FCS EXPRESS VERSION 4 RUO EDITION USERS MANUAL

FCS Express 4 RUO Edition

The Future of FCM Analysis

De Novo Software

FCS Express 4 adds the power and ease of use of the Microsoft Ribbon Interface.

One great product for both flow cytometry and image analysis.

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Chapter

1 Welcome to FCS Express Version 4

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 4.07.0020.

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 **Application Button** 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.

In the Windows operating system, the designation "left mouse button" refers

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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 new Ribbon interface where most menus and toolbars are replaced with the **Application Button** as well as **Tabs**, **Groups**, and **Commands**. The drop-down list of commands which appear when selecting the **Application Button** 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 Version 4 are as follows:

- Windows 2000*/2003/XP/Vista/Windows 7
- Pentium III - class PC, 500 MHz
- 1 GB RAM
- 50 MB Hard drive space

Recommended system: 4 GB RAM

Note: FCS Express 4 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.

*To use FCS Express on Windows 2000, GDI+ must be installed. You can obtain GDI+ from the Microsoft web site at http://www.microsoft.com/downloads/en/details.aspx?FamilyId=6A63AB9C-DF12-4D41-934C++-BE590FEAA05A&displaylang=en

To use FCS Express on Windows 2000 with the SQL Server security system, MDAC must be installed. You can obtain MDAC from the Microsoft web site at http://www.microsoft.com/downloads/en/details.aspx?FamilyID=78cac895-efc2-4f8e-a9e0-3a1afbd5922e

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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:

De Novo Software
3250 Wilshire Blvd., Suite 803
Los Angeles, CA 90010
USA

We accept checks, money orders, wire transfers, purchase orders, Visa™ and MasterCard™. 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 4 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 http://www.denovosoftware.com/site/WhichLicenseRight.shtml.

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.

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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 24 and written 24 tutorials, an online forum 25, and email support 25.

2.1 Tutorial Videos

A good place to start learning FCS Express is by watching online videos. These can be reached by selecting on the **Application Button→Help→Video Tutorials** . They are also available at http://www.denovosoftware.com/site/TutorialVideos.shtml. These videos show an overview of many of the features available in FCS Express and will allow you to get a brief glimpse at the wide variety of possibilities you have when using 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 590 are included in this manual, and are also available by selecting **Application Button→Help→Tutorials**, and at http://www.denovosoftware.com/site/manual/index.html?tutorials.htm. The following tutorials are currently available (check on our web site for additional tutorials as they become available):

- Using Objects 591
- Inserting Plots 607
- Formatting Plots 620
- Gating 650
- Text Boxes and Tokens 675
- Quadrants 712
- Quadrant/Marker to Gate Linking 722
- Compensation 736
- Batch Processing 767
- Creating and Using Panels 821
- Docking 845
- Data Navigator 866
- Gate Navigator 889
- Layout Navigator 904
- Creating and Using Custom Data 924
- Bar and Scatter Plots 963
- Titration 986
- High Content Flow 1027
- DNA Analysis 1069
- Proliferation 1105
- Kinetics 1127

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- Cell Concentration 1150
- Channel Calibration 1169
- CellProfiler Export 1183
- Exporting from IsoCyte 1300
- Picture Plots 1315
- Linear Regression Analysis 1342

2.3 Integrated Help System

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

2.4 Comprehensive Manual

The FCS Express manual is a detailed explanation of all of the functions in FCS Express. The manual is available by selecting **Application Button→Help→FCS Express Manual**. You can also obtain the latest version of the manual on the De Novo Software web site.

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. This is the preferred method for technical support and we encourage you to post questions, comments, and suggestions to this forum. You can access the forums by selecting **Application Button→Help→Online Forum** or at http://www.denovosoftware.com/forums/.

2.6 Email Support

If you have any questions pertaining to FCS Express, please do not hesitate to contact us at support@denovosoftware.com.

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Chapter

3 A Quick Tour of FCS Express

FCS Express Version 4 has a brand new interface. The toolbars and menus have been replaced with a Ribbon, similar in design to the familiar Microsoft Office suite of programs (Office 2007 and later). The Ribbon helps you quickly find commands that you need to complete a task. The commands are organized in groups, which are collected together under tabs. Each tab relates to a type of activity in FCS Express, such as inserting plots, gating, and batch processing.

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. 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 preferences for each user.

The **Import...** button at the bottom left of the **User Selection** window is used for importing FCS Express Version 3 user preference files (.fcg) from the FCS Express V3 installation folder.

For details on changing user preferences, see the section on Customizing Default Preferences 117. *Note:* An individual user's preferences can be restored to the default settings (those chosen when FCS Express is shipped), by selecting **Application Button→System→Restore Preferences 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 31 (from **Application Button →System**).

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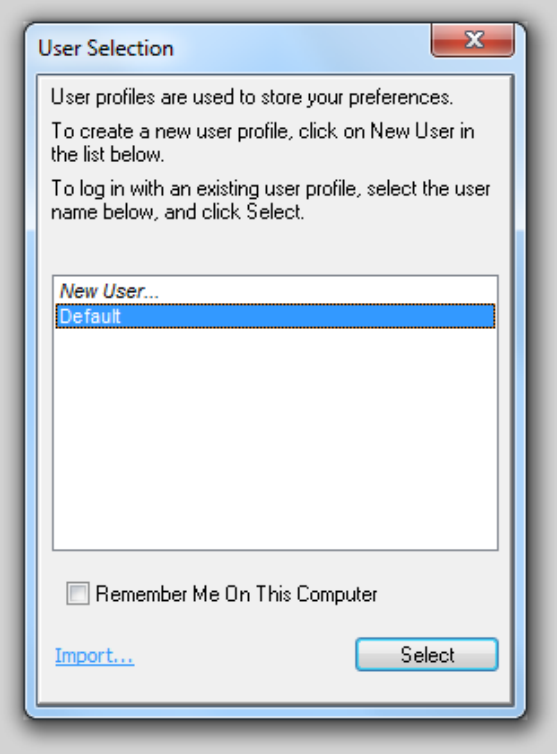

Figure 3.1 User Selection Dialog

When a user is selected, the preferences previously saved for that user are activated. Therefore, multiple users sharing the same computer can customize their preferences without affecting other users.

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 preferences for that user cannot be recovered*.

Note: If you are running the security system 520, 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 a blank document (layout) will be displayed. Layouts (.fey) are the document format of FCS Express. The default layout is a single page with a white background.

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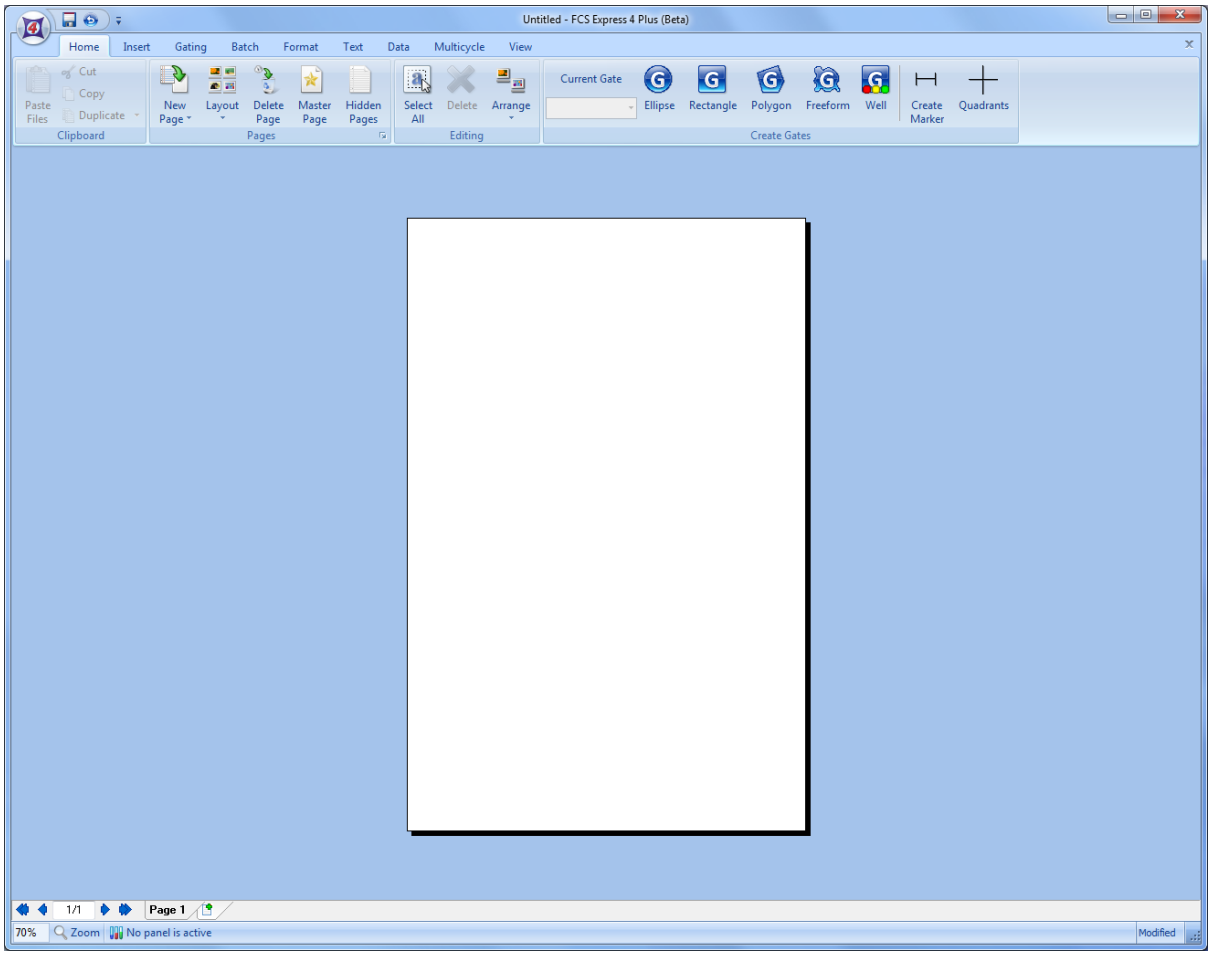

Figure 3.2 Startup Screen

You can open an existing layout using **Application Button→Open Layout →Open Layout** to bring up a **Load Layout** dialog box. 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 52.

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, 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.

3.2 A Quick Walk through the Ribbon

The Ribbon (Figure 3.3) is at the top of the page. You can find the Application Button 31 and the **Quick Access Toolbar** at the top, left corner of the Ribbon.

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The **Quick Access Toolbar** is a place to put frequently used functions. You can customize 34 the functions on the **Quick Access Toolbar** (as well as the entire Ribbon itself 48). 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.

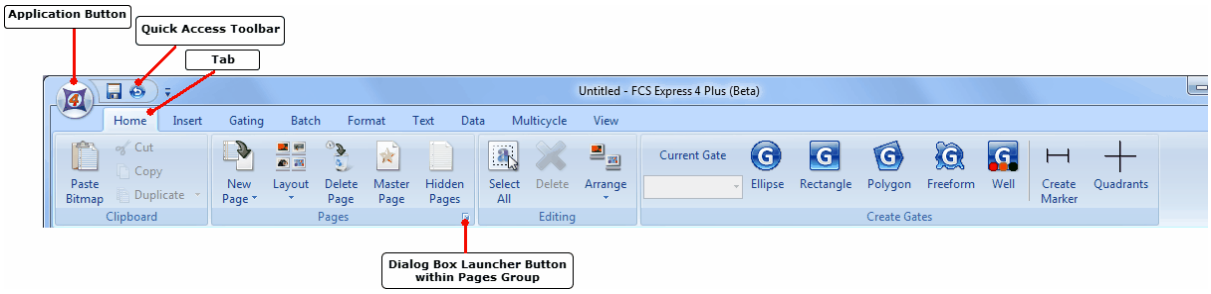

Figure 3.3 The Ribbon

At the very bottom left of the application window is the **Layout Navigation Toolbar** (Figure 3.4) used for navigating through the layout 63. Beneath the **Layout Navigation Toolbar** is the Zoom Toolbar 64. To the right of the **Zoom Toolbar** is a **Status Bar** showing the active panel, described in Working with Panels 422.

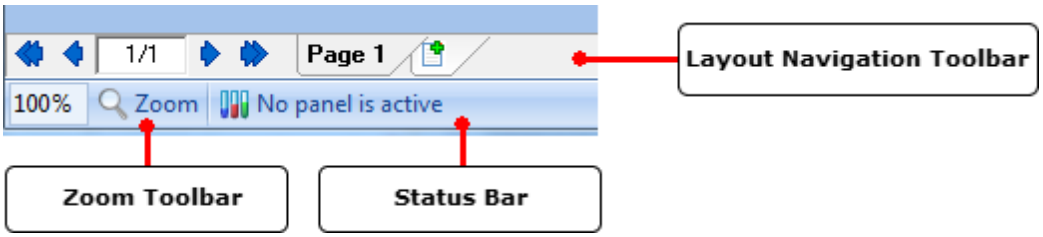

Figure 3.4 Layout Navigation Toolbar with Zoom Toolbar and Status Bar

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

3.2.1 The Application Button

The **Application Button** gallery (Figure 3.5) contains the following items in three sections. It also contains two buttons (lower right), **Edit Preferences** and **Exit**.

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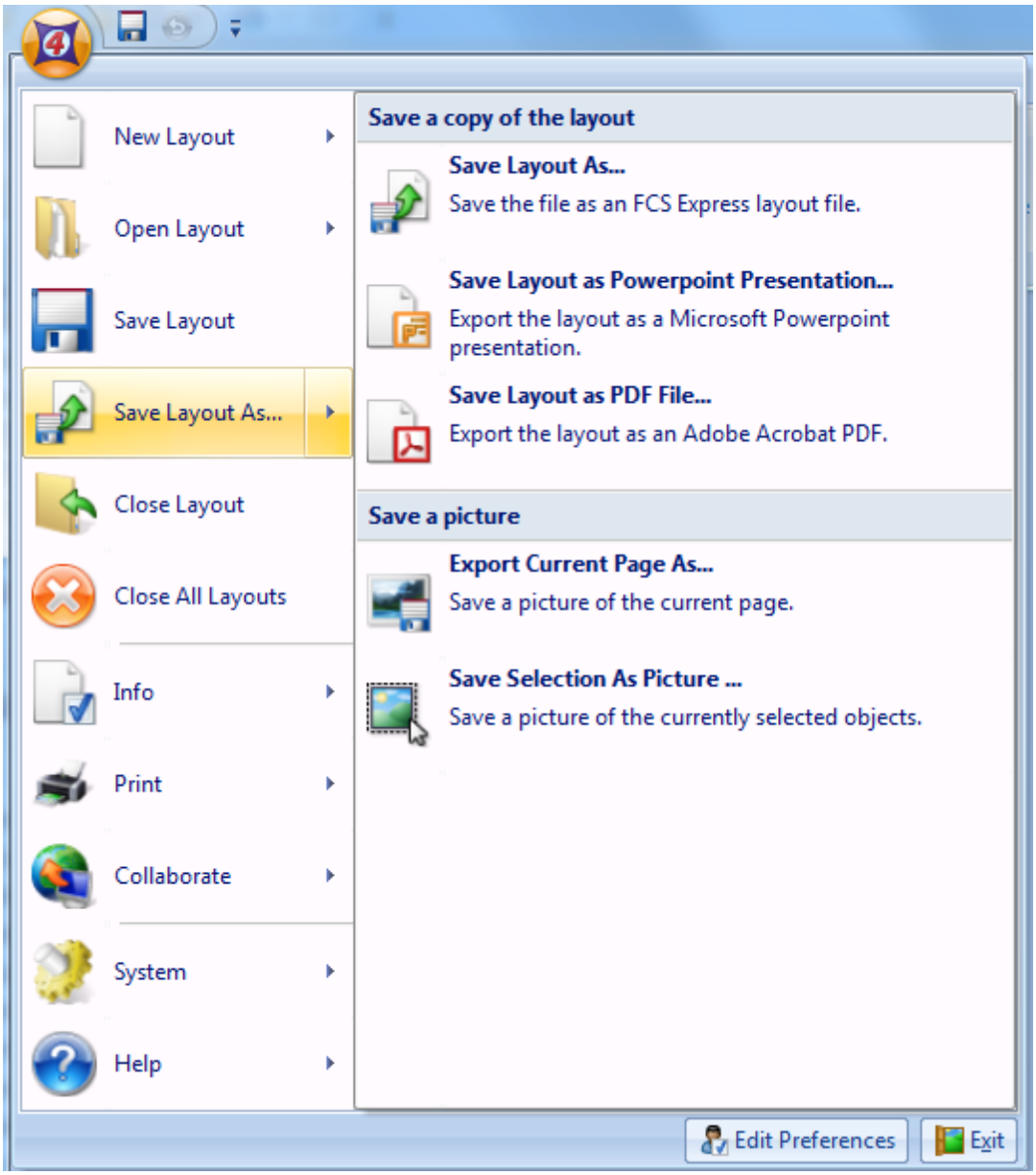

Figure 3.5 Application Button Gallery (with Save Layout As... selected)

- **New Layout, Open Layout, Save Layout, Save Layout As..., Close Layout, Close All Layouts**.

  These items allow the user to create, open, save, and close layouts. The **Open Layout** command also allows the user to import BD FACSDiva 430 and BD Accuri C6 437 files. The **Save Layout As...** item allows you to export your layout to a PowerPoint presentation or a PDF. In addition, you can save the current page or selected items as a Picture.

- **Info**

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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 Audit Trails add-on).

- **Print**

  This item is used to:

  - Select a printer, which pages to print, and other options before printing.
  - Change the page size, orientation, and margins.
  - Manage print jobs, and downloads.

- **Collaborate**

  This item is for:

  - Publishing your layout using the De Novo Software publishing service.
  - Managing the layouts you have published.
  - Connecting with other users and working together in real-time.

- **System**

  With this command, you can:

  - Edit information for the current user.
  - Restore preferences to default values.
  - Reset toolbars to default settings.
  - Logout the current user.
  - Change your configuration.
  - Enter a countercode to activate your copy of FCS Express.
  - Manage tasks that are running in the background.

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- **Help**

  With this item, you can:

  - Open the integrated help system and/or the PDF manual.
  - Navigate to the De Novo Software web site to view written and video tutorials.
  - Visit the online forum.

*Note: If security is activated, there will be an additional item available labeled Security.*520

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

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

3.2.2 The Quick Access Toolbar

With the default **Quick Access Toolbar**, there are icons (Figure 3.6) to **Save** the current layout or **Undo** 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 the state you were in before you started changing your layout.

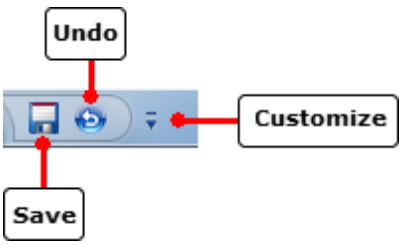

Figure 3.6 Quick Access Toolbar Icons

Clicking the **Customize** command (tooltip "More Buttons"), brings up a gallery (Figure 3.7) which allows you to fully customize the interface.

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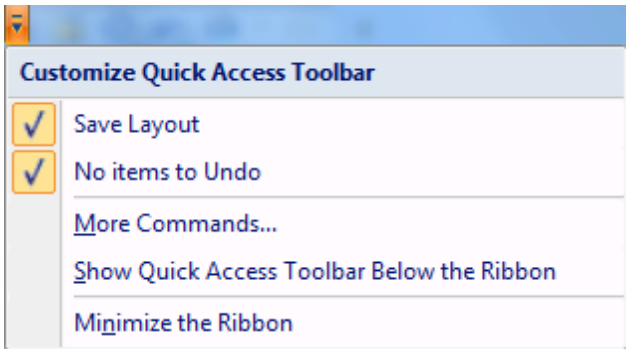

Figure 3.7 Customize Menu

The **More Commands...** item brings up a **Customize** dialog (Figure 3.8) with **Toolbars**, **Commands**, and **Options** tabs, where you can hide or reorganize any of the Ribbon groups and the individual commands that appear in the groups.

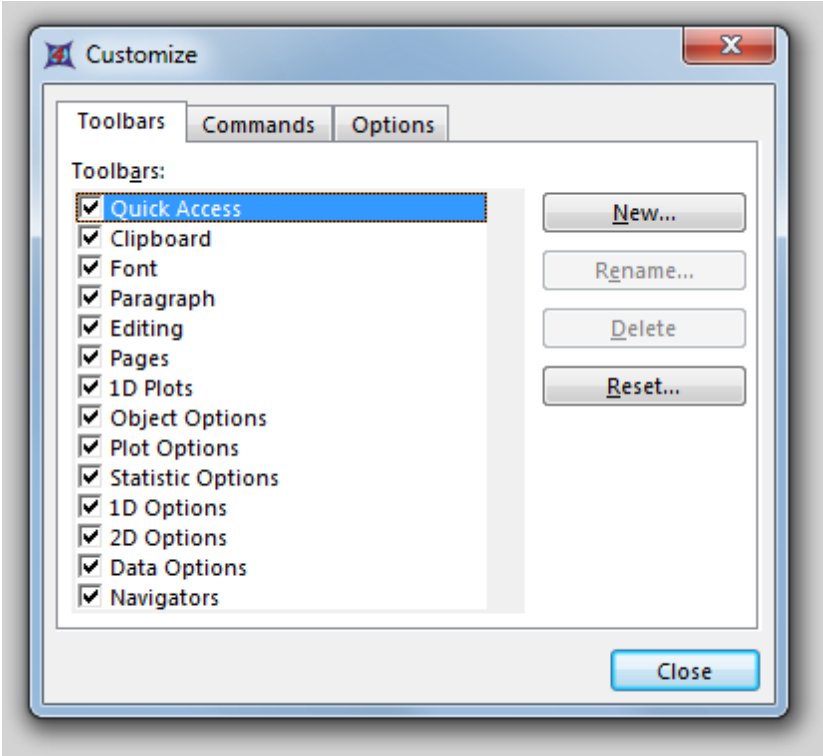

Figure 3.8 Customize Dialog

Under **Application Button→System**, the **Reset Toolbars to Default Settings** command will revert your changes.

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3.2.3 The Home Tab

The **Home** Tab (Figure 3.9) contains the following **groups**:

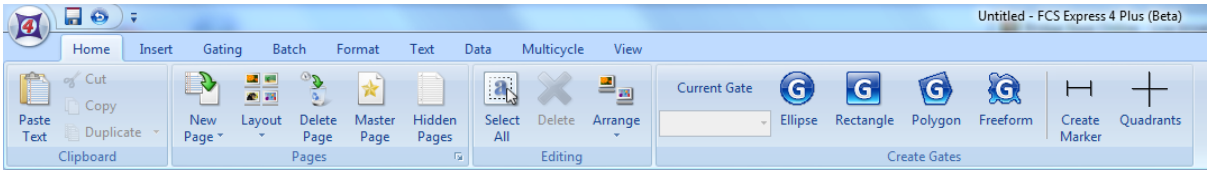

Figure 3.9 Home Tab

- **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 item. 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 changes 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. The positioning of the duplicated objects can be set in the user preferences 118.
  - 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 layouts using the **View→Windows→ Switch Windows** command.
- **Pages**
  - 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 **Layout** command opens a gallery for adding Place Holders for predefined text boxes, plots, and statistics to the current page.

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- The **Delete Page** command deletes the current page.
- The **Master Page** command shows the Master Page 87.
- The **Hidden Pages** command is used to show pages that the user has previously chosen to hide in the layout 58.

- **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**, **Group Objects**, and **Position 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 objects together, so that selecting any one of the objects will automatically select all items in the group. **Position Objects** is used for aligning the sides, tops, bottoms, centers, heights and/or widths of the selected objects.

- **Create Gates**
  - The **Create Gates** group contains commands for creating gates (**Ellipse**, **Rectangle**, **Polygon**, **Freeform**, **Well**), Markers (**Create Marker**) and **Quadrants** on a plot. It also 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 192.

3.2.4 The Insert Tab

The **Insert** Tab (Figure 3.10) contains four **groups** for inserting objects on a page:

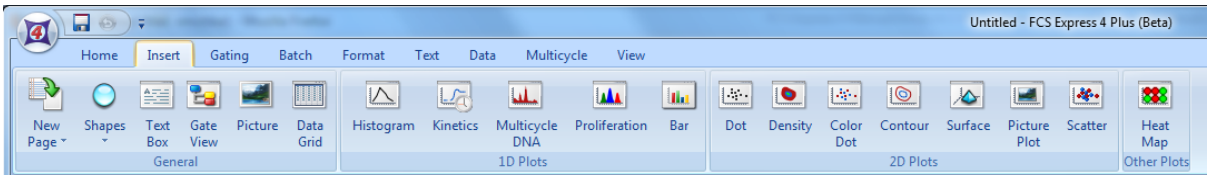

Figure 3.10 Insert Tab

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- **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, to view and change the properties of all your gates.
  - The **Picture** command inserts a picture from a file.
  - The Data Grid command 320 allows the user to view data in a table (spreadsheet) format or work with custom data 234.
- **1D Plots**
  - The five commands in the **1D Plot** group insert Histograms 110, Kinetics plots 474, Multicycle DNA histograms 445, Proliferation histograms 463, and Bar charts 241.
- **2D Plots**
  - The seven commands in the 2D Plot group insert two-dimensional **Dot** plots, **Density** plots, **Color Dot** plots, **Contour** plots, **Surface** plots, and Scatter plots 241.
- **Other Plots**
  - The Heat Map 482 command inserts a **Heat Map** which is used to view statistical values across an entire microtiter plate.

3.2.5 The Gating Tab

The **Gating** Tab (Figure 3.11) contains two **groups** for working with gates:

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Figure 3.11 Gating Tab

- **Create Gates**

  The **Create Gates** group contains commands for creating gates (**Ellipse**, **Rectangle**, **Polygon**, **Freeform**, Well[488]), Markers (**Create Marker**), and **Quadrants** on a plot. It also 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[192].

- **Gates**

  The **Load** and **Save** commands allow you to save and import gates into another layout[203].

3.2.6 The Batch Tab

The **Batch** Tab (Figure 3.12) contains two **groups**.

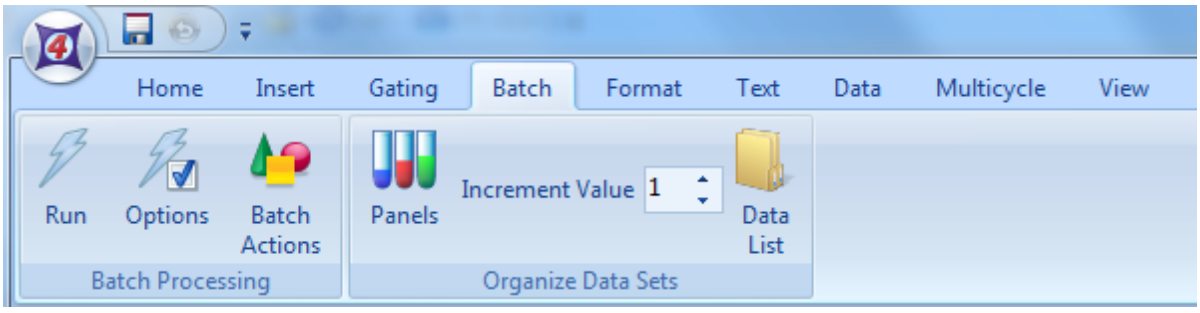

Figure 3.12 Batch Tab

All of the commands associated with this Tab are described in detail in the chapter on Batch Processing[372].

- **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,

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saving, and reporting.

- The **Options** command brings up the **Batch Processing Options** 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.
  - Restore the original files on the layout after batch processing.
- The **Batch Actions** command brings up the **Batch Actions** 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 422.
  - 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 373 window to process the list of current files (or custom data).

3.2.7 The Format Tab

The **Format** Tab (Figure 3.13) contains seven **groups**. The commands allow you to change the properties of one or more selected objects.

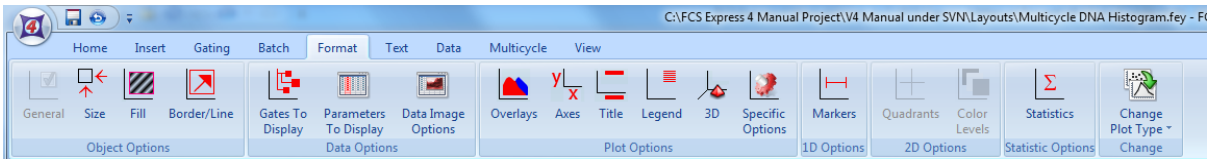

Figure 3.13 Format Tab

- **Object Options**: Each command brings up a dialog box to set object properties.
  - The **General** command is for assigning or changing the object name.

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- With the **Size** command, you can change or scale the objects height and width. 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).

- **Data Options**

  - The **Gates to Display** command is used for choosing which gates to display in a plot.

  - 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 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.

- **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**

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- 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, the **Statistics** command allows you to format the properties of the statistics.

- **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.

3.2.8 The Text Tab

The **Text** Tab (Figure 3.14) contains four **groups**.

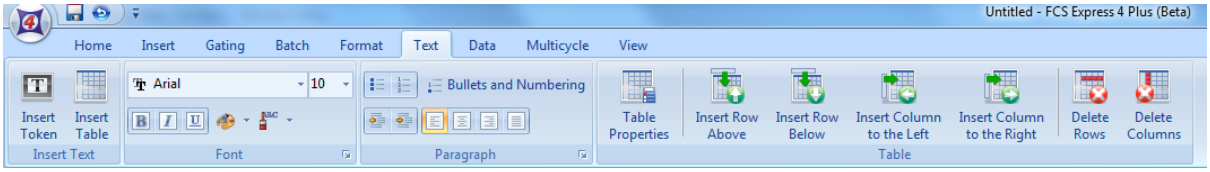

Figure 3.14 Text Tab

- **Insert Text**

  If a text box is selected, the **Insert Token** command allows you to choose the type of predefined token you want 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 (little arrow icons at the bottom right) of the **Font** and **Paragraph groups**. While the font and paragraph commands usually apply to

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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.

3.2.9 The Data Tab

The **Data** Tab (Figure 3.15) contains six **groups**:

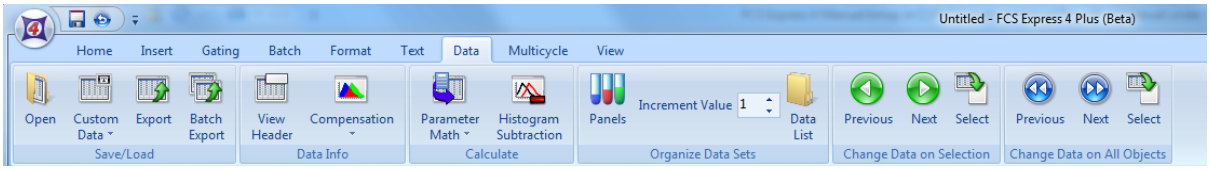

Figure 3.15 Data Tab

- **Save/Load**

  - The **Open** command brings up the **Select a Data File** dialog (Figure 3.16) which allows you to select a data set (e.g., an FCS file) and choose which type of plot(s) to open from the **Select Plot Types...** dialog (Figure 3.17). The **Select a Data File** dialog contains a **File List** tab which allows you to see which files/data sets you are working with in the current experiment and an **Active Files** tab (i.e., the files currently open in the layout). Using the **Files of type:** drop-down list, you can choose the file extension, e.g., files from different instrument manufacturers, or comma/character separated value (.csv) files.

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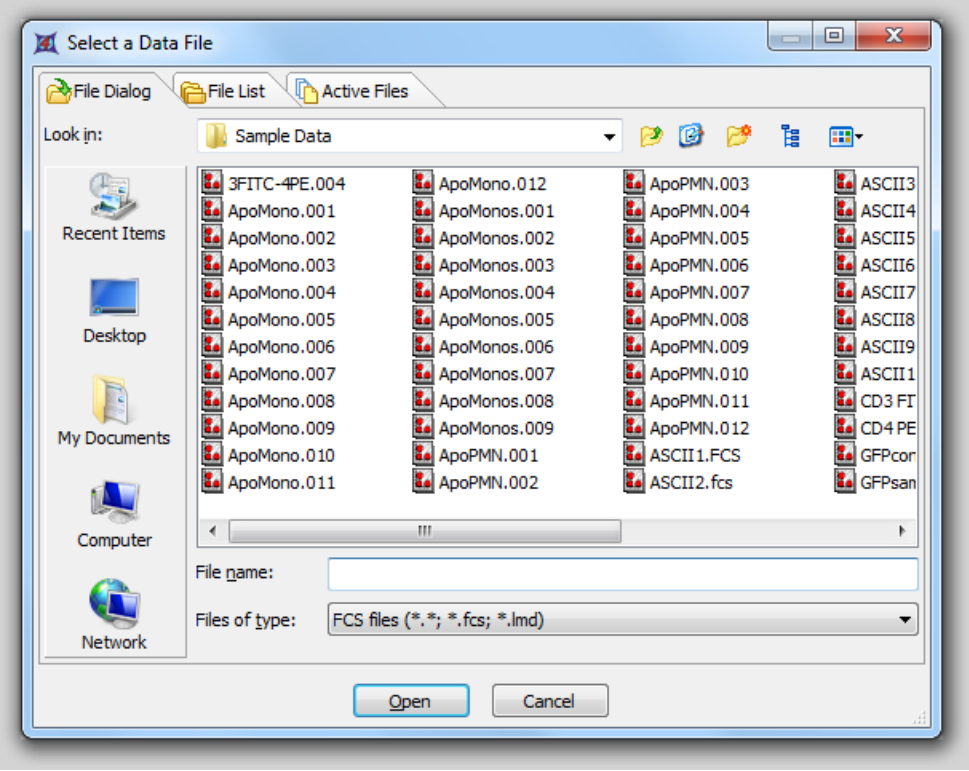

Figure 3.16 Select a Data File Dialog

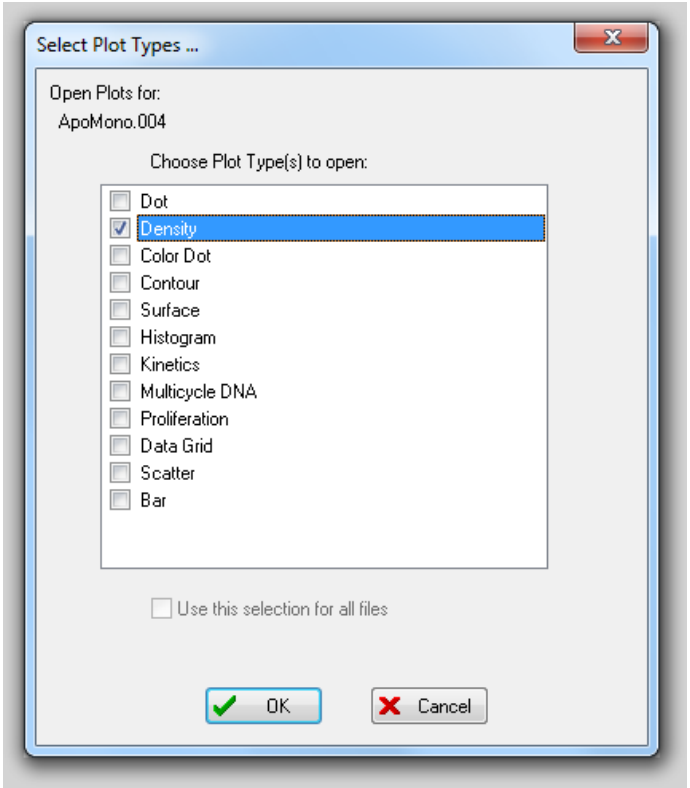

Figure 3.17 Select Plot Types Dialog

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- The **Custom Data** command allows you to create new custom data 234.
- With the **Export** command, you export the data from a single open file 314.
- You can export data from multiple files consecutively using the Batch Export 316 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 that you can choose which header information to display.
  - Clicking on the **Compensation** command brings up a gallery which allows you to display the compensation information on the selected file, and load or save compensation information.

- **Calculate**
  - **Parameter Math** opens a gallery containing **Single Calculation**, **Sequence Setup**, **Run Sequence**, **Load Sequence**, and **Save Sequence** commands. These items allow you to define a single or sequence of math functions, save the functions, and apply the functions to data files 260.
  - The **Histogram Subtraction** command allows you to apply four mathematical methods of comparing sample and control histograms 274.

- **Organize Data Sets**
  - The **Panels** command brings up the Panel Information dialog for working with panels 422.
  - 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.
  - The **Data List** command brings up the dockable Data List window to process the list of current data sets 373.

- **Change Data on Selection**

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- **Previous** changes the selected FCS file to the previous file in the **Data List.**
- **Next** changes the selected FCS file to the next file in the **Data List.**
- **Select** brings up the **Select a Data File** dialog so that you can choose an FCS file.

- **Change Data on All Objects**
  - **Previous** changes all FCS files to the previous file in the **Data List.**
  - **Next** changes all FCS files to the next file in the **Data List.**
  - **Select** brings up the **Select a Data File** dialog so that you can choose an FCS file.

3.2.10 The Multicycle Tab

The Multicycle Tab449 (Figure 3.18) allows you to work with the Multicycle AV plugin from Phoenix Flow Systems. It contains two groups:

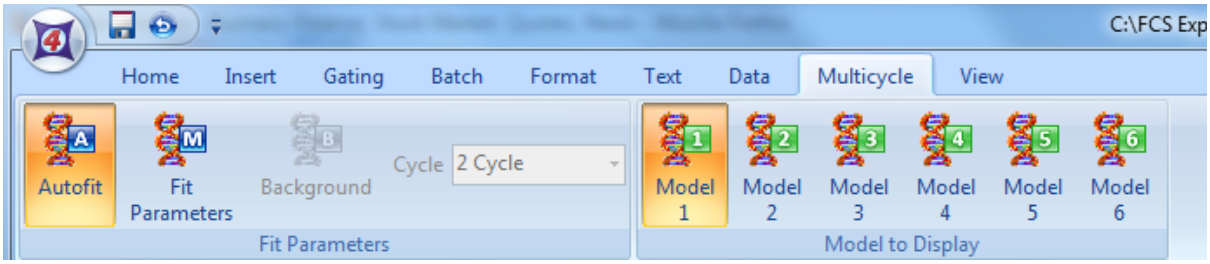

Figure 3.18 Multicycle Tab

- **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.

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3.2.11 The View Tab

The **View** Tab (Figure 3.19) contains five **groups**:

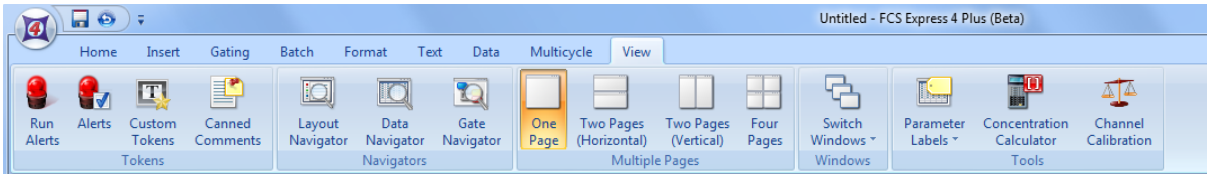

Figure 3.19 View Tab

- **Tokens**
  - The **Custom Tokens** command brings up a dockable **Custom Tokens** window where you can create new custom tokens[349] to generate your own custom statistics.
- **Navigators**
  - The Layout Navigator[75] command brings up a dockable **Layout Navigator** window listing each item in the layout.
  - The Data Navigator[76] command brings up a dockable **Data Navigator** window listing the parameters of each file referenced in the layout.
  - The Gate Navigator[77] 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[64].
- **Windows**

  If there are multiple layouts open, the **Switch Windows** command allows you to choose which layout is active.
- **Tools**
  - The **Parameter Labels** opens a gallery containing **Modify Labels**, **Load Labels**, and **Save Labels** commands. These items allow the user to easily change, recall, and save alternate parameter labels[230].

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- The **Concentration Calculator** command brings up a **Concentration Calculator** window where you can calculate the concentration of events in a sample 268.

- The **Channel Calibration** command brings up the Channel Calibration window 284 where you can change the axis fluorescence intensity scales from a linear or logarithmic scale to one indicating either the absolute number of antigen molecules or the number of binding sites on the surface of a cell.

3.2.12 Customizing the Ribbon

In addition to customizing the interface using the customize command to the right of the Quick Access Toolbar 34, right-clicking on an individual command within a group, or right-clicking on the name of a group, brings up a gallery (Figure 3.20) which includes the command to **Add the command** (or group of commands) to the Quick Access Toolbar 34.

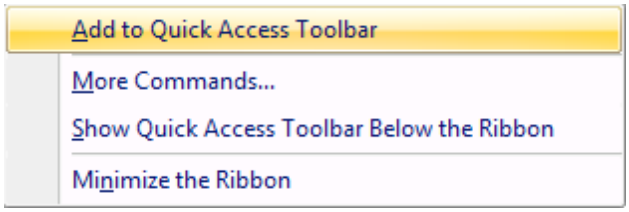

Figure 3.20 Add Command to Quick Access Toolbar

The **More Commands...** item brings up a **Customize** dialog (Figure 3.21) with **Toolbars**, **Commands**, and **Options** tabs, where you can hide or reorganize any of the Ribbon groups and the individual commands that appear in the groups.

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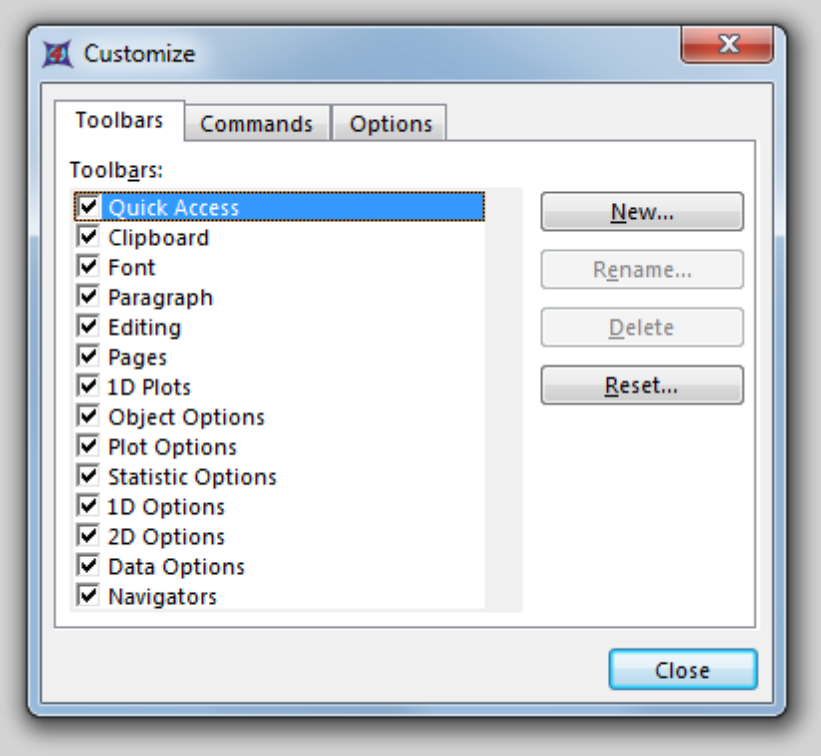

Figure 3.21 Customize Dialog

When the **Customize** dialog is open, you can perform many customizations to the Ribbon. To remove items from the Ribbon: click on the item to remove, hold down the mouse button, drag the item to an area that is off the Ribbon, 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.

Under **Application Button→System**, the **Reset Toolbars to Default Settings** command will restore all toolbars to their default settings.

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Chapter

4 Working with Layouts

Layouts are the basic building block of FCS Express.

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 individual 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.

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 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:

- By selecting **Application Button→New Layout**, 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 (Figure 4.1).

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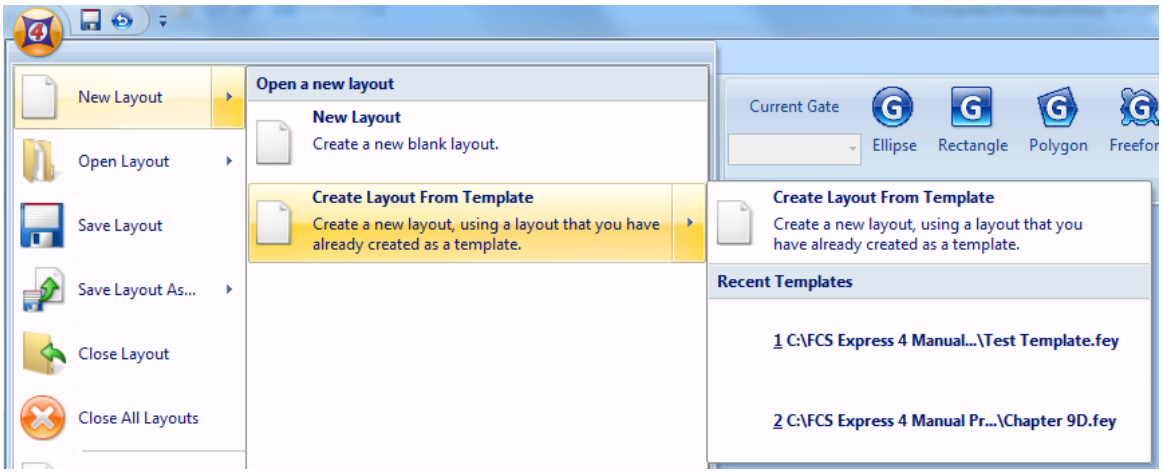

Figure 4.1 Recent Templates

- Templates are very useful for setting up standard document settings, like margins, page size, plots, statistics, and page orientation. 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 **Application Button→New Layout→Create Layout From Template** command. You can also designate a saved layout file as your default template 135. When a default template is present, FCS Express opens that template file instead of a blank layout when using the **Application Button→New Layout** 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 and FCS Express will "forget" that the new layout file was based on the template. Therefore, when you save the new layout, you will be prompted for a new filename to prevent overwriting the original template.

- Select **Home→Pages→Layout**. With this method, you can choose a blank layout or select from a number of predefined layout styles.

4.1.2 Saving a Layout

Layouts can be saved in any of the following ways:

- **Application Button→Save Layout**.

- Click the **Save Current Layout** icon in the Quick Access Toolbar 34.

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- Use the **Ctrl+S** keyboard shortcut.

Using any of these methods will bring up the **Save Layout** dialog (Figure 4.2) where you can name the layout file to be saved and choose the location to save it.

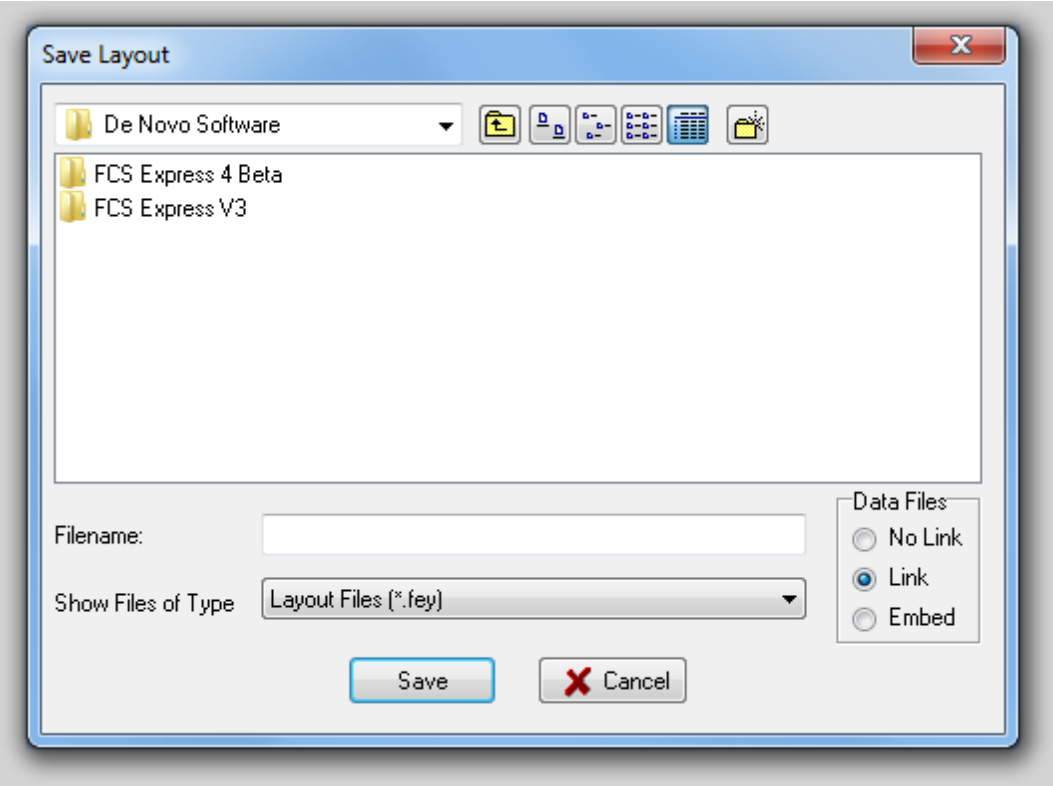

Figure 4.2 Save Layout Dialog

When saving a layout, you have the option to save without a link, save with a link, or embed the data file within the saved layout according to the radio button you choose in the **Data Files** group box on the lower right of the dialog.

- Save without data link (**No Link** radio button)

  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.

- Save with data link (**Link** radio button)

  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

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files, no data will appear in the layout plots or statistics. Linked layout files are smaller than embedded layout files.

- Save with embedded data file(s) [**Embed** radio button]

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

4.1.3 Opening a Previously Created Layout

A previously saved layout can be opened by using the **Application Button→ Open Layout** command. This brings up the **Load Layout** dialog. Recently used documents will appear on the right when you press the Application Button (Figure 4.3). To open a recently used document, simply select the document.

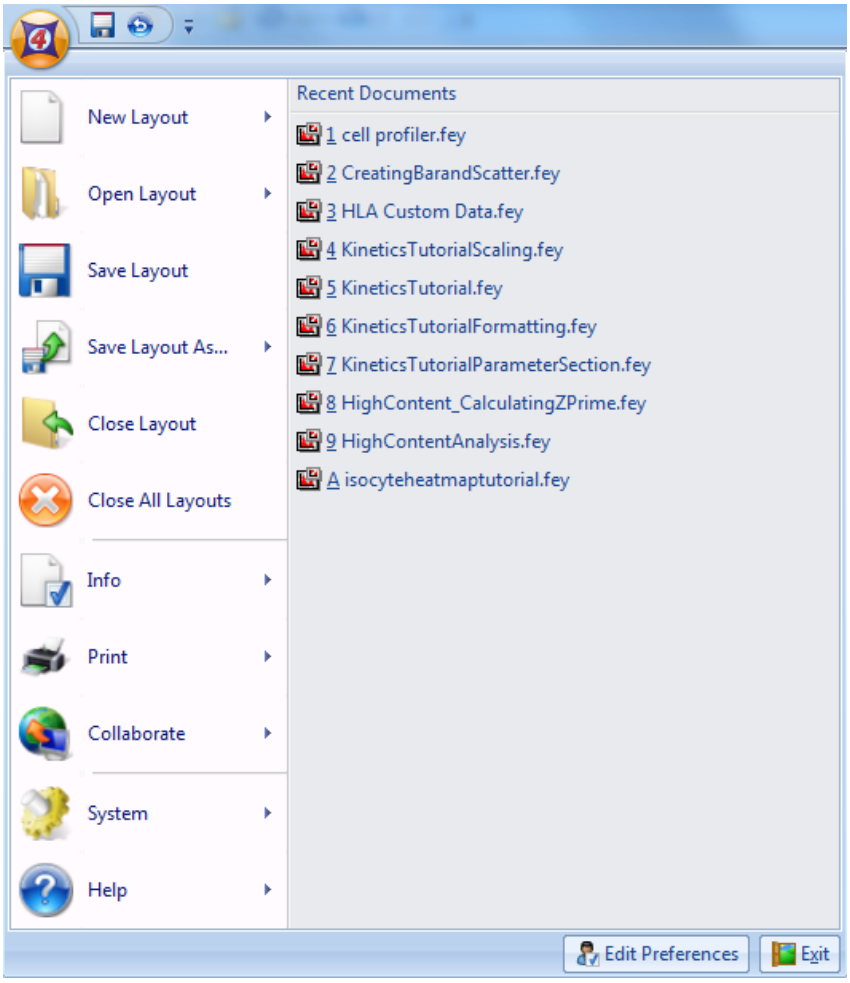

Figure 4.3 Recent Documents Appear on the Right

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Navigate to the file you wish to open. 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 in the selected layout file, and the file linking status[53] (Figure 4.4).

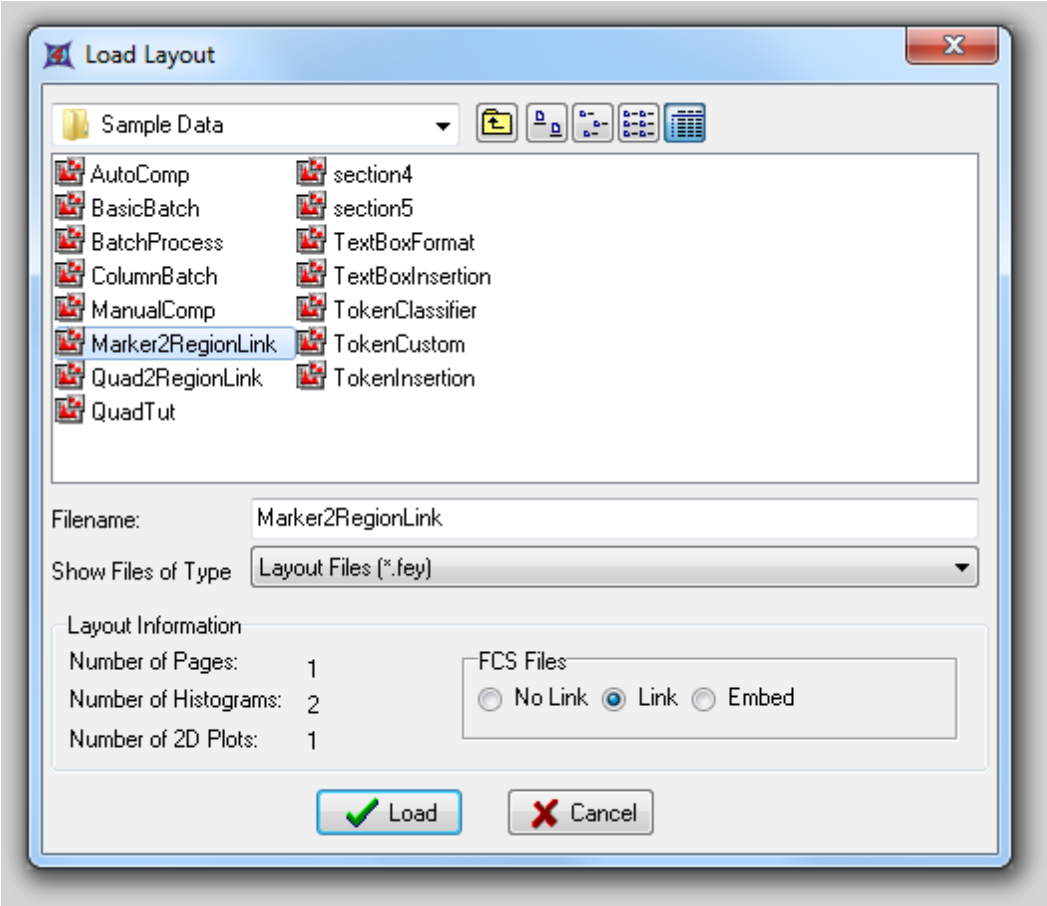

Figure 4.4 Load Layout Dialog

If the layout you are opening has linked data and FCS Express cannot find the file (s), you will be notified in a **Select a Data Directory** dialog, and will be given the opportunity to load the file(s) from a different folder (Figure 4.5).

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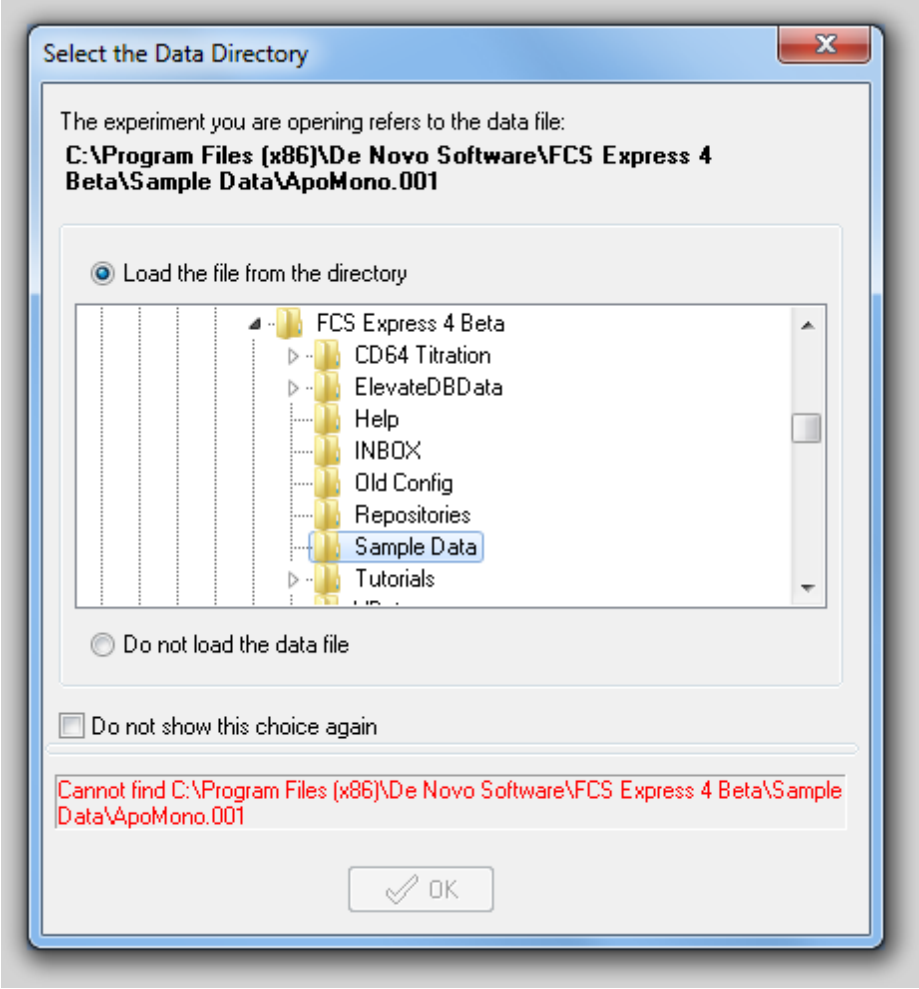

Figure 4.5 Missing Linked Data

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→Switch Windows**, if necessary) and select **Application Button→Close Layout**. 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 either of the two "x" icons in the upper right of the application window.

Note: If more than one layout is open, both the Close Program "x" icon, located at the extreme upper right corner of the window at the same level as the window title, and the Close Layout "x" icon, located on the level of the Tab names, act the same way (Figure 4.6). Only the current layout will be closed. However, if only one layout is open, clicking on the Close Program "x"

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will close (Exit) FCS Express, while clicking on the lower (Close Layout) "x" will only exit the program if the layout you are closing has not been changed.

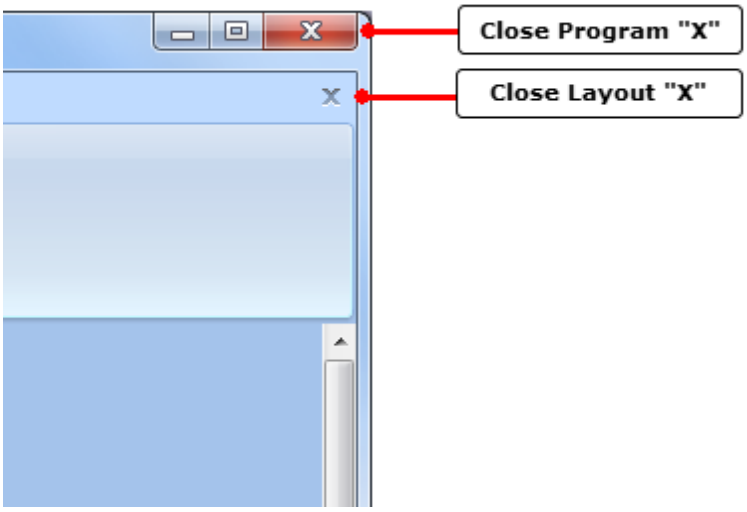

Figure 4.6 Close icons

- To close all of the open layout files, select **Application Button→Close All Layouts**.

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 user preferences[137] 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 gallery.
- Right-click on the layout and select **New Page** from the pop-up menu.
- Click on the **Add Page** tab (Figure 4.7) of the **Layout Navigation Toolbar** (near the bottom of the application window on the left).

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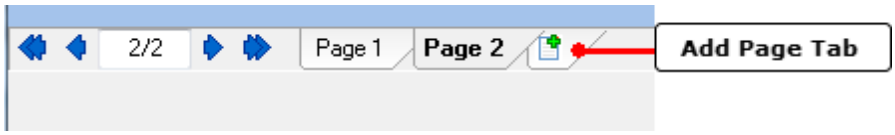

Figure 4.7 Add Page Tab

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.
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 4.8). 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 362 (by clicking on the T button), and the page will only be visible if the token evaluates to True.

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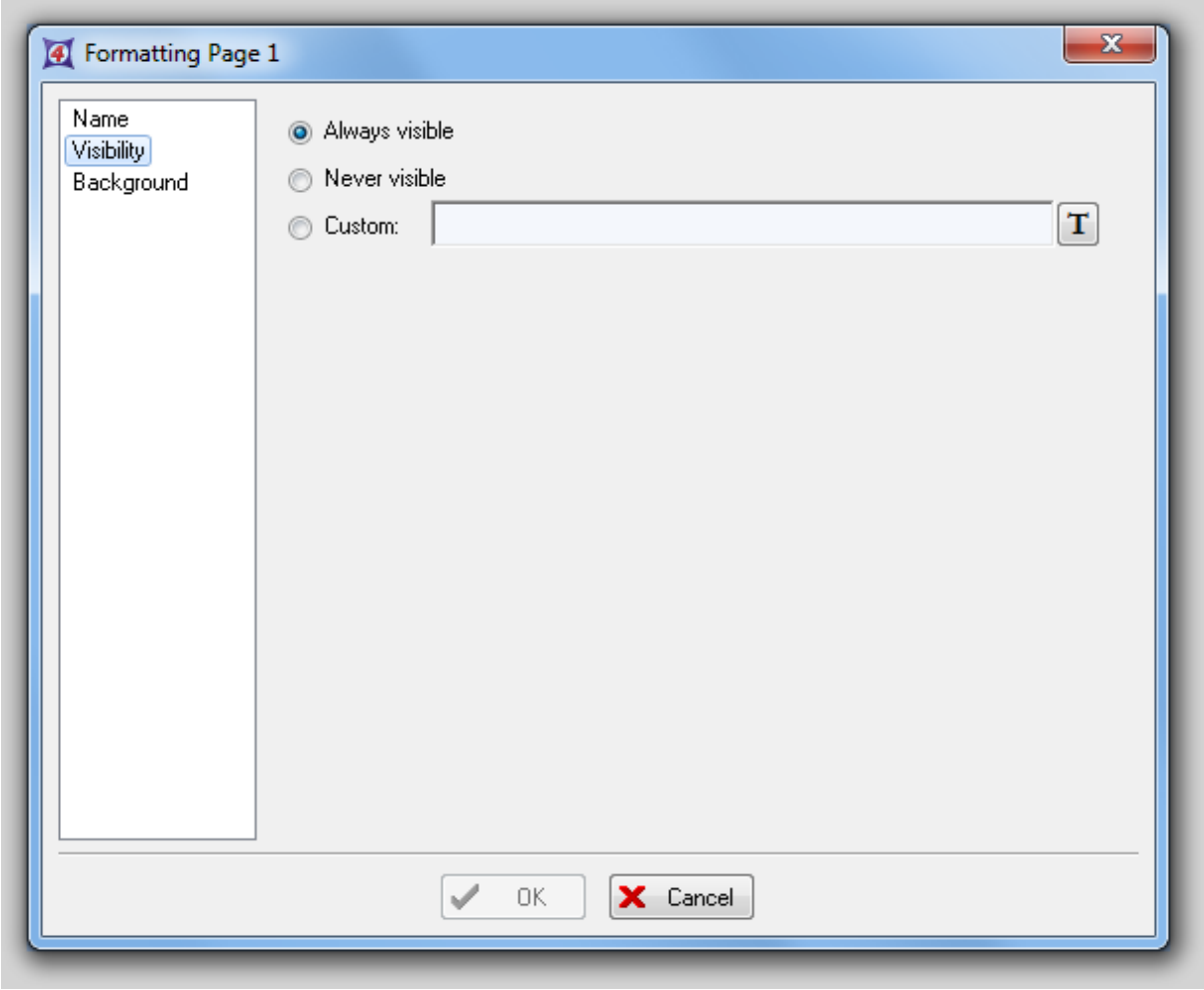

Figure 4.8 Formatting Page Visibility

The default background of a page is the background of the master page87. 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 4.9).
3. Click on the **Custom** radio button.
4. Choose the **Color**/**Fill Pattern**, **Gradient**, or **Image**.

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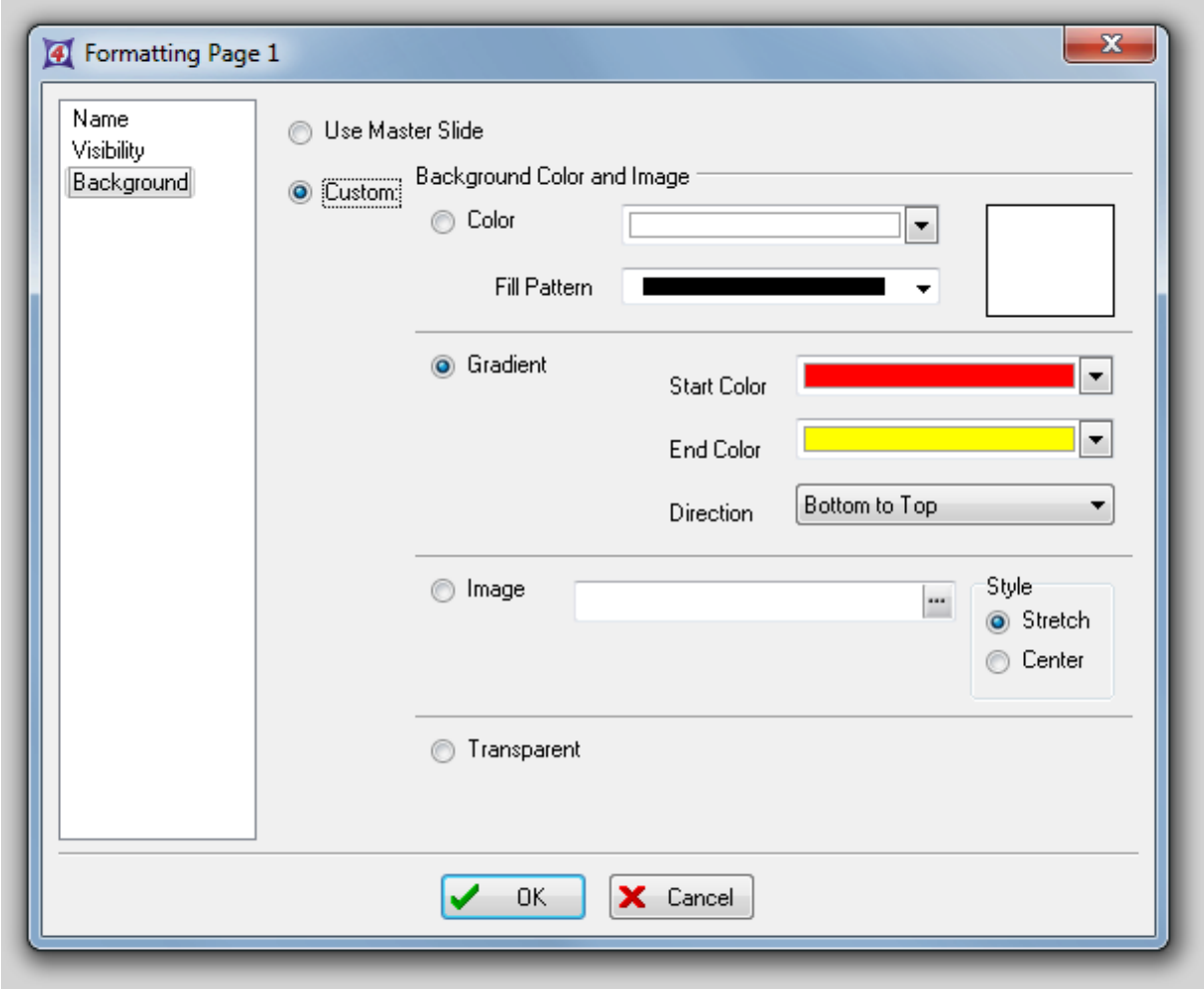

Figure 4.9 Changing an Individual Page Background

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 **Application Button →Print**. A standard **Print** dialog box 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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To change the current printer and settings, select **Application Button→Print →Page Setup** to bring up a **Print Setup** dialog (Figure 4.10). In the **Print Setup** dialog, you can change printers, page margins, and page orientation. To lock the aspect ratio for each object on the page when you change the orientation, check **Maintain the size of all objects on the layout**.

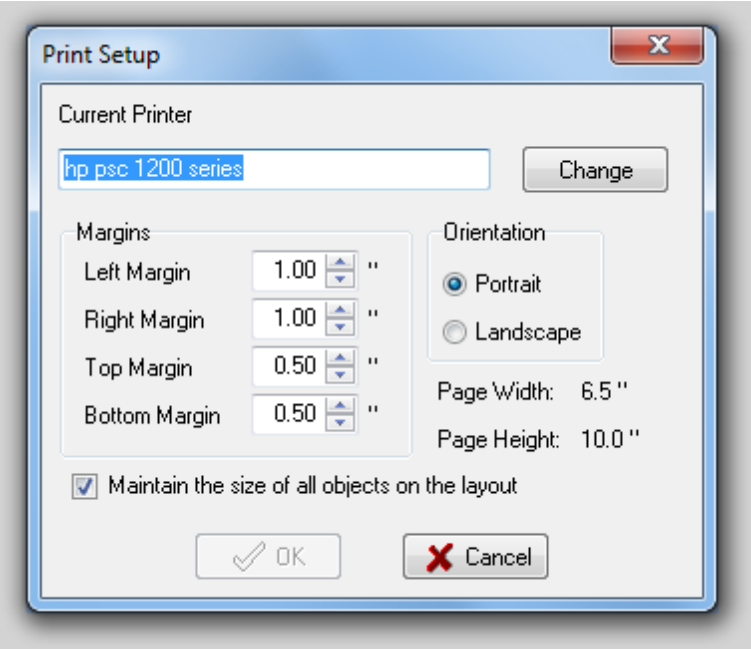

Figure 4.10 Print Setup Dialog

4.1.7 Exporting a Page as a Picture from a Layout

A page can be exported as a picture by selecting **Application Button→Save Layout As...→Export Current Page As...**.

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. The resulting PowerPoint presentation will appear exactly as it looked in FCS Express, i.e., a five page layout in FCS Express will become a five slide layout in PowerPoint. All plots and text boxes are inserted into the PowerPoint presentation as Windows Metafiles and can be moved, resized, and manipulated within PowerPoint.

To save as a PowerPoint presentation, select **Application Button→Save Layout As...→Save Layout as PowerPoint Presentation...**.

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A dialog box will appear that will allow you to:

- Name the presentation.
- Choose which layout pages you wish to export.
- Decide whether or not to include the background.

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.

4.1.9 Exporting a Layout as a PDF File

A layout can be exported directly as an Adobe PDF file. The resulting PDF file will appear exactly as it looked in FCS Express.

To save as a PDF file, select **Application Button→Save Layout As...→Save Layout as PDF File...**.

A dialog will appear that will allow you to:

- Name the PDF.
- Designate the PDF quality [**Low**, **Medium**, **High**, **Highest**]. Higher quality documents will have better graphics, but larger file sizes. PDF quality primarily effects the look of statistics windows and gate views.
- Choose which pages you wish to export.

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 30 (always located at the bottom left

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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 4.11).

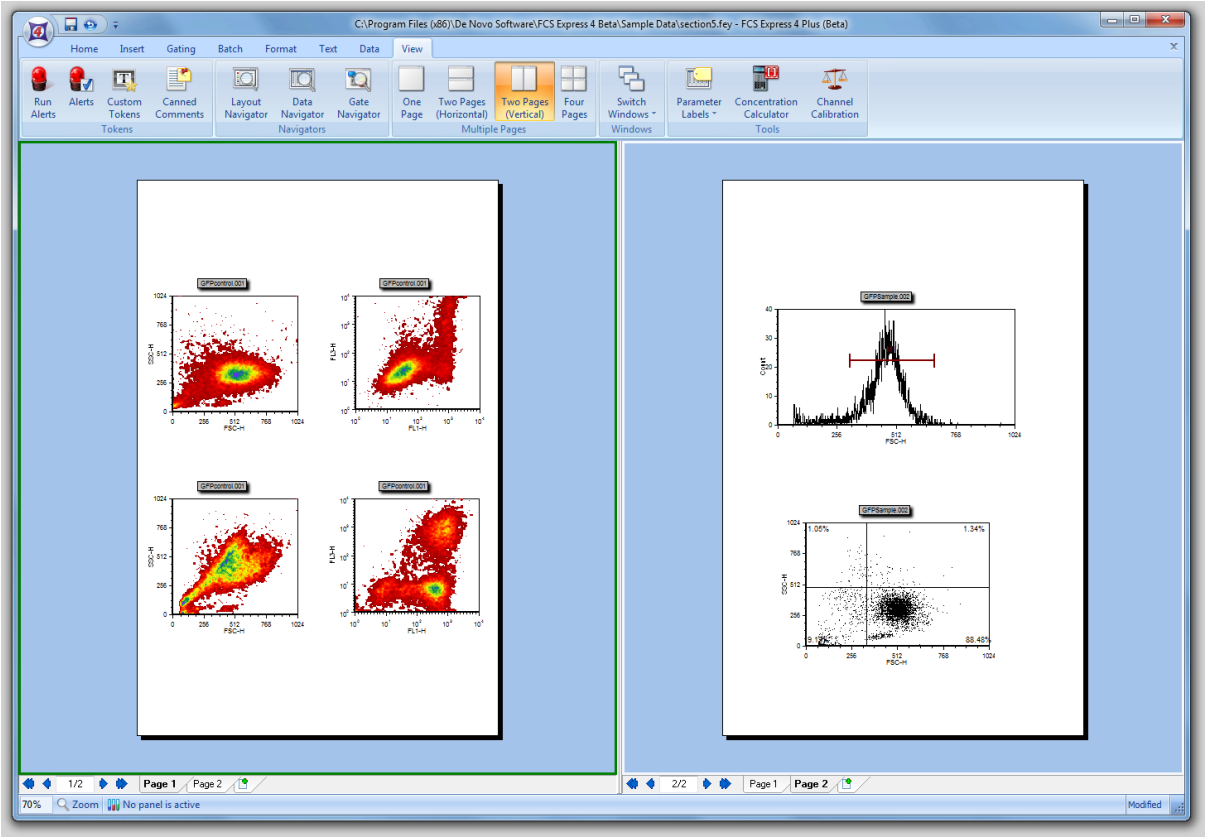

Figure 4.11 Two Pages Vertical View

4.1.12 Zooming the Layout

It is possible to change the current zoom factor (in percentages) by using the Zoom Toolbar 30 located below the Layout Navigation Toolbar 63. You can enter the desired zoom percentage directly into the toolbar edit field on the left or

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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 preference, you can turn off this feature, or change the interval between saves, by editing the Save page of the Edit Preferences 137 dialog. If FCS Express is terminated suddenly, without having the chance to ask if you want to save the layout, then the next time you start the program, you will be asked if you want to resume using the AutoSave 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 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.

4.1.15 Layout Properties

It is possible to access the layout properties by selecting **Application Button →Info→Layout Properties**.

The **Layout Properties** dialog (Figure 4.12) displays information about the layout and allows you to assign a password to open the layout.

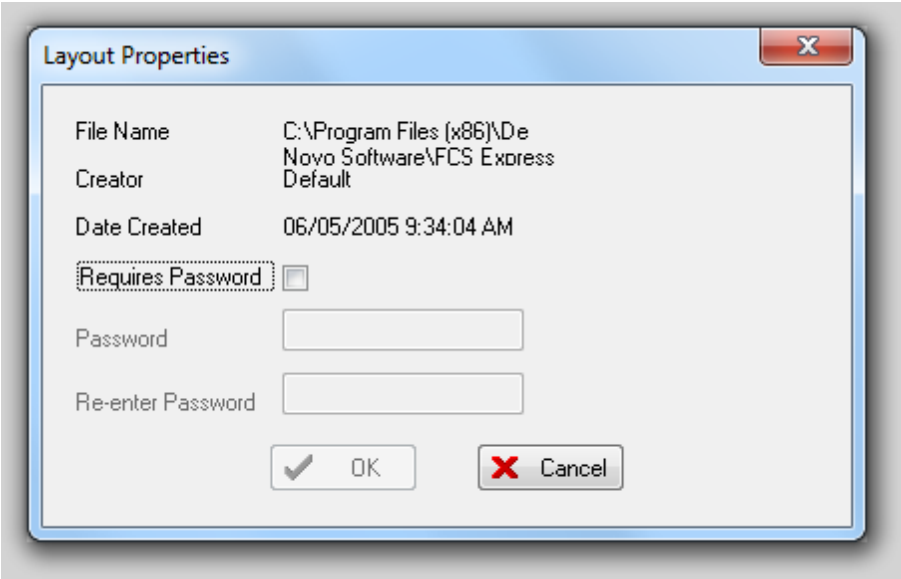

Figure 4.12 Layout Properties Dialog

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

If you have security enabled[520], additional properties will be available on the **Layout Properties** dialog.

4.2 Introduction to Docking

FCS Express Version 4 has a very flexible user interface. A new feature, dockable windows, gives you the ability to customize your desktop to fit your needs.

The following windows are dockable:

- Data List
- Layout Navigator
- Data Navigator
- Gate Navigator
- Batch Actions
- Custom Tokens

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

4.2.1 Opening a Dockable Window

Dockable window are opened by clicking on the appropriate Ribbon[30] commands from the Batch Tab[39], the Data Tab[43], or the View Tab[47].

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**
Layout Navigator	**View→Navigators→Layout Navigator**
Data Navigator	**View→Navigators→Data Navigator**
Gate Navigator	**View→Navigators→Gate Navigator**
Batch Actions	**Batch→Batch Processing→Batch Actions**
	View→Tokens→Alerts
Custom Tokens	**View→Tokens→Custom Tokens**
	View→Tokens→Canned Comments

When opened, the window will first appear as a floating window, undocked, in the center of the screen (Figure 4.13). You can leave it as a floating window, or

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proceed to dock it in the location of your choice 67.

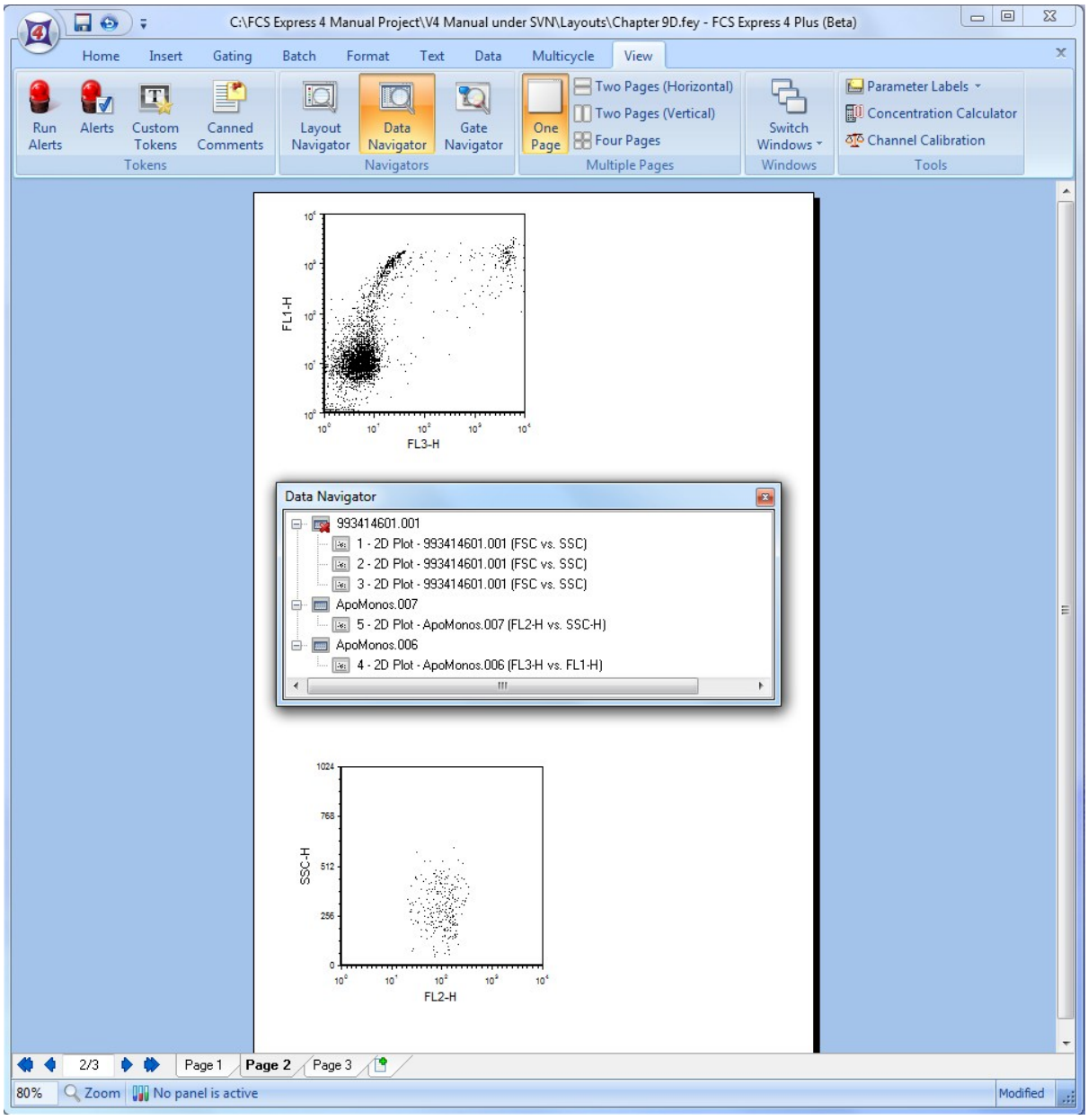

Figure 4.13 Data Navigator, Floating (Undocked)

4.2.2 Docking a Window

To dock a floating window:

- Move the cursor over the title bar of the window (Figure 4.14).

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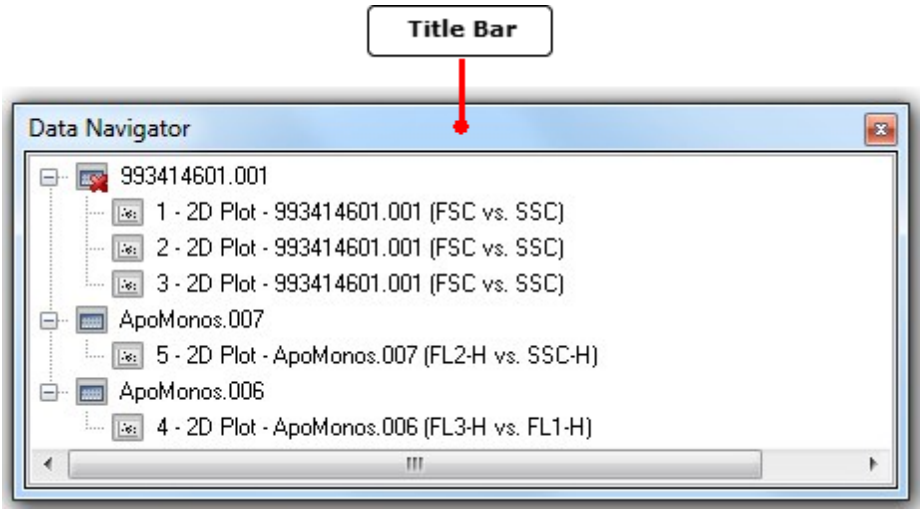

Figure 4.14 Title Bar of Floating Window

- 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 4.15).

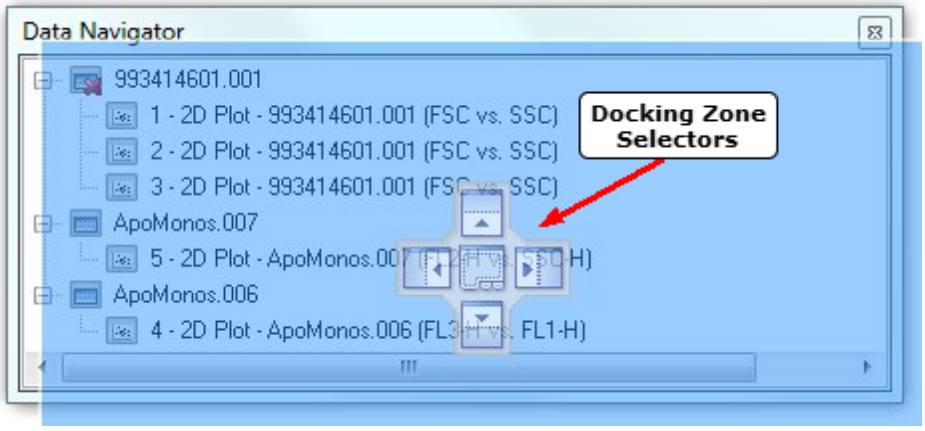

Figure 4.15 Docking Zone Selectors

- 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 4.16), 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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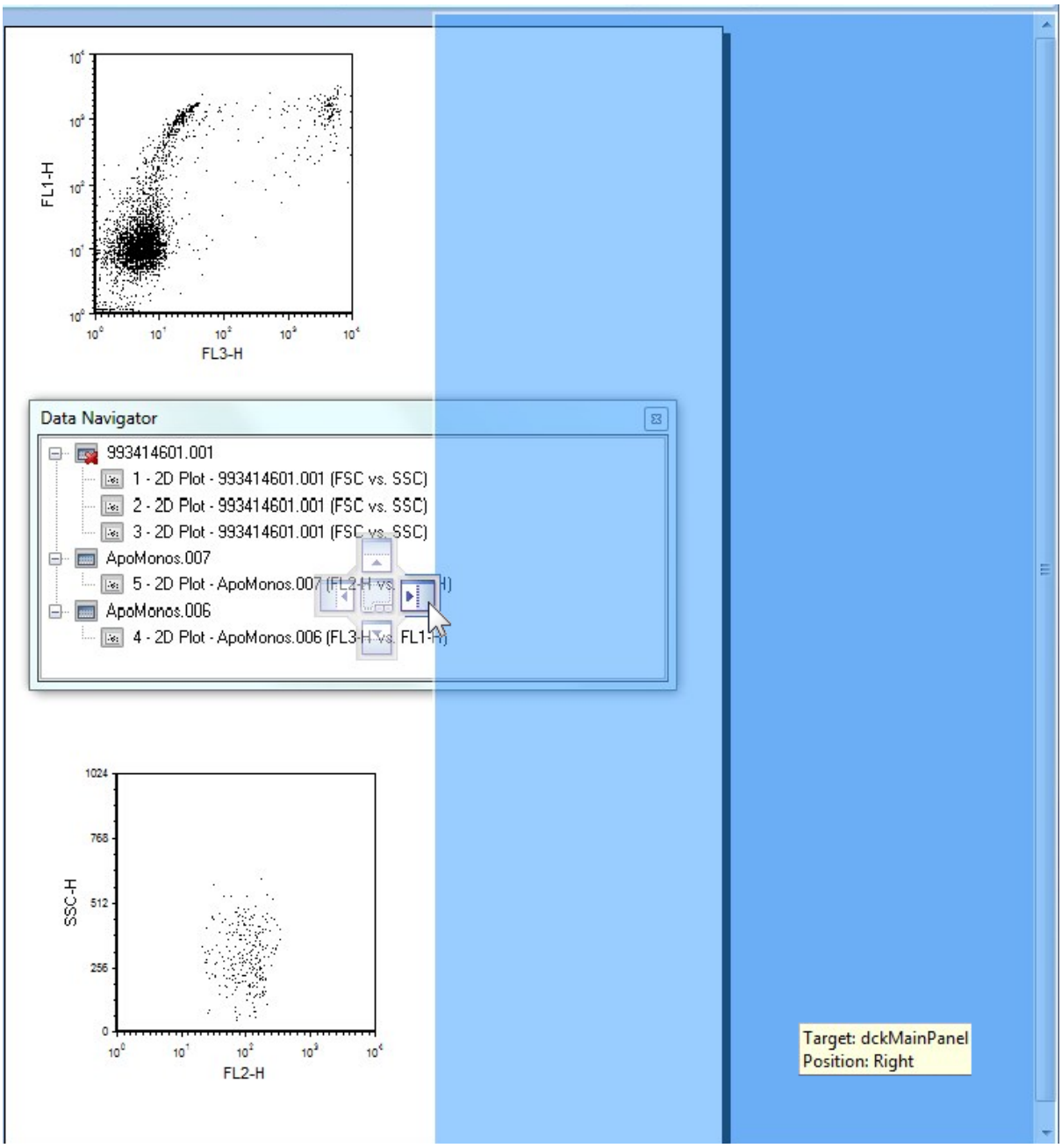

Figure 4.16 Cursor Positioned over Right Docking Zone Selector

- When you release the button with the cursor positioned over a docking selector, the window will dock to the location you selected (Figure 4.17).

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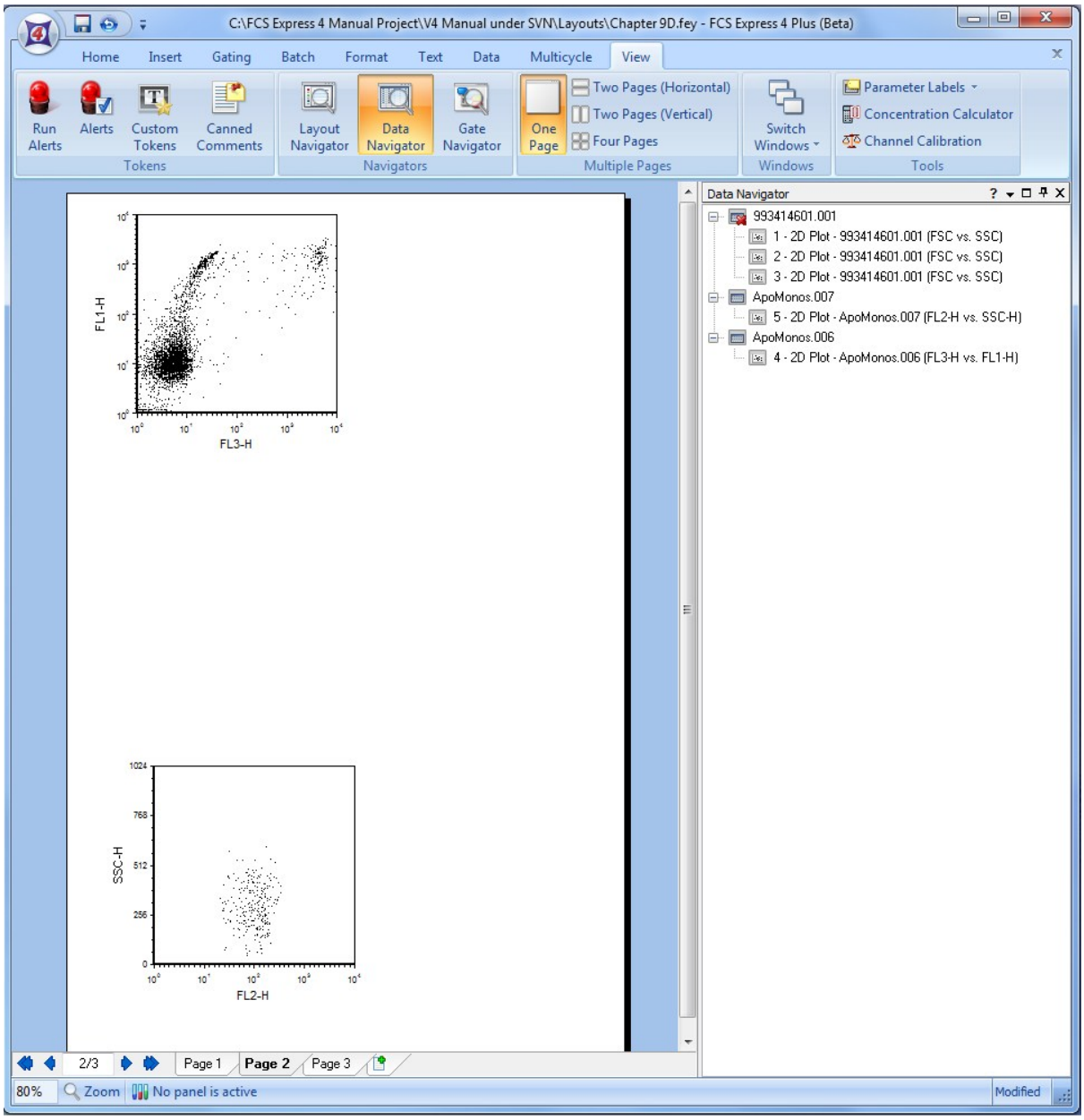

Figure 4.17 Data Navigator Window Docked to the Right

- 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 4.18). 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 4.19). 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 4.20).

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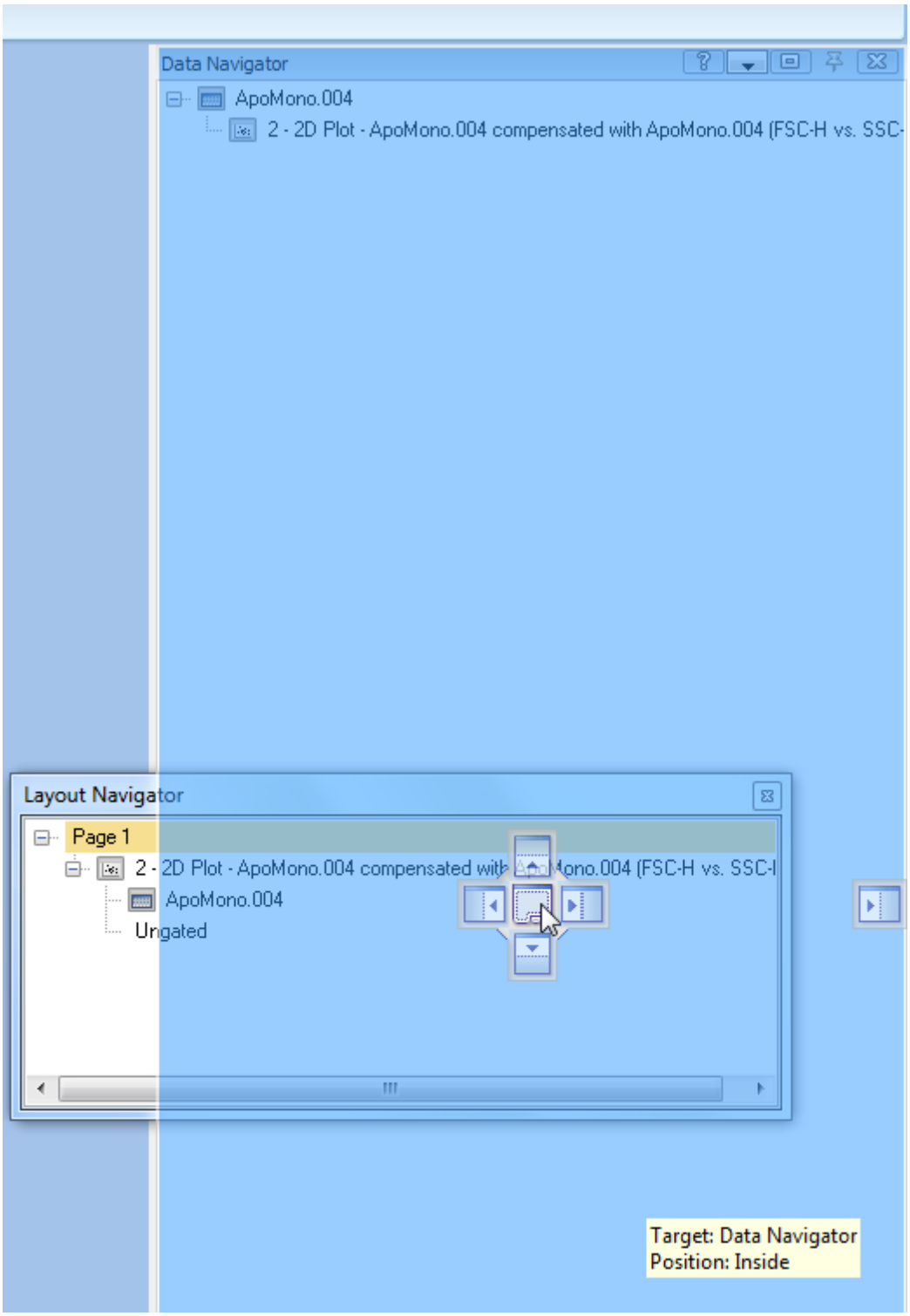

Figure 4.18 Docking the Layout Navigator inside the Data Navigator

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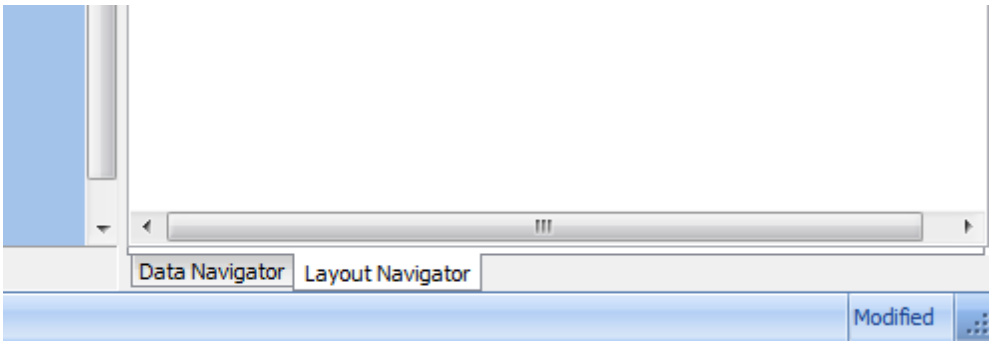

Figure 4.19 Docked Windows Identified by Tabs

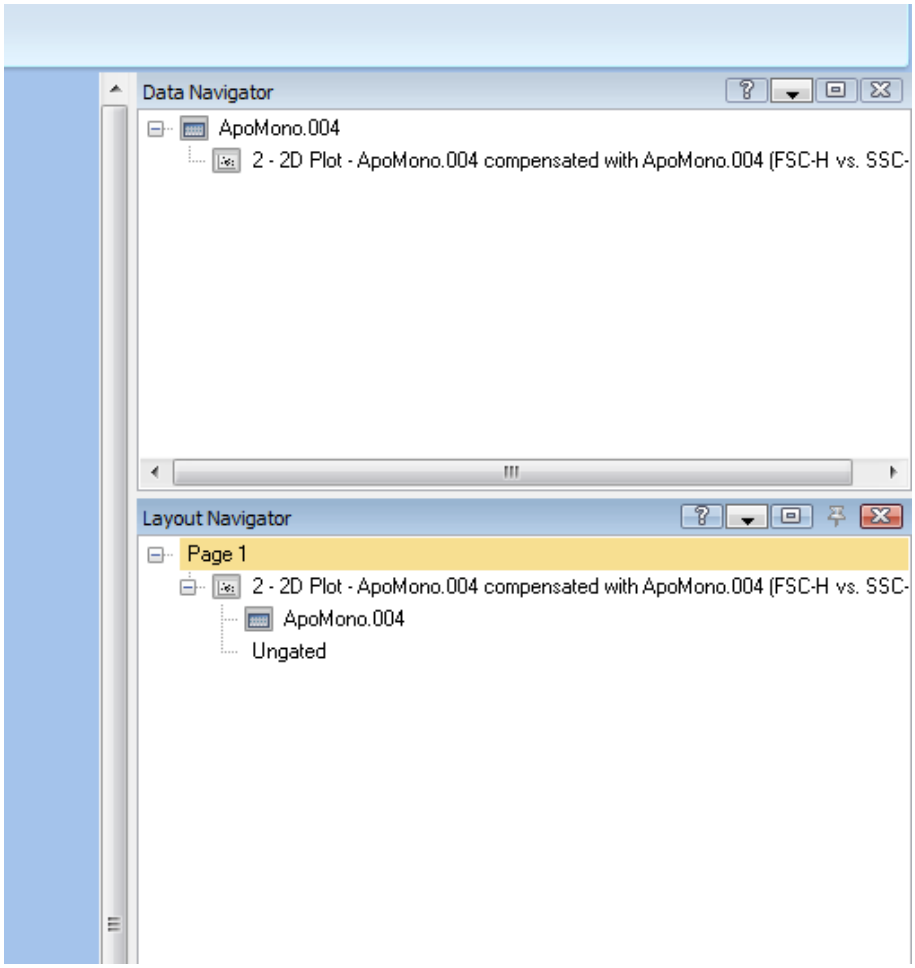

Figure 4.20 Docked Windows Seperated by a Horizontal Splitter Bar

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Note: Although docked windows are not part of the layout page, 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 4.21) controls the *Auto Hide* feature.

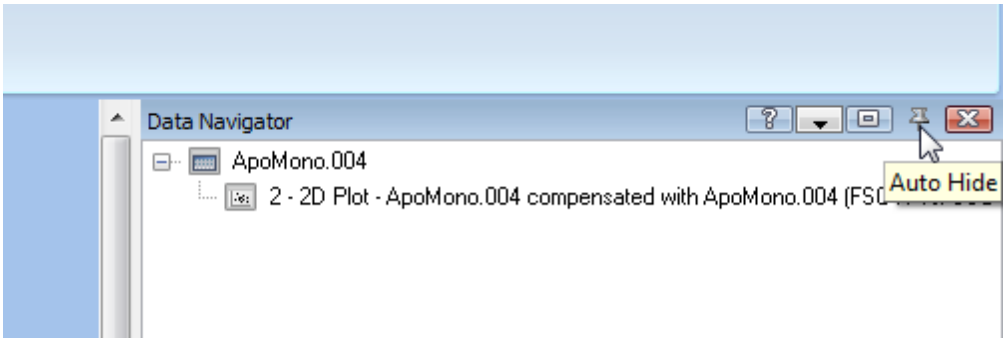

Figure 4.21 Auto Hide Pin Icon in a Docked Window

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* 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 4.22).

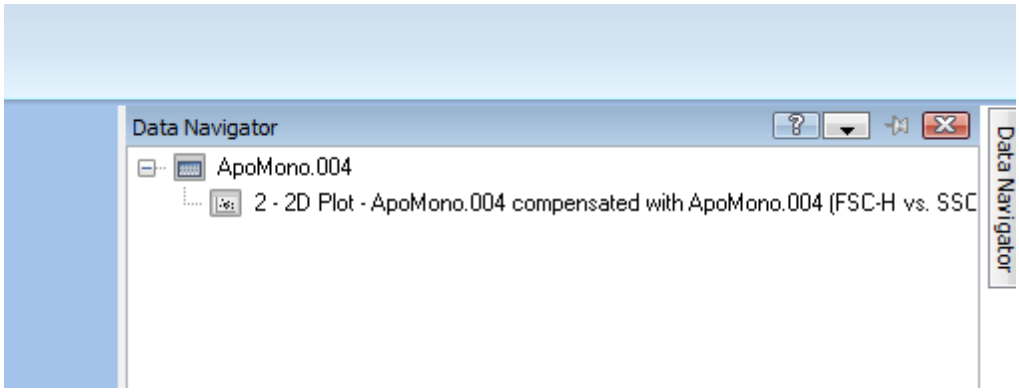

Figure 4.22 Docked Window with Auto Hide Activated

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

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tab will be visible (Figure 4.23). When a window is pinned, you can also hide it by clicking on the X icon. To open a hidden window, hover the cursor over the tab.

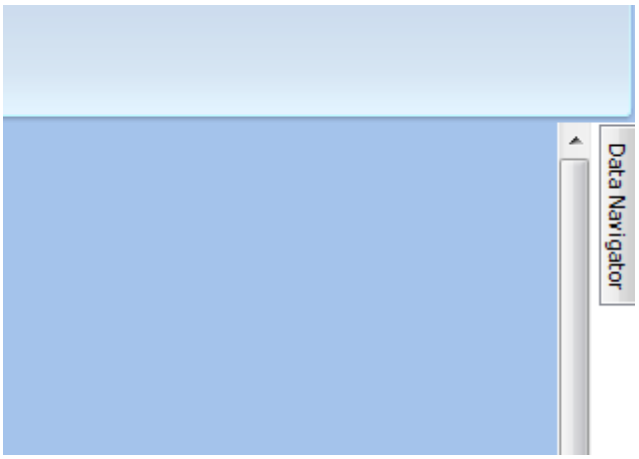

Figure 4.23 Pinned Data Navigator Hidden from View

Note: A window that has been pinned cannot be undocked[74] or closed[74] 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[73].

To undock an unpinned window:

- Move the cursor over the title bar[67] of the window.
- 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[73].

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[66] with drag and drop functionality to help you manipulate the objects in your layout[75], the data sources[76] connected to your objects, and the gates[77] on your plots.

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4.3.1 Layout Navigator

The **Layout Navigator** shows a hierarchical view of all of the objects in your layout by page, plot, and data source (Figure 4.24).

