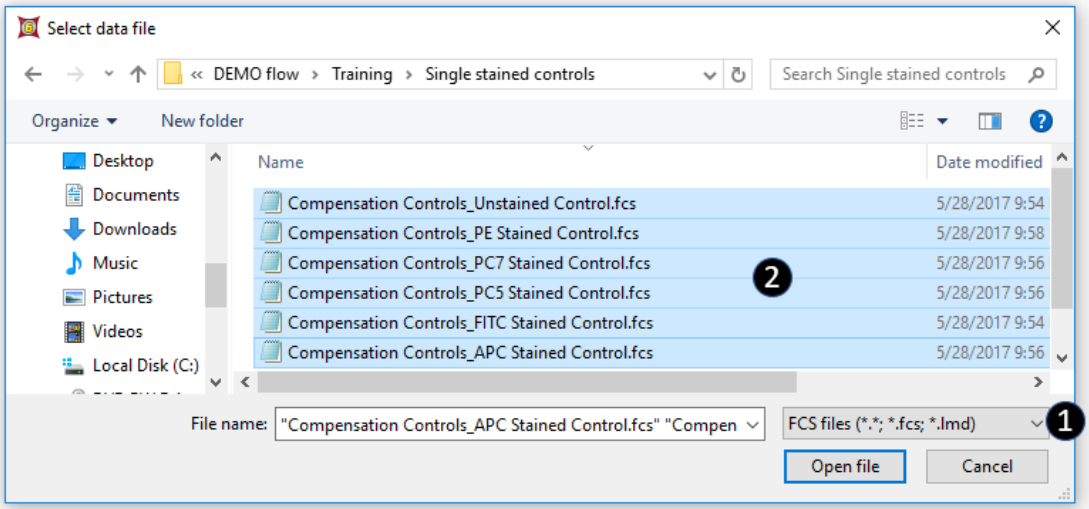

Figure T8.8 - Use the Select Data File window to select all of the files for use in the Automatic Compensation Setup.

7. Click **Open file**.

Notice that all of the single-stain control files and the unstained control file have been brought into the **Select single-stain control** window (Figure T8.9). We will now assign the correct parameter for each individual file by matching the single-stain control name in the file name to the **Parameter** in the **-select parameter-** drop down list.

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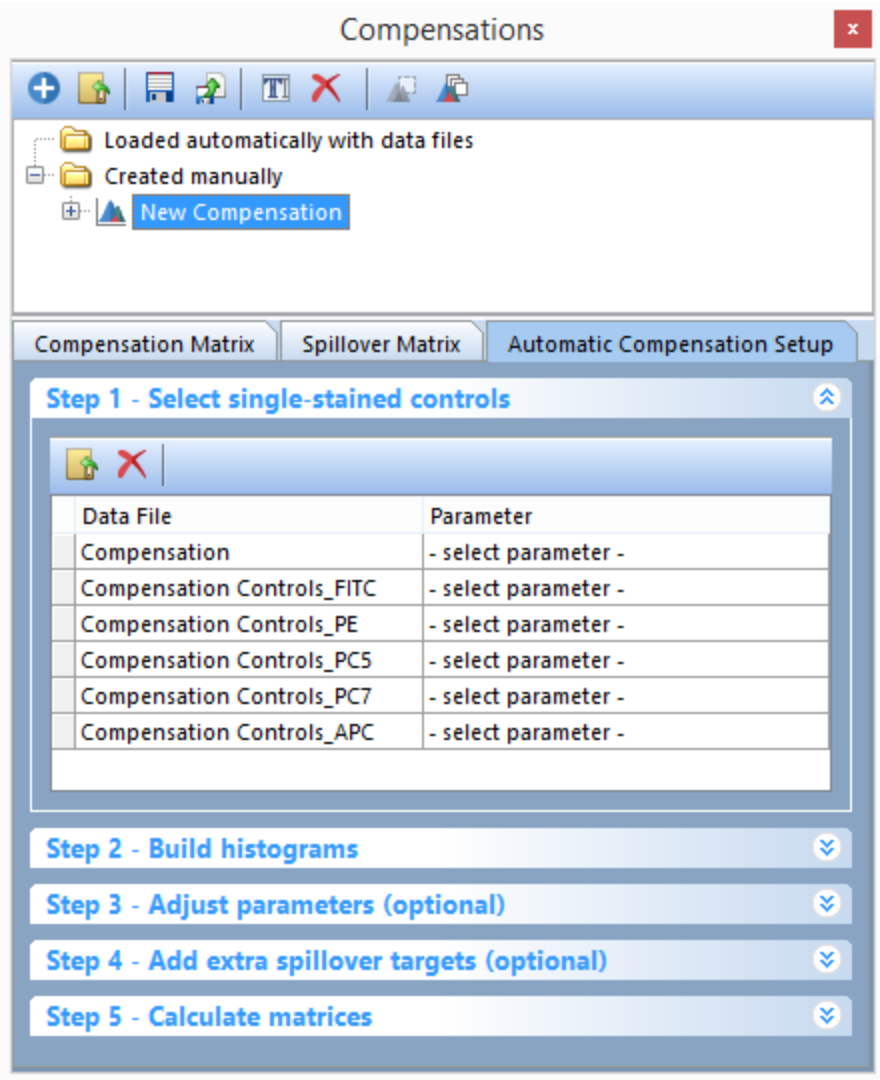

Figure T8.9 - Files have been added to Step 1 in the Automatic Compensation Setup. The Parameter column drop downs will be used to match the single stained sample data control files to the respective parameter for compensation.

8. Click on **-select parameter-** for the first file (Compensation Controls_Unstained Control.fcs) (Figure T8.10).

9. Select **Universal Negative** from the bottom of the **-select parameter-** drop down list (Figure T8.10).

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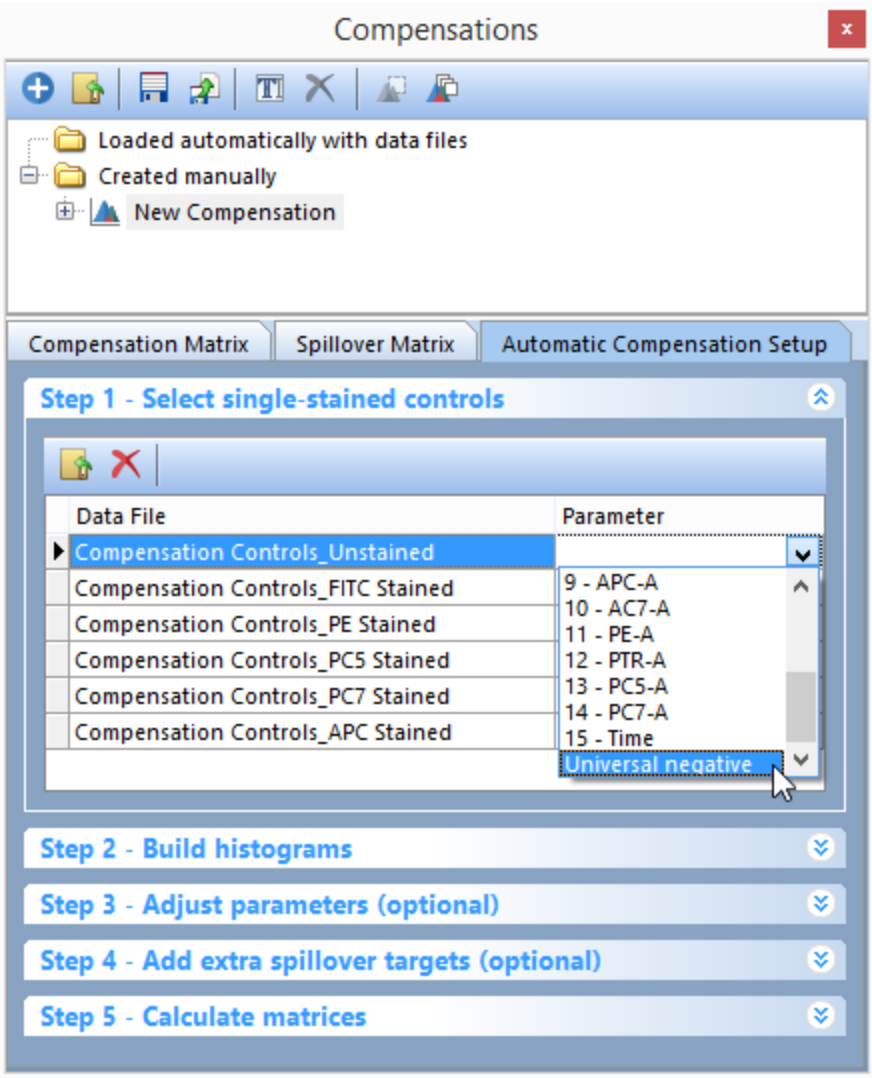

Figure T8.10 - Selecting the Universal negative parameter to match the Unstained Control.fcs file.

This will assign the Compensation Controls_Unstained Control.fcs file as the universal negative control for the rest of the single stained controls.

10. Click on **-select parameter-** for the second file (Compensation Controls_FITC Stained Control.fcs) (Figure T8.11).
11. Select **7- FITC-A** from the **-select parameter-** drop down list (Figure T8.11).

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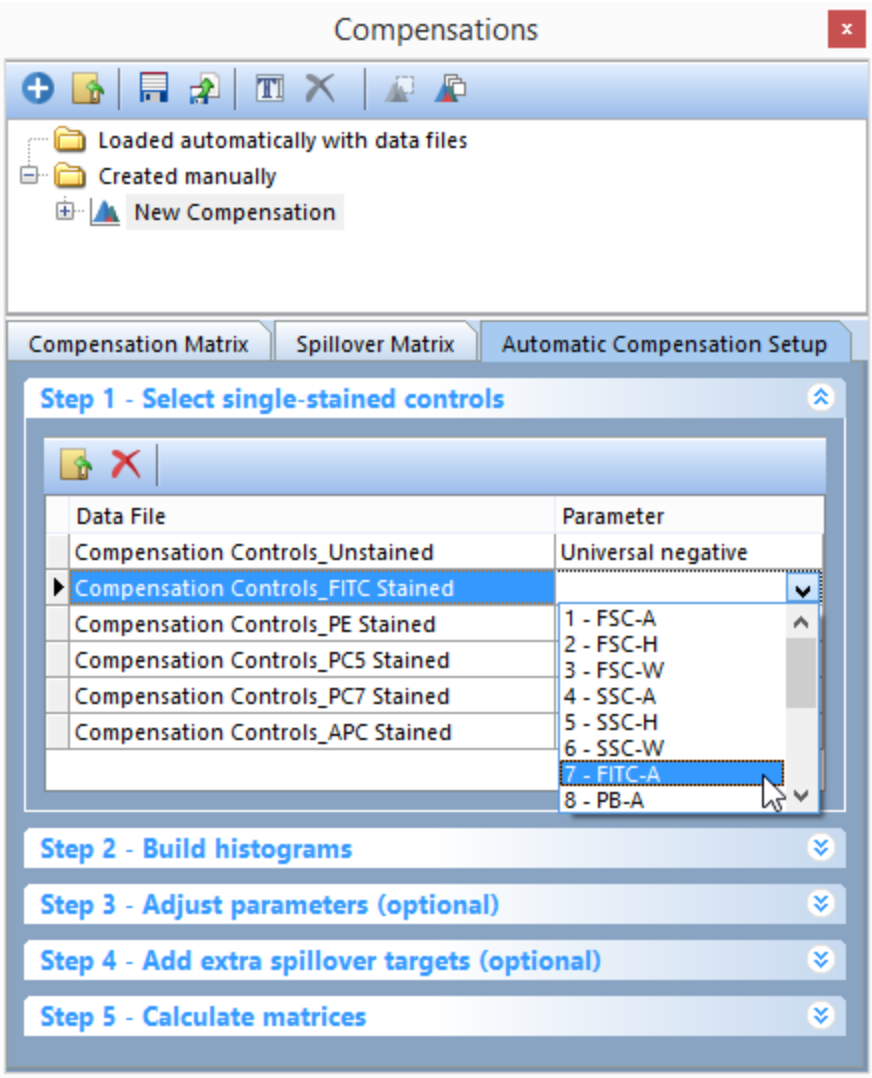

Figure T8.11 - Selecting the FITC-A parameter to match the FITC Stained Control.fcs file.

12. Repeat steps 11 and 12 for each of the single stained controls and parameters. For example, PE Stained Control.fcs should be matched with **11 -PE-A**. The **Step 1 - Select single-stained controls** window should look like figure Figure T8.12 when you are done.

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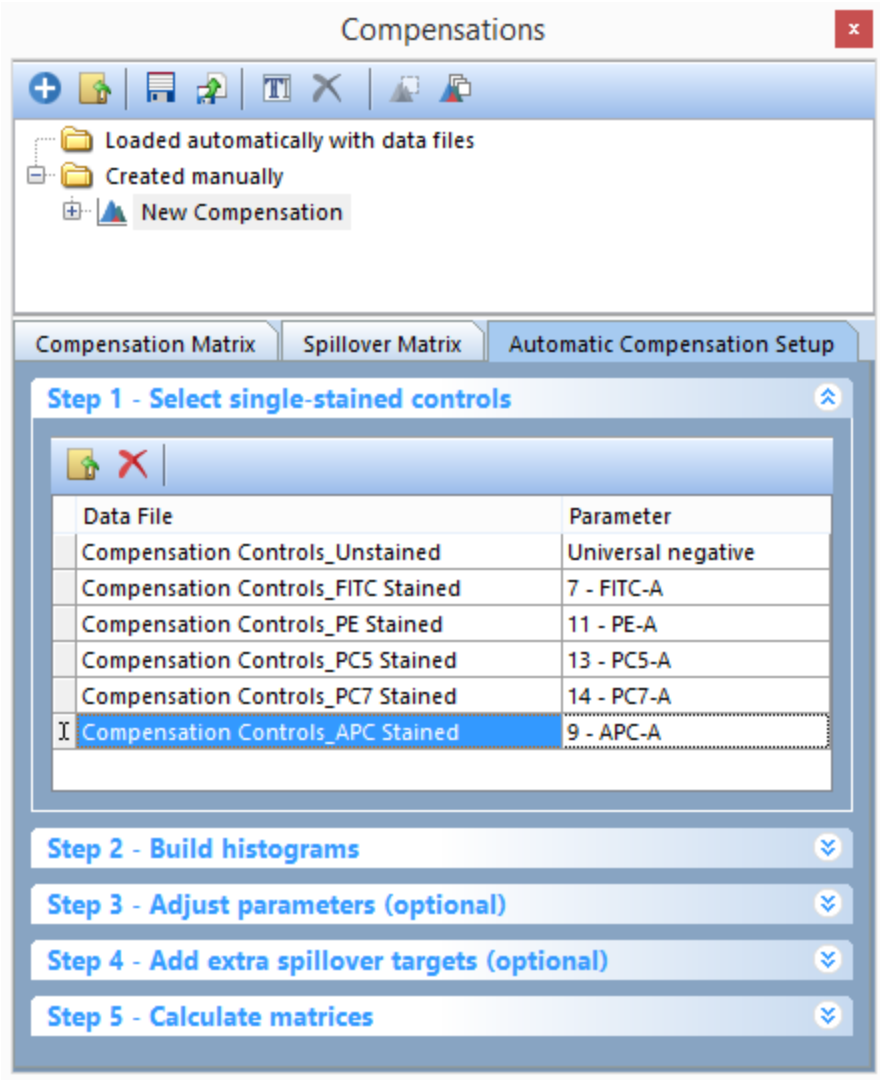

Figure T8.12 - All Parameters have been matched to their respective single stain control.fcs file.

13. Click on **Step 2 - Build histograms** to expand the build histograms window (T8.13).

14. Click on the **Build Histograms** button (T8.13).

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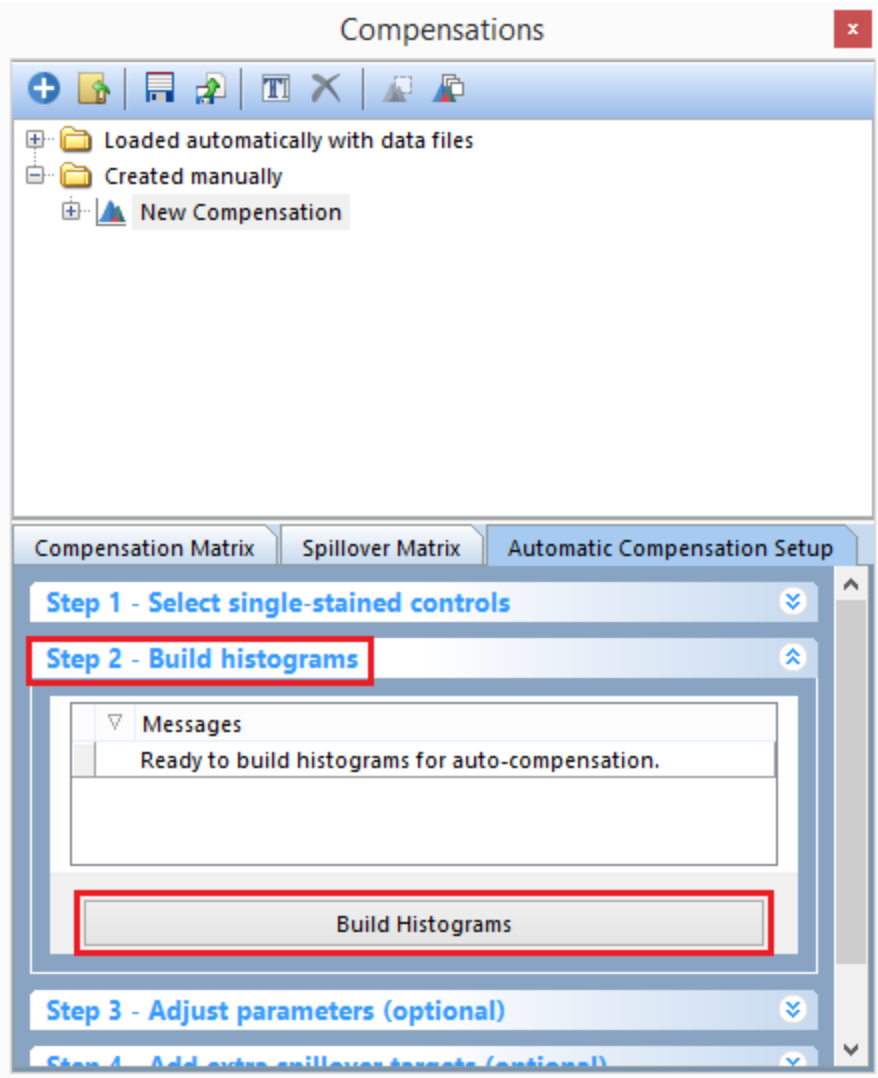

Figure T8.13 - The Build Histograms button will create a new page for scatter parameters and a new page containing a postive control and negative control histogram for each individual stain.

Six new pages will be created (Figure T8.14):

- **Scatter Plot**
- **FITC-A control**
- **PE-A control, PC5-A control**
- **PC7-A control**
- **APC-A control**

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The **Scatter Gate** page contains a plot based on the first two parameters of the data while the rest of the pages (single stain control pages) contain two histograms for each parameter compensated. The top histogram shows the positive population from the single stain control for the parameter while the bottom histogram shows the negative population from the universal negative control. A histogram marker has automatically been created for each positive and negative population and each histogram marker has also been automatically converted and linked to a gate. All of the histogram markers/gates are children of the **Scatter Gate** which means adjusting the **Scatter Gate** will cause the histogram markers/gates to update in real time.

We will now make some adjustments to the **Scatter Gate** to refine the automatically marked/gated single stain and negative populations.

To better appreciate plot adjustments, turn on the **Multiple Page** view by:

15. Click on the **View** tab→**Multiple Page**→**Two Pages (Vertical)**.
16. Select the **Scatter Plot** page for the left page.
17. Select the **FITC-A control** page for the right page.

your screen should now look as in Figure T18.14

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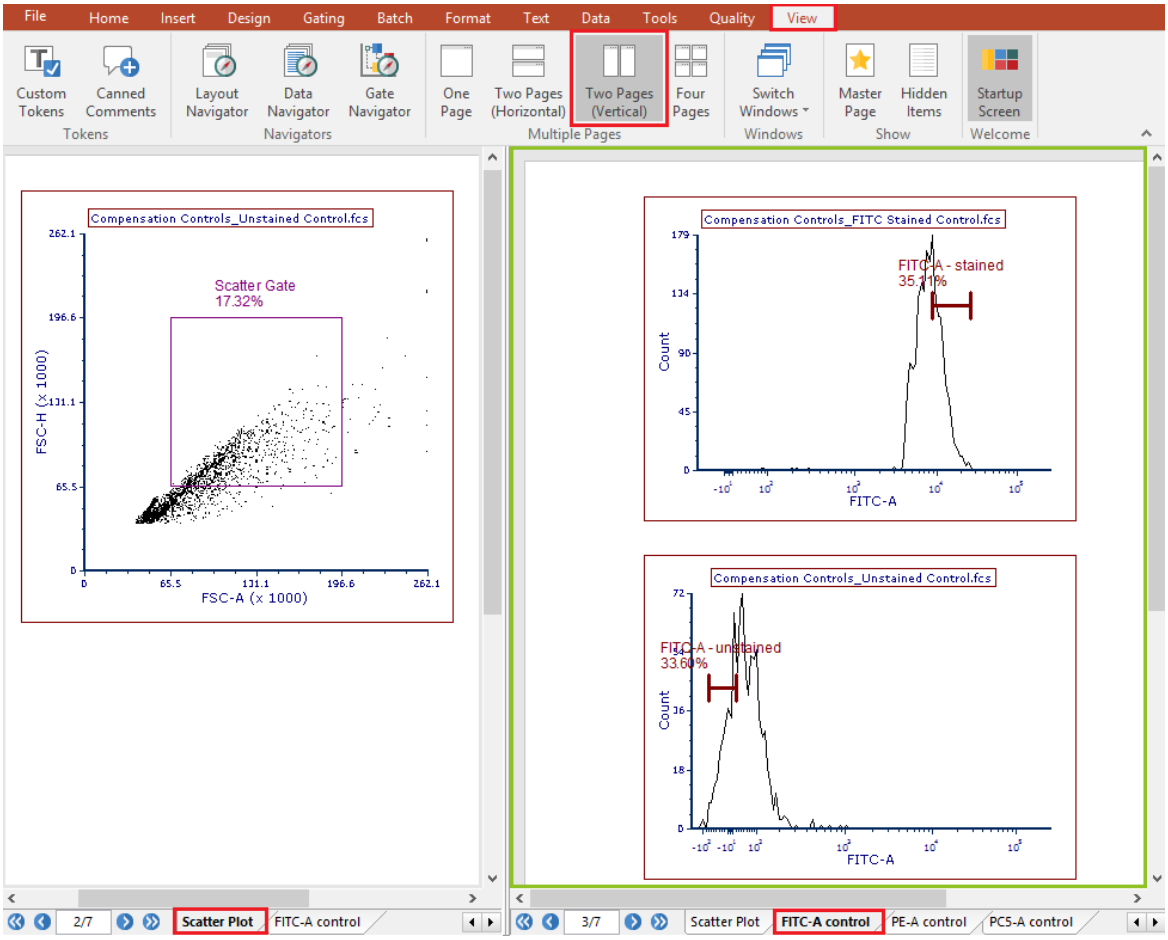

Figure T8.14 - Clicking on the build histograms button results in a Scatter Plot page and a new page with every single stained control.

In this instance FSC-H vs FSC-A are not the correct parameters for defining scatter so we will replace the **Scatter Gate** with a more appropriate one.

18. Change the X-Axis parameter on the Unstained Control dot plot (left page) to **SSC-H** (Figure T8.15)

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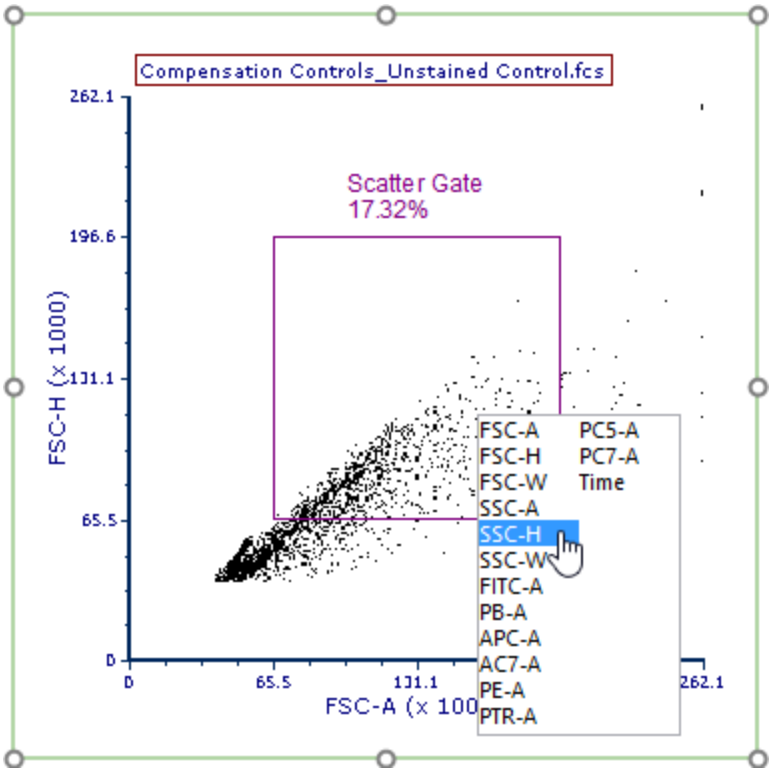

Figure T8.15 - Changing the X-Axsi parameter to SSC-H.

19. Choose the **Gating** tab→**Create Gates**→**Rectangle**.

20. Draw a gate around the beads population like the one in Figure T8.16 (left panel).

21. A **Create New Gate** dialog appears.

22. Chose the **Replace an existing gate** radio button (Figure T8.16).

23. Choose **Scatter Gate** from the bottom of the drop down list (Figure T8.16).

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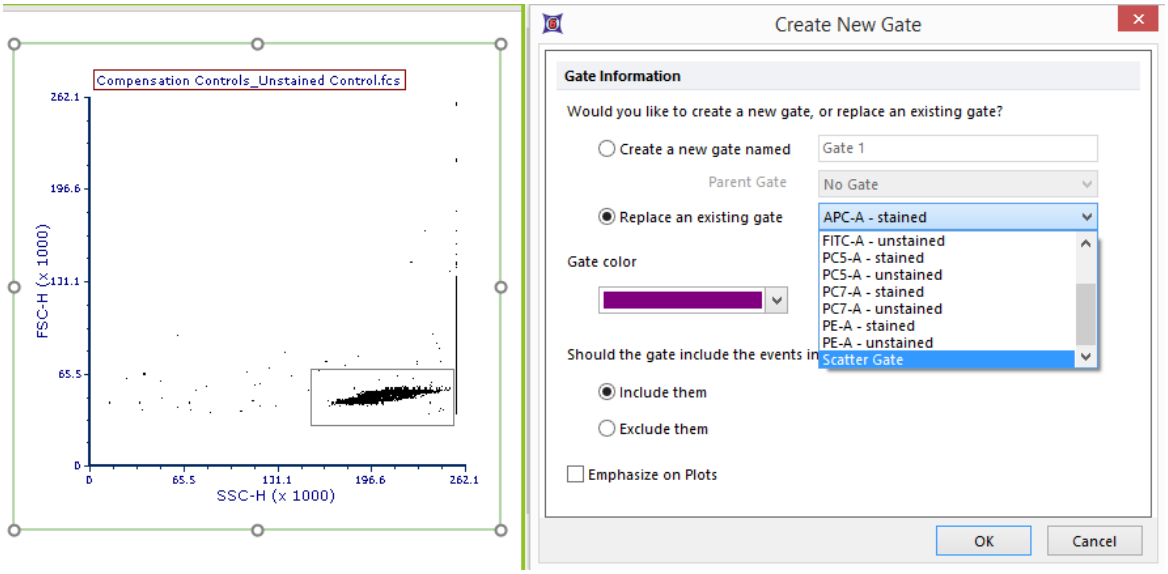

Figure T8.16 - Creating a gate around the bead population on the plot and choosing to replace the Scatter Gate with the newly created gate.

24. Click **OK**.

Notice that the original scatter gate has been replace with the new one you defined. It is convenient to replace the original scatter gate because all the single stained control histograms in the layout are already using that gate. If we had created a new gate, we would have had to apply that new gate to all the single stained control histograms. Notice that the subsequent single stain control histogram pages have updated to use this new gate.

User may also decide to refine markers position in the following pages of the layout (the ones containing single staining control histograms).

We will now complete the **Automatic Compensation Setup** in **Step 5**. **Steps 3** and **4** of the **Automatic Compensation Setup** are optional and we will come back to them at the end of this tutorial 1399.

25.Click on **Step 5- Calculate matrices** in the **Compensations** navigator.

26. Click on the **Calculate matrices** button (Figure T8.17).

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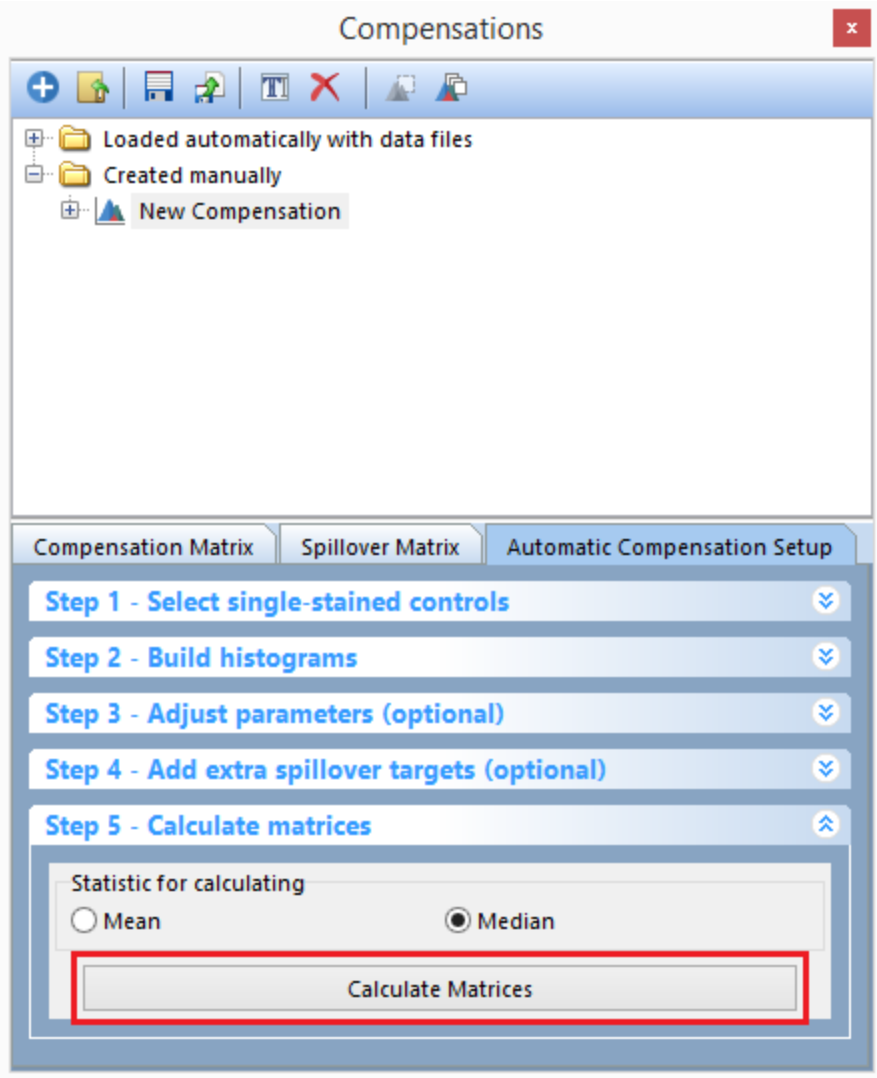

Figure T8.17 - Click the Calculate Matrices button to complete your automatic compensation setup.

All of the compensation and spillover matrix's will be automatically calculated and displayed in the **Compensation Matrix** and **Spillover Matrix** tabs of the **Compensations** navigator. The **Editor** view (highlighted in red) can be changed from **Matrix Editor** to **List Editor** to view the compensation definitions in matrix or list form (this may also be done in the **Spillover Matrix** tab). (Figure T8.18 below)

Note: Your values may differ slightly from figure T8.18 due to differences in how the scatter gate was manually setup in steps 20-25 of this tutorial.

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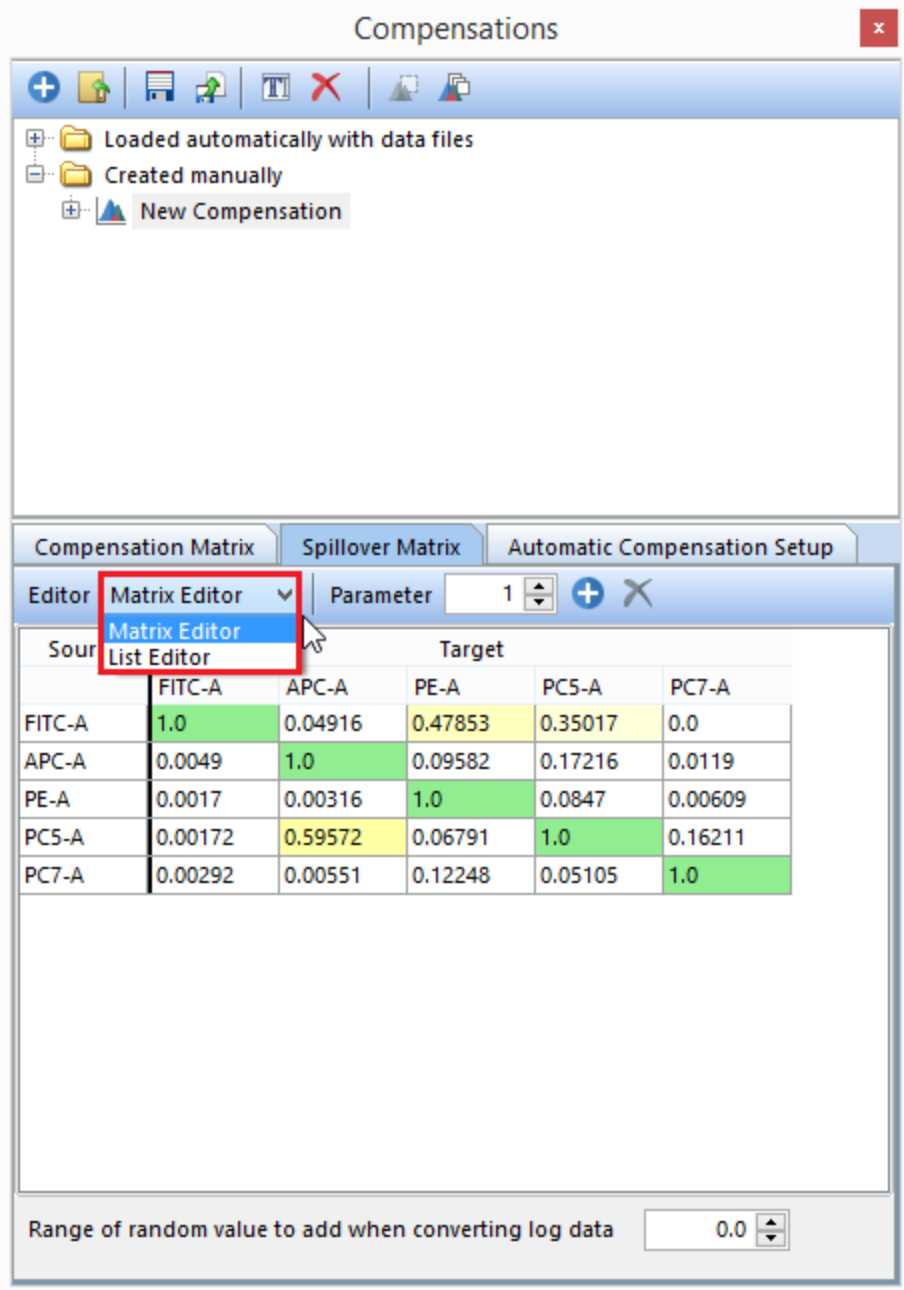

Figure T8.18 - The Spillover Matrix created from the Automatic Compensation Setup. The red box indicates you can change from Matrix Editor to List Editor view. The Spillover Matrix tab will display the spillover matrix for your compensation definition.

We will now switch back to a one page view and apply our new automatic compensation to a multicolor control.

27. Click on the **View** tab→**Multiple Page**→**One Page**.

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28. Click on the **Compensation Samples** page tab to move on the first page of the layout (Figure T8.6).

29. Click on the **New Compensation** compensation in the **Compensations** navigator to select it (Figure T8.19).

30. Drag and drop (by click and holding the left mouse button) the **New Compensation** on top of the Compensation Controls_All Colors.fcs plot in the upper right of the layout (see red arrow in Figure 8.19).

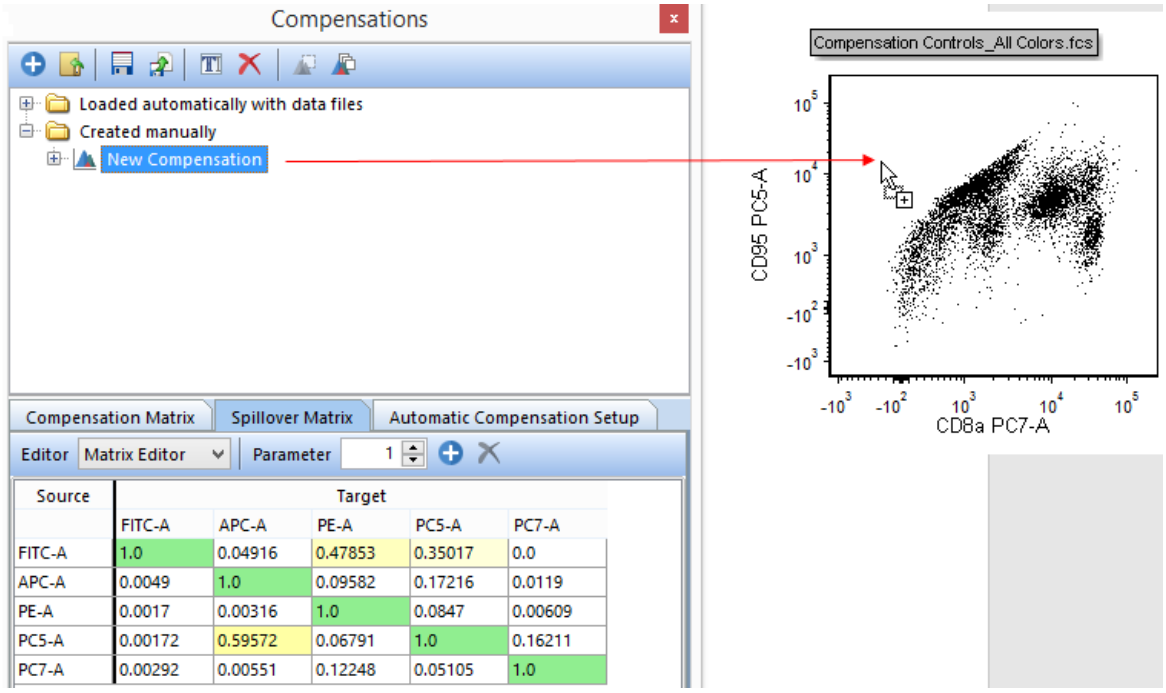

Figure T8.19 - Dragging the New Compensation definition from the Compensations navigator and dropping it on a plot will apply that compensation definition to the plot.

The plot will update and show the data properly compensated as in figure T8.20 below.

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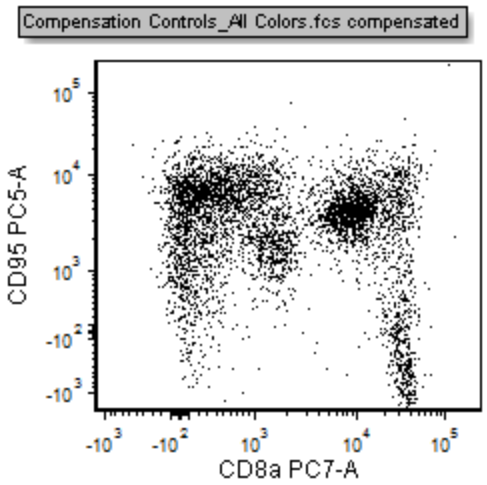

Figure T8.20 - The All Colors control plot with the compensation defined in the Automatic Compensation Setup feature applied.

Compensation and **Spillover** matrices may also be added anywhere in your layout or Microsoft Excel in a text box.

31. Click **Compensation Matrix** within the **New Compensation** definition to select it (FigureT8.21).

32. Drag the **Compensation Matrix** to an empty space in the layout and drop it (FigureT8.21).

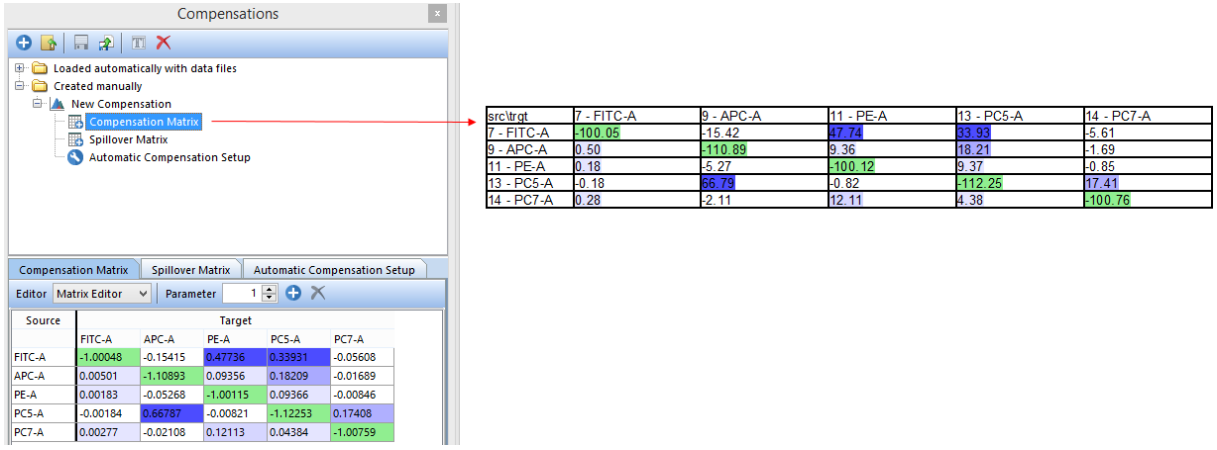

Figure T8.21 - Drag and drop the Compensation Matrix to a blank space in the layout (red arrow) to insert a text displaying all of the matrix values.

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The compensation matrix will now be displayed in a text box (FigureT8.21). Steps 32 and 33 can be repeated with a drag and drop to Microsoft Excel if you would like the matrices displayed there.

At this point the Automatic Compensation Setup is complete although **Step 3 - Adjust parameters (optional)** 638 and **Step 4 - Add extra spillover targets (optional)** 640 may also be used to refine your single stain populations (**Adjust parameters** 638) or to have other targets defined without defining a source (**Add extra spillover targets** 640). If you would like to learn more about these advanced features please see the **Automatic Compensation- Optional Steps 3 and 4** 1400 tutorial.

Please see the Manual Compensation 1377 tutorial or the Compensation 626 section of the manual for more details on compensation in FCS Express.

Note: *Sometimes when working with your own data digitization errors may occur as in Figure T8.22 below. This happens primarily if you are using log converted FCS 2.0 data. If you wish to correct for the digitization errors please see the brief Compensation Digitization Errors* 1421 *tutorial.*

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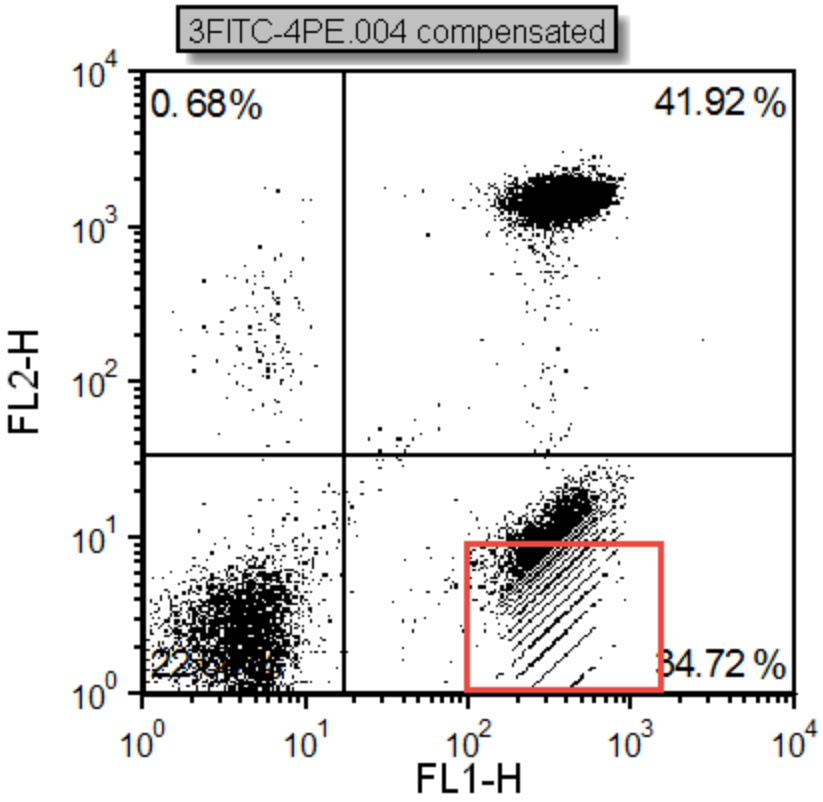

Figure T8.22 - Digitization errors after compensation can be seen in the red box. Please refer to the Compensation Digitization Errors tutorial to correct this type of error.

38.8.2.1 Automatic Compensation - Optional Steps 3 and 4

To adjust parameters and extra spillover targets:

Once you have completed Step 2 of the **Automatic Compensation Setup** you may proceed directly to Step 5 to calculate the matrices or you can go through Step3 and/or Step 4. This tutorial will walk you through a sample compensation setup that uses also Step 3 and Step 4. If you are not yet familiar with automatic compensation in FCS Express please complete the **Automatic Compensation** 1381 tutorial before starting this section.

- **Step 3 - Adjust parameters (optional)** 638 allows you to base your single stained controls on gates other than the default marker gates that were created during the automatic compensation setup.
    - **Adjust parameters** 638 is useful when you would like to refine the populations that are used for calculating compensation. For instance, if the **Automatic Compensation Setup** does not make a good

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estimate of the population or if you would like to use a population from another file, gate, gating hierarchy, or plot **Step 3** would prove very useful. Parameters may be adjusted or changed all together by dragging and dropping a plot or gate onto the parameter for the **Stained** and **Unstained** columns. If a gate is dropped the compensation definition will be based on that gate and plot it originated from (keep in mind any gating hierarchy you have defined with a gate will be used) . If a plot is dropped, the compensation definition will be based on the plot that you just dropped, with the gating being unchanged. Dragging and dropping new plots, files, or gates in this section will allow you to override the automatic setup and use gates and plots of your choice.

- **Step 4 - Add extra spillover targets (optional)** will allow you to have other fluorescence targets defined without defining a source fluorescence.
  - **Adding extra spillover targets** is useful if you would like to have an idea of what spillover would look like if an extra parameter was added in future experiments. To add an extra spillover target simply check the box for the extra target you would like to add. Note. Extra spillover targets will only appear for parameters that were acquired with the data.

The layout that will be used for this section of the tutorial is **AutoCompAdditionalSteps.fey** and it can be found in the Tutorial Sample Data archive. In this layout an automatic compensation setup definition (named **New Automatic Compensation)** has already been created using the standard default setup described in the previous section. The layout contains 4 pages as follows:

- **Gating Strategy and Sample** page: The Gate View on this page shows the gating strategy that was created automatically by the compensation setup (purple gates) and the one that we would like to use (red and blue gates). The plots are arranged starting from the top left to show how the gating hierarchy has been applied. Follow the green arrows next to each plot to see how the gates have been applied and defined. The plot labeled **SAMPLE COMPENSATION** has the **New Automatic Compensation** definition applied to it. The plot off the page to the right is labeled **Data for New Compensation Gates** and has no compensation applied to it. The **Data for New Compensation Gates** plot has gates drawn on the PE positive, PerCPCy5-5 positive, and negative populations. These gates will be used later in the tutorial for the adjust parameters steps.
- **Scatter Plot** page: This page was created by the Automatic Compensation Setup tool and displays the stained and unstained populations used to calculate the compensation matrix.

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- **CD 62L YG PE-A control** page: This page was created by the Automatic Compensation Setup tool and displays the current gate used to calculate the compensation matrix for the PE parameter.
- **CD 4 PerCP-Cy5-5-A control** page: This page was created by the Automatic Compensation Setup tool and displays the stained and unstained populations used to calculate the compensation matrix for the PerCPCy5-5 parameter.

In the following steps we will:

- Adjust the parameters to define new gates for the single stained and unstained controls.
- Change the gating hierarchy to make the Scatter Gate (defined by the Automatic Compensation Setup) a child of manually defined gates.
- Add an extra spillover target to view the potential spillover of another target.

1. Select **File** tab→**Open** (Figure T3.1 1237).
2. Open the layout **AutoCompAdditionalSteps.fey** found in the Tutorial Sample Data archive 1210 (see above for a description of the layout 1401).
3. Click on the **Created manually** folder→**New Automatic Compensation**→**Automatic Compensation Setup** in the **Compensations** navigator (figure T8.22 red box).
4. Click on the Add data for compensation button, , in the **Automatic Compensation Setup**→**Step 1 - Select single-stained controls** window (Figure T8.7).
5. Click on the **Data List** tab (Figure T8.8).
6. Multiple-select the following files in the Data List (Figure T8.8):
   - Specimen_001_PE_CD62L.fcs
   - Specimen_001_PerCP Cy5-5 CD4.fcs
   - Specimen_001_PE_unstained.fcs
7. Chose the parameter for each data file based on the table below:

Data File	Parameter
Specimen_001_PE_CD62L.fcs	9 - YG PE-A

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Specimen_001_PerCP Cy5-5 CD4.fcs	10 - PerCP-Cy5-5-A
Specimen_001_PE_unstained.fcs	Universal Negative

8. Click on **Step 2 - Build histograms** to expand the build histograms window (T8.13).
9. Click on the **Build Histograms** button (T8.13).
10. A window asking for deleting the layout pages and gates previously created opens. Click **Yes**.

As in the previous tutorial section we will now make some adjustments to the **Scatter Gate** to refine the automatically marked/gated single stain and negative populations.

In this instance FSC-H vs FSC-A are not the correct parameters for defining scatter so we will replace the **Scatter Gate** with a more appropriate one.

11. Select the **Scatter Plot** page and change the X-Axis parameter of the displayed dot plot to **SSC-H**.
12. Choose the **Gating** tab→**Create Gates**→**Rectangle**.
13. Draw a gate around the cell population like the one in the Figure below (Figure T8.22a, left panel).
14. In the **Create New Gate** dialog that appears chose the **Replace an existing gate** radio button and select **Scatter Gate** from the bottom of the drop down list (Figure T8.22a, right panel).

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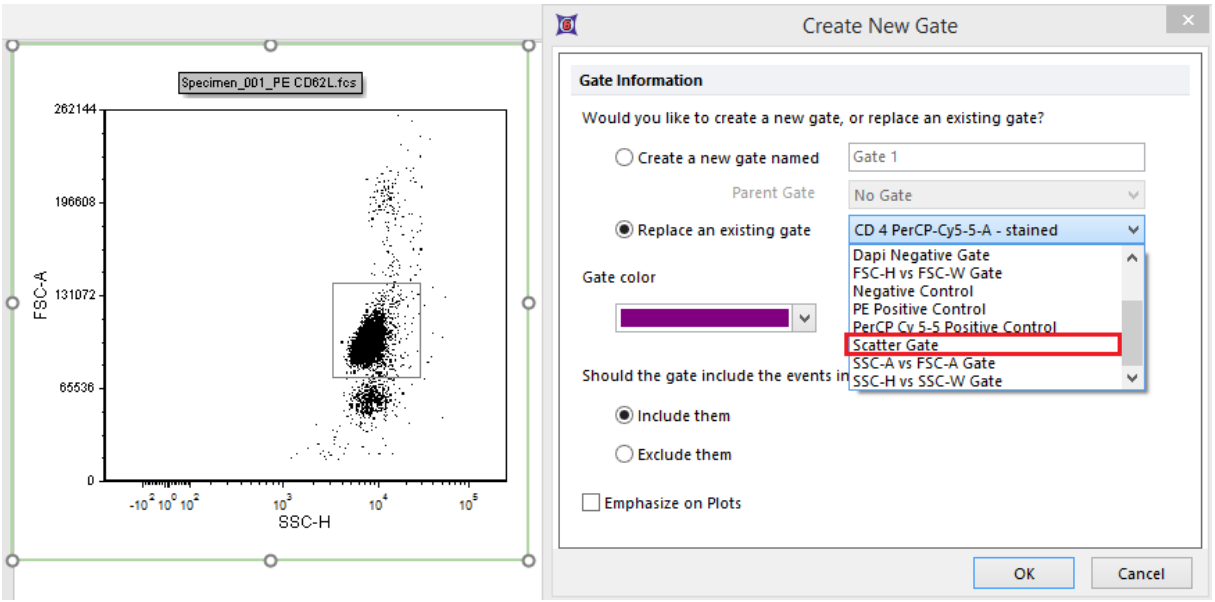

Figure 8.22a - A new Scatter Gate is drawn and used as replacement for the original one.

15. Click **OK**.

Notice that the original scatter gate has been replace with the new one you defined. It is convenient to replace the original scatter gate because all the single stained control histograms in the layout are already using that gate. If we had created a new gate, we would have had to apply that new gate to all the single stained control histograms. Notice that the subsequent single stain control histogram pages have updated to use this new gate.

User may also decide to refine markers position in the following pages of the layout (the ones containing single staining control histograms).

16. Click on **Step 3- Adjust parameters (optional)** (figure T8.22b).

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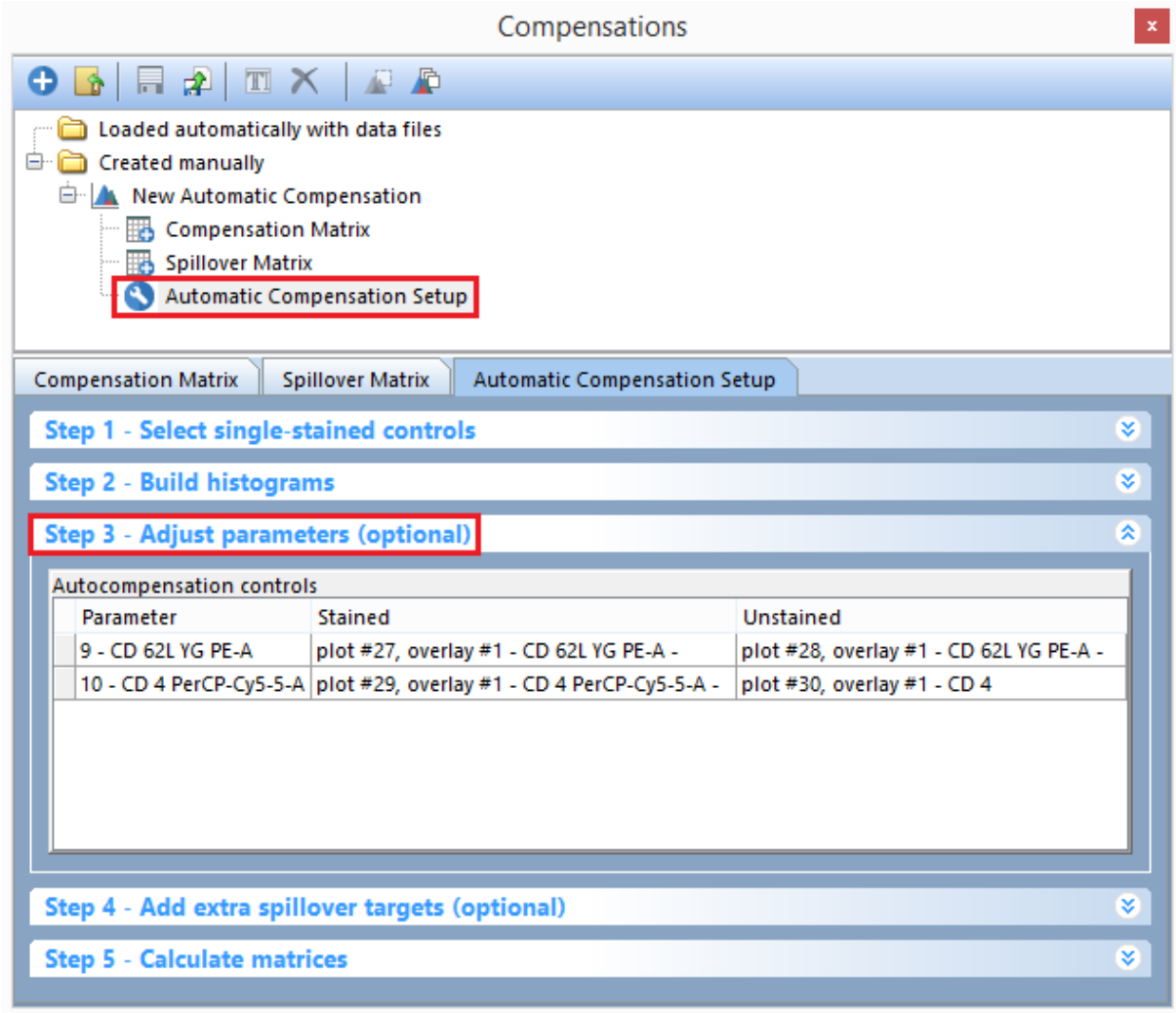

Figure T8.22b - Selecting the Automatic Compensation setup and Step 3 for the New Automatic Compensation definition.

We will now replace the gates that were defined in the Automatic Compensation Setup with gates we have defined manually. These manually defined gates represent the following:

- A gating hierarchy has been defined with all of the FSC and SSC parameters and a Dapi Negative gate. This hierarchy is used to exclude debris and other unwanted events.
- **Negative Control** Gate: This gate is a child of the **Dapi Negative Gate** hierarchy. It defines a negative or unstained control population.
- **PE Positive Control** gate: This gate is a child of the **Dapi Negative Gate** hierarchy. It defines a PE single color control only population.
- **PerCP CY5-5 Positive Control** gate: This gate is a child of the **Dapi Negative Gate** hierarchy. It defines a PerCP CY5-5 single color only population.

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17. Click and hold on the **Negative Control** gate in the gate view (Figure T8.23).
18. Drag the **Negative Control** gate onto the **Unstained** column in the **CD62L YG PE-A** row and drop it here.
19. Click and hold on the **Negative Control gate** in the gate view.
20. Drag the **Negative Control gate** onto the **Unstained** column in the **CD4 PerCP-Cy5-5-A** row and drop it here.

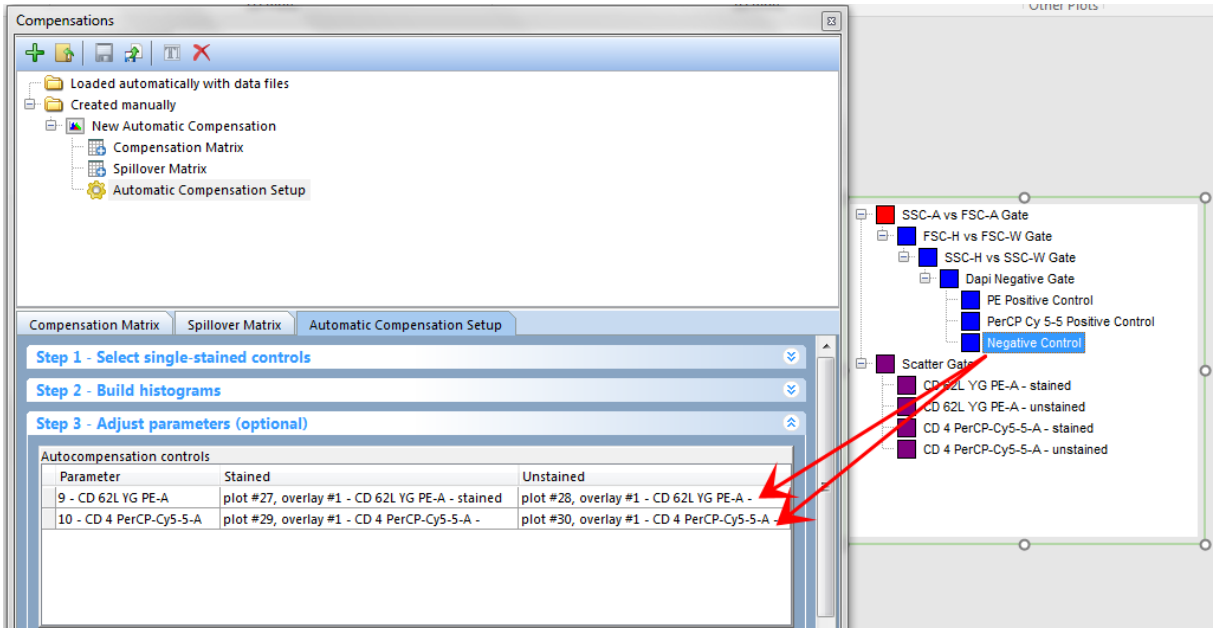

Figure T8.23 - Dragging the Negative Control gate from the gate view to the Unstained column for both parameters.

Now the **Step3 - Adjust Parameters (Optional)** section should look as in Figure T823b.

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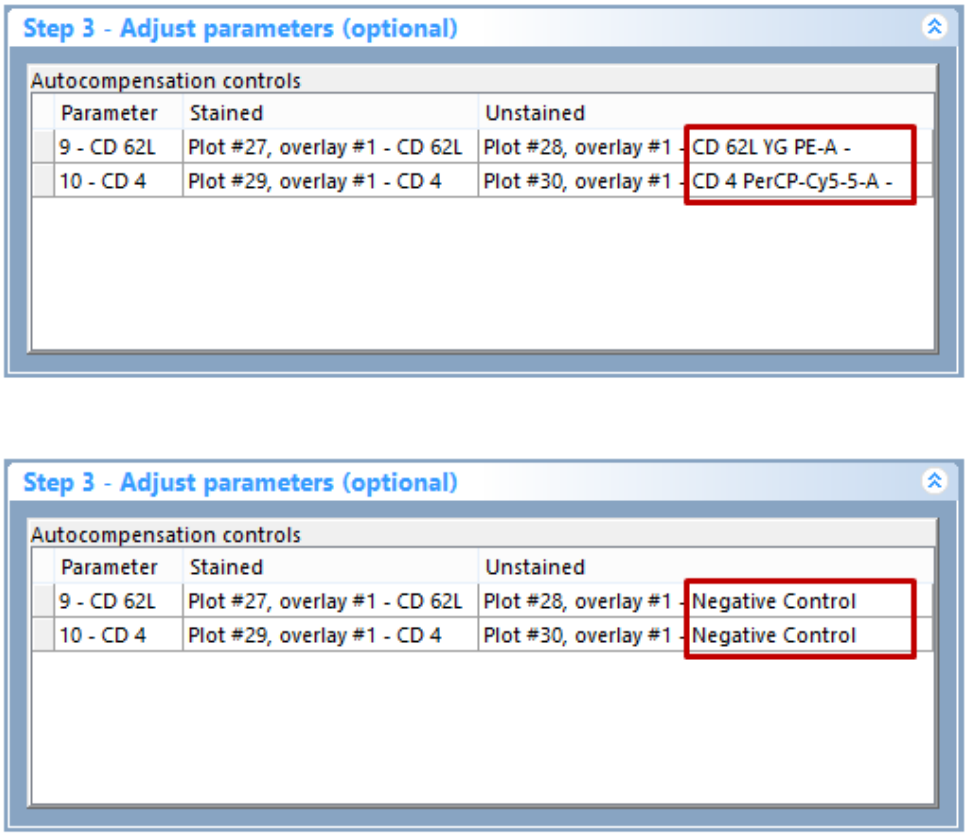

Figure T8.23b The Step3 section after the drag&drop

We previously dragged and dropped the **Negative Control** gate from the **Gate View**. We will now use a plot a source for the drag and drop (Figure T8.24).

21. Click and hold on the **PE Positive Control** gate in the **Data for New Compensation Gates plot** (off the right hand side of page).

22. Drag and drop it onto the **Stained** column in the **CD62L YG PE-A** row and drop it here.

23. Click and hold on the **PerCPCY5-5 Positive Control** gate in the **Data for New Compensation Gates** plot (off the right hand side of page) (Figure T8.24).

24. Drag and drop it onto the **Stained** column in the **CD4 PerCP-Cy5-5-A** row and drop it here.

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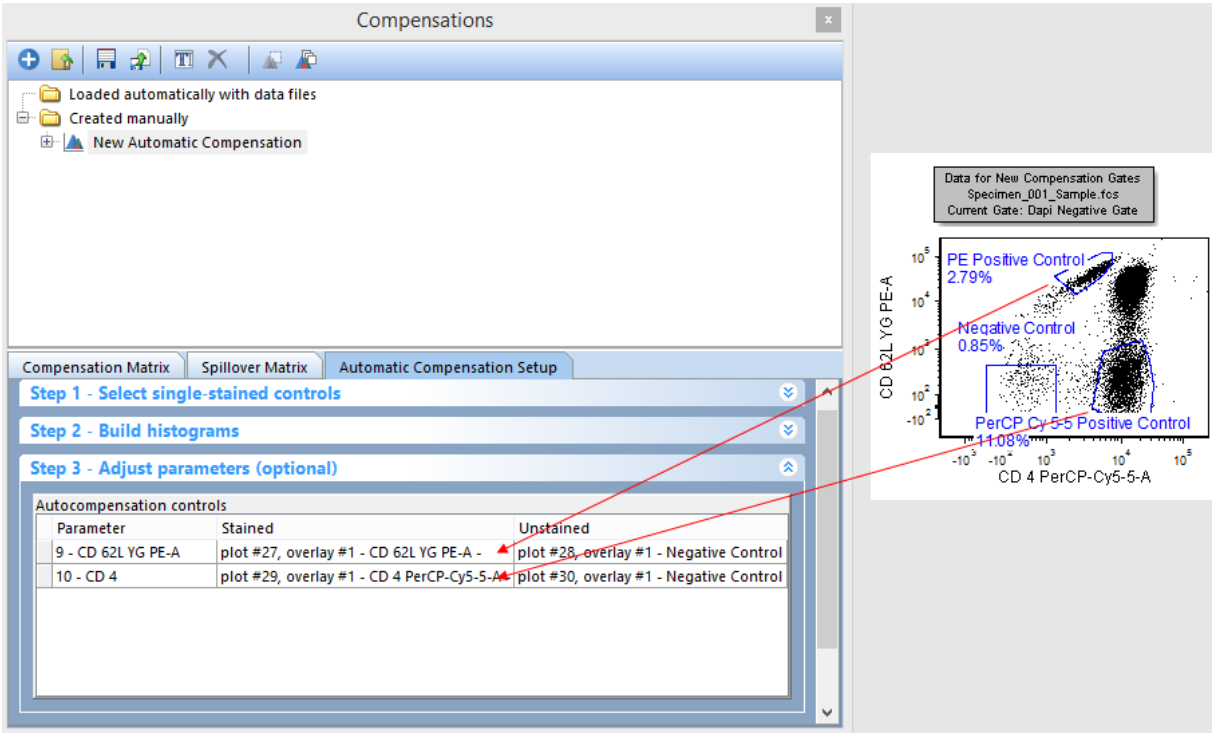

Figure T8.24 - Dragging and dropping gates from a plot to the Stained column in step 3 for each parameter.

Step 3 - Adjust parameters (optional) will look like figure T8.25 below when you are done.

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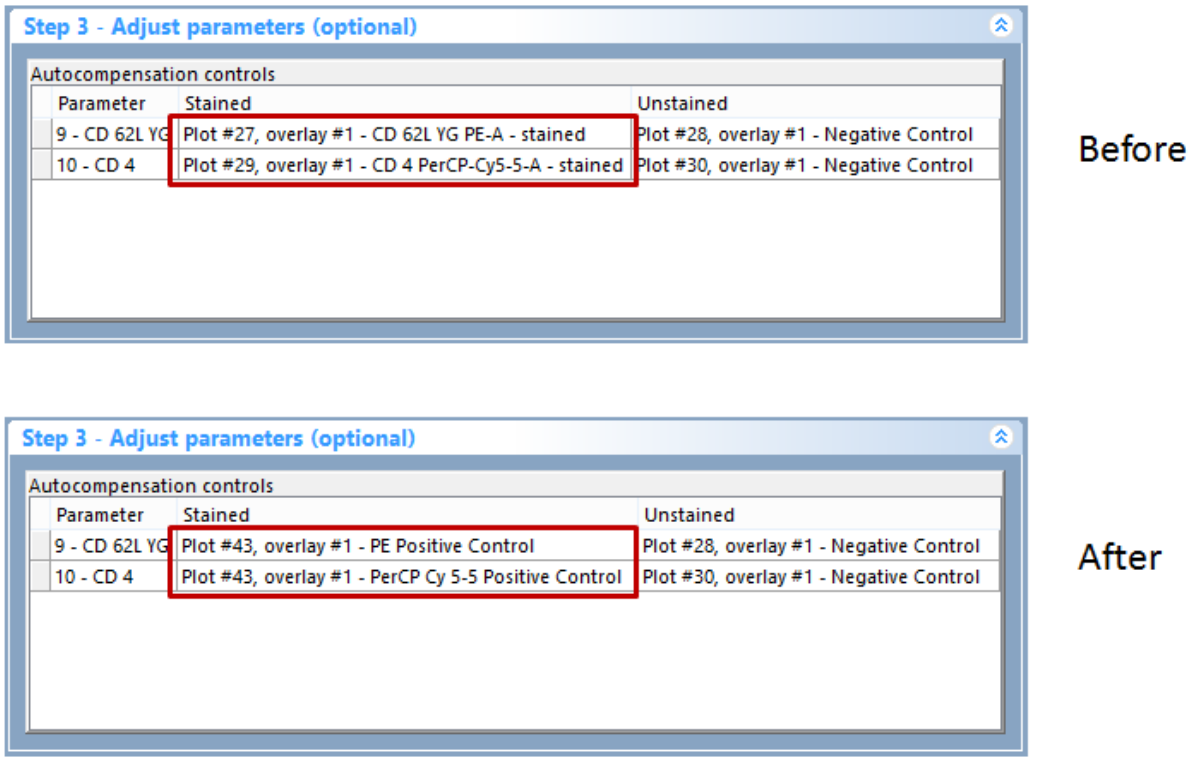

Figure T8.25 - Step 3 - Adjust parameters (optional) after dragging and dropping gates from the gate view and plots onto the stained and unstained population columns.

25. Click now on **Step 5 - Calculate matrices**.

26. Click the **Calculate Matrices** button.

Notice that the spillover matrix for the New Automatic Compensation definition has changed (see Figure T8.26 below) to reflect the new gating changes. You can drag and drop any gate or an entire plot to the **Stained** and **Unstained** columns in this fashion to customize your **Automatic Compensation Setup**.

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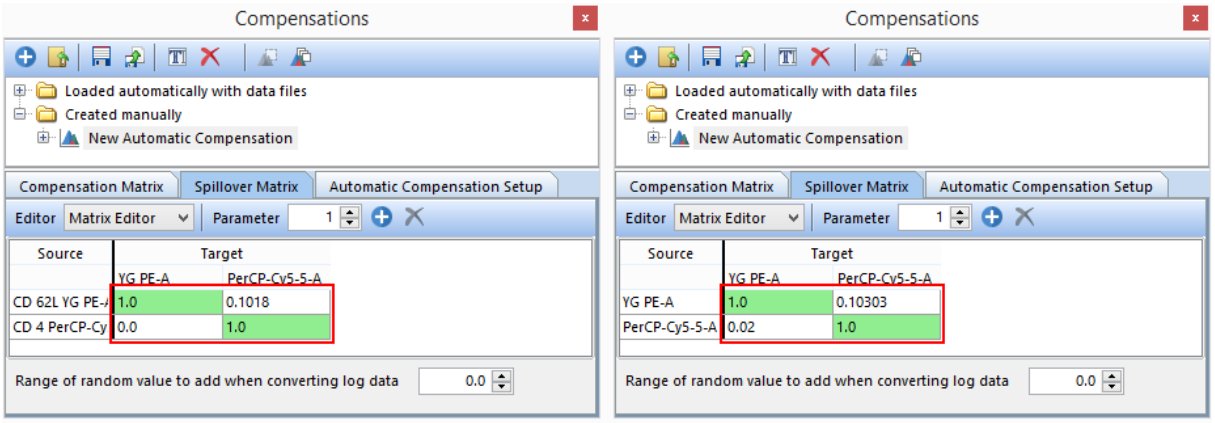

Figure T8.26 - The original Automatic Compensation Setup definition (left) compared to the Automatic Compensation Setup definition (right) that is based on a gating hierarchy and manually defined positive and negative controls.

Another useful trick is to make the Scatter Gate and its children (defined by the Automatic Compensation Setup) children of another gate that you have defined. In the following steps we will make the Scatter Gate a child of the Dapi Negative gate. When the matrix is recalculated the manually entered gating hierarchy for debris exclusion will be taken into account with the Automatic Compensation Setup. We will first close the layout and open it again to reset the changes we have made to the compensations.

27. Select **File** tab→**Close**. (Do not save any changes).

28. Select **File** tab→**Open**.

29. Open the layout **AutoCompAdditionalSteps.fey** found in the Tutorial Sample Data archive.

30. Click on the **Created manually** folder→**New Automatic Compensation**→**Automatic Compensation Setup** in the **Compensations** navigator (figure T8.22 red box).

31. Click on the Add data for compensation button, , in the **Automatic Compensation Setup**→**Step 1 - Select single-stained controls** window (Figure T8.7).

32. Click on the **Data List** tab (Figure T8.8).

33. Multiple-select the following files in the Data List (Figure T8.8):
    - Specimen_001_PE_CD62L.fcs

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- Specimen_001_PerCP Cy5-5 CD4.fcs
- Specimen_001_PE_unstained.fcs Click and hold on the **Scatter Gate** in the Gate View

33. Chose the parameter for each data file based on the table below:

Data File	Parameter
Specimen_001_PE_CD62L.fcs	9 - YG PE-A
Specimen_001_PerCP Cy5-5 CD4.fcs	10 - PerCP-Cy5-5-A
Specimen_001_PE_unstained.fcs	Universal Negative

34. Drag the **Scatter Gate** on top of the **Dapi Negative Gate** and drop it here (Figure T8.27).

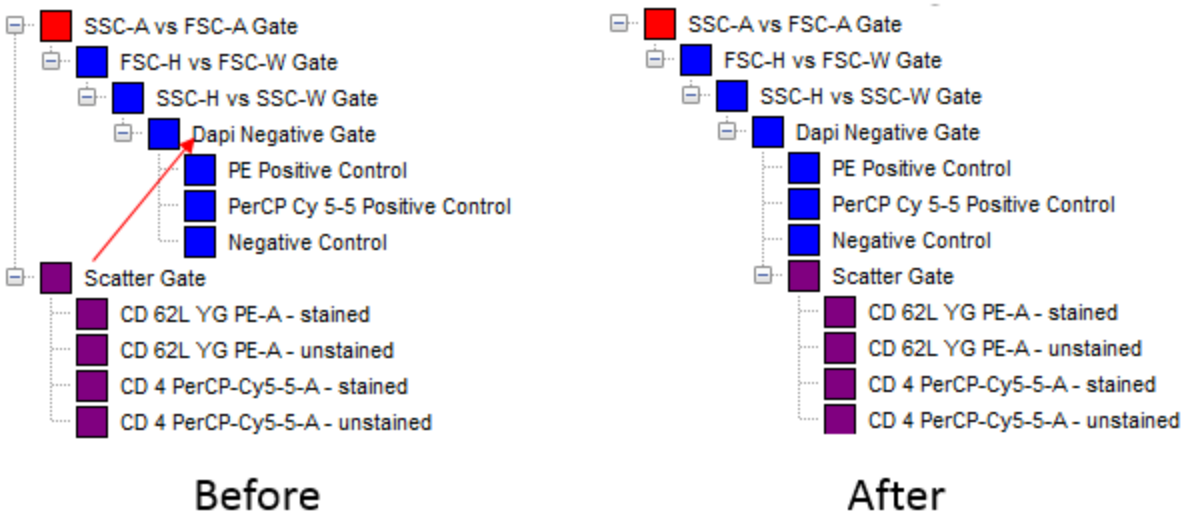

Figure T8.27 - Dragging and dropping the Scatter Gate onto the Dapi Negative Gate to create a new gating hierarchy

Click on **Step 5 - Calculate matrices**.

35. Click on **Step 2 - Build histograms** to expand the build histograms window (T8.13).

36. Click on the **Build Histograms** button (T8.13).

37. A window asking for deleting the layout pages and gates previously created opens. Click **Yes**.

38. Click the **Calculate Matrices** button.

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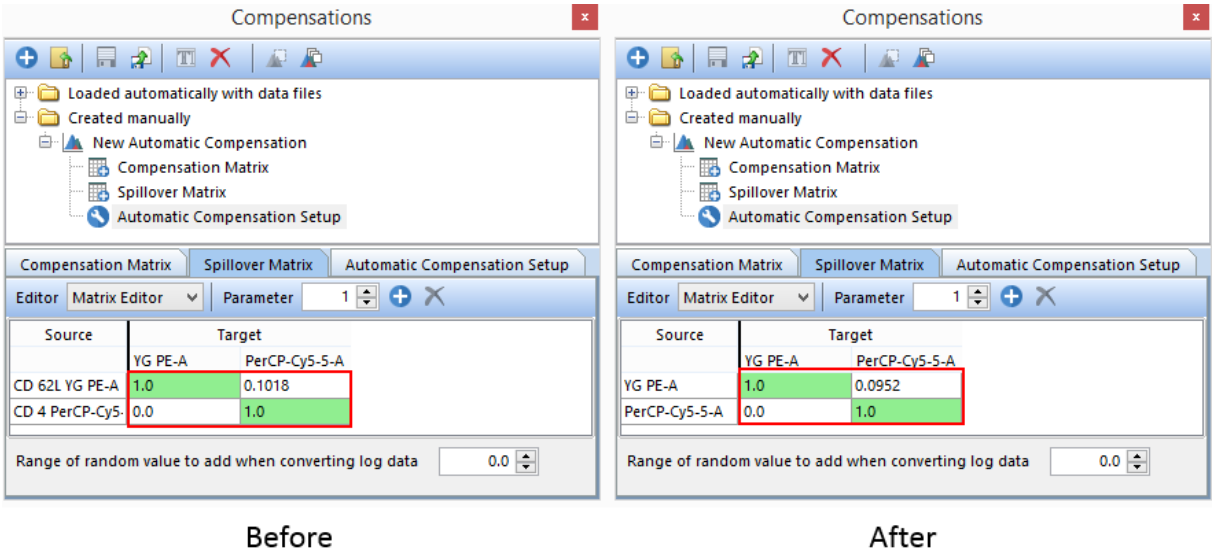
Notice that the spillover matrix for the New Automatic Compensation definition has changed (see Figure T8.28 below) to reflect the new gating changes. This is because the plots that were created by the Automatic Compensation Setup were gated on the **Scatter Gate** so by moving the **Scatter Gate** in the gating hierarchy the statistics based on these plots were automatically updated.



Before After

Figure T8.28 - The original Automatic Compensation Setup definition (left) compared to the Automatic Compensation Setup definition (right) that is based on the new gating hierarchy of the Scatter Gate.

We will now use **Step 4 - Add extra spillover targets (optional)** to determine the theoretical spillover for the FITC parameter.

39. Click on **Step 4 - Add extra spillover targets (optional)** to expand the window.

40. Check the **FITC-A** check box (Figure T8.28).

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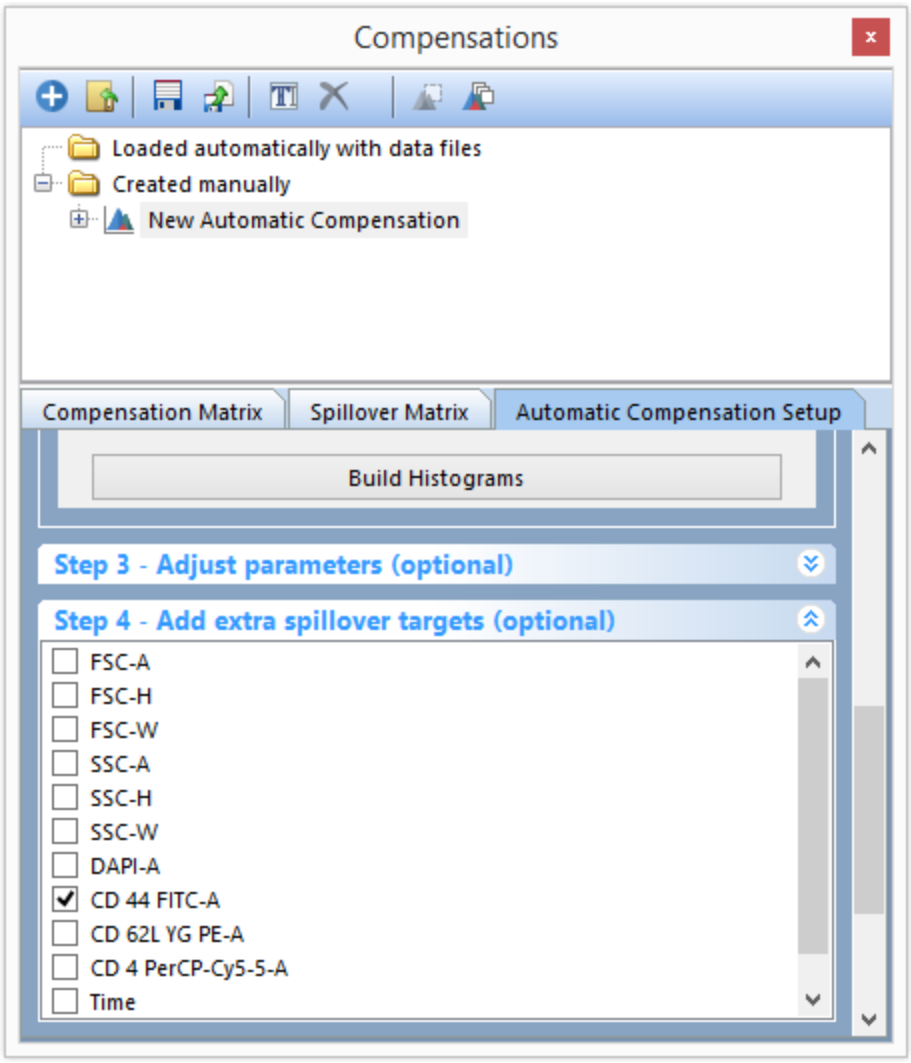

Figure T8.28 - Adding an extra spillover target in Step 4.

41. Click the **Calculate Matrices** button in **Step 5 - Calculate matrices**.

The **Spillover Matrix** tab will now display all of your original parameters along with the **FITC-A** parameter that you selected in **Step 4- Add extra spillover targets (optional)** (Figure T8.29). The column for **FITC-A** in the matrix is displaying the theoretical compensation needed against all other targets.

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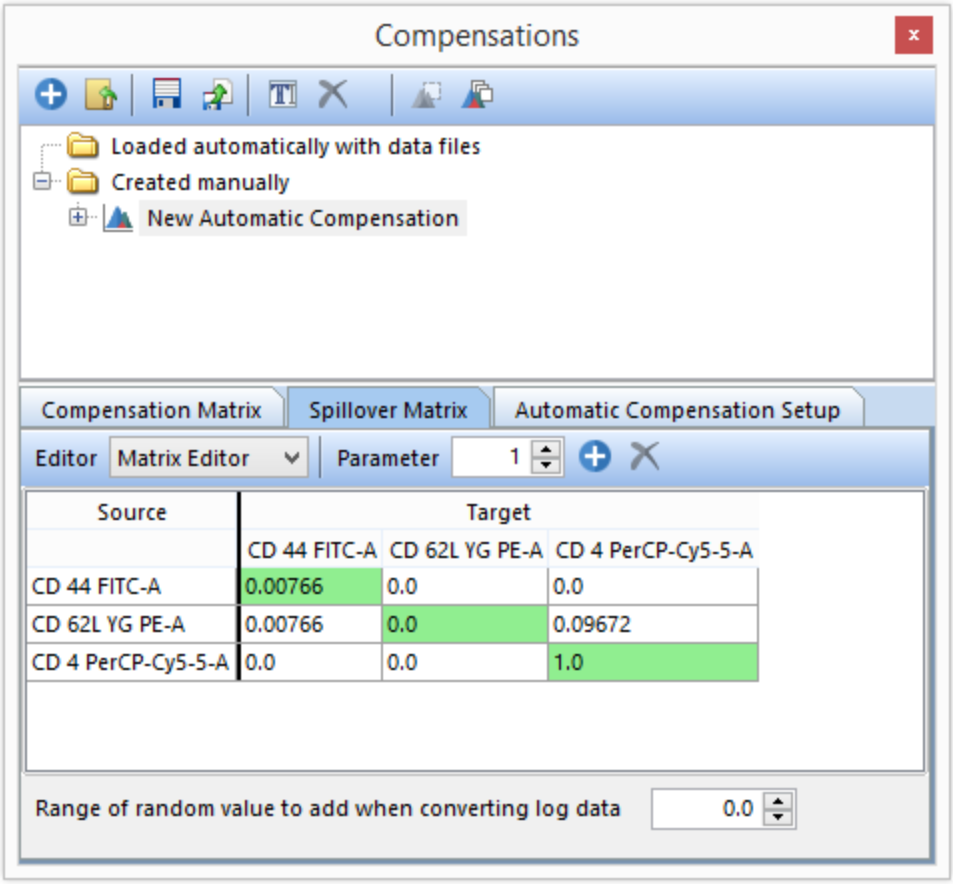

Figure T8.29 - The compensation matrix displaying the extra spillover target that was added. The compensation values for CD 44 FITC-A vs other sources/targets is the theoretical value.

This brings us to the end of the Automatic Compensation Optional Steps 3 and 4 tutorial. Please see the Manual Compensation 1377 tutorial or the Compensation 626 section of the manual for more details on compensation in FCS Express.

38.8.2.2 Automatic Compensation and Batch Processing

In FCS Express, you may define a post-acquisition compensation matrix using single-stained controls, apply it to your experimental samples, and batch process your results to Microsoft PowerPoint, PDF, and Word, all within a single layout. There is no need to export and import compensation into separate layouts.

In this section, we will:

- Change our default Options to use a post-acquisition compensation matrix as default
- Set an automatic compensation as default for the entire layout
- Insert plots of experimental files in the same layout

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- Batch process the results to Microsoft Office PowerPoint

We'll begin with a layout in which an automatic compensation was already defined, using single-stain controls.

1. Select **File** tab→**Open**.
2. Open the layout **AutoCompWithBatchProcess.fey** from the Tutorial Sample Data archive 1210.

An automatic compensation has already been defined in this layout, according to Steps 1-27 of this section 1381 of this tutorial. We will now set this compensation as default for the entire layout.

3. Select **File** tab→**Options**.
4. Select **Compensation Default, then FCS File** from the **Default Compensation to Use** dropdown menu (Fig. 1, ❶). Learn more about Compensation default Options 645.

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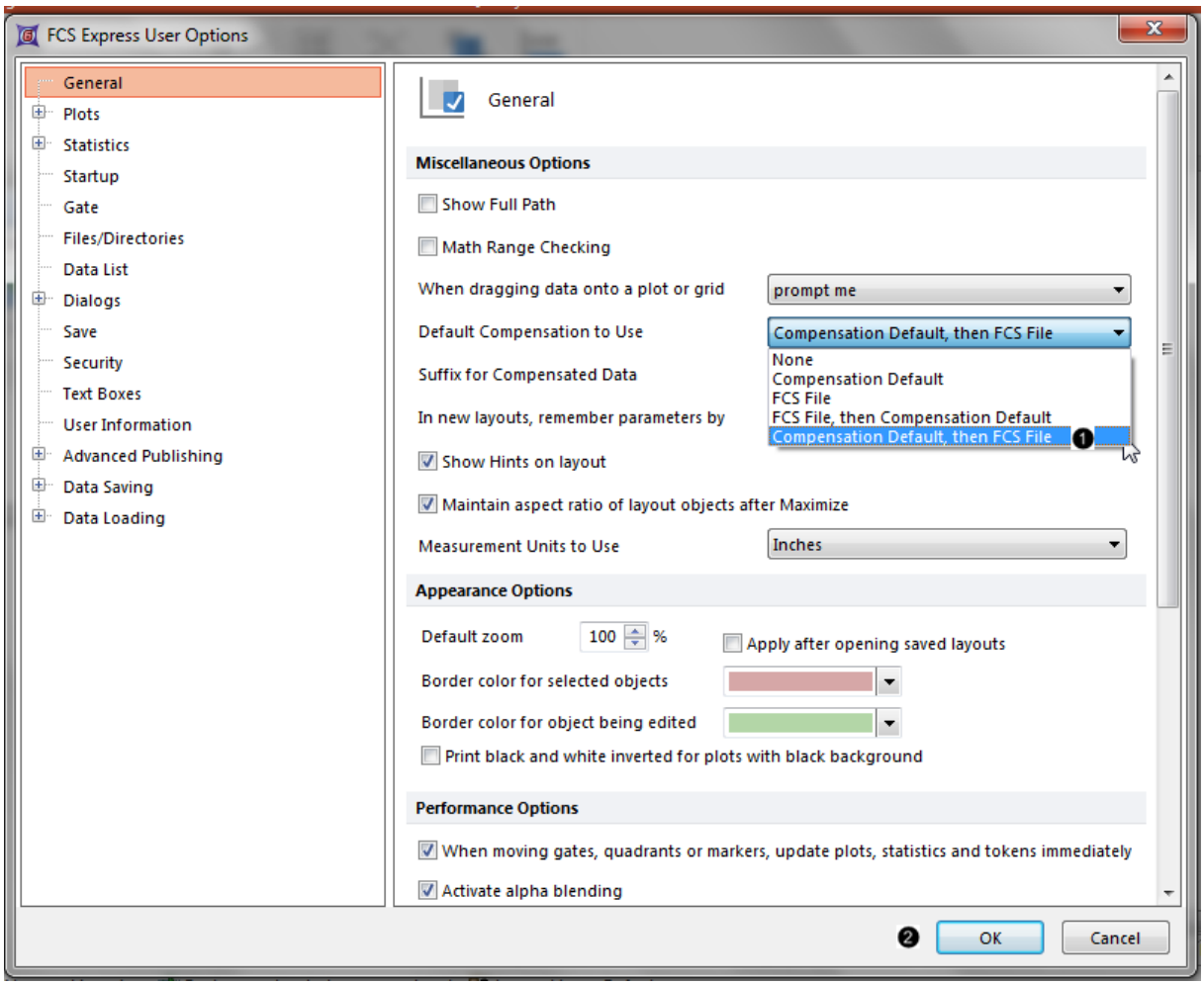

Figure 1. Changing the default Compensation Option.

5. Click **OK** (Fig. 1, ❷).

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6. Click to expand the **Created manually** node within the Compensations window (Fig. 2, ❶).

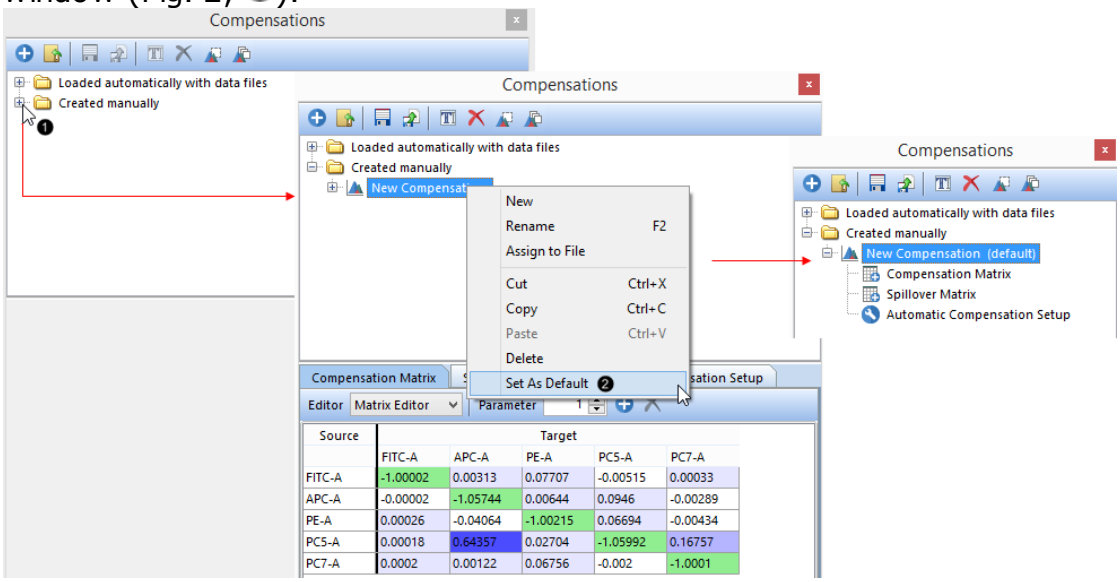

Figure 2. Setting a compensation matrix as default for the layout.

7. Right-click on the "New Compensation" matrix to **Set As Default** (Fig. 2, ❷). This matrix will now be marked as *default* (Fig. 2, *right*).

We will now insert plots of experimental files in the same layout, and watch as the Compensation Default matrix is automatically applied.

8. Drag the first file from the Data List to an empty spot on the layout (Fig. 3, ❶).

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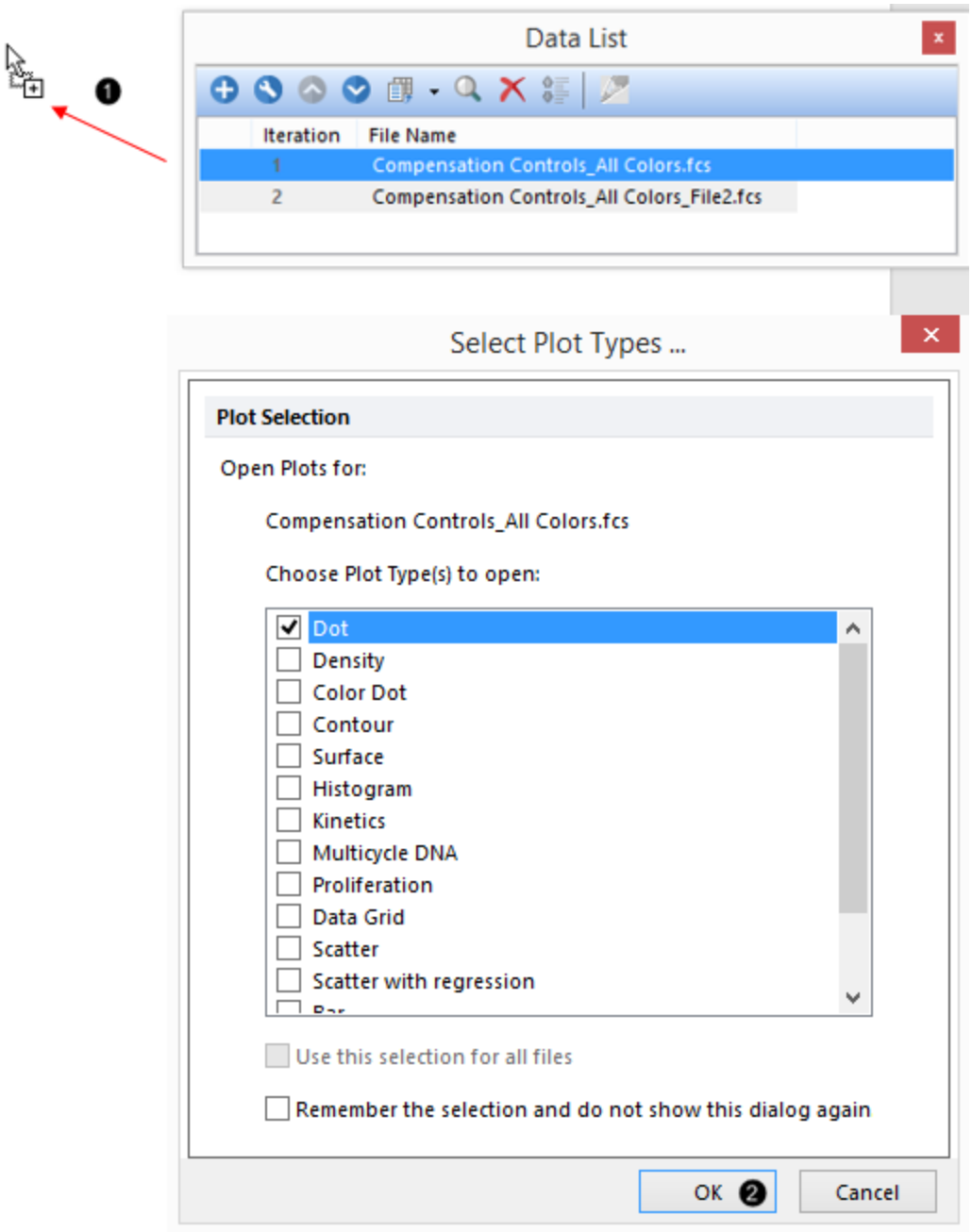

Figure 3. Inserting a plot on the layout.

9. Click **OK** (Fig. 3, ❷).

10. Click and hold the X- and Y-axis labels to change the parameters of the newly inserted plot to "CD95 PC5-A" (Fig. 4, ❶) and "CD8a PC7-A" (Fig. 4, ❷), respectively.

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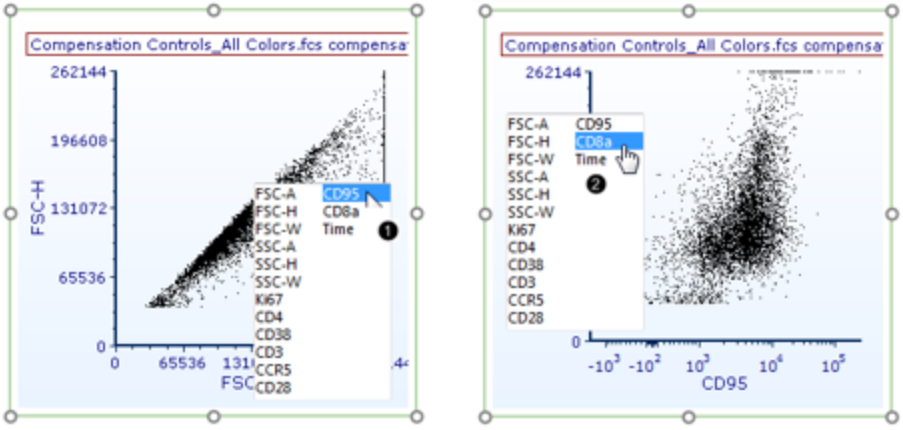

Figure 4. Changing axis parameters.

The Compensation Default matrix has been applied automatically to the plot. You may verify this by manually changing the values in the Compensation Matrix from the Compensations window (Fig. 5, mouse arrow) *and watching the population in the plot update*.

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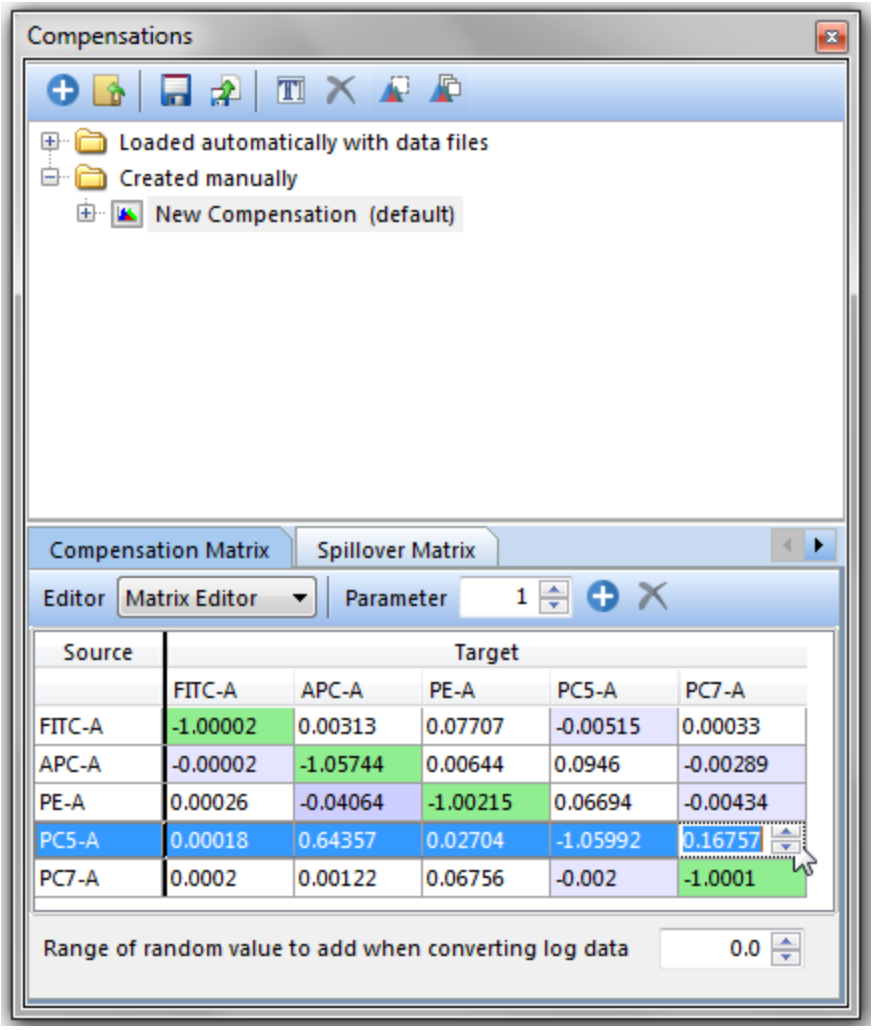

Figure 5. Editing a compensation matrix manually.

We will now perform a Batch Process to export the results from all the files in our Data List to PowerPoint. The Batch Process Action has already been set up for convenience. To learn more about this functionality, see the Batch Processing tutorial 1445 or the short Batch Processing video on YouTube.

11. Click **Batch** tab→**Run** (Fig. 6).

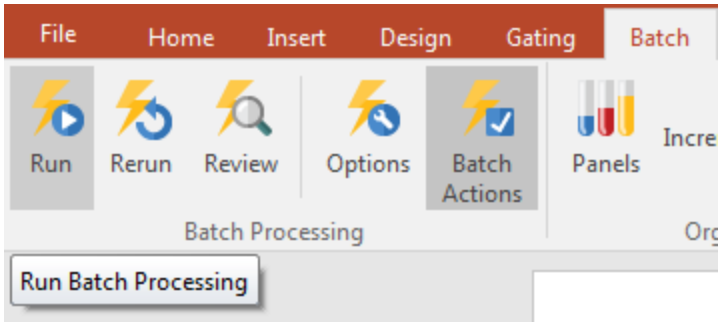

Figure 6. Running a Batch Process.

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A PowerPoint file with the results from all the files in your Data List has now been saved on your Desktop. If the PPT file does not open automatically, please change the Export to PowerPoint's ***Output file options*** *save path to a valid one for your computer. See the PowerPoint section of the Batch Processing tutorial 810 for details.*

38.8.3 Compensation Digitization Error

Sometimes compensation will introduce a digitization error as seen in the red box in the figure below. The *digitization error* of the FL1-H (+) population in the lower right-hand quadrant after compensation (figure T8.30) can easily be corrected by adding a range of random values. Digitization errors and adding a range of random values is further discussed in Editing a Compensation Definition 631 topic. Please follow the steps below to learn how to correct for digitization errors.

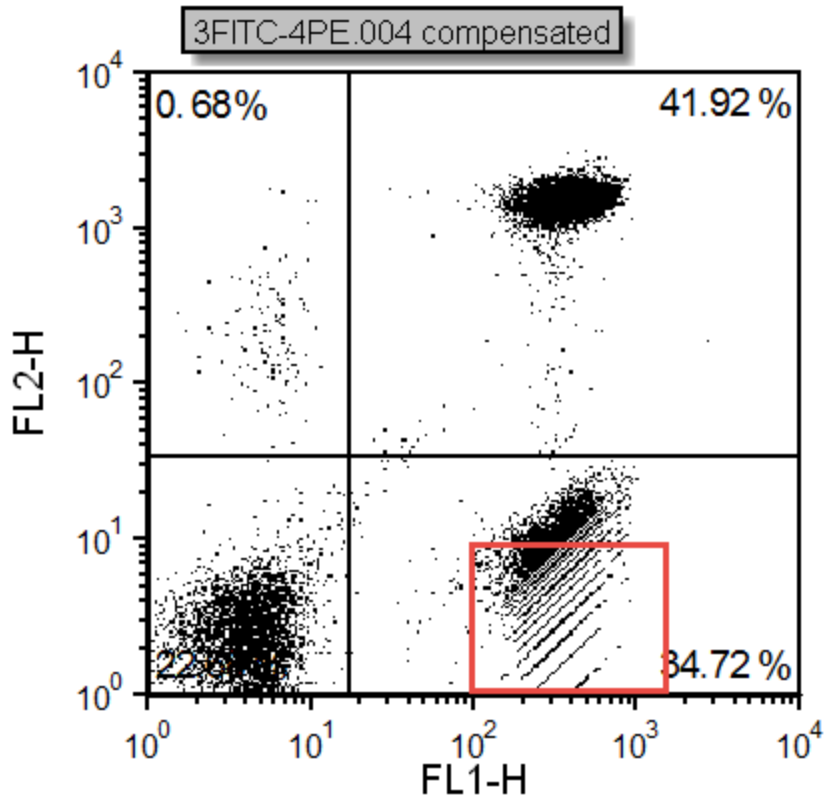

Figure T8.30 - Digitization errors can be seen highlighted in the red box.

1. Select **File** tab→**Open Layout**.
2. Open the layout **ManualComp.fey** found in the Tutorial Sample Data archive 1210.

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3. Click on the **Digitization Errors** page tab.

Notice the *digitization error* of the FL1-H (+) population in the lower right-hand quadrant after compensation. To correct for the digitization error:

4. Select the **Tools→Transformations→Compensations** command
5. Select the **Create Manually** folder→**DigitizationError** compensation definition (figure T8.31)

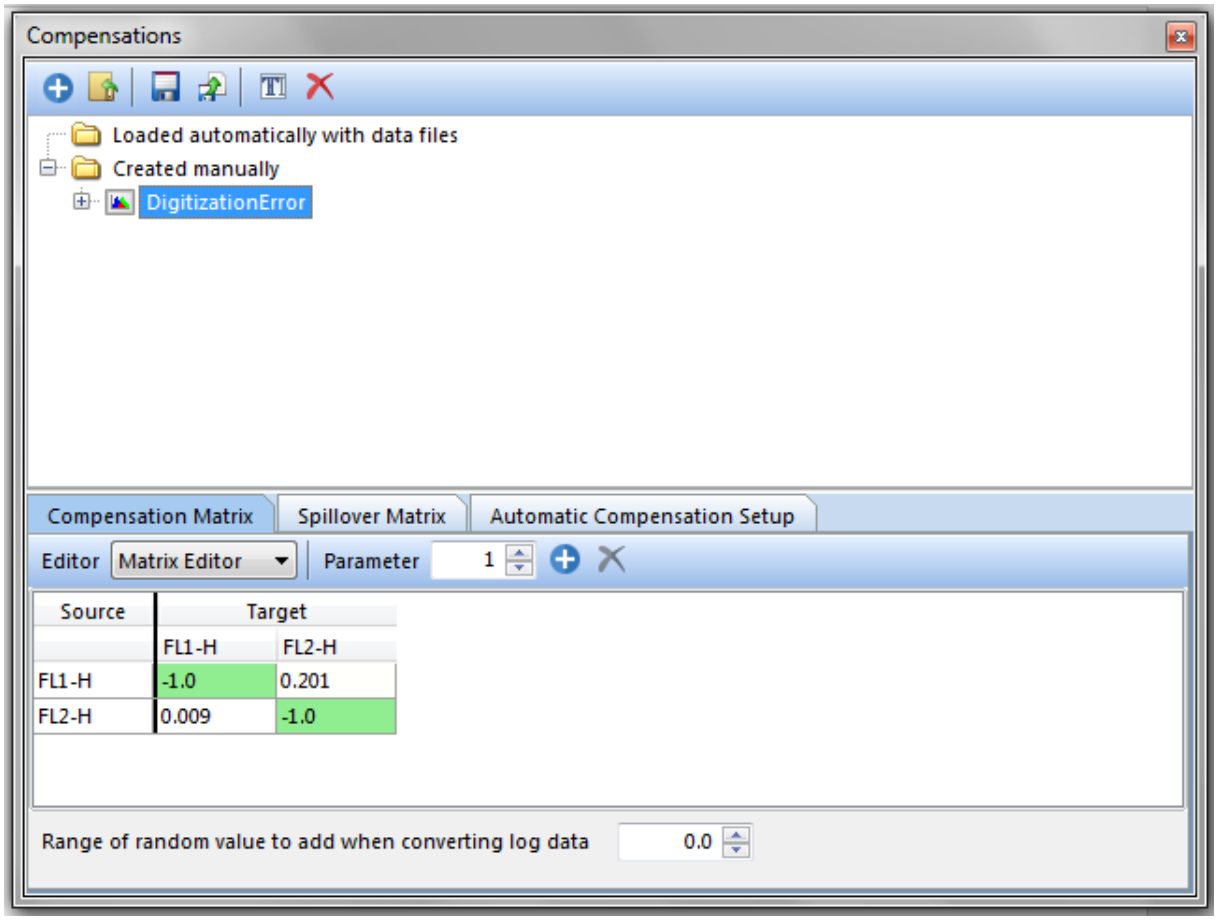

Figure T8.31 - Selecting the DigitizationError compensation definition for editing.

6. Select the **Create Manually** folder→**DigitizationError** compensation definition (figure T8.31).
7. Type **2.0** in the **Range of random value to add when converting log data** field (indicated by red box in Figure T8.32).

The plot will update in real time to reflect the change and the FITC(+) population in the lower right quadrant will now appear without the digitization error. The random value entered above was added to visually correct the data displayed in the 2D plot.

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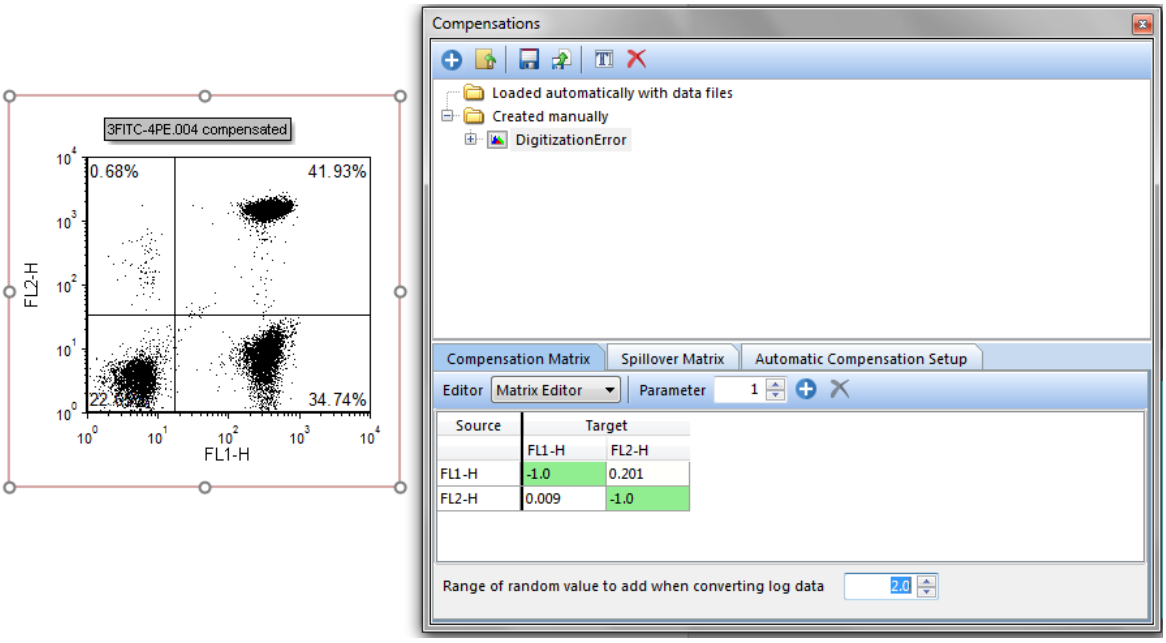

Figure T8.32 - Changing the Range of random value to add when converting log data to 2.0.

38.9 Snapshots

Snapshots 372 provide a convenient way to easily save multiple gate, quadrant, or marker positions and to apply these positions for quick access across samples in your analysis.

Snapshots can be applied during Batch Processing 852, Iteration Navigation 376, and independently through the Snapshots window 1423.

These tutorials will highlight these tools and how they can be utilized during analysis.

- Snapshot Window Tutorial 1423
- Iteration Snapshots Tutorial 1429
- Batch Processing Snapshots Tutorial 1436

We will start by focusing on the Snapshot Window 1423.

38.9.1 Snapshots Window

In this tutorial:

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- We will learn how to take Snapshots through the use of the Snapshots window.
- We will learn how to apply Snapshots to the data in the layout.
- Although any data files of your own can be used to follow along in this tutorial, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive 1210.

1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open.**

2. Open the layout **SnapshotWindow.fey**.

This layout contains 2 color dot plots, 2 density plots and a 1D histogram. There are also 3 data files loaded into the Data List, which is docked 115 to the right side of the layout. We will navigate and analyze through these 3 files and make adjustments to the various gates, quadrants and markers that are to be used to analyze the data. Once we adjust the coordinates of our gates, we will take a snapshot.

3. Open the **Snapshots** navigator from **Tools→Snapshots→Snapshots** (Figure 1).

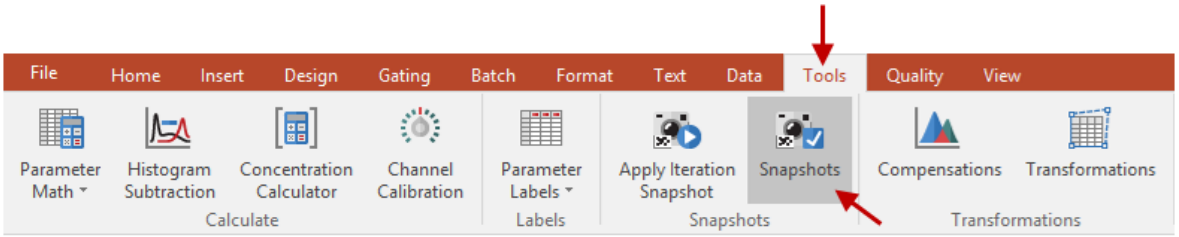

Figure 1 - The Snapshots Tool

We will begin by taking a snapshot of the current state of the layout.

4. Click on the **Create a new Snapshot** button ( ) in the **Snapshots** navigator.

5. Replace the text "New Snapshot" with "Baseline" in the Snapshot name text area.

Once the snapshot is taken, FCS Express will show a time stamp for that snapshot (Figure 2).

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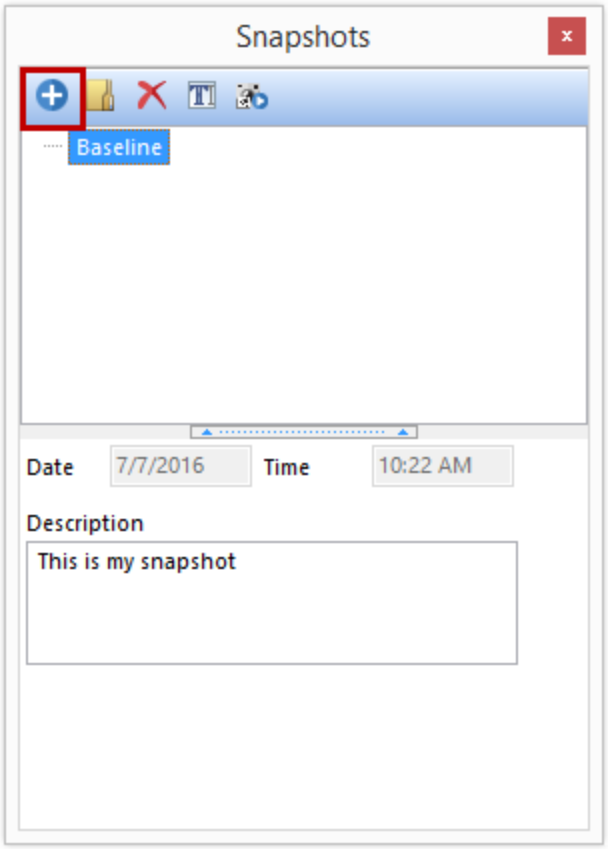

Figure 2 - Baseline Snapshot with a Timestamp

6. Navigate to the next iteration by clicking on iteration #2 in the Data List, as seen in Figure 3.

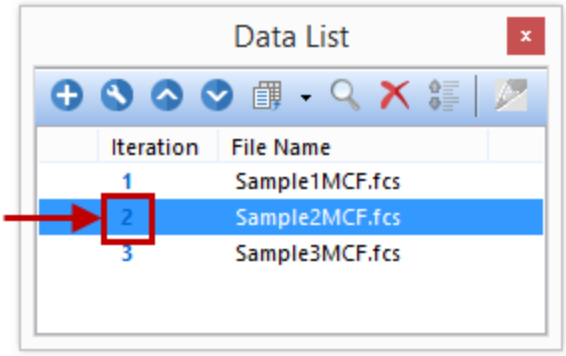

Figure 3 - Navigate to the Next Iteration By Clicking on the Iteration Number

The steps below are highlighted in Figure 4.

7. Adjust the Red gate to include the majority of the cells in the cluster. (Figure 4 ❶)

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8. Right click on the BV605 vs BV421 plot. (Figure 4 ❷)

9. Select **Show Quadrants**.

10. Move the quadrant in the APC-H7 vs APC plot to a new position. (Figure 4 ❸)

11. Adjust the Marker in the 1D histogram of APC. (Figure 4 ❹)

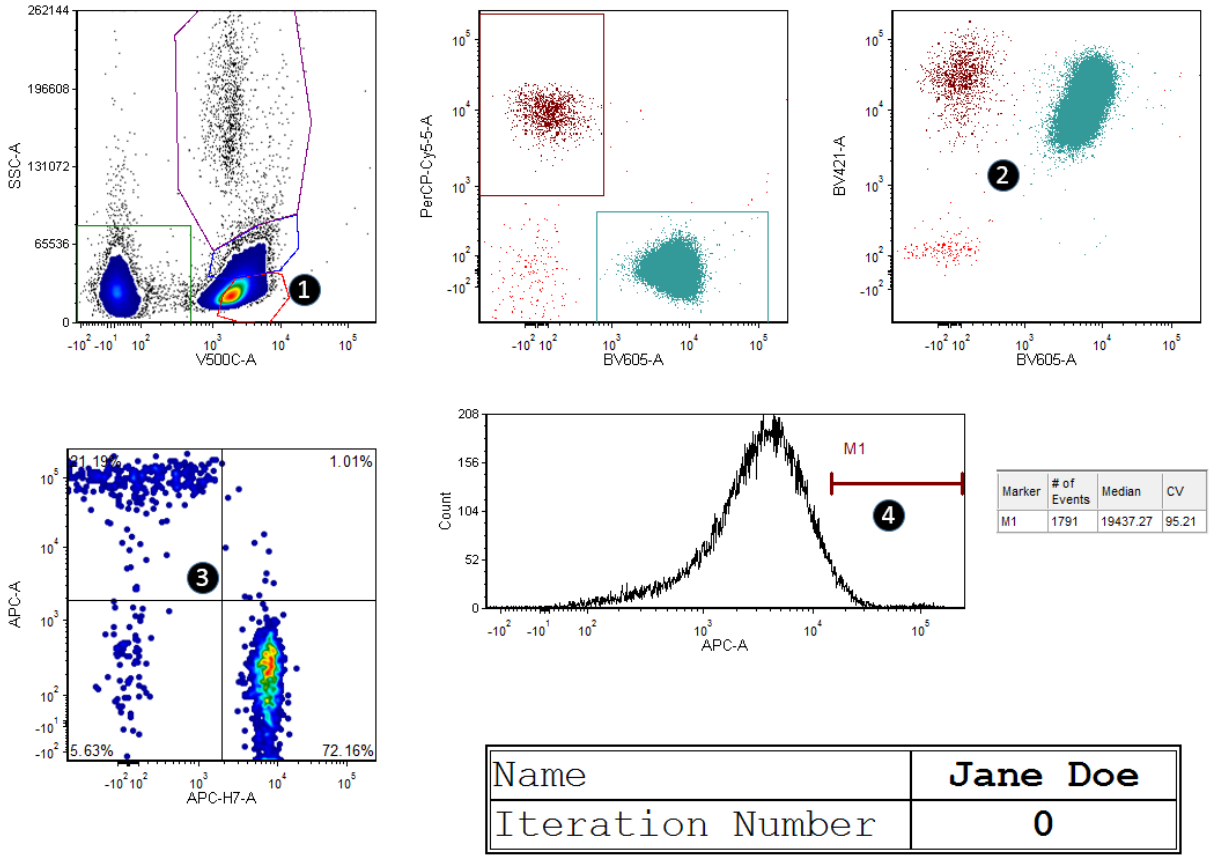

Figure 4 - Making Adjustments Before Taking A Snapshot

After adjustments, the layout should look similar to Figure 5.

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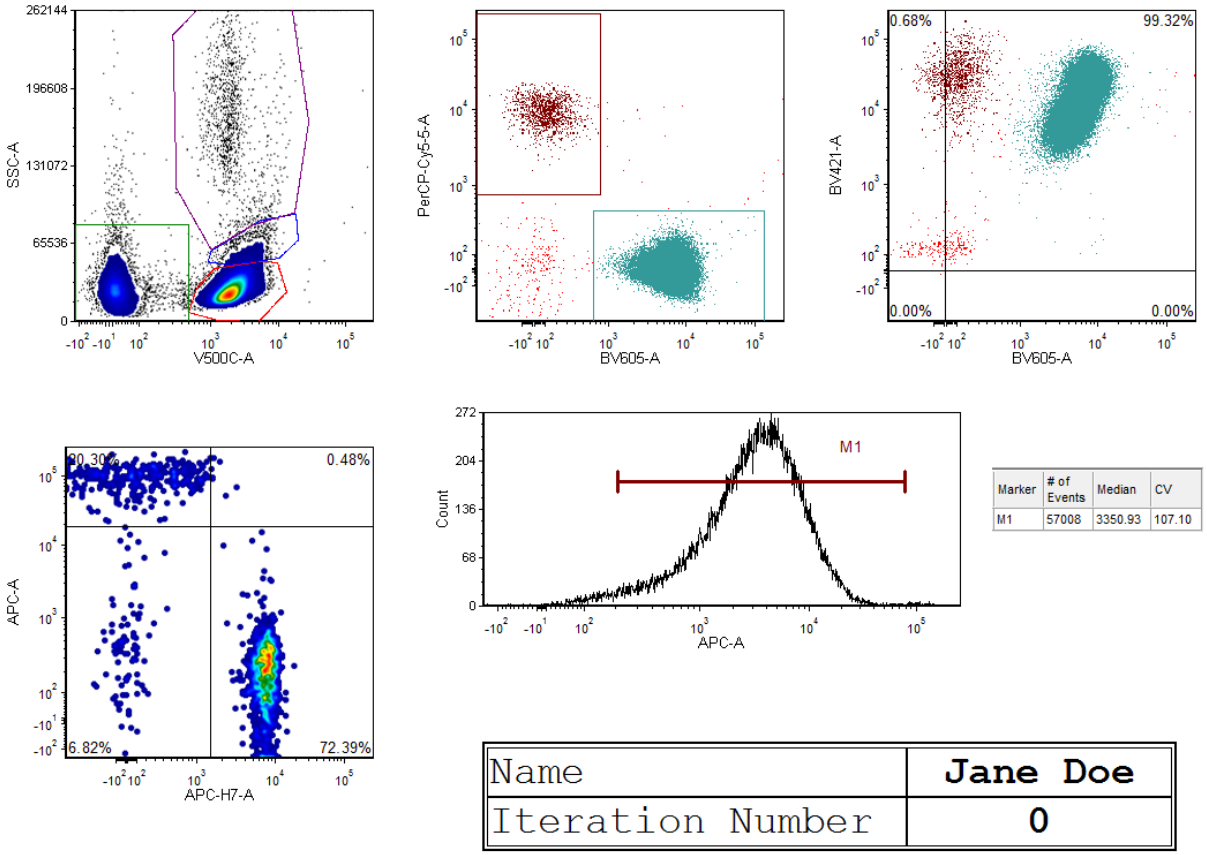

Figure 5 - After Making Gating Adjustments

12. Repeat Step 4 to record a new Snapshot.

13. Replace the text "New Snapshot" with "Adjusted" in the Snapshot name text area.

14. Navigate to the next iteration by clicking on iteration #3 in the Data List.

We will now apply the Baseline snapshot from step 5 to Iteration 3.

15. Click on the text "Baseline" in the **Snapshots** navigator to select the snap shot.

16. Drag the "Baseline" Snapshot from the Snapshots window to the layout, as seen in Figure 6a.

Alternatively, you may apply the snapshot by selecting the snapshot as in step 15 and clicking on the ***Apply Snapshot*** *button* *in the* ***Snapshots*** *navigator*.

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Once the snapshot has been applied, all gates and coordinates that applied to that snapshot will be applied to the data, as seen in Figure 6b. Repeating steps 15 and 16 with the "Adjusted" snapshot will apply that snapshot to the layout.

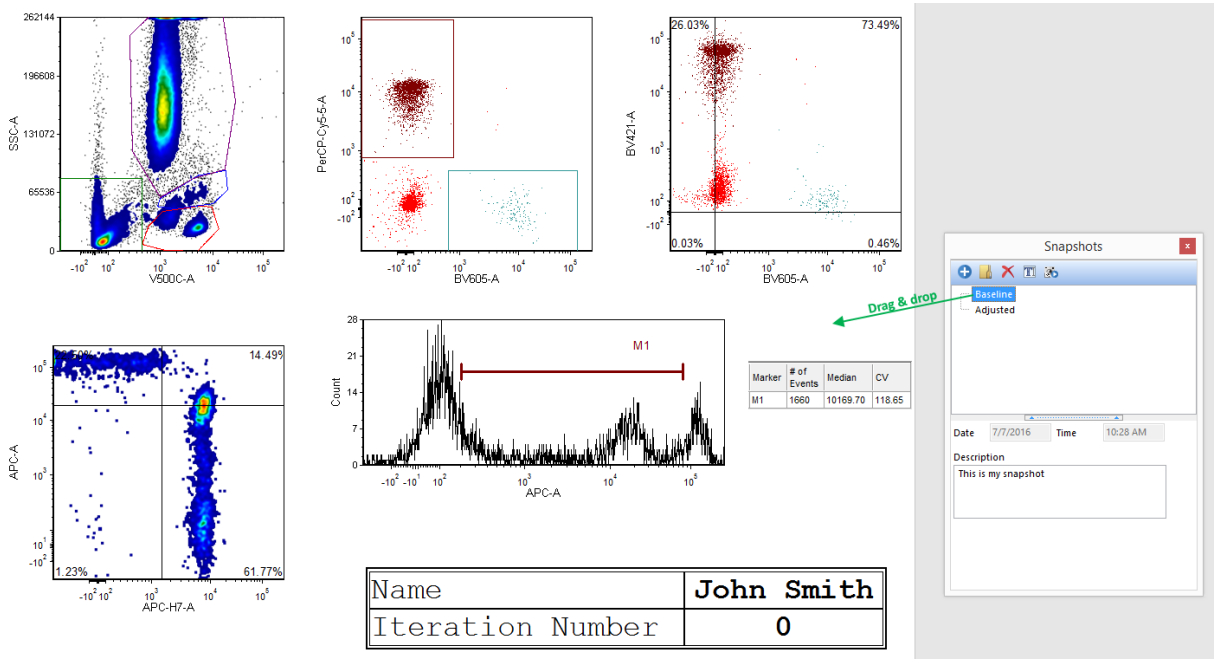

Figure 6a - Dragging and Dropping the Baseline Snapshot

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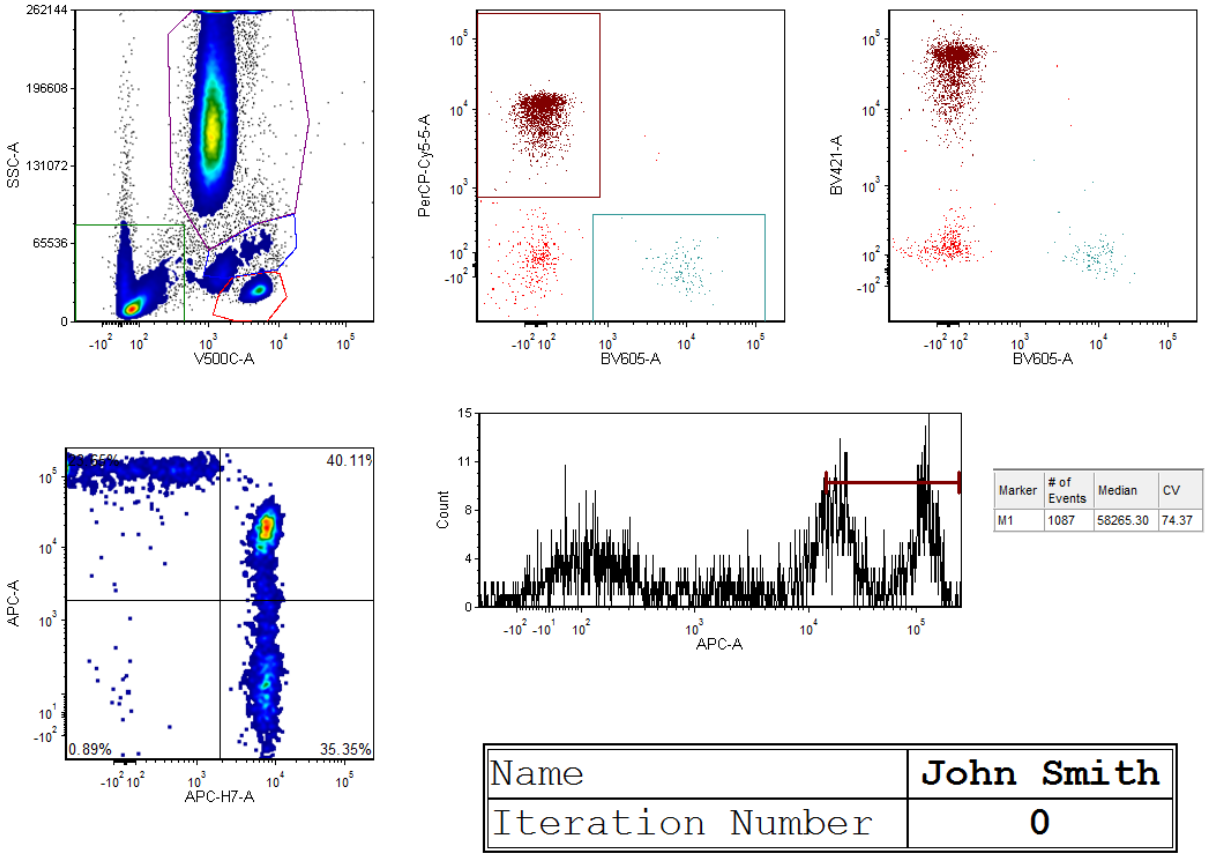

Figure 6b - The layout as it appear after the drag and drop of the Baseline snapshot.

In the next section, we discuss on how to automatically apply snapshots will navigating thru each iteration 1429.

38.9.2 Iteration Snapshots

In this tutorial:

- We will learn how to take Snapshots through the use of the **Apply Iteration Snapshot** tool.
- We will learn how to navigate through our data by using the **Previous/Next** buttons or thru the **Iteration** drop down menu on the **Data** tab.
- We will learn how to Flag an iteration for review later on during analysis.
- Although any data files of your own can be used to follow along in this tutorial, you have the option of using the same sample data files used in this

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tutorial. The sample data files are located in the Tutorial Sample Data archive.

1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open.**
2. Open the layout **IterationSnapshots.fey**.

This layout contains 4 density plots and a 1D histogram and is currently displaying the 1st iteration from 3 iterations that are contained in the Data List.

3. Select the **Tools** tab→**Snapshots**→**Apply Iteration Snapshot** button (Figure 8).

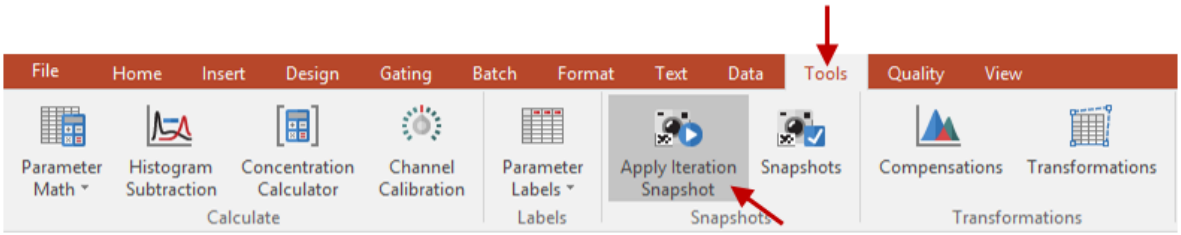

Figure 8 - Apply Iteration Snapshot Tool

4. Click the **Next** button (double right hand arrow) in the **Data** tab→**Change Data on All Objects** category to advance to the next iteration.

   Alternatively, you can advance to the next iteration by selecting **2** from the **Data** tab→**Change Data on All Objects**→**Iteration** drop down menu ***or*** by selecting **2** from the **Iteration** column in the **Data List**.

The content of the layout should now be similar to Figure 9a.

We will now make the adjustments for the 2nd iteration according to the steps below and as seen in Figure 9a:

5. Adjust the red gate around the cluster on the SSC/V500 plot (Figure 9a ❶).
6. Adjust the purple gate around the cluster in the right of the BV421/BV605 plot (Figure 9a ❷).

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7. Right click on the FITC/BV605 plot (Figure 9a ❸).

8. Choose **Show Quadrants**.

9. Adjust the M1 marker to fully accommodate the peak on the APC 1D histogram (Figure 9a ❹).

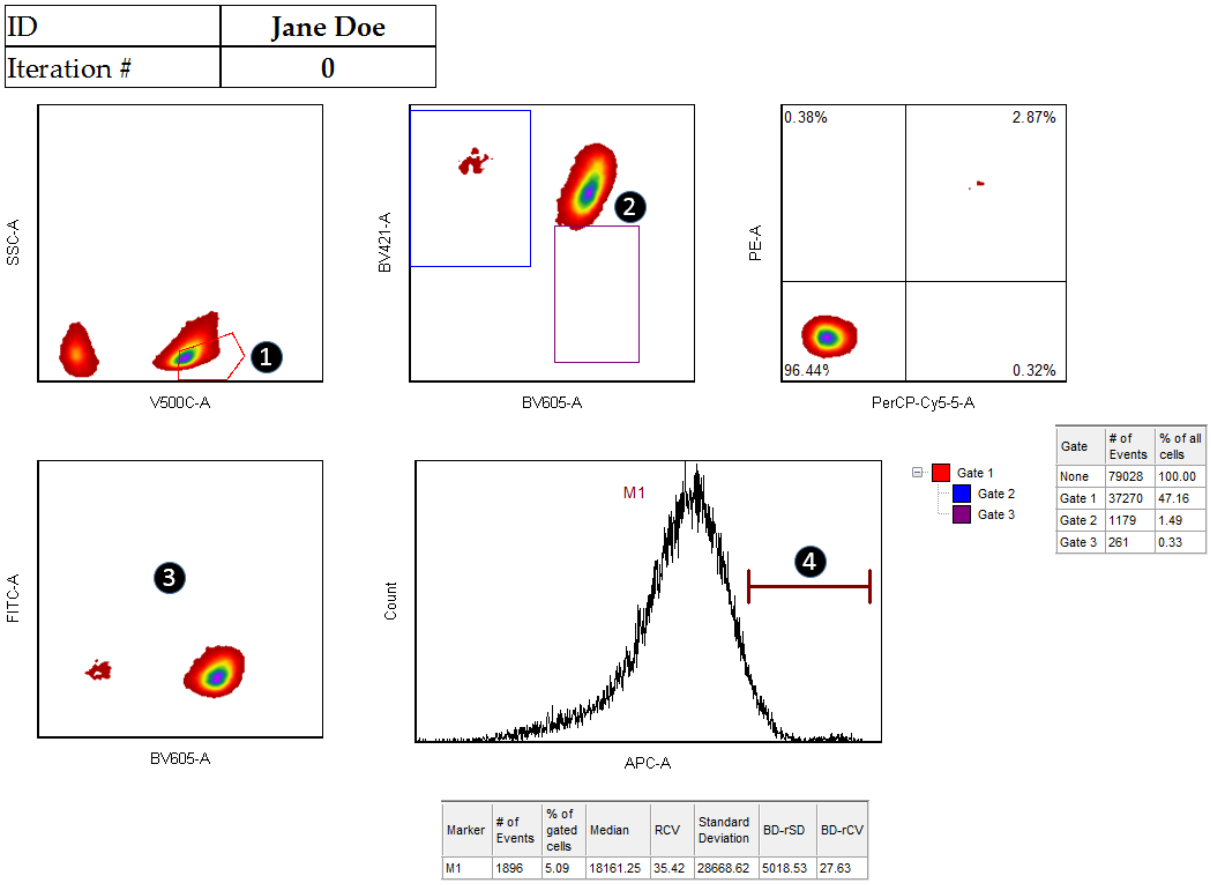

Figure 9a - Steps for the 2nd Iteration

After these adjustments the layout should be similar to Figure 9b.

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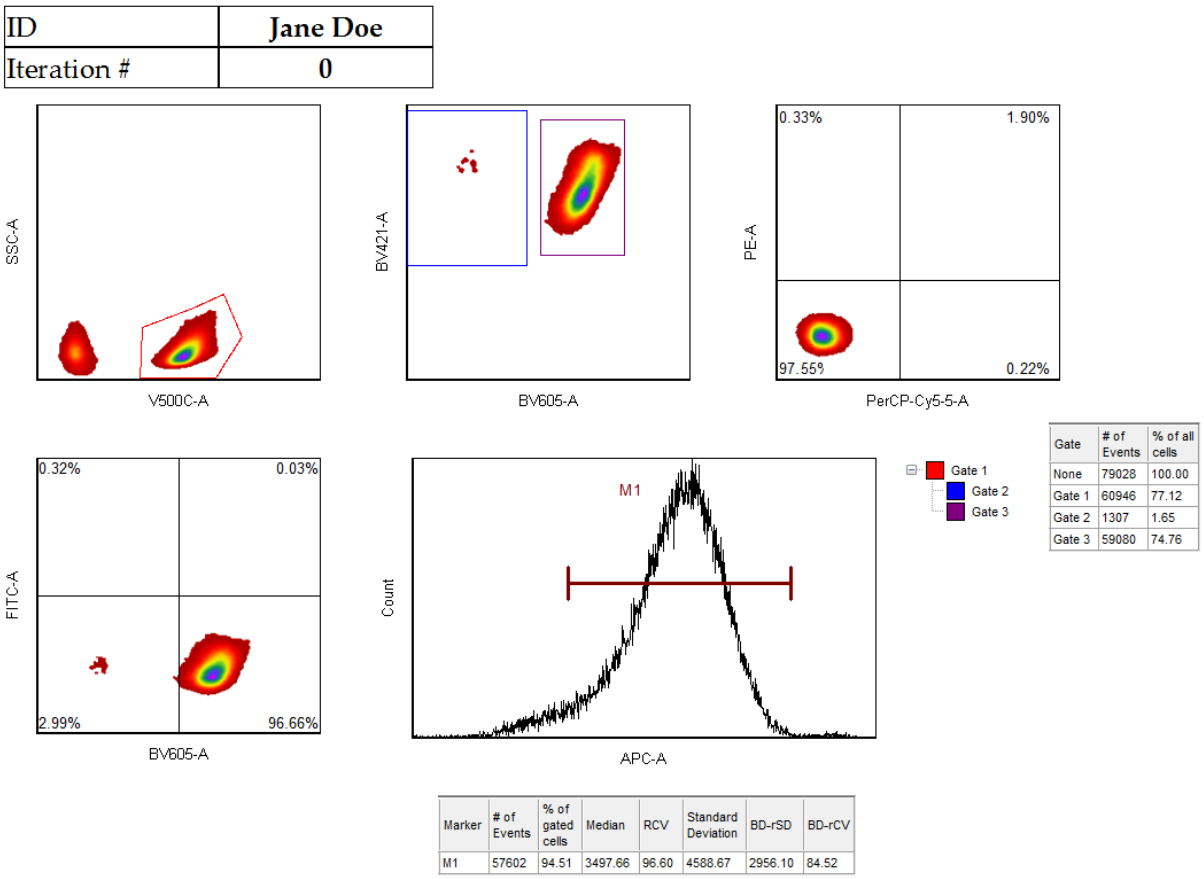

Figure 9b - The layout as it should appear after the adjustments reported in Steps 5-9.

We will now Flag the iteration for use later in the tutorial.

10. Click on the **Flag this Iteration** button in the **Data** tab→**Change Data On All Objects** section(Figure 10).

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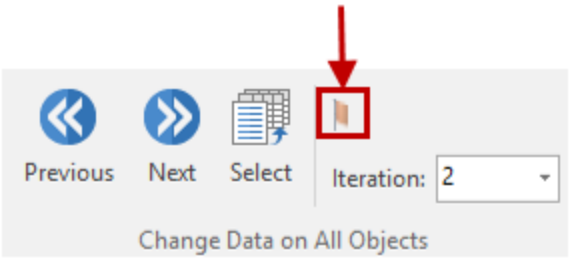

Figure 10 - Flag the 2nd Iteration

Once the 2nd iteration is flagged, you will see a new icon within the Data List, next to the 2nd iteration: 2 Sample2MCF.fcs.

We will come back to the Flagged iteration in the next tutorial section, Batch Processing Snapshots 1436.

11. Click the **Next** button (double right hand arrow) in the **Data** tab→**Change Data on All** category to advance to the third iteration.

We will now make the adjustments for the third iteration according to the steps below and as seen in Figure 11a:

12. Adjust the red gate around the cluster on the SSC/V500 plot (Figure 11a ❶)

13. Adjust the purple gate around the cluster in the lower right of the BV421/BV605 plot (Figure 11a ❷)

14. Move the floating quadrant on the PE/PerCPCy5.5 plot to a new position (Figure 11a ❸)

15. Move the quadrant on the FITC/BV605 plot to a new position (Figure 11a ❹)

16. Move the M1 marker on the APC 1D histogram (Figure 11a ❺).

17. Create a new marker 353 to accommodate the second peak on the APC 1D histogram (Figure 11a ❺).

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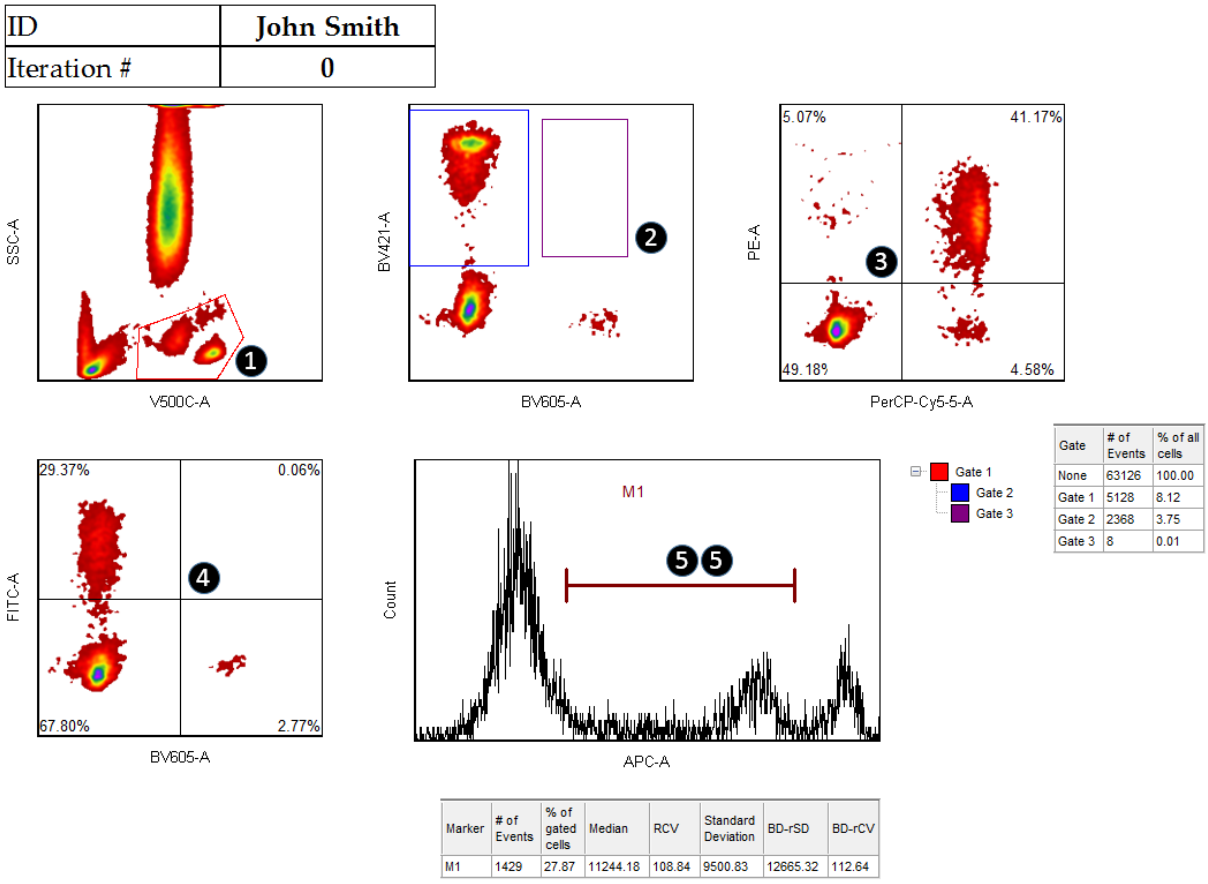

Figure 11a - Steps for 3rd Iteration

The layout should now appear similar to Figure 11b:

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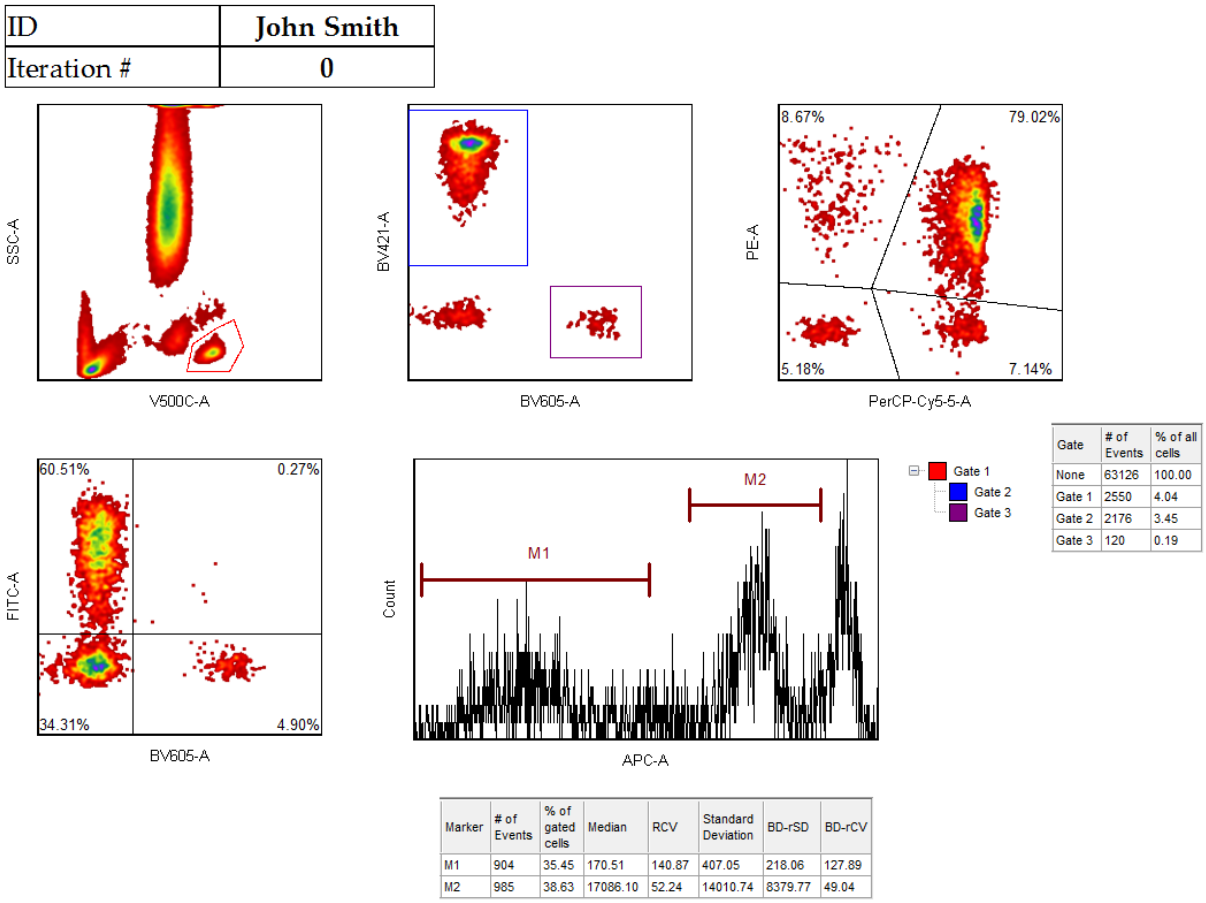

Figure 11b - The layout as it should appear after the adjustments reported in Steps 12-17.

We will now navigate back through our data to observe the Iteration Snapshot feature in effect.

18. Click the **Previous** button (double left hand arrow) in the **Data** tab→**Change Data on All Objects** category to move back to the second iteration.

19. Click the **Previous** button (double left hand arrow) in the **Data** tab→**Change Data on All Objects** category to move back to the first iteration.

Notice that FCS Express has automatically saved and applied a snapshot for each iteration.

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20. Click on **File→Save As** to save your work.

21. Navigate to your desktop.

22. Name the layout file a name of your choice.

23. Choose **Linked** as the **Data Link Style**.

24. Click **Save**.

You can now double click on the layout file you saved in steps 20-24 to open and pick up your analysis where you left off. After opening the saved layout you will notice that clicking the **Previous/Next** buttons will automatically apply the iteration snapshots saved with the layout.

In the next section, we will further investigate how Snapshots are integrated with Batch Processing.

38.9.3 Batch Processing Snapshots

In this tutorial:

- We will learn how to analyze data and take Snapshots through Batch Processing.
- We will learn how to **Run**, **Rerun** and **Review** the analysis during batch processing.
    - **Run** - will run batch processing and perform all batch actions that are selected for each iteration. Snapshots for each iteration will automatically be taken.
    - **Rerun** - will rerun the previous batch process and perform all batch actions that are selected, for each iteration. Any Snapshots that were previously saved by FCS Express will automatically be re-applied during Rerun. This will also be the opportunity where the user can regate and have FCS Express overwrite the previously saved Snapshot with the new gating.
    - **Review** - will rerun the previous batch process, without performing any batch actions that were created and selected for each iteration.
- We will learn about the new Batch Processing Options that are available and how they are used to play back the snapshots taken during analysis.
- We will learn how to Flag an iteration for further review after batch processing has been completed.

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- The layout used in this tutorial contains a batch action to **Save to Powerpoint** 810. This course assumes that the user knows how to create Batch Processing Actions 803 to be performed each iteration of data.

To Run Batch Processing and Automatically Save Snapshots

1. Navigate to the *Tutorial Sample Data* folder from the **File** tab→**Open.**

2. Open the layout **BPSnapshots.fey**.

The layout contains 3 iterations of data already in the Data List. The layout also contains a Save to PowerPoint 810 batch action that is designated to save to the Desktop of the Current Windows User 717. For this tutorial, we will focus on how to set up the Batch Processing Options to provide the you with an opportunity to optimize gates during the batch process.

3. Click on the **Batch** tab→**Batch Processing**→**Options** (Figure 12).

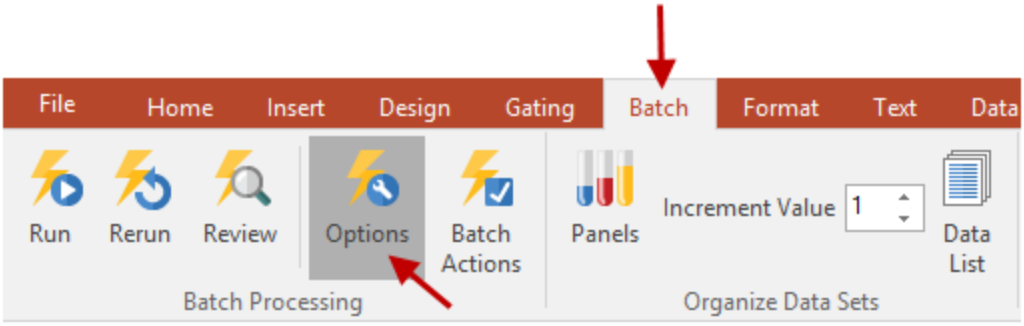

Figure 12 - Batch Processing Options Button

Towards the middle of the **Batch Processing Options** window there are several pause options that will determine whether or not FCS Express will pause between each iteration during analysis and when FCS Express will pause during analysis.

4. Select **Unconditionally pause between iterations** (Figure 13).

5. Click **OK**.

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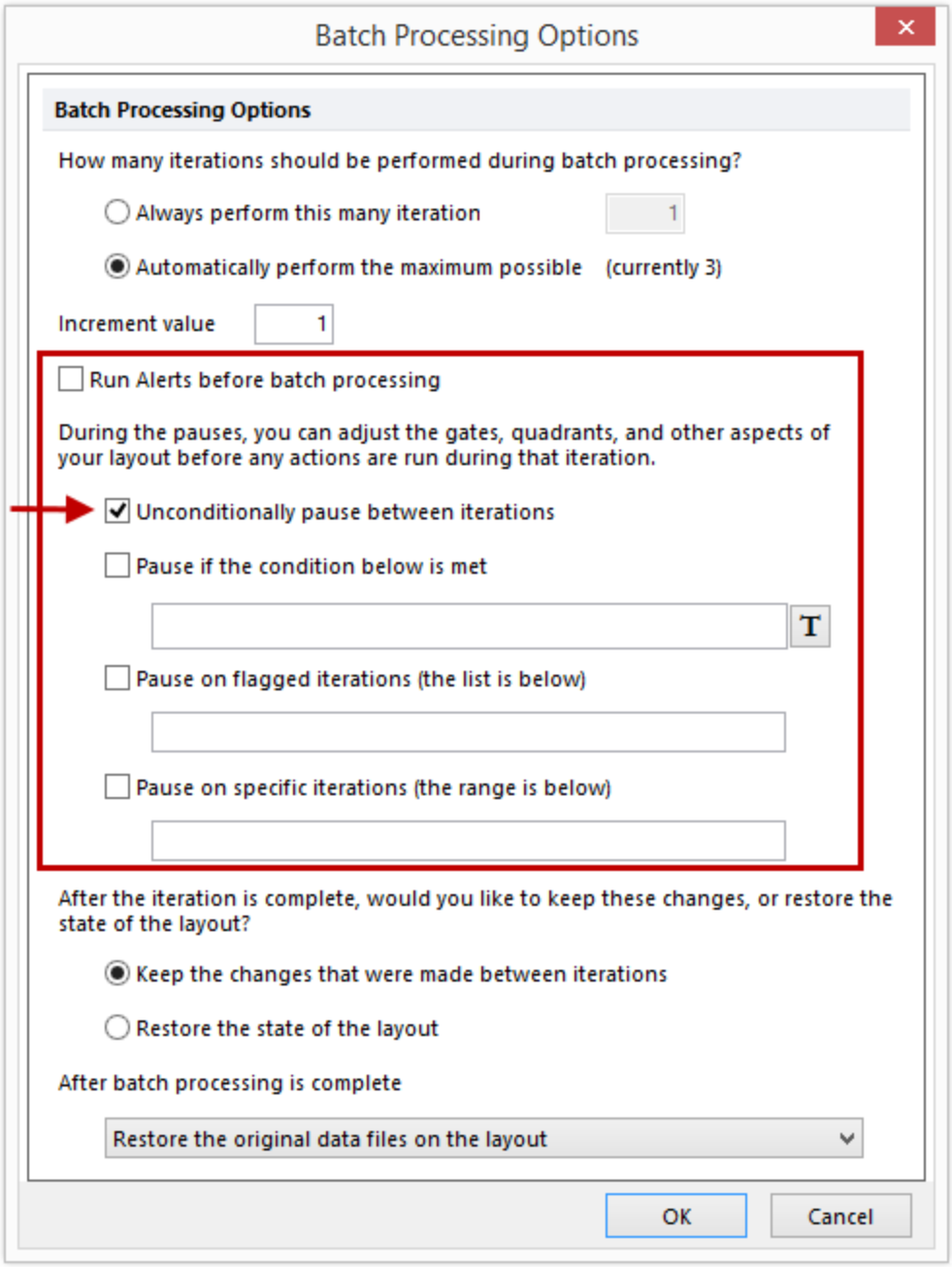

Figure 13 - Batch Processing Options - Unconditionally Pause Between Iterations

6. From **Batch→Batch Processing**, click the **Run** button.

Microsoft PowerPoint will automatically open. You can minimize PowerPoint while the batch process completes. The **Batch Processing** window will also

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appear showing the status of the current action, current iteration progress, and overall progress.

At the 1st pause, for Iteration #2, make the following adjustments (Figure 14):

7. Aadjust the Red gate 316 around the cluster of cells in the SSC/V500 plot. (Figure 14 ❶).

8. Adjust the Purple gate around the cluster of cells on the right side of the BV421 vs BV605 plot. (Figure 14 ❷).

9. Add a Quadrant 291 to the FITC vs BV605 plot (Figure 14 ❸).

10. Adjust the Marker 272 around the peak in the APC 1D histogram (Figure 14 ❹).

11. Click on the Flag 849 icon in the Batch Processing progress window (Figure 14 ❺).

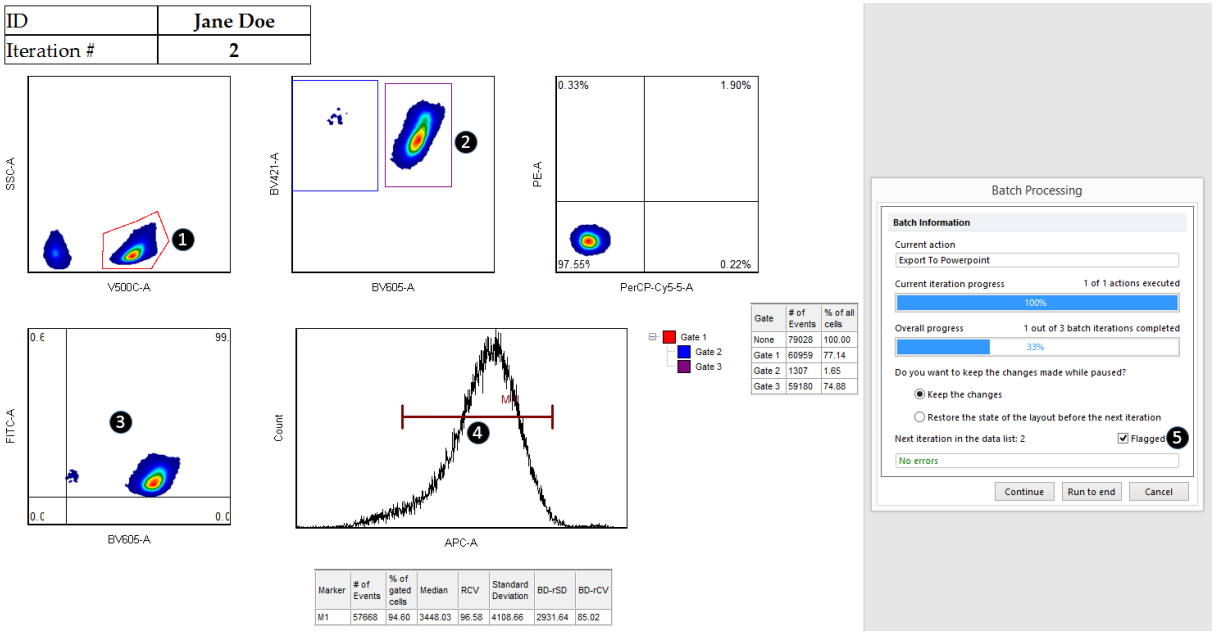

Figure 14 - Steps for 2nd Iteration Analysis

12. In the Batch Processing progress window, click **Continue** (Figure 15).

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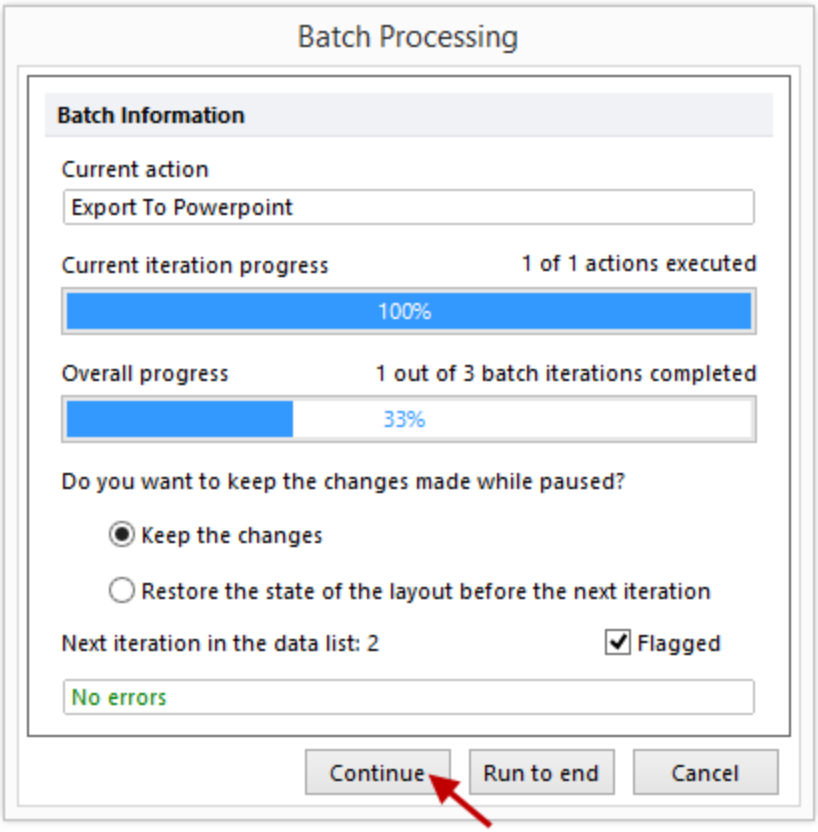

Figure 15 - Continuing with Batch Processing

At the 2nd pause, make the following adjustments (Figure 16):

13. Aadjust the Red gate around the cluster of cells in the SSC/V500 plot (Figure 16 ❶).

14. Adjust the Blue gate around the cluster of cells in the upper left portion, and the Purple gate around the cluster of cells in the lower right portion of the BV421 vs BV605 plot (Figure 16 ❷).

15. Adjust the floating quadrant as seen in figure 5 in the PE vs PerCP Cy5.5 plot (Figure 16 ❸).

16. Adjust the quadrant as seen in the image in the FITC vs BV605 plot (Figure 16 ❹).

17. Adjust marker M1 around one peak and create a new marker, M2, around the 2nd peak in the APC 1D histogram (Figure 16 ❺).

18. Click **Continue** within the Batch Processing progress window. At the conclusion of the batch process, the PowerPoint export will open (Figure 16 ❻).

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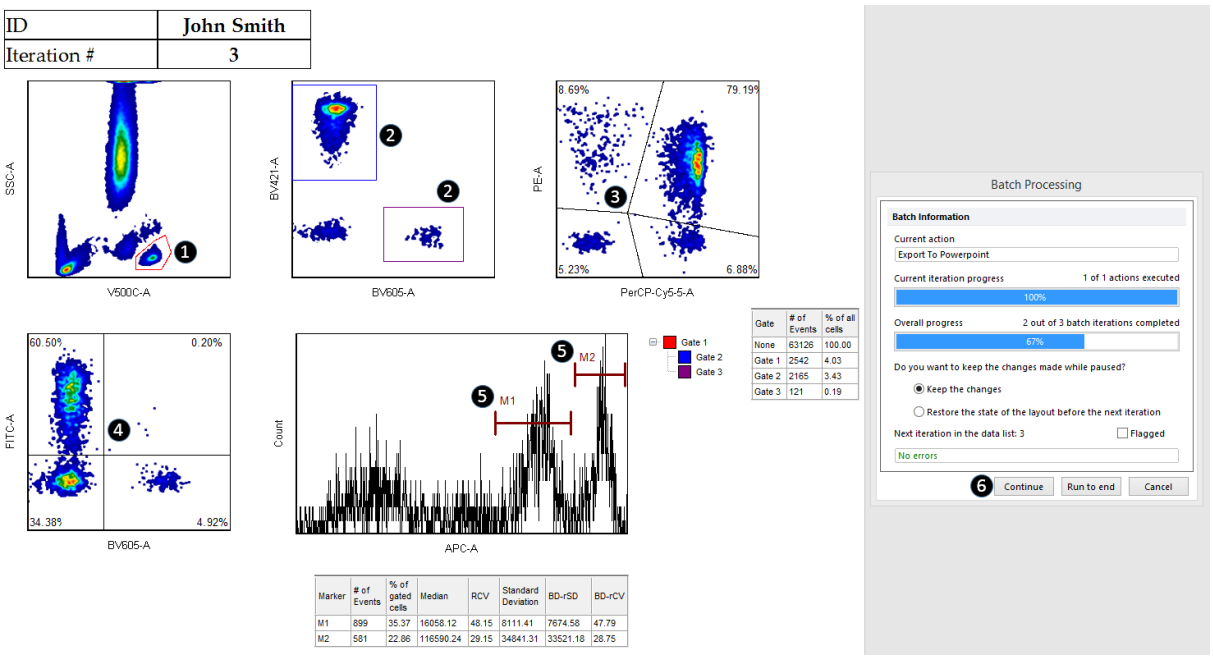

Figure 16 - Steps for 3rd Iteration Analysis

FCS Express has automatically taken Snapshots of each iteration analysis.

a. To view the Snapshots that were taken, you must either **Rerun** 1441 the batch process or **Review** 1442 the data.

b. **Using the Previous/Next buttons will remove whatever Snapshots were taken during batch processing.**

c. The following steps will detail how to view the Snapshots that were taken during batch processing.

Rerunning Batch Processing

1. From **Batch→Batch Processing**, click the **Rerun** button (Figure 17).

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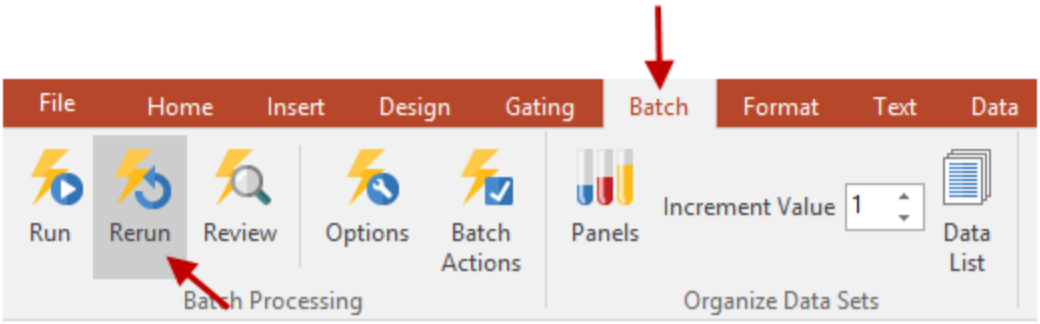

Figure 17 - Rerunning Batch Processing

- The same process as before will occur. Since we still have the Batch Processing Pause Option set to Unconditionally pause between iterations, FCS Express will continue to pause after each iteration. However, if you would like to experiment with different options, you can find a summary of the Batch Processing Options here 843. Please note that FCS Express will still process all iterations. FCS Express will only *pause* for the iterations that are determined in Options.

2. Click **Continue** with the progress window to continue with the **Rerun** process.

Make any changes to gates, markers and quadrants that you deem necessary. If you do not wish to make any changes, click **Run to end**.

- If any changes were made to gating, marker and/or quadrant placement, FCS Express will record a new Snapshot for that iteration and will overwrite any previously existing Snapshot for that iteration.
- During the Rerun process, FCS Express will also perform any batch actions that were set up as part of any process.

Reviewing Data

Review will give the user the opportunity to look at their data and make any necessary changes to gate, marker or quadrant positions for each iteration. However, FCS Express will not perform any batch processing actions that were set up when in review mode.

As before, the user can control when FCS Express pauses during batch processing. For the earlier exercise, we chose the option to have FCS Express unconditionally pause between each iteration. This time, we will tell FCS Express that we only want to review any iterations that were flagged during analysis. In this exercise, we flagged the 2nd iteration, so, FCS Express will pause only for that iteration.

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1. From **Batch→Batch Processing** click the **Options** button.

2. Deselect Unconditionally pause between iterations (if selected).

3. Select the option to **Pause on flagged iterations (the list is below)** (Figure 18).

FCS Express will automatically add any iterations that were flagged by the user during batch processing. In our example, you should the number 2 listed for that pause option.

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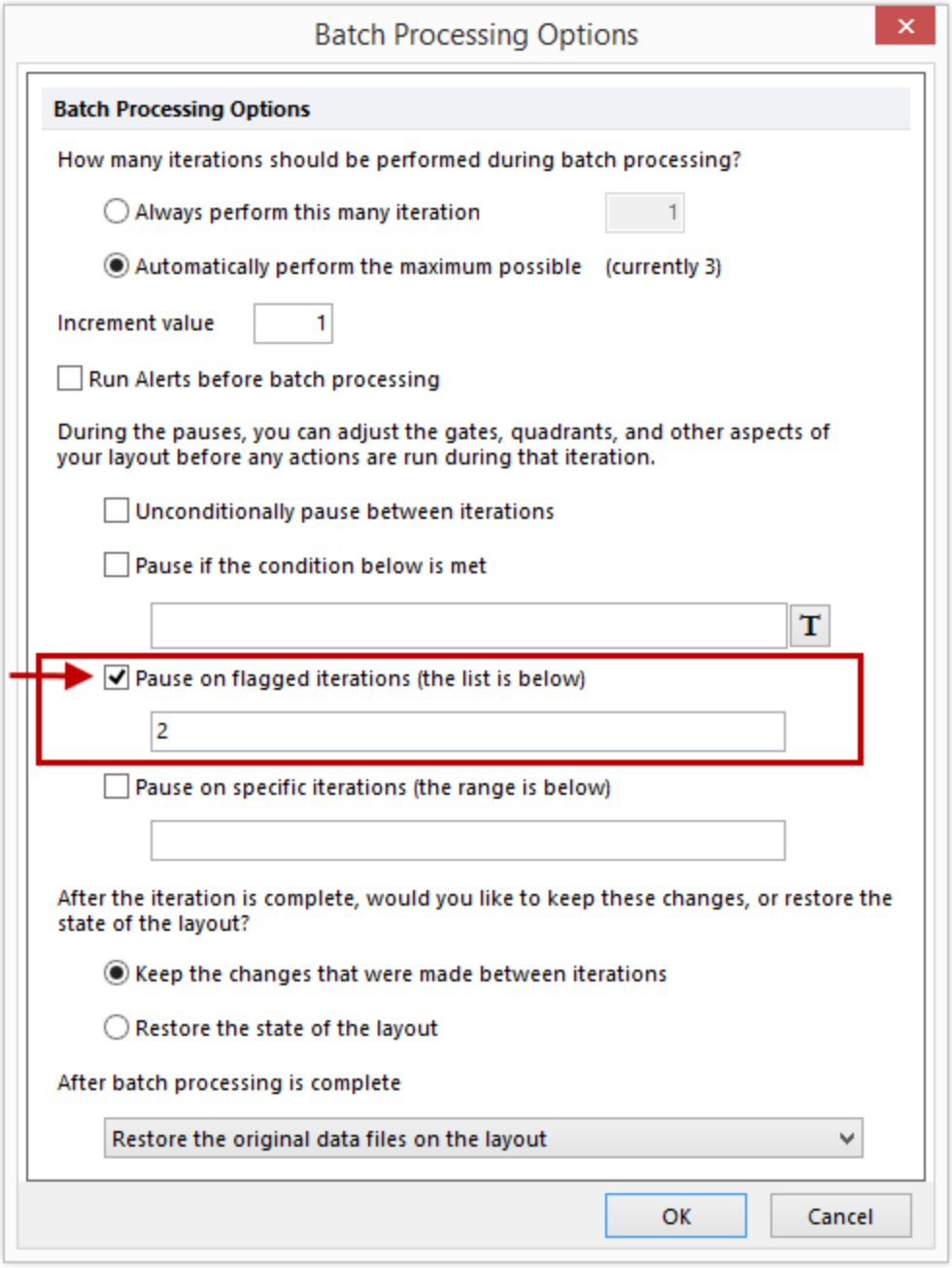

Figure 18 - Pause on Flagged Iterations

We will now review the data and the snapshots that were taken at the time of analysis without completing any of the batch actions through the Review command., go to **Batch→Batch Processing**.

4. From **Batch→Batch Processing**, click the **Review** button (Figure 19).

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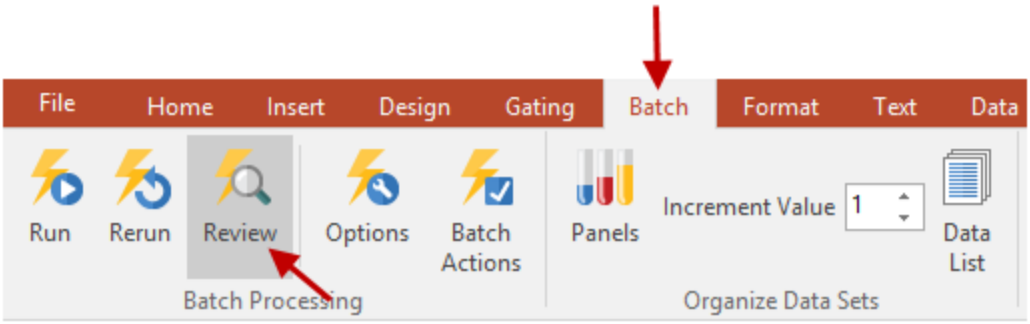

Figure 19 - Review

5. Once the Review process begins, another Batch Processing progress window will appear.

6. Make any changes that you would like to make. Click **Continue** or **Run to end** to complete the process.

38.10 Batch Processing

Batch processing allows you to analyze many data files consecutively, without having to set up the plots for each file. Batch processing also gives you the ability to export your layout and statistics in many different formats, including: printing, saving, and exporting to PowerPoint, PDF, Excel, a text file, Microsoft Word, or LabKey Server 1483.

In the first example, we will export plots to a PowerPoint file 1445.

38.10.1 Exporting Plots to PowerPoint

In the course of this example, we will:

- Create a Data List of files to be batch processed.
- Export plots to a PowerPoint file.

To Run a Batch Processing Action:

1. Select the **File** tab and click on **Open**.
2. Open the layout **BatchProcess.fey** found in the Tutorial Sample Data archive 1210.

This layout contains two 2D density plots and a histogram of the file **TCellSample1.fcs** (Figure T9.1). The left plot is of SSC-A vs. PerCP-Cy5.5-A

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with a gate on **Lymphs**. The right plot is of PE-A vs. APC-A gated on **Lymphs** with quadrants. The histogram of FITC-A is also gated on **Lymphs**.

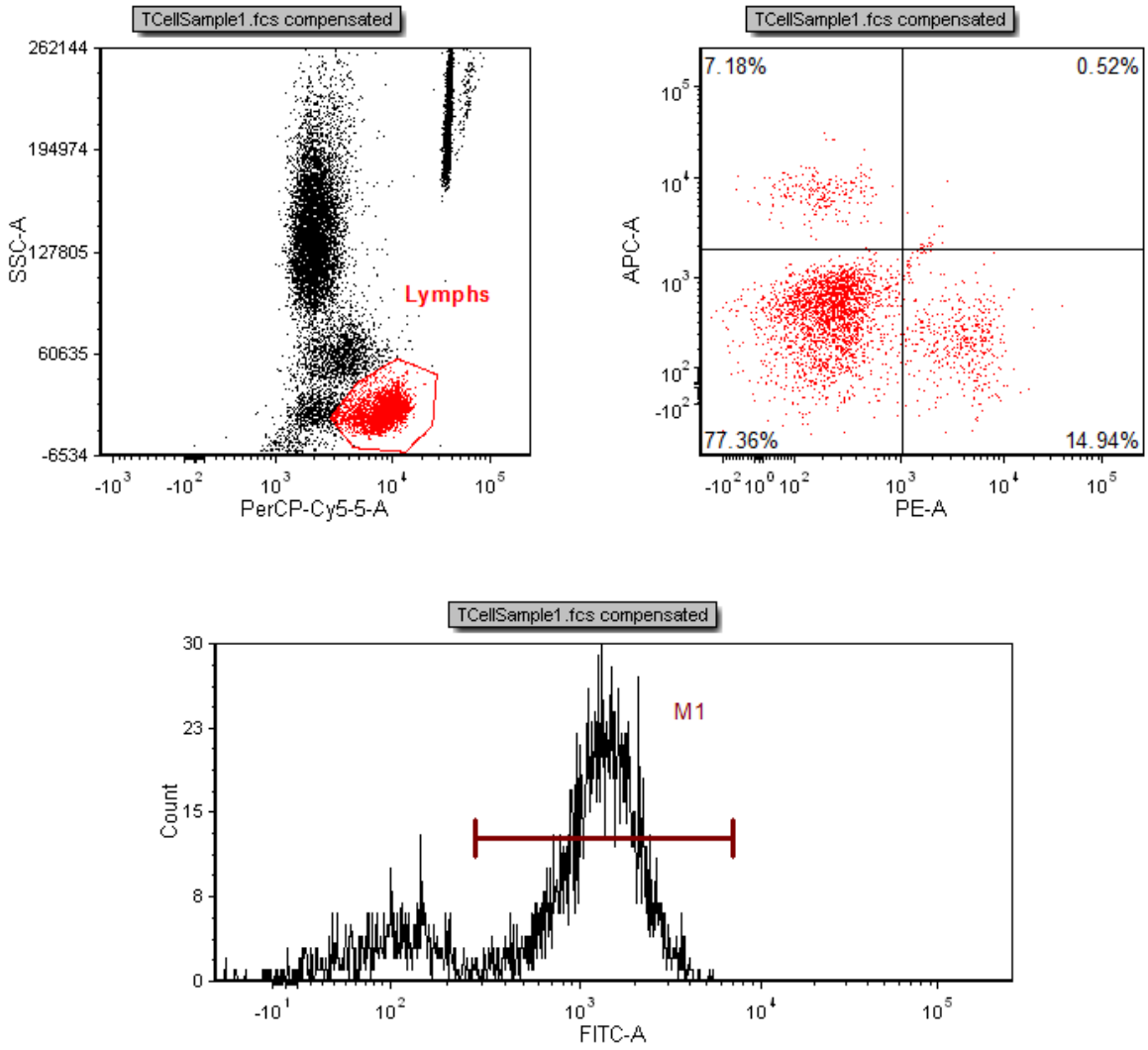

Figure T9.1 Batch Layout (BatchProcess.fey)

In order to run a batch process, you need to put files in the **Data List**.

3. Select the **Batch→Organize Data Sets→Data List** command (Figure T9.2).

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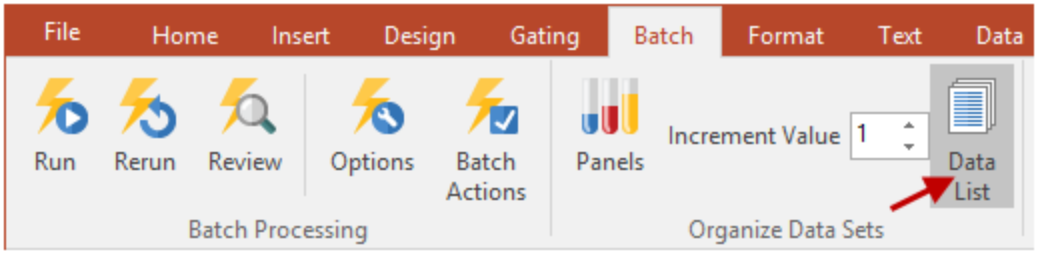

Figure T9.2 Selecting the Data List Command

A **Data List** window will appear (Figure T9.3).

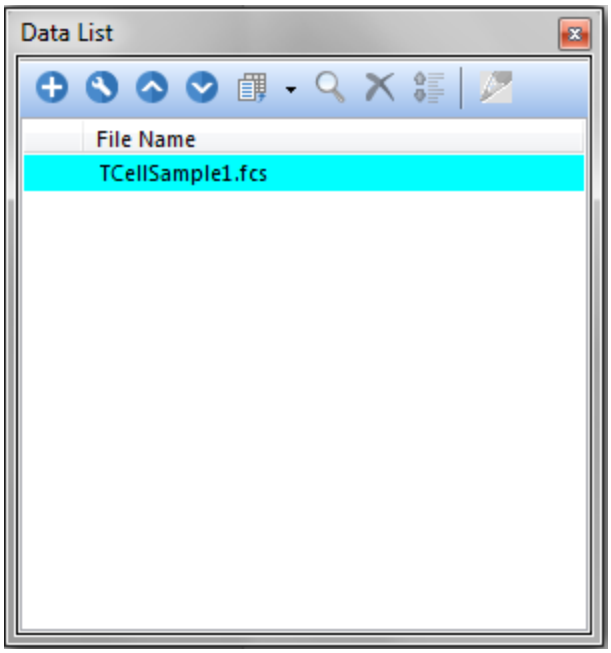

Figure T9.3 Data List Window

4. Click the green plus to add files .

A **Select a Data File** dialog will appear (Figure T9.4).

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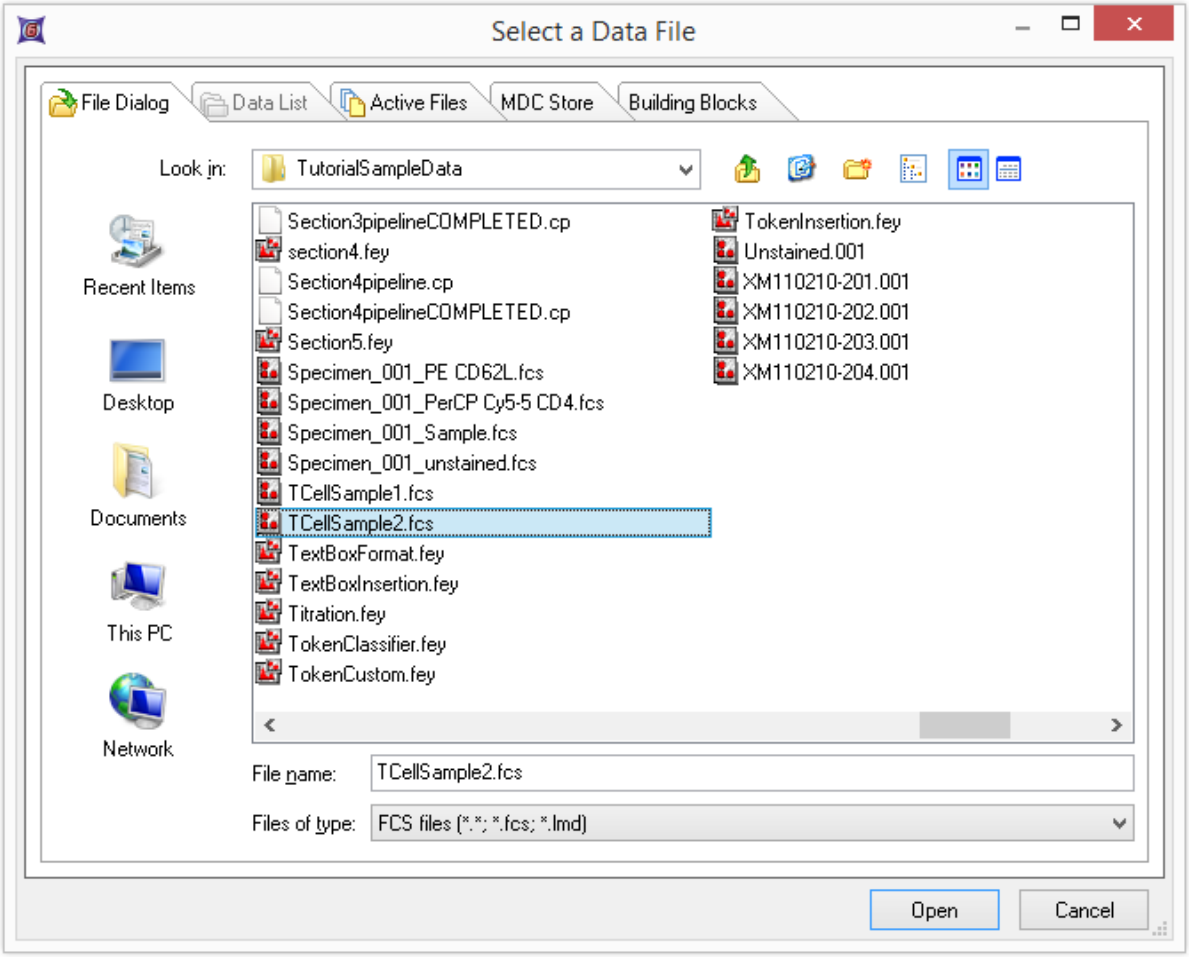

Figure T9.4 Select a Data File Dialog

5. Navigate to the location where the tutorial sample is saved and select the file **TCellSample2.fcs**.

6. Click **Open**.

The **Data List** window will show the two files (Figure T9.5).

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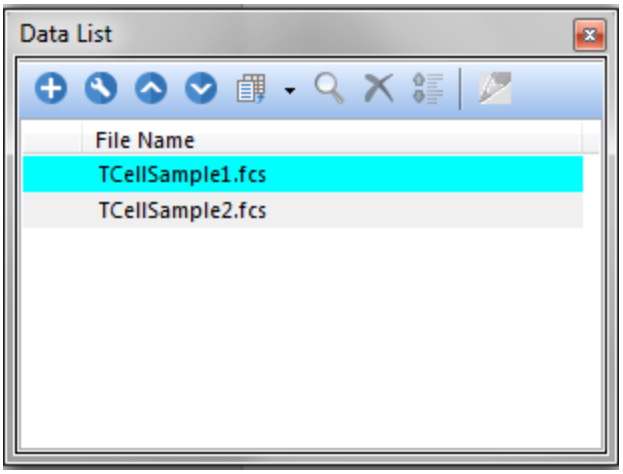

Figure T9.5 Data List with Two Files

Note: Files are processed during a batch process in the order they appear in the Data List.

So far we have told FCS Express that we want to take the **BatchProcess.fey** layout and use it to analyze the two files: TCellSample1.fcs and TCellSample2.fcs. The next step is to tell FCS Express what we want to do every time we analyze a different file.

7. Select the **Batch→Batch Processing→Batch Actions** command (Figure T9.6).

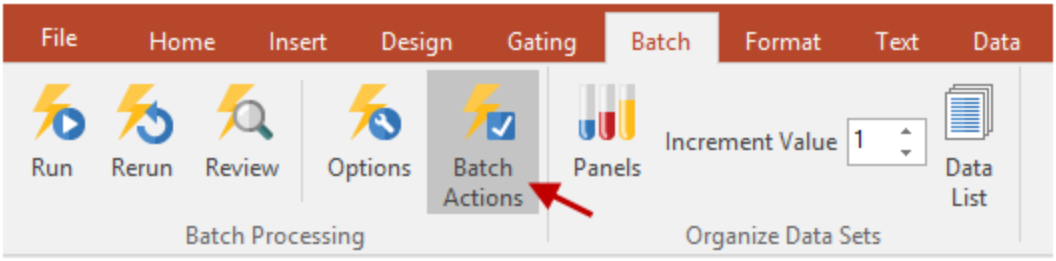

Figure T9.6 Selecting the Batch Actions Command

A **Batch Actions** window will appear (Figure T9.7). This is where you tell FCS Express what actions you want to perform during the batch process. We will now tell FCS Express that we want to save a PowerPoint file during each iteration of the batch process.

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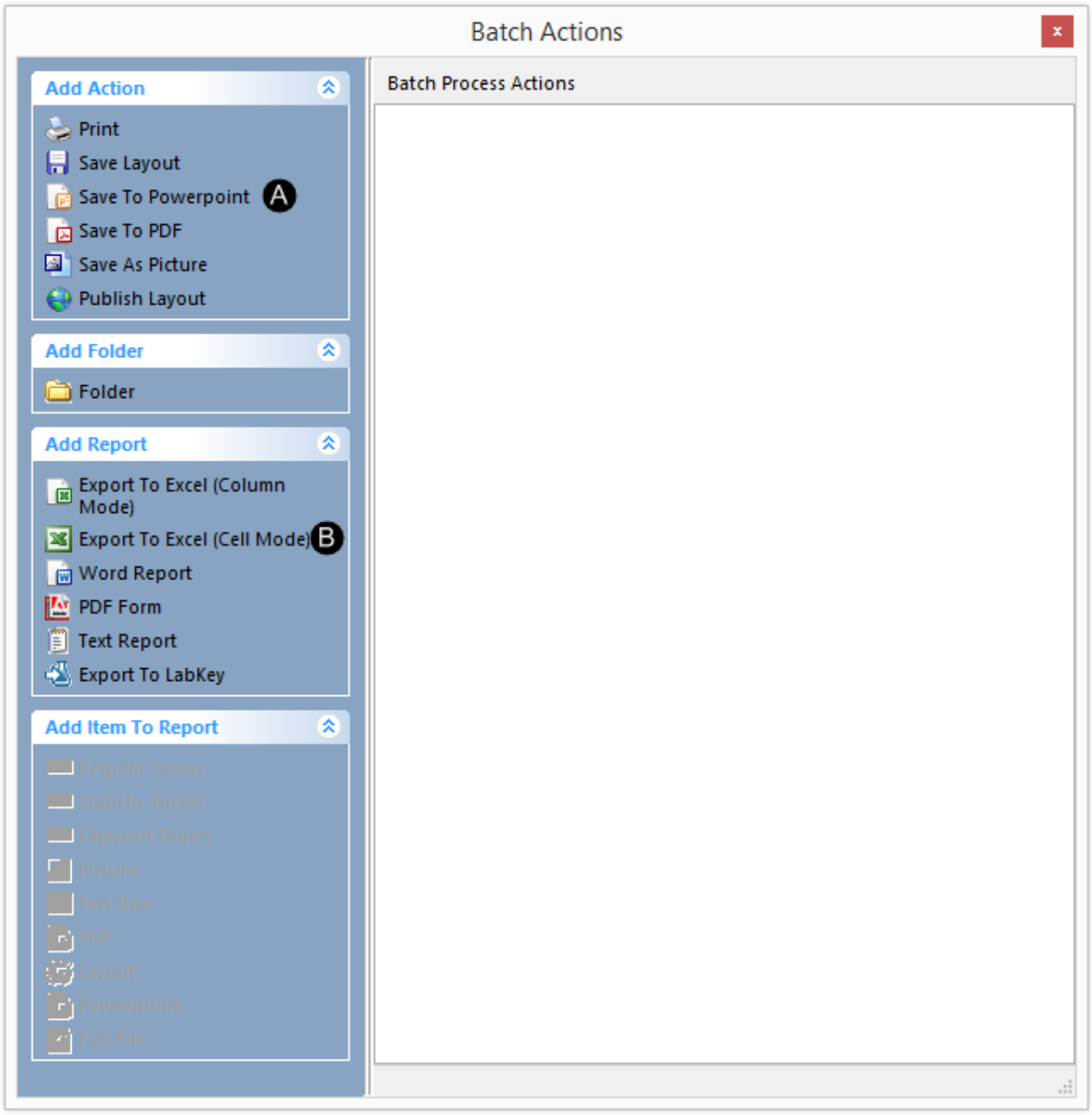

Figure T9.7 Batch Actions Window

8. Select **Save To PowerPoint** Ⓐ from the **Add Action** category (Figure T9.7).

A **Create Export To Powerpoint Action** dialog will appear (Figure T9.8).

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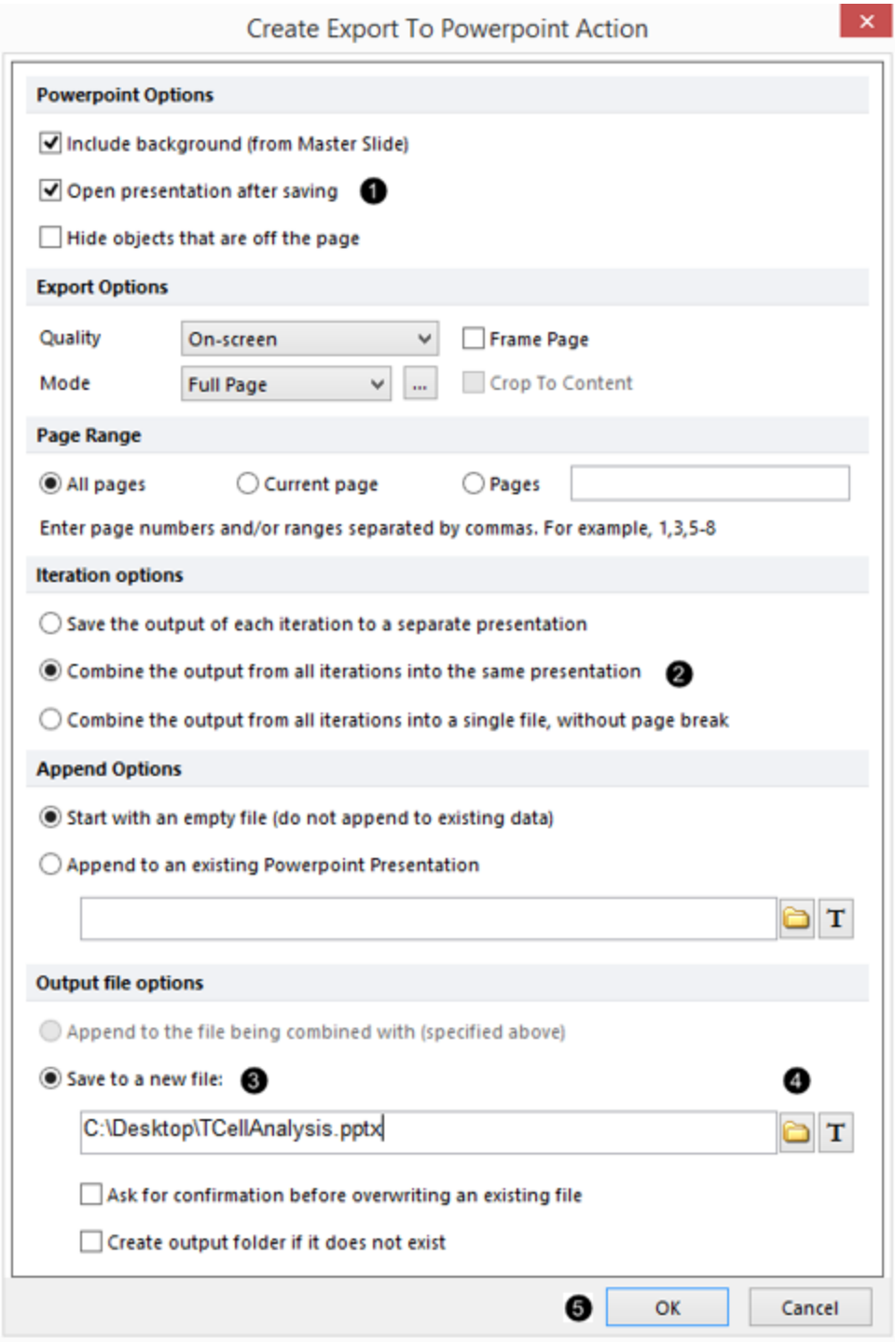

Figure T9.8 Create Export to PowerPoint Action Dialog

9. Select the option to **Open presentation after saving** ❶.

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10. Select **Combine the output from all iterations into the same presentation** from the **Iteration options** section ❷ .
11. Select **Save to a new file** ❸ from the **Output file options** section and click on the folder icon ❹ to choose where the file will be saved, such as your Desktop, and give your file a name.
12. Click **OK** ❺.

The **Batch Actions** window will now be updated to reflect the export to PowerPoint (Figure T9.9) from the **Add Action** category (Figure T9.7).

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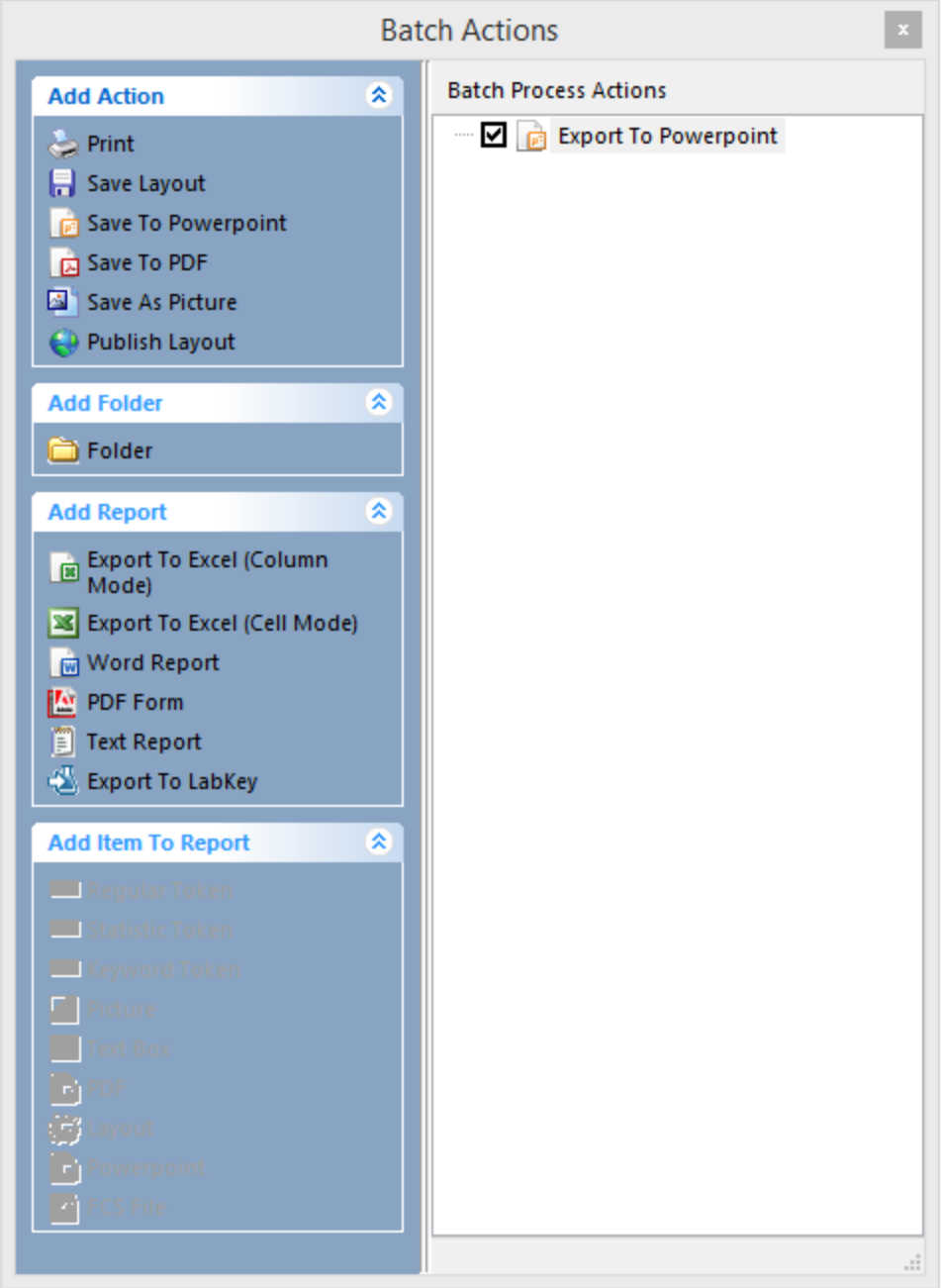

Figure T9.9 Batch Actions Window Updated with Export to PowerPoint Action

13. Select the **Batch→Batch Processing→Run →Run** command (Figure T9.10).

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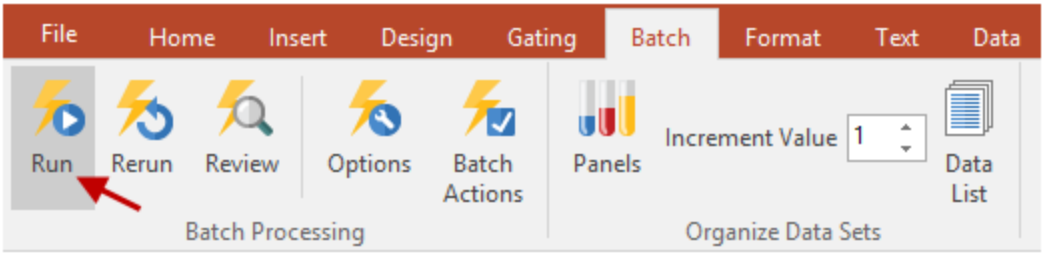

Figure T9.10 Selecting the Run Batch Process Command

FCS Express will now run the batch process. After a series of automated steps, PowerPoint will launch and open the file **TCellAnalysis.pptx**. The file will consist of two pages: the first will be a slide showing the layout with the data file **TcellSample1.fcs** (Figure T9.11) and the second the layout with the data file **TcellSample2.fcs** (Figure T9.12).

Note about ungrouping objects in PowerPoint for PC versus PowerPoint for Mac

Once exported to PowerPoint, layout objects can be further ungrouped into single components in the PC version of Microsoft PowerPoint. Ungrouping objects saved by FCS Express in PowerPoint for Mac is not supported. However, presentations saved on a Mac may be opened on a PC and ungrouped.

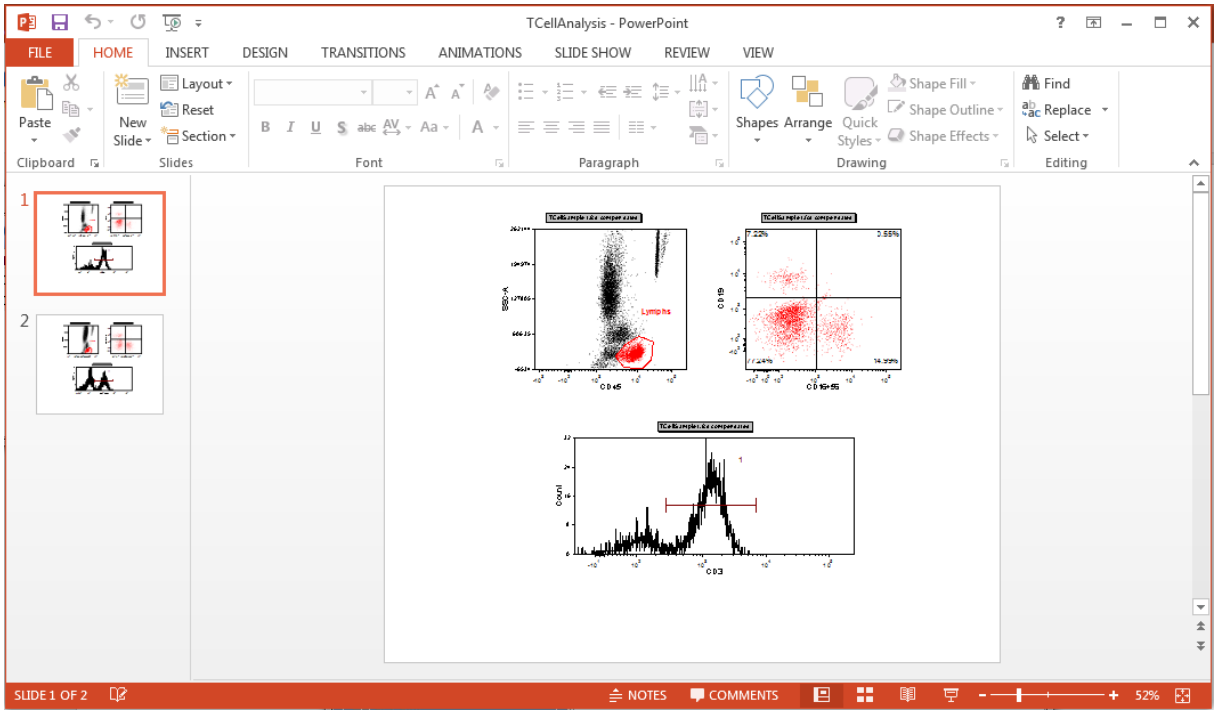

Figure T9.11 Slide 1 in PowerPoint

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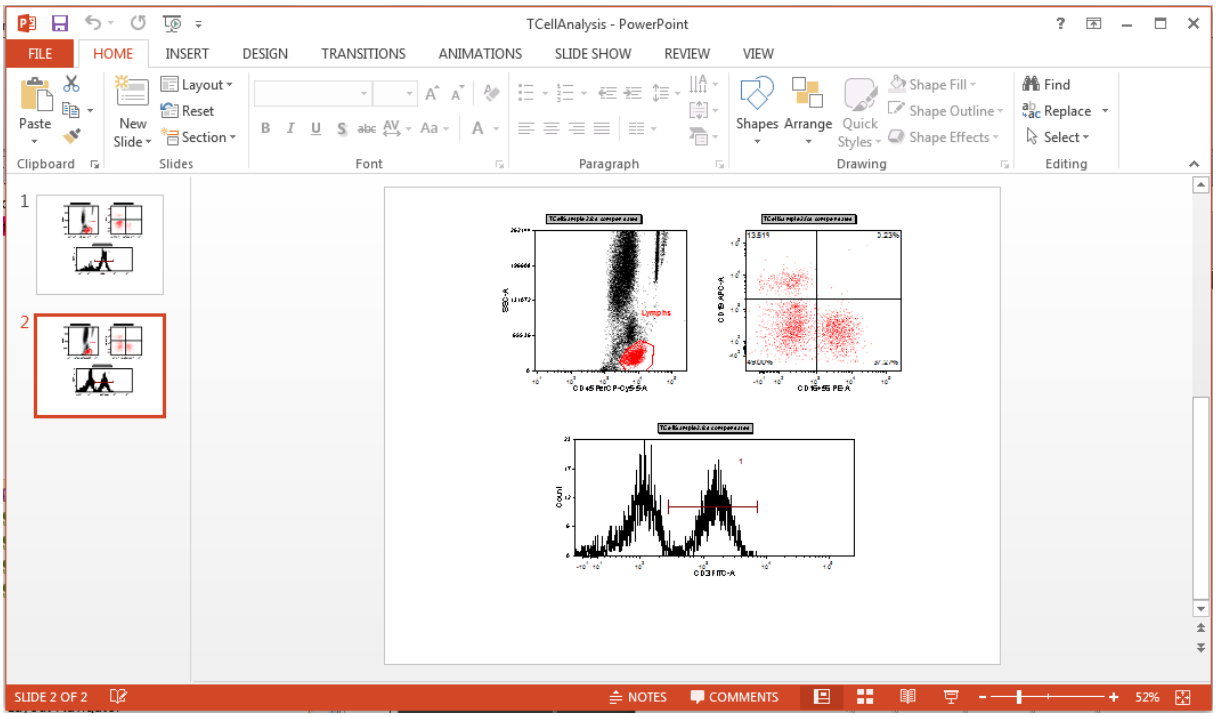

Figure T9.12 Slide 2 in PowerPoint

The next section will continue using this layout, so you can keep it open. Otherwise, please feel free to save it so that you can start the next section where you left off.

In the next section, we will export to an Excel report (Cell Mode 1455).

38.10.2 Excel Report (Cell Mode)

In the course of this example we will:

- Create an Excel – Cell Mode Report.
- Export the $FIL keyword.
- Export the $DATE keyword.
- Export a Statistic.
- Export a Picture.
- Run a batch process.

For convenience, we are going to start with the same layout as before. The images contained within this section will continue with the layout from the previous section. However, you can add reports to layouts that already have actions defined. If you have the previous layout still open, please jump to Step 5.

1. Select the **File** tab and click on **Open**.

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2. Open the layout **BatchProcess.fey** found in the Tutorial Sample Data archive 1210.

To Perform an Excel Report Batch Process:

3. Create a **Data List** with files **TCellSample1.fcs** and **TCellSample2.fcs** (see the previous section 1445).

4. Select the **Batch→Batch Processing→Batch Actions** command (Figure T9.6 1445).

A **Batch Actions** window will appear (Figure T9.7).

5. Select **Export to Excel (Cell Mode)** (Figure T9.7 1445) from the **Add Report** category.

An **Excel (Cell Mode) Options** dialog will appear (Figure T9.13). We will use this dialog to set up the batch export of files in the **Data List** to Excel.

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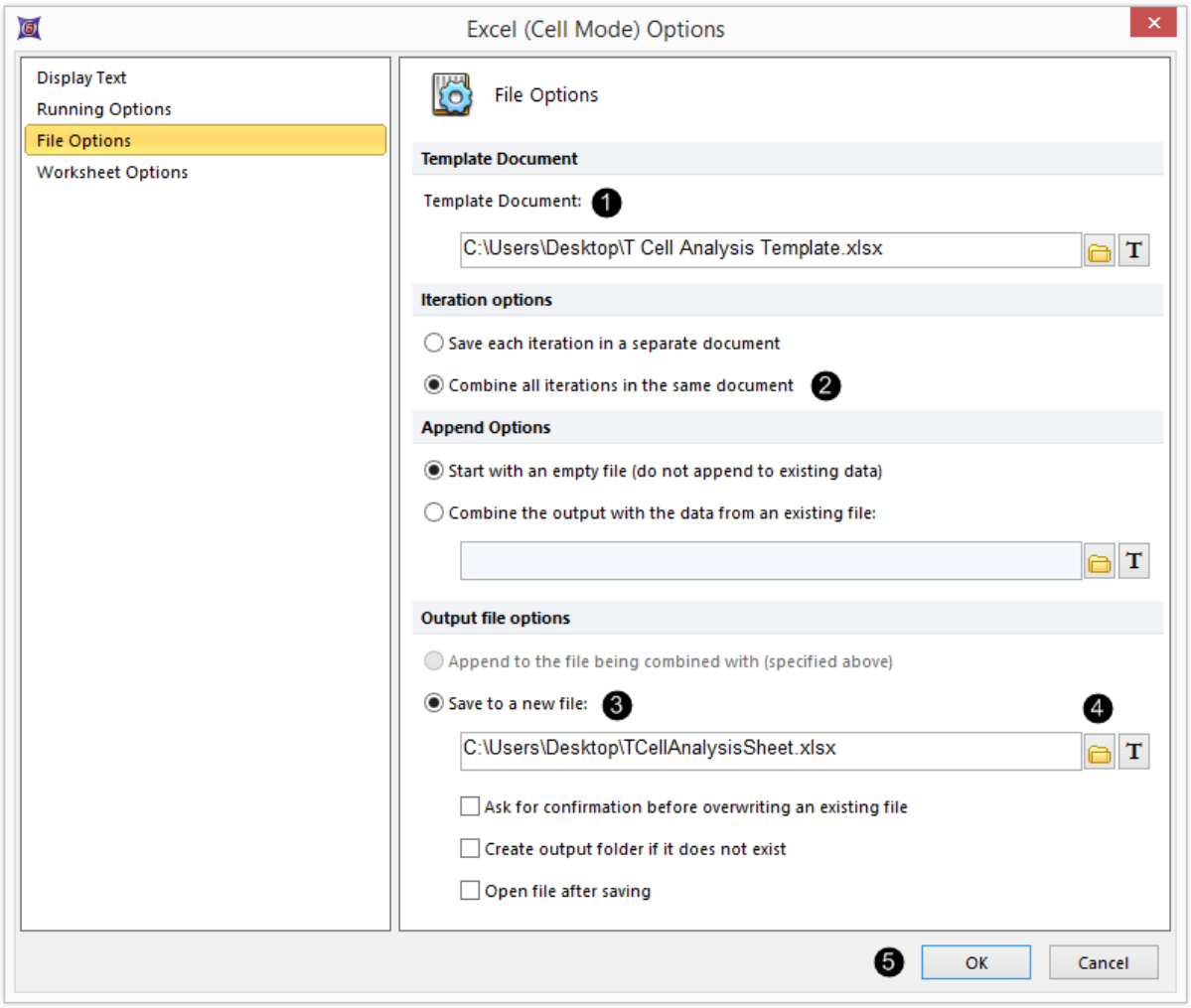

Figure T9.13 Excel (Cell Mode) Options Dialog

6. Click on the Open File Icon in the **Template Document:** field ❶ (Numbers refer to Figure T9.13).

7. Select **T Cell Analysis Template.xlsx** from *FCS Express Tutorial Sample Data* folder.

T Cell Analysis Template.xlsx is an Excel file that contains the background for your report. The Excel template we are using is shown in Figure T9.14.

8. Click on **Combine all iterations in the same document** in the **Iteration options** section ❷.

9. Click on **Save to a new file** from the **Output file options** section ❸.

10. In the **Save to a new file** field, click on the Folder icon to specify the output location of your Excel Report ❹, such as your desktop.

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11. Click **OK** ❺.

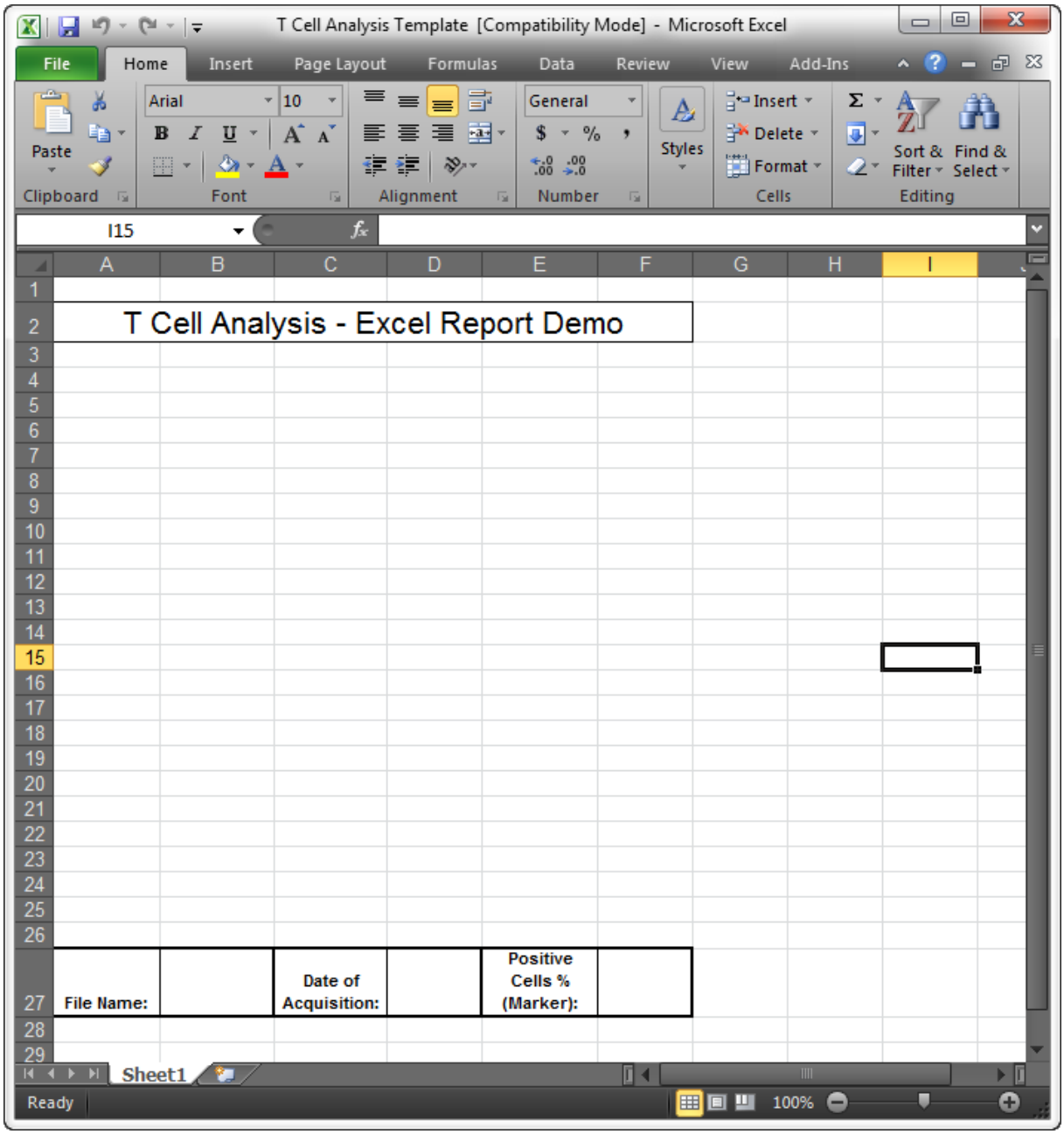

Figure T9.14 T Cell Analysis Excel Template

The **Batch Actions** window will show the export to **Excel (Cell Mode)** action listed (Figure T9.15). This image also shows the **Save To Powerpoint** Batch Action from the last section.

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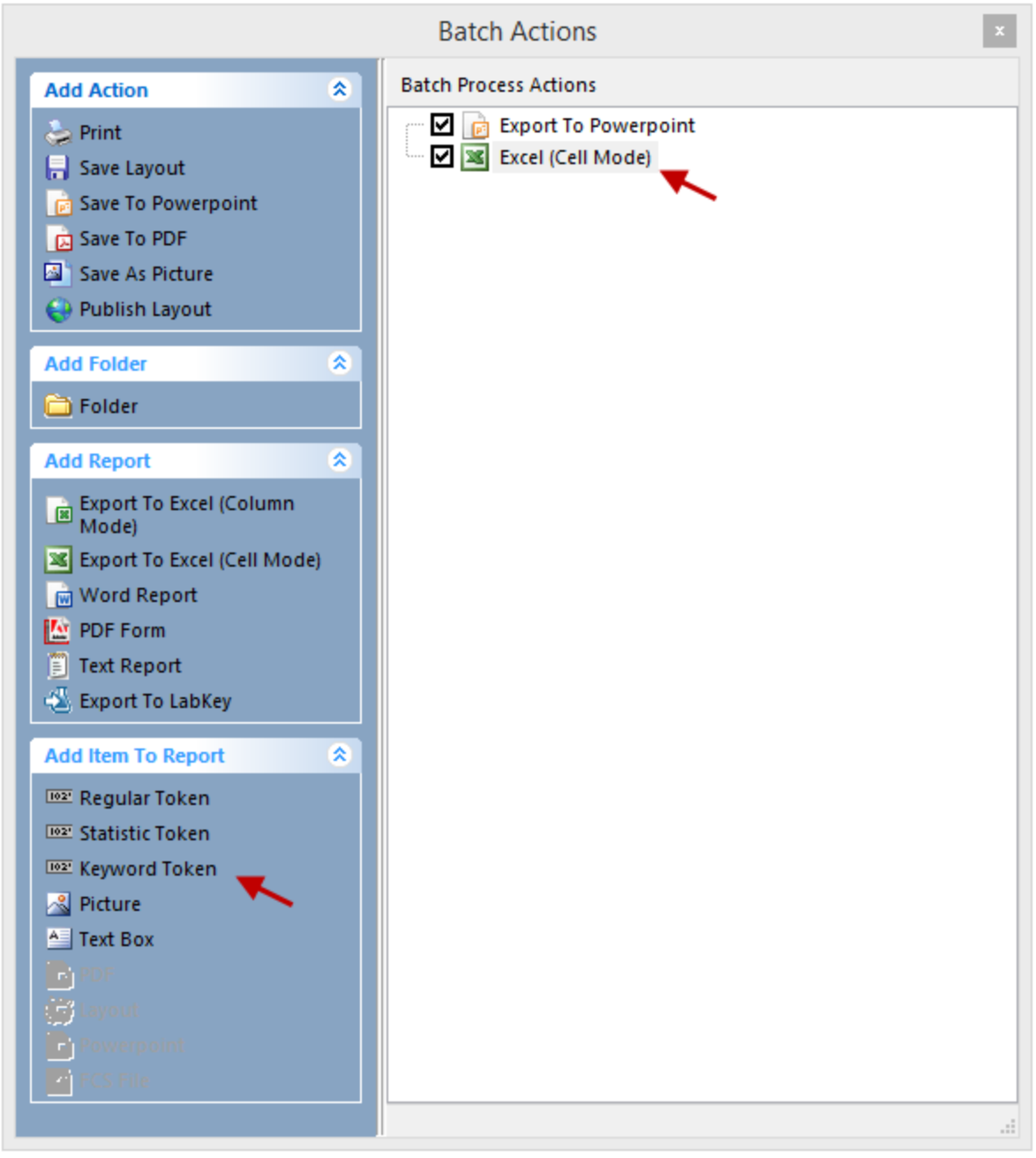

Figure T9.15 Batch Actions Window with the Export To Excel Action

12. Select **Keyword Token** from the **Add Item to Report** category (cursor in Figure T9.15).

An **Excel (Cell Mode) field "Keyword" options** dialog will appear showing the **Data Source** category (Figure T9.16). This is where we will define the "keywords" to export to Excel.

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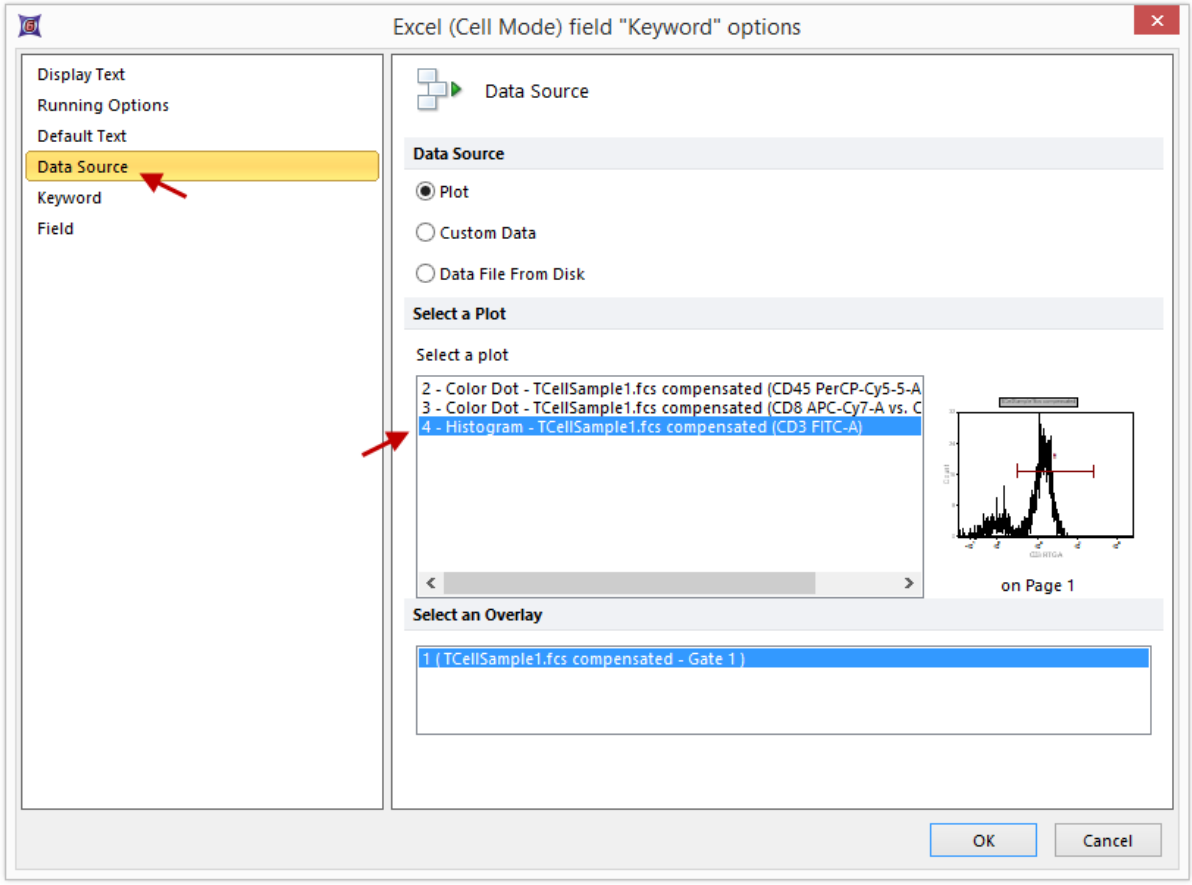

Figure T9.16 Excel (Cell Mode) field "Keyword" options Dialog

13. Under the Data Source category, select **4 - Histogram - TCellSample1.fcs compensated (FITC-A)**.

14. Click on the **Keyword** category. (Figure T9.16).

The **Keyword** category page will appear (Figure T9.17).

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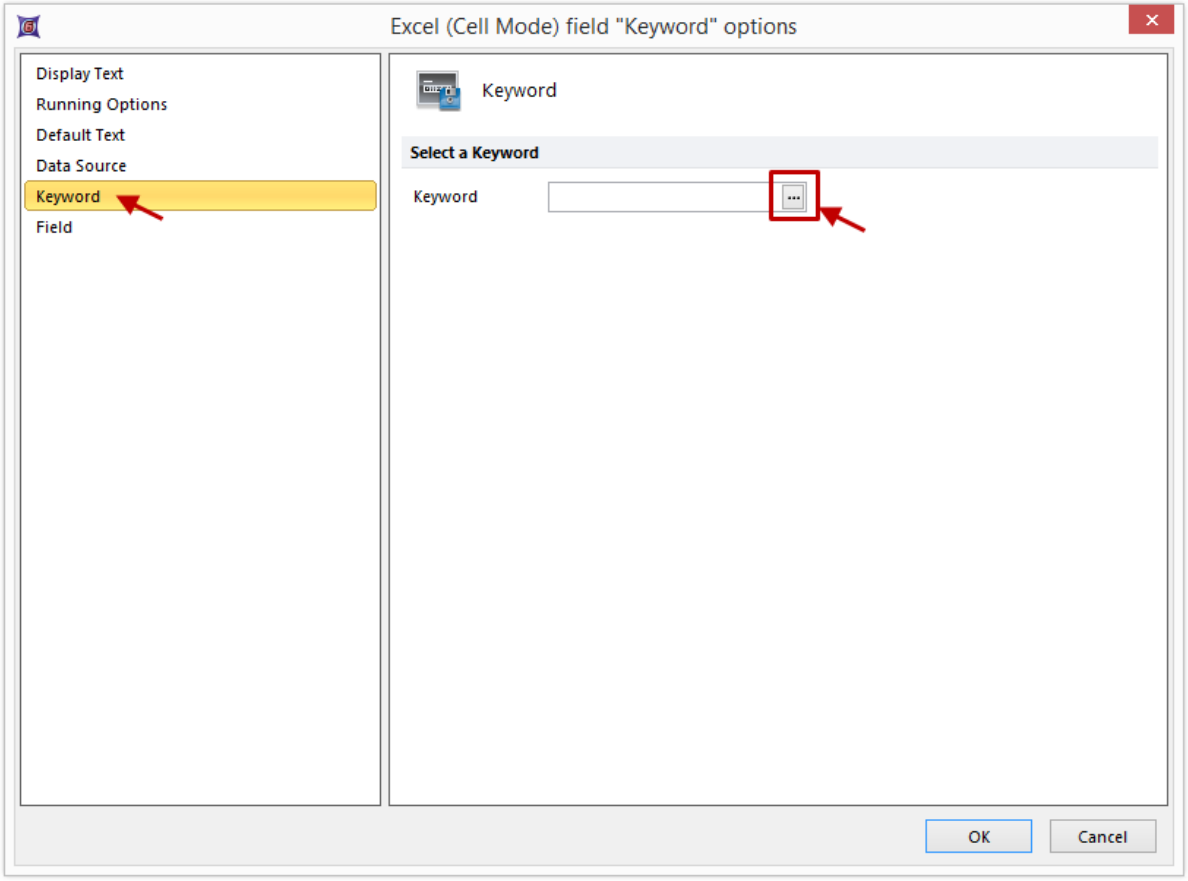

Figure T9.17 Excel (Cell Mode) field "Keyword" options Dialog -- Keyword Category

15. Click on the ellipsis (**...**) button to the right of the field **Select a keyword** (Figure T9.17).

The **Please select a keyword** dialog will appear (Figure T9.18). This dialog displays the keywords found in the FCS file.

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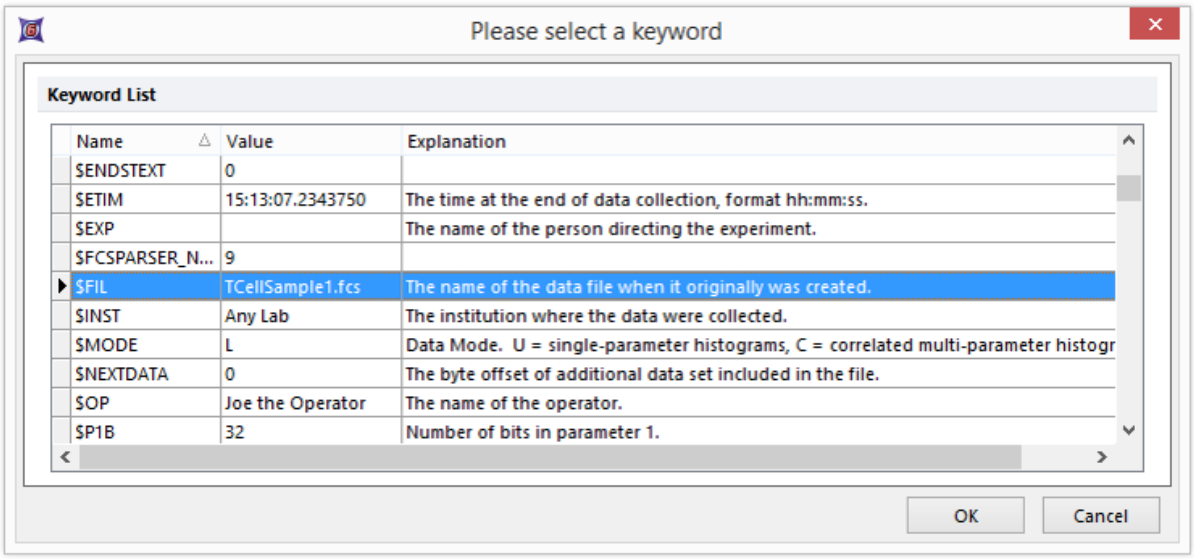

Figure T9.18 Dialog Displaying Keywords Found in the FCS File

16. Select the keyword **$FIL** (Figure T9.18), which is the filename keyword stored in the FCS File.

17. Click **OK**.

18. Click on the **Field** category of the **Excel (Cell Mode) field "Keyword" options** dialog (Figure T9.19).

The **Field** page of the **Excel (Cell Mode) field "Keyword" options** dialog will appear. This is where we select the Excel cell address that the **$FIL** keyword data will be batch exported to.

19. Type **B27** in the **Cell Address** field (Figure T9.19). This tells FCS Express that we want to put the FCS file name in cell B27 of the Excel template (Figure T9.14).

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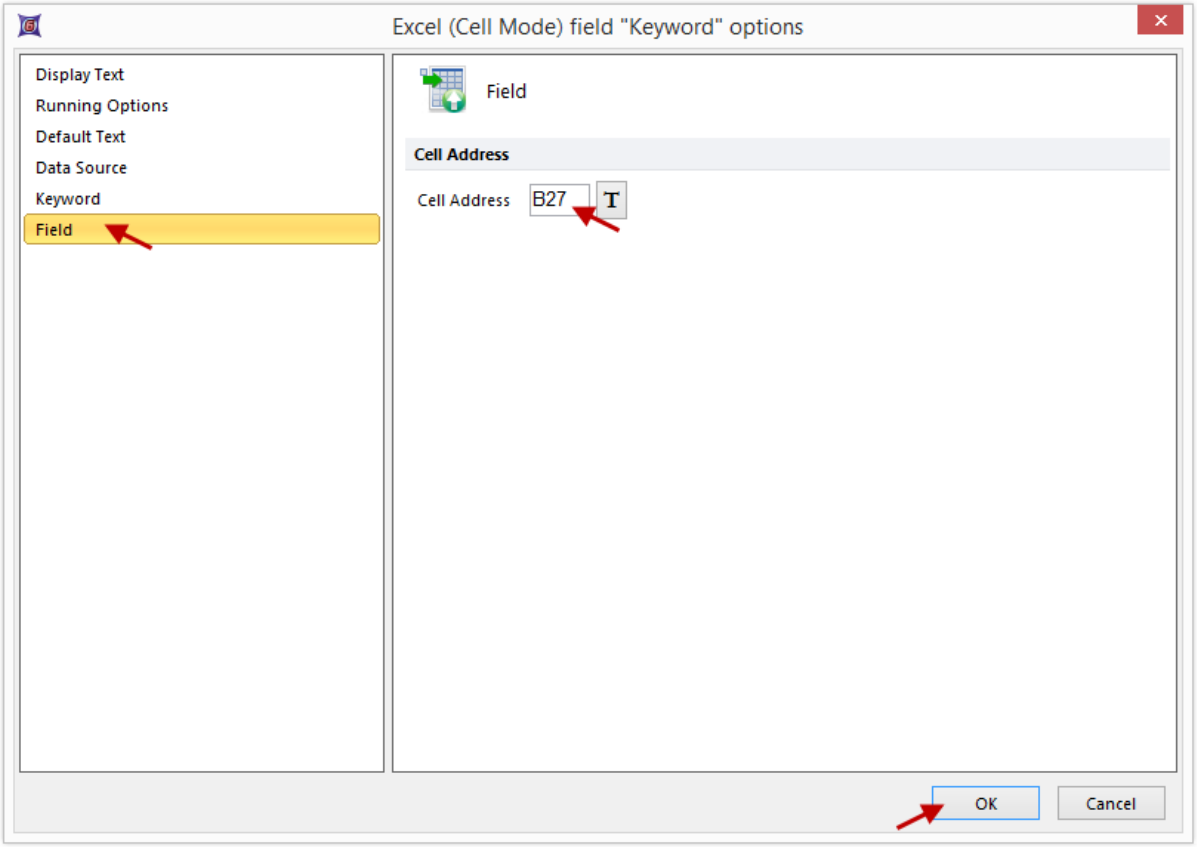

Figure T9.19 Setting the Excel Report Cell Address

20. Click **OK**.

The **Batch Actions** window will show the action **Keyword: $FIL** added (Figure 9.20A).

You can also select a token associated with a plot to batch export to Excel by dragging and dropping the plot or gate of interest from the layout page onto the **Batch Process Actions** section of **the Batch Actions** dialog.

21. Select the bottom histogram plot by clicking on the edge of the plot. You will know it is selected because it will be outlined in red.

22. Hold down the left mouse button and drag the histogram plot over to the **Batch Process Actions** section of the **Batch Actions** dialog (Figure T9.20A).

23. Release the mouse button when the plot is on top of the **Excel (Cell Mode)** text inside the **Batch Process Actions** section (Figure T9.20A). A **Paste Special** dialog box will appear (Figure T9.20B).

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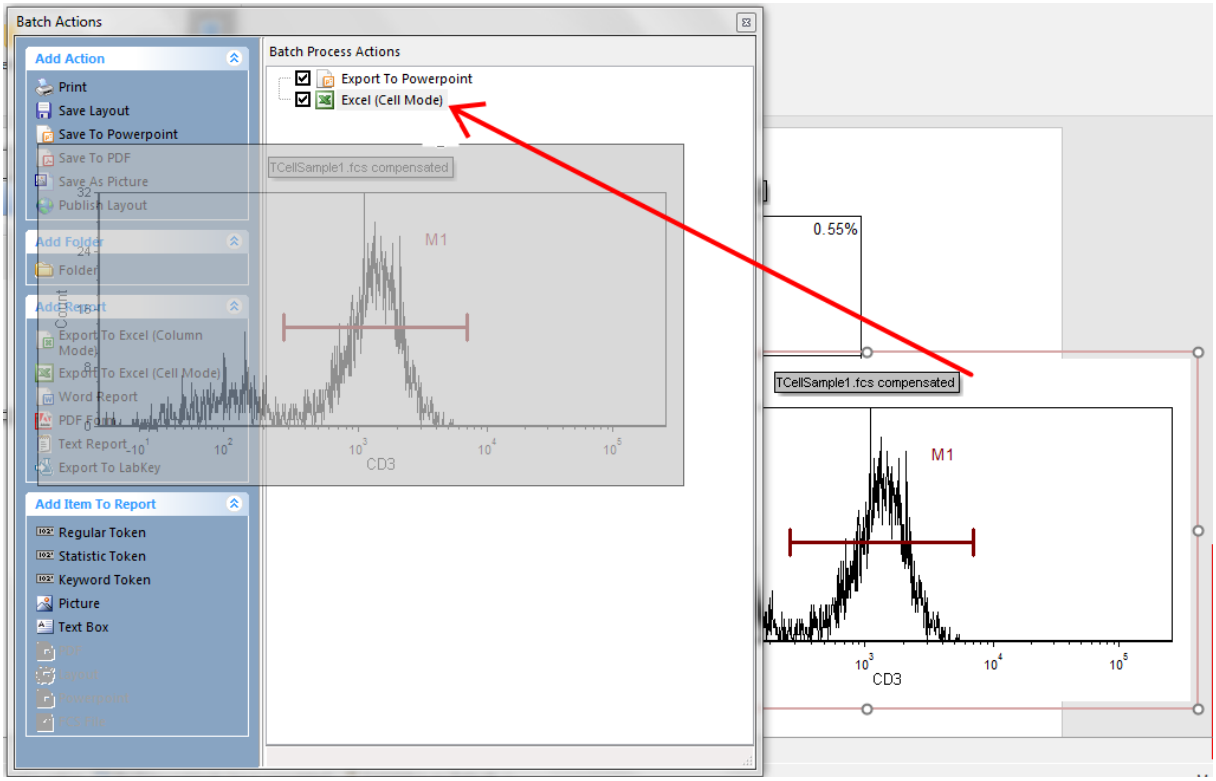

Figure T9.20A Dragging and Dropping a Histogram onto the Batch Actions Window

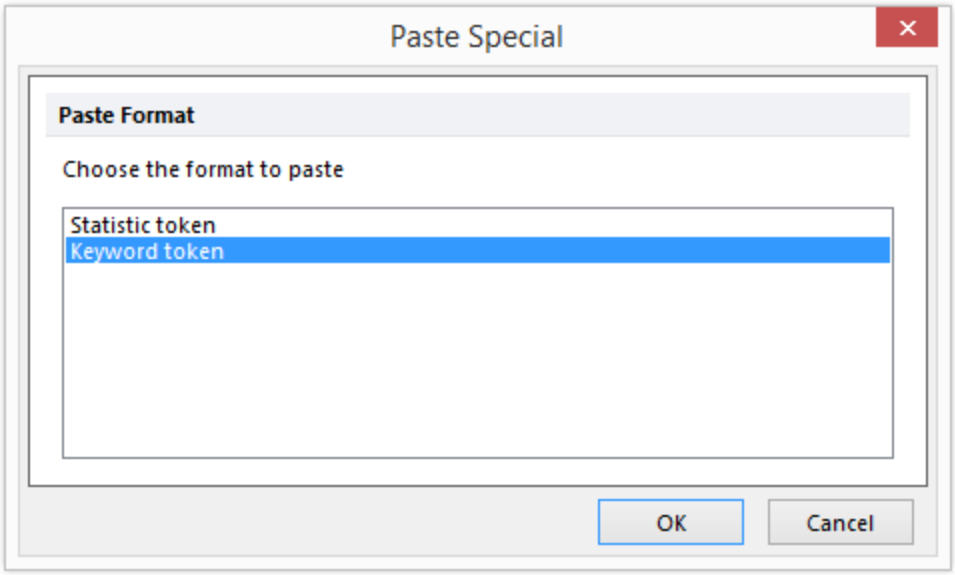

Figure T9.20B Paste Special Dialog

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24. Select the **Keyword token** item and click **OK** (Figure T9.20B).
25. The **Keyword** category page will appear (Figure T9.17)
26. Click on the **Keyword** category.
27. Click on the ellipsis (…) button to the right of the field **Select a keyword** (Figure T9.17).

A **Please select a keyword** dialog will again appear (Figure T9.21).

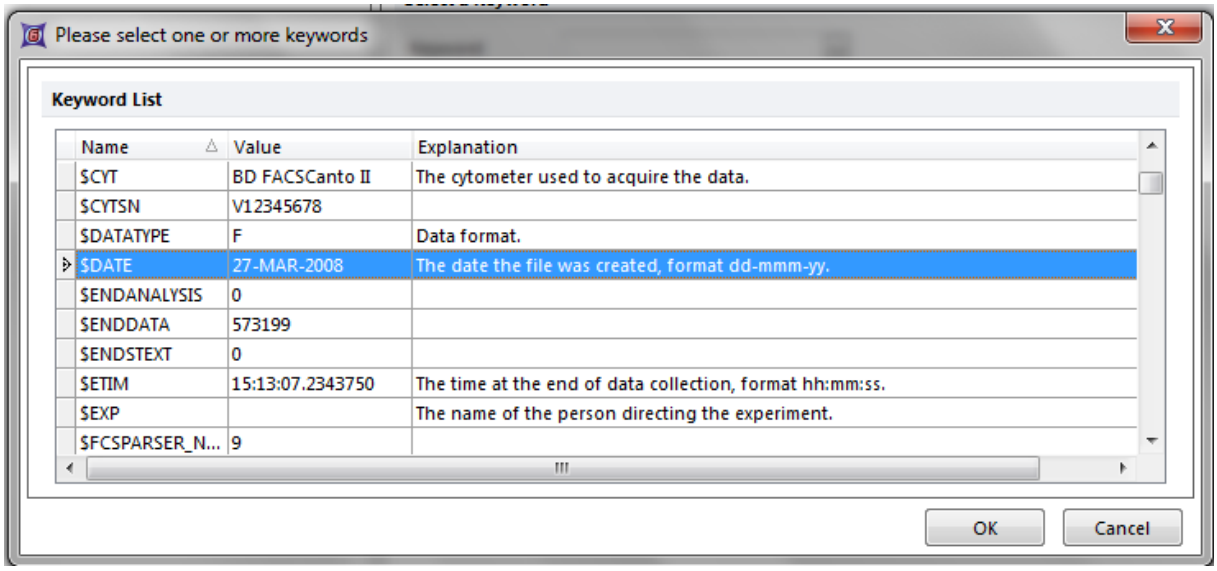

Figure T9.21 Inserting the $DATE Keyword

28. Select the keyword **$DATE** (Figure T9.21)**.**
29. Click **OK** (Figure T9.21).
30. Click on the **Field** category from the **Excel (Cell Mode) field "Keyword" options** dialog (Figure T9.22).

The **Field** page of the **Excel (Cell Mode) field "Keyword" options** dialog will appear again.

31. Type **D27** in the **Cell Address** field (Figure T9.22).

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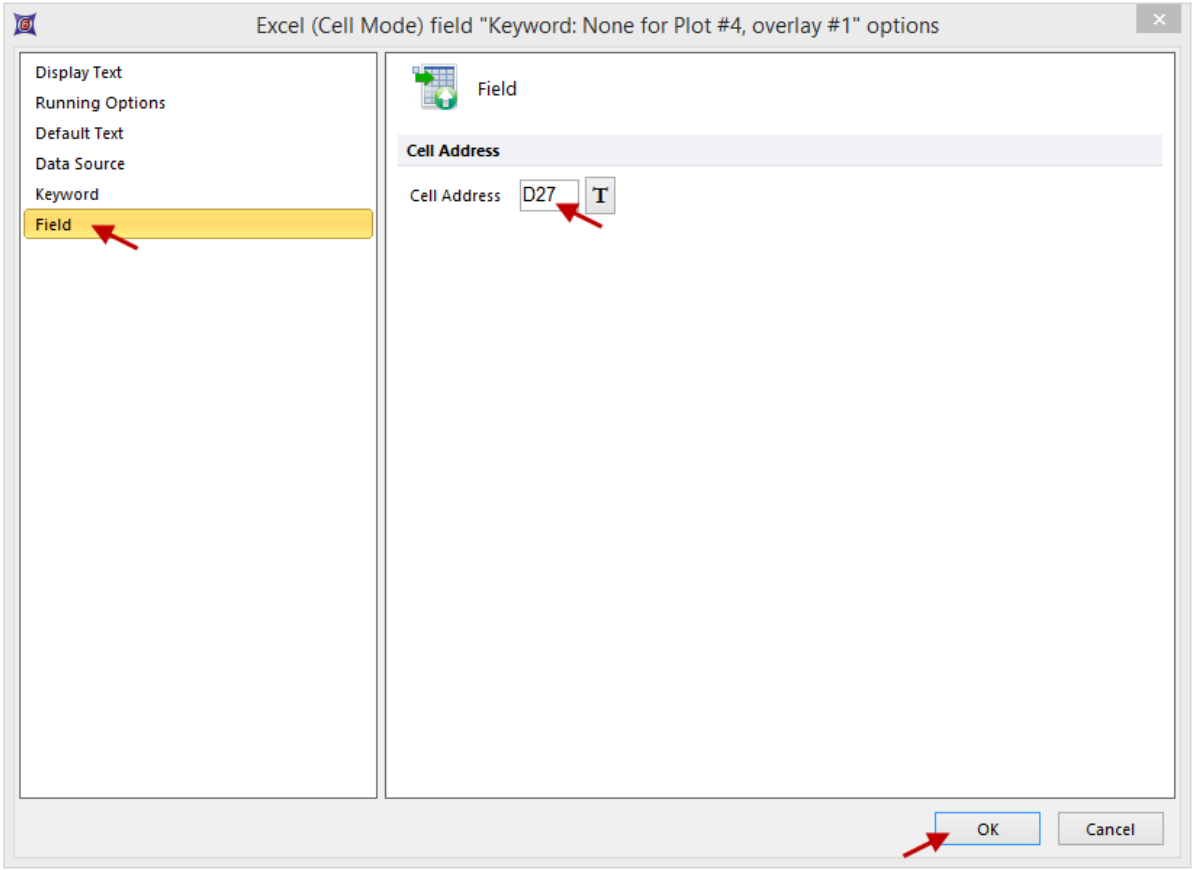

Figure T9.22 Cell Address Field Defined for Batch Export of $DATE Keyword to Excel

32. Click **OK** (Figure T9.22).

The **Batch Actions** window will show the action **Keyword: $DATE** added (Figure T9.23).

This means that if the batch analysis were run now, using the files in the Data List, the file name, saved in the FCS keyword $FIL, and the date, saved in the FCS keyword $DATE, would be exported to cells B27 and D27, respectively, of the Excel file previously specified in Figure T9.13.

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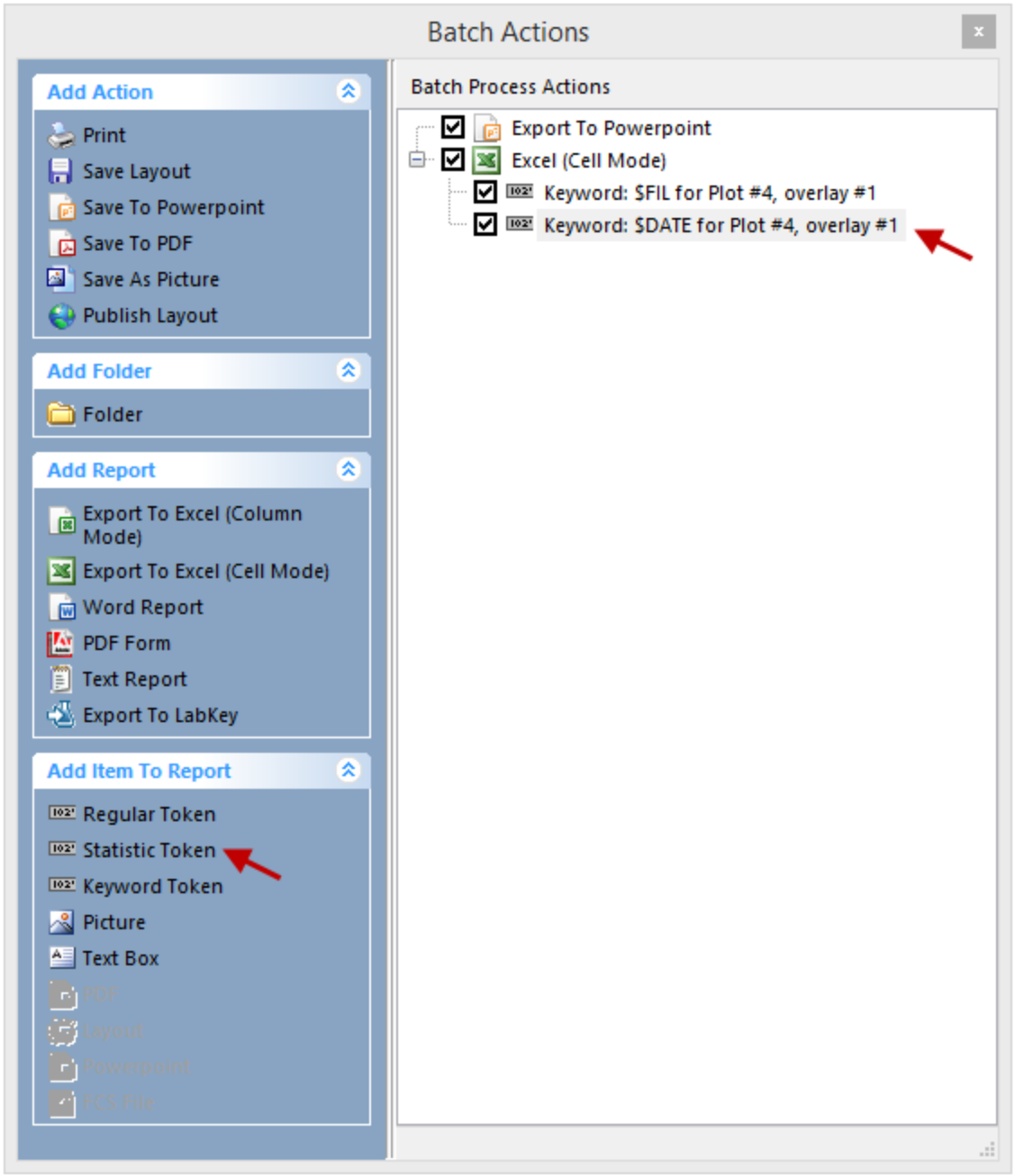

Figure T9.23 Batch Actions Window with $DATE Keyword Exported

We are now going to select a Statistic to batch export to the Excel file.

33. Select **Statistic Token** from the **Add Item to Report** category of the **Batch Actions** window (Figure T9.23).

An options dialog will appear, as previously shown in Figure T9.16.

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34. Click on the **Data Source** category (Figure T9.16).

35. Select **4 - Histogram - TCellSample1.fcs compensated (FITC-A)** from the **Select a plot** field (Figure T9.16).

36. Click on the **Statistic** Category (on the left). The **Statistic** page of the **Excel (Cell Mode) field "Statistics" options** dialog will appear (Figure T9.24). This is where we will select a Statistic for the histogram plot that we want to batch export to Excel.

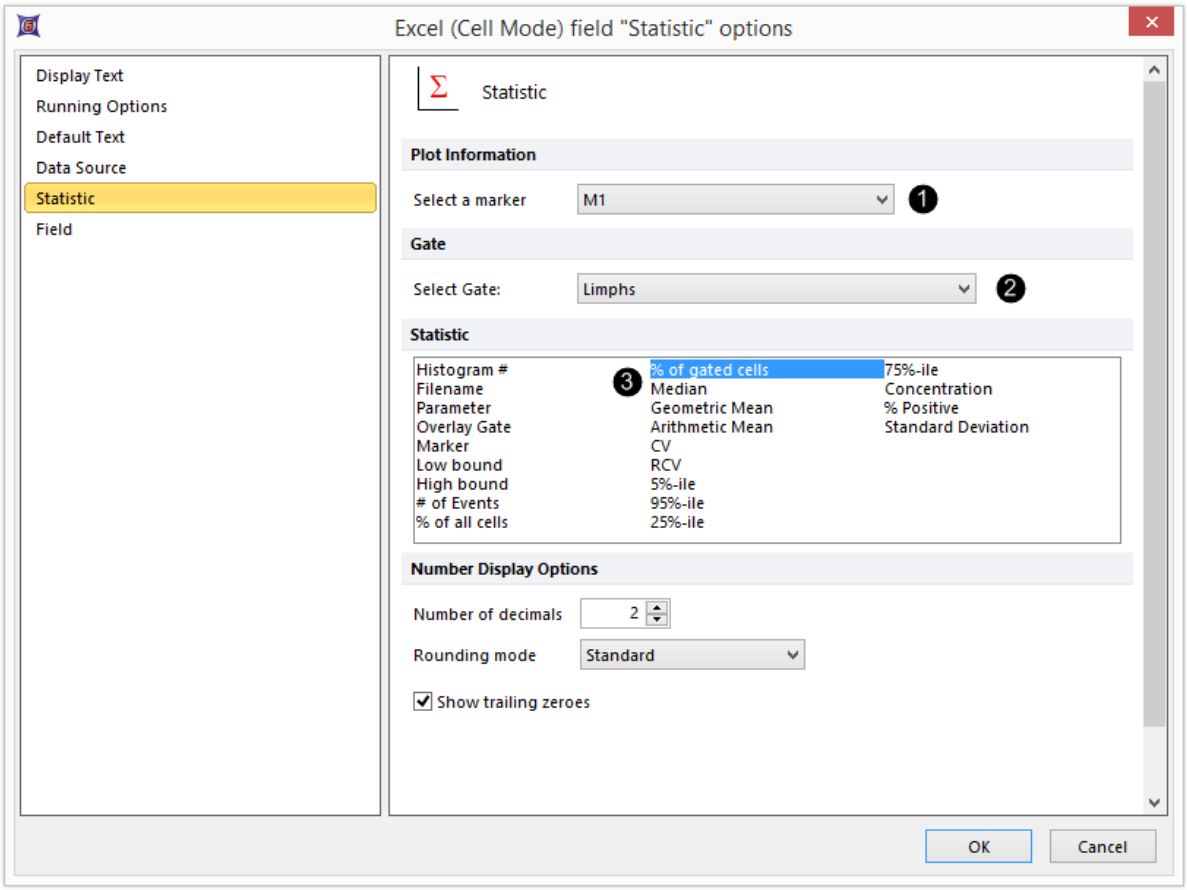

Figure T9.24 Selecting a Statistic to Export

37. Select the marker **M1** from the **Plot Information** drop-down list ❶.

38. Select the Gate **Lymphs** from the **Gate** drop-down list ❷.

39. Select the Statistic **% of gated cells** from the **Statistic** list box ❸.

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40. Click on the **Field** category (on the left). The **Field** category of the **Excel (Cell Mode) field "Statistics" options** dialog will appear (Figure T9.25). This is where we select the Excel cell address that the statistic data will be batch exported to.

Type **F27** in the **Cell Address** field (Figure T9.25). This is telling FCS Express that we want to put the statistic in cell F27 of the Excel template (Figure T9.14).

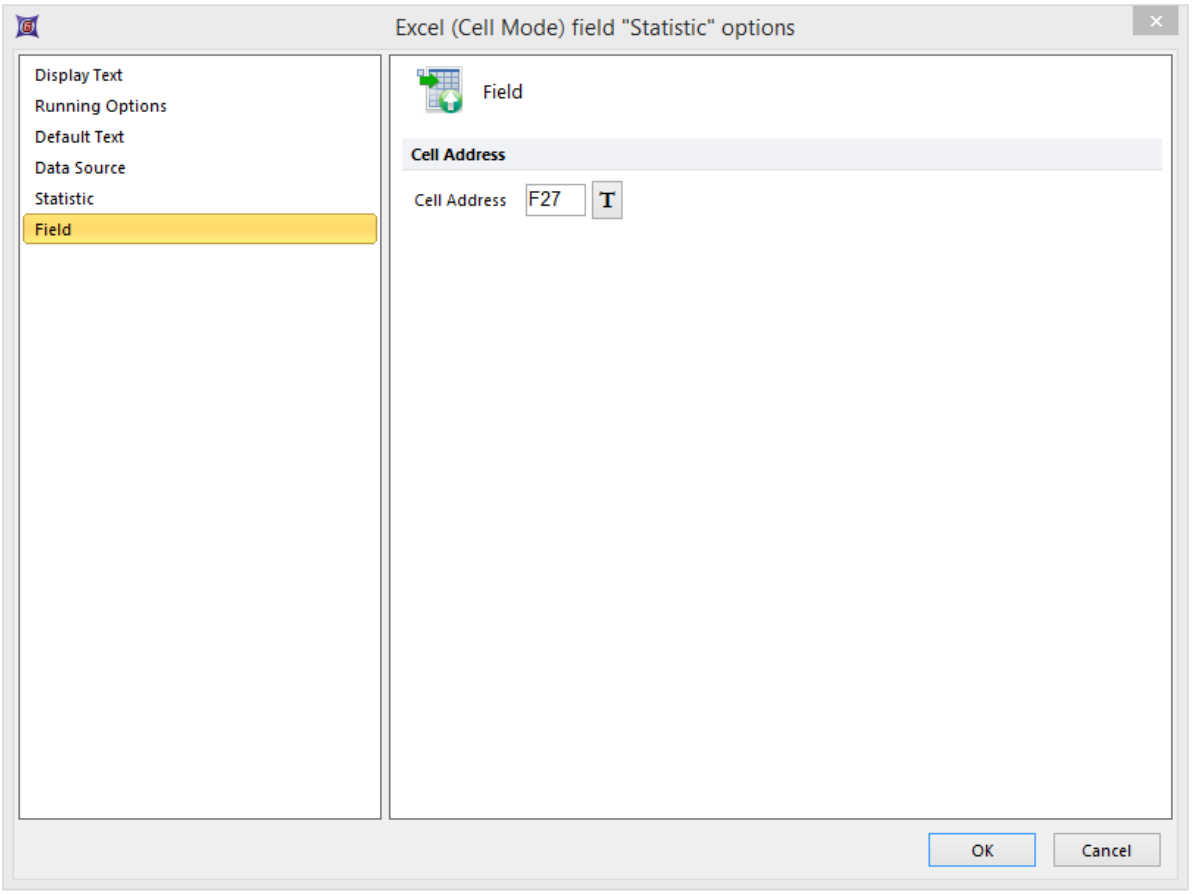

Figure T9.25 Setting the Excel Cell Address

41. Click **OK** to accept the changes.

The **Batch Actions** window will show the action **Statistic: % of Gated Cells** added (Figure T9.26).

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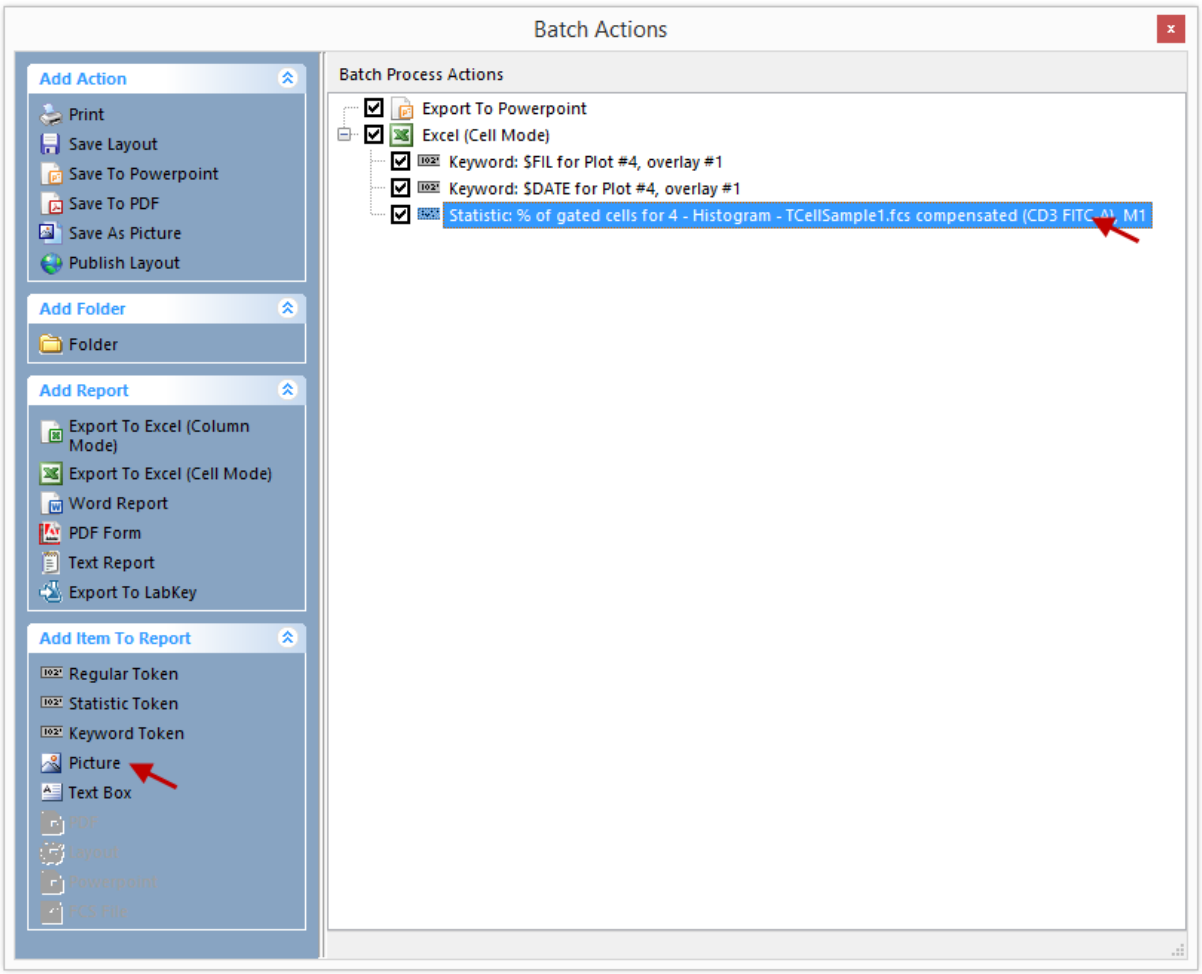

Figure T9.26 Batch Actions with Statistic % of Gated Cells to be Exported to Excel

We will now select a picture to batch export to the Excel file.

42. Select **Picture** from the **Add Item to Report** section (Figure T9.26).

An **Excel (Cell Mode) field "Picture" options** dialog will appear (Figure T9.27). This is where we will define the details of the picture that will be batch exported to Excel.

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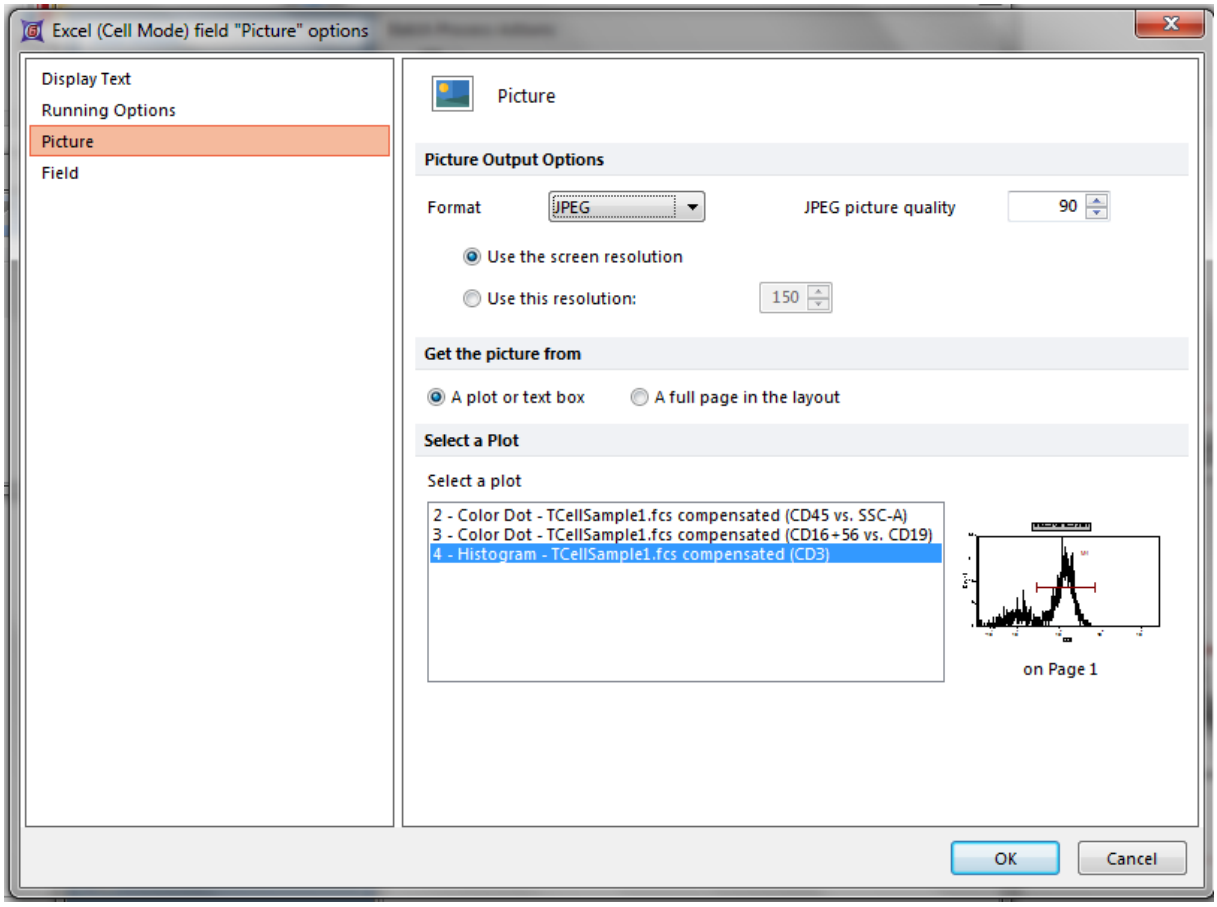

Figure T9.27 Excel (Cell Mode) field "Picture" options Dialog

43. Select **4 - Histogram – TCellSample1.fcs compensated (FITC-A)** in the **Select a Plot** field (Figure T9.27).

44. Click on the **Field** category (Figure T9.28).

The **Field** category of the **Excel (Cell Mode) field "Picture" options** dialog will appear (Figure T9.28). This is where we select the Excel cell address that the picture will be batch exported to.

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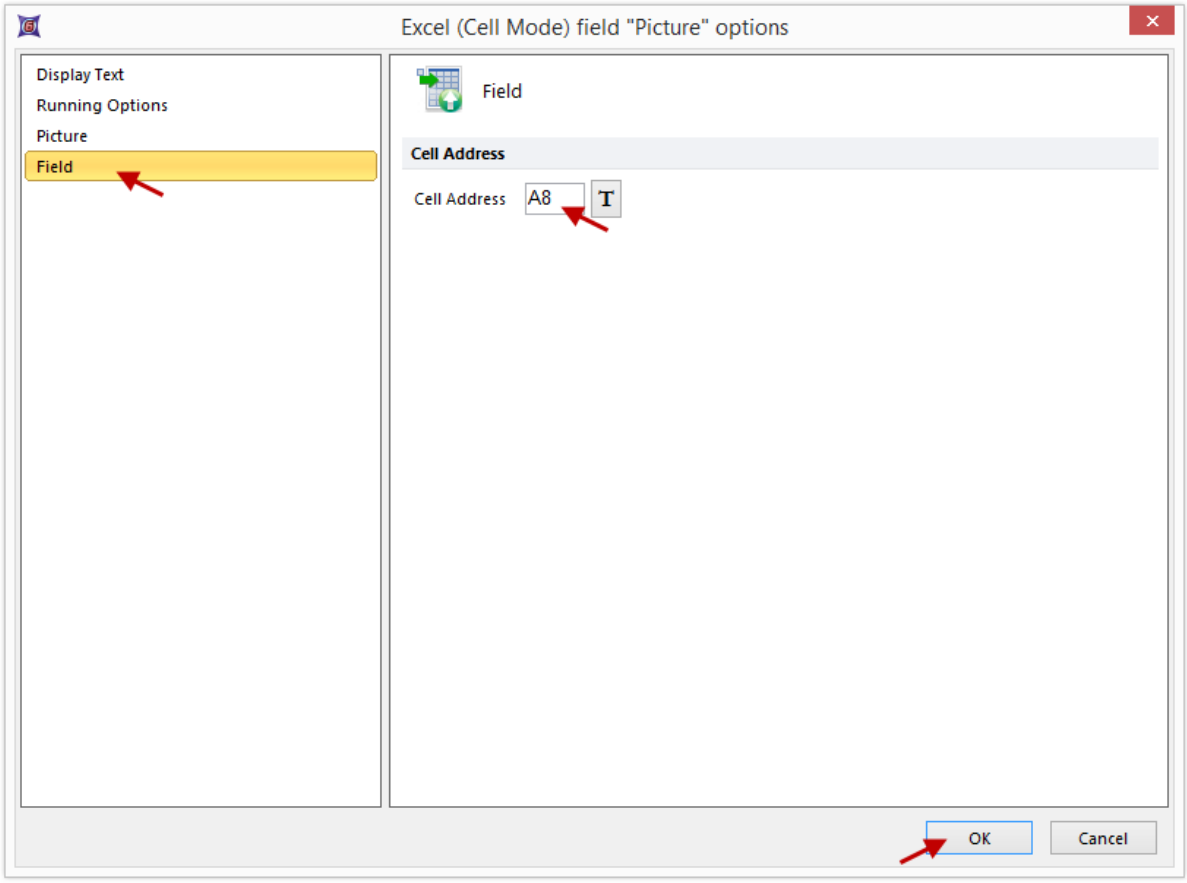

Figure T9.28 Setting the Excel Cell Address for the Picture Field

45. Type **A8** in the **Cell Address** field (Figure 28).

46. Click **OK**.

We have now told FCS Express to export a picture of **4 - Histogram – TCellSample1.fcs compensated (FITC-A)** to cell A8 of the Excel report template shown in Figure T9.14.

The **Batch Actions** window will show the action **Picture** added (Figure T9.29).

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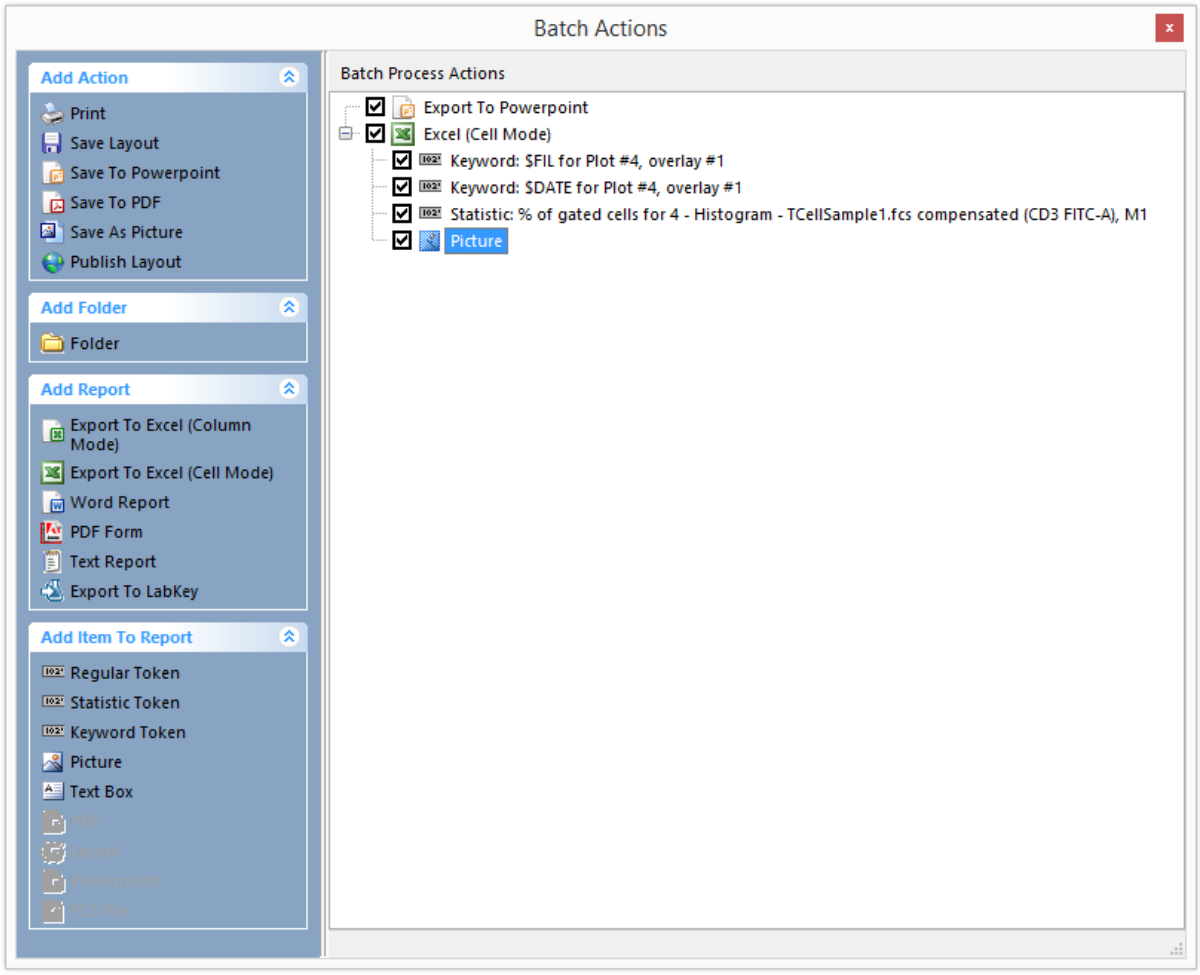

Figure T9.29 Batch Actions with Picture to be Exported to Excel

47. Click the Close button (**red x**) in the upper right corner of the window to close the Batch Actions window (Figure T9.29).

We will now return to the layout page and run the Batch Processing.

48. Select the **Batch→Batch Processing→Run or Replay→Run** command (Figure T9.10 1445).

FCS Express will now execute the Batch Process. When the Batch Process is complete, an Excel file will be created (Figure T9.30) at the location you specified, with two worksheets, one for each of the two files **TCellSample1.fcs** and **TCellSample2.fcs.**

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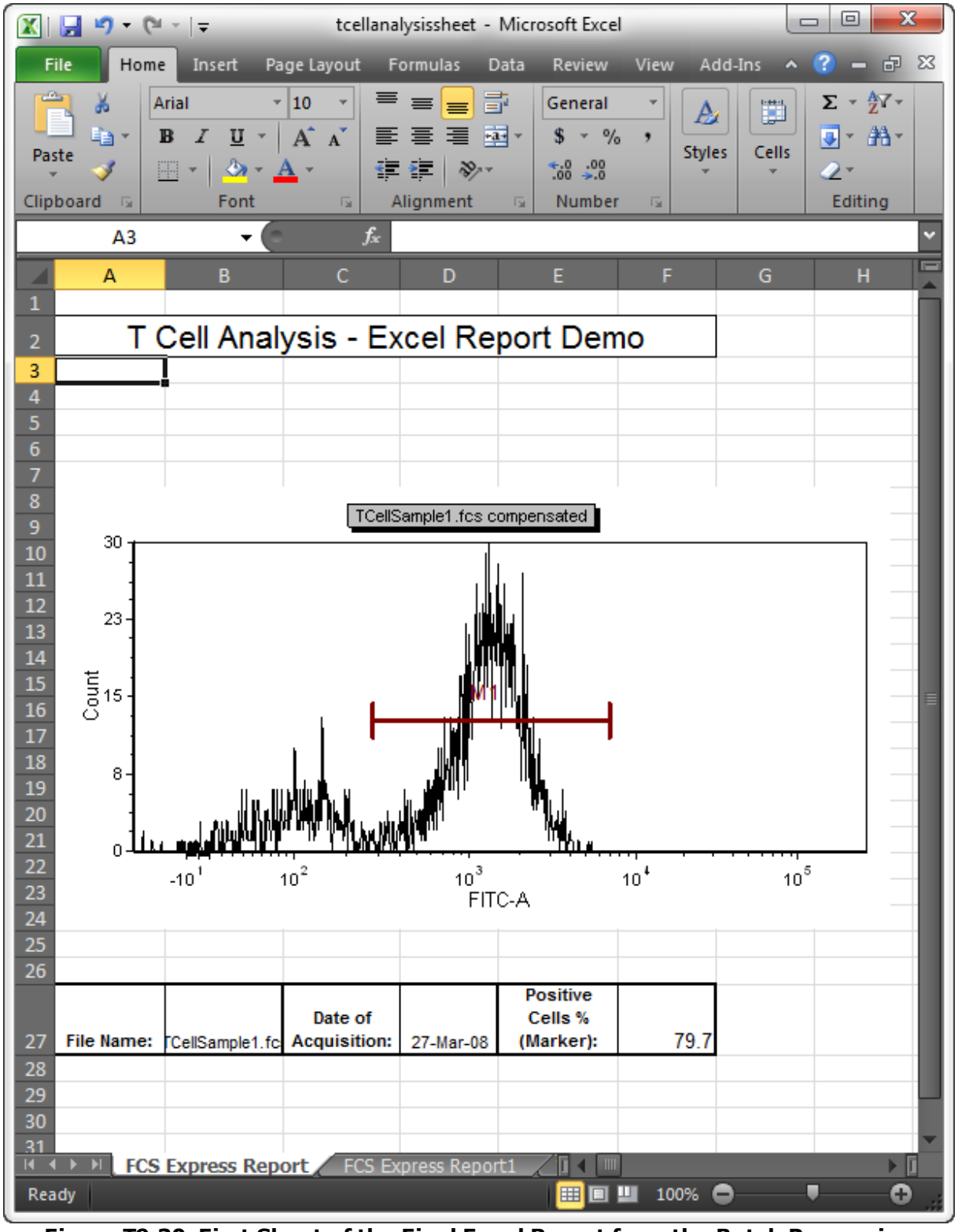

Figure T9.30 First Sheet of the Final Excel Report from the Batch Processing

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We will continue to use this same layout in the next exercise.

Next, we will batch process an Excel report in column mode[1475].

38.10.3 Excel Report (Column Mode)

A **Column Mode** batch analysis report can be created in a similar fashion as the **Cell Mode.**

In this exercise we will:

- Create an Excel Column Mode batch analysis report.
- Add a statistic token to the report.
- Add a keyword token to the report.
- Add a picture to the resulting Excel spreadsheet.

For convenience, we are going to start with the same layout as before. The images contained within this section will continue with the layout from the previous section. However, you can add reports to layouts that already have actions defined. If you have the previous layout still open, please jump to Step 5.

1. Select the **File** tab and click on **Open**.
2. Open the layout **BatchProcess.fey** found in the Tutorial Sample Data archive[1210].

To Perform an Excel Report Batch Process:

3. Create a **Data List** with files **TCellSample1.fcs** and **TCellSample2.fcs** (see Steps 4-6 of the previous section[1447]).
4. Select the **Batch** tab→**Batch Processing**→**Batch Actions** command (Figure T9.6[1445]).

A **Batch Actions** window will appear (Figure T9.7[1445]). Please note that if you have the previous layout still open the **Batch Actions** window will also shows the Batch Actions from the (Figure T9.29[1455])[1445]

We will now dock the **Batch Actions** window to the left side of the layout. Docking the **Batch Actions** dialog makes it easier to drag and drop plots and tokens while the rest of the layout remains in view, allowing you to set up the batch process actions very quickly. Please refer to the Docking Tutorial[1537] for more details on using the docking feature in FCS Express.

5. Move the **Batch Actions** window so that you see a four-headed icon in the center of the FCS Express window (Fig. T. 9.31)

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6. Release the mouse on the left side of the icon so that both it and the left side of the FCS Express window become highlighted (Fig. T. 9.31). The Batch Actions window is now docked to the left side of the FCS Express window (Fig. T. 9.31, *right*).

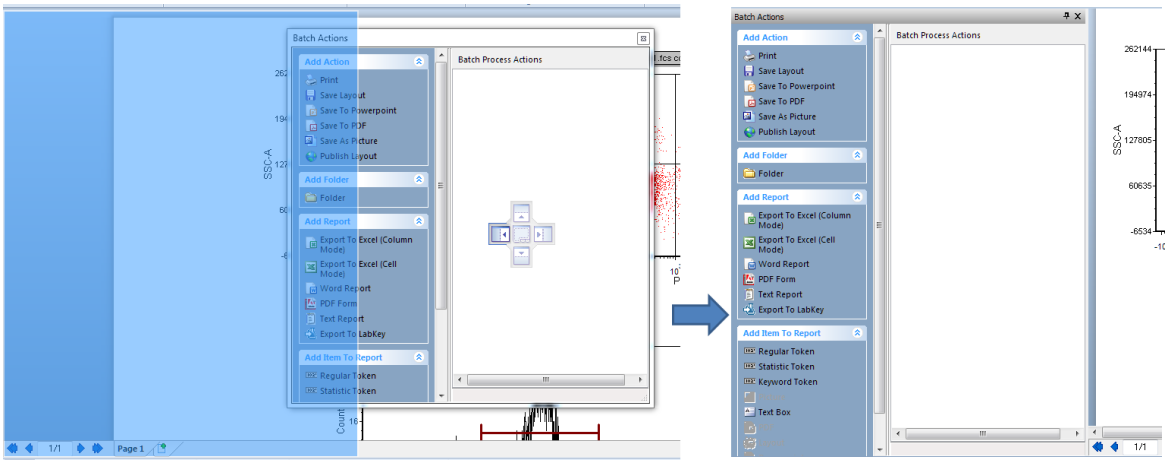

Figure T9.31 Docking the Batch Actions window.

7. Select **Export to Excel (Column Mode)** from the **Add Report** category (Figure T9.32, ❶).

An **Excel (Column Mode) Options** dialog appears (Figure T9.32, *right*).

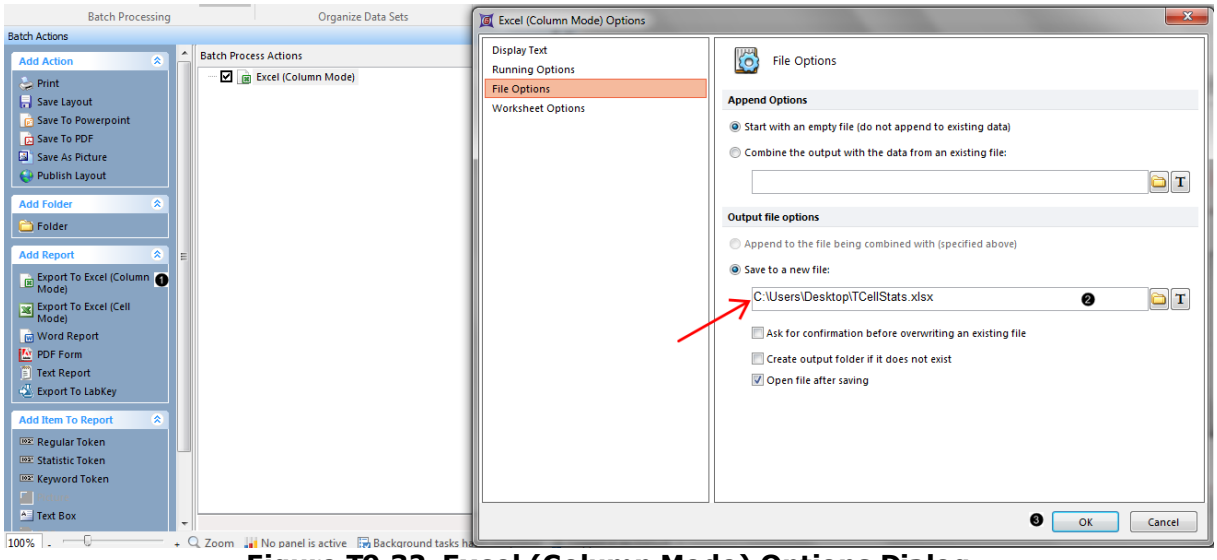

Figure T9.32 Excel (Column Mode) Options Dialog

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8. In the **Save to a new file** field, click on the Folder icon (Figure T9.32, ❷) to navigate and specify where you would like to save your file and give it a name (e.g., "TCellStats" saved on the Desktop in Figure T9.31).

9. Click **OK** (Figure T9.32, ❸).

An **Excel (Column Mode)** process will now appear in the **Batch Process Actions** section of the window (Figure T9.33, mouse pointer). We will now add three items to the report (a keyword token, a statistic token, and a picture of a plot from our layout).

10. Drag and drop the border of the upper-left plot on the layout onto the **Excel (Column Mode)** action (Figure T9.33).

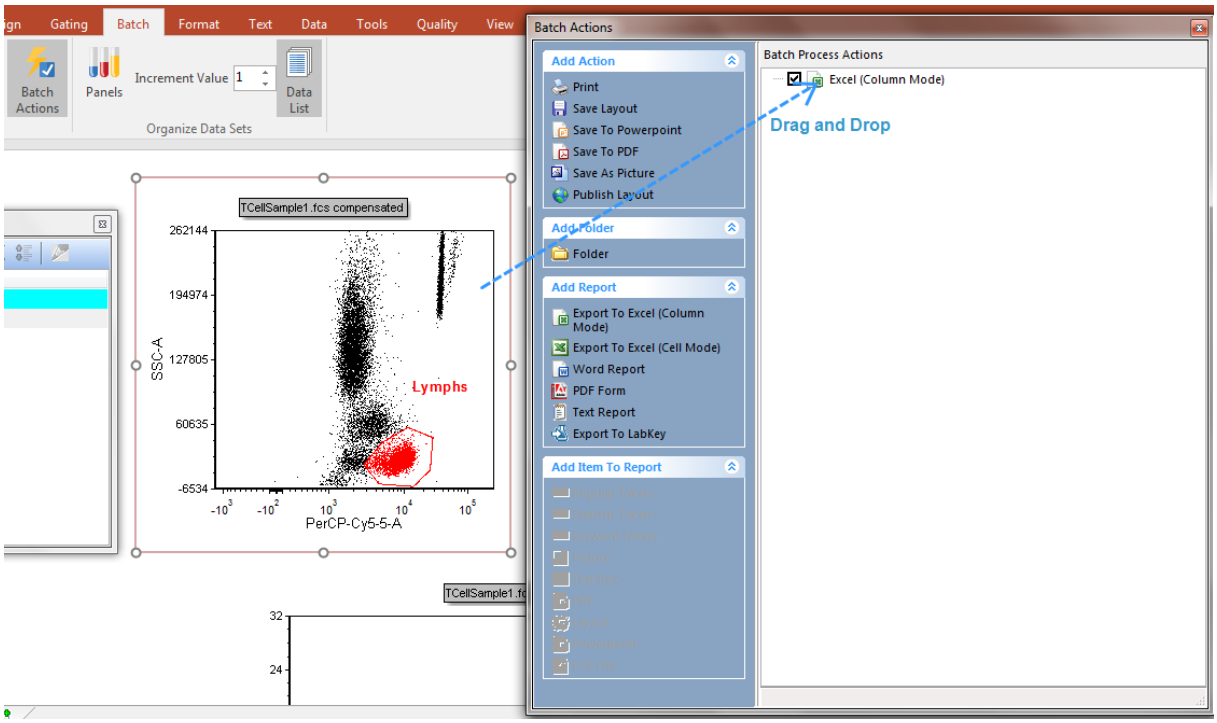

Figure T9.33 Dragging and dropping a plot to serve as data source for a token to be added to the report

11. Double-click **Keyword token** from the **Paste Special** dialog (Figure T9.34, ❶).

12. Click on the ellipsis in the **Excel (Column Mode) field "Keyword..." options** dialog (Figure T9.34, ❷).

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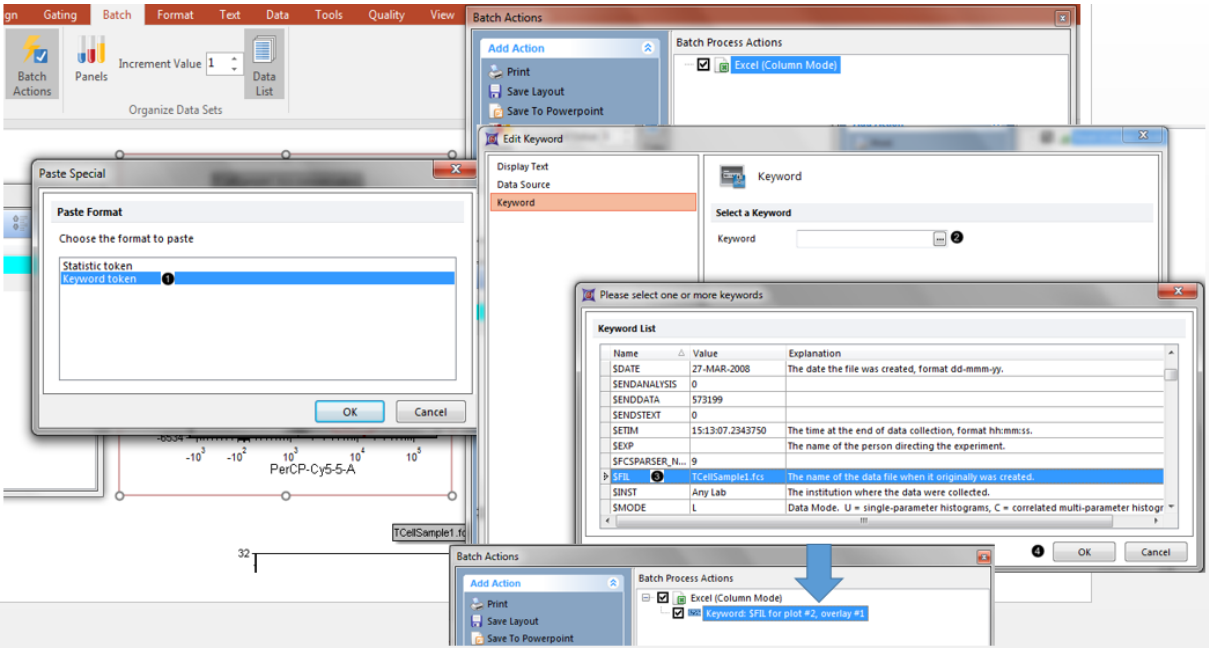

Figure T9.34 Adding a keyword token to the report

13. Scroll to and select **$FIL** in the **Please select a keyword** dialog (Figure T9.34, ❸).

14. Click **OK** in the **Please select a keyword** dialog (Figure T9.34, ❹).

15. Click **OK** in the **Excel (Column Mode) field "Keyword..." options** dialog (Figure T9.33). The Keyword token has now been added as an item to the Excel (Column Mode) report (Figure T9.34, *bottom*).

16. Drag and drop the border of the histogram on the layout onto the **Excel (Column Mode)** action (similar to Figure T9.33, but we are dragging a different plot).

17. Double-click **Statistic token** from the **Paste Special** dialog (Figure T9.35, ❶).

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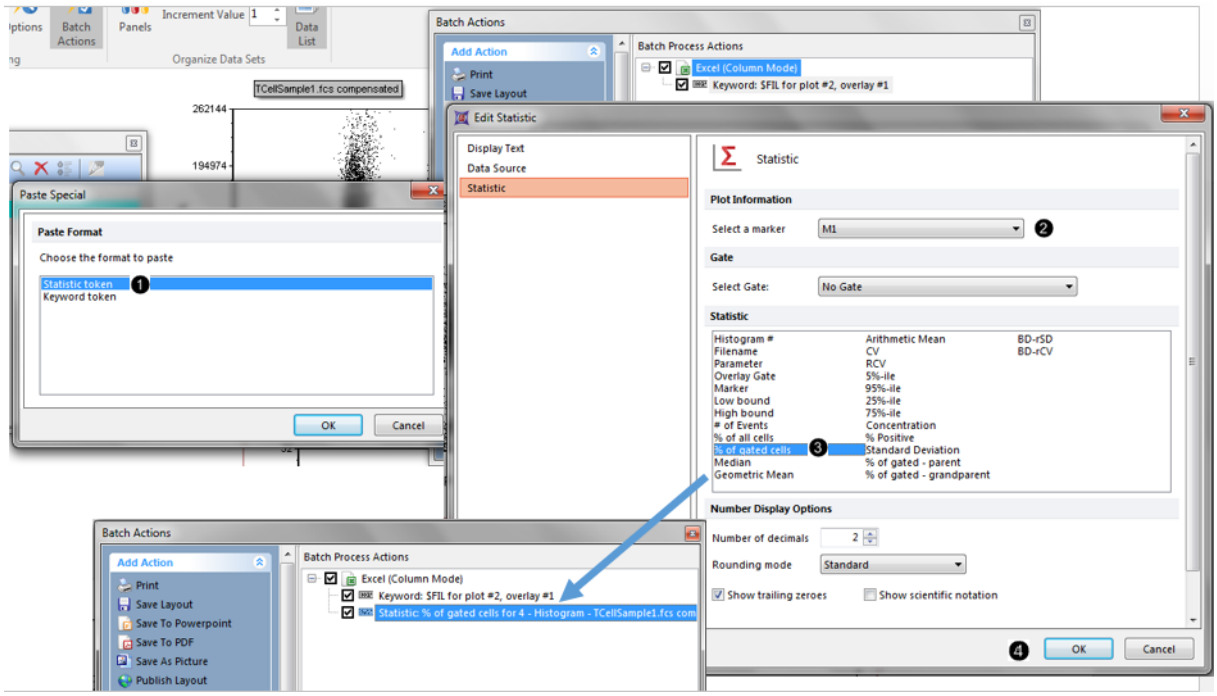

Figure T9.35 Adding a statistic token to the report

18. Select "M1" from the **Select a marker** dropdown menu of the **Excel (Column Mode) field "Statistic..." options** dialog (Figure T9.35, ❷).

19. Select **% of gated cells** from the **Statistic** list box (Figure T9.35, ❸).

20. Click **OK** in the **Excel (Column Mode) field "Statistic..." options** dialog (Figure T9.35, ❹). The Statistic token has now been added as an item to the Excel (Column Mode) report (Figure T9.34, *bottom*).

    Note: you can also drag and drop gates directly from plots onto the Excel Batch Action.

21. Deselect the check boxes next to the **Export to Powerpoint** and **Excel (Cell Mode)** batch actions, if these exist on your layout (Figure T9.36).

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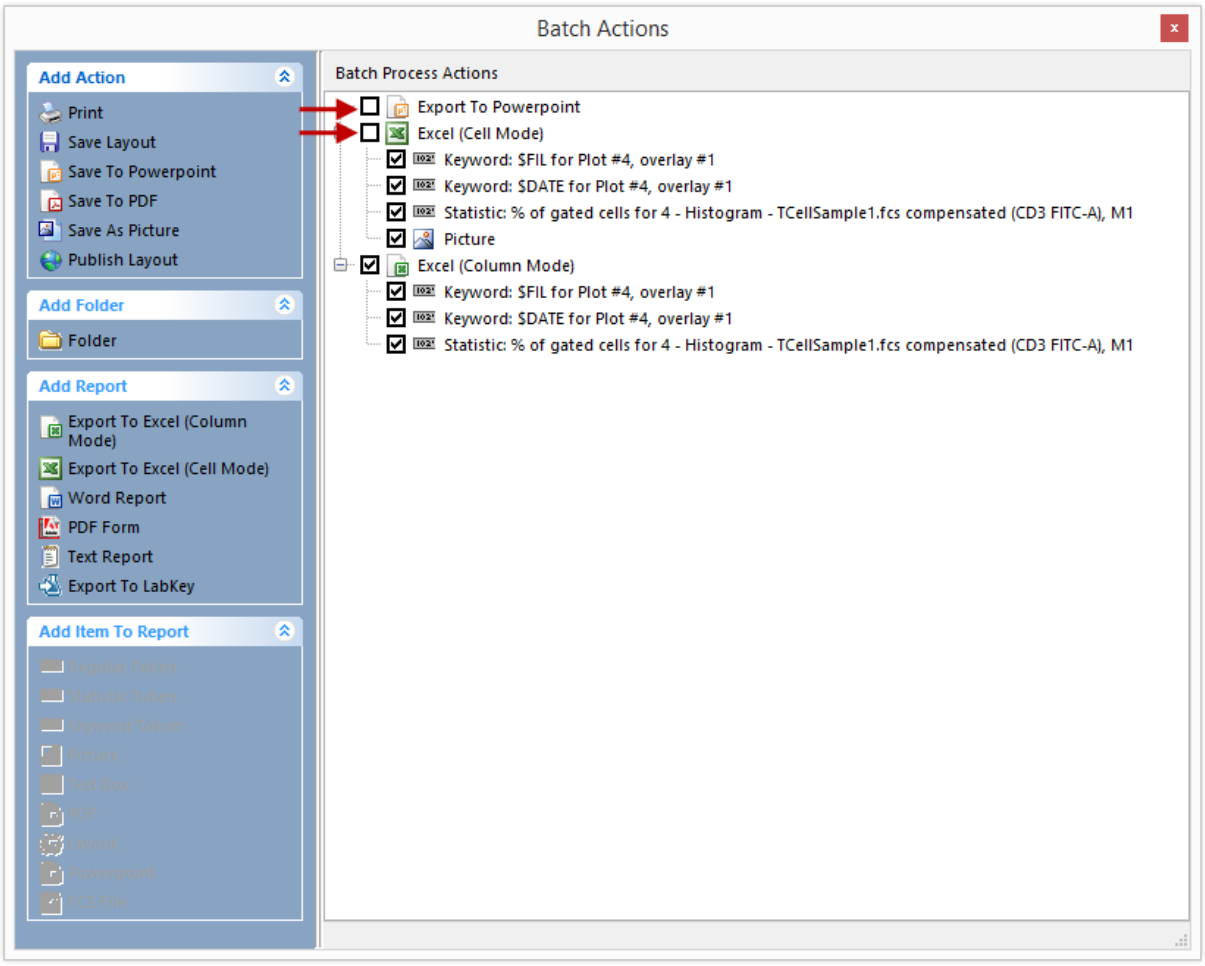

Figure T9.36 Deselect Excel (Cell Mode) to Batch Process Only Excel (Column Mode)

Now we are ready to run the Batch Processing to export to **Excel (Column Mode)**.

22. Select the **Batch** tab→**Batch Processing**→**Run**→**Run** command (Figure T9.10 1445).

An Excel (Column Mode) report will appear with the results of the batch process (Figure T9.37).

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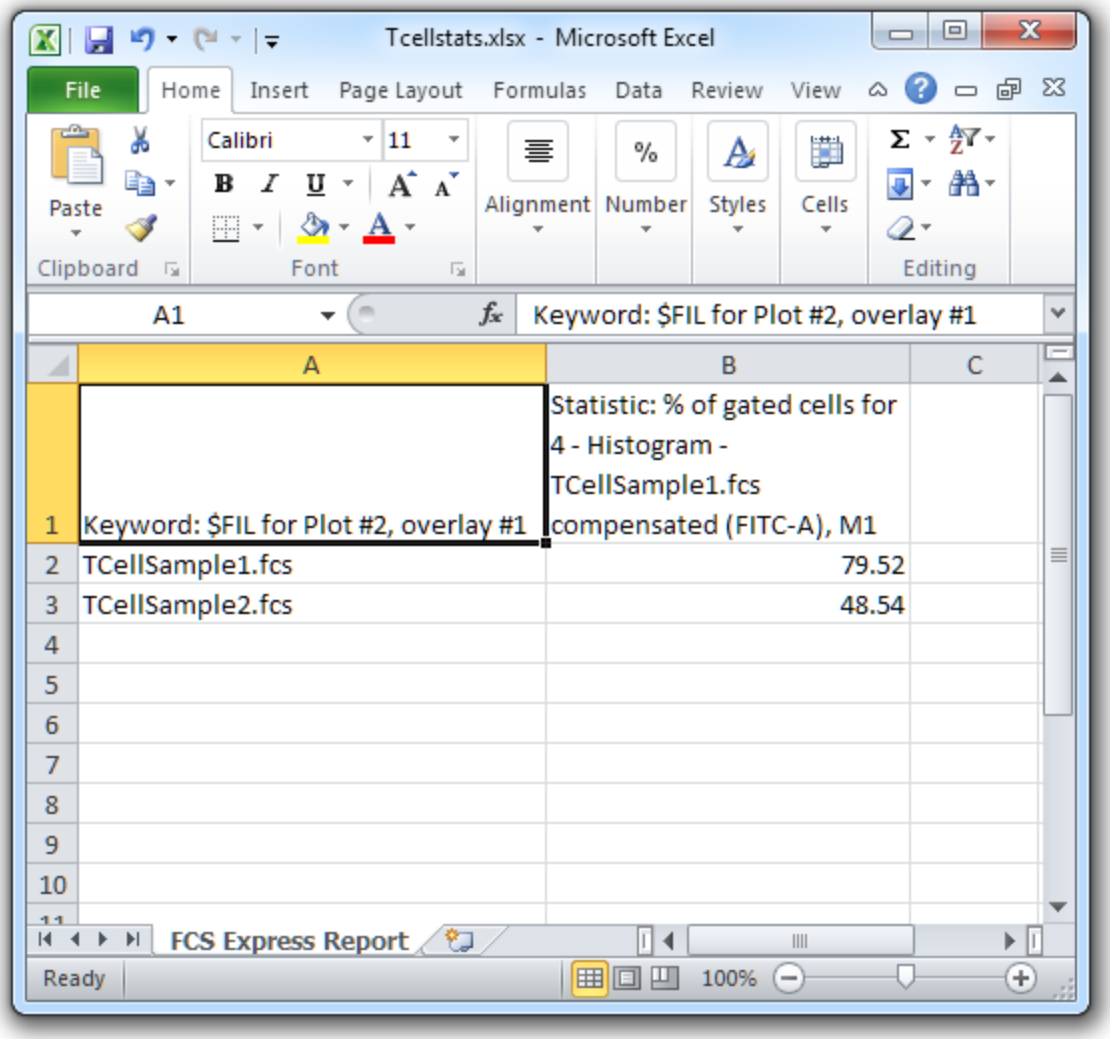

Figure T9.37 Excel (Column Mode) Report

We will now insert the image of a plot from FCS Express onto the Excel spreadsheet using drag and drop.

23. Drag and drop the border of the histogram on the layout onto an empty part of the Excel spreadsheet (Figure T9.38).

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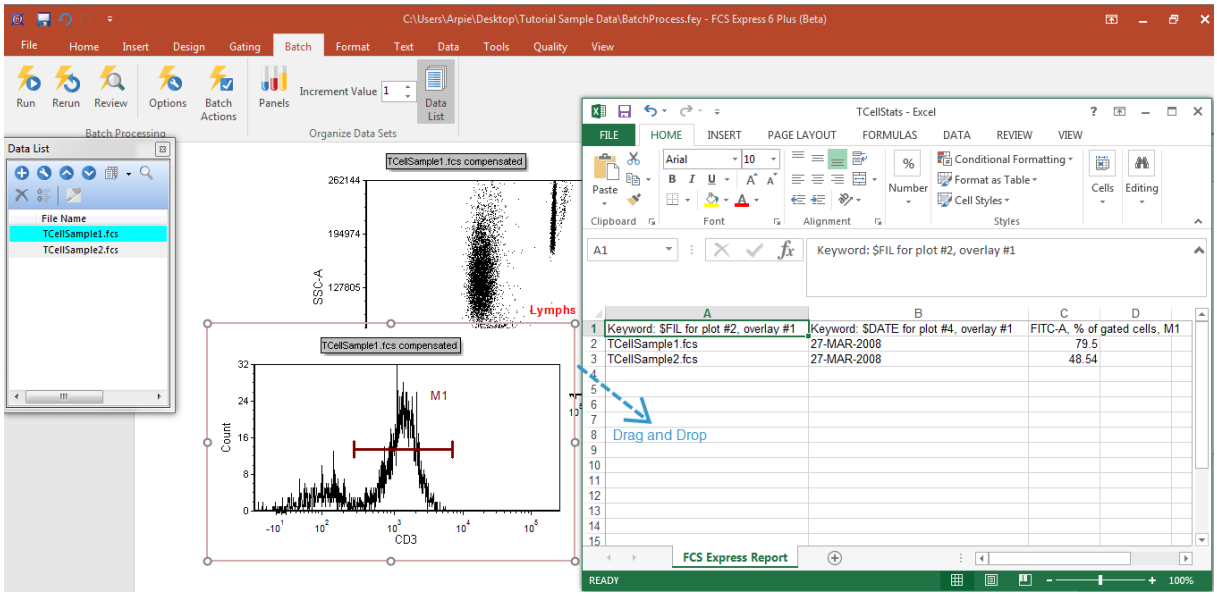

Figure T9.38 Dragging and Dropping a Plot Image from FCS Express to an Excel Spreadsheet

An image of the histogram plot will now appear in the Excel spreadsheet (Figure T9.39). You can drag and drop items from FCS Express to Word and PowerPoint in a similar manner.