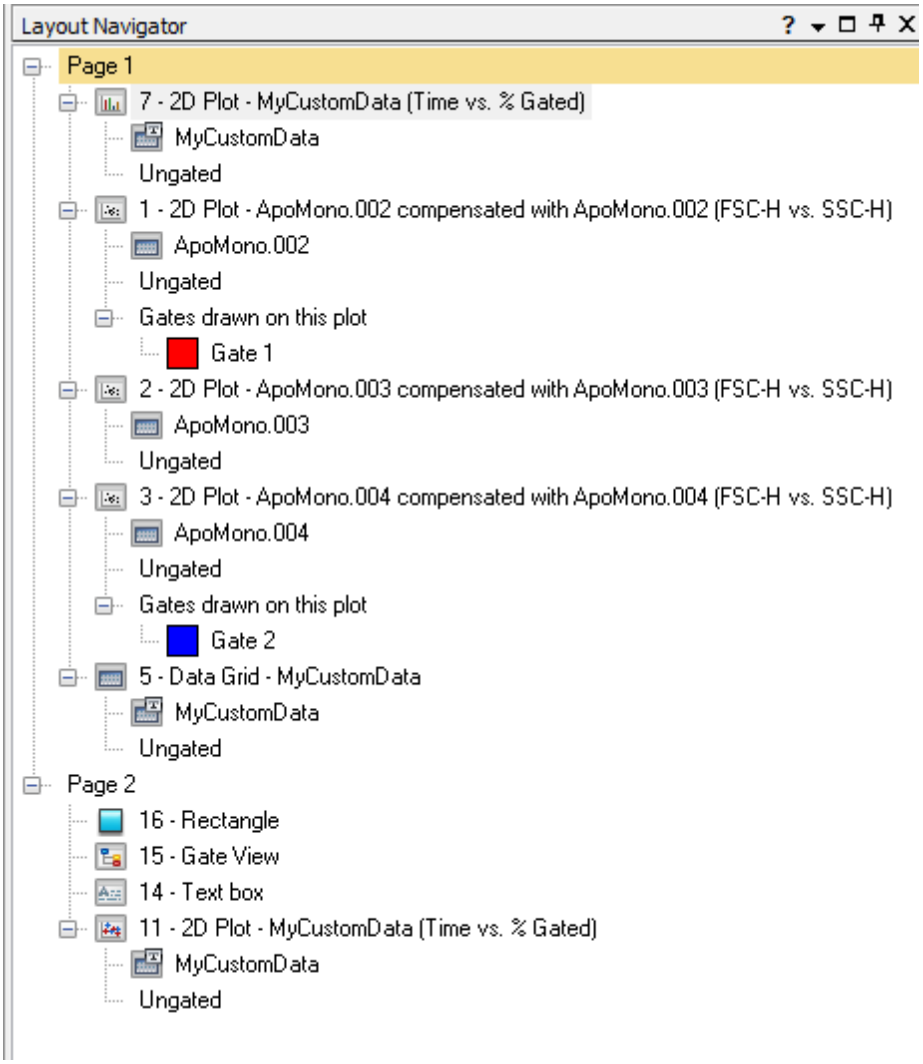

Figure 4.24 Layout Navigator

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, 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 **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

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visible layout page to the page where the corresponding object is located.

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 an object node to an empty part of the layout	Moves the object to the new location.
Drag an object node 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.
Drag a data set node 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 data set node to an empty part of the layout	Brings up the Select plot types... dialog to open plot (s) using this data set.
Drag a gate to a plot	Applies the gate to the plot.
Drag a plot to a text box	Prompts you to create a keyword or statistics token from that plot in the text box.

4.3.2 Data Navigator

The **Data Navigator** shows a hierarchical view of the data sets in your layout, and the plots and data grids associated with those data sets (Figure 4.25).

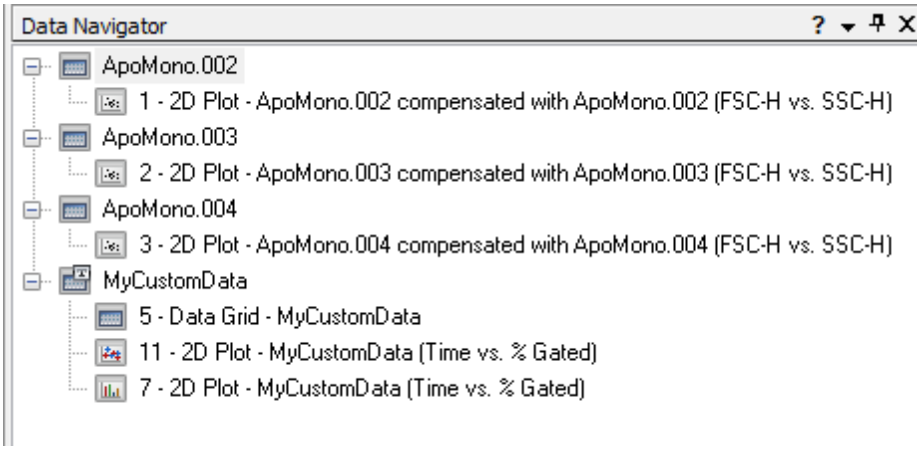

Figure 4.25 Data Navigator

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 the **Data Navigator** displays a thumbnail view of the object.

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

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operations are described in the table below.

Drag and Drop Operation	Function
Drag a data set node 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 data set node to an empty part of the layout	Brings up the Select plot types... 43 dialog to open plot (s) using this data set.
Drag an object node to an empty part of the layout	Moves the object to the new location.
Drag an object node 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.
Within the navigator, drag a plot onto another plot	Brings up a **Paste Special** dialog to insert data or overlays.

4.3.3 Gate Navigator

The **Gate Navigator** shows a hierarchical view of the gates that have been defined in your layout, and the plots on which those gates have been applied (Figure 4.26).

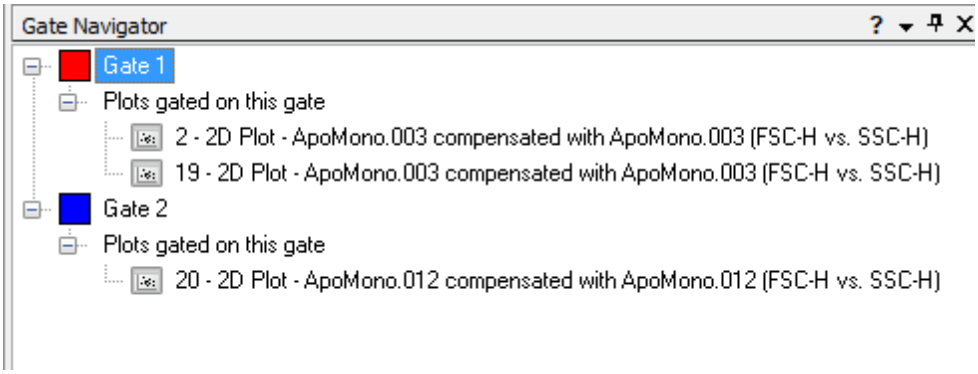

Figure 4.26 Gate Navigator

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 **Gate Navigator** displays a thumbnail view of the plot.

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 node to a plot	Applies the gate to the plot.
Drag a plot node to an empty part of the layout	Moves the plot to the new location.

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Drag and Drop Operation	Function
Drag a plot node onto an existing plot in the layout	Adds the appropriate data to the existing object. You will be prompted to choose special paste format options.

Note: The Gate Navigator is a complementary tool to the Gate View 202. The gate view allows similar functionality in terms of dragging and dropping to/from plots, but allows additional gating functionality.

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 dialog for any object, place 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 37 to insert objects on the current layout page. The objects you can insert are located in four groups:

- **General**

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

- **1D Plots**

  Histogram, Kinetics, Multicycle DNA, Proliferation, Bar

- **2D Plots**

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

- **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

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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.

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 4.27 shows one item in selected mode and one item in edit mode.

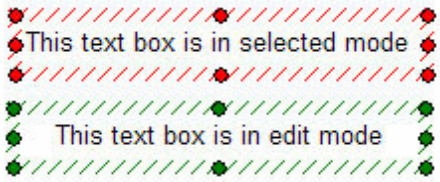

Figure 4.27 Selected and Edit Modes

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 105, Using Gates 192, and Using Markers 207.

To put a single item into selected mode, move the cursor over the border of the

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

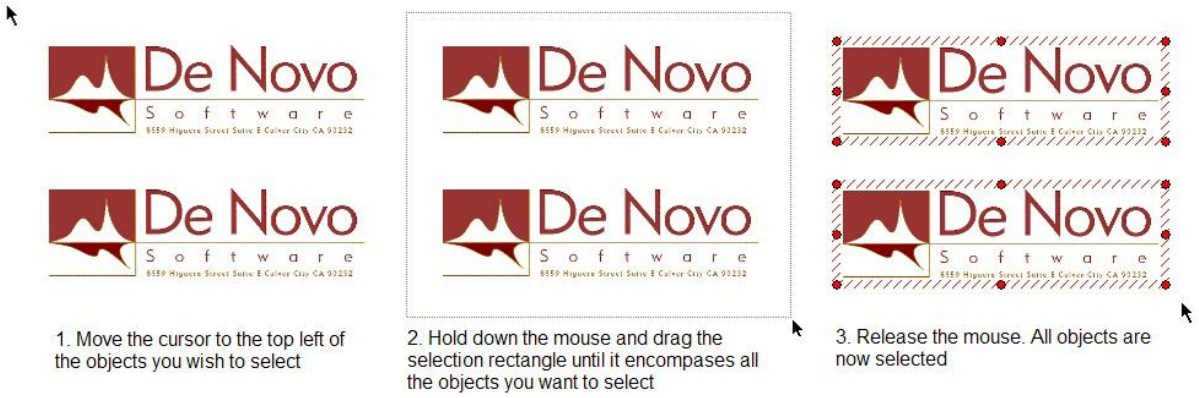

Figure 4.28 Selecting Multiple Objects with Click and Drag

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).

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4.4.3 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.

In order to create a group, select the objects you want to group as described under Selecting Items on a Page 79, then, either:

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

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

After selecting one or more objects as described under Selecting Items on a Page 79, use the **Home→Clipboard** commands 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.

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 changes to the next or previous file in your data list. The

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Multiple commands allow you to make more than one copy of the selected item (s). The positioning of the duplicated objects can be set in the user preferences 118.

Note: In FCS Express 4, objects and text can be copied into another application simply using the copy and paste functions, without loss of formatting information.

4.4.5 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 79).

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.

4.4.6 Resizing Objects

To resize selected object(s), position the cursor over the one of the resizing rectangles (black squares on the object border) 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.

4.4.7 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 select the **Home→Editing→Arrange→Align** command. This will bring up an **Alignment** dialog (Figure 4.29).

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

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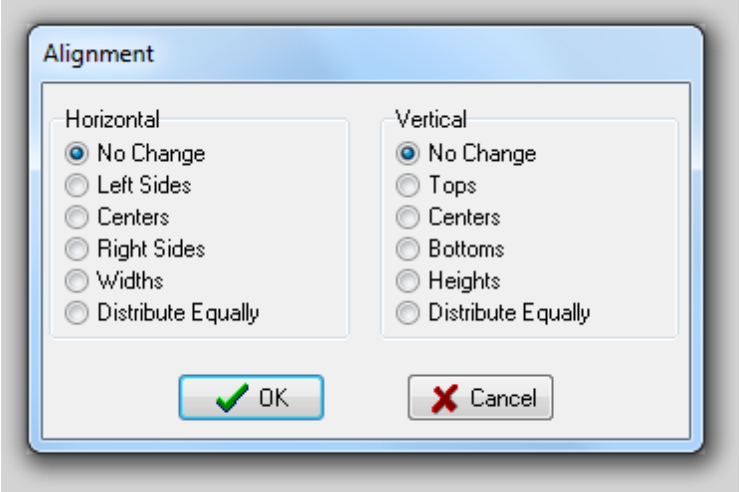

Figure 4.29 Alignment Dialog

Objects are always aligned with respect to the first item selected. For example, if you select several items, use the **Home→Editing→Arrange→Align** command, and then choose **Tops**, 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 4.30.

In addition, to make multiple objects the same size:

1. Select the object you wish to resize to.
2. **Ctrl + Click** on the objects to be resized.
3. Use the **Home→Editing→Arrange→Align** command and choose both **Widths** and **Heights**. All objects will then be resized to the first object you selected.

Figure 4.30 Horizontal Center Alignment

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4.4.8 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 Backwards**

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

Figure 4.31 demonstrates the results when you rearrange the order of the red circle and the green rectangle.

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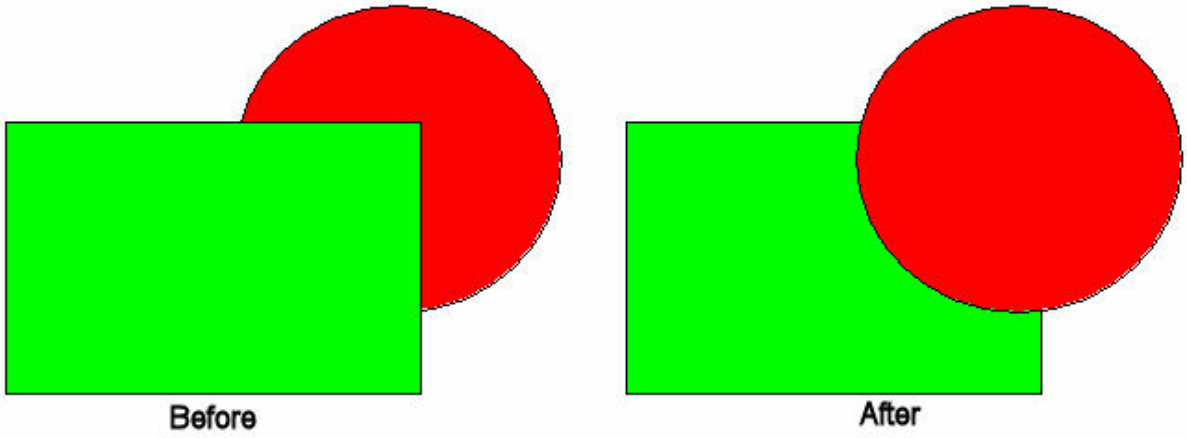

Figure 4.31 Ordering Objects

4.4.9 Undoing Changes

FCS Express allows an unlimited number of operations to be undone.

To undo any changes made to a layout, select (Undo) from the Quick Access Toolbar 34. 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.

4.4.10 Page Layout Using Predefined Placeholders

On the gallery associated with the **Home→Pages→Layout** command (also present under **Home→Pages→New Page**) you can select and apply predefined layouts to the current page (Figure 4.32).

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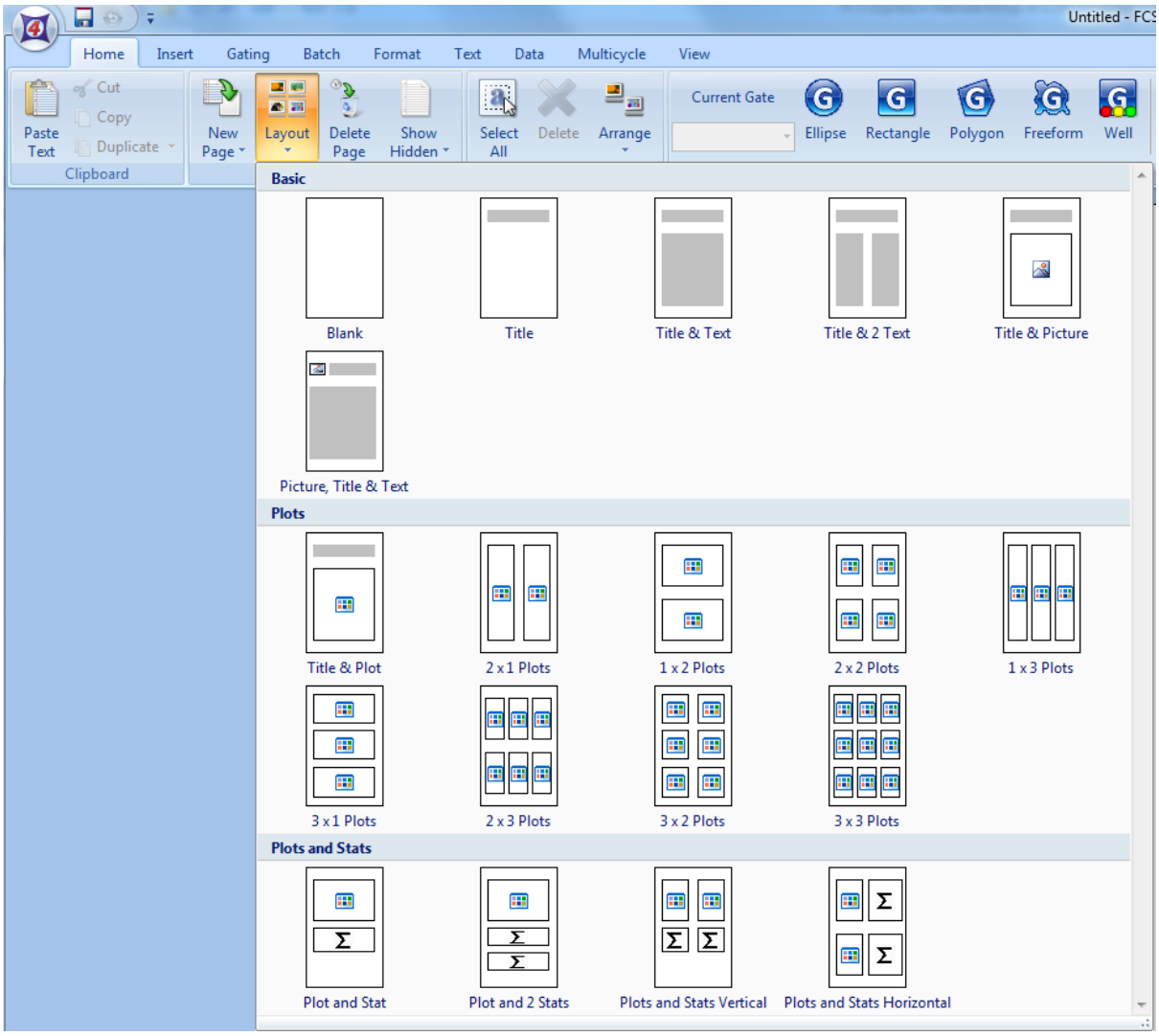

Figure 4.32 Layouts Using Predefined Placeholders

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 4.33.

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Figure 4.33 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 on an individual page basis 58, to not have the master page appear as the background of that page.

To view the Master Page, select the **Home→Pages→Show Hidden Show→Master Page** command.

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 87.

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 87, 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 list box on the left (Figure

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4.34).

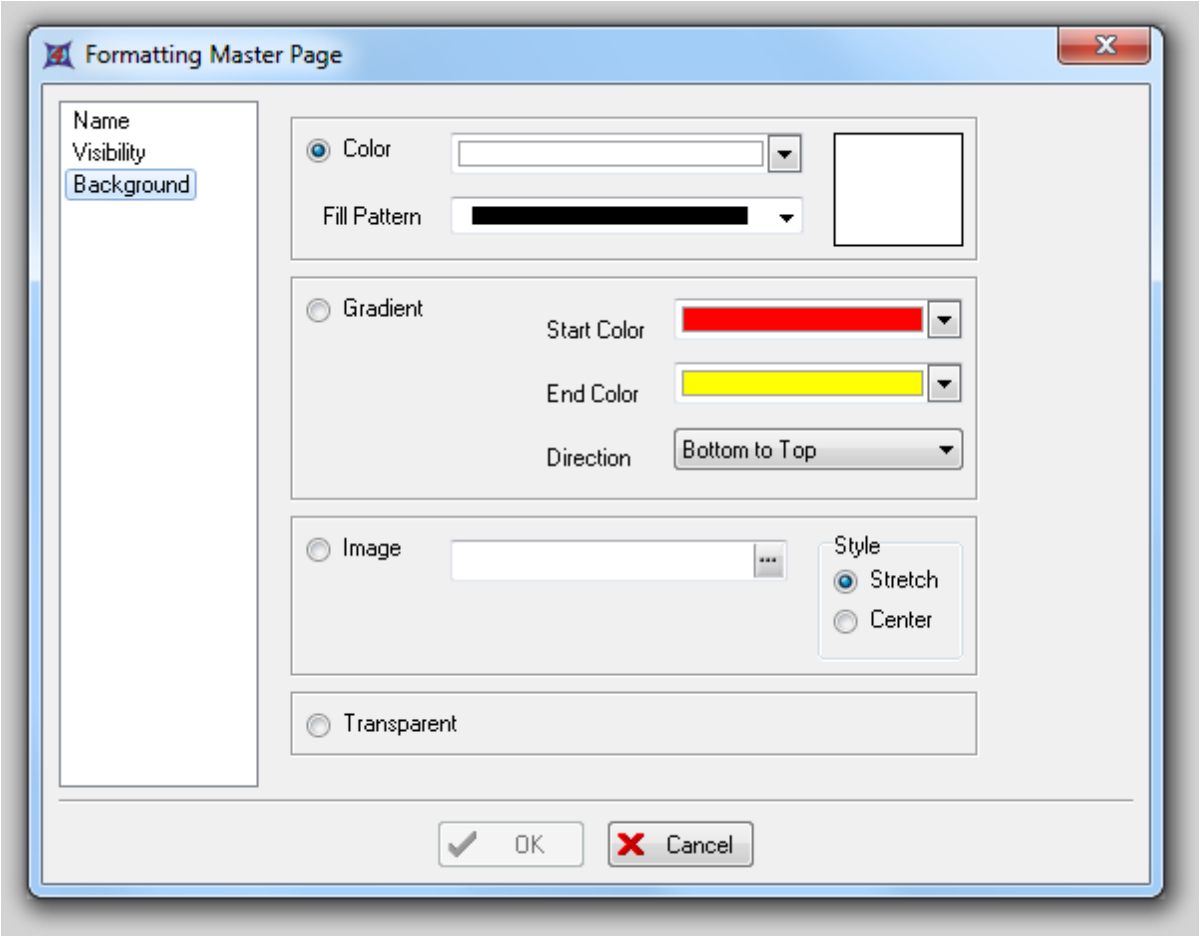

Figure 4.34 Formatting Master Page Background Screen

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 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 trasparent 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 Version 4 contains many shortcut keystroke combinations to make navigating and working within the software easier. The table below summarizes

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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+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
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	Page Setup	Any layout
Ctrl+W	Close current layout	Any layout
F1	Show help	Any layout
Up	Scroll up	Layout or selected object

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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	Layout users
Ctrl+F	Edit format	Objects, Text Boxes, Plots, Data Grids, and Shapes
1-9, a-z	Change x parameter for selected plot	Plots
Shift+1-9, Shift+a-z	Change y parameter for selected plot	Plots
Ctrl+M	Maximize/Restore	Plots

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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.

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 **Insert** button (to the right of the file name), and choose either **Insert** or **Link to File**. In both cases, the picture will be placed on the current page.

If you choose **Insert**, the picture will be saved as part of the layout file. If you choose **Link to File**, 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.

The following graphics file types are supported: Windows bitmaps (*.bmp), JPEG files (*.jpg), Windows Metafiles (*.wmf), Windows Extended Metafiles (*.emf), Portable Network Graphics (*.png), GIF (*.gif), and TIFF (*.tif).

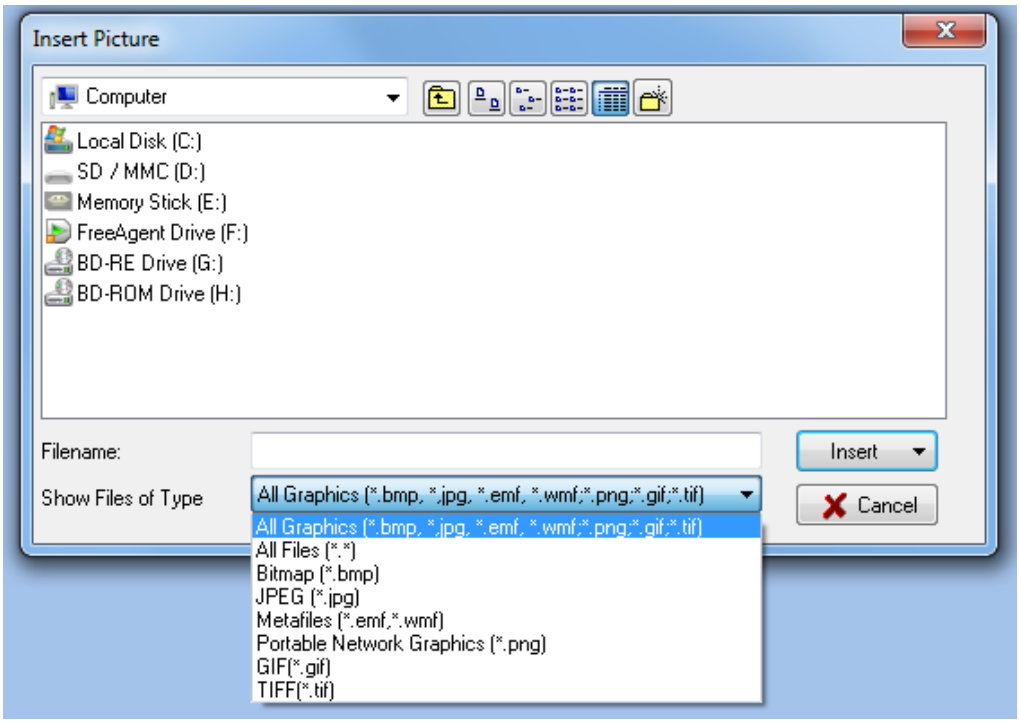

Figure 5.1 Insert Picture Dialog

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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 will allow you to edit the border and size 150 of the image.

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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 more.

6.1 Inserting Text Boxes onto a Page

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

6.2 Entering and Formatting Text in a Text Box

In order to enter text in a Text Box, place it in edit mode 79 by clicking anywhere inside it.

The Text Box can be used just like a typical word processor. This means that 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.

The most common formatting options are available on the Text Tab 42. 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.

This **Font** dialog (Figure 6.1) allows you to change all font properties. With the Layout Tab options, you can change the character spacing and subscript/ superscript offset.

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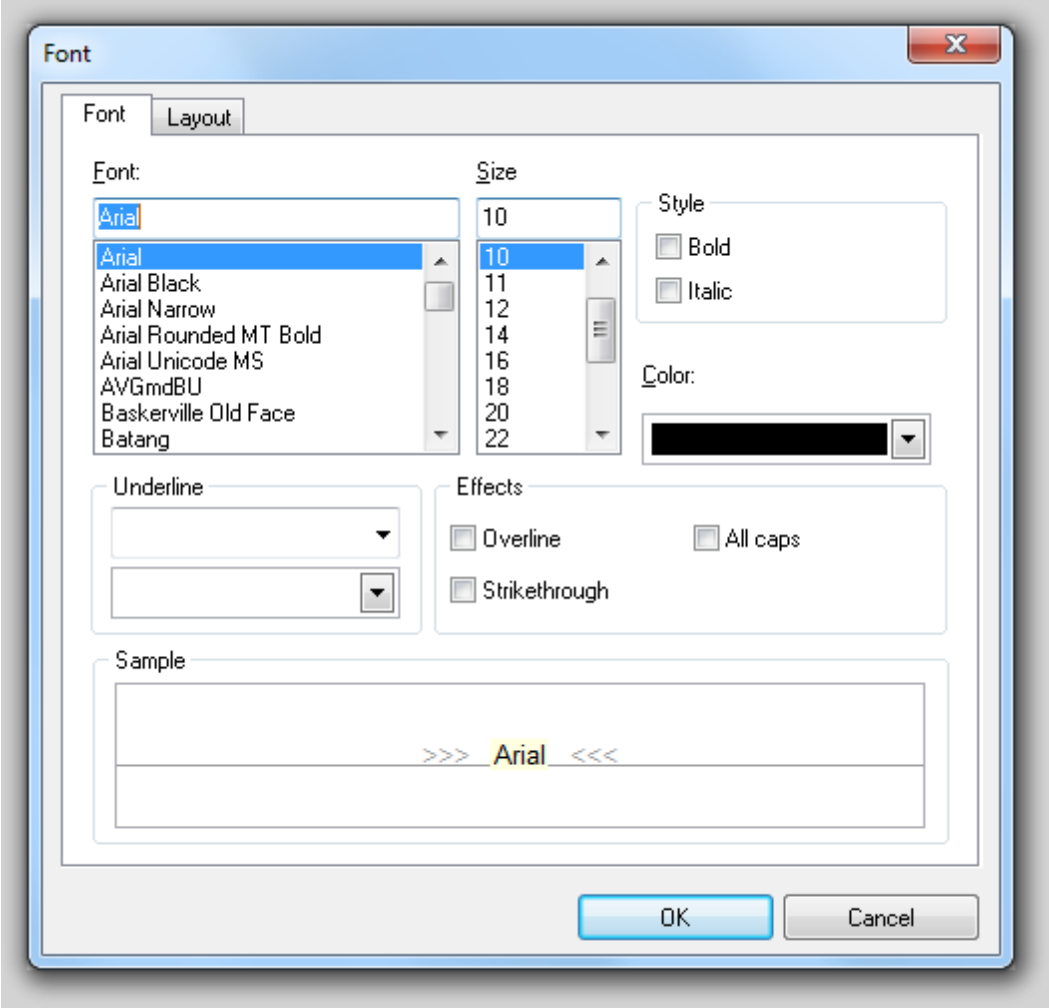

Figure 6.1 Font Dialog

To control the detailed aspects of paragraph formatting, use the **Paragraph** dialog box launcher. The **Paragraph** dialog (Figure 6.2) allows you to change paragraph indentation and spacing settings, including text alignment, line spacing, and first-line indentation.

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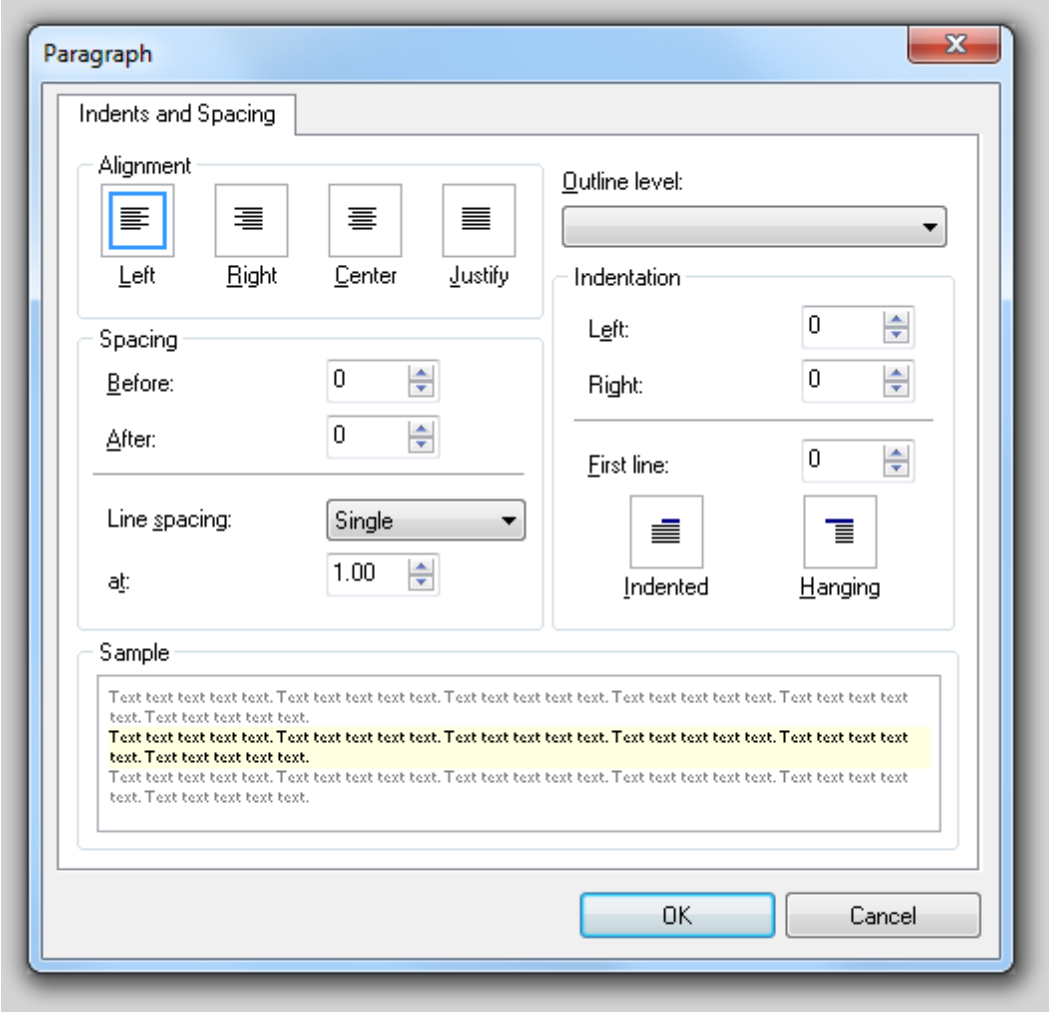

Figure 6.2 Paragraph Dialog

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 6.3).

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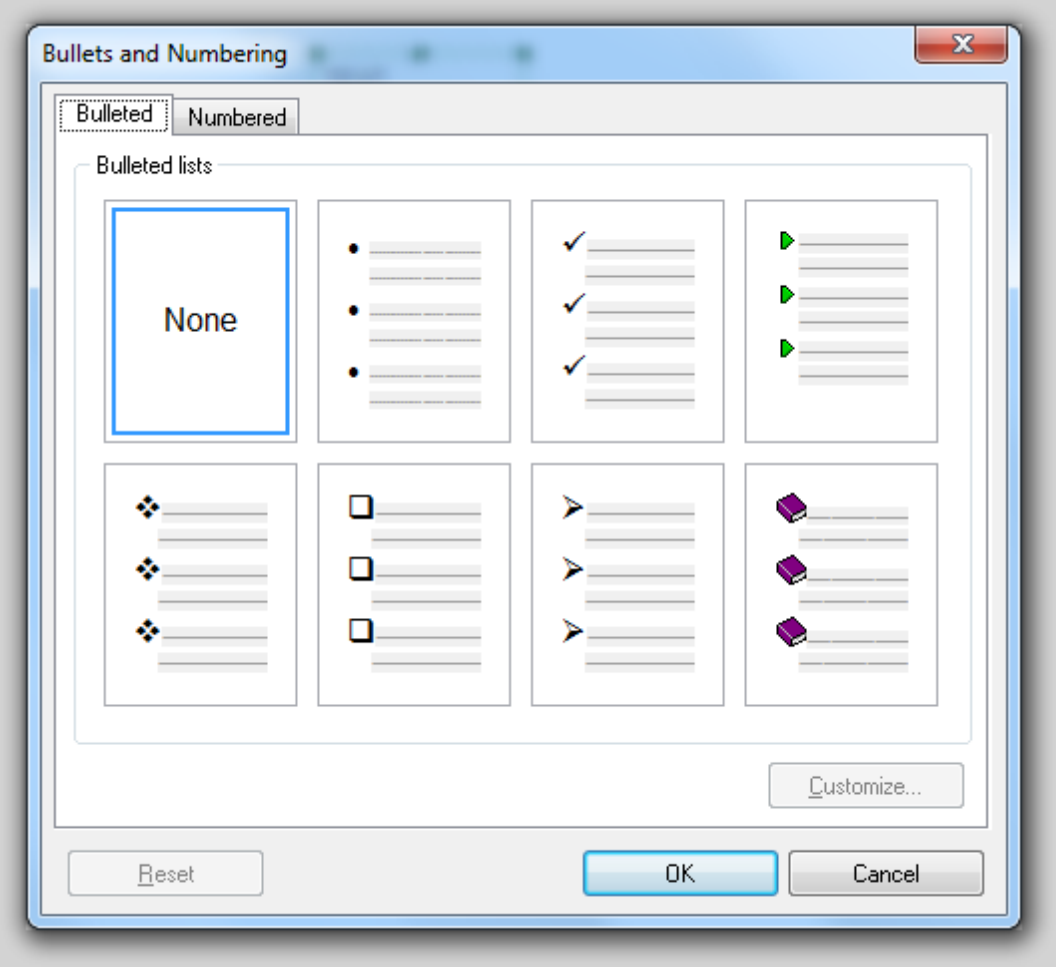

Figure 6.3 Bullets and Numbering Dialog

6.2.1 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→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 6.4) 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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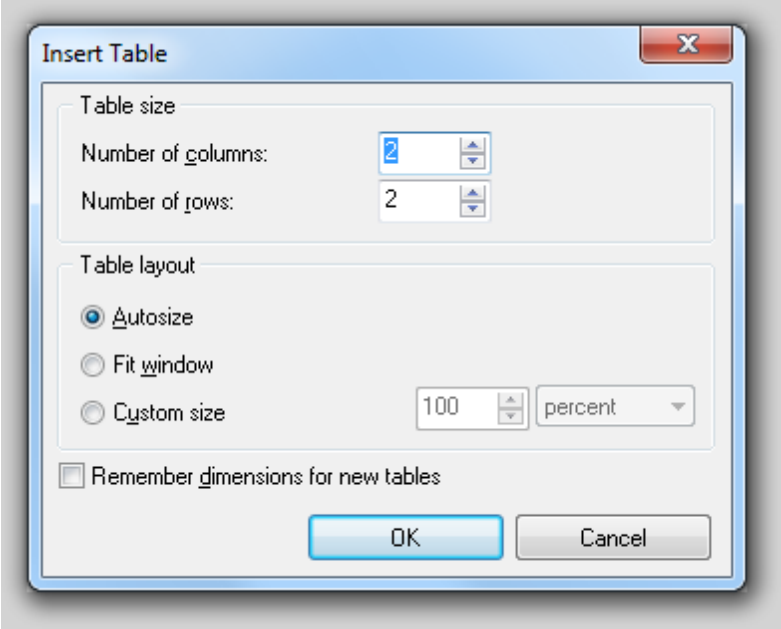

Figure 6.4 Insert Table Dialog

Tables can also be pasted from Microsoft Word. Simply copy the table to the clipboard in Word, then select the **Home→Clipboard→Paste Metafile** command (or use the **Ctrl+V** keyboard shortcut).

6.2.2 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.

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.

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Command	Explanation
Delete Columns	Delete the current column or the selected columns.
Table Properties 101	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.

6.2.2.1 Table Properties

The Table **Properties** window is divided into 3 tabs:

- **Table** Tab.

  These settings apply to the whole table (Figure 6.5):

  - **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**

    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**

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Set the color and width of the individual cell borders.

- **Rows** Tab

  These options apply to the vertical alignment of the selected row(s).

- **Cells** Tab

  These settings apply to the selected cell(s) (Figure 6.6).

  - **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: T*o align a full Row, use the Table **Properties** - **Rows** Tab.)

  - **Border**

    Set the color of the current or selected cell borders.

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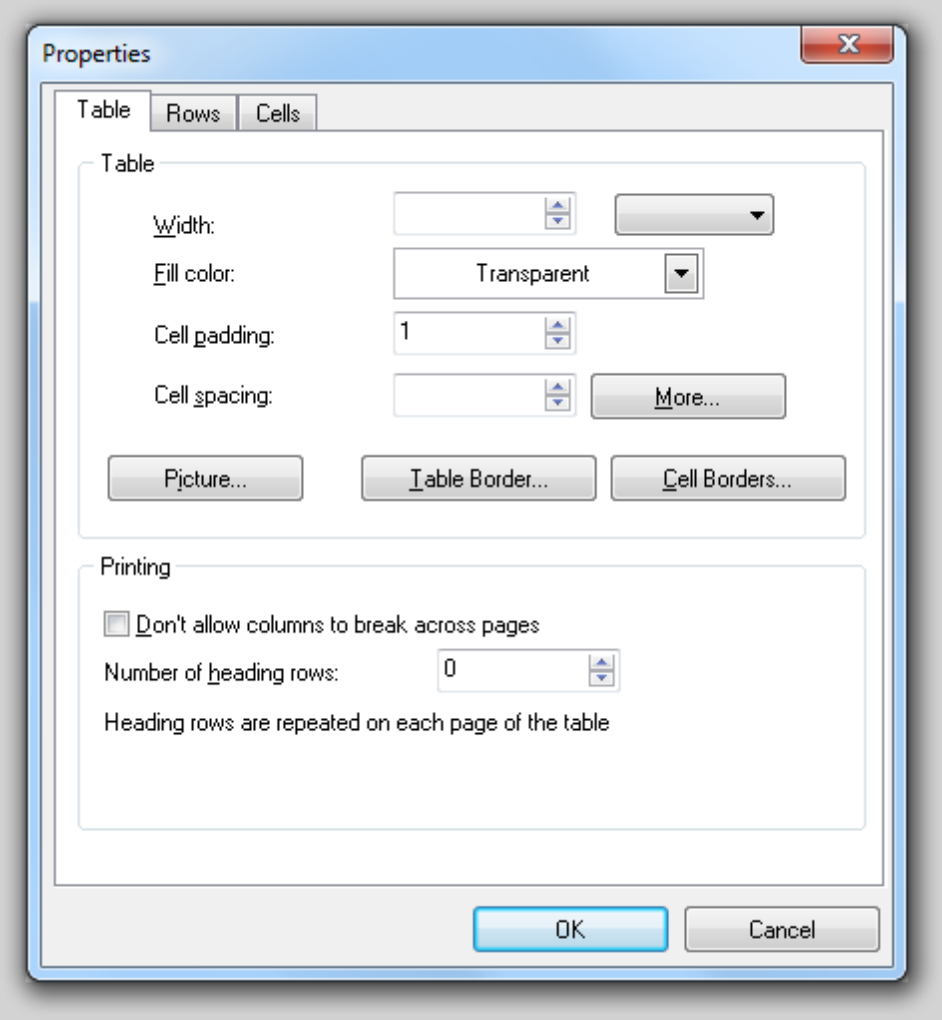

Figure 6.5 Table Properties - Table Tab

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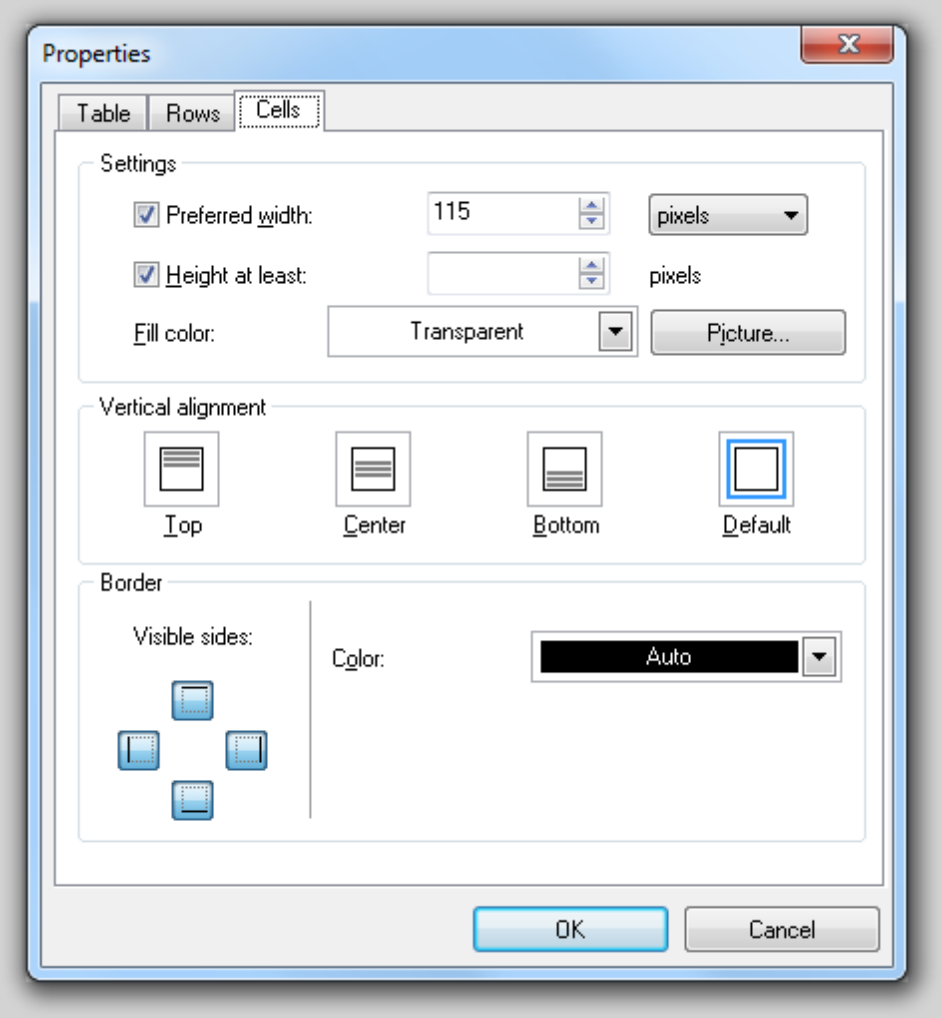

Figure 6.6 Table Properties - Cells Tab

6.3 Resizing Text Boxes

You can change the text box size as described in Formatting Text Box Features 106. Alternately, you can resize text boxes the same way all items are resized in FCS Express 82, 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

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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.

6.4 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 6.7).

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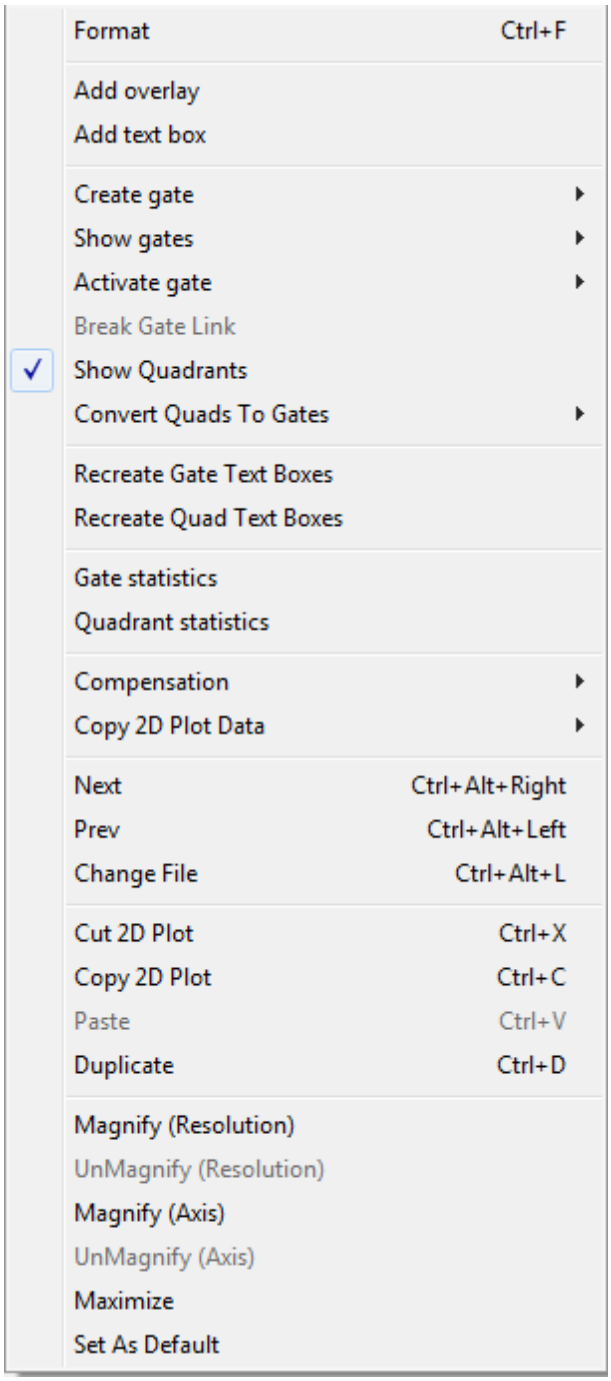

Figure 6.7 Right-click Pop-up Menu wth Recreate Gate and Quad Text Boxes Commands

6.5 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 border150, size150, and background140 of the text box. The default background for a text box is transparent.

6.6 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 Tokens326.

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Chapter

7 Displaying Plots

FCS Express supports many different ways of displaying your data. You can view the raw data in a Data Window 320, or display the data in many different types of plots described in this chapter.

7.1 Data Display Plot Types

FCS Express has 12 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 474 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 445 are a subset of one-dimensional plots that fit DNA cell cycle statistics.
    - Proliferation histograms 463 are a special subset of one dimensional plots that automatically model proliferation statistics.
    - Bar charts 241 can be used to graphically display custom data 234.
- **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.
    - **Density** plots. In a density plot, the color of an area reflect how many events are in that position of the plot.
    - **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.

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- **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.

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

- Picture Plots 490 are used to display the location of cell (or other) images in two dimensions across an entire area.

- Scatter plots 241 can be used to graphically display custom data (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 482 are displays of a microtiter plate which show statistical values (using colors) across all the wells of the plate.

7.2 Inserting Plots

To insert plots on a layout page in the default size, 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 112.

To change the default size for a plot, see Plot Statistic Preferences 126.

To change the file in a plot see Changing Data Files 113.

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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.

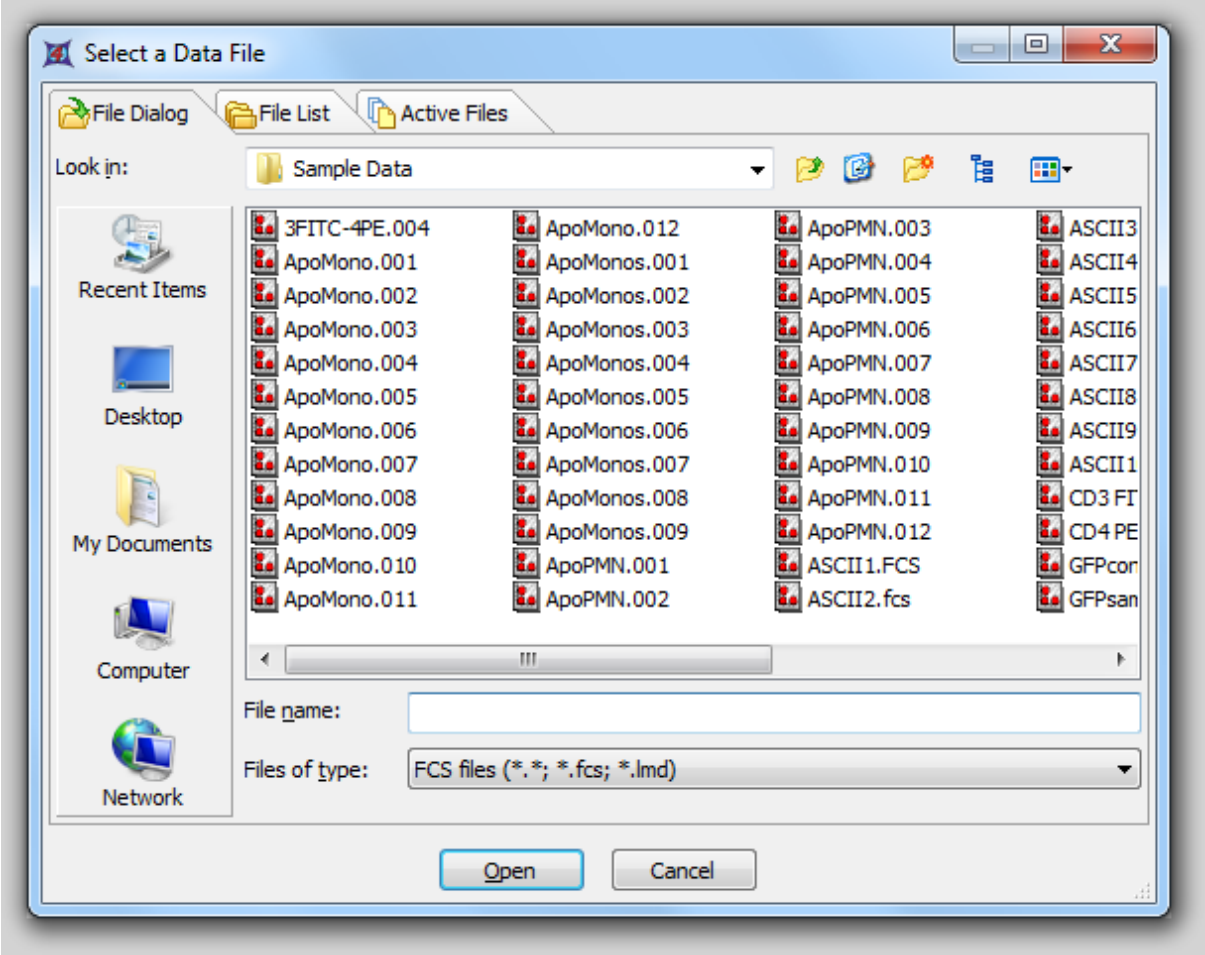

Figure 7.1 Select a Data File Dialog

You can also configure FCS Express to prompt you for a data file every time you open a plot. To do this, go to the Application Button[31] and click on the **Edit Preferences** button on the lower right. In the **Edit Preferences** dialog, choose **General** from the items on the left and click on the **Always show a file selection dialog when opening new plots** checkbox. With this configuration, the **Select a Data File** dialog will open every time you create or select a plot.

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If a data file has been changed during your analysis (i.e., you have performed parameter math to create or modify a parameter), then before that data file is removed from memory, you will be prompted whether or not to save the file. If you do not want this prompt to appear, open the **Edit Preferences** dialog (using the **Application Button→Edit Preferences** button), select the **Save** category, and choose the **Don't save modified data files on close** (see Save Preferences 137) 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:

- Dragging and dropping one plot from the layout (or a plot node from a Navigator 74) onto another plot in the layout. This brings up a Paste Special dialog (Figure 7.2).

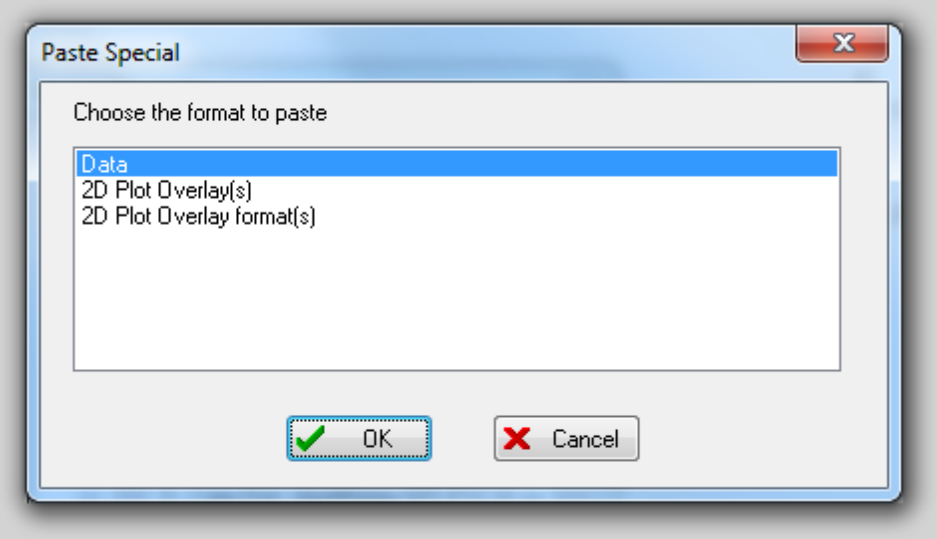

Figure 7.2 Paste Special Dialog

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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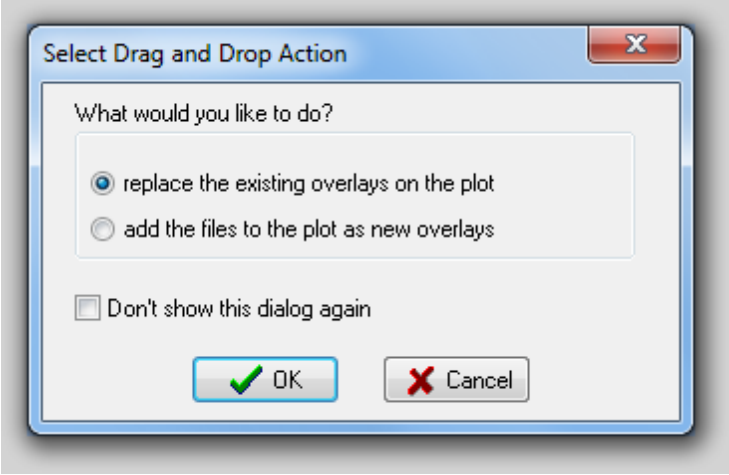

Figure 7.3 Select Drag and Drop Action Dialog

- 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 Selection** (or **Change Data on All Objects**) groups to change the file on the selected object (or on all objects).

In the 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 is extremely flexible and offers you many choices 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 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 40.

All formatting windows work in a similar manner. An example is shown in Figure 8.1. The area on the left, denoted by the 1, 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 2.

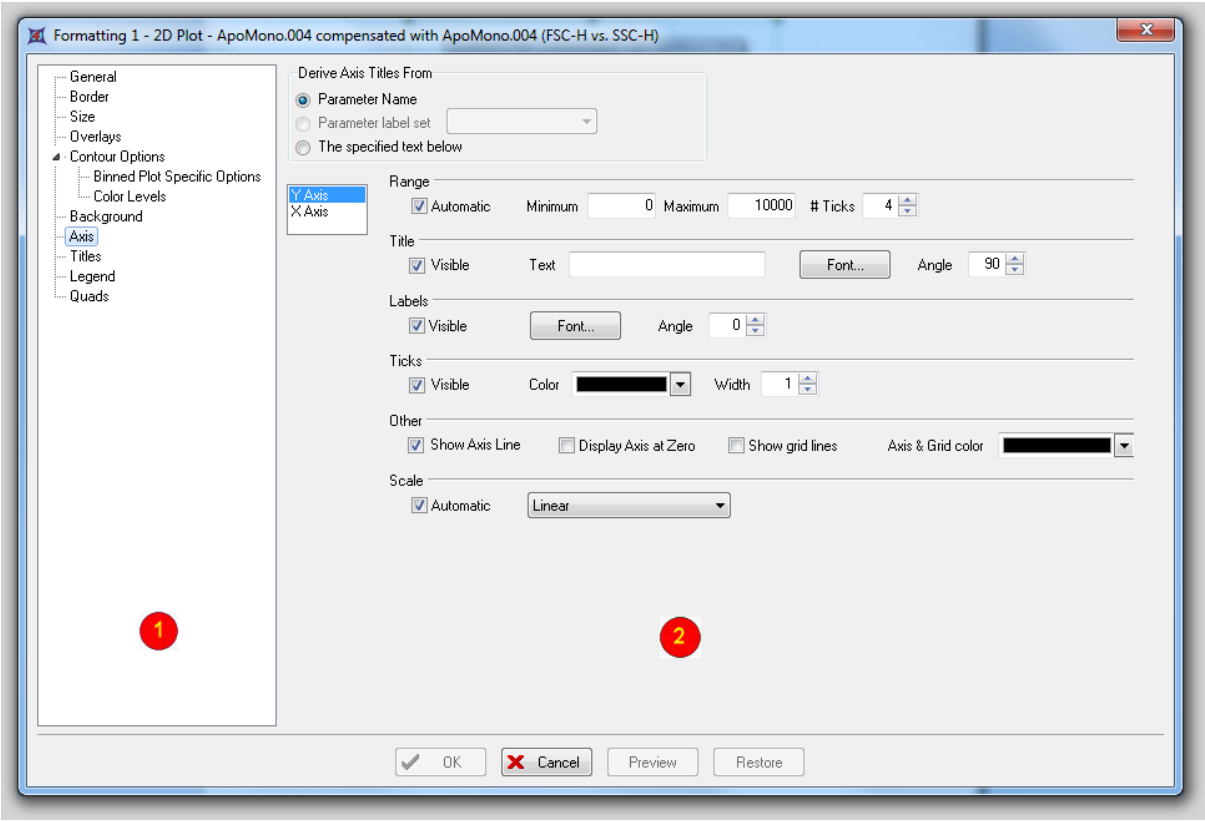

Figure 8.1 Formatting Dialog

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8.2 Customizing Default Preferences

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 Preferences. These preferences range from default sizes and formatting options for plots to the way FCS Express copies data to the clipboard.

All Preferences can be customized and saved under a unique user name. Any changes to the preferences 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 **Application Button→Edit Preferences Button**. The **Edit Preferences** dialog (Figure 8.2) contains many categories 1, each of which groups several types of preferences together 2. The specific categories are discussed in other sections of this chapter.

A tutorial 1513 exists with step-by-step instructions for changing commonly edited **Preferences**.

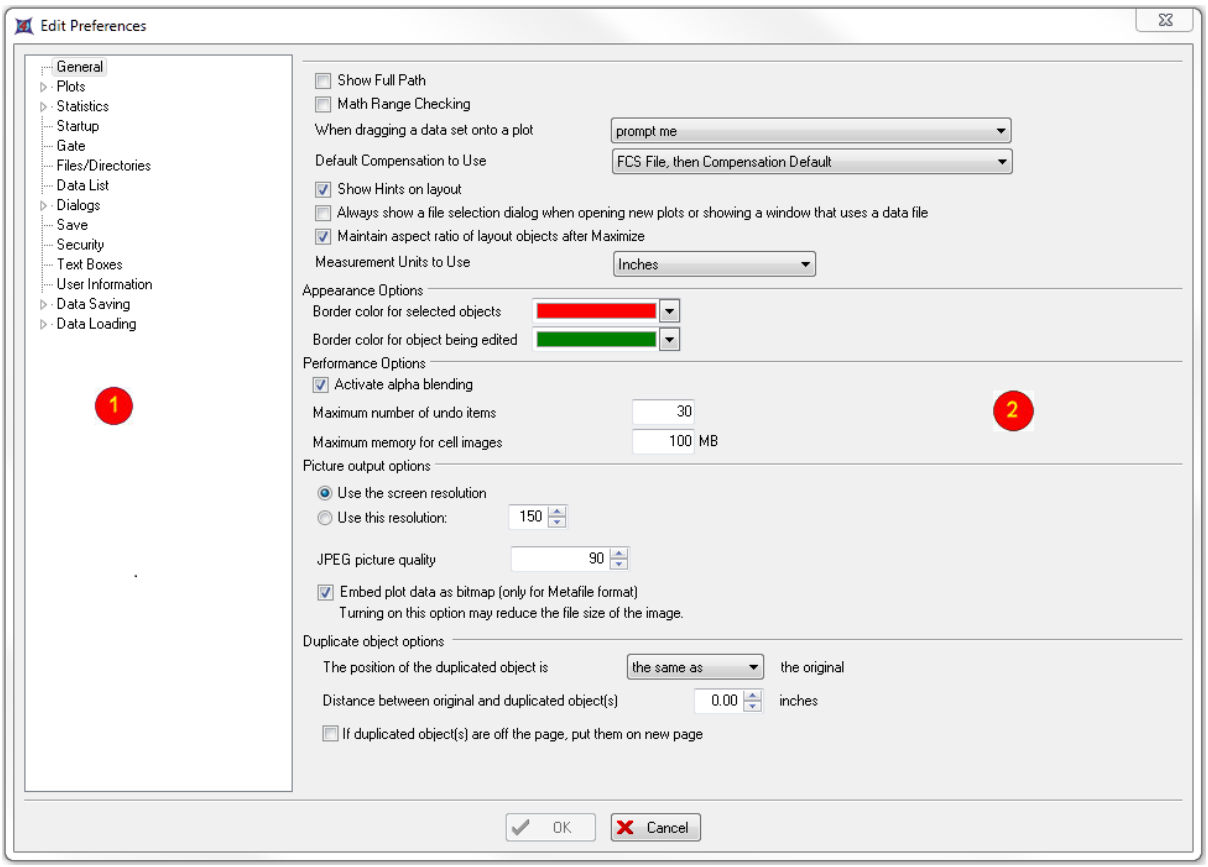

Figure 8.2 Edit Preferences Dialog Overview

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8.2.1 General Preferences

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

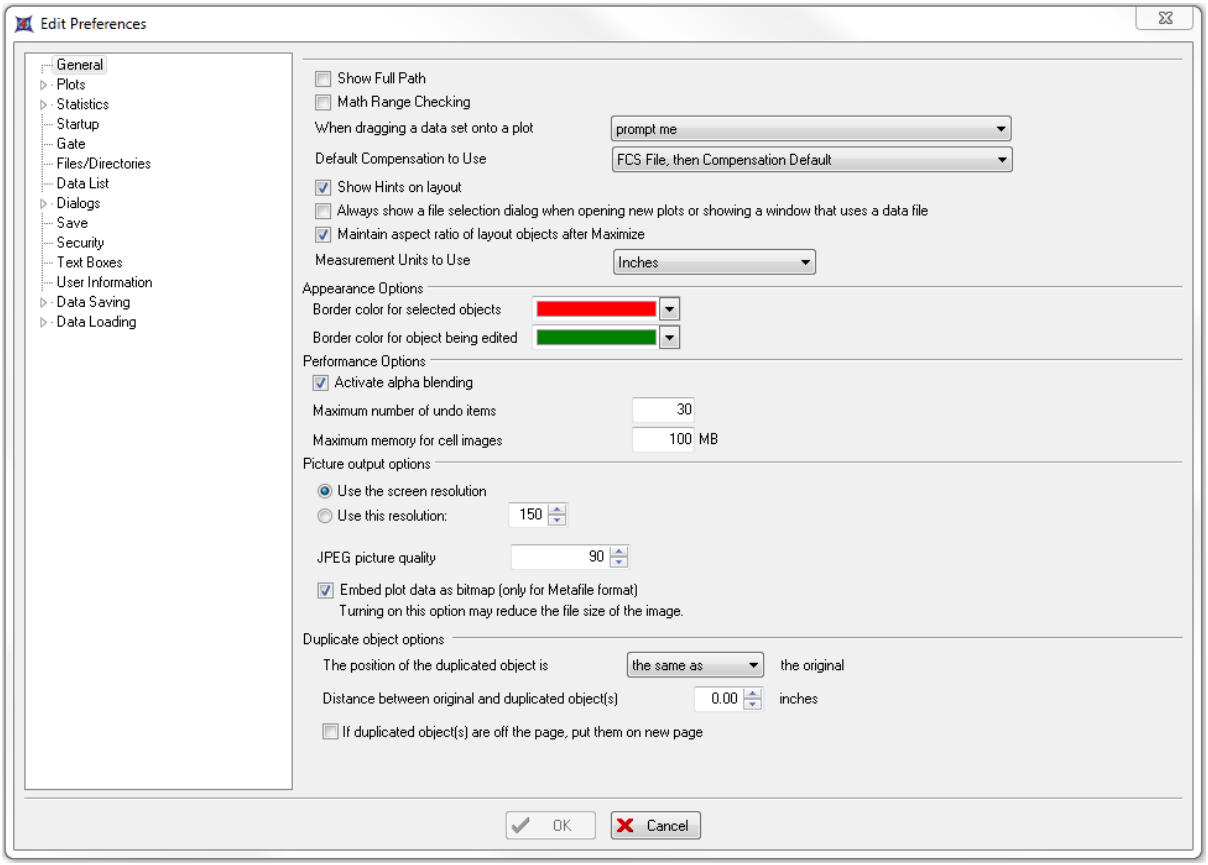

Figure 8.3 General Preferences

The specific preferences are described in the table below.

Preference Name	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	When dragging a dataset onto a plot, one of three possible actions will occur: **replace existing overlays** with the file being dragged

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Preference Name	Explanation
plot	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 these options: • **None**. No compensation will be used. • **Compensation Default**. The Compensation Default is selected in the Compensation Info window 289. • **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.
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.
Always show a file selection dialog when opening new plots	Configures FCS Express to prompt you for an FCS file every time you open a 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.
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.
Performance Options	These options allow you to define preferences that may increase the performance speed of FCS Express.
Activate Alpha Blending	Alpha Blending describes two features in FCS Express: • When selecting multiple plots a semi-transparent blue background is shown to highlight which objects are being selected. • When plots are being moved or resized, a semitransparent image shows how the plots will look at their new position/size. Turning off alpha blending may speed up drawing on slower computers.
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	Uses the resolution of the picture on the screen for an exported or

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Preference Name	Explanation
resolution	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).
Embed plot data as bitmap (only for Metafile format)	When pictures of plots are being saved in Metafile format (EMF), including being saved to PowerPoint or PDF, this option will cause the plots to be saved as a bitmap inside the metafile. Note that turning on this option will reduce the size of the image for certain kinds of plots, such as dot plots with many events.
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 Preferences

The Plot preferences category lets the user select 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 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 preferences for all plots opened in the future.

In most cases, the general preference settings mirror the specific plot preference options, as described in Formatting Features Shared by All Plots 152. The option for Fill overlay area is discussed in Overlays 167 (Formatting Single Parameter Histograms). Other discrepancies are described below.

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 preferences 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 hyperlog scale.

There is a full set of axis options for each type of data file (Figure 8.4). Therefore, when you open a new plot on your layout, the default settings for

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each axis will depend on what type of data file it is.

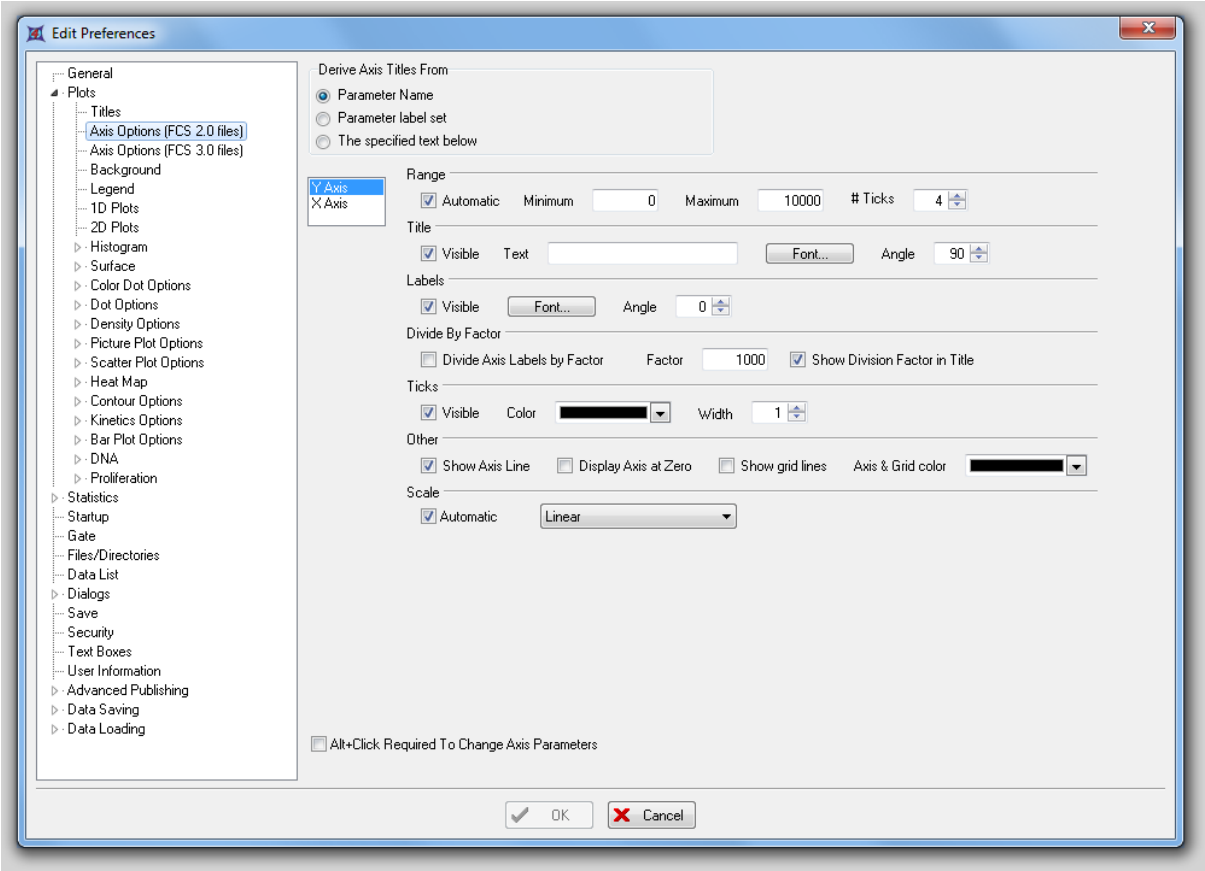

Figure 8.4 Edit Preferences - Axis Options

By default, clicking on the axis label of a plot will allow you to change the parameter for that axis[188]. While this is convenient for users who are designing layouts, it can make it easy to accidentally change the parameters, as well. You can force the **Alt** key to be required to change parameters by checking the **Alt+Click Required To Change Axis Parameters** checkbox.

8.2.2.2 1D Plot Preferences

The 1D Plot Preferences options are shown in Figure 8.5.

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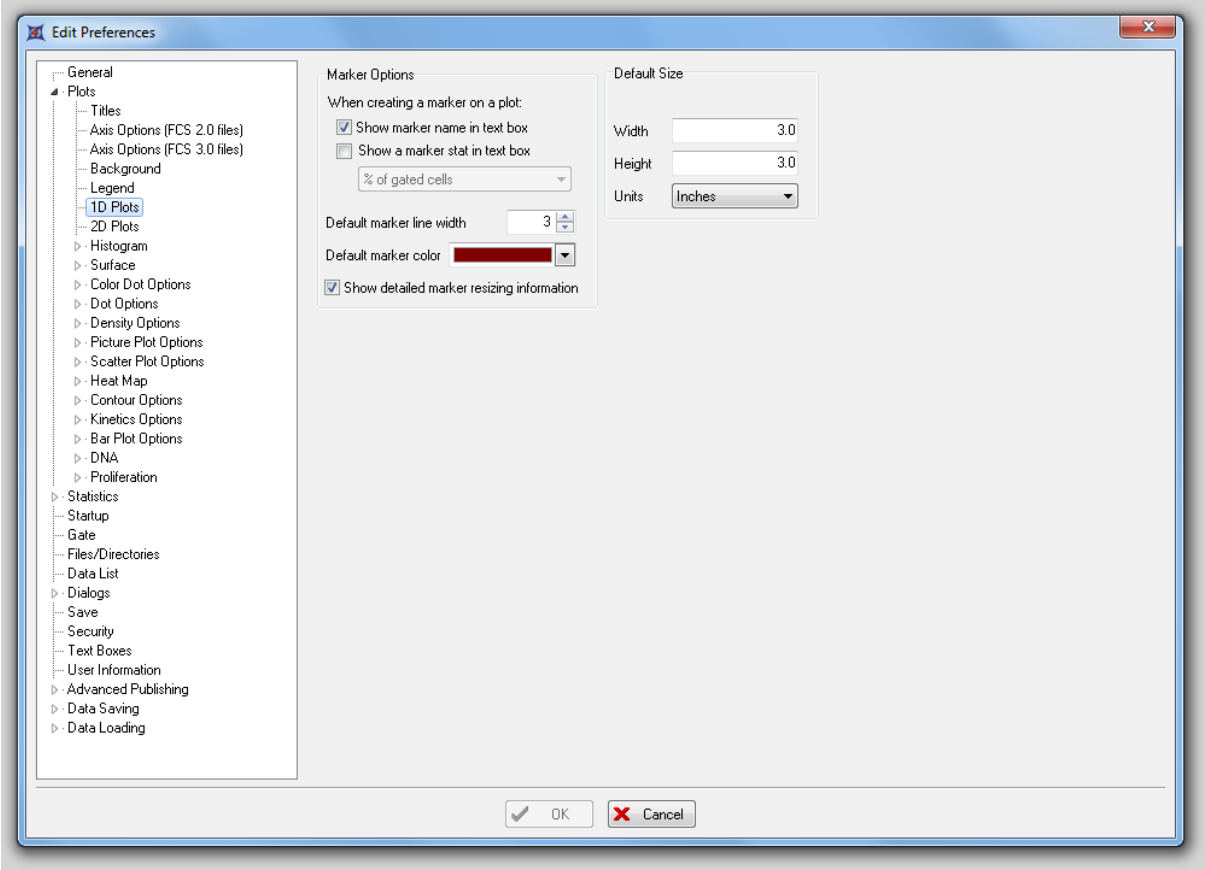

Figure 8.5 1D Plot Preferences

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

Preference Name	Explanation
Marker Options	The default options when markers are inserted on a 1D Plot.
Default Size	The default size of 1D Plots.

8.2.2.3 2D Plot Preferences

The 2D Plot preferences category (Figure 8.6) contains items that are specific for 2D plots.

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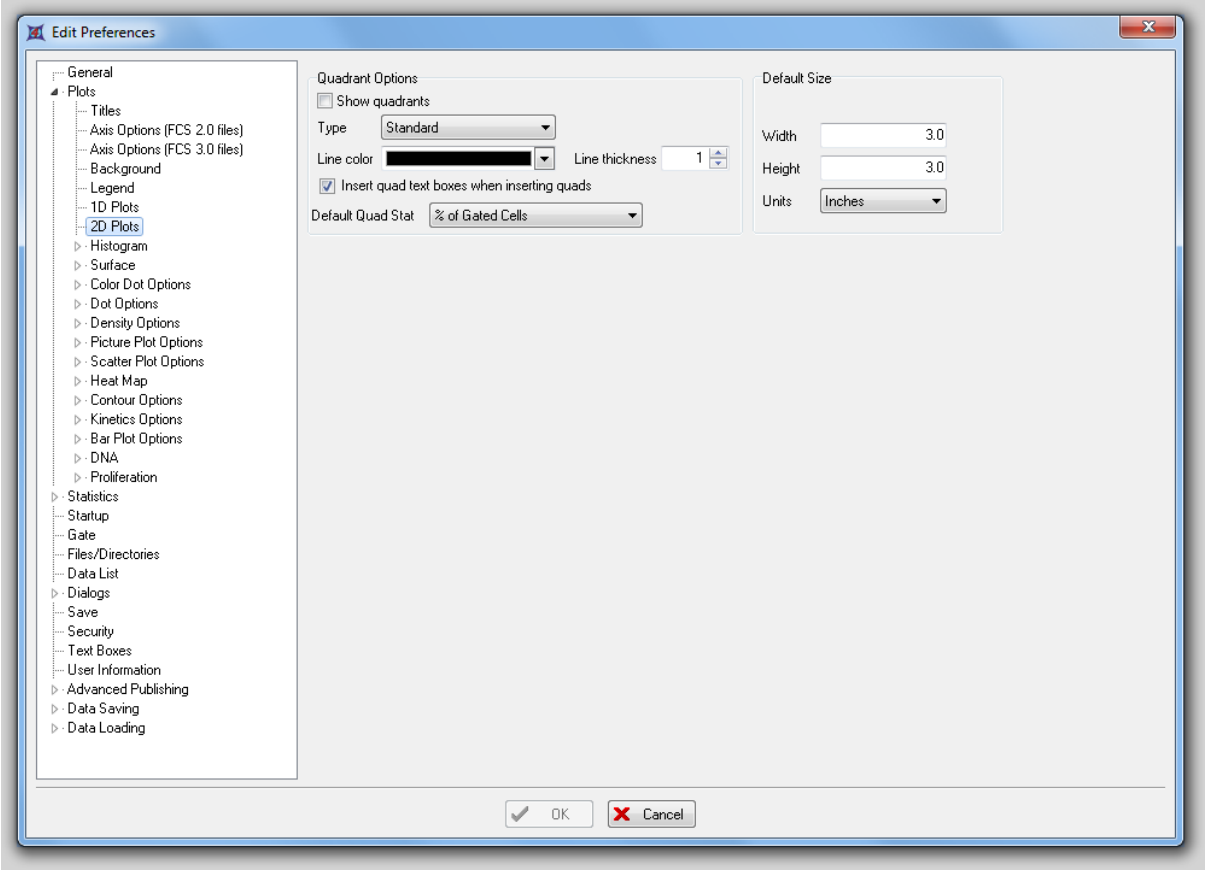

Figure 8.6 2D Plot Preferences

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

Preference Name	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.
Default Size	The default size of 2D Plots.

8.2.2.4 DNA Preferences

The DNA preferences refer to general preferences, overlay preferences, and position marker preferences for performing DNA analysis using Multicycle 444.

- **General**

  The General category of DNA preferences (Figure 8.7) are miscellaneous properties dealing with the Multicycle fit model and the parameter to plot on DNA histograms.

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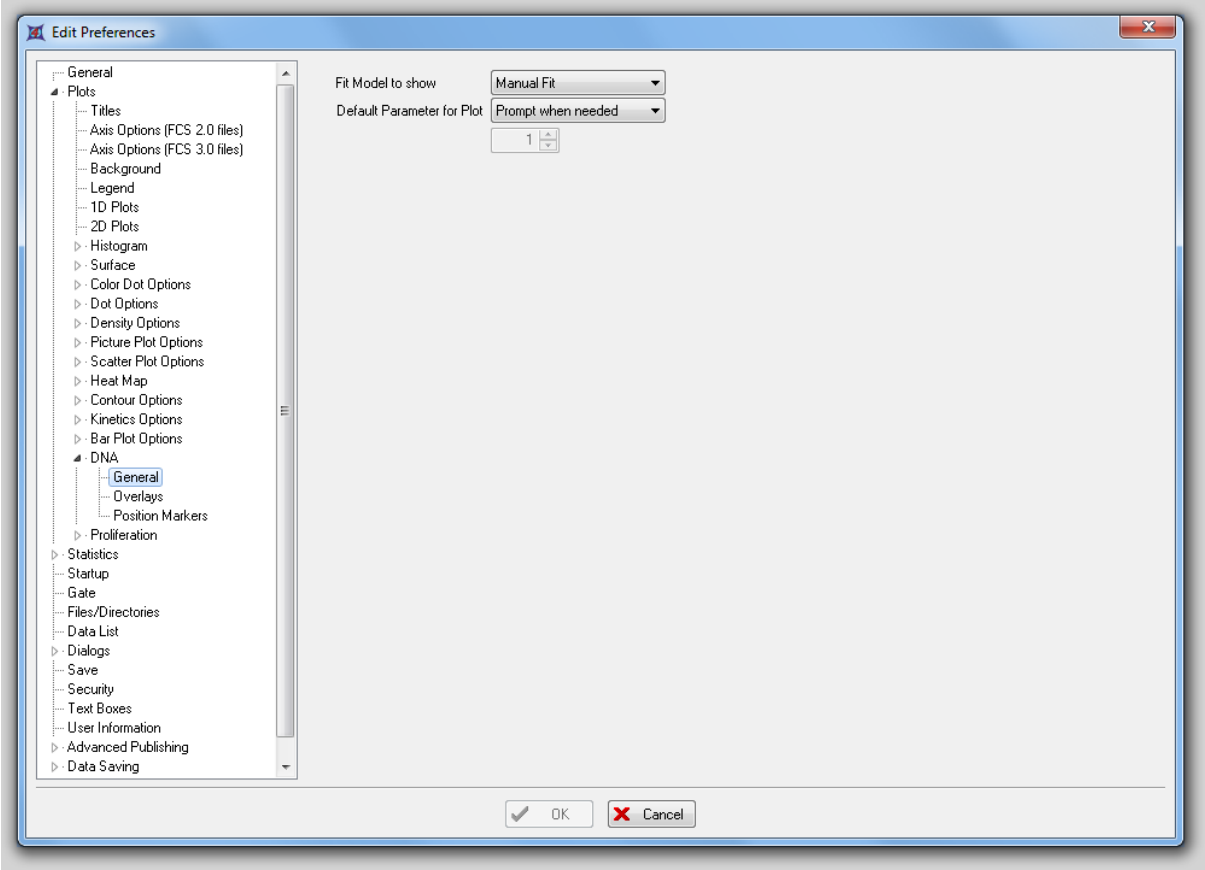

Figure 8.7 DNA General Preferences

The properties are described in the table below.

Preference	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.
Default Parameter for Plot	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 8.8) allows the user to set up how the calculated overlays look on the DNA histogram.

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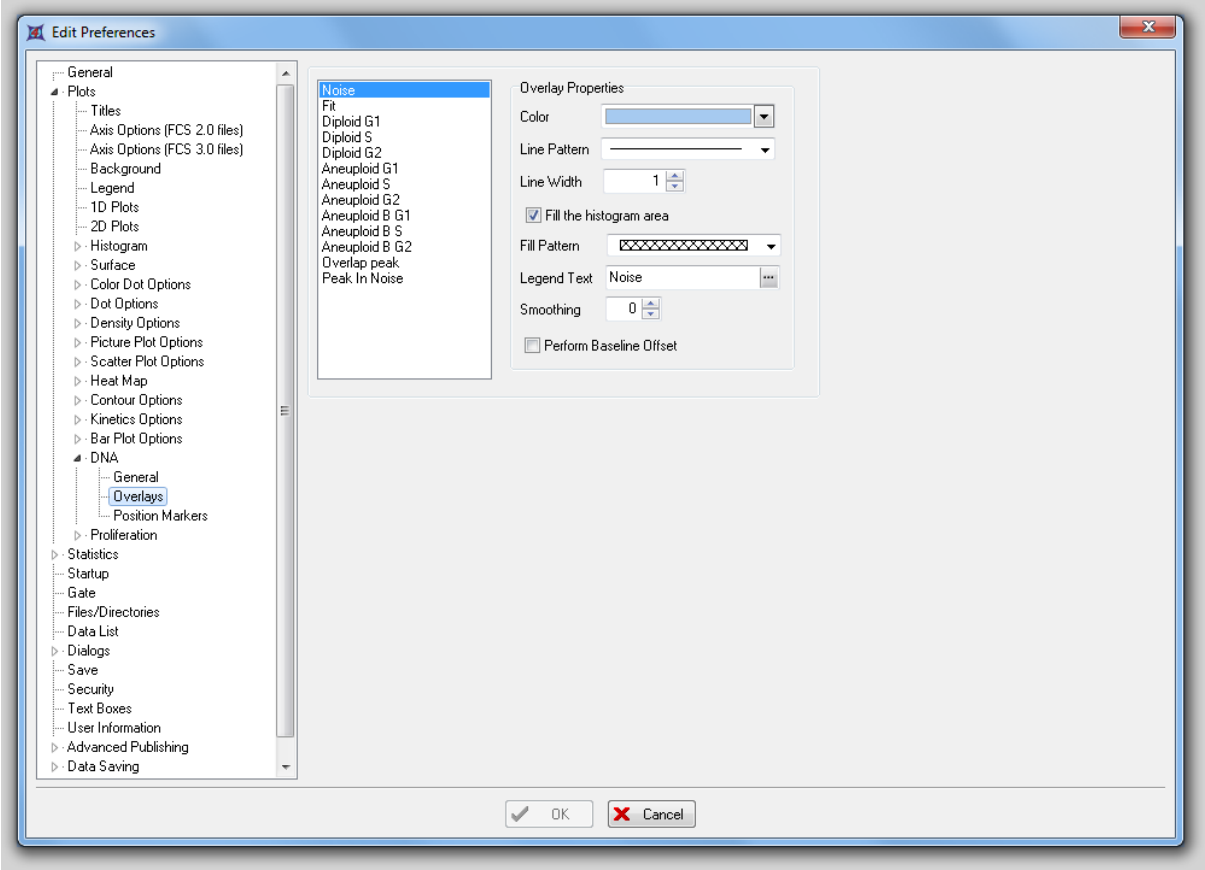

Figure 8.8 DNA Overlays Preferences

Select one or more overlays from the list box on the left of the Overlays page. The visual properties that can be changed are similar to those for regular histogram overlays[167].

- **Position Markers**

  Position markers[452] can be displayed on DNA histograms to mark the position of cell cycle parameters such as the G1 mean or the background region. The Position Markers category for DNA Preferences allows the user to set the default properties for these markers.

  Select one or more categories from the list box on the left (Figure 8.9) and choose line options for the markers on the right.

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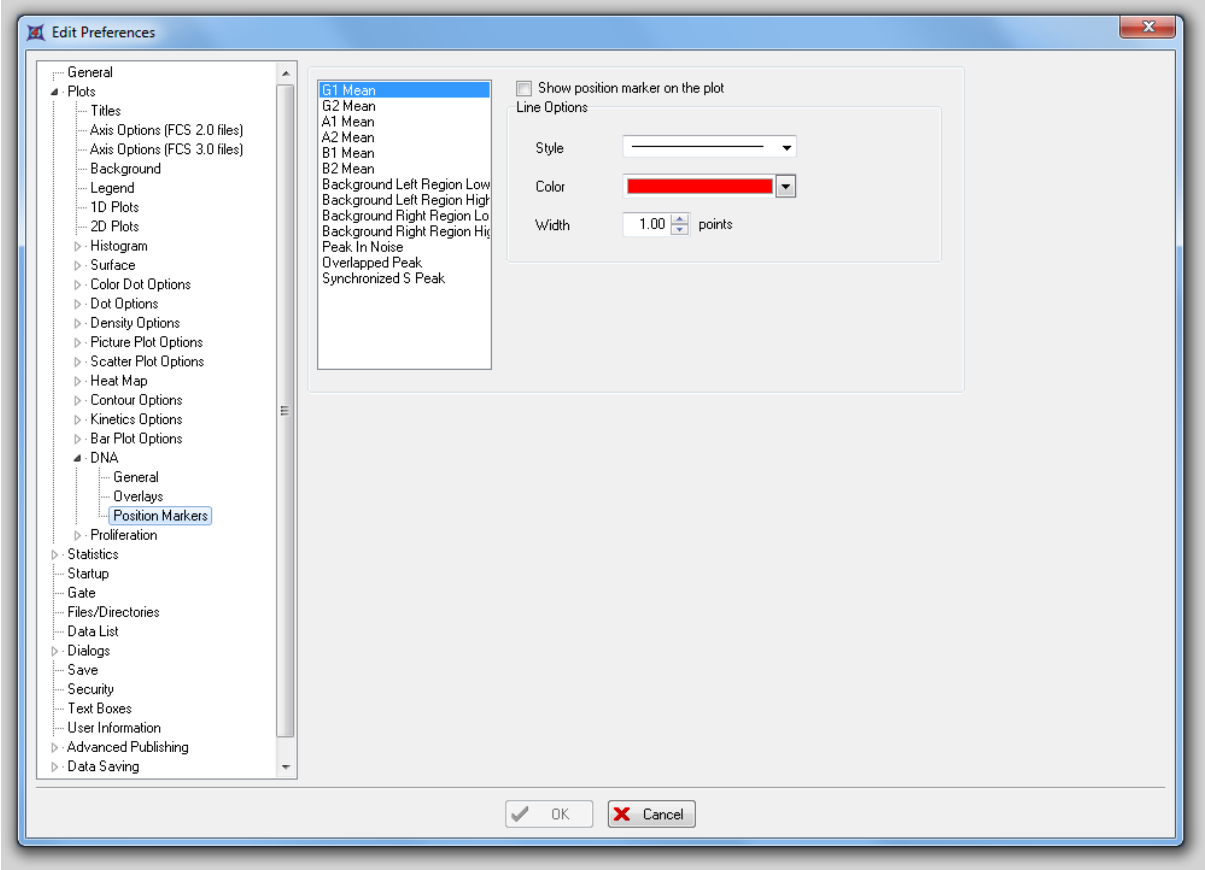

Figure 8.9 DNA Position Markers Preferences

8.2.3 Statistic Preferences

The subcategories of the **Statistics** preferences 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.

8.2.3.1 Plot Statistic Preferences

The **Plot Statistics** preferences (Figure 8.10) determine the default visibility and display for 1D and 2D plot statistics.

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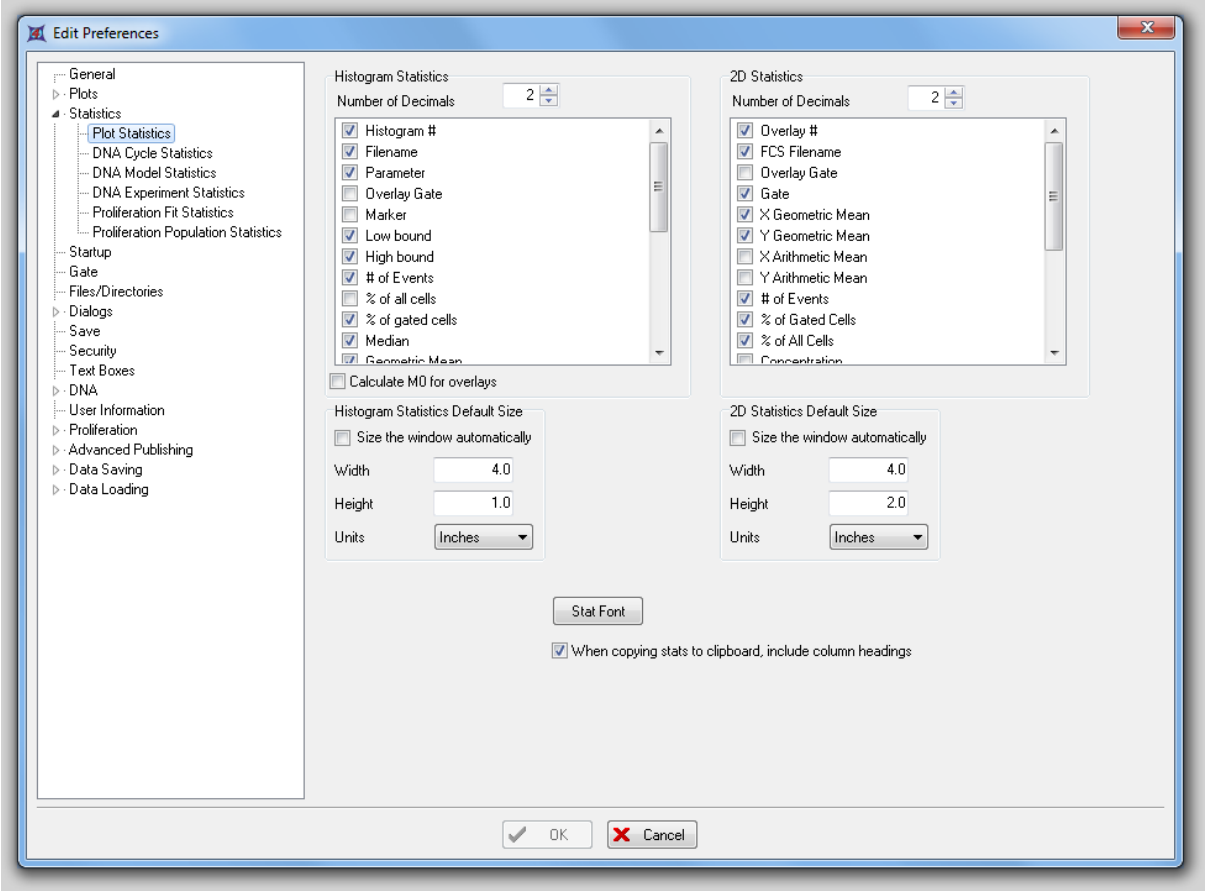

Figure 8.10 Plot Statistics Preferences

Toggling individual statistics on or off 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 214. Preferences that are specific to the statistics window that are not simply regular statistics are explained in the table below.

Preference Name	Explanation
Number of Decimals	Indicates the number of decimal places listed to the right of any decimal point.
Calculate M0 for overlays	The statistics window lists statistics for every marker on a histogram. By checking **Calculate M0 for overlays**, statistics will also be calculated for the entire histogram as if there were no markers present.
Histogram or 2D Statistics Default Size	Sets the default size of the statistics window. The size will automatically adjust if data is added that extends beyond the window borders.
Stat Font	The default font to use when displaying the statistics.

8.2.3.2 DNA Cycle Statistics

The **DNA Cycle Statistics** preferences (Figure 8.11) control which statistics are displayed by default when DNA Cycle Statistics windows are opened.

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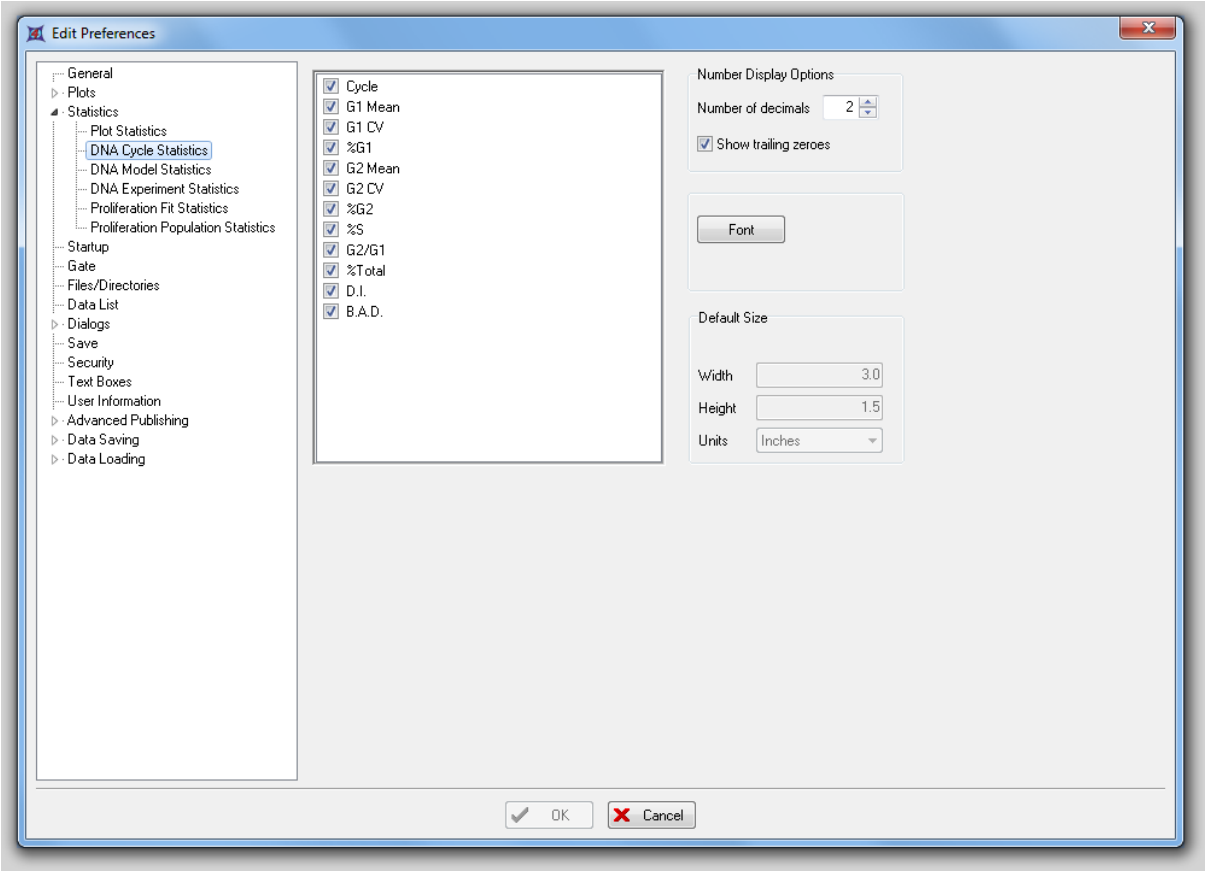

Figure 8.11 DNA Cycle Statistics Preferences

Editing the preferences for each of the 12 statistics in the list box works in the same manner:

- If the checkbox to the left of the name of the statistic to checked, that statistic will be visible when the statistics window opened.
    - In **Number Display Options**, change the **Number of decimals** to control how many decimal places are displayed for that statistic and choose whether or not you want to display trailing zeroes.
    - Click on the **Font** button to open a font selection dialog that will allow you to set the font used to display the statistic.
    - The **Default Size** preferences control the size of the statistics window when it is first opened.

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8.2.3.3 DNA Model Statistics

The **DNA Model Statistics** preferences (Figure 8.12) control which statistics are displayed by default when DNA Model Statistics windows are opened.

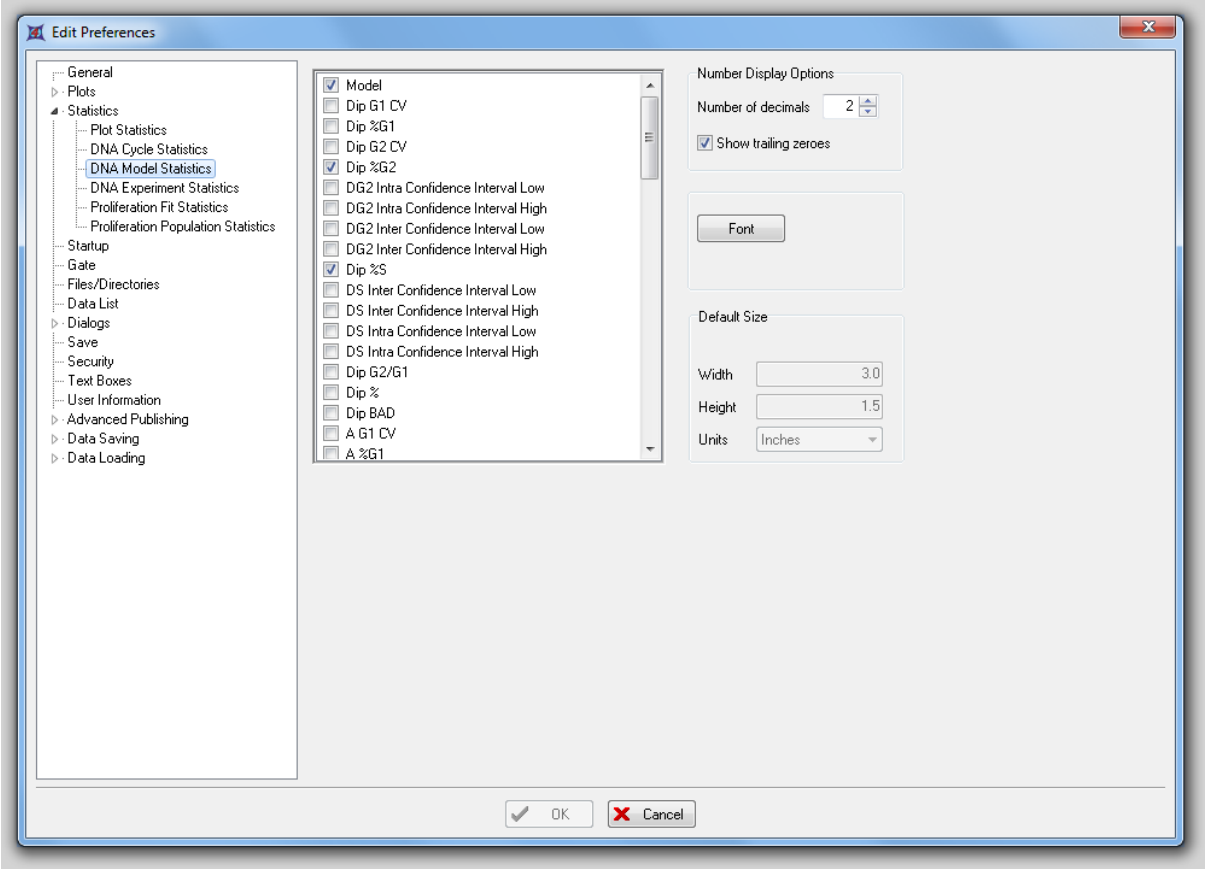

Figure 8.12 DNA Model Statistics Preferences

DNA model statistics are only available when a DNA analysis package is active. Editing the preferences for each of the 72 statistics in the list box works in the same manner:

- If the checkbox to the left of the name of the statistic to checked, that statistic will be visible when the statistics window opened.

  - In **Number Display Options**, change the **Number of decimals** to control how many decimal places are displayed for that statistic and choose whether or not you want to display trailing zeroes.

  - Click on the **Font** button to open a font selection dialog that will allow you to set the font used to display the statistic.

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- The **Default Size** preferences control the size of the statistics window when it is first opened.

8.2.3.4 DNA Experiment Statistics

The **DNA Experiment Statistics** preferences (Figure 8.13) control which statistics are displayed by default when DNA Experiment Statistics windows are opened.

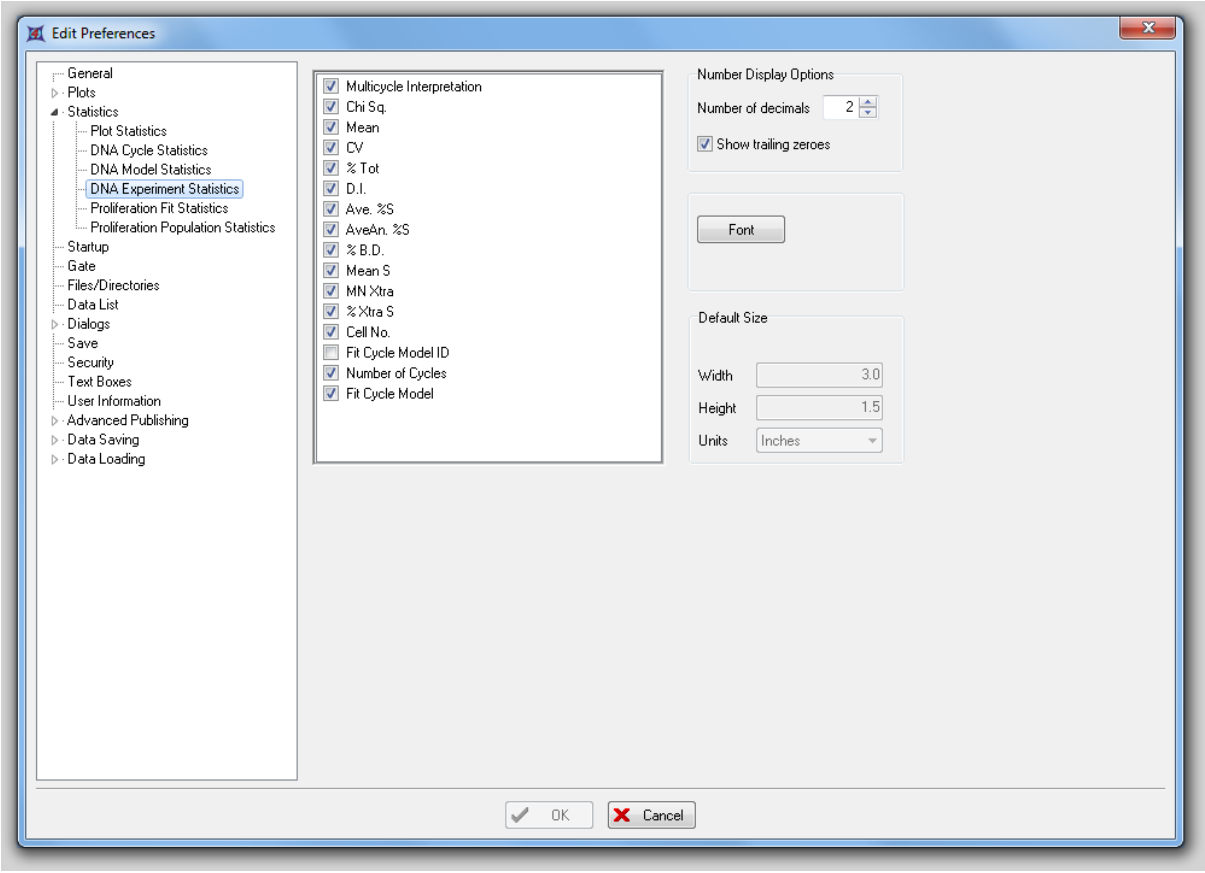

Figure 8.13 DNA Experiment Statistics Preferences

Editing the preferences for each of the 16 statistics in the list box works in the same manner:

- If the checkbox to the left of the name of the statistic to checked, that statistic will be visible when the statistics window opened.

- In **Number Display Options**, change the **Number of decimals** to control how many decimal places are displayed for that statistic and choose whether or not you want to display trailing zeroes.

- Click on the **Font** button to open a font selection dialog that will allow you

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to set the font used to display the statistic.

- The **Default Size** preferences control the size of the statistics window when it is first opened.

8.2.3.5 Proliferation Fit Statistics

The **Proliferation Fit Statistics** preferences (Figure 8.14) control which statistics are displayed by default when Proliferation Fit Statistics windows are opened.

Note: Please refer to the Proliferation Statistics 467 topic for explanations of each statistic and for a list of statistical formulas 469.

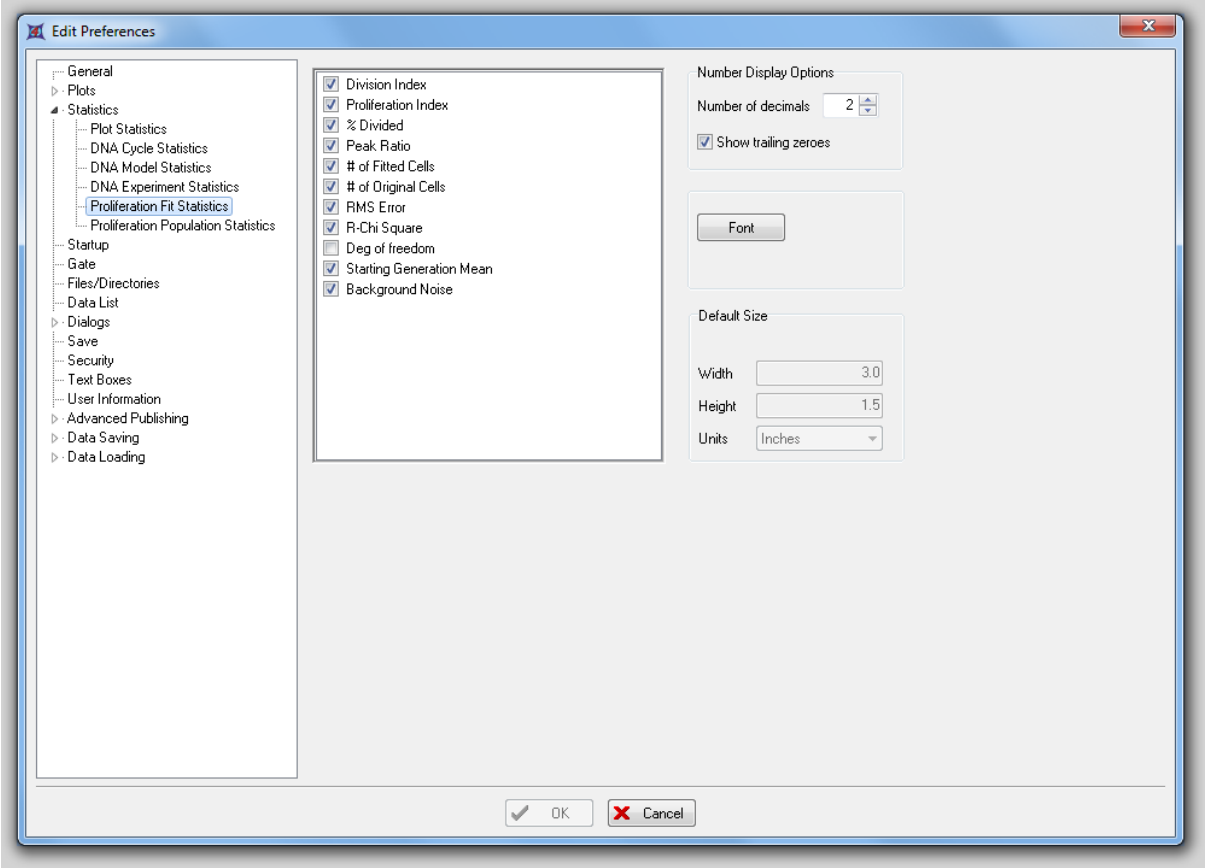

Figure 8.14 Proliferation Fit Statistics Preferences

Editing the preferences for each of the 11 statistics in the list box works in the same manner:

- If the checkbox to the left of the name of the statistic to checked, that statistic will be visible when the statistics window opened.

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- In **Number Display Options**, change the **Number of decimals** to control how many decimal places are displayed for that statistic and choose whether or not you want to display trailing zeroes.

- Click on the **Font** button to open a font selection dialog that will allow you to set the font used to display the statistic.

- The **Default Size** preferences control the size of the statistics window when it is first opened.

Note: Please refer to the Proliferation Statistics[467] topic for explanations of each statistic and for a list of statistical formulas[469].

8.2.3.6 Proliferation Population Statistics

The **Proliferation Population Statistics** preferences (Figure 8.15) control which population statistics[468] are displayed when the proliferation population statistics window is opened.

Note: Please refer to the Proliferation Statistics[467] topic for explanations of each statistic and for a list of statistical formulas[469].

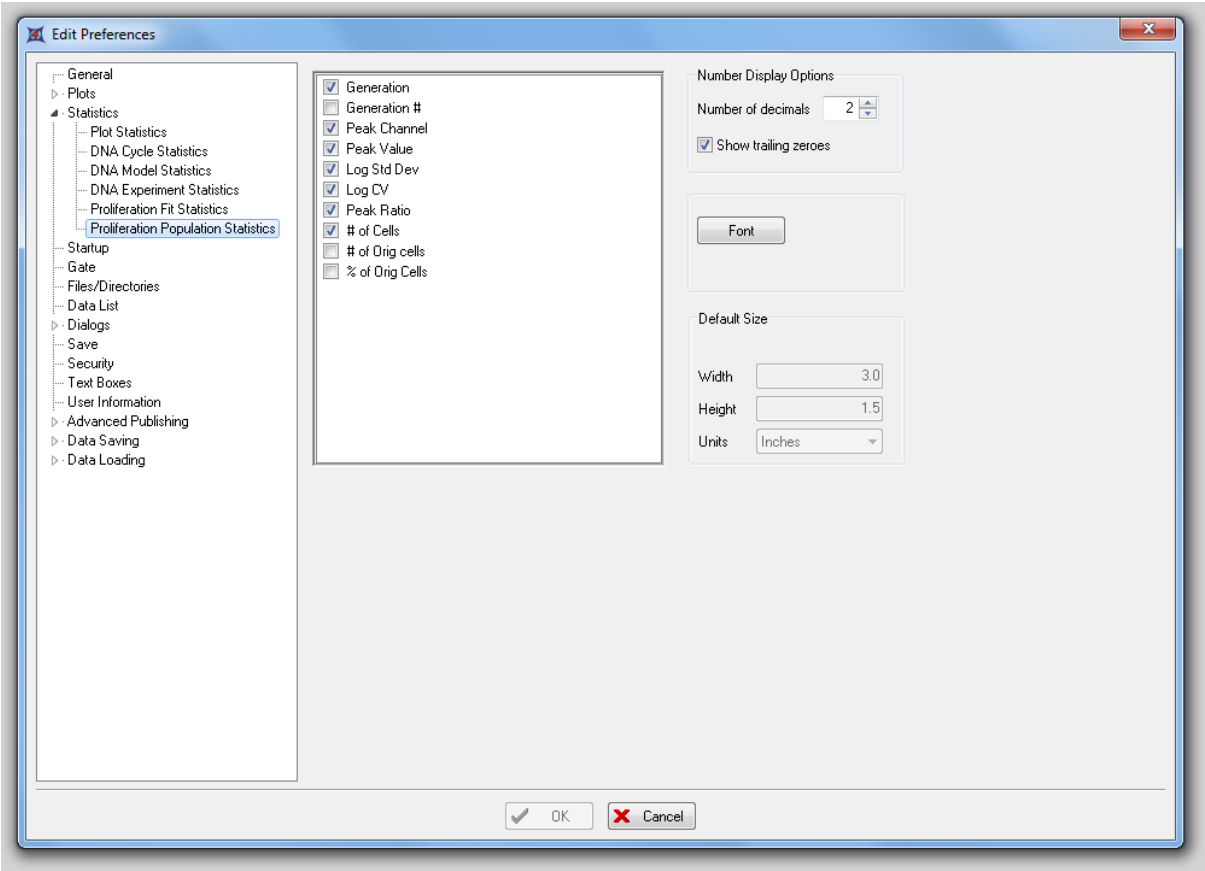

Figure 8.15 Proliferation Population Statistics Preferences

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Editing the preferences for each of the 10 statistics in the list box works in the same manner:

- If the checkbox to the left of the name of the statistic to checked, that statistic will be visible when the statistics window is opened.
- In **Number Display Options**, change the **Number of decimals** to control how many decimal places are displayed for that statistic and choose whether or not you want to display trailing zeroes.
- Click on the **Font** button to open a font selection dialog that will allow you to set the font used to display the statistic.
- The **Default Size** preferences control the size of the statistics window when it is first opened.

8.2.4 Startup Preferences

The **Startup** preferences allow the user to control the behavior of FCS Express at startup.

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

- Associate the .fey extension with FCS Express on startup.

If the checkbox is checked, then when FCS Express starts up, it will make sure that Windows associates the extension .fey with FCS Express. Fey is the extension that FCS Express uses for layouts. That means that when you double-click on an FCS Express layout, FCS Express will automatically open up with the layout. Note that if you are not an administrator on your computer, you may see a warning message on startup, because Windows may not give enough permission to create the .fey association. In that case, you can create the association yourself, by right-clicking on the layout file, and selecting Open With... In the subsequent dialog, you can browse to the FCS Express.exe, located in a subfolder under C:\Program Files\De Novo Software, or C:\Program Files (x86)\De Novo Software.

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8.2.5 Gate Preferences

The Gate preferences category (Figure 8.16) lets the user select the default preferences for gates.

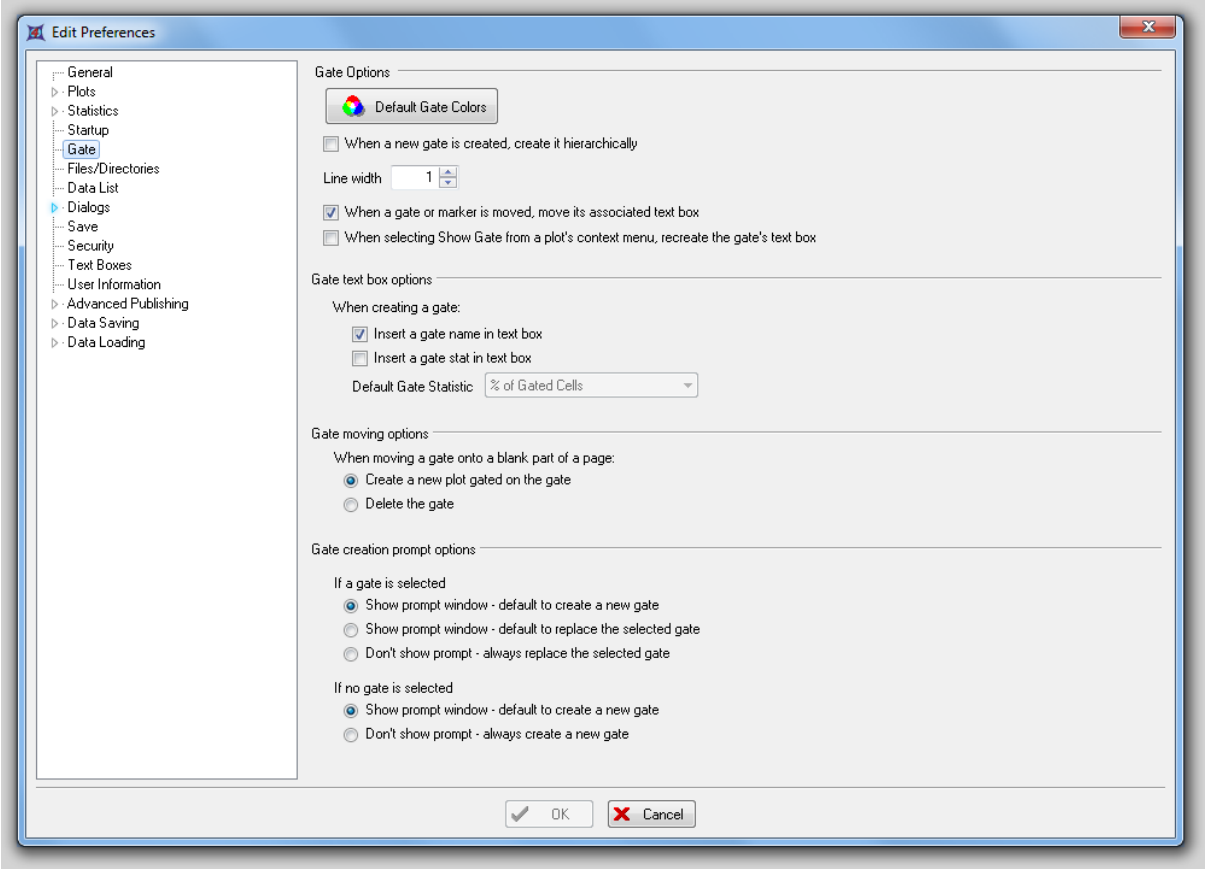

Figure 8.16 Gate Preferences

These preferences are described in the table below.

Preference Name	Explanation
Gate Options	Set the default gate colors and the width of the line used to draw the outline of the gate on a plot. Determine whether or not to: • Use hierarchical gating 206 as the default for new gates. • 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 from the plot's context menu.
Gate text box options	Define the **Default Gate Statistic**. Determine whether or not to insert a gate name or gate stat in the text box and chose which gate statistic to show.
Gate moving options	Choose whether or not to **Delete the gate** or **Create a new plot on the gate** when moving a gate onto a blank part of the page.

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Preference Name	Explanation
Gate creation prompt options	Choose prompt window options depending on whether a gate is selected or not.

8.2.6 Files/Directories

The **Files/Directories** preferences category allows the user to select default directories and files for various aspects of FCS Express (Figure 8.17). 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.

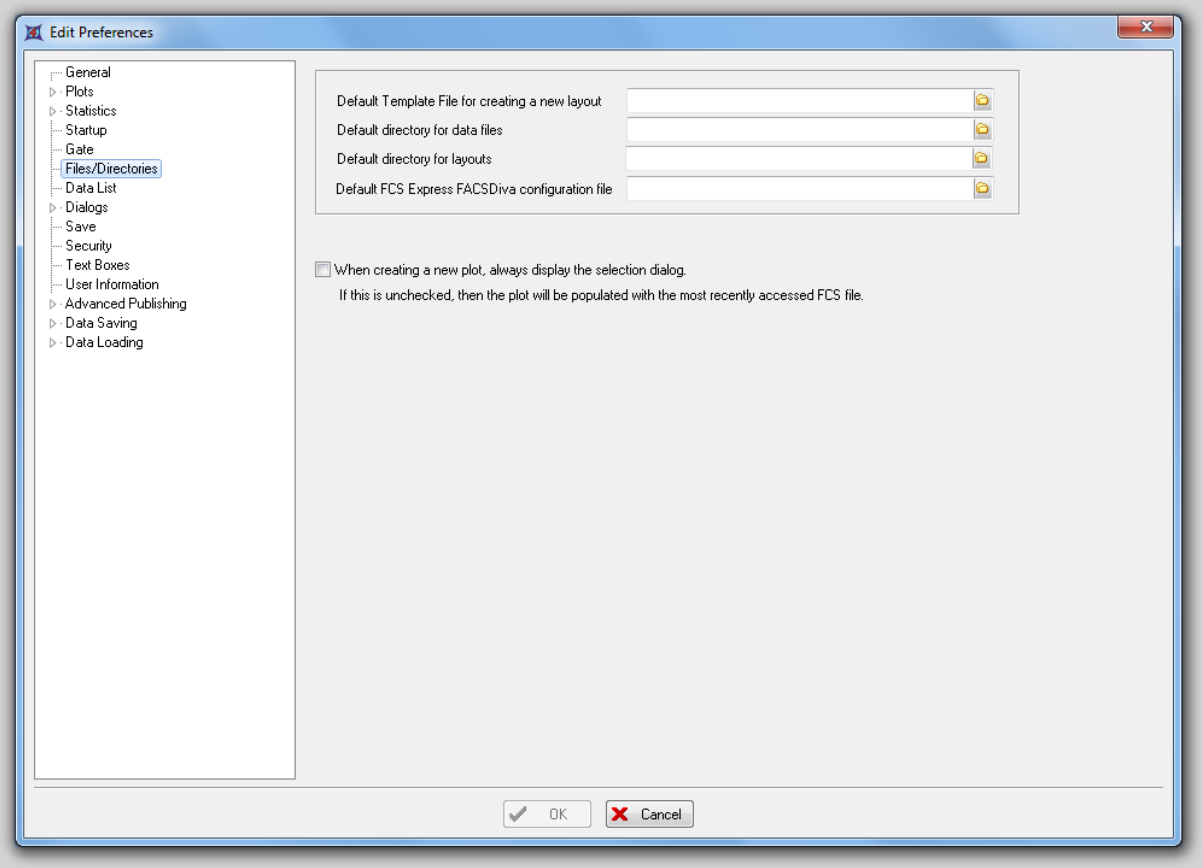

Figure 8.17 Files/Directories Preferences

These preferences are described in the table below.

Preference Name	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 428 for more information.
Default directory for	When an FCS Express layout is first opened and a data file is first

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Preference Name	Explanation
data files	required, FCS Express will look in this directory for the data files.
Default directory for layouts	When you first open a layout 55, the Open Layout dialog will first start in this directory.
Default FCS Express FACSDiva configuration file	This is the default FACSDiva configuration file 430.
When creating a new plot, always display the selection dialog	If the checkbox is enabled, you will always be prompted to choose the file when creating a new plot.

8.2.7 Dialogs Preferences

The keyword dialog is used in many places throughout FCS Express whenever you are required to select a keyword that is going to be evaluated and displayed as text. For instance, keywords can be inserted in plot titles 154 and keyword tokens 342.

The **Dialogs→Keyword Dialog** preferences allow you to specify which keywords appear in the keyword dialog (Figure 8.18).

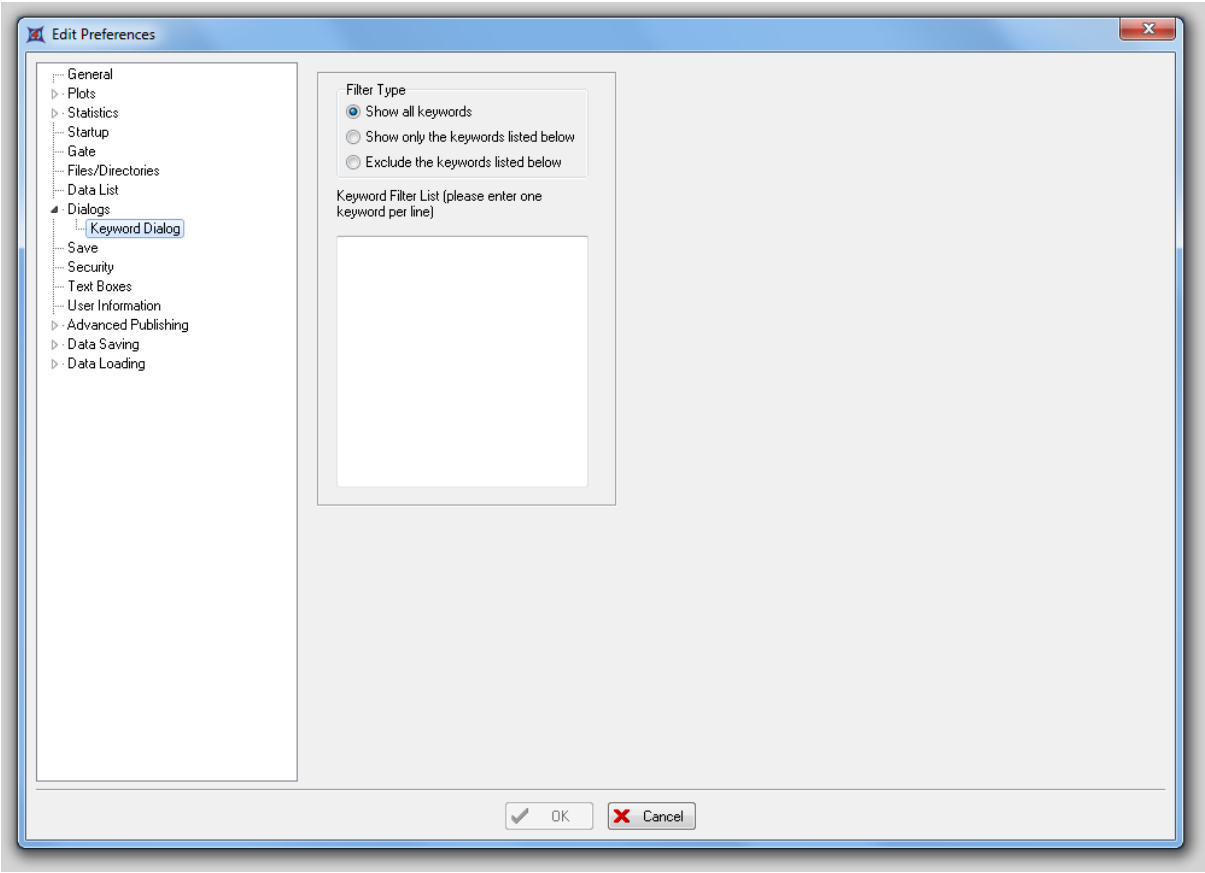

Figure 8.18 Keyword Dialog Preferences

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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 list box at the bottom. Enter one keyword per line. Case/capitalization are ignored. You can also use the wildcard "*" 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 Save Preferences

The **Save** category (Figure 8.19) lets the user select how layout files are saved. These preferences are described in the table below.

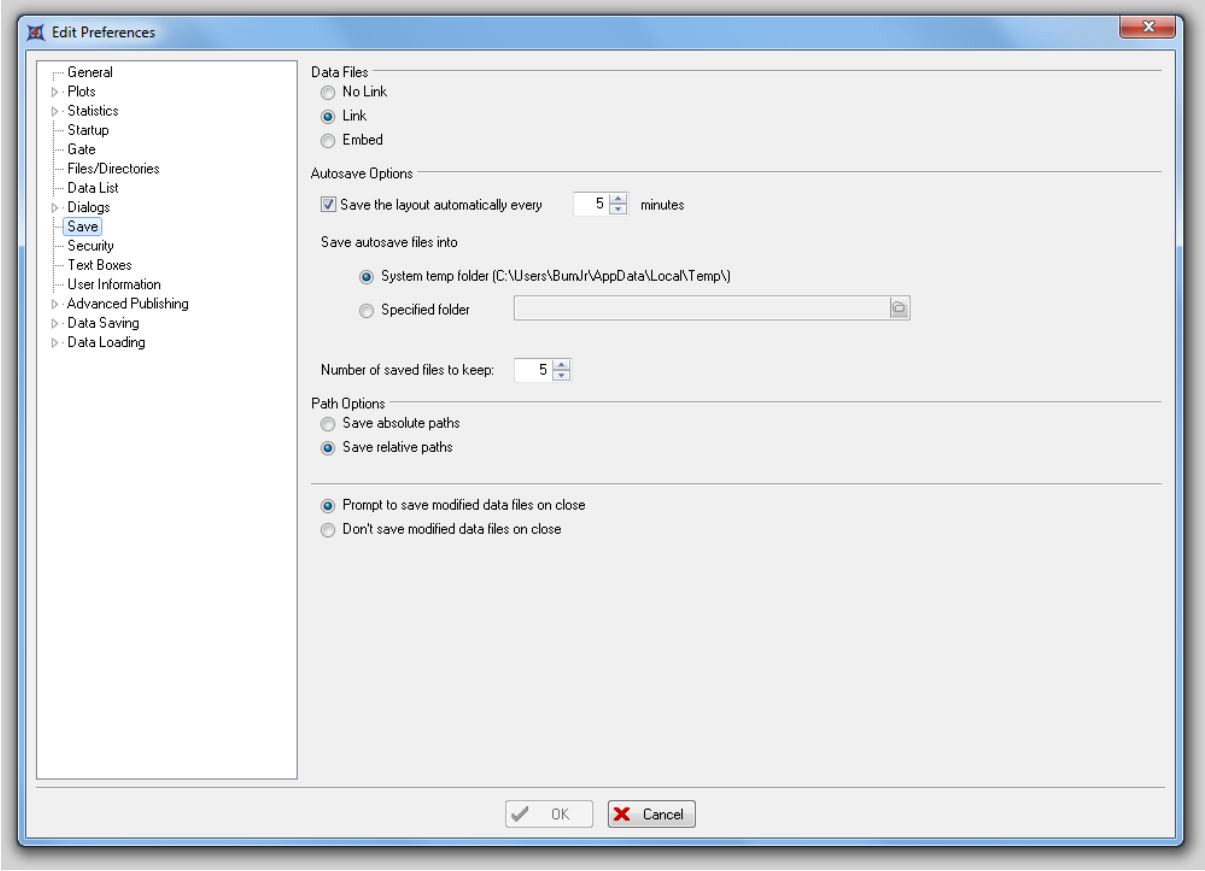

Figure 8.19 Save Preferences

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Preference Name	Explanation
Data Files	Layout linking and embedding are described in Saving a Layout 53.
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 65.
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 53). 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.
Prompt to save (or Don't save) modified data files on close	Data file header information can be changed and new parameters can be created using Parameter Math 260. These actions create a modified data file different from the

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Preference Name	Explanation
	original. Choosing the **Prompt to save modified data files on close** radio button will prompt you 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.

8.2.9 Security Preferences

The Security preferences allow the user to set a password to prohibit someone from modifying the preferences of the current user.

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

8.2.10 Text Box Preferences

The Text Box preferences category allows the user to configure the default appearance for text boxes (Figure 8.20).

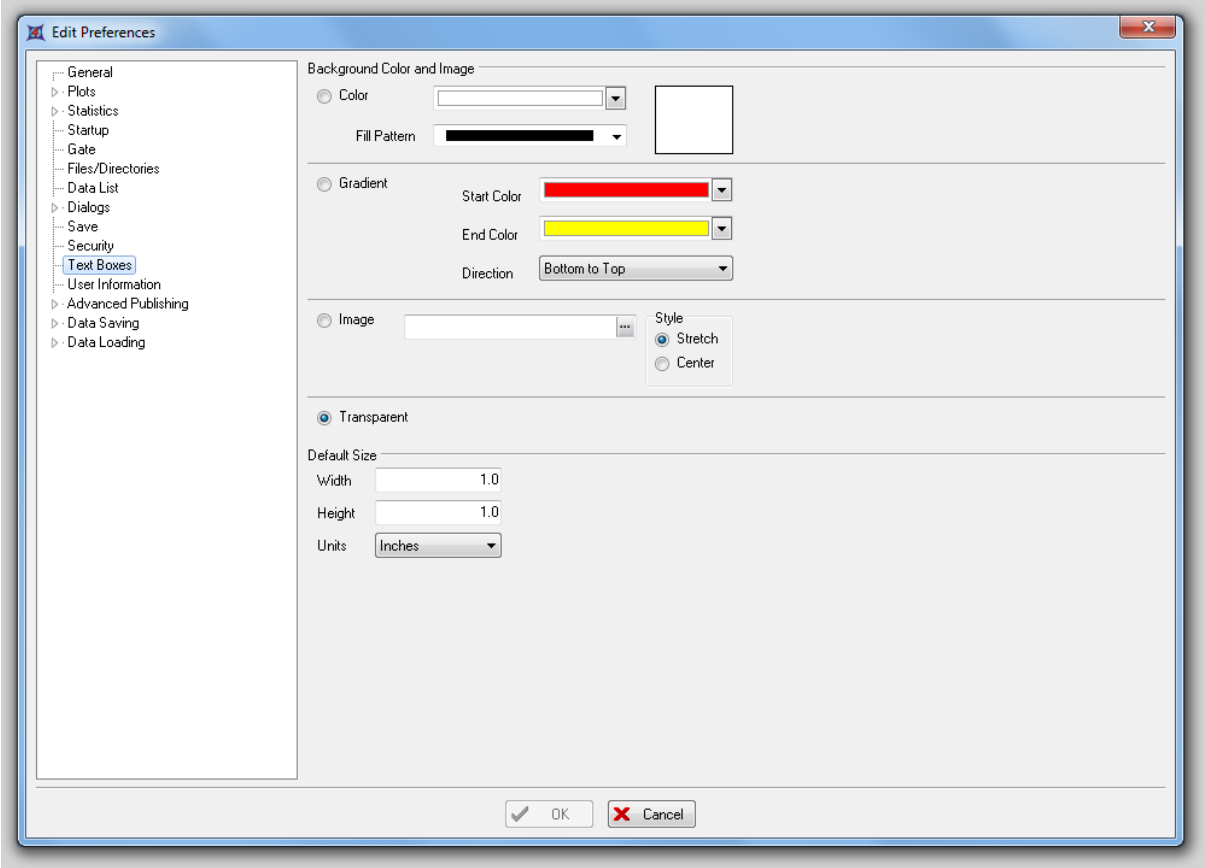

Figure 8.20 Edit Preferences - Text Boxes

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

8.2.11 User Information

Under user information (Figure 8.21), you can set an e-mail address, author name, and organization for the user.

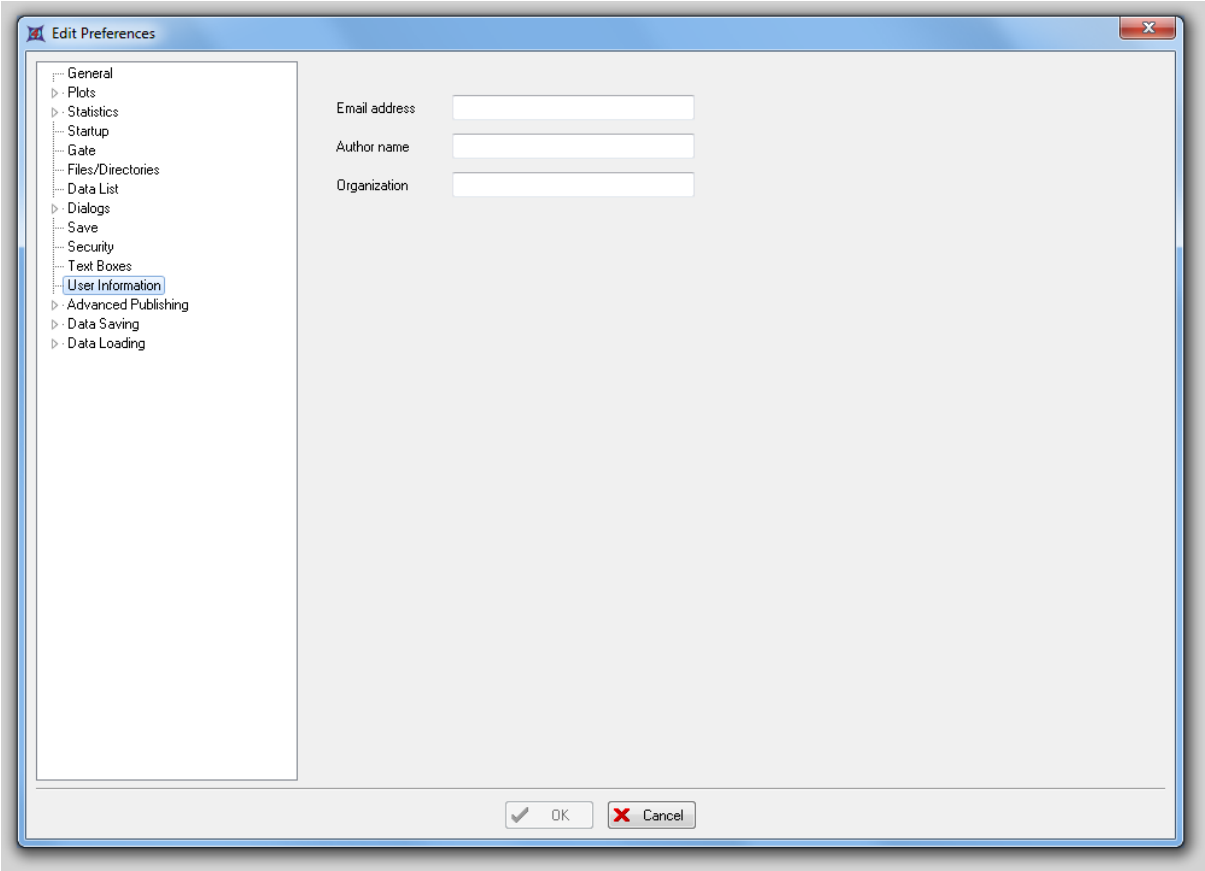

Figure 8.21 Edit Preferences - User Information

8.2.12 Advanced Publishing Preferences

There are two subcategories for Advanced Publishing[583] preferences: **Default Permissions** and **Publishing Options**.

The Default Permissions page allows you to limit what people viewing you 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 8.22).

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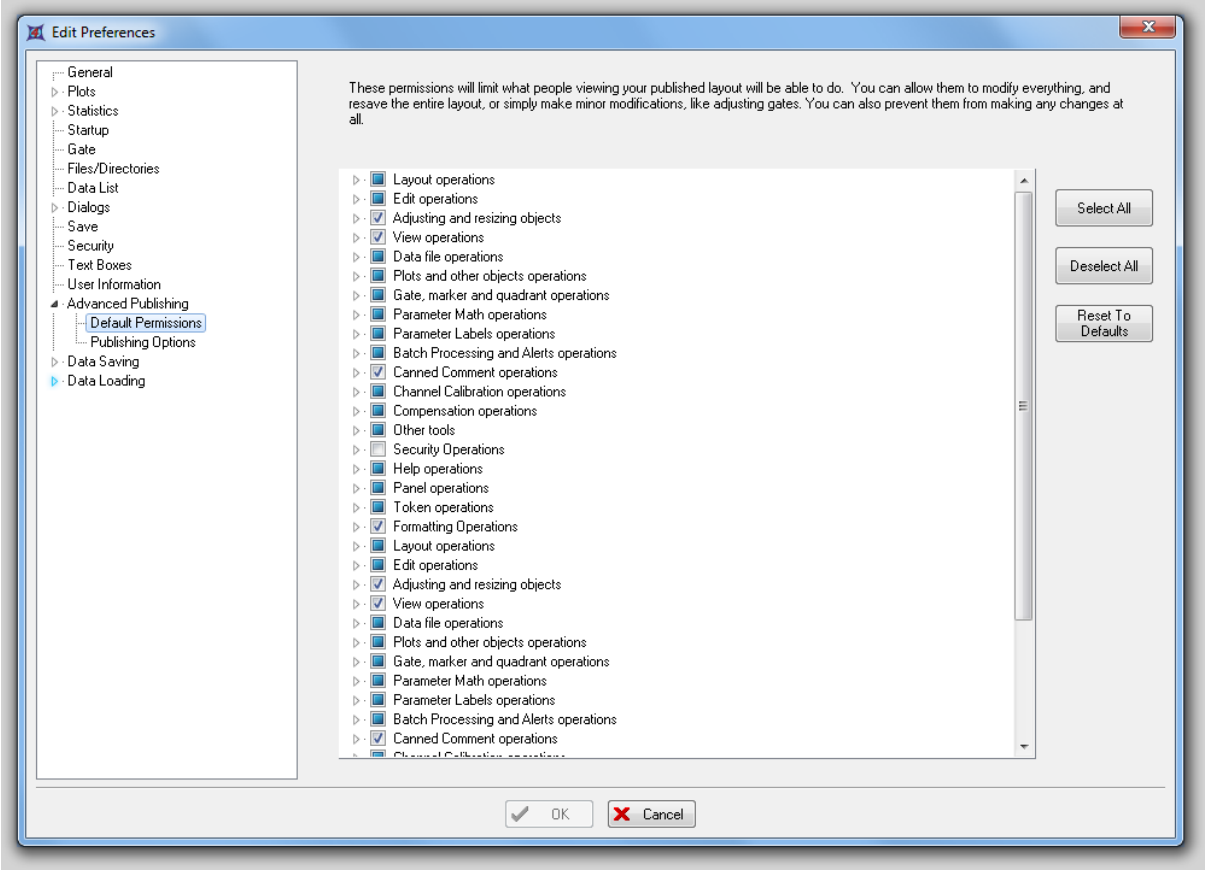

Figure 8.22 Advanced Publishing Default Permissions

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 8.23).

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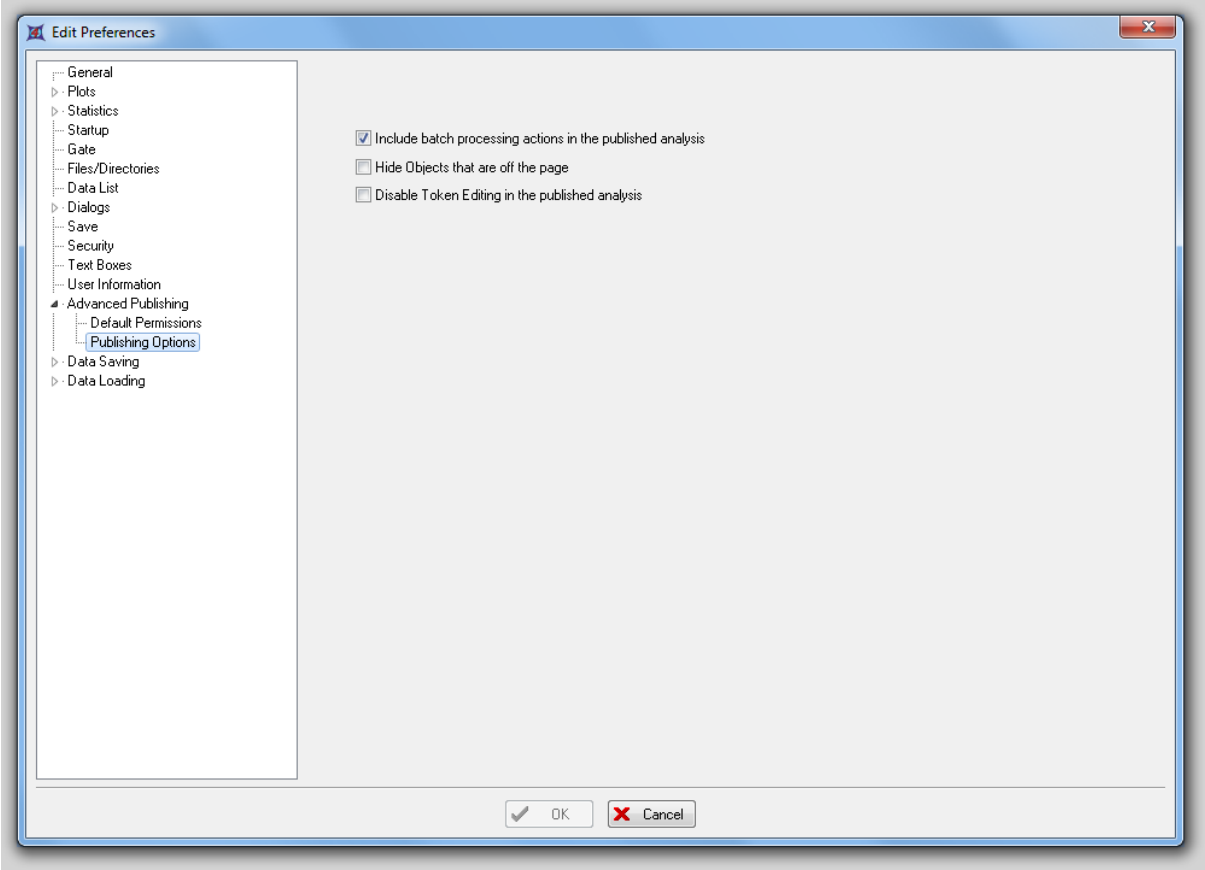

Figure 8.23 Advanced Publishing Options

8.2.13 Data Saving

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

When saving data as a text file, the **Save as Text Format** default preferences allow you to choose a delimiter ('comma' is the default) and decide whether to **Save Headers** (Figure 8.24).

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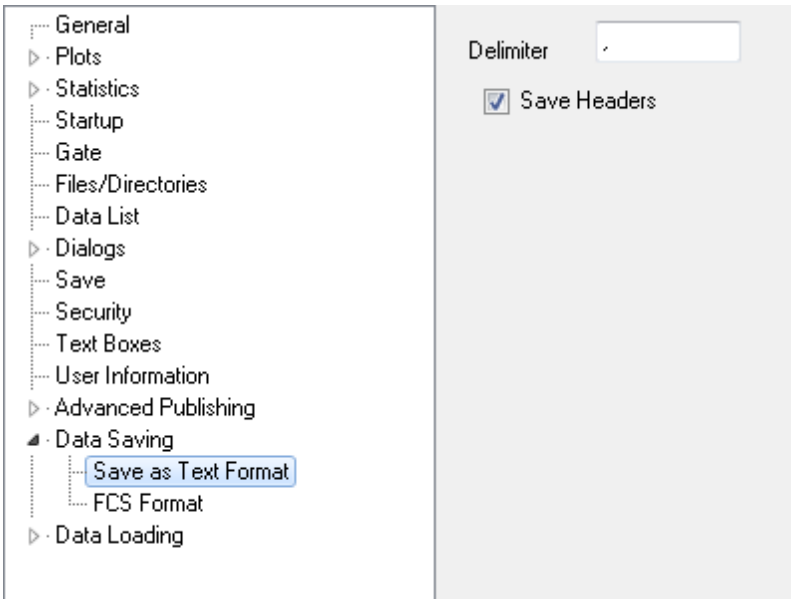

Figure 8.24 Data Saving - Save as Text Format Preferences

You can also set default preferences for **Data Format**, **Endian Format**, and **FCS Version** when saving files in **FCS Format** (Figure 8.25).

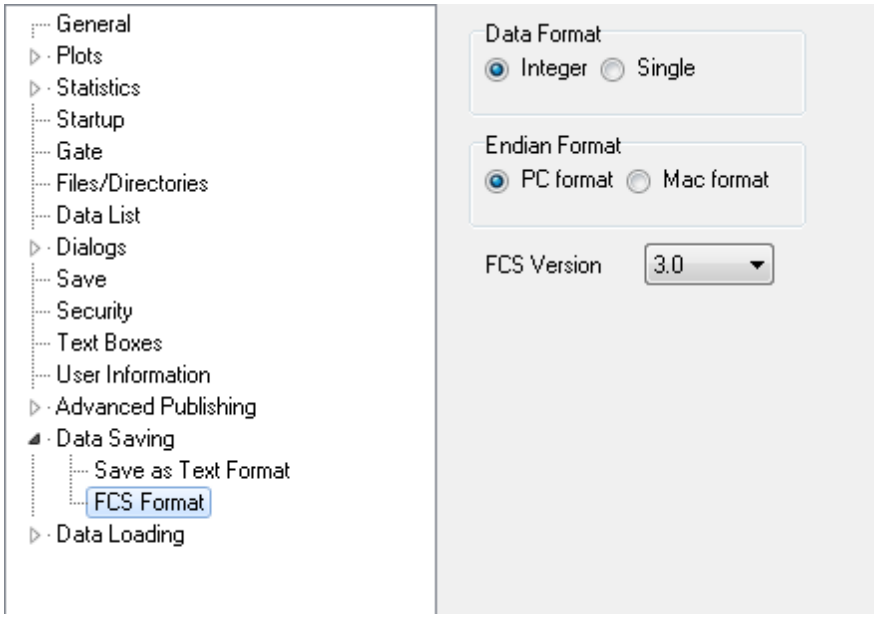

Figure 8.25 Data Saving - FCS Format Preferences

8.2.14 Data Loading

The **Data Loading** category contains **FCS File Options** as well as properties for different instrument data sets including, **IsoCyte options** and **Cell Profiler**

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Options.

Note: Different choices may appear in the data loading options category depending on whether you are using FCS Express 4 Flow, FCS Express 4 Image, or FCS Express 4 Plus.

8.2.14.1 FCS File Options

This option only appears if you are using FCS Express 4 Flow or FCS Express 4 Plus.

The FCS File Options category (Figure 8.26) allows you to select how FCS Files will appear when they are being browsed for in data selection dialogs (e.g., when changing files113, adding files to the Data List374, or inserting new plots112. The preferences are described in the table below.

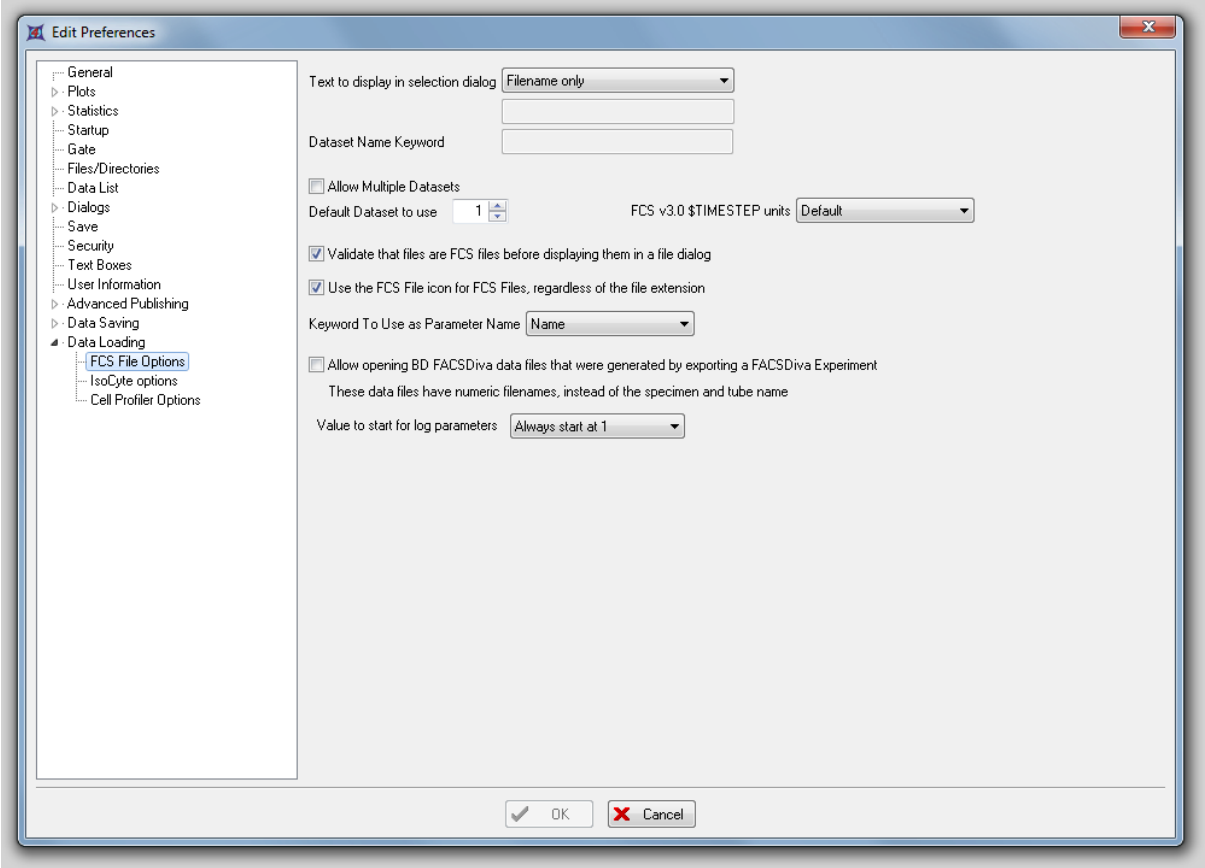

Figure 8.26 Edit Preferences - FCS File Options

Preference	Explanation
Text to display in selection dialog	FCS Express allows you to display keyword values (e.g., the SAMPLE ID) as well as the file name. For a list of keywords, use the Data Data

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Preference	Explanation
	Info View Header command, choose an FCS file, and click on the **All Keywords...** button. Choose **The text below** from the drop-down list to unlock the edit field below. Enter the keyword (s) you wish to view using **<** and **>** to bracket the keyword. For example, *<$SAMPLE ID>* will display the Sample ID as the file name.
Dataset Name Keyword	Select the keyword to use as the default dataset name when selecting from multiple datasets.
Allow Multiple Datasets	Some FCS files contain multiple data sets. If this option is checked, you can select one of the embedded datasets.
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 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.
Use the FCS File icon for FCS Files, regardless of the file extension	FCS files may not be named with consistent extensions. If this option is checked, FCS Express will check each file to determine if it is an FCS file (irrespective of its extension) and display FCS Files with a consistent icon. *Note*: This option may slow down the file dialog display.
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 open numerically-named files exported from BD FACSDiva. See http://www.denovosoftware.com/site/FAQDivaInvalidDataMessage.shtml#QInvalidDivaDataMessage 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.
Value to start for log parameters	Choose the starting value for parameters displayed in the log scale from the drop-down list.

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8.2.14.2 IsoCyte Options

This option only appears in FCS Express 4 Image Cytometry and FCS Express 4 Plus.

When loading IsoCyte data, you can choose whether to **Load cell images**, and/or **Load as Plate** (Figure 8.27).

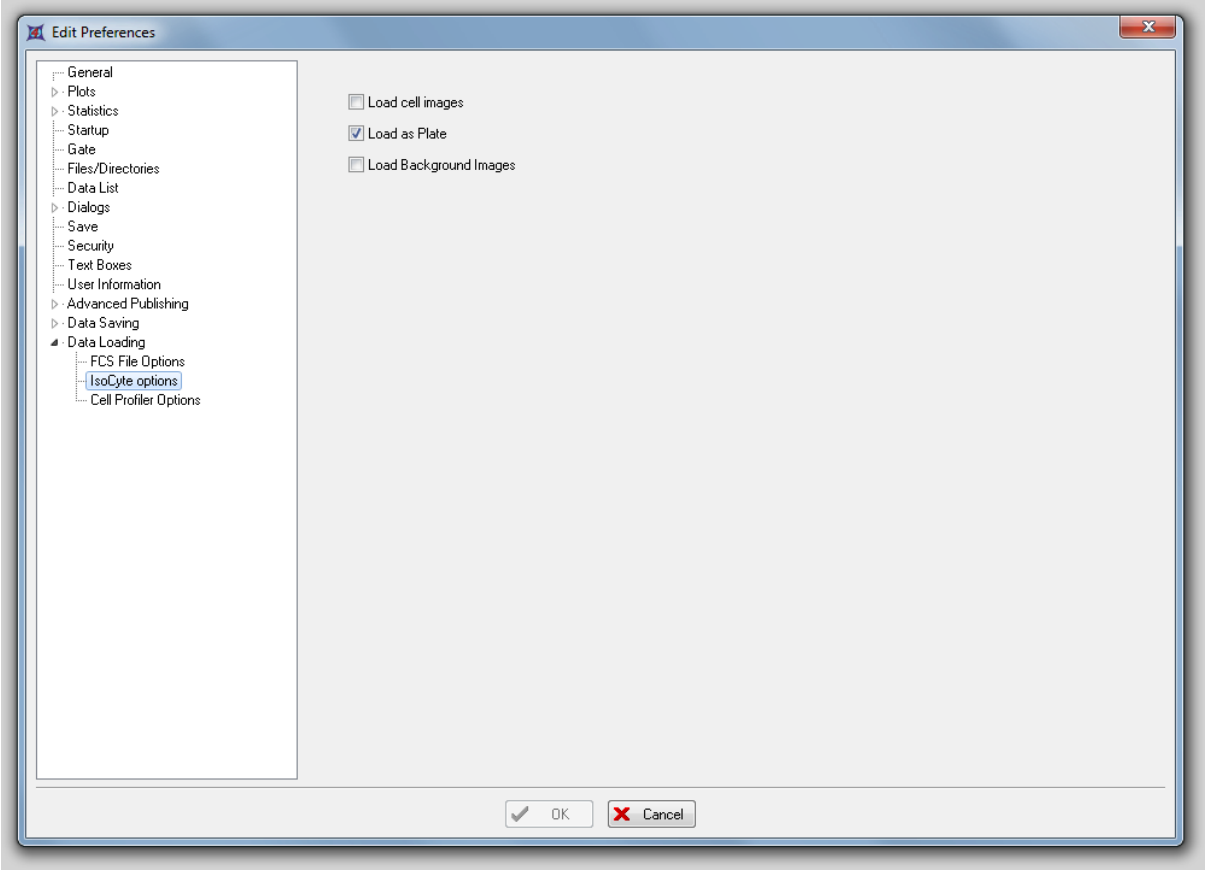

Figure 8.27 IsoCyte Data Loading Options

8.2.14.3 Cell Profiler Options

When loading files from CellProfiler, you can choose the format (**File delimiter**), either character separated values (.csv) or tab-delimited files. You can also choose whether to **Load Images, Load path names from Cell Profiler table of contents file,** and/or **Load Background Images** (Figure 8.28).

If **Load path names from Cell Profiler 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.

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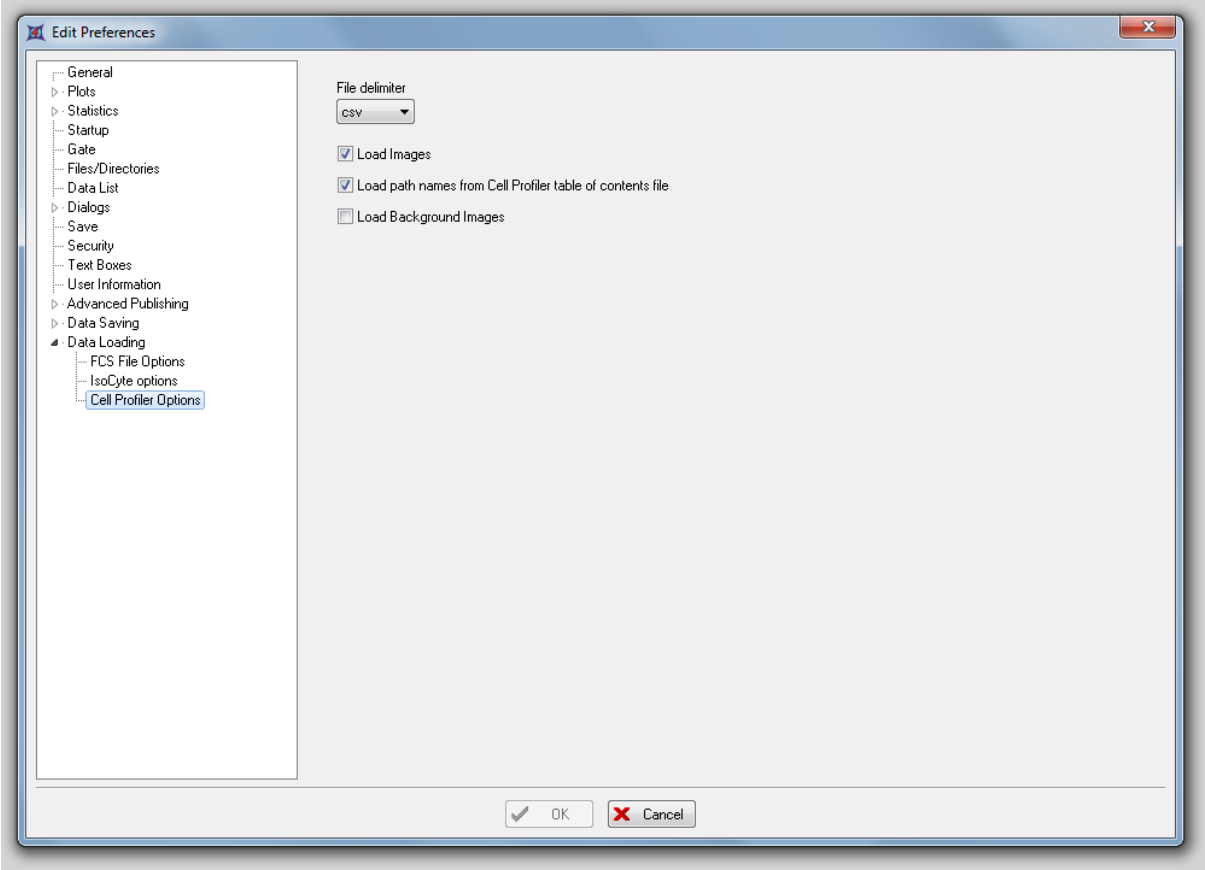

Figure 8.28 Cell Profiler Data Loading Options

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 **Background**, **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 34.

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8.3.1 General

For most objects, the only general formatting option is the object's name. 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[74]. 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.

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.

Data Grids have some extra general options (Figure 8.29).

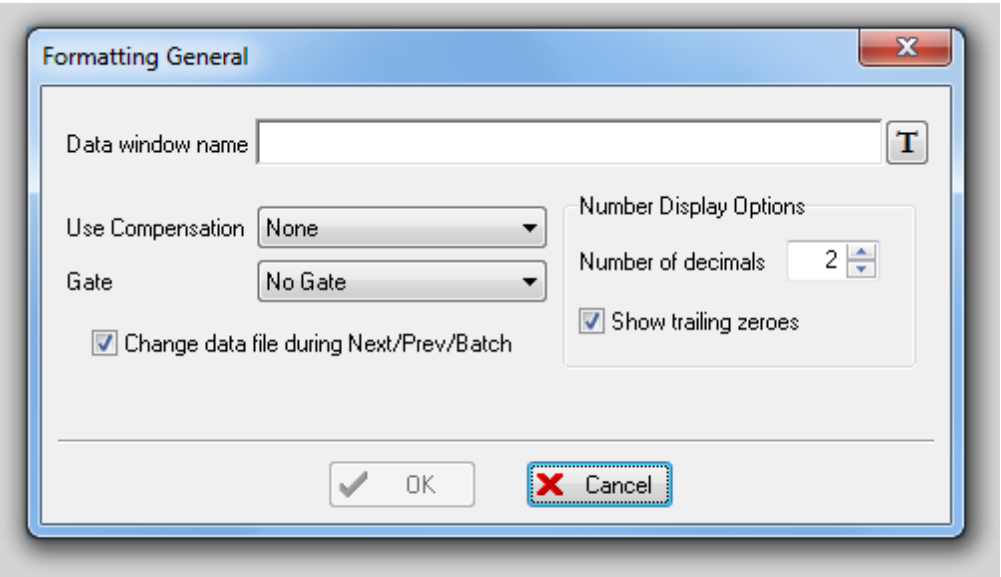

Figure 8.29 Formatting General Dialog for Data Grids

Besides the usual name property (referred to as **Data window name**), you can also choose a compensation[288] definition, which 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[372] or in response to a Next or Previous command.

8.3.2 Background

The Background options (Figure 8.30) allow you to change the background appearance of an object. To customize the background, click on the object or

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

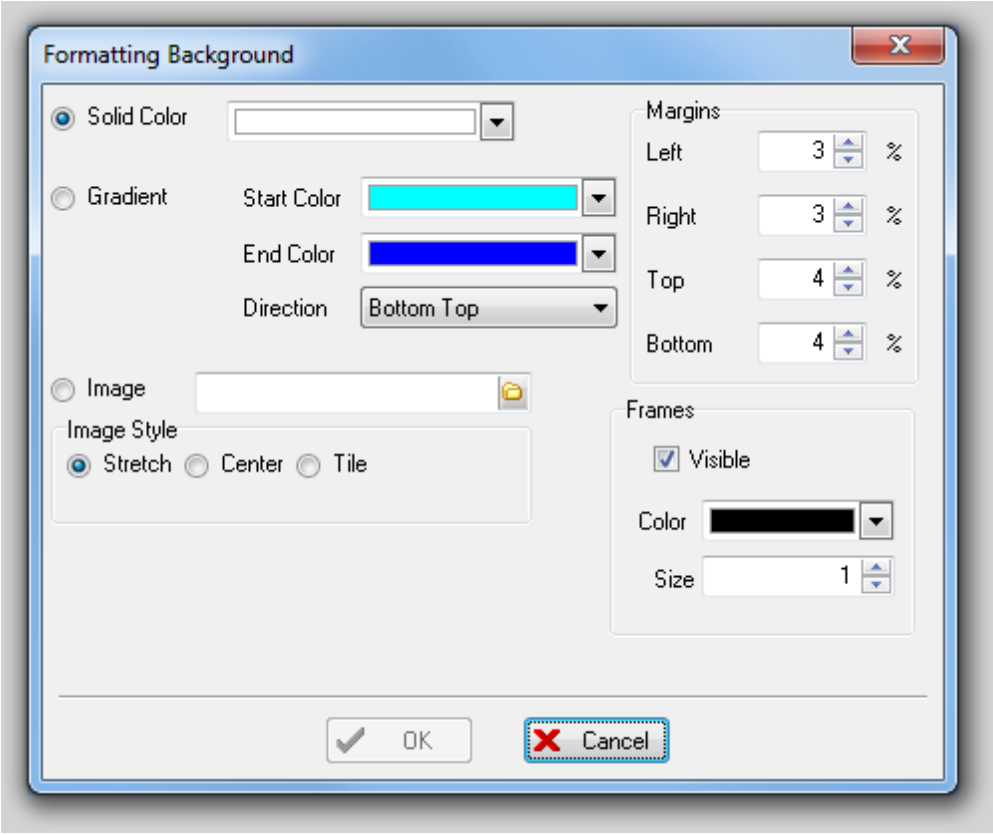

Figure 8.30 Formatting Background Dialog for a Plot

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

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

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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 and Size

The **Border/Line** options (Figure 8.31) 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 an object in edit or selected mode.
- Right-click on the object, select **Format** from the pop-up menu, and choose the **Border** (or **Line**) category.

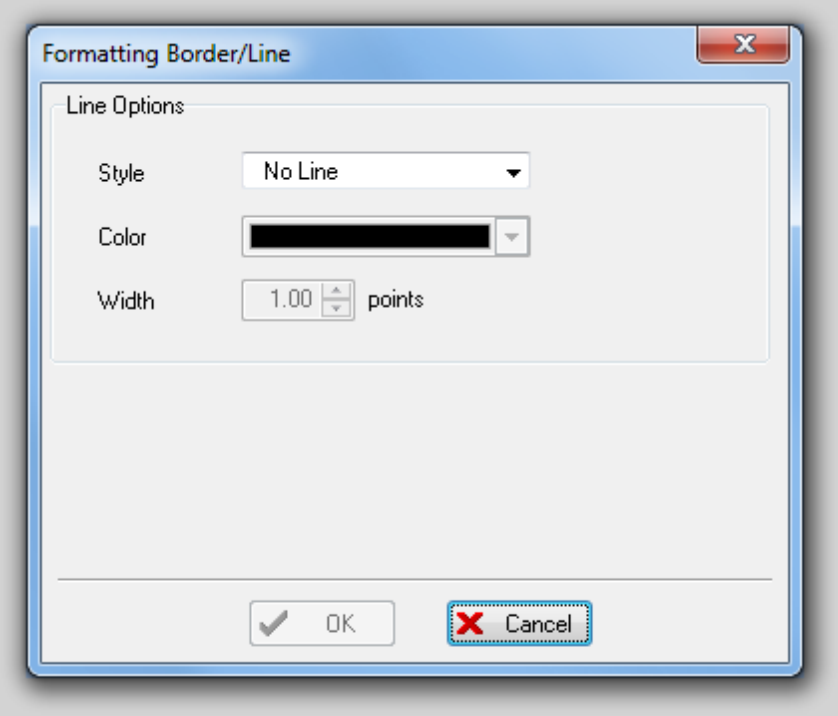

Figure 8.31 Formatting Border/Line Dialog

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The **Size** category (Figure 8.32) 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 104).

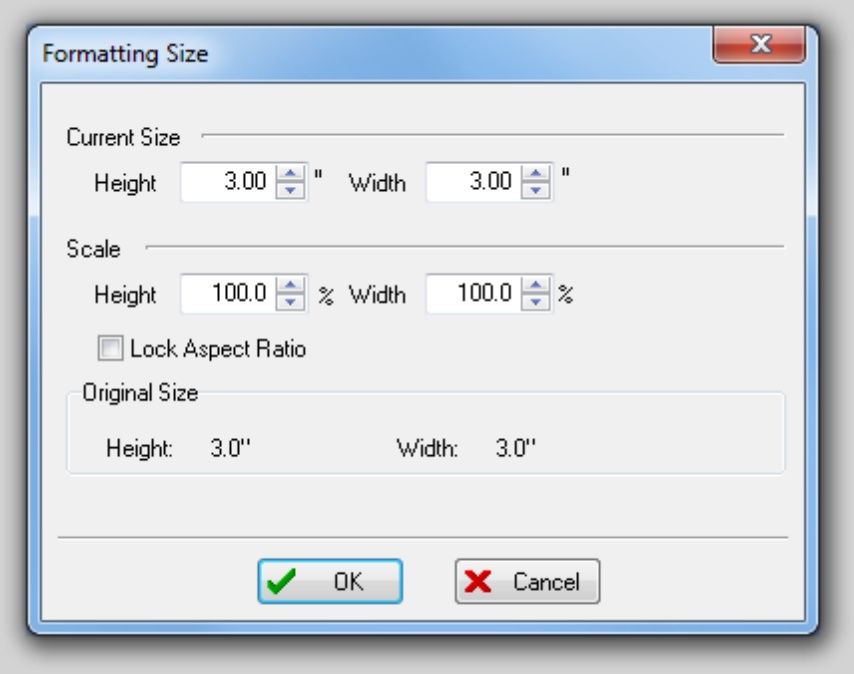

Figure 8.32 Formatting Size Dialog

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8.4 Formatting Features Shared by All Plots

In addition to the formatting options common to all objects 147, all plots in FCS Express share certain basic features, e.g., title, axes, legend, and 3D properties. 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 preferences 120.

You can always format a plot by right-clicking on the plot, selecting **Format** from the pop-up menu, and choosing a category to format.

After formatting a plot:

- Press **OK** to apply the changes.
- 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.
- Press **Restore** to ignore 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 40. Preview and restore options are not available using this method, however.

8.4.1 Resolution Overview

The resolution options are located in the **Formatting** dialog in the Histogram→Histogram Specific Options 172 category for histograms 167, and the Dot Options→Binned Dot (or Plot) Specific Options 184 category for 2D plots 175. 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 lowered. The way FCS Express puts the data into the bins is determined by

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the scale[219].

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 8.33), 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.

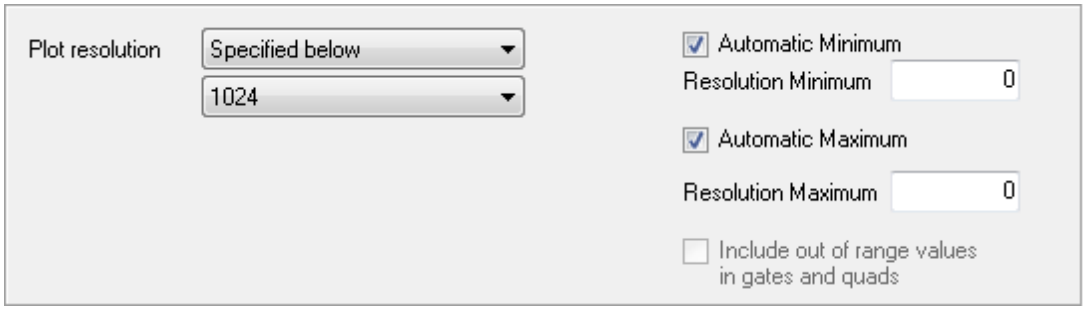

Figure 8.33 Histogram Resolution Options

  - For 2D plots (Figure 8.34), choose from the drop-down list (32 x 32 to 1024 x 1024).

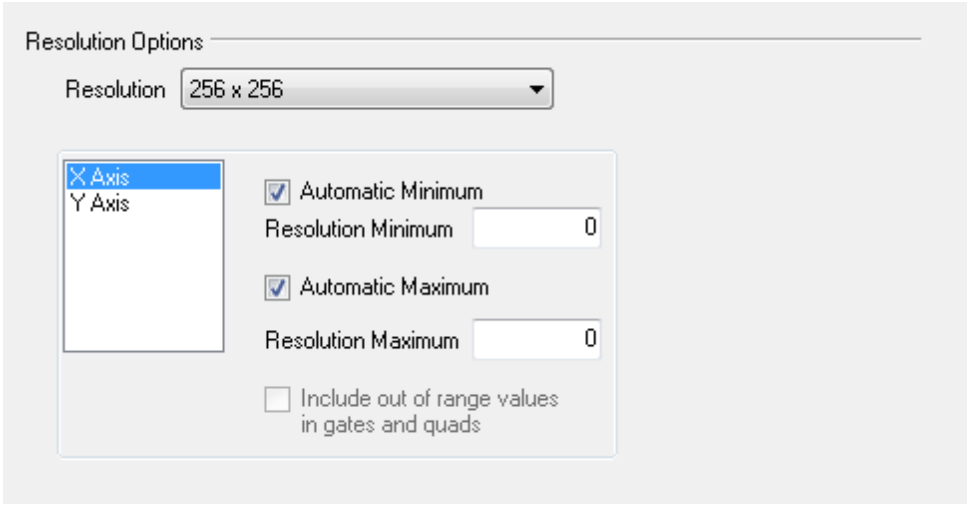

Figure 8.34 2D Plot Resolution Options

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

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 and use the **Format→Plot Options→Title** command (Figure 8.35).

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

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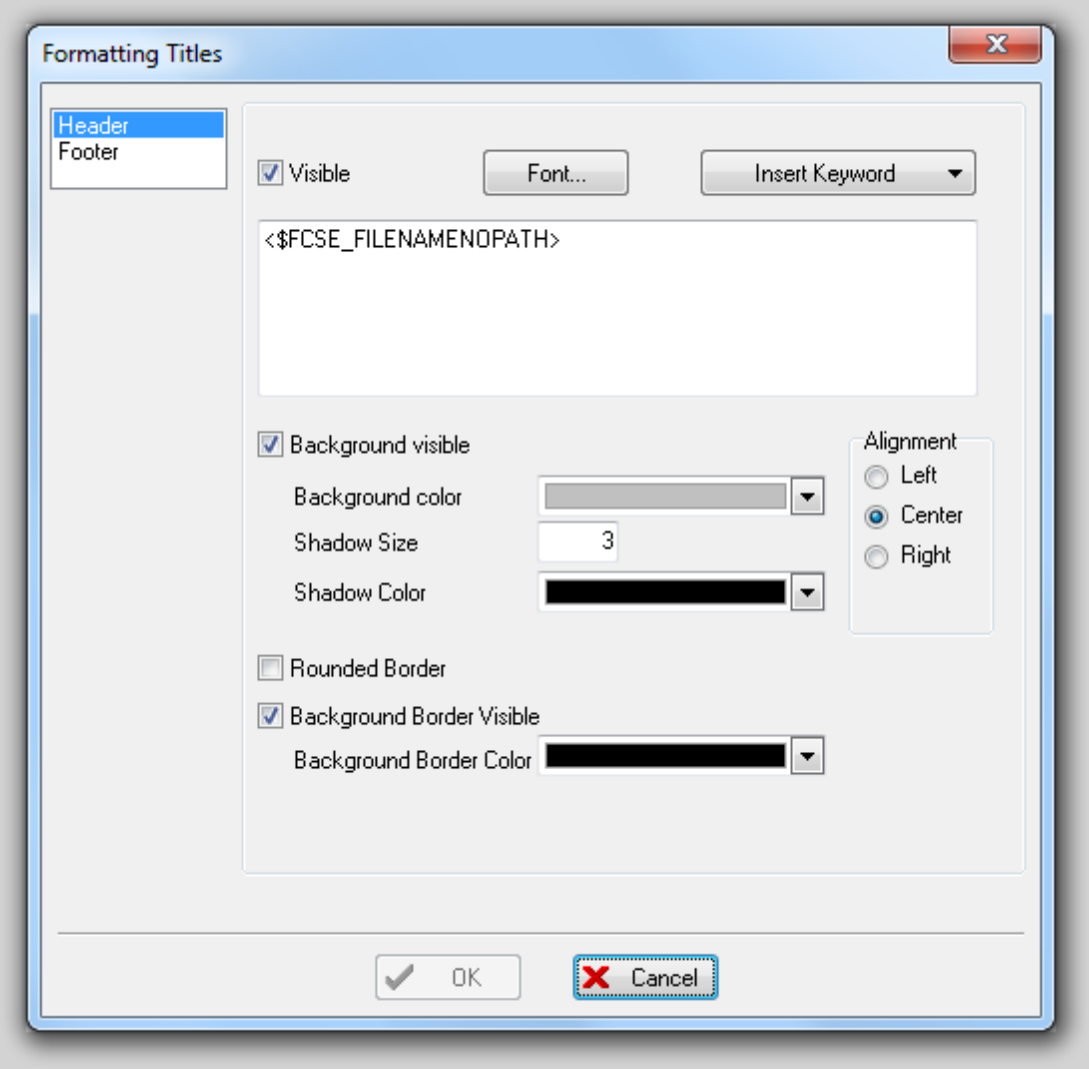

Figure 8.35 Formatting Titles

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 **<$FCSE_FILENAMENOPATH>** which represents the local file name (no drive\folder) with compensation information.

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

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Option	Explanation
	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: • **FCS keyword**. Opens the FCS keyword list for the current file. Select one keyword from the list to insert in the header/footer. Repeat to add more keywords. • **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. • **Current Gate**. Displays the name of the gate that is applied to the base overlay.

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 8.36).

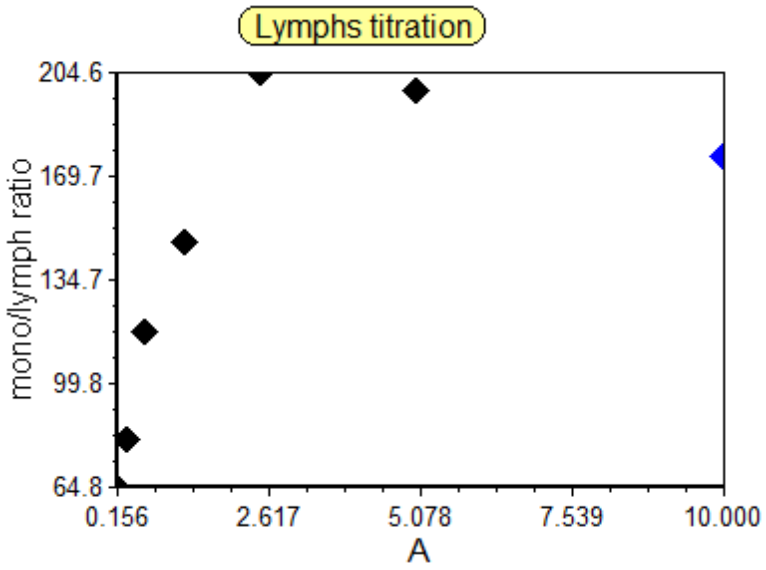

Figure 8.36 Header Title with Rounded Border

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:

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- Select the plot and use the **Format→Plot Options→Legend** command (Figure 8.37).
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Legend** category.

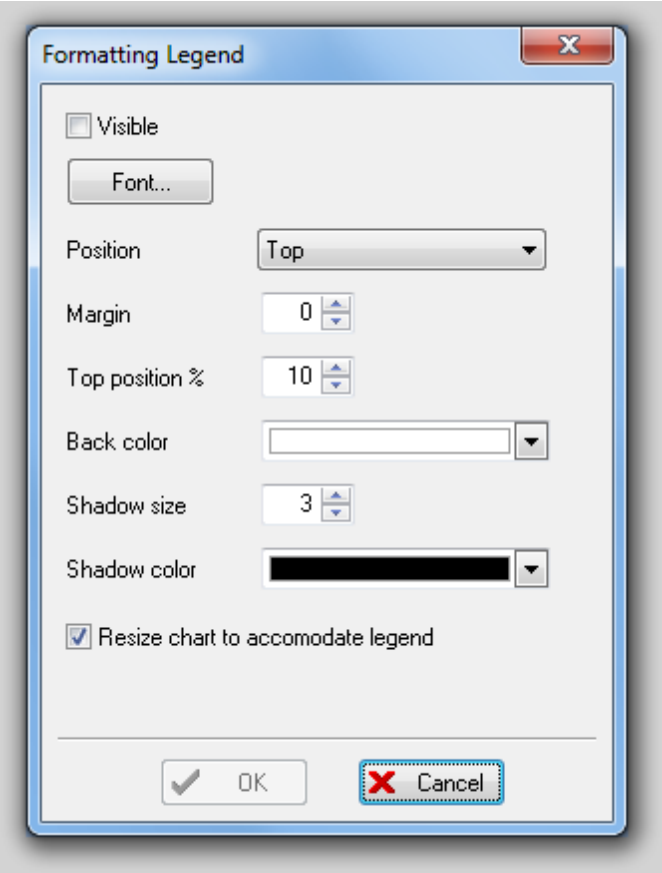

Figure 8.37 Formatting Legend Dialog

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.
Font...	Customize the Legend font style, size, and text color.
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	The number sets the size of the shadow; use 0 for no shadow.

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Option	Explanation
color	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.

8.4.4 Axes

Axes options control the appearance and text of the plot axes.

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

- Select the plot and use the **Format→Plot Options→Axes** command (Figure 8.38).
- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Axis** category.

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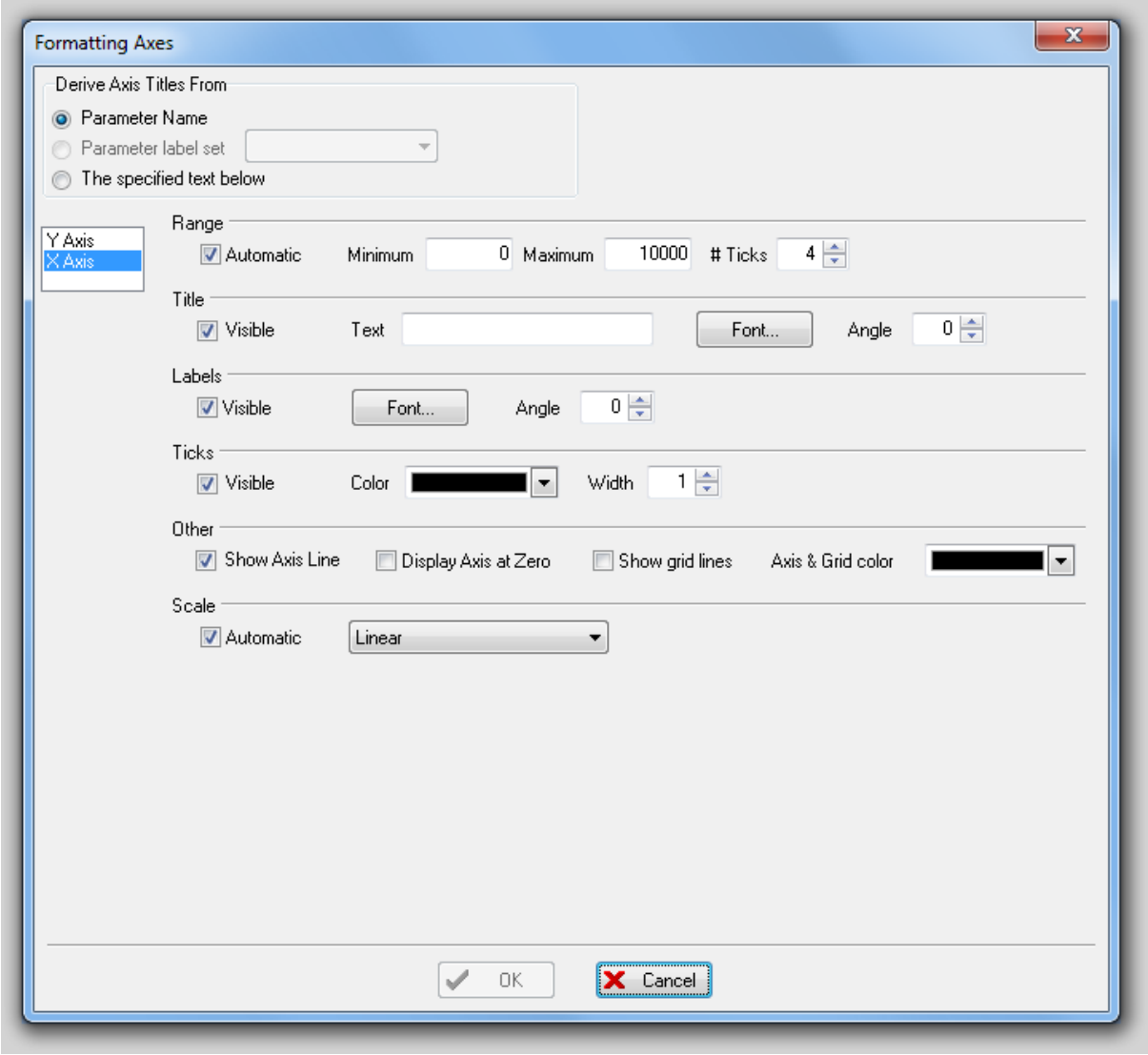

Figure 8.38 Formatting Axes Dialog

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 230 (if a label set has been defined), or the text specified in the **Text** field (under **Title**).
Y Axis/X Axis	Select the **Y Axis** or **X Axis** on the left of the dialog to determine which axis is currently being edited.

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Option	Explanation
Range	For all axes displaying data: • If **Automatic** Range is checked and the resolution options are set to automatic 152, 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.
Labels	Set the visibility, font style, size, color, and angle for the numbers which label the scale.
Ticks	Set the visibility, color, and width for the linear/logarithmic tick markers.
Other	Choose whether or not to show axis and grid lines, along with formatting their color.
Scale	If **Automatic** scaling is checked, the axis will be shown in the scale specified by the FCS file for that parameter (from the $PnE keyword). You can override automatic scaling, and choose the axis scaling 219.

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. Histograms with multiple overlays that are filled in may look better in 3D 167.

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 and use the **Format→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 8.39).

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 152.

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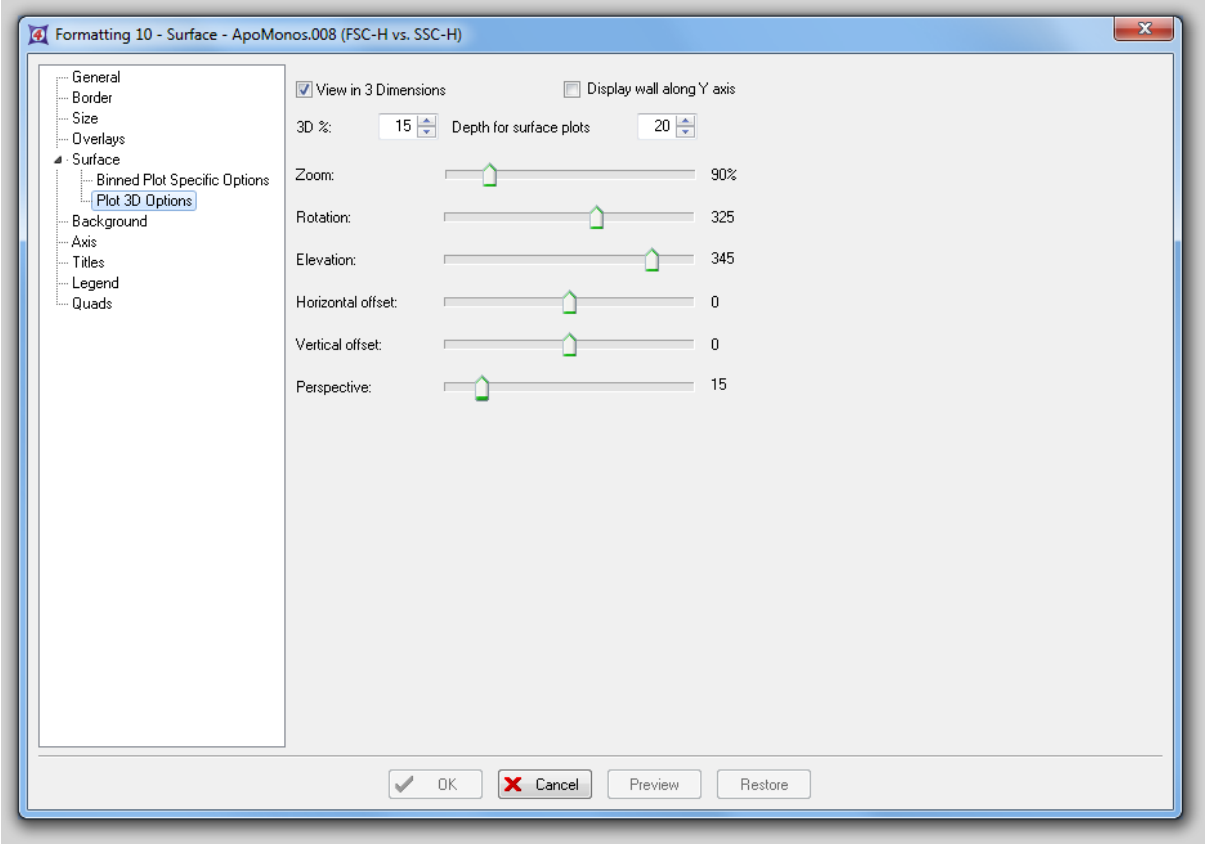

Figure 8.39 Formatting 3D Options for a Surface Plot

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

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

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.

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- **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.

8.4.6 Magnifying a Plot

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

Magnifying a histogram is equivalent to setting the minimum and maximum X-axis scales.

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.
- 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 8.40.

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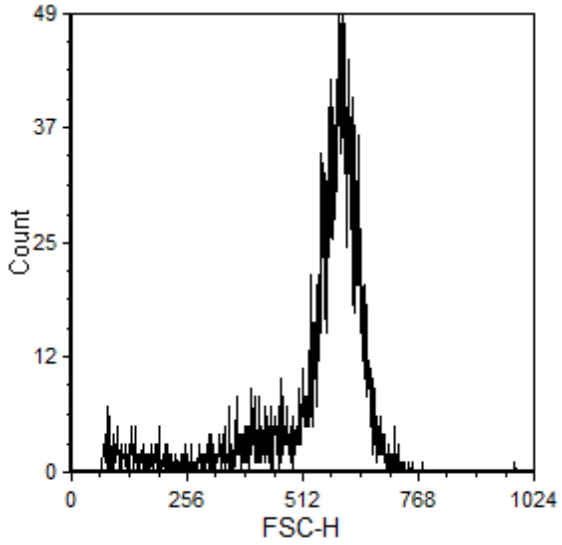

Figure 8.40 An Example Histogram Before Magnification

The result for **Magnify (Resolution)** for the region from channel 445.8 to 776.8 is shown in Figure 8.41.

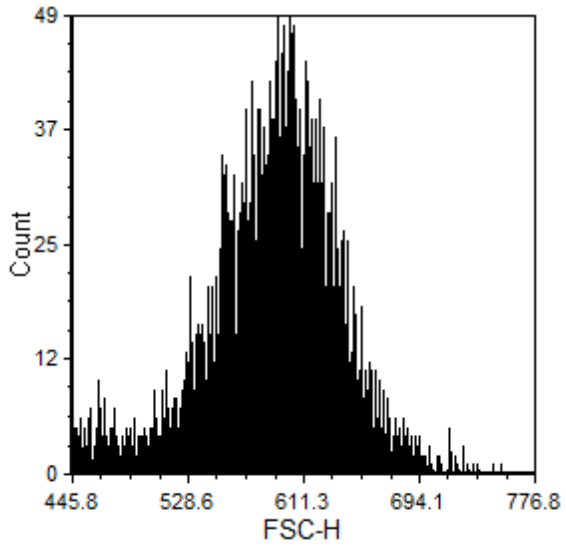

Figure 8.41 Magnify Resolution from Channel 445.8 to 776.8

The result for **Magnify (Axis)** for the region from channel 445.8 to 776.8 is shown in Figure 8.42.

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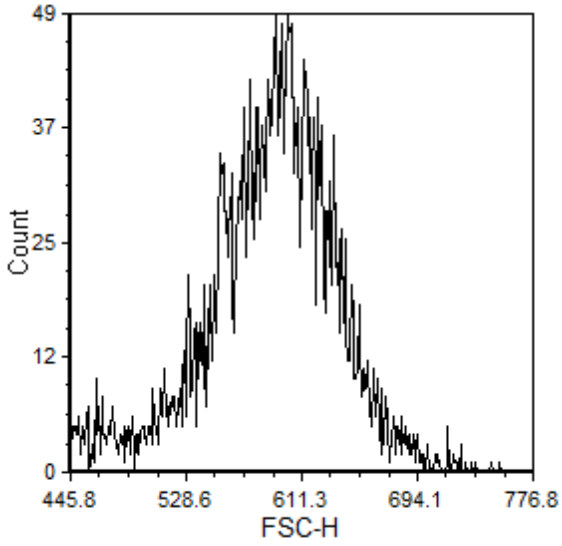

Figure 8.42 Magnify Axis from Channel 445.8 to 776.8

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 8.43.

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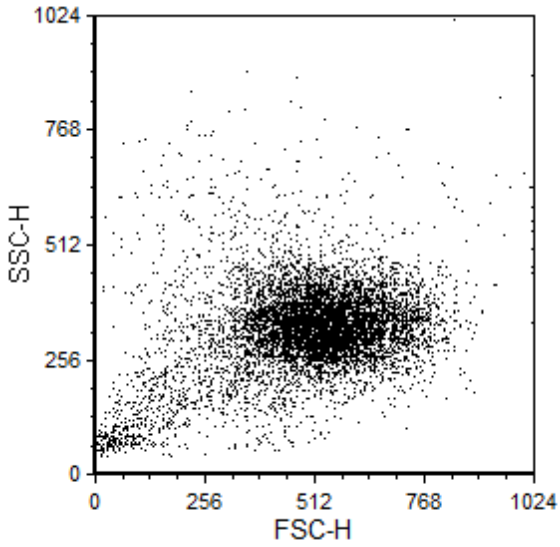

Figure 8.43 An Example 2D Plot Before Magnification

The result for **Magnify (Resolution)** for the region with the majority of cells is shown in Figure 8.44.

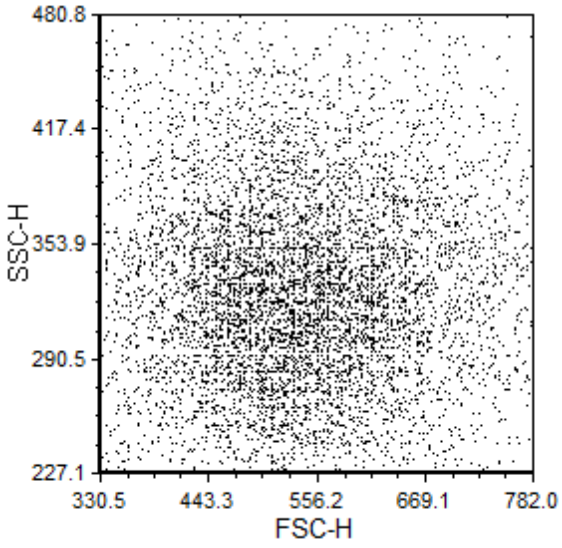

Figure 8.44 Magnify Resolution for a 2D Plot

The result for **Magnify (Axis)** for the region with the majority of cells is shown in Figure 8.45.

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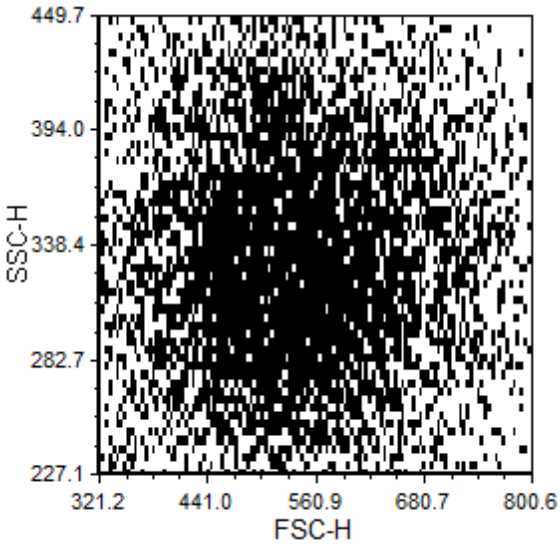

Figure 8.45 Magnify Axis for a 2D Plot

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)[597] and using the **Change Plot Type** button on the **Format** tab (Fig. 46.)

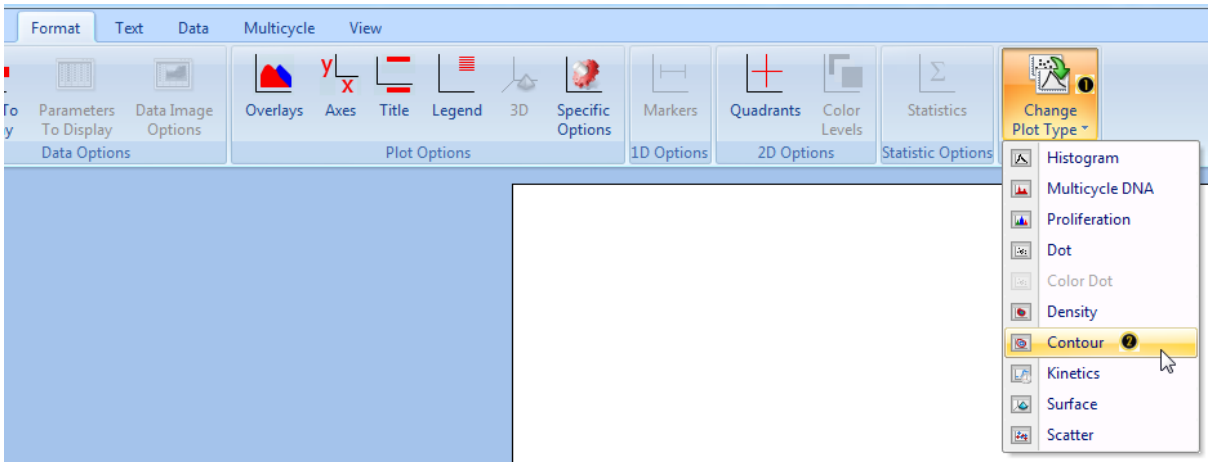

Figure 8.46 Changing the plot type from color dot to contour.

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8.5 Formatting Single Parameter Histograms

In addition to the formatting options common to all Objects[147], and those common to all Plots[152], single parameter histograms have certain properties, such as overlays[167], markers[170], magnification[162], and other specific options[172] which can be formatted.

Overlays 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 may contain one or more overlays. Overlays can be added or deleted from a histogram, but the first overlay, also known as the base overlay, cannot be removed. Furthermore, while any number of data files with parameters different from 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 becomes invaluable for making sense of complex histograms with multiple overlays.

Markers are regions defined along a single parameter.

Magnification of a histogram allows you to reset the minimum and maximum axis scale to restrict your view to a certain region of the histogram.

The histogram specific options 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 applying the changes. Alternatively, once the histogram is selected, you can choose the desired category from the Format Tab of the Ribbon[40].

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, or
- Drag-and-drop file(s) from the **Data List**[373] onto the plot you wish to overlay, or
- Drag-and-drop a plot from the layout onto the plot you wish to overlay.

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You can edit overlays in one of two ways:

- Select the histogram and use the **Format→Plot Options→Overlays** command (Figure 8.47).
- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Overlays** category.

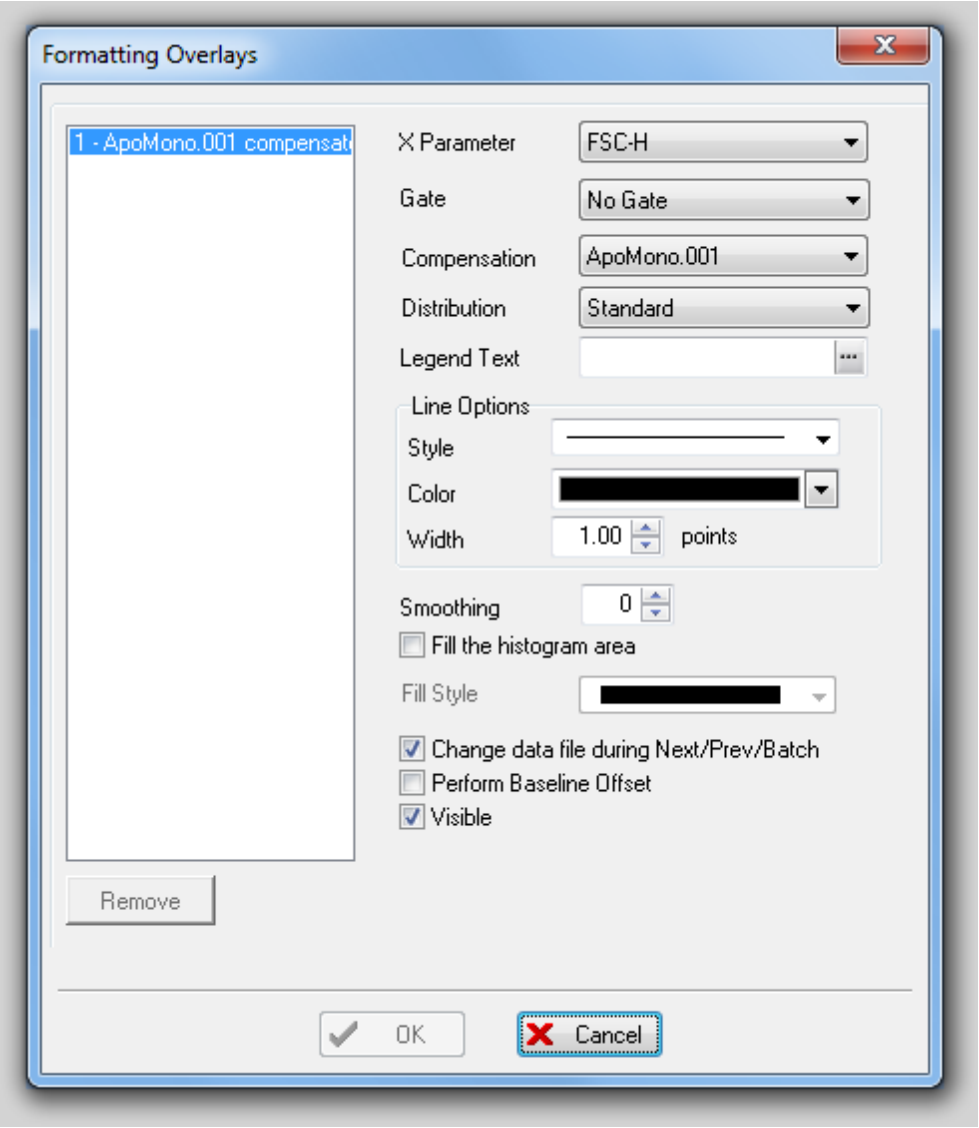

Figure 8.47 Formatting Histogram Overlays Dialog

In the dialog, a list of all the overlays on the histogram will appear in the list box to the left. You can select one or more overlays using standard Microsoft Windows techniques, including **Shift**-Click and **Ctrl**-Click. The properties on the

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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. To remove an overlay from a plot, select the overlay (or multiple overlays) and press the **Remove** button.

Overlay properties are explained in the table below.

Option	Explanation
X Parameter	Change the parameter displayed on the histogram.
Gate	If gates are defined 192, select the gate that will be used to limit the data that is displayed on the histogram.
Compensation	If compensation definitions 288 are set, select the definition to use when compensating the data in the 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. 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 inserting <GateFormula> into the legend text.
Line Options	Choose the overlay line style, color, and/or width.
Smoothing	Set the degree of smoothing for the overlay.
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 160 (Figure 8.48).
Fill Style	The pattern that will be used to fill the histogram.
Change data file during Next/Prev/ Batch	If this option is checked, the data file for this overlay will change when doing batch processing 372, or selecting Previous File or Next File 39. 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.

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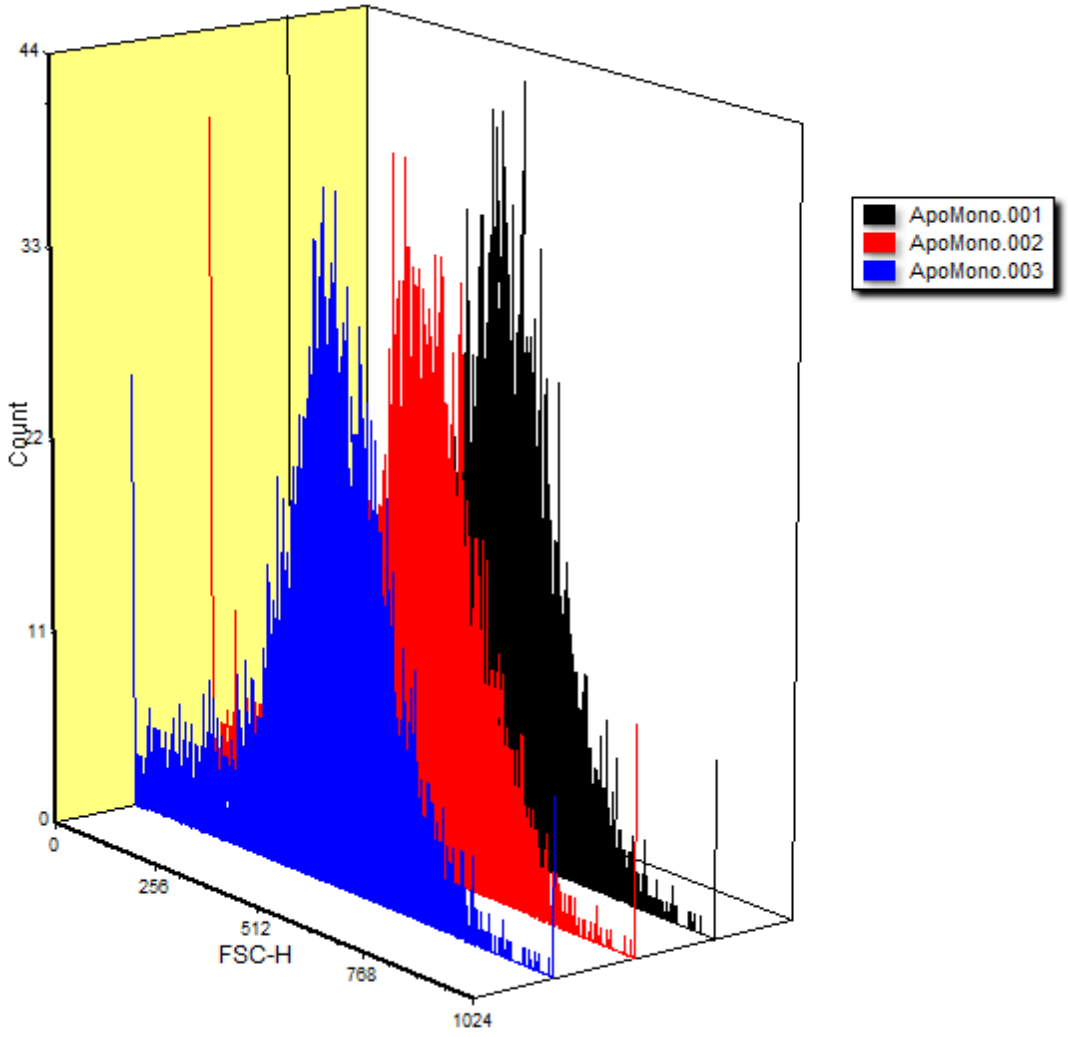

Figure 8.48 Histogram Overlays in 3D

8.5.2 Histogram Markers

The **Markers** option allows the user to control the formatting of histogram markers.

You can edit markers in one of two ways:

- Select the histogram and use the **Format→1D Options→Markers** command (Figure 8.49).
- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Markers** category.