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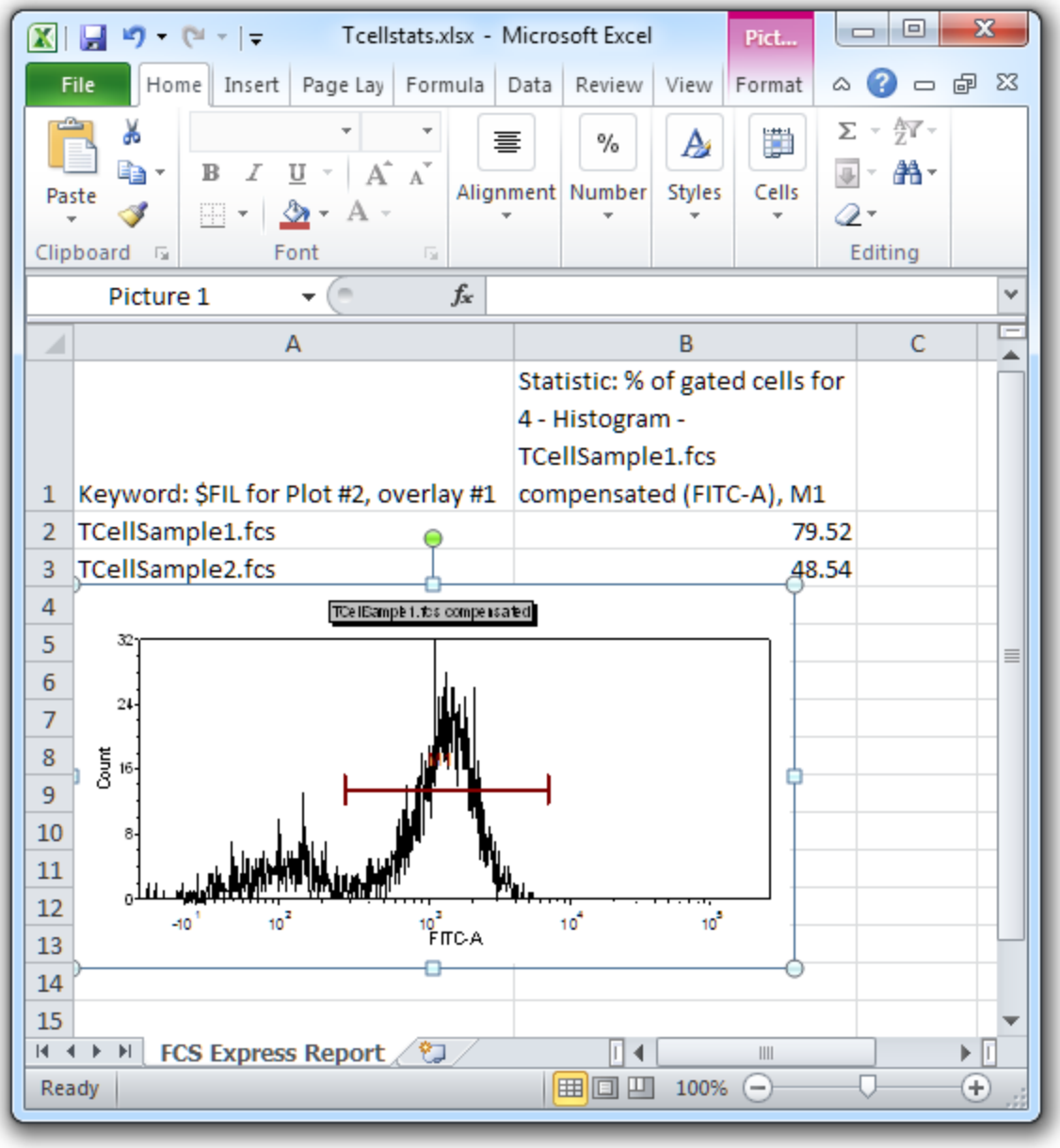

Figure T9.39 Excel (Column Mode) Report with a Plot Image Added

38.10.4 Exporting to LabKey

LabKey Software and De Novo Software have worked together to provide direct export capabilities from FCS Express 6 Flow Cytometry to LabKey Server. The collaboration will allow high volume flow cytometry users a unique combination of report generation and analysis with FCS Express and an online data

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environment, extra quality controls, and workflow tools from LabKey Server. Various items including tokens, pictures, files, and even your entire FCS Express analysis can be exported to LabKey Server, simply by adding a **LabKey Report** as a Batch Process Action.

In this exercise we will:

- Download the free LabKey Server to your local computer.
- Create a LabKey Server Report with FCS Express Batch Actions 803.
- Add the following items to the LabKey Server Report Batch Actions 803:
  - Keyword Tokens 708
  - Statistic Tokens 703
  - FCS Express Layout (.FEY) file
  - .FCS files
  - Picture 839 of a 2D plot (JPEG format)

To export to LabKey Server:

The first step in exporting to LabKey Server is to register and install LabKey Server to your local machine. If you have already completed this step, please proceed to step 2.

1. Download LabKey Server to your local computer and register with LabKey Server by following the directions at https://www.labkey.org/wiki/home/Documentation/page.view?name=installServerDemo. Alternatively, write to us at support@denovosoftware.com if you would like to try out this tutorial using De Novo Software's own LabKey server for public use.

2. Set up an Assay Design in LabKey Server to which you will export FCS Express data, by following the directions in the **LabKey Setup: Folder and Assay Design/Protocol** section of the accompanying LabKey tutorial.

   Now that the LabKey Server is ready to receive your data from FCS Express, you may proceed to step 3 below to complete the rest of the tutorial in FCS Express.

3. Select the **File** tab and click on **Open**.

4. Open the layout **LabKeyServerExportTutorial.fey** found in the "LabKey Server Export" folder within the Tutorial Sample Data archive 1210.

   The layout is currently displaying 2D density and dot plots and a histogram of the file **Sample 1.fcs** (Figure T9.40). The layout contains a data list with a total of 7 .fcs files which are also located in the "LabKey Server Export" folder to be used in the batch export process. The table in the

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upper right displays Participant ID and Date Keyword Tokens from the sample .fcs in the plots, the number of events in Gate 1 from the 2D density plot, and the percent of gated events in M1 from the histogram.

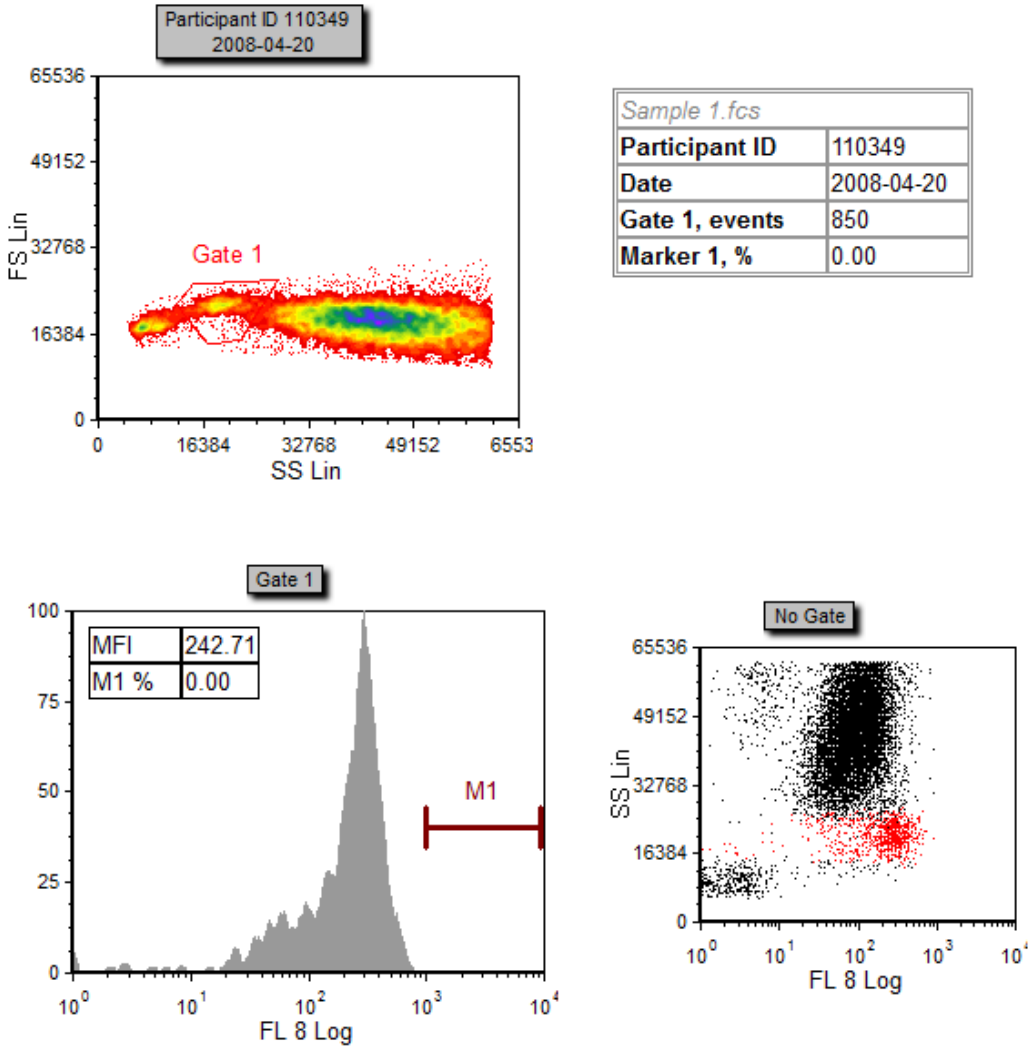

Figure T9.40 Example of the LabKeyServerExportTutorial.fey layout

5. Select the **Batch→Batch Processing→Batch Actions** command (Figure T9.41).

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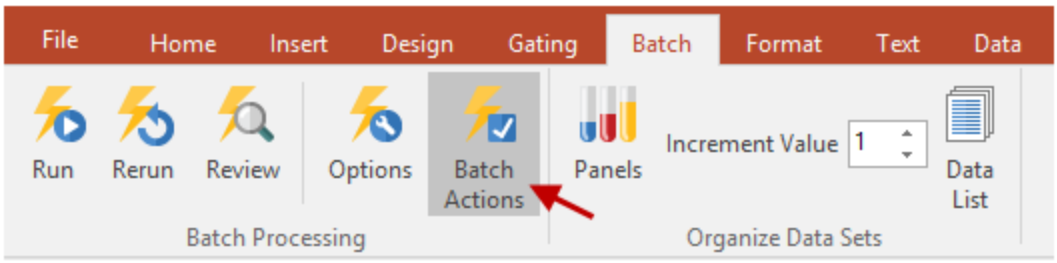

Figure T9.41 Selecting the Batch Actions command

6. Click on **Export to LabKey** under the **Add Report** category of the **Batch Actions** window (Figure T9.42).

   The **Export to LabKey Options** dialog box opens (Figure T9.43).

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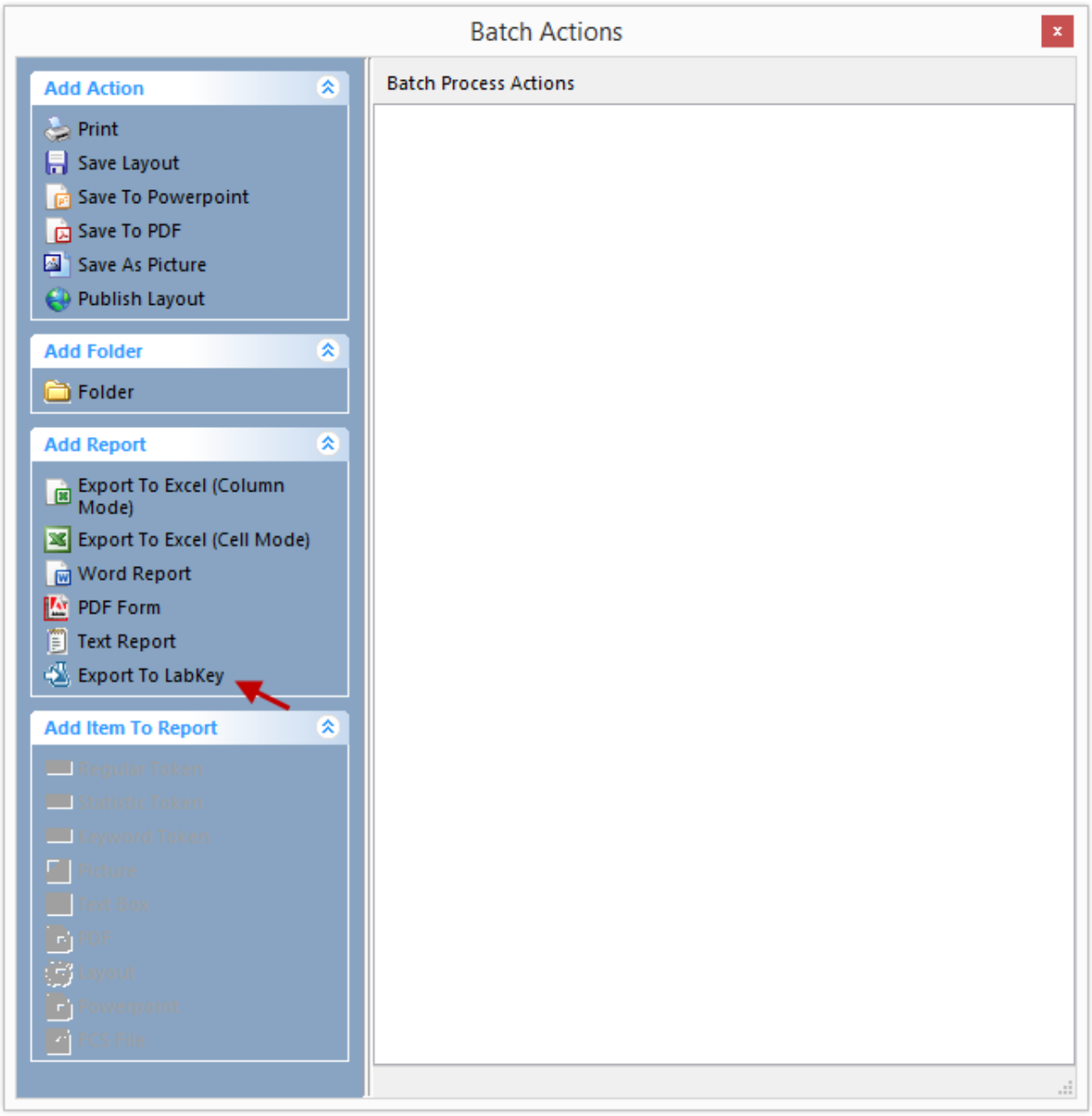

Figure T9.42 Adding a LabKey Report

7. Type (or copy and paste) the URL generated by LabKey Server into the **LabKey Server URL** field (Figure T9.43 ❶) of the **Export to LabKey Options** dialog box. This URL is generated automatically by LabKey Server and can be found by following the directions in the **FCS Express Set Up: Prepare the FCS Express Report** section of the accompanying LabKey tutorial.

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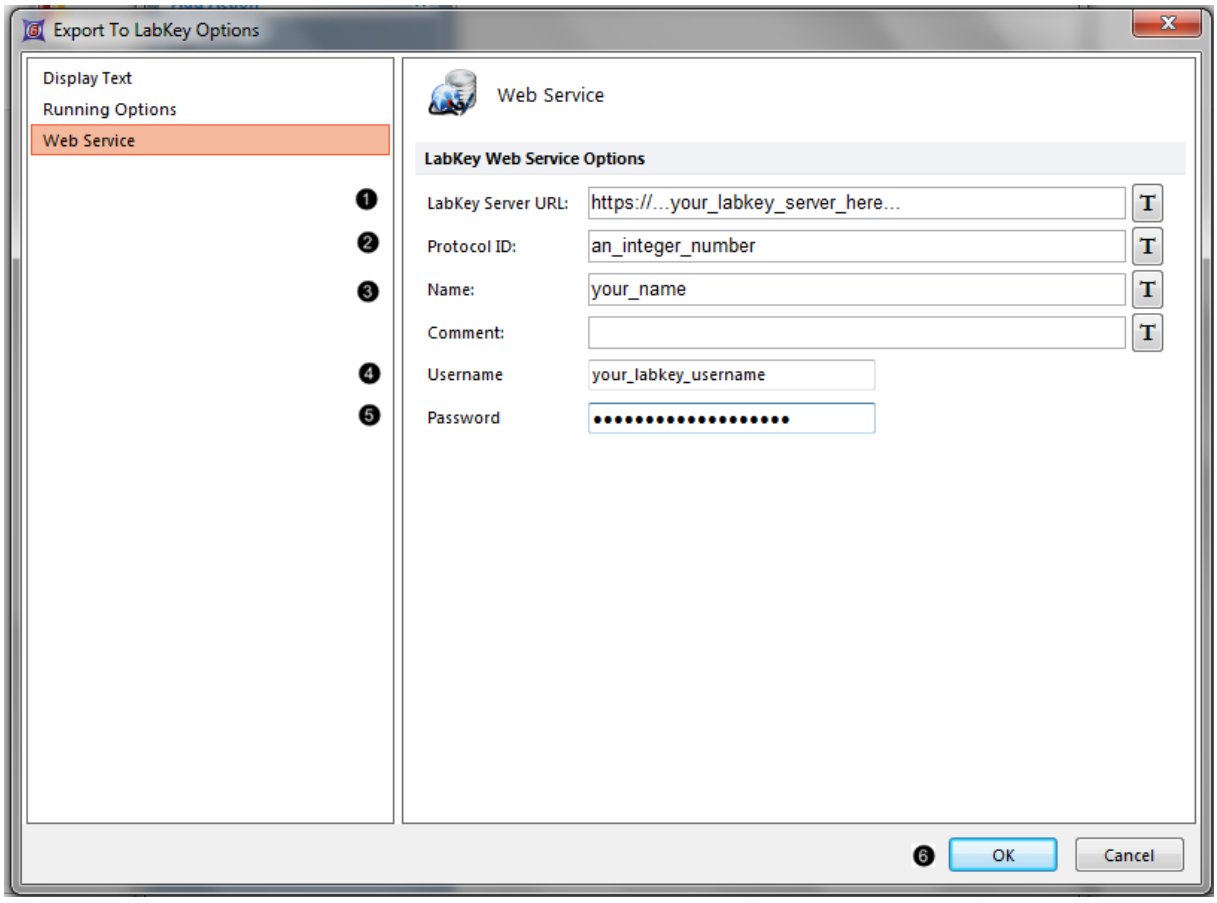

Figure T9.43 Export to LabKey Options dialog box

8. Type the Protocol ID number into the **Protocol ID** field of the **Export to LabKey Options** dialog box (Figure T9.43 ❷). The Protocol ID is an integer generated automatically by LabKey Server and can be found by following the directions in the **FCS Express Set Up: Prepare the FCS Express Report** section of the accompanying LabKey tutorial.

9. Type your name into the **Name** field of the **Export to LabKey Options** dialog box (Figure T9.43 ❸). This entry will be shown as the "Assay Id" in the table of Experimental Runs in LabKey Server.

10. Type the email address you used to register with LabKey Server in step 1 into the **Username** field (Figure T9.43 ❹).

11. Type the password you used to register with LabKey Server in step 1 into the **Password** field (Figure T9.43 ❺).

12. Click **OK** to close the dialog window (Figure T9.43 ❻).

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13. Highlight the Participant ID Token in the second column of the Table in the **Layout** (Figure T9.44 ❶) so that the Token text ("110349") appears white.

14. Drag and drop the highlighted Token on top of the **Export to LabKey** action which has just appeared in the **Batch Actions** window (Figure T9.44, red arrow).

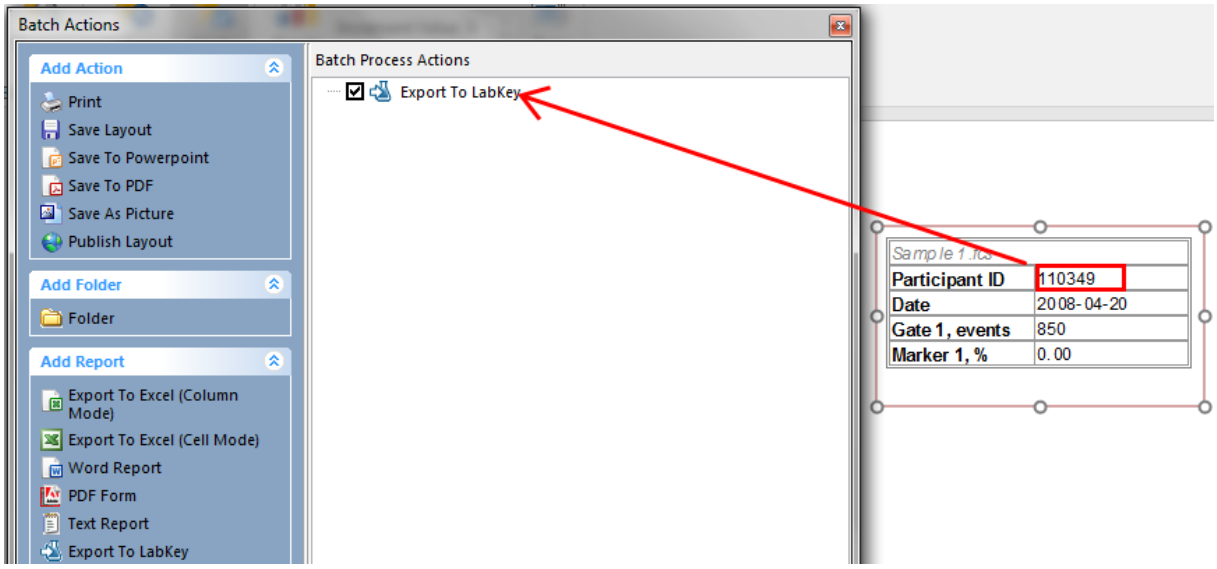

Figure T9.44 Dragging and dropping Tokens from the Table into the Batch Actions Window

15. Repeat steps 13 and 14 for the next three Tokens in the Table (i.e., *Date* ❷, *Gate 1 events* ❸, *and Marker 1 %* ❹) (Figure T9.44 ❷,❸,❹).

Note that you can also drag drop the whole table directly on top of the **Export to LabKey** action. In this case the file name "Sample 1.fcs" will also be included in the list of **Export to LabKey** items.

16. Double-click on the item **Keyword: $PARTICIPANTID for Plot #3, overlay #1** in the list of **Export to LabKey** items (Figure T9.45 ❶).

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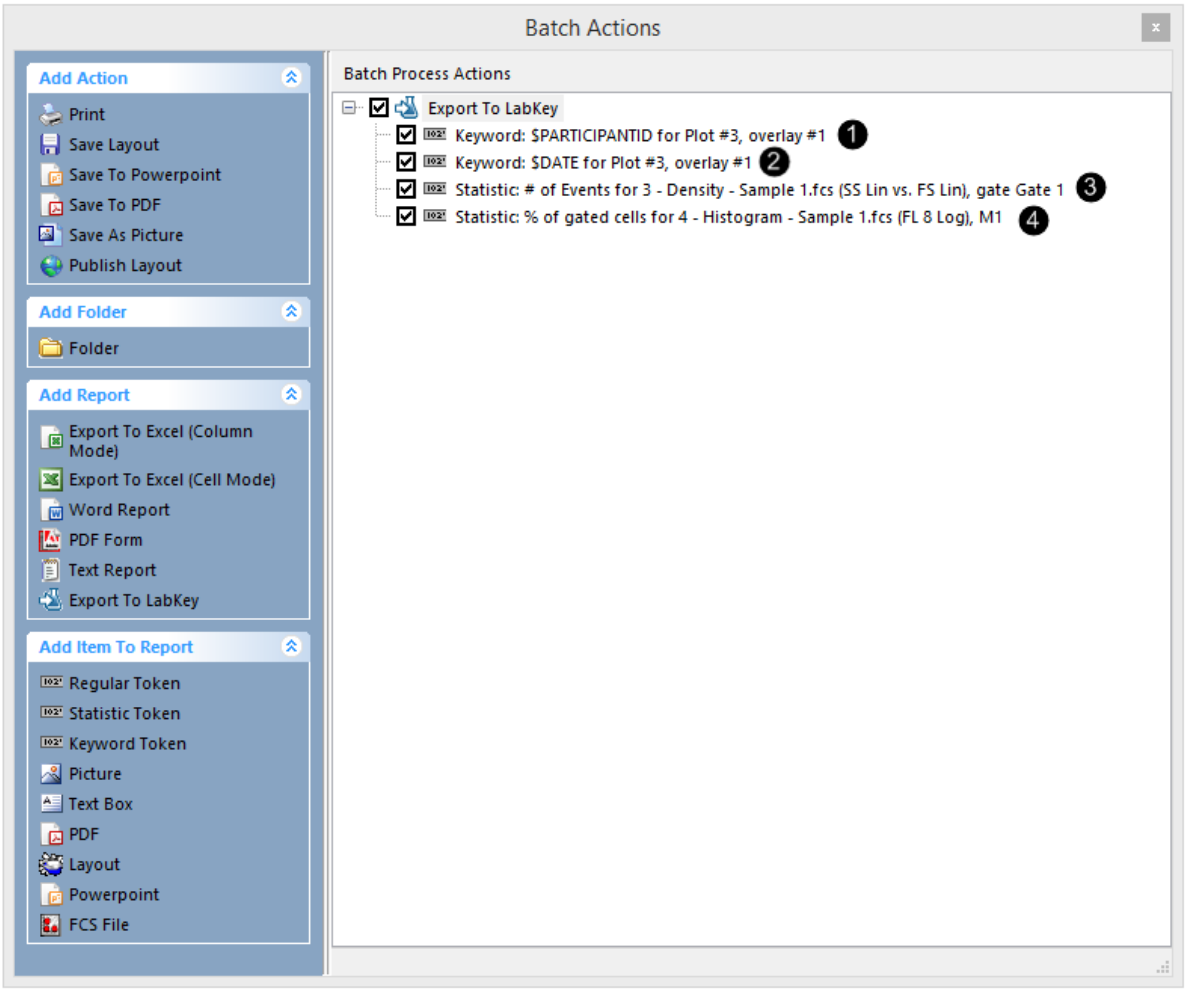

Figure T9.45 Export to LabKey Batch Action with Tokens added to Report

The Export To LabKey options window will appear.

17. Click on the **Field** category on the left (Figure T9.46 ❶).

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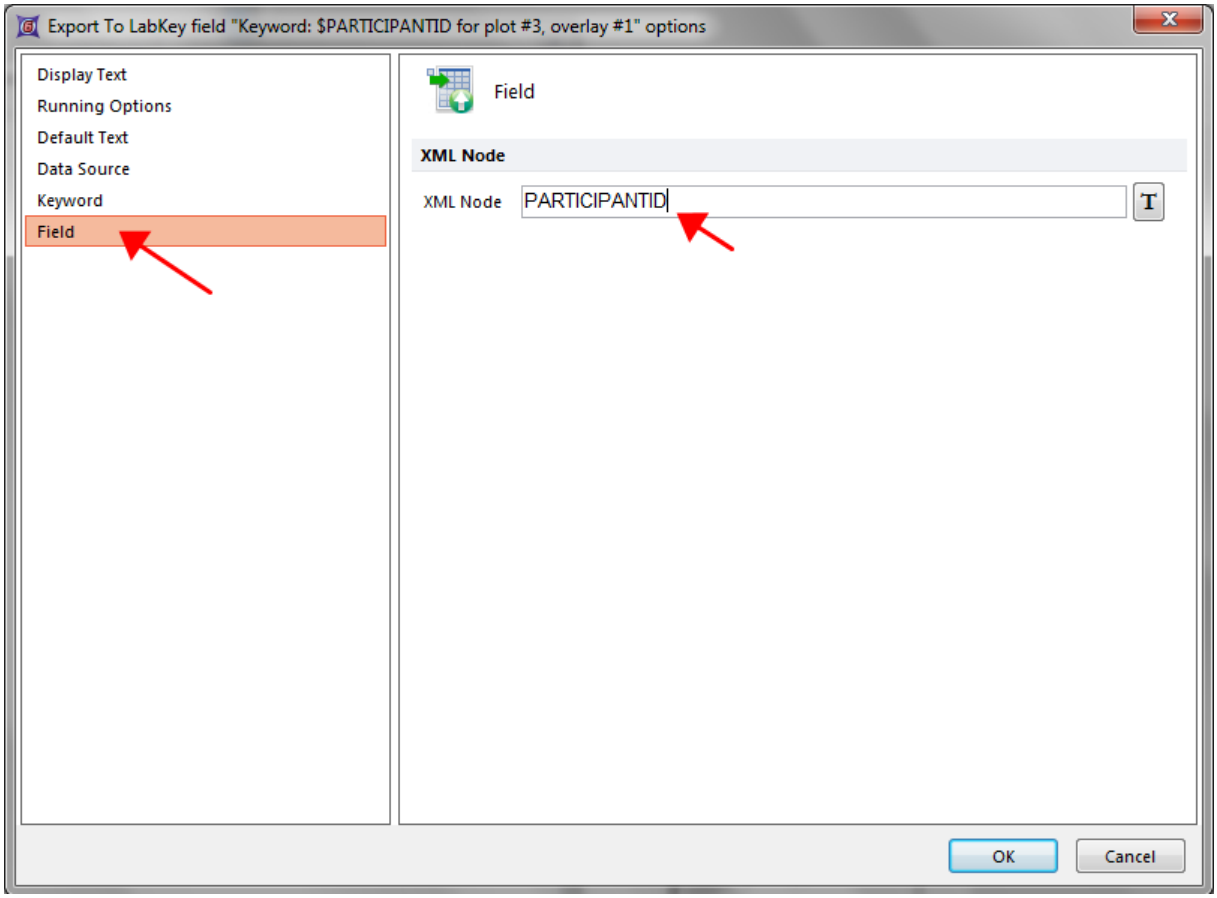

Figure T9.46 Entering text into the XML node field

18. Type the text "PARTICIPANTID" in the **XML Node** field (Figure T9.46). The **XML Node** field ensures that this data is exported to the correct Data Field in LabKey Server.

19. Click **OK**.

20. Repeat steps 16-19 for the next three items in the Export list (see bullets below) but in the **XML Node** field (step 18) type "DATE", "Events", and "Percent", respectively. (Figure T9.45 ❷,❸,❹):

    - **Keyword: $DATE for Plot #3, overlay #1** ❷
    - **Statistic: # of Events for Plot #3, overlay #1 Additional Gate: Gate 1** ❸
    - **Statistic: % of gated cells for Plot #4, overlay #1, in marker1** ❹

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21. Click on the **Export to LabKey** action in the **Batch Actions** window (Figure T9.47 ❶) to highlight it.

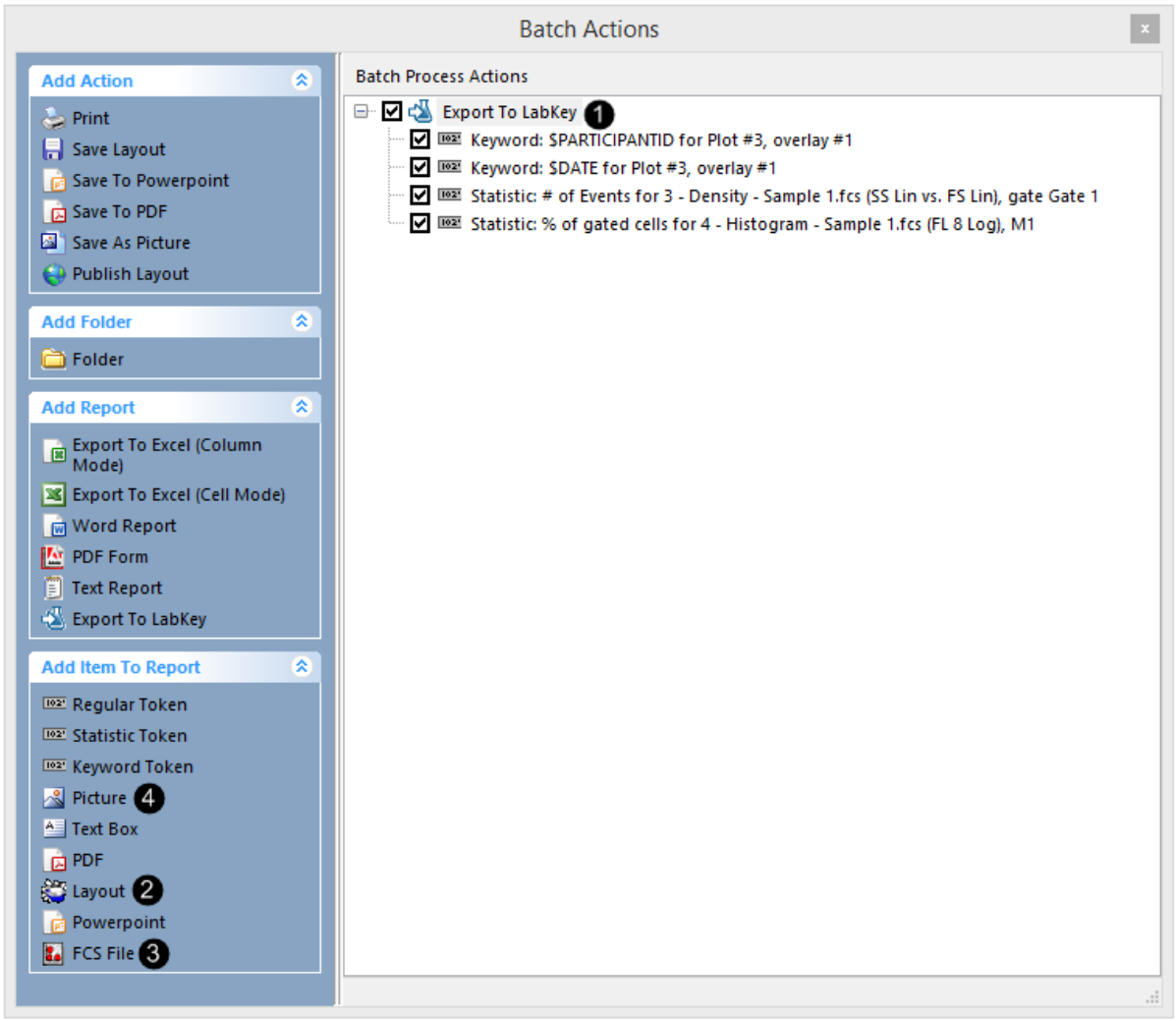

Figure T9.47 Adding items to the LabKey Report

22. Click on the **Layout** icon under the **Add Item to Report** category of the **Batch Actions** window (Figure T9.47 ❷). Leave the default option in the Layout category so that the Layout is exported linked to your data files 93.

23. Click on the **Field** category on the left of the dialog window which opens (Figure T9.48).

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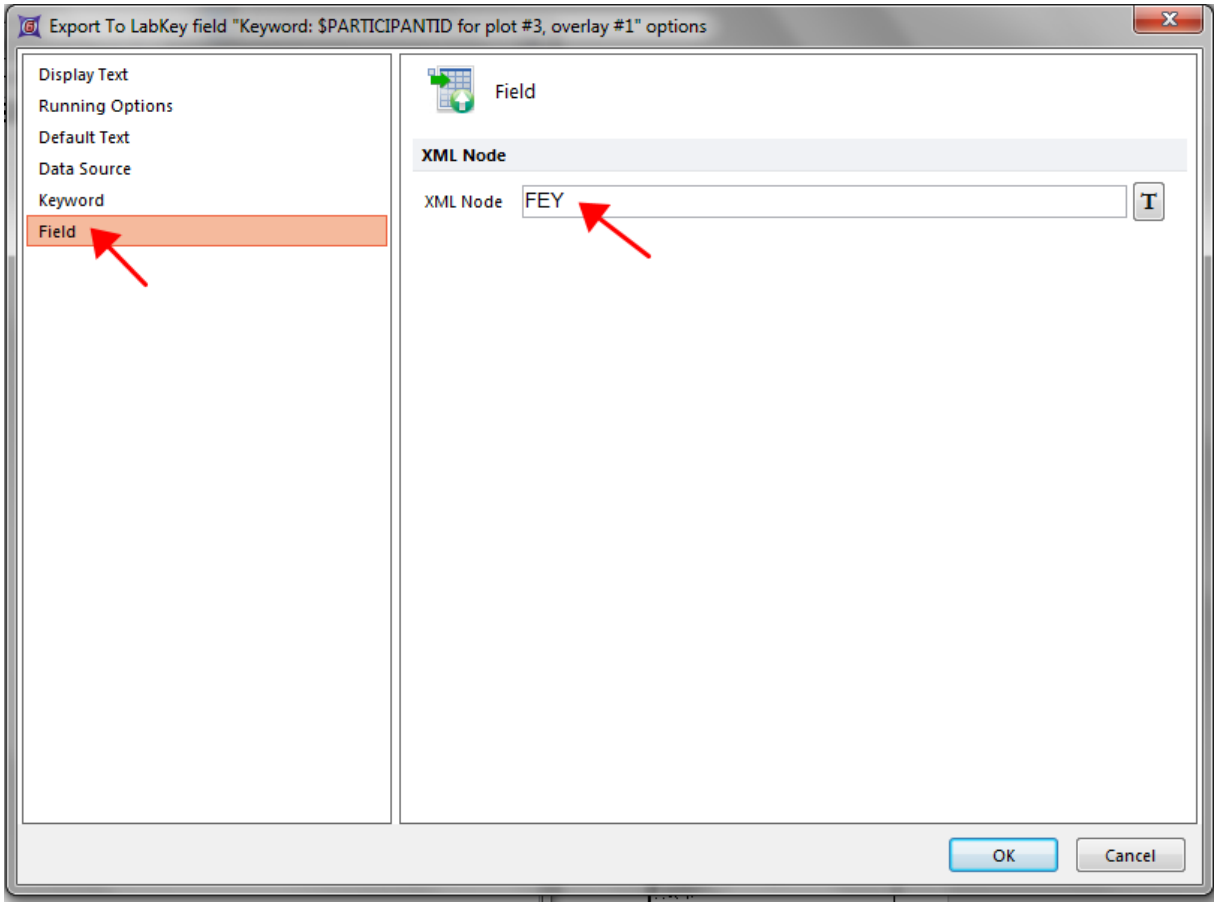

Figure T9.48 Entering text into the XML Node field

24. Type "FEY" in the **XML Node** field and click **OK** (Figure T9.48).

25. Click on the **FCS File** icon under the **Add Item to Report** category of the **Batch Actions** window (Figure T9.47 ❸).

26. Highlight plot 3, "**3-Density-Sample1.fcs(SS Lin vs. FS Lin)**", under **Select a Plot** to specify it as the **Data Source** for the FCS file to be exported (Figure T9.49 ❶).

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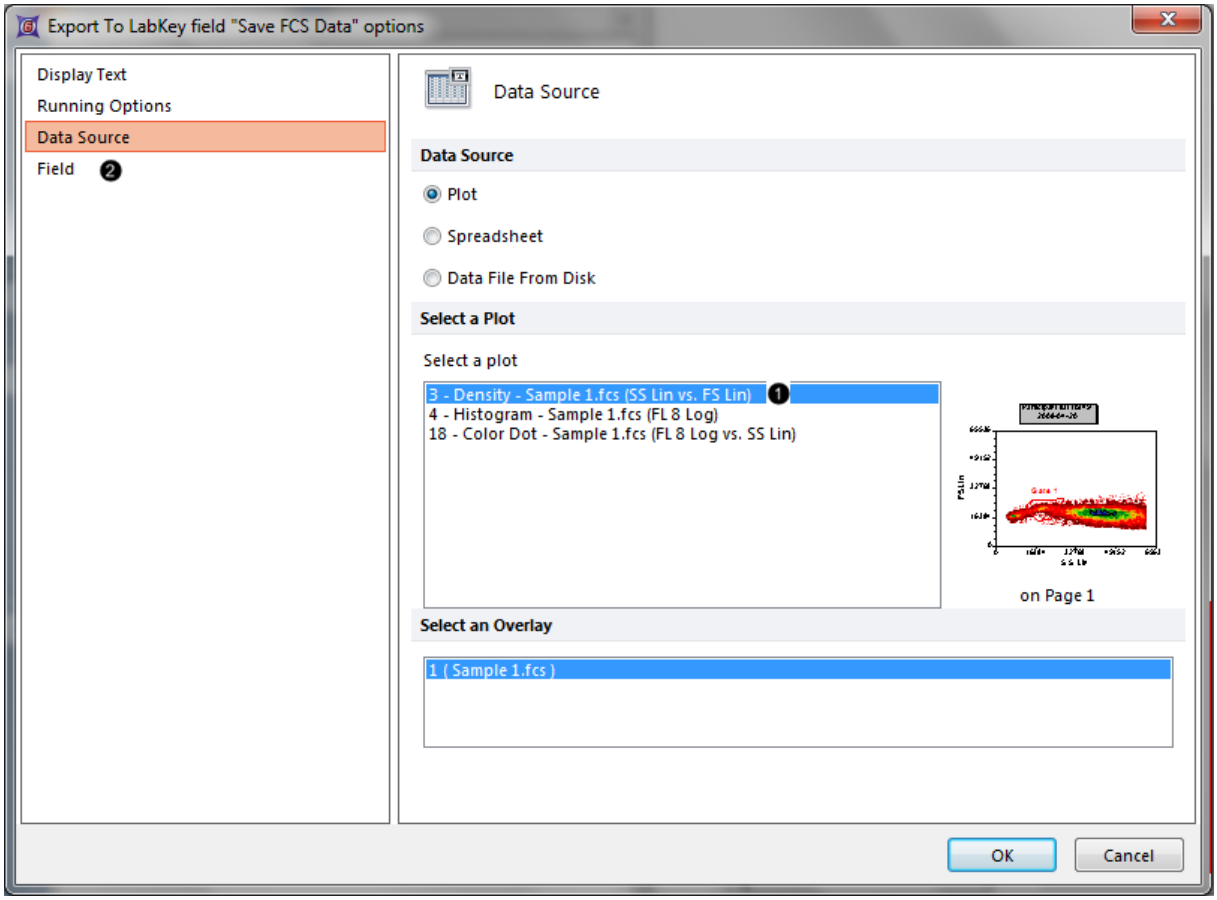

Figure T9.49 Data Source options for exported FCS files

27. Click on the **Field** category on the left of the dialog window (Figure T9.49 ❷).

28. Type "FCS" in the **XML Node** field (Figure T9.50 ❶).

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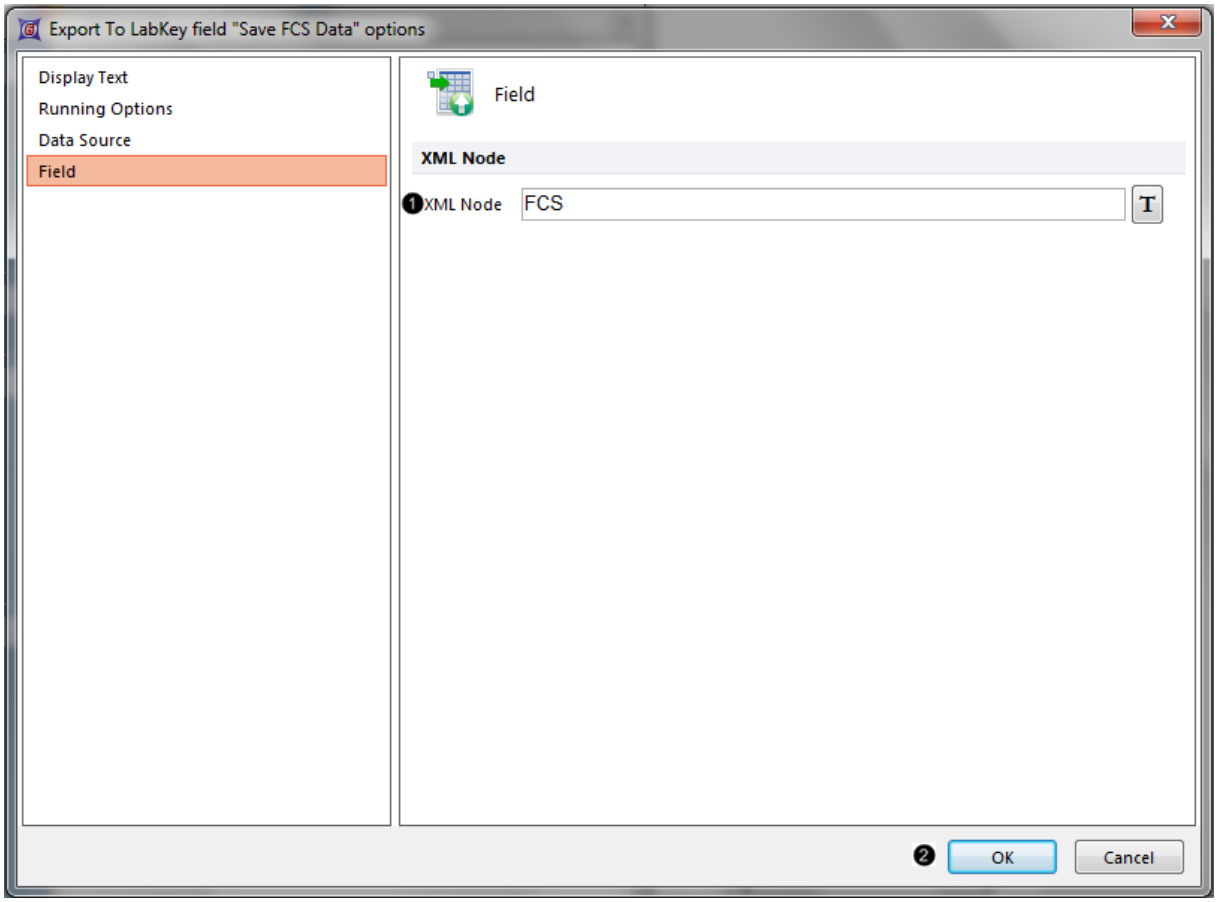

Figure T9.50 Entering text into the XML Node field

29. Click **OK** to close the dialog window (Figure T9.50 ❷).

30. Click on the **Picture** icon under the **Add Item to Report** category of the **Batch Actions** window (Figure T9.47 ❹).

31. Select **JPEG** from the **Format** drop-down menu at right, in the window that opens (Figure T9.51 ❶).

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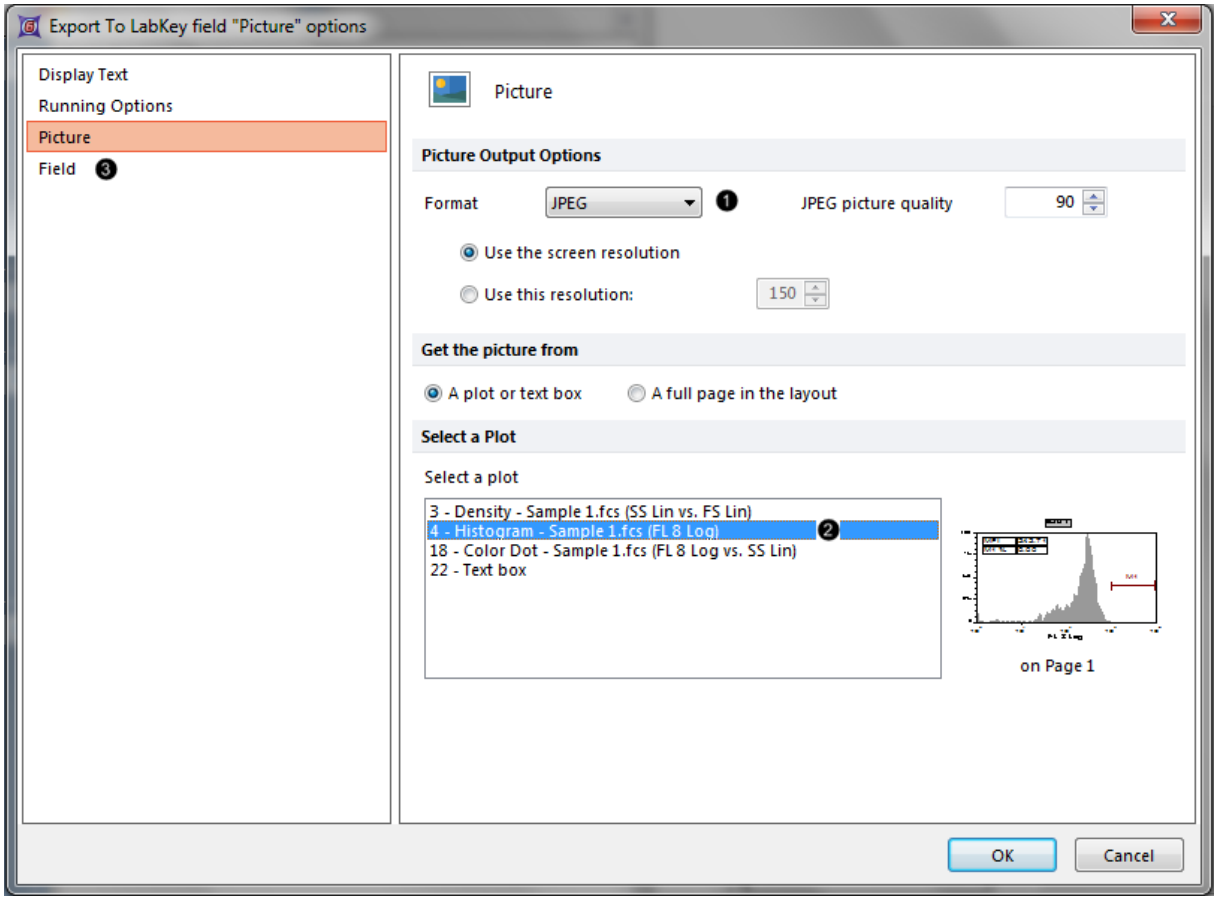

Figure T9.51 Format and data source options for exported pictures

32. Select plot 4, "**4-Histogram - Sample1.fcs(FL8 Log)"** under **Select a plot** to specify it as the plot to be exported (Figure T9.52 ❷).

33. Click on the **Field** category on the left of the dialog window (Figure T9.51 ❸).

34. Type "JPEG" in the **XML Node** field (Figure T9.52 ❶)

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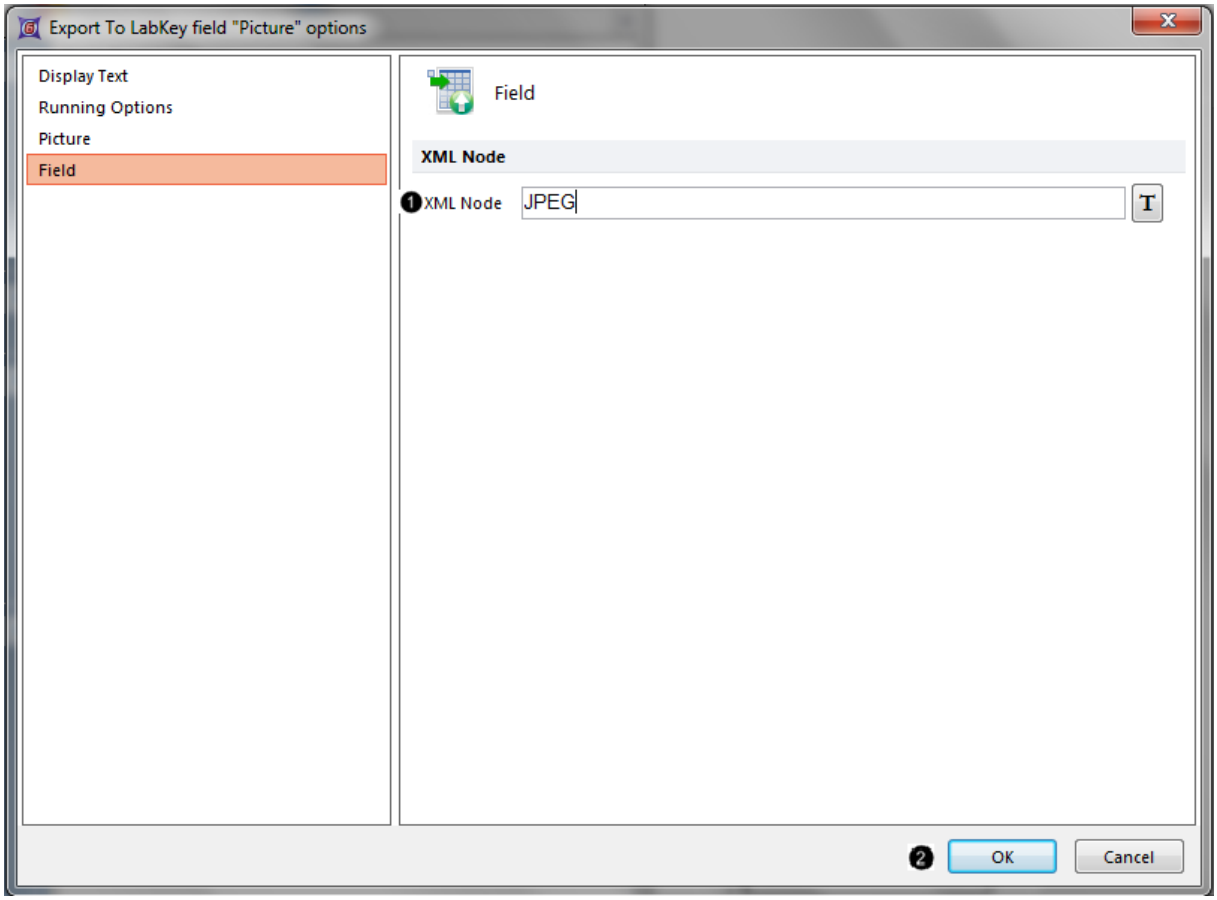

Figure T9.52 Entering text into the XML Node field

35. Click **OK** to close the dialog window (Figure T9.52 ❷).

36. Select the **Batch→Batch Processing→Run or Replay→Run** command (Figure T9.53). FCS Express will now export the items to the LabKey Server.

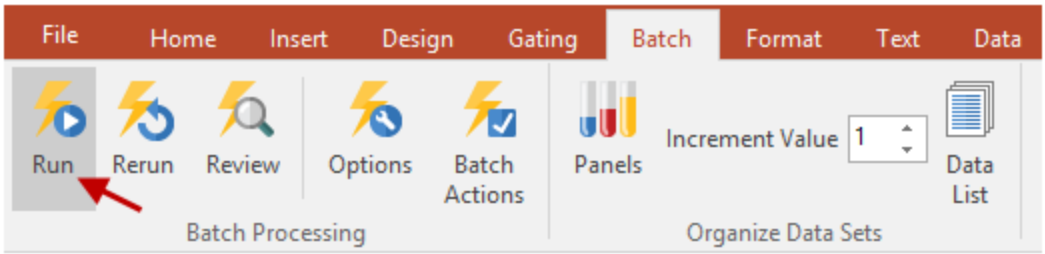

Figure T9.53 Running the Batch Process

37. Follow the directions in the **View the Exported Data** section of the accompanying LabKey Server tutorial to view the data you have just exported from FCS Express to LabKey Server.

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You have now set up a **LabKey Report** in the **Batch Actions** window from within FCS Express. You have added Keyword and Statistic Tokens, and files including an FCS Express Layout, FCS files, and pictures to the **LabKey Report**. Please see the accompanying LabKey Server tutorial for more information on sharing your flow cytometry analysis with your colleagues via LabKey Server.

38.10.5 Snapshots

FCS Express will automatically take Snapshots during Batch Processing.

The tutorial for Batch Processing Snapshots is located in the Snapshots 1423 section and can be found the Batch Processing Snapshots section 1436.

38.11 Creating and Using Panels

It is very common in the day-to-day work with flow cytometry to use the same analysis layout to analyze many different data files (for example, CD3-CD4-CD8 determinations in a clinical setting, where this panel of stains is repeated for different patients, or to test for the expression of the same surface markers after different treatments). In order to facilitate this type of analysis, FCS Express has the **Panels** option. Panels allow you to rapidly change all of the data on a particular layout to a new set of data.

In order to use the panels feature in FCS Express, you will first set up the number of tubes that you are working with, and then create the plots and other elements on the layout. Once that is done, you will be able to load another set of data with just a few mouse clicks.

You can also set up a panel 860 after the layout has already been created.

In the first section, we will set up the panel 1498.

38.11.1 Setting Up the Panel

In the course of this example, we will:

- Learn how to set up a panel.
- Learn how to set up a file list with the experimental files to be analyzed.

In this tutorial we will be analyzing 2 samples, each comprised of a set of 2 tubes. The panel will consist of the tubes that comprise the "set", which will repeat itself.

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We will begin by defining the panel and then setting up the data list.

1. Open a new, blank layout.
2. Select the **Batch→Organize Data Sets→Panels** command (Figure T10.1). Alternatively, select the **Data→Organize Data Sets→Panels** command.

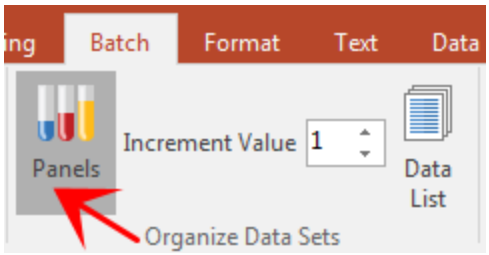

Figure T10.1 Selecting the Panels Command

The **Panel Information** dialog appears as shown in Figure T10.2.

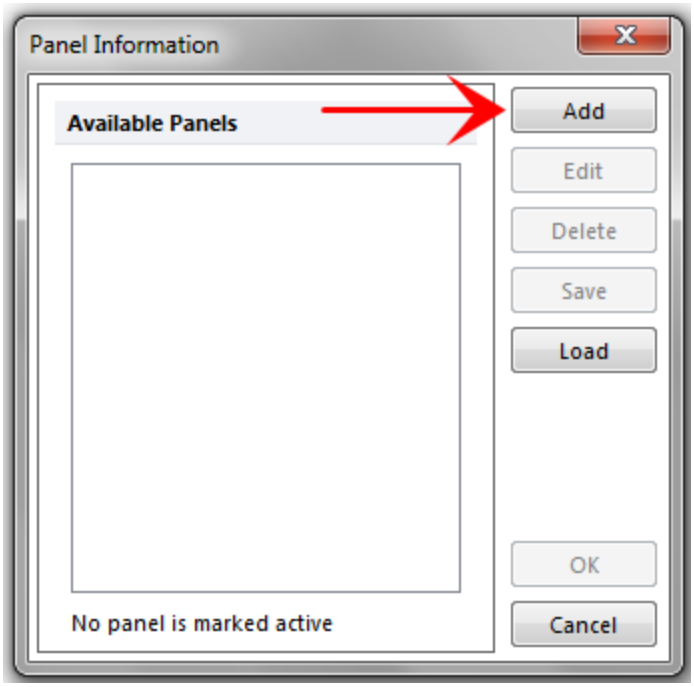

Figure T10.2 Panel Information Dialog

3. Click the **Add** button (Figure T10.2).

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The **Add Panel** dialog will appear as shown in Figure T10.3A.

4. Enter "**My Panel**" in the **Panel Name** field. ❶
5. Click on the upward triangle in the **Number of Tubes** spin control until it reaches **2**. The dialog should now look like Figure T10.3B. ❷
6. In the **Name** column, name Tube #1 "T Cell markers" and Tube #2 "B Cell markers" The dialog should now look like Figure T10.3C. ❸
7. Click **OK** to close the **Add Panel** dialog. ❹

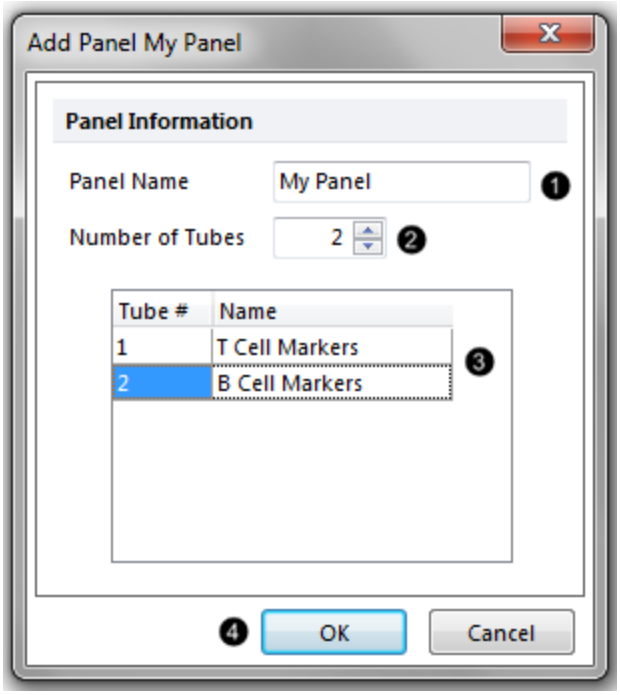

Figure T10.3 Setting up a Panel using the Add Panel Dialog

The **Panel Information** dialog will now appear updated with the newly added panel information, as shown in Figure T10.4.

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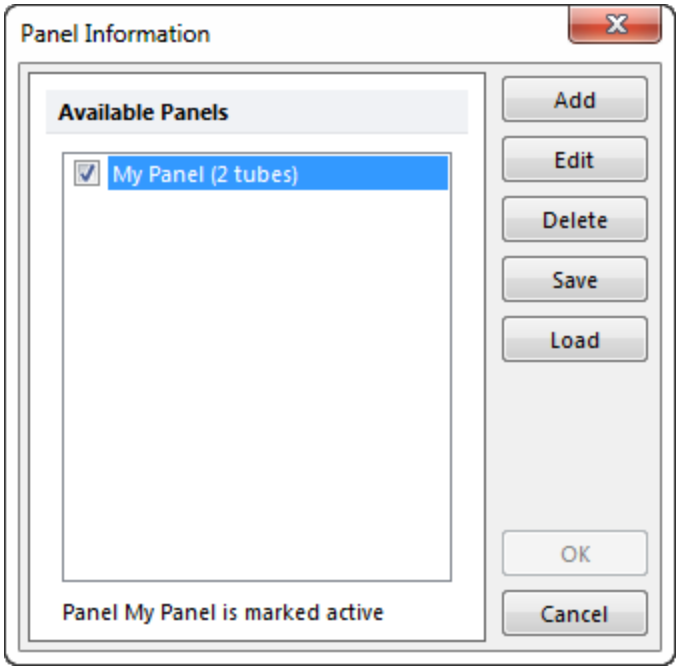

Figure T10.4 Updated Panel Information Dialog

8. Click **OK** to close the **Panel Information** dialog (Figure T10.4).

The next step consists of using the **Data List** to set up a list of the files we want to analyze using the panel.

9. Select the **Data→Organize Data Sets→Data List** command (Figure T10.5). Alternatively, select the **Batch→Organize Data Sets→Data List** command.

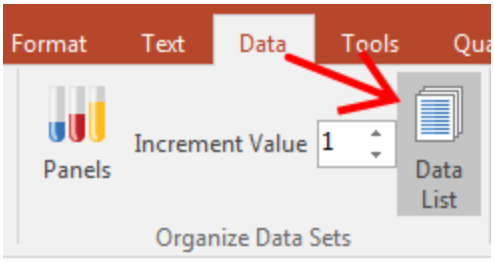

Figure T10.5 Selecting the Data List Command

A **Data List** window opens as shown in Figure T10.6.

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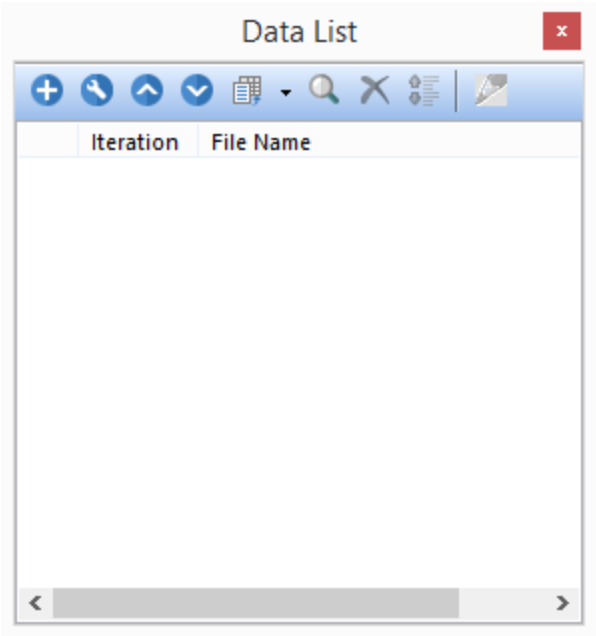

Figure T10.6 Data List Window

10. Click the **Add** button on top of the **Data List** window to add files.

The **Select a Data File** dialog opens (Figure T10.7).

11. Navigate to where the **Tutorial Sample Data** folder is located.

12. Select the 4 files named **Normal2_Tube1, Normal2_Tube2, Normal3_Tube1 and Normal3_Tube2** as shown in Figure T10.7. (To select multiple files at once, **Ctrl +** click each file or click on the first file, **Normal2_Tube1**, press and hold the **Shift** key on the keyboard, and then click on the last file, **Normal3_Tube2**.)

13. Click **Open** (Figure T10.7).

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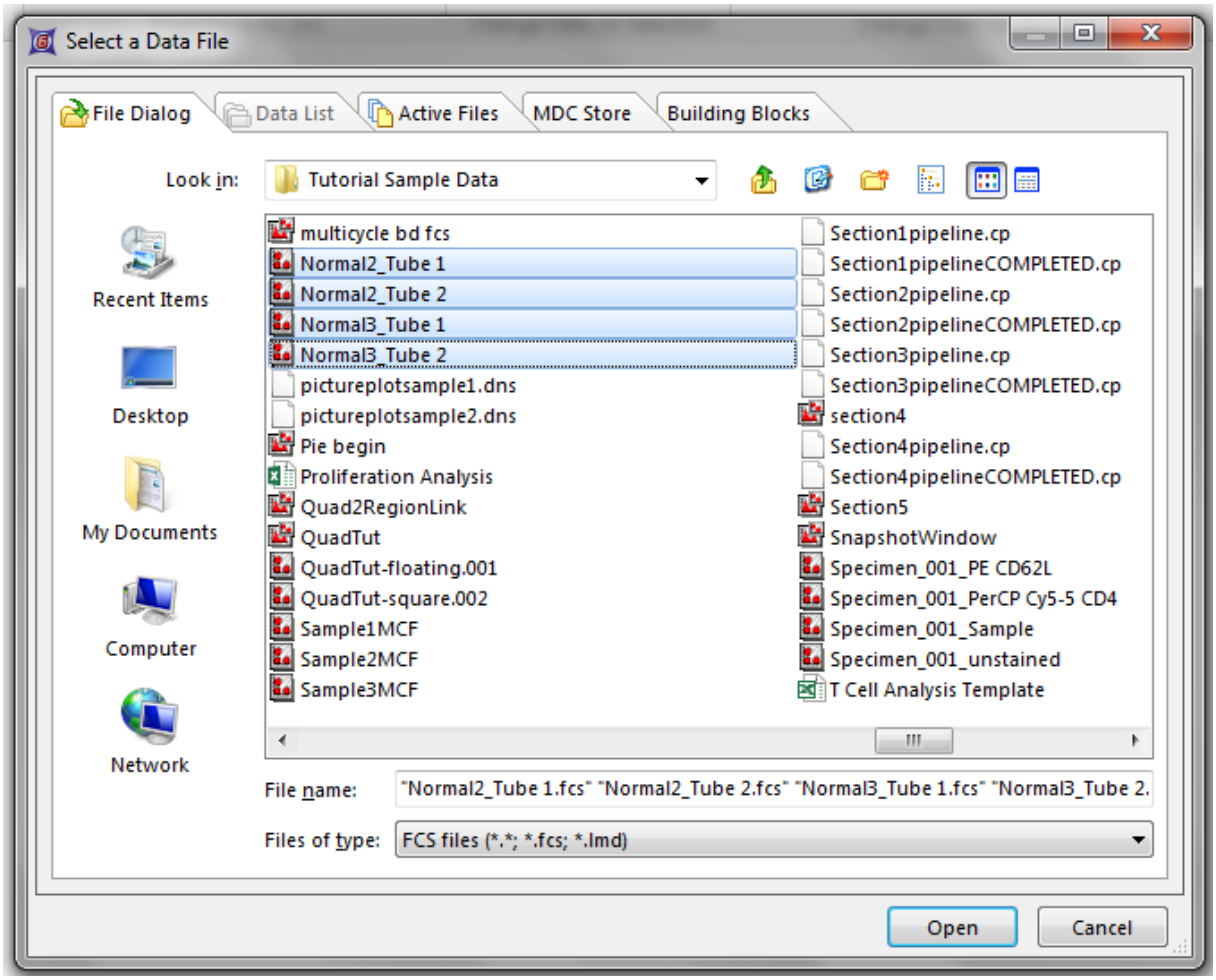

Figure T10.7 Selecting Data Files to insert into the Data List.

The **Data List** window now lists the data files selected, each with its **Iteration** number, **Tube** from the panel, and **File Name** displayed. The **Data List** should appear as shown in Figure T10.8.

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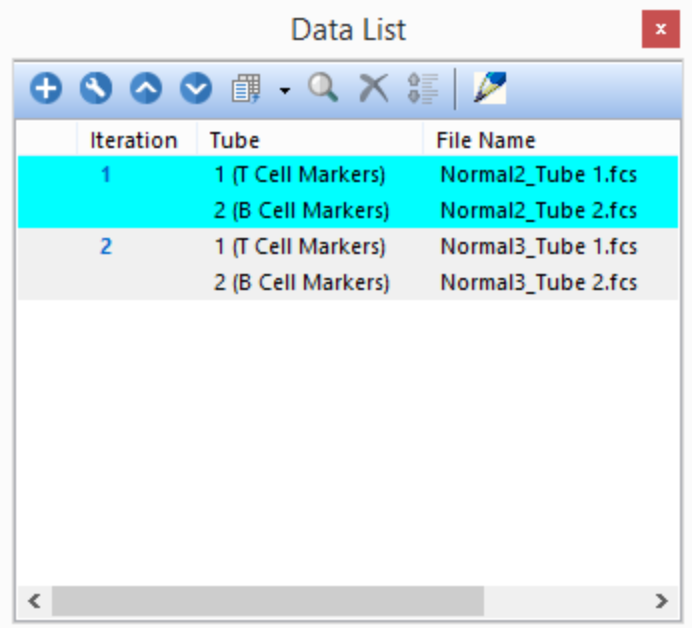

Figure T10.8 Updated Data List with Corresponding Tubes for the Panel

As shown in Figure T10.8, the **Data List** window shows the tubes from the panel you created in the left column, and the files you added in the right column. This greatly helps to organize your files into the corresponding tubes for further analysis. To further aid organizing the data files, FCS Express allows you to add keywords from the FCS file to the information displayed in the Data List. Keywords that are descriptive indicators of sample identification can be added to the Data List to easily identify which data files corresponds to each entry in the Data List. There are many keywords that can be used to help identify the data files within the Data List. In this tutorial, we will add the keyword **$SRC** to the Data List.

14. Click on a sample in the Data List to highlight it.

15. Click on the **Format** button shown at the top of the **Data List** in Figure T10.8.

The **Format File List** dialog appears, as shown in Figure T10.9.

16. Select **Add** from the **Format Data List** dialog.

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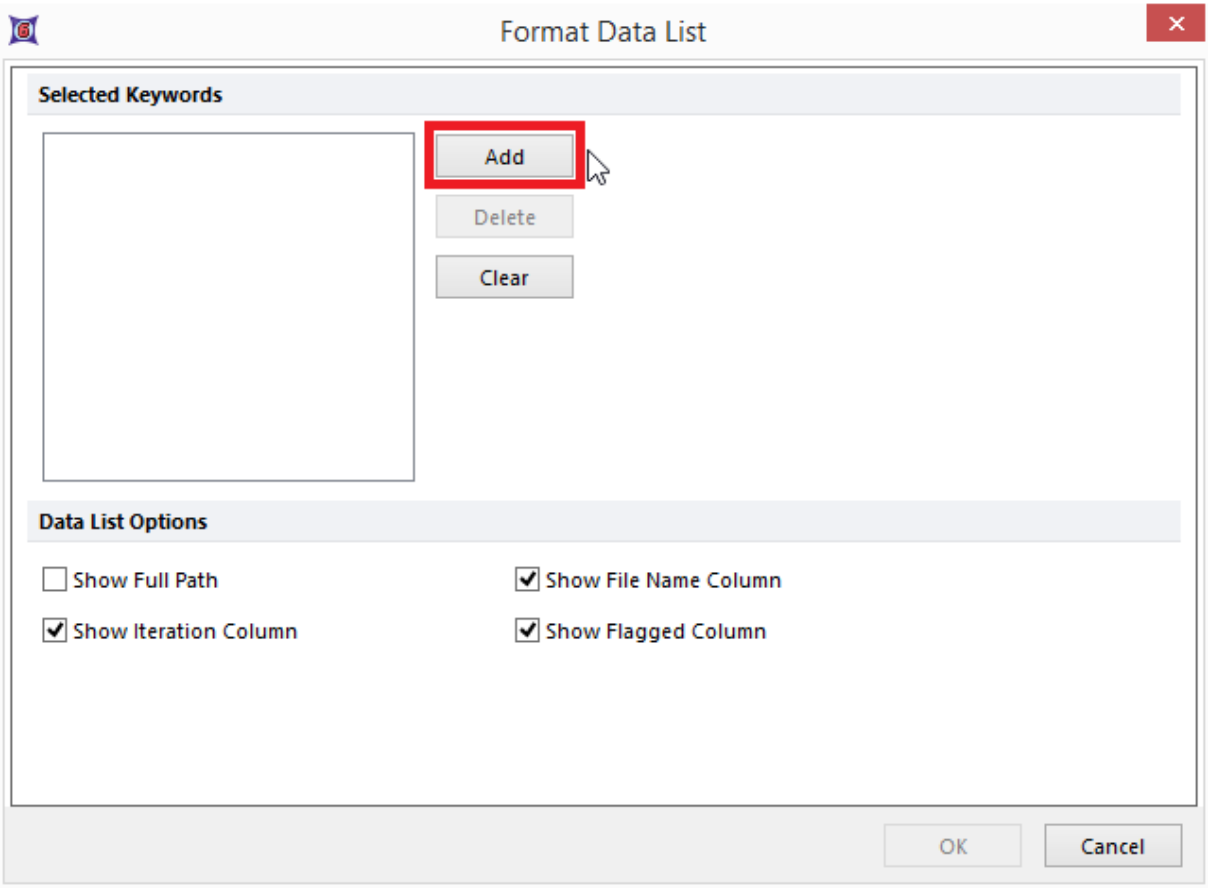

Figure T10.9 Format File List Dialog

The **Please select one or more keywords** dialog appears, as shown in Figure T10.10. This is where we will select the keyword **$SRC** to display in the **Data List**.

17. Click on the down arrow to scroll down to the bottom of the list.

18. Click on **$SRC**, highlighted in Figure T10.10. If you want to display more than one keyword, you can select multiple adjacent keywords by holding down the **Shift** key on the keyboard or by selecting multiple non-adjacent keywords by holding down the **Ctrl** key while clicking on each keyword. For this example, we will only select **$SRC**.

19. Click **OK** (Figure T10.10).

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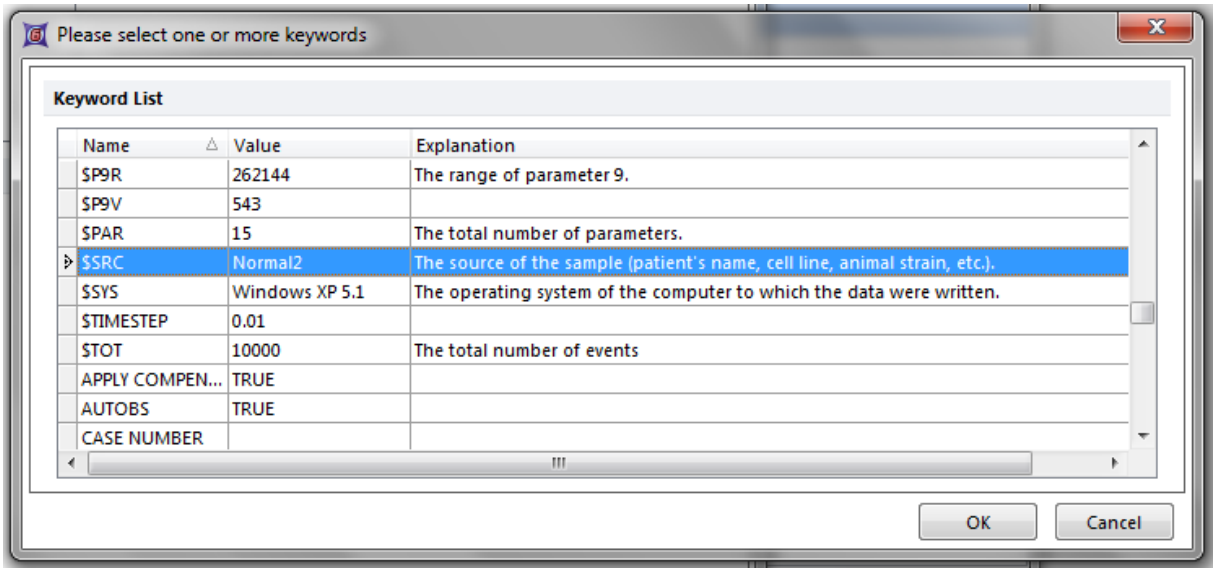

Figure T10.10 Selecting an FCS Keyword to use in the Data List

The **Format File List** dialog will be updated with **$SRC** in the **Selected Keywords** field, as shown in Figure T10.12. The Data List can also be formatted to display your data in Groups.

20. Select **OK** on the **Format File List** dialog.

The **Data List** dialog will now appear updated showing the grouping of the data files, the file name without the full path and with the column **$SRC**, as shown in Figure T10.13.

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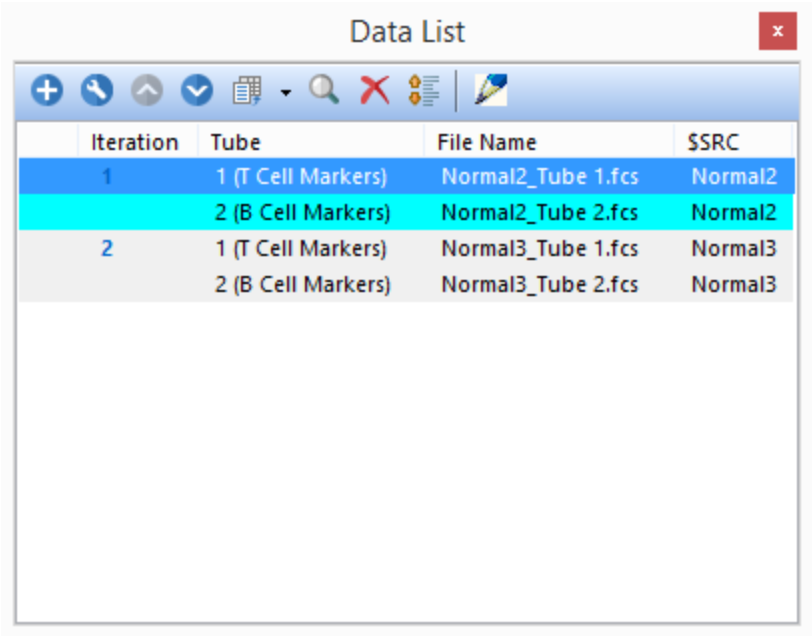

Figure T10.13 Data List Window Updated with Groups, File Names and $SRC Column.

You can use the icons at the top of the **Data List** dialog, (Figure T10.13) to manipulate the data files in the list.

As previously demonstrated, you can add more files to the Data list by selecting the Add button and you can format the list to include FCS keywords by selecting the Format button. To move a file up or down in the **Data List** select the data file and then select the Up or Down arrows. To change a file, select the Change File button, and to search for a file select the Search button. To delete a file from the list, select the file, and then select the Delete button.

In a previous version, FCS Express 4, any changes made to the order of the tubes in the Data List would automatically change in which plots those data files were being viewed as they are being moved. If your files are large, it could take some time for your layout to refresh as each tube is moved up or down the Data List.

However, in FCS Express 6, the user can now defer those changes until the files have been moved and placed in the required order. By clicking the **Defer Layout Changes** icon, the user can makes changes to the order of tubes, then, deselect the icon and the data would automatically refresh in the analysis layout.

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We will now proceed to creating the layout 1508.

38.11.2 Creating the Layout

In the course of this example, we will:

- Create a layout to use for our panel.

We will now proceed to create a layout for the analysis of our experiment using the panel we created. We will be using plots and gates. Please refer to previous tutorials for more information on inserting plots 1225, formatting plots 1236, and using gates 1266.

Using the layout we started in the previous section, Setting Up the Panel 1496, we will create four plots for our first set of samples.

1. Open the **Data List** window, if it is not already open.
2. Select the first two files from the list (click on the first file, hold down the **Shift** key and click on the second file).
3. Drag the selected files outside of the **Data List** dialog.
4. Release the mouse button to drop the files onto a blank place on the layout.

The **Select Plot Types...** dialog will now appear, as shown in Figure T10.14.

5. Check the **Color Dot** option and uncheck all other plot types.
6. Check the **Use this selection for all files** option.
7. Click **OK** (Figure T10.14).
8. Note that now in Version 5, FCS Express can remember your selection and use this selection as the default plot type for this layout when creating new plots by dragging and dropping from the Data List.

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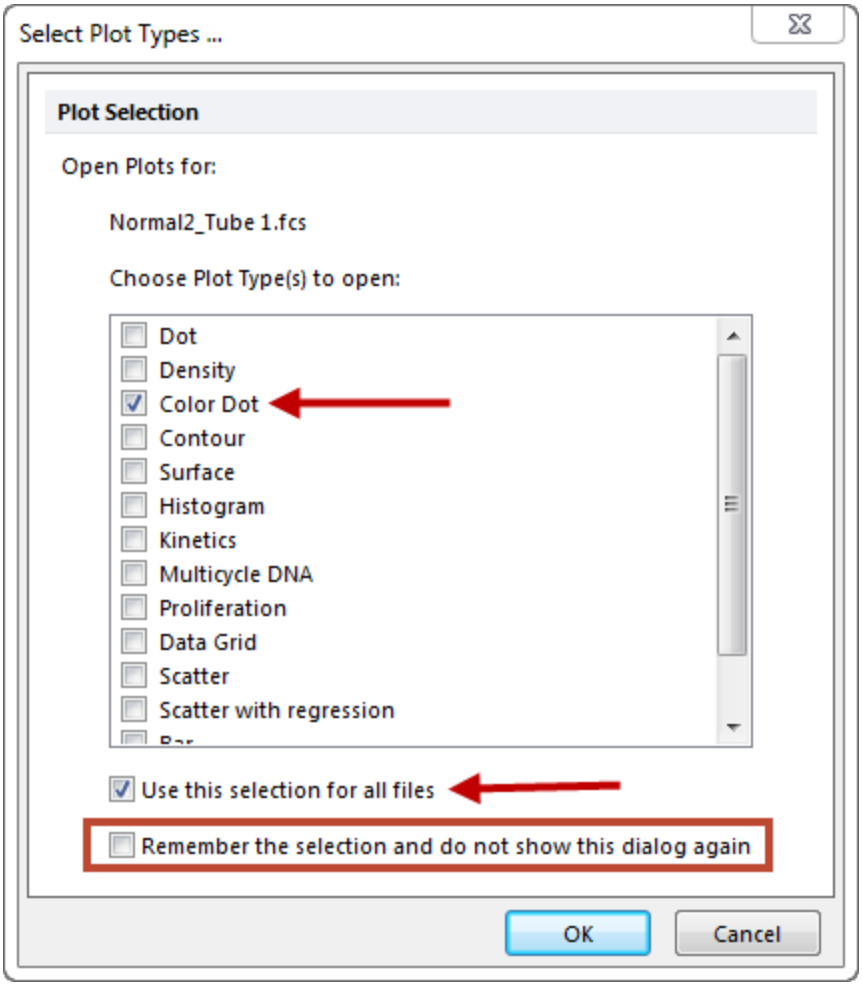

Figure T10.14 Select Plot Types... Dialog

Two color dot plots, using the first two data files selected in the **Data List**, are now created on the layout. We now need to arrange them in a column on the left side of the layout.

8. Select the plots by holding down the **Ctrl** button on the keyboard and clicking on each one. It maybe necessary to move the density plot to be able to individually select each plot. Alternatively, click and hold the mouse button down and drag a box that encompasses all four plots, or select the **Home→Editing→Select All** command.

9. From **Home → Editing → Align**, select the **Align** command (Figure T10.15). This tool will help align and distribute your object by arranging them according to the first object that was selected while holding the **Ctrl** key.

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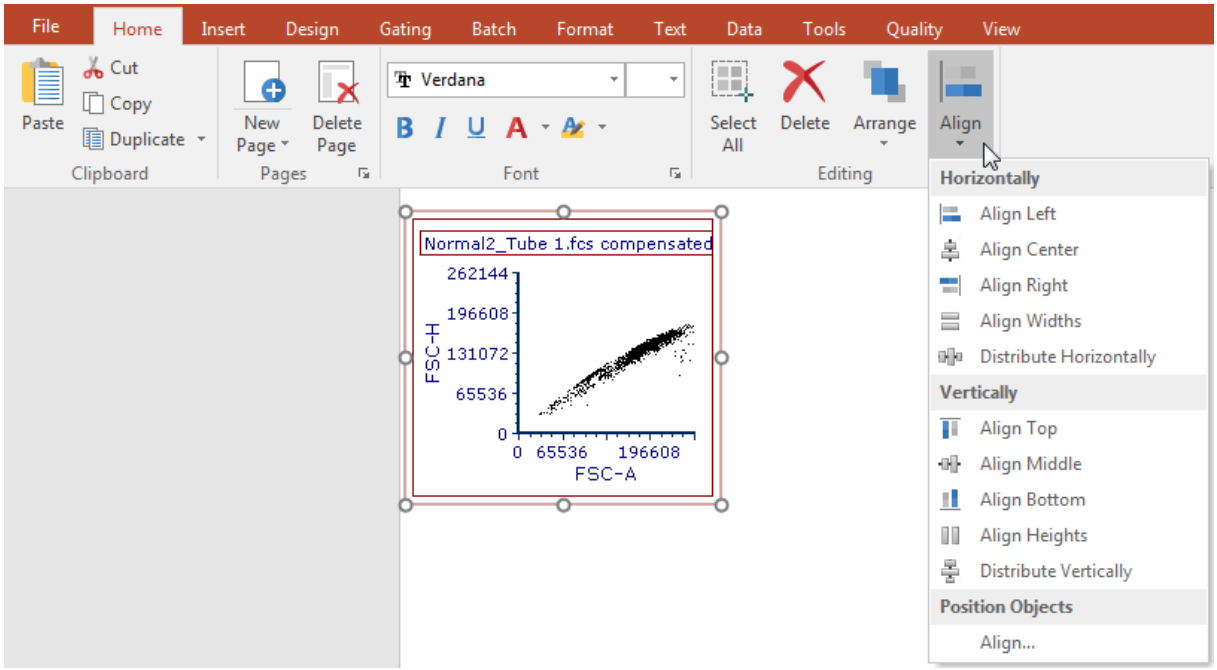

Figure T10.15 Aligning Plots

The **Alignment** window similar to Figure T10.16 will appear.

10. Under the **Horizontal** column, select **Left Sides**. Under the **Vertical** Column, select **No Change**.

11. Click **OK**.

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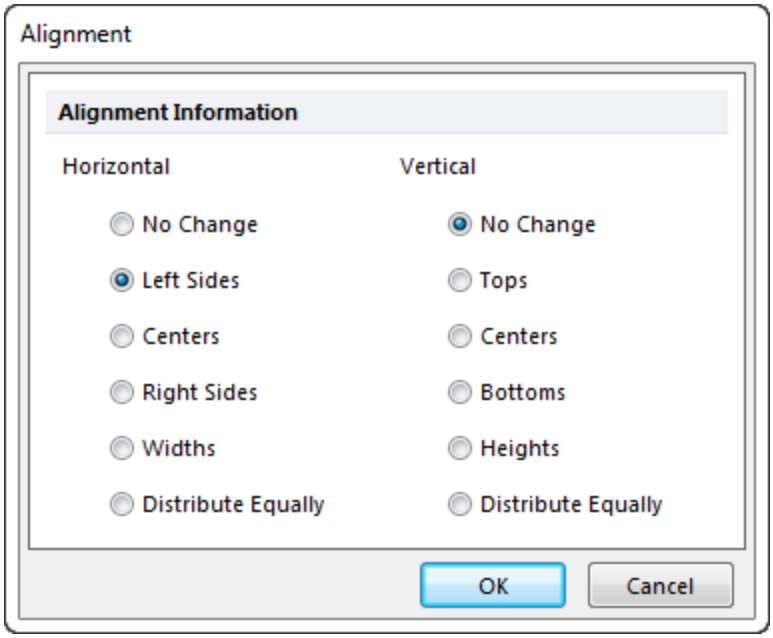

Figure T10.16 Aligning Plots on the Left Side

12. Select the plots. Right click on them and select **Duplicate**. (Figure T10.17).

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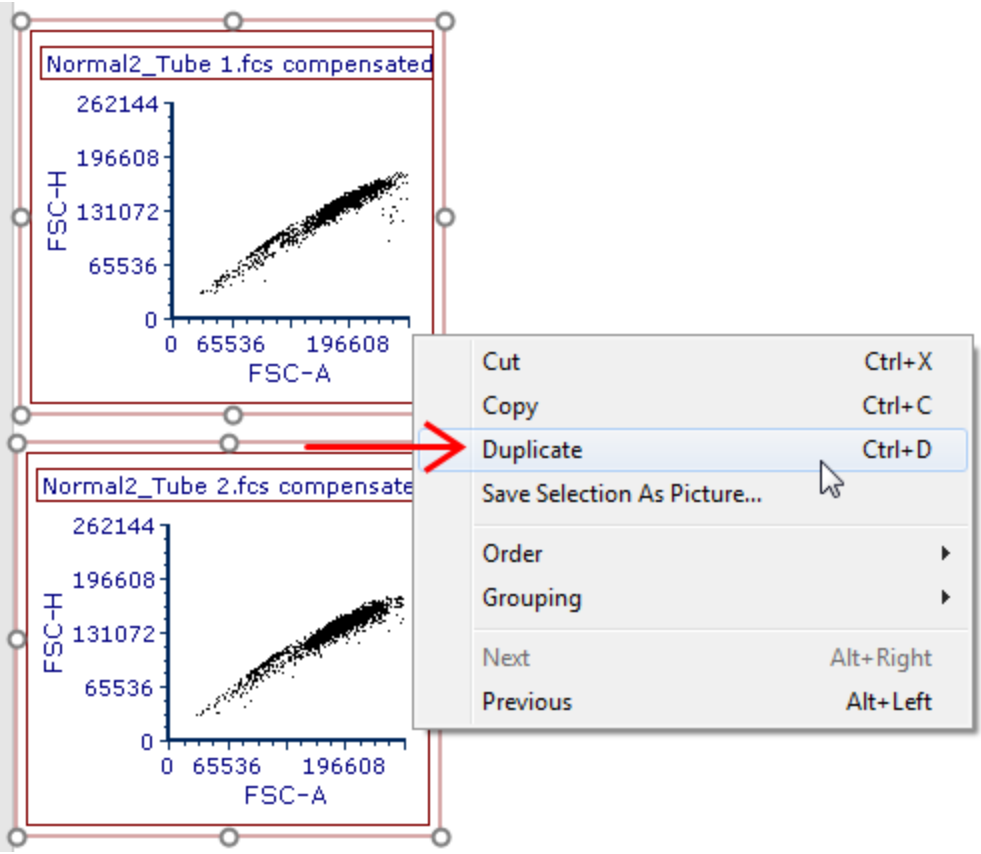

Figure T10.17 Duplicating Plots

The duplicated plots will appear. (Figure T10.18).

13. Rearrange the duplicated plots by placing them to the right of the original plots.

14. Use the Align tool, if needed.

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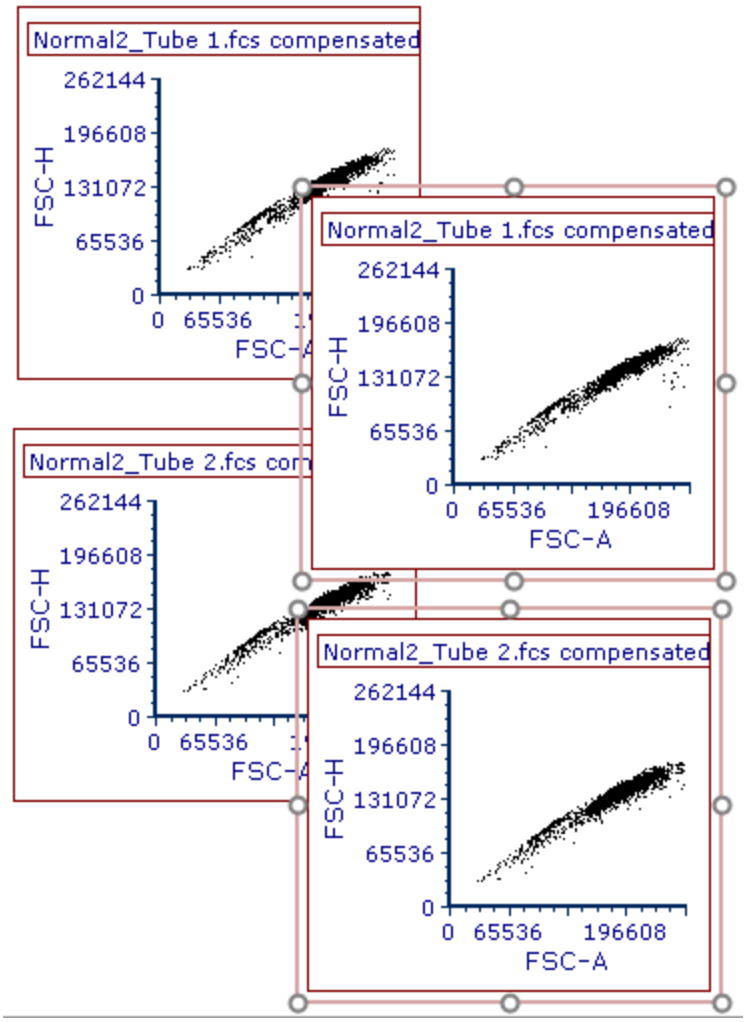

Figure T10.18 Duplicated Plots

The page of the layout should now appear similar to Figure T10.19. For more information on manipulating objects in FCS Express please refer to the tutorial on Using Objects 1213.

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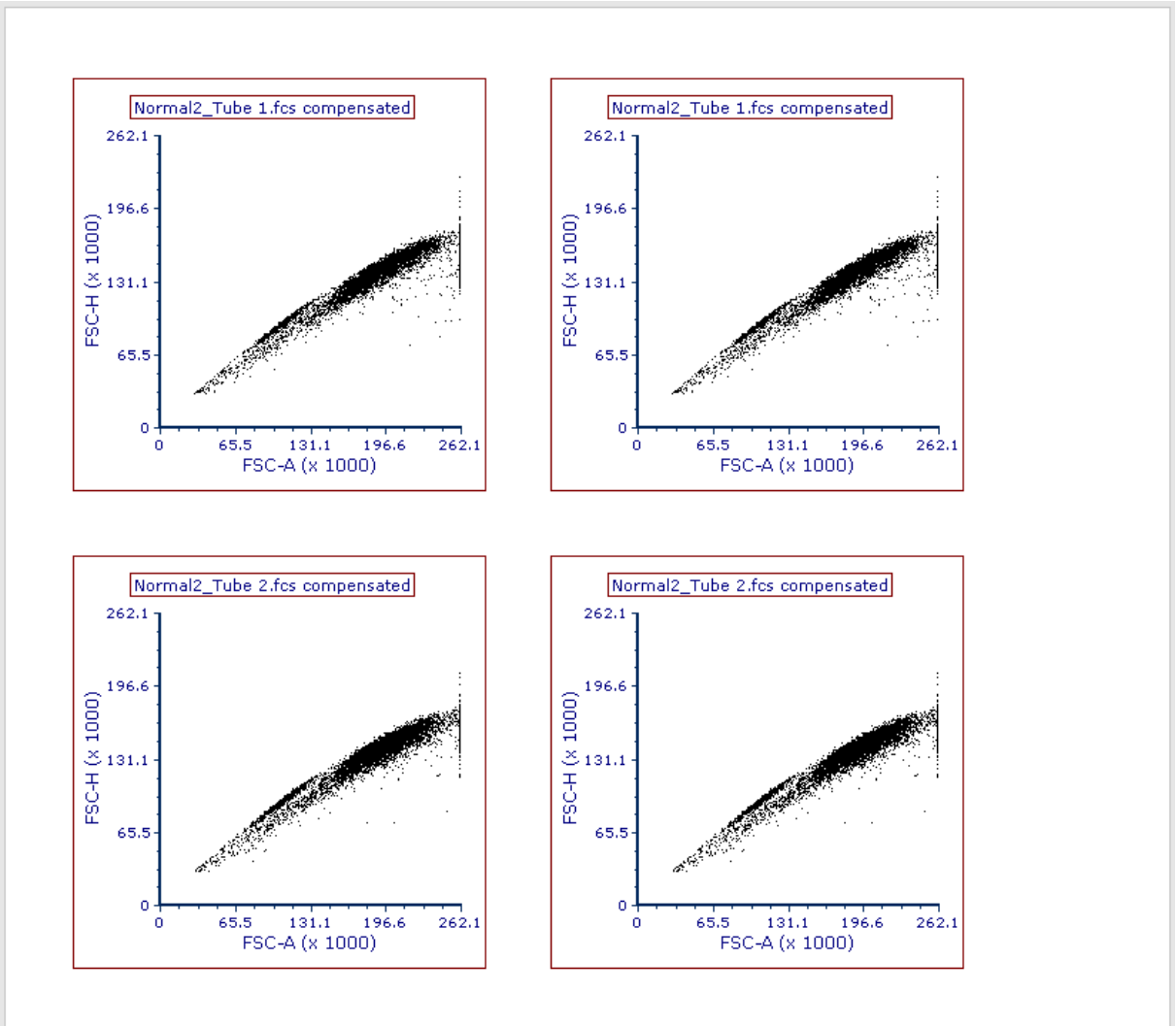

Figure T10.19 Plots Arranged on Page

We will now adjust the X and Y axis parameters[284], create gates[318], create statistics[381] and create a 1D histogram[1227] to which we will add an overlay[268].

16. On the 1st plot in the upper left corner, click on the X axis and choose **V-500-A**. Click on the Y axis and choose **SSC-A**.

17. On the 2nd plot in the upper right corner, click on the X axis and choose **V450-A**. Click on the Y axis and choose **PerCP-Cy5-5-A**.

18. For the 3rd plot, in the lower left, set the X axis to **APC-H7-A** and the Y axis to **V450-A**.

19. For the 4th plot in the lower right, set the X axis to **APC-A** and the Y axis to **PE-A**.

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20. The layout should similar to Figure T10.20.

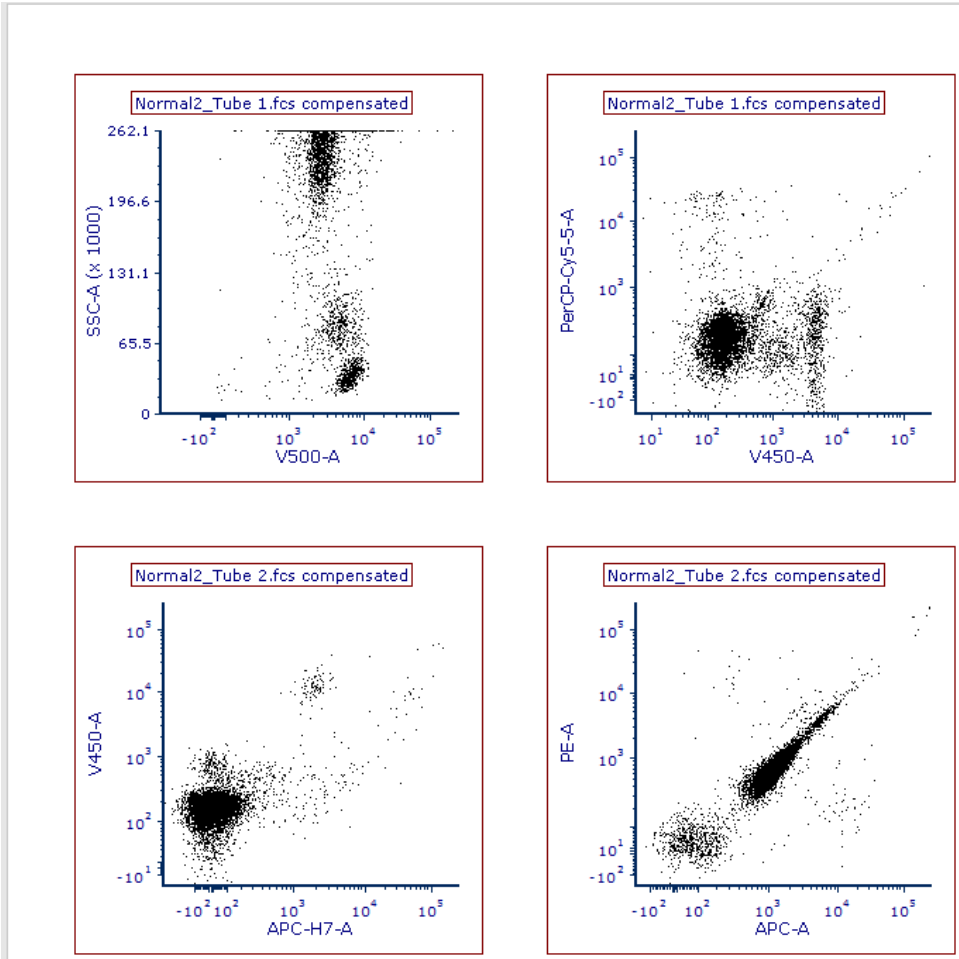

Figure T10.20 Plots with X and Y axis set to Parameters

21. We will also format the titles of the plot to include the Current Gate for that plot.

22. Select all plots by selecting **Select All** from the **Home** tab or by clicking **Ctrl+A**.

23. From the Format tab, select **Titles**. If you are using Default preferences, most likely the keyword <$FCSE_FILENAMENOPATH> will already be entered as a Title. Position your cursor after <$FCSE_FILENAMENOPATH> and click on Insert Keyword. Select Current Gate from the dropdown menu, as seen in Figure T10.21. Click **OK**.

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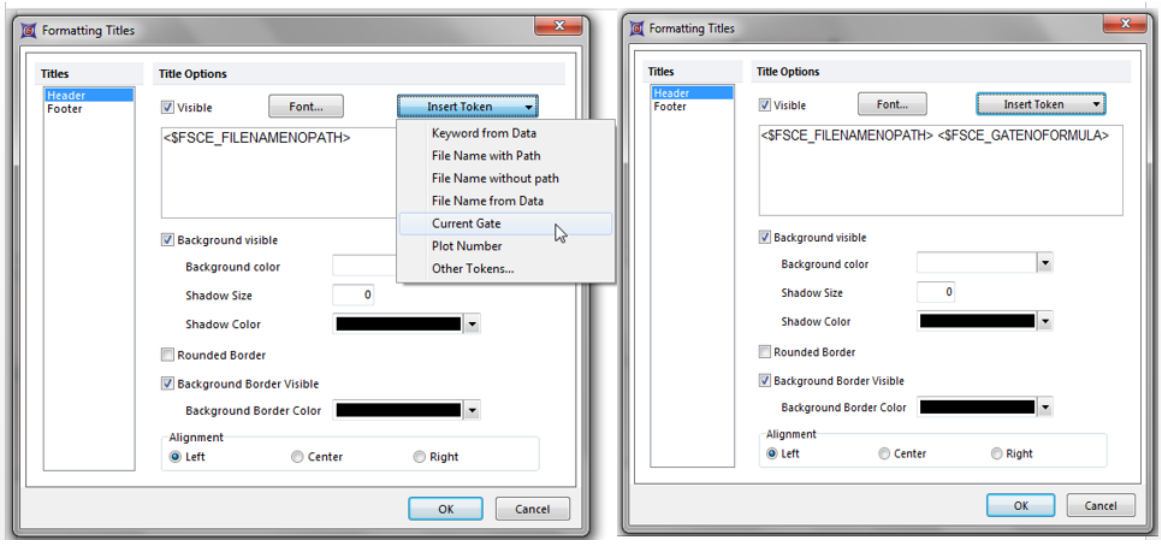

Figure T10.21 - Before and After Adding Current Gate to the Plot Title

24. We will now create gates 318. On the SSC/V500-A plot, we will create a polygon gate around the lymphs. From the Gating tab, select Polygon and create a gate around the lymphs. Name the gate **Lymphs**, as seen in Figure T10.21.

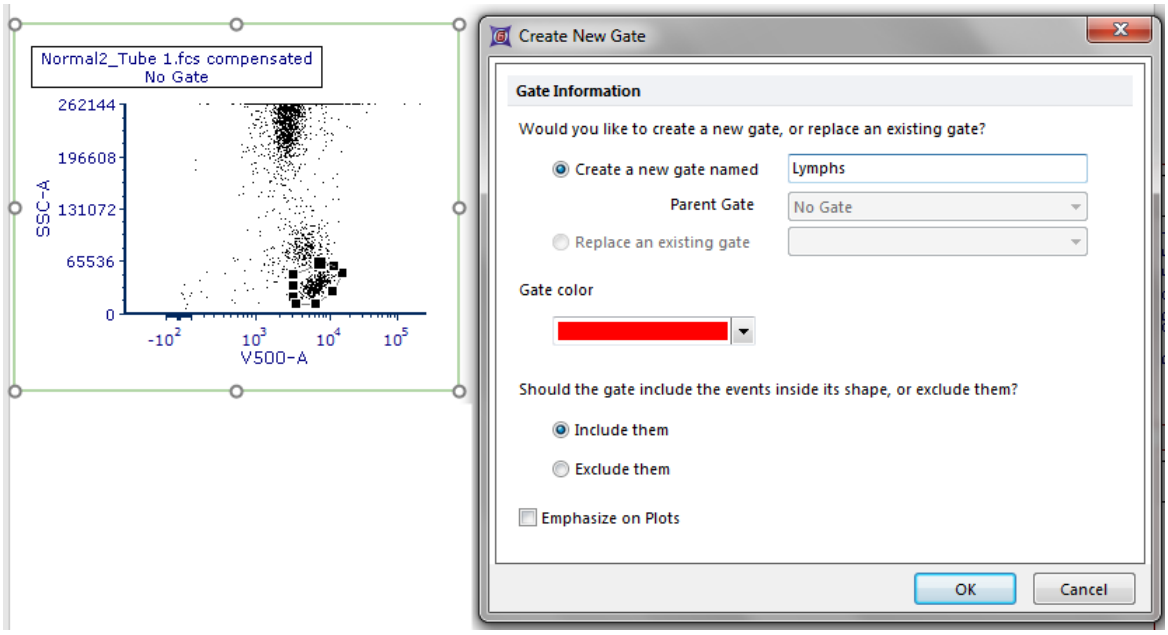

Figure T10.21 - Creating a Lymphs gate

25. We will apply the **Lymphs** gate to the 2nd plot in the upper right by dragging and dropping the Lymphs gate on top of the 2nd plot. The Before and After can be see in Figure T10.22.

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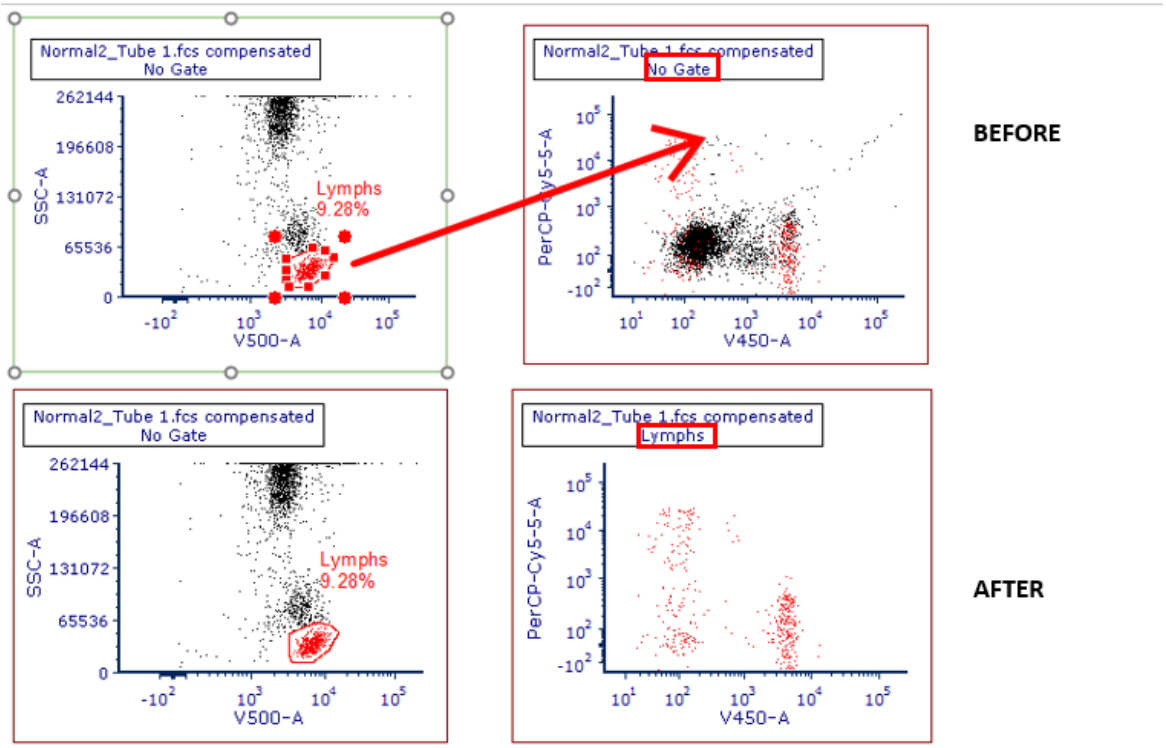

Figure T10.22 - Before and After Dragging and Dropping the Lymphs Gate

26. Select the 2 plots at the bottom of the page. From the **Gating** tab, select **Lymphs** from the **Current Gate** drop down menu, as seen in Figure T10.23.

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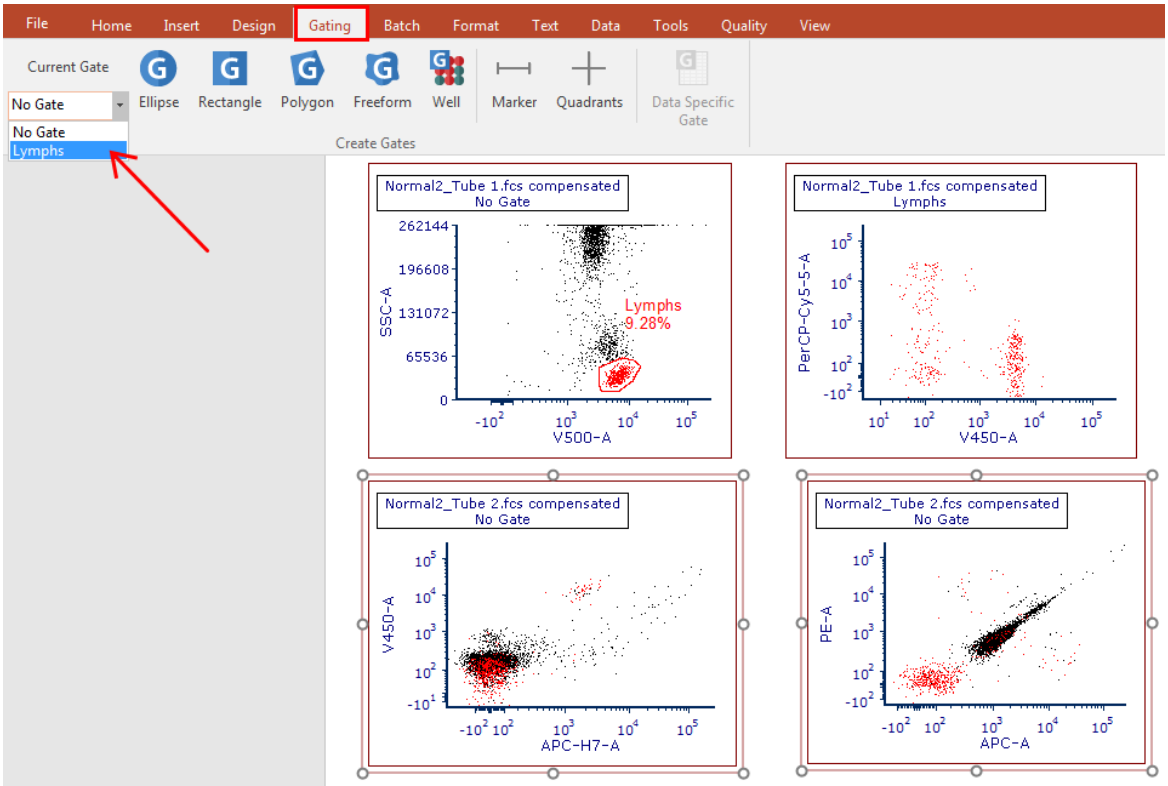

Figure T10.23 - Selecting Lymphs from the Current Gate Drop Down Menu

27. In the 3rd plot in the lower left, create a Rectangle gate around the cluster of cells that are APC-H7/V450 dual positive. Name the gate **Cells of Interest**.

28. Apply the Cells of Interest gate to the 4th plot in the lower right by dragging and dropping the gate on top of it.

29. Edit the Gates to Display in the top 2 plots by selecting them, then selecting from the **Format** tab, **Gates to Display**.

30. Under **Gate Options**, choose **Only the gates checked below**. Choose **Lymphs**.

31. For the question **When a new gate is created on the layout, should the gate color be displayed?**, select the option **Only if the gate was create on a plot showing the same data file as this plot**, as seen in Figure T10.24. Click **OK**.

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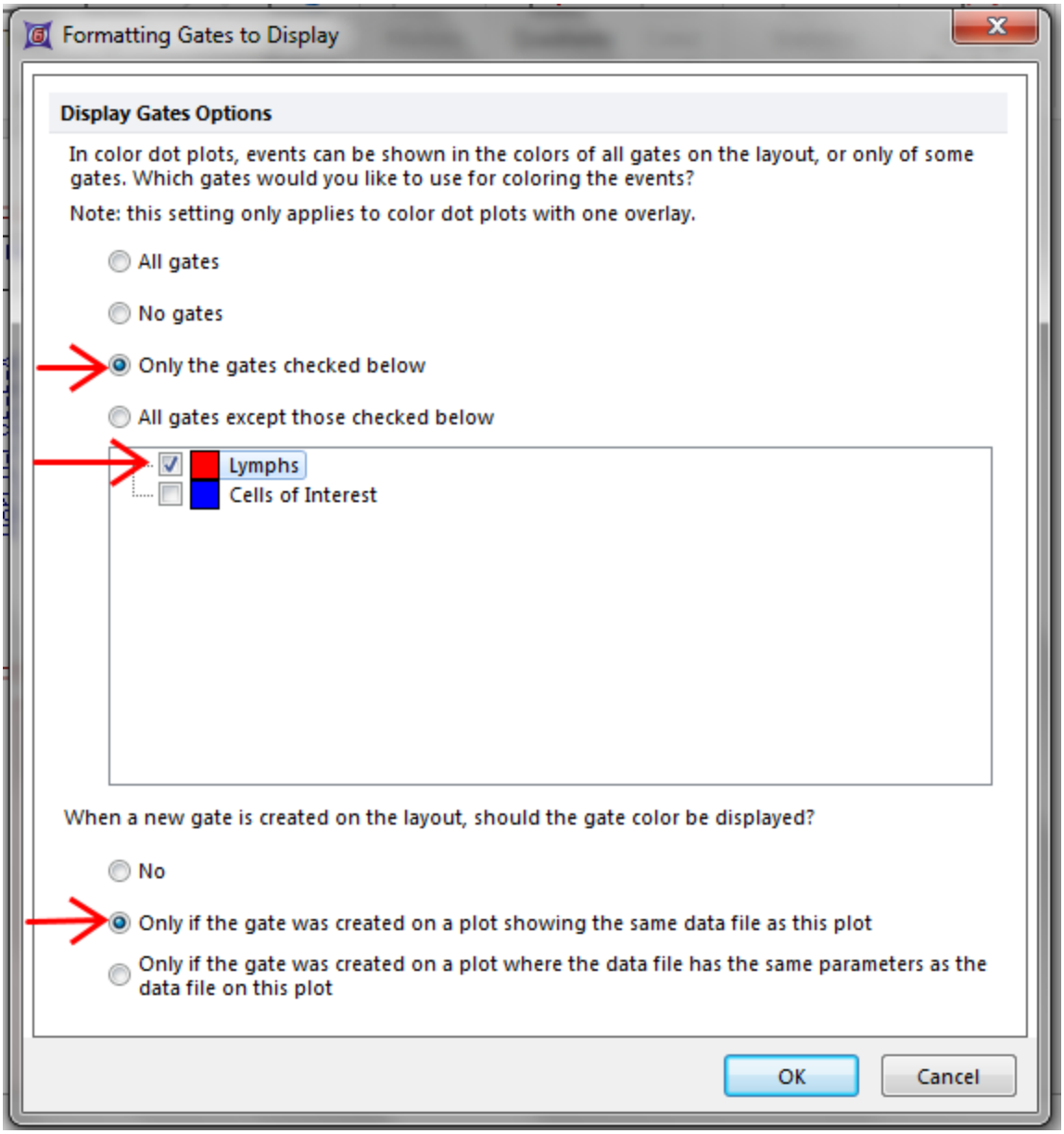

Figure T10.24 - Editing the Gates to Display

32. Your layout should look similar to Figure T10.25.

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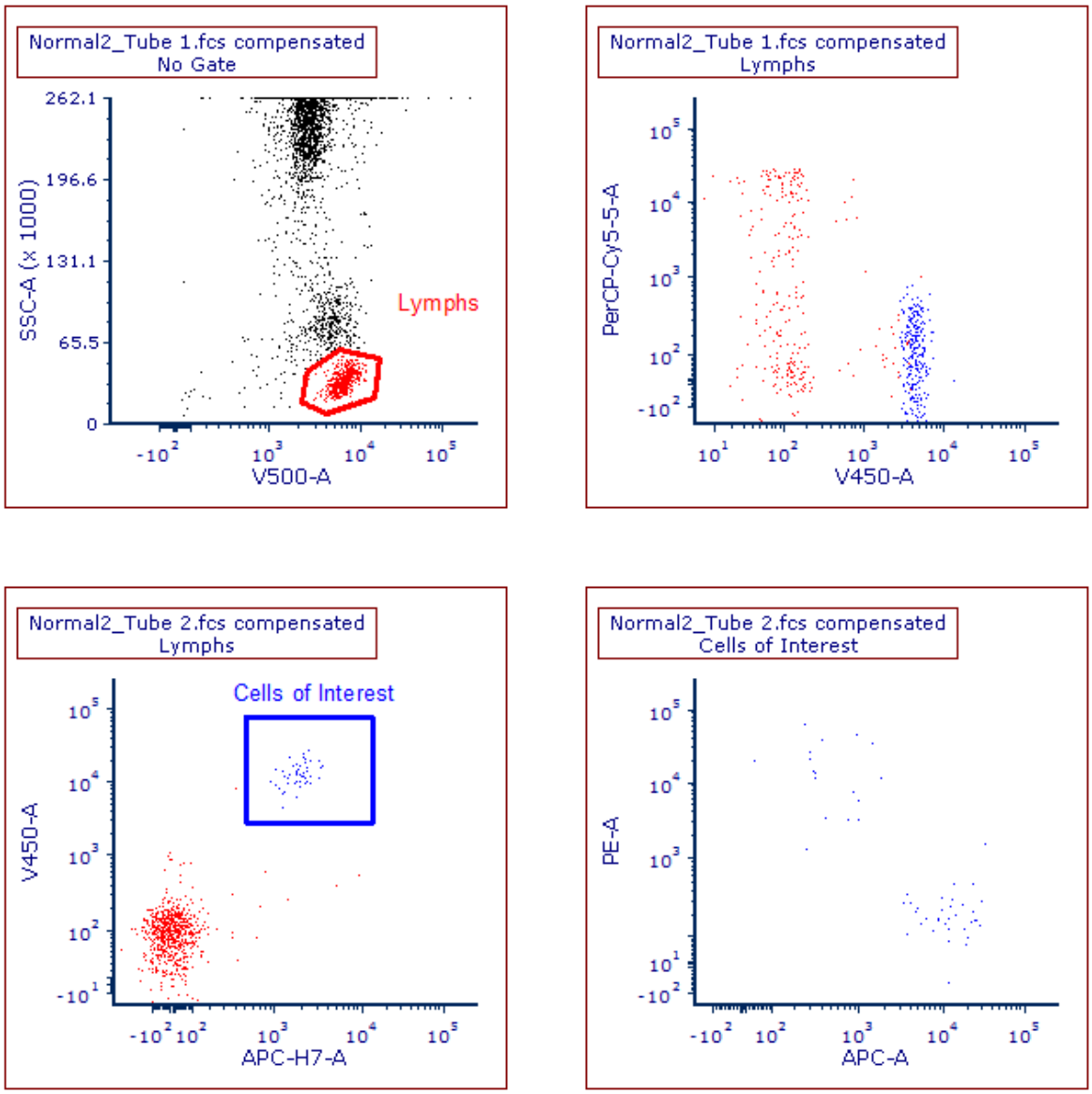

Figure T10.25

33. We will now add Quadrants to the upper right and lower right plots. From the **Gating** tab, click on **Quadrants**, then click on the V450 vs PerCPCy5.5 plot. Quadrants can be moved by clicking on the center point of the quadrant and then moving the quadrant to the desired position.

34. Repeat step 33 for the APC vs PE plot.

35. Your layout should appear as in Figure T10.26.

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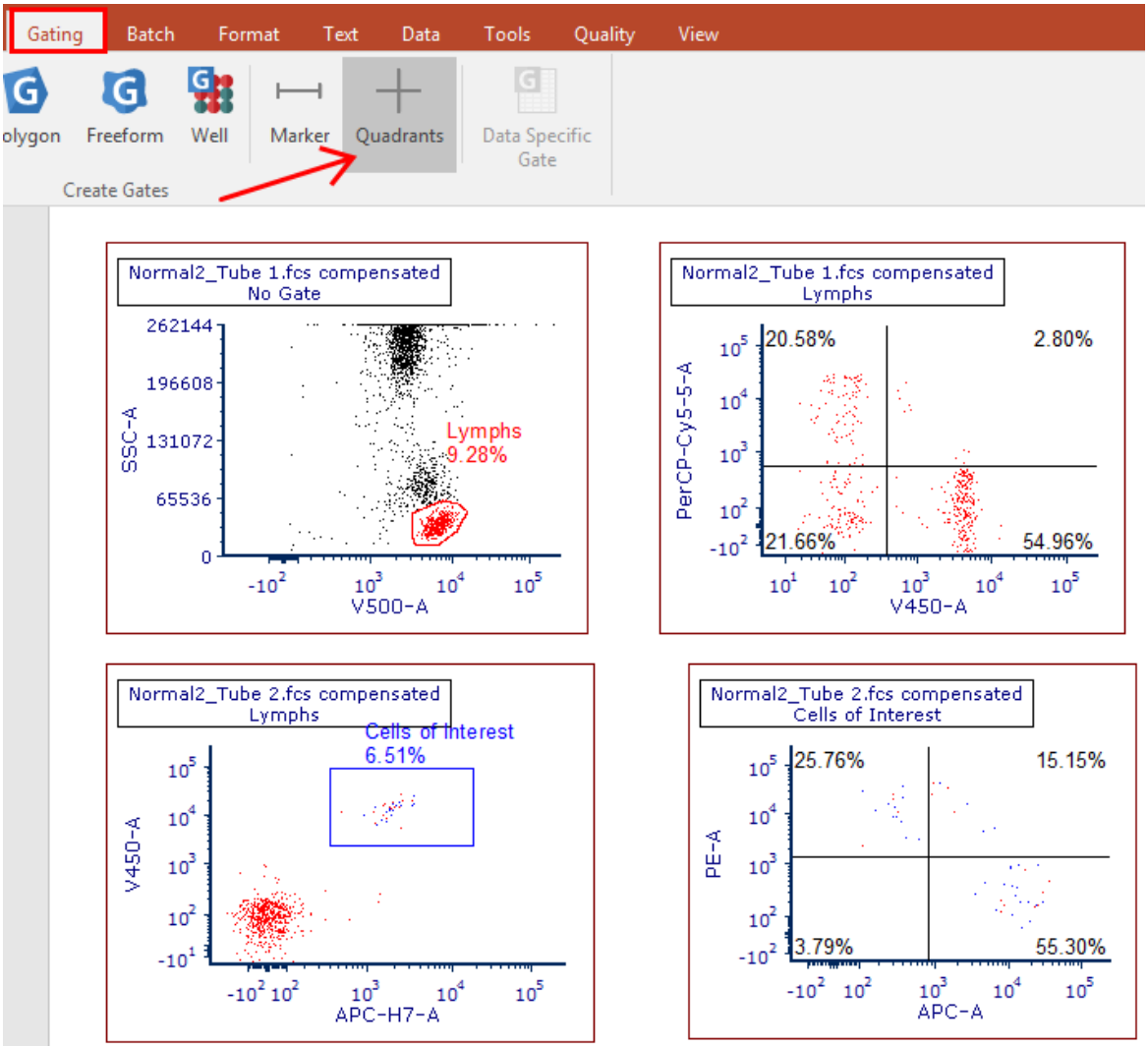

Figure T10.26

36. Now we will add quadrant statistics for the 2 plots that contain quadrants.

37. On the V450 vs PerCPCy5.5 plot, right click and select **Statistics>Quadrant Statistics**. Right click on the statistics grid and de-select everything but the columns **Gate**, **# of Events**, **% of Gated Cells** and **% of All Cells**, as seen in Figure T10.27.

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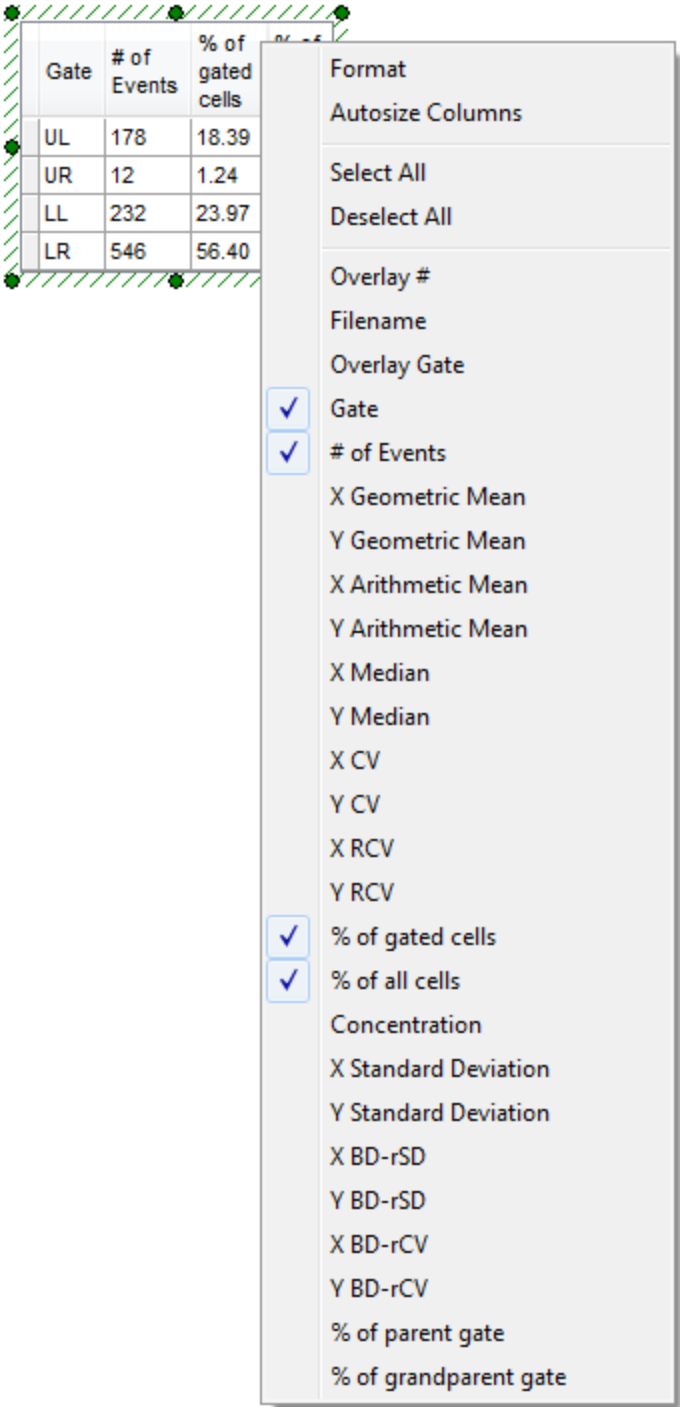

Figure T10.27 - Formatting Statistics

38. From the right click menu, you can also choose **Format>Stats** 389 to edit the columns to be viewed.

39. Repeat steps 36&37 for the APC vs PE plot.

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40. You can also edit 196 the FCS Express User Options to reflect these changes, so each element does not need to edited manually.

41. We will now add another page and add a 1D histogram to this page.

42. From the **Insert** tab, select **New Page**. We can also add a new page by selecting the "+" sign next to the Page 1 tab at the bottom of the layout.

43. On Page 2, drag and drop the 2nd tube from the Data List.

44. Select **Histogram**. Click **OK**.

45. Change the X axis to **PE-A** and change the Current Gate to **Lymphs**.

46. Right click on the histogram and select **Add Overlay using Advanced Open Data Dialog**, as seen in Figure T10.28.

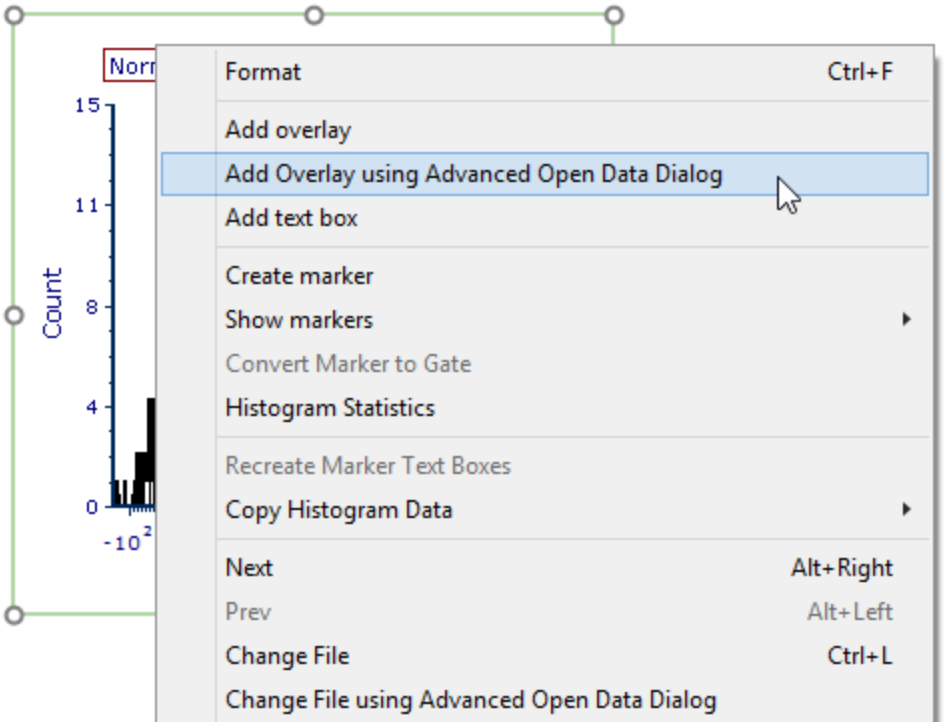

Figure T10.28 - Adding an Overlay

47. In the **Advanced Open Data File** window, select the **Data List** tab and choose the 2nd tube. Click **Open**, as seen in Figure T10.29.

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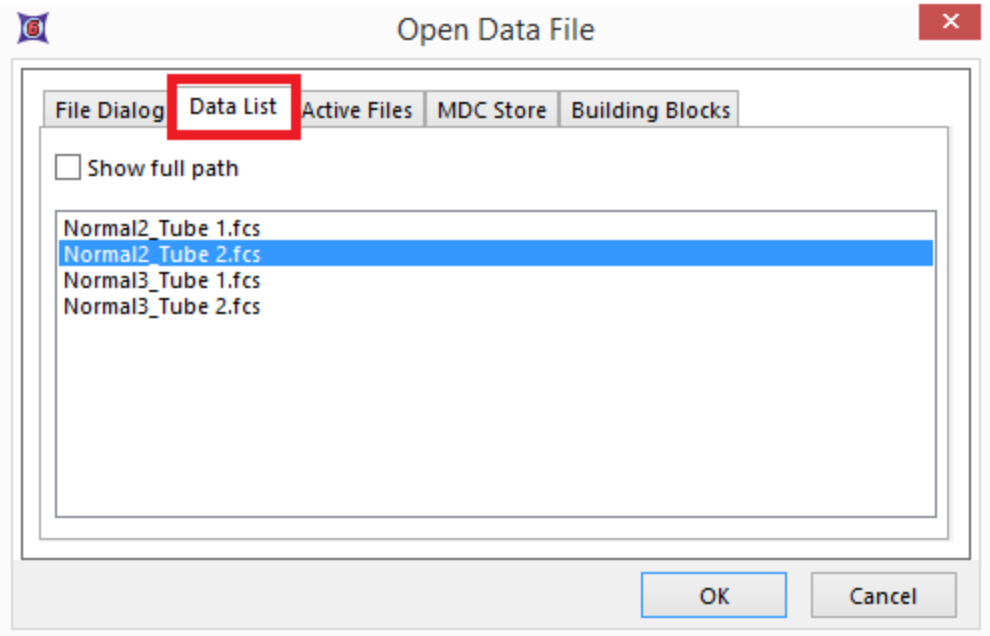

Figure T10.29 - Selecting a Data File for Overlay

48. In the **Add Overlay** window, in the **Parameter** section, select **APC-A**. If not already chosen, select the Lymphs gate, as seen in Figure T10.30. Click **OK**.

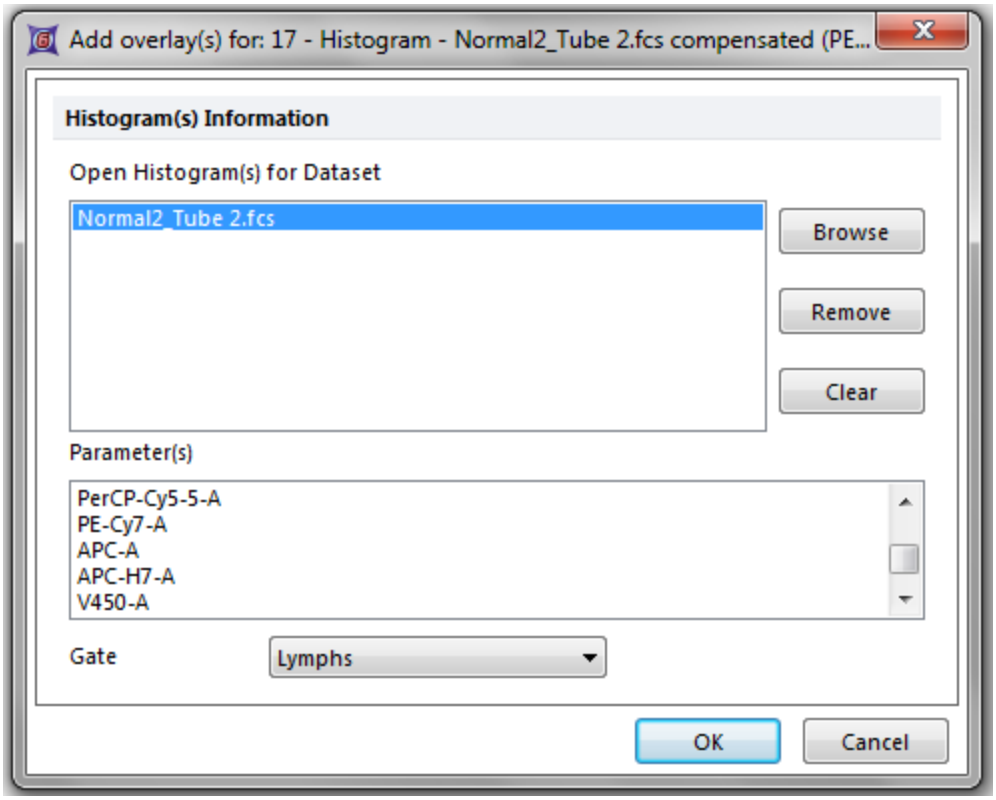

Figure T10.30 - Adding the Overlay

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49. Your histogram should look similar to Figure T10.31.

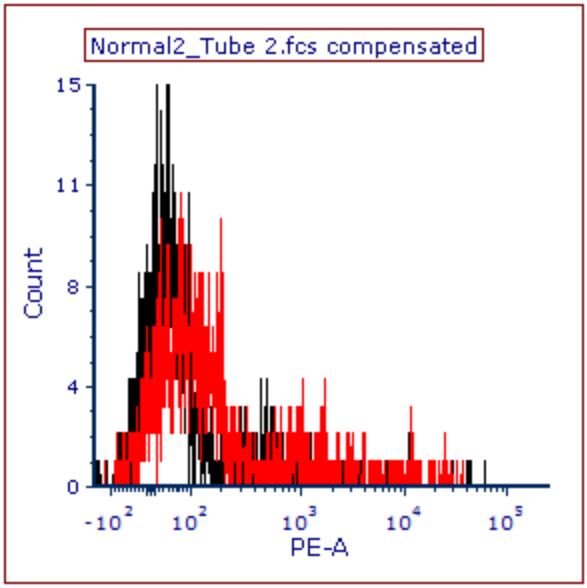

Figure T10.31 - Histogram with Overlay

50. At this point, we will save the layout. From the File tab, select Save As. Once you have chosen a location where you can save your layout, choose **Unlinked** from the **Data Link Style** section, as seen in Figure T10.32. This will save your layout as empty template. Click **Save**.

51. Close the layout.

In the next section, we will use the panel 1525.

38.11.3 Using the Panel

In the course of this example, we will:

- Load new data into the layout we just created.
- Use the panel to analyze the other similar samples.
- Extend the layout easily using the panel set up in the previous steps.

1. Open the layout that you created in the previous lesson, **Creating the Layout** 1508.
2. Open the Data List 209 window, if it is not already open.

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3. Click on the blue "+" sign in the Data List toolbar to add data files to be analyzed.

4. Navigate to the tutorial sample folder and select file *Normal2_Tube1*. Make sure the **Files of type** is set to **FCS files**. Select **Open**.

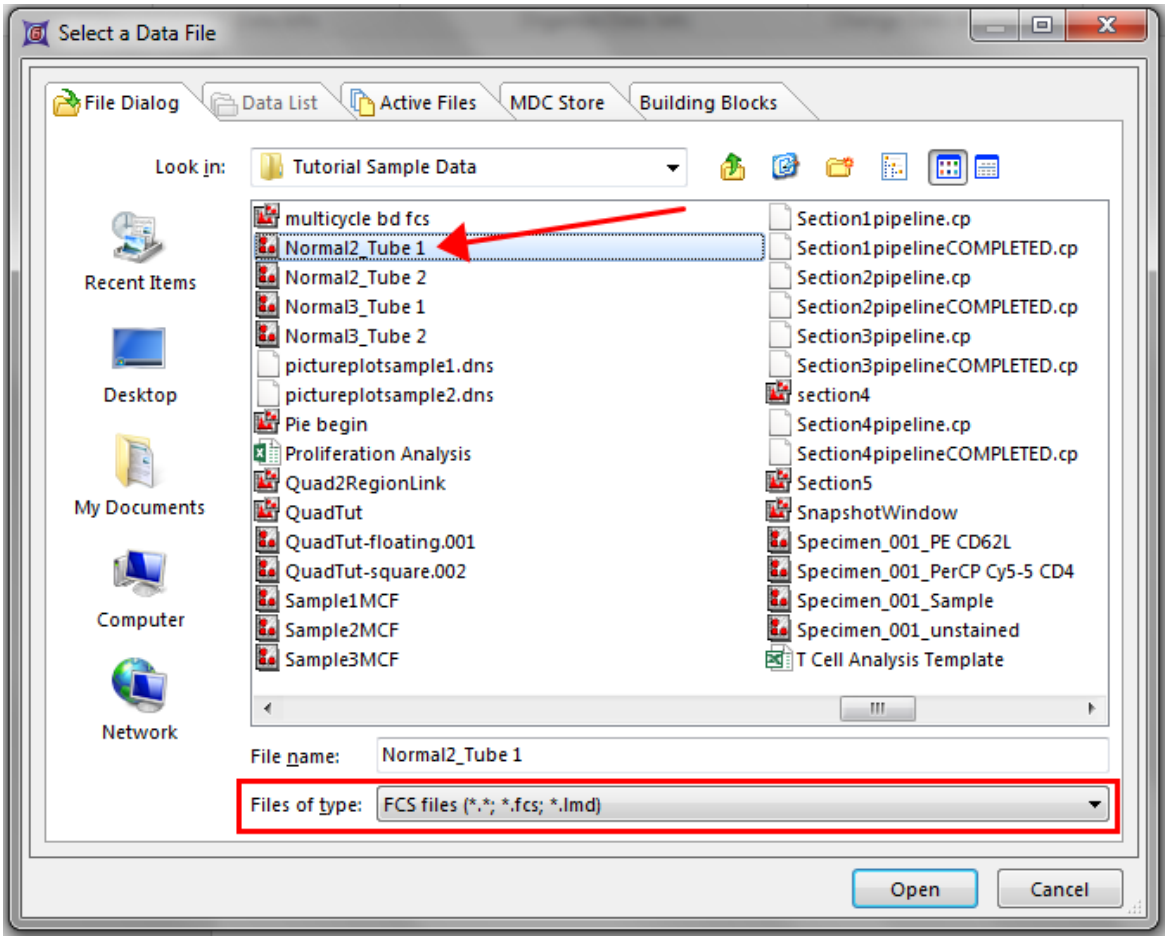

Figure T10.32 Selecting Panel Data for the Data List

5. Since the Panel that was created for this layout was defined as a 2-tube panel, FCS Express will prompt you to whether or not you would also like to add the next tube to the Data List to complete the 2-tube panel, as seen in Figure T10.33.

6. Click **Yes**.

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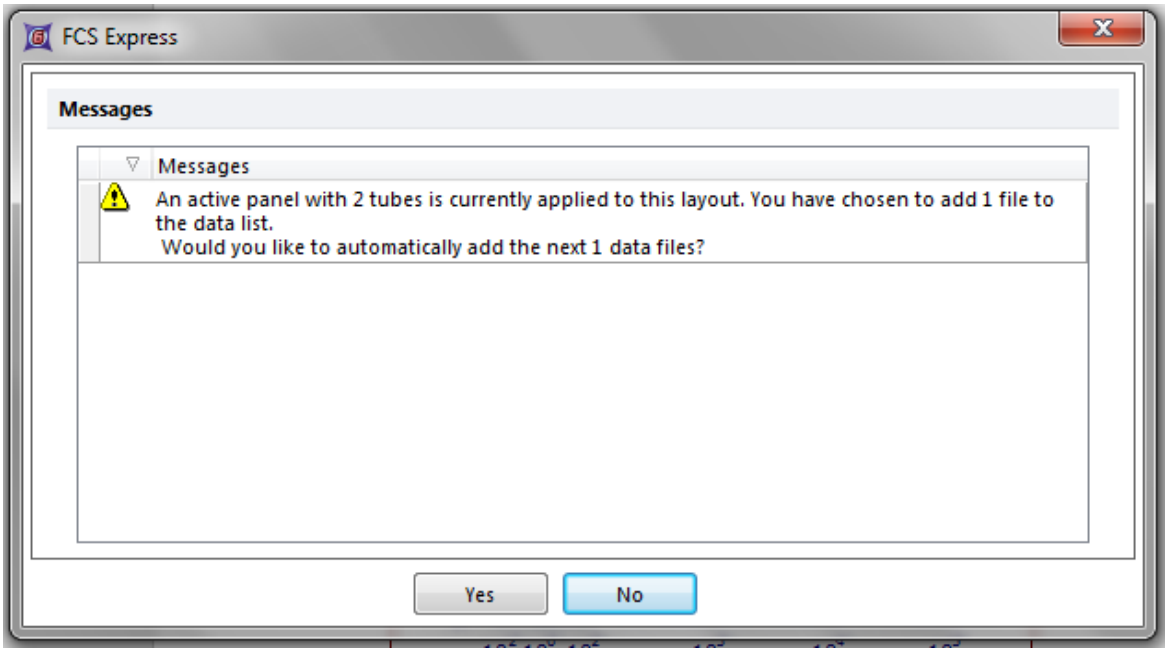

Figure T10.33 - Automatically Add Data File to Data List

7. FCS Express will load the 2 tubes, *Normal2_Tube1* and *Normal2_Tube2* into the Data List. FCS Express will also automatically populate all the plots in the layout with the appropriate data files that were assigned to each plot during the creation of the layout, including the histogram that contains the overlay on Page 2.

8. To analyze another sample that fits the Panel criteria, click on the green "+" sign in the Data List toolbar.

9. Navigate to the tutorial sample folder and, while holding the **Ctrl** key, select files *Normal3_Tube1* and *Normal3_Tube2.*

10. Because you have selected 2 tubes, FCS Express will not prompt whether you would like to automatically add the next data files to the Data List. Also, the same would hold true if all 4 data files were selected for addition to the Data List.

11. After adding the 4 data files, the Data List should look like Figure T10.34.

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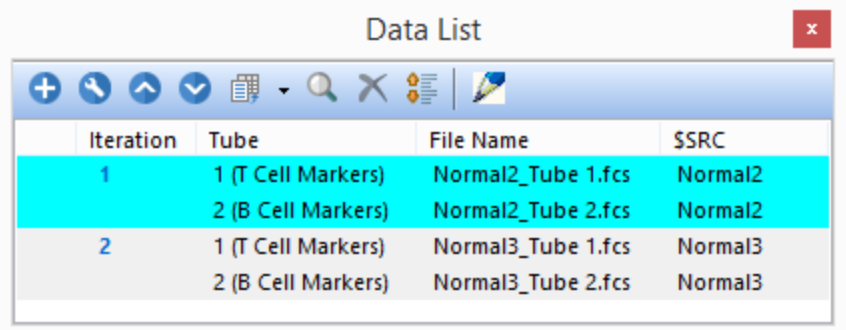

Figure T10.34 - Data List Containing More Than One Panel Iteration

12. Keep the Data List open. Feel free to dock and pin the Data List. Later on, we will discuss how we can rearrange data files within the Data List.

Advancing Through Panel Iterations in the Data List

13. To advance from one iteration to another iteration to be analyzed, use the **Next** button, which can be found under **Data>Change Data On All Objects,** as seen in Figure T10.35.

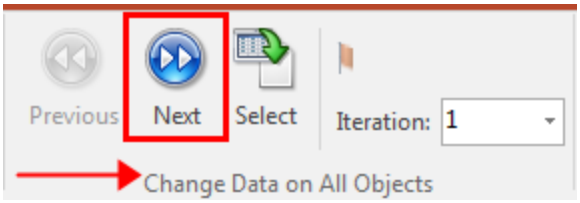

Figure T10.35 - Change Data on All

14. Also, to navigate through your data, select the **Iteration** number from the drop down menu, as seen in Figure T10.36.

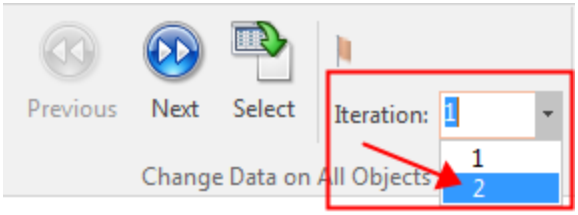

Figure T10.36 - Navigating by Using the Iteration Dropdown Menu

15. The number of iterations available will match the number of iterations seen in the Data List, as seen in Figure T10.34.

16. Also, to navigate between iterations within the Data List, you can select the Iteration within the Data List, as seen in Figure T10.37.

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17. The iteration that is currently displayed within the plots of the layout will be highlighted within the Data List.

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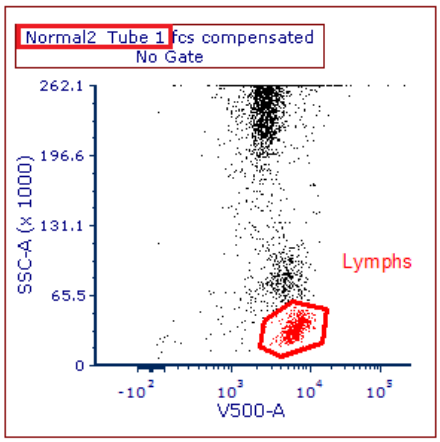
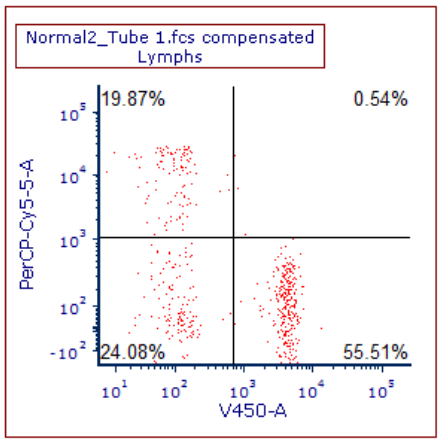
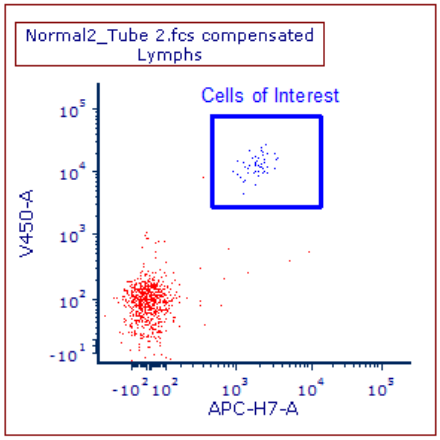
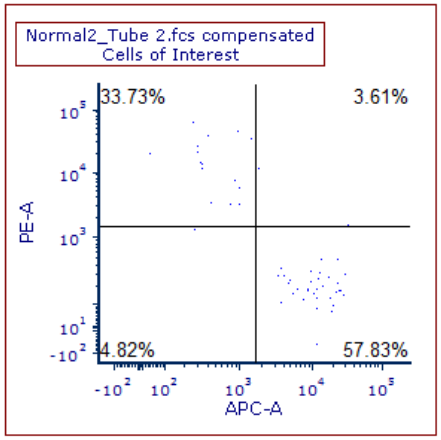


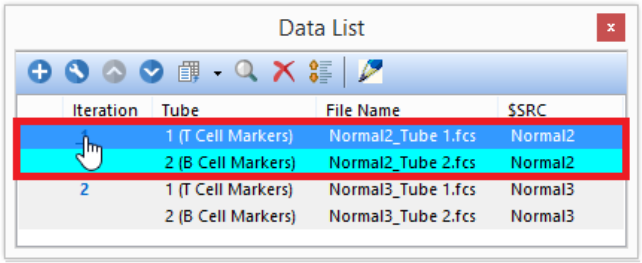


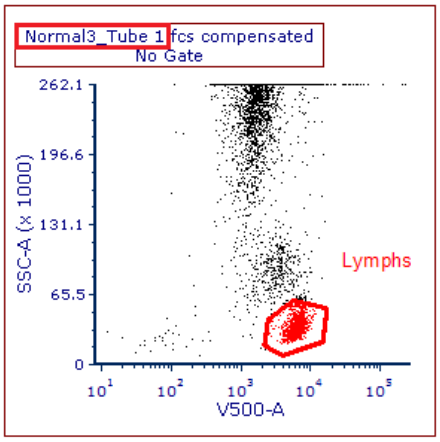
Gate	# of Events	% of gated cells	% of all cells
UL	184	19.87	1.84
UR	5	0.54	0.05
LL	223	24.08	2.23
LR	514	55.51	5.14

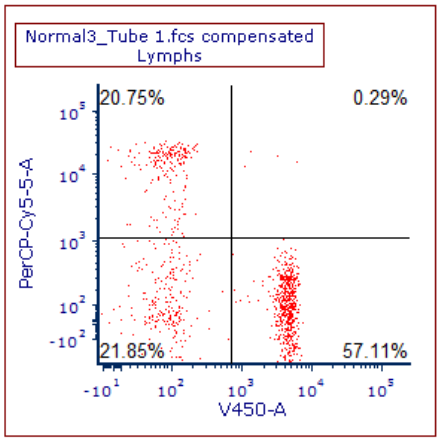




Gate	# of Events	% of gated cells	% of all cells
UL	28	33.73	0.28
UR	3	3.61	0.03
LL	4	4.82	0.04
LR	48	57.83	0.48







Gate	# of Events	% of gated cells	% of all cells
UL	359	20.75	3.59
UR	5	0.29	0.05
LL	378	21.85	3.78
LR	988	57.11	9.88

Rearranging Data Files within the Data List

18. For the tubes that comprise the Panel to be correctly displayed in their properly assigned plots, the order of the tubes within the Data List is critical.

19. If tubes are in the incorrect order, the tubes can be moved up or down the Data List by select the tube, then clicking the Up or Down arrow within the Data List toolbar until the tube is in its proper order.

20. In Figure T10.38, the tubes of the 1st iteration are in the wrong order. As a result, incorrect data is being displayed in the incorrect plots.

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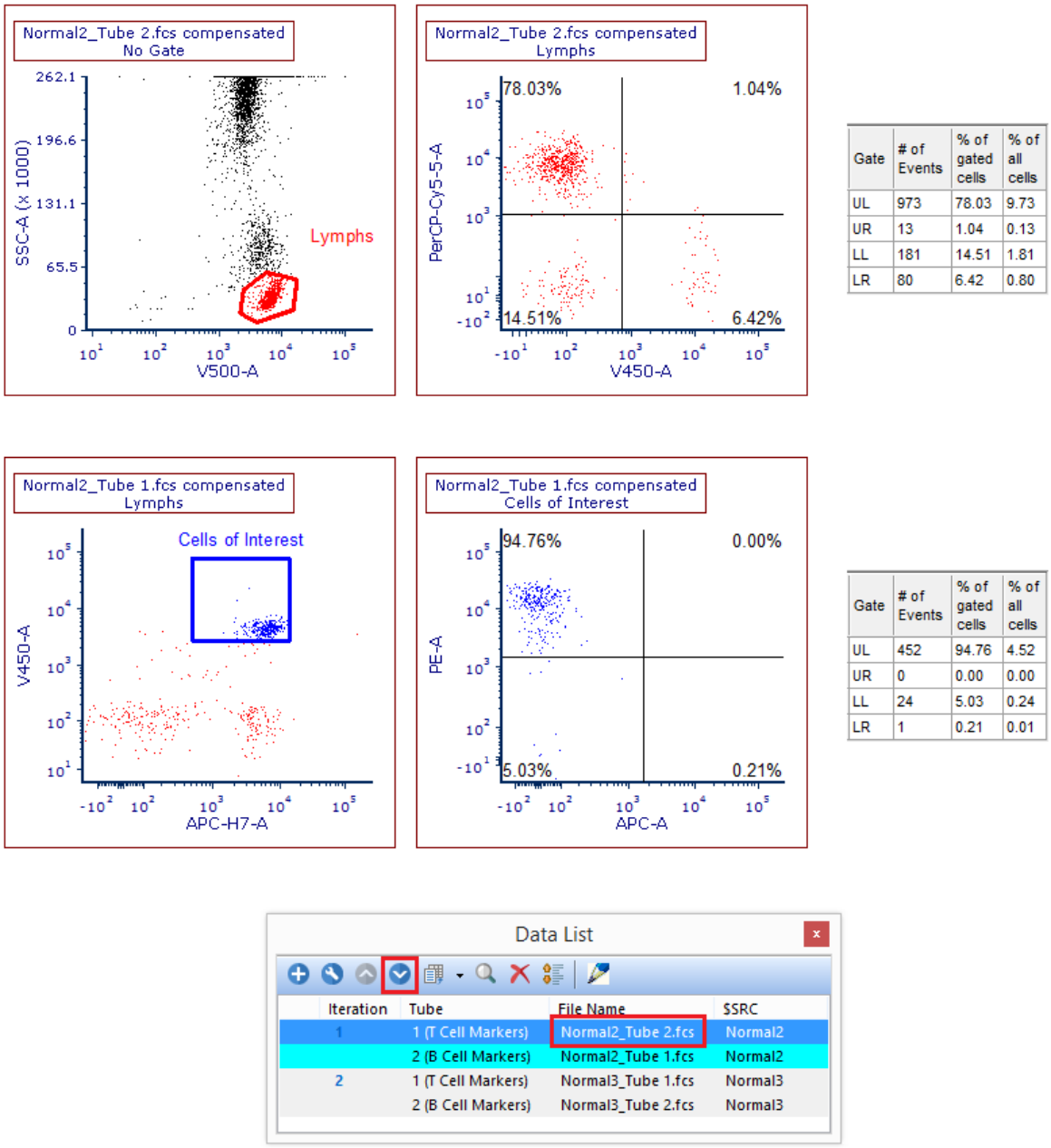

Gate	# of Events	% of gated cells	% of all cells
UL	973	78.03	9.73
UR	13	1.04	0.13
LL	181	14.51	1.81
LR	80	6.42	0.80

Gate	# of Events	% of gated cells	% of all cells
UL	452	94.76	4.52
UR	0	0.00	0.00
LL	24	5.03	0.24
LR	1	0.21	0.01

Figure T10.38 - Incorrect Order Of Tubes Within the Data List. Notice How Tube 2 is Ahead of Tube 1.

21. By selecting a tube of the 1st iteration, we can correct this by moving the tube using the Up and Down arrows, as seen in Figure T10.38.

22. As the tubes are moved, the data will refresh and recalculate within the layout in real time, as seen in Figure T10.39.

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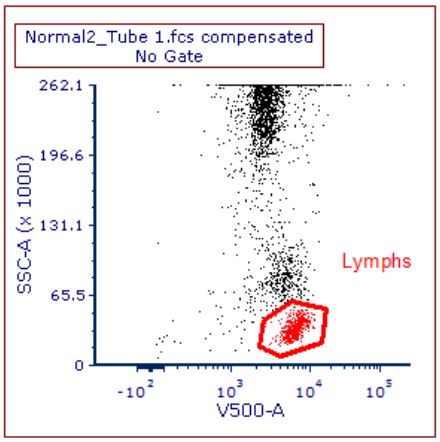
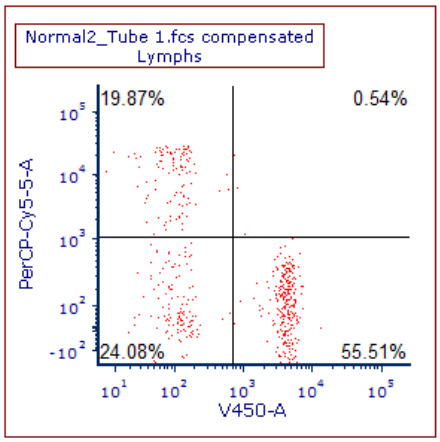
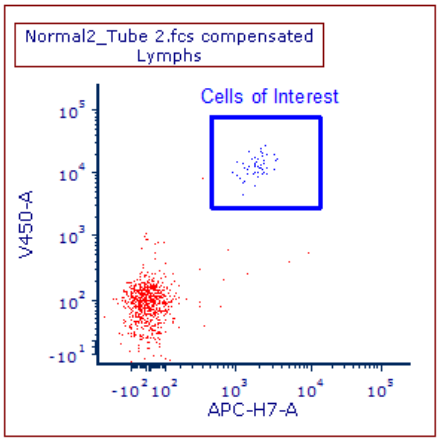
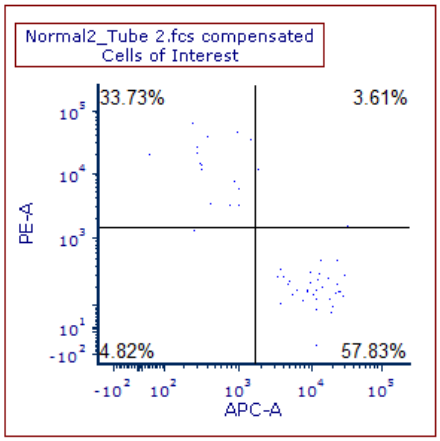




Gate	# of Events	% of gated cells	% of all cells
UL	184	19.87	1.84
UR	5	0.54	0.05
LL	223	24.08	2.23
LR	514	55.51	5.14





Gate	# of Events	% of gated cells	% of all cells
UL	28	33.73	0.28
UR	3	3.61	0.03
LL	4	4.82	0.04
LR	48	57.83	0.48

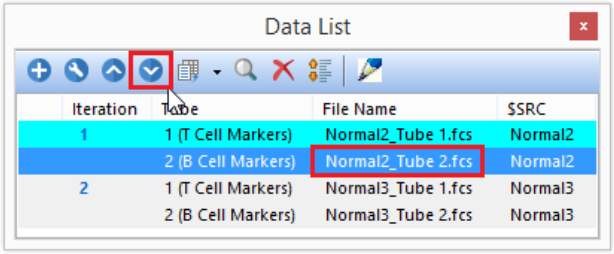


Figure T10.39 - Corrected Tube Order in Data List

23. However, if the files are large, there may be a noticeable delay in the refresh time as the tubes are moved within the Data List.

24. Users can defer the layout changes by selecting the Defer Layout Changes button in the Data List toolbar.

25. As seen in Figure T10.40, users can:

    a. Select Defer Layout Changes ❶.

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b. Select the file to be moved ❷.

c. Click the Up or Down arrow ❸.

d. After all the changes have been made, click the green check mark ❹.

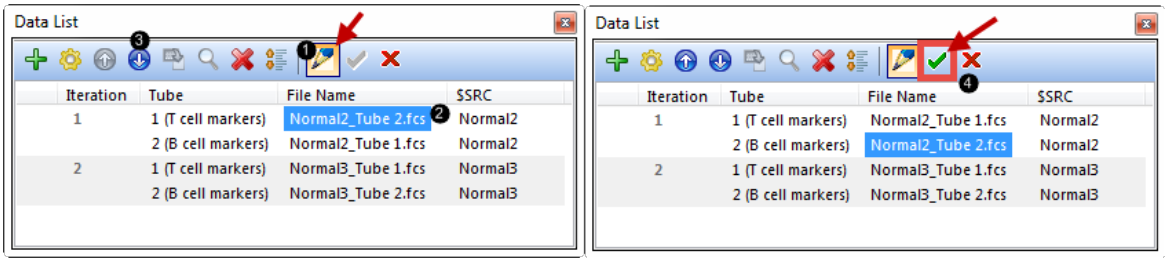

Figure T10.40 - Deferring Layout Changes - Before and After

26. After clicking the green check mark, the changes will take effect.

Adding plots to the layout

27. After a Panel has been added to a layout, the layout can be easily edited.

28. On Page 1, select the following plots while holding the **Ctrl** key:

    a. V450 vs PerCP Cy5.5

    b. APC-H7 vs V450

    c. APC vs PE

29. Right click on the selected plots and choose **Copy**. Also, the keyboard shortcut **Ctrl+C** will work.

30. Go to Page 2. Right click on the Page and select **Paste**. Also, the keyboard shortcut **Ctrl+V** will work.

31. Rearrange the plots around the 1D histogram with the overlay.

32. Change the parameters for these pasted plots to any pairing desired.

33. Add a Quadrant to the plot that was previously the APC-H7 vs V450 plot.

34. Save your layout by choosing **File** tab>**Save**, or by clicking the Save button in the layout toolbar at the top of your computer screen.

Finally, we will see examples of saving and loading panels 1535.

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38.11.4 Saving and Loading Panels

In the course of this example, we will:

- Work with the layout that was created in **Creating the Layout**.
- Learn how to save and load panels for future use.

We will now proceed to save and load the panel we created for future use.

1. Select the **Data→Organize Data Sets→Panels** command. Alternatively, select the **Batch→Organize Data Sets→Panels** command.

The **Panel Information** dialog appears with **My Panel** listed and marked as active, as shown in Figure T10.41.

2. Click the **Save** button on the **Panel Information** dialog.

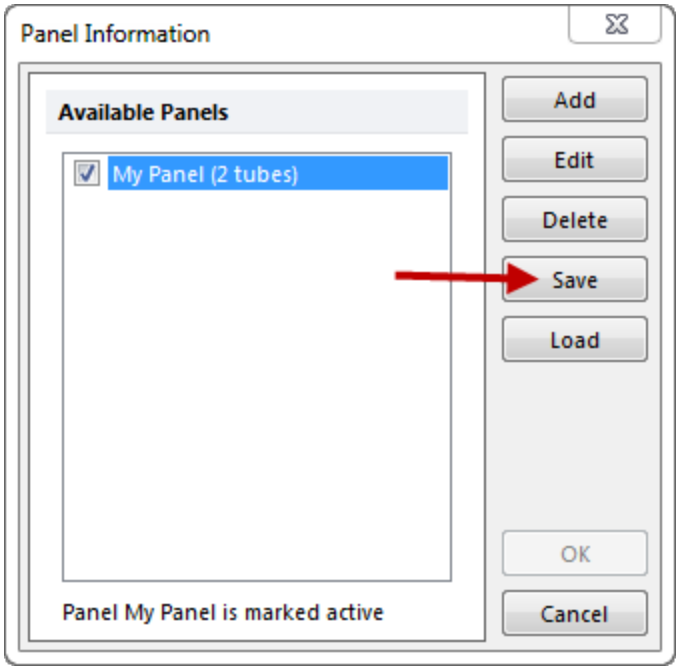

Figure T10.41 - Saving a Panel

The **Save As** dialog appears.

3. Navigate to the **Tutorial Sample Data** folder and enter "**My Panel**" in the File Name field.

4. Click **Save** on the **Save As** dialog.

We have now saved **My Panel**. We will now save the current layout, and then load the saved panel into a new layout.

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5. Click on **Cancel** to close the **Panel Information** dialog.

6. Select the **File** tab →**Save**, to save the current layout. Alternatively, select the **Save** icon located in the Quick Access Toolbar 65.

If you have not previously saved the layout then the **Save Layout** dialog appears.

7. Navigate to the **Sample Data** folder and enter "**Panel Demo Layout**" in the File Name field.

8. Click **Save** on the **Save Layout** dialog.

9. Select the **File** tab →**New Layout**, to open a new blank layout.

A new blank layout will now appear.

10. Select the **Data→Organize Data Sets→Panels** command. Alternatively, select the **Batch→Organize Data Sets→Panels** command.

The **Panel Information** dialog will now appear and it will be empty.

11. Click the **Load** button on the **Panel Information** dialog, as seen in Figure T10.42.

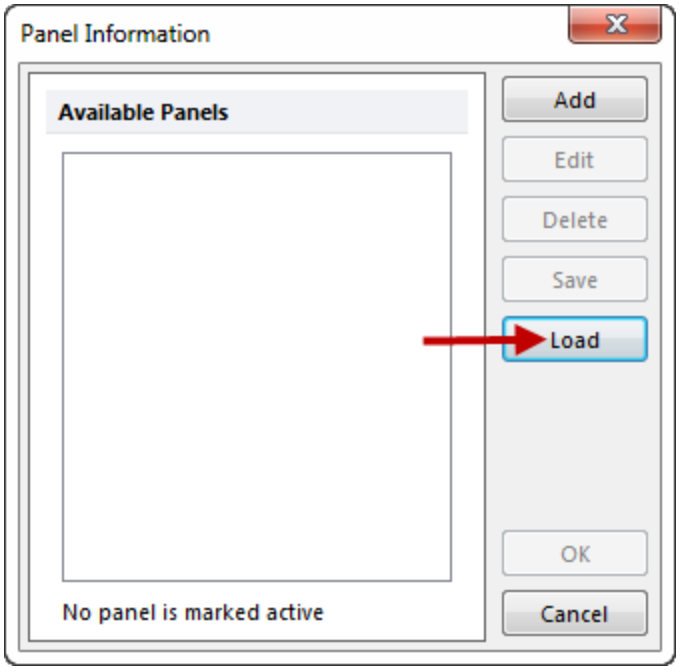

Figure T10.42 - Loading a Panel

The **Open** dialog will now appear.

12. Navigate to the **Sample Data** folder and select the "**MyPanel.PanelInfo**" file.

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13. Click **Open**.

The **Panel Information** dialog will now show **My Panel**. Saving a panel allows you to avoid having to redefine a panel over and again for every new layout you create.

14. Click on **OK** to activate **My Panel** and close the dialog.

My Panel is now active on this layout and you could choose to create a new layout of plots to analyze with this panel.

38.12 Docking

Many of the functional windows within FCS Express are dockable. This means that they can be embedded within the layout and becoming a more permanent fixture within the layout. Docked windows can also be Pinned, which enables an Auto Hide feature for that docked window.

The docking feature allows you to place any of the Navigator windows (Layout, Data and Gate), the Token windows (Custom Token, Canned Comments, and Alerts), and the Data List window, onto the layout and then "dock" the window, or windows, to a specific fixed location on the outside of the layout page. Docking has many powerful features which we will explore in this tutorial.

Note: We are going to open a lot of interesting Navigators in this tutorial, but will only focus on the docking functionality. For more information on how these Navigators work, please see the tutorials on the Layout Navigator 1597, Data Navigator 1561, and Gate Navigator 1582.

In the course of this example, we will:

- Learn how to dock a window.
- Learn how to use the many docking features.
- Learn how to use docking features with multiple windows.
- Learn how to undock a window.

To Dock a window:

1. Select **File** tab→**Open Layout**.
2. Open the layout **Docking.fey** found in the *FCS Express Sample Data* folder.

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The following layout will appear, shown in Figure T11.1. This layout may look familiar as a similar layout was used in the Tokens tutorial.

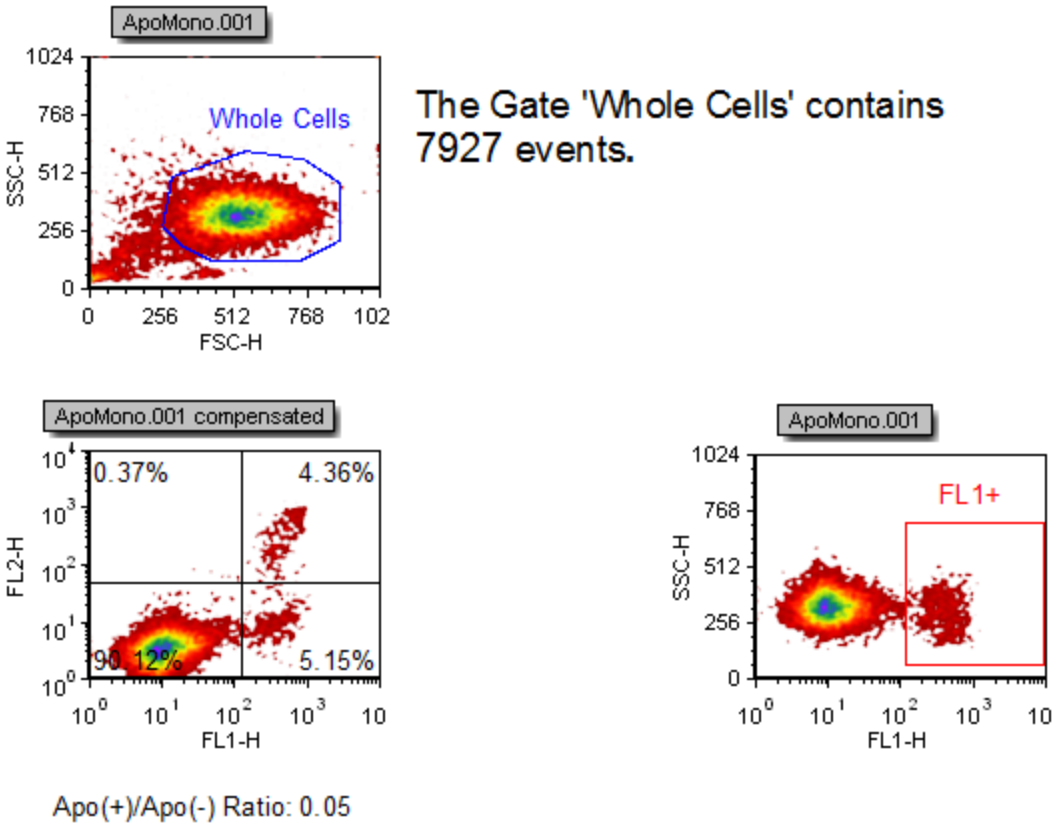

Figure T11.1 Docking Layout

3. Select the **View→Navigators→Gate Navigator** command (Figure T11.2).

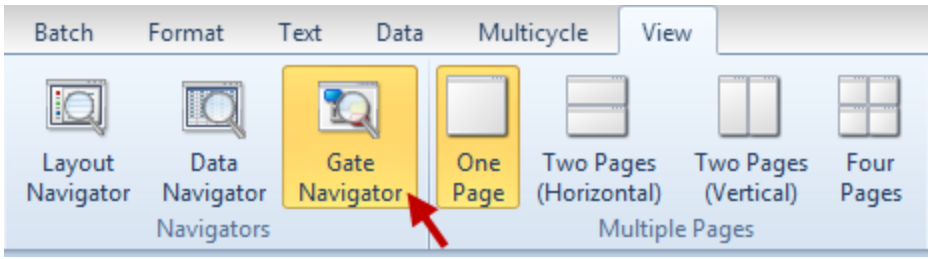

Figure T11.2 Selecting the Gate Navigator Command

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The **Gate Navigator** window will appear on the layout. When dockable windows are first opened, they always appear in the "floating" state. This means that they can be moved anywhere you like, even outside the layout itself. We will now dock the **Gate Navigator** window to the left side of the layout.

4. Place the mouse on the top border of the **Gate Navigator** window.

5. Press and hold the left mouse button.

Continue to hold the left mouse button and move the **Gate Navigator** window. As soon as you start moving the **Gate Navigator** window, an icon with four outward facing arrows will appear in the center of the layout, as shown in Figure T11.3; this is a docking icon. The docking icon, allows you to choose the docking location for the **Gate Navigator** (or any other dockable window). As you drag the window and the cursor moves over each of the four arrows of the docking icon, a blue rectangular area will appear to indicate the proposed position on the layout where the window will be docked. However, you cannot dock a window directly on the layout page itself, but rather to the sides of the layout page.

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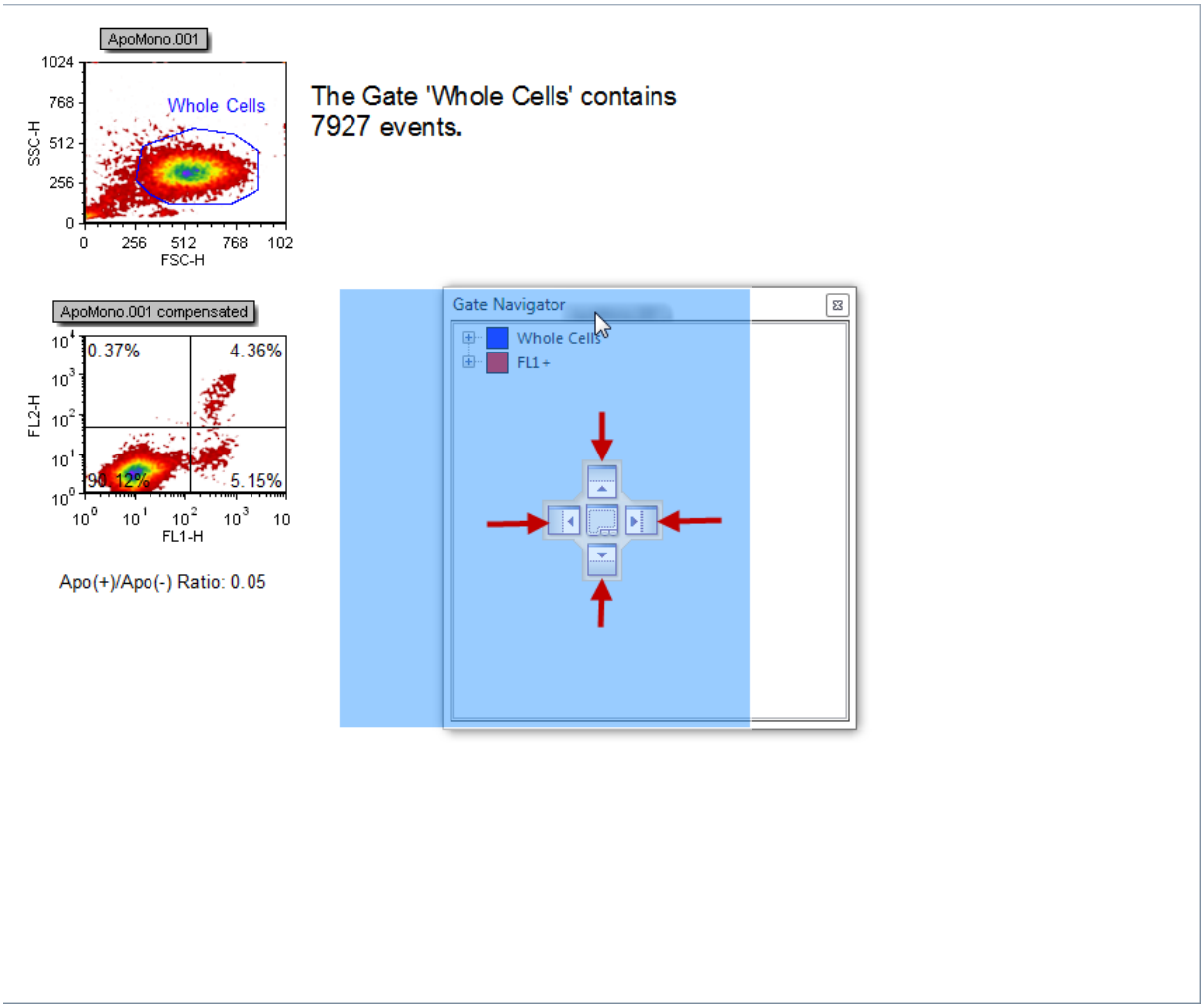

Figure T11.3 Docking Icon in the Center of the Layout

Continue to hold down the mouse button and drag the **Gate Navigator** towards the central docking icon.

6. Move the **Gate Navigator** towards the right arrow of the docking arrow.

7. Continue to hold down the mouse button and place the cursor over the right arrow, indicated by the red circle in Figure T11.4A.

When the cursor is over the right arrow of the docking icon, a blue rectangular window appears on the right side of the layout indicating the proposed docking location for the window, as shown in Figure T11.4A.

8. Continue to hold down the mouse button and Repeat steps 7 and 8, but place the cursor over the bottom docking arrow, indicated by the red arrow in Figure T11.4B.

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Again, a blue rectangle appears at the proposed docking location at the bottom under the page (Figure T11.4B).

9. Continue to hold down the mouse button but place the cursor over the left docking arrow, indicated by the red arrow in Figure T11.4C.

10. Release the mouse button when the cursor is over the left arrow of the docking icon, as indicated by the red circle in Figure T11.4C.

Again, a blue rectangle appears at the proposed docking location to the left of the page (Figure T11.4C).

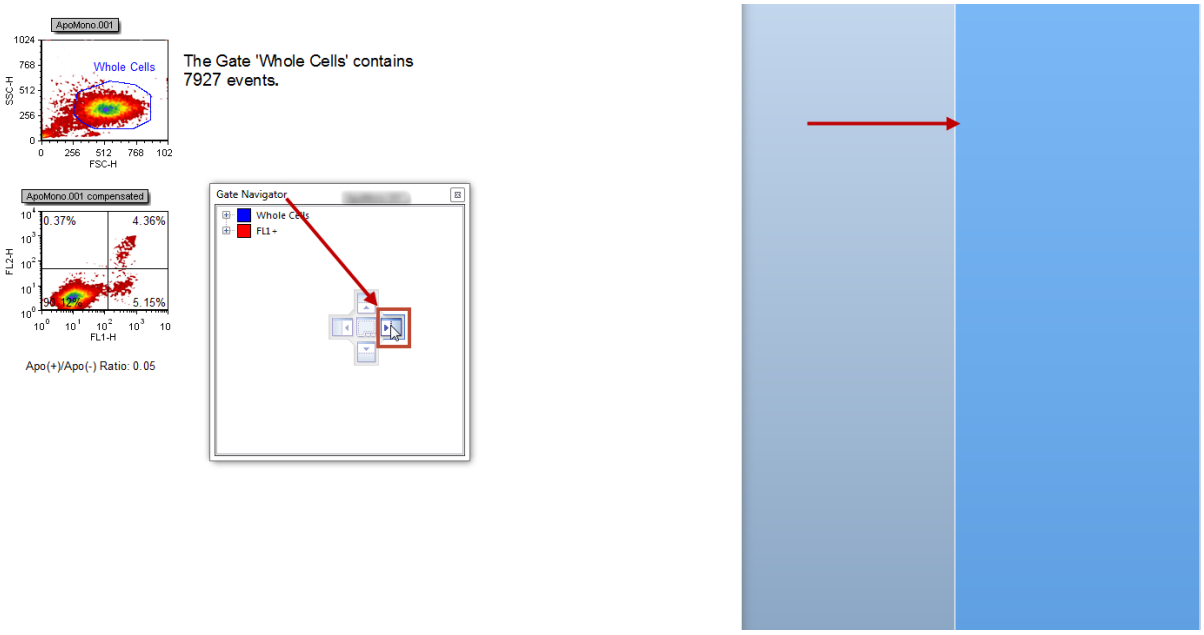

Figure T11.4A Gate Navigator -- Cursor on the Right Arrow of the Docking Icon

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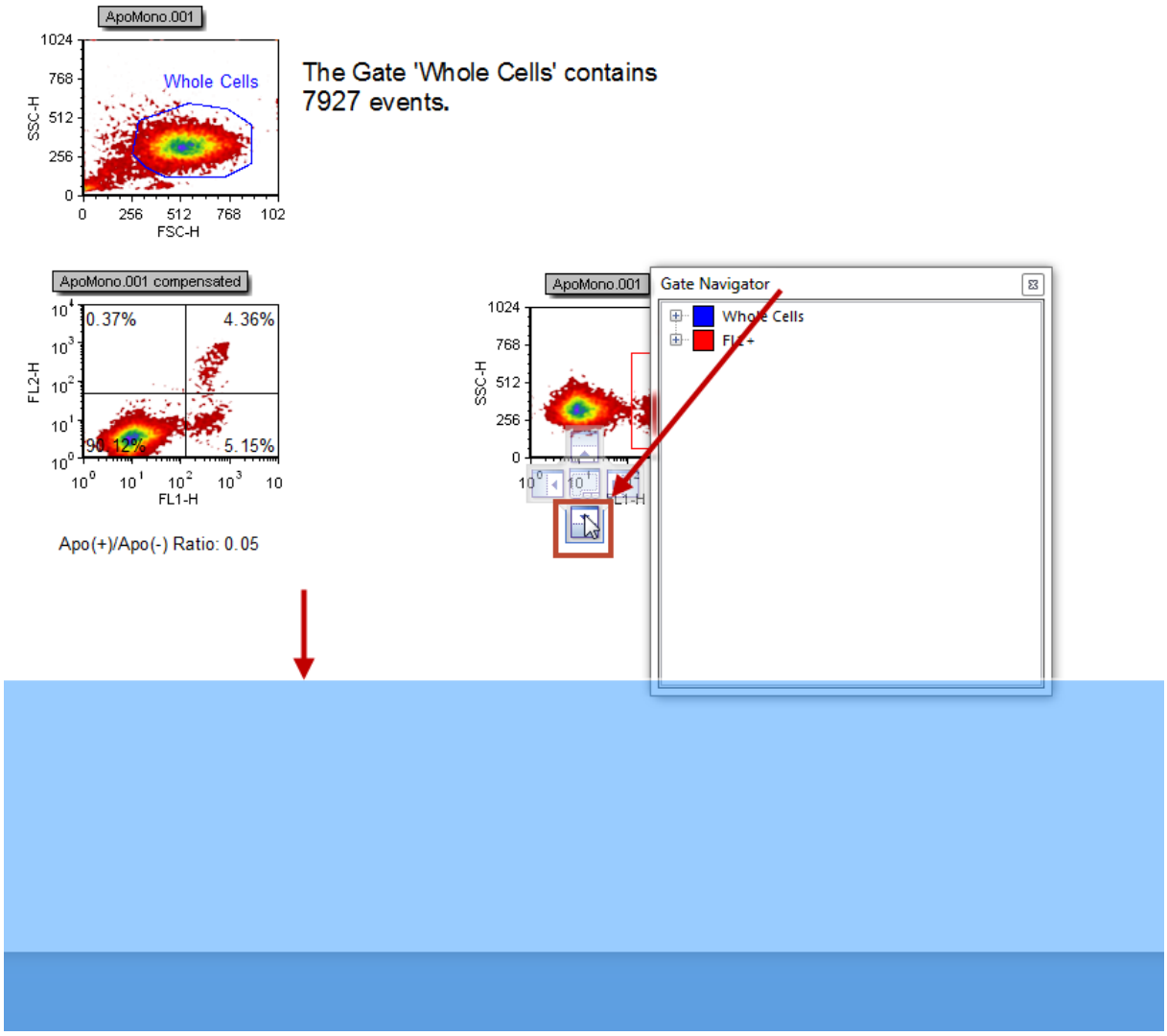

Figure T11.4B Gate Navigator -- Cursor on the Bottom Arrow of the Docking Icon

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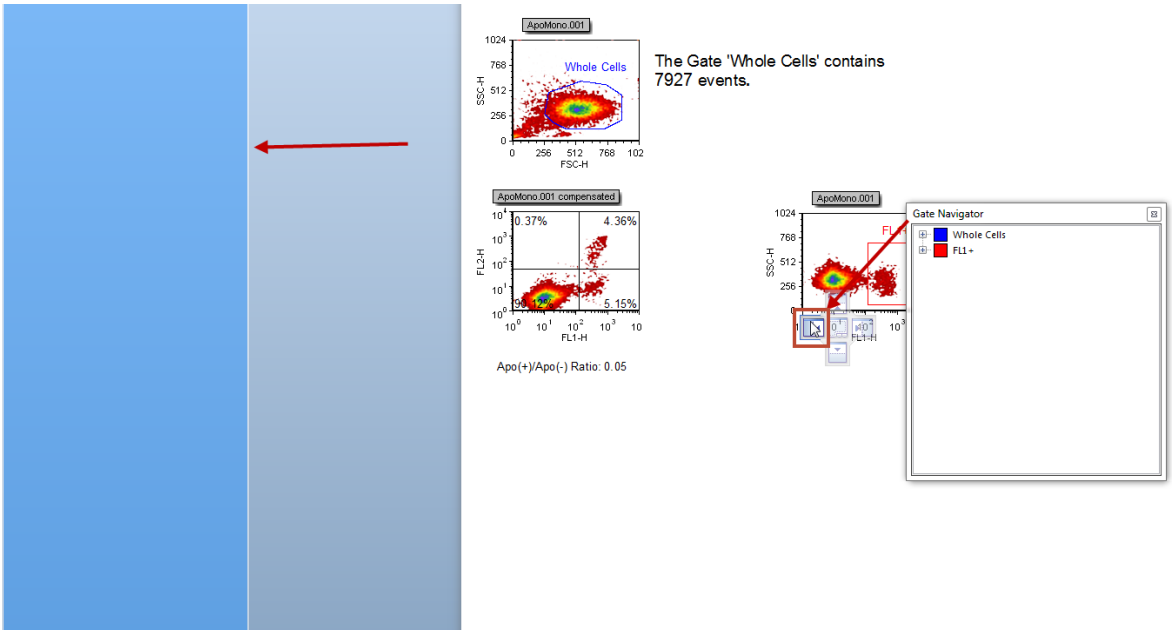

Figure T11.4C Gate Navigator -- Cursor on the Left Arrow of the Docking Icon

The **Gate Navigator** is now docked on the left side of the layout as shown in Figure T11.5.

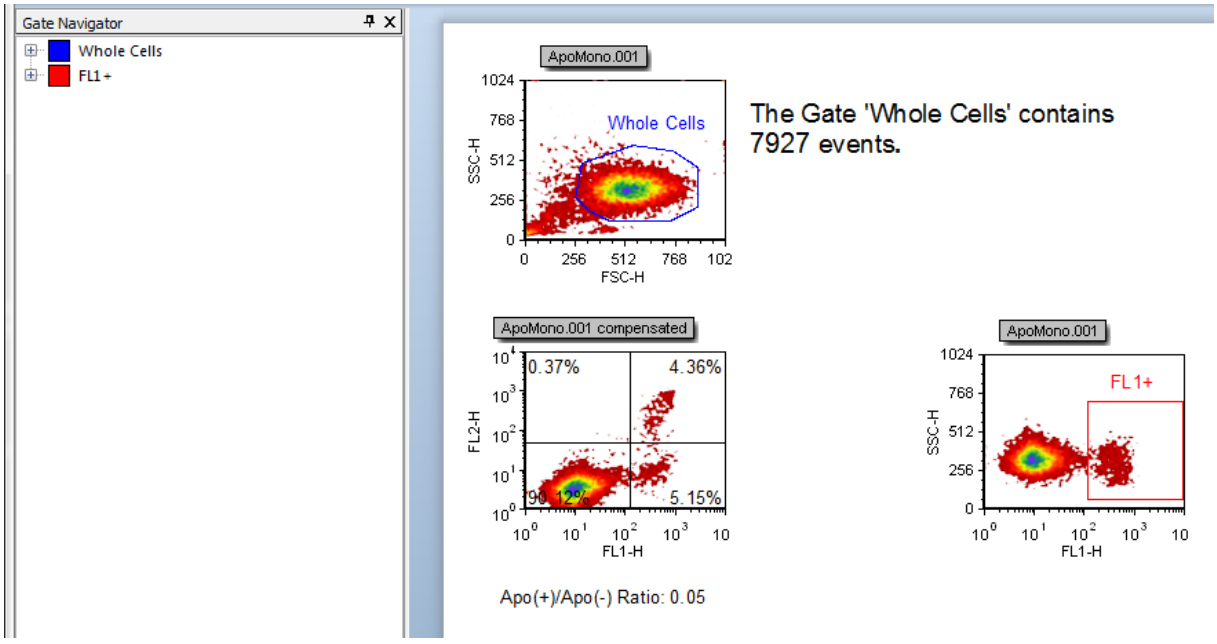

Figure T11.5 Gate Navigator Docked on the Left Side of the Layout

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In the next section, we will learn the features of a docked window 1544.

38.12.1 Features of a Docked Window

Once a window is docked it can be resized, undocked, redocked to a new location, and you can Auto Hide it. Note that when a layout is saved, the corresponding docking configuration is also saved with the layout, so that it will be reloaded when you load the layout. We will now explore the various features of a docked window.

1. Place the cursor on the top border of the docked **Gate Navigator**.
2. Press the left mouse button and continue to hold it while dragging the **Gate Navigator** to a new location on the layout.

As you move the **Gate Navigator** on the layout docking icon arrows appear on the four outer sides of the layout, indicated by the red circles in Figure T11.6. If you continue to move the **Gate Navigator** towards the middle of the layout the central docking icon will re-appear, as previously shown in Figure T11.3 1537.

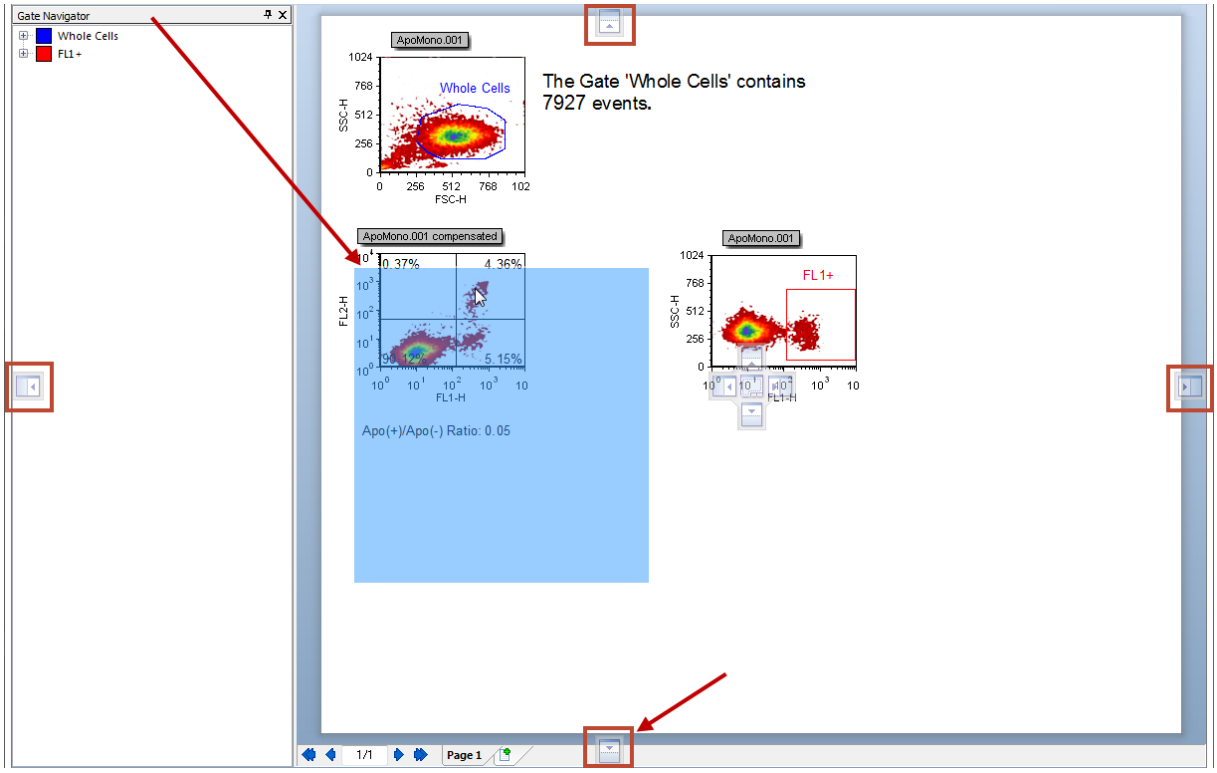

Figure T11.6 Four Outer Docking Icon Arrows Circled in Red

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3. Continue to hold down the mouse button and move the **Gate Navigator** towards the bottom docking arrow.

4. Release the mouse button when the cursor is on top of the bottom docking arrow, as indicated by the red circle in Figure T11.7.

Notice that a blue rectangle appears at the bottom of the layout, again to indicate the proposed docking location for the window.

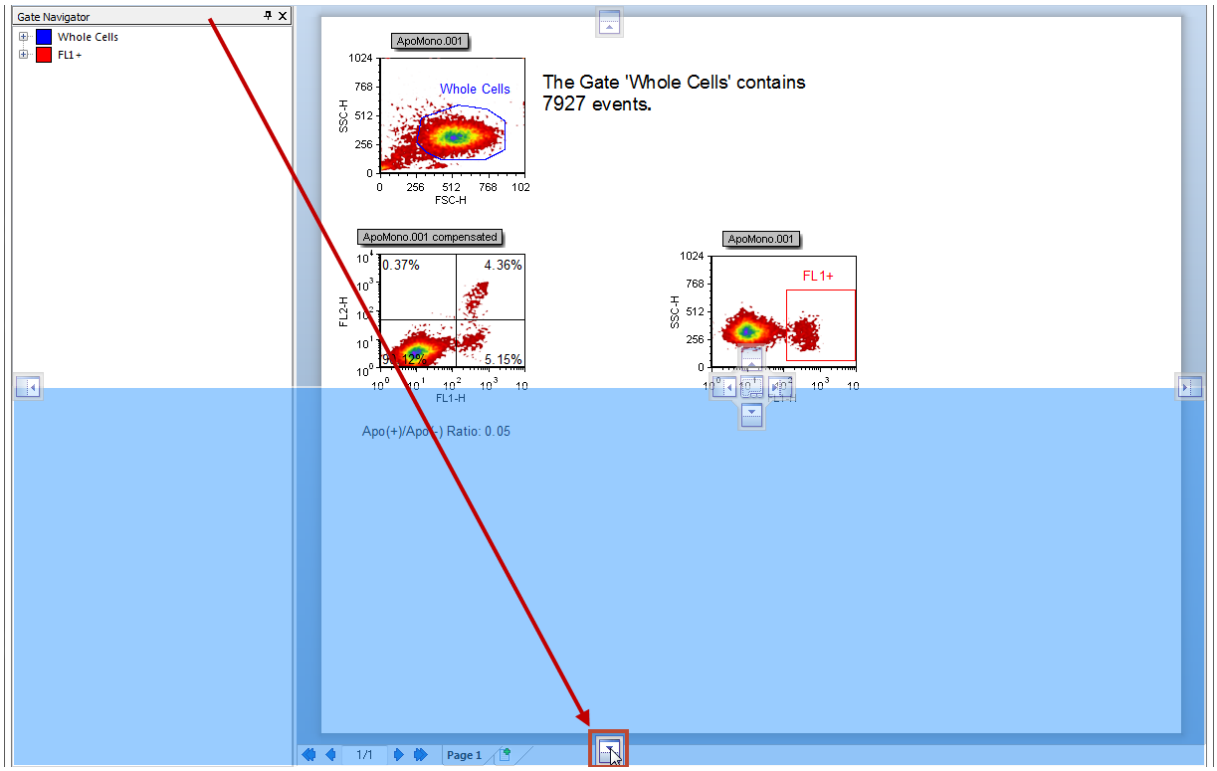

Figure T11.7 Bottom Docking Icon Arrow

The **Gate Navigator** is now docked at the bottom of the layout, as shown in Figure T11.8. However, the current size of the docked Navigator is too large and covers part of the plots on the layout. We will now resize the docked window to a more appropriate size.

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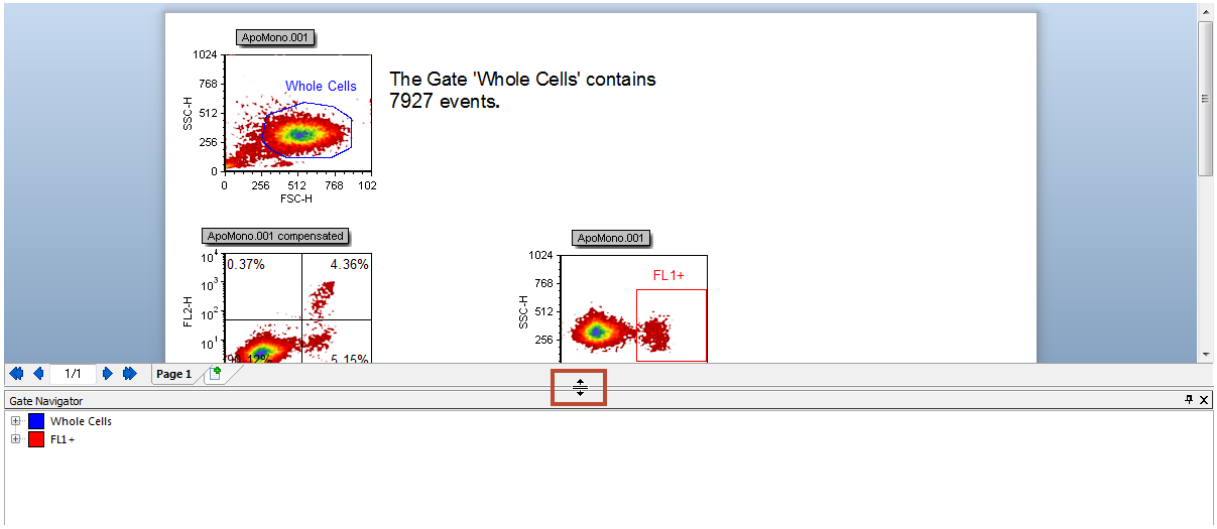

Figure T11.8 Gate Navigator Docked at the Bottom of the Layout

5. Place the cursor over the top border of the docked **Gate Navigator**.
6. Move the cursor slightly until it changes to a resizing icon (two horizontal lines with an arrow on either side of the lines facing outward), indicated by the red circle in Figure T11.8.
7. Press the left mouse button and continue to hold it while dragging the border of the **Gate Navigator** down.
8. Release the mouse button when the docked **Gate Navigator** is no longer covering the plots on the layout.

Rather than resize the docked Navigator, we could use the **Auto Hide** feature. **Auto Hide** allows the window to remain docked, but hidden, except for a visible tab with the name of the docked window on it. When the cursor is placed on the tab the docked window re-appears and remains visible as long as the cursor remains on the docked window. If the cursor is moved off the docked window then the window is again hidden.

9. Click on the **Auto Hide** icon from the upper right corner of the docked **Gate Navigator**, indicated by the cursor in Figure T11.9.

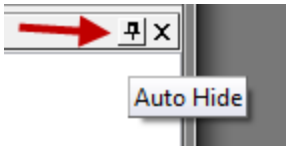

Figure T11.9 Auto Hide Icon

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10. Move the cursor off the docked **Gate Navigator**.

The docked **Gate Navigator** is now hidden on the layout, except for a tab on the bottom of the layout, indicated by a red circle in Figure T11.10.

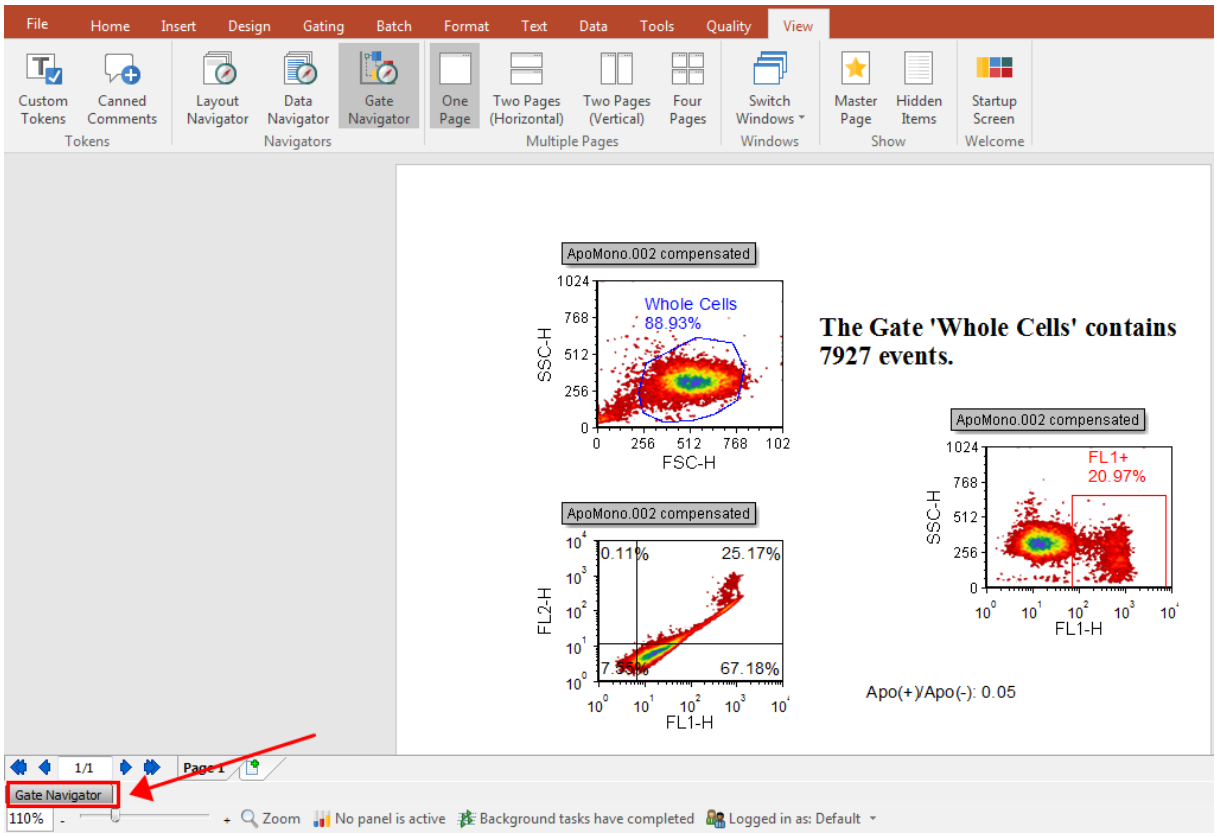

Figure T11.10 Gate Navigator Hidden using the Auto Hide Feature

11. Move the cursor over the **Gate Navigator** tab, indicated by the red circle in Figure T11.10.

The docked **Gate Navigator** will now reappear. We will now remove the **Auto Hide** feature so that the **Gate Navigator** will remain visible.

12. Click on the **Auto Hide** icon located in the upper right corner of the docked **Gate Navigator**, indicated by the cursor in Figure T11.11, to remove the **Auto Hide** feature from the docked window.

Note that the **Auto Hide** icon has changed from its original design, shown in Figure T11.9, to the design shown in Figure T11.11. The **Auto Hide** design, shown is Figure T11.11, indicates that the **Auto Hide** feature is on. However

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after clicking on the **Auto Hide** icon it will go back to the original design, shown in Figure T11.9, which indicates that the **Auto Hide** feature is off. The **Gate Navigator** is now visible, docked at the bottom of the layout.

Figure T11.11 Removing Auto Hide

In the next section, we will continue using the **Docking.fey** layout and cover docking features with multiple windows docked 1548.

38.12.2 Docking Features (Multiple Windows)

We will now open more Navigators and the Custom Tokens window, and dock them to demonstrate the features of docking with multiple windows. Please refer to the beginning of this tutorial for details on docking.

1. Select the **View→Navigators→Data Navigator** command.

The **Data Navigator** window will appear on the layout.

2. Dock the **Data Navigator** window to the left of the layout.

The layout now has two windows docked, **Gate Navigator** on the bottom and **Data Navigator** on the left, as shown in Figure T11.12.

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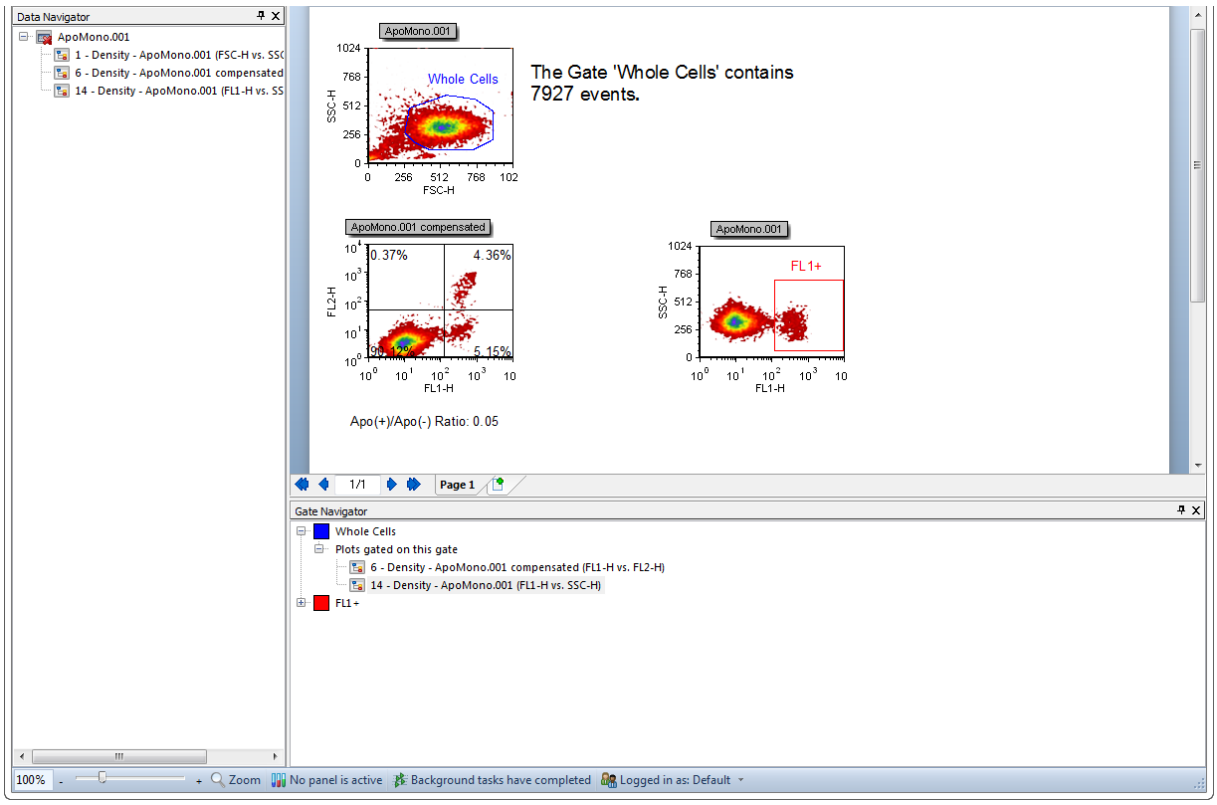

Figure T11.12 Layout with Gate Navigator and Data Navigator Docked

3. Select the **View→Navigators→Layout Navigator** command.

The **Layout Navigator** appears on the layout. We will now dock the **Layout Navigator** to the left side of the layout.

4. Drag the **Layout Navigator** toward the left side of the layout.

When you first start to drag the **Layout Navigator**, the four-headed arrow and the single arrow docking icons, appear at all the possible docking locations (Figure T11.13). The four-headed arrows are indicated by red circles, and the single arrows by blue circles in Figure T11.13. Once you begin moving the **Layout Navigator**, the docking icons fade away and the only docking icons visible are those that indicate the current docking site. The four-headed arrow docking icon will only appear within a specific docking site and indicates the possible docking locations at that site. The single docking arrows, indicated by the blue circles in Figure T11.13, always appear to the left, bottom, right, and top of the layout page and indicate that these docking sites are always available.

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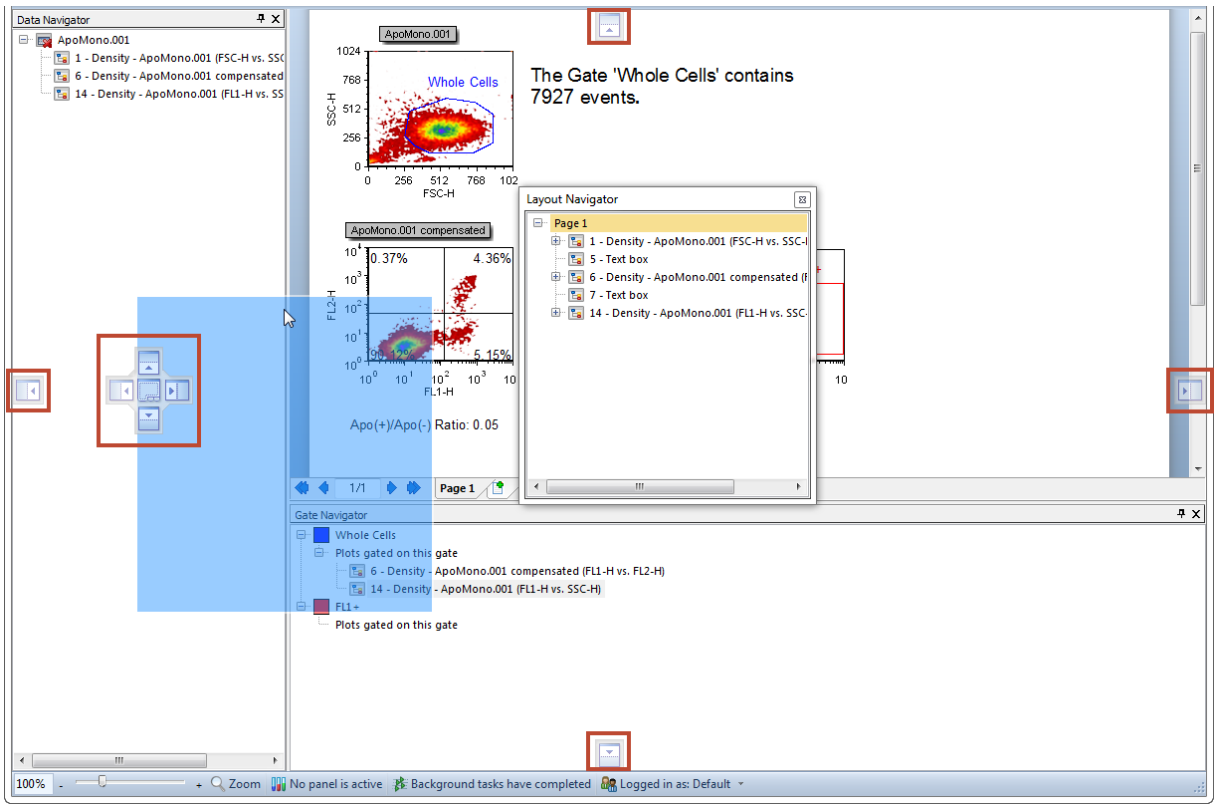

Figure T11.13 Initial Docking Icons

As you move the **Layout Navigator** towards the left side of the layout, the four-headed arrow docking icon appears at the left docking site, indicated by the red circle in Figure T11.14. The four-headed arrow docking icon indicates the possible docking locations for the **Layout Navigator**. The single docking arrow on the far left indicates that the window can be docked all the way to the left of the currently docked **Data Navigator**.

5. Continue to hold down the left mouse button and drag the **Layout Navigator** towards the docking icon with the four arrows on the left side of the layout, circled in red in Figure T11.14.

6. Release the mouse button when the **Layout Navigator** is over the right arrow, indicated by the blue circle in Figure T11.14.

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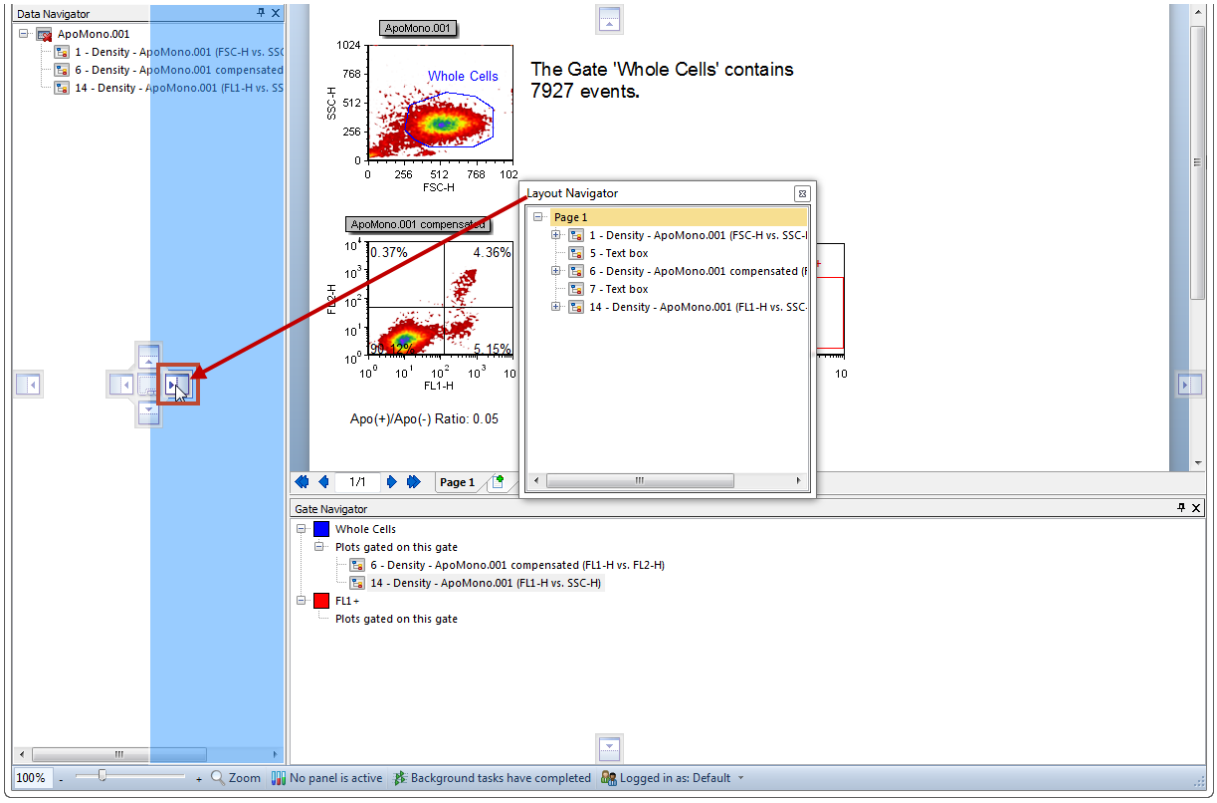

Figure T11.14 Docking Layout Navigator

The **Layout Navigator** is now docked to the right of the **Data Navigator**, on the left side of the layout, as shown by Figure T11.15. Note that it was docked inside the previous **Data Navigator,** i.e., both new docked windows take up the same amount of space as the **Data Navigator** did.

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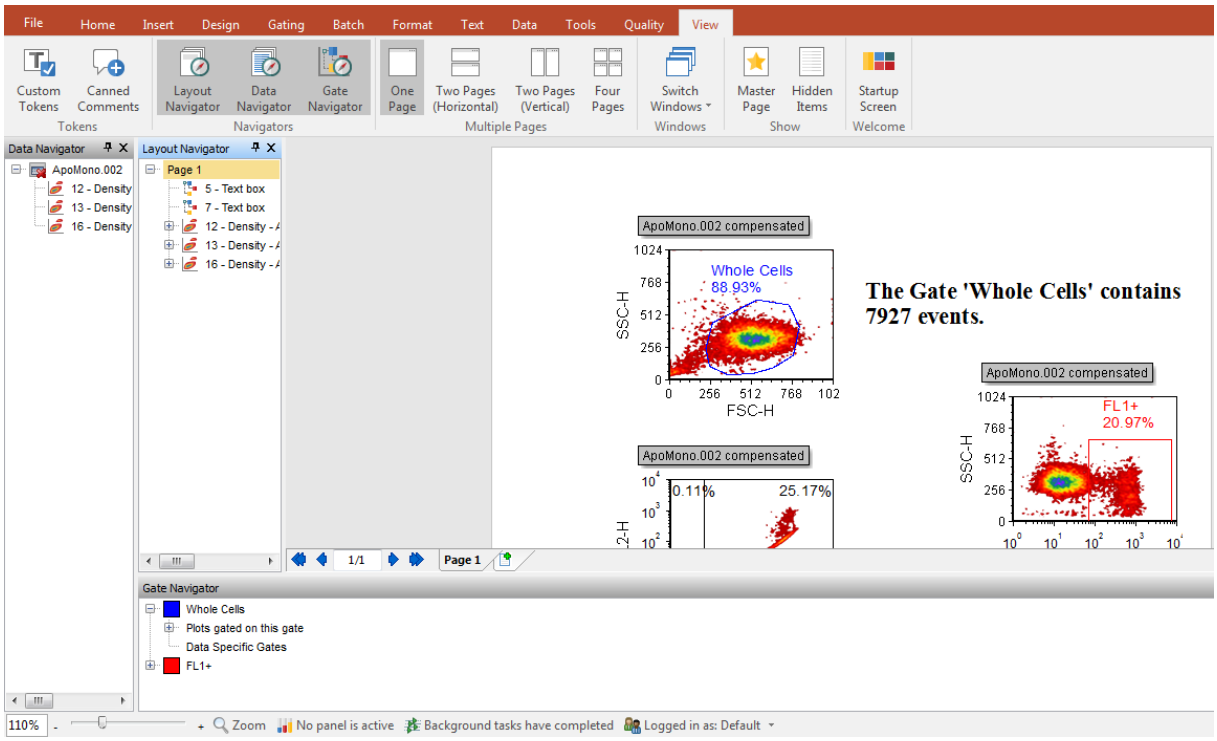

Figure T11.15 Layout Navigator Docked to Right of the Data Navigator

Note that the windows docked on the layout are highlighted on the ribbon (Figure 15). We will now dock the **Custom Tokens** window on the layout. However, this time we will dock it on top of the **Layout Navigator**.

7. Select the **View→Tokens→Custom Tokens** command.

The **Custom Tokens** window appears on the layout.

8. Drag the **Custom Tokens** towards the left side of the layout.

9. Continue to hold down the left mouse button and drag the **Custom Tokens** window towards the middle of the docked **Layout Navigator**.

When the **Custom Tokens** is on top of the **Layout Navigator** the four-headed arrows docking icon appears (Figure T11.16).

10. Release the mouse button when the cursor is on top of the central area of the docking icon on top of **Layout Navigator**, indicated by the cursor in Figure T11.16.

You will know you are in the correct place because the blue rectangle indicates the proposed docking location.

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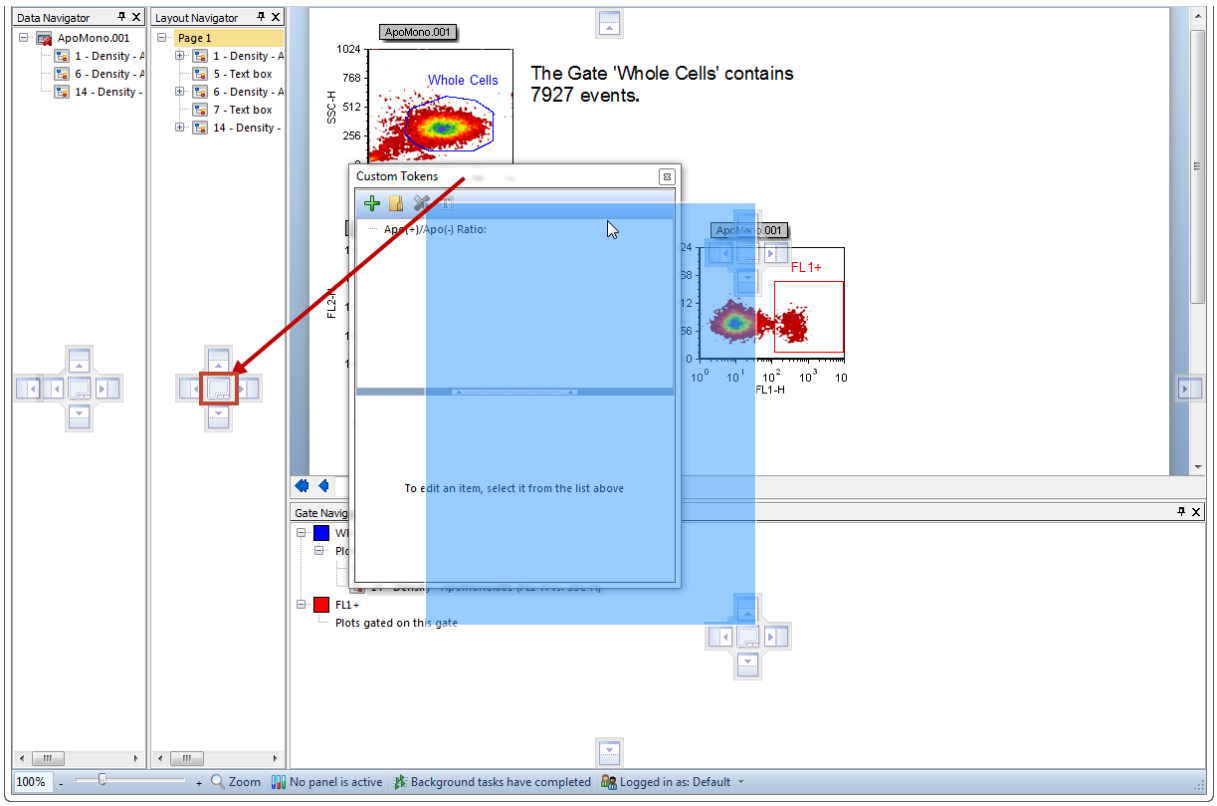

Figure T11.16 Docking Custom Tokens on top of the Layout Navigator

The **Custom Tokens** window is now docked on the left side of the layout, on top of the **Layout Navigator**, as shown in Figure T11.17. Notice that at the bottom of the docked **Custom Tokens** window there is a tab labeled **Custom Tokens**. However, we want to see the tabs for both docked windows so we will now resize the docked windows to make them larger.

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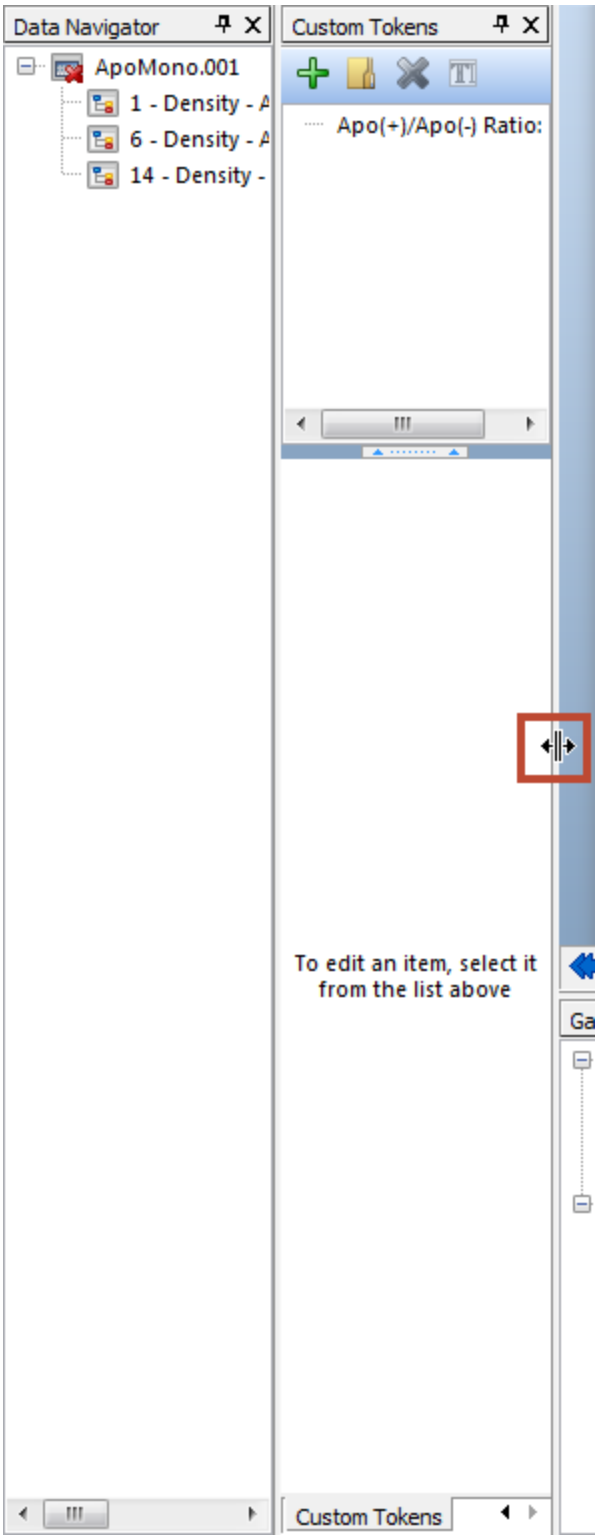

Figure T11.17 Custom Tokens Docked on top of the Layout Navigator

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11. Place the mouse over the outer right border of the docked **Custom Tokens**.

12. Move the mouse slightly over the border until the resizing icon appears, indicated by the red square in Figure T11.17.

13. Press and hold down the left mouse button and continue to hold it while dragging the border to the right.

14. Release the mouse button when the docked window is large enough to show the tabs for both **Custom Tokens** and **Layout Navigator** at the bottom (Figure T11.18).

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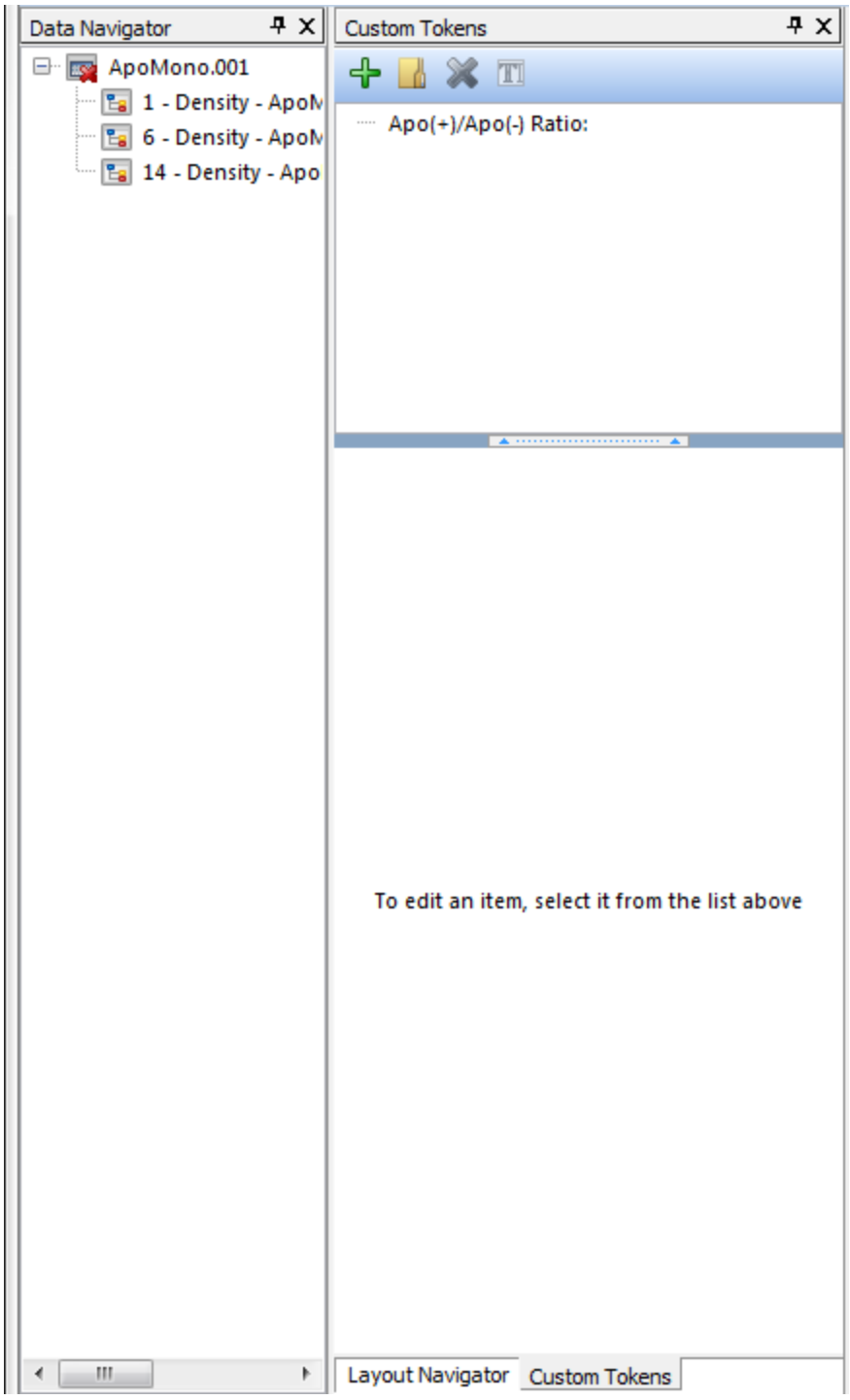

Figure T11.18 Custom Tokens and Layout Navigator Tabs

We will now explore the benefits of using **Auto Hide** when multiple windows are docked in one location.

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15. Select the docked **Data Navigator**.

16. Drag the **Data Navigator** and dock it on top of **Custom Tokens**.

The **Data Navigator** is now docked on top of both **Custom Tokens** and **Layout Navigator**, as shown in Figure T11.19. If you can't see all three tabs, then resize the docked windows to make them wider.

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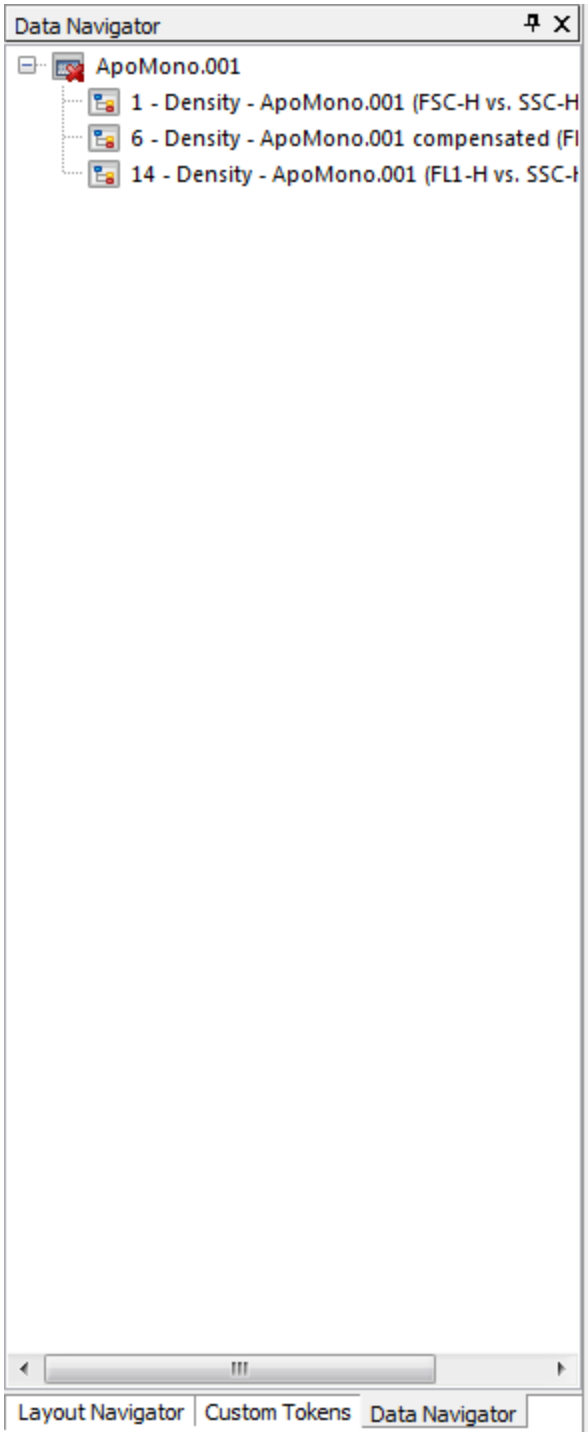

Figure T11.19 Data Navigator Docked on top of Custom Tokens and Layout Navigator

17. Click on the **Auto Hide** icon in the upper right corner of **Data Navigator**, previously shown in Figure T11.9 1544.

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18. Move the cursor off the docked **Data Navigator**.

The **Auto Hide** feature is now applied to all three docked windows, and only the tabs for the docked windows are visible, as shown in Figure T11.20. To see a docked window, move the cursor over the respective tab of the window you wish to see.

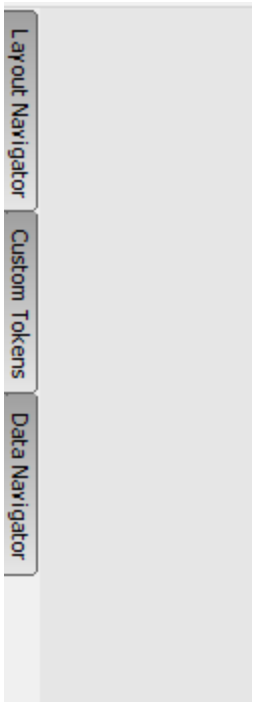

Figure T11.20 Docked Windows using Auto Hide Feature

In the last section, we will undock a window 1559.

38.12.3 Undocking a Window

To undock a window:

1. Place the cursor on the top border of the docked **Gate Navigator,** which is docked on the bottom of the layout.
2. Press and hold the left mouse button while dragging the **Gate Navigator** up and away from its current docked location.

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3. Release the mouse button when the **Gate Navigator** is at the desired location. Be sure the cursor is not on any of the docking icons.

To undock the docked windows that are using **Auto Hide**, we need to first turn off the **Auto Hide** feature; there are two ways of doing this.

4. Move the cursor over the **Data Navigator** tab to make the docked **Data Navigator** visible.

5. Click on the **Auto Hide** icon in the upper right corner of the docked **Data Navigator**, as previously shown in Figure T11.11 1544, to turn off the **Auto Hide** feature. Alternatively,

   - Place the cursor on the top border of the docked **Data Navigator**.
   - Right-click to bring up the associated pop-up menu.
   - Deselect **Auto Hide** from the pop-up menu, indicated by the cursor in Figure T11.21.

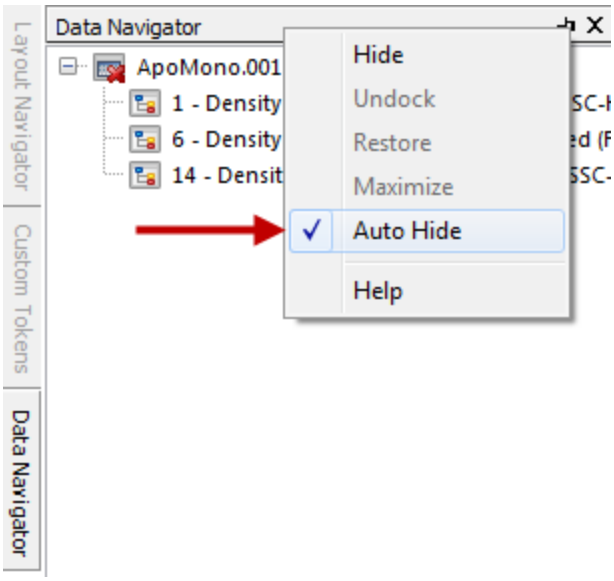

Figure T11.21 Removing Auto Hide using the Pop-up Menu

The **Auto Hide** feature has now been turned off for all three docked windows, and they can now be undocked.

6. Right-click on the top border of the docked **Data Navigator** to bring up the associated pop-up menu.

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7. Select **Undock** from the pop-up menu.

Alternatively, the docked windows can be undocked by dragging and dropping them back onto the layout as previously shown.

38.13 Data Navigator

The **Data Navigator** is designed to give you a useful overview of all the data files used in the entire layout. It shows you a hierarchical view of all the data, as all the plots using that data. The **Data Navigator** also allows you to manipulate the data and plots on the layout. For example, by simply dragging and dropping the data icon on the **Data Navigator** you can replace or overlay the data on a plot.

In the course of this example, we will:

- Open and dock the **Data Navigator**.
- Learn about the **Data Navigator** features and how to use them. Specifically we will use the **Data Navigator** to:
  - Explore the use of the thumbnail image of a plot associated with a data file.
  - Insert new data onto a plot.
  - Add an overlay to a histogram.
  - Insert a new plot onto the layout.

In the first exercise, we will open and dock the Data Navigator 1561.

38.13.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on two pages.

1. Select **File** tab→**Open**.
2. Open the layout **DataNavigator.fey** found in the Tutorial Sample Data archive 1210.

The first page of the layout appears, as shown in Figure T12.1.

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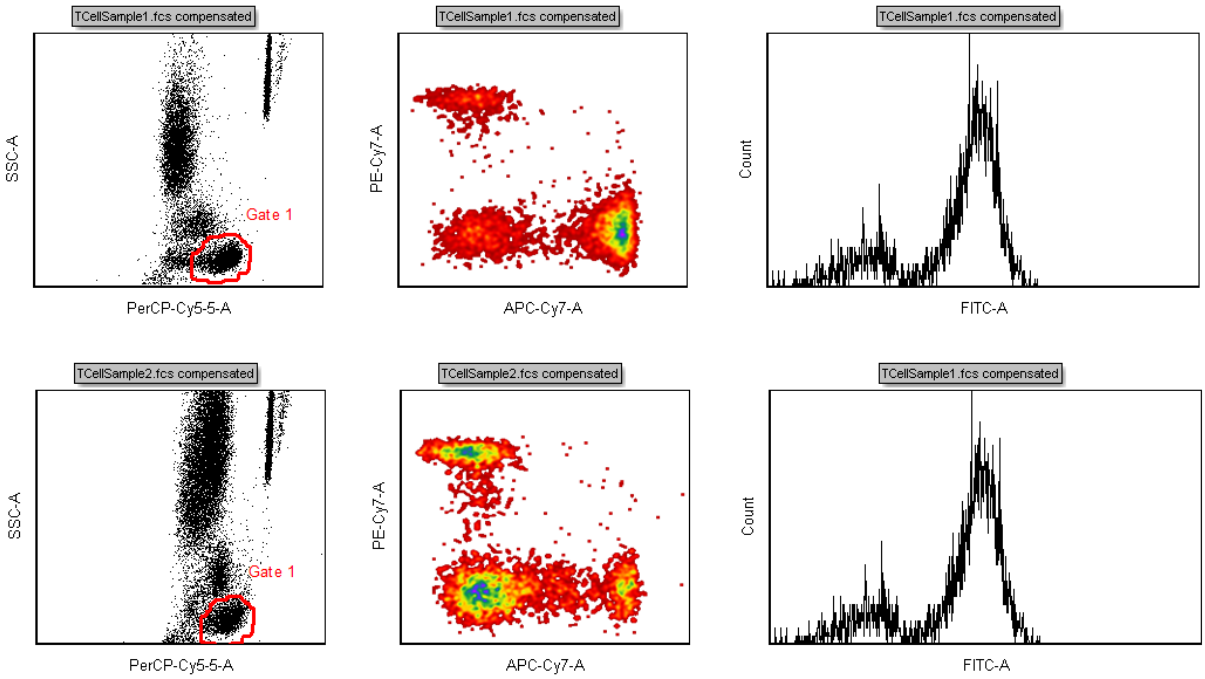

Figure T12.1 Page 1 of the Data Navigator Layout (DataNavigator.fey)

We will now open the **Data Navigator.**

3. Select the **View→Navigators→Data Navigator** command (Figure T12.2).

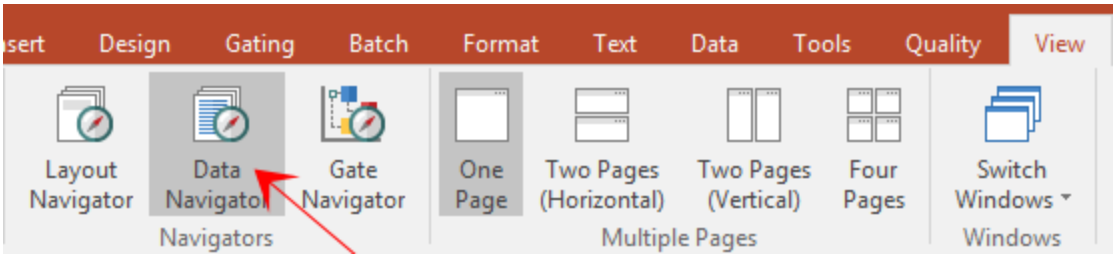

Figure T12.2 Selecting the Data Navigator Command

The **Data Navigator** appears, as shown in Figure T12.3. Right click within the Data Navigator and select **Expand All** or click on the "+" sign next to each file to expand the view. Under each file, you will see a list of plots that are currently showing data from that file.

We will now dock it to the left side of the layout. For more information on docking, please refer to the Docking tutorial 1537.

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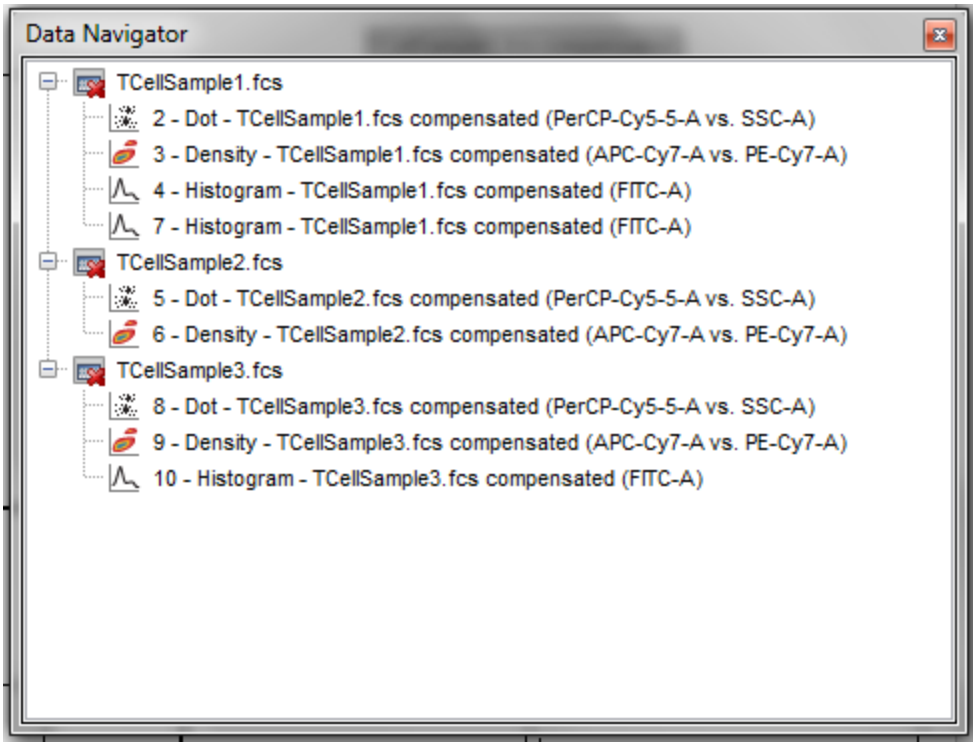

Figure T12.3 Data Navigator

4. Place the mouse on the top border of the **Data Navigator** window.
5. Press and hold the left mouse button and move the **Data Navigator** window.

As soon as you start moving the **Data Navigator** window the four-headed arrows of the docking icon appears in the center of the layout, as shown in Figure T12.4.

6. Continue to hold the mouse button and move the **Data Navigator** toward the left arrow of the docking icon.
7. Release the mouse button when the cursor is over the left arrow of the docking icon, indicated by the cursor in Figure T12.4. You will know you are in the correct place because the blue rectangle, indicating the proposed docking location, will appear on the left side of the layout.

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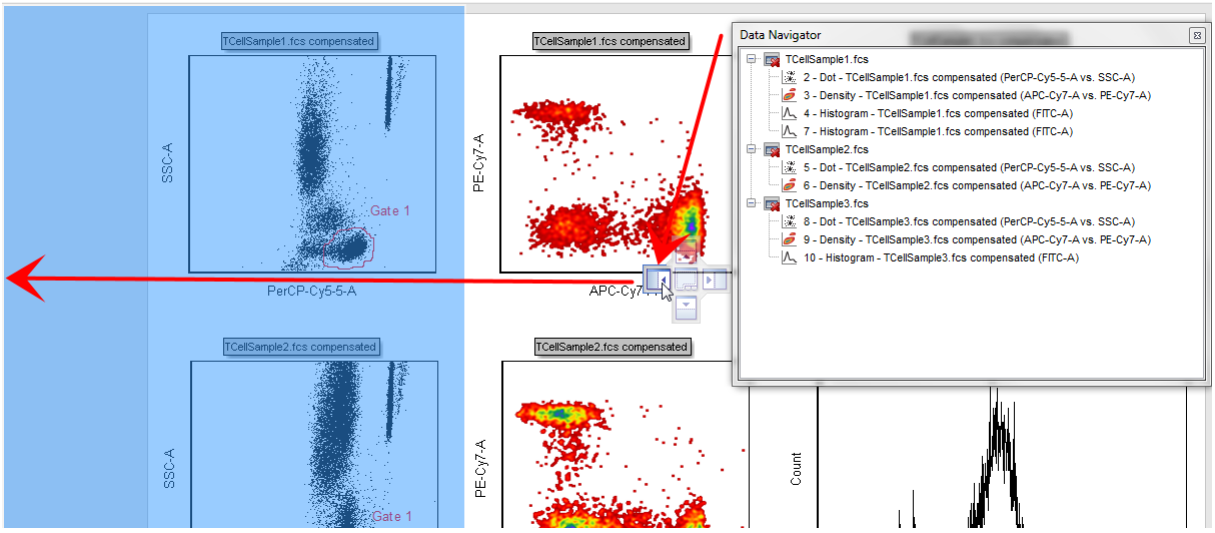

Figure T12.4 Docking the Data Navigator to the Left Side

The **Data Navigator** is now docked on the left side of the layout. However, all the text might not be visible so we will now resize the docked window it to make it wider.

8. Place the mouse over the outer right border of the docked **Data Navigator**.

9. Move the mouse slightly over the border until the resizing icon appears, indicated by the red square in Figure T12.5.

10. Press and hold down the left mouse button and continue to hold it while dragging the border to the right.

11. Release the mouse button when the docked window is large enough to show all the text of the **Data Navigator**, as shown in Figure T12.6.

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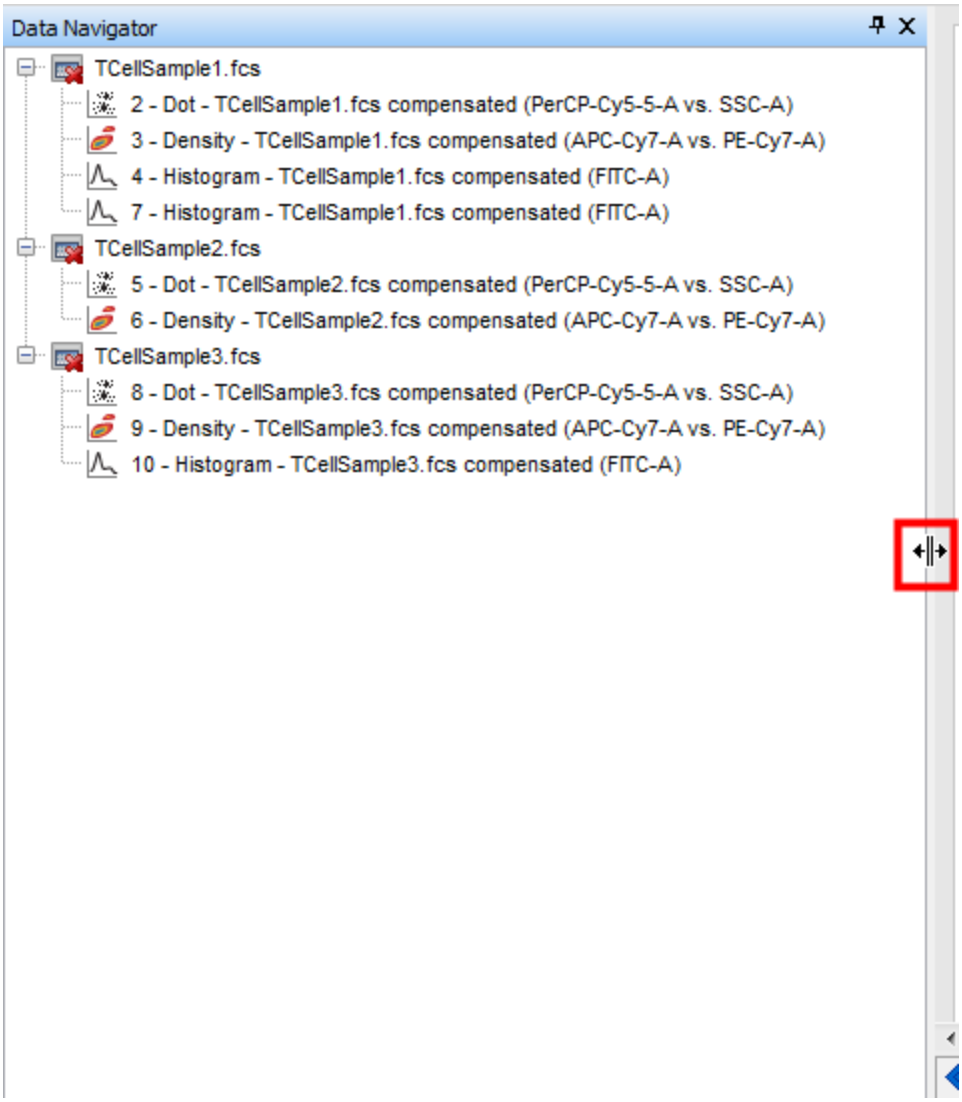

Figure T12.5 Resizing the Data Navigator

The docked **Data Navigator** should appear similar to Figure T12.6.

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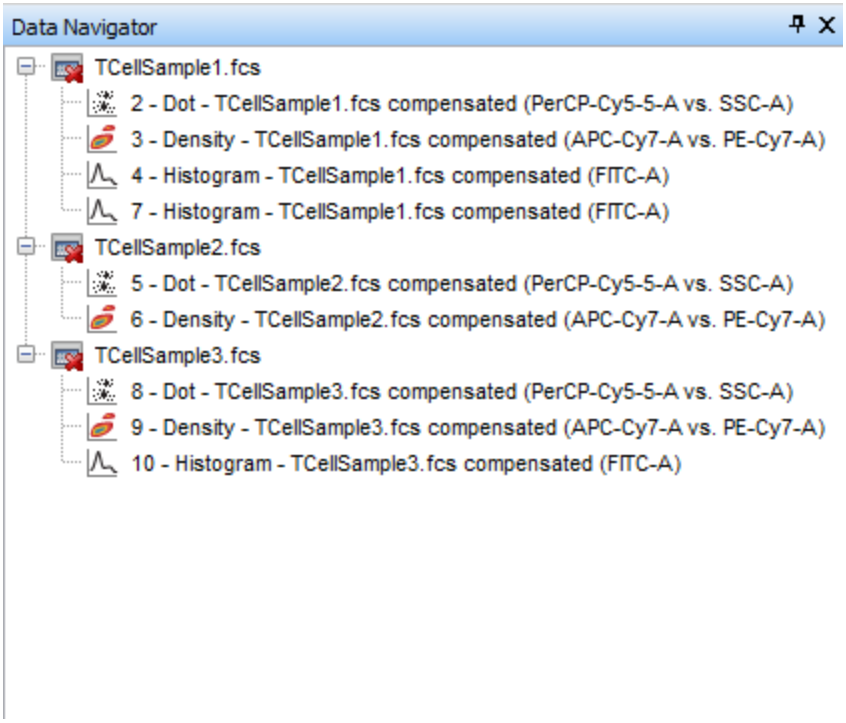

Figure T12.6 Docked Data Navigator

In the next section, we will explore the features of the Data Navigator 1566.

38.13.2 Data Navigator Features

As shown in Figure T12.6 1561, the **Data Navigator** is organized by the data files in the layout. Under each data filename, is the list of the plots which are using that data file. We will now explore the many features of the **Data Navigator**.

1. Hover the mouse over the **9 - Density – TCellSample3.fcs compensated (APC-Cy7-A vs PE-Cy7-A)**, under the data file listing for **TCellSample3.fcs**, within the **Data Navigator**, as indicated by the cursor in Figure T12.7.

A thumbnail image of the actual 2D plot will appear, as shown in Figure T12.7. This is a helpful quick reminder of which plot is associated with a data file, particularly if that plot is not currently visible. This feature applies to any of the plots listed in the **Data Navigator**.

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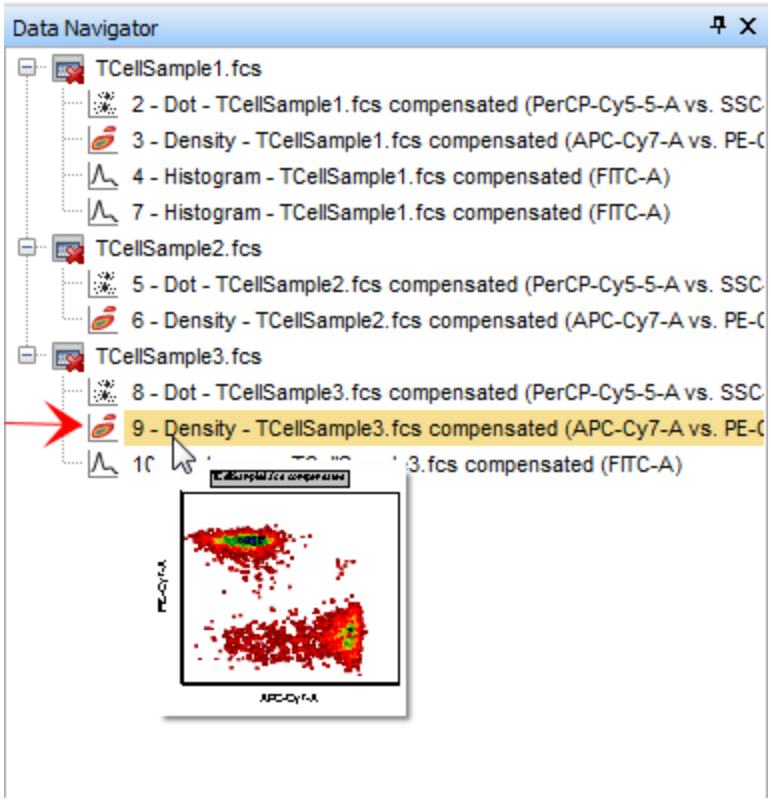

Figure T12.7 Thumbnail Image of a 2D Plot on the Data Navigator

We will now use the **Data Navigator** to change the data displayed in a 2D plot. We will replace the **TCellSample1.fcs** data in the 1D plot in the lower right of the layout with the **TCellSample2.fcs** data using just the **Data Navigator**.

2. Place the cursor over data file **TCellSample2.fcs** in the **Data Navigator**, highlighted in blue in Figure T12.8.

3. Press and hold the left mouse button.

4. Drag the **TCellSample2.fcs** data file up to the plot listing **7 – Histogram – TCellSample1.fcs compensated (FITC-A)**, indicated by the cursor and highlighted in blue in Figure T12.8.

5. Release the mouse button when the **TCellSample2.fcs** data file is on top of the listing **7 – Histogram – TCellSample1.fcs compensated (FITC-A)**.

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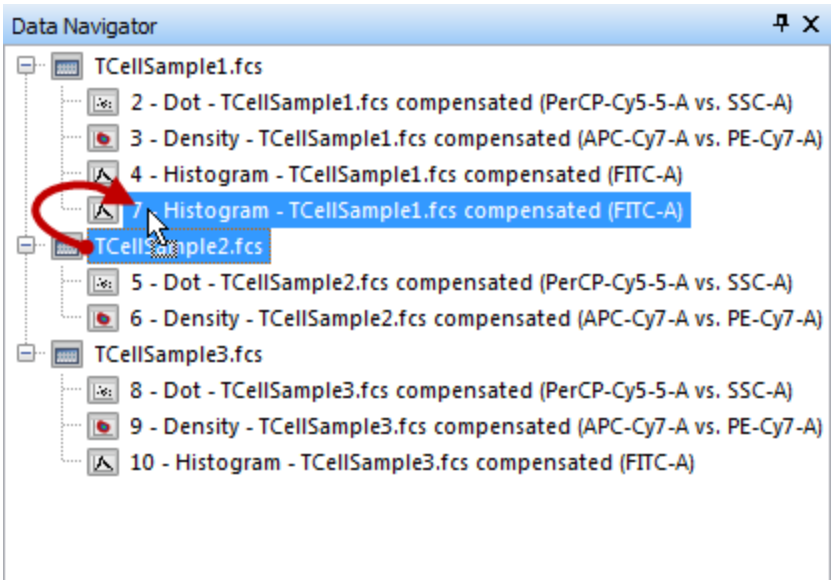

Figure T12.8 Dragging TCellSample2.fcs data file onto a 1D plot in the Data Navigator

The **Select Drag and Drop Action** dialog appears, as shown in Figure T12.9.

6. Select **replace the existing overlays in the plot** (Figure T12.9).
7. Click **OK**.

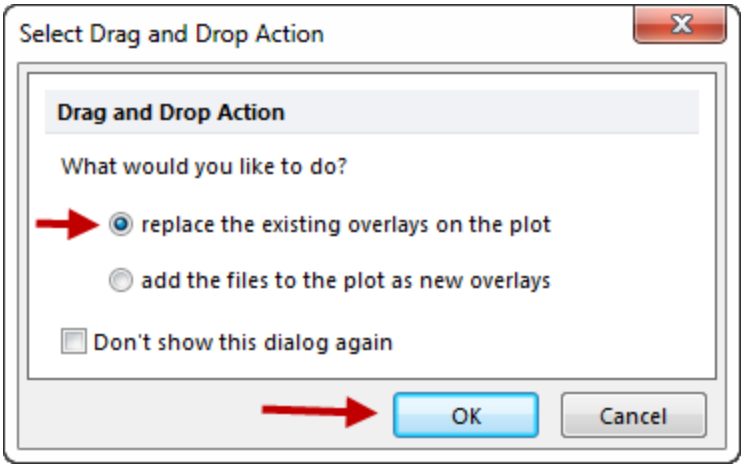

Figure T12.9 Select Drag and Drop Action Dialog

The 1D histogram in the lower right of the layout is now selected and updates to reflect the **TCellSample2.fcs** data, outlined in green in Figure T12.10.

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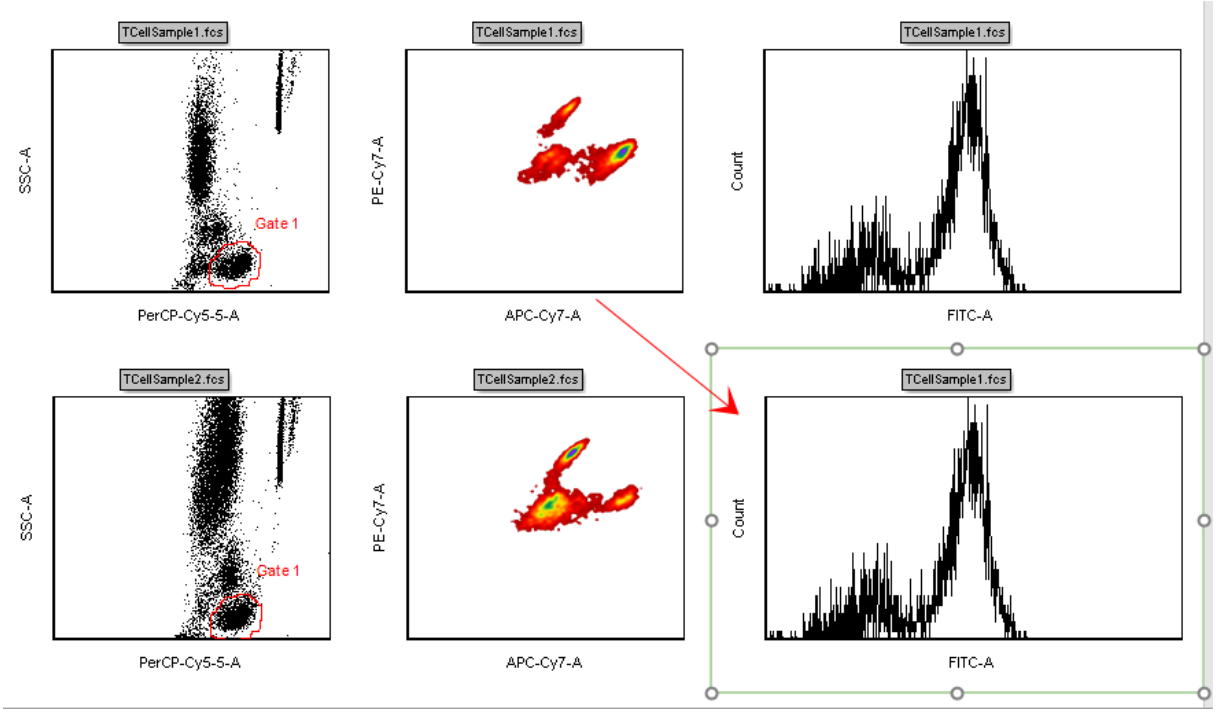

Figure T12.10 Selected and Updated 1D Histogram with TCellSample2.fcs Data

The **Data Navigator** is also updated to reflect the changes, as shown in Figure T12.11. The plot **7 – Histogram – TCellSample2.fcs compensated (FITC-A)** appears under the **TCellSample2.fcs** data file listing, as indicated by the cursor and the thumbnail image in Figure T12.11.

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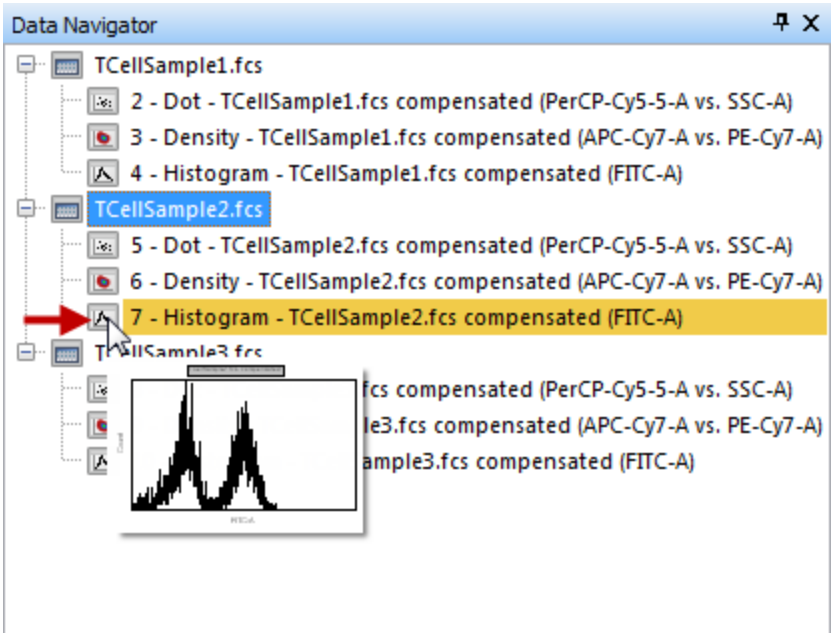

Figure T12.11 Data Navigator Updated to Reflect TCellSample2 Plot Changes

We will now use drag and drop in the **Data Navigator** to overlay data on a histogram.

8. Place the cursor over data file **TCellSample2.fcs** in the **Data Navigator**, highlighted in blue in Figure T12.12.

9. Press and hold the left mouse button.

10. Drag the **TCellSample2.fcs** data file up to the histogram plot listing **4 – Histogram – TCellSample1.fcs compensated (FITC-A)**, indicated by the cursor and highlighted in blue in Figure T12.12.

11. Release the mouse button when the **TCellSample2.fcs** data file is on top of the histogram listing.

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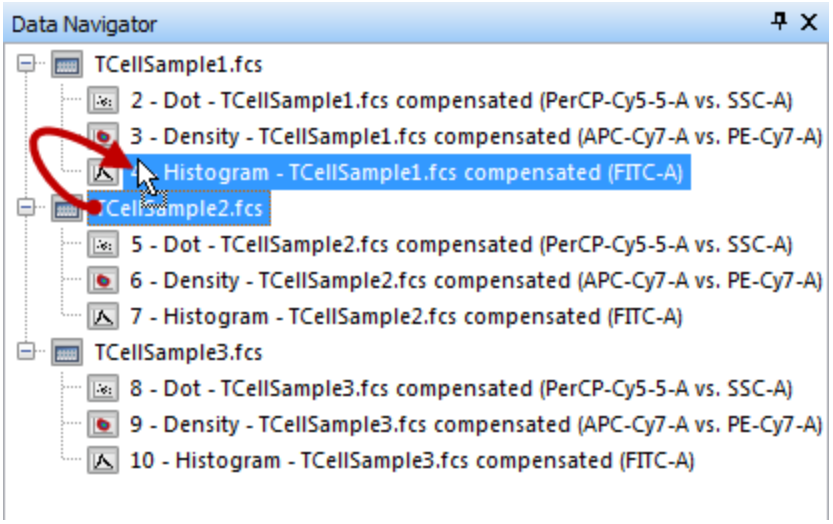

Figure T12.12 Dragging TCellSample2 onto a Histogram Plot in the Data Navigator

The **Select Drag and Drop Action** dialog appears, as previously shown in Figure T12.9. However, this time we will change the selection on the dialog to allow the data to be overlaid on the histogram.

12. Select "**add the files to the plot as new overlays**".
13. Click **OK**.

The histogram in the upper right of the layout is now updated to reflect the overlay of the TCellSample2.fcs data. The histogram plot is shown outlined in green and the new overlay data is shown in red in Figure T12.13.

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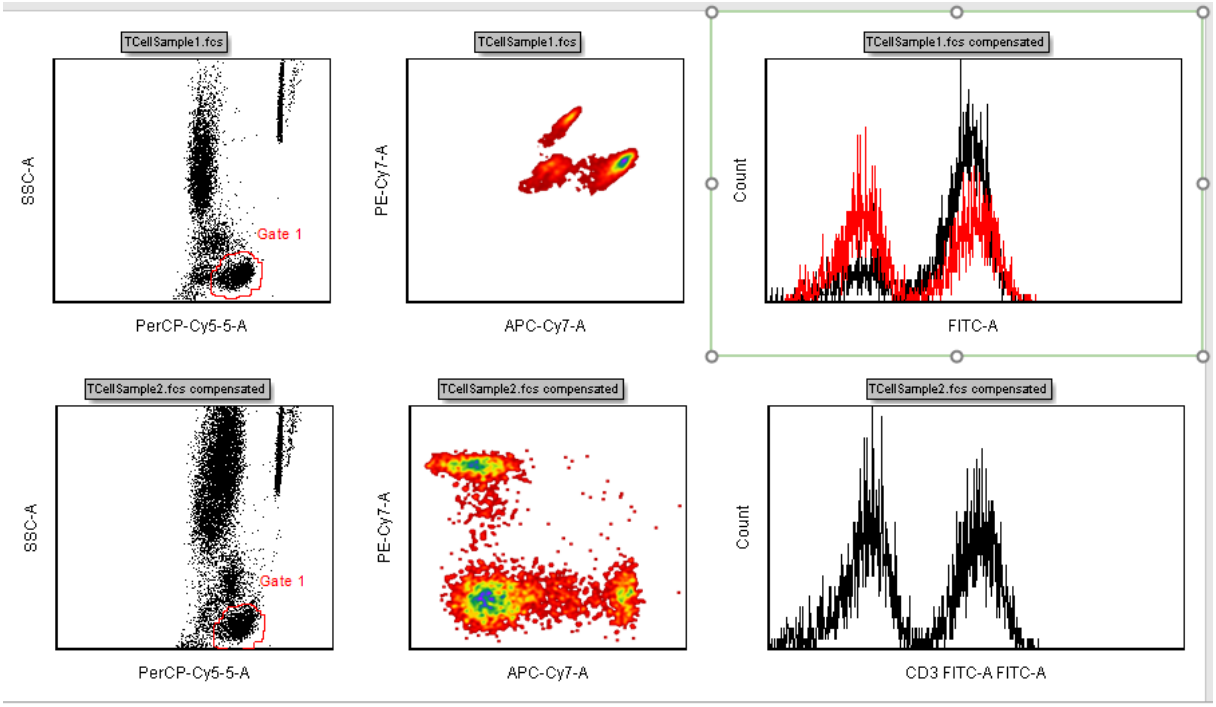

Figure T12.13 Histogram Updated to Show the Overlay of TCellSample2 Data

Again, the **Data Navigator** also updates to reflect the changes as shown in Figure T12.14. Under the data file listing for **TCellSample2.fcs**, a listing now appears for **4 – Histogram – TCellSample1.fcs compensated (FITC-A)**, as indicated by the cursor and the thumbnail image in Figure T12.14. The sub-listing **Overlay 2** also appears under the histogram listing to indicate that the data file **TCellSample2.fcs** is the second overlay on the **TCellSample1.fcs** histogram. Note that under the **TCellSample1.fcs** data file listing, the histogram **4 – Histogram – TCellSample1.fcs compensated (FITC-A)** listing has also been updated with the sub-listing **Overlay 1** to indicate that the data ApoMono.001 is the base data.

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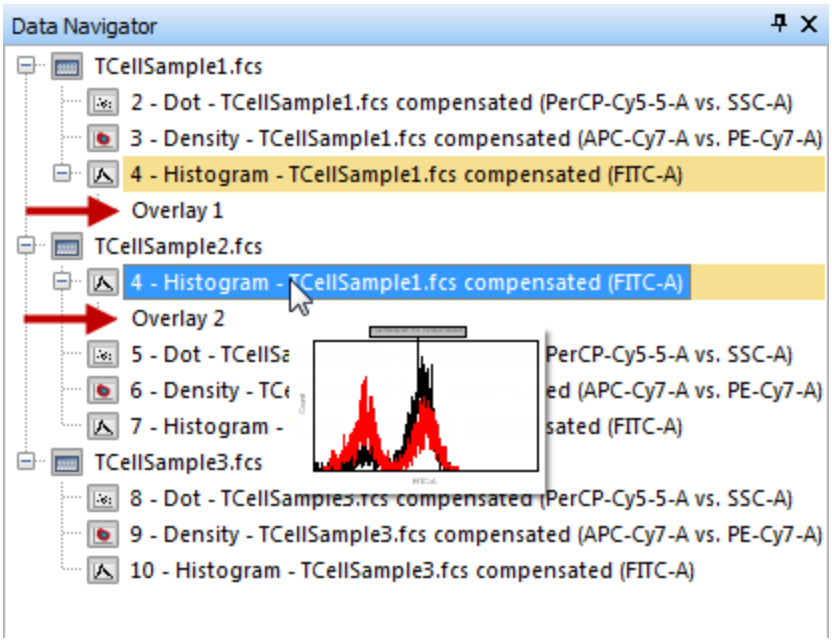

Figure T12.14 Updated Data Navigator with Overlay of TCellSample2 Data

All the previous actions of dragging and dropping data in the **Data Navigator** could also have been done using the **Data Navigator** pop-up menu. We will now use the **Data Navigator** pop-up menu to add data as another overlay to the histogram in the upper right of the layout.

14. Place the cursor over the **TCellSample3.fcs** data file listing on the **Data Navigator**, shown highlighted in blue in Figure T12.15.

15. Right-click to bring up the associated pop-up menu.

16. Select **Copy** from the pop-up menu, indicated by the cursor in Figure T12.15.

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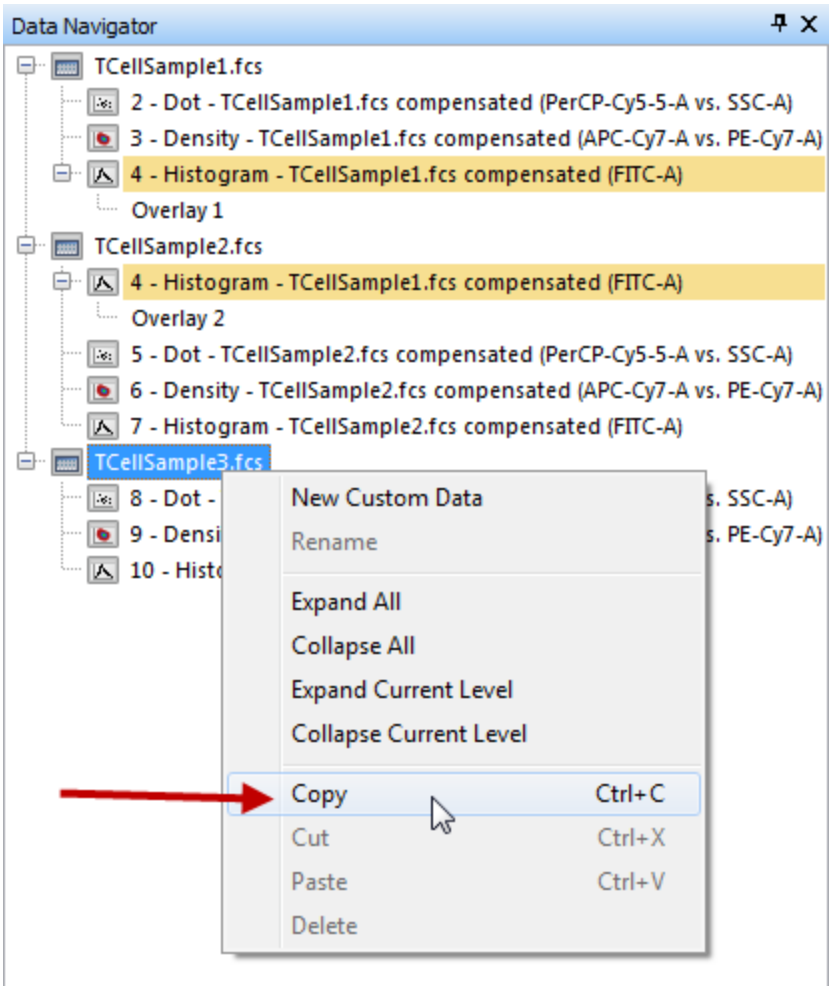

Figure T12.15 Copy Using Data Navigator Pop-up Menu

17. Place the cursor over the listing **4 – Histogram – TCellSample1.fcs compensated (FITC-A)** listed under **TCellSample1.fcs** data on the **Data Navigator**, shown highlighted in blue in Figure T12.16.

18. Right-click to bring up the associated pop-up menu.

19. Select **Paste** from the pop-up menu, indicated by the cursor in Figure T12.16.

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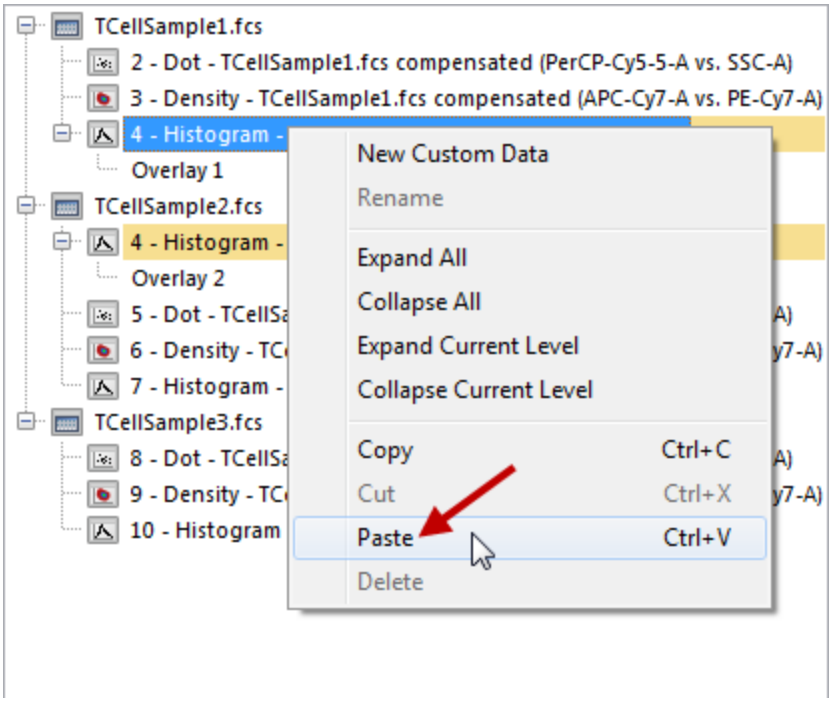

Figure T12.16 Paste Using Data Navigator Pop-up Menu

The **Select Drag and Drop Action** dialog appears, as previously shown in Figure T12.9. However, we will change the selection on the dialog to allow the data to be overlaid on the histogram.

20. Select "**add the files to the plot as new overlays**".

21. Click **OK**.

The histogram in the upper right of the layout is now updated to reflect the overlay of the ApoMono.003 data. The histogram is shown outlined in green and the new overlay data is shown in blue in Figure T12.17.

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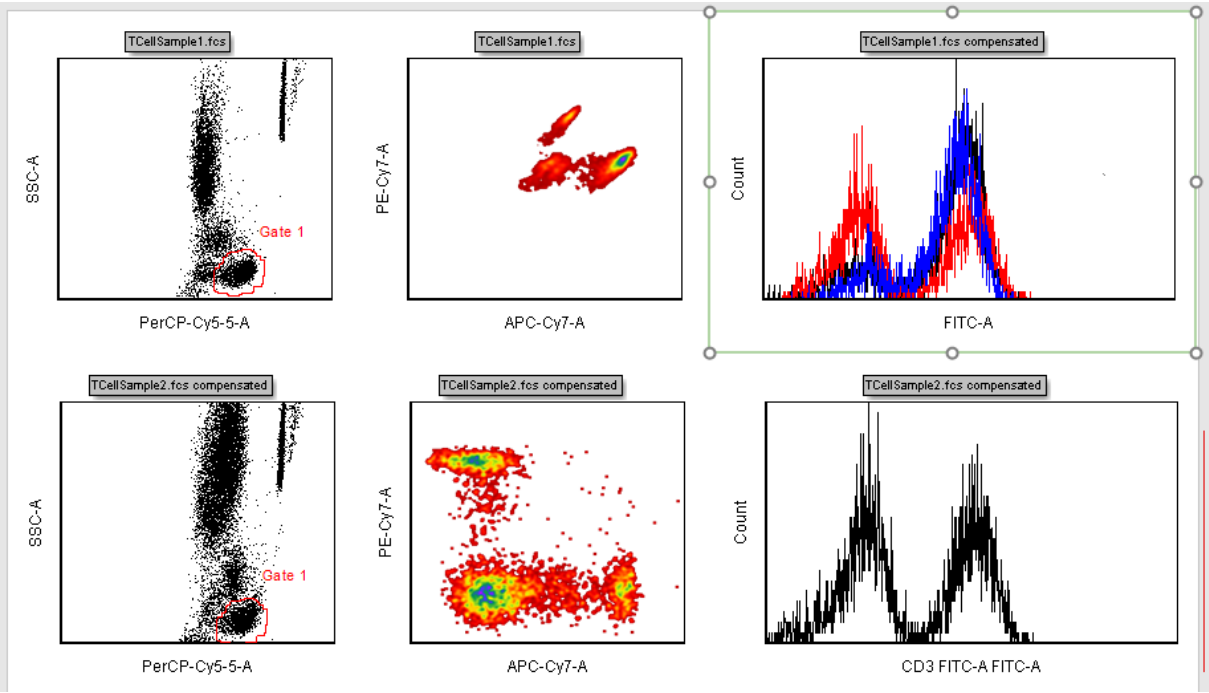

Figure T12.17 Histogram Updated to Show the Overlay of TCellSample3 Data

Again, the **Data Navigator** updates to reflect the changes as shown in Figure T12.18. Under the data file listing for **TCellSample3.fcs**, a listing now appears for **4 – Histogram – TCellSample1.fcs compensated (FITC-A)**, as indicated by the cursor and the thumbnail image in Figure T12.18. The sub-listing **Overlay 3** also appears under the histogram listing to indicate that the data file **TCellSample3.fcs** is the third overlay on the **TCellSample1.fcs** histogram.

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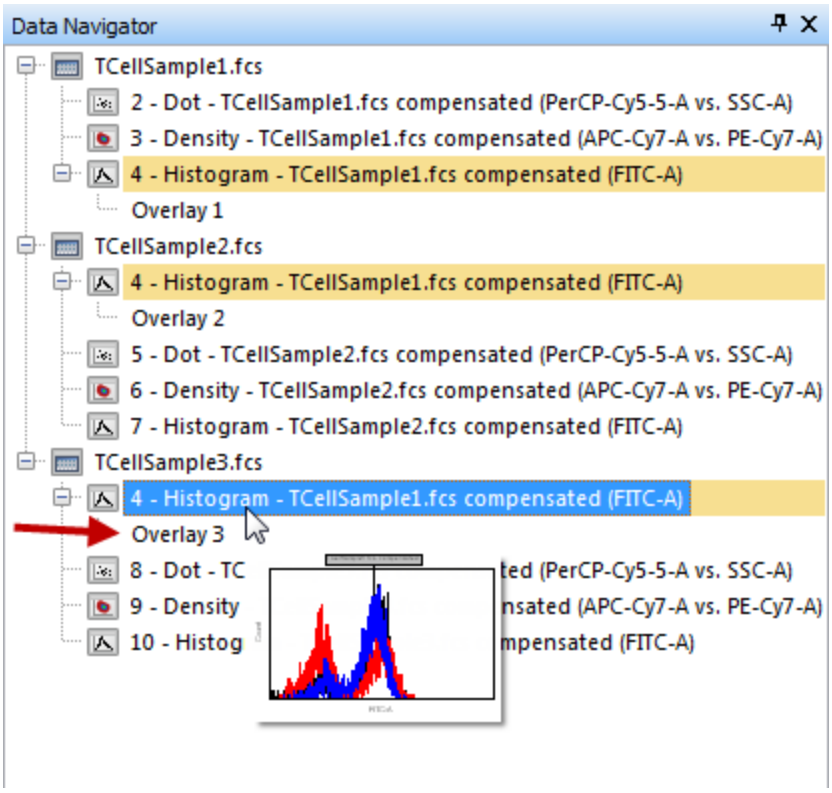

Figure T12.18 Updated Data Navigator with Overlay of TCellSample3 Data

We can also drag and drop data from the **Data Navigator** to either replace or overlay data within the plots on the pages of our layout.

We will drag a data file from the **Data Navigator** onto a plot on the layout. On the layout, the dot plot of SSC-A vs. PerCP Cy5.5-A, in the lower left corner of Page 1, is using the data **TCellSample2.fcs**. We will overlay that with **TCellSample1.fcs** data.

22. Place the cursor over data file **TCellSample1.fcs** in the **Data Navigator**, highlighted in blue in Figure T12.19.

23. Press and hold the left mouse button.

24. Drag the data file **TCellSample1.fcs** onto the layout.

25. Release the mouse button when **TCellSample1.fcs** is on top of the dot plot of SSC-A vs. PerCP Cy5.5-A, in the lower left corner of Page 1, indicated by the cursor and outlined in blue in Figure T12.19.

26. In the Select Drag and Drop Action window, select **add the files to the plot as new overlays**.

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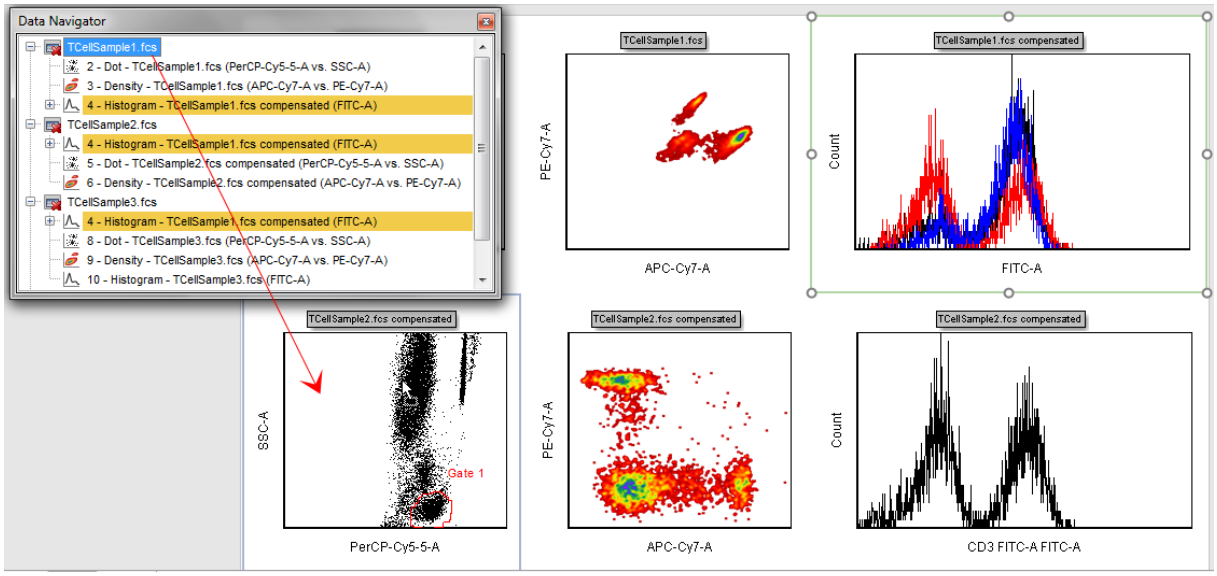

Figure T12.19 Dragging and Dropping TCellSample1 from the Data Navigator onto the Dot Plot on the Layout

Both the dot plot on the layout, in the lower left, and the **Data Navigator** have updated to reflect the data change, as shown in Figure T12.20. The cursor and thumbnail image, shown in Figure T12.20 indicate the changes in the **Data Navigator**.

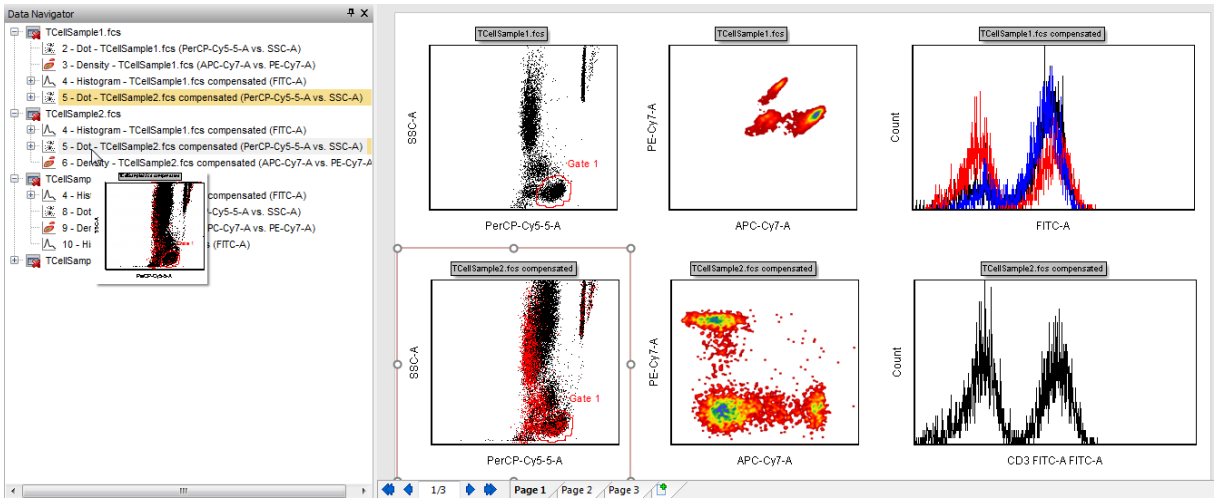

Figure T12.20 Updated Layout and Data Navigator with TCellSample1 Overlay

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In addition to dragging the data file from the **Data Navigator** and dropping it onto the dot plot on the layout, we could have used the **Data Navigator** pop-up menu to copy the data object, and then used the pop-up menu on the layout to paste the data onto the dot plot. Just like all the other pop-up menus, the **Data Navigator** pop-up menus are accessed by right-clicking when the cursor is located over an item in the **Data Navigator**. We will now use the **Data Navigator** pop-up menu to insert a new data plot onto the layout.

26. Place the cursor over **TCellSample3.fcs** in the **Data Navigator**.
27. Right-click to bring up the associated pop-up menu.
28. Select **Copy** from the pop-up menu, as previously shown in Figure T12.15.
29. Click on **Page 2** at the bottom of the layout, as shown in Figure T12.21.

Figure T12.21 Selecting Page 2

Page 2 of the layout will now appear as shown in Figure T12.22.

30. Place the cursor on an empty part of the layout.
31. Right-click to bring up the associated pop-up menu, shown in Figure T12.22.
32. Select **Paste** from the pop-up menu.

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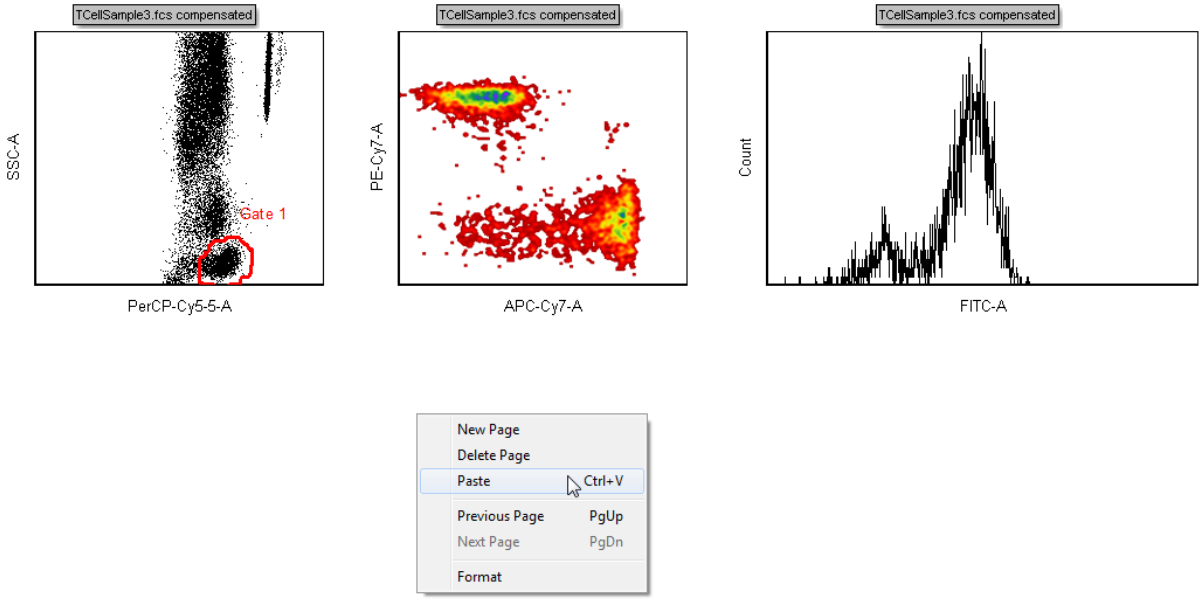

Figure T12.22 Page 2 of the Layout with the Pop-up Menu

The **Select Plot Types...** dialog appears, as shown in Figure T12.23.

33. Select **Density** as the types of plots to open (Figure T12.23).
34. Click **OK**.

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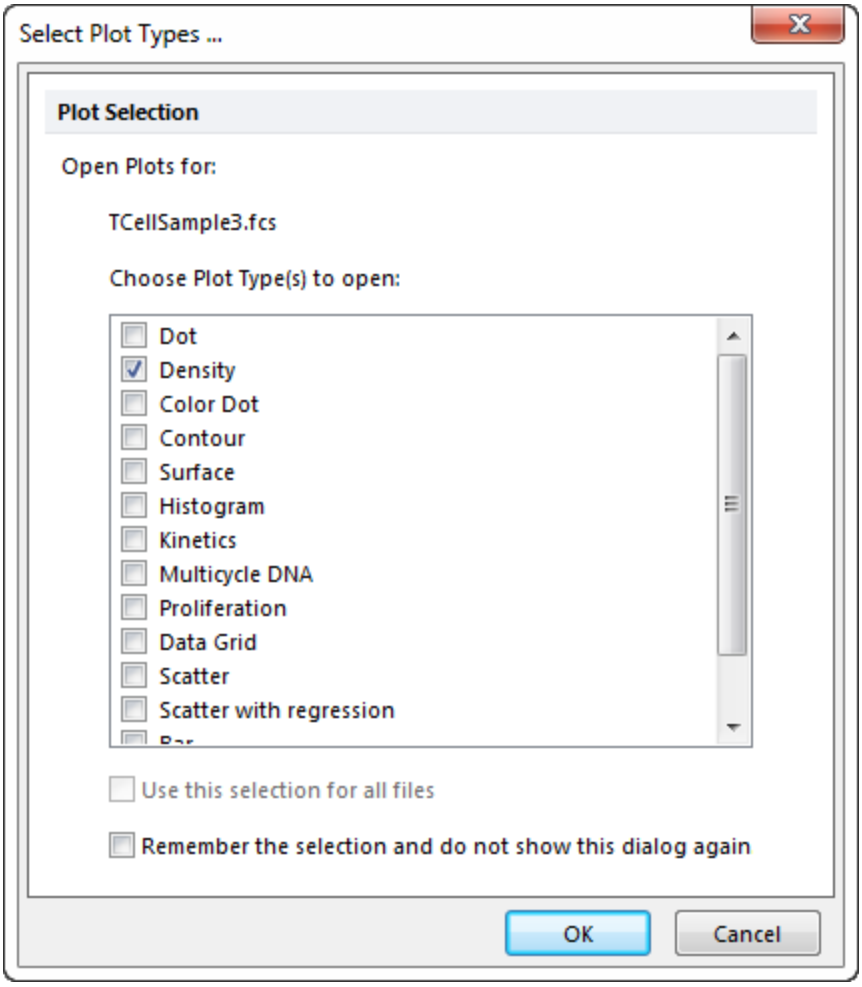

Figure T12.23 Select Plot Types... Dialog

A density plot of **TCellSample3.fcs** appear, using the default parameters of Time vs. FSC-H, as shown in Figure T12.24. Feel free to format this plot any way you wish.

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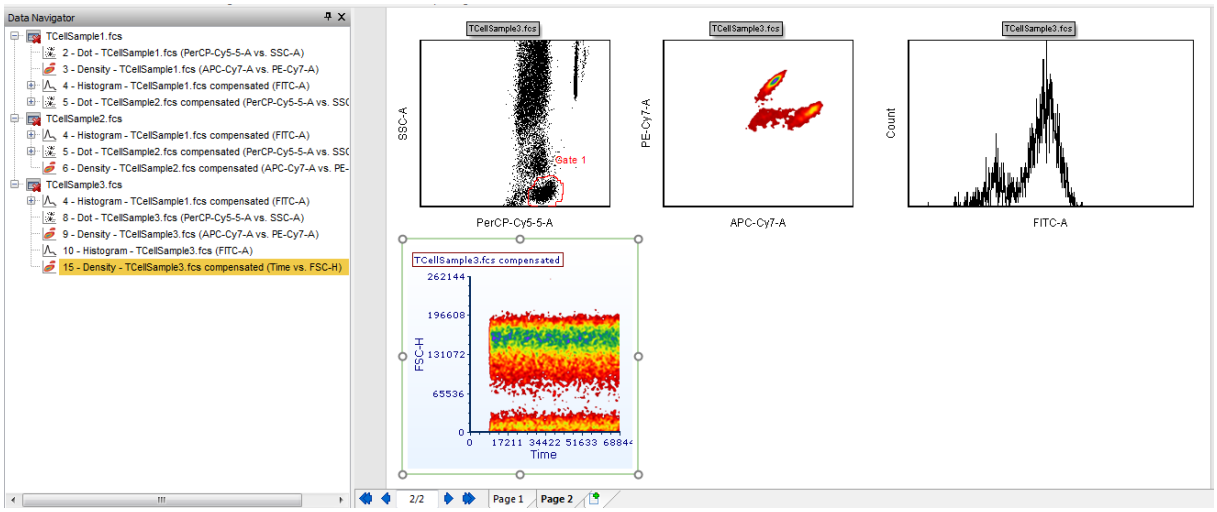

Figure T12.24 Contour Plot of TCellSample3.fcs Data

38.14 Gate Navigator

The **Gate Navigator** is designed to give you a useful overview of all the gates used in the layout. It shows a hierarchical view of the gates and the plots associated with those gates. The **Gate Navigator** also allows you to manipulate the gates and plots on the layout. For example, by simply dragging and dropping the gate icon on the **Gate Navigator** you can change the gate a plot is gated on.

In the course of this example, we will:

- Open and dock the **Gate Navigator**.
- Learn about the **Gate Navigator** features and how to use them. Specifically, we will use the **Gate Navigator** to:
  - Apply gates to plots on the layout.
  - Insert a plot onto the layout.

In the first exercise, we will open and dock the Gate Navigator 1582.

38.14.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on two pages.

1. Select **File** tab→**Open**.
2. Open the layout **GateNavigator.fey** found in the *FCS Express Sample Data* folder.

The layout appears, as shown in Figure T13.1.

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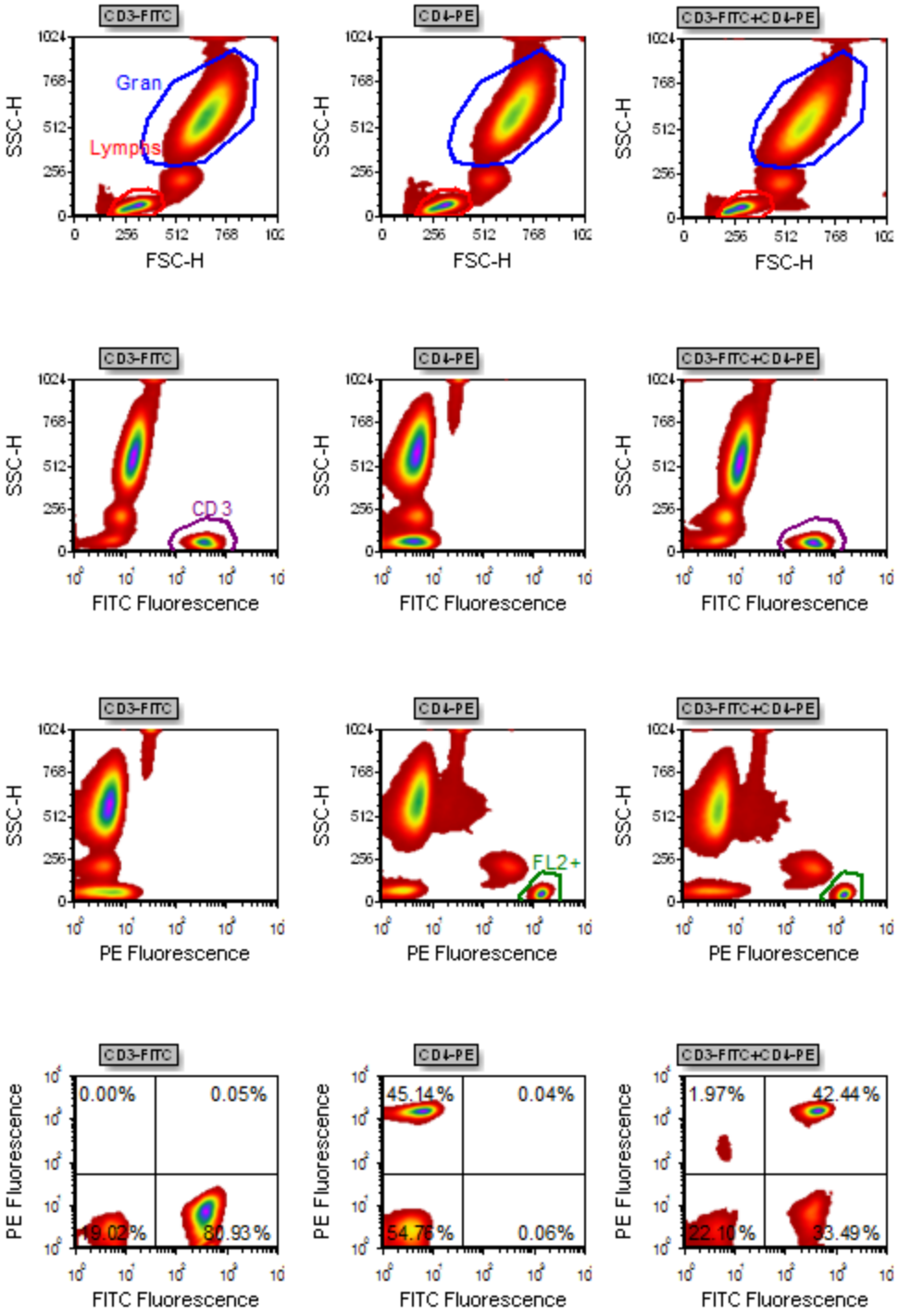

Figure T13.1 Gate Navigator Layout (GateNavigator.fey)

We will now open the **Gate Navigator.**

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3. Select the **View→Navigators→Gate Navigator** command (Figure T13.2).

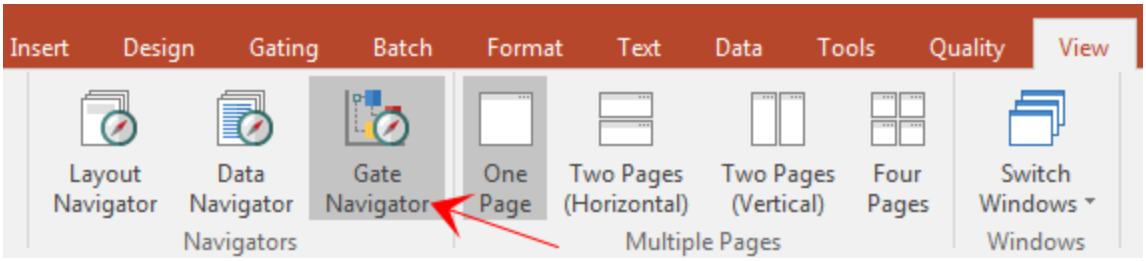

Figure T13.2 Selecting the Gate Navigator Command

The **Gate Navigator** appears, as shown in Figure T13.3. We will now expand the menu to view all the plots using these gates.

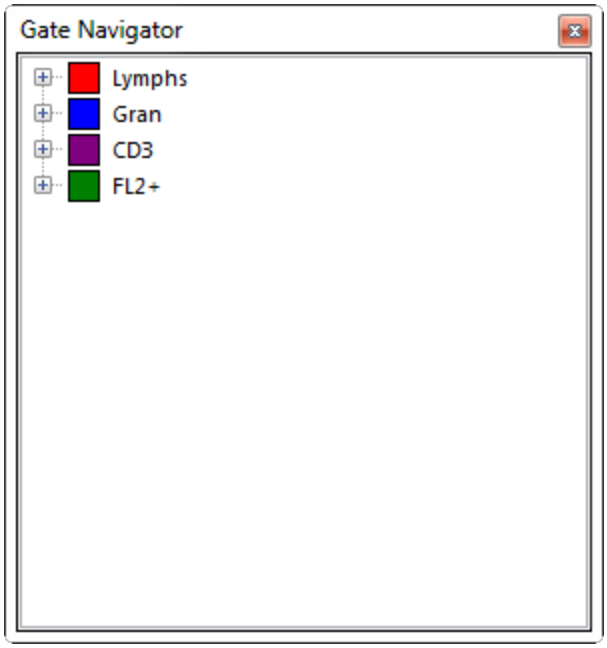

Figure T13.3 Gate Navigator Window

4. Click on the "+" icon to the left the **Lymphs** gate, indicated by the cursor in Figure T13.3, to expand the menu.

5. Continue to click on the "+" icons until all gate listings are expanded.

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Alternatively, you can use the pop-up menu to expand the **Gate Navigator** menu.

- Place the cursor on the Gate Navigator.
- Right-click to bring up the pop-up menu (Figure T13.4).
- Select **Expand All**, indicated by the cursor in Figure T13.4.

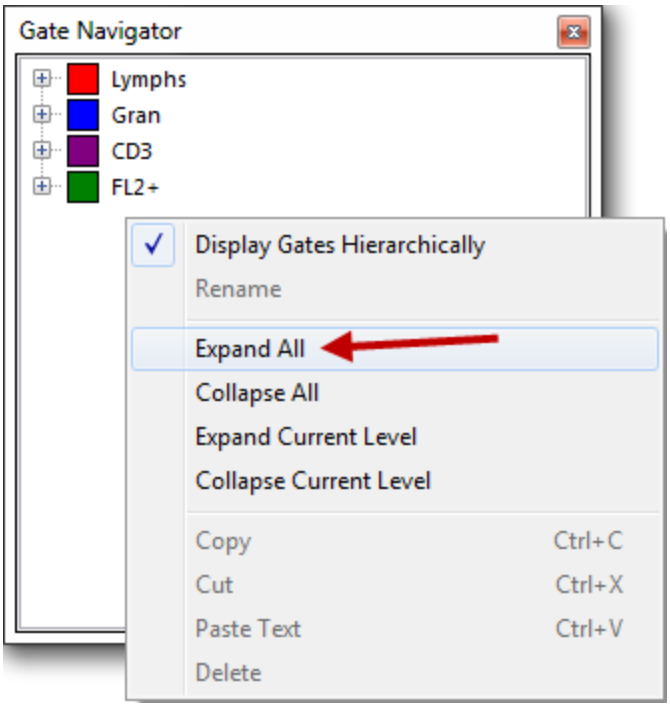

Figure T13.4 Gate Navigator Pop-up Menu

The **Gate Navigator** should now appear similar to Figure T13.5.

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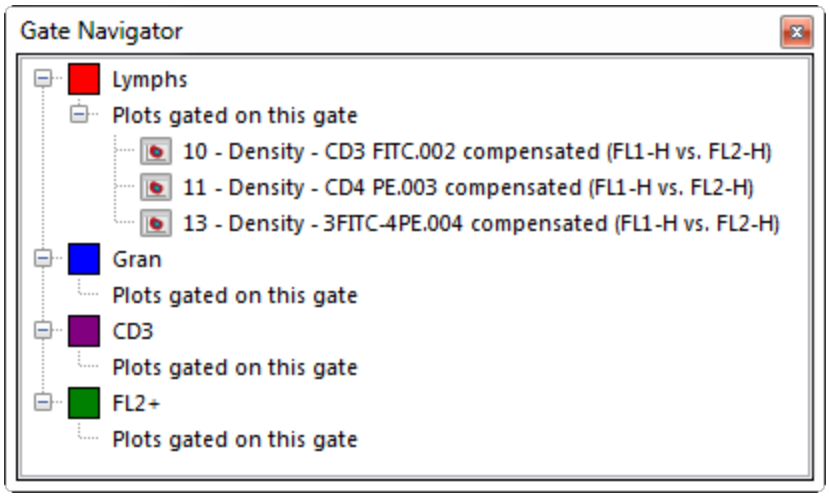

Figure T13.5 Gate Navigator with Listings Expanded

We will now dock the **Gate Navigator** to the left side of the layout. For more information on docking, please refer to the Docking tutorial 1537.