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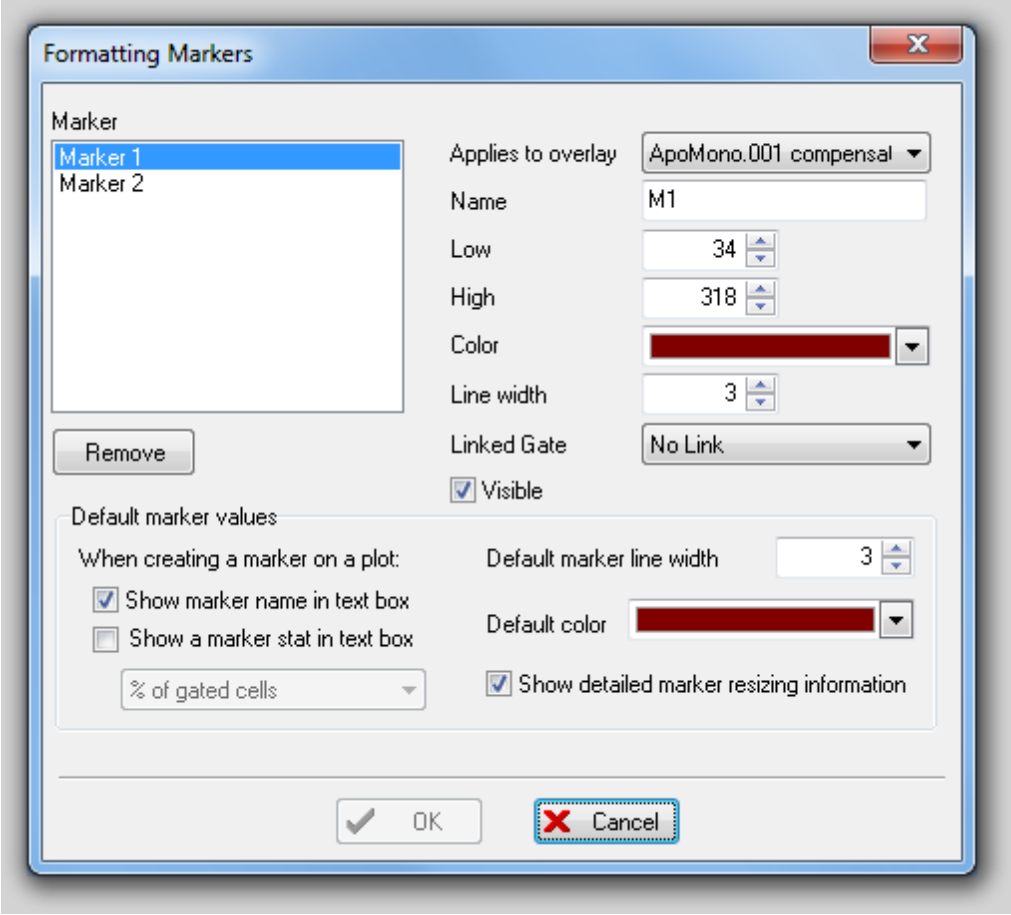

Figure 8.49 Formatting Markers Dialog

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 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.

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Option	Explanation
Low	The low channel boundary of the marker.
High	The high channel boundary of the marker.
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 210. To break the link between the marker and a gate select **No Link**.
Visible	You can hide a marker without removing it. Select the marker from the list and uncheck **Visible**. Check **Visible** to show the marker again.
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 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.

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 and use the **Format→Plot Options→Specific Options** command (Figure 8.50).
- Right-click on the histogram, select **Format** from the pop-up menu, and choose the **Histogram→Histogram Specific Options** category.

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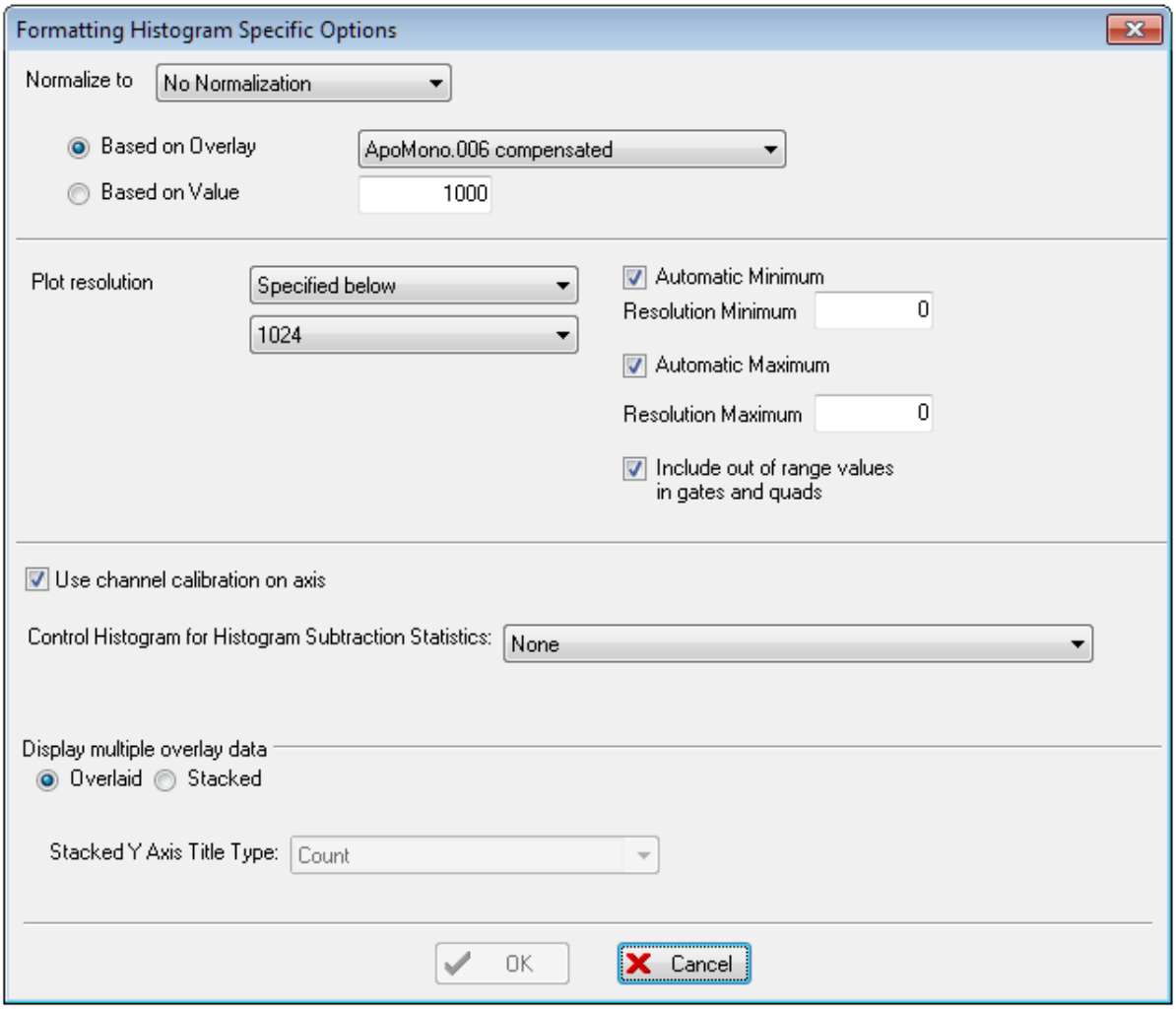

Figure 8.50 Formatting Histogram Specific Options

The options are described in the table below. See Resolution Overview 152 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. 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.
Plot resolution	In the first drop-down list, choose whether to use the resolution specified in the data or select your own resolution from the second drop-down list. 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

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Option	Explanation
	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 280. 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 274 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 8.51.
Stacked Y Axis Title Type	If multiple overlay data is displayed Stacked, choose the Y Axis Title to use.

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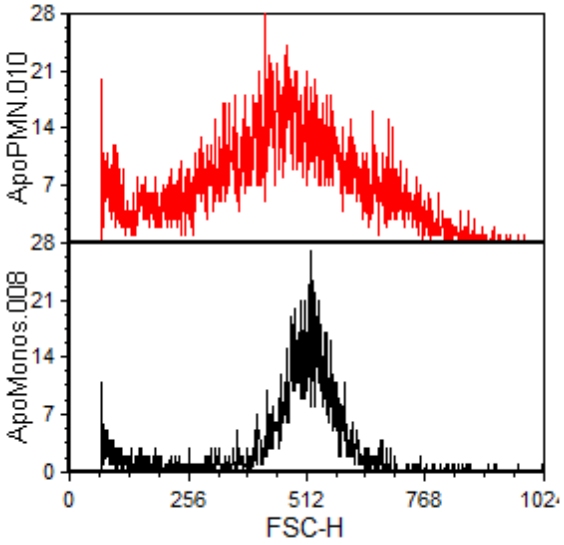

Figure 8.51 A Histogram with Two Overlays in Stacked View

8.6 Formatting 2D Plots

In addition to the formatting options common to all Objects[147], and those common to all Plots[152], 2D plots have certain properties, such as overlays[175], quadrants[178], gates to display[183], color levels[181] for density and contour plots, and other specific options[184] 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 applying the changes. Alternatively, once the 2D plot is selected, you can choose the desired category from the Format Tab of the Ribbon[40].

8.6.1 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, or
- Drag-and-drop file(s) from the **Data List**[373] onto the plot you wish to

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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 8.52).
- Right-click on the 2D plot, select **Format** from the pop-up menu, and choose the **Overlays** category.

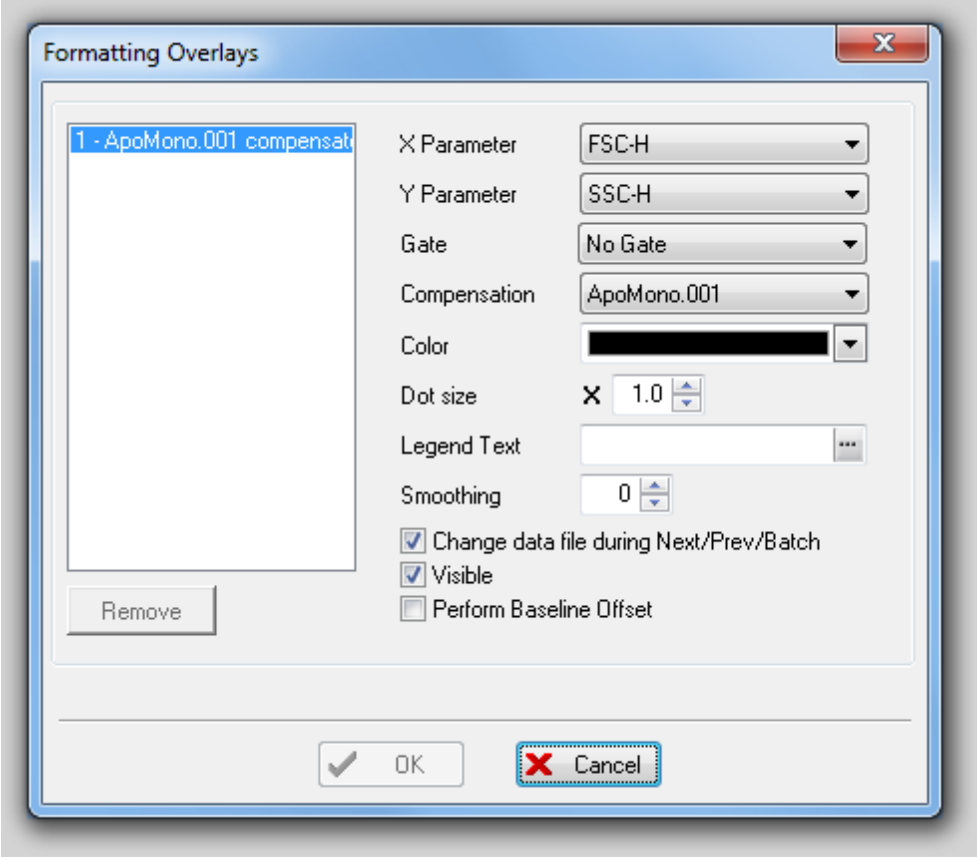

Figure 8.52 Formatting Overlays Dialog for 2D Dot Plots

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

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(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.

Overlay properties for dot plots are explained in the table below.

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 192, select the gate that will be used to limit the data that is displayed on the 2D plot.
Compensation	If compensation definitions 288 are set, select the definition to use when compensating 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 Edit Preferences Dialog 122.
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 184.
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 <GateFormula> into the legend text.
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 372, or selecting Previous File or Next File 39. 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.

For Contour and Density plots, you can choose whether or not to display **Outliers** and control the **Outlier Dot Color** and/or **Outlier Dot size** (Figure 8.53).

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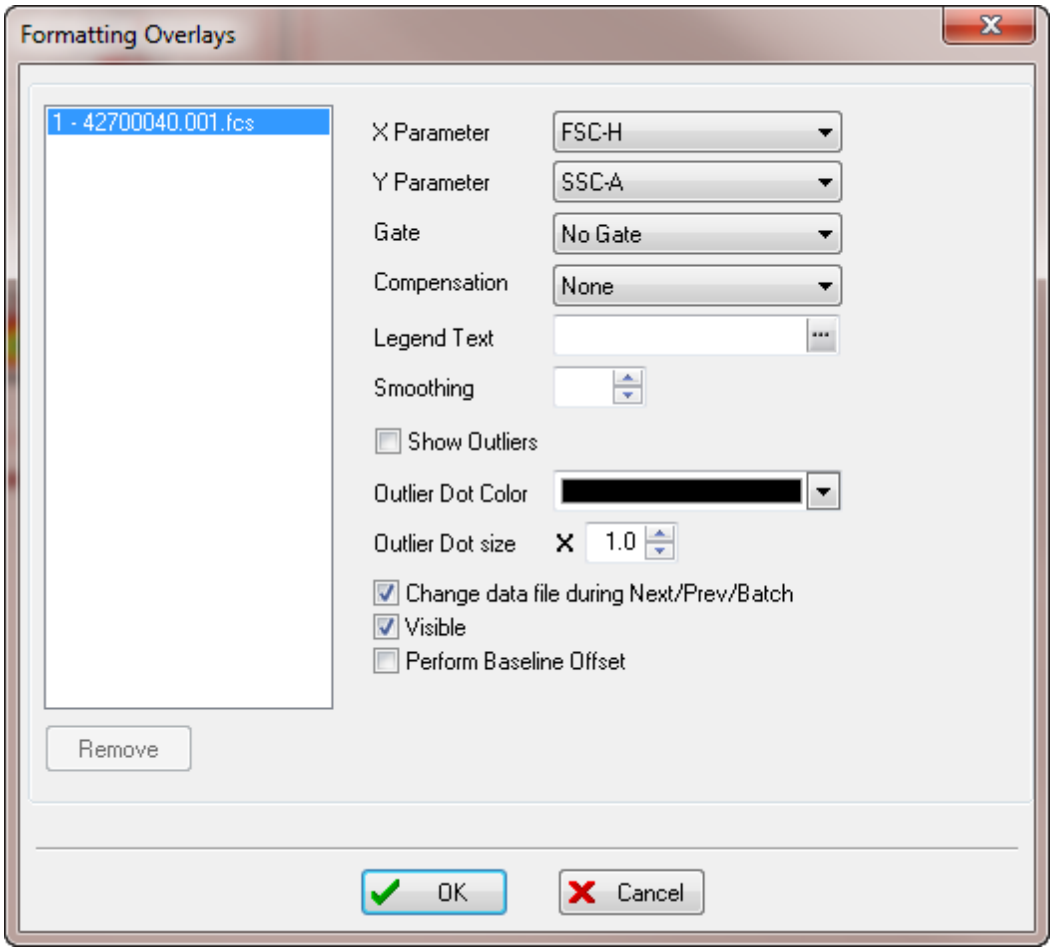

Figure 8.53 Formatting Contour or Density Plots

8.6.2 Quadrants

The **Quadrants** category allows you to manipulate the quadrants on a 2D plot. Quadrants can also be created and manipulated directly from the plot 210.

You can edit quadrants in one of two ways:

- Select the 2D plot and use the **Format→2D Options→Quadrants** command (Figure 8.54).
- Right-click on the 2D plot, select **Format** from the pop-up menu, and choose the **Quads** category.

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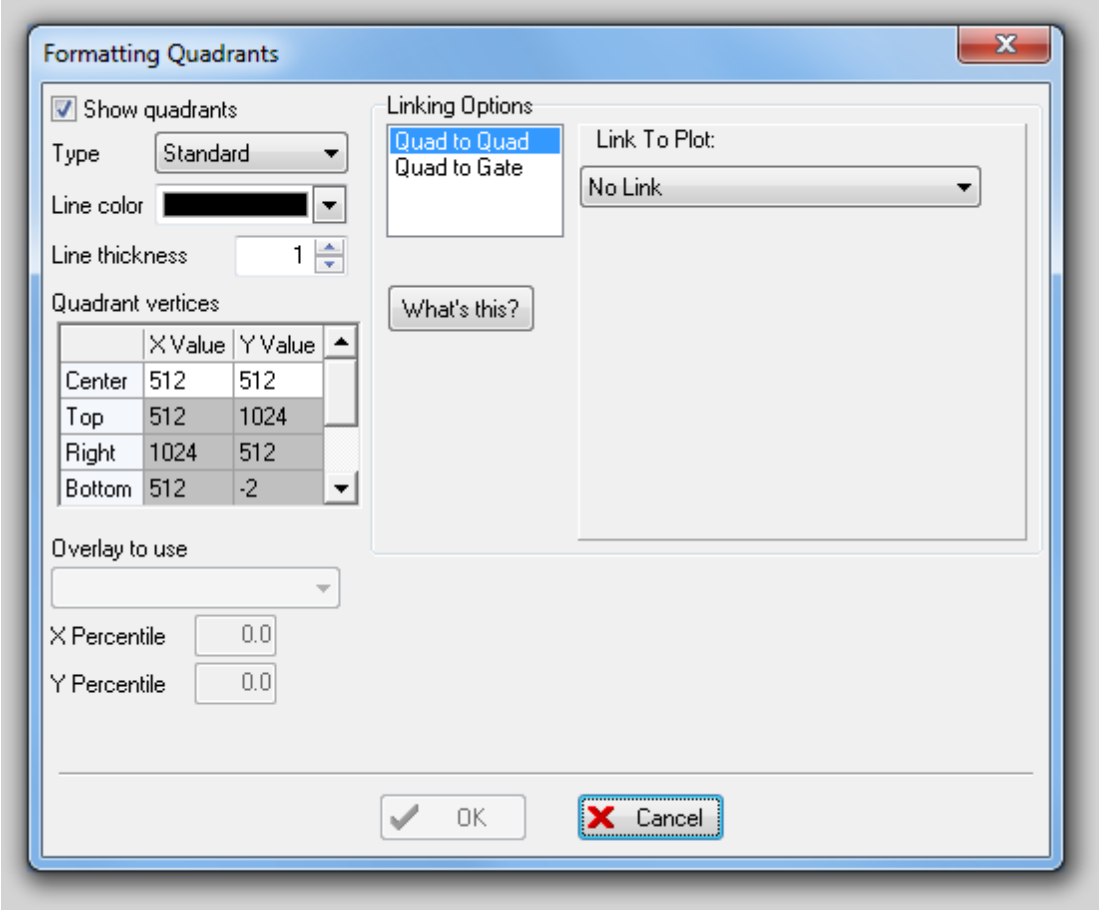

Figure 8.54 Formatting Quadrants Dialog

The options on the left side of the dialog are explained in the table below.

Option	Explanation
Show quadrants	Choose whether to display the quadrants on the plot.
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. • **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. • **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.
Line color and **thickness**	The color and thickness that the quadrant lines will be drawn in.

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Option	Explanation
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 quadrants213 or to gates214. 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.

To change the quadrant to gate link, select **Quad to Gate**. The dialog will now appear similar to Figure 8.55.

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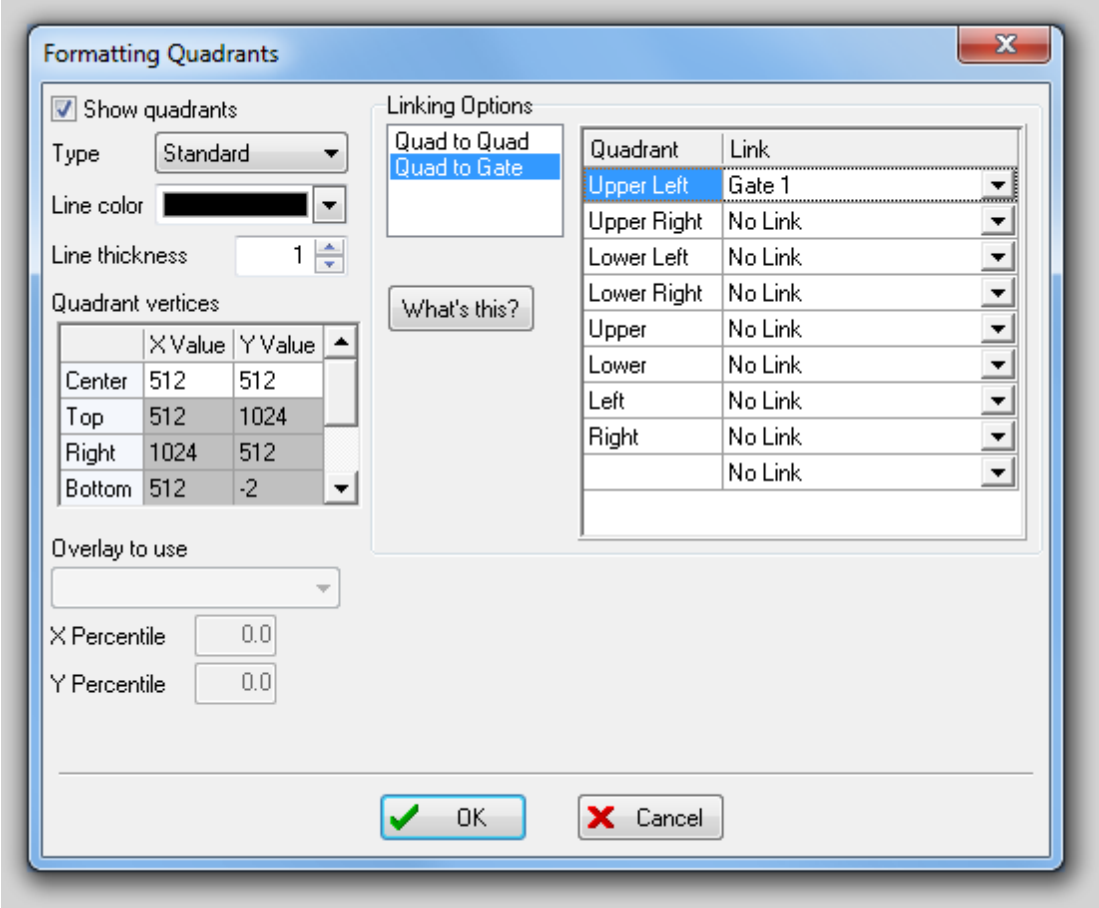

Figure 8.55 Linking Quad to Gate

In order to change the link, select the down arrow to the right of the drop-down list. The first entry is always **No Link** which allows you to break the link between the quadrant and the region. Below the **No Link** item will be a list of available gates to link to the current 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, either the gate is not a polygon/freeform gate, or that gate is already linked to another quad on the current plot or to another plot in the layout.

The **What's this?** button provides useful information on linking options.

8.6.3 Color Levels

The **Color Levels** options are used to control which colors are displayed at different density levels on a **Density** or **Contour** plot. You can edit the **Color Levels** for **Density** or **Contour** plots in one of two ways:

- Select a **Density** or **Contour** plot and use the **Format→2D Options →Color Levels** command (Figure 8.56).

- Right-click on a **Density** or **Contour** plot, select **Format** from the pop-up

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menu, and choose **Density Options→Color Levels** or **Contour Options→ Color Levels**, as appropriate.

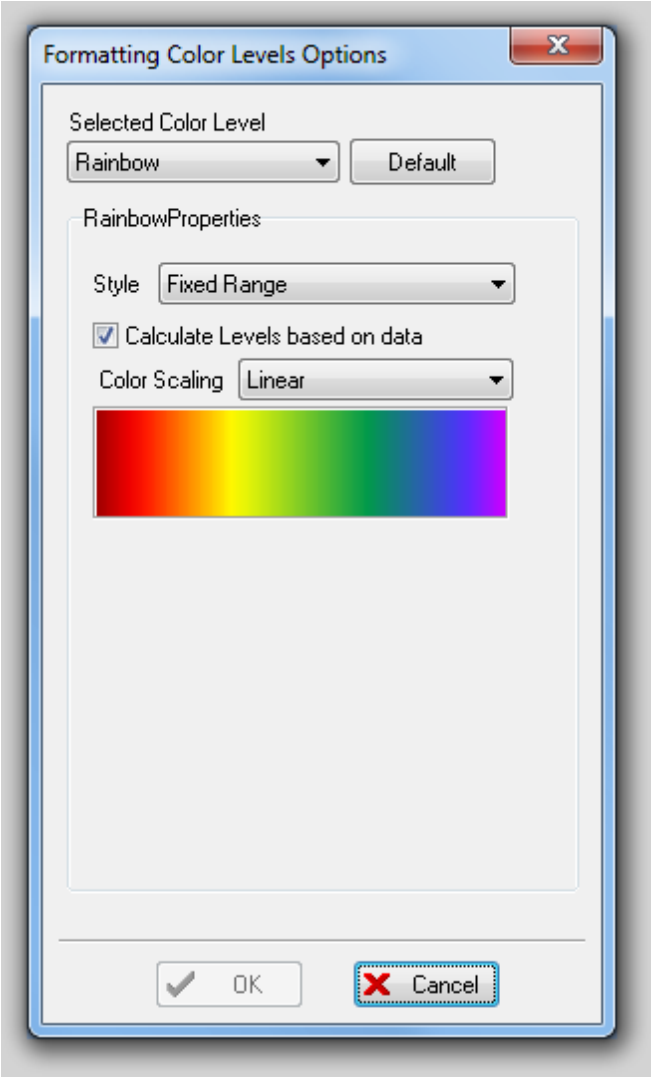

Figure 8.56 Contour Level Options

Select the color spectrum from the **Selected Color Level** drop-down list. **Rainbow** is the default for both **Density** and **Contour** plots. However, the choices in the list differ slightly for each type of plot. When you select a color level category, the colors in the chosen spectrum will be displayed in the bottom half of the dialog. You can return to the rainbow style by clicking on the **Default** button.

Contour Plots

The following choices exist in addition to the variety of color spectrums available for both types of plots:

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- **Absolute**

  The density line color will depend on whether the number of cells is less than the value in the value field.

- **Percentile**

  The density line color will depend on whether the number of cells is less than x percent of the total number of cells, where x is the value in the value field.

The color for an individual level can be changed by selecting the color and pressing 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.

The default way to display the contour levels is in color, but you can also choose to display them in grayscale using the **Selected Color Level** drop-down list. If you select grayscale, then the colors of each level will automatically be converted to shades of gray from light to dark according to the total number of levels. If you select black, then all contour levels will be black lines.

When selecting grayscale, the shades of gray are automatically displayed for you. You will not be able to edit the colors for an existing level. If you press to add a level while in grayscale, you will be prompted to assign a color from the full range of colors (not grayscale levels) available on your computer.

8.6.4 Gate Display

The gate 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 320.

You can edit gate display in one of two ways:

- Select the plot and use the **Format→Data Options→Gates to Display** command (Figure 8.57).

- Right-click on the plot, select **Format** from the pop-up menu, and choose the **Display Gate Options** category (located under the Plot Options category for each specific type of plot).

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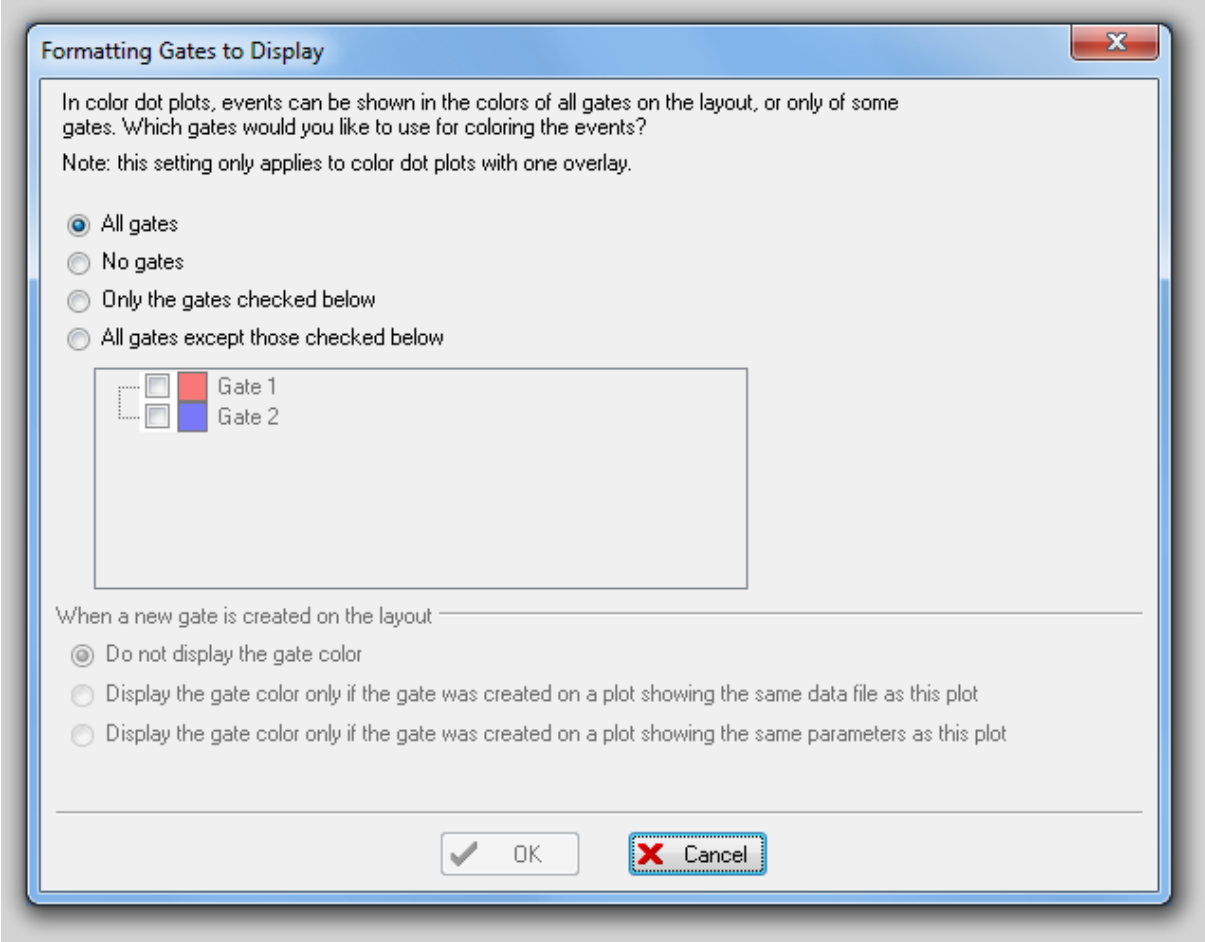

Figure 8.57 Formatting Gates to Display Dialog

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.

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.

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).

8.6.5 Specific Options

The Specific Options category contains properties to control the way data is displayed on 2D plots.

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You can edit 2D plot specific options in one of two ways:

- Select the 2D plot and use the **Format→Plot Options→Specific Options** command. For dot plots this will bring up the **Formatting Binned Dot Specific Options** dialog (Figure 8.58) or the **Formatting Binned Plot Specific Options** dialog (Figure 8.59).

- 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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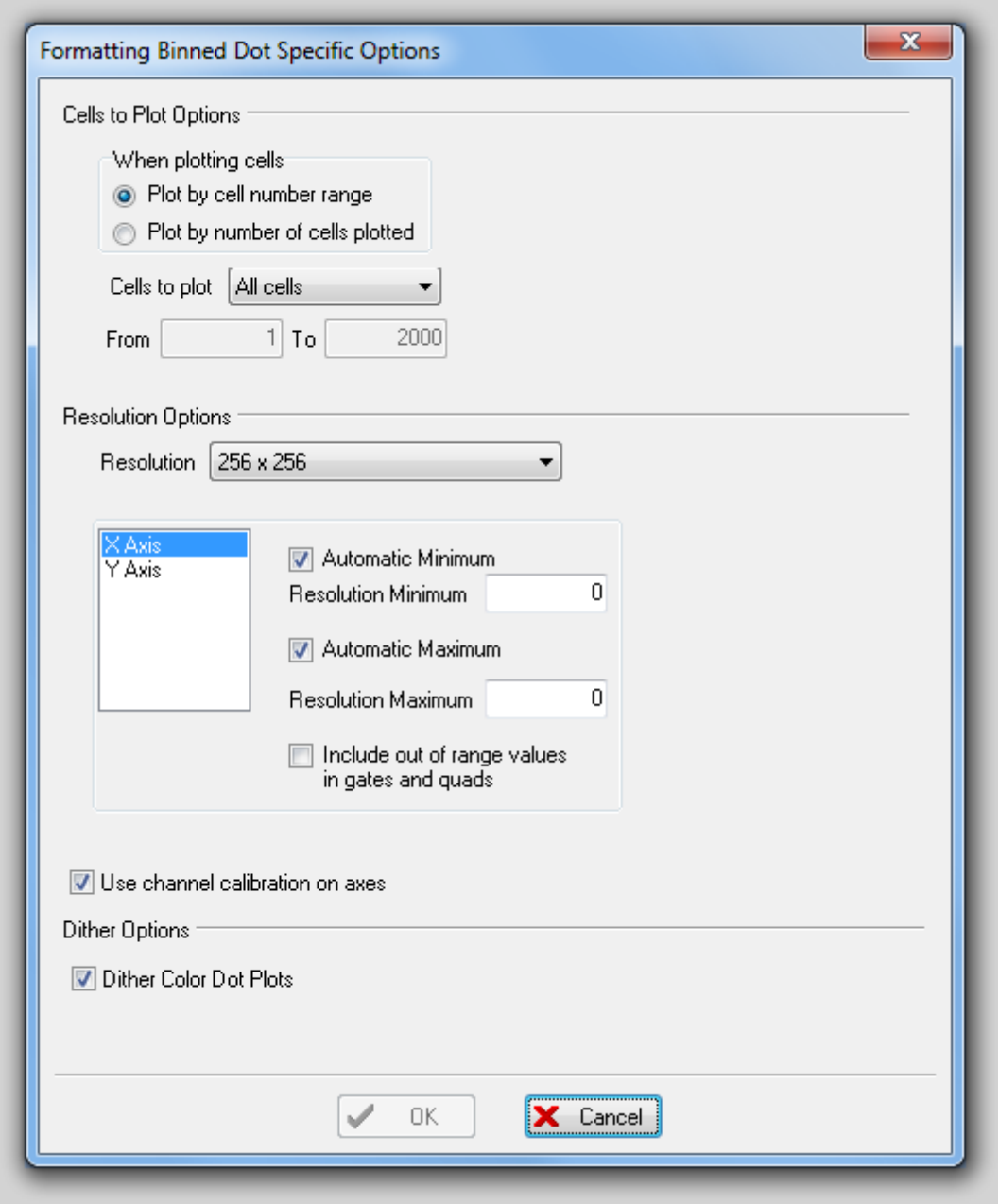

Figure 8.58 Formatting Binned Dot Specific Options Dialog

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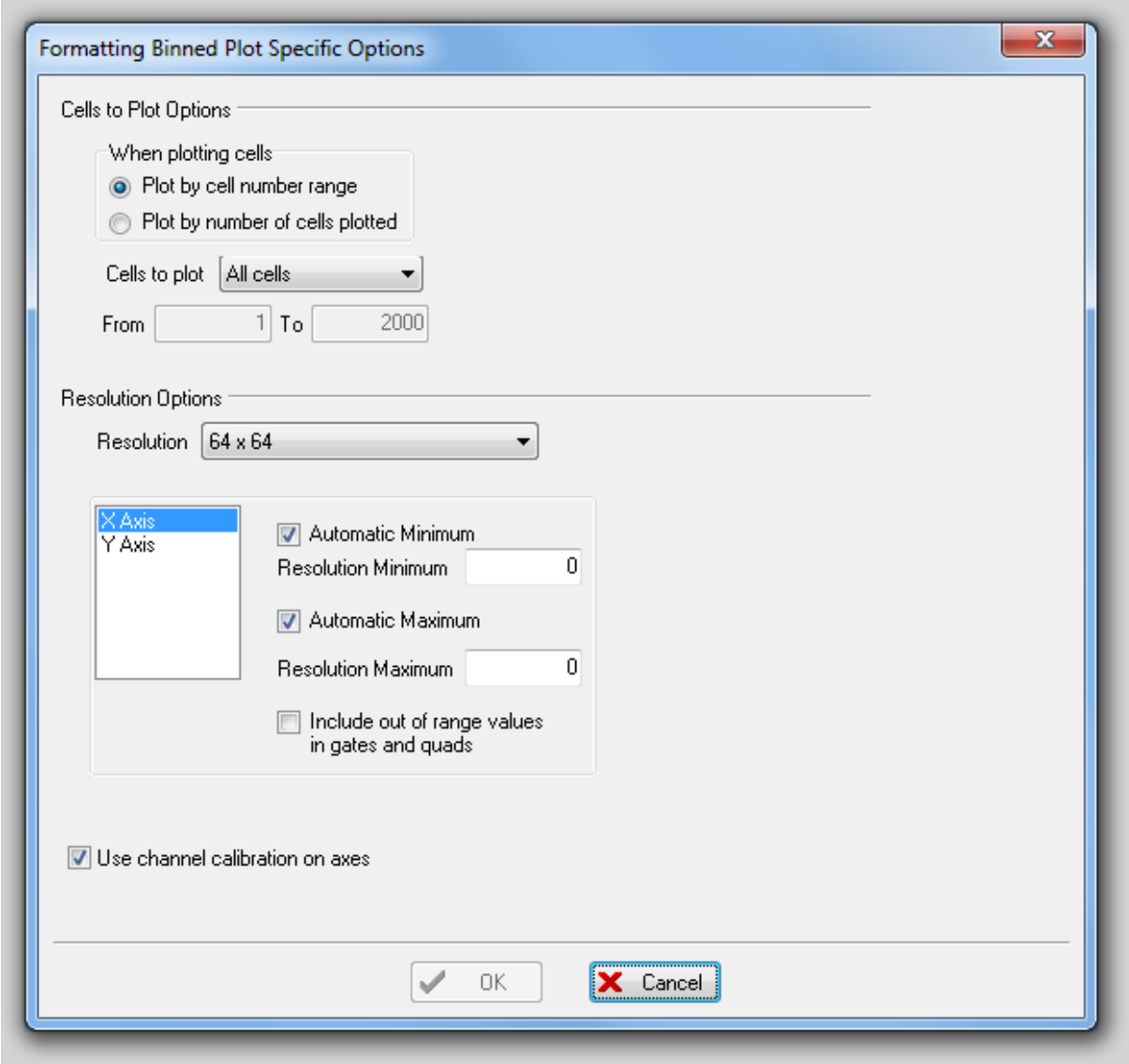

Figure 8.59 Formatting Binned Plot Specific Options Dialog

The options are described in the table below. See Resolution Overview 152 for a further description of resolution options.

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 **number range** or **number of cells plotted** option. Then, select a value from the drop-down list. Statistics are always

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Option	Explanation
	calculated based upon all of the cells in the data set, irrespective of how many are plotted.
X Axis/Y Axis	The Resolution options can be changed for the X and Y Axes separately, 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).
Resolution	Choose a resolution to display the data from the drop-down list. Lowering the resolution effectively smoothes the 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 axes	FCS Express can establish calibrated values for the axes 280. 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.
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).

8.7 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 167 and 2D plot overlays 175. You can also change the parameters of the base overlay on each plot using the keyboard or the mouse.

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Note: For either of these methods to work, you must make sure that the plot is in edit mode 79.

- **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 until it changes to the shape of a hand. When the hand appears, press and hold the mouse button and a pop-up menu will appear containing the available parameters. (You will also have to press the **Alt** key while clicking, if you have set the Alt+Click Required To Change Parameters option 120.) While holding the mouse button down, move the cursor over the new parameter in the list and release the mouse button. If you do not wish to change the parameter after the list has appeared, simply move the cursor anywhere outside the list and release the mouse button.

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Chapter

9 Analysis of FCS Data

FCS Express provides many tools, such as gates, markers, quadrants, and statistics, for flow cytometry analysis. 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, however. 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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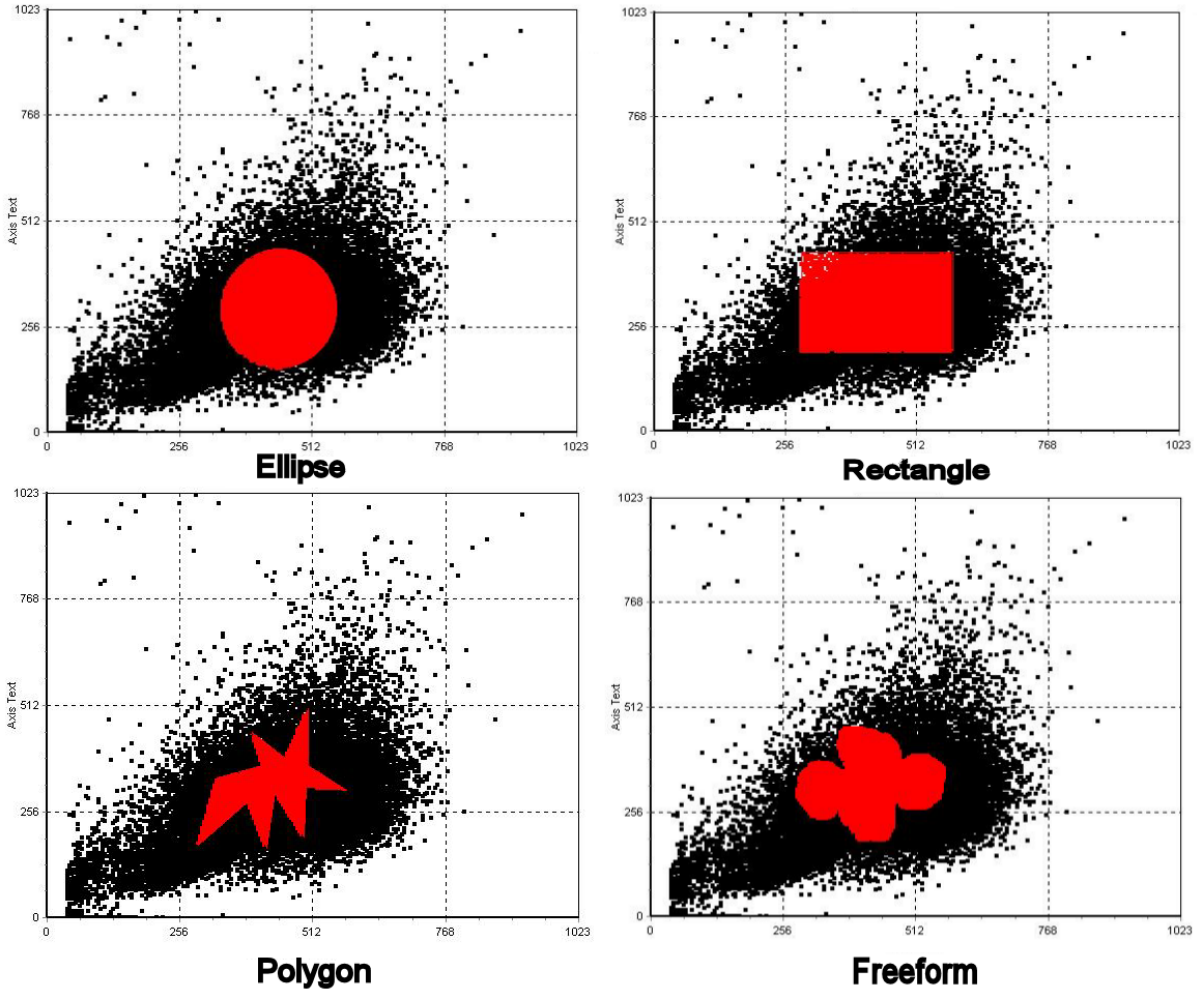

Figure 9.1 Examples of Different Gate Types

9.1.1 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**

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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 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 of all the vertices.

- 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 488 (microtiter plate).

- 1D Gates

  Create a **Marker** on a histogram as described in Creating Markers 208. 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

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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.
- 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 9.2) asking you to name the gate, choose a color, and decide if a gate should include or exclude the events contained within it. 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 drop-down list.

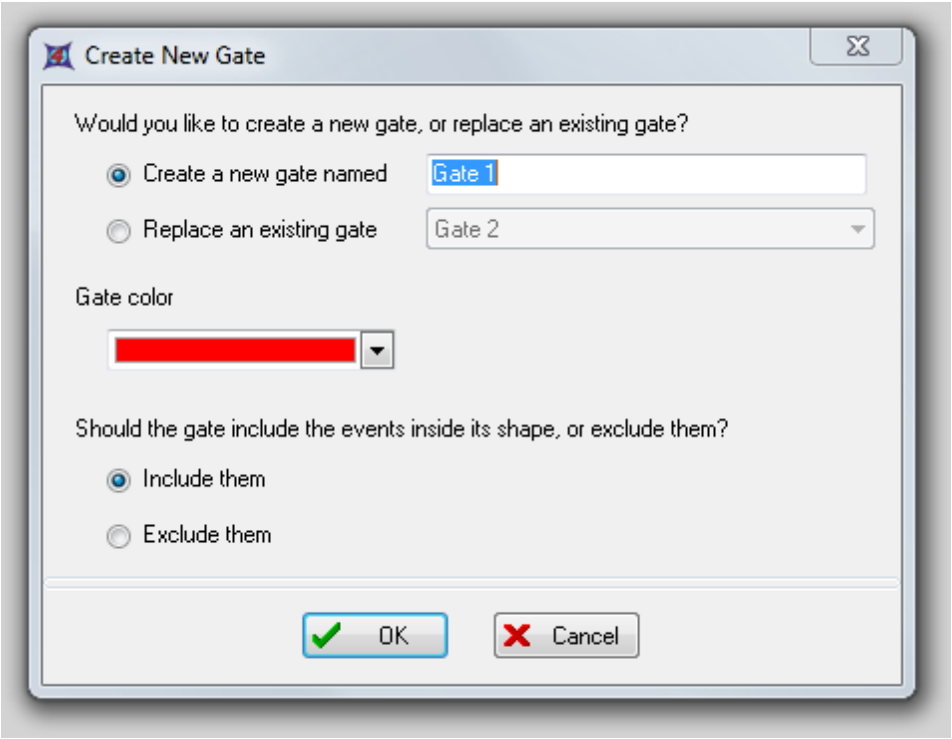

Figure 9.2 Create New Gate Dialog

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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**

  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.

  Technical Note: Certain words and letters are reserved as operators for combination gates. If gates are named as any of these specific labels a combination gate cannot be created. For example, a gate named "A" cannot be used in a combination gate and you may consider renaming the gate "A gate" to proceed with creation of a combination gate.

  Letter and Word Gate Names Not Compatible with Combination Gates (Case-Insensitive):

  - **And**
  - **Not**
  - **Or**

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- **XOR**
- **A**
- **N**
- **O**
- **X**

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 202 onto the layout (**Insert→General→ Gate View**), right-click on the **Gate View** and select **Add Combination Gate** from the pop-up menu.

A dialog box (Figure 9.3) 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

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complete the word for you. 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.

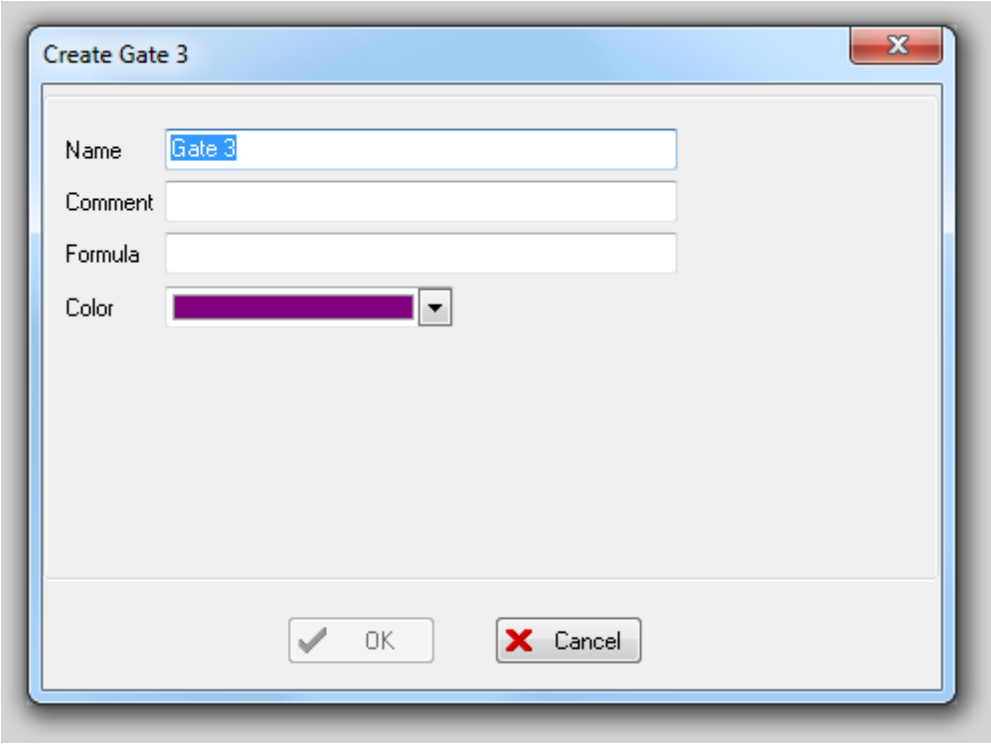

Figure 9.3 Creating a Combination Gate

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 9.4):

- CD4+/CD25+
- CD4-/CD25+
- CD4+/CD25-
- CD4-/CD25-

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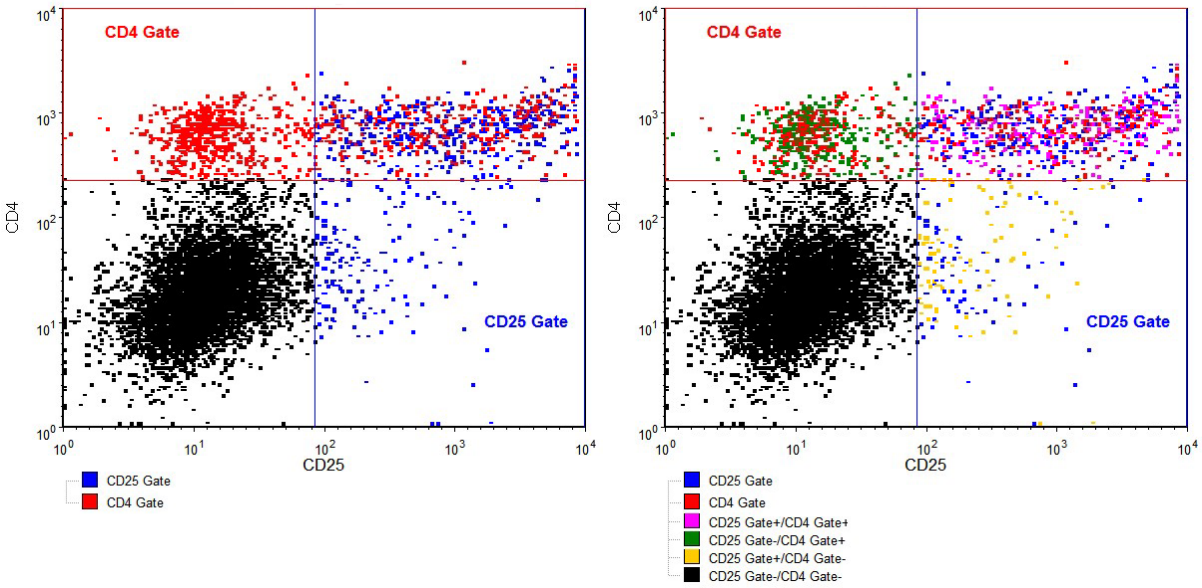

Figure 9.4 Combination Gate Matrix Example. Left: Gates applied to select all CD4+ or CD25+ cells. Right: Combination Gate Matrix applied to create gates for all four combinations.

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 202 onto the layout (**Insert→General→ Gate View**), select the gates that you want to use, and then right-click on the **Gate View** and select **Add Combination Gate Matrix** from the pop-up menu (Figure 9.5).

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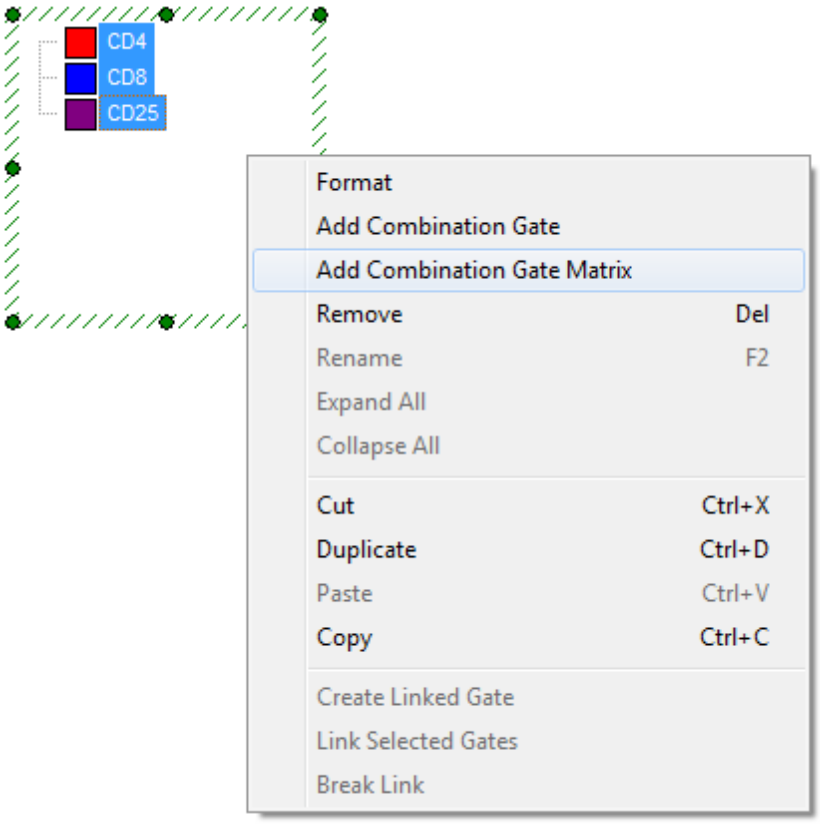

Figure 9.5 Adding a Combination Gate Matrix

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 9.6.

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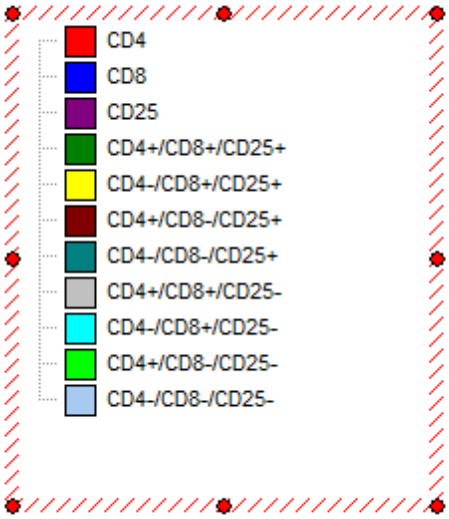

Figure 9.6 After Adding a Combination Gate Matrix

9.1.2 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 the mouse button down anywhere inside the gate. Move the mouse to move the cursor, and the gate will move with the cursor. Release the mouse to drop the gate into the new position.

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 preference you set in the Gate moving options section on the Gate page of the Edit Preferences dialog 134.

9.1.3 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 change to the drag cursor and resizing handles will appear on the vertices of the gate. 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

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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 **Esc** key before you release the mouse button.

9.1.4 Deleting Gates

A gate can be deleted in one of two ways:

- Select the gate from a Gate View object 202 and either: Right-click and choose **Remove** from the pop-up menu; press the **Delete** key on the keyboard; or select the **Home→Editing→Delete** command.

- 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 or select the **Home→Editing→Delete** command. A dialog box will appear asking whether you want to delete the gate completely, or just hide the gate 202 on that plot.

9.1.5 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. Click the mouse button when the cursor is in the gate that you wish to hide to activate the gate.
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.

9.1.6 Gate View Object

The **Gate View** object allows you to place a list of the gates on a page. 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 147.

To insert a **Gate View**, select the **Insert→General→Gate View** command.

Many of the actions can be performed directly from the **Gate View** as follows:

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- To delete a gate from the entire layout, select the gate in the **Gate View** and press the **Delete** key.
- To rename a gate, select the gate in the **Gate View** and press **F2**. Type in the new gate name 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 9.7). If you check **Alt+Click required to select this 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.
- To rearrange the hierarchy of the gates 206, 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.
- To gate a plot on a gate, drag the gate from the **Gate View** onto that plot.

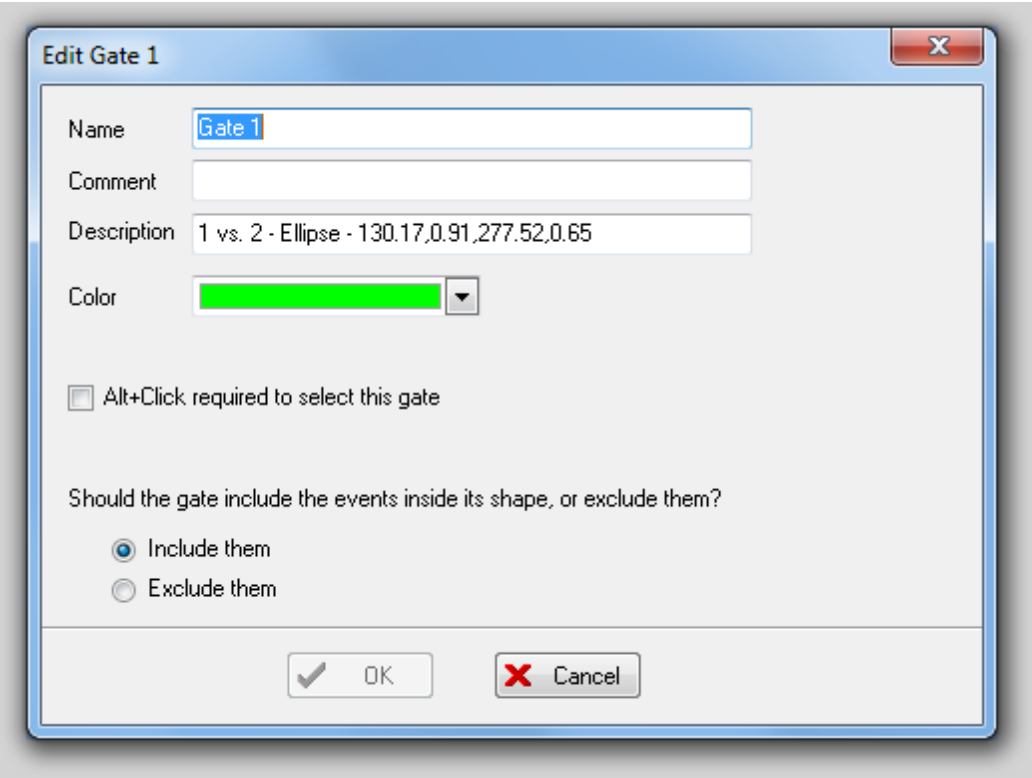

Figure 9.7 Edit Gate Dialog

9.1.7 Saving and Loading Gates

Gates can be saved and loaded so that gates created in one layout can be imported into another layout. Saving the gates saves all information pertaining to the gates, and will also preserve any gating hierarchy. To save and load gates,

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select the **Gating→Gates→Save** and **Gating→Gates→Load** commands, respectively.

9.1.8 Gates and the Clipboard

Gates can be copied to and pasted from the clipboard for use within FCS Express.

- **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 202 and select **Home→Clipboard→Copy Gate** or press **Ctrl+C** from the keyboard.

- **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 202 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.

9.1.9 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. Not all overlays on a plot have to be gated with the same gate.

- Create a Gate View 202 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.

- Select a gate on a plot. While holding the mouse button down, drag and drop the gate to another plot.

- Select a plot or multiple plots 597 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** page (see Formatting Single Parameter Histograms 167 or Formatting 2D plots 175) and choose the gate you want to apply from the drop-down list.

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9.1.10 Linking Gates

In FCS Express Version 4, 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 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 9.8).

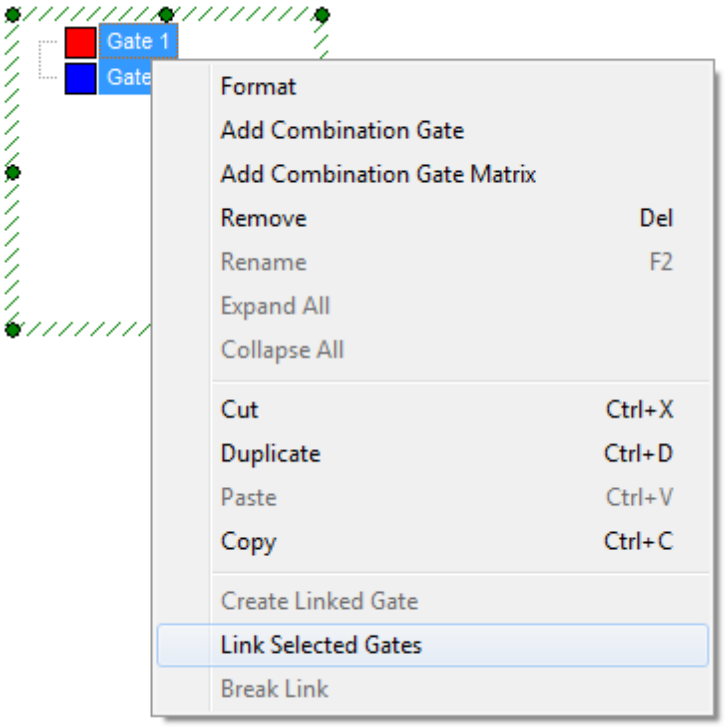

Figure 9.8 Linking Gates

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 9.9).

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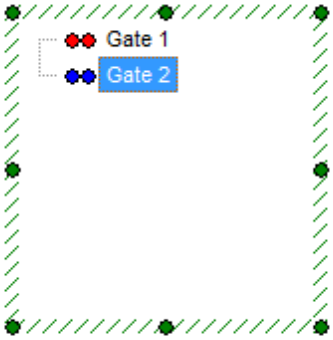

Figure 9.9 Linked Gates with Chain Icon

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.

9.1.11 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, 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:

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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. 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 a Gate View 202.

In Figure 9.10, the hierarchical gating strategy for the gates in our example are displayed.

Figure 9.10 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.

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 preferences 134, the new gate will become a child of the plot's current gate. Note that the default for this option is off, but a tutorial 1517 with step-by-step instructions for turning on this **Preference** is available.

9.2 Using Markers

Markers are areas (sometimes known as "regions" in other data analysis software) that you set on a one-dimensional plot.

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9.2.1 Creating Markers

To create a marker on a plot do one of the following:

- Select the **Gating** (or **Home**)→**Create Gates**→**Create Marker** command.
- 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. Position the cursor where you want the right marker boundary to be, and release the mouse button.

9.2.2 Moving Markers

Markers can be moved in one of the following ways:

- Click anywhere on the marker to select it. When the marker is selected, three resizing handles will appear, one on each end, and one in the center. The middle handle is used for moving the marker. When the mouse cursor is over the central handle, the cursor will turn into a four-headed arrow. Press and hold the mouse button when the cursor is the four-headed arrow and move the marker to a new position. Release the mouse when the marker is in the new position. To cancel moving the marker, press the **Esc** key before releasing the mouse button.
- With the plot containing the marker you want to move in selected or edit mode, use the **Format→1D Options→Markers** command. This brings up a **Formatting Markers** dialog. In the list box, highlight the marker you want to move. The low and high parameters represent the channel numbers of the left and right marker boundaries, respectively. Change the low and high parameters by either typing in new numbers or using the arrow buttons to incrementally change the numbers. Click on **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 you want to move in the list box. The low and high parameters represent the channel numbers of the left and right marker boundaries, respectively. Change the low and high parameters by either typing in new numbers or using the arrow buttons to incrementally change the numbers. Click on **OK**.

9.2.3 Resizing Markers

Markers can be resized in one of the following ways:

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- Click anywhere on the marker to select it. When the marker is selected, three resizing handles will appear, one on each end, and one in the center. The resizing handles on the ends are used for resizing the marker. When the mouse cursor is over one of the resizing handles, the cursor will turn into a two-headed arrow. Press and hold the mouse button when the cursor is the 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.

- 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 list box, highlight the marker you want to resize. The low and high parameters represent the channel numbers of the left and right marker boundaries, respectively. Change the low and high parameters by either typing in new numbers or using the arrow buttons to incrementally change the numbers. Click on **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 you want to resize in the list box. The low and high parameters represent the channel numbers of the left and right marker boundaries, respectively. Change the low and high parameters by either typing in new numbers or using the arrow buttons to incrementally change the numbers. Click on **OK**.

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 resizing handles will appear, one on each end, and one in the center. 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 list box, highlight the marker(s) you want to delete. Click on the **Remove** button. Click on **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 on **OK**.

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9.2.5 Using Markers and the Clipboard

- Copying/Cutting a marker to the clipboard

  Click anywhere on the marker to select it (three resizing handles will appear). Use the **Home→Clipboard→Cut Marker** (or **→Copy Marker**) command.

- Pasting a marker

  Select the plot where you want the marker to be pasted. Use the **Home→ Clipboard→Paste Marker** command.

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 170.

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. If the Show prompt window radio button is set in Gate creation prompt options 134 (Gate Preferences category of Edit Preference dialog), you will be prompted to enter a name for the new gate.

To break a linked gate:

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**.

9.3 Using Quadrants

FCS Express supports three different kinds of Quadrants 178: 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 center point. Moving the center point of the quadrants will move all four quadrants.

Floating quadrants allow the four quadrants to be non-rectangular. When the

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quadrants are floating, each quadrant can have a different shape. The end points of the quadrants are no longer linked to the center of the quadrants and each vertex of the quadrants can be manipulated individually (see Floating Quads example, below). Floating quadrants allow the user to quickly and easily get statistics on populations that are not perpendicular to the x or y axes. This can eliminate the need for compensation when only percentages are needed.

Examples of standard quadrants and floating quadrants are shown in Figure 9.11.

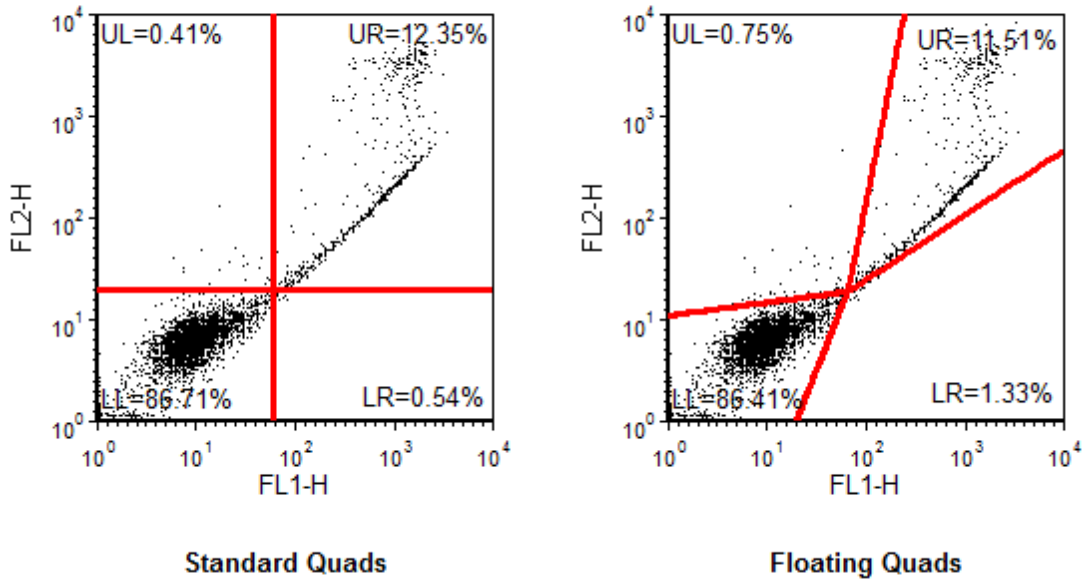

Figure 9.11 Standard vs Floating Quads

Percentile quadrants are similar to standard quadrants except that the vertex of the quadrant is automatically calculated based on the percentile entered in the Quads page of the format dialog[178]. 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 is very 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[213].

9.3.1 Selecting Quadrants

To select the quadrants, click on the vertex where all the lines meet. If a standard quadrant is selected, a resizing handle will appear at the central vertex. If floating quads are selected, resizing handles will appear over all the vertices.

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9.3.2 Showing Quadrants

Only one set of quadrants can exist for a plot. They are automatically created when the plot is created. You can control whether quadrants are automatically displayed by enabling the **Show quadrants** checkbox in the 2D Plots page of the Edit Preferences 122 dialog. The default behavior is for the quadrants to be hidden when the plot is created.

You can show the quadrants 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.

9.3.3 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.

- **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 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. Side 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 178. *Note:* Format windows where you can enter vertex coordinates can be reached in one of two ways, either: Right-click a

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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.

Note: If quadrants are linked to the quadrants on another plot 213, the quadrants on the other plot will move automatically. In addition, you cannot move percentile quadrants (see the Quadrants section under Formatting 2D Plots 178).

9.3.4 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** checkbox.
- Select the plot and use the **Format→2D Options→Quadrants** command. Uncheck the **Show quadrants** checkbox.
- Select the quadrants by clicking on the central vertex. Press the **Delete** key on the keyboard, or select the **Home→Editing→Delete** command.

9.3.5 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 negative quadrant on your control, link the control quad to your sample data quads, and then update the quads on your sample data automatically when you adjust the control quad. To set up linked quadrants, see Quadrant Linking Options 178.

9.3.6 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:

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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 214.

9.3.7 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.

You can create a linked gate by:

- Right-click on a plot and select the Convert and Link from the Convert Quads to Gates 213 item in the pop-up submenu (after choosing the quadrant from the submenu).

- Right-click on the plot and select **Format** from the pop-up menu. Select the **Quads** category. Choose **Quad to Gate** from the list box. Select a quadrant and an existing polygon or freeform gate from the drop-down list. An explanation of quadrant linking is available by clicking in the **What's this?** button and further information is available in the Quadrants section under Formatting 2D Plot 178. Alternatively, you can reach the format window by selecting a plot and using the **Format→2D Options→Quadrants** command.

9.3.8 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. *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.

9.4 Statistics

FCS Express can generate any statistic that one would need for analysis of flow cytometry data. Statistics can be calculated either from a histogram or a 2D plot. Statistics are only calculated for cells that are accepted by the gating formula for the overlay.

FCS Express calculates statistics to be accurate to the IEEE single precision

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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.

More information on the default statistics available for 1D- and 2D-plots, as well as using and formatting statistics are in the subsections below:

- Histogram statistics 215
- 2D plot statistics 216
- Using statistics and the clipboard 218
- Formatting statistics 218

9.4.1 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 208 and Histogram Markers 170).

To view the histogram statistics, right-click on the plot and select **Statistics** from the pop-up menu.

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 167 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 **Edit Preferences** Dialog under Data Loading - FCS File Options 144.
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 170).
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.
% 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

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	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.
Peak Value	The number of events that are in the peak channel.
Peak Channel	The channel in which the greatest number of events are located.
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 268.
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 172.
Standard Deviation	A measure of the variation from the average for the events. The square root of the variance.

When statistics have been added to your layout, selecting the **Format→ Statistics Options→Statistics** command will bring up a dialog box where you can format the statistics. The option **Calculate M0 for overlays** is used to display statistics for the entire overlay with no marker applied in addition to the statistics calculated for a marker.

9.4.2 2D Plot Statistics

There are two types of statistics that can be displayed for a 2D plot. The same statistics are calculated in either case. However, the way the events on the plot are divided for statistical purposes are different.

- **Gate Statistics**

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The statistics are calculated for every gate designate in the **Gates to Display** data option, even if the gate is not being displayed on the plot. The Gates to Display can be chosen from a dialog reached by using the **Format→Data Options→Gates To Display** command, or in the Formatting Statistics 218 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.

In order to open a statistics window, right-click on a 2D Plot and choose **Gate Statistics** or **Quadrant Statistics** (if Quadrants are not being shown, this option is not enabled) from the pop-up menu.

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 175 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.
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	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

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Statistic	Explanation
Variation)	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 Gate 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 268.
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.

9.4.3 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. You can then paste the statistics into a text file or a spreadsheet. Cutting cells from the statistics window is not allowed.

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, 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.

9.4.4 Formatting Statistics

There are several options to control which statistics get displayed in any individual statistics window.

You can change the displayed statistics in one of the following ways:

- Right-click on the statistics window. In the pop-up menu, there will be a list

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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.

- Right-click on the statistics window and select **Format** from the pop-up menu. Individually select each statistic you want to display by enabling the appropriate checkbox in the list box on the **Stats** page.

- Select the statistics window and use the **Format→Statistics Options→ Statistics** command. Select each statistic you want to display by enabling the appropriate checkbox in the list box.

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.

To set the default statistics that appear when you first open a statistics window, use the Plot Statistics category 126 of the **Edit Preferences** dialog. This feature is convenient if you routinely prefer to see the same set of statistics during data analysis.

9.5 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.

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- 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.

$$T(x) = \begin{cases} Log(x), x > 0 \\ -Log(-x), x < 0 \\ 0, x = 0 \end{cases}$$

  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.

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$$T(x) = \begin{cases} ax, x < t \\ Log(cx), x > t \end{cases}$$

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.

9.5.1 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.

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[219].

An example of setting the *Lin/Log* scaling with a Transition Point of 200 is shown in Figure 9.12. Transition values are entered in channel units, and do not have to be scaled as described in the Bagwell paper.

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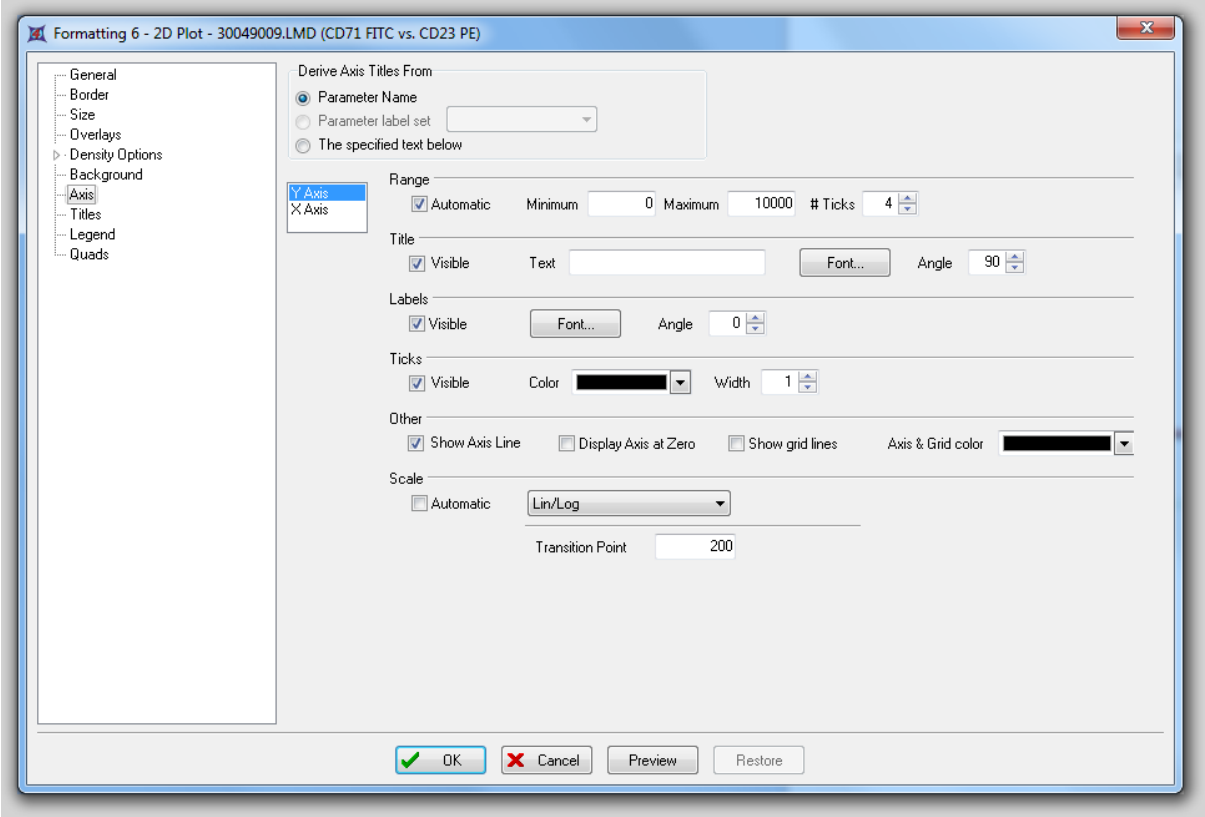

Figure 9.12 Setting the Transition Point for the Y-axis in Lin/Log Scaling

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 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 9.13).

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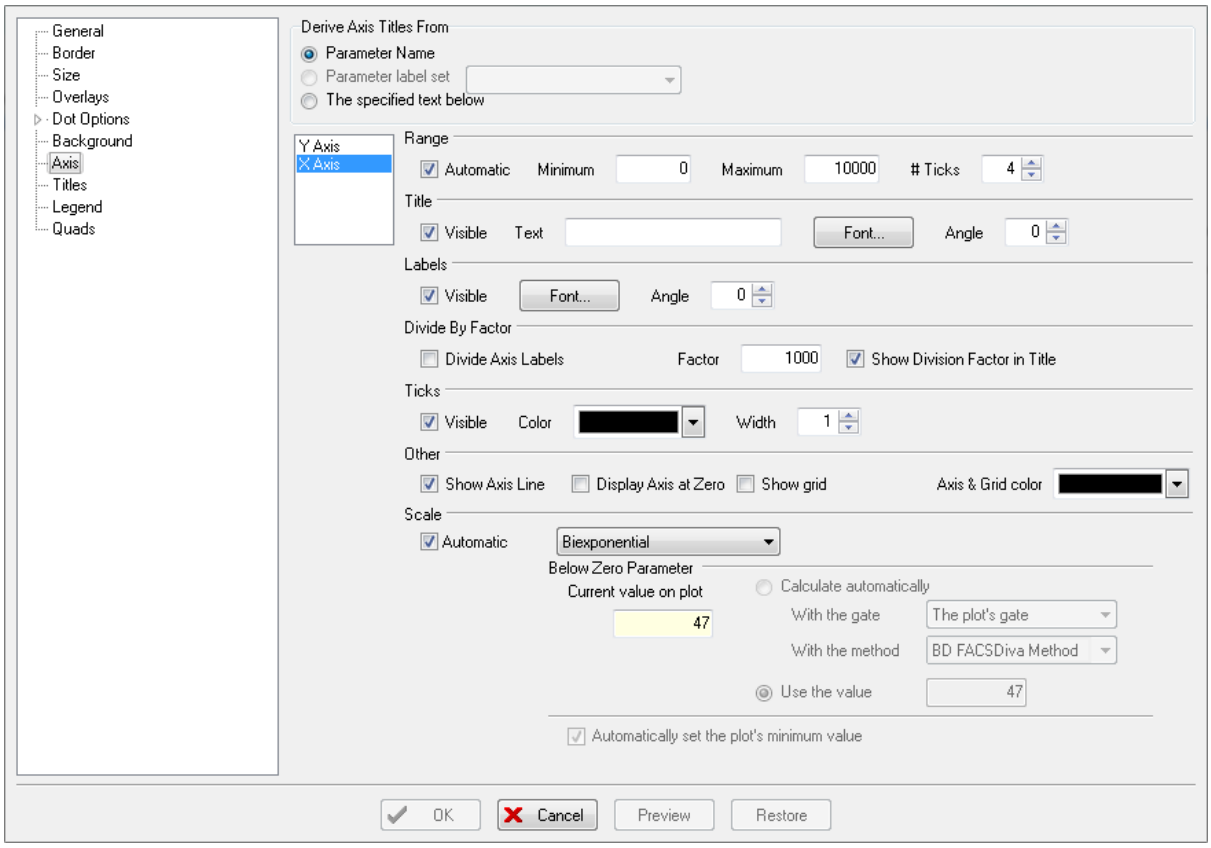

Figure 9.13 Automatic biexponential scaling in FCS Express of a file acquired in BD FACSDiva.

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 transition point is set to optimize the display by not displaying outliers. When unchecked the entire range of data, including outliers, will be displayed (Figure 9.14).

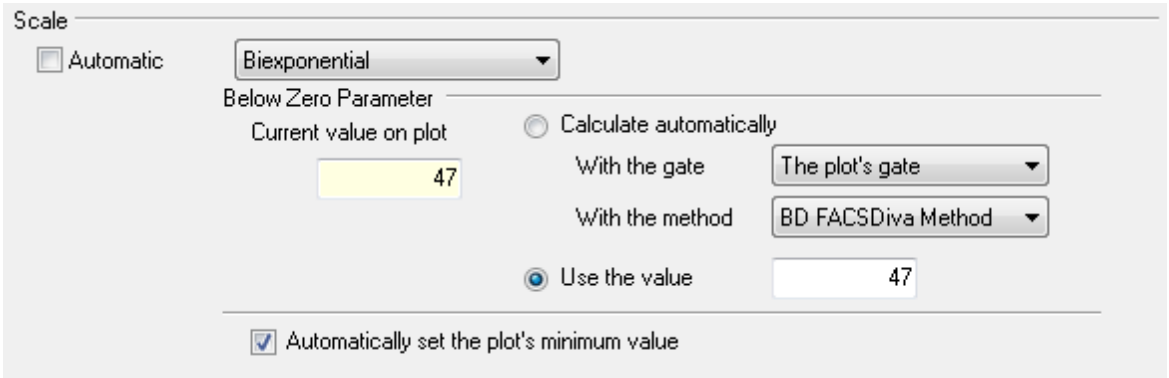

Figure 9.14 Manually entering a Below Zero Parameter value with Biexponential scaling.

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The **Calculate automatically** radio button may also be checked if you would like to change the gate for calculating the display (figure 9. 15) or if you would like to change the method for calculation to 5th percentile (figure 9. 16). The Fifth percentile method sets the plots minimum such that the bottom 5% of the data within the selected gate is not visible.

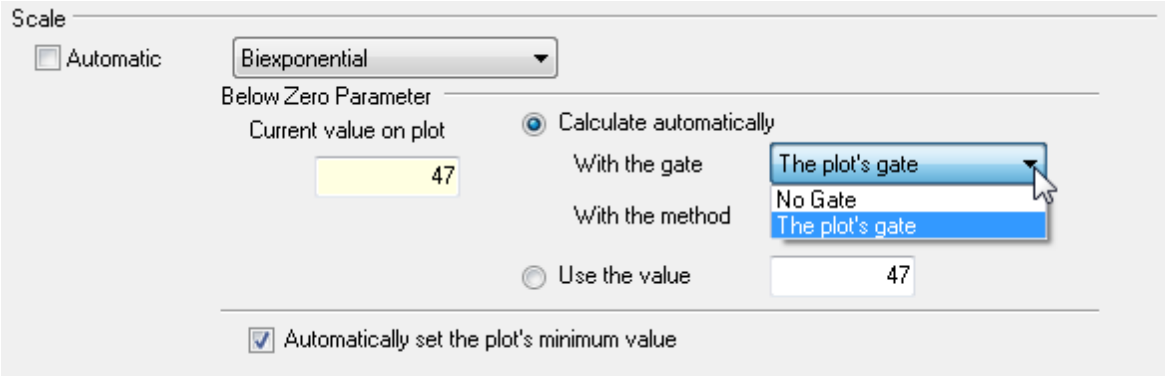

Figure 9.15 Choosing a gate for calculating the biexponential display.

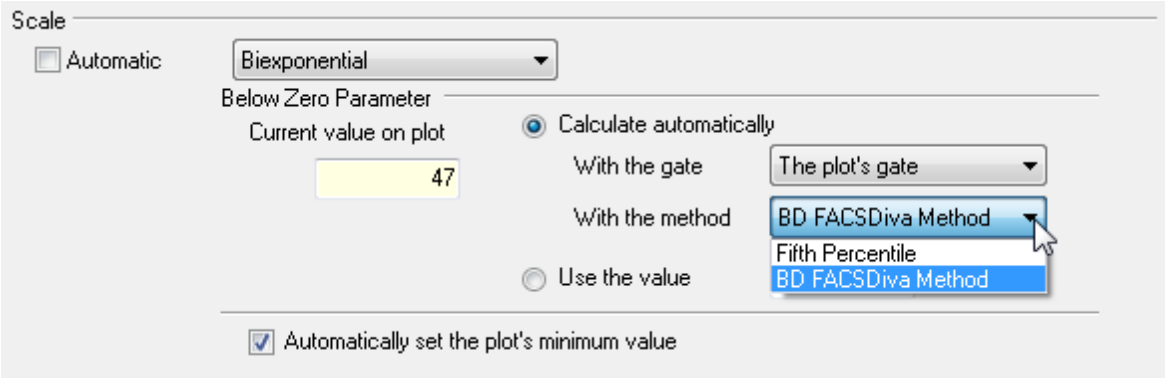

Figure 9.16 Choosing the BD FACSDiva Method or the Fifth Percentile method for display.

9.5.2 Gating

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. 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 9.17 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 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 distortion introduced by the

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log scaling.

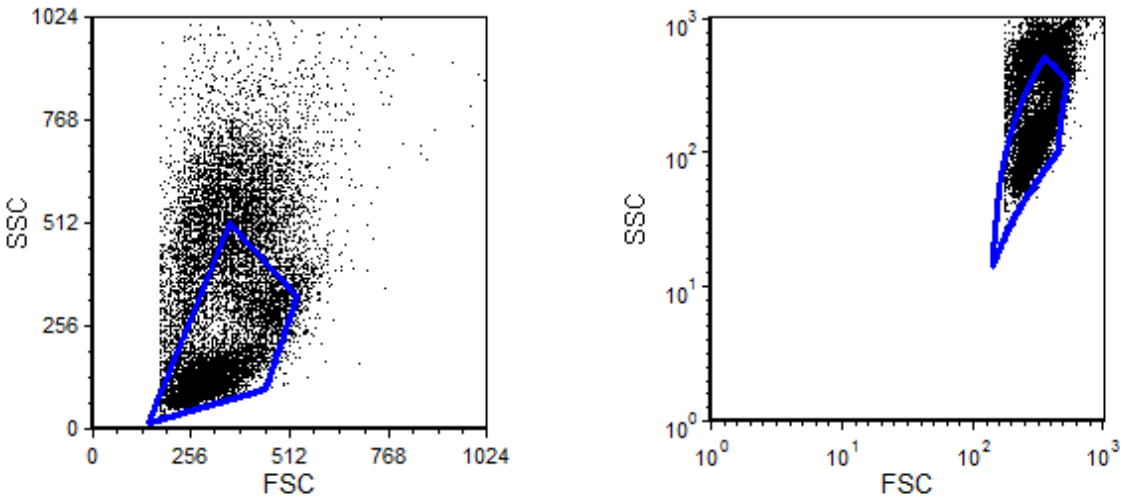

Figure 9.17 Warped Gates in Different Scalings

If you move a gate[201] 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 error 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 9.18. It is obvious that the gate no longer appears as a four-sided polygon.

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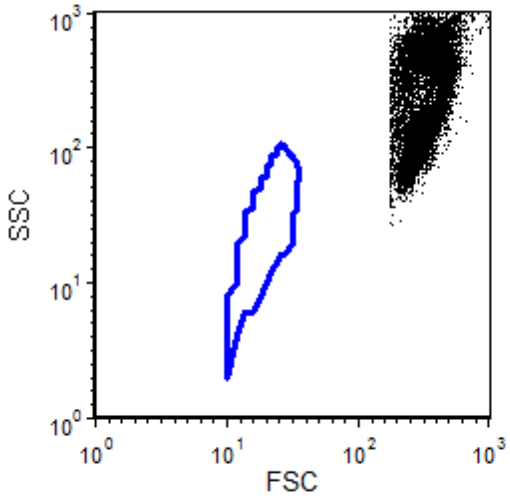

Figure 9.18 Distorted Gate in Non-native Space

The explanation for this can be appreciated by examining the gate in the native space, seen in Figure 9.19. 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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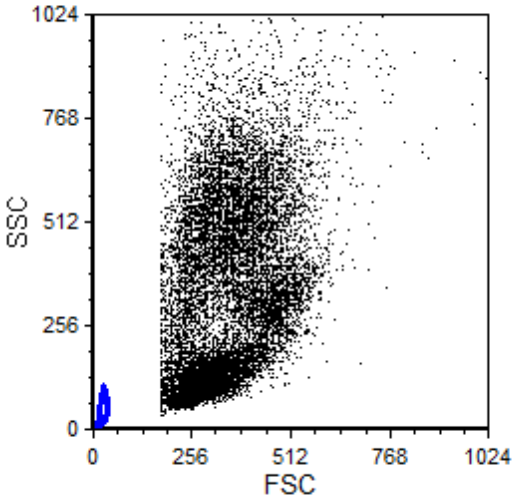

Figure 9.19 Not Enough Resolution in Native Space

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 193).

9.5.3 Quadrants

Linked quadrants 213 will also appear distorted if they are displayed on plots with different scales, as shown in Figure 9.20.

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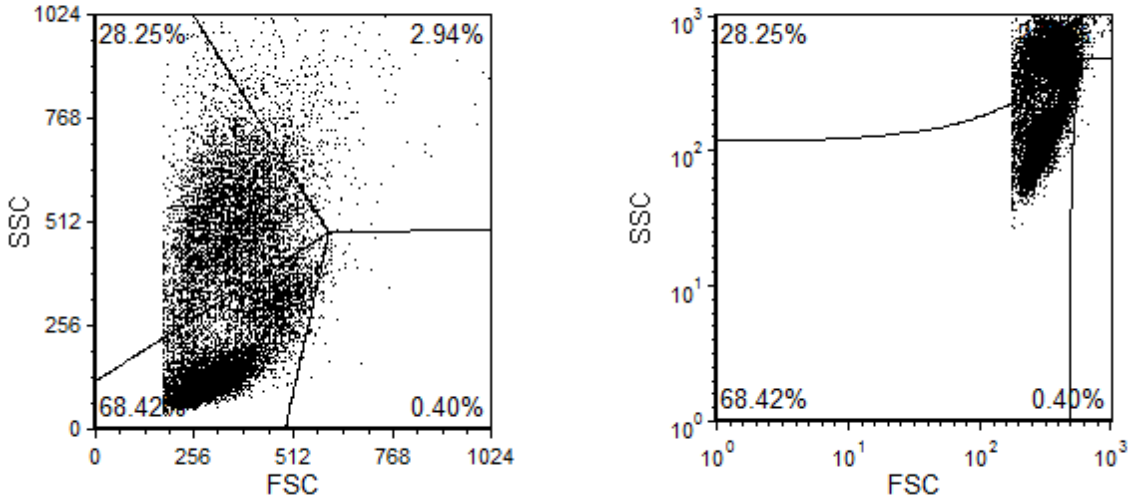

Figure 9.20 Linked Quads Displayed in Non-native Space

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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 158 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 **View→Tools→Parameter Labels→Modify Labels** command.
2. Click the **Add Set** button.
3. Enter a unique name for the new parameter set and click OK.
4. Click the **Add** button.
5. Select the parameter to modify (parameter 1 through 25).
6. Enter the alternate text in the **Label** field.
7. Click **OK**.
8. 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).
9. Click **OK** to save the set

Figure 10.1 shows a parameter label set named **Lymphs** that has been created with three alternate labels.

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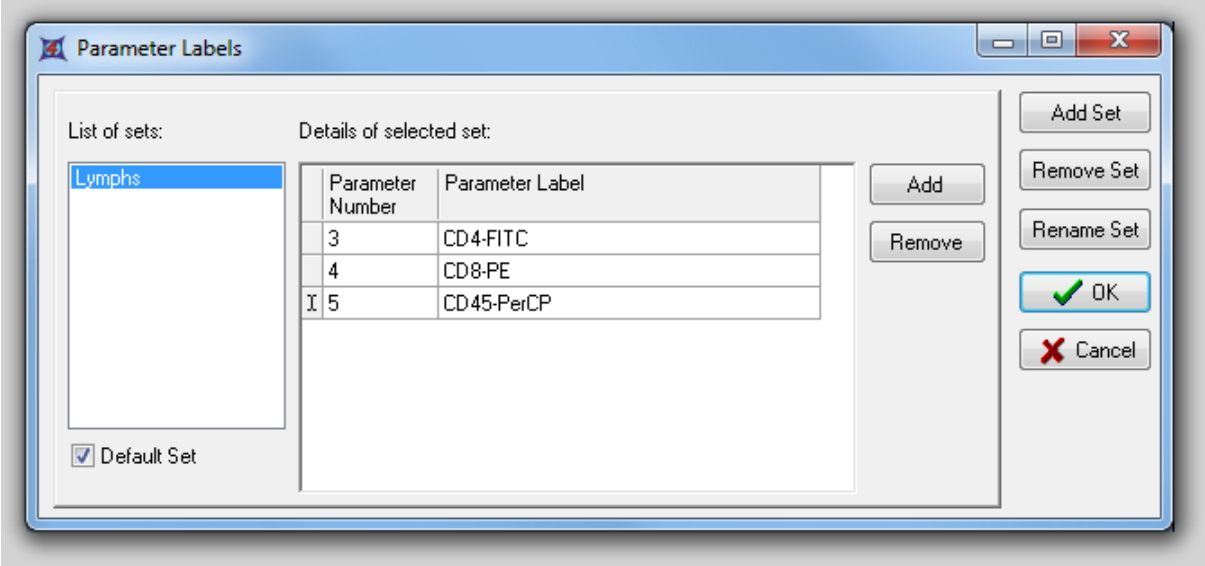

Figure 10.1 Parameter Label Sets

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 CD4-FITC 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 158.

Parameter Label Set Lists can be saved to and loaded from a file by selecting the **View→Tools→Parameter Labels→Save Labels** or **View→Tools→ Parameter Labels→Load Labels** commands, respectively. Parameter labels are automatically saved with the layout file 53.

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Chapter

11 Custom Data

Custom Data takes the form of a table/data grid (also known as a spreadsheet) where you:

- Define columns which represent the parameters of your experiment.
- Fill the columns of your table with derived data based on formulas and/or tokens (statistics and other tokens available in FCS Express).

Each row in the table represents one "cell/event" from the data set (analogous to each cell in an FCS file).

Custom Data can be used like any other kind of data set. It will appear as a node in the Data Navigator 76. You can view the header to see parameter definitions and add keywords.

Once the data is tabulated, you can display it graphically, using Bar Charts and Scatter Plots 241. For example, Custom Data can be used to automate the process of generating titration curves.

11.1 Adding Custom Data

To define **Custom Data**, use the **Data→Save/Load→Custom Data→New Custom Data** command to bring up the **Create new custom data** dialog (Figure 11.1). Alternatively, if a Data Navigator 76 is visible, you can right-click on the Data Navigator and choose **New Custom Data** from the pop-up menu.

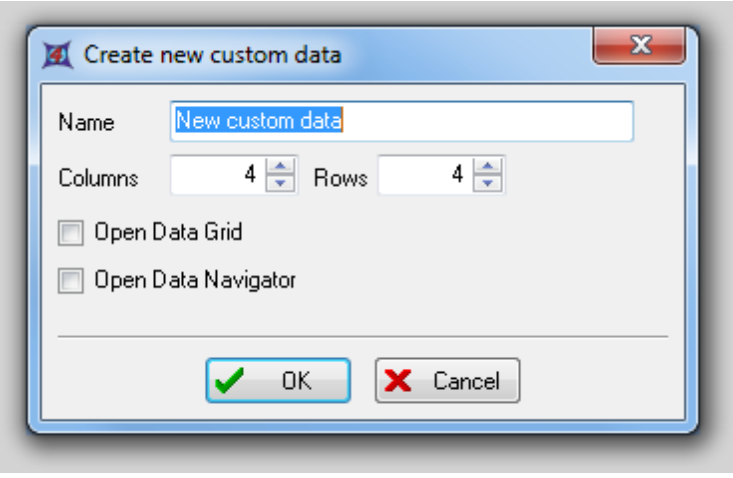

Figure 11.1 Create New Custom Data Dialog

To create the table (spreadsheet) elements for the custom data:

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- Assign a name to the data set.
- Set the number of columns and rows using the spin controls. The number of rows represents the number of data elements. The columns represent the parameters of your data.
- You can choose to automatically open a **Data Grid** and/or **Data Navigator** .
- When you are finished, click **OK**. The **Create new custom data** dialog will disappear. Unless a Data Navigator is visible, nothing will appear on your screen. However, the custom data will be defined.

11.2 Working with Custom Data

To work with **Custom Data** efficiently, you should have a Data Navigator 76 open.

If you have added Custom Data 234, a node will appear in the **Data Navigator** with the name you assigned (Figure 11.2).

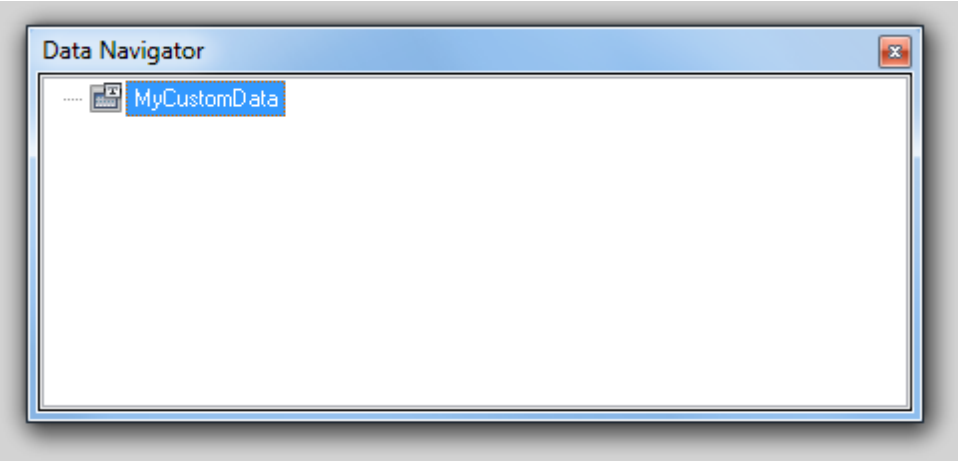

Figure 11.2 Data Navigator with Custom Data Node

You work with custom data in a **Data Grid**. To add a **Data Grid** to your layout:

1. Click on your Custom Data Node and while holding the mouse button down, drag the node to a layout page. A **Select Plot Types...** dialog will appear.
2. Check **Data Grid** (Scatter Plots, and Bar Charts can also be used in to graphically display Custom Data).
3. Click **OK**. A **Data Grid** will appear with the number of rows and columns that you previously defined (Figure 11.3).

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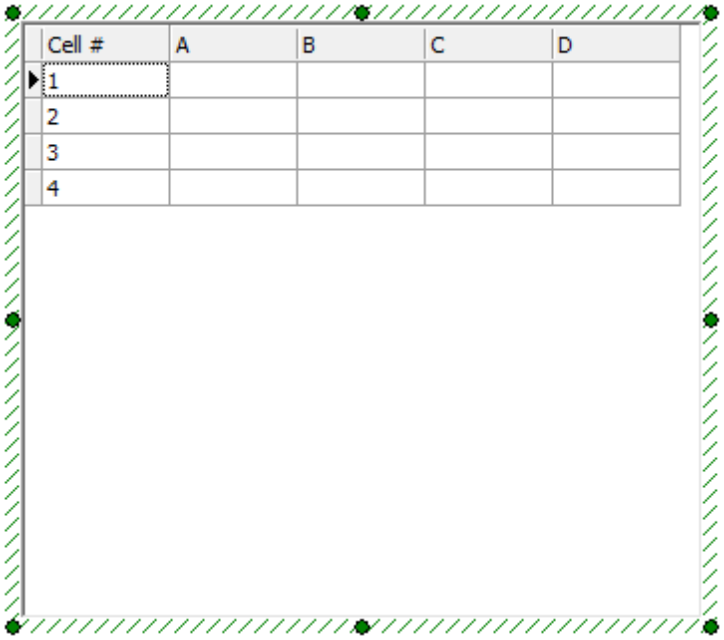

Figure 11.3 Empty Data Grid (Custom Data)

The default column names are **Cell #**, **A**, **B**, **C**, **D**, etc. The **Cell #** is fixed (it cannot be renamed) and the rows (representing the events/"cells" in your custom data) are automatically numbered for you. The remaining columns represent the parameters in your custom data. You can rename a column as follows:

1. Click on the column header (e.g., **A**) to select/highlight the entire column.
2. Right-click anywhere in the highlighted column and choose **Rename Selected Column** from the pop-up menu.
3. Rename the column in the **Enter the new name for column** field (Figure 11.4) and then click **OK**.

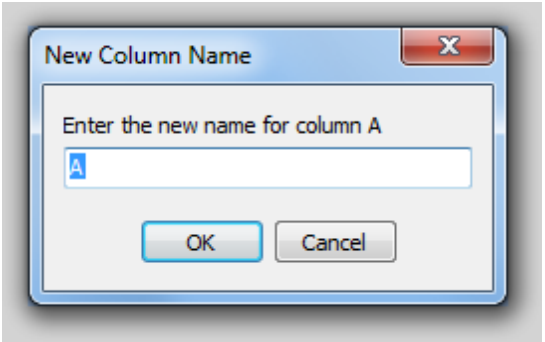

Figure 11.4 New Column Name

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To edit the value of a cell:

- Click on any cell (outside of the **Cell #** column) to select it. An edit bar will appear above the data grid (Figure 11.5).

Figure 11.5 Custom Data -- Data Grid with Edit bar

  - To enter *Text*, simply start typing. Focus will automatically move to the edit field, and the text you type will be displayed. Alternatively, you can click in the edit field and start typing. (Figure 11.6).

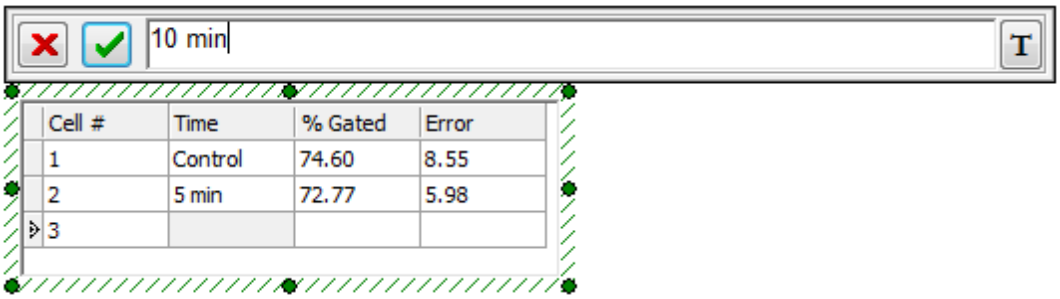

Figure 11.6 Custom Data -- Entering Text

  - To enter a *mathematical formula*, type '=' followed by the formula (Figure 11.7).

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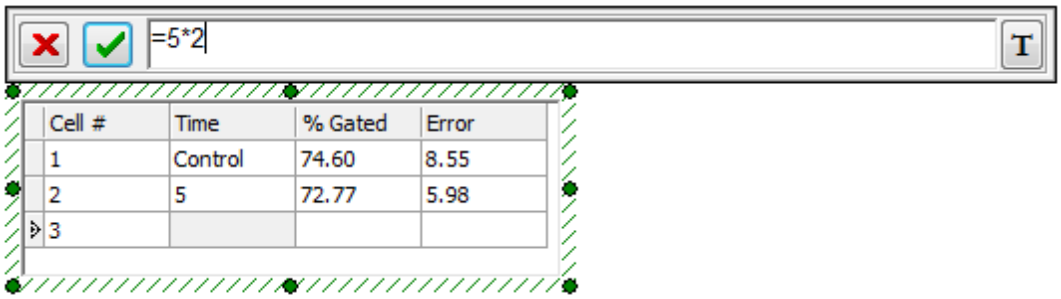

Figure 11.7 Custom Data -- Entering a Formula

- To insert a statistic or Custom *Token*, click on the T button, and select a token to insert in the same manner as inserting a token into a text box[328] (Figure 11.8).

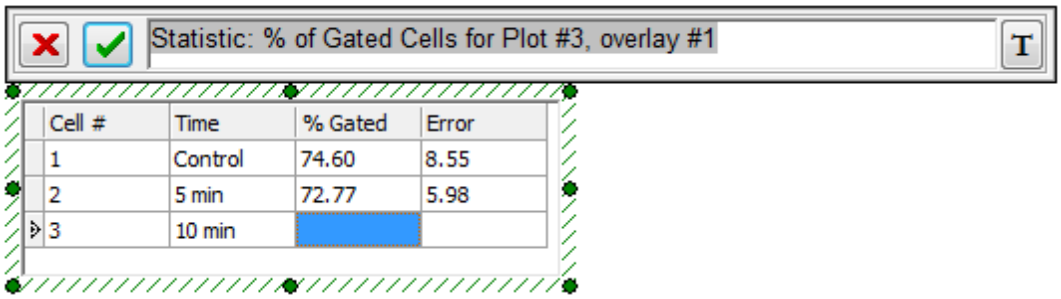

Figure 11.8 Custom Data -- Inserting a Statistic Token

- To accept the entry in the edit field, click the ✓ button. To cancel the changes, click the ✗ button.

11.2.1 Adding Rows or Columns

To add rows or columns to your **Custom Data** table (data grid/spreadsheet).

1. Click on a cell that is adjacent to where you want to add a new row or column to select/highlight it.
2. Right-click on the highlighted cell.
3. Select **Insert** from the pop-up menu (Figure 11.9).
4. Choose one of the submenu items described in the table below.

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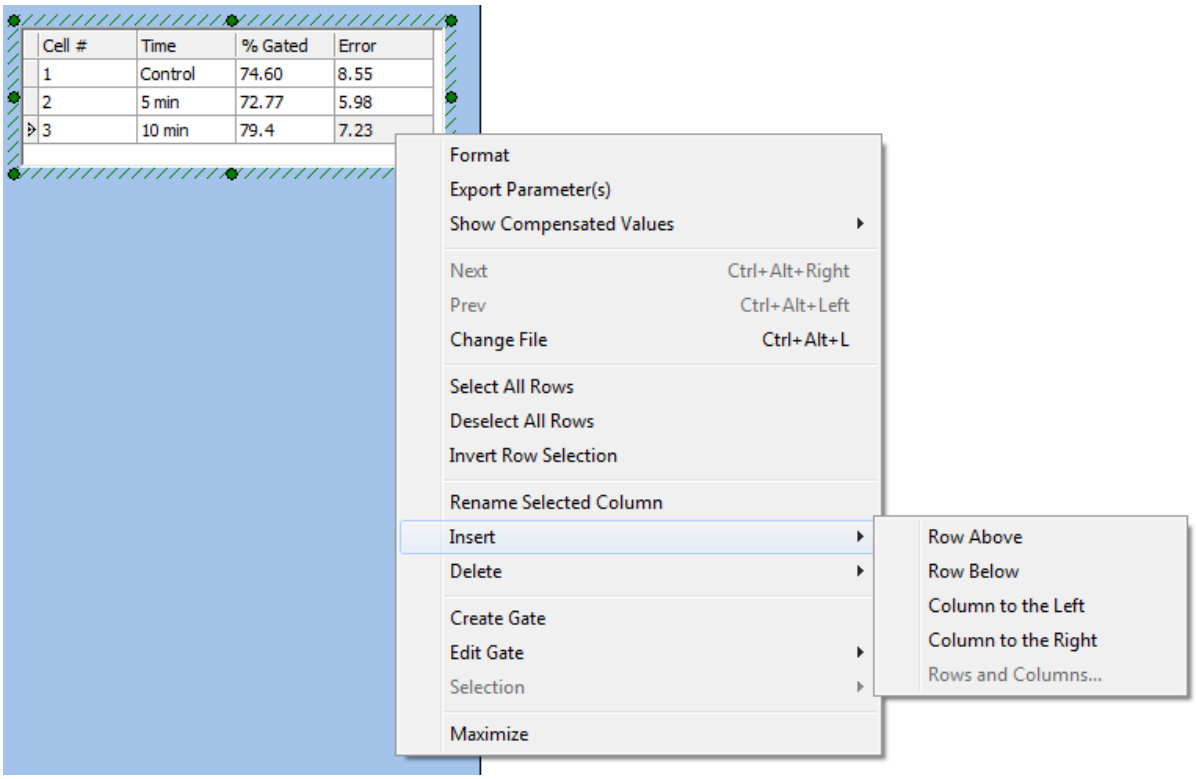

Figure 11.9 Custom Data -- Add Rows or Columns

Menu Item	Explanation
Row Above	Add a row above the selected row. In the example, the new row would be row 3, and the existing row 3 would become row 4.
Row Below	Add a row below the selected row. In the example, the new row would be row 4.
Column to the Left	Add a column to the left of the selected column. In the example, the new column would appear between **% Gated** and **Error**. You will be prompted for a column name.
Column to the Right	Add a column to the right of the selected column. In the example, the new column would be the 5th column (to the right of **Error**). You will be prompted for a column name.

11.2.2 Deleting Rows or Columns

To delete rows or columns from your **Custom Data** table (data grid/ spreadsheet):

1. Click on a cell in the row or column you want to delete to select/highlight it. To select two or more adjacent rows or columns, select the first and then **Shift**-click on the second.
2. Right-click on a highlighted cell.

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3. Select **Delete** from the pop-up menu (Figure 11.10).
4. Choose one of the submenu items described in the table below.

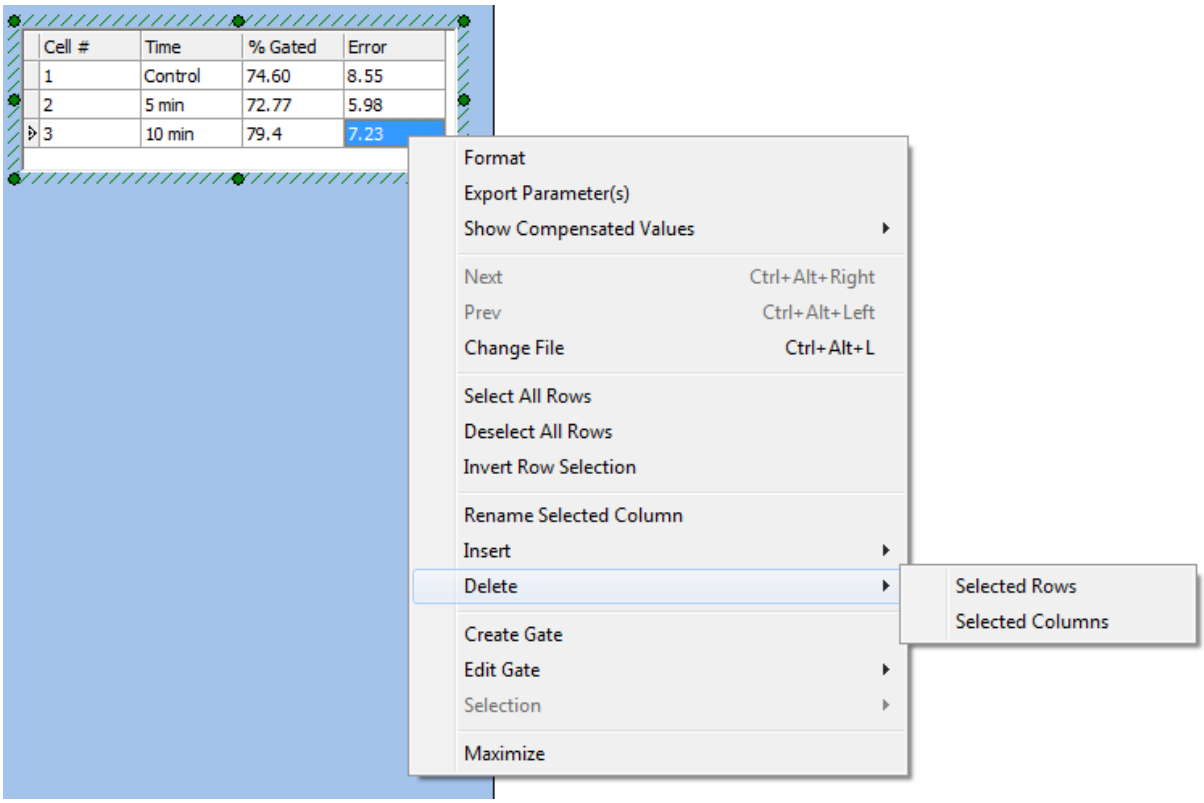

Figure 11.10 Custom Data -- Delete Rows or Columns

Menu Item	Explanation
Selected Rows	Delete the entire row of the selected cell. In the example, row 3 would be deleted.
Selected Columns	Delete the entire column of the selected cell. In the example, the **Error** column would be deleted.

11.3 Renaming Custom Data

To rename your **Custom Data**:

1. Select **Data→Save/Load→Custom Data→Rename**. An entry will appear to the right for each set of custom data that is defined.
2. Choose the Custom Data Set to rename to bring up a **Rename data** dialog (Figure 11.11). In the figure, the *Lymphs titration* custom data set is being renamed.
3. Enter the new name in the edit field and click **OK**.

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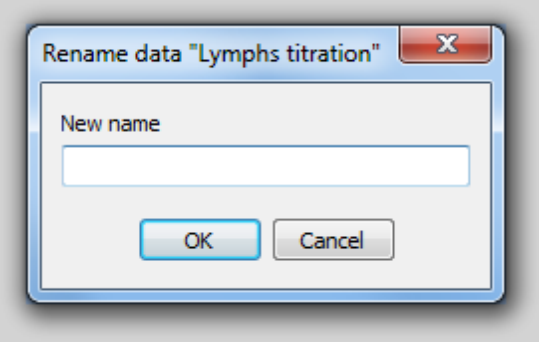

Figure 11.11 Rename Data Dialog

Alternatively, right-click on the custom data set in the Data Navigator and choose **Rename** from the pop-up menu. An in-place editor will appear, allowing you to type the new name of the custom data.

11.4 Deleting Custom Data

To delete a **Custom Data** set:

1. Select **Data→Save/Load→Custom Data→Delete**. An entry will appear to the right for each set of custom data that is defined.
2. Choose the **Custom Data** set to delete. You will not be prompted to confirm your choice. If you want to immediately restore a custom data set that was deleted by mistake, use the Undo command in the Quick Access Toolbar 34.

Alternatively, right-click on the custom data set in the Data Navigator and choose **Delete** from the pop-up menu, or highlight your custom data and press Delete on your keyboard.

11.5 Graphing Custom Data

Custom Data can be graphed using Bar Charts and Scatter Plots.

To graph custom data on a Bar Chart:

1. Use the **Insert→1D Plots→Bar** command.
2. Select the Bar Chart and click on **Format→Plot Options→Overlays** (Figure 11.12).
3. Set the **X** and **Y** parameters to columns in your Custom Data. One of the columns in your data can be used for error bars on the Bar Chart (Figure 11.13).

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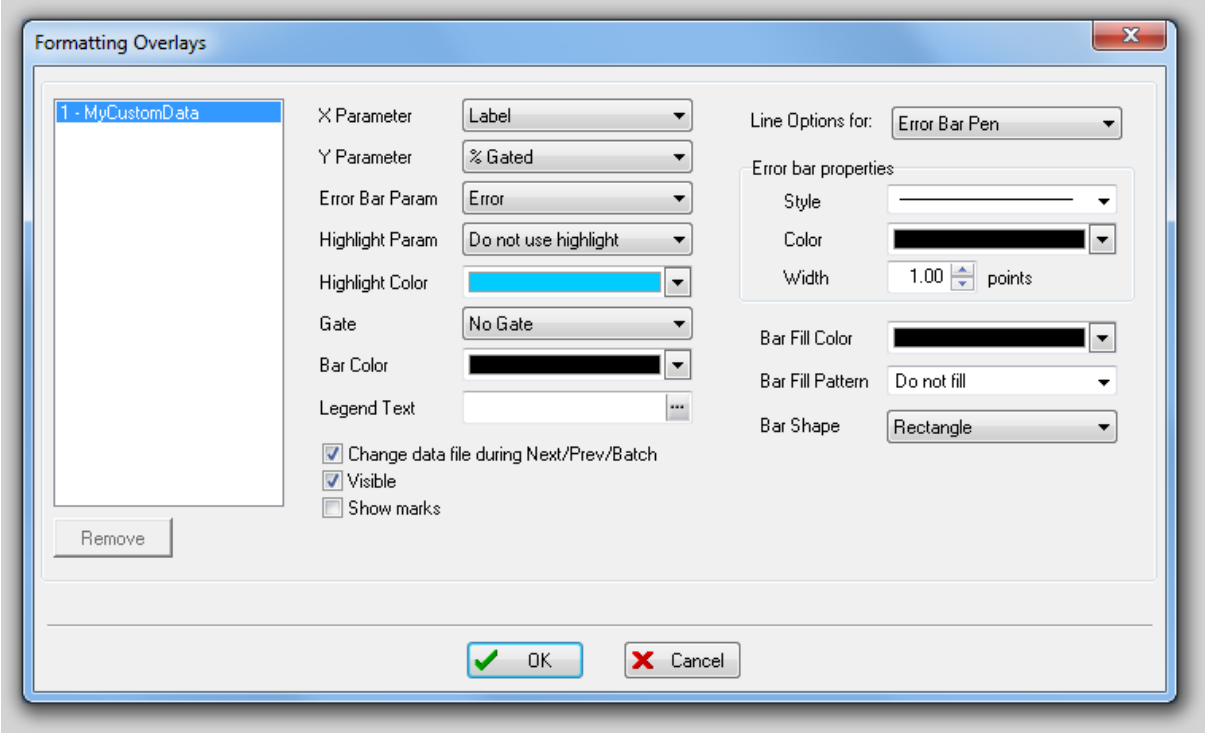

Figure 11.12 Bar Chart -- Formatting Overlays

Cell #	Time	% Gated	Error
1	Control	74.60	8.55
2	5 min	72.77	5.98
3	10 min	79.41	7.23

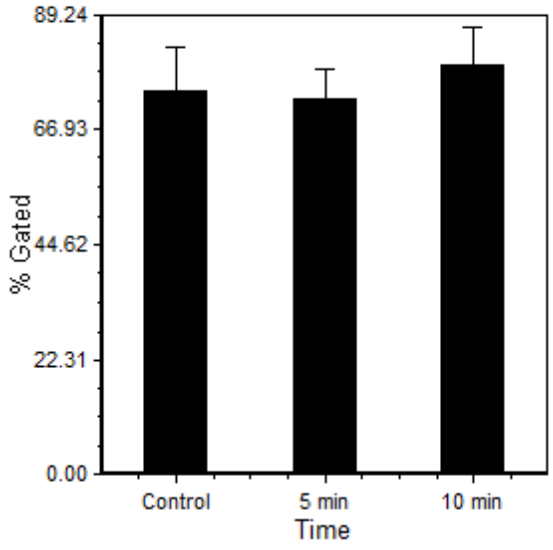

Figure 11.13 Bar Chart with Error Bars

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FCS Express provides a feature to highlight a bar in a preset color if the data meets certain criteria. To use this feature:

1. Add a column to your Custom Data (e.g., Highlight).
2. In each cell, put a formula that evaluates to *TRUE* or *FALSE*. In the example (Figure 11.14), the formula was *=% Gated < 40*. A '*0*' in the Highlight column for a cell indicates that the formula evaluates to *FALSE*. The number '*-1*' in row 3 indicates *TRUE*.
3. Select the Bar Chart and click on **Format→Plot Options→Overlays**.
4. Set the **Highlight Param** to the column with the TRUE/FALSE formulas using the drop-down list (in the example, it is the Highlight column).
5. Set the **Highlight Color** using the drop-down list (in the example, the color is **Light Blue**).

Cell #	Time	% Gated	Error	Highlight
1	Control	74.60	8.55	0
2	5 min	72.77	5.98	0
3	10 min	29.41	7.23	-1

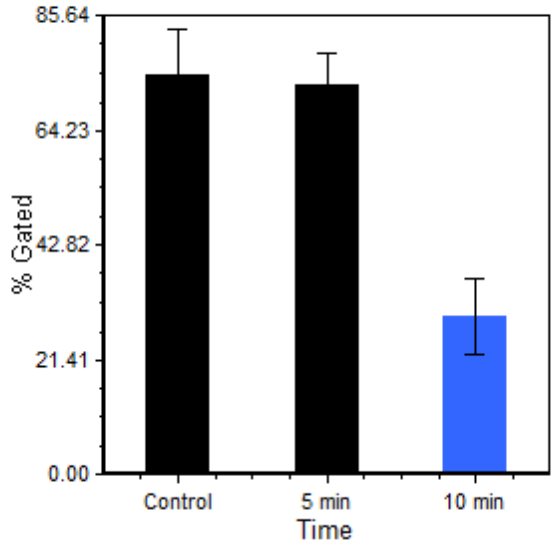

Figure 11.14 Bar Chart with Highlight

To graph custom data on a Scatter Plot (e.g., a titration curve):

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1. Use the **Insert→2D Plots→Scatter** command, or drag the custom data from the Data Navigator to an empty part of the page, and select **Scatter** from the **Select Plot Types...** dialog.
2. Select the Scatter Plot and click on **Format→Plot Options→Overlays** (Figure 11.15).
3. Set the X and Y parameters to columns in your Custom Data. Set the **Point Color**, **fill color**, **fill style**, **size**, and **shape**. Click **OK**. Your data will be displayed (Figure 11.16).

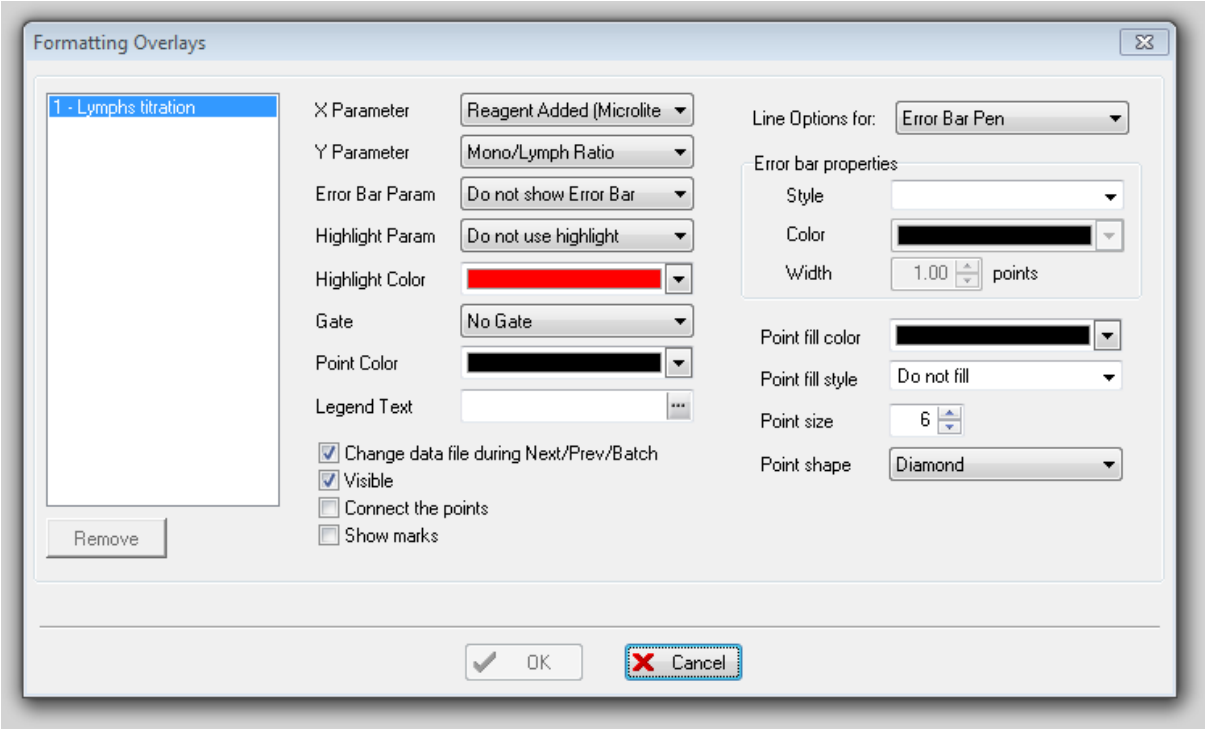

Figure 11.15 Formatting a Scatter Plot

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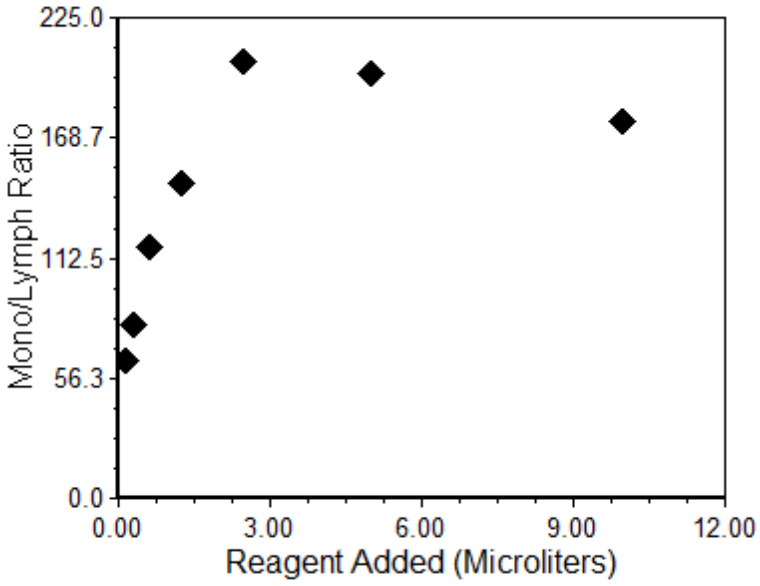

Figure 11.16 Scatter Plot (Titration Curve)

11.6 Regression Analysis

FCS Express Version 4 has the ability to perform regression analysis on your data.

FCS Express provides the user with many mathematical functions to create custom tokens 348 and custom data 234 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 the red italic, *xxx*, representing the FCS Express Function name for a particular equation 256 (The FCS Express Function names can be found in the Available Equations 256.). 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	([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 326. Where **Y Values** is a comma delimited list of Y	Comma delimited list of all of the fit

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	a particular equation.	values, generally specified by a series of tokens[326]. **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[256] 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[326]. 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.	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[256] (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[326] 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 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.	The value of the specified variable.
xxx XtoY	Uses the X values of a given data set and the "Model Parameters" or "Fit" of a regression to return	*xxx*XToY(X, [modelParameters]) Where *xxx* represents an equation[256](ie. Linear, Sigmoidal, Exponential, etc...). Where **X** is a comma delimited list of X values, generally specified by a token[326]. Where **modelParameters** represents a comma delimited list of all of the fit variables and their fit values. Generally specified by a token[326] result of the Perform *xxx*Fit function.	Comma delimited list of all of the Y values calculated from the associated equation, X value, and

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	an unknown Y Value.		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 256 (ie. Linear, Sigmoidal, Exponential, etc...) Where **Y** is a comma delimited list of Y values, generally specified by a token 326. Where **modelParameters** represents a comma delimited list of all of the fit variables and their fit values. Generally specified by a token 326 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 256. To see an example of using these equations please see the topic of Regression Analysis Example 247 or visit the Linear Regression Analysis 1342 tutorial.

11.6.1 Regression Analysis Example

In order to perform regression analysis, you normally do it on 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 326, representing some statistic from your analysis.

In Figure 11.17, we have inserted a 2-column, 4-row custom data set 234 (which we named Linear Regression Data), re-named the columns X and Y 235, and inserted some values in the cells.

Cell #	X	Y
1	5	51
2	10	101
3	20	201
4	30	301

Figure 11.17 Custom Data for Regression Analysis

With this data, a linear regression should yield a slope of 10 and a Y-intercept of 1.

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Regression analysis is performed using Custom Tokens[348].

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 11.18).

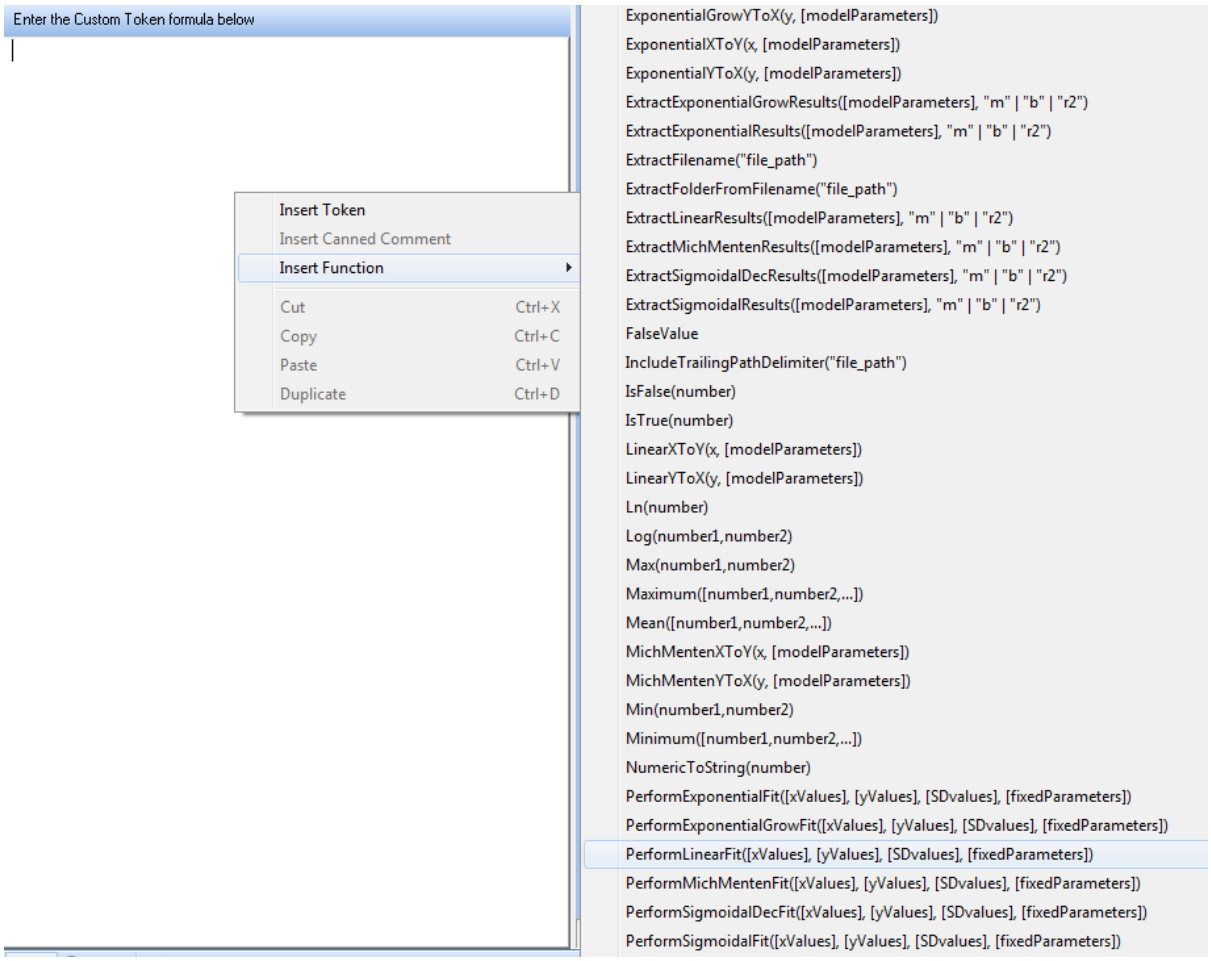

Figure 11.18 Regression Functions

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 11.19).

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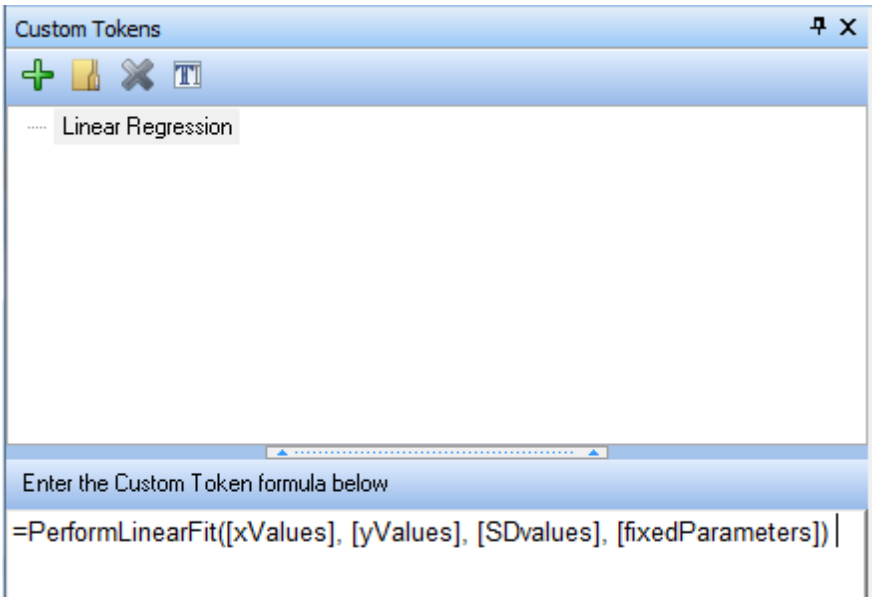

Figure 11.19 Linear Regression Formula

3. In the formula, highlight **xValues**, right-click to bring up the pop-up menu, and choose (Figure 11.20).

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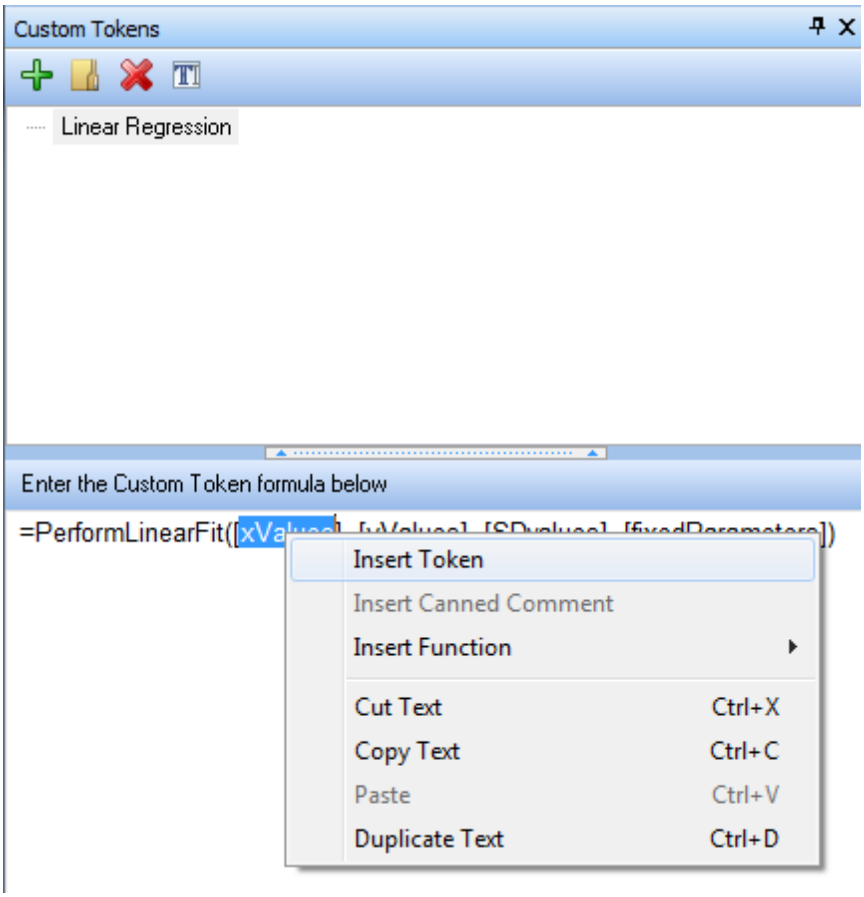

Figure 11.20 Inserting a Token into a Linear Regression Formula

An **Insert a Token** dialog will appear that contains a **Data Parameter** token (Figure 11.21).

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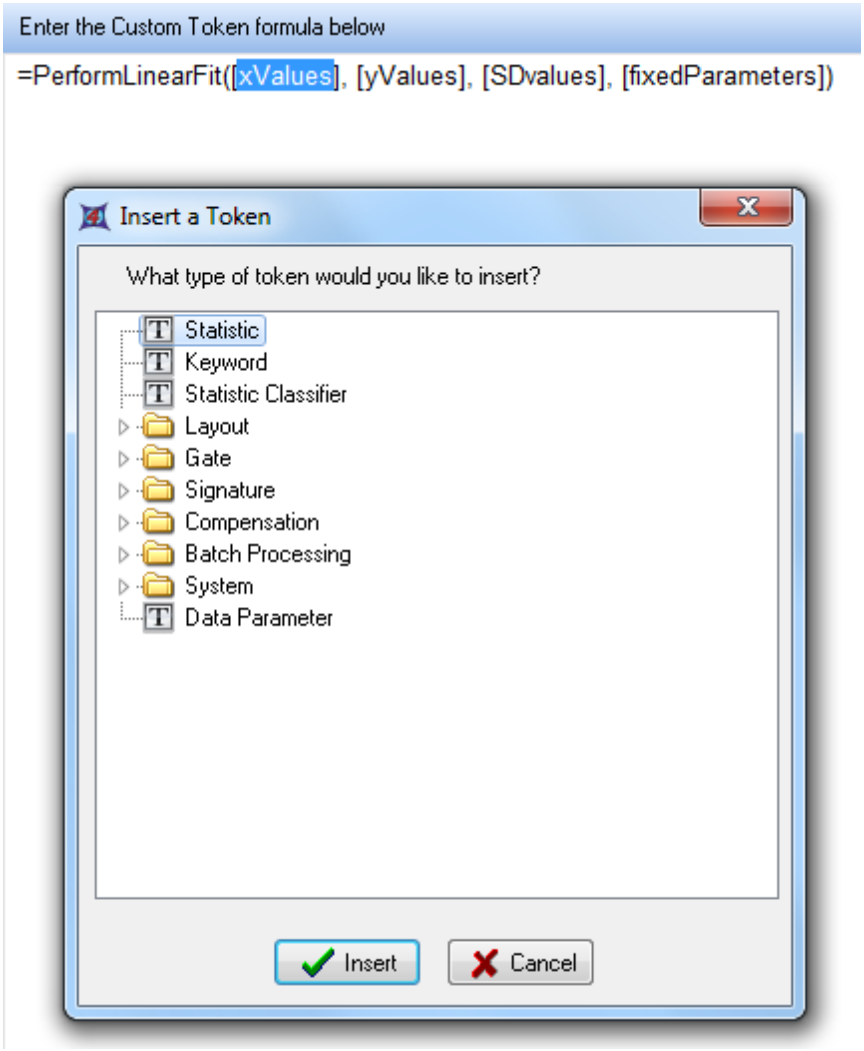

Figure 11.21 Data Parameter Token

4. Select the **Data Parameter** token and click **Insert**.

5. In the **Create Data Parameter** dialog which appears, choose the **Custom Data** radio button as a **Data Source**, and select **Linear Regression Data** (Figure 11.22).

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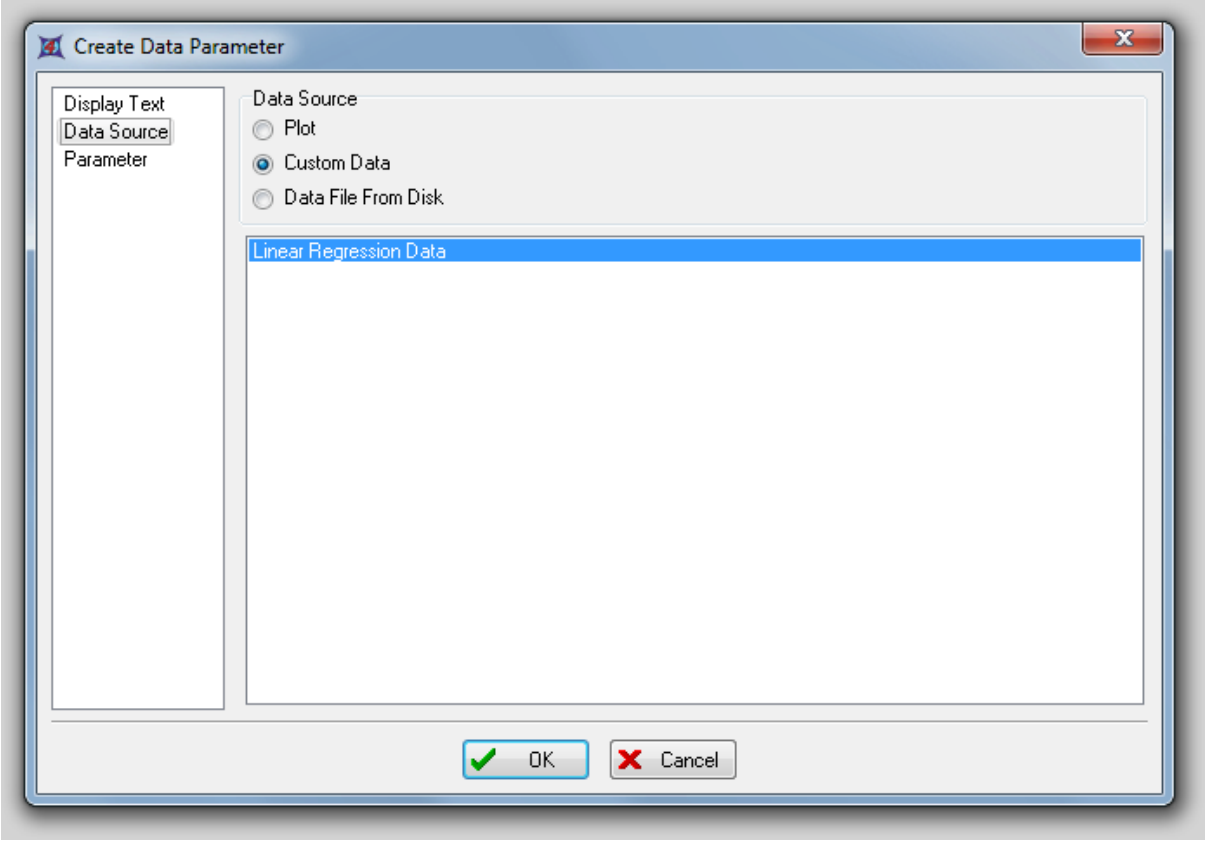

Figure 11.22 Create Data Parameter -- Data Source

6. Then, go to the **Parameter** page and from the **Parameter** drop-down list, choose the data in column **X** (Figure 11.23). 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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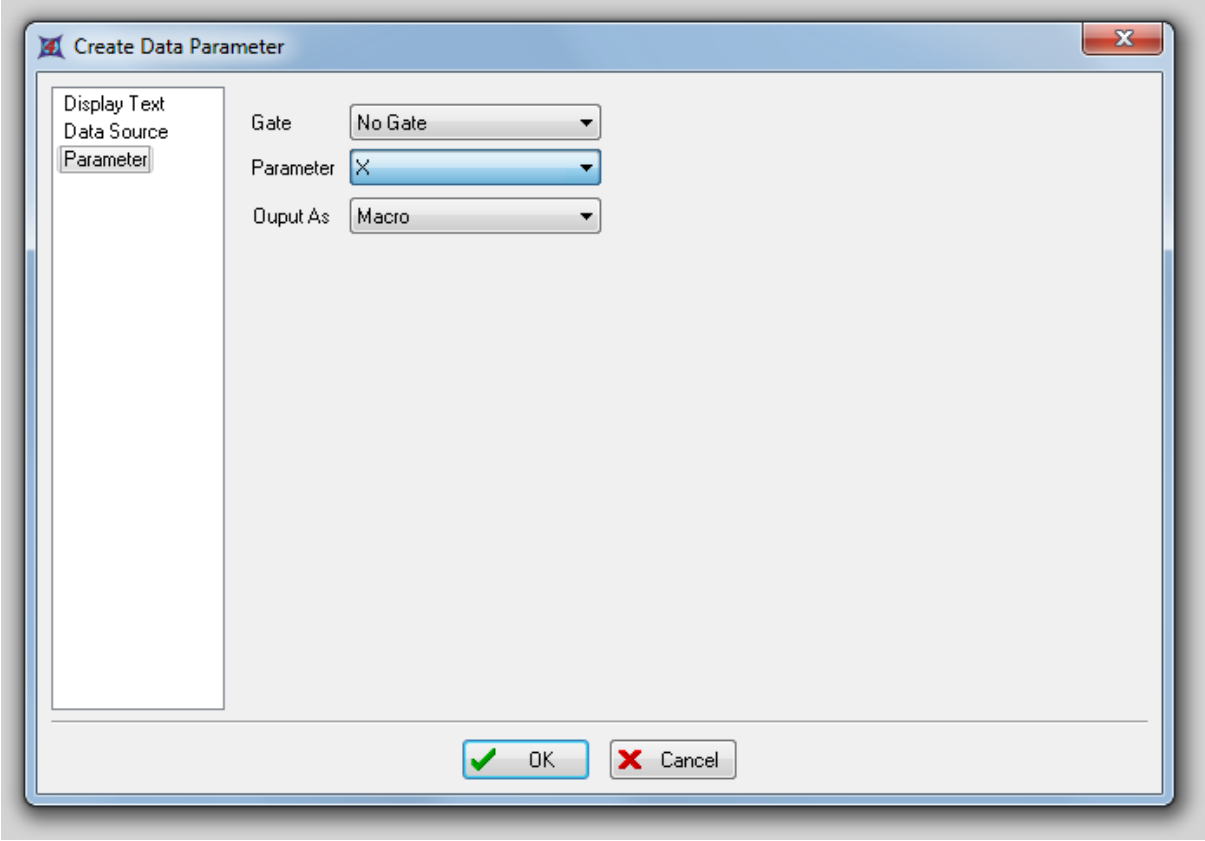

Figure 11.23 Create Data Parameter -- Parameter Page

7. Repeat steps 3-6 using **yValues** in the formula and choosing Parameter **Y** .

8. Highlight **SDvalues** and press the Del key (leaving the area between the brackets blank. Do the same for **fixedParameters** (Figure 11.24).

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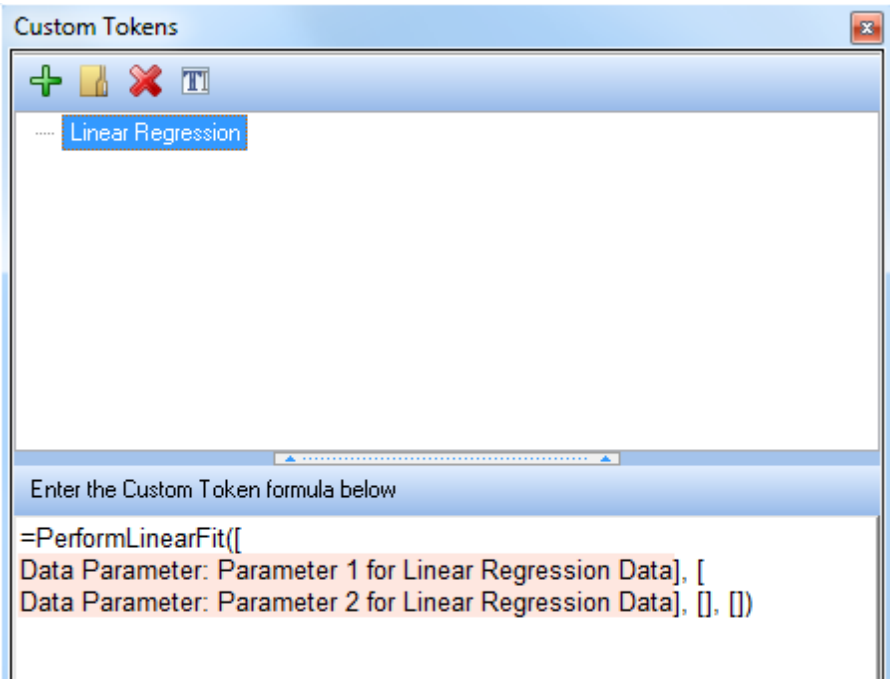

Figure 11.24 Completed Linear Regression Formula

If the measurement in your Y 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 formula. The model will weight the points with the lower standard deviation moe 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 361 (they will be highlighted in blue instead of pink).

10. Insert a Text Box 96 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 11.25).

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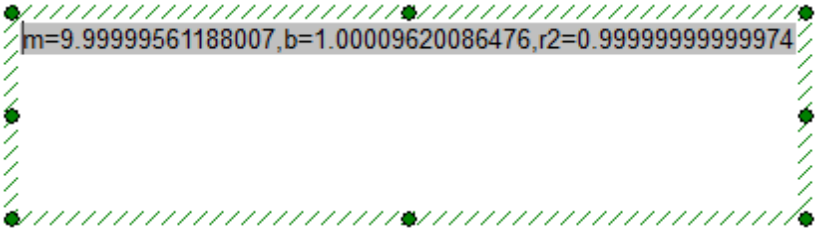

Figure 11.25 Linear Regression Token inserted into a Text Box

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 11.26) 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 11.27).

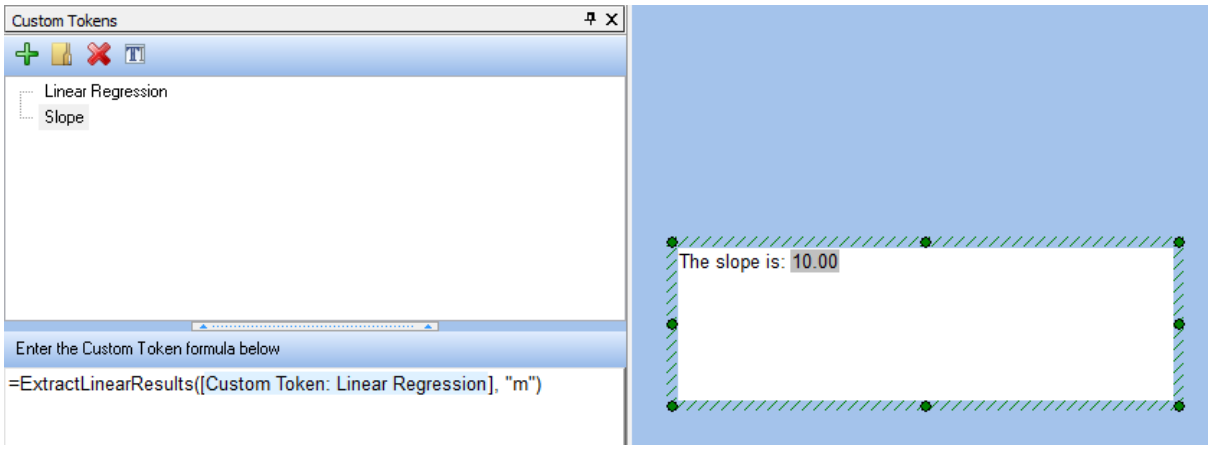

Figure 11.26 Slope Parameter of Linear Regression Analysis Inserted into a Text Box as a Token

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 form that fit.

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11.6.2 Available Equations

FCS Express Version 4 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
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

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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	$y = B + A \operatorname{Sin}(Fx + \varphi)$	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

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Chapter

12 Using Parameter Math

Parameter Math is a tool that allows you to create new parameters (or change current parameters) based on mathematical combinations of currently existing parameters. Parameter Math also allows you to create a sequence of equations that can be applied to an FCS file, or saved and then applied to data files automatically as they are loaded.

Continue to:

- Performing Parameter Math 260
- Creating a Parameter Math Sequence 263
- Running a Parameter Math Sequence 264

12.1 Performing Parameter Math

To perform parameter math on a data file, select the **Data→Calculate→ Parameter Math→Single Calculation** command, which brings up the dialog shown in Figure 12.1. If no data files are open, you will be prompted to select an data file.

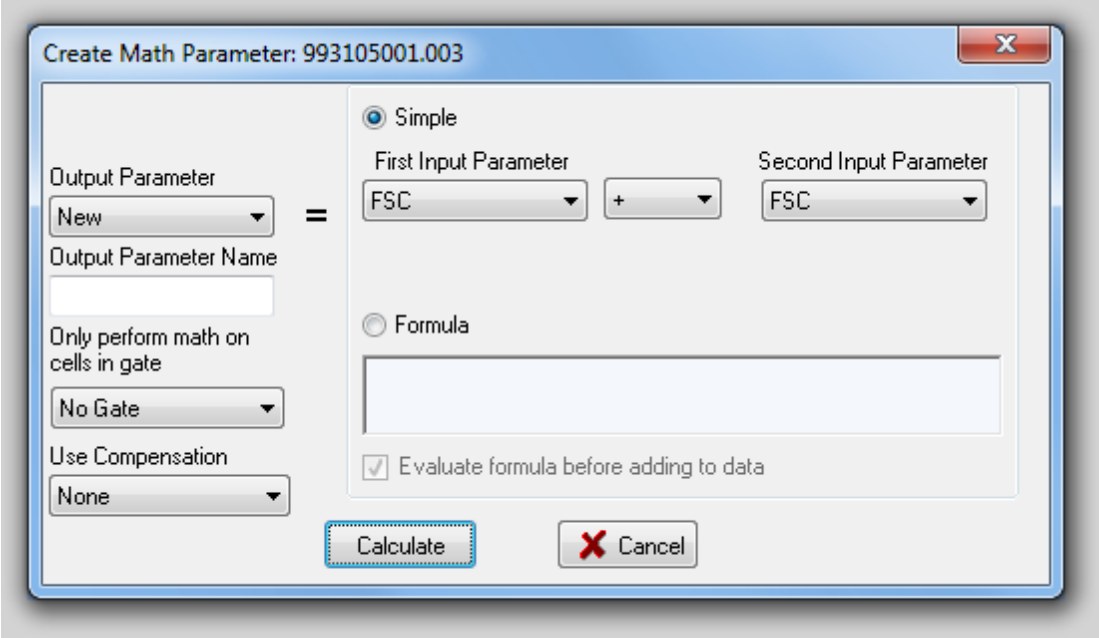

Figure 12.1 Parameter Math Dialog

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. If you are creating a new parameter, you can enter the name of the

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new parameter in the **Output Parameter Name** field.

Parameter Math will only be performed on cells that are accepted by the gating formula listed under **Only perform math on cells in gate**. If this field says "**No Gate**", the calculation will be performed on all cells in the file.

The **Use Compensation** field allows you to choose whether or not the mathematical calculations will be performed on compensated or uncompensated 288 data. To use uncompensated data, select "**None**" in this field, otherwise choose the compensation matrix you wish to use.

There are two ways to set up a Parameter Math calculation. 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 326, 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)

To enter a token into your equation, right-click on the **Formula** field and choose **Insert Token** from the pop-up menu (Figure 12.2).

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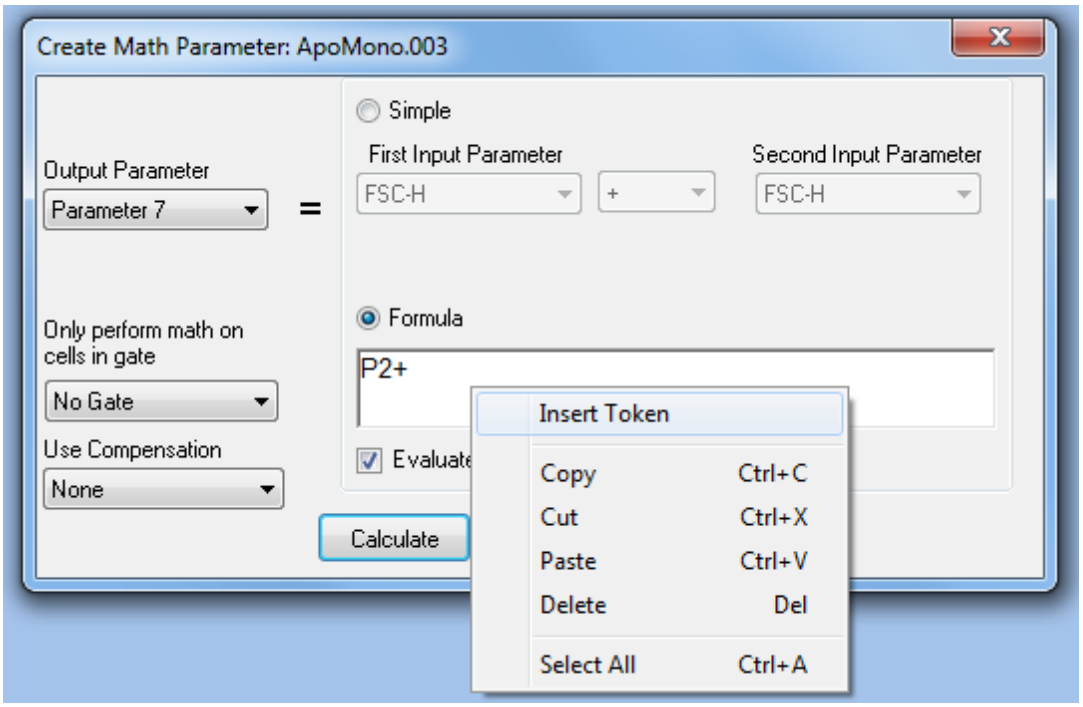

Figure 12.2 Inserting a Token into a Parameter Math Formula

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 320. 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** check box 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.

If you choose to enter a formula, make sure to use the "=" sign in the front to allow the formula to be evaluated properly.

A comprehensive set of all formula operators and functions 364 is available. This set is the same as is available 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.

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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 Edit Preferences dialog 118.

To perform the calculation, press the **Calculate** button.

12.2 Creating a Parameter Math Sequence

There may be situations where you want to perform a series of Parameter Math calculations every time an FCS file is loaded. Although it is possible to perform many calculations in a single formula 260, sometimes it is necessary to store and plot the intermediate results of a calculation, which makes parameter math sequences very useful.

To create a parameter math sequence, select the **Data→Calculate→ Parameter Math→Sequence Setup** command, which brings up the dialog shown in Figure 12.3.

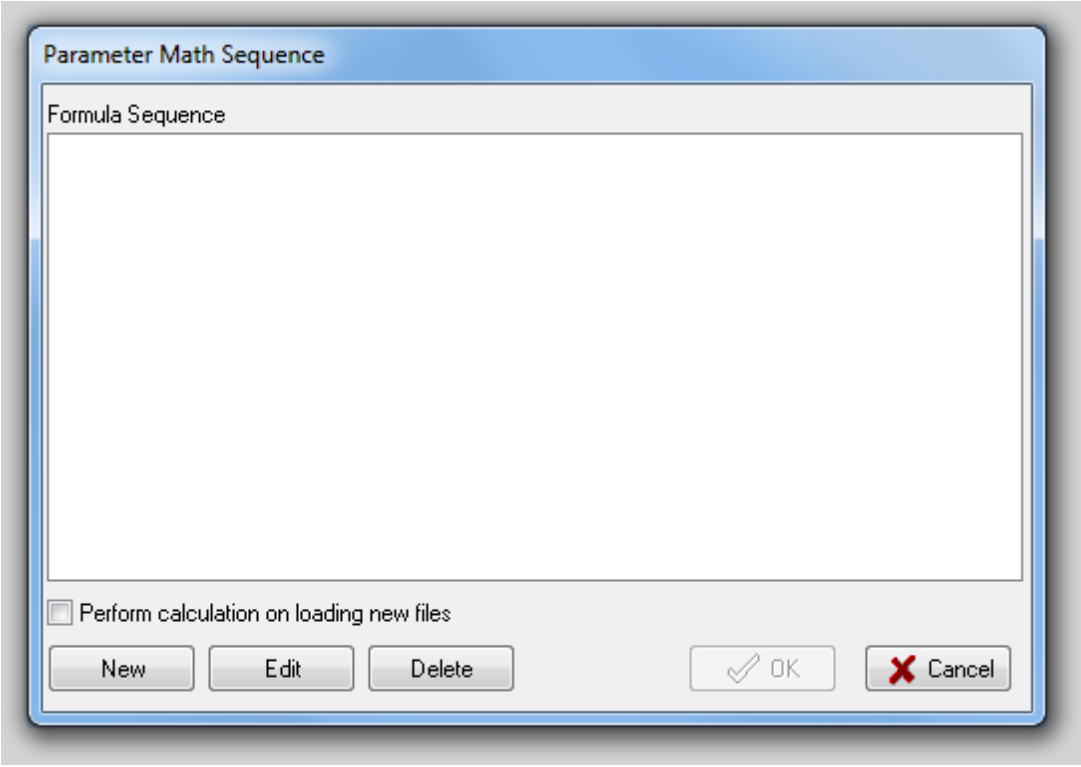

Figure 12.3 Parameter Math Sequence Window

To create a new calculation in the sequence, select the **New** button. If there are no data files open, FCS Express will ask you to select a data file to use as a template for the Parameter Math sequence. A **Create New Sequence Item**

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dialog (Figure 12.4) will appear and you can enter a single calculation as described above 260.

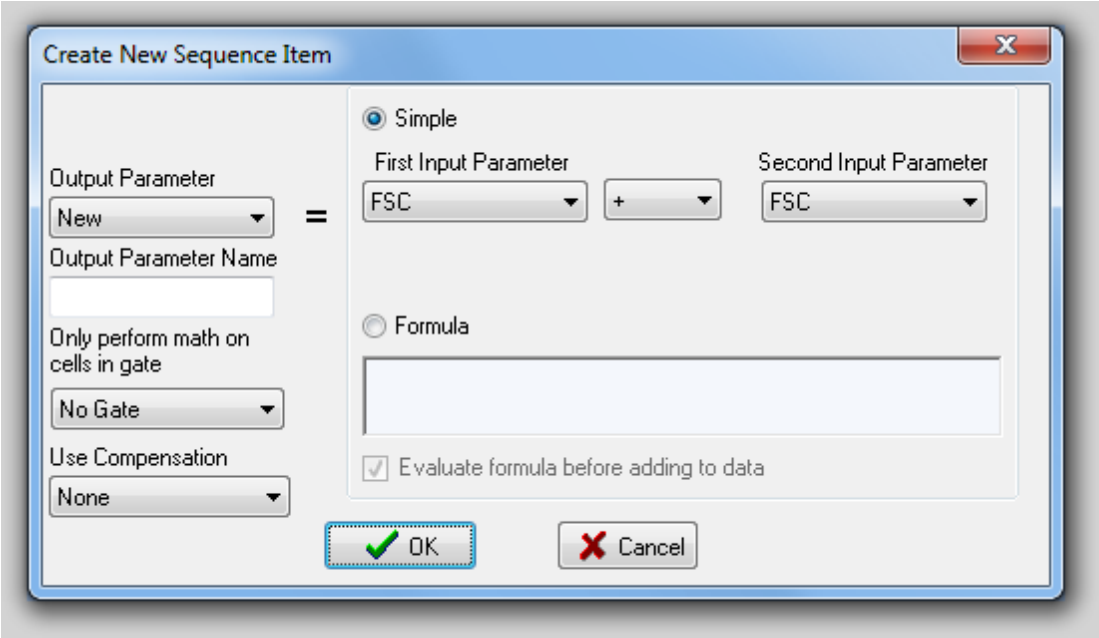

Figure 12.4 Parameter Math Create New Sequence Item Dialog

You can delete a calculation in the sequence by selecting the parameter and pressing the **Delete** button. To edit a calculation in the sequence, select the parameter and press the **Edit** button.

If you wish this sequence of calculations to be performed on new files as you load them, enable the **Perform calculation on loading new files** checkbox. If you are going to run a sequence every time you load a file, you may not want FCS Express to keep prompting you to save the FCS files when they are closed. To stop this prompting, disable the Prompt to save modified data Files on close checkbox in the dialog Save preferences 137.

The **Data→Calculate→Parameter Math→Save Sequence** command brings up a standard dialog to save your Parameter Math Sequence (*.fcf).

The **Data→Calculate→Parameter Math→Load Sequence** command brings up a standard dialog to load a saved formula sequence file.

12.3 Running a Parameter Math Sequence

In addition to running a Parameter Math sequence when new data files are loaded, you can also run a Parameter Math sequence at any time. To run a sequence, select the **Data→Calculate→Parameter Math→Run Sequence** and

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choose **On Top File** or **On All Files**.

The **Top File** is the most recently accessed file. If a plot is in edit mode, then its data will be the top file. If no plot is in edit mode, then the most recently opened file will be the top file.

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Chapter

13 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.

FCS Express allows you to:

- Calculate individual concentrations.
- Have the concentration calculated automatically and appear directly on the statistics window.

13.1 Calculating a Single Concentration

To set up the proper parameters for the concentration calculator, select the **View→Tools→Concentration Calculator** command.

To calculate a single concentration:

- Go to the **Individual** tab of the **Concentration Calculator** dialog (Figure 13.1).

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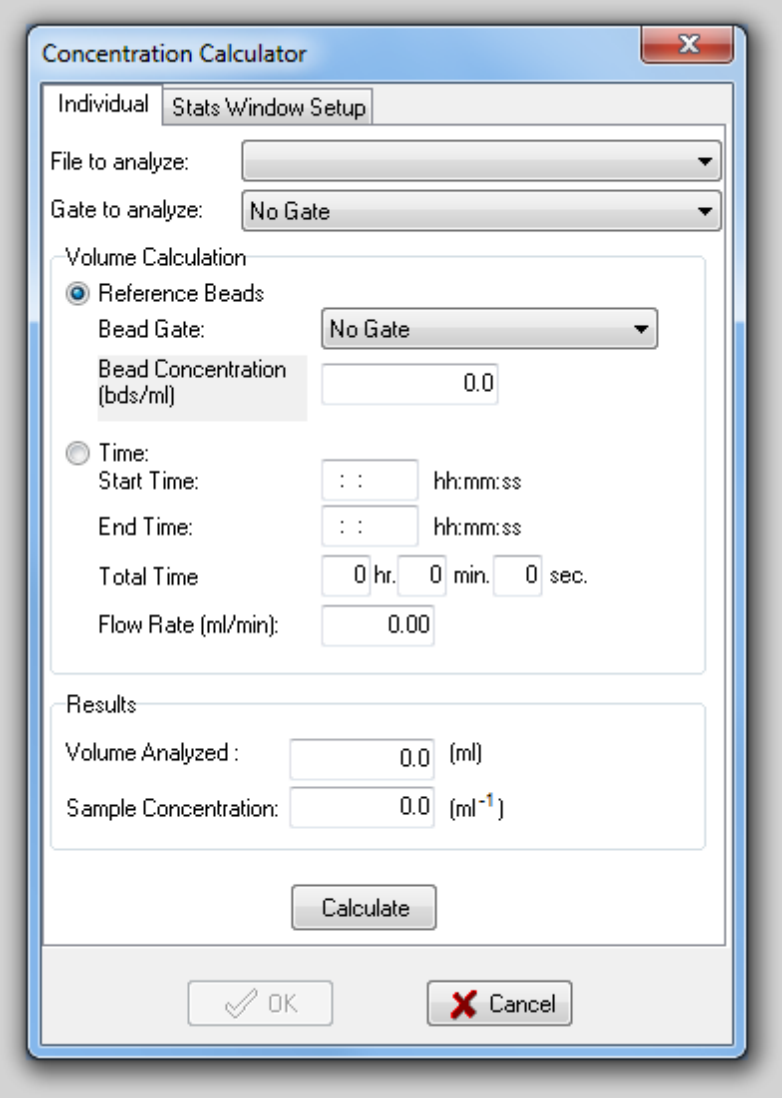

Figure 13.1 Concentration Calculator - Individual Tab

- 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.

- Select a method to calculate volume using **Reference beads** or acquisition **Time** (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**

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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
- Enter the gate number in the **Bead Gate** field.
- 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.

13.2 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 13.2) and enter the volume parameters (by **Reference Beads** or **Time**) in a similar manner as for a single concentration 268.

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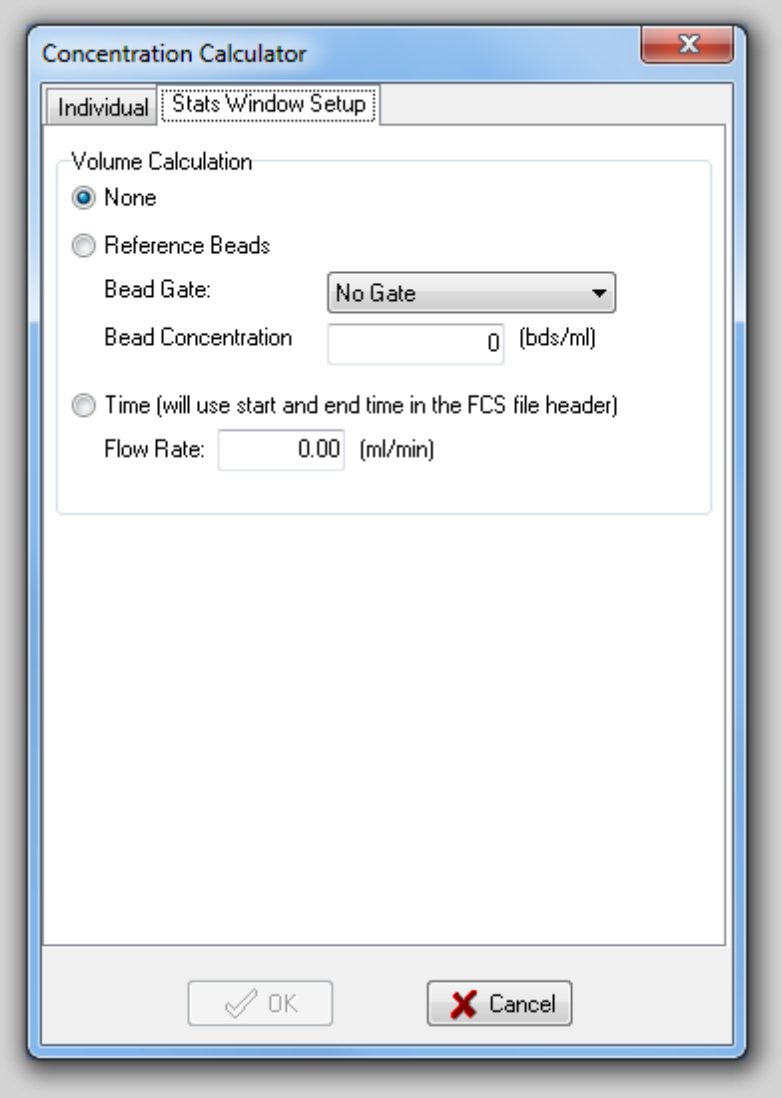

Figure 13.2 Concentration Calculator Dialog - Stats Window Setup

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Chapter

14 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 performed either on an individual basis, or set up to be automatically calculated and appear on the statistics window for a series of histograms.

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 218.
- 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 14.1). Statistics histogram subtraction always uses the *Overton Subtraction* method, described below.

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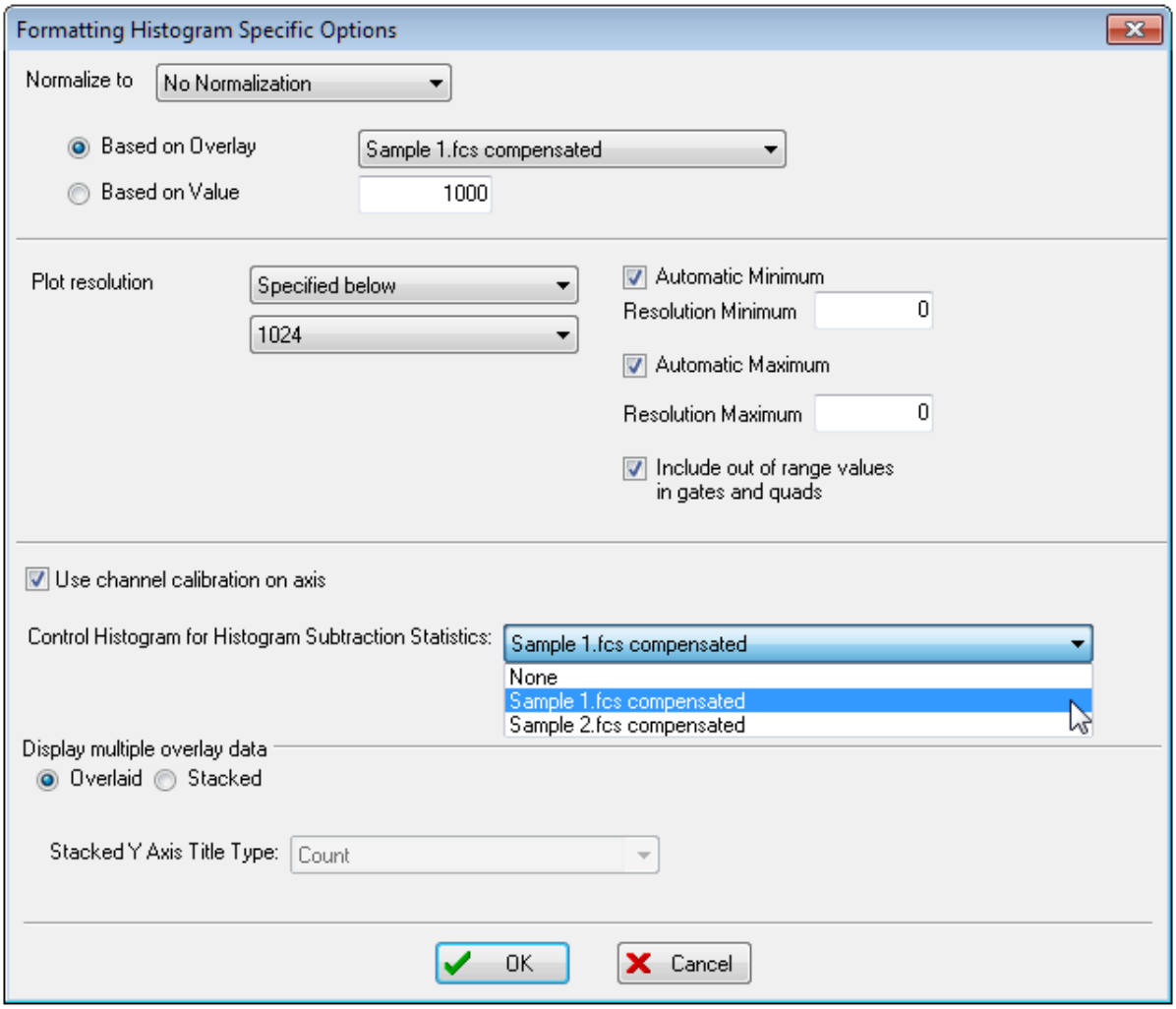

Figure 14.1 Setting the Control Histogram in the Formatting Hisotgram Specific Options Dialog

To perform an individual histogram subtraction, select the **Data→Calculate→ Histogram Subtraction** command, which displays the **Histogram Subtraction** window (Figure 14.2).

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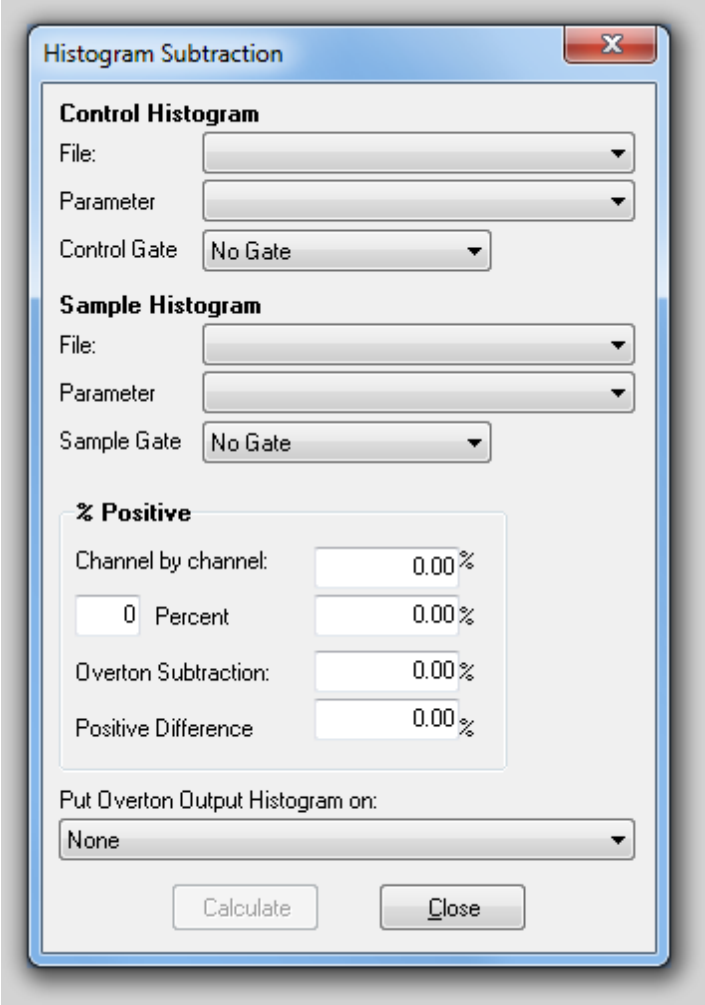

Figure 14.2 Histogram Subtraction Window

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**

  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

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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

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. To set up a channel calibration 280, 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 208 bounding each bead as shown in Figure 15.1.

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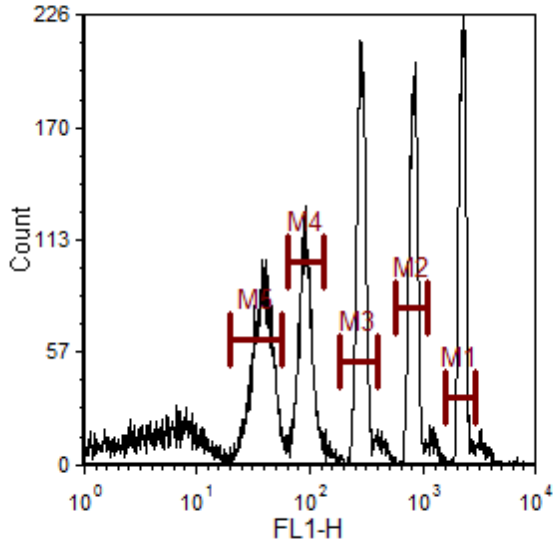

Figure 15.1 Setting Up Markers for Channel Calibration

- Select the **View→Tools→Channel Calibration** command. The Channel Calibration window 284 will appear (Figure 15.2).

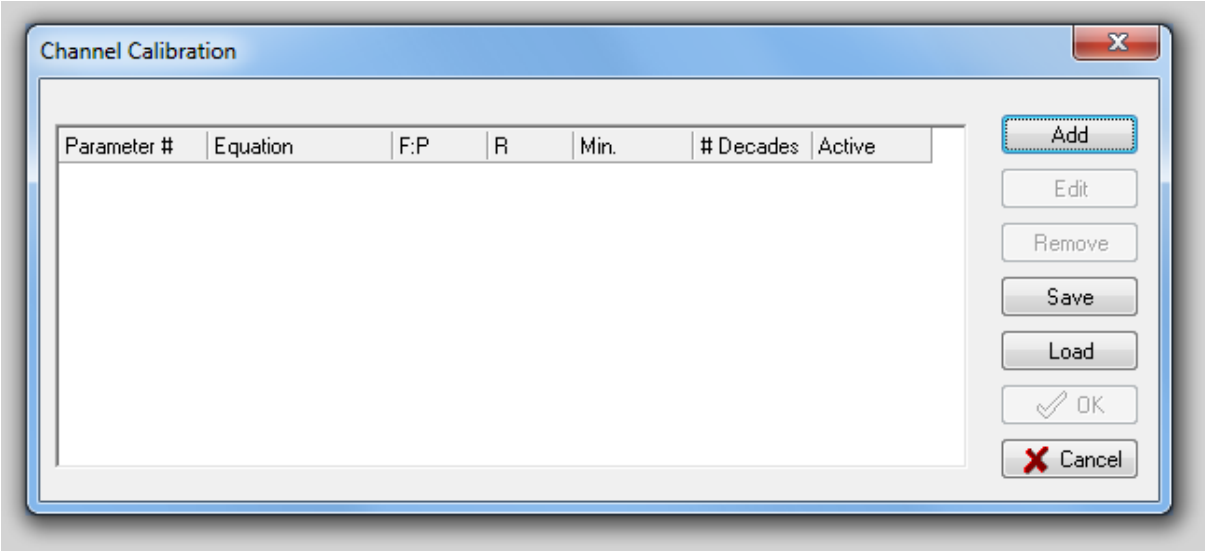

Figure 15.2 Channel Calibration Window

Press the **Add** button to create a new calibration. The **New Calibration**

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dialog will appear (Figure 15.3).

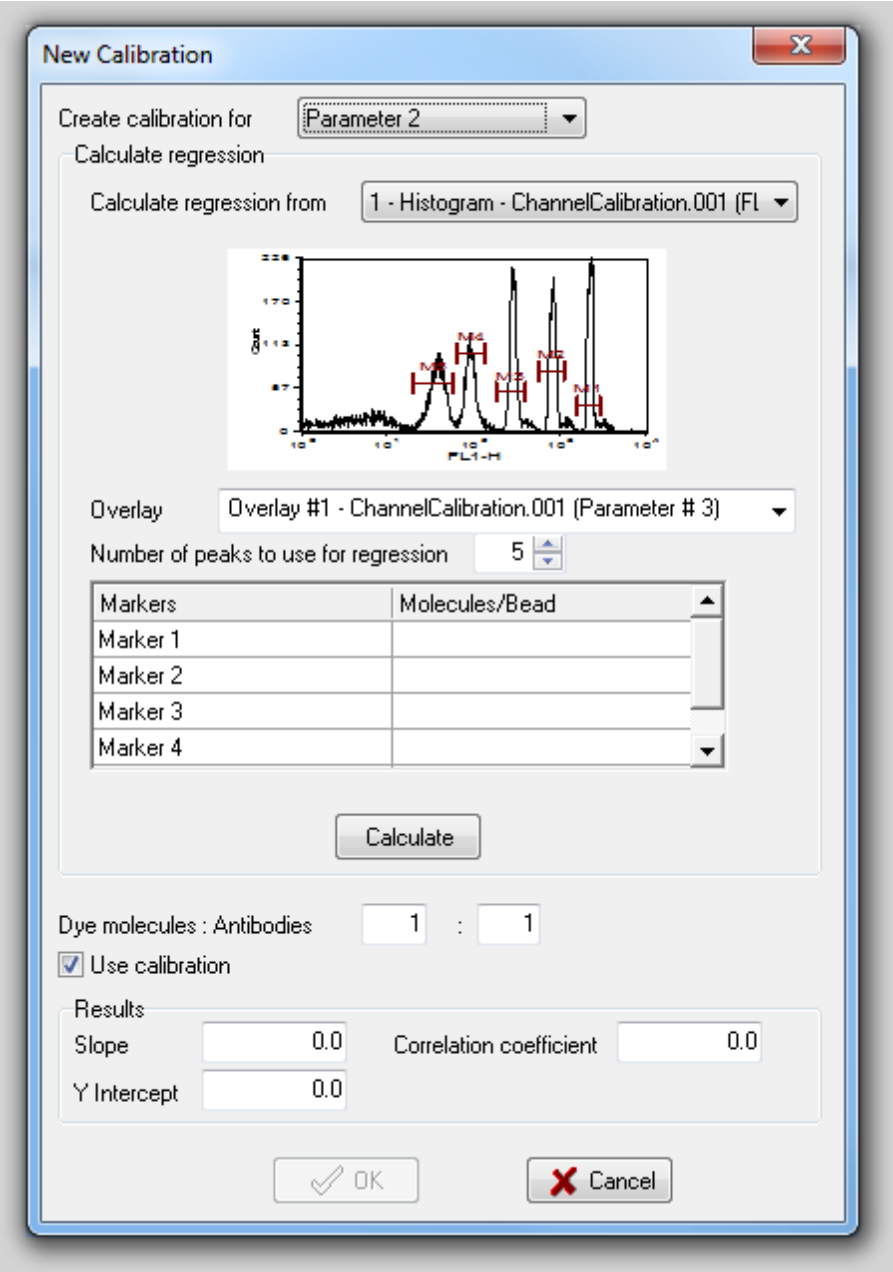

Figure 15.3 New Calibration Dialog

- Select the parameter you wish to create the calibration for in the **Create calibration for** drop-down list.
- Choose which histogram you wish to use in the **Calculate regression from** drop-down 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.

- Press the **Calculate** button 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.

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 **View→ Tools→Channel Calibration** command and uncheck the **Active** checkbox beside that calibration (Figure 15.4).

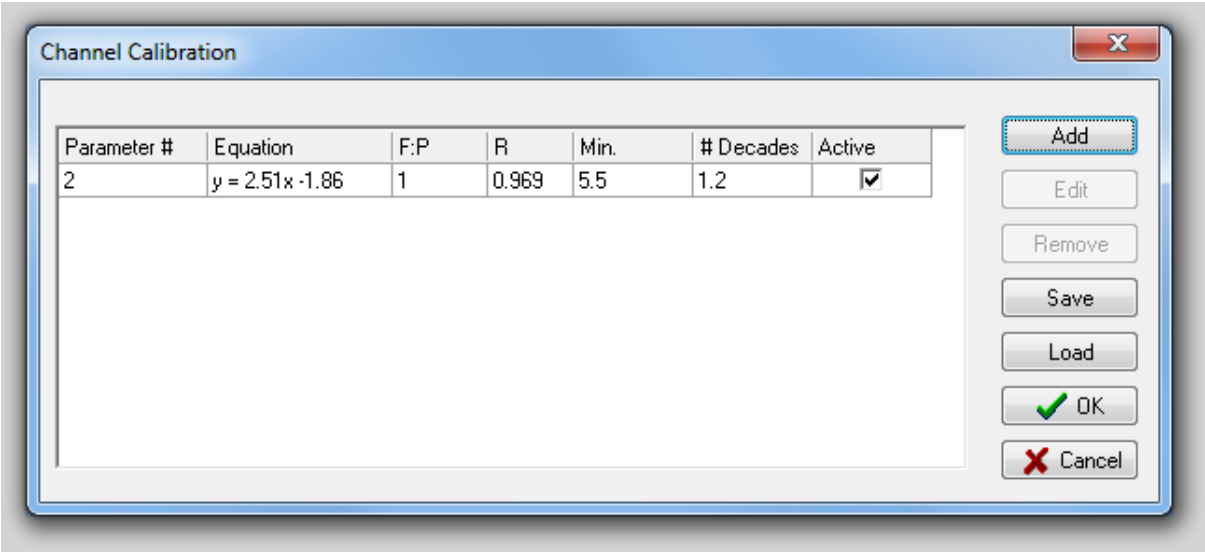

Figure 15.4 Channel Calibration Window with Active Checkbox

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You can also choose which individual plots use the calibrations. The **Specific Options** page of the **Format** window for both histograms 172 and 2D Plots 184 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.

15.3 The Channel Calibration Window

The Channel Calibration window (Figure 15.5) shows all the current calibrations. It is possible to have different calibrations defined for different parameters. FCS Express will automatically use the appropriate calibration.

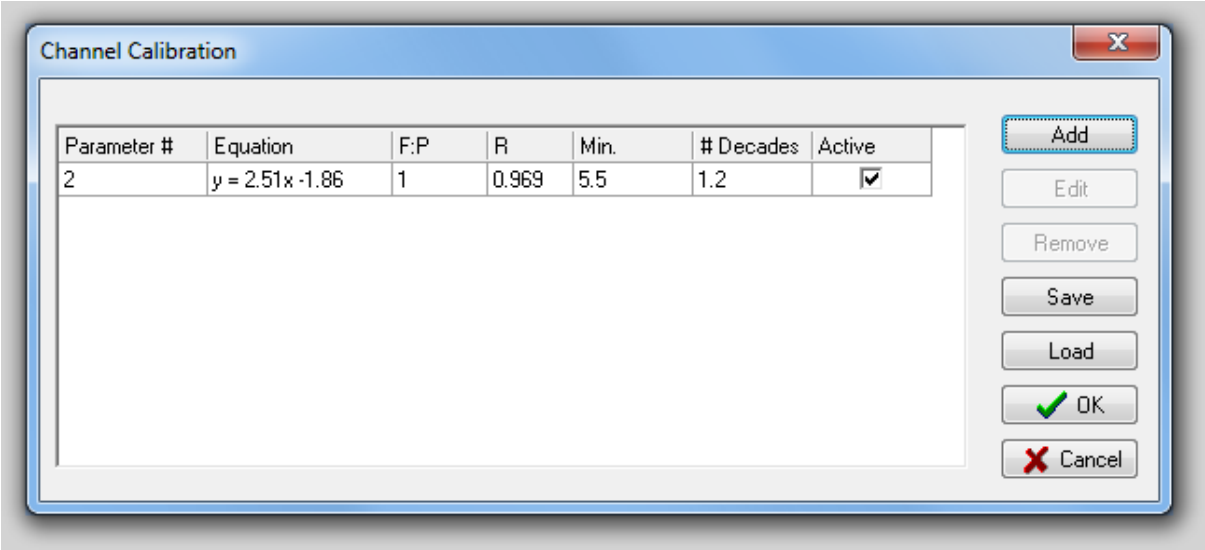

Figure 15.5 Channel Calibration Window

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.

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- **# 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 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.

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 **Data→Data Info→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 extracted from a data file (and will be automatically removed when the data file is no longer used in the layout) and the **Created manually** folder shows compensation definitions that have been created manually within FCS Express.

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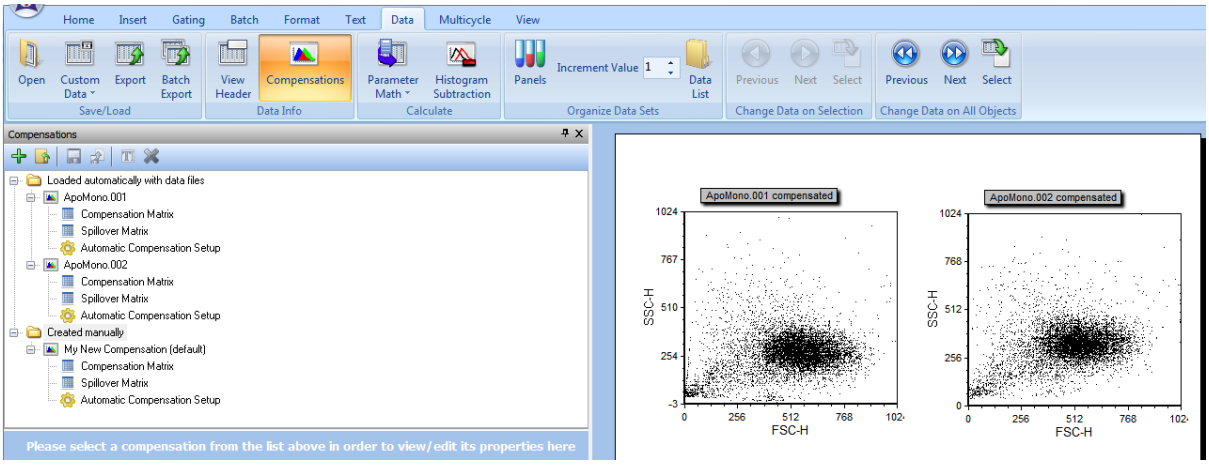

Figure 16.1 Compensations Navigator showing compensations available in the layout.

The **Compensations** window (Figure 17.1) is dockable and pinnable 66 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 or by right-clicking on a compensation folder or sub-item. The actions are summarized in the table below:

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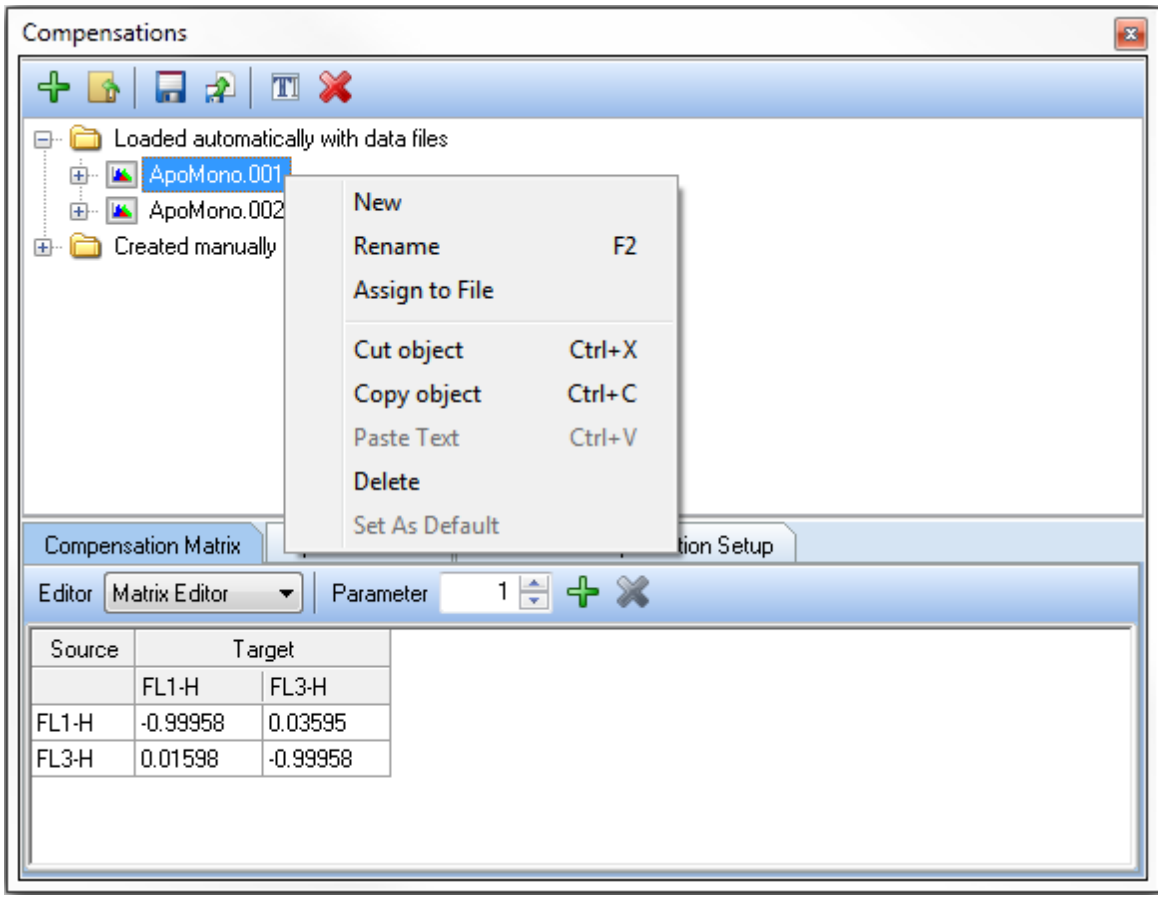

Figure 17.1 Compensations Navigator showing available commands and actions.

Action	Command	Explanation
Create a new compensation	or Right click on Folder or Compensation	Add a new compensation definition. A new definition will be created
Import a compensation from a file		Import a previously saved compensation (*. compensation) file.
Export the selected compensation		Exports and saves a compensation (*. compensation) file.
Assign this compensation to a data file	or right click on Folder or Compensation → **Assign to file**	Assigns and saves 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.
Delete	or right click on Folder or Compensation → **Delete**	Deletes a compensation definition from the compensation window.

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Cut Object	Right click on compensation→ **Cut Object**	Performs a cut command on the compensation definition. Matrix can then be pasted as a text box.
Copy Object	Right click on compensation **→ Copy Object**	Performs a copy command on the compensation definition. Matrix can then be pasted as a text box.
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, c ompensation 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 296 (Figure 18.1). The **Compensation Matrix** and **Spillover Matrix** tabs allow you to view and Edit a Compensation Definition 293 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 18 left) or to view a list of **Target - % Source** (Figure 18 right). Please see the **Automatic Compensation Setup** 296 section for details on this tab.

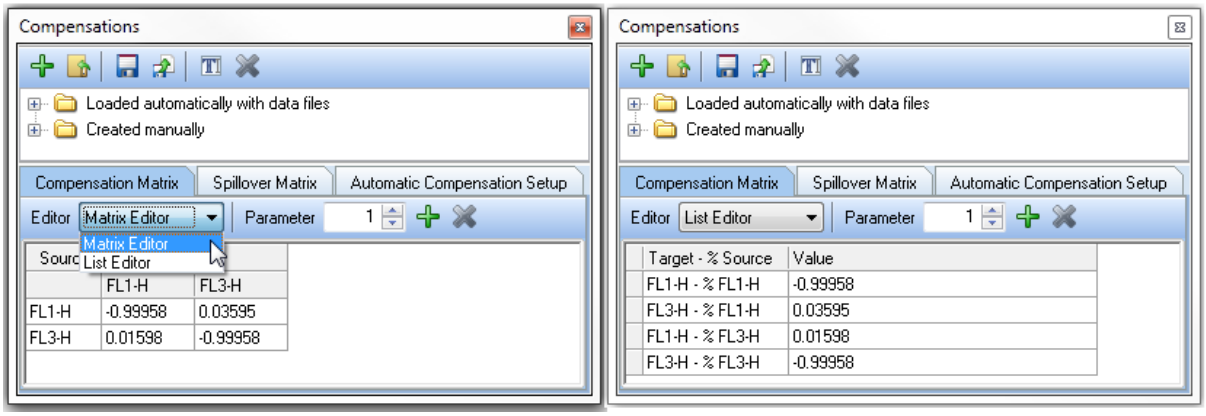

Figure 18.1 Compensations Navigator showing available tabs. Matrix Editor View (left) and List Editor View (right).

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 19.1).

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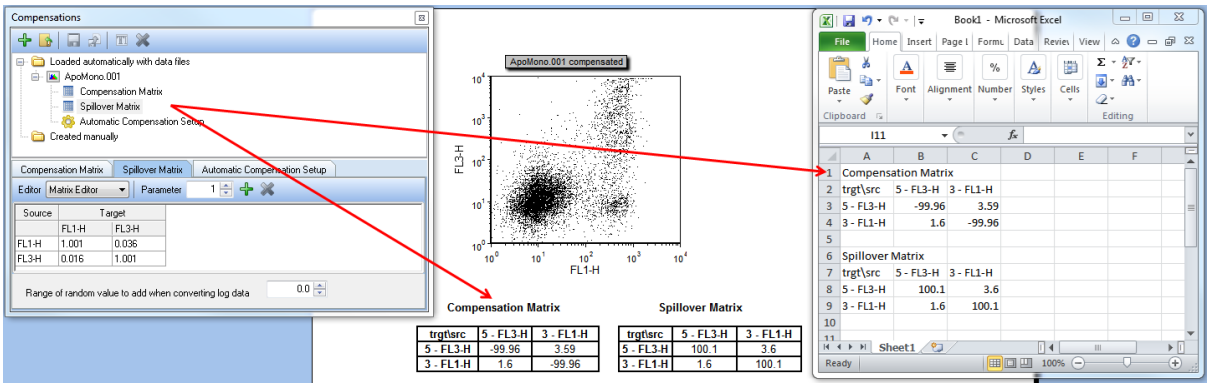

Figure 19.1) - Drag and drop a compensation matrix from the Compensations navigator onto a layout, into a text box, or directly into Microsoft Excel to display the values.

16.3 Editing a Compensation Definition

Selecting a file within the **Compensations** navigator displays the compensation definition in the tabs below. Clicking on the **+** button next to the compensation file will enable an expanded view in which you can directly select the **Compensation Matrix**, **Spillover Matrix**, and **Automatic Compensation Setup** 296. Clicking on items in the expanded view acts just like choosing the different tabs below (Figure 20.1).

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**.

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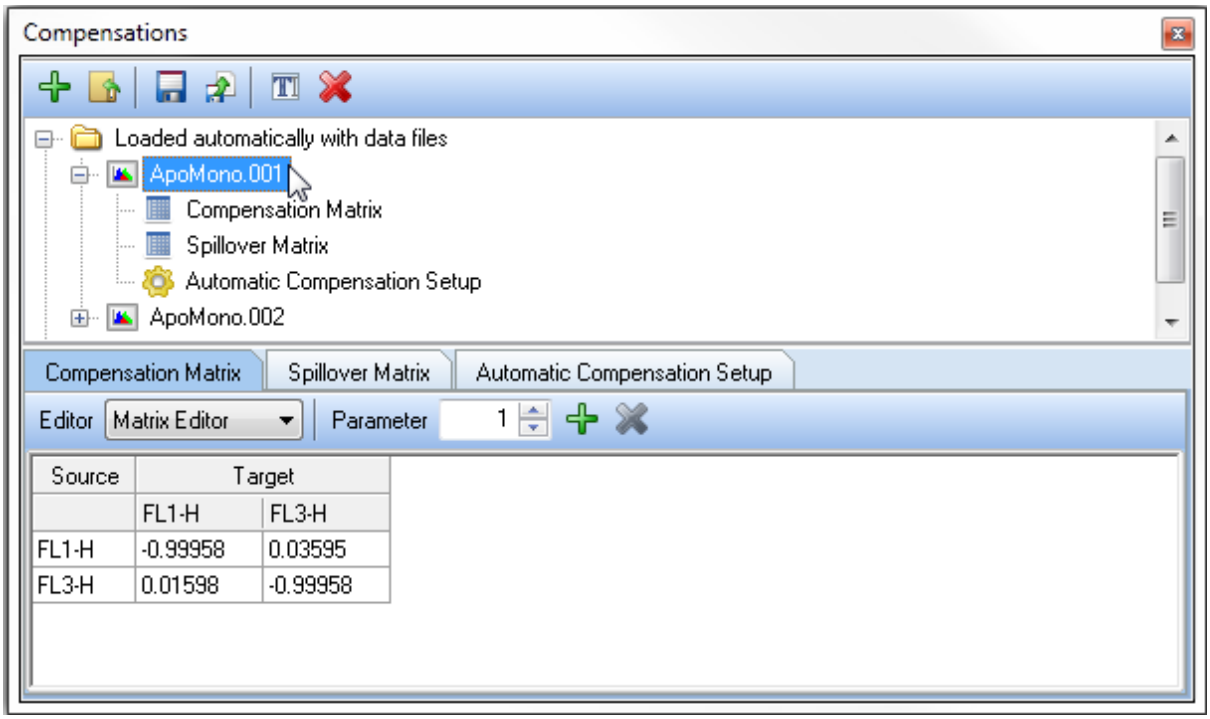

Figure 20.1 The Compensations Navigator displaying the Compensation Matrix in Matrix Editor View.

Compensations can be edited directly from the **Compensation Matrix** tab and **Spillover Matrix** tab by clicking on the **Target** (**Matrix Editor**) (Figure 21.1 left) or **Value** (**List Editor**)(Figure 21 right) and using the up and down arrows or entering a value to adjust the compensation.

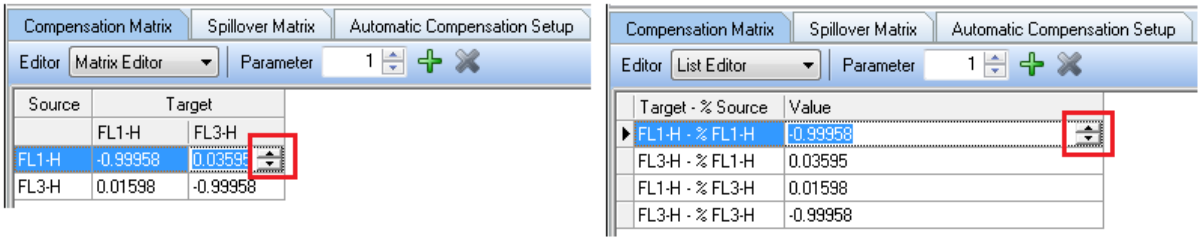

Figure 21.1 Adjusting compensation values using the compensation Matrix (left) and List (right) Editors. Use the up and down arrows or manually enter a value to adjust the compensation.

Every compensation must have a unique name that is entered as the c compensation 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

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matrix by clicking the or buttons (Figure 22.1).

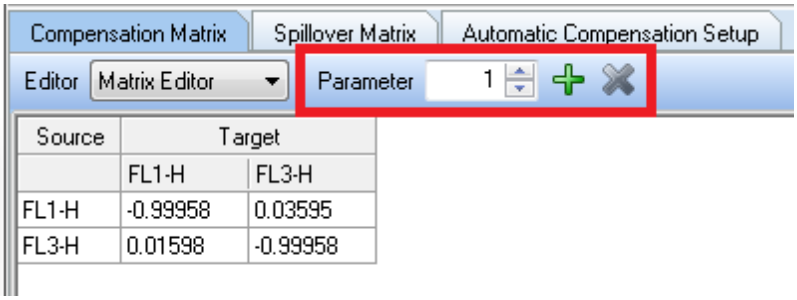

Figure 22.1 The Parameter field allows you to add or remove parameters to your compensation matrix.

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 23.1). Typically, this value should be less than one. This will add a small random value to the linear value prior to it being reconverted back to log. It is recommended to leave this value at zero unless these digitization errors become apparent.

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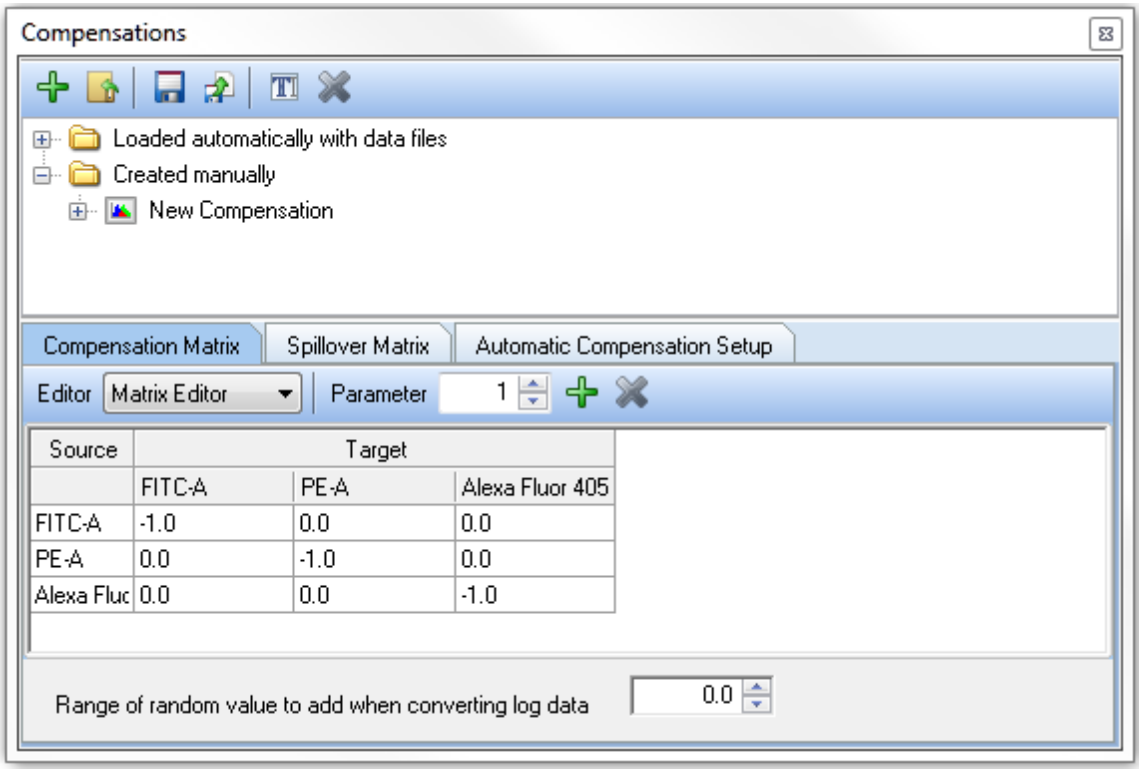

Figure 23.1 The Range of randow value to add when converting log data field allows you to easily correct for digitization errors.

16.4 Automatically Calculating Compensation Definitions

Instead of manually entering every percent to subtract for every target-source pair, 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**741. To get started, use the **Data→Data Info→Compensations** command, open the **Compensations** navigator, and add a new compensation with the **Create a new compensation button** . You can then click on the **Automatic Compensation Setup** tab to access the automatic settings (Figure 24.1).

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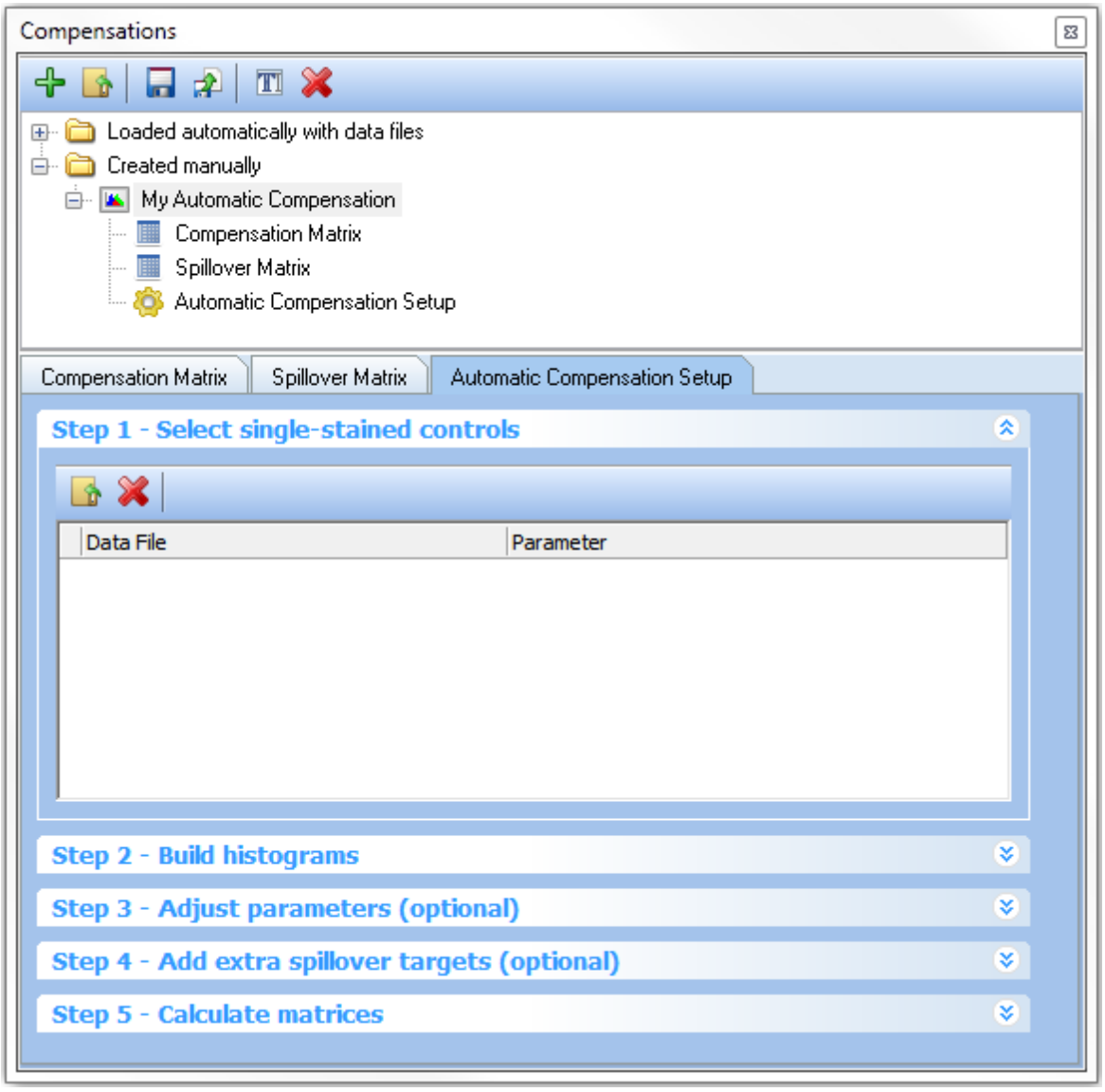

Figure 24.1 The Automatic Compensation Setup tab.