6. Place the mouse on the top border of the **Gate Navigator** window.

7. Press and hold the left mouse button and move the **Gate Navigator** window.

As soon as you start moving the **Gate Navigator** window the four-headed arrows of the docking icon appears in the center of the layout, as shown in Figure T13.6.

8. Continue to hold the left mouse button and move the **Gate Navigator** toward the left arrow of the docking icon.

9. Release the mouse button when the cursor is over the left arrow of the docking icon, indicated by the cursor in Figure T13.6. You will know you are in the correct place because the blue rectangle, indicating the proposed docking location, will appear on the left side of the layout.

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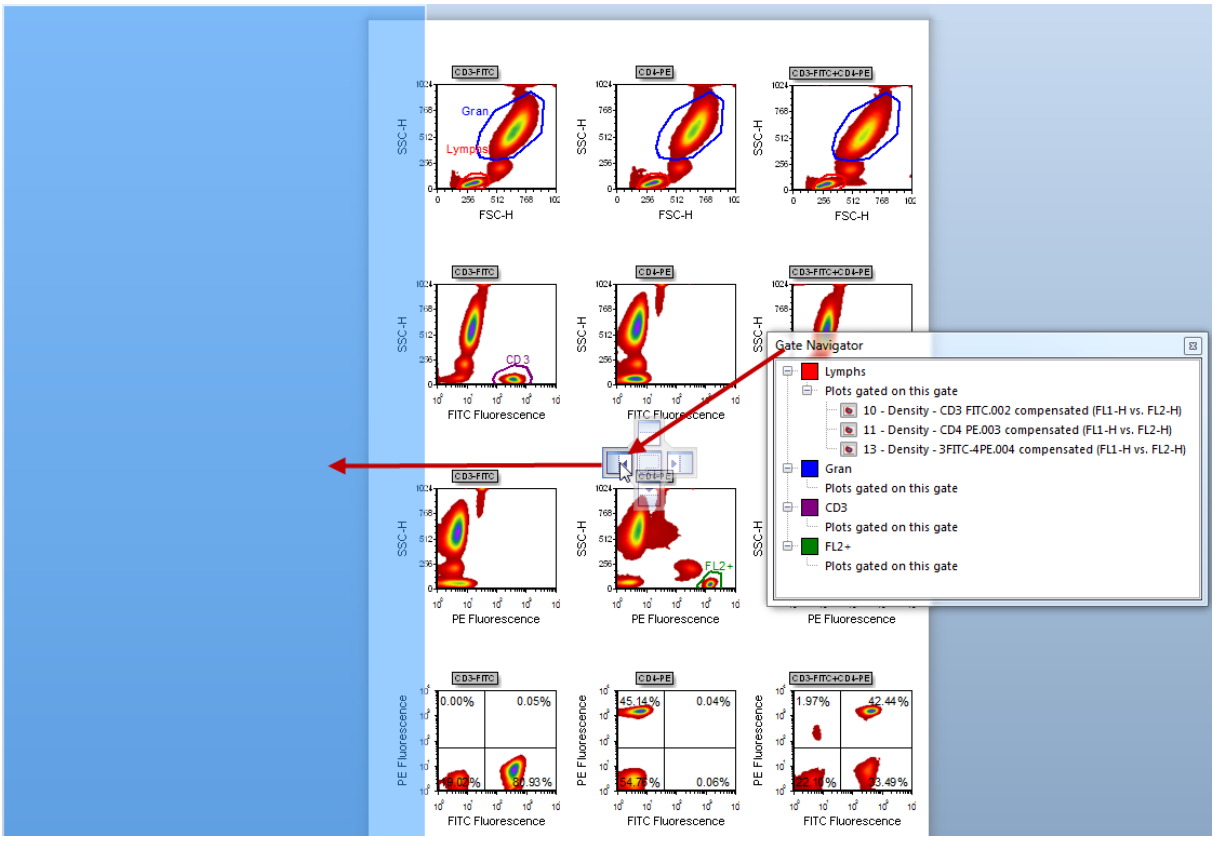

Figure T13.6 Docking the Gate Navigator to the Left Side of the Layout

The **Gate Navigator** is now docked on the left side of the layout. However, not all the text is visible, so we will now resize the docked window it to make it wider.

10. Place the mouse over the outer right border of the docked **Gate Navigator**.

11. Move the mouse slightly over the border until the resizing icon appears, indicated by the red circle in Figure T13.7.

12. Press and hold down the left mouse button and continue to hold it while dragging the border to the right.

13. Release the mouse button when the docked window is large enough to show all the text of the **Gate Navigator**, as shown in Figure T13.7.

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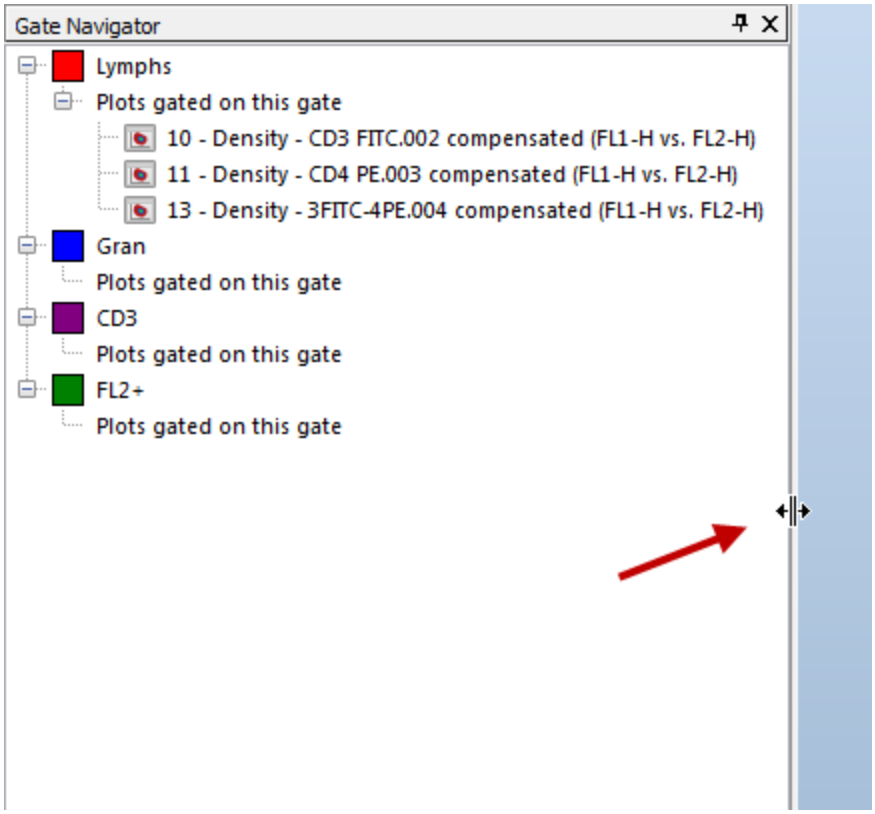

Figure T13.7 Resizing the Gate Navigator

In the next section, we will explore the features of the Gate Navigator 1588.

38.14.2 Gate Navigator Features

As shown in Figure T13.7 1582, the **Gate Navigator** is organized by the gates used in the layout. Under each gate, is the list of the plots gated on this gate. As you move the cursor over the plots listed in the **Gate Navigator**, thumbprint images of the plots appear. This is a helpful quick reminder of which plot is gated on a gate, and is particularly useful if that plot is not currently visible.

We will now explore the features of the **Gate Navigator**, beginning with applying a gate to a plot on the layout using the **Gate Navigator**. Specifically, we will apply the **CD3** gate and then the **FL2+** gate to 2D plots on the layout.

1. Place the cursor over the **CD3** gate in the **Gate Navigator**, highlighted in blue in Figure T13.8.
2. Press and hold the left mouse button.
3. Drag the **CD3** gate onto the layout.

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4. Release the mouse button when the **CD3** gate is on top of the 2D density plot of CD3-FITC (FITC fluorescence vs. SSC-H) in the second row of the left column of the layout, outlined in blue and indicated by the cursor in Figure T13.8.

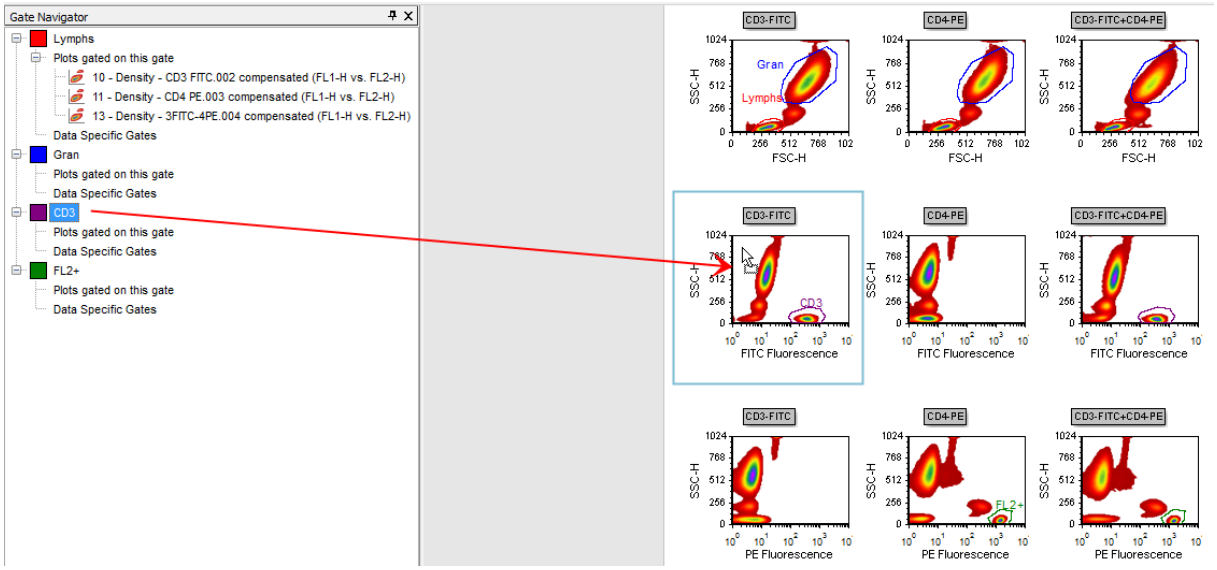

Figure T13.8 Dragging and Dropping the CD3 Gate from the Gate Navigator onto a Density Plot on the Layout

The 2D density plot of CD3-FITC (FITC vs. SSC-H) will now update to reflect that is gated on the **CD3** gate, as shown in Figure T13.9. The **Gate Navigator** also updates, and under the **CD3** gate is a new listing for the 2D density plot CD3-FITC (FL1-H vs. SSC-H), indicated by the cursor and the thumbnail image in Figure T13.9.

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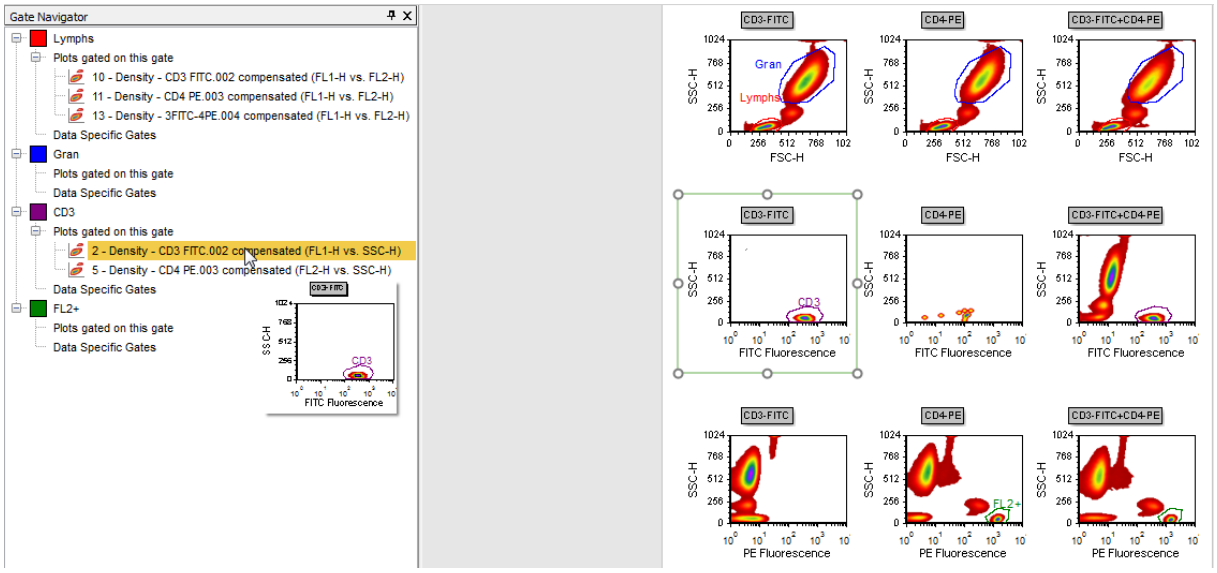

Figure T13.9 Application of the CD3 Gate to the 2D Density Plot of CD3-FITC (FL1-H vs. SSC-H)

We will now use drag and drop to apply the **CD3** gate from the **Gate Navigator** to the other FITC Fluorescence vs. SSC-H 2D plots on the layout.

5. Repeat steps 1 through 3.

6. Release the left mouse button when the **CD3** gate is on top of the 2D density plot of CD4-PE (FITC fluorescence vs. SSC-H), located in the second row of the middle column of the layout.

Both the 2D plot and the **Gate Navigator** update to reflect the application of the **CD3** gate to the CD4-PE (FITC fluorescence vs. SSC-H) plot, as shown in Figure T13.10.

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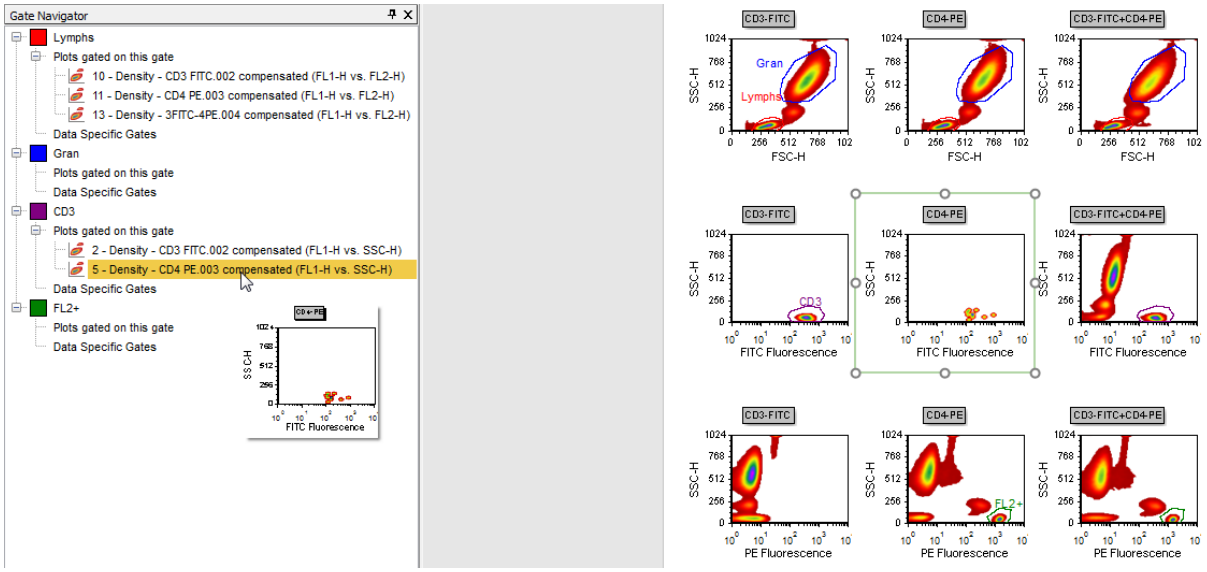

Figure T13.10 Application of the CD3 Gate to the 2D Density Plot of CD4-PE (FL1-H vs. SSC-H)

7. Repeat steps 1 through 3.

8. Release the left mouse button when the **CD3** gate is on top of the 2D density plot of CD3-FITC+CD4-PE (FITC fluorescence vs. SSC-H), located in the second row of the last column of the layout.

Both the 2D plot and the **Gate Navigator** update to reflect the application of the **CD3** gate to the CD3-FITC+CD4-PE (FITC fluorescence vs. SSC-H) plot, as shown in Figure T13.11.

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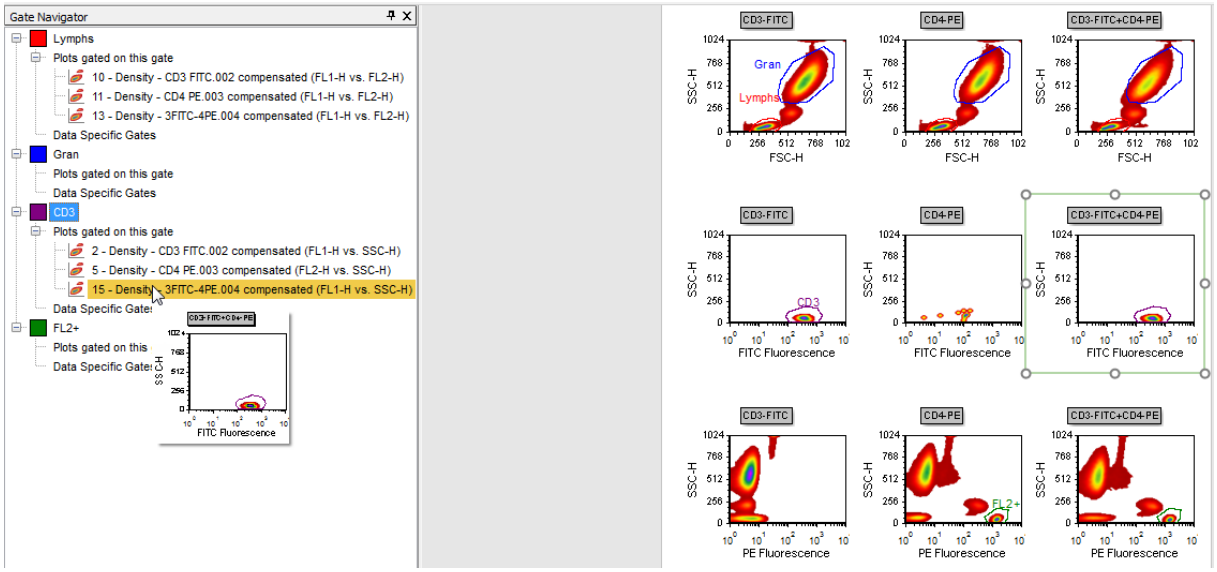

Figure T13.11 Application of the CD3 Gate to the 2D Density Plot of CD3-FITC+CD4-PE (FL1-H vs. SSC-H)

We will now apply the **FL2+** gate to the 2D plots of PE Fluorescence vs. SSC-H in the third row of the layout.

9. Place the cursor over the **FL2+** gate in the **Gate Navigator**.

10. Press and hold the left mouse button.

11. Drag the **FL2+** gate onto the layout.

12. Release the mouse button when the **FL2+** gate is on top of the 2D density plot of CD3-FITC (PE fluorescence vs. SSC-H), located in the third row of the first column of the layout.

Both the 2D plot and the **Gate Navigator** update to reflect the application of the **FL2+** gate to the CD3-FITC (PE Fluorescence vs. SSC-H) plot. We will now apply the **FL2+** gate to the other 2D plots of PE Fluorescence vs. SSC-H).

13. Repeat steps 9 through 11.

14. Release the mouse button when the **FL2+** gate is on top of the 2D density plot of CD4-PE (PE fluorescence vs. SSC-H), located in the third row of the middle column of the layout.

15. Repeat steps 9 through 11.

16. Release the mouse button when the **FL2+** gate is on top of the 2D density plot of CD3-FITC+CD4-PE (PE fluorescence vs. SSC-H), located in the third row of the right column of the layout.

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The 2D plots on the layout and **Gate Navigator** should now look similar to Figure T13.12.

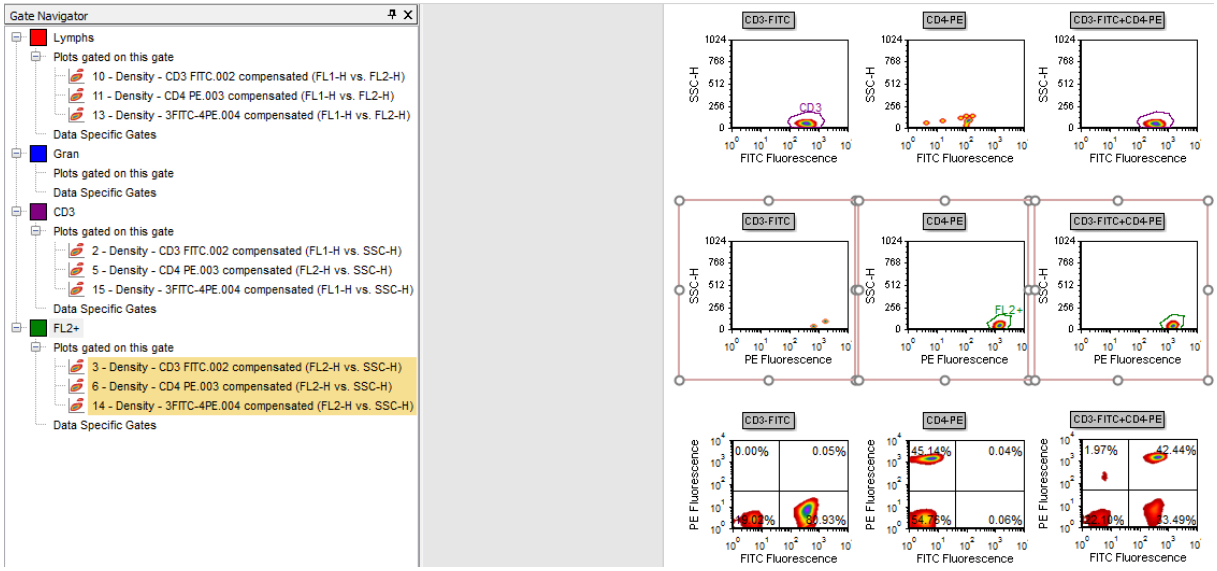

Figure T13.12 2D Plots and Gate Navigator Updated to Reflect the Application of Gates

Now that we have lots of 2D plots using gates, we can drag and drop gates on the **Gate Navigator** itself to change how plots are gated. We will now apply the **Lymphs** gate to a 2D plot that is currently being gated on the **CD3** gate using only the Gate Navigator.

17. Place the cursor on the **Lymphs** gate, shown highlighted in blue in Figure T13.13.

18. Press and hold the left mouse button.

19. Drag the **Lymphs** gate down the **Gate Navigator**.

20. Release the mouse button when the **Lymphs** gate is on top of the listing for **2 - Density – CD3 FITC.002 compensated (FL1-H vs. SSC-H)**, indicated by the cursor and highlighted in yellow in Figure T13.13.

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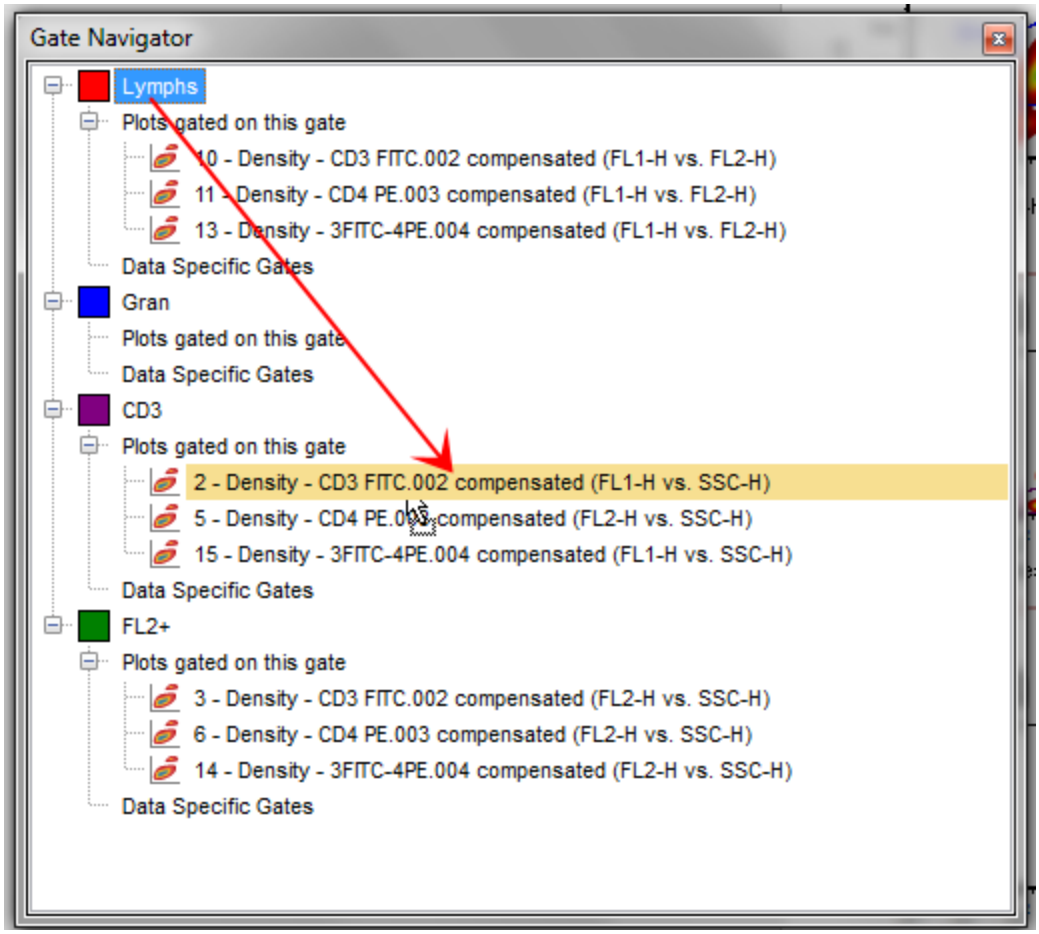

Figure T13.13 Dragging and Dropping the Lymphs Gate on the Gate Navigator

The **Lymphs** gate has now been applied to the 2D plot of the CD3-FITC.002 (FL1-H vs. SSC-H) by simply dragging and dropping the gate on the **Gate Navigator**. The updated 2D plot is shown highlighted in green, and the updated **Gate Navigator** listing of the plot is indicated by the cursor in Figure T13.14.

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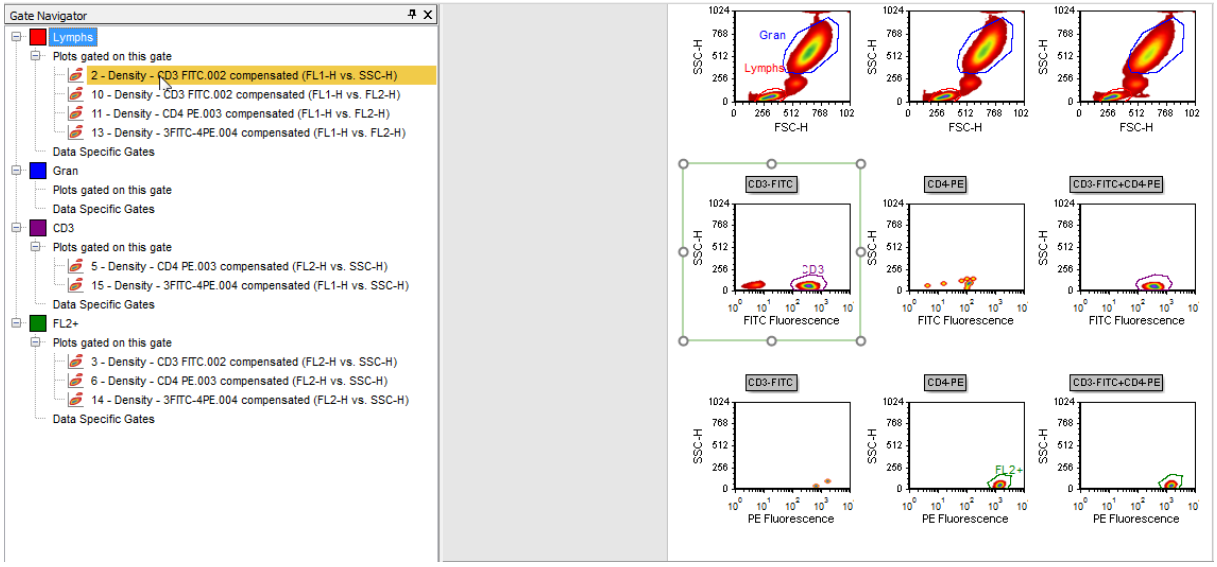

Figure T13.14 Updated Gate Navigator and Layout Reflect the Application of the Lymphs Gate

Instead of dragging and dropping the **Lymphs** gate onto the plot, we could have used the associated pop-up menu.

We will now use the **Gate Navigator** to move a plot onto a new page in the layout. First, we will insert a new page on the layout.

21. Click on the insert new Page icon, located on the bottom border of the layout, indicated by the cursor in Figure T13.15.

Figure T13.15 Insert New Page

Alternatively, you can insert a new Page using the Insert command.

- Select the **Insert→General→New Page** command, indicated by the cursor in Figure T13.16.

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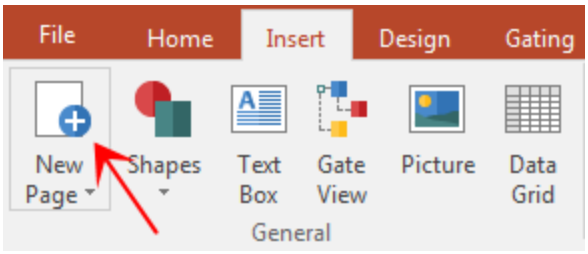

Figure T13.16 Selecting the Insert New Page Command

A new blank page appears on the layout.

22. Place the cursor over the listing for 2D density plot **CD3FITC.002 (FL1-H vs. FL2-H)** listed under the **Lymphs** gate on the **Data Navigator**, shown highlighted in blue in Figure T13.17.

23. Press and hold the left mouse button.

24. Continue to hold down the mouse button and drag the plot listing from the **Gate Navigator** onto page 2 of the layout.

25. Release the mouse button when the plot listing is on page 2, indicated by the cursor in Figure T13.17.

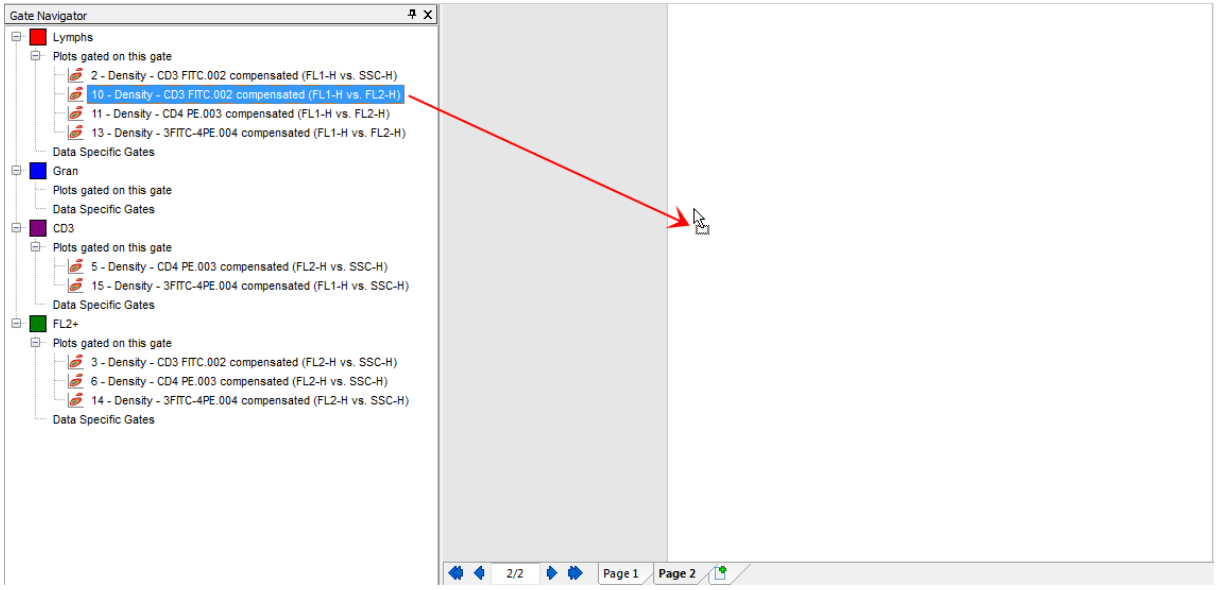

Figure T13.17 Dragging and Dropping a 2D Density Plot Listing onto Page 2 of the Layout

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A new density plot, **19 - CD3FITC.002 compensated (FL1-H vs. FL2-H)**, appears under the **Lymphs** gate and on the new page of the layout, similar to Figure T13.18.

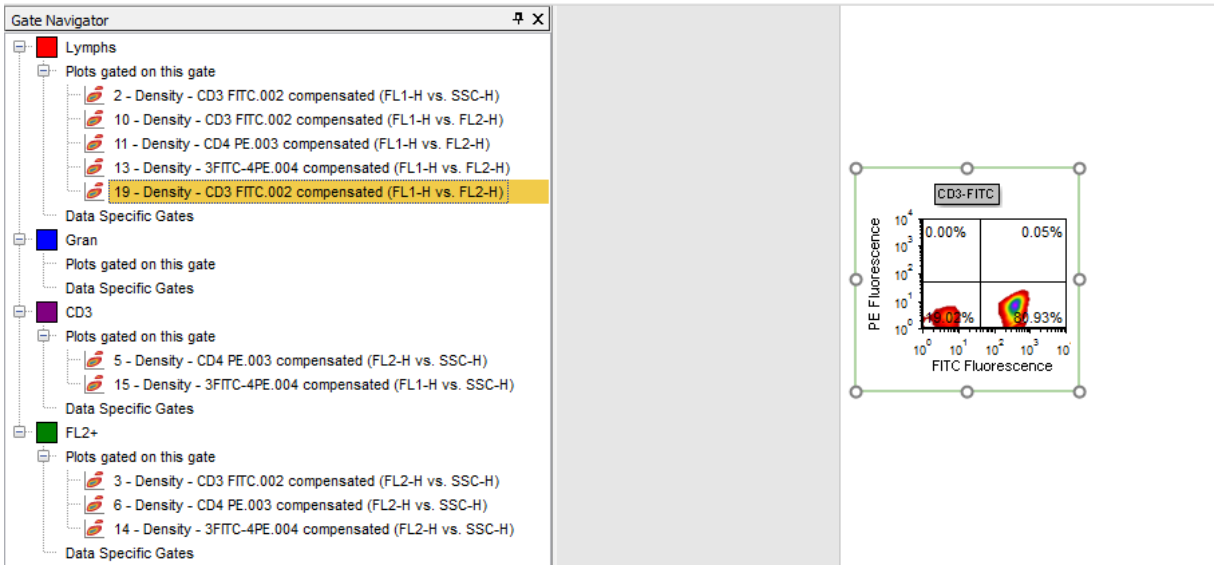

Figure T13.18 2D Plot on Page 2 of the Layout

38.15 Layout Navigator

The **Layout Navigator** is designed to give you a useful overview of your entire layout. It shows you a hierarchical view of all the pages in your layout, along with which plots and data files are being displayed on each page. The **Layout Navigator** also allows you to manipulate the plots and their associated gates and data files within the layout. For example, plots can be moved from one page to another by simply dragging and dropping the plot icons on the **Layout Navigator**.

In the course of this example, we will:

- Open and dock the **Layout Navigator**.
- Learn about the **Layout Navigator** features and how to use them. Specifically, we will use the **Layout Navigator** to:
  - Apply a gate to a plot.
  - Add an overlay to a histogram.
  - Insert a new plot.

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- Move a plot from one page to another.

In the first exercise, we will open and dock the Layout Navigator 1598.

38.15.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on multiple pages.

1. Select **File** tab→**Open**.
2. Open the layout **LayoutNavigator.fey** found in the Tutorial Sample Data archive 1210.

The layout appears. However, part of the layout is not visible, so we will now use zoom to view the whole layout page on the screen.

3. Click on the **Zoom** icon located in the Zoom Toolbar at the lower left corner of the screen (indicated by the cursor in Figure T14.1).

Figure T14.1 Zoom Icon

The **Zoom** dialog appears, as shown in Figure T14.2. Please note that the **Percent** number in the field at the bottom of the dialog window will be dependent on your screen and screen resolution.

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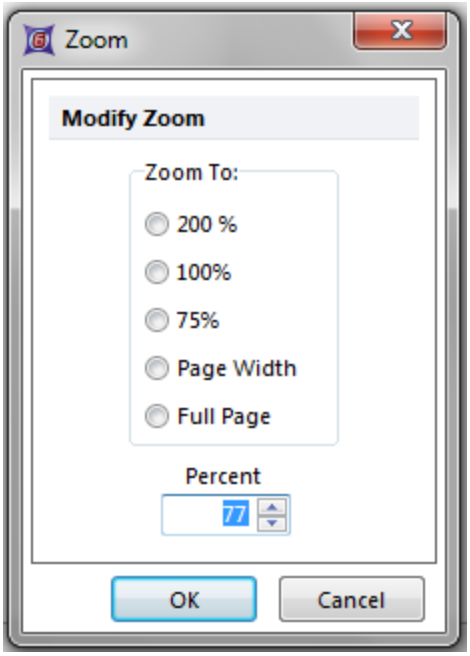

Figure T14.2 Zoom Dialog

4. Select **Full Page**.
5. Click **OK**.

The layout page will update to display the full page of the layout as shown in Figure T14.3. Notice the tabs at the bottom of the screen (Figure T14.1) indicating there are two pages in the layout.

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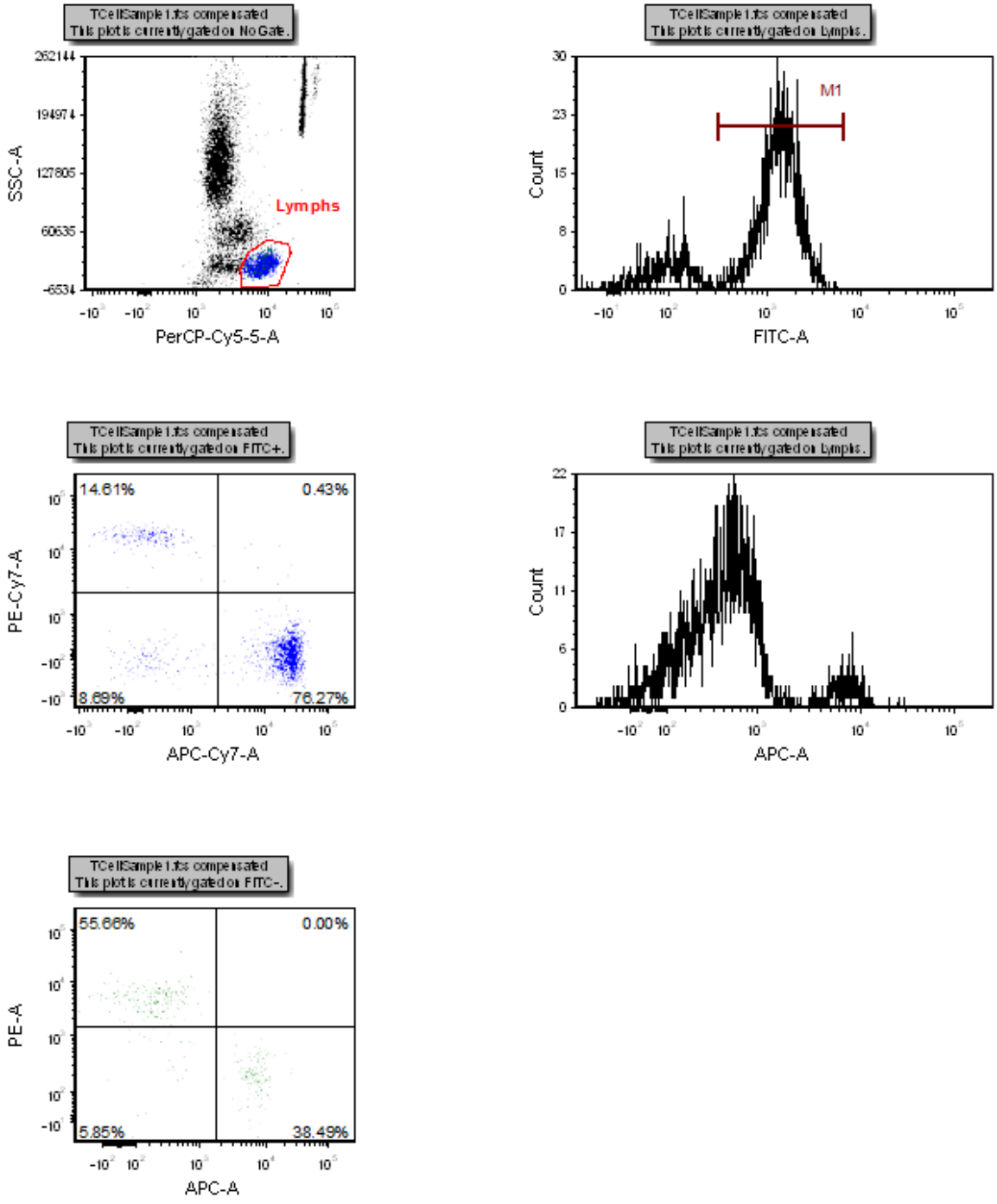

Figure T14.3 Layout Navigator - Page 1 Plots

We will now open the **Layout Navigator.**

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6. Select the **View→Navigators→Layout Navigator** command (Figure T14.4).

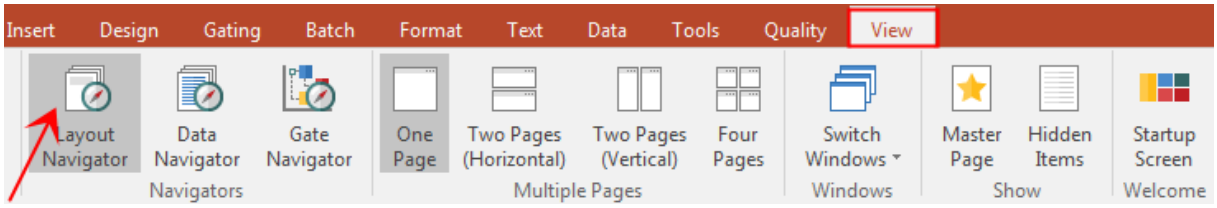

Figure T14.4 Selecting the Layout Navigator Command

The **Layout Navigator** appears; we will now dock it to the right side of the layout. For more information on docking please refer to the Docking tutorial 1537.

7. Place the mouse on the top border of the **Layout Navigator** window.

8. Press and hold the left mouse button and move the **Layout Navigator** window.

As soon as you start moving the **Layout Navigator** window the four-headed arrows of the docking icon appears in the center of the layout, as shown in Figure T14.5.

9. Continue to hold the mouse button and move the **Layout Navigator** toward the right arrow of the docking icon.

10. Release the mouse button when the cursor is over the right arrow of the docking icon, indicated by the cursor in Figure T14.5. You will know you are in the right place because the blue rectangle, indicating the proposed docking location, will appear on the right side of the layout.

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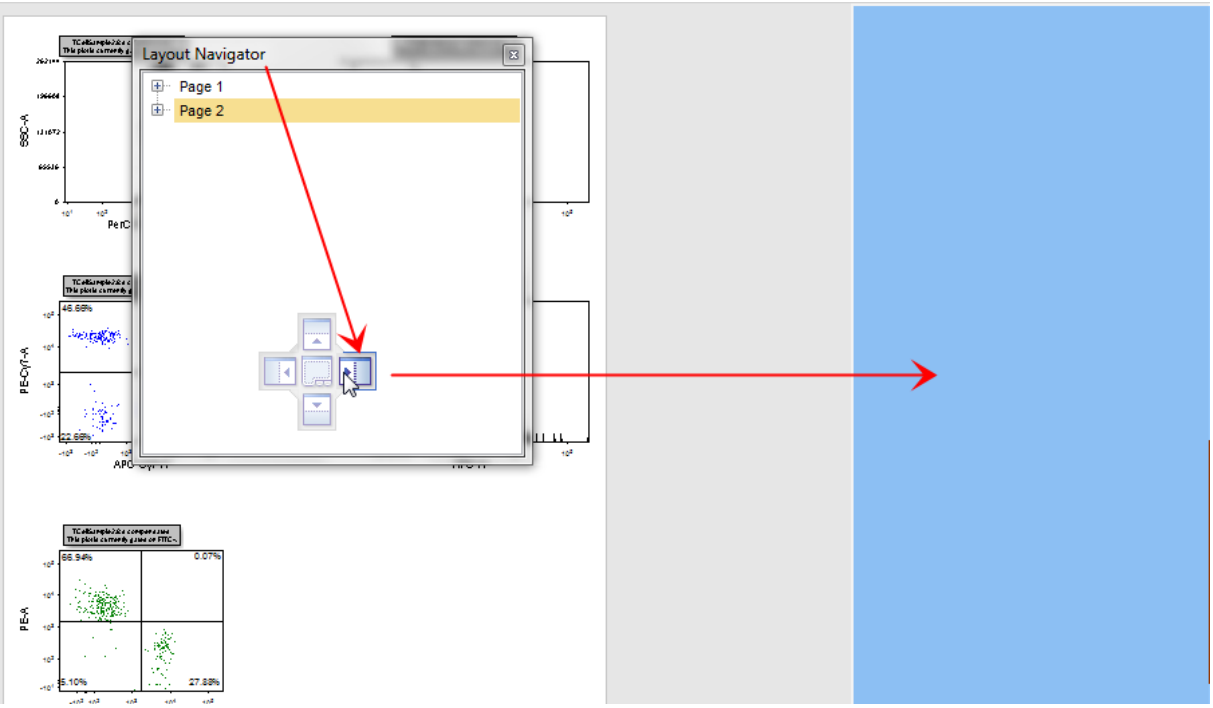

Figure T14.5 Docking the Layout Navigator

The **Layout Navigator** is now docked on the right side of the layout. However, not all the text is visible so we will now resize the docked window it to make it wider.

11. Right click within the Layout Navigator and select **Expand All**.
12. Place the mouse over the outer left border of the docked **Layout Navigator**.
13. Move the mouse slightly over the border until the resizing icon appears.
14. Press and hold down the left mouse button and continue to hold it while dragging the border to the left.
15. Release the mouse button when the docked window is large enough to show all the text of the **Layout Navigator**, as shown in Figure T14.6.
16. If necessary, use the scroll bar on the right side of the docked window to scroll up and down the **Layout Navigator**.

The docked **Layout Navigator** should appear similar to Figure T14.6.

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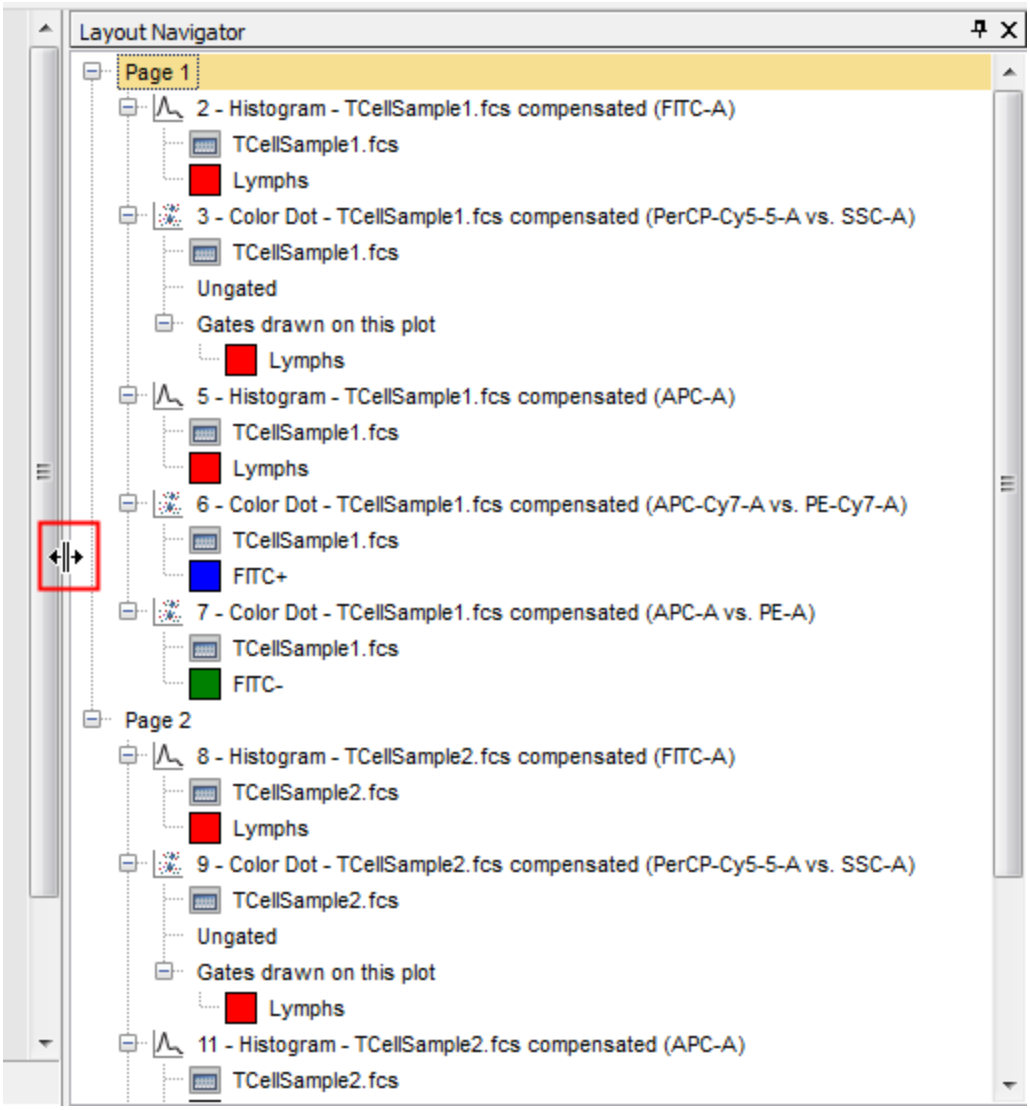

Figure T14.6 Docked Layout Navigator

In the next section, we will learn the features of the Layout Navigator.

38.15.2 Layout Navigator Features

The **Layout Navigator** is organized by the Pages of the layout. Notice that the current page, Page 1, is highlighted, as shown in Figure T14.7. Under the Page all the plots on that page are listed. Under each plot the filename, gating, and other information is listed. We will now explore the many features of the **Layout Navigator**.

1. Hover the mouse over the **Page 2** listing on the **Layout Navigator**, as indicated by the cursor in Figure T14.7.

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A thumbnail image of the actual layout page will appear, as shown in Figure T14.7. This is a helpful, quick reminder of the layout of plots on a page that may not be currently visible. This feature also applies to the plot listings in the **Layout Navigator**. If you hover the mouse over any of the plot listings in the **Layout Navigator**, a thumbnail of the plot will appear.

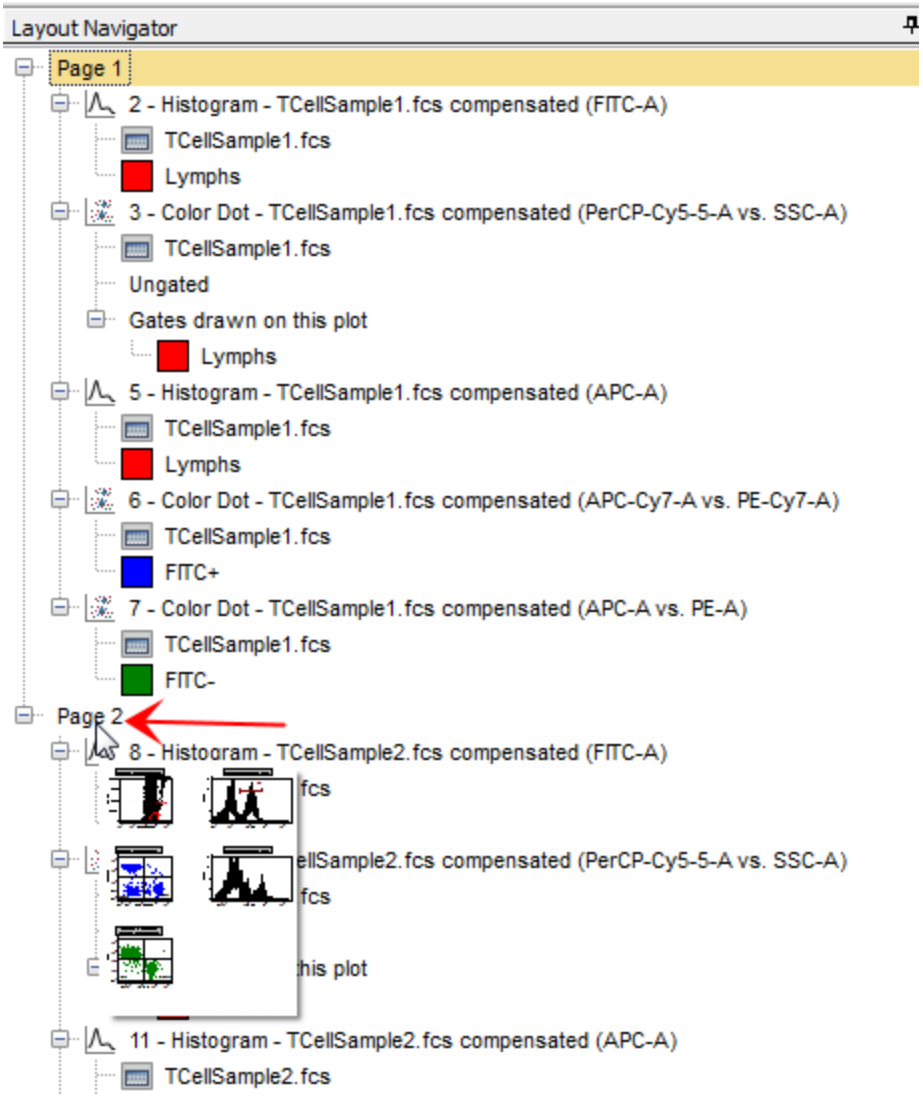

Figure T14.7 Image of Page 2 on the Layout Navigator

2. Click on **Page 2** in the **Layout Navigator**.

Page 2 of the layout now appears with five plots, as shown in Figure T14.8. This demonstrates another way of changing pages within the layout. This page looks just like the thumbnail that was previously displayed on the **Layout Navigator**,

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shown in Figure T14.7. Also notice that Page 2 is now highlighted on the **Layout Navigator** indicating that this is the currently displayed page.

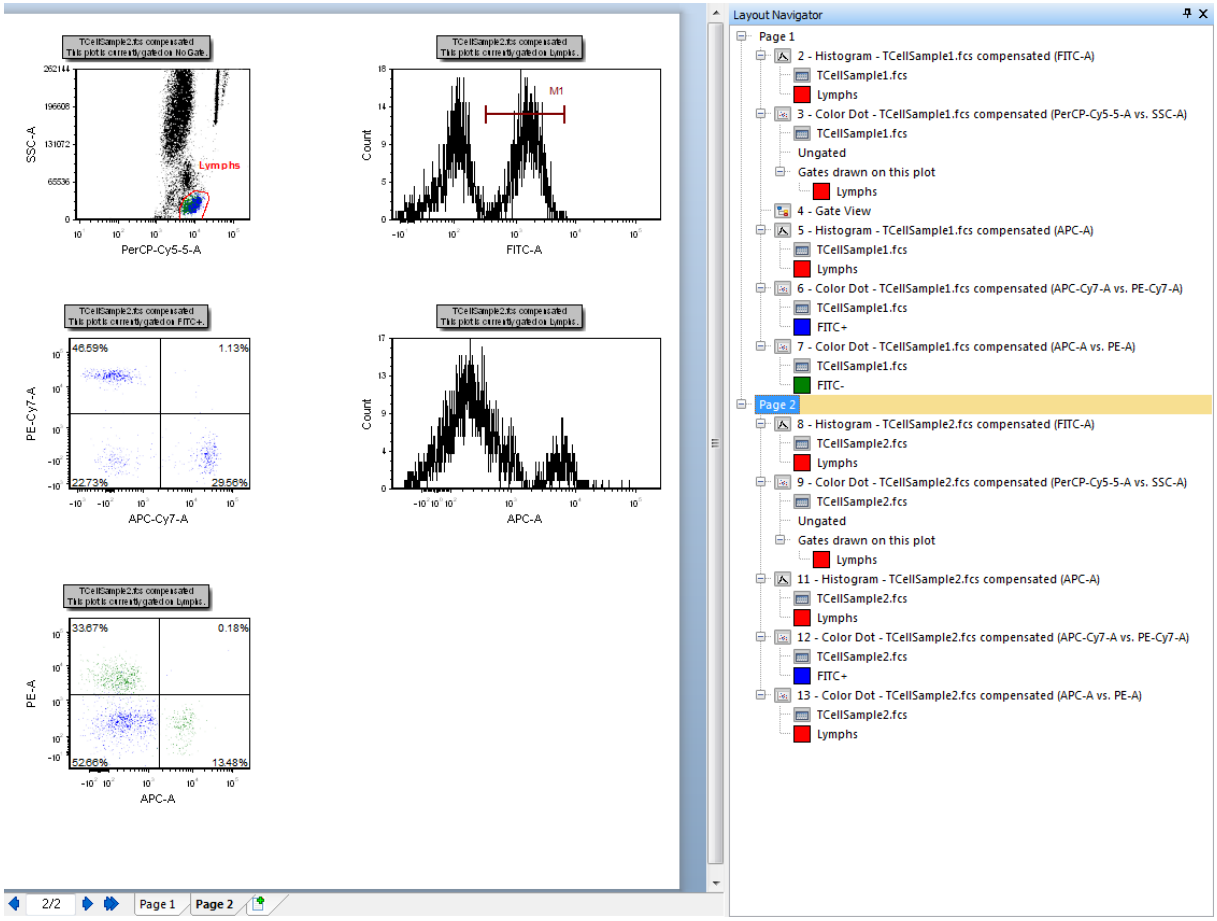

Figure T14.8 Page 2 of the Layout

3. Select the TCellSample2.fcs APC-A vs PE-A color dot plot, located in the lower left of the layout (Figure T14.9).

Notice that the listing for the **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)** is now highlighted in the **Layout Navigator**, as shown in Figure T14.9.

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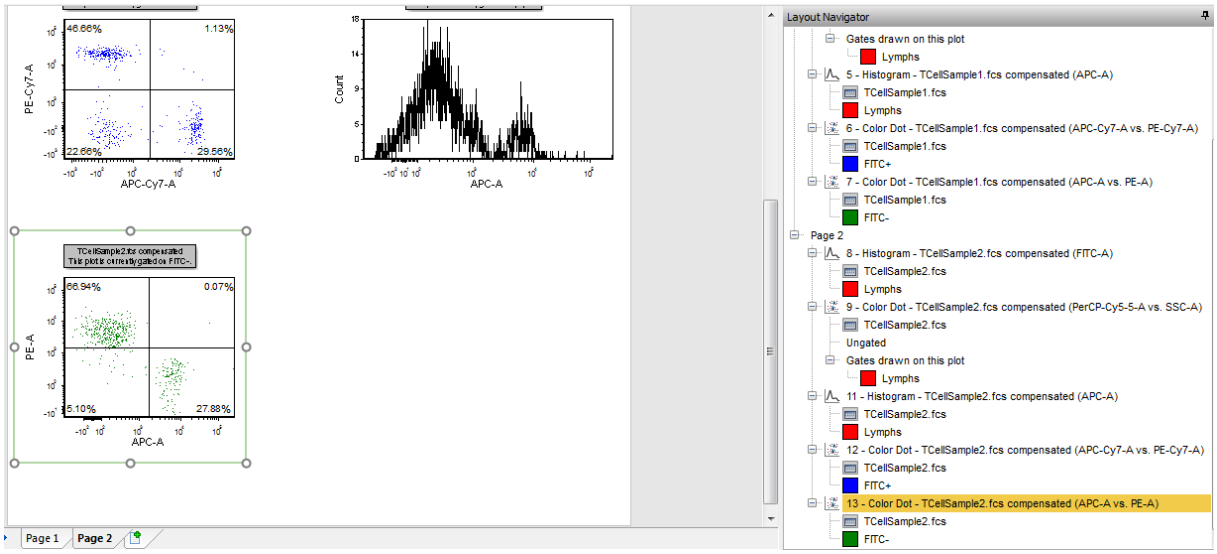

Figure T14.9 TCellSample2.fcs Color Dot plot Selected

This plot is currently gated using the **Lymphs** gate. We will now use the **Layout Navigator** to apply the **FITC-** gate to **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)**.

4. Place the cursor over the **FITC-** gate, listed under **7 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)** on Page 1 of the **Layout Navigator**, highlighted in blue in Figure T14.10.

5. Press and hold the left mouse button.

6. Continue to hold the left mouse button and drag the **FITC-** gate down towards the listing for **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)**, on Page 2 of the **Layout Navigator**.

7. Release the mouse button when the **FITC-** gate is over the **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)** listing, highlighted and indicated by the cursor in Figure T14.10.

8. Press Undo to remove the gate from the histogram.

Alternatively, you can directly drag the gate from the **Layout Navigator** onto the plot on the layout.

A. Click on **Page 2** in the **Layout Navigator** to display that layout page.

B. Place the cursor over the **FITC-** gate, listed under **7 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)** on Page 1 of the **Layout Navigator**, highlighted in blue in Figure T14.10.

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C. Press and hold the left mouse button.

D. Continue to hold the mouse button and drag the **FITC-** gate onto the histogram plot of **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)** that is visible on Page 2.

E. Release the mouse button when the FL+ gate is on the histogram plot of **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)**.

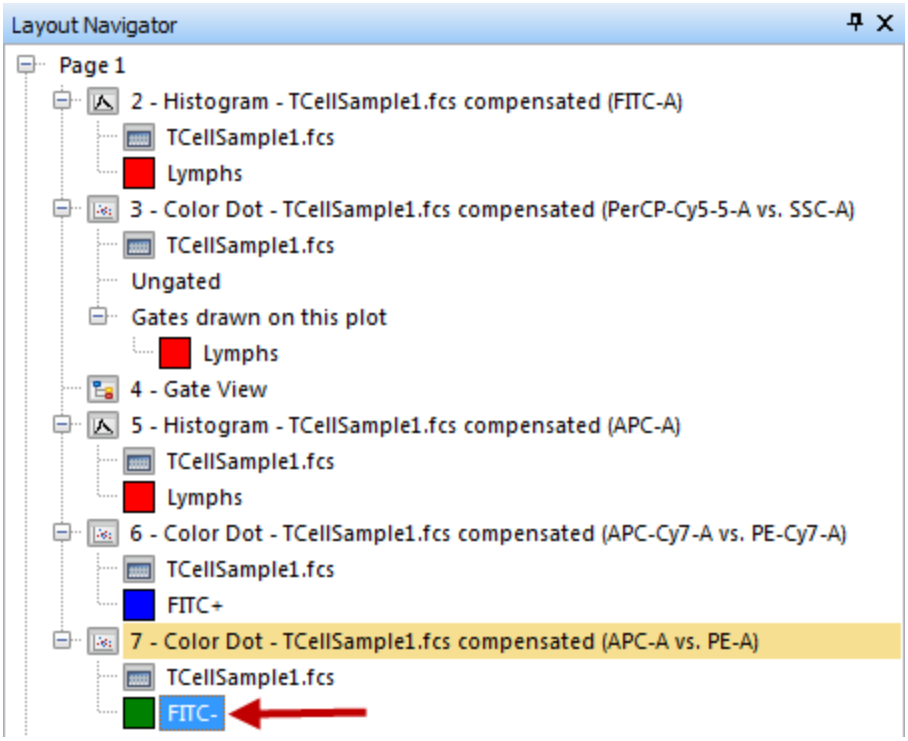

Figure T14.10 Dragging and Dropping the FITC- Gate onto TCellSample1.fcs Color Dot plot with the Layout Navigator

13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A) updates to reflect the application of the **FITC-** gate, as shown in Figure T14.11. The **Layout Navigator** also updates to reflect the **FITC-** gate on the **13 - Color Dot - TCellSample2.fcs compensated (APC-A vs PE-A)**.

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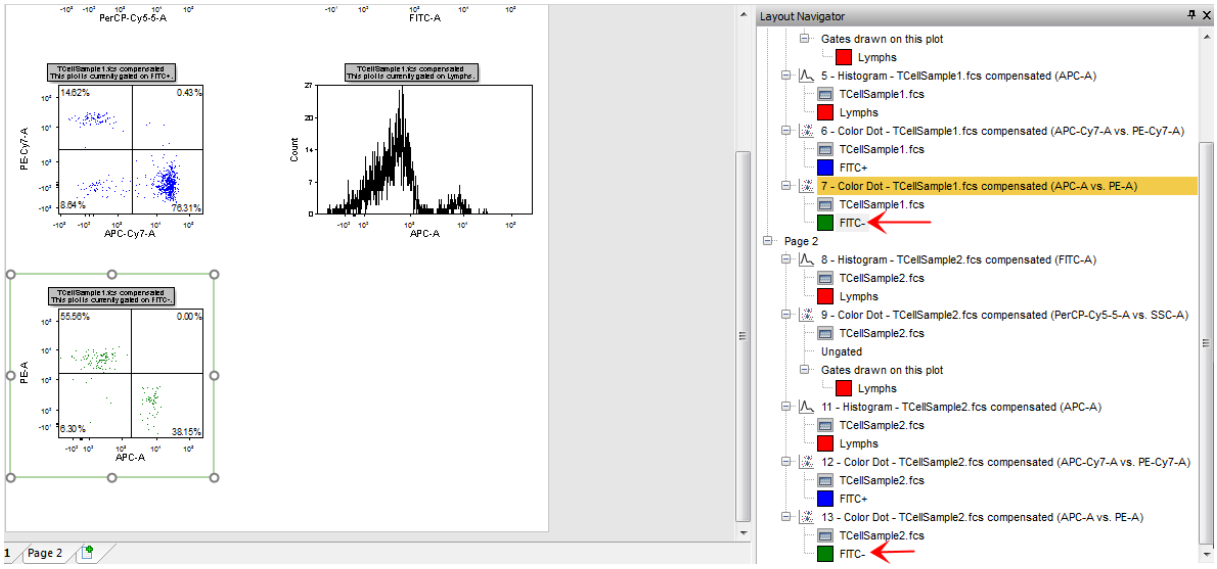

Figure T14.11 TCellSample2.fcs Color Dot plot Histogram with FITC- Gate

A very powerful feature of the **Layout Navigator** is the ability to drag and drop plots, gates, or data within the **Layout Navigator** or directly onto the layout. We have just showed two ways you can apply a gate to a plot using the **Layout Navigator**. Now we will use the **Layout Navigator** to add an overlay to a histogram.

9. Click on **Page 2** in the **Layout Navigator** to display that layout page.

10. Place the cursor over the listing for the **TCellSample1.fcs** data from Page 1 in the **Layout Navigator** (it doesn't matter which plot it's listed under) indicated by the cursor in Figure T14.12.

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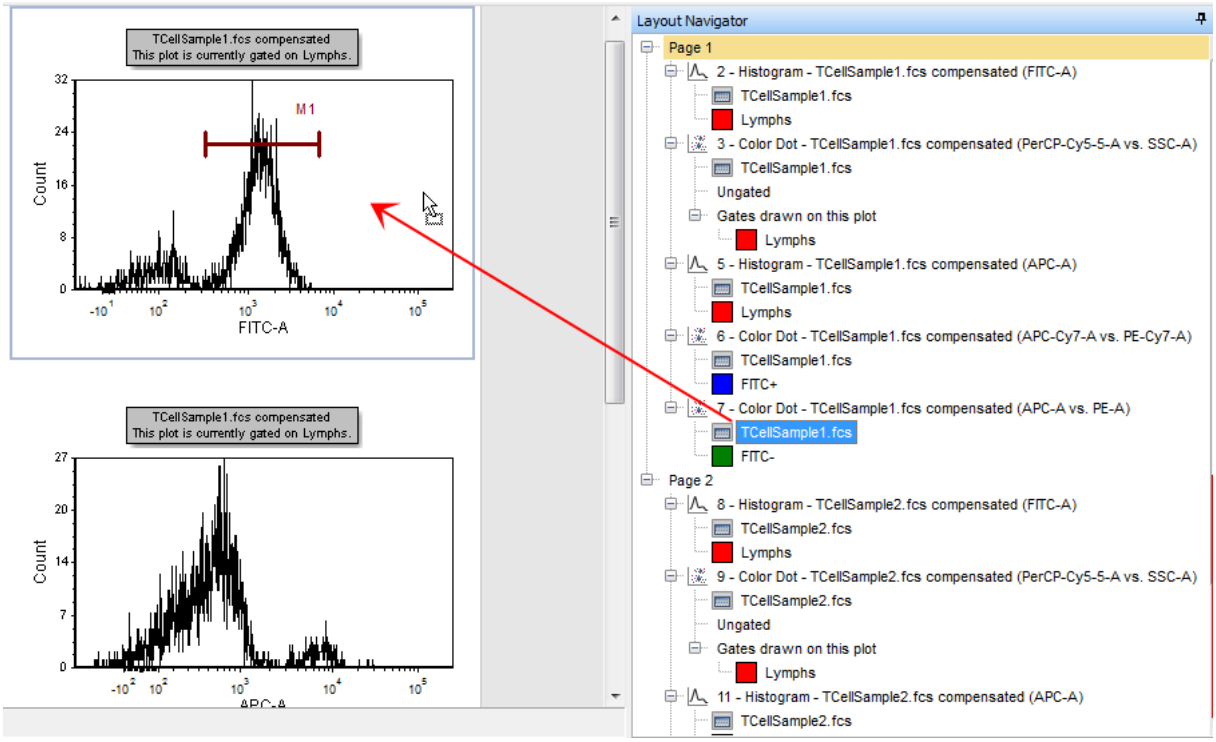

Figure T14.12 TCellSample1 Data Listing in the Layout Navigator

11. Press and hold the left mouse button.
12. Continue to hold down the mouse button and drag the **TCellSample1.fcs** data onto the layout.
13. Release the mouse button when the cursor is over the histogram of **TCellSample2.fcs compensated (FITC-A)** on **Page 2**.

Alternatively, you can drag and drop the data within the **Layout Navigator**.

A. Place the cursor over one of the listings for the **TCellSample1.fcs** data in the **Layout Navigator**, indicated by the cursor in Figure T14.12.

B. Press and hold the left mouse button.

C. Continue to hold down the left mouse button and drag the **TCellSample1.fcs** data down the **Navigator Layout** towards **8 - Histogram - TCellSample2.fcs compensated (FITC-A)**.

D. Release the mouse button when the cursor is over **8 - Histogram - TCellSample2.fcs compensated (FITC-A)**, highlighted and indicated by the cursor in Figure T14.13.

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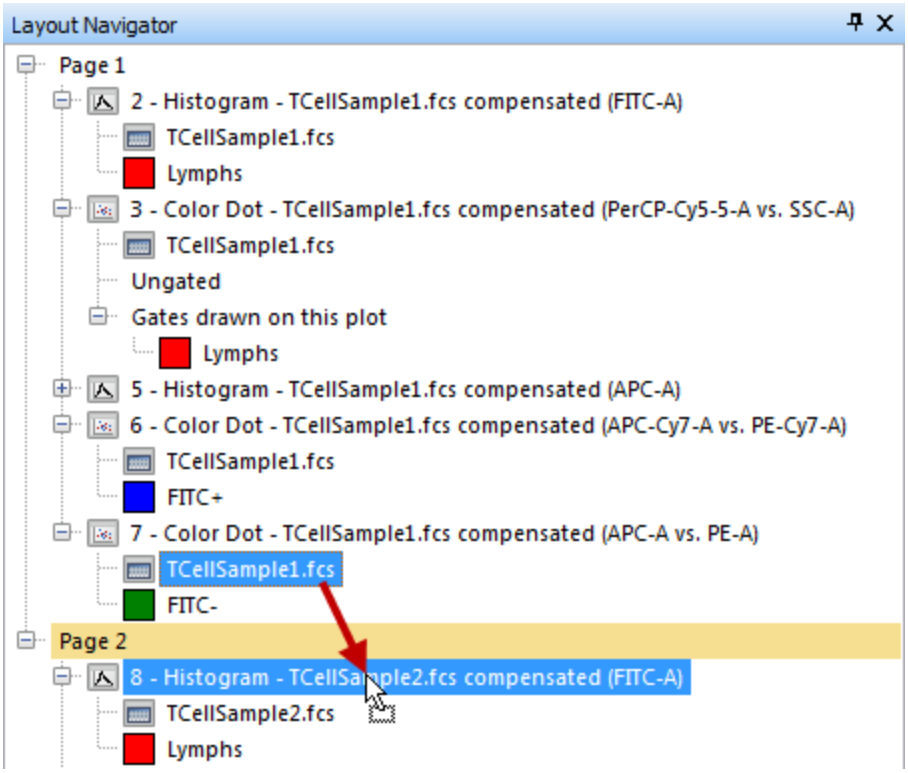

Figure T14.13 Dragging and Dropping TCellSample1.fcs Data within the Layout Navigator

The **Select Drag and Drop Action** dialog appears, as shown in Figure T14.14.

14. Select **add the files to the plot as new overlays**.
15. Click **OK**.

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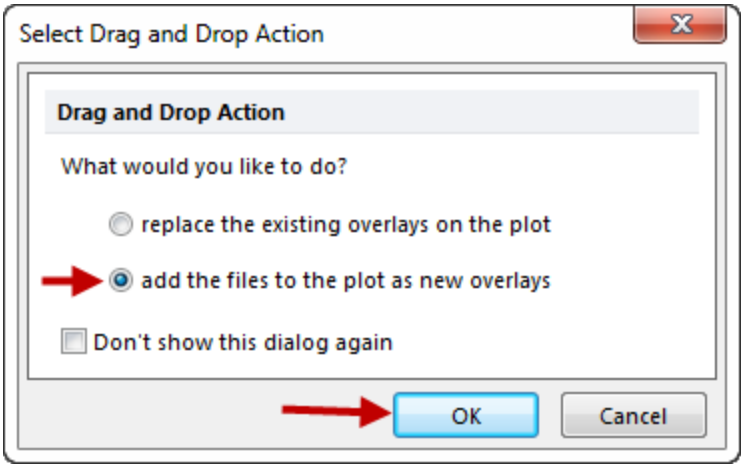

Figure T14.14 Select Drag and Drop Action Dialog

The **8 - Histogram - TCellSample2.fcs compensated (FITC-A)** plot now updates to reflect the overlay of the **TCellSample1.fcs** data as shown in Figure T14.15. The **Layout Navigator** also updates to show that there is now overlay data on **8 - Histogram - TCellSample2.fcs compensated (FITC-A)**, highlighted in Figure T14.15.

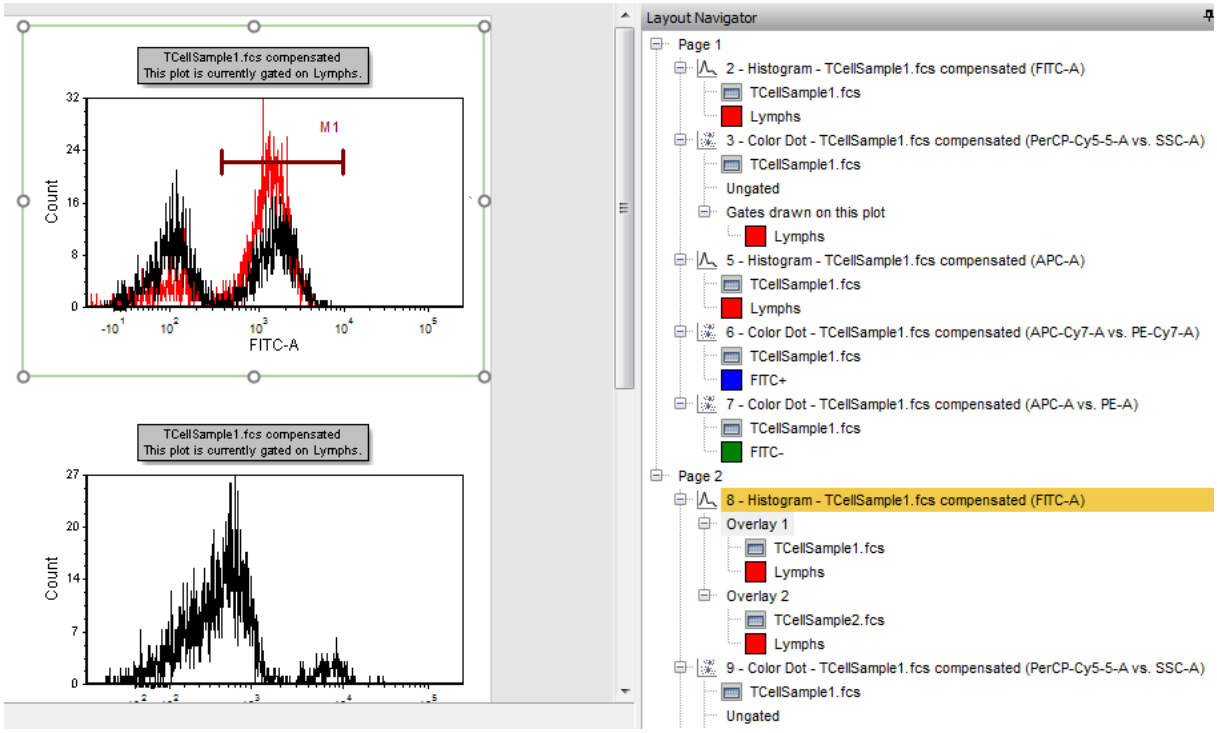

Figure T14.15 Overlay of TCellSample1.fcs and TCellSample2.fcs

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We will now undo the overlay on the histogram.

16. Click on the **Undo** icon on the **Quick Access Toolbar** (Figure T14.16).

Figure T14.16 Undo Drag and Drop

The **8 - Histogram - TCellSample2.fcs compensated (FITC-A)** now updates to reflect the removal of the overlay.

We will now use the **Layout Navigator** to insert a new plot onto page 2 of the layout.

17. Click on **Page 2** in the **Layout Navigator**, if page 2 is not already being displayed.

18. Place the cursor over one of the listings for the **TCellSample2.fcs** data in the **Layout Navigator** (it doesn't matter which plot it's listed under) shown highlighted in blue in Figure T14.17.

19. Press and hold the left mouse button.

20. Continue to hold down the mouse button and drag the **TCellSample2.fcs** data onto page 2 of the layout.

21. Release the mouse button when the cursor is on a blank area of the layout, indicated by the cursor in Figure T14.17.

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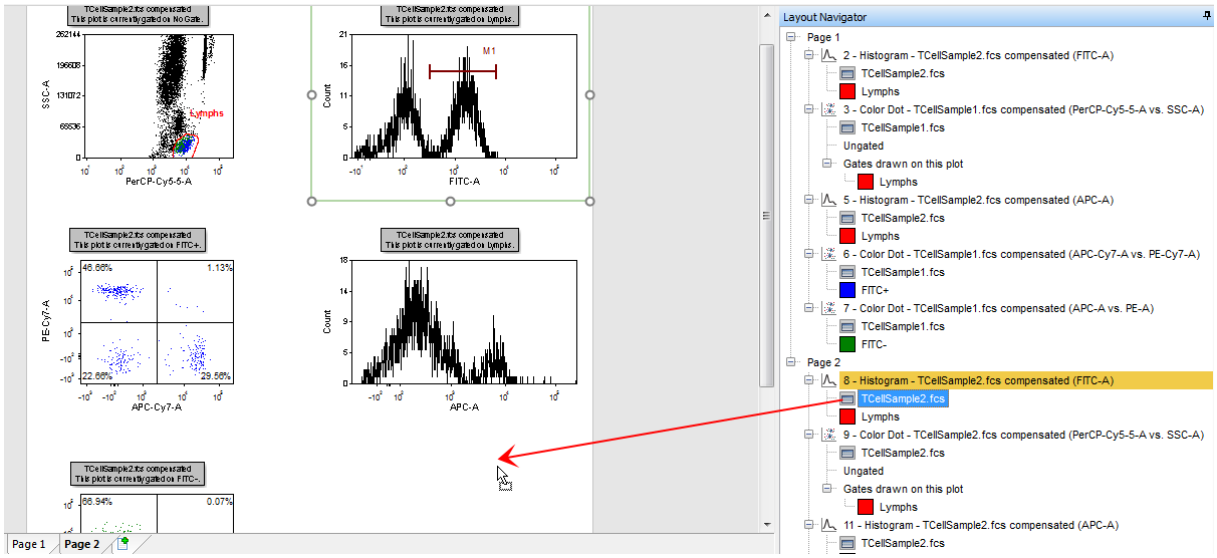

Figure T14.17 Dragging and Dropping TCellSample2.fcs Data onto the Layout

The **Select Plot Types...** dialog appears, as shown in Figure T14.18.

22. Select **Density** as the type of plot to open for the **TCellSample2.fcs** data (Figure T14.18).

23. Click **OK**.

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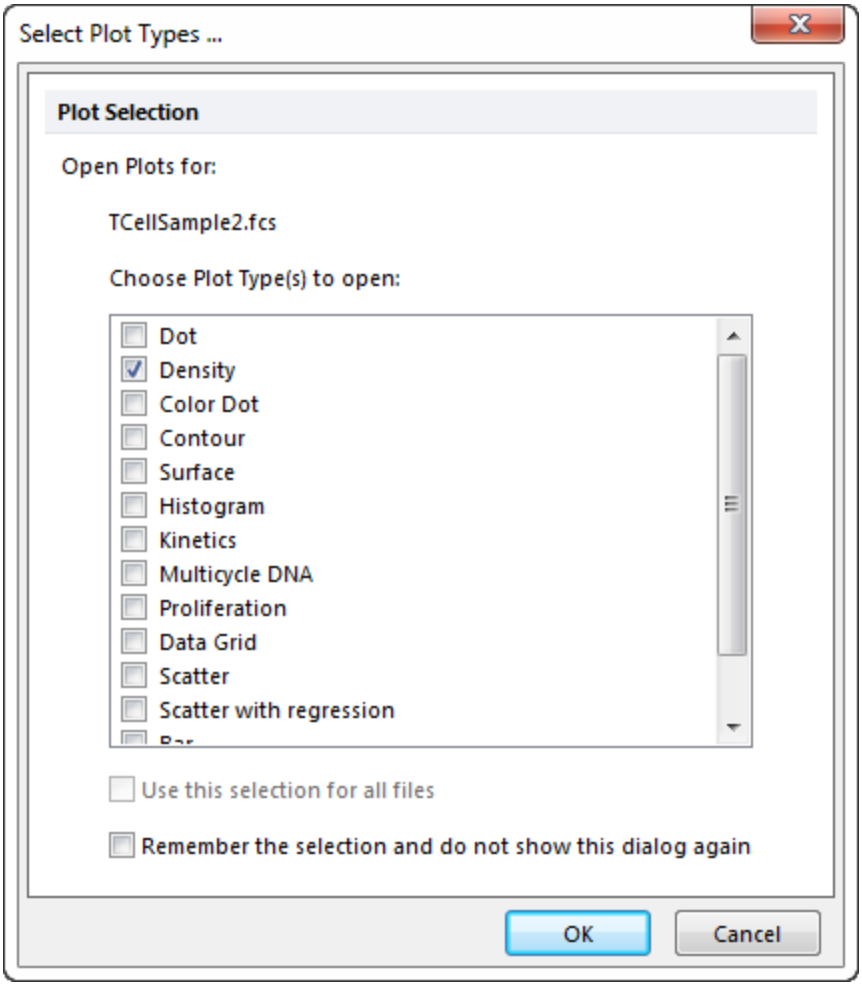

Figure T14.18 Select Plot Types... Dialog

A density plot of the **TCellSample2.fcs** data now appears on the layout, with Time vs. FSC-H as the default parameters. The **Layout Navigator** also updates to reflect the insertion of a 2D plot onto page 2 of the layout, indicated by the cursor in Figure T14.19.

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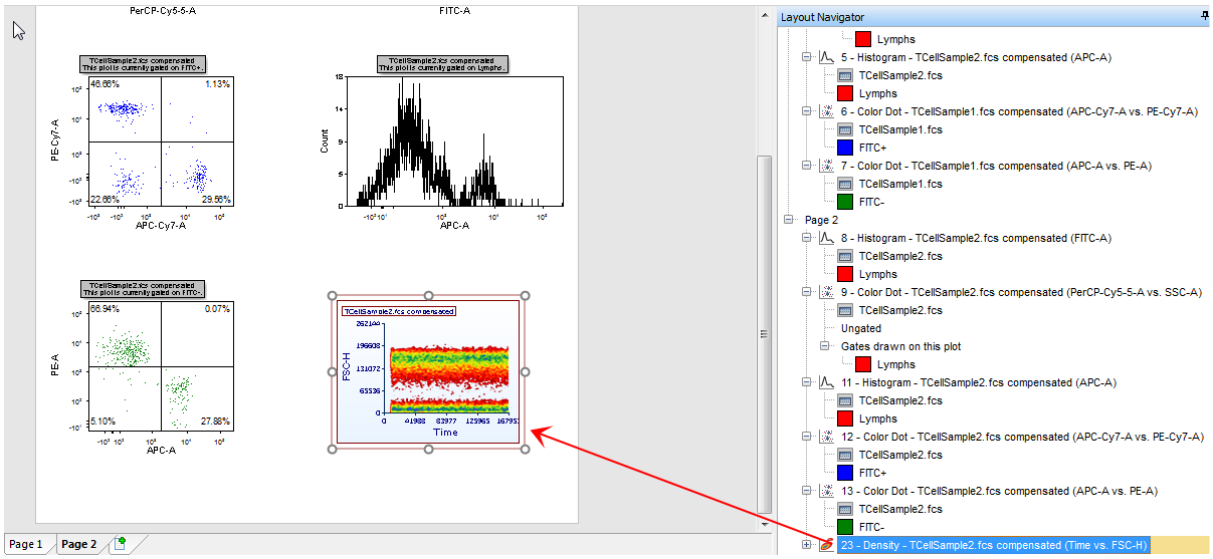

Figure T14.19 Density plot of TCellSample2.fcs with Updated Layout Navigator

We will now undo the insertion of the 2D plot.

24. Click the **Undo** icon in the **Quick Access Toolbar**, as previously shown in Figure T14.16.

We will now use the **Layout Navigator** to move a plot from one page to another.

25. Place the cursor over the listing for **7 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)**, shown highlighted in blue in Figure T14.20.

26. Press and hold the left mouse button.

27. Continue to hold down the mouse button and drag the 2D plot down the **Layout Navigator**.

28. Release the mouse button when the cursor is over **Page 2** on the **Layout Navigator**, highlighted and indicated by the cursor in Figure T14.20.

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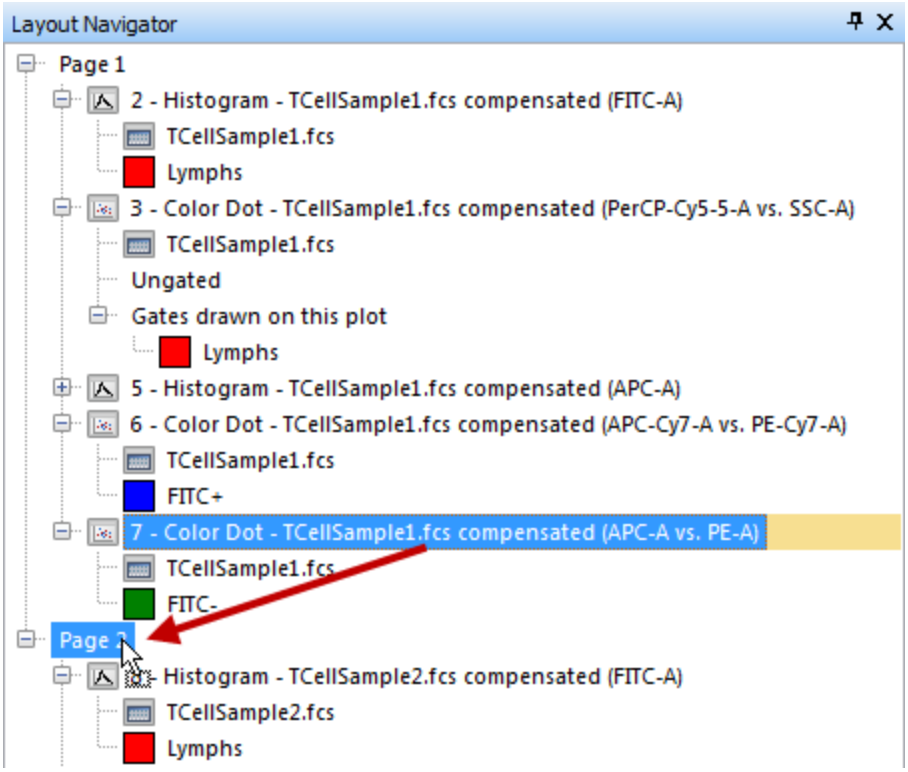

Figure T14.20 Moving a Plot to Page 2 in the Layout Navigator

Both the layout and the **Layout Navigator** update to reflect the new location of the density plot.

29. Click on **Page 1** in the **Layout Navigator**, highlighted in yellow in Figure T14.21.

Page 1 of the layout appears, as shown in Figure T14.21. Notice that **7 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)** is now missing, as it was moved to its same location on page 2 of the layout and renamed **18 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)**.

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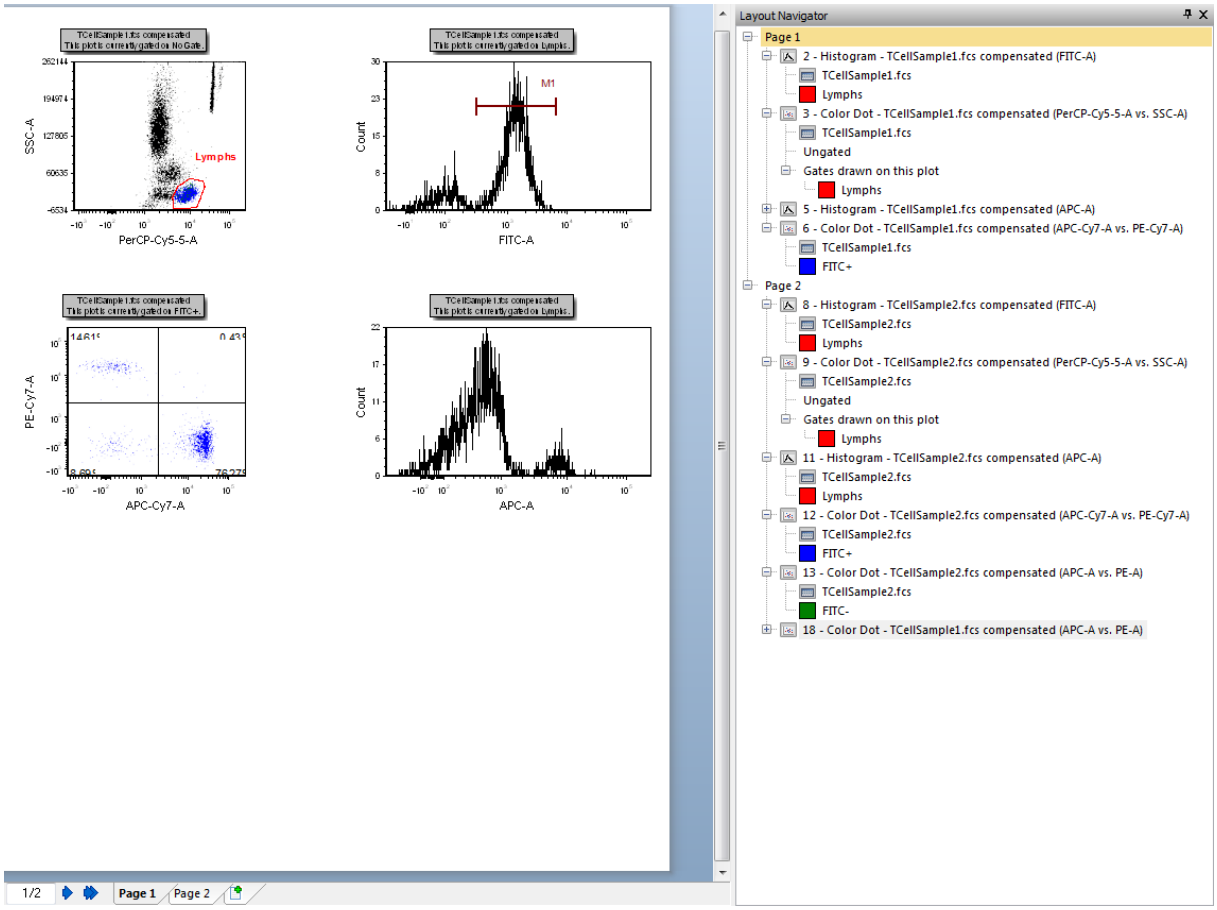

Figure T14.21 Page 1 of the Layout without the TCellSample1 2D Plot

30. Click on **Page 2** in the **Layout Navigator**, highlighted in yellow in Figure T14.22.

Page 2 of the layout appears, as shown in Figure T14.22. Notice that **18 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)** is now added to the bottom left corner of the layout, and the **Layout Navigator** now reflects the addition of this plot to Page 2. You can move the new plot to an open part of the layout.

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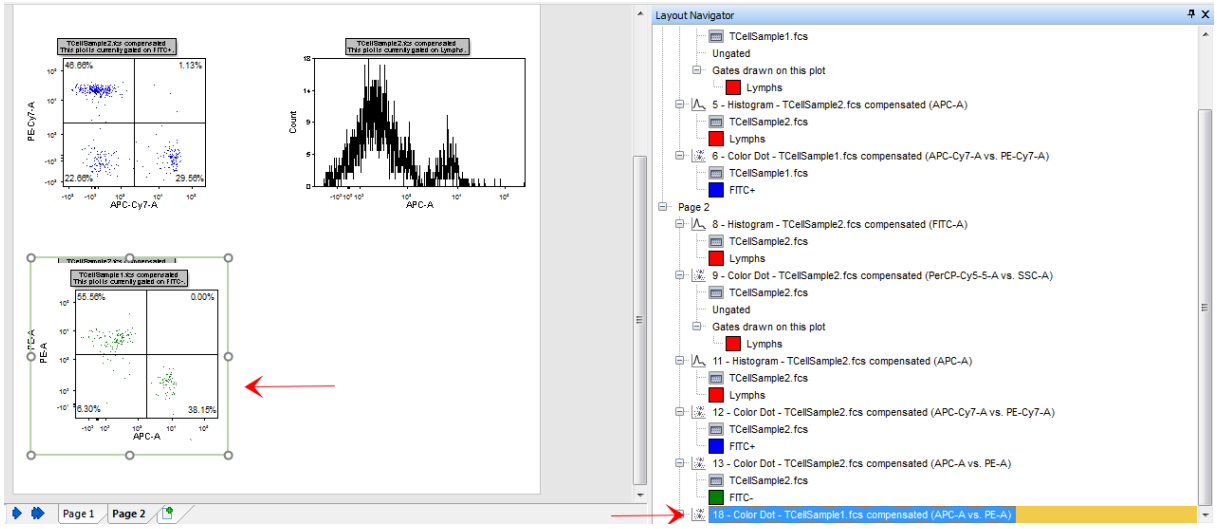

Figure T14.22 Page 2 of the Layout with the Addition of the new 2D Color Dot Plot

Alternatively, the 2D plot could have been moved by dragging and dropping the plot listing in the **Layout Navigator** directly onto page 2 of the layout.

All of the previous manipulations of data, gates, and plots with the **Layout Navigator** involved dragging and dropping the items. However, another way to accomplish these actions would have been to use the pop-up menu associated with the different items in the **Layout Navigator**. Just like all the other pop-up menus, the **Layout Navigator** pop-up menus are accessed by right-clicking when the cursor is located over an item in the **Layout Navigator**. We will now use the **Layout Navigator** to move the 2D plot from page 2 back to page 1 of the layout.

31. Select **18 - Color Dot - TCellSample1.fcs compensated (APC-A vs PE-A)** listed on Page 2 of the **Layout Navigator**.

32. Right-click on the color plot on Page 2 of the layout to bring up the menu, shown in Figure T14.23.

33. Select **Cut** from the menu (Figure T14.23).

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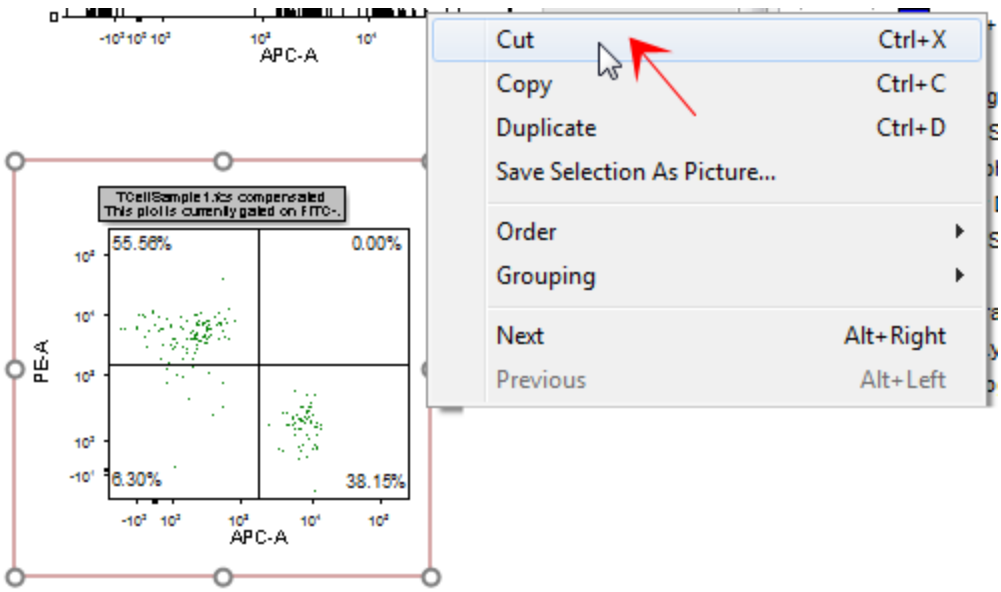

Figure T14.23 Layout Navigator 2D Plot Pop-up Menu

The density plot is now cut from page 2 of the layout.

34. Select **Page 1** on the **Layout Navigator**.

35. Right-click to bring up the **Layout Navigator** pop-up menu (Figure T14.24).

36. Select **Paste** from the menu (Figure T14.24).

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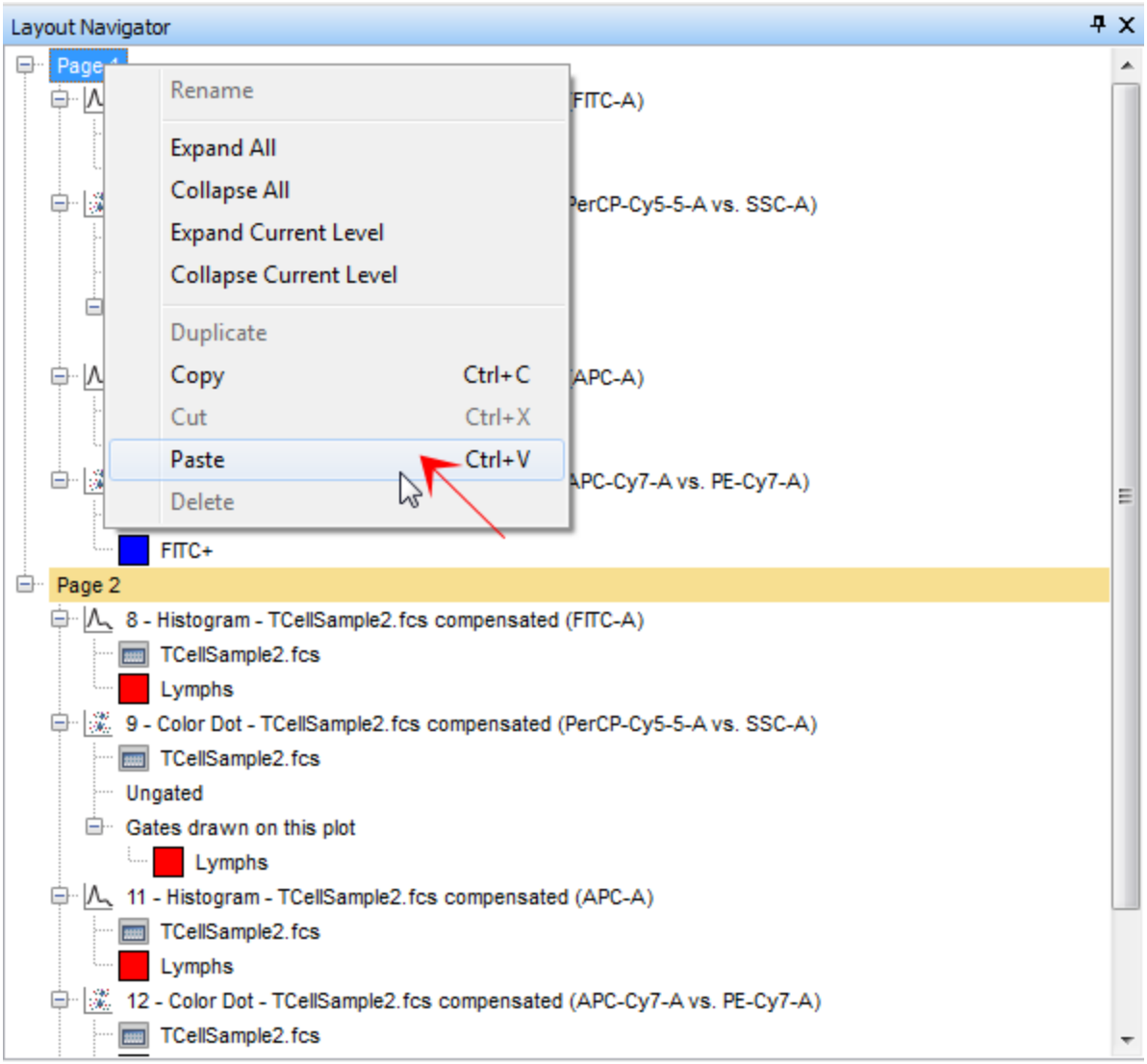

Figure T14.24 Paste 2D Plot Menu Item

Alternatively, after following steps 31 through 33 to cut the color dot plot, you can place the cursor directly onto page 1 of the layout, right-click to bring up the pop-up menu and select **Paste** to paste the color dot plot.

The color dot plot is now pasted back onto page 1 of the layout and the **Layout Navigator** updates to reflect this change.

38.16 HLA Cross Matching - Spreadsheets Use Case 1

FCS Express provides a number of tools that allow you to create and use spreadsheets to graph your data. Your spreadsheets can contain data from many sources including, but not limited to, tokens such as keyword, statistics,

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custom tokens, and token classifiers. In previous versions of FCS Express, custom data grids were used to create and graph data sets. However, in FCS Express version 6, the custom data grid is replaced with the new spreadsheet function. The advantage of creating a spreadsheet containing tokens is that when your layout changes (e.g., by making a gate or displaying a new set of data) your data within that spreadsheet will change as well. The use of a spreadsheet allows you to easily demonstrate the important results from your experiments using a spreadsheet very similar to excel, by graphing the results using a bar chart or scatter plot. In this tutorial, we will focus on creating and using spreadsheets.

Note: Spreadsheets replace **Custom Data grids** from previous versions of FCS Express but have the same and more advanced capabilities. If you have **Custom Data** tables saved from previous versions of FCS Express in your layout, then FCS Express 6 will allow you to open them for use and formatting. New custom data may not be created as Spreadsheets will replace all functions of the previous Custom Data grid functionality.

We will create a custom spreadsheet to aid in the analysis of an HLA cross-match experiment. Flow cytometry is frequently used to predict how much soluble IgG, from a potential recipient's serum, will bind to the cells of an engrafted organ. The assumption is that most of the IgG binding is directed against the HLA molecules on the donor cells. A negative cross-match means that the soluble IgG in the potential recipient's serum did not bind to a significant number of lymphocytes from the donor cells. This negative cross-match correlates with an improved graft survival. Specifically, we will examine the T- and B-cells from the donor to determine the amount of IgG binding from the potential recipient using an anti-human FITC labeled IgG.

In the course of this example, we will:

- Load and examine a layout with all the plots needed to perform an HLA cross-match.
- Create and insert various kinds of tokens and data into a new Spreadsheet.
- Explore various ways of entering information into a Spreadsheet.
- Create Conditional Formatting in Spreadsheet cells.

First, we will load a layout in preparation for creation of a custom dataset.

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38.16.1 Loading a layout for analysis

In this section, we will:

1. Load and examine a layout with all the plots needed to perform an HLA cross-match.

We will begin by loading a two page layout of HLA cross-match data. The layout contains data plots from two different negative controls, a positive control, and serum from a possible recipient patient.

2. Select **File** tab→**Open**.
3. Open the layout **HLA Custom Data.fey** found in the *FCS Express Sample Data* folder.

The two pages of the layout should appear as shown in Figure T15.1, with both pages being displayed next to each other.

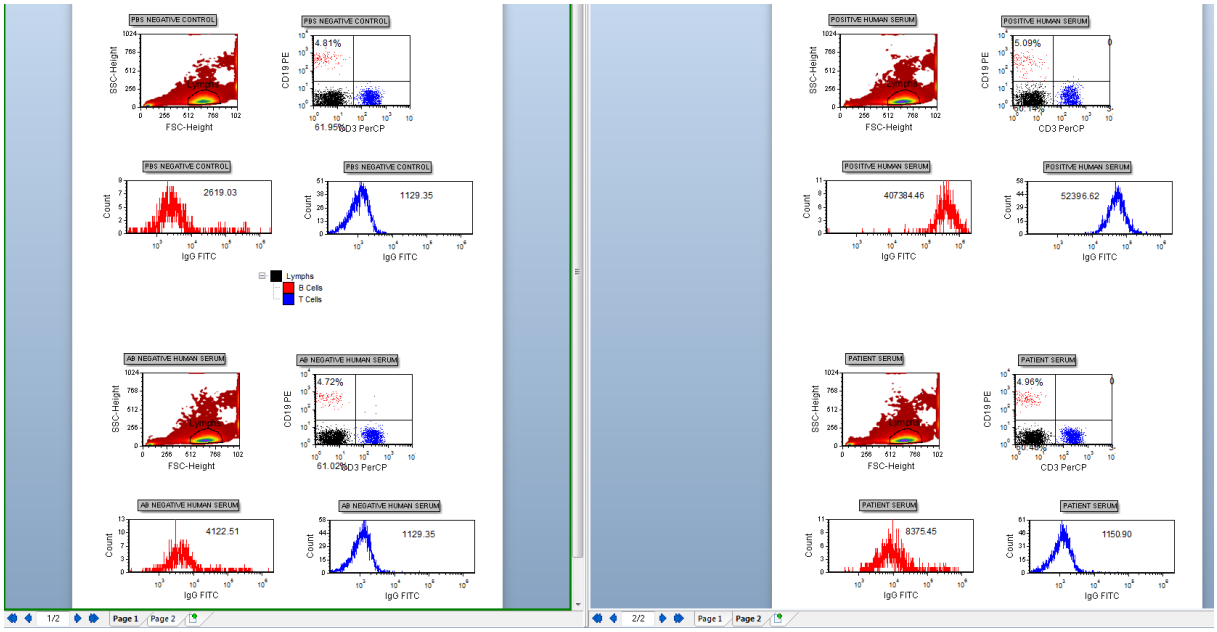

Figure T15.1 HLA Custom Data.fey

Let's begin by examining the first four plots on the top of Page 1, all labeled 'PBS NEGATIVE CONTROL', shown in Figure T15.2. The plot in the upper left corner is of the cells from the donor; a gate has been drawn around the lymphocyte population and labeled 'Lymphs'. The density plot in the upper right is gated on only the lymphocyte population and displays the CD3 positive T-cells, in the lower right quadrant (blue) and the CD19 positive B-cells, in the upper left quadrant (red). The two quadrants for the T- and B-cells have been converted to

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gates and are sub-gates, or children, of the Lymphs gate, as shown by the Gate View window. The two histograms on the bottom, from left to right, are gated on the B-cell population (red) and T-cell population (blue), respectively. The numbers inside the histograms are the median MESF values of the IgG FITC parameter of the T- and B-cells.

The other plots on the layout are duplicates of these four plots displaying the data from three other data files.

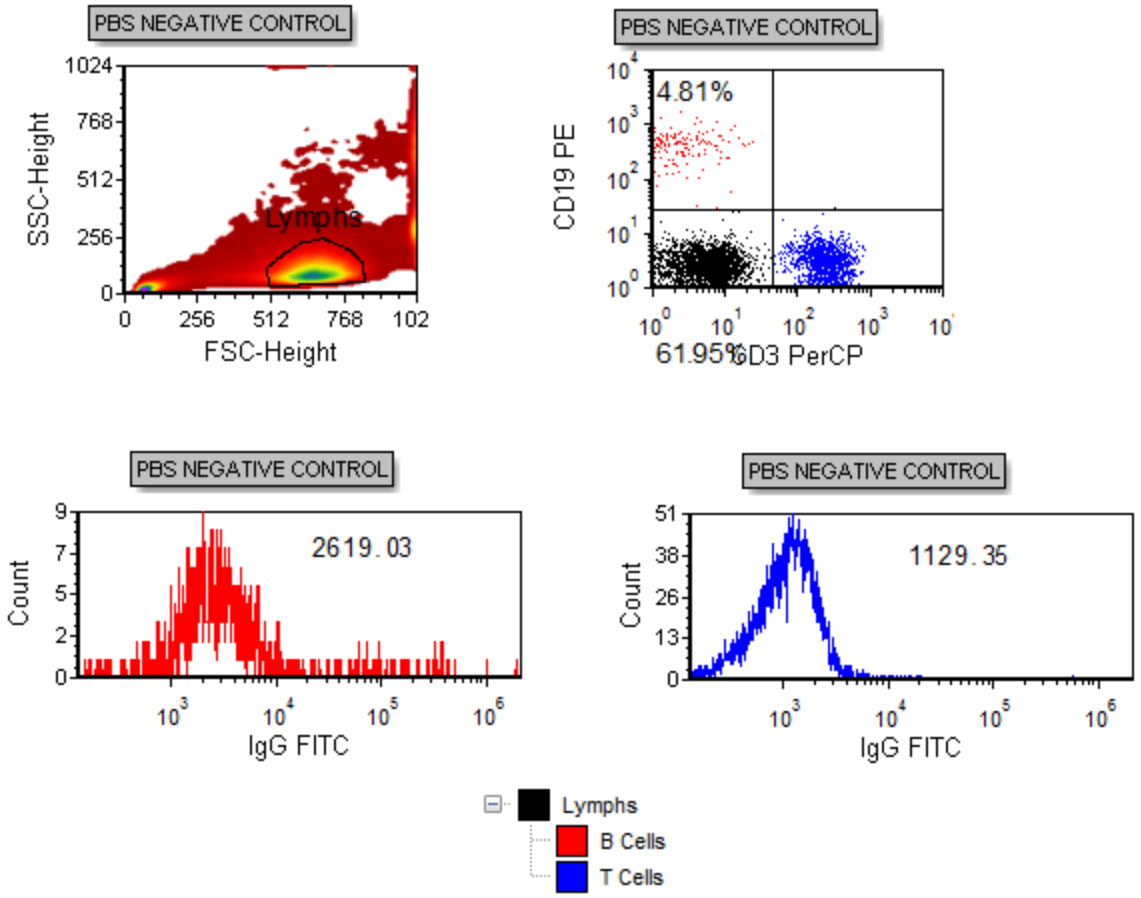

Figure T15.2 Layout of PBS NEGATVE CONTROL Plots and Gate View

Rather than using arbitrary channel values, the layout uses a Channel Calibration regression that was previously created from FITC MESF beads. This means the IgG FITC histograms use a calibrated FITC MESF scale. For more information on using beads in this way, please refer to the tutorial on Calibrating Fluorescence Using Beads. As shown in Figure T15.2, the median value from the PBS NEG CONTROL histogram of the B-cells is 2619.03. This means that the median

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intensity of the IgG FITC signal, from the soluble IgG bound to the B-cells of the donor cells, is approximately 2,619 MESF (Molecules of Equivalent Soluble Fluorochrome) of fluorescein.

Next, we will create a spreadsheet 1624.

38.16.2 Creating a Spreadsheet for analysis

In this section, we will:

- Create a Spreadsheet.
- Create and insert tokens and data into the Spreadsheet.

Because the IgG histogram scales are calibrated, we can use the MESF median values from the B- and T-cell histograms to construct a custom data set. Ultimately, we want the custom data set to tell us if serum from a patient sample is considered a "positive", "negative" or "weak" positive cross-match against the donor cells. To be able to do this, we have to define what is considered 'positive' or 'negative'. To aid in these definitions, multiple negative control serum samples are analyzed against the donor cells to determine the average FITC MESF value. The standard deviation is used to determine a significant "cut-off" value to use. We will assume that the FITC MESF values that define 'positive', 'negative' and 'weak' positive have already been determined.

We will begin by inserting a new blank page in the **HLA Custom Data.fey** layout from the previous "loading a layout for analysis" tutorial section, and then inserting a spreadsheet.

1. Select the **Insert→General→New Page** command.

Alternatively, click on the New Page Icon at the bottom of Page 2 of the layout, as indicated by the cursor in Figure T15.3.

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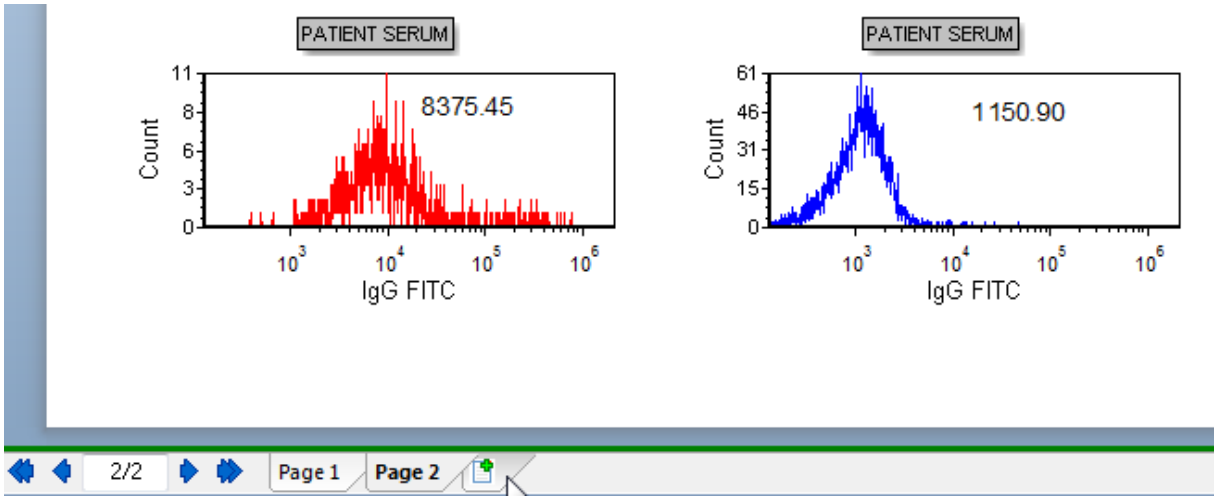

Figure T15.3 New Page Tab/Icon

A new blank page appears.

2. Select the **Insert** tab→**Spreadsheet**→**New** command (Figure T15.4).

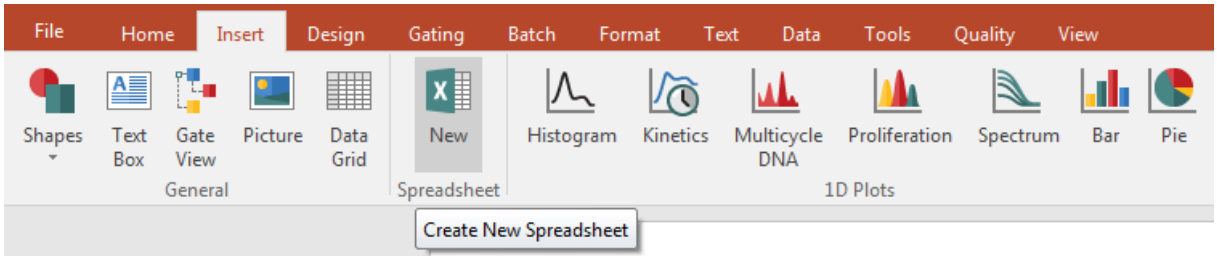

Figure T15.4 New Spreadsheet Command

The **Create new spreadsheet** dialog appears. Please refer to Figure T15.5 for the following steps.

3. Type 'HLA Cross-match' in the **Name** field.

4. Click **OK**.

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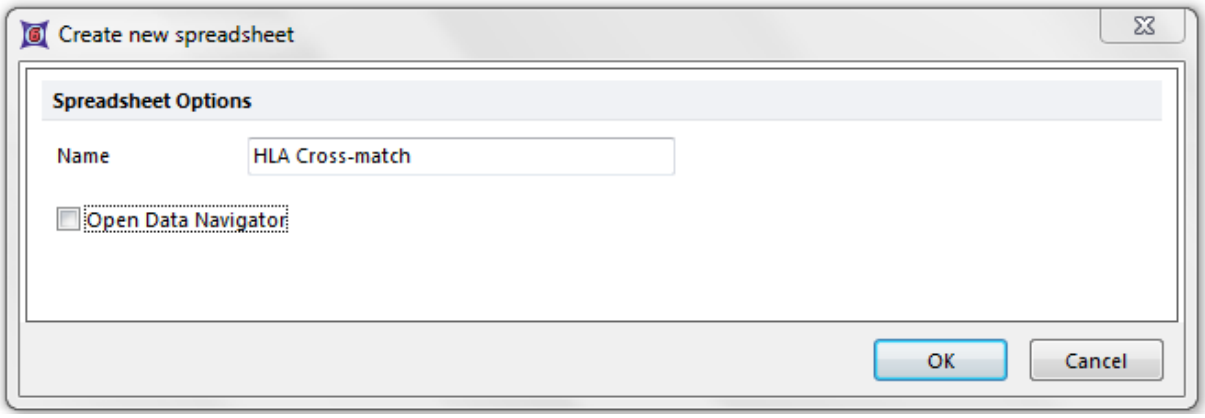

Figure T15.5 Create New Spreadsheet Dialog

An empty spreadsheet will appear on Page 3 of the layout, as shown in Figure T15.6. If the spreadsheet appears on another page, you can cut and paste it onto Page 3 of the layout.

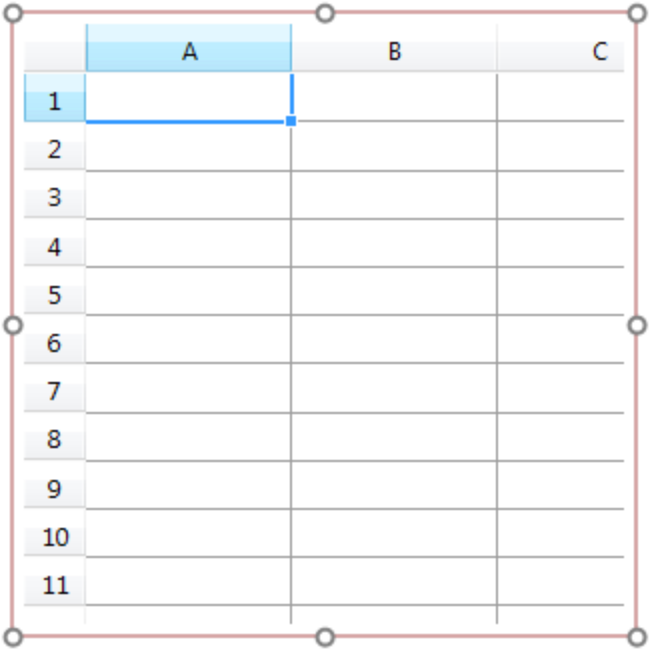

Figure T15.6 Empty Spreadsheet

In the next exercise, we will Populate the spreadsheet for analysis 1627.

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38.16.3 Populating a Spreadsheet with data

In this section, we will:

- Insert keyword and statistic tokens into the Spreadsheet.
- Explore various ways of entering information into the Spreadsheet.

We will insert a keyword token in the cells of 'A' column of the **HLA Custom Data.fey** can be found in the *FCS Express Sample Data* folder. The keyword *$SRC* will be used because it has a good description of the sample. In fact, the plot titles are all using the keyword *$SRC* rather than the filename.

1. Click inside the top cell in the 'A' column, shown highlighted in blue in Figure T15.10.

We are now going to insert the keyword token into the cell. Find the Spreadsheet Tab in the Top Center of the screen, then Click on **Layout→Tokens→Insert Token.**

2. Click on the **Spreadsheet** tab→**Layout** tab→**Tokens**→Insert **Token** command, indicated by the red arrow in Figure T15.10.

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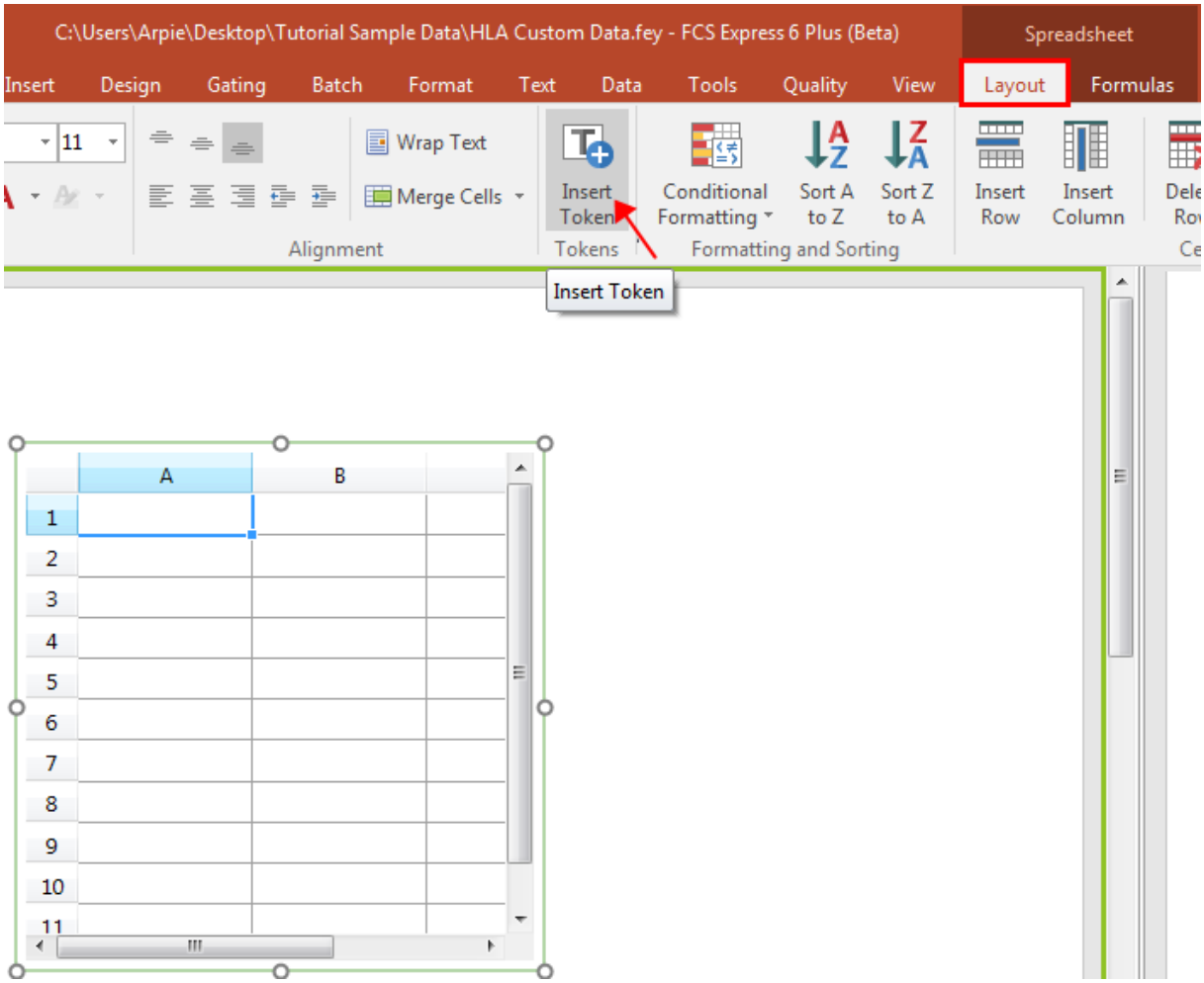

Figure T15.10 Spreadsheet with Insert Token

The **Insert a Token** dialog appears, as shown in Figure T15.11.

3. Select **Keyword**.
4. Click **Insert**.

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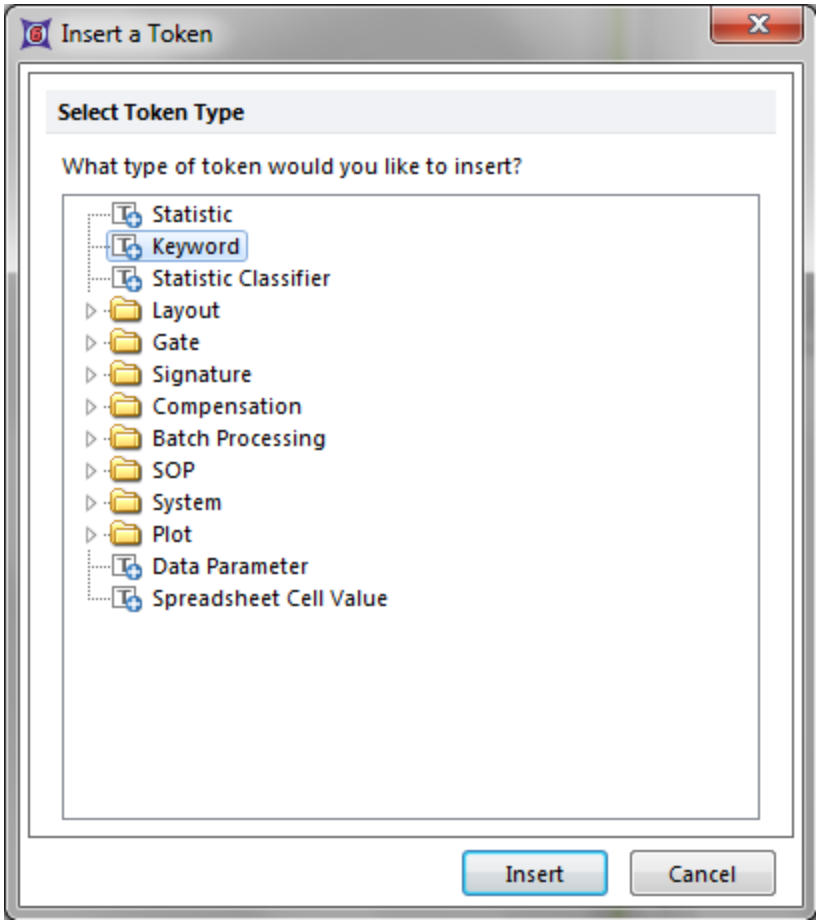

Figure T15.11 Insert a Token Dialog

The **Create Keyword** dialog appears, with the **Data Source** category displayed, similar to Figure T15.12.

5. Select any of the plots associated with the data file XM110210-201.001 (PBS NEGATIVE CONTROL), shown highlighted in blue in Figure T15.12.

6. Select the **Keyword** category on the left.

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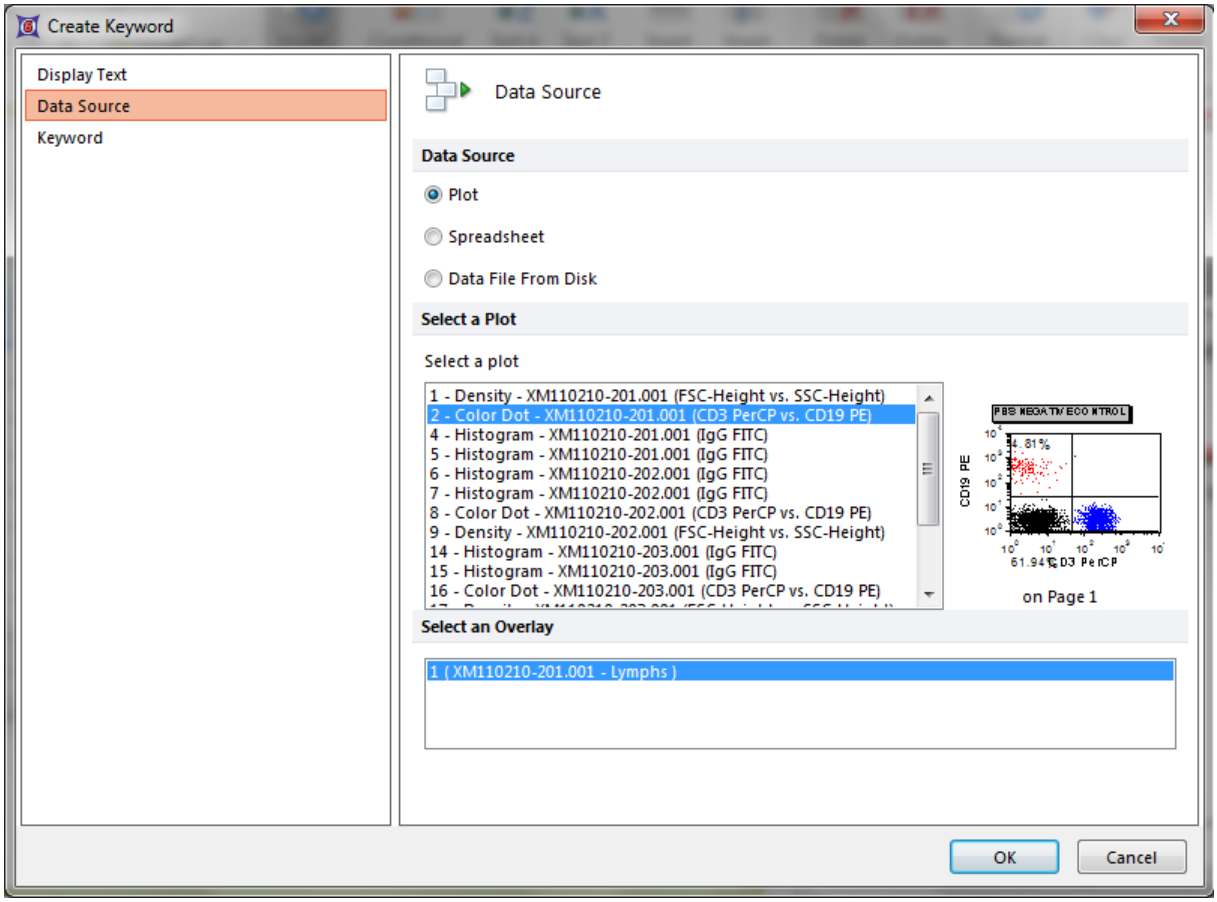

Figure T15.12 Create Keyword Dialog

The **Keyword** category of the **Create Keyword** dialog appears, similar to Figure T15.13.

7. Type '$SRC' in the **Select a keyword** text field.
8. Click **OK**.

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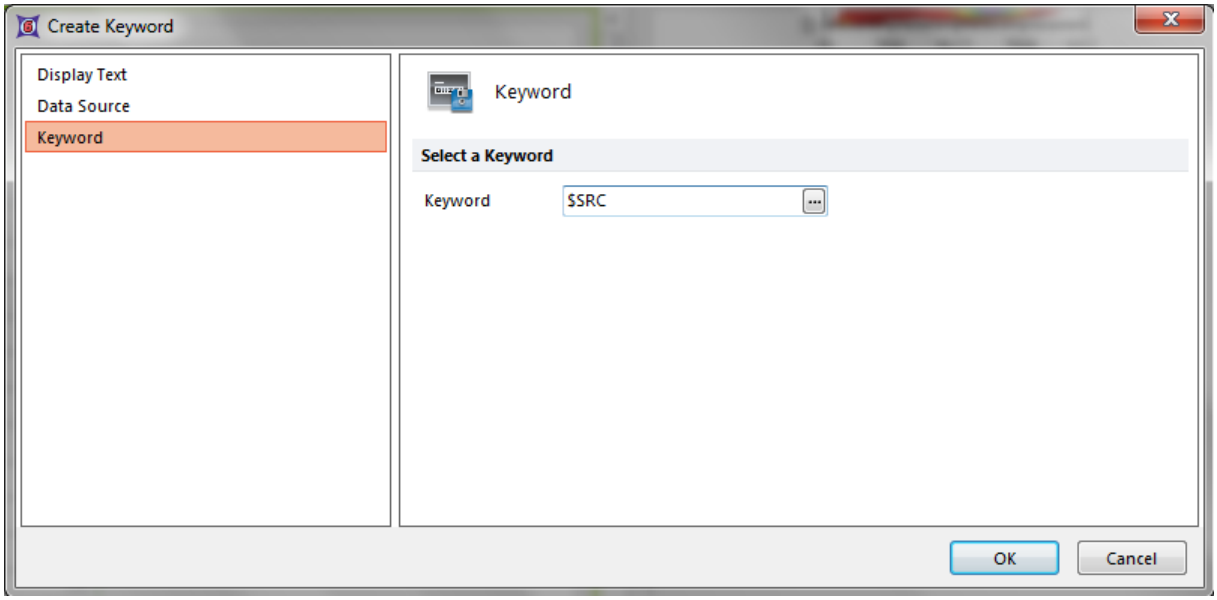

Figure T15.13 Create Keyword Dialog -- Keyword Category

Alternatively, click on the ellipsis icon, indicated by the cursor in Figure T15.13. The **Please select a keyword** dialog appears, as shown in Figure T15.14.

- Scroll down and select **$SRC**, shown highlighted in blue in Figure T15.14.
- Click **OK**.
- Click **OK** in the **Create Keyword** dialog.

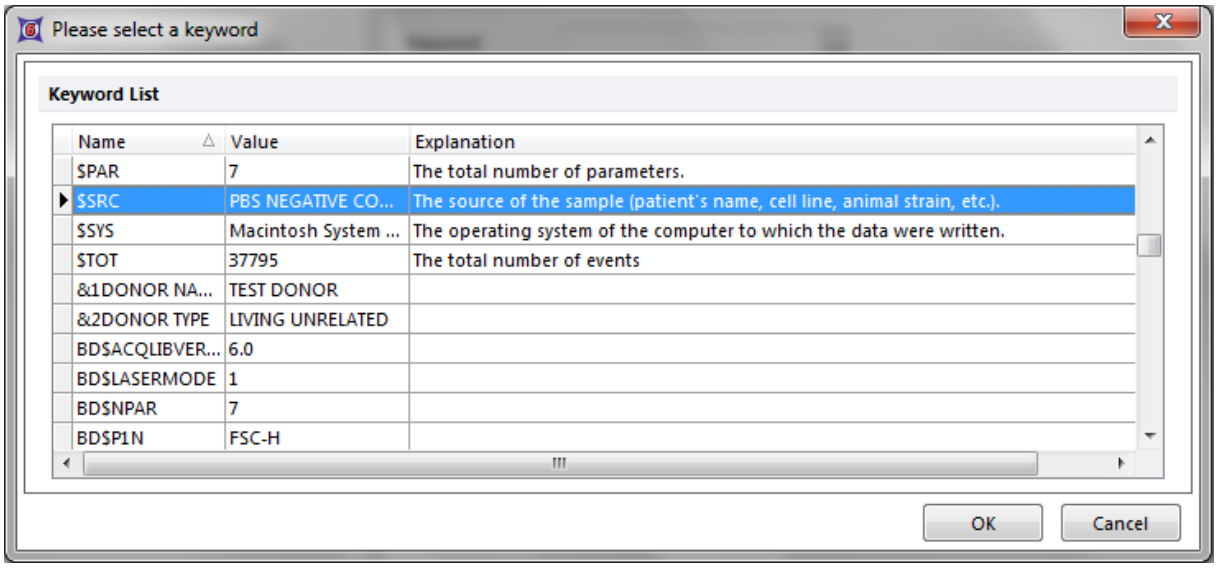

Figure T15.14 Please Select a Keyword Dialog

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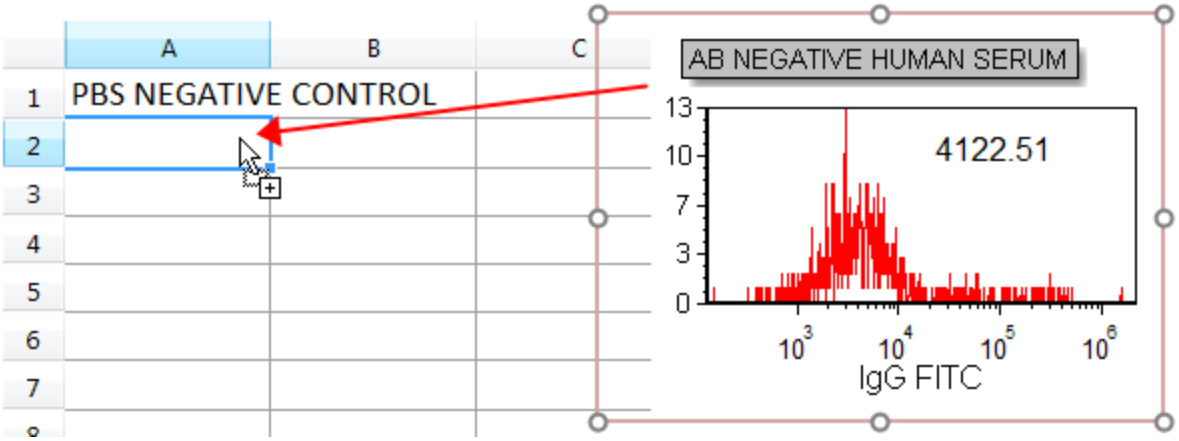
The Spreadsheet updates to reflect the insertion of the keyword in the 'A1' cell.

We will now fill in the rest of the 'A' Column. However, rather than repeating all the previous steps, we will simply drag and drop the appropriate plot onto the correct cell in the data grid.

10. Place the cursor over one of the 'AB HUMAN NEGATIVE SERUM ' 2D plots from page 1, shown highlighted in red in Figure T15.16.

11. Press and hold the left mouse button and drag the plot towards the Spreadsheet, as shown in Figure T15.16.

12. Release the left button when the plot is over the second cell of the A column, indicated by the cursor in Figure T15.16.



Figre T15.16 Dragging and Dropping the AB NEGATIVE HUMAN SERUM Plot onto the Spreadsheet

The **Paste Special** dialog appears, similar to Figure T15.17.

13. Select **Keyword token**, shown highlighted in blue in Figure T15.17.

14. Click **OK**.

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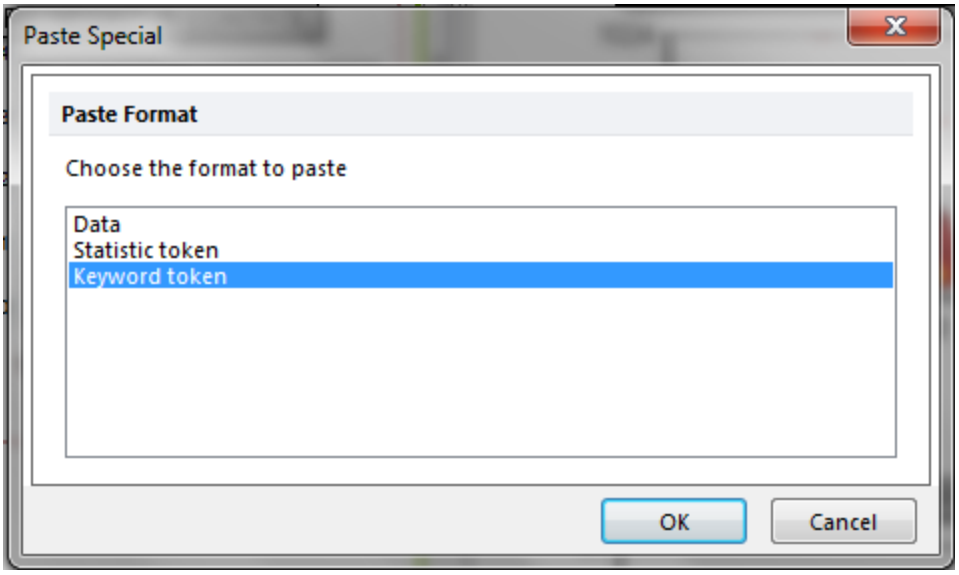

Figure T15.17 Paste Special Dialog

The **Edit Keyword** dialog appears, similar to the Keyword category of the **Create Keyword** dialog previously shown in Figure T15.13.

15. Type '$SRC' in the **Select a keyword** text field.

16. Click **OK**.

Note: Dragging a plot onto a place in your layout that can accept a token is a very convenient way of creating that token, since you do not have to manually select the data source for the token like you did in step 5 above.

The second cell of the **A** column updates to reflect the addition of the keyword for the AB NEGATIVE HUMAN SERUM plot. We will now fill the rest of the cells in the **A** column.

17. Repeat steps 10 through 16 for the third cell in the **A** column using a POSITIVE SERUM plot from page 2.

For the 4th cell in the **A** column, we will manually type in the sample information.

18. Type 'PATIENT SERUM'.

Alternatively, you can click anywhere outside the Spreadsheet to accept the entry into that particular cell. If you do not wish to save the data typed in the editor, you can select the Delete icon, or hit **Esc** on the keyboard and the changes will be canceled.

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The Sample column of the Spreadsheet is now filled in and should appear similar to Figure T15.20.

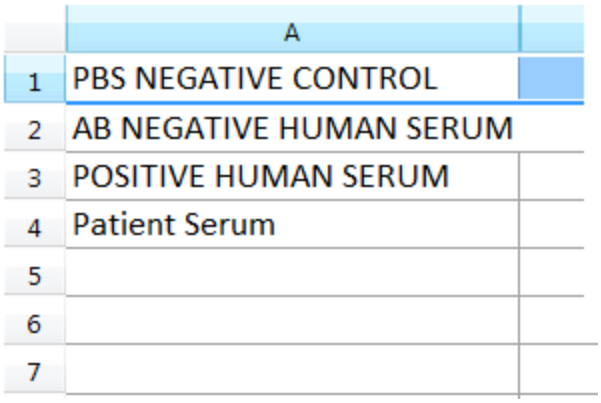

Figure T15.20 Sample Column Completed

We will now insert the 'B' column as the results of the 'Bcell Median', and the 'C' Column as the results of 'Tcell Median' column values as token statistics. Because these median values are already being displayed as tokens in the histograms, we will simply copy and paste these tokens into the appropriate cells of the 'B' and 'C' columns.

19. Place the cursor at the beginning of the B-Cell token value in the PBS NEGATIVE CONTROL histogram (page 1), shown in red on the layout.

20. Select the B-Cell token text. You will know it is selected because the text will be white, indicated by the cursor in Figure T15.21.

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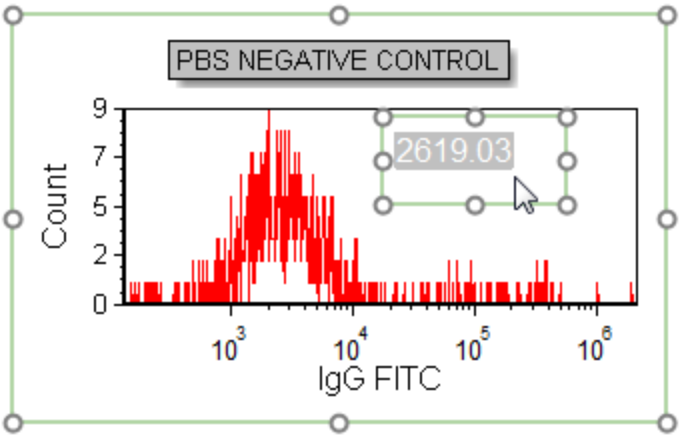

Figure T15.21 Token Value Selected

23. Copy the text using **Ctrl+C** on the keyboard.

Alternatively, after Step 21, the B-cell token text can be copied using the pop-up menu.

- Right click to bring up the pop-up menu.
- Select **Copy** from the pop-up menu, shown highlighted and indicated by the cursor in Figure T15.22.

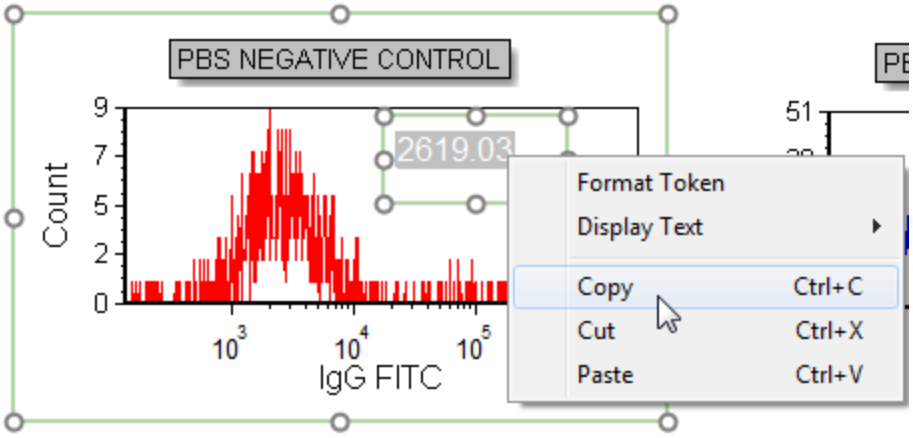

Figure T15.22 Copying a Token Using the Pop-up Menu

We will now paste the B-cell token into the appropriate cell of the 'B' column of the Spreadsheet.

24. Click inside the first cell of the 'B' column to select it.

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25. Paste the text using **Ctrl+V** on the keyboard.

26. Click anywhere outside the Spreadsheet to accept the entry.

Alternatively, after Step 24, the token text can be pasted using the pop-up menu.

- Right-click inside the first cell of the 'B' column to bring up the pop-up menu.
- Select **Paste Text** from the pop-up menu.
- Click anywhere outside the Spreadsheet to accept the entry.

The rest of the 'B' column can now be filled using copying and pasting by repeating Steps 20 through 26 using the B-cell histograms of the AB NEGATIVE HUMAN SERUM, POSITIVE SERUM, and PATIENT SERUM. The 'C' column of the Data Grid can be filled in by copying the token median text displayed in the T-cell histograms, shown in blue on the layout. Again, repeat Steps 20 through 26, substituting the T-cell tokens for the B-cell tokens. All the cells of the 'B' and 'C' columns of the Spreadsheet should be filled in and appear similar to Figure T15.23.

	A	B	C
1	PBS NEGATIVE CONTROL	2619.03	1129.35
2	AB NEGATIVE HUMAN SERUM	4122.51	1129.35
3	POSITIVE HUMAN SERUM	407384.46	52396.62
4	Patient Serum	8375.45	1150.9
5			
6			
7			

Figure T15.23 Spreadsheet with B- and T-Cell Median Values, in Column B and Column C, Respectively

In the next section, we will create and use custom tokens in a spreadsheet.

38.16.4 Working with Custom Tokens and Formula References in spreadsheets

In this section, we will:

- Creating a custom token and insert it in a spreadsheet.

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- Learn how to use spreadsheet formulas to replace custom token formulas. (Jump to this section 1642)

We will begin by using the **HLA Spreadsheet.fey** layout found in the *FCS Express Sample Data* folder.

The layout contains plots and statistics related to an HLA cross matching data set. In this tutorial we will first learn how to create a custom token formula and insert the result in a spreadsheet. We will then learn how to use spreadsheets to create the formula in spreadsheet cells to contrast the two different methods for calculating custom statistics in FCS Express. Using spreadsheets for custom calculations (rather than custom tokens) allows for quick access to additional chart types for the data sets of interest and may also be more efficient for calculating certain formulas. For instance, with the particular data set we are using it would take an additional amount of effort to create the necessary custom token formula(s) whereas using a spreadsheet allows you to quickly copy and paste cells to derive statistics and use the copy down feature to calculate formulas for multiple cells.

To begin, we will first complete a series of custom tokens that normalize a 'samples' median fluorescence values based on a 'negative' value. We will use the median MESF FITC values from the B cells of the PBS NEGATIVE CONTROL as the 'negative' value and subtract this value from the median MESF FITC value of the corresponding samples. We will begin by inserting the **Custom Tokens** window.

1. Select **File tab→Open**.
2. Load the **HLA Spreadsheet.fey** layout from the **FCS Express Sample Data folder.** 1210

The layout will appear with the **Custom Tokens** window docked on the right side of the layout. For more information on docking, please see the Docking tutorial 1537.

Three custom tokens that calculate the shift in median fluorescent values between a negative control and a corresponding sample are already created in the **Custom Token** Dialog as seen in **Figure T15.24**. We will create a final custom token that represent the MESF channel shift of a "patient" sample relative to the PBS NEGATIVE CONTROL sample and place the value into a spreadsheet table. The following steps serve as an example of how to create and use a custom token formula in a spreadsheet. Later in the tutorial we will examine how to define the formula solely in a spreadsheet.

Note: For more information on creating custom tokens, refer to the Custom Token Tutorial 1324.

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3. Click on the symbol on the top border of the **Custom Tokens** window, indicated by the red box in **Figure T15.24**.

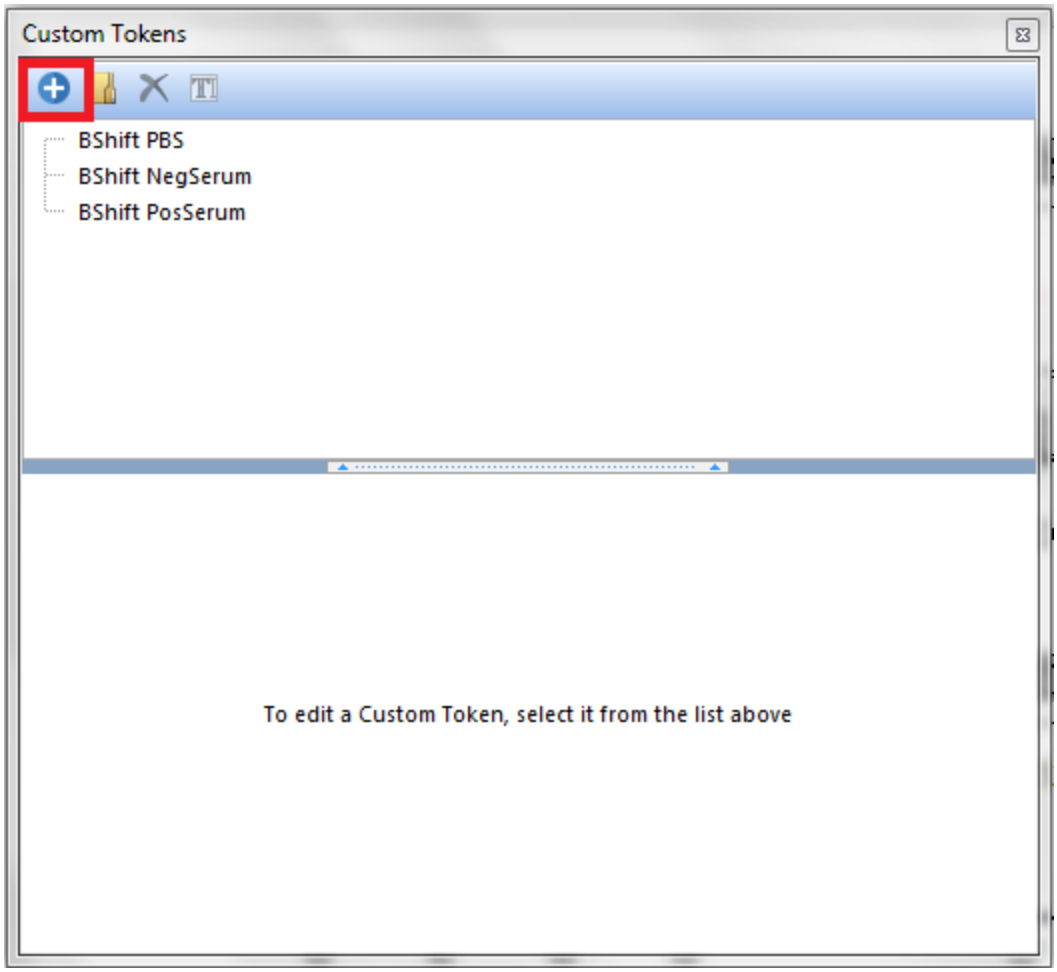

Figure T15.24 Creating a new Custom Token.

4. Type 'BShift Patient' for the Custom Token name.
5. Click in the bottom of the Custom Token window under **Enter the Custom Token formula below**.
6. Type '=' in the Custom Token definition area.
7. Right click to the right of the '=' sign.
8. Choose **Insert Token**.
9. Double click on the **Statistic** category.
10. Click **Insert**.

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11. Choose **20-Histogram XM110210-210.001** in the **Select a Plot** dialog, as in **Figure 15.25a.**

12. Click on the **Statistic** category on the left as seen in **Figure 15.25b**.

13. Choose the B Cells gate from the **Select Gate** drop down.

14. Click on **Median** under **Statistic**.

The Create Statistic dialog should appear as in **Figure 15.25b** below.

15. Click **OK**.

The median token value will appear in the custom token definition.

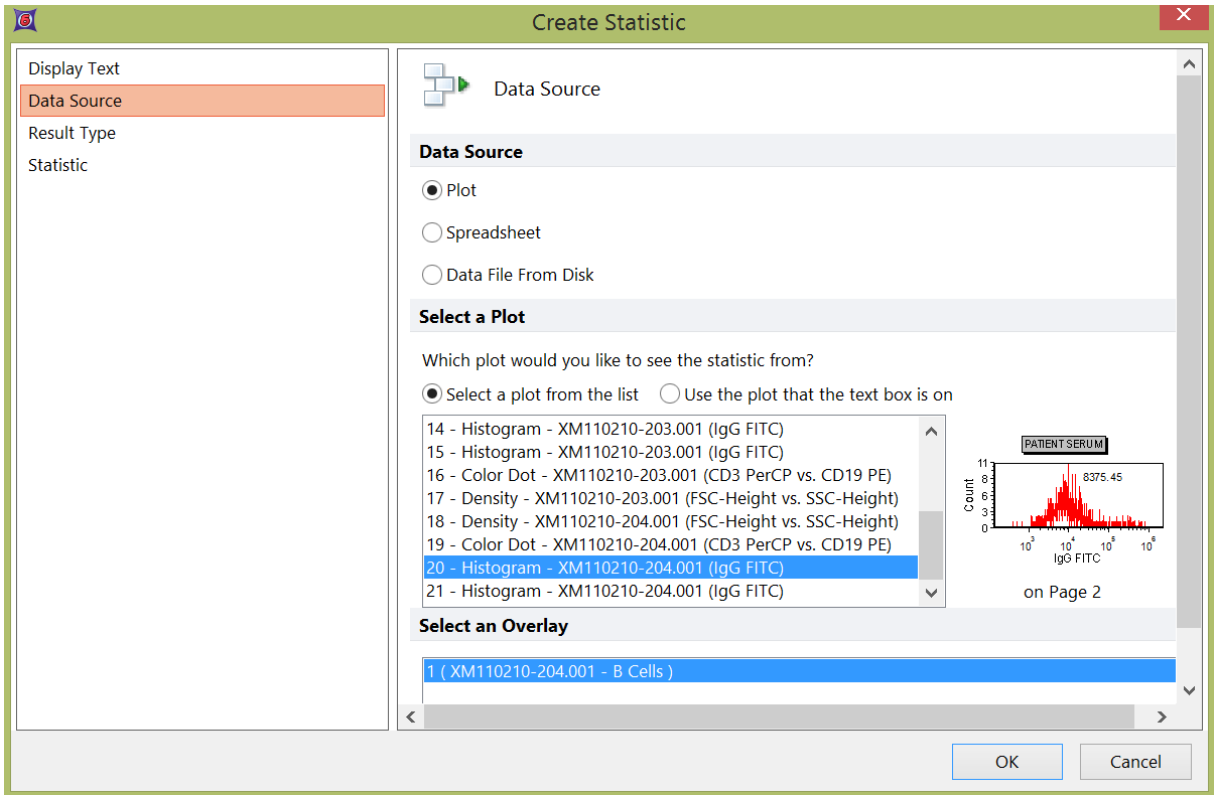

Figure 15.25a - Create Statistic Dialog, Selecting a Token from the appropriate histogram.

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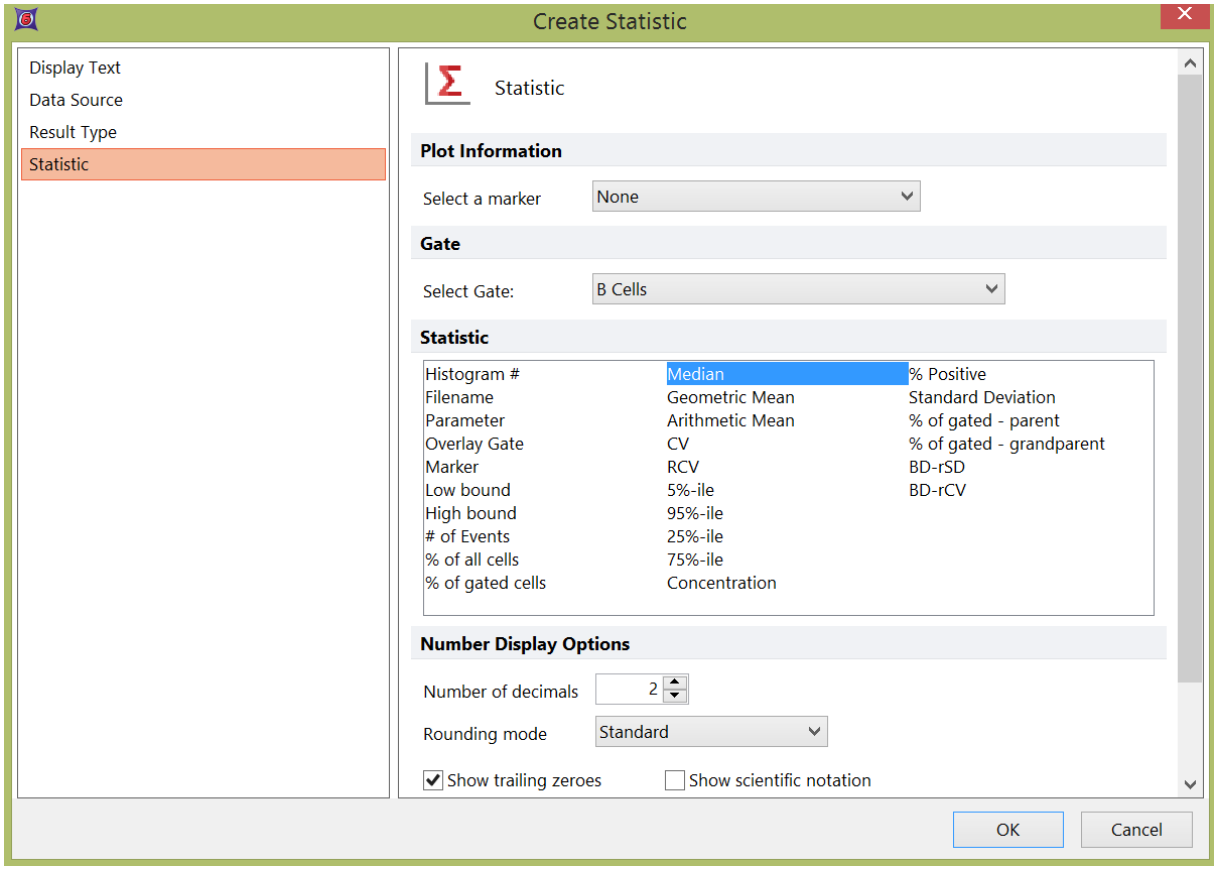

Figure 15.25b Choosing a Statistic for the Custom Token.

We will now complete the complete the BShift Patient custom token, as it looks in **Figure T15.26.**

16. Type '-' after the token defined in the previous steps (red arrow in **Figure T15.26** below).

17. Repeat steps 7-15 after the minus sign choosing '**4-Histogram XM110210-210.001'** instead of '**20-Histogram XM110210-210.001'**.

The Custom Token formula will appear as in **Figure 15.26** below. Notice the custom token defines the median for plot 20 minus the median for plot 4.

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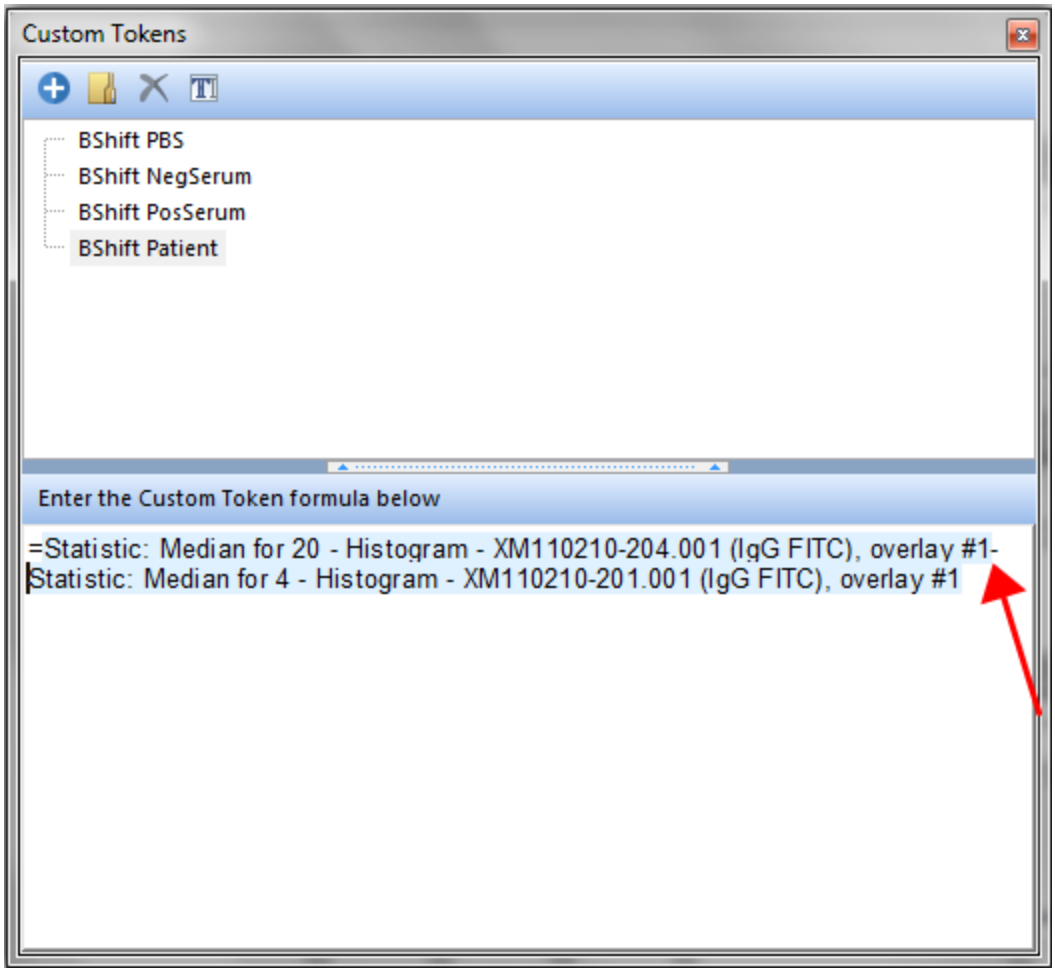

Figure T15.26 Creating the B-cell Median Shift Custom Token Definition

We will now complete the column of B Cell Shift custom tokens in the spreadsheet on Page 3 by dragging and dropping the BShift Patient Custom Token into the spreadsheet.

Please refer to **FigureT15.27** for the steps below.

18. Click on the **Page 3** page tab
19. Place the cursor over the 'BShift Patient' Custom Token, shown highlighted in blue.
20. Left-click and hold the mouse button.
21. Drag the 'BShift Patient' Custom Token toward cell B4 in the Spreadsheet.
22. Release the mouse button when the 'BShift Patient' Custom Token is over cell B4 in the Spreadsheet, (indicated by the cursor and the red arrow in **Figure T15.27**.)

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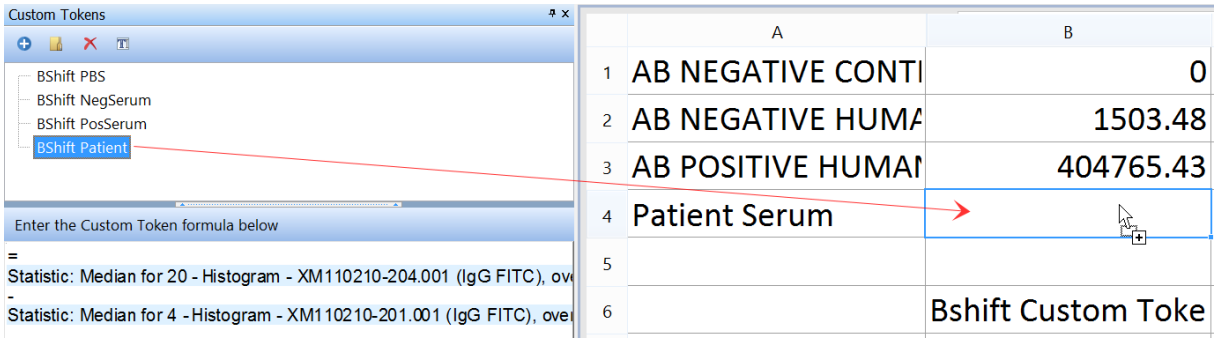

Figure T15.27 Dragging and Dropping the 'BShift PBS' Custom Token into the Spreadsheet.

The spreadsheet should now appear as in **Figure T15.28**.

	A	B	C	D	E
1	AB NEGATIVE CONTROL	0			
2	AB NEGATIVE HUMAN SERUM	1503.48			
3	AB POSITIVE HUMAN SERUM	404765.43			
4	Patient Serum	5756.42			
5					

Figure T15.28 Spreadsheet with BShift Custom Tokens in 'B', and Cells to be filled in Columns 'C', 'D' and 'E'

We will now complete a series of custom tokens for corresponding T cell median fluorescent shift values by using tokens and formulas in the spreadsheet. The method described below will contrast the method of using custom tokens. The TShift Negative Control Tokens that we will bring into Column 'D', will be subtracted from the TShift Median Tokens in Column 'C'. The result will be returned from a formula Column 'E' which can serve to replace a series of custom token values such as the previous way we defined the BShift custom token series in the steps above.

Let's start with Dragging and Dropping the Histogram from the Data Navigator to Column 'C'. First Open up the Data Navigator. We have more information about the Data Navigator in our Tutorials and Manuals.

23. Click on the **View** tab→**Navigators**→**Data Navigator**.

24. Expand the **XM110210-201.001** node.

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25. Click on **5-Histogram-XM110210-201.001 (IgG FITC)**. (It will be highlighted in blue as in Figure T15.29).

26. Drag and Drop **5-Histogram-XM110210-201.001 (IgG FITC)** to cell C1.

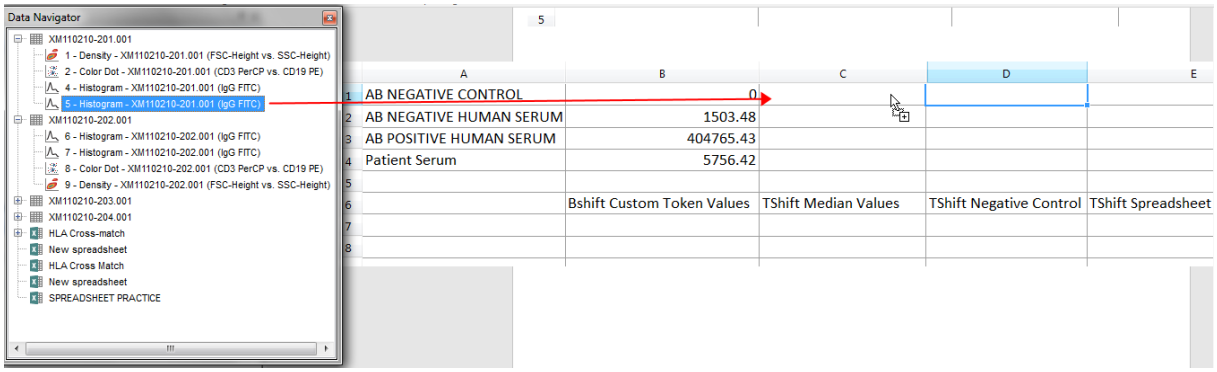

Figure T15.29 Drag and Drop a Histogram to retrieve a statistical token and populate a cell.

A **Paste Special Dialog** will appear.

27. Click on **Statistic Token** in the Paste Special as in **Figure T15.30** below.

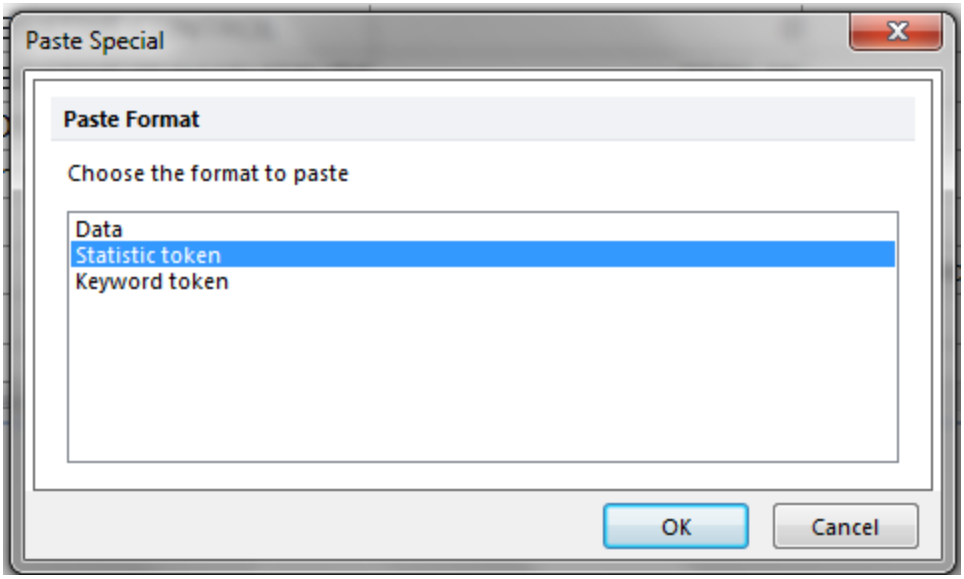

Figure T15.30 The Paste Special dialog.

28. Select the **T Cells** gate from the **Select Gate** dropdown menu.

29. Select **Median** from the **Statistic** list.

30. Click **OK**.

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We will now repeat the steps above to insert the rest of the TShift Median values by dragging and dropping the histograms from the Data Navigator to cells C2, C3, and C4, respectively.

31. Expand the **XM110210-202.001** node in the Data Navigator.

32. Drag and Drop **6-Histogram-XM110210-201.001 (IgG FITC)** to cell C2 and repeat steps 24-30.

33. Expand the **XM110210-203.001** node in the Data Navigator.

34. Drag and Drop **14-Histogram-XM110210-201.001 (IgG FITC)** to cell C3 and repeat steps 24-30.

35. Expand the **XM110210-204.001** node in the Data Navigator.

36. Drag and Drop **21-Histogram-XM110210-201.001 (IgG FITC)** to cell C4 and repeat steps 24-30.

We will now use copy and paste to copy the baseline TShift Negative Control value to the corresponding cells in Column D. The value will be subtracted from the Column C median values in a subsequent formula resulting in the TShift series of values within Column E of the Spreadsheet.

37. Select Cell C1.

38. Press **Ctrl+C** to Copy cell C1. Alternatively, Right-Click and choose Copy as in Figure **T15.31**.

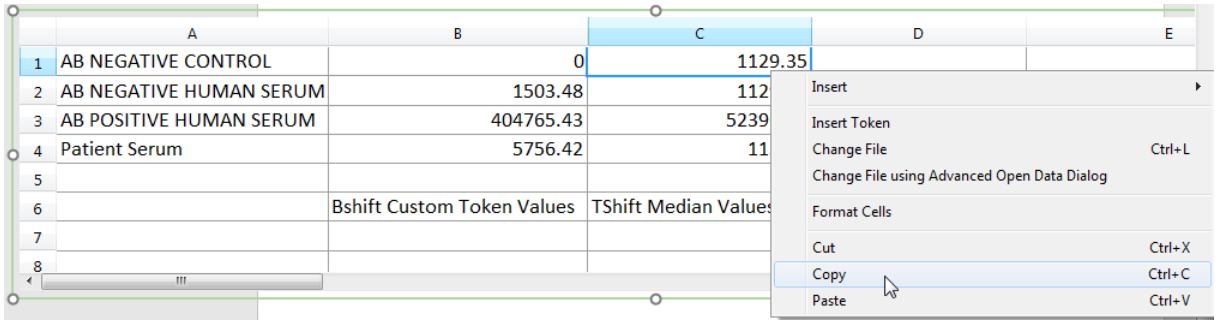

Figure T15.31 Using the copy command to copy cells in a spreadsheet.

39. Multiple select Cells D1 through D4 by left clicking in cell D1 and mousing down as you would multiple select cells in Excel.

40. Press **Ctrl+V** to Paste to cells D1-D4.

The spreadsheet should now appear as in **Figure T15.32** below.

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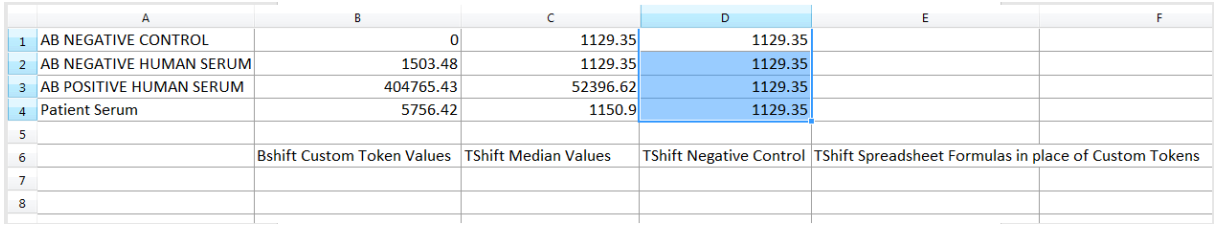


	A	B	C	D	E	F
1	AB NEGATIVE CONTROL	0	1129.35	1129.35		
2	AB NEGATIVE HUMAN SERUM	1503.48	1129.35	1129.35		
3	AB POSITIVE HUMAN SERUM	404765.43	52396.62	1129.35		
4	Patient Serum	5756.42	1150.9	1129.35		
5						
6		Bshift Custom Token Values	TShift Median Values	TShift Negative Control	TShift Spreadsheet Formulas in place of Custom Tokens	
7						
8						

Figure T15.32 Using the multiple select and paste command in a spreadsheet.

We will now complete the spreadsheet by defining the formula of the TShift series in column in E using a spreadsheet formula reference and using the copy down feature to quickly complete the formula for all rows.

41. Click on cell E1.

42. Enter an '=' sign.

43. Click on cell C1.

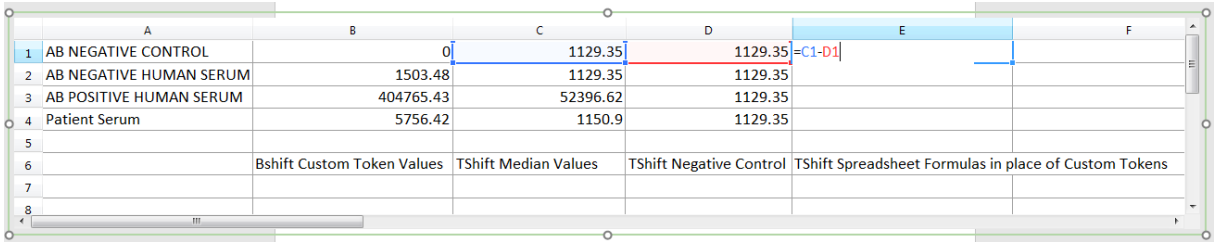
44. Enter a '-' sign.

45. Click on cell D1.

The formula should appear as in **Figure T15.33**

46. Press **Enter**.

The formula for C1 minus D1 will result in the value of 0 in cell E1.



	A	B	C	D	E	F
1	AB NEGATIVE CONTROL	0	1129.35	1129.35	=C1-D1	
2	AB NEGATIVE HUMAN SERUM	1503.48	1129.35	1129.35		
3	AB POSITIVE HUMAN SERUM	404765.43	52396.62	1129.35		
4	Patient Serum	5756.42	1150.9	1129.35		
5						
6		Bshift Custom Token Values	TShift Median Values	TShift Negative Control	TShift Spreadsheet Formulas in place of Custom Tokens	
7						
8						

Figure T15.33 Defining a formula in a spreadsheet

We will now use the copy down feature to quickly copy the formula down to through the remaining cells and complete the spreadsheet.

47. Select cell E1.

48. Press **Ctrl+C** to Copy.

49. Multiple select Cells E1 through E4 by left clicking in cell E1 and mousing down as you would multiple select cells in Excel.

50. Press **Ctrl+V** to Paste.

The formula will be copied down the range of cells selected in Step 49 and the spreadsheet will be completed as in **Figure T15.34**.

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	A	B	C	D	E
1	AB NEGATIVE CONTROL	0	1129.35	1129.35	0
2	AB NEGATIVE HUMAN SERUM	1503.48	1129.35	1129.35	0
3	AB POSITIVE HUMAN SERUM	404765.43	52396.62	1129.35	51267.27
4	Patient Serum	5756.42	1150.9	1129.35	21.55

Figure T15.34 Spreadsheet with formulas.

Next, we will create and use token classifiers in the spreadsheet for IVD use.

38.16.5 Create Token Classifiers

Classifier Tokens are only available in the Clinical Edition (IVD version) of FCS Express. Please contact support@denovosoftware.com if you would like more information on FCS Express Clinical Edition and access to the Creating Token Classifiers tutorial.

38.16.6 Using Conditional Formatting to Classify Data

- Create Conditional Formatting using custom tokens.
- Insert Conditional Formatting into the new Spreadsheet.

Using the 'BShift' and Custom Token data created in the last section, we will now define conditional formatting ('NEG', 'POS', and 'WEAK') in the Spreadsheet. We will use conditional formatting to classify our samples and ultimately determine the status of the patient serum cross-match. To begin, we will insert a new Spreadsheet.

1. Open the **HLA Conditional Formatting.fey** found in the *FCS Express Sample Data* folder.

We will now begin defining Conditional Formatting to classify the results for each sample in the spreadsheet.

2. Click on the **Page 3** tab.

3. Click on the header of the 'B' column on the spreadsheet.

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4. Click on the **Spreadsheet** Tab→**Layout** Tab→**Formatting and Sorting→Conditional Formatting.**

5. Mouse over **Icon Sets.**

6. Mouse to the right to reveal the available icon set.

7. **Left Click** the first set of arrows icons in the first row, shown by the red box as in **Figure T15.40** below.

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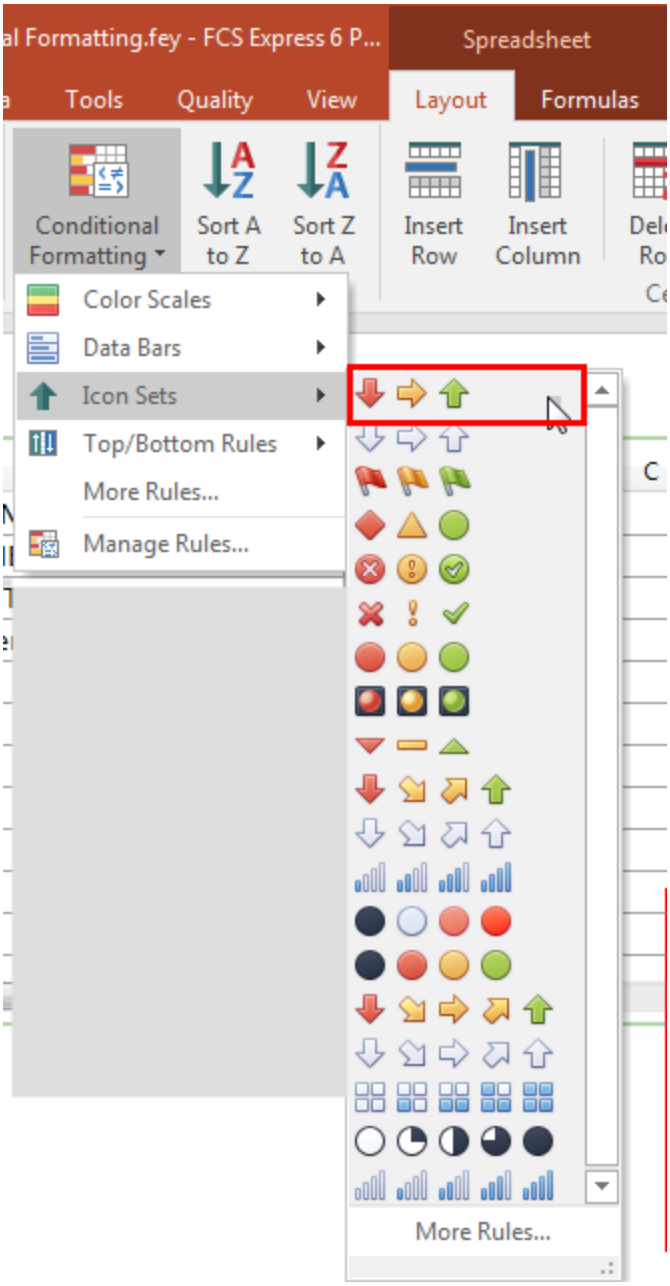

Figure T15.40 Icon Set with veritcal and horizontal arrows to define conditional formatting

You will notice that the data set has now been conditionally formatted based to use the icon set based on the default rules. We will now adjust the rules to perform a custom classification using the icon sets.

6. Click on the **Spreadsheet** Tab→**Layout** Tab→**Formatting and Sorting→Conditional Formatting.**

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7. Click on the drop down menu to **Managing Rules** (as seen enclosed in the red box in **Figure T15.41**).

Note: Here we will define the rules for the conditional formatting seen in **Figure T15.41**. Before using the **Manage Rules** feature make sure the column containing the data set to classify in the spreadsheet is selected. For example; in this tutorial, it will be column 'B'.

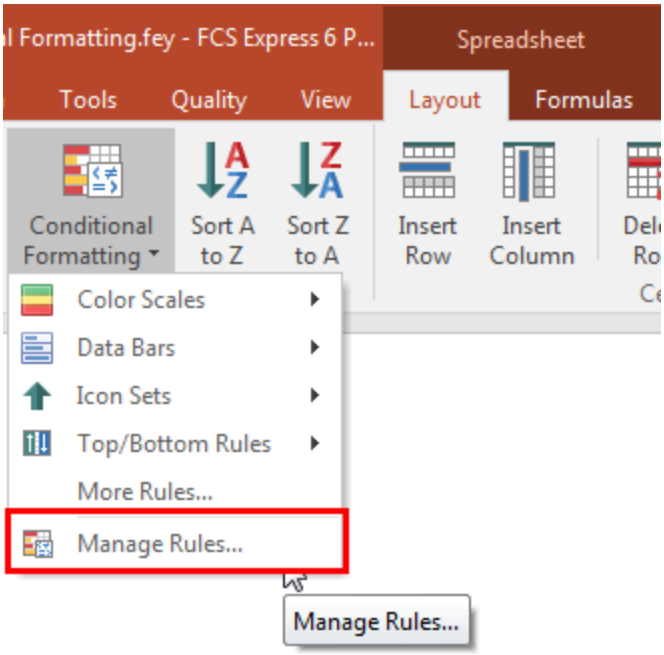

Figure T15.41 Managing Conditional Formatting Rules

8. Click on the words **Icon Set** underneath the **Rule** column in **Figure T15.42**

9. Click on the **Edit Rule...** button (shown with the cursor on it in **Figure T15.42**).

Note: During conditional formatting, we can also add additional rules. In this particular case, we will edit the rule as it is already started in this particular layout (shown by the cursor). This rule will apply to cells B1-B4 as is shown in **Figure T15.42**.

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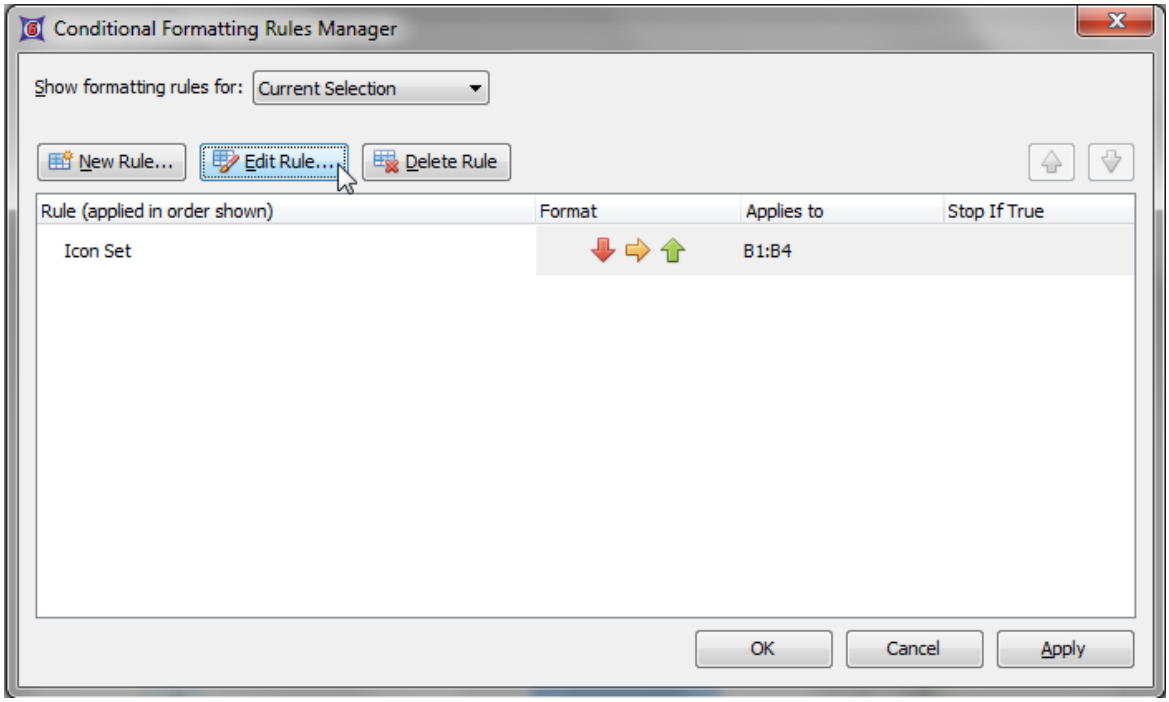

Figure T15.42 Conditional Formatting Rules Manager Dialog

10. Click on **Format all cells based on their values via icon set** as in **Figure T15.43** (shown highlighted in blue).

Note: Here you will add values according to each rule displayed by the icon set. Make sure you choose the rule type. In this tutorial, we are using **Format all cells based on their values via icon set**.

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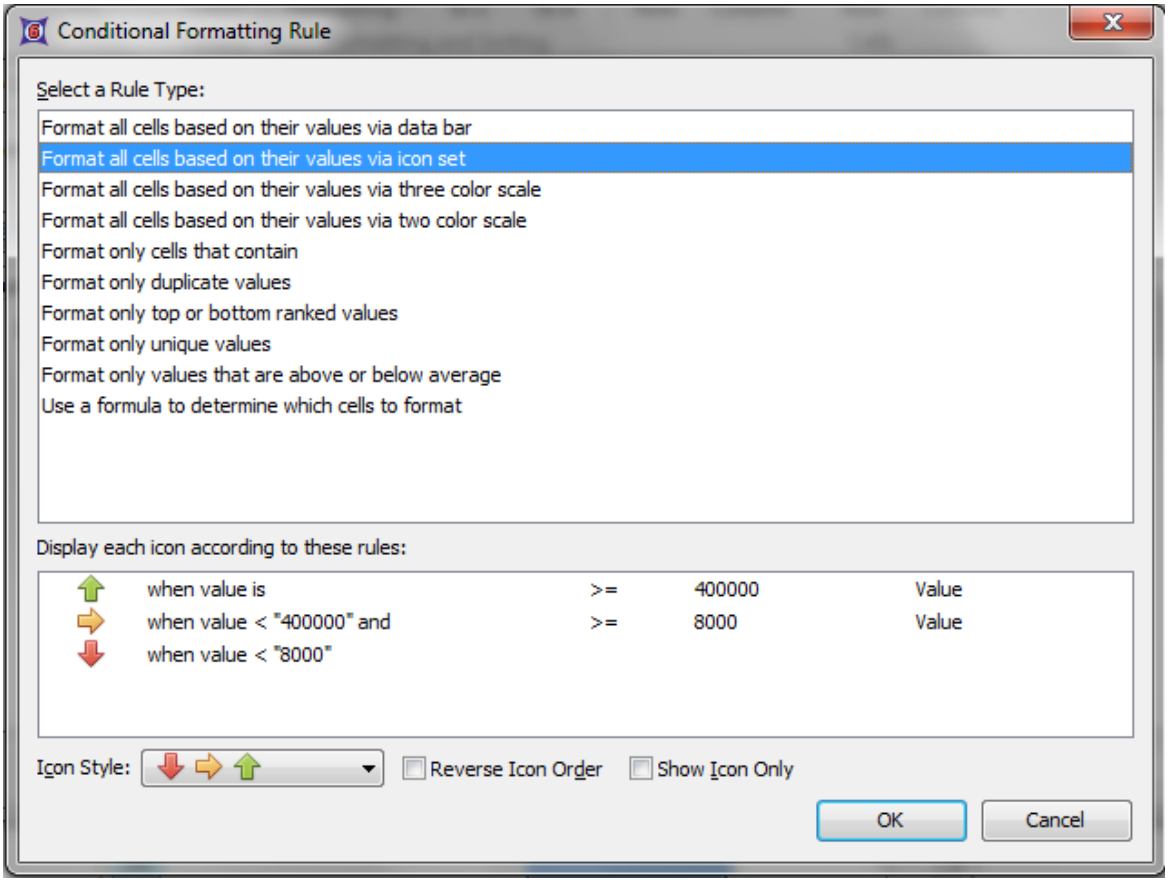

Figure T15.43 Conditional Formatting Rule Range Definitions

11. Click on the **percent** drop down in the Up Arrow category **Figure T15.45**.

12. Choose **value** as in **Figure T15.45.**

13. **Click** on the "0" in order to enter a new value.

14. Enter the value of **40000** to replace "0".

Note: In this case, we entered 40000 which defines greater than or ">" 40000 for the green arrow.

In order to define the second line for the right facing yellow arrow in the **Display each icon according to these rules:** window;

15. Click on **percent** in the second row.

16. Choose **value** from the drop down**.**

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17. Click on the "0" from the second line in the **Display each icon according to these rules:** window.

18. Enter **8000** as the numerical value for the yellow arrow.

Note: The yellow rule is defined by less than or "<" 40000 but greater than of ">" 8000.

Also Note: **The red arrow is then automatically defined** as less than or "<" 8000.

19. Click **OK**. The formatting rules dialog will appear as in **Figure T15.45**.

20. Click **Apply**.

21. Click **OK**.

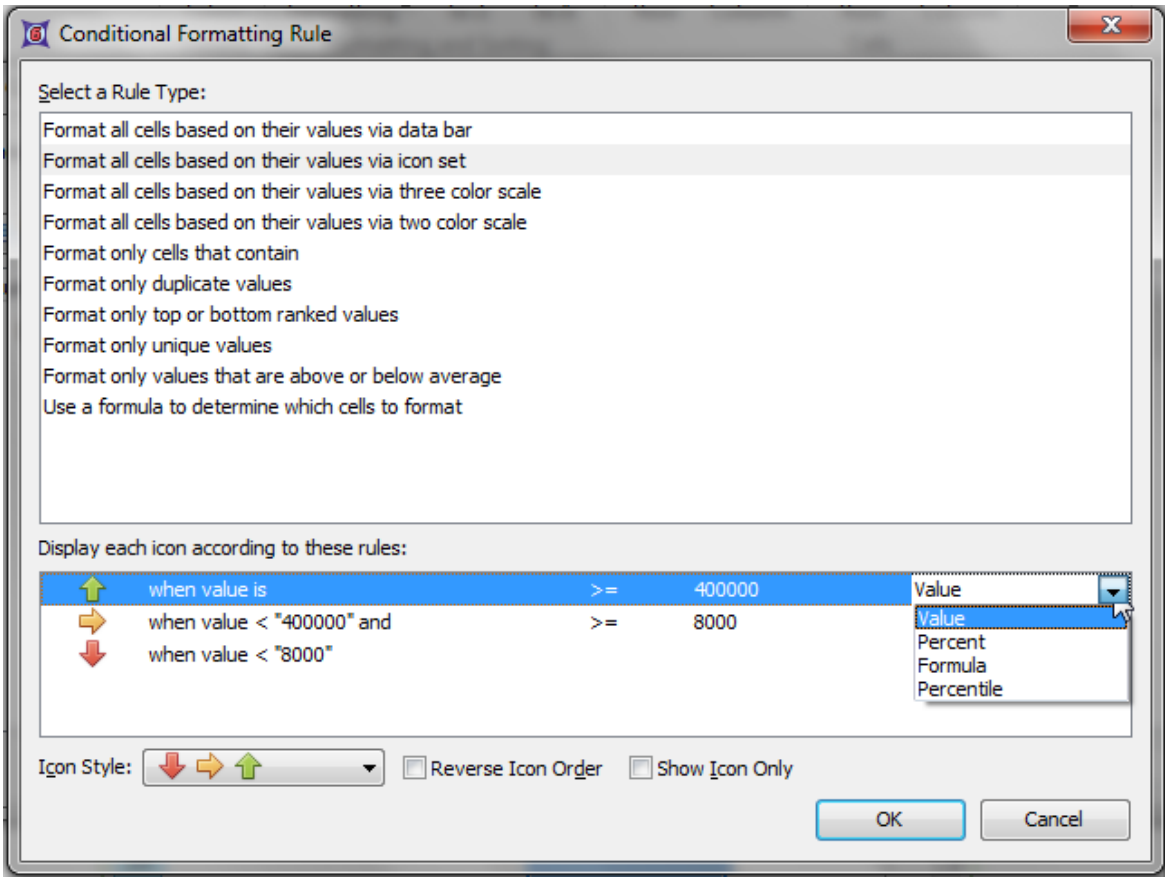

Figure T15.45 Conditional Formatting Rules Dialog

Note: you will see the conditional formatting defined in the spreadsheet shown in **Figure T15.46.** To see the changes in real-time you may type in a

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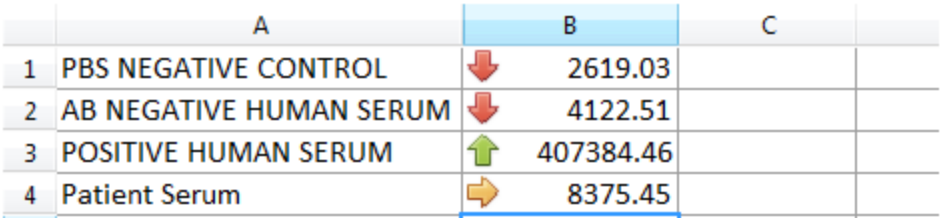
different value in column B or update the Lymphs gate on Page 1 or Page 2. The conditional formatting will change due to the values in the cells updating.



	A	B	C
1	PBS NEGATIVE CONTROL	2619.03	
2	AB NEGATIVE HUMAN SERUM	4122.51	
3	POSITIVE HUMAN SERUM	407384.46	
4	Patient Serum	8375.45	

Figure T15.46 Spreadsheet with conditional formatting

38.17 Antibody Titration - Spreadsheets Use Case 2

FCS Express 6 allows you to easily create summary data and plots that show your entire experimental results using **Spreadsheets** 414. Your spreadsheet(s) can contain information from data files, plots, or gates. When your gates change, so will the data in spreadsheets and any plot derived from them. Formulas 440 can be used also to calculate custom data as well.

In this tutorial, we will use spreadsheets and plots to aid in the visualization of data from antibody titration experiments. This tutorial will demonstrate, by selecting the ideal antibody concentration the best separation between positive and negative cells will be achieved.

We will examine data from a titration experiment where seven different amounts of CD64 were used to stain peripheral blood mononuclear cells. Because CD64 is constitutively expressed on only macrophages and monocytes, we will examine the ratio of the intensity of fluorescence staining on the monocyte versus lymphocyte population. The negative population will be defined by the lymphocytes and the positive population by the monocytes.

In the course of this example, we will:

- Create a new spreadsheet and populate it by manually entering information as well and dragging and dropping statistics from gates and data files and calculate a CD64 signal to noise ratio.
- Insert a scatter plot displaying the data calculated within the spreadsheet.

In the first exercise, we will create a spreadsheet 1653.

38.17.1 Creating an analysis Spreadsheet

For this tutorial, we will be using a layout file that has 2D density plots with defined gates from seven data files, each having a different amount of CD64 antibody. Please note you may skip ahead to the next section to insert a scatter with regression plot 1667 using a completed spreadsheet.

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1. Select the **File** tab→**Open From**→**Load Layout** dialog.
2. Open the layout **Titration.fey** found within the *FCS Express Sample Data* in a subfolder called **Spreadsheet and Titration**.

The following layout should appear as shown in Figure T17.1.

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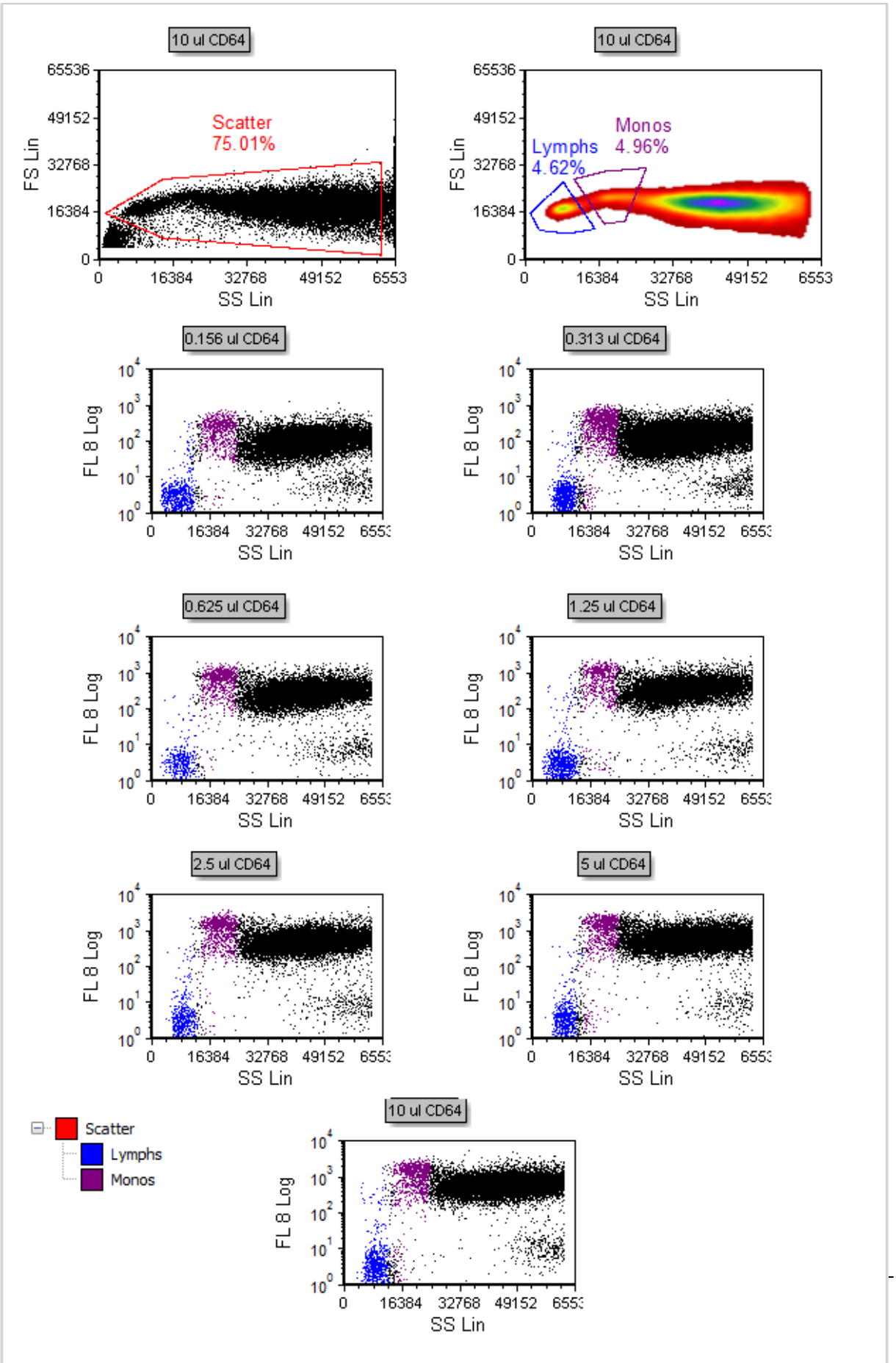

Figure T17.1 - Titration Layout

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In the upper left corner of the layout is a dot plot of Side Scatter versus Forward Scatter with a scatter gate, drawn in red. The *Scatter* gate has been defined to exclude very low and very high scatter signals. All the other plots on the layout are gated using this *Scatter* gate. Seven different volumes of CD64 were added to peripheral blood mononuclear cells and are shown in the seven color dot plots. To define the negative population for this titration experiment, a gate has been drawn on the lymphocyte population, shown in blue as the *Lymphs* gate, in the 2D plot of Side Scatter (SS Lin) versus CD64 (FL 8 Log). The positive population is the monocyte population which is defined in purple as the *Monos* gate. In the lower left corner of the layout is the **Gate View** window showing that the *Lymphs* and *Mono* gates are children of the *Scatter* gate, meaning the data is first gated using the *Scatter* gate and then the *Lymphs* and *Mono* gates are applied.

We will use a spreadsheet to calculate the values of CD64 signal to noise ratio for each of the seven volumes of CD64 used. The values will be defined as the ratio of the median CD64 fluorescent intensity of the monocyte population (the positive population) divided by the median CD64 fluorescent intensity of the lymphocyte population (the negative population).

3. Select the **Insert→Spreadsheet→New** command (Figure T17.2).

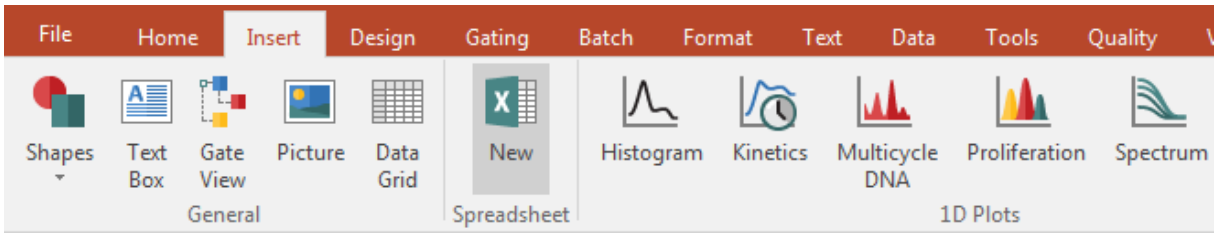

Figure T17.2 - Opening new spreadsheet from ribbon

The **Create new spreadsheet** window now appears on the layout.

4. Enter "Titration" in **Name** field.
5. Click **OK** to close window.

The new spreadsheet appears on the layout.

6. Click and drag spreadsheet border to reposition.
7. Release the button when spreadsheet is positioned where desired.

For this exercise, we will populate the spreadsheet with the following information:

Column A - amount of antibody added,

Column B - median CD64 fluorescent intensity of the monocyte population, and

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Column C - median CD64 fluorescent intensity of the lymphocyte population.

8. Left-click on cell **A1** of spreadsheet.
9. Type "Ab Added".
10. Press Enter. Cell **A2** will be outlined in blue (Figure T17.3).

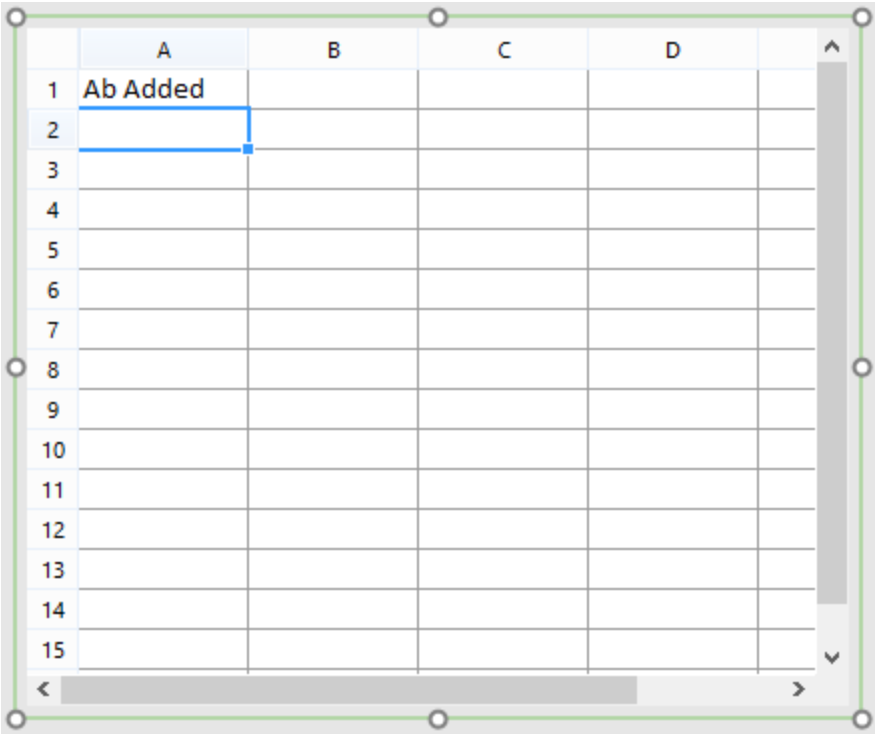

Figure T17.3 - Populating cell A1 of spreadsheet

11. Type "0.156".
12. Press Enter. Cell **A3** will be outlined in blue.
13. Type "0.313".
14. Press Enter. Cell **A4** will be outlined in blue.
15. Type "0.625".
16. Press Enter. Cell **A5** will be outlined in blue.
17. Type "1.25".
18. Press Enter. Cell **A6** will be outlined in blue.
19. Type "2.5".
20. Press Enter. Cell **A7** will be outlined in blue.

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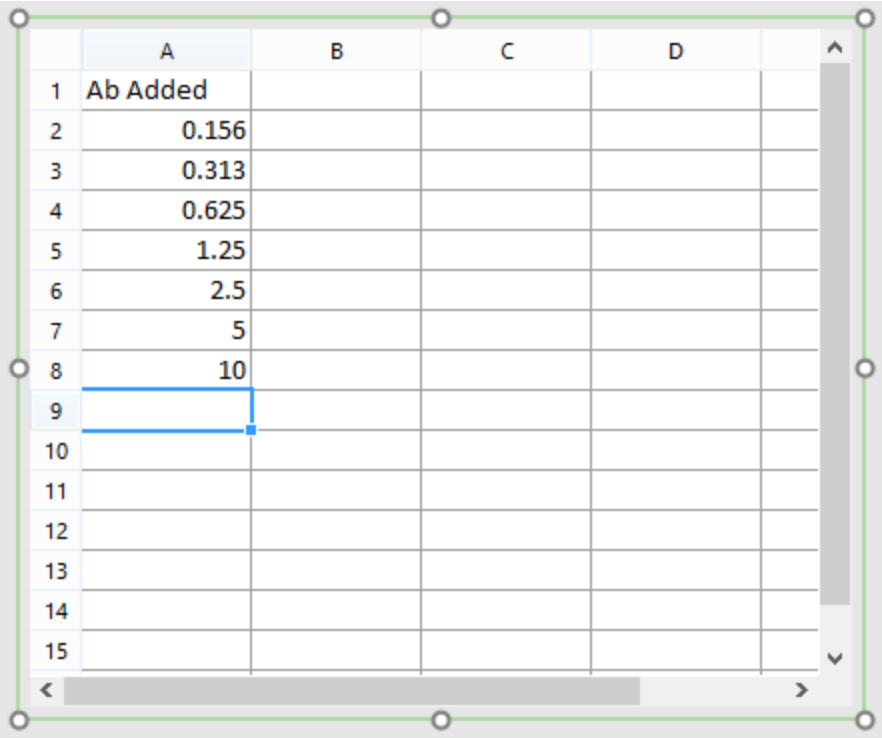
21. Type "5".
22. Press Enter. Cell **A8** will be outlined in blue.
23. Type "10".
24. Press Enter. Cell **A9** will be outlined in blue.

Cells A2-8 should be populated with antibody amounts (Figure T17.4).



	A	B	C	D
1	Ab Added			
2	0.156			
3	0.313			
4	0.625			
5	1.25			
6	2.5			
7	5			
8	10			
9				
10				
11				
12				
13				
14				
15				

Figure T17.4 - Column A populated properly

To populate cells from columns B, we will drag and drop the **SS Lin vs FL8 Log Color Dot** plots for each respective antibody amount.

25. Type "Monos Median".
26. Press Enter. Cell **B2** will be outlined in blue.
27. Click plot for **0.156 ul CD64**
28. Click border of plot to cell **B2** of spreadsheet (Figure T17.5).

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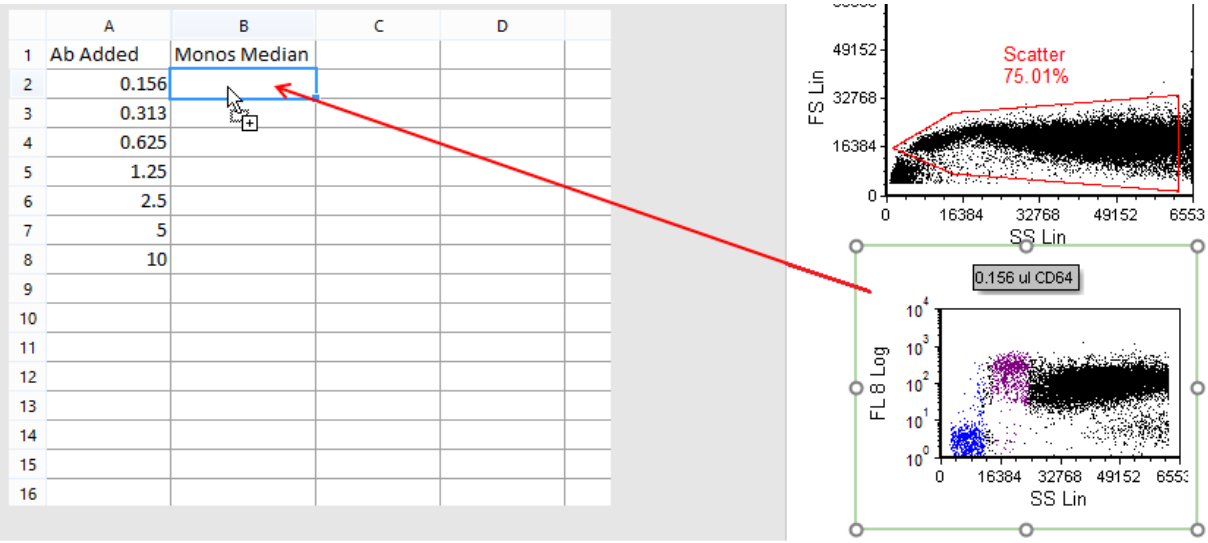

Figure T17.5 - Drag and Drop Plot to cell B2 of spreadsheet

29. Release mouse button.

30. Select **Statistic token** from **Paste Special** window (Figure T17.6).

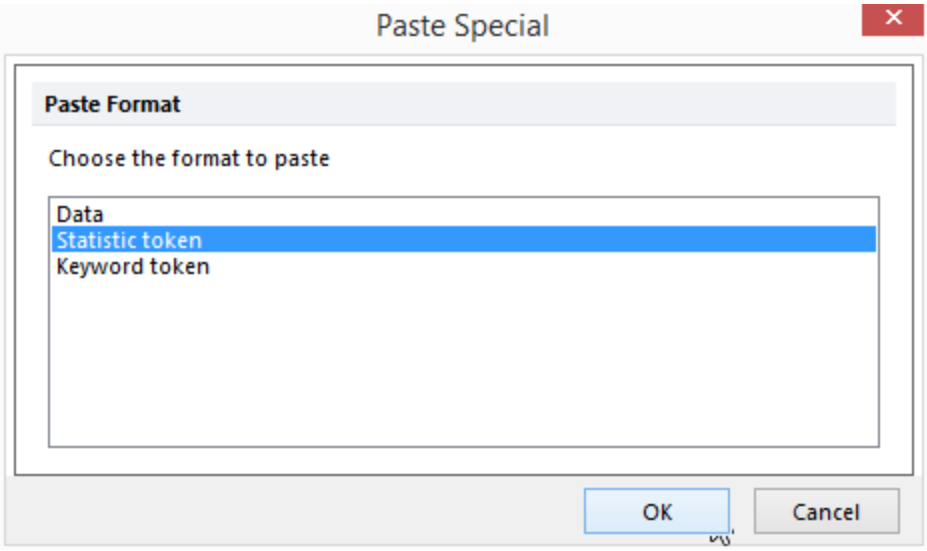

Figure T17.6 - Paste Special window to insert Statistic token

The **Statistic** page of the **Edit Statistic** dialog now appears (Figure T17.7).

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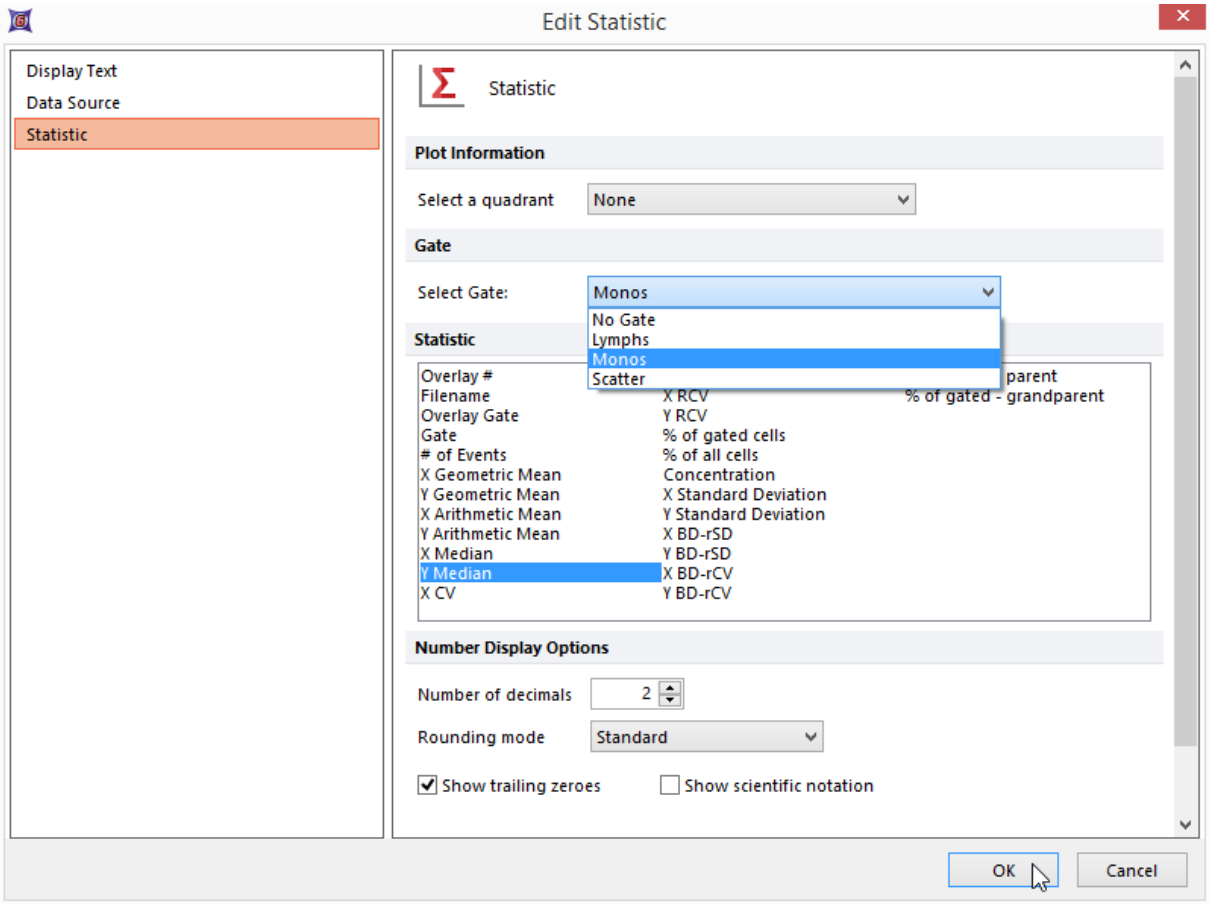

Figure T17.7 - Edit Statistic information for Monos

31. Click **OK.**
32. Select **Monos** from the **Select Gate:** drop down list.
33. Select **Y Median** from the **Statistics** list box.
34. Select **OK**.
35. Repeat **Steps 27 - 34** for the following color dot plots and spreadsheet cells:

0.313 ul CD64 to **B3**, **0.625 ul CD64** to **B4**, **1.25 ul CD64** to **B5**, **2.5 ul CD64** to **B6**, **5 ul CD64** to **B7**, and

10 ul CD64 to **B8**.

The spreadsheet should now appear as in Figure T17.8.

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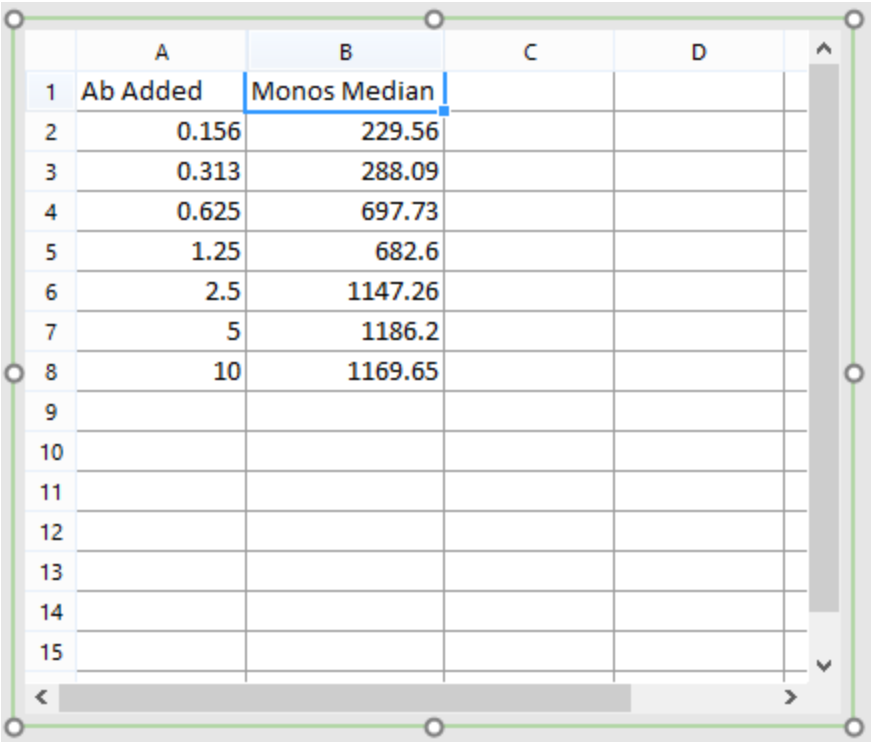

	A	B	C	D
1	Ab Added	Monos Median		
2	0.156	229.56		
3	0.313	288.09		
4	0.625	697.73		
5	1.25	682.6		
6	2.5	1147.26		
7	5	1186.2		
8	10	1169.65		
9				
10				
11				
12				
13				
14				
15				

Figure T17.8 - Columns A and B populated properly

Column C will be populated by dragging and dropping data files from the data list onto the spreadsheet.

36. Click **Data** tab→**Organize Data Sets**→**Data List** command.
37. Press **Ctrl+A** to select all data files.
38. Click and hold mouse button on any data file in data list.
39. Drag data files to cell **C1** in spreadsheet (Figure T17.9).

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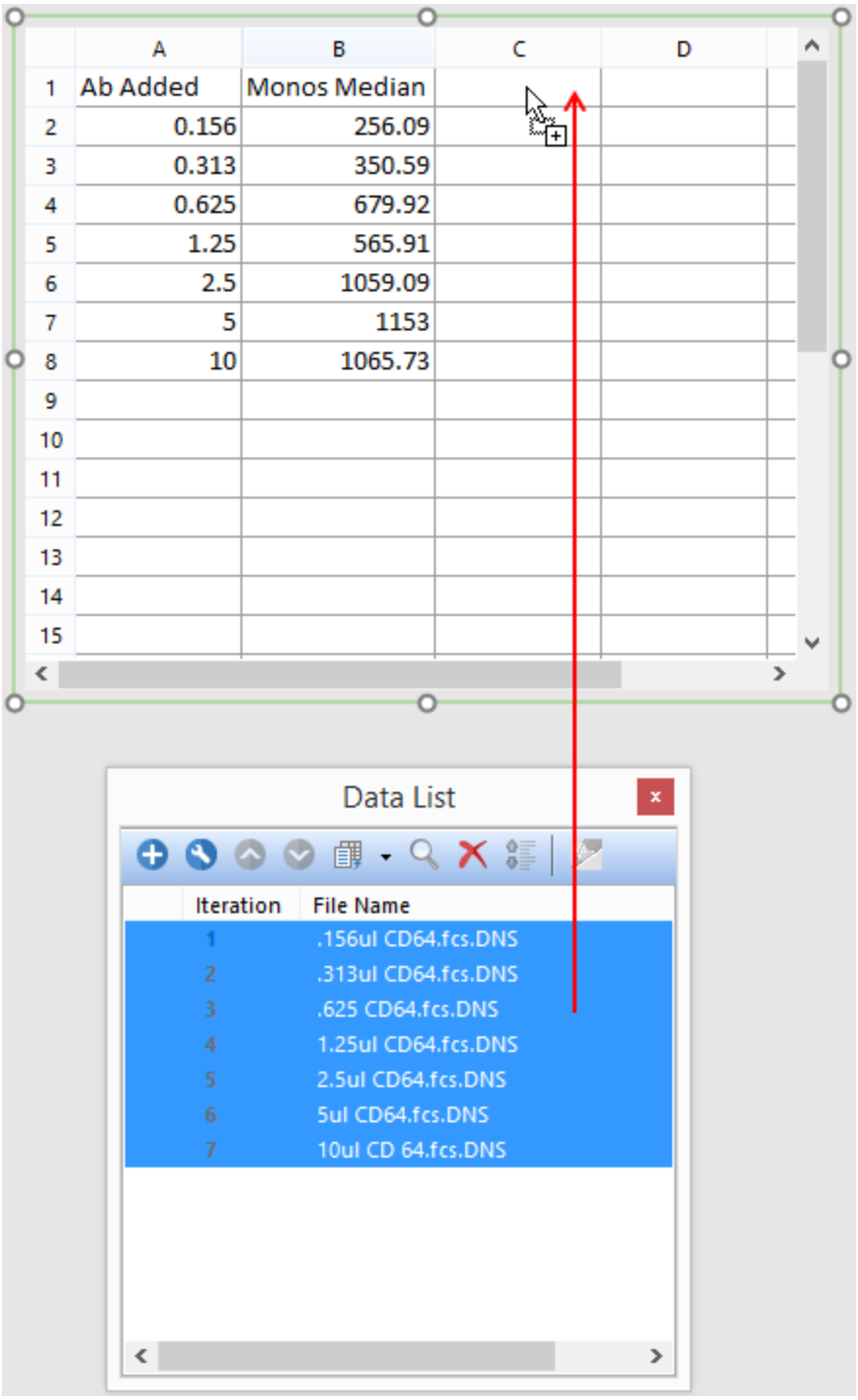

Figure T17.9 - Drag and Drop Files from Data List to spreadsheet

40. Release mouse button.

The **Statistic** category of the **Edit Statistic** dialog now appears.

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41. Select **FL 8 Log** from **Parameter** drop down list under **Data File Information**.
42. Select **Lymphs** from **Select Gate:** drop down list.
43. Select **Median** from **Statistic** list box.
44. Click **OK** (Figure T17.10).

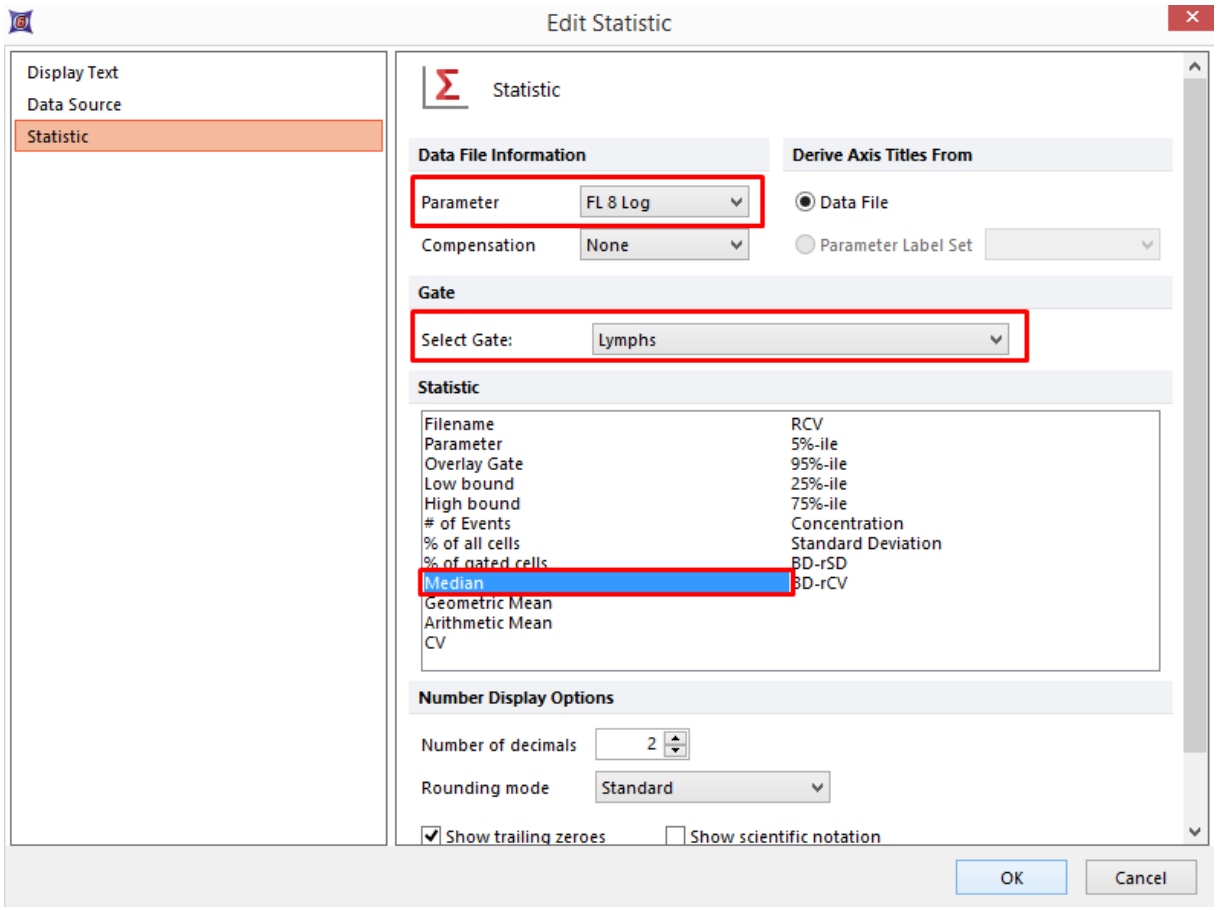

Figure T17.10 - Edit Statistic window for Lymphs

45. Click cell **C1**.
46. Type "Lymphs Median".
47. Press Enter.

The spreadsheet should appear similar to Figure T17.11 below.

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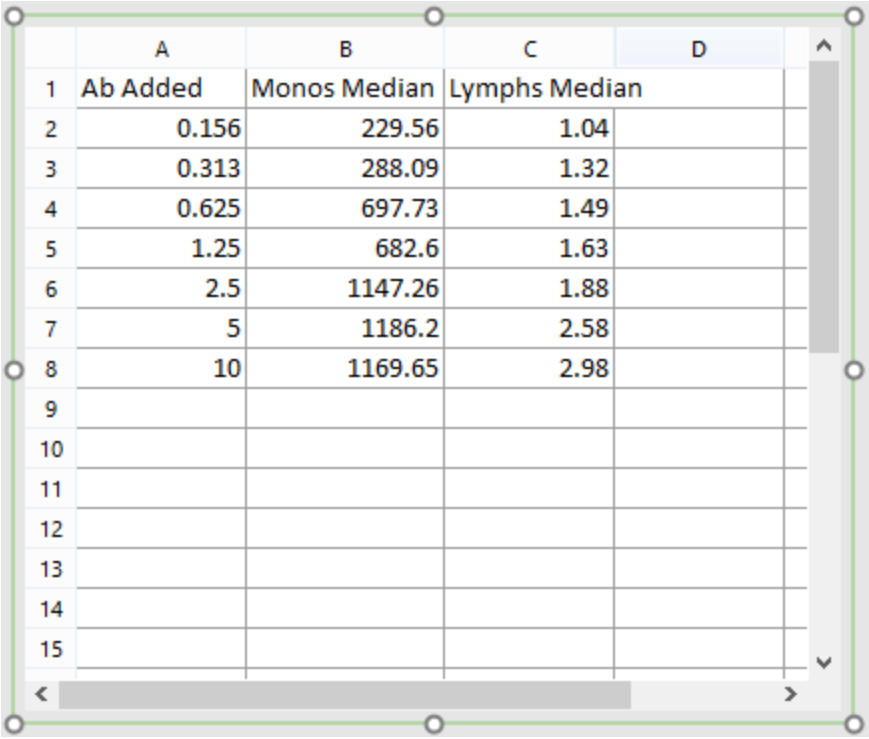

	A	B	C	D
1	Ab Added	Monos Median	Lymphs Median	
2	0.156	229.56	1.04	
3	0.313	288.09	1.32	
4	0.625	697.73	1.49	
5	1.25	682.6	1.63	
6	2.5	1147.26	1.88	
7	5	1186.2	2.58	
8	10	1169.65	2.98	
9				
10				
11				
12				
13				
14				
15				

Figure T17.11 - Columns A, B, and C populated properly

Alternatively, gates may be dragged and dropped from each dot plot to the spreadsheet.

- Click gate of interest from plot and drop onto appropriate cell.
    - For example, click and drag purple **Monos** gate from color dot plot - **0.156 ul CD64 SS Lin vs FL 8 Log** to cell **B2** of spreadsheet.
- Release mouse.
- Select **Y Median** from **Statistic** list box.
- Click **OK**.

Values in the spreadsheet will update in real-time as gating is changed.

- Click and hold red **Scatter** gate in upper left plot on layout - **10 ul CD64 SS Lin vs FS Lin**.
- Move mouse and observe values changing in spreadsheet as **Scatter** gate is moved.
- Release mouse.
- Press **Ctrl+Z** to undo gate move.

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To resize column width(s), follow one of the two commands below:

- Hover mouse cursor over right border of column header until resizing cursor appears ( )→double click (left in Figure T17.13 below).
- **Spreadsheet** tab group→**Layout** tab→**Format** drop down list→**AutoFit Column Width** (right in Figure T17.12 below).

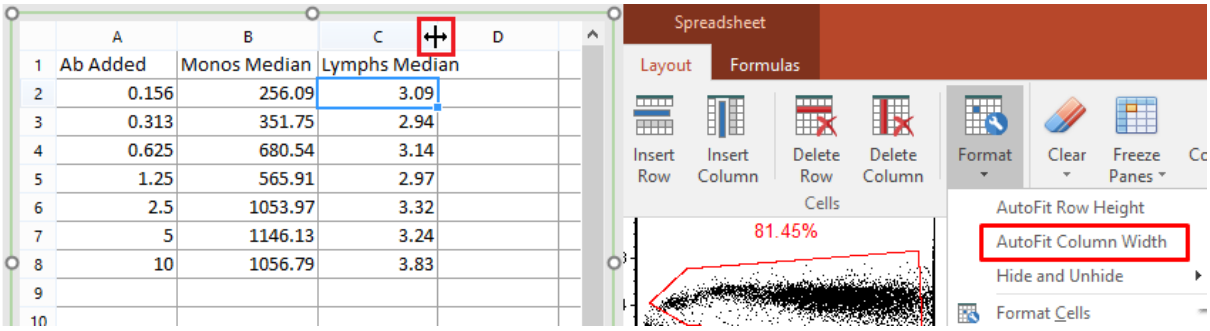

Figure T17.12 - Resizing single column width (left) or autofitting widths for entire spreadsheet (right)

We will now calculate the ratios of the CD64 staining for the Monocyte and Lymphocyte populations within the spreadsheet.

48. Add page to layout by using **Insert→New Page** command.
49. Use **View→Navigators→Layout Navigator** command to open **Layout Navigator**.
50. Drag and drop spreadsheet "Titration" from Page 1 to Page 2 within Layout Navigator (Figure T17.13).

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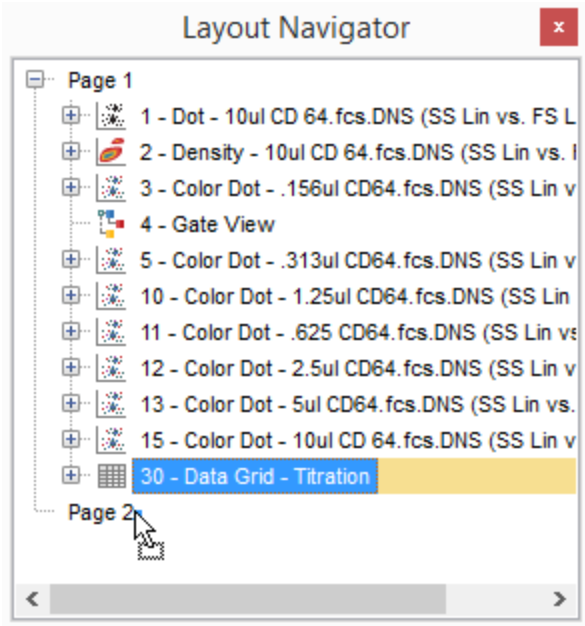

Figure T17.13 - Moving Spreadsheet to Page 2

51. Select the **View→Multiple Pages→Two Pages (Vertical)** command.
52. Go to Page 2 of layout.
53. Select the spreadsheet.
54. Click cell **D1**.
55. Type "M/L Ratio".
56. Press Enter. Type "=" into cell **D2**.
57. Click cell **B2.**
58. Type "/".
59. Click cell C2.
60. Press Enter.
61. Click cell **D2**.
62. Hold **Shift** and click cell **D8**.
63. Release mouse button.
64. **Ctrl+C**.

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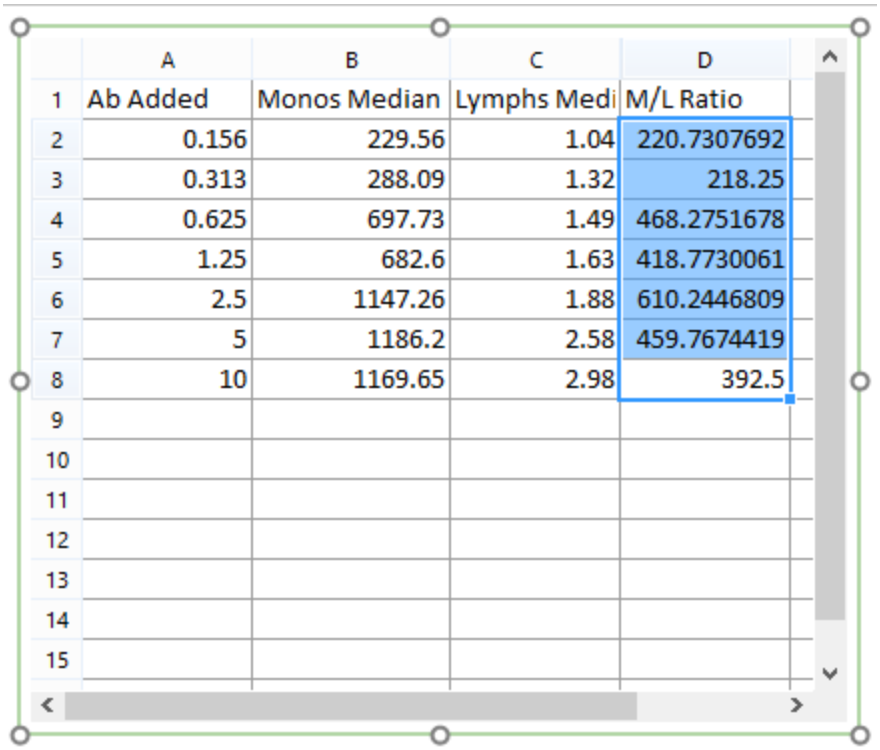
65. **Ctrl+D**.

The spreadsheet should now appear similar to Figure T17.14.



	A	B	C	D
1	Ab Added	Monos Median	Lymphs Medi	M/L Ratio
2	0.156	229.56	1.04	220.7307692
3	0.313	288.09	1.32	218.25
4	0.625	697.73	1.49	468.2751678
5	1.25	682.6	1.63	418.7730061
6	2.5	1147.26	1.88	610.2446809
7	5	1186.2	2.58	459.7674419
8	10	1169.65	2.98	392.5
9				
10				
11				
12				
13				
14				
15				

Figure T17.14 - Columns A, B, C, and D properly populated

In the next section, we will create a regression curve from a spreadsheet.

38.17.2 Creating a regression curve from a Spreadsheet

The remainder of this tutorial will focus on creating a regression analysis of the data by using a scatter plot with regression plot with Spreadsheet based data. You can use the layout you created in the previous section or open **Titration with Complete Spreadsheet.fey** in *FCS Express Sample Data* within the sub folder called **Spreadsheet and Titration**.

1. Click on spreadsheet.
2. **Insert→2D Plots→Scatter with regression**.
3. Click on blank space on page 2 of layout.

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Plot should now appear where clicked similar to Figure T17.15 below.

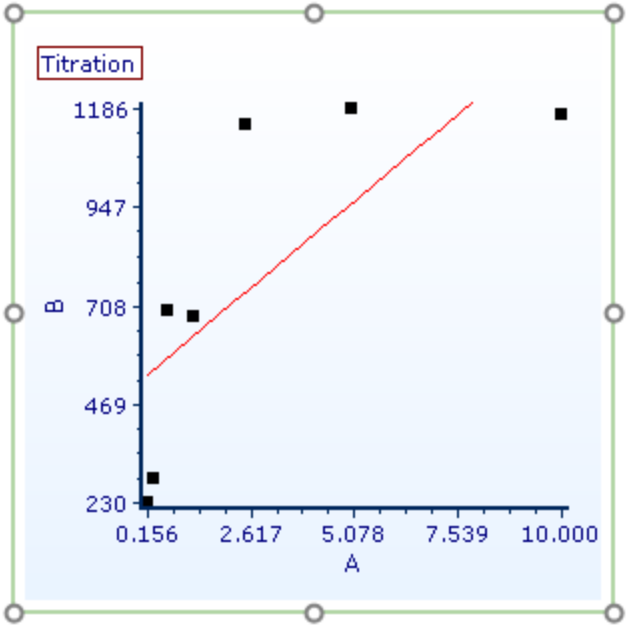

Figure T17.15 - Scatter Plot with Regression Unedited

The 2D scatter with regression plot using the titration data in the spreadsheet appears. Notice that the plot contains points in black that represent the points from Columns A (X-axis) and B (Y-axis) of the spreadsheet. The red line overlay is a regression overlay. The default regression fit for all scatter with regression plots is a linear fit. In the following steps we will adjust the plot to display the correct data from the spreadsheet and a more appropriate Michaelis-Menten regression fit. We will also start by adjusting the axis range to more appropriately fit the points within the plotted region.

4. Double-click plot to open **Formatting** dialog.

5. In **Overlays**, change **Y Parameter** to **D** for **2D Unbinned Overlay Options**. This will change the data used from Column A "Monos Median" to Column D "M/L Ratio"(Figure T17.16).

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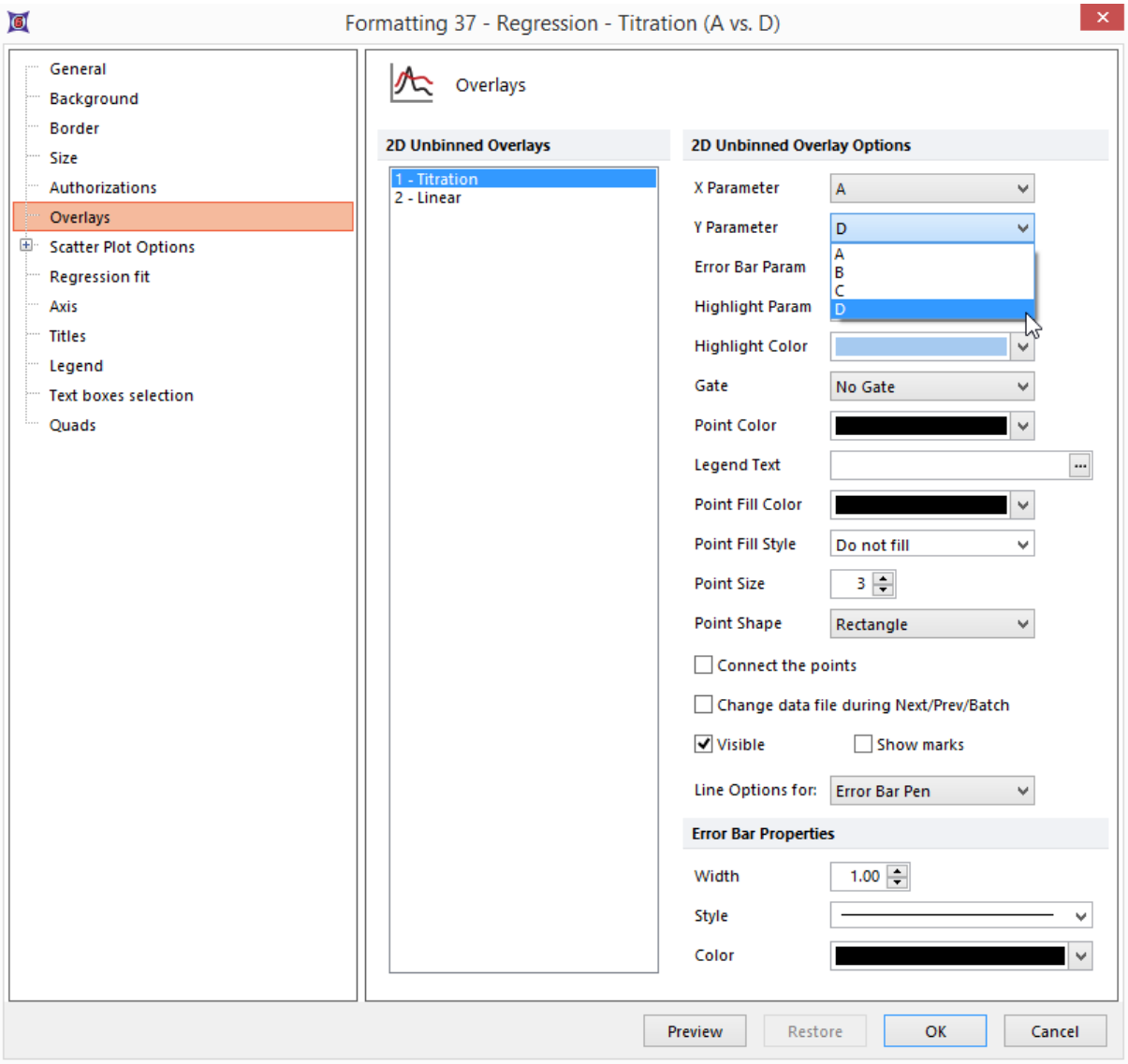

Figure 17.16 - Changing Y Parameter

6. Select the **Axis** category.

7. Select **Y axis**, shown highlighted in blue in Figure T17.17A.

8. Enter "**150**" in the **Minimum Range** and "**750**" in the **Maximum Range** fields, shown highlighted in blue (Figure T17.17A).

9. Select **X axis**, shown highlighted in blue in Figure T17.17B.

10. Enter "**12**" in the **Maximum Range** window, shown highlighted in blue (Figure T17.17B).

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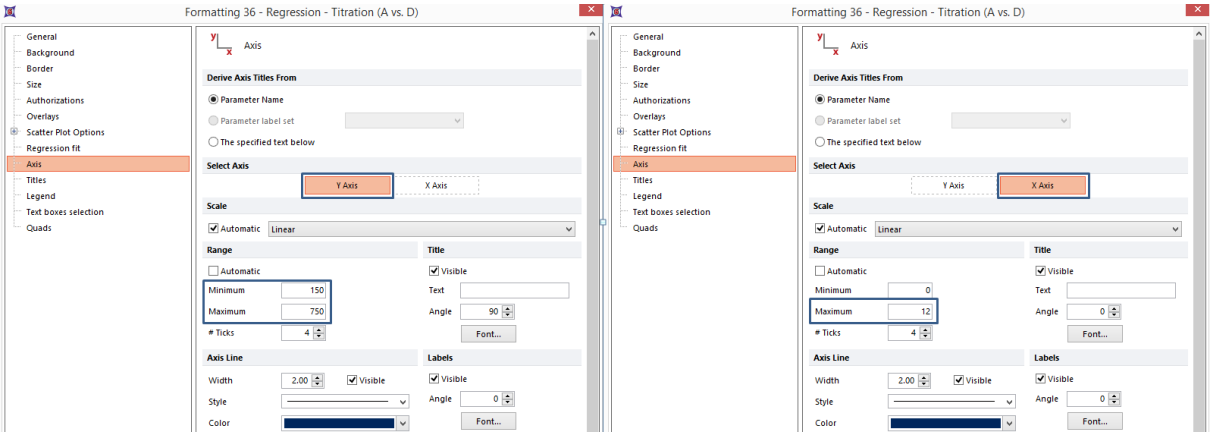

Figure T17.17A - Changing Y-Axis Scale (left); B - Changing X-Axis Scale

11. Select **Regression fit** category.
12. Choose **MichMenten** from **Regression Type** drop down list.
13. Confirm **Number of regression dots** is set to **30** (Figure T17.18).

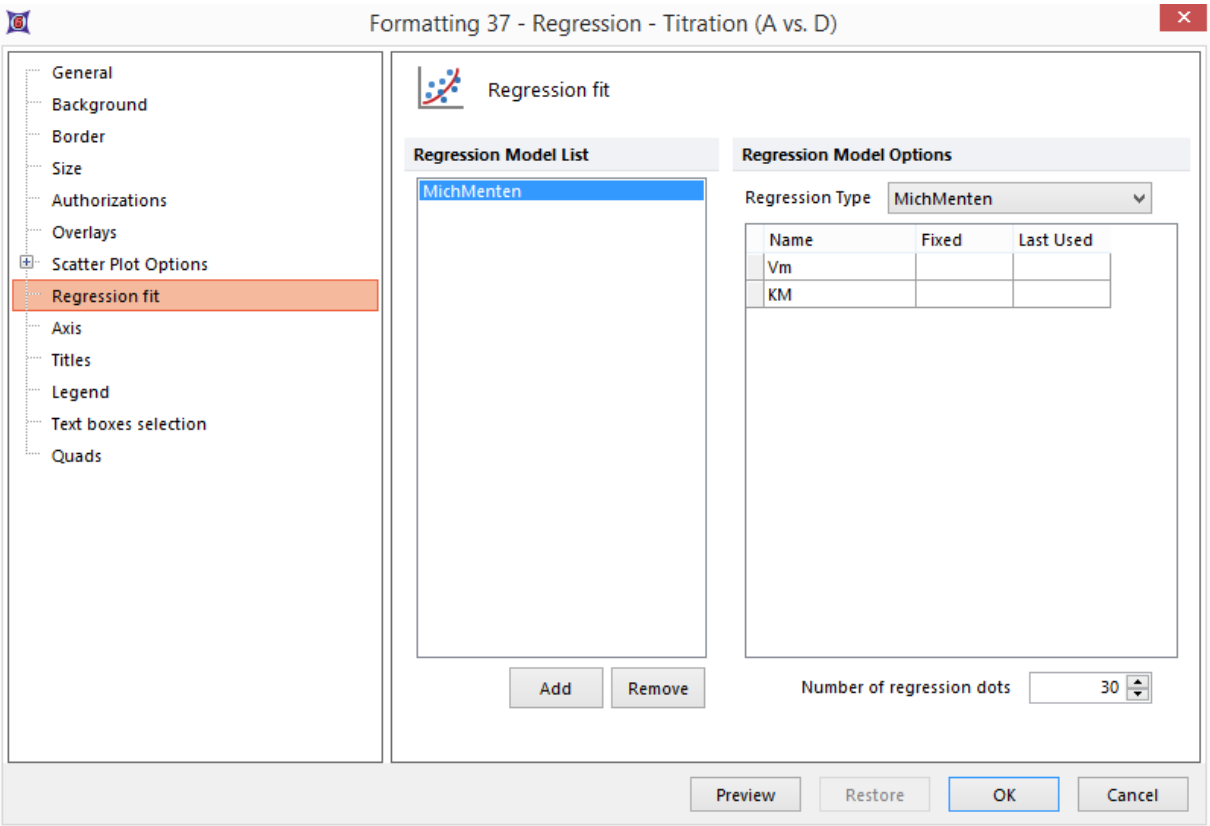

Figure T17.18 - Regression Fit Settings

14. Click **OK**.

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The scatter plot should now appear similar to Figure T17.19

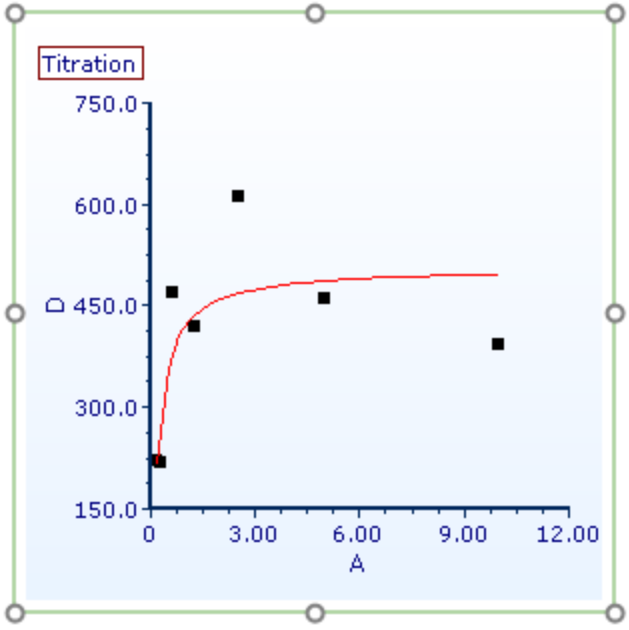

Figure T17.19 - Modified Scatter with Regression Plot

15.Click cell **E1**.

16. In **Data List**, **Ctrl+A**.

17. Drag and drop data files to **E1** (Figure T17.20).

18.Choose **Statistic Token** from the Paste Special dialog.

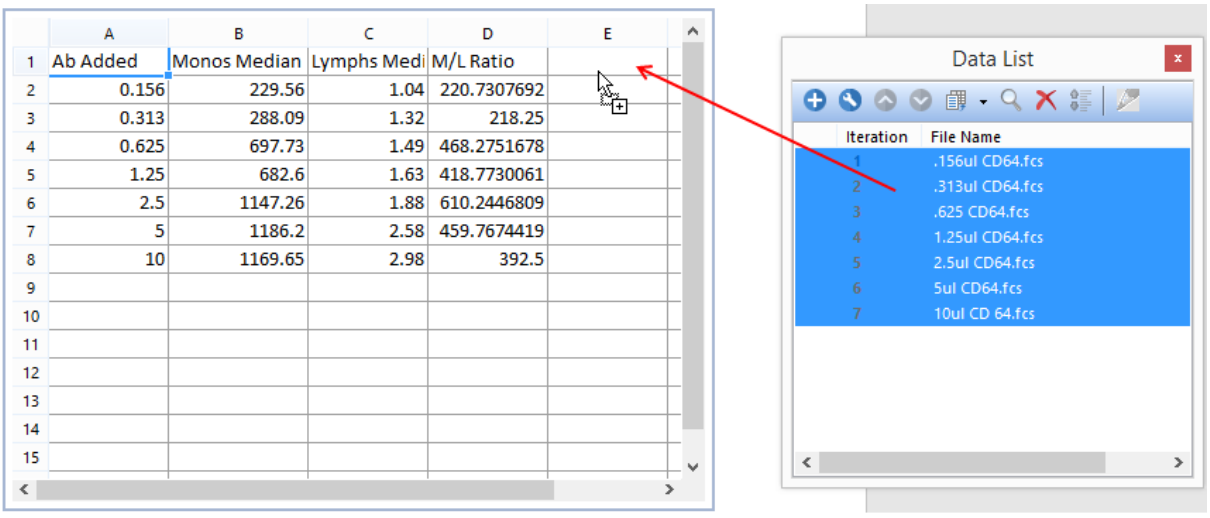

Figure T17.20 - Drag and Drop Data Files to E1

The **Statistic** page of the **Edit Statistic** dialog now appears.

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19. Select **FL 8 Log** from **Parameter** drop down list under **Data File Information**.
20. Select **Monos** from **Select Gate:** drop down list.
21. Select **CV** from **Statistic** list box.
22. Click **OK** (Figure T17.21).

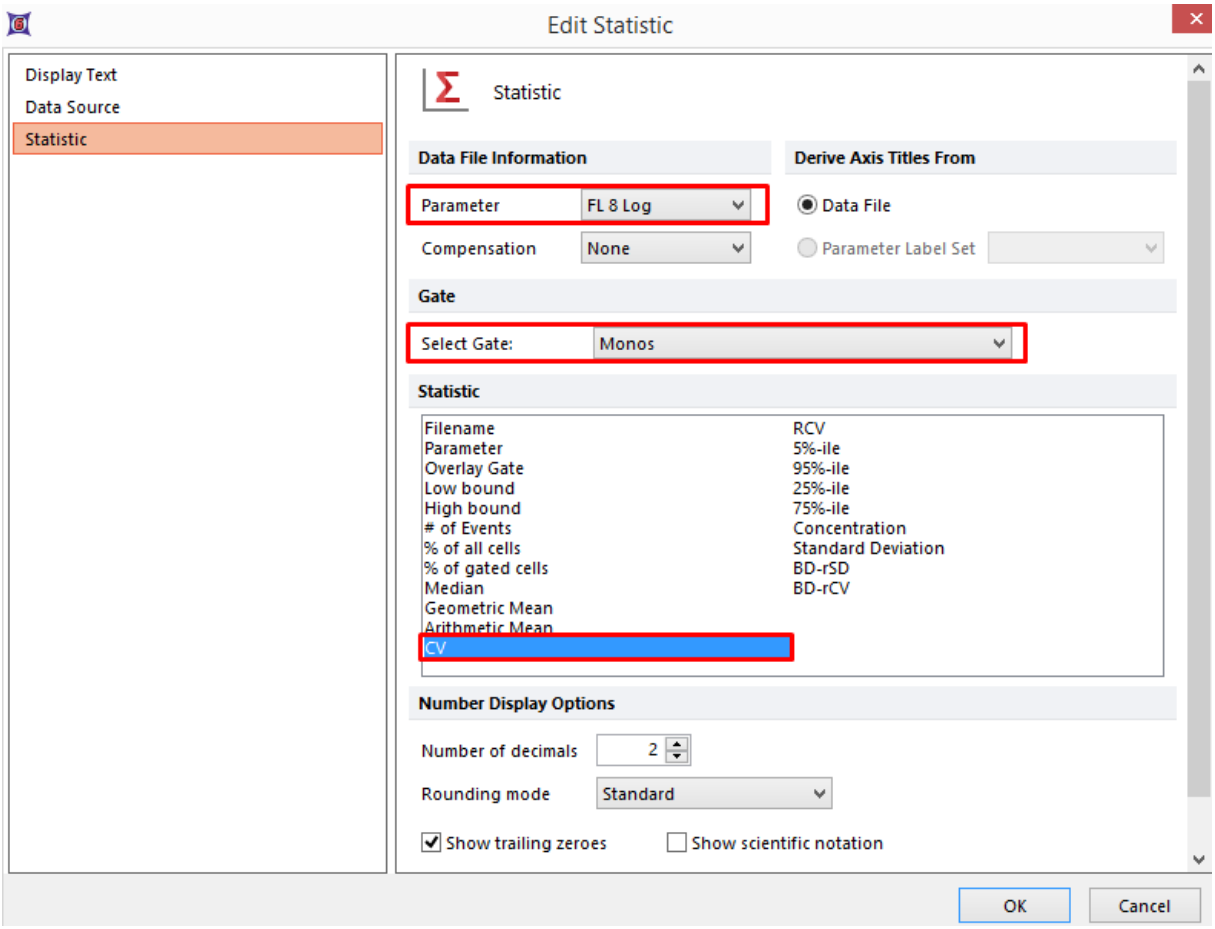

Figure T17.21 - Edit Statistics for Monos CV

23. Click cell **E1**.
24. Type "Monos CV".
25. Press Enter.

The spreadsheet should now appear as Figure T17.22 below.

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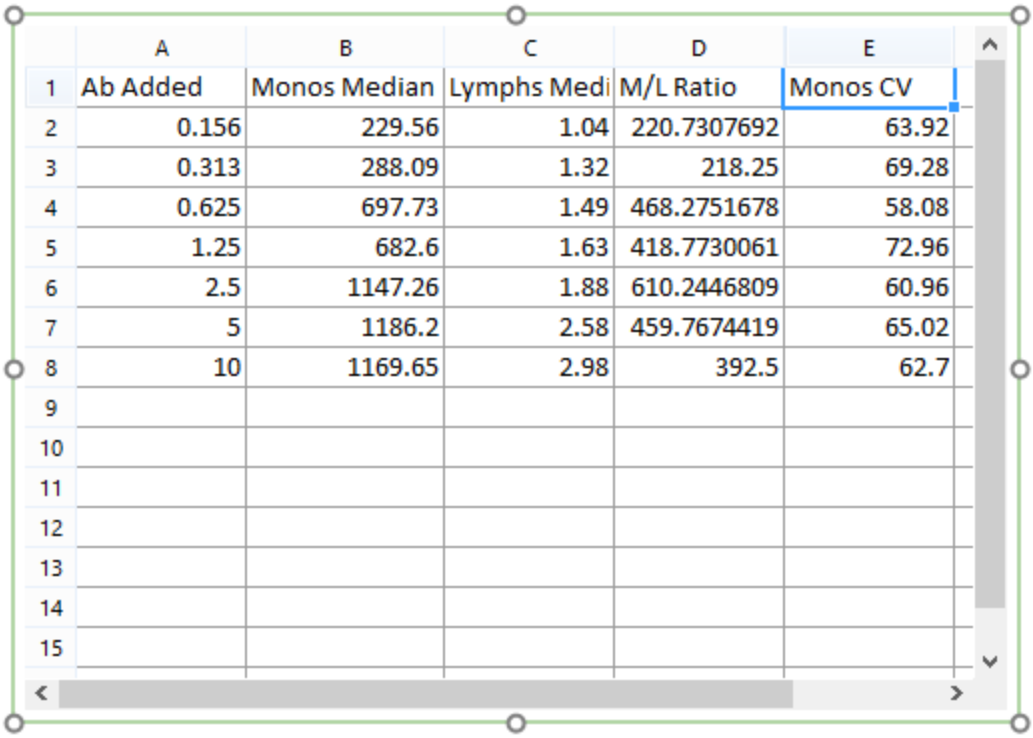

	A	B	C	D	E
1	Ab Added	Monos Median	Lymphs Medi	M/L Ratio	Monos CV
2	0.156	229.56	1.04	220.7307692	63.92
3	0.313	288.09	1.32	218.25	69.28
4	0.625	697.73	1.49	468.2751678	58.08
5	1.25	682.6	1.63	418.7730061	72.96
6	2.5	1147.26	1.88	610.2446809	60.96
7	5	1186.2	2.58	459.7674419	65.02
8	10	1169.65	2.98	392.5	62.7
9					
10					
11					
12					
13					
14					
15					

Figure T17.22 - Columns A, B, C, D, and E populated properly

28. Double-click plot.

29. Select the **Overlays** category.

30. Select **E** from the **Error Bar Param** drop-down list. This will change the data used from Column A "Monos Median" to Column E "Monos CV". (Figure T17.23).

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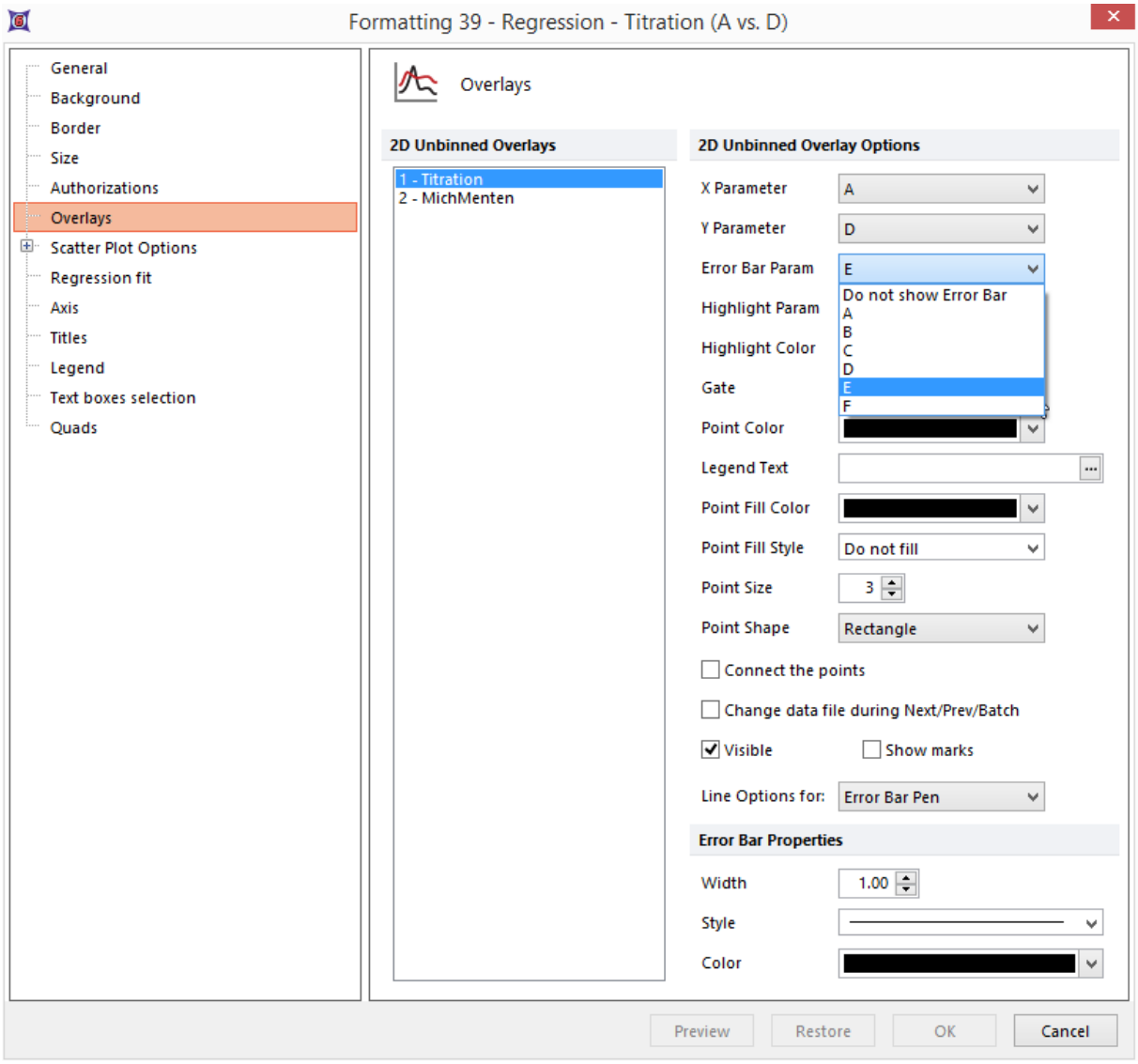

Figure T17.23 - Adding Error Bar from Overlays

31. Click **OK**.

The titration Scatter with Regression plot updates to reflect the addition of black error bars using the Monos CV data, as shown in Figure T17.24. Note that as you adjust any of the gates in the layout the raw data points, regression line, and error bars will all update accordingly and in real-time.

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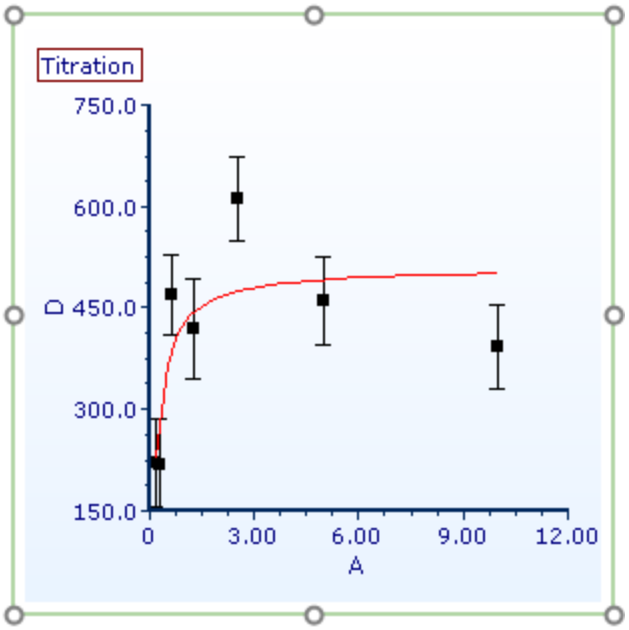

Figure T17.24 - Scatter with Regression Plot, error bars added

We will now add a Regression Parameter Statistics default window to the layout, to obtain the Vmax (Vm in Fig. T17.24b, below) and summarize the fit.

32. Right-click on the Scatter with Regression plot.

33. Select **Statistics**> **Regression Parameter Statistics** from the pop-up menu.

A Regression Parameter Statistics window has now been inserted on the layout (Figure T17.24b).

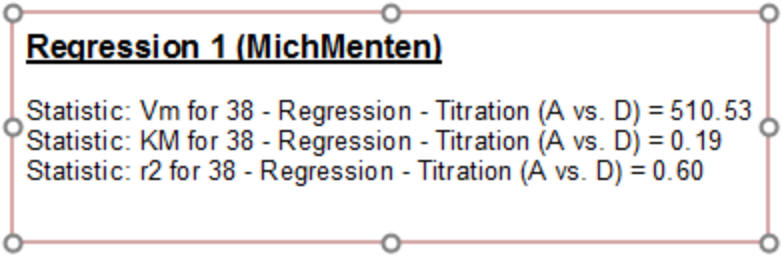

Figure T17.24b - Regression Parameter Stats Window inserted from Scatter with Regression Plot

38.17.3 Interpolating values from the regression

We will now interpolate the X-value for the Vmax calculated from the Scatter with Regression Plot in the previous section, using the "RegressionPlotYToX" Custom Token function.

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Please ensure that the previous section of the tutorial has been completed before proceeding.

1. Select the **View** tab→**Tokens**→**Custom Tokens** command.
2. Click on the blue plus in the Custom Tokens window to Create a new Custom Token (Fig. T17.25, ❶).
3. Type "X from Vmax" to descriptively title the new Custom Token (Fig. T17.25, ❷).
4. Type an equals sign ("=") in the Custom Token formula pane of the Custom Tokens window (Fig. T17.25, ❸).
5. Right-click and select **Insert Function> Regression > RegressionPlotYtoX** from the pop-up menu (Fig. T17.25, ❹).

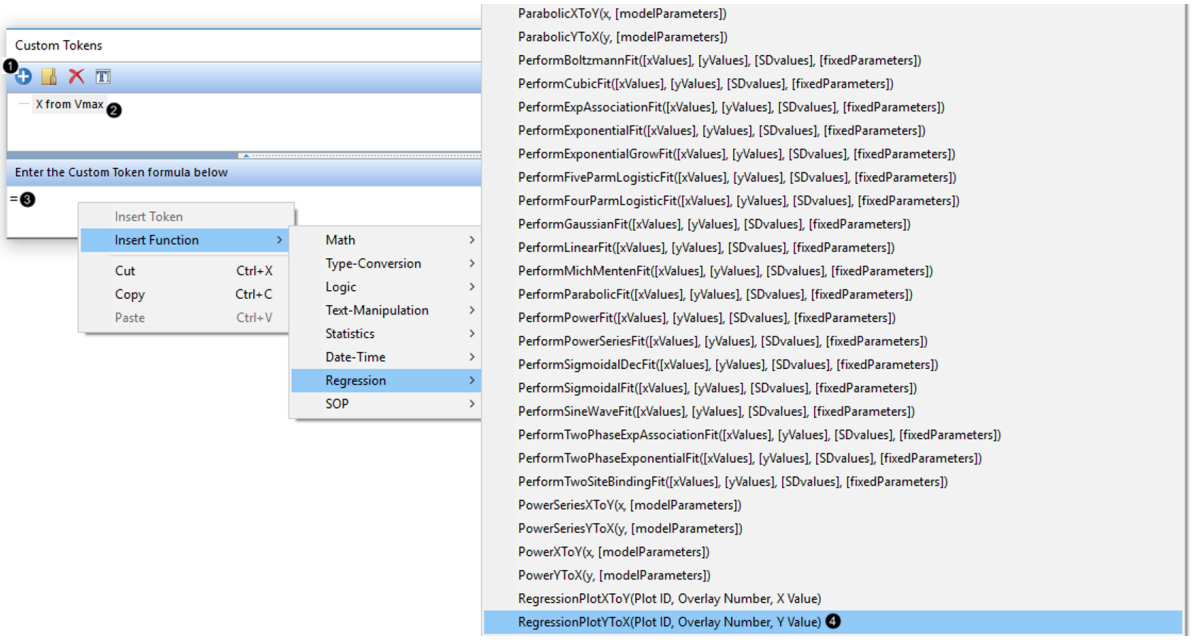

Figure T17.25. Inserting a Custom Token function to interpolate X from Y from the regression

The Custom Token formula pane now appears as in Fig. T17.26.

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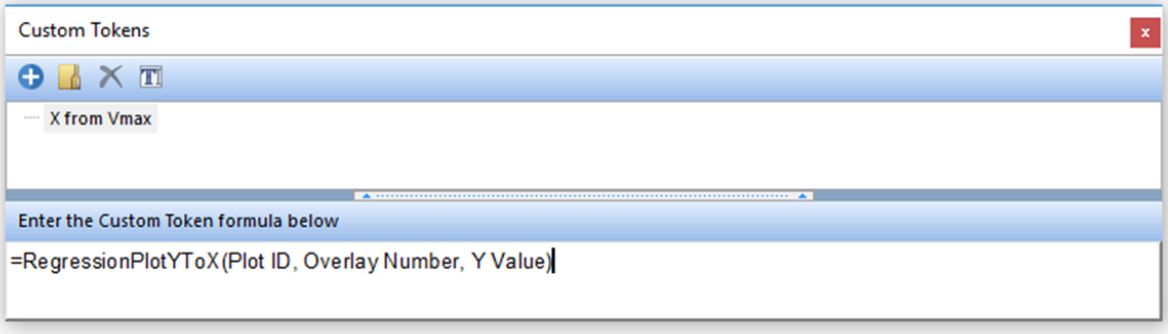

Figure T17.26. "RegressionPlotYtoX" Interpolation Custom Token formula

We will now replace the components of this Custom Token with specific information from our layout.

6. Highlight the "Plot ID" text of the Custom Token (Fig. T17.27, ❶).
7. Right-click on Plot ID and select **Insert Token** (Fig. T17.27, ❷).
8. Select **Plot Information** from the **Plot** node of the Insert a Token dialog (Fig. T17.27, ❸).
9. Click **Insert** (Fig. T17.27, ❹).

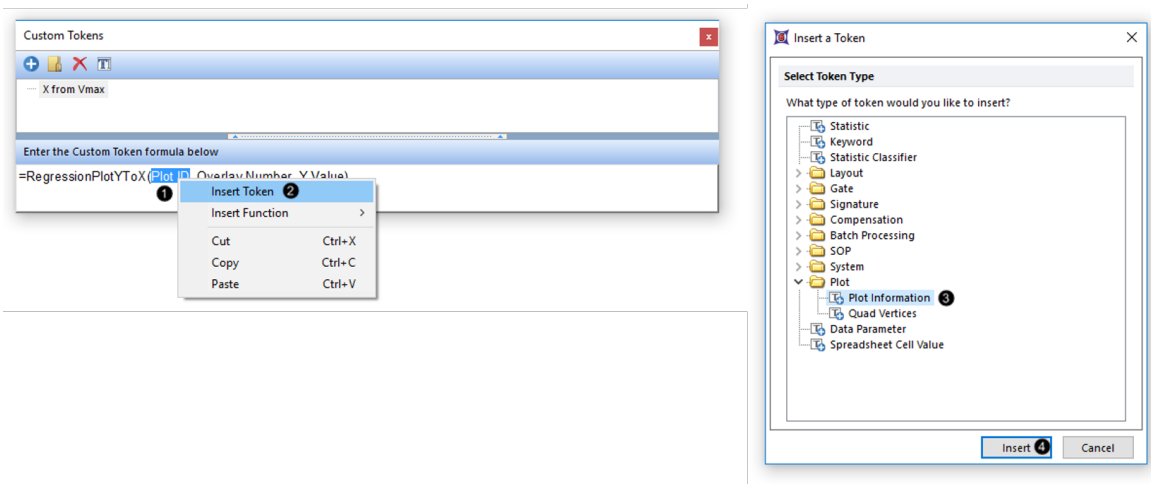

Figure T17.27. Filling components of the Custom Token

10. Scroll to and select the Regression plot under the **Select a Plot** box of the **Edit Plot Information Token** dialog window (Fig. T17.28, ❶). A thumbnail image at right aids in selection of the correct plot.
11. Select the **Plot Elements** category at left (Fig. T17.28, ❷).
12. Select **Plot Number** from the Select a Plot Token Element box (Fig. T17.28, ❸).

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13. Click **OK** (Fig. T17.28, ❹).

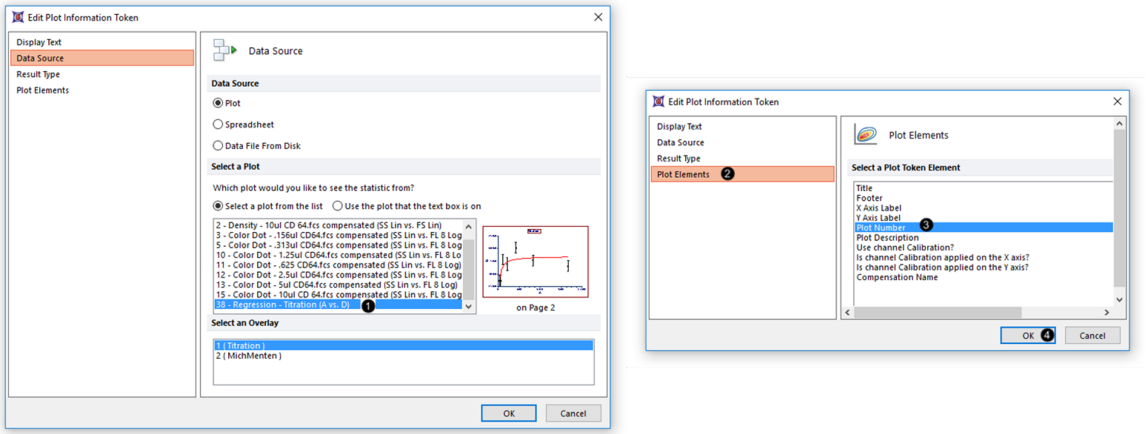

Figure T17.28. Filling components of the Custom Token, continued

14. Highlight the "Overlay Number" text of the Custom Token (Fig. T17.27, above).

15. Type "2", as the Regression line is the second overlay in the Scatter with Regression Plot.

16. Highlight the "Y value" text of the Custom Token (Fig. T17.27, above).

17. Type a value close to the Vmax, e.g., "500".

Note: typing in a value too close or equal to the Vmax will result in a non-useful, very high X-value, as the Michaelis-Menten equation asymptotically approaches the Vmax. As an alternative to typing in an arbitrary freetext value, you could create a Custom Token 728 that is equal to a percentage (e.g., 90%) of the Vmax regression token 585, and enter this Custom Token in place of the "Y value" text.

The "X from Vmax" Custom Token formula now appears as below (Fig. T17.29, *left*). We will now insert the result as a Custom Token onto our layout.

18. Drag and drop the "X from Vmax" Custom Token from the upper pane of the Custom Tokens window to an empty spot on your layout, or to the Scatter with Regression plot.

The Custom Token's mathematically calculated value is now displayed in a Text Box (Fig. T17.29, *center*). We will now add descriptive freetext to the Text Box.

19. Click inside the Text Box so that it enters edit mode 132 (its border appears green as in Fig. T17.29, *right*).

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20. Type "The amount of antibody to use is" before the Custom Token, and "uL" or "microliters" for units after the Custom Token.

The interpolated X-value for the close-to-Vmax Y-value chosen in Step 17 is now displayed on the layout (Fig. T17.29, *right*).

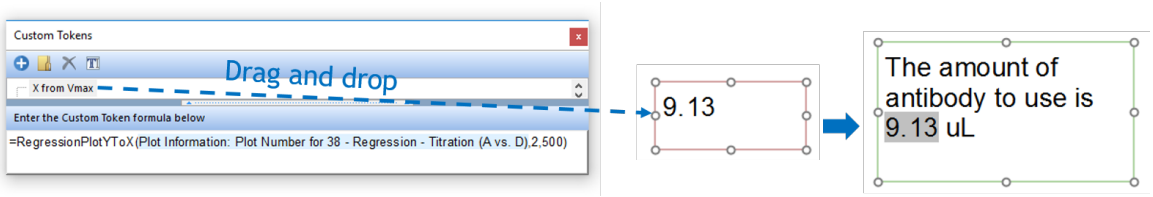

Figure T17.29. Inserting the completed Custom Token for interpolated X value onto the layout

38.18 Calibration via Regression Analysis - Spreadsheets Use Case 3

FCS Express provides the user with many mathematical functions to create custom tokens and custom data sets that can be plotted in Scatter with Regression and Scatter Plots to provide live updating regression curves and fit statistics as gates or data files change.

In this tutorial, we will complete a Spreadsheet 414, insert a scatter with regression plot, and derive model fit statistics from the data. You will also learn how to create a regression overlay on a scatter plot, how to apply a regression fit to an FCS file, as well as how to save a regression fit for later use.

As an example, we will be performing our analysis on a FITC MESF bead data set in order to obtain an equation that returns the FITC MESF value for a data channel value. Because FCS Express also provides the user with an easy to use Channel Calibration Tool 614 that uses MESF data from a set of beads to calibrate the axes to MESF values, this tutorial is most useful in demonstrating the ways in which custom tokens and custom data sets can be created and plotted and how you can use regression analysis to fit your data in real time right within FCS Express.

In the course of this example, we will:

- Load a layout of MESF beads with histogram markers to complete a Spreadsheet for regression fitting. 1680

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- Insert a Scatter with Regression Plot of the Spreadsheet values and automatically generated best-fit regression curve. 1680
- Add a regression fit token to the Scatter with Regression Plot. 1689
- Apply the linear regression to an .fcs data file to obtain MESF data. 1695
- Save the linear regression formula in a text file usable by other .fcs data files and layouts. 1699

First, we will complete the Spreadsheet 1680 and insert a scatter with regression plot.

38.18.1 Complete a Spreadsheet and Insert a Scatter with Regression Plot

For this tutorial we will be using a layout file that has plots of FITC MESF beads and a custom data set, with custom tokens.

1. Open the layout **FITC MESF Beads.fey** found in the **Calculating MESF From Regression** folder of the Tutorial Sample Data archive 1210.

The following layout should appear as shown in Figure T27.1.

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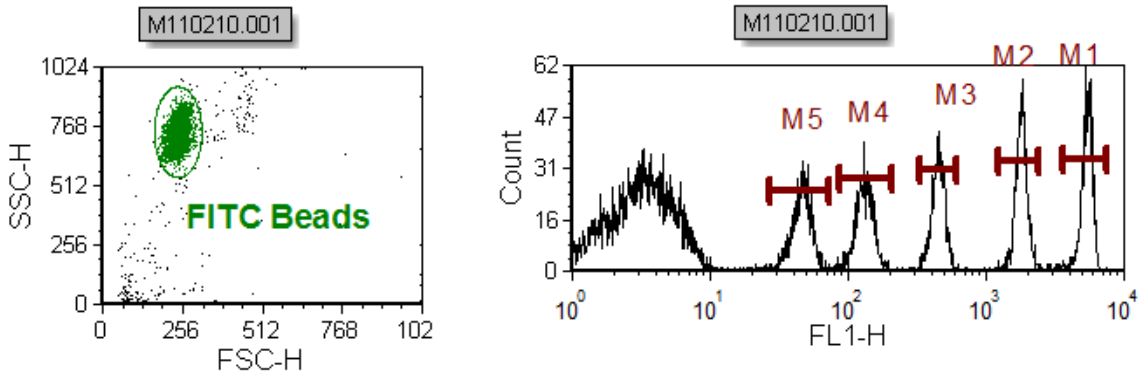

	A	B	C	D	E	F
1	Marker	Median	MESF	LogMedian	LogMESF	
2	M1	5424.69	1106971	3.73	6.04	
3	M2	1810.56	346613	3.26	5.54	
4	M3	449.1	83175	2.65	4.92	
5	M4	132.16	22464	2.12	4.35	
6	M5	46.56	7409	1.67		
7						
8						

Figure T27.1 The FITC MESF Beads.fey Layout

In the upper left corner of the layout, is a Color Dot plot of the FITC beads with a gate around the single bead events. To the right of this plot, is the FITC histogram plot, gated with the 'FITC Beads' gate, with markers defining each of the five FITC bead populations.

Under both plots, is a spreadsheet, named 'FITC MESF'. For more details on how to create a spreadsheet, please see our creating a spreadsheet for analysis tutorial 1624.

Please take a moment and double click on an entry in each column of the spreadsheet and examine the data entry. The first column is the marker name and is a manually entered data value. The second column is the median value for each of the defined markers on the histogram, and is defined using a statistics token (see the Inserting a Token or Custom Token in a cell 420 chapter of the manual to learn more about this topic).

The third column is the MESF FITC value assigned to each of the bead populations, and is a manually entered data value. The fourth and fifth columns

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are the Log values of the median and MESF bead populations, and are defined by formula inserted directly in the spreadsheet. In this case, the formula for calculating the Log, using log base 10, is **=LOG(num)**, where **num** can be both a manually inserted number, a numeric token or, as in this case, a cell reference.

Notice that the bottom right cell of the spreadsheet (Cell E6), under the 'LogMESF' column, is empty. We will now fill this cell with the required value by copy-pasting the content of one of the other cells in that column.

2. Select Cell E3, as shown in Figure T27.2, ❶. Please note that any cells among E2 and E5 can actually be copied in this step.

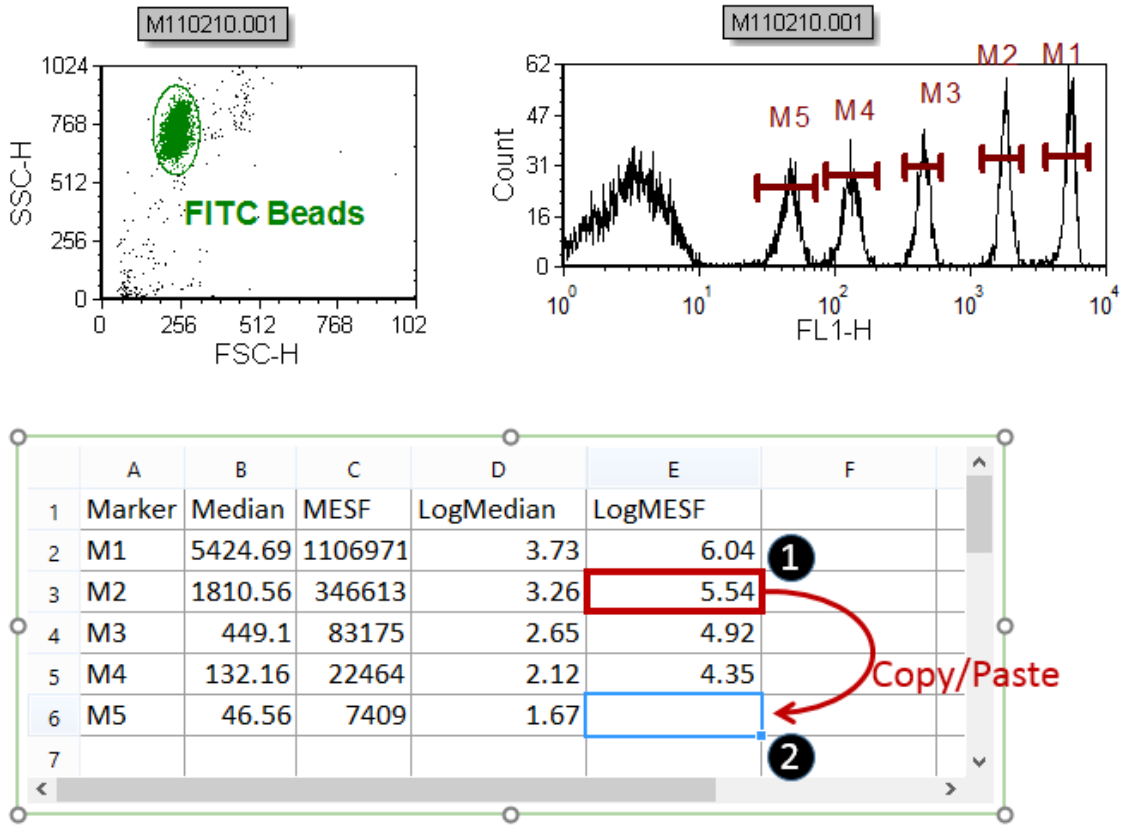

Figure T27.2 Copying and pasting the E3 cell content into the E6 cell to complete the spreadsheet.

3. Press **Ctrl+C** to copy the cell content.

4. Select Cell E6 as show Figure T27.2, ❷.

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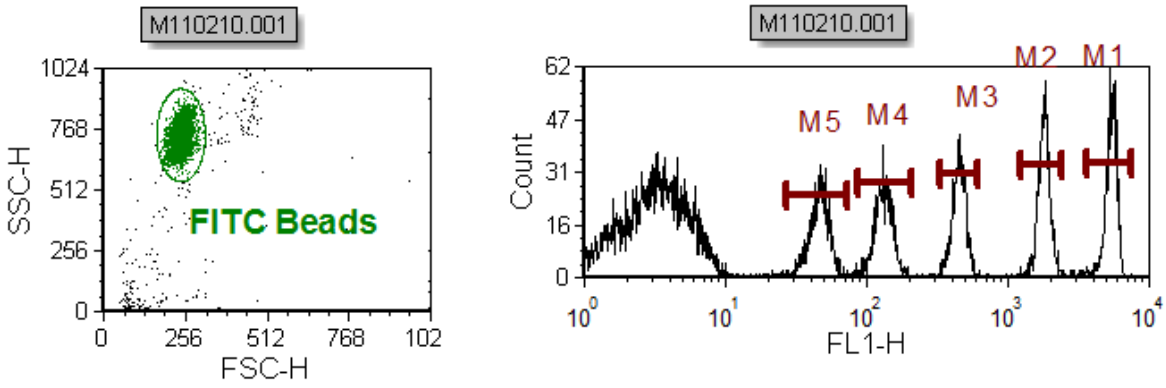
5. Press **Ctrl+V** to paste the cell content .

We have now completed the spreadsheet as shown in Figure T27.3.



	A	B	C	D	E	F
1	Marker	Median	MESF	LogMedian	LogMESF	
2	M1	5424.69	1106971	3.73	6.04	
3	M2	1810.56	346613	3.26	5.54	
4	M3	449.1	83175	2.65	4.92	
5	M4	132.16	22464	2.12	4.35	
6	M5	46.56	7409	1.67	3.87	
7						

Figure T27.3 The spreadsheet successfully complete.

Now, we will insert a Scatter with Regression plot using the FITC bead data in the spreadsheet.

4. Select the spreadsheet; it should now have a green border.
5. Select the **Insert** tab→**2D Plots→Scatter with Regression** command.
6. Click on a blank area of the layout below the spreadsheet to insert the Scatter with Regression plot.

An empty plot using the data from the first two columns, 'A' and 'B', of the custom spreadsheet appears.

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We will now format the Scatter with Regression plot to display the Median and MESF data from the spreadsheet (i.e. columns 'B' and 'C' respectively).

7. Place the cursor over the Y-axis 'B' label.
8. Press and hold the left mouse button and the list of columns from the spreadsheet appears, as shown in Figure T27.4.
9. Release the button when the cursor is over 'C', shown highlighted in blue in Figure T27.4.
10. Repeat 7 to 9 for X-axis selecting 'B' as column to display.

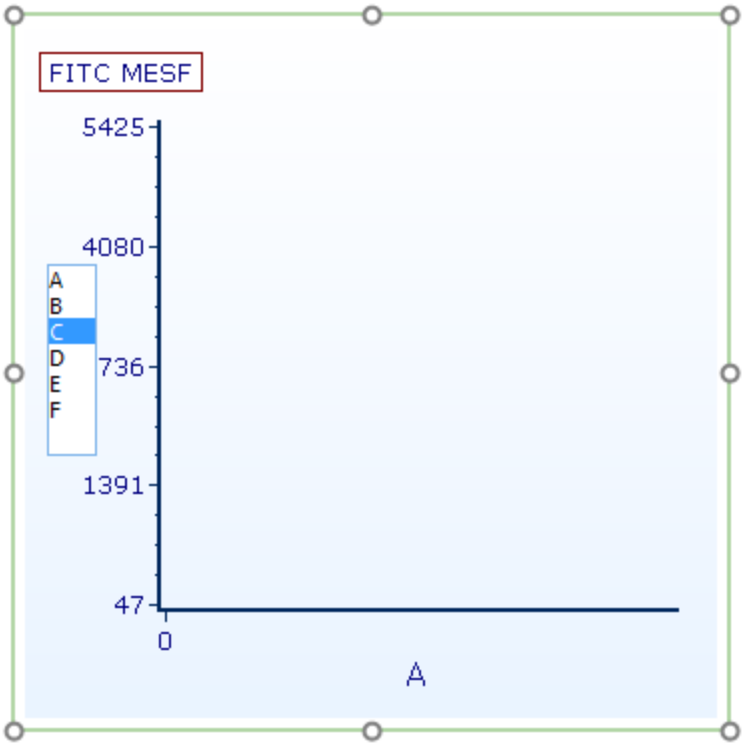

Figure T27.4 Selecting 'LogMESF' Data for the Y-axis

The empty plot is now populated with the data from column B and C of the spreadsheet in **black**, along with a best-fit regression curve in **red**, as shown in Figure T27.5.

Note: The regression curve type has defaulted to the first option (Linear), which is appropriate for this dataset. When working with other datasets, the Regression Type can be chosen from the Formatting options of the Scatter with

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Regression plot. The smoothness of the curve can also be adjusted. See [this section of the manual](#) 580 for details.

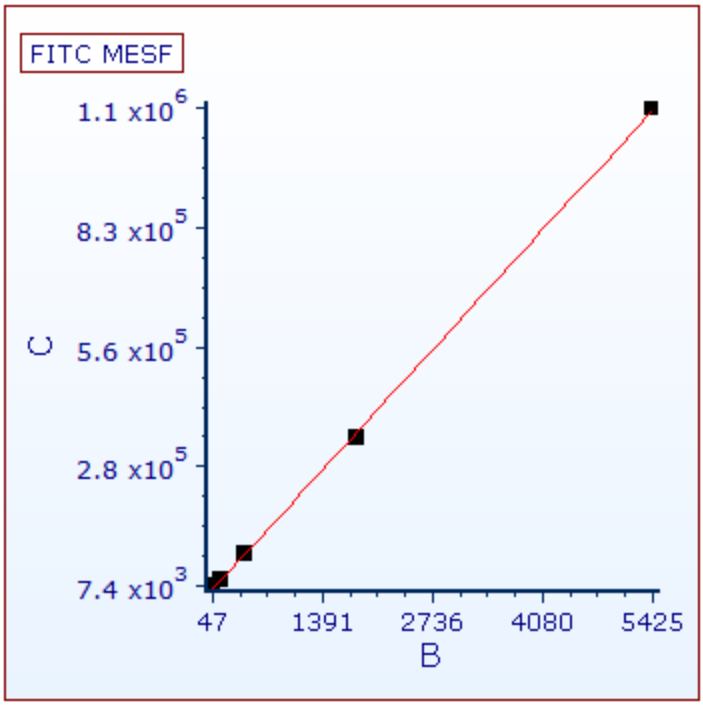

Figure T27.5 Scatter with Regression Plot of Median and MESF Data from the Data Grid

We will now format the Scatter with Regression plot to display the 'Log MESF' (i.e. column 'E') data of the spreadsheet on the Y-axis.

11. Place the cursor over the Y-axis 'C' label.
12. Press and hold the left mouse button and the list of columns from the Spreadsheet appears.
13. Release the button when the cursor is over 'E'.

The Scatter with Regression plot now updates to reflect the selection of the 'Log MESF' (i.e. column 'E') data for the Y-axis.

We will now change the X-axis parameter to the 'LogMedian' (i.e. column 'D') data of the spreadsheet on the X-axis.

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10. Place the cursor over the X-axis 'B' label.

11. Press and hold the left mouse button and the list of columns from the Spreadsheet appears.

12. Release the button when the cursor is over 'D'.

The Scatter with Regression plot now updates to reflect the selection of the 'LogMedian' (i.e. column 'D') data for the X-axis and appears as in Figure T27.6.

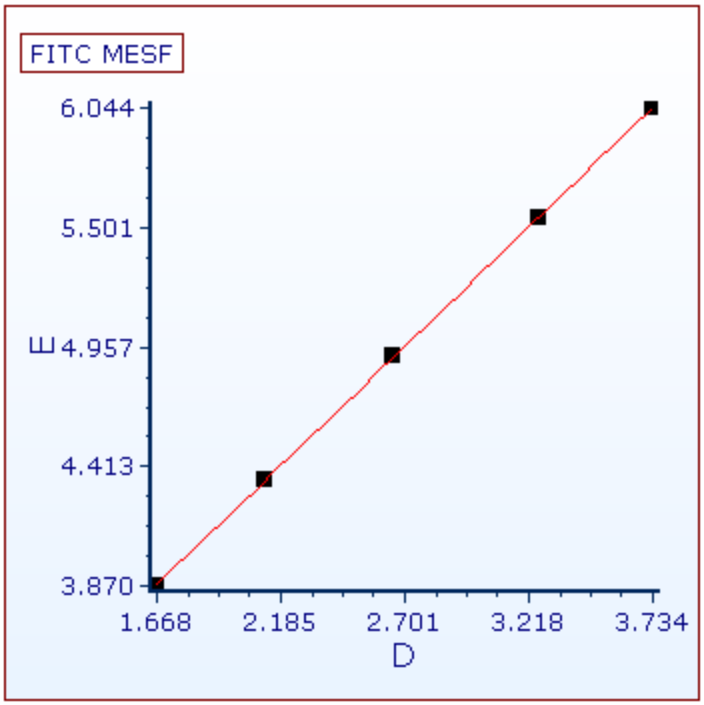

Figure T27.6 Scatter with Regression Plot with Log Median and Log MESF Values from the Spreadsheet.

We will now format the Scatter with Regression plot to better visualize the five data points.

13. Right-click on the Scatter with Regression Plot.

14. Choose **Format** from the pop-up menu.

15. Choose the **Axis** category (Figure T27.7a, ❶).

16. Change the **Y Axis Range Minimum** to 3 and **Maximum** to 7 (Figure T27.7a, ❷).

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17. Click on **X Axis** under **Select Axis** (Figure T27.7a, ❸).

18. Change the **X Axis Range Minimum** to 1 and **Maximum** to 4.

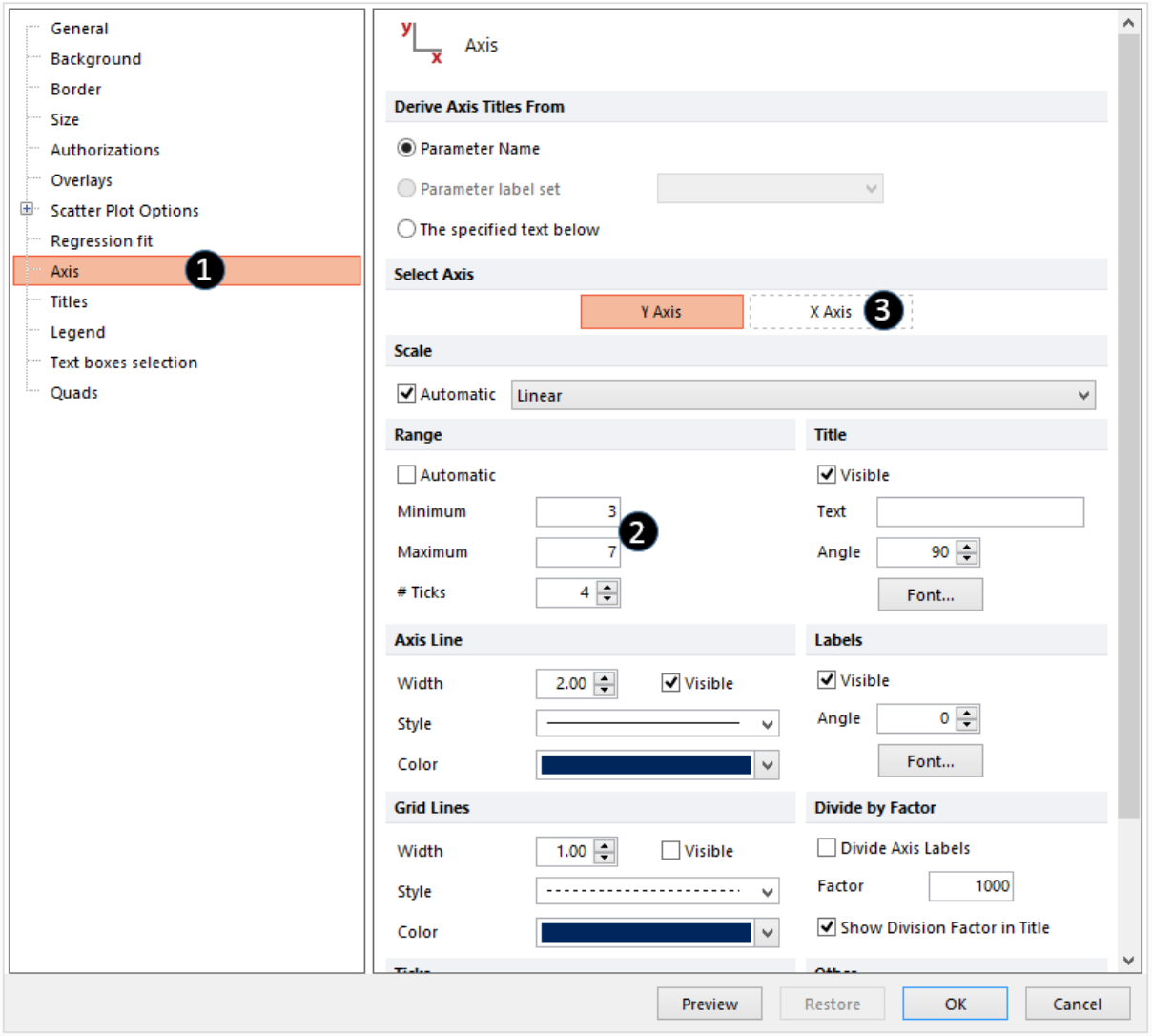

Figure T27.7a Formatting axis for Scatter with Regeression plot.

19. Click on the **Overlays** category (Figure T27.7b, ❹).

20. Click on the second overlay (*"2-Linear"*) from the **2D Unbinned Overlays** list (Figure T27.7b, ❺).

21. Choose **Connecting Lines** from the **Line Options for** dropdown menu (Figure T27.7b, ❻).

22. Set the line with to **1.5** (Figure T27.7b, ❼).

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23.Click **OK** (Figure T27.7b, 8).

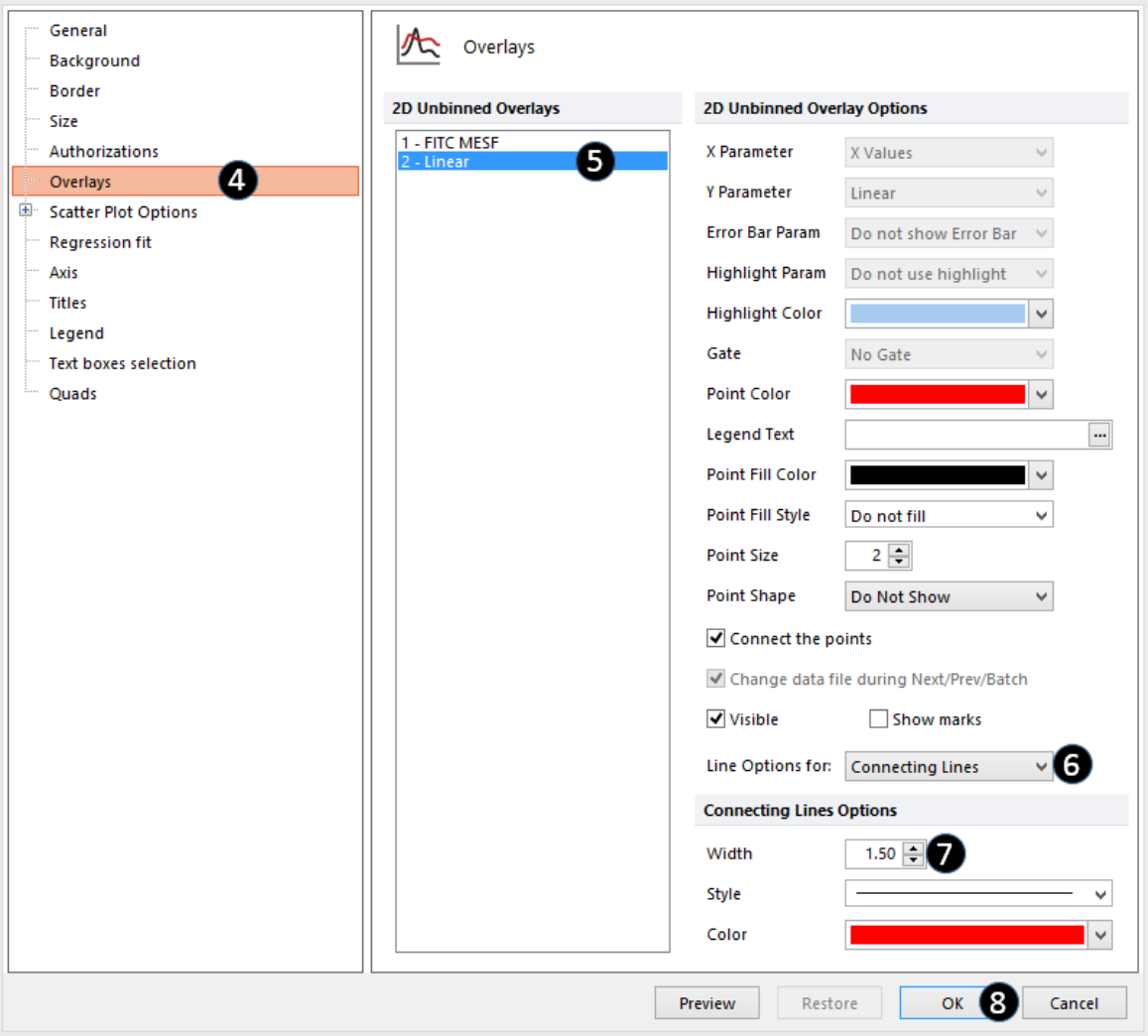

Figure T27.7b Formatting fitting line in Scatter Plot with Regression.

The Scatter with Regression plot will now look like Figure T27.7c. It has been formatted to bring all of the data points into range and to increase the width of the red regression curve.

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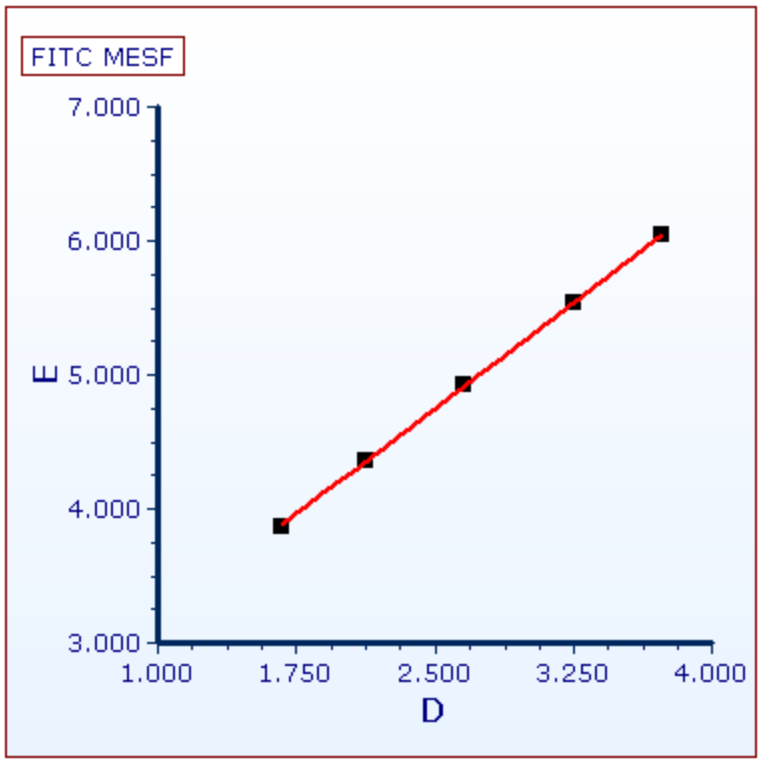

Figure T27.7C Scatter Plot with Regression reformatted.

Next, we will insert and view a linear regression token for the data set.