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 25.1)

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 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 gates in **Step 3: Adjust parameters**.

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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** drop down.

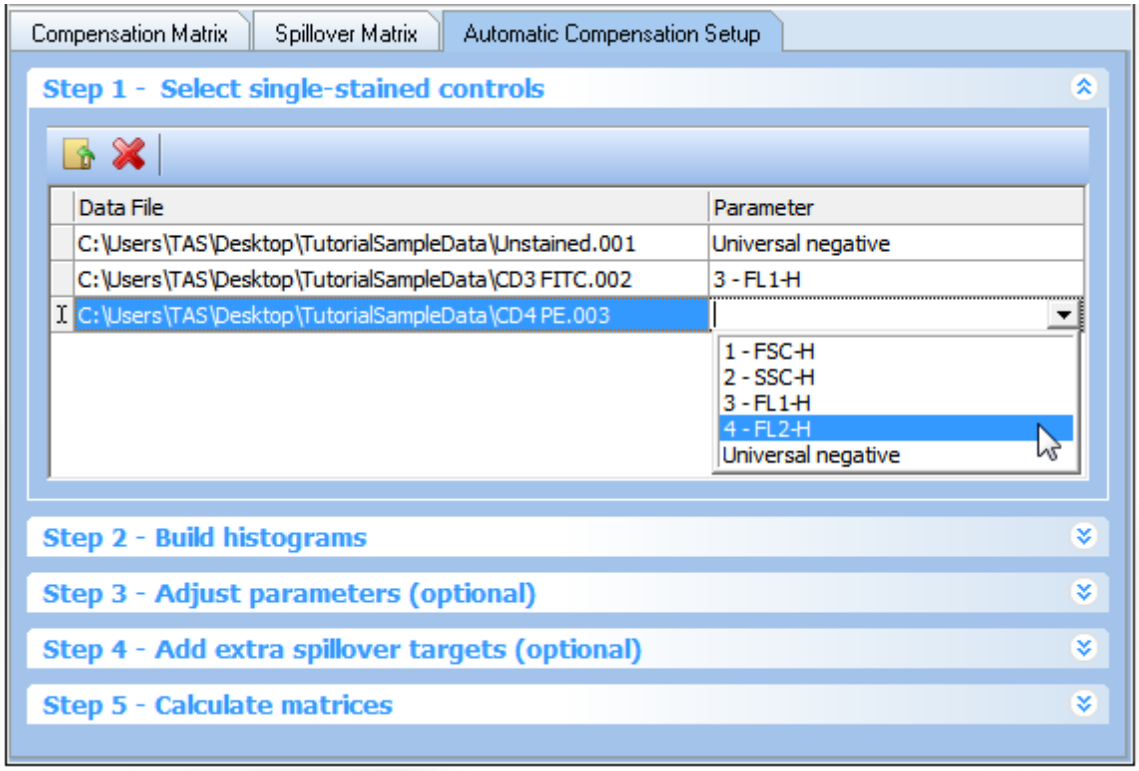

Figure 25.1 Step 1 - Select single-stained controls. Add compensation control data files and choose their respective channels from the Parameter drop down list.

- **Step 2: Build Histograms** (Figure 26.1)

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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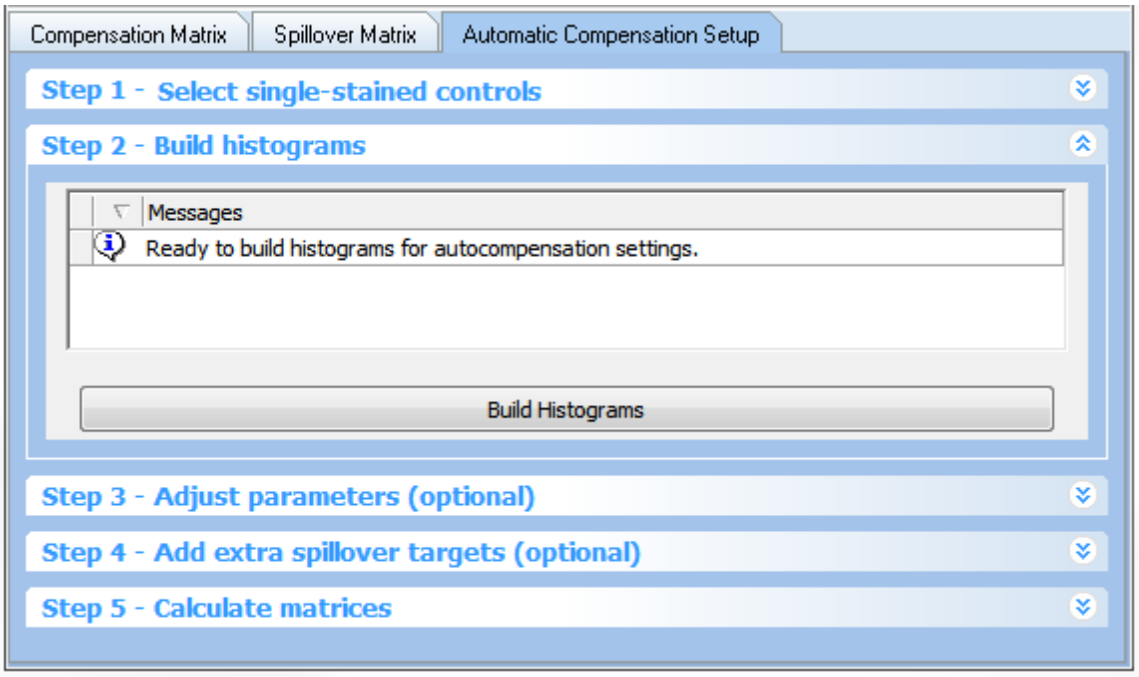

Figure 26.1 Step 2: Build Histograms

If there were no errors, the **Ready to build histograms for autocompensation settings message** in the **Build histograms** dialog. **The Build histograms** step will automatically create the following items (Figure 27.1):

- Scatter Plot 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 gates are children of the **Scatter Gate** which means adjusting the **Scatter Gate** will cause all other gatesto update in real time. If the **Scatter Gate** does not appear on the right parameters (ie...SSC-H vs FSC-H) you may change the gate using the replace gate feature 195. To replace the **Scatter Gate** with a new gate:

    . Change the X and Y axis to the correct parameters.
    . Draw a gate around the population of interest.
    . Choose the **Replace an existing gate** radio button.
    . Select the **Scatter Gate** from the drop down menu.
    . Click **OK**.
    (Note: These steps are also described in the Automatic Compensation Setup 741 tutorial.)

- Single Stained Control Pages: A new page will be created for every parameter you are compensating (ie. FL1-H control, FL2-H 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. The initial position of the markers will be placed automatically, based on the peak positions.

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. *(Note: A gate view will not automatically be inserted.)*

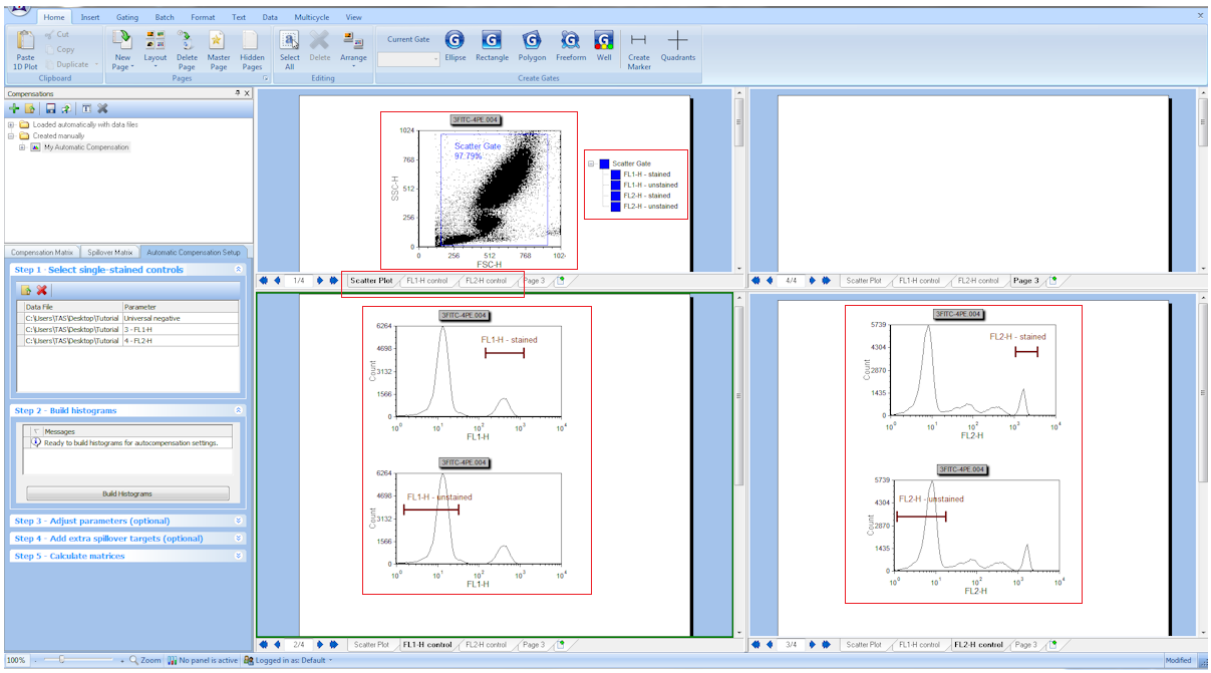

Figure 27.1 Layout after the Build histogram step was compeleted. Note the Scatter Plot, FL1-H control, and FL2-H control pages. Also notice that a plot for SSC vs FSC has been created with a Scatter Gate automatically applied. Histograms with markers for the stained and unstained populations have also been automatically create. All gates have been added to the gate list and markers have been converted and linked to gates.

- **Step 3: Adjust Parameters (optional)** (Figure 28.1)

This step is rarely used, but can be helpful with certain kinds of data. Once you have determined your initial compensation parameters via step 1 and step 2 (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. For instance, if the **Automatic Compensation Setup** does not make a good estimate of the

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population or if you would like to use a population from another file, gate, 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 (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. 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.

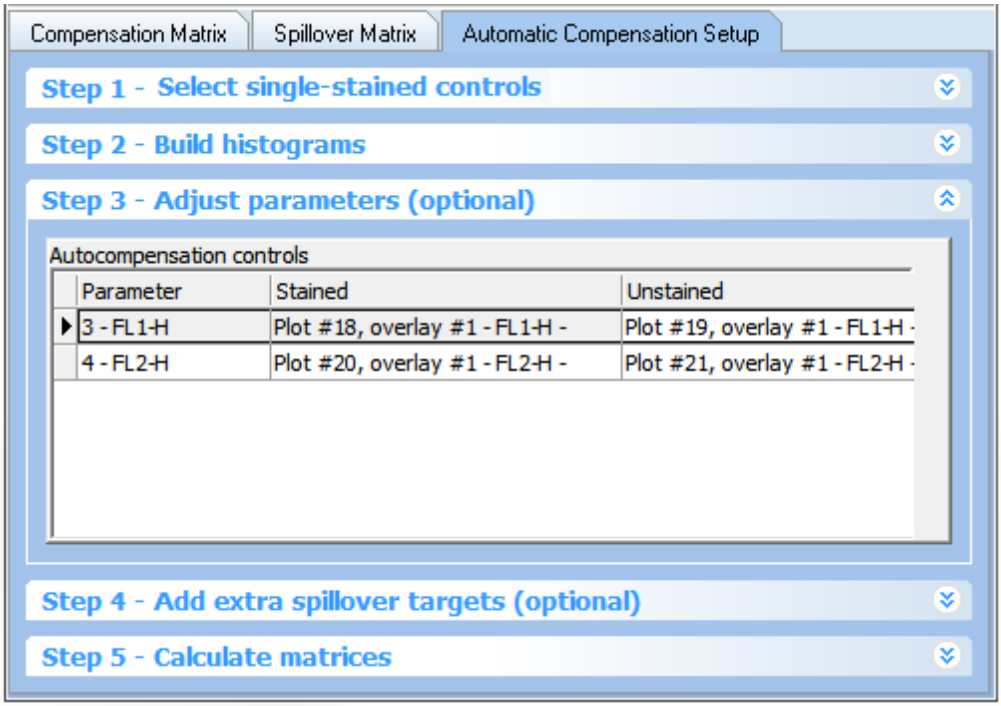

Figure 28.1 The Adjust parameters dialog displaying the currently defined parameters, plots, and gates.

Instructions for adjusting parameters (Figure 29.1):

1. Click and hold on a gate or plot.
2. Drag the gate and plot onto the Parameter/Column combination for the control (Red Arrow).
3. Release the mouse button.

The new gate and or plot will appear where the plot appeared in the Adjust parameters dialog (Green Arrow).

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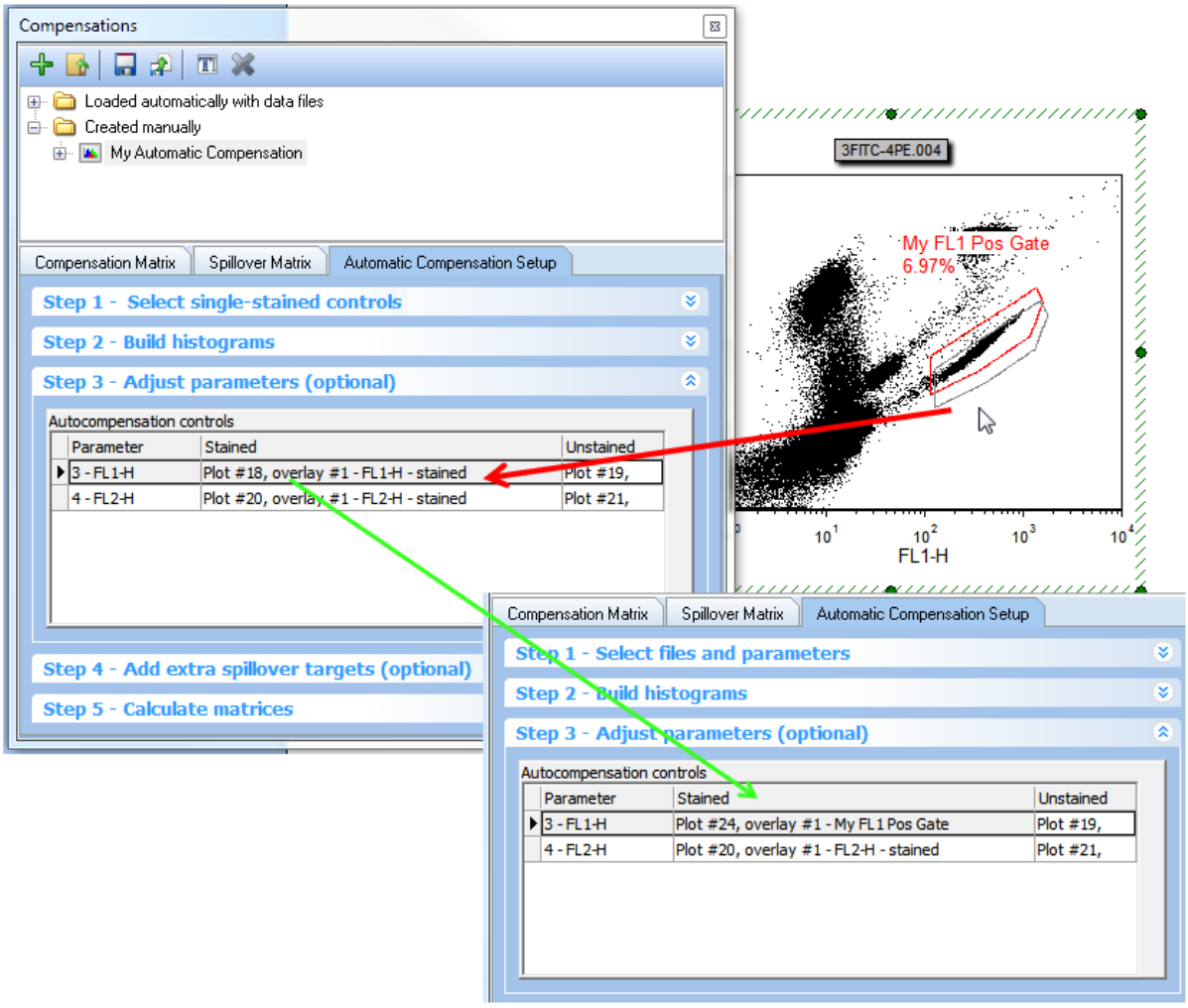

Figure 29.1 To change a parameter plot or gate drag the gate or plot onto the plot you wish to change (red arrow). The plot information will then update to the newly choosen gate or plot (green arrow).

• **Step 4: Add extra spillover targets (optional)** (Figure 30.1)
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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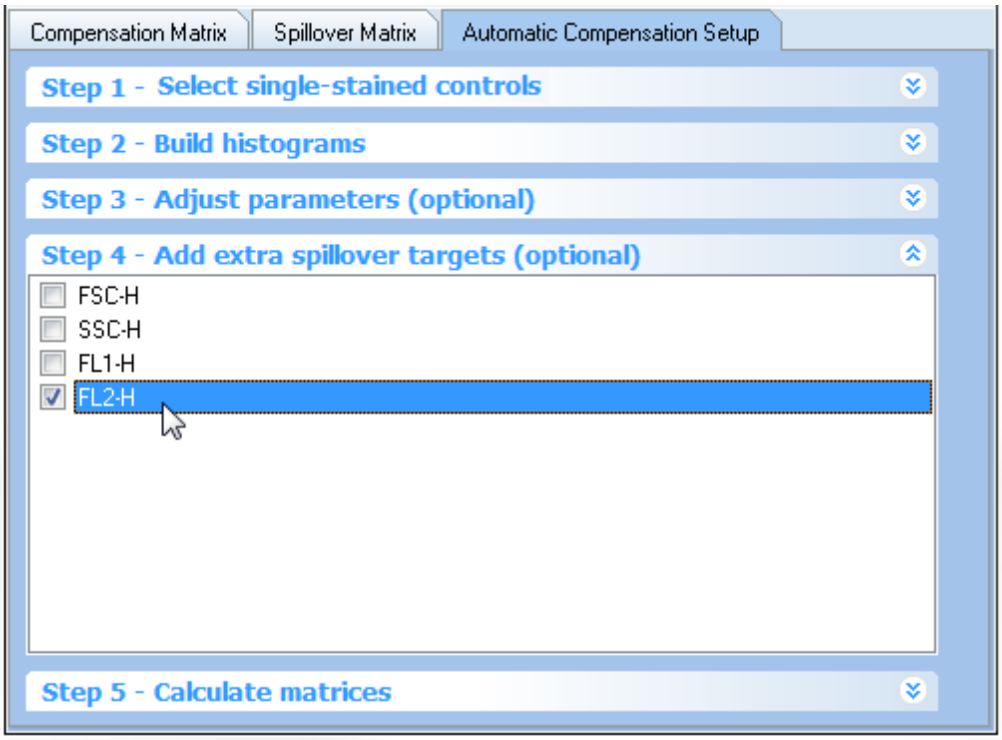

Figure 30.1 The Add extra spillover targets (optional) dialog. Checking a box will calculate the spillover for targets that have no source.

• **Step 5: Calculate matrices** (Figure 31.1)
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.

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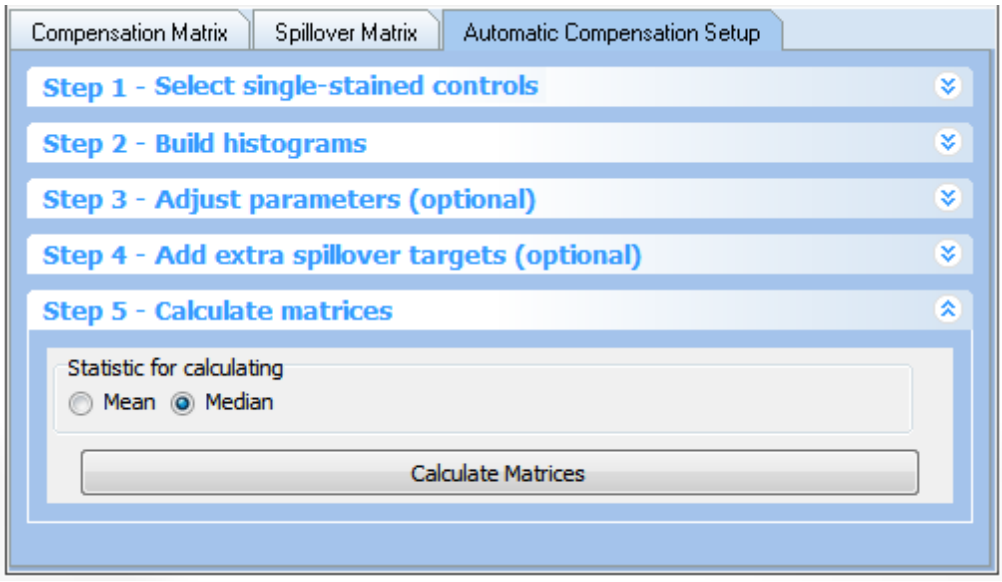

Figure 31.1 Step 5: Calculate Matrices button and choosing Mean or Median.

Your **Compensation Matrix** will then appear in the **Compensations** navigator (Figure 32.1 and you may edit any of the source-target pairs[293] through the **Compensation Matrix**, **Spillover Matrix**, or make new adjustments in the **Automatic Compensation Setup**.

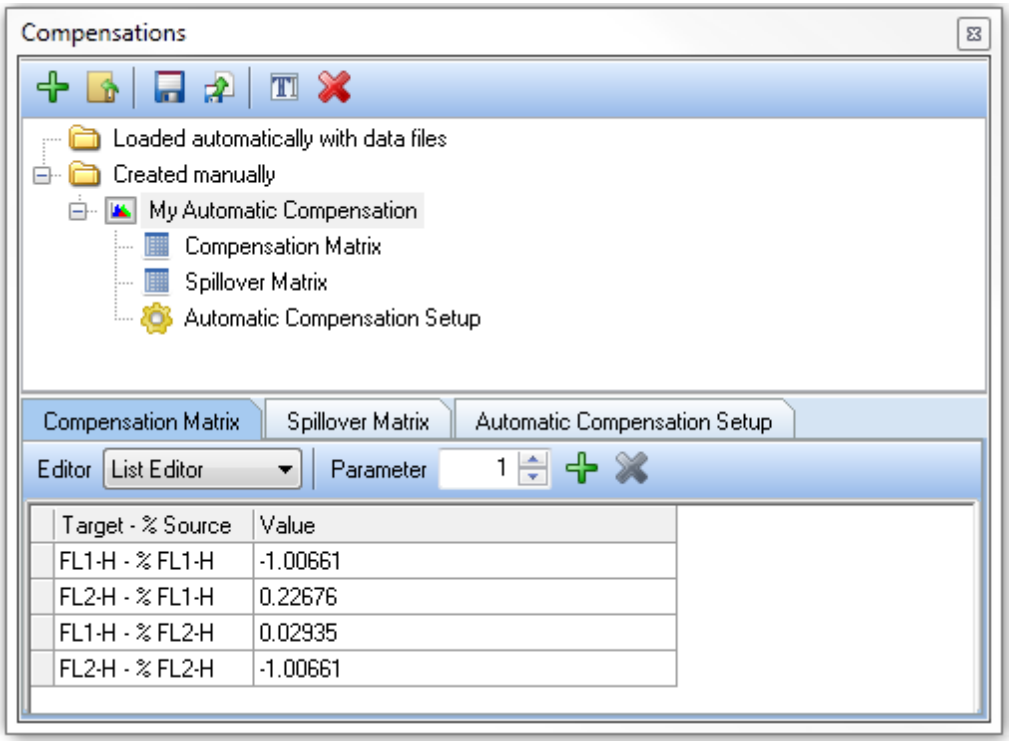

Figure 32.1 The Compensation Matrix after the Automatic Compensation Setup is complete.

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The automatic compensation setup settings are saved with the layout 309. To create a new compensation matrix from a different set of data, simply change the **Data File** in **Step 1,** readjust the markers if necessary, and then press the **Calculate Matrices** button in **Step 5**.

We also suggest that you use the compensation determined in the **Automatic Compensation Setup** and apply it to different layouts and files 305 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** 736.

16.5 Applying Compensation Definitions

Every overlay on a plot, and every file loaded as a plate, can have a different compensation definition. To change the compensation for an overlay, right-click on the plot and select **Format** from the pop-up menu. Go to the **Overlays** page and select the compensation definition you want to use. Alternatively, you can use the **Format→Plot Options→Overlays** command.

There are also many drag and drop commands for compensations 289. 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 33.1).

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 onto the plot you want to apply it to (Red arrow).
   Blue borders around the plot will indicate you can drop the compensation.
3. Drop the compensation by releasing the left mouse button.
   The compensation will then be applied to the plot (green arrow).

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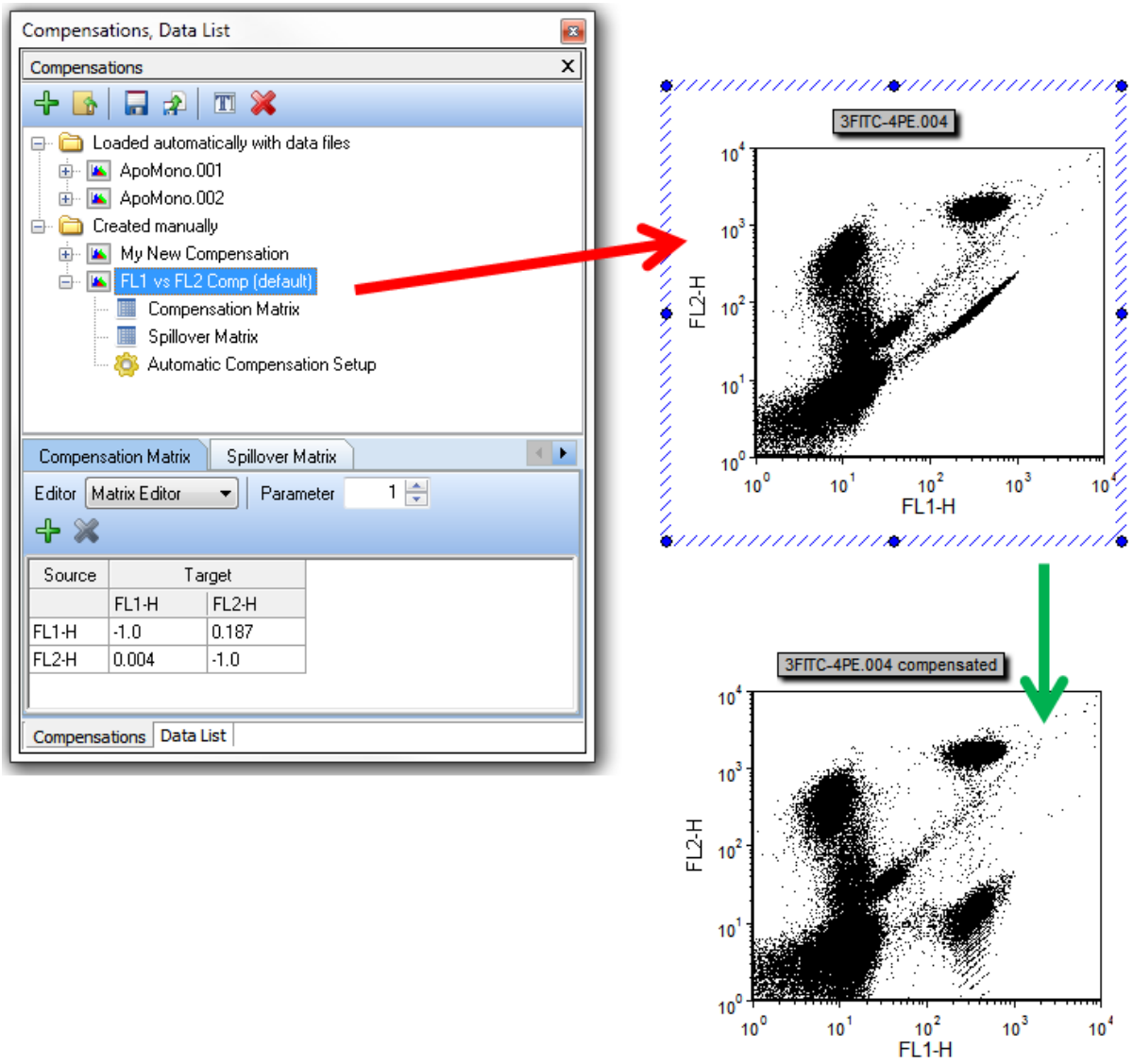

Figure 33.1 Dragging and dropping a compensation from the Compensations navigator onto a plot to apply the compensation.

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 through 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 save compensation definitions to folders 309, and have them automatically apply to the FCS files in the folder.

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Additionally, copy and paste commands (Ctrl+C/Ctrl+V or right click Copy/Cut/Paste Object) may be used to move matrices from one compensation definition to another within the **Compensations** navigator.

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 **Edit Preferences** dialog (**Application Button→Edit Preferences Button**) in the **Default Compensation to Use** drop-down list. The options are listed 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 before the change will continue to be used.
Compensation Default	The default compensation definition will be used. To set the definition, see Setting Up Compensation Definitions 289.
FCS File	The compensation definition stored with the file is automatically used when the data file is displayed on a plot.
FCS File, then Compensation Default	See FCS, above. However, if there is no definition, the default compensation definition will be applied (see Setting Up Compensation Definitions 289).

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. For step by step instructions on using the this feature please see our **Manual Compensation Tutorial** 737.

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 34.1). To edit an existing compensation definition, select the appropriate submenu item.

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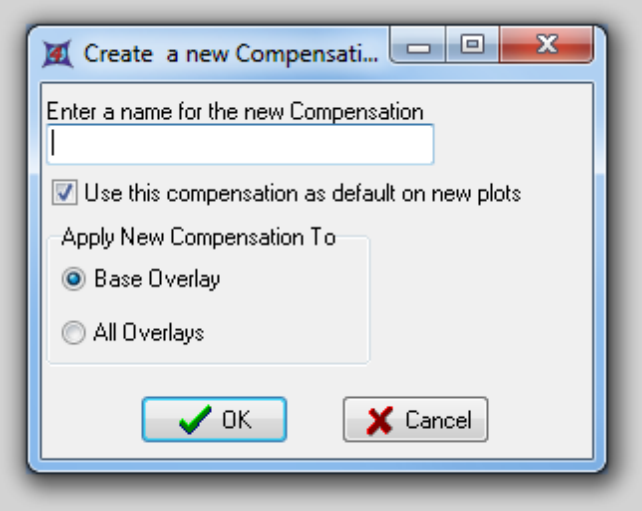

Figure 34.1 Creating a New Compensation from a 2D Plot

After naming a new compensation and pressing the **OK** button, or selecting a compensation definition to edit, the **Edit Compensation** dialog will appear (Figure 35.1). 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 every time you change the compensation. If the **Compensations** navigator is open you to the compensation you are adjusting the Compensation Matrix and Spillover Matrix 293 values will update in real time. If the compensation you are editing is applied to other plots they will also update in real time.

Press **Cancel** to reject the compensation changes. Press **Restore** to ignore the changes but continue editing the plot. Once you press **OK**, the compensation settings for the entire layout will be changed to the new values.

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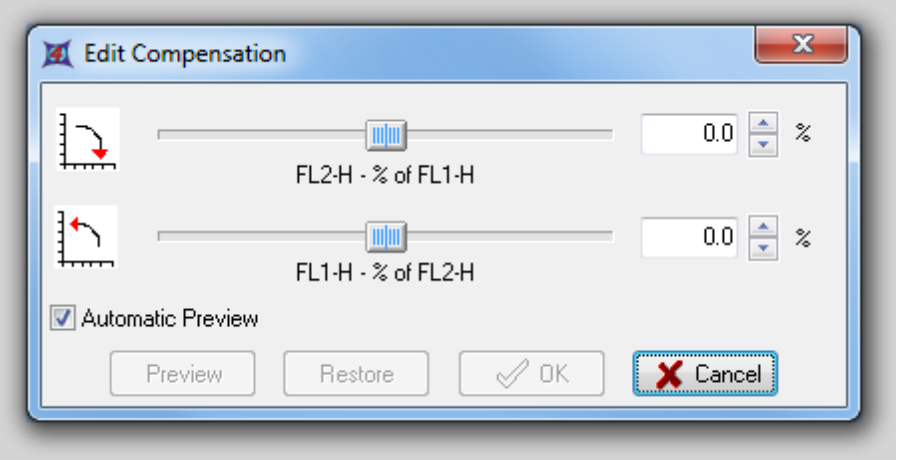

Figure 35.1 Edit Compensation Dialog

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** 289 navigator.

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.

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 settings are also saved with each compensation.

Save an individual compensation definition (**.compensation*) by selecting the **Data→Data Info→Compensations** command, select a compensation definition by clicking on it, and press the **Export the selected compensation** command .

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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 by choosing the **Import a compensation from a file** command.

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Chapter

17 Working with FCS Data Files

FCS Express gives you the power to modify the FCS data files directly. You will be able to make changes to the keywords in the headers, add your own keywords, and export the data to a table.

17.1 Viewing and Changing FCS File Headers

To view the header details of the current FCS file, use the **Data→Data Info→ View Header** command. The View Header window will appear (Figure 17.2). If you do not have any open FCS files, you will be presented with a dialog to select the desired file.

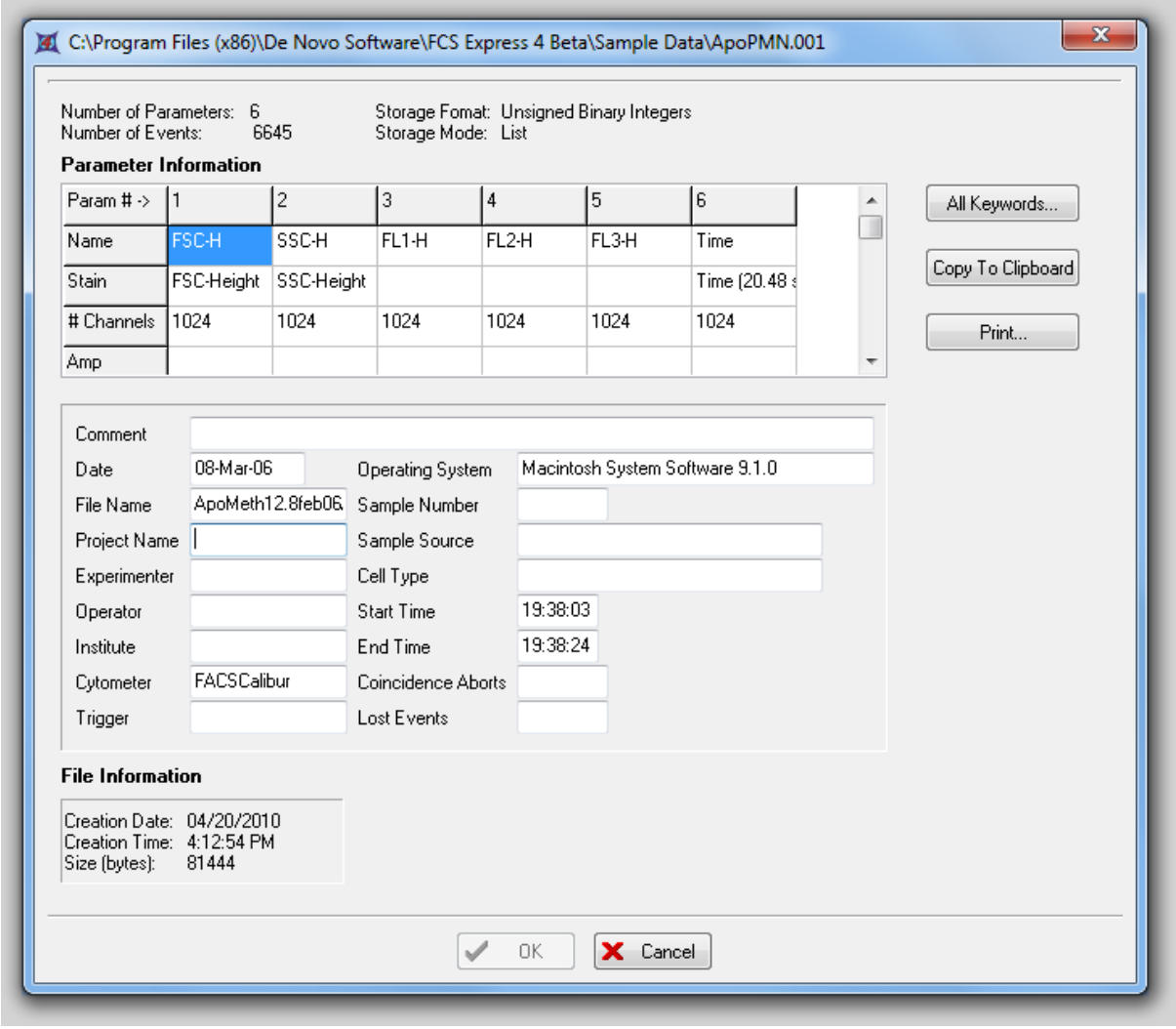

Figure 17.2 View Header Window

Some of the more common keywords associated with each parameter are

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displayed in the **Parameter Information** field. You can modify all the values in this table except the **# Channels** and **Word Size** entries. You can also modify any of the values in the editable fields in the middle of the window.

To copy all of the keywords to the clipboard in the $Keyword=Value format (for pasting into a text document), press the **Copy to Clipboard** button.

To print the keywords in the $Keyword=Value format, press the **Print...** button.

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 17.3). You can modify the values of the keywords by clicking in the value column and typing in the new value. *Note:* Not all values can be modified.

To add keywords, press 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 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.

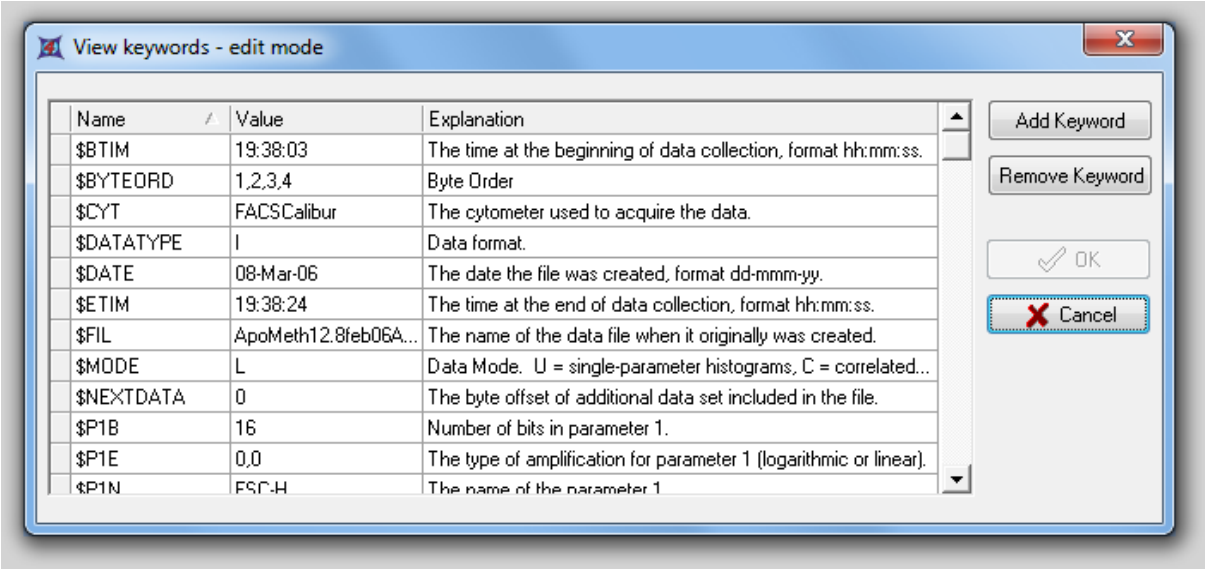

Figure 17.3 View Keywords - Edit Mode

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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:

- If there is a keyword text file with the name **<filename>.extrakeywords.txt** in the same folder as a data file with the name **<filename>**, then FCS Express will add those extra keywords to the data file when loading.
- If there is a keyword text file with the name *.**allextrakeywords.txt** in a data folder, then those keywords will be added to all files in the folder when the files are loaded.

If you are loading a list of FCS files collected together in a microtiter plate, or an BD Accuri C6 file as a plate, choose **FCS Plate folder** from the **Files of type** drop-down list in the **Select a Data File** dialog. Then choose a folder that contains the files.

When reading in data as a plate, you must specify the size of the plate in an extra keywords file. Create a keywords file containing the keywords $PlateRows and $PlateColumns. In a typical 96 well plate, that file would contain the following text:

$PlateRows=8
$PlateColumns=12

17.3 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 314 or you can "batch export" data from several files 316 at one time.

17.3.1 Exporting FCS File Data from a Single File

To export the data from an open FCS file, use the **Data→Save/Load→Export** command to bring up the **Export Data** dialog (Figure 17.4).

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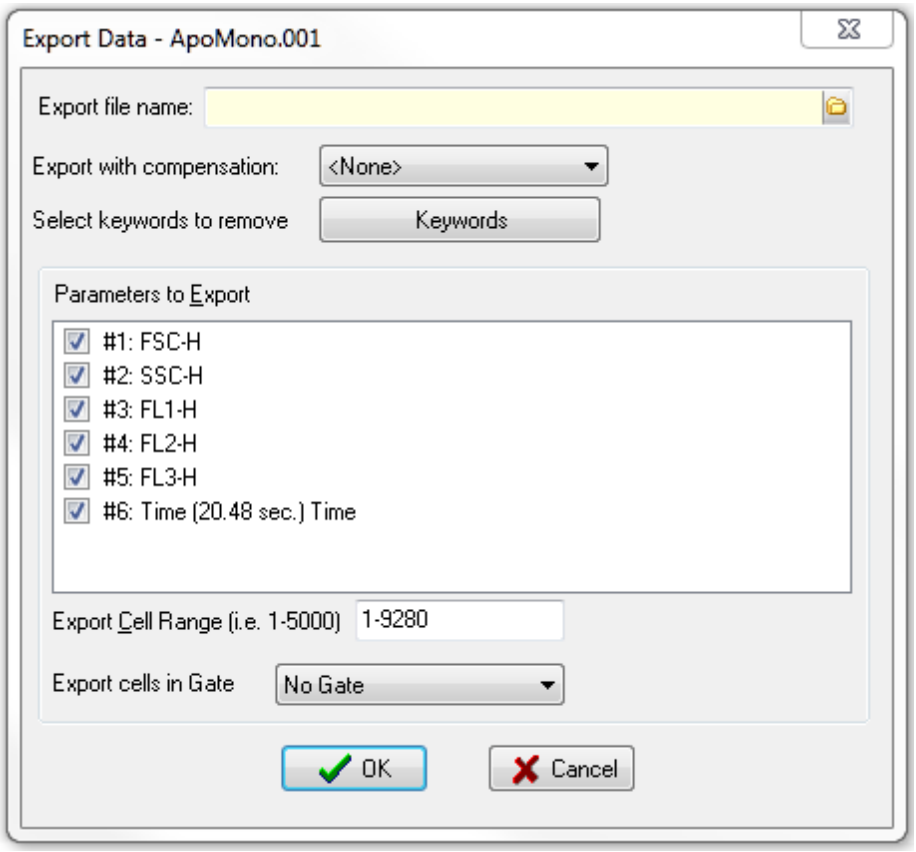

Figure 17.4 Export Data Dialog

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 where you can choose the folder to save the data in, enter a file name, and choose the file type. You can export the data as a text file (*.txt), comma separated values file (*.csv), tab separated values file (*.tab), space separated values file (*.prn), FCS file (*.fcs), or a DNS data stream file (*.dns). After assigning the file name and type, Press **Save**.

You have a choice of exporting the data with or without compensation using the **Export with compensation** drop-down list. If compensation definitions have been defined for the file you are exporting, they can be selected from the list. The keywords to export can also be edited.

There are many options to choose exactly which data to export:

- Select which parameters to export in the **Parameters to Export** list box.
- 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.
- Use the **Export cells in Gate** drop-down list, to export cells within a gate.

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When you are ready to export, press **OK**.

17.3.2 Exporting FCS File Data from Multiple Files: Batch Export

You can export data from multiple files consecutively using batch export.

1. Select the **Data→Save/Load→Batch Export** command to bring up the Batch Export dialog (Figure 17.5).
2. Press **Add** to select the files to export. You can remove files from the export list by pressing the **Remove** button (or clear all files with the **Clear** button).
3. Use the **Export as Individual Files** option to export all files separately and the **Export as Merged File** option to export all files merged into a single file.

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 314.

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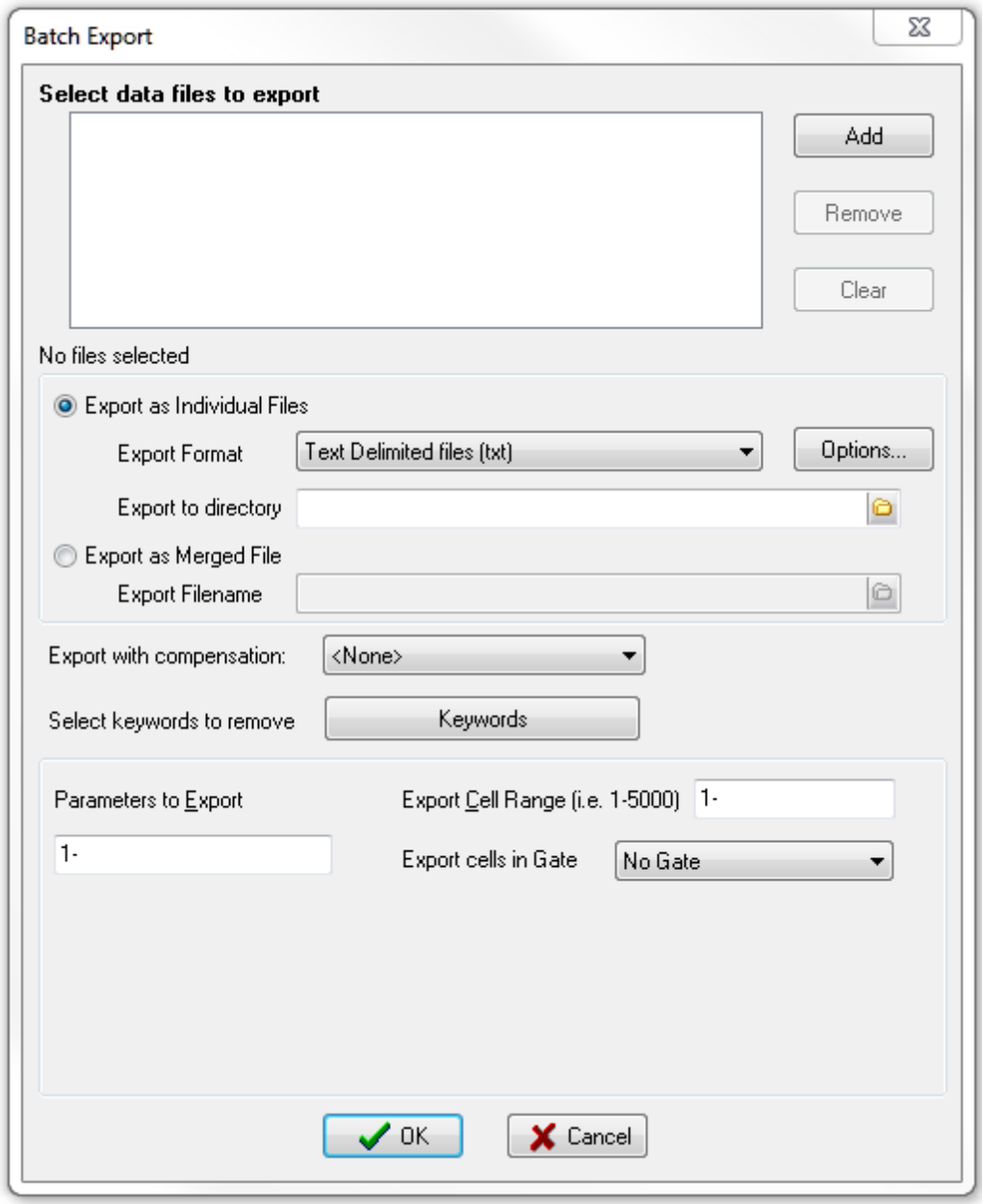

Figure 17.5 Batch Export Dialog

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Chapter

18 Data Grids

FCS Express allows you to view the raw data acquired for every event or "cell" in a data set.

Select the **Insert→General→Data Grid** command to insert a **Data Grid** in the layout to show the data from the top data set 264 (Figure 18.2). 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 18.2 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 314.

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18.1 Formatting Data Grids

In order to format a **Data Grid**, right-click on it 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 18.3). To show selected columns, choose **Only the items checked below** or **All items except those checked below**, and then check the appropriate items.

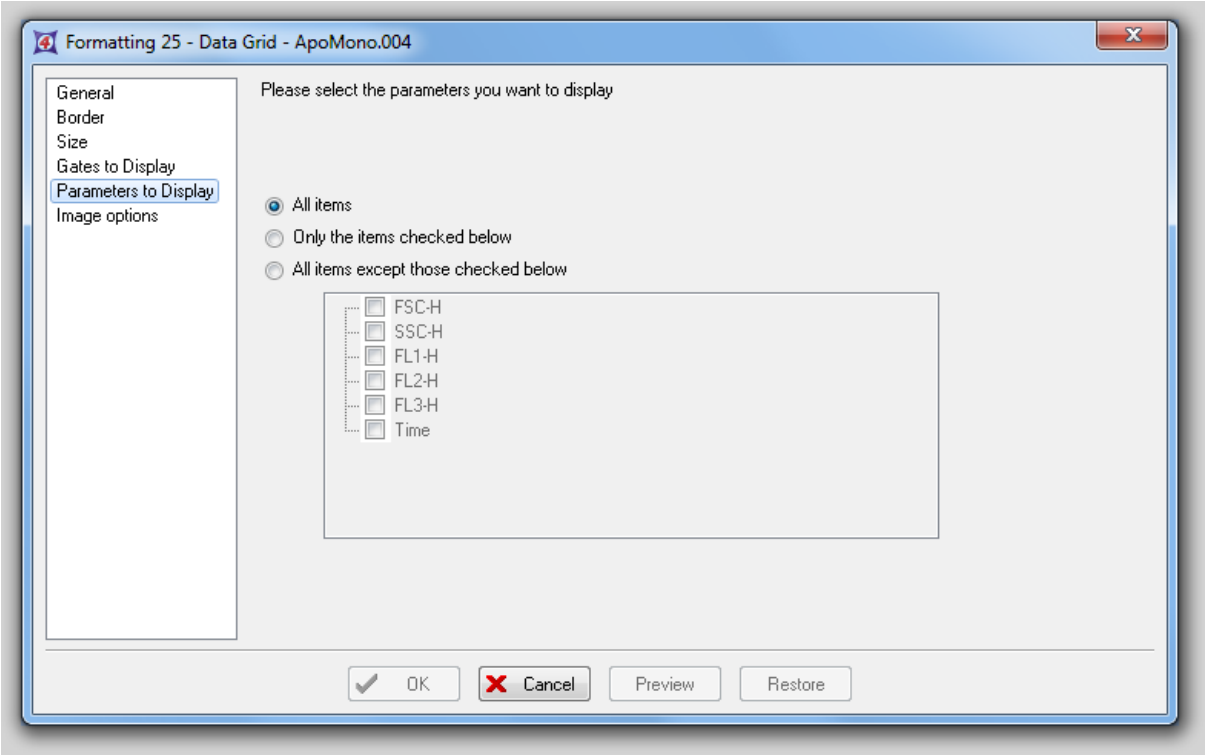

Figure 18.3 Showing and Hiding Columns in a Data Grid

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** (or **Home**)→**Create Gates** group and choose a gate from the **Current Gate** drop-down list.

18.3 Back Gating on a Data Grid

A **Data Grid** can also be *back-gated* using the formatting options:

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 18.4).

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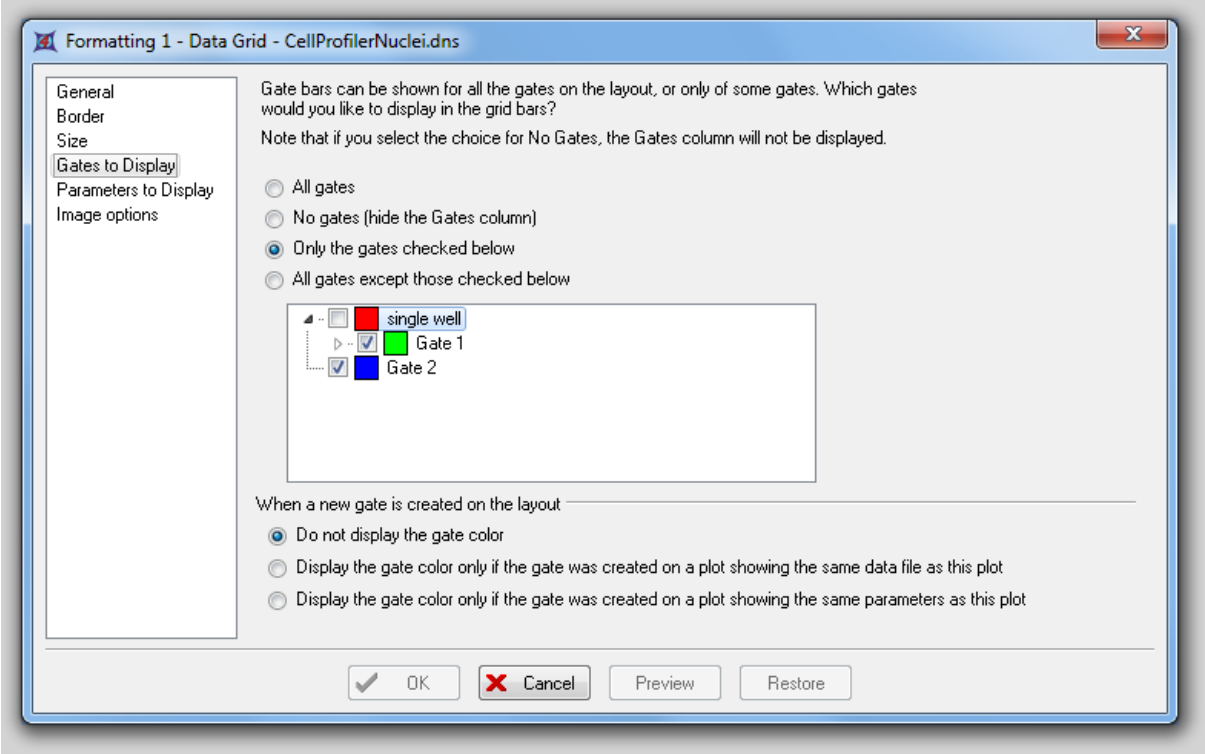

Figure 18.4 Formatting a Data Grid - Gates to Display

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 18.5).

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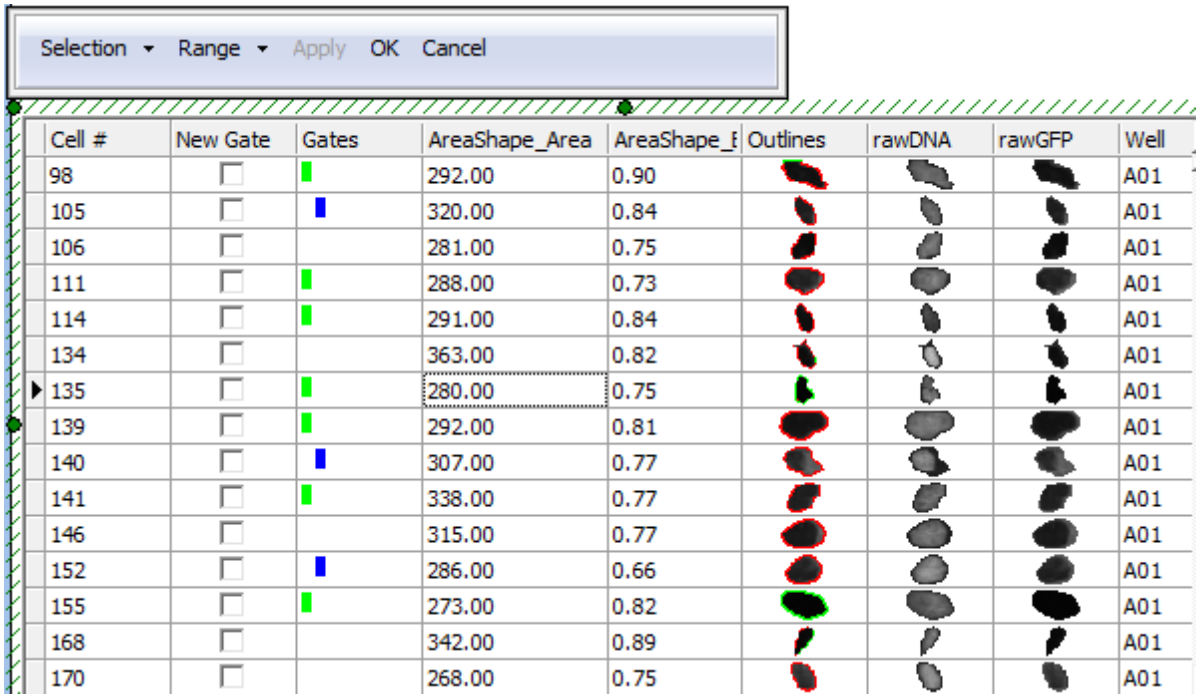
Cell #	Gates	AreaShape_Area	AreaShape_E	Outlines	rawDNA	rawGFP	Well
1		106.00	0.75				A01
2		170.00	0.44				A01
3		216.00	0.83				A01
4		344.00	0.84				A01
5		223.00	0.93				A01
6		266.00	0.73				A01
7		364.00	0.87				A01
8		139.00	0.86				A01
9		305.00	0.59				A01
10		169.00	0.68				A01
11		149.00	0.95				A01
12		212.00	0.85				A01
13		207.00	0.70				A01
14		140.00	0.89				A01
15		223.00	0.89				A01
16		153.00	0.72				A01

Figure 18.5 Back-Gated Data Grid. Bars in the Gates column of some events show which gate(s) the cells fall in.

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 Gate** column will appear in the grid (Figure 18.6).



Cell #	New Gate	Gates	AreaShape_Area	AreaShape_E	Outlines	rawDNA	rawGFP	Well
98	☐		292.00	0.90				A01
105	☐		320.00	0.84				A01
106	☐		281.00	0.75				A01
111	☐		288.00	0.73				A01
114	☐		291.00	0.84				A01
134	☐		363.00	0.82				A01
135	☐		280.00	0.75				A01
139	☐		292.00	0.81				A01
140	☐		307.00	0.77				A01
141	☐		338.00	0.77				A01
146	☐		315.00	0.77				A01
152	☐		286.00	0.66				A01
155	☐		273.00	0.82				A01
168	☐		342.00	0.89				A01
170	☐		268.00	0.75				A01

Figure 18.6 Creating a Gate on a Data Grid

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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 193.

You can also create a gate containing a **Selection** or **Range** using the appropriate drop-menu items.

18.5 Image Galleries

If you are using FCS Express 4 Image Cytometry or FCS Express Plus, it is possible to use the Data Grid as an Image Gallery. Select only to display the parameters with the image data from the Data Grid format screen 321 Figure 18.7.

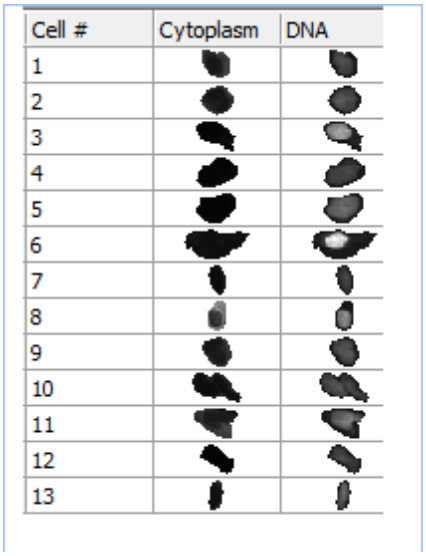

Figure 18. 7 An image gallery displaying only the image parameters.

To view images of only the cells within a particular gate apply a gate 321 to the Data Grid.

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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 a Page Layout Token

The following example is a tutorial on inserting a Page Layout Token:

1. Create a new layout.
2. Insert a Text Box on the page and type in "The number of pages on this layout is " (with the <space> after the word "is"). 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. The **Insert a Token** dialog will appear (Figure 19.2).

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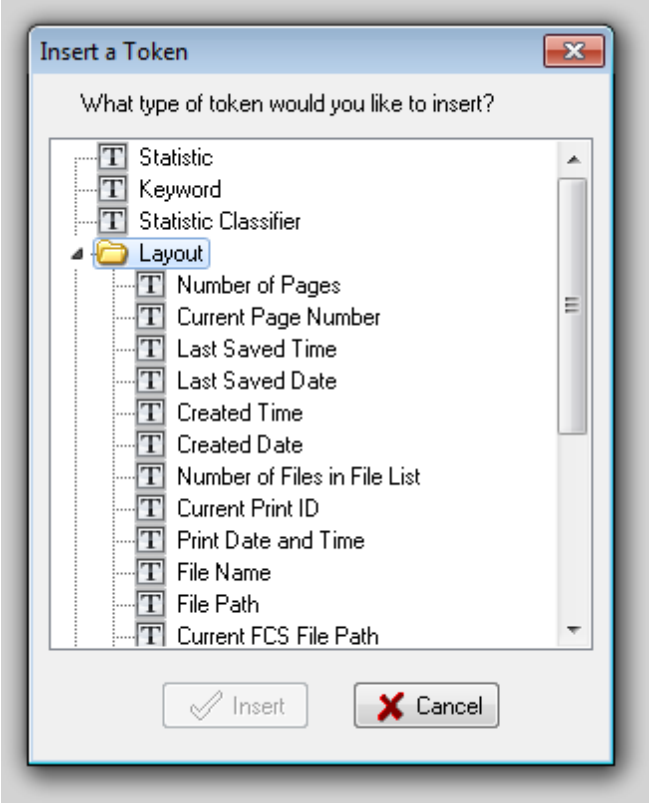

Figure 19.2 Insert a Token Dialog

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 19.3. Note how the background of the number 1 is grey. In edit mode, this signifies that the text is actually a token rather than normal text.

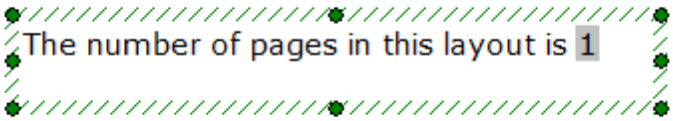

Figure 19.3 Number of Pages Token in a Text Box

6. Insert a new page on the layout by using the **Insert→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 19.4).

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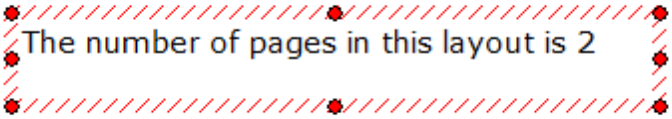

Figure 19.4 Token Automatically Updates

19.2 Inserting Tokens

To insert a Token in a Text Box (or any other edit field that accepts tokens) right-click on 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 (Figure 19.5).

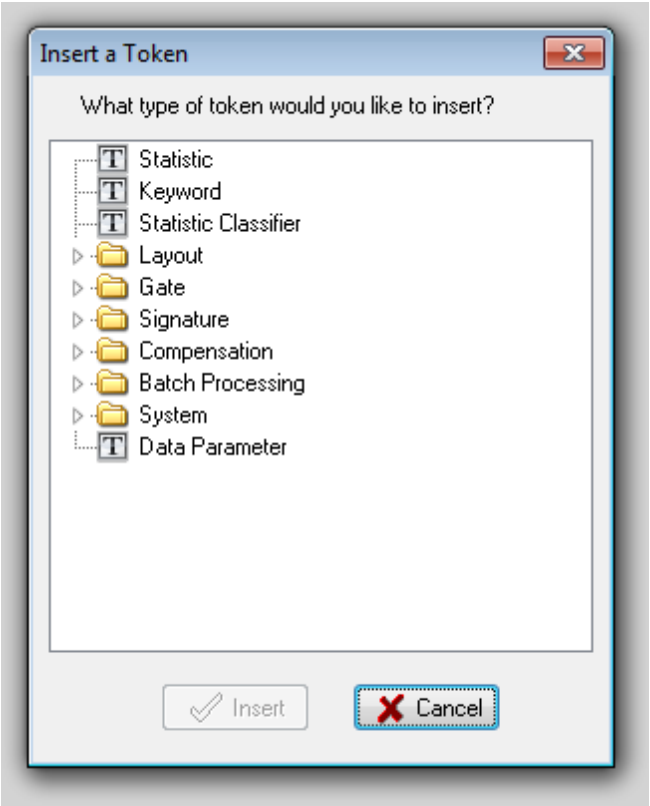

Figure 19.5 Insert a Token Dialog

There are many kinds of tokens that can be inserted:

- **Statistic**

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Displays plot, quadrant, and gate statistics. The value of a statistic token updates automatically, whenever a change pertaining to that statistic is made.

- **Keyword**

  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**

  Displays information related to the layout, including the number of pages, the layout creation date, compensation settings, batch processing, and gate information.

- **System**

  Displays information related to the current user and other system-related data.

- Custom 348

  These are tokens, which can contain user-defined formulas, that are created from the Custom Token window 349. A custom token formula can contain arithmetic operations, such as division and multiplication, and conditional operations, such as IF … THEN … ELSE. See Custom Tokens 348 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 330.

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19.3 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.

19.3.1 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**

  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 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 348. 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.

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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 326.
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 19.6). Note that the three options are listed with the **Token Value** selected by default.

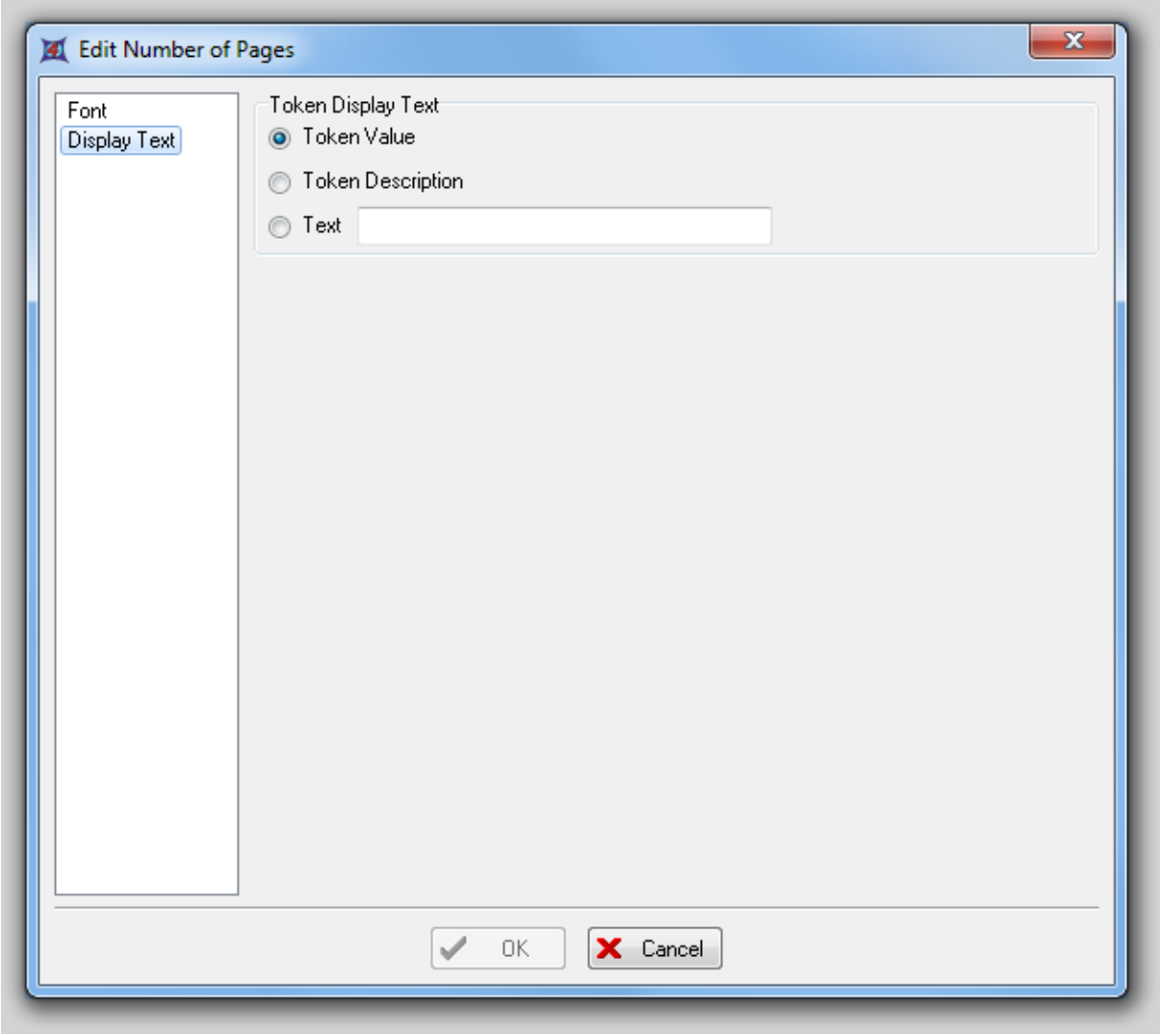

Figure 19.6 Edit Number of Pages Dialog

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

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19.7 rather than the token value "1".

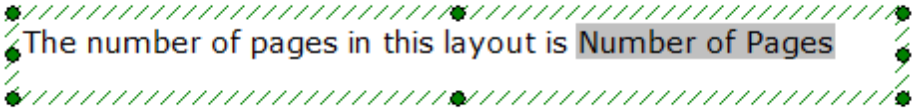

Figure 19.7 Display Text - Token Description

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 19.8).

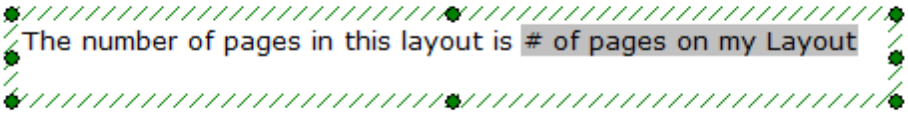

Figure 19.8 Display Text - User Defined Text

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 19.9). The user-defined **Text** option is primarily useful when creating custom tokens 348.

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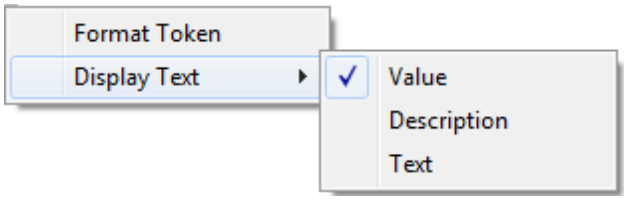

Figure 19.9 Display Text Pop-up Menu

19.3.2 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 FCS data. Each FCS file contains multiple keywords which can also be viewed from the View Header window 312.

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 52.
2. Insert a Text Box 96 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 19.10).

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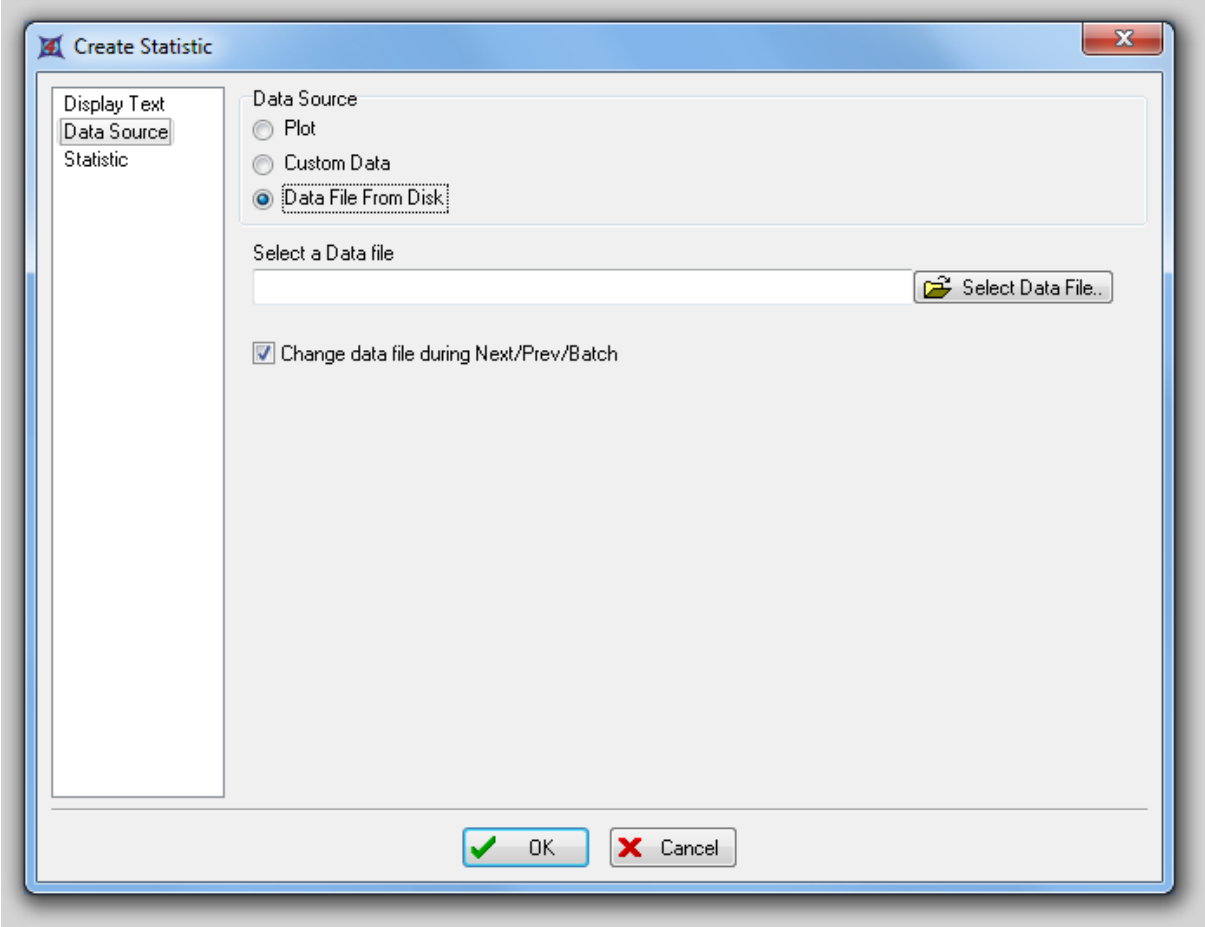

Figure 19.10 Create Statistic Dialog - Data Source - Data File from Disk

5. Click on the **Select Data File** button to locate an FCS file. When you have chosen an FCS file, the filename will appear in the field under **Select a Data file**.
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.
7. Click on **Statistic** from the list of token properties on the top-left of the **Create Statistic** dialog.
8. Select a Statistic option, such as **# of Events** (Figure 19.11).

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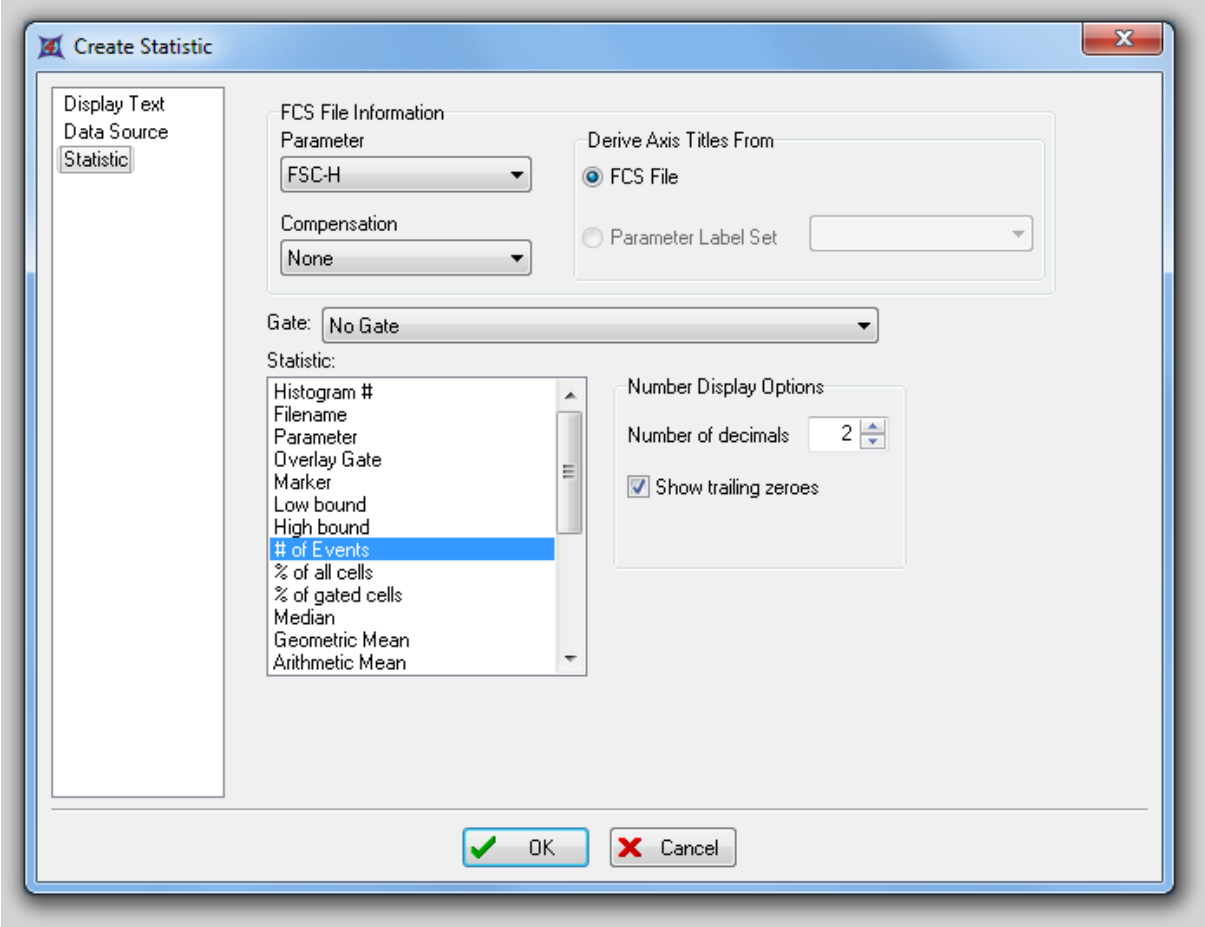

Figure 19.11 Create Statistic Dialog - Statistic Page

9. Click **OK**. The text box will display the text entered in step 2 followed by the statistical data from step 8 for the FCS file selected in step 5.

To configure a **Data Source** token property for a **Statistic** token using a plot:

1. Create a new layout 52.
2. Insert a 2D Dot Plot 111 on the page.
3. Insert a Text Box 96 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** (Figure 19.12).

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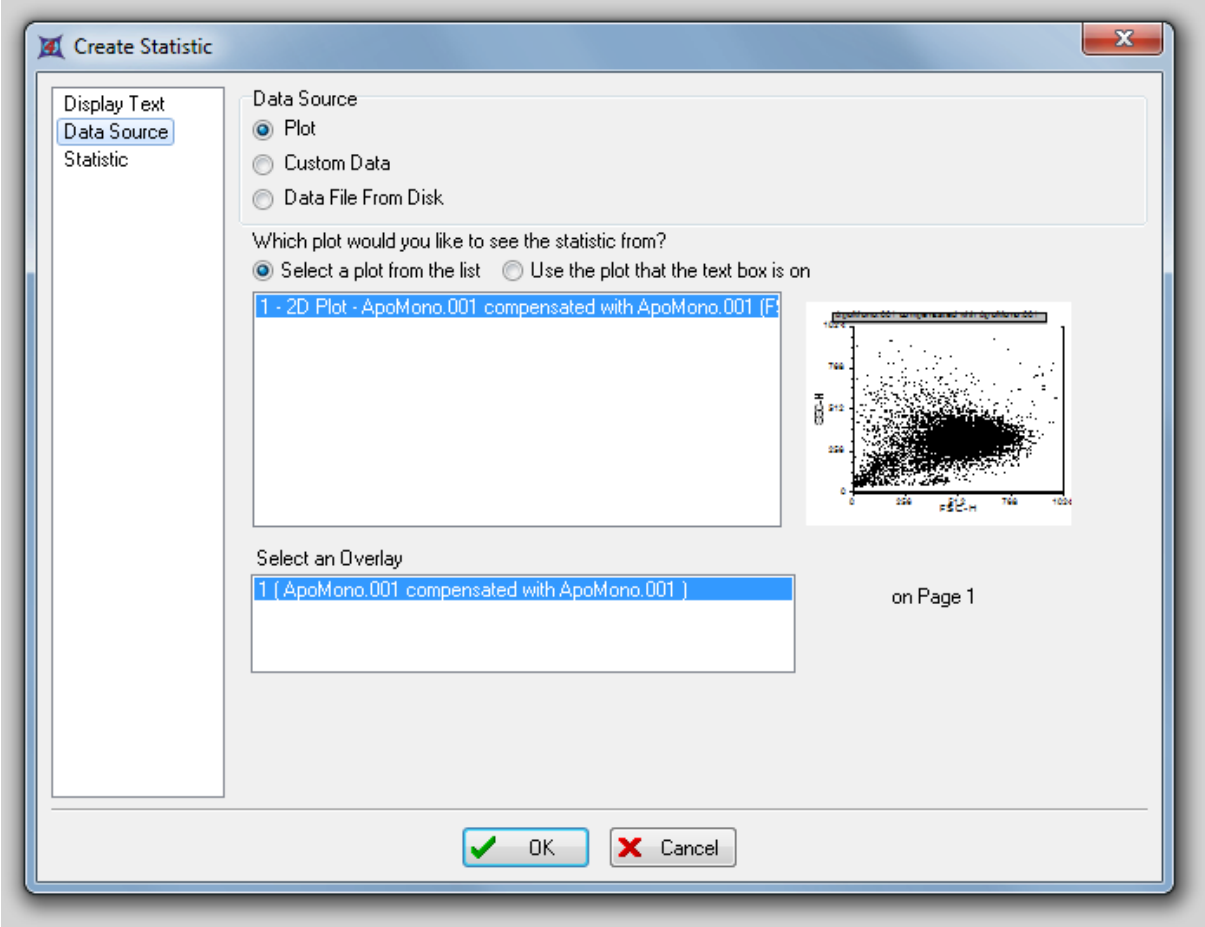

Figure 19.12 Create Statistic Dialog - Data Source Page

6. The **Select a plot from the list** field lists the plots on the layout. The **Select an Overlay** field lists all overlays for the selected plot. In this example, there is only plot with one overlay so you do not have to make selections in these fields.
7. Click on **Statistic** from the list of token properties on the top-left of the **Create Statistic** dialog.
8. Select a Statistic option, such as **# of Events**.
9. Click the **OK** button.

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 was as **Statistics** tokens, with the following exceptions:

- To insert a keyword token, select **Keyword** after selecting **Insert Token**.

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- 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 type in a keyword, or select one from the list of default keywords (see Editing Keyword Tokens 342).

19.4 Editing Tokens

Each of the different categories of tokens contains a number of properties which can be edited.

19.4.1 Editing Statistic Tokens

To insert or edit a **Statistic** token, the following properties need to be set:

- Display Text 330 to select the token value, description, or user-defined text.
- Data Source 333 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.

19.4.1.1 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 19.13).

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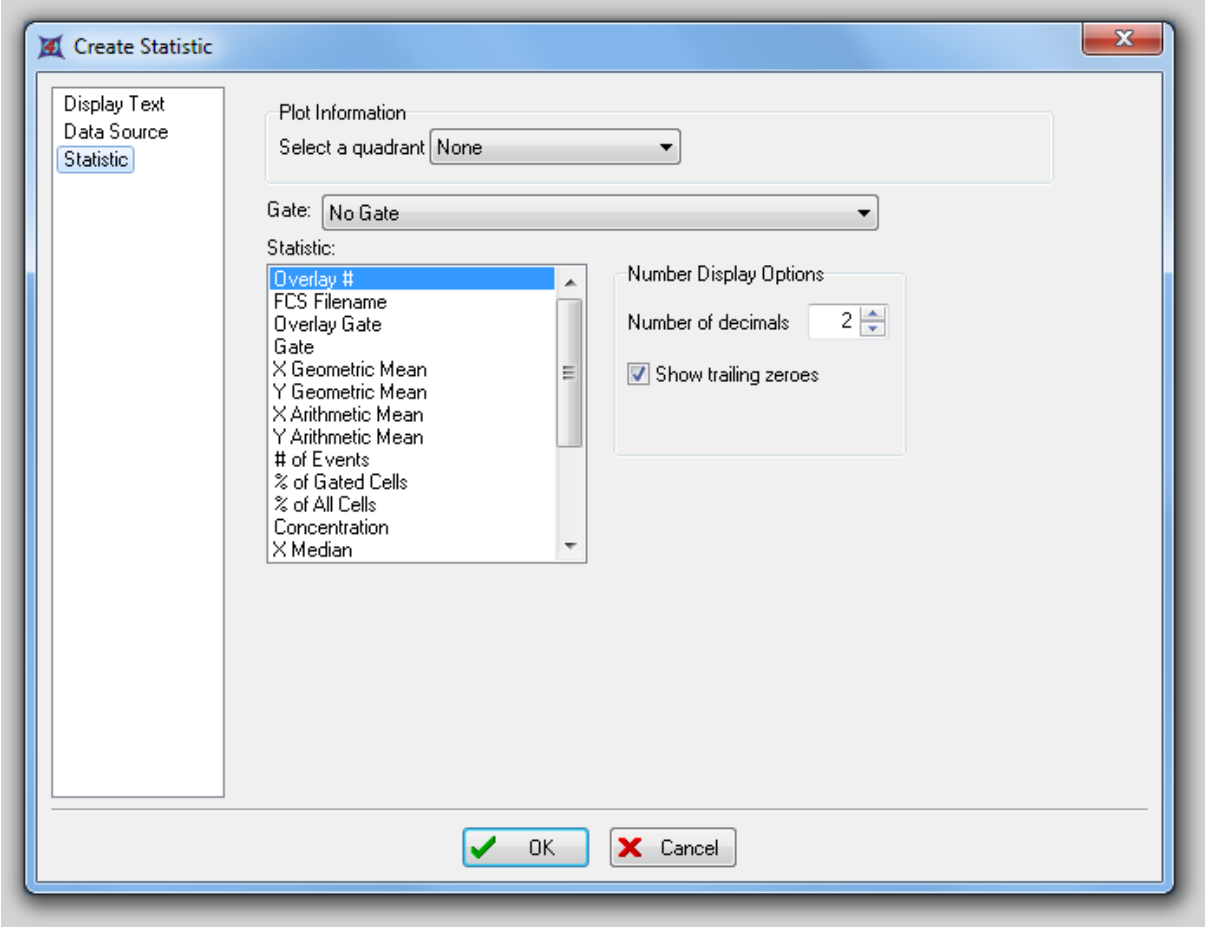

Figure 19.13 Statistic Options - 2D Plot as Data Source

- **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**.

- **Statistic**

  Select the statistic to display. See 2D Plot Statistics 216 for explanations of the options.

- **Number Display Options**

  Select the number of decimals to be displayed for this statistic. The default value is 2 decimal places. You can also choose whether or not to display

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trailing zeroes.

To insert or edit a **Statistic** token from an FCS file, these properties need to be set (Figure 19.14):

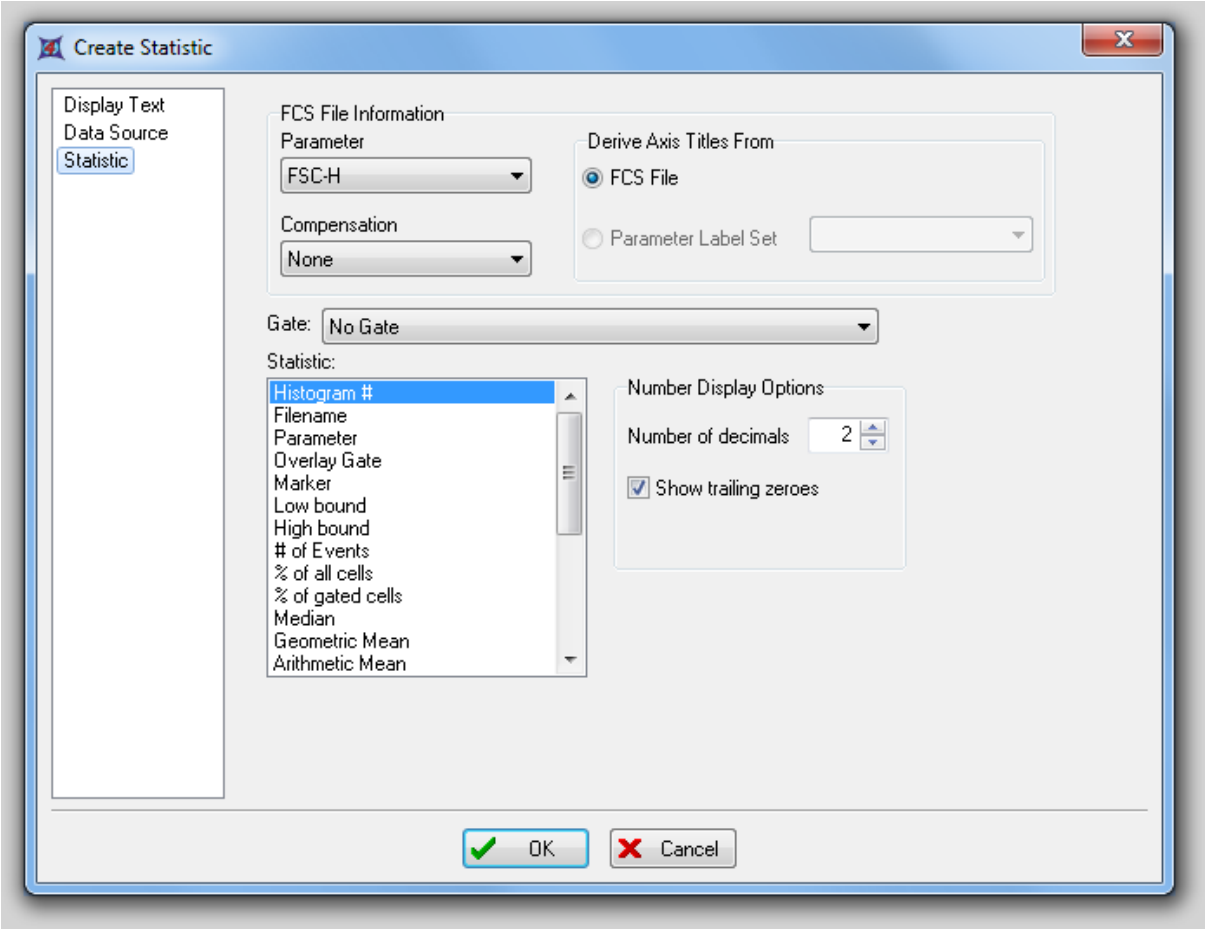

Figure 19.14 Statistic Options - FCS File as a Data Source

- **FCS File information**
  - **Parameter**

    Select the parameter data to use from the drop-down list.

  - **Compensation**

    Select a compensation definition 289 for the data. The default is **None**.

  - **Derive Axis Titles From**

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Choose from the **FCS File** axis titles, or axis titles from a predefined Parameter Label Set 230.

- **Gate**

  Choose a gate from the drop-down list to 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 default is 2 decimal places.

19.4.1.2 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 19.15).

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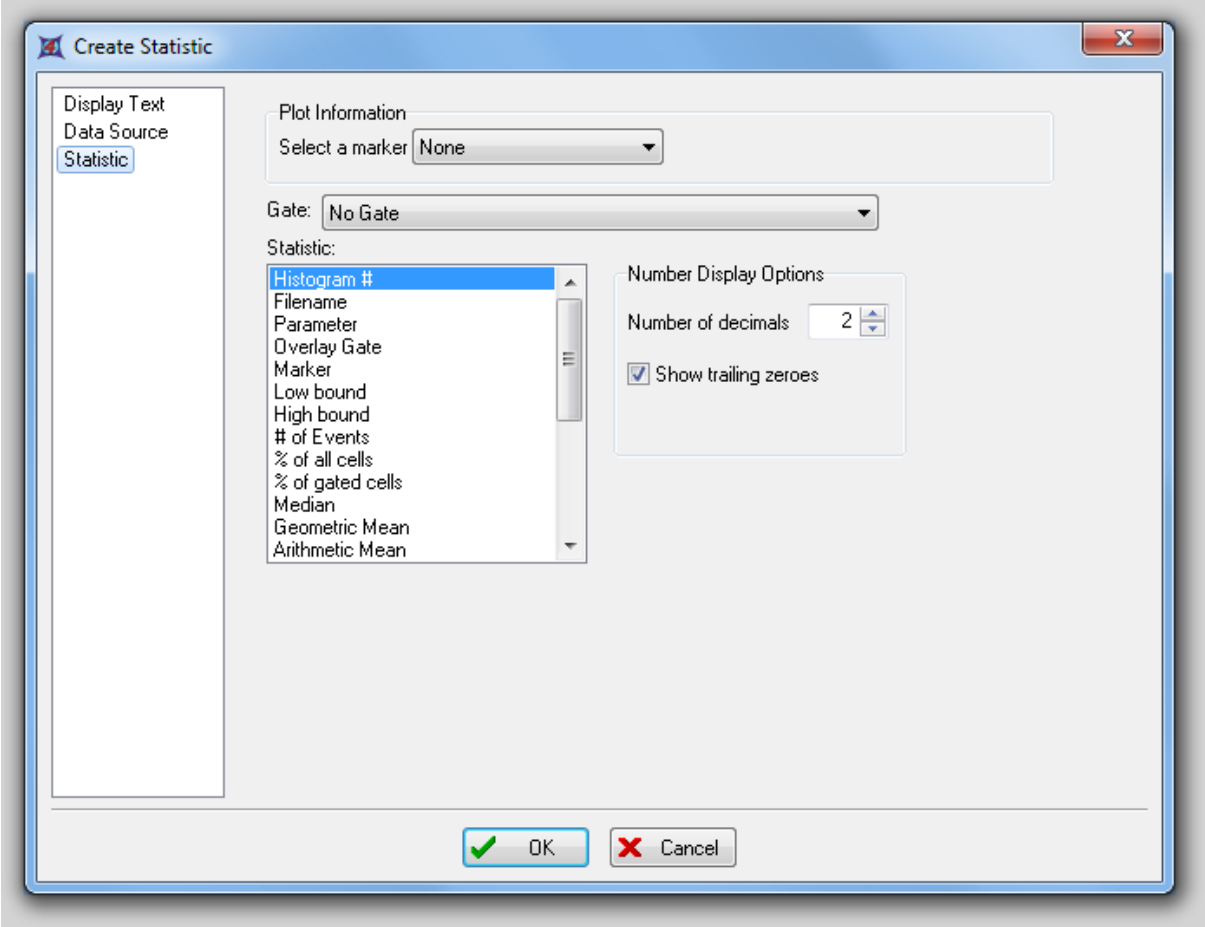

Figure 19.15 Statistic Options - Histogram as Data Source

- **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 215 for explanations of the options.

- **Number Display Options**

  Select the number of decimals to be displayed for this statistic. The default value is 2 decimal places. You can also choose whether or not to display

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trailing zeroes.

19.4.2 Editing Keyword Tokens

When you insert or edit a **Keyword** token, the following properties need to be set:

- Use the Display Text 330 page of the **Create Keyword** dialog to choose a token value, description, or user-defined text to display in the token.
- Use the Data Source 333 page 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 19.16). *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.

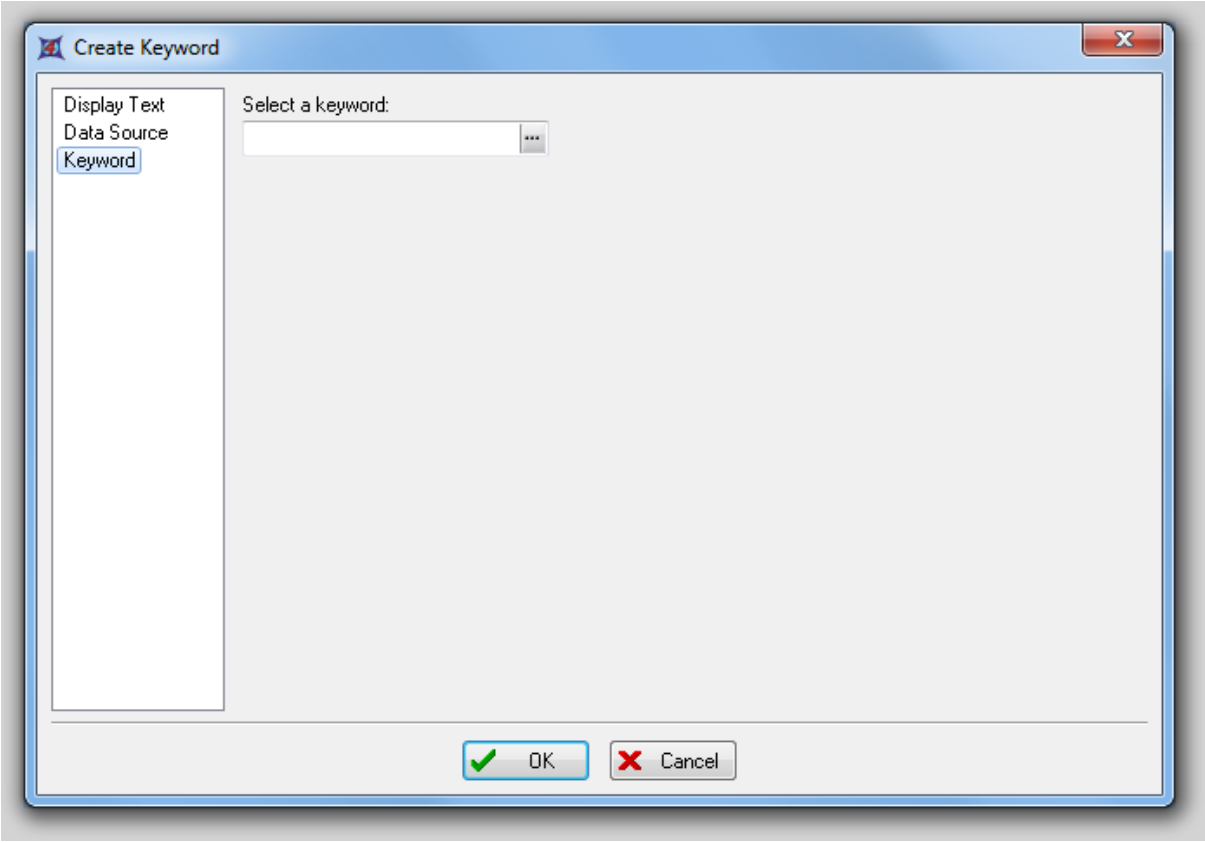

Figure 19.16 Keyword Properties

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- Select a keyword. Click on the ellipsis to display the **Please select a keyword** dialog (Figure 19.<%19F++++%>). If necessary, scroll down the list to view all the **Keyword** items. Click on the **Keyword** 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.

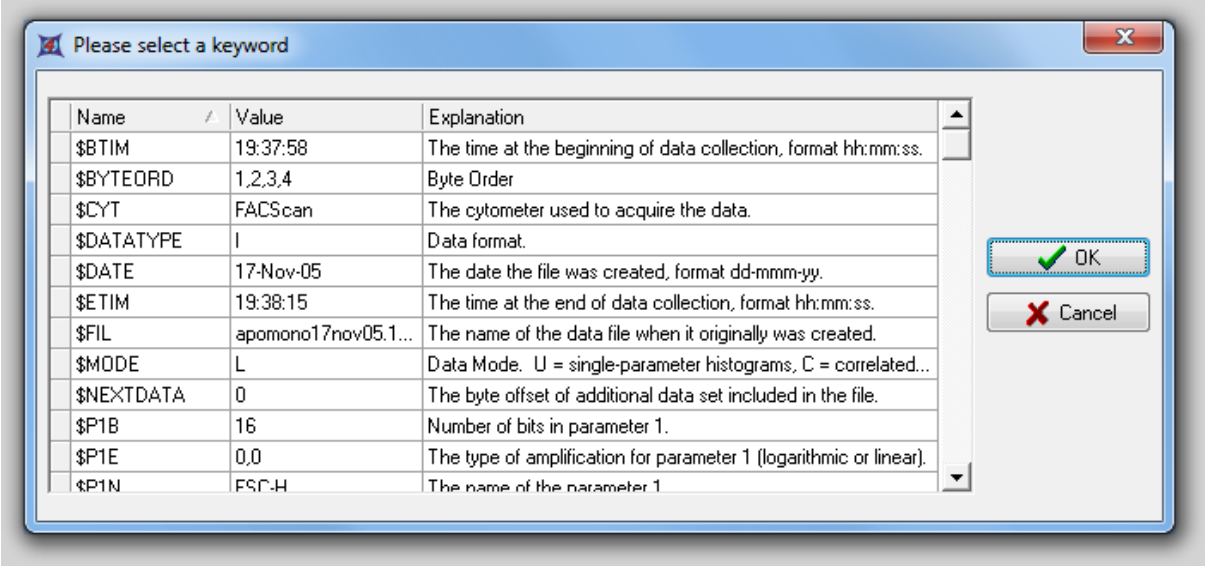

Figure 19.<%19C++++%> Select a Keyword

For more information regarding Keywords, please visit our YouTube site at http://youtu.be/rkgkXHhAbEE for a short video that goes into greater detail about what Keywords are and how to use them within your analysis layout.

19.4.3 Editing 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.

Layout Token	Definition
Number of	The overall number of pages in the current layout.

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Pages	
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.
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.
Plot Information	This token will show 1 of 4 plot elements, originating from a Data Source that the user selects while setting up the token. The elements are: 1. **Title** - will show the currently selected value of the Title preference, whether it's visible or not visible. 2. **Footer** - will show the currently selected value of the Footer preference, whether it is visible or not. 3. **X Axis Label** - the current x axis label in the Data Source chosen. 4. **Y Axis Label** - the current y axis label in the Data Source chosen.

19.4.3.1 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 19.17).

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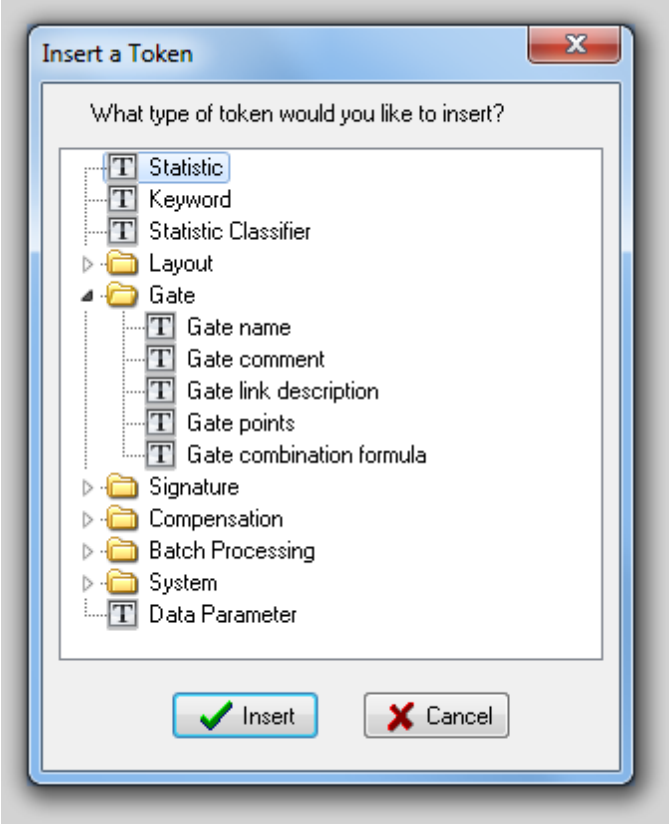

Figure 19.17 Choose a Gate Token

4. Select a **Gate** token and click the **Insert** button.

When you insert or edit a **Gate** token, the **Create Gate Token** dialog appears.

Use the Display Text 330 page of the **Create Gate Token** dialog to choose a token value, description, or user-defined text to display in the token.