38.18.2 Working with Regression Tokens and Fit Equations

To learn working with regression we will pick up from where we left off in the previous section of this tutorial.

If you didn't go along the previous section, you can directly start with the **FITC MESF Beads - Part2.fey** layout stored in the **Calculating MESF From Regression** folder of the Tutorial Sample Data archive.

As a first step we will display a regression token on the Scatter with Regression Plot.

1. Right-click on the Scatter with Regression plot and select **Add text box** from the pop-up menu (Fig. T27.8, ❶).

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2. Right-click in the text box on the plot and select **Insert Token** from the pop-up menu (Fig. T27.8, ❷).

3. Double-click **Statistic** in the **Insert a Token** dialog (Fig. T27.8, ❸).

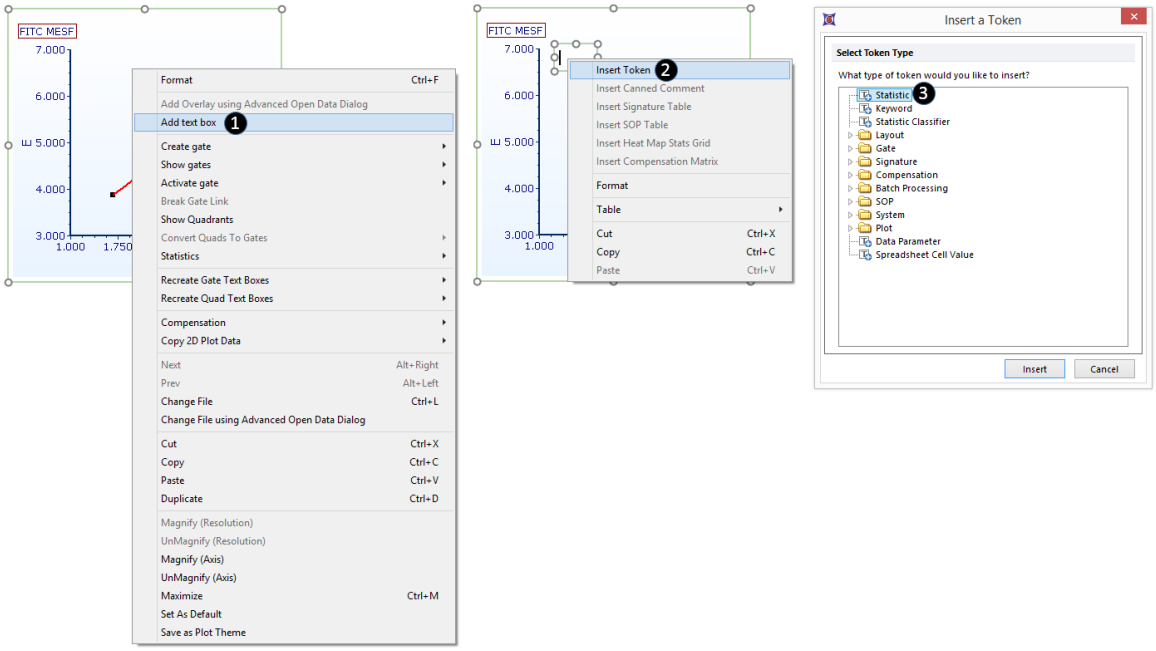

Figure 27.8 Adding a regression token to the plot

4. Click the **Statistic** category in the **Create Statistic** dialog (Fig. T27.9, ❶).

5. Select **Regression Parameter Statistics** from the **Select a Statistic** dropdown menu (Fig. T27.9, ❷).

6. Select **m** (slope) from the **Statistic** list (Fig. T27.9, ❸).

7. Increase the **Number of decimals** to "4" (Fig. T27.9, ❹).

8. Click **OK** (Fig. T27.9, ❺). The slope (m) of the regression curve is now displayed on the plot.

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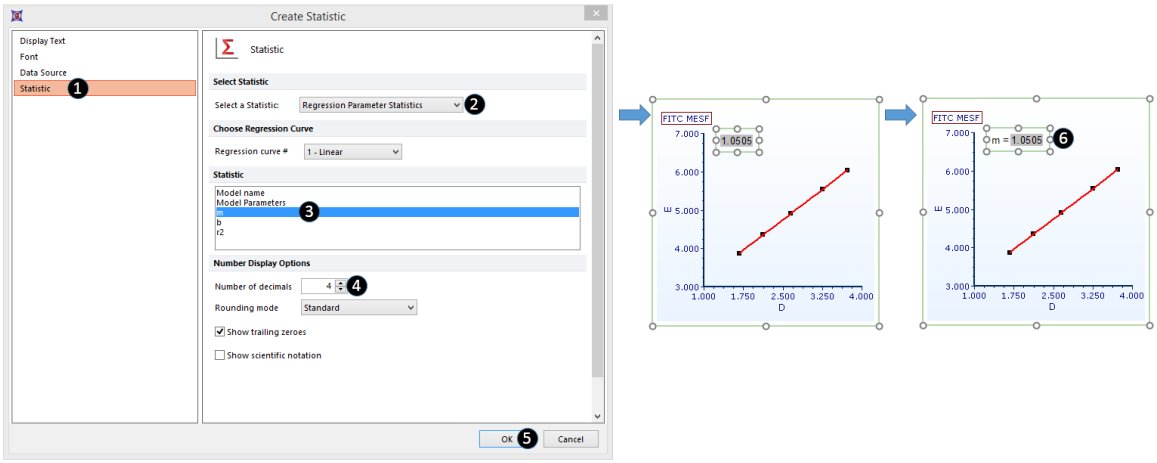

Figure 27.9. Adding a regression token to the plot, continued

9. Click inside the text box to put it into edit mode. The border will be green.

10. Use the arrow keys on the keyboard or click within the text box to move the cursor to the left of the token.

11. Type "m = " (Fig. T27.9, ❻). The token has now been annotated with this descriptive freetext within the text box. *Note: if necessary, the text box can be enlarged by dragging its border, so that all the text fits on one line as in the figure.*

We will now create a Custom Token for the linear regression equation, so that we can apply the regression to other .fcs files (including those in other layouts). Each token in the Regression Parameter Statistics list (Fig. 27.9 above) will be used as a component of this Custom Token.

12. Open the Custom Token window by clicking on **View→Token→Custom Tokens** in the Ribbon bar.

13. Click the blue plus ( ) in the Custom Tokens window (Fig. 27.10, ❶).

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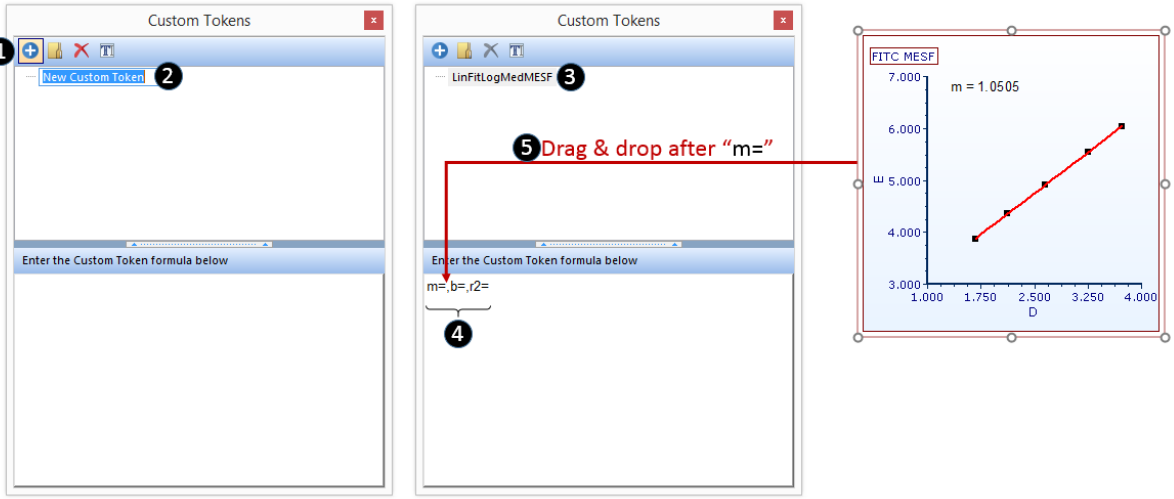

Figure T27.10 Creating the Linear Regression Fit Custom Token

14. Highlight the "New Custom Token" text in the Custom Tokens window (Fig. 27.10, ❷).

15. Type 'LinFitLogMedMESF' (Fig. 27.10, ❸).

16. Type "m=,b=,r2=" in the formula (lower) pane of the Custom Tokens window (Fig. 27.10, ❹).

17. Click on the Scatter with Regression plot so its border becomes highlighted.

18. Drag and drop the border of the Scatter with Regression plot to the position after the "m=" and before the "," (comma) in the formula pane of the Custom Tokens window (Fig. 27.10, ❺).

19. Double-click **Statistic token** from the **Paste Special** dialog (Fig. 27.11, ❶).

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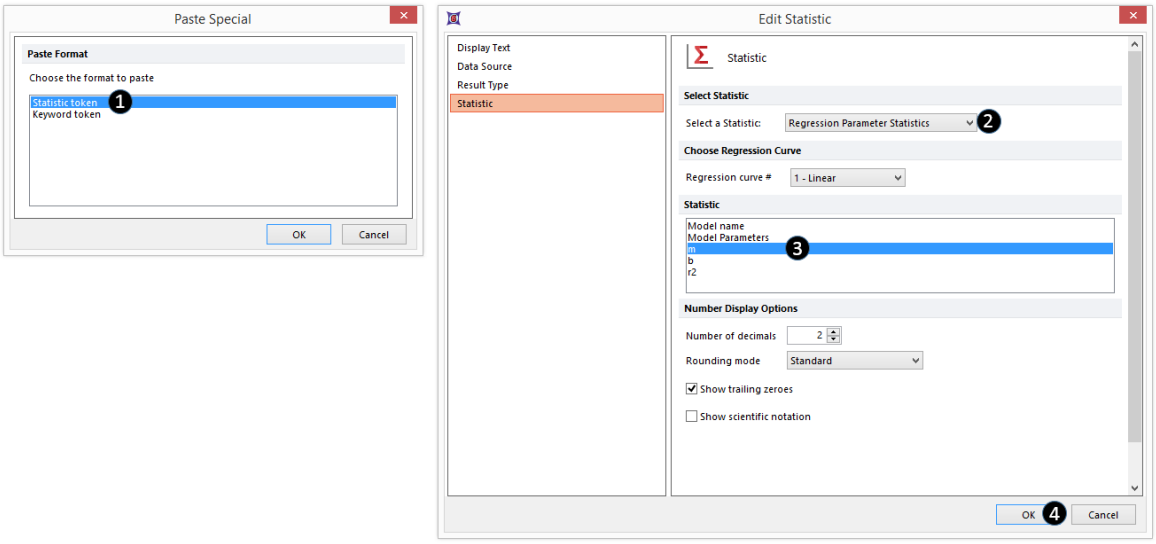

Figure 27.11 Inserting a regression parameter token as a component of the linear regression fit custom token

20. Choose **Regression Parameter Statistics** from the **Select a Statistic** dropdown menu (Fig. 27.11, ❷).

21. Choose **m** from the **Statistic** list (Fig. 27.11, ❸).

22. Click **OK** (Fig. 27.11, ❹).

23. Repeat Steps 17-21, with the following modifications:

    - Step 17: Drop the plot after the "b=" in the formula pane of the Custom Tokens window
    - Step 20: Choose **b** from the **Statistic** list

24. Repeat Steps 17-21, with the following modifications:

    - Step 17: Drop the plot after the "r2=" in the formula pane of the Custom Tokens window
    - Step 20: Choose **r2** from the **Statistic** list

25. Drag the LinFitLogMedMESF Custom Token from the Custom Token window onto a blank area of the layout. A new text box containing the linear regression fit token will be automatically created.

Note: an r^2 value of exactly 1 denotes a perfect fit, which is rarely attained with experimental data. The Custom Token initially displays a value of 1.00 for r^2 because its value here is greater than 0.995, and statistics in FCS Express are rounded to two decimal places by default.

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We will now edit the r^2 component of the Custom Token to display five digits after the decimal point.

26. Double-click the component r^2 token in formula pane of the Custom Tokens window (Fig. 27.12, ❶).

27. Click the **Statistic** category in the **Edit Statistic** dialog (Fig. 27.12, ❷).

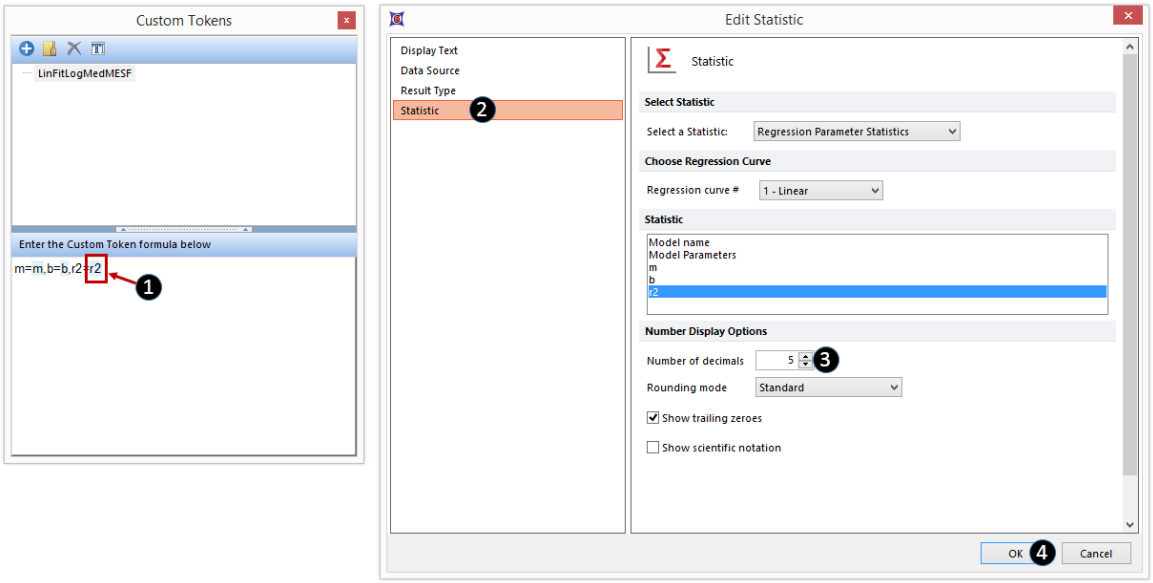

Figure 27.12 Increasing the number of digits after the decimal displayed for the r-squared component of the Custom Token

28. Increase the **Number of decimals** to **5** (Fig. 27.12, ❸).

29. Click **OK**.

The linear fit token will be displayed as in Figure T27.13.

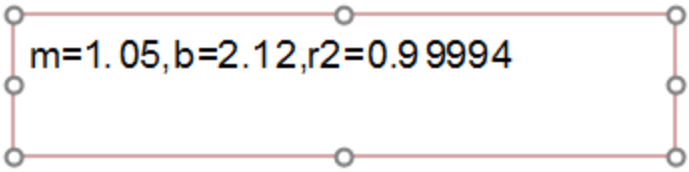

Figure T27.13 The LinFitLogMedMESF Custom Token displayed in a Text Box, after editing the number of digits displayed for r-squared

Once a linear regression is calculated it can be used as model for fitting unknown samples. This can be done directly within the same layout in which the regression has been calculated (see the Apply Regression to an FCS File 1695

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section of this tutorial) or in any new layout. To use the regression in a new layout see the Save a Regression 1699 topic of this tutorial.

38.18.3 Apply Regression to an FCS File

We will now use the linear regression formula calculated in the previous section 1689 to calculate MESF data for four unknown samples (i.e. four FCS files). The goal of this analysis is to assess the amount of IgG that is present in the patient's serum that is binding to the B- and T-cells of the potential donor.

The layout we will be using contains five pages. The first page displays the linear regression calculated in the previous section using beads. Page 2 and 3 define the negative and positive controls along with the patient sample. The **MESF Calculations** page is where we will be working to complete the linear regression and MESF data. The **Bar Plots** page will be used to easily visualize the data once it is complete.

1. Open the **HLA CrossMatch.fey** layout from the **Calculating MESF From Regression** folder in the Tutorial Sample Data archive 1210.
2. Click on the **MESF Calculations** page tab of the layout.

This page contains two spreadsheets (Table 1 and Table 2), one for calculating MESF for B cells and one for calculating MESF for T cells. Each spreadsheet contain the "m" and "b" regression parameters calculated on Page 1 (Figure T27.14). This way, both parameter will be available in each spreadsheet using cell reference. Moreover, since they have been inserted as statistic tokens, they will automatically update if the linear regression change.

Note: as we will see in the Save a Regression 1699 section of this tutorial, linear regression parameters can also be added to the data files as a keyword. This allows to use the regression parameter even in a brand new layout.

	A	B	C
1	**Linear Regression parameters**		
2	m	1.050509	
3	b	2.122613	
4			
5			

Figure T27.14 - "b" and "m" parameter have been inserted into the spreadsheet.

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Spreadsheets on **MESF Calculations** page also contain the Name, the Median fluorescence intensity and the log10-transformed Median fluorescence intensity of each of the four sample.

To calculate the MESF for the said unknown samples using the linear regression model previously calculated we will need to:

- Use the log10-transformed Median intensity (i.e. the X variable of the linear regression) to calculate the log10MESF value (i.e. the Y variable of the linear regression) following the linear regression formula Y=mX+b;
- Elevate the log10MESF value to the tenth in order to calculate the actual MESF value.

3. Select the G2 cell of Table 1 (i.e. the first available cell of the **B Cell Log MESF** column).
4. Type "**=$B$2*F2+$B$3**" (without quotes), where $B$2 is an absolute cell reference for the "m" parameter, F2 is a relative cell reference for the "X" parameter and $B$3 is an absolute reference for the "b" parameter.

Note: A cell reference can also be inserted in the formula by mouse selection of the cell of interest. Please see the referencing cells in a spreadsheet section of the manual.

5. Press **Return** on your keyboard. The formula is calculated in real time and the spreadsheet looks like the one of Figure T27.22.

	A	B	C	D	E	F	G	H
1	**Linear Regression parameters**			**Sample Name**	**B Cell Median**	**B Cell Log Med**	**B Cell Log MESF**	**B Cell MESF**
2	m	1.050509		PBS NEGATIVE CONTROL	17	1.23	3.415210666	
3	b	2.122613		AB NEGATIVE HUMAN SERUI	26.18	1.42		
4				PATIENT SERUM	51.4	1.71		
5				POSITIVE HUMAN SERUM	2072.08	3.32		

Figure T27.22

Note: if you double click on cell G2, cell references will highlight as displayed in figure T27.23.

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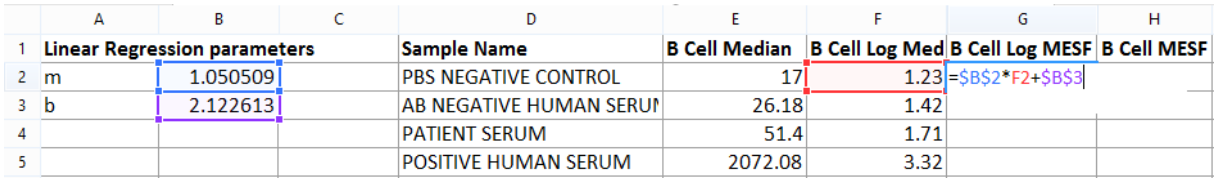


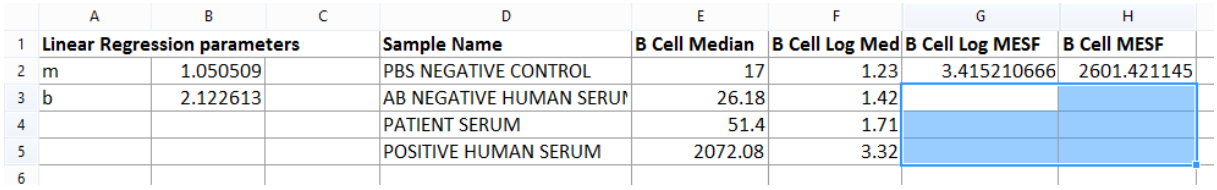
	A	B	C	D	E	F	G	H
1	**Linear Regression parameters**			**Sample Name**	**B Cell Median**	**B Cell Log Med**	**B Cell Log MESF**	**B Cell MESF**
2	m	1.050509		PBS NEGATIVE CONTROL	17	1.23	=$B$2*F2+$B$3	
3	b	2.122613		AB NEGATIVE HUMAN SERUI	26.18	1.42		
4				PATIENT SERUM	51.4	1.71		
5				POSITIVE HUMAN SERUM	2072.08	3.32		

Figure T27.23

6. Select the H2 cell of Table 1 (i.e. the first available cell of the **B Cell MESF** column).
7. Type "**=10^G2**" (without quotes), where G2 is the relative reference to the **B Cell Log MESF** of the **PBS NEGATIVE CONTROL SAMPLE**.
8. Press **Return** on your keyboard. The formula is calculated in real time.

We will now fill the remaining cells of column G and H by copy-pasting the content of cells G2 and H2.

9. Multiple select both cell **G2** and cell **H2**.
10. Press **Ctrl+C** on your keyboard or, alternatively, right click and select **Copy** from the right-click menu.
11. Multiple select cells from **G3** to **H5** as shown in Figure T27.24.



	A	B	C	D	E	F	G	H
1	**Linear Regression parameters**			**Sample Name**	**B Cell Median**	**B Cell Log Med**	**B Cell Log MESF**	**B Cell MESF**
2	m	1.050509		PBS NEGATIVE CONTROL	17	1.23	3.415210666	2601.421145
3	b	2.122613		AB NEGATIVE HUMAN SERUI	26.18	1.42		
4				PATIENT SERUM	51.4	1.71		
5				POSITIVE HUMAN SERUM	2072.08	3.32		
6								

Figure T27.24 - Multiple cell selections.

12. Press **Ctrl+V** on your keyboard or, alternatively, right click and select **Paste** from the right-click menu. The cell are filled with the corresponding value and the spreadsheet looks like the one displayed in figure T27.25.

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	A	B	C	D	E	F	G	H
1	**Linear Regression parameters**			**Sample Name**	**B Cell Median**	**B Cell Log Med**	**B Cell Log MESF**	**B Cell MESF**
2	m	1.050509		PBS NEGATIVE CONTROL	17	1.23	3.415210666	2601.421145
3	b	2.122613		AB NEGATIVE HUMAN SERUI	26.18	1.42	3.612202871	4094.518813
4				PATIENT SERUM	51.4	1.71	3.919995155	8317.544923
5				POSITIVE HUMAN SERUM	2072.08	3.32	5.606527896	404136.3323
6								

Figure T27.25 - The B Cell table completed.

We will now fill the **T Cell Log MESF** and the **T Cell MESF** columns of Table 2 by copy/pasting the content of the content of the **B Cell Log MESF** and the **B Cell MESF** columns respectively from Table 1.

13. Multiple select cells from **G2** to **H5** as shown in Table 1 (i.e. the B cell Table) as shown in Figure T27.26.
14. Select the **G2** cell in Table 2 (i.e. the T Cell table).
15. Press **Ctrl+V** on your keyboard or, alternatively, right click and select **Paste** from the right-click menu.

Table 2 is now completed and looks like Figure T27.27.

	A	B	C	D	E	F	G	H
1	**Linear Regression parameters**			**Sample Name**	**T Cell Median**	**T Cell Log Med**	**T Cell Log MESF**	**T Cell MESF**
2	m	1.050509		PBS NEGATIVE CONTROL	7.64	0.88	3.050310521	1122.820986
3	b	2.122613		AB NEGATIVE HUMAN SERUI	7.64	0.88	3.050310521	1122.820986
4				PATIENT SERUM	7.77	0.89	3.058008294	1142.900161
5				POSITIVE HUMAN SERUM	294.27	2.47	4.71605288	52005.9316

Figure T27.27 - The T Cell table completed.

To see the results of the table expressed in a bar plot (Figure T27.28), please click on the **Bar Plots** page tab (for instructions on creating bar plots, please visit the Bar and Scatter Plots tutorial 1703). You will see that the B-Cell MESF values for the Patient Serum are slightly elevated over negative controls yet well below the positive control. The T-Cell MESF values for the Patient Serum fall within the negative control range and are also well below the positive control value.

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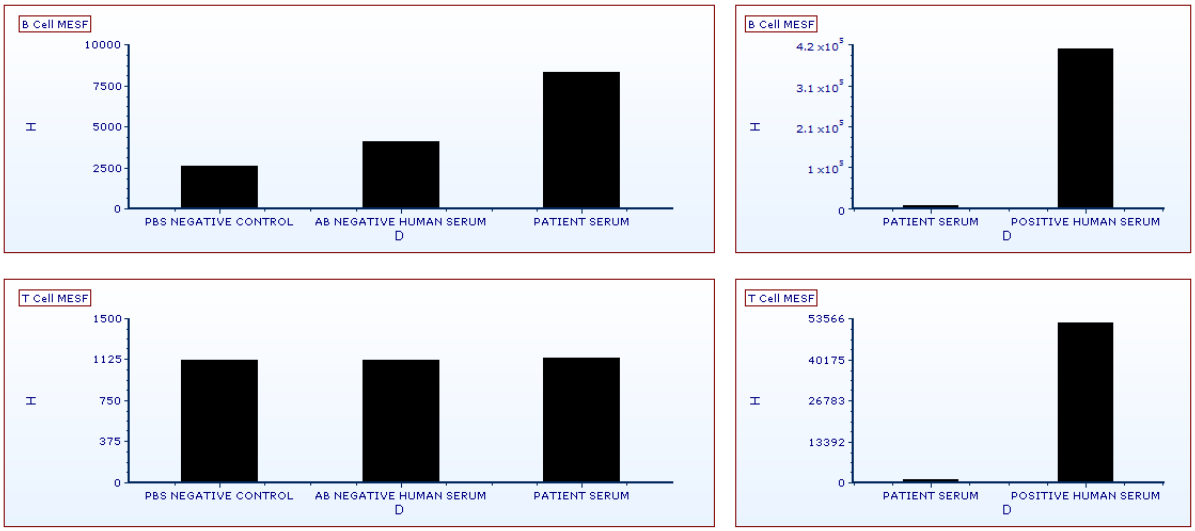

Figure T27.28 Bar Plots Displaying the FITC MESF HLA Cross Match Data from B- and T-cells

Next, we will save a previously created regression for use in another layout 1699.

38.18.4 Save a Regression

Once a formula for a linear regression has been defined, we can display it in our layout, use it to overlay a line fit on the scatter plot, and even save the formula as a keyword for use in future experiments and analysis. Since it is typical to have one layout for calibration and another for analysis, it is useful to save a regression for use in any layout. In this section, you will learn how to save a regression formula as a keyword for use in any layout.

1. Load the **FITC MESF Beads Completed.fey** layout located in the **Regression Analysis** folder within the Tutorial Sample Data archive 1210.
2. Drag the **LinFitLogMedMESF** custom token from the **Custom Tokens** window onto a blank area of the layout.

A new text box will be automatically created and the linear fit token will be inserted in it. The linear fit token will be displayed as in Figure T27.28.

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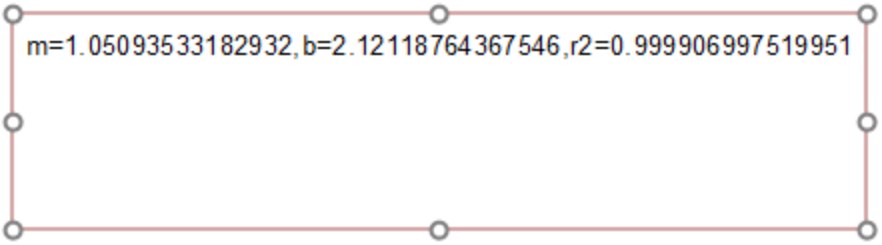

Figure T27.28 LinFitLogMedMESF Custom Token Formula Output Displayed in a Text Box

3. Right-click on the text box.
4. Choose **Copy** from the pop-up menu.
5. Open a text editor application such as Notepad for Microsoft Windows.
6. Paste the text box in the text editing software.
7. Enter a '$' symbol followed by the name you would like the keyword displayed as before the formula followed by an equals sign (Figure T27.29). In this case, use: '$FITCMESFCalib='

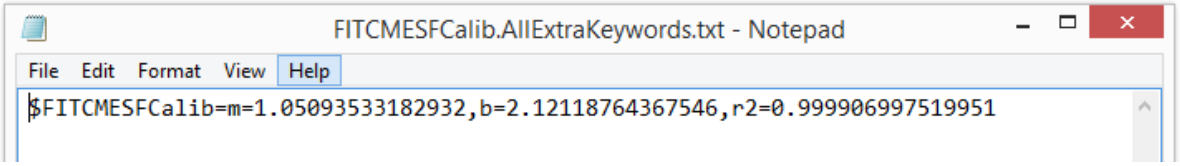

Figure T27.29 Creating an Extra Keyword for the Regression Formula in Notepad

8. Save the text document as FITCMESFCalib.AllExtraKeywords.txt (Figure T27.30) in a location that will be easy to access such as your Desktop.

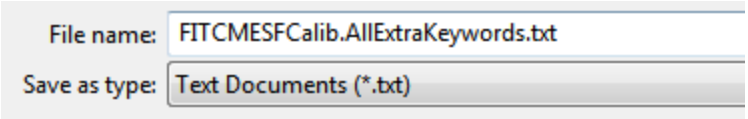

Figure T27.30 Saving the Keyword Text Document

FCS Express has the capability to load keywords from external files as part of loading a data file[652]. These keywords are treated within FCS Express as if they were originally part of the data file itself. This is a convenient way to introduce externally derived data into your analysis template. We will make the regression keyword available for use in another layout in the following steps.

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9. Copy the FITCMESFCalib.AllExtraKeywords.txt file you just created and paste it into the **Regression Analysis** folder within the *FCS Express Sample Data* directory (Figure T27.31).

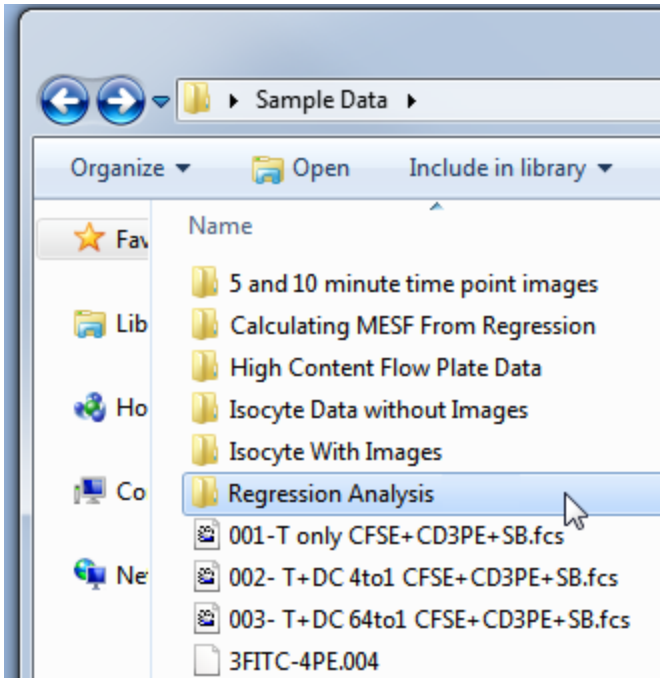

Figure T27.31 Paste the FITCMESFCalib.AllExtraKeywords.txt File into the Linear Regression Analysis Folder

10. Load the **HLA CrossMatch Keyword Regression.fey** layout located in the **Regression Analysis** folder within the *FCS Express Sample Data* Directory.

This layout contains sample files and a page for **MESF Calculations**. We will now use the regression formula saved as a keyword to complete the table.

11. Click the **Tcell LogMESF Patient** Custom Token to select it.

12. Right-click in **Enter the Custom Token formula below** area of the **Custom Tokens** window.

13. Choose **Insert Function→Regression→LinearXtoY(x, [modelParameters])** from the pop-up menu.

14. Highlight the text 'x' in the formula brackets.

15. Right-click on the highlighted text.

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16. Chose **Insert Token** from the pop-up menu.
17. Double-click the **Tcell LogPatient** token from the **Custom** category.
18. Highlight the text **[modelParameters]** in the formula (*Note: the brackets must be selected in this instance*).
19. Right-click on the highlighted text.
20. Choose **Insert Token** from the pop-up menu.
21. Double-click on **Keyword**. The **Create Keyword** dialog opens.
22. Choose the **Keyword** category in the **Create Keyword** dialog (Figure T27.32, ❶).

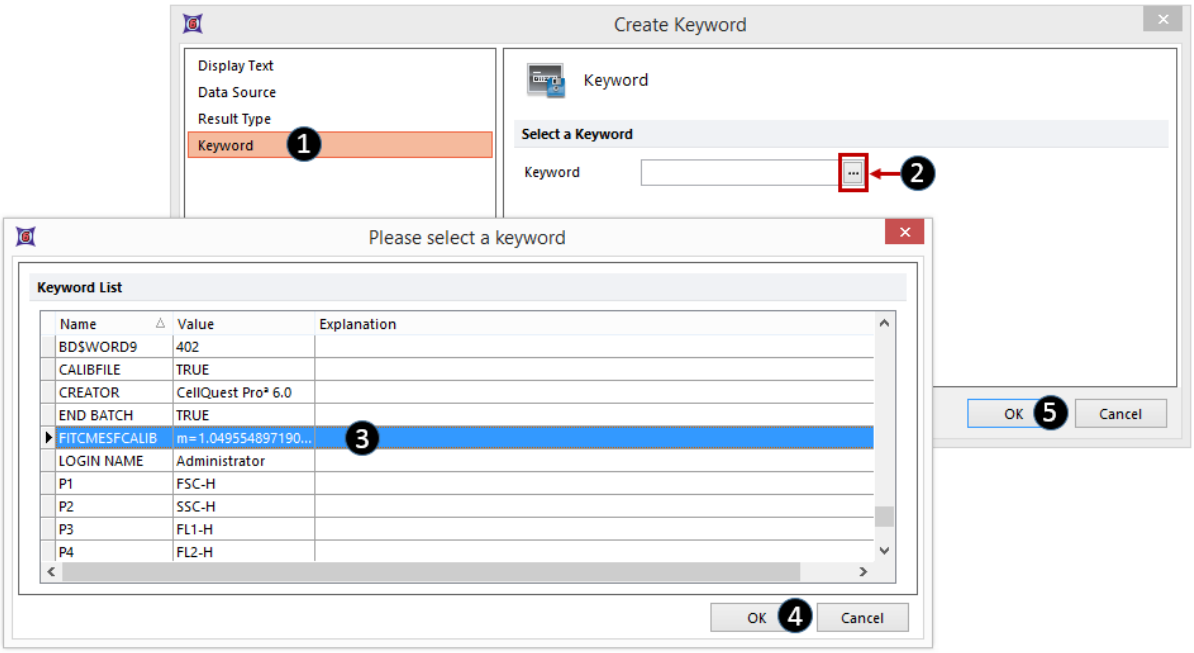

Figure T27.32 Choosing the $FITCMESFCALIB Keyword Token

23. Click on the ellipsis (Figure T27.32, ❷).
24. Scroll to and click on the **$FITCMESFCALIB** keyword (Figure T27.32, ❸).
26. Click **OK** in the **Please select a keyword** dialog (Figure T27.32, ❹).
27. Click **OK** in the **Create keyword** dialog (Figure T27.32, ❺).
28. Enter an "=" sign before the formula.

The formula will now look like Figure T27.33.

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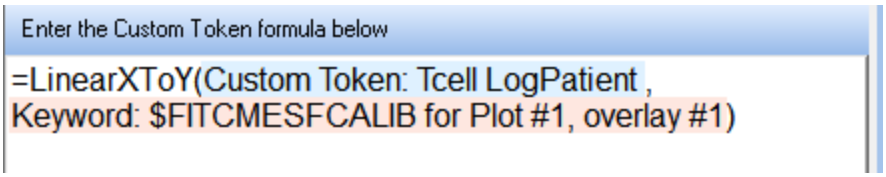

Figure T27.33 Completed Custom Token Formula for Tcell LogMESF Patient

Tables or spreadsheets for your analysis may now be populated using the custom tokens you have defined. The formulas use the regression stored as a keyword in the data.

In summary, when trying to create any kind of calibration regression to apply to your sample data, the best practice is to have a layout that is used for creating the regression. This will contain all of your controls. You then save the final regression as an "allextrakeywords.txt" file. Create another analysis layout that is used to analyze the actual data and apply the regression you created. The analysis layout will get the regression from the keyword that you defined in the "allextrakeywords.txt" file. By placing the "allextrakeywords.txt" file into the folder with the appropriate data files, you ensure that all the data files actually contain the regression that you wish to use.

38.19 Working with Pie, Bar, and Scatter Plots - Spreadsheets Use Case 4

Introduction:

In FCS Express 6, data can be displayed in Bar Plots, Scatter Plots, or Pie Charts that update in real-time as you advance through your dataset or adjust your gates. This eliminates intermediate steps in your workflow such as creating tables, exporting and reassembling data in a spreadsheet program, and manually generating new graphs to evaluate additional data or a change in your gating positions or hierarchy. Bar Plots, Scatter Plots, and Pie Charts can be formatted and exported to PowerPoint and PDF, just like any other plot in FCS Express--so you can summarize your results and generate publication-quality graphics in record time.

In the course of this tutorial you will learn how to:

- Plot your data in a Bar Plot, Scatter Plot, or Pie Chart 1704
- Create tokens for use in Bar Plots, Scatter Plots, or Pie Charts 1704

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- Manually enter values for Bar Plots, Scatter Plots, or Pie Charts 1704
- Use tokens for creating error bars on your plots 1724
- Create conditional formulas to emphasize interesting data sets 1730
- Display additional datasets (as Overlays on Bar and Scatter Plots, or a Ring on Pie Charts) 1734
- Format the appearance of Bar and Scatter Plots and Pie Charts 1724

Please see the table below for an overview of the options for working with each plot type.

	Add Data to Plots by		*Formatting Feature*		
	Drag and Drop	**Spreadsheet**	**Error Bars**	**Data Emphasis** (according to user-defined criteria)	**Display Additional Datasets**
Bar Plots	✓	✓	✓	✓ (Highlight)	✓ (as Overlay)
Scatter Plots		✓	✓	✓ (Highlight)	✓ (as Overlay)
Pie Charts	✓	✓		✓ (Explode)	✓ (as Ring)

In the first exercise, we will create bar and scatter plots 1704.

38.19.1 Creating Bar and Scatter Plots and Pie Charts

Bar Plots, Scatter Plots, and Pie Charts graph data from Spreadsheets. The cells of the spreadsheet can contain statistical tokens based on your plotted data, or freetext. You may want to familiarize yourself with Spreadsheets prior to beginning this tutorial by visiting the Spreadsheet tutorial 1620.

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Note that Bar Plots and Pie Charts can be populated by drag-and-drop directly, but the Spreadsheet must be accessed to add formatting elements such as Error Bars and Data Emphasis features (i.e., Highlighting and Exploding).

In this section of the tutorial, we will learn to:

- Insert a Spreadsheet onto the layout and populate it with freetext and statistical tokens.
- Create a Bar Plot 1705.
- Create a Pie Chart 1711.
- Create a Scatter Plot 1718 (also see the Regression Analysis tutorial 1679 for more details on creating live updating regression analysis).

38.19.1. Creating Bar Plots 1

In this section we will learn to populate a Bar Plot with data from a Spreadsheet. Please also see our Bar Plots webpage for additional videos and tips on creating Bar Plots.

1. Select **File** tab→**Open**.
2. Open the layout titled "Bar Pie Scatter begin.fey" within the Tutorial Sample Data archive.

   The layout contains Color Dot Plots and a Spreadsheet from which the Bar Plot will be derived (Fig. 1). See this section of the manual to see how the Spreadsheet was added to the layout 415.

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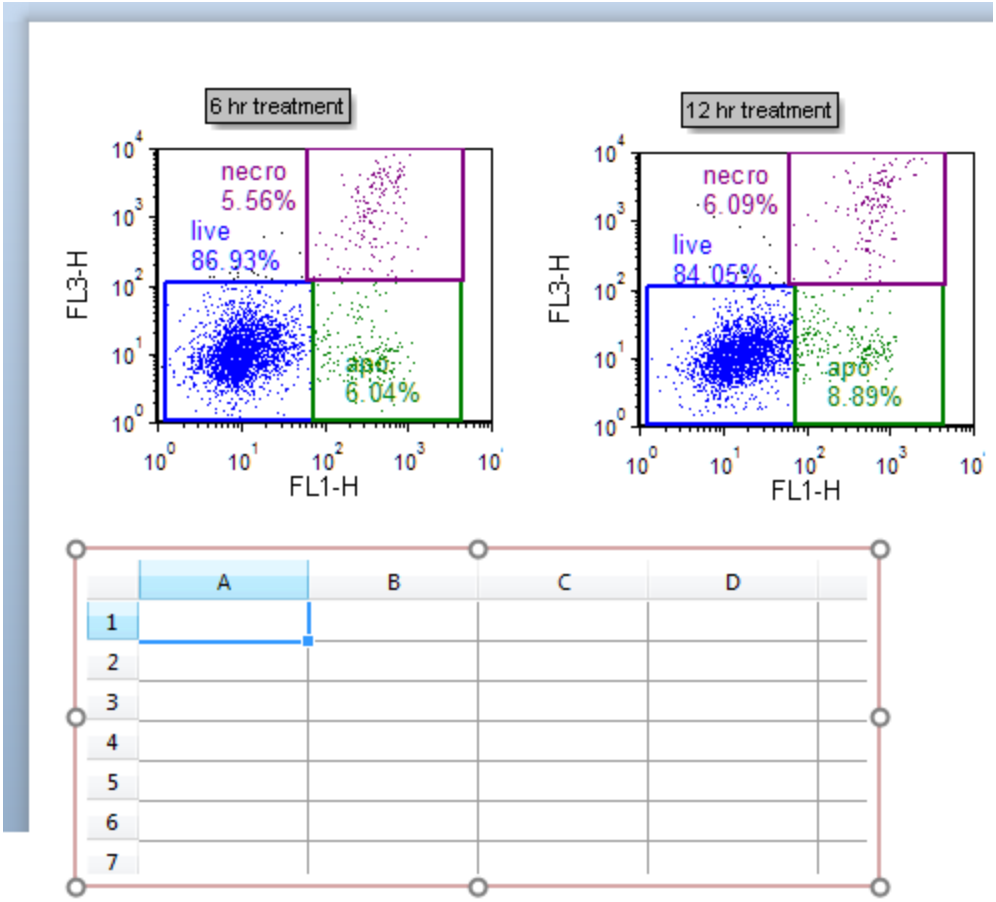

Figure 1. Spreadsheet on the layout

We will now fill the Spreadsheet with both freetext and live-updating tokens. First, we will manually enter freetext values that will be used for our bar plot axis labels.

3. Click on Cell A1 in the Spreadsheet to highlight it.
4. Type "live" (Fig. 2, ❶).
5. Press **Enter** on the keyboard.
6. Repeat steps 3-5 for Cells A2 and A3 in the Spreadsheet, typing in "apo" and "necro", respectively. The Spreadsheet will now appear as below (Fig. 2).

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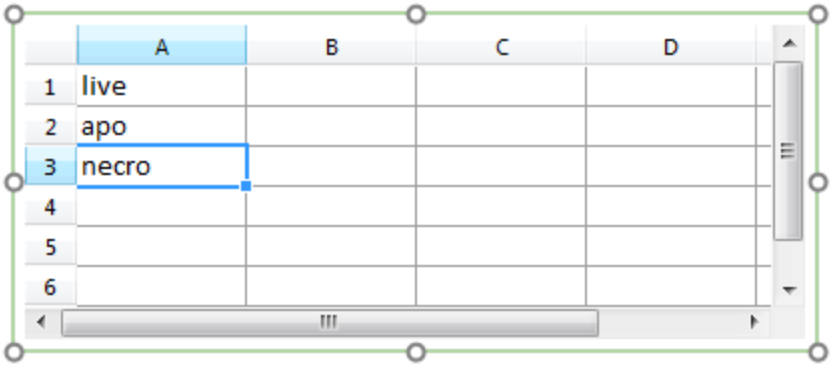

Figure 2. Entering freetext into a Spreadsheet

We will now select statistic tokens to plot in the Bar Plot or Pie Chart and place them in the Spreadsheet. Using tokens will allow live-updating of plots as you adjust your gates or advance through your dataset.

7. Drag and drop the "live" gate from the *6 hr treatment* plot at upper left into **Cell #** B1 of the Spreadsheet. While dragging, the gate outline will move (Fig. 3, ❶).

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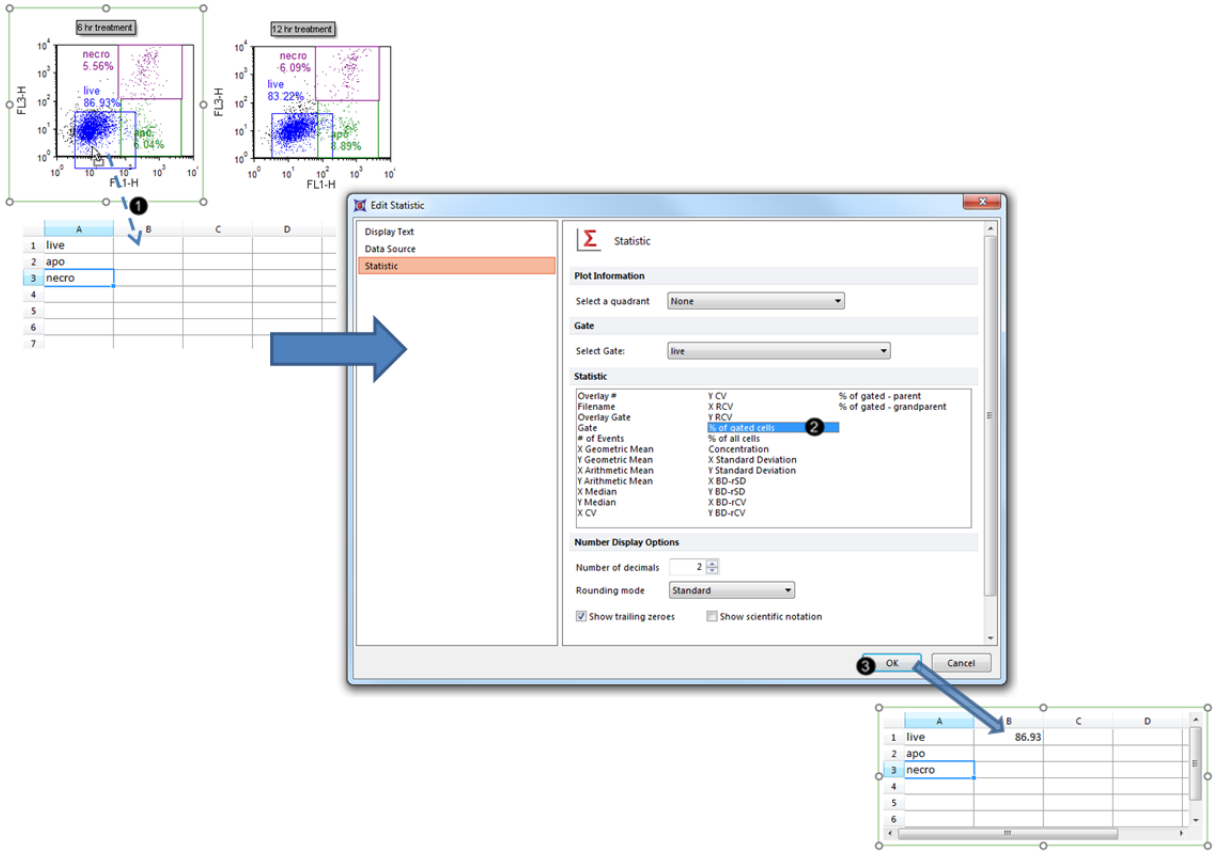

Figure 3. Dragging and dropping a statistic token into the Spreadsheet.

8. Select **% of gated cells** from the **Statistic** list box in the **Edit Statistic** dialog (Fig. 3, ❷).

9. Click **OK** (Fig. 3, ❸).

The B1 cell of the Spreadsheet will now contain the %-gated statistic for the "live" gate, as shown in Fig. 3, *bottom*.

10. Repeat steps 7-9 for the "apo" and "necro" gates of the *6 hr treatment* plot respectively into **Cell #s** B2 and B3. The Spreadsheet will now appear as in Fig. 4.

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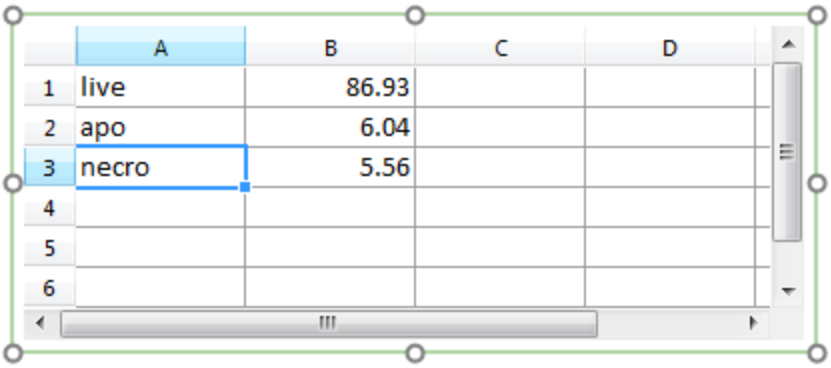

Figure 4. Spreadsheet with B column filled with %-gated statistic tokens

Note: Steps 1-10 can also be accomplished via drag-and-drop of statistics, gates, or plots directly to the Bar Plot 549, without using a Spreadsheet. Please see this section of the manual for a pictorial guide 549.

11. Select the **Insert** tab → **1D Plots**→ **Bar** command (Fig. 5).

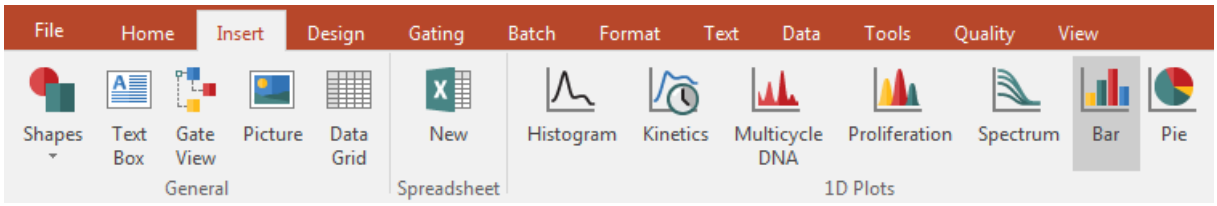

Figure 5. Inserting a Bar Plot

12. Click on an empty space on the layout (e.g., to the right of the Spreadsheet) to insert a Bar Plot (Fig. 6).

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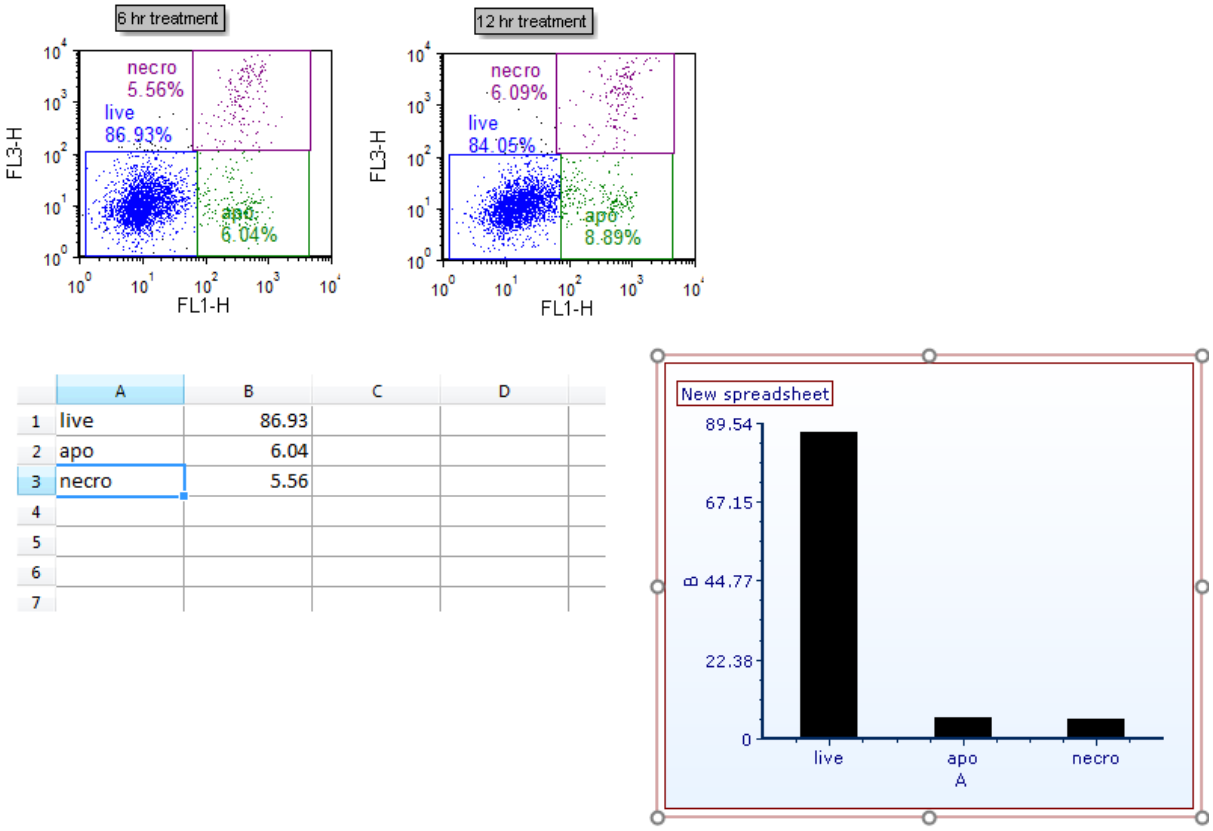

Figure 6. Bar Plot inserted on the layout

We will now rename the Bar Plot axes.

13. Double-click the Bar Plot to open the **Formatting** dialog.
14. Click on the Axis category (Fig. 7, ❶).
15. Click the **The specified text below** radio button (Fig. 7, ❷).
16. Type "%-gated" into the **Text** box under **Title** (Fig. 7, ❸).
17. Click **X Axis** under Select Axis (Fig. 7, ❹).
18. Type "subset" into the **Text** box under **Title**.
19. Click **OK** (Fig. 7, ❺).

The Bar Plot will now appear as in Fig. 7, *right*.

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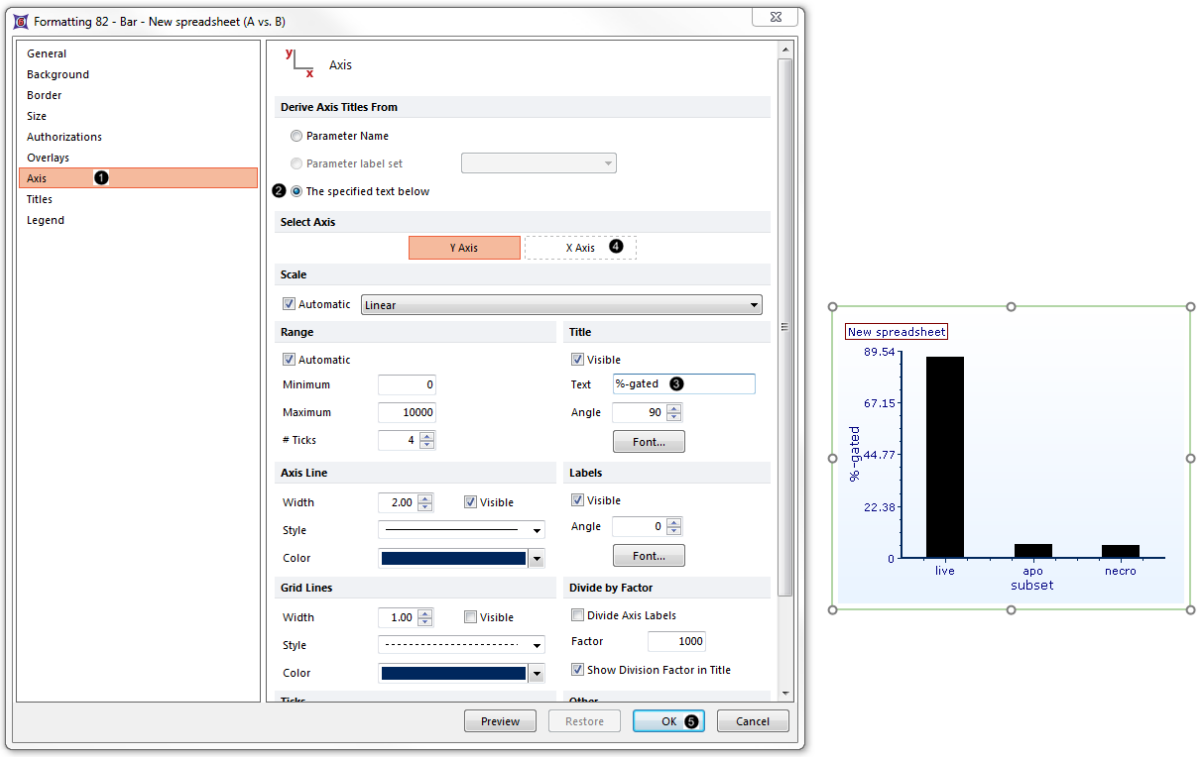

Figure 7. Bar Plot with Renamed Axes.

Note: Bar color, style, and other attributes of a Bar Plot can be customized. See the Formatting Bar Plots 556 section of the manual for details.

20. *Optional:*

- Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Bar Plot updates with the new data.
- Adjust the gates on the plots and watch the Bar Plot update in real-time.

Go on to the next section to create a Pie Chart 1711 from the same raw data. Or, jump to the section on Formatting Bar Plots 1724.

38.19.1. Creating Pie Charts 2

Creating Pie Charts in FCS Express is much like creating Bar Plots, in that they can be populated via a Spreadsheet or by drag-and drop.

1. Select **File** tab→**Open**.
2. Open the layout titled "Pie begin.fey" within the Tutorial Sample Data archive.

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This layout contains Color Dot Plots and a Spreadsheet that has already been filled with one row of tokens and freetext.

3. Select the **Insert** tab → **1D Plots**→ **Pie** command (Fig. 8).

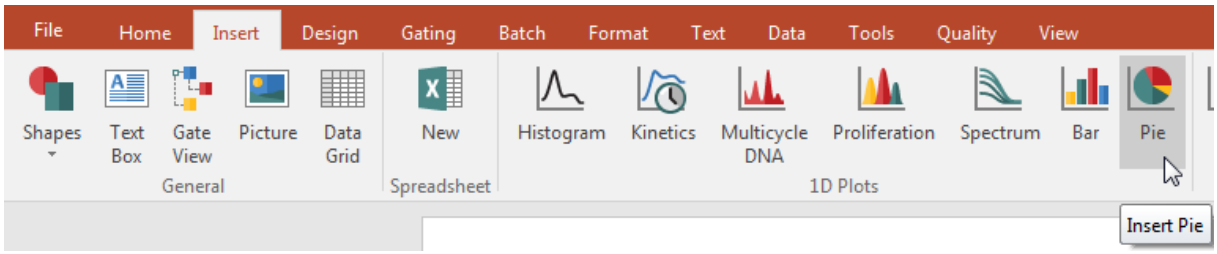

Figure 8. Inserting a Pie Chart

4. Click on an empty space on the layout (e.g., to the right of the Spreadsheet) to insert a Pie Chart (Fig. 9).

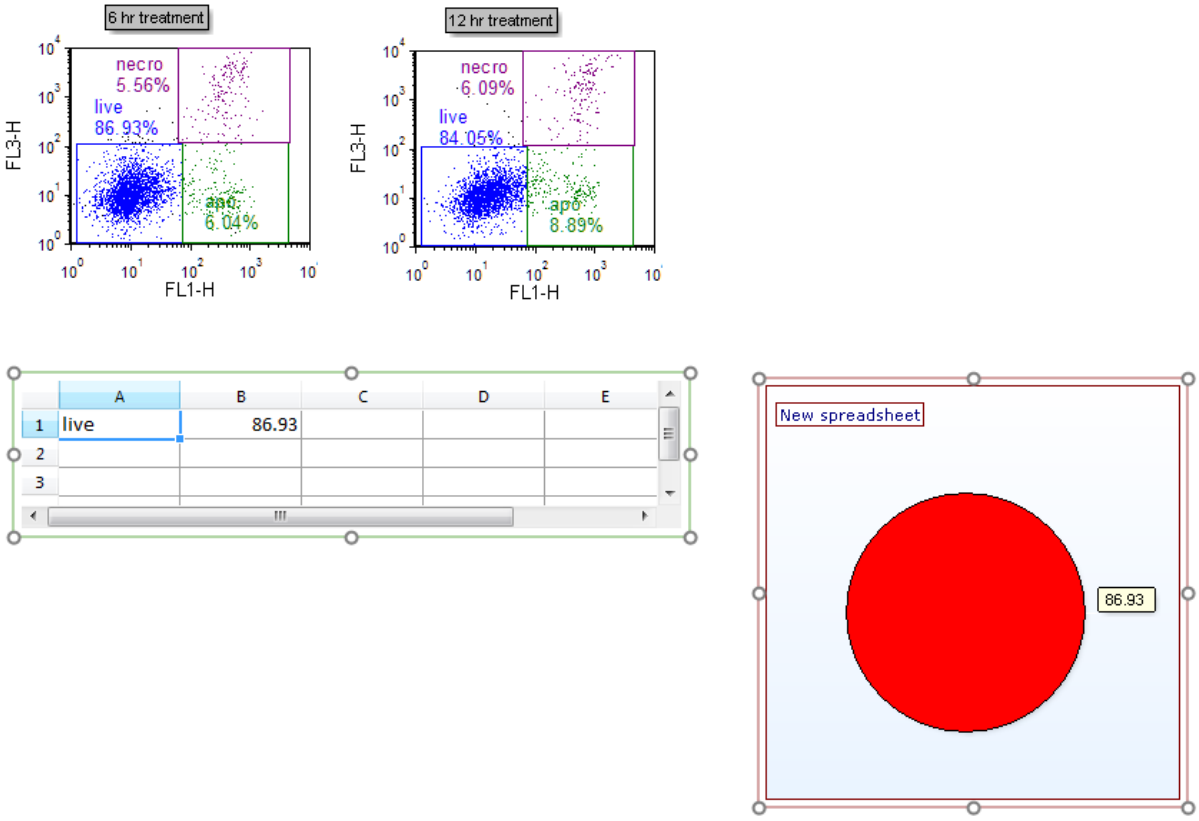

Figure 9. A Pie Chart inserted on the layout

We will now use the drag-and-drop functionality of FCS Express 6 to add new sectors (slices) and replace old ones.

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5. Drag and drop the "apo" gate from the *6 hr treatment* plot at left to an empty spot on the Pie Chart (not on the pie sector itself) (Fig. 10).

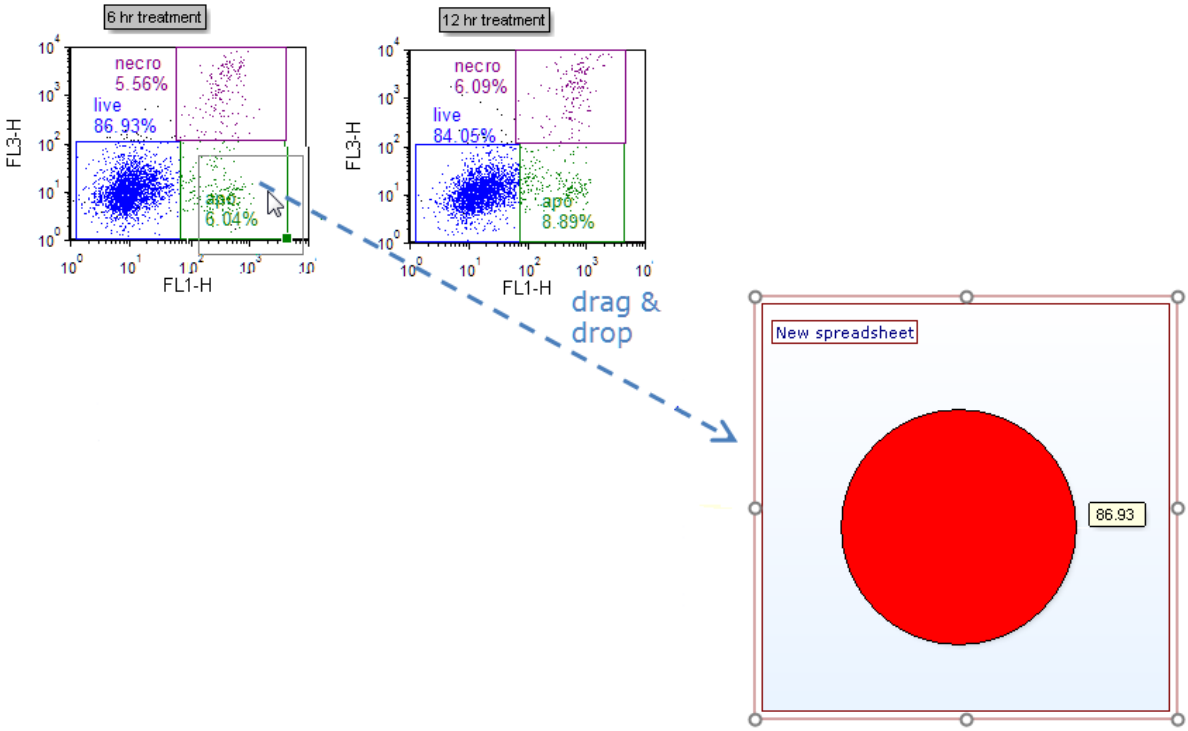

Figure 10. Dragging a gate to a Pie Chart to create a new sector (slice)

6. Select "% of gated cells" from the **Statistic** box in the **Edit Statistic** dialog (Fig. 11).

7. Click **OK**. A new sector (slice) displaying this statistic now appears as in Fig. 11, *right*.

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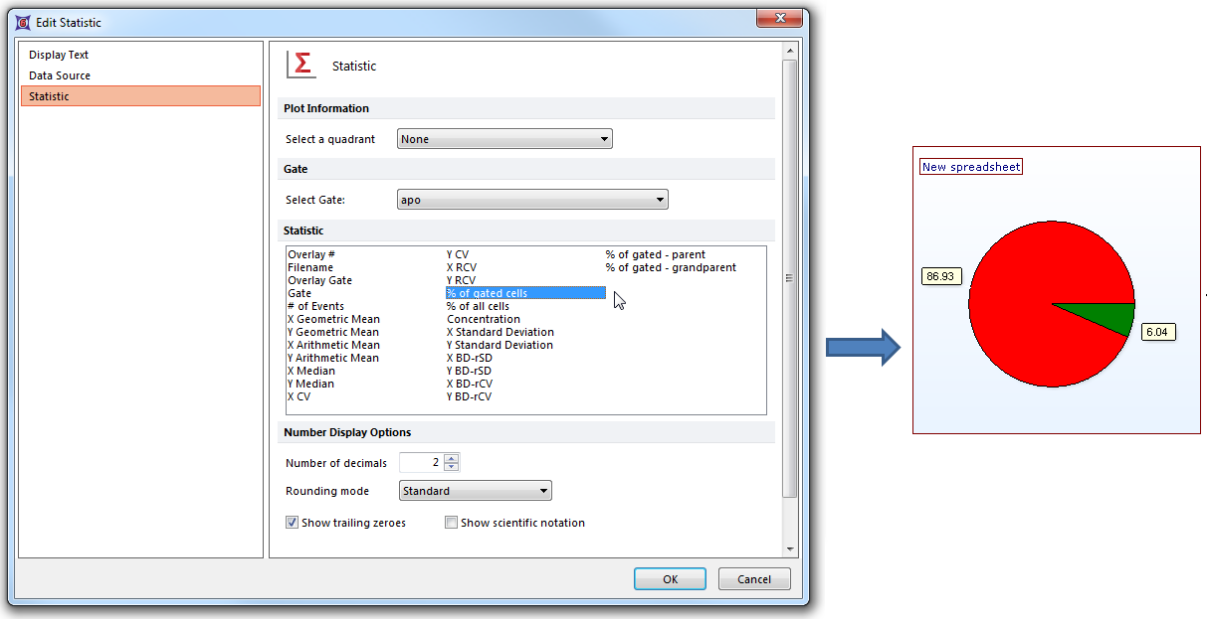

Figure 11. Choosing a statistic to plot on the Pie Chart after dragging a gate

8. Repeat Steps 5-7 for the "necro" gate of the *6 hr treatment* plot at left, so that the Pie Chart will have a total of three sectors (slices).

We will now Format the Pie Chart.

9. Right-click on the Pie Chart.

10. Choose **Format** from the pop-up menu (Fig. 12).

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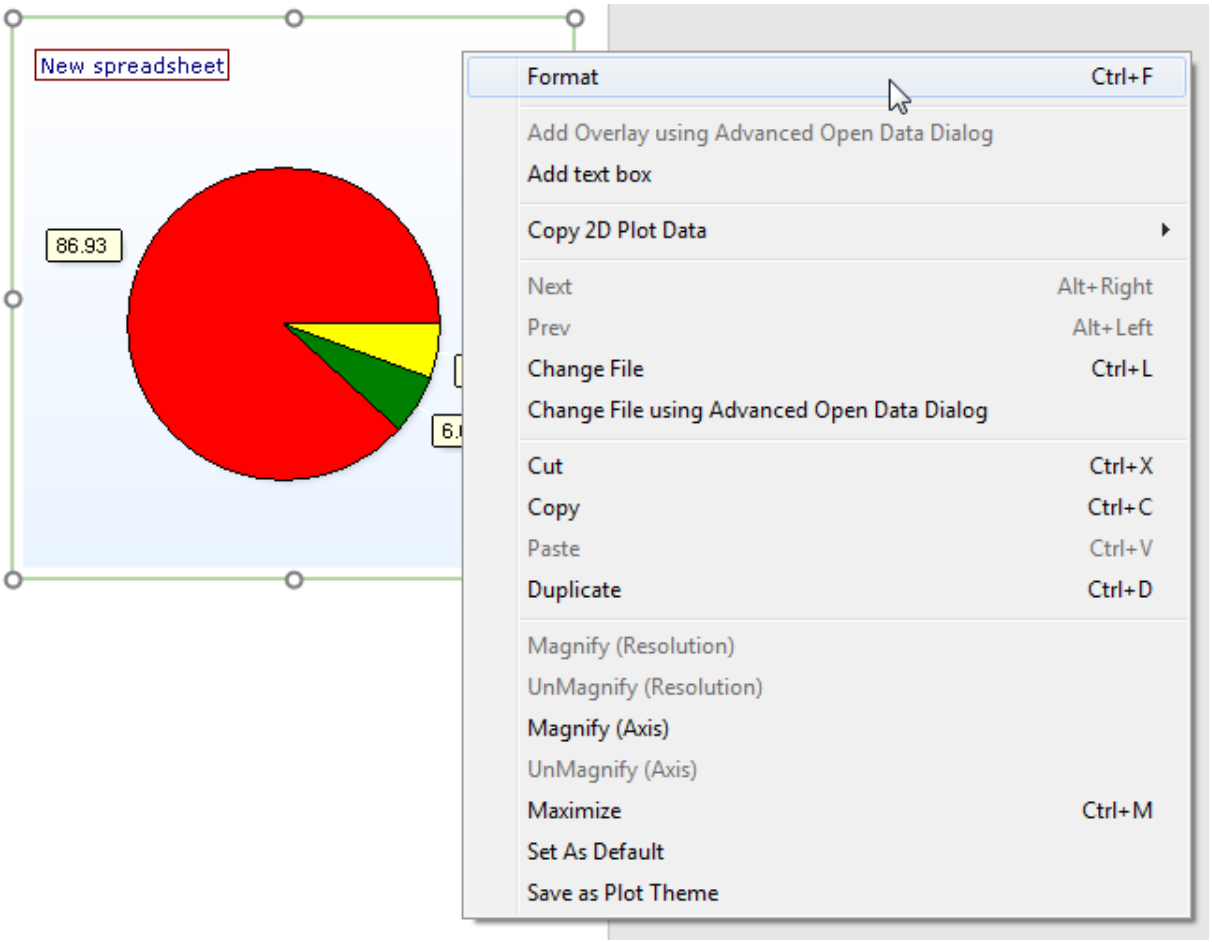

Figure 12. Accessing Formatting options of the Pie Chart

11. Choose the **Legend** category (Fig. 13, ❶).

12. Check the **Visible** box (Fig. 13, ❷).

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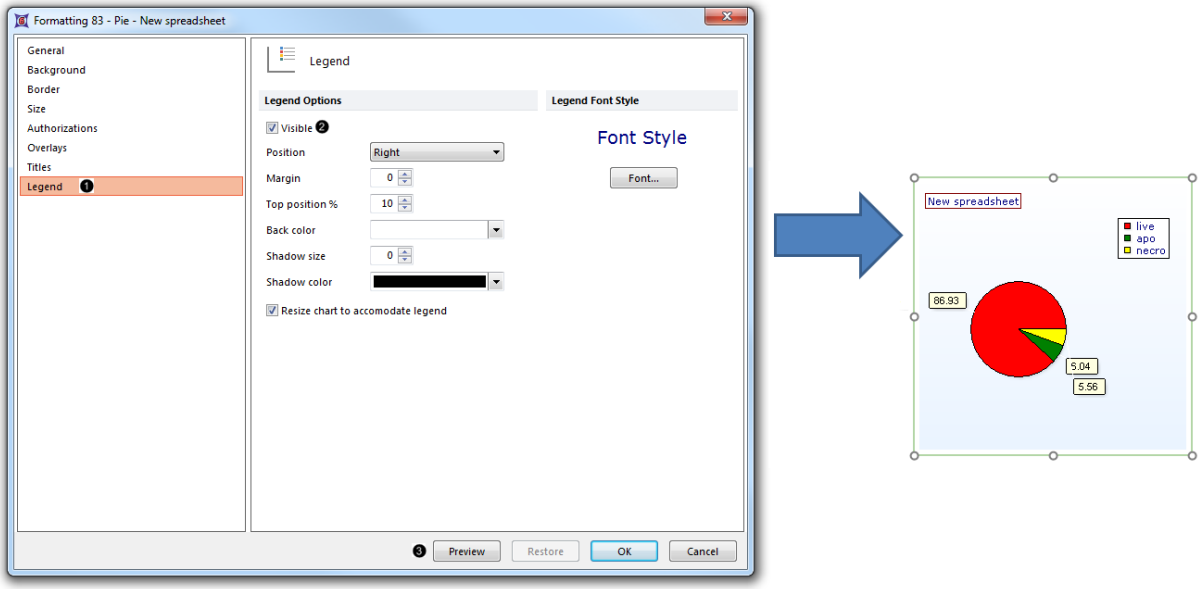

Figure 13. Adding a Legend to the Pie Chart

13. Click **Preview** (Fig. 13, ❸). The Pie Chart will now display the name of each subset (listed in the first column of the Custom Data Grid) in the Legend (Fig. 13, *right*).

14. Choose the **Overlays** category (Fig. 14, ❶).

The Name Parameter is derived by default from the first column of the Custom Data Grid on your layout. It appears as "subset" or as "A", depending on the appearance of your Custom Data Grid.

The Value parameter is derived by default from the second column of the Custom Data Grid. It appears as "%-gated" or as "B", depending on the appearance of your Custom Data Grid. It is displayed by default as a Caption for each slice of the Pie Chart (Fig 18, *upper right*). The Caption can be formatted to display any combination of Name, Value, or Percentage for each sector (slice) of the Pie.

15. Check *any combination* of **Value, Percentage,** and **Name** from the **Caption Style** section of the **Overlays** category to customize the Caption with the desired information (Fig. 14, ❷).

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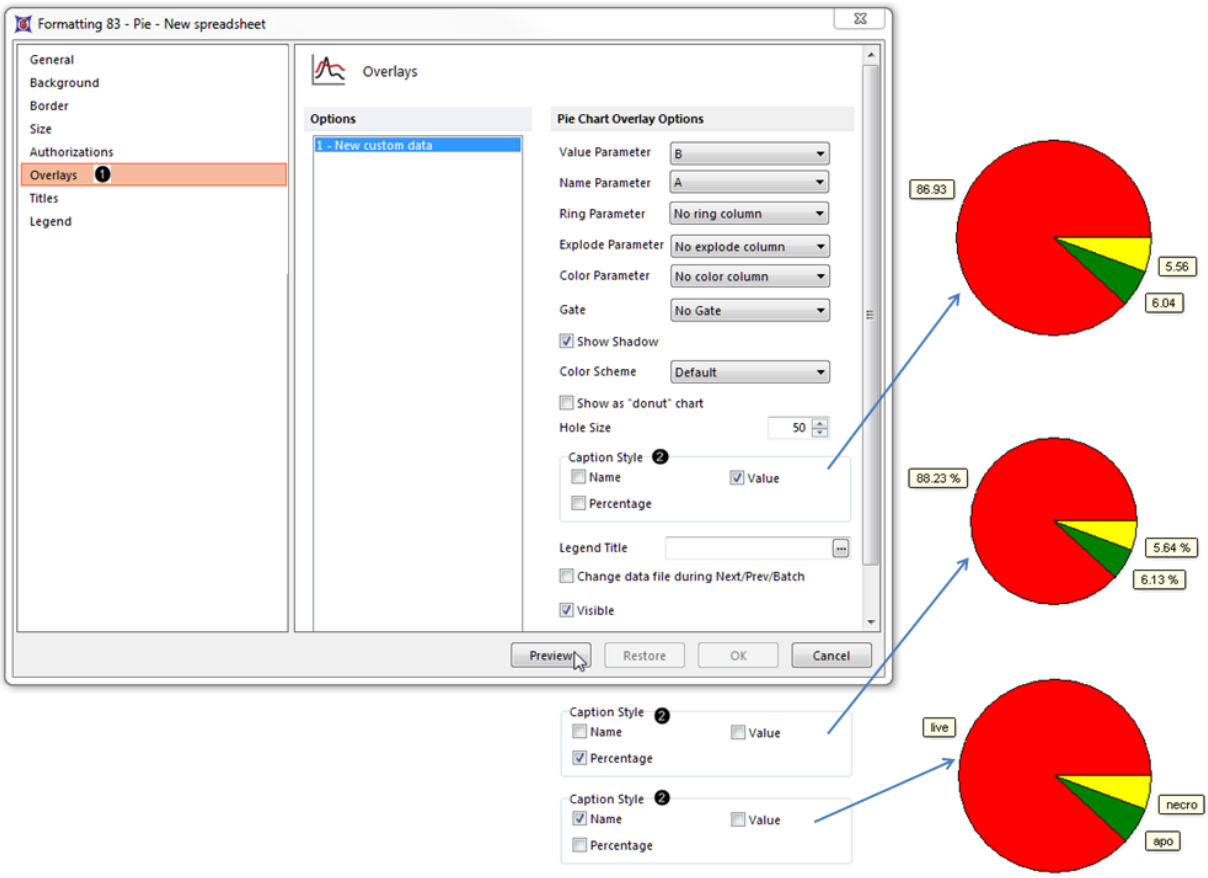

Figure 14. Editing the Caption Style of the Pie Chart

16. Click **Preview** to view the Caption Style change (Fig. 14, mouse arrow).

    Note: Clicking on the Caption in the Pie Chart allows for direct editing of the Captions with freetext or tokens of your choice 547. Pie shadow, donut vs. pie appearance, color scheme, and other attributes can also be customized. See the Formatting Pie Charts 544 section of the manual for details.

17. *Optional:*

    - Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Pie Chart updates with the new data.
    - Adjust the gates on the plots and watch the Pie Chart update in real-time.

Optional: Explore Pie Chart color formatting options in Steps 18-28. Or, go to the Formatting section of this tutorial 1724 to learn how to Explode slices for emphasis and display an additional dataset as a Ring at the periphery of the Pie.

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18. Select **Modern** from the **Color Schemes** dropdown list (Fig. 15, ❶). *Note:* there are many predefined color schemes that may be chosen for display.

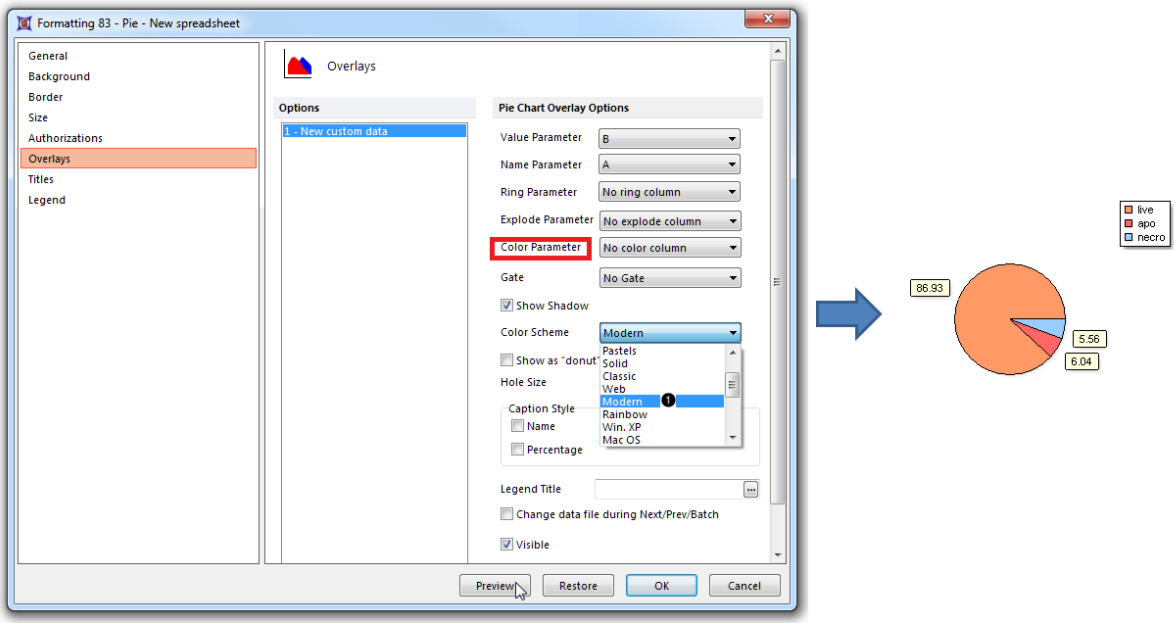

Figure 15. Changing the Color Scheme

19. Click **Preview** (Fig. 15, mouse arrow).

The Pie Chart colors have now been reformatted with the **Modern** color scheme. (Fig. 15, *right*)

Go on to the next section to create a Scatter Plot 1718 from the same Color Dot Plots on this layout. Or, jump to the Formatting section of this tutorial 1724 to learn how to Explode Pie slices for emphasis and display an additional dataset as a Ring at the periphery of the Pie.

38.19.1. Creating Scatter Plots 3

1. Select **File** tab→**Open**.

2. Open the layout titled "Bar Pie Scatter begin.fey" within the Tutorial Sample Data archive.

   The layout contains Color Dot Plots and a Spreadsheet from which the Scatter Plot will be derived (Fig. 22). See this section of the manual to see how the Spreadsheet was added to the layout 415.

   We will now fill the Spreadsheet with both freetext and live-updating tokens. First, we will enter the independent parameters for the Scatter Plot. Note

that in contrast to Bar Plots and Pie Charts, the x-axis values in a Scatter Plot must be numeric. We will enter the *hours of treatment* indicated in the title for each Color Dot Plot as this parameter.

3. Click on Cell A1 in the Spreadsheet (Fig. 1, ❶) to highlight it.

4. Type "0" in the Custom Data Grid formula editor (Fig. 1, ❷).

5. Press **Enter** on the keyboard.

6. Repeat steps 3-5 for Cells A2 and A3 in the Spreadsheet, typing in "6" and "12", respectively. The Spreadsheet will now appear as in Fig. 1, *right*.

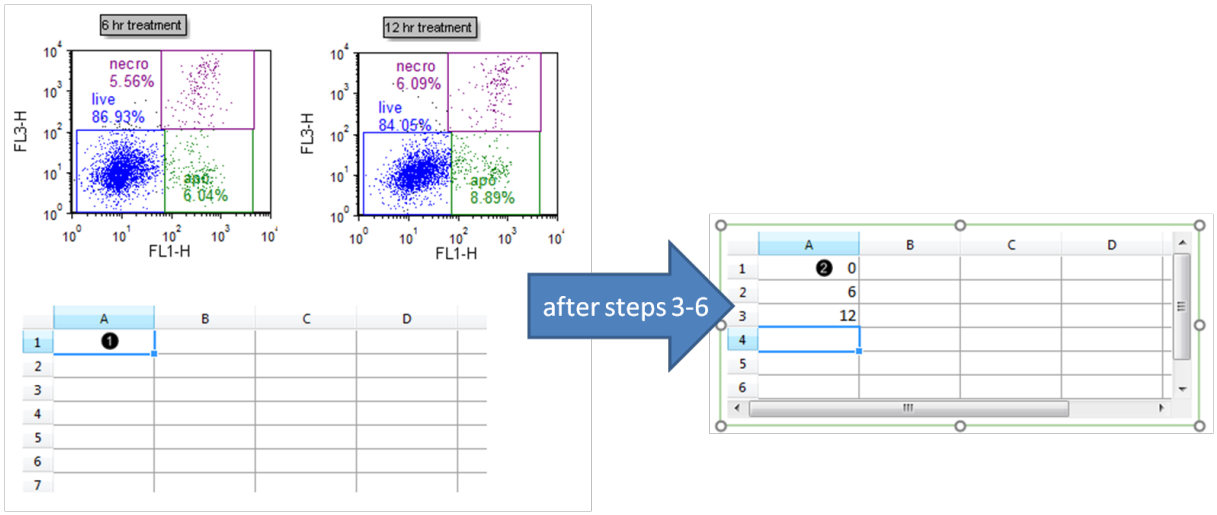

Figure 1. Typing freetext into a Spreadsheet

We will now insert tokens into the Spreadsheet to display on the Scatter Plot.

7. Drag the "live" gate from the *6 hr treatment* plot onto cell B2 of the Spreadsheet. While dragging, the gate outline will move (Fig. 2, ❶).

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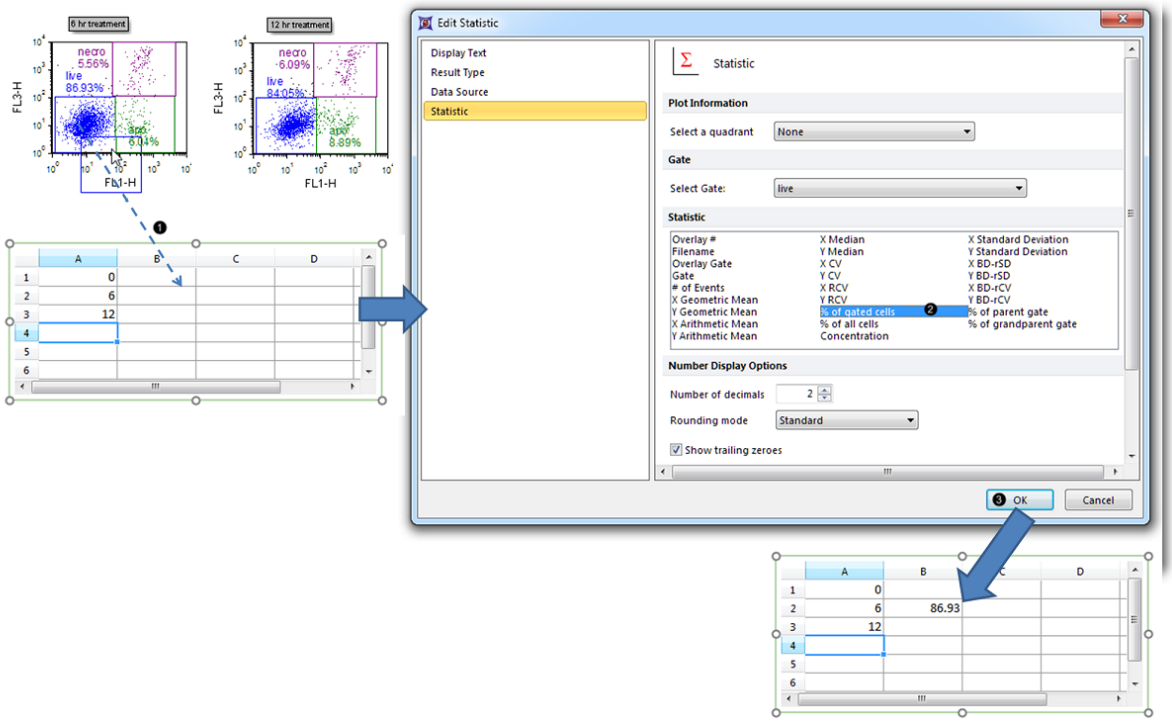
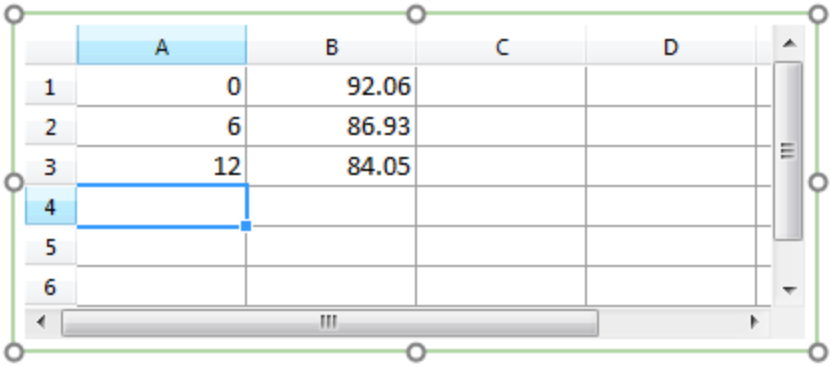


Figure 2. Dragging and dropping a statistic token into a Spreadsheet

8. Select **% of gated cells** from the **Statistic** list box in the **Edit Statistic** dialog (Fig. 2, ❷).

9. Click **OK** (Fig. 2, ❸). The B2 cell of the Spreadsheet will now contain the **%-gated statistic** for the "live" gate of the *6 hr treatment* Color Dot Plot (Fig. 2, *bottom*).

10. Repeat steps 7-9 for the *12 hr treatment* and *Control (no treatment)* Color Dot Plots, dragging the "live" gate from each plot into cell #s B1 and B3, respectively. The Spreadsheet will now appear as in Fig. 3.



	A	B	C	D
1	0	92.06		
2	6	86.93		
3	12	84.05		
4				
5				
6				

Figure 3. Completed Spreadsheet

11. Select the **Insert** tab → **2D Plots**→ **Scatter** command (Fig. 4).

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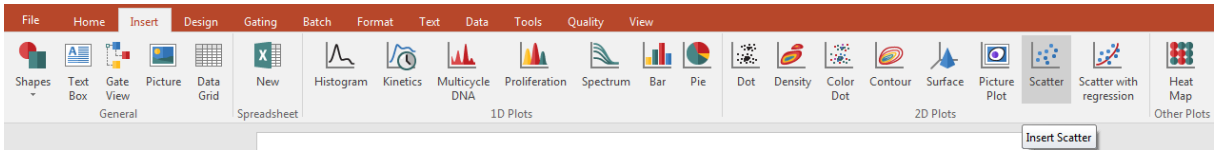

Figure 4. Inserting a Scatter Plot

12. Click on an empty space on the layout (e.g., to the right of the Spreadsheet) to insert a Scatter Plot (Fig. 5).

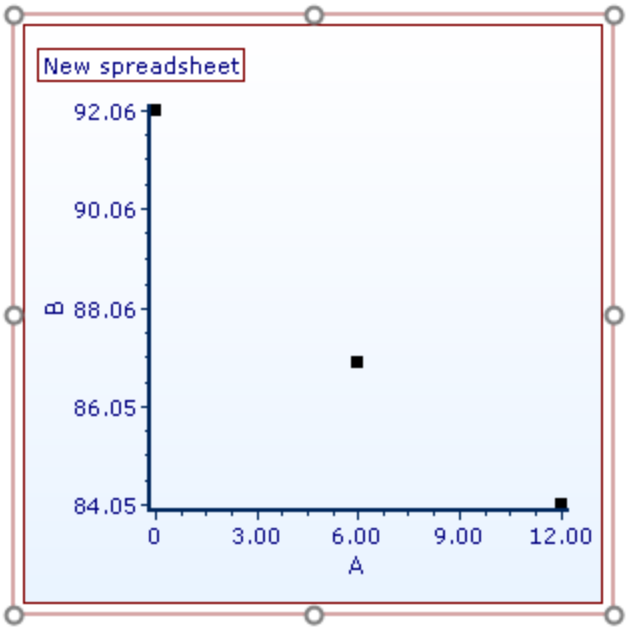

Figure 5. Inserted Scatter Plot

We will now rename the Scatter Plot axes.

13. Double-click the Scatter Plot to open the **Formatting** dialog.
14. Click on the Axis category (Fig. 6, ❶).
15. Click the **The specified text below** radio button (Fig. 6, ❷).
16. Type "%-gated" into the **Text** box under **Title** (Fig. 6, ❸).
17. Click **X Axis** under Select Axis (Fig. 6, ❹).
18. Type "treatment time (hr)" into the **Text** box under **Title**.
19. Click **OK** (Fig. 6, ❺).

The Scatter Plot will now appear as in Fig. 6, *right*.

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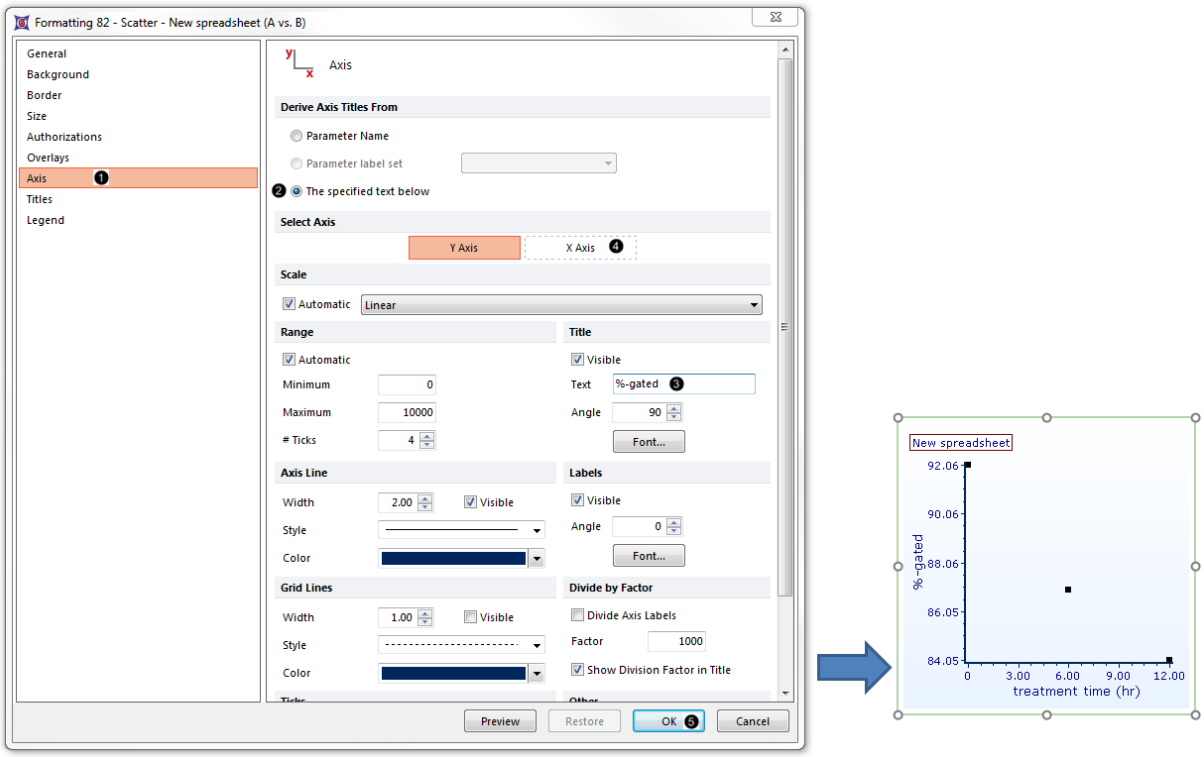

Figure 6. Scatter Plot with Renamed Axes

We will now adjust the range of the Scatter Plot so that the points are more readily visible.

20. Double-click the Scatter Plot to open the **Formatting** dialog from the pop-up menu.

21. Select the **Axis** category (Fig. 7, ❶).

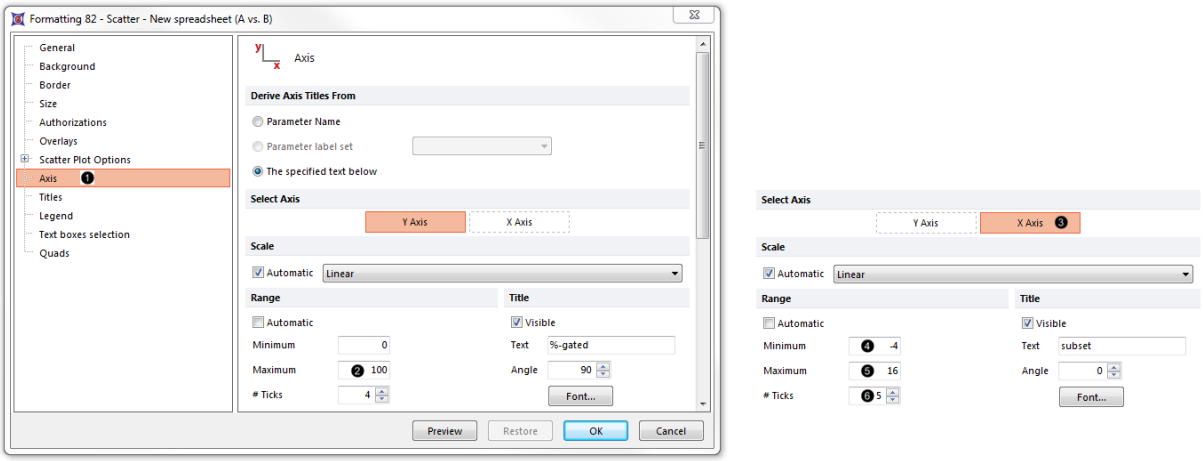

Figure 7. Adjusting range of the Scatter Plot from the Formatting dialog

22. Enter "100" as the **Maximum** (Fig. 7, ❷).

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23. Click on **X-axis** of the **Select Axis** section (Fig. 7, ❸).

24. Enter "-4" as the **Minimum** (Fig. 7, ❹).

25. Enter "16" as the **Minimum** (Fig. 7, ❺).

26. Increase the **# Ticks** to 5 (Fig. 7, ❻).

27. Click **OK**.

The Scatter Plot will now appear as in Fig. 8.

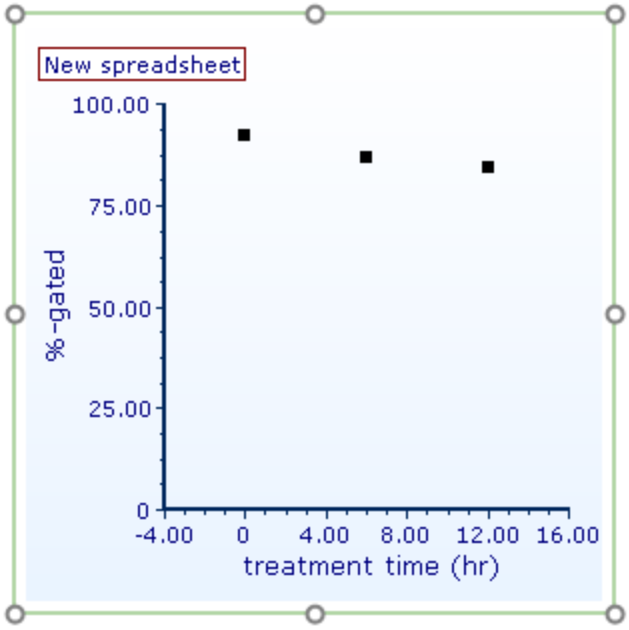

Figure 8. Completed Scatter Plot with adjusted range

Note: Point color, shape, connecting lines, and other attributes of a Scatter Plot can be customized. See the Formatting Scatter Plots 563 section of the manual for details.

28. *Optional:*

- Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Scatter Plot updates with the new data.
- Adjust the gates on the Color Dot Plots and watch the Scatter Plot update in real-time.

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38.19.2 Formatting Bar and Scatter Plots and Pie Charts

Like any other plot in FCS Express, Bar Plots, Scatter Plots, and Pie Chart axes, titles, legends, backgrounds, and more are fully formattable[246]. In this tutorial section we will focus on learning how to apply the special formatting features listed below. Please click on the links below or in the table to jump to a tutorial topic.

- Adding Error Bars to Bar and Scatter Plots[1724]
- Formatting Highlighting and Explode data emphasis features[1730]
- Adding an additional dataset to a Spreadsheet[1734]
- Creating Overlays on Bar and Scatter Plots[1736]
- Creating a Ring on a Pie Chart[1738]

	Add Data to Plots by		*Formatting Feature*		
	Drag and Drop	**Spreadsheet**	**Error Bars**[1724]	**Data Emphasis**[1730] (according to user-defined criteria)	**Display Additional Datasets**[1734]
Bar Plots	✓	✓	✓	✓ (Highlight[1730])	✓ (as Overlay[1736])
Scatter Plots		✓	✓	✓ (Highlight[1730])	✓ (as Overlay[1736])
Pie Charts	✓	✓		✓ (Explode[1730])	✓ (as Ring[1738])

38.19.2.1 Adding Error Bars

Error Bars and Highlighting can be added to Bar Plots and to Scatter Plots. In this example, we will add these features to a Bar Plot.

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1. Select **File** tab→**Open**.
2. Open the layout titled "Error Bars.fey" within the Tutorial Sample Data archive.

A Spreadsheet and its associated Bar Plot without Error Bars or Highlighting already exist on the layout. The Bar Plot is graphing the means of the "apo" and "necro" %-gated statistic for each of the three Color Dot Plots. This is accomplished through the use of array formulae[748] within the Spreadsheet, which can be seen when clicking on Cell E1 (Fig. 1).

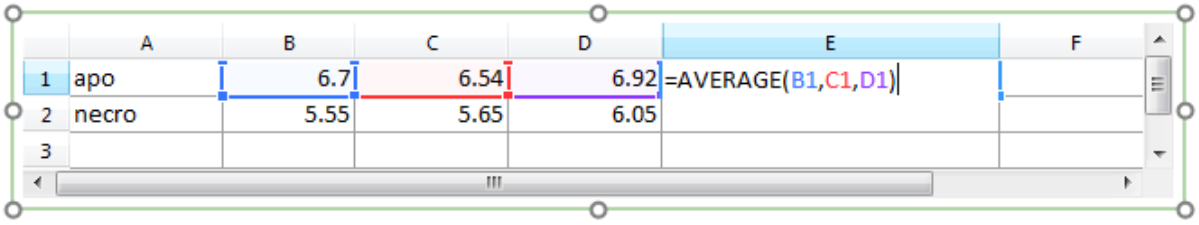

Figure 1. Array formula for calculating the mean of three tokens in a Spreadsheet

We will also employ an array formula[748] to calculate the error bars in this example; however, error bars may also derive from default Statistic Tokens or from freetext.

3. Click on Cell F1 of the Spreadsheet (Fig. 2, ❶) to select it.
4. Click the **Formulas** tab, which has now been revealed in the formerly hidden Spreadsheet tab family (Fig. 2, ❷).

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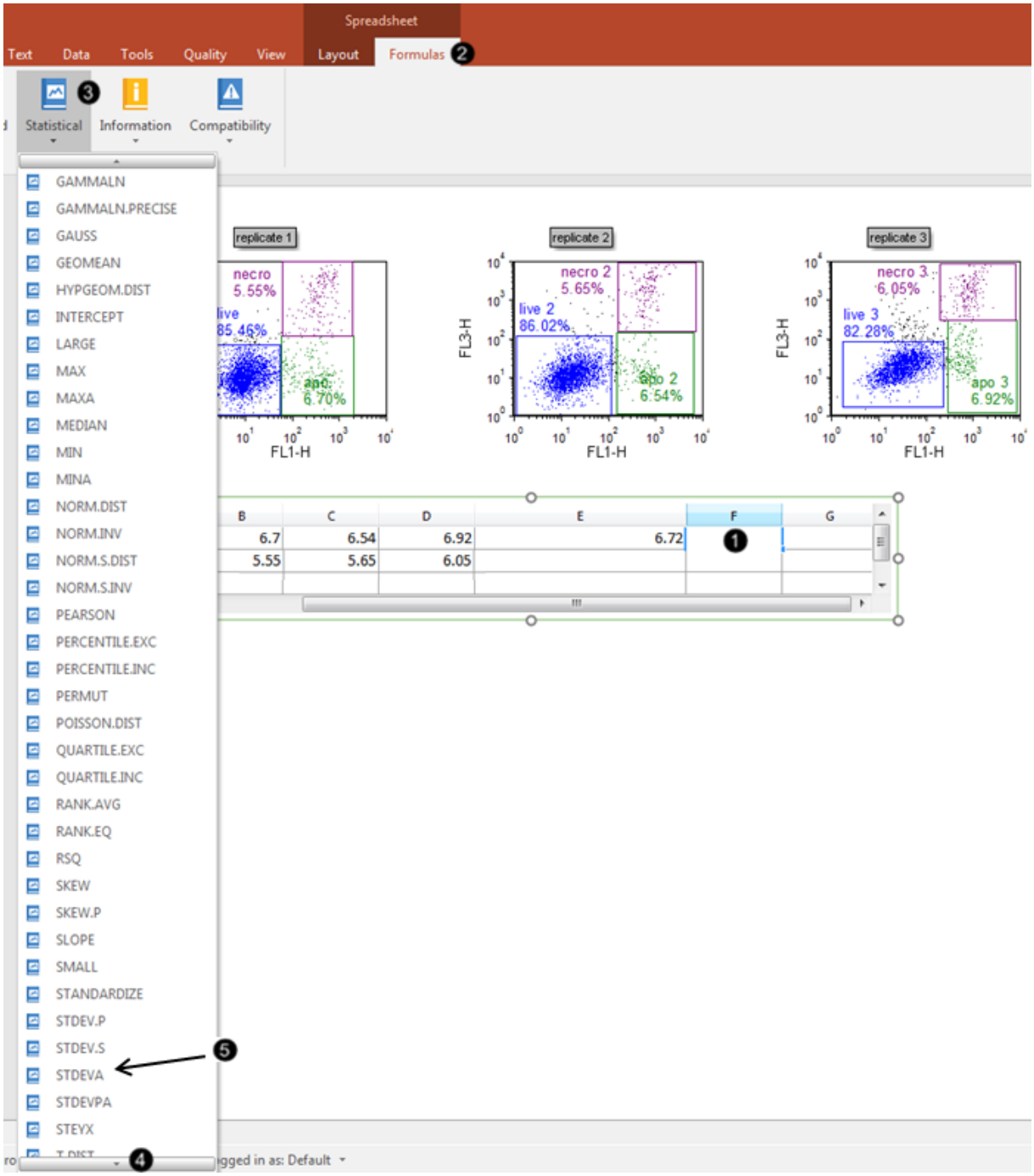

Figure 2. Inserting a function into the Spreadsheet cell

5. Click the **Statistical** command (Fig. 2, ❸).

6. Scroll down through the menu of Statistical formulas (Fig. 2, ❹) until "STDEVA" is visible.

7. Select "STDEVA" from the menu (Fig. 3, ❶).

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We will now enter Cells B1-D1 as components of the *StdDev* array formula.

8. Click Cell B1 of the Spreadsheet (Fig. 3, ❶), *while the cursor is within the parentheses of the "STDEVA" formula, as shown by the green arrow*.

9. Type a comma (") (Fig. 3, ❷).

10. Repeat Steps 8-9 for Cells C1-D1 of the Spreadsheet. When complete, the filled-in formula will appear as in Figure 3, *bottom*.

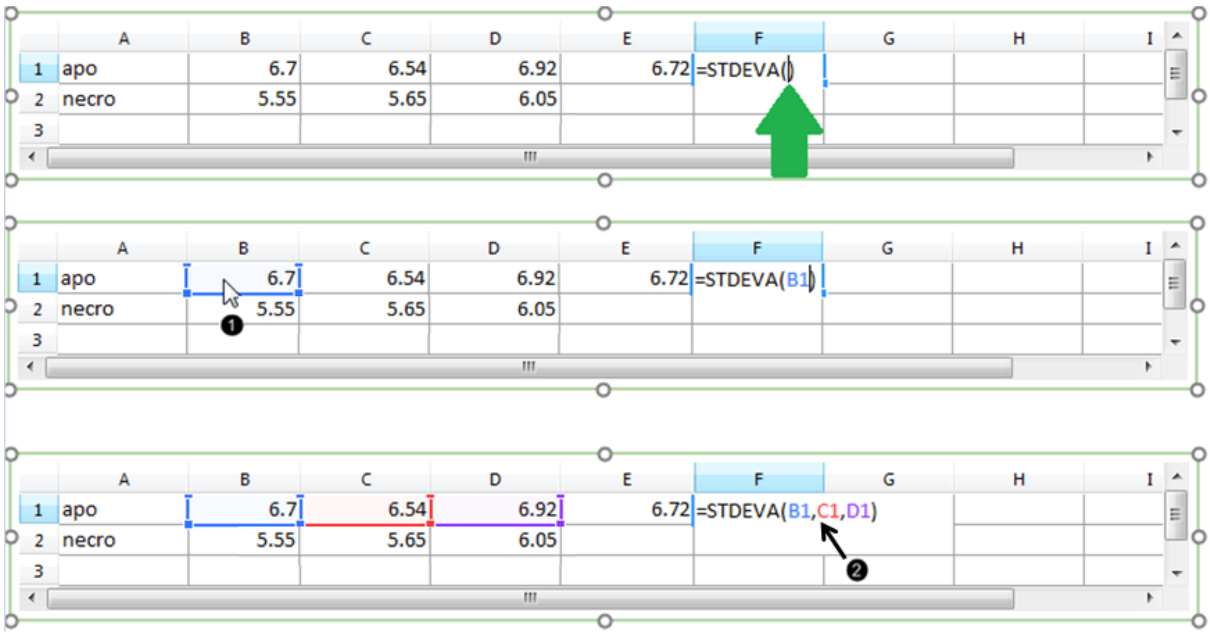

Figure 3. Entering components of the Standard Deviation formula

The Custom Data Grid "apo" row now contains a complete *StdDev* array formula for the error bar, which has been evaluated with the result shown in Cell F1 (Fig. 4).

	A	B	C	D	E	F	G	H	I
1	apo	6.7	6.54	6.92	6.72	0.19078784			
2	necro	5.55	5.65	6.05					
3									
4									

Figure 4. Numerical result of completed array formula within the Spreadsheet

We will now enter the corresponding mean and error bar formulas for the "necro" row of the Custom Data Grid, by dragging the appropriate Cells within the Spreadsheet to fill the series.

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11. Click on Cell E1 in the Spreadsheet, which contains the complete AVERAGE formula that was created for you in the layout, prior to beginning the tutorial.

12. Hover the mouse pointer over the lower-right corner of Cell E1 in the Spreadsheet, until the cursor forms a crosshair (Fig. 5, ❶).

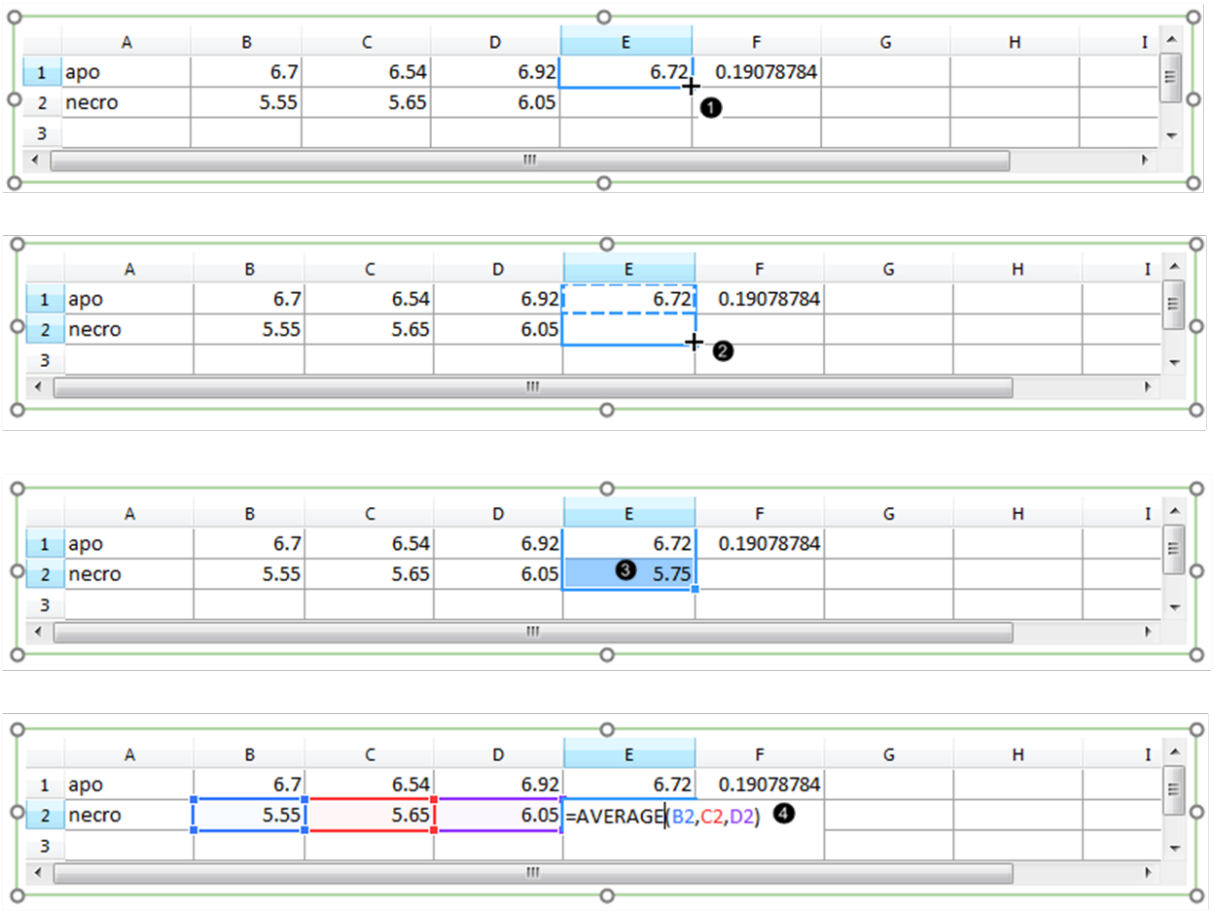

Figure 5. Filling a series in the Spreadsheet.

13. Drag the mouse to the lower-right corner of Cell E2 in the Spreadsheet, while keeping the mouse button pressed (Fig. 5, ❷).

14. Release the mouse button (Fig. 5, ❸). A numerical value appears in Cell E2 as a result of filling the series.

15. Double-click Cell E2 to view the formula and its components (Fig. 5, ❹). Note that the formula was extended faithfully, but refers to the "necro" %-gated values from Row 2 in the Spreadsheet.

16. Repeat Steps 11-15 for Cell F1, to fill the formula for the Standard Deviation of the "necro" %-gated values. The Spreadsheet will then appear as in Figure 6 below.

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	A	B	C	D	E	F	G	H	I
1	apo	6.7	6.54	6.92	6.72	0.19078784			
2	necro	5.55	5.65	6.05	5.75	0.264575131			
3									
4									

Figure 6. The Standard Deviation for the "necro" row values has been calculated by filling the appropriate cell in the Spreadsheet.

We will now format the Bar Plot to derive the error bars from the appropriate column in the Spreadsheet.

17. Double-click the Bar Plot to open the **Formatting** dialog.

18. Click on the **Overlays** category (Fig. 7, ❶).

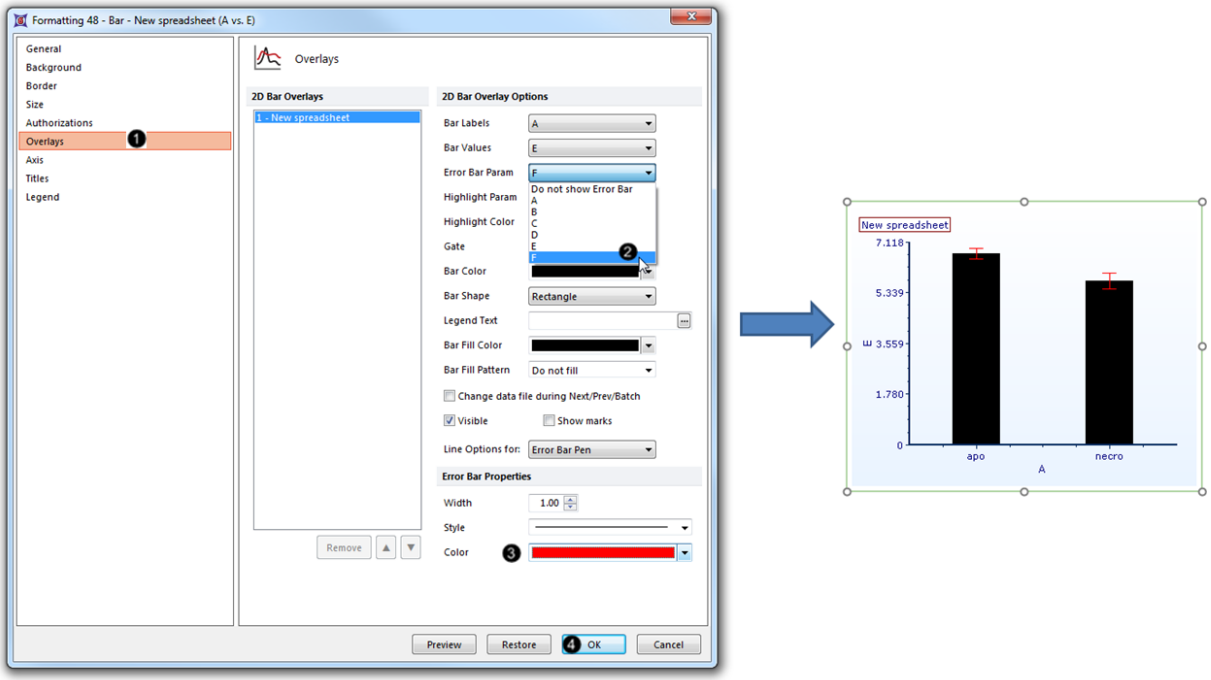

Figure 7. Adding error bars to a Bar Plot in the Formatting dialog

19. Choose "F", which refers to the column F of the Spreadsheet, from **2D Bar Overlay Options→Error Bar Param** dropdown list (Fig. 7, ❷).

20. Select red from the **Color** dropdown menu of the **Error Bar Properties** section (Fig. 7, ❸).

21. Click **OK** (Fig. 7, ❹).

The Bar Plot will now appear as in Fig. 7, *right.*

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22. *Optional:*

- Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Bar Plot and Scatter Plot update with the new data.
- Adjust the gates on the plots and watch the Bar Plot and Scatter Plot update in real-time.

In the next section we will add highlighting and exploding parameters to Bar, Scatter, and Pie Plots.

38.19.2. Adding Highlighting or Exploding 2

We will now add a Highlight or Explode parameter to a Bar Plot and Pie Chart. The Highlight/Explode parameter must be a formula in the Spreadsheet evaluating to "TRUE" or "FALSE"; if "TRUE," the bar will be highlighted in a selected color or the pie sector (slice) will explode away from the Pie for emphasis. The Layout used in this section contains a Spreadsheet and an associated Bar Plot and Pie Chart, to which we will now add a Highlight or Explode rule, respectively.

Note: Highlighting may be added to Scatter Plots in an identical manner to Bar Plots, but for simplicity, we have omitted the Scatter Plot from this section.

1. Select **File** tab→**Open**.
2. Open the layout titled "**Highlighting and Exploding.fey**" within the Tutorial Sample Data archive. The Bar Plot and Pie Chart are graphing the mean of the three subsets ("live", "apo", and "necro") for the three replicates in the Dot Plots.
3. Click on Cell D3 of the Spreadsheet (Fig. 1, ❶).
4. Click the **Formulas** tab, which has now been revealed in the formerly hidden Spreadsheet tab family (Fig. 1, ❷).
5. Click the **Logical** command (Fig. 1, ❸).
6. Select "IF" from the menu (Fig. 1, ❹). The Spreadsheet will now appear as below (Fig. 1, *bottom*).

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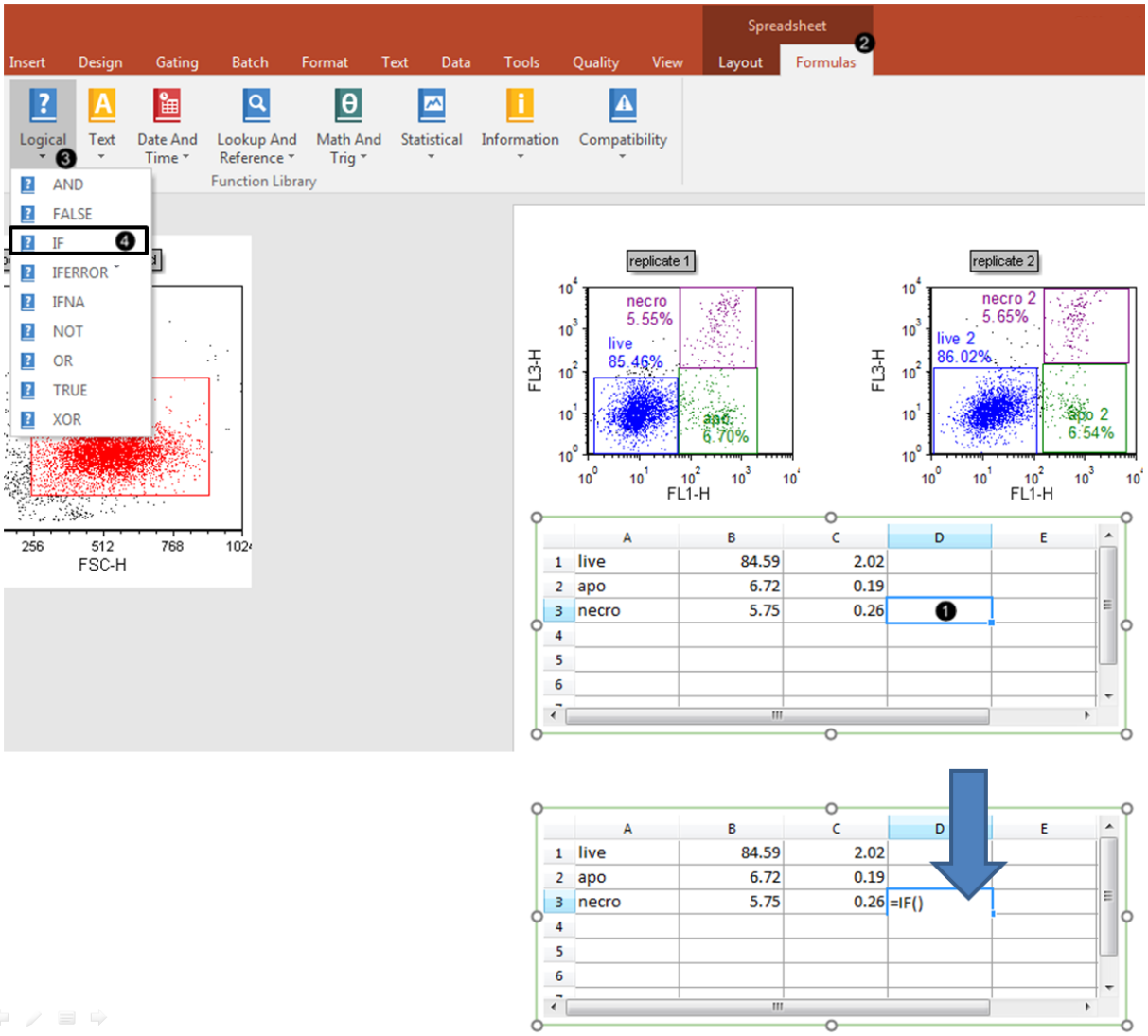

Figure 1. Entering a Highlighting rule containing the Logical operator "IF" into the Spreadsheet.

We will now enter Cell B3 as a component of the highlighting formula.

7. Click Cell B3 of the Spreadsheet (Fig. 2, ❶), *while the cursor is within the parentheses of the "IF" formula, as shown by the green arrow*.

8. Type ">5.5" (Fig. 2, ❷).

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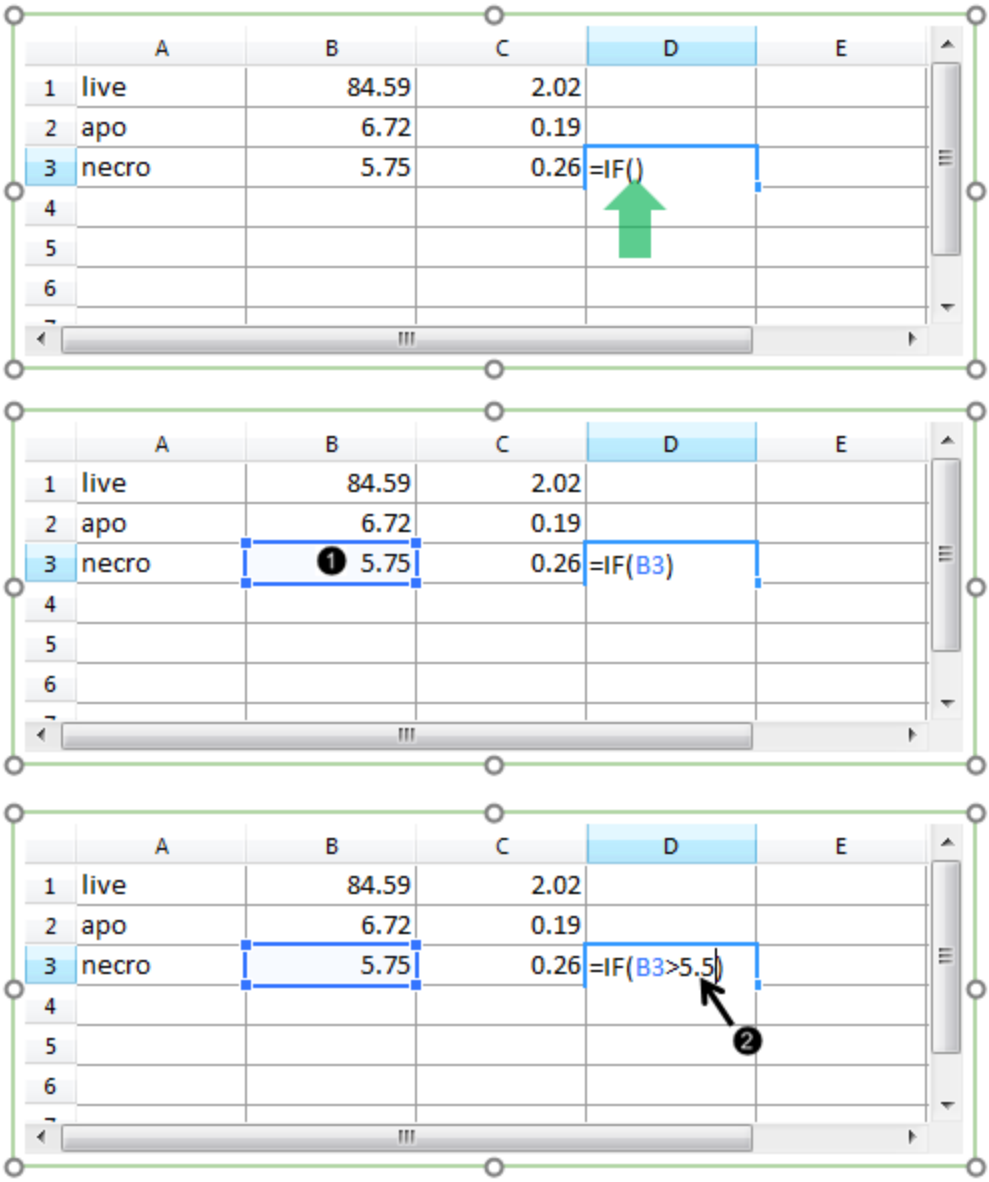

Figure 2. Entering a Spreadsheet cell as a component of the Highlighting rule.

9. Click on the Layout outside of the Spreadsheet. The result of the Logical formula will appear as "TRUE" (Figure 3).

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	A	B	C	D	E
1	live	84.59	2.02		
2	apo	6.72	0.19		
3	necro	5.75	0.26	TRUE	
4					
5					
6					
7					

Figure 3. Result of the Logical "IF" fourmula.

If desired, an analogous procedure to Steps 3-8 could be followed to create a Highlight or Explode rule for the "live" and "apo" rows of the Spreadsheet.

We will now format the Bar Plot or the Pie Chart to derive the Highlight or Explode rule from the appropriate column of the Spreadsheet.

10. Double-click the Bar Plot or the Pie Chart to open the **Formatting Bar** or **Pie** dialog (Fig. 4, *upper* or *lower pane*, respectively).

11. Click the **Overlays** category (Fig. 4, ❶).

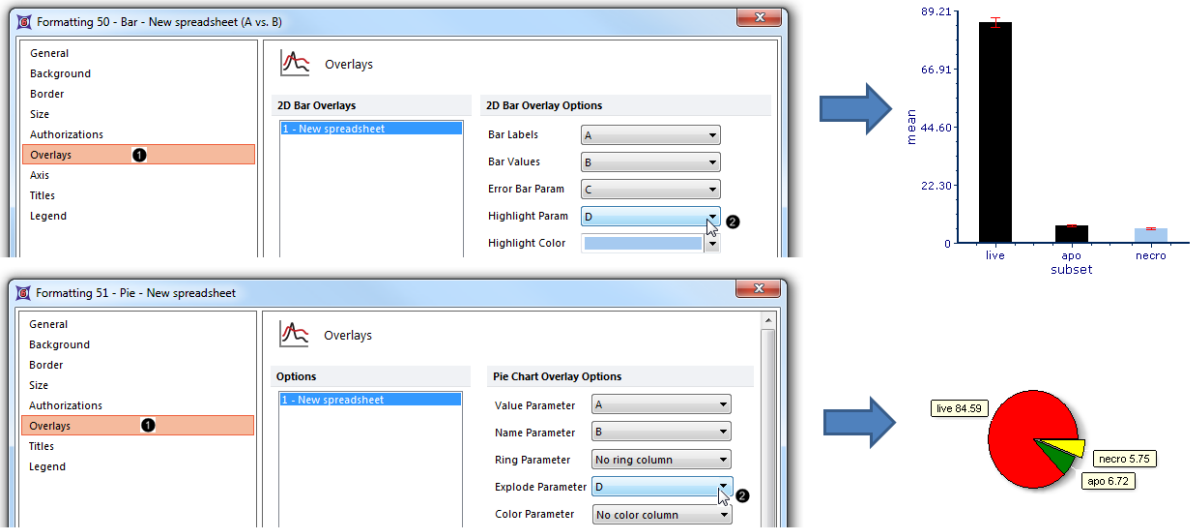

Figure 4. Designating the Custom Data Grid column from which the Highlighting or Explode rule will be derived

12. Choose "D" from the **Highlight Param** or **Explode Parameter** dropdown list (Fig. 4, ❷) of the **Formatting Bar** or **Formatting Pie** dialog, respectively.

13. Click **OK** in the **Formatting Bar** or **Pie** dialog (Fig. 5).

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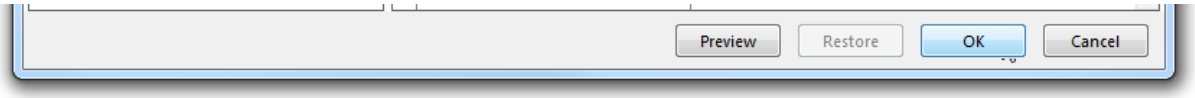

Figure 5. Clicking OK in the Formatting dialog.

The Bar Plot or Pie Chart will now appear as in Fig. 4, *right*. Note the following change:

- in the Bar Plot, the "necro" bar is highlighted. The **Highlight Color** and other visual formatting elements of the Bar Plot may be customized from the Overlays category. See this section of the manual 557 for details.
- the Pie Chart, the "necro" sector (slice) has exploded away from the rest of the Pie for emphasis. Sector (slice) **Color** and other visual formatting elements of the Pie Chart may be customized from the Overlays category. See this section of the manual 545 for details.

14. *Optional:*

- Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Bar Plot and Pie Chart update with the new data.
- Adjust the gates on the plots and watch the Bar Plot and Pie Chart update in real-time.

Go on to the next section to learn how to display additional datasets 1734 as Overlays on Bar and Scatter Plots, and as a Ring on a Pie Chart.

38.19.2. Displaying Additional Datasets 3

Additional datasets and parameters can be displayed on Bar and Scatter Plots as well as on Pie Charts. Bar and Scatter Plots can display multiple additional datasets as Overlays, while Pie Charts can display one additional dataset as a Ring.

In this section of the tutorial, we will enter an additional dataset into a Spreadsheet which can be subsequently displayed on a Bar Plot, Scatter Plot, or Pie Chart.

1. Select **File** tab→**Open**.
2. Open the layout titled "**Displaying Additional Datasets.fey**" within the Tutorial Sample Data archive.

   The layout a contains a Spreadsheet and its associated Bar Plot, Scatter Plot, and Pie Chart. The %-gated statistic for the "apo" gate is being plotted.

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We will now add a %-gated statistic for the "necro" gate to the Spreadsheet.

3. Drag and drop the "necro" gate from the *6 hr treatment* Color Dot Plot to cell C1 of the Spreadsheet. While dragging, a ghost of the gate outline will move (Fig. 1, ❶).

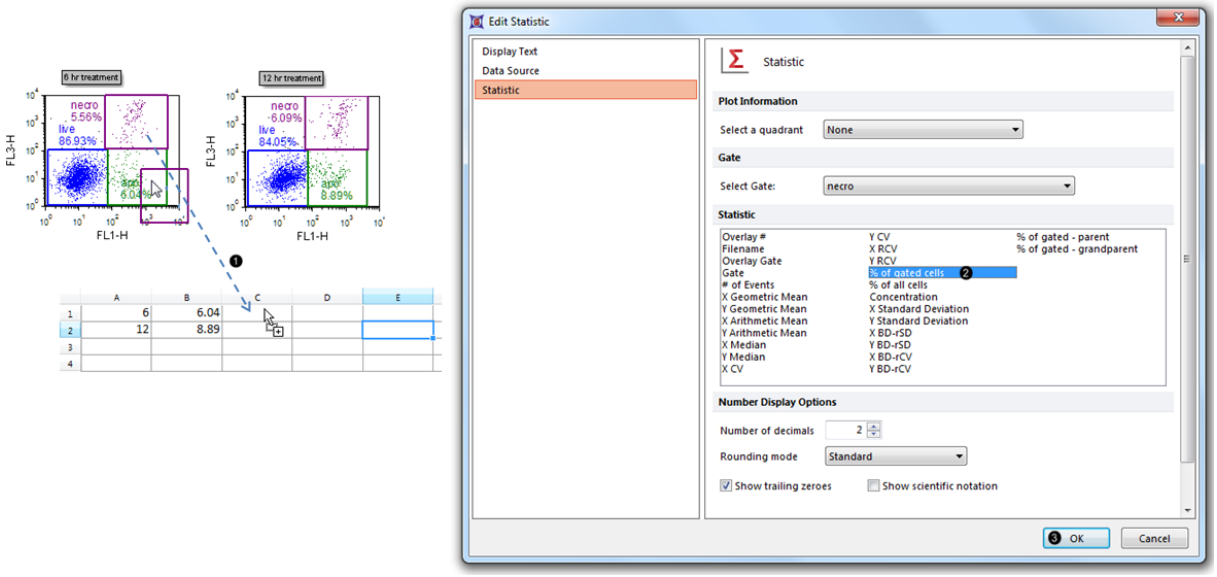

Figure 1. Entering the first member of an additional dataset into an empty column in the Spreadsheet.

4. Select **% of gated cells** from the **Statistic** list box in the **Edit Statistic** dialog (Fig. 1, ❷).

5. Click **OK** (Fig. 1, ❸). The C1 cell of the Spreadsheet will now contain the %-gated statistic for the "necro" gate, as shown in Fig. 2, *left*.

6. Repeat steps 3-5 for the "necro" gate of the *12 hr treatment* plot. The Spreadsheet will now appear with the %-gated statistic in Cell C2 (Fig. 2, *right*).

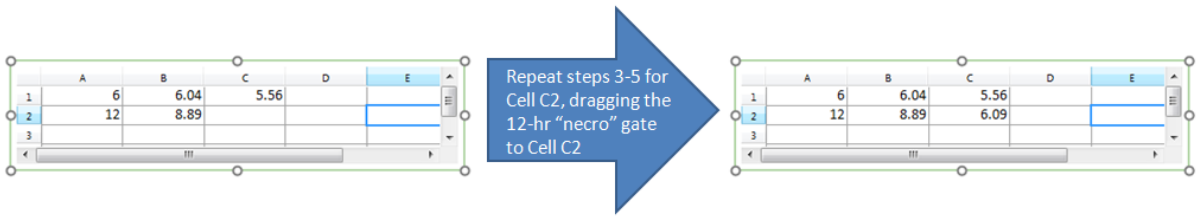

Figure 2. Populating the Spreadsheet with statistics from the 12-hr treatment plot.

The "necro" dataset is now complete within Column C of the Spreadsheet (Fig. 2, *right*). We will now format the Bar Plot, Scatter Plot 1736, and/or Pie Chart 1738 to display this additional dataset from the appropriate column of the Spreadsheet.

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38.19.2.3.1 Overlays on Bar and Scatter Plots

In this section of the tutorial, we will display an additional dataset as an Overlay on a Bar Plot and/or Scatter Plot. Please complete the preceding section of the tutorial (Displaying Additional Datasets 1734) before proceeding.

1. Click on the border of the Spreadsheet so it is highlighted in red.
2. Drag and drop the Spreadsheet onto the Bar Plot or Scatter Plot (Fig. 1, ❶).

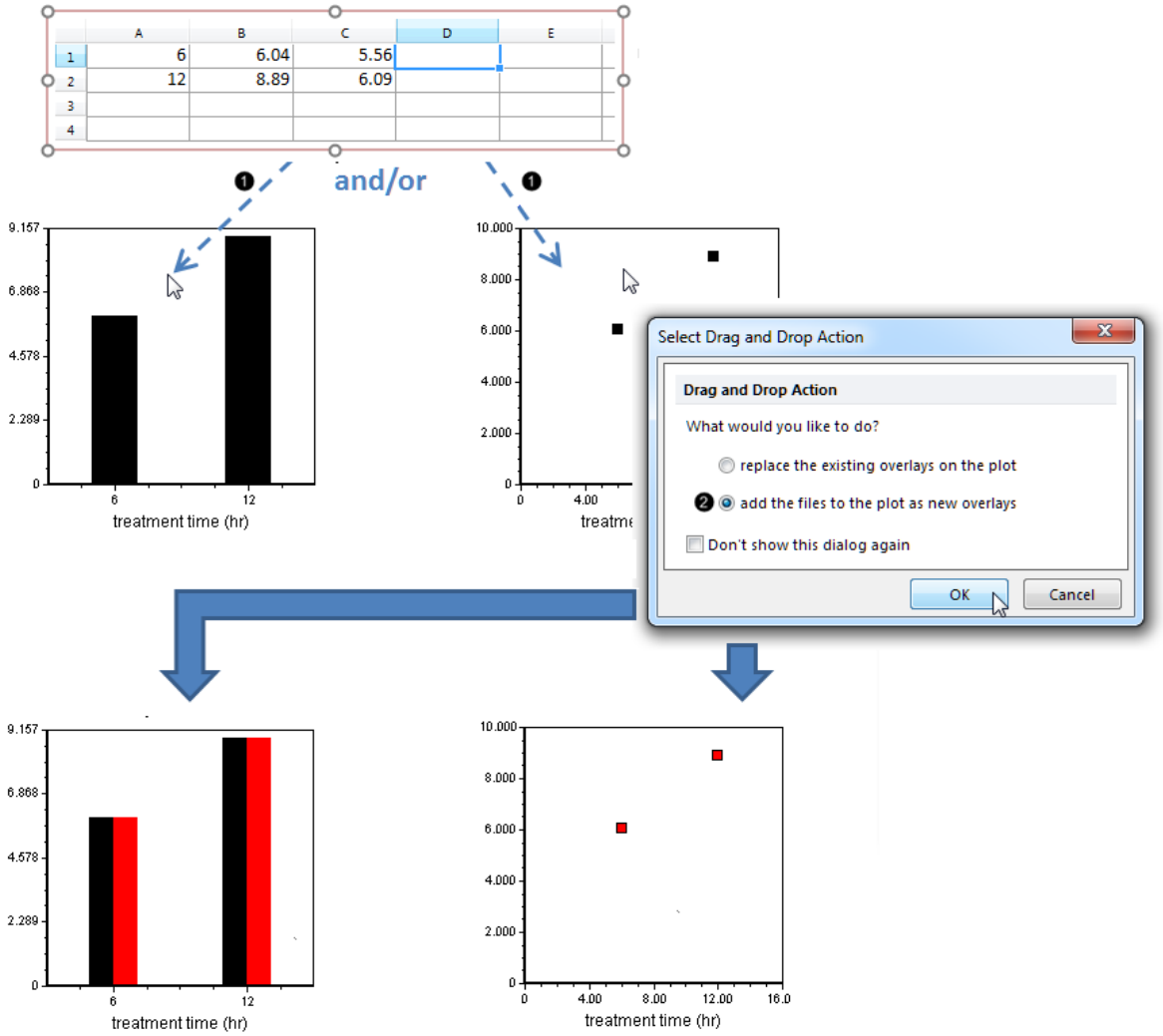

Figure 1. Dragging and dropping a Spreadsheet onto a Bar or Scatter Plot to create an overlay

3. Click the **add the files to the new plot as new overlays** radio button (Fig. 1, ❷).
4. Click **OK** (Fig. 1, mouse pointer).

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The Bar Plot or Scatter Plot will now appear as in Fig. 1, *bottom*.

The new Overlay has defaulted to the second ("apo") column of the Spreadsheet for its values. We will now reformat the plot to derive the values from the third ("C") column, in order to plot the "necro" gate-derived values.

5. Double-click the Bar or Scatter Plot to open the **Formatting Bar** or **Formatting Scatter** dialog (Fig. 2, *upper* or *lower pane*, respectively).

6. Click the **Overlays** category (Fig. 2, ❶).

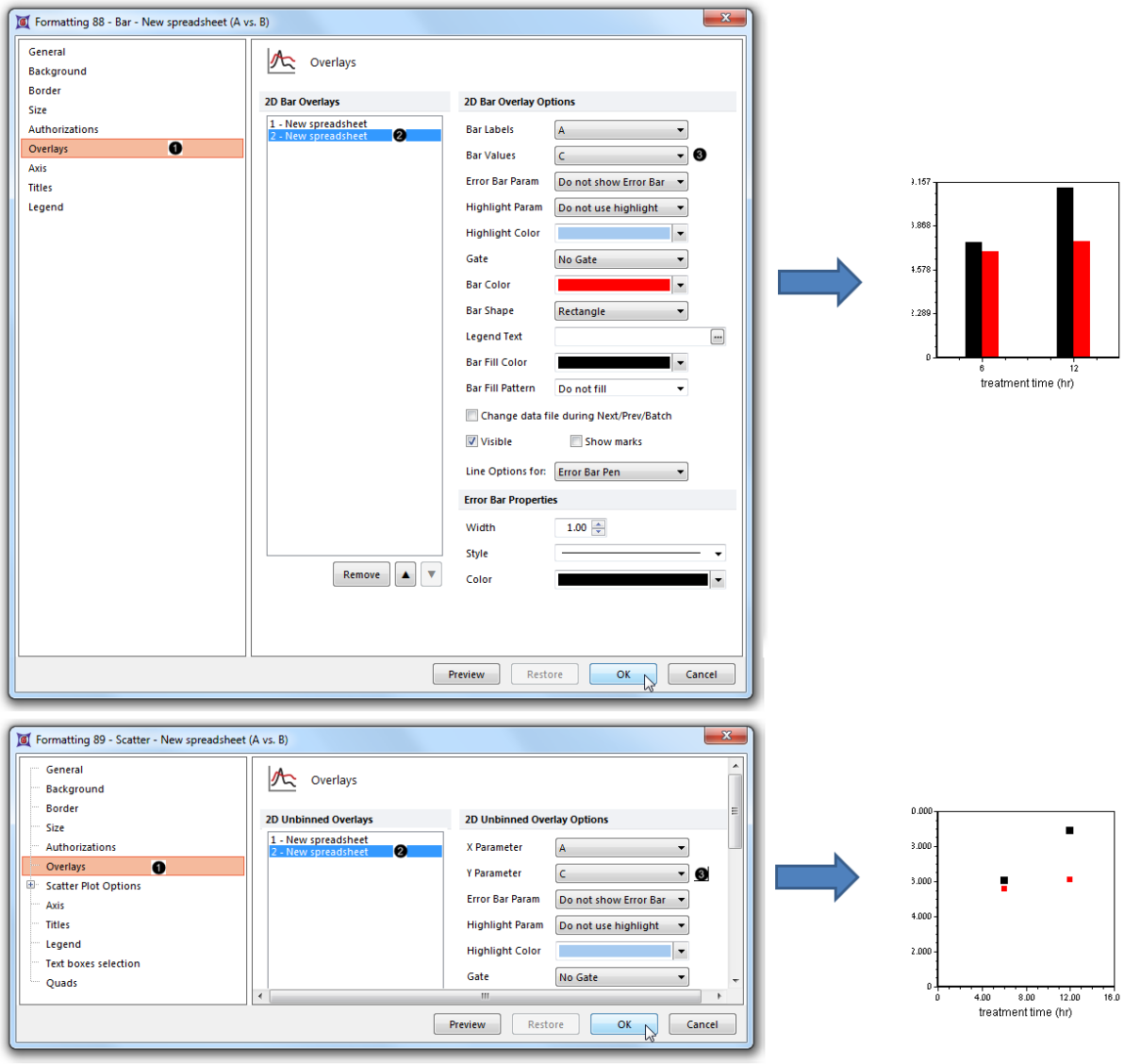

Figure 2. Designating the Spreadsheet column from which the new overlay will be derived

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7. Click *2- New spreadsheet* from the **2D Bar Overlays** or **2D Unbinned Overlays** list (Fig. 2, ❷)

8. Choose "C" from the **Bar Values** or **Y Parameter** dropdown list of the **Formatting Bar** or **Formatting Scatter** dialog, respectively (Fig. 2, ❸).

9. Click **OK** (Fig. 2, mouse pointer). The Bar Plot or Scatter Plot now appears as in Fig. 2, *right*.

   Note: Color, style, size, and other attributes of bars on a Bar Plot and points on a Scatter Plot can be customized independently for each overlay. See the following sections of the manual for details:
   - Formatting Bar Plots 556
   - Formatting Scatter Plots 563

10. *Optional:*

- Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Bar Plot, Scatter Plot, and Pie Chart update with the new data.
- Adjust the gates on the plots and watch the Bar Plot, Scatter Plot, and Pie Chart update in real-time.

38.19.2.3.2 Ring on Pie Chart

In this section of the tutorial, we will display an additional dataset as a Ring on a Pie Chart. Please complete the Displaying Additional Datasets 1734 section of the tutorial before proceeding.

1. Double-click the Pie Chart to open the **Formatting** dialog.

2. Click the **Overlays** category (Fig. 1, ❶).

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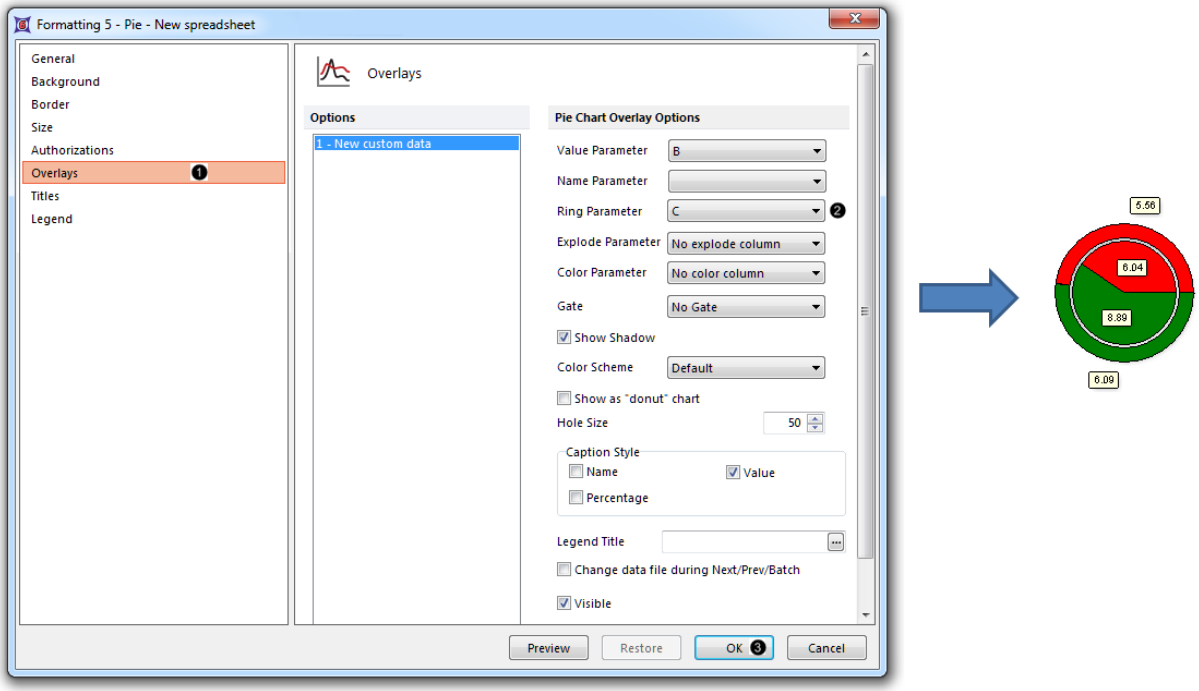

Figure 1. Displaying an additional dataset as a Ring on a Pie Chart

3. Choose "C" from the **Ring Parameter** dropdown list of the **Formatting Pie** dialog (Fig. 1, ❷).

4. Click **OK** (Fig. 1, ❸). The Pie Chart now appears with the "necro" dataset as a ring at the periphery of the pie (Fig. 1, *right*).

5. *Optional:*

   - Click the **Data** tab→**Change Data on All**→ **Next/Previous** buttons to advance through the set of files in the Data List and watch as the Bar Plot, Scatter Plot, and Pie Chart update with the new data.
   - Adjust the gates on the plots and watch the Bar Plot, Scatter Plot, and Pie Chart update in real-time.

38.20 High Content Flow

FCS Express continues a legacy of innovation in the field by providing analysis capabilities for 96 well plate format flow cytometry data. In the course of this tutorial you will learn how to:

- Load data as an FCS plate folder file
- Insert heat maps
- Format heat maps

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- Gate on heat maps
- View plots from multiple and individual wells
- Create overlays from well data
- Batch process for multiple plates
- Use the Z-Prime function to calculate the Z-Factor for screening

In the first exercise, we will load, insert, and format heat map data 1740.

38.20.1 Load, Insert, and Format Heat Maps

In this section, we will learn how to:

- Load data in the FCS plate folder format
- Insert a Heat Map
- Change the orientation of the Heat Map
- Choose the statistic/parameter to display in the wells
- Change the color levels of the Heat Map
- Adjust the style of display
- Show only events gated on a plot in the Heat Map

We will be using the **HighContentFormatting.fey** layout for this section which is located in the Tutorial Sample Data archive 1210. This layout contains one 2D Plot that will be used towards the end of this section.

In this section you will also learn how to load a folder that contains data from a 96 well plate-based experiment. FCS Express can accommodate any number wells in any order on the plate. The data that we will be using is located in the Tutorial Sample Data archive 1210. For convenience, only one row plus one well of data has been included.

In order to load most types of 96 well plate flow data, you must choose the folder that contains all the data files from the plate, as opposed to one of the data files themselves.

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1. Load the **HighContentFormatting.fey** layout from the Tutorial Sample Data archive 1210.
2. Select the **Data→Organize Data Sets→Data List** command (Figure T9.2 1445).
3. Click the symbol to add data files to the data list.
4. Choose **FCS Plate folder** from the **Files of type:** dropdown list (Figure T18.1, outlined in red).

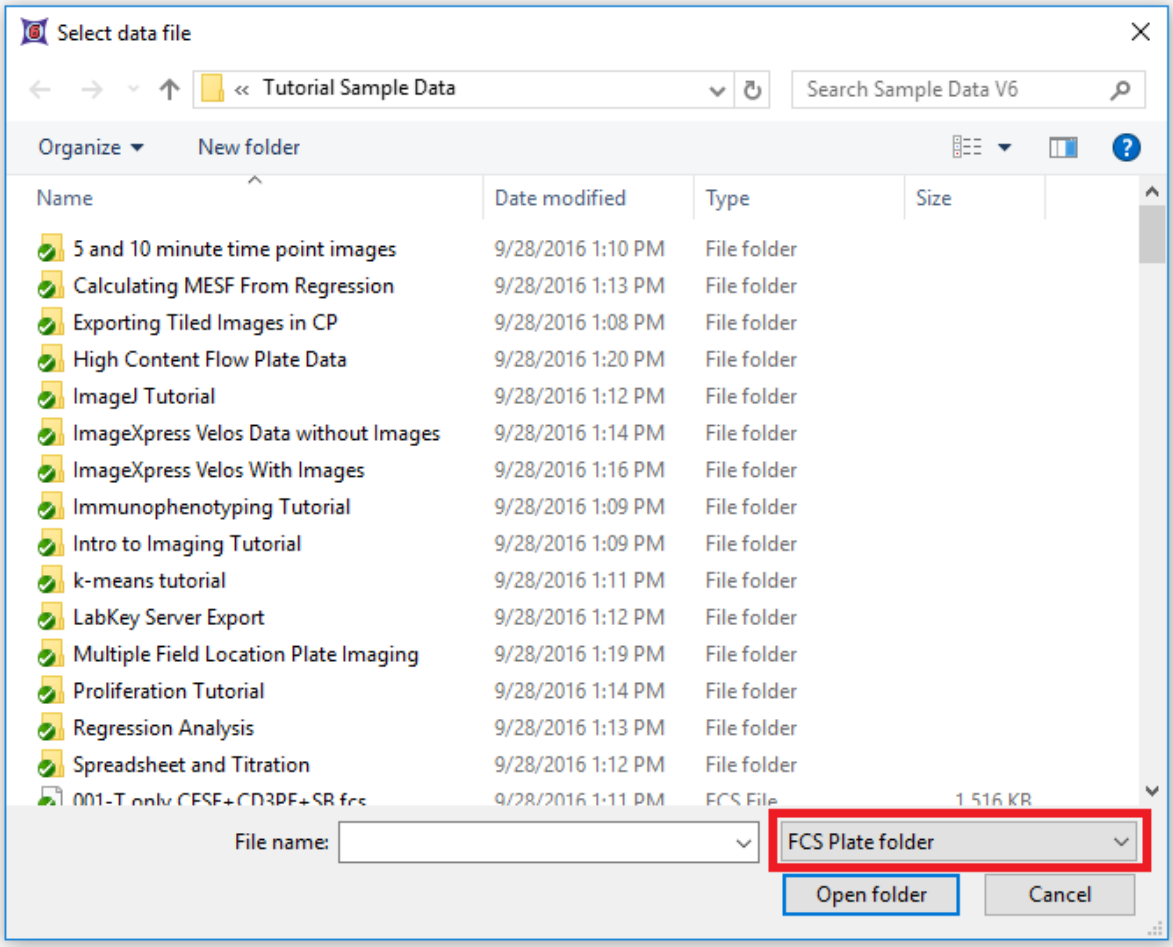

Figure T18.1 Choosing FCS Plate Folder from the Files of Type: Drop-down List

5. Navigate to the **High Content Flow Plate Data** folder in the Tutorial Sample Data archive 1210 (Figure T18.2).

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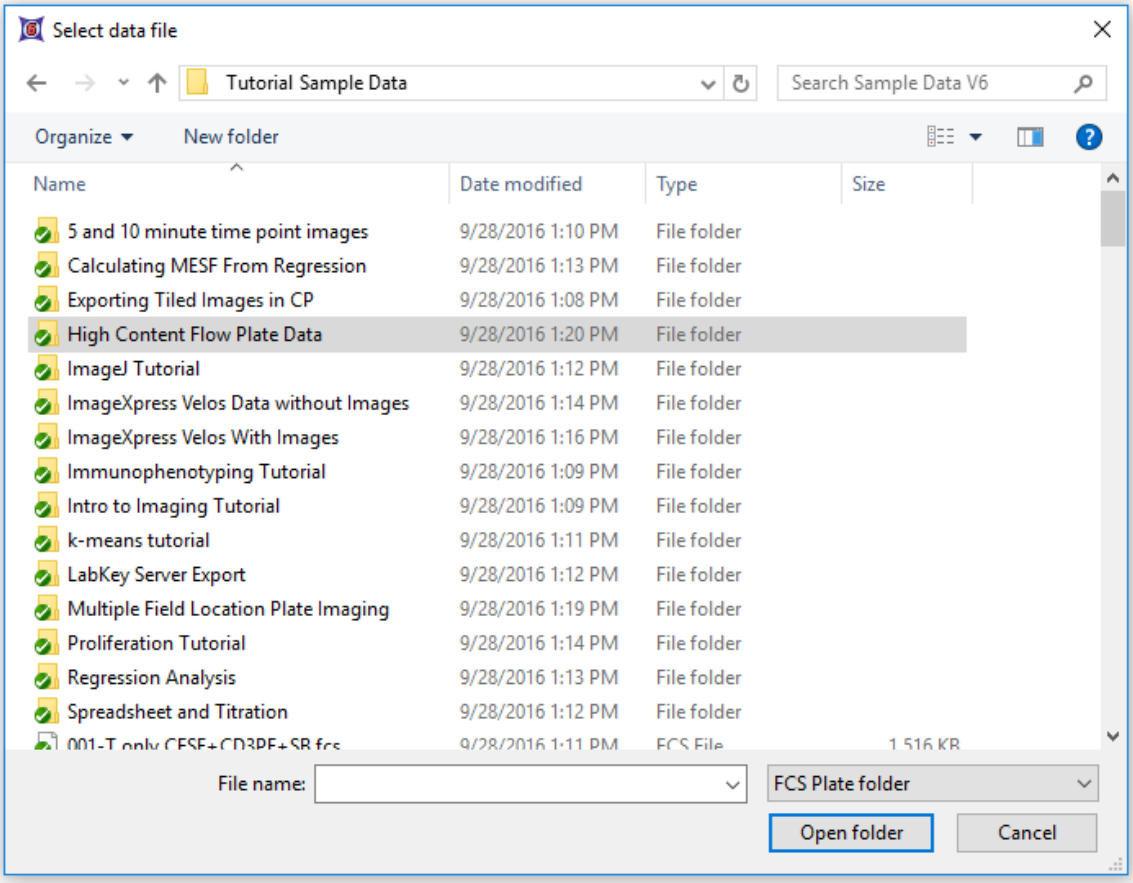

Figure T18.2 - Choosing the High Content Flow Plate Data Folder

6. Select the folder and click **Open folder**.
7. Select any .fcs data file within the folder and click **Open Folder**.

The folder will now appear in the Data List as a single file. This single file contains the .fcs files and data from all wells in the plate.

Note: In order for FCS Express to know what size plate is being used for analysis (i.e., 24, 48, 96, 384 wells) the folder must contain a file which defines the plate. For this example, the file has been included in the **High Content Flow Plate Data** folder and is named "**plate.AllExtraKeywords.txt**" (Figure T18.2b). This uses a special feature of FCS Express that allows you to add extra keywords to a data file without modifying the file. Please see chapter 17.2 of the FCS Express manual, *Extra Keywords*, for more details.

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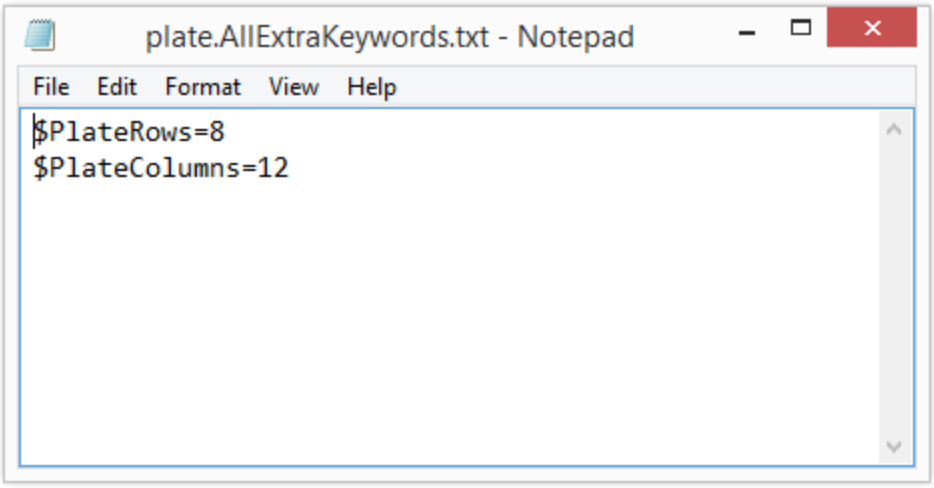

Figure T18.2b - Content of "plate.AllExtraKeywords.txt" file

8. Drag and drop the "High Content Flow Plate.Data" from the Data List to a blank spot on the page within the layout (Figure T18.3, ❶).

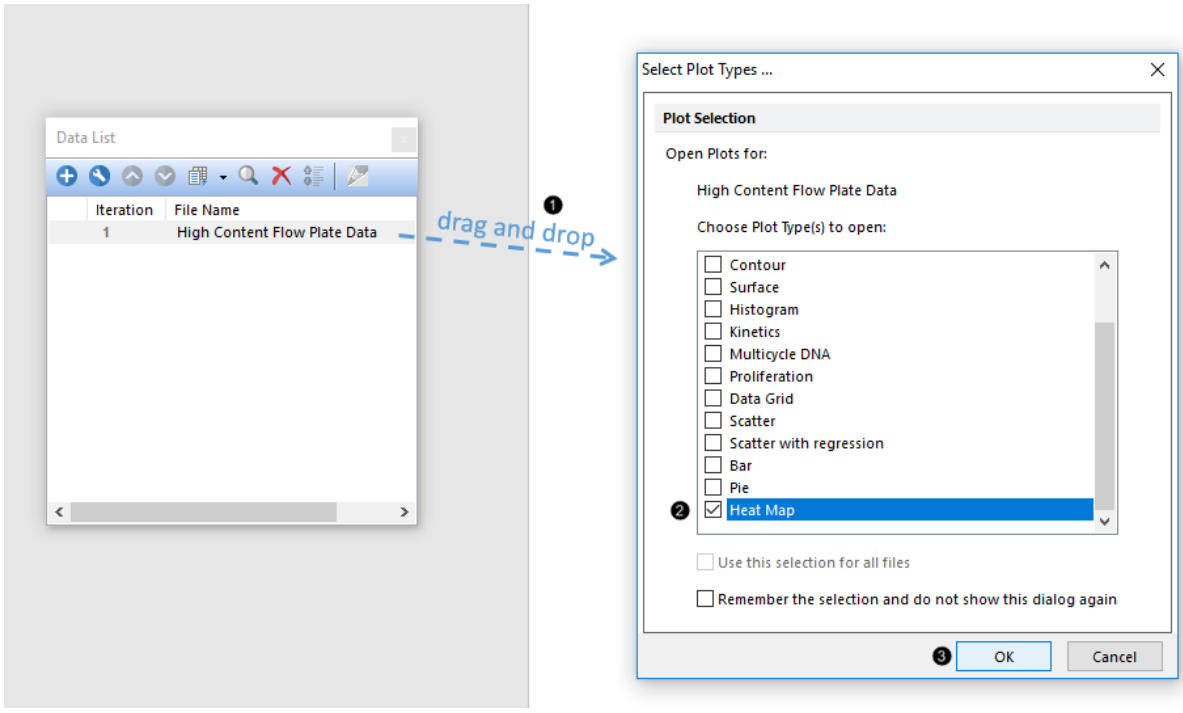

Figure T18.3 Dragging and Dropping a Data File from the Data List Tab and Choosing Heat Map as Plot Type

9. Check the **Heat Map** box in the **Select Plot Types...** dialog (Figure T18.3, ❷).

10. Click **OK** (Figure T18.3, ❸).

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A heat map will appear in your layout like the one below in Figure T18.4. The heat map is only displaying wells A1-12 and B1 because we have only included these wells in the sample data. The parameter we are concerned with in this data set is FL2-A. We will now change the axis to display this parameter.

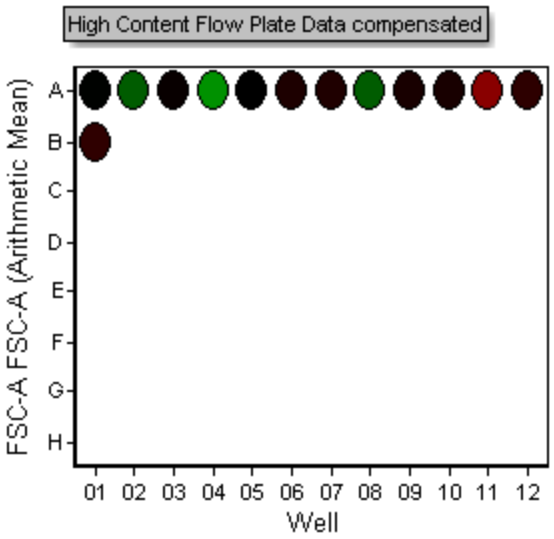

Figure T18.4 Inserted Heat Map

11. Click on the axis parameter along the row labels (Figure T18.5).

12.Choose **FL2-A** from the pop-up menu.

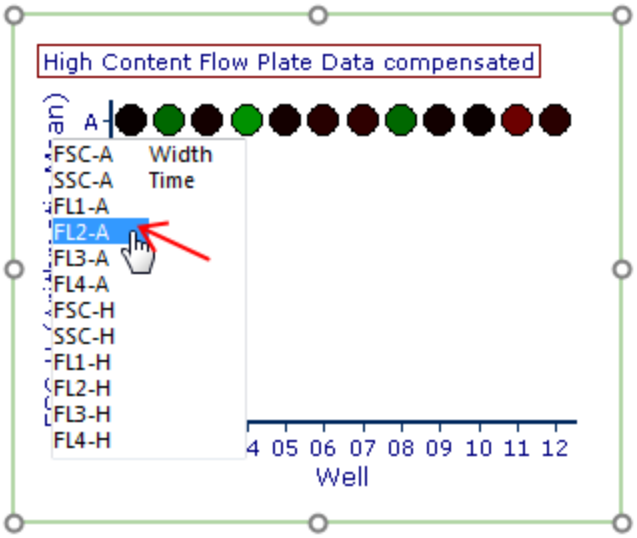

Figure T18.5 Changing the Heat Map Parameter

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Note: The colors in the plot above now reflect the Arithmetic mean of the FL2-A channel of each well of heat map. We will show how to change the statistic that is plotted later in the tutorial.

We will now duplicate the Heat Map and change the orientation of the newly created Heat Map.

13. Select the Heat Map.

14. Choose **Home→Clipboard→Duplicate** (Figure T18.5b). Alternatively, use the **Ctrl+D**D command.

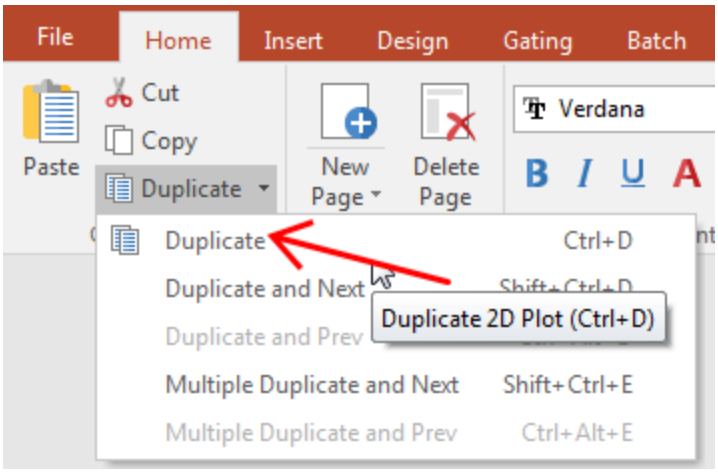

Figure T18.5b - Duplicate command

A copy of the original heat map will appear on the layout. Arrange the plots so they look like Figure T18.6.

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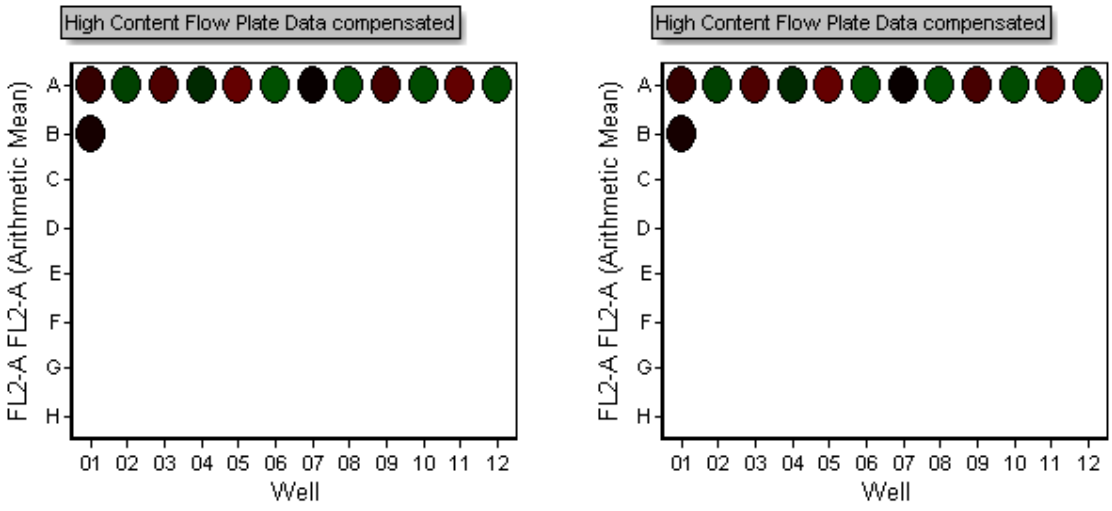

Figure T18.6 Duplicate Heat Maps

15. Right-click on the newly created Heat Map on the right side of the layout.
16. Choose **Format** from the pop-up menu.
17. Choose the **Heat Map→Heat Map Specific Options** category.
18. Change the Orientation from **Landscape** to **Portrait** (Figure T18.7).
19. Click **OK**.

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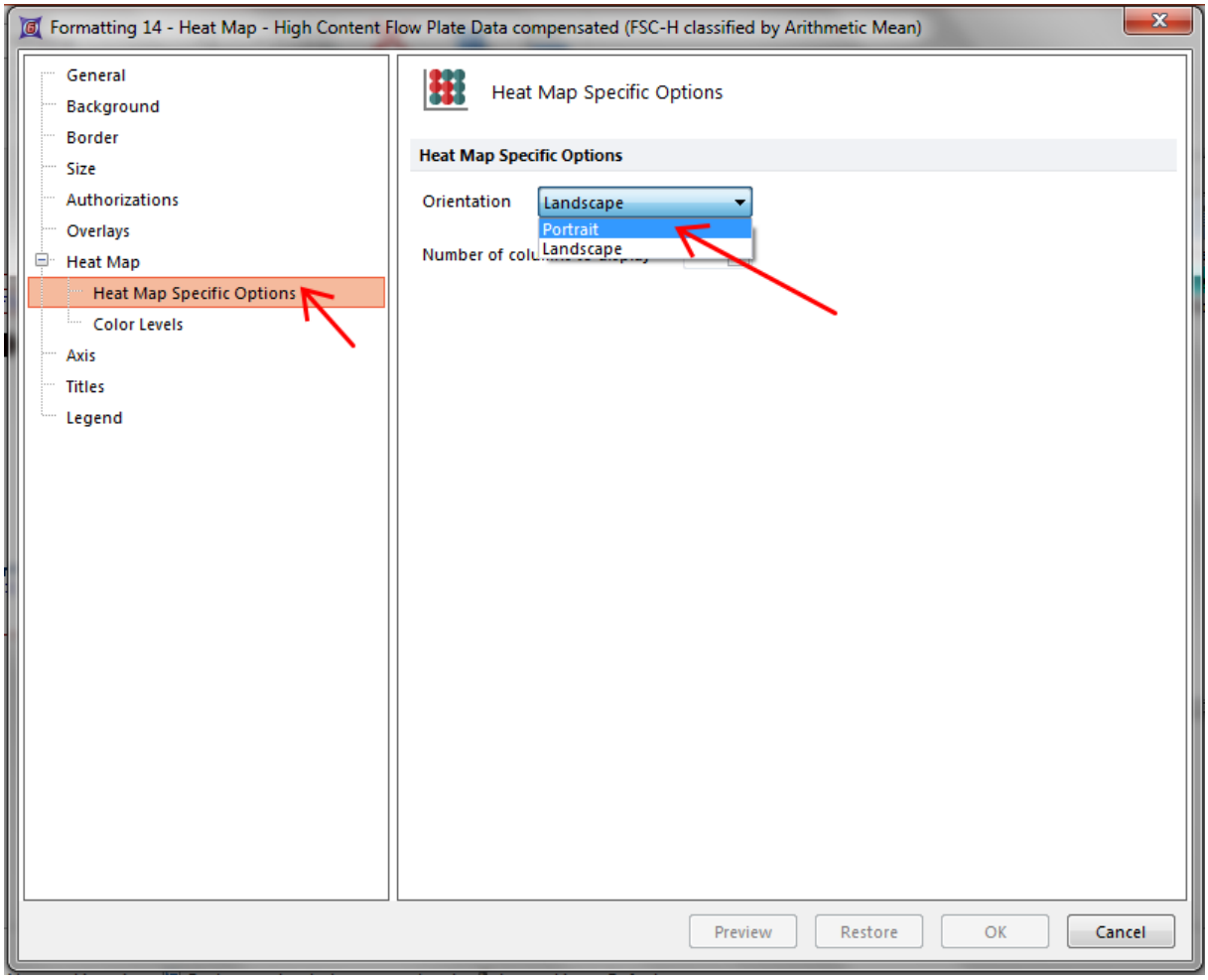

Figure T18.7 Changing Orientation from Landscape to Portrait

The layout will now look like Figure T18.8. Changing the orientation of the Heat Map may be useful depending on the layout of your experiment or your display/publication needs.

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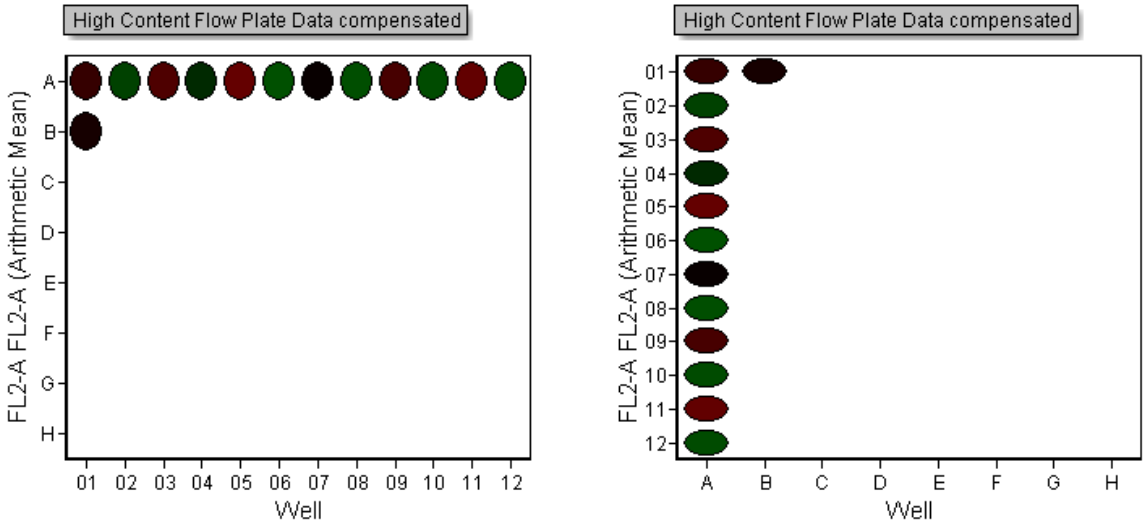

Figure T18.8 Heat Maps Displayed in Landscape and Portrait Modes

We will now change the statistic that is being represented by colors in the heat map.

20. Hit **Ctrl+Z** to undo the orientation change.

21. Right-click on the left plot.

22. Choose **Format** from the pop-up menu.

23. Choose the **Overlays** category.

The heat map is currently displaying the "Arithmetic Mean" of all the events in the well as indicated in the "Statistic to show" field.

24. Change the **Statistic to show** to **Median** from the drop-down list and click **OK** (Figure T18.9).

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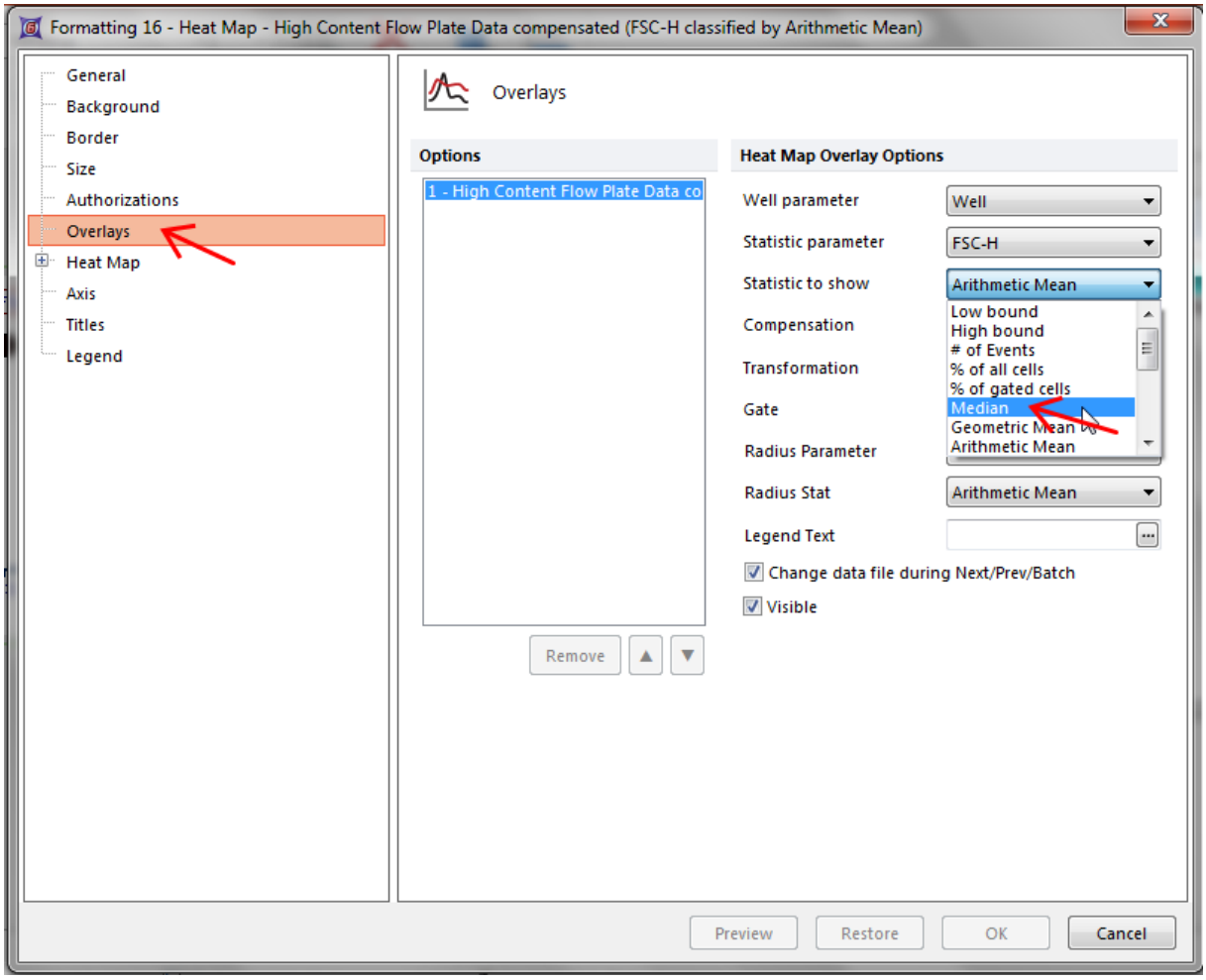

Figure T18.9 Changing the Statistic to Show in the Heat Map

The layout will now look like Figure T18.10. Notice how the plot on the left side now shows a very positive "hot" well in position A4. In this example, Median provides better discrimination between hot (red) and cold (green) wells than does Arithmetic Mean (plot on right side).

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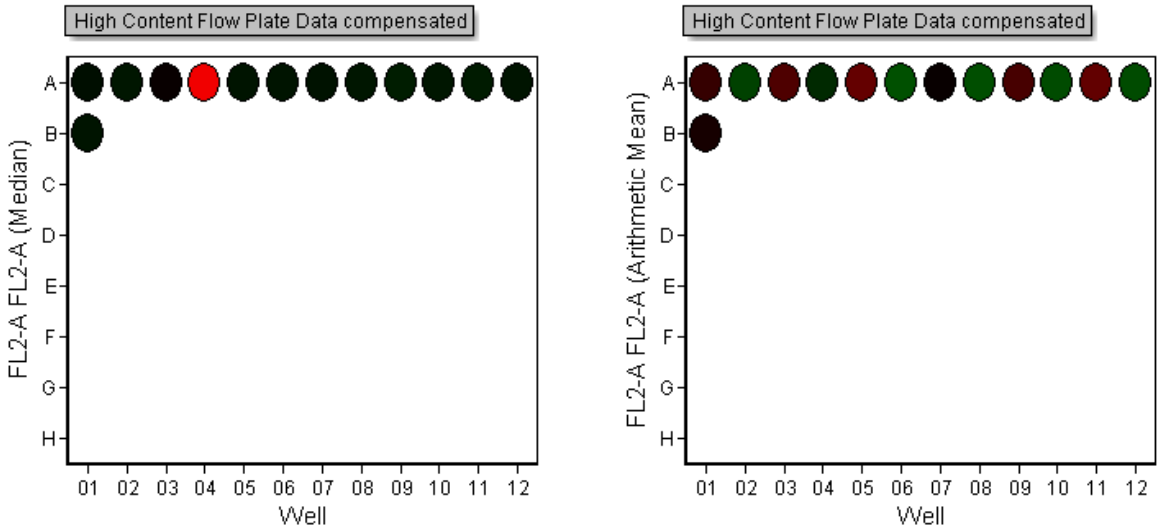

Figure T18.10 Heat Maps Displaying Statistics for Median (Left) and Arithmetic Mean (Right).

We will now adjust the color levels of the Heat Map which is useful for display purposes and for helping to further discriminate positive from negative samples.

25. Right-click on the left plot.
26. Choose **Format** from the pop-up menu.
27. Choose the **Heat Map→Color Levels** category.
28. Change the **Selected Color Level** from the drop-down list to **Rainbow** and click **Preview** (Figure T18.11).

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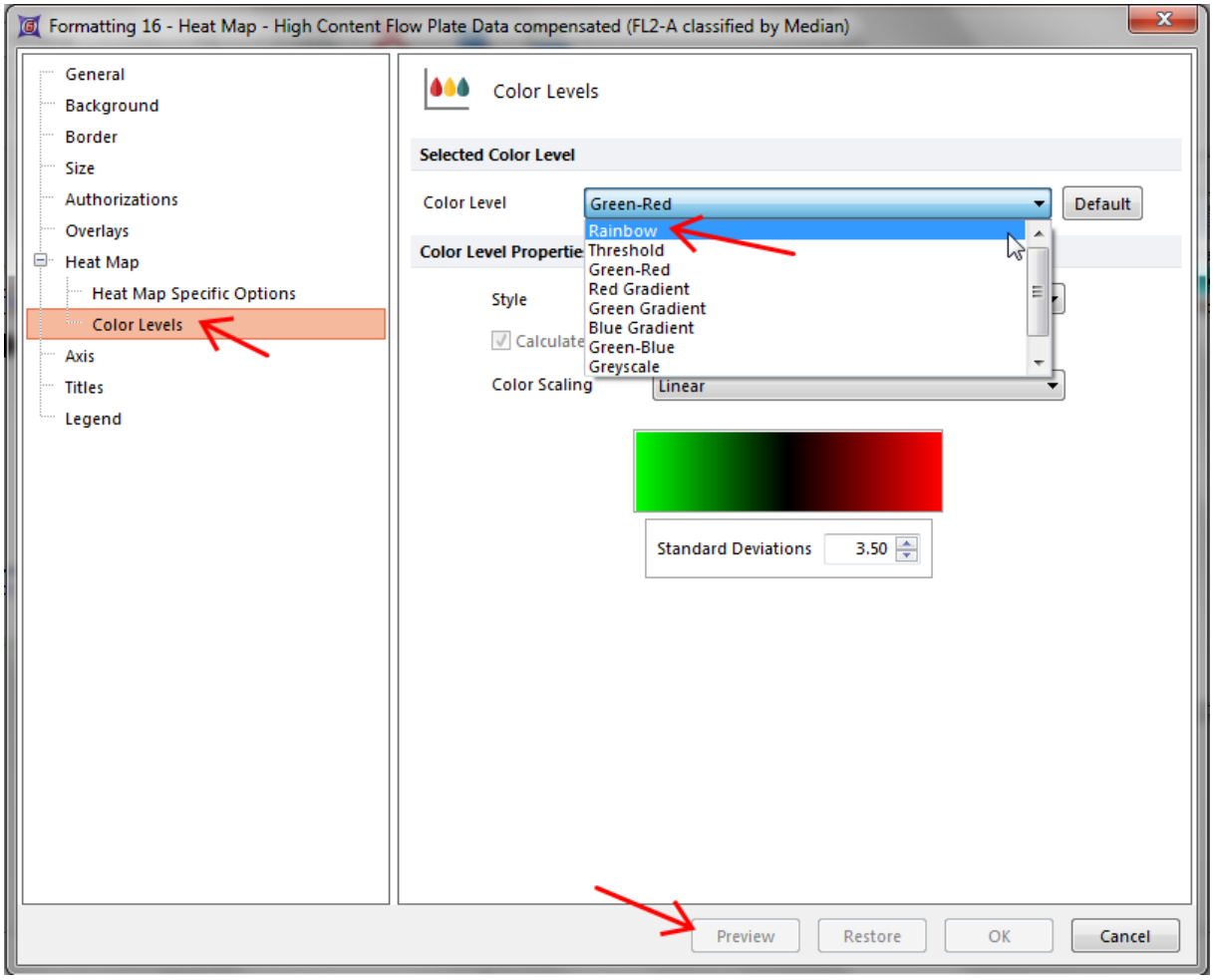

Figure T18.11 Changing the Color Level of a Heat Map

Move the formatting dialog to the side so you can see the changes to the heat map. Try changing and previewing the color levels for Green-Red, Green-Blue, Greyscale, and Inverse Greyscale, to see the differences. The Threshold color level assigns a color to any wells above the defined threshold level. We will now enter threshold to view this feature as in Figure T18.12.

29. Change the **Selected Color Level** from the drop-down list to **Threshold**.
30. Enter "1000" in the **Threshold Value** field.
31. Change the **Below Threshold Color** to Yellow.
32. Change the **Above Threshold Color** to Orange.

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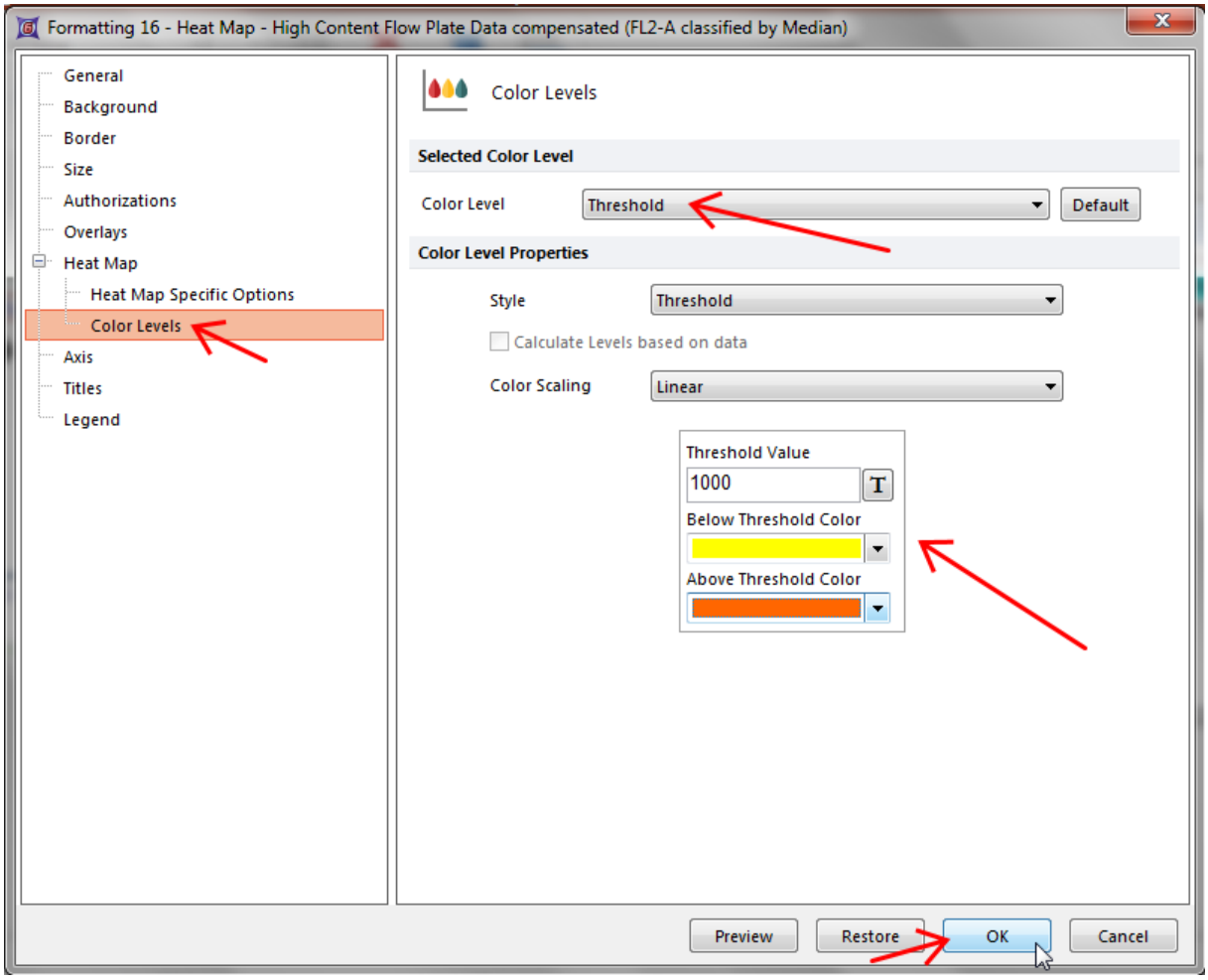

Figure T18.12 Defining a Threshold to Display as a Color Level in a Heat Map

33. Click **OK**.

The heat map will now look like Figure T18.13. Wells in which the median FL2-A value is above 1000 are displayed as orange and wells which are less than 1000 are displayed in yellow.

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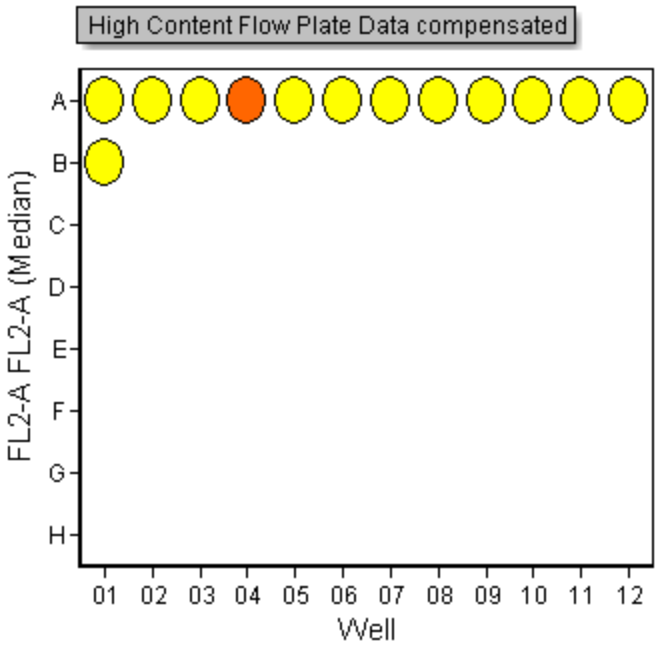

Figure T18.13 Heat Map with Threshold Color Level

Note: See Choosing a Color Scheme 1057 for additional information about coloring heat maps.

We will now change the Color Level Style. The Style refers to the method of assigning colors to different values in the heat map. The default style is "mean +/-SD". This assigns wells whose values are the mean of all the values on the plate to be the color black. Wells whose values deviate from the mean are colored one of two colors, depending on whether they are above or below the mean. The degree to which a well is shaded towards a particular color depends on the user defined standard deviation value, which defaults to 3.5 SD. For the Red-Green Color level, wells that are 3.5 SD lower than the mean are green, and wells that are 3.5 SD above the mean are red. There are three other choices in this dialog; fixed range, percentile, and threshold.

34. Select both heat maps on the layout.

35. Choose the **Format→Plot Options→Overlays** command.

36. Change the **Statistic to show** to **Median** and click **OK** (Figure T18.13b).

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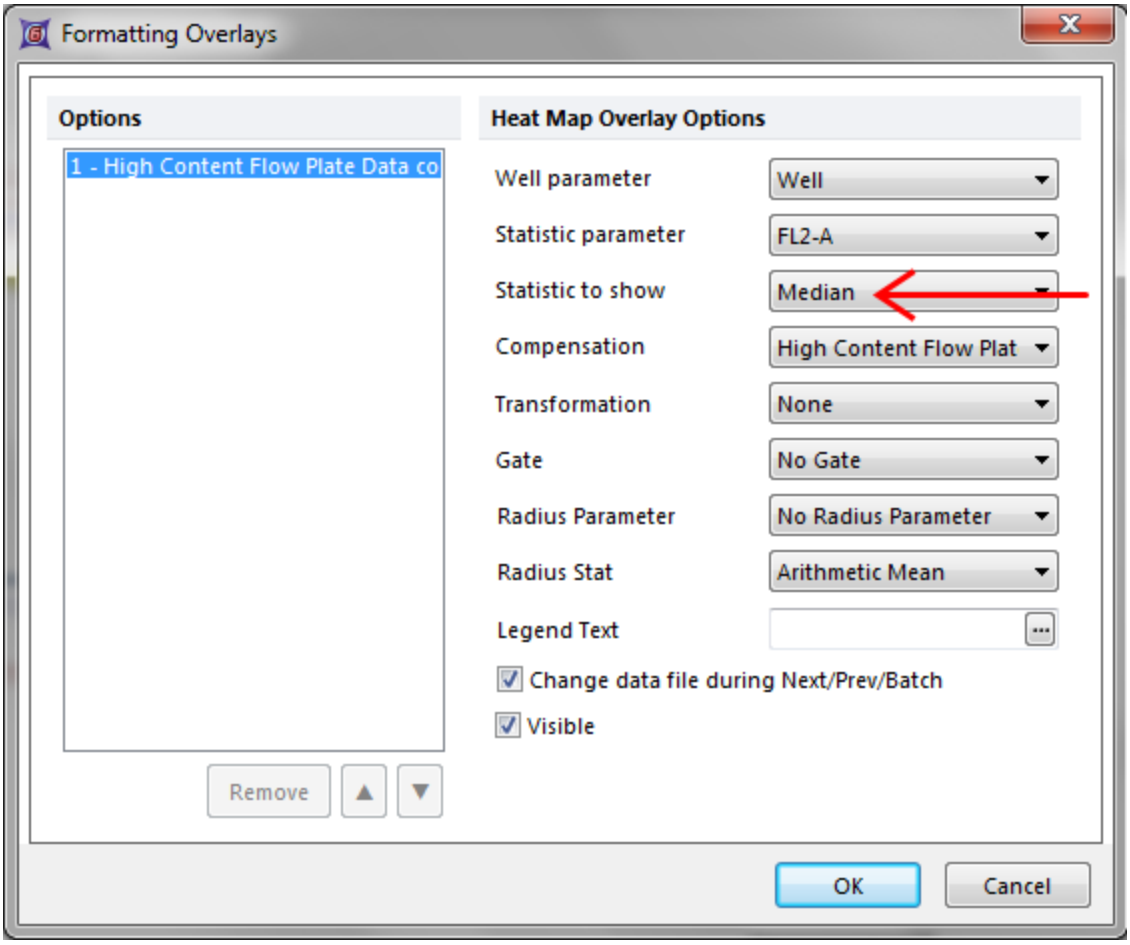

Figure T18.13b - Formatting Overlays window

37. Right-click on the left plot.
38. Choose **Format** from the pop-up menu.
39. Choose the **Heat Map→Color Levels** category (Figure T18.11).
40. Change the **Selected Color Level** to **Green-Red**.
41. Change the **Style** to **Percentile** from the drop-down list and click **OK** (Figure T18.14).

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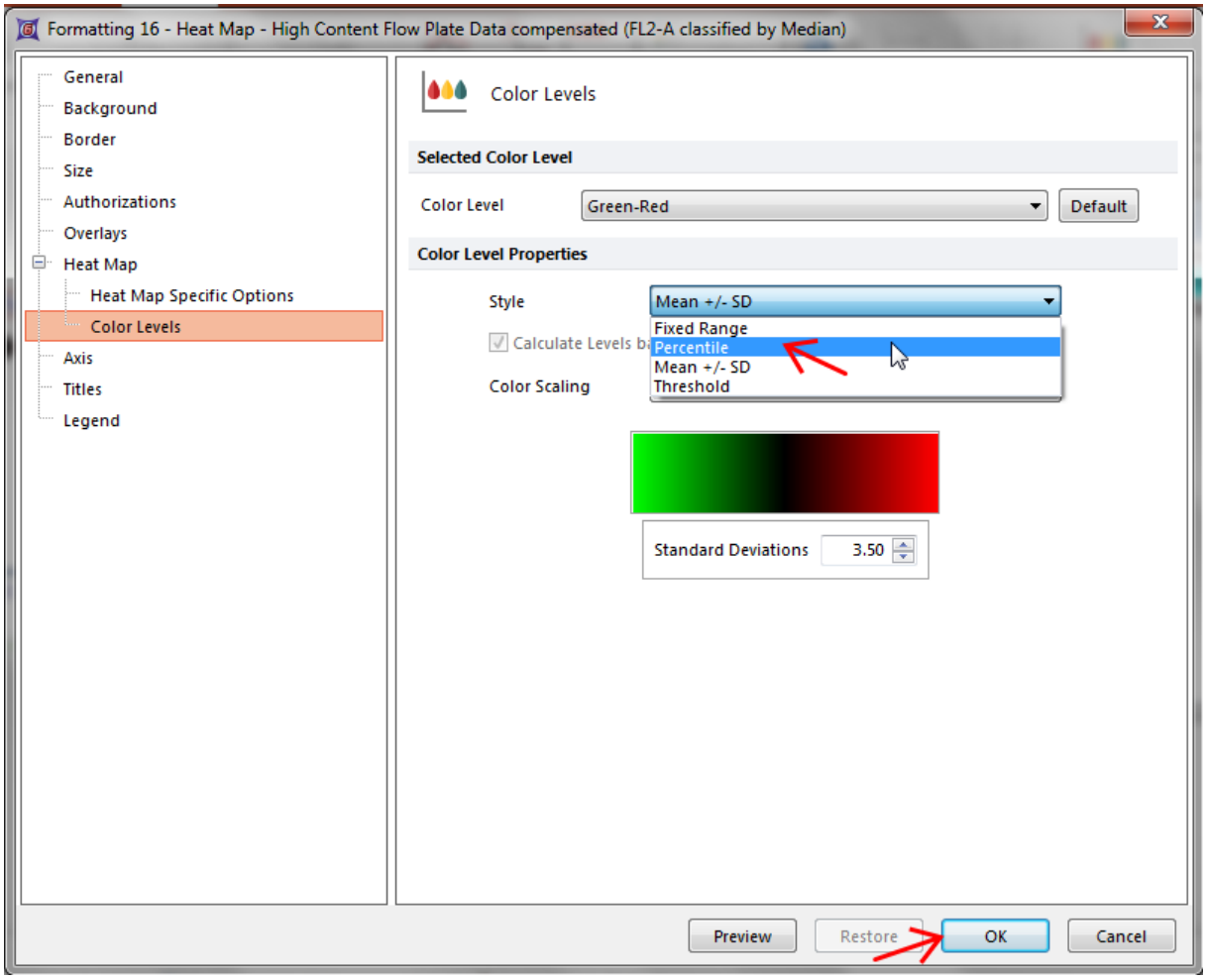

Figure T18.14 Changing the Style of the Heat Map Color Levels

42. Repeat steps 37-40 on the right plot.

43. Change the **Style** to **Fixed Range** (on the right plot).

44. Change the **Color Scaling** to **Logarithmic** from the drop-down list (on the right plot).

45. Click **OK**.

The layout and heat maps will now look like Figure T18.15. Notice how the color levels have changed as a result of changing the color level **Style** from **Mean +/- SD** to **Percentile**, and **Fixed Range**.

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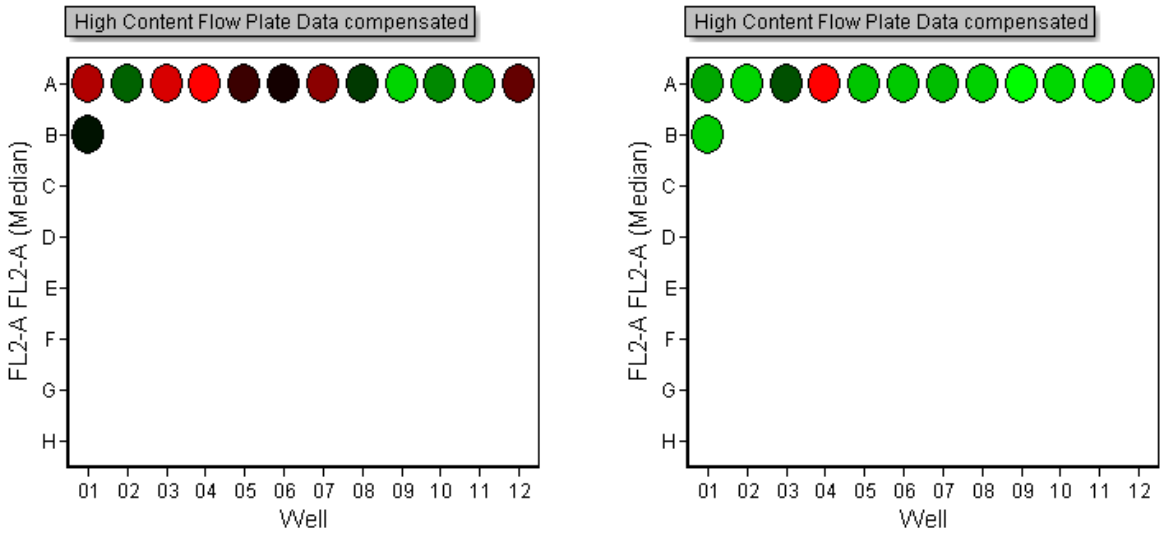

Figure T18.15 Adjusting Color Styles

We will now load the FCS Plate folder data into the 2D plot on the layout, gate the plot, and apply the gates to the heat map to display only the gated events.

46. Select the plot labeled "**2D Plot for Gating**".
47. Choose the **Data→Change Data on Selection→Select** command.
48. Choose the **Data List** tab.
49. Select **High Content Flow Plate Data**.
50. Click **Open**.

Note: Alternatively you can drag and drop the "High Content Flow Plate Data" from the Data List directly onto the "2D Plot for Gating" (Figure T18.15b).

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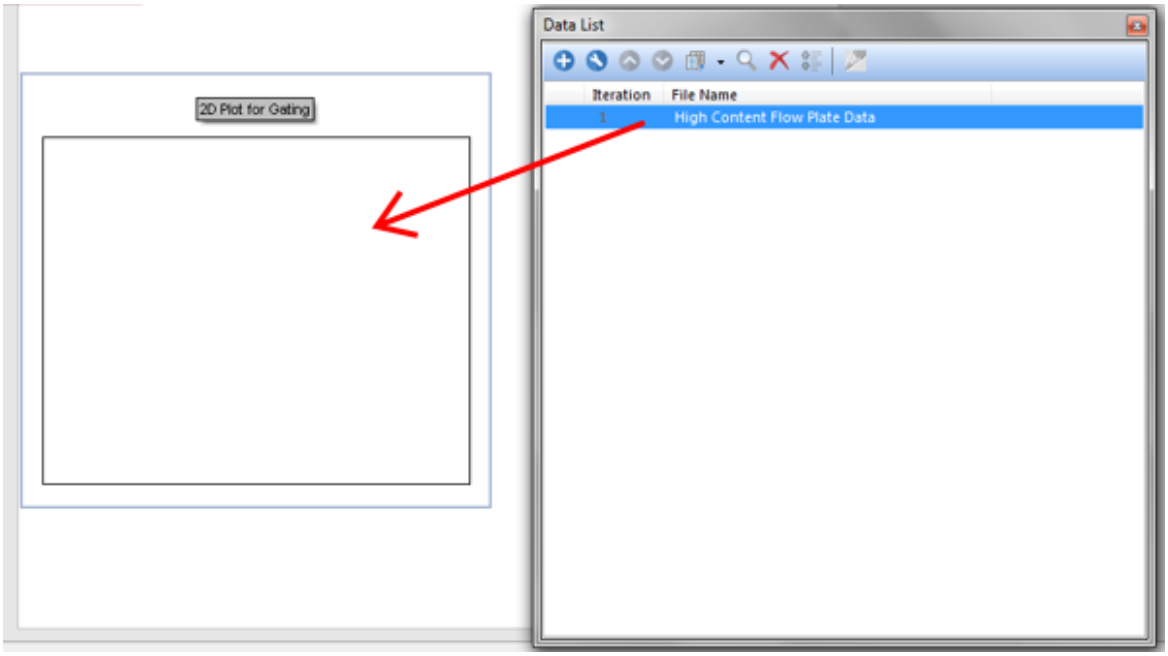

Figure 18.15b - Drag&Drop from Data List

51. Create two gates[318] on the 2D plot as in Figure T18.16 below.

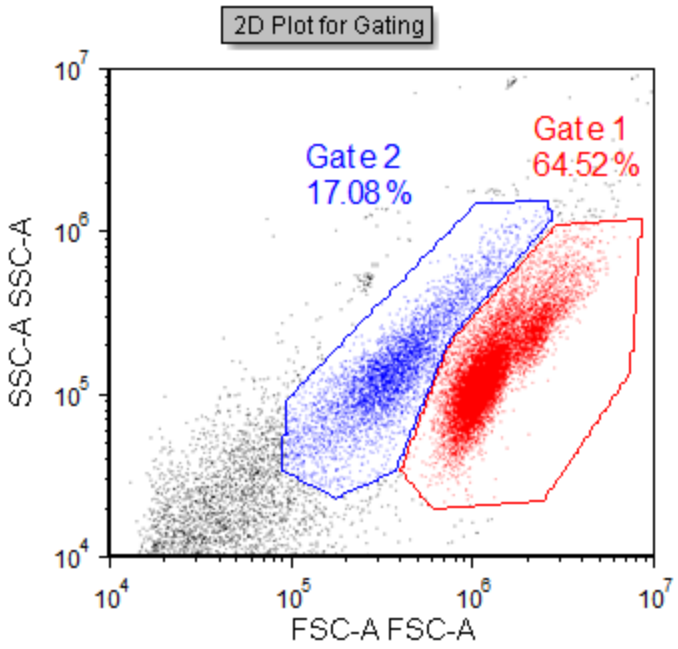

Figure T18.16 A Gated 2D Plot Created from the High Content Flow Plate Data

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Note: Since the plot above is ungated, it is showing all the events from all the wells in the data file.

52. Select the Heat Map on the left.

53. Choose **Gate 1** from the **Gating→Create Gates→Current Gate** drop-down list (Alternatively you can drag and drop Gate 1 from the 2D Plot directly onto the Heatmap).

54. Select the Heat Map on the right.

55. Choose **Gate 2** from the **Gating→Create Gates→Current Gate** drop-down list (Alternatively you can drag and drop Gate 2 from the 2D Plot directly onto the Heatmap).

Notice how the heat map updates to only display cells that fall within the gate you applied. Try moving the gates and watch the heat maps update.

Next, we will analyze data in a heat map.

38.20.2 Analyzing Data in Heat Maps

In this section you will learn how to:

- Create a gate on a heat map
- Use well gates to view events in 2D plots from single and multiple wells
- Create heat map overlays to view events from multiple wells on a single plot as an overlay

We will be using a data set that is looking for wells (hits) that are positive in the FL2-A channel. We will be using the **HighContentAnalysis.fey** layout and the **HighContent96wellData.dns** data file (.dns is special file format created by FCS Express), which are both located in the Tutorial Sample Data archive. The original data set contains 96 FCS Files acquired on a plate-based acquisition system.

1. Load the **HighContentAnalysis.fey** layout.
2. Choose the **Data** tab→**Organize Data Sets→Data List** command (Figure T9.2).
3. Click the symbol to Add Data Files to the data list.
4. Choose **DNS data Stream files (*.dns)** from the **Files of type:** drop-down list (Figure T18.17).

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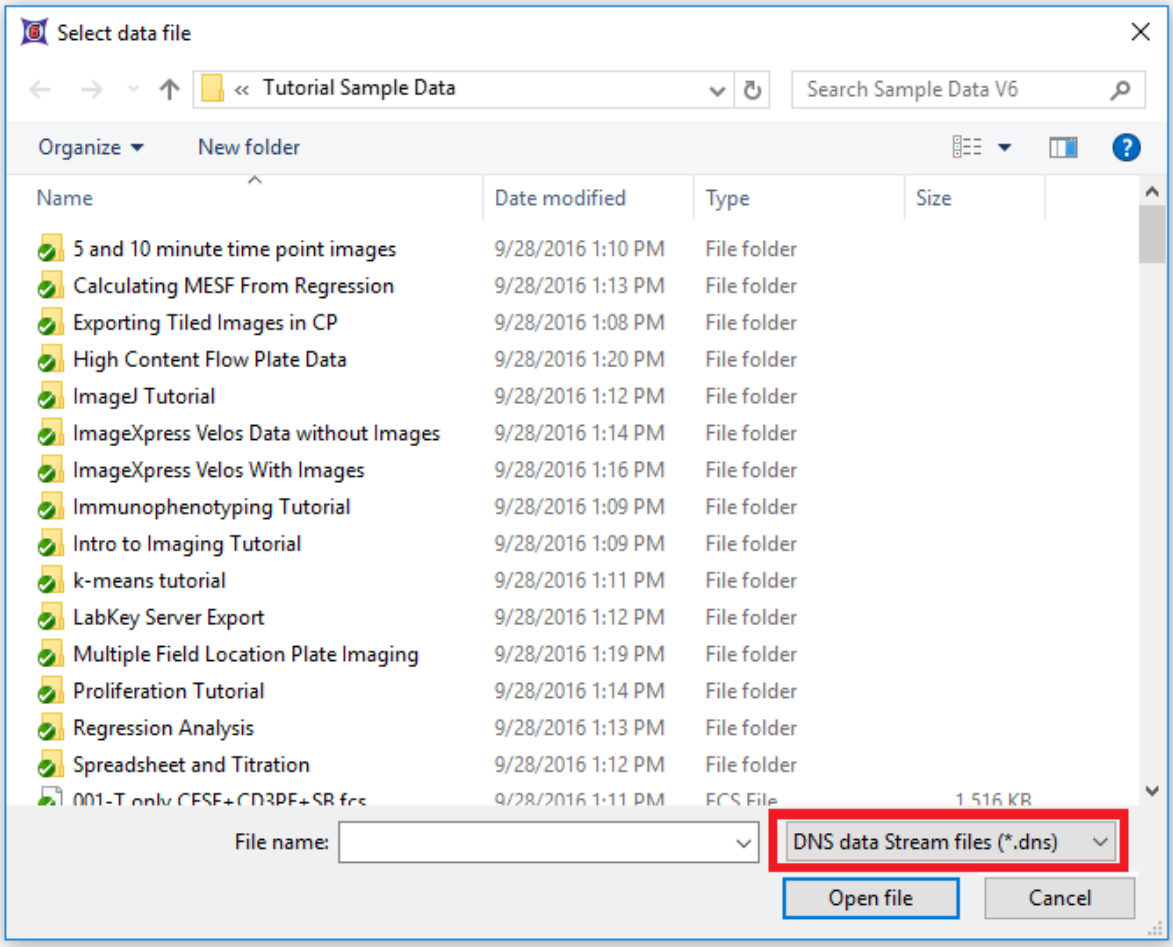

Figure T18.17 Choosing DNS Data Stream Files

5. Navigate to the FCS Express *Tutorial Sample Data* directory (Figure T18.18).

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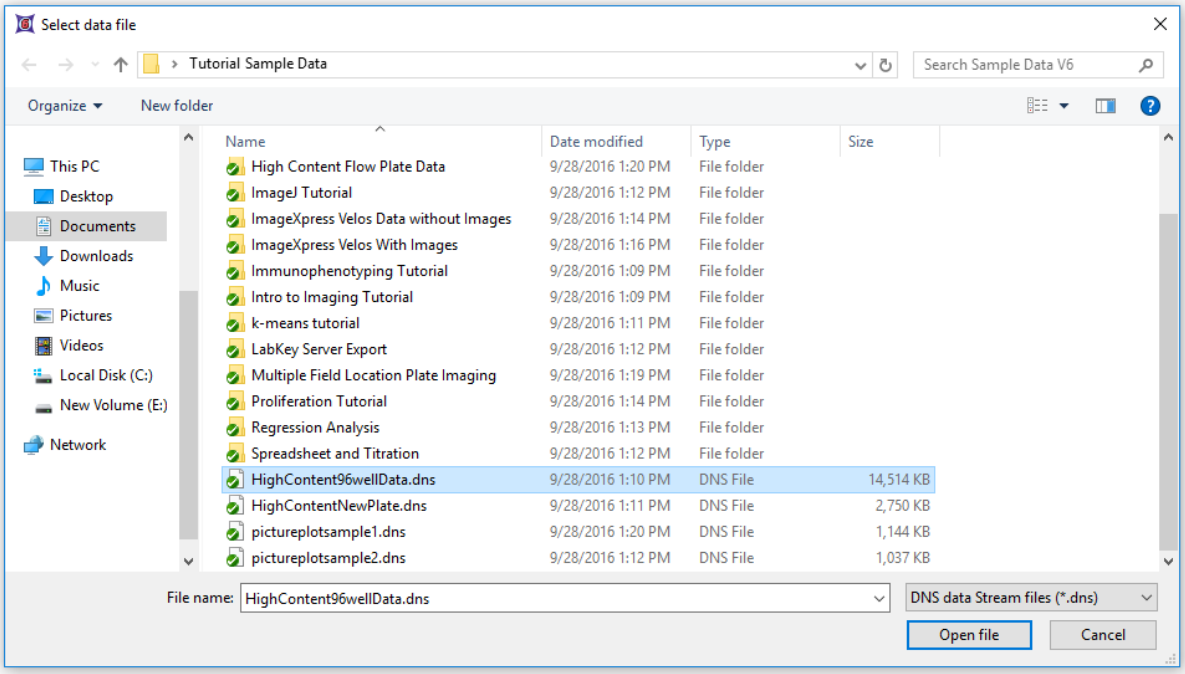

Figure T18.18 Choosing the HighContent96wellData.dns file

6. Choose the file "**HighContent96wellData.dns**"
7. Click **Open file** (Figure T18.18, above).

The file will now appear in the data list as a file. This file contains data from all 96 wells stored in .dns format. We will now load the data into the plots on the layout before we insert a heat map.

8. Choose the **Data** tab→**Change Data on All**→**Select**→**Select using Advanced Open Data Dialog** command (Figure T18.18a).

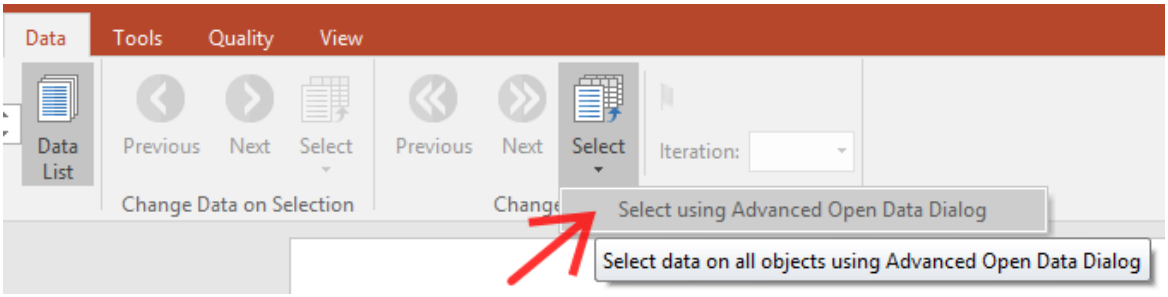

Figure T18.18a. Select Using Advanced Open Data Dialog command.

9. Choose the **Data List** tab in the resulting **Change Data File** dialog.
10. Highlight the **HighContent96wellData.dns** file and click **OK**.

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Note: Alternatively you can select the "HighContent96wellData.dns" item in the Data List and click on "Change Data On All Plots" (Figure T18.18b)

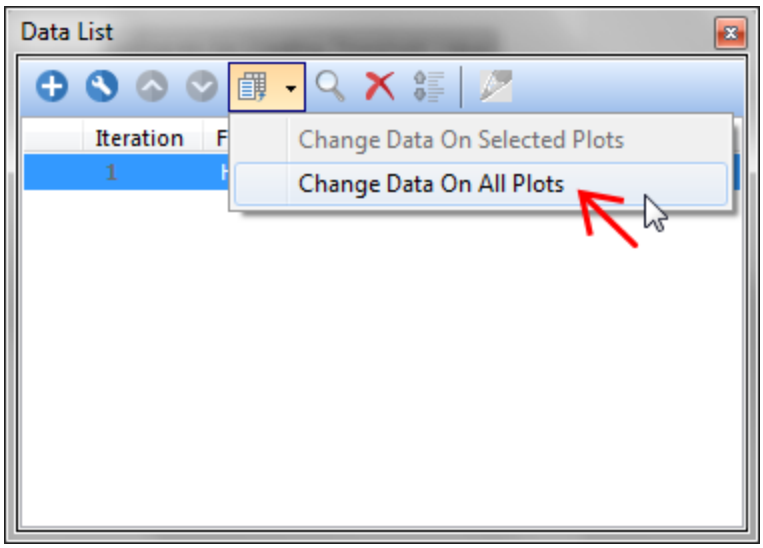

Figure T18.18b - Change Data On All Plots button

11.The layout will now look like Figure T18.19.

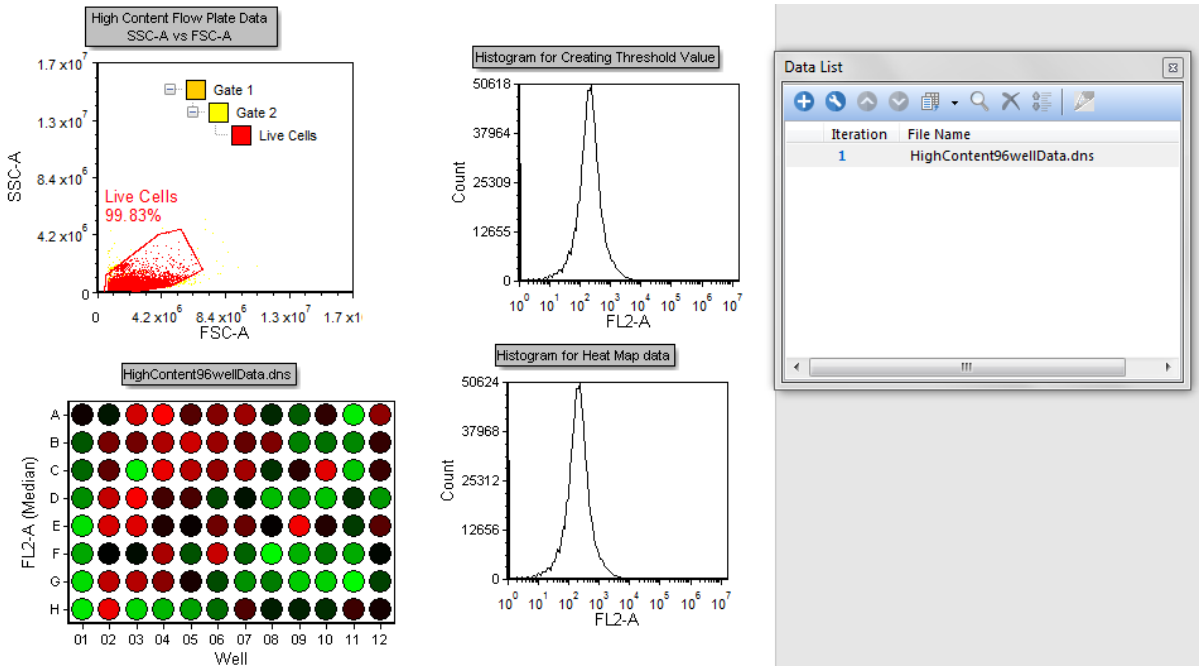

Figure T18.19 High Content Analysis Layout after Loading HighContent96wellData.dns File

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The color dot plot in the layout is showing all the events within the .dns file (Figure T18.19). The Live Cells gate is applied to the heat map so it will only display cells falling within the gate, defined on the 2D plot.

Wells that are "hot" for FL2-A are displayed in red and wells that are "cool" for FL2-A are displayed in green. Varying degrees of heat are represented by the transition from Red to Black to Green.

We will now create gates into the heat map.

12. Right-click on the Heat Map.
13. Choose **Create Gate→Well** from the pop-up menu.
14. Click in the white space surrounding the plate but within the green selection as in Figure T18.20 (clicking directly on a well will immediately select the well for gating).

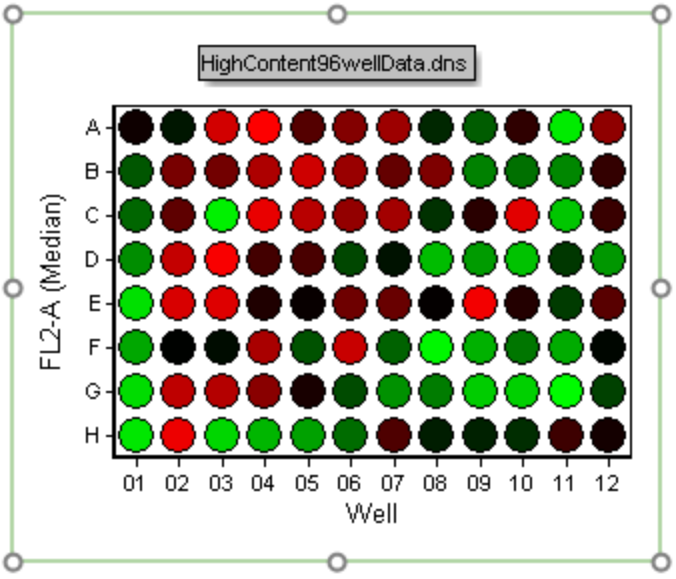

Figure T18.20 Creating a Well Gate

The Editing New Well Gate window will appear (Figure T18.21).

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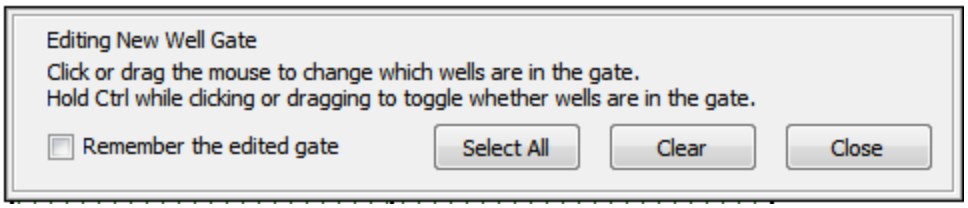

Figure T18.21 Editing a New Well Gate

15. Click on Well H12. The **Create New Gate** dialog will appear (Figure T18.21b).

16. Enter "Negative Control" for the gate name, change the gate color to "Green" and click **OK** (Figure T18.21b).

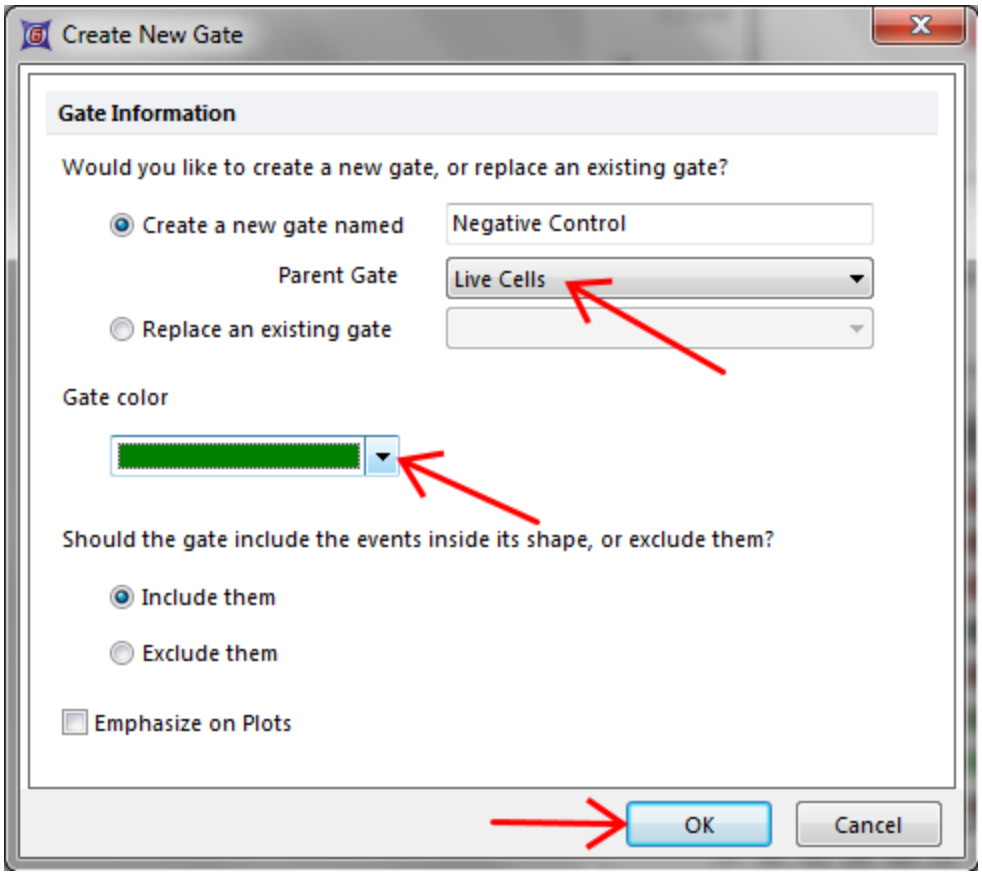

Figure T18.21b - Create New Gate window

We will now create a second gate into the heat map called "Experimental". For training purpose we will use an alternative procedure to steps 12-16.

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17. Choose the **Gating→Create Gates→Well** command (Figure T18.22).

Figure T18.22 Selecting the Create Well Gate Command

18. Click directly on Well A4. The **Create New Gate** dialog will appear (similar to Figure T18.21b).

19. Enter "Experimental" for the gate name, change the gate color to Blue and click **OK.**

The layout and gate view should now look like Figure T18.23.

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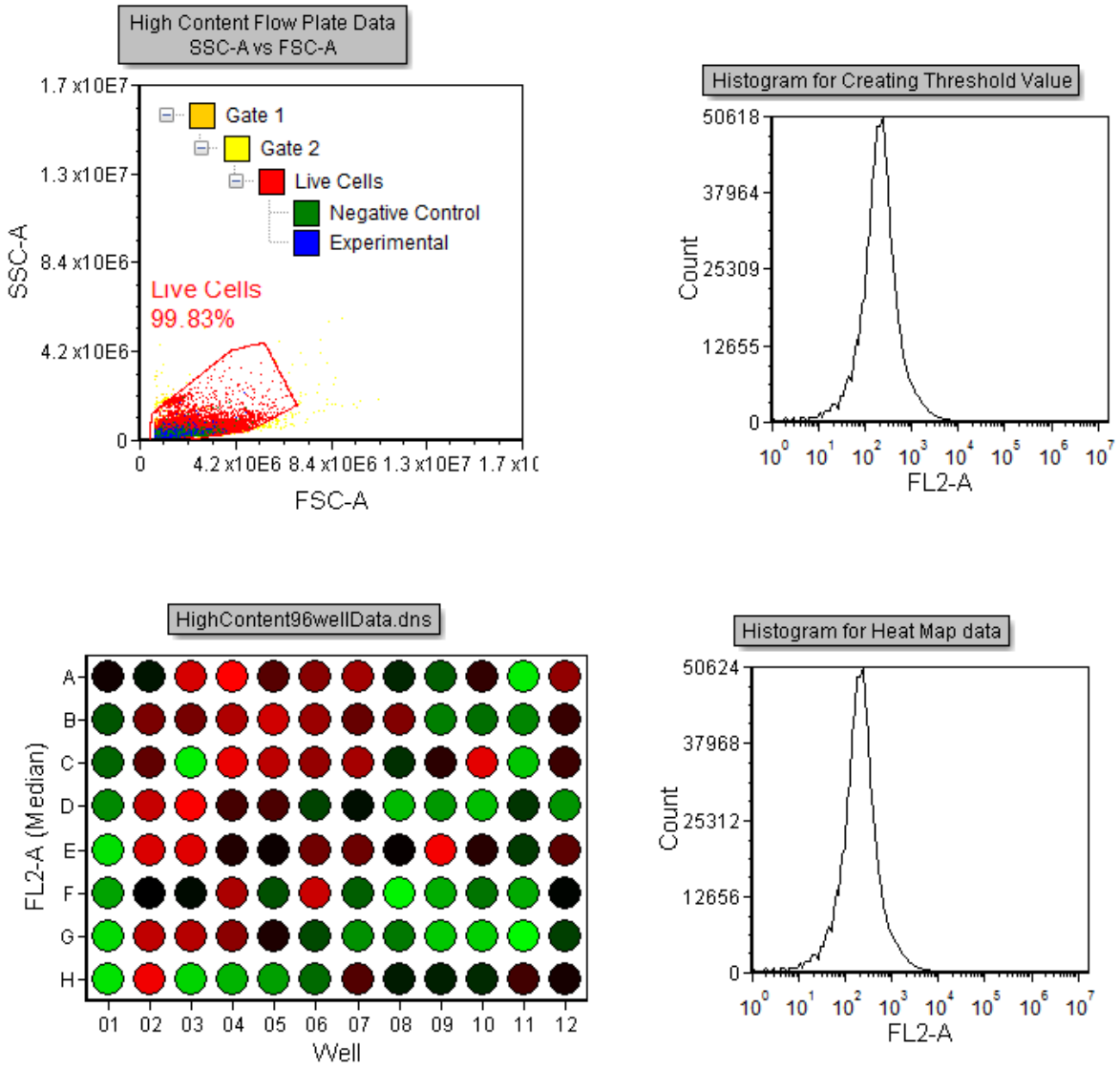

Figure T18.23 Layout with Gate View Indicating Hierarchical Gating of the Negative Control and Experimental Gates

We will now create a histogram overlay to view data from separate wells.

20. Drag and drop the entire Heat Map to the plot labeled **Histogram for Heat Map data** on the layout (Figure T18.23b).

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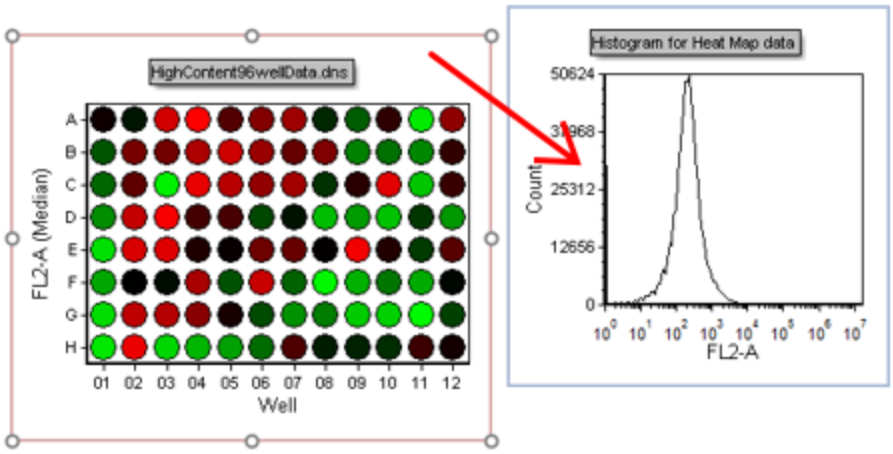

Figure T18.23b - Drag&Drop action to create overlay

21. The **Select Drag and Drop Action** window appears (Figure T18.23c)

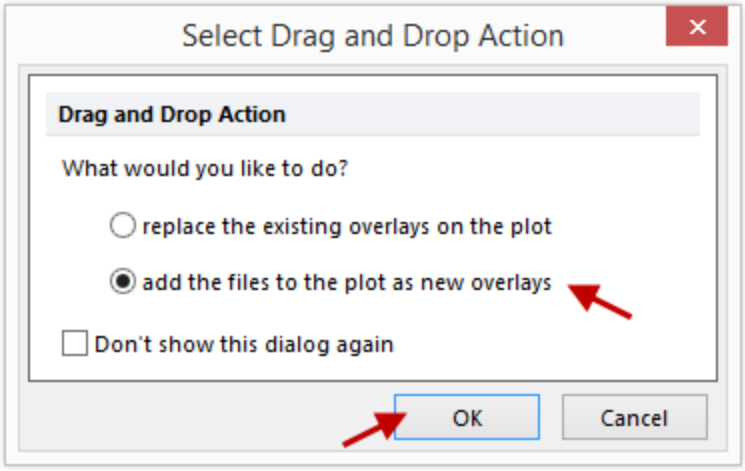

Figure T18.23c - Select Drag and Drop Action window

22. Select the option to **Add the files to the plot as new overlays** and click **OK** (Figure T18.23c).

23. Right-click on the histogram.

24. Choose **Format** from the pop-up menu and select the **Overlays** category.

Two overlays for the same FCS Plate folder file will be displayed in the **Overlay list**.

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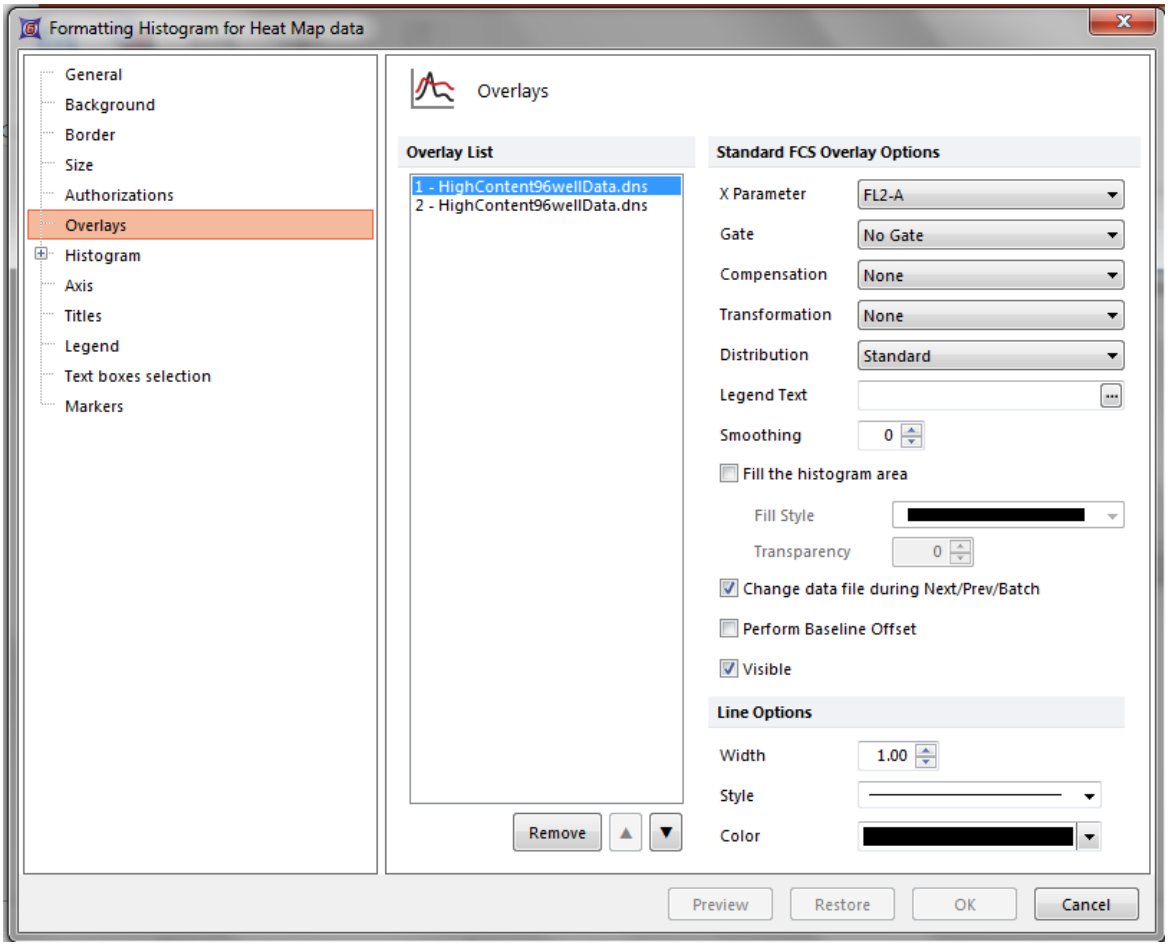

Figure T18.23d - Formatting Histogram for Heat Map data windows

25. Highlight the first overlay in the **Overlay List**.

26. Change the **Gate** to "Negative Control".

27. Change the **Color** under **Line Options** to Green.

28. Highlight the second overlay in the list.

29. Change the **Gate** to "Experimental".

29. Change the **Color** under **Line Options** to Blue.

30. Click **OK**.

The Histogram will now look like Figure T18.24, displaying the cells in the "Negative Control" well gate as green, and the cells from the "Experimental" well gate as blue.

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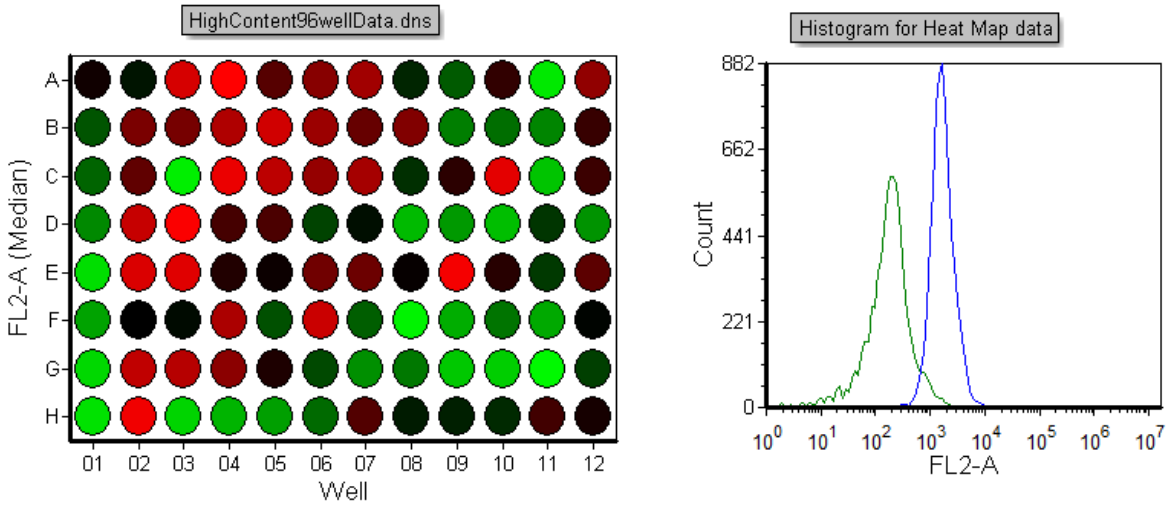

Figure T18.24 Histogram Displaying Events from Well Gates

We will now edit the well gates to select different wells.

31. Right-click on the Heat Map.

32. Choose **Edit Gate→Experimental** from the pop-up menu. The **Editing** well gates window will appear and well A4 will be highlighted in blue (Figure T18.25).

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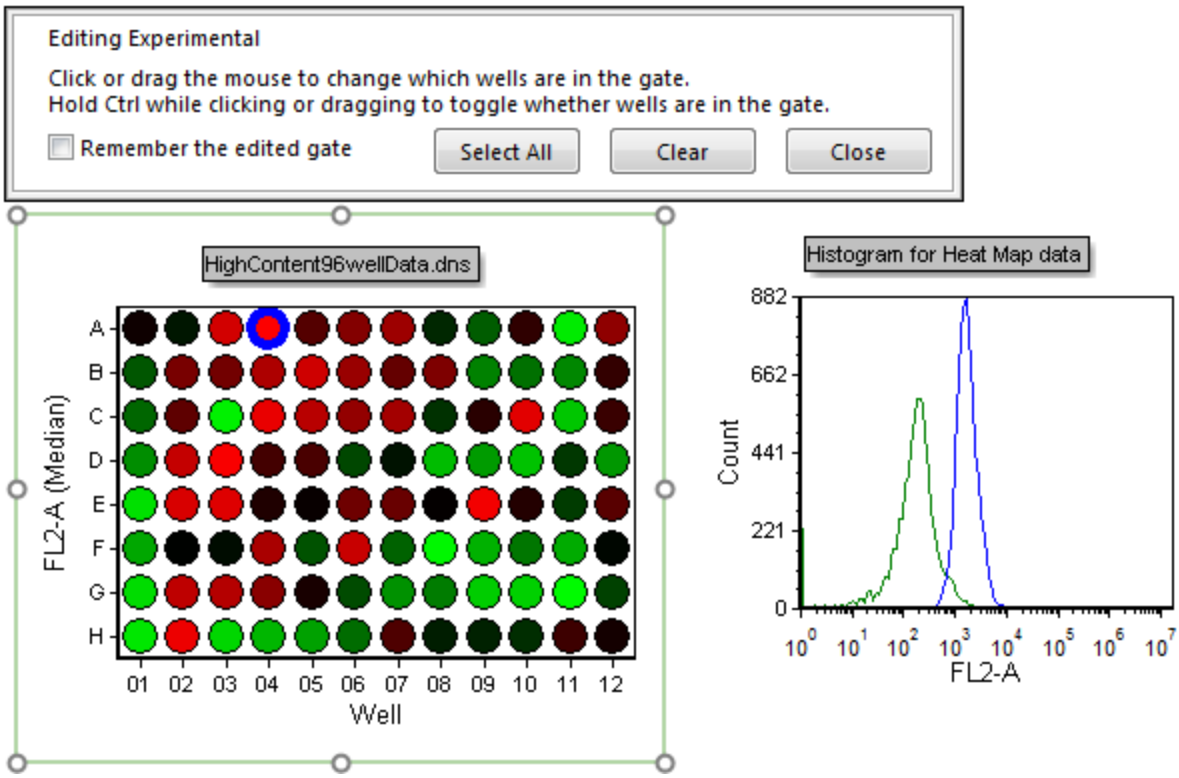

Figure T18.25 Editing the Experimental Well Gate (A4)

33. Click on well C12.

Notice how the experimental histogram peak shifts showing only the cells in the newly selected well (Figure T18.26).

Well C12 shows heterogeneous staining for FL-2A while "cooler" wells such as G9 (click on well G9 to view) overlap with the negative control. We will now select multiple "Negative" wells to create a better picture of the negative population on the histogram.

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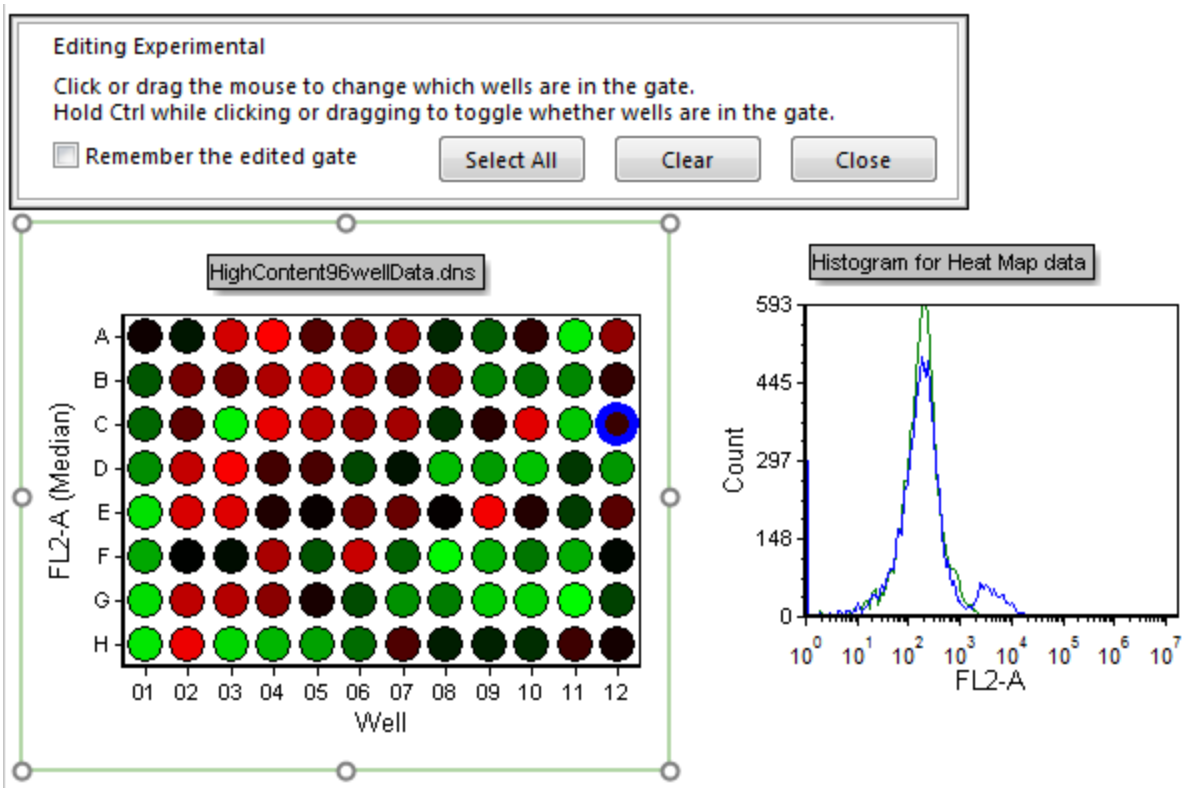

Figure T18.26 Experimental Well Gate Changed to C12

34. Hit **Ctrl+Z** to undo the gate change.

35. Right-click on the Heat Map**.**

36. Choose **Edit Gate→Negative Control** from the pop-up menu.

37. Press and hold down the **Ctrl** button to allow multiple non-consecutive selection.

38. Click on wells C1 and F1 (Figure T18.27).

39. Release the **Ctrl** button.

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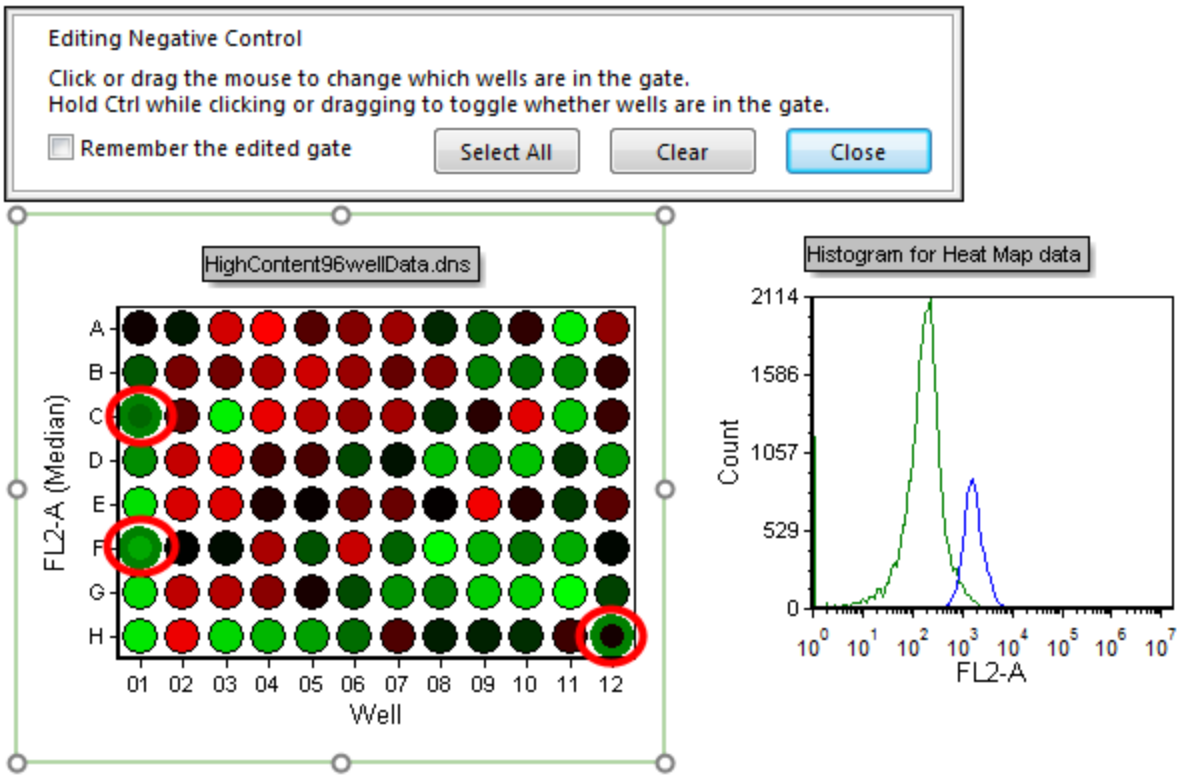

Figure T18.27 Selecting Multiple Non-consecutive Wells (C1, F1, and H12)

Multiple non-consecutive wells can be chosen in this manner. Notice how the histogram updates to reflect selection of the multiple wells (C1, F1, and H12 in Figure T18.27). We will now select multiple wells using a selection box.

40. Left-click and hold the mouse button down just above and to the right of well B1.

41. Drag the mouse down and to the left to select wells B1-F1. Notice how the selection box appears and indicates which wells are being selected (Figure T18.28).

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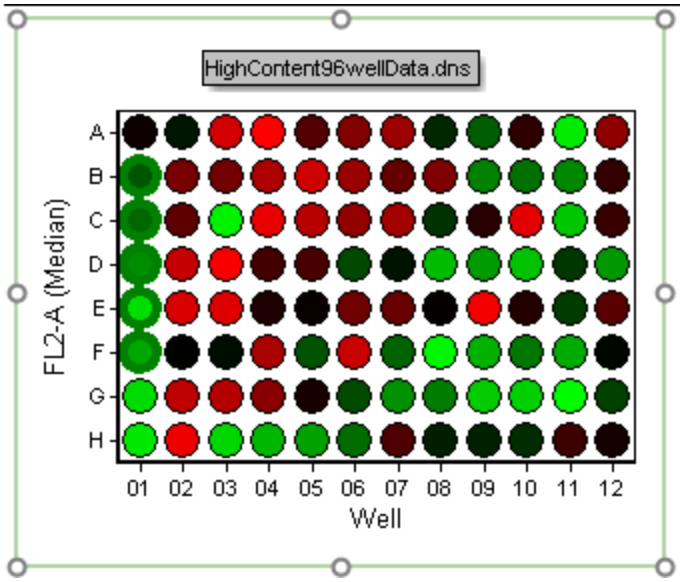

Figure T18.28 Selecting Multiple Continuous Wells

42. Release the mouse button to select the wells.

Multiple wells can also be selected by a combination of using the selection box and **Ctrl**+Clicking.

43. Hold down the **Ctrl** key.

44. Select wells B1-F1.

45. Click on well G9 to select it (continue holding down the **Ctrl** key).

46. Create a new selection box (with the **Ctrl** key still being held down) to encompass wells A8-A11.

47. Release the **Ctrl** key.

48. Click **Close** from the Editing Well Gate window.

Wells B1-F1, A8-A11, and G9 are now selected and contributing to the Negative Control histogram peak (Figure T18.29).

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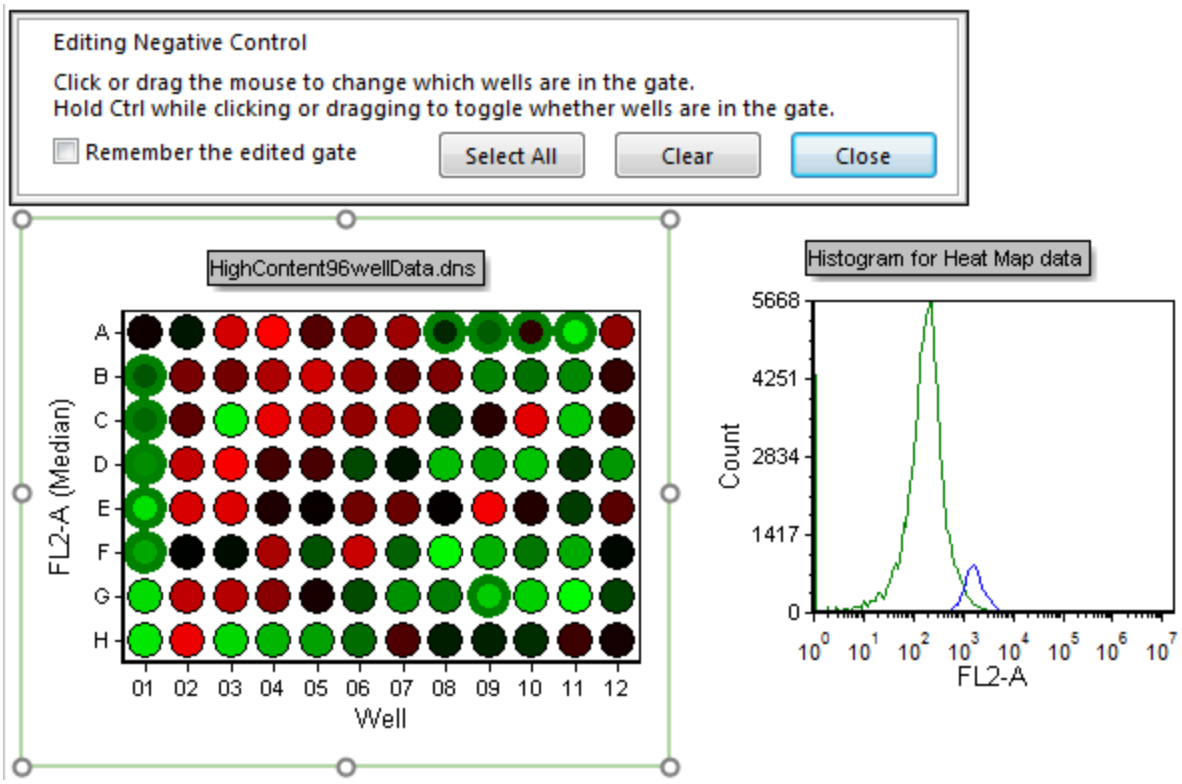

Figure T18.29 Multiple Well Selection

Gates may also be deselected from the well gating dialog in a similar manner.

49. Right-click on the Heat Map.
50. Choose **Edit Gate→Negative Control** from the pop-up menu.
51. **Ctrl+Click** on well A8. Notice how the well gate is removed.
52. **Ctrl+Drag** a selection box over wells B1-F1. The continuous column well gates will be removed.

Although selecting wells for gating and displaying them in histograms or 2D plots is useful, some users may be only interested in which wells contain the highest channel values. We will now format the heat map to display wells that are above a threshold value of interest.

53. Right-click on the **Heat Map.**
54. Choose **Format** from the pop-up menu.
55. Choose the **Heat Map→Color Levels** category.

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56. Change the **Style** to **Threshold** from the drop down window (as in Figure T18.12).
57. Click on the T symbol in the **Threshold Value** field to insert a token.
58. Double-click on **Statistic** from the **Insert a Token** dialog.
59. Choose the "Histogram for Creating Threshold Value" in the **Select a plot** list box.
60. Choose the **Statistic** category on the left of the **Create Statistic** dialog.
61. Set the **Gate** to **Live Cells** from the drop-down list.
62. Highlight **Median** in the **Statistic** list box.
63. Click **OK**.
64. Change the **Below Threshold Color** to Black, **Above Threshold Color** to Red and click **OK**.

The heat map will now look like Figure T18.30. The red wells indicate which wells have median FL2-A fluorescence that are above the median FL2-A fluorescence of the entire plate.

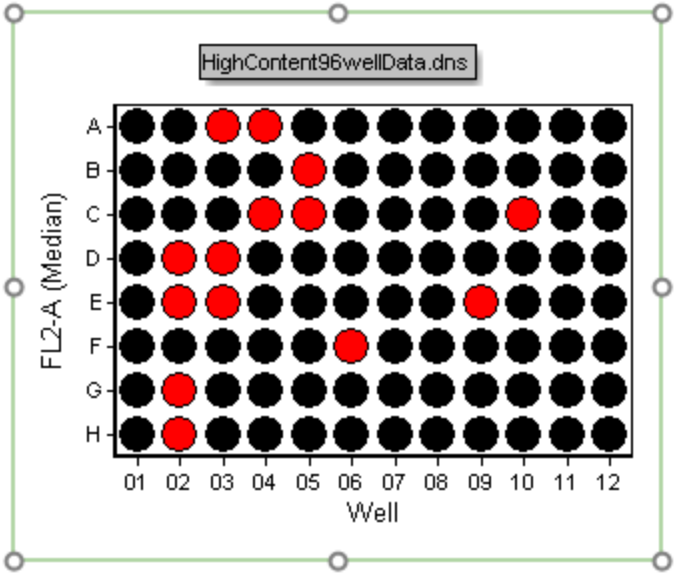

Figure T18.30 Threshold Defined as Median Value

In the next section, we will use batch analysis to analyze multiple plates.

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38.20.3 Using Batch Analysis

We will now learn how to set up a batch process to analyze data from multiple plates. The batch functions for heat maps are the same as for any other plot in FCS Express. For a more detailed introduction to batch processing, we recommend you view the Batch Processing tutorial 1445 before you begin.

We will be using the HighContentBatch.fey layout, along with the HighContent96wellData.dns and HighContentNewPlate.dns data files (.dns is an extension created in FCS Express that allows for special compression of the data).

1. Open the **HighContentBatch.fey** layout located in the *FCS Express Sample Data* directory.

The layout is saved as unlinked and we must load our plate data into the Data List before we can begin adding batch actions.

2. Select the **Data→Organize Data Sets→Data List** command (Figure T9.2 1445).

3. Click the symbol to add data files to the data list. The **Select a Data File** window appears.

4. Choose **DNS data Stream files (*.dns)** from the **Files of type:** drop-down list (Figure T18.17 1758).

5. Choose the files **HighContent96wellData.dns** and **HighContentNewPlate.dns** and click **Open** (Figure T18.31a).

Note: you can select multiple files by pressing and holding down the **Ctrl** button on the keyboard.

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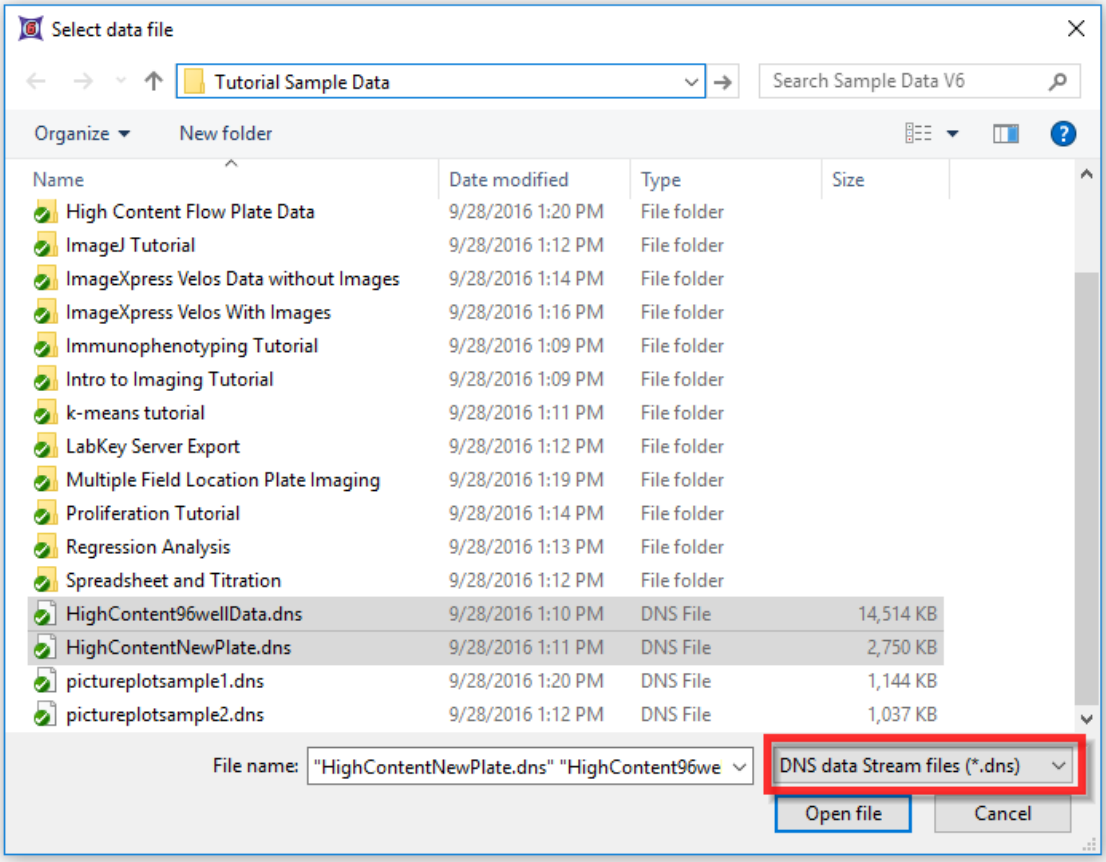

Figure T18.31a - Multiple file selection in the Select a Data File window

6. Choose the **Data** tab →**Change Data on All**→**Select**→**Select using Advanced Open Data Dialog** command (Figure T18.13b, *left*, ❶). The **Change Data File** window appears (Figure T18.13b, *right*).

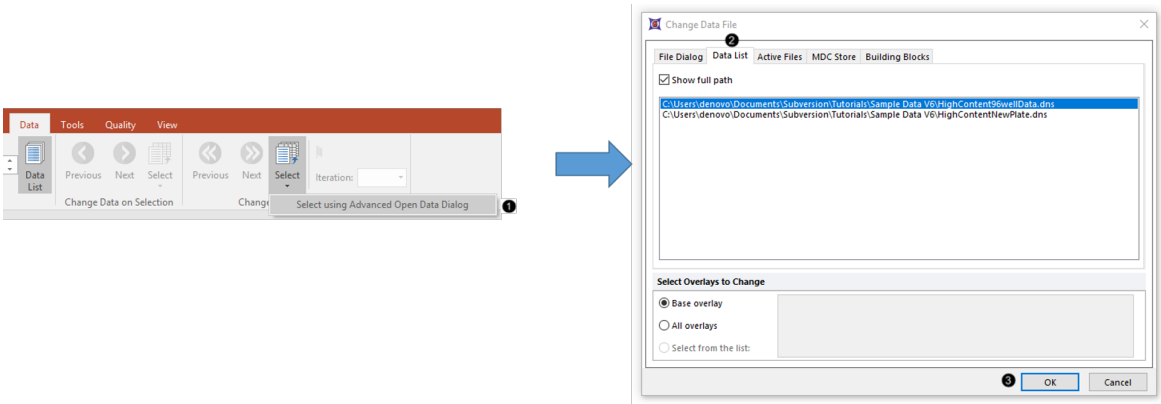

Figure T18.13b - Selecting a .dns file via the Advanced Open Data dialog

7. Click the **Data List** tab (Figure T18.13b, ❷).

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8. Choose **HighContent96wellData.dns** and click **OK** (Figure T18.13b, ❸).

The layout will now look like Figure T18.31b. There are three differently styled views of the heat map data and a table containing the statistics for each well in the heat map. We will now define batch actions to export the two plate files in the data list.

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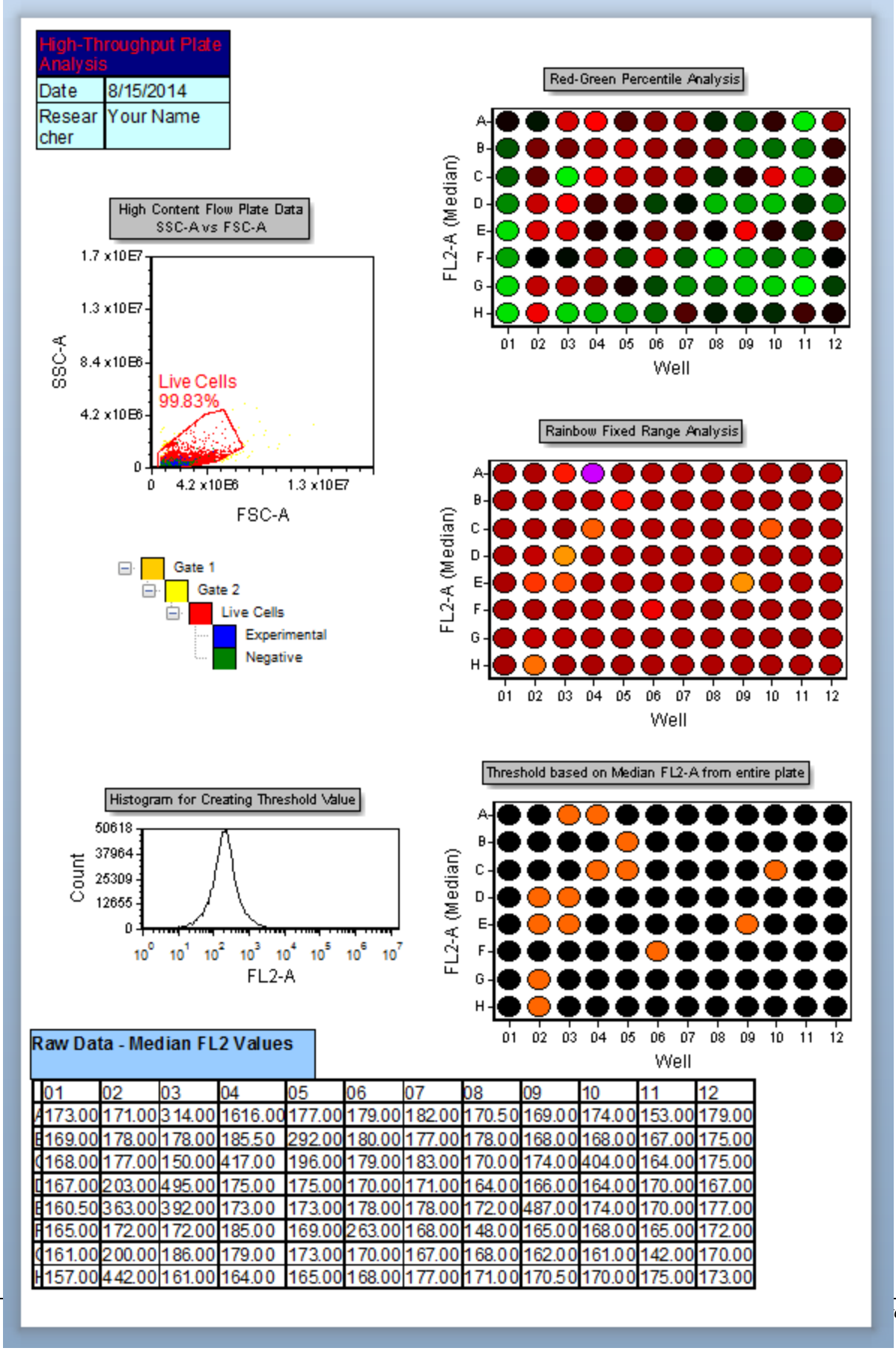

	01	02	03	04	05	06	07	08	09	10	11	12
A	173.00	171.00	314.00	1616.00	177.00	179.00	182.00	170.50	169.00	174.00	153.00	179.00
B	169.00	178.00	178.00	185.50	292.00	180.00	177.00	178.00	168.00	168.00	167.00	175.00
C	168.00	177.00	150.00	417.00	196.00	179.00	183.00	170.00	174.00	404.00	164.00	175.00
D	167.00	203.00	495.00	175.00	175.00	170.00	171.00	164.00	166.00	164.00	170.00	167.00
E	160.50	363.00	392.00	173.00	173.00	178.00	178.00	172.00	487.00	174.00	170.00	177.00
F	165.00	172.00	172.00	185.00	169.00	263.00	168.00	148.00	165.00	168.00	165.00	172.00
G	161.00	200.00	186.00	179.00	173.00	170.00	167.00	168.00	162.00	161.00	142.00	170.00
H	157.00	442.00	161.00	164.00	165.00	168.00	177.00	171.00	170.50	170.00	175.00	173.00

Figure T18.31b - High Content Batch Layout with Data

9. Choose the **Batch→Batch Processing→Batch Actions** command (Figure T9.6 1445). The **Batch Actions** window appears.

10. Choose **Save to PDF** from the **Add Action** area (on the left). The **Create Export to PDF Action** window appears (Figure T18.32a).

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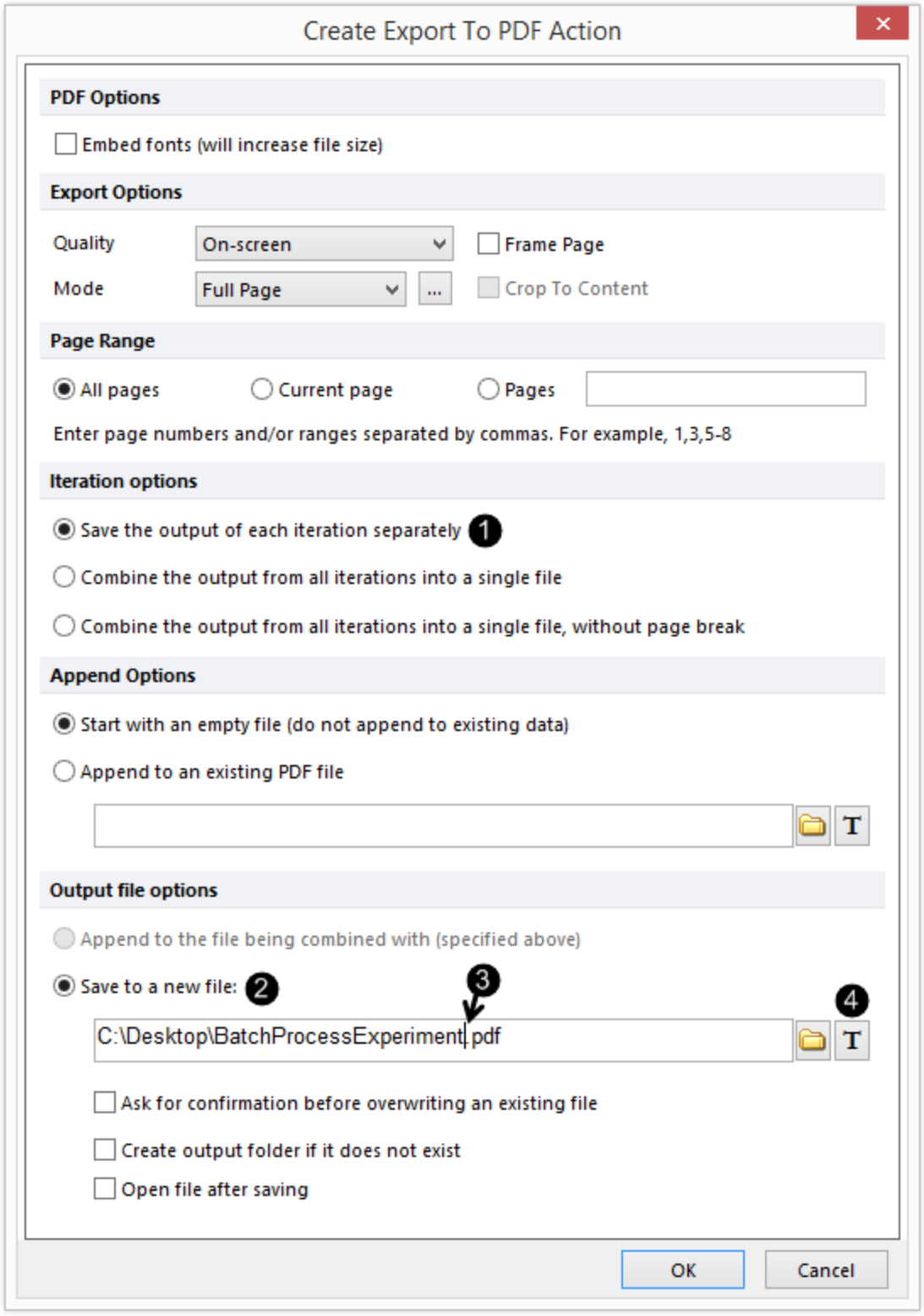

FIgure T18.32a - The Create Export to PDF Action window

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11. Click the **Save the output of each iteration separately** radio button under **Iteration options** (❶ Figure T18.32a).

12. Choose a file name and location for saving the pdf in the **Save to a new file** field (❷ Figure T18.32a).

13. Click directly after the file name and before the extension ".pdf" (❸ Figure T18.32a).

14. Click on the T symbol to insert a token (❹ Figure T18.32a).

15. Choose **Batch Processing→Current iteration** in the **Insert a Token** dialog (Figure T18.32b).

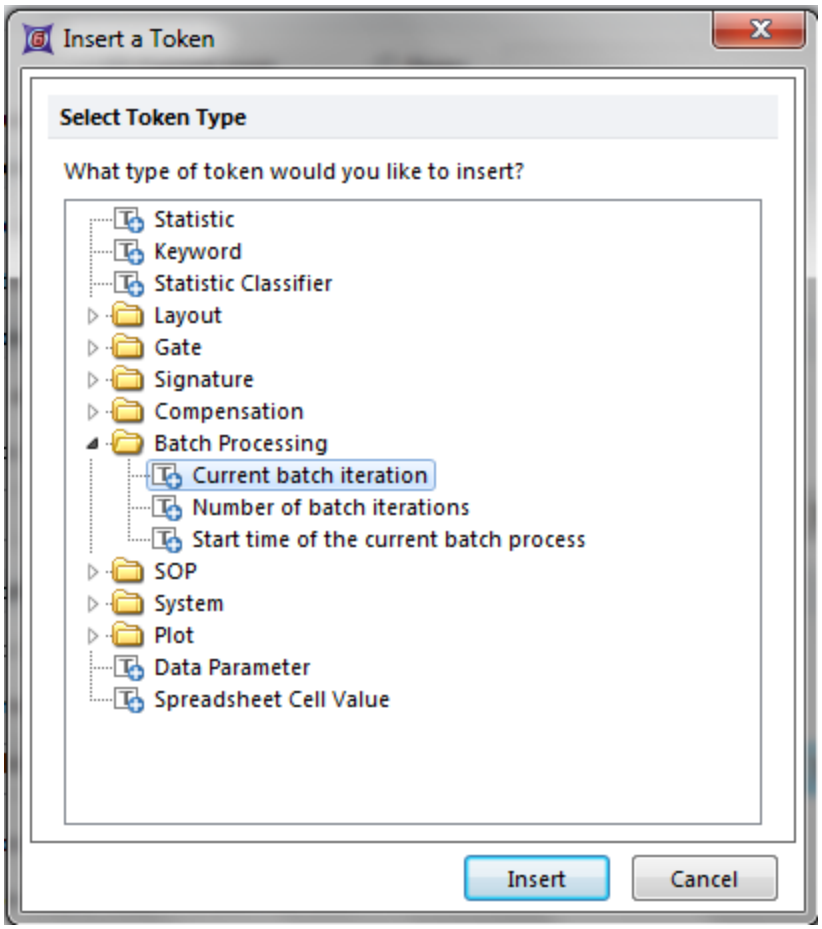

Figure T18.32b - The insert Token dialog

16. Click **Insert**.

The **Create Export To PDF Action** dialog should look similar to Figure T18.32c.

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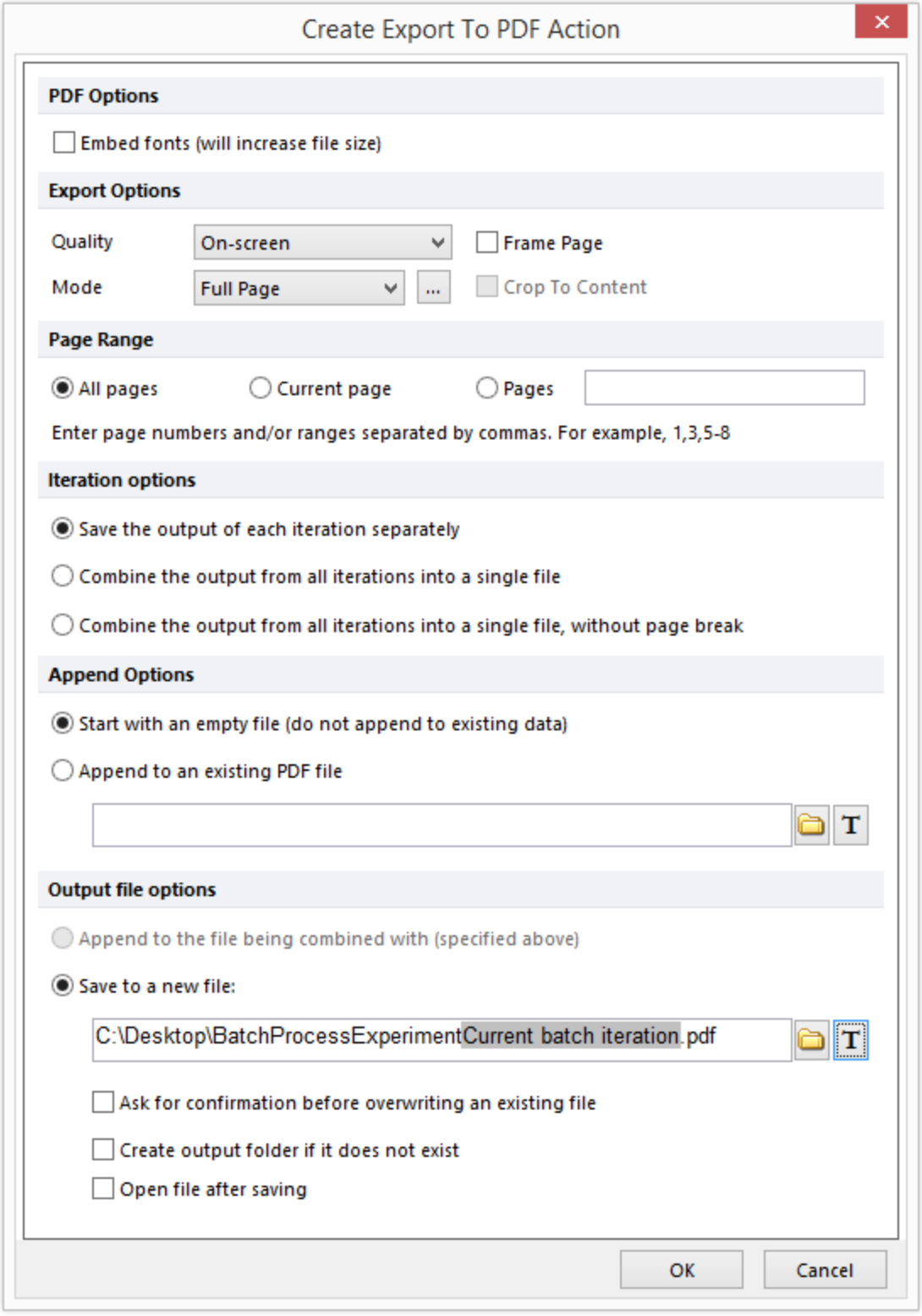

Figure T18.32c - Create Export To PDF Action Dialog

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17. Click **OK**.

18. Choose **Save to Powerpoint** from the **Add Action** area (Figure T9.7 1445). The **Create Export to Powerpoint Action** window appears (Figure T9.8 1445).

19. Click the **Combine the output from all iterations into a single file** radio button under **Iteration options**

20. Choose a file name and location for saving the Powerpoint file in the **Save to a new file** field.

The **Create Export To Powerpoint Action** dialog should look similar to Figure T18.33.

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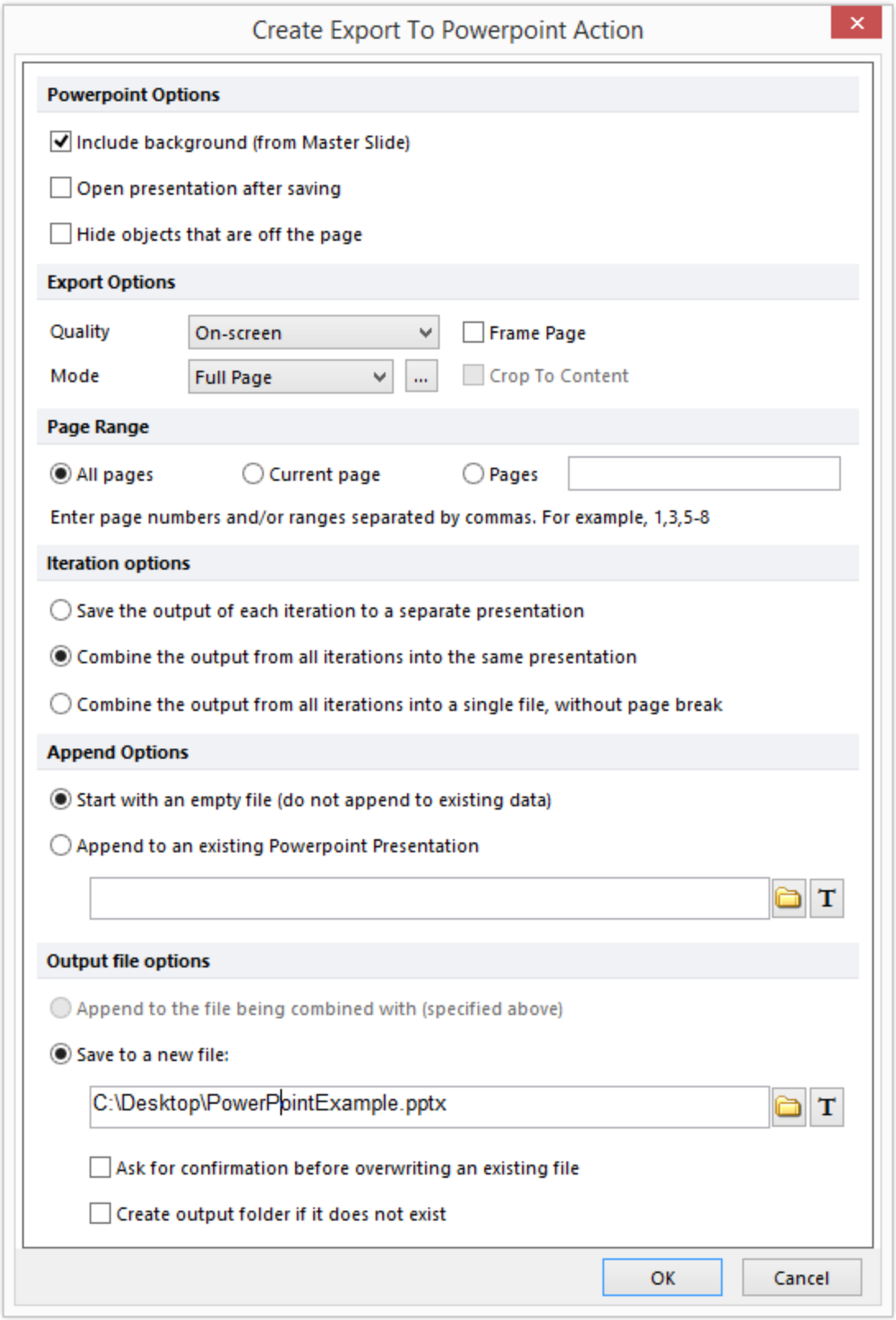

Figure T18.33 Create Export To Powerpoint Action Dialog

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21. Click **OK**.

The **Batch Actions** window will now look like Figure T18.34. Running this batch will create one PDF file with each file located on a new page and a new PowerPoint file for each file.

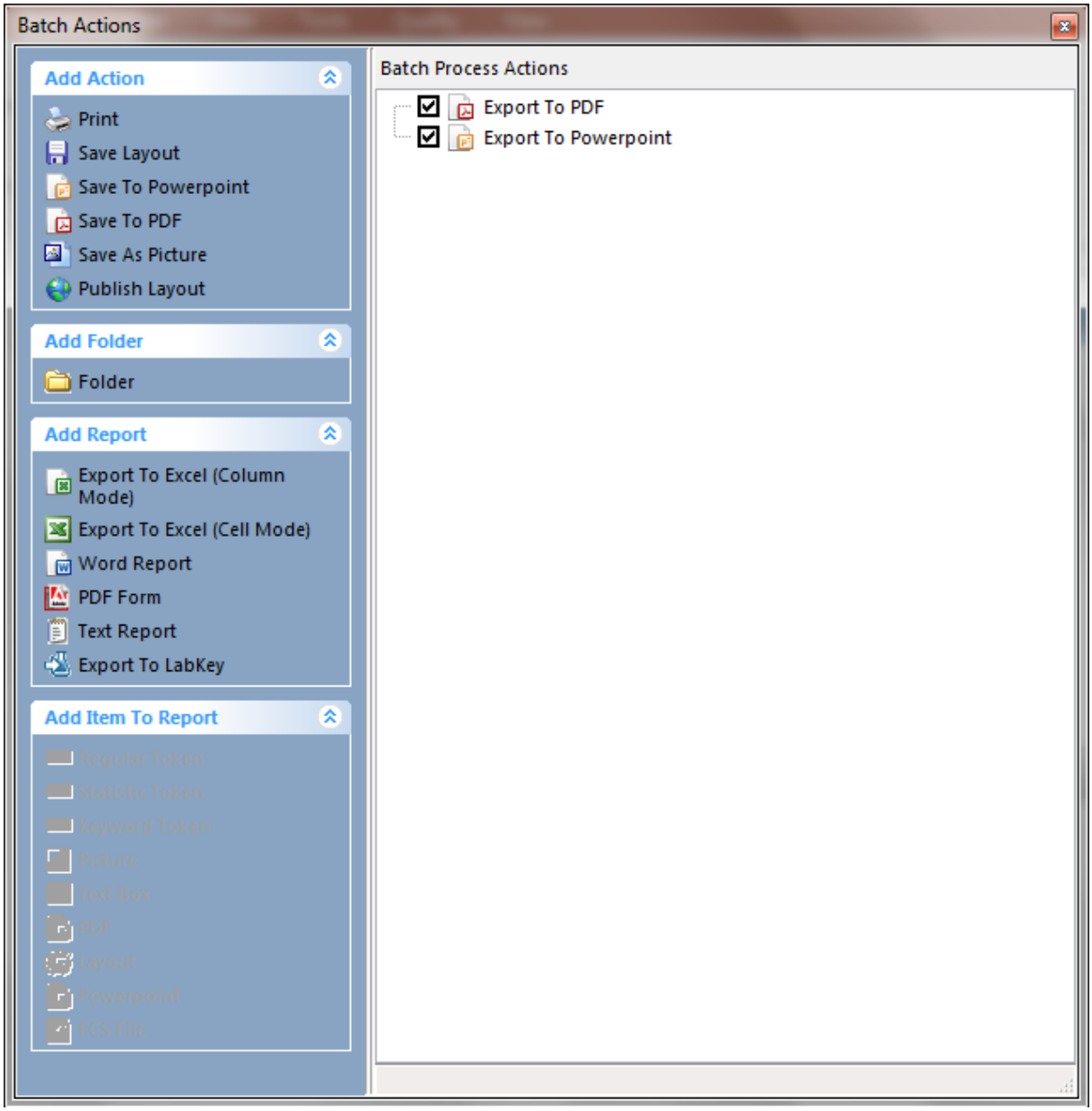

Figure T18.34 Batch Actions Window

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22. Choose the **Batch→Batch Processing→Run** command.

The batch process will iterate over each data file in the data list and export the actions we have added to the directory you selected. Since there are only two files in the data list, there should be:

- Two PDF files containing one iteration each (since we choose to **Save the output of each iteration separately**). File names contain a common part ("BatchProcessExperiment") and a variable part (the token referring to the iteration number). The latter allow you to distinguish among the different PDF files generated by the batch process.

- One Power Point file containing all the iterations (since we choose to **Combine the output from all iterations into a single file**). Since file is unique the file does not need any token.

In the last exercise, we will use the Z-Prime function.

38.20.4 Using the Z-Prime Function

In high-throughput screens, experimenters often compare a large number (hundreds of thousands to tens of millions) of single measurements of unknown samples to positive and negative control samples. Large screens are expensive in time and resources. Therefore, prior to starting a large screen, smaller test (or pilot) screens are used to assess the quality of an assay, in an attempt to predict if it would be useful in a high-throughput setting. The Z-factor is an attempt to quantify the suitability of a particular assay for use in a full-scale, high-throughput screen.

FCS Express now provides a Z-Prime function for use in Custom tokens to make calculating the Z-Factor easy.

We will be using the **HighContent_CalculatingZPrime.fey** layout which is embedded with positive and negative control .fcs files. We will be utilizing tokens and custom tokens in this section. For a more detailed explanation of tokens, please view the Using Text Boxes and Tokens tutorial.

1. Open the **HighContent_CalculatingZPrime.fey** layout located in the *FCS Express Sample Data* directory.

The layout will look like Figure T18.35. It contains a negative control file on the left and a positive control file on the right. There is also a text box where we will place the formula for the Z-Factor and a classification.

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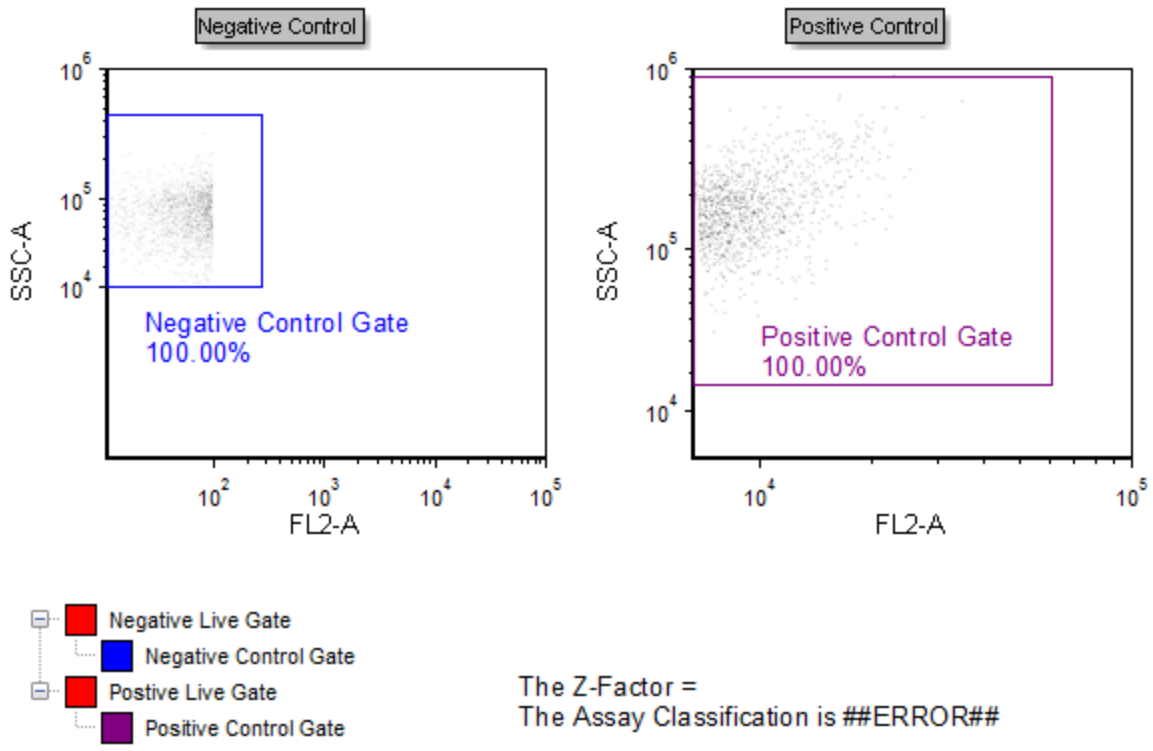

Figure T18.35 High Content Calculating Z-Prime Layout

2. Click the symbol in the **Custom Tokens** window.
3. Name the new custom token "Z Factor".
4. Click in the **Enter the Custom Token formula below** field.
5. Enter an equals sign ("=").
6. Right-click after the equals sign.
7. Choose **Insert Function→Statistics** from the pop-up menu.
8. Choose **ZPrime([mean negative, SD negative, mean positive, sd positive])** from the **Statistics** list (Figure T18.36).

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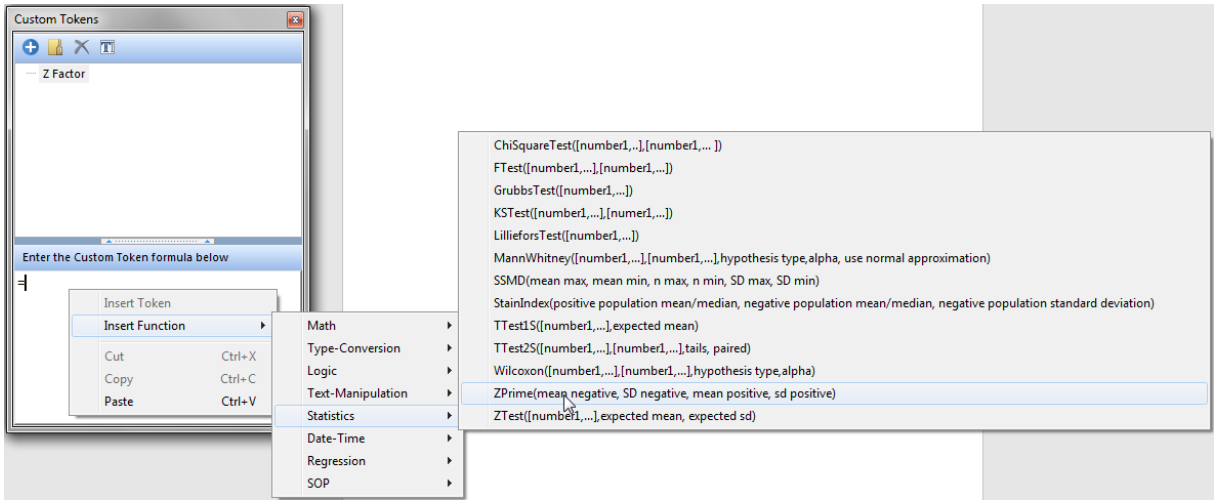

Figure T18.36 Choosing the ZPrime function from the Functions List

9. Highlight **mean negative** in the formula field (Figure T18.36b).

10. Right-click with the text highlighted and choose **Insert Token** (Figure T18.36b).

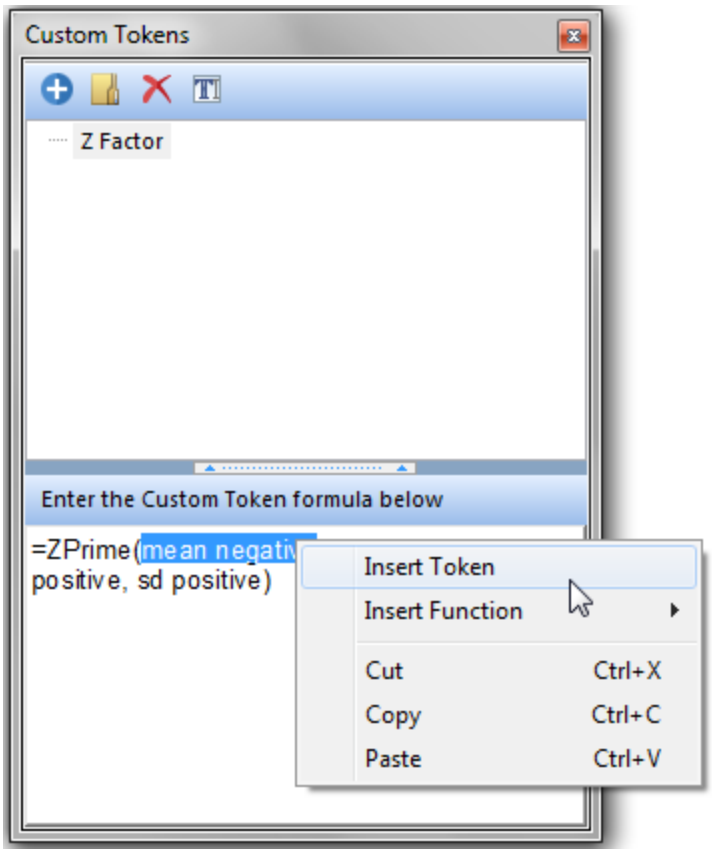

Figure T18.36b - Insert Token option

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11. Double-click on **Statistic**. The **Create Statistics** window appears (Figure T18.36c).

12. In the Data Source section choose the **Negative Control** plot as the data source (Figure T18.36c).

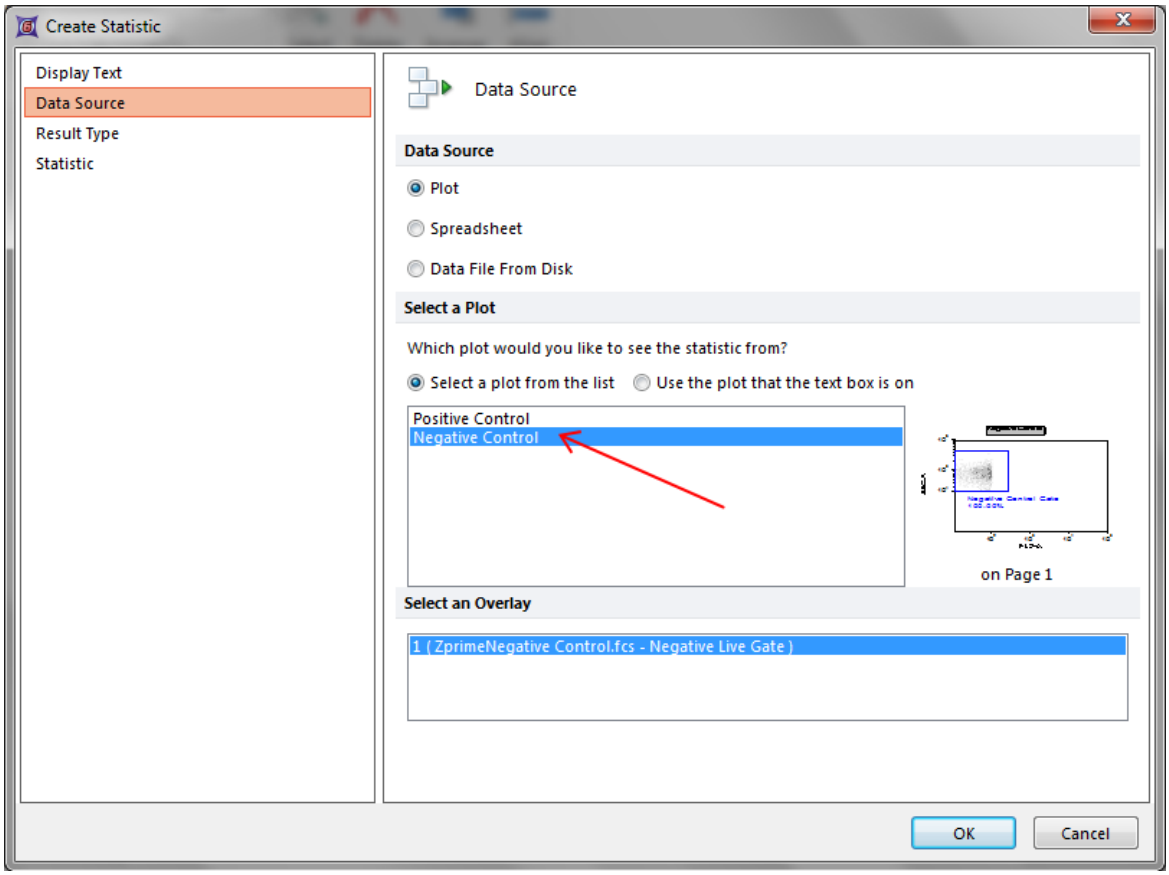

Figure T18.36c - The Create Satistics windows

13. Click on **Statistic** on the left.

14. Choose **Negative Control Gate** in the **Gate** section and **X Arithmetic Mean** from the **Statistic** section (Figure T18.37).

15. Click **OK**.

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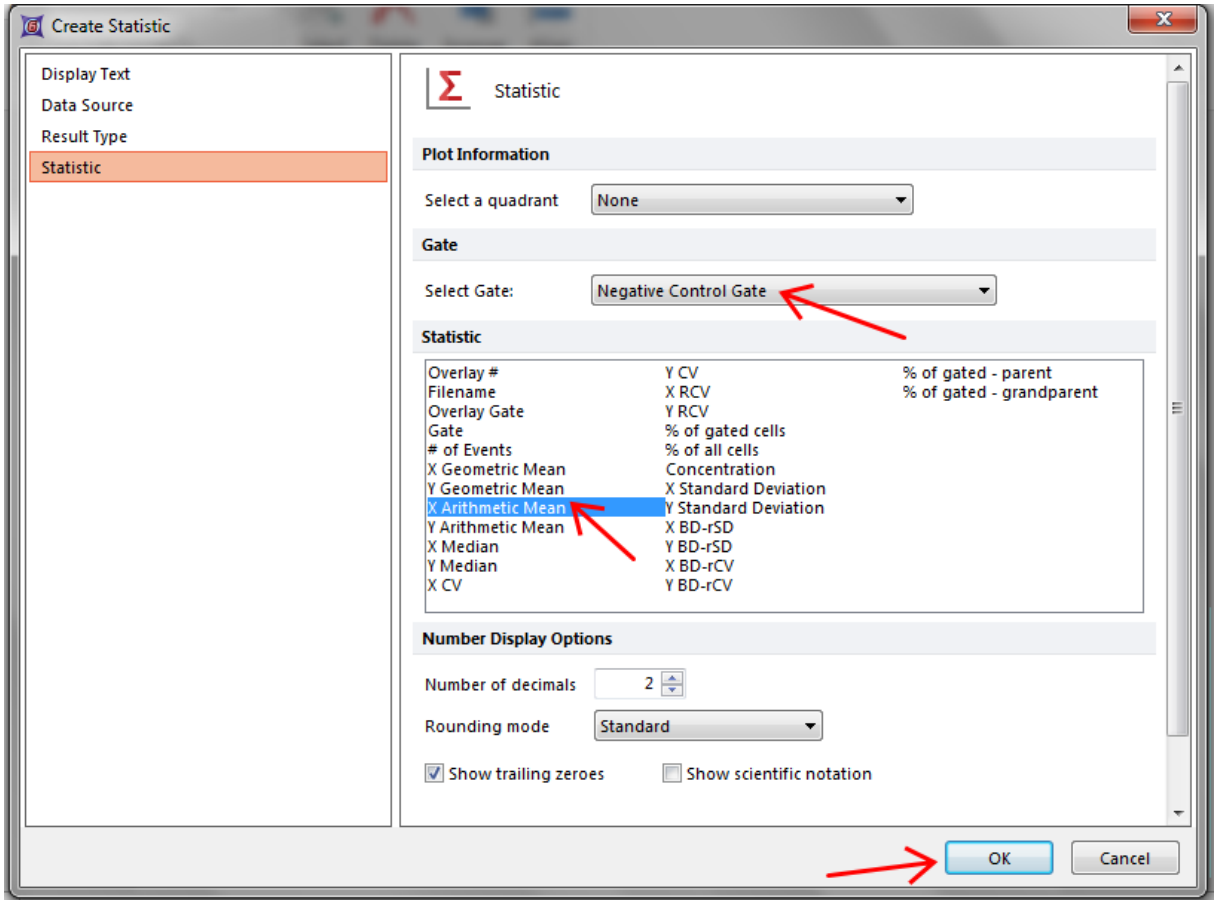

Figure T18.37 Replacing Text in a function with a Token

16. Highlight **SD negative** in the formula field.
17. Right-click with the text highlighted and choose **Insert Token**.
18. Double-click on **Statistic**.
19. Choose the **Negative Control** plot as the data source.
20. Click on **Statistic** on the left.
21. Choose the **Negative Control Gate** from the drop-down gate list
22. Choose **X Standard Deviation** from the **Statistic** list box.
23. Click **OK**.
24. Repeat steps 11-25 with "**mean positive**" and "**SD positive**" using the **Positive Control** plot as Data Source and the **Positive Control Gate** as gate.

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The formula for the Z Factor custom token will now look like Figure T18.38 where each of the original text placeholders have been replaced with the representative token value.