Use the **Gate information** page (Figure 19.18) to:

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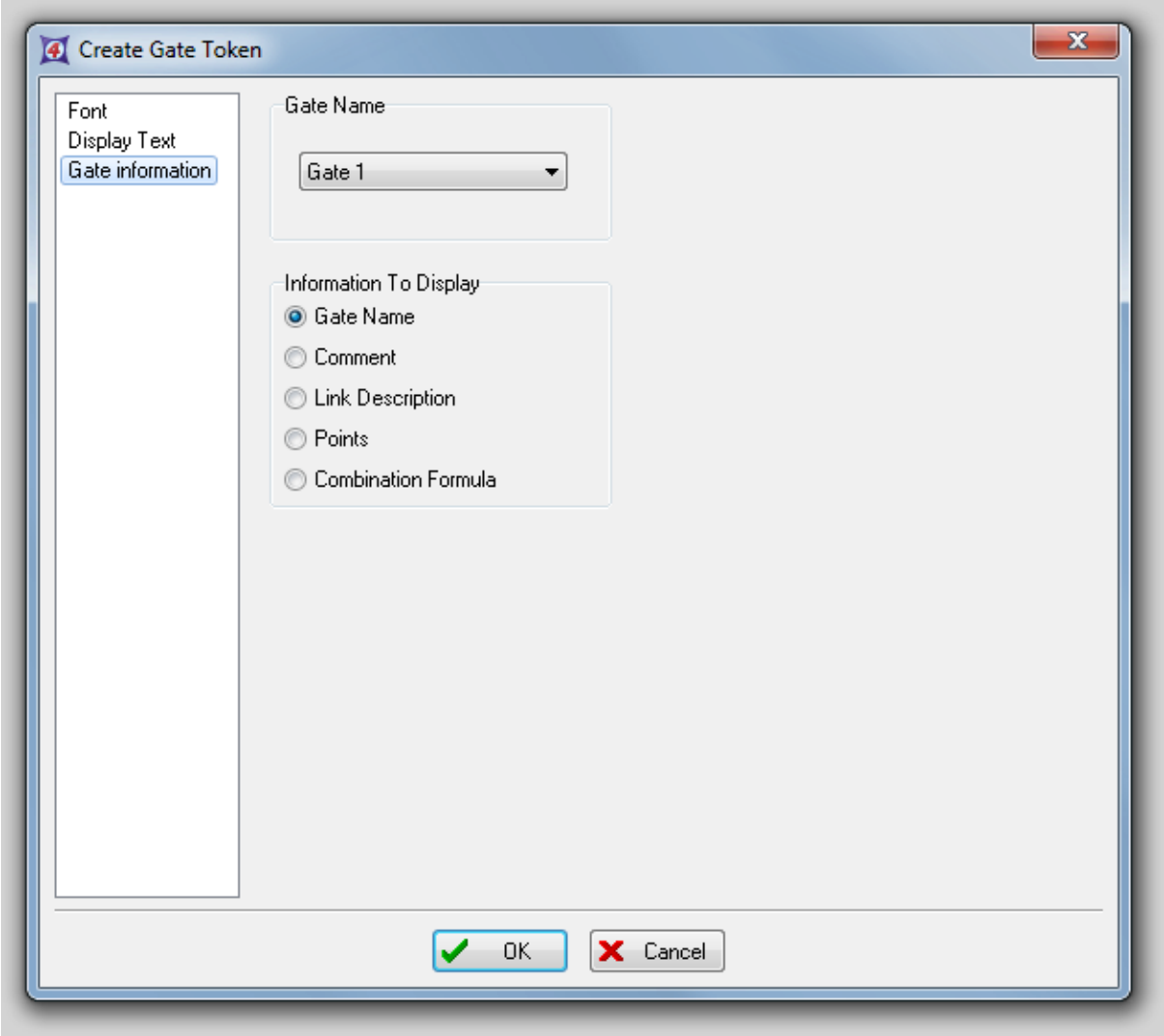

Figure 19.18 Gate Token Options

- 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 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 202.

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- **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.

19.4.3.2 Compensation Tokens

Compensation tokens allow you to insert information pertaining to the current compensation 288 settings. You can choose different source and target parameters and display either the crossover or spillover matrix value.

19.4.3.3 Batch Processing Tokens

Batch processing tokens allow you to insert information relating to the current state of the batch process. 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 387).

19.4.4 System Tokens

System tokens allow you to insert information pertaining to the current computer session. The current system tokens are: Current FCS Express User, Current Date, Current Time, and Current Windows User.

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 19.19).

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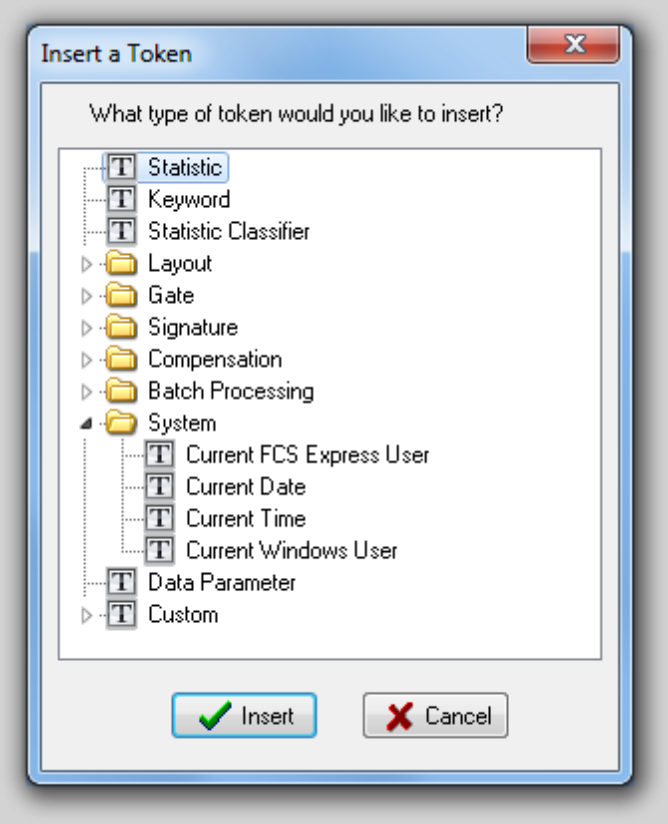

Figure 19.19 System Tokens

4. Choose one of the **System** tokens and click the **Insert** button.

19.5 Custom Tokens

Custom Tokens contain user-defined combinations of tokens and text to create custom statistics. The custom combinations are created using mathematic 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 326. During data analysis, the custom token formula is evaluated by FCS Express and the result is displayed or returned to 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[364] is also provided.

Note: FCS Express Version 4 uses Excel-style formula evaluation. Therefore, formulas must start with "=".

19.5.1 Creating Custom Tokens

To create a **Custom Token**:

1. Click on the **View→Tokens→Custom Tokens** command to bring up the dockable **Custom Tokens** window.
2. Make sure that the **Custom Tokens** window is docked[67] to the left or right of the screen so that both panels of the window are visible (Figure 19.20). The two areas of the **Custom Tokens** window, the Custom Token List and the Custom Token Formula Editor, are separated by a splitter which can be moved to resize the areas.

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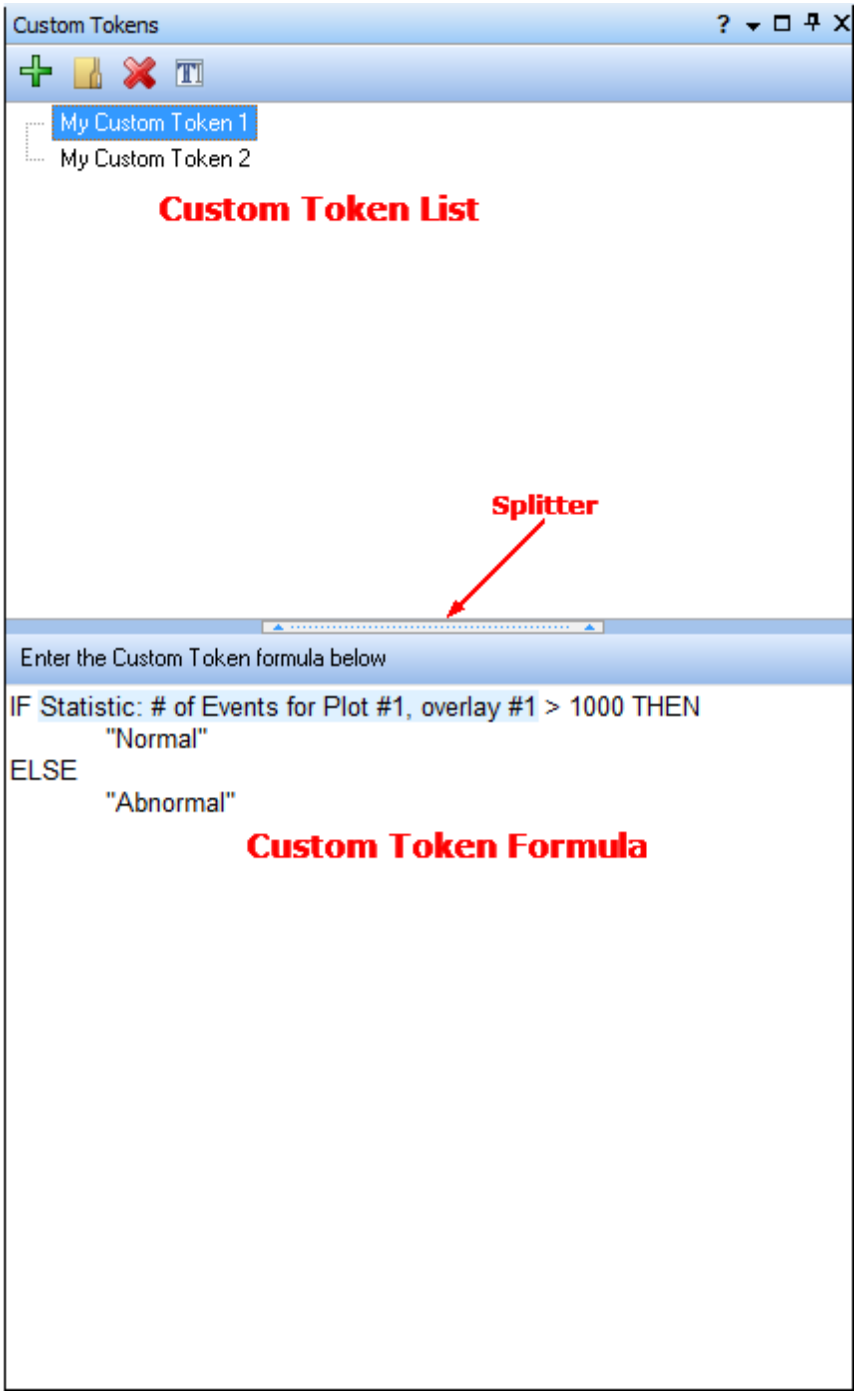

Figure 19.20 Docked Custom Tokens Window

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:

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- Right-click the formula area and select **Insert Token** from the pop-up menu.
- Choose a token from the **Insert a Token** dialog.
- Modify the formula. You can use any of the operators and functions that are available for custom tokens 364.

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 Tool Bar* 34.
	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.

19.5.2 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 349.
2. Edit **New Custom Token** to read *Custom1*.
3. In the Formula area below the splitter 349, enter the following: *=100 * 30*.
4. Click on **Custom1** to highlight/select it. Your **Custom Token** window should look like Figure 19.21.

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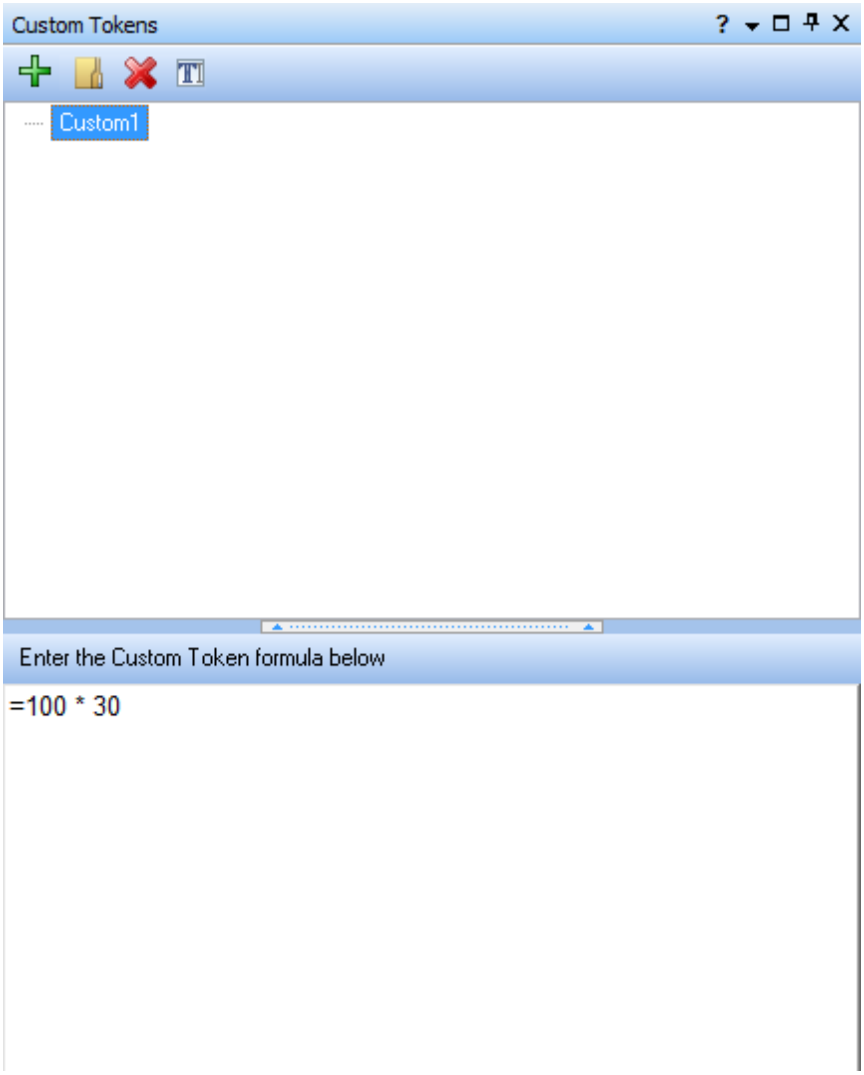

Figure 19.21 Custom Token Example 1

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 19.22), make sure that the **Custom** category is open (click on the triangle next to **Custom**, if necessary), and select **Custom1.**

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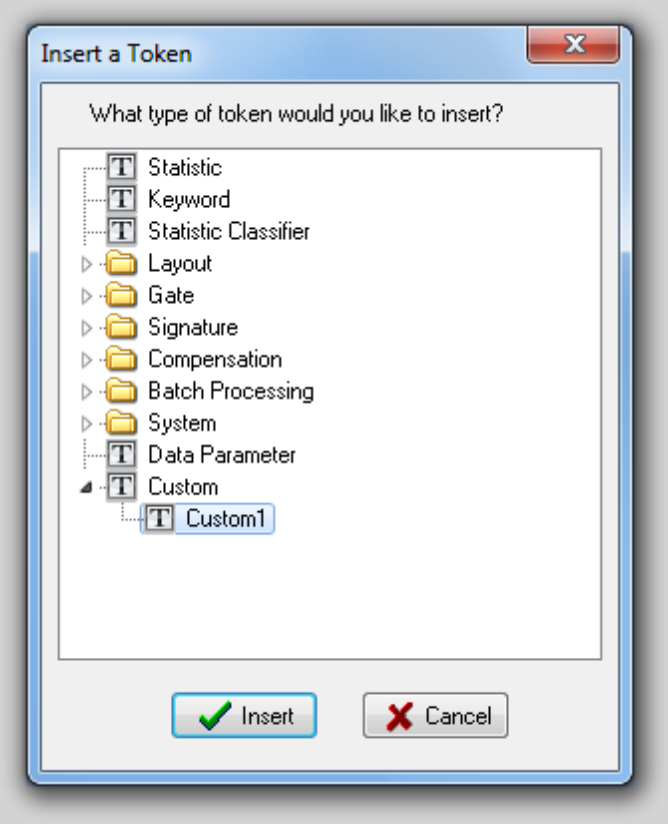

Figure 19.22 Insert a Token Dialog

9. With **Custom1** 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 *Custom1* token will display its result without a grey background.

This example demonstrates that when a **Custom Token** is inserted into a Text Box, its formula is calculated and the result is displayed.

19.5.3 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 111 and a text box 96 on a layout page. Open the Custom Token window 349 and dock 67 it.
2. Click on (Add) in the docked Custom Token window.
3. Edit **New Custom Token** to read *Custom2*.
4. Enter = in the Formula area below the splitter 349. *Note: FCS Express 4 uses Excel-style formula evaluation. Therefore, formulas must start with "=".*

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5. Right-click in the Formula area and choose **Insert Token** from the pop-up menu. Select **Statistic** and click on the **Insert** button.
6. Choose a plot on the **Data Source** page, then select the **Statistic** category and highlight **# of Events** as the statistic. Click **OK**.
7. In the Formula area, enter *<space>/<space>2* to the right of the token.
8. Click on **Custom2** to highlight/select it. Your **Custom Token** formula should look like Figure 19.23.

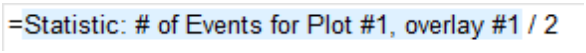

Figure 19.23 Custom Token Example 2

9. Right-click on the Text Box and choose **Insert Token** from the pop-up menu. At the bottom of the **Insert a Token** dialog, make sure that the **Custom** category is open (click on the triangle next to **Custom**, if necessary), select **Custom2**, and click the **Insert** button.

The Text Box should now display the number of events in your dataset divided by 2.

19.5.4 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 111 and a text box 96 on a layout page. Open the Custom Token window 349 and dock 67 it.
2. Click on (Add) in the docked Custom Token window.
3. Edit **New Custom Token** to read *Custom3*.
4. Right-click in the Formula area and choose **Insert Token** from the pop-up menu. Select **Keyword** and click on the **Insert** button.
5. Choose a plot on the **Data Source** page, select the **Keyword** category, and click on 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 5 and 6). The Formula field should resemble Figure 19.24.

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Keyword: $FIL for Plot #1, overlay #1 was created on
Keyword: $DATE for Plot #1, overlay #1

Figure 19.24 Custom Token Example 3 Formula

9. Right-click on the Text Box and choose **Insert Token** from the pop-up menu. At the bottom of the **Insert a Token** dialog, make sure that the **Custom** category is open (click on the triangle next to **Custom**, if necessary), select **Custom3**, and click the **Insert** button.

The Text Box should now resemble Figure 19.25.

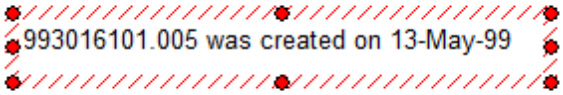

Figure 19.25 Custom Token Example 3

19.5.5 Custom Token Example 4: Conditional Statements

This example demonstrates using conditional IF..THEN..ELSE statements in a Custom Token formula.

1. Insert a plot 111 and a text box 96 on a layout page. Right-click on the plot and select **Show Quadrants**. Open the Custom Token window 349 and dock 67 it.
2. Click on (Add) in the docked Custom Token window.
3. Edit **New Custom Token** to read *Custom4*.
4. In the Formula field enter: *=IF <space>*
5. Right-click in the Formula area and choose **Insert Token** from the pop-up menu. Select **Statistic** and click on the **Insert** button.
6. Choose a plot on the **Data Source** page, then select the **Statistic** category.
7. Select Lower Right from the drop-down list in the Select a quadrant field. Highlight **# of Events** as the statistic. Click **OK**.
8. Type the following in the formula area: *>3000 THEN "Normal" ELSE "Abnormal"*. The Formula field should resemble Figure 19.26.

=IF Statistic: # of Events for Plot #1, overlay #1,LR quad>3000
THEN "Normal" ELSE "Abnormal"

Figure 19.26 Custom Token Example 4 Formula

9. Right-click on the Text Box and choose **Insert Token** from the pop-up menu. At the bottom of the **Insert a Token** dialog, make sure that the **Custom** category is open (click on the triangle next to **Custom**, if necessary), select

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Custom4, and click the **Insert** button.

The token will display **Normal** if the number of events in the Lower Right quadrant is greater than 3000 and **Abnormal** if the number of events is less than or equal to 3000. If you move the quadrants 212, the Custom Token formula is recalculated. Therefore, the token will display a different result if the number of events changes from less than 3000 to greater than 3000 (or vice versa).

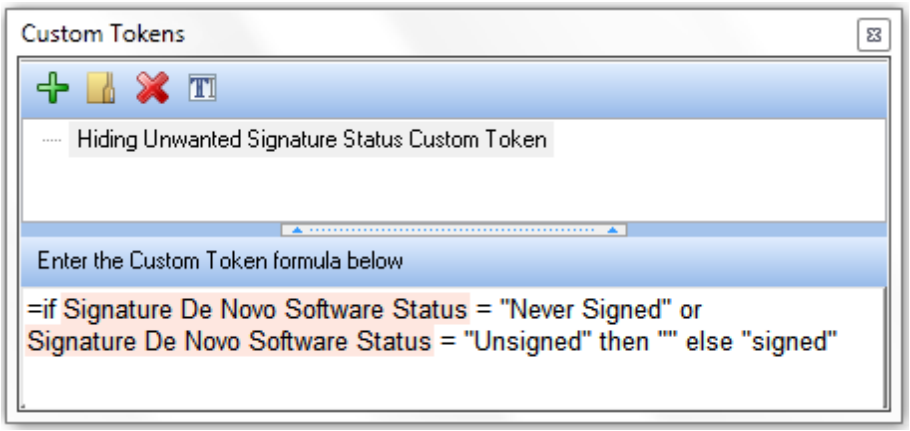
19.5.6 Custom Token Example 5: Hiding Signature Tokens

Once you have created and set up your signatures 565 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 328 will accomplish the hiding effect.

The custom token will take the form of a conditional statement 355 (IF, THEN, ELSE statement) as follows where <signature XXXXX status> represents your signature status 574 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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19.5.7 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 19.27 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 19.27 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 19.28 categorizes the fluorescence of a histogram based on the percentage of events within marker regions (Figure 19.29). 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 19.28 Custom Ton Example 6 Formula

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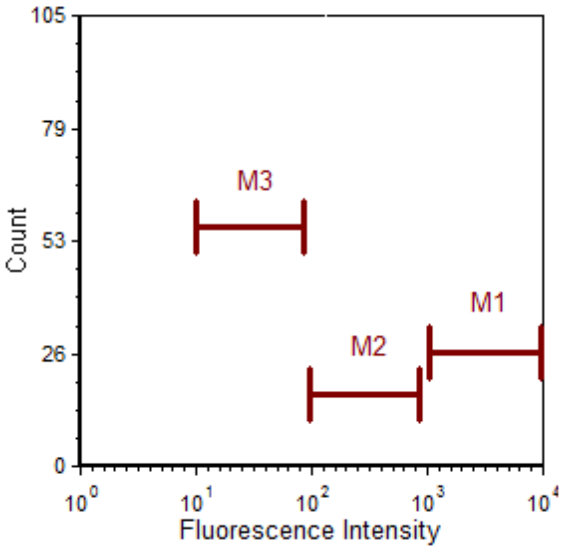

Figure 19.29 Fluorescence Intensity Marker Example

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.

19.5.8 Inserting Functions into Formulas

A number of mathematical functions[364] 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 19.30).

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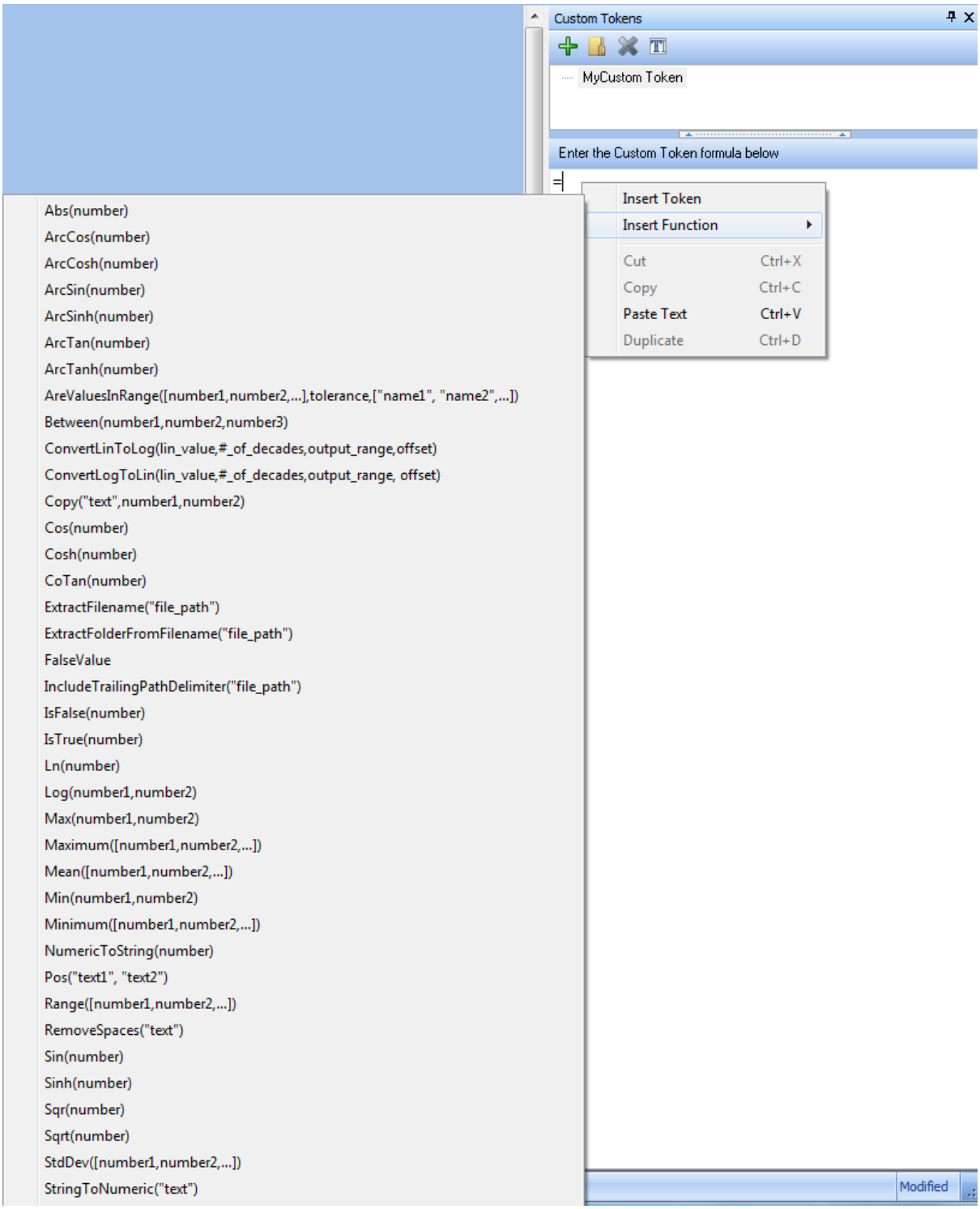

Figure 19.30 Inserting a Function into a Formula

19.5.9 Editing Custom Tokens

You can edit the formula of a Custom Token directly in the Custom Tokens window 349 by simply clicking on an available Custom Token from the list.

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If you have already inserted a Custom Token into a text box, you can open an **Edit** dialog (Figure 19.31) in one of two ways:

- Right-click on the Custom Token in the Text Box and choose **Format Token** from the pop-up menu.
- Double-click on the token in the text box.

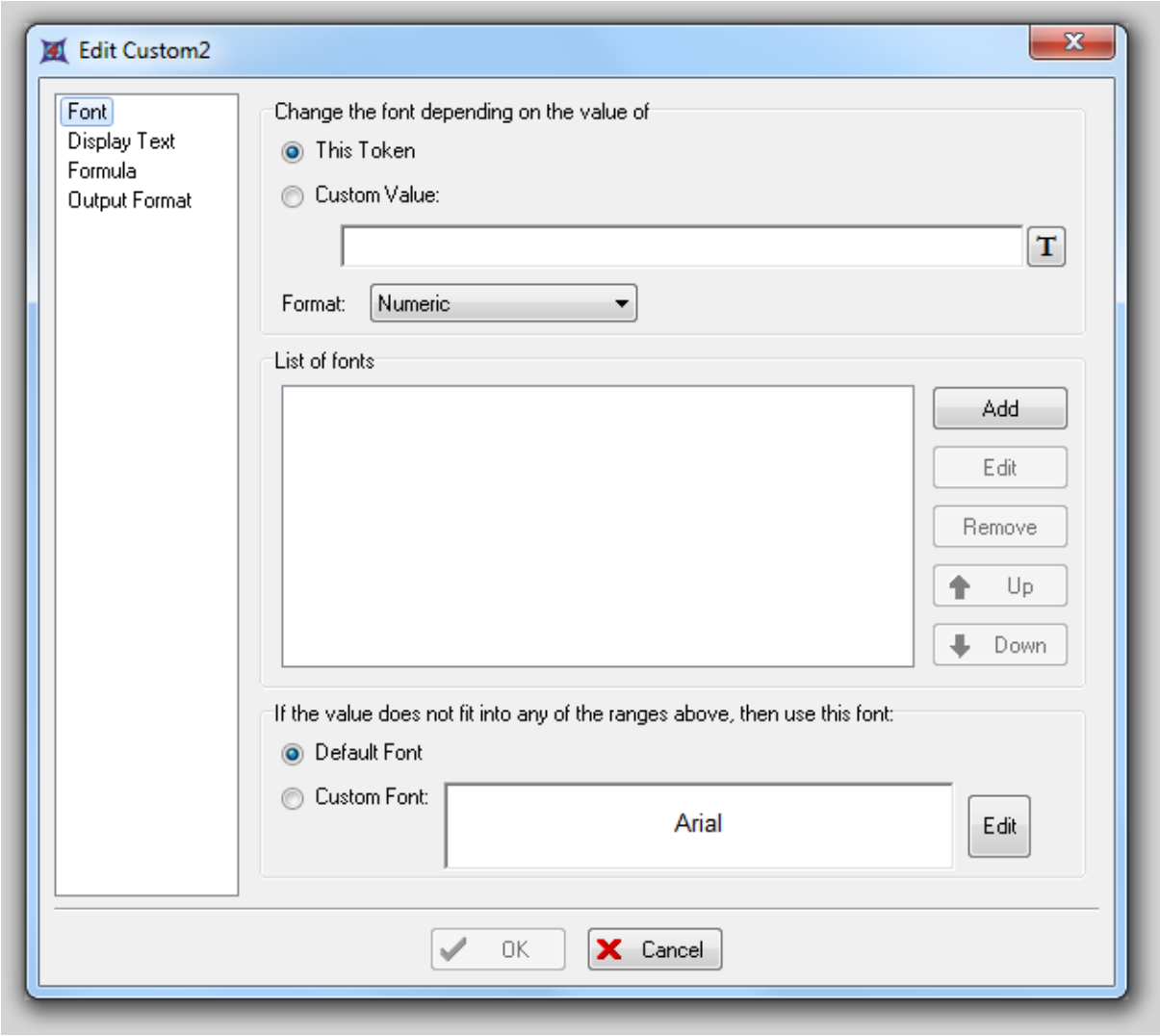

Figure 19.31 Editing a Custom Token -- Font Page

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 19.32). 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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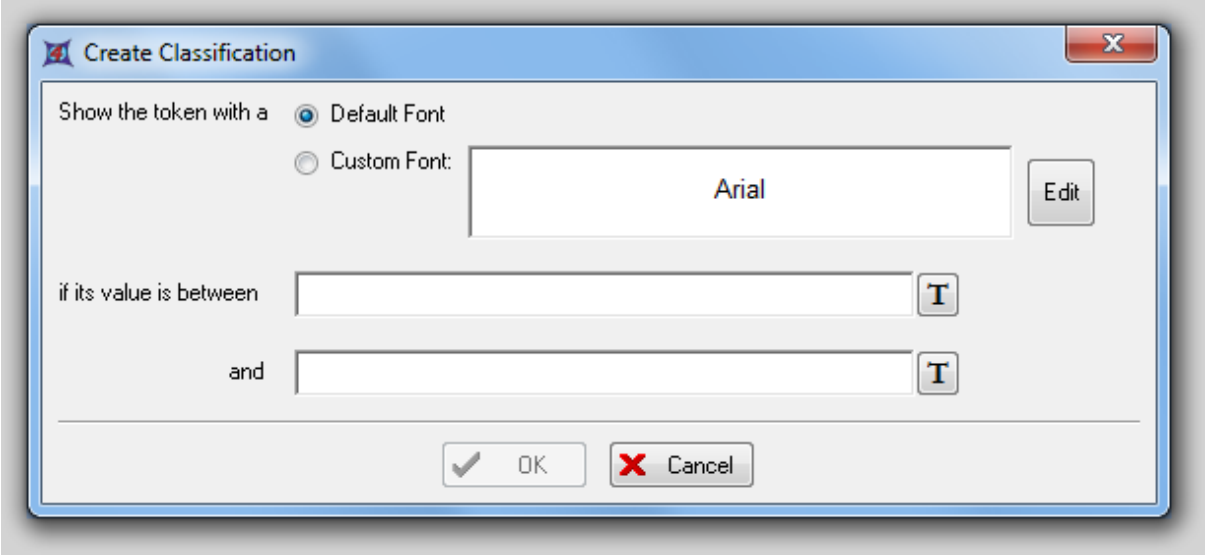

Figure 19.32 Create Classification Dialog

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 change 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.

19.5.10 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, either: 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 edited. It is possible to change the result type of a token by right-clicking

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on the token and selecting the alternate result type from the drop-down menu (Figure 19.33).

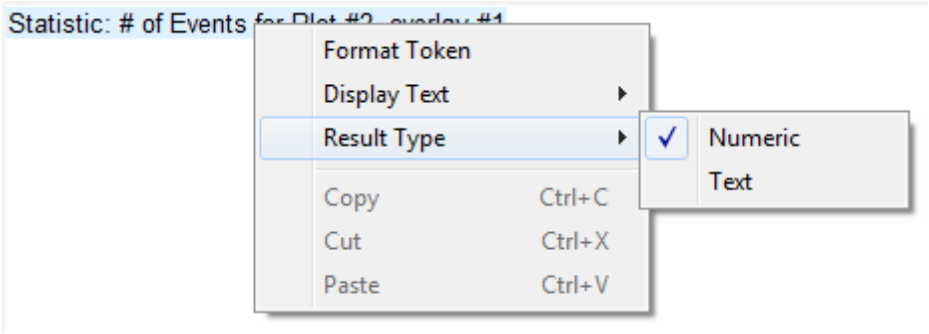

Figure 19.33 Changing the Result Type in a Custom Token Formula

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.

19.5.11 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 19.34, 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

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lymphocyte gate.

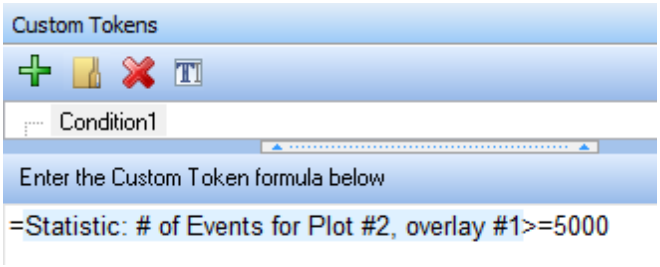

Figure 19.34 Token that Evaluates to TRUE or FALSE

You can use the **Condition1** token inside another token. To do so, set the result type of the token to Numeric and use the IsTrue or IsFalse function 364, as shown in Figure 19.35.

```
=IF IsTrue(Custom Token: Condition1) THEN
"There are at least 5000 cells in the lymphocyte gate." ELSE
"There are fewer than 5000 cells in the lymphocyte gate."
```

Figure 19.35 Using a Boolean token

19.5.12 Using Array Formulas in Custom Tokens

Certain of the mathematical functions described in the Custom Token Formula Reference 364, 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 19.36 will return the mean of the X Median values across four plots.

```
=Mean([Statistic: X Median for Plot #2, overlay #1,
       Statistic: X Median for Plot #5, overlay #1,
       Statistic: X Median for Plot #6, overlay #1,
       Statistic: X Median for Plot #7, overlay #1])
```

Figure 19.36 Using Tokens in an Array Formula

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19.5.13 Deleting Custom Tokens

To delete a custom token, right-click on the token in the Custom Token window list[349] 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.

19.6 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[349] and with Parameter Math[260].

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.

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

Mathematical Functions

Function	Description
Log(x,y)	Logarithm of y in base x
Ln(x)	Natural logarithm 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.

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Function	Description
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.
Sin(x)	Sine of x
Cos(x)	Cosine of x
ArcSin(x)	Arcsine of x
ArcCos(x)	Arccosine of x
Sinh(x)	Hyperbolic Sine of x
Cosh(x)	Hyperbolic Cosine of x
ArcSinh(x)	Hyperbolic Arcsine of x
ArcCosh(x)	Hyperbolic Arccosine of x
Tan(x)	Tangent of x
CoTan(x)	Cotangent of x
ArcTan(x)	Arctangent of x
ArcTanh(x)	Hyperbolic Arctangent of x
Sqr(x)	x squared
Sqrt(x)	Square root of x
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.
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.
Minimum([x,y,z...])	Returns the minimum value in the array ([x,y,z,…]). The array can hold an unlimited number of elements.
Maximum([x,y,z...])	Returns the maximum 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,…])).
Abs(x)	Absolute value of x
Max(x,y)	Returns the higher value of x and y.
Min(x,y)	Returns the lower value of x and y.
AreValuesInRange ([x,y,z...],tolerance, [xName,yName, zName...])	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 within tolerance % of each other, it returns text indicating which ones are not.

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	

Converter Functions. These are used in more advanced formulas, to convert a

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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 361.

Function	Description
stringToNumeric(x)	Converts the string x to a number.
numericToString(x)	Converts the number x to text (also known as a string).

Boolean (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.
IsTrue(x)	Returns True if x has a value of -1.
IsFalse(x)	Returns True if x has a value of 0.
TrueValue	Returns -1
FalseValue	Returns 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.

Text Manipulation Functions. These functions can be used to manipulate or get information out of existing text values.

Function	Description
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.
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**.
Copy(x,y,z)	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.
x+y	If x and y are both text (i.e., strings), returns the concatenation

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Function	Description
	of x with y.
ExtractFilename ("file_path")	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.
ExtractFolderFromFilename("file_path")	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.

Date and Time Manipulation Functions

Function	Description
FormatDateTime (const Format: string, DateTime: TDateTime): string	FormatDateTime formats the TDateTime value given by DateTime using the format given by Format. DateTime needs to be passed in either as StrToDate or StrToTime. Example: =FormatDateTime("yyyy-mm-dd",StrToDate(<Current Date token>)) will return the Current Date in a format showing Year, Month,Day =FormatDateTime("hh:mm:ss",StrToTime(<Current Time token>)) will return the Current Time in a format showing Hour: Minute:Second **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 Displays the date using the format given by the LongDateFormat global variable. e

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Function	Description
	(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 Displays the time using the format given by the ShortTimeFormat global variable. tt Displays the time using the format given by the LongTimeFormat

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Function	Description
	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.

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)

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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 380 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 418, or the currently active panel 422.

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 167 or 2D plots 175. 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 333.

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 386

  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 397

  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 374.
2. Add Batch Action and Report processes to the Batch Processing Action Screen 382.
3. Specify the Batch Processing Options 418.
4. Run the Batch Process 420.

20.1 Working with the Data List

Once you have created a layout to analyze your initial data, you can begin to 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 20.2). The **Data List** is a dockable window 66.

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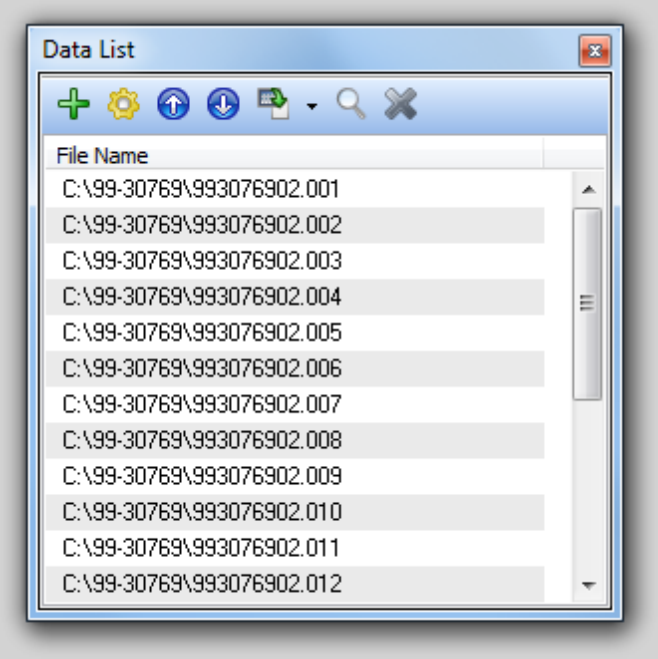

Figure 20.2 Data List Window

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 43.
	Format. Brings up the **Format File List** dialog where you can add keywords to be used for sorting, and show or hide filepaths. You can also hide filepaths by default by adjusting your Preferences 1513.
	Move selected file(s) up.
	Move selected file(s) down.
	Change file.
	Search for files using Find Data Files 374 dialog.
	Delete the selected file(s).

20.1.1 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 373.
2. Click on the **+** button to bring up the **Select a Data File** dialog.
3. Select the appropriate files and click on the **Open** button to add the

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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).

Note: If you have set an active Panel422, 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**.

  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: FCS Express Version 4 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 command373.
  2. Click on the Search button373 to bring up the **Find Data Files** dialog (Figure 20.3).

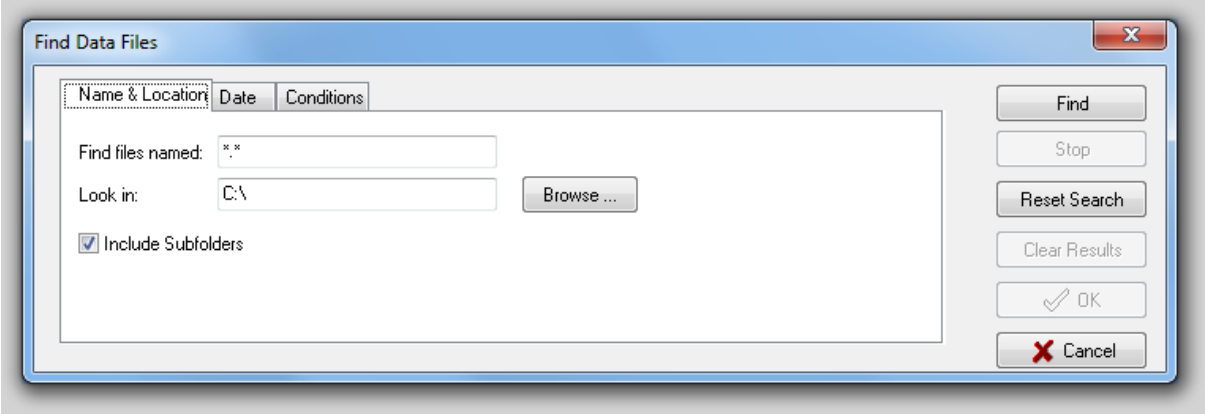

Figure 20.3 Find Data Files Dialog

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

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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 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. In the **Relation** and **Value** columns, select the keyword range to search. For example, to search for files with less than 50000 events, select **<** (for less than) in the **Relation** column, then enter 50000 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 20.4 shows a search for all files in the "C:\99-31206 CLL" directory with fewer than 50,000 cells acquired.

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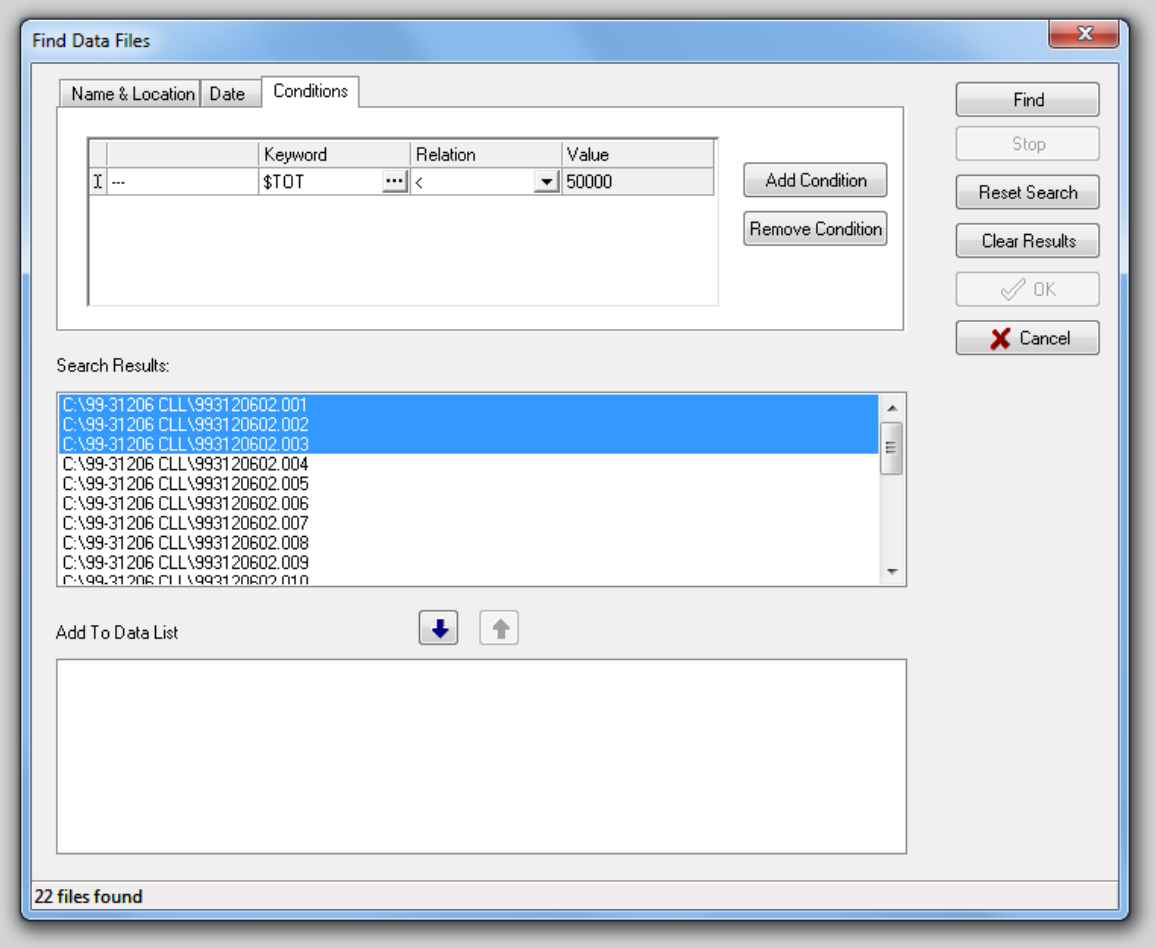

Figure 20.4 Search Files by Keyword

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 Panel422 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.

20.1.2 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 373.
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 (Figure19.5).

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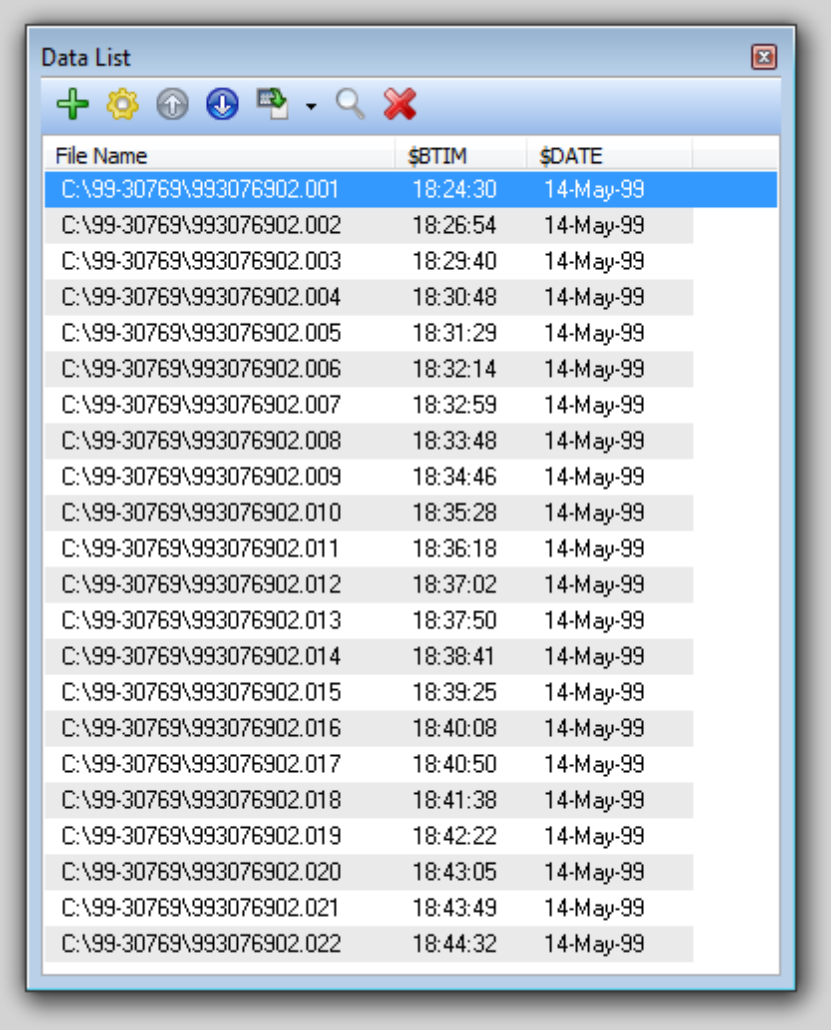

Figure 20.5 Data List with Keyword Columns

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 using standard Windows techniques for selecting multiple objects (**Shift**-Click, **Ctrl**-Click), then pressing the or buttons.

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20.1.3 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. Once a **Data List** has been created, you can also use the list to change the data set in plots, or change the Data Source in tokens.

20.1.3.1 Next/Previous

The Next and Previous functions will change the data set on the following objects:

- Plots
- Data Windows
- Tokens in Text Boxes
- Custom Tokens
- Batch Processing Actions

In order to use the Next/Previous functions to change data sets, each of the following conditions must be met:

- A **Data List** has been defined;
- The object to change (e.g., a plot) is selected or in edit mode;
- The selected object displays or is linked to a data set in the list, and finally;
- The selected object(s), e.g., a histogram 167, 2D plot 175, and/or Token 333, must have the **Change data file during Next/Prev/Batch** option enabled.

To change the data set on one or more selected objects to the next or previous data set:

1. Select the plots and/or token Text Boxes you wish to change.
2. Use one of the three following methods:
   - Use the **Data→Change File on Selection→Next File** (or **Previous File**) command.
   - Use the keyboard shortcuts **Alt+Right arrow** for Next or **Alt+Left arrow** for Previous.
   - Right-click on the selected object and choose **Next** or **Prev** from the pop-up menu.

Note: To change to the next or previous data set in the **Data List** for every appropriate object in your layout, use the **Data→Change File on All Objects→ Next File** (or **Previous File**) command.

20.1.3.2 Dragging and Dropping

Like other dockable windows 66 in FCS Express Version 4, 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 an existing plot or create a new plot.

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The **When dragging from the file list to a plot** drop-down list in the General category of the Edit Preferences dialog[118] 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 20.6).

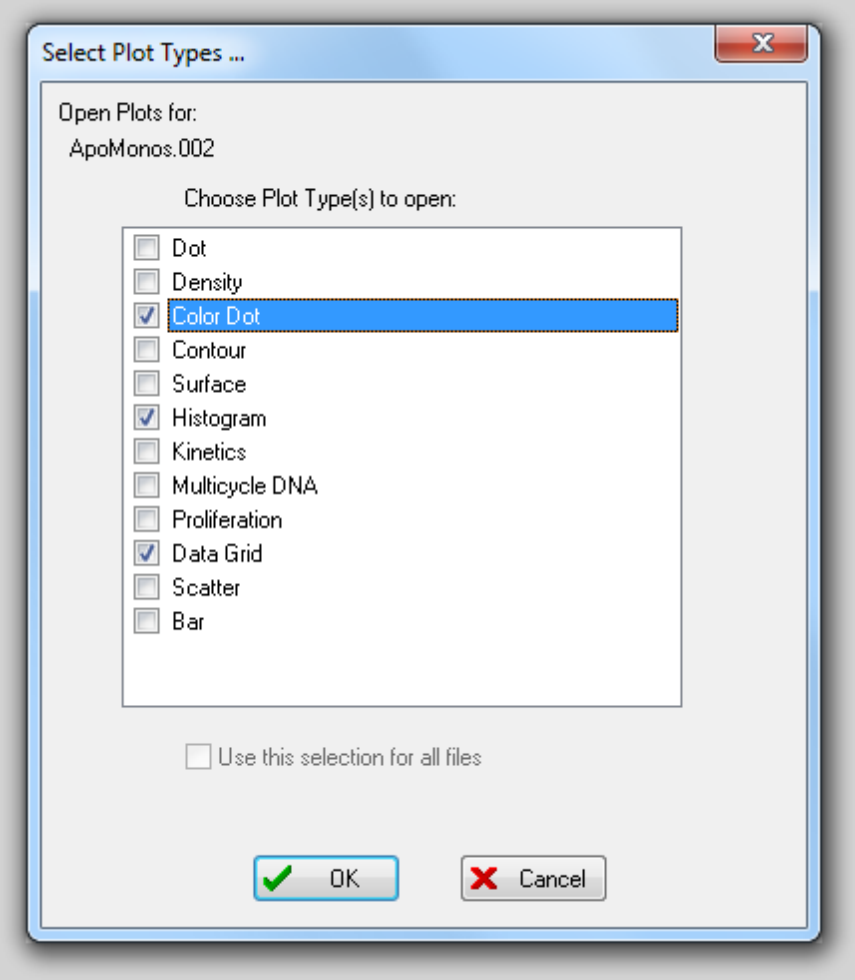

Figure 20.6 Select Plot Types... Dialog

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**.

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20.1.3.3 The Change File Button

To use the **Data List** to change the files/data sets on your plots:

1. Click on a dataset in the Data List to highlight it.
2. Click on the (Change File) icon and choose either **Change Data On Selected Plots** or **Change Data On All Plots** from the drop-down menu.

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 372.

To add actions to the batch process, use the **Batch→Batch Processing→Batch Actions** command to bring up the dockable **Batch Actions** window (Figure 20.7).

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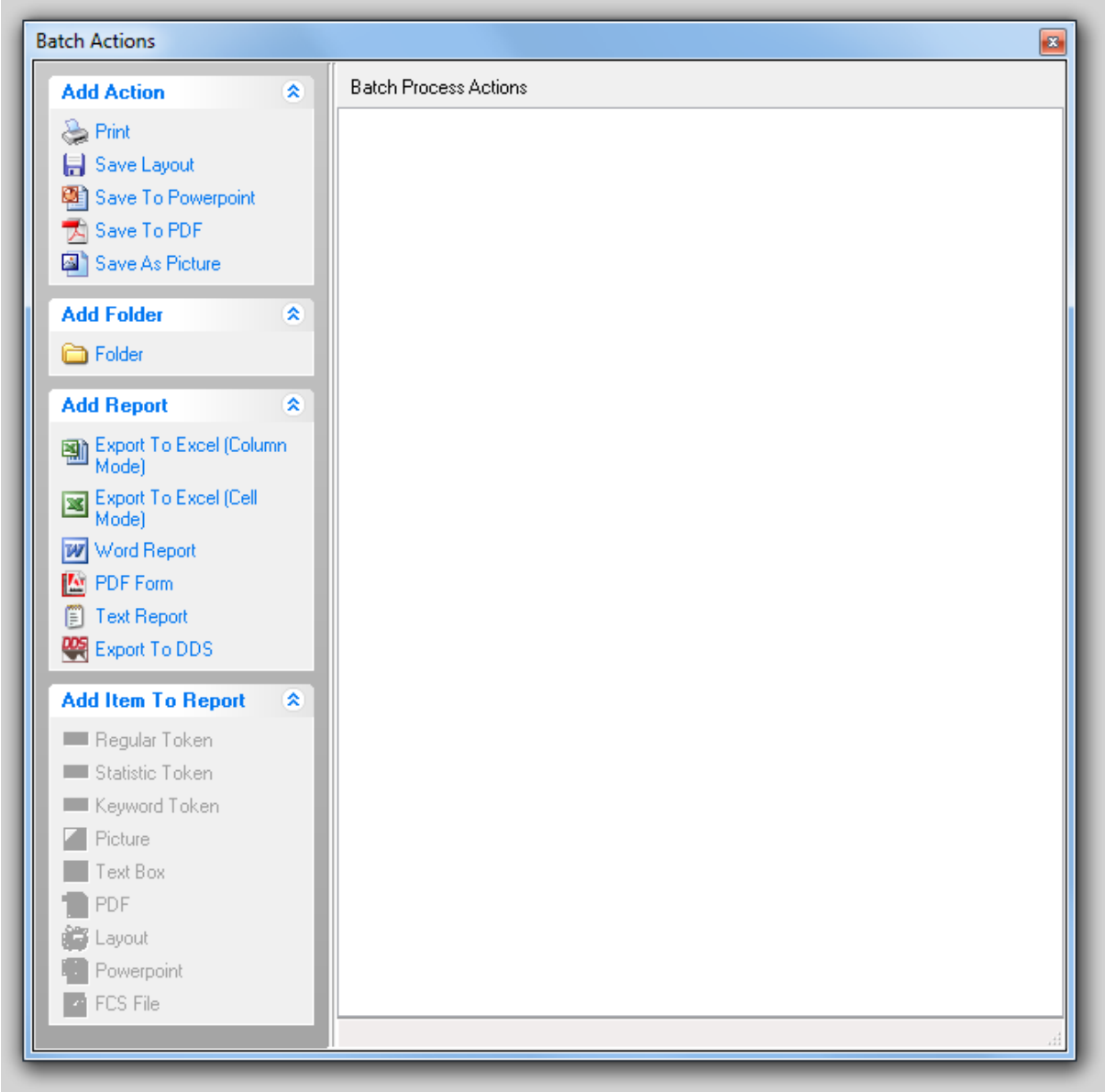

Figure 20.7 Batch Actions Window

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 386 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.

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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:

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 20.8 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.

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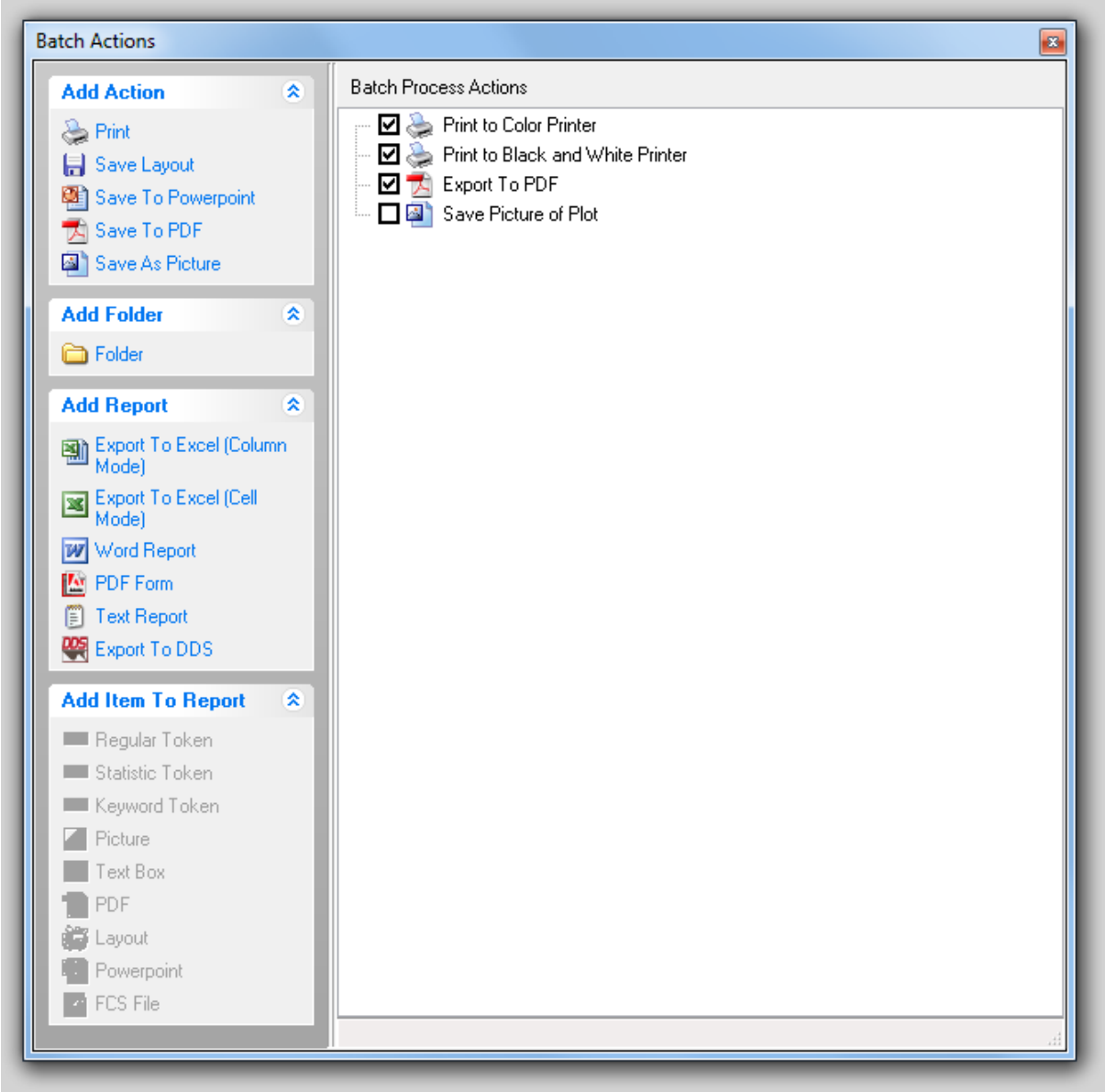

Figure 20.8 Batch Process Actions List with Actions

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.
- **Properties**: Display the dialog box for the selected action to allow changes to the action properties.

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20.2.1 Batch Processing Actions

The Batch Processing Actions include printing a layout386, saving a layout387, saving to PowerPoint390, saving to PDF392, saving as a picture394. You can also group your actions using a folder (action container)396.

In FCS Express Version 4, editing Batch Processing Actions has been streamlined. Changes to Batch Processing Actions are automatically applied. There is no need to press OK.

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 20.9), is used to set up how the layout will be printed with each iteration of the batch process.

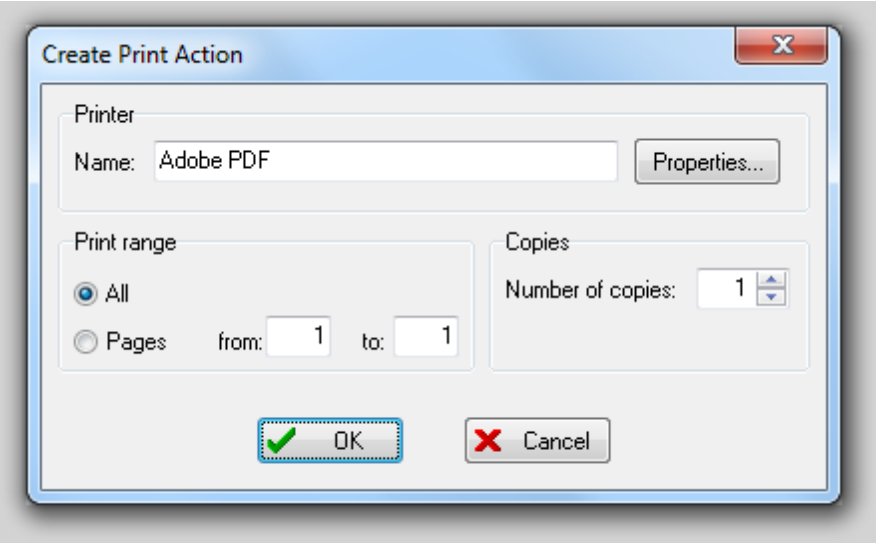

Figure 20.9 Create Print Action Dialog

The properties in the **Create Print Action** dialog are divided into three sections:

- **Printer**

  Select which printer to use to print the layout.

- **Print range**

  Choose which page(s) of the layout to print.

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- **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 20.10) is used to set up how the layout will be saved after each iteration.

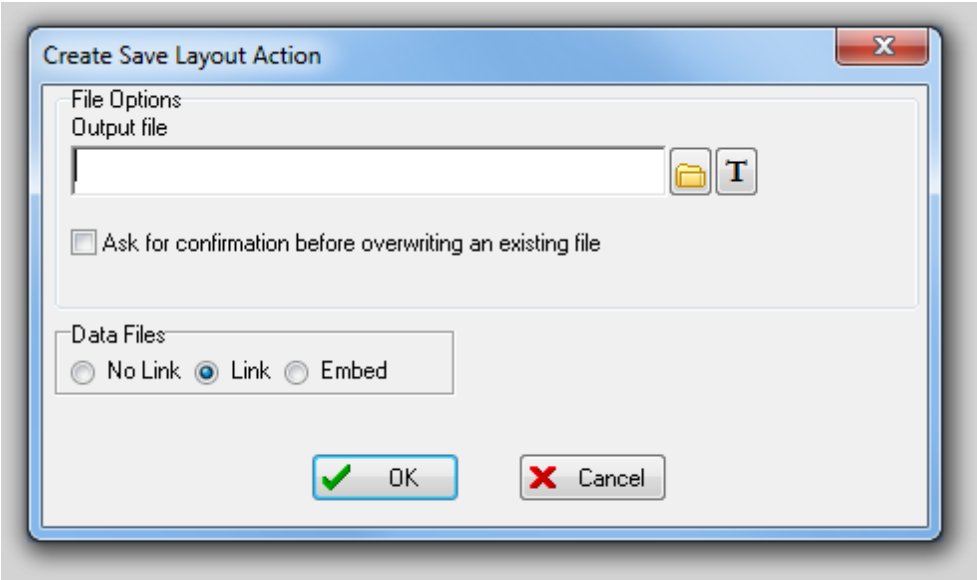

Figure 20.10 Create Save Layout Action Dialog

The properties are divided into two sections:

- **File Options**

  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[326] 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

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want the token text to appear. To insert a Current Iteration token:

1. Click on the button to bring up the **Insert a Token** dialog.
2. Click on the triangle next to **Batch Processing**.
3. Select **Current iteration** (Figure 20.11).
4. Click **Insert**.

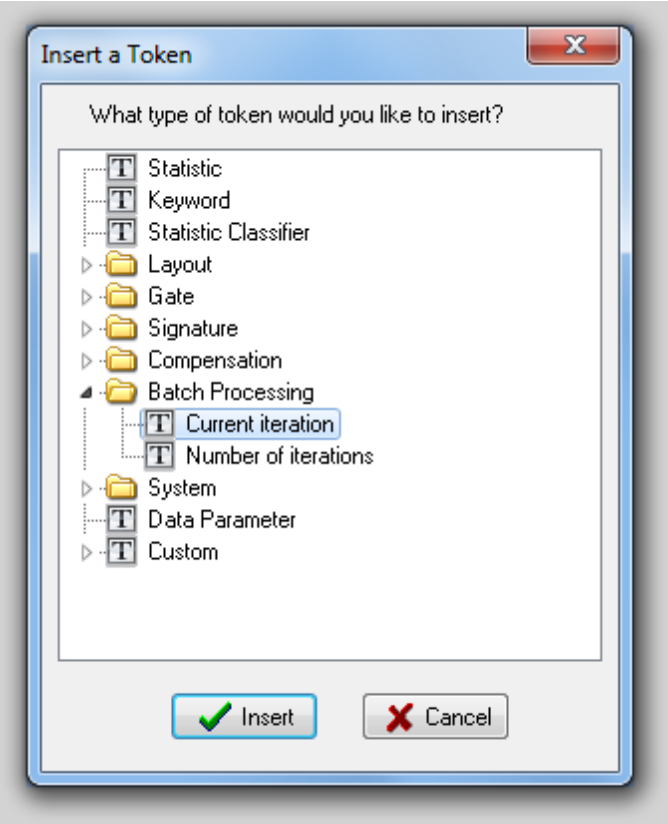

Figure 20.11 Insert a Current Iteration Token

Figure 20.12 shows an example of Output file, which will create a dynamic filename using the Current iteration token.

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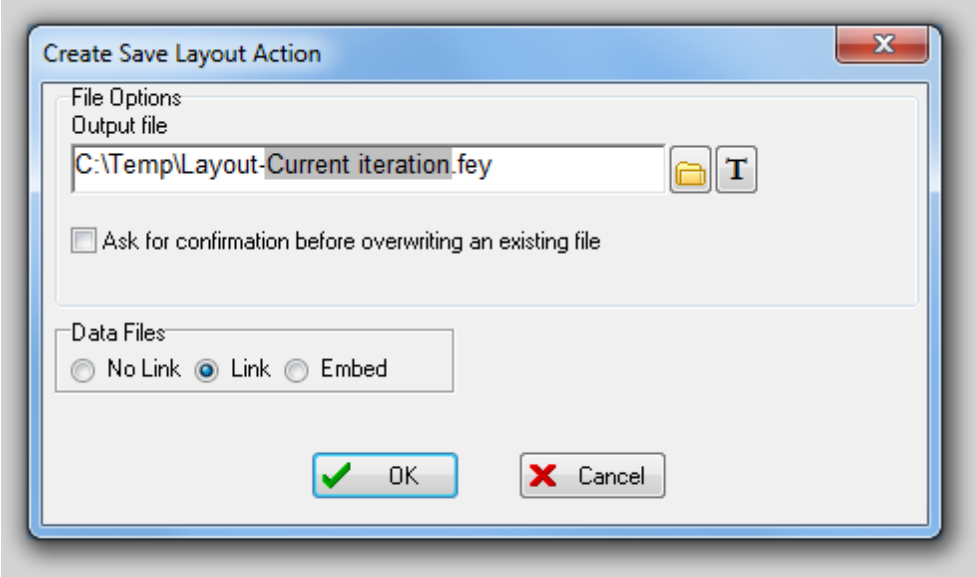

Figure 20.12 Example Output File with Current Iteration Token

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 Files**

  The options are the same as in the Save Layout dialog 53.

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 20.13).

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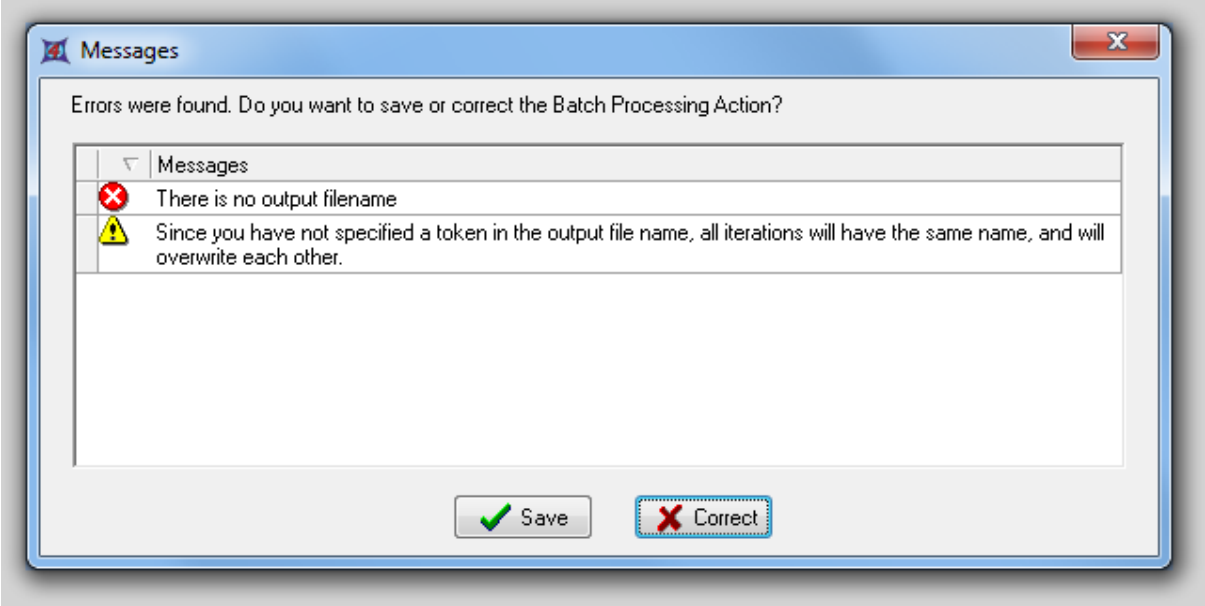

Figure 20.13 Batch Processing Error Messages

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 20.14) is used to set up how each iteration of the layout will be saved as a PowerPoint presentation file.

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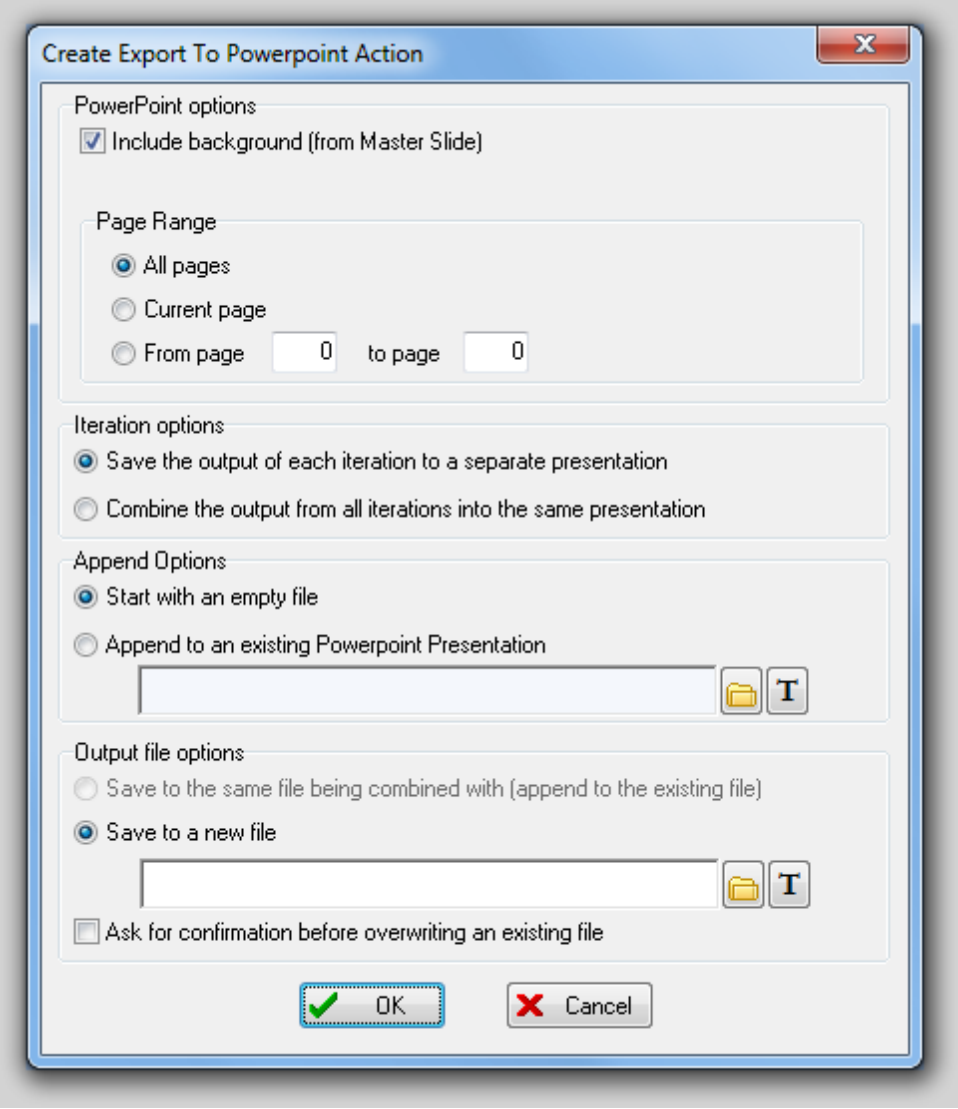

Figure 20.14 Create Export to PowerPoint Action Dialog

The properties are divided into four sections:

- **PowerPoint options**
  - **Include background (from Master Slide)**

    When checked, the objects on the master page will be placed on the PowerPoint master slide.
  - **Page Range**

    Choose which layout pages to export.
- **Iteration options**

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Choose whether to save the output of each iteration to a separate file or combine all the output into a single presentation.

- **Append Options**

  Choose whether to export to a new file or append the output to an existing PowerPoint presentation.

- **Output file options**

  Enter the filename for the exported PowerPoint file(s). See Save Layout - File Options for a description of batch generated filenames 387.

Click **OK** when you are finished editing the properties.

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 20.15) is used to set up how each iteration of the layout will be saved as a PDF file.

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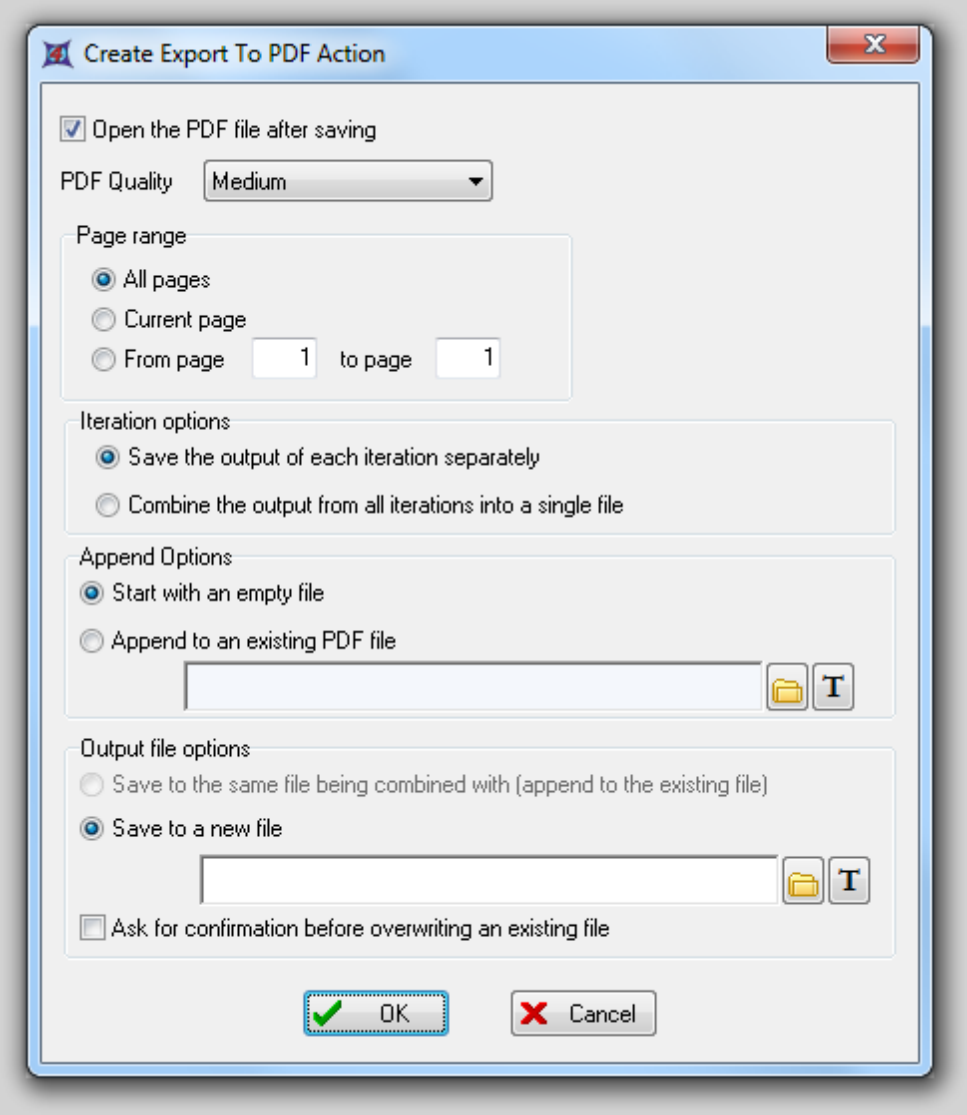

Figure 20.15 Create Export to PDF Action Dialog

You can choose to Open the PDF file after saving. The properties are divided into six sections:

- **Open the PDF file after saving**

  When checked, the PDF file will open at the end of each iteration.

- **PDF Quality**

  Set the PDF quality (**Low**, **Medium**, **High**, **Highest**) from the drop-down list.

- **Page Range**

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Choose which layout pages to export.

- **Iteration options**

  Choose whether to save the output of each iteration to a separate file or combine all the output into a single file.

- **Append Options**

  Choose whether to start with a new file or append the output to an existing PDF file.

- **Output file options**

  Enter the filename for the exported PDF file. See Save Layout - File Options for a description of batch generated filenames 387.

Click **OK** when you are finished editing the properties.

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 20.16) is used to set up how, in each iteration, plots or layout pages will be saved as picture files.

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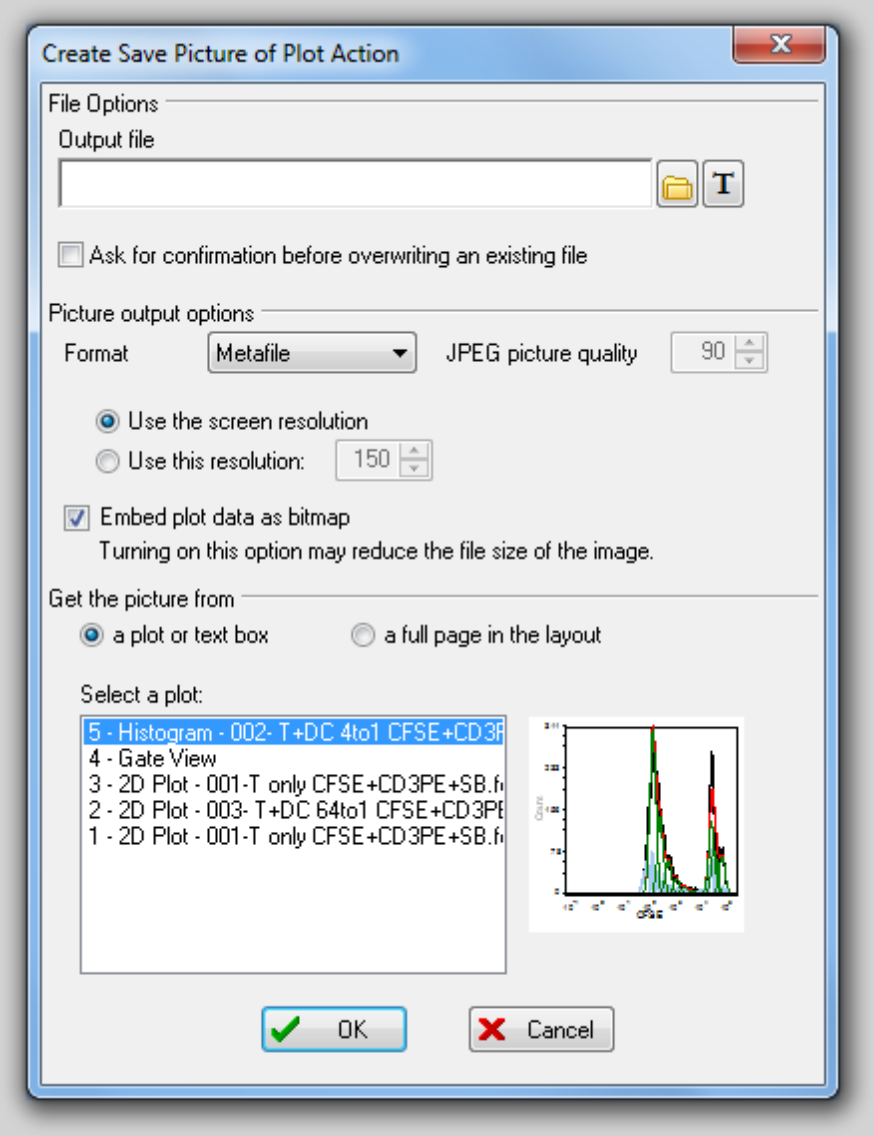

Figure 20.16 Create Save Picture of Plot Action Dialog

The properties are divided into three sections:

- **File Options**

  Enter the filename for the exported file. See Save Layout - File Options for a description of batch generated filenames 387.

- **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.

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- **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 20.17, 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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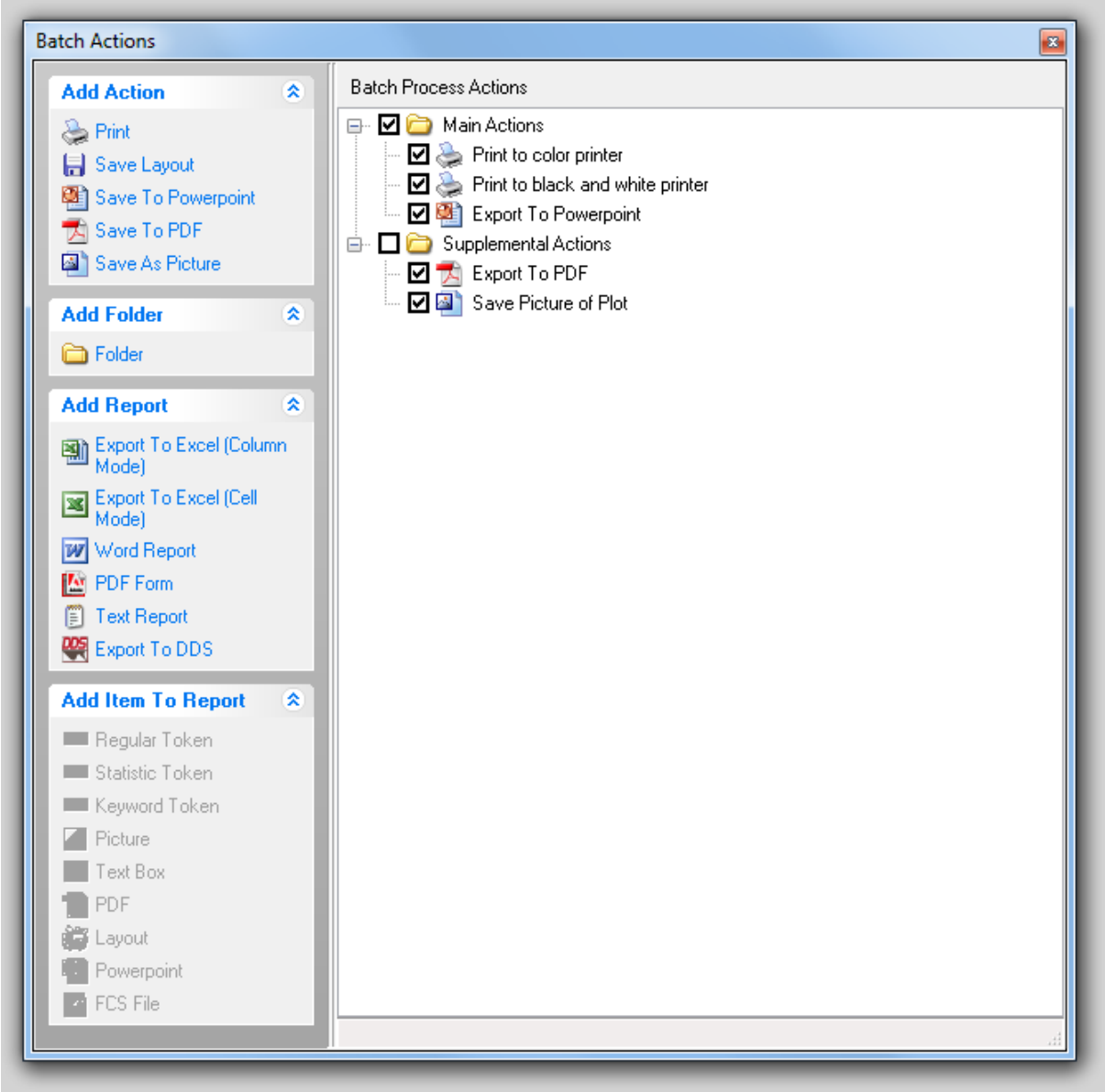

Figure 20.17 Folders (Action Containers)

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 De Novo Data Services (DDS) or to LabKey Server. 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**

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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 401 (text)
- Excel - Cell Mode 404 (text and images)
- Word Report 406 (text and images)
- PDF Form 408 (text)
- Text Report 409 (text)
- DDS 409 [*De Novo Data Services*] (tokens, images, PDF files, PowerPoint Presentations, data files)
- LabKey Server 410 (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 326 (all available tokens, including Custom tokens 348)
- Statistic Token 337
- Keyword Token 342
- Picture 414 (a plot in the layout, or an entire page of the layout, only used in Word, Excel-Cell Mode, and DDS reports)
- Text Box 96 (text in a text box (e.g., canned comments) can be exported to a column in an Excel worksheet or other location)
- PDF 63
- Layout 52
- PowerPoint 767
- FCS File

An example of the Batch Process Actions for an Excel (Cell Mode) report is shown in Figure 20.18.

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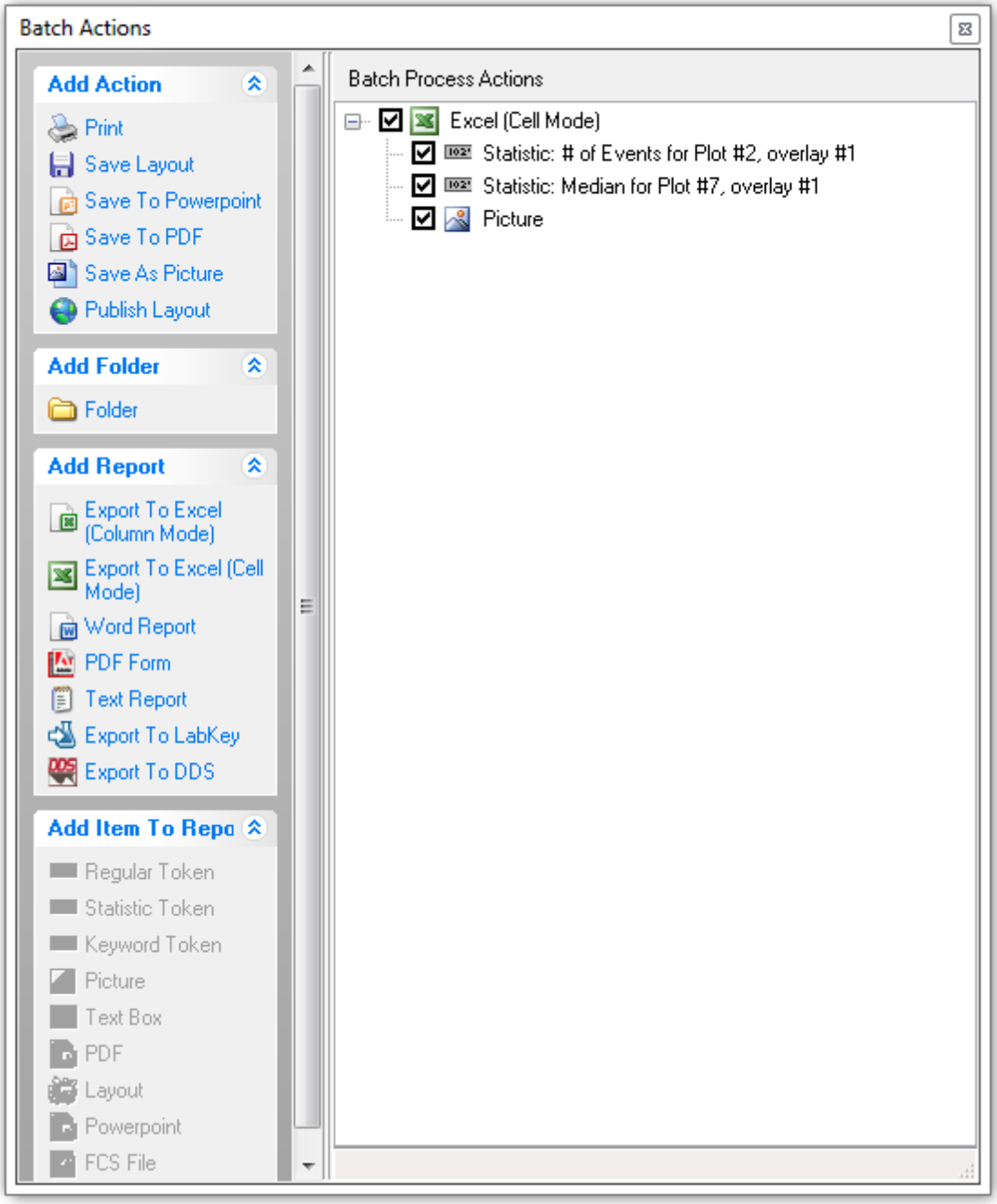

Figure 20.18 Batch Process Actions for Excel Report

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

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menu), you can navigate to the **Running Options** page of the **Options** dialog to control the activation state of an action (Figure 20.19). 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.

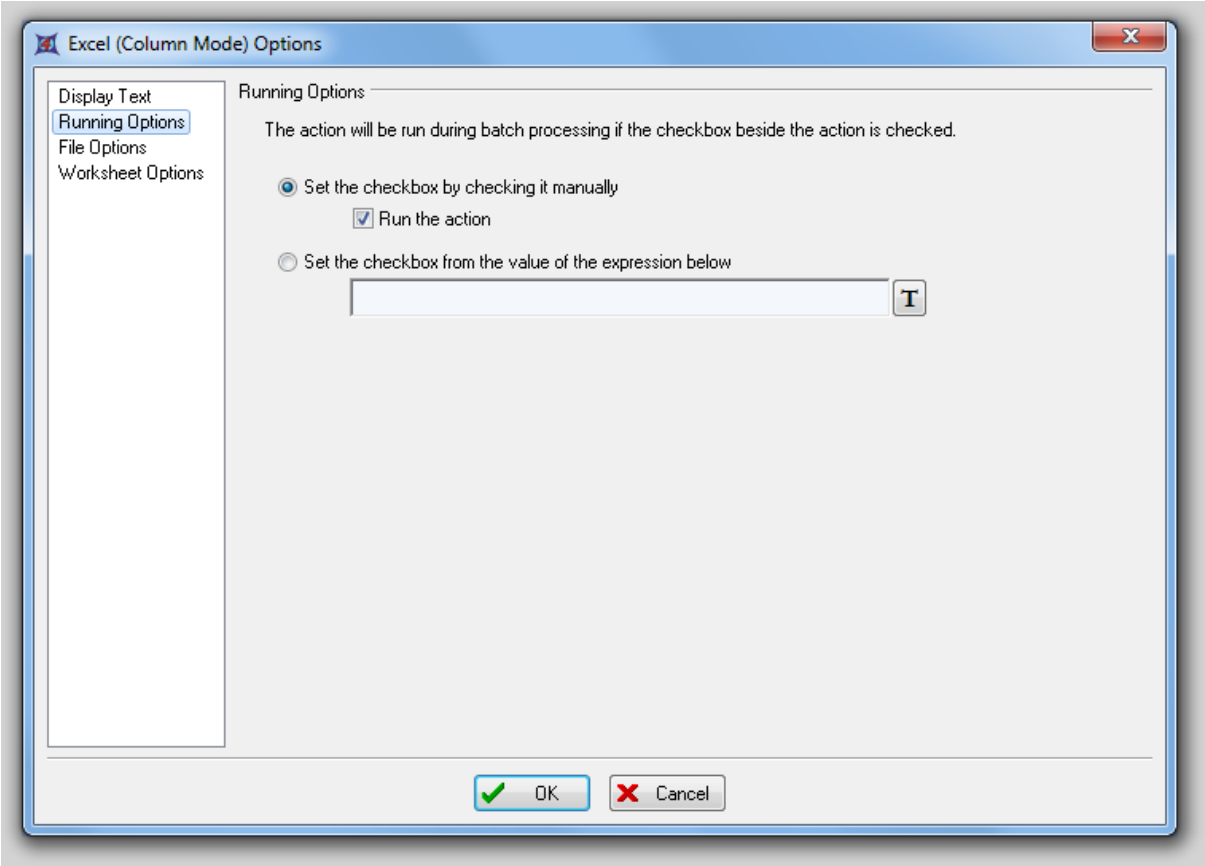

Figure 20.19 Running Options

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) 401

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- Excel (Cell Mode) 404
- Word 406
- PDF form 408
- Text 409
- DDS 409
- LabKey Server 410

- **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 20.20.

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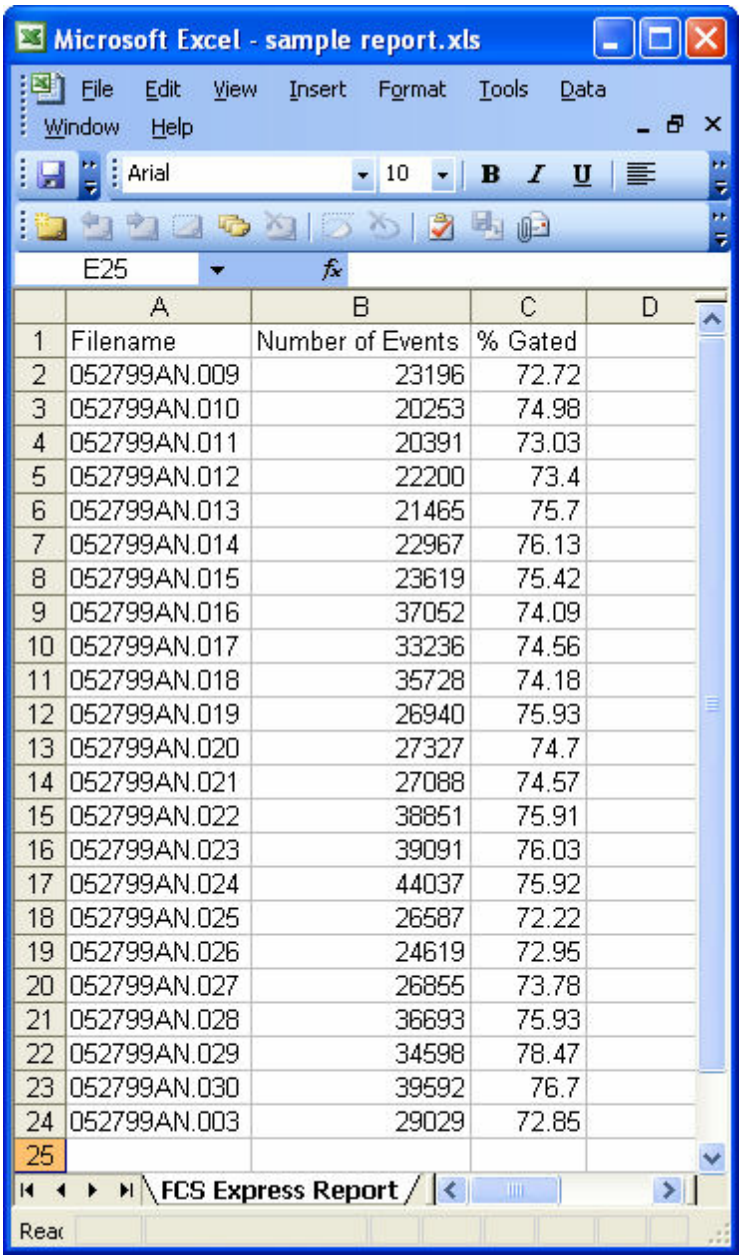

	A	B	C	D
1	Filename	Number of Events	% Gated	
2	052799AN.009	23196	72.72	
3	052799AN.010	20253	74.98	
4	052799AN.011	20391	73.03	
5	052799AN.012	22200	73.4	
6	052799AN.013	21465	75.7	
7	052799AN.014	22967	76.13	
8	052799AN.015	23619	75.42	
9	052799AN.016	37052	74.09	
10	052799AN.017	33236	74.56	
11	052799AN.018	35728	74.18	
12	052799AN.019	26940	75.93	
13	052799AN.020	27327	74.7	
14	052799AN.021	27088	74.57	
15	052799AN.022	38851	75.91	
16	052799AN.023	39091	76.03	
17	052799AN.024	44037	75.92	
18	052799AN.025	26587	72.22	
19	052799AN.026	24619	72.95	
20	052799AN.027	26855	73.78	
21	052799AN.028	36693	75.93	
22	052799AN.029	34598	78.47	
23	052799AN.030	39592	76.7	
24	052799AN.003	29029	72.85	
25				

Figure 20.20 Excel (Column Mode) Sample Report

The **File Options** page of the **Excel (Column Mode) Options** dialog (Figure 20.21) 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 (.

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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.

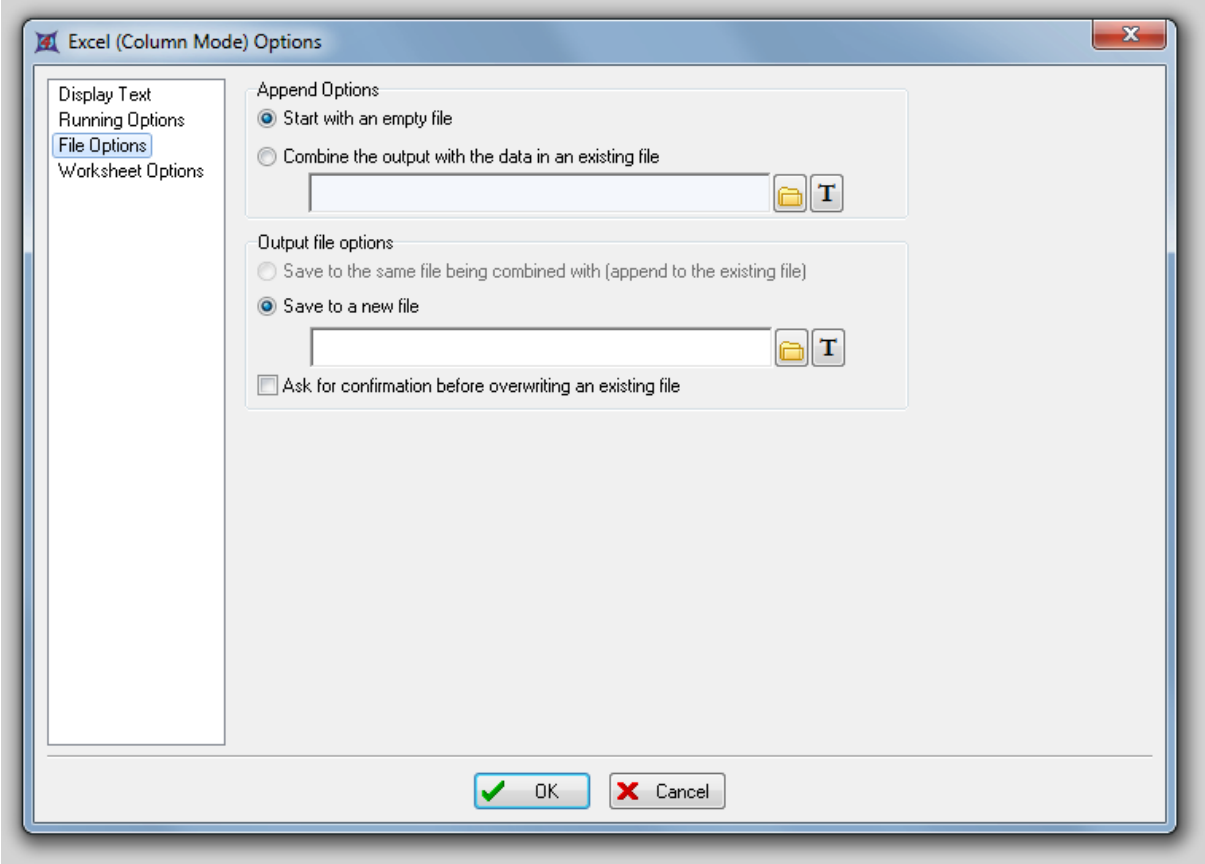

Figure 20.21 Excel (Column Mode) File Options

The **Worksheet Options** 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**, **Column** (e.g., start at the first available row or column or start at a particular row or column), and or **Header Options** (Figure 20.22).

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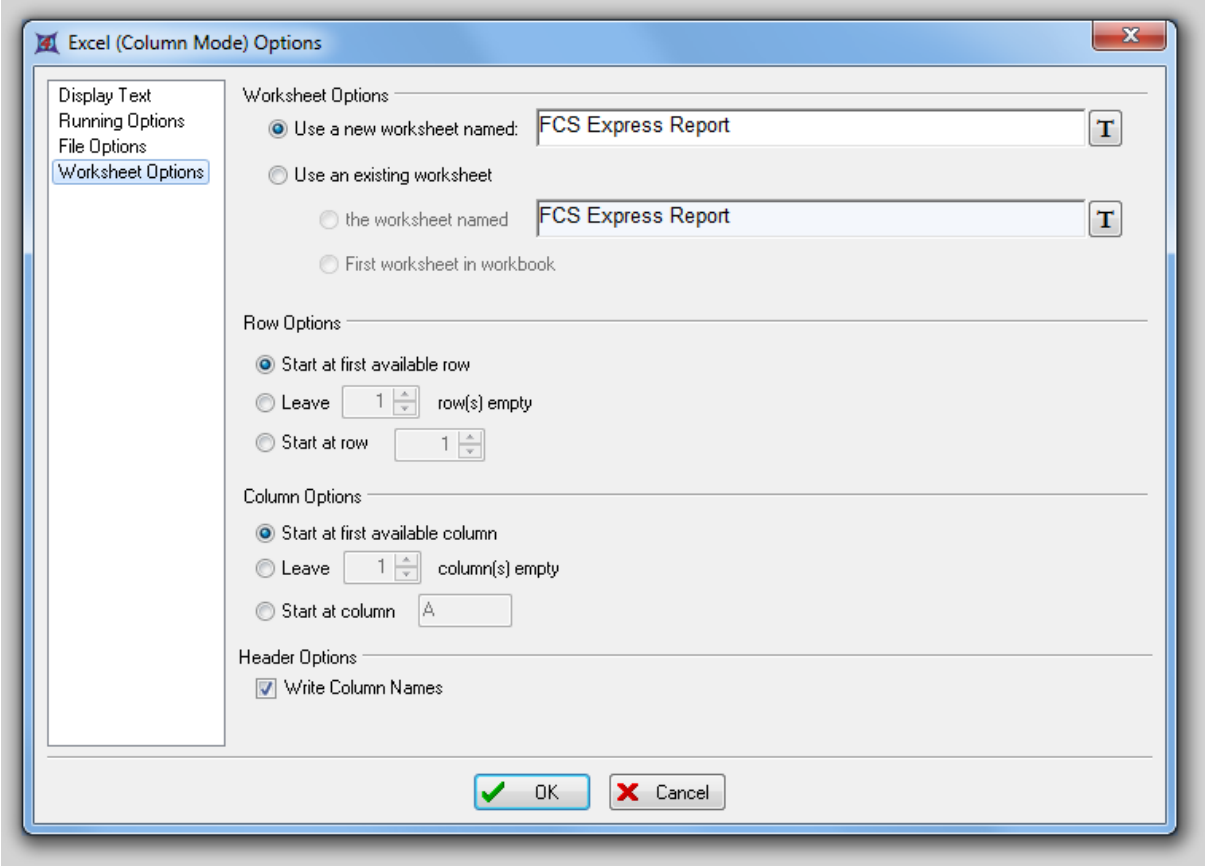

Figure 20.22 Worksheet Options

- **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 20.23.