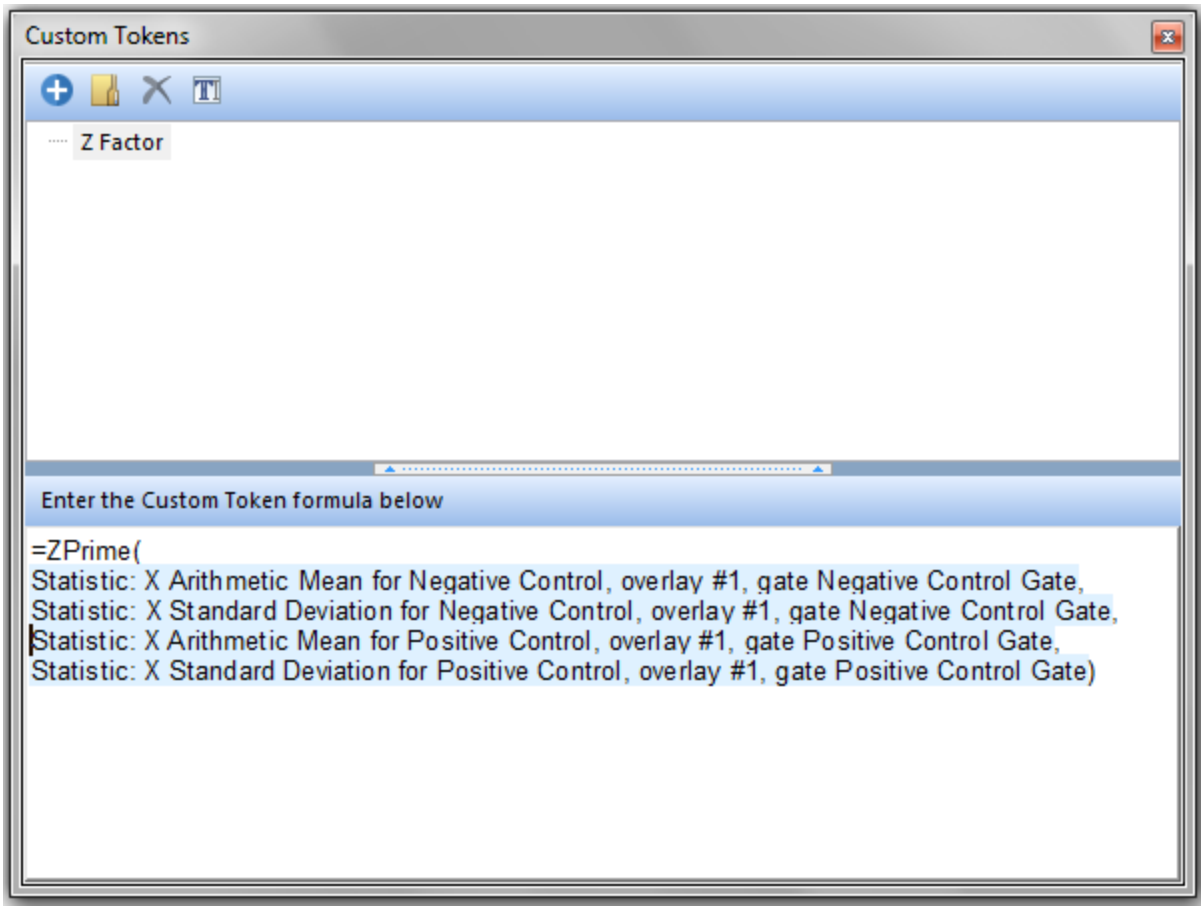

Figure T18.38 Z Factor Represented by the Z-Prime Function in FCS Express

25. Right-click after "**The Z-Factor =**" in the text box on the layout.
26. Choose **Insert Token** from the pop-up menu.
27. Double-click on **Z Factor** under **Custom**.

The Z-Factor custom token will now appear in the text box with a value of **0.00**.

To interpret the Z-factor , we will use the guidelines from Zhang JH, Chung TD, Oldenburg KR, A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen*. 1999;4(2):67-73.

- A Z-factor of 1, ideal. Z-factors can never be greater than 1.0.

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- A Z-factor between 0.5 and 1.0 is an excellent assay.
- A Z-factor between 0 and 0.5 is marginal.
- A Z-factor less than 0 means that the signal from the positive and negative controls could overlap, making the assay not very useful or screening purposes.

A Custom Classification Token has already been defined on this layout to classify the Z-Factor. To enable the classification token:

28. Right-click on **##ERROR##** next to "**The Assay Classification is**".
29. Choose **Format Token** from the pop-up menu. The **Edit Statistic Classifier** window appears.
30. Choose the **Classification** category.
31. Click on the symbol in the **Enter a value to classify** field.
32. Double-click on **Z Factor** under **Custom**.

The **Edit Statistic Classifier** dialog should look like Figure T18.39.

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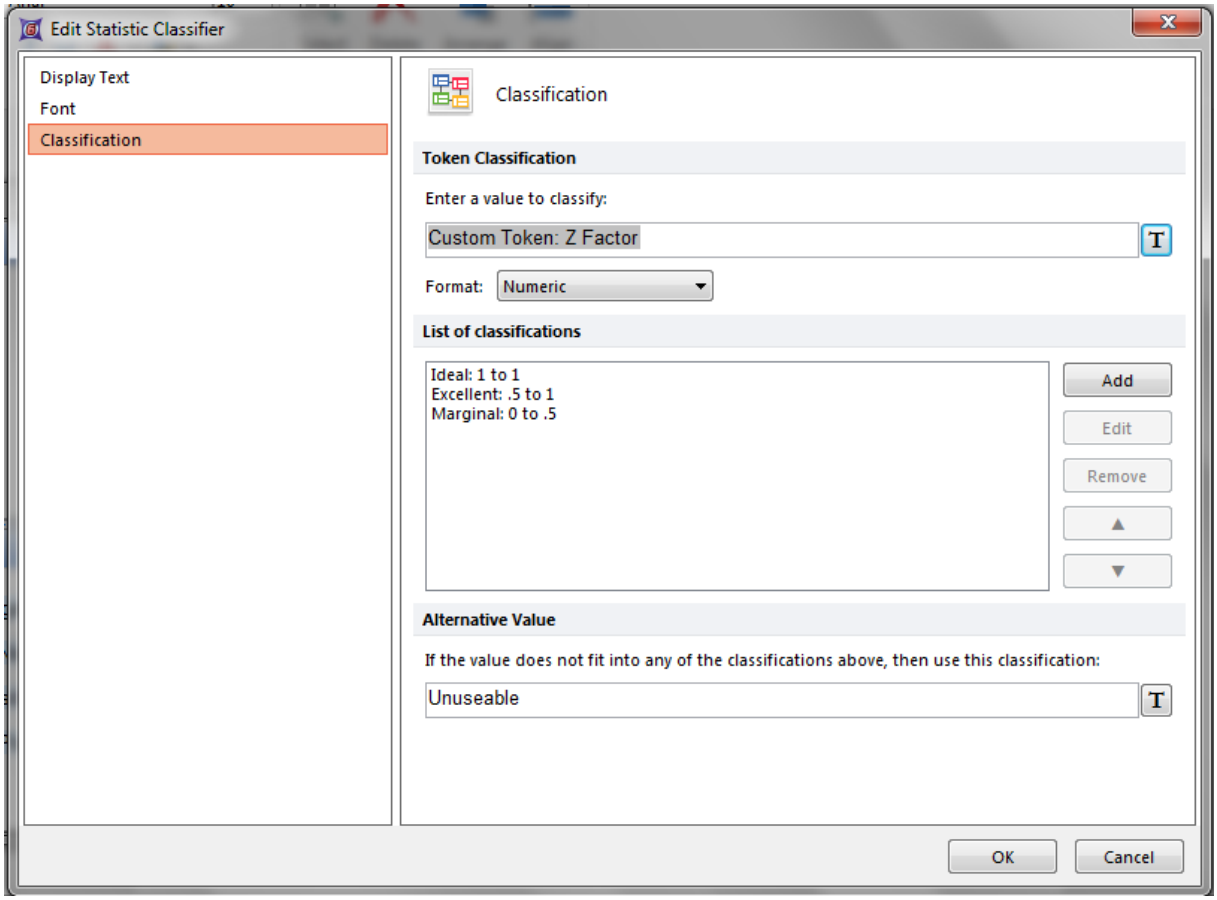

Figure T18.39 Changing the Classification Token to Z Factor

33. Click **OK**.

The layout will look like Figure T18.39b. The Assay Classification will read **Marginal** since the value is 0.00.

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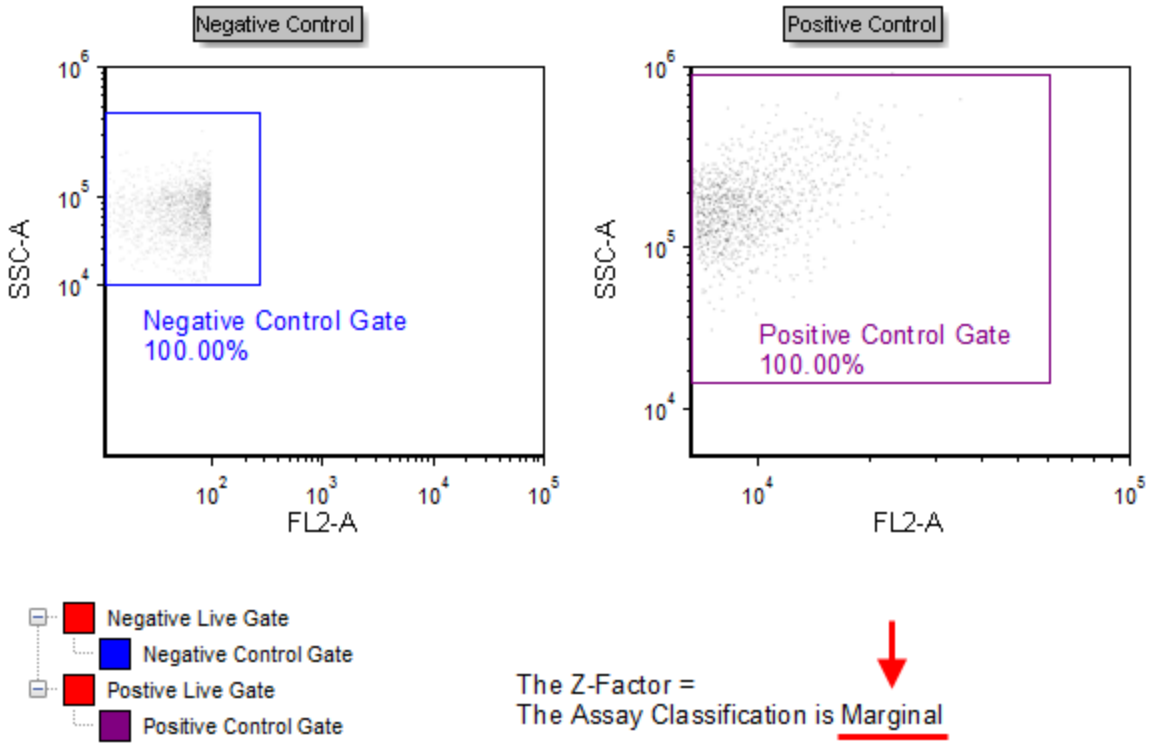

Figure T18.39b - The High Content Calculating Z-Prime Layout with the Classification Token

Adjusting the Positive Control gate to contain different portions of the sample will cause the Z Factor and Assay Classification to update in real time (Figure T18.40).

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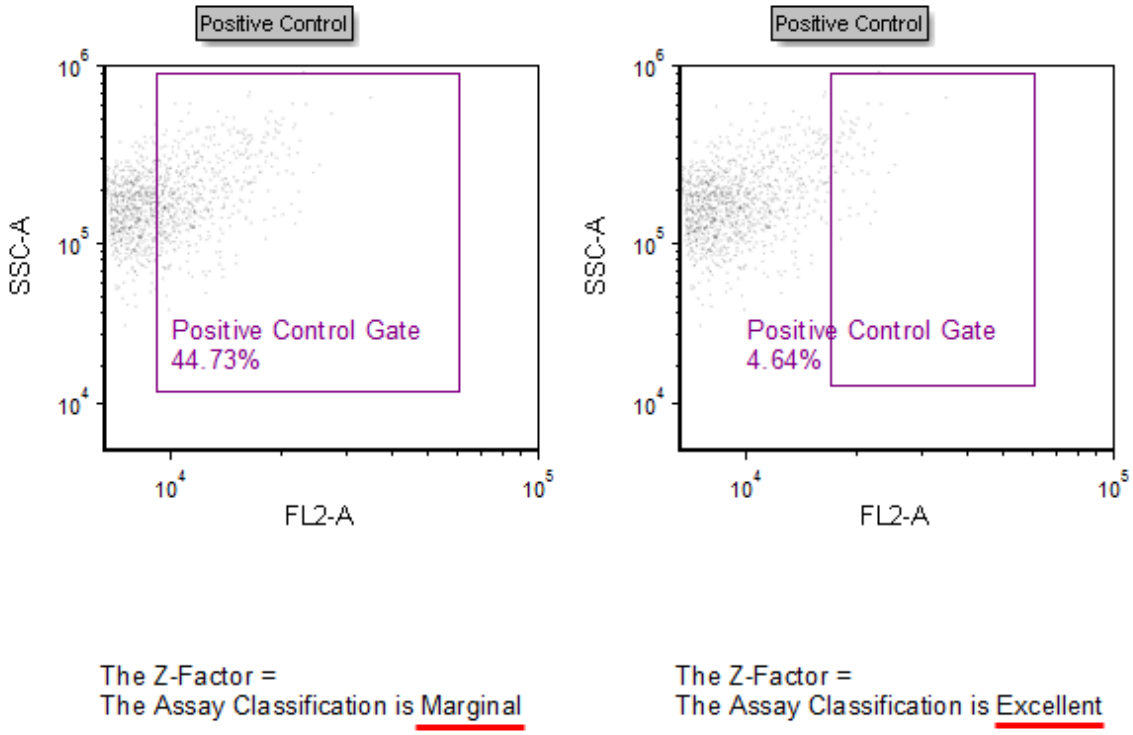

Figure T18.40 Gating on the Sample Data Causes the Z Factor and Classification to Update in Real Time

38.21 DNA Analysis

In this tutorial, you will learn how to use MultiCycle to analyze different types of cell cycle histograms. There are ten different histogram files named ASCII1.fcs through ASCII10.fcs, located in the *FCS Express Sample Data* folder, that show different DNA cell cycles to explore MultiCycle.

The true strength of MultiCycle is its ability to analyze cell cycle histograms with multiple models coupled with the batch processing ability of FCS Express.

FCS Express includes a basic version of MultiCycle. The basic version will fit a single cycle model to your data without background or aggregate/debris correction. A single cycle analysis means only one population of cells will be modeled. The advanced version can fit up to two aneuploid populations (considered a 3-cycle model) as well as apply sophisticated background and aggregate/debris correction. The advanced version must be purchased separately.

In the course of this example, we will:

- Use gating to exclude doublets.

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- Use Autofit in MultiCycle to analyze a DNA histogram.
- Format MultiCycle histograms.
- Use MultiCycle without Autofit to analyze a DNA histogram.

In the first exercise, we will use autofit selection for a single cycle analysis.

38.21.1 Autofit Selection (1 Cycle Analysis)

Setting gates to exclude doublets:

In order to exclude doublets, we will examine a 2D dot plot of the DNA fluorescence height versus the DNA fluorescence area signal and draw a gate on the single cell events. Many times, users will also use the plot of the DNA fluorescence width signal versus the DNA fluorescence area signal. The Height signal corresponds to the peak intensity of fluorescence pulse from the DNA dye. The Area signal is the calculated area under the fluorescence pulse emitted by the DNA dye. The Width signal represents the length of time of the fluorescence pulse emitted by the DNA dye. The specific geometry of the laser beam and other instrument factors determine which parameters are best suited to discriminate single cell events from doublets. In our example, DNA fluorescence area signal is the FL2-A parameter, DNA fluorescence width is the FL2-W parameter, and DNA fluorescence height is the FL2-H parameter.

If a new blank layout is already open then proceed to step 2.

1. Select the **File** tab→**New**→**New**.
2. Select the **Insert** tab→**2D Plots**→**Dot** command.
3. Click anywhere inside the blank layout.

The **Select a Data File** dialog appears (Figure T19.1).

4. Navigate to the *Tutorial Sample Data* folder.
5. Make sure that **FCS files (*.*, *.fcs, *.lmd)** is chosen as the *Files of type*.
6. Select the file **BD.LMD.FCS** (Figure T19.1).
7. Click **Open**.

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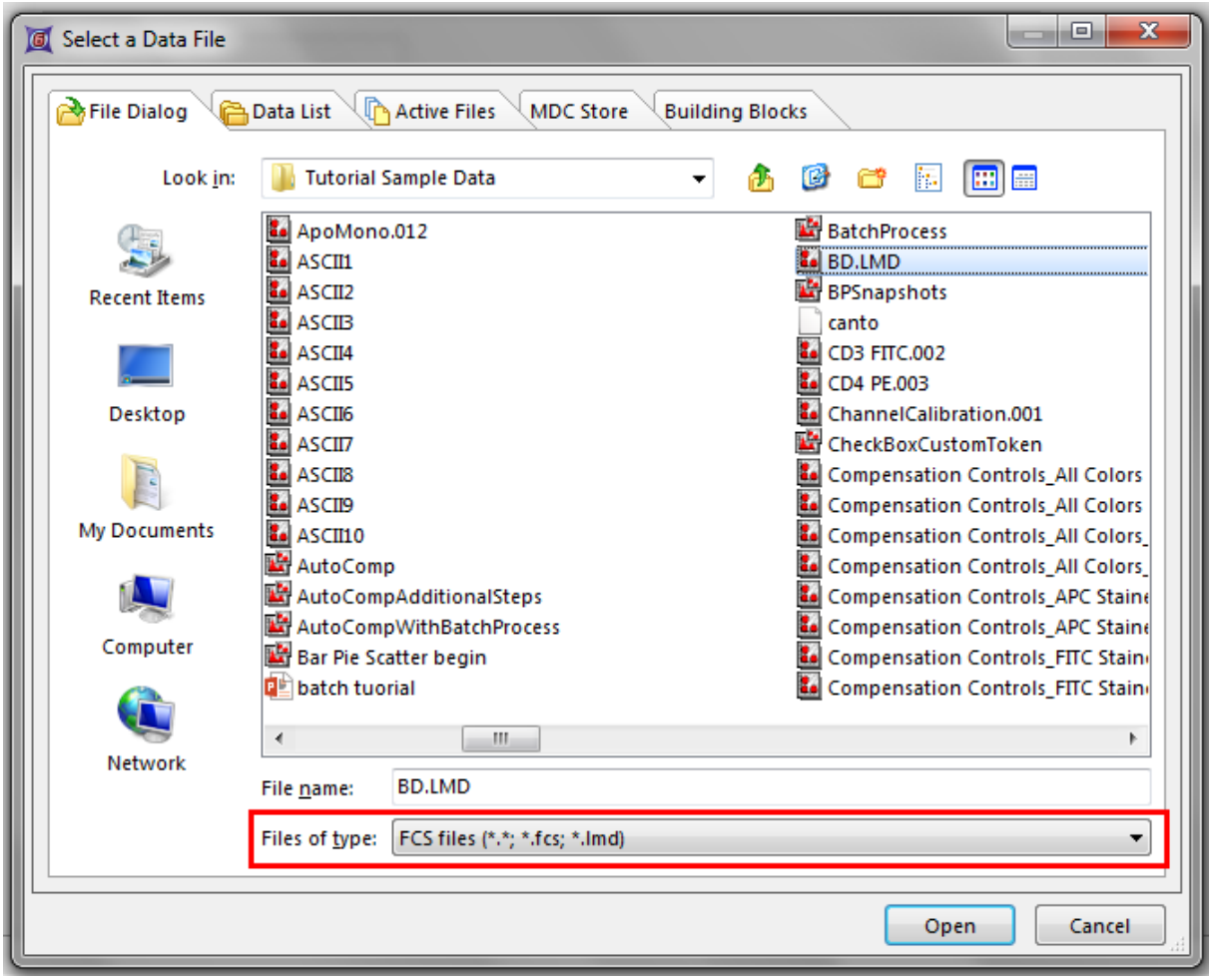

Figure T19.1 Select a Data File Dialog

A dot plot appears on the layout

7. Left-click on the x-axis and select **FL2-H** from the pop-up menu.
8. Left-click on the y-axis and select **FL2-A** from the pop-up parameter menu. The dot plot should appear similar to Figure T19.2.

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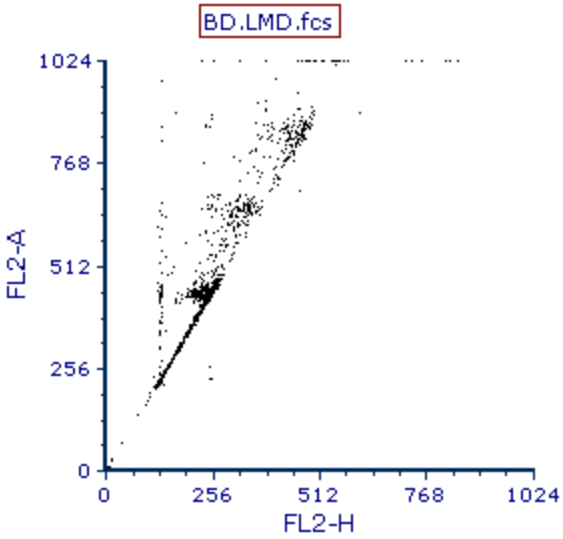

Figure T19.2 Dot Plot of FL2-H vs FL2-A

We will now create a gate on the 2D plot to exclude the doublets as well as to exclude what appear to be tetraploid amounts of DNA. We are excluding these cells with higher amounts of DNA because we want to perform a single cycle DNA analysis. Please refer to Figure T19.3 during the following steps. For more information on creating gates, please refer to the Gating tutorial.

9. Select the **Gating** tab →**Create Gates**→**Polygon** command.

10. Create a polygon gate similar to the gate shown in Figure T19.3.

The **Create a New Gate** dialog appears.

12. Name the gate 'singlets HA'.

The gate should look similar to Figure T19.3.

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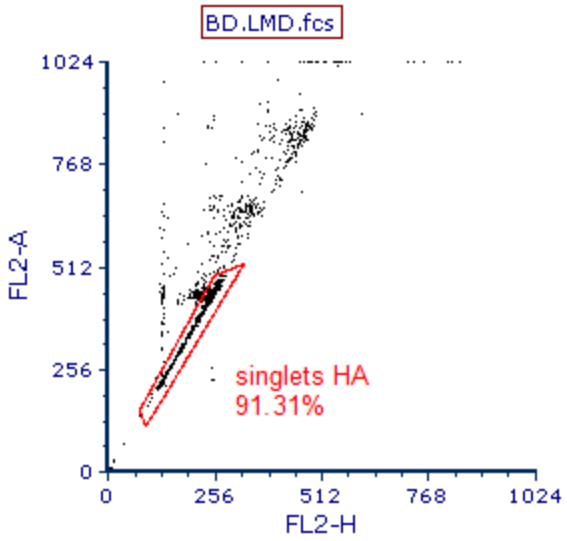

Figure T19.3 Gate to Exclude Doublets and Cells iwth High DNA Content

We are now ready to use the Autofit function in MultiCycle to perform a DNA cell cycle analysis.

Inserting a MultiCycle histogram:

13. Select the **Insert** tab→**1D Plots**→**MultiCycle DNA** command (Figure T19.4).

14. Click on a blank area of the layout.

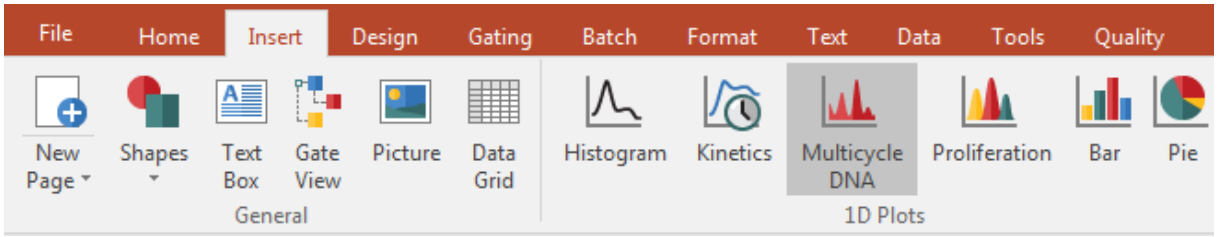

Figure T19.4 Selecting the Insert Muticycle DNA Command

The **Select DNA parameter** dialog appears, as shown in Figure T19.5. Please refer to Figure T19.5 for the following steps.

18. Select **FL2-A** as the Parameter, shown highlighted in blue.

19. Click **OK**.

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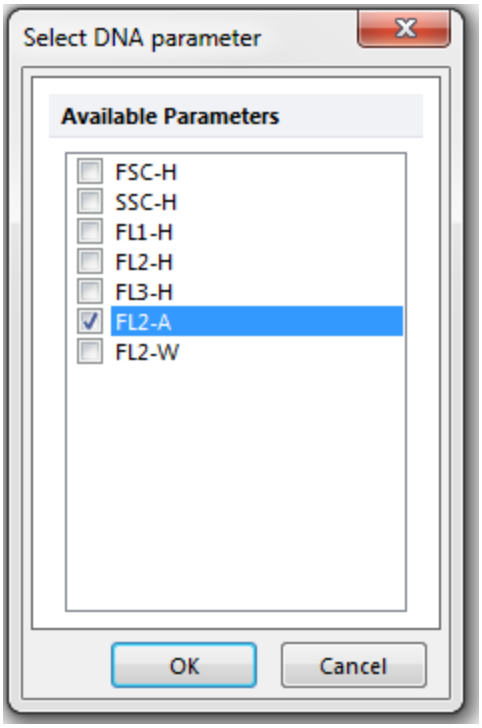

Figure T19.5 Select DNA Parameter Dialog

The histogram of FL2-A, gated on **singlets HA**, is analyzed by MultiCycle using the Autofit function and appears on the layout (Figure T19.6). Note that your plot may differ from the figure depending on your singlets gate shape.

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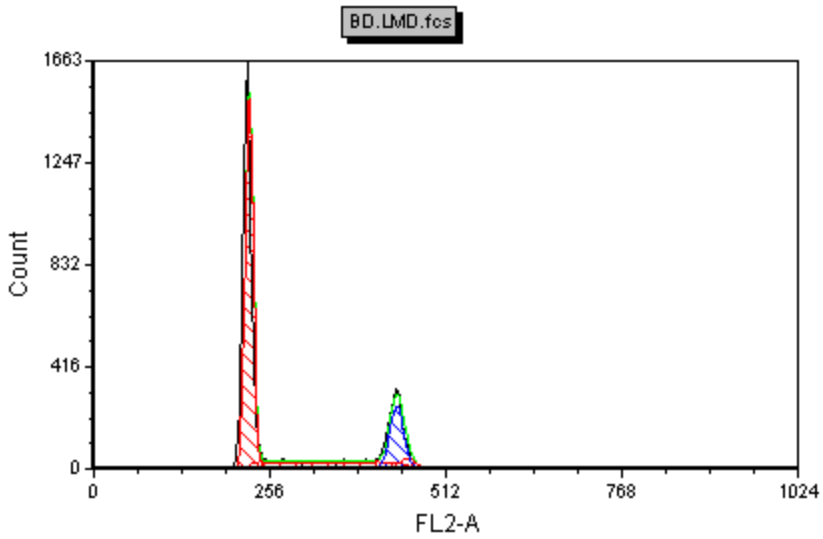

Figure T19.6 MultiCycle Analysis of FL2-A Using Singlets HA Gate

We will now zoom into the region of interest on the Multicycle plot to improve the fit.

20. Right-click on the Multicycle plot and select **Magnify (Resolution)** from the pop-up menu.

21. Drag the cursor, which is now a crosshair, around the region of interest as shown (Fig. T 19.7, *left*).

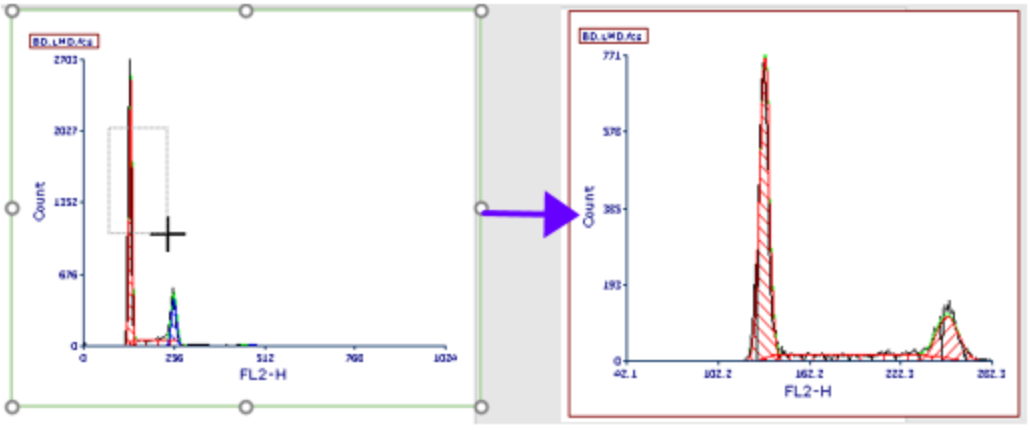

Figure T19.7 Magnifying resolution on a MultiCycle plot to improve the fit of the model

22. Release the cursor. The Multicycle plot's fit has updated, and only one cycling population is now detected, depicted in red (Fig. T 19.7, r*right*).

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We will now display the legend information associated with the MultiCycle DNA analysis.

21. Right-click on the histogram to bring up the associated pop-up menu.
22. Select **Format** from the pop-up menu.
23. Select **Legend** from the categories section of the **Formatting** dialog (Figure T19.8).
24. Click on the **Visible** checkbox.
25. Click **OK** (Figure T19.8).

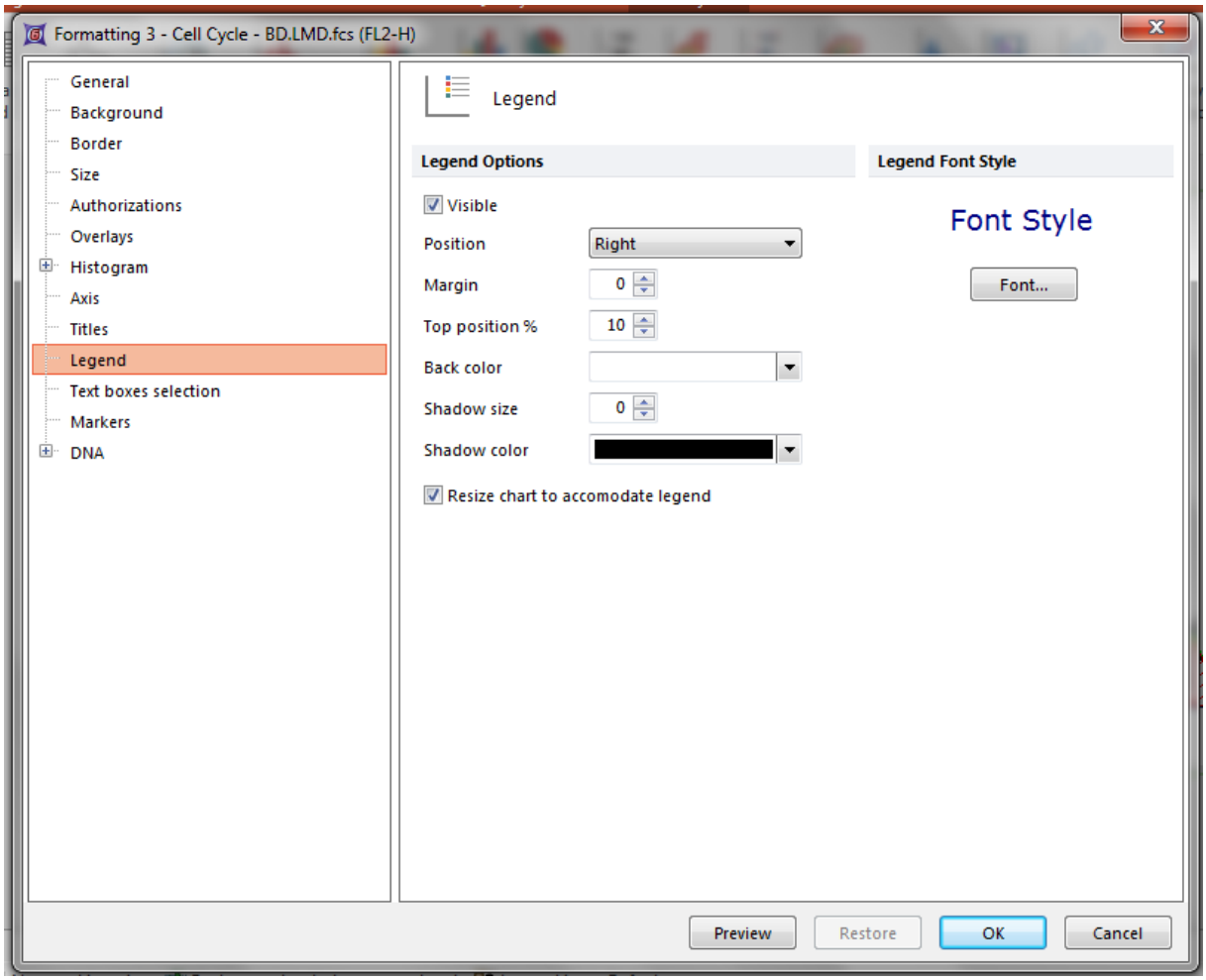

Figure T19.8 Formatting the DNA histogram to Display the Legend Associated with MultiCycle Analysis

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Alternatively, the histogram legend information can be displayed using the **Format Legend** command, as follows:

- Left-click to select the histogram; it should be outlined in green.

Select the **Format→Plot Options→Legend** command (Figure T19.9).

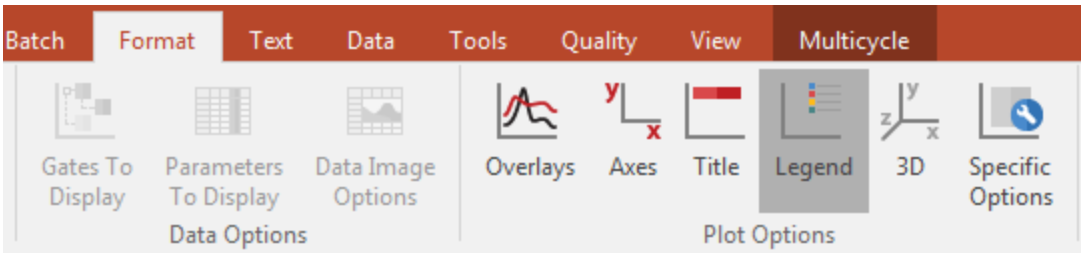

Figure T19.9 Selecting the Format Legend Command

The **Formatting Legend** dialog appears (Figure T19.10).

- Click on the **Visible** checkbox in the **Formatting Legend** dialog.
- Click **OK**.

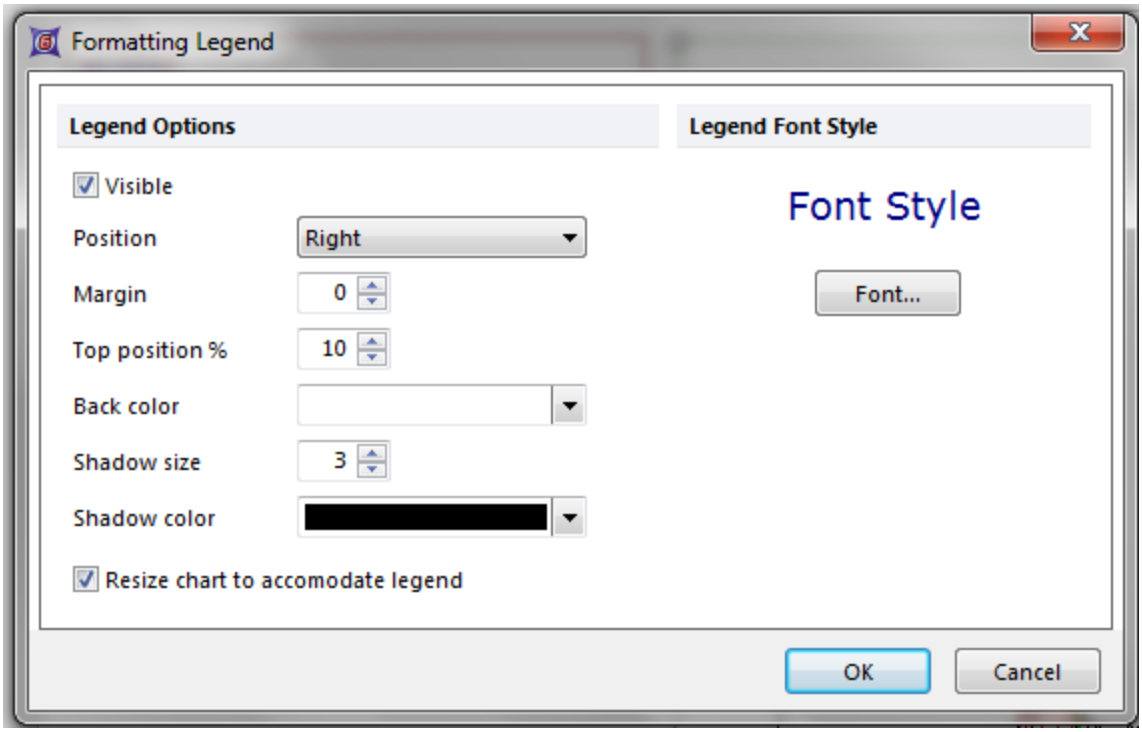

Figure T19.10 Formatting Legend Dialog

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The legend associated with the MultiCycle DNA analysis will appear to the right of the histogram (Figure T19.11).

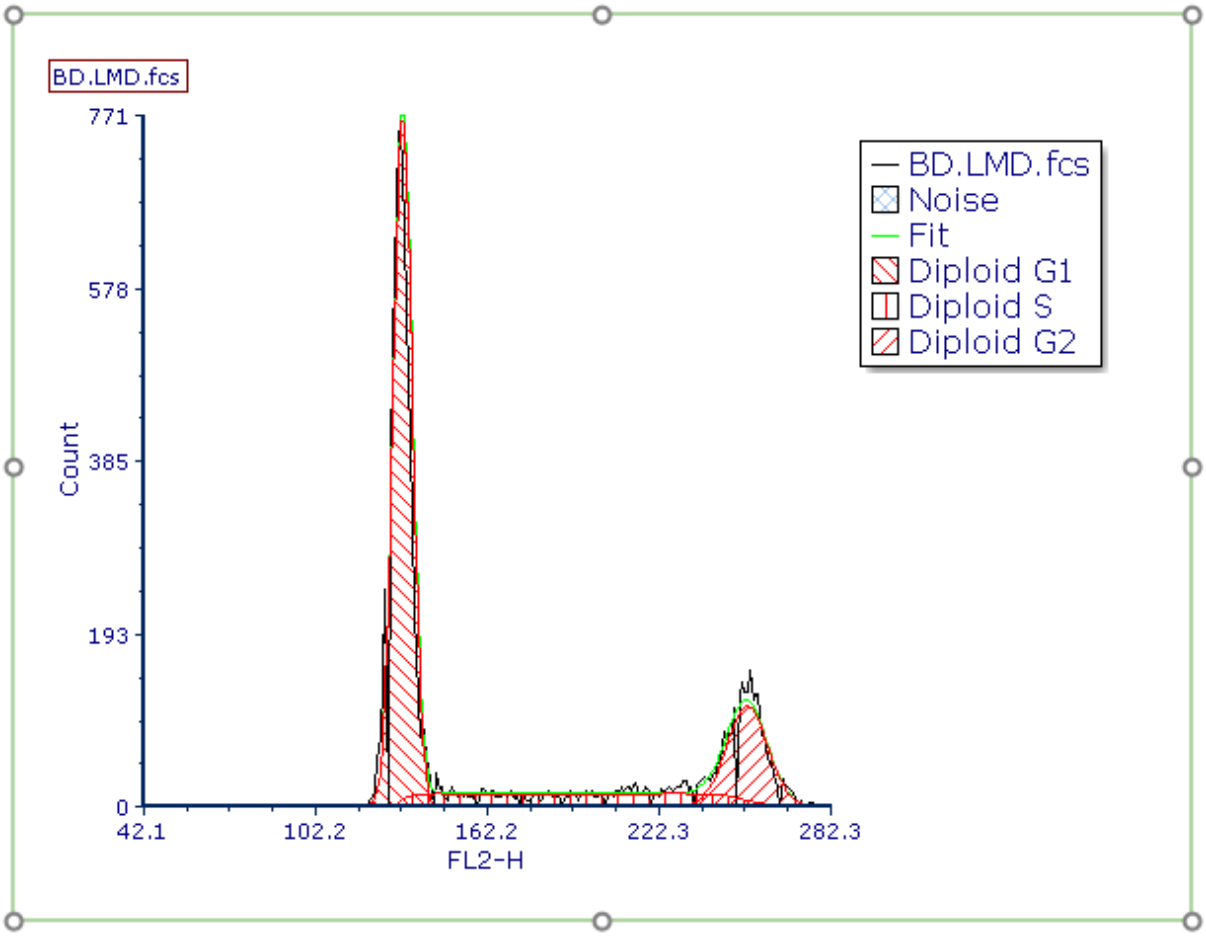

Figure T19.11 MultiCycle DNA Histogram Cell Cycle Analysis with Associated Legend

As you can see from the legend, this histogram contains only diploid cells and therefore a single cycle analysis was performed by the Autofit function of MultiCycle. We can confirm this by examining the MultiCycle information on the ribbon.

26. Left-click to select the histogram, it should be outlined in green (Figure T19.11).

27. Select the **MultiCycle** tab (Figure T19.12).

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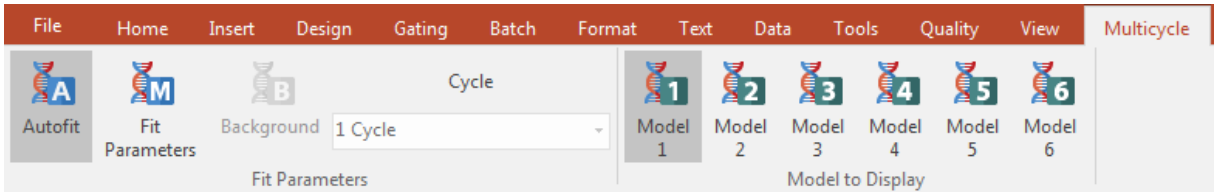

Figure T19.12 The Multicycle Tab

Notice that **Autofit** and **Model 1** are highlighted in grey (Figure T19.12). This indicates that the MultiCycle Autofit function and that Model 1 are being used to analyze this DNA histogram. Also notice that 1 Cycle, indicated by the cursor in Figure T19.11, was used in the analysis.

Note: For an explanation of the differences between all the models, please see the De Novo Software DNA/Multicycle AV knowledgebase page.

We will now view all the different statistics for the DNA analysis of this histogram.

28. Right-click on the DNA histogram to bring up the associated pop-up menu.

29. Select **Statistics→DNA Cycle Statistics** from the pop-up menu (Figure T19.13).

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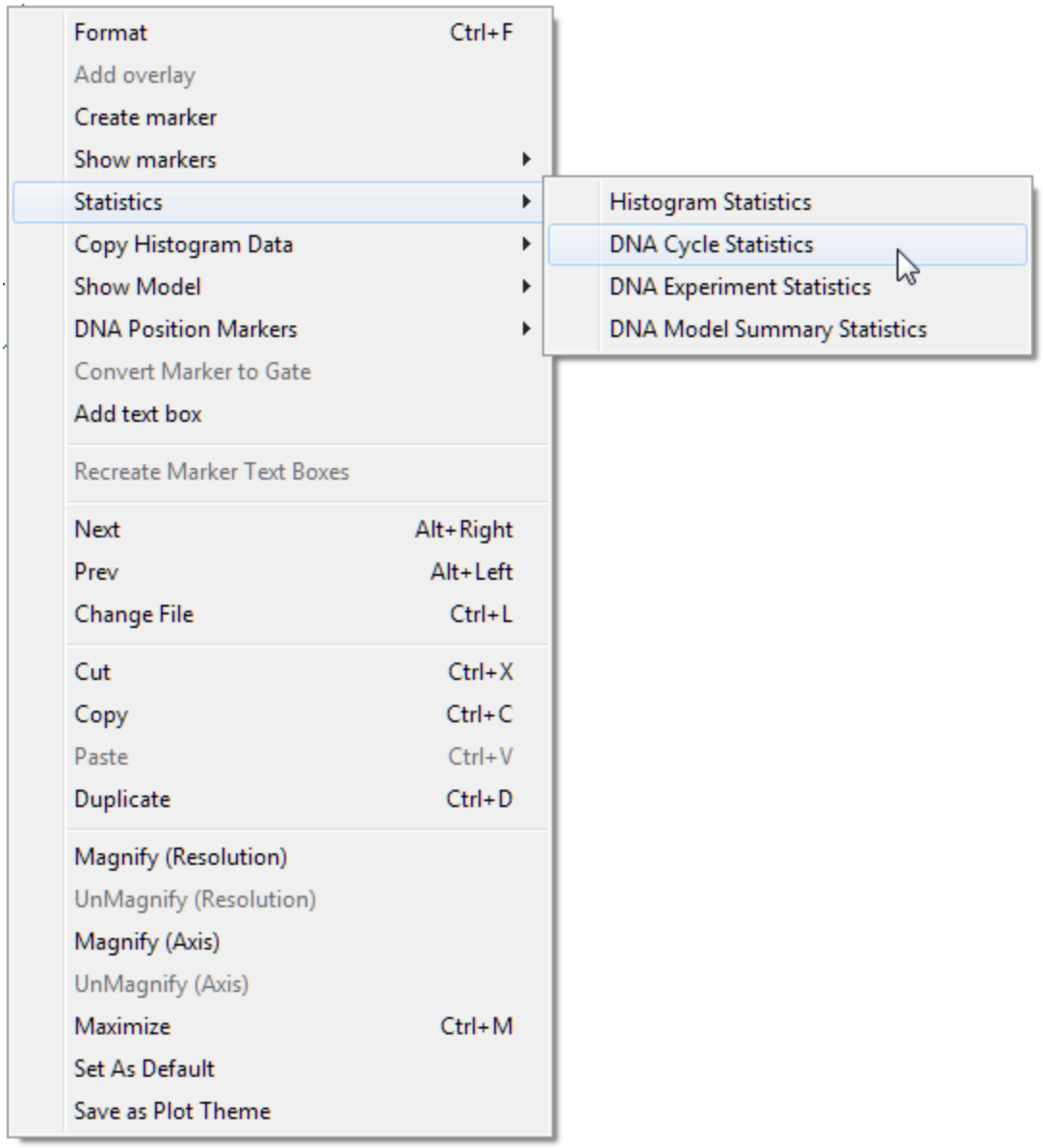

Figure T19.13 DNA Cycle Statistics Pop-up Menu Item

The **DNA Cycle Statistics** window of the current model (**Model 1**) will now appear (Figure T19.14). Note that you will have slightly different values for the statistics depending on how your singlet gate was drawn.

30. Move the **DNA Cycle Statistics** window beneath the plot (Figure T19.14).

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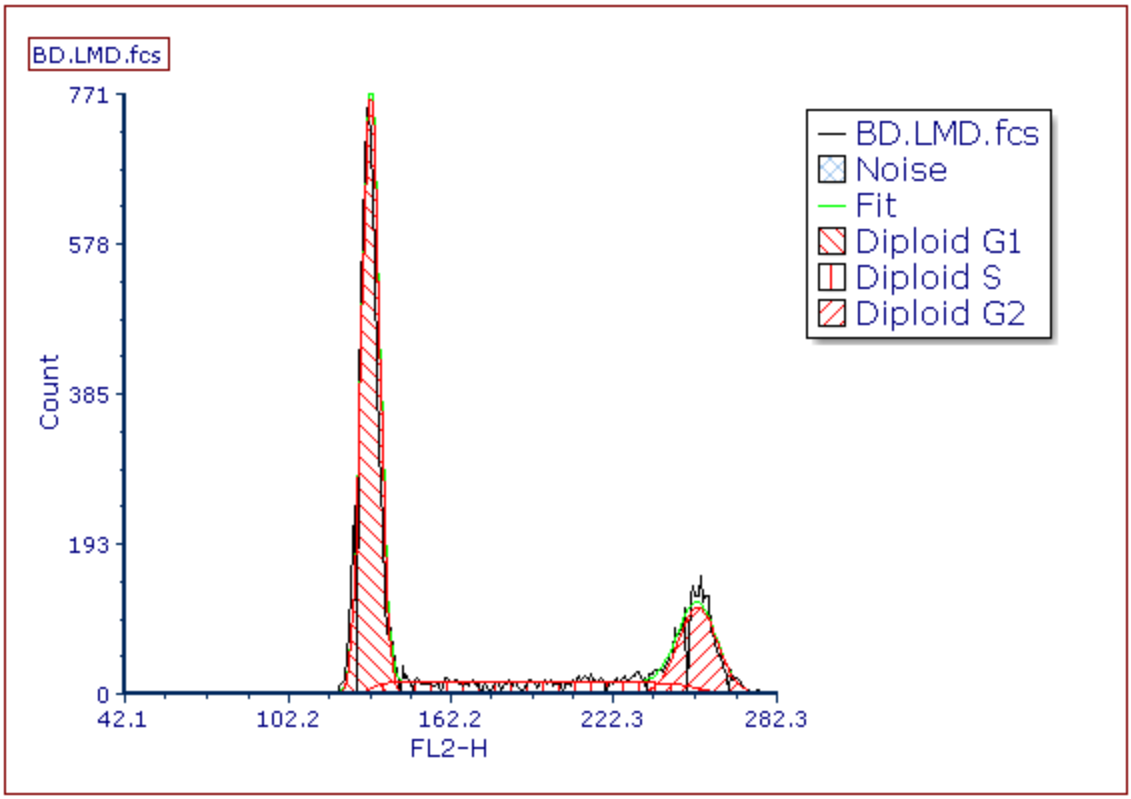

Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	133.20	2.44	62.85	254.16	2.83	19.96	17.19	1.91	100.00	0.26

Figure T19.14 DNA Cell Cycle Statistics from the MultiCycle Model 1 Analysis of the Histogram

31. Right-click on the DNA Histogram to bring up the associated pop-up menu.

32. Select **Statistics→DNA Model Summary Statistics** from the pop-up menu, previously shown in Figure T19.13.

The **DNA Model Summary Statistics** window, showing all of the six defined MultiCycle models applied to the current histogram, appears. The six models shown in the **DNA Model Summary Statistics** window correlate respectively with the six models shown in the **MultiCycle→Model to Display** group (Figure T19.12). The **DNA Model Summary Statistics** displays the default statistics set in FCS Express preferences which we will change later in the tutorial.

33. Move the **DNA Model Summary Statistics** window under the **DNA Cell Cycle Statistics** window (Figure T19.15).

34. Right-click on the DNA Histogram to bring up the associated pop-up menu.

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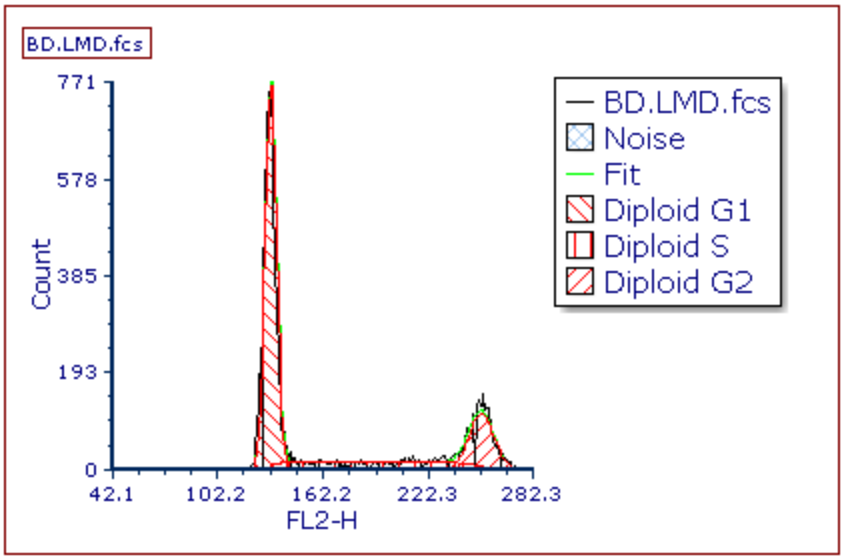
35. Select **Statistics→DNA Experiment Statistics** from the pop-up menu, previously shown in Figure T19.13.

The **DNA Experiment Statistics** window with the interpretation of the DNA model and the experiment statistics will appear.

36. Move the **DNA Experiment Statistics** window next to the **DNA Model Summary Statistics** window (Figure T19.15).



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	133.20	2.44	62.85	254.16	2.83	19.96	17.19	1.91	100.00	0.26

Model	Dip %G2	Dip %S	Chis
SL S0	19.96	17.19	4.67
SL CL S0	19.42	17.40	4.67
+G2/G1 Fixed	7.64	23.10	9.82
+Aggregates	10.26	22.24	10.45
+S Order = 1	19.40	17.61	4.63
+CVs Fixed	18.69	18.14	4.65

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=17.2, %G2=20.
The S Phase confidence is good

Experiment Statistics

Chi sq: 4.67
BAD: 0.26
Number of cells: 9955.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Figure T19.15 DNA Histogram with All of the MultiCycle Statistics Windows

In the next section, we will perform an expanded analysis with Autofit 1809.

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38.21.2 Expanded Analysis with Autofit

We will now adjust the 'singlet HA' gate to include the cells with higher DNA content. Autofit in the basic version of MultiCycle will continue to perform a one cycle analysis. However, Autofit in the advanced version of MultiCycle, will mathematically decide whether to apply a one, two, or three cycle fit to the histogram data.

Please refer to Figure T19.16 for the final location of the gate.

1. Activate the 'singlet HA' gate for editing by clicking inside the gate.
2. Move the cursor to the top upper right resizing handle of the gate; you will know you are in the correct place when the cursor changes to a two-headed arrow.
3. Press and hold down the left mouse button while dragging the corner of the gate upwards.
4. Release the button when the corner of the gate is in the desired location (Figure T19.16).
5. Repeat steps 2 through 4 for the top upper left corner of the gate.

The gate should now appear similar to Figure T19.16, modified to include cells with higher content DNA, but still excluding most of the doublets.

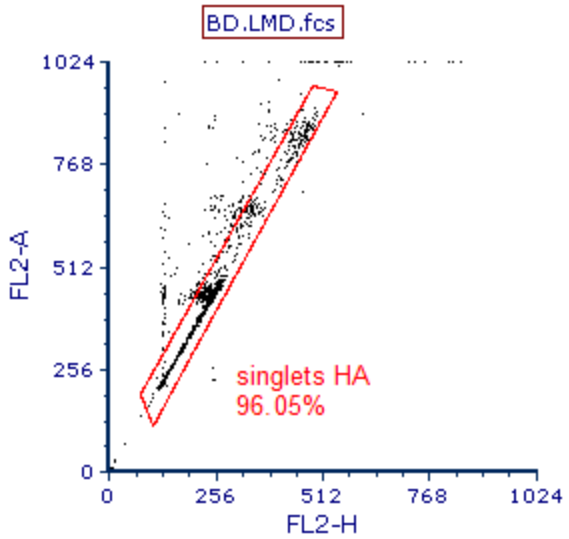

Figure T19.16 Gate Modified to Include Cells with Higher DNA Content

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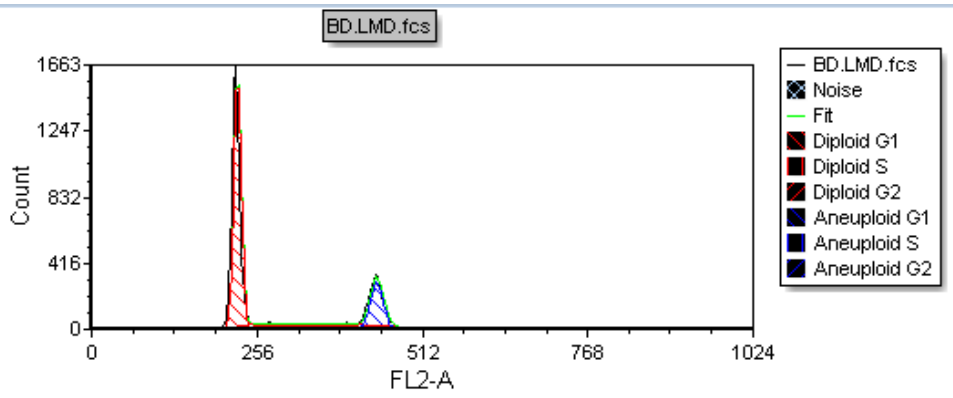
The Autofit function of MultiCycle updates the cell cycle analysis, as shown in Figure T19.17. *Note:* Figure T19.17 shows the MultiCycle analysis using the advanced version of Multicycle. The basic version of MultiCycle will continue to apply a one cycle model to the histogram.

For the rest of this tutorial we will use the advanced version of MultiCycle for the DNA analysis. However, all the formatting features of the DNA histogram and related statistics are also available in, and operate in the same way, in the basic version of MultiCycle.

Note that the DNA analysis now shows an aneuploid population, shown in blue in Figure T19.17. In fact, the MultiCycle Interpretation suggests that this is most likely a tetraploid population. Also of note is that a 2-cycle model was used. This information is shown in the Experiment Statistics window and can also be found by selecting the DNA histogram and then selecting the **Multicycle** tab.



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	226.96	2.56	75.89	460.87	2.56	2.29	21.81	2.03	77.97	n/a	0.06
Aneuploid A	443.13	2.23	84.32	852.74	2.23	6.09	9.60	1.92	22.03	1.95	0.04

Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	2.29	21.81	6.09	9.60	1.75
SL CL S0	2.26	21.85	6.25	8.08	1.71
+G2/G1 Fixed	9.63	19.27	4.24	16.29	2.22
+Aggregates	0.00	20.36	5.15	7.74	1.89
+S Order = 1	2.14	21.67	6.02	9.34	1.74
+CVs Fixed	0.01	22.32	5.83	8.64	1.80

Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=9.6, %G2=6.09
The S Phase confidence is fair
Note: % cells, inter-model error,

Experiment Statistics

Chi sq: 1.75
Average %S: 19.12
Average %S aneuploid: 9.60
BAD: 0.06
Number of cells: 10100.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Figure T19.17 Autofit MultiCycle Analysis with Modified gate

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We will now select the specific statistics we want to display in the **DNA Model Summary Statistics** table. There multiple ways of selecting the statistics shown in the table and these same methods can be used to select which statistics are shown for any of the DNA-associated statistics shown in the pop-up menu on Figure T19.13 1806. For more information on formatting, please see the tutorial on Formatting Plots 1236. First, we will close the **DNA Experiment Statistics** window so that we have room to expand the **DNA Model Summary Statistics** window.

6. Hover the mouse over the border of the **DNA Experiment Statistics** window.
7. Left-click when the cursor changes to a four-headed arrow; you will know it is selected for editing because it will have a red border.
8. Press **Del** on the keyboard or select delete from **Home→Editing**.
9. Left-click inside the **DNA Model Summary Statistics** window to select it.
10. Select the **Format→Statistic Options→Statistics** command.

The **Formatting Stats Options** dialog appears (Figure T19.18).

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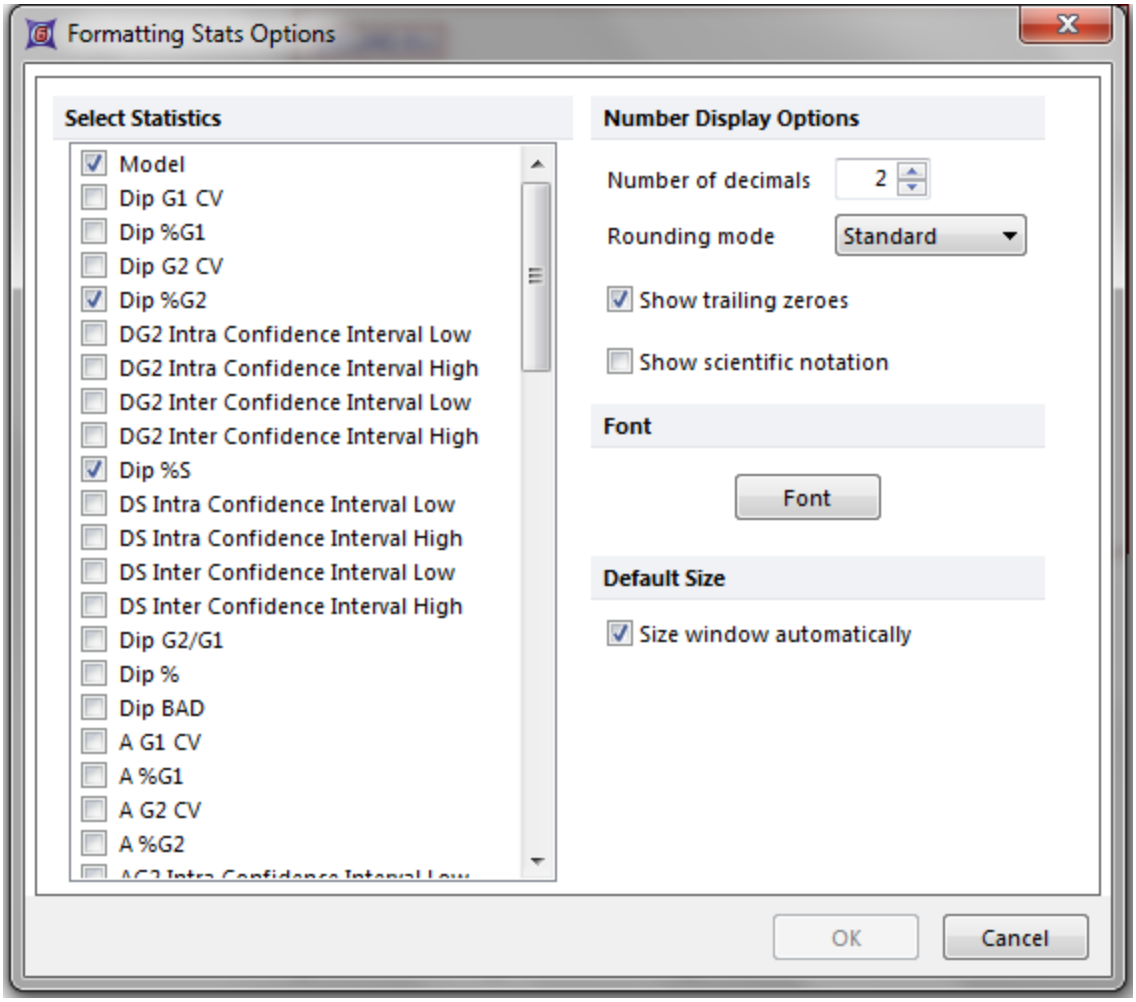

Figure T19.18 Formatting Stats Options Dialog for DNA Model Summary Statistics

11. Select the down arrow, shown by the cursor on Figure T19.18.
12. Continue to hold down the left mouse button to scroll down to the bottom of the list.
13. Click on the box next to **Clump** and **Debris** as shown in Figure T19.19.
14. Click **OK**.

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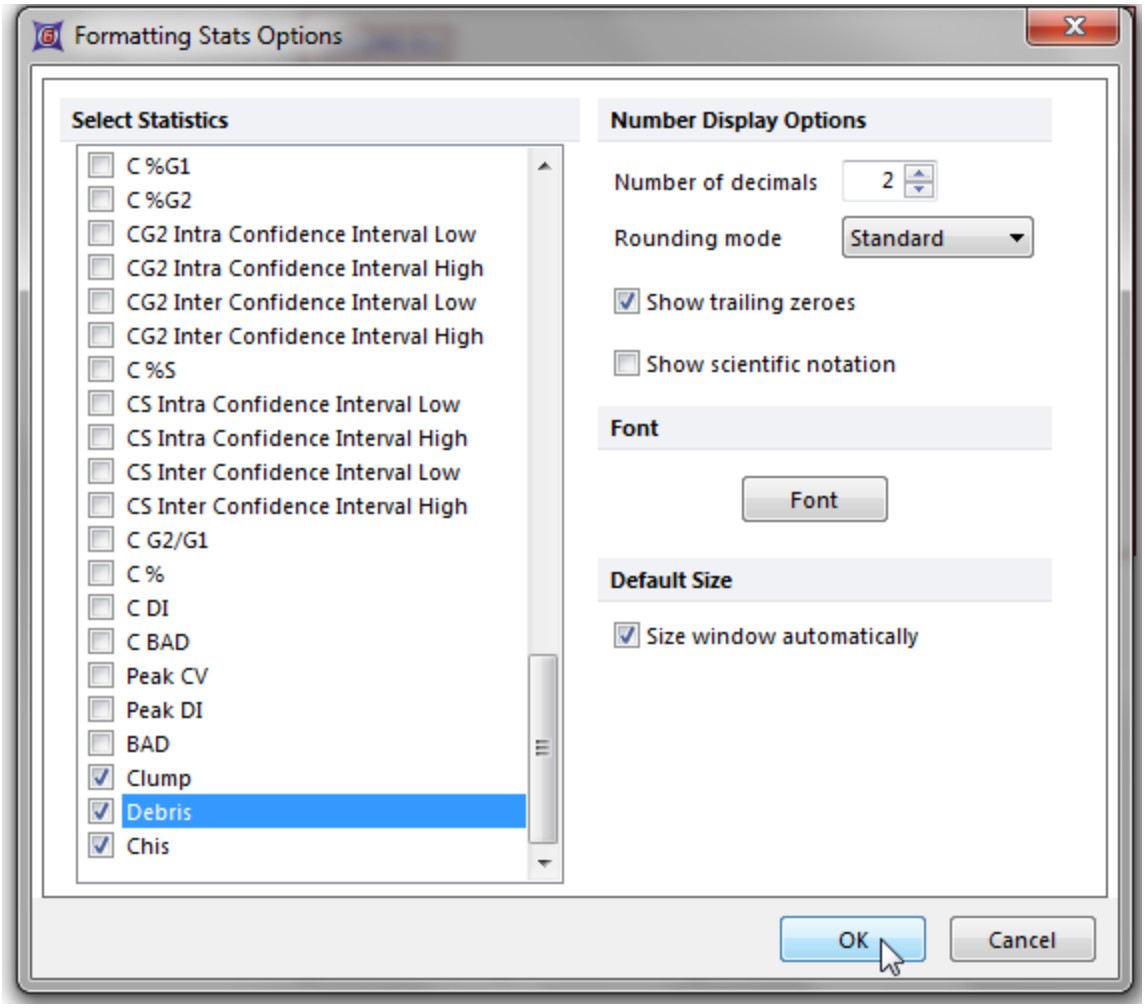

Figure T19.19 Selecting Clump and Debris from the Formatting Stats Options Dialog

The **DNA Model Summary Statistics** table will now update to show the selected statistics as shown in Figure T19.20.

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Model	Dip %G2	Dip %S	A %G2	A %S	Clump	Debris	Chis
SL S0	2.29	21.81	6.09	9.60	-1.00	0.07	1.75
SL CL S0	2.26	21.85	6.25	8.08	0.72	0.08	1.71
+G2/G1 Fixed	9.63	19.27	4.24	16.29	-1.00	0.07	2.22
+Aggregates	0.00	20.36	5.15	7.74	0.54	0.08	1.89
+S Order = 1	2.14	21.67	6.02	9.34	-1.00	0.08	1.74
+CVs Fixed	0.01	22.32	5.83	8.64	-1.00	0.10	1.80

Figure T19.20 DNA Model Summary Statistics Table Updated to Show to Clump and Debris

Alternatively, you can use the **DNA Model Summary Statistics** associated pop-up menu to select, or De-select, the specific statistics to display.

- Right-click on the **DNA Model Summary Statistics** window to bring the associated pop-up menu.

The **DNA Model Summary Statistics** pop-up menu appears (Figure T19.21).

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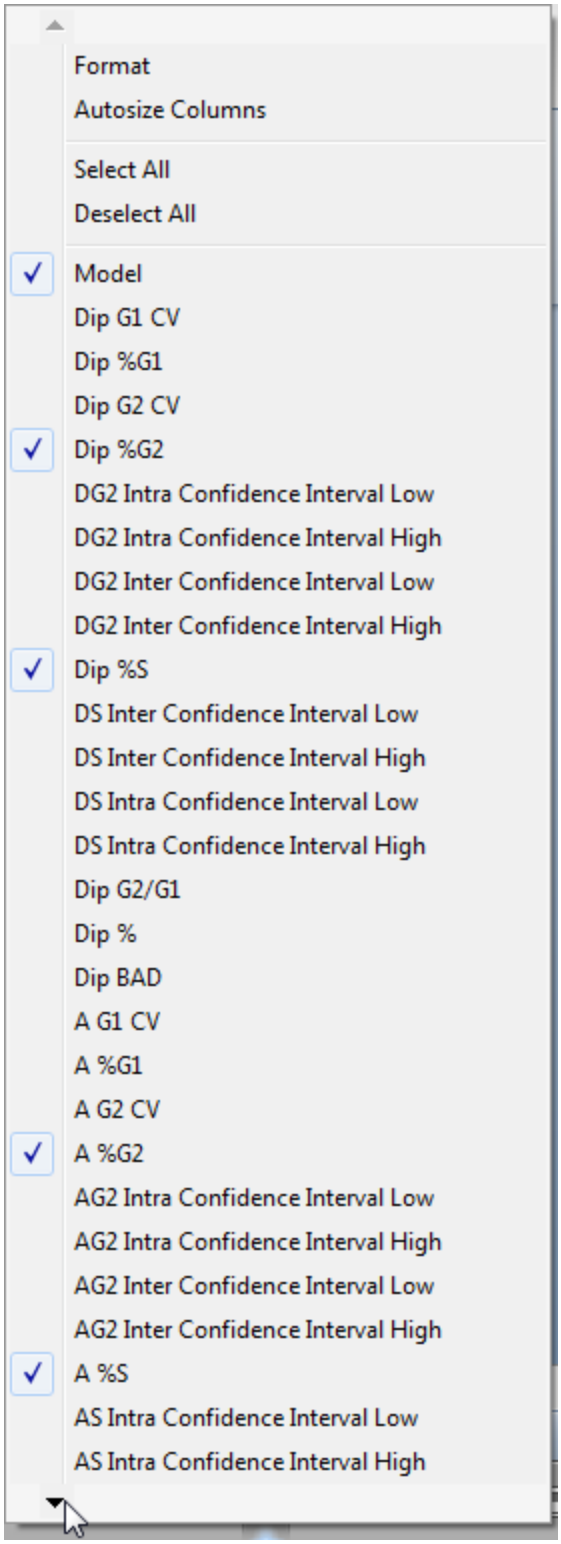

Figure T19.21 DNA Model Summary Statistics Pop-up Menu

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- Select the arrow in the lower left corner of the menu to scroll down, indicated by the cursor in Figure T19.21.
- Continue to hold down the left mouse button until the menu scrolls all the way down (Figure T19.22).
- Left-click next to **Clump**, as indicated by the cursor on Figure T19.22, to de-select the item.

The pop-up menu will now close and the **DNA Model Summary Statistics** table will update to reflect the removal of the item **Clump**.

- Repeat the above steps to de-select **Debris**, located under **Clump** in Figure T19.22.

The pop-up menu will now close and the **DNA Model Summary Statistics** table will update to reflect the removal of the item **Debris**.

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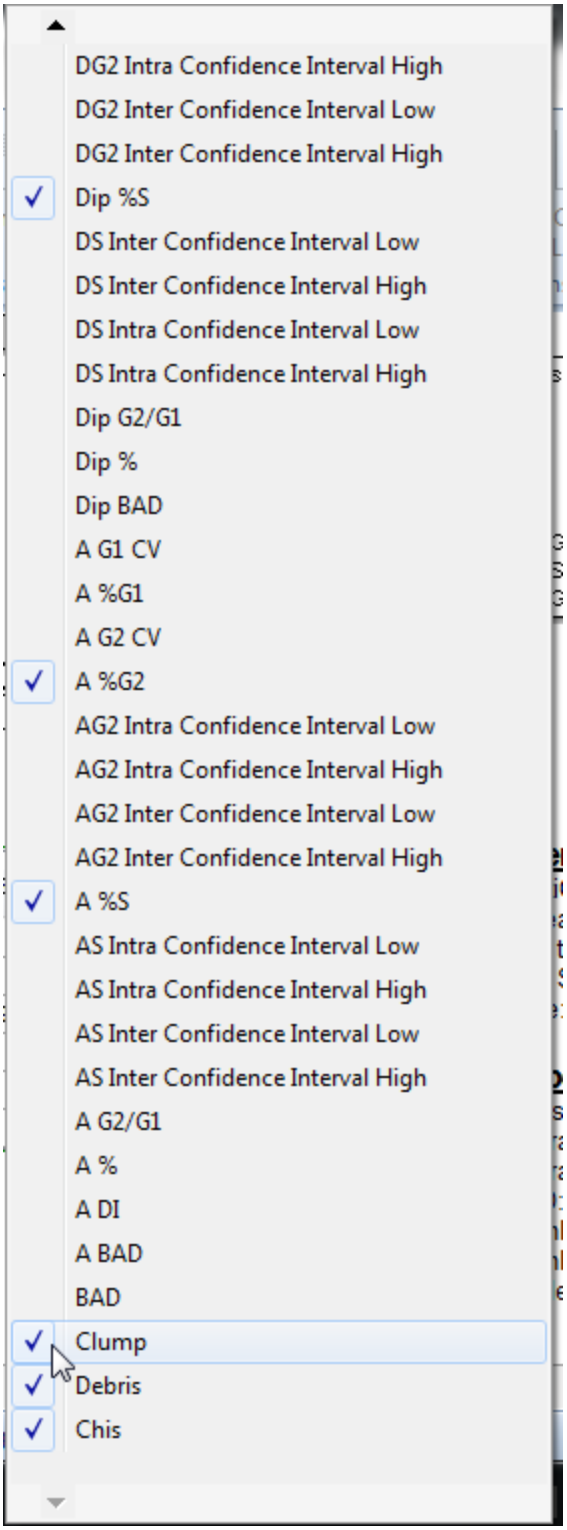

Figure T19.22 Deselecting Clump from the DNA Model Summary Statistics Pop-up Menu

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You can select different DNA cell cycle models in the **Multicycle→Model to Display** group, and the DNA cell cycle statistics window will update to reflect the model selected. Try selecting different models from the **Multicycle** tab using **Autofi**t and observe how the results change in the DNA cell cycle statistics window. When using the full version of MultiCycle, the histogram display will also update to reflect the fit to the model selected. The different models vary by the complexity of the model (applying constraints, or not, on the CV or G2/G1 mean ratio) and the inclusion of software aggregate compensation. For more information on the different models refer to the MultiCycle documentation.

A quick visual examination of the DNA Model is often useful. However, the model with the lowest **Chis** (Chi-square) value is usually the best fit, shown circled in red in Figure T19.23. In this case **Model 2 (SL CL S0)** has the lowest **Chis;** however, two other models have very similar low **Chis** values.

Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	2.29	21.81	6.09	9.60	1.75
SL CL S0	2.26	21.85	6.25	8.08	1.71
+G2/G1 Fixed	9.63	19.27	4.24	16.29	2.22
+Aggregates	0.00	20.36	5.15	7.74	1.89
+S Order = 1	2.14	21.67	6.02	9.34	1.74
+CVs Fixed	0.01	22.32	5.83	8.64	1.80

Figure T19.23 DNA Cell Cycle Model with the Lowest Chis (Chi-square) Value

In the next section, we will format a DNA histogram 1818.

38.21.3 Formatting a DNA Histogram

There are many formatting features in FCS Express that aid the user in the visual assessment of a MultiCycle analysis of a DNA histogram. The different cell cycle populations can be shaded in different ways and markers can be displayed indicating the location of the mean of a specific population. In addition, the user can zoom in on a specific area on the histogram for a more detailed view of the analysis.

We will begin by changing the pattern of the diploid population on the MultiCycle histogram to a solid color.

1. Click inside the MultiCycle histogram to select it.

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2. Select the **Format→Plot Options→Overlays** command.

The **Formatting Overlays** dialog window appears, shown in Figure T19.24. Please refer to Figure T19.24 for the following steps.

3. Select **Diploid G1** from the category on the left, shown highlighted in blue.
4. Select the solid fill pattern from the Fill Style drop down menu, indicated by the cursor.
5. Click **OK**.

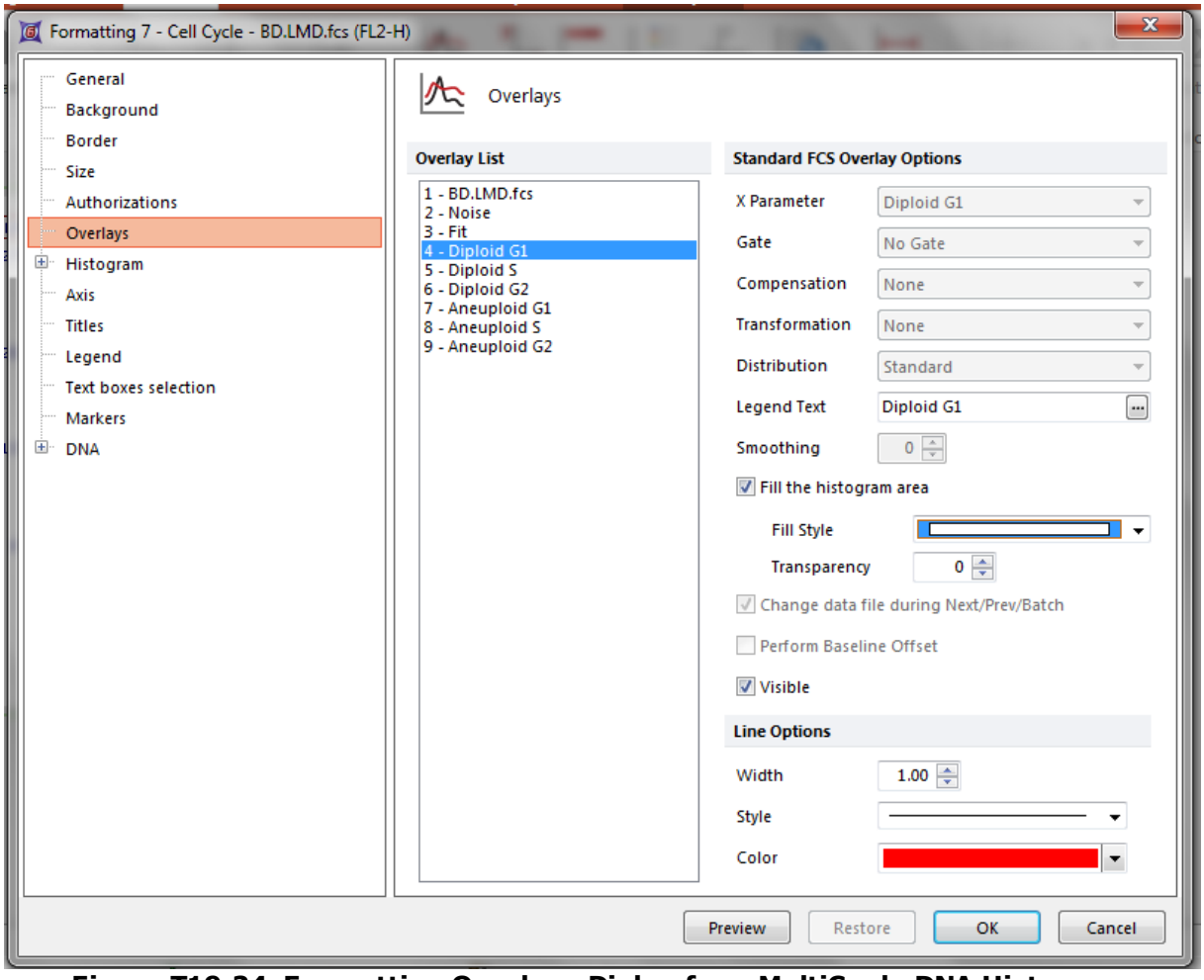

Figure T19.24 Formatting Overlays Dialog for a MultiCycle DNA Histogram

The MultiCycle histogram will now update to reflect the changes as shown in Figure T19.25. Alternatively, we could have accessed the histogram Overlay

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menu by right-clicking on the DNA histogram to bring up the pop-up menu and selecting **Format** and then **Overlays**.

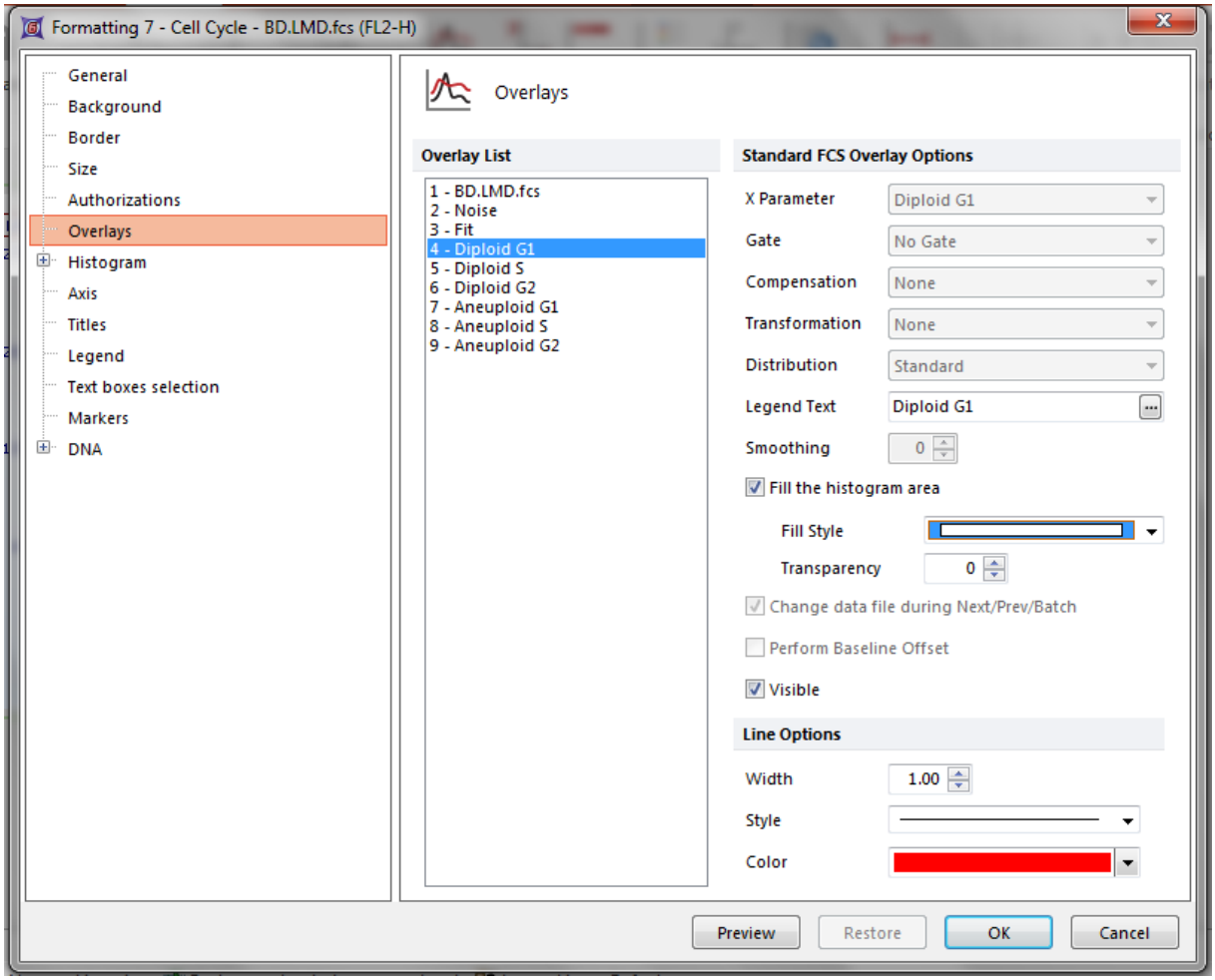

Figure T19.25 MultiCycle DNA Histogram Updated with Solid Fill on the Diploid Peak

We will now add a marker on the histogram to indicate the location of the aneuploid G2 peak.

6. Right-click inside the DNA histogram to bring up the pop-up menu.
7. Select **DNA Position Markers→A2 Mean** from the menu, indicated by the cursor in Figure T19.26.

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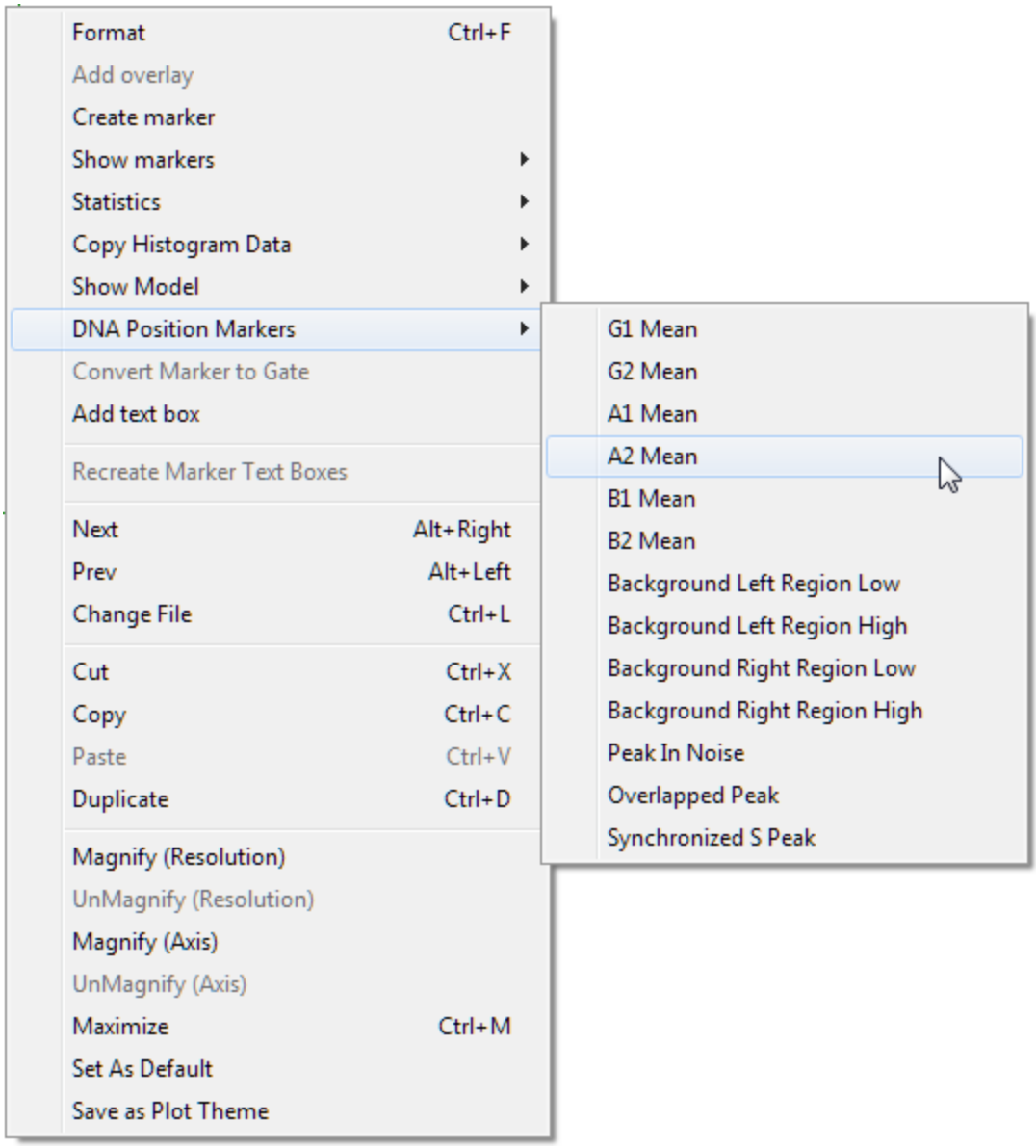

Figure T19.26 Selecting DNA Position Markers on the Pop-up Menu

Alternatively, the DNA Position Markers can be accessed from the **Format** item on the pop-up menu.

- Right-click inside the histogram to bring up the pop-up menu.
- Select **Format** from the menu.

The histogram formatting window appears, please refer to Figure T19.27 for the following steps.

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- Select **DNA→Position Markers** from the category on the left, shown outlined in blue.
- Select the box **Show position marker on the plot**, indicated by the cursor.
- Select **A2 Mean**, shown highlighted in blue.
- Click **OK**.

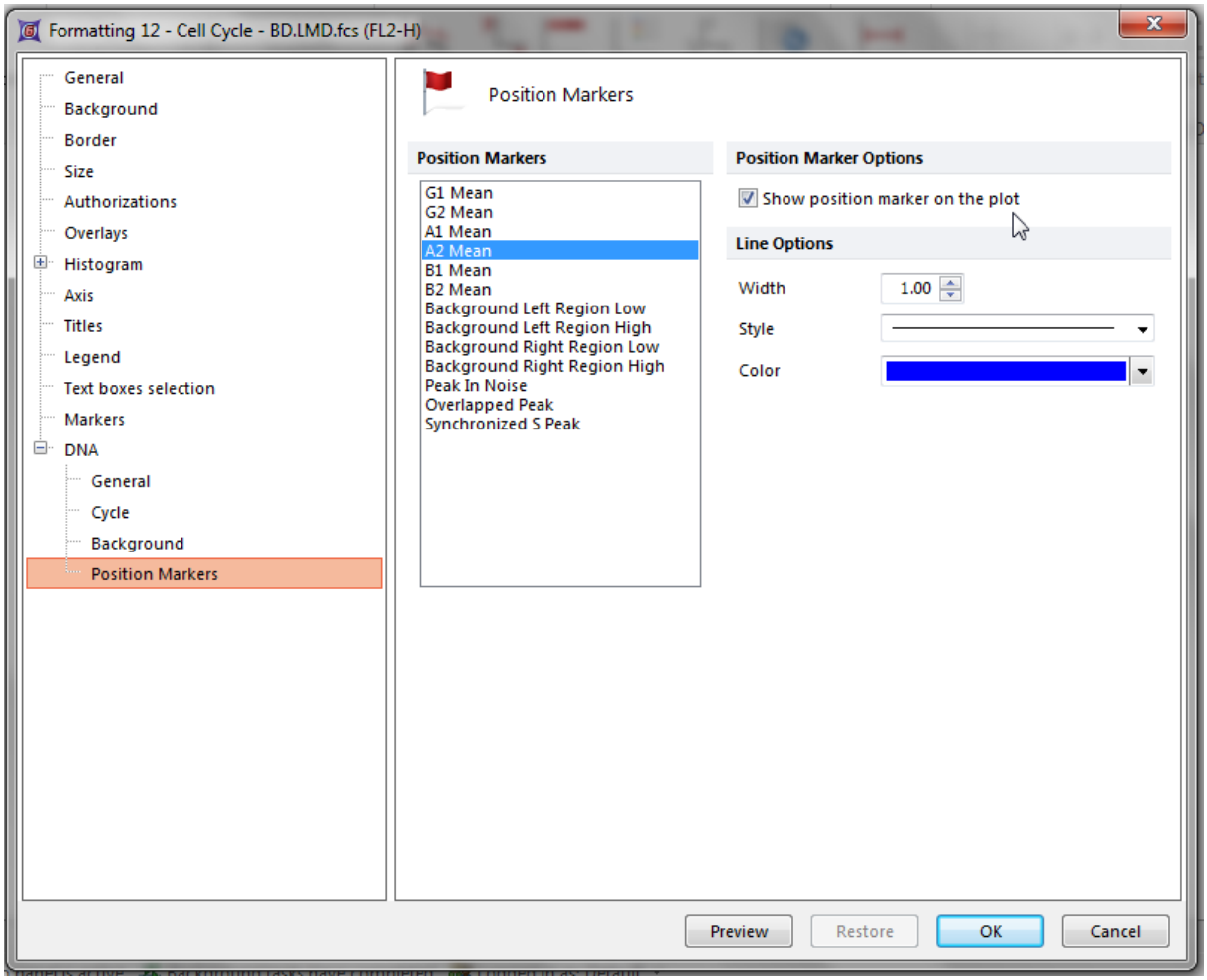

Figure T19.27 Formatting DNA Histogram Position Markers

A vertical line marking the location of the mean of the aneuploid G2 peak appears on the Multicycle DNA histogram (Figure T19.28).

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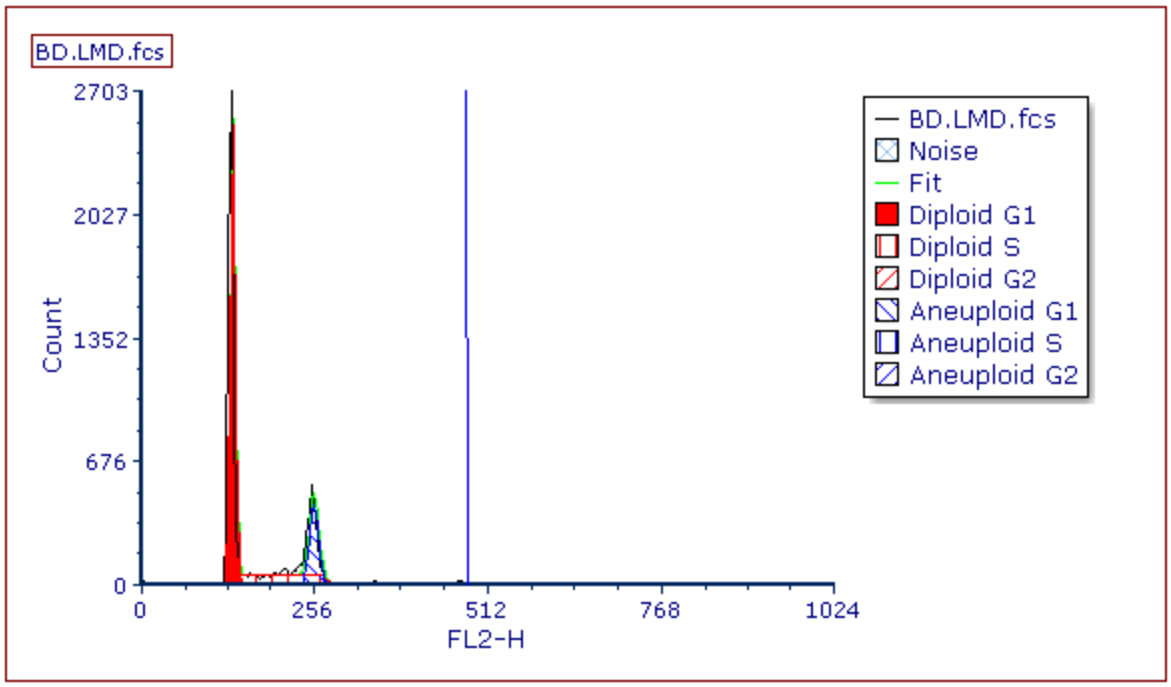

Figure T19.28 MultiCycle Histogram Updated with a DNA Position Marker on the Aneuploid G2 Mean

We will now zoom in to the DNA histogram to more closely examine the aneuploid population.

8. Right-click inside the DNA histogram to bring up the pop-up menu.
9. Select **Magnify (Axis)** from the pop-up menu (Figure T19.26).

Please refer to Figure T19.29 for the following steps.

10. Place the cursor to the left of the aneuploid G1 peak on the DNA histogram, shown by left arrow.
11. Press and hold the left mouse button and move the mouse to the right.
12. Release the left button when the right arrow is to the right of DNA Position Marker, indicated by the cursor.

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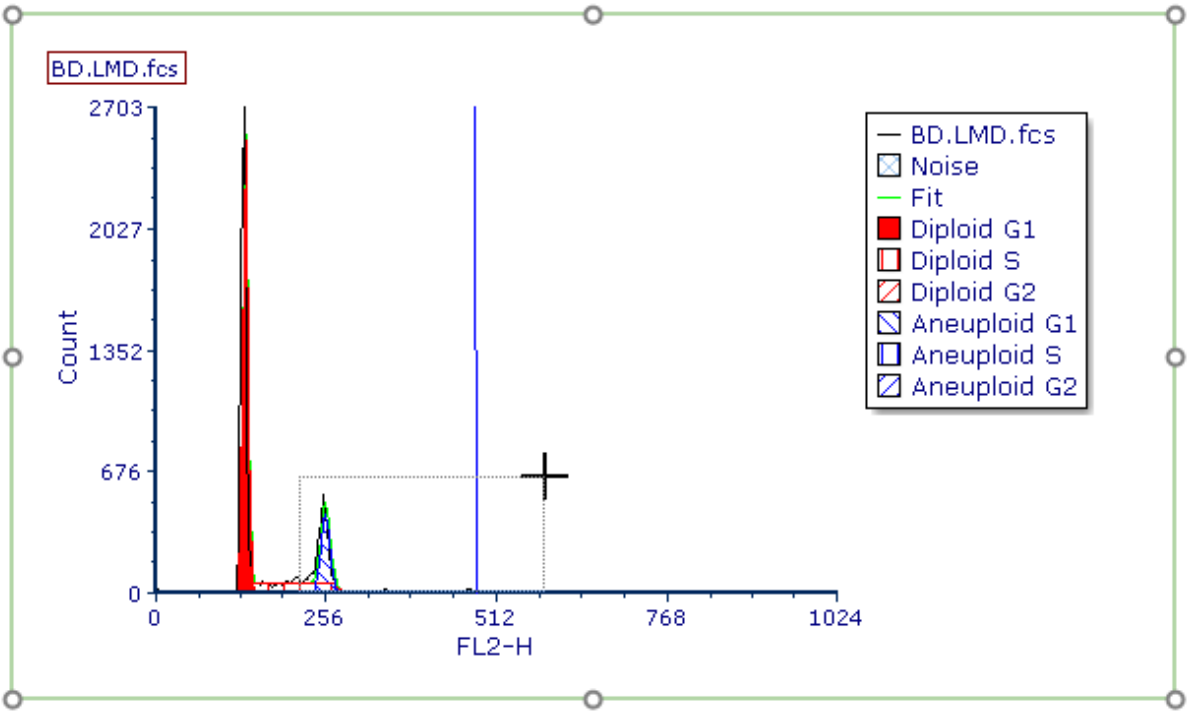

Figure T19.29 Selecting an Area on the X-axis to Zoom

The histogram now updates to zoom in on the area of the x-axis that was selected in the previous steps, as shown in Figure T19.30.

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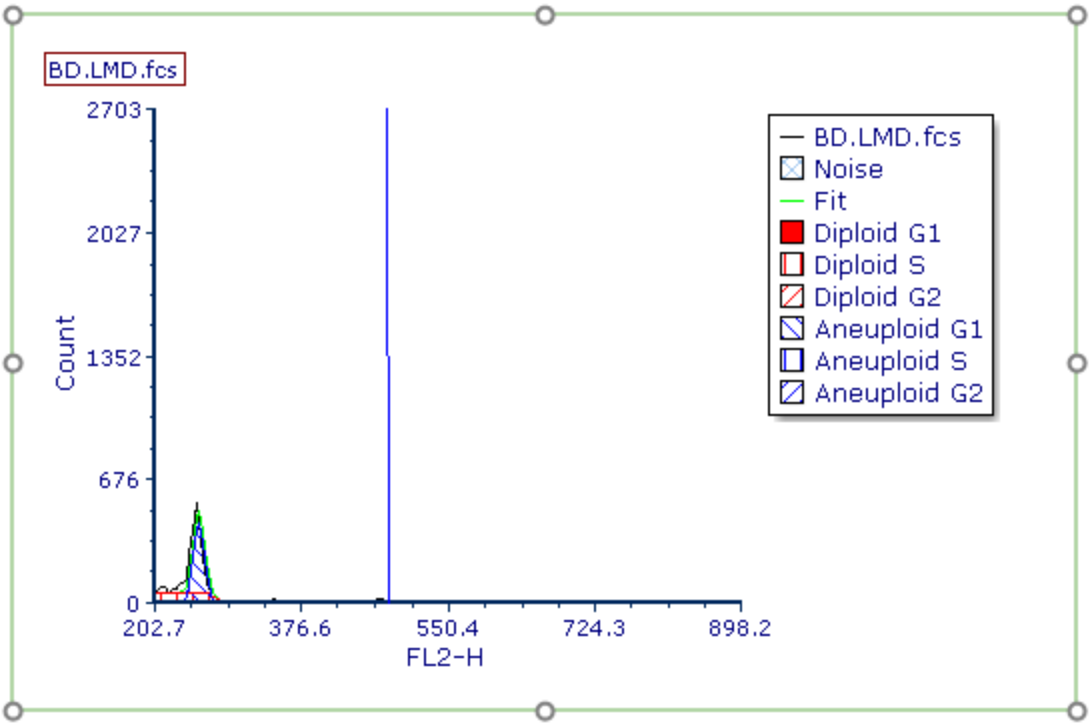

Figure T19.30 Histogram Zoomed

We will now change the y-axis scale on the histogram.

13. Click inside the histogram to select it.
14. Select the **Format→Plot Options→Axes** command.

The **Formatting Axes** dialog appears. Please refer to Figure T19.31 for the following steps.

15. Select **Y-Axis**, highlighted in blue.
16. Deselect the box next to **Automatic**, indicated by the cursor.
17. Enter '300' as the maximum value for the Y-axis, shown highlighted in blue.
18. Click **OK**.

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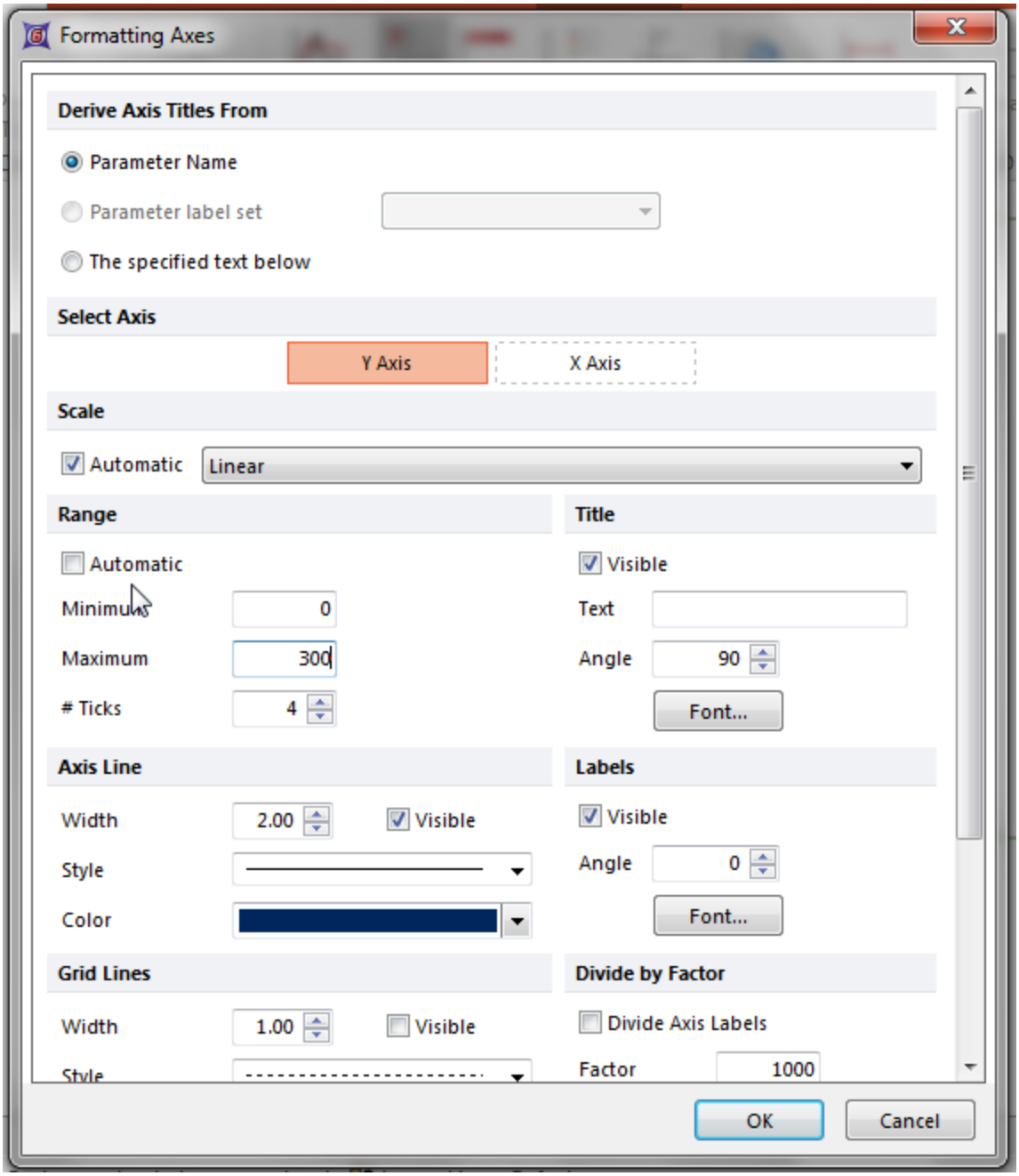

Figure T19.31 Formatting Axes Dialog

The MultiCycle histogram updates, scaling the y-axis from 0 to 300 as shown in Figure T19.32.

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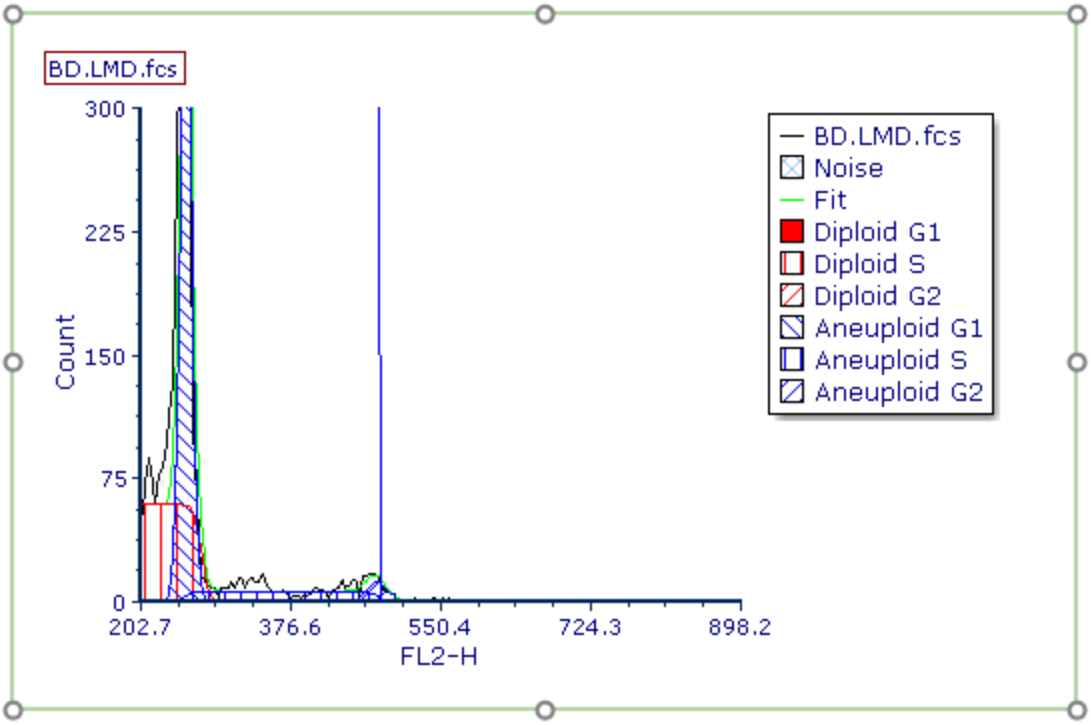

Figure T19.32 MultiCycle Histogram with Rescaled Y-axis

Next, we will perform MultiCycle analysis of 3 cycle data 1827.

38.21.4 Analysis of 3 Cycle Data

Analysis of DNA histogram data that contains multiple cycling populations requires the advanced MultiCycle version which must be purchased separately. We will begin by opening a new blank Layout page and inserting a DNA histogram that has multiple aneuploid populations.

1. Select **File** tab→**New**→**New**.
2. Select the **Insert** tab→**1D Plots**→**MultiCycle DNA** command.
3. Click on a blank area of the layout.

The **Select a Data File** dialog appears, as previously shown in Figure T19.1 1796.

4. Navigate to the *FCS Express Sample Data* folder.
5. Select the file **ASCII4.fcs**.
6. Click **Open**.

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The MultiCycle DNA histogram now appears as shown in Figure T19.33. Note the two aneuploid populations, shown in blue and green, that make up a majority of this histogram.

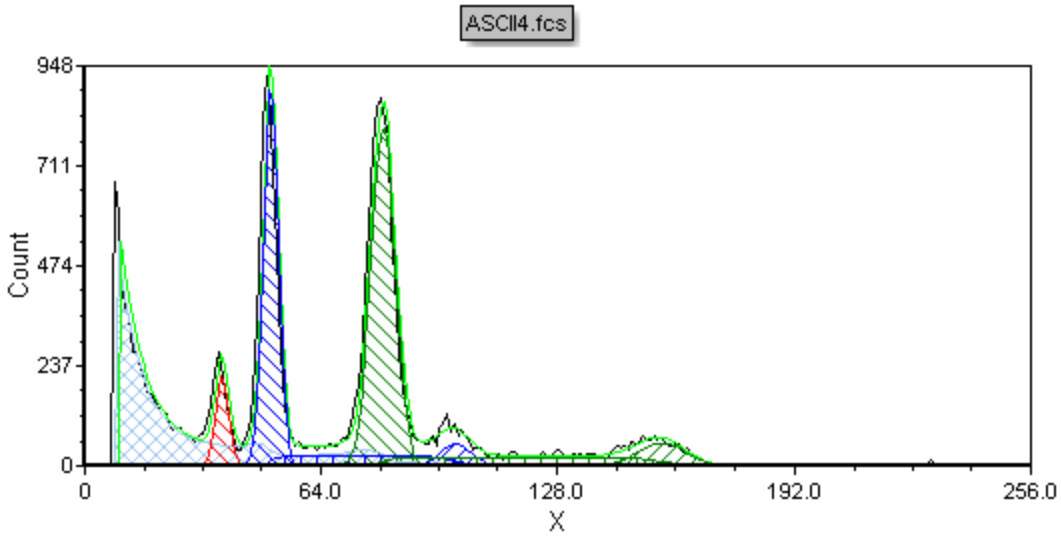

Figure T19.33 MultiCycle DNA Histogram with Two Aneuploid Populations

You could insert the **DNA Cycle**, **DNA Model Summary**, and **DNA Experiment Statistics** windows as previously shown in the tutorial to further study this histogram. However, it is apparent that this DNA histogram has multiple cycling populations, which MultiCycle appears to have correctly identified.

We will now look at a histogram that is more difficult to analyze because the aneuploid populations overlap, and in fact the Autofit function of MultiCycle does not correctly identify the two aneuploid populations, requiring us to use manual settings. We will begin by inserting just the histogram.

7. Select the **Insert** tab→**1D Plots**→**Histogram** command.

8. Click on a blank area of the layout.

A histogram of the current data file appears on the layout. We will now change the data in the plot.

9. Right-click on the histogram and select **Change file** from the pop-up menu.

10. Select the file **ASCII8.fcs** from the **Select a Data File** dialog which appears.

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11. Click **Open**.

The histogram changes to display the ASCII8.fcs data file, as shown in Figure T19.34. Note that there is an aneuploid peak, shown circled in red. However, upon closer examination it appears that there may actually be two aneuploid peaks with very similar amounts of DNA. We will now use the Autofit function of MultiCycle to analyze this DNA histogram, and show that it does not yield correct results in this case.

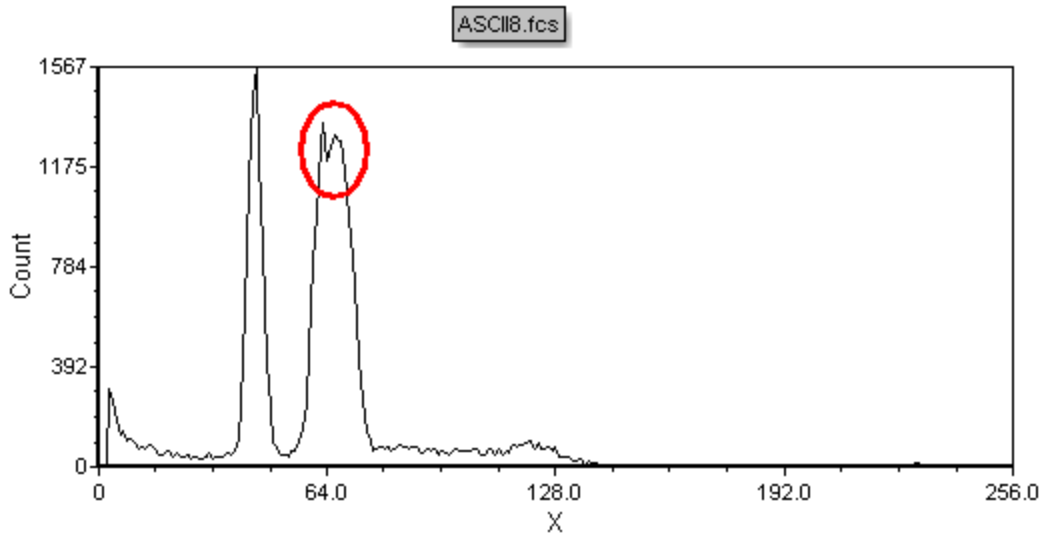

Figure T19.34 ASCII8.fcs Histogram

12. Select the **Insert** tab→**1D Plots**→**MultiCycle DNA** command (Figure T19.4 1796).

13. Click on a blank area of the layout.

The **Select a Data File** dialog appears, as previously shown in Figure T19.1 1796.

The MultiCycle DNA histogram now appears as shown in Figure T19.35. The Autofit function of MultiCycle has analyzed this histogram using a two cycle model, thereby assuming there is one aneuploid population, shown in blue. We will now use MultiCycle to analyze the DNA histogram without using Autofit.

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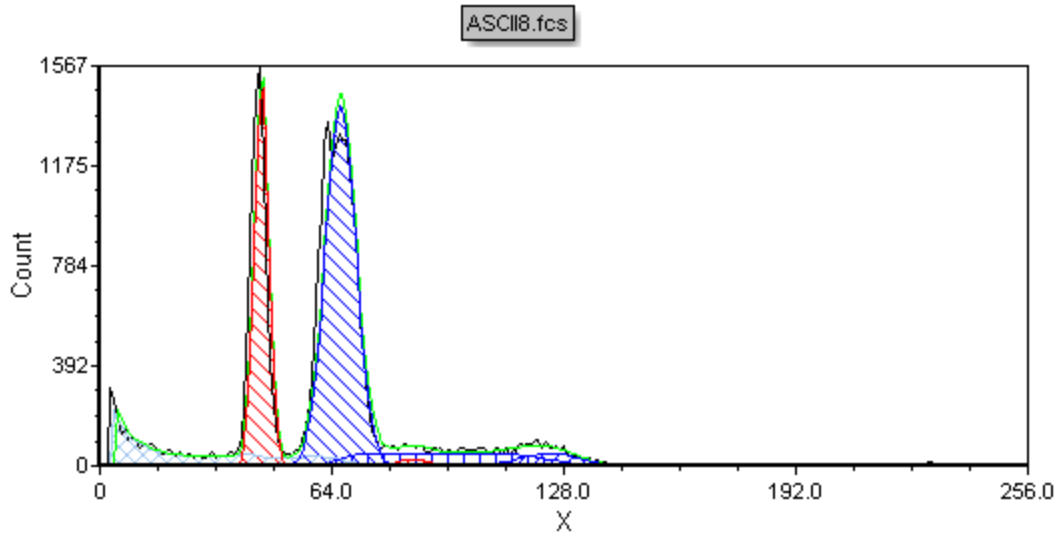

Figure T19.35 Autofit MultiCycle Analysis of ASCII8.fcs

17. Click inside the MultiCycle histogram of ASCII8.fcs to select it.
18. Select the **MultiCycle→Fit Parameters** command.

The **Formatting Fit Model Options** dialog appears. Please refer to Figure T19.36 for the following steps.

19. Deselect the box Ⓐ next to **Detect fit type automatically**, this turns off Autofit.
20. Select **3 Cycle** Ⓑ from the **Fit Type** drop-down menu.
21. Select the box Ⓒ next to **Diploid CV = Aneuploid CV**.
22. Click **OK**.

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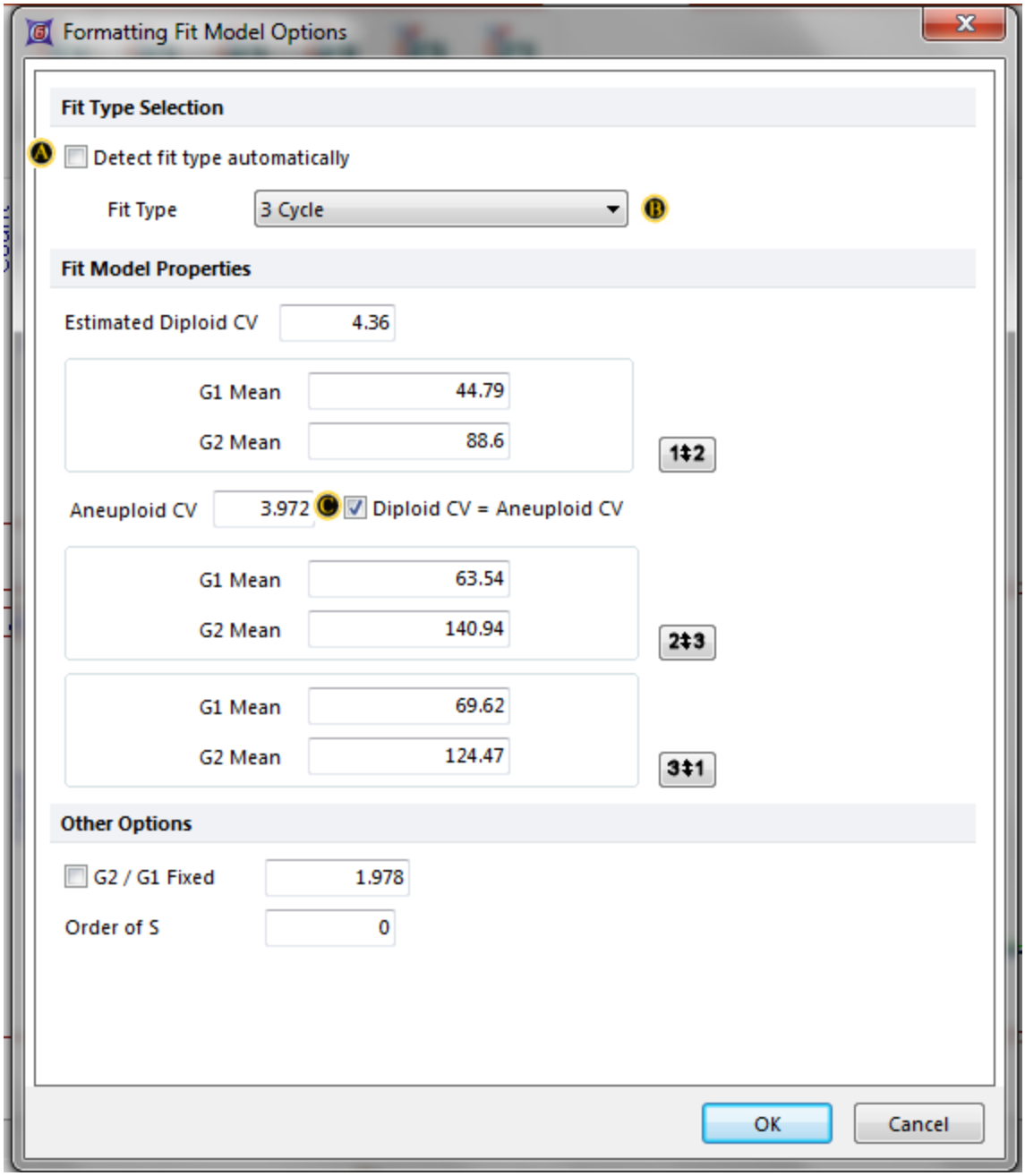

Figure T19.36 Formatting Fit Model Options Dialog

We have now turned off Autofit and told MultiCycle to apply a 3 Cycle model. We have also specified the additional constraint on the model that the CV of the diploid peak should equal the CV of the aneuploid peaks. The MultiCycle analysis of the histogram using the manual fit options appears, as shown in Figure T19.37.

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Alternatively, you can turn off Autofit by deselecting it on the **MultiCycle** tab and specifying a 3 Cycle model.

- Select the **MultiCycle→Fit Parameters→Autofit** command, to turn off Autofit.
- Select **MultiCycle→Fit Parameters→3 Cycle** from the **Cycle** drop-down list.
- Right-click on the ASCII8 MultiCycle histogram to bring up the pop-up menu.
- Select **Format** from the pop-up menu.

The **Formatting Cell Cycle** window appears.

- Select the **DNA→Cell Cycle** category from the left side of the window.

The **DNA Cell Cycle** category of the **Formatting Cell Cycle** dialog appears, similar in appearance to the **Formatting Fit Model Options** shown in Figure T19.36.

- Select the box next to **Diploid CV = Aneuploid CV** (Figure T19.36).
- Click **OK**.

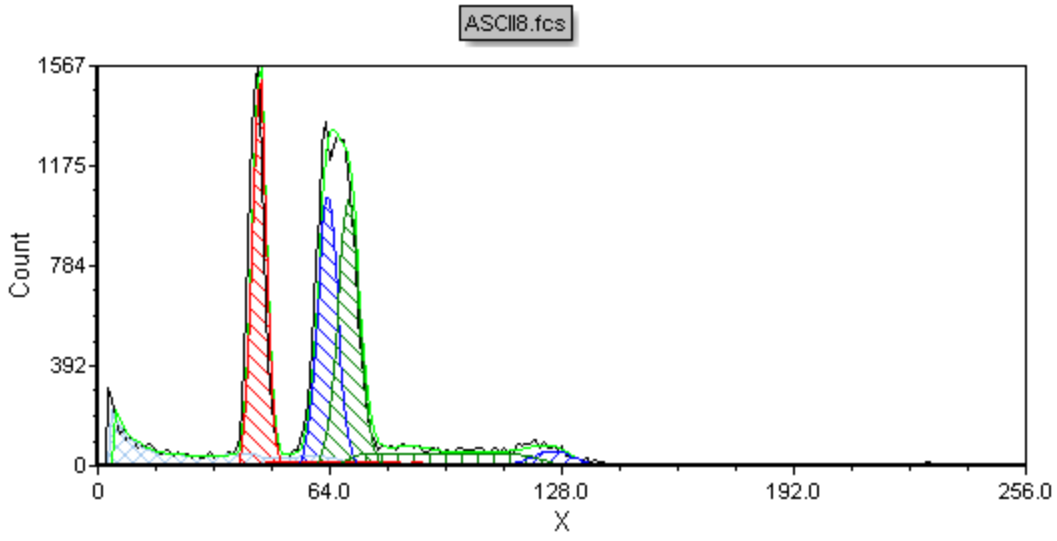

Figure T19.37 Manual MultiCycle Fit

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38.22 Proliferation

One of the most common methods used to study cell proliferation[902] induced by different treatments is the dye dilution technique. In this technique, the cells of interest (usually T-cells) are labeled uniformly with tracking dyes such as CFSE or the CellTrace and PKH series of dyes. With each generation, the dye content of the cells decreases by half as the dye is equally divided among the daughter cells. Thus, as time progresses, cell populations that have divided can be identified by their characteristic distribution of peaks with diminished fluorescence as compared to the undivided control. The proliferation module[902] in FCS Express performs sophisticated curve fitting in order to quantify the proliferation characteristics of your population. This allows for the easy determination of the proportion of cells that proliferated, as well as other derived statistics.

In this tutorial we will learn how to apply such algorithms, create proliferation statistics, format the plotted data, and generate publication quality reports in a batch process.

Layout Design and Gating Strategy Notes:

The layouts for use in this tutorial are listed below can be found in the Proliferation Tutorial folder within the Tutorial Sample Data folder.

- Proliferation Tutorial Fitting Proliferating Cells Section.fey
- Proliferation Tutorial Formatting Section.fey
- Proliferation Tutorial for Batch Processing.fey

The layouts have been optimized for ease of use while assuming that you are familiar with the basics of data analysis in FCS Express. The samples for the data set are listed below and were stained with SYTOX AADvanced (live/dead marker), CD4 Alexa 488, and CellTrace Violet (proliferation dye).

- Unstained Control.fcs
- 10 uM CellTrace Violet Control.fcs
- 10 uM CellTrace Violet Stimulated Control.fcs
- 10 uM CellTrace Violet Stimulated Sample.fcs

In the Gating page of the layouts, the top two plots define the live cells in the assay using a hierarchy of gates from the SSC-H, FSC-H, and SYTOX AADvanced-H parameters. The plots in the middle row of the Gating page are an inverse greyscale density plot (left) and a color dot plot (right). These plots are used to help visualize generational data and are useful for further downstream analysis of the proliferation data. The final plots for the analysis are two histograms of the CellTrace Violet-H parameter: one which contains a marker that is linked to the Non Proliferating Control gate (M1) and is labeled Proliferation Histogram in the title; the other, "Background Noise Histogram,"

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contains a marker for Background Noise. (Note: The Background Noise histogram is only visible in the *Proliferation Tutorial Fitting Proliferating Cell.fey* while in the other layouts it can be found on a hidden page[98]). The Proliferation Data page contains a pre-inserted proliferation plot of the data. The Proliferation Tutorial for Batch Processing.fey layout has completed all of the steps from the first three sections of the proliferation tutorial so you may proceed directly to batch processing the data.

We will begin by learning how to insert a proliferation plot and how to convert a histogram to a proliferation plot[1834].

38.22.1 Inserting Proliferation Plots

A proliferation plot can be created just like any other plot in FCS Express, and regular histograms[1227] can easily be changed to a proliferation plot.

In the course of this section we will:

- Insert a new proliferation plot
- Change a histogram to a proliferation plot

1. Open the "Proliferation Tutorial Fitting Proliferation Cells Section.fey" layout in the Proliferation Tutorial folder within the Tutorial Sample Data folder.
2. Click on the **Proliferation Data** page tab.
3. Click on the **Insert Tab→1D Plots→Proliferation** button (Fig. T20.1).

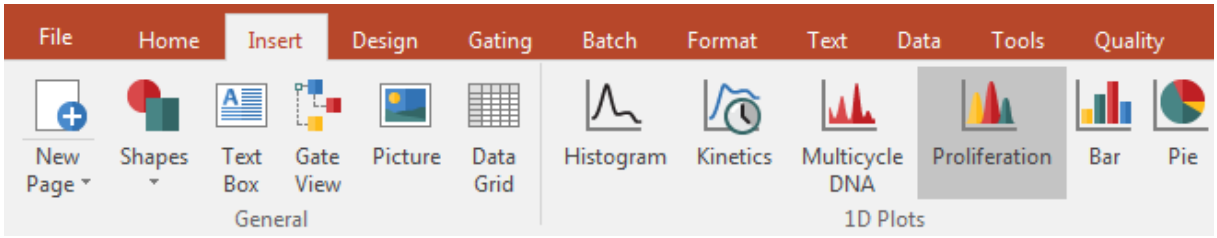

Figure T20.1 Inserting a Proliferation Plot

4. Click below the current proliferation plot to insert a new proliferation plot. The plot will look like figure T20.2a and we must change the X Axis parameter and gate to display the data correctly.

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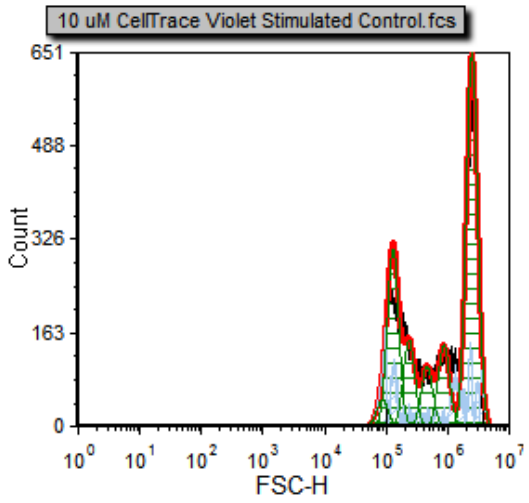

Figure T20.2a Proliferation plot before formatting the X Axis parameter and applying the Sytox gate.

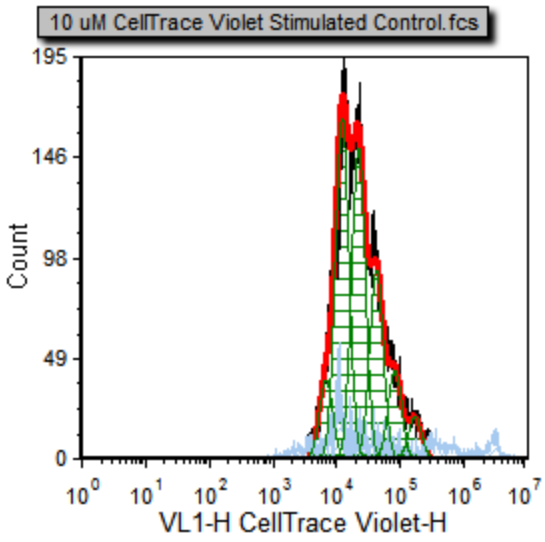

Figure T20.2b Proliferation plot after formatting the X Axis parameter and applying the Sytox gate.

5. Choose the **Format** tab from the ribbon→**Plot Options→Overlays** (Fig. T20.3, ❶).

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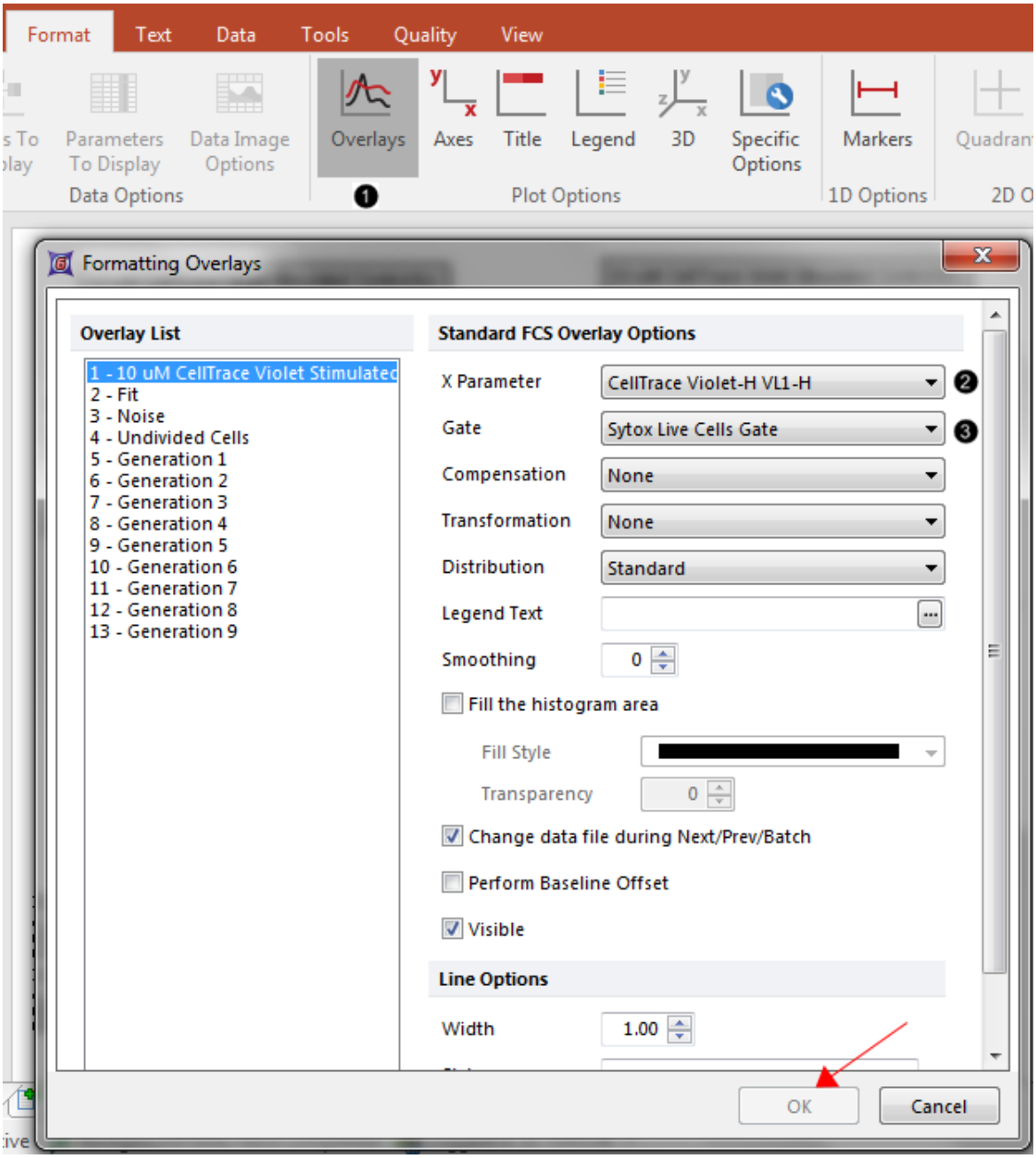

Figure T20.3 Formatting overlay options on a proliferation plot.

6. Change the **X Parameter** to **CellTrace Violet-H** (Fig. T20.3, ❷).

7. Change the **Gate** to **Sytox Live Cells Gate** (Fig. T20.3, ❸).

8. Click **OK** (Fig. T20.3, mouse pointer).

The proliferation plot for the sample is now present on the page and has been formatted to display the correct parameters and gates (Figure T20.2b).

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We will now change the histogram on the Gating page to a proliferation plot.

9. Click on the **Gating** page tab at the bottom of the program to make the **Gating** page visible.
10. Click on the histogram labeled Proliferation Histogram in the title, located towards the bottom of the Gating page, to select it.
11. Click on the **Format** tab→**Change**→**Change Plot Type** button.
12. Select **Proliferation** from the drop down menu (Figure T20.4).

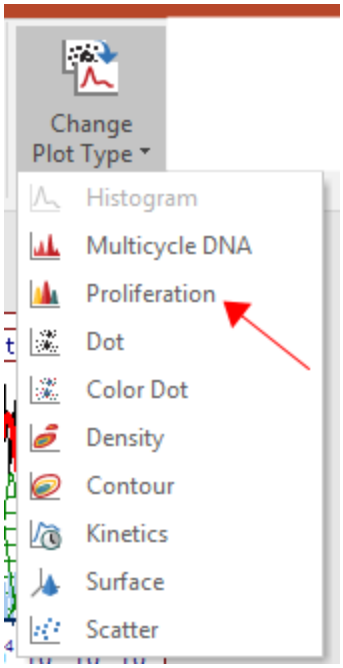

Figure T20.4 Changing plot type from a histogram to a proliferation plot.

You will now notice that the histogram has been changed to a proliferation plot that is ready for analysis.

The Inserting Proliferation Plots section is now complete. Please close the layout and do not save the changes before proceeding to the next section.

In the next section we will learn how to further modify the default fit for use with the data.

38.22.2 Formatting a Proliferation Histogram

In the course of this section we will :

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- Learn how to adjust the proliferation overlays to better visualize the populations of interest.
- Learn how to produce the curve fitting statistics.
- Learn how to smooth the proliferation histogram.
- Learn how to use a batch process for analyzing multiple proliferation samples.

1. Open the "Proliferation Tutorial Formatting Section.fey" layout in the "Proliferation Tutorial" folder within the Tutorial Sample Data folder.
2. Click on the "Proliferation Data" page.
3. Click once on the proliferation histogram to select it.
4. Select the **Format** tab→ **Plot Options**→ **Overlays** command.

   The **Formatting Overlays** dialog will appear (Fig. T20.11).
5. Select the **Noise** overlay from the left (Fig. T20.11a, ❶).
6. Uncheck the **Visible** box (Fig. T20.11a, ❷).
7. Select the **Generation 6** overlay from the left (Fig. T20.11b, ❶).
8. Change the **Color** to Blue (Fig. T20.11b, ❷).
9. In the **Fill Style** drop down select the 1^{st} option for a completely filled rectangle (Fig. T20.11b, ❸).
10. Select the **Generation 8** overlay from the left (Fig. T20.11c, ❶).
11. In the **Fill Style** drop down Select the 1^{st} option for a completely filled rectangle (Fig. T20.11c, ❷).
12. Click **OK** (Fig. T20.11c, ❸).

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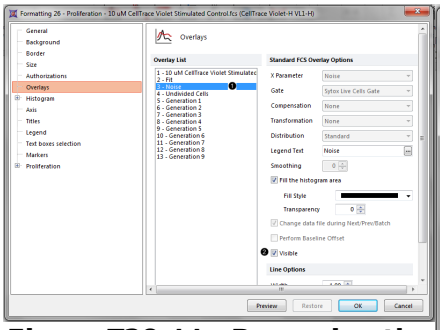

Figure T20.11a Removing the noise overlay from the proliferation plot.

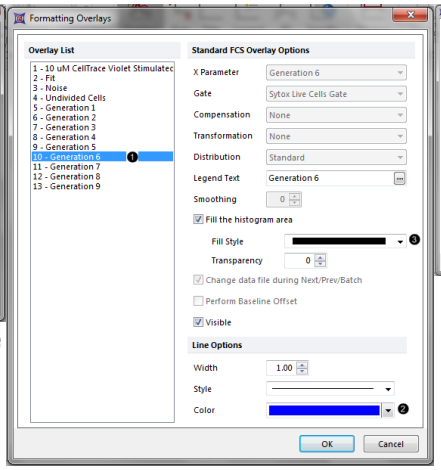

Figure T20.11b Coloring generation 6 in the proliferation plot blue.

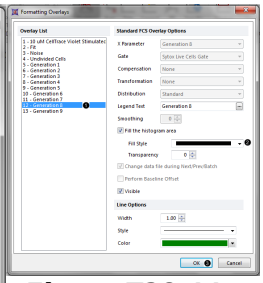

Figure T20.11c Coloring generation 8 in the proliferation plot green.

The proliferation histogram now appears without displaying the noise, with the peak corresponding to generation 6 filled in blue, and the peak corresponding to generation 8 filled in green (Fig. T20.12). This allows for easier confirmation and visualization of each generation and fitted peak. You can change the formatting of any and all of the different fitted elements and overlays in this way.

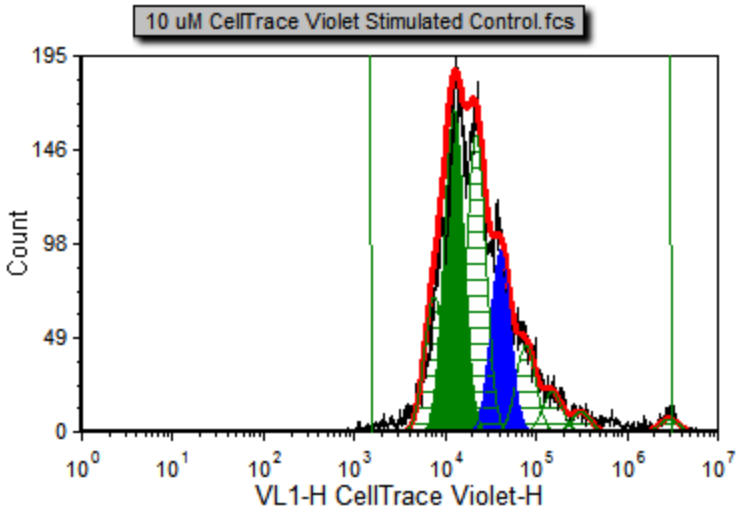

Figure T20.12 The formatted proliferation plot displaying different generations in different colors with the noise overlay removed.

Both the **RMS error** and **R-Chi Square** are measures of the goodness of the fit. When setting the proliferation model fit parameters, it is important to check how changing these parameters affect these two statistics. The combination of settings that provides the least **RMS error** and **R-Chi Square** is mathematically

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the best fit. We will now show the associated statistics for this proliferation model.

12. Right-click on the proliferation histogram to bring up the associated pop-up menu.
13. Select **Statistics→Proliferation Fit Statistics** from the pop-up menu.

    The **Proliferation Fit Statistics** window will now appear.
14. Right-click on the proliferation histogram to bring up the associated pop-up menu.
15. Select **Statistics→Proliferation Population Statistics**.

    The **Proliferation Population Statistics** window will now appear.
16. Move both statistic tables so that the proliferation histogram and tables are all visible as in Fig. T20.13.

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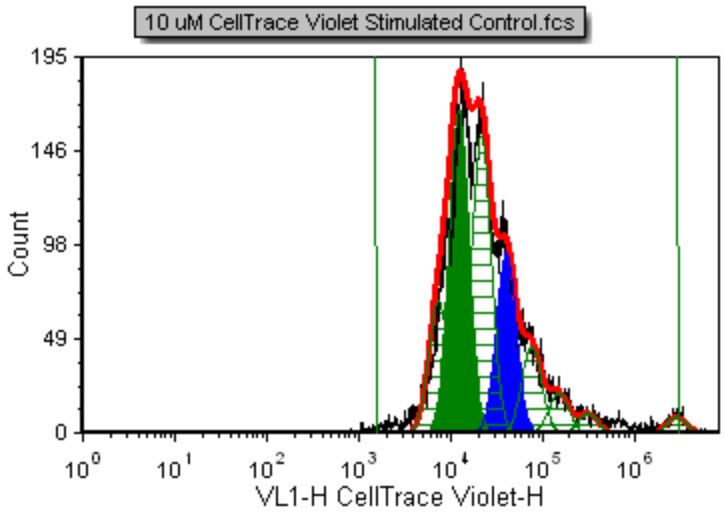

Division Index	Proliferation Index	% Divided	Peak Ratio	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square	Starting Generation Mean	Background Noise
76.98	39.23	50.31	0.51	22319	565.47	8.66	4.82	2913319.39	1489.57

Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells
Undivided Cells	2913319.39	8.00	15.84	1.66	0.00	281
Generation 1	1162853.57	0.00	0.00	0.00	0.40	0
Generation 2	636509.24	0.00	0.00	0.00	0.55	0
Generation 3	309221.53	10.00	15.84	1.95	0.49	353
Generation 4	146460.92	21.00	15.84	2.07	0.48	801
Generation 5	74986.12	45.00	15.84	2.19	0.50	1750
Generation 6	40227.61	93.00	15.84	2.32	0.52	3661
Generation 7	21580.80	154.00	15.84	2.47	0.52	6065
Generation 8	12321.30	167.00	15.84	2.61	0.54	6608
Generation 9	7371.06	68.00	15.84	2.76	0.55	2662

Figure T20.13 Proliferation Histogram with the Proliferation Fit Statistics and Proliferation Population Statistics

We will now smooth the proliferation histogram.

17. Click on the proliferation histogram to select it.
18. Select the **Format Tab→Plot Options→Overlays** command.

    The **Formatting Overlays** dialog will appear, similar to Figure T20.11.

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Note that, by default, the first overlay is selected in the left hand side. This is the overlay we will smooth since the rest of the overlays are calculated from the fitting algorithm.

19. Increase the **Smoothing** to **6**.

20. Click **OK.**

The Proliferation histogram and the statistics are now updated to reflect the smoothing (Figure T20.14). The black contour of the raw data is much smoother, but the fit changes significantly (note that the proliferation fit and proliferation population statistics have changed). Although this provides for a nicer visual presentation, this option should be used with caution since it affects the statistics obtained when using the raw data, and can thus affect the interpretation of the data. Note that the R-Chi Square 908 value has increased from 4.82 to 17.76 as a result of smoothing the raw data, indicating an inferior fit. Thus it is advisable to cull statistics from an unsmoothed (raw) proliferation plot, while applying smoothing to a duplicate Proliferation plot or regular histogram for visual presentation only, if desired.

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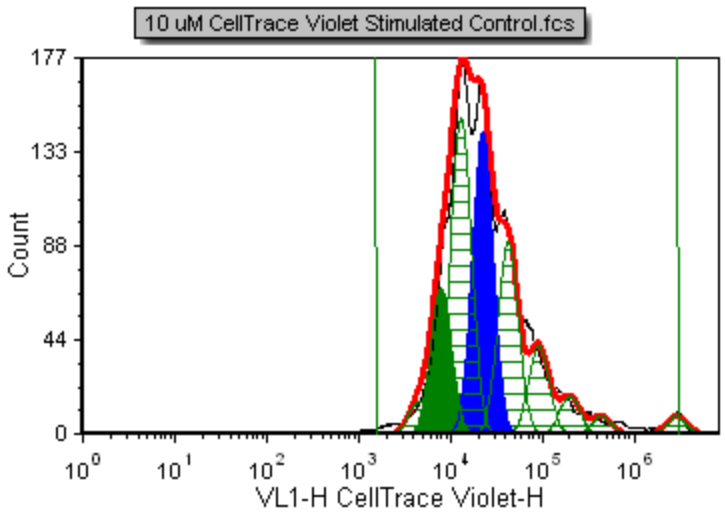

Division Index	Proliferation Index	% Divided	Peak Ratio	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square	Starting Generation Mean	Background Noise
40.52	26.16	63.67	0.47	22287	845.09	5.86	17.76	2913319.39	1489.57

Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells
Undivided Cells	2913319.39	8.00	16.56	1.73	0.00	307
Generation 1	965961.37	0.00	0.00	0.00	0.33	0
Generation 2	396691.03	8.00	16.56	1.98	0.45	315
Generation 3	196874.14	18.00	16.56	2.11	0.45	701
Generation 4	88993.12	41.00	16.56	2.26	0.45	1678
Generation 5	42151.06	90.00	16.56	2.42	0.45	3681
Generation 6	22263.35	142.00	16.56	2.57	0.51	5859
Generation 7	12910.44	149.00	16.56	2.72	0.55	6156
Generation 8	7844.69	69.00	16.56	2.87	0.56	2817
Generation 9	4341.52	15.00	16.56	3.06	0.48	594

Figure T20.14 Proliferation Histogram and Associated Statistics with Smoothing

Now that we have formatted our plots for the experiment and inserted proliferation statistics we will create a batch process 784 in the next section 1852 to summarize the three sample experiment.

38.22.2. Fitting Proliferating Cells 1

In the course of this section we will:

- Learn how the curve fitting algorithm and parameters are fitted to a histogram.

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- Use tokens to define a Fixed Starting Generation and Background.
- Insert proliferation position markers.

We will begin by navigating through the data to get an idea of what the experiment looks like and what the color coded overlays represent.

1. Open the "Proliferation Tutorial Fitting Proliferating Cells Section.fey" layout in the "Proliferation Tutorial" folder within the Tutorial Sample Data folder.
2. Navigate to the **Proliferation Data** page tab.

You will notice a proliferation plot on the **Proliferation Data** page tab for the 10 uM CellTrace Violet Stimulated Control.fcs sample (Figure T20.5a). This is the curve fitting result on the positive control, proliferating cells. There are four samples in the data list.

We will now switch to our control sample in which cells are not proliferating.

3. Select the **Data** tab→ **Change Data on All**→ **Previous** command to load the previous file in the Data List, i.e., 10 uM CellTrace Violet Control.fcs (Figure T20.5b).

Notice that the 10 uM CellTrace Violet Control.fcs file represents the stained yet undivided control sample and can serve as our starting generation peak.

We will now switch to our Stimulated Sample.

4. Select the **Data** tab→ **Change Data on All**→ **Next** command to advance two files to 10 uM CellTrace Violet Stimulated Sample.fcs (Figure T20.5c).

Notice that the 10 uM CellTrace Violet Stimulated Sample.fcs file represents our stimulated (dividing) sample in which we would like to measure the degree of proliferation.

We will now switch to our final Unstained Control sample.

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5. Select the **Data** tab→ **Change Data on All**→ **Next** command to the end of the Data List to Unstained Control.fcs (Figure T20.5d).

Notice that the Unstained Control.fcs file represents the sample as it appears without any proliferation staining and can serve as our background noise peak. Please note that FCS Express is fitting the background peak as it was a population of proliferating cells. We will modify the fitting parameters later on to tell FCS Express to treat these as the background.

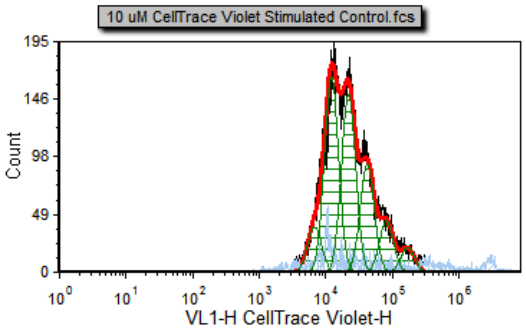

Figure T20.5a Proliferation positive control

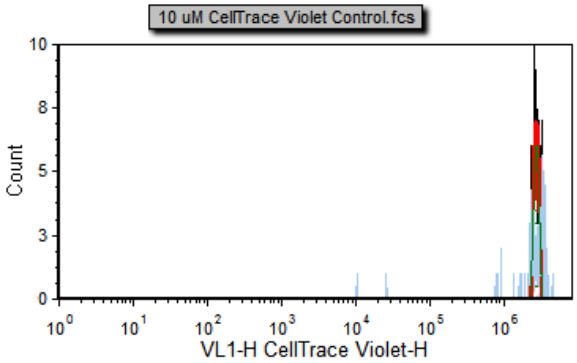

Figure T20.5b Proliferation negative control

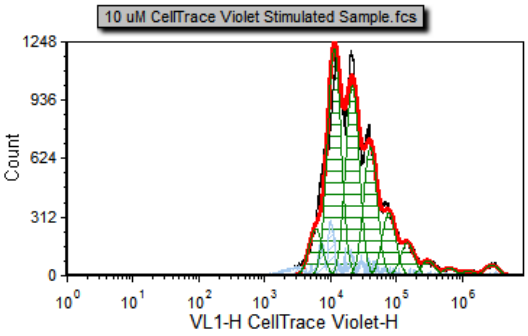

Figure T20.5c Proliferation stimulated sample

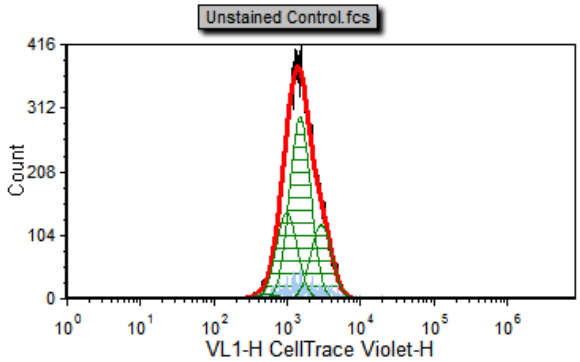

Figure T20.5d Proliferation unstained control

You will notice many different color overlays on the different proliferation samples. The default colors for the proliferation curve fitting are as follows:

Black – Raw histogram contour. This includes all the data and does not represent an output of the fitting algorithm and would be the same histogram you would see if you plotted the data on a regular histogram.

Red – Overall contour of the fitted data. This excludes what the algorithm determines to be noise.

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Light blue – Noise. This is the data that the algorithm determines to be noise, and is not included in the statistics.

Green – Modeled populations of proliferating cells. These are the actual curves fitted to the data, representing the parent, undivided population, and all of the proliferating generations.

Note that there are cells in the proliferation stimulated sample (Figure T20.5c) in the 10^6 to 10^7 range, shown in light blue, that have been determined by the model to be noise. We will now modify the **Proliferation Fit Parameters** to see if we can improve the model's fit by specifying where the starting generation is located. Notice on the Gating page there is a histogram with the marker M1 visible. M1 defines the starting generation based on the negative control and we will use a token representing the geometric mean of the data under the marker to define our starting generation more accurately.

6. Change the data using the **Data** tab→ **Change Data on All**→ **Next/Previous** buttons until you reach the 10 uM CellTrace Violet Stimulated Control.fcs sample.

7. Right-click on the proliferation plot on the **Proliferation Data** page.

8. Select **Format** from the pop-up menu.

The **Formatting** dialog now appears (Figure T20.6).

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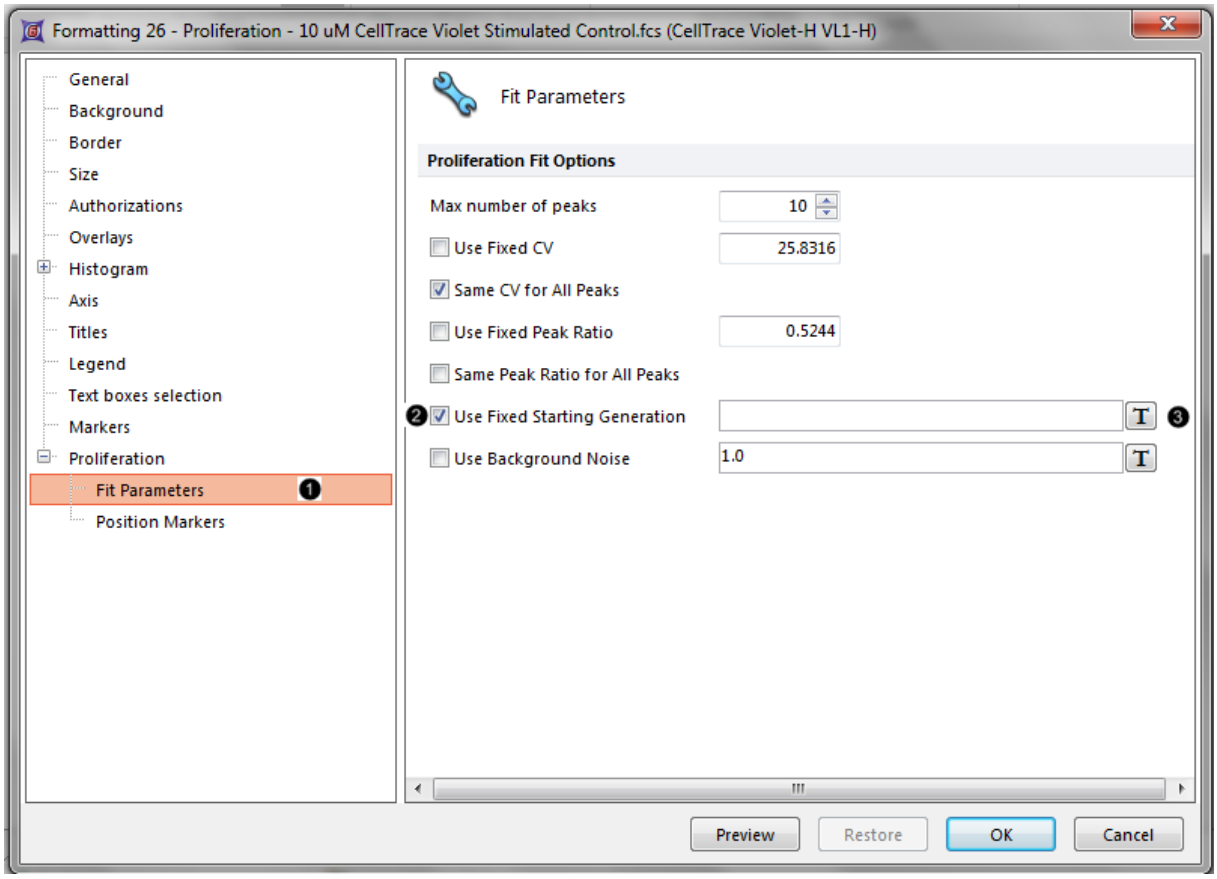

Figure T20.6 Adjusting proliferation fit parameters in the formatting dialog.

9. Navigate to the **Proliferation→Fit Parameters** category (Figure T20.6, ❶).

10. Check the **Use Fixed Starting Generation** check box (Figure T20.6, ❷).

11. Delete the numbers in the white text box next to the **Use Fixed Starting Generation** option (Figure T20.6).

12. Click on the T token symbol next to the text box (Figure T20.6, ❸).

    The **Insert a Token** dialog will appear.

13. Double click on **Statistic** from the **Insert a Token** dialog.
14. Choose Plot **4 - Histogram** - as the **Data Source** as shown highlighted in blue below (Figure T20.7, ❶).

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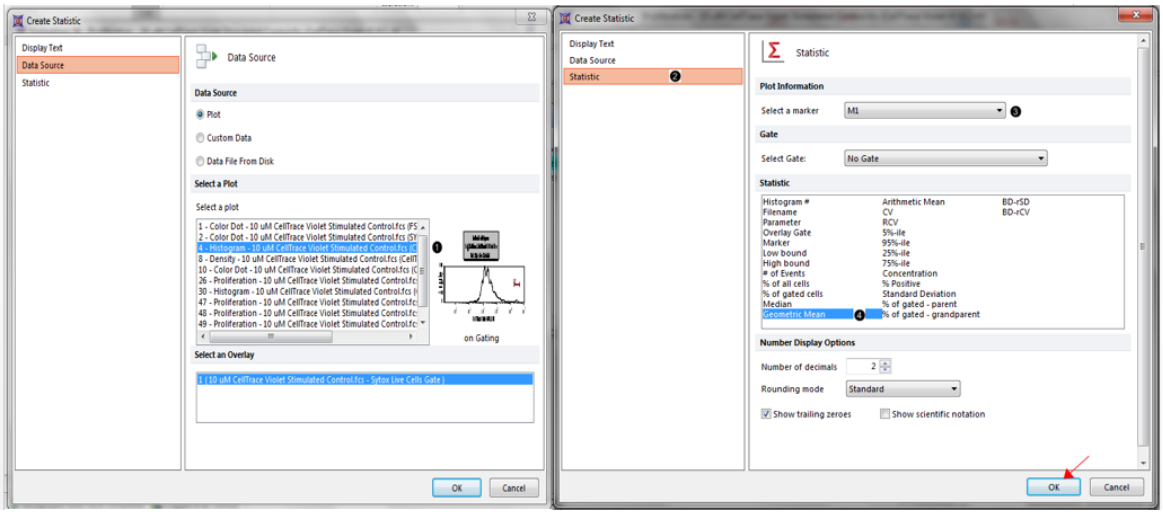

Figure T20.7 Adjusting proliferation fit parameters, continued.

15. Click on the **Statistic** category on the left (Figure T20.7, ❷).

16. Set the **marker** to M1 (Figure T20.7, ❸).

17. Select **Geometric Mean** from the **Statistic** list (Figure T20.7, ❹).

18. Click **OK** (Figure T20.7, mouse pointer).

19. Click on the **Proliferation→Position Markers** category (Figure T20.8, ❶).

20. Check the box for **Show position marker on the plot** with **Undivided cells peak** highlighted in blue (Figure T20.8, ❷).

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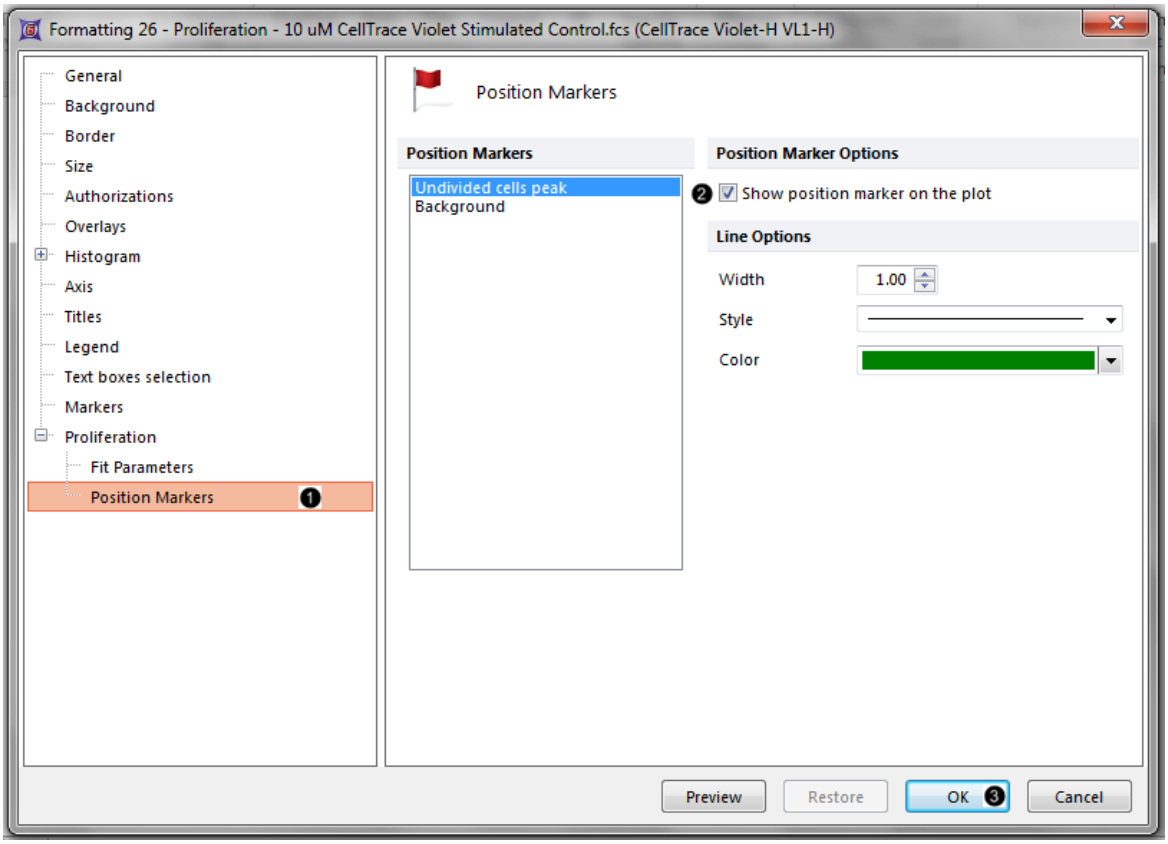

Figure T20.8 Checking the Show position marker on the plot box for the Undivided cells peak.

21. Click **OK** (Figure T20.8, ❸).

The proliferation histogram should now look like Figure T20.9.

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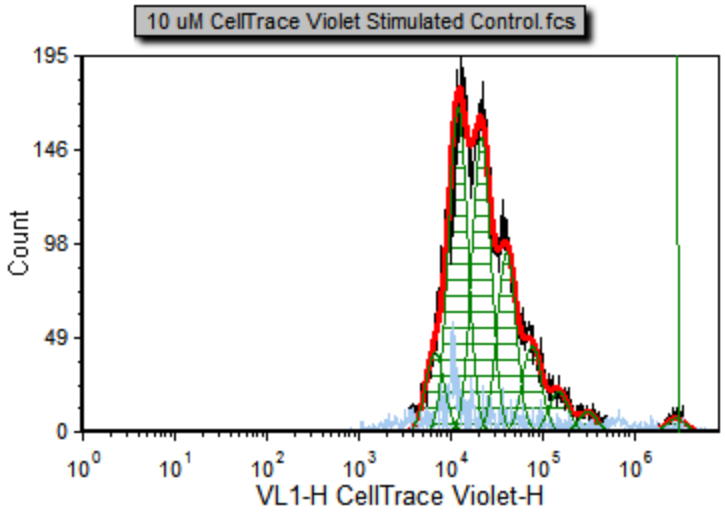

Figure T20.9 The proliferation plot after adjusting the starting generation and choosing to display the Undivided cells peak marker.

Notice on the Gating page there is a histogram labeled "Background Noise" with a marker of the same name. The area under this marker has been defined as the background noise based on the Unstained Control.fcs sample which is being displayed in the histogram. We will now use steps similar to those above for defining a starting generation to define the background noise.

22. Right-click on the proliferation plot on the **Proliferation Data** page.
23. Select **Format** from the pop-up menu.

The **Formatting** dialog will appear as in Figure T20.6.

24. Navigate to the **Proliferation→Fit Parameters** category.
25. Check the **Use Background Noise** check box.
26. Delete the numbers in the white text box next to the **Use Background Noise** option.
27. Click on the T token symbol next to the text box.

    The **Insert a Token** dialog will appear.
28. Double click on **Statistic** from the **Insert a Token** dialog.
29. Choose Plot **30 - Histogram** - as the **Data Source**.
30. Click on the **Statistic** category on the left.
31. Set the **marker** to **Background Noise**.
32. Select **Geometric Mean** from the **Statistic** list.
33. Click **OK**.

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34. Click on the **Proliferation→Position Markers** category.
35. Check the box for **Show position marker on the plot** with **Background** highlighted in blue.
36. Click **OK**.

The proliferation histogram should now look like Figure T20.10a with the Starting Generation and Background noise based on the markers from the histogram in Figure T20.10b.

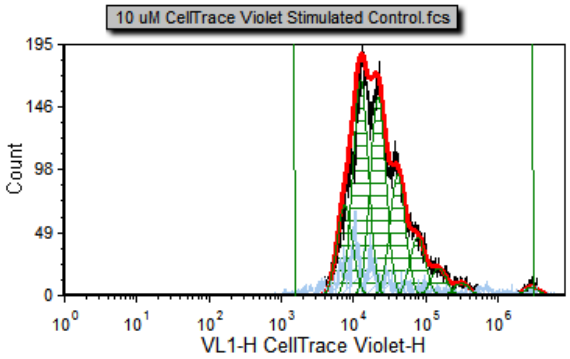

Figure T20.10a Proliferation plot with Background Noise Position and Starting Generation Markers defined.

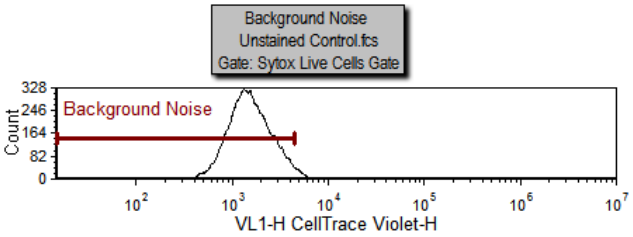

Figure T20.10b Histogram with markers that define the starting generation and background noise for proliferation analysis.

Now the fit is better since the starting generation and background noise (now indicated by the green vertical lines on the right and left respectively) are based on cells that fall within the Sytox Live Cells gate in M1 or the Background Noise marker. Notice how the red, fitted contour, more closely follows the black, raw data contour towards the high end of the data; the R-Chi Square value 908 is also lower. Although the fit is better for the high end of the data you can see for most of the curve it has changed very little. That is because the curve fitting algorithm correctly found a good approximation of the starting generation before adding the more correct token value for the starting generation. Nevertheless, in some cases, it is important to manually set the starting generation and background from control samples. On the other hand, sometimes in proliferation experiments, the control sample can have a slightly different peak fluorescence value than the undivided cells from the experimental samples. These differences can be enough to preclude the use of control histograms to set the position of the undivided cells.

You will also notice that if you adjust the markers to make them broader or narrower the proliferation plot fit will update in real time since the starting generation and background noise are now based on the statistics for the geometric mean under the M1 and Background noise markers. For a description of how the fit is being calculated please see the technical note below.

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Technical Note: This is a brief technical note to describe how the fitting process works. This information is to help you have an understanding of how adjusting the parameters affects your data. If you wish to skip this, you may do so.

- The fitting process starts by determining where the population of cells that have not divided is located. The fitting process attempts to find this peak automatically, although you can specify it manually, as described above. In some cases, you have to specify it manually because your cell population may no longer include cells that have not divided.
- Once the undivided population is found, the model attempts to find populations that have decreased in fluorescence by 50% from each other. Since not all experiments work according to theory, the model starts looking at the 50% point but can move left or right to find the true peak. You can control this by selecting the **Same Peak Ratio for All Peaks** option (Figure T20.6).
- In order to find a peak, the model makes certain assumptions about the width of each peak compared to the undivided control. You can also control these settings, selecting or deselecting the **Same CV for All Peaks** option, as shown above (Figure T20.6).
- The model can also subtract background fluorescence from all the calculations, if you provide a value for the background fluorescence.
- Since the algorithm uses the actual histogram contour to model the fit, the histogram data should not be smoothed. Only in special cases, when the original data is very noisy, should a little smoothing be applied to allow the model to better fit the data.
- These options exist in order to accommodate the wide range of data sources and biological behaviors that the model has to encompass. Thus, in order to obtain the best fit, it is important that the correct initial settings be selected. In practice, this is usually performed by trial and error until the settings providing the best fit are found.
- There are two ways to asses the goodness of the fit; the first is visual inspection, which gives a rough measure, and the second is fit statistics, which are discussed below.

Next, we will format the proliferation histogram and show statistics.

38.22.3 Batch Processing Proliferation Data

In the previous sections we have inserted proliferation plots, optimized the proliferation fit, formatted the proliferation plot, and inserted proliferation fit

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and population statistics 1837. We will now combine these efforts to create a final presentation and excel export.

We will be using the Proliferation Tutorial for Batch Processing.fey layout which incorporates all of the steps from the previous three sections so we can focus on the batch process.

1. Open the "Proliferation Tutorial for Batch Process.fey" layout.
2. Click on the **Batch** tab→ **Batch Processing**→ **Batch Actions** command.

   The **Batch Actions** window will open.
3. Click on **Save to Powerpoint** in the **Add Action** category.

   The **Create Export To Powerpoint Action** window will open (Fig. T20.15).
4. Check the **Open presentation after saving box** ❶ (Fig. T20.15).
5. Check the **Combine the output from all iterations in to the same presentation** radio button ❷ (Fig. T20.15).
6. Click on the folder icon under the **Output file options category**→ **Save to a new file** ❸ (Fig. T20.15).

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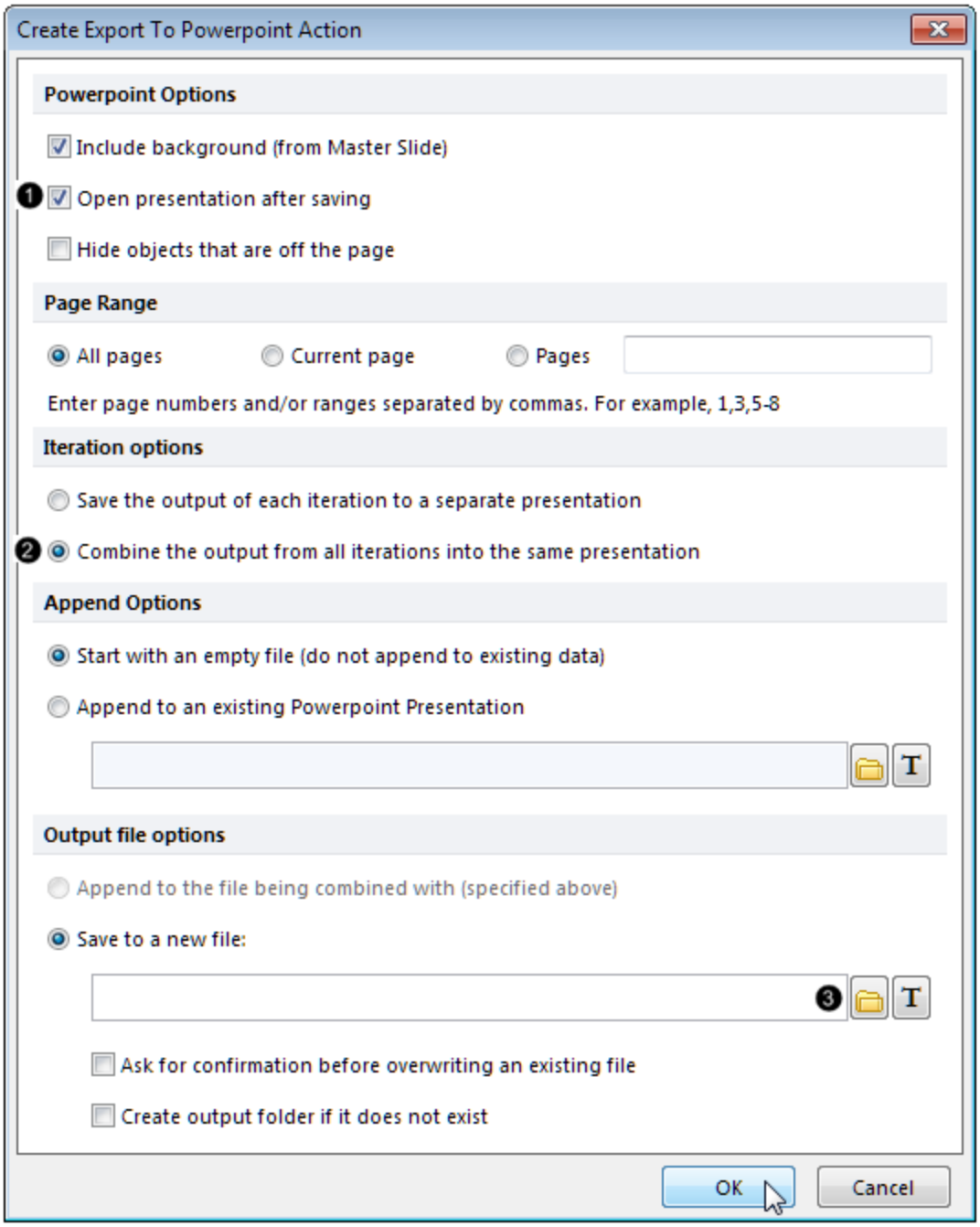

Figure T20.15 Editing the Create Export To Powerpoint Action options.

7. Choose a location and a file name for the Powerpoint file that will be created.
8. Click **Save**.
9. Click **OK**.
10. Click on the **Export to Excel (Column Mode)** in the **Add Report** category.

    The **Excel (Column Mode) Options** window will open.

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11. Click on the folder icon under the **Output file options category→Save to a new file**.
12. Choose a location and a file name for the Excel file that will be created.
13. Click **Save**.
14. Click **OK**.
15. Drag the text box containing the Sample ID from the top of the Gating page on top of the **Excel (Column Mode)** icon in the **Batch Process Actions** window (Fig. T20.16).

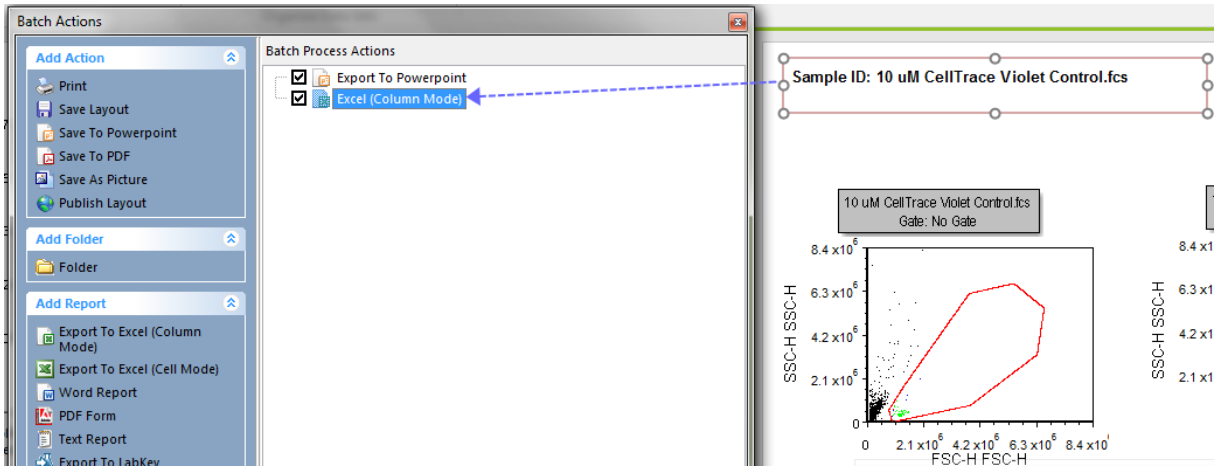

Figure T20.16 Dragging and dropping the sample ID text box to the Excel (Column Mode) batch action.

16. Select all the statistics in the Proliferation Fit Statistics Table so they appear highlighted in blue (Fig. T20.17).
17. Drag the selection on top of the **Excel (Column Mode)** icon in the **Batch Process Actions** window (Fig. T20.17).

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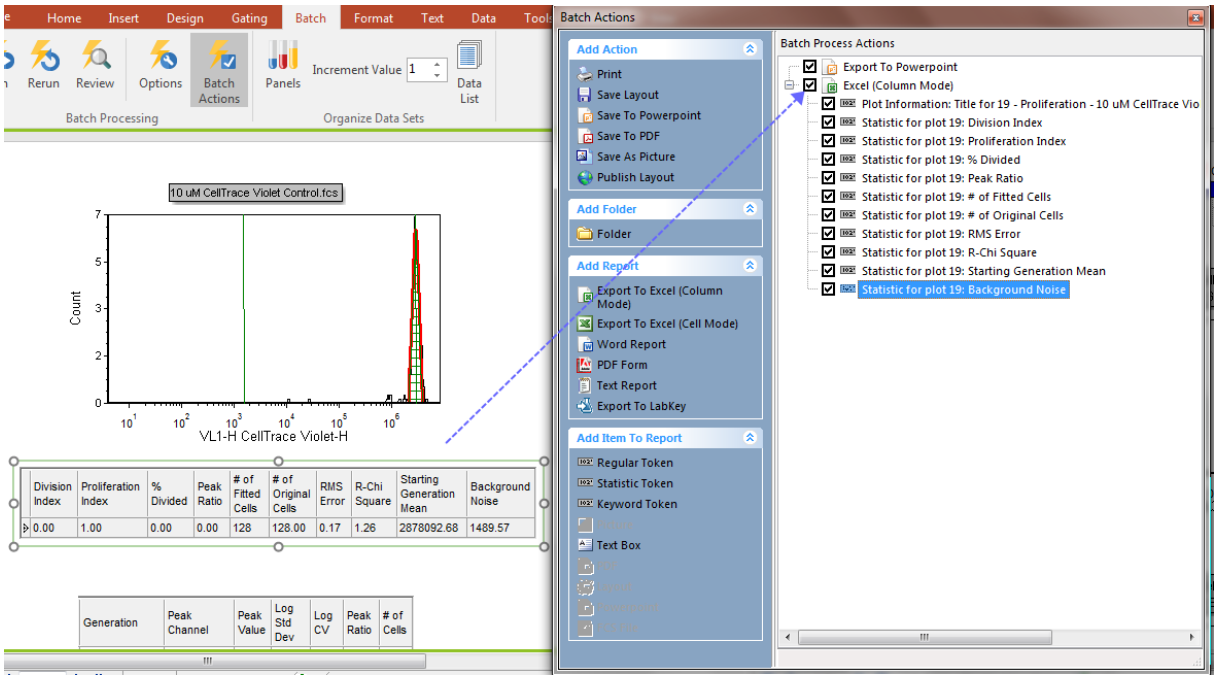

Figure T20.17 Dragging and dropping the Proliferation Fit Statistics table to the Excel (Column Mode) batch action.

Dragging and dropping the sample ID text box and table data to the **Batch Process Actions window→Excel (Column Mode)** icon has added the sample ID and all of the statistics from the table to the excel report.

18. Click the **Batch** tab→ **Batch Processing**→ **Run** button to begin the batch process.

The batch process will proceed through the 4 samples in the data list and generate an 8-slide Powerpoint file which will include 2 slides per sample and an Excel document that will have four rows of data for each of the three samples in the data list.

38.23 Kinetics

Many flow cytometers are capable of recording a time parameter in the .fcs file, allowing for kinetics experiments to be performed. Kinetics experiments are particularly useful for examining changes in DNA content, antibody binding, and calcium flux over time. By adding a compound (usually a drug) to a sample and monitoring influx, efflux, or binding of your probe over time, unique information can be gathered. FCS Express offers the capability to display and analyze kinetics data in an easy and user friendly manner while updating your plots as your analysis changes.

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In the course of this tutorial we will show how to:

- Create parameter math formulas to view ratios between two parameters.
- Create gates and display statistics for **Kinetics Plots**.
- Plot Mean, Geometric Mean, Median, % Above Threshold, and Count.
- Overlay multiple **Kinetics Plots**.
- Format a **Kinetics Plot** to view the data at the correct resolution.

In the first exercise, we will create parameter math formulas for use in kinetics plots 1857.

38.23.1 Using Parameter Math

It is often useful to define a ratio when examining kinetics data. This can be a ratio of two different dyes, or the ratio of the same dye in two different emission wavelengths. The parameter math feature of FCS Express allows the user to define mathematical operations from simple ratios to complex formulas for use in your analysis. FCS Express will also calculate and display the **actual ratio values** rather than an arbitrarily scaled multiplied integer as some other software packages do.

In this section you will learn how to create parameter math formulas for use in Kinetics Plots.

Although any kinetics data files of your own can be used to follow along in this tutorial, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive 1210. The **KineticsTutorial.fey**, **kinetics10.010**, and **kinetics25.025** data files have been embedded and will be used here.

1. Select **File** tab→**Open Layout**.
2. Select the **KineticsTutorial.fey** layout file located in the *FCS Express Sample Data* directory from the **Load Layout** dialog.

In the KineticsTutorial. fey layout the two samples (**kinetics10.010**, and **kinetics25.025**) are already displayed as kinetics plot 914.

The following steps open the transformation window and create a new Parameter Math transformation:

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3. Open the **Transformation** window by selecting the **Tools** tab→**Transformations** →**Transformations** command ❶ (Figure T21.1).

4. Click on the drop down arrow adjacent to the blue plus button ❷ in the transformations dialog(Figure T21.1).

5. Click on **Parameter Math** in the drop down list ❸ (Figure T21.1).

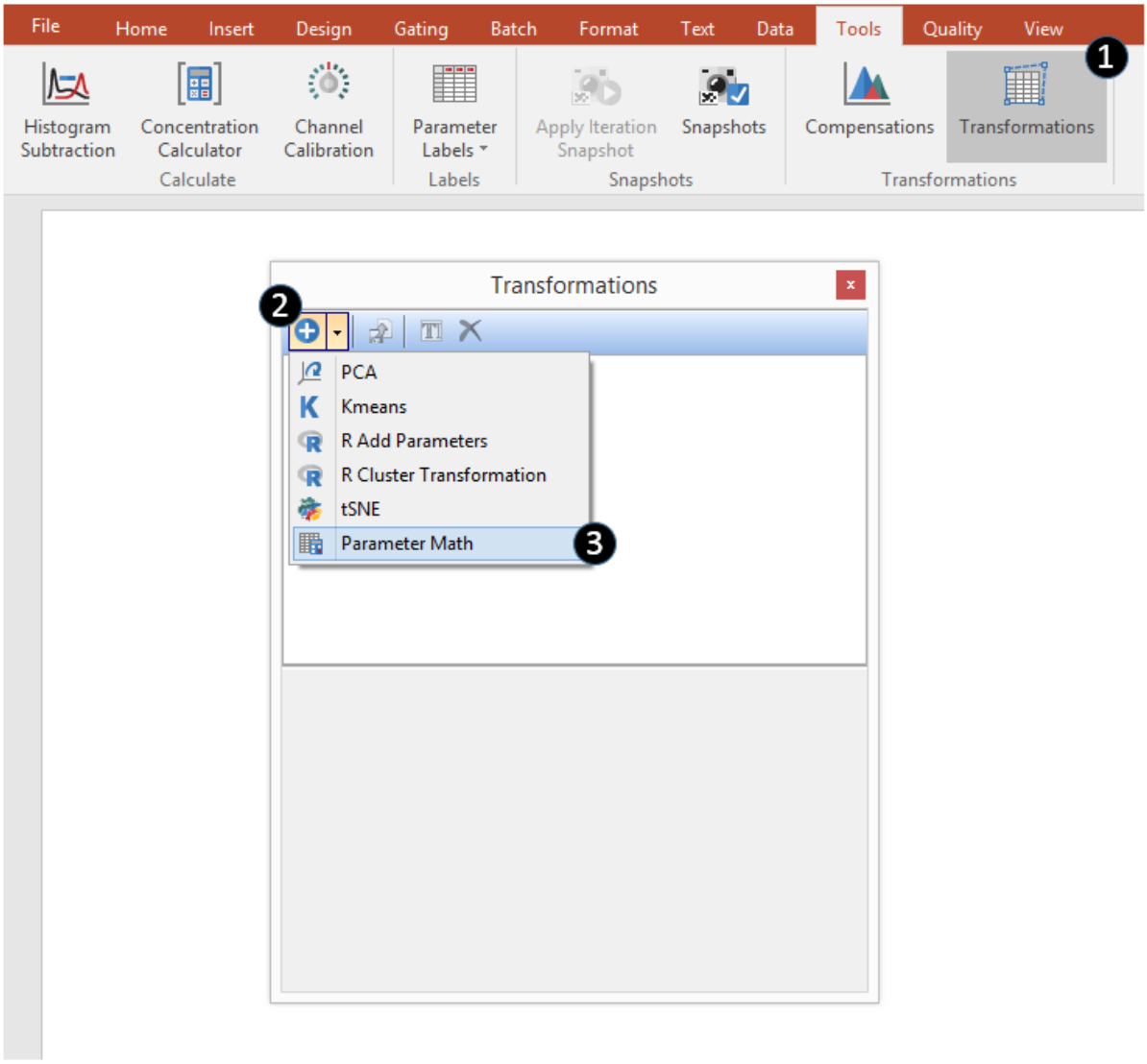

Figure T21.1 Selecting the Parameter Math transformation.

A **New Parameter Math** transformation is created and listed in the upper part of the **Transformation** Window. The **Parameter Math** dialog now appears in

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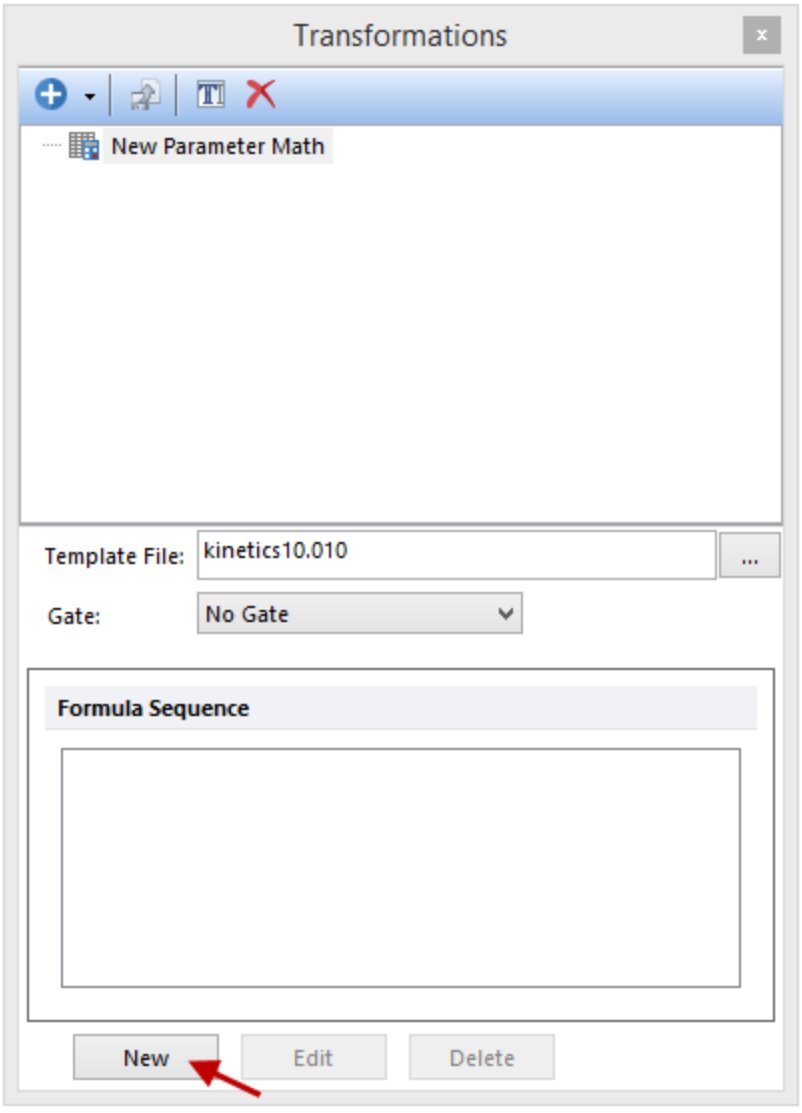
the lower part of the **Transformation** window as seen in Figure T21.2. Note that the **kinetics10.010** sample has been automatically selected as template file in this example but this can be changed by clicking on the ellipsis ... at the right of the Template File field.

6. Choose **New** in the **Parameter Math** dialog (Figure T21.2) to define a new formula.



T21.2 The New Parameter Math dialog as it appear in the Transformation window after Step 5.

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A **Create New Sequence Item** window similar to the one represented in Figure T 21.3 will open.

The following instructions refer to Figure T21.3.

5. Enter **Ca++ Ratio** in the **Output Parameter Name** box ❶.
6. Change the **First Input Parameter** to **Fluo 3** ❷.
7. Change the mathematics operator to "**/**" for division ❸.
8. Change the **Second Input Parameter** to **Fura Red** ❹.
9. Click **OK** ❺.

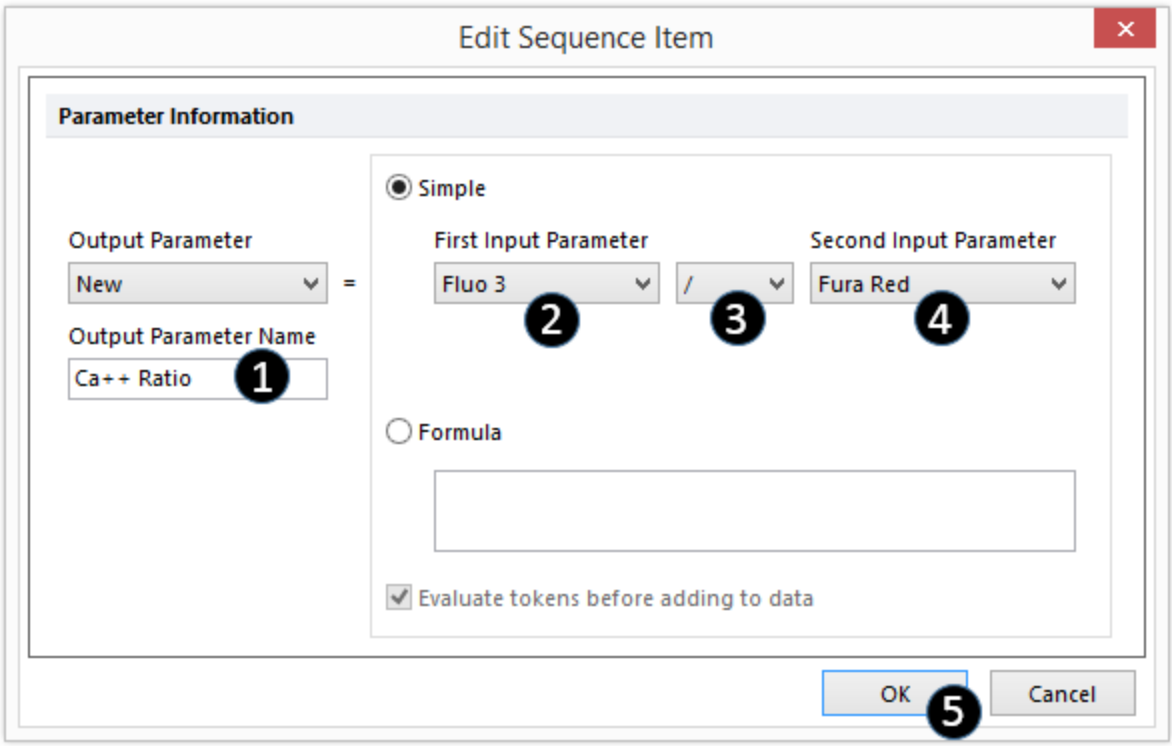

Figure T21.3 Defining a Parameter Math Sequence Item Formula

The **Parameter Math** dialog in the **Transformation** window now displays the formula sequence we defined (Figure T21.4). Please note that, although in this example the **New Parameter Math** transformation contain only the **Ca++ Ratio** formula, a Parameter Math transformation can contain more than one formula.

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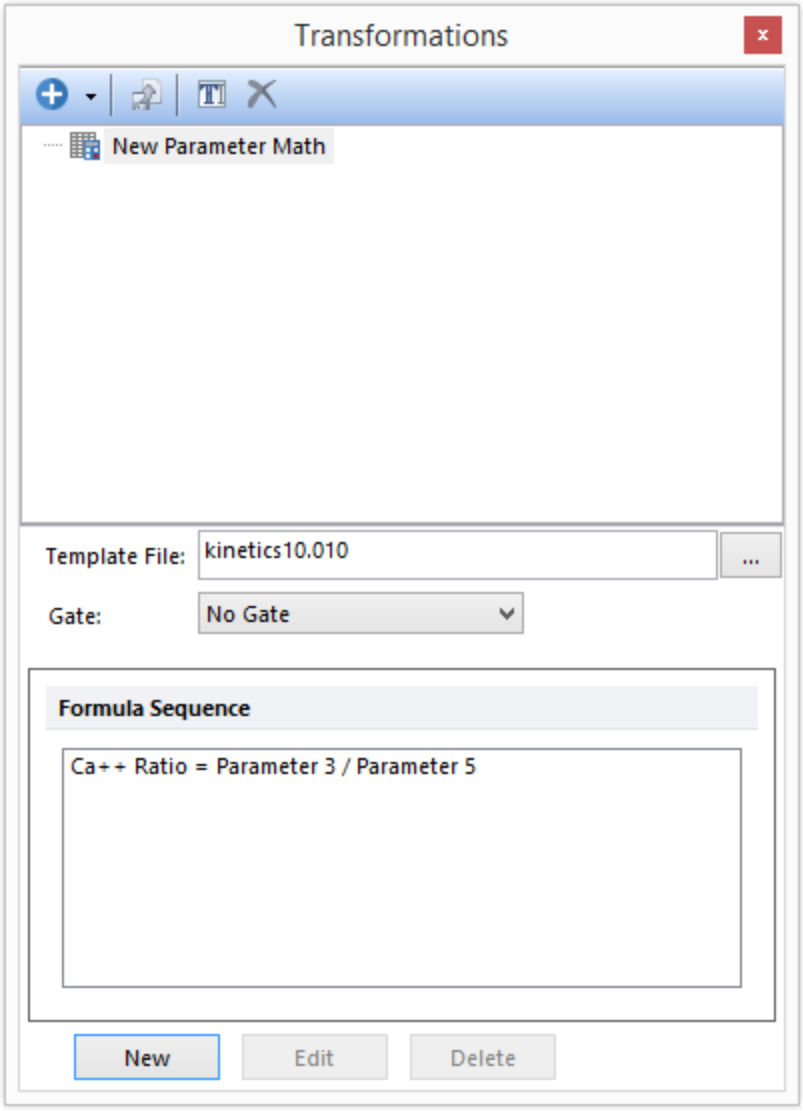

Figure T21.4 Parameter Math Sequence Dialog with Formula Displayed

The Parameter Math transformation has now been defined and can now be applied to plots for display and analysis.

We will now apply the **New Parameter Math** transformation (containing the Ca++ Ratio formula) to the histograms displayed in the layout.

Please note that the **New Parameter Math** transformation has been created using the **kinetics25.025** sample as Template File. Though, since the

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kinetics10.010 sample has the exact same parameter in the same exact order, we can apply the **New Parameter Math** transformation to this sample as well.

10. Left click and hold on the **New Parameter Math** transformation from the **Transformation** window.

11. Drag the **New Parameter Math** transformation out of the Transformation window and release it on top of each of the 1D plot (Figure T21.5).

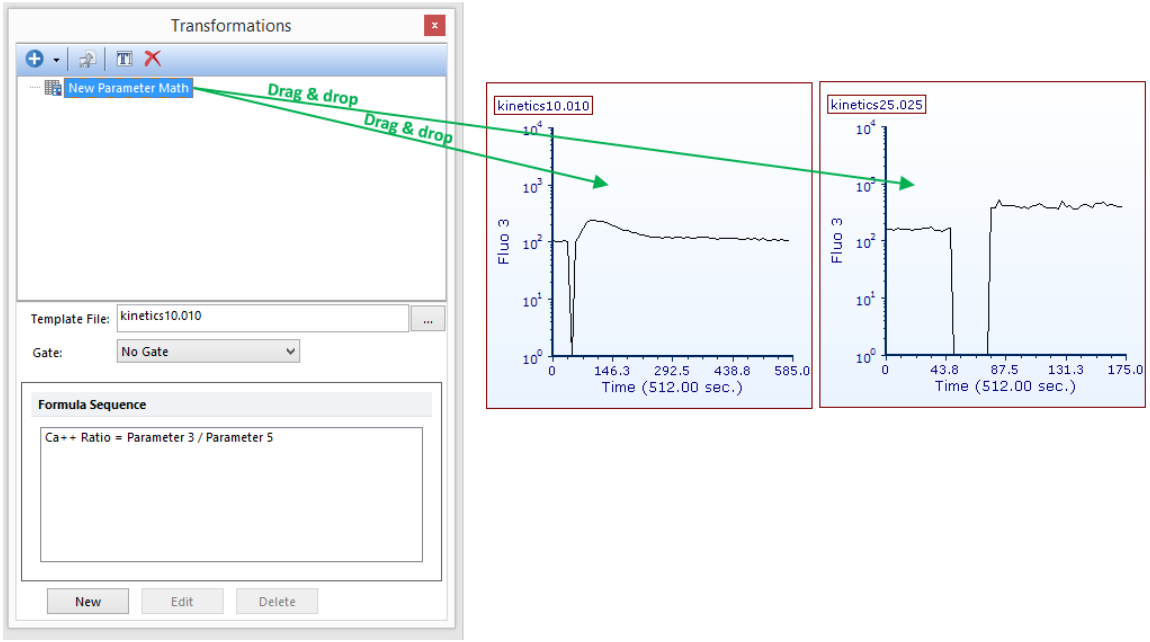

Figure T21.5. Use Darg&Drop to apply the Parameter Math transformation to the plots of the layout.

The two 1D plots should now look as the ones displayed in figure T21.5b. Note the suffix **Parameter Math applied** automatically added to the plot title.

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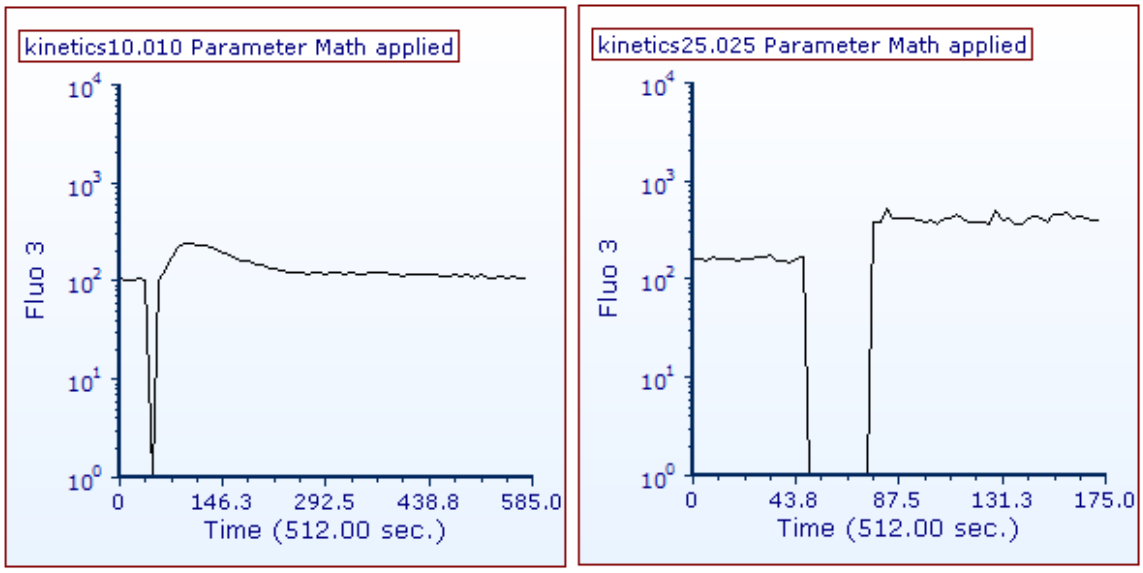

Figure T21.5b The two kinetics plot as they appear when the New Parameter Math transformation has been applied to them.

The newly calculated **Ca++ Ratio** parameter can now be accessed as a standard parameter from the axis labels.

We will now display the **Ca++ Ratio** parameter on the Y axis of both 1D plot simultaneously via the formatting windows.

12. Hit **Ctrl+A** to select all plots.

13. Choose the **Format → Plot Option → Overlay** command from the Ribbon.

14. Change the **Y Parameter** to **Ca++ Ratio** (*i.e.* the formula defined in the **New Parameter Math** transformation) from the drop-down list (Figure T21.6)

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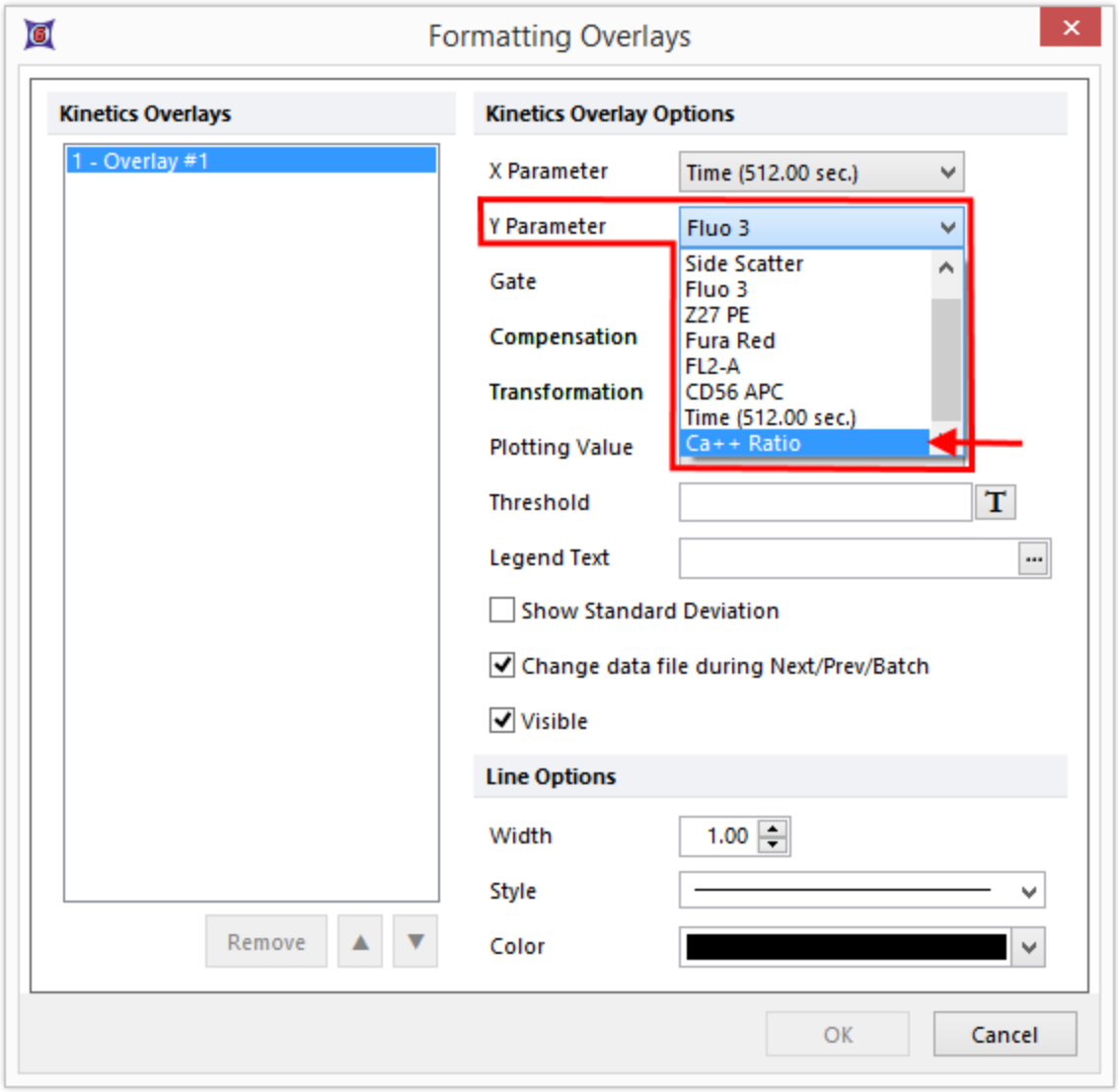

Figure T21.6 Changing the Y Axis to the Ratio Defined by the Parameter Math

The plots on the layout should now look like Figure 21.7. Since the first parameter of the parameter math calculation was a logarithmic parameter, FCS Express assumed the newly created parameter should be a log parameter as well. This is typically the case, but in this situation it is not correct. Since the plots display the real ratio value, we will have to adjust the axis settings to display the data on a linear scale.

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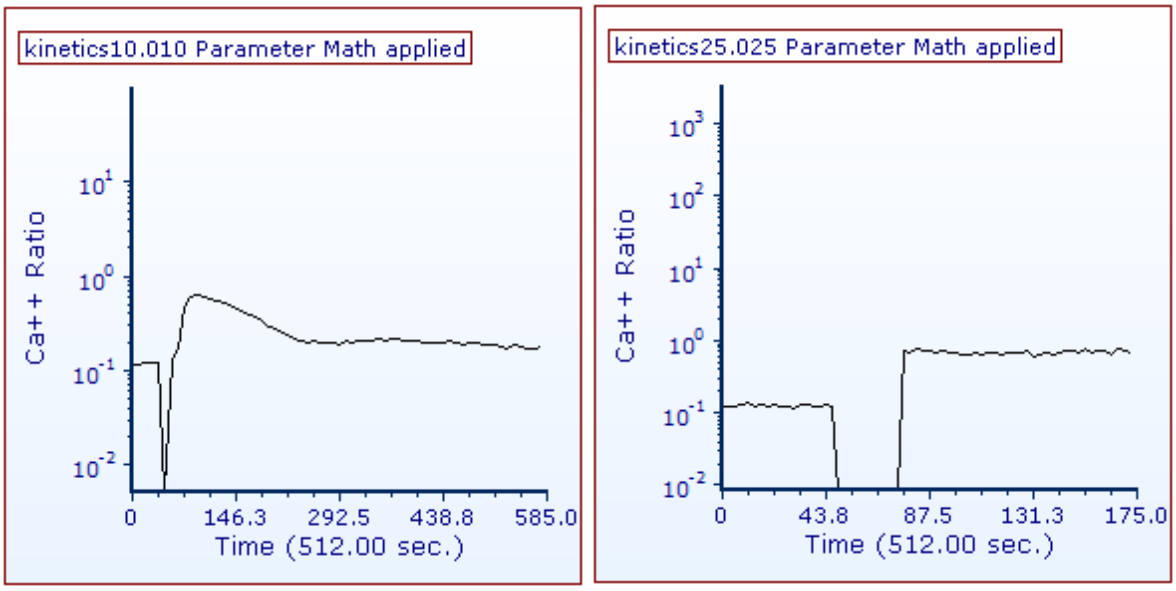

Figure T21.7 Ca++ Ratio vs Time

15. Hit **Ctrl+A** to select all plots.

16. Choose the **Format→Plot Options→Axes** command from the Ribbon.

Refer to Figure T21.8 for the following steps.

17. Under the **Select Axis** section, select **Y-Axis** ❶.

18. Under the **Scale** section, un-check **Automatic** ❷.

19. Change the Scale to **Linear** ❸.

20. Under the **Range** section, un-check **Automatic** ❹.

21. For **Minimum**, enter 0 ❺.

22. For **Maximum**, enter 1 ❻.

23. Click **OK** ❼.

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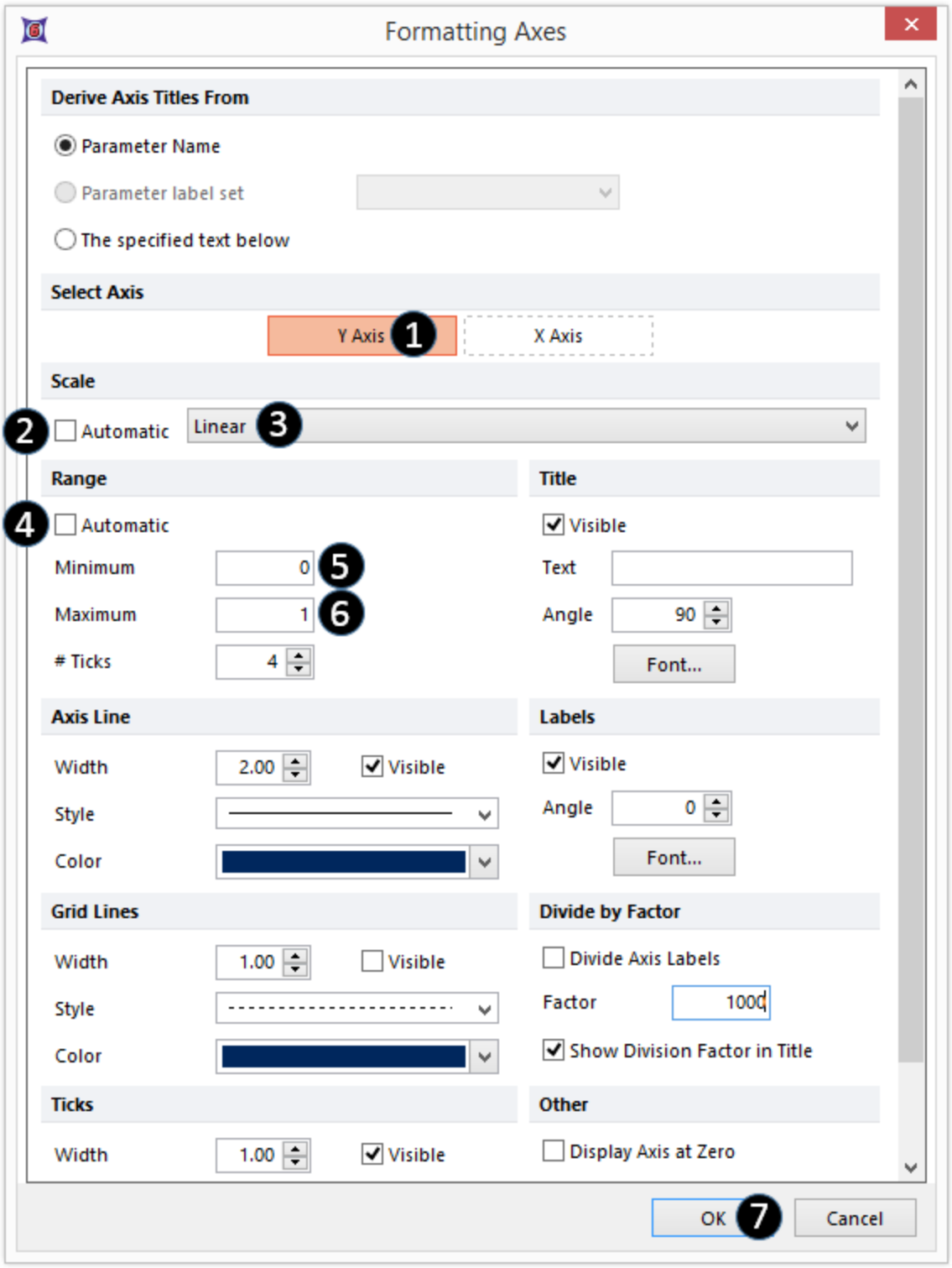

Figure T21.8 Formatting Axes Dialog

The plots in the layout should now look like Figure T21.9.

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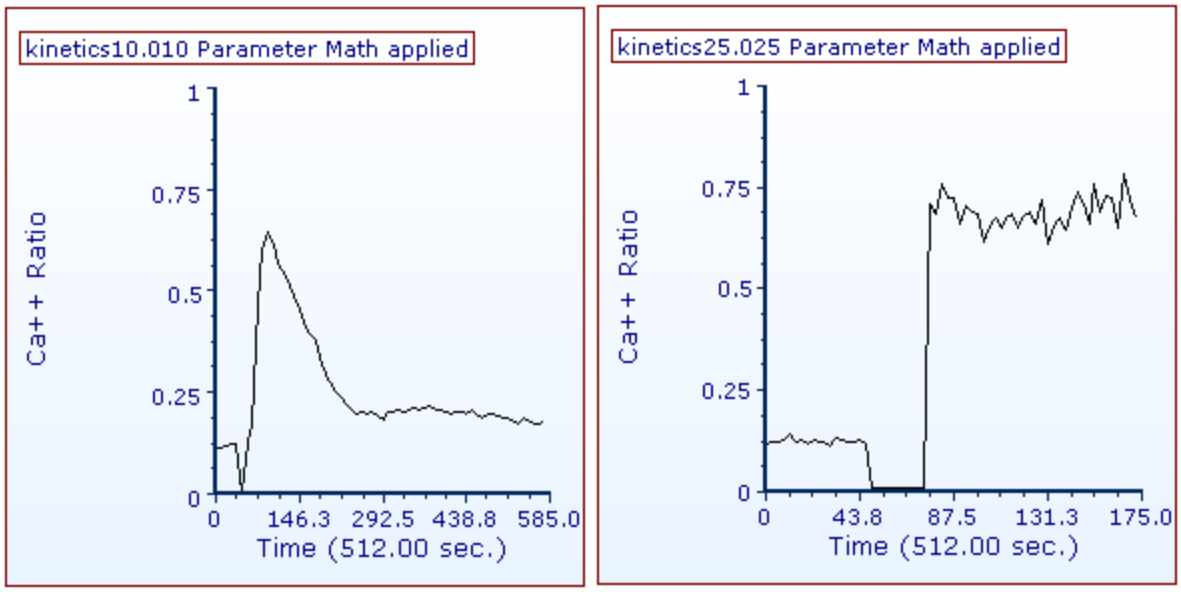

Figure T21.9 Plots Scaled Correctly to Display the Ca++ Ratio

In the next section, we will apply gating and statistics to kinetics plots.

38.23.2 Applying Gating and Statistics

In this section, you will learn how to apply a gate to a kinetics plot and view kinetics statistics for the gate.

Although any kinetics data files of your own can be used to follow along, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive. The **KineticsTutorial.fey**, **kinetics10.010**, and **kinetics25.025** data files will be used here. Please complete the steps in the Using Parameter Math section before beginning.

1. Choose the **Gating→Create Gates→Create Marker** (Figure T21.10).

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Figure T21.10 Creating a Marker

2. Create a 1D (marker) gate on sample **kinetics25.025** similar to the one in Figure T21.11, so that its left boundary defines the baseline and its right boundary is after the peak has subsided.
3. Name the gate "Marker Gate 1".
4. Click **OK**.
5. Choose the **Gating→Create Gates→Create Marker** command.
6. Create a 1D (marker) gate on sample **kinetics10.010** similar to the one in Figure T21.11, so that its left boundary defines the baseline and its right boundary is after the peak has subsided.
7. Name the gate "Marker Gate 2".
8. Click **OK**.

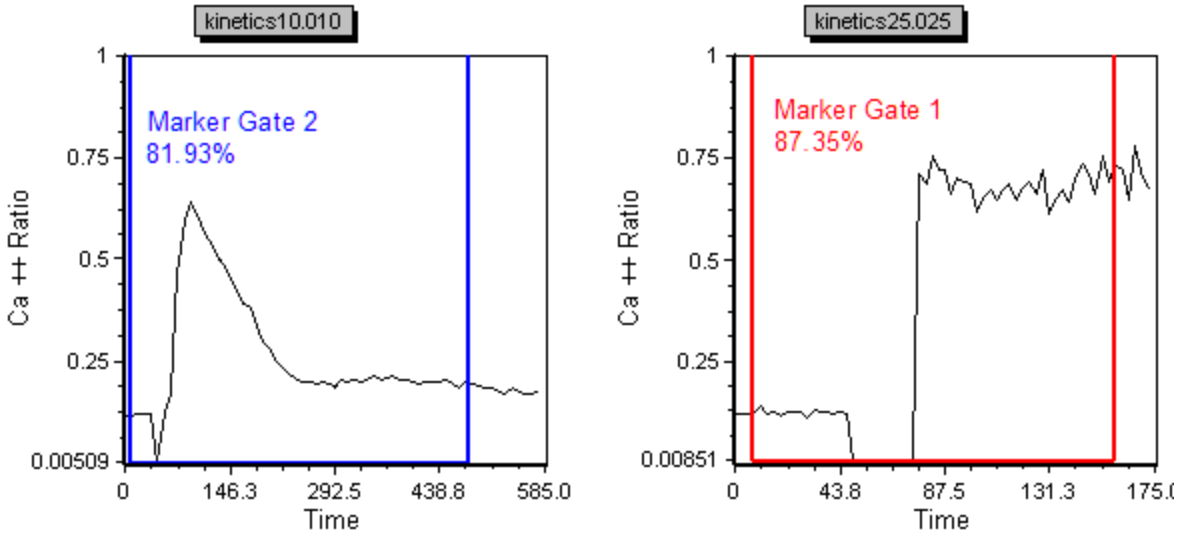

Figure T21.11 Kinetics Plots with 1D Gates Applied

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7. Right-click on the **kinetics25.025** plot.

8. Choose **Kinetics Statistics** from the pop-up menu. A **Kinetics Statistics** window will appear on the layout.

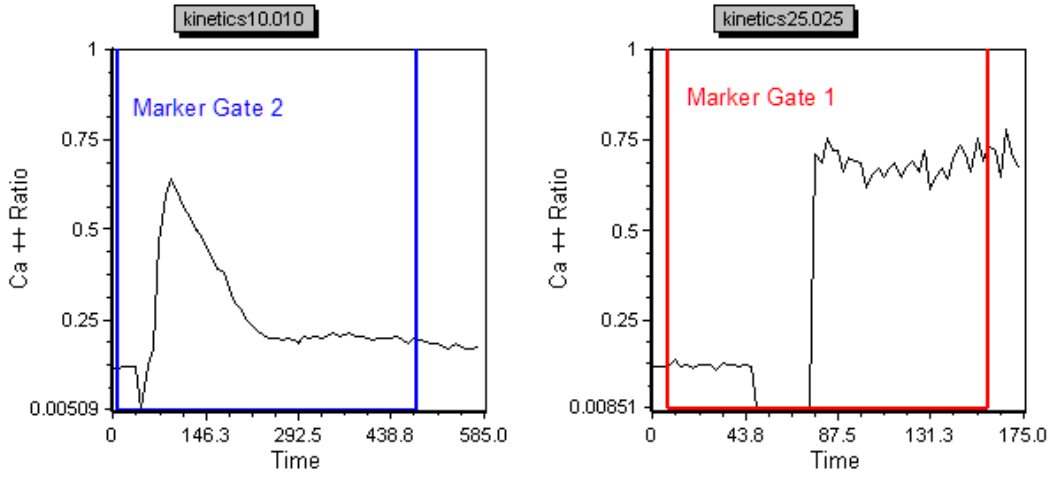
9. Move the **Kinetics Statistics** window to a convenient position on the layout.

10. Right-click on the **kinetics10.010** plot.

11. Choose **Kinetics Statistics** from the pop-up menu. A **Kinetics Statistics** window will appear on the layout.

12. Move the **Kinetics Statistics** window to a convenient position on the layout.

The layout should now look like Figure T21.12. By right-clicking on the **Kinetics Statistics** window, you may choose to view up to 14 different kinetics statistics (Figure T21.13). These values will change dynamically with adjustments to the gates.



Overlay Number	Gate	Start Time	End Time	Elapsed Time	Peak Value	Peak Time
1	Marker Gate 2	9.14	475.31	466.17	0.64	91.41

Overlay Number	Gate	Start Time	End Time	Elapsed Time	Peak Value	Peak Time
1	Marker Gate 1	8.20	155.86	147.66	0.76	82.03

Figure T21.12 Plots with Kinetics Statistics Windows

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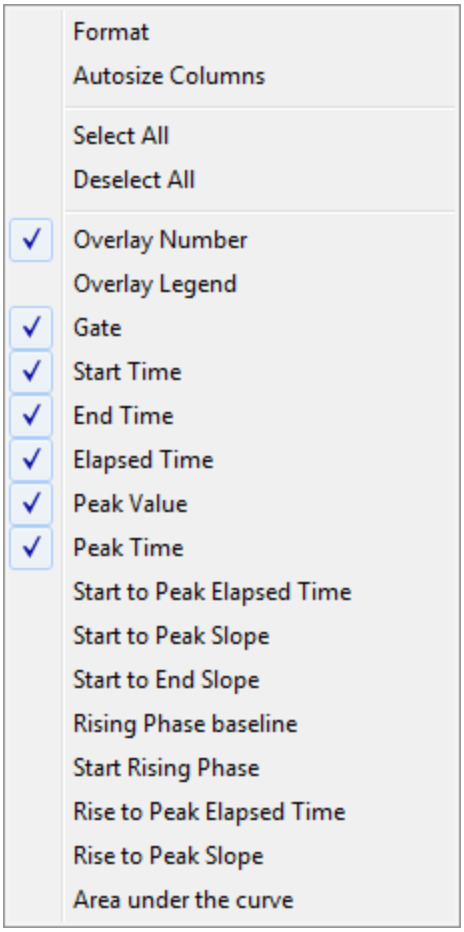

Figure T21.13 Kinetics Statistics Right Click Pop-up Menu

Next, we will explore different plotting values.

38.23.3 Plotting Values

The default parameter for plotting kinetics data is Median, but often times it is useful to look at other values such as Mean, Geometric Mean, Count, and percentage of events above a threshold. In this section, you will learn how to change these plotting values and define a threshold based on gates/tokens.

The sample data files for this section are located in the Tutorial Sample Data archive. The **KineticsTutorialParameterSection.fey** layout will be used here. The layout is embedded with the **kinetics10.010**, and **kinetics25.025** sample data, and has a **Ca++ Ratio** parameter math formula applied.

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1. Click on the **kinetics10.010** plot to select it.
2. Right-click on the plot and choose **Format** from the pop-up menu.
3. Select the **Overlays** category.
4. Change the **Plotting Value** from **Median** to **Mean** (Figure T21.14).

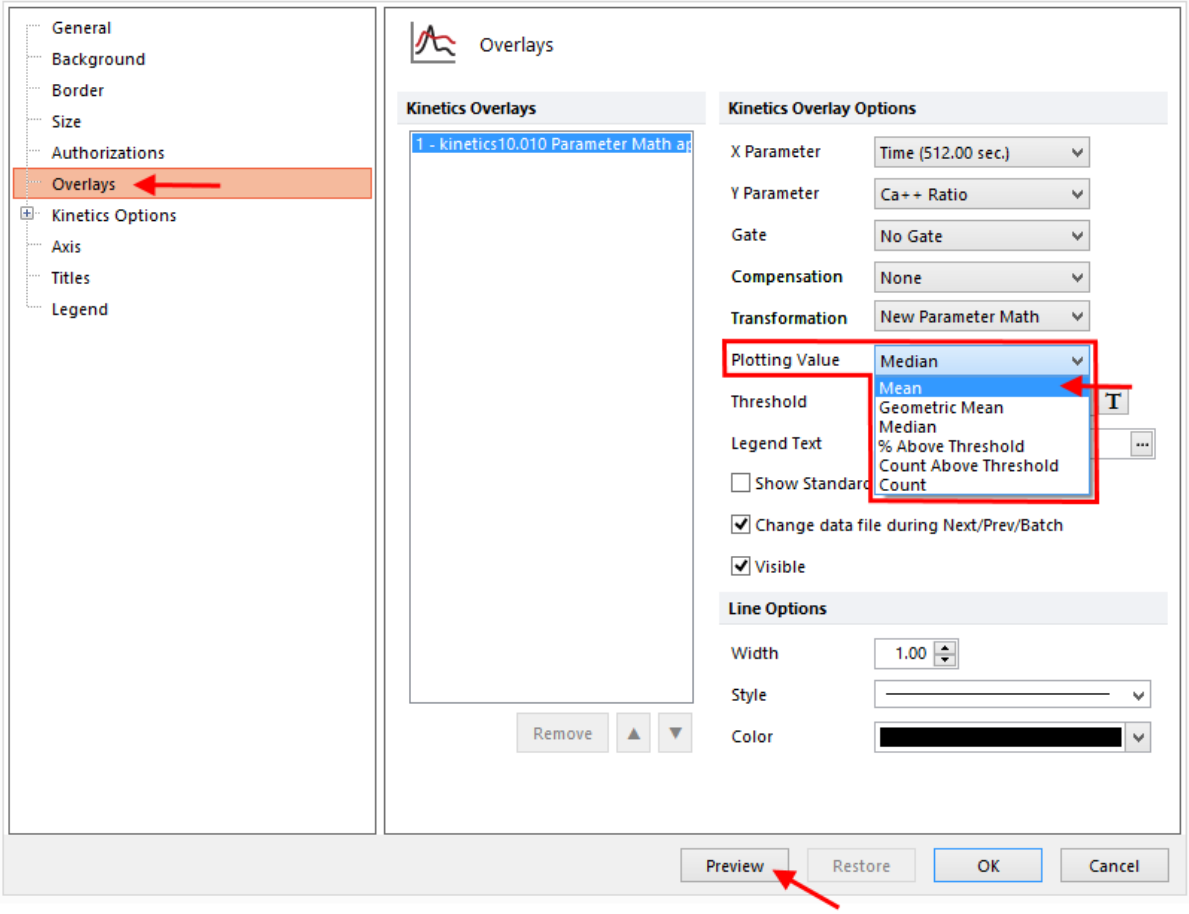

Figure T21.14 Changing the Plotting Value

5. Move the **Format** dialog window so that you can see the **kinetics10.010** plot.
6. Click **Preview**.

The plot will change to display the Mean Ca++ Ratio on the Y-Axis. Steps 1-6 may be repeated to view any of the plotting values.

7. Change the **Plotting value** to **Count**.

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8. Click **Preview**.

Selecting Count will plot the number of events acquired per unit time. This is very useful for quality control, to ensure that the event rate over time is consistent. Notice how the data has many peaks and valleys due to low cell numbers. We will now change the resolution to view the **#Events/Time** at the appropriate resolution.

9. Choose the **Kinetics Options→Binned Plot Specific Options** category (in the **Formatting** dialog).

10. Change the Y-Axis Resolution to **32x32** from the **Resolution** drop-down list (make sure that Y-axis is selected in the list box).

11. Click **OK**.

The plot should now look like Figure T21.15. Count vs. Time is a useful way to view the flow rate of your sample (it can reveal when a sample was removed or placed on the instrument, or if the flow rate decreased or increased for any reason). Note the large valley during the beginning of acquisition in this example when the sample was removed to add a drug.

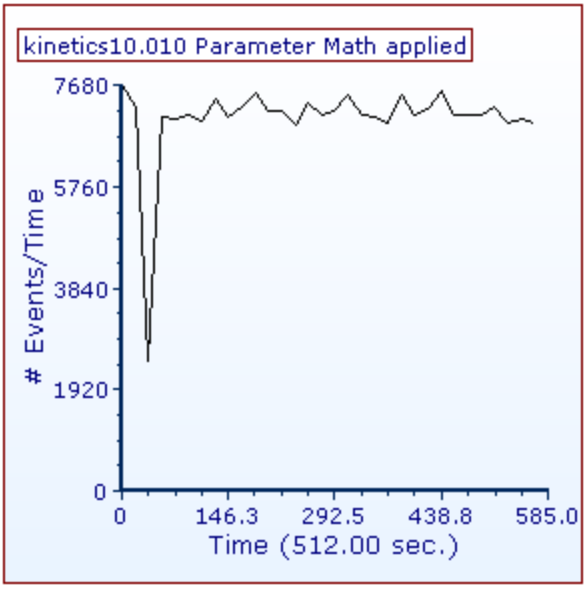

Figure T21.15 Kinetics Plot Displaying Count vs. Time

We will now assign a threshold to view **% Above Threshold** as a plotting value on a new page and plot.

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12. Choose **% Above Threshold** from the page tabs.

You will see a **Kinetics Plot** and a **Density Plot** for **kinetics10.010** with gates applied to the **Density Plot** (Figure T21.16). The gates are applied such that the **Baseline Gate** encompasses events before addition of a drug. Statistics derived from the gate will serve as baseline values for plotting % Above Threshold. The **Post Treatment Gate** includes events after the addition of a drug. We will plot the percentage of cells within this gate that responded with a fluorescence signal above the baseline.

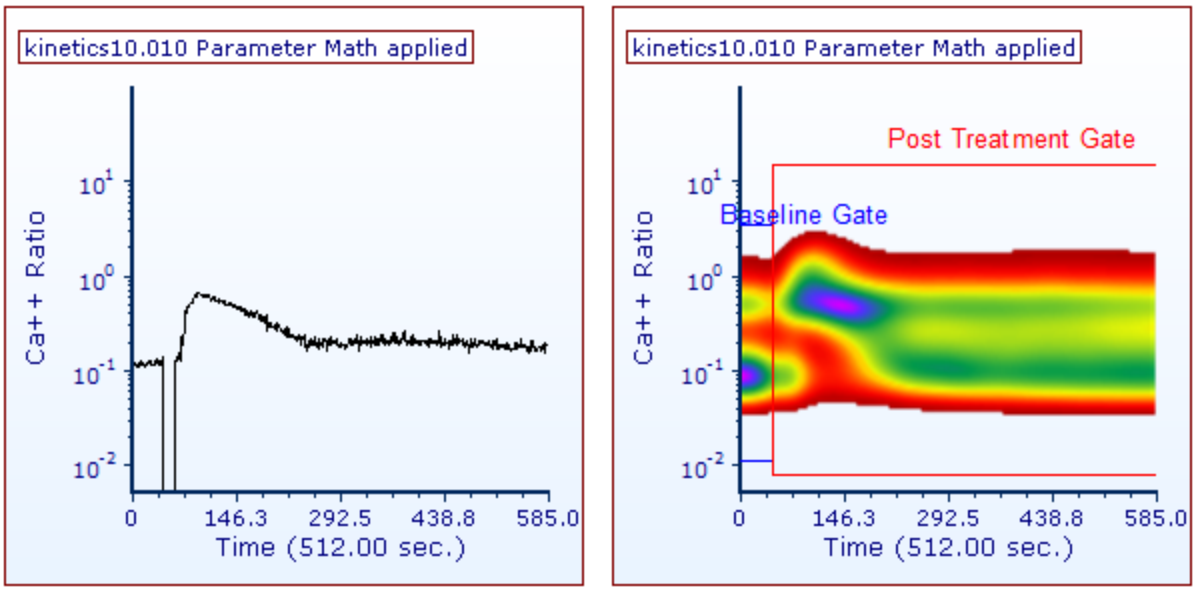

Figure T21.16 Kinetics and Density Plots with Thresholding Gates Applied

13. Select the Kinetics Plot on the left.
14. Right-click on the Kinetics Plot.
15. Choose **Format** from the pop-up menu.
16. Choose the **Overlays** category.
17. Select "Post Treatment Gate" from the **Gate** drop-down list.
18. Choose **% Above Threshold** from the **Plotting Values** drop-down list.
19. Click on the Token Symbol T after the **Threshold** field.
20. Choose "**Y Median + 2 * SD**" from **Custom** in the **Insert a Token** dialog (Figure T21.17). *(The Y Median + 2* SD custom token represents*

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the Y median of the baseline gate plus two standard deviations of the Y median in the baseline gate.)

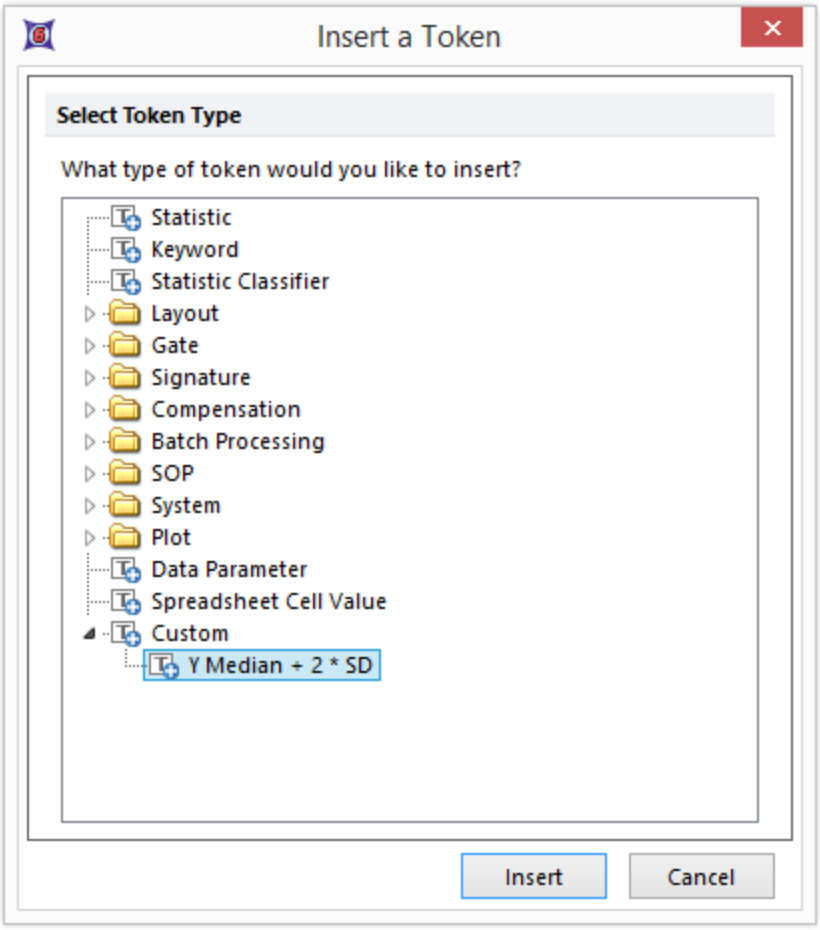

Figure T21.17 Choosing a Custom Token as a Threshold Value

21. Click **OK**.

The plots in the layout should now look like Figure T21.18. The kinetics plot on the left is now displaying the percentage of events in the Post Treatment Gate that are above the Y-Median plus two standard deviations of the baseline gate for the Ca++ Ratio parameter.

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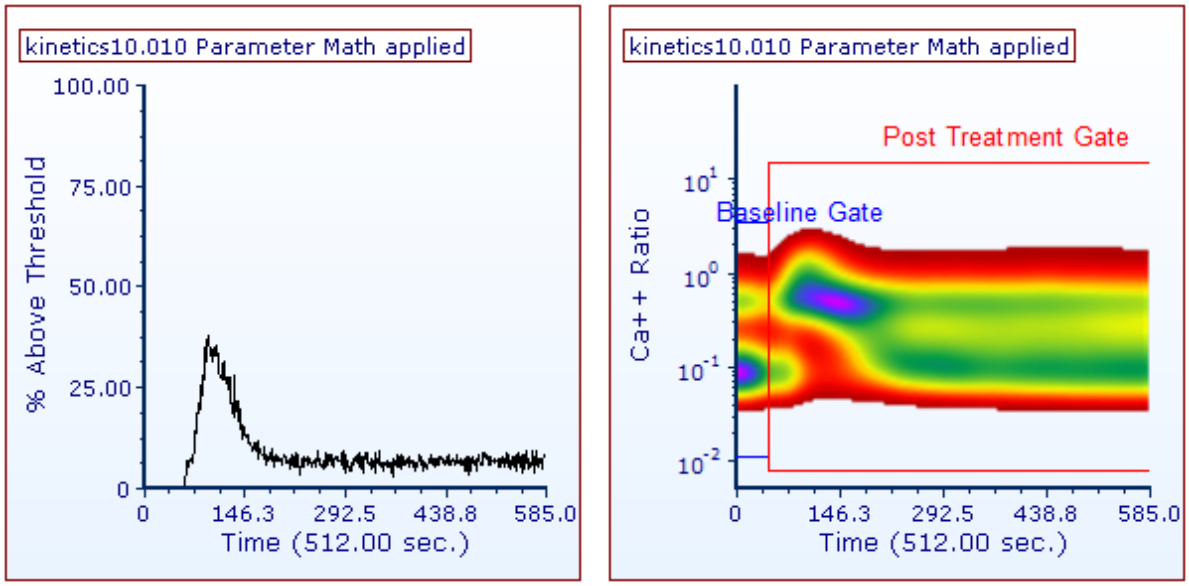

Figure T21.18 Kinetics Plot Displayed as % Above Threshold

In the next exercise, we will overlay and format kinetics plots.

38.23.4 Overlay and Format Kinetics Plots

It is often useful to look at many samples on the same plot to see sample variation and to compare experimental samples against a control. In this section, you will learn how to overlay multiple kinetics samples, apply parameter math to the overlays, and format the overlays to make the data easier to view.

Although any kinetics data files of your own can be used to follow along, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive. The **KineticsTutorialFormatting.fey**, **kinetics8.008**, **kinetics9.009, kinetics10.010**, and **kinetics25.025** data files will be used here.

1. Select **File** tab→**Open Layout**.
2. Select the **KineticsTutorialFormatting.fey** layout file located in the *FCS Express Sample Data* directory from the **Load Layout** dialog.

The layout contains one kinetics plot displaying Time vs Fluo 3 for sample kinetics25.025. We will now add the other three samples to the kinetics plot as overlays.

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3. **Ctrl+Click** on samples **kinetics8.008**, **kinetics9.009**, and **kinetics10.010** to select them in the data list.
4. Drag the files from the data list onto the Kinetics Overlay plot.
5. Release the mouse button to drop them on the plot.
6. Choose **add the files to the plot as new overlays**.
7. Click **OK**.

The four sample are now displayed simultaneously in the kinetics plot. Though, the Parameter Math transformation is not yet applied to the plot. We will now apply the Parameter Math transformation to the plot via drag and drop.

8. Left click and hold on the **New Parameter Math** transformation from the **Transformation** window.
9. Drag the **New Parameter Math** transformation out of the Transformation window and release it on top of the kinetics plot (Figure T21.5). The Parameter Math applied suffix will be added to the plot title as a confirmation of this action.
10. Change he Y-Axis parameter on the **Kinetics Overlay** plot to **Ca++ Ratio**.
11. Select the **Live Cells** gate from **Gating→Create Gates→Current Gate** section of the Ribbon bar.

The plot now contains overlays for each sample and should look like Figure T21.19.

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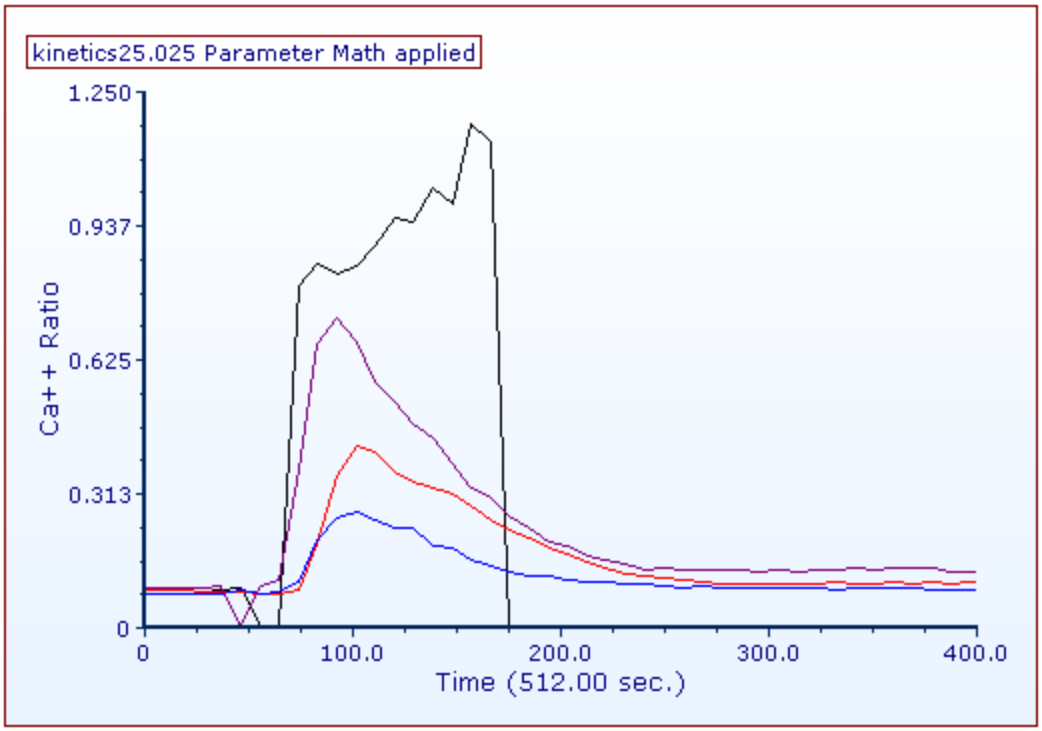

Figure T21.19 Multiple Kinetics Overlays

We will now format the plot to make it more visually appealing.

18. Right-click on the plot.
19. Choose **Format** from the pop-up menu.
20. Choose the **Overlays** category.
21. **Ctrl+Click** on all of the plots in the list box to select them.
22. Change **Line Options→Width** to **1.50**.
23. Choose the **Legend** category.
24. Click on the **Visible** check box to select it.
25. Change the **Position** from **Right** to **Bottom**.
26. Click **OK**.

The plot should now look like the Figure T21.20.

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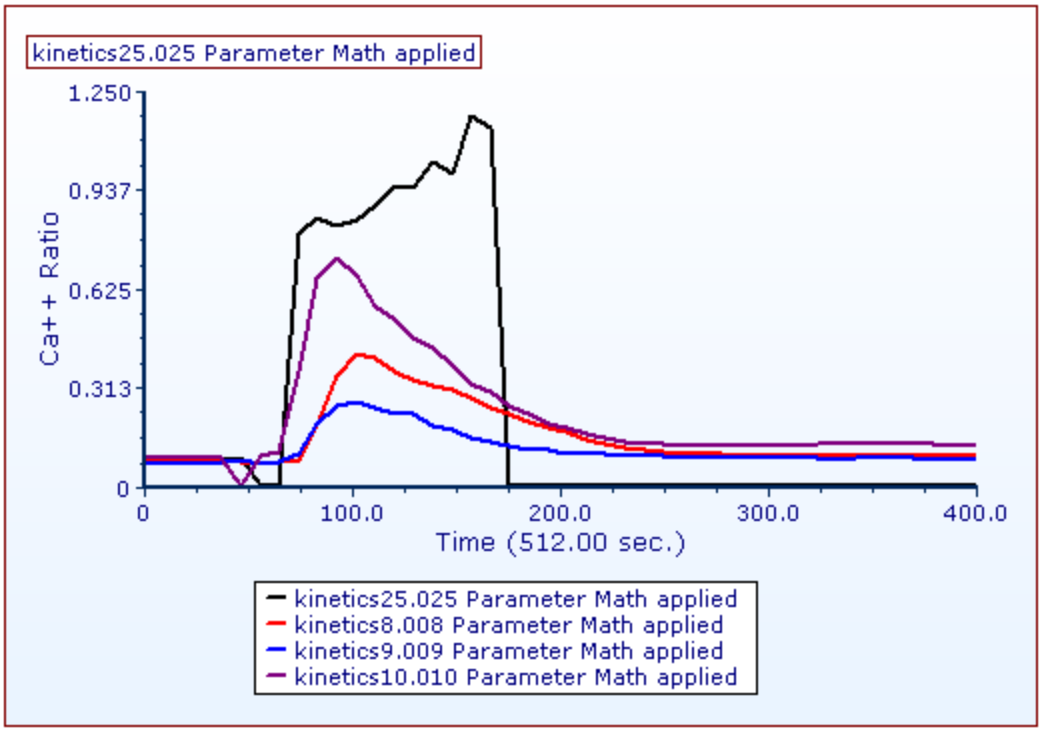

Figure T21.20 Kinetics Plots Formatted

Finally, we will change the resolution of a kinetics plot.

38.23.5 Format to Change Resolution

From time to time your kinetics data files may not be displayed suitably with your default settings from the preferences. In this section, you will learn how to adjust resolution to view your data correctly, and how to show the standard deviation of your kinetics curves.

Although any kinetics data files of your own can be used to follow along, you have the option of using the same sample data files used in this tutorial. The sample data files are located in the Tutorial Sample Data archive. The **KineticsTutorialScaling.fey**, **kinetics10.010**, and **kinetics25.025** data files will be used here.

1. Select **File** tab→**Open Layout**.
2. Select the **KineticsTutorialScaling.fey** layout file located in the *FCS Express Sample Data* directory from the **Load Layout** dialog.

The layout should look like Figure T21.21 below. The red highlighting is showing the standard deviation of the sample while the black line is displaying the Median

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of Fluo 3. Sample **kinetics10.010** is scaled correctly and is not showing standard deviation. Notice how sample **kinetics25.025** has a very jagged black line and is displaying standard deviation. The jagged black line is occurring because the resolution on the plot is set too high. We will now format the kinetics25.025 plot to remove the standard deviation highlighting and adjust resolution to display the plot correctly.

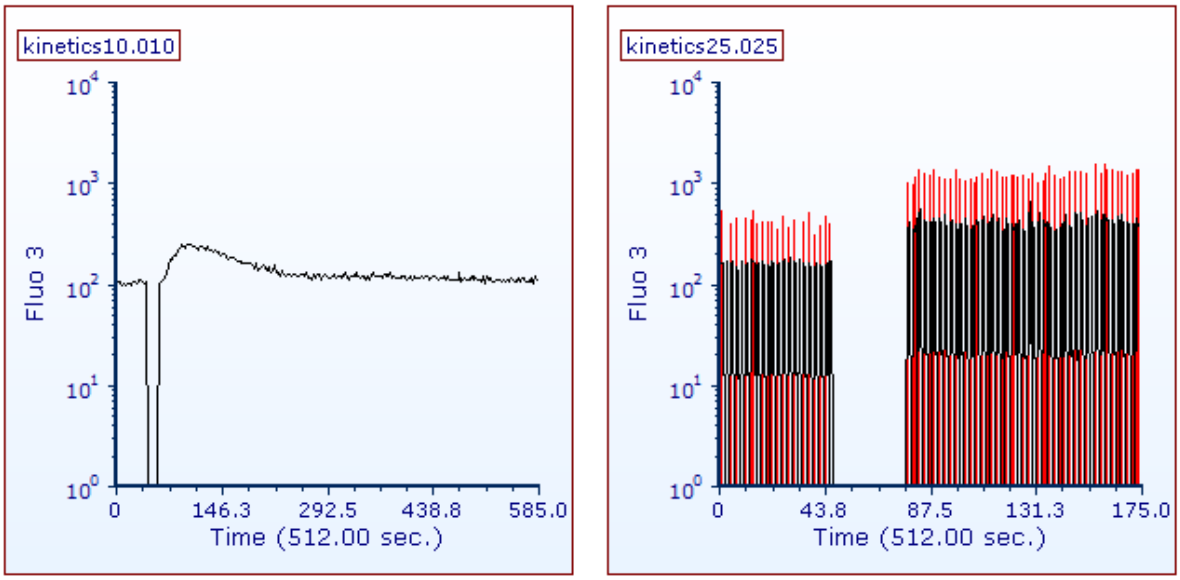

Figure T21.21 Kinetics Tutorial Layout After Adding Sample Data

3. Click on the **kinetics25.025** plot to select it.
4. From the Ribbon Bar choose the **Format→Plot Options→Overlays** command.
5. Uncheck the **Show Standard Deviation** check box (Figure T21.22).

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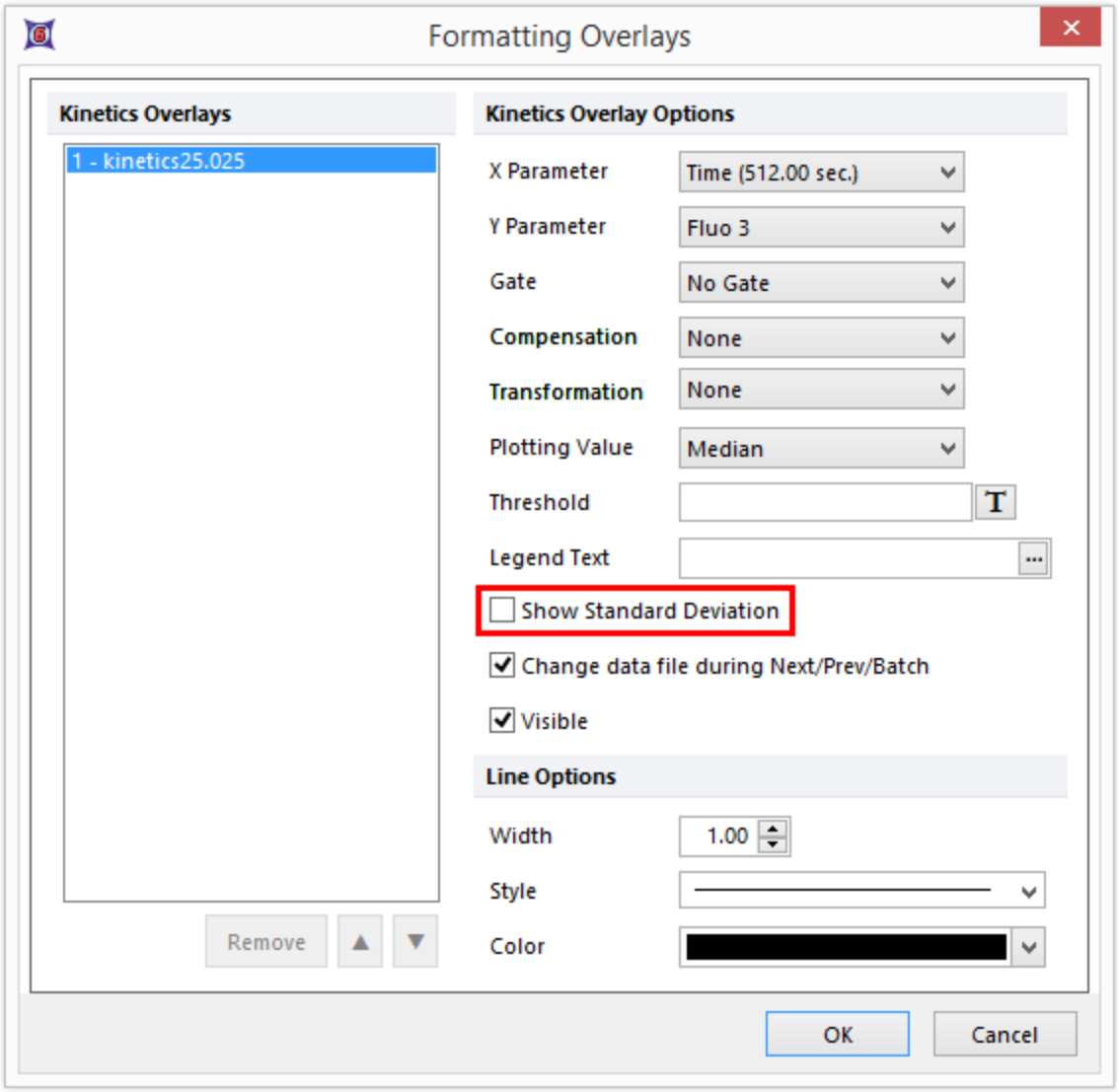

Figure T21.22 Unchecking Show Standard Deviation

6. Click **OK**.

The red standard deviation highlighting will now be removed as in Figure T21.23.

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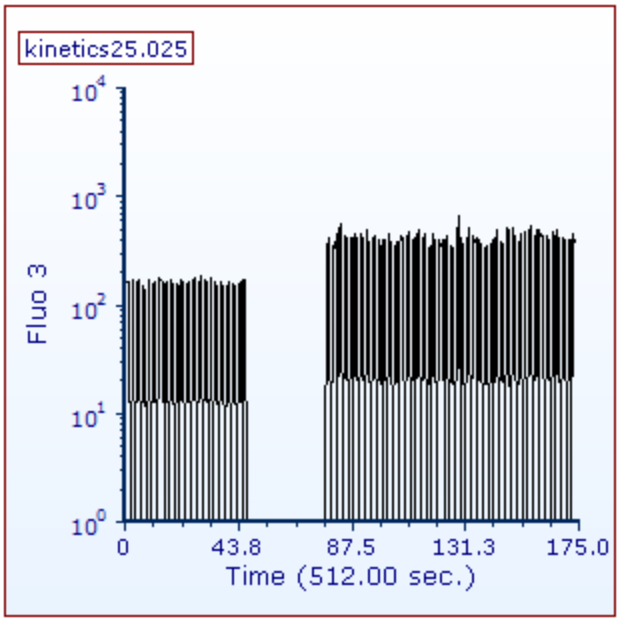

Figure T21.23 Sample Plots with Standard Deviation Highlighting Removed

We will now change the resolution of sample **kinetics25.025** to display the plot properly.

7. Right-click on the **kinetics25.025** plot.
8. Choose **Format** from the pop-up menu.
9. Choose the **Kinetics Option→Binned Plot Specific Options** category.
10. Click on the **Resolution** drop-down list under **Resolution Options** (Figure T21.24).

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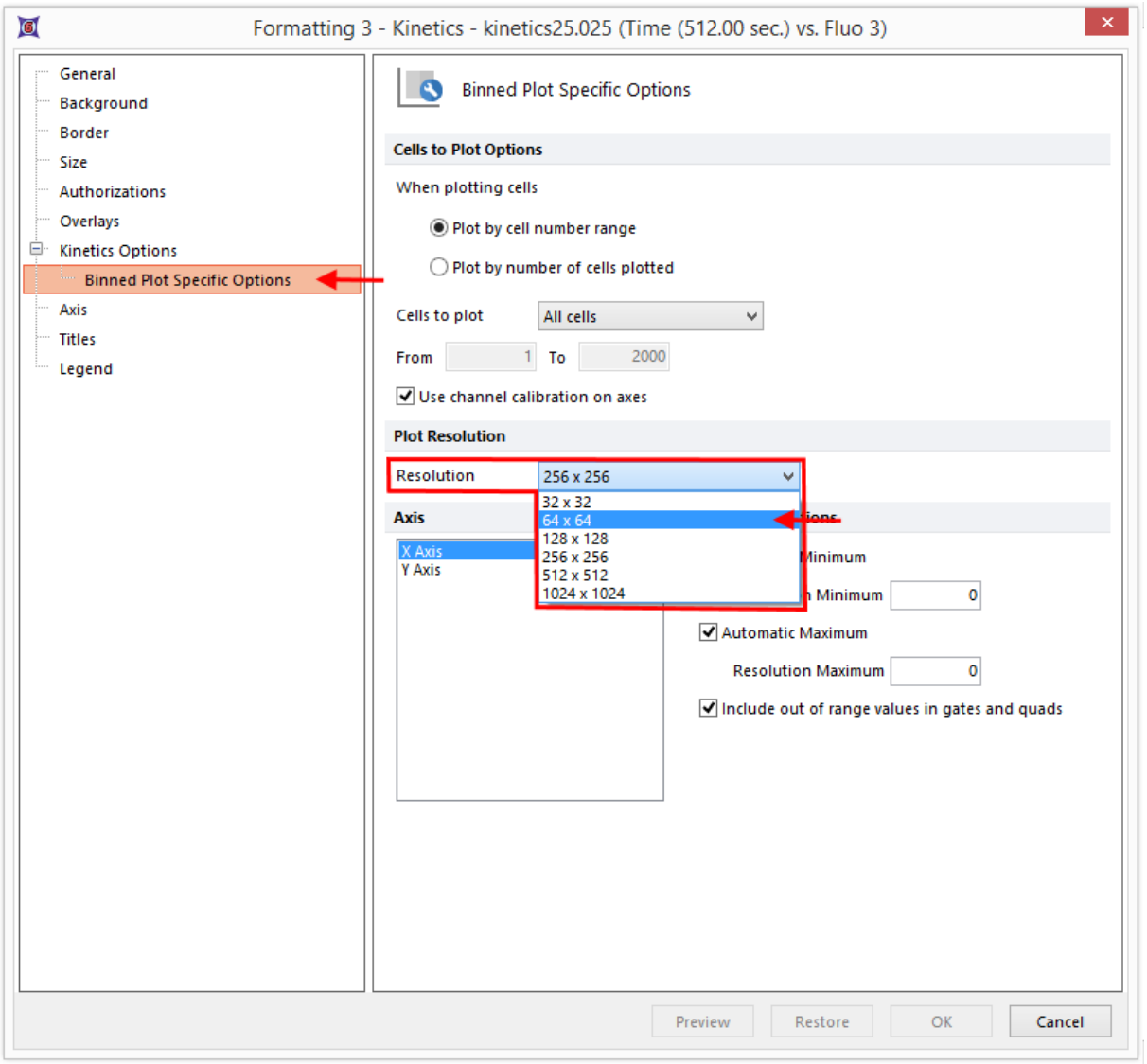

Figure T21.24 Changing the Resolution on a Plot

11. Select the **64x64** resolution option.
12. Click **OK.**

The plot will now change and be scaled properly as seen in Figure T21.25.

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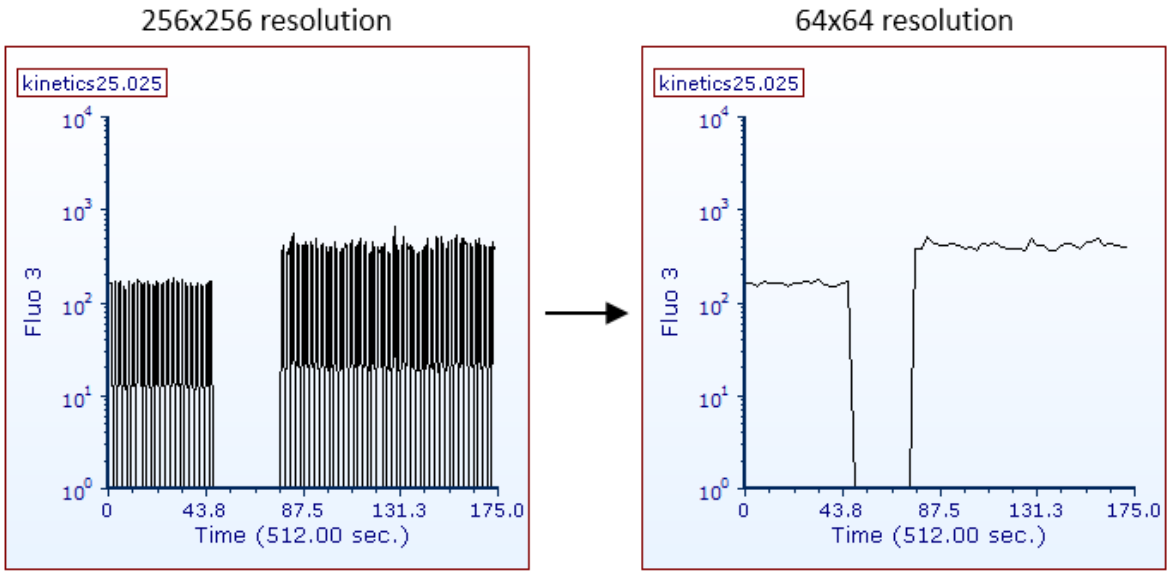

Figure T21.25 Changing the Resolution: 256x256 (Left) to 64x64 (Right)

After following the instruction above, the layout should look like Figure T21.26.

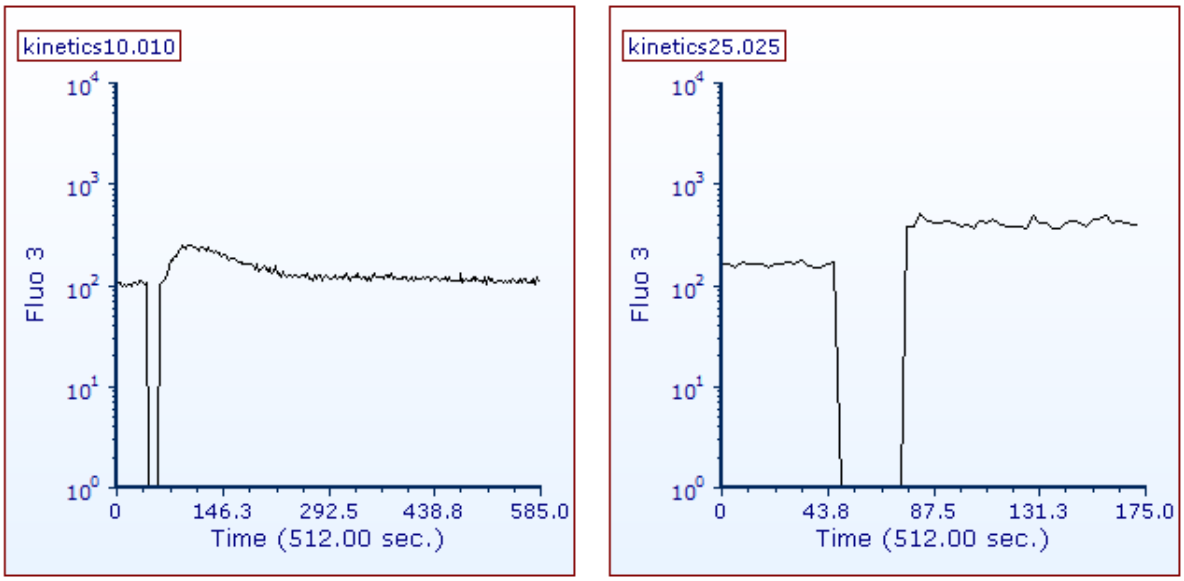

Figure T21.26 Kinetics Plots Scaled Properly

We will now apply a gate and the standard deviation highlighting to both of the plots.

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13. Hit **Ctrl+A** to select all plots.
14. Choose the **Format→Plot Options→Overlays** command.
15. Change the **Gate** to **Gate 1**.
16. Check the **Show Standard Deviation** check box.
17. Click **OK**.

The layout should now look like Figure T21.27.

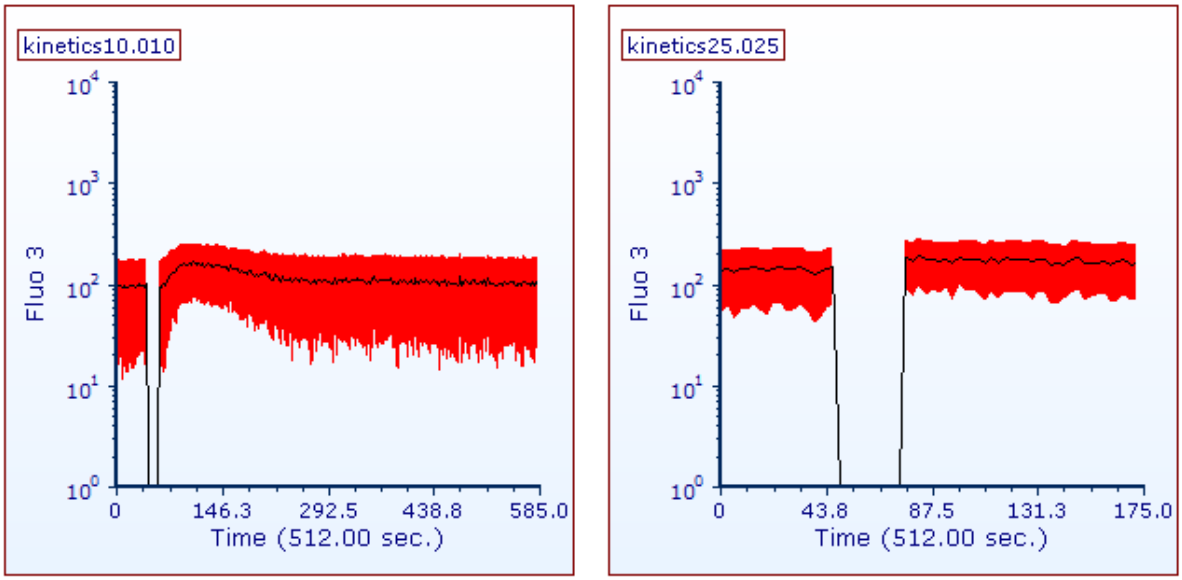

Figure T21.27 Kinetics Plots with Standard Deviation Highlighting Applied

38.24 Cell Concentration

FCS Express can calculate the absolute concentration of the cells in a sample. FCS Express does so by automatically calculating the volume of the sample that was used during acquisition. The volume can be calculated by a) using beads that were present in the sample tube at a known concentration, b) using the sample flow rate and acquisition time, or c) using the volume keyword from FCS 3.1 files, or the equivalent in Amnis DAF files, if available. FCS Express can display the concentration as a one time calculation, in a statistics table, or as a token.

To begin, we will set up the bead gates 1885.

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38.24.1 Setting up the Bead Gates

In the course of this example, we will:

- Learn how to gate beads using hierarchical gating.

For more information about inserting a plot[1225], see the tutorial on that subject.

1. Open a new, blank layout.
2. Insert a forward (FSC-H) vs side scatter (SSC-H) dot plot[172] of the file **Monocytes.002** found in the *FCS Express Sample Tutorial Data* folder.
3. Create a polygonal gate around the monocytes, calling it **Monocytes** (Figure T22.1).
4. Create a rectangular gate around the beads, calling it **Beads1** (Figure T22.1).

The gates should look similar to the ones shown in the plot on the left side of Figure T22.1. For more information on creating gates, please refer to the Gating tutorial[1266].

5. Create a second dot plot, this time showing FL1-H vs SSC-H (Figure T22.1).
6. Apply the **Beads1** gate to the new plot. For more information about applying gates, please refer to the Gating tutorial[1266].
7. Draw a new rectangular gate around the beads, calling it **Beads2**, as shown on the plot on the right side of Figure T22.1.

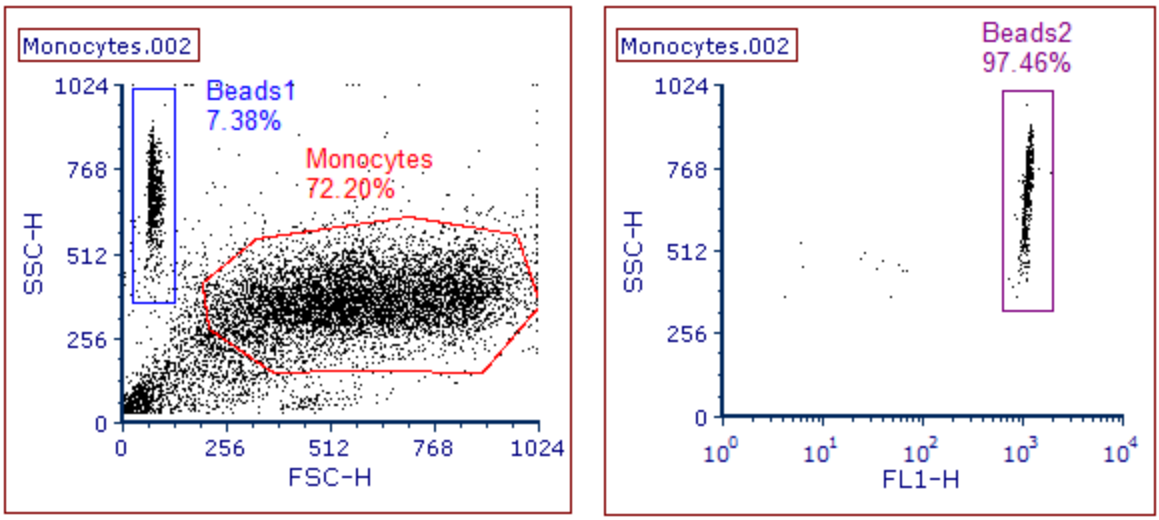

Figure T22.1 Gates for Monocytes and Beads

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The **Beads2** gate allows for added discrimination between the cells and the beads. Depending on how well separated the beads are from your sample, you can use an approach consisting of only one gate, but applying two gates generally allows for cleaner isolation of the bead events to exclude doublets and higher order aggregates.

In the next section, we will use the Concentration Calculator 1886 tool.

38.24.2 Concentration Calculator (Beads)

In the course of this example, we will:

- Identify the variables needed for the concentration calculation.
- Set up the concentration calculator using beads.
- Obtain a one time result.

In order to calculate the concentration, we need the following four parameters:

A. The file to be analyzed.

B. The cells of interest.

C. The gate containing the beads that were added to the sample tube.

D. The concentration of the beads in the sample tube.

Now that the gating strategy for the cells and beads is ready, we will proceed to do the actual calculations. (Note that the following sections build upon steps 1 through 7 in the Setting up the Beads Gates chapter 1885)

1. Select the **Tools→Calculate→Concentration Calculator** command (Figure T22.2).

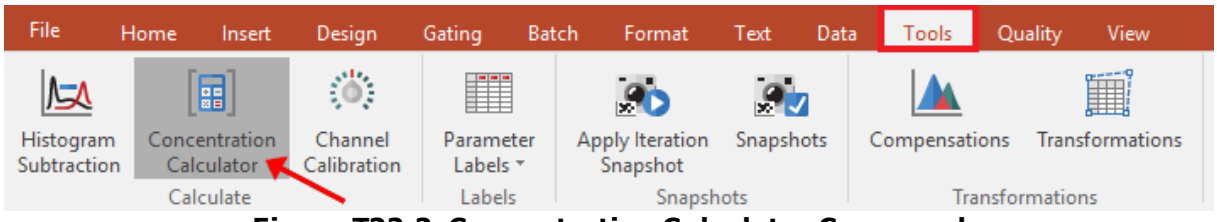

Figure T22.2 Concentration Calculator Command

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The **Concentration Calculator** dialog now appears as shown in Figure T22.3. The following steps all refer to Figure T22.3.

2. Select the **File to analyze**, in this case **Monocytes.002**, from the drop-down list ❶.
3. Select **Monocytes** as the **Gate to analyze**, from the drop-down list ❷.
4. Select the **Reference Beads** radio button ❸ to specify the **Volume Calculation** type.
5. Select **Beads2** for the **Bead Gate** ❹.
6. Input the concentration of the beads in the sample tube. In this case we will use a value of **100,000** beads/ml ❺.
7. Click the **Calculate** button.

In the **Results** section of the **Concentration Calculator**, the volume of the sample analyzed appears in the **Volume Analyzed** Ⓐ field and the concentration of the cells in the **Monocytes** gate appears in the **Sample Concentration** Ⓑ field (Figure T22.3).

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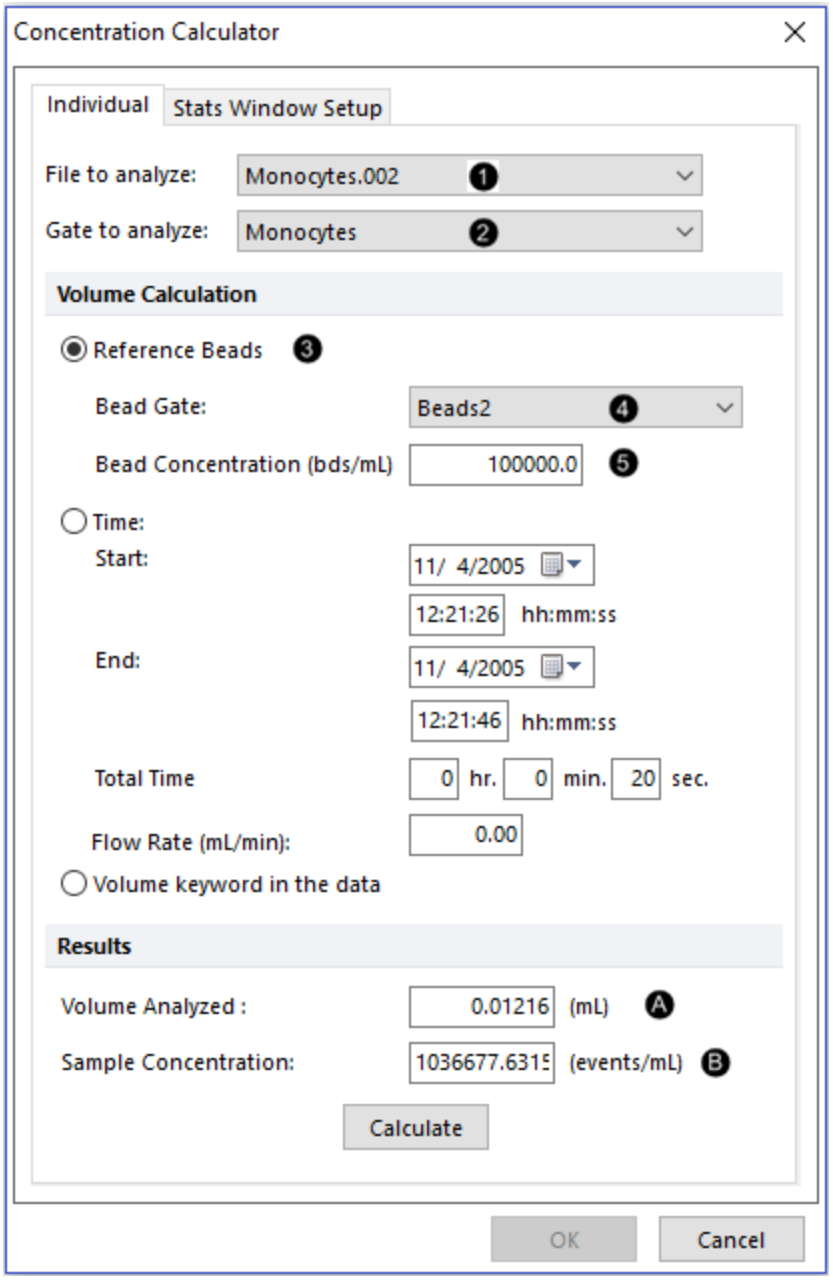

Figure T22.3 Concentration Calculator Dialog Using Beads

8. Click **Cancel** to close the **Concentration Calculator** dialog.

In the next example, we will calculate the concentration using time and flow rate 1889.

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38.24.3 Concentration Calculator (Time/Flow Rate)

In the course of this example, we will:

- Set up the concentration calculator using the time of acquisition and the flow rate.
- Obtain a one time result.

(Note that the following sections build upon steps 1 through 7 in the Setting up the Beads Gates chapter 1885)

1. Select the **Tools→Calculate→Concentration Calculator** command (Figure T22.2 1886).

The **Concentration Calculator** dialog appears again as shown in Figure T22.2. The following steps all refer to Figure T22.4.

2. Select the **File to analyze**, in this case **Monocytes.002**, from the drop-down list❶.
3. Select **Monocytes** as the **Gate to analyze**, from the drop-down list❷.
4. Select the **Time** radio button❸ to specify the **Volume Calculation** type.

The start and end times embedded in the FCS data file appear by default, and the **Total Time** is calculated automatically. You can change the start or end time manually, or enter a specific **Total Time** directly.

5. Input the **Flow Rate** used when acquiring the sample. In this case, since the data was collected on a cytometer that used a flow rate of 60 µl/min, we will use a value of **0.06** mL/min❹.
6. Click the **Calculate** button.

The **Results** section now displays the volume of the sample🅐 analyzed and the concentration of cells in the **Monocytes** gate🅑 (Figure T22.4).

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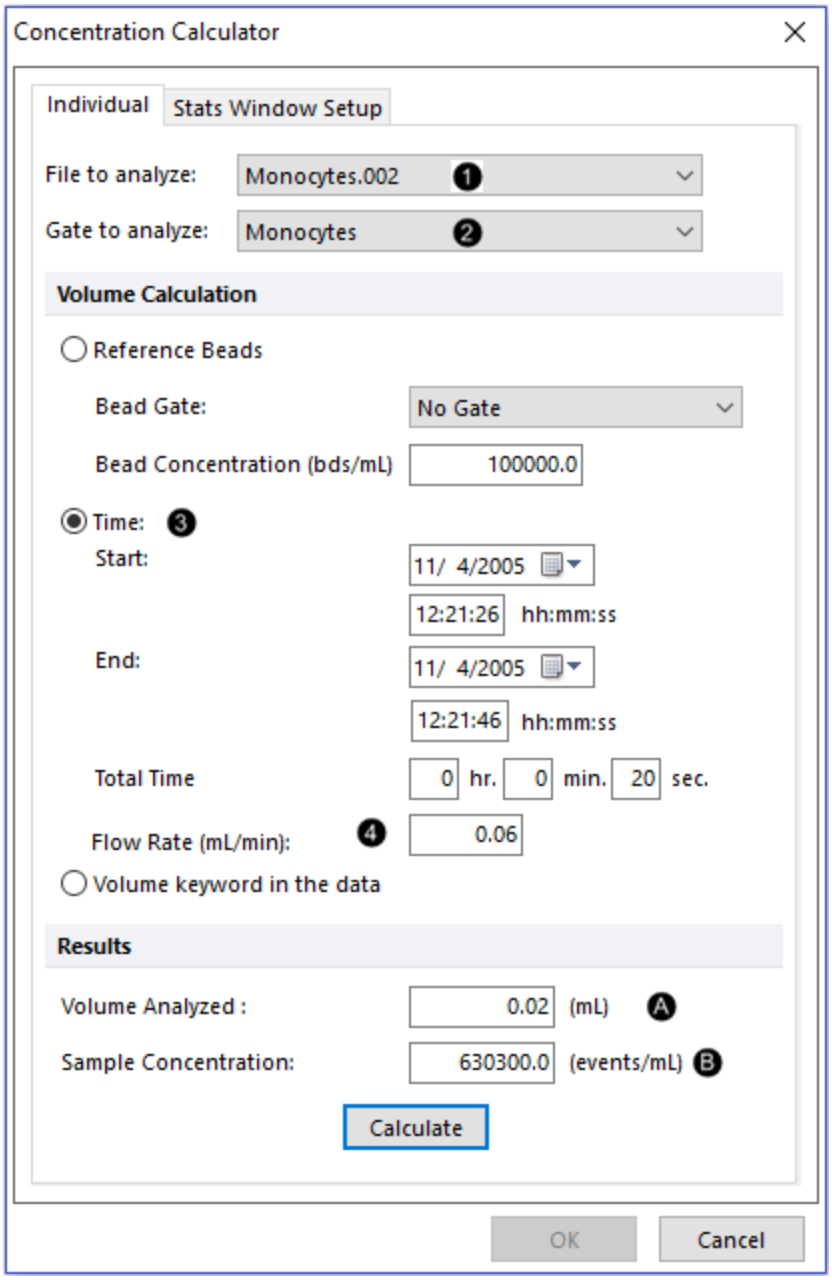

Figure T22.4 Concentration Calculator Dialog Using Time and Flow Rate

7. Click **Cancel** to close the **Concentration Calculator** dialog.

In the next example, we will calculate the concentration using volume ($VOL) keyword 1891.

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38.24.4 Concentration Calculator ($VOL keyword)

In the course of this example, we will:

- Set up the concentration calculator using the volume ($VOL) keyword in the data.
- Obtain a one time result.

(Note that the following sections build upon steps 1 through 7 in the Setting up the Beads Gates chapter 1885)

1. Select the **Tools→Calculate→Concentration Calculator** command (Figure T22.2 1886).

The **Concentration Calculator** dialog appears again as shown in Figure T22.2. The following steps all refer to Figure T22.5.

2. Select the **File to analyze**, in this case **Monocytes.002**, from the drop-down list ❶.
3. Select **Monocytes** as the **Gate to analyze**, from the drop-down list ❷.
4. Select the **Volume keyword in the data** radio button ❸ to specify the **Volume Calculation** type.
5. Click the **Calculate** button.

In the **Results** section of the **Concentration Calculator**, the volume of the sample analyzed appears in the **Volume Analyzed** Ⓐ field and the concentration of the cells in the **Monocytes** gate appears in the **Sample Concentration** Ⓑ field (Figure T22.5).

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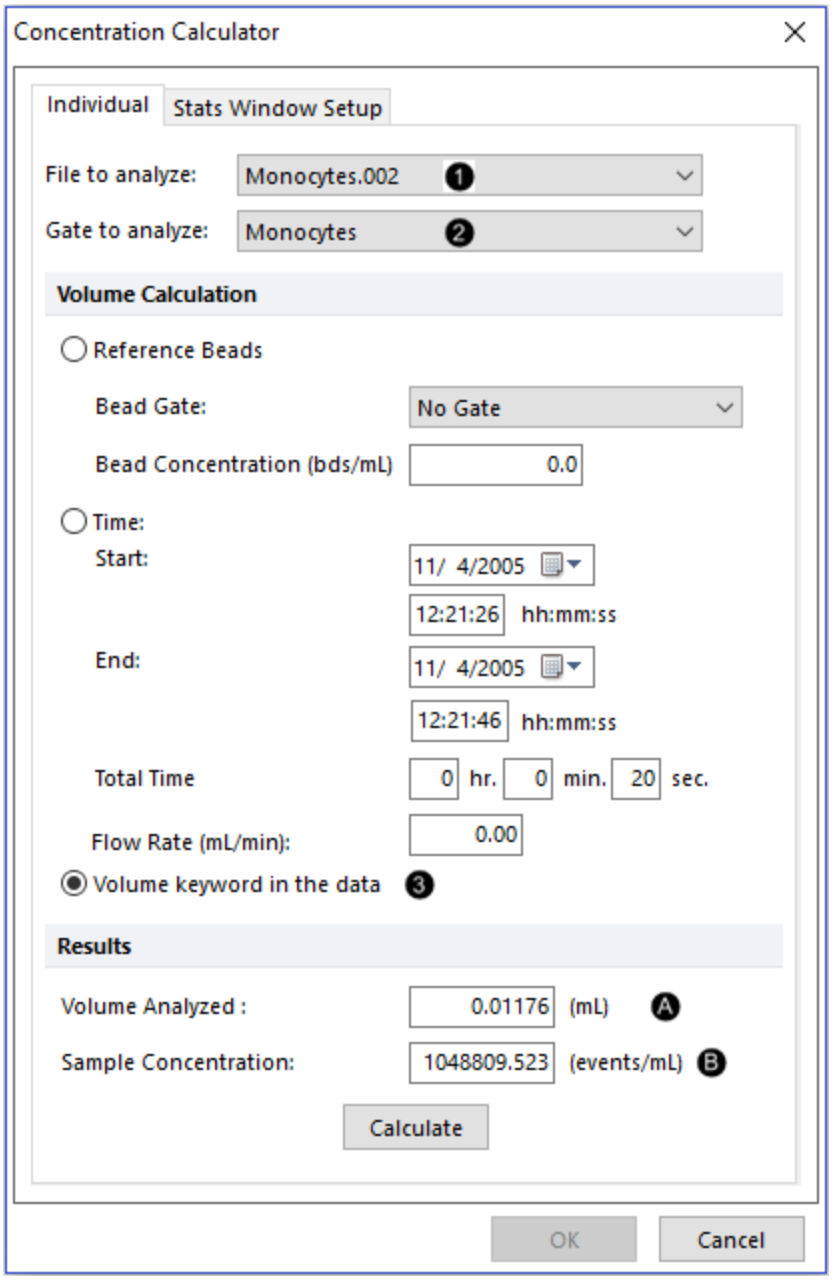

Figure T22.5 Concentration Calculator Dialog Using the $VOL keyword

8. Click **Cancel** to close the **Concentration Calculator** dialog.

Next, we will show the calculated concentration in a statistics window.

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38.24.5 Showing the Concentration in a Statistics Window

In the course of this example, we will:

- Set up the concentration calculator for displaying the results in the layout using beads or time and flow rate.
- Show the concentration calculated as a statistic in a **statistics window**.

We have learned how to obtain one-time calculations of the cell concentration in a sample. We will now learn how to present these results in the layout using the **statistics window**.

1. Right-click in the forward vs. side scatter plot containing the **Beads1** and **Monocytes** gates to bring up the associated pop-up menu.
2. Select **Statistics→Gate Statistics** from the pop-up menu.

A **Gate Statistics window** should appear (Figure T22.6). Move it to a convenient place in the layout.

Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Monocytes.002	None	17070	n/a	302.94	100.00	100.00
1	Monocytes.002	Monocytes	12479	543.81	364.08	73.10	73.10
1	Monocytes.002	Beads1	1244	n/a	677.65	7.29	7.29
1	Monocytes.002	Beads2	1214	n/a	680.58	7.11	7.11

Figure T22.6 Statistics Window

3. Click once on the **statistics window** to select it; it should have a green border (Figure T22.6).
4. Select the **Format→Statistics Options→Statistics** command (Figure T22.7).

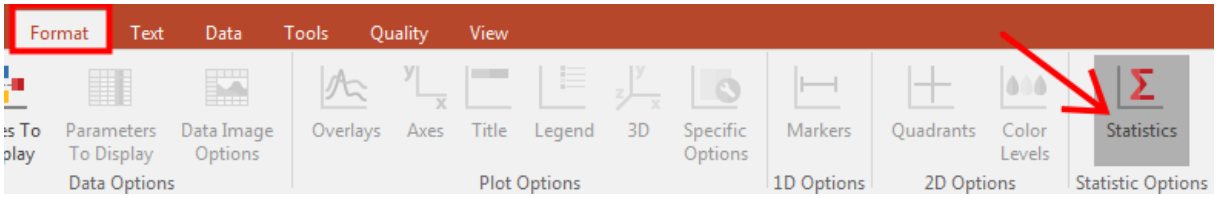

Figure T22.7 Selecting Format Statistics Command

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The **Formatting Statistics** dialog now appears (Figure T22.8). We are now going to select which statistics to display in the **statistics window** on the layout.

5. Select only the following items on the **Formatting Statistics** dialog (Figure T22.8):
    - **Gate**❶
    - **Number of Events**❷
    - **% of Gated Cells**❸
    - **% of All Cells**❹
    - **Concentration**❺
6. Click **OK** to close the **Formatting Statistics** dialog.

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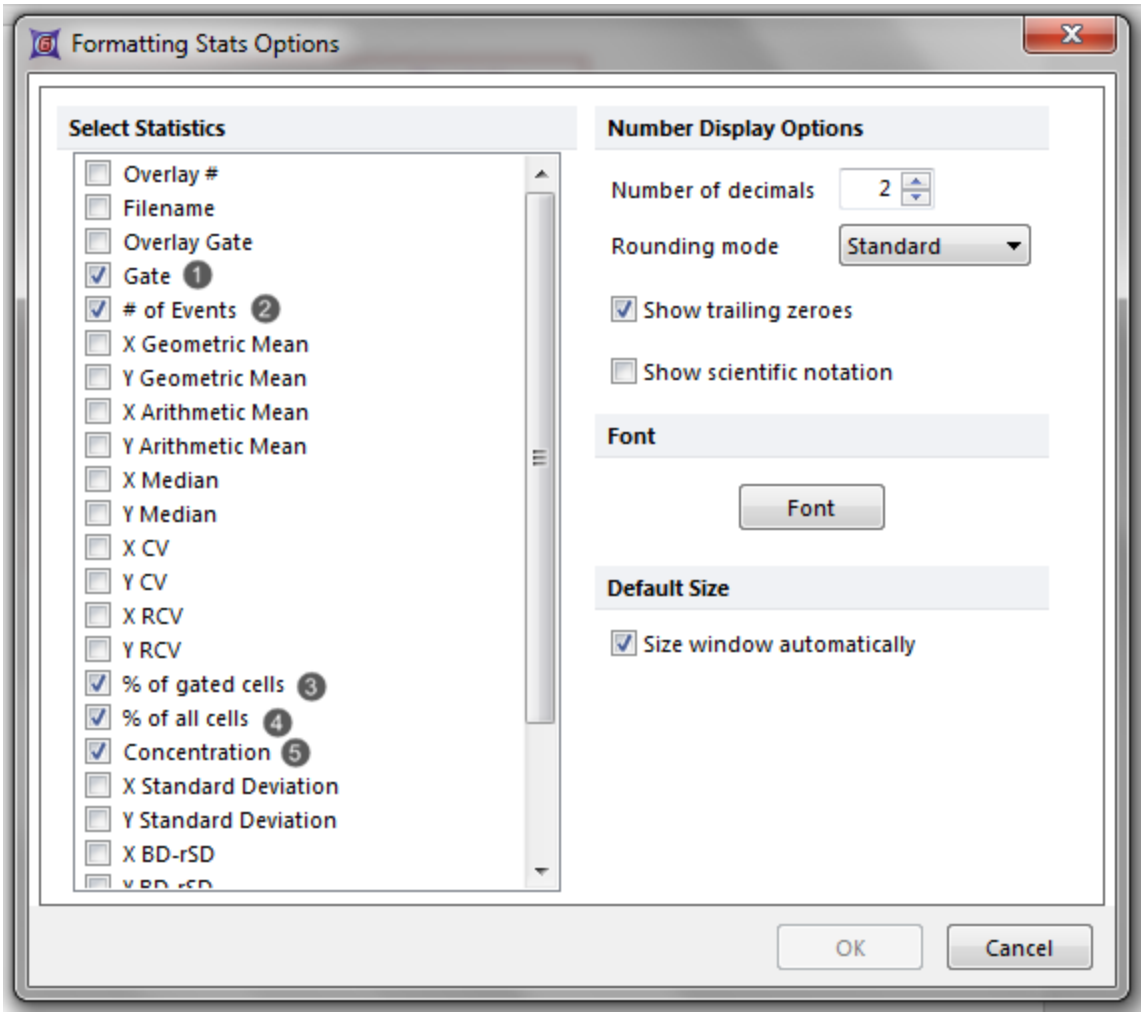

Figure T22.8 Formatting Statistics Dialog

The **statistics window** will now appear as shown in Figure T22.9 displaying only the items selected in the **Formatting Statistics** dialog.

Gate	# of Events	% of Gated Cells	% of All Cells	Concentration
None	17070	100.00	100.00	n/a
Monocytes	12479	73.10	73.10	n/a
Beads1	1244	7.29	7.29	n/a
Beads2	1214	7.11	7.11	n/a

Figure T22.9 Statistics Window after Formatting

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There are two other ways to format the **statistics window** to show only the statistics you want:

- Choosing the statistics to be displayed using the **statistics window** pop-up menu:

  A. Right-click on the **statistics window** to bring up the associated pop-up menu (Figure T22.9).

  B. Left-click on the individual statistics you want displayed.

  C. Left-click on the items you want removed (Figure T22.10).

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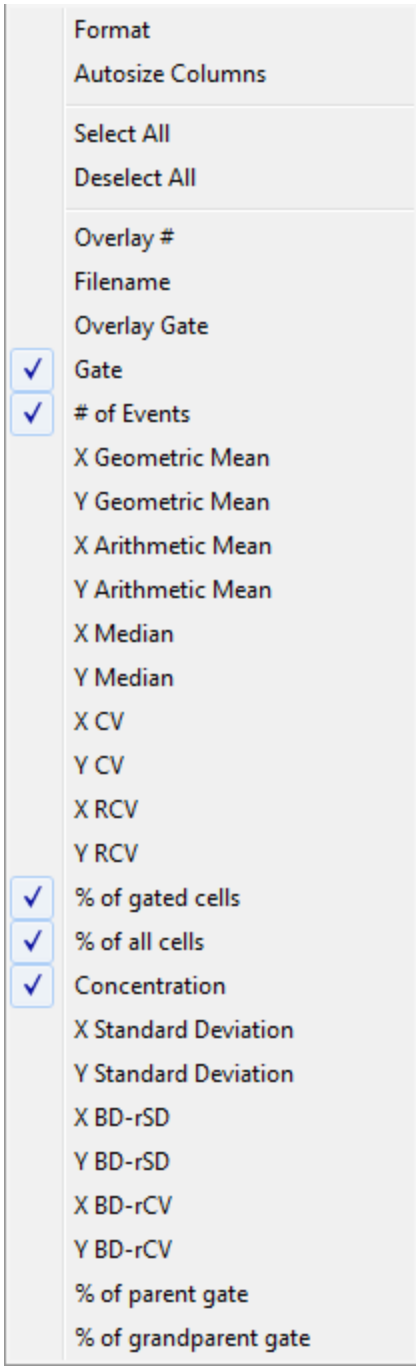

Figure T22.10 Statistics Window Pop-up Menu

- Choosing the statistics to be displayed using the **statistics window Format** dialog:

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A. Right-click on the **statistics window** to bring up the associated pop-up menu (Figure T22.10).

B. Select **Format** from the pop-up menu.

C. Select the **Stats** category from the left panel of the **Format** dialog (Figure T22.11).

D. Set the statistics you want displayed and then click **OK** (Figure T22.11).

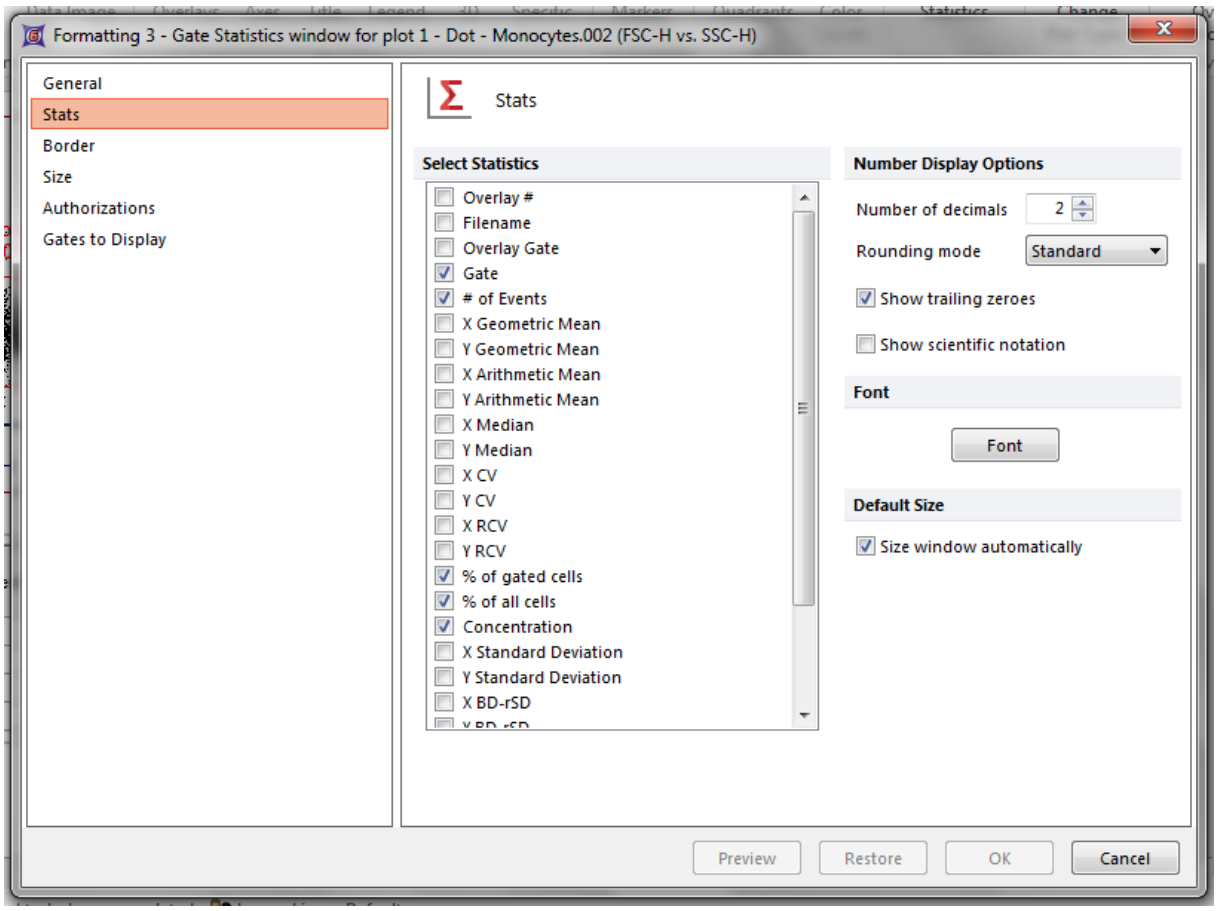

Figure T22.11 Stats Category of the Statistics Window Format Dialog

As you can see in Figure T22.9, the **statistics window** has a **Concentration** column, but it is undefined and shows only **n/a**. We will now setup FCS Express to calculate these concentrations.

7. Select the **View→Tools→Concentration Calculator** command (Figure T22.2 1886).

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The **Concentration Calculator** dialog appears as previously shown in Figure T22.4 1889.

8. Click on the second tab titled **Stats Window Setup** (Figure T22.12).

As before, there are two ways to do this: a) specifying a **beads gate** and giving the concentration of the beads in the sample, or b) using **time** together with the sample's **flow rate** when acquired. We are going to specify the Reference Beads as shown in Figure T22.12.

9. Select the **Reference Beads** radio button.
10. Select **Beads2** in the Bead Gate drop-down list.
11. Enter **100000** in the **Bead Concentration** field.
12. Click **OK** (Figure T22.12).

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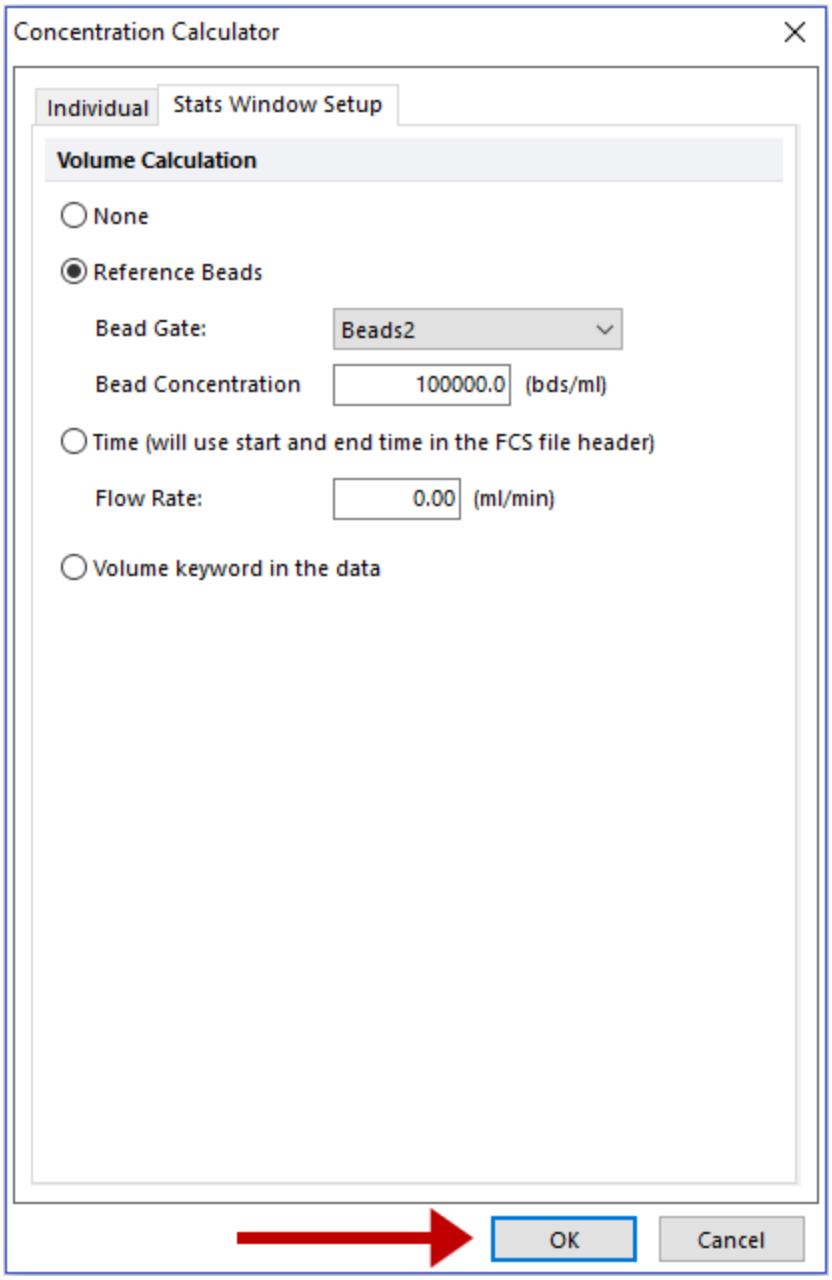

Figure T22.12 Concentration Calculator Dialog -- Stats Window Setup

Now, the **statistics window** will show the results in the **Concentration** column for every gate in the plot using the parameters you specified in the previous step (Figure T22.13). This setup will apply to the whole layout and will be used for the concentration statistics of every plot in the layout.

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Gate	# of Events	% of Gated Cells	% of All Cells	Concentration
None	17070	100.00	100.00	1406095.55
Monocytes	12479	73.10	73.10	1027924.22
Beads1	1244	7.29	7.29	102471.17
Beads2	1214	7.11	7.11	100000.00

Figure T22.13 Statistics Window with Concentration Results by Gate

Now, we will show the concentration as a token 1901.

38.24.6 Showing the Concentration as a Token

In the course of this example, we will:

- Show the concentration as a token.

You can use FCS Express tokens to insert statistics in a text box to present your results. For more information, see the tutorial on using text boxes and tokens 1298. In case you haven't setup the concentration calculation, please do so now in the previous section of this tutorial 1893.

1. Create a blank text box on the layout.
2. Enter the following text: "**Beads were added to this sample at a final concentration of 50,000 beads per mL. The final concentration of Monocytes in the sample is <blank space>.**" in the text box.
3. Right-click on the blank space you left in the text box to bring up the associated pop-up menu (Figure T22.14).
4. Select **Insert Token** from the pop-up menu (Figure T22.14).

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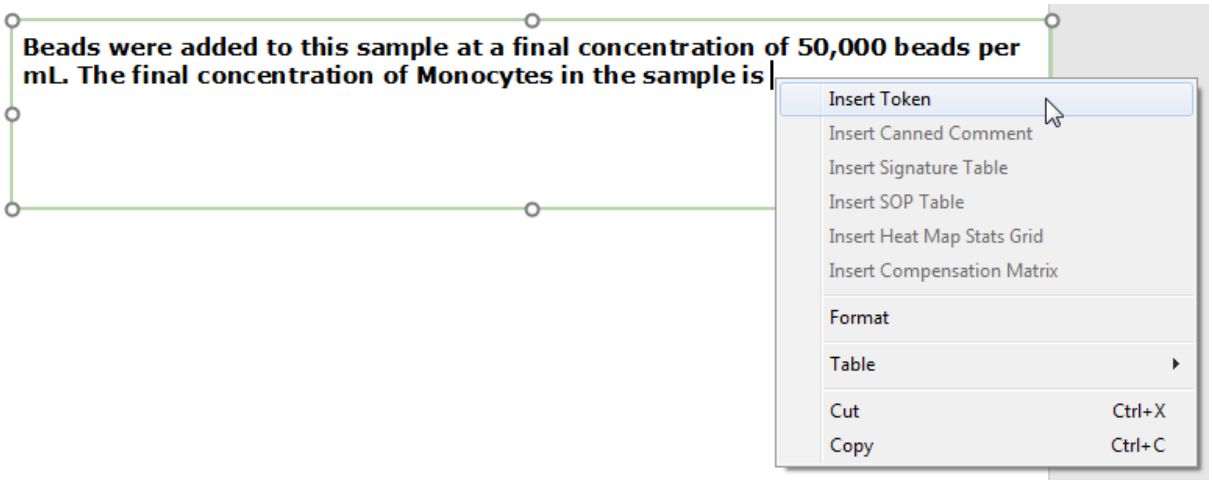

Figure T22.14 Inserting a Token in a Text Box

The **Insert a Token** dialog now appears (Figure T22.15).

5. Select **Statistic** from the **Insert a Token** dialog.
6. Click **Insert** (Figure T22.15).

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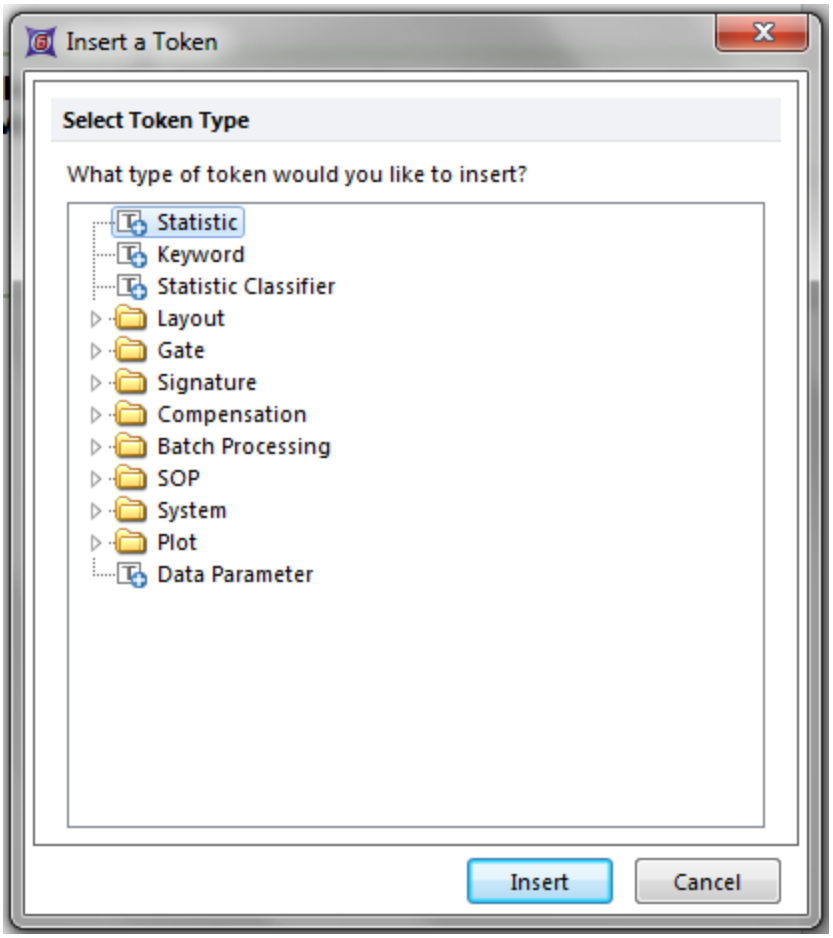

Figure T22.15 Insert a Token Dialog

The **Create Statistic** dialog now appears with the **Data Source** category displayed (Figure T22.16). This is where you choose which plot the token will refer to; that is, which plot's data will be used for the concentration calculation. If the plot contains multiple overlays, you can also pick the specific overlay you want to use for the calculation. Since we have created a very simple layout during this tutorial, there is nothing to change on this dialog.

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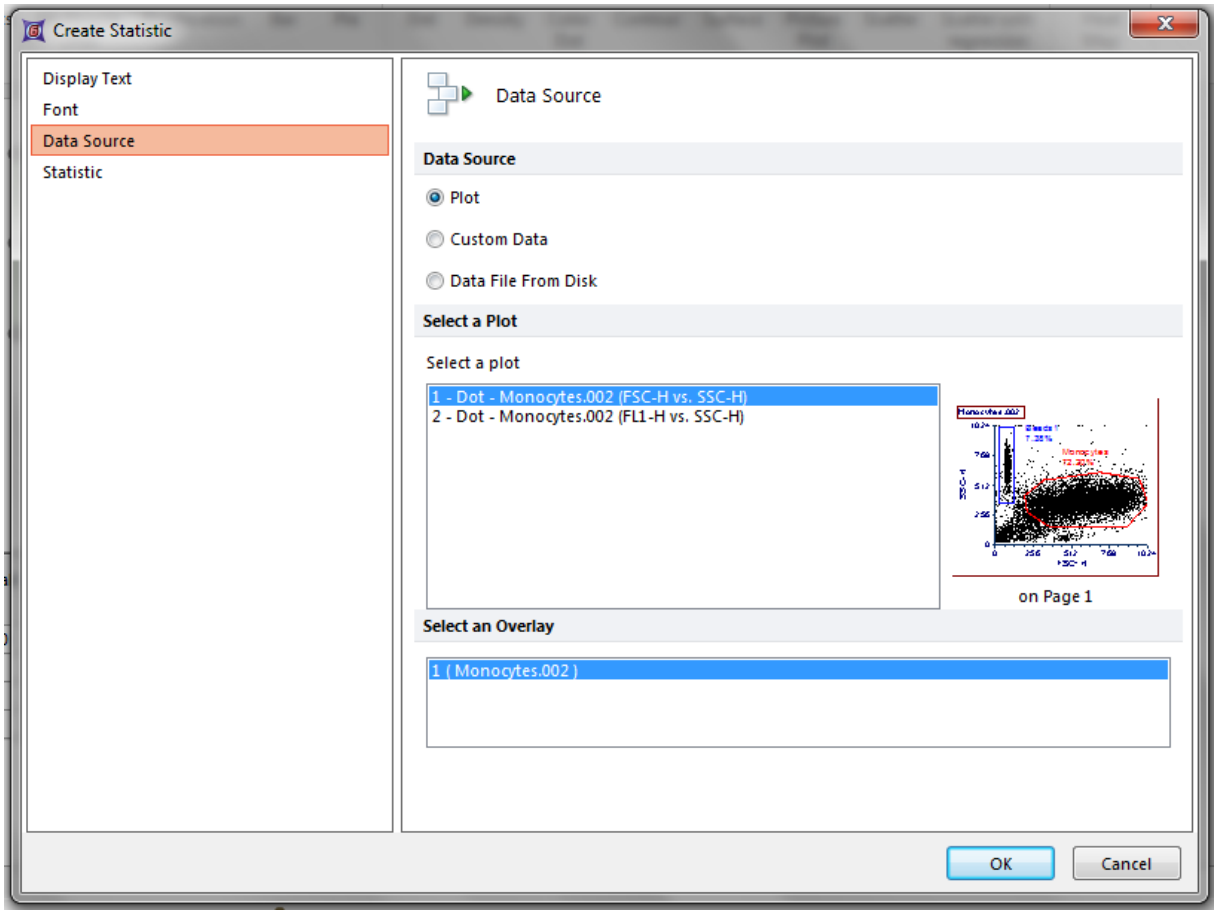

Figure T22.16 Create Statistic Dialog -- Data Source

7. Click on the **Statistic** category in the left panel (Figure T22.17).
8. Select **Monocytes** from the **Gate** drop-down list.
9. Select **Concentration** from the **Statistic** list.
10. Click **OK** (Figure T22.17).

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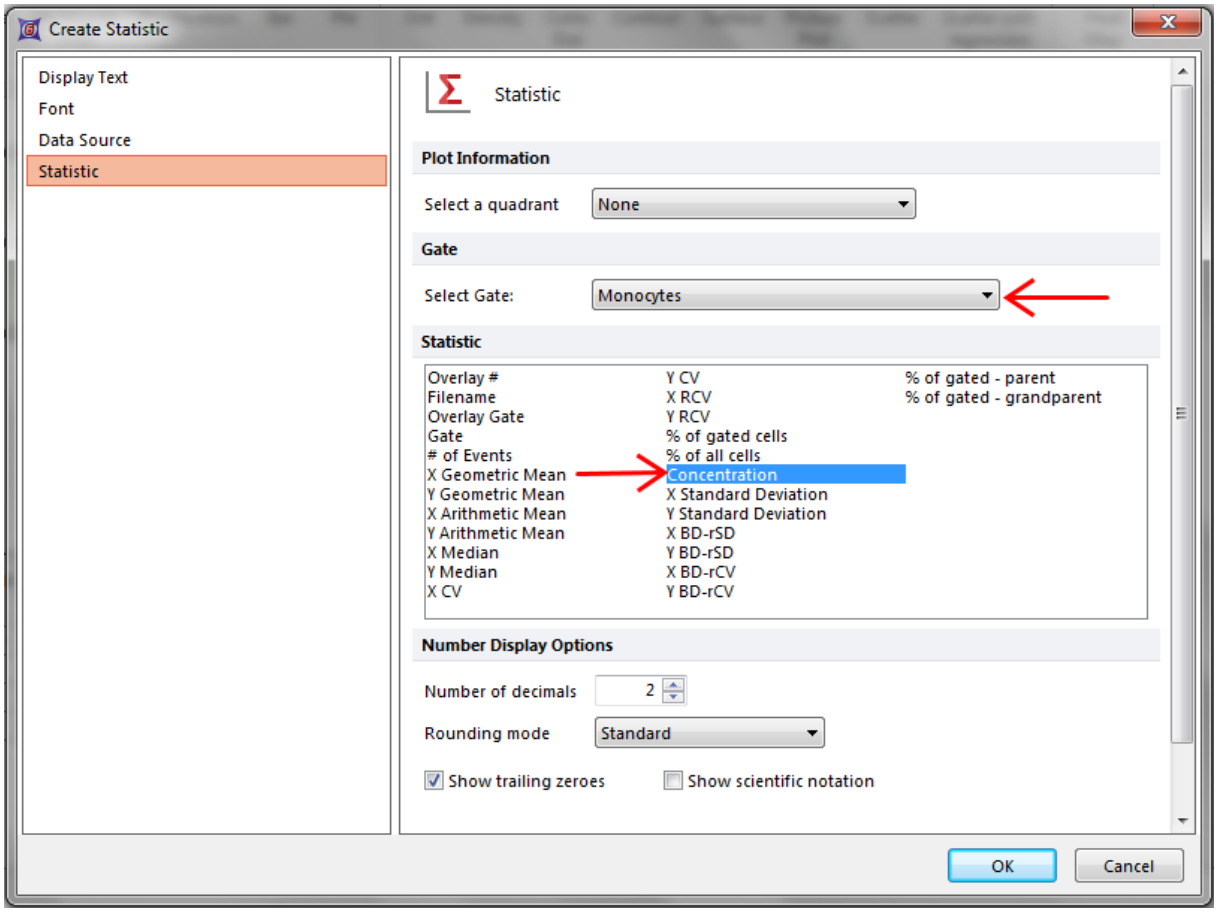

Figure T22.17 Selecting Concentration as the Statistic Token

The text box is now updated with the concentration of the monocytes using the token we just defined, shown highlighted in grey in Figure T22.18.

Beads were added to this sample at a final concentration of 100,000 beads per mL.

The final concentration of Monocytes in the sample is 1027924.22

Figure T22.18 Text Box with the Concentration Token Value

You can now appreciate that the number in the token is the same as the number that appears in the **statistic window** for the "Monocytes" gate. What you have done is specify the [four parameters needed for calculating the cell concentration](1886), in two separate places: the volume was determined in the **Stats Window Setup** of the **Concentration Calculator**, and the number of cells was determined in the token setup itself.

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38.25 Channel Calibration

FCS Express can ease the task of determining the absolute fluorescence values of your samples using calibration particles. Using the integrated Channel Calibration tool, you can setup FCS Express, using calibration particles, to have your plots and statistics display corresponding MESF fluorescence values.

We will start by setting up the bead gates and markers 1906.

38.25.1 Setting Bead Gates and Markers

In the course of this example, we will:

- Learn how to gate the beads.
- Learn how to apply markers to the fluorescence peaks of the beads.
- Set up channel calibration defaults for FCS Express.

1. Open a new, blank layout.
2. Insert a Forward vs Side scatter dot plot of the file **ChannelCalibration.001** located in the Tutorial Sample Data archive 1210. For information on how to do this, please see the tutorial on Inserting Plots 1225.
3. Create a polygonal gate around the beads.

The gate should look similar to the one shown in Figure T23.1. For more information on creating gates, please refer to the Gating tutorial 1266.

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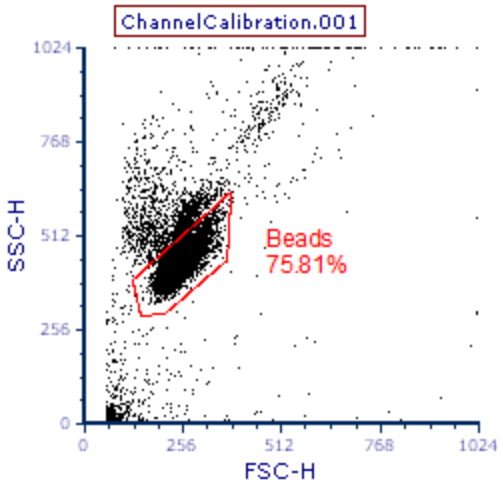

Figure T23.1 Polygonal Gate

4. Insert a new histogram of the same file.
5. Set it to show channel FL3-H.
6. Apply the **Beads** gate to the new histogram.

The histogram should appear similar to Figure T23.2.

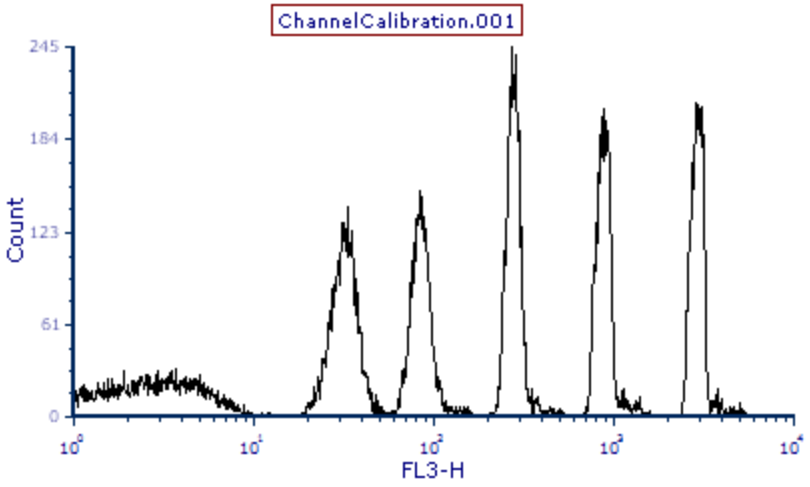

Figure T23.2 Histogram of FL3-H with Beads Gate Applied

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The histogram shows the five fluorescent peaks, plus a "blank", of the calibration particles. We will now proceed to delineate the peaks by setting individual markers on each peak.

7. Select the **Gating→Create Gates→Create Marker** command.
8. Place the cursor inside the histogram and to the left of the brightest peak.
9. Press and hold the left mouse button down and drag the mouse to the right side of the peak.
10. Release the mouse button when you are on the right side of the brightest peak. Take care to avoid the "tail" which represents the doublets, indicated by the red circle in Figure T23.3.

You can move and resize the marker freely once it has been created by selecting the marker and moving either the ends of the marker or the whole marker. For more information on resizing markers please refer to the tutorial section explaining Marker to Gate Linking.

Repeat steps 7 through 9 for each of the other four peaks, moving from the brightest to the dimmest peak, excluding the lowest peak associated with the "blank" beads. You should now have five markers, one for each fluorescent peak, as shown in Figure T23.3.

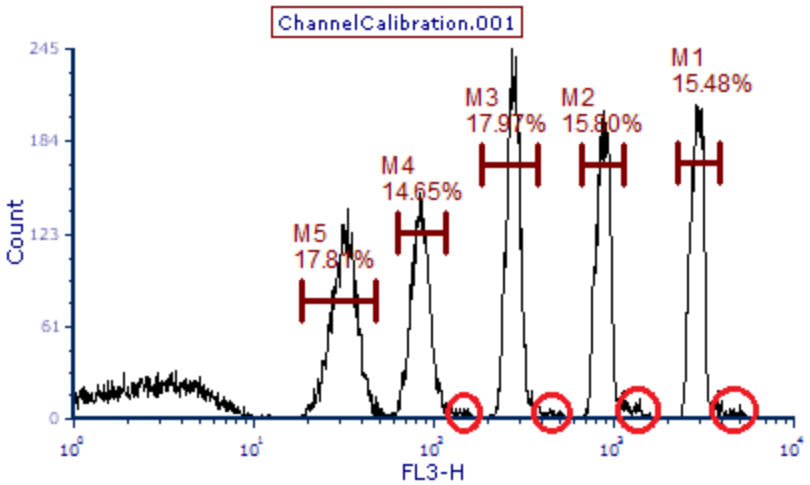

Figure T23.3 Markers M1 through M5 for the five Fluorescent Peaks (red circles indicate doublets)

Note: The default setting for FCS Express is to use channel calibration on the axes. Therefore, before we set up the channel calibration, it is important to set

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the histogram, showing the beads, to not use channel calibration, since that is our original reference.

11. Right-click on the histogram showing the calibration beads in the FL3 channel to bring up the associated pop-up menu.

12. Select **Format** from the pop-up menu.

The **Formatting** dialog for the histogram appears.

13. Select **Histogram→Histogram Specific Options** from the categories listed on the left of the **Formatting** dialog.

The Histogram Specific Options category of the **Formatting** dialog will appear (Figure T23.4).

14. Unselect the **Use channel calibration on axis** option, indicated by the cursor (Figure T23.4).

15. Click **OK**.

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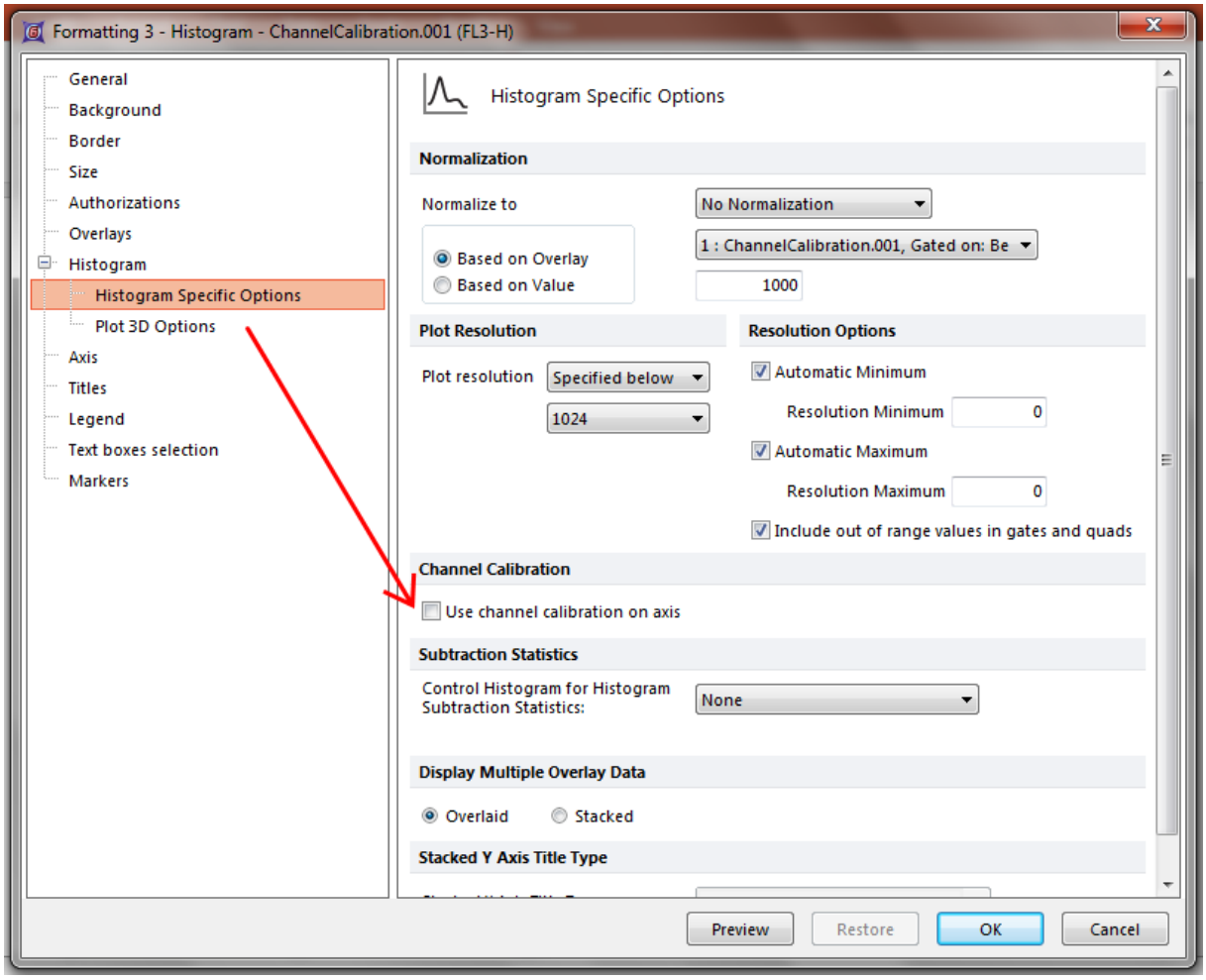

Figure T23.4 Formatting Dialog -- Unselecting Use channel calibration on axis

In the next section, we will set up the channel calibration tool 1910.

38.25.2 Channel Calibration Tool Setup

In the course of this example, we will:

- Identify the variables needed for calibrating the channels.
- Set up the Channel Calibration tool using the beads and markers prepared in the previous section.
- Obtain the calibration information.

Before we can begin our setup, it is important to clarify a detail on how flow cytometry FCS data files are formatted. Typically, in an FCS file, the first

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parameter described is Forward scatter, followed by Side scatter, and then the fluorescent channels in order – FL1, FL2, FL3, etc. Some instruments embed the name of the parameter directly in the FCS file and you can see it in the Channel Calibration tool (see instructions below). In the file we will be using for this tutorial, that is not the case. Nevertheless, we know that "Parameter 5" corresponds to FL3, which is the channel we want to calibrate. In case you do not know which parameter corresponds to a fluorescent channel, you can view the FCS text header information by selecting the **Data→DataInfo→View Header** command. A dialog displaying the FCS data file header information will appear displaying all the parameter information in a grid.

We will now proceed to set up the Channel Calibration tool.

1. Select the **Tools→Calculate →Channel Calibration** command (Figure T23.5).

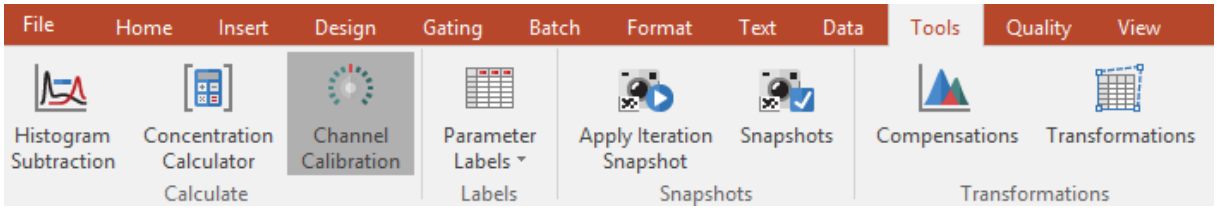

Figure T23.5 Selecting the Channel Calibration Tool Command

The **Channel Calibration** dialog appears (Figure T23.6).

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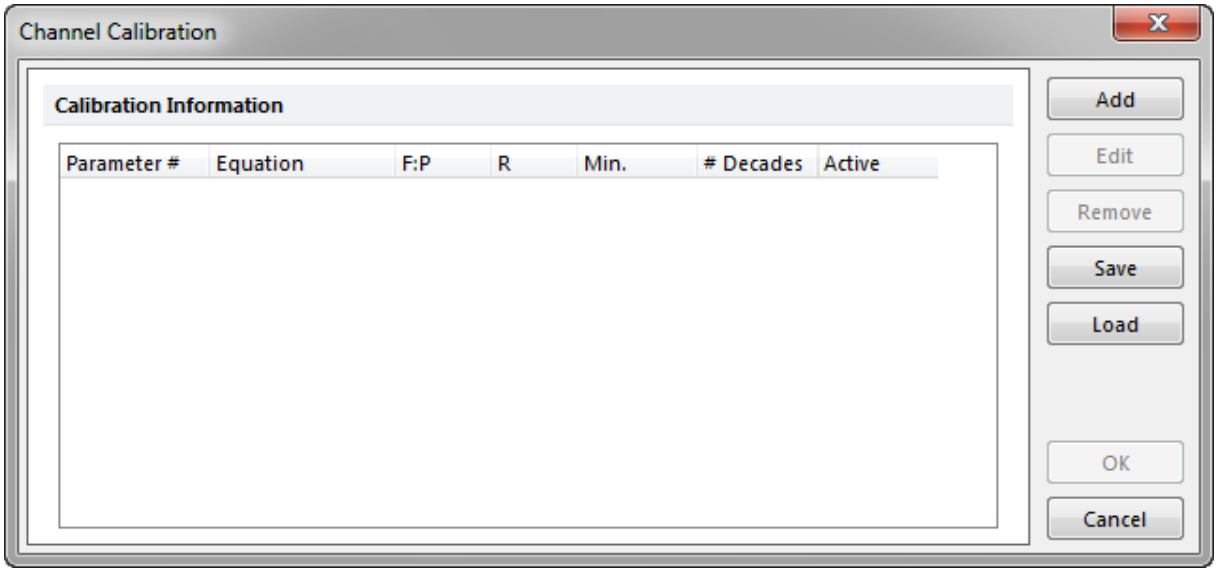

Figure T23.6 Channel Calibration Dialog

2. Click the **Add** button on the **Channel Calibration** dialog (Figure T23.6).

A **New Calibration** dialog now appears, similar to Figure T23.7.

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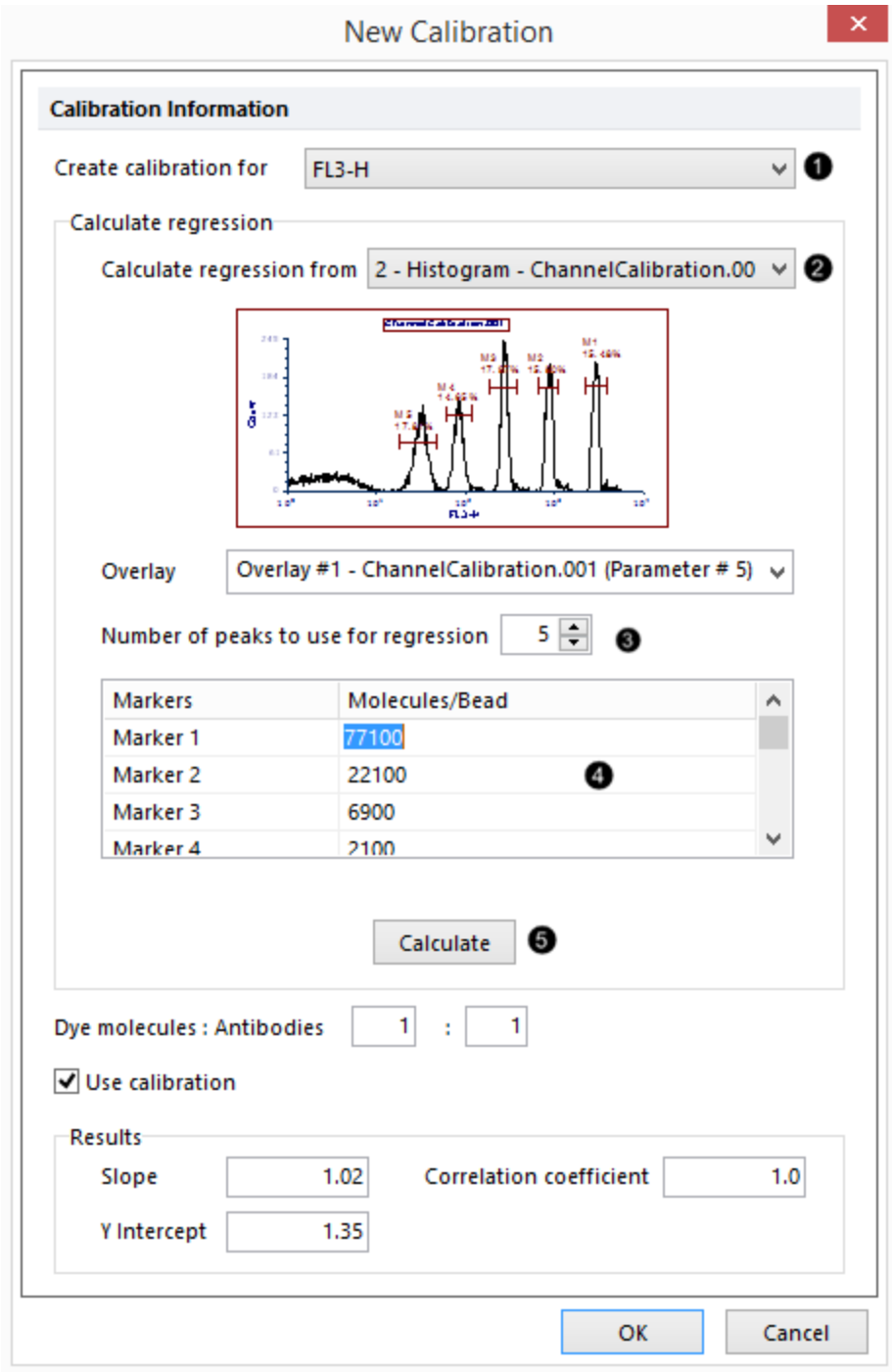

Figure T23.7 New Calibration Dialog

3. Select **FL3-H** from the **Create calibration for** ❶ drop-down list (Figure T23.7).

4. Select the **Histogram - ChannelCalibation.001 (FL3)** ❷ with the calibration beads from the **Calculate regression from** drop-down list.

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Note: If there are multiple overlays in your histogram, you can select which one to use in the **Overlay** option. In this case it is a simple histogram. Therefore, there are no other overlays and nothing has to be changed.

5. Enter "**5**" ❸ in the **Number of peaks to use for regression** field.

Usually, it is best to use all available peaks (except the blank one); but if for some reason you want to omit one of the peaks, you can do so. You must use a minimum of three peaks to calculate a regression.

6. Enter the following fluorescence values, shown in Figure T23.7, for each peak, in the **Molecules/Bead**❹ column beginning with Marker 1; **77100**, **22100**, **6900**, **2100**, **750**.

These values are provided by the manufacturer of the beads, usually quoted as MESF – Mean Equivalent of Soluble Fluorochrome. In this case, the reported MESF are for PE-Cy5 molecules.

If you specified fewer peaks than the number of markers in the histogram, for example, four peaks out of five markers, make sure you select the correct markers in the left column.

7. Click the **Calculate** button ❺ (Figure 7).

The **New Calibration** dialog and results, i.e., **Slope** Ⓐ, **Y intercept** Ⓑ, and **Correlation coefficient** Ⓒ, should look similar to Figure T23.7. FCS Express calculates the regression using the logarithmic values. The minimal detection threshold is the fluorescence obtained by setting the channel to "1" in the regression equation. The number of decades (the dynamic range of the machine) is calculated as the inverse of the slope.

8. Click **OK** to close the **New Calibration** dialog.

The **Channel Calibration** dialog will now appear similar to Figure T23.8. The channel calibration is now ready for use.

As you define calibrations for more than one parameter, they will all appear here. If the **Active** option is set, then that calibration set will be used in the layout (in those plots that have the **Use channel calibration on axis** checked. You can only have one active calibration defined for a particular parameter.

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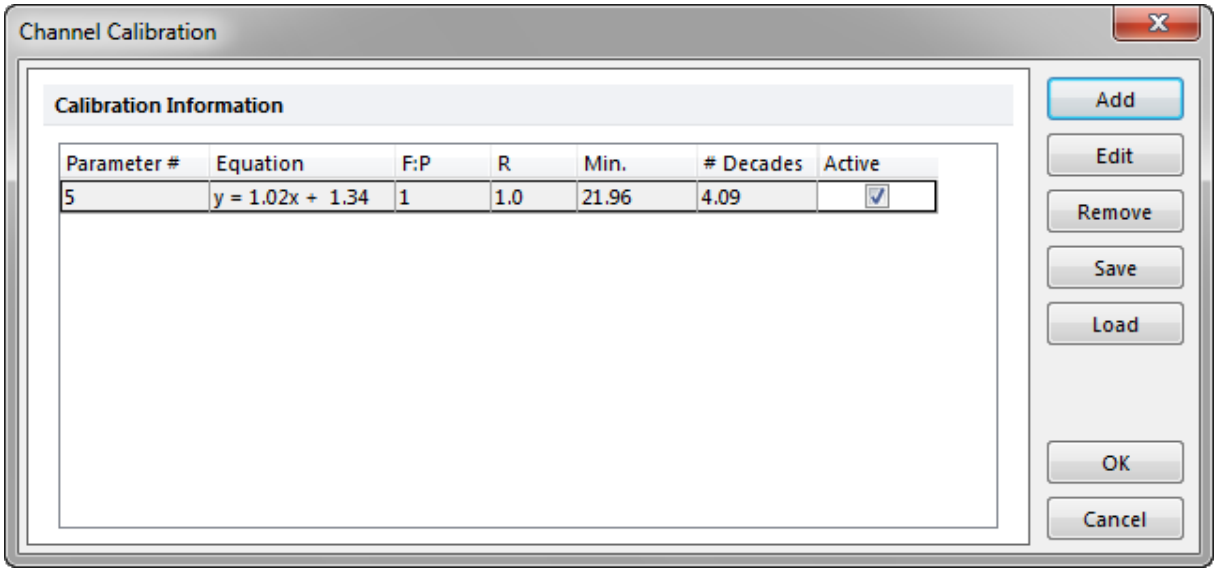

Figure T23.8 Updated Channel Calibration Dialog

Note: Although we won't show it in the tutorial, you can also edit existing calibrations for a particular parameter by selecting the parameter in the Channel Calibration list and pressing the **Edit** button.

9. Click **OK** to close the **Channel Calibration** dialog and return to the layout.

Next, we will demonstrate channel calibration.

38.25.3 Channel Calibration Demonstration

In the course of this example, we will:

- Demonstrate the effect of channel calibration on the data display.

We will now proceed to open a new histogram to show how the calibration affects how the data is displayed.

1. Insert a new histogram using the file **Monocytes.002** in the Tutorial Sample Data archive.
2. Set the new histogram to display channel FL3-H.
3. Make sure the histogram is ungated.

The histogram should look similar to Figure T23.9. Note that the x-axis extends from less than 10^2 to more than 10^5. The axis is shown as calibrated, expressing

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the mean equivalent number of soluble PE-Cy5 molecules. This is because the default setting for all plots is to use calibrated axes.

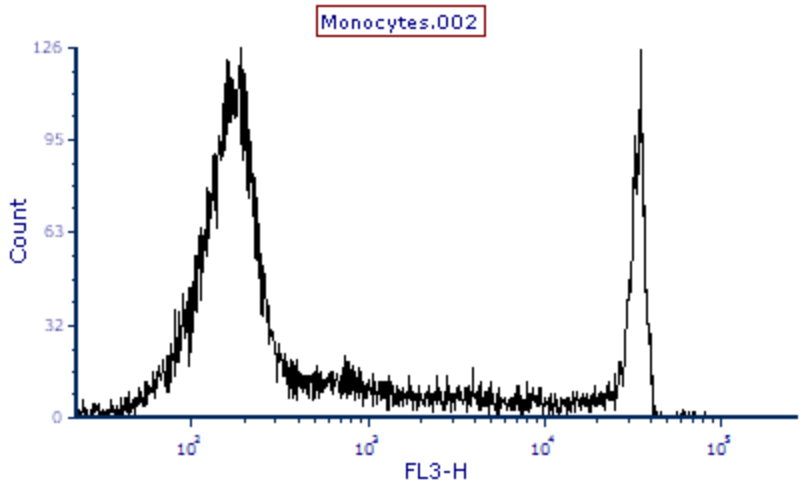

Figure T23.9 Histogram with Calibrated Axis

4. Right-click on the histogram to bring up the associated pop-up menu.
5. Select **Histogram Statistics** from the pop-up menu.

The **Statistics window** associated with the histogram will appear.

6. Move the **Statistics window** under the histogram.
7. Click inside the **Statistics window** so that it is selected; it should have a green border.
8. Select the **Format→Statistics Options→Statistics** command (Figure T23.10).

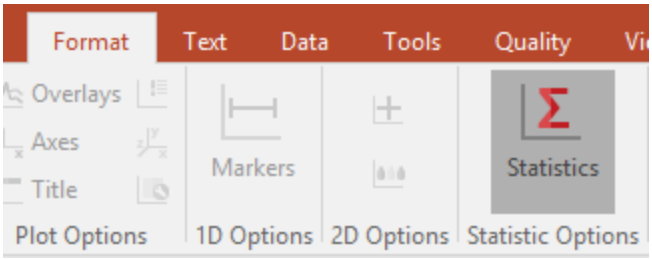

Figure T23.10 Selecting the Format Statistics Command

The **Formatting Statistics** dialog now appears.

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9. Select the following items on the **Formatting Statistics** dialog: **Median**, **Geometric Mean**, **Arithmetic Mean**, **CV**.

10. Deselect all other items from the **Formatting Statistics** dialog.

11. Click **OK**.

The Statistics window should appear similar to Figure T23.11.

Median	Geometric Mean	Arithmetic Mean	CV
8.98	24.63	242.85	204.76

Figure T23.11 Statistics Window for Histogram with Calibrated Axis

12. Right-click on the histogram to bring up the associated pop-up menu.

13. Select **Format** from the pop-up menu.

The **Formatting** dialog now appears.

14. Move the **Formatting** dialog so that both the histogram and the statistics window are visible.

15. Select **Histogram→Histogram Specific Options** from the categories listed on the left of the **Formatting** dialog, as previously shown in Figure T23.4 1906.

Notice that the **Use channel calibration on axis** is checked.

16. Deselect the **Use channel calibration on axis** option (Figure T23.4 1906).

17. Click the **Preview** button.

Note that the histogram curve itself does not change, but the axis reverts to the usual 10^0 to 10^4 uncalibrated scale. Notice that the values in the statistics window change when the axis changes from calibrated to uncalibrated. When calibration is active, the units represent the MESF of PE-Cy5. When calibration is not used the units are the arbitrary values obtained from the raw data in the FCS file.

18. Click **Cancel** to close the **Formatting** dialog.

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In the next section, we will cover saving and loading channel calibrations 1918.

38.25.4 Saving/Loading Channel Calibrations

In the course of this example, we will:

- Learn how to save and load channel calibration sets.

Now that you have set up this calibration (or in the future, multiple calibration sets), you can save them in order to use them for other layouts. For example, if you acquired multiple samples which you are analyzing in separate layouts, you can set up the calibration in one layout, save it, and then load it in all the other layouts. That way you avoid the need to repeat the calibration process in every layout. Bear in mind that the calibration is applicable only to the samples acquired during the same experimental run using the exact same cytometer setup.

We will now proceed to save the calibration setup.

1. Select the **Tools→Calculate→Channel Calibration** command (Figure T23.5 1910).

The **Channel Calibration** dialog will appear as previously shown in Figure T23.8 1910.

2. Select the calibration you created. There should only be one.
3. Click the **Save** button.

The **Save As** dialog appears.

4. Navigate to the *FCS Express Sample Data* folder and enter "**CalibDemo**".
5. Click **Save**.

We will now proceed to load the calibration setup into a new layout.

6. Click on **Cancel** to close the **Channel Calibration** dialog.
7. Select **Save Layout** from the application button to save your current layout.
8. Press **Ctrl+N** on the keyboard, to open a new blank layout.
9. Select the **Tools→Calculate→Channel Calibration** command.

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The **Channel Calibration** dialog appears empty, with no calibrations defined, as previously shown in Figure T23.6 1910.

10. Click the **Load** button.

The **Open** dialog appears where we can select the calibration file to load.

11. Navigate to the *FCS Express Sample Data* folder and select the "**CalibDemo**" file that you just saved.

12. Click **Open**.

The **Channel Calibration** dialog will now look like Figure T23.8 1910, with the same calibration information that you saved.

13. Click on **Cancel** to close the **Channel Calibration** dialog.

14. Close the current blank layout to return to the original layout we were working on before.

Finally, we will set up channel calibration defaults 1919.

38.25.5 Channel Calibration Defaults

In the course of this example, we will:

- Learn how to change channel calibration default settings for FCS Express plots.

As previously mentioned, the default setting is for FCS Express to use channel calibration on all plots. However, you have the option of turning the channel calibration option off, or on, for a specific plot type. We will now show how you can turn the channel calibration option off for all 2D dot plots.

1. Select **File Tab→Options**.

The **FCS Express User Options** dialog now appears (Figure T23.12).

2. Select the **Plots→Dot Options→Binned Dots Specific Options** subcategory on the left side of the **FCS Express User Options** dialog, shown highlighted in grey in Figure T23.12.

3. Uncheck the **Use channel calibration on axes** box, indicated by the cursor in Figure T23.12.

4. Click **OK** to close the **FCS Express User Options** dialog.

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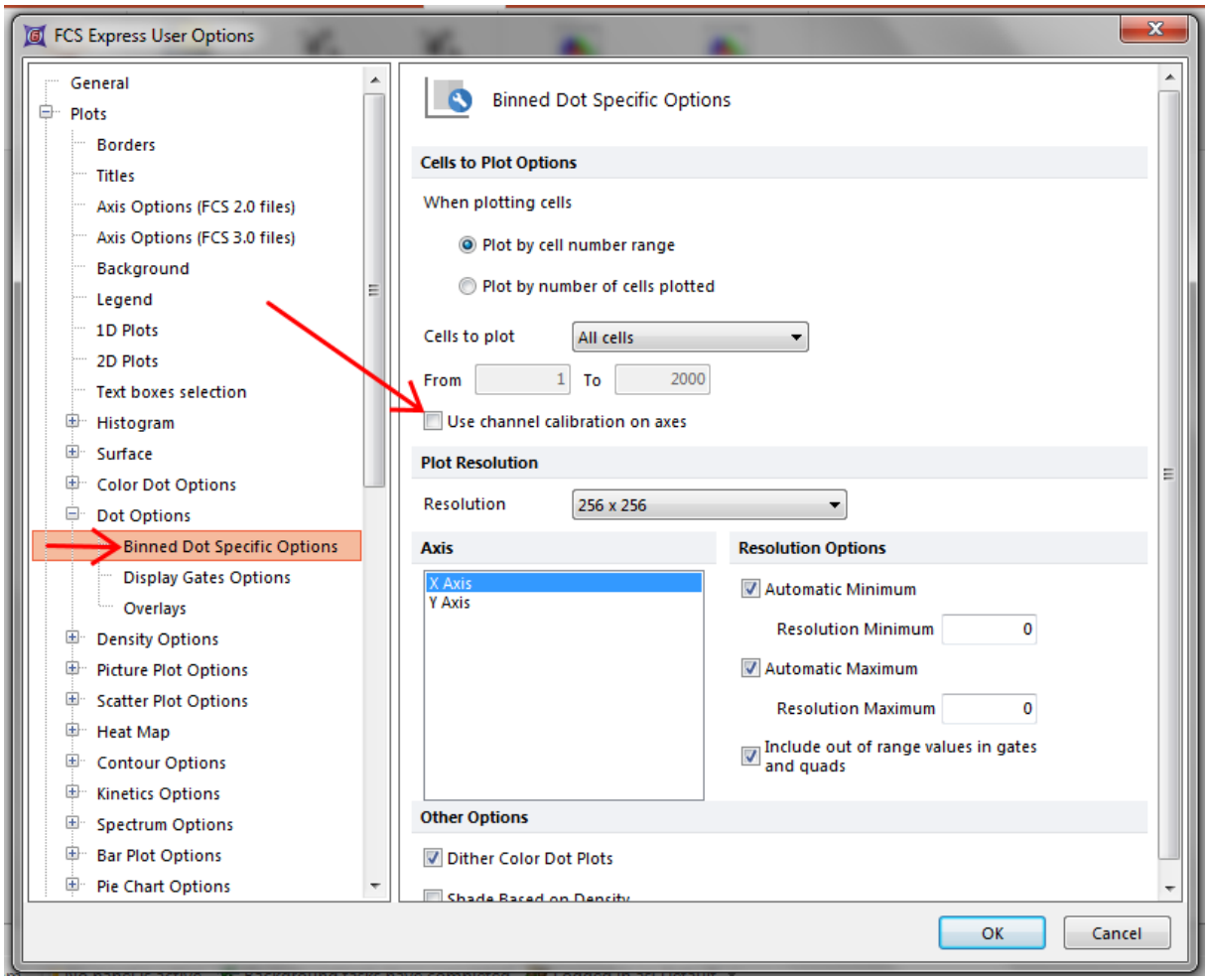

Figure T23.12 FCS Express User Options Dialog -- Unselecting Use channel calibration on axes for 2D Dot Plots

This setting will now ensure that all new 2D dot plots created will not use channel calibration. If we would like to remove the channel calibration for other types of 2D plots, we would need to go to the specific subcategory for that type of 2D plot under the **Plots** category on the **FCS Express User Options** dialog.

Note that you always have the option of setting or removing the channel calibration for a specific plot, rather than for all plots, using either the **Format→Plot Options→Specific Options** command, or using the **Format** dialog from the plot pop-up menu as shown in the previous steps. When the **Formatting** dialog associated with the plot appears, remember to select **Dot Options→Binned Dot Specific Options** for 2D dot plots or **Histogram→Histogram Specific Options** for histograms. Then, select or unselect the **Use channel calibration on axes** option.

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Remember, it is important to uncheck the **Use channel calibration on axes** option on any histograms you are going to use to set up a new bead channel calibration.

38.26 CellProfiler Export

CellProfiler is free, open-source software designed to enable biologists to quantitatively measure cell phenotypes from thousands of images. CellProfiler can automatically find all the cells within a given image and measure parameters from each cell. De Novo Software has teamed up with the developers of CellProfiler to allow analysis of high throughput imaging data in FCS Express. Once your data is properly exported from CellProfiler, FCS Express can import the data and allow visualization of your data set with all of our familiar 2D plots and new plotting features such as picture plots and heat maps.

In the first two sections of this tutorial you will learn:

- How to export CellProfiler data from a 96 well plate imaging experiment.
- How to import the data into FCS Express.

Note: This tutorial will not focus on how to create and use pipelines in CellProfiler; please refer to CellProfiler manual for extensive information in building pipelines. The focus of this tutorial will be how to modify an existing pipeline in CellProfiler 3.0 in order to export the data in a format that FCS Express can read. If you are using CellProfiler 2.2 or earlier, please visit this page and download the 3.0 release and follow the steps outlined here in this tutorial.

First, we will setup to export 96 well data from CellProfiler 1921.

38.26.1 Exporting 96 Well Format Images

In addition to extracting metadata from the image files and providing names for different channels (if applicable), the following CellProfiler analysis modules are required or must be amended in the pipeline order for export to FCS Express: Convert Objects to Images, Save Images, and Export to Spreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive 1210 and is named **Section1pipelineCOMPLETED.cpproj**. The completed

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pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

This tutorial will use representative files from the Broad Institute example data set quantifying features of interest, **Human cytoplasm-nucleus translocation assay (SBS Vitra)**, which is available in the Example Vitra Images folder within Tutorial Sample Data archive. More details about this experiment can be found here.

The steps for exporting data from CellProfiler have been outlined by illustrating how to add images, extract metadata from files, and modify the required modules according to the **Section1pipeline.cpproj**. After loading the **Section1pipeline.cpproj**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folder

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for the **Default Input Folder** (Figure T24.1).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, you can select *Do not write MATLAB or HDF5 files* from the **Output file format** drop-down menu.

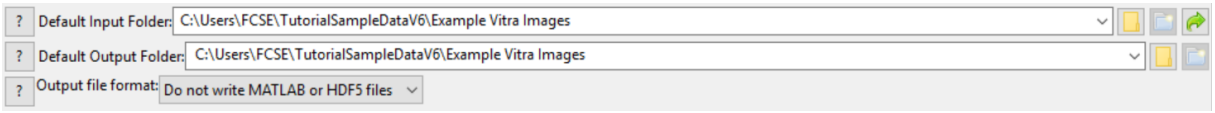

Figure T24.1 Set the Default Input and Output Folders to the Same Location

A. Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler (Figure T24.2):

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **Example Vitra Images** folder into designated area.

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3. Click **Apply filters to the file list** to remove non-image files. Font for **Experiment.ini** should become gray.
4. Click **Metadata** category directly below **Images**.
5. Click **Yes** radio button in the **Extact metadata?** box.
6. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.
7. Confirm **Metadata source** drop-down list is set to **File name**.
8. Click the magnifying glass icon to the right of **Regular Expression to** field.
9. Erase all text from **Regex:** field.
10. Copy and paste following text into **Regex:** field, which will define the Channel Number, Well Number, Row of the plate with a letter, and Column of the plate with a number:

    Channel (?P<ChannelNumber>[0-9]{1,2})-(?P<WellNumber>[0-9]{2})-(?P<Row>[A-H]{1})-(?P<Column>[0-9]{2})-00.Bmp
11. Click **Submit** in **Regular expression editor** window.
12. Click **Update** button in lower portion to see information extracted for each of the images.
13. Click **NamesandTypes** directly below **Metadata**.
14. Change **Assign a name to** drop-down list to **Image matching rules**.
15. Enter **Channel 2-** in blank field to right of **Contain** drop-down list.
16. Confirm **DNA** is entered in **Name to assign these** field.
17. Click **Add another image** button.
18. For second image set, enter **Channel 1-** in blank field to right of **Contain** drop-down list.
19. Replace **GFP** in **Name to assign these** field with **Cytoplasm**.
20. Click **Update** button in lower portion.
21. Screen should resemble Figure T24.2 below for Metadata module.

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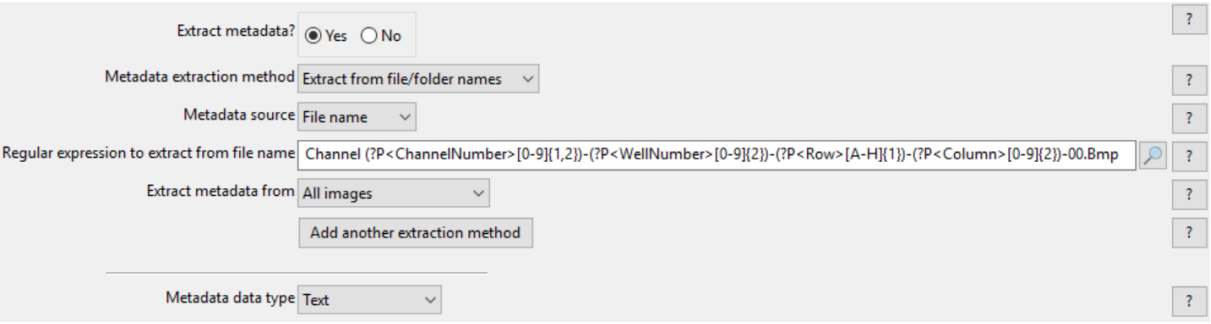

Figure T24.2 Setting up a Regular Expression in the LoadImages Module for a 96 Well Plate Experiment

Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export.

B. ConvertObjectsToImage Modules

In these modules, the objects you have defined through the "Identify Primary/Secondary Objects" module will be converted to image masks that FCS Express will use during import and analysis. See Figure T24.3 for an example of the module window.

1. Select the first **ConvertObjectsToImage** module in the pipeline.
2. Choose **Nuclei** from **Select the input objects** drop-down list.
3. Enter **Nuclei** in the **Name the output image** field.
4. Select **uint 16** from the **Select the color type** drop-down list (Figure T24.3 below).
5. Select the second **ConvertObjectsToImage** module in the pipeline.
6. Choose **Cells** from **Select the input objects** drop-down list.
7. Enter **Cells** in the **Name the output image** field.
8. Select **uint 16** from the **Select the color type** drop-down list.

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Note: Repeat steps 5-8 for as many objects you have defined through the Identify Primary/Secondary Objects module when defining your own pipeline.

*Note: In order for FCS Express to properly import the data the **Name the output image** name must be the same as the **Select the input objects** name and is case sensitive.*

Figure T24.3 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

C. SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** modules must now be saved and related to the original images/data.

1. Select the first **SaveImages** module in the pipeline.
2. Set the **Select the type of image to save** drop-down list to **Image**.
3. Choose **Cells** for the **Select the image to save** drop-down list.
    a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
4. Set the **Select method for constructing file names** drop-down list to **Single name**.
5. Enter **CellLabel_** in the **Enter single file name** field.
6. Right-click after **CellLabel_**, choose **Row,** and enter an underscore **"_"**after it.
7. Right-click after **Row_** and choose **Column**.
8. Select the second **SaveImages** module in the pipeline**.**
9. Set the **Select the type of image to save** drop-down list to **Image**.
10. Choose **Nuclei** for the **Select the image to save** drop-down list.
    a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.

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11. Set the **Select method for constructing file names** drop-down list to **Single name**.
12. Enter **NucleiLabel_** in the **Enter single file name** field.
13. Right-click after **NucleiLabel_**, choose **Row,** and enter an underscore **"_"**after it.
14. Right-click after **Row_** and choose **Column**.
    a. *Note: Row and Column are values you defined as regular expressions for the load images module. Using regular expressions allows every output file to be assigned a unique file name based on the text from the input file name. This will prevent output file names from overwriting each other and allow FCS Express to identify the unique image mask associated with each image file.*
    b. Perform steps 15-22 on both **SaveImages** modules set up for **CellLabel** and **NucleiLabel**.
15. Confirm **tiff** from the **Saved file format** drop-down list.
16. Set **Image bit depth** drop-down list to **16-bit integer**.
17. Choose **Default Output Folder** from **Output file location** drop-down list**.**
18. Click **Yes** radio button in the **Overwrite existing files without warning?** box.
19. Set **When to save** as **Every cycle** from the drop-down list.
20. Click **Yes** radio button in **Record the file and path information to the saved image?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.4.

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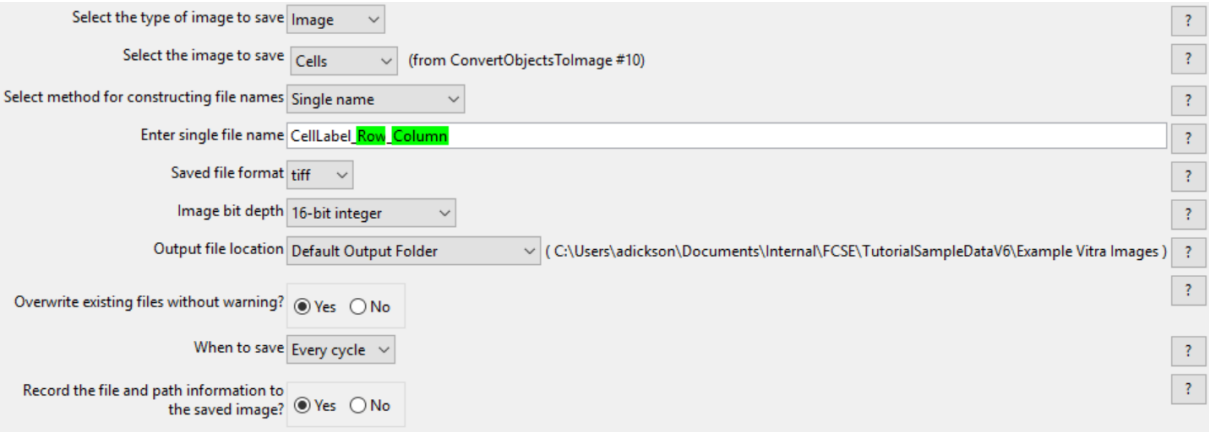

Figure T24.4 Defining the SaveImages Module

D. ExportToSpreadsheet Module

Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export 1976 section)

1. Select the **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image.cptoc** in **File name** field**.**
9. Click **Add another data set** button.
10. Choose **Nuclei** from the second **Data to export** drop-down list.

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11. Click **No** radio button in the **Use the object name for the file name?** box.
12. Replace **DATA.csv** with **nuclei.cptoc** in **File name** field**.**
13. Click **Add another data set** button.
14. Repeat steps 10-12 for **"Cells"** and set the file name as **cells.cpout**.

The **ExportToSpreadsheet** module options should look like Figure T24.5 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual listmode data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

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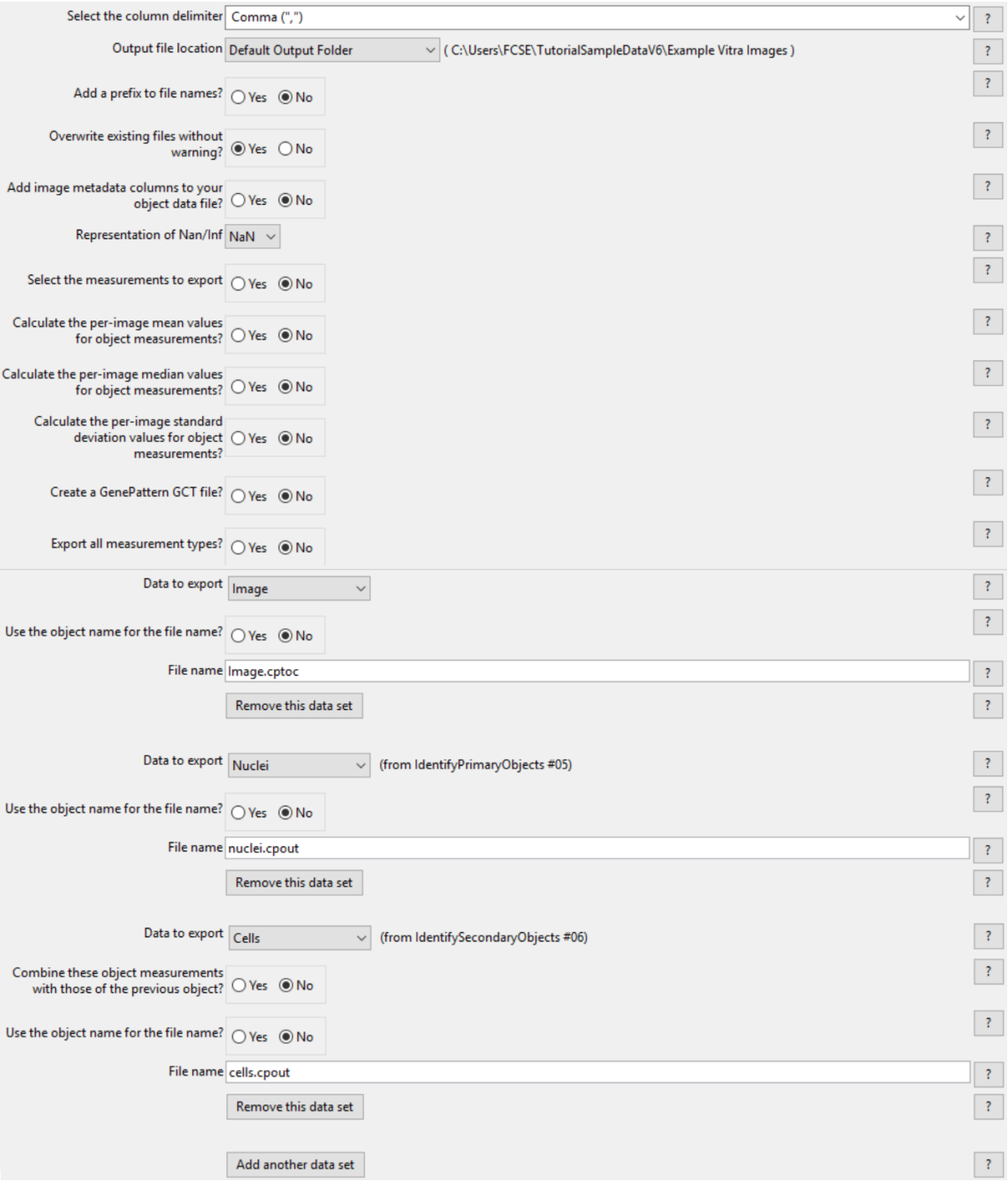

Figure T24.5 ExportToSpreadsheet Module Set Up to Export Image.cptoc, nuclei.cpout, and cells.cpout

15. Click **Analyze Images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout and Image.cptoc files

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(Figure T24.6A). There will also be a CellLabel and NucleiLabel image associated with every original image/well from the plate (Figure T24.6B).

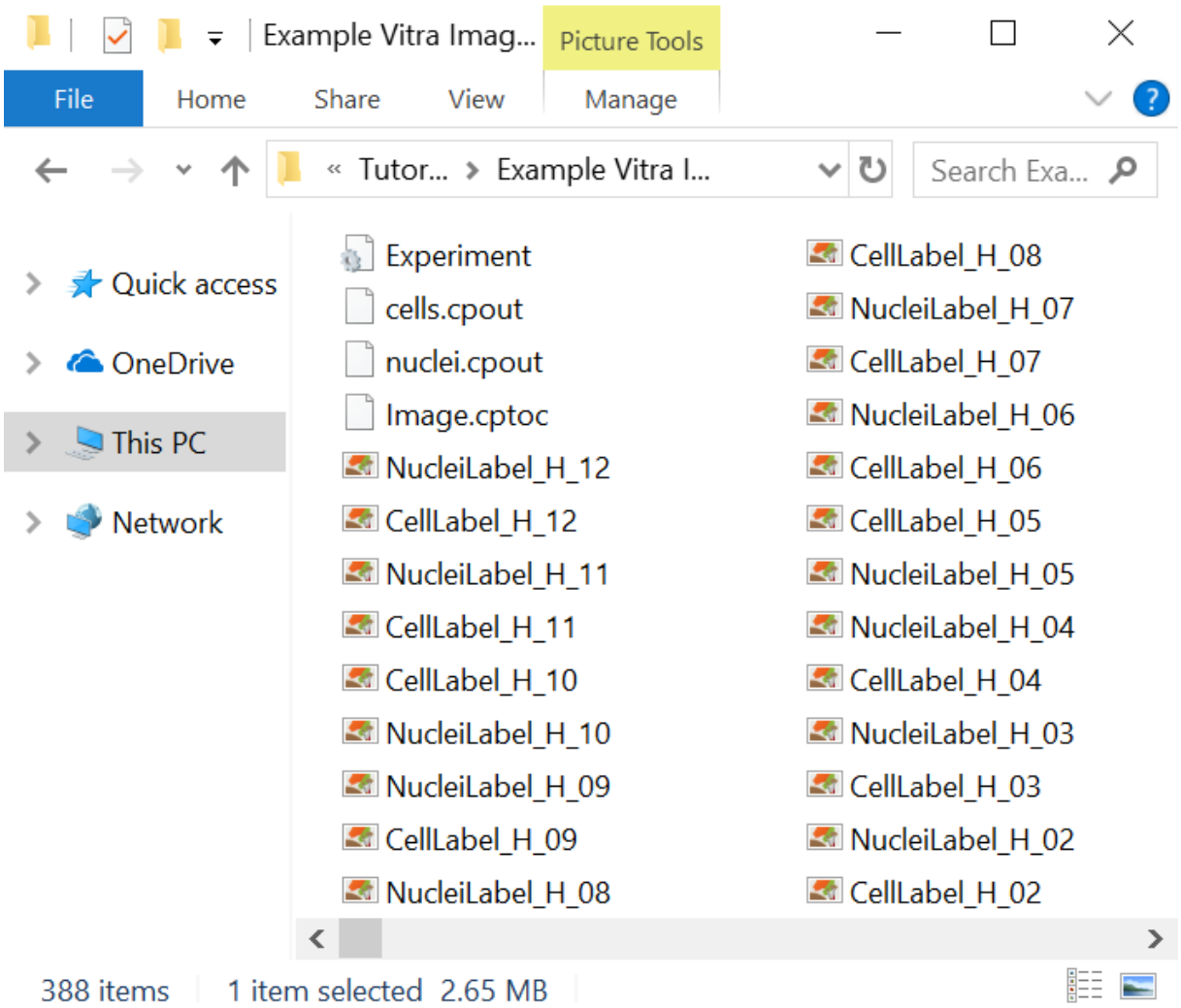

Figure T24.6A cells.cpout, nuclei.cpout, and Image.cptoc Files were Created. Experiment.ini was Downloaded.

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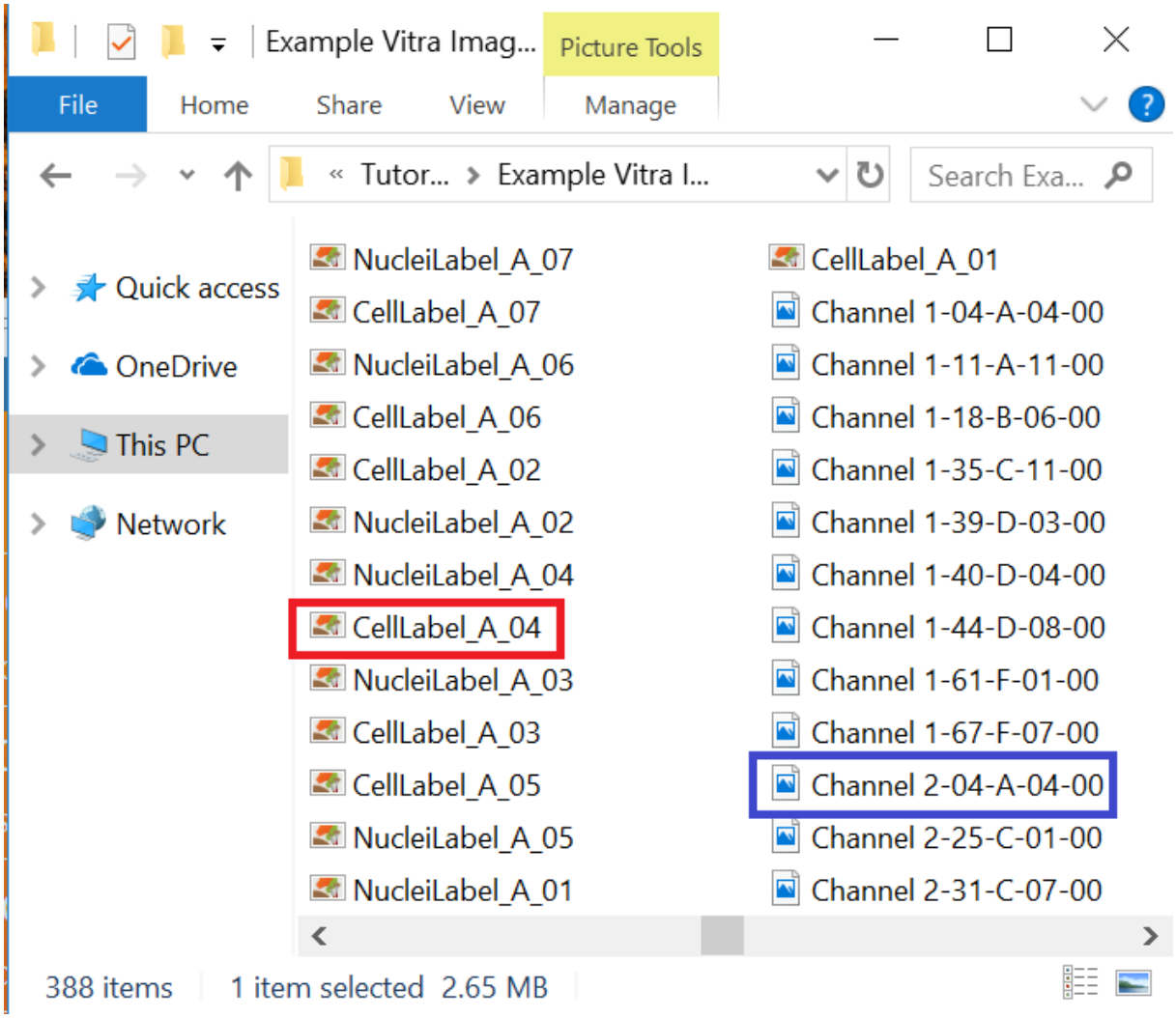

Figure T24.6B NucleiLabel and CellLabel image mask .tiff Have Been Created for Every Well of the Plate. The File Names Reflect the Row and Column Numbers for the CellLabels (red rectanglel) and Channels (blue rectangle).

In the next section, we will import and analyze the 96 well image data.

38.26.2 Import and Analyze 96 Well Images

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to import the data into FCS Express. FCS Express only needs one additional piece of information regarding the setup of your experiment. CellProfiler currently does not export the plate format that was used for your experiment. A series of standard plate format definitions can be downloaded from the FCS Express website. For this example, we will be using a 96 well template which can found in the Tutorial Sample Data archive and is labeled **Experiment.ini**. The plate format definition file must be called **Experiment.ini**

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and placed into the **Default Output Folder** you defined in CellProfiler (Figure T24.6A 1921).

1. Load the **Section1pipelineCOMPLETED.cpproj** pipeline into CellProfiler 3.0.
2. Drag and drop the folder called **Example Vitra Images** into **Images** module and click **Apply filters to file list** button.
3. Confirm the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.1 1921.
4. Click the **Analyze Images** button in the lower left of the in CellProfiler window.

Importing the 96 Well Plate Imaging Experiment to FCS Express

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial.)

5. Open FCS Express.
6. Open the **FCS Express User Options** by choosing **File→Options**.
7. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.7).
8. Select **csv** from the **delimiter** drop-down list.
9. Check the **Load Images** check box.
10. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
11. Click **OK** to close the **FCS Express User Options** dialog.

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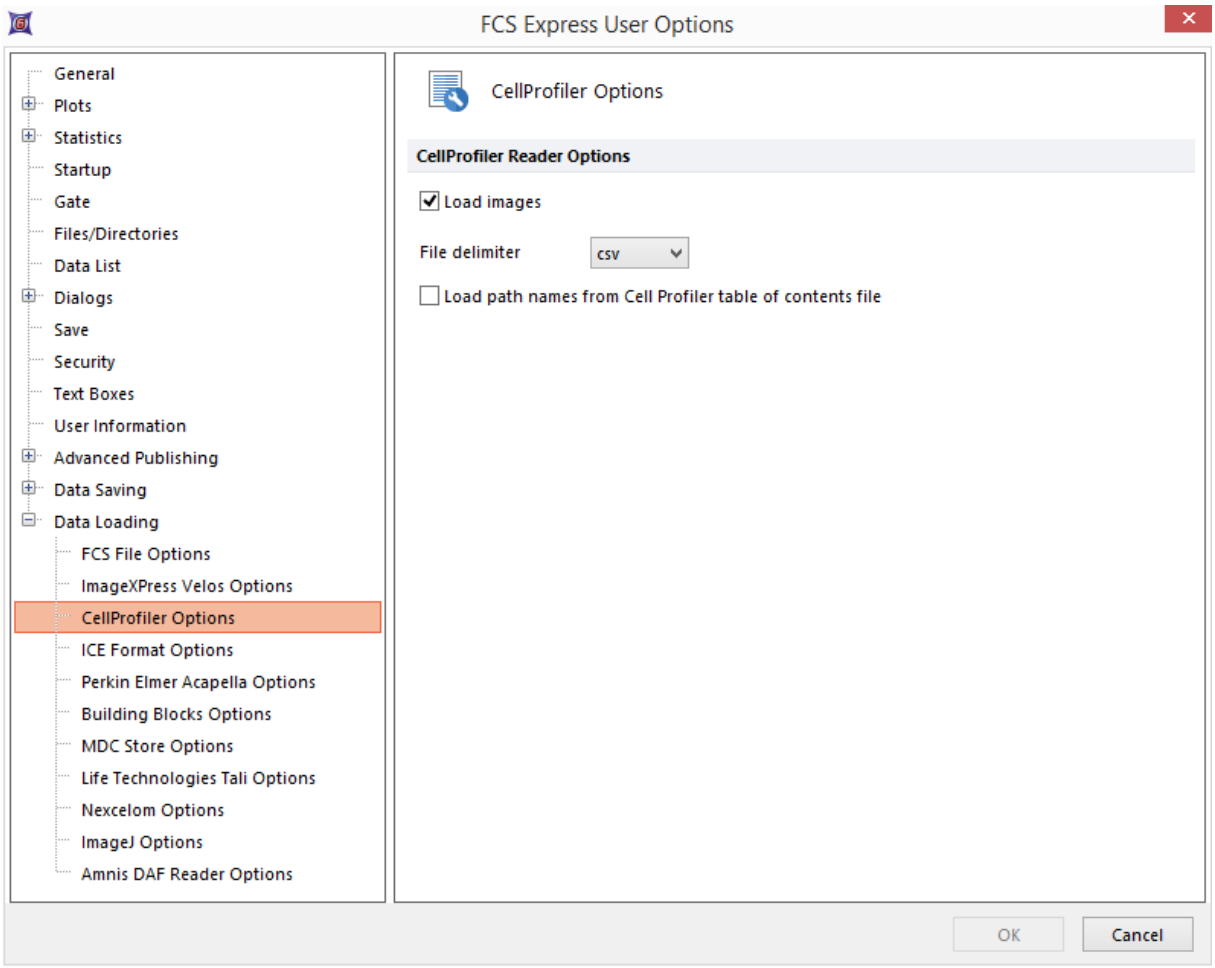

Figure T24.7 CellProfiler Data Loading Options for a 96 Well Plate Experiment with Images

12. Select the **Insert→Other Plots→Heat Map** command (Figure T24.8).

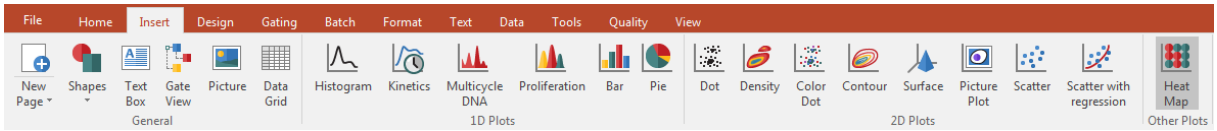

Figure T24.8 Choosing to Insert a Heat Map

13. Click anywhere on the layout and the **Select a Data File** dialog will appear.

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14. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

15. Change the **Files of type:** drop-down list to **Cell Profiler (*.cpout)** (Figure T24.9). Icons for the documents output by CellProfiler will appear. For this example, they will be labeled **cells.cpout** and **nuclei.cpout**.

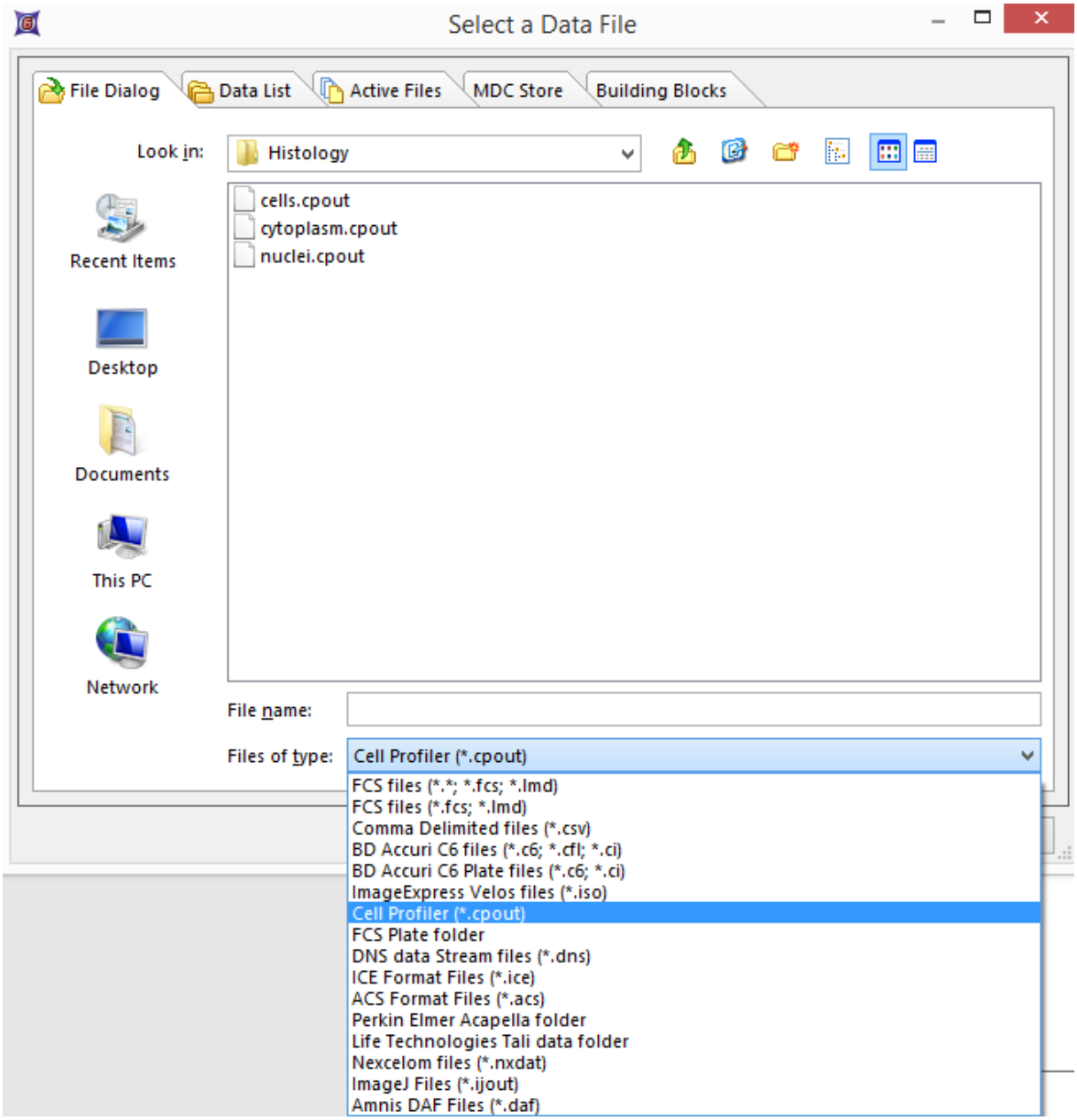

Figure T24.9 Choosing CellProfiler Files

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16. Highlight **cells.cpout**.

17. Click **Open**.

Note: For this example, we will use cells.cpout which will display data from objects classified in the *IdentifySecondaryObjects* module of CellProfiler which corresponds to the entire cell area. The file, nuclei.cpout, will display data from objects classified in the *IdentifyPrimaryObjects* module of CellProfiler, which corresponds only to the nuclear area of the cells.

18. Highlight the **Y-Axis Parameter** you wish to view in the heat map from the **Open 2D Plots** dialog. For this example, choose **Intensity_MeanIntensity_Cytoplasm** (Figure T24.10).

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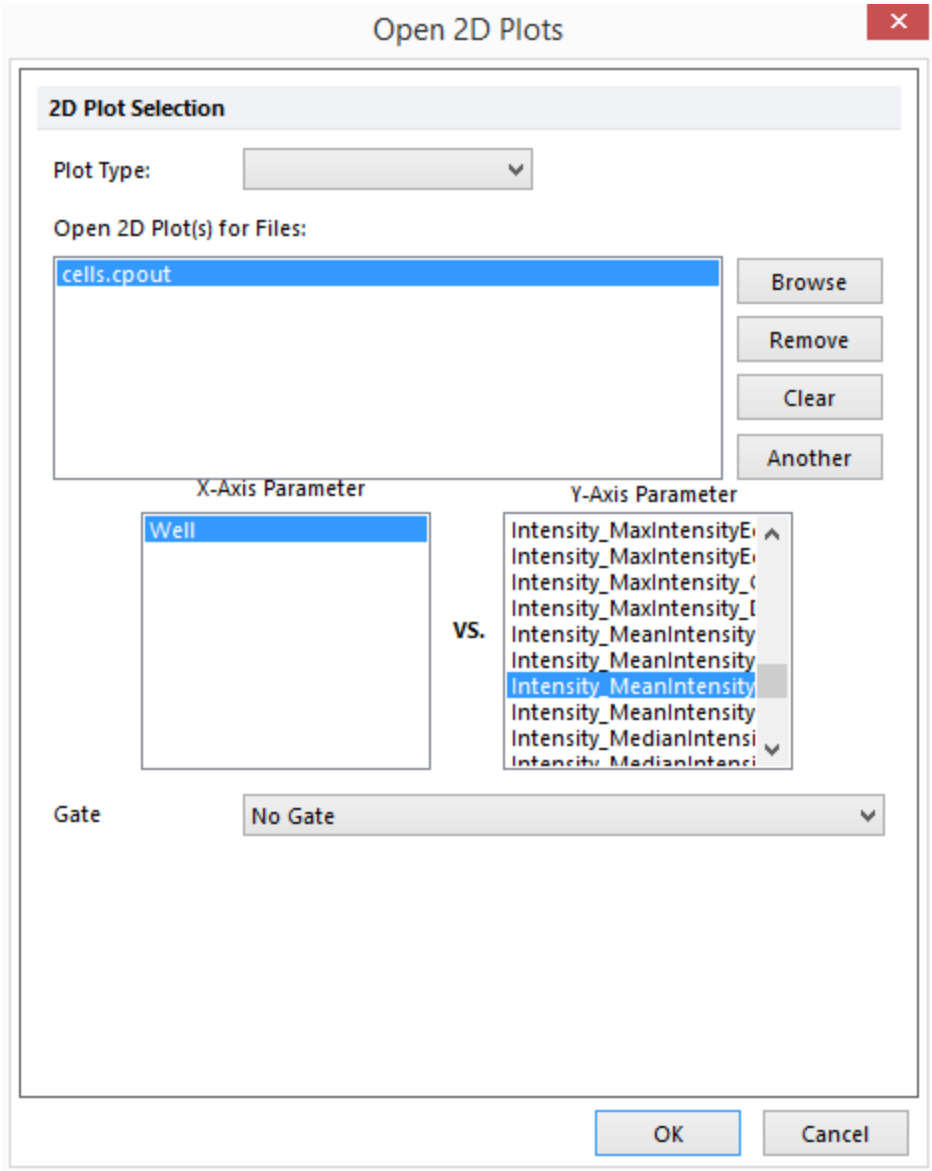

Figure T24.10 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

19. Click **OK**.

A Heat Map will now appear on your layout indicating that the data was successfully imported (Figure T24.11).

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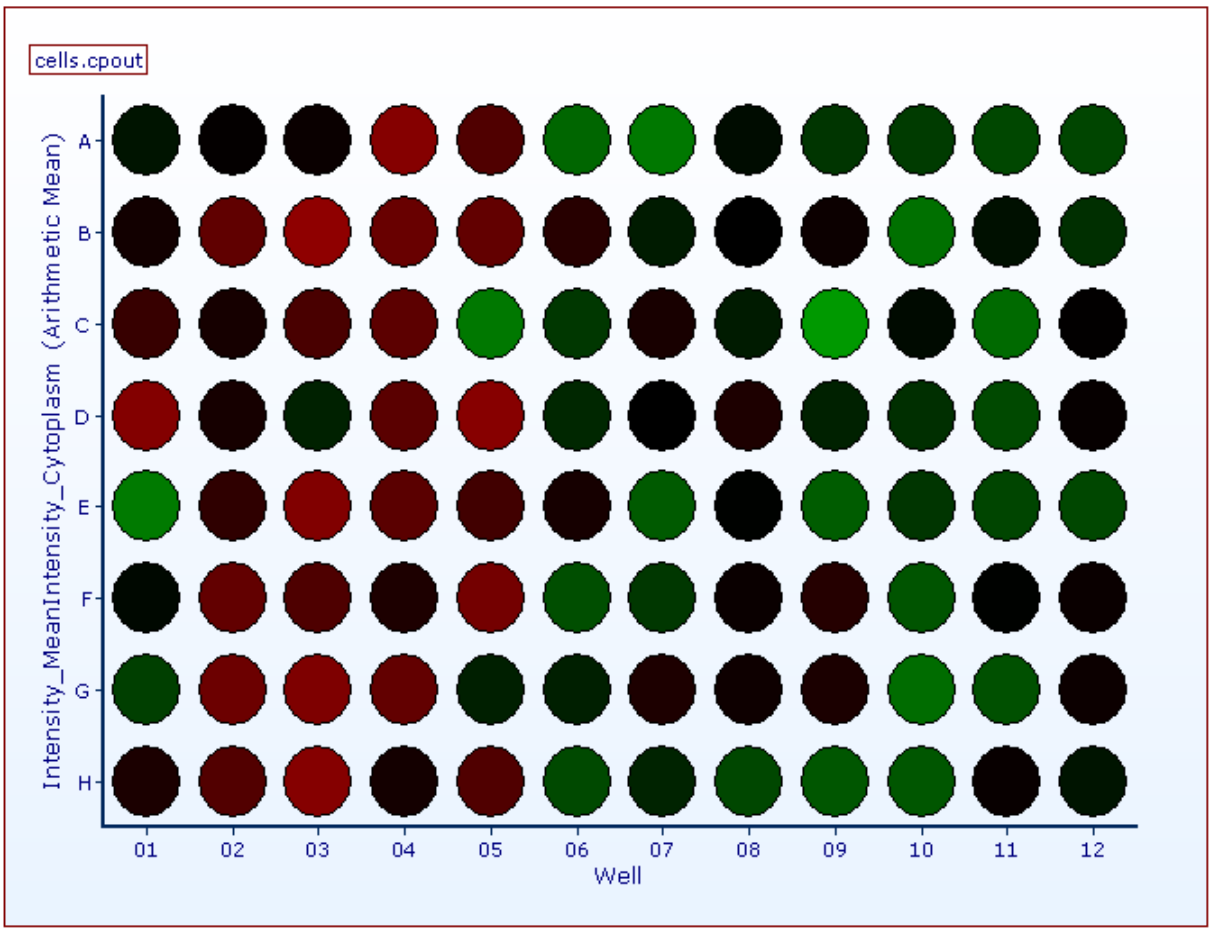

Figure T24.11 Example of a Heat Map Generated from CellProfiler Data

The Heat Map can now be gated using the **Gating→Create Gates→Well** command. Inserting a new 2D plot based on the well gate will allow you to view the data as familiar dot plots or view the image if using a Picture Plot (Figure T24.12).

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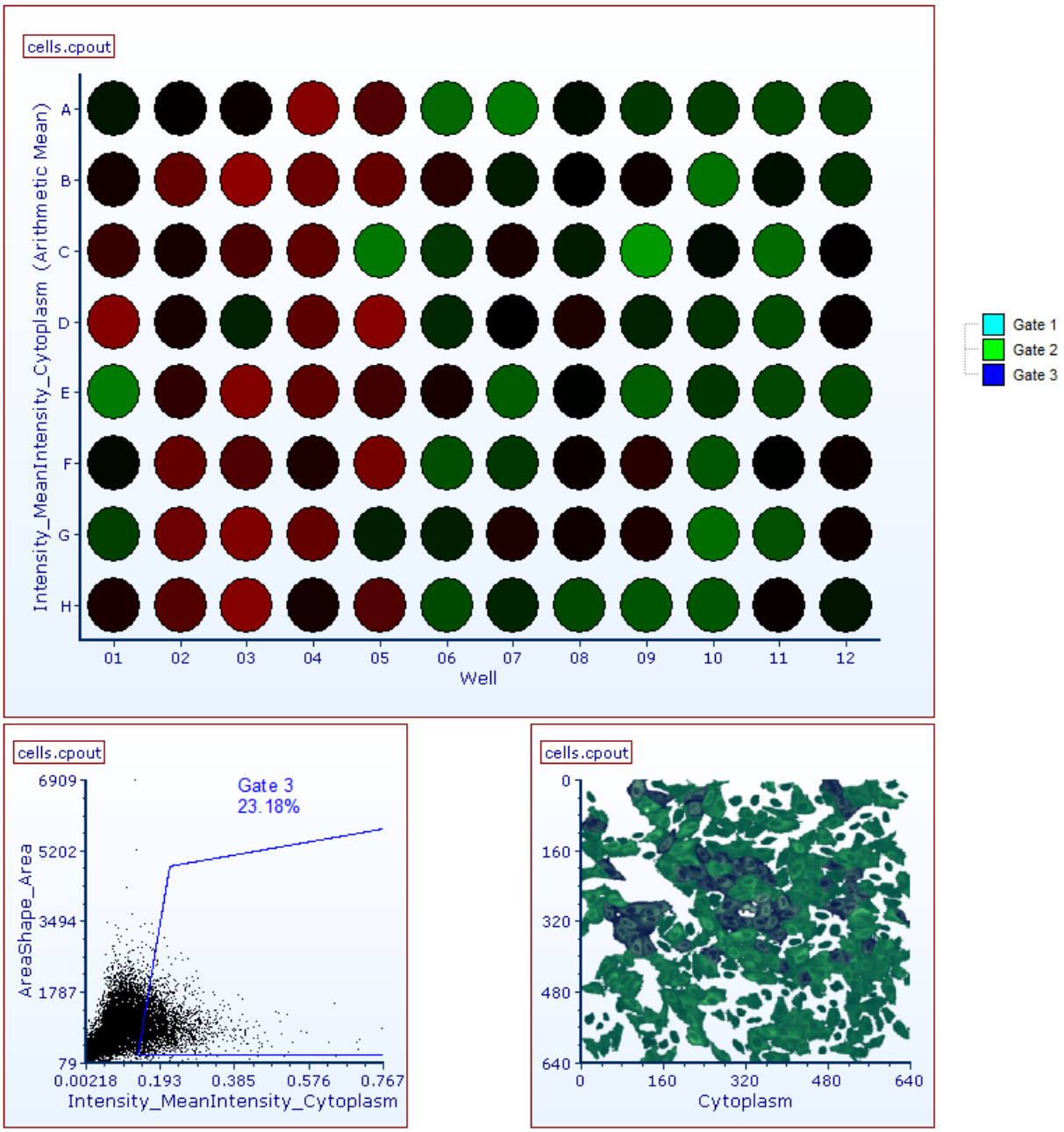

Figure T24.12 A Heat Map from CellProfiler Data with Corresponding Dot and Picture Plots

Next, we will export single images from CellProfiler 1938.

38.26.3 Exporting Single Images

In the next two sections, you will learn:

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- How to export CellProfiler data from a set of single images that were not obtained from a 96 well plate.
- How to import the data into FCS Express.

This section pertains to data from a set of single images that were not obtained from a 96 well plate such as images obtained from a slide.

In addition to extracting metadata from the image files and providing names for different channels (if applicable), the following CellProfiler analysis modules are required or must be amended in the pipeline order for export to FCS Express: Convert Objects to Images, Save Images, and Export to Spreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive and is named **Section2pipelineCOMPLETED.cpproj**. The completed pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

The sample images in the example data set we will be using can be found in the Tutorial Sample Data archive and are the **5 and 10 minute time point images** folder. These images are based on the "**Human cytoplasm-nucleus translocation assay (Vitra Image)"** from the previous module.

The steps for exporting data from CellProfiler have been broken down by module. After loading the **Section2pipeline.cpproj**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folder

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for **Default Input Folder** (example in Figure T24.13 below).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, you can select *Do not write MATLAB or HDF5 files* from the **Output file format** drop-down menu.

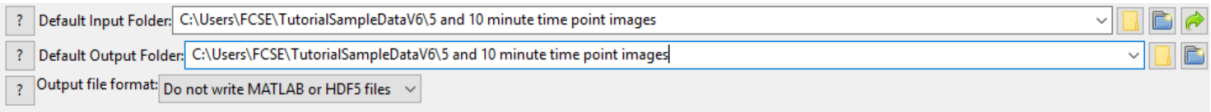

Figure T24.13 Set the Default Input and Output Folders to the Same Location

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A. Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler (Figure T24.14):

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **5 and 10 minute time point images** folder from **archive folder** into designated area in CellProfiler.
3. Click **Metadata** category directly below **Images**.
4. Click **Yes** radio button in the **Extact metadata?** box.
5. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.
6. Confirm **Metadata source** drop-down list is set to **File name**.
7. Click the magnifying glass icon to the right of **Regular Expression to** field.
8. Erase all text from **Regex:** field.
9. Copy and paste following text into **Regex:** field, which will define the Channel Number and time with a number:

   Channel(?P<ChannelNumber>[0-9]{1,2})-(?P<time>[0-9]{2})-min.tif
10. Click **Submit** in **Regular expression editor** window.
11. Click **Update** button in lower portion to see information extracted for each of the four images.
12. Click **NamesandTypes** directly below **Metadata**.
13. Change **Assign a name to** drop-down list to **Image matching rules**.
14. Enter **Channel2-** in blank field to right of **Contain** drop-down list.
15. Confirm **DNA** is entered in **Name to assign these** field.
16. Click **Add another image** button.
17. For second image set, enter **Channel1-** in blank field to right of **Contain** drop-down list.
18. Replace **GFP** in **Name to assign these** field with **Cytoplasm**.
19. Click **Update** button in lower portion.

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Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- Setting up metadata and regular expressions only has to be done for one image in the set. For this example, it has been set up for channel 2.
- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export.

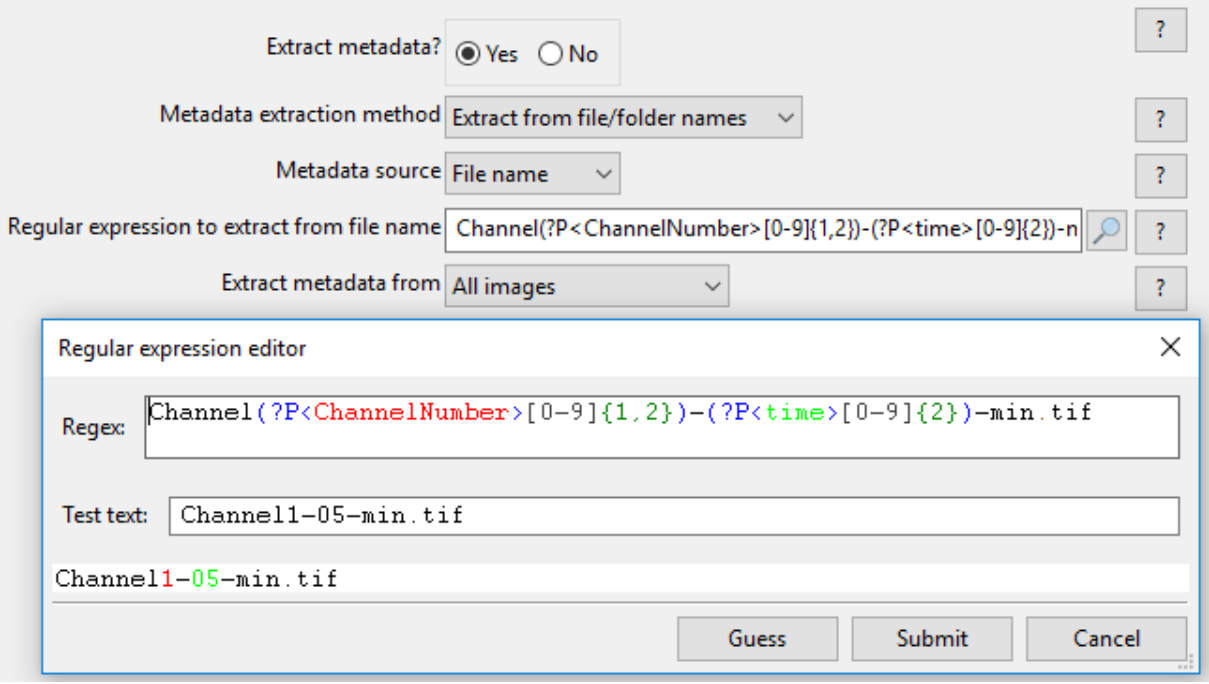

Figure T24.14 Setting Up a Regular Expression in the LoadImages Module

B. ConvertObjectsToImage Modules

In these modules, the objects you have defined through the "Identify Primary/Secondary Objects" module will be converted to image masks that FCS Express will use during import and analysis. See Figure T24.15 for an example of the module window.

1. Select the first **ConvertObjectsToImage** module in the pipeline.

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2. Choose **Nuclei** from **Select the input objects** drop-down list.
3. Enter **Nuclei** in the **Name the output image** field.
4. Select **uint 16** from the **Select the color type** drop-down list.
4. Select the second **ConvertObjectsToImage** module in the pipeline.
5. Choose **Cells** from **Select the input objects** drop-down list.
6. Enter **Cells** in the **Name the output image** field.
5. Select **uint 16** from the **Select the color type** drop-down list.

Note: Repeat steps 4-7 for as many objects you have defined through the Identify Primary/Secondary Objects module when defining your own pipeline.

*Note: In order for FCS Express to properly import the data the **Name the output image** name must be the same as the **Select the input objects** name and is case sensitive.*

C. SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** module must now be saved and related to the original images/data.

1. Select the first **SaveImages** module in the pipeline.
2. Set the **Select the type of image to save** drop-down list to **Image**.
3. Choose **Cells** for the **Select the image to save** drop-down list.
    a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
4. Set the **Select method for constructing file names** drop-down list to **Single name**.
5. Enter **CellLabel_** in the **Enter single file name** field to replace **OrigBlue**.
6. Right-click after immediately after the underscore of **CellLabel_** and choose **time**.
7. Select the second **SaveImages** module in the pipeline**.**
8. Set the **Select the type of image to save** drop-down list to **Image**.
9. Choose **Nuclei** for the **Select the image to save** drop-down list.

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a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.

10. Set the **Select method for constructing file names** drop-down list to **Single name**.

11. Enter **NucleiLabel_** in the **Enter single file name** field to replace **OrigBlue**.

12. Right-click after immediately after the underscore of **NucleiLabel_ and** choose **time**.

    a. Perform steps 13-18 on both **SaveImages** modules set up for **CellLabel** and **NucleiLabel**.

13. Confirm **tiff** from the **Saved file format** drop-down list.

14. Set **Image bit depth** drop-down list to **16-bit integer**.

15. Choose **Default Output Folder** from **Output file location** drop-down list**.**

16. Click **Yes** radio button in the **Overwrite existing files without warning?** box.

17. Set **When to save** as **Every cycle** from the drop-down list.

18. Click **Yes** radio button in **Record the file and path information to the saved image?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.15 below.

Figure T24.15 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

D. ExportToSpreadsheet Module

Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the

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measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export section)

1. Select the **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image_.cptoc** in **File name** field**.**
9. Right-click between the underscore and period; click **time**.
10. Click **Add another data set** button.
11. Choose **Nuclei** from the second **Data to export** drop-down list.
12. Click **No** radio button in the **Use the object name for the file name?** box.
13. Replace **DATA.csv** with **nuclei_.cptoc** in **File name** field**.**
14. Right-click between the underscore and period; click **time.**
15. Click **Add another data set** button.
16. Repeat steps 10-12 for **"Cells"** and set the file name as **cells_.cpout**.
17. Right-click between the underscore and period; click **time**.

The **ExportToSpreadsheet** module options should look like Figure T24.16 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual

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listmode data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

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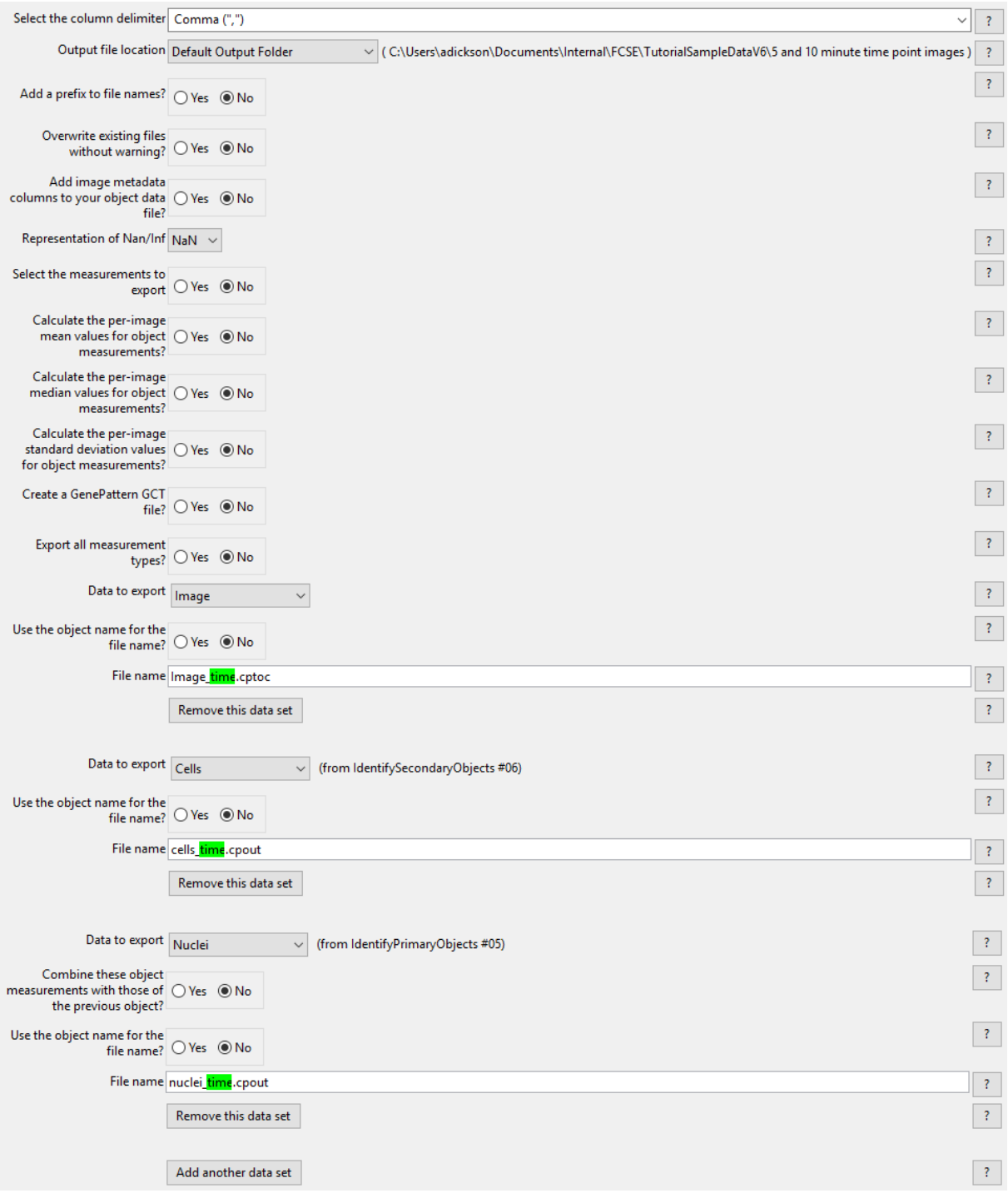

Figure T24.16 ExportToSpreadsheet Module

18. Click **Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout and Image.cptoc files for each time point. There will also be a CellLabel and NucleiLabel

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image associated with every original image from the experiment (Figure T24.17).

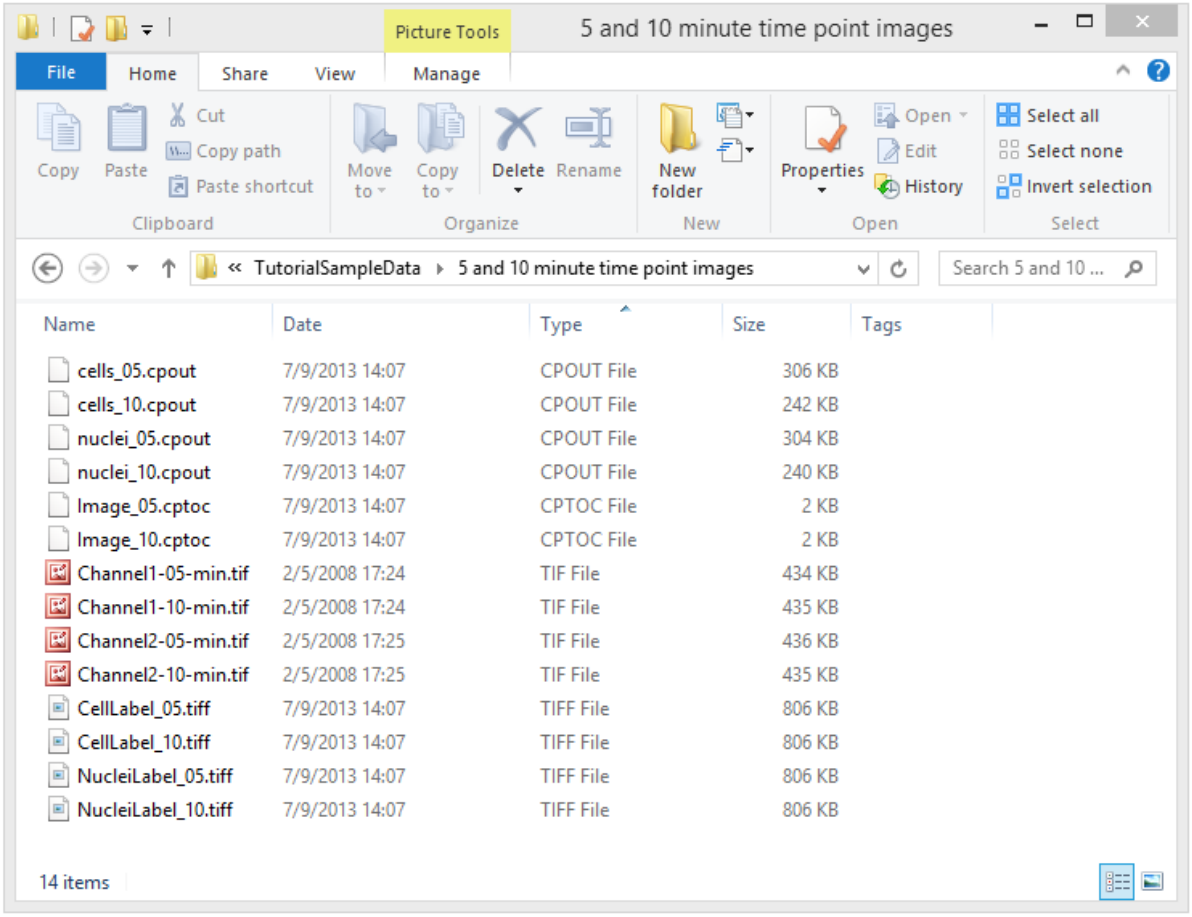

Figure T24.17 Example of Output Folder once CellProfiler Analysis is Complete

In the next section, we will import the single image experiment into FCS Express.

38.26.4 Importing Single Image Data

This section pertains to data from a set of single images that were not obtained from a 96 well plate, such as images obtained from a slide.

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to analyze your images and import the data into FCS Express.

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1. Load the **Section2pipelineCOMPLETED.cpproj** pipeline into CellProfiler 3.0.
2. Drag and drop the folder called **5 and 10 minute time point images** folder into **Images** module.
3. Confirm the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.13 1947.
4. Click the **Analyze Images** button in the lower left of the in CellProfiler window.

Importing the Single Image Experiment to FCS Express

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

5. Open FCS Express.
6. Open the **FCS Express User Options** by choosing **File→Options**
7. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.18).
8. Select **csv** from the **delimiter** drop-down list.
9. Check the **Load Images** check box.
10. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
11. Click **OK** to close the **FCS Express User Options** dialog.

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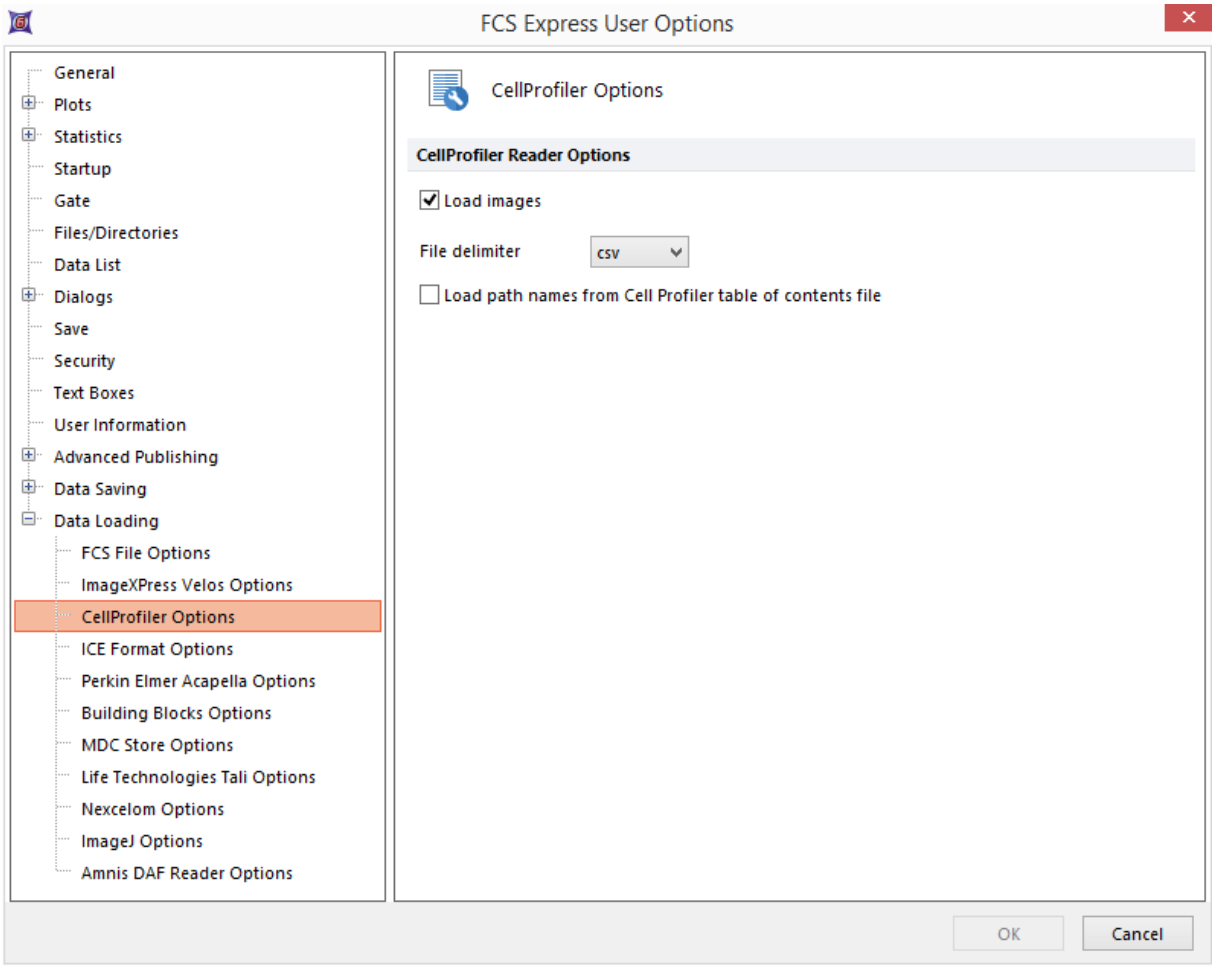

Figure T24.18 CellProfiler Data Loading Options

12. Select the **Insert→2D Plots →Picture Plots** command (Figure T24.19).

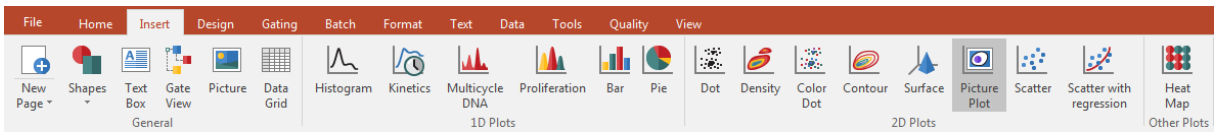

Figure T24.19 Choosing a Picture Plot to Insert

13. Click anywhere on the layout and the **Select a Data File** dialog will appear.

14. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

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15. Change the **Files of type:** drop-down list to **CellProfiler (*.cpout)** (Figure T24.20)**.** Icons for the documents outputted by CellProfiler will appear. For this example, they will be labeled cells_05.cpout and nuclei_05.cpout.

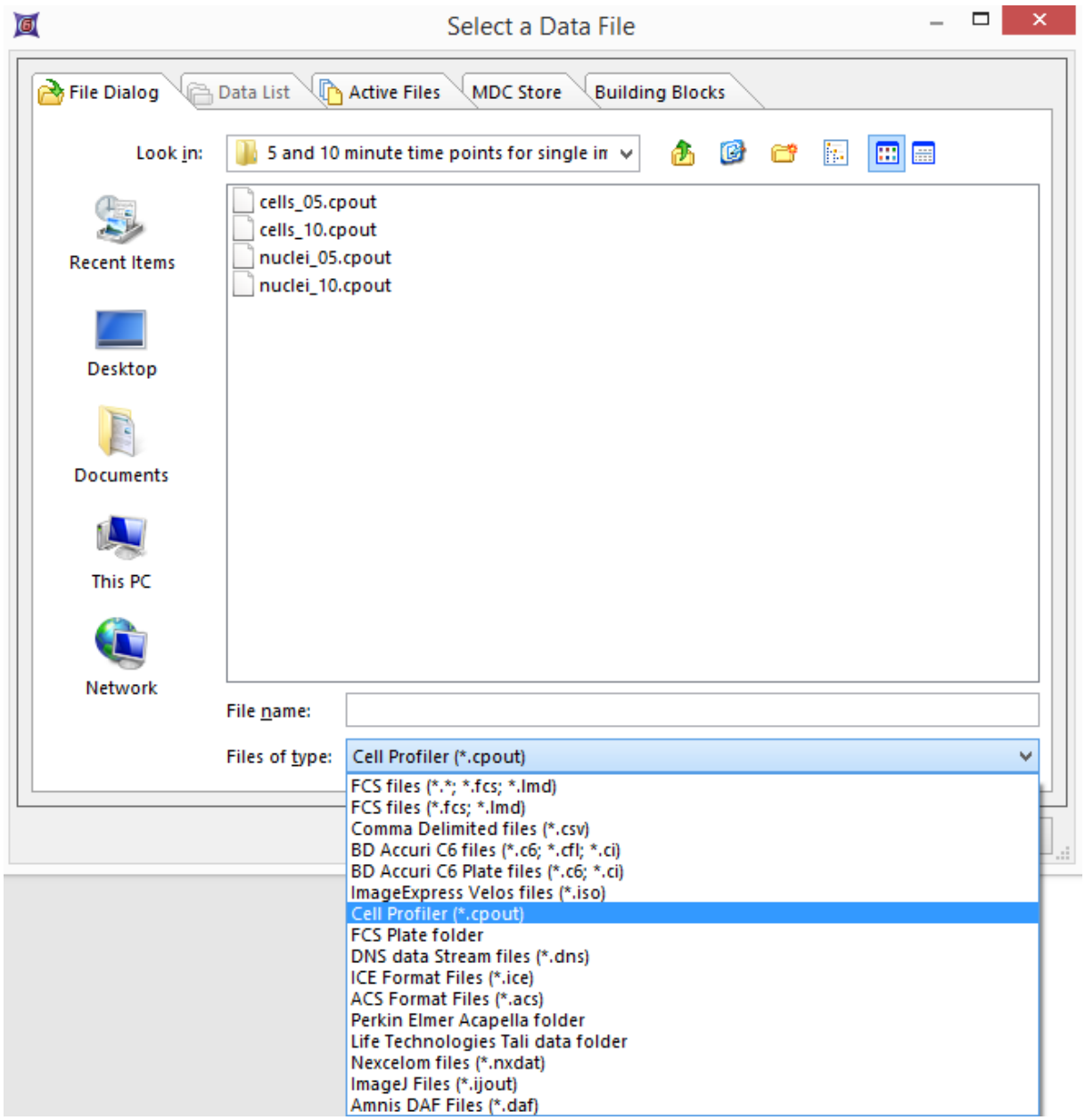

Figure T24.20 Select a Data File Dialog

16. Highlight **cells_05.cpout** and click **Open**.

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Note: For this example, we will use **cells_05.cpout** which will display data from the 5 minute time point objects classified in the IdentifySecondaryObjects module of CellProfiler which corresponds to the entire cell area. **Nuclei_05.cpout** will display data from the 5 minute time point objects classified in the IdentifyPrimaryObjects module of CellProfiler which corresponds only to the nuclear area of the cells.

17. Highlight **Cytoplasm** from the **Image Parameter** list box of the **Open 2D Plots** dialog (Figure T24.21) to view the cytoplasmic image.

18. Click **OK**.

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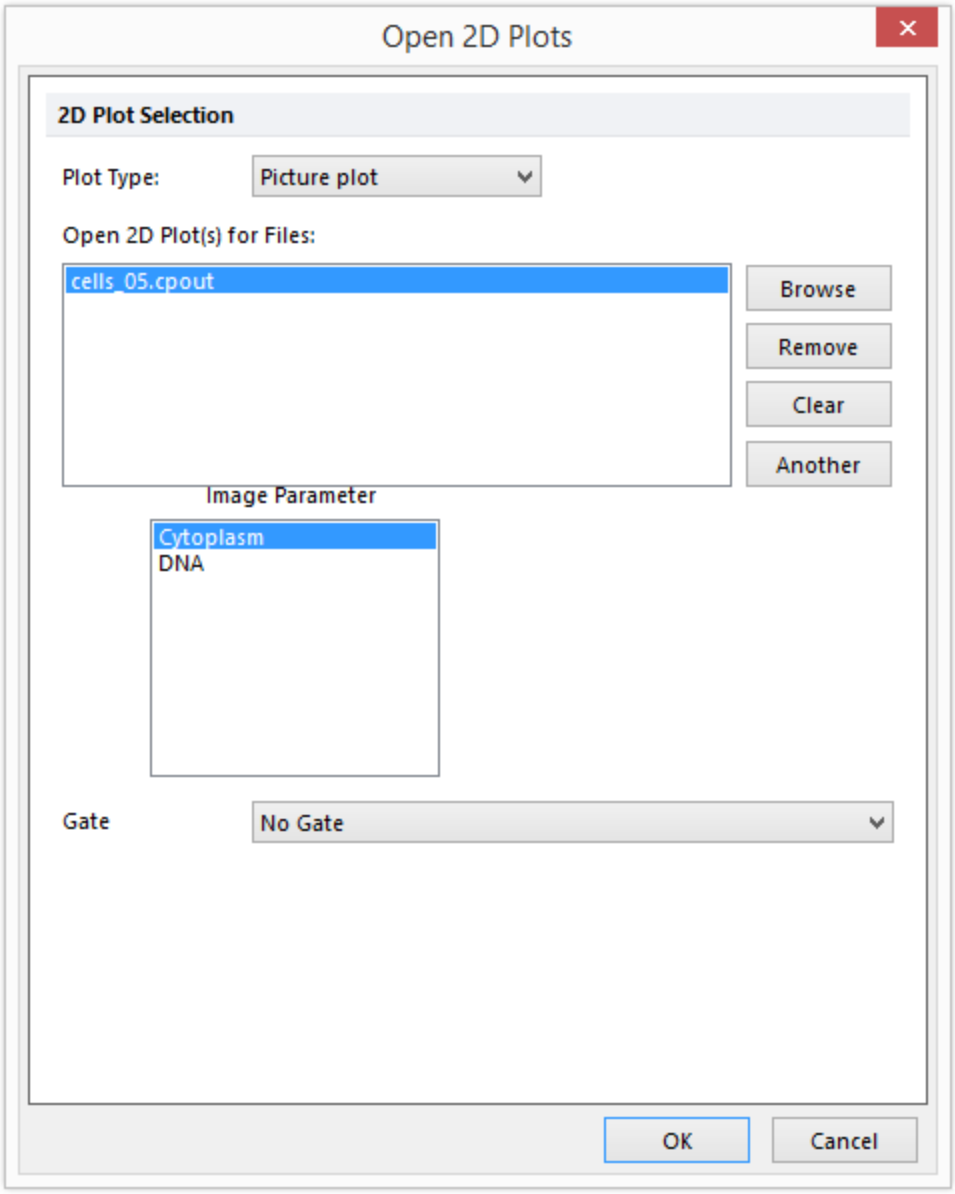

Figure T24.21 Choosing Image Parameters from the Open 2D Plots Dialog

A **Picture Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.22).

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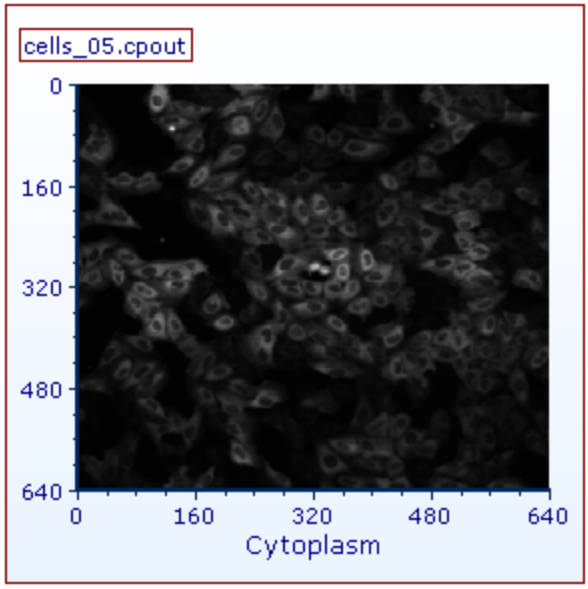

Figure T24.22 Example of the Cells (5 Minute Time Point) Picture Plot

19. Repeat steps 13-18 but highlight **DNA** as **Image Parameter** in the **Open 2D Plots** dialog.

Note: alternatively to step 17 you can duplicate 2D plot previously created and then change the visualized parameter by clicking on the X axis and choosing **DNA** instead of **Cytoplasm**.

The layout should now looks like Figure T24.23a.

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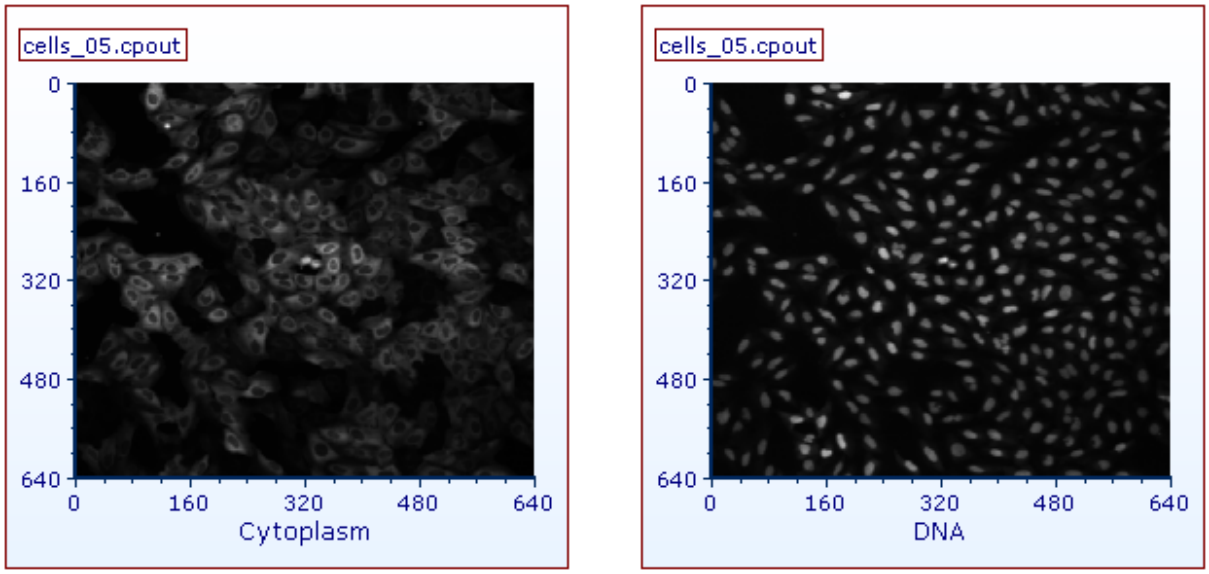

Figure T24.23a Picture plots of the cells_05.cpout file depicting cytoplasm and DNA

18. Any type of 2D plot can be created by following the previous step. For training purpose, the user can try opening two **Color Dot Plots** of the same file (i.e. cell_05.cpout) depicting:

- **AreaShape_Area** with **Intensity_MeanIntensity_Cytoplasm**

- **AreaShape_Area** with **Intensity_MeanIntensity_DNA**

The layout should now looks like Figure T24.23b.

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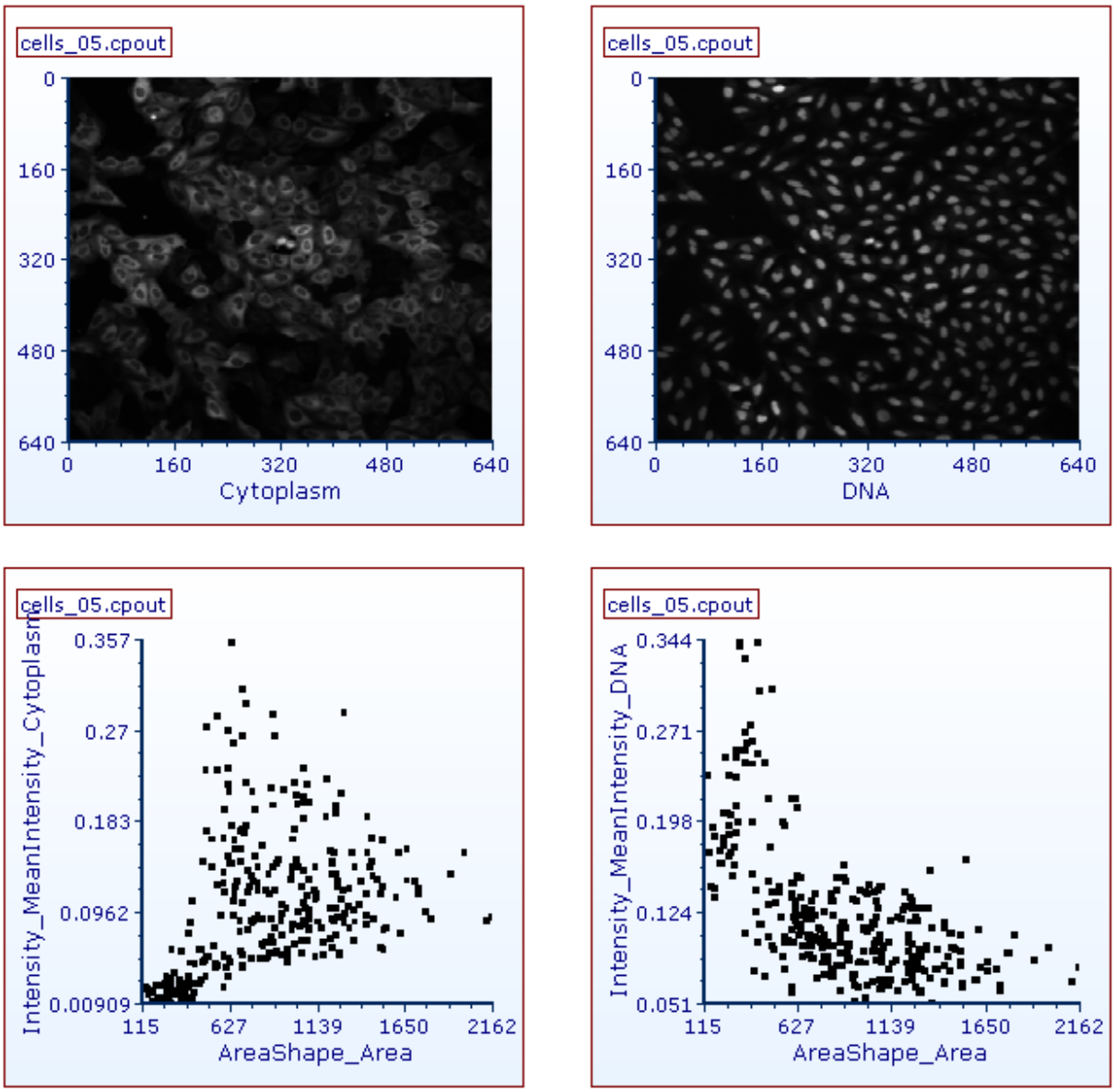

Figure T24.23b Picture plots and Scatter plots of the cell_05.cpout file

Any 2D gate can easily be created on the **Picture Plots** and on the **Scatter Plots** as well. Inserting a new 2D plot gated on the picture plot gate will allow you to view the data as familiar dot plots, or view gated portions of the image (Figure T24.24).

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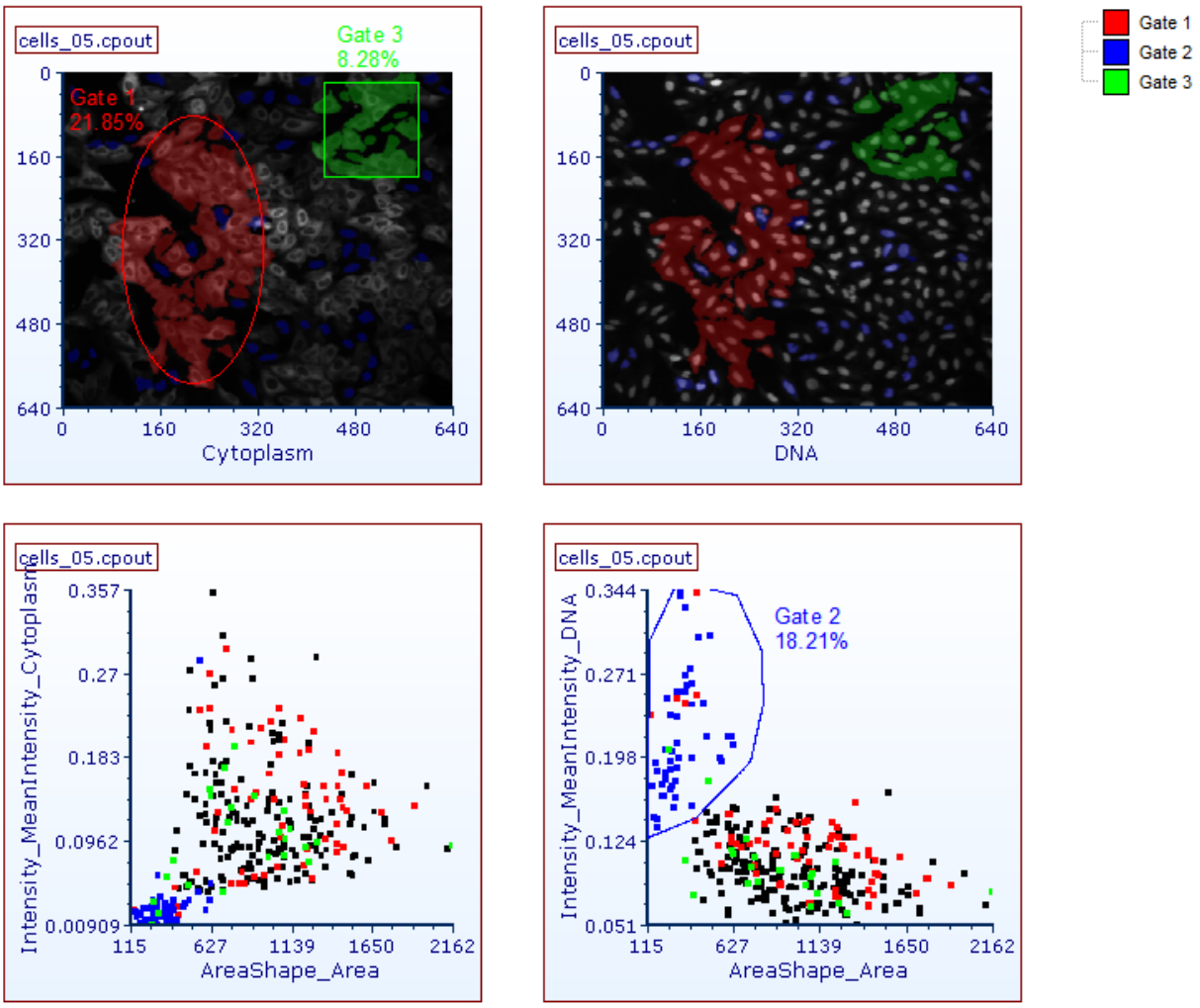

Figure T24.24 Example of Gating on a Picture Plot and Backgating from a Dot Plot to a Picture Plot

In the next exercise, we will export numeric data only from a 96 well plate.

38.26.5 Exporting Numeric Data (96 Well)

In this section of the tutorial you will learn:

- How to export only the numeric data from a 96 Well Plate CellProfiler pipeline.

In addition to extracting metadata from the image files and providing names for different channels (if applicable), the Export to Spreadsheet module must be amended. The completed pipeline for this example can be found in the Tutorial

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Sample Data archive and is named **Section3pipeline.cpproj**. The completed pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

The example data set we will be using can be downloaded from the CellProfiler Examples website and is labeled "**Human cytoplasm-nucleus translocation assay (Vitra Image)**".

The steps for exporting data from CellProfiler have been broken down by module according to the Section3pipeline.cp. After loading the **Section3pipeline.cpproj**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folder

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for the **Default Input Folder** (Figure T24.25).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, you can select *Do not write MATLAB or HDF5 files* from the **Output file format** drop-down menu.

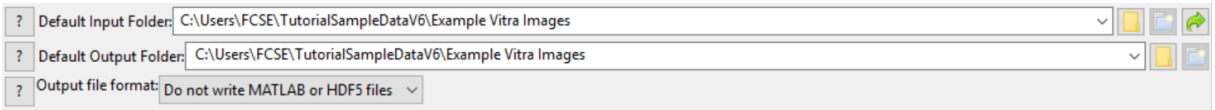

Figure T24.25 Set the Default Input and Output Folders to the Same Location

A. Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler (Figure T24.2):

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **Example Vitra Images** folder from **archive folder** into designated area.

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3. Click **Apply filters to the file list** to remove non-image files. Font for **Experiment.ini** should become gray.
4. Click **Metadata** category directly below **Images**.
5. Click **Yes** radio button in the **Extact metadata?** box.
6. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.
7. Confirm **Metadata source** drop-down list is set to **File name**.
8. Click the magnifying glass icon to the right of **Regular Expression to** field.
9. Erase all text from **Regex:** field.
10. Copy and paste following text into **Regex:** field, which will define the Channel Number, Well Number, Row of the plate with a letter, and Column of the plate with a number:

    Channel (?P<ChannelNumber>[0-9]{1,2})-(?P<WellNumber>[0-9]{2})-(?P<Row>[A-H]{1})-(?P<Column>[0-9]{2})-00.Bmp
11. Click **Submit** in **Regular expression editor** window.
12. Click **Update** button in lower portion to see information extracted for each of the images.
13. Click **NamesandTypes** directly below **Metadata**.
14. Change **Assign a name to** drop-down list to **Image matching rules**.
15. Enter **Channel 2-** in blank field to right of **Contain** drop-down list.
16. Confirm **DNA** is entered in **Name to assign these** field.
17. Click **Add another image** button.
18. For second image set, enter **Channel 1-** in blank field to right of **Contain** drop-down list.
19. Replace **GFP** in **Name to assign these** field with **Cytoplasm**.
20. Click **Update** button in lower portion.
21. Screen should appear as Fig.T24.2 1921 for Metadata module.

Notes:

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- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export.

B. ExportToSpreadsheet Module

Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export 1976 section.)

1. Select the **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image.cptoc** in **File name** field**.**
9. Click **Add another data set** button.
10. Choose **Nuclei** from the second **Data to export** drop-down list.
11. Click **No** radio button in the **Use the object name for the file name?** box.
12. Replace **DATA.csv** with **nuclei.cptoc** in **File name** field**.**
13. Click **Add another data set** button.
14. Repeat steps 10-12 for **"Cells"** and set the file name as **cells.cpout**.

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The **ExportToSpreadsheet** module options should look like Figure T24.5 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual listmode data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

15. Click **Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout and Image.cptoc files (Figure T24.66). There will also be a CellLabel and NucleiLabel image associated with every original image/well from the plate (Figure T24.26).

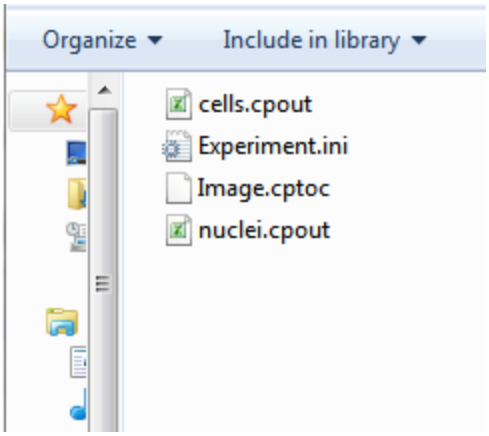

Figure T24.26 Data Only Output Files

Next, we will import and analyze the numeric data.

38.26.6 Importing Numeric Data (96 Well)

In this section you will learn:

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- How to import the data into FCS Express to view Heat Maps, 2D Plots, and 1D plots.

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to import the data into FCS Express. FCS Express only needs one additional piece of information regarding the setup of your experiment. CellProfiler currently does not export the plate format that was used for your experiment. A series of standard plate format definitions can be downloaded the FCS Express website. For this example, we will be using a 96 well template which can found in the Tutorial Sample Data archive and is labeled **Experiment.ini.** The plate format definition file must be called **Experiment.ini** and placed into the **Default Output Folder** you defined in CellProfiler.

1. Load the **Section3pipelineCOMPLETED.cpproj** pipeline into CellProfiler 3.0.
2. Drag and drop the folder called **Example Vitra Images** into **Images** module and click **Apply filters to file list** button.
3. Confirm the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.1.
4. Click the **Analyze Images** button in the lower left of the in CellProfiler window.

Importing 96 Well Plate Numeric Data Only

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

5. Open FCS Express.
6. Open the **FCS Express User Options** by choosing **File→Options**

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7. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.27).
8. Select **csv** from the **delimiter** drop-down list.
9. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
10. Click **OK** to close the **FCS Express User Options** dialog.

Note: In this example there are no images associated with the data so the **Load Images** check box must remain unchecked.

The dialog should look like Figure T24.27 when you are done.

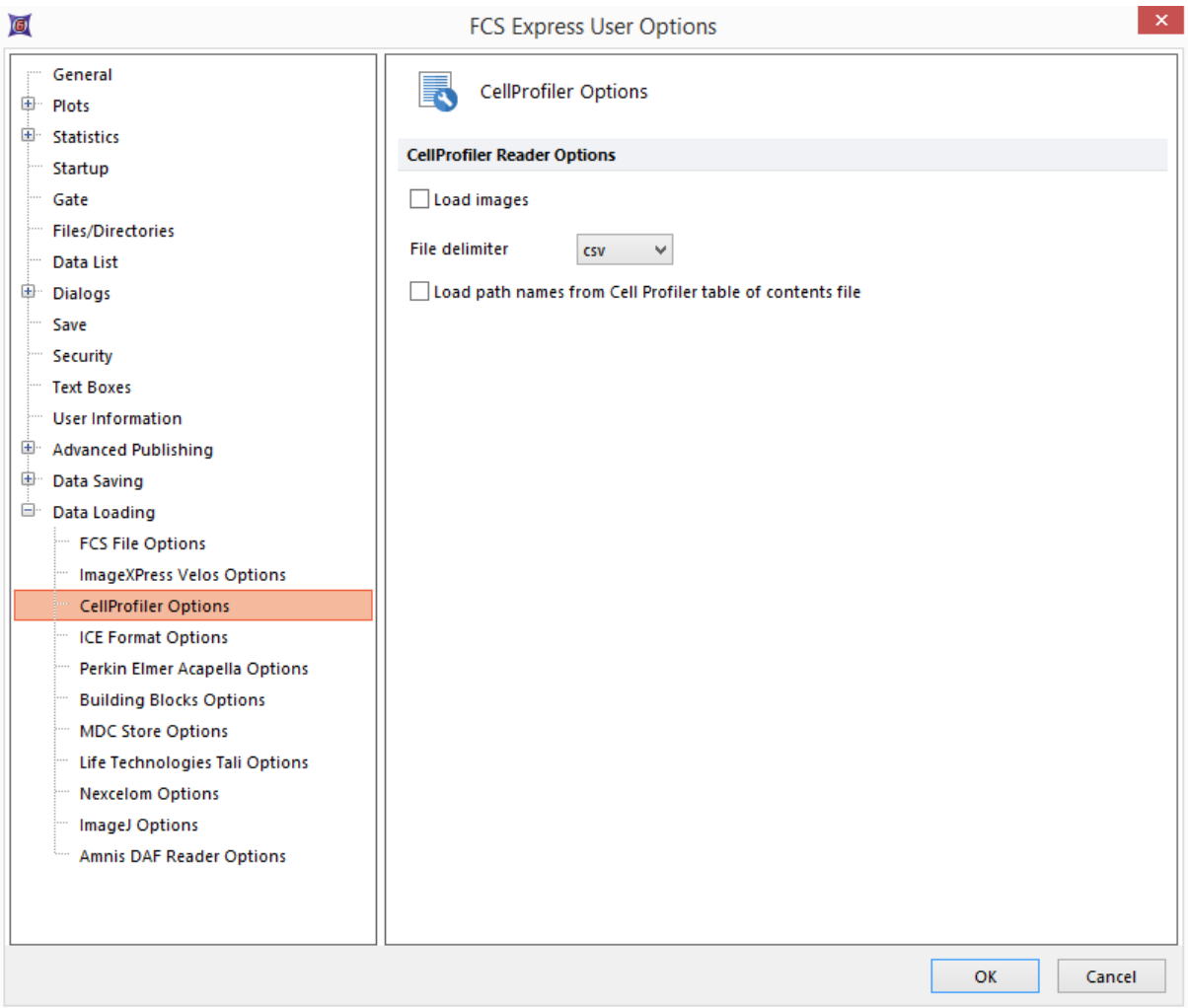

Figure T24.27 CellProfiler Data Loading Options for Data Only

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11. Select the **Insert→Other Plots→Heat Map** command (Figure T24.28).

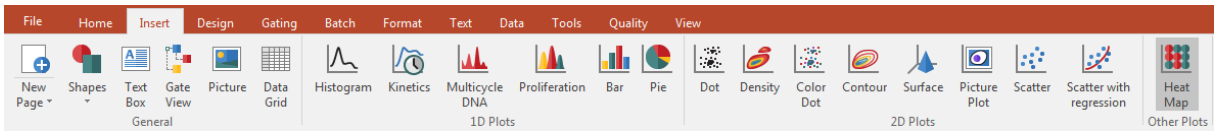

Figure T24.28 Choosing to Insert a Heat Map

12. Click anywhere on the layout and the **Select a Data File** dialog will appear.

13. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

14. Change the **Files of type:** drop-down list to **Cell Profiler (*.cpout)** (Figure T24.29). Icons for the documents output by CellProfiler will appear. For this example, they will be labeled **cells.cpout** and **nuclei.cpout**.

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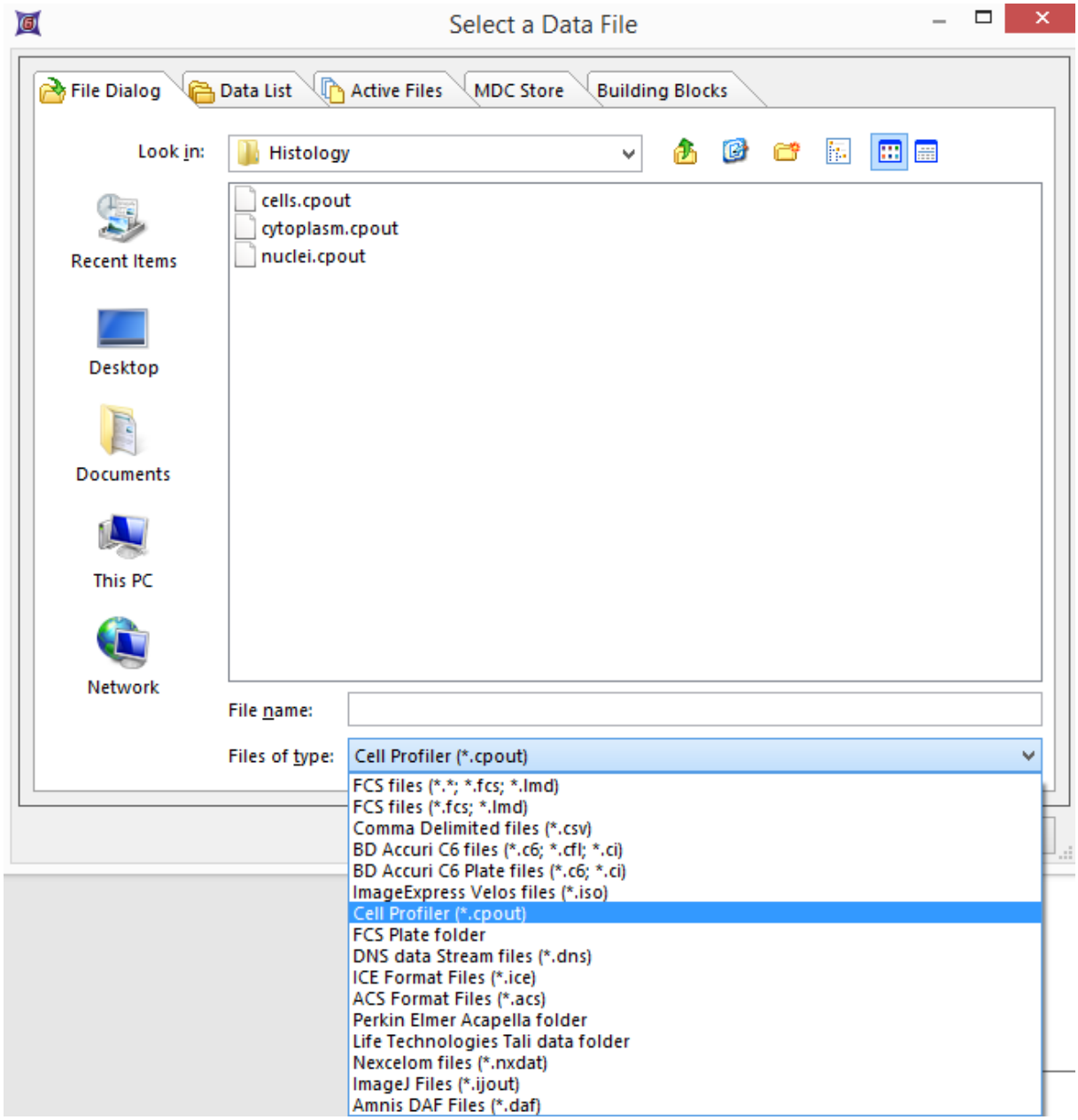

Figure T24.29 Choosing CellProfiler Files

15. Highlight **cells.cpout** and click **Open**.

Note: For this example, we will use cells.cpout which will display data from objects classified in the *IdentifySecondaryObjects* module of CellProfiler which corresponds to the entire cell area. The file, nuclei.cpout, will display data from objects classified in the *IdentifyPrimaryObjects* module of CellProfiler which corresponds only to the nuclear area of the cells.

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16. Highlight the **Y-Axis Parameter** you wish to view in the heat map from the **Open 2D Plots** dialog. For this example, choose **Intensity_MeanIntensity_Cytoplasm** (Figure T24.30).

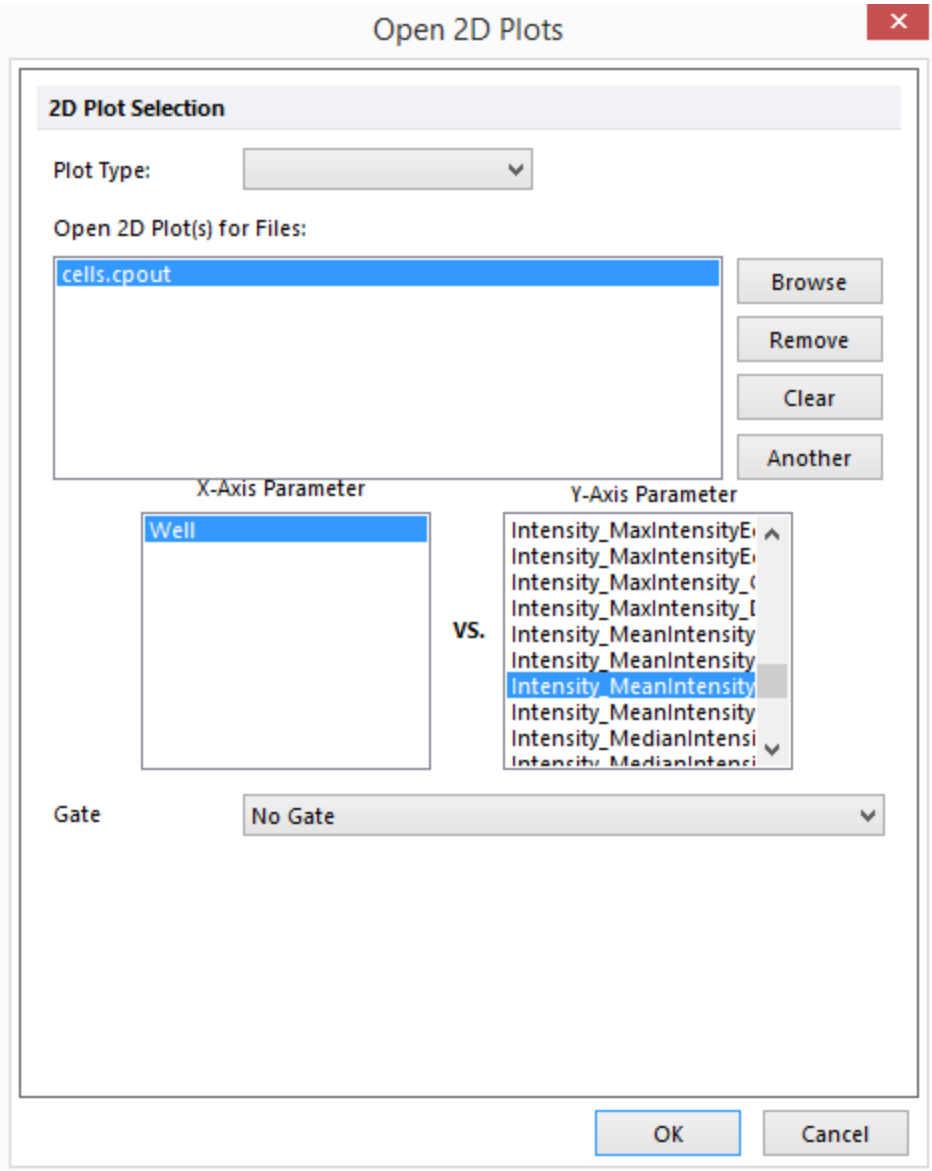

Figure T24.30 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

17. Click **OK**.

A Heat Map will now appear on your layout indicating that the data was successfully imported (Figure T24.31).

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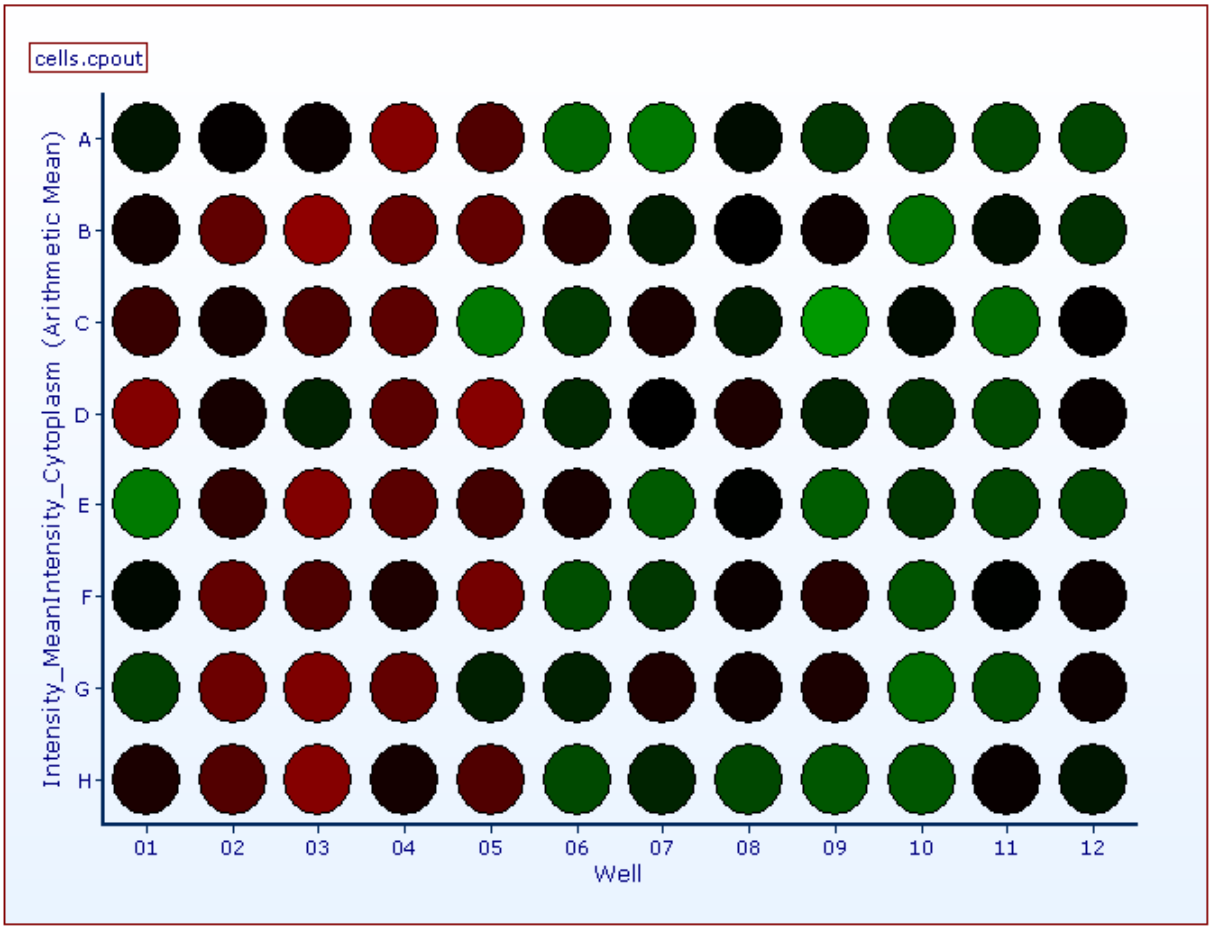

Figure T24.31 Example of a Heat Map Generated from CellProfiler Data

In the next exercise, we will export single image numeric data only 1966.

38.26.7 Exporting Single Image Numeric Data

In this section, you will learn:

- How to export CellProfiler data from a set of single images that were not obtained from a 96 well plate in numeric form.

This section pertains to data from a set of single images that were not obtained from a 96 well plate such as images obtained from a slide.

In addition to extracting metadata from the image files and providing names for different channels (if applicable), the following CellProfiler analysis modules are

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required or must be amended in the pipeline order for export to FCS Express: Convert Objects to Images, Save Images, and Export to Spreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive and is named **Section4pipeline.cpproj**. The completed pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

The sample images in the example data set we will be using can be found in the Tutorial Sample Data archive and are the **5 and 10 minute time point images** folder. These images are based on the "**Human cytoplasm-nucleus translocation assay (Vitra Image)"** from the previous module.

The steps for exporting data from CellProfiler have been broken down by module. After loading the **Section4pipeline.cpproj**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folder

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for **Default Input Folder** (Figure T24.13).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, you can select *Do not write MATLAB or HDF5 files* from the **Output file format** drop-down menu.

Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler:

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **5 and 10 minute time point images** folder from **archive folder** into designated area in CellProfiler.
3. Click **Metadata** category directly below **Images**.
4. Click **Yes** radio button in the **Extact metadata?** box.
5. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.

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6. Confirm **Metadata source** drop-down list is set to **File name**.
7. Click the magnifying glass icon to the right of **Regular Expression to** field.
8. Erase all text from **Regex:** field.
9. Copy and paste following text into **Regex:** field, which will define the Channel Number and time with a number:

   Channel(?P<ChannelNumber>[0-9]{1,2})-(?P<time>[0-9]{2})-min.tif
10. Click **Submit** in **Regular expression editor** window.
11. Click **Update** button in lower portion to see information extracted for each of the four images.
12. Click **NamesandTypes** directly below **Metadata**.
13. Change **Assign a name to** drop-down list to **Image matching rules**.
14. Enter **Channel2-** in blank field to right of **Contain** drop-down list.
15. Confirm **DNA** is entered in **Name to assign these** field.
16. Click **Add another image** button.
17. For second image set, enter **Channel1-** in blank field to right of **Contain** drop-down list.
18. Replace **GFP** in **Name to assign these** field with **Cytoplasm**.
19. Click **Update** button in lower portion.

Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- Setting up metadata and regular expressions only has to be done for one image in the set. For this example, it has been set up for channel 2.
- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export.

ExportToSpreadsheet Module

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Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export 1976 section)

1. Select the **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image_.cptoc** in **File name** field**.**
9. Right-click between the underscore and period; click **time**.
10. Click **Add another data set** button.
11. Choose **Nuclei** from the second **Data to export** drop-down list.
12. Click **No** radio button in the **Use the object name for the file name?** box.
13. Replace **DATA.csv** with **nuclei_.cptoc** in **File name** field**.**
14. Right-click between the underscore and period; click **time.**
15. Click **Add another data set** button.
16. Repeat steps 10-12 for **"Cells"** and set the file name as **cells_.cpout**.
17. Right-click between the underscore and period; click **time**.

The **ExportToSpreadsheet** module options should look like Figure T24.16 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information

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about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual listmode data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

18. Click **Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout and Image.cptoc files for each time point. There will also be a CellLabel and NucleiLabel image associated with every original image from the experiment (Figure T24.33).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB, if you do not wish to use this file we recommend deleting it to conserve disk space.

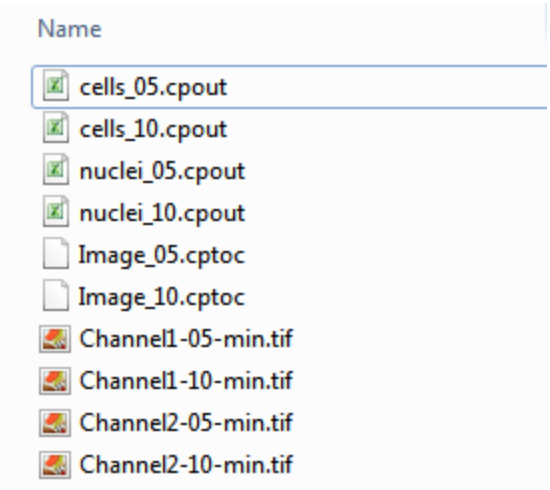

Figure T24.33 Example of CellProfiler Output Folder When Analysis is Complete

In the next exercise, we will import and analyze single image numeric data.

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38.26.8 Import Single Image Numeric Data

In this section, you will learn:

- How to import the data into FCS Express to view 2D Plots and 1D plots.

This section pertains to data from a set of single images that were not obtained from a 96 well plate, such as images obtained from a slide.

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to analyze your images and import the data into FCS Express.

1. Load the **Section4pipelineCOMPLETED.cpproj** pipeline into CellProfiler 3.0.
2. Drag and drop the folder called **5 and 10 minute time point images** folder into **Images** module and click **Apply filters to file list** button.
3. Confirm the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.13 1947.
4. Click the **Analyze Images** button in the lower left of the in CellProfiler window.

Importing the Single Image Experiment to FCS Express

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

4. Open FCS Express.
5. Open the **FCS Express User Options** by choosing **File→Options**
6. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.35).

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7. Select **csv** from the **delimiter** drop-down list.
8. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
9. Click **OK** to close the **FCS Express User Options** dialog.

Note: In this example there are no images associated with the data so the **Load Images** check box must remain unchecked.

The dialog should look like Figure T24.34 when you are done.

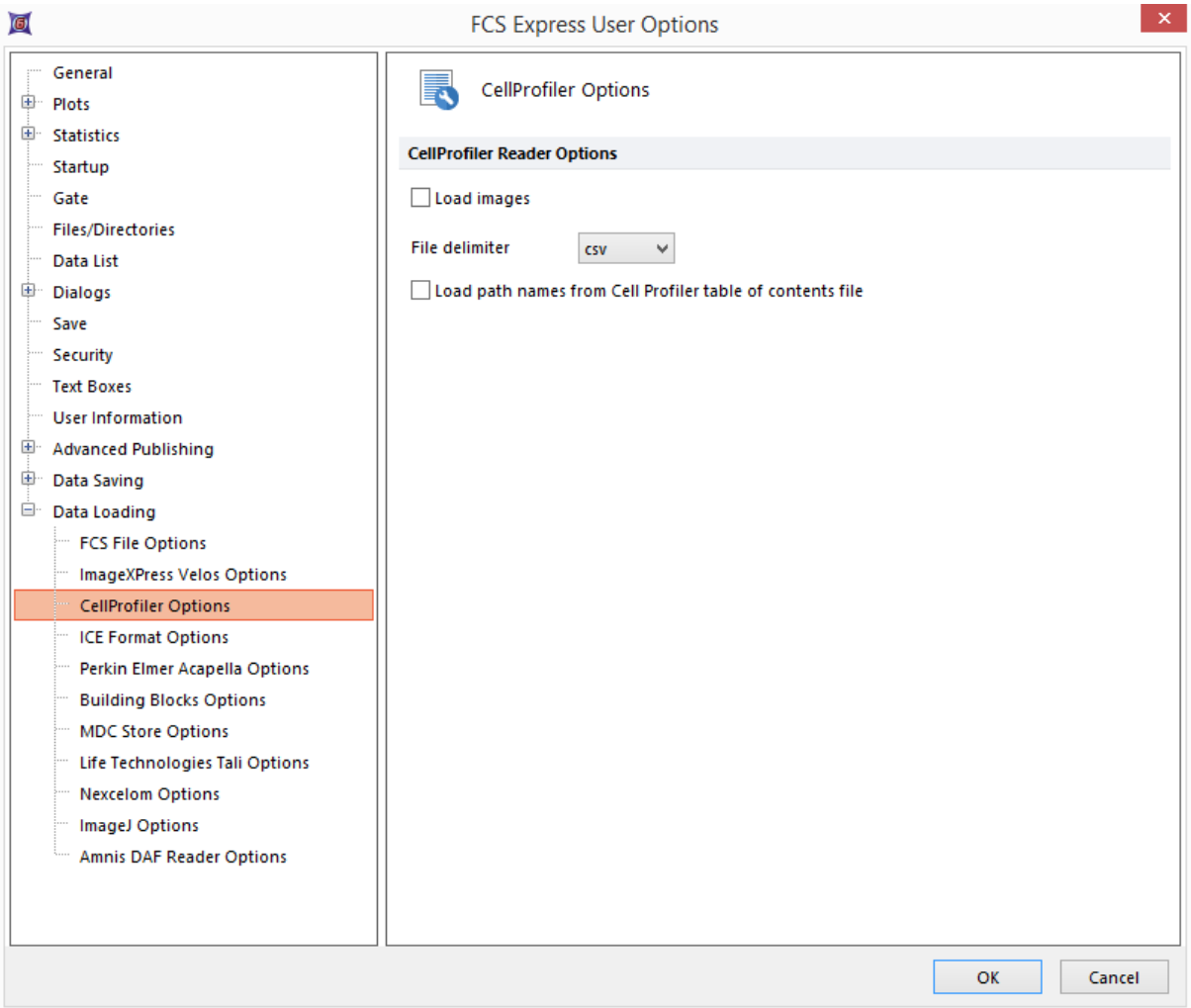

Figure T24.34 Data Loading Options for Data Only Importing

10. Select the **Insert→2D Plots→Dot** (Figure T24.35) command.

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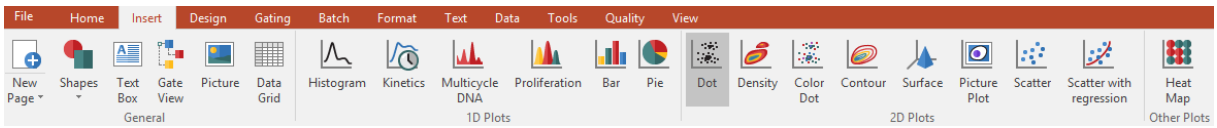

Figure T24.35 Inserting a Dot Plot

11. Click anywhere on the layout and the **Select a Data File** dialog will appear.

12. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

13. Change the **Files of type:** drop-down list to **CellProfiler (*.cpout).** For this example, they will be labeled **cells_05.cpout** and **nuclei_05.cpout** (Figure T24.36).

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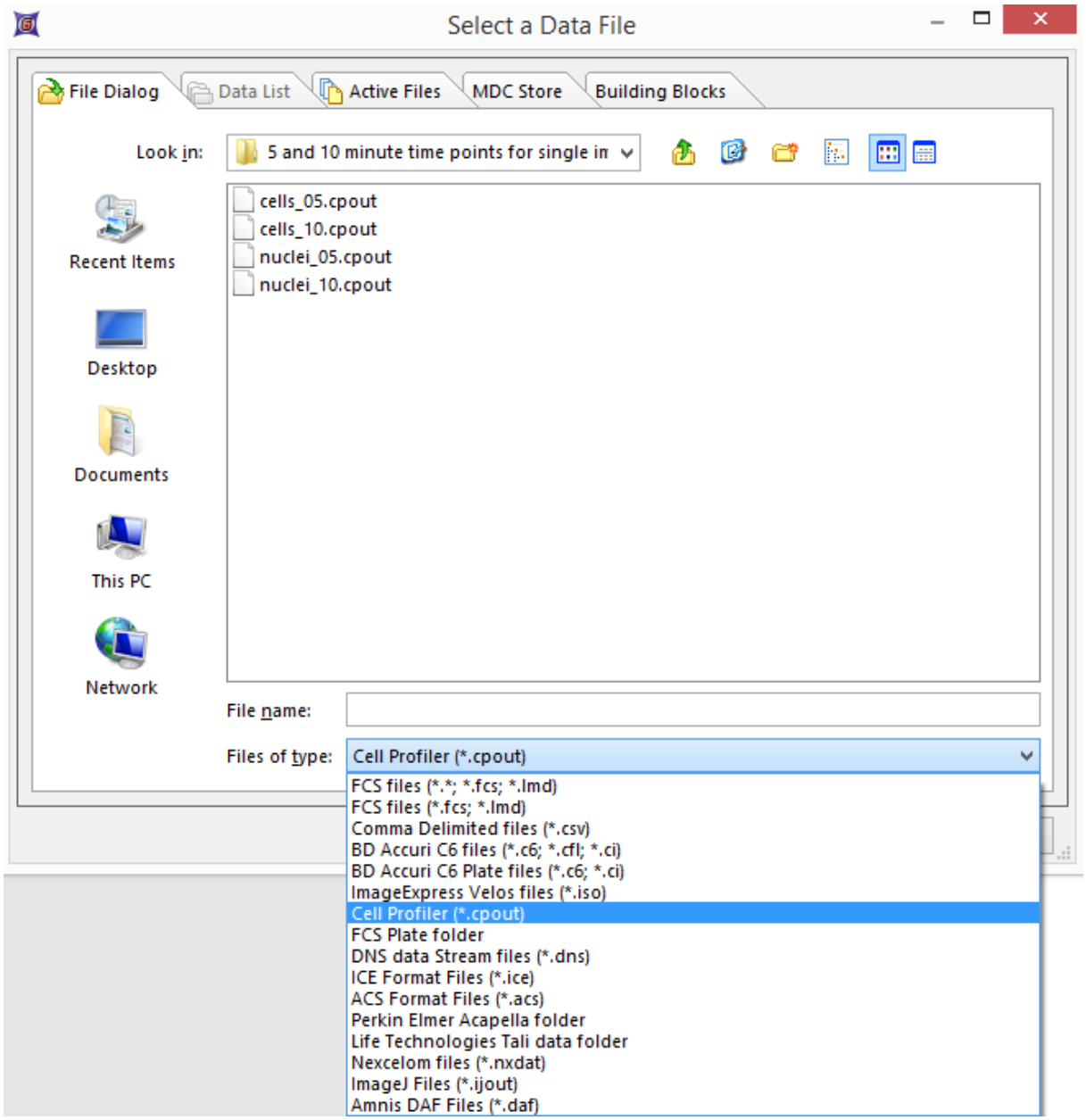

Figure T24.36 Select a Data File Dialog

14. Highlight **cells_05.cpout**.

15. Click **Open**.

Note: For this example we will use cells_05.cpout which will display data from the 5 minute time point objects classified in the IdentifySecondaryObjects module of CellProfiler which corresponds to the entire cell area. The file, nuclei_05.cpout, will display data from the 5 minute time point objects classified

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in the IdentifyPrimaryObjects module of CellProfiler which corresponds only to the nuclear area of the cells.

16. Select **AreaShape_Area** for the **X-Axis parameter** (Figure T24.37).
17. Select **Intensity_MeanIntensity_Cytoplasm** for the **Y-Axis parameter** (Figure T24.37).
18. Click **OK**.

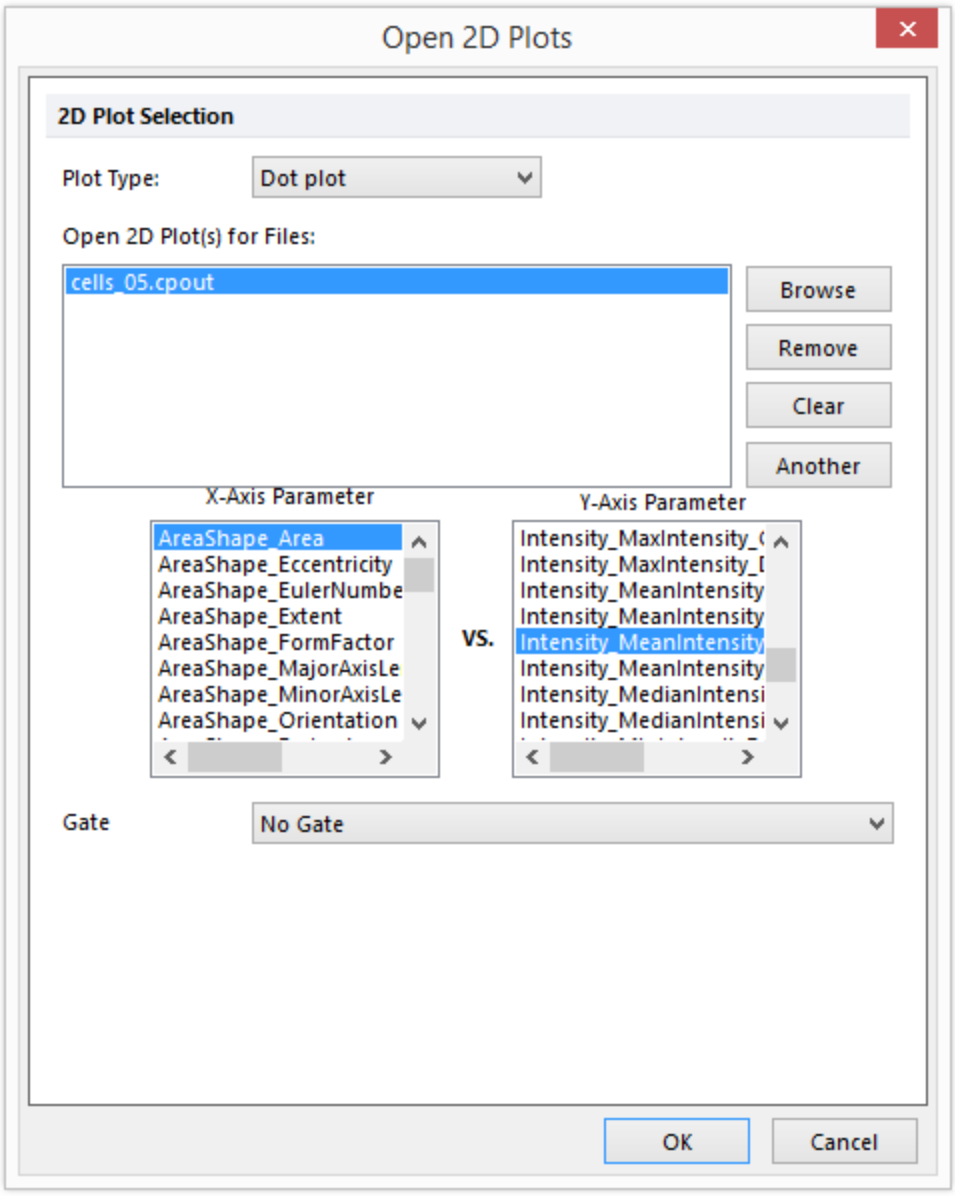

Figure T24.37 Open 2D Plots Dialog

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A **Dot Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.38).

Note: you can easily change dot size by selecting **Plot Options→Overlays** within the **Format** ribbon bar.

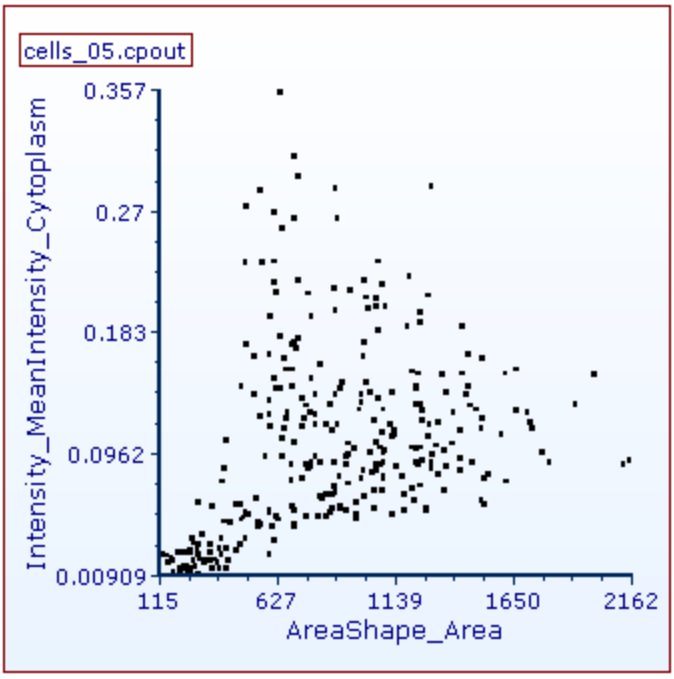

Figure T24.38 Dot Plot Created for cells_05.cpout

In the next exercise, we will Select Individual Parameters for CellProlifer Export 1976 .

38.26.9 Selecting Individual Parameters for CellProfiler Export

In this section, you will learn:

- How to export a selected number of parameters of interest from CellProfiler rather than all of the measured values.

In the previous export sections we have chosen to export all of the object size, shape, and intensity parameters that are generated by CellProfiler. While some of these parameters may be useful for your experiment not every parameter defined by CellProfiler is useful for every experiment. In order to specifically choose which parameters you would like to export you must split the export

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process into two modules: one for the Image.CPTOC and one for the selected parameters in the .CPOUT files. This tutorial will guide you through the process by modifying the **Section5pipeline.cpproj** pipeline located in the Tutorial Sample Data archive 1210. The pipeline as been designed for use with the Exporting Single Images 1938 tutorial which should be completed before beginning this tutorial.

1. Load the **Section5pipeline.cpproj** pipeline in CellProfiler from the FCS Express Tutorial Sample Data folder.
2. Confirm the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.13 1947.
3. Right click on **ExportToSpreadsheet** module.
4. Choose **Duplicate** from the dialog.
5. Click on the first **ExportToSpreadsheet** module to select it.
6. Click on the **Remove this data set** button in the **Data to export Nuclei** (nuclei_time.cpout) data set.
7. Click on the **Remove this data set** button in the **Data to export Cells** (cells_time.cpout) data set.

(Note: Only the **Data to export Image** (Image_time.cptoc) dataset will be remaining in this module as in Figure T24.39)

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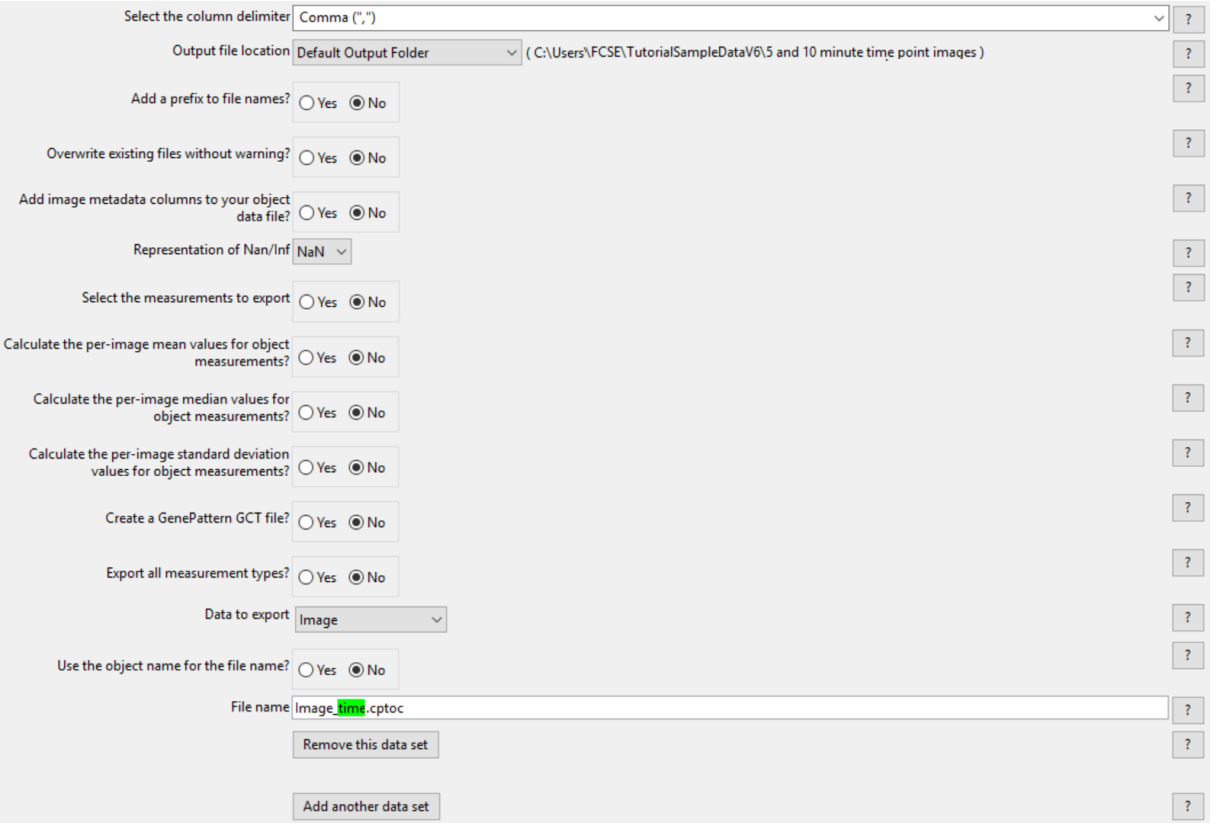

Figure T24.39 The first ExportToSpreadsheet module after the Data to export Nuceli and Cells has been removed.

8. Click on the second **ExportToSpreadsheet** module to select it.
9. Click on the **Remove this data set** button in the **Data to export Image** (Image_time.cpout) data set.

(Note: Only the **Data to export Nuclei** (nuclei_time.cpout) data set and **Data to export Cells** (cells_time.cpout) data set will be remaining in this module as in figure T24.40)

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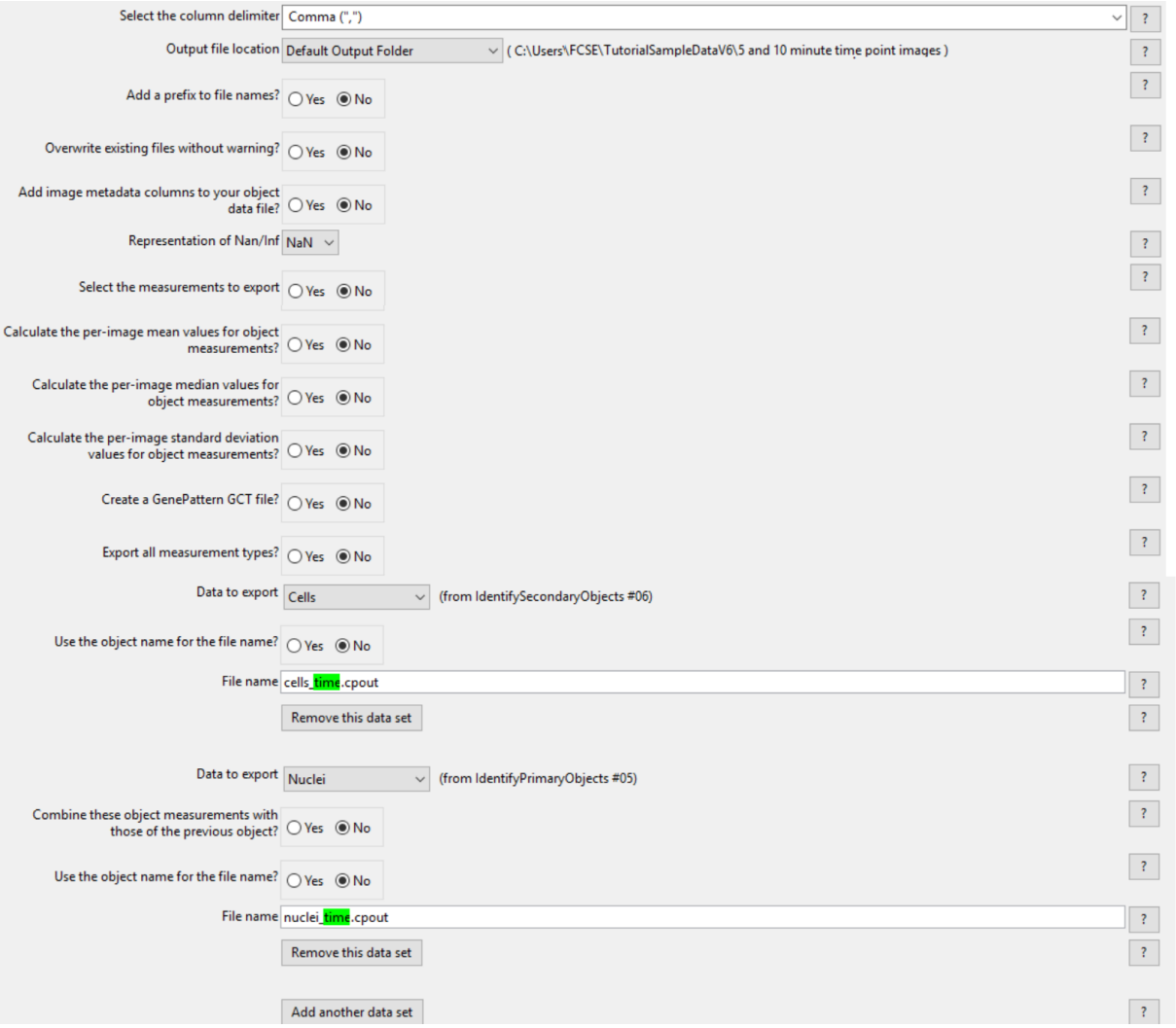

Figure T24.40 The second ExportToSpreadsheet module after the Data to export Image has been removed.

10. Click **Yes** radio button in the **Select the measurements to export** (Figure T24.41).
11. Click the **Press button to select measurements** (Figure T24.41).
12. Expand the **Cells→AreaShape** categories (Figure T24.41).
13. Check the box for **Area** (Figure T24.41).
14. Check the box for the **Cells→Intensity→MeanIntensity** category (Figure T24.41).

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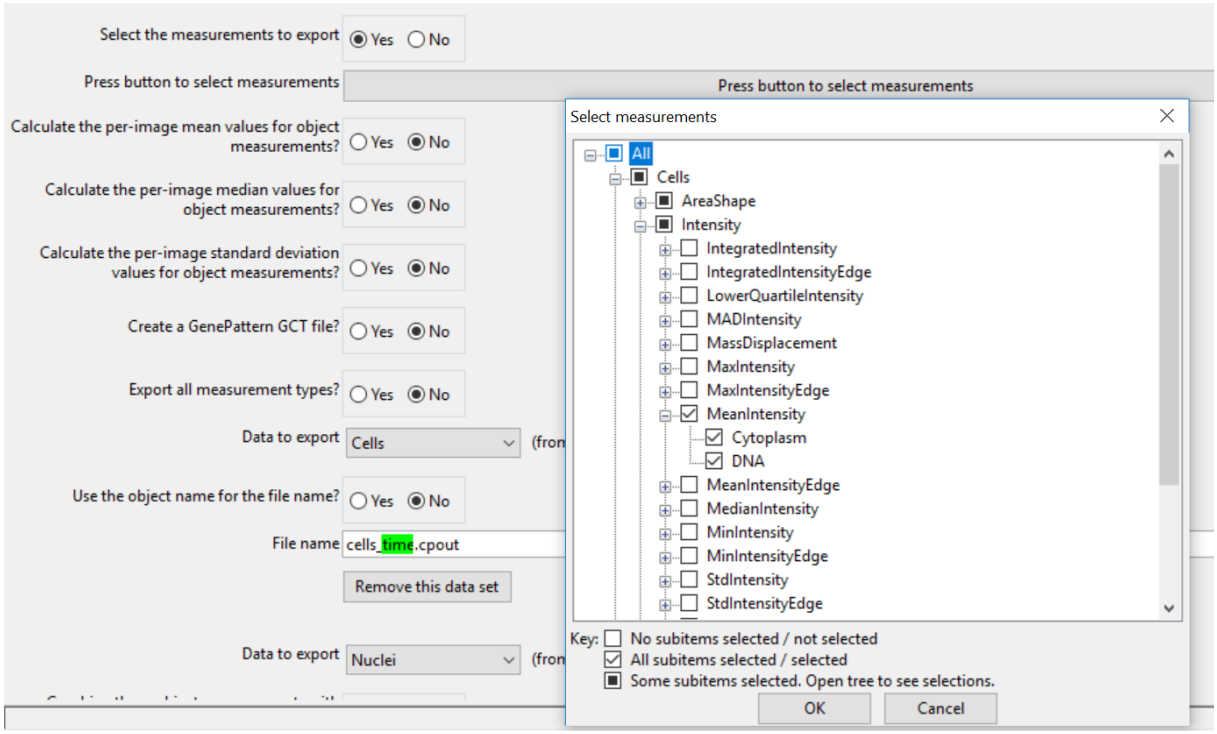

Figure T24.41 Choosing the Select the columns of measurements to export check box, clicking on the Press to select measurements button, and selecting the parameters to export for the Cells objects.

15. Expand the **Nuclei→AreaShape** categories
16. Check the box for **Area**.
17. Expand the **Nuclei→Intensity→MeanIntensity** category.
18. Check the box for **Cytoplasm**.
19. Check the box for **DNA.**
20. Click **OK**.
21. Click **Analyze images** to run the pipeline.

The **ExportToSpreadsheet** module has now been broken down into a module for the Image.cptoc and a module for the cells.cpout and nuclei.cpout. The Image.cptoc will use the default export process to export all the information FCS Express needs to import your data. The .cpout files will now only contain the parameters for AreaShape_Area, Intensity_MeanIntensity_Cytoplasm, and Intensity_MeanIntensity_DNA. After analysis is complete you may open the

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cells.cpout and nuclei.cpout files in FCS Express to view the new data set as you did in the Importing Single Image Data section 1947.

38.26.1 Exporting Tiled Images From CellProfiler 0

Exporting Large Tiled Images

Often high resolution imaging of large objects or specimens is accomplished by tiling many smaller images to create one large high resolution image (also referred to as a montage image). These images are usually provided from the acquisition instrument or software in one of two ways. Often, they are automatically stitched together and provided as one large image. In this case, they must be broken into smaller images for CellProfiler to analyze (due to memory limitations of CellProfiler) and then FCS Express will reassemble the larger image. Alternatively, they are provided as many smaller images. In that case, CellProfiler can analyze the smaller images as they are, and FCS Express will (optionally) reassemble them into a single large image.

In the course of this tutorial we will explain:

- Image naming conventions needed for FCS Express to import the tiled data processed by CellProfiler.
- How to split large images into tiles for segmentation in CellProfiler.
- Exporting tiled images in CellProfiler for use in FCS Express

Naming Conventions for Tiled Images

When reconstructing a series of tiled images FCS Express will use information stored in the filename to piece the images back together. In particular the words "row" and "col" followed by a number indicate the position of the tile in the image. Below are a few examples of this naming convention:

mytiledimage_row00_col00.tif

mytiledimage_row00_col01.tif

mytiledimage_row00_col02.tif

The above file names represent the first three tiles (from left to right) of a tiled image as indicated by their "row" and "col" positions. The words "row" and "col" and the associated tile position should be added at the time of acquisition if

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possible or post acquisition by manually renaming the files or using a file renaming program.

Starting with a Large Image

Large images may also be analyzed by Cell Profiler and FCS Express. Due to the memory limits of most computers these images must first be "split" into smaller images for image segmentation. We recommend splitting larger images using Total Image Slicer to 500 x 500 pixel squares and setting the File name template as: **[name]_row%row_col%col.tiff** . (Total image slicer will simultaneously and automatically split the image to discrete squares and rename them with the "rowXX" "colXX" naming convention).

Exporting Tiled Images in CellProfiler for use in FCS Express

The CellProfiler modules that will be discussed in this section are Load Images, Convert Objects to Images, Save Images, and Export to Spreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive within the **Exporting Tiled Images in CP** folder is named **CellProfilerTiledExportCOMPLETED.cpproj**. The completed pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

The example data set we will be using can be found in the Tutorial Sample Data archive within the **Exporting Tiled Images in CP** folder is named "**In_Situ_Cerebellum_Staining**". The sample data set (*kindly provided by Dragan Maric, Ph.D*) represents a 3 color + bright field experiment in which a portion of a rat cerebellum was imaged. To access the final product of the pipeline and a layout of the data please visit our Applications Examples webpage.

The steps for exporting data from CellProfiler have been broken down by module according to the CellProfilerTiledExport.cp pipeline. After loading the **CellProfilerTiledExport.cpproj** pipeline in CellProfiler, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folders

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for the **Default Input Folder** (Figure T24.42).

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Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, we recommend deleting it to conserve disk space.

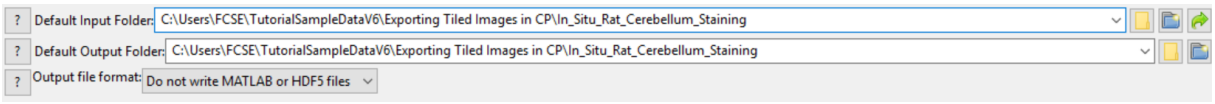

Figure T24.42 Set the Default Input and Output Folders to the Same Location

A. Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler (Figure T24.2):

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **In_Situ_Rat_Cerebellum_Staining** subfolder of **Exporting Tiled Images in CP** into designated area.
3. Click **Metadata** category directly below **Images**.
4. Click **Yes** radio button in the **Extact metadata?** box.
5. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.
6. Confirm **Metadata source** drop-down list is set to **File name**.
7. Click the magnifying glass icon to the right of **Regular Expression to** field.
8. Erase all text from **Regex:** field.
9. Copy and paste following text into **Regex:** field, which will define the row tile and column tile:

   _0000_350_DAPI_row(?P<rowtile>[0-9]{1,3})_col(?P<coltile>[0-9]{1,3}).tiff

10. Click **Submit** in **Regular expression editor** window.
11. Click **Update** button in lower portion to see information extracted for each of the images.
12. Click **NamesandTypes** directly below **Metadata**.

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13. Change **Assign a name to** drop-down list to **Image matching rules**.
14. Confirm **CellMask** in blank field to right of **Contain** drop-down list.
15. Confirm **CellMask** is entered in **Name to assign these** field.
16. Click **Add another image** button.
17. For second image set, enter **GLAST** in blank field to right of **Contain** drop-down list.
18. Confirm **GLAST** in **Name to assign these** field.
19. Click **Add another image** button.
20. For third image set, enter **Bright** in blank field to right of **Contain** drop-down list.
21. Confirm **Bright** in **Name to assign these** field.
22. Click **Add another image** button.
23. For fourth image set, enter **DAPI** in blank field to right of **Contain** drop-down list.
24. Confirm **DAPI** in **Name to assign these** field.
25. Click **Update** button in lower portion.
26. Screen should resemble Figure T24.43 below for Metadata module.

Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- Setting up metadata and regular expressions only has to be done for one image in the set. For this example, it has been set up for the DAPI channel.

- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export.

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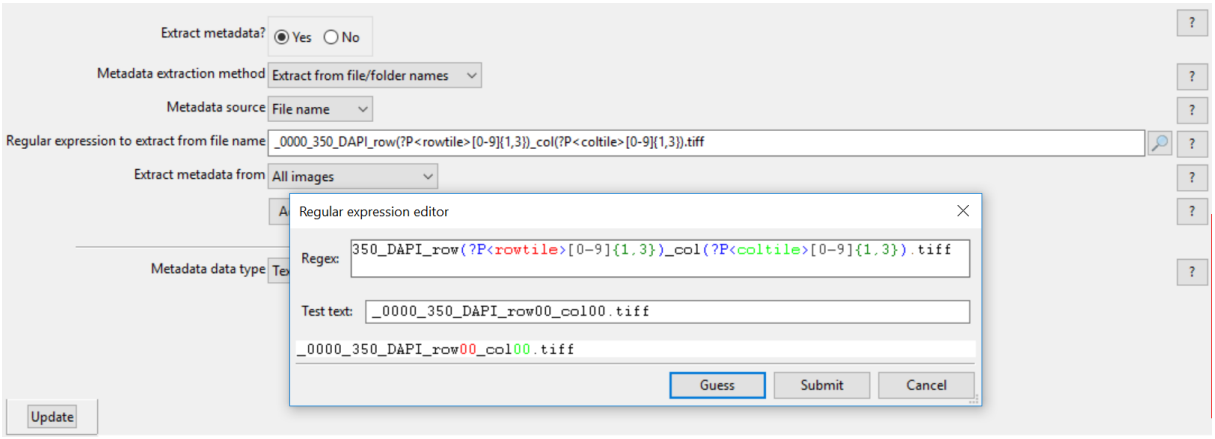

Figure T24.43 Setting up a Regular Expression in the LoadImages Module for a tiled image experiment.

B. ConvertObjectsToImage Modules

In these modules, the objects you have defined through the "Identify Primary/Secondary/Tertiary Objects" modules will be converted to image masks that FCS Express will use during import and analysis. See Figure T24.43 for an example of the module window.

1. Select the first **ConvertObjectsToImage** module in the pipeline.
2. Choose **Nuclei** from **Select the input objects** drop-down list.
3. Enter **Nuclei** in the **Name the output image** field.
4. Select **uint 16** from the **Select the color type** drop-down list (see Figure T24.43 below).
5. Select the second **ConvertObjectsToImage** module in the pipeline.
6. Choose **Cells** from **Select the input objects** drop-down list.
7. Enter **Cells** in the **Name the output image** field.
8. Select **uint 16** from the **Select the color type** drop-down list.
9. Select the third **ConvertObjectsToImage** module in the pipeline.
10. Choose **Cytoplasm** from **Select the input objects** drop-down list.
11. Enter **Cytoplasm** in the **Name the output image** field.
12. Select **uint 16** from the **Select the color type** drop-down list.

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Note: Repeat steps 1-4 for as many objects you have defined through the Identify Primary/Secondary Objects module when defining your own pipeline.

*Note: In order for FCS Express to properly import the data the **Name the output image** name must be the same as the **Select the input objects** name and is case sensitive.*

Figure T24.43 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

C. SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** modules must now be saved and related to the original images/data.

1. Select the first **SaveImages** module in the pipeline.
2. Set the **Select the type of image to save** drop-down list to **Image**.
3. Choose **Cells** for the **Select the image to save** drop-down list.
    a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
4. Set the **Select method for constructing file names** drop-down list to **Single name**.
5. Enter **CellLabel_** in the **Enter single file name** field.
6. Right-click after **CellLabel_**, choose **rowtile,** and enter an underscore **"_"**after it.
7. Right-click after **rowtile_** and choose **coltile**.
8. Select the second **SaveImages** module in the pipeline**.**
9. Set the **Select the type of image to save** drop-down list to **Image**.
10. Choose **Nuclei** for the **Select the image to save** drop-down list.
11. Set the **Select method for constructing file names** drop-down list to **Single name**.

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12. Enter **NucleiLabel_** in the **Enter single file name** field.
13. Right-click after **NucleiLabel_**, choose **rowtile,** and enter an underscore **"_"**after it.
14. Right-click after **rowtile_** and choose **coltile**.
15. Select the third **SaveImages** module in the pipeline**.**
16. Set the **Select the type of image to save** drop-down list to **Image**.
17. Choose **Cytoplasm** for the **Select the image to save** drop-down list.
18. Set the **Select method for constructing file names** drop-down list to **Single name**.
19. Enter **CytoplasmLabel_** in the **Enter single file name** field.
20. Right-click after **CytoplasmLabel_**, choose **rowtile,** and enter an underscore **"_"**after it.
21. Right-click after **rowtile_** and choose **coltile**.

Perform steps 22-27 on both **SaveImages** modules set up for **CellLabel**, **NucleiLabel**, and **CytoplasmLabel** .

22. Confirm **tiff** from the **Saved file format** drop-down list.
23. Set **Image bit depth** drop-down list to **16-bit integer**.
24. Choose **Default Output Folder** from **Output file location** drop-down list**.**
25. Click **Yes** radio button in the **Overwrite existing files without warning?** box.
26. Set **When to save** as **Every cycle** from the drop-down list.
27. Click **Yes** radio button in **Record the file and path information to the saved image?** box.

Note: rowtile and coltile are values you defined as regular expressions for the load images module. Using regular expressions allows every output file to be assigned a unique file name based on the text from the input file name. This will prevent output file names from overwriting each other and allows FCS Express to identify the unique image mask associated with each image file.

The **SaveImages** module for **CellLabel** should now look like Figure T24.45.

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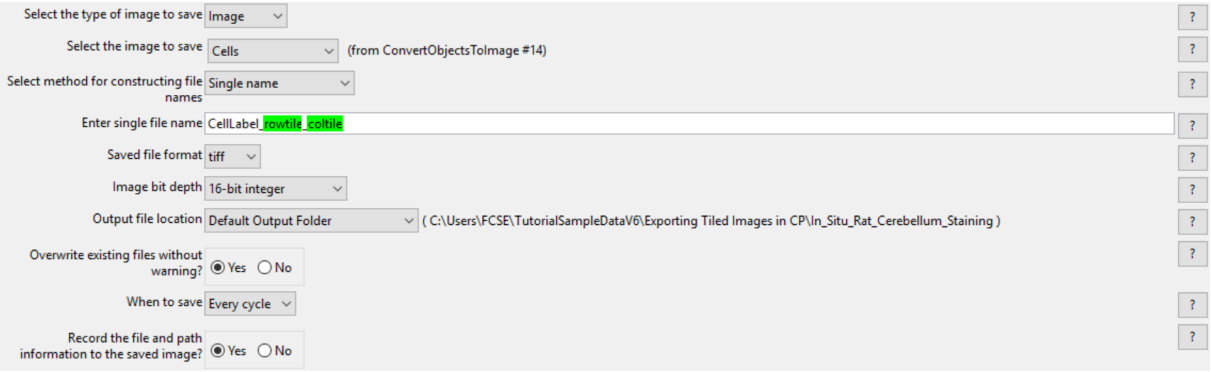

Figure T24.45 Defining the SaveImages Module

D. ExportToSpreadsheet Module

Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export 1976 section)

1. Select the first **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image.cptoc** in **File name** field**.**
9. Select the second **ExportToSpreadsheet** module.
10. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
11. Choose **Default Output Folder** from the **Output file location** drop-down list.

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12. Click **No** radio button in the **Add a prefix to file names?** box.
13. Click **No** radio button in the **Export all measurement types?** box.

    Note: In this case, measurements have already been chosen for you and the nuclei.cpout file has been setup. Click on the **Press to select measurements button** to review which measurements have been selected.

14. Click **Add another data set** button.
15. Choose **Cells** from the second **Data to export** drop-down list.
16. Click **No** radio button in the **Use the object name for the file name?** box.
17. Replace **DATA.csv** with **cells.cptoc** in **File name** field**.**
18. Click **Add another data set** button.
19. Repeat steps 14-18 for **"Cytoplasm"** and set the file name as **cytoplasm.cpout**.

The second **ExportToSpreadsheet** module options should look like Figure T24.46 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual raw data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

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Figure T24.46 The second ExportToSpreadsheet Module Set Up to Export nuclei.cpout, cells.cpout, and cytoplasm.cpout.

20. Select **Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout, cytoplasm.cpout and Image.cptoc files. There will also be a CellLabel, NucleiLabel, and CytoplasmLabel image associated with every original image/well from the tiled data set.

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In the next section, we will import and analyze the Tiled or Montage Image 1991.

38.26.1 Importing Tiled Images 1

This section pertains to importing data that was exported from CellProfiler and generated from a set of tiled images. Please see Exporting Tiled Images 1981 for instructions how to export the data from CellProfiler.

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to analyze your images and import the data into FCS Express.

1. Load the **CellProfilerTiledExportCOMPLETED.cpproj** pipeline in CellProfiler.
2. Select the default input and output folders in CellProfiler to load and store the data as in the Exporting Tiled Images 1981 tutorial.
3. Drag and drop **In_Situ_Rat_Cerebellum_Staining** subfolder of **Exporting Tiled Images in CP** folder into the designated area.
4. Click the **Analyze Images** button in the lower left of the in CellProfiler window.

Importing the Tiled Images Experiment to FCS Express

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

5. Open FCS Express.

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6. Open the **FCS Express User Options** by choosing **File→Options**
7. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.47).
8. Select **csv** from the **delimiter** drop-down list.
9. Check the **Load Images** check box.
10. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
11. Click **OK** to close the **FCS Express User Options** dialog.

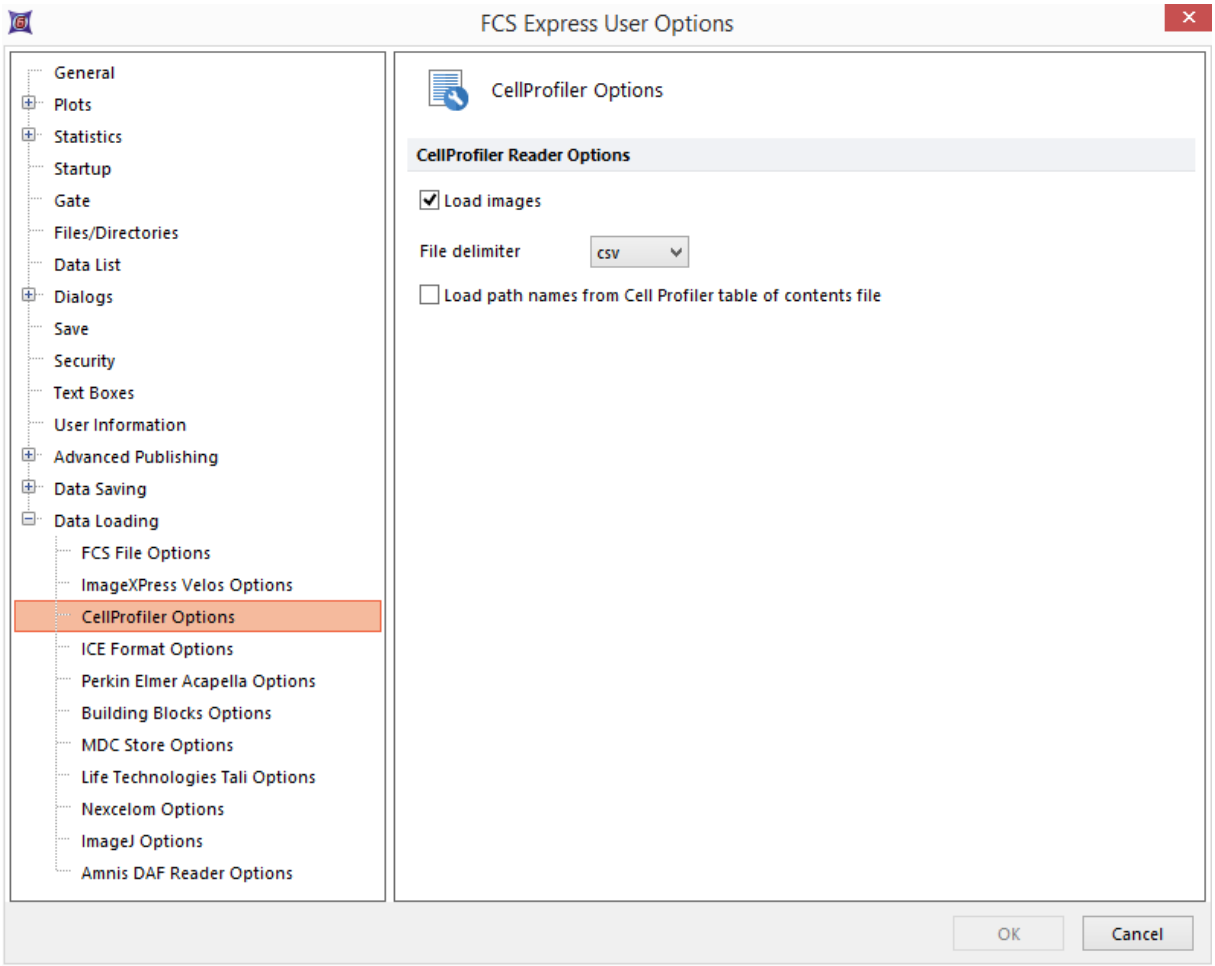

Figure T24.47 CellProfiler Data Loading Options

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12. Select the **Insert→2D Plots →Picture Plots** command (Figure T24.48).

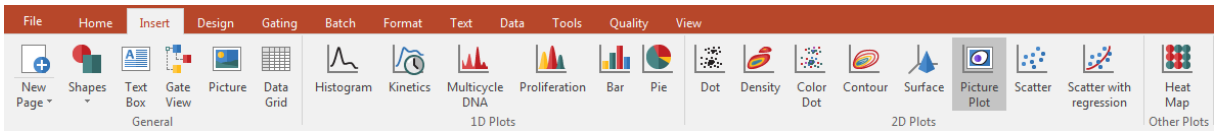

Figure T24.48 Choosing a Picture Plot to Insert

13. Click anywhere on the layout and the **Select a Data File** dialog will appear.

14. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

15. Change the **Files of type:** drop-down list to **CellProfiler (*.cpout)** (Figure T24.49)**.** Icons for the documents outputted by CellProfiler will appear. For this example, they will be labeled cell.cpout, cytoplasm.cpout, and nuclei.cpout

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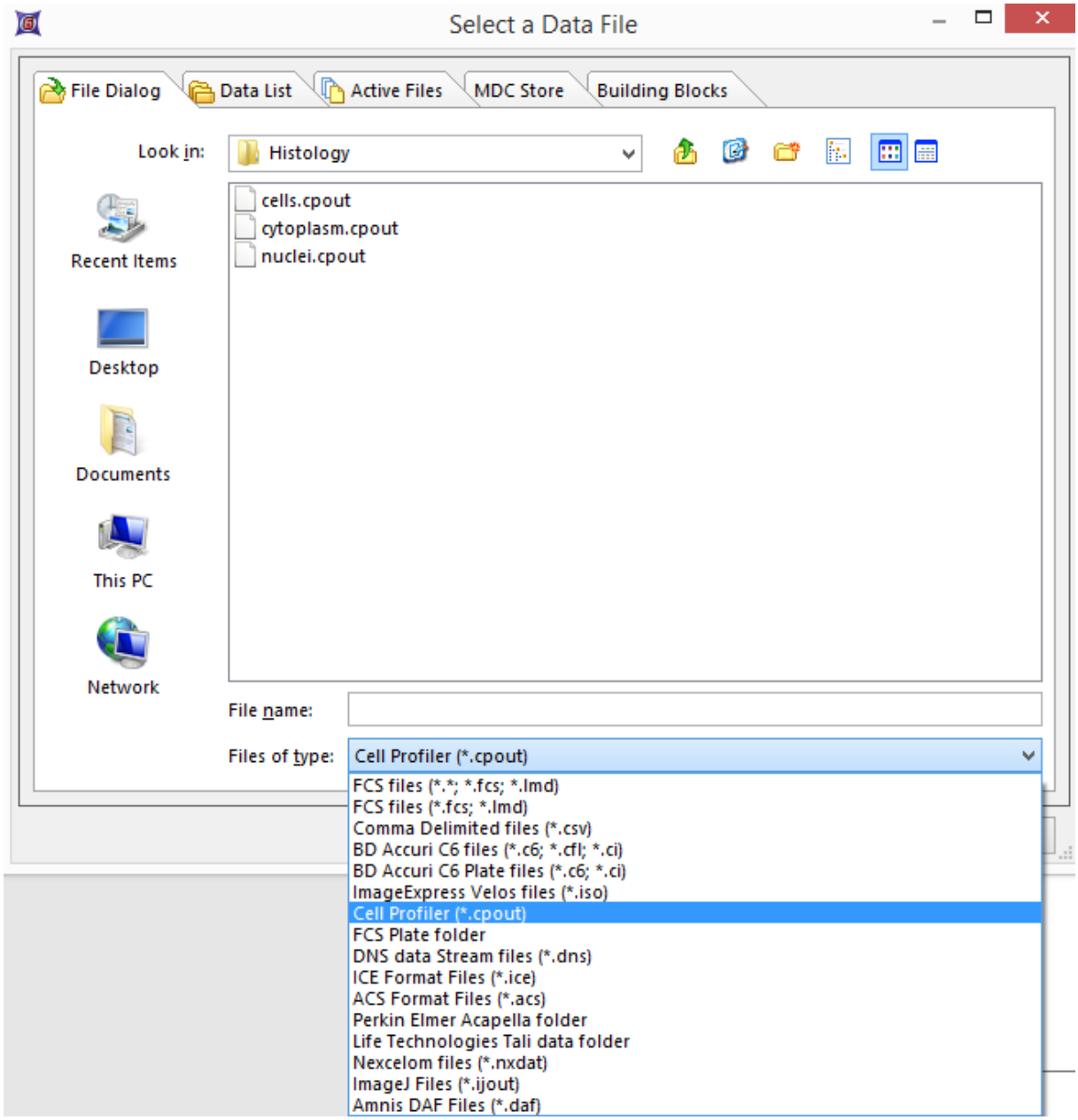

Figure T24.49 Select a Data File Dialog

16. Highlight **cells.cpout** and click **Open**.

Note: For this example, we will use **cells.cpout** which will display data from the cellular objects classified in the IdentifySecondaryObjects module of CellProfiler which corresponds to the entire cell area. **Nuclei.cpout** will display data from the objects classified in the IdentifyPrimaryObjects module of CellProfiler which corresponds only to the nuclear area of the cells. The **Cytoplasm.cpout** file will display data from the objects classified in the

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IdentifyTertiaryObjects module of CellProfiler which corresponds to the area between the nuclear object and the cell boundary.

17. Highlight CellMask from the **Image Parameter** list box of the **Open 2D Plots** dialog (Figure T24.50) to view the cellular image.

18. Click **OK**.

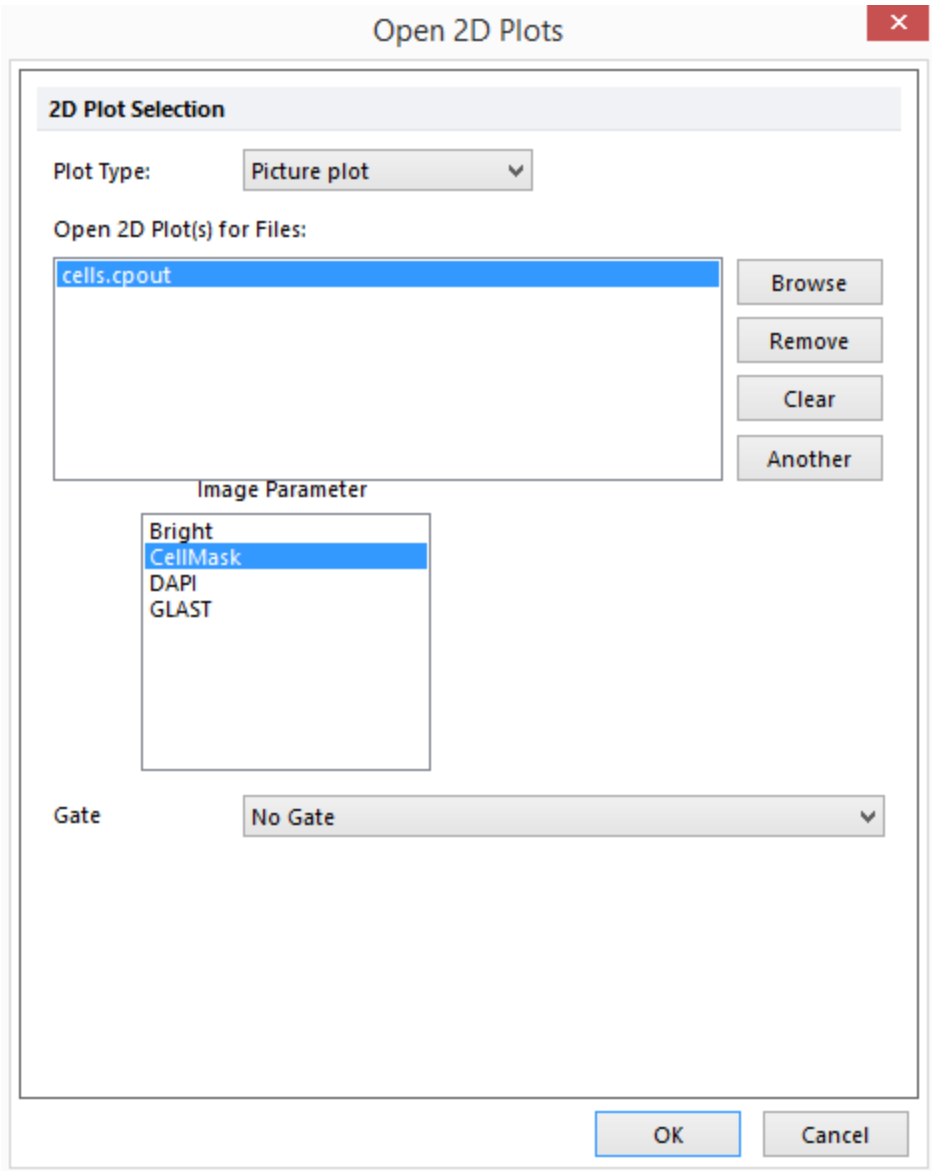

Figure T24.50 Choosing Image Parameters from the Open 2D Plots Dialog

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A **Picture Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.51).

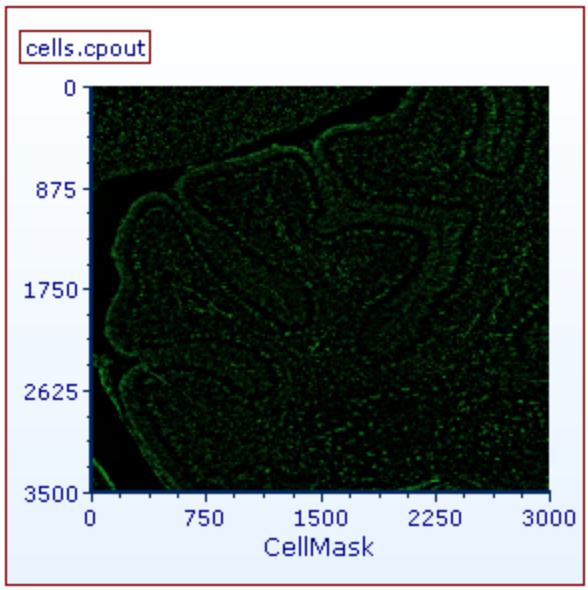

Figure T24.51 Example of the Cells.cpout Picture Plot

Notice that the image is only displaying the object "cut-outs" since we have decided to not load the background images. To make the display look more appropriate we will manually color the background.

19. Right click on the picture plot.

20. Choose **Format** from the drop down list.

21. Choose the **Background** category in the formatting dialog (Figure T24.52).

22. Click on the **Solid Color** drop down box (Figure T24.52).

23. Choose **Black** as the background color (Figure T24.52).

24. Click **OK**.

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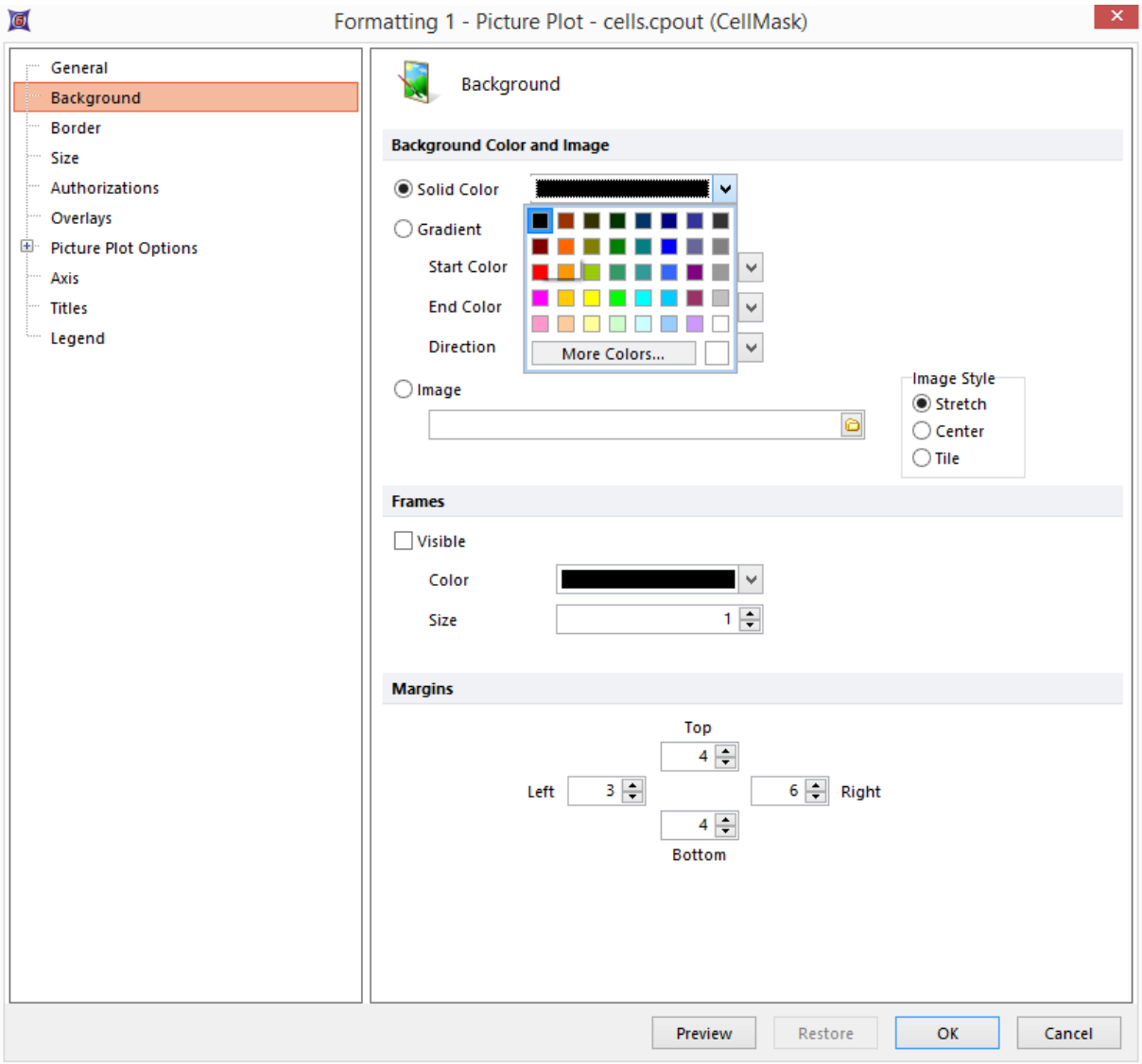

Figure T24.52 The Background formatting options dialog.

The picture plot will now have a black background as the original image did (Figure T24.53)

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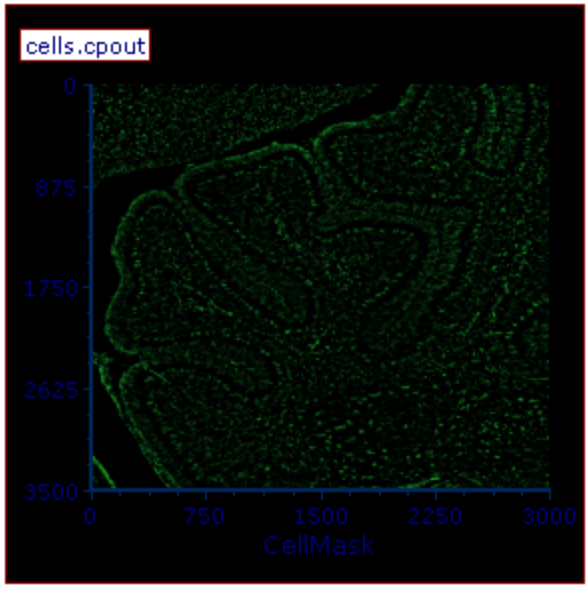

Figure T24.53 The Picture Plot has been formatted to use a black background.

To continue your analysis of the data please visit the Picture Plots 2060 or Introduction to FCS Express Image Cytometry 2098 tutorials which describe working with your data in FCS Express.

38.26.1 Exporting Plate Based Data That Contains Multiple Fields Per Well

Exporting Plate Based Data That Contains Multiple Fields Per Well

Often images of multiple fields within a single well of a plate format experiment are taken. CellProfiler can segment all of the individual field images and pool the data as a single well for export to FCS Express Image Cytometry. The resulting data sets may then be opened in FCS Express to review and analyze the data in Heat Maps, Picture Plots, Dot Plots, and Histograms.

In the course of this tutorial we will explain:

- Image naming conventions needed for FCS Express to import the multi well, multi field, imaging data processed by CellProfiler.
- Exporting Multiple Well, Multiple Field Location, Imaging data in CellProfiler for use in FCS Express.

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Naming Conventions for Plate Based Multiple Field Location Image Data Sets

When reconstructing a series of plate based data containing multiple fields per well FCS Express will use information stored in the filename to assign well locations and to merge the multiple field images into a single image. In particular the regular expression names *Row and Column (for well locations) and rowtile and coltile (for the field location within the well)* should be used when exporting plate based data with multiple image locations per well. These names define the plate location of the well by Row Letter and Column Number and the location of the field within the well by *rowtile* and *coltile* position. To successfully use regular expression for processing the data in CellProfiler and analyzing the data in FCS Express the information for Well numbers and field locations must be stored somewhere within the filename of the image. In Figure T24.54 below the image name is: Channel1-01-A-01_00_00. The red box highlights the well Row and Column information (well A01), and the blue box highlights the field location (position 00, 00).

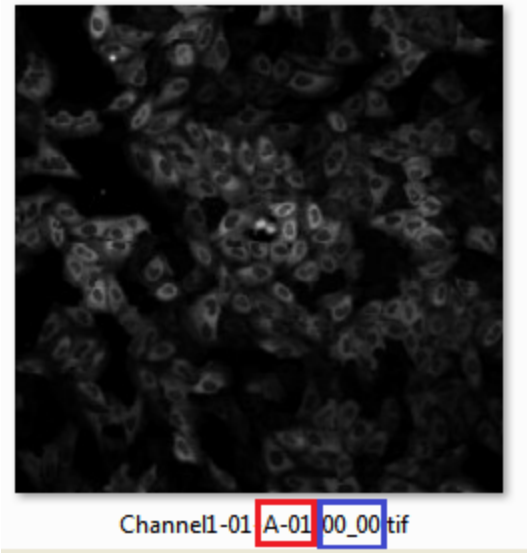

Figure T24.54 The image name is: Channel1-01-A-01_00_00. The red box highlights the well Row and Column information (well A01), and the blue box highlights the field location (position 00, 00).

Exporting Multiple Field Location Plate Imaging Data in CellProfiler for use in FCS Express

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The CellProfiler modules that will be discussed in this section are Load Images, Save Images, and Export to Spreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive within the **Multiple Field Location Plate Imaging** folder is named **MultipleFieldLocationPipelineCOMPLETED.cpproj**. The completed pipeline is meant to be used as a template for comparison to your pipeline and represents the finished product of this tutorial.

The example data set we will be using can be found in the Tutorial Sample Data archive within the **Multiple Field Location Plate Imaging** folder. The example data set contains 16 images which are broken down into two channels of images (channel 1 and channel 2) and two wells (A1 and A2) with 4 fields per well.

The steps for exporting data from CellProfiler have been broken down by module according to the **MultipleFieldLocationPipeline.cpproj** pipeline. After loading the **MultipleFieldLocationPipeline.cpproj** pipeline in CellProfiler, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Output Folder

To organize your data correctly for FCS Express, ensure the **Default Output Folder** is the same folder where your images are stored and used for the **Default Input Folder** (Figure T24.55 below).

Note: The DefaultOUT.mat file will also be exported to the Output Folder. This file is for use in MATLAB. If you do not wish to use this file, you can select *Do not write MATLAB or HDF5 files* from the **Output file format** drop-down menu.

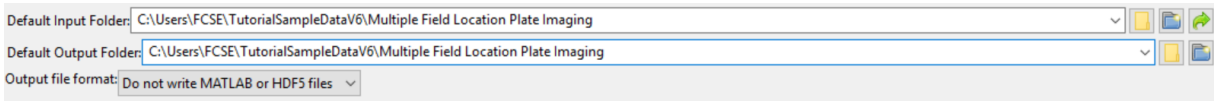

Figure T24.55 Set the Default Input and Output Folders to the Same Location

A. Loading Images, Extracting Metadata, Assigning Names

In order for FCS Express to recognize your data is in 96 well format, metadata from the image file names must be extracted. A Regular Expression

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must be defined to find the metadata in the file name or path of the data by following these steps in CellProfiler (Figure T24.2):

1. Click **Images** category in left column of CellProfiler window.
2. Drag and drop **Multiple Field Location Plate Imaging** folder into designated area.
3. Click **Apply filters to the file list** to remove non-image files. Font for **Experiment.ini** should become gray.
4. Click **Metadata** category directly below **Images**.
5. Click **Yes** radio button in the **Extact metadata?** box.
6. Confirm **Metadata extraction** drop-down list is set to **Extract from file/folders**.
7. Confirm **Metadata source** drop-down list is set to **File name**.
8. Click the magnifying glass icon to the right of **Regular Expression to** field.
9. Erase all text from **Regex:** field.
10. Copy and paste following text into **Regex:** field, which will define the Channel Number, Well Number, Row of the plate with a letter, Column with a number, Row Tile with a number, and column tile with a number:

    Channel1-[0-9]{2}-(?P<Row>[A-H])-(?P<Column>[0-9]{2})_(?P<rowtile>[0-9]{1,2})_(?P<coltile>[0-9]{1,2}).tif

11. Click **Submit** in **Regular expression editor** window.
12. Click **Update** button in lower portion to see information extracted for each of the images.
13. Click **NamesandTypes** directly below **Metadata**.
14. Change **Assign a name to** drop-down list to **Image matching rules**.
15. Enter **Channel2-** in blank field to right of **Contain** drop-down list.
16. Confirm **DNA** is entered in **Name to assign these** field.
17. Click **Add another image** button.
18. For second image set, enter **Channel1-** in blank field to right of **Contain** drop-down list.
19. Replace **GFP** in **Name to assign these** field with **Cytoplasm**.

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20. Click **Update** button in lower portion.

21. Screen should resemble Figure T24.56 below for Regular expression editor window of Metadata module.

Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.
- Setting up metadata and regular expressions only has to be done for one image in the set. For this example, it has been set up for Channel2.
- The regular expression output names: *Well*, *Row*, *Column*, *rowtile* and *coltile* may only be used with plate based or montage imaging experiments. These output names are reserved, and should not be used for anything aside from denoting the well and/or field positions.

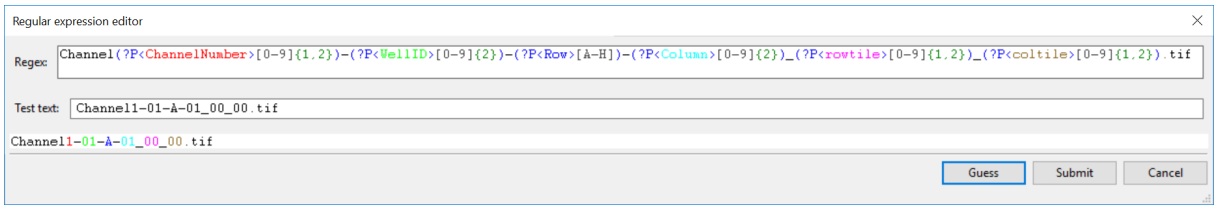

Figure T24.56 Setting up a Regular Expression in the LoadImages Module for a multiple field plate based experiment.

B. SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** modules must now be saved and related to the original images/data.

1. Select the first **SaveImages** module in the pipeline.
2. Set the **Select the type of image to save** drop-down list to **Image**.
3. Choose **Cells** for the **Select the image to save** drop-down list.
   a. Note: when defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
4. Set the **Select method for constructing file names** drop-down list to **Single name**.
5. Enter **CellLabel_** in the **Enter single file name** field.

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6. Right-click after **CellLabel_**, choose **Row,** and enter an underscore **"_"**after it.
7. Right-click after **Row_** and choose **Column**.
8. Right-click after **Column_**, choose **rowtile,** and enter an underscore **"_"**after it.
9. Right-click after **rowtile_** and choose **coltile**.
10. Select the first **SaveImages** module in the pipeline.
11. Set the **Select the type of image to save** drop-down list to **Image**.
12. Choose **Nuclei** for the **Select the image to save** drop-down list.
13. Set the **Select method for constructing file names** drop-down list to **Single name**.
14. Enter **NucleiLabel_** in the **Enter single file name** field.
15. Right-click after **NucleiLabel_**, choose **Row,** and enter an underscore **"_"**after it.
16. Right-click after **Row_** and choose **Column**.
17. Right-click after **Column_**, choose **rowtile,** and enter an underscore **"_"**after it.
18. Right-click after **rowtile_** and choose **coltile**.

*Note: Row, Column, rowtile and coltile are values you defined as regular expressions for the load images module. Using regular expressions allows every output file to be assigned a unique file name based on the text from the input file name. This will prevent output file names from overwriting each other and allows FCS Express to identify the unique image mask associated with each image file.*Perform steps 15-22 on both **SaveImages** modules set up for **CellLabel** and **NucleiLabel**.

19. Confirm **tiff** from the **Saved file format** drop-down list.
20. Set **Image bit depth** drop-down list to **16-bit integer**.
21. Choose **Default Output Folder** from **Output file location** drop-down list**.**
22. Click **Yes** radio button in the **Overwrite existing files without warning?** box.
23. Set **When to save** as **Every cycle** from the drop-down list.

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24. Click **Yes** radio button in **Record the file and path information to the saved image?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.57

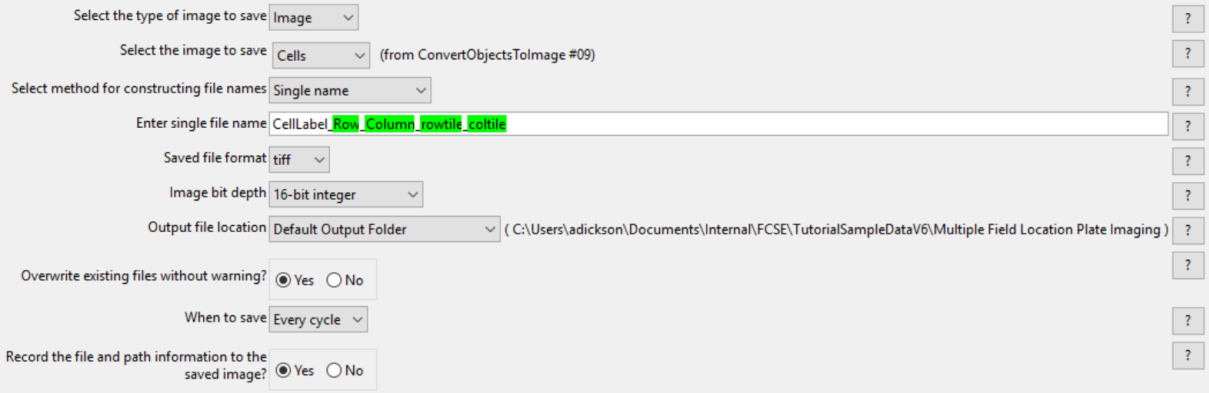

Figure T24.57 Defining the SaveImages Module

C. ExportToSpreadsheet Module

Now that all of the images and object image masks have been defined and saved for analysis, you must now ask CellProfiler to export the measurements you wish to view in FCS Express. For this example, we will export all measurements.

(Note: If you would like to only export certain parameters please see the Selecting Individual Parameters for CellProlifer Export 1976 section)

1. Select the **ExportToSpreadsheet** module.
2. Confirm the **Select the column delimiter** drop-down list is set to **Comma (",")**.
3. Choose **Default Output Folder** from the **Output file location** drop-down list.
4. Click **No** radio button in the **Add a prefix to file names?** box.
5. Click **No** radio button in the **Export all measurement types?** box.
6. Choose **Image** from the first **Data to export** drop-down list.
7. Click **No** radio button in the **Use the object name for the file name?** box.
8. Replace **DATA.csv** with **Image.cptoc** in **File name** field**.**
9. Click **Add another data set** button.

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10. Choose **Nuclei** from the second **Data to export** drop-down list.
11. Click **No** radio button in the **Use the object name for the file name?** box.
12. Replace **DATA.csv** with **nuclei.cptoc** in **File name** field**.**
13. Click **Add another data set** button.
14. Repeat steps 10-12 for **"Cells"** and set the file name as **cells.cpout**.

The **ExportToSpreadsheet** module options should look like Figure T24.58 when you are done.

Note 1: on file extensions: ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "Nuclei.cpout" and "Cells.cpout" store the actual raw data associated with individual objects and analysis. There should be a separate ".cpout" file defined for each object mask defined in CellProfiler. Only one "Image.cptoc" file is needed.

Note 2:The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names ***nuclei.cpout*** *and* ***nuclei_<regular express>.cpout*** *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

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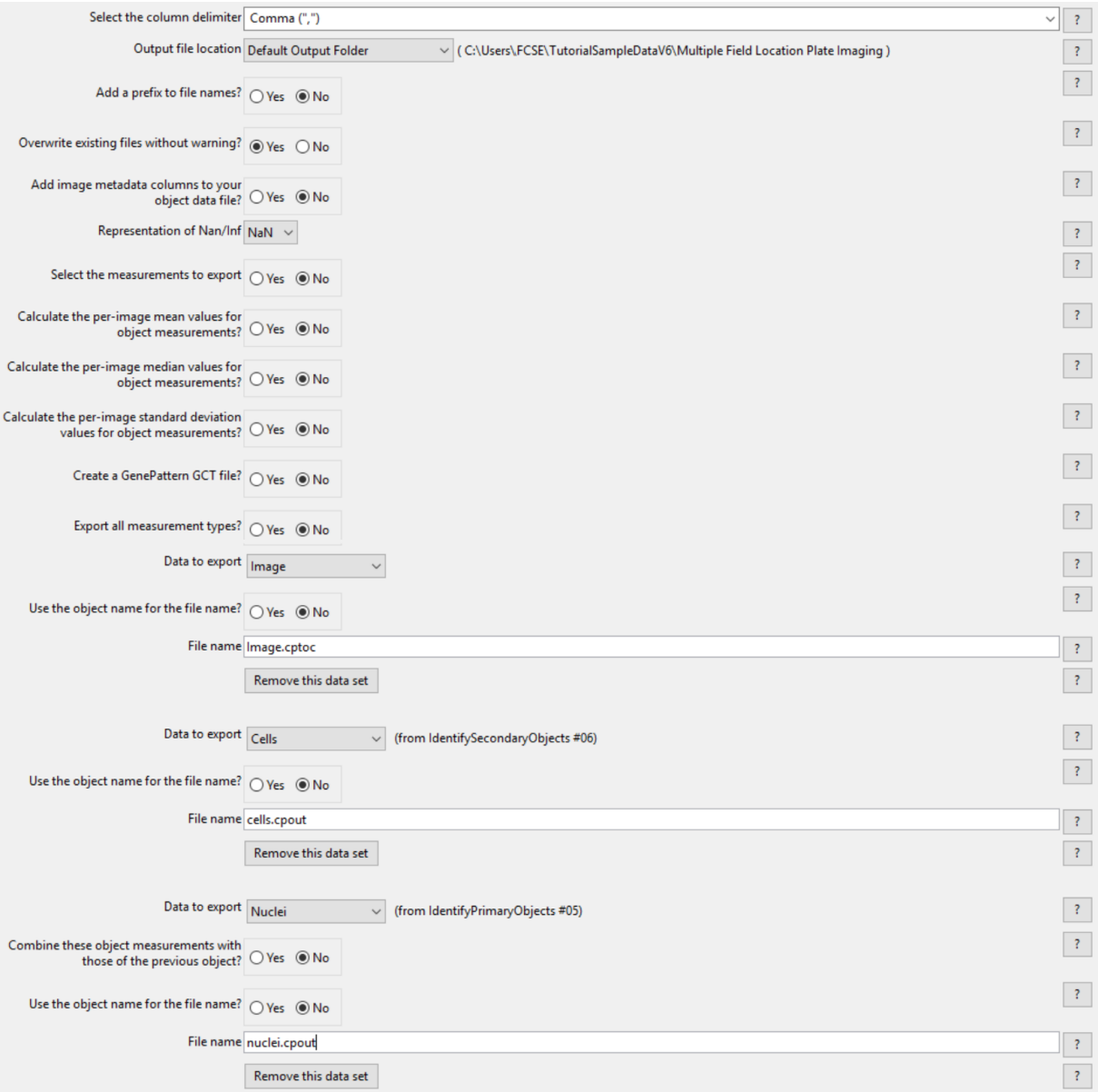

Figure T24.58 The ExportToSpreadsheet Module Set Up to Export image.cptoc, nuclei.cpout and cells.cpout.

15. Select **Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout, and Image.cptoc files. There will also be a CellLabel and NucleiLabel image associated with every original image from the data set.

In the next section, we will import and analyze the plate based multiple field location imaging data 2007

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38.26.1 Importing Plate Based Multiple Field Location Imaging Data 3

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to import the data into FCS Express. FCS Express only needs one additional piece of information regarding the setup of your experiment. CellProfiler currently does not export the plate format that was used for your experiment. A series of standard plate format definitions can be downloaded from the FCS Express website. For this example, we will be using a 6 well template which can found in the Tutorial Sample Data archive within the Multiple Field Location Plate Imaging folder and is labeled **Experiment.ini**. The plate format definition file must be called **Experiment.ini** and placed into the **Default Output Folder** you defined in CellProfiler.

1. Download the example data from the Tutorial Sample Data archive within the **Multiple Field Location Plate Imaging** folder.
2. Load the **MultipleFieldLocationPipelineCOMPLETED.cpproj** pipeline into CellProfiler.
3. Select the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.56.
4. Drag and drop **Multiple Field Location Plate Imaging** folder into the designated area of the **Images** module.
5. Click the **Analyze Images** button in the lower left of the in CellProfiler window.
6. Ensure the **Experiment.ini file**, located in the Tutorial Sample Data archive within the Multiple Field Location Plate Imaging folder, is placed in the folder containing your exported CellProfiler data.

Importing the Plate Based Multiple Field Location Imaging Experiment to FCS Express

Note on FCS Express Preferences before starting these steps of the tutorial:

The *File selection* dialog must be enabled:

A. Open the **FCS Express User Options** by choosing **File→Options**.

B. Check the **When creating a new plot, always display the selection dialog** box in the **Files/Directories** category.

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(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

6. Open FCS Express.
7. Open the **FCS Express User Options** by choosing **File→Options**
8. Select **Cell Profiler Options** from the **Data Loading** category (Figure T24.59).
9. Select **csv** from the **delimiter** drop-down list.
10. Check the **Load Images** check box.
11. Uncheck the **Load path names from Cell Profiler table of contents file** check box.
12. Click **OK** to close the **FCS Express User Options** dialog.

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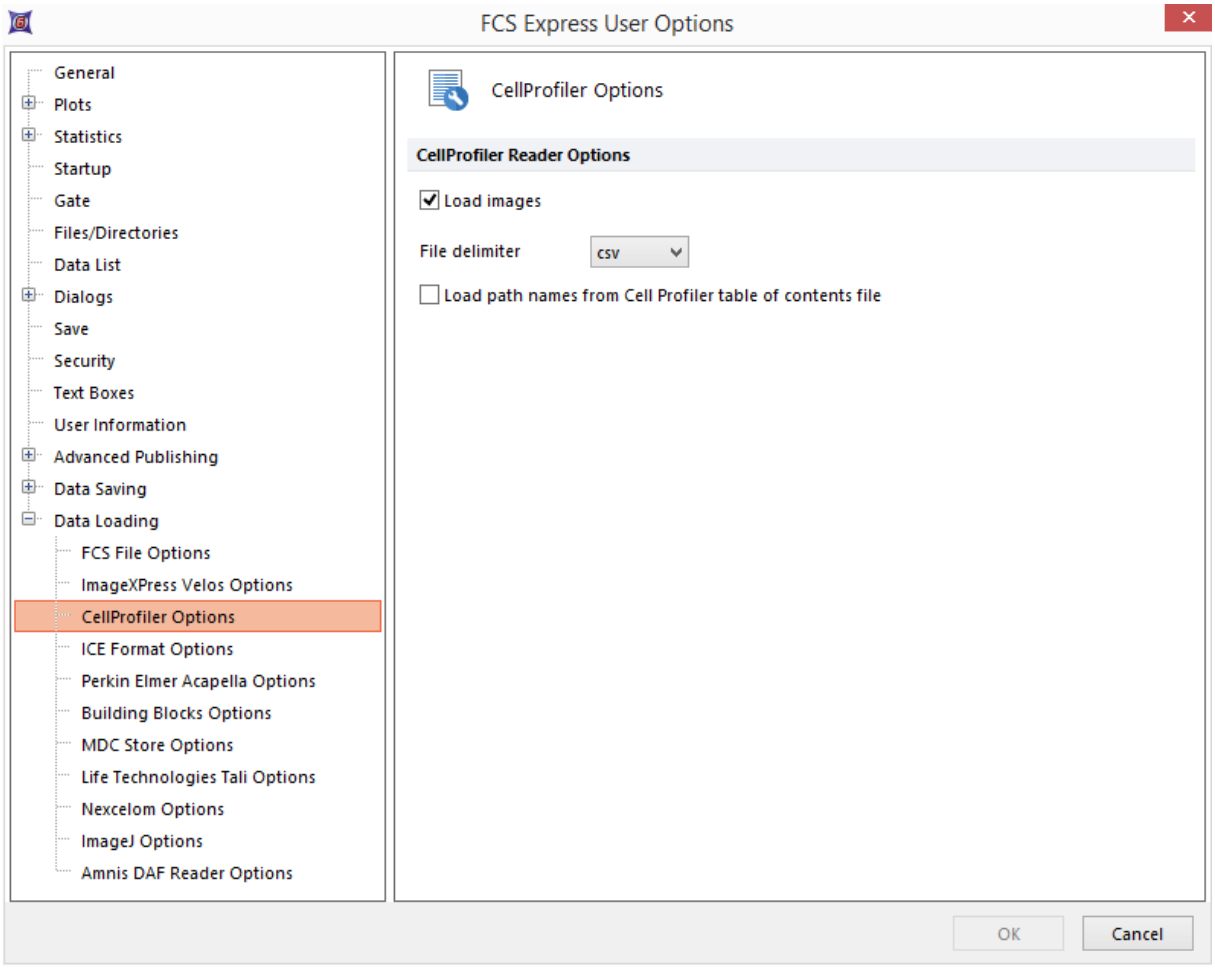

Figure T24.59 CellProfiler Data Loading Options for Importing Plate Based Multiple Field Location Imaging Data.

11. Select the **Insert→Other Plots→Heat Map** command (Figure T24.60).

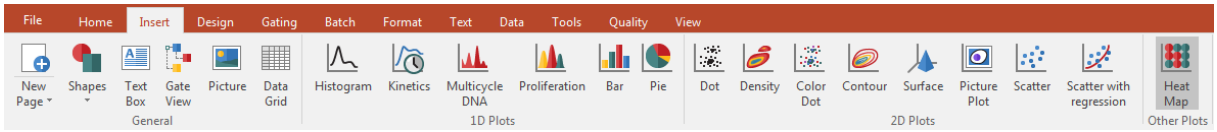

Figure T24.60 Choosing to Insert a Heat Map

12. Click anywhere on the layout and the **Select a Data File** dialog will appear.

13. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

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14. Change the **Files of type:** drop-down list to **Cell Profiler (*.cpout)** (Figure T24.61). Only documents output by CellProfiler will appear. For this example, they will be labeled **cells.cpout** and **nuclei.cpout**.

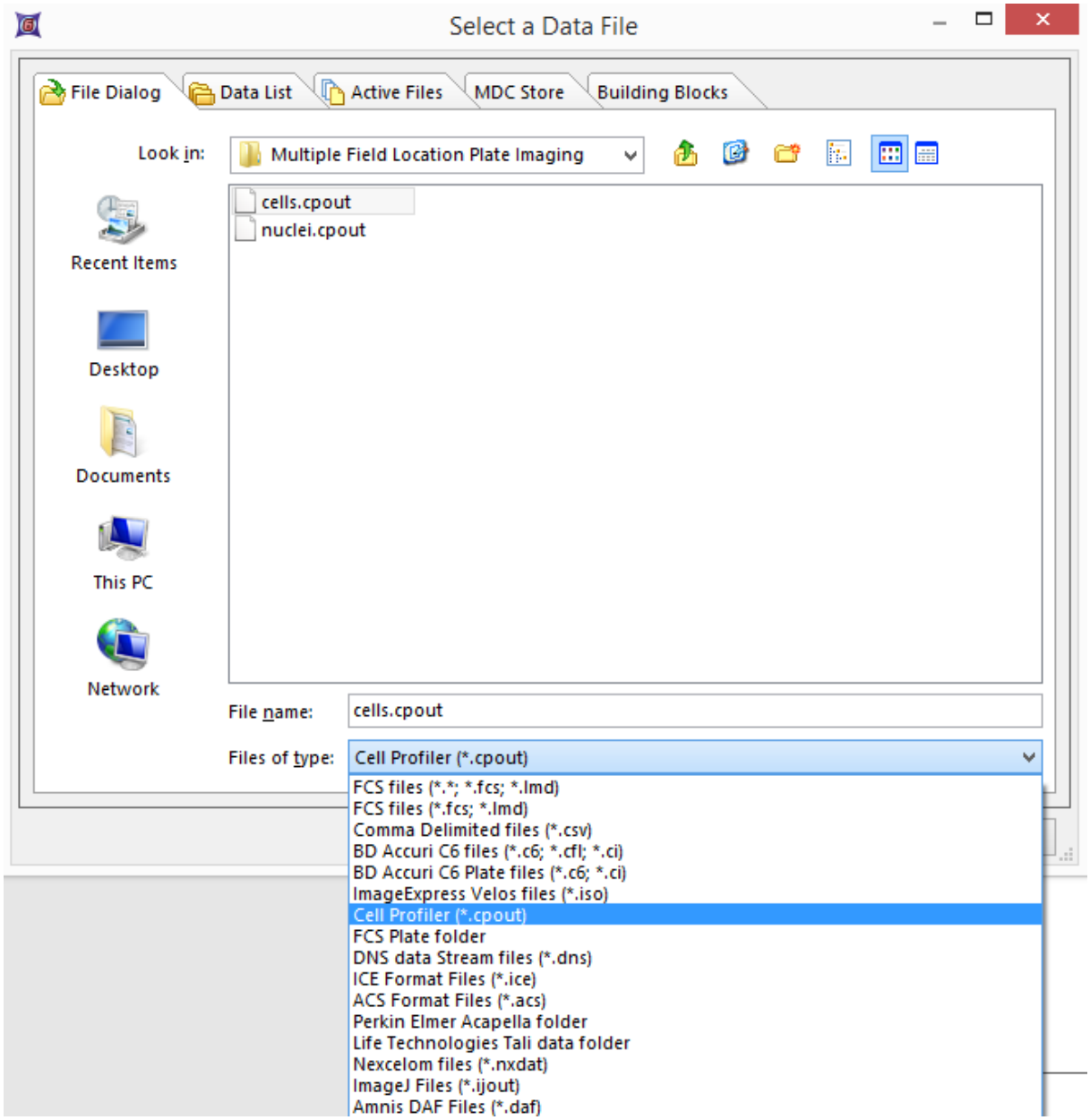

Figure T24.61 Choosing CellProfiler Files

15. Highlight **cells.cpout**.

16. Click **Open**.

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Note: For this example, we will use cells.cpout which will display data from objects classified in the *IdentifySecondaryObjects* module of CellProfiler which corresponds to the entire cell area. The file, nuclei.cpout, will display data from objects classified in the *IdentifyPrimaryObjects* module of CellProfiler which corresponds only to the nuclear area of the cells.

17. Highlight the **Y-Axis Parameter** you wish to view in the heat map from the **Open 2D Plots** dialog. For this example, choose **Intensity_MeanIntensity_Cytoplasm** (Figure T24.62).

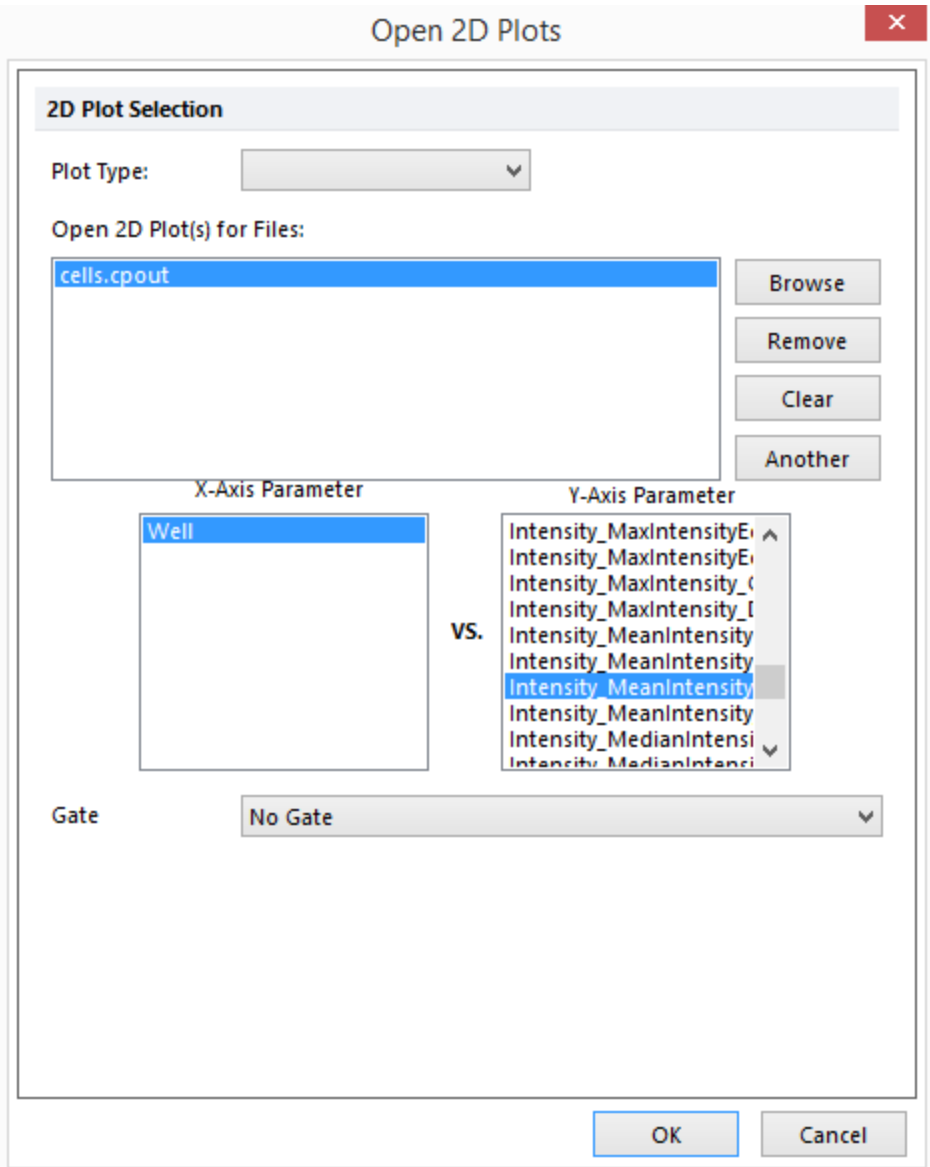

Figure T24.62 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

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18. Click **OK**.

A Heat Map will now appear on your layout indicating that the data was successfully imported (Figure T24.63).

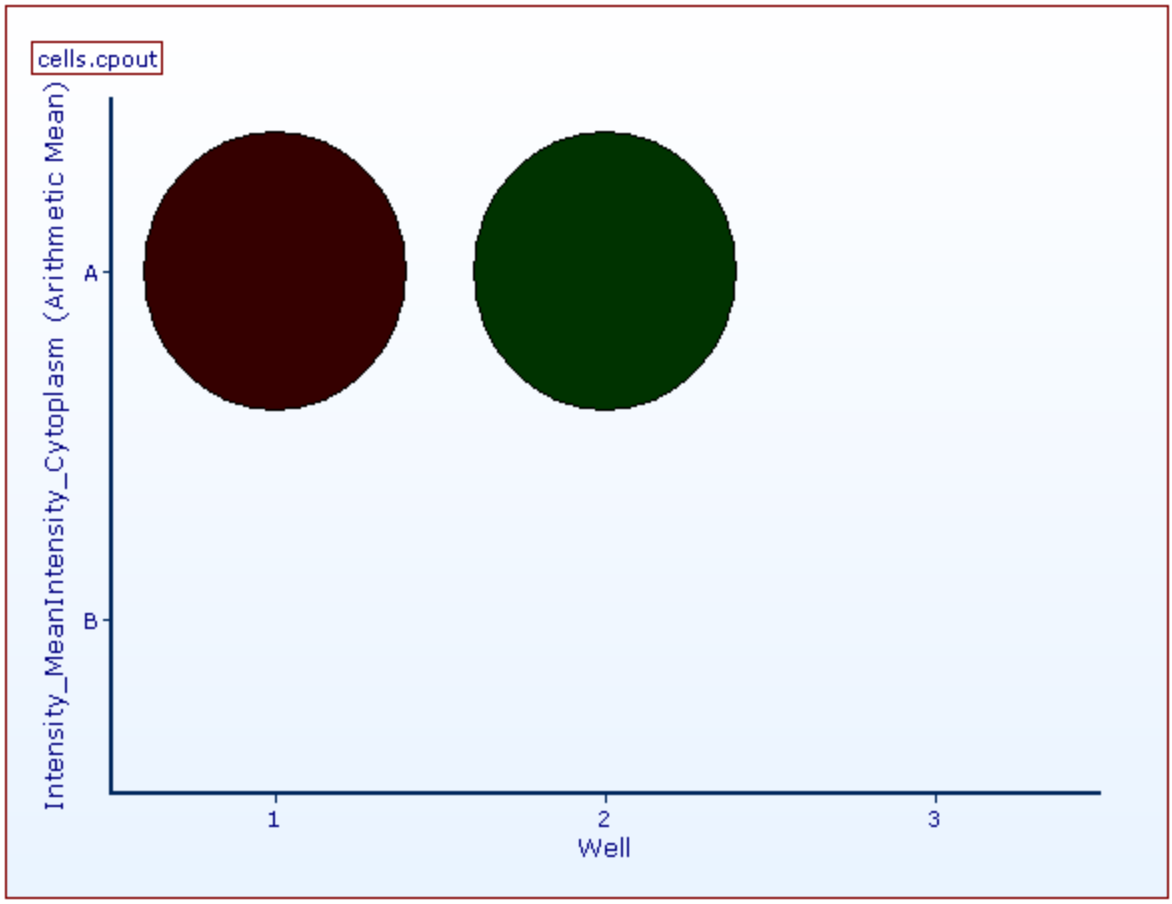

Figure T24.63 Example of a Heat Map Generated from a 6 Well Plate Based Multiple Field Location Imaging Data.

19. Right click on the heat map.
20. Choose **Create gate→Well**.
21. Create a well gate on Well A 01 named **Gate 1**.
22. Choose the **Insert Tab→2D Plots→Picture Plot**
23. Click anywhere on the layout to insert a picture plot.

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24. Choose the cells.cpout file from the select a data file dialog.
25. Click **Open**.
26. Select **Gate 1** from the **Gate** drop down.
27. Click **OK**.

The picture plot will now display the four fields from well A01 as defined by the well gate, Gate 1 (Figure T24.64).

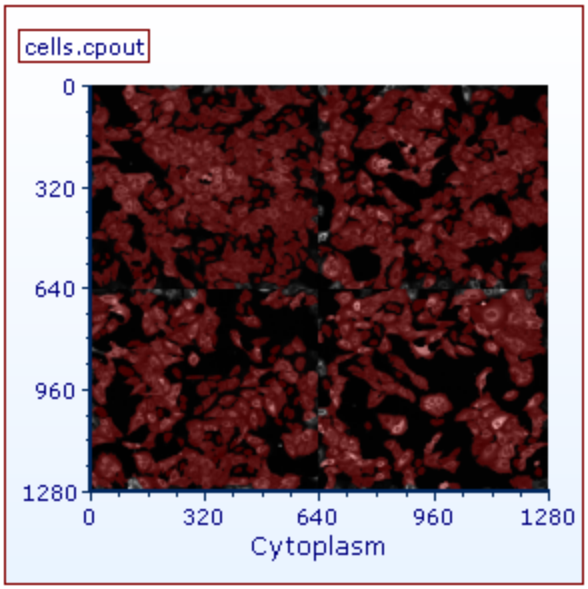

Figure T24.64 The picture plot has been gated on Gate 1, well A01, of the heat map. Four image fields may be observed in the image.

Next, we will discuss frequently asked questions in the CellProfiler FCS Express Knowledge Base.

38.26.1 CellProfiler for FCS Express Knowledge Base 4

We understand it can be difficult to learn new software such as CellProfiler. Below are some of the common questions and export errors that users may experience when exporting for FCS Express Image Cytometry. If you have specific questions related to creating pipelines in CellProfiler please see the CellProfiler website at http://www.cellprofiler.org/ or visit their user support

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forum at http://cellprofiler.org/forum/. You may also find a webinar covering the specifics of CellProfiler segmentation at: CellProfiler Webinar

File Output and Organization:

File extensions:

- The file extension ".cptoc" stands for CellProfiler Table of Contents and ".cpout" stands for CellProfiler Output. The "Image.cptoc" file stores information about the location and number of images for all objects processed in the pipeline, while the separate "xxxxxx.cpout" files store the actual raw data associated with individual objects and analysis.
- There should be a separate ".cpout" file defined for each object mask defined in CellProfiler and only one "Image.cptoc" file.

Folder Structure:

After images are processed in CellProfiler, FCS Express must be able to access the original images (Figure T24.67 below).

- The original image files should be stored in the same folder that contains your .cptoc, cpout, and image mask files.
- An Experiment.ini file must be found in the same data folder as described immediately above if you wish to view the multiwell plate data using the **Heat Maps** feature in FCS Express. This file describes the plate format.

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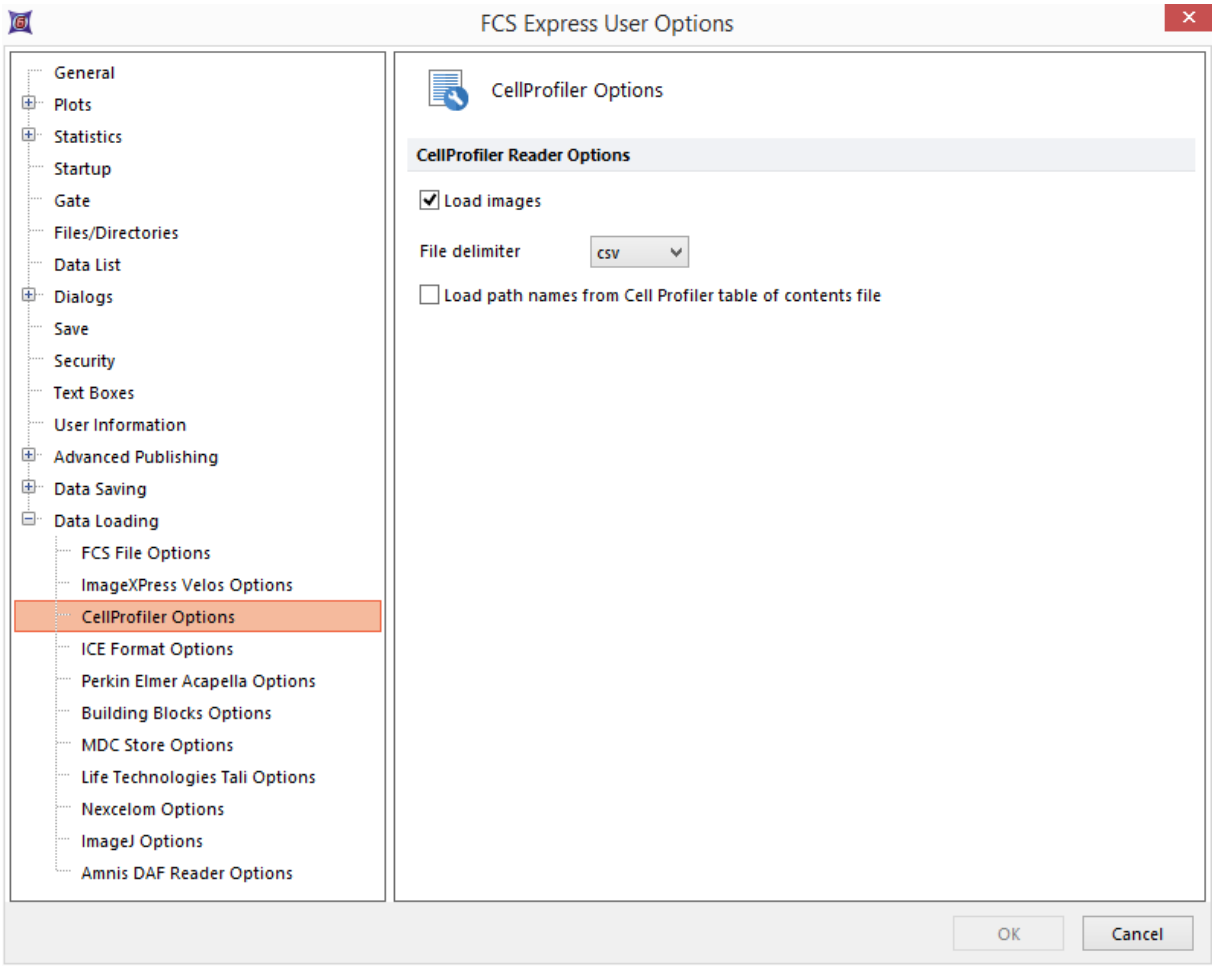

Figure T24.67 CellProfiler Data Loading Options

Loading Images and IdentifyPrimaryObjects Module:

- Please note the value entered in **Name to Assign these** field for **NamesandTypes** cannot be the same as the value entered in the **Name the primary objects** field for the **Identify Primary Objects** module.

Using the ConvertObjectsToImage Module:

- In order for FCS Express to properly import image masks from the **ConvertObjectsToImage** module the **Name the output image** name must be the same as the **Select the input objects** name and is case sensitive as in Figure T24.68 below.

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Figure T24.68 ConvertObjectsToImage Module: Naming the output image the same as the input object.

Naming Files and Regular Expressions:

- The regular expression output names: Well, Row, Column, and Col may only be used with plate based or montage imaging experiments. Using any of these names for a single image experiment will result in a non-working export. Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.

- The name of your .cpout file must begin with the name of the object data you are exporting and may only be include the object name and regular expressions. For example: the file names **nuclei.cpout** and **nuclei_<regular express>.cpout** will result in a proper export while **dataset1nuclei.cpout** or **<regularexpression>_nuclei.cpout** will resulting in a non-working export.

- **Working with Plate Based Data**
  - The regular expression names *Row* and *Column* should be used when exporting plate based data. These names define the plate location of the image by Row Letter and Column Number found in the image name. In Figure T24.69 below the original image name is found in the blue circle while the image mask name, based on the Row and Column regular expressions, can be found in the red circle.

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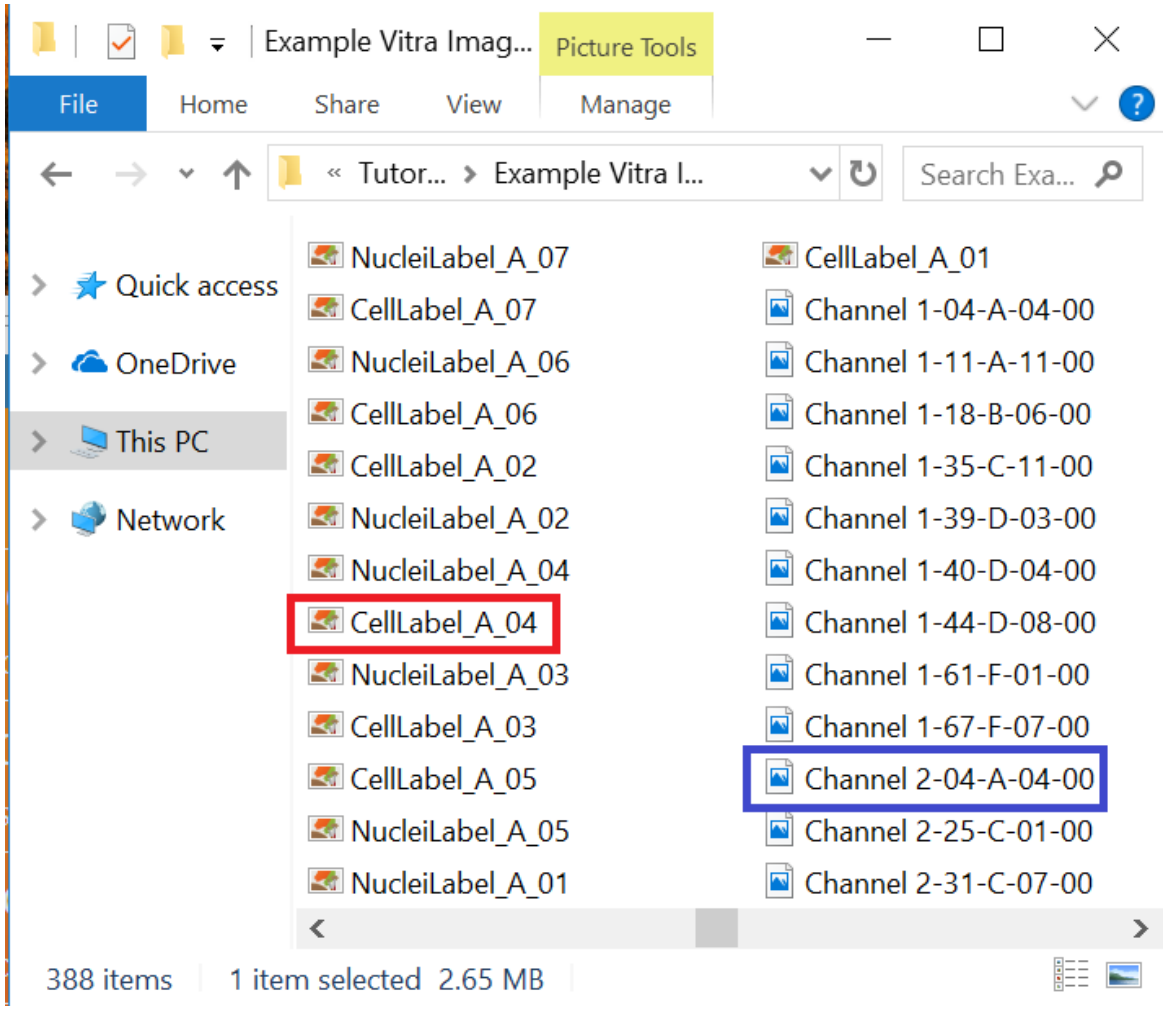

Figure T24.69 NucleiLabel and CellLabel image mask .tiff files have been created for every well of the plate. The file names reflect the Row and Column numbers for the CellLabels (Red Oval) based on the original images (Blue Oval).

- **Working with Tiled Images:**
  - When reconstructing a series of tiled images FCS Express will use information stored in the filename to piece the images back together. In particular the words "row" and "col" followed by a number indicate the position of the tile in the image. Numbering for tiled images must begin at 0_0, for instance row00_col00. Below are a few examples of this naming convention:

    mytiledimage_row00_col00.tif

    mytiledimage_row00_col01.tif

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mytiledimage_row00_col02.tif

The above file names represent the first three tiles (from left to right) of a tiled image as indicated by their "row" and "col" positions. The words "row" and "col" and the associated tile position should be added at the time of acquisition if possible or post acquisition by manually renaming the files or using a file renaming program.

- **Working with Plate Based Data That Contains Images from Multiple Field Locations Within the Well**
  - The regular expression names *Row and Column (for the well locations) and row and col (for the well image location)* should be used when exporting plate based data with multiple image locations per well. These names define the plate location of the well by Row Letter and Column Number and the image location within the well by row and col found in the image name. In Figure T24.70 below the original image name is found in the blue circle while the image mask name, based on the Row, Column, row and col regular expressions, can be found in the red circle. Numbering for multiple well image locations must begin at 0_0, for instance row00_col00.
  -

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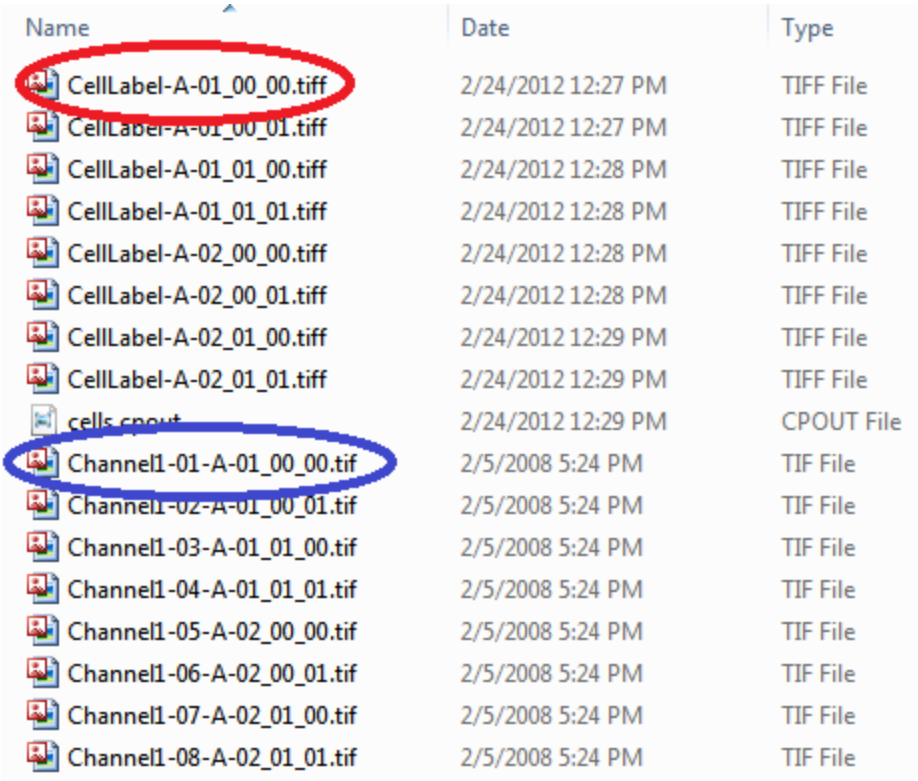

Figure T24.70 CellLabel image mask .tiff files have been created for every well of the plate. The file names reflect the Row and Column letters and numbers as well as the image location Row and Col numbers for the CellLabels (Red Oval) based on the original images (Blue Oval).

38.27 ImageJ Export

ImageJ is free open-source software designed to process and analyze images. Through the use of macros and plugins users can automate tasks and create custom tools. De Novo Software has created macros and plugins for ImageJ that allow you to export many types of imaging experiments from ImageJ to FCS Express Version (see our Example Webpage for specific examples). Once your data is properly exported from ImageJ, FCS Express Image Cytometry will allow you to perform a flow cytometry style data analysis on your data with all of our familiar 2D plots and new plotting features 2098 such as picture plots and heat maps.

The tutorial will use sample data to:

- Export a single image from ImageJ to FCS Express 2033
- Create a .ijx Analysis Configuration file 2025

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- Use a .ijx Analysis Configuration file to export a group or set of images from ImageJ to FCS Express 2037

For an overview and further descriptions regarding the De Novo Software ImageJ Macros please see the links below:

- General workflow for exporting ImageJ data to FCS Express 2040
- ImageJ Macros for FCS Express General Overview 2020
- Overview for the DNSMacroExample.ijm macro used to export data from ImageJ to FCS Express 2021
- Overview for the DNSAnalysisCreator.ijm macro to create an .ijx Analysis Configuration File 2023
- ImageJ for FCS Express Knowledge Base 2040

Note: The tutorials will not focus on how to create macros or use ImageJ but will focus on the macros De Novo Software has provided. For more help with ImageJ please see the ImageJ website at http://rsbweb.nih.gov/ij/index.html or write to the very helpful and informative ImageJ Mailing List.

38.27.1 ImageJ Macros Overview

De Novo Software has provided two ImageJ macros that will help you export your image analysis from ImageJ to FCS Express Image Cytometry. The macros can be downloaded from the De Novo Software website at www.denovosoftware.com/site/imageJ.shtml.

General Overview:

The macros provided by De Novo Software are used for the following:

- **DNS Macro Example Macro:**
  - The DNSMacroExample.ijm macro 2021 will process your images and export your segmentation and single cell image analysis results in a way that FCS Express Image Cytometry can read. The macro will process images and generate:

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1. Object by object numerical results
2. Mask images of the segmented objects
3. An ***.ijout** file that FCS Express Image Cytometry will load to examine the object by object data while relating results back to the original image

The macro can analyze images from many use case listed below (please see full details on use cases in the DNS Macro Example Overview)

- A set of single standalone images
- An image with multiple channels (ie. Red, Green, and Blue, or DAPI, CD4, and Protein X etc.)
- Images from multiple wells in multi well plate format (ie. 96 well plate)
- Images from multiple fields of view (ie. multiple fields of one well in a multi-well format or a tiled/montage image)
- Any combination of multiple channels, multiple wells, and multiple fields of view

- **DNS Analysis Creator Macro:**
  - Creates an *.IJX analysis configuration file that ImageJ can use to order, group, and process images in the above mentioned sets. (please see full details on use cases in the DNS Analysis Creator Overview)

When images belong to a grouping the user must generate an Analysis Configuration file with an .IJX extension. The Analysis Configuration file tells FCS Express how the different images are related to each other. For example, if two images represent channel 1 and channel 2 from a single field or if 96 separate images make up the contents of a 96 well plate based experiment. The DNSAnalysisCreator.ijm macro has been designed to assist the user in creating the Analysis Configuration file.

In the next section, we will give a general overview of the DNS Macro Example

38.27.1. DNS Macro Example Overview 1

The DNS Macro Example Macro will process your images and export your segmentation and single cell image analysis results in a way that FCS Express Image Cytometry can read. The macro can process and export images from ImageJ in the five use cases listed below.

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A. A set of single standalone images:

1. **A set of single standalone images in a folder:** For example, a series of single images (one channel) from different samples. If the user selects a folder, this macro will generate one .ijout file per valid image file in that folder. (If you would like to add any other image format that ImageJ supports please check and/or update the "function validImageFileName" and "function MaskImageFile" sections of the macro) This means one subfolder with masks/results per file. The name of the .ijout file will be the name of each of the original image files. This is the only use case that does NOT require an analysis configuration file.

2. **A set of single standalone images where the image locations are defined by an .IJX file. For example, a series of single images from different samples.** If the user selects an .IJX analysis configuration file, which contains ONLY filenames (may be only one), then same as A above, but only for the files in the .IJX file.

B. A set of images with multiple channels. For example, a series of images from different samples that contain more than one channel, ie DAPI and FITC stains. If the user selects an .IJX file that has multiple groups of channels, with NO wells, then this macro will generate one IJOUT file per group, named with the same name as the selected mask. This macro will ask for the channel to use to create the masks.

C. A set of images from a multiple well plate based assay. The .IJX file can accommodate multiple channel images and multiple field locations. For example, a 96 well plate with two channel images and four fields per well. If the user selects an .IJX file with multiple wells of a plate, which has to include the channels, then this macro will generate only one .IJOUT file for the entire plate, using the same name as the .IJX file for the .IJOUT file. This macro will ask for the channel to use to create the masks.

D. A set of images that makes up a larger tiled or montage image. For example, a large thin section that has been imaged in many 20x fields. If the user selects an .IJX file with multiple channels, but no wells. This is the case when a large image has been sliced into smaller segments (or originates from an unstitched group of montage image tiles), to perform the analysis on each of them.

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If this macro file is installed in ImageJ (Plugins→Macros→Install), a red rectangle with three color blobs button with the hint "**De Novo Software Exporter**" will be shown in the toolbar (figure T28.92).

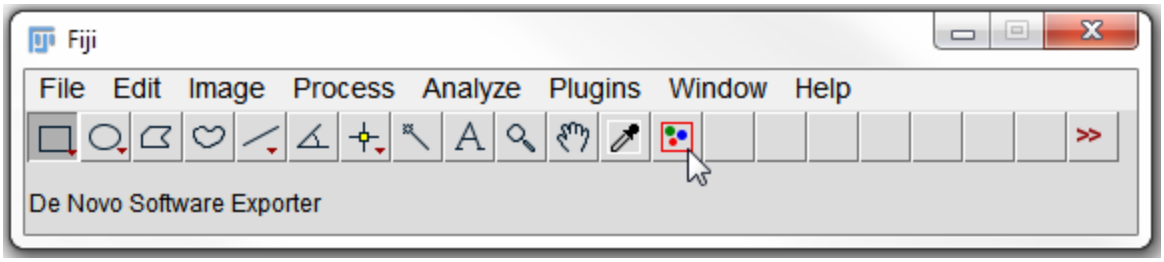

Figure T28.92 The ImageJ tool bar with the DnsExampleMacro.ijm (De Novo Software Exporter) installed.

The functions to customize the macro are at the last lines of the macro following the text:

// - - - - - - - - - The functions below this line are the ones that the user can customize - - - - - - - - - - -

In the next section, we will give a In the next section, we will give a general overview of the DNS Analysis Creator Macro 2023

38.27.1. DNS Analysis Creator Macro Overview 2

The DNS Analysis Creator Macro creates an *.ijx analysis configuration file that ImageJ can use to order, group, and process images in sets described in the DNS Macro Example Overview 2021.

The analysis configuration file is a comma separated value (.csv) text file, with an .ijx extension. The first line of this file must contain a column header(s). For example: Filename, Channel.

The valid column headers for the .ijx file are:

Filename: It must be the first field, and must be the fully qualified path to the file, this is, including the full path to it. It can not be empty. For example, c:\image data\img004.jpg.

Channel: (*Optional)* If present, it must be the second field. It can not be empty. For example, it can be: green. The channels can be any name you like but must repeat for different wells, fields of view etc..

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Well: (*Optional)* If present, it must be the third field. It can not be empty. The format is one or two letters, in the range a..z, A..Z, followed by at most four digits in the range 0..9. For example, it can be B07.

FOV_row: *(Optional)* This is the number of the row for the field of view or tile/stitch position in a montage image. Fields of view are reassembled into a grid according to your specifications. If present, it must be the fourth field if "wells" are present, or the third field if "wells" are not present. It can not be empty. It must be an integer number, denoting the row of the field of view, starting at 0 (zero). For example, 0

FOV_col: *(Optional)* This is the number of the column for the field of view or tile/stitch position in a montage image. Fields of view are reassembled into a grid according to your specifications. If present, it must be the fifth field if wells are present, or the fourth field if "wells" are not present. It can not be empty. If FOV_row column is present, this column must be present, too. It must be an integer number, denoting the column of the field of view, starting at 0 (zero). For example, 2

These are examples of headers:
Filename
Filename,Channel
Filename,Channel,Well
Filename,Channel,FOV_row,FOV_col
Filename,Channel,Well,FOV_row,FOV_col

Additional Notes:

a. There must be at least one column for the image file name with full path. If subsequent columns are present, all intervening columns must be also present. For example, if you choose **Filename** and **Well** you must also have a column for **Channel** present, resulting in **Filename,Channel,Well**.
b. Header keywords are case-sensitive.
c. There are no default values for the fields. This means that two consecutive delimiters are not allowed.

How to create an .IJX file

The analysis configuration file (*.IJX file) may be created by:

- Using the DNS Analysis Creator Macro
- Using the DNS IJX writer in Excel utilizing Excel based macros 2026
- Manually editing the .IJX file in ImageJ 2030 or Excel 2031

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In the next section, we will complete the Creating a .ijx Analysis Configuration File section 2025 of the tutorial

38.27.2 Creating a .ijx Analysis Configuration File

Introduction:
When images belong to a set or group the user must generate an analysis configuration file with an .ijx extension. The analysis configuration file (.ijx file) will allow ImageJ to determine how different images are related to each other. For example, if two images represent channel 1 and channel 2 from a single field of view or if 96 separate images make up the contents of a 96 well plate based experiment. A full list of possible sets and use cases that can be represented with an .ijx file can be found in the DNS Macro Example Overview 2021.

We will be using the macros and images from the Tutorial Sample Data archive 1210 within the ImageJ Tutorial folder. The following files and folders will be used to create the analysis configuration file in the tutorial:

- The DNS-IJXwriter.xlsm Excel macro
- The DNSAnalysisCreator.ijm macro
- Images found in the Multi Channel Image Experiment folder

The images for the data set represent a two color (two channel) imaging experiment where nuclei were stained with DAPI and a cytoplasmic protein was stained with FITC.

We will begin the tutorial by creating an .ijx file from the DNS-IJXwriter.xlsm Excel Macro* (click below to continue).

- DNS-IJXwriter.xlsm, a macro-enabled Excel file that reads your filenames and generates an .ijx file automatically 2026 *(recommended)*.

Or go to the section for creating an .ijx file using the DNS Analysis Creator:

- DNSAnalysisCreator.ijm, a macro that has been designed to assist the user in creating the Analysis Configuration file 2028

Note: This tutorial will not focus on how to create macros or use ImageJ The focus of this tutorial will be on how to use the macros De Novo Software has provided. For more help with ImageJ please see the ImageJ website at http://rsbweb.nih.gov/ij/index.html or write to the very helpful and informative ImageJ Mailing List.

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*DNS-IJXwriter.xlsm Excel macro written by Peter Haub

In the next section, we will use the DNS-IJXwriter.xlsm macro-enabled Excel file to automatically generate an .ijx Analysis Configuration file.

38.27.2. Macro-enabled Excel method for creating ijx file 1

In this section, you will learn how to use the DNS-IJXwriter.xlsm Excel macro to automatically generate an .ijx analysis configuration file. The DNS-IJXwriter.xlsm Excel macro is an Excel file containing a macro, written by Peter Haub, that will automatically read filenames and generate an .ijx file from a folder of images.

1. Open the DNS-IJXwriter.xlsm macro-enabled Excel file, located in the ImageJ Tutorial→ImageJ DNS Macros folder of the Tutorial Sample Data Archive.
2. Click on **Enable Content** button if not already enabled (Fig. T28.102)

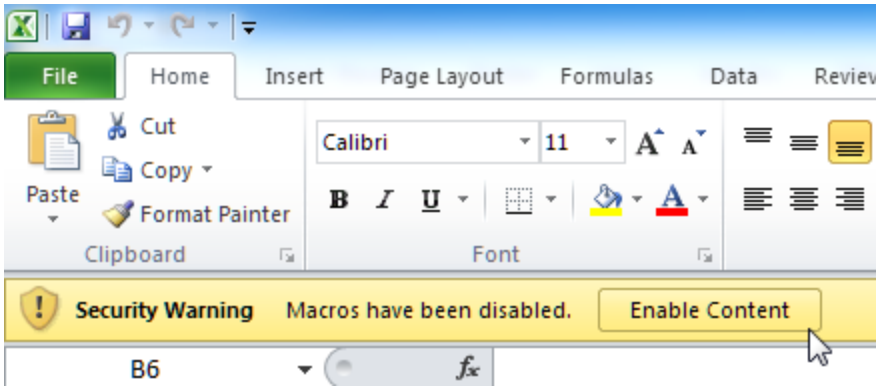

Figure T28.102 Enabling macros in a Microsoft Excel file.

3. Click on the **Read File Names** button (Fig. T28.103)

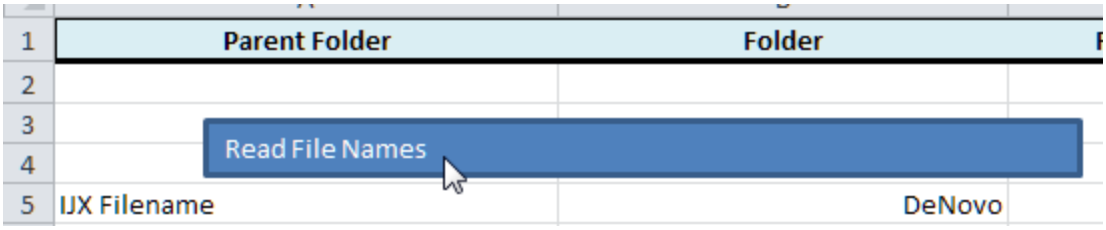

Figure T28.103 Clicking on the Read File Names macro button

4. Navigate to the "Mutli Channel Image Experiment" directory folder within the Tutorial Sample Data Archive→ImageJ tutorial folder.
5. Click **OK** (Fig T28.104).

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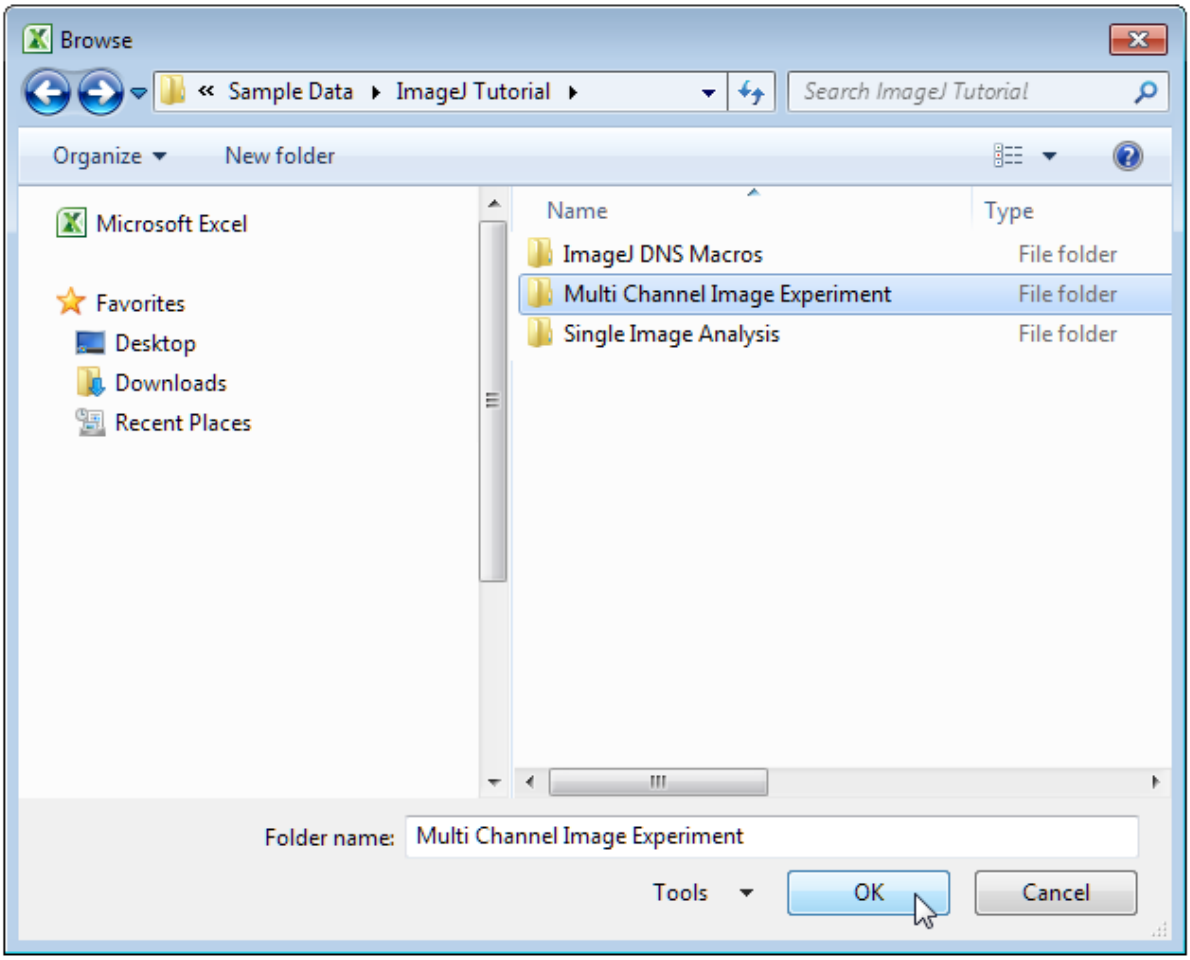

Figure T28.104 Navigating to the ImageJ Tutorial folder within the Tutorial Sample Data Archive

6. Type the corresponding channel name (i.e., "DAPI" or "FITC") for each image into the "**Channel**" column (Column D) of the spreadsheet. Your spreadsheet should now resemble Fig. T28.105.

	A	B	C	D	E	F	G
1	Parent Folder	Folder	Filename	Channel	Well	FOV_row	FOV_col
2						Peter Haub 08-06-2013	
3		Read File Names		Write IJX File			
4							
5	IJX Filename	DeNovo					
6	Directory	C:\Users\kaya\Documents\Subversion\Tutorials\Sample Data\ImageJ Tutorial					
7	Plate Row	3					
8	Plate Column	5					
9							
10	C:\Users\kaya\Documents\Subversion\Tutorials\Sample Data\ImageJ Tutorial	Multi Channel Analysis	Image1DAPI.jpg	DAPI			
11	C:\Users\kaya\Documents\Subversion\Tutorials\Sample Data\ImageJ Tutorial	Multi Channel Analysis	Image1FITC.jpg	FITC			
12	C:\Users\kaya\Documents\Subversion\Tutorials\Sample Data\ImageJ Tutorial	Multi Channel Analysis	Image2DAPI.jpg	DAPI			
13	C:\Users\kaya\Documents\Subversion\Tutorials\Sample Data\ImageJ Tutorial	Multi Channel Analysis	Image2FITC.jpg	FITC			
14							

Figure T28.105 DNS-IJXwriter.xlsm Excel file after file names have been read and channel names entered into the appropriate column.

7. Click the **Write IJX File** button (Fig. T28.105, outlined in red)

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"DeNovo.IJX", an Analysis Configuration file has now been written to the *TutorialSampleData/ImageJ Tutorial/Multi Channel Image Experiment* directory folder. This .ijx file may now be processed in ImageJ using the DNS MacroExample.ijm macro 2037 in order to render the .ijout files which are readable by FCS Express.

In the next section, we will use the newly created .ijx file to analyze and export results from ImageJ to FCS Express 2037

38.27.2. Creating an analysis configuration file using the DNS Analysis Creator Macro 2

In this section, you will learn how to create an .ijx Analysis Configuration file using the DNSAnalysisCreator.ijm macro. For an in depth description of this macro please see the De Novo Software ImageJ Macro General Overview 2023.

1. Open ImageJ.
2. Install the DNSAnalysisCreator.ijm macro in ImageJ.

   When this macro file is installed in ImageJ 2041 (Plugins→Macros→Install), the **De Novo Software Analysis Configuration File Creator** button (a blue rectangle with the hint "De Novo Software Analysis Configuration File Creator") will be shown in the toolbar. (figure T28.106).

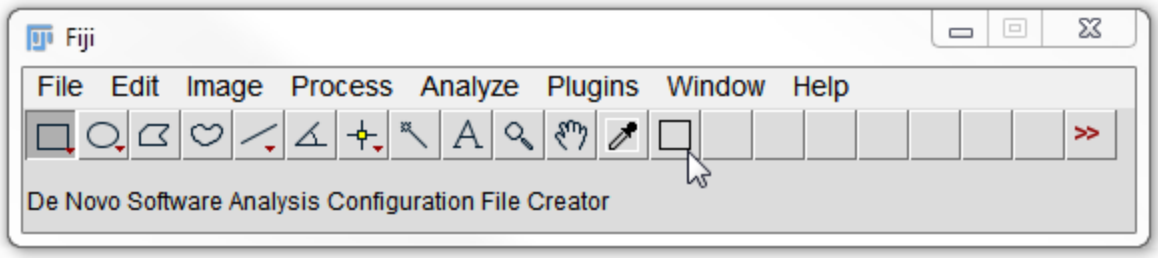

Figure T28.106. The ImageJ tool bar with the DNSAnalysisCreator.ijm macro installed. Notice the blue rectangle button that indicates the De Novo Software Analysis Configuration File Creator.

3. Click on the **De Novo Software Analysis Configuration File Creator** button (figure T28.106).
4. Choose the **Multi Channel Analysis** folder within the **Tutorial Sample Data** folder→**ImageJ Tutorial** folder→**Multichannel Image Experiment** folder (figure T28.107). This selects the folder in which the original images are found.
5. Click **Select**.

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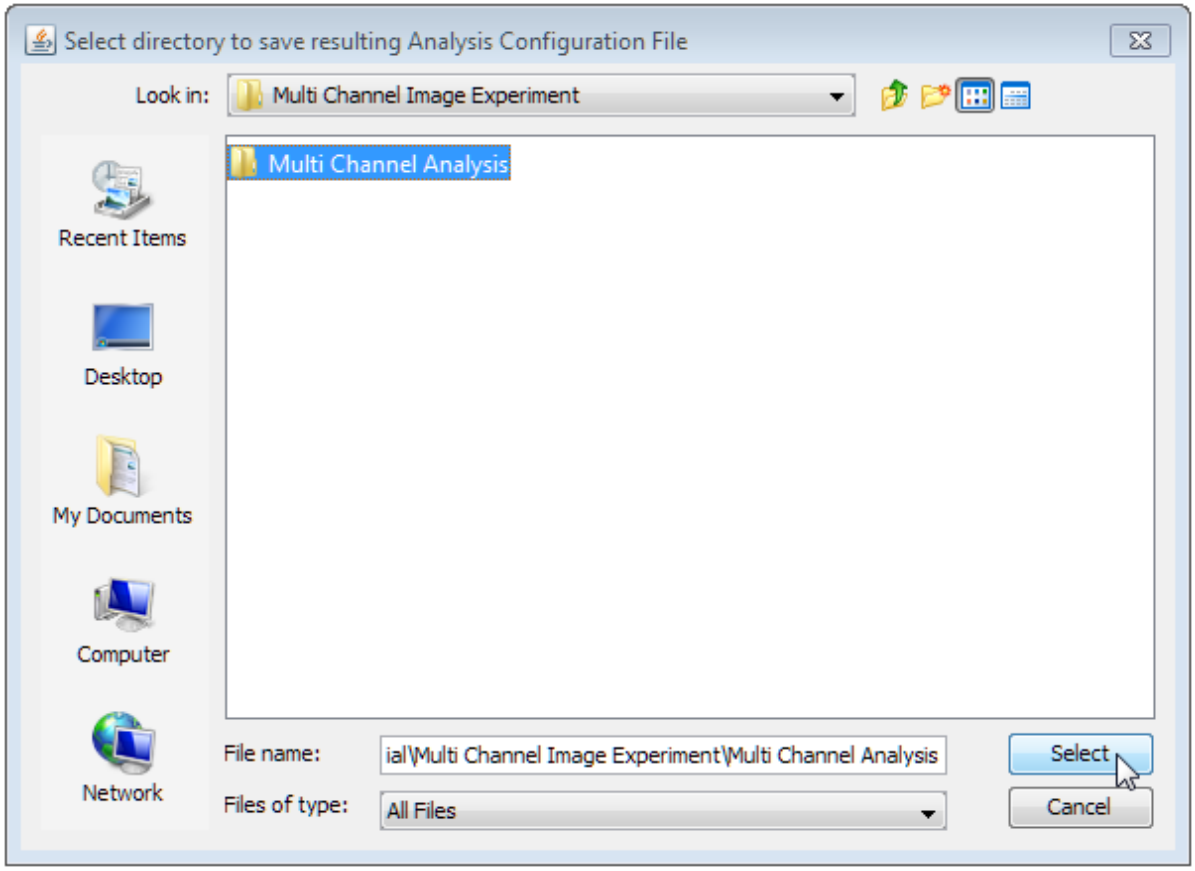

Figure T28.107 Selecting the Multi Channel Analysis folder directory which contains our images for the Analysis Configuration file.

6. Choose the **Multichannel Image Experiment** folder within the **Tutorial Sample Data** folder→**ImageJ Tutorial** folder. This chooses a directory to save the resulting Analysis Configuration File.
7. Click **Select**.

At this point a file named **FileListForDnsExporter.ijx** will be found in the Multi Channel Image Experiment folder. The .ijx file is the analysis configuration file that is needed to define grouping of multiple images. For more details on the different types of groupings and conventions for defining them please see the DNS Analysis Creator.

You must now edit the analysis configuration file to further define how the images have been grouped for an export to FCS Express Image Cytometry. For this experiment we have two images that both contain two channels (DAPI and FITC) The .ijx file is simply a comma separated value file (.csv) with an .ijx extension. We will now edit the file to define the file path and channel for each of the images in the .ijx file.

There are many ways to edit the .IJX file and we will cover two in this section:

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- Editing directly in ImageJ 2030
- Editing in Microsoft Excel 2031

38.27.2.2.1 Editing an .IJX file in ImageJ

ImageJ can be used to directly edit a .IJX file created by the DNS Analysis Creator Macro 2023.

Editing the .ijx file in ImageJ

1. Click on File→Open in ImageJ.
2. Navigate to the **Multichannel Image Experiment** folder within the **Tutorial Sample Data** folder→**ImageJ Tutorial** folder.
3. Double click on the FileListForDnsExporter.ijx file to open it.

The FileListForDnsExporter.ijx file will open in the ImageJ text editor as seen below in figure T28.108. The default file that is created automatically creates column headers for all possible situations and rows with place holders (represented as text between less than and equal to symbols/brackets, ie. <WellID>) for the information. You will also notice a section in line 6 (at the bottom) that contains information for plate based assays if needed. We now need to edit the file so it only contains Filename and Channel as column headers and the relevant channel information in each row.

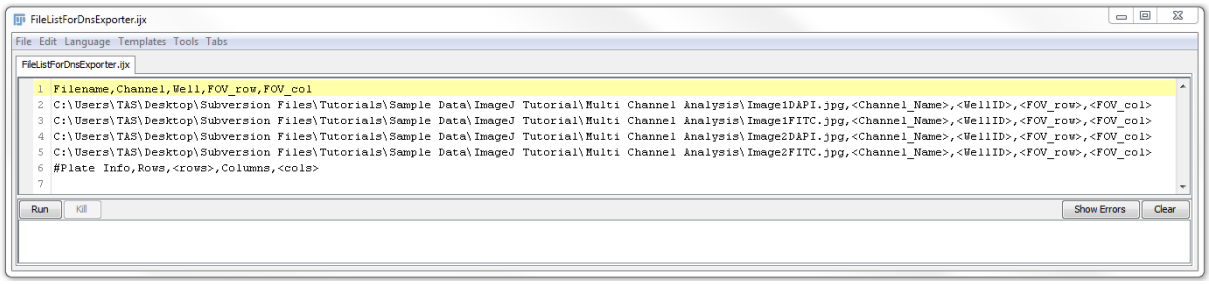

Figure T28.108 The FileListForDnsExporter.ijx file opened in ImageJ that was created in the previous steps.

4. Delete **Well**, **FOV_row**, and **FOV_col** from line 1.
5. Delete **#Plate Info**, **Rows**, **<rows>**, **Columns**, **<cols>** from the last line..
6. Delete all instances of **<WellID>**, **<FOV_row>**, and **<FOV_col>**.
7. Replace the text **<Channel_Name>** in each row with the respective channel name from the image name.

For instance: in row 2 the image name is Image1DAPI.jpg. In this case replace **<Channel_Name>** with **DAPI**. Similarly, replace the <Channel_Name> in the alternate rows with FITC.

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When you have completed the editing the FileListForDnsExporter.ijx should look like figure T28.109 below.

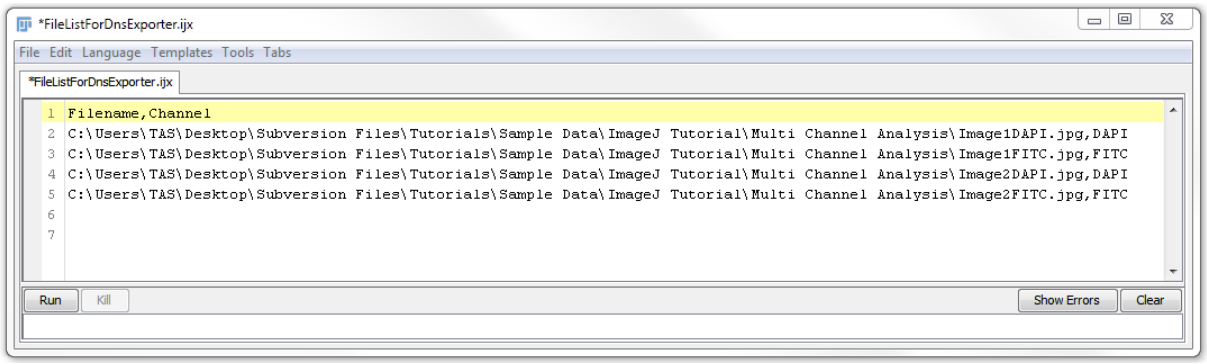

Figure T28.109 The FilelistForDnsExpoter.ijx file in ImageJ after editing to remove extra column headers, plate information, and adding the channel information.

8. Click on **File→Save** in the ImageJ text editor.

You have now edited and saved the analysis configuration file for this two channel two image experiment.

In the next section, we will use the newly created .ijx file to analyze and export results from ImageJ to FCS Express 2037

38.27.2.2.2 Editing an .IJX file in Excel

Microsoft Excel can be used to directly edit a .IJX file created by the DNS Analysis Creator Macro 2023.

Editing the .ijx file in Excel

To edit the .ijx file in Excel you have two options:

A. The file can be renamed with a .csv extension (this is the option we will use for the tutorial and it is outlined in step 16 below).

B. Choose Excel as the default program to open the .ijx file. Right click on the .ijx file and choose **open with**>choose **default program**. From the list of available programs choose Excel, or use the **Browse** button to navigate to Excel. When Excel is chosen check the **Always use the selected program to open this kind of file** box. From now on, any time you double click on a .ijx file it will open with Microsoft Excel. (Please note these instructions are for Windows 7. (Please see the Microsoft Windows website for instructions for other Windows Operating Systems)

1. Rename the **FileListforDnsExporter.ijx** file to **FileListforDnsExporter.csv**. (Right click on the file in a windows browser and choose rename)

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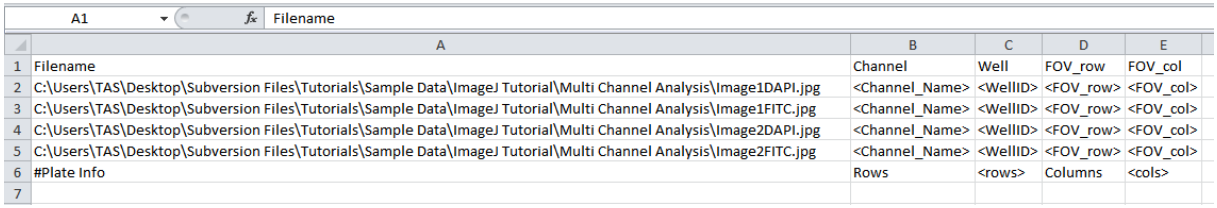
2. Open the **FileListforDnsExporter.csv** file with Microsoft Excel.

The File will open and the excel document will look like figure T28.110 below:



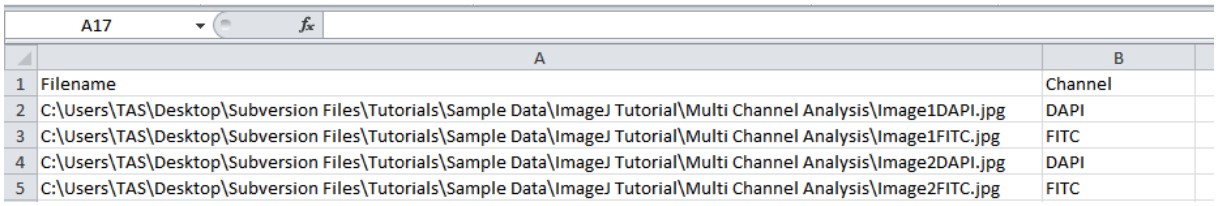
	A	B	C	D	E
1	Filename	Channel	Well	FOV_row	FOV_col
2	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image1DAPI.jpg	<Channel_Name>	<WellID>	<FOV_row>	<FOV_col>
3	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image1FITC.jpg	<Channel_Name>	<WellID>	<FOV_row>	<FOV_col>
4	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image2DAPI.jpg	<Channel_Name>	<WellID>	<FOV_row>	<FOV_col>
5	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image2FITC.jpg	<Channel_Name>	<WellID>	<FOV_row>	<FOV_col>
6	#Plate Info	Rows	<rows>	Columns	<cols>
7					

Figure T28.110 The FileListforDnsExporter.csv file opened with Microsoft Excel.

3. Delete the columns for **Well**, **FOV_row**, and **FOV_col**.
4. Delete row 6 that contains **#Plate Info**, **Rows**, **<rows>**, **Columns**, **<cols>**.
5. Replace the text **<Channel_Name>** in each row with the respective channel name from the image name.

For instance: in row 2 the image name is Image1DAPI.jpg. In this case replace **<Channel_Name>** with **DAPI**.

When you have completed the editing the FileListForDnsExporter.csv should look like figure T28.111 below.



	A	B
1	Filename	Channel
2	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image1DAPI.jpg	DAPI
3	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image1FITC.jpg	FITC
4	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image2DAPI.jpg	DAPI
5	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image2FITC.jpg	FITC

Figure T28.111 The FilelistForDnsExpoter.csv file in Excel after editing to remove extra column headers, plate information, and adding the channel information.

There are many ways to automate the task of pulling the channel name from the path of your file name in Excel. We will not be covering these methods in the tutorial but an example may be found below where the "MID" function was used to automatically pull out the word **DAPI** from the file name from cell A2 in figure T28.111 above. The formula "**=MID(A2,106,4)**" indicates perform the MID function (get characters from the middle of a text string), text is located in cell A2, starting at character 106, and is 4 characters long (figure T28.112).

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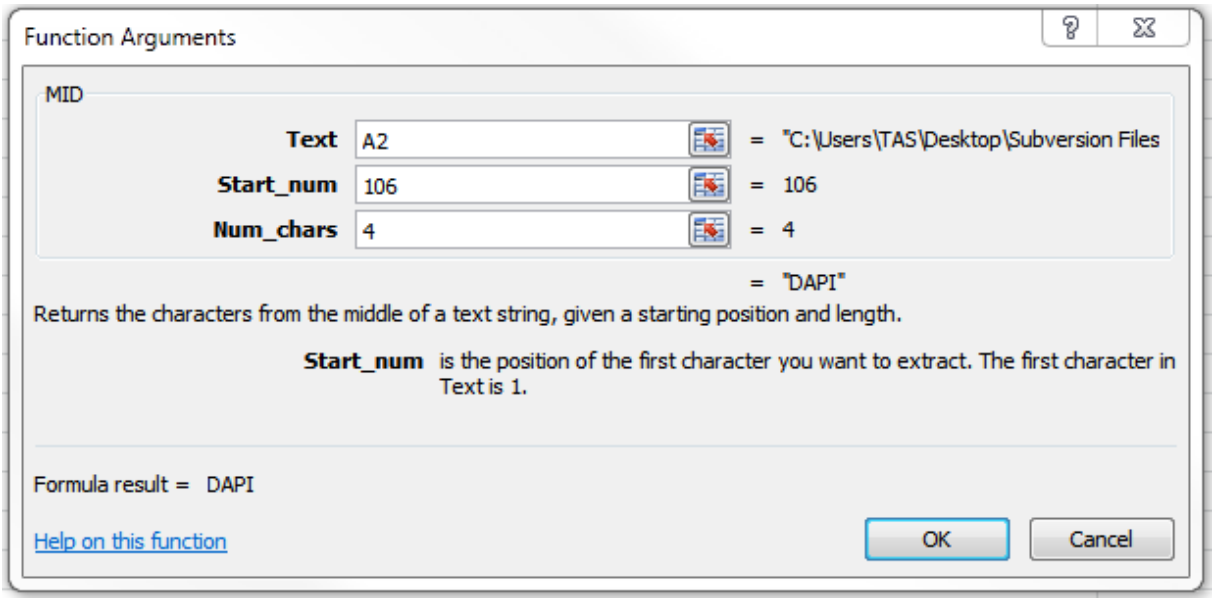

Figure T28.112 An example of using functions or formulas in Excel to automatically fill columns in the Analysis Configuration file.

6. Save the FileListForDnsExporter.csv file that you just edited.
7. From a windows explorer window rename the **FileListforDnsExporter.csv file** to **FileListforDnsExporter.ijx**

If you would like to create a .ijx file for other groupings such as multiple field based data, montage/tile imaging, plate based data, or multiple field plate based data please edit your .ijx file as in the steps above but applying the formatting for each particular type of grouping defined in the ImageJ Macro 2021 help topic.

In the next section, we will use the newly created .ijx file to analyze and export results from ImageJ to FCS Express 2037

38.27.3 Analyzing and Exporting Individual Image Results with ImageJ

In this section you will learn how to analyze and export individual image results from ImageJ using the DNSMacroExample (a set of single standalone images in a folder 2021 use case) . We will be using the macros and images from the Tutorial Sample data set→ImageJ Tutorial folder. The DNSMacroExample.ijm macro will be used along with the image found in the Single Image Analysis folder (DNAImage1.jpg and DNAImage2.jpg).

Note: This tutorial will not focus on how to create macros or use ImageJ. The focus of this tutorial will be on how to use the macros De Novo Software has provided. For more help with ImageJ, please see the ImageJ website at http://rsbweb.nih.gov/ij/index.html or write to the very helpful and informative ImageJ Mailing List.

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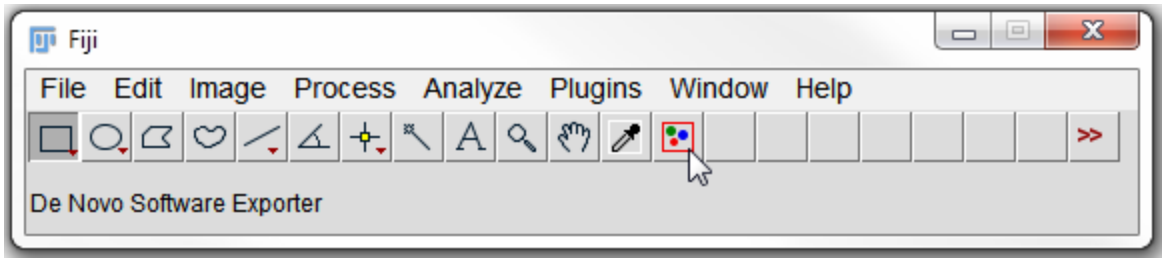
1. Open ImageJ.
2. Install the DNSMacroExample in ImageJ.

   When this macro file is installed in ImageJ (Plugins→Macros→Install), the **De Novo Software Exporter** button (a red rectangle with three colored circles button with the hint "De Novo Software Exporter") will be shown in the toolbar (figure T28.97).



FigureT28.97. The ImageJ tool bar with the DNSMacroExample.ijm macro installed. Notice the red rectangle with three color blobs button that indicates the De Novo Software Exporter.

3. Click on the **De Novo Software Exporter** button (figure T28.97).
4. Click on the **Source** drop down button (figure T28.98).
5. Choose "**From a folder**" in the drop down list (figure T28.98).

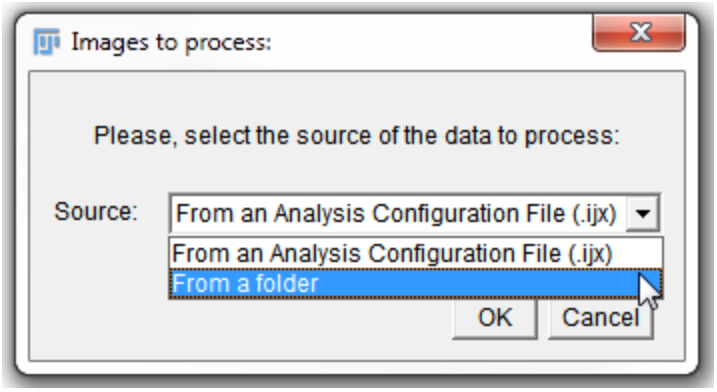


Figure T28.98 Selecting Images to process from a folder in ImageJ.

6. Click **OK**.
7. Choose the **DNA Experiment** folder found within the **Tutorial Sample Data folder→ImageJ Tutorial→Single Image Analysis**.
8. Click **Select** (figure T28.99).

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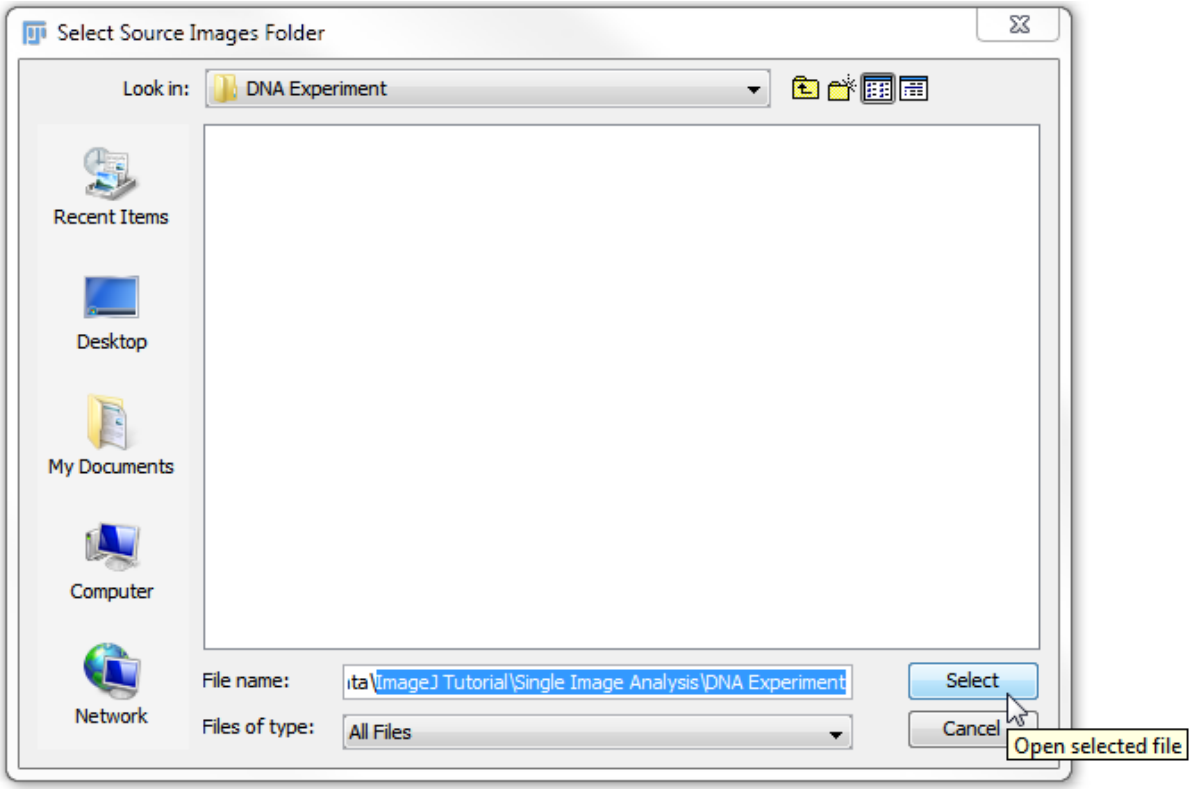

Figure T28.99 Selecting the DNA Experiment folder which contains our images of interest and pressing select.

ImageJ will now automatically process all of the images within the Single Image Analysis folder. The images will be process based on the functions to customize the macro in the last lines of the macro following the text:

// - - - - - - - - - The functions below this line are the ones that the user can customize - - - - - - - - - - -

This will include thresholding and object identification/segmentation. *For more information on customizing macros and adjusting settings in ImageJ please see the ImageJ website at http://rsbweb.nih.gov/ij/index.html or write to the very helpful and informative ImageJ Mailing List.*

The exported .ijout files will be named DNAImage1.ijout and DNAImage2.ijout. Two folders that contain other exported information that are named according to the original file name will also be present (figure T28.100)

Note: that the name of the .ijout file and the folder that contains the results must always be the same. Multiple segmentations, and therefore multiple .ijout and results folders may also be generated from the same images. In the case of running multiple segmentations the same set of original images may be used without duplicating the image data set.

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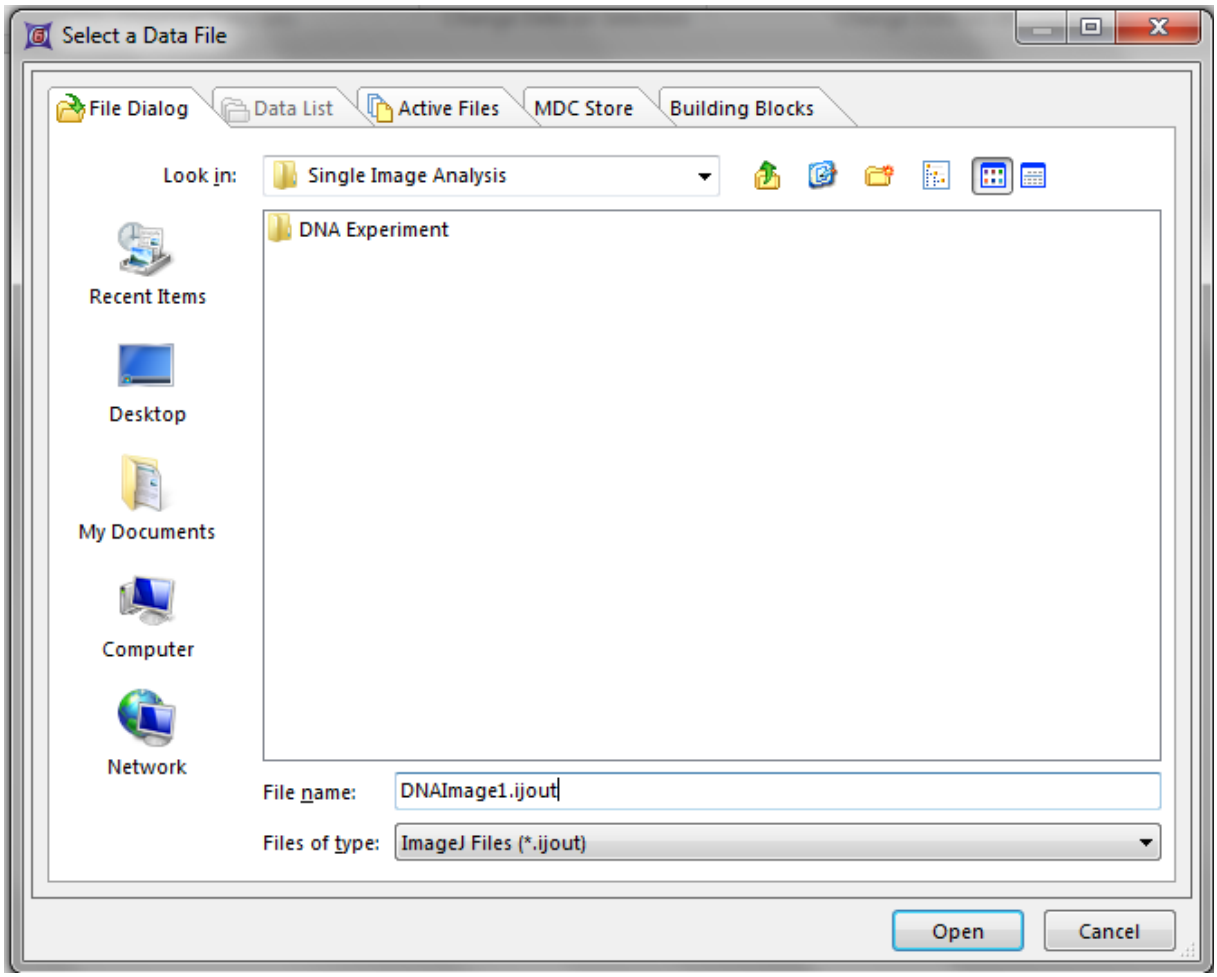

Figure T28.100 The Single Image Analysis folder after processing the images. There will be separate .ijout files for each image and folders that contain the results and masking files.

We will now open the exported data with FCS Express.

9. Open FCS Express Image Cytometry.
10. Click on the **Insert Tab→2D Plots→Dot**.
11. Click anywhere on the layout to insert the Dot plot.
12. Choose **ImageJ Files (*ijout)** from the **Files of type** drop down menu.
13. Navigate to the **Tutorial Sample Data folder→ImageJ Tutorial→Single Image Analysis** folder.
14. Choose **DNAImage1.ijout**.
15. Click **Open**.
16. Click on the **Insert Tab→2D Plots→Picture Plot**.
17. Click anywhere on the layout to insert the picture plot.

The layout should now contain a Dot plot and Picture Plot for the data exported from ImageJ (figure T28.101). Using the **Next** and **Previous** buttons from the **Data Tab→Change Data on All Objects** section will change between the data from the first and second DNA images that were processed. Please see the

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further resources section below for more information about working with plots and imaging data in FCS Express.

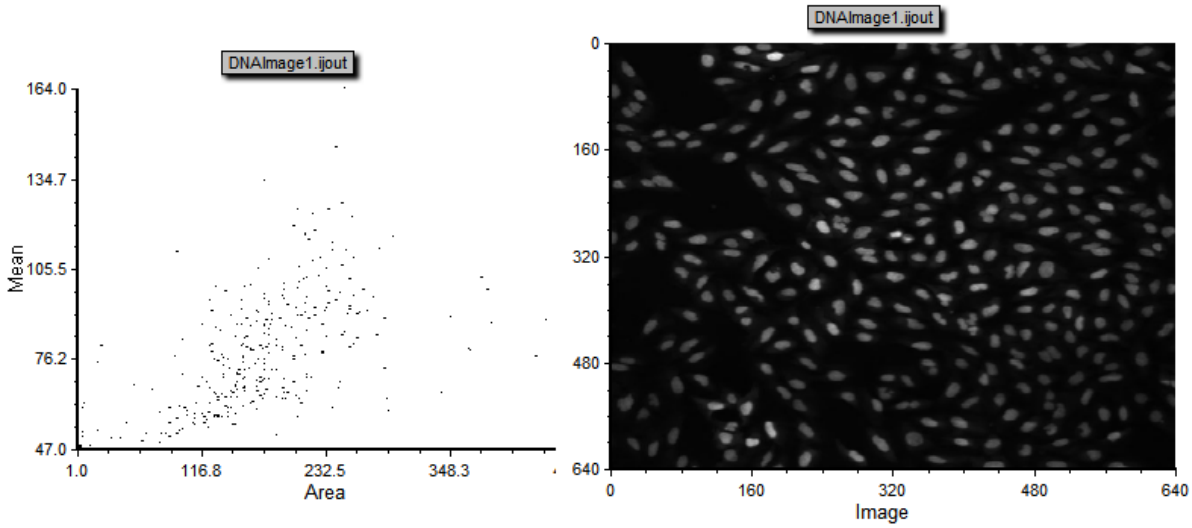

Figure T28.101 A dot plot and picture plot that were create from the DNAImage1.ijout file.

Further Resources:

- **Introduction to FCS Express Image Cytometry Tutorial** 2098
- **Picture Plots Tutorial** 2060
- **ImageJ for FCS Express Knowledge Base** 2040

38.27.4 Analyzing and Exporting Multi Channel Image Results with an .ijx file

In this section you will learn how to analyze and export a group of multi channel image results from ImageJ using the DNSMacroExample and an .ijx analysis configuration file, which was created in the previous section 2025 with the macro-enabled Excel worksheet 2026* or manually edited in ImageJ 2030 or Excel 2031.

We will be using the macros and images from the tutorial sample data set→ImageJ Tutorial folder. The DNSMacroExample.ijm macro will be used along with the images found in the Multi Channel Analysis folder (Images 1 and 2 for the DAPI and FITC channels).

1. Open ImageJ.
2. Install the DNSMacroExample in ImageJ (Note: if the DNSMacroExample has already been installed you may skip step 2).

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When this macro file is installed in ImageJ (Plugins→Macros→Install), a red rectangle with three color blobs button with the hint "De Novo Software Exporter" will be shown in the toolbar (figure T28.113).

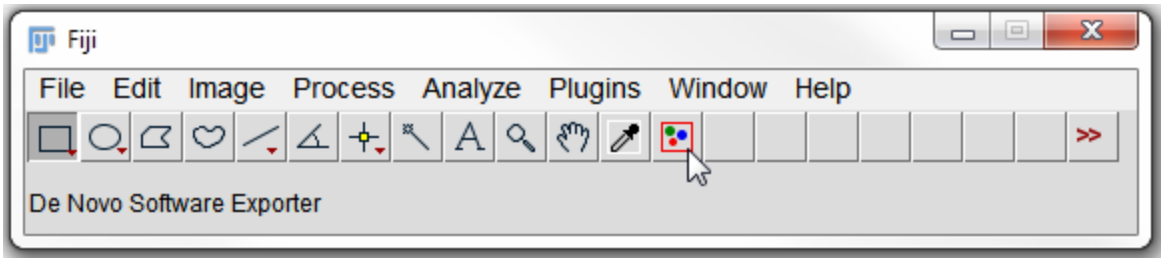

Figure T28.113. The ImageJ tool bar with the DNSMacroExample.ijm macro installed. Notice the red rectangle with three color blobs button that indicates the De Novo Software Exporter.

3. Click on the **De Novo Software Exporter** button (red rectangle with three color blobs) in the Image J tool bar (figure T28.113).
4. Choose **From an Analysis Configuration File (.ijx)** (figure T28.114).

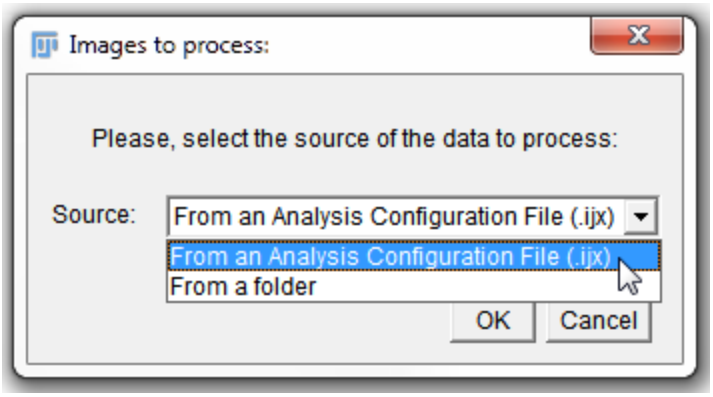

Figure T28.114 Choosing an .ijx file to process images.

5. Click **OK**.
6. Choose either **DeNovo.ijx** (if your .ijx file was created with the macro-enabled Excel worksheet) or **FileListForDnsExporter.ijx** (if your .ijx file was manually edited in ImageJ or Excel) from the **Multi Channel Image Experiment** folder within the **Tutorial Sample Data** folder→**ImageJ Tutorial** directory folder.
7. Click **Open**.
8. Choose **dapi** as the **Channel**.

   Choosing the channel in step 8 will define which channel should be used for image segmentation. In this case we are using the dapi stain which is defining the nuclear area of the cells for segmentation.

9. Click **OK**.

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ImageJ will now process all of the files listed in the .ijx Analysis Configuration file and create a .ijout file for each set of two channel images and a folder for each of the matching .results.txt and .mask.tif files.

We will now open the exported data with FCS Express.

10. Open FCS Express Image Cytometry.
11. Click on the **Insert Tab→2D Plots→Dot**.
12. Click anywhere on the layout to insert the Dot plot.
13. Choose **ImageJ Files (*ijout)** from the **Files of type** drop down menu.
14. Navigate to the **Tutorial Sample Data folder→ImageJ Tutorial→Multi Channel Image Experiment** folder.
15. Choose **Image1DAPI1.ijout**.
16. Click **Open**.
17. Click on the **Insert Tab→2D Plots→Picture Plot**.
18. Click anywhere on the layout to insert the picture plot.

The layout should now contain a Dot plot and Picture Plot for the data exported from ImageJ (figure T28.115). Using the **Next** and **Previous** buttons from the **Data Tab→Change Data on All Objects** section will change between the data from the first and second images that were processed. By clicking on the Channel Label in the picture plot (DAPI) you will be able to choose between the DAPI and FITC channels (Figure T28.115 right). Please see the further resources section below for more information about working with plots and imaging data in FCS Express.

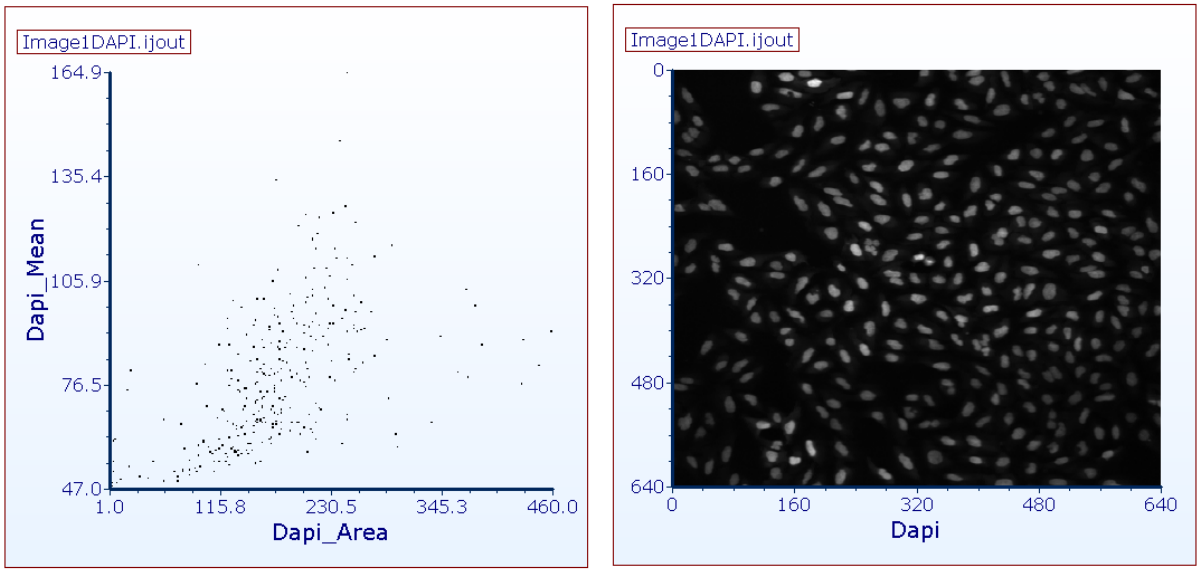

Figure T28.115 A dot plot and picture plot created from the .ijout files that were exported using the .ijx analysis configuration file. Since there are multiple channels you can change the axis of the picture plot to choose which channel to view.

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Further Resources:

- Introduction to FCS Express Image Cytometry Tutorial 2098
- Picture Plots Tutorial 2060
- ImageJ for FCS Express Knowledge Base 2040

*macro written by Peter Haub

38.27.5 ImageJ for FCS Express Knowledge Base

Workflow for analyzing ImageJ data in FCS Express:
The flowchart below indicates how the DNSMacroExample.ijm macro can be used to export ImageJ data as .ijout files readable by FCS Express (Fig. T28.116). The DNS-IJXwriter.xlsm macro (written by Peter Haub) can be used to define the relationship between grouped images.

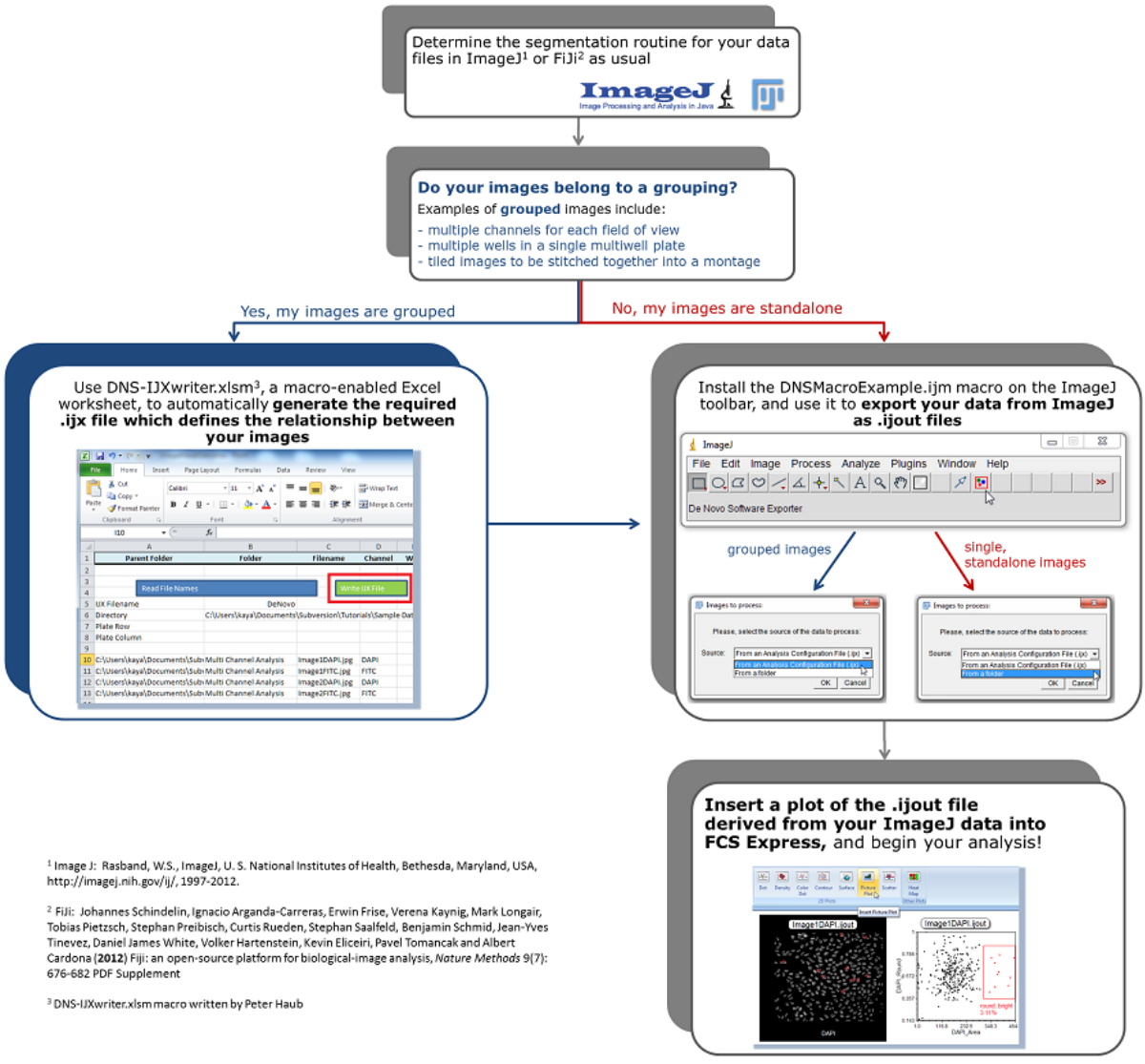

Figure T28.116 Workflow for analyzing single or grouped images segmented in ImageJ within FCS Express

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Installing an ImageJ Macro:
ImageJ Macros for use with FCS Express can be downloaded from http://www.denovosoftware.com/site/imageJ.shtml.
Macros are installed in ImageJ with the following steps:
1. Open ImageJ
2. Click on **Plugins→Macros→Install** or press control+shift+M
3. Navigate to the location of the macro
4. Click **Open**.

The macro will now be installed in the ImageJ tool bar. For more help with ImageJ Macros please see the ImageJ website at *http://rsbweb.nih.gov/ij/index.html*.

Folder Structure and File Naming:

File Names:
The DNSExampleMacro will name any exported file names (.ijout files) and folder names with the name of the original image. For instance, if your original image was named DapiImage#1.tif, the resulting ImageJ export file will be named DapiImage#1.ijout and a folder will be created name DapiImage#1 that contains a .results.txt file named DapiImage#1.results.txt and a mask image named DapiImage#1.mask.tif. Note that the name of the .ijout file and the folder that contains the results must always be the same.

Loading Files (Folder Structure):
There are options in **FCS Express User Options→Data Loading→ImageJ Options** for choosing how to load your data (figure T28.117).

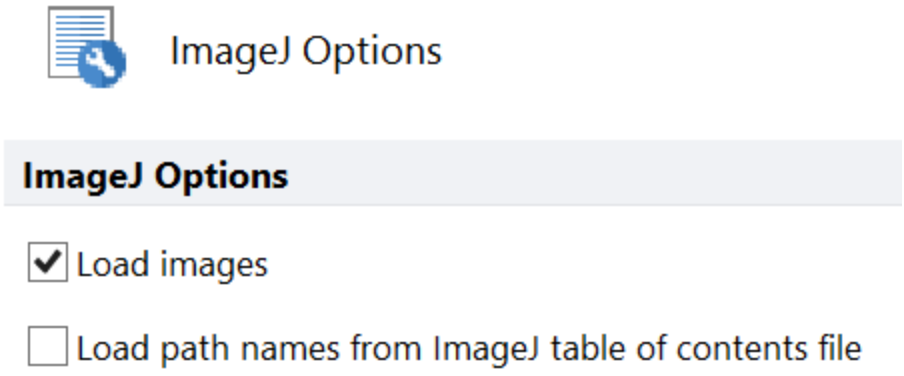

Figure T28.117 The ImageJ Data Loading options available from Edit Preferences in FCS Express Image Cytometry.

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Choosing **Load images** will determine if you want to load just raw data (**Load images** unchecked), or raw data plus the original images (**Load images** checked).

"**Load path names from ImageJ table of contents file**" indicates that you would like to load the data files with the absolute file path that was determined at the time of exporting the data from ImageJ. If you have subsequently moved the data files or folder containing the data you will need to uncheck this button. To work with data without loading the path names from the ImageJ table of contents file, the following files must be within the same folder at the same level as in figure T28.118 below:

- The original image (DNAImage1.jpg below)
- The exported .ijout file (DNAImage1.ijout below)
- The folder containing the .results.txt file and the .mask.tif file (DNAImage1 folder below)

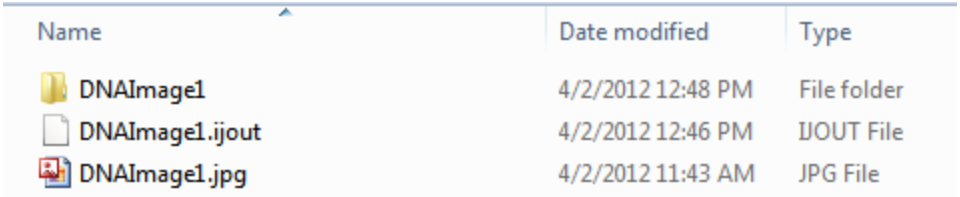

Figure T28.118 When moving .ijout files to another location on your computer the .ijout file, original image file, and folder containing the .results.txt and .masks.tif files must be at the same level.

Note that the name of the .ijout file and the folder that contains the results must always be the same. Multiple segmentations, and therefore multiple .ijout and results folders may also be generated from the same images. In the case of running multiple segmentations the same set of original images may be used without duplicating the image data set.

Working with Plate Based Data:

If the .ijx file represents a plate, the lines of the file that are related to a well must be grouped consecutively. For example, if the file is about a plate with wells D05 and H04, all the channels for well D05 must be in a group of lines, followed by another group of lines with the channels of well H04.

If the ijx file represents a plate, the last line must have the plate information, starting at column 1 with a # and the words 'Plate Info'. Then it is expected a field with the constant 'Rows', the number of rows in the plate, the constant field 'Columns', and the number of columns of the plate. For example: #Plate Info,Rows,8,Columns,12 . See figure Figure T28.119 for an example.

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	A	B	C	D	E
1	Filename	Channel	Well		
2	C:\Users\TAS\Desktop\Image J Testing\Plate Data New Macro Testing\Images\Channel1-05-min-A01.tif	chn1	A01		
3	C:\Users\TAS\Desktop\Image J Testing\Plate Data New Macro Testing\Images\Channel2-05-min-A01.tif	chn2	A01		
4	C:\Users\TAS\Desktop\Image J Testing\Plate Data New Macro Testing\Images\Channel1-10-min-A02.tif	chn1	A02		
5	C:\Users\TAS\Desktop\Image J Testing\Plate Data New Macro Testing\Images\Channel2-10-min-A02.tif	chn2	A02		
6	#Plate Info	Rows	8	Columns	12
7					

Figure T28.119 An .IJX Analysis Configuration file for a plate based assay that contains two wells, one image per well, and each image contains two channels. The text #Plate Info, Rows, 8, Columns, 12 indicate the plate is 8 x 12 rows, or a 96 well plate.

The name of the .ijx schema can be customized. At the bottom of the DnsAnalysisCreator.ijm you will find:

```
// This function returns the name of the automatically generated .ijx file.
function getExporterFilename() {
  return "FileListForDnsExporter";
```

Modify the text in, '**return "XXXX;**' where the X's represent your desired filename, to change to a customized file name.

If this macro file is installed in ImageJ (Plugins→Macros→Install), a blue rectangle with the hint "**De Novo Software Analysis Configuration File Creator**" will be shown in the toolbar (figure T28.120).

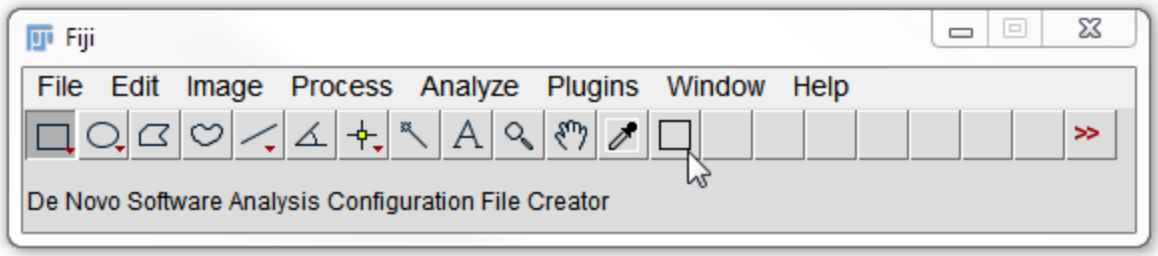

Figure T28.120 The ImageJ tool bar with the DnsAnalysisCreator.ijm macro installed. It is represented by a black box with the hint "De Novo Software Analysis Configuration File Creator".

38.28 Exporting from Image Xpress Velos

The **ImageXpress Velos**™ Imaging Cytometer enables robust real-time assays for microarray, bead, cell, and colony classification and enumeration in user-defined or commercially available formats. The **ImageXpress Velos** Imaging Cytometer is ideally suited for high throughput screening of many cell-based assays which makes for a great pairing with the high content and batch

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processing abilities of FCS Express. De Novo Software has teamed up with Molecular Devices to allow analysis of high throughput imaging in FCS Express. Once your data is properly saved within the **ImageXpress Velos** software, FCS Express can import the data and allow visualization of your data set with all of our familiar 2D plots and new plotting features, such as picture plots and heat maps.

In the first exercise, we will export an experiment from ImageXpress VelosTM 2044.

38.28.1 Export an Image Xpress Velos Experiment

In the course of this tutorial you will learn :

- How to save **ImageXpress Velos** data with images from an experiment.
- How to import the data into FCS Express.

ImageXpress Velos Setup – Saving Images and Object Data

1. Select **Instrument Setup** from the menu bar.
2. Click on the **Analysis Tab**.
3. Click the **Produce individual iso files** check box. This saves the analyzed object data for each well.
4. Click the **Produce MDCStore Compatible files** check box. This saves the raw tiff image from each well.
5. Ensure the **Auto. Correct Saturated Data** check box is checked.
6. Click **OK**.

The Instrument Setup window should look like Figure T25.1 when you are done.

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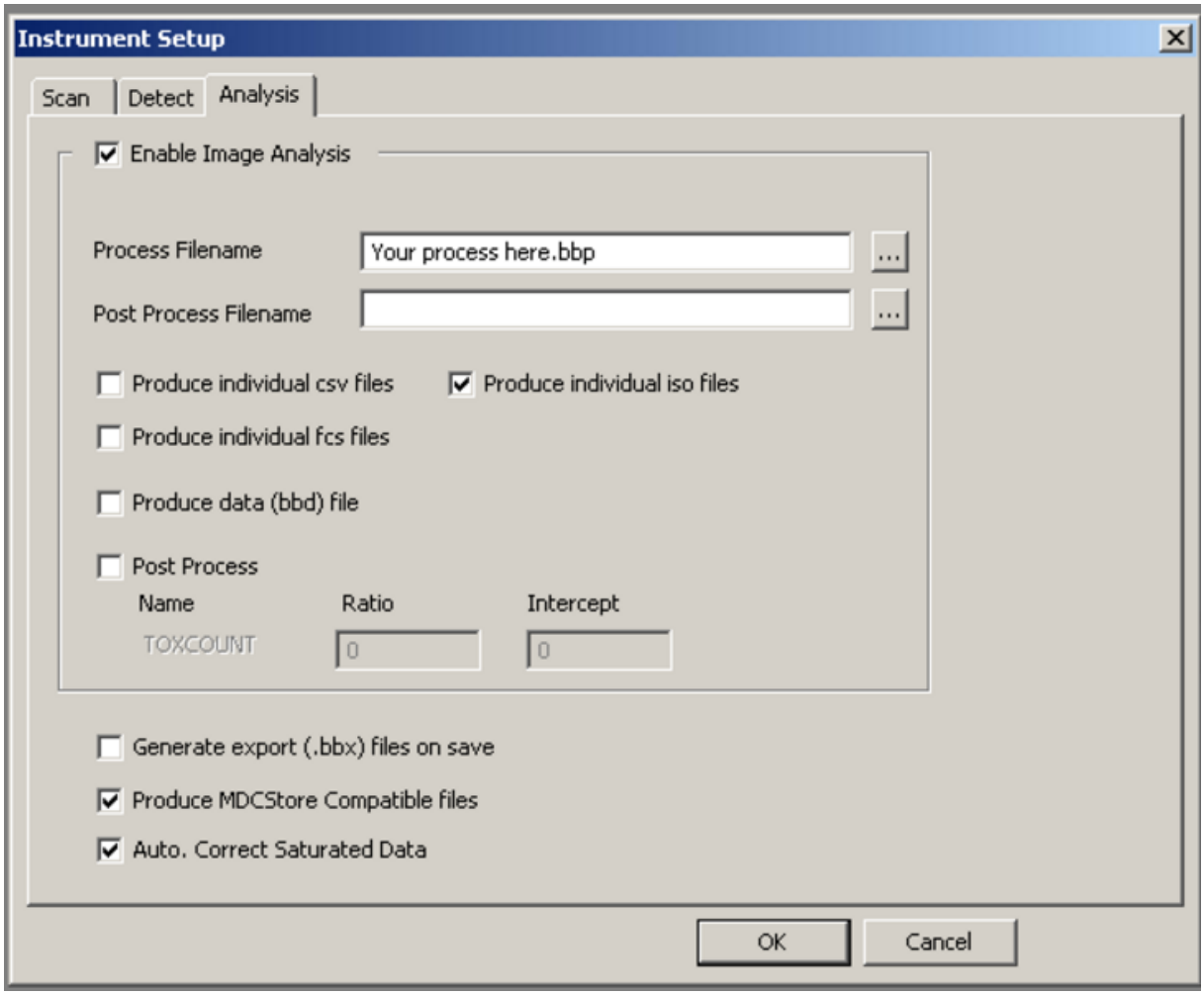

Figure T25.1 Instrument Setup for ImageXpress VelosTM

7. Analyze your experiment in **ImageXpress Velos**.
8. Save your experiment in **ImageXpress Velos**.

In the next section, we will import ImageXpress VelosTM Data with images into FCS Express 2045.

38.28.2 Import Image Xpress Velos Data with Images

After adjusting the export settings and analyzing your data in **ImageXpress Velos**, you may proceed to import the data into FCS Express.

The **ImageXpress Velos** files used for this section are located in the Tutorial Sample Data archive 1210.

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1. Select **File→Options→Data Loading→ImageXpress Velos Options**.
2. Check the **Load images** check box.
3. Check the **Load as Plate** check box.
4. Click **OK**.

The **ImageXpress Velos options** page of the **Data Loading** preferences should look like Figure T25.2.

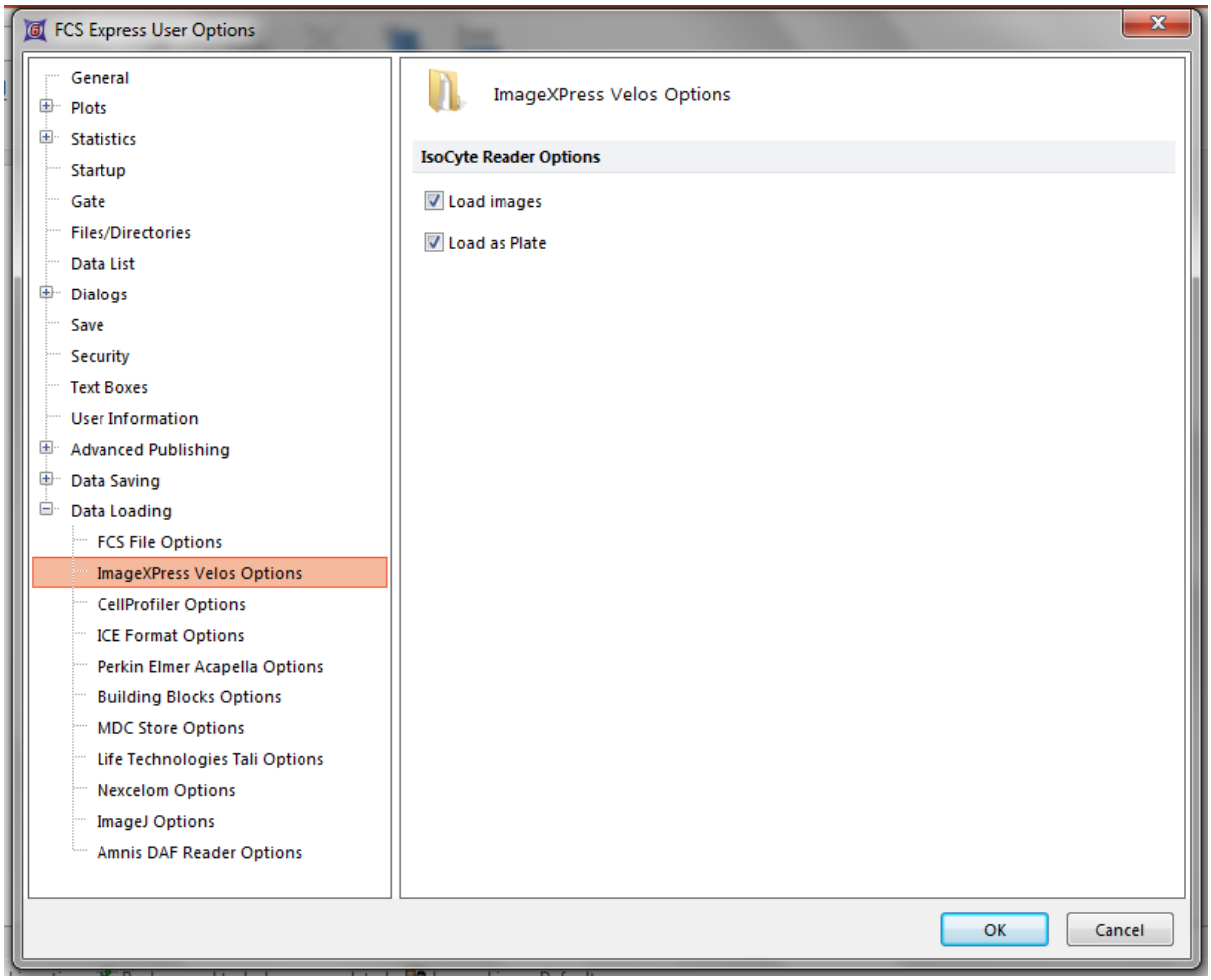

Figure T25.2 Setting up ImageXpress Velos Data Loading Preferences in FCS Express

5. Select the **Insert→Other Plots→Heat Map** command (Figure T25.3).

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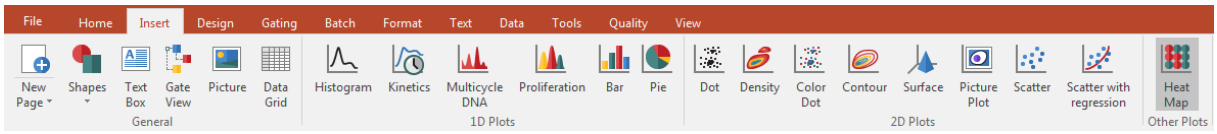

Figure T25.3 Inserting a Heat Map

6. Click anywhere on the layout.

7. Choose **ImageXpress Velos files (*.iso)** from the **files of type:** drop-down list in the **Select a Data File** dialog (Figure T25.4).

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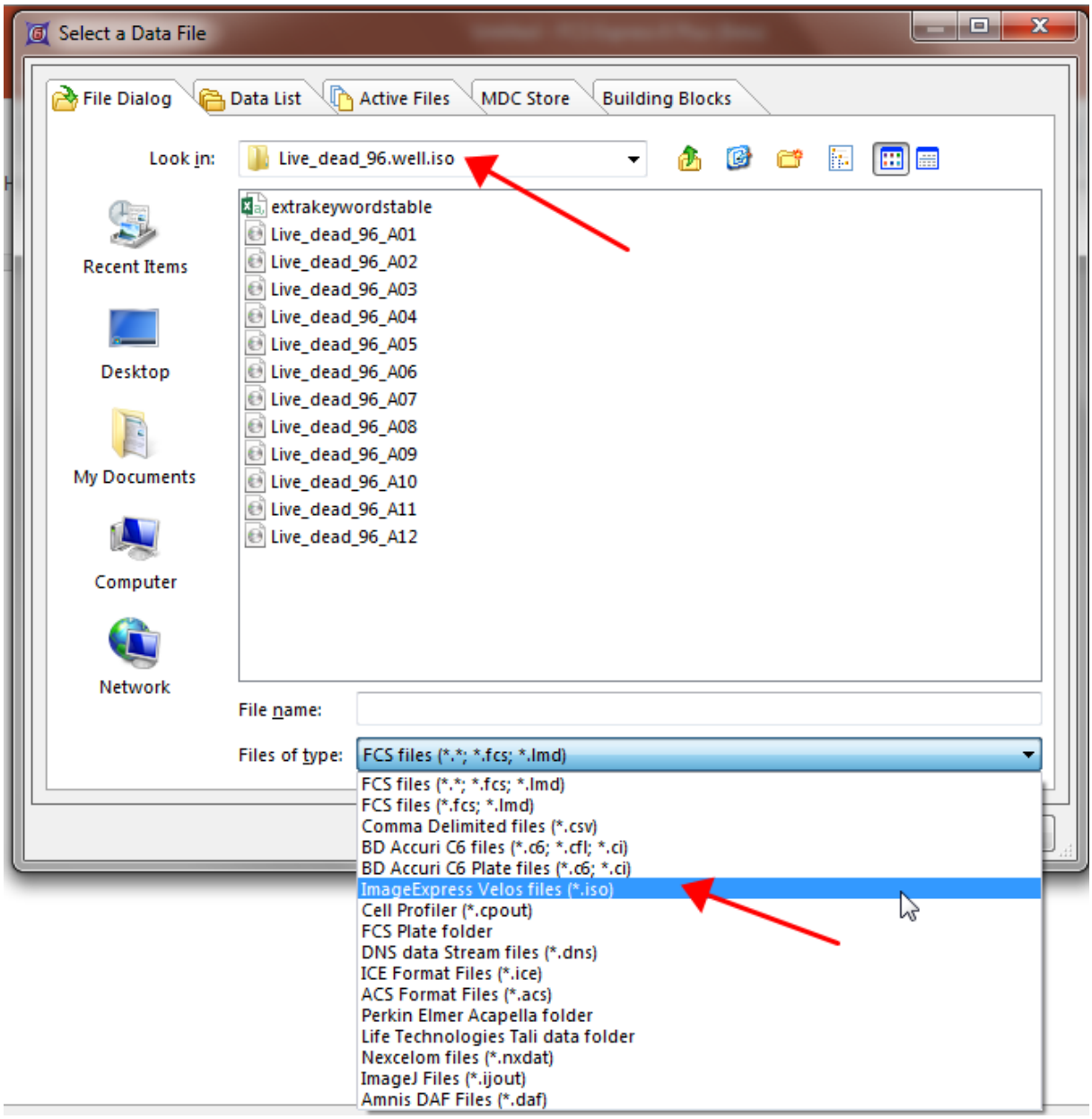

Figure T25.4 Choosing an ImageXpress Velos file for a Heat Map

8. Navigate to the **Sample Data→ImageXpress Velos With Images→Live_dead_96.well.iso** folder.
9. Highlight the **Live_dead_96_A01.iso** file in the list.
10. Click **Open**.

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A heat map containing your data set will appear indicating your import was successful (Figure T25.5). The data set we are using in this section only contains one row of samples.

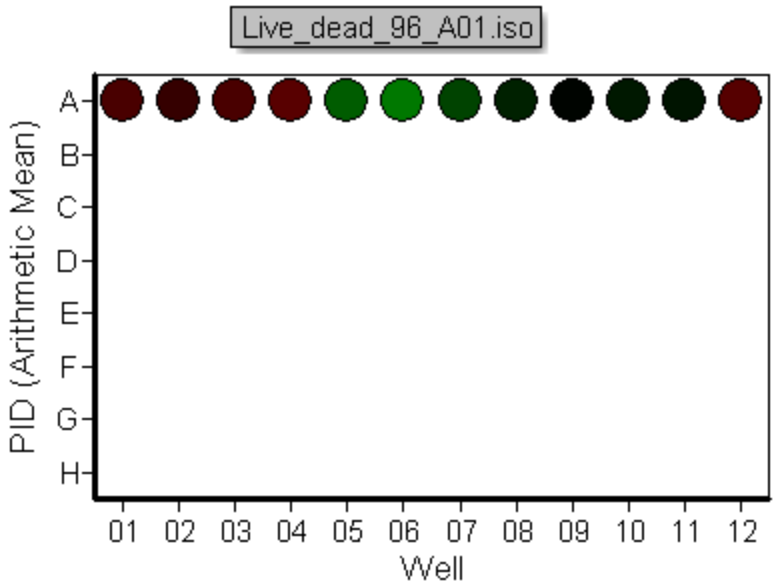

Figure T25.5 A Heat Map Generated from ImageXpress Velos Data

To view the images associated with each well

11. Insert a **Picture Plot** by choosing **Insert→2D Plots→Picture Plot** (Figure T25.6).

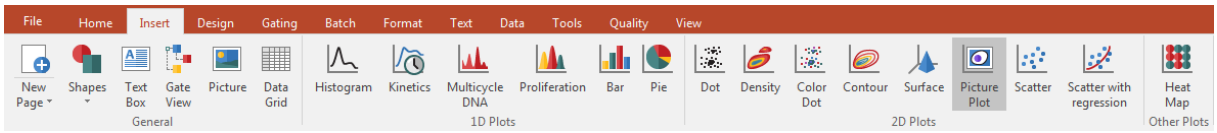

Figure T25.6 Insert a Picture Plot

12. Click anywhere on the layout.

A Picture Plot will appear on the layout (Figure T25.7). The picture plot is now displaying all the image from the first well in the plate. We will now create a well

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gate and apply it to the picture plot to display the image associated with a particular well.

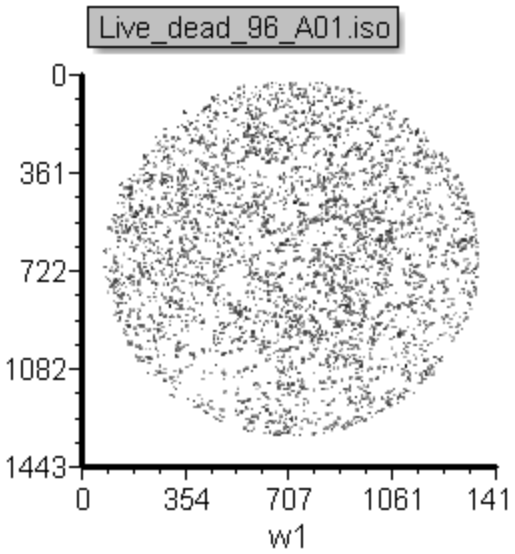

Figure T25.7 A Picture Plot displaying the image from the first well in the plate.

13. Create a Well Gate for well A6 on your **Heat Map** (Figure T25.8).

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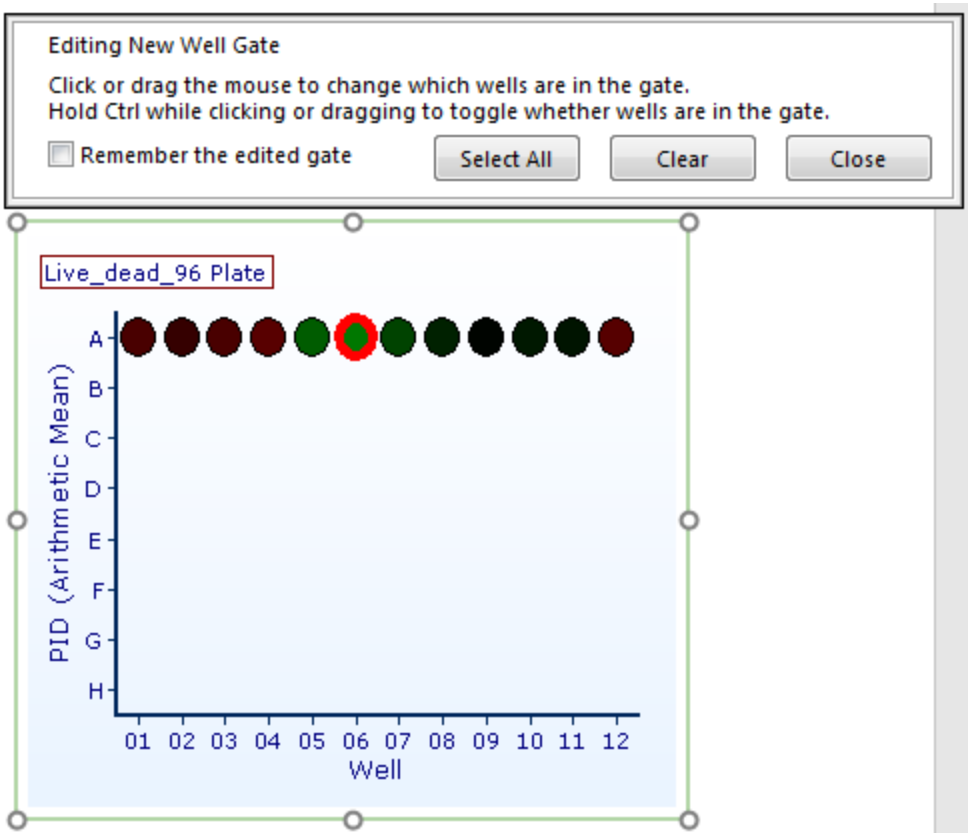

Figure T25.8 Gating a Heat Map

14. Apply the **Well Gate** from the last step to the **Picture Plot** (Figure T25.9). For more information about applying gates, see the Gating tutorial.

Picture plot should now looks as in Figure T25.9 (right panels).

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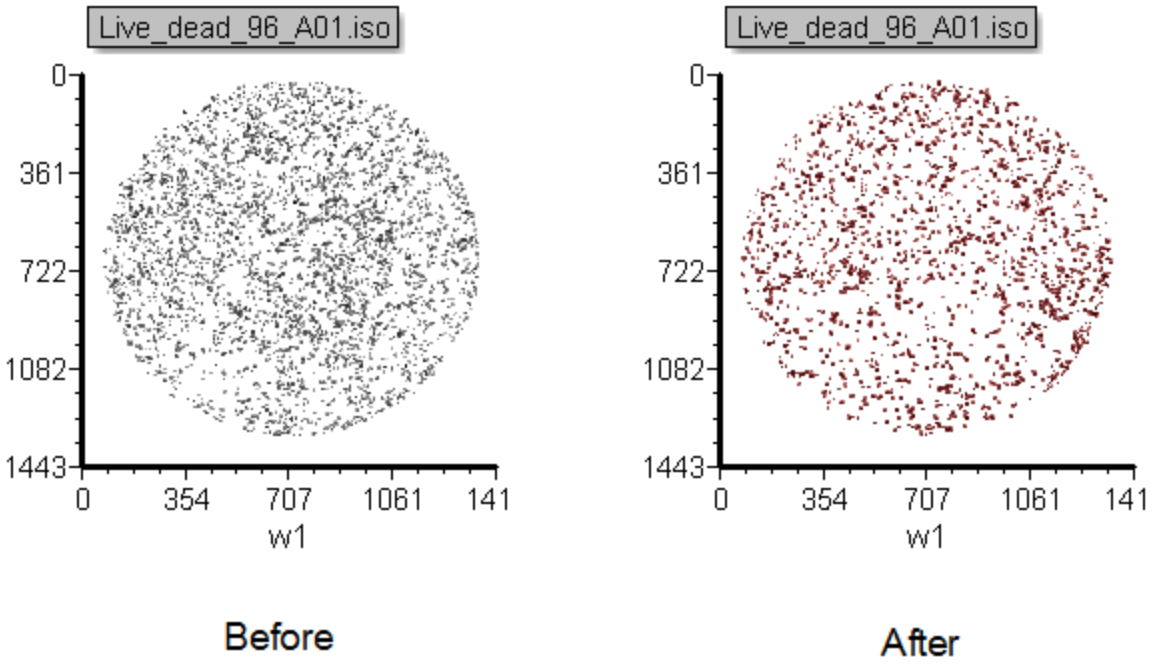

Figure T25.9 Picture Plot with Well Gate Applied

The image displayed in the **Picture Plot** is now based on the well gate that was defined. Editing the **Well Gate** and selecting different wells will change the image in the **Picture Plot**.

In the next section, we will set up **ImageXpress Velos** to export an experiment in numeric form 2059 (data only).

38.28.2. Import Numeric Form Data 1

After adjusting the export settings and analyzing in **ImageXpress Velos** you may proceed to import the data into FCS Express.

The ImageXpress Velos files used for this section are located in the Tutorial Sample Data archive 1210.

1. Select **File→Options→Data Loading→ImageXpress Velos Options**.
2. Unchecked the **Load images** check box.

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3. Click **OK**.

*Note: In this example, there are no images associated with the data. Therefore, the **Load Images** check box can remain checked or unchecked without affecting the import.*

The ImageXpress Velos data loading options should look like Figure T25.11 when you are done.

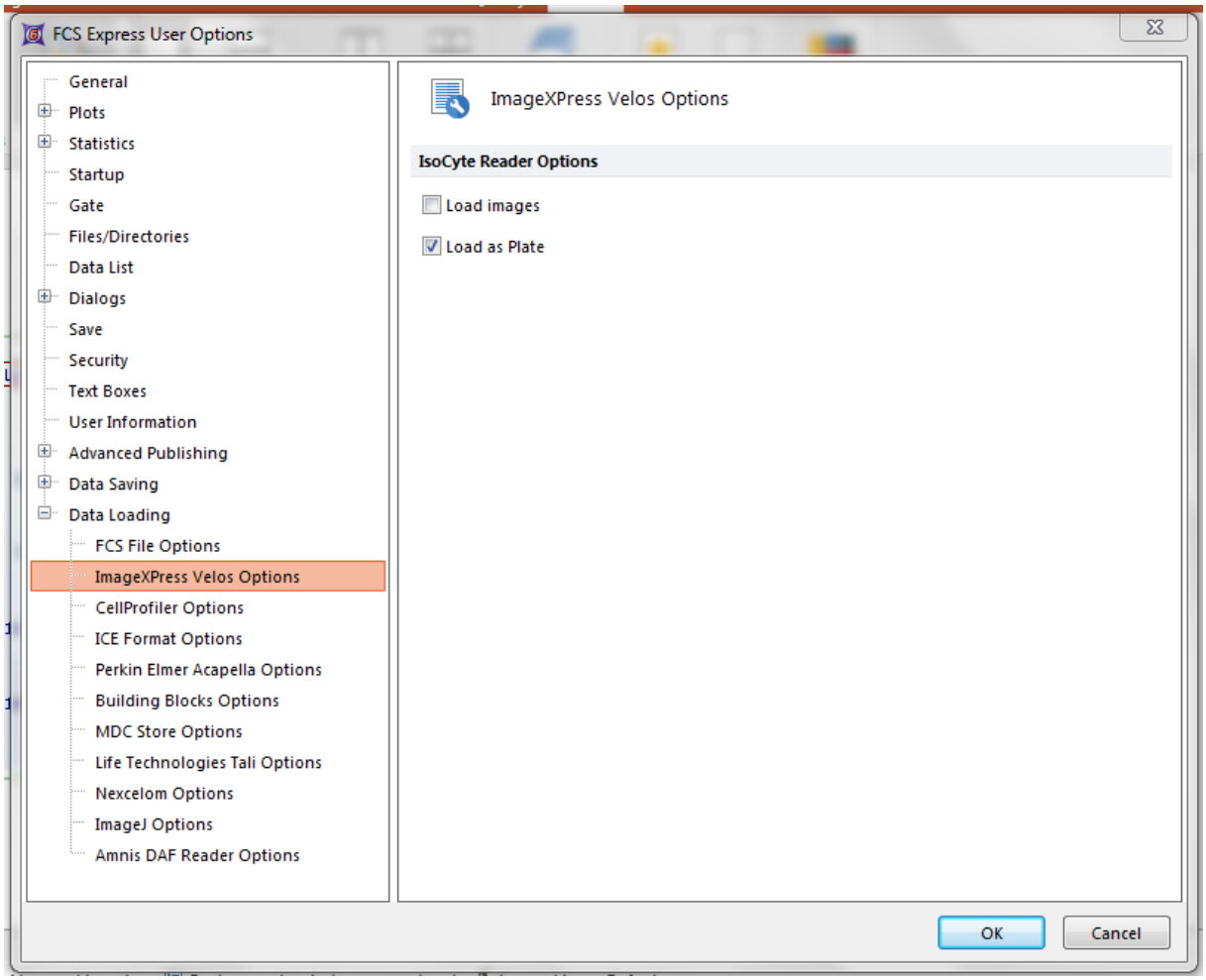

Figure T25.11 IsoCyte Data Loading Options for Numeric Data Only

4. Select the **Insert→Other Plots→Heat Map** command (Figure T25.12).

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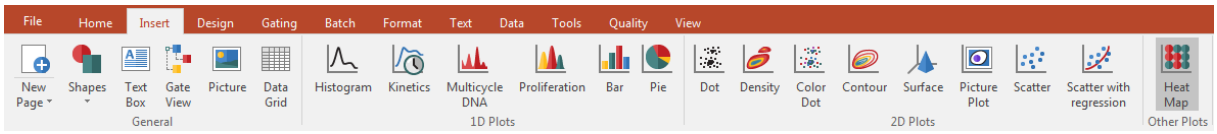

Figure T25.12 Insert a Heat Map

5. Click anywhere on the layout.

6. Choose **ImageXpress Velos files (*.iso)** from the **files of type:** drop-down list within the **Select a Data File** dialog (Figure T25.13).

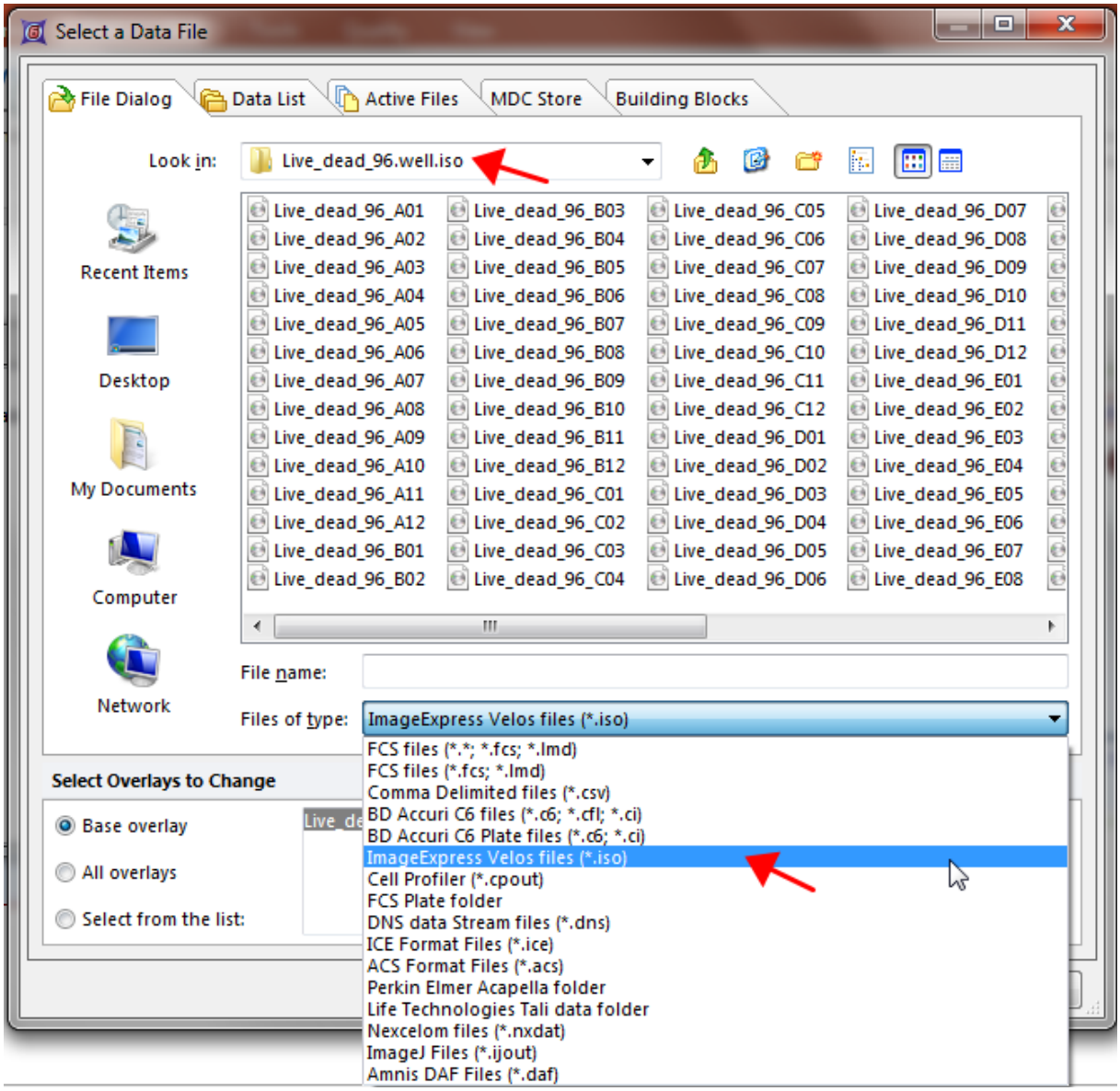

Figure T25.13 Choosing an IsoCyte File for a Heat Map

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7. Navigate to the **Sample Data→ImageXpress Velos Data Without Images→Live_dead_96.well.iso** folder.
8. Highlight the **Live_dead_96_A01.iso file** in the list.
9. Click **Open**.

A heat map containing your data set will appear indicating your import was successful (Figure T25.13b).

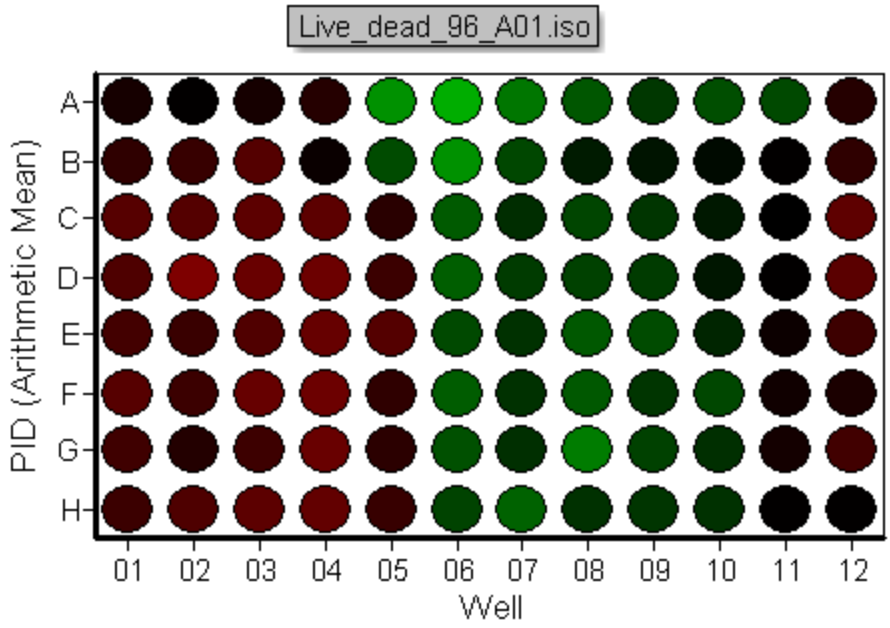

Figure T25.13 The Heatmap of the Live_dead_96_A01.iso file

To view the data associated with each well

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10. Insert a **2D Dot Plot** by choosing **Insert→2D Plots→Dot Plot** (Figure T25.14). *Note:* Histogram, Dot, Density, Color Dot, Contour, or Surface Plots can also be inserted.

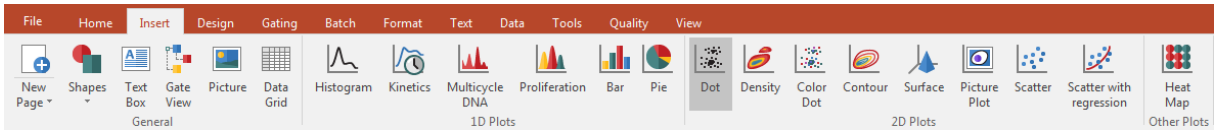

Figure T25.14 Insert a 2D Plot

11. Click anywhere on the layout.

A plot containing all of the events in the data set/**Heat Map** will appear (Figure T25.15).

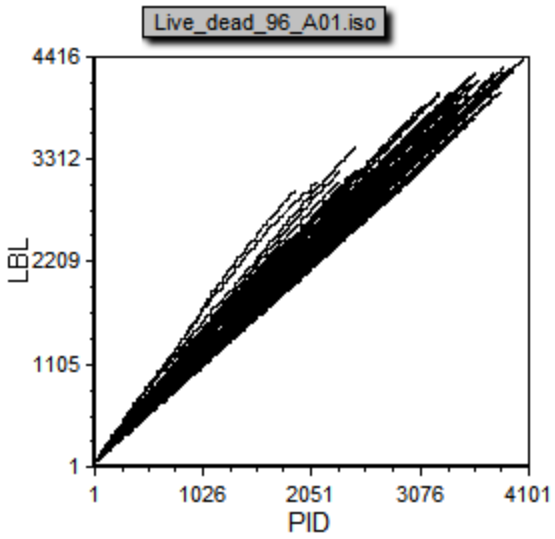

Figure T25.15 Dot Plot created from IsoCyte Data

Note: The plot is defaulting to PID vs LBL because those are the first two parameters in the data file.

In our example, we will change the axes to **I-MEAN1 vs I-MEAN3** to view relevant data (Figure T25.16).

12. Change the Y-Axis parameter to IMEAN-1.

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13. Change the X-Axis parameter to IMEAN-3.

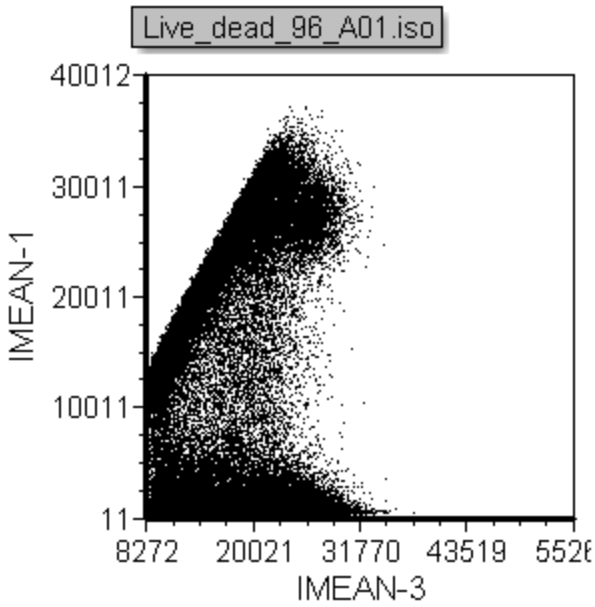

Figure T25.16 Changing the Plot Axes

14. Create a Well Gate for well C3 on your **Heat Map** (Figure T25.17).

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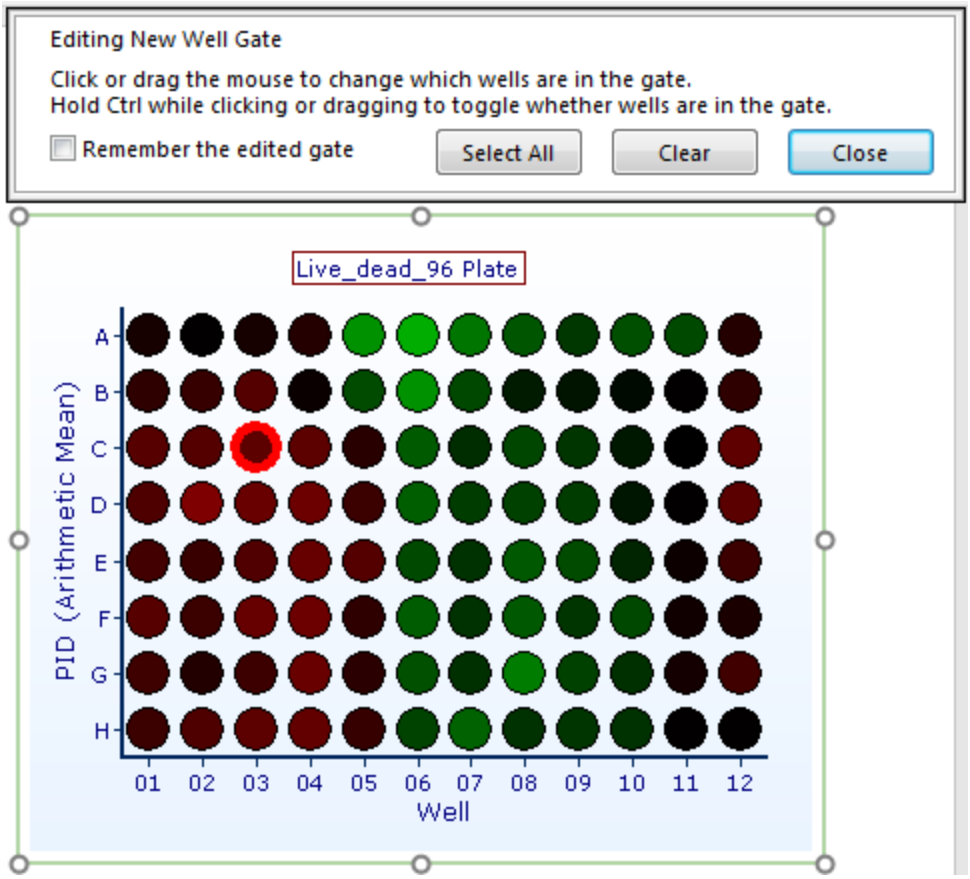

Figure T25.17 Create a Well Gate on the Heat Map

15. Apply the **Well Gate** from the last step to the **Dot Plot**.

The data displayed in the **Plot** is now based on the data within the well you gated (Figure T25.18). Editing the **Well Gate** and selecting different wells or multiple wells will change the data in the **Plot**.

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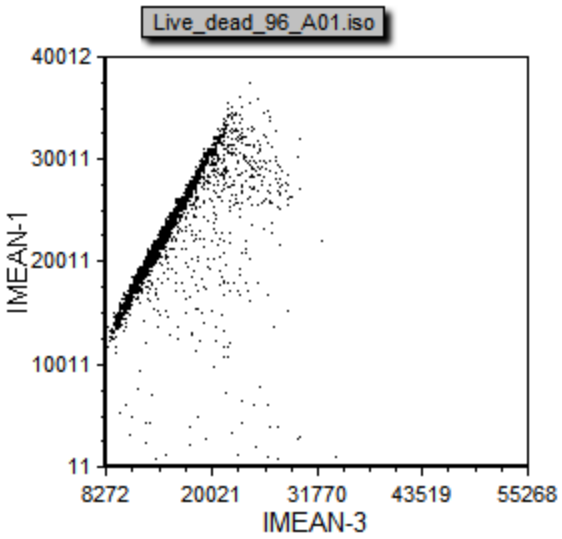

Figure T25.18 Dot Plot Gated by Well

38.28.3 Export in Numeric Form (Data Only)

ImageXpress Velos Setup – Saving Object Data

1. Select **Instrument Setup** from the main menu in the ImageXpress Velos software.
2. Click on the **Analysis Tab**.
3. Click the **Produce individual iso files** check box. This saves the analyzed object data for each well.
4. Ensure the **Auto. Correct Saturated Data** check box is checked.

The Instrument Setup dialog should look like Figure T25.10.

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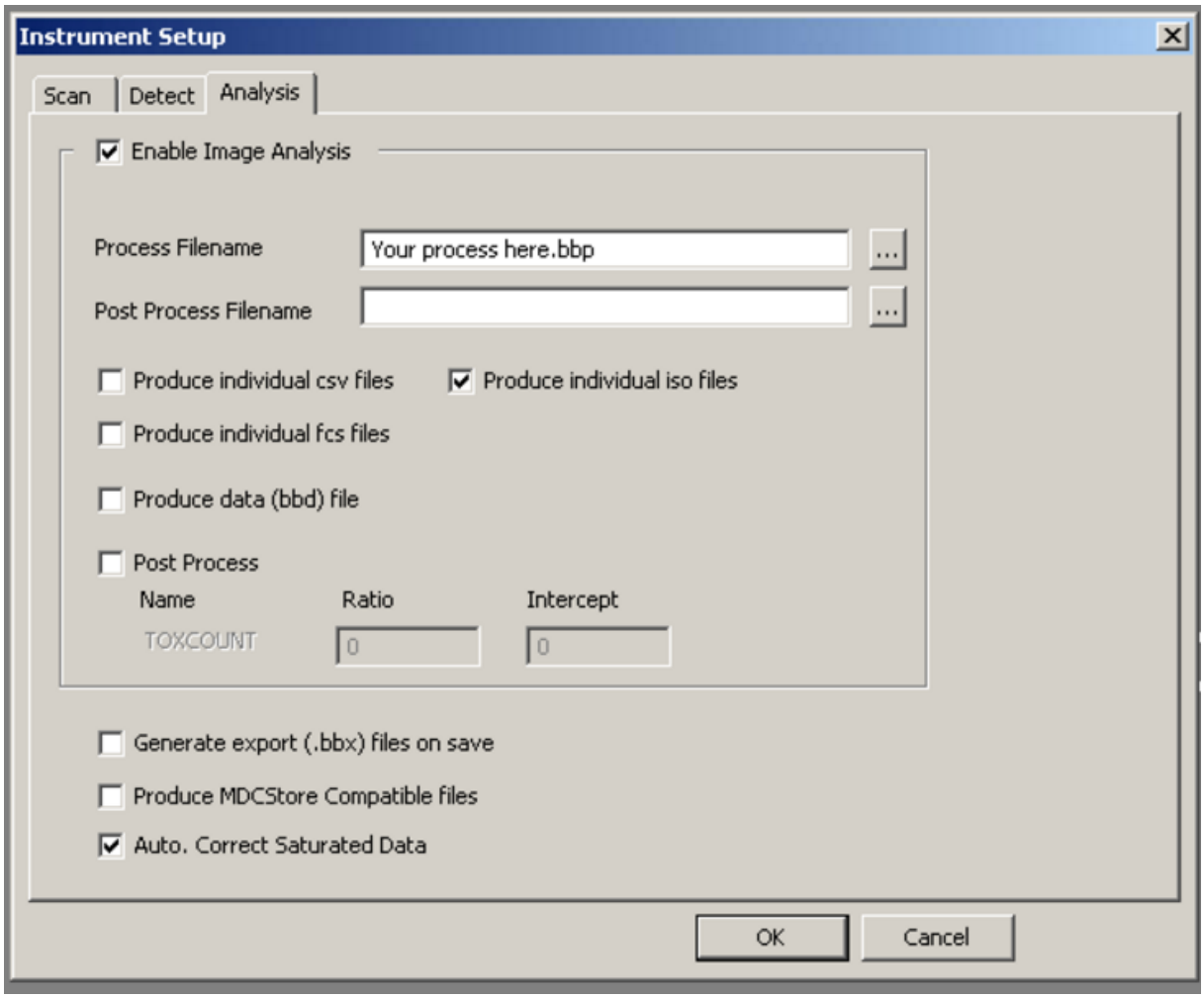

Figure T25.10 Instrument Setup for Data Only Exporting

5. Click **OK**.
6. Analyze your experiment in ImageXpress Velos.
7. Save your experiment in ImageXpress Velos.

Next, we will import ImageXpress Velos data in numeric form into FCS Express 2052.

38.29 Picture Plots

FCS Express now supports picture plots, which allow the user to import images for analysis. Picture plots display the imported images and allows the user to gate directly on an image to perform any downstream analysis available in FCS

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Express. Individual cells identified in a plot can further be isolated in a cell gallery, allowing the user to perform single cell analysis.

In the course of this tutorial, you will learn how to:

- Insert and format a picture plot
- Create multi-channel overlays to merge images
- Use gates with picture plots
- Generate statistics from picture plots
- Use cell galleries for gating and viewing individual cells

In the first exercise, we will insert and format picture plots.

38.29.1 Insert and Format Picture Plots

In the course of this section, you will learn how to:

- Define settings for viewing picture plots
- Choose the channel to display
- Create multi-channel overlays to create a merged image

Note: In this section we will be using files ending in the .dns file format. Picture plots may be inserted using a variety of file formats depending on your analysis instrument and export format.

1. Open a new layout.
2. Choose the **Data→Organize Data Sets→Data List** command. A **Data List** window will appear (Figure T9.3).
3. Click the blue plus to add files .
4. Choose **DNS data Stream files (*.dns)** from the **Files of type:** drop-down list

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5. Navigate to the FCS Express Tutorial Sample Data archive.

6. Select *pictureplotsample1.dns* and *pictureplotsample2.dns* and click **Open** to load them into the **Data List**. Now, the Data List should appear as in Figure T26.0.

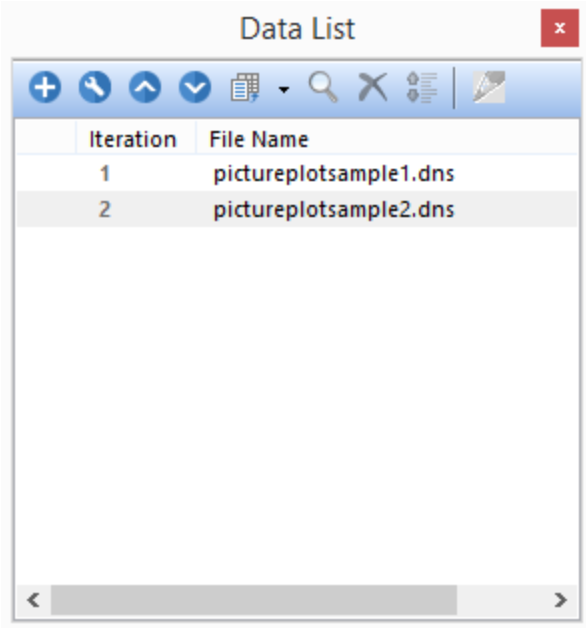

Figure T26.0 The Data List after file loading

5. Drag and drop *pictureplotsample1.dns* from the **Data List** to the layout.

6. Select **Picture Plot** in the **Select Plot Types...** dialog (Please ensure no other boxes are checked) (Figure T26.1).

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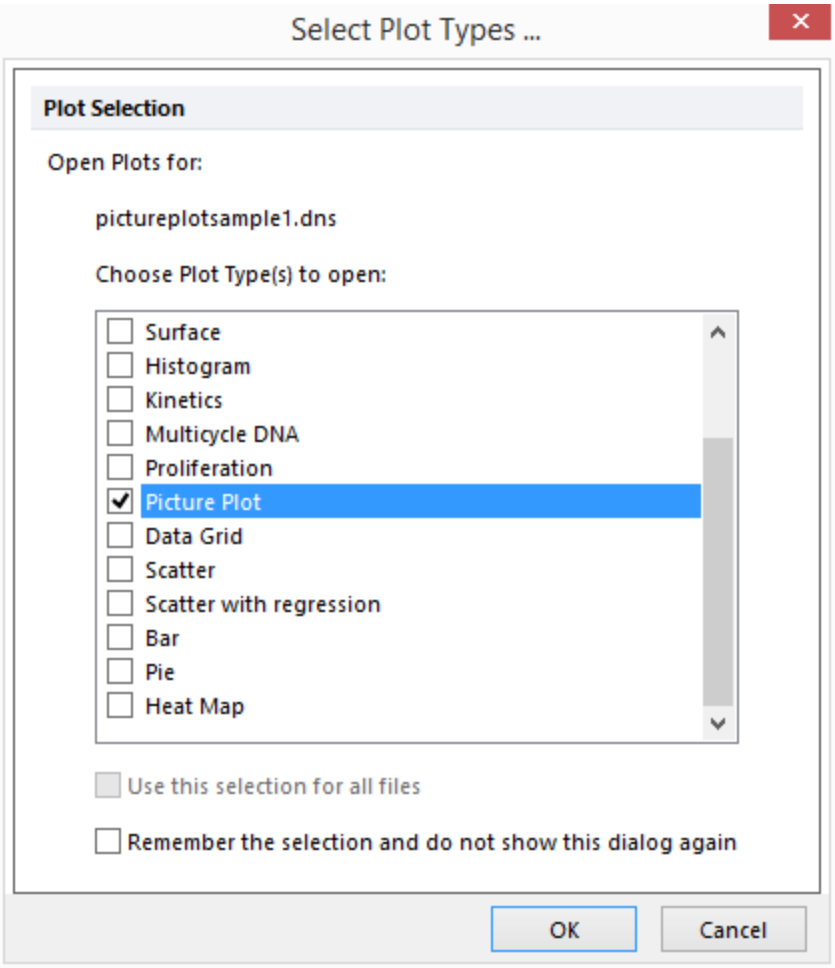

Figure T26.1 Choosing to Insert a Picture Plot

6. Click **OK**.

A Picture Plot will appear in the layout as in Figure T26.2.

We will now change the view background options to display only the masks for the individual cells and not the background.

Note: The labels on the axes reflect the pixel coordinates. You can turn these off by changing the axis formatting options.

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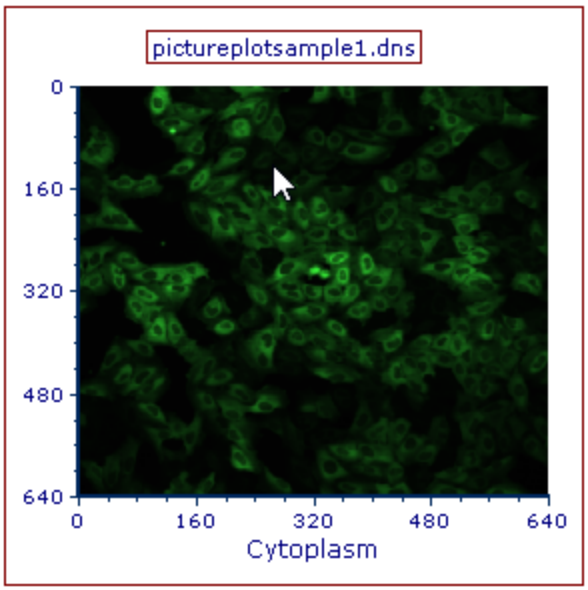

Figure T26.2 Picture Plot for pictureplotsample1.dns Inserted in the Layout

7. Right-click on the picture plot.
8. Choose **Format** from the pop-up menu.
9. Choose the **Overlays** category.
10. Uncheck **Show Background Image** (Figure T26.3).

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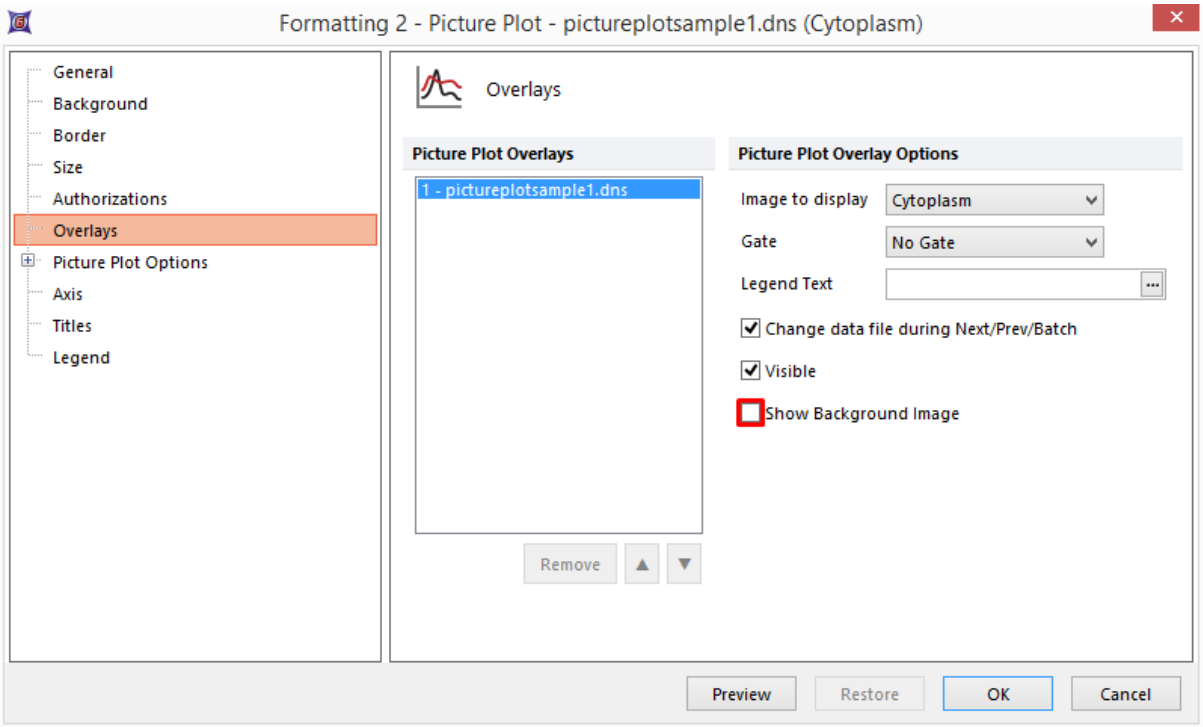

Figure T26.3 Deselecting the Show Background Images Option

11. Click **Preview**.

Move the formatting window aside so you can see the picture plot. Notice how the black background has been removed and only individual cells are now displayed (Figure T26.4).

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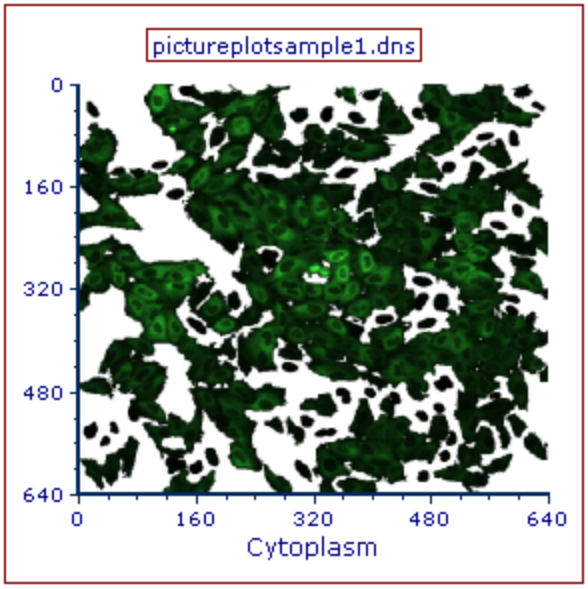

Figure T26.4 Before (Left) and After (Right) Removing the Background Image

We will now look at the other channel associated with the image and create a two channel merged image.

12. Click **Cancel** to revert to the image with background.

13. Copy and paste the picture plot anywhere in the layout.

14. Click the X-Axis label of the new plot (Figure T26.5).

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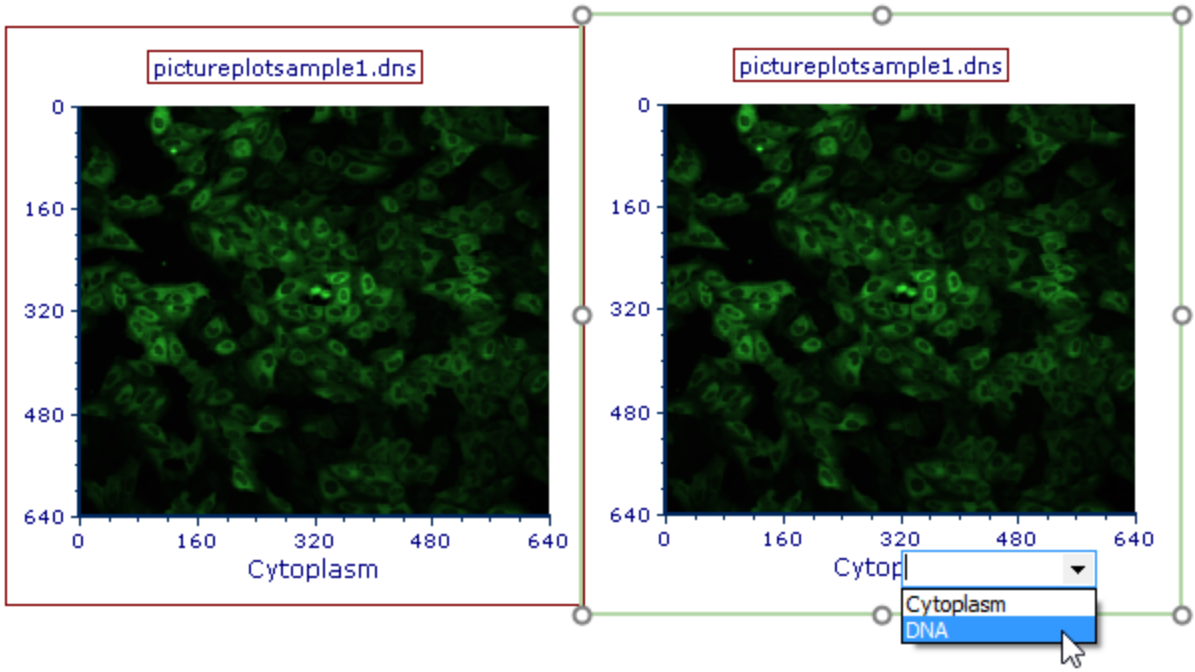

Figure T26.5 Choosing the DNA Imaging Channel

15. Choose **DNA** to view the image of the DNA channel (Figure T26.5).

The picture plot will now look like Figure T26.6, displaying the DNA image associated with the picture plot.

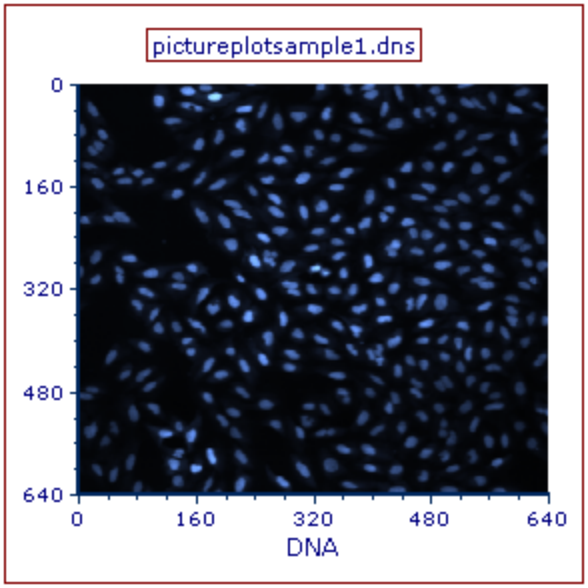

Figure T26.6 The DNA Image Channel Associated with pictureplotsample1.dns

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16. Drag and drop the plot displaying the DNA channel on top of the plot displaying the cytoplasm channel.

17. Choose **2D Plot Overlay(s)** from the **Paste Special** dialog.

18. Click **OK**.

The picture plot will now look like Figure T26.7, displaying the DNA channel overlaid on the Cytoplasm channel to create a merged image.

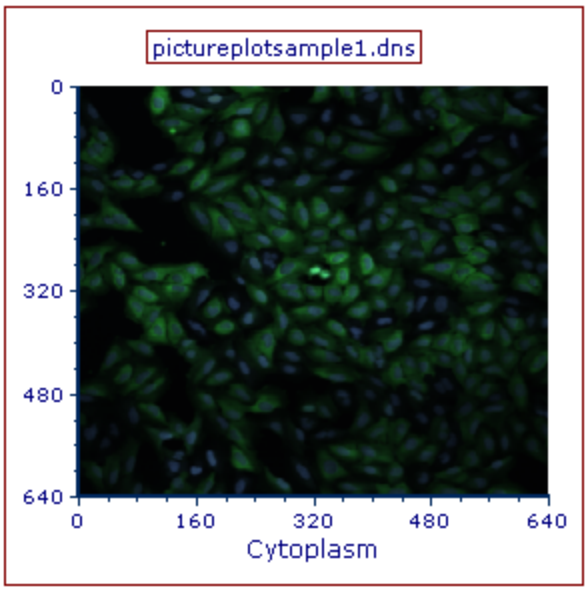

Figure T26.7 Picture Plot Overlay Displaying the Cytoplasmic and DNA Channels as a Merged Image

Just like any other plot in FCS Express, data from picture plots can be scrolled through using the **Next** and **Previous** commands. We will now change the image being displayed in the picture plot with these commands.

19. Select the newly overlaid picture plot by clicking on it.

20. Choose the **Data→Change Data on Selection→Next** command (Figure T26.8).

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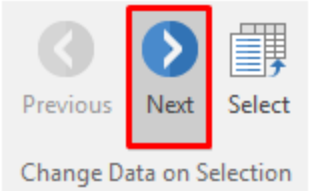

Figure T26.8 The Next button

The picture plot will change to display the next sample/image in the **Data List**. FCS Express can handle color and grey scale images (as seen with the grey scale images associated the pictureplotsample2.dns).

In the next section, we will learn about gating with picture plots 2069.

38.29.2 Gating with Picture Plots

In the course of this section, you will learn how to:

- Gate on a picture plot
- Adjust picture plot options for viewing gates
- Backgate from picture plots to scatter plots and from scatter plots to picture plots
- View statistics from picture plot gates

Note: In this section we will be using files ending in the .dns file format. Picture plots may be inserted using a variety of file formats depending on your analysis instrument and export format.

Gating on and applying a gate to a picture plot is similar to using a gate on any other plot in FCS Express. For an introduction to gating please see the Gating tutorial 1266.

1. Open a new layout.
2. Choose the **Data→Organize Data Sets→Data List** command. A **Data List** window will appear.

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3. Click the blue plus to add files .
4. Choose **DNS data Stream files (*.dns)** from the **Files of type:** drop-down list.
5. Navigate to the FCS Express Tutorial Sample Data archive.
6. Select *pictureplotsample1.dns.*
7. Click **Open** to load it into the **Data List**.
8. Drag and drop pictureplotsample1.dns from the **Data List** to the layout.
9. Select **Color Dot** and **Picture Plot** in the **Select Plot Types...** dialogue (Please ensure no other boxes are checked) (Figure T26.8).

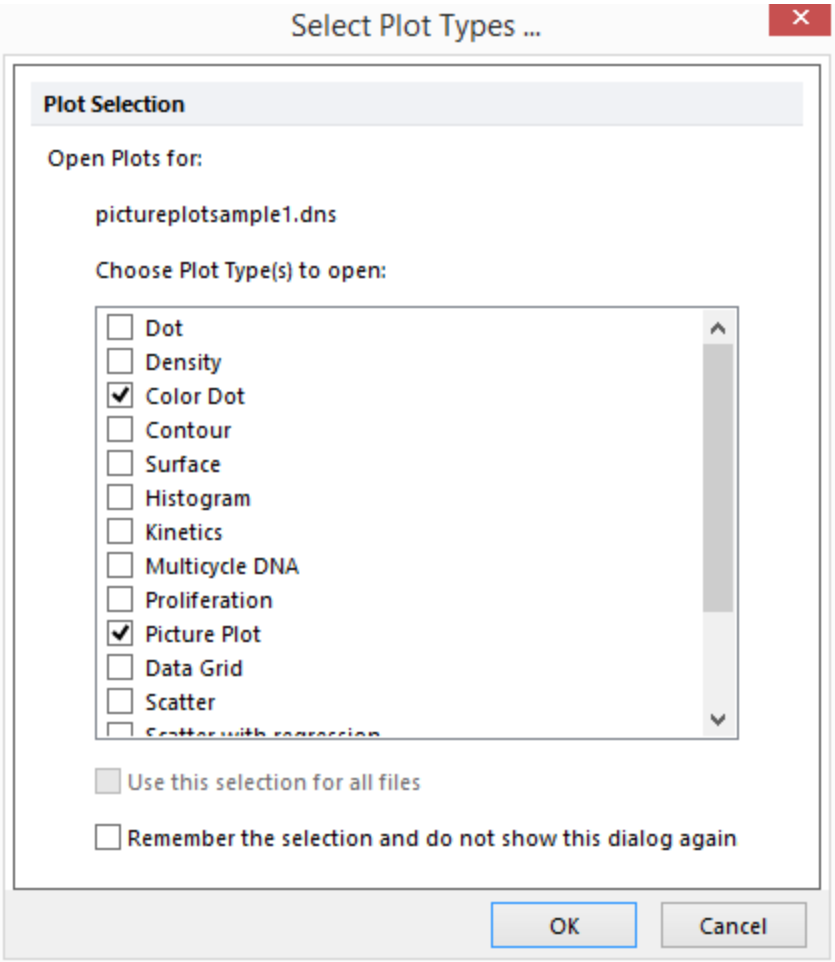

Figure T26.8 Choosing to Insert a Picture Plot and Color Dot Plot

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6. Click **OK**.

A color dot plot and a picture plot for the file will appear on the layout. Arrange the plots side by side as in Figure T26.9.

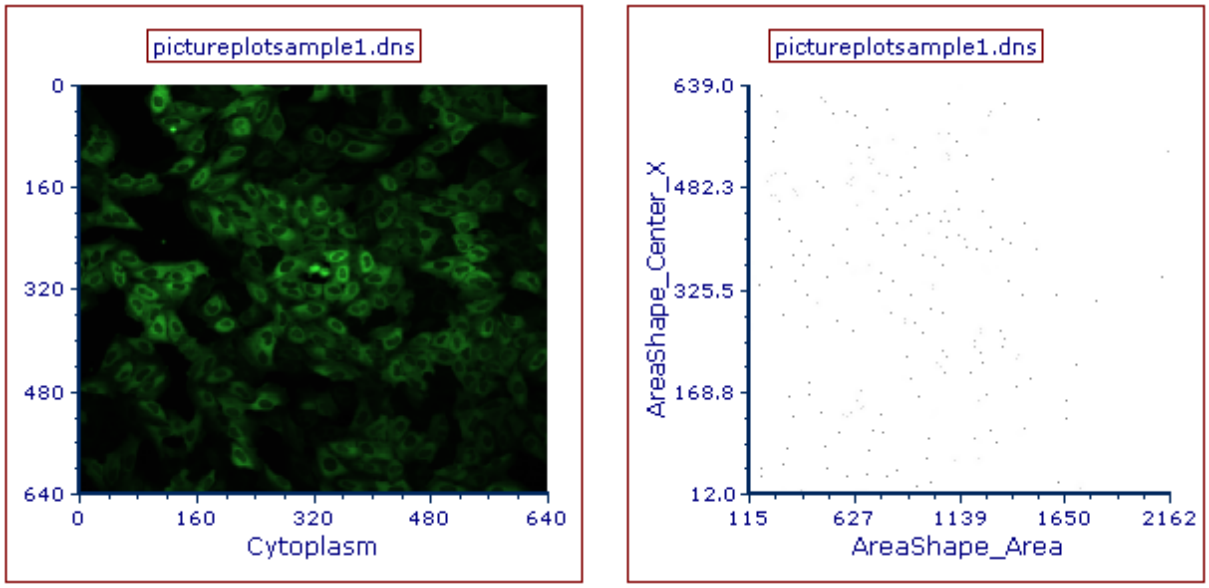

Figure T26.9 Arrangement of the Picture Plot and Color Dot Plot on the Layout

7. Create a rectangle gate on the picture plot similar to the one in Figure T26.10 and name it **Picture Plot Gate 1**.

8. Create another gate (ellipse gate) on the picture plot similar to the one in Figure T26.10 and name it **Picture Plot Gate 2**.

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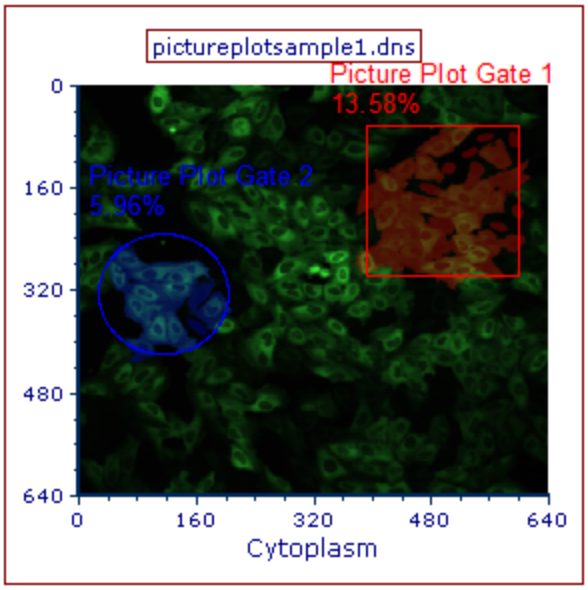

Figure T26.10 Creating Gates on the Picture Plot

Notice how the cells on the picture plot that fall within the gate are highlighted the same color as the gate (Figure T26.10). Also note that the color dot plot is now displaying the cells that fall within picture plot gates in different colors. At times, it may be difficult to differentiate colors or events on the dot plot due to the nature of a single image experiment having a limited number of events. We will now format the dot plot to make it easier to view and analyze.

9. Right-click on the color dot plot.
10. Choose **Format** from the pop-up menu.
11. Choose the **Overlays** category.
12. Change the **Dot size** to 5 (Figure T26.10b).

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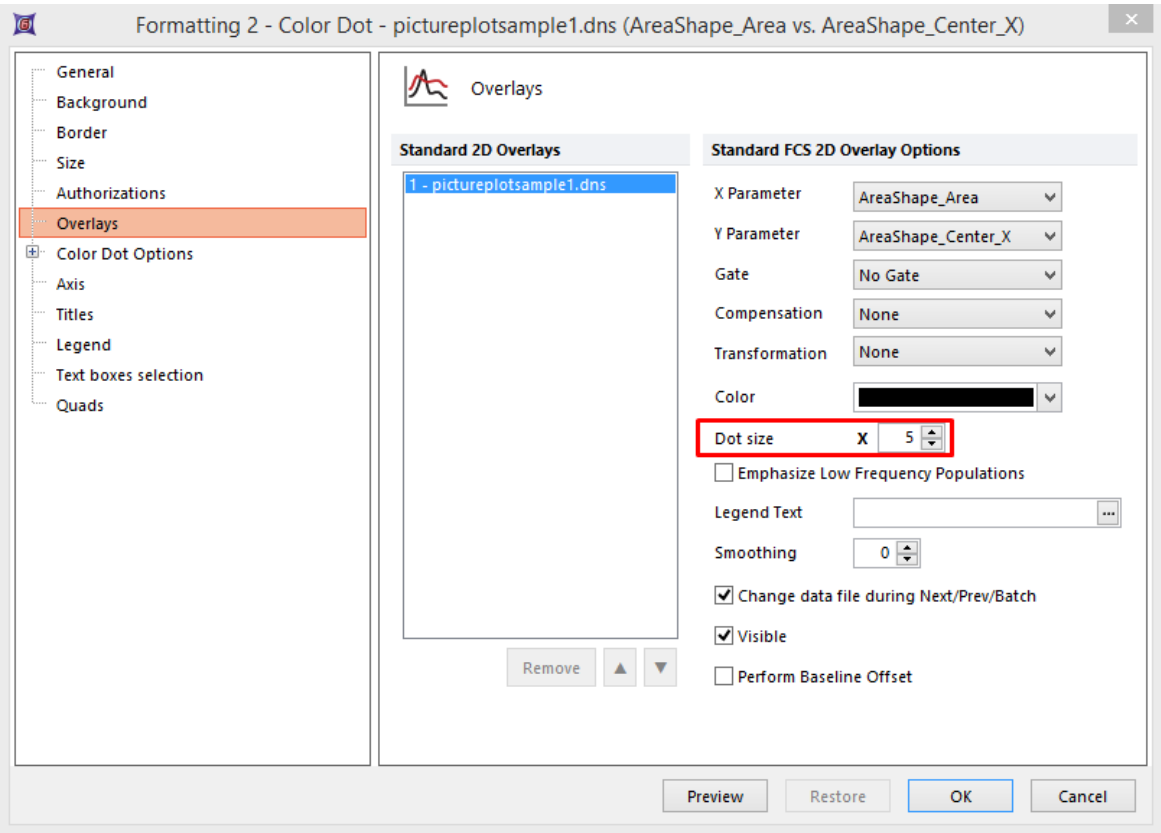

Figure T26.10b Formatting Dot Size

13. Click **OK**.

The dot plot will now look like Figure T26.11 and the events that fall within each picture plot gate are more obvious.

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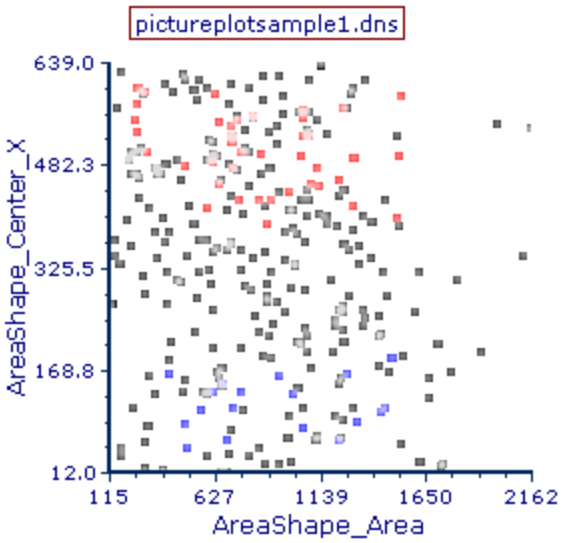

Figure T26.11 Color Dot Plot with Dots Enlarged

We will now format the picture plot to only highlight cells that fall within one of the two picture plot gates.

14. Right-click on the picture plot.
15. Choose **Format** from the pop-up menu.
16. Choose the **Picture Plot Options→Display Gates Options** category.
17. Choose the **All gates except those checked below** radio button (Figure T26.12).
18. Check the box for **Picture Plot Gate 2** (Figure T26.12).

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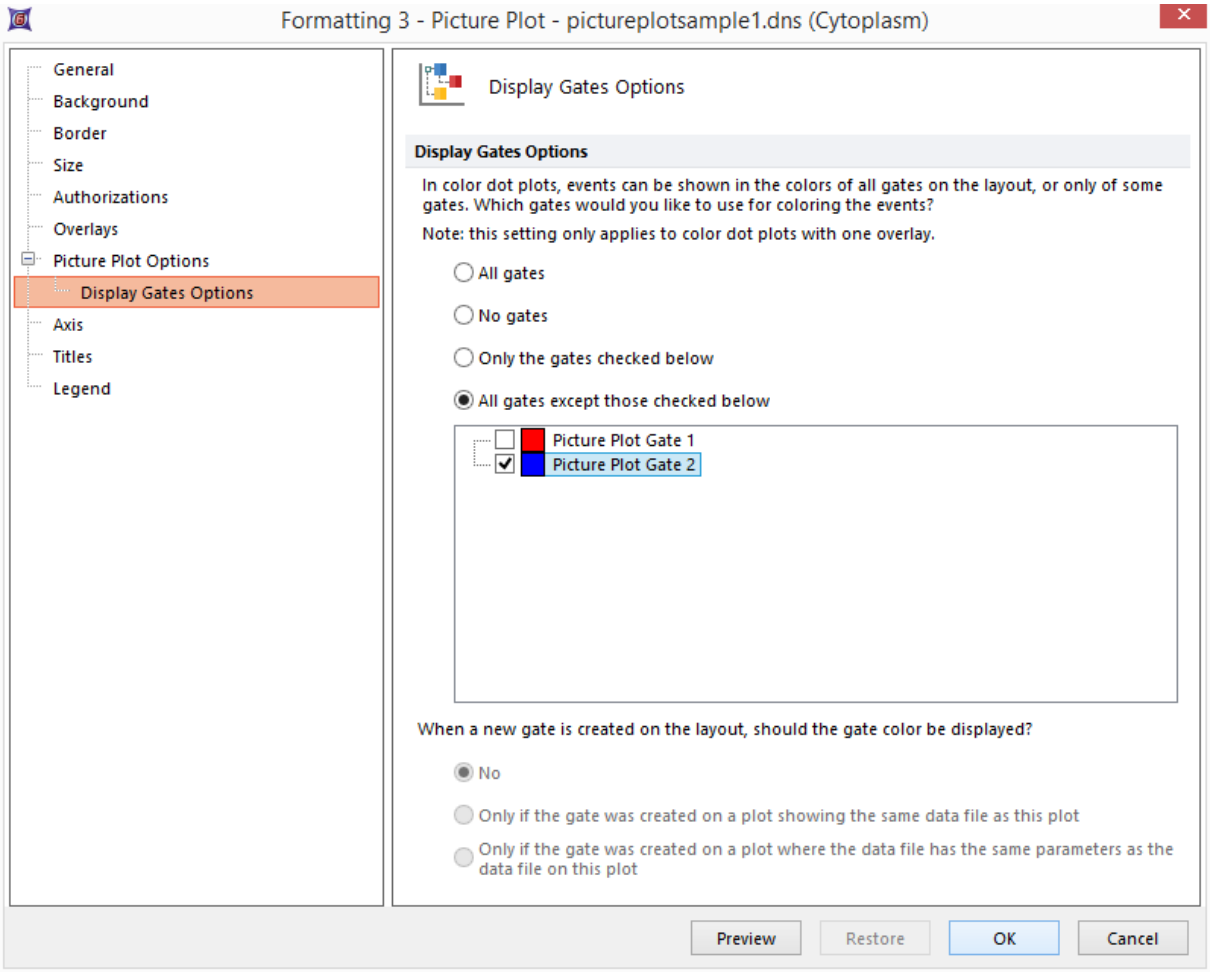

Figure T26.12 Choosing to Display All Gates Except Picture Plot Gate 2

19. Click **OK**.

The picture plot will now look like Figure T26.13, only highlighting the cells found within Picture Plot Gate 1.

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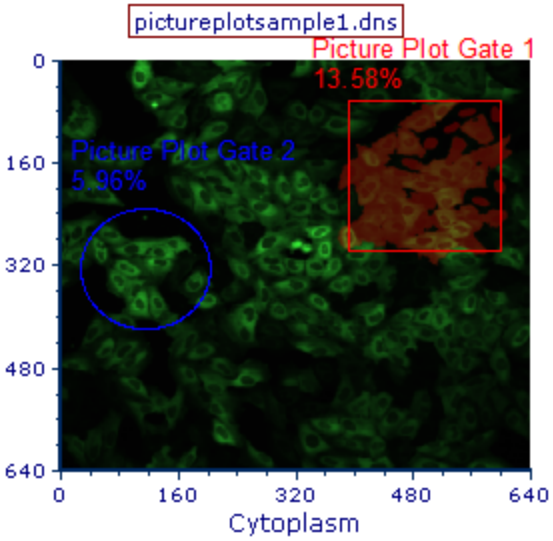

Figure T26.13 Picture Plot Highlighting Only Cells Found Within Picture Plot Gate 1

We will now gate the color dot plot based on the picture plot gates.

20. Click on the color dot plot to select it.

21. Navigate to **Gating→Create Gates→Current Gate** and choose **Picture Plot Gate 1** from the **Current Gate** drop-down list (Figure T26.13b).

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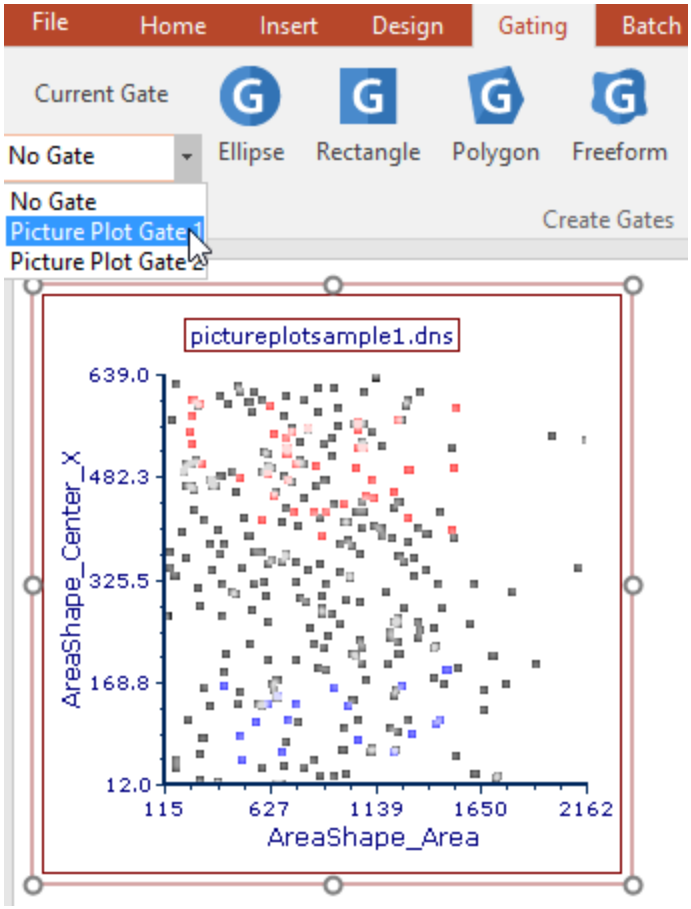

FIgure T26.13b Applying Picture Plot Gate 1

Notice how the color dot plot has changed to only display events that fall within the gate defined on the picture plot (Figure T26.14).

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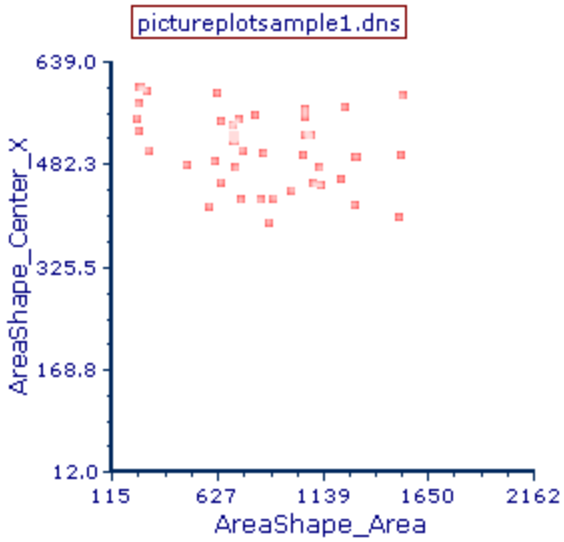

Figure 27.14 Color Dot Plot Displaying Cells from Picture Plot Gate 1

We will now set a gate on the color dot plot and apply it to the picture plot.

22. Press **Ctrl+Z** to undo the gating change (The plot will look as in Figure T26.11).

23. Create a rectangle gate on the color dot plot similar to Figure T26.15 and name it **Dot Plot Gate**.

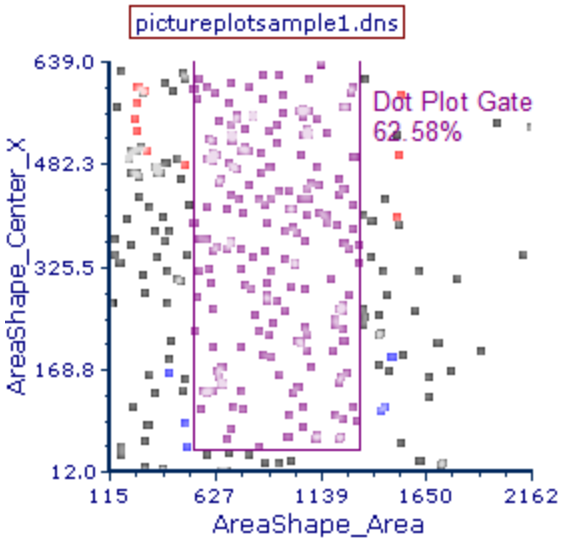

Figure T26.15 Applying a Gate to the Dot Plot

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Notice how the picture plot is now highlighting cells in the color of the dot plot gate that was just created (Figure T26.16). Adjust the Dot Plot gate and watch as the highlighting changes on the picture plot to indicate events in the gate. This gating strategy may be particularly useful for identifying interesting populations of cells in an image based on numerical results.

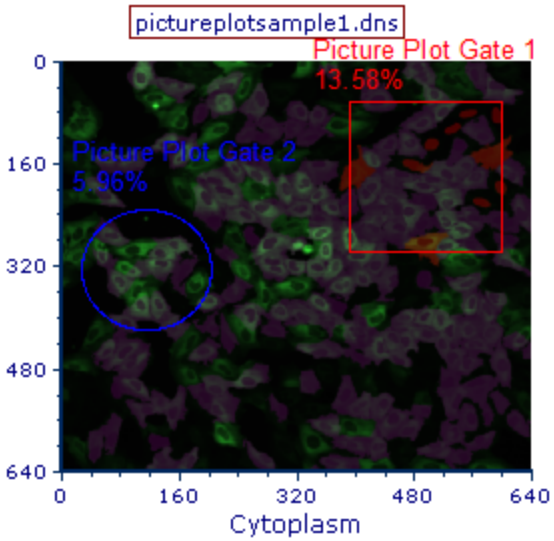

Figure T26.16 Picture Plot Displaying the Dot Plot Gated Cells in Purple

The individual cells that fall within the dot plot gate can also be shown in the picture plot by removing the background image.

24. Right-click on the picture plot.
25. Choose **Format** from the pop-up menu.
26. Choose the **Overlays** category.
27. Uncheck **Show Background Image** (Figure T26.3 2061).
28. Click **OK**.
29. Navigate to **Gating→Create Gates→Current Gate** and choose **Dot Plot Gate** from the **Current Gate** drop-down list.

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The picture plot will now look similar to Figure T26.17, displaying only the cells found within the Dot Plot Gate. Try adjusting the Dot Plot Gate and watch as the cells found within the gate update in real time.

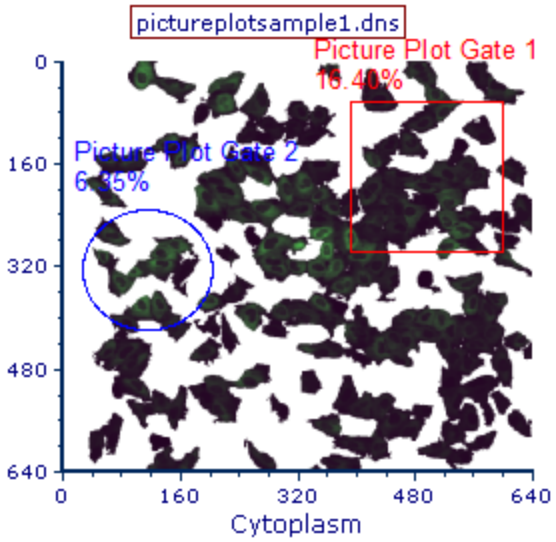

Figure T26.17 Picture Plot with Dot Plot Gate Applied

Next, we will use data grids for image analysis 2080.

38.29.3 Using Data Grids for Analysis

FCS Express allows the user to insert a data grid for any plots. Data grids typically display cell-by-cell parameter data. In the context of image cytometry, the data grid can be used as an image gallery to view images of individual cells and their associated parameters.

In the course of this section, you will learn how to:

- Insert a data grid/cell gallery
- Format a data grid/cell gallery to display individual cell images and parameters
- Apply gates to a data grid/cell gallery
- Create gates in a data grid/cell gallery

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1. Open a new layout.
2. Choose the **Data→Organize Data Sets→Data List** command. A **Data List** window will appear (Figure T9.3 1445).
3. Click the blue plus to add files .
4. Choose **DNS data Stream files (*.dns)** from the **Files of type:** drop-down list (Figure T18.17 1758).
5. Navigate to the FCS Express Tutorial Sample Data archive 1210.
6. Select *pictureplotsample1.dns.*
7. Click **Open** to load it into the **Data List**.
8. Drag and drop pictureplotsample1.dns from the **Data List** to the layout.
9. Select **Color Dot**, **Picture Plot** and **Data Grid** in the **Select Plot Types...** dialogue (Please ensure no other boxes are checked) (Figure T26.1).

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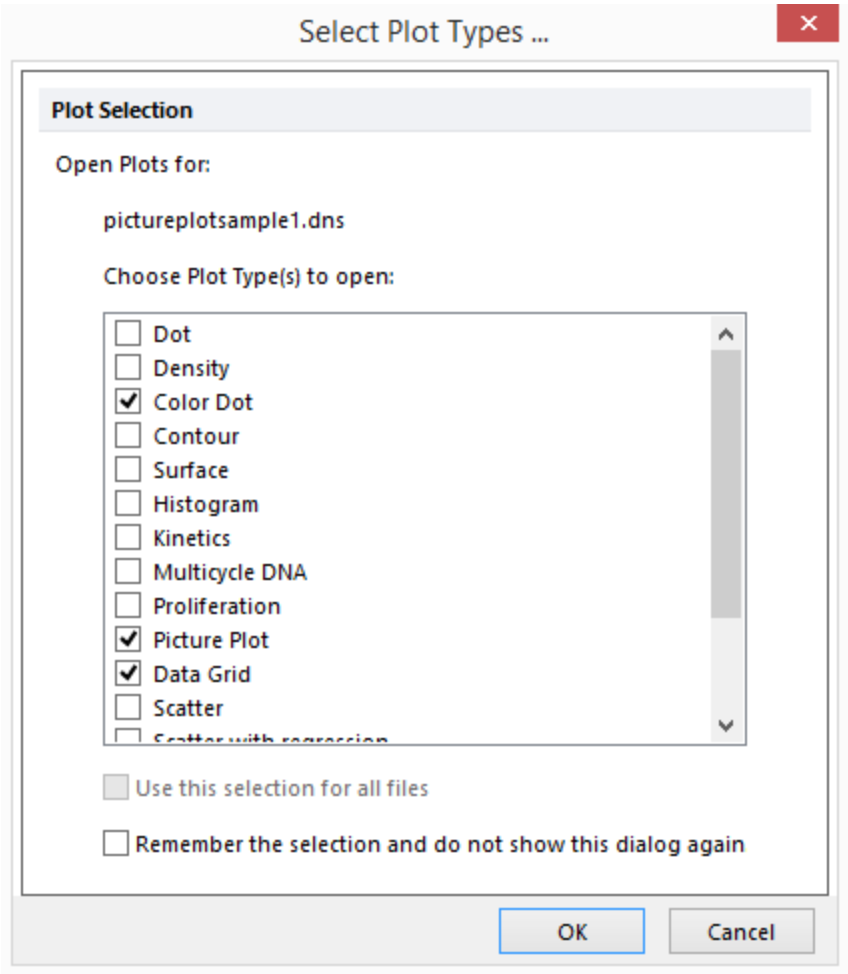

Figure T26.18 Inserting a Data Grid and Two Plots

10. Click **OK**.

A Picture plot, data grid, and color dot plot will now appear on the layout. Arrange these plots and grids as in Figure T26.19.

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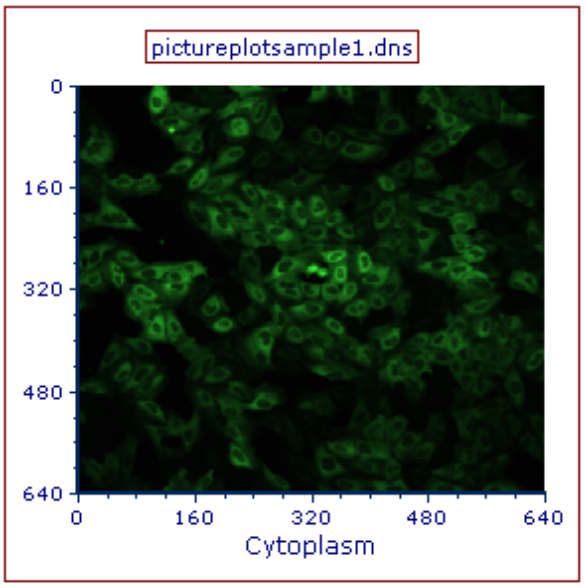

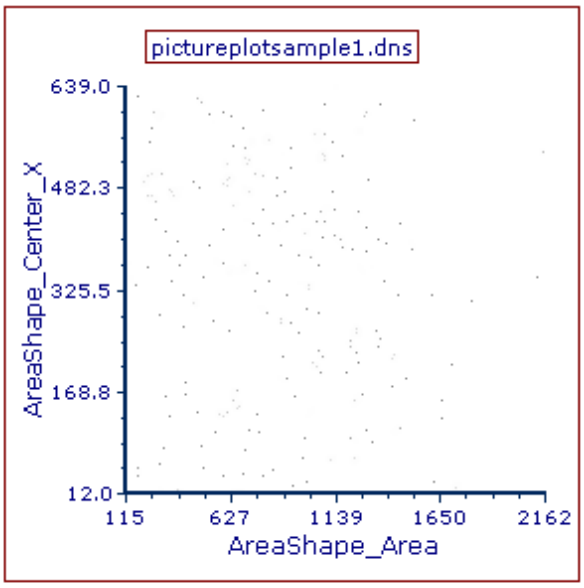

Cell #	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_
1	551.00	368.00	14.00	1.21	0.74	1.00	0.59	0.49
2	695.00	483.00	5.00	1.24	0.81	1.00	0.62	0.61
3	483.00	152.00	11.00	1.27	0.83	1.00	0.58	0.56
4	381.00	606.00	11.00	1.11	0.64	1.00	0.55	0.34
5	371.00	399.00	17.00	1.28	0.83	1.00	0.59	0.33
6	1541.00	190.00	26.00	1.41	0.72	1.00	0.40	0.24
7	1090.00	565.00	16.00	1.26	0.82	1.00	0.58	0.47
8	1296.00	109.00	23.00	1.07	0.67	1.00	0.72	0.69
9	626.00	498.00	26.00	1.07	0.49	1.00	0.59	0.60
10	2162.00	537.00	36.00	1.16	0.77	1.00	0.61	0.46
11	659.00	144.00	36.00	1.23	0.75	1.00	0.48	0.49
12	172.00	624.00	32.00	1.34	0.88	1.00	0.57	0.66
13	870.00	421.00	37.00	1.33	0.84	1.00	0.55	0.44
14	641.00	165.00	43.00	1.24	0.73	1.00	0.56	0.44
15	1250.00	228.00	38.00	1.30	0.85	1.00	0.60	0.57

Figure T26.19 A Picture Plot, Data Grid, and Color Dot Plot Arranged on the Layout for Analysis

The data grid can be used as a cell gallery to view individual cells from the picture plot and gates. We will now format the grid to show cell cutouts, a few parameters, and gates from the data.

11. Right-click on the data grid.

12. Choose **Format** from the pop-up menu.

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13. Choose the **Parameters to Display** category (Figure T26.20).
14. Click the **Only the items check below** radio button (Figure T26.20).
15. Check the following boxes found by scrolling to the bottom of the list (Figure T26.20):
    a. Intensity_UpperQuartileIntensity_DNA
    b. Number_Object_Number
    c. Cytoplasm
    d. DNA

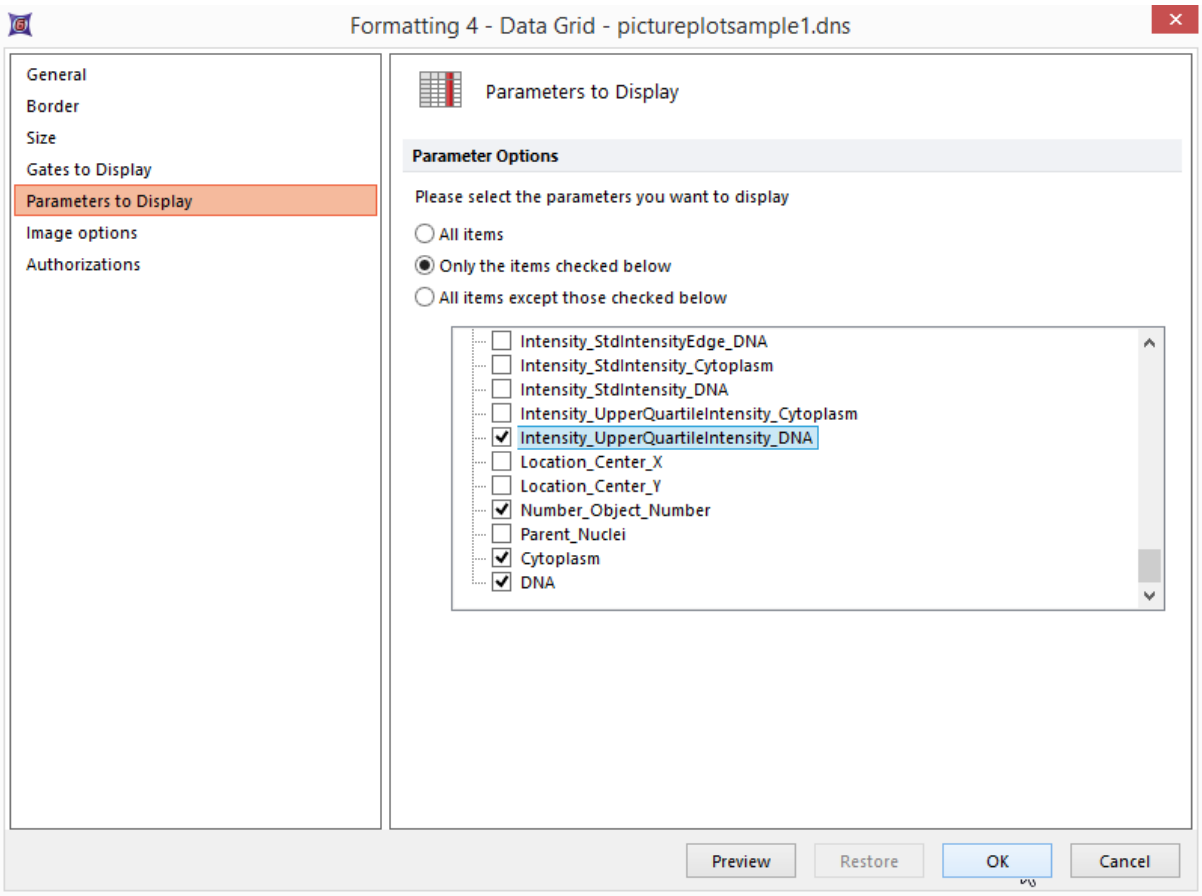

Figure T26.20 Formatting the Parameters to Display for the Data Grid

16. Choose now the **Gates to Display Category**.
17. Click on the **All gates** radio button.

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18. Click **OK**.

The data grid is now displaying parameter values from each cell, a cutout of the Cytoplasmic and DNA image of each cell, and a column for Gates (Figure T26.21).

We will now create gates on the dot plot and picture plot, and use these gates to view individual events within the data grid.

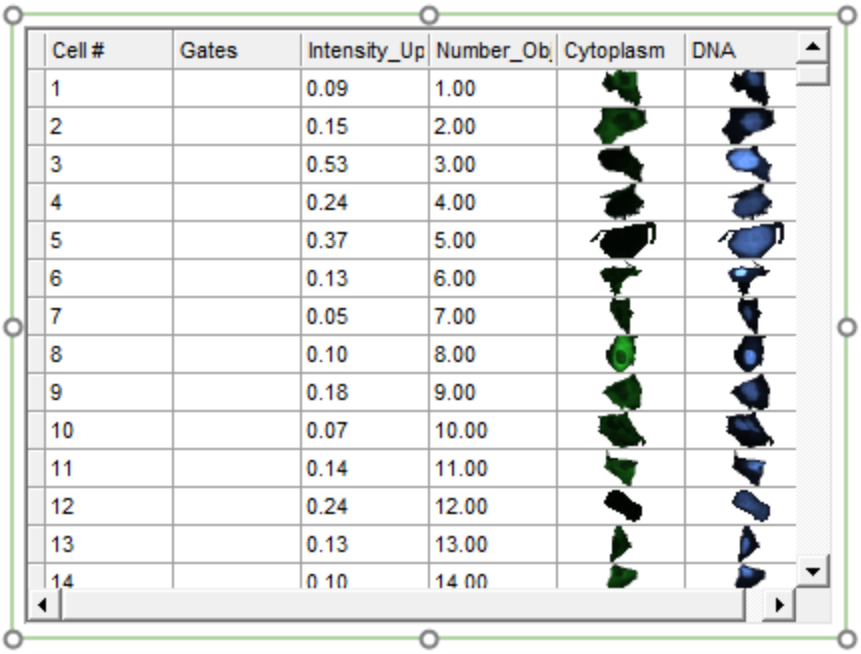

Figure T26.21 Data Grid Displaying the Chosen Parameters, Individual Cell Images, and a Column for Gates

19. Create a gate on the picture plot similar to the one in Figure T26.22 and name it "Picture Plot Gate".

20. Create a gate on the dot plot similar to the one in Figure T26.22 and name it "Dot Plot Gate".

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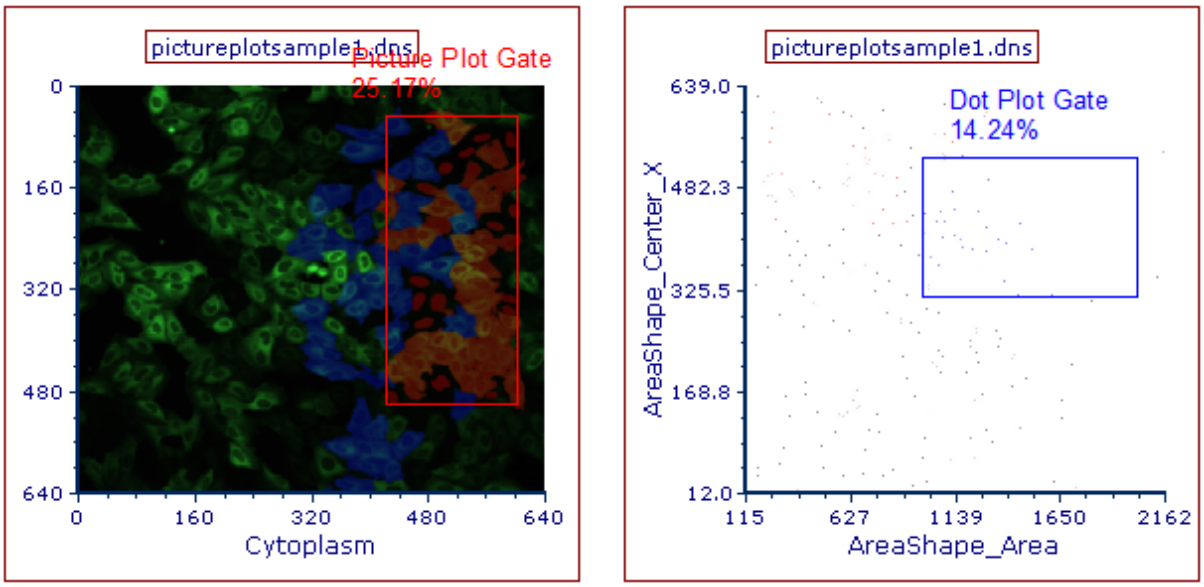

Figure T26.22 Examples of Gates to Create on the Picture and Dot Plots

Scroll down through the data grid and notice that it is now displaying colored rectangular boxes in the gates column (Figure T26.23). These boxes represent which gates the individual cells fall within. If a cell falls into multiple gates, then multiple colored bars will appear next to the cells in the "Gates" column. If the "Gates" column is empty, then that cell does not fall into any gates that are currently being displayed.

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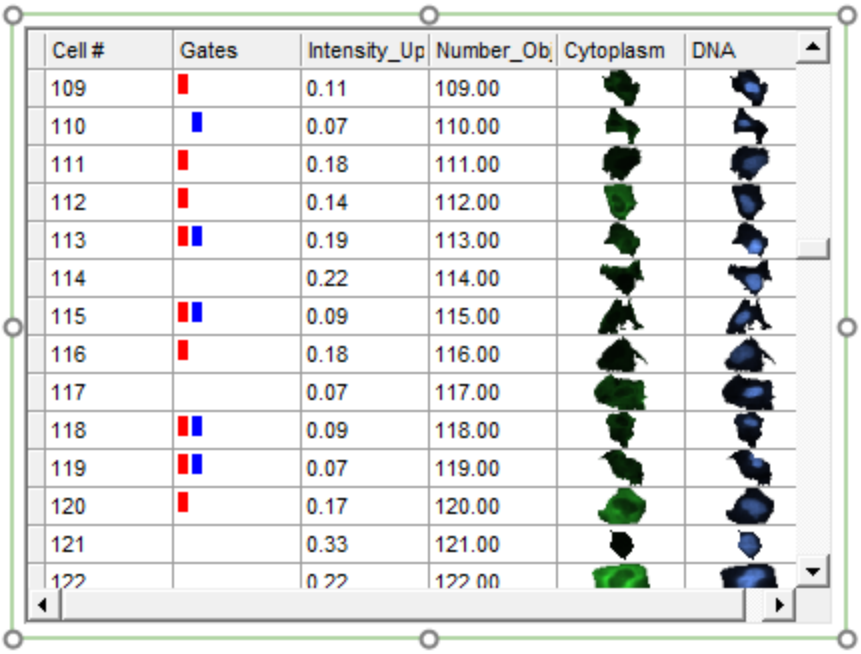

Cell #	Gates	Intensity_Up	Number_Ob	Cytoplasm	DNA
109		0.11	109.00		
110		0.07	110.00		
111		0.18	111.00		
112		0.14	112.00		
113		0.19	113.00		
114		0.22	114.00		
115		0.09	115.00		
116		0.18	116.00		
117		0.07	117.00		
118		0.09	118.00		
119		0.07	119.00		
120		0.17	120.00		
121		0.33	121.00		
122		0.22	122.00		

Figure T26.23 Data Grid Displaying Dot Plot and Picture Plot Gates as Colored Rectangular Boxes

We will now apply the **Picture Plot Gate** and **Dot Plot Gate** to the data grid to view the individual cells that fall within these gates.

21. Click on the data grid to select it.

22. Navigate to **Gating→Create Gates→Current Gate** and choose **Picture Plot Gate** from the **Current Gate** drop-down list (Figure T26.24). Alternatively you can drag and drop the **Picture Plot Gate** directly from the Picture Plot onto the Data Grid.

23. Adjust the **Picture Plot Gate** to select fewer cells as in Figure T26.24.

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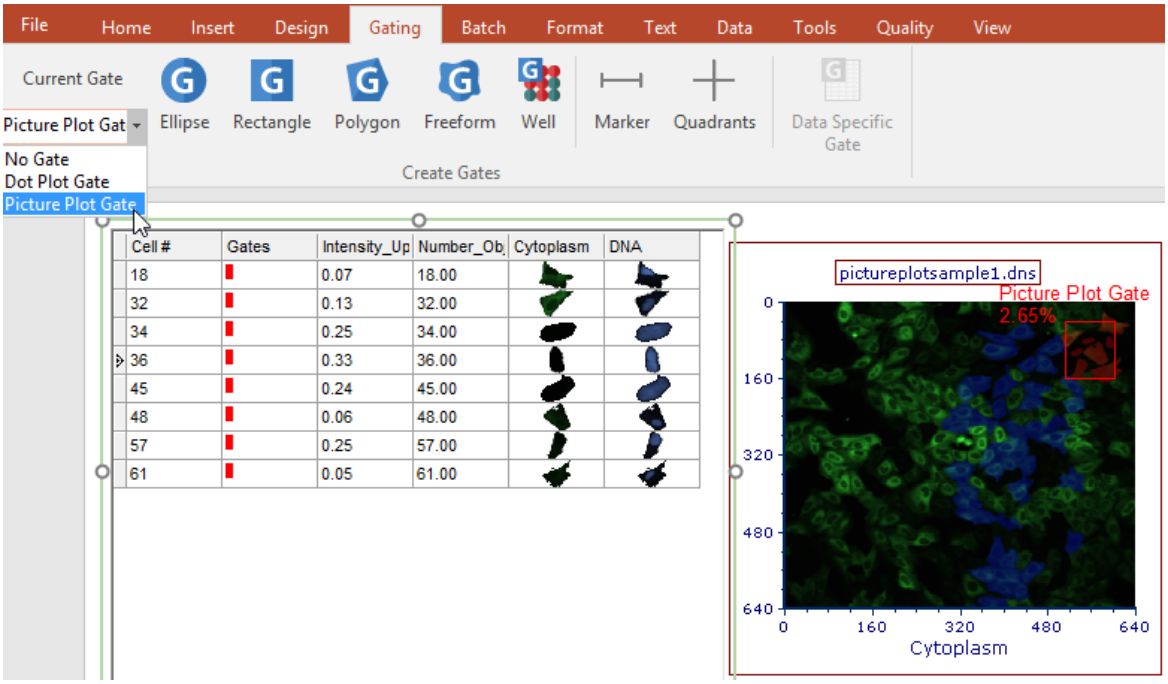

Figure T26.24 Apply Picture Plot Gate to the Data Grid

Notice how the cell gallery updates in live time to display only the events found within the chosen **Picture Plot Gate**. This is an example of "drilling down" to cells of interest.

24. Click on the data grid to select it.

25. Navigate to **Gating→Create Gates→Current Gate** and choose now the **Dot Plot Gate** from the **Current Gate** drop-down list (Figure T26.25). Alternatively you can drag and drop the **Dot Plot Gate** directly from the Dot Plot onto the Data Grid.

26. Adjust the **Dot Plot Gate** to select fewer cells as in Figure T26.25.

Notice how the cell gallery updates to display only the events found within the chosen dot plot gate. This is an example of "drilling down" to cells of interest.

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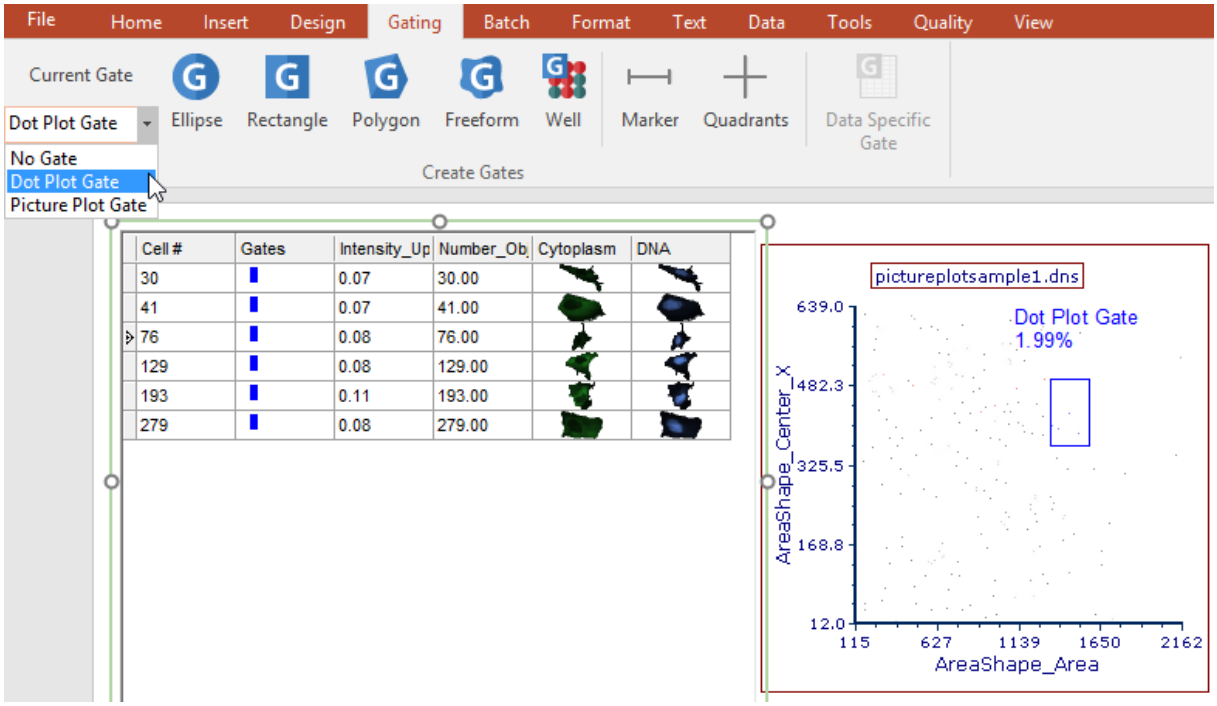

Figure T26.25 Apply Dot Plot Gate to Data Grid

We will now create a gate directly on the data grid to display those events in the picture plot and scatter plot.

27. Click on the data grid to select it.

28. Navigate to **Gating→Create Gates→Current Gate** and choose **No Gate** from the **Current Gate** drop-down list.

29. Right-click on the data grid.

30. Choose **Create Gate** from the pop-up menu. A Range Gate selection area will appear as in Figure T26.26.

31. Click on **Range**.

32. Select **Add Range to Gate...** from the drop-down list.

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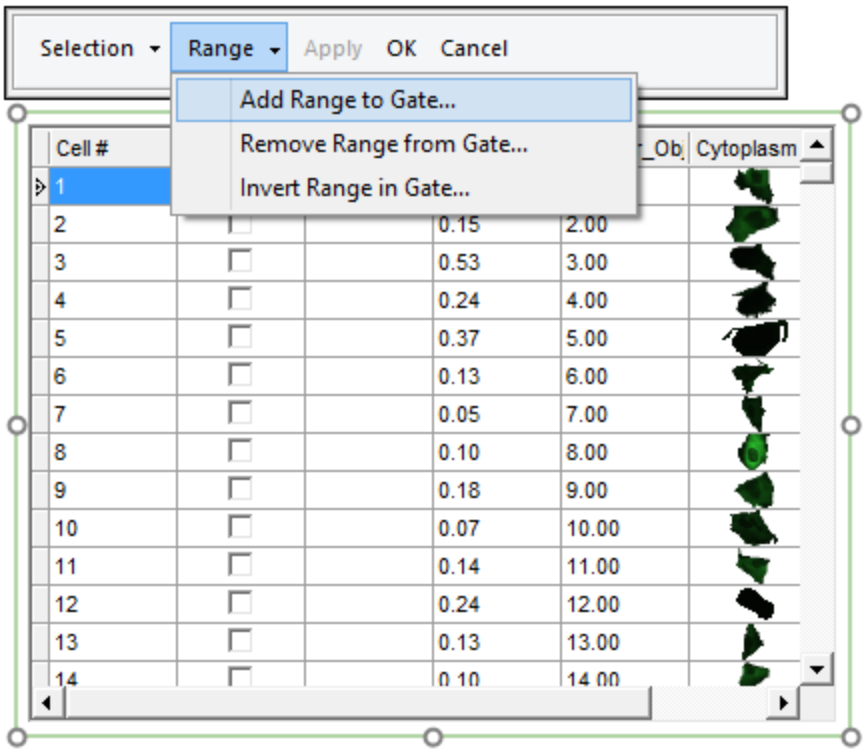

Figure T26.26 Range Gate Selection

33. Enter the range as **From 1 To 100** in order to select the first 100 cells of the sample (Figure T26.27).

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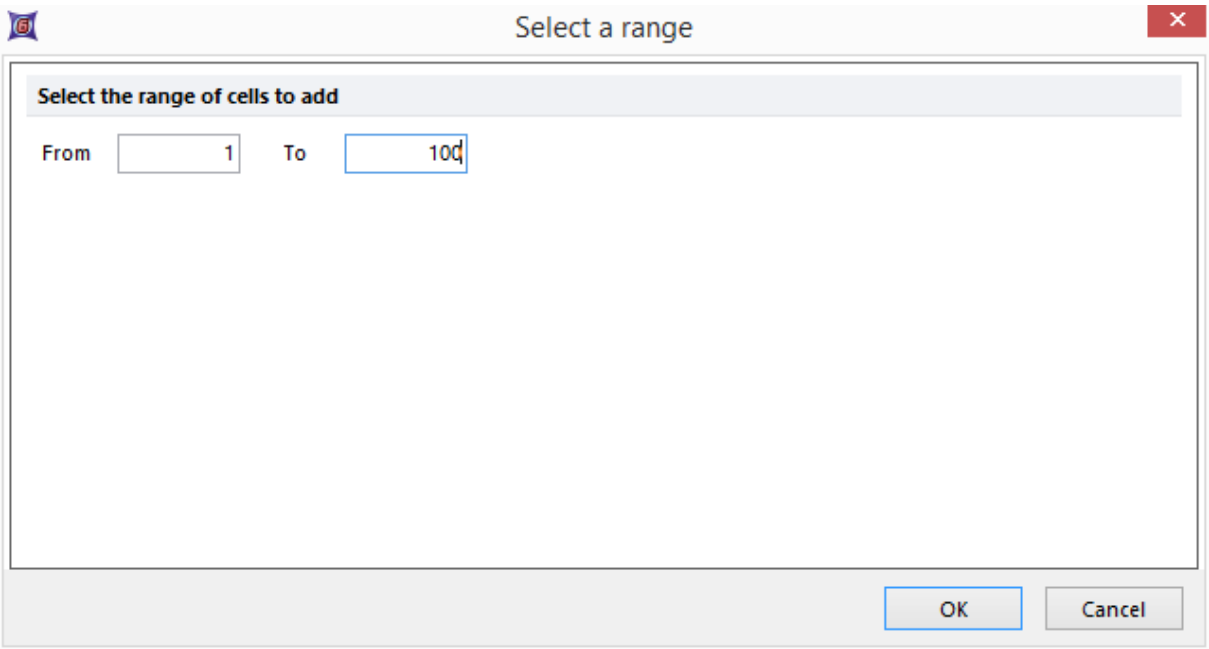

Figure T26.27 Select a Range Dialog

34. Click **OK**.

35. After the **Select a range window** closes, click **OK** in the Range Gate selection area above the data grid (figure T26.28).

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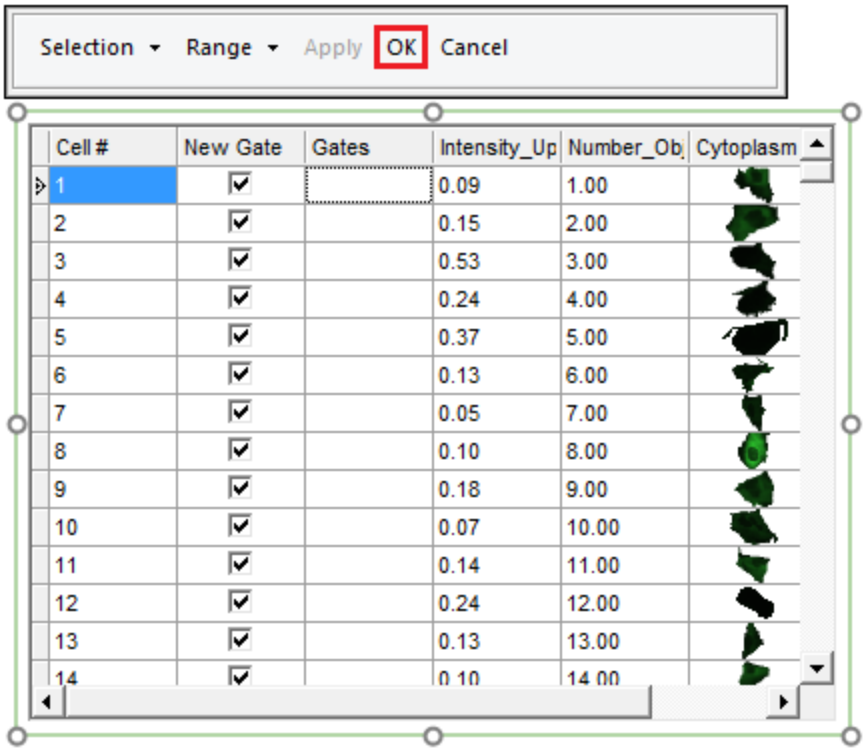

FIgure T26.28 Click OK in the Range Gate Selection area

36. Name the new gate **Range Gate**.

37. Click **OK**.

The new gate is now visible in the **Gates** column of the Data Grid (Figure T26.29).

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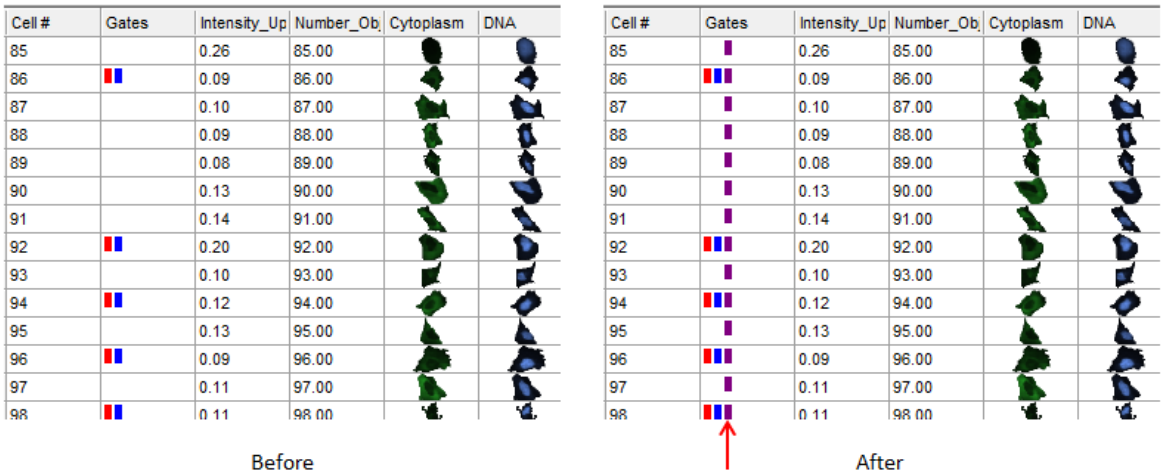

Figure T26.29 - Before and after creating the range gate.

Let's now add more events to the **Range Gate**.

38. Right click on the data grid and select **Edit Gate→Range Gate**.
39. Click on **Range** in the Range Gate selection area above the data grid.
40. Select **Add Range to Gate...** from the drop-down list.
41. Enter the range as **From** 180 **To** 250.
42. Click **OK**.
43. Click **OK** in the Range Gate selection area.

Note that now the **Range Gate** contains cells 1-100 and cells 180-250.

Notice that the picture plot and scatter plot are now displaying the events that were selected in the range gate as dots or cells highlighted by the range gate color, purple in this example (Figure T26.30).

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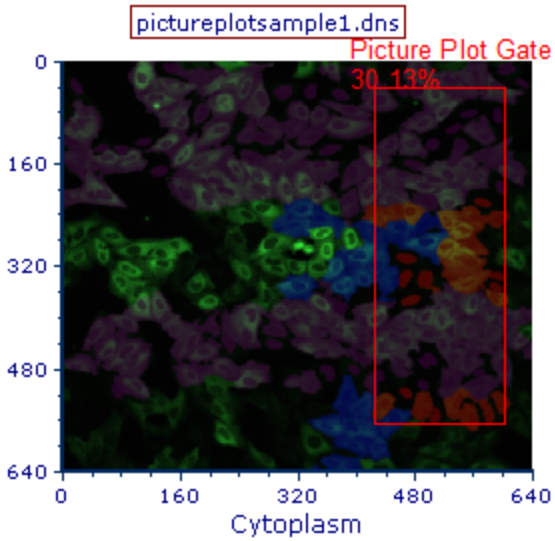

Figure T26.30 Picture Plot Displaying the Eevents in the Range Gate as Purple Highlighted Cells

Individual cells may also be selected in a range.

44. Right-click on the data grid.
45. Choose **Create Gate** from the pop-up menu.
46. Select cells 5 through 10 in the data grid by checking the boxes in the **New Gate** column ❶ (Figure T26.31).

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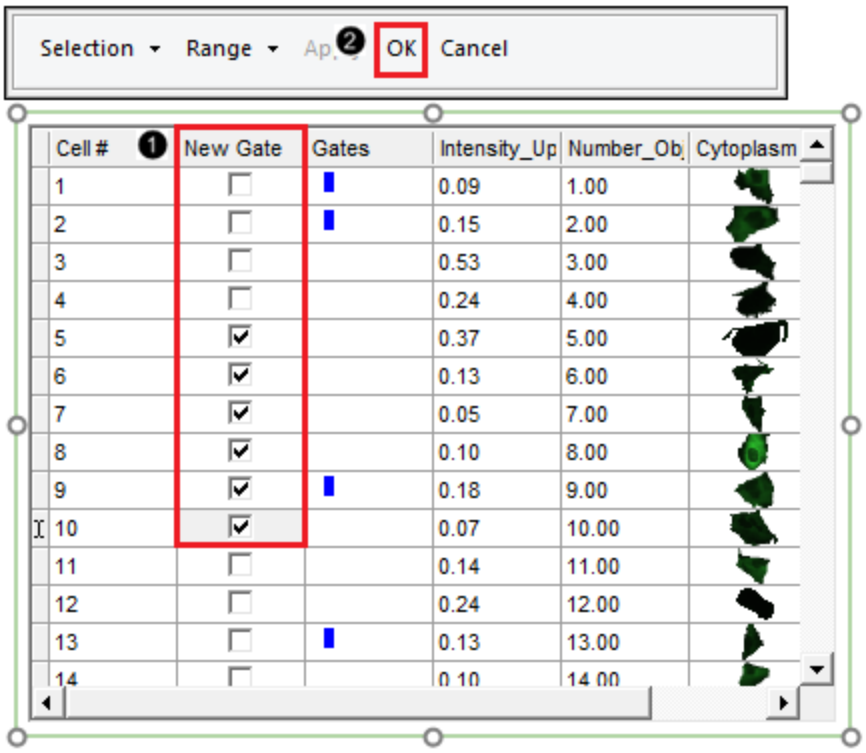

Figure T26.31 Selecting Individual Cells for Gating by Checking the Boxes in the New Gate Column

47. Click **OK** ❷ in the Range Gate selection area.
48. Name the gate "Selected Cells".
49. Click **OK**.

A new range gate will be created for these cells (Figure T26.32).

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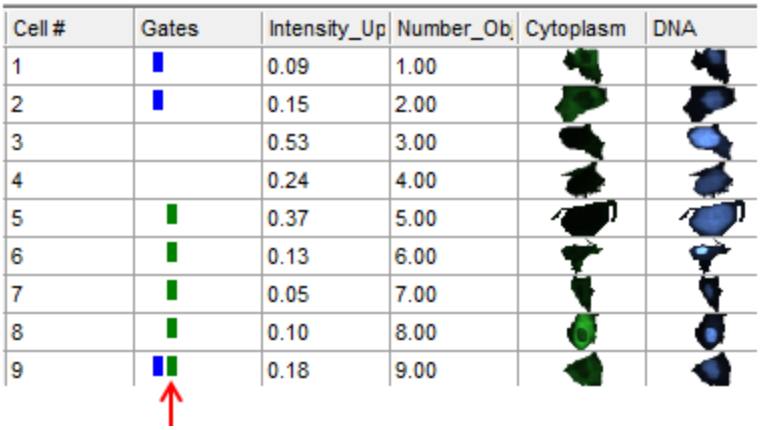

Cell #	Gates	Intensity_Up	Number_Ob	Cytoplasm	DNA
1		0.09	1.00		
2		0.15	2.00		
3		0.53	3.00		
4		0.24	4.00		
5		0.37	5.00		
6		0.13	6.00		
7		0.05	7.00		
8		0.10	8.00		
9		0.18	9.00		

Figure T26.32 Selected Cells Range Gate Displayed as a Green Box in the Gates Column

To view the individual cells in this gate on the picture plot and dot plot, we will quickly format both plots to make it easier to view a small number of cells.

50. Right-click on the picture plot.
51. Choose **Format** from the pop-up menu.
52. Choose the **Overlays** category.
53. Uncheck the **Show Background Image** box.
54. Click **OK**.
55. Right-click on the color dot plot.
56. Choose **Format** from the pop-up menu.
57. Choose the **Overlays** category.
58. Change the **Dot size** to 5.
59. Click **OK**.

We will now gate both plots on the Selected Cells gate to view only these events in each plot.

60. Select both the dot plot and picture plot.
61. Navigate to **Gating→Create Gates→Current Gate** and choose **Selected Cells** from the **Current Gate** drop-down list.

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The dot plot and picture plot will now display only cells that fall within the manually checked range boxes (Figure T26.33). A new range or individual cells may be added to the gate at any time.

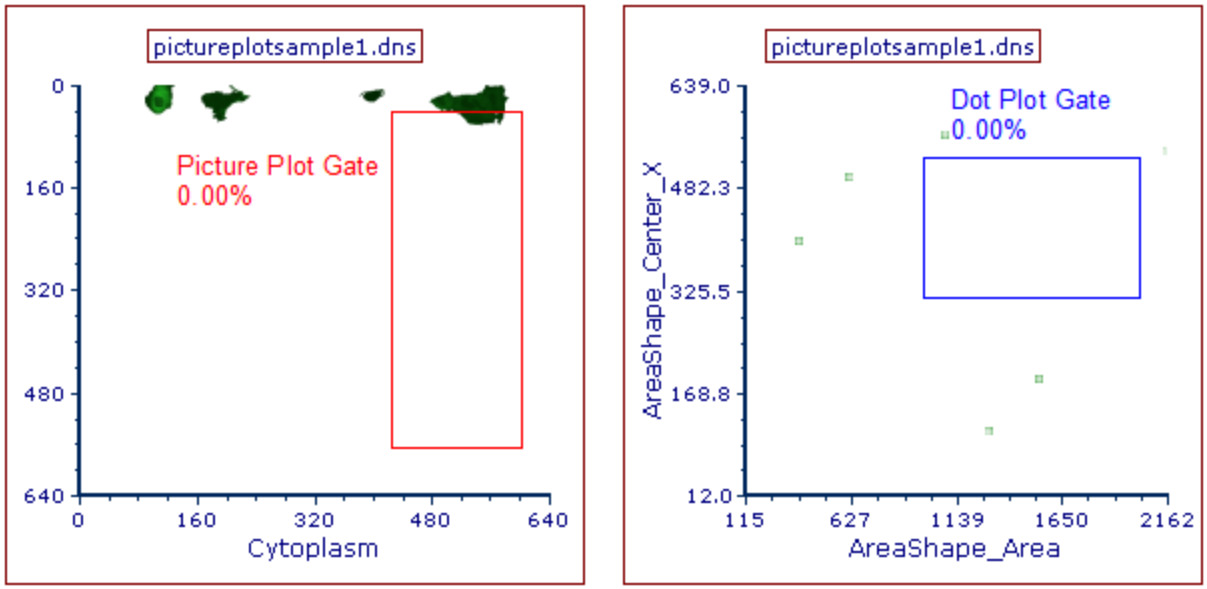

Figure T26.33 Plots Gated on the Range Gate

62. Right-click on the data grid.
63. Choose **Edit Gate→Selected Cells** from the pop-up menu.
64. Click on **Range**.
65. Select **Add Range to Gate** from the drop-down list.
66. Enter the range as **From** 1 **To** 50.
67. Click **OK**.
68. Click **Apply** in the Range Gate selection area to see the changes to the gate in real time.
69. Click **OK** in the range gate selection area.

The picture plot and dot plot will now display the newly edited range that was entered (Figure T26.34). Also notice that since events are now falling within the Dot Plot Gate, the percent gate statistic automatically updates.

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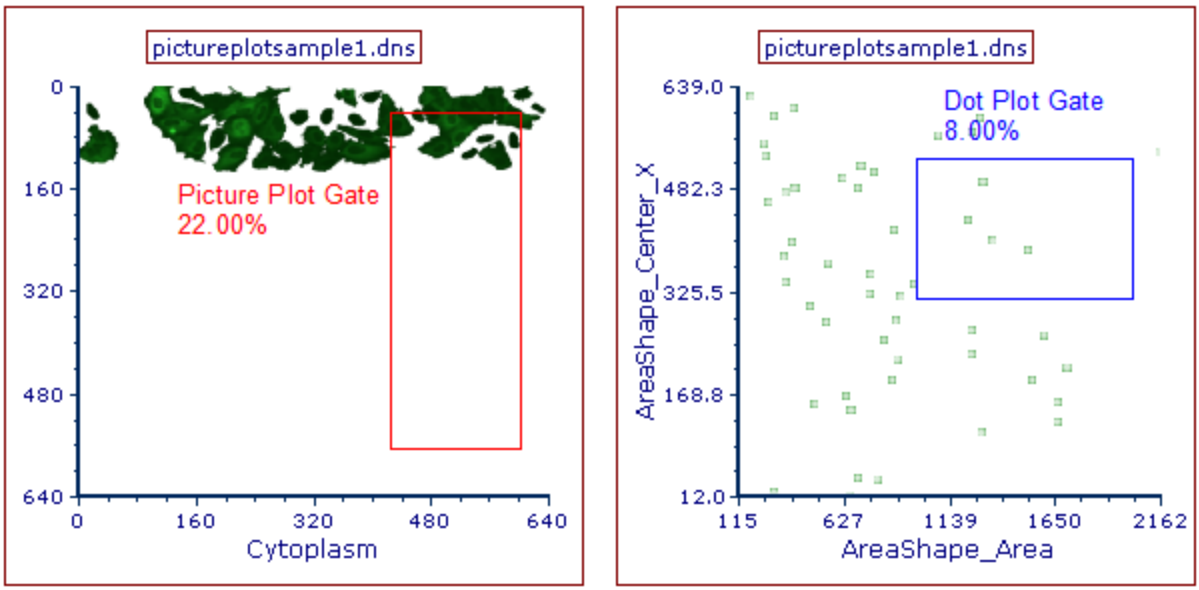

Figure T26.34 Picture Plot and Dot Plot After Adding a New Range to the Selected Cells Gate

38.30 Introduction to FCS Express 6 Image Cytometry

A Flow Cytometry environment for Image Cytomtery Data

FCS Express 6 Image Cytometry is a new version of FCS Express that contains exciting and unique tools for working with Image Cytometry data.

This tutorial is designed to provide an overview of many powerful new tools that allow you to work with image data in much the same way as flow cytometry data. Now you can combine population based analysis and single cell interrogation of your image cytometry results in one software package!

This three section tutorial is designed to take you through prepared examples demonstrating the use of FCS Express 6 Image Cytometry.

- **Getting Started with Image Cytometry** 2099
- **Using the High Content Module with Image Data** 2130
- **Batch Processing and Reporting for Image Data** 2152

This tutorial closely follows the "Introduction to FCS Express 6 Image Cytometry" video on the recorded webinars page of the De Novo Software website. It is recommended you watch the video **PRIOR** to starting the tutorial. You will better understand the overview and biology behind the data by doing so.

In the course of the tutorial you will load and review image datasets, establish and modify gating strategies and view individual cells in cell galleries, use the High

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Content Add-on 1050 to optimize and view a 96-well plate nuclear translocation assay. The High Content Add on enables review of multi-parametric results and images from an entire plate.

A brief example of Batch analysis and FCS Express reporting tools will also be provided. This tutorial will not expand on the details of many common features and tools in FCS Express 6 for which there are existing detailed tutorials 1210. When relevant, tutorials designed to drill down and teach those commonly used functions will be referenced.

In the course of this multi-part tutorial, we will specifically:

- Demonstrate loading image cytometry data and working with images
- Review gated populations in data grid with cell image galleries
- Review analysis and reporting tools for image cytometry
- Optimize the assay settings for a nuclear translocation assay
- Use the "High Content Analysis" module to work with an image-based assay in a 96-well plate
- Review batch analysis and reporting option for image cytometry data

Due to the large size of image data, we have only worked with a several example images for each part of the tutorial. The images are embedded in the example layouts. We do not provide the image data from an entire 96-well plates in our Tutorial Sample Data Archive for examples using the High Content Add on. If you would like to move beyond this introductory tutorial and work with data from the whole study please use our step by step instructions in the CellProfiler Export Tutorial 1921.

Next Topic 2099

38.30.1 Getting Started with Image Cytometry

In this section, we will learn how to:

- Understand the required components for importing image data
- Load a layout with image data
- Modify and insert new data plots and images using picture plots
- Apply example image gating approaches
- Create a data grid to and include cell images to create a "cell gallery"
- Visually examine and optimize control settings for a Nuclear Translocation assay

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- Modify settings and gating for the data grid displaying a cell gallery

Understanding Image Data Import with FCS Express Image Cytometry

When working with image data, we have several components of data to consider: the raw image data, the segmentation maps used to specify individual objects within the image, and derived object-based multi-parametric results. Ideally, the data you will work with has all of these components available to FCS Express. With only the cell by cell multi-parametric data you can still review results with all of the familiar flow analysis tools without interactive image options.

If the images and segmentation maps are available, FCS Express can link your multi-parametric results back to the source images. The individual cells can then be highlighted in Picture Plots, images of single cells can be excised and displayed in cell galleries, and meaningful multi-parametric data can be reviewed on a cell by cell basis. FCS Express Image Cytometry is able to import data from a number of imaging platforms so you can easily import and work with your data.

Section 1: Working with Image Cytometry Data

We will be using the **IntroToImage1.fey** layout for this section which is located in the Tutorial Sample Data archive→**Intro to Imaging Tutorial** folder. This layout contains a detailed pre-formatted approach to working with image data. As an introduction to use of the image cytometry features we will not go into detail for operations that are detailed in our other general tutorials. Relevant detailed tutorials will be referenced as applicable.

1. Load the **IntroToImage1.fey** layout from within the "Intro to Imaging Tutorial" folder within the Tutorial Sample Data archive. The multi-parametric data and images are embedded in this layout.

This is a 2-page layout, please confirm you are viewing page **Example page.** This page contains three Picture Plots and a Color Dot Plot of the cellular data (Figure T28.1). It also has the "Data List" indicating data used in the layout, and a "Data Grid" displaying single cell information to the right side of the work area on the page. You can place plots and grids anywhere in the layout on the **Example page.** The layout also contains a **Blank page**.

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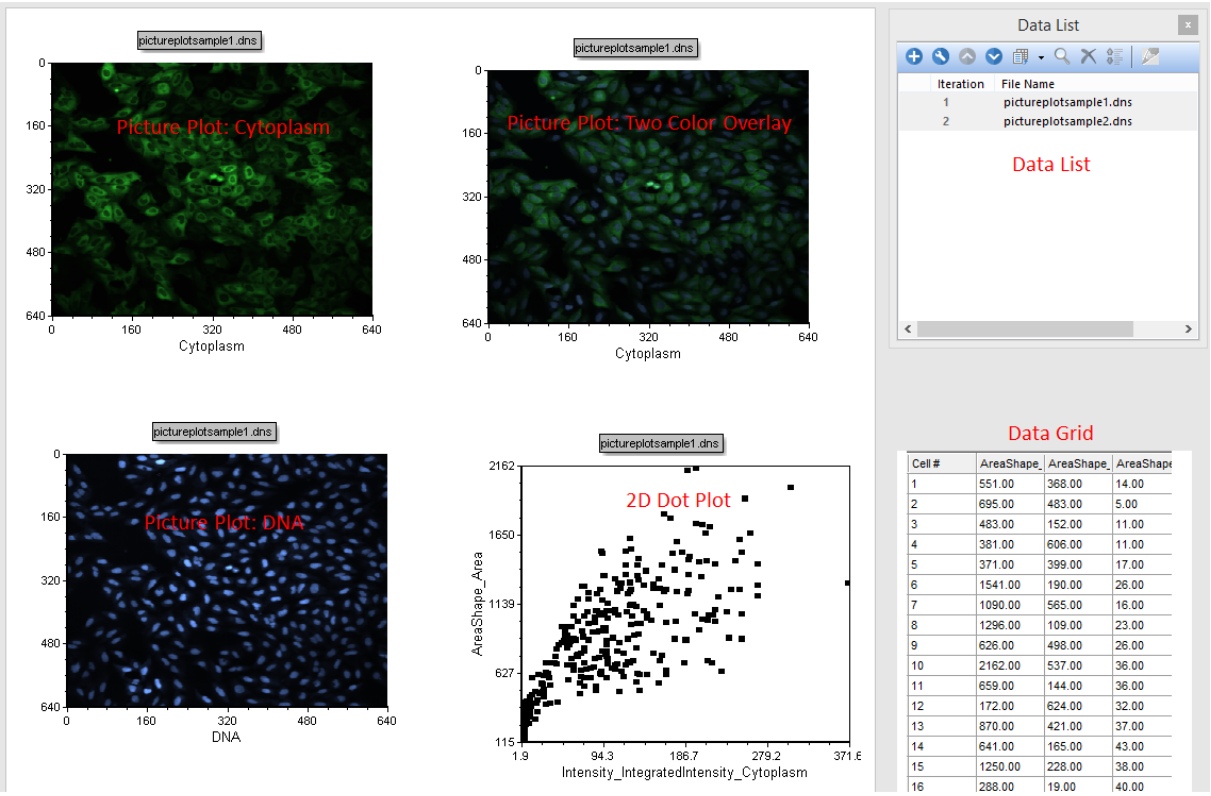

Figure T28.1 Overview of the IntroToImage1.fey layout

The **Color Dot Plot** displays two of the many parameters measured and saved by CellProfiler plotted against each other on the X-Y Axes. You can change the parameters displayed on the **Color Dot Plot** easily. We will proceed by changing the X-Axis parameter.

2. Click the X-Axis label of the Color Dot Plot. You will see a menu of parameters appear (Figure T28.2).

3. Scroll through the list of parameters left or right or begin typing the name of the parameter in the empty field at the top of the list.

4. Click on parameter you wish to display.

The new parameter will now be selected and displayed on the X-axis. This will work on all plot types with multi-parametric data.

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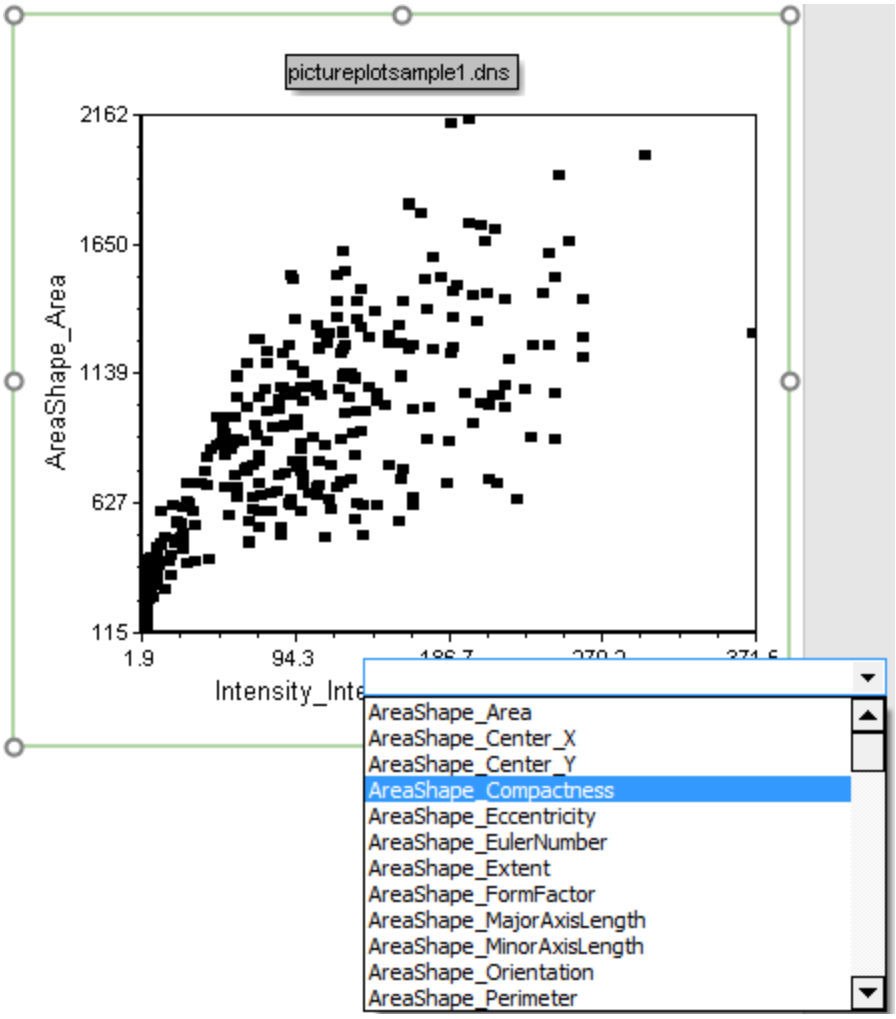

Figure T28.2 Selecting a New Parameter on a 2d Plot.

Undo is a powerful feature in FCS Express. Note that once you have made and change you can use **Ctrl+Z** to undo your changes any time you like. FCS Express supports unlimited undo commands.

6. Type **Ctrl+Z** to undo the new parameter for the X-Axis.

There is an alternative way to change X-Y Axes parameter display and have access to change other qualities of the Dot Plot:

7. Right click on the Color Dot Plot.

8. Select **Format** from the pop-up menu (Figure T28.3).

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This is the general format screen for the plot. All plots and many other objects in FCS Express may be formatted similarly.

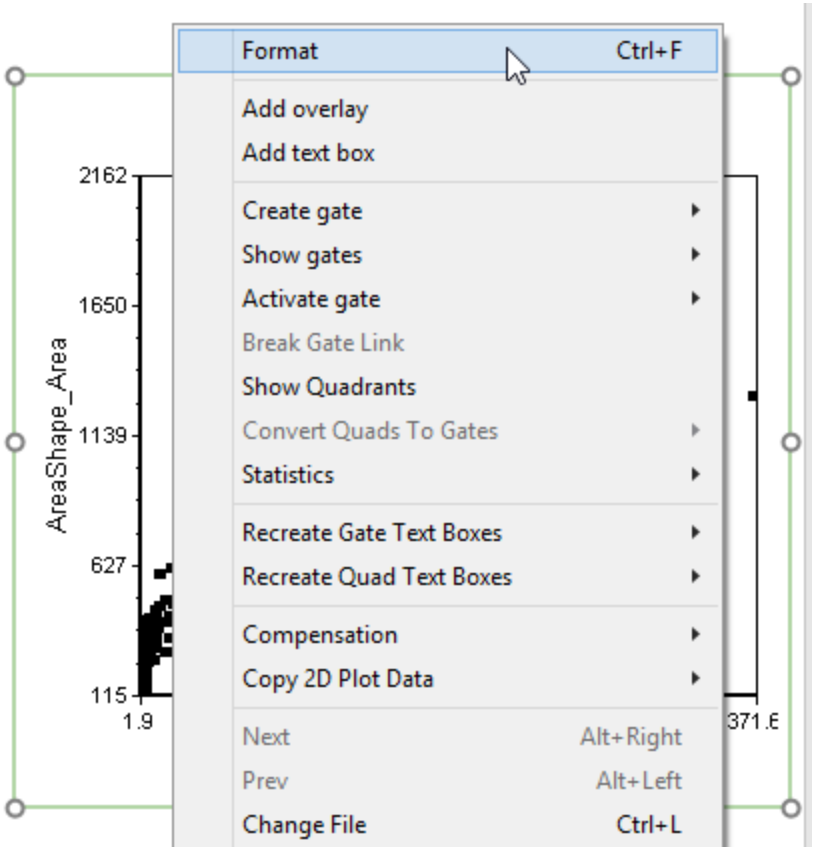

Figure T28.3 Launching the Format Menu for a Plot

Observe that you now have a number of options for changing the plot. Formatting all characteristics of the plot are possible.
The **X and Y Parameters** can be changed in this manner as well. We will access many features in the **Formatting** menu during this tutorial, so please familiarize yourself with the various items in the menu, but do not make any changes at this time.

9. Choose the **Overlays** category (Figure T28.4). X and Y Parameters can be easily changed here (red rectangle in Figure T28.4).

For the moment let's take advantage of this windows to change dot size to 8 (Figure T28.4).

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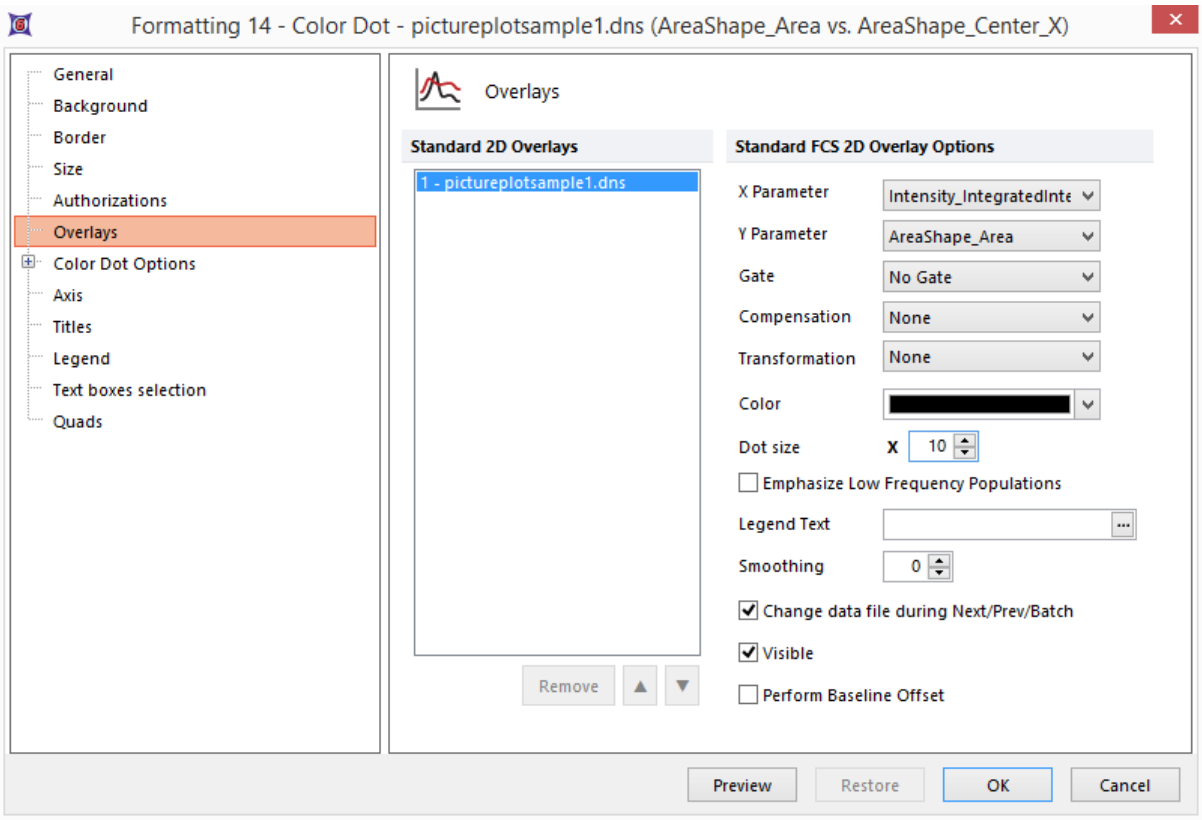

Figure T28.4 The Format Sub Menus

10. Increase the **Dot size** to **8**.

11. Click **OK** to accept the change.

12. Click the **Gating** tab→**Create Gates**→**Rectangle** command. (Figure T28.5)

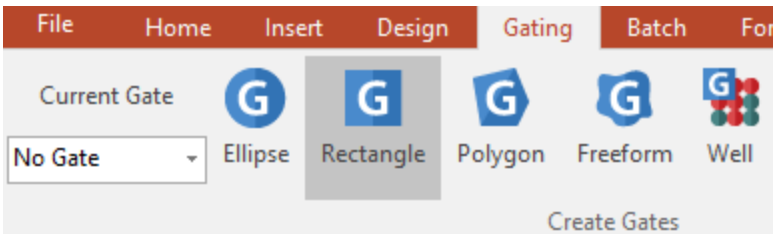

Figure T28.5 Selection of Gate Type

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12. Press and hold the left mouse button down on the center of the Color Dot Plot.

13. While holding the left mouse button down, drag to the right and down to draw a rectangular gate on the color dot plot (Figure T28.6).

14. Release the mouse button when the gate is the desired size.

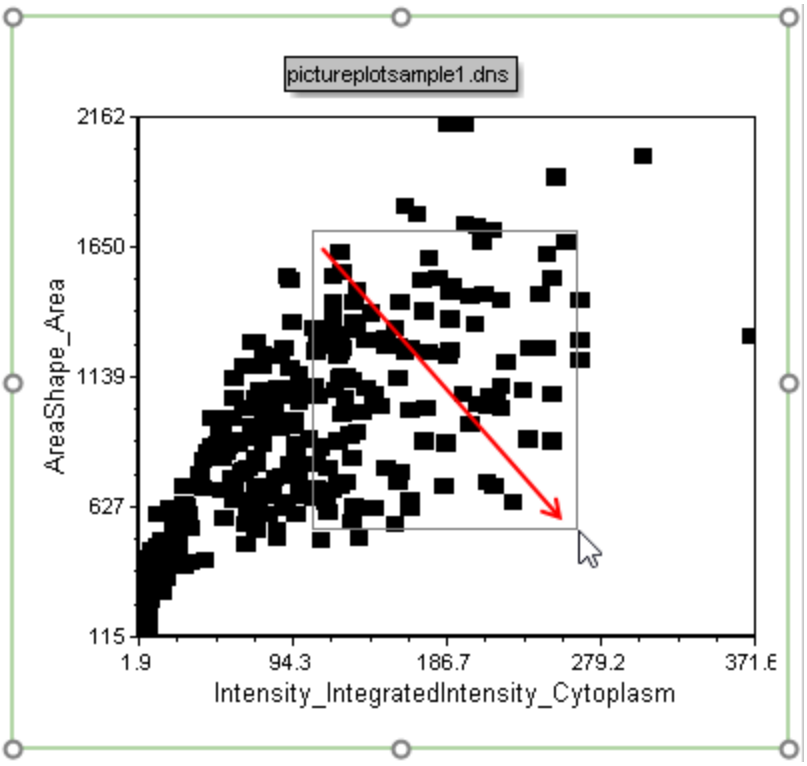

Figure T28.6 Drawing a Rectangular Gate

15. Enter the name "**Cells Gate 1**" when the **Create New Gate** dialog appears (Figure T28.7).

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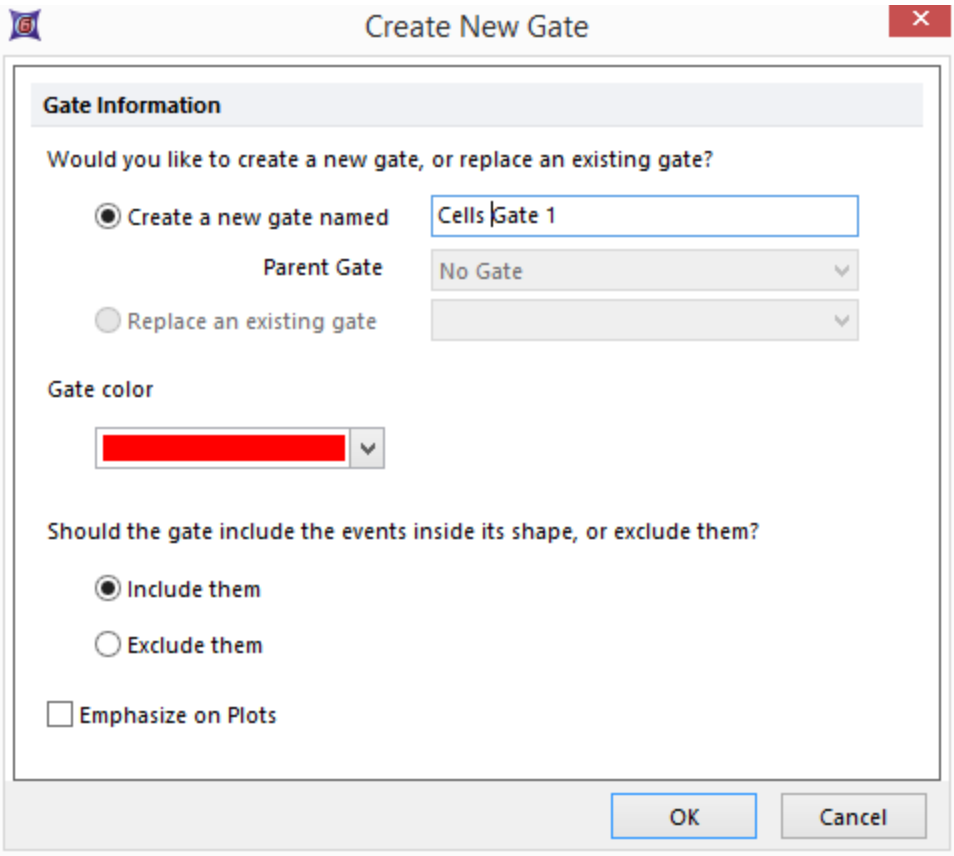

Figure T28.7 Create New Gate

16. Click **OK.** Notice that the cells included in the gate change to appear in red in both picture plots (Figure T28.8).

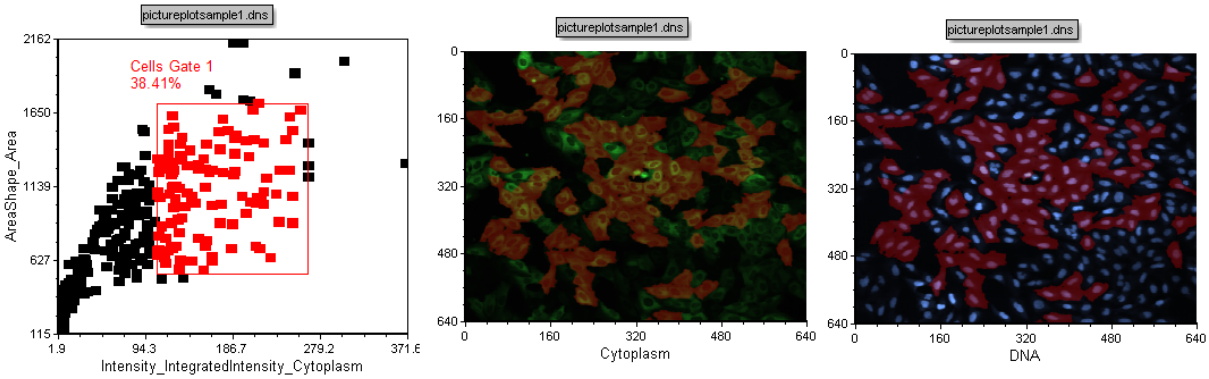

Figure T28.8 Highlighting of Cells Included in Cells Gate 1

Recall that **Unlimited Undo and Redo** is a powerful feature in FCS Express 6. Note that once you have made changes you can use **Ctrl+Z** to undo your changes if you like.

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17. Type **Ctrl+Z** to undo the addition of **Cells Gate 1.**

18. Type **Ctrl+Y** to redo the addition of **Cells Gate 1**.

19. Click once inside the **Cells Gate 1** on the dot plot.

    This selects the gate. In a selected rectangle gate, the corners will appear with blocks on them. These blocks are "vertices" and they are the point you can grab to change the gate dimensions.

20. Press and hold the left mouse button over one of the vertices of **Cells gate 1**.

21. Move the mouse leftward and downward (in the direction of the blue arrow in Figure T28.9) until the gate is similar in size to that shown in Figure T28.9 below.

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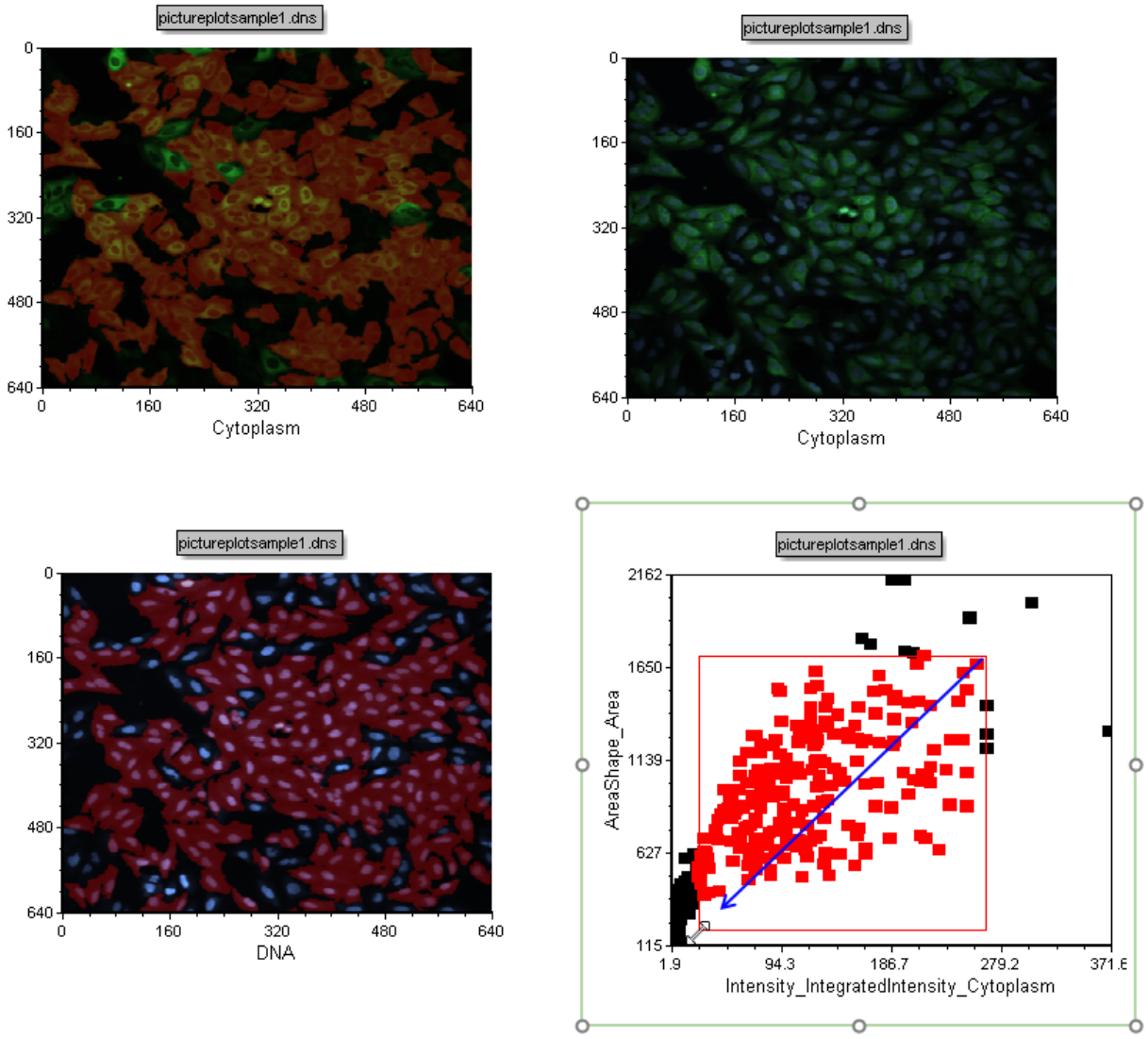

Figure T28.9 Increasing the Size of Cells Gate 1

22. Release the mouse button. Notice the increased gate size include more cells in the gate which also means more cells become highlighted in red in the images (Figure T28.9).

    You can also move a gate.

23. Press and hold the left mouse button inside the gate (but not over one of the vertices) Figure T28.10.

24. Move the mouse with the left button depressed to place the gate in a new location.

25. Release the mouse button when the gate is in the desired position.

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Observe the highlighted cells in the picture plots changing to, or from, a red color as you modify or move gates to indicate which cells are in the gate.

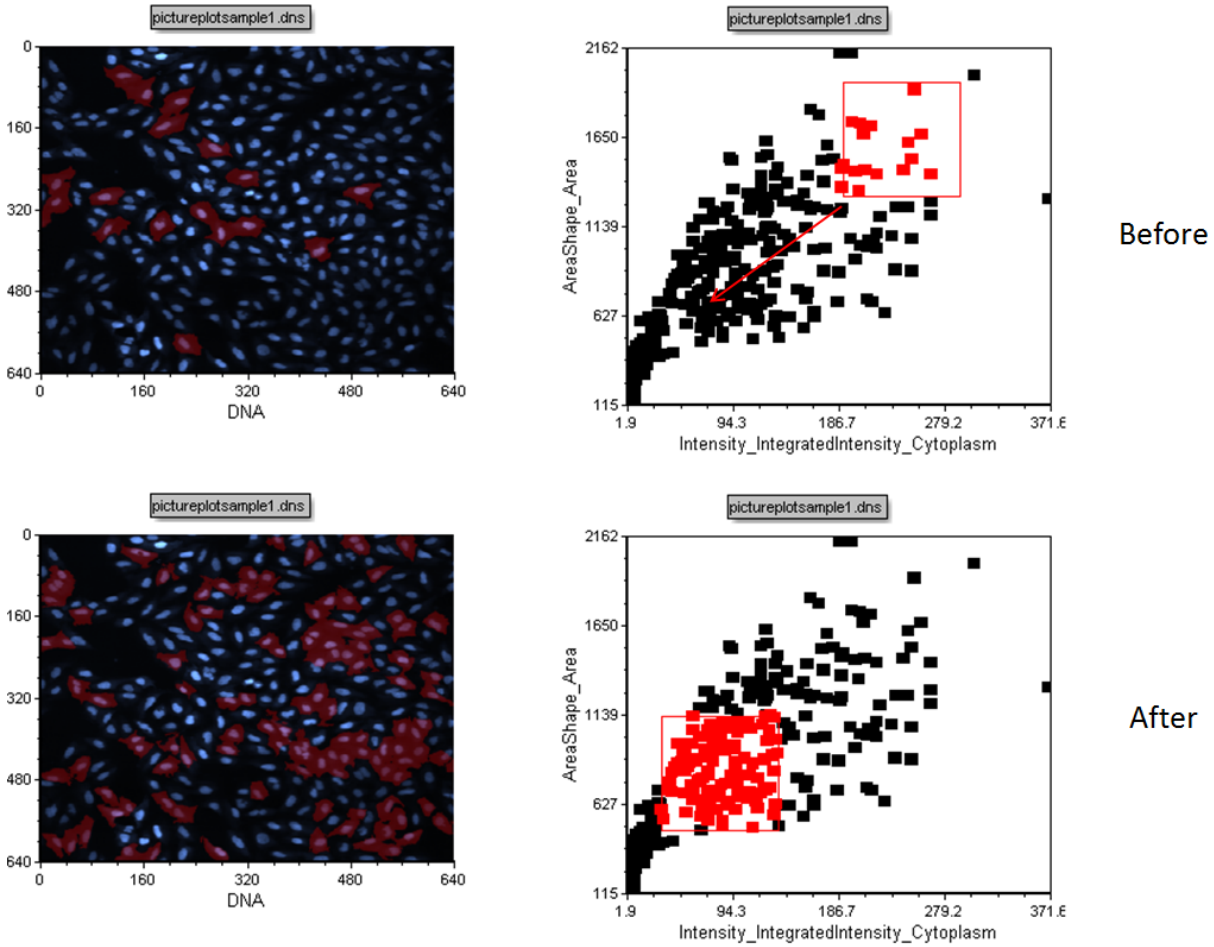

Figure T28.10 Moving Cells Gate 1

26.Close the layout and **do not** save changes.

Section 2: Building a Layout Page From Scratch

27. Open **IntroToImage1.fey**. We will build up the Example page from scratch.

28. Click on the **Blank Page** Tab. (Figure T28.11)

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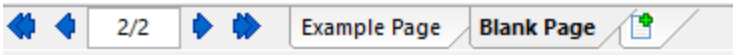

Figure T28.11 Selecting the Blank Page Tab

29. Click **Insert tab→Insert 2D plots→Picture Plot** command.

30. Press and hold down the left mouse button to drag and drop a square anywhere on the blank page. This will insert a Picture Plot with images of the cells (Figure T28.12).

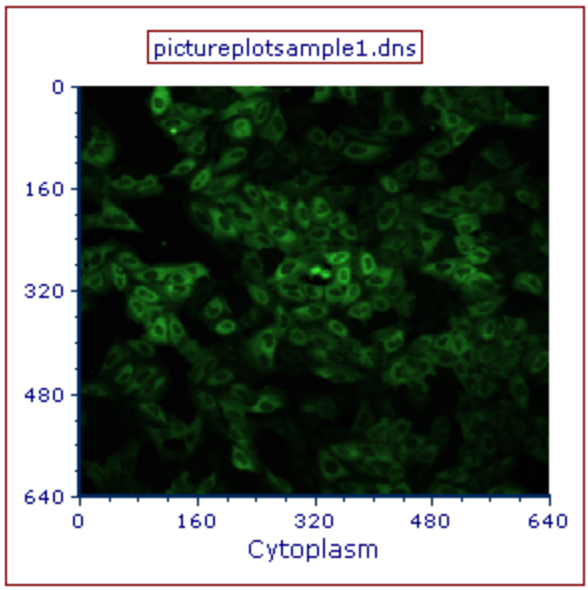

Figure T28.12 The Inserted Picture Plot

The picture plot will be called **pictureplotsample1.dns**. This is because this data is embedded in the layout as indicated by the Data List shown in the layout (Figure T28.13). (Note that "pictureplot1.dns" may not be highlighted in dark blue on your layout, and that is fine)

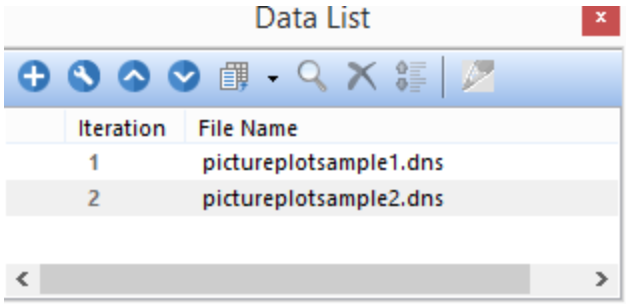

Figure T28.13 The Data List

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31. Select the **pictureplotsample1.dns** Picture Plot by clicking on it.
32. Copy (**Ctrl+C**) the **pictureplotsample1.dns** Picture Plot
33. Paste (**Ctrl+V**) **pictureplotsample1.dns** and reposition just below the original on the same page.

You will have two identical plots one above the other as shown in Figure T28.14.

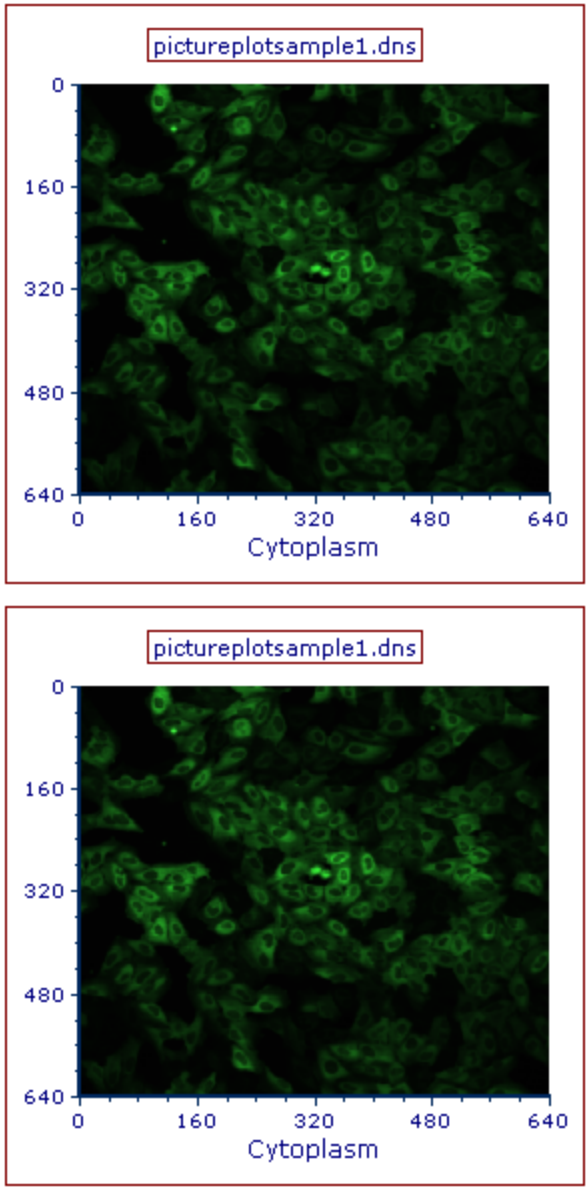

Figure T28.14 Copy and Paste a Picture Plot

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34. Change the X-Axis label on the pasted Picture Plot from **Cytoplasm** to **DNA** (Figure T28.15) as described previously in step 2 or steps 7-9.

You should now have the Cytoplasm (green) and Nuclear (Blue) image as in Figure T28.15 below.

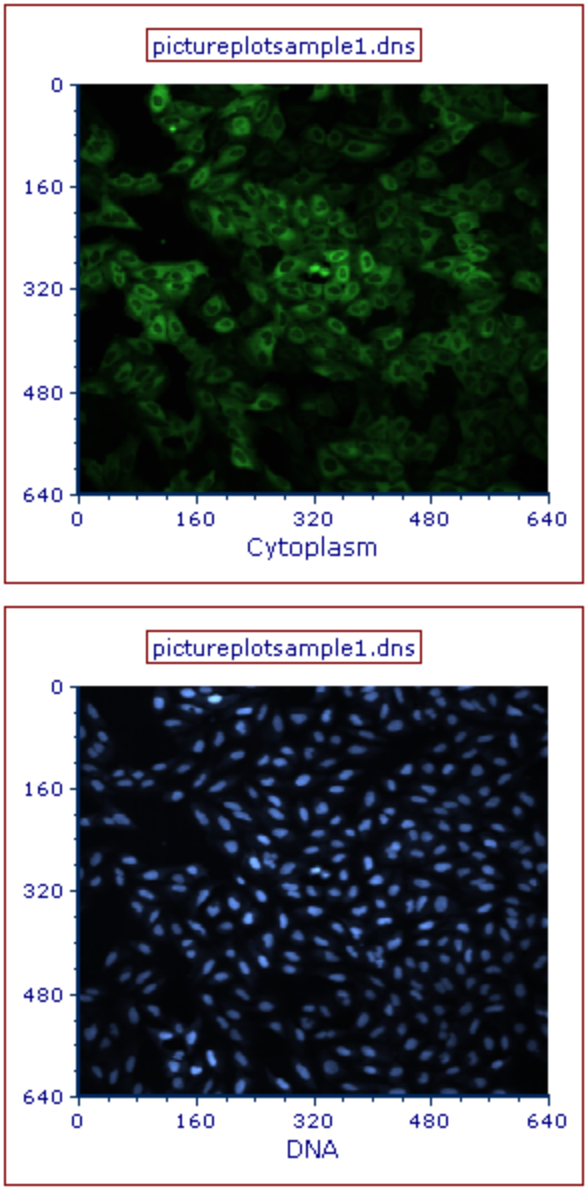

Figure T28.15 Changing the Image Parameter of a Picture Plot

35. Click anywhere that is empty on the layout. Paste **pictureplotsample1.dns** plot again in the upper right part of the page.

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36. Click on the Picture Plot in the lower left that is displaying the **DNA** image plot to place it in edit mode. (Ensure the green selection box surrounds the plot, as shown in Figure T28.15 above).

37. Click on the green border of the Picture Plot to place it in Selected mode (the border became red colored). When plots are in selected mode, you can move then or resize them etc.

38. Press and hold the left mouse button while the mouse is over the red border of the Picture Plot displaying the DNA image.

39. Move the mouse until the outline of the DNA plot is over the top right cytoplasmic plot. You will know you are in the right spot when the **"+"** appears next to the cursor and the borders of the plot turn blue (Figure T28.16).

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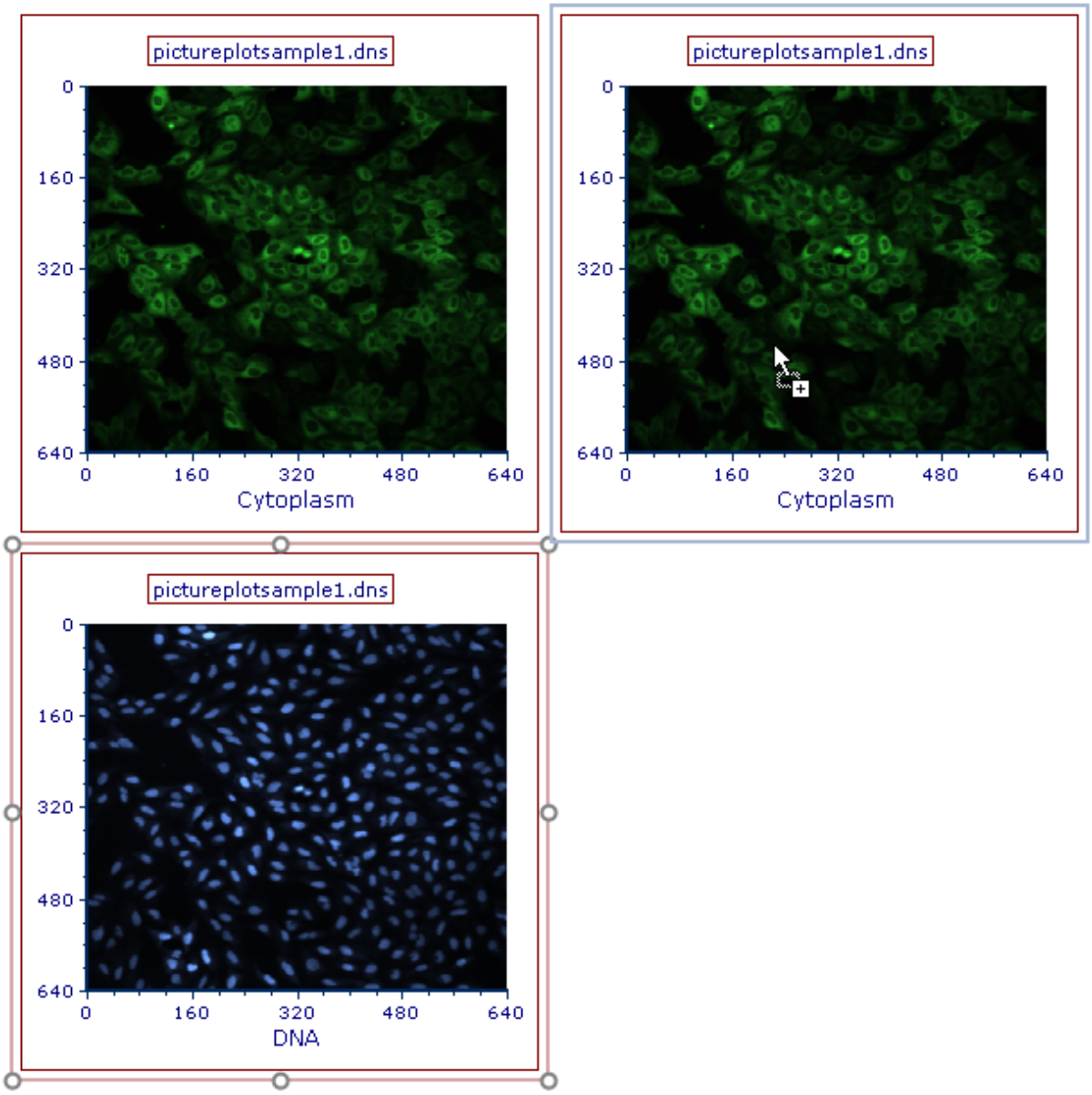

Figure T28.16 Drag and Drop a Picture Plot onto Another Picture Plot to Create a 2d Overlay

A **Paste Special** dialog will appear as see in Figure T28.17 below.

40. Select "**2D Plot Overlay(s)**".

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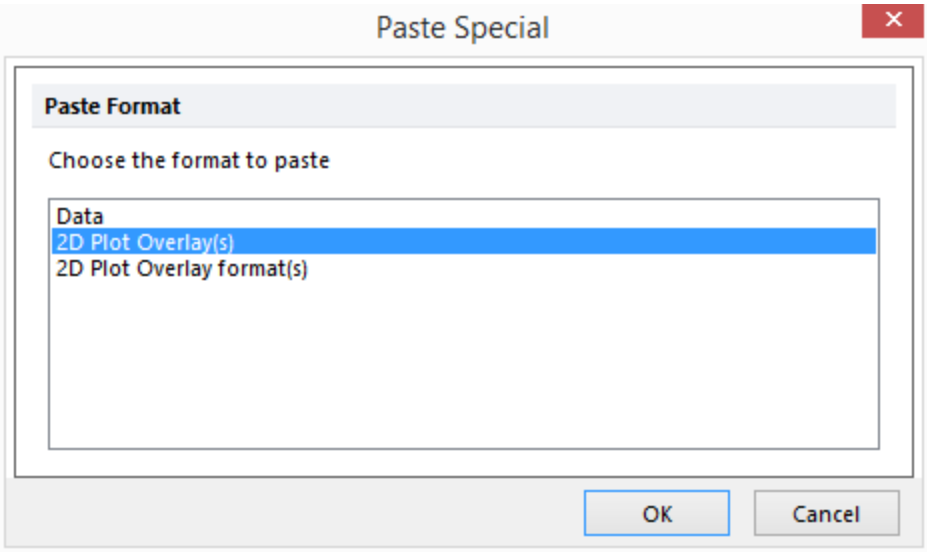

Figure T28.17 Selecting 2D Plot Overlay(s)

41. Click **OK**.

This will create a two channel overlay as shown in Figure T28.18.

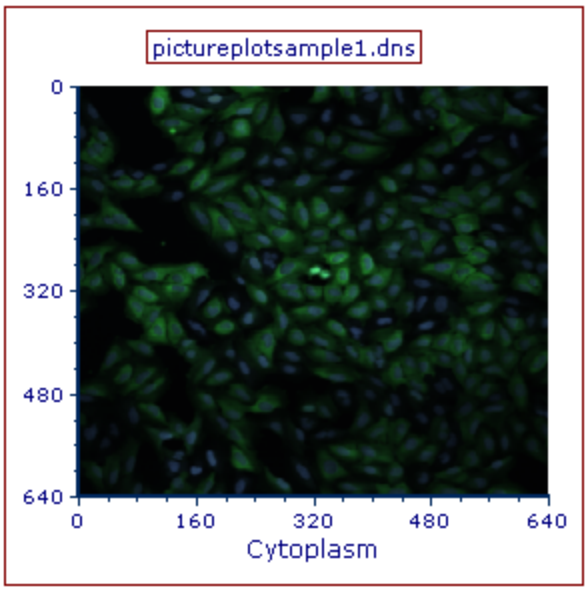

Figure T28.18 Completed 2D Overlay

Notes:

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To learn other ways to load a new image data set and insert image plots see the Picture Plots 2060 tutorial.

To learn more ways to modify the data and image plots see the Formatting Plots 1236 tutorial.

We will now insert a **2D Dot Plot** in the layout

42. Click on **Insert** Tab→**Insert 2D Plots→Color Dot** (Figure T28.19).

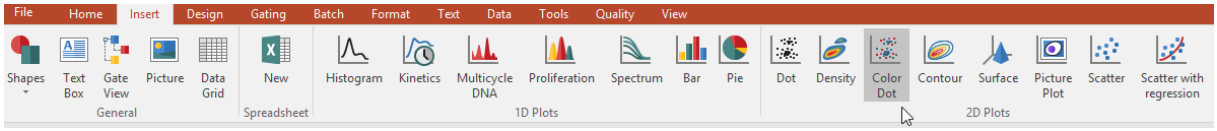

Figure T28.19 Selecting the Dot Plot Icon

43. Right click on the Color Dot Plot to open the **Format** Window in order to change from the default display (Figure T28.20).

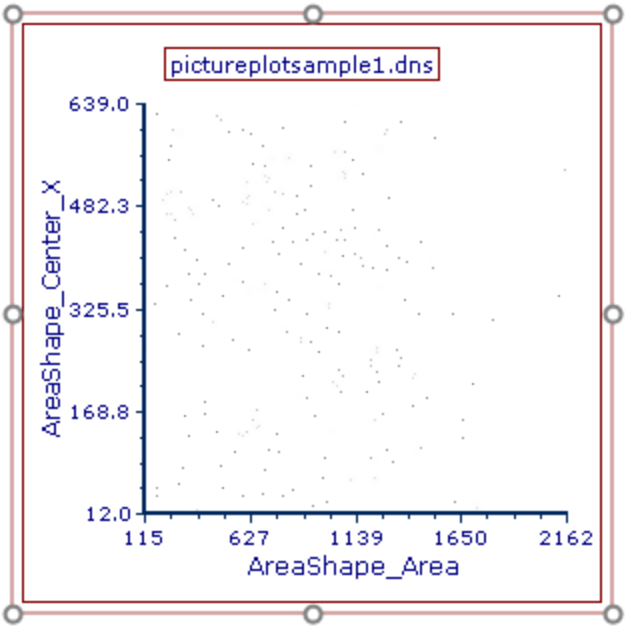

Figure T28.20 Default Configuration of Inserted Color Dot Plot

44. Select **Format**.

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45. Choose the **Overlays** category.
46. Set the **X Parameter** to **Intensity_IntegratedInstensity_Cytoplasm** as shown in Figure T28.21 below.
47. Set the **Y Parameter** to **AreaShape_Area** also as shown below in Figure T28.21.
48. Increase the **Dot size** from **1** to **10** (Figure T28.21).
49. Click **OK**.

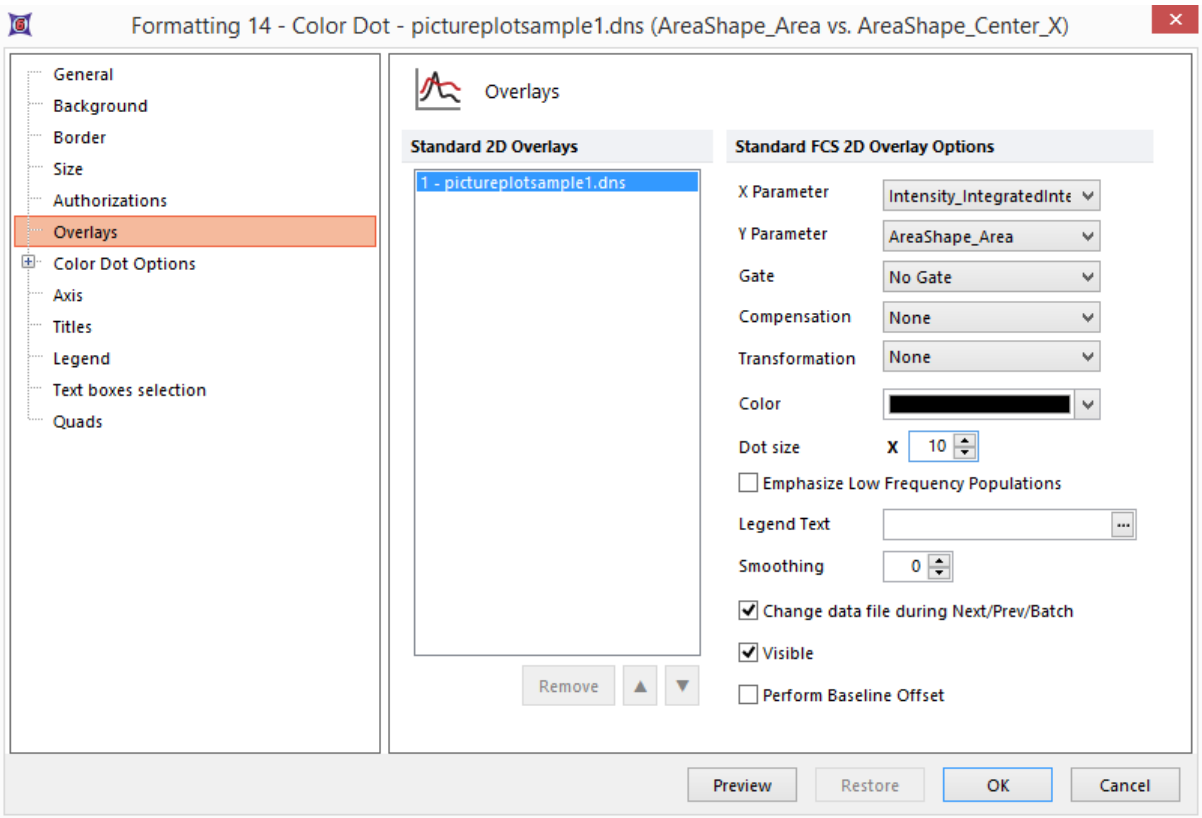

Figure T28.21 Format Settings for New Dot Plot

The Dot plot will now appear as in Figure T28.22.

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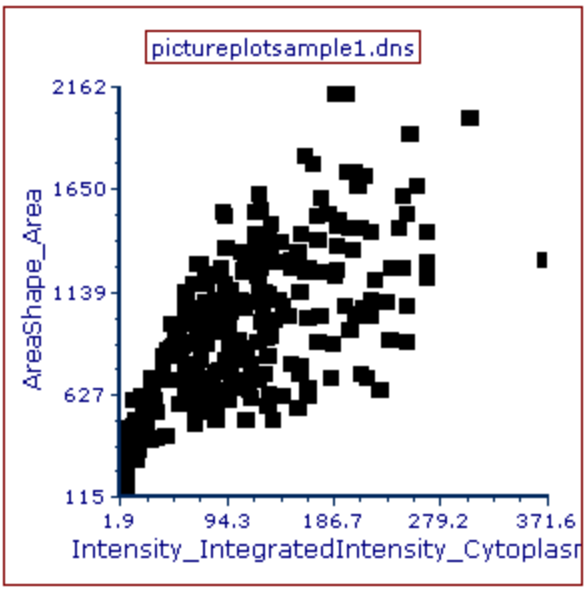

Figure T28.22 Formatted Color Dot Plot

50. Insert a gate by selecting **Gating** tab→**Create Gates**→**Rectangle** as you have done previously in the tutorial (Steps 11-14).

51. Name the gate **Cells Gate 1**.

52. Click **OK**.

Your **Blank page** will now resemble the Example Page as in Figure T28.23.

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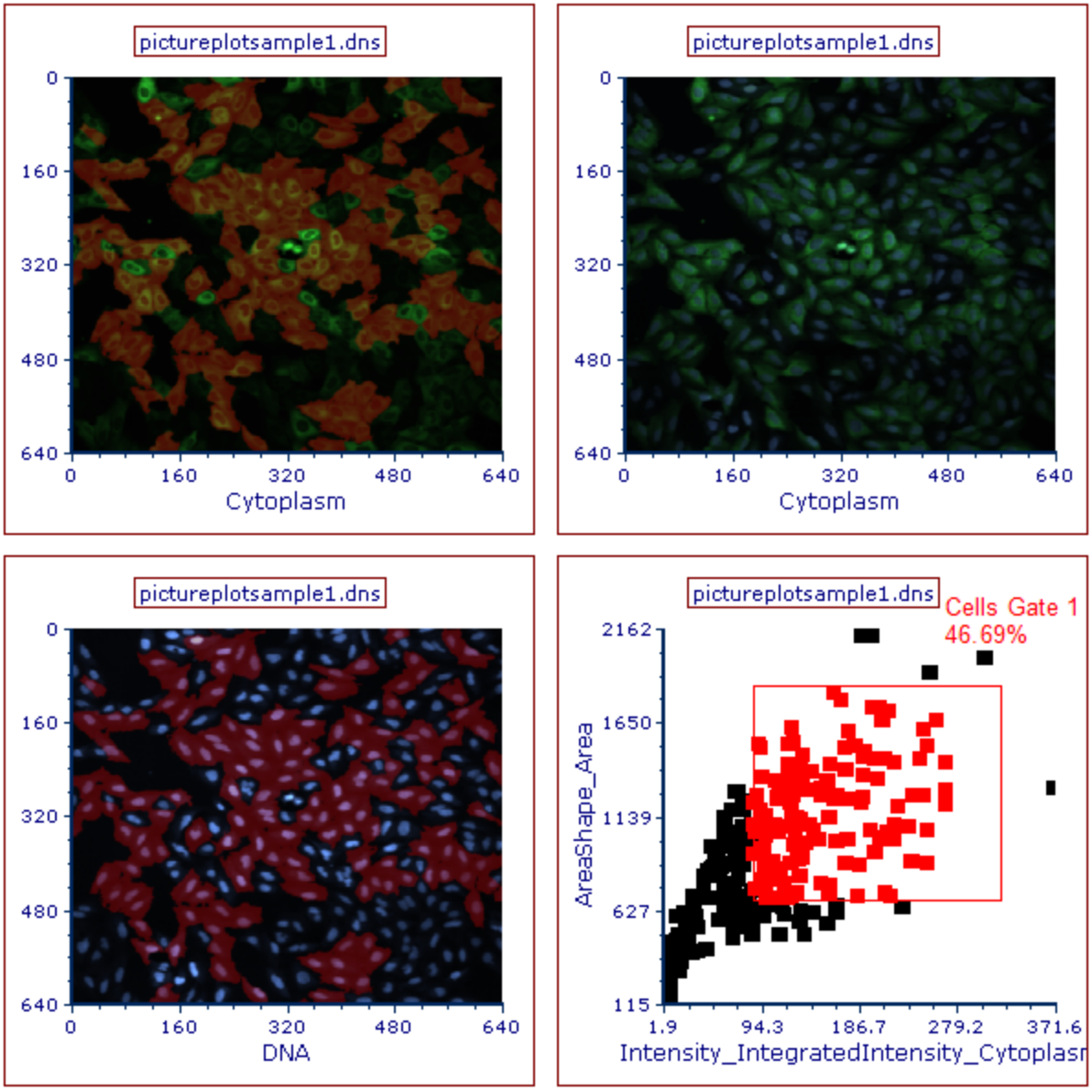

Figure T28.23 Completed Example Page

53. Close the layout, and **do not save** changes.

Section 3: Image Gating Strategies

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When using image cytometry data it can be very useful to apply gates to dot pots representing cellular data and review the gated cells in the original images. Reviewing the actual images of cell helps confirm that the gated populations represent the expected changes in cell morphology. In this section we will 1) gate on cells in an image and update plots and 2) gate on plots to update highlighted cells in images.

54. Load the **IntroToImage2.fey** layout from the Tutorial Sample Data archive 1210 →**Intro to Imaging Tutorial** folder.

This layout has a two Picture Plots 2060 and two color Dot Plots 1225 of the cellular data from a nuclear translocation assay. The images represent the nuclear stain and cytoplasmic stains of the same cells (Figure T28.24). Notice the gates on the cytoplasm image. The gates appear as a green square and red circle. Notice the red and green dots in the color dot plot below the image which indicates the cells that are within the gates on the Picture Plot 2060. Note that we have also included a Gate View 345 and Cell Gallery in the layout. These help us keep track of activity in the layout. Items not yet discussed are labeled in red. Figure T28.26 below displays the complete layout.

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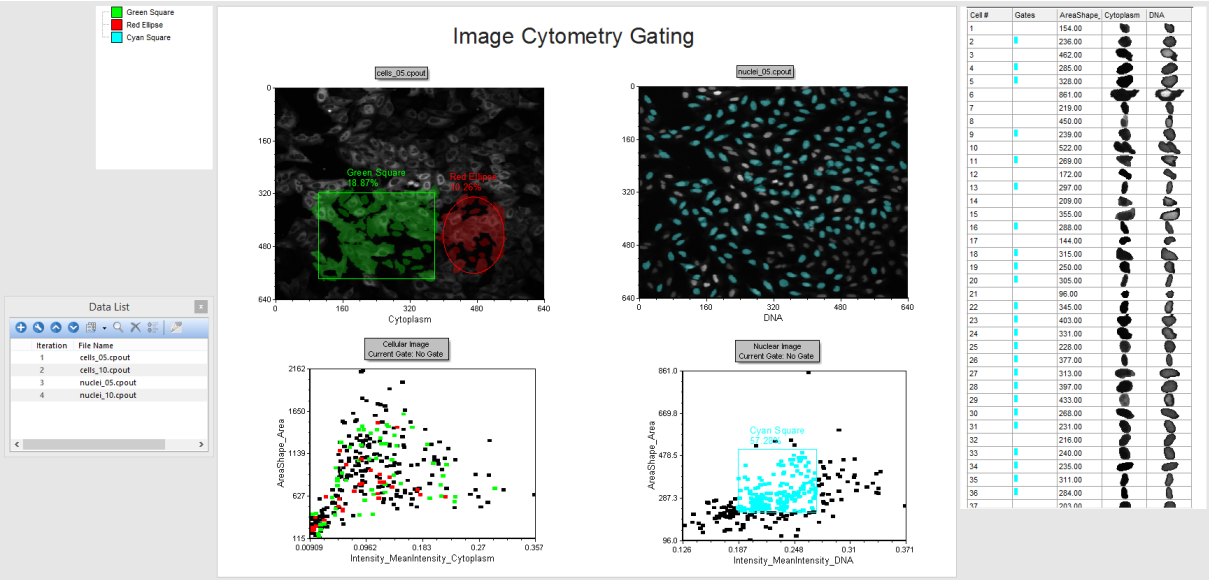

Figure T28.24 Complete Layout view for IntroToImage2.fey

55. Select and move the green square and red circle gates shown in Figure T28.25. Observe the red and green dots updating in real time on the Dot Plot 1225 below the image to indicate which cells are in the gates 400.

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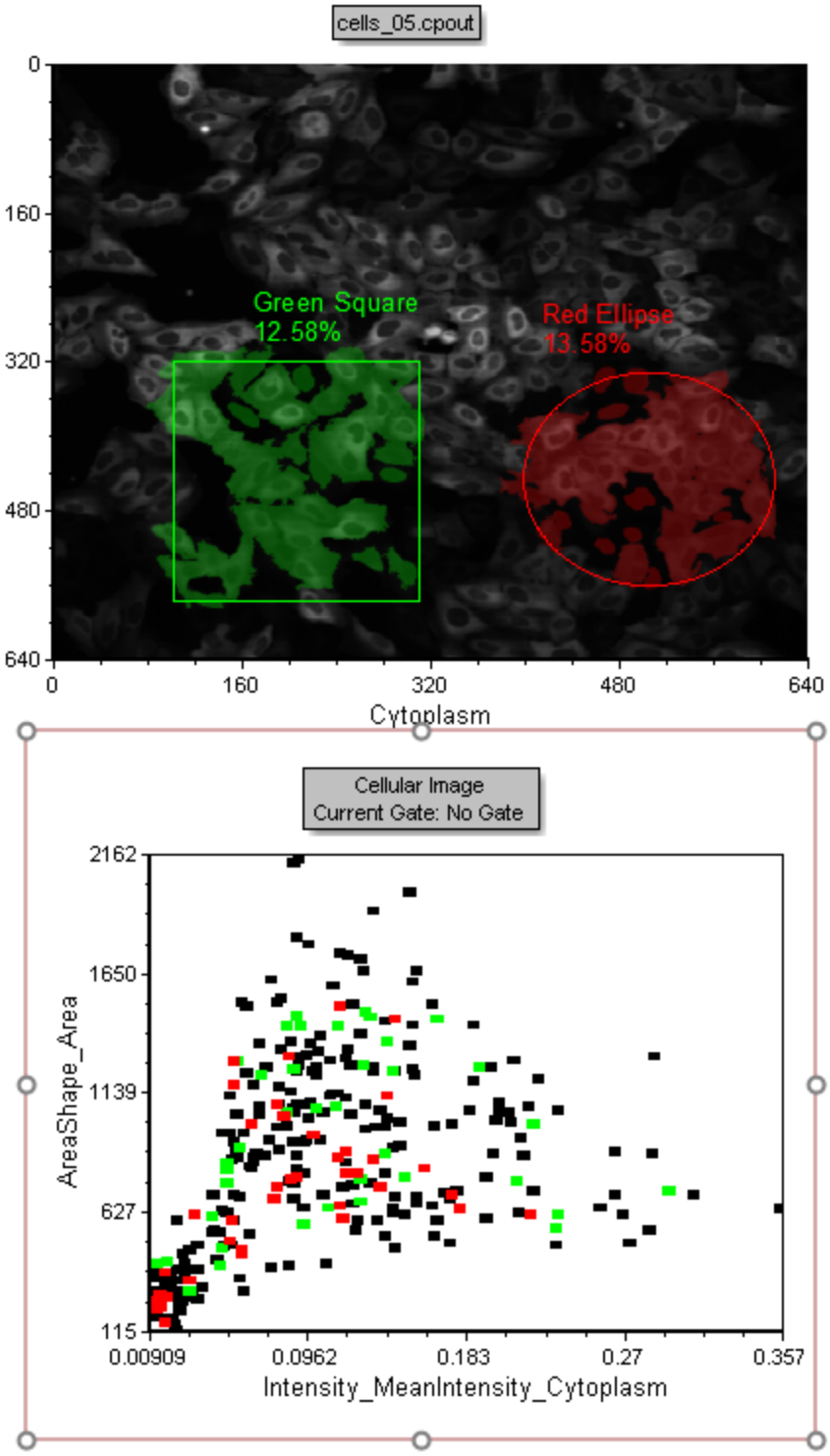

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Notice the **Cyan Square** gate on the **Nuclear Image** dot plot on the lower right side of the page (Figure T28.26). Notice how the cells in the **nuclei_05.cpout** Picture Plot are highlighted in cyan when they fall within the cyan square gate.

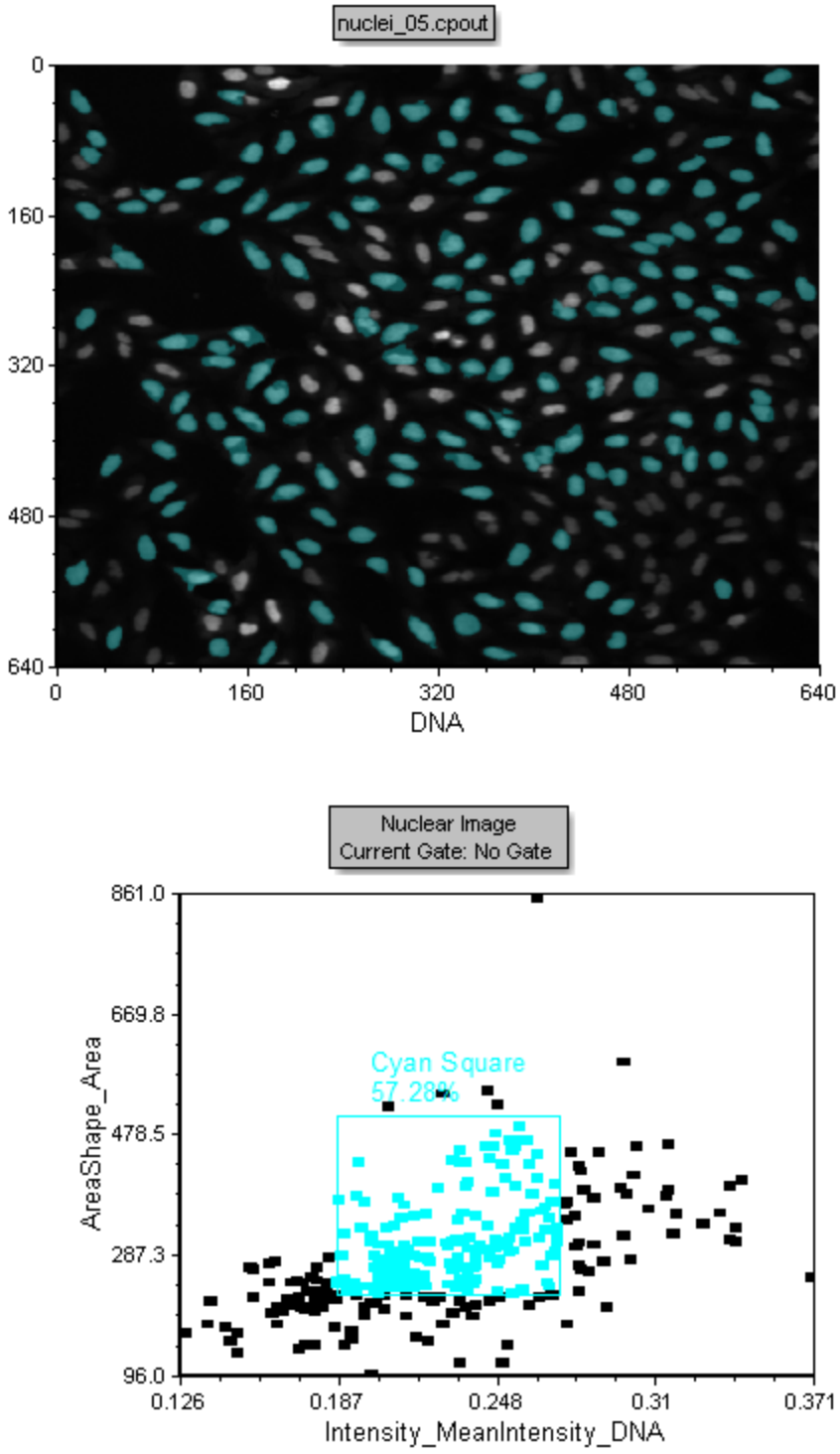

Figure T28.26 Cyan Square Gate and Nuclear Image

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56. Select, move, and increase the size of the **Cyan Square Gate** on the plot.

Notice the cyan cells updating in the **DNA** image in real time.

57. Drag and drop the **Green Square** gate from the **Cytoplasm** Picture Plot onto the **DNA** Picture Plot with cyan cells as shown in Figure T28.27.

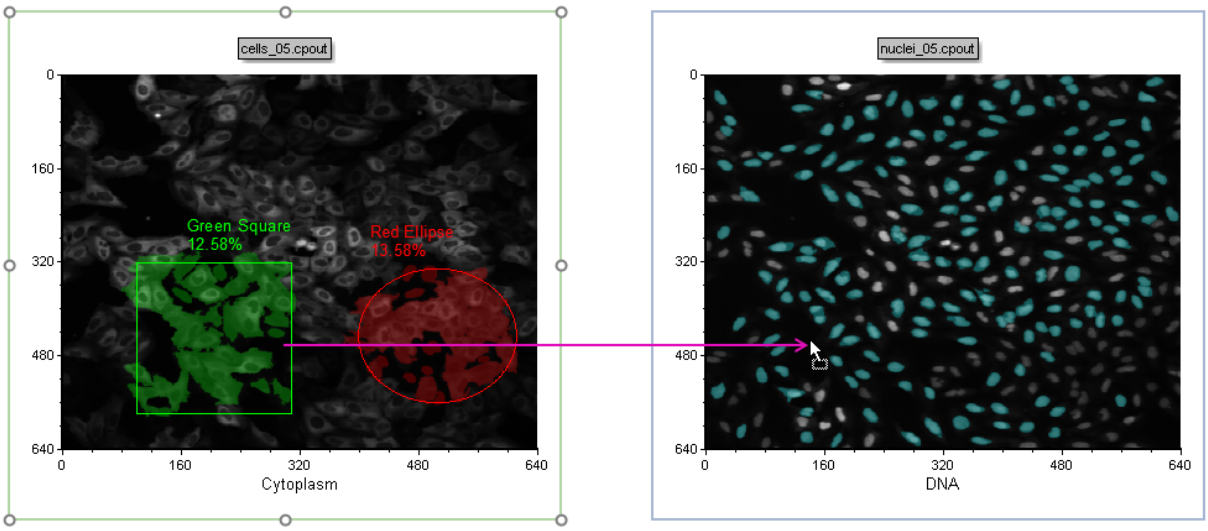

Figure T28.27 Drag and Drop a Gate to Another Plot

Notice that the only cells that appear cyan are those that fall in the **Green Square** gate. Cells that are only in the **Cyan Square** gate will appear white. The **DNA** picture plot is only highlighting cells within the cyan gate and since the Green Square gate has been applied the plot now only displays highlighted cells that fall within the combined cyan gate and the green square gate (Figure T28.28a and b).

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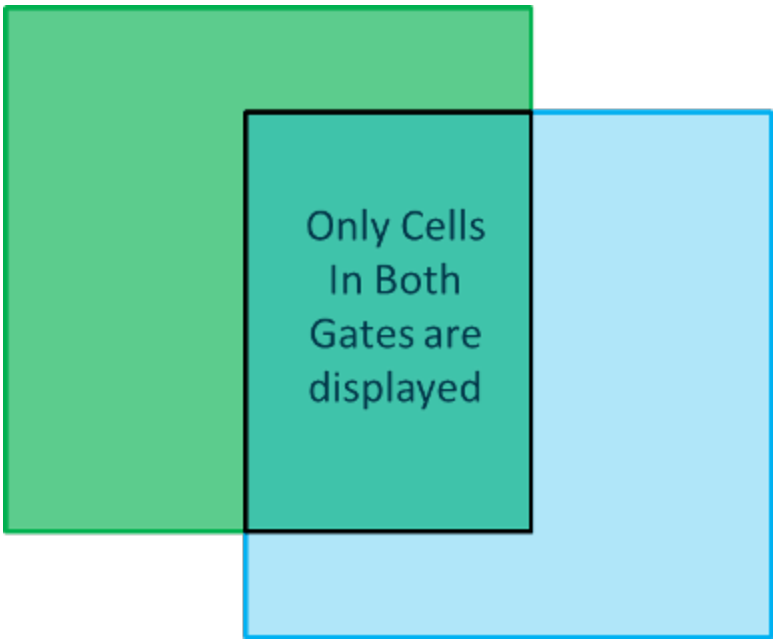

Figure T28.28a Venn Diagram of Combined Green Square and Cyan Gates

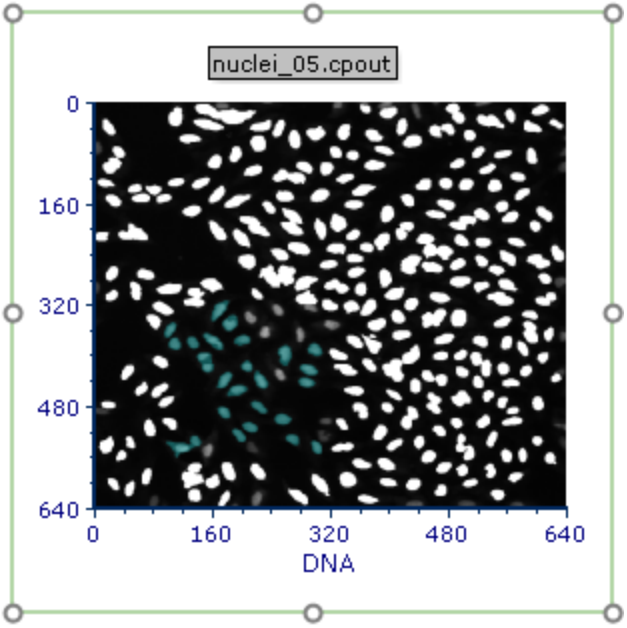

Figure T28.28b Highlighted Cells Are Within Combined Gates

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58. Press **Ctrl+Z** to undo applying the **Green Square** gate to the **nuclei_05.cpout** picture plot.
59. Drag and drop the **Cyan Square** gate from the Nuclear Image dot plot onto the **DNA** picture plot to reset the gate applied to be Cyan Square.

Notice that only the gated cells remain visible in the image (Figure T28.29). (The display may not change since the cyan gate was applied prior to adding the green gate)

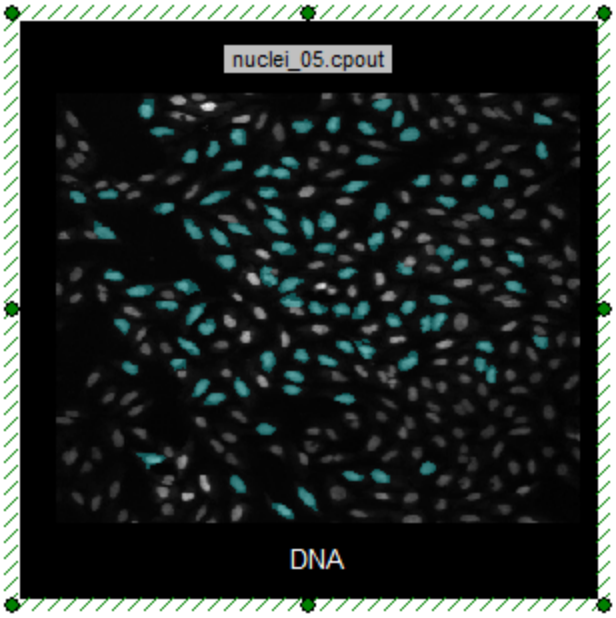

Figure T28.29 Cells within Cyan Gate Highlighted on Picture Plot

Notice the Data Grid displaying cell images. The images of cells provide a "Cell Gallery" on the right side of the layout displaying all cells in the image with the selected parametric data. Notice that the gate within which a cell resides is

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displayed by a colored rectangle in the **Gates** column (See Figure T28.30 below).

60. Drag and drop the **Cyan Square** gate from the Nuclear Image dot plot onto the Data Grid.

Notice the cell gallery component of the Data Grid now only displays the cells in the **Cyan Square** gate. Cells in the **Cyan Square** gate that are also in the **Green Square** and/or **Red Circle** are also indicated (you may have to scroll down to see them). Note that depending on the last position of the gates in the demo layout your Data Grid may not appear exactly as in Figure T28.30.

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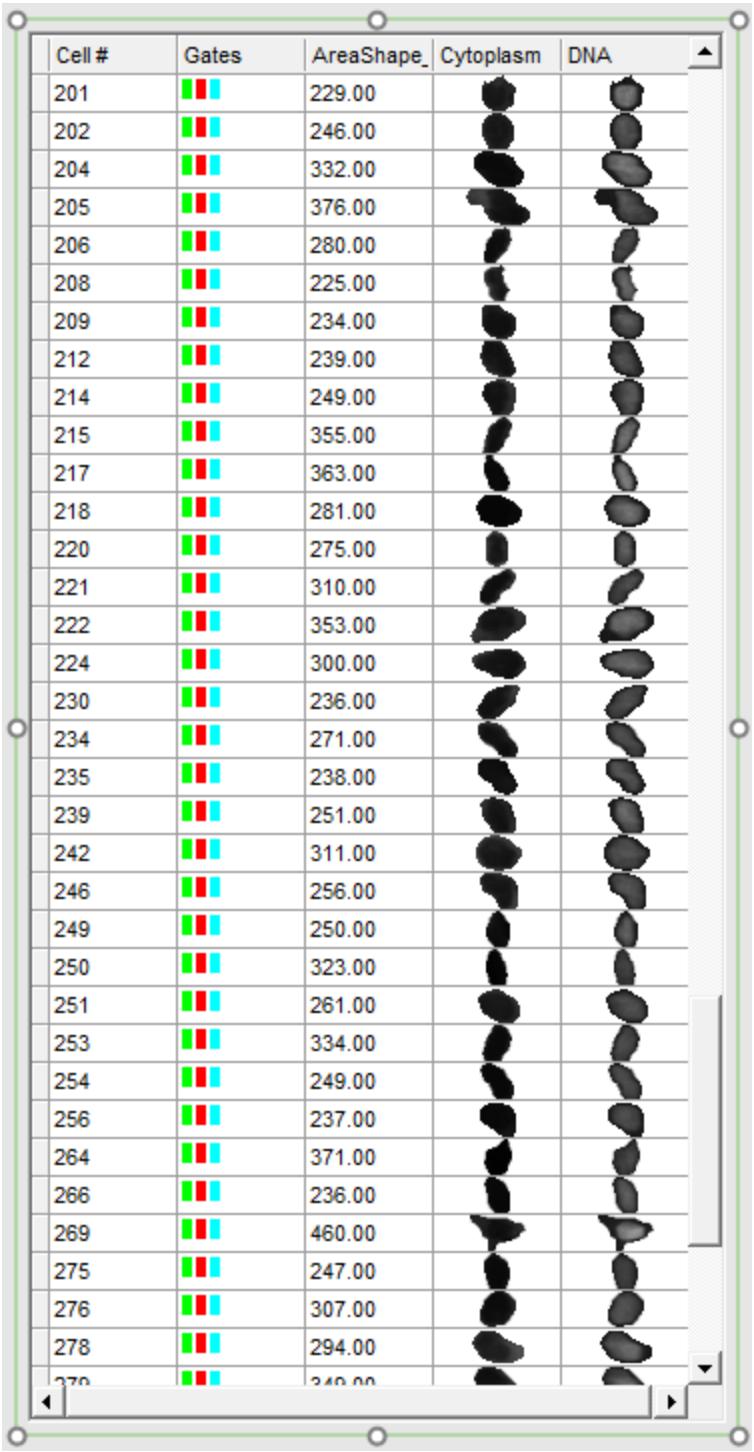

Cell #	Gates	AreaShape_	Cytoplasm	DNA
201		229.00		
202		246.00		
204		332.00		
205		376.00		
206		280.00		
208		225.00		
209		234.00		
212		239.00		
214		249.00		
215		355.00		
217		363.00		
218		281.00		
220		275.00		
221		310.00		
222		353.00		
224		300.00		
230		236.00		
234		271.00		
235		238.00		
239		251.00		
242		311.00		
246		256.00		
249		250.00		
250		323.00		
251		261.00		
253		334.00		
254		249.00		
256		237.00		
264		371.00		
266		236.00		
269		460.00		
275		247.00		
276		307.00		
278		294.00		

Figure T28.30 Example of Data Grid with Cyan Gate Applied

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61. Move the **Cyan Square** gate to a new location on the **DNA** picture plot and watch the cells in the gallery and picture plot update.
62. Left Click on the Data Grid displaying the cell gallery to select it.
63. Right click on the Data Grid and select **Format** to open the format window for the cell gallery.
64. Click **Parameters to Display**.
65. Scroll down the list and Click the check boxes next to **Location_Center_X** and **Location_Center_Y** to select them to be displayed in the cell gallery (Figure T28.31).

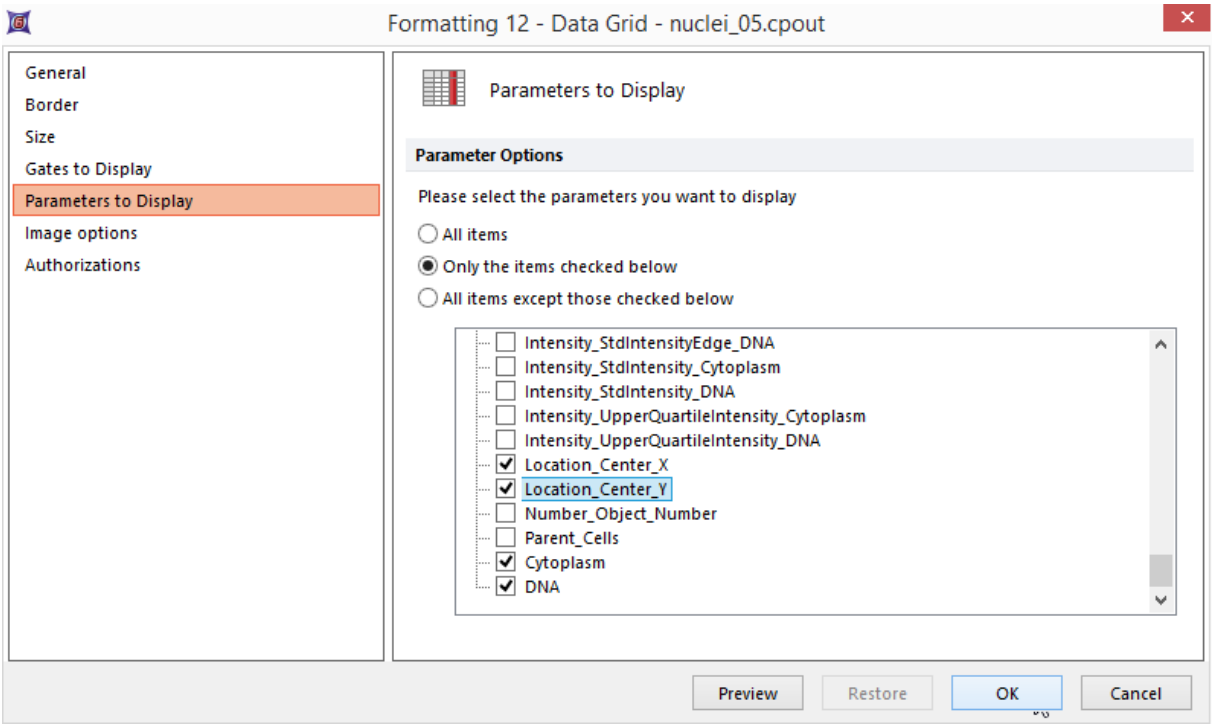

Figure T28.31 Selecting Parameters to Display in Data Grid

66. Click **OK**.

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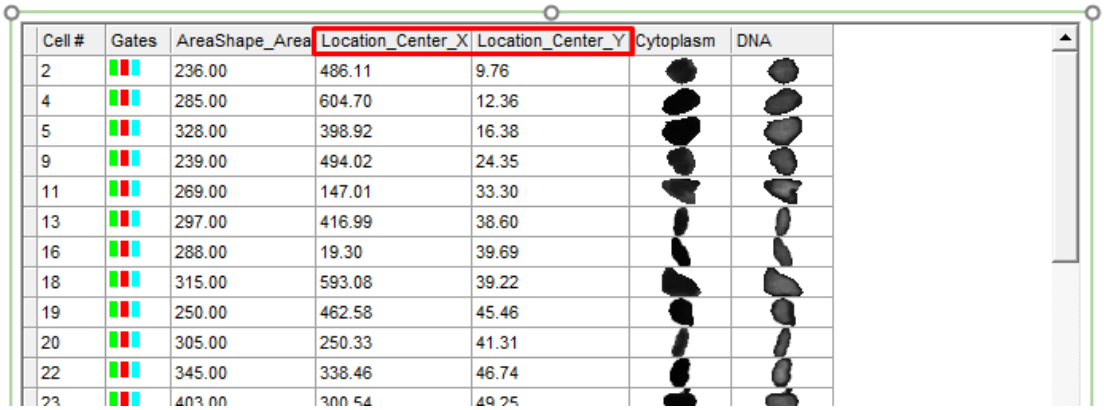
Notice that the cell gallery is now displaying the selected variables. The expand the size of the data grid to see everything. You may expand column headers can be expanded in the live software view if names are long. The cell images are displayed as a result of selecting the check boxes for the image names **Cytoplasm** and **DNA** at the bottom of the list (Figure T28.32). We will refer to adding cell images to a data grid as adding a "Cell Gallery" to a data grid.



Cell #	Gates	AreaShape_Area	Location_Center_X	Location_Center_Y	Cytoplasm	DNA
2		236.00	486.11	9.76		
4		285.00	604.70	12.36		
5		328.00	398.92	16.38		
9		239.00	494.02	24.35		
11		269.00	147.01	33.30		
13		297.00	416.99	38.60		
16		288.00	19.30	39.69		
18		315.00	593.08	39.22		
19		250.00	462.58	45.46		
20		305.00	250.33	41.31		
22		345.00	338.46	46.74		
23		403.00	300.54	49.25		

Figure T28.32 Formatted Data Grid With Added Parameters

67. Close the layout **do not save** changes.

Notes: To learn more about Gating options see the tutorial called "Gating 318".

Continue to the next topic - Using the High Content Module with Image Data. 2130

38.30.2 Using The High Content Module with Image Data

In this section, we will learn how to:

- Load a layout form a nuclear translocation assay run on a 96-well plate
- Review layouts and examine control well results with picture plots and dot plots
- Use Heat Maps to review key variables measured on cells in a plate view
- Use multiple heat maps and data grids to display results in a report

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- Optimize gate settings for the nuclear translocation assay
- See how FCS Express 6 "Tokens" can be used to enhance reporting
- Create and manipulate a Data Grid to display a "Cell Gallery"

We will be using the "**IntroToImage3.fey**" layout for this section which is located in the Tutorial Sample Data archive 1210 →**Intro to Imaging Tutorial** folder. This layout contains a detailed pre-configured template. Please complete the Getting started with Image Cytometry 2099 section of this Tutorial before beginning if you are not familiar with FCS Express 6 Image Cytometry.

Section 1: Image Gating Strategies for Data Analysis with the High Content Module

Image cytometry is often carried out on many images acquired from slides or multi-well plates to interrogate various conditions, compounds or multiple sample sources. Cellular Imaging studies carried out under multiple conditions is typically called "High Content Analysis". The **High Content add-on** for FCS Express enables working with and viewing of data from multiple samples in heat maps 1050. This data is typically from multi-well plates (from 6 up to 1536 well plates). Heat maps can display variables measured from the cells in the well encoded as a color map.

We have not provided data for all wells in the plate in the examples used here because of the large size of the original data set. If you would like to work with the full data set, or create your own, please visit the Exporting 96 Well Format Images 1921 section of the **CellProfiler Export** tutorial. The instructions there will provide you with a link to the original data set, a completed CellProfiler pipeline for analysis, and instructions for importing and viewing the data in FCS Express 6 Image Cytometry.

1. Load the **IntroToImage3.fey** layout from the Tutorial Sample Data archive 1210 →**Intro to Imaging Tutorial** folder.

Make sure you are viewing the **Experiment Design** page. This page contains results for a nuclear translocation assay from a 96 well plate. A Heat Map 1050 displays a representation of wells in the plate along with a measured parameter as the Y-Axis (**Intensity_IntegratedIntensity_Cytoplasm** in this case) on a green (low) to red (high) color scale.

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Below the Heat Map 1050 is a plate map of the compounds added to each well (Figure T28.33) which was inserted as an image. Column 1 contains replicates of 4 negative and 4 positive control wells. The Picture Plot 2060 in the upper right displays an image of the cytoplasmic stain from well A-01. and the Dot Plot 1225 below it displays the integrated cytoplasmic intensity plotted against area for the cells in that image. On the far left a "Gate View 345" shows a summary of all of the pre-configured gates used in this layout. We will not address all of the gates in this tutorial but you may find them useful when investing the layout further.

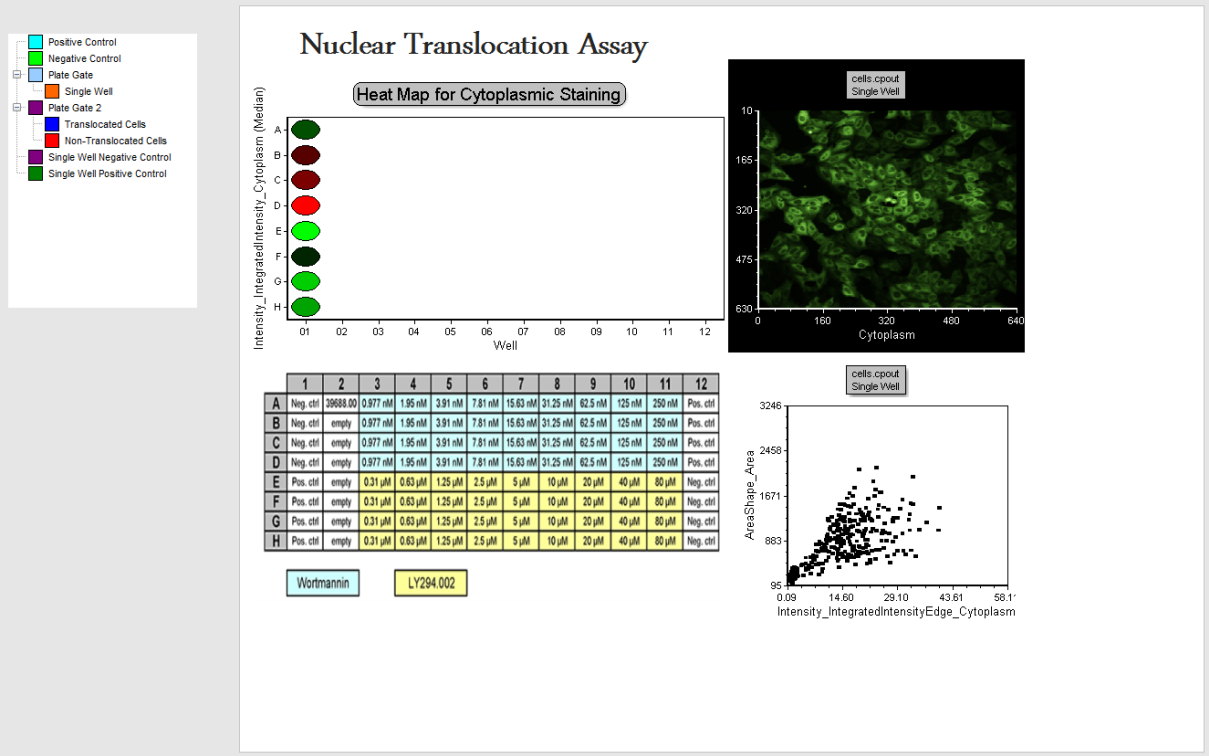

Figure T28.33 The Experiment Design Layout Page

2. Right click on the Heat Map 1050.

3. Select **Format**.

4. Choose the **Overlays** category (Figure T28.34).

Notice you can to plot any parameter that was measured for cells in each well by changing the **Statistic parameter** drop down and you can choose the displayed

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statistic of the parameter with the **Statistic to show** drop down. In this case we are plotting the **Median** value for all of the cells. Review variable options, but do not change from **Intensity_IntegratedIntensity_Cytoplasm**.

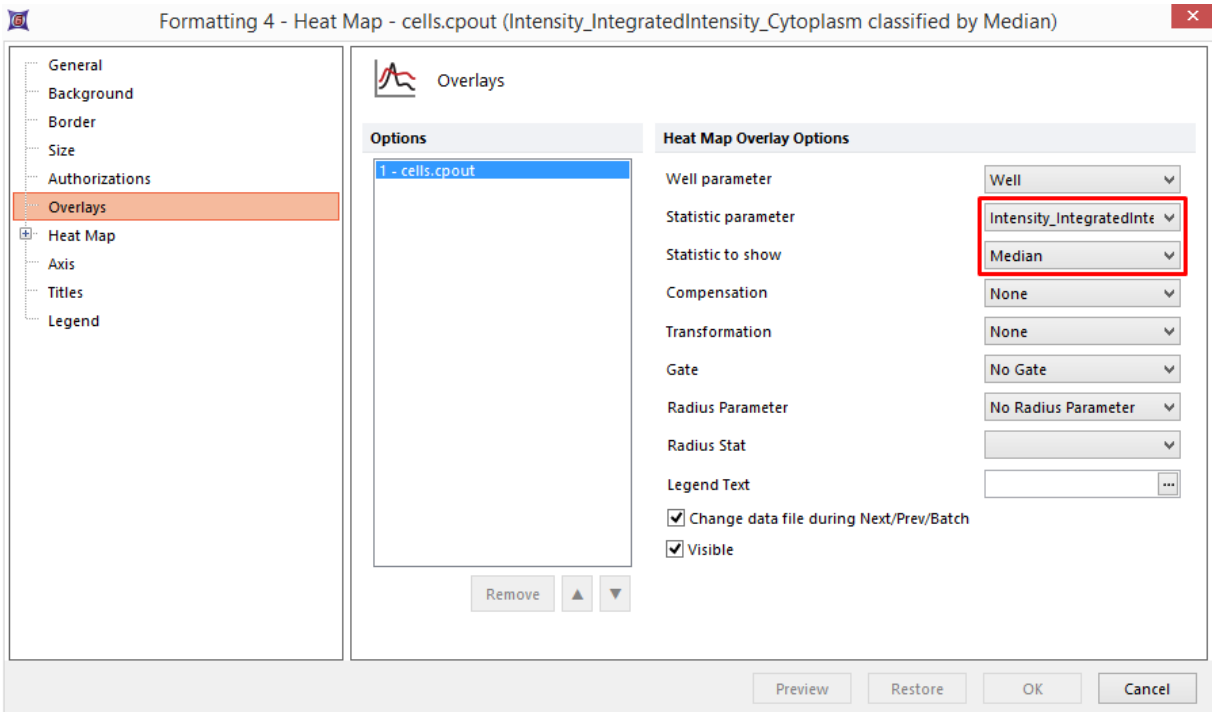

Figure T28.34 Format Options for Heat Map

5. Left Click on the "**+**" sign next to Heat Map 1050 category to expand options for the heat map display (Figure T28.35).
6. Select the **Color Levels** category.

This category lets you configure the coloring scheme used on the Heat Map. Observe that you can change the color scheme with the **Selected Color Level** drop down, the type of color display formatting with the **Style** drop down, and choose to display **Color Scaling** in a **linear** or **logarithmic** manner.

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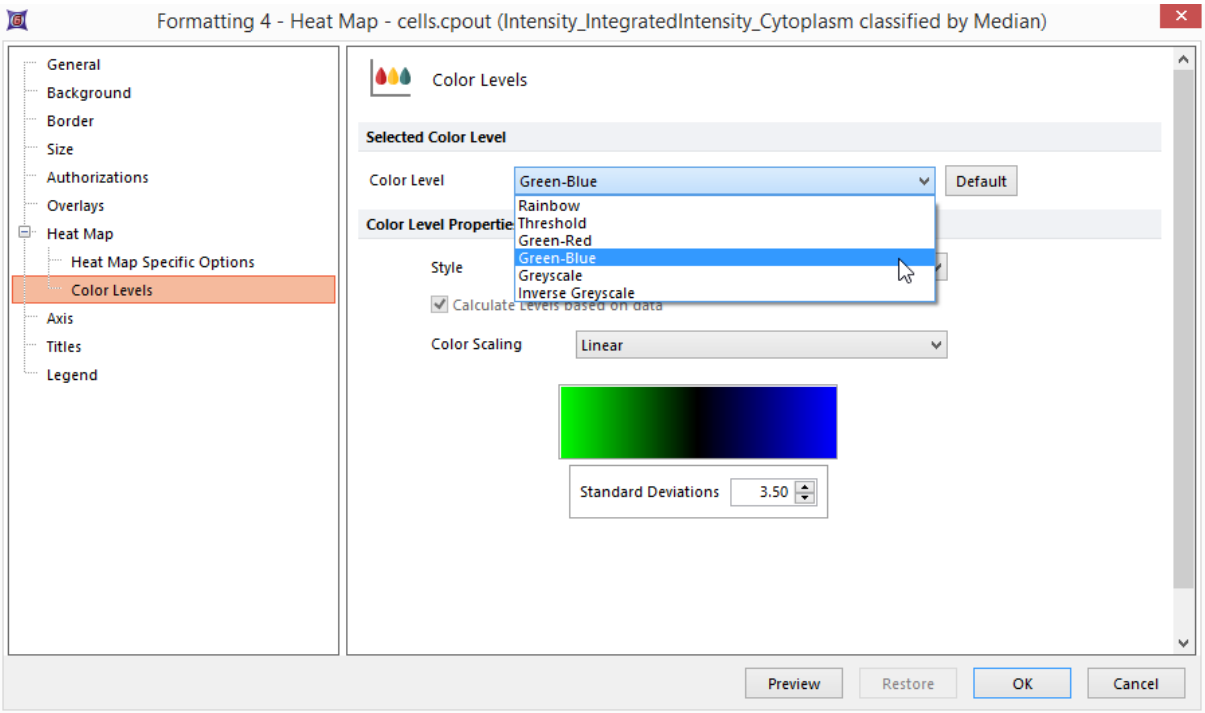

Figure T28.35 Color Formatting Option for Heat Maps

7. Change the **Selected Color Level** to **Green-Blue**.

8. Click **OK**. The heat map will now look like Figure T28.36 below.

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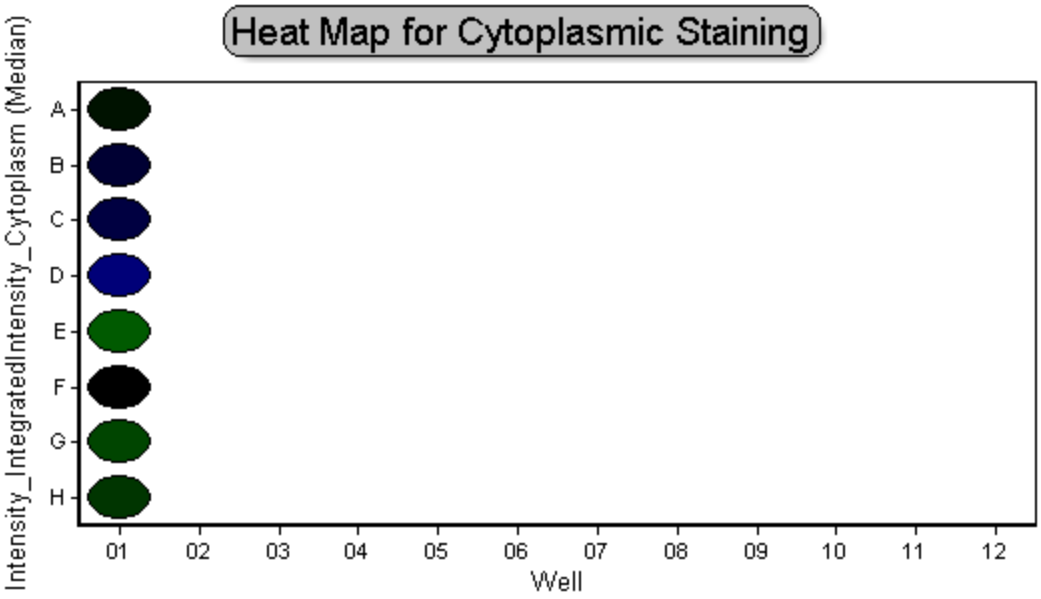

Figure T28.36 Green-Blue Scaled Heat Map

9. Right click on the Heat Map 1050.

10. Select **Edit Gate→Single Well** from the popup menu that appears.

This will allow us to edit the "single well" gate that had been created previously. Notice an orange circle appears to highlight well A-01 on the Heat Map (Figure T28.37a) indicating that well A-01 is the only member of the "single well" gate. Looking at the plate map of compounds you will notice that this is one of the four negative control wells (Figure T28.37b).

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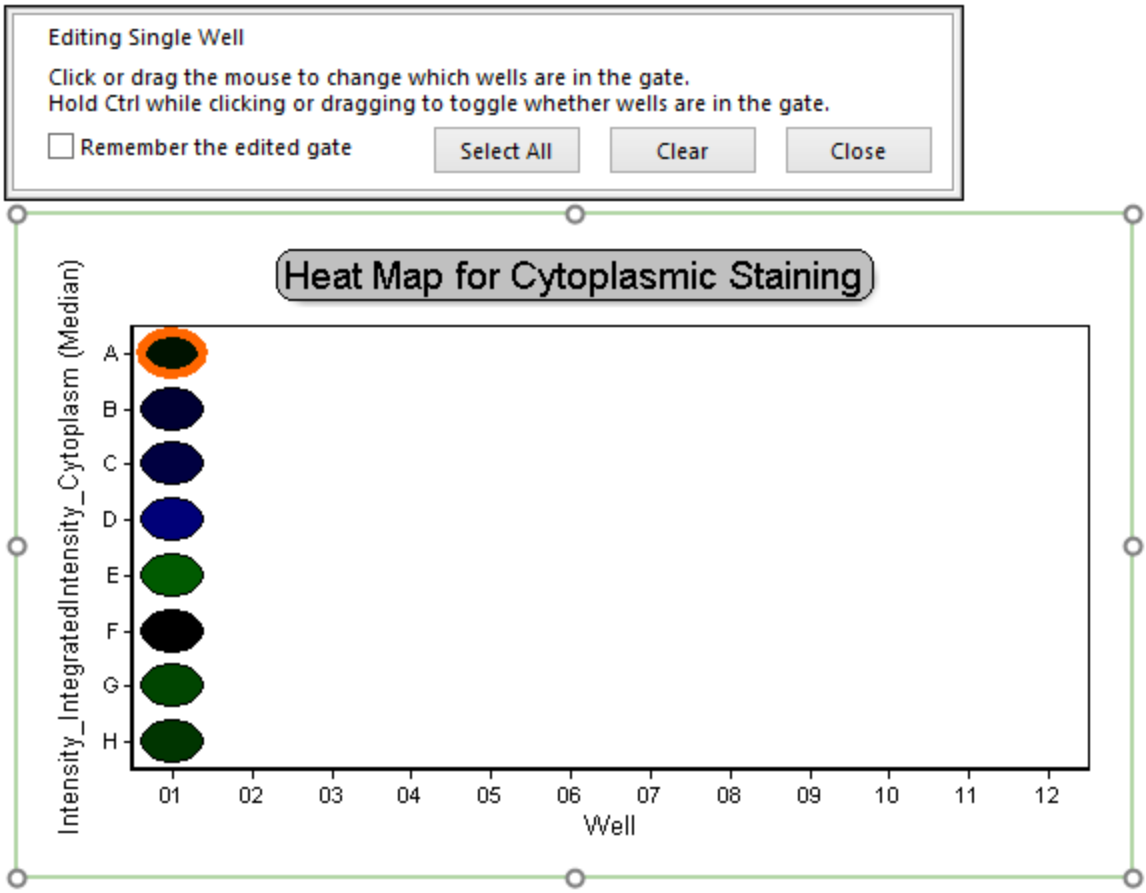

Figure T28.37a Activating Single Well Gate on Heat Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg. ctrl	39688.00	0.977 nM	1.95 nM	3.91 nM	7.81 nM	15.63 nM	31.25 nM	62.5 nM	125 nM	250 nM	Pos. ctrl
B	Neg. ctrl	empty	0.977 nM	1.95 nM	3.91 nM	7.81 nM	15.63 nM	31.25 nM	62.5 nM	125 nM	250 nM	Pos. ctrl
C	Neg. ctrl	empty	0.977 nM	1.95 nM	3.91 nM	7.81 nM	15.63 nM	31.25 nM	62.5 nM	125 nM	250 nM	Pos. ctrl
D	Neg. ctrl	empty	0.977 nM	1.95 nM	3.91 nM	7.81 nM	15.63 nM	31.25 nM	62.5 nM	125 nM	250 nM	Pos. ctrl
E	Pos. ctrl	empty	0.31 µM	0.63 µM	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	Neg. ctrl
F	Pos. ctrl	empty	0.31 µM	0.63 µM	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	Neg. ctrl
G	Pos. ctrl	empty	0.31 µM	0.63 µM	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	Neg. ctrl
H	Pos. ctrl	empty	0.31 µM	0.63 µM	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	Neg. ctrl

Wortmannin LY294.002

Figure T28.37b Compound Map For 96-Well Plate

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11. Click on **well B-01** (another negative control well).

By clicking on **well B-01** you have changed the "**single well**" gate to contain only the **well B-01** data, and no longer contain the **well A-01** data.

Notice that the picture plot and dot plot on the page update to display image and measured data from **well B-01** (Figure T28.38a).

12. Click on **well E-01** (positive control). Notice that the picture plot and dot plot on the page update to display image and measured data from **well E-01** (Figure T28.38b).

Notice that the image of **well E-01** displays a more concentrated morphology of the nucleus. Notice the events in the dot plot distribution are concentrated towards show smaller and dimmer cytoplasmic staining. This is consistent with the fluorescence moving from the cytoplasm to the nucleus. These changes in distribution can be used to help establish gates.

Well B-01 (Negative Control) **Well E-01 (Positive Control)**

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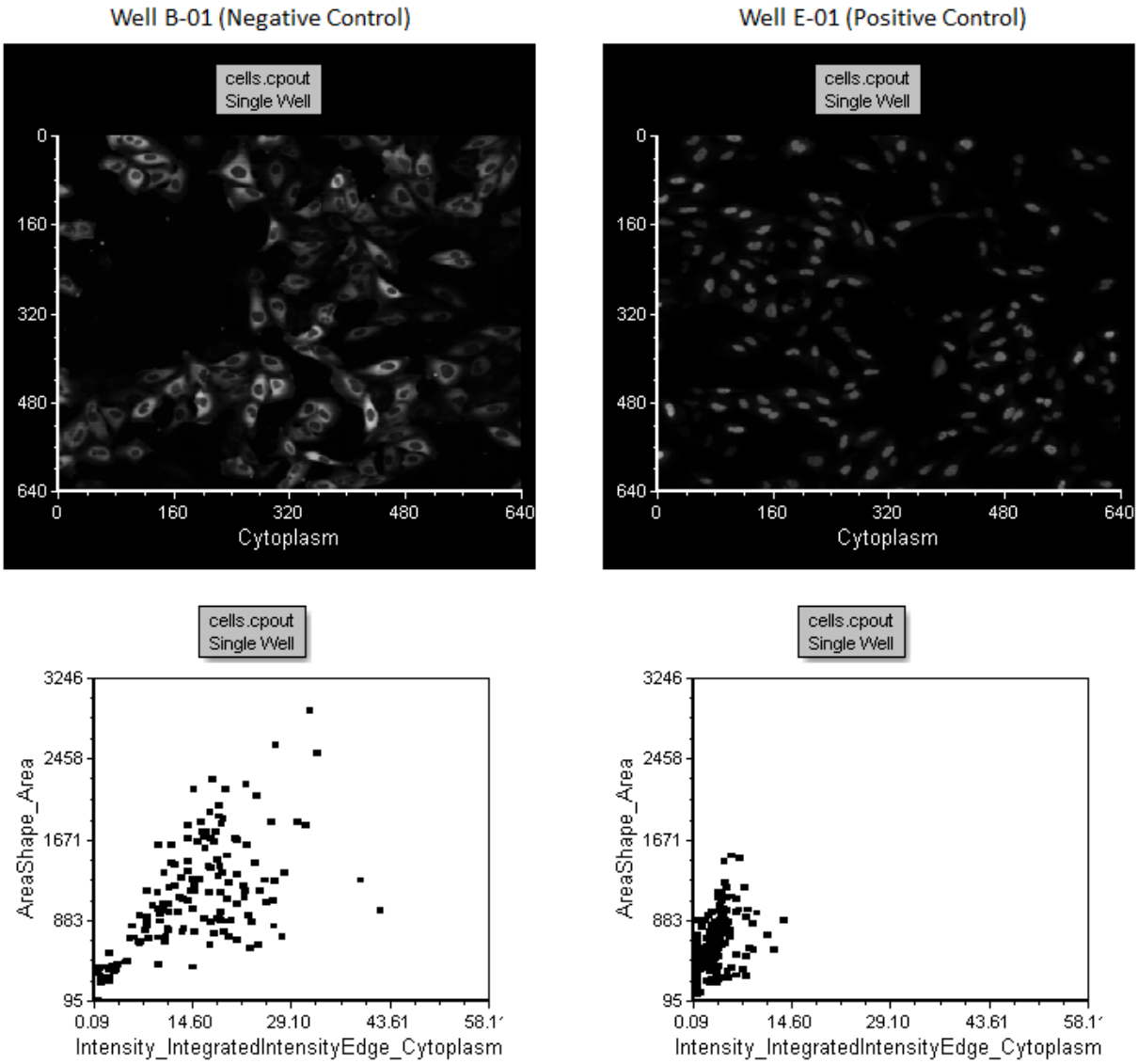

Figure T28.38 Wells B-01 and E-01

Choosing Optimal Parameters

13. Select the **Choosing Parameters** page.

Notice there are three dot plots on this page. The top dot plot "**All Cells No Gate"** displays the distribution of all cells by plotting

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Intensity_IntegratedIntensity_Cytoplasm against **Intensity_IntegratedIntensity_DNA**. On this plot cells tend to be a loosely clustered distribution (Figure T28.39).

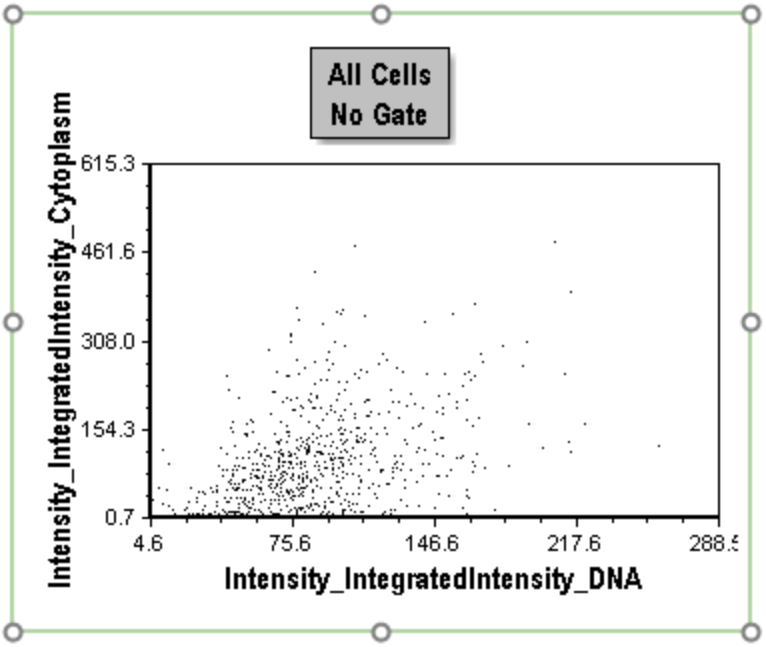

Figure T28.39 Choosing Parameters Using a Dot Plot

14. Right click on the dot plot "**All Cells No Gate**".
15. Choose **Format**.
16. Select the **Overlays** category.
17. Change the **X Parameter** to

    Intensity_IntegratedIntensityEdge_Cytoplasm.
18. Click **OK.**

View Figure T28.40 Below. Notice this plot is a distribution of all cells in the first column of the plate. This is optimal for creating gates because of the significant

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separation of cells throughout positive and negative control conditions. The positive control cells are clustered toward the origin of the plot (Figure T28.40). We have now identified two parameters to use that give good separation of the positive and negative controls. During the onset of protein translocation from the cytoplasm to the nucleus the outer edge of the cytoplasm change most rapidly. Thus, comparing the fluorescence of the outer edge of cytoplasm to the whole cytoplasm is a sensitive indicator of the redistribution of the protein toward the nucleus. We will proceed using this combination of parameters for the rest of the tutorial.

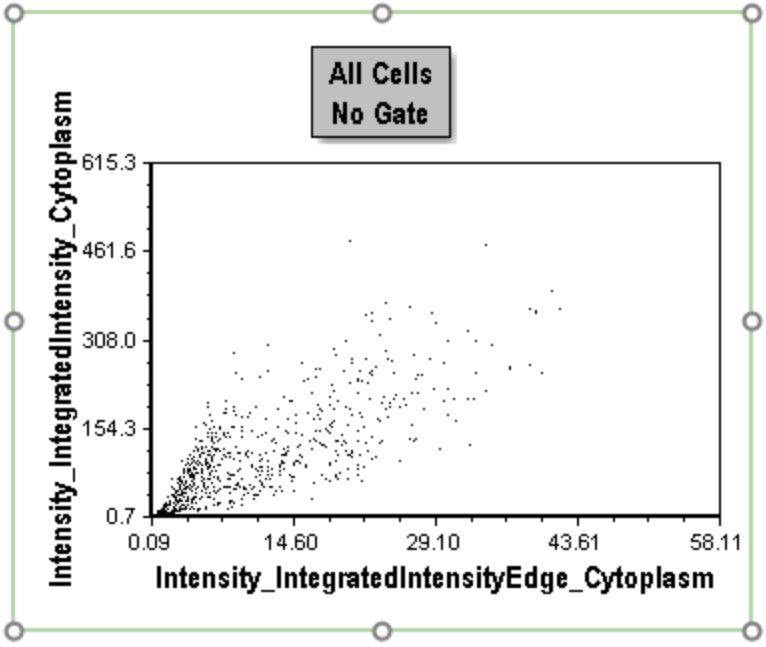

Figure T28.40 Choosing Alternate Parameters

Notice that the lower two dot plots (Figure T28.41) are displaying cells for either the positive control wells only or the negative control wells only, we see very

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different population distributions. These have been used previously to help define gates for the tutorial.

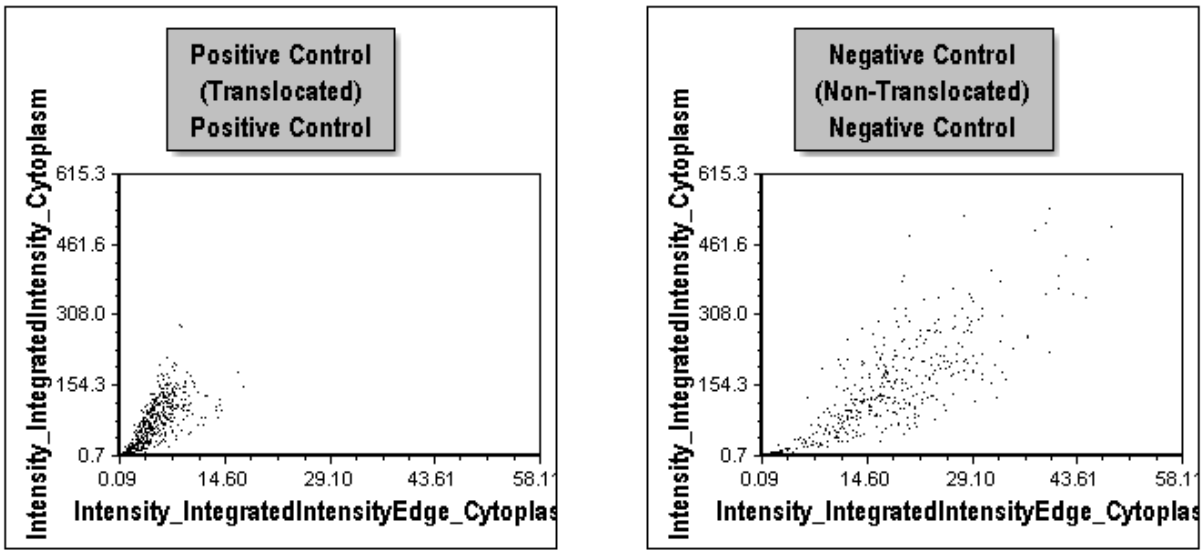

Figure T28.41 Comparison of Distributions of Positive and Negative Control Cells

19. Select the **Applying Gates** page.

Notice in Figure T28.42 the picture plots are displayed for both a positive and negative control well, with corresponding dot plots below them. Gates have been applied to all plots for **Non-Translocated Cells** shown in red and **Translocated Cells** shown in blue.

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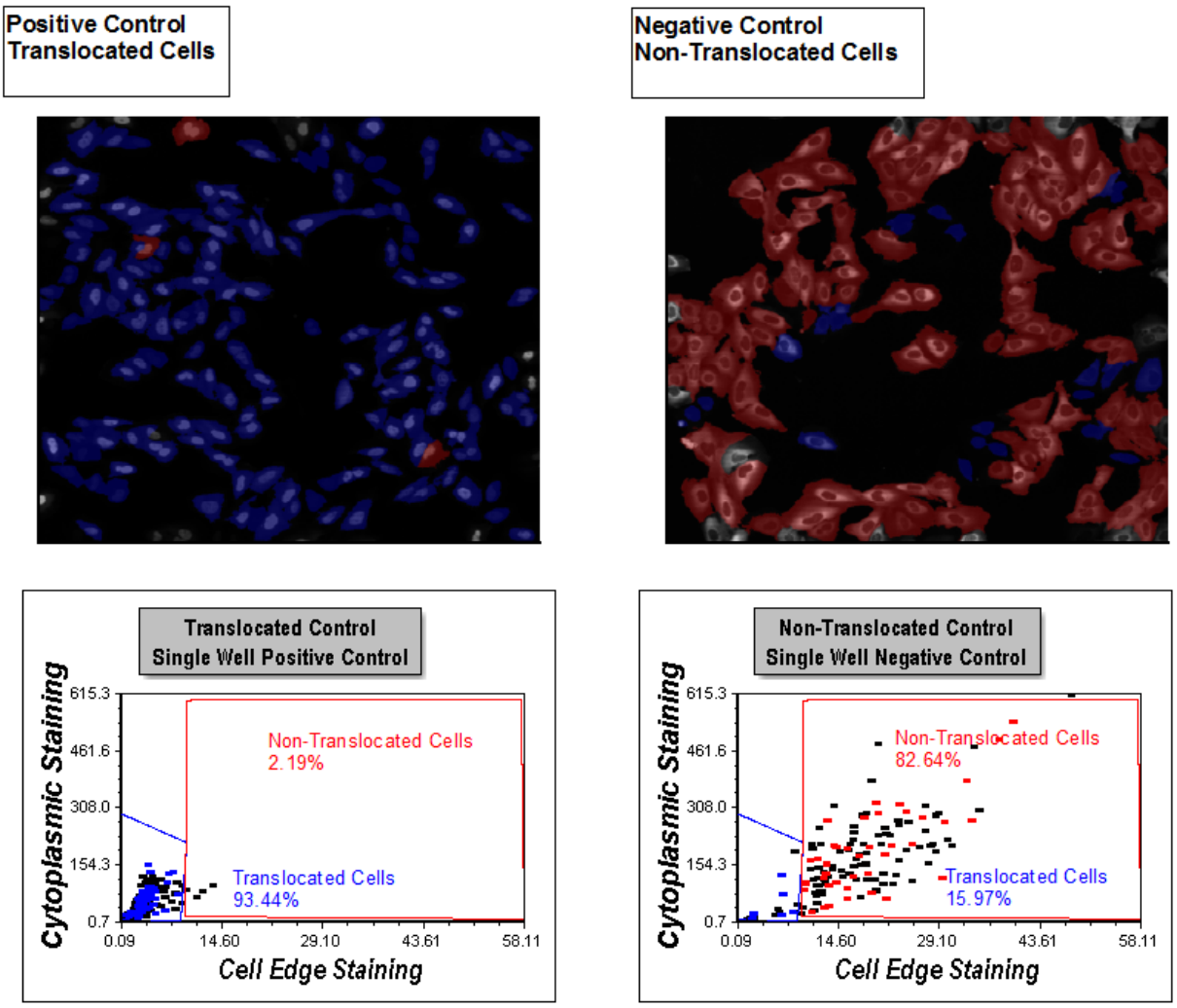

Figure T28.42 Display of Gates on Dot Plots and Gated Cell in Picture Plots

Examine the picture plots. You can see the distinct morphological differences in cells that are highlighted red or blue. Review the dot plots. You can see single positive and single negative control well dot plots with gates applied for **Translocated Cells** and **Non-Translocated Cells** respectively. The gating was completed with guidance from viewing distributions in the **Choosing Parameters** page and also by viewing the picture plots to be sure the gates select for the right morphology in this page. The picture plots are back gated to

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highlight cells within the **Translocated Cells** and **Non-Translocated** gates as blue and red, respectively. Notice that the percentage of cells in each gate is displayed in a text box on each plot. 93.44% of cells are **Translocated Cells** (blue) in the Positive Control picture plot on the left and only 2.19% cells are **Non-Translocated Cells** (red). The cells in the **Non-Translocated Cells** gate still have significant cytoplasmic edge staining and have not translocated, even though they are in the positive control well.

In the **Non-Translocated** picture plot 82.64% of the cells reside in the **Non-Translocated** gate and in the image they primarily exhibit cytoplasmic staining with an absence of nuclear staining. 15.97% of the cells reside in the **Translocated Cells** gate. This is the expected behavior for the controls, because compound that drives translocation can be nearly 100% effective while there is always some auto activation in the negative control condition, resulting in a noticeable presence of translocated cells. Using this gating strategy we can account for the auto-activation in negative controls in subsequent analysis on a cell-by-cell basis. Accounting for these cell sub-populations can improve the assay quality.

Note: In this example the **Translocated Cells** and **Non-Translocated** gate **names** are displayed on the dot plots as well as the specific **percentage of cells** within a gate. This is accomplished easily by using "Tokens" in FCS Express. Tokens are very powerful and allow you to insert a statistic (and many other

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pieces of information) in a text box either on a page or on plots. Tokens can be used in many locations in a layout to display information from other locations in the layout. The values or text of Tokens update automatically as gates are repositioned or new data sets are loaded. We will not discuss Tokens in detail here. For more information on creating and using "Tokens" see the tutorial "Text Boxes and Tokens" or review the Manual section entitled "Using Tokens".

20. Select the blue **Translocated Cells** gate on either dot plot by clicking on it with the left mouse button.
21. Hold the left mouse button down and drag to move the gate a small amount to the right (Figure T28.43).
22. Release the mouse when the gate is in the desired position.

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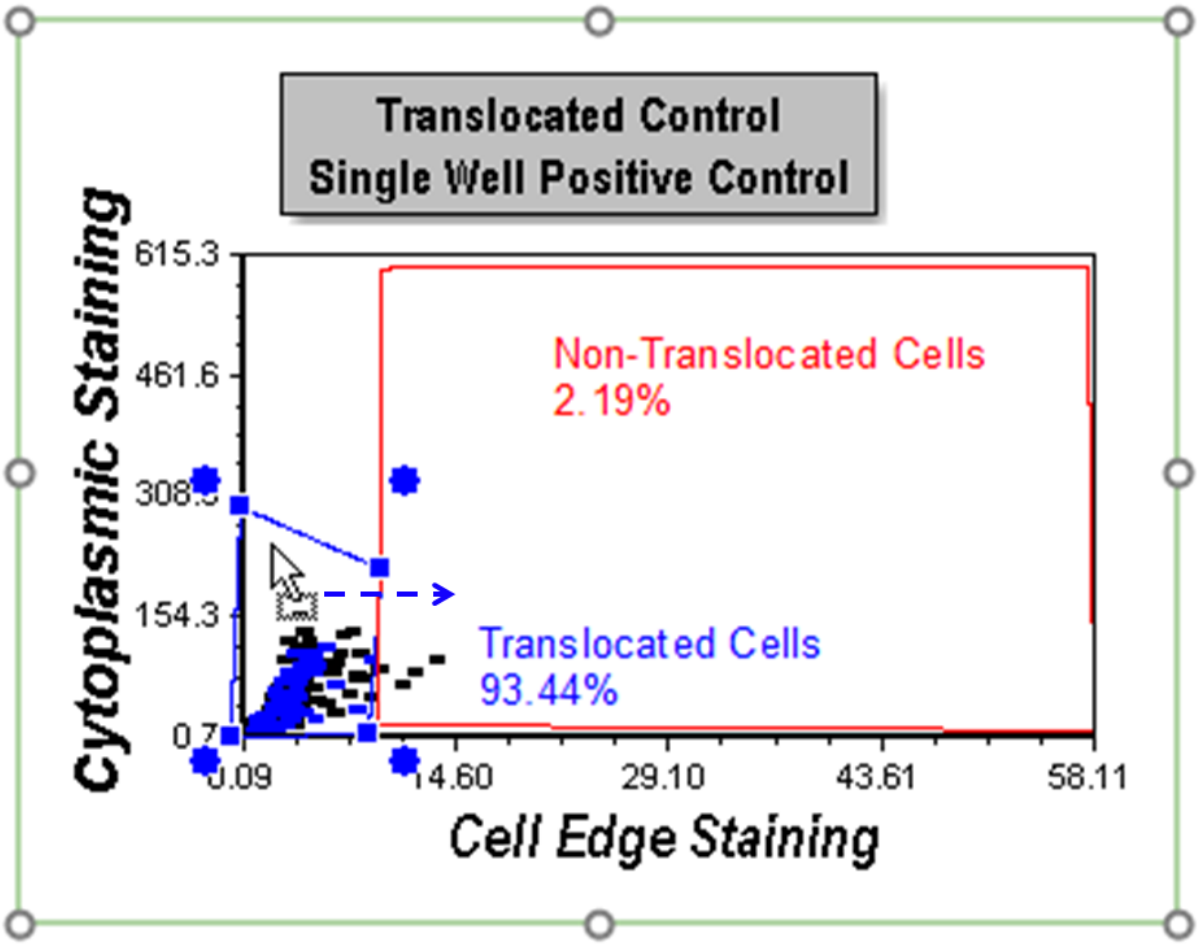

Figure T28.43 Moving the Translocated Cells Gate

Notice that the back gating has adjusted to show the cells in colors based on the new position of the gate (Figure T28.44). Any cells not residing in either gate have no color. Notice that all plots and statistics displayed for gates update. Note: your layout may not appear exactly at in Figure T28.44 because you make not move the **Translocated Cells** Gate to the exact same position as in the example figure.

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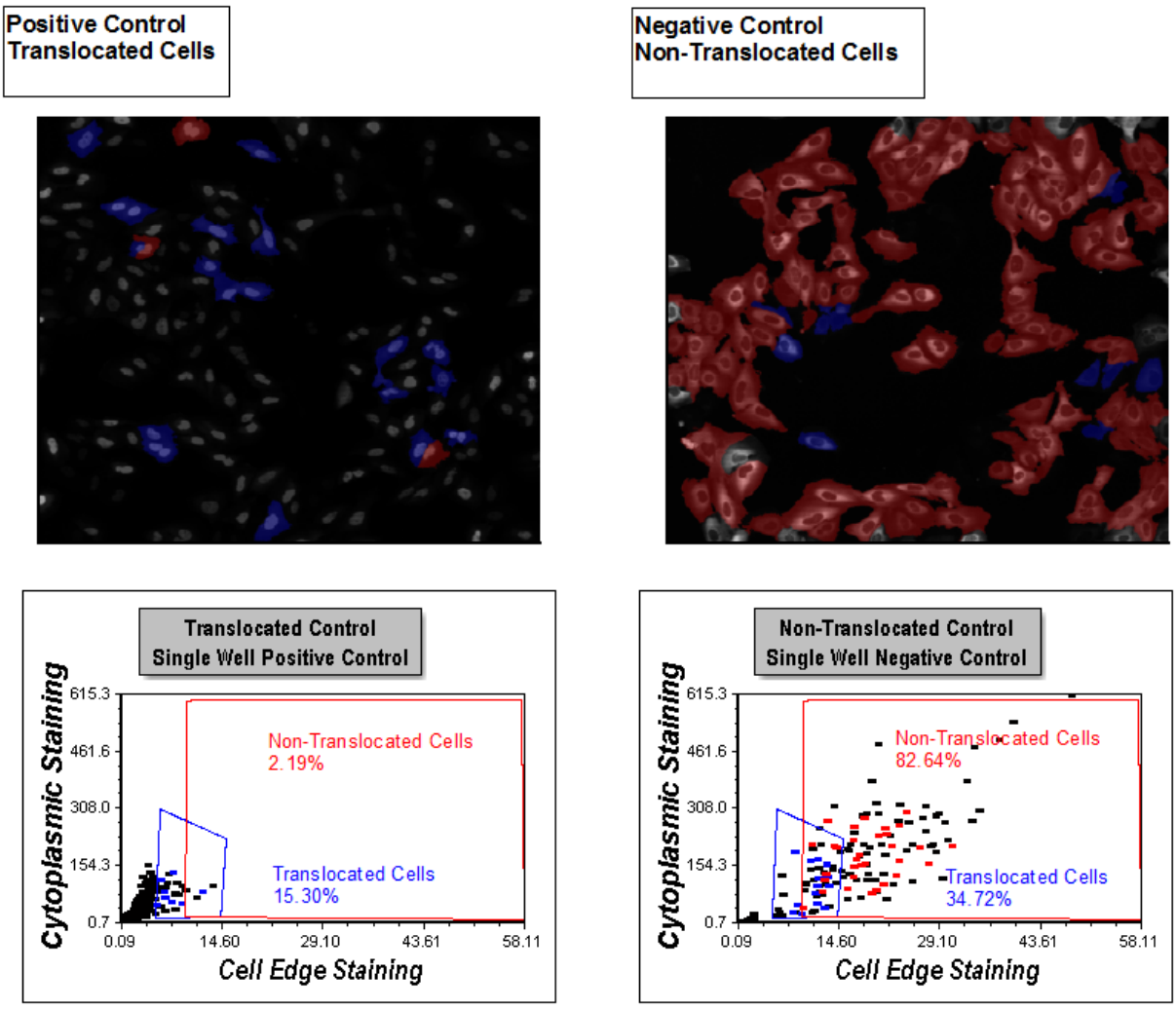

Figure T28.44 All Plots Update Following Repositioning of Translocated Cells Gate

23. Undo the gate repositioning by pressing **Ctrl+Z**.

Section 2: Using Data Grids To Create a Cell Gallery View

24. Select the **Insert** tab→**General**→**Data Grid** (Figure T28.45).

25. Insert a **Data Grid** anywhere on the layout where you have space.

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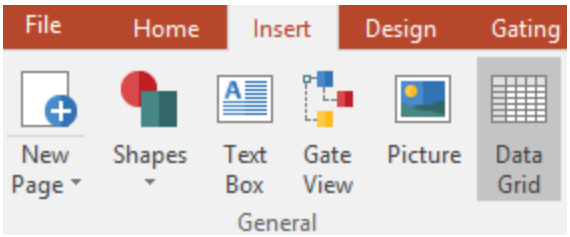

Figure T28.45 Inserting a Data Grid

26. Right click on the **Data Grid**.
27. Select **Format**. The **Formatting** dialog will appear (Figure T28.47).
28. Select the **Parameters to Display** category.
29. Select the radio button to display "**Only the items checked below**".
30. Scroll down to the bottom of the parameter list and check the boxes next to: **Cytoplasm, DNA,** and **Well** (Figure T28.47).

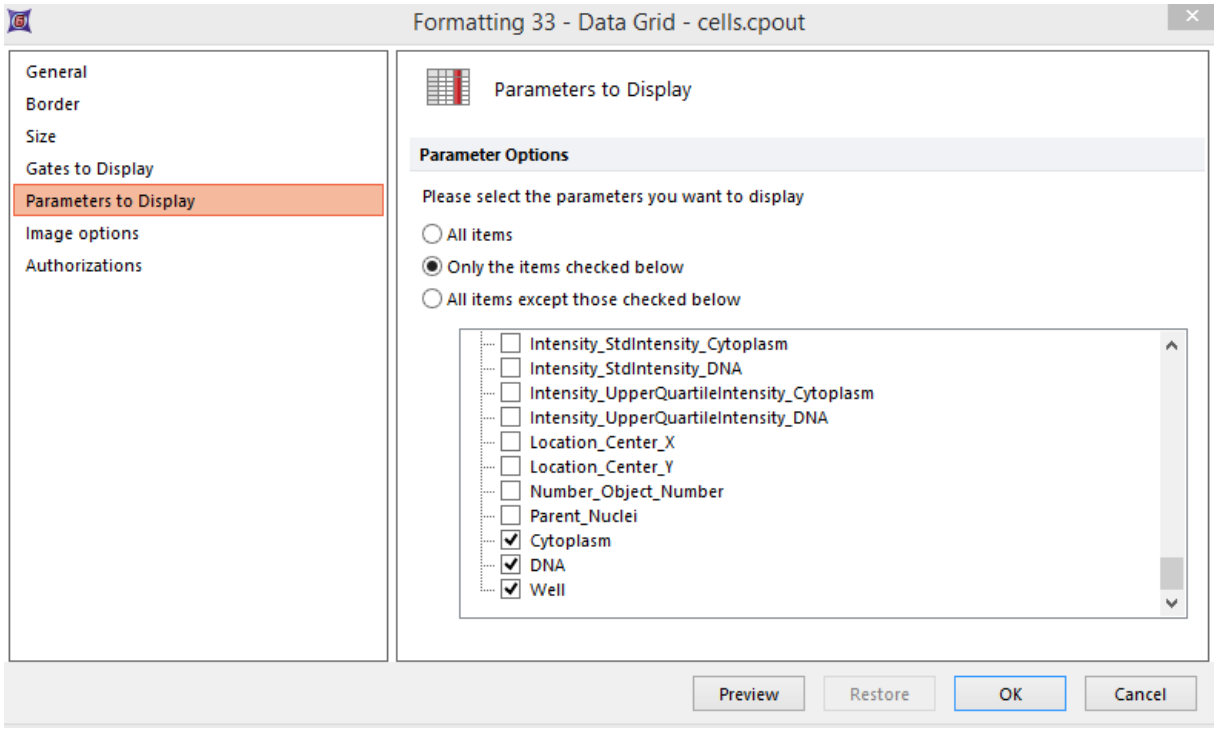

Figure T28.47 Configuring Parameters to Display

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31. Click **OK**.

You will now see a Data Grid displaying images of the cells with their parametric information. We will refer to this configuration of a Data Grid as including a "Cell Gallery" display (see Figure T28.48).

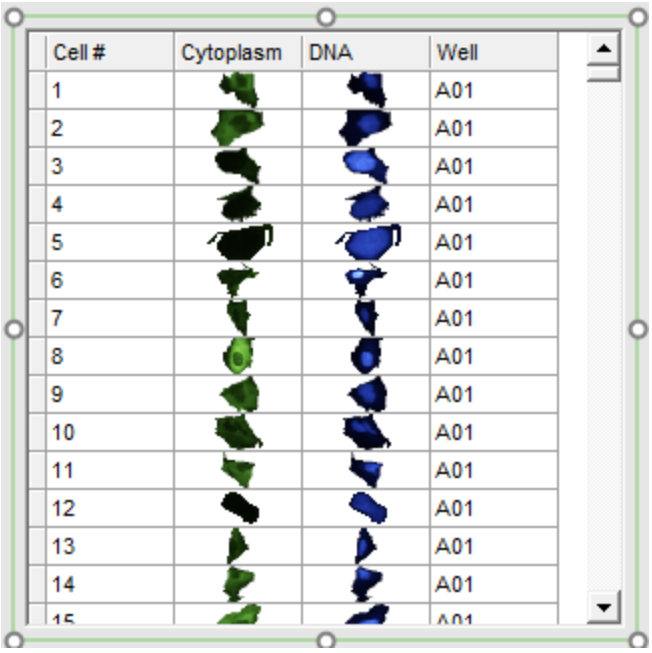

Figure T28.48 Data Grid Configured to Display Cell Images in a Cell Gallery

32. Click on the border of the Data Grid displaying the cell gallery to be sure it is selected. A red border will appear so you know it is selected properly.

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33. Select the **Gating** tab. Observe in the **Current Gate** box on the left that is says "**No Gate**" (Figure T28.49). This means that all cells in the 8 wells are being shown in Data Grid as no events are being gated out.

Figure T28.49 Current Gate Display

34. Select the **Translocated Cells** Gate in the **Current Gate** menu. Observe that the Data Grid with the cell gallery updates to show only the translocated cells.
35. Select the **Non-Translocated Cells** Gate in the **Current Gate** menu. Observe that the Data Grid with the cell gallery updates to show only the non-translocated cells.
36. Drag the **Translocated Cells** gate from either dot plot onto the Data Grid with the cell gallery. Observe that changes the cells displayed to only those in the **Translocated Cells** gate and the morphology of the displayed cells is clearly different.
37. Alternatively, you may drag the gates from the dot plots onto the Data Grid with the cell gallery for either the translocating or non-translocating cells. You will see the Data Grid updates in the same way.
38. Close the layout and **do not save** changes.

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Section 3: Reviewing an Assay with the High Content Module

39. Open the **IntroToImage3.fey** layout from the Tutorial Sample Data archive 1210.

40. Click on the "**Overall Review**" page.

Notice that this tab is a summary of the entire assay. The Heat Map on the left shows the plate and the 8 wells of data we have loaded for it (Figure T28.50).

There is a dot plot depicting results from **well A-01** and the **Translocated Cells** and **Non-Translocated Cells** gates. There are picture plots from the same well and a Data Grid with a cell gallery displaying all cells in the well with parametric data. Lastly, there is a Gate View 345 which is showing all of the saved gates that are used in the template. Note that some of the gates displayed in the Gate View 345 may not have been used in the examples we have walked through in this tutorial. We will use this page to review the assay as a whole and get a sense of what is happening in each well.

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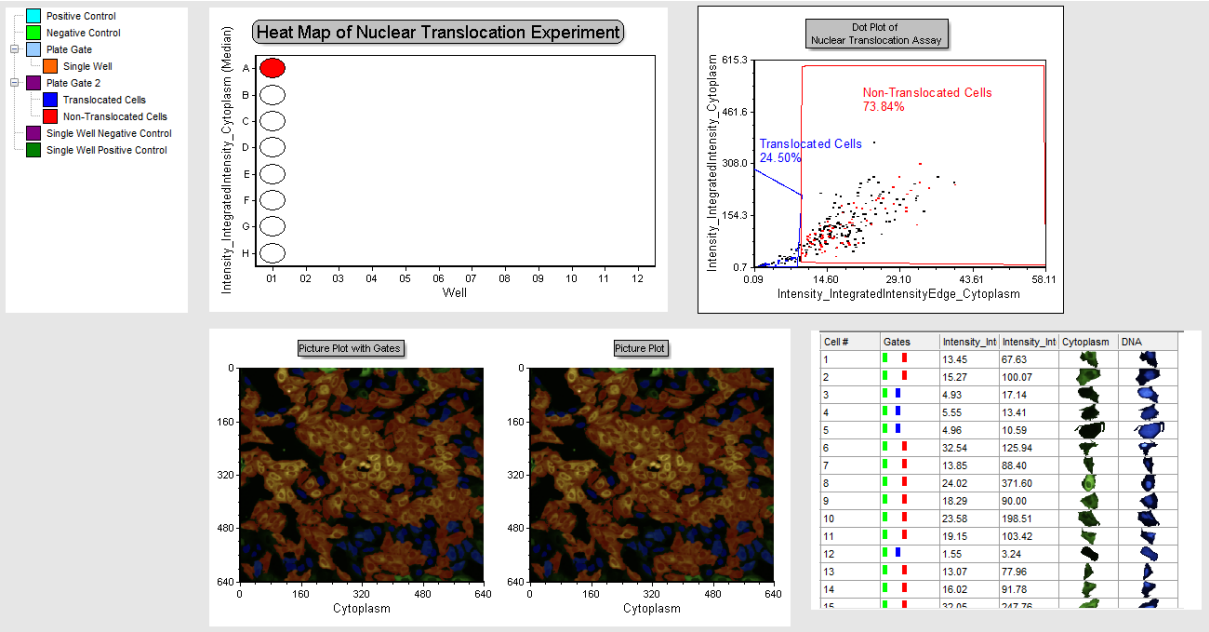

Figure T28.50 Review Page of IntroToImage3.fey layout

41. Right click on the Heat Map.

42. Select **Edit Gate→Single Well** as we have done previously in this tutorial.

Well A-01 will appear red because **well A-01** is the only member of the **"Single well"** Gate. You can now click on any well and all plots will update for the selected well. Note that if you click on any other plot you will have to repeat the edit gate process to change well displays.

43. Starting with **well A-01** click on the various wells moving down toward **well H-01**. Observe the changes in the plots.

Give the layout time to fully update when selecting a well. You will find that **well A-01** through **well D-01** are negative controls and have higher percentage of

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non-translocated cells. Notice in the images that most of the cells are red indicating that they are not translocating. In the dot plot see the large distribution in the plot, and that cells reside in both gates. In the Data Grid displaying a cell gallery you can see what gates individual cells reside in, and the corresponding morphology in the images matching their translocated or non translocated designation.

44. Click on wells **well E-01** through **well A-H01** and observe the dot plots and picture plots for each well. These are the positive control wells.

Notice the change in the images to predominantly nuclear staining in blue, the dot plot depicts cells clustered predominantly within the positive control gate. The Data Grid with a cell gallery indicates translocated morphology with staining predominantly in the nucleus. The gate display in the data grid indicates all cells in the positive control gate. It is easy to review your data for the whole plate in this layout page and view analytical results combined with images and other relevant information from the original data all in one place!

45. Close the layout and **do not save** changes .

Continue to the next topic - Batch Processing and Reporting for Image Data.

38.30.3 Batch Processing and Reporting for Image Data

In this section, we will learn how to:

- Open a layout and configure a batch process for several wells in a 96-well plate

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- Configure the **Batch Processing Actions** to export the batch results as reports
- Pause between wells to confirm the current iteration of the batch is being analyzed correctly
- Reconfigure the batch to run in an automated fashion
- Export a high resolution image from the layout for publication purposes
- Export the layout to a **PowerPoint**
- Export the layout to **.PDF**

We will be using the **IntroToImage4.fey** layout for this section which is located in the Tutorial Sample Data archive→**Intro to Imaging Tutorial** folder. This layout contains a detailed pre-formatted approach to batch processing and report generation with image data.

For this section of the tutorial we will use data generated on the Molecular Devices ImageXpress Velos system (previously called "IsoCyte"). We will first open FCS Express 6 to check our preferences and then close and open the **IntroToImage4.fey** layout to learn how to import and load data into a data list.

The ImageXpress Velos System is a high-speed cytometer that conducts whole well imaging for an entire plate. Cells are typically 10-20 pixels each and appear as small dots within a whole well image. This system can scan plates in as little as 3 minutes enabling high throughput image-based cytometry. It is ideal for cytometric analysis of adherent cells that are difficult to study in suspension with Flow Cytometers.

The ImageXpress Velos data is fully compatible with FCS Express 6 Image Cytometry. As we will show in this tutorial, preferences can be set to immediately import and open data from the ImageXpress Velos into FCS Express. You will see images and plots from an Live-Dead experiment run on a 96-well plate. We will review one row of data from the plate for this experiment using a pre-configured layout for the tutorial. This single row includes wells treated with a dose response dilution series that result in all live to all dead. Live cells are stained with a dye identified in the software as "IMEAN-1" and dead cells with another dye identified as "IMEAN-3".

Section 1: Checking Preference Settings and Preparing a Batch Analysis

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1. Start **FCS Express 6**.
2. Select **Default** in the user selection menu and click **OK** (Figure T28.51).

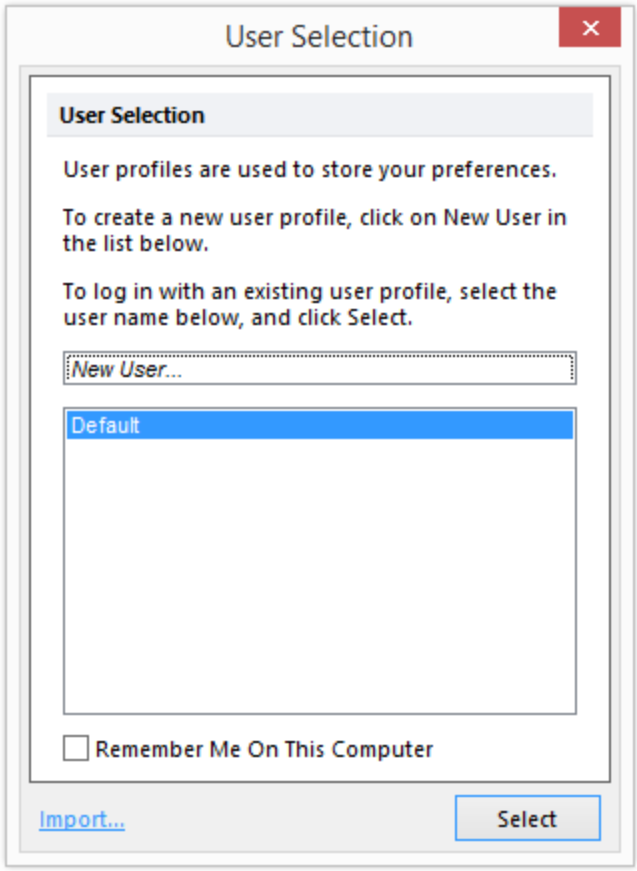

Figure T28.51 Selecting the Default User

3. Select **New Layout** from the Startup screen (Figure T28.52).

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Figure T28.52 The Startup screen of FCS Express V6.

FCS Express 6 will open with a single blank page and the Home tab of the ribbon bar selected. We will now confirm that the data loading preferences are set correctly.

4. Click on the **File** tab→**Options** .

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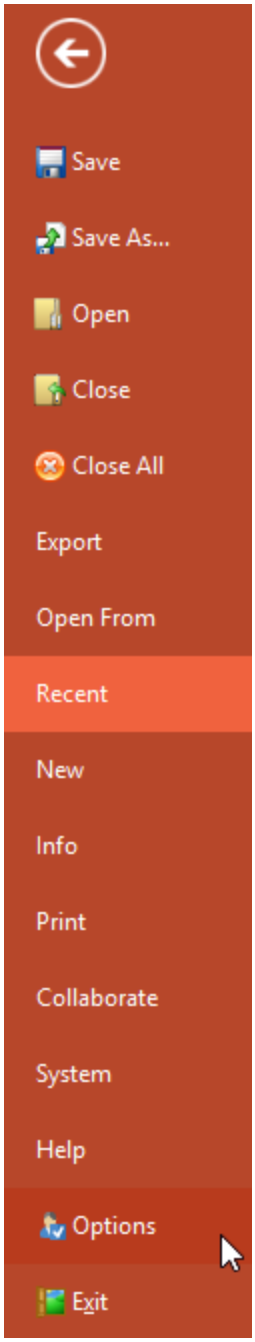

Figure T28.53 The FCS Express User Options button in the File tab.

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5. Select the **Data Loading→ImageXpress Velos options** category (Figure T28.54).

6. Make sure that "**Load images**" is the only box with a check in it. It is only necessary to load data as a plate when working with the High Content module[1050]. Do not load data as a plate when planning to run a batch analysis, unless you are planning on batching through plates, and do not set to load background images unless you need them.

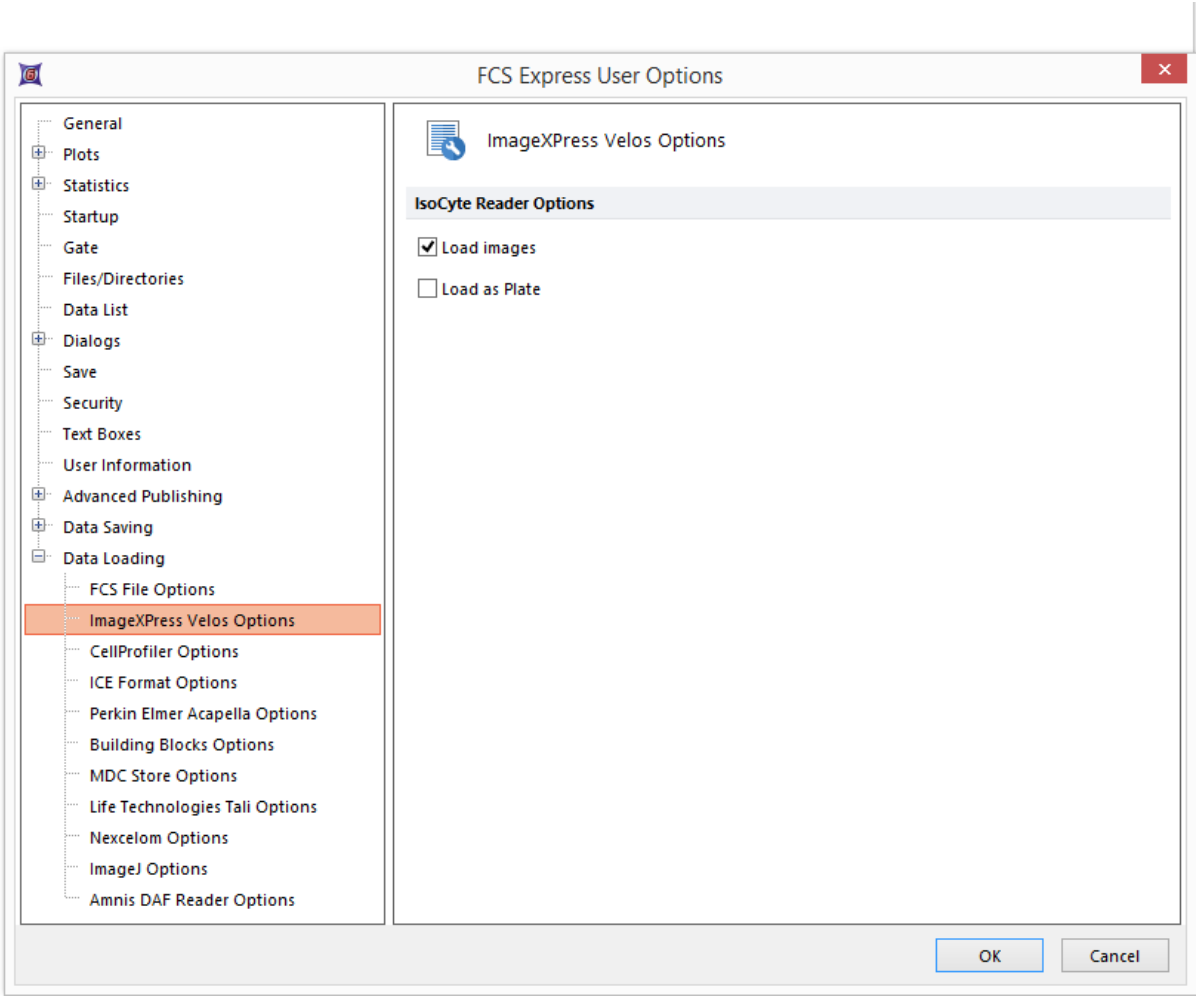

Figure T28.54 Correct Settings for Data Loading

8. Click **OK**.

9. Select **File Tab→Open** (Figure T3.1[1237]).

10. Load the **IntroToImage4.fey** layout from the Tutorial Sample Data archive[1210]→**Intro to Imaging Tutorial** folder.

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You will see a pre-formatted template open for analysis and reporting of Data from Live-Dead Assays run on the IsoCyte / Image Express Velos. The **Batch Setup** page of the layout will have an empty Data List, an array of 4 unpopulated plots, with an empty summary data table at the top and a Gate View 345.(Figure T28.55). We will now import the data from the Tutorial Sample Data archive 1210.

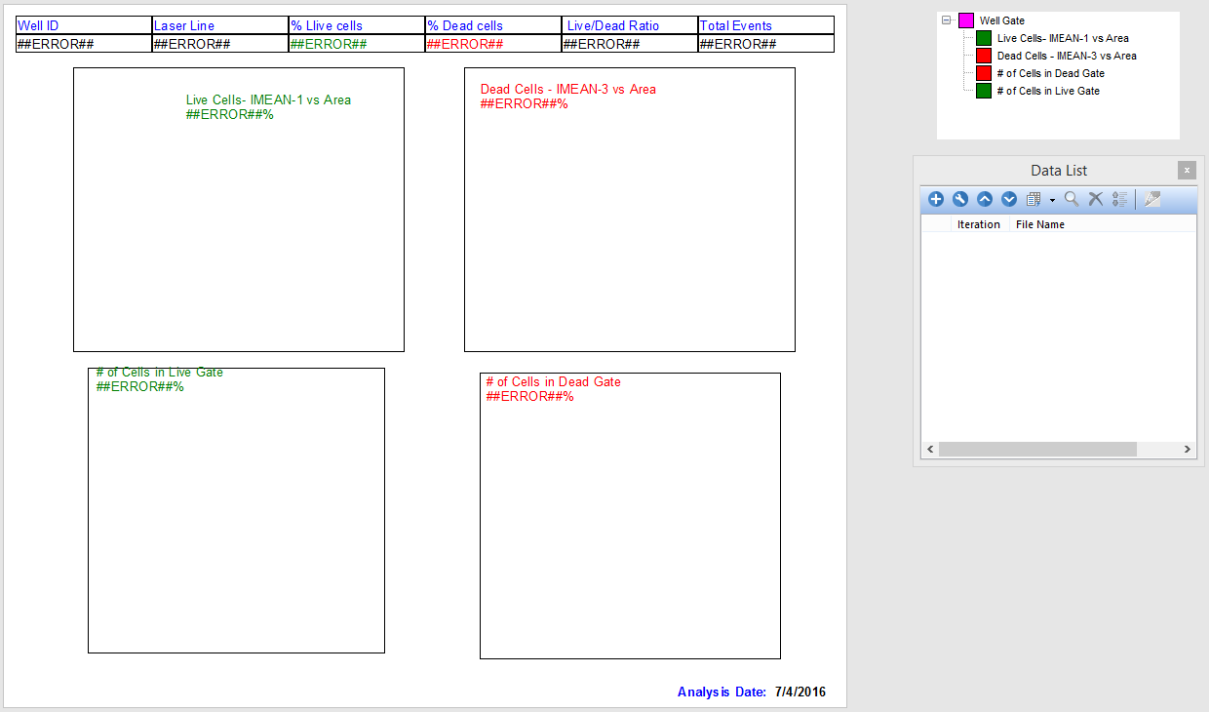

Figure T28.55 The Empty Layout Template

10. Click the button of the empty **Data List** to add data to the template (Figure T28.56).

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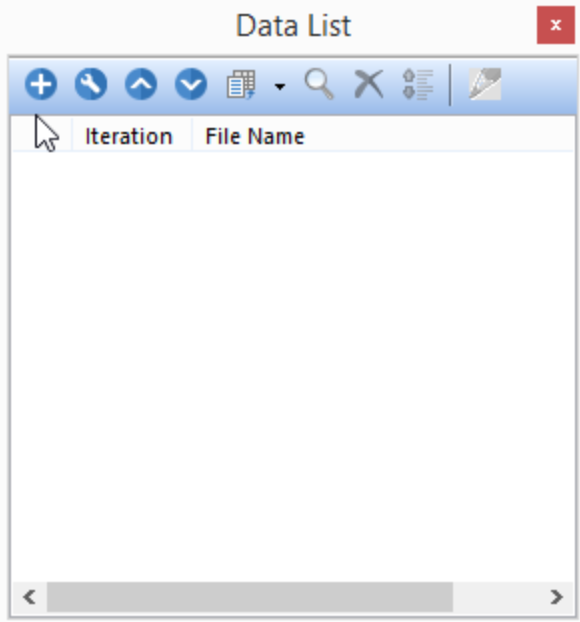

Figure T28.56 Empty Data List

11. Choose **ImageXpress Velos files (*.iso)** from the **files of type:** drop-down list in the **Select a Data File** dialog (Figure T28.57).

12. Navigate to the **Sample Data→ImageXpress Velos With Images→Live_dead_96.well.iso** folder (Figure T28.57).

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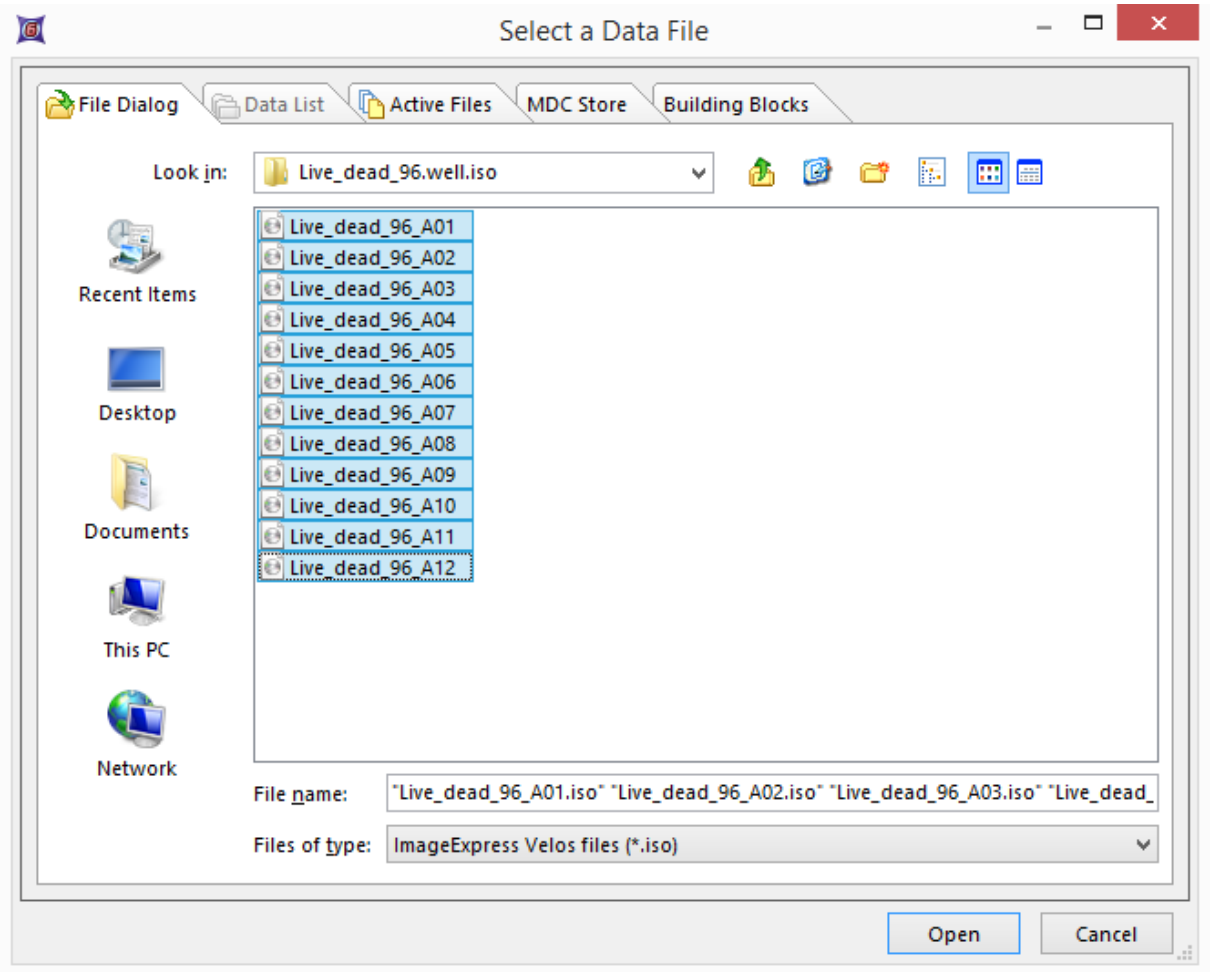

Figure T28.57 Selecting the Data Files to Import

13.Select all the **Live_dead_96...** files

14. Click **Open**.

The Data List will now populate with data from the 12 wells (A01-A12) of the Live-Dead Dataset (Figure T28.58).

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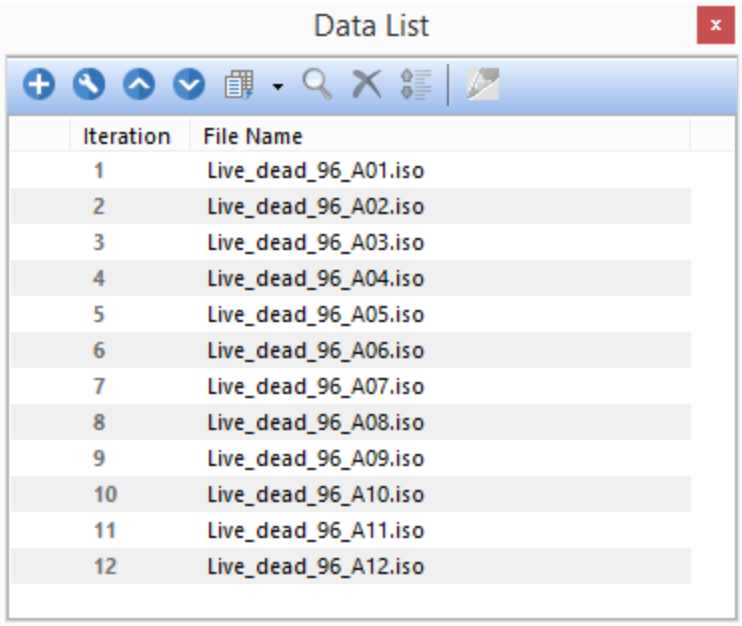

Figure T28.58 Data List showing Data Files

14. Click to select the first live-dead file (it will highlight in dark blue) (Figure T28.59 below).

15. Be sure that no objects are selected in the layout (click on any empty area to be sure of this)

16. Click on the **Change data icon** in the Data List menu and select **Change Data On All Plots** (Figure T28.59).

Note: the **Change data icon** would behaves differently if one or more object are selected in the layout.

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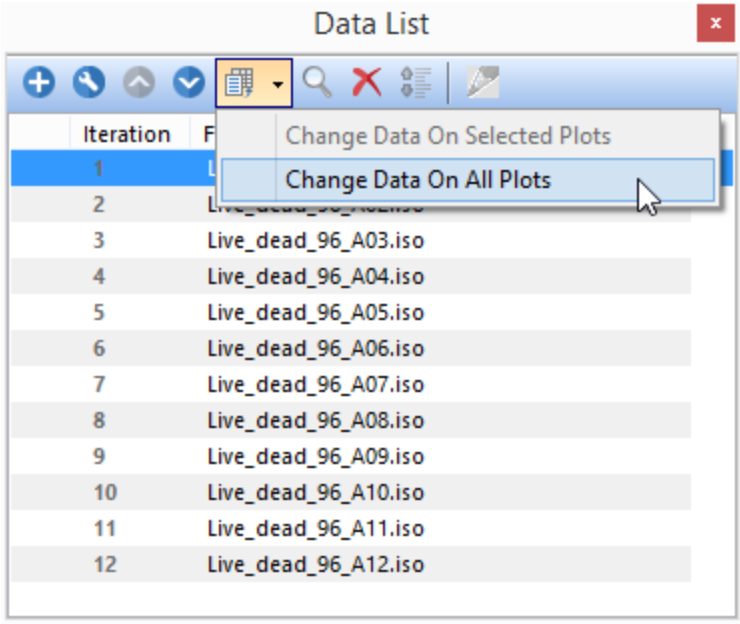

Figure T28.59 Selecting the Data File to Load Into Layout

All plots and tables in the layout page will populate with data from **well A01** (Figure T28.60).

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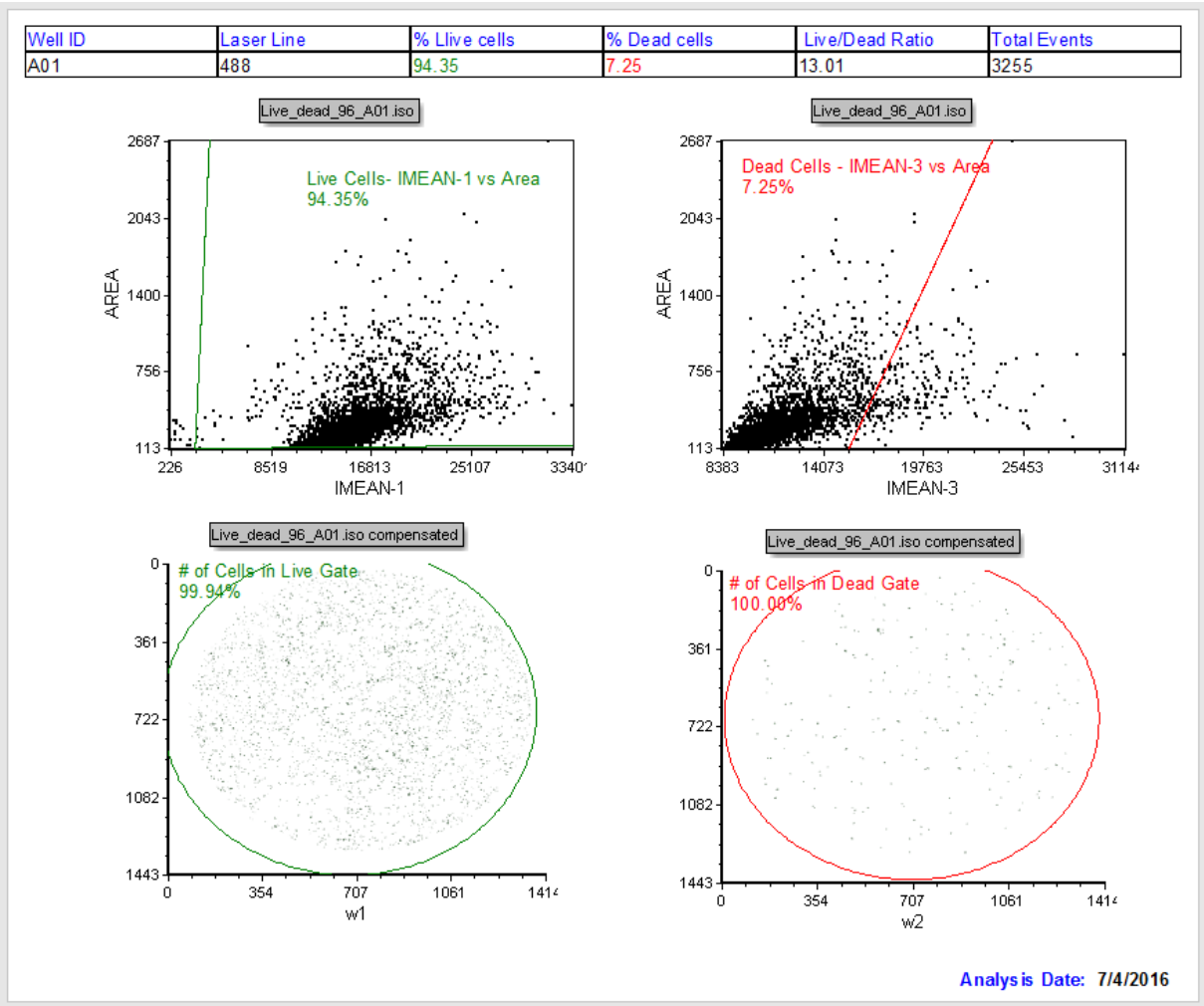

Figure T28.60 Batch Setup Page of IntroToImage4.fey Showing Data from Well A01

Let's review the entire **Batch Setup** page of **IntroToImage4.fey** again. Notice the page is configured with a summary table at the top and four plots (Figure T28.60). Two dot plots[796] on the top indicate the Live and Dead cell channels, respectively for the given well. The picture plots[121] on the bottom of the page are the live and dead images respectively. **Live Cells** and **Dead Cells** gates have been set in advance. Tokens[692] on the dot plots calculate the percentage of live and dead cells. Additional gates (**# Cells in Live/Dead**) count total number of events in each of the fluorescent channels.

Observe in the data table at the top of the page that we are viewing data from **well A-01** as noted in the top left cell in the table (Figure T28.61).

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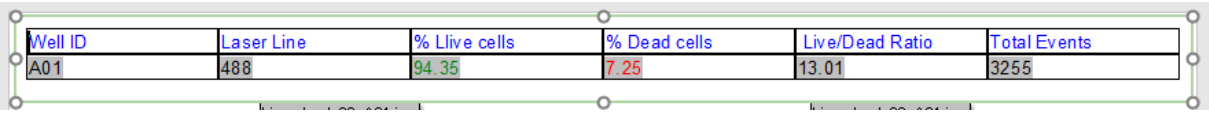

Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	7.25	13.01	3255

Figure T28.61 The Data Summary Table

Now we will go through our data list sample by sample

17. Click on the **Data** tab in the ribbon (Figure T28.62).

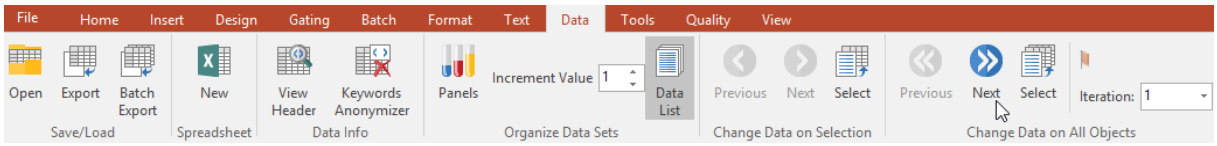

Figure T28.62 The Data List Tab

18. Click on the **Change Data on All Objects→Next** blue arrow to move to subsequent wells (Figure T28.61). Notice that all of the four plots update to show data and images from **well A-02**.

19. Step through the data for each of the wells using the **Next** blue arrow in the **Change Data on All Objects** menu. Observe the picture plots and dot plots updating and changing from mostly live cells to predominantly dead cells as you move through the series of wells. Observe that the data in the summary table also updates for each well.

20. Return to **well A-01** by pressing the **Previous** blue arrow in the **Change Data on All Objects** menu repeatedly.

Now we will learn how to close and reopen the **Data List** window.

21. Observe the **Data list** in the layout (Figure T28.58).

22. Close the **Data List** by clicking the **"x**" in the upper right hand corner of the data list window.

23. Reopen the **Data List** by clicking the **Data Tab→Organize Data Set→Data List** icon on the ribbon (Figure T28.63).

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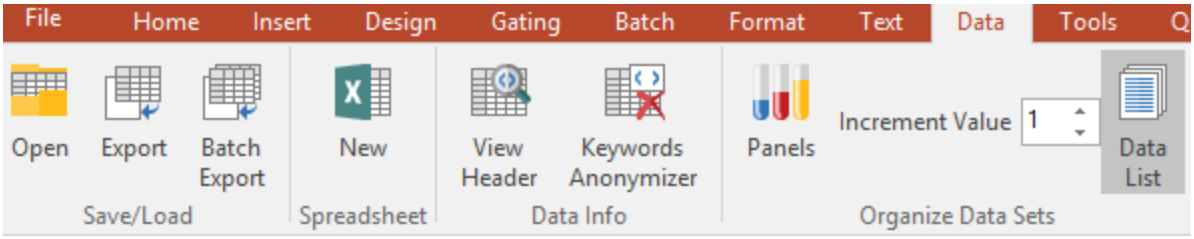

FIgure T28.63 The Data List button within the Data tab

Modifying the Summary Table

24. Click on the **Insert tab→General→Text Box** in the ribbon (Figure T28.66).
25. Insert a new text box by left clicking on anywhere on the layout.

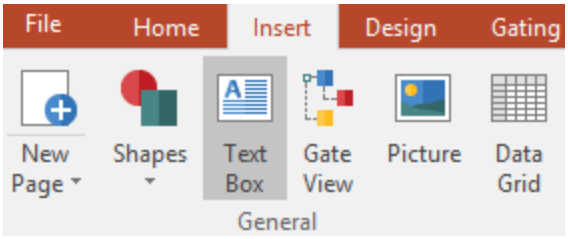

Figure T28.66 The Insert Tab

26. Right click on the text box to open the drop-down menu.
27. Select **Table→Insert Table** in the submenu that appears (Figure T28.67).

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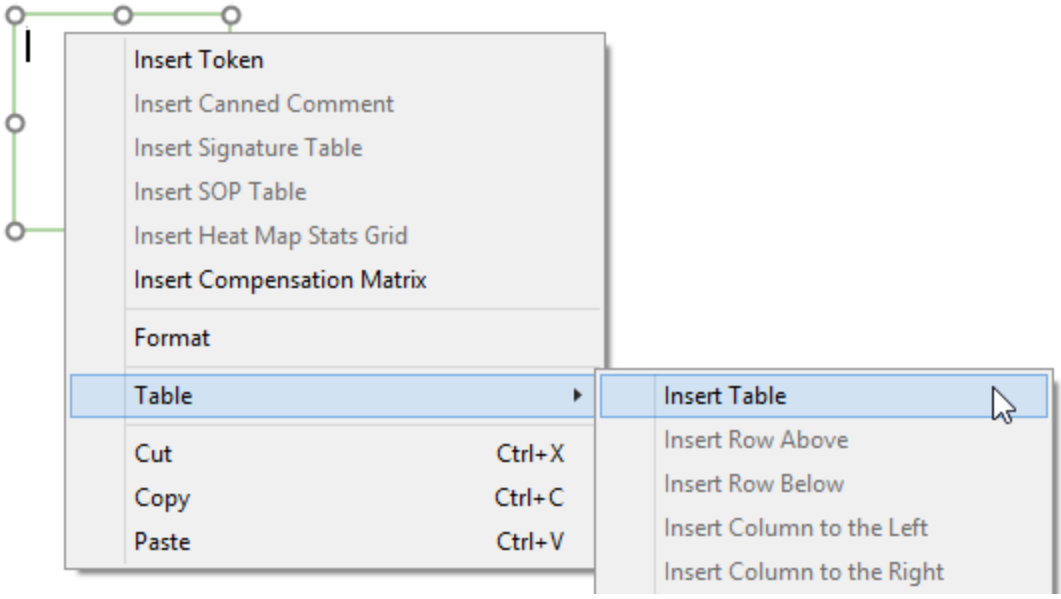

Figure T28.67 Inserting a Table

A new window will appear allowing you to create a table of any size and dimension (Figure T28.68).

28. Change the **Number of columns**: box to "**5**".

This demonstrates how the summary table already in the layout page was created. We will edit the pre-existing table in this tutorial instead of creating a new one. Click **Cancel** to avoid creating a new table.

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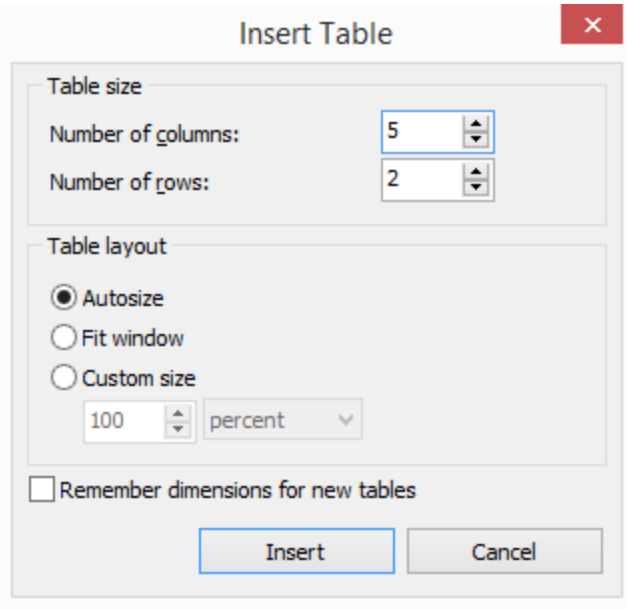
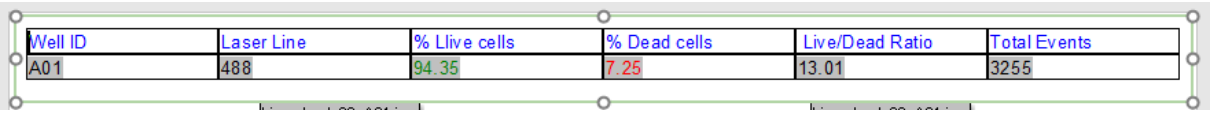


Figure T28.68 Insert Table Configuration

29. Delete the text box, by selecting it with a left mouse click and then right click and select **Cut Text Box**. Alternatively you can select the text box and use the **delete** key.

30. Click on the "**Well ID**" text in the cell in the upper left hand corner of the summary table (Figure T28.69).



Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	7.25	13.01	3255

Figure T28.69 Summary Data Table

31. Edit the "**Well ID**" text as you would in Word, Excel or PowerPoint.

32. Undo your edits with as many **Ctrl+Z** commands as required to restore the "**Well ID**" text.

33. Select the **A01** text in the lower left hand box of the summary data table using standard Windows selection techniques (click and hold the mouse to the right of the A01 text and drag the mouse over the entire text. Release the mouse when the text is selected). The grey background of the A01 text

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denotes that it is a Token. Notice the text turns white when selected (Figure T28.70).

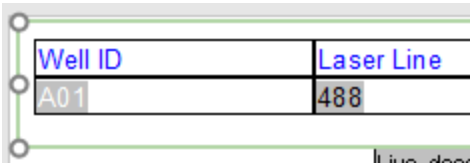

Figure T28.70 Selecting the Token

33. Double click on the A01 Token. A formatting window will appear (Figure T28.71).
34. Select the **Keyword** category to choose a token displaying a Keyword from the data set (Figure T28.72).
35. Click on the ellipsis (**...**).

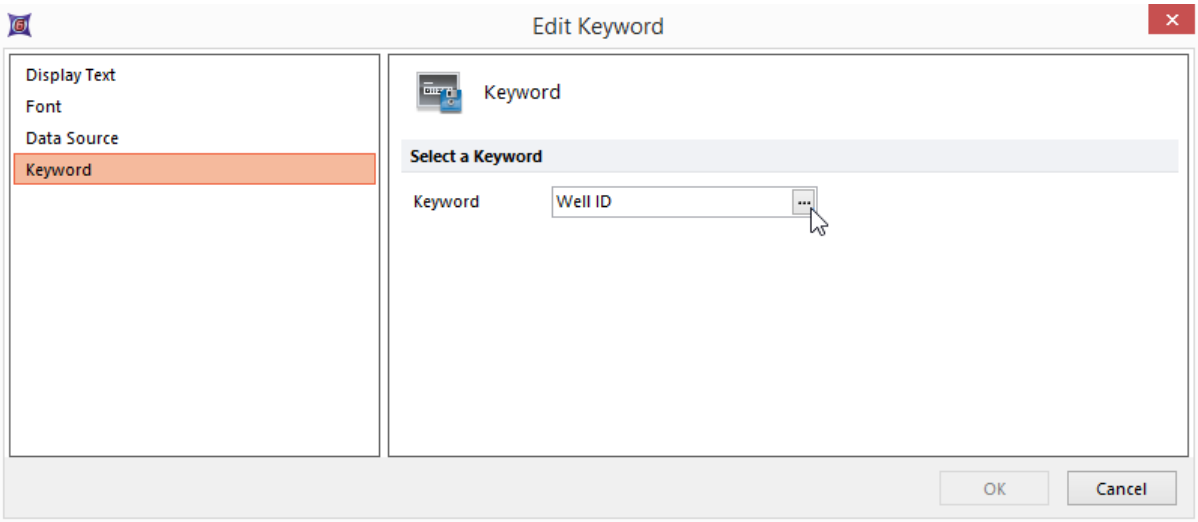

Figure T28.71 Edit Token Dialog Box

36. The list of the available keywords appears (Figure T28.72).
37. Select **Well ID** from the **Please select a keyword** dialog.

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38. Click **OK**. You have just confirmed the **Well ID** as keyword to be printed in the Well ID column of our Summary table.

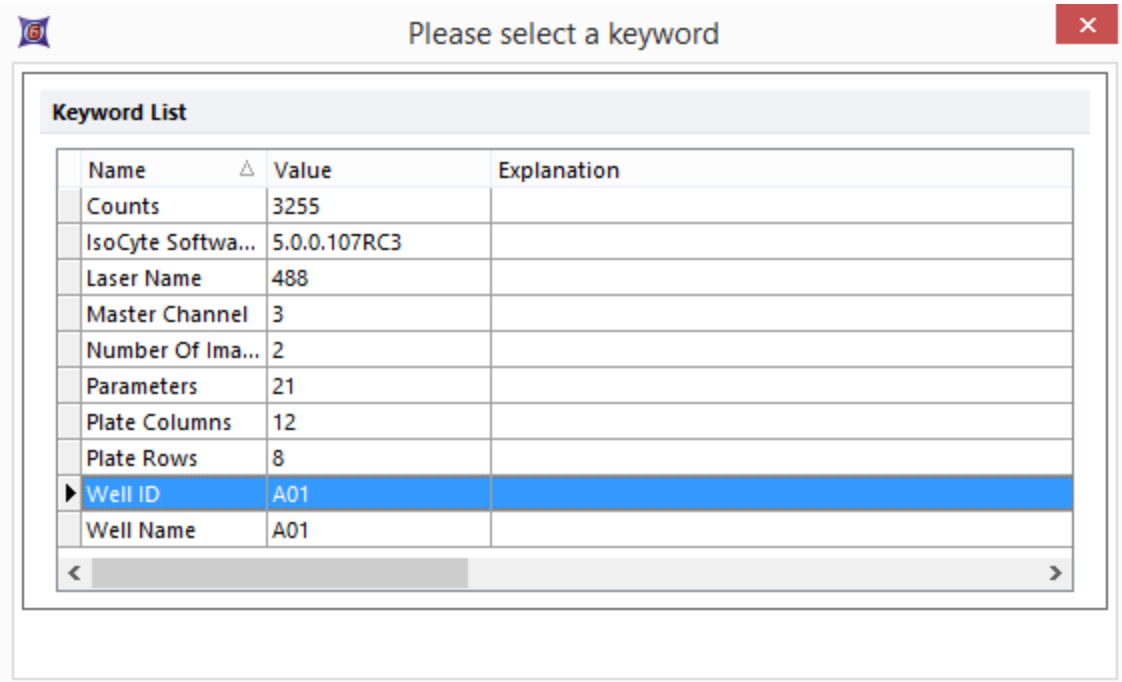

Figure T28.72 Selecting a Token To Display Well Name in the Summary Data Table

Optional: You may repeat 31-37 and go back to edit the "**Laser Line**" text heading in the summary data table with "**Master Channel**" and choose **Master Channel** for the Token to display. This will give you more experience with inserting a Keyword Token. Please undo your changes if you do this optional exercise.

In this report layout all of the values in the lower row of the Summary Data Table are Tokens which have been placed in the cells to insert dynamic data into the table. We have inserted the keywords for **Well Name** and **Laser Name** from the ImageXpress Velos data into the first 2 cells.

Note that we are able to label the summary data table titles any way we choose because they are simply text. They indicate "**Well ID**" and "**Laser Line**" for added clarity in the report from the default names (Well Name and Laser Name) reported by the instrument. The other values in the table are tokens that indicate calculated values from specific gates.

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For example, the **% Live cells** and **% Dead cells** values are Tokens displaying the percentage of cells residing in the respective **Live Cells** or **Dead Cells** gates. The **Live/Dead Ratio** Token is a "custom token" in which we have used the custom token tools in FCS Express 4 to create a ratio of two existing values (% live / % dead) that are derived from the gates. We will not discuss Custom Tokens in detail here. For a complete tutorial on using Tokens please see "Text Boxes and Tokens" or review the "Using Tokens" section of the manual. We will not make any additional changes to Tokens used in the table.

38. Select all of the text in the Summary Data Table by clicking and dragging over the entire table. Alternatively, click in any cell of the table and use **Ctrl+A** to select all text in the table (Figure T28.73).

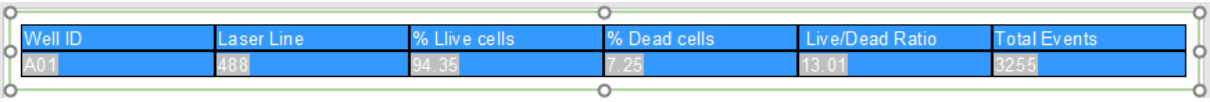

Figure T28.73 Selecting all Text in the Summary Data Table

39. Select the **Text** tab as shown in Figure T28.74.

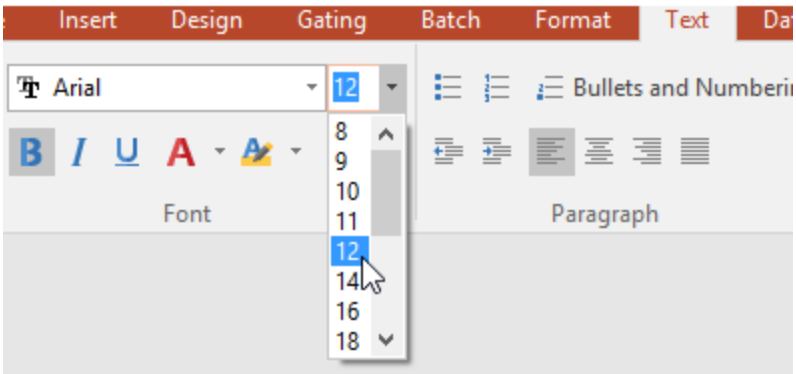

Figure T28.74 Text Tab Font and Bold Selection

40. Change the size of the font in the Table from **10pt** to **12pt** as shown in Figure T28.74 above.

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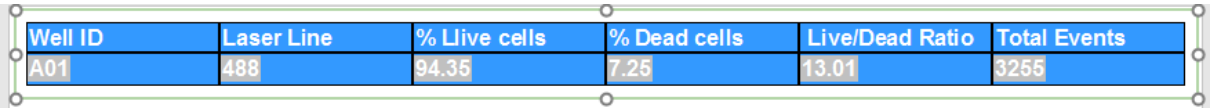
41. Click on the "**B**" (bold) button to change all to **Bold** as shown in Figure T28.74 above.

Notice you have similar options for editing text in a layout page as you would expect in any Microsoft product. This allows layouts to be customized in any way you require for custom reporting. The Summary Data Table will now appear as in Figure T28.75a and Figure T28.75b below.



Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	7.25	13.01	3255

Figure T28.75a Summary Data Table All Cells Selected

42. Click anywhere on the summary data table to remove the highlighting for the selection and view changes (Figure T28.75b).

Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	7.25	13.01	3255

Figure T28.75b Summary Data Table All Cells Modifed to Bold and 12pt Font

Section 2: Configuring a Batch Analysis for Image Data

We will now configure a batch analysis and run through it to create a report to export from FCS Express 6. In this example the settings for the batch are pre-configured and we will review them, and then run the batch several ways. Please see the tutorial on Batch Processing 784 for detailed instructions on configuring the advanced batch and reporting functions.

43. Click on the **Batch tab→Batch Processing→Batch Actions** (Figure T28.76).

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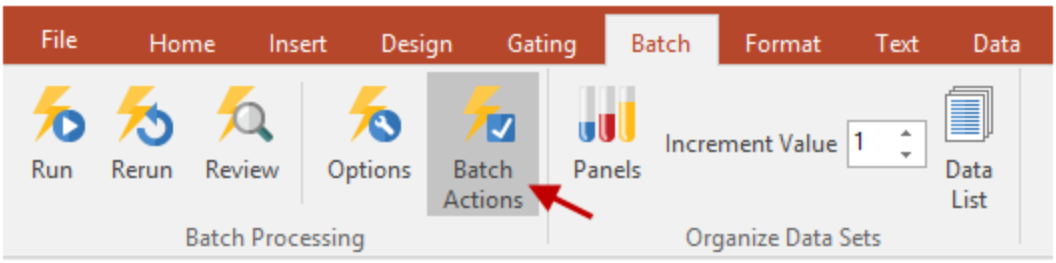

Figure T28.76 Opening the Batch Actions Window

44. The Batch Actions window will appear as shown in Figure T28.77.

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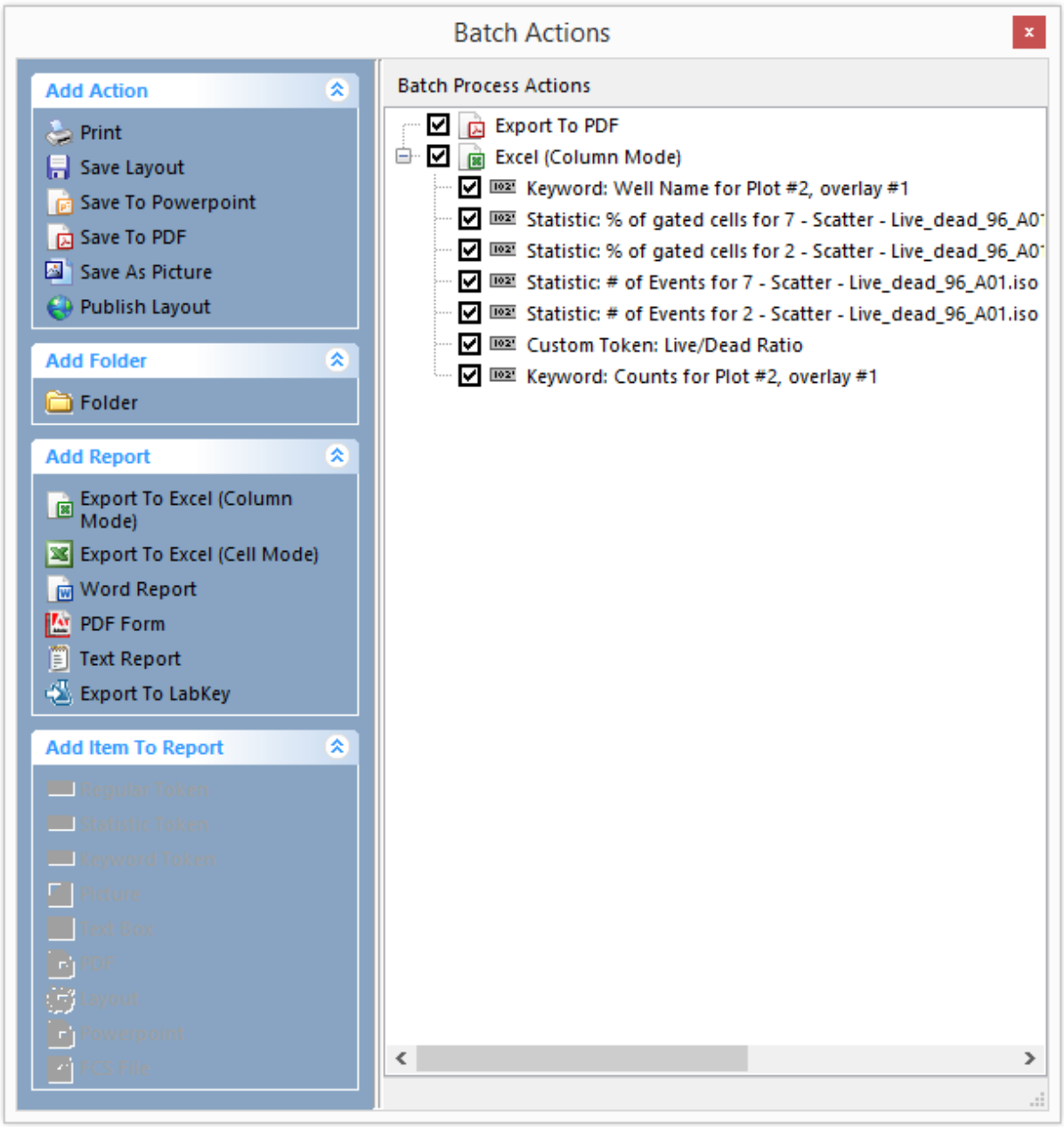

Figure T28.77 Batch Analysis Options and Configuration for IntroToImage4.fey

Notice in the **Batch Actions** menu that there are many options for setting up a batch analysis and report (Figure T28.77). Please review the items for familiarity, but we will not cover building from scratch in this tutorial. Once configured, batch analysis of data and report generation occur simultaneously and can be exported to Excel, PowerPoint or PDF files. For addition information on building reports refer to the Batch Processing 784 tutorial.

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Observe in the **Batch Process Actions** window that it is pre-configured to run batch analysis for each well in the Data List, and export information to both a PDF and an Excel spreadsheet. The PDF will show what we see in the Batch Setup Page, and the Excel file has been configured to log specific values to the spreadsheet automatically during the batch run. Items to be exported to Excel are listed.

To demonstrate the ease of configuring batch output, we will add one additional variable to the output for the Excel spreadsheet using drag and drop capabilities for tokens.

44. Select the token for **Total Events** in the summary data table on the **Batch Setup** page of the layout. Recall from that the Token will appear in white when selected properly (Figure T28.78).

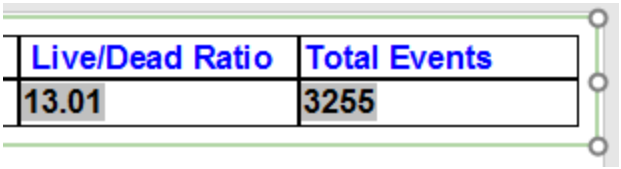

Figure T28.78 Selecting the Token for Total Events

45. Click on the highlighted token; while holding the left mouse button down, drag it onto the Excel (Column Mode) icon in the **Batch Process Actions** window. When you see a **"+"** sign appear, release the left mouse button. As shown below in Figure T28.79.

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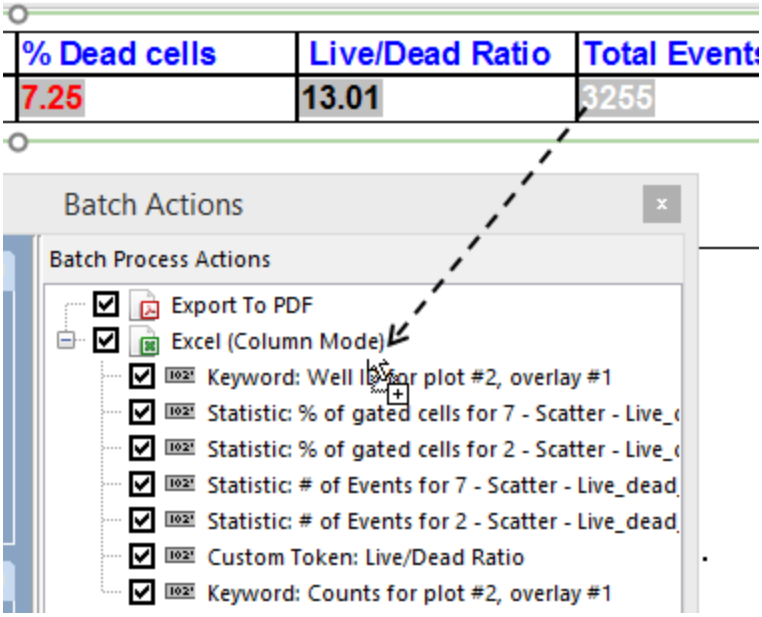

Figure T28.79 Drag and Drop Onto the Batch Actions List

46. Note that a new action has appeared in the Batch Process Action section (Figure T28.80).

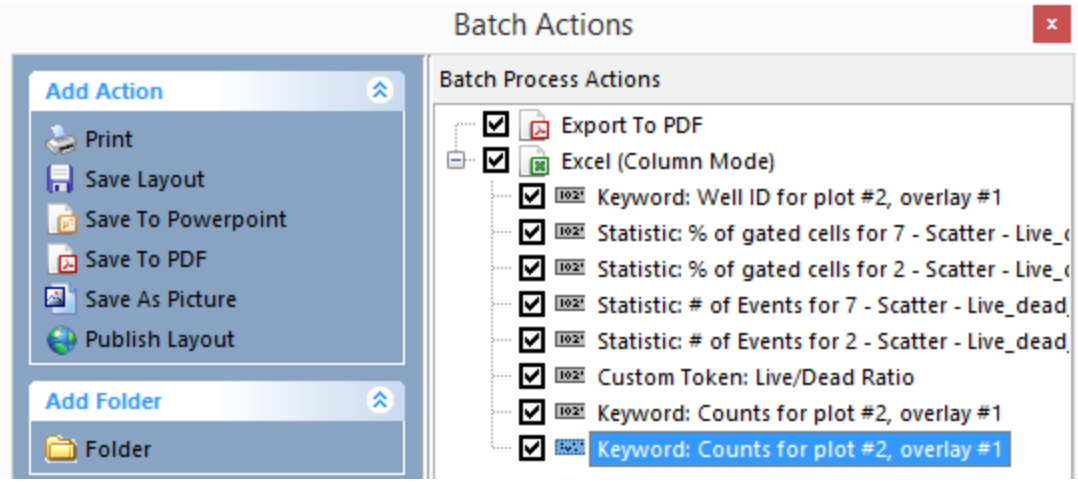

FIgure T28.80 The just added Batch Action is blue highlight

Please note that the action **Keyword: Counts for Plot #2, overlay #1** is repeated twice since the exact same action was already present in the list.

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In order to avoid replicate let's remove the first **Keyword: Counts for Plot #2, overlay #1** action (the one NOT selected in Figure T28.80) by left clicking on it and pressing DEL on your keyboard.

Now we will modify the action just added in step 45-46.

47. Double click on the last item in the list, **Keyword: Counts for Plot #2, overlay #1**. A new window will appear.

48. Select the **Field** category on the left (Figure T28.81).

47. The **Column Name** Edit box will be empty and we will edit it by entering "**Total Events**" (Figure T28.81).

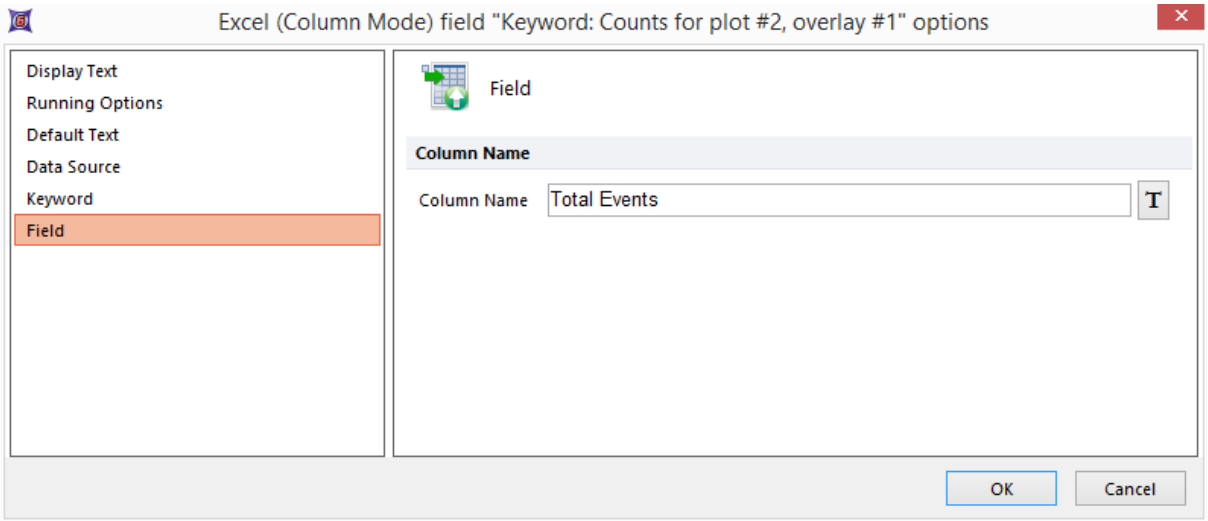

Figure T28.81 Editing the Column Name Box

49. Click "**OK**". You have now given the column name for the Excel file to log the Total Counts.

This Token has now been added to the excel export report. For each well the actual values will be logged into an Excel table, and we have edited the column header that will appear as "Total Events" in the report. You may turn off any Batch Action unchecking the checkbox next to it **Batch Process Actions** window list. We will not discuss configuring batch analysis in more detail in this overview tutorial. For a more detailed tutorial on Batch Processing please see

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the tutorial titled Batch Processing 1445. There is also a detailed section in the Manual on Batch Processing 784.

We will now configure the paths to save our PDF and Excel reports to the desktop when the batch process runs.

50. Double Click on **Export to PDF** in the **Batch Actions** window (Figure T28.82).

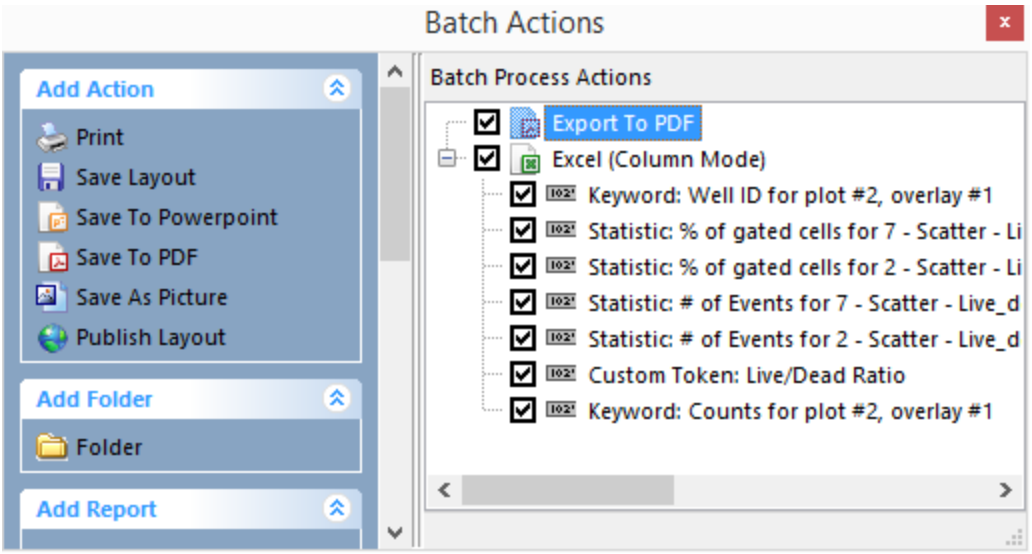

Figure T28.82 Editing the PDF Export Settings

A menu will open with Output file configuration options (Figure T28.83 below).

51. In the **Output file options section** click the Folder icon to select the saving location. A file dialog will open.

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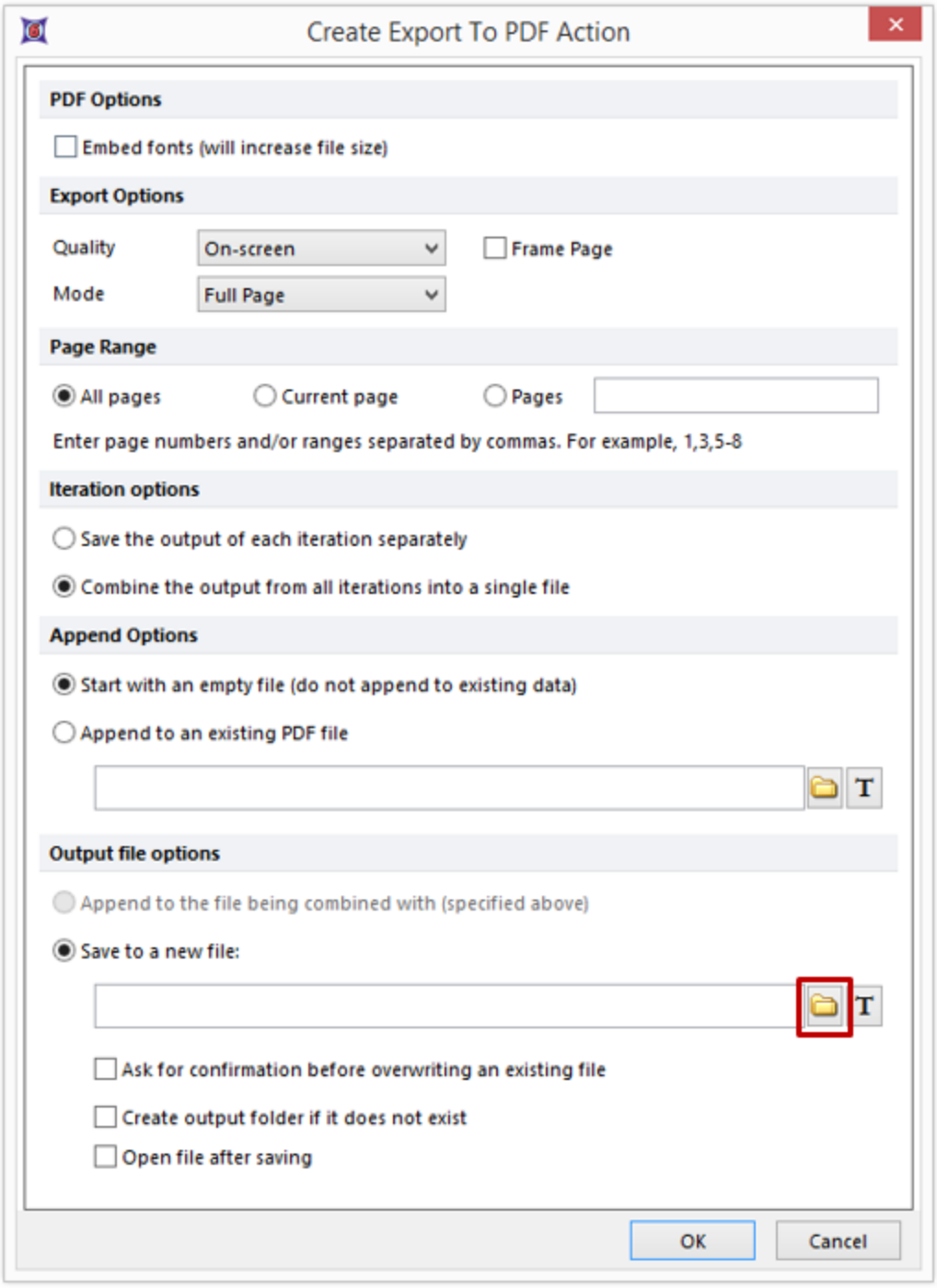

Figure T28.83 Editing the PDF Export Saving Path

52. Select your desktop and name your PDF "**MyBatchReport**".

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53. Click **Save** and the path will appear in the empty Output file options window.

54. Click **OK.**

55. Double click on **Excel (Column Mode)** in the **Batch Actions** window (Figure T28.84).

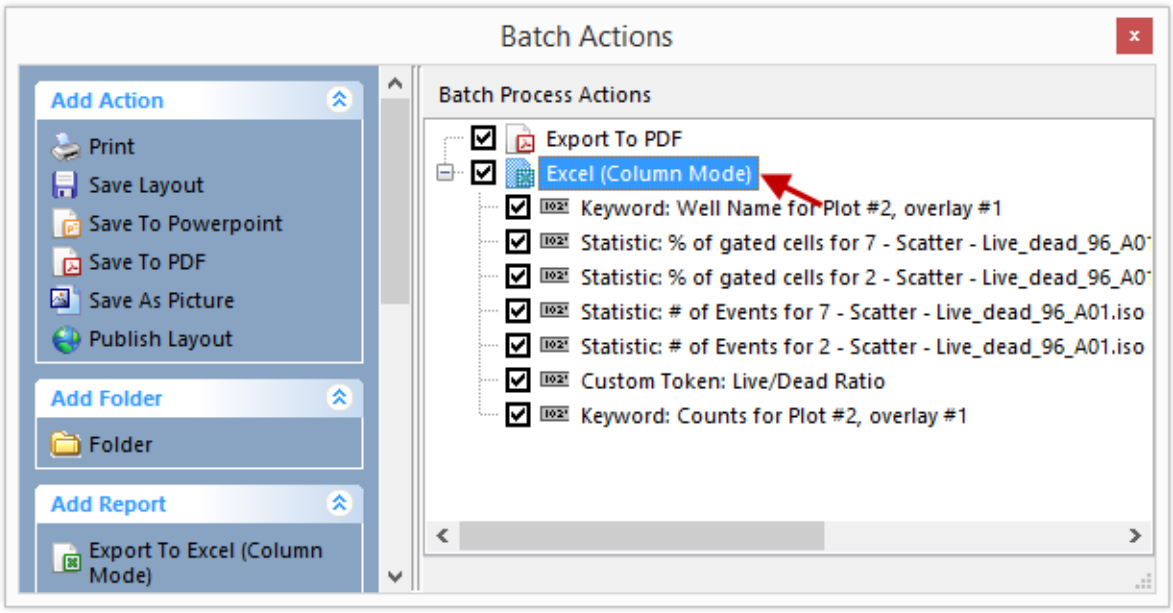

Figure T28.84 Editing the Excel Export Settings

A menu will open with Output file configuration options (Figure T28.85) below.

56. Just as you did for the PDF report, set the path to the your desktop and name your Excel file "**MyBatchReport**". (See Figure T28.85).

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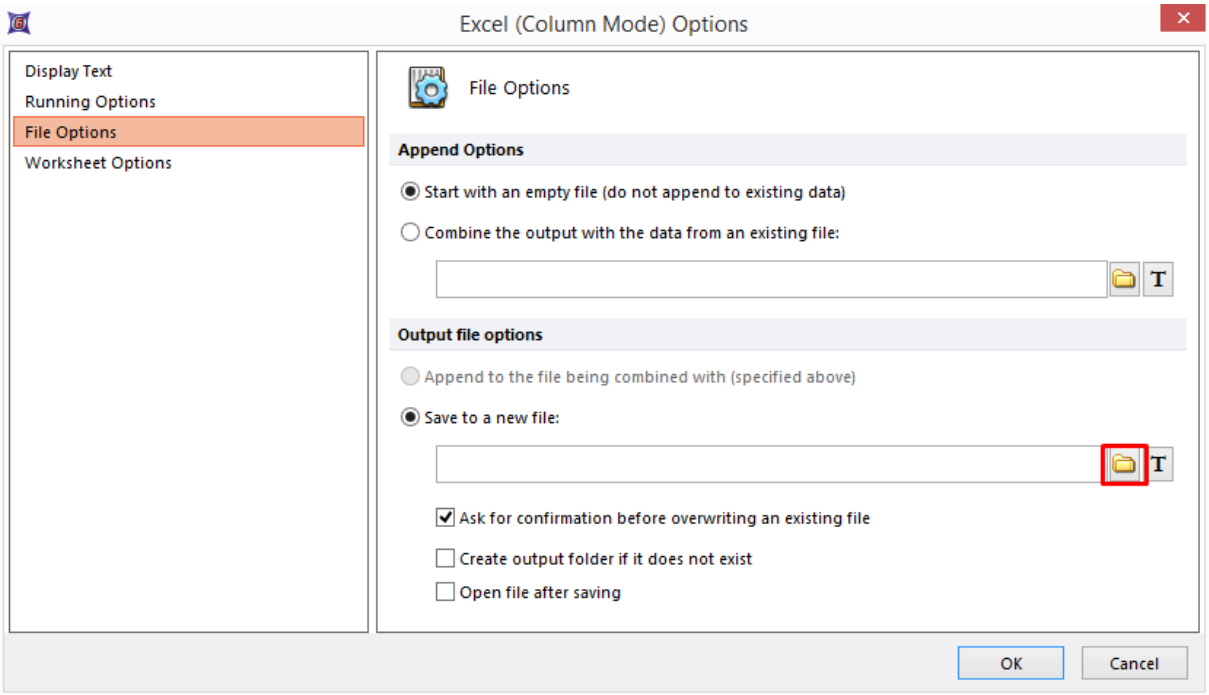

Figure T28.85 Editing the Excel Export Saving Path

Section 3: Running the Batch and Reviewing Reports

57. Click on the **Batch tab→Batch Processing→Options**. A configuration window will appear (Figure T28.86).

Notice that you have many options when configuring a batch process. Do not change any of the settings already configured. However, notice that we have selected the check box to **Unconditionally pause between iterations**. This way we do not need to run all wells to test a batch run. In this case we have only 12 wells and will allow the batch to run completely. Pausing between iterations is a great way to test a batch run through several steps without committing to a complete run. For a comprehensive view of all the options please refer to the Batch Processing Options 843 manual.

58. Uncheck the box for **Unconditionally pause between iterations**.

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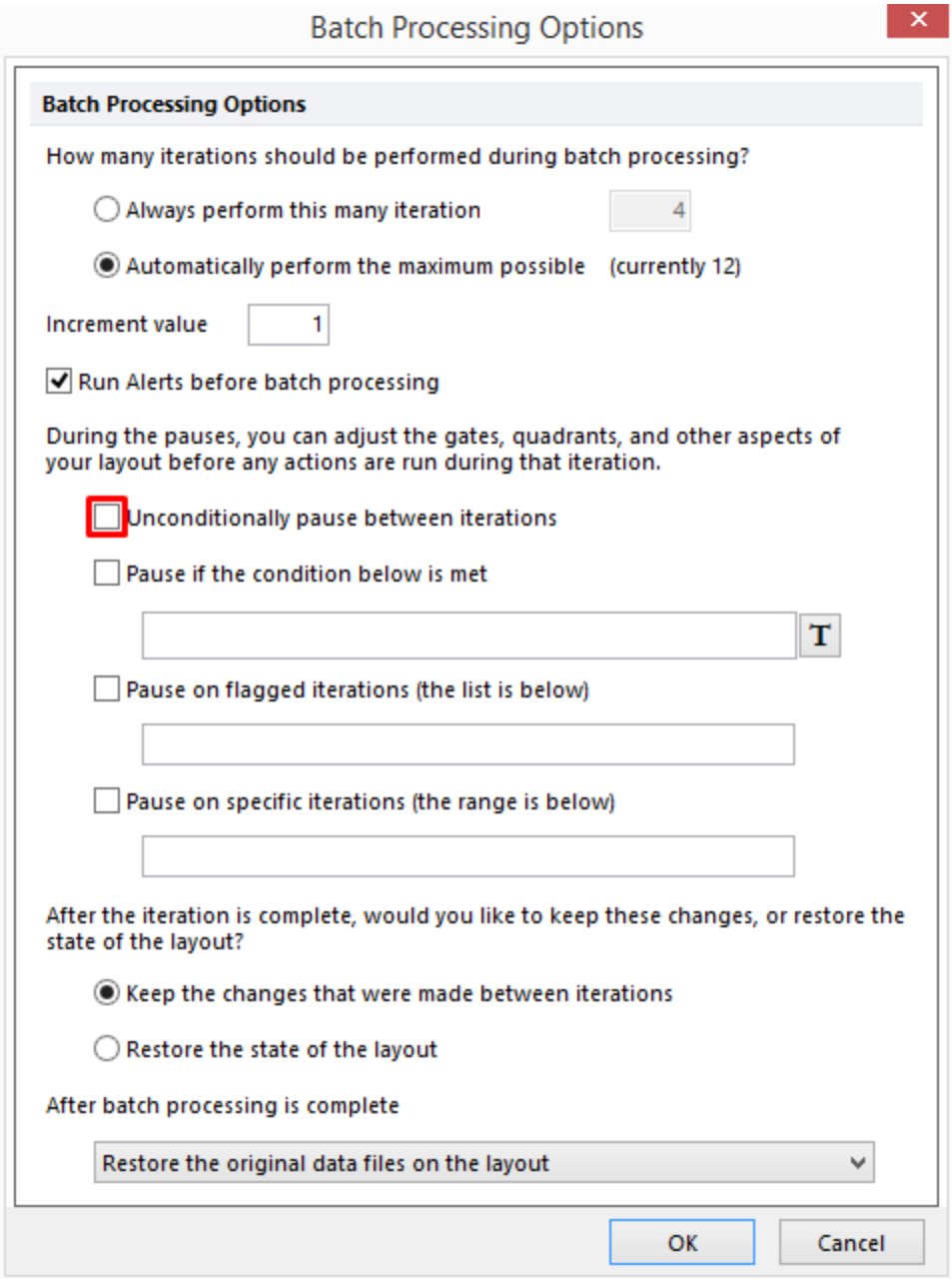

Figure T28.86 Configuring Batch Processing Options

59. Click **OK**.
60. Click on **Batch tab→Batch Processing→Run**.

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The batch process will run for roughly less than one minute. A status window will appear during the batch process showing how far the batch has progressed (Figure T28.87).

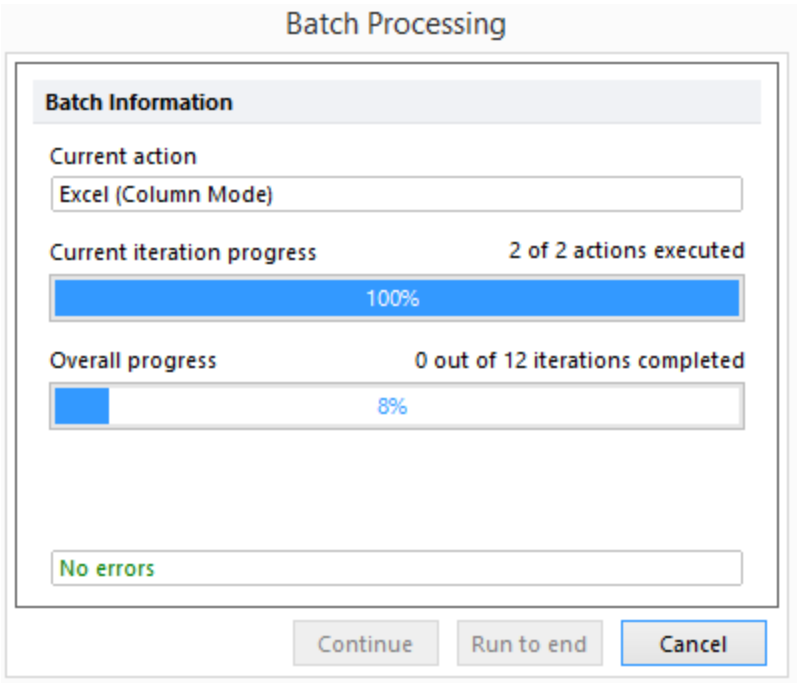

Figure T28.87 Batch Processing Status Window

When finished A PDF file called **MyBatchReport.pdf** will open automatically with the report. Similarly, an Excel file called **MyBatchReport.xls** will open. (Figure T28.88).

61. Review the PDF document and confirm there are 12 pages of data, one for each well processed (Figure T28.88)

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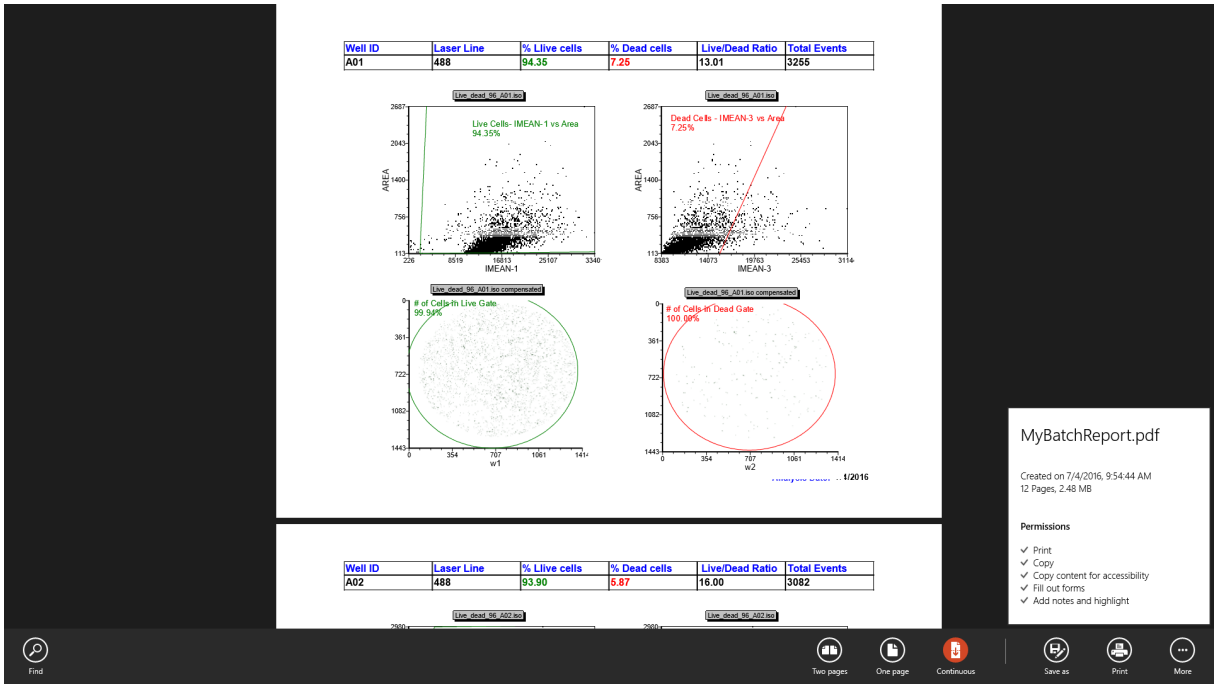

Figure T28.88 "MyBatchReport" PDF Report from Batch Actions

An Excel file will open (Figure T28.89). This Excel spreadsheet will have 12 rows of data set to export visible with the designated column labels. Notice that **Total Events** is now a column with the appropriate title.

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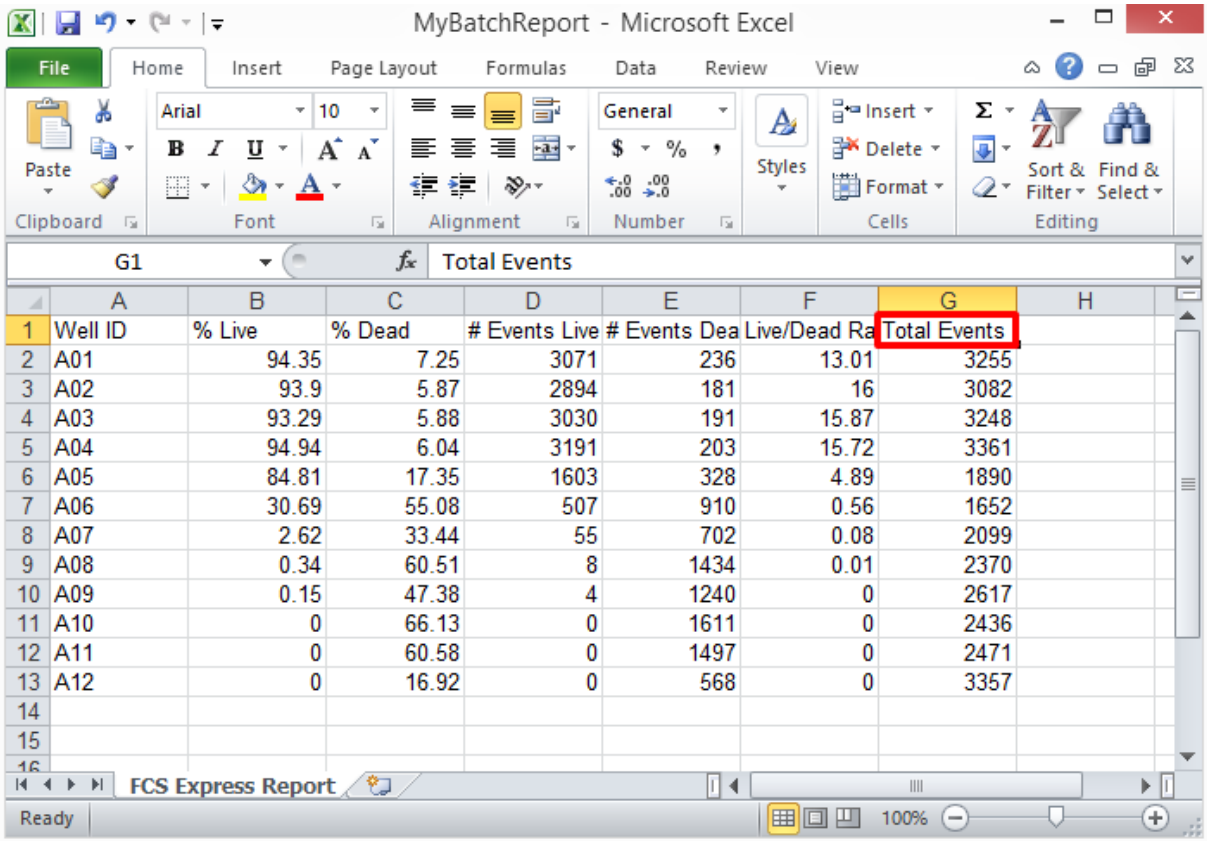

	A	B	C	D	E	F	G	H
1	Well ID	% Live	% Dead	# Events Live	# Events Dea	Live/Dead Ra	Total Events	
2	A01	94.35	7.25	3071	236	13.01	3255	
3	A02	93.9	5.87	2894	181	16	3082	
4	A03	93.29	5.88	3030	191	15.87	3248	
5	A04	94.94	6.04	3191	203	15.72	3361	
6	A05	84.81	17.35	1603	328	4.89	1890	
7	A06	30.69	55.08	507	910	0.56	1652	
8	A07	2.62	33.44	55	702	0.08	2099	
9	A08	0.34	60.51	8	1434	0.01	2370	
10	A09	0.15	47.38	4	1240	0	2617	
11	A10	0	66.13	0	1611	0	2436	
12	A11	0	60.58	0	1497	0	2471	
13	A12	0	16.92	0	568	0	3357	
14								
15								

Figure T28.89 "MyBatchReport" Excel Spread Sheet Report from Batch Actions

62. Close the **Batch Actions** window and all PDF and Excel files. You do not need to save the reports.

Exporting a high resolution picture from FCS Express

You can also save out any selected components of a layout page as a high resolution graphic or publication figure.

63. Select the Live Cells **Live_dead_96_A01.iso** picture plot as shown in Figure T28.90.

Note, the picture plot will have a green border of you select by clicking on the center body of the plot. It will have a red border if you select by clicking on the edge of the plot or a green border. Either state of selection is fine for exporting an image.

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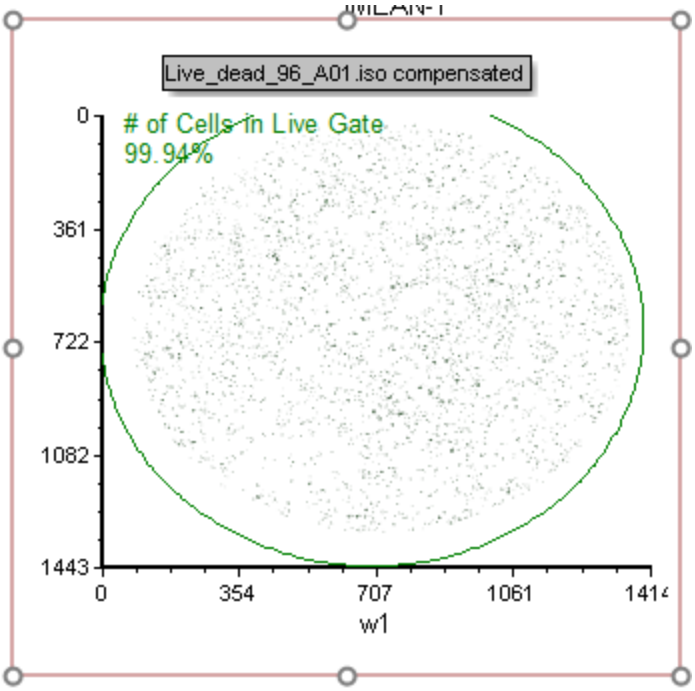

Figure T28.90 The Live_dead_96_A01.iso compensated" Picture plot

64. Click exactly on the border (no matter whether it is green or red) of the plot and select **Save selection As Picture** (Figure T28.91)

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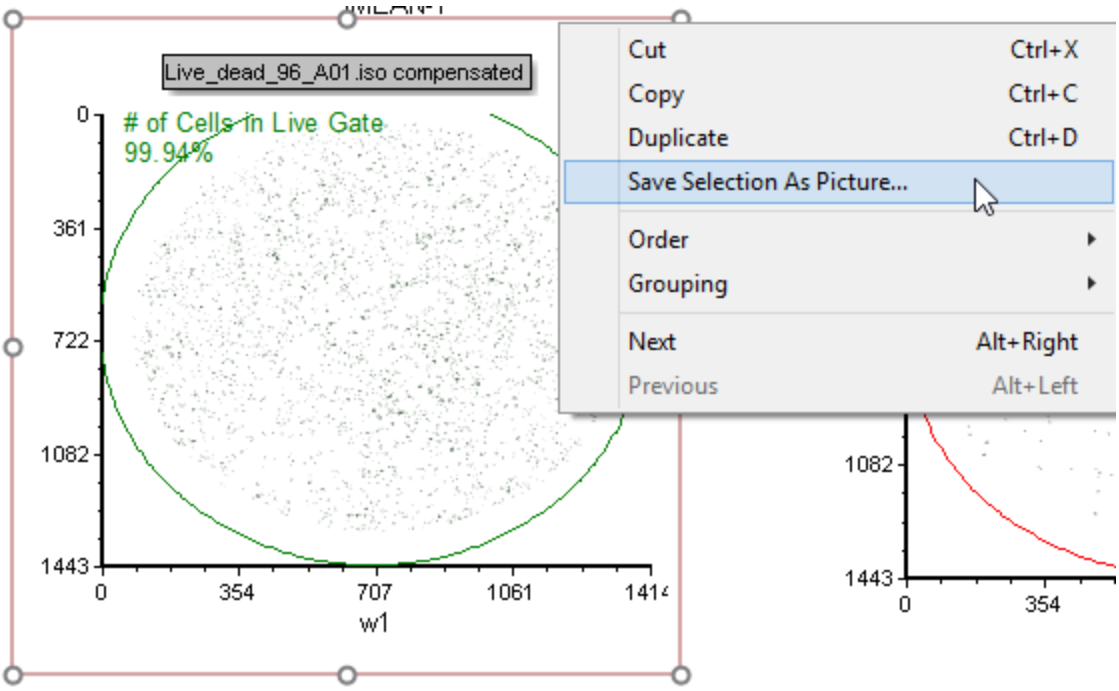

Figure T28.91 Saving a Selected Plot as a Picture

65. Select the Desktop as a destination and input "**MyExportedImage**" for the filename.

66. Select **.TIF** format to save the image in .tif format.

67. Click **Save**.

68. Review the .tiff image on your Desktop. Notice it is a high resolution image. You can change the precise resolution in the FCS Express preferences.

69. Close the Layout and **do not save** changes.

Congratulations! You have completed the **Introduction to FCS Express 6 Image Cytometry Tutorial.**

38.31 Immunophenotyping

FCS Express makes it easy for you to analyze your flow cytometry data and generate reports. In this Immunophenotyping Tutorial, rather than going into depth on any particular procedure, we'll touch on a variety of software features in the context of a natural work flow for analyzing flow cytometry data.

In the course of completing this tutorial, you will insert[1225] and format plots[1236]; create gates[316], markers[353], and quadrants[368]; add default[1315] and custom

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statistics[1324] to your layout; and, batch export[784] your results to PowerPoint™ and Excel™. After completing the tutorial, you will have become familiar with many of the basic yet powerful features of FCS Express.

This tutorial is organized into the three sections, listed below, which together comprise one complete work flow. Each section stands as an independent exercise, so you may complete each section of the tutorial separately if you wish.

- Section 1: Begin building your layout[2187] with plots and other analysis elements (e.g., gates, markers, quadrants), on the next page.
- Section 2: Add default and custom statistics[2210] to your layout, or to the provided layout.
- Section 3: Batch process your results to PowerPoint and Excel[2221], using your own layout or the provided layout.

You may also view the Immunophenotyping Analysis recorded webinar which accompanies this tutorial.

38.31.1 Building your layout

In this section, we will:

- Load an .fcs file[173] into the plots on the layout.
- Insert[1225] and format plots[1236].
- Create[318] and apply gates[1291], markers[353], and quads[368].
- Change our axis labels[2234].

Note: There are three Save Points in this section of the tutorial, so that you may save your progress and return to these Save Points in the tutorial later:

- *Save Point #1 (Step 21)*[2198]
- *Save Point #2 (Step 39)*[2203]
- *Save Point #3 (Step 69, end of this section)*[2210]

1. Select **File** tab→**Open**.
2. Open the layout **Immunophenotyping Tutorial.fey** found in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive.

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The layout has been saved unlinked to any .fcs files, so the plots will appear empty (Fig. T29.1). Unlinked .fey files (layouts) are convenient to use as templates for similar experiments in the future. Learn more about the three options for saving layouts.

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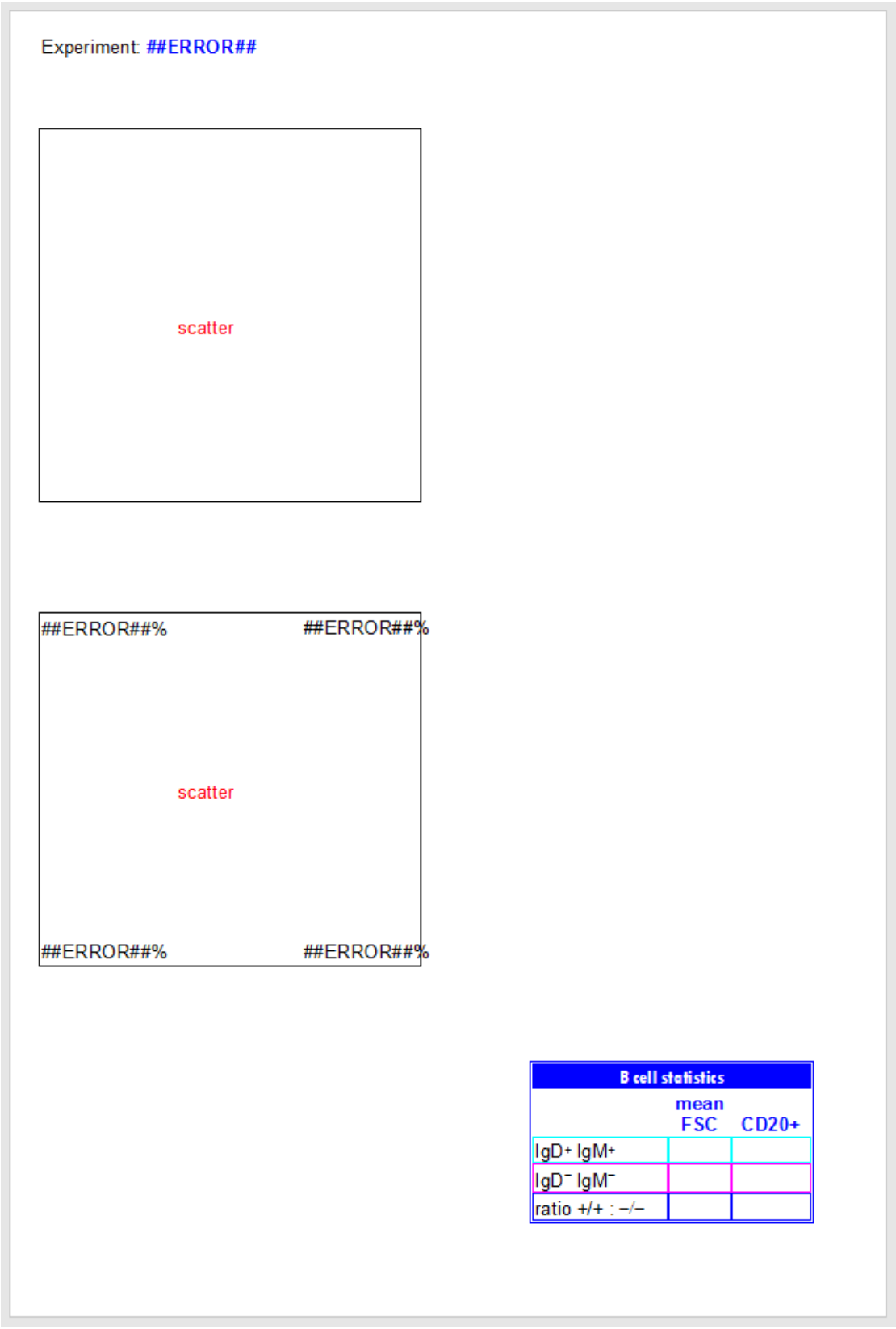

Figure T29.1 Immunophenotyping Tutorial.fey. Note that plots appear empty because the layout is saved as "unlinked".

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We will first change our Default Options[179] so that our .fcs file-derived Stain and Name keywords[708] appear on our plot axes[254].

3. Follow the directions in the Changing Axis Labels section[2234] of the Changing Common Default Options tutorial[2232].

   Now, your plots' axes[254] for .fcs files subsequently loaded into this layout will be labeled with these descriptive keywords[708] that were saved with your .fcs files at the time of acquisition.

We will now load an .fcs file into the plots[173].

4. Select the **Data** tab→**Change Data on All Objects→Select** from the Ribbon (Fig. T29.2, ❶).

5. Navigate to the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive[1210], in the **Select a Data File** dialog box which appears (Fig. T29.2, ❷).

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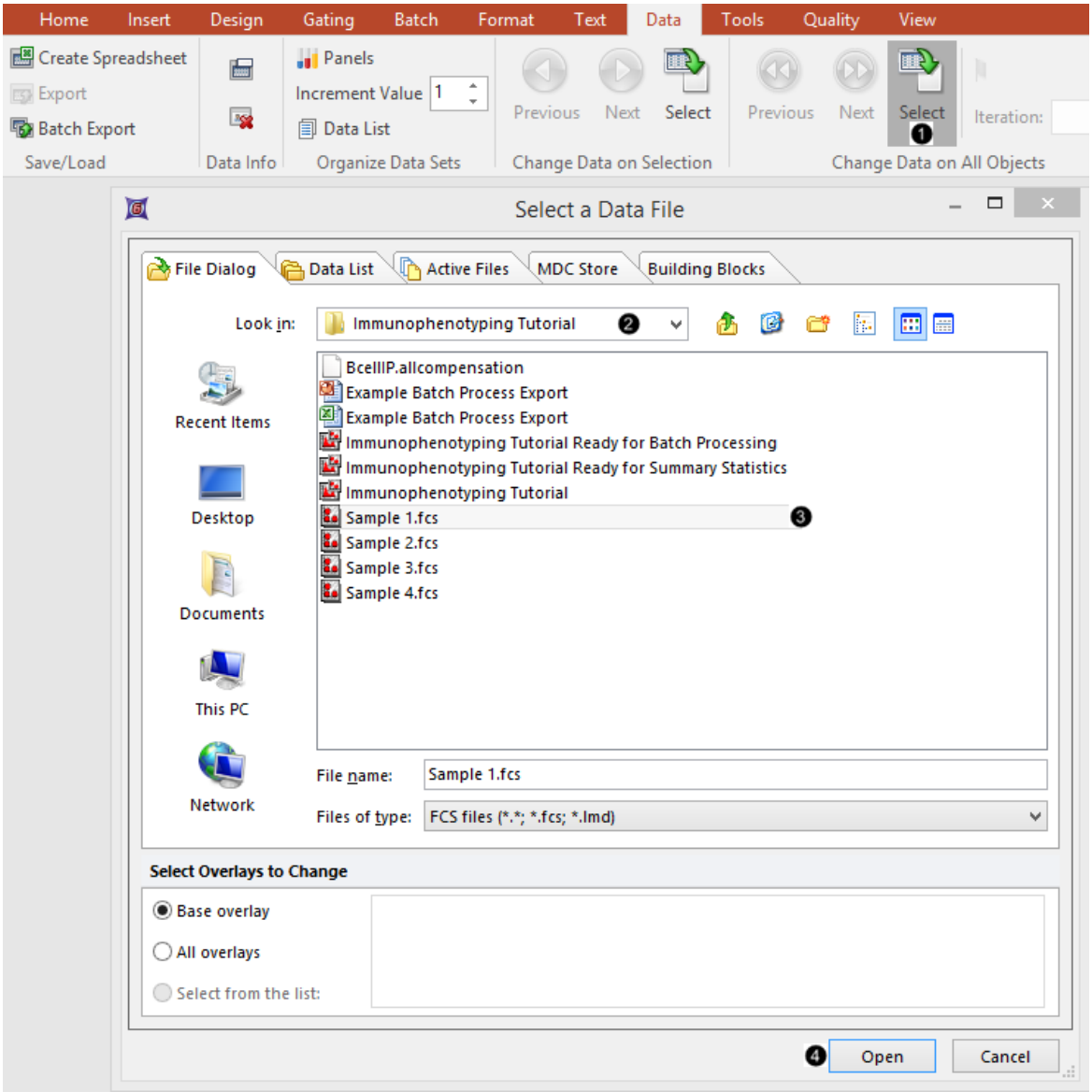

Figure T29.2 Loading .fcs files into the plots

6. Select "Sample 1.fcs" in the **Select a Data File** dialog (Fig. T29.2, ❸) (Note that the advanced open dialog 212 is used in the above screen shot. The standard open dialog may be used much in the same way).

7. Click **Open** (Fig. T29.2, ❹).

   The plots now display the data from the Sample 1.fcs file with a predefined "scatter" gate 318 and quadrants 368 (Fig. T29.3).

   Note that the *Experiment* keyword 708 derived from the .fcs file is now displaying in the text box 152 at the top of the Layout, as are the Current Windows User and Current Date. All of these were inserted into a text box 152 as live-updating tokens 1298.

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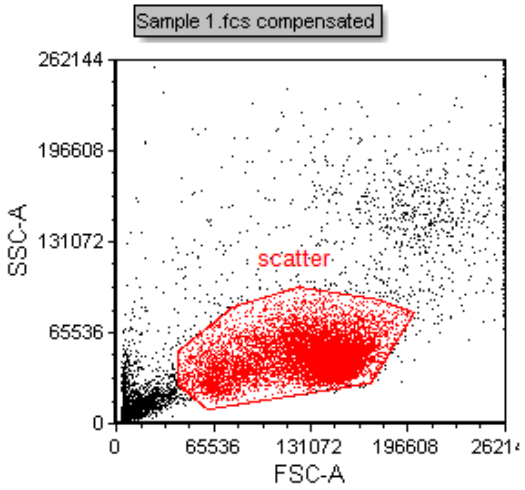
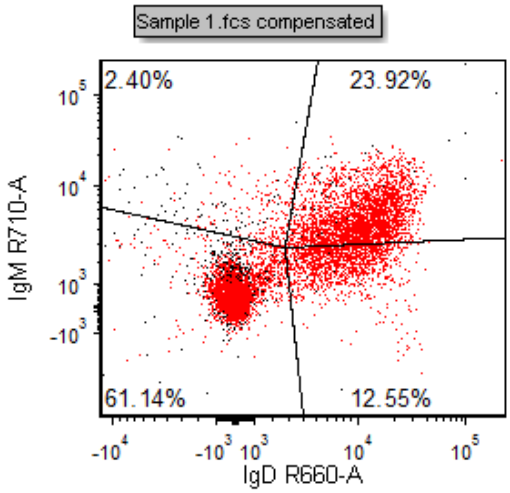
Experiment: B cell immunophenotyping study #A245-6AD
Analyzed by Jane Doe on 6/30/2016





B cell statistics	mean FSC	CD20+
IgD+ IgM+		
IgD− IgM−		
ratio +/+ : −/−		

Figure T29.3 2D plots after changing file

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8. Press and hold anywhere inside the boundaries of the red "scatter" gate (Fig. T 29.4).

9. Drag the "scatter" gate out of the plot to the empty space at right (Fig. T29.4, ❶), while continuing to hold the mouse button down.

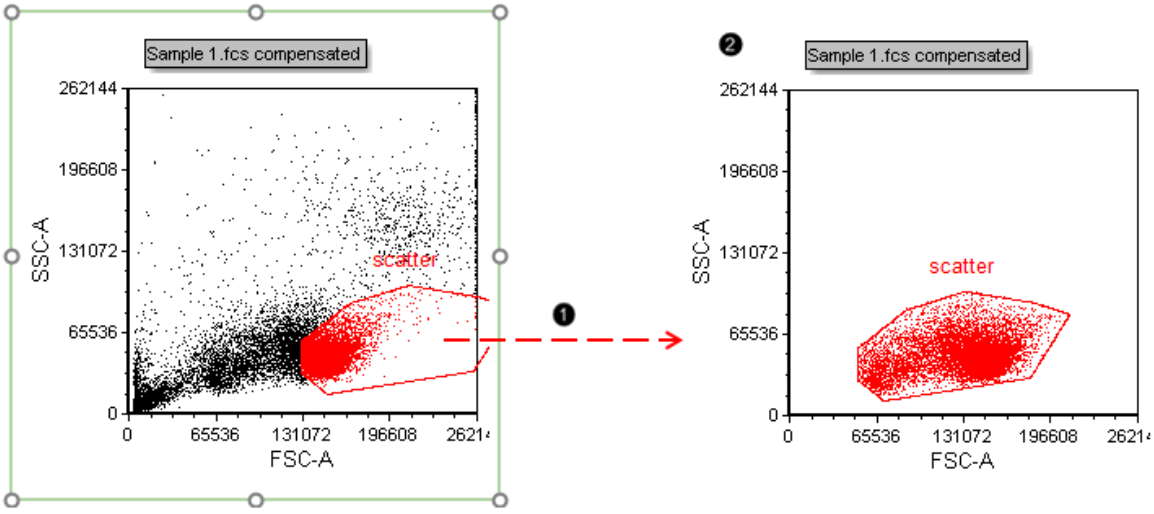

Figure T29.4 Dragging and dropping the "scatter" gate out of a plot to make a new plot gated on "scatter"

10. Release the mouse button when your cursor is on an empty part of the layout to the right of the original plot.

    A new plot gated on this gate will appear at the conclusion of the drag and drop action commenced in Step 8 (Fig. T29.4, ❷).

11. Click the X-axis of the new plot.

12. Click "**CD45 V800-A**" to select it as the parameter 309 from the pop-up list. You may also type **CD45 V800-A** in the empty field at the top of the list (Fig. T29.5).

The X-parameter of the plot has now been changed to "CD45 V800". Note that Step 3 of this tutorial permitted both the Stain (CD45) and Name (V800-A) keywords 708 to appear on the axis. This change in your **Preferences** will affect all future layouts made under the current FCS Express User Profile 50.

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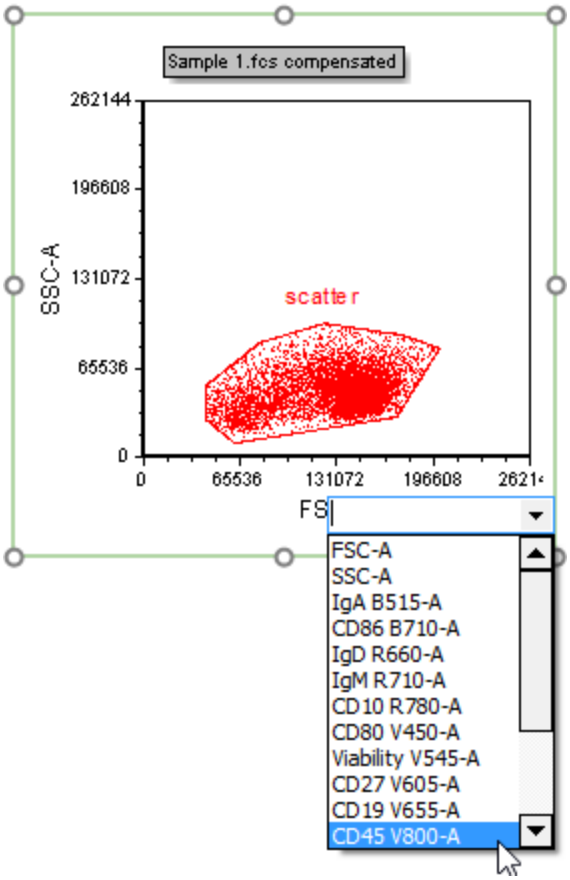

Figure T29.5 Changing the X-parameter of a plot

13. Repeat steps 11 and 12 for the Y-axis of the new plot, selecting "**CD19 V655-A**" as the new parameter. The plot will now appear as in Fig. T29.6.

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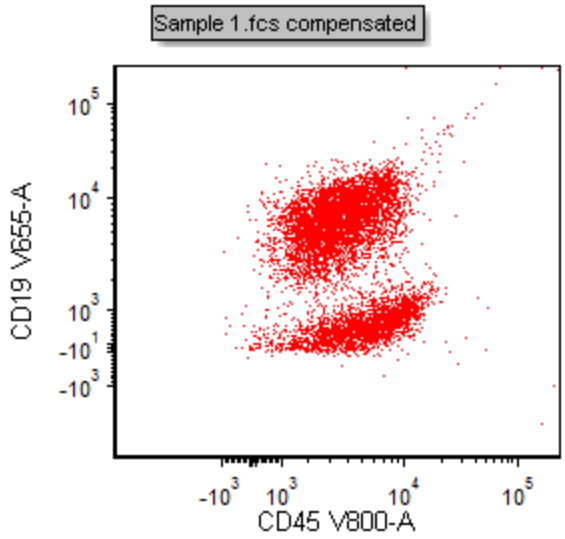

Figure T29.6 Plot with X- and Y-parameters changed to CD45 V800-A vs CD19 V655-A

14. Select the **Gating** tab→**Create Gates**→**Polygon** from the Ribbon (Fig. T29.7).

Figure T29.7 Selecting a gate shape from the Gating tab of the ribbon

15. Click once on each vertex to create a gate around the periphery of the CD45+CD19+ population (as in the figure below) in the plot at right (Fig. T29.8).

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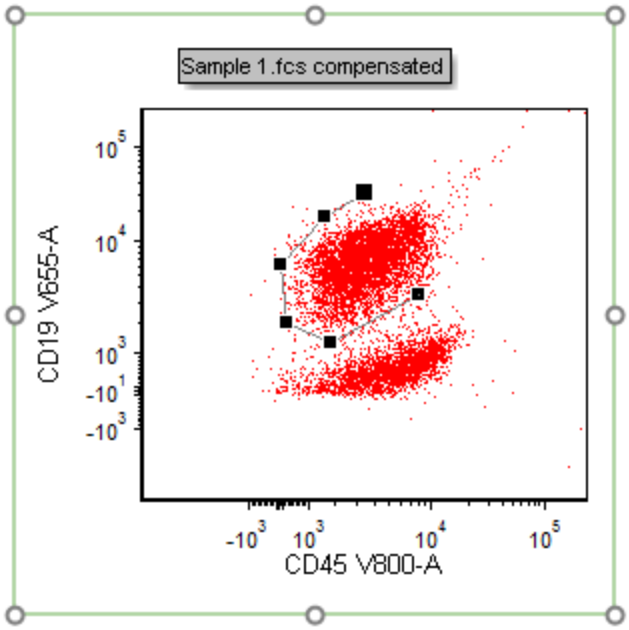

Figure T29.8 Drawing a polygon gate

16. Close the gate by clicking on the first vertex you defined (Fig. T29.9, ❶).

A dialog box 318 appears in which the gate name, color, and inclusion/exclusion property may be defined.

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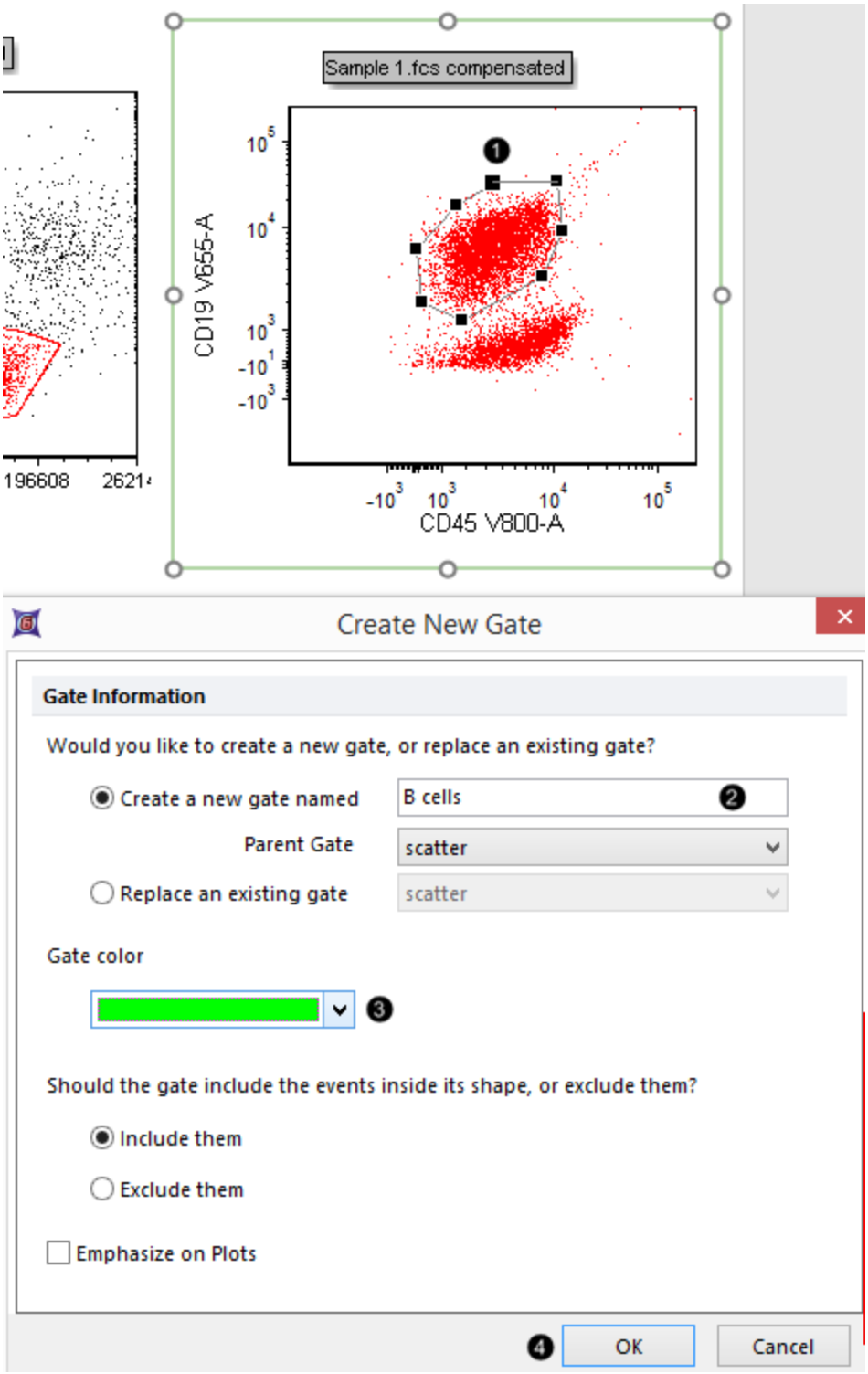

Figure T29.9 Closing and naming a polygonal gate

17. Type "B cells" in the **Create a new gate name** field (Fig. T29.9, ❷)

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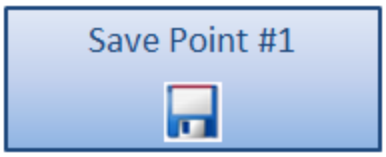
18. Select *bright green* from the **Gate color** drop down menu. (Fig. T29.9, ❸).

19. Click **OK** (Fig. T29.9, ❹).

20. Select **File** tab→**Save As** and save the layout **linked** (first option from drop down menu shown in Fig. 4.2). You may then return to this Step in the tutorial at a later time by clicking on Save Point #1 at the top of this page.



Save Point #1 (Step 21)

We will now examine the phenotype of the B-cell population in more detail by gating the plot in the lower-left corner on the "B cells" gate.

21. Press and hold anywhere inside the boundary of the green "B cells" gate (Fig. T29.10).

22. Drag the gate toward the plot in the lower-left corner. You will start to see a "ghost" of the gate outline move along the dragging path (Fig. T29.10, ❶).

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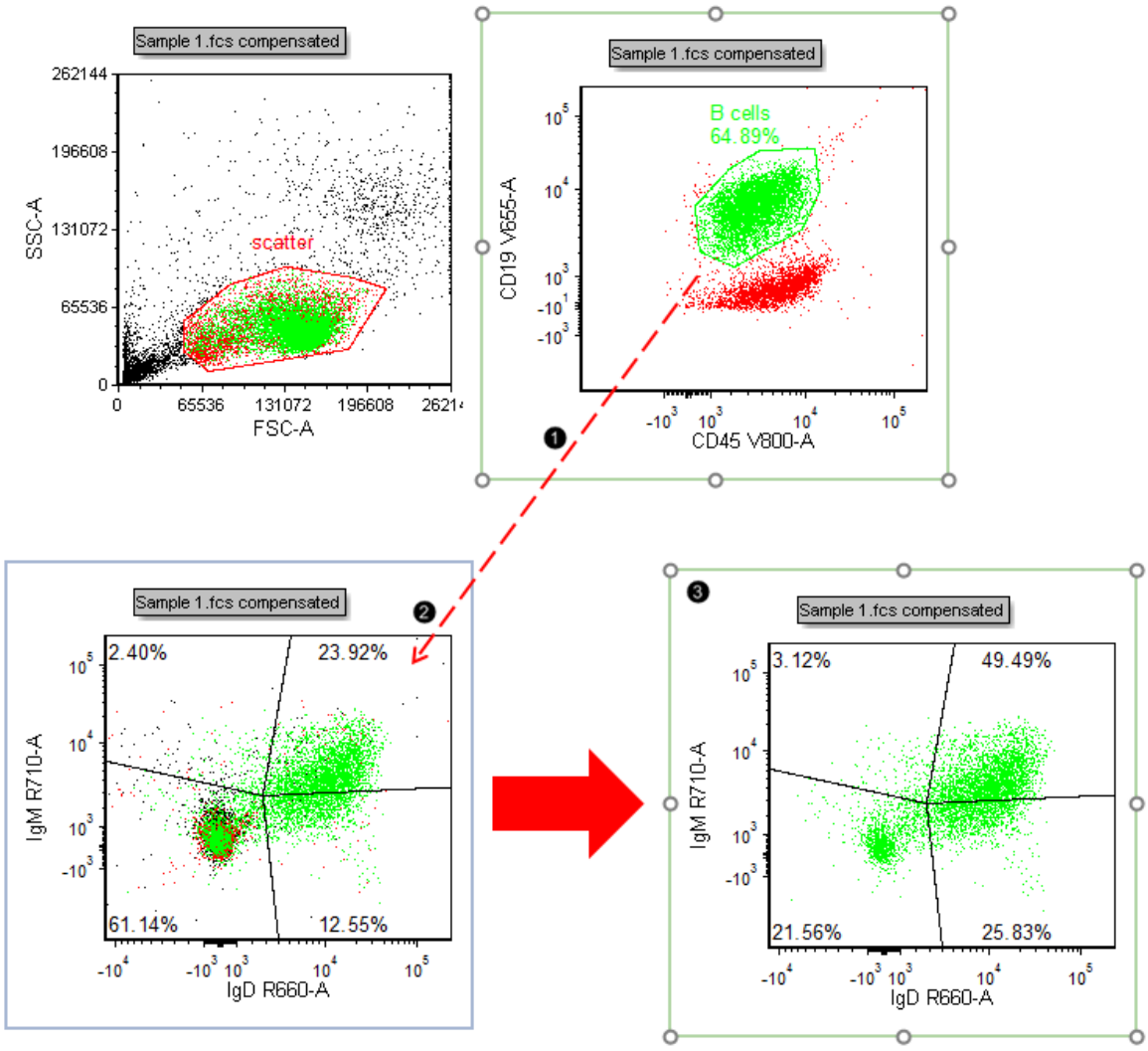

Figure T29.10 Gating a plot by dragging and dropping a gate from another plot

23. Release the mouse button when the recipient plot outline turns blue (Fig. T29.10, ❷).

    The recipient plot is now gated on "B cells" and appears similar to Fig. T29.10, ❸.

    Note: Dragging and dropping the "B cells" gate from the Gate View 345 to the recipient plot would achieve the same action.

24. Select **Format** tab→**Change**→**Change Plot Type** from the Ribbon (Fig. T29.11) while the lower-left plot is still selected.

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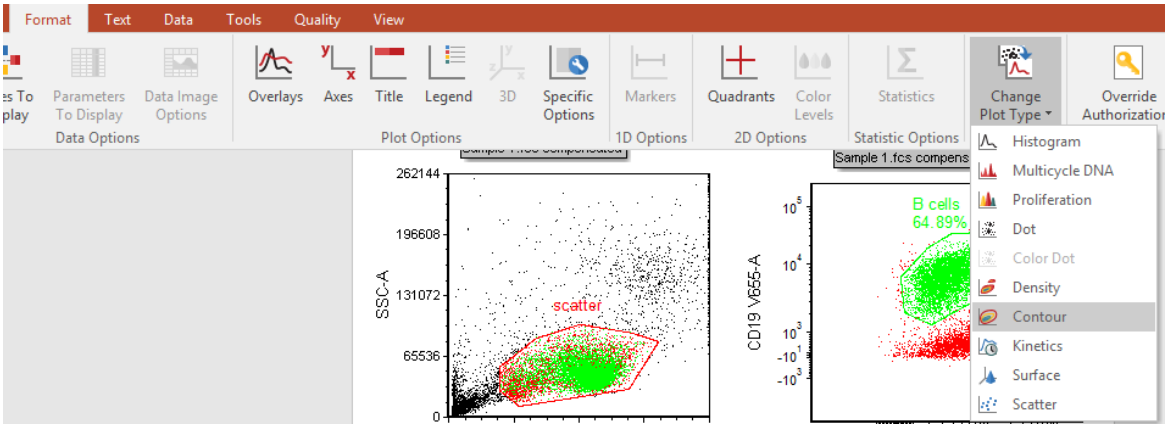

Fig. T29.16 Changing plot type

25. Click on **Contour** (Fig. T29.11).

    This plot now appears similar to the highlighted plot in Fig. T29.12.

Optional: Add outlier dots 284 to the Contour Plot, in Steps 27 to 32. Otherwise, proceed to Step 33.

26. Right-click on the **Contour Plot**.

27. Select **Format** from the associated pop-up menu (Fig. T29.12, mouse pointer).

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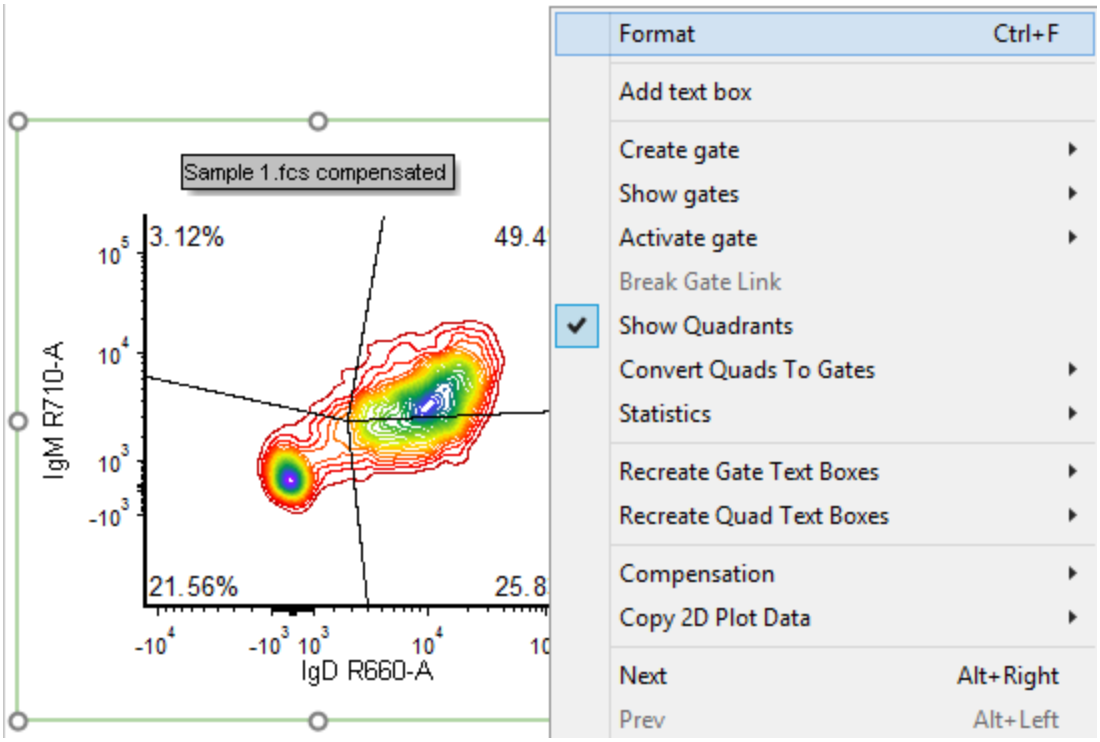

Figure T29.17 Right-clicking on a plot to reformat its appearance

28. Click on the **Overlays** category at the left (Fig. T29.13, ❶).

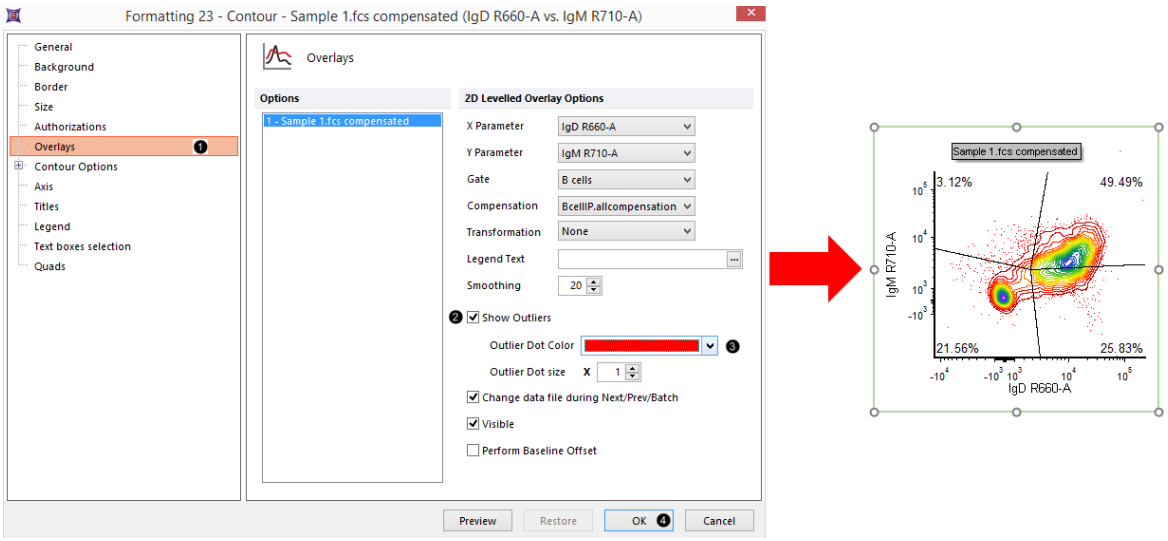

Figure T29.18 Adding outlier dots to a contour plot

29. Check the **Show Outliers** box (Figure T29.13, ❷).

30. Change the **Outlier Dot Color** to red (Figure T29.13, ❸).

31. Click **OK** (Figure T29.13, ❹). The plot now appears similar to Fig. T29.13, *right*.

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We will now examine the phenotype of the IgD/IgM-double-negative and -double-positive populations of B-cells in more detail.

32. Press and drag the central Quadrant vertex (e.g., as shown in Fig. T29.14) to adjust its position so that the double-positive and double-negative populations are better divided.

    Note: Outer vertices of these Floating Quadrants 1349 may also be adjusted, if desired.

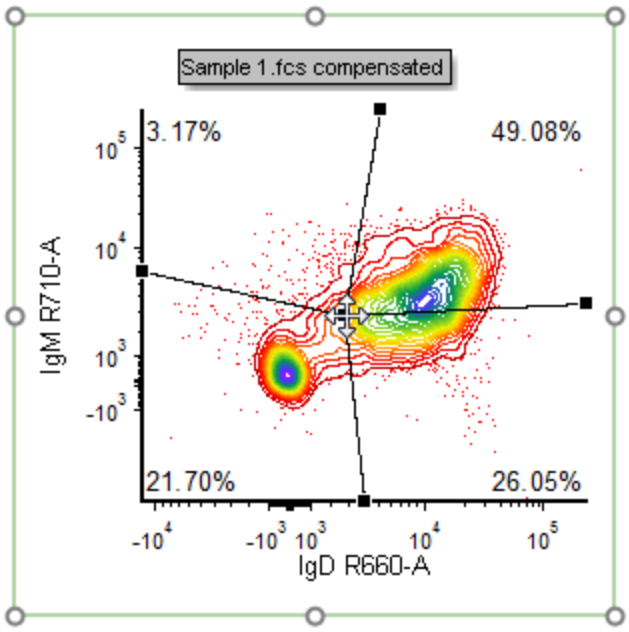

Figure T29.14 Adjusting the central quadrant vertex

33. Right-click on the contour plot (Fig. T29.15).

34. Select **Convert Quads to Gates→Upper Right→Convert and Link** (Fig. T29.15, mouse pointer).

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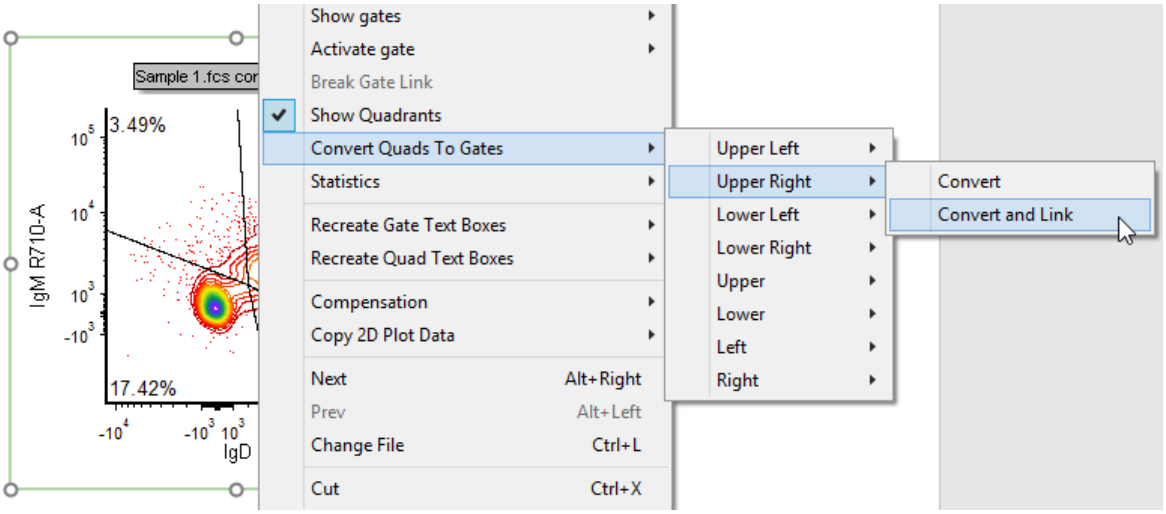


Fig. T29.15 Converting and linking the upper right quadrant to a gate

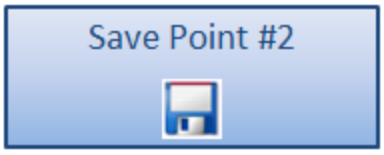
A dialog box similar to that in Fig. T29.9 2197 appears.

35. Repeat steps 18-20 2197 but name the gate "Double Positive" and assign it a turquoise color to match the pre-formatted text table in the lower right corner of the layout.

36. Repeat steps 34-36 in order to **Convert and Link** the **Lower Left Quadrant** to a Gate.

37. Repeat steps 18-20 2197 but name the gate "Double Negative" and assign it a pink color to match the pre-formatted text table in the lower right corner of the layout.

38. Select **File** tab→**Save As** and save the layout **linked** 93 (second radio button as shown in Fig. 4.2) 93, or simply press **Ctrl+S** on your keyboard if you already saved this layout linked 93 as in Save Point #1. You may then return to this Step in the tutorial at a later time by clicking on Save Point #2 at the top of this page.



Save Point #2 (Step 39)

39. Select **Insert** tab→**1D Plots**→**Histogram** from the Ribbon (Fig. T29.16, outlined in red).

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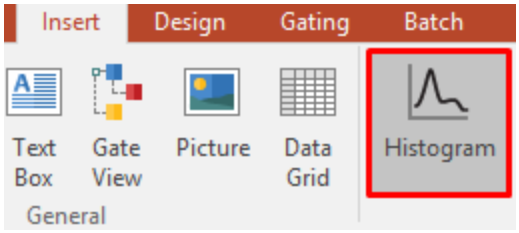

Figure T29.22 Selecting histogram from the Insert tab of the Ribbon

40. Press and hold on the blank space of the layout above the pre-formatted table (Fig. T29.17).

41. Drag the cursor until the histogram is of the desired size, while continuing to hold the mouse button (Fig. T29.17, ❶).

    Note: Any plot, text box, or other [object](239) on your layout may be [resized](1213) at any time.

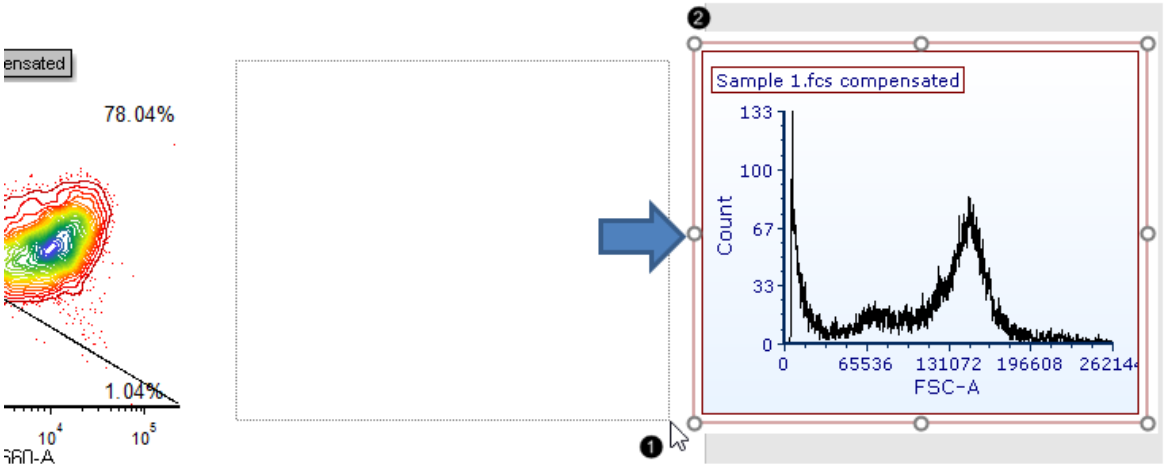

Fig. T29.17 Inserting a histogram onto the layout

42. Release the mouse button. A new [histogram](267) appears, similar to Fig. T29.17, ❷.

We will now [overlay](268) the "double-positive" and "double-negative" populations onto the histogram to compare their CD20-expression.

43. Right-click on the [histogram](267) (Fig. T29.18).

44. Select **Add overlay** in the associated pop-up menu (Fig. T29.18).

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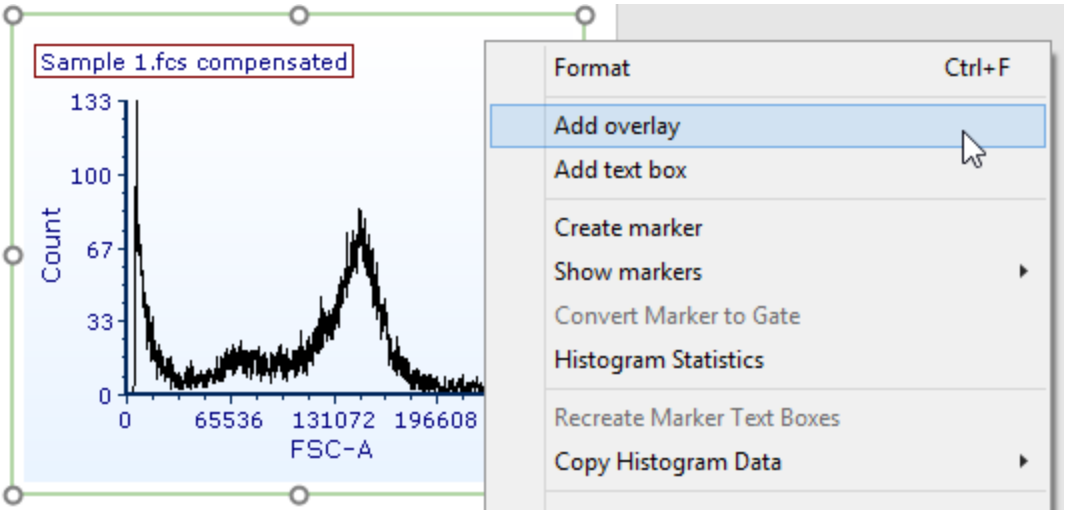

Figure T29.18 Adding an overlay to the histogram by right clicking on the plot and choosing "Add Overlay"

45. Repeat Steps 5-7 as in Fig. T29.2 to add "Sample 1.fcs" from the Data List tab as a second overlay to the histogram.

46. Click **OK** on the additional dialog box that appears (Fig. T29.19).

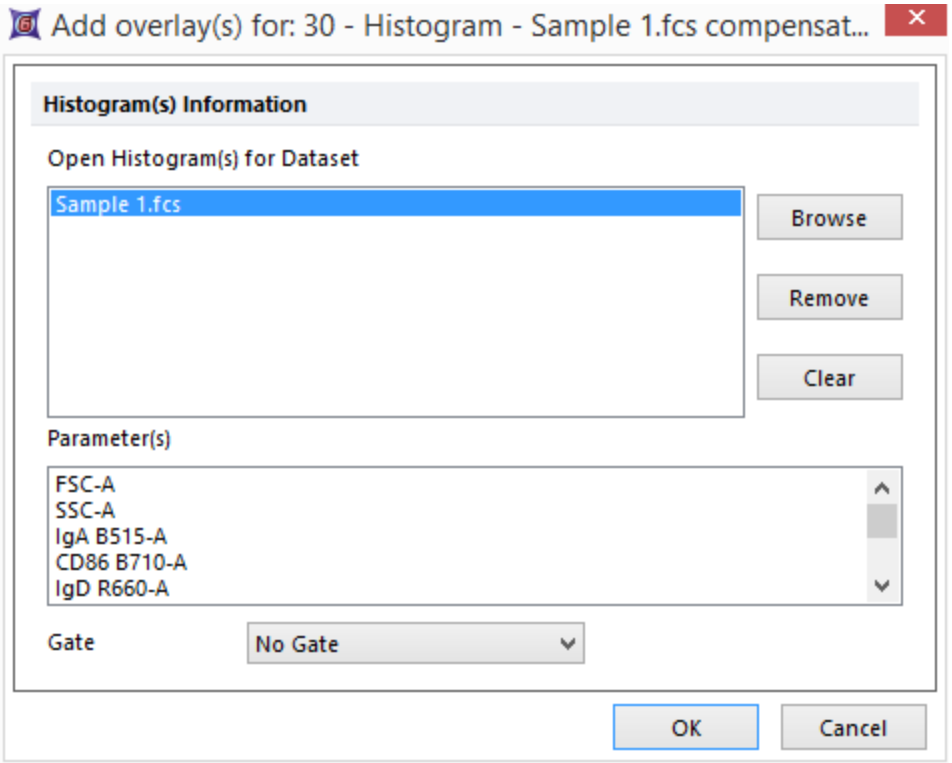

Fig. T29.19 Additional dialog box for adding overlays

47. Right-click on the histogram again (Fig. T29.20).

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48. Select **Format** on the associated pop-up menu (Fig. T29.26).

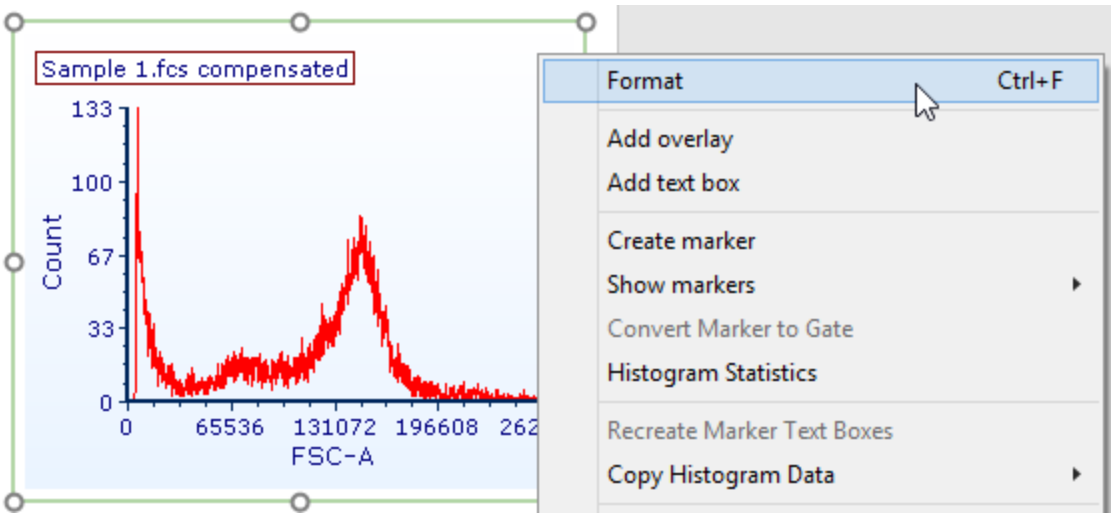

Figure T29.20 Right-clicking on the 1D-plot to access Format options

49. Select the **Overlays** category at the left (Fig. 29.27, ❶).

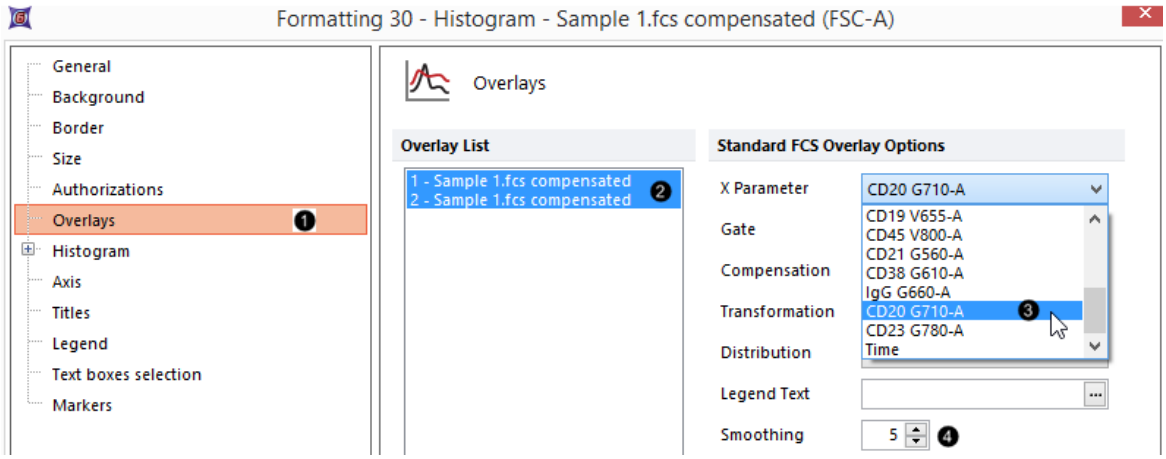

Figure T29.21 Formatting histogram overlays

50. Select both overlays by clicking on each while holding down **Ctrl** (Control) on the keyboard (Fig. 29.21, ❷).

51. Select "**CD20 G710-A**" from the **X Parameter** drop-down menu (Fig. 29.21, ❸).

52. Increase **Smoothing** 268 to "**5**" (Fig. 29.21, ❹).

53. Click on the first overlay ("**1-Sample 1.fcs compensate...**") so it alone is selected (Fig. 29.22, ❶).

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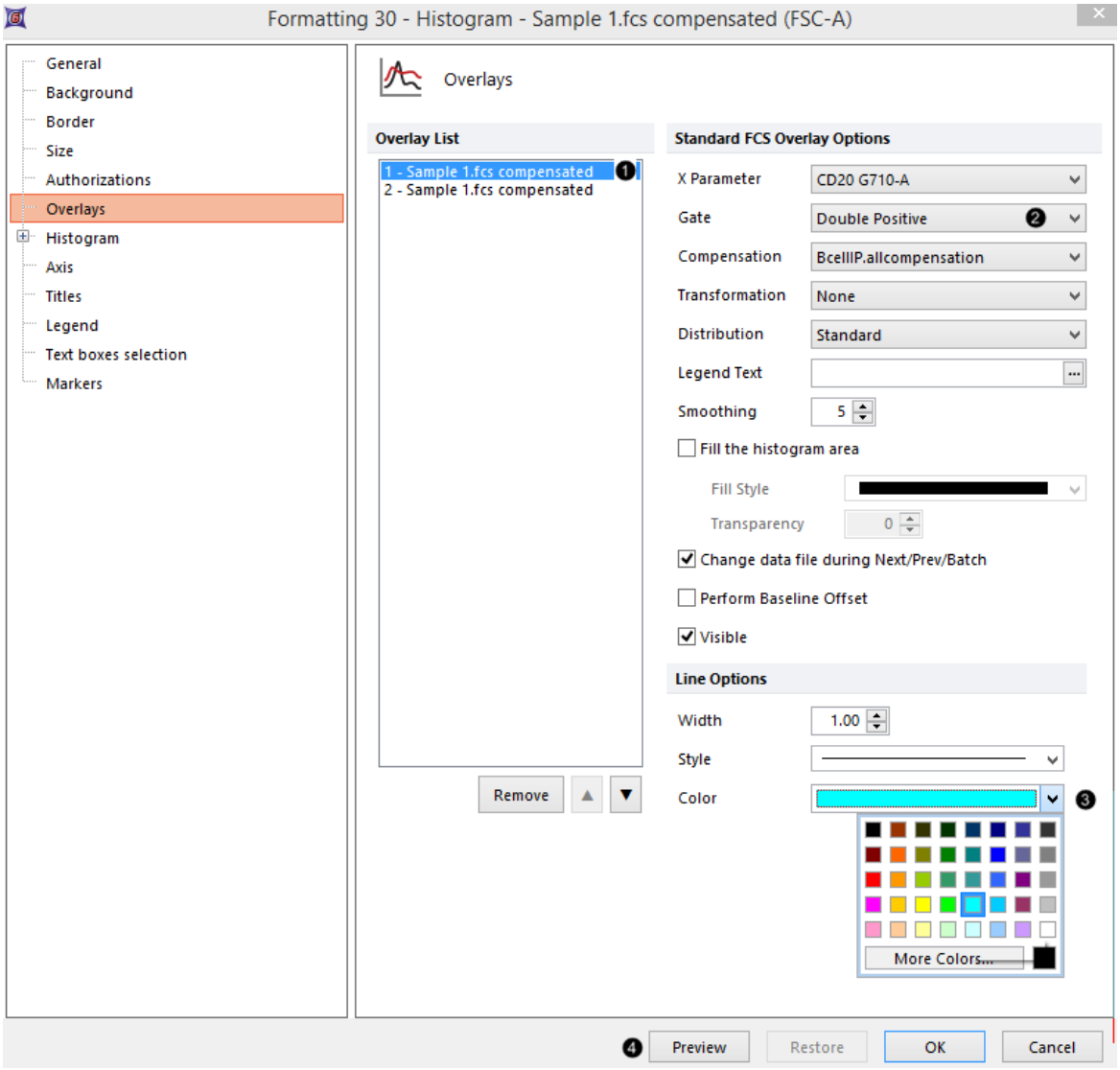

Figure T29.22 Formatting histogram overlays, continued

54. Select "**double positive**" from the **Gate** drop-down menu (Fig. 29.22, ❷).

55. Select turquoise as the **Color** under **Line Options** (Fig. 29.22, ❸).

56. Repeat Steps 54-56, but this time click instead on the second overlay ("**2-Sample 1.fcs compensate...**"), and choose "double negative" as the **Gate** and pink as the **Color**, respectively.

57. Click **Preview** (Fig. 29.22, ❹). *(Note: Move the formatting window to the side to preview the formatting changes)*

    The histogram now appears similar to Fig. T29.23.

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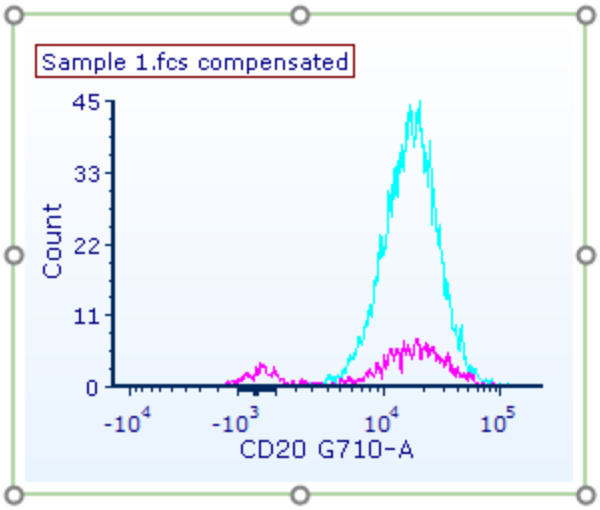

Figure T29.23 Preview of gates, colors, and smoothing applied to both histogram overlays

Optional: Normalize the histogram, in Steps 59-62. Otherwise, proceed to Step 63 2208.

58. Expand the Histogram, on the category at left (Fig. T29.24, ❶).

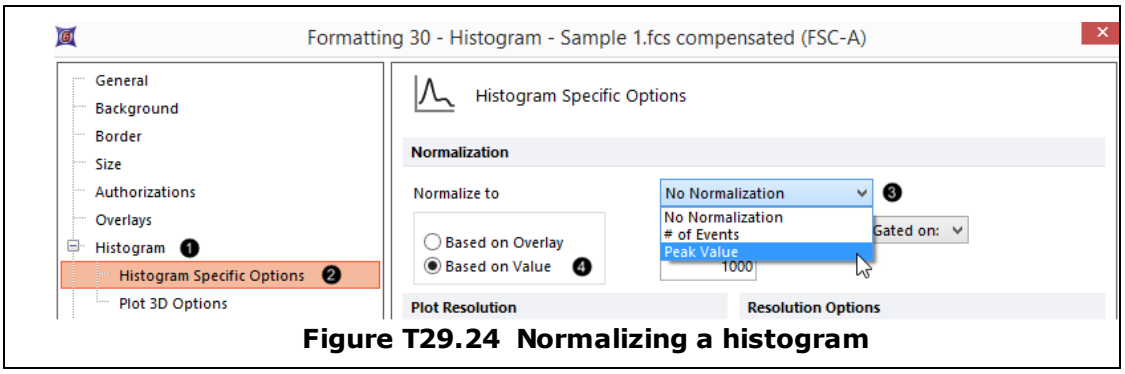

Figure T29.24 Normalizing a histogram

59. Click on **Histogram Specific Options** (Fig. T29.24, ❷).

60. Select **Peak Value** from the **Normalize to** drop-down menu (Fig. T29.24, ❸).

61. Select the **Based on Value** radio button (Fig. T29.24, ❹).

62. Click **OK** to close the **Formatting** pop-up window (Fig. T29.25). Post-normalization, the histogram will appear similar to Fig. T29.30, *right*; if you chose not to normalize, it will appear similar to Figure T29.29.

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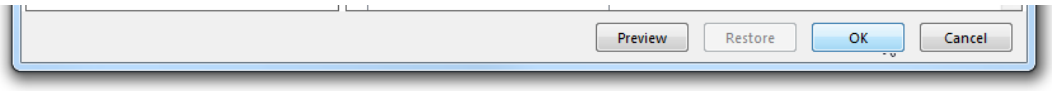

Figure T29.25 Clicking OK to accept the formatting changes.

We will now look at the differences in CD20-expression between the IgD/IgM-double-positive and -double-negative populations by creating a marker on the CD20-positive population.

63. Right-click on the histogram (Fig. T29.26).

64. Select **Create marker** on the associated pop-up menu (Fig. T29.26).

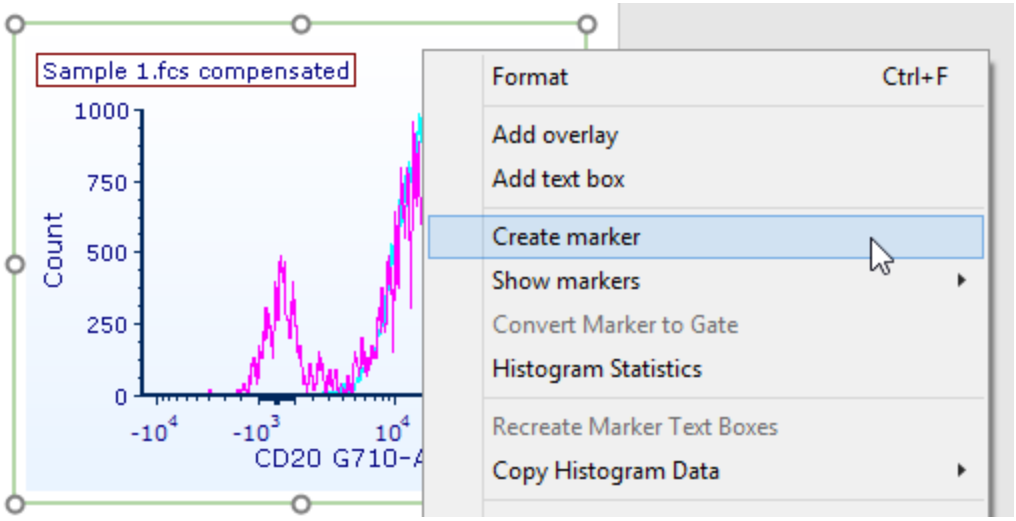

Figure T29.26 Right-clicking on the histogram to add a marker

65. Press and hold on the histogram to the left of the positive population peak (Fig. T29.27).

66. Drag the mouse pointer to the right until the marker spans the peak (Fig. T29.27, ❶).

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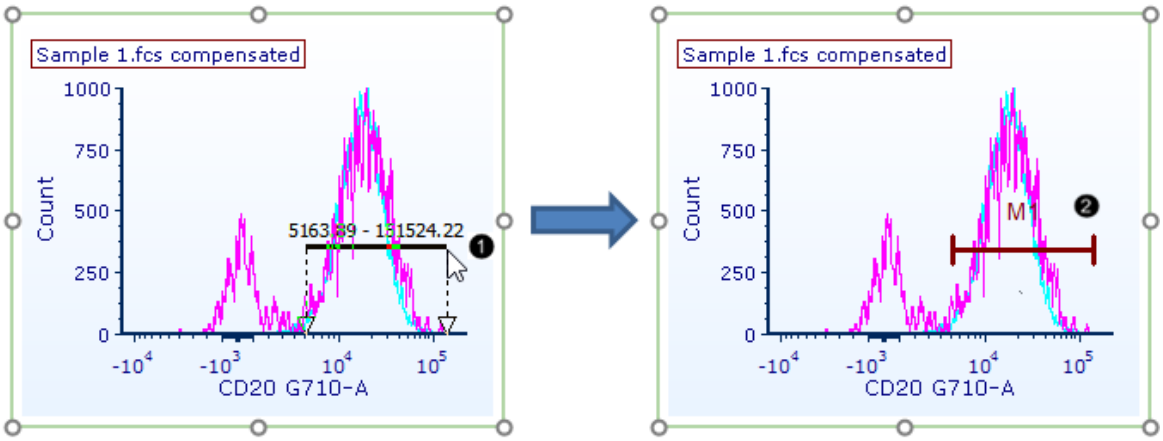
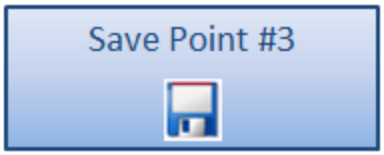


Figure T29.27 Creating a marker

67. Release the mouse. The marker ("**M1**") has now been added to the plot (Fig. T29.27, ❷).

68. Select **File** tab→**Save As** and save the layout **linked** (second radio button as shown in Fig. 4.2), or simply press **Ctrl+S**on your keyboard if you already saved this layout linked as in Save Point #1 or #2. You may then return to this Step in the tutorial at a later time by clicking on Save Point #3 at the top of this page.



Save Point #3 (Step 69, end of this section)

We have now created a Layout with 2D-plots, histograms, gates, quadrants, and markers. Next, we will add summary statistics to our layout.

38.31.2 Adding statistics to your layout

In this section, we will:

- Add a default statistics table to our layout.
- Fill a custom text-table with selected statistics.
- Define a custom ratio using a Custom Token.

It is easy to add default statistics tables as well as custom-made tables and statistics to your FCS Express Layout. We will now add one example of each to this Immunophenotyping Tutorial Layout.

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1. Select **File** tab→**Open**.

2. Open the layout **Immunophenotyping Tutorial Ready for Summary Statistics.fey** found in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive.

   The Layout has been saved linked 93 to the .fcs files in the same folder, and should resemble your Layout after completing the previous section of the tutorial 2187. You may use this Layout or your own Layout to proceed to Step 3.

3. Right-click on the contour plot in the lower-left corner of the Layout (Fig. T29.28; *note: the plot pictured is from the provided layout for this section and shows red outlier dots added as in optional Steps 27-32 from Section 1 2200. If you are using your own layout from the previous section and skipped those steps, your plot will look slightly different.)*

4. Select **Quadrant statistics** from **Statistics** on the associated pop-up menu (Fig. T29.28).

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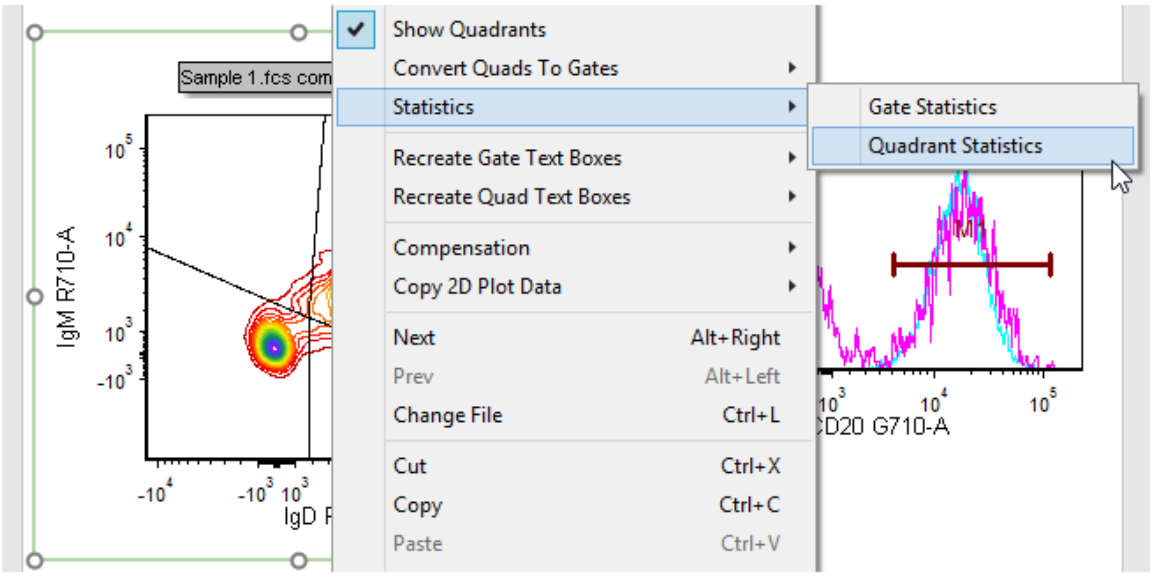

Overlay #	Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of gated cells	% of all cells
1	Sample 1.fcs compensated	UL	265	n/a	3879.65	3.20	1.48
1	Sample 1.fcs compensated	UR	6336	9105.08	n/a	76.61	35.31
1	Sample 1.fcs compensated	LL	1433	n/a	n/a	17.33	7.99
1	Sample 1.fcs compensated	LR	237	3969.61	n/a	2.87	1.32

Figure T29.28 Adding a default quadrant statistics window to the layout

A quadrant statistics window appears, similar to Fig. T29.28. *Note: you may move this statistics window to a convenient part of your layout, such as below the contour plot.*

Note: Default statistics windows may also be similarly added for gates, markers, and histograms. You may right-click on the quadrant statistics window to add or remove various statistics.

A Default statistics window is a convenient way to quickly display summary statistics for a given plot. Summarizing the results of your entire experiment, however, often requires data from multiple plots on the layout. This is easily accomplished in FCS Express by adding statistical and keyword tokens to custom-made tables in a text box. We will now add statistical tokens to the text table in the lower-right corner of the layout, which was custom designed within a text box. The chief

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advantages of these custom tables are: statistics[381] from disparate plots may be displayed; an endless variety of statistics[381] including user-defined statistics[1324] may be defined; and, the appearance of the table itself is fully formattable[1311].

5. Click on the border of the histogram to select it (Fig. T29.29)

   ***Note**: the histogram pictured is from the provided layout for this section and shows a normalized histogram as formatted in optional Steps 59-62 from the previous section[2206]. If you are using your own layout from the previous section and skipped those steps, your histogram will instead resemble Fig. T29.23[2208].)*

6. Drag the plot toward the upper-right cell in the table (Fig. T29.29, ❶).

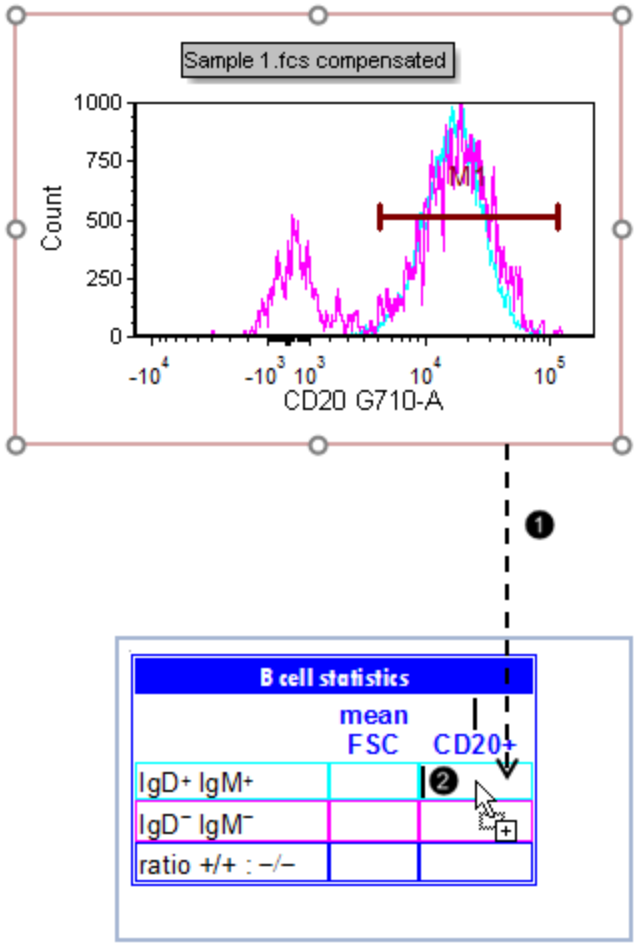

Figure T29.29 Dragging and dropping a marker-derived statistical token into a text table

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7. Release the mouse when the cursor is visible within the upper-right, turquoise cell in the table (Fig. T29.29, ❷). Go on to Step 8 or to Step 11 2214 if you were referred here from a more advanced point in this tutorial.

8. Double-click **Statistic token** in the **Paste Special** dialog (T29.30, ❶).

9. Select "M1" in the **Select a marker** drop-down menu of the **Edit Statistic** dialog box that appears (Fig. T29.30, ❷).

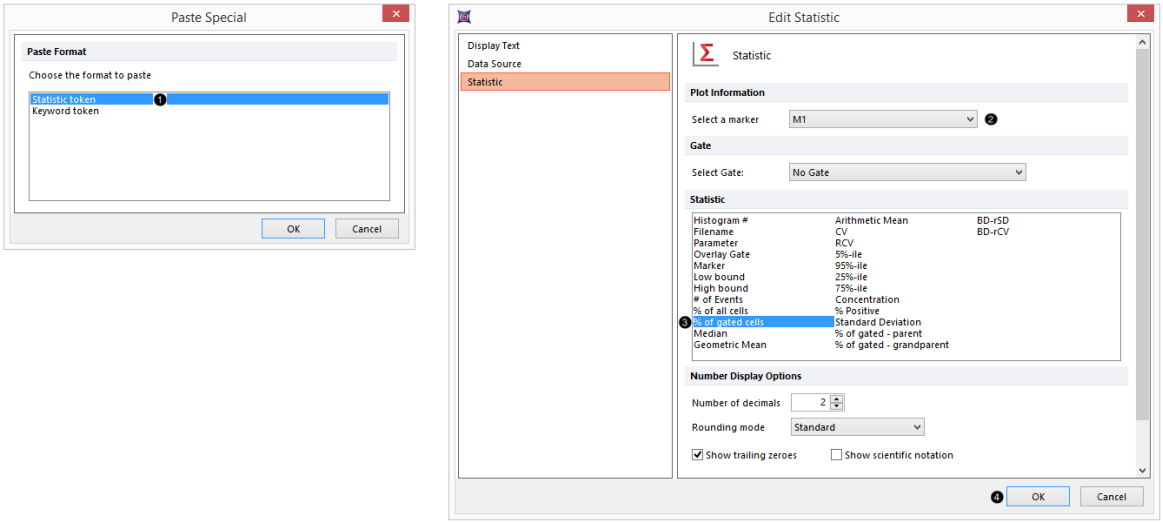

Figure T29.30 Selecting a marker-derived statistical token

10. Select **% of gated cells** in the **Statistic** list (Fig. T29.30, ❸).

11. Click **OK** (Fig. T29.30, ❹).

12. Repeat Steps 5-9 2213, but drag the marker into the pink cell reserved for the double-negative, CD20-positive population (immediately below the cell indicated in Fig. T29.30).

13. Click on **Data Source** in the category at left (Fig. T29.31, ❶).

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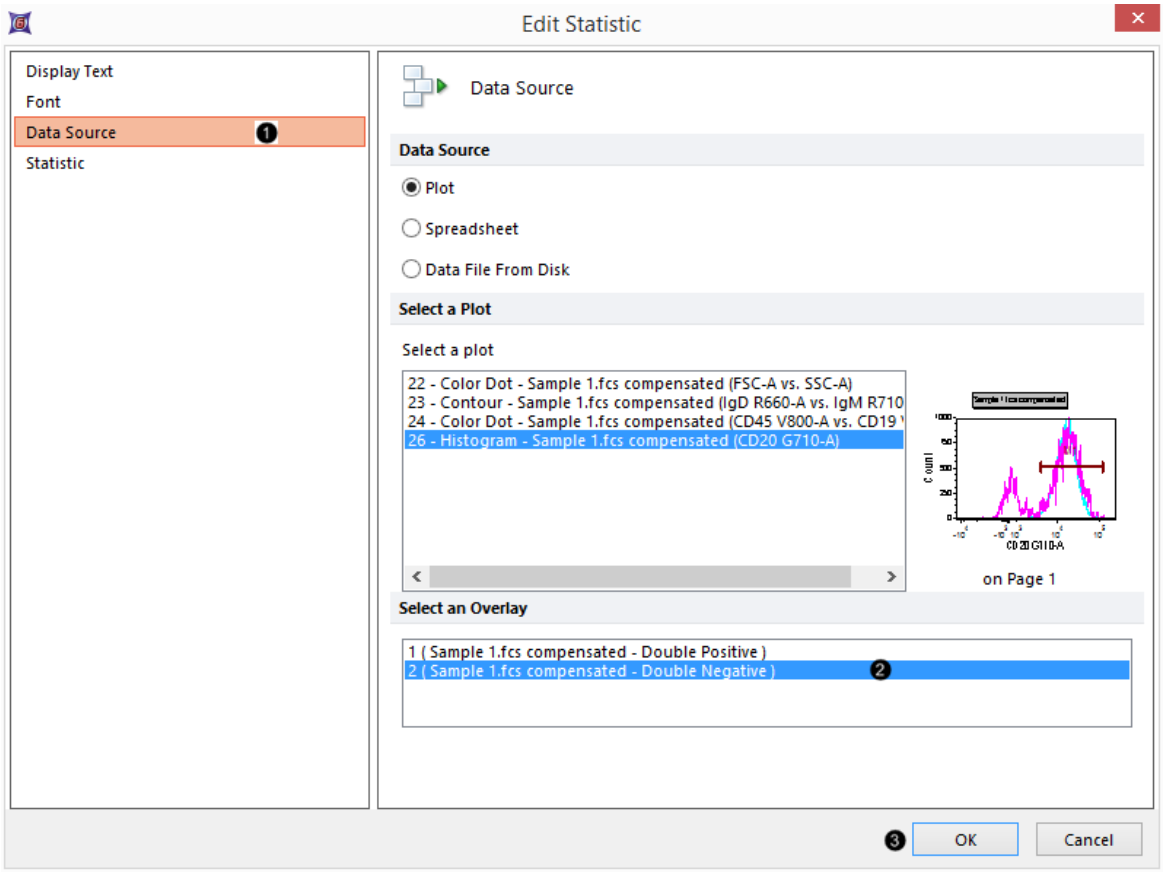

Figure T29.31 Defining the second overlay in the histogram as the data source for a statistical token

14. Click on the second overlay to select it as the **Data Source** for this statistic (Fig. T29.31, ❷).

15. Click **Statistic** and select **% of gated cells**.

16. Click **OK** (Fig. T29.31, ❸). The two tokens now appear in the text table as shown in Fig. T29.32. *Note: exact values may vary in your layout due to your placement of gates, markers, and quadrants. You may adjust any of these and watch the tokens update accordingly.*

17. Press and hold the mouse button inside the boundaries of the red "scatter" gate in the upper-left color dot plot (Fig. T29.32).

18. Drag it toward the table (Fig. T29.32, ❶).

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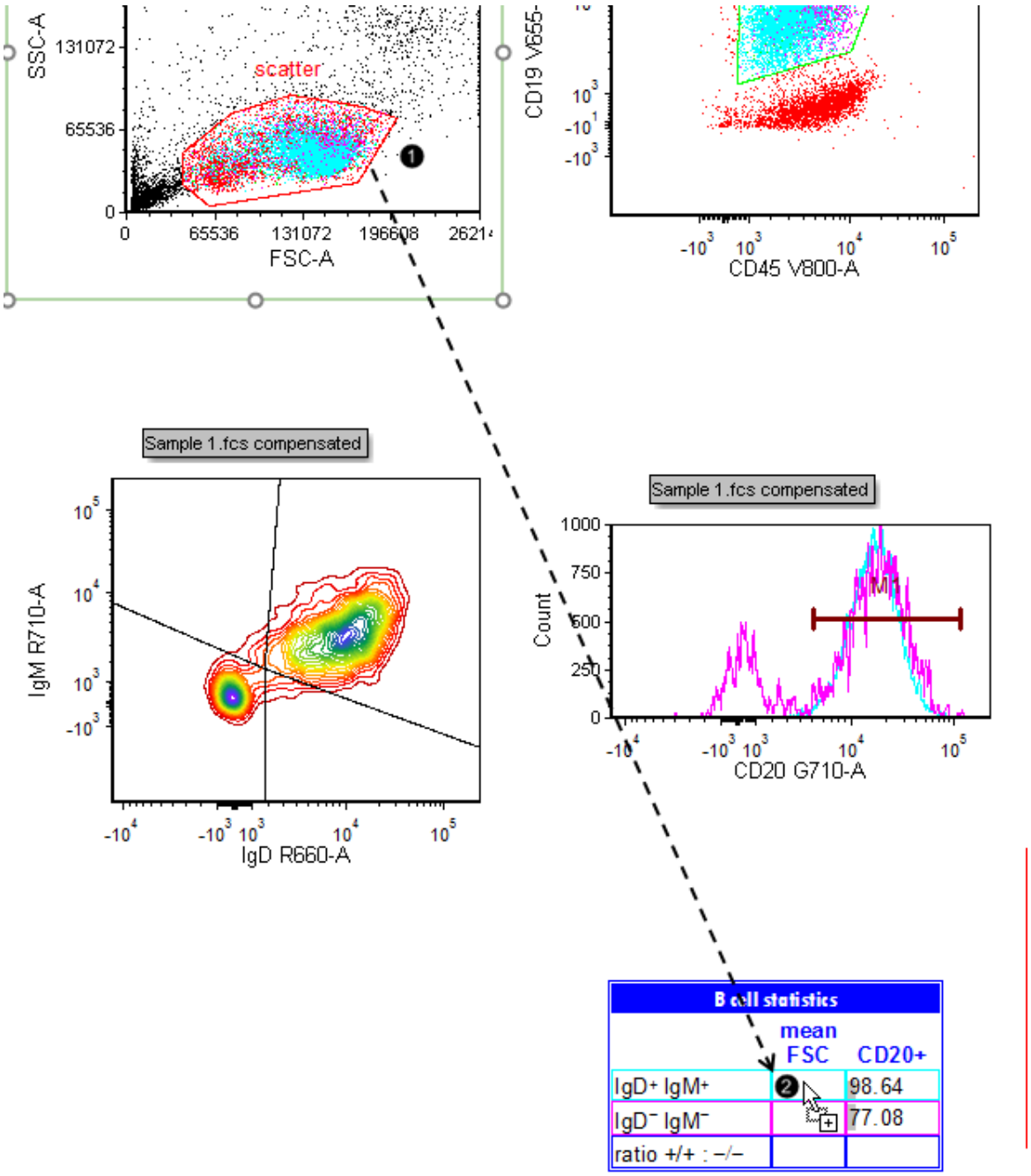

Figure T29.32 Dragging and dropping a gate-derived statistical token into a text table

19. Release the mouse when the cursor is visible inside the turquoise cell pictured in Fig. T29.32, ❷.

20. Select "double positive" from the **Gate** drop-down menu of the **Edit Statistic** dialog box that appears (Fig. T29.33, ❶).

21. Select **X Arithmetic Mean** in the **Statistic** category (Fig. T29.33, ❷).

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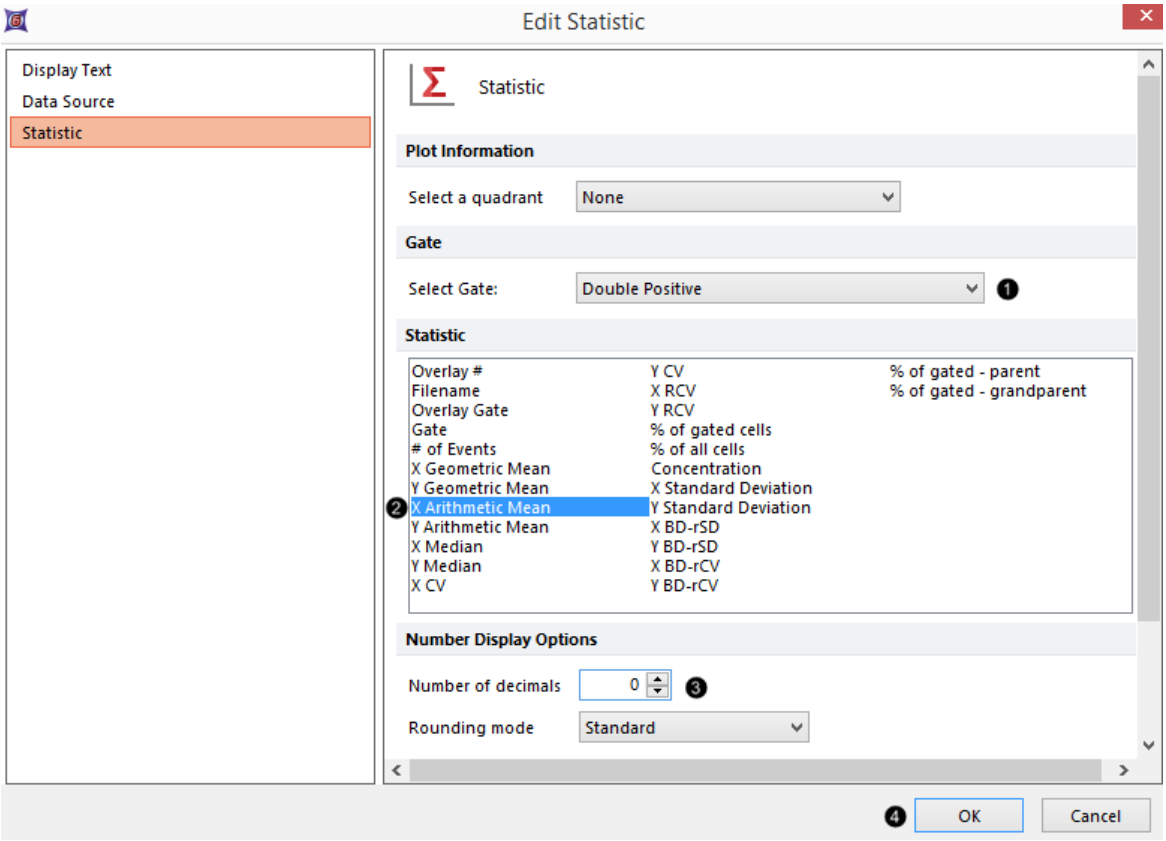

Figure T29.33 Selecting a gate-derived statistical token

22. Reduce the **Number of decimals** to "0" (Fig. T29.33, ❸).

23. Click **OK** (Fig. T29.33, ❹).

24. Repeat Steps 16-22 2215, but drag the "scatter" gate into the pink cell reserved for the double-negative population (immediately below the cell indicated in Fig T29.32), and select "double negative" in the **Gate** drop-down menu.

We will now define a ratio between the CD20-positive populations of the IgD/IgM-double-positive and -double-negative B cells using Custom Tokens 1324, which we will subsequently enter into the lower-right blue cell in the table reserved for "ratio +/+ : –/–".

25. Select the **View** tab →**Tokens**→**Custom Tokens** (Fig. T29.34).

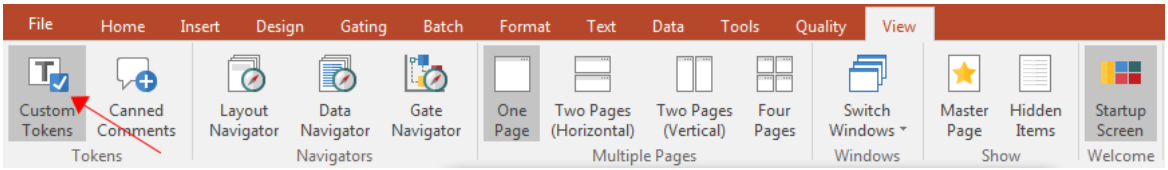

Figure T29.34 Opening the Custom Tokens window

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26. Move the floating **Custom Tokens** window to the side of the page so the plots are visible.

27. Click the blue Plus button ( ) for (**Create a new Custom Token**) (Fig. T29.35, ❶).

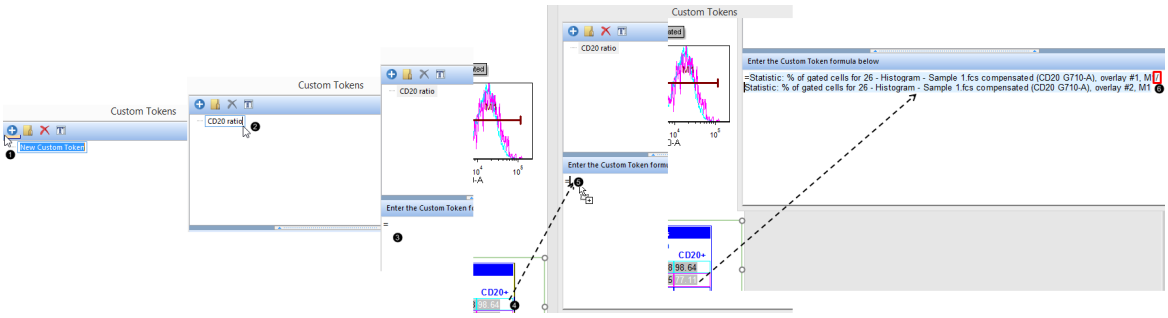

Figure T29.35 Defining a new custom token as a ratio between two regular statistical tokens

28. Type "CD20 ratio" as a descriptive name for the Custom Token (Fig. T29.35, ❷).

We will now define a Formula for a simple ratio, using a procedure similar to that used in Microsoft Excel®. However, instead of typing static numbers into the Formula, we will enter the Tokens we defined in the text table on the layout. Thus, as the values of the tokens change among different samples in our experiment, the value of the "CD20 ratio" Custom Token will update accordingly.

29. Type an equals sign ("=") in the **Enter the Custom Token formula below** pane of the **Custom Tokens** window (Fig. T29.35, ❸).

30. Highlight the Token in the upper right, turquoise cell of the Layout (Fig. T29.35, ❹).

31. Drag it toward the **Enter the Custom Token formula below** pane, while keeping the mouse button pressed.

32. Release the mouse when the cursor appears after the equals sign ("=") (Fig. T29.35, ❺). The Token Description will appear there, highlighted in blue.

33. Type a division sign ("/") in the **Enter the Custom Token formula below** pane of the **Custom Tokens** window (Fig. T29.35, ❻).

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34. Repeat Steps 29-31 for the Token in the pink cell corresponding to the CD20-positive, IgD/IgM double-negative population. The complete "CD20 ratio" Custom Token Formula is pictured in Fig. T29.35, *extreme right*.

We will now drag and drop this "CD20 ratio" Custom Token into the appropriate cell, to complete the "CD20+" column of the text table on our Layout.

35. Press and hold on the "CD20 ratio" Custom Token in the **Custom Tokens Window** (Fig. T29.36, ❶).

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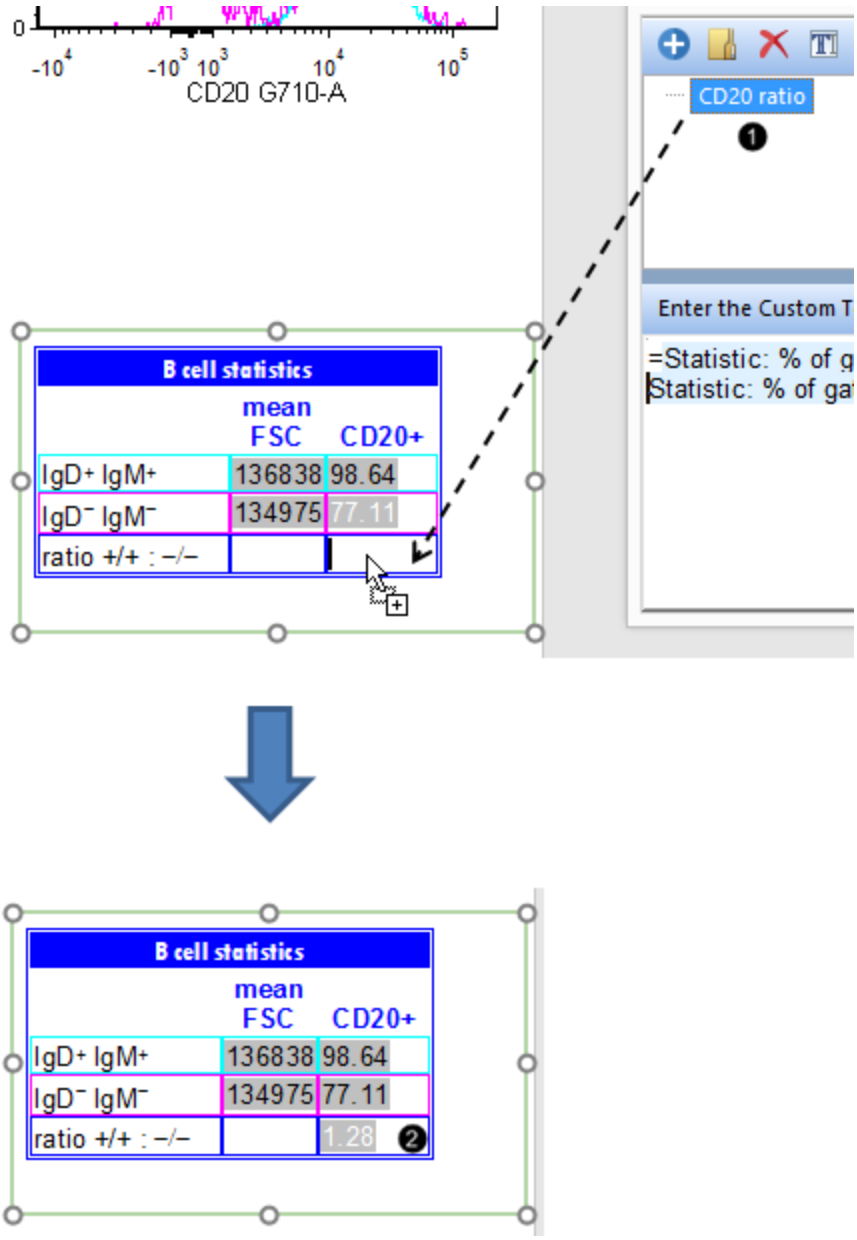

Figure T29.36 Dragging and dropping a custom token from the custom tokens window into the summary text table on the layout

36. Drag it toward the lower-right cell in the text table.

37. Release the mouse when the cursor is visible in the appropriate cell (Fig. T29.42, dashed arrowhead). The value of the "CD20 ratio" Custom Token will appear as in (Fig. T29.36, ❷). *Note: the value may differ slightly from that shown, depending on your placement of gates, markers, and quadrants. You may adjust these to watch the value of the "CD20 ratio" Custom Token update in real-time.*

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You have now added a default statistics table to your layout, and have filled a custom text table[1311] with selected statistics[381], including a custom ratio between two statistics[1324].

Learn even more about using text boxes and tokens to take advantage of the powerful statistical features in FCS Express by viewing a recorded webinar and completing a separate tutorial[1298] dedicated to this topic.

In the final section of this tutorial, we will batch process our data[2221] to generate Microsoft PowerPoint™ and Excel™ reports.

38.31.3 Batch processing your analysis

In this final section of the tutorial, we will create Microsoft PowerPoint™ and Excel™ exports using the powerful Batch Processing[784] function of FCS Express.

1. Select **File** tab→**Open**.
2. Open the layout **Immunophenotyping Tutorial Ready for Batch Processing.fey** found in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive[1210].

   The layout has been saved linked[93] to the .fcs files in the same folder, and should resemble your own layout after completing the previous section of the tutorial[2210]. You may use this provided layout or your own layout to proceed to Step 3.
3. Select the **Batch** tab→**Batch Processing**→**Batch Actions** (Figure 29.82).

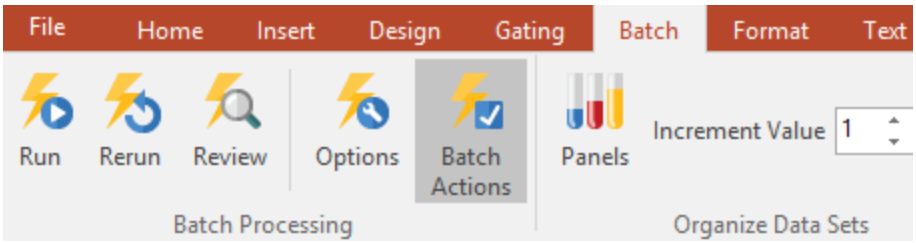

Figure 29.<%C++%> - Opening Batch Actions

4. Move the **Batch Actions** window to the side of the page so that your plots are visible.

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5. Click **Save to Powerpoint** in the **Add Action** category of the **Batch Actions** window (Fig. T29.38, ❶).

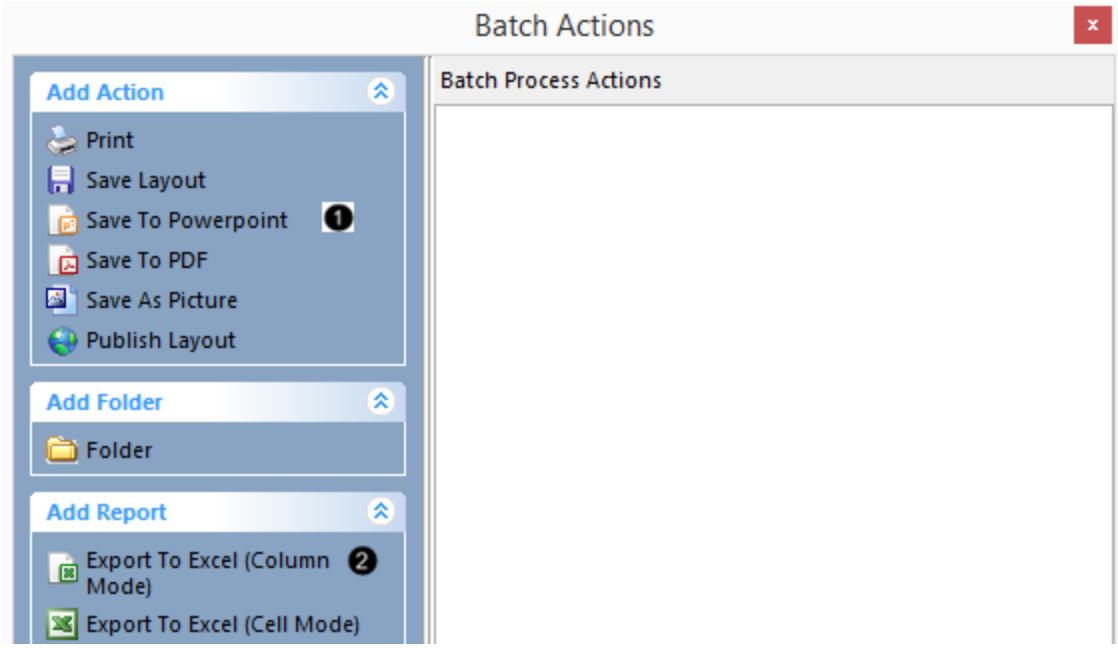

Figure T29.38 Batch actions window

6. Check the **Open presentation after saving** box in the **Create Export to Powerpoint Action** dialog box which opens automatically (Fig. T29.39, *left,* ❶).

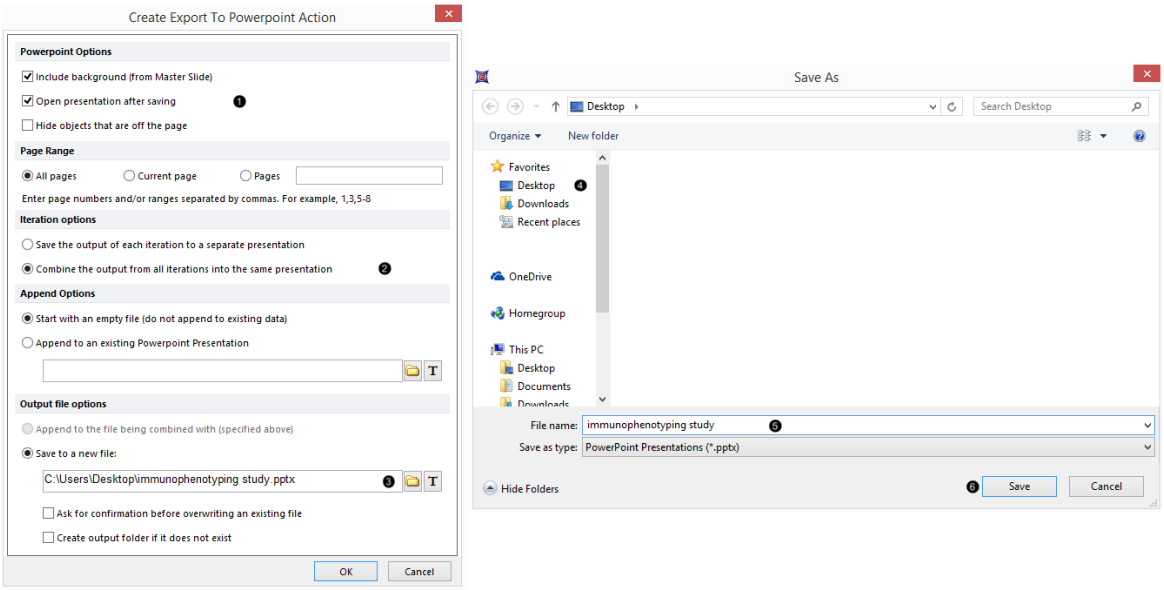

Figure T29.39 Adding a PowerPoint action to Batch Process Actions

7. Click the **Combine the output from all iterations into the same presentation** radio button in the **Iteration options** section (Fig. T29.39,

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left, ❷). *Note: the first radio button, if selected, would result in each sample being saved to its own .PPT file.*

8. Click on the Folder icon under **Save to a new file** in the **Output file options** section (Fig. T29.39, *left,* ❸).

9. Navigate to the directory folder to which the exported file will be saved (e.g., Desktop), in the **Save As** dialog window (Fig. T29.39, *right,* ❹).

10. Type "immunophenotyping study" in the **File name** field (Fig. T29.39, *right,* ❺).

11. Click **Save** (Fig. T29.39, *right,* ❻).

12. Click **OK** in the **Create Export to Powerpoint Action** dialog box (Fig T29.39, *left*, mouse pointer).

13. Click on **Export to Excel (Column Mode)** in the **Add Report** category of the **Batch Actions** window (Fig T29.38, ❷).

14. Click on the Folder icon under **Save to a new file** in the **Output file options** section of the **Excel (Column Mode) Options** dialog box (Fig. T29.40, ❶).

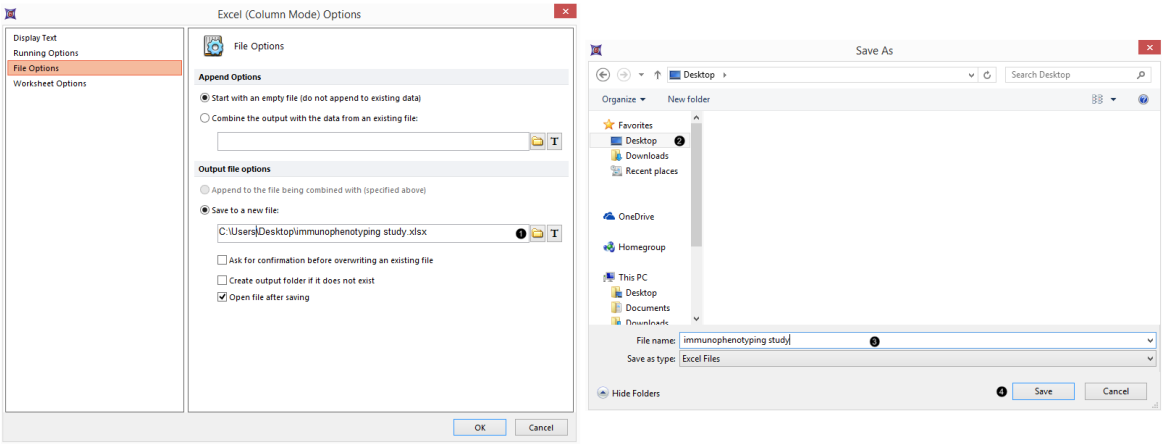

Figure T29.40 Adding an Excel (Column Mode) report to Batch Process Actions

15. Navigate to the directory folder to which the exported file will be saved, such as the Desktop, in the **Save As** dialog window (Fig. T29.40, ❷).

16. Type "immunophenotyping study" in the **File name** field (Fig. T29.40, ❸).

17. Click **Save** (Fig. T29.40, ❹).

18. Click **OK** in the **Excel (Column Mode) Options** dialog box (Fig T29.40, ❺).

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We will now choose which tokens to include in our Excel export.

19. Click on the text table in the lower-right corner of the layout so that its border is highlighted (Fig. T29.41, ❶).

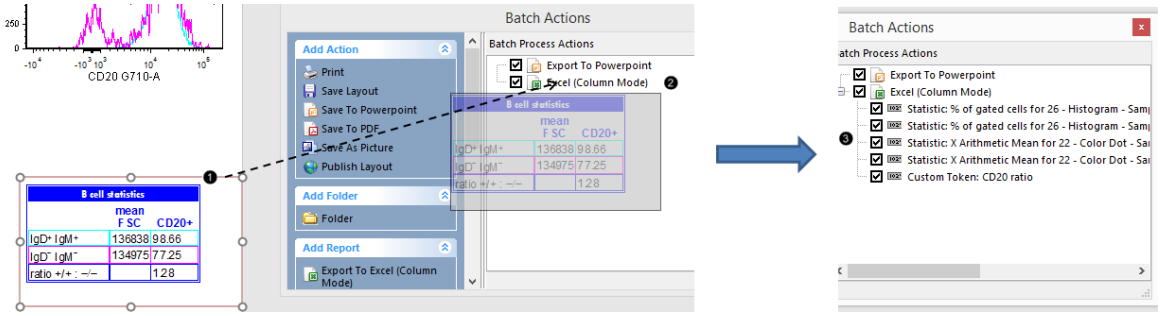

Figure T29.41 Dragging and dropping the tokens within a text table into the Excel export in the Batch Process Actions window.
(Note: the layout pictured has had the optional steps completed for adding red outlier dots to the contour plot, and for normalizing the histogram. If you are using your own layout from the previous section and skipped those steps, those two plots will look slightly different.)

20. Drag the text table toward the **Batch Actions** window, while keeping the mouse button pressed.

21. Release the mouse when the cursor is on the **Excel (Column Mode)** icon in the list of **Batch Process Actions** (Fig. T29.41, ❷). You may notice a ghost of the table appearing in the **Batch Process Actions** before you release the mouse. All of the statistical tokens in the text table have now been added to the **Excel (Column Mode)** export (Fig. T29.41, ❸).

Note: You may also add tokens to your Excel exports 1455 by dragging and dropping plot elements, including gates, gate/marker/quadrant text boxes, and entire plots; individual tokens from default Statistics Windows and text tables may be added in the same manner as well. We will now add a Token to the report by dragging and dropping a plot.

22. Click on the upper-left plot on the layout so that its border becomes highlighted, indicating that it is selected (Fig. T29.42, ❶).

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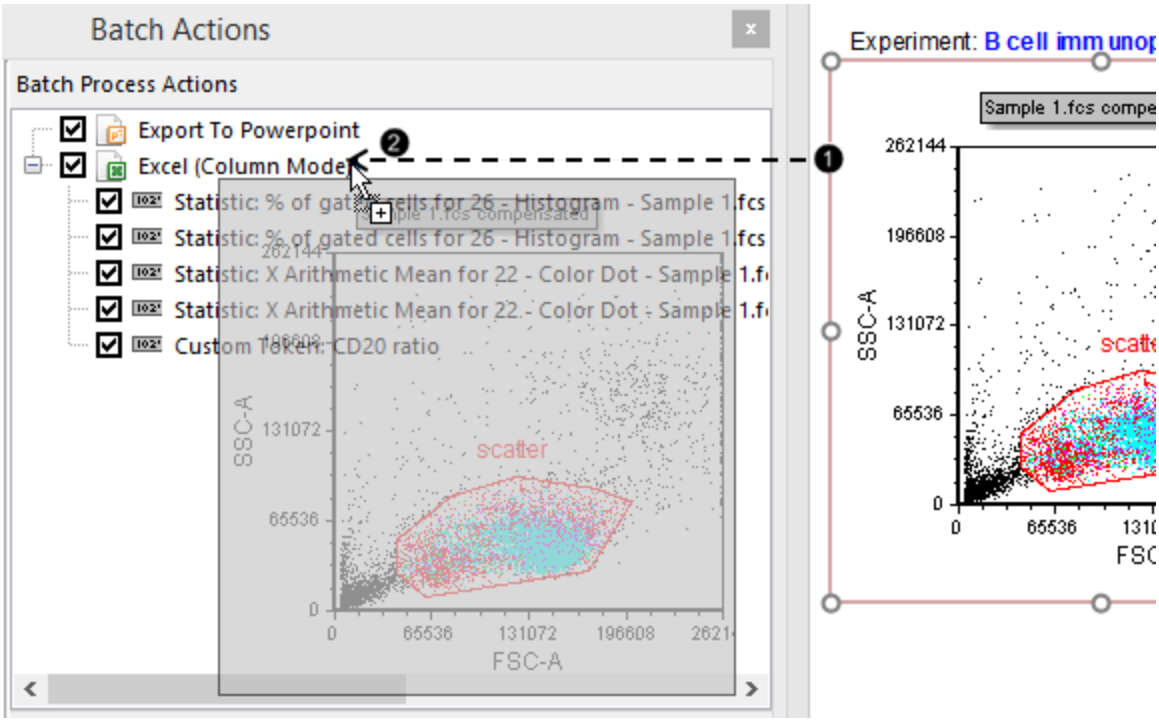

Figure T29.42 Dragging and dropping a plot-derived element into the Excel export of the Batch Process Actions window

23. Drag the plot toward the **Batch Actions** window, while keeping the mouse button pressed.

24. Release the mouse when the cursor is on the **Excel (Column Mode)** icon in the list of **Batch Process Actions** (Fig. T29.42, ❷). You may notice a ghost of the plot appearing in the **Batch Process Actions** before you release the mouse.

25. Click on **Keyword Token** in the **Paste Special** dialog box (Fig. T29.43, ❶).

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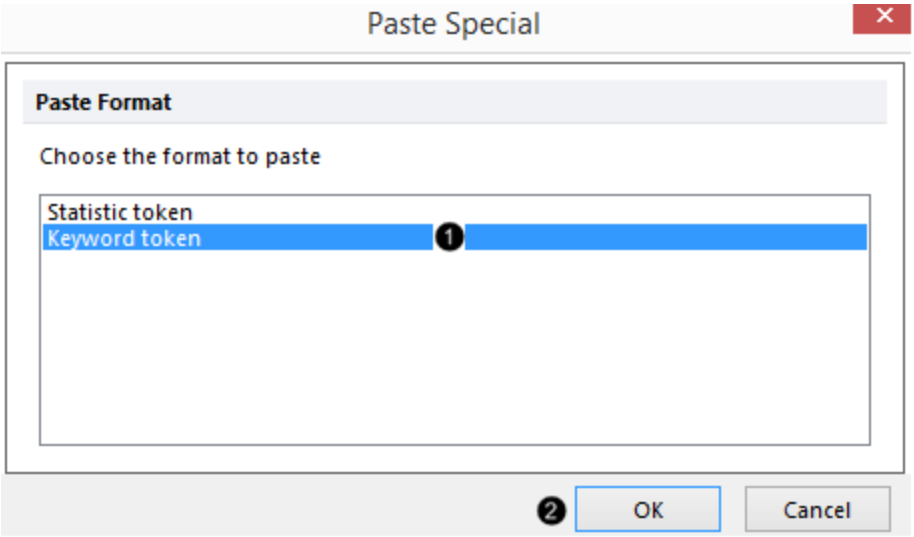

Figure T29.43 Selecting a keyword token

26. Click **OK** (Fig. T29.43, ❷).

27. Click on the ellipsis in the **Select a keyword** dialog box (Fig. T29.44, ❶).

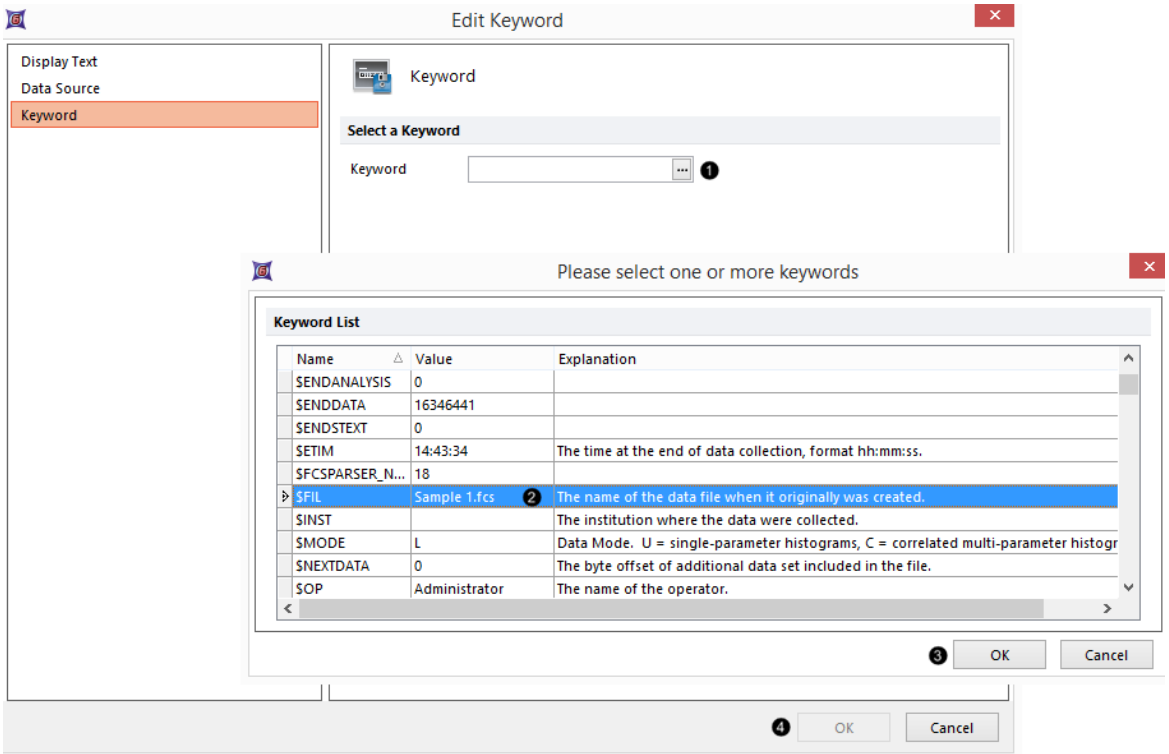

Figure T29.44 Selecting a specific keyword token

28. Scroll to and select **$FIL** in the **Please select a keyword** dialog box (Fig. T29.44, ❷).

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29. Click **OK** in the **Please select a keyword** dialog box (Fig. T29.44, ❸).

30. Click **OK** in the **Excel (Column Mode) field "Keyword..."** dialog box (Fig. T29.44, ❹).

Several statistical[692] and keyword tokens[692] are now ready to export to Excel. We will now reorder the list so the keyword token is exported to the first column in your Excel worksheet.

31. Press and hold on the "**Keyword: $FIL for Plot#...**" token (Fig. T29.45, ❶).

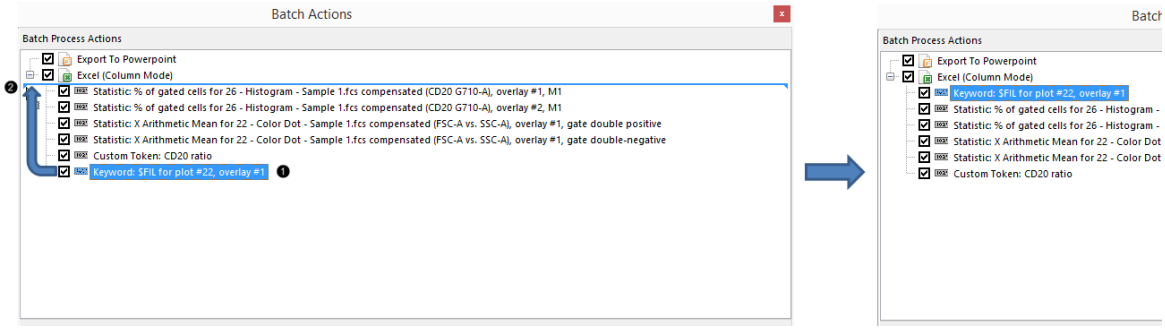

Figure T29.45 Reordering the tokens in the list to be exported to Excel

32. Drag this token to be directly below the **Excel (Column Mode)** icon (Fig. T29.45, ❷).

33. Release the mouse when the blue bar spanning the window width is at the position shown in Fig. T29.45, ❷. The tokens have now been re-ordered as in Fig. T29.45, *right*. *Note: if the token does not move to the new position in the list, expand the width of the* ***Batch Process Actions*** *window (simply by dragging its border to be wider, so there is white space beyond the end of each token descriptor) and try again.*

Although we are now ready to Batch Process[784] our analysis, let's first take a look at all of our samples within the layout itself. This is easily accomplished in FCS Express; there is no need to make replicate plots and tables for each of our samples. And, since in FCS Express "What you see is what you get," you could even print the layout[102] now or add a Print action[803] to the list of Batch Process Actions, and the printout would appear exactly as the layout does on the screen.

34. Select the **Data** tab→**Organize Data Sets**→**Data List** (Fig T29.46, *left*). The Data List[785] appears (Fig. T29.46, *right*), which contains the four .fcs

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files in the directory folder from which "Sample 1.fcs" was loaded. When we run the Batch Process, there will be four iterations, and we will have four slides in our PowerPoint file and four rows of data in our Excel export.

***Note**: If you generally prefer to define a list of .fcs files to work with at the start of your analysis, you may add your files to the Data List before beginning to build your layout. In this tutorial, we simply loaded "Sample 1.fcs" into our plots and allowed the other three files in the directory folder to be added to the Data List automatically.*

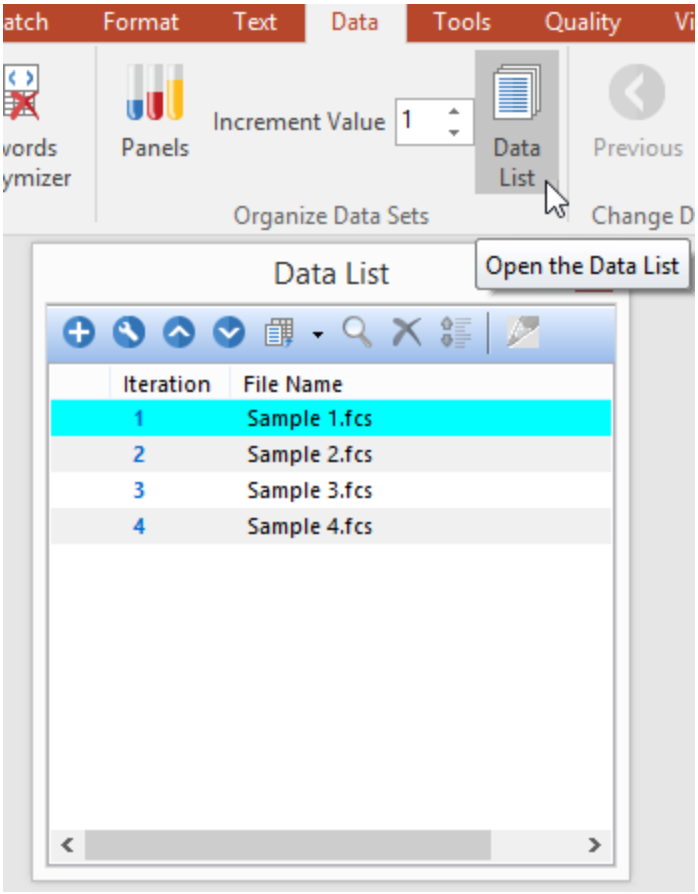

Figure T29.46 Opening the Data List

35. Select the **Data** tab→ **Change Data on All Objects**→ **Next** (Fig. T29.47). Watch as each of the plots and statistics tables on your layout update to the next file every time **Next** is clicked. When "Sample 4.fcs" is displaying on the plots, it will no longer be possible to click **Next**, as it is the last file in the Data List.

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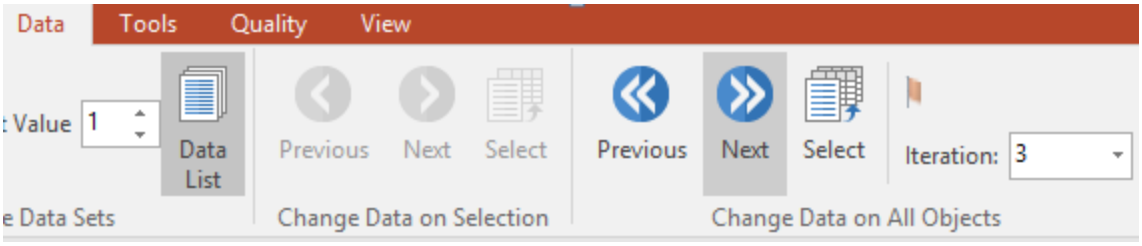

Figure T29.47 Changing data on all objects to the next iteration in the Data List

36. Click on the **Data** tab→**Change Data on All Objects** →**Previous** (Fig. T29.47, to the left of **Next**) until "Sample 1.fcs" is displaying on the layout again.

As you click **Next** to advance through your Data List[785], you may notice that the populations shift in position, and you may wish to adjust gates, markers, or quadrants accordingly. We will now add a pause between iterations to our Batch Options[843] so that the software will pause before exporting the data for each sample. During this pause, we can adjust the "B cells" gate for "Sample 2.fcs" (for example), before the PowerPoint and Excel exports are generated for that sample and prior to the layout advancing to the "Sample 3.fcs" iteration[843]. *Note: you need not access Batch Options (as described in Steps 41-44 and 46-48) for batch processing[784] FCS Express layouts in which no adjustment between iterations[843] is required.*

37. Select the **Batch** tab→**Batch Processing**→**Options** (Fig. T29.48).

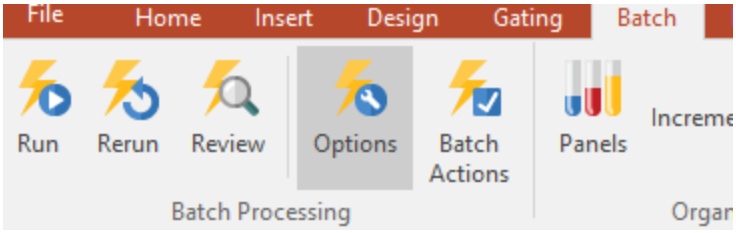

Figure T29.48 Accessing Batch Processing Options

38. Check the **Unconditionally pause between iterations** box in the **Batch Processing Options** dialog (Fig. T29.49, ❶).

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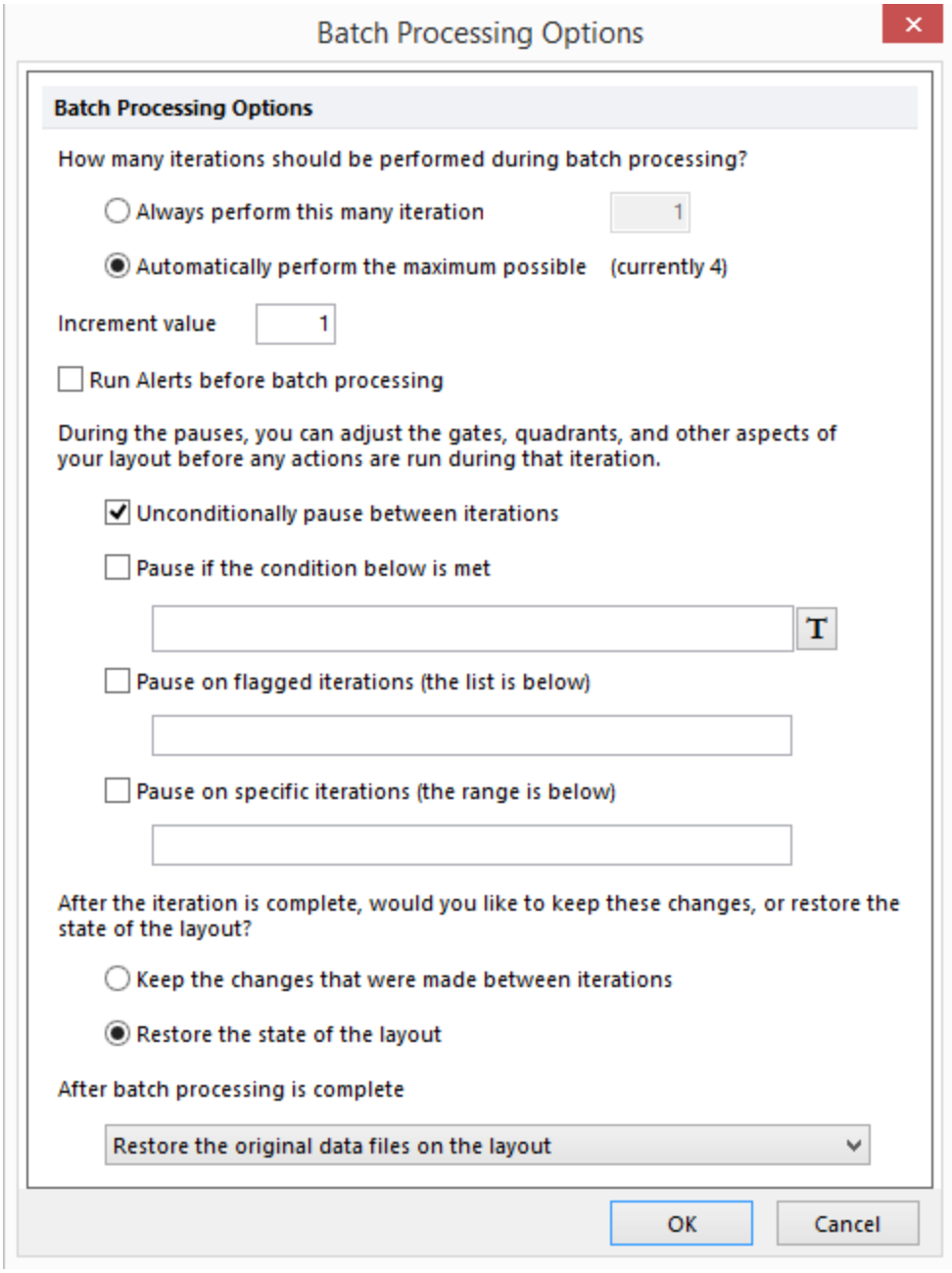

Figure T29.49 Adding a pause between iterations to our batch process

39. Click the **Restore the state of the layout** radio button (Fig. T29.49, ❷).

40. Click **OK** (Fig. T29.49, ❸).

41. Select the **Batch** tab→**Batch Processing**→**Run** (Fig. T29.50).

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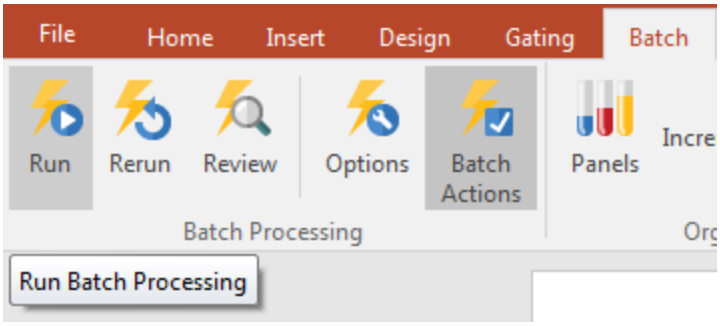

Figure T29.50 Running the batch process

The **Batch Processing** status window appears (Fig. T29.51), indicating that 2 of 2 actions[803] (i.e., one PowerPoint slide + one Excel row of statistics) have been completed for 1 of 4 iterations[843] in the Data List[785].

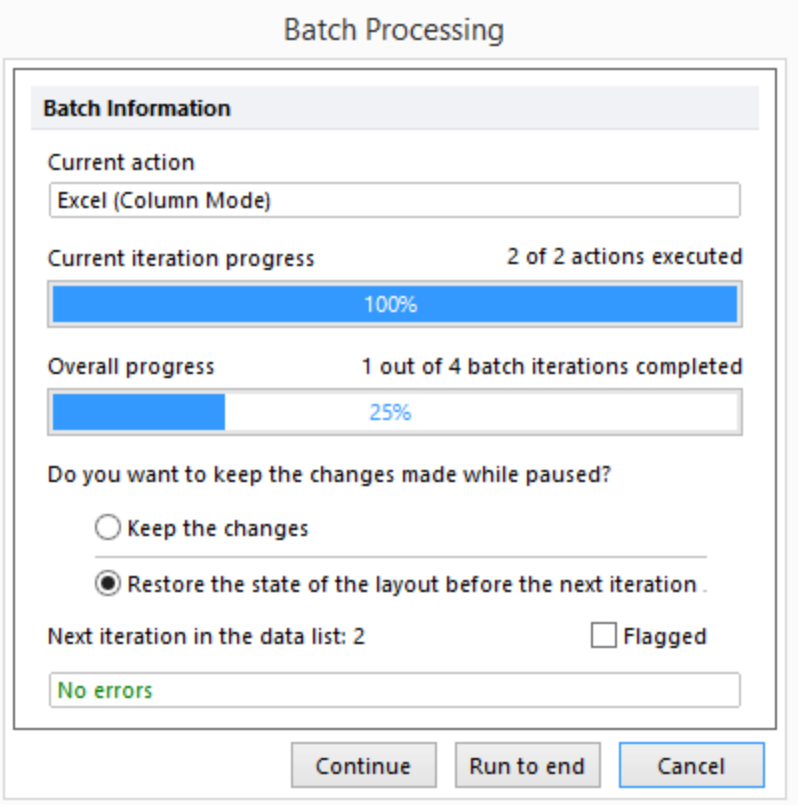

Figure T29.51 Status of the batch process (currently in progress)

42. Adjust the "B cells" gate, quadrants on the contour plot, and/or the marker "M1", if desired. You may also make other changes to your layout during this pause, such as rescaling plot axes, or reformatting, adding, or deleting plots, text boxes, or any other object.

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43. Click **Continue** (at the bottom of the window pictured in Fig. T29.51).

44. Repeat Steps 42-43 until the end of the Batch Process; alternatively, click **Run to End** (at the bottom of the window pictured in Fig. T29.51) if no more changes need be made.

45. Examine your PowerPoint export, noting that each of the four slides looks exactly like your layout in FCS Express in which you performed your analysis, and that the adjustments made to gates, markers, and quadrants are recorded in the slides. Each plot and statistics window on the slide is exported as a high-resolution object which can be moved and resized as you wish.

Note about ungrouping objects in PowerPoint for PC versus PowerPoint for Mac

Once exported to PowerPoint, layout objects can be further ungrouped into single components in the PC version of Microsoft PowerPoint. Ungrouping objects saved by FCS Express in PowerPoint for Mac is not supported. However, presentations saved on a Mac may be opened on a PC and ungrouped.

Note: an example PowerPoint export of this layout is saved in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive.

46. Examine your Excel export and note that the exported statistics for each of the four samples reflect the adjustments made to gates, markers, and quadrants which were recorded in the Powerpoint export. *Note: an example Excel export of this layout is saved in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive.*

View the Immunophenotyping Analysis recorded webinar accompanying this tutorial.

38.32 Changing Common Preferences

FCS Express provides many options that allow you to completely customize your working environment. The settings for these preferences are called User Options 179. You can save time by creating a new user name 50 to save your common options rather than changing the default settings each time you start the program.

This tutorial explains step-by-step how to change the following commonly edited preferences:

- Displaying filenames without path in the Data List 2233
- Changing axis labels to stain and name 2234

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- Creating gates hierarchically and adding a %-gated statistic to gate text boxes 2237

38.32.1 Displaying Filenames Without Path in Data List

Here we will change our FCS Express User Options 179 so that only the .fcs filenames will appear by default in our Data List 785.

1. Select **File** tab→**Options** (Fig. T30.1, outlined in red).

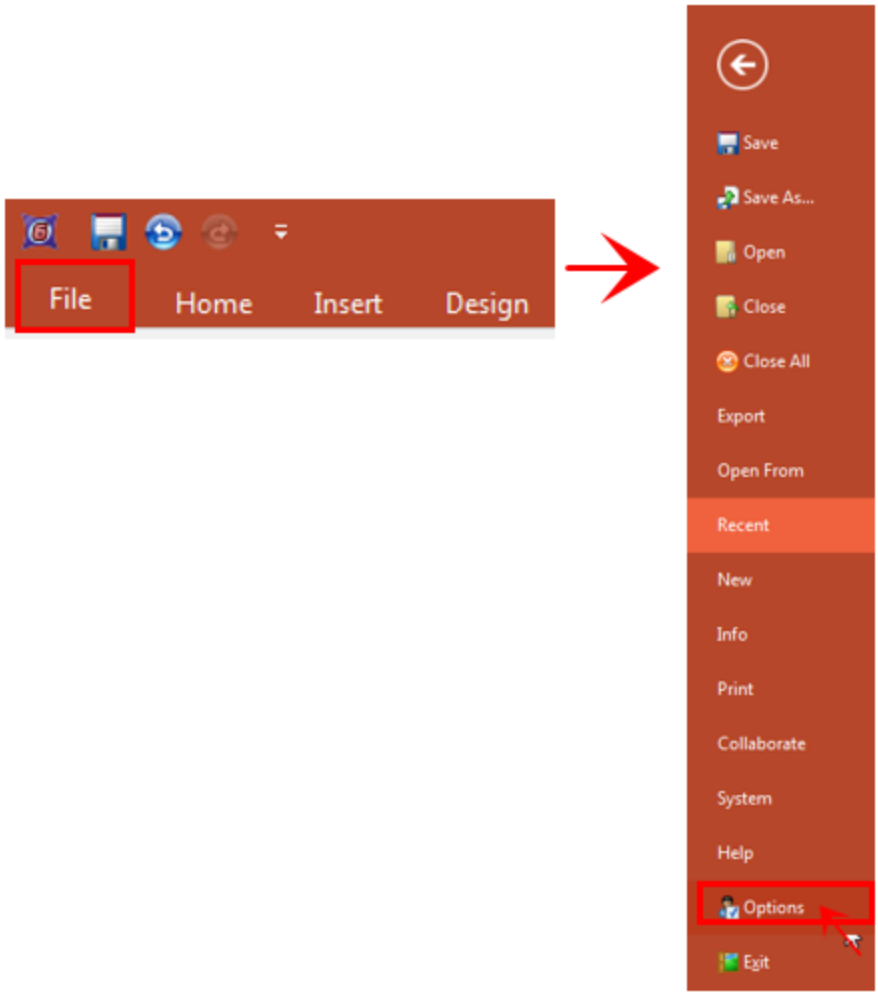

Figure T30.1 Accessing Preferences from the Application Button

2. Click on **Data List** in the category at left (Fig. T30.2, ❶).

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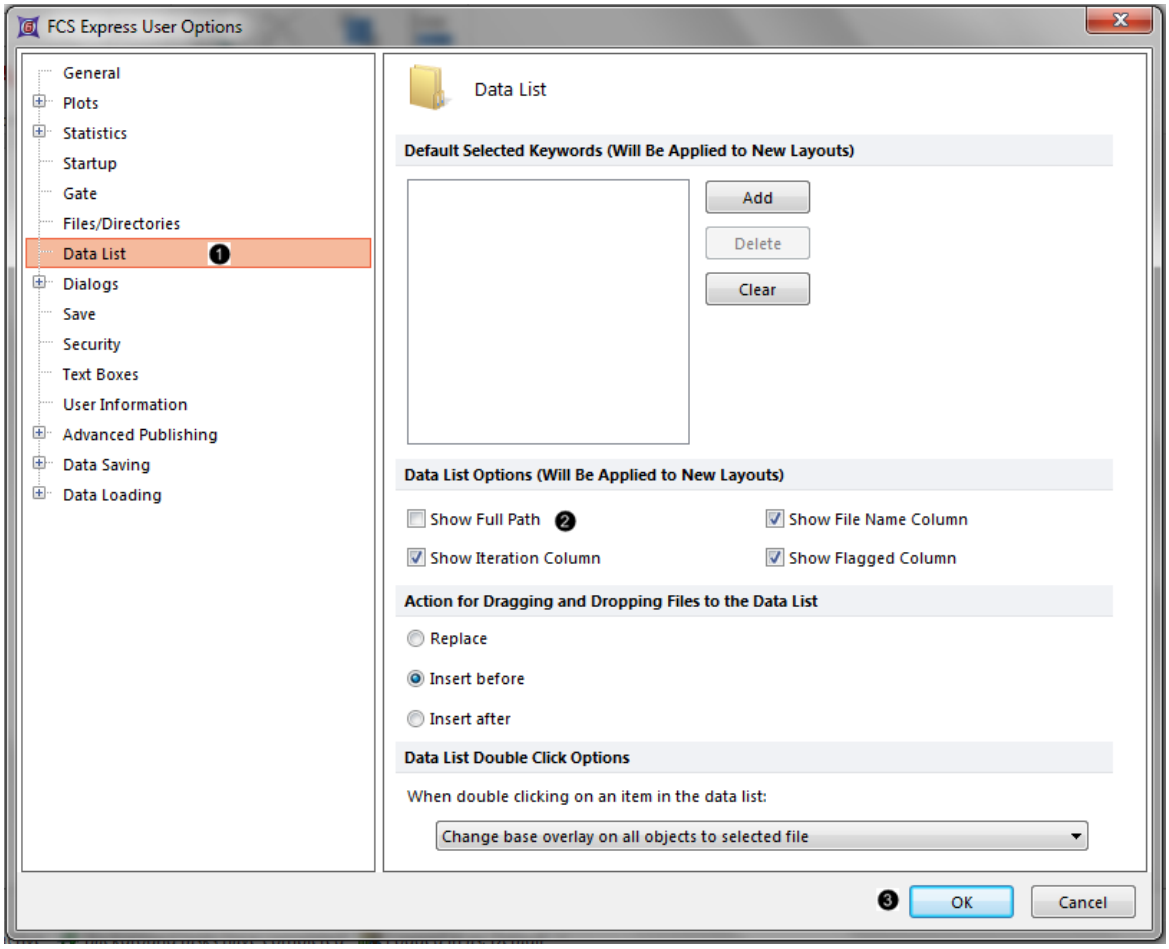

Figure T30.2 Data List preferences

3. Uncheck **Show Full Path** (Fig. T30.2, ❷).

4. Click **OK** (Fig. T30.2, ❸).

Note: the current Option for showing or hiding file paths may be overridden on a per-layout basis at any time. In addition, keywords may be added to the Data List and the files may be sorted on these keywords by Formatting the Data List 791.

38.32.2 Changing Axis Labels

Here we will change our User Options 179 so that data file-derived Stain and Name keywords appear on our plot axes 1254.

1. Select **File** tab→**Options** (Fig. T30.3, outlined in red).

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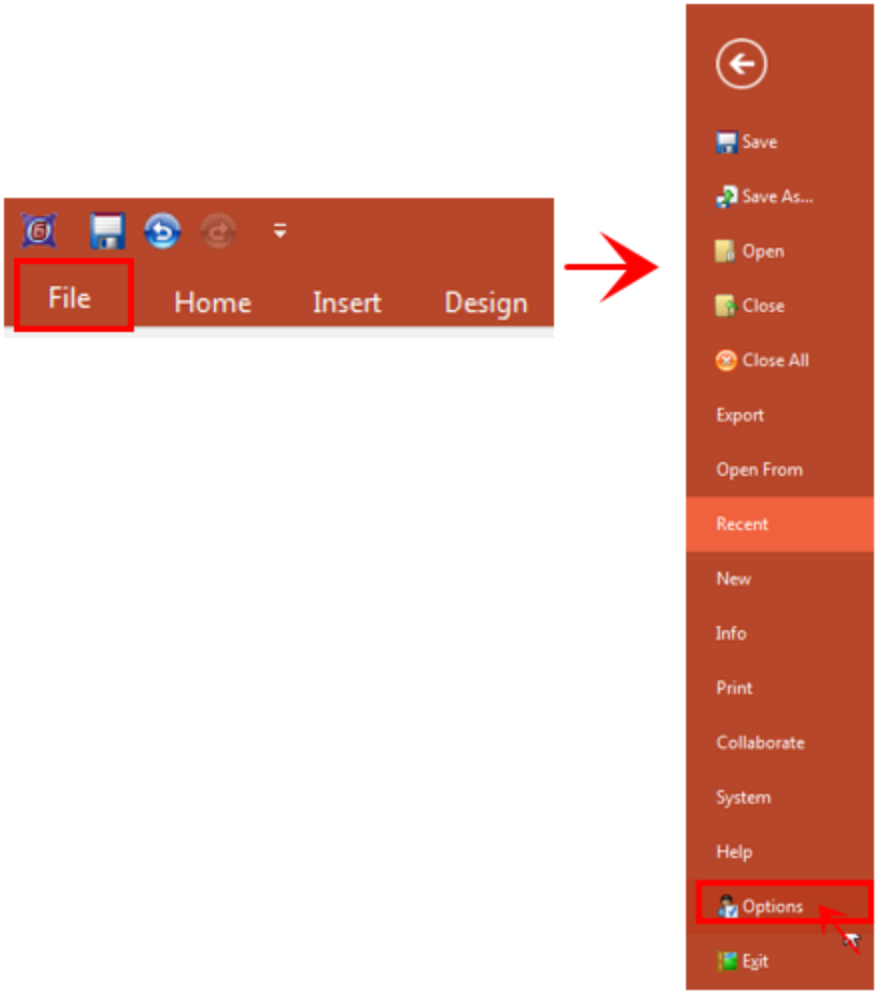

Figure T30.3 Accessing Preferences from the Application Button

2. Click on the **Data Loading** category → **FCS File Options** at the left (Fig. T30.4, ❶).

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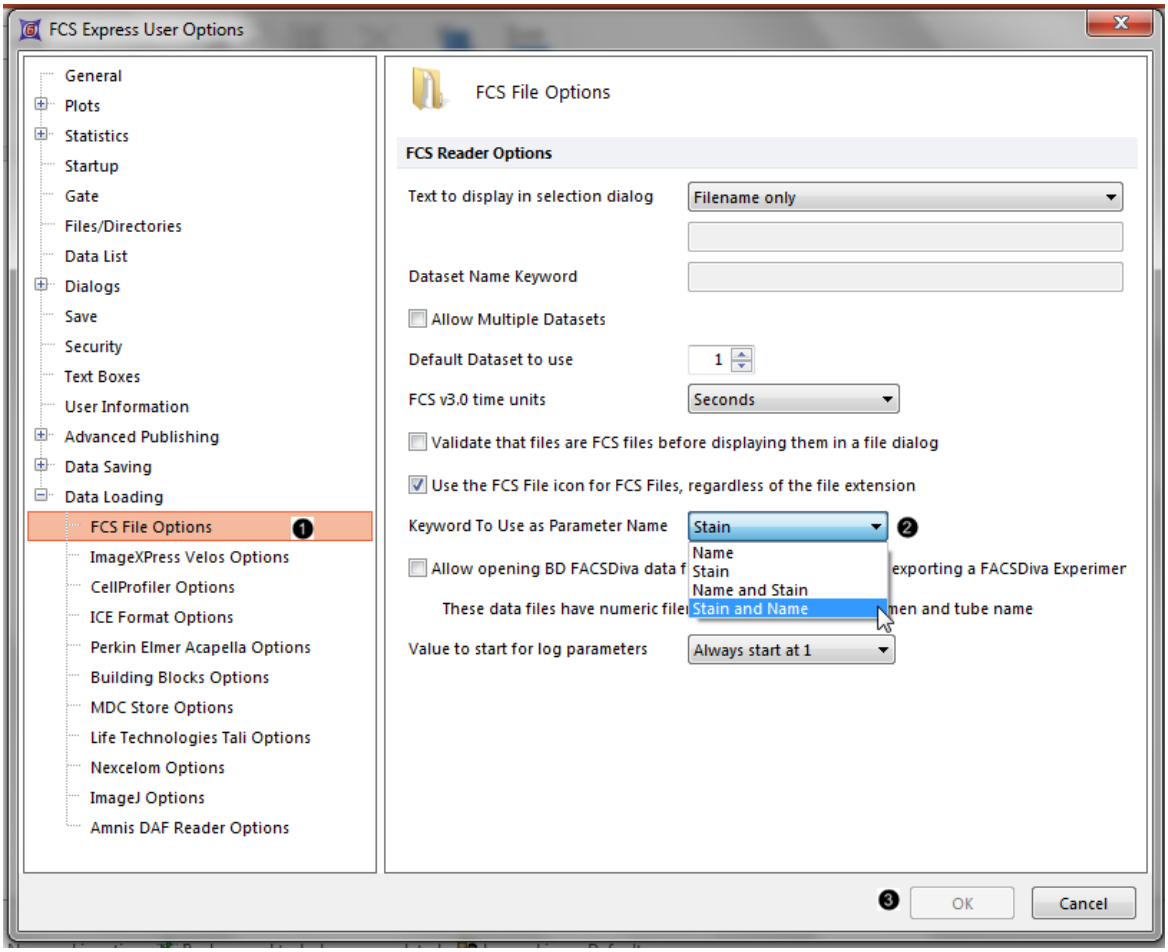

Figure T30.4 Selecting Stain and Name as the Parameter Name for plot axes

3. Select **Stain and Name** from the **Keyword to Use as Parameter Name** drop-down menu (Fig. T30.4, ❷).

4. Click **OK** (Fig. T30.4, ❸).

   Now, as you load in new data files, your plot axes 254 in FCS Express will be labeled with the values for the keywords that you have selected. These keywords values were saved with your data at the time of acquisition, however, you can edit keywords 650 in FCS Express.

   - Please note that different manufacturers will use different bits of information for each keyword value.
   - For example:
     - on a BD analog instrument (FACScan, FACSCalibur)
       - Stain keyword = CD3 FITC, Name Keyword = FL-H
     - on a BD digital instrument (anything which uses BD FACSDiva)

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- Stain keyword = CD3, Name keyword = FITC-A

Go back to Immunophenotyping Tutorial. 2190

38.32.3 Creating Gates Hierarchically and Adding Statistics to Gate Text Boxes

Here we can change our User Options 179 to determine whether or not gates 318 are created hierarchically. In FCS Express 6, Gates are automatically created hierarchically by default. From this preference, you can also choose whether or not to include a statistic and the type of statistic within the gate text box. With this option selected, a statistic 692 is automatically added to gate text boxes 180 upon creation of that gate.

1. Select **File** tab→**Options** (Fig. T30.5, outlined in red).

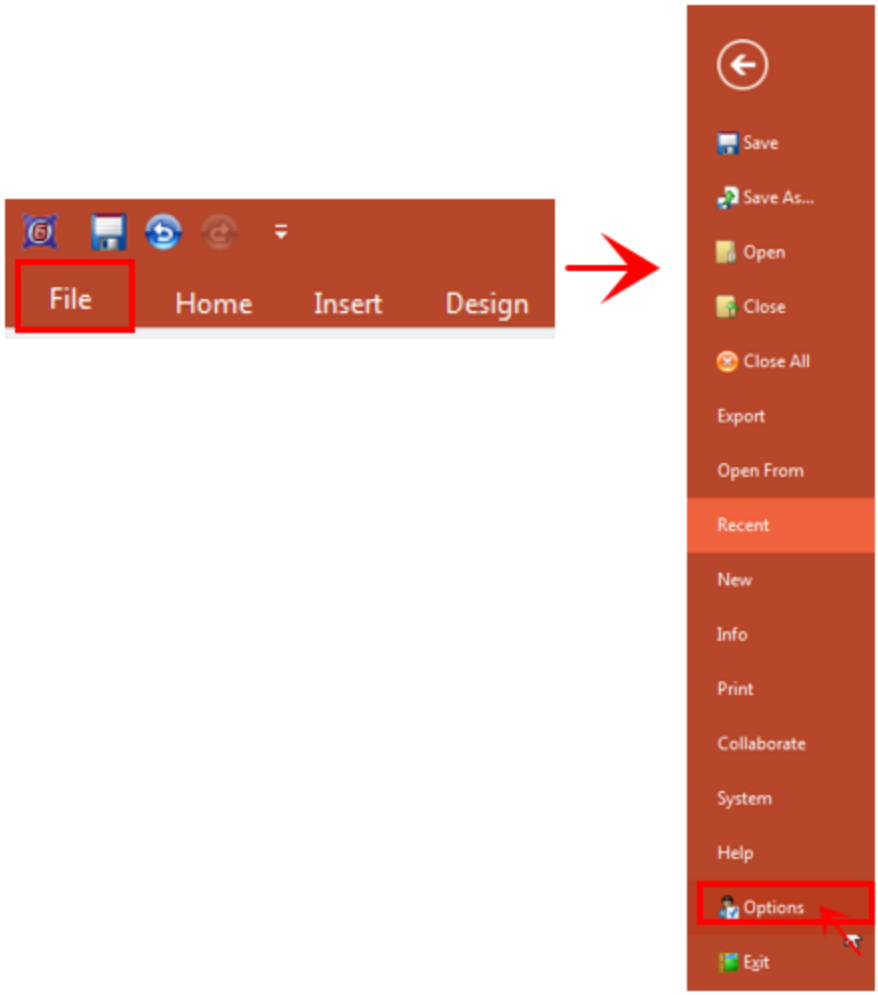

Figure T30.5 Accessing Preferences from the Application Button

2. Click on the **Gate** category at the left (Fig. T30.6, ❶).

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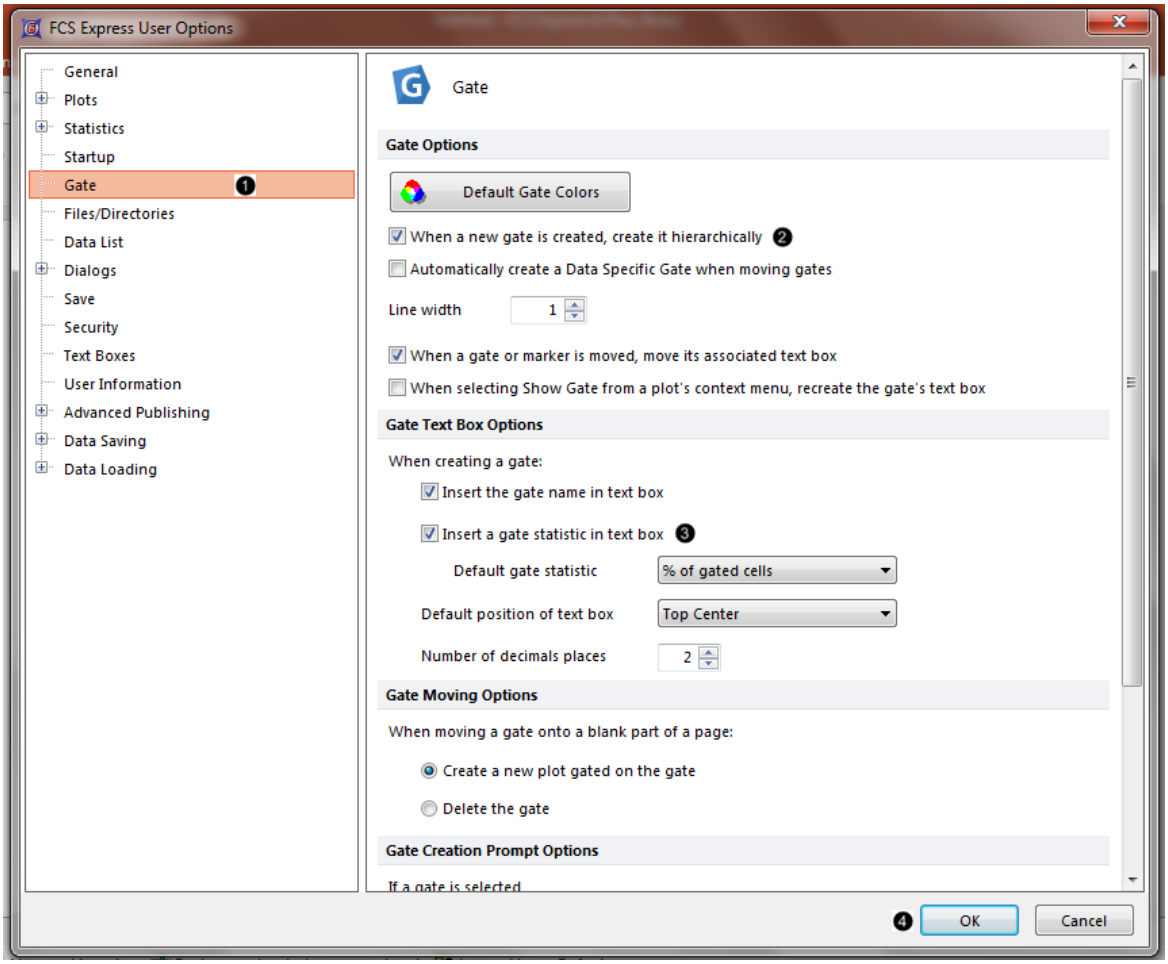

Figure T30.6 Adjusting gate preferences

3. To de-select the option for hierarchical gating, uncheck the "**When a new gate is created, create it hierarchically**" box (Fig. T30.6, ❷).

4. Check the "**Insert a gate stat in text box**" box (Fig. T30.6, ❸). *Note: You may choose a different statistic from the drop-down menu to be added by default to **gate text boxes**; see gate 207 preferences 207 for more details.*

5. Click **OK** (Fig. T30.6, ❹).

Go back to Immunophenotyping Tutorial. 2187

38.33 Cluster Analysis

FCS Express provides users with many different cluster analysis tools which are referred to as Transformations 932 to help you in analyzing high dimensional data.

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Example of these tools are k-means Clustering 958, Principal Component Analysis 946 and the R integration 973 tool.

In this tutorial we will:

- create a *k*-means clustering
- apply a *k*-means clustering
- work with a *k*-means clustering

As an example we will be performing our analysis on a sample of Peripheral Blood stained for different surface markers. However, please note that transformations can be performed and applied on any type of sample.

38.33.1 Defining a k-means clustering

For this tutorial, we will be using a layout file that has 2D density plots with a defined gate. The layout contain a single FCS data file obtained from Peripheral Blood stained for different surface markers. The sample has two physical parameters (FSC-H and SSC-A) and six fluorescence parameters.

To open the layout

1. Select the **File** tab→**Open**.
2. Navigate to the *Tutorial Sample Data* folder and open the **Defining k-means.fey** layout stored in the *k-means tutorial* subfolder.

The layout should appear as shown in Figure T33.1.

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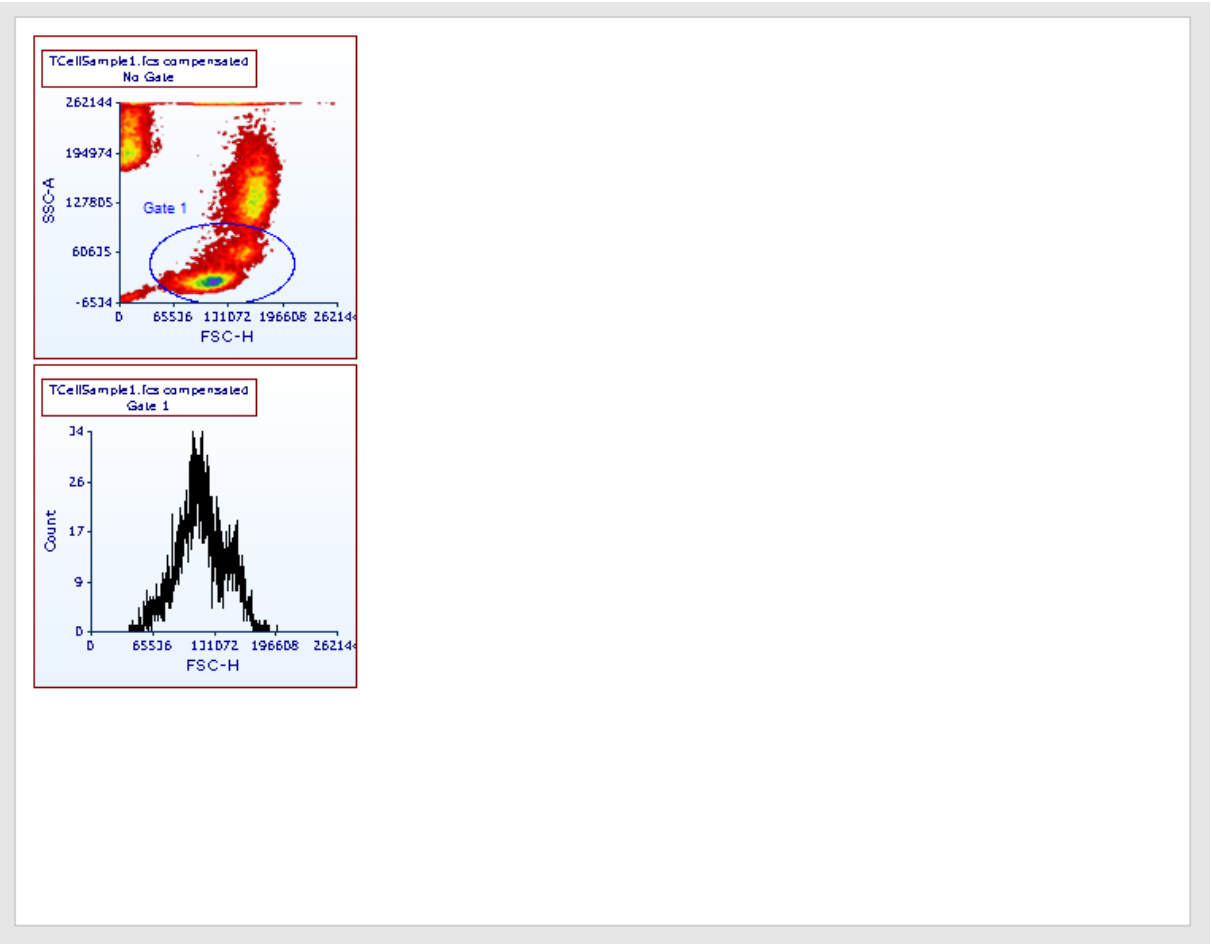

The aim of this tutorial is to create k-means clustering on Gate 1, events are based on 3 out of the 6 fluorescence parameters (i.e. CD3, CD4 and CD8).

The following steps open the transformation window and create a new k-means transformation:

1. Open the **Transformation** window by selecting the **Tools** tab→**Transformations** →**Transformations** command ❶.
2. Click on the drop down arrow adjacent to the blue plus button ❷.
3. Click on **Kmeans** in the drop down list ❸ (Figure T33.2).

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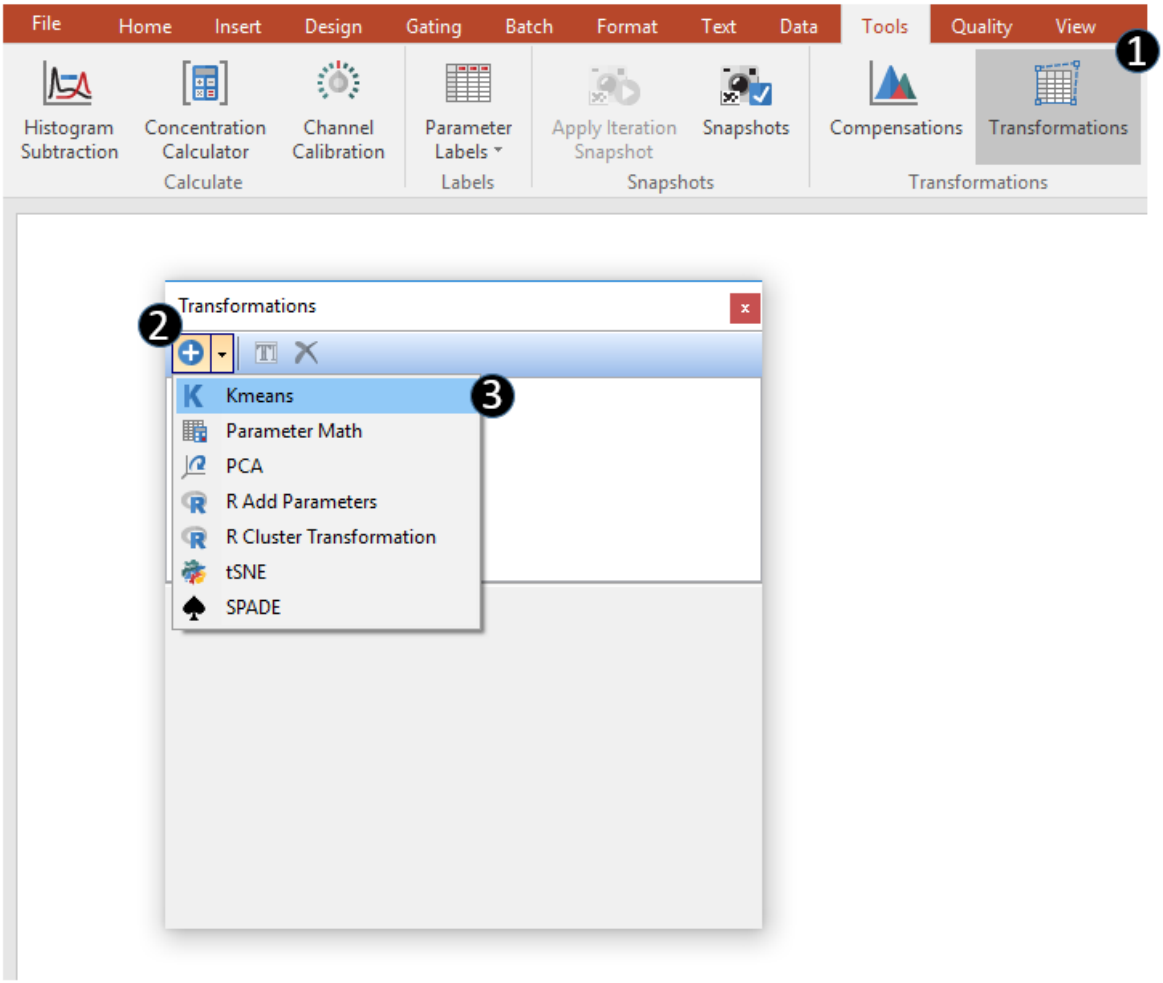

Figure T33.2. k-means transformations are created via the Transformation navigator.

The **Kmeans** dialog will now appear in the **Transformation** window as seen in Figure T33.3.

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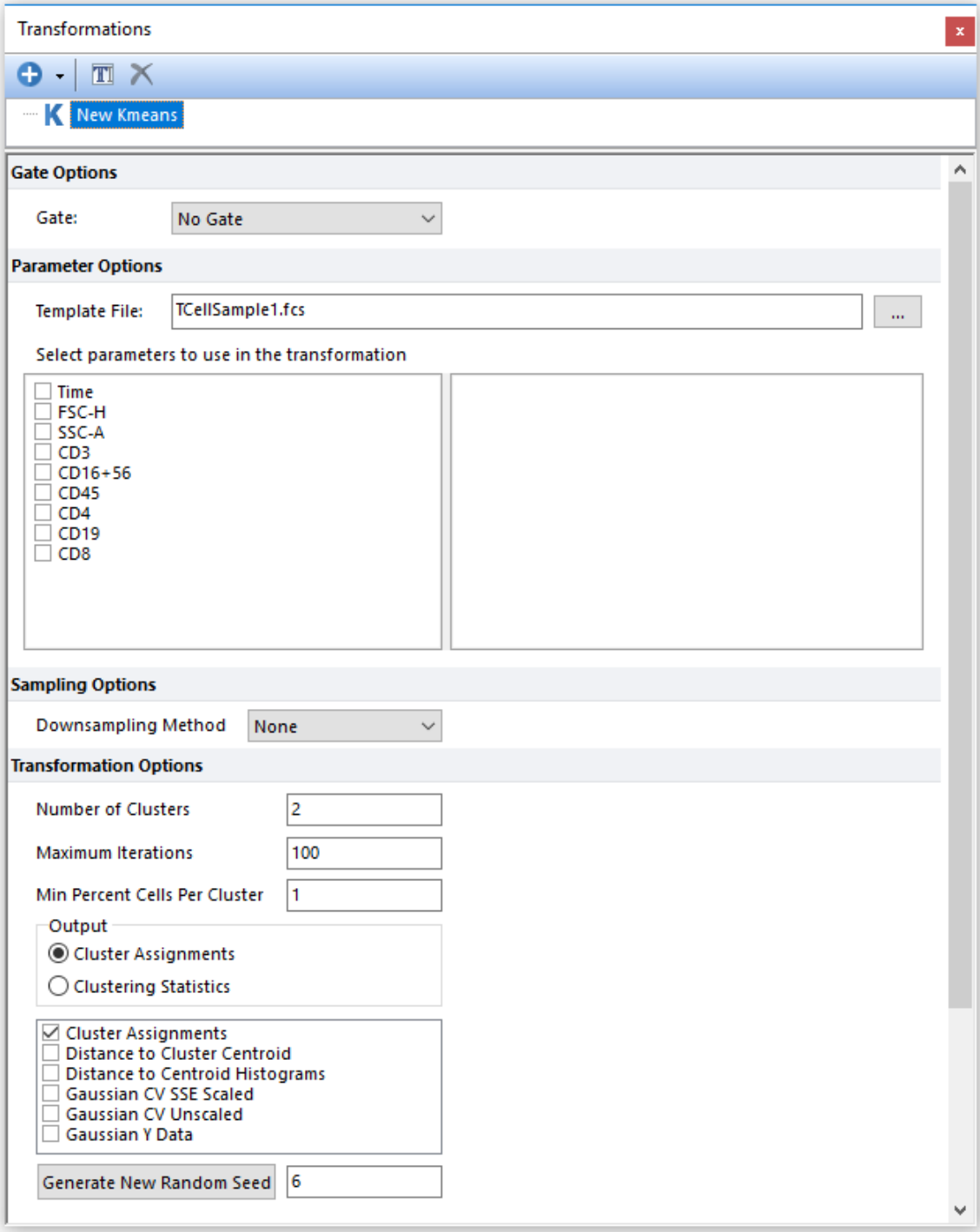

Figure T33.3. The Trasnformation window as it appears after creating a new K-means transformation.

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Note: the **Template File** field already contains the **TCellSample1.fcs** file, thus the file already loaded into the layout. When a transformation is created before any file is loaded into the layout, a template file will need to be selected by the user via the ellipsis button ( ) at the right of the **Template File** field.

We will now rename the new k-means transformation as **My first k-means clustering**:

4. Select the **New Kmeans** in the Transformation window.
5. Select the button from the Transformation window.
6. Type **My first k-means clustering** as the new name.

Please Note: other ways of renaming a k-means transformation are listed in the Defining a k-means cluster analysis 959 topic of the manual.

We will now have to define the k-means clustering arguments. We will start by selecting the parameters the cluster will be based on, and the gate containing the events we want to cluster. Then we will specify additional required arguments such as the number of clusters, the maximum number of iterations, and the cluster minimum size. For a detailed description of each argument, please refer to the Defining a k-means cluster analysis 959 topic of the manual.

7. Select **Gate 1** from the gate drop down menu of the **Transformation** dialog.
8. Flag **CD3**, **CD4** and **CD8** parameters in the parameter list of the **Transformation** dialog.

   Please note that a scale can be specified for each parameter via the **Parameter Scaling Options** section of the **Transformation Dialog**.

As for all Transformations in FCS Express, a **Downsampling Method** 1036 can be selected. We will use all events in this tutorial (i.e. **Downsampling Method** set to **None**).

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9. Type **3** in the **Number of Clusters** field. This is the number of centroids and thus the number of clusters that will be created.
10. Type **100** in the **Maximum Iterations** field. This is the maximum number of iterations FCS Express will perform upon quitting the clustering algorithm.
11. Type **10** in the **Min Percent Cells Per Cluster** field. Iterations ending up with clusters containing less then 1% of events will be discharged and recalculated. This applies to all iterations except the last one.

At this point, the Transformation dialogue should appear as in the following Figure T33.4:

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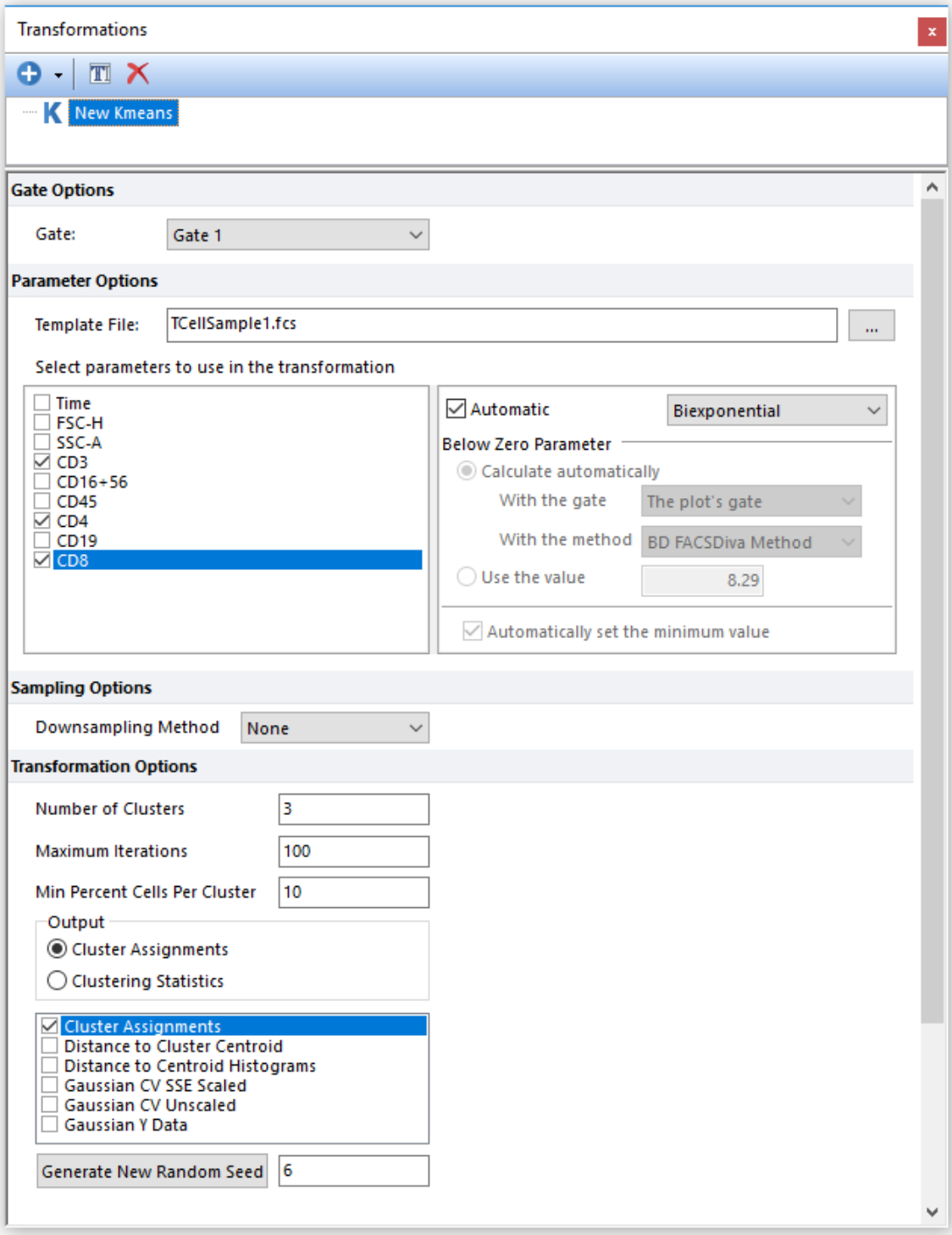

Figure T33.4. The Transforamtion dialogue partially filled for a new k-means clustering transformation.

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Now that the input parameters are selected we need to define the output style we want to use for the clustering result. FCS Express provides two output styles: **Cluster Assignment** and **Clustering Statistics**.
In this tutorial we will select the **Cluster Assignment** output style, which allows several graphical representations of the clustering results. For more details about the two different output styles, please refer to the Defining a k-means cluster analysis 959 topic of the manual.

13. Select **Cluster Assignment**.
14. Flag **Cluster Assignments**, **Gaussian CV SSE Scaled**, **Gaussian Y Data** from the list located below the output styles. These will be the new clustering parameters that will be directly available in a 1D/2D plot and in Heat Maps.

The Transformation dialogue should now appear as in the following Figure T33.5:

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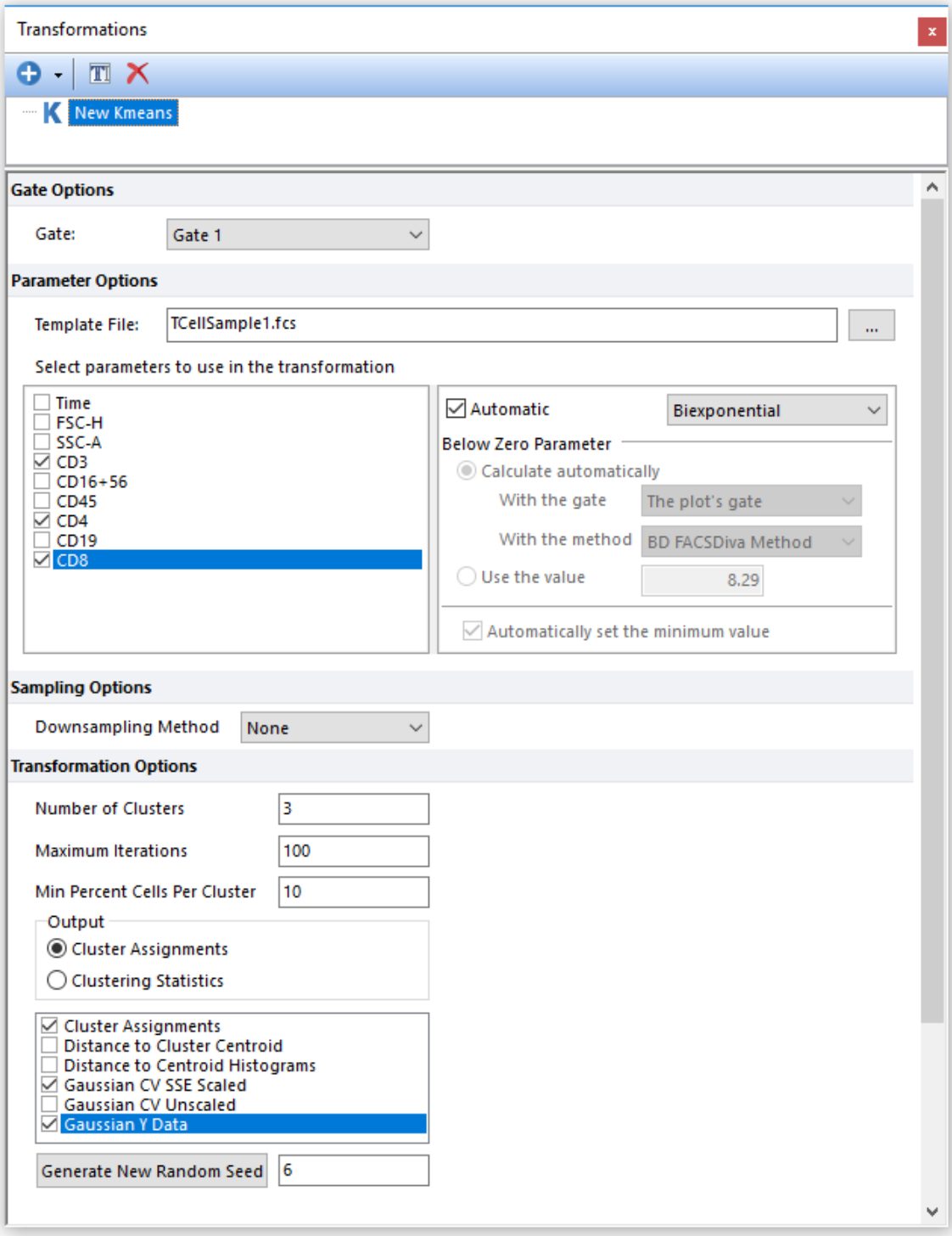

Figure T33.5. The Transformation dialogue filled for a new k-means trasnformation.

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The *k*-means clustering has now been defined and automatically calculated. *k*-means clustering can now be applied to plots for display and analysis. Please proceed to the next part of this tutorial to see how to apply the k-means transformation to plots and heat maps 2248.

38.33.2 Applying a k-means clustering

Once *k*-means clustering has been performed, results can be accessed as 1D plots, 2D plots 172, Heatmaps 1050 and Data Grids 680 depending on the selected output style (see the Defining a k-means cluster analysis 959 topic of the manual for details). In the previous part of this tutorial, we selected **Cluster Assignment** as the output style that allowed us to create three clustering parameters; **Cluster Assignments**, **Gaussian CV SSE Scaled**, and **Gaussian Y Data**. These new parameters 970 may be accessed on plots on which the transformation has been applied.
In this tutorial we will apply the k-means transformation to 1D plots, 2D plots and Heatmaps.

Please Note: although different methods can be exploited in applying a transformation to plots, (please refer to the Applying a k-means Cluster Analysis 968 topic of the manual for further info on this subject), in this tutorial, we will use the drag&drop method.

To open the layout required for this tutorial:

1. Select the **File** tab→**Open**.
2. Navigate to the *Tutorial Sample Data* folder and open the **Applying k-means.fey** layout stored in the *k-means tutorial* subfolder.

The layout should appear as shown in Figure T33.6. A Transformation window with the **My first k-means clustering** should also been opened.

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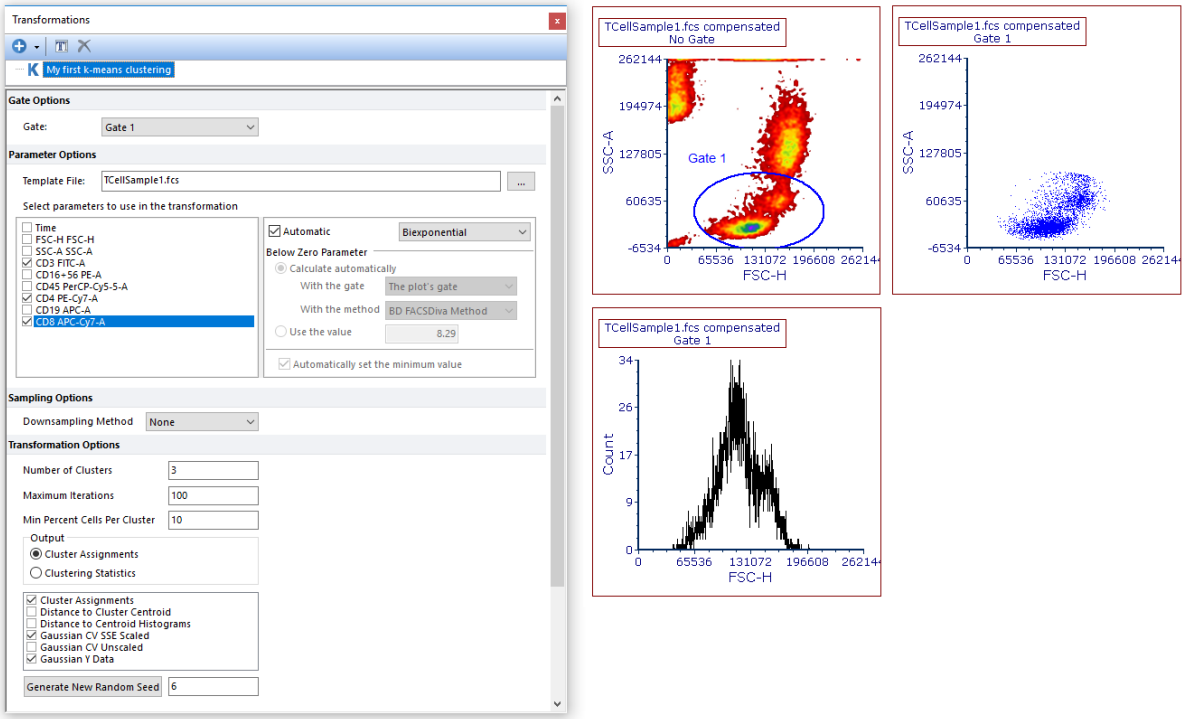

Figure T33.6. The Applying k-means.fey layout as it appear after opening it.

Since we selected **Cluster Assignments** among the new clustering parameters, we will be able to display clusters using Heat Maps. Thus, let's open a new heatmap in the layout.

3. From the ribbon bar select **Insert→Other Plots→Heat Map**.
4. Click in the place on the layout where you wish to insert the Heat Map.
5. A message saying, there is no parameter compatible with Heat Maps available..., will open (Figure T33.7). Click **Continue**.

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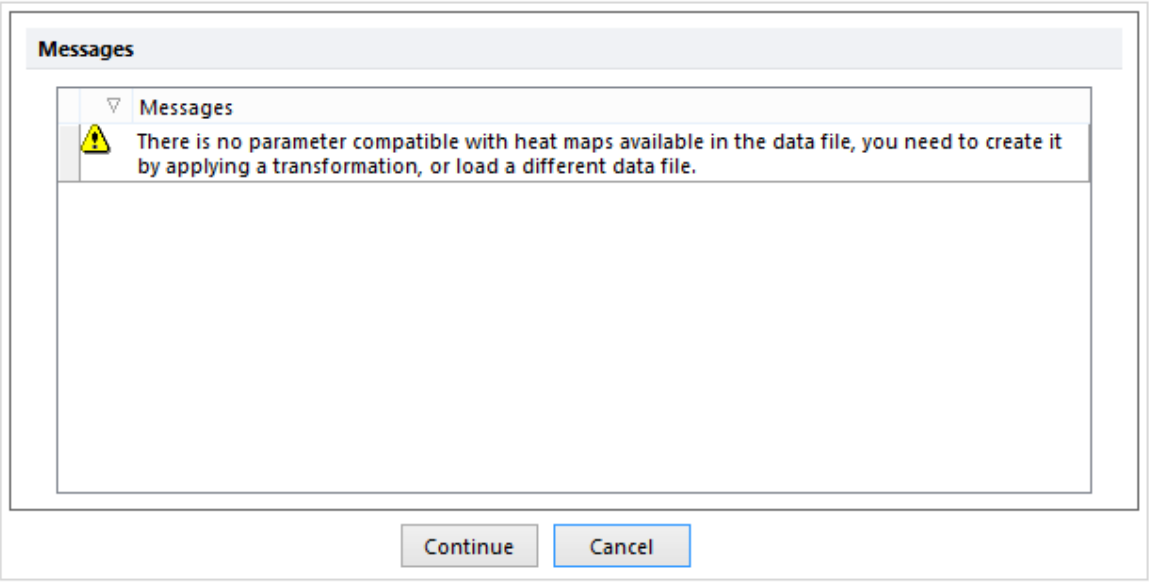

Figure T33.7. The message saying that not heat map compatible parameter is currently available.

6. Select the Heat Map and then select **Gate 1** from the **Gating→Create Gates→Current Gate** drop down menu. The Heat Map where the cluster is displayed, will be gated on Gate 1, thus the same gate used for the k-Means clustering.

We will now apply the k-means transformation to all the plots present in the layout by drag & drop (Figure T33.8):

7. Left click and hold on *k*-means clustering from the **Transformation** window.
8. Drag the k-means transformation out of the Transformation window and release it on top of each of the 2D plot.
9. Repeat step 7 and release the transformation on top of the newly created Heat Map.
10. Repeat step 7 and release the transformation on top of the histogram.

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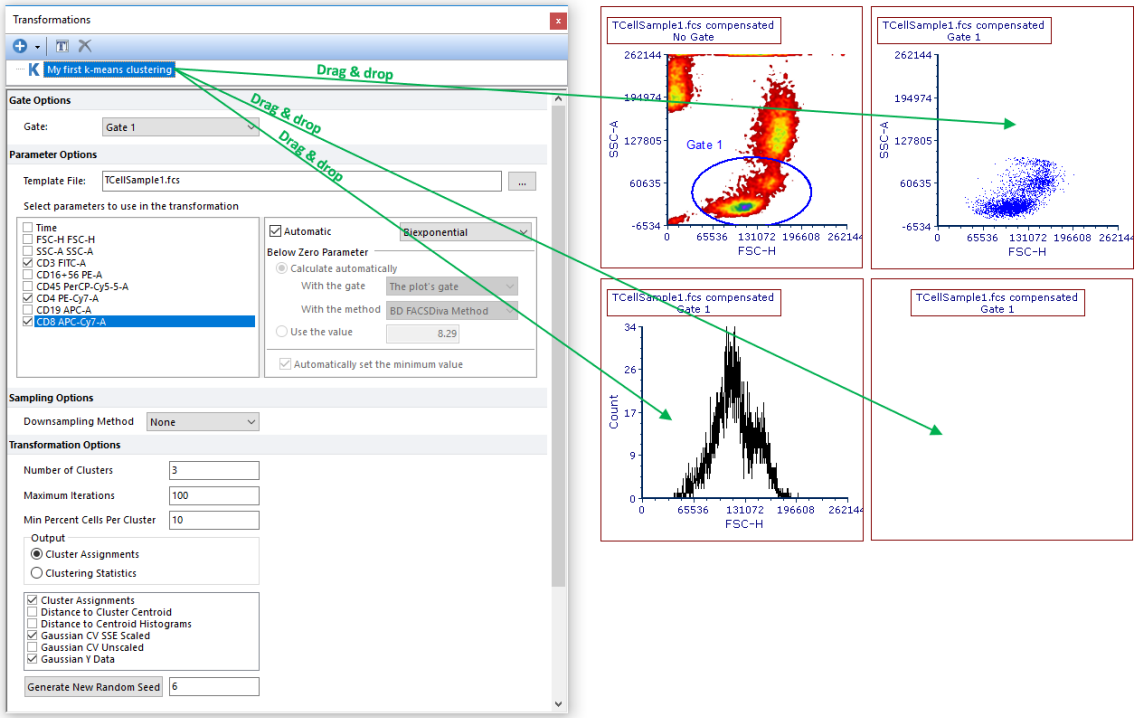

Figure T33.8. Use Darg&Drop to apply the k-means transformation to the objects of the layout.

The k-means is now applied to all the objects in the layout and the title of the plot will now include the text "Kmeans clustered" 970 to indicate the transformation has been applied (Figure T33.9).

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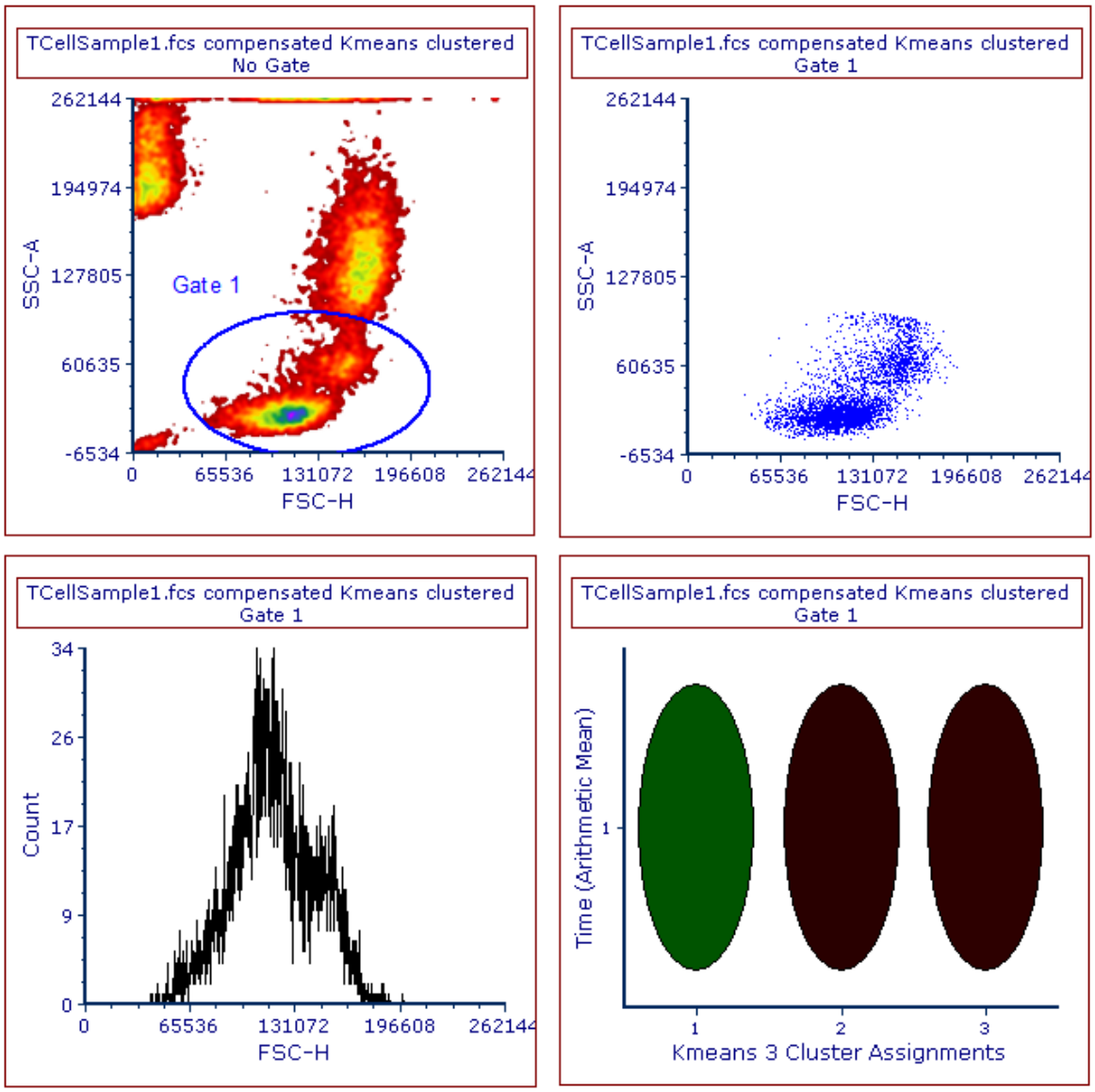

Figure T33.9. When a k-means transformation is applied to plots, the suffix "Kmeans clustered" is automatically added to the title.

Please Note: When a k-means transformation with **Cluster Assignments** parameter is applied to a Heat Map, the latter automatically displays the **Cluster Assignments** parameter by plotting each cluster as a well. When a k-means transformation with parameters other than **Cluster Assignments** is applied to 1D or 2D plots, the clustering parameters can be accessed as standard flow cytometry parameters.

In the next section we will see how to work with clustering parameters.

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38.33.3 Working with k-means using 1D-2D plots

When a k-means transformation includes parameters other than **Cluster Assignments**, and these are applied to 1D or 2D plot, the clustering parameters can be accessed as standard flow cytometry parameters.
In this tutorial we will access clustering parameters on 1D and 2D plots, and we will use gates and markers to select clustered events. These gates and markers will then be available for downstream analysis.

For this tutorial we will use the **k-means on plots.fey** layout stored in the *k-means tutorial* subfolder of the *Tutorial Sample Data* folder.

To open the layout required for this tutorial:

1. Select the **File** tab→**Open**.
2. Navigate to the *Tutorial Sample Data* folder and open the **k-means on plots.fey** layout stored in the *k-means tutorial* subfolder.

The layout should appear as shown in Figure T33.10:

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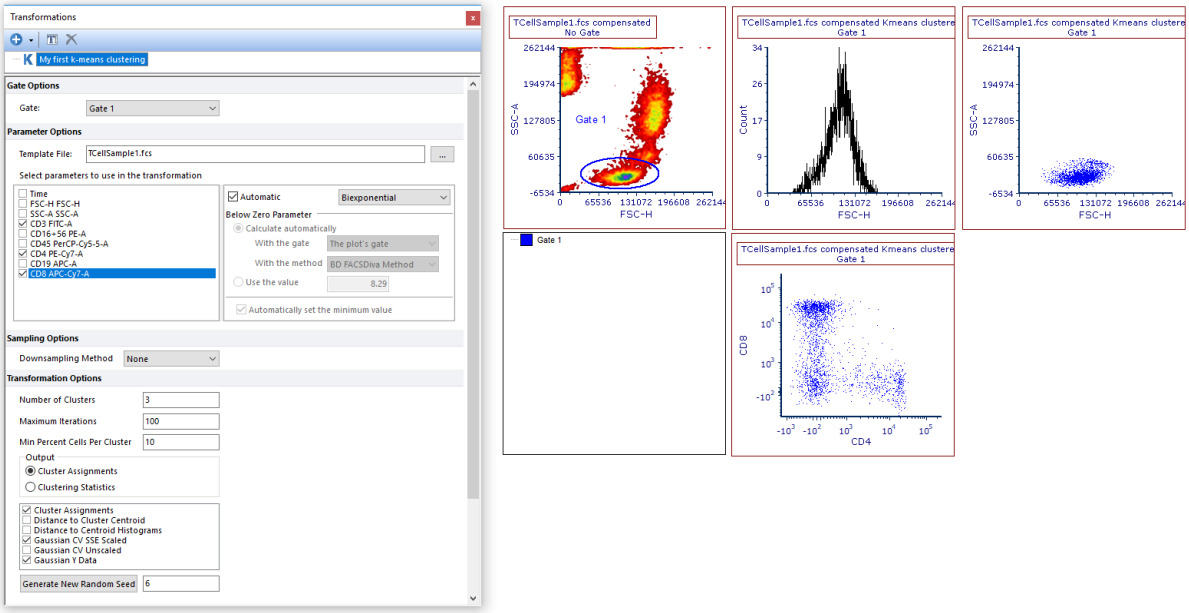

Figure T33.10. The k-means on plots.fey layout as it appear after opening it.

In this layout a k-means transformation has been created[2239] and applied[2248] to a histogram and to 2 color dot plots, (please note the **Kmeans clustered** suffix in the plot title). In order to simplify gate visualization, and to create a cluster gate with the correct hierarchy, Gate 1 (thus the gate on which the clustering has been performed), has already been applied to all this objects.

In this example, the following clustering parameters have been selected, and thus automatically created as output results for the k-means clustering: **Cluster Assignments** (which is suitable for Heat Maps, see the next section of this tutorial[2258]), **Gaussian CV SSE Scaled** and **Gaussian Y Data** (which can be both accessed on 1D and 2D plots). To learn more about the available clustering parameters, please refer to the Defining a k-means cluster analysis[959] topic of the manual.

We will now select clustered events using markers on 1D plots.

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3. Change the parameter displayed on the 1D plot to be **Kmeans 3 Gaussian CV SSE Scaled**. Three peaks will appear in the 1D plot, one for each clusters (Figure T33.11).
4. Select the peak on the left (i.e. cluster 1) by creating a marker 353.
5. Convert the marker to a gate, by right clicking on it and selecting **Convert Marker to gate → Marker #1 (M1) → Convert and Link**. A **Create a new gate** dialogue opens.
6. Replace **Gate 2** with **Marker on Cluster** as gate name and click **OK**.

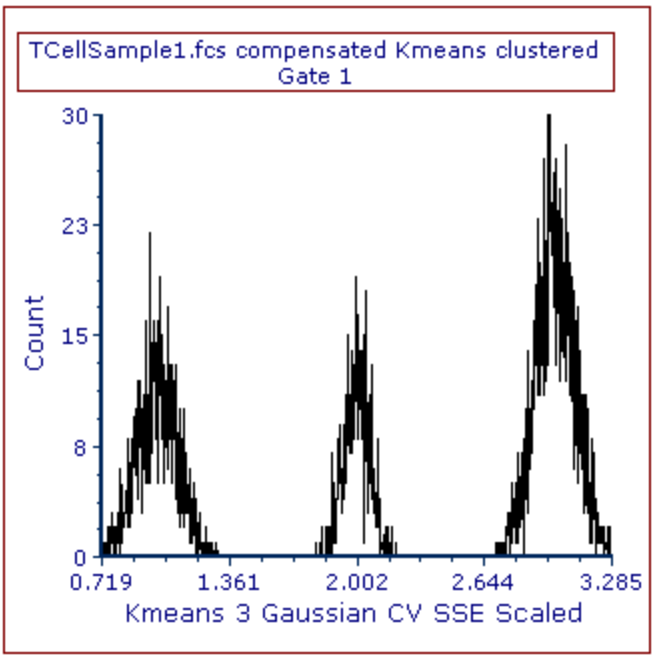

Figure T33.12. A 1D plot displaying a clusering parameter.

The content of the layout should now be similar to Figure T33.12. Please note that the new gate is now listed in the gate view, and that the events belonging to **Marker on Cluster** are now shown in green in both of the color dot plots.

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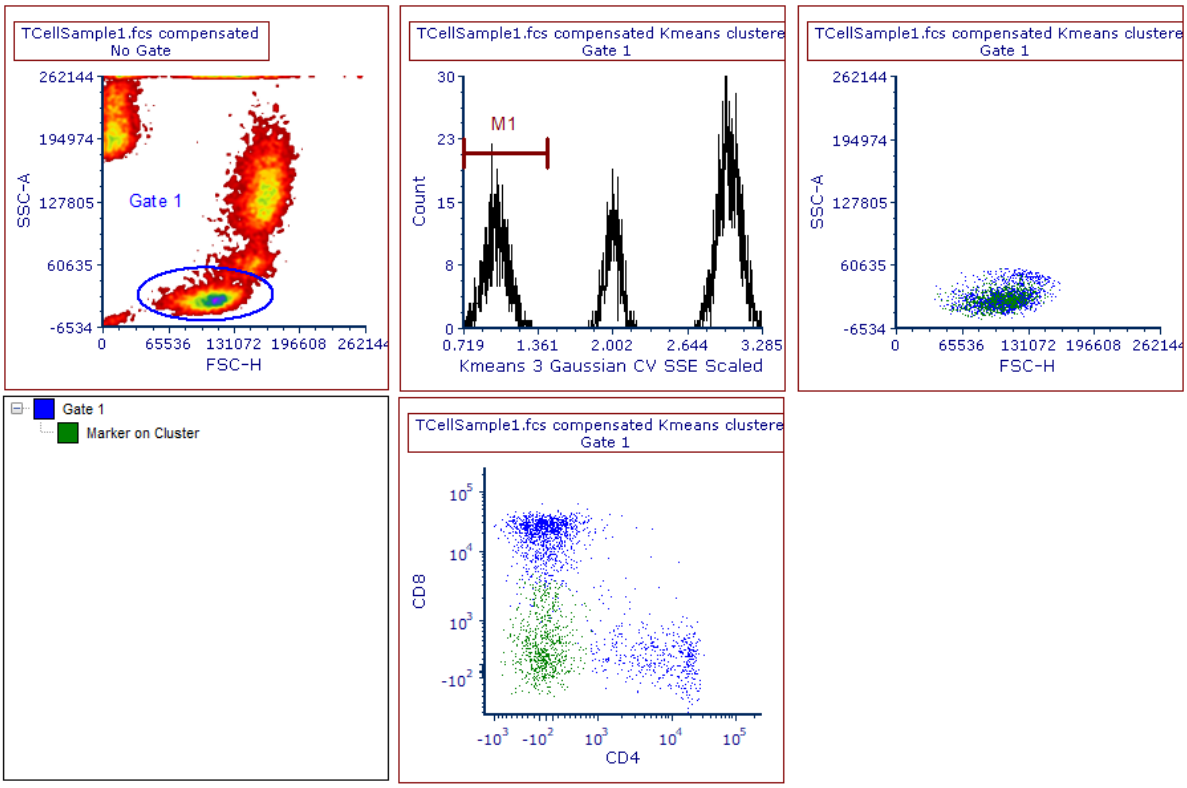

Figure T33.12. A marker has been created to select cluster 1. The marker has then been converted and linked to be a gate. The gate view and the color dot plots reflect this change.

7. Move marker M1 to select a different peak in the histogram, thus a different cluster. Please take note that the color dot plot is updated based on these changes.

Also, please note: Since the **M1** marker has been converted to a gate, now it can also be applied to a plot 352 and to other objects in the layout. The **M1** gate will have the same functionality as any other gate.

We will now select clustered events using gates on 2D plots.

8. Change the parameter displayed on the 2D color dot plot in the upper part of the layout to be **Kmeans 3 Gaussian CV SSE Scaled** on the X axis, and **Kmeans 3 Gaussian Y Data** on the Y axis (Please also note that clustering parameters can be used together with any other non-clustering parameter as well on 2D plots).

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The content of the plot will be similar to the one displayed in Figure T33.13. Please note that the coloring may be different depending on the position of marker M1 (see step 7).

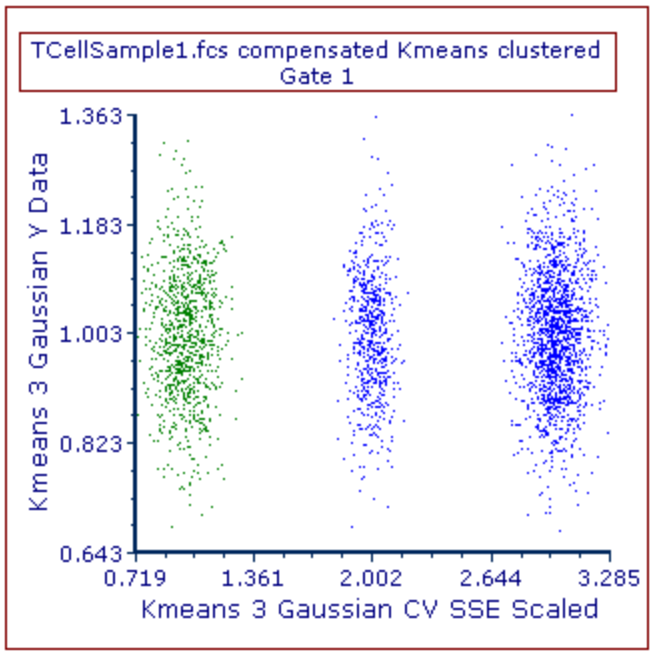

Figure T 33.13. A 2D plot displaying two clustering parameters.

9. Select one of the blue clusters by creating a 1D Gate 318 (select **Gating→Create Gates→Markers** from the ribbon bar) around it. A **Create New Gate** dialogue opens.

Note: although any type of gate can be used to select clustered events on 2D plots, 1D Gates are particularly useful in this cases as they automatically extend from the bottom to the top of the 2D plot.

10. Replace **Gate 2** with **Gate on Cluster** as gate name, change color to be red and click **OK**.

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The layout should now appear similar to the one displayed in Figure T33.14. Please note that the new gate (i.e **Gate on Cluster**) is now listed in the gate view, and that the events belonging to it are now red, in both of the color dot plots.

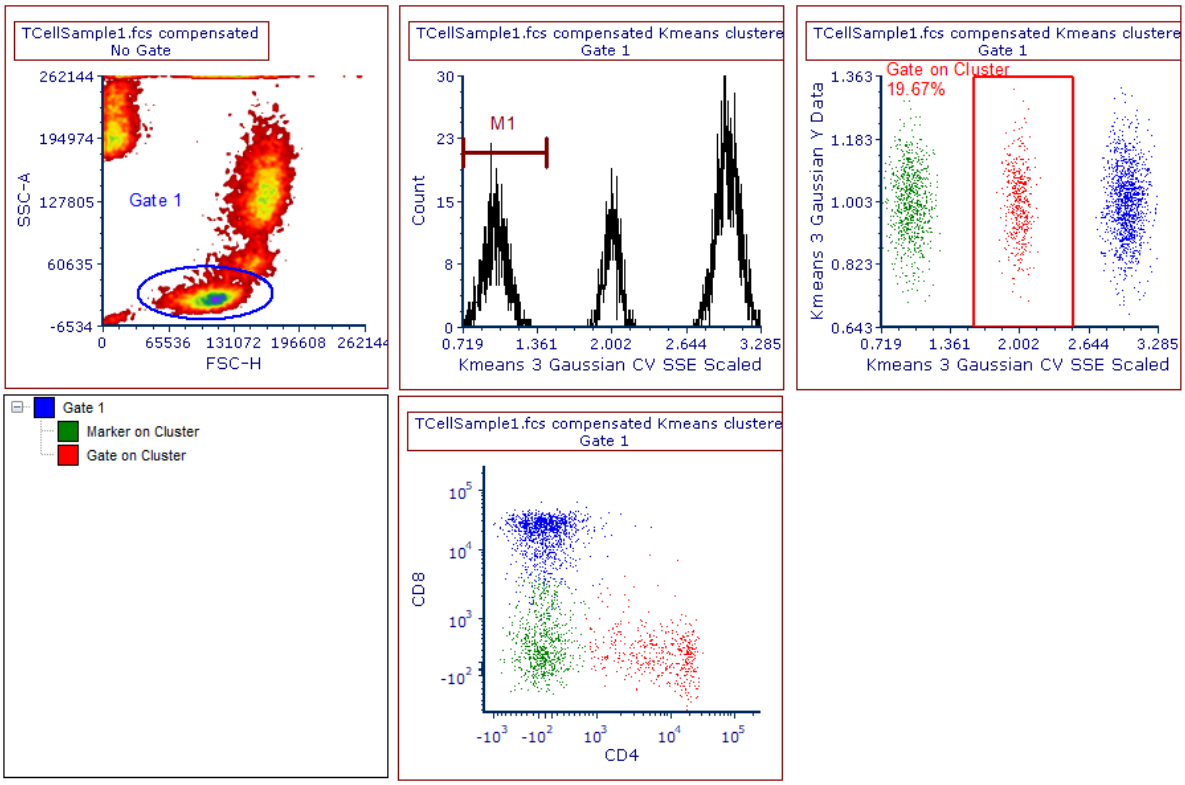

Figure T33.14. A gate has been created to select cluster 2. The gate view and the color dot plots reflect this change.

11. Move the **Gate on Cluster** gate to select a different cluster. Note that the color dot plots update their content based on these changes. Note: the **Gate on Cluster** gate can also be applied to a plot 352 and to other objects in the layout. The **Gate on Cluster** gate will have the same functionality as any other gate.

38.33.4 Working with k-means parameters

When a k-means transformation with a **Cluster Assignments** parameter is applied to a Heat Map, it automatically displays the **Cluster Assignments**

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parameter by plotting each cluster as a well. The k-means transformation available in this layout is defined by three clusters, thus 3 wells are displayed in the Heat Map.

In this tutorial we will access the clustering parameter on the Heat Map, and we will use the well gates to select clustered events. These well gates will then be available for downstream analysis.

For this tutorial we will use the **k-means on heatmap.fey** layout stored in the *k-means tutorial* subfolder of the *Tutorial Sample Data* folder.

To open the layout required for this tutorial:

1. Select the **File** tab→**Open**.
2. Navigate to the *Tutorial Sample Data* folder and open the **k-means on heatmap.fey** layout stored in the *k-means tutorial* subfolder.

The layout should appear as shown in Figure T33.15:

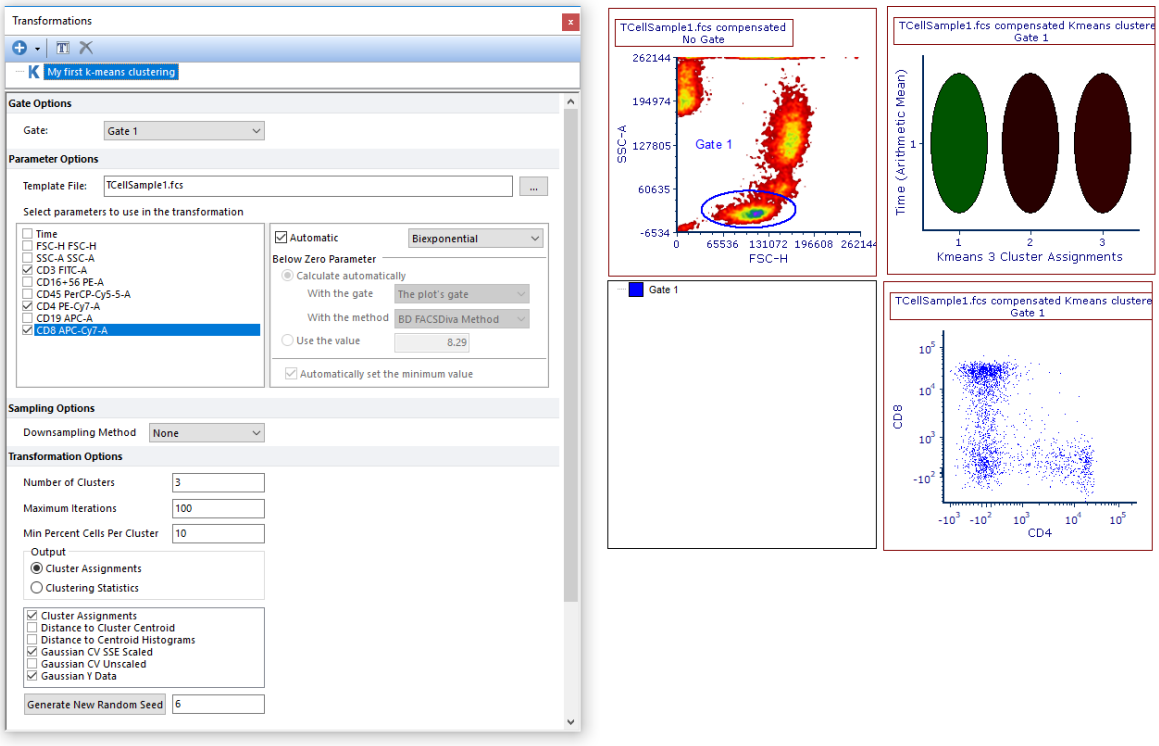

Figure T33.15. The k-means on heatmap.fey layout as it appear after opening it.

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In this layout a *k*-means transformation has been created and applied to a Heat Map and to a color dot plot. In order to simplify the gate visualization, and to create a cluster gate with the correct hierarchy, **Gate 1** (thus the gate on which the clustering has been performed) has already been applied to the color dot plot.
In this example, the following clustering parameters have been selected and thus automatically created as output results for the k-means clustering; **Cluster Assignments** (which is suitable for Heat Maps), **Gaussian CV SSE Scaled** and **Gaussian Y Data** (which can be both accessed on 1D and 2D plots, see the previous section of this tutorial). To read more about the clustering parameters, please refer to the Defining a k-means cluster analysis topic of the manual.

We will now select clustered events using well gates on Heat Map.

3. Right click on the Heat Map and select **Create gate→Well** from the right click menu.
4. Select cluster 1 by left clicking on it. A **Create New Gate** dialog opens.
5. Create **Gate 2** and name it as **Cluster 1 on Heat Map** as gate name, and have Gate 1 as the Parent Gate and click **OK**.
6. Click **Close** in the **Edit new gate** dialogue. The new gate named **Cluster 1 on Heat Map** is now listed in the gate view.
7. Right click on the Heat Map and select **Show Gates→Cluster 1 on Heat Map** from the right click menu. The gate is now visible on the Heat Map as a border around cluster 1.

The content of the layout should now be similar to the one displayed in Figure T33.16. Please note that the new gate is now listed in the gate view, and that the events belonging to cluster 1 are now green in the color dot plot.

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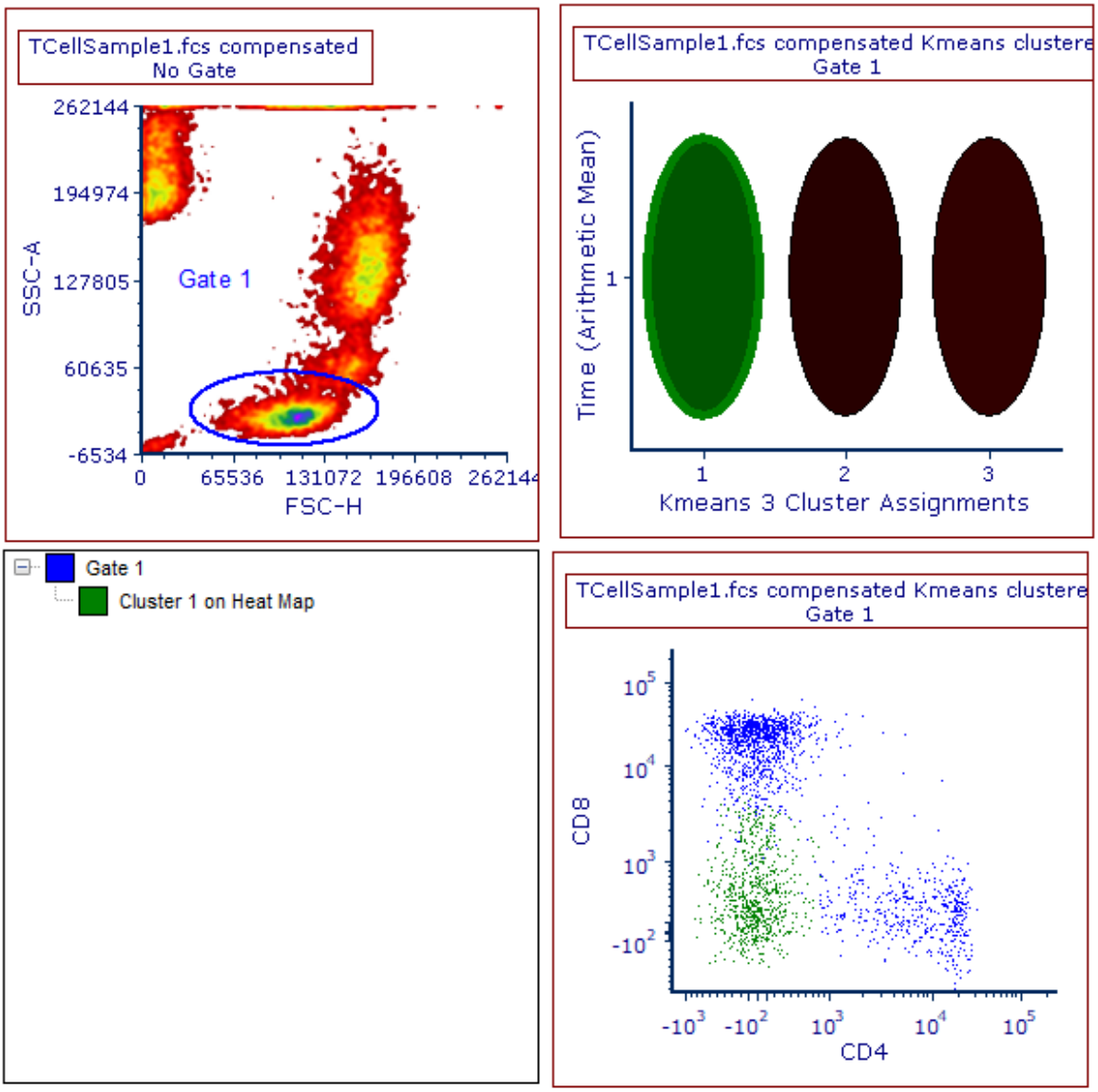

Figure T33.16. A well gate has been created to select cluster 1. The gate view and the color dot plot reflect this change.

Note: The **Cluster 1 on Heat Map** gate can also be applied to a plot 352, and to other objects in the layout just as any other gate.

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