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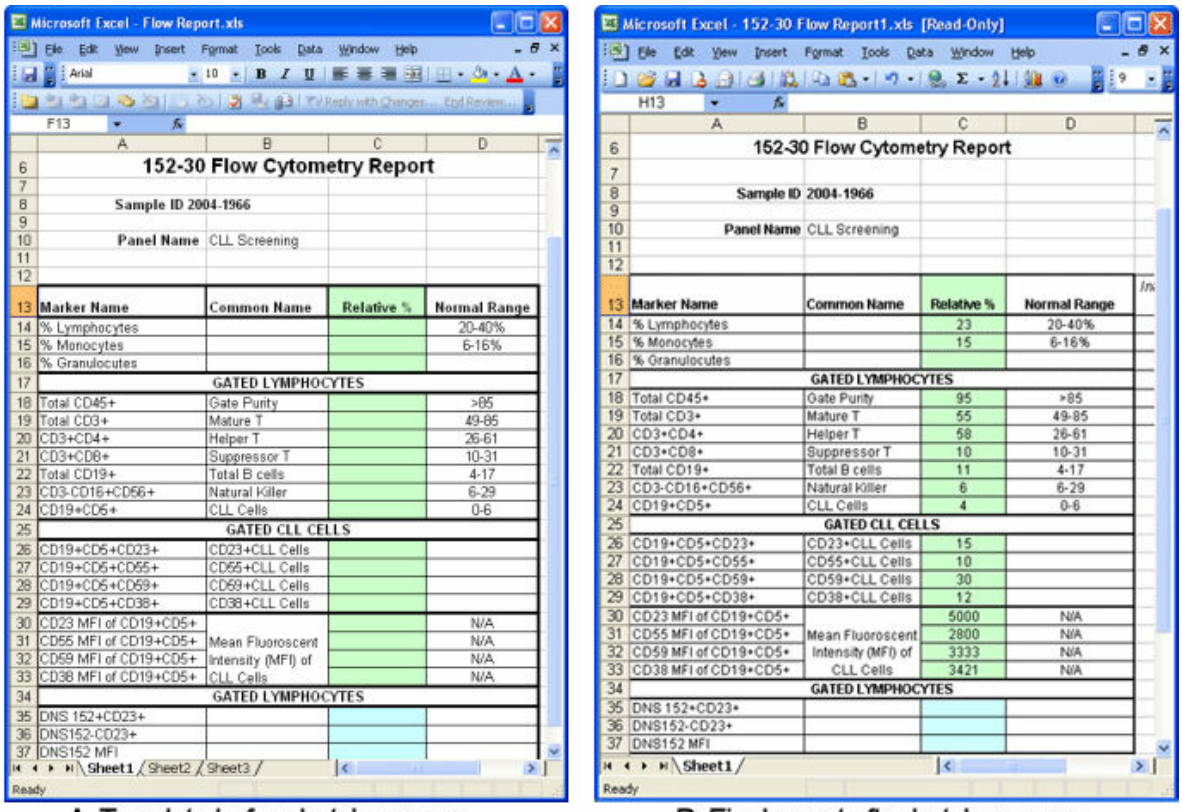

Figure 20.23 An Example Excel (Cell Mode) Report

The **File Options** page of the **Excel (Cell Mode) Options** dialog (Figure 20.24) 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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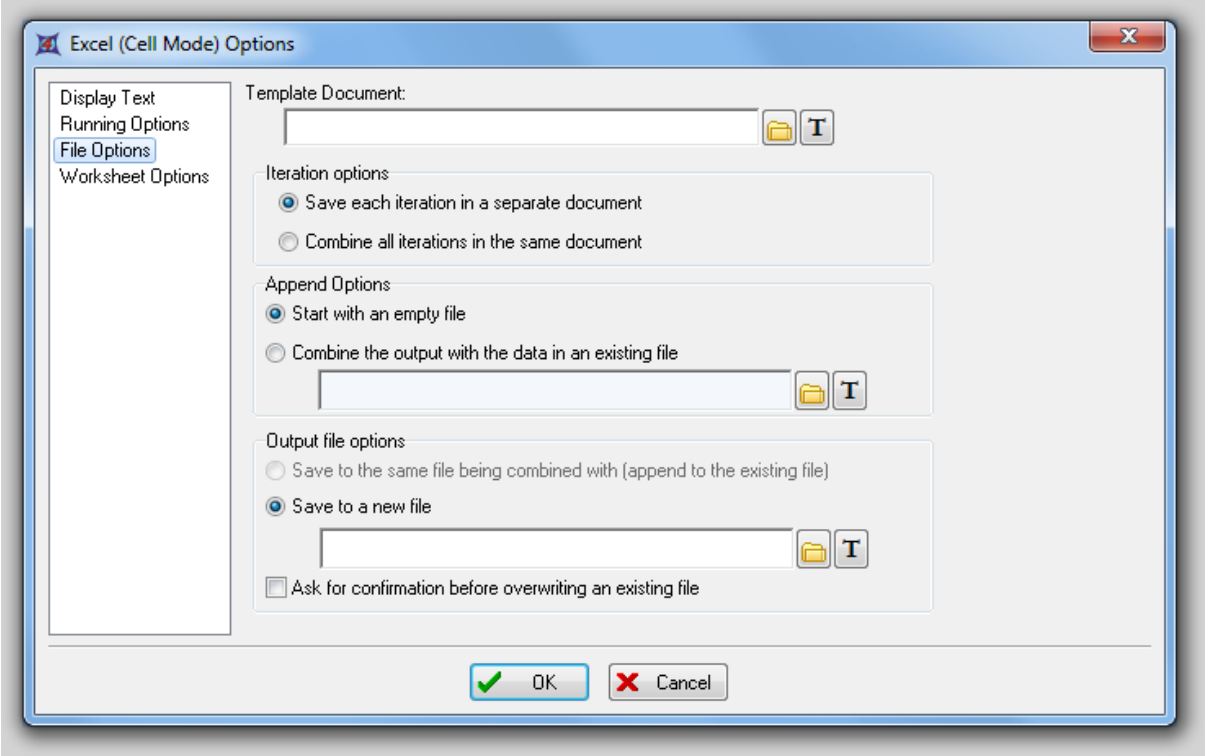

Figure 20.24 Excel (Cell Mode) File Options

- **Word Report**

  With this report type you can create Word files resembling Figure 20.25. 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 Name413, 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 Reports411.

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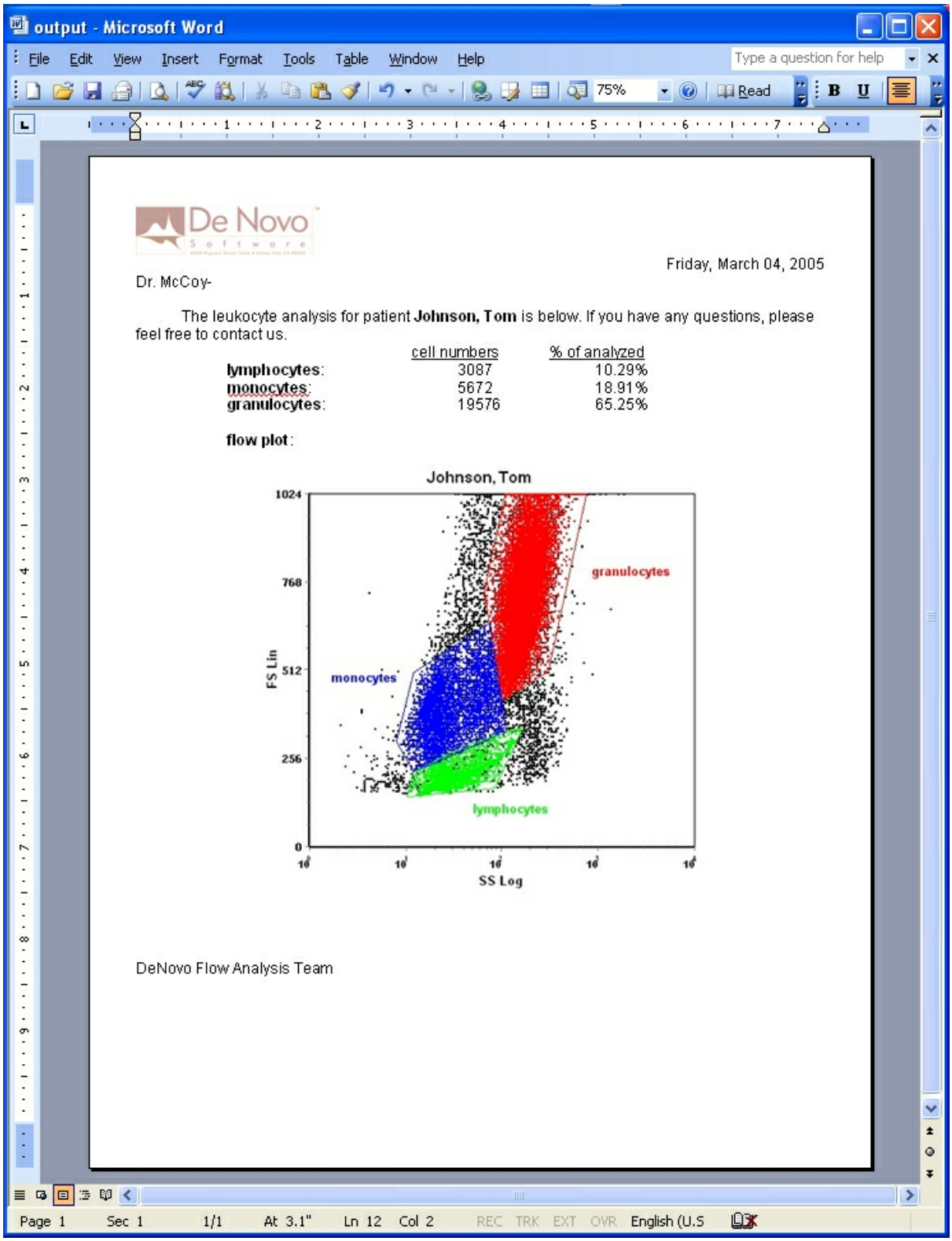

Figure 20.25 Example Word Report

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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 file63, 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 Name413, 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, in columns, separated by commas. The first row of the text file will contain the column header of each exported item. 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 (.txt) file entered in the text box. The final output (new iterations plus existing data) will be written to another text file.

  - **Output file options**

    When combining data with an existing file, the destination file can be a new file (.txt) or the existing text file.

- **DDS Report**

  The De Novo Software Data Services (DDS) is an easy-to-use online database that allows you to store your experimental results. Data is placed into the DDS through the Export to DDS Batch Processing report. Once you set up your layout with the DDS integration, you simply run through your data using the batch processing function and it will be automatically sent up to the DDS. See http://www.denovosoftware.com/site/dataservices.shtml for more details.

  The **DDS** page of the **Export to DDS Options** dialog (Figure 20.26) is used for entering your User Name, Password, Experiment Name, and choosing whether to upload to the De Novo Software DDS server (or an **Alternate URL**).

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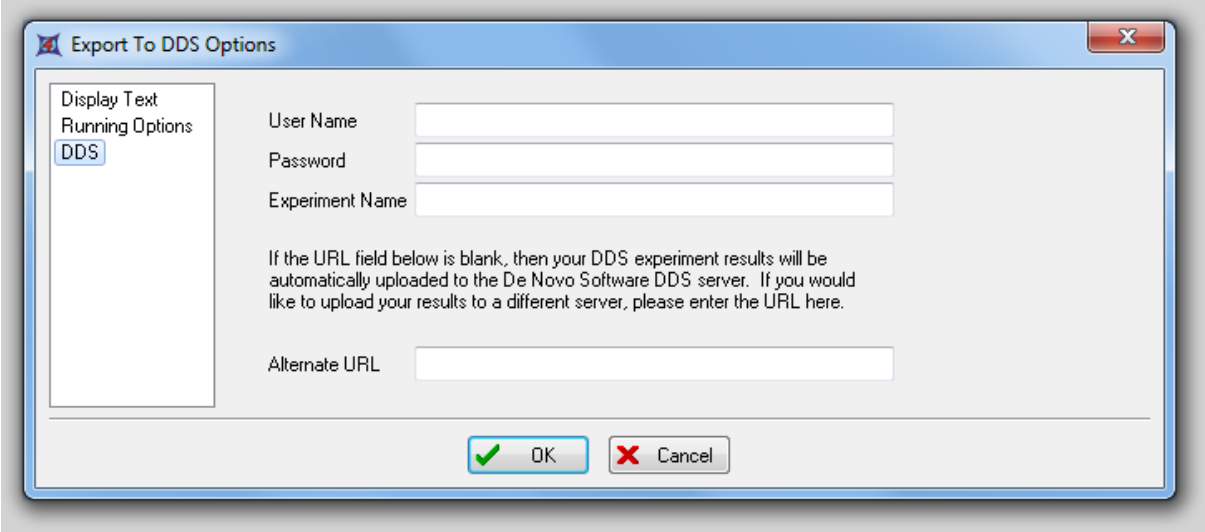

Figure 20.26 Export to DDS Options

- **LabKey Server Report**

  LabKey Server is free, open source software that supports automated quality control, centralized data management, and web-based data 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 20.27). 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.

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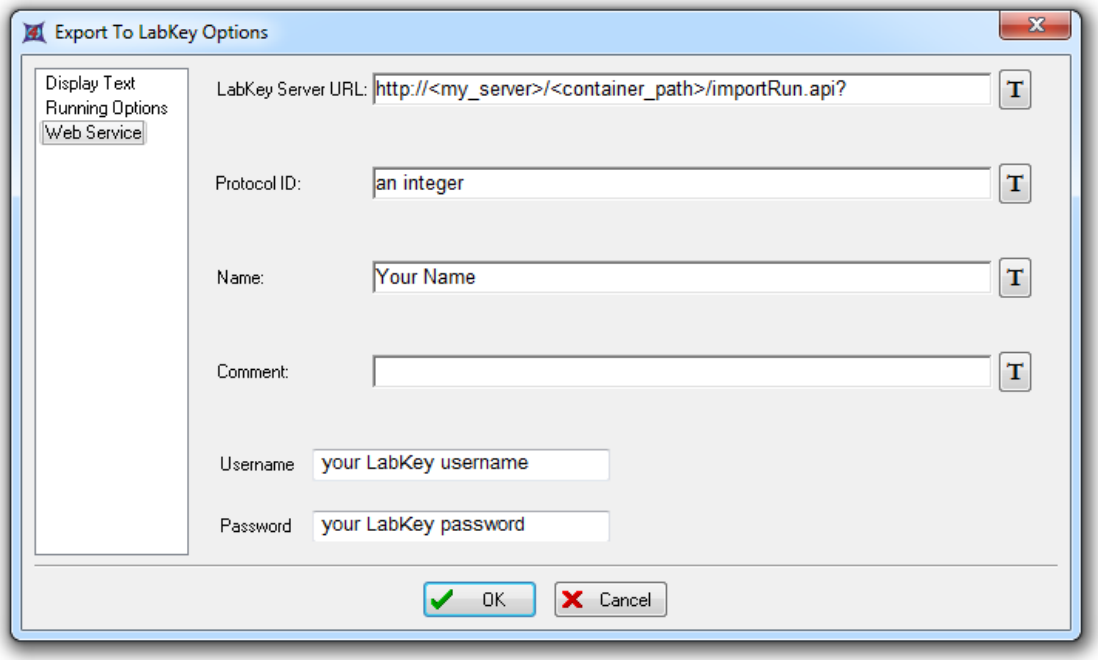

Figure 20.27 Export to LabKey Options

20.2.2.2 Creating Templates for Reports

You can create templates for Excel (Cell Mode) reports, as well as PDF and Word reports.

- *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*

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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 416 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 two methods to add items to reports:

- Adding items from a Text Box or a Statistics window.

  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 the Add Item to Report list.

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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.
4. Once an item has been added to a report, you will need to configure the item's "report-specific" property 413. This property tells FCS Express where to insert the item in the final report.

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 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*

  For Excel (Column Mode) and Text reports, the report-specific property is set using the **Column Name** property (Figure 20.28). Enter a **Column Name** in the edit field.

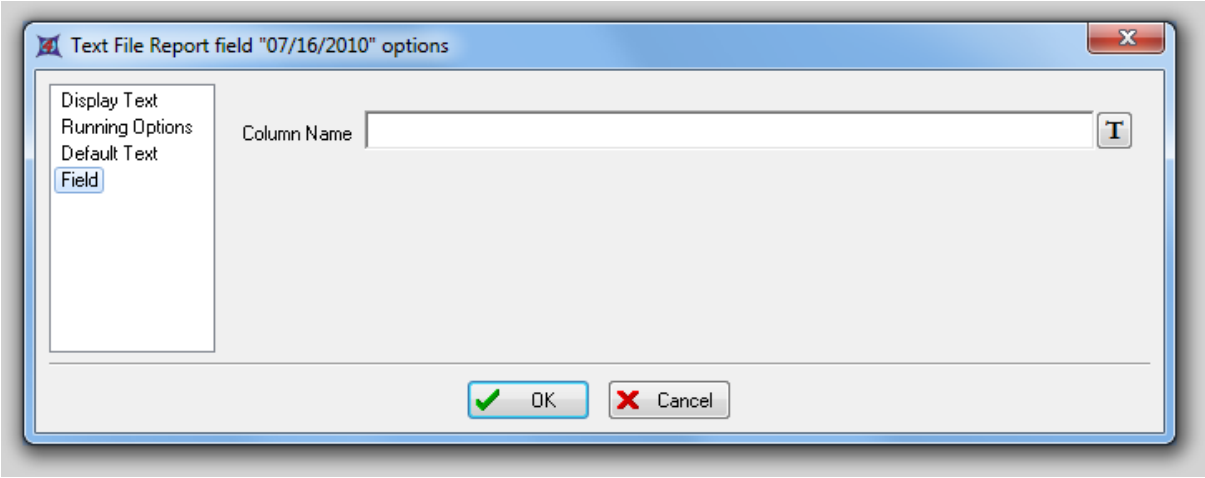

Figure 20.28 Column Name Property

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The **Column Name** specifies the text that will appear in the report at the top of the column containing data from your layout. 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*. The default Column Name is always the name of the item in your layout.

- *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 411 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 411 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, DDS, and LabKey Server reports. To add a picture to a report:

1. Click on the appropriate report type in the Add Report section of the Batch Actions window 397.
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 20.29). This page has two sections:

  - **Picture output options**

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Choose the file format and resolution 394. *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.

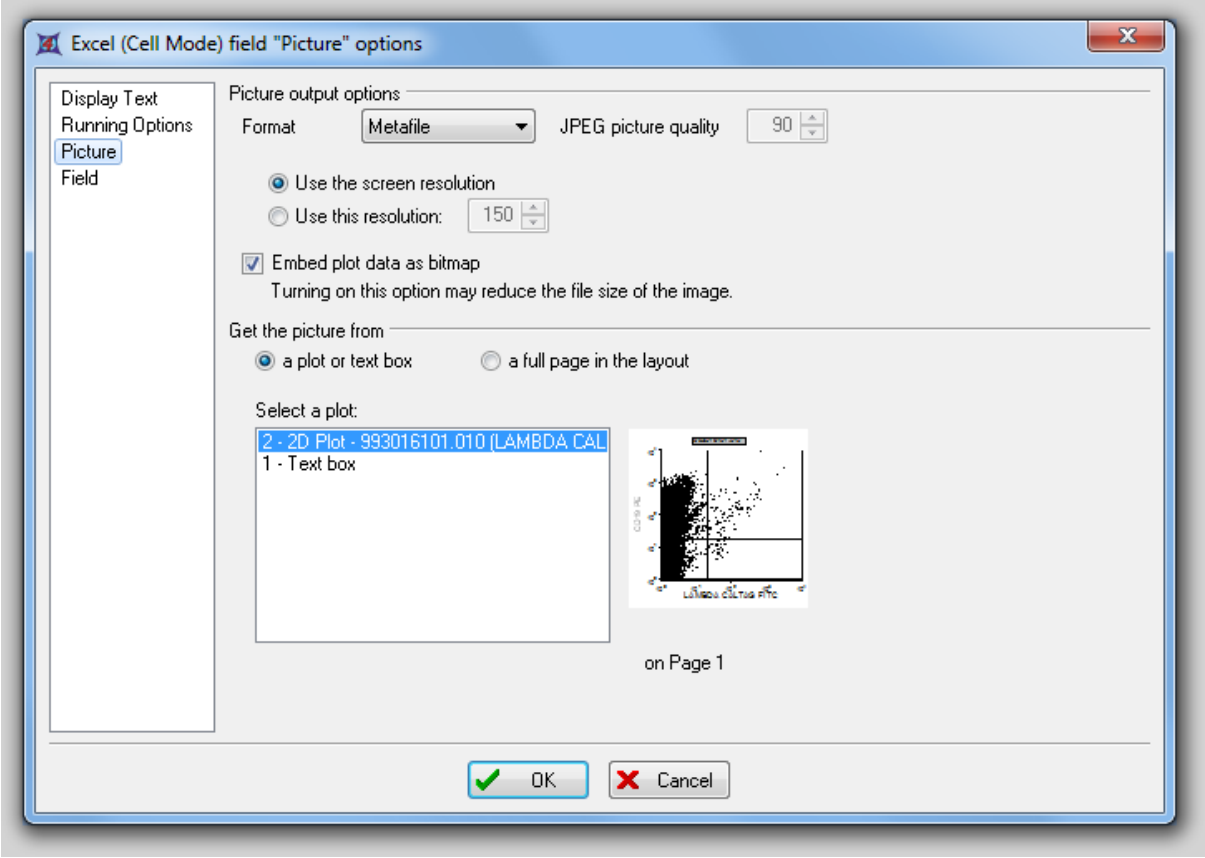

Figure 20.29 Excell (Cell Mode) Picture Options

- *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

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the section on report-specific properties 413 for a description of the Field Name property.

- *DDS*

  The **Picture** page of the **Export to DDS field "Picture" options** dialog is similar to the Excel (Cell Mode) dialog described above. See http://www.denovosoftware.com/site/dataservices.shtml for a detailed description of the De Novo Software Data Services option and a tutorial on this service.

- *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 808 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 20.30). 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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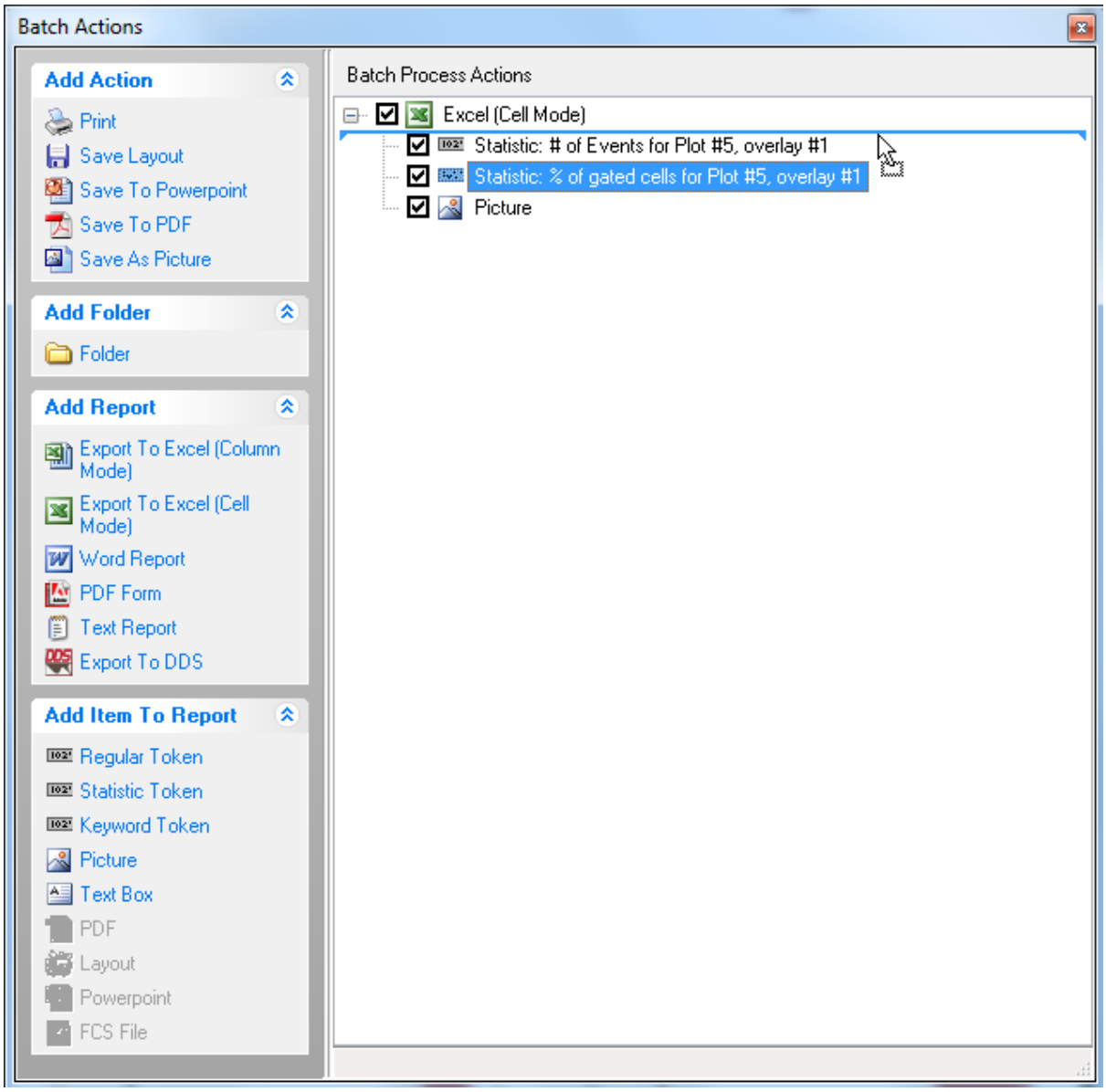

Figure 20.30 Repositioning a Token in a Report

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.

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Note: After you have moved or copied an item from one report to another, it is important to ensure that the report-specific properties[413] 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.

20.3 Batch Processing Options

You can control the iterations when running a batch process. To set batch processing options, use the **Batch→Batch Processing→Options** command to bring up the **Batch Processing Options** dialog (Figure 20.31).

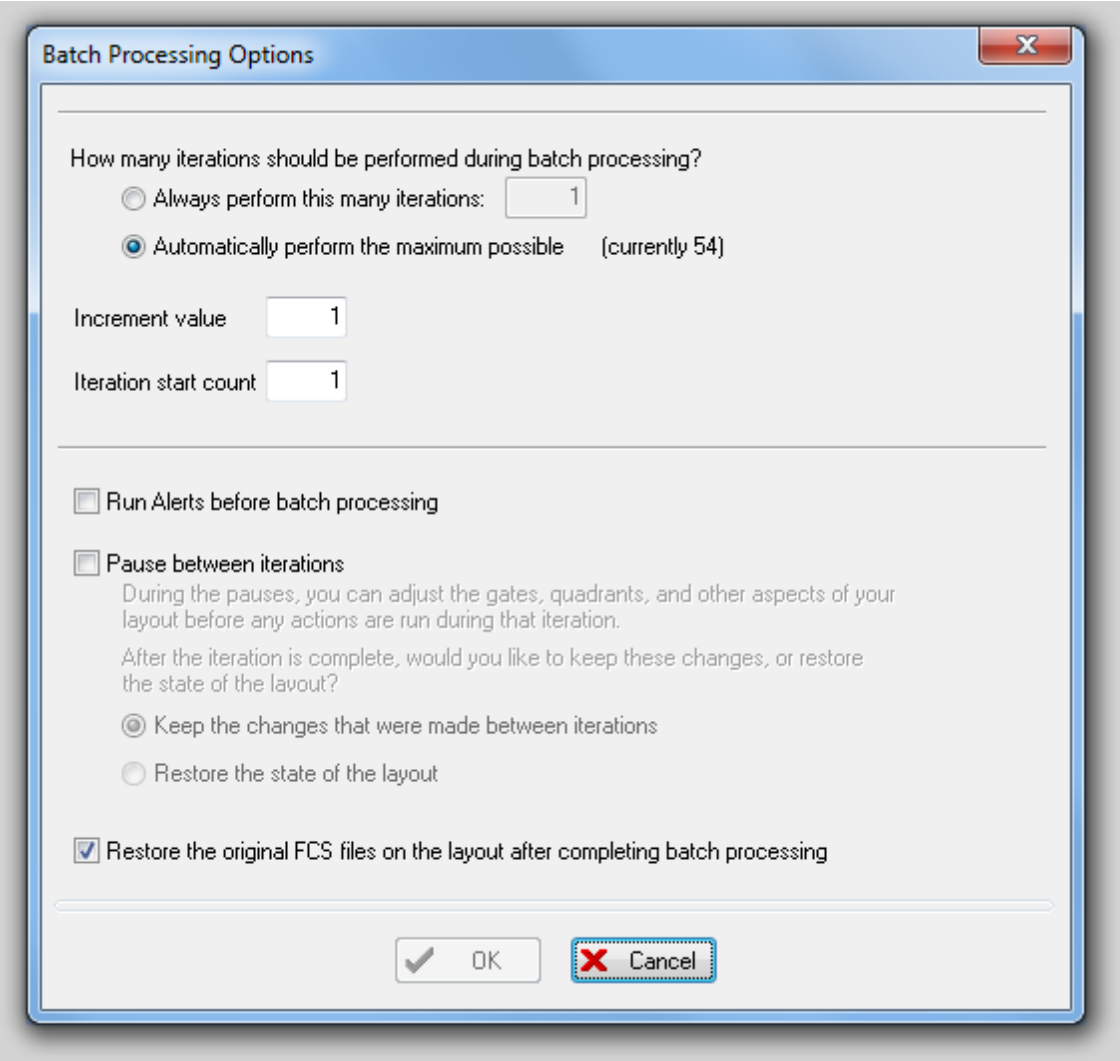

Figure 20.31 Batch Processing Options Dialog

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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 422 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 387 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 up will be run before Batch Processing is started. You will have the option of aborting or continuing the batch process if any alerts are triggered.

- **Pause between iterations**

  If this property is enabled, you will be prompted to click a **Continue** button in the Batch Run progress window at the beginning of each iteration 420. This setting allows you to adjust gates and quadrants as needed before the next iteration. The Batch will run uninterrupted if this property is unchecked. Using the radio buttons, you can choose to **Keep**

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the changes that were made between iterations or **Restore the state of the layout** for the next iteration.

- **Restore the original FCS files on the layout after completing batch processing**

  When this property is checked, 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.

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.

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 a **Continue** button allowing you to adjust the analysis for the current iteration prior to continuing.

If the Batch Process aborts for any reason, FCS Express will now save all of the reports up until the abort.

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Chapter

21 Using Panels

In a typical experiment, you repeat the same set of staining protocols many different times, with different samples or using different conditions. A staining protocol is 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 List373, 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**, the plots will know what tubes they are displaying data for. 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 21.2, will appear.

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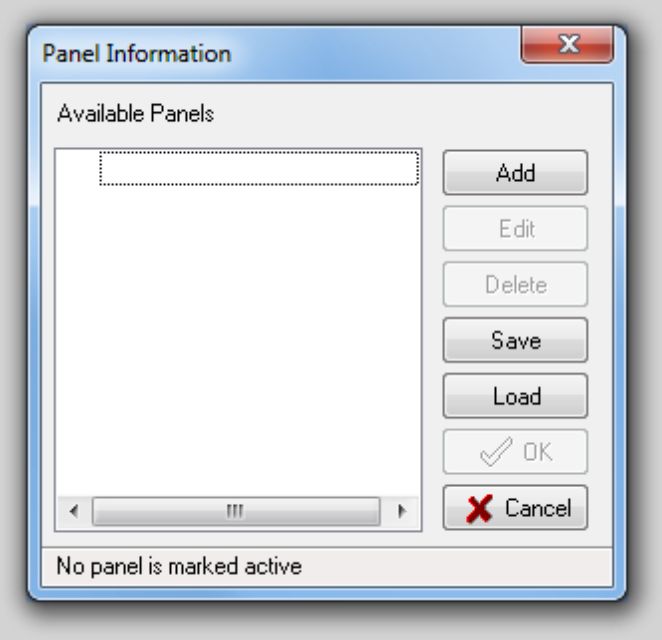

Figure 21.2 Panel Information Dialog

2. Click on the **Add** button to create a blank Panel. This will bring up the Add Panel dialog (Figure 21.3).

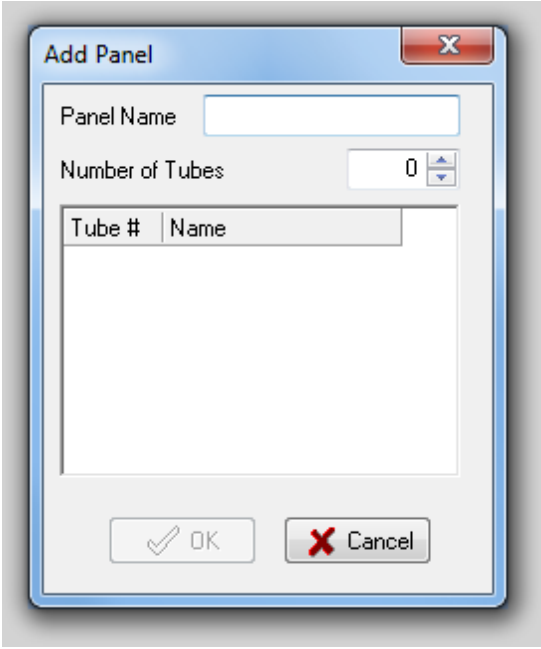

Figure 21.3 Add Panel Window

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.

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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 21.4). 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.

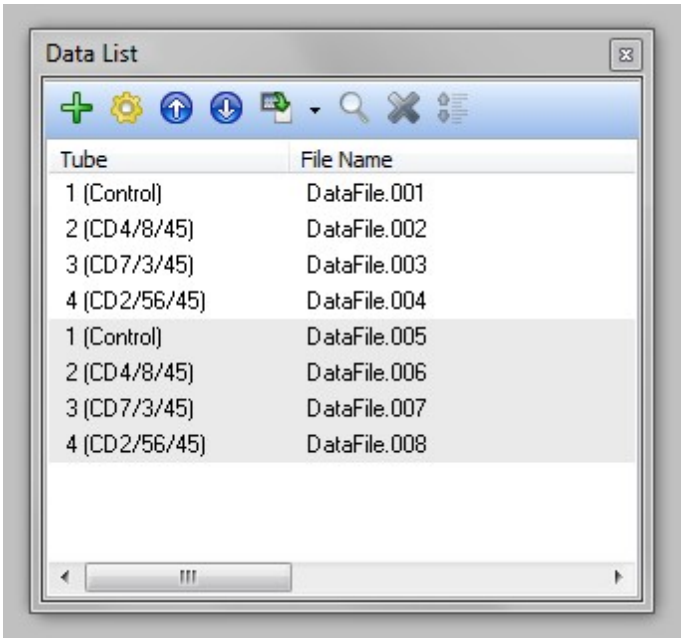

Figure 21.4 Data List with 4-tube Example Panel

To use the **Data List** in conjunction with the panel to control the data files in the

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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 21.5).

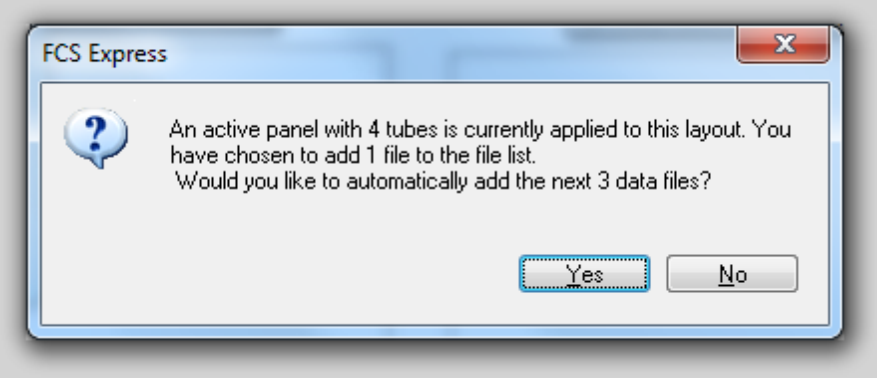

Figure 21.5 Automatically Populating a Panel

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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. Otherwise, it is just like any other layout. FCS Express remembers the layout as a template when you select it using the **Application Button→New Layout→Create Layout From Template** command/gallery. Clicking on **Create Layout From Template** 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 Templates area of the gallery 52.

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 **Application Button→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 135.

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Chapter

23 Importing External Documents

FCS Express supports importing two external layout types:

- BD FACSDiva experiments.
- BD Accuri C6 experiments.

23.1 BD FACSDiva Files

The standard 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 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 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 configuration for use again with other FACSDiva experiment files. The configuration is saved to an FCS Express FACSDiva configuration file431.

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23.1.1 How to Import FACSDiva Experiment Files

To import a FACSDiva experiment file:

1. Use the **Application Button→Open Layout→Import BD FACSDiva File...** command.

2. Browse your directories to select the FACSDiva experiment file (.xml) to import. Click **Load** when you have located it.

3. The **Import BD FACSDiva File Wizard** appears, beginning with the **Import Options** page (Figure 24.2). 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.

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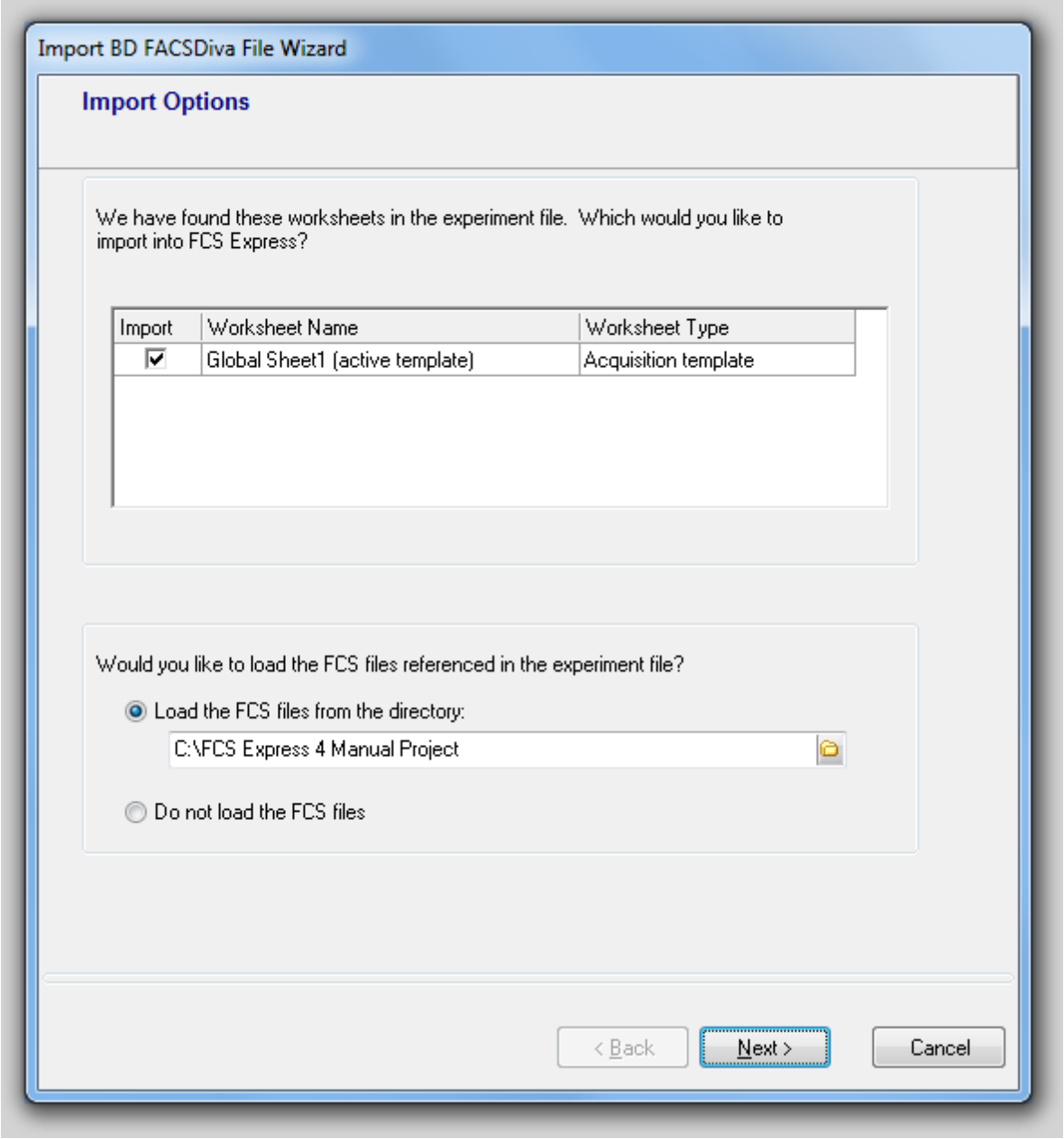

Figure 24.2 Import FACSDiva File Wizard - Import Options

4. Towards the bottom of the window, choose whether you want to load the FCS files corresponding to the experiment. 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 24.3). 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

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the **Create a new configuration** radio button, and click **Next**.

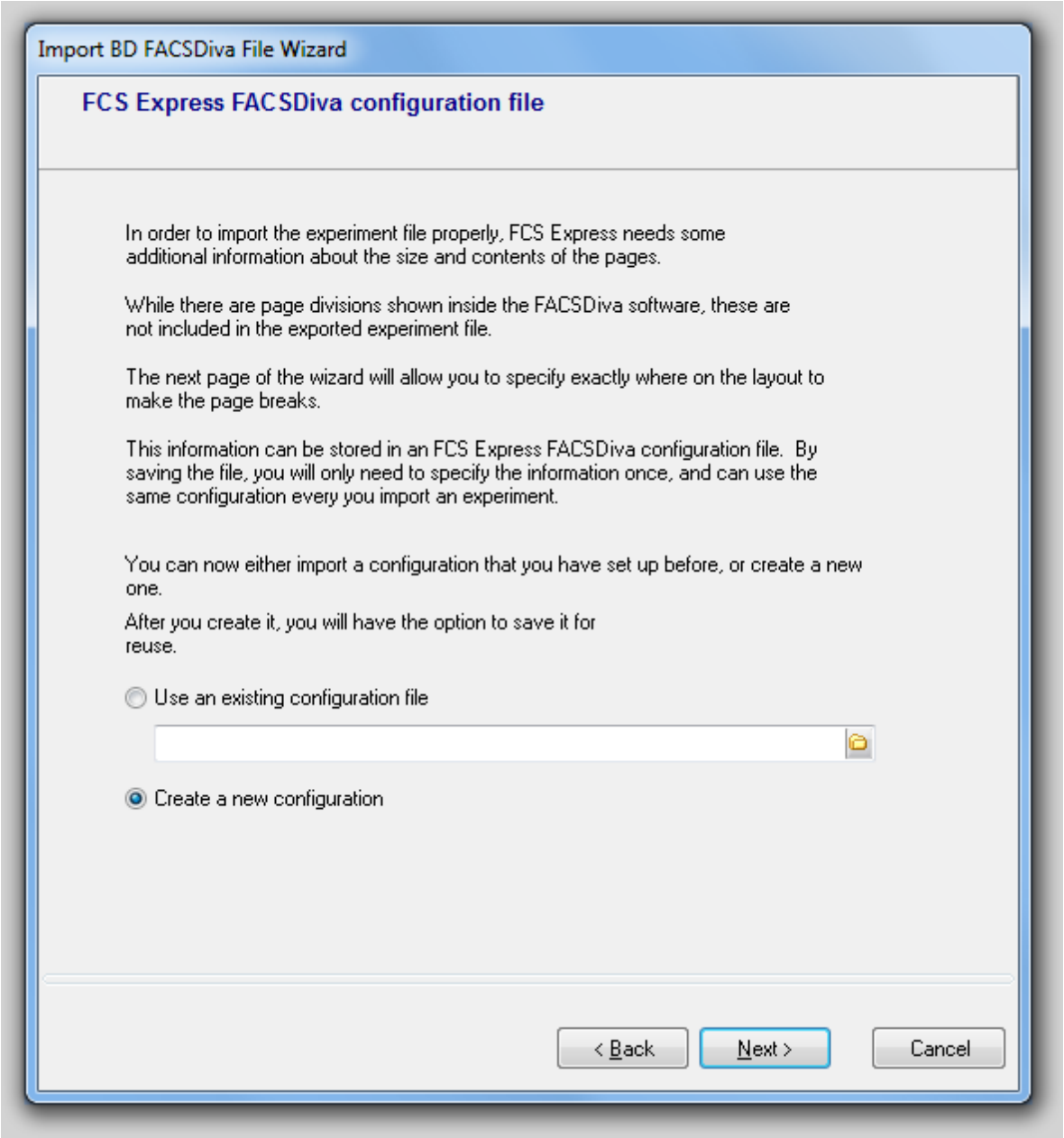

Figure 24.3 Import FACSDiva File Wizard - Configuration File

6. If you decided to create a new configuration, the **Setting up the page size for the layout in FCS Express** (Figure 24.4) will appear. Here, you set up how the plots from the FACSDiva experiment will be imported into the FCS Express layout.

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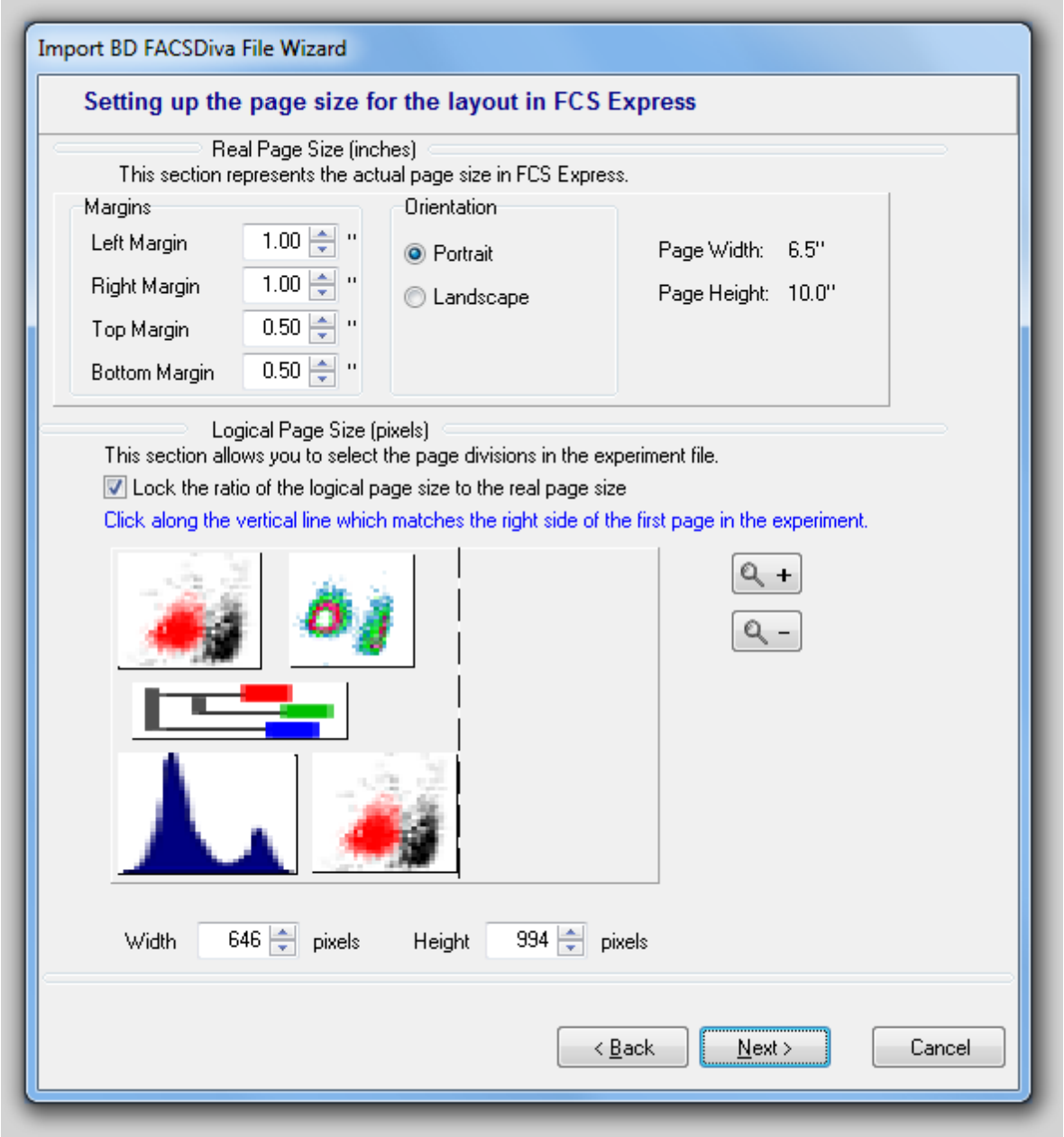

Figure 24.4 Import FACSDiva File Wizard - Setting Page Size

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)**

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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 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 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 selected 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 a Data File** dialog will appear (Figure 24.5). 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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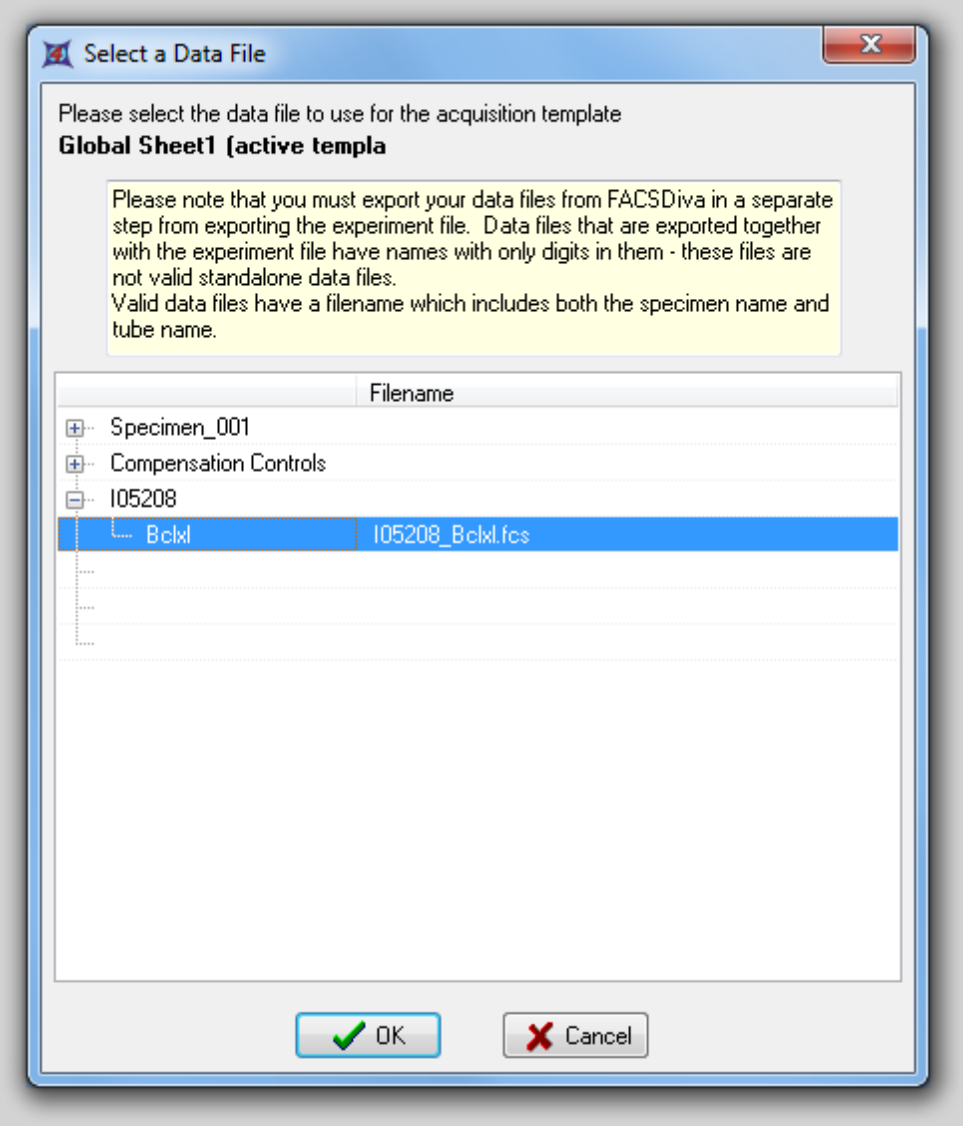

Figure 24.5 Selecting FCS File Data for an Imported FACSDiva Experiment

9. For each worksheet with an acquisition template you selected in Step 3, you will see a **Select a data file** dialog to load FCS data file.

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

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experiment will be recreated on your layout. Although each plot can be reformatted using standard FCS Express techniques[152], it is useful to remember that default format settings (i.e., fonts, axes labels, plot resolution, overlay settings, etc.) can be customized before a plot is created, using the Edit Preferences dialog[117]. To insure that imported plots are displayed in the manner that you prefer, it is recommended that you take advantage of the default preferences options before you import FACSDiva experiments.

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 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[219].

23.2 Accuri CFlow Files

The BD Accuri ™ C6 is a bench-top system that delivers performance and features similar to those of the market-leading flow cytometers, yet the instrument 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.

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- 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.

23.2.1 How to Import CFlow Experiment Files

To import a CFlow experiment file:

1. Use the **Application Button→Open Layout→Import BD Accuri C6 File...** command.

2. Browse your directories to select the C6 experiment file (.c6) to import. Click **Load** when you have located it.

3. The **Import BD Accuri C6 File Wizard** appears, beginning with the **Import Selections** page (Figure 24.6). 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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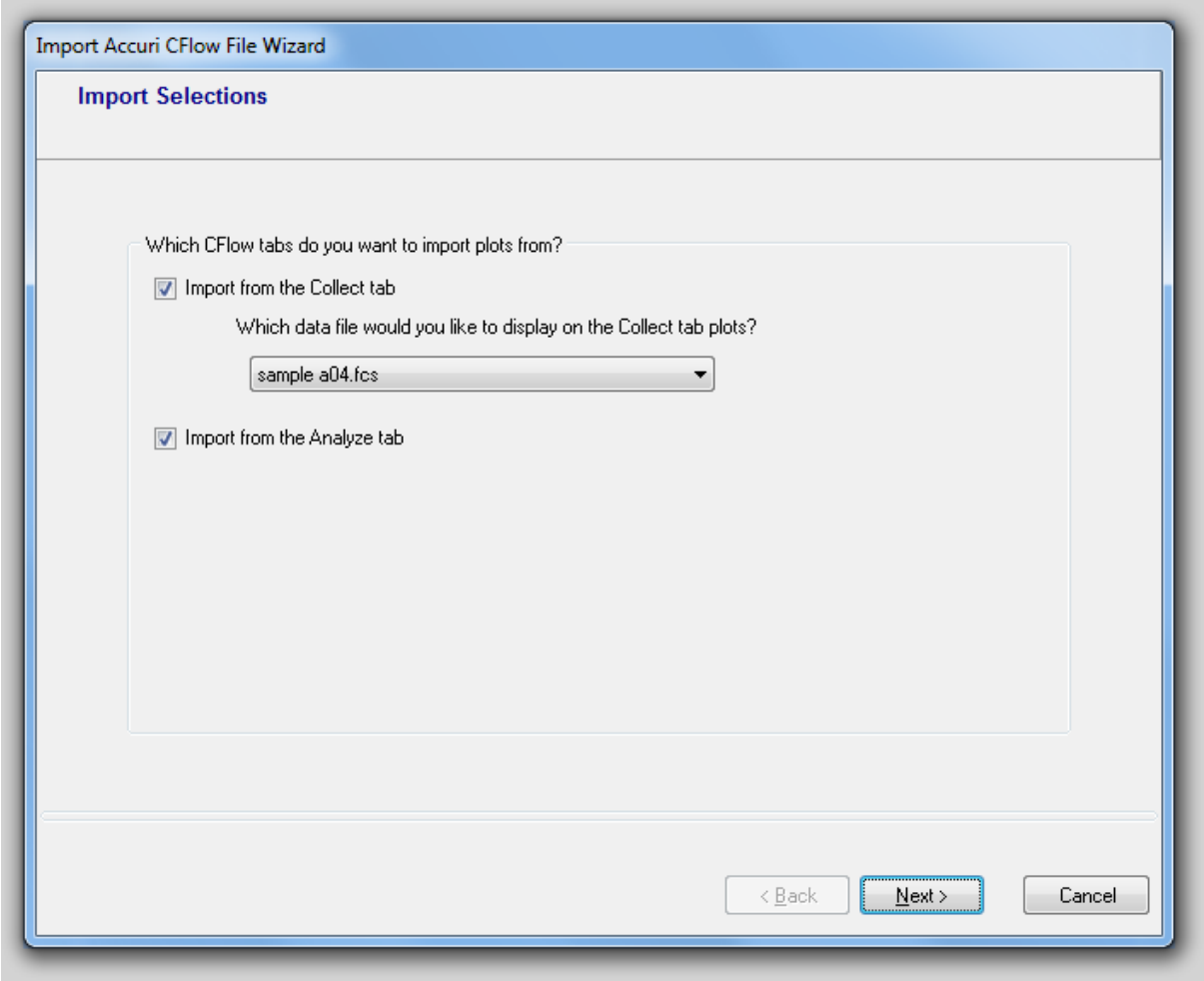

Figure 24.6 Import Accuri CFlow File Wizard - Import Selections

The **Page Layout Options** page (Figure 24.7) will appear. Here, you configure how the plots from the C6 experiment will be imported into FCS Express.

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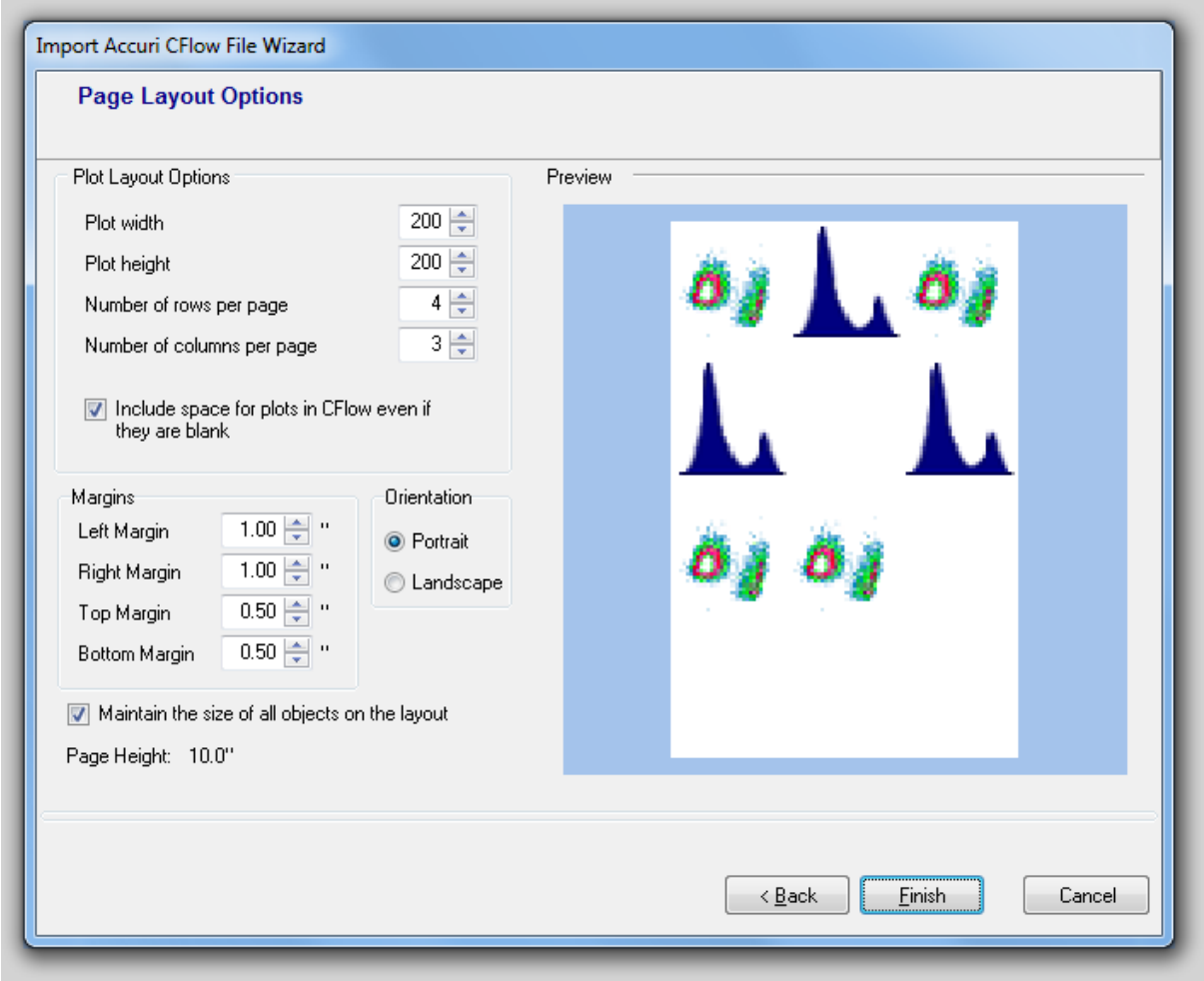

Figure 24.7 Import Accuri CFlow File Wizard - Page Layout Options

6. On the left you can set the **Plot width**, **Plot height**, **Number of rows per page**, and **Number of columns per page** using the spin controls, along with the margins and orientation of the 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:

- Recommended settings in FCS Express to help improve performance when importing BD Accuri C6 experiments.

  1. Open the **Edit Preferences** dialog using the Application Button→Edit

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Preferences Button[117] command.

2. Select the **Data Load→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.

3. Uncheck **Validate that files are FCS Files before displaying them in a file dialog**. This change, while not necessary for loading a C6 data file, will speed up adding files into the file list.

- Loading CFlow data into FCS plots.

1. Insert a plot, just as you would with any other data file using the commands from the Insert Tab. When the Select a Data File dialog opens, change the file type to **C6 files (*.c6; *.cfl)**.

2. Navigate to the CFlow 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**.

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Chapter

24 DNA Analysis

FCS Express provides a complete DNA analysis solution based upon the widely used MultiCycle™ DNA analysis package written by Dr. Peter Rabinovich 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 4. 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 if there is more than one cycle present, 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 must be purchased as an add-on. Multicycle can be purchased from Phoenix Flow Systems (http://www.phoenixflow.com) or from De Novo Software.

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.

- Rabinovitch, P.S. Practical considerations for DNA content and cell cycle

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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.

Use the **Insert→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 can be displayed when the standard histogram features are formatted (Figure 25.2).

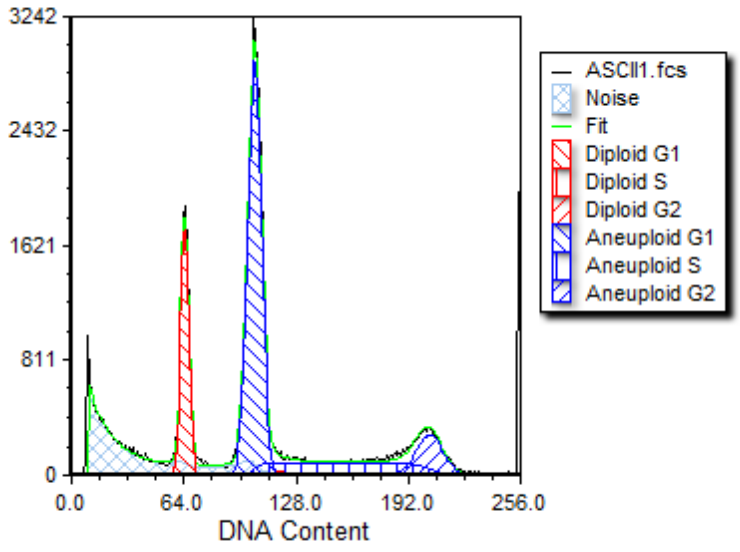

Figure 25.2 Multicycle DNA Histogram with Legend

FCS Express can fit DNA curves from both standard FCS list mode data files as well as uncorrelated FCS histogram files.

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24.3 Formatting a DNA Histogram

To format a Multicycle DNA histogram:

- Right-click on the histogram, select **Format** from the pop-up menu, click on the triangle next to **DNA**, and choose one of the four DNA-specific categories: General446, Cycle447, Background448, or Position Markers452.

The other categories which can be formatted are similar to the formatting options for standard histograms167.

24.3.1 General Options

The **General** sub-category of DNA formatting options contains propterties that control how the data on the DNA histogram is presented (Figure 25.3).

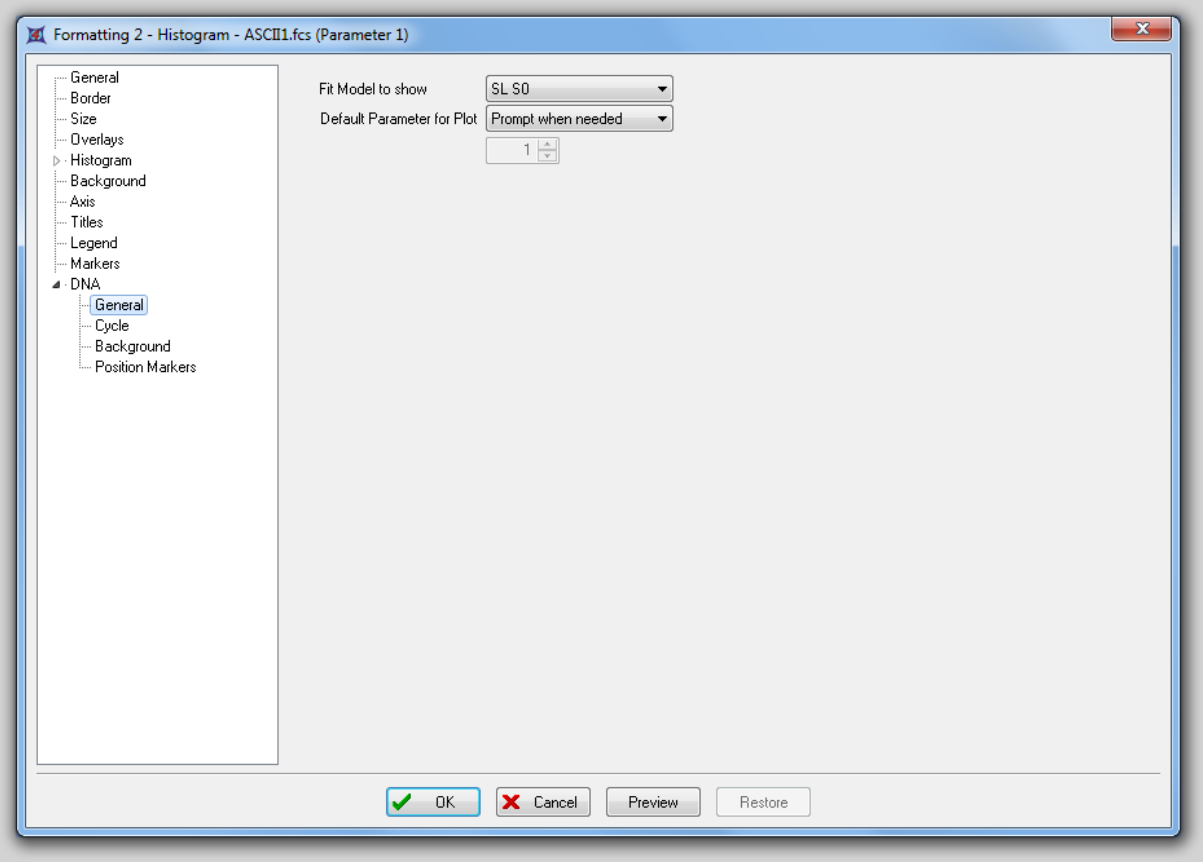

Figure 25.3 Formatting a DNA Histogram - General Options

The options are described in the table below.

Property name	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.
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.

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 become enabled (Figure 25.4).

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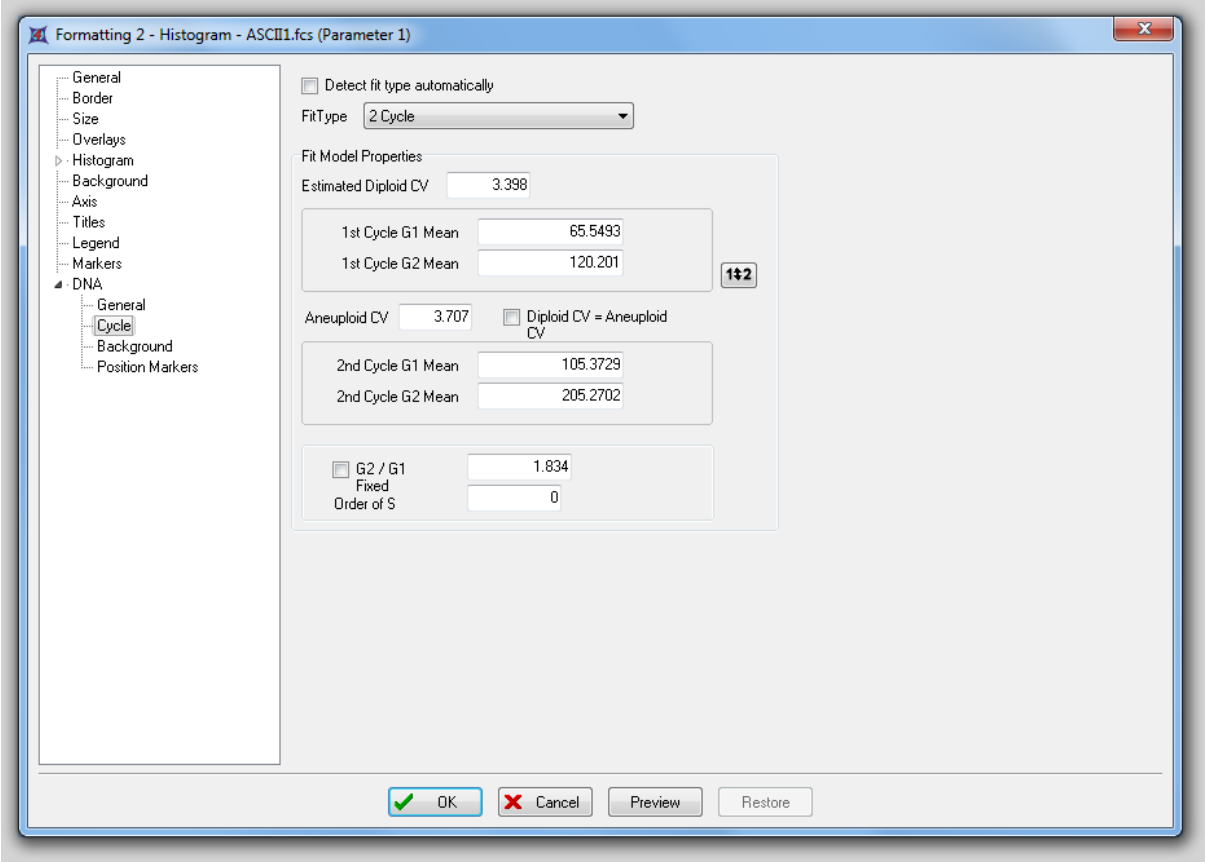

Figure 25.4 Formatting a DNA Histogram - Cycle Options

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. A complete listing of all the supported fit types and the various parameters that apply for those fits can be found in the Multicycle documentation.

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 455 or create Text Boxes with DNA specific tokens 457.

24.3.3 Background Options

The **Background** category (Figure 25.5) 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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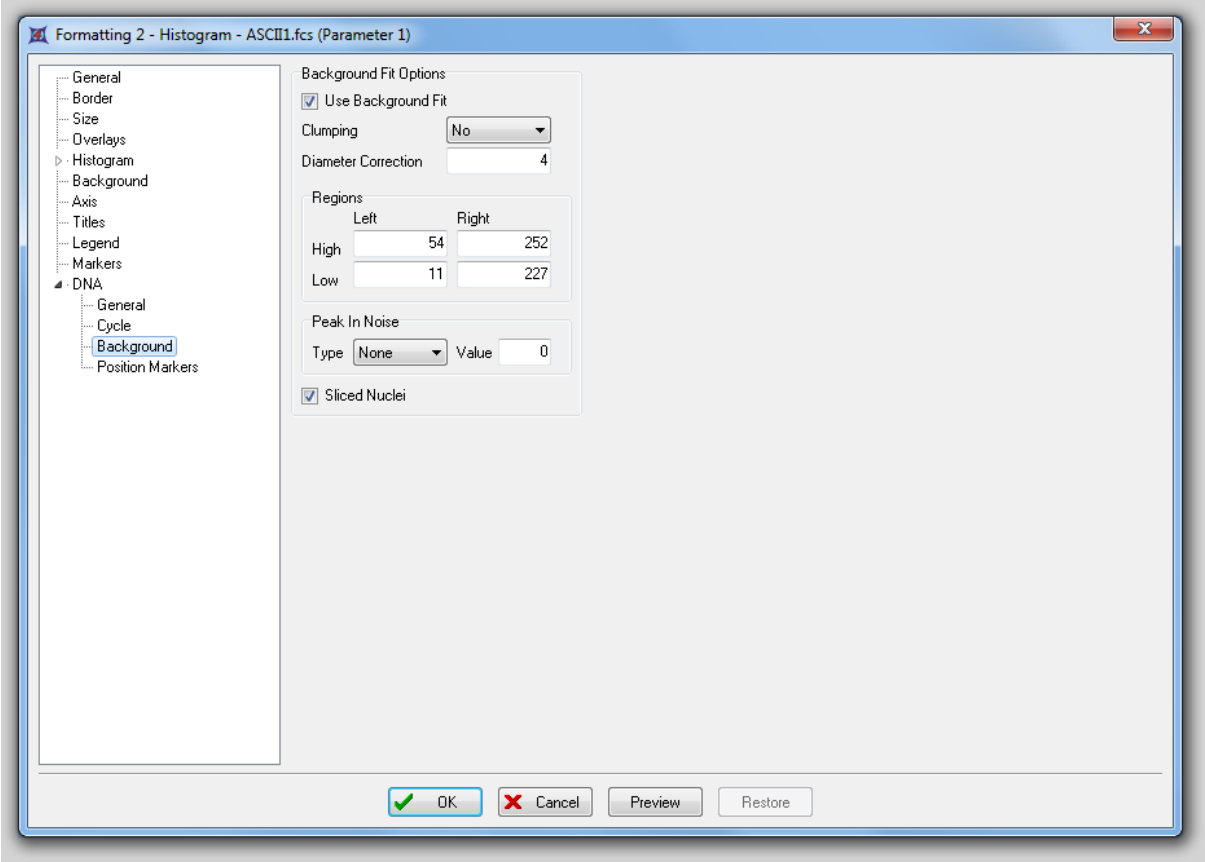

Figure 25.5 Formatting a DNA Histogram - Background Options

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.

24.4 Setting DNA Preferences

The DNA preferences 123 including general preferences, overlay preferences, and position marker preferences for performing DNA analysis using Multicycle 444 are described in the appropriate section of the chapter on formatting objects 123.

24.5 The Multicycle Tab

Multicycle can automatically fit and analyze cell cycle histograms with multiple models simultaneously. You can control the number and type of populations to fit, the currently displayed model, and the background options using the Multicycle Tab (Figure 25.6).

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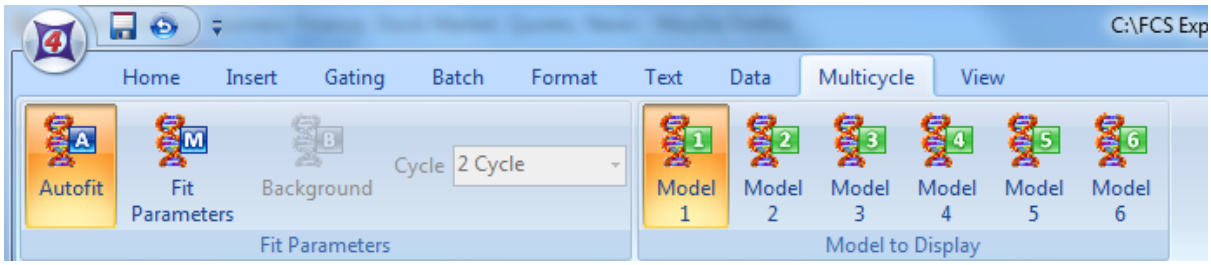

Figure 25.6 The Multicycle Tab

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 25.7). 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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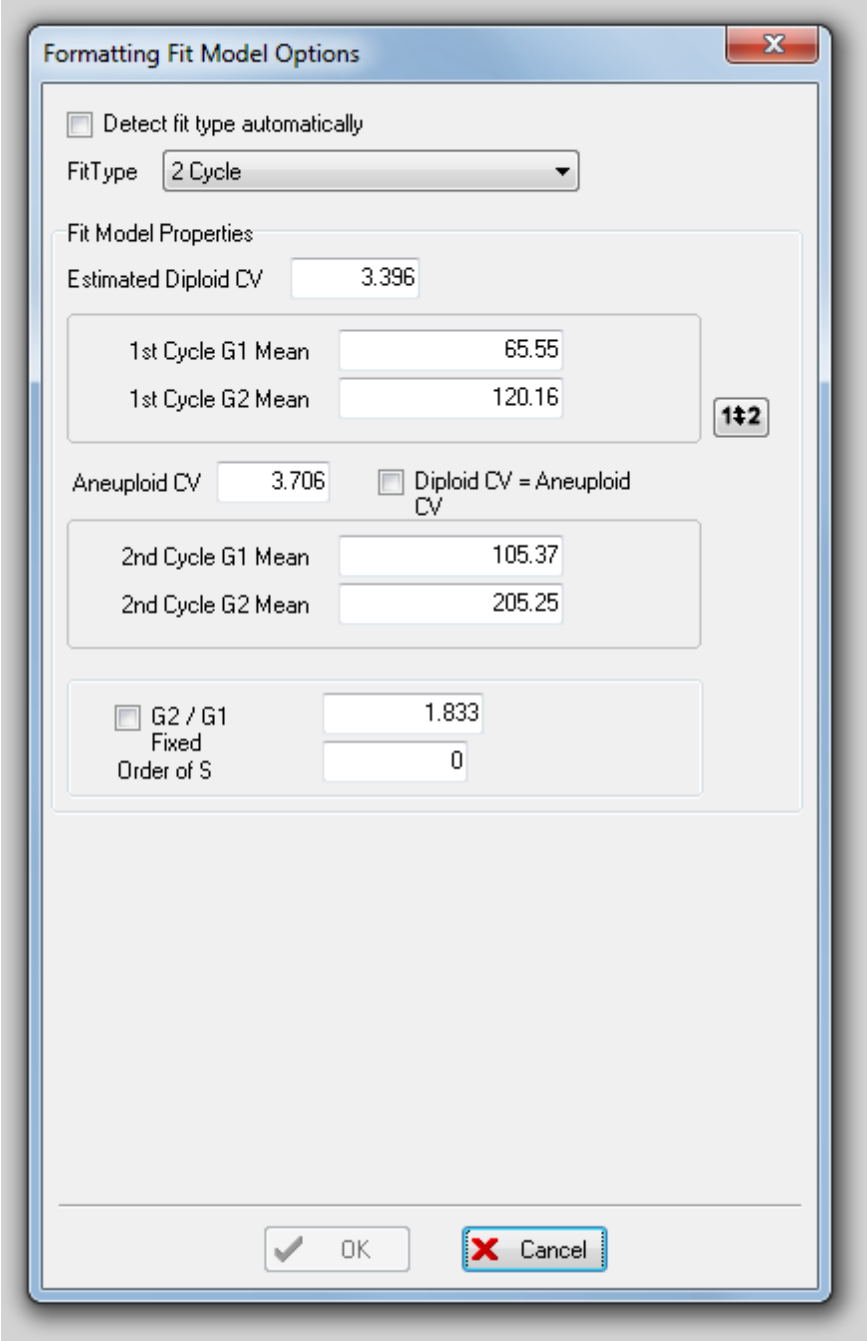

Figure 25.7 Formatting Fit Model Options Dialog

- To change background parameters, select the **Multicycle→Fit Parameters→Background** command to bring up the **Formatting Background Options** dialog (Figure 25.8). The parameters are described in the Multicycle manual.

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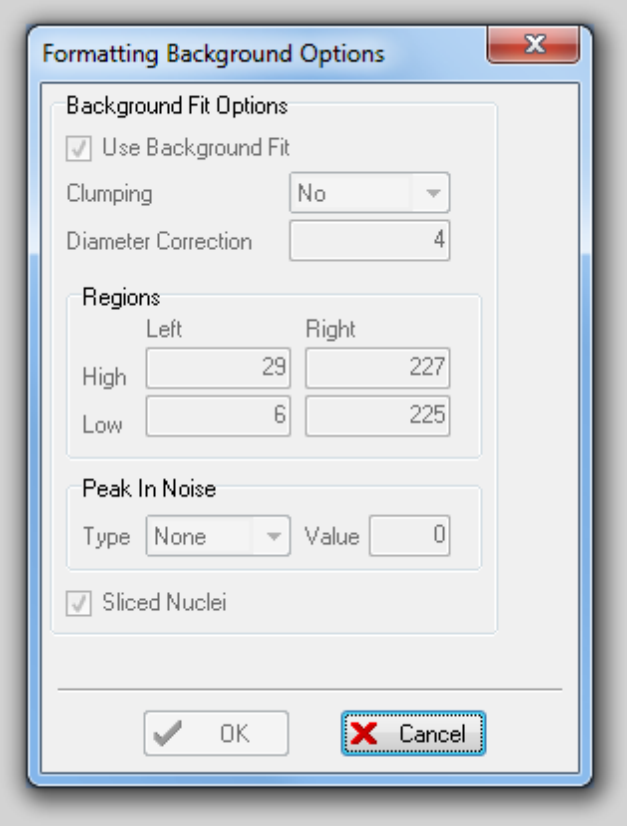

Figure 25.8 Formatting Background Options Dialog

24.6 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 25.9).

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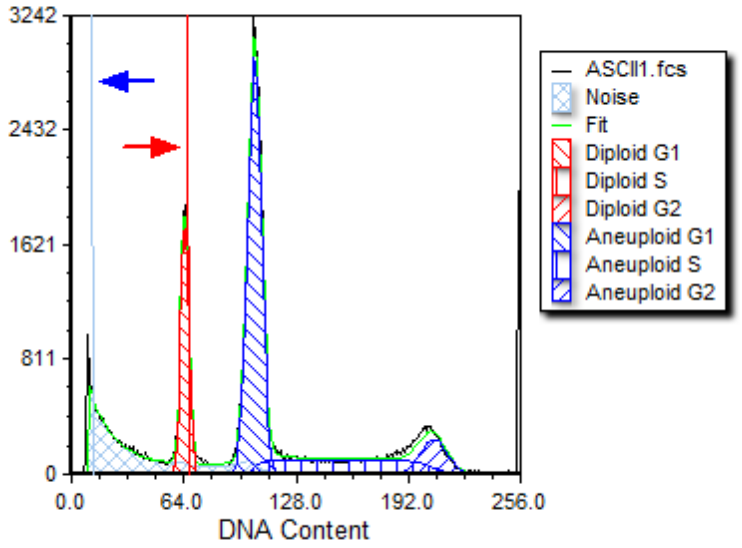

Figure 25.9 DNA Histogram showing Background Region Low (blue) and G1 Mean (red) Position Markers

You can show or hide a position marker by right-clicking on the DNA Histogram, selecting **DNA Position Markers** from the pop-up menu, and choosing the appropriate position marker from the submenu (Figure 25.10). Select a marker to show it and deselect the marker to hide it.

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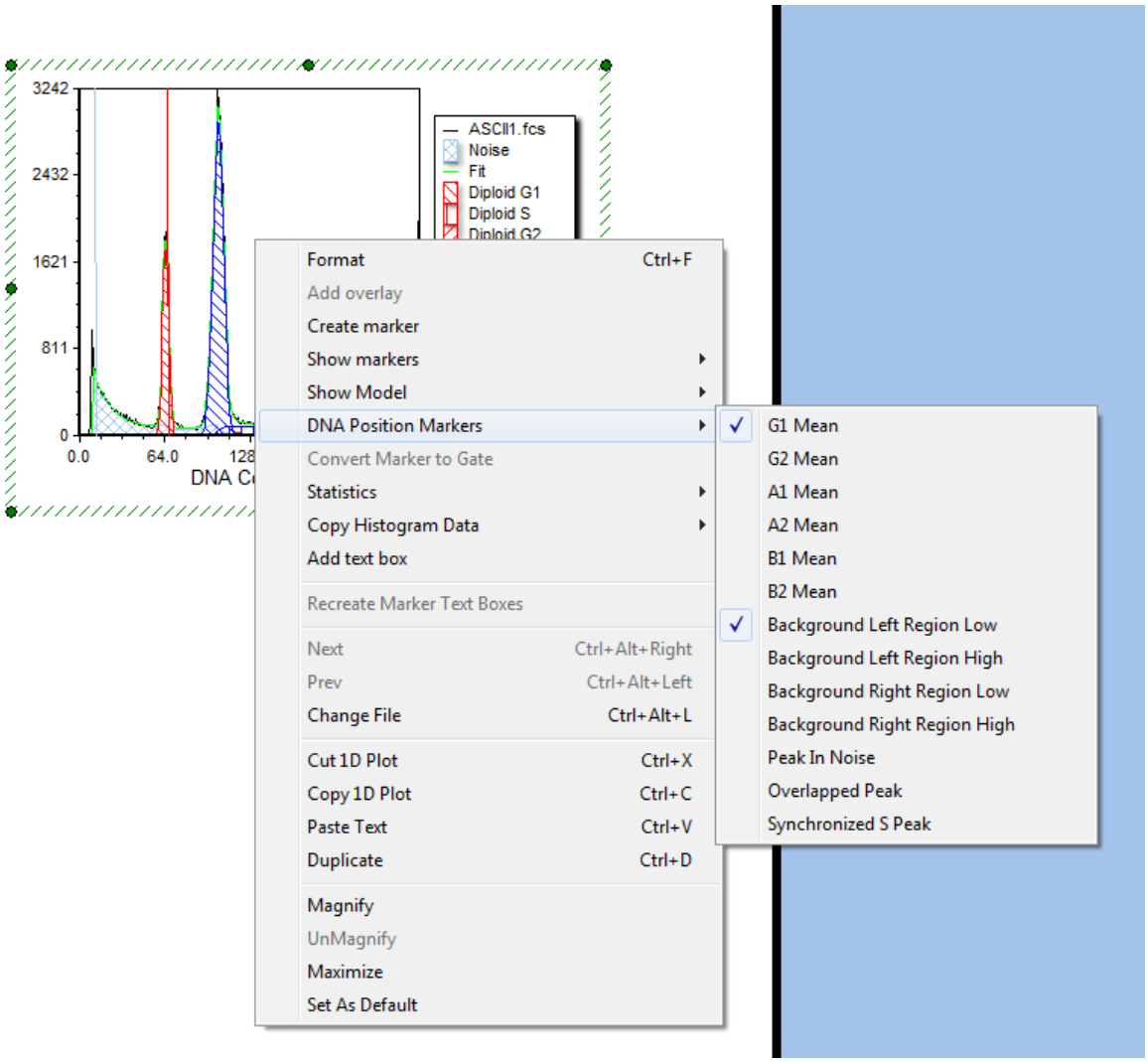

Figure 25.10 Showing or Hiding DNA Position Markers

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, click on the triangle next to **DNA**, and choose the **Position Markers** page (Figure 25.11). 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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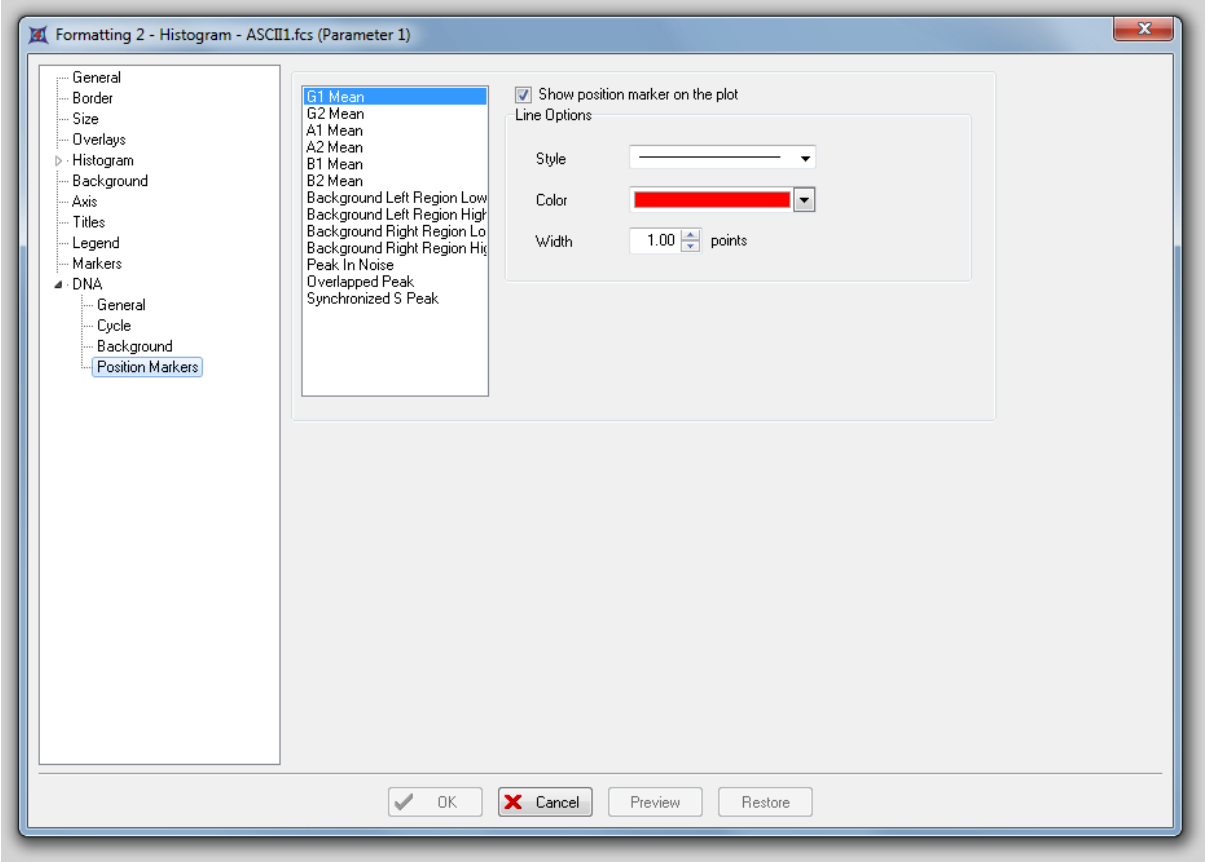

Figure 25.11 Formatting a DNA histogram - Position Markers

24.7 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 25.12).

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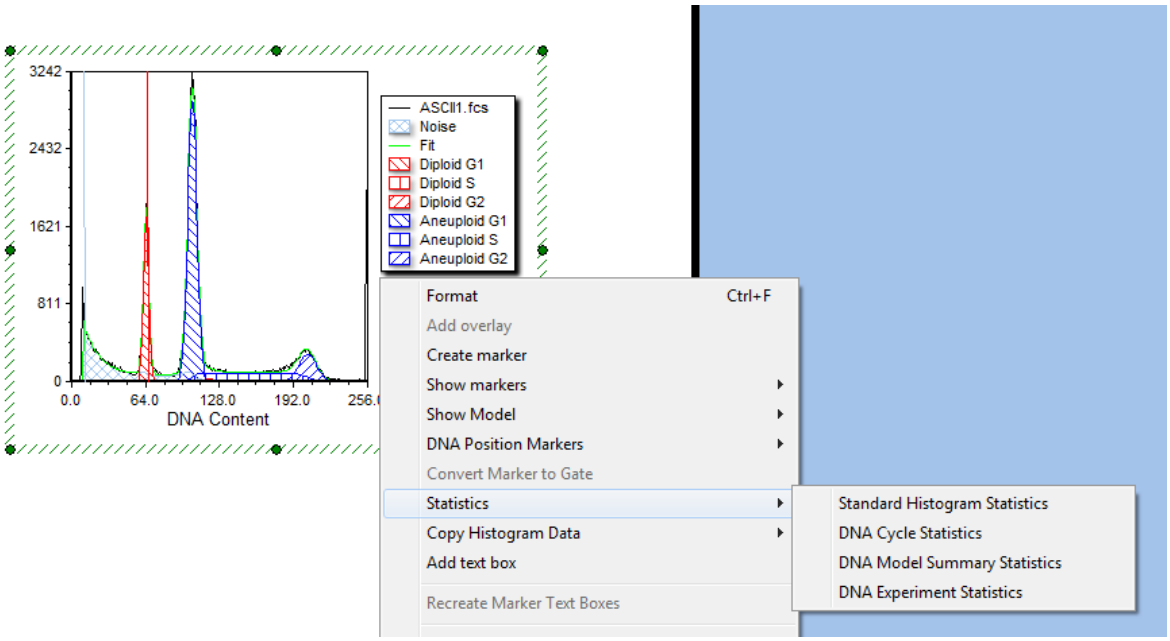

Figure 25.12 Choosing DNA Statistics

The first choice, **Standard Histogram Statistics**, represents the statistics available for non-DNA histograms 215.

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 25.13 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	65.55	3.40	91.60	120.16	3.41	2.43	5.97	1.83	19.99	n/a	8.38
Aneuploid A	105.37	3.71	66.75	205.25	3.71	12.60	20.65	1.95	80.02	1.61	5.58

Figure 25.13 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 25.14).

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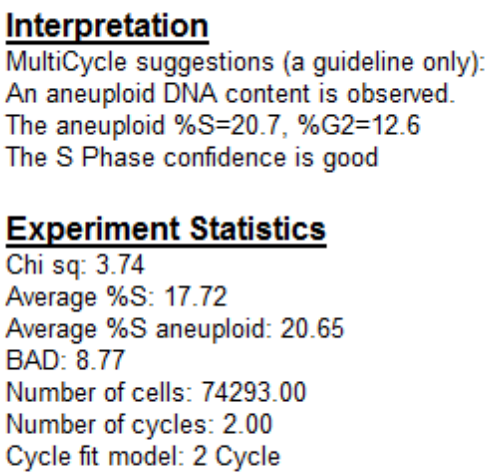

Figure 25.14 DNA Interpretation and Experiment Statistics

24.8 DNA-Specific Tokens

All of the DNA statistics can be used as tokens 326. DNA tokens can be inserted into text boxes 328, used in Custom Token 348 or Alert formulas, and exported to reports during batch processing 412.

To insert a DNA 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 DNA Histogram as a **Data Source**.
5. Open the **Statistic** page.
6. Choose a DNA statistic 455 from the **Select a Statistic** drop-down list. If you choose DNA Cycle Statistics, the dialog will look like Figure 25.15. 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.

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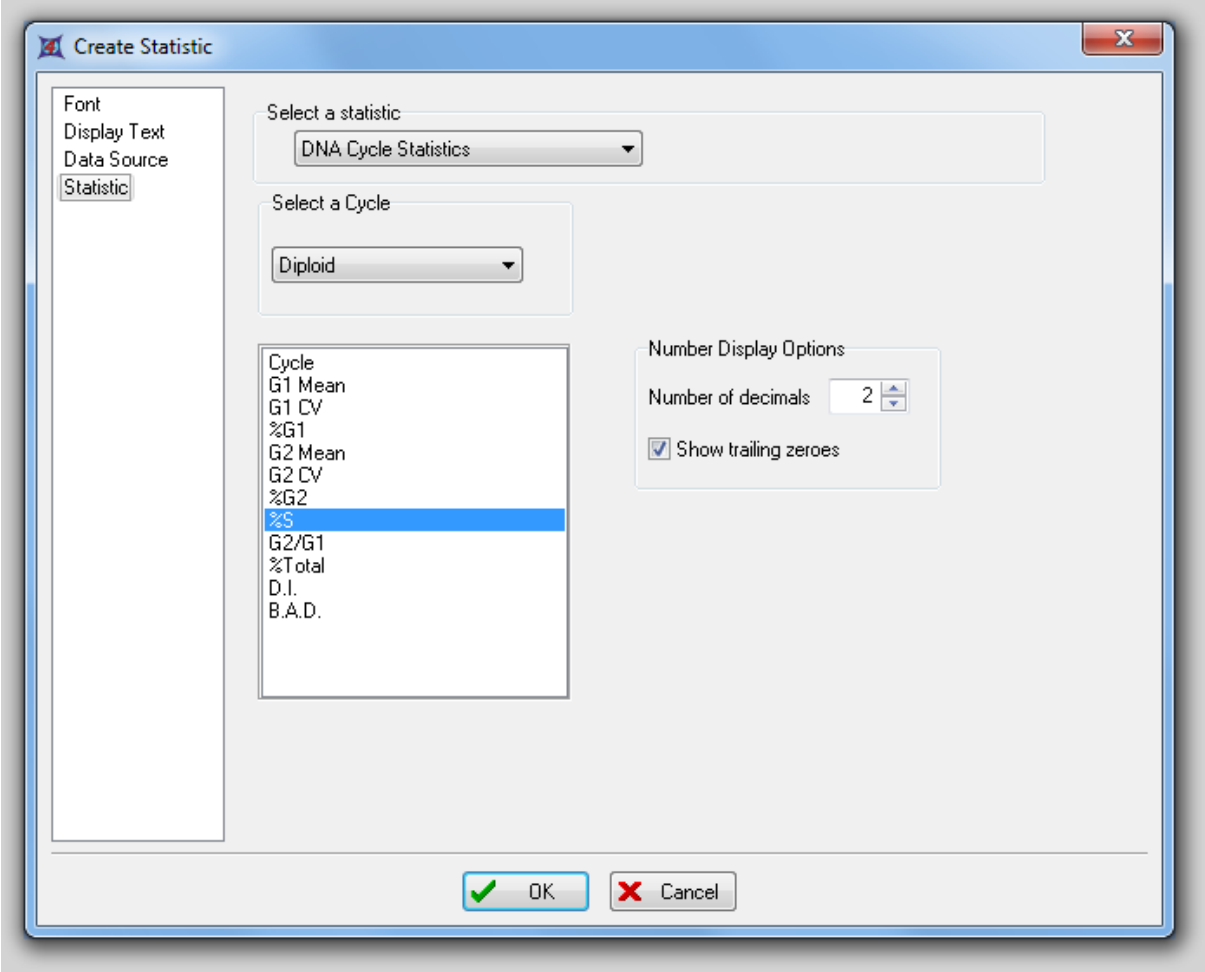

Figure 25.15 Create Statistics Dialog - DNA Cycle Statistics

24.9 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.

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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 Overlay Peak	This cycle is performed when there is a Diploid peak that does not contain a full cell cycle.
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 6 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 6 different models.

Model	Dip %G2	Dip %S	BAD	Chis
SL S0	2.00	2.08	0.65	2.06
SL CL S0	0.08	1.85	4.33	1.98
+G2/G1 Fixed	2.00	2.07	0.65	2.06
+Aggregates	0.00	1.78	3.99	1.97
+S Order = 1	2.32	3.01	0.65	1.89
+CVs Fixed	2.29	2.99	0.65	1.89

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

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	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-chosed fitting model.
+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 zero order S, 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 Knowledge Base 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#ModelErrors.

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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 allows for easy determination of the fraction of proliferating cells, as well as

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other derived statistics.

The Cell Proliferation Analysis add-on module is included with any Professional license of FCS Express 4 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[158] 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[1105] for further information.

To insert a Proliferation histogram, use the **Insert→1D Plots→Proliferation** command. If necessary, change the x-axis parameter[188] to reflect the CFSE fluorescence.

An example of a Proliferation histogram is shown in Figure 26.2.

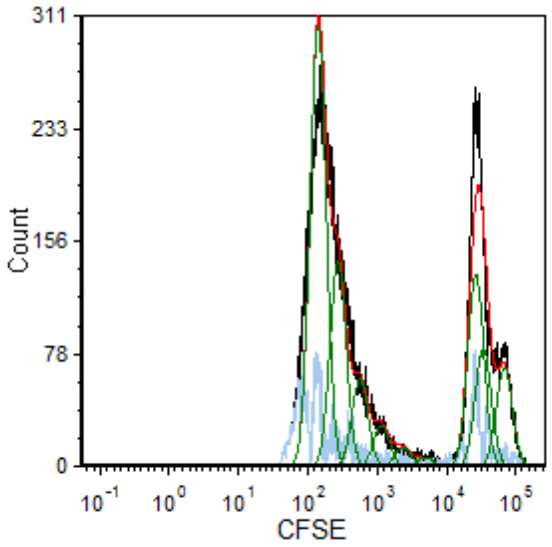

Figure 26.2 Proliferation Histogram

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 167, Proliferation Histograms have two additional property categories which can be formatted: Fit Parameters 464 and Proliferation Position Markers 466.

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 26.3).

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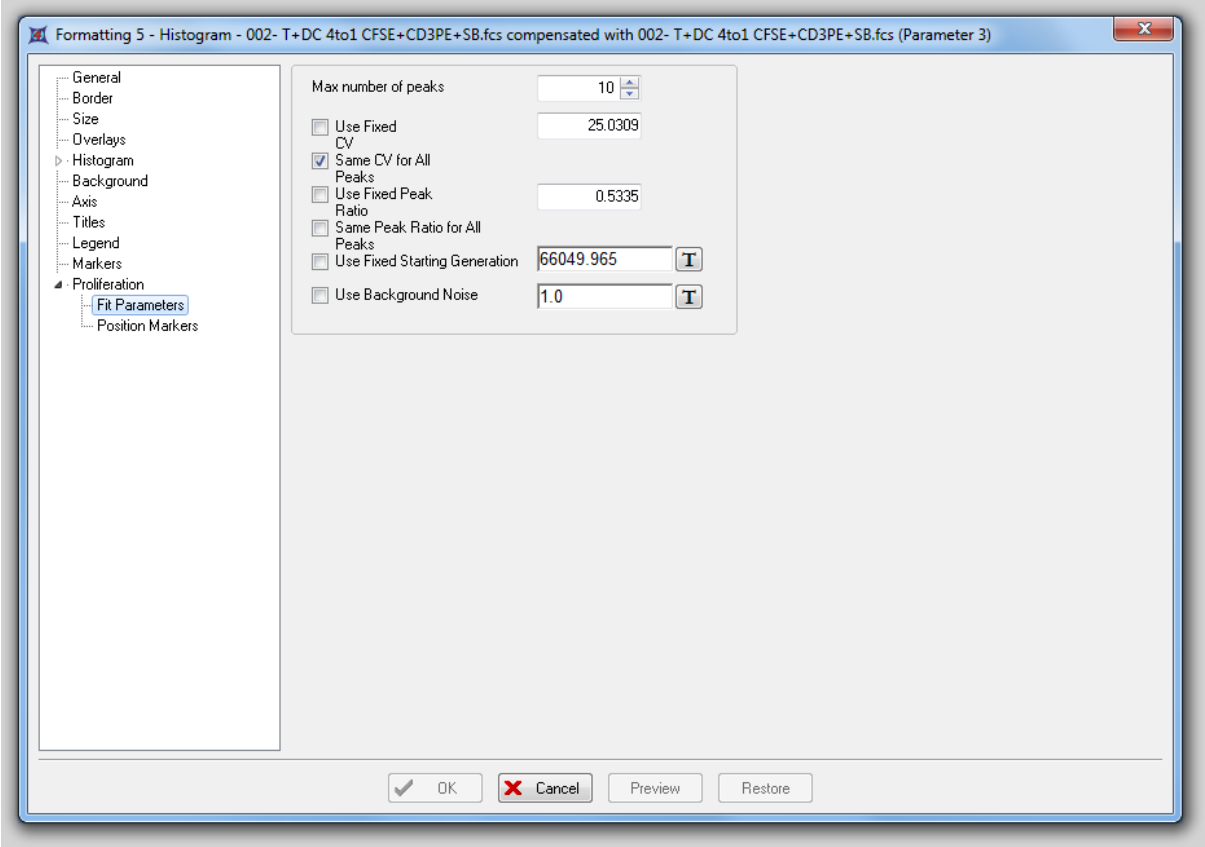

Figure 26.3 Formatting Proliferation Fit Parameters

On the **Fit Parameters** page, you can modify a number of properties related to the fit algorithm, including:

- Choose the **Maximum number of peaks** to model.
- Decide whether or not to use a **Fixed CV** and/or the **Same CV for All Peaks**.
- Use a **Fixed Peak Ratio** and/or the **Same Peak Ratio for All Peaks**.
- Set parameters for a **Fixed Starting Generation** and/or **Background Noise**.

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.

- 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 467.

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.

Select the **Undivided cells peak** and/or **Background** properties from the list box on the left (Figure 26.4) and choose line options for the selected markers on the right.

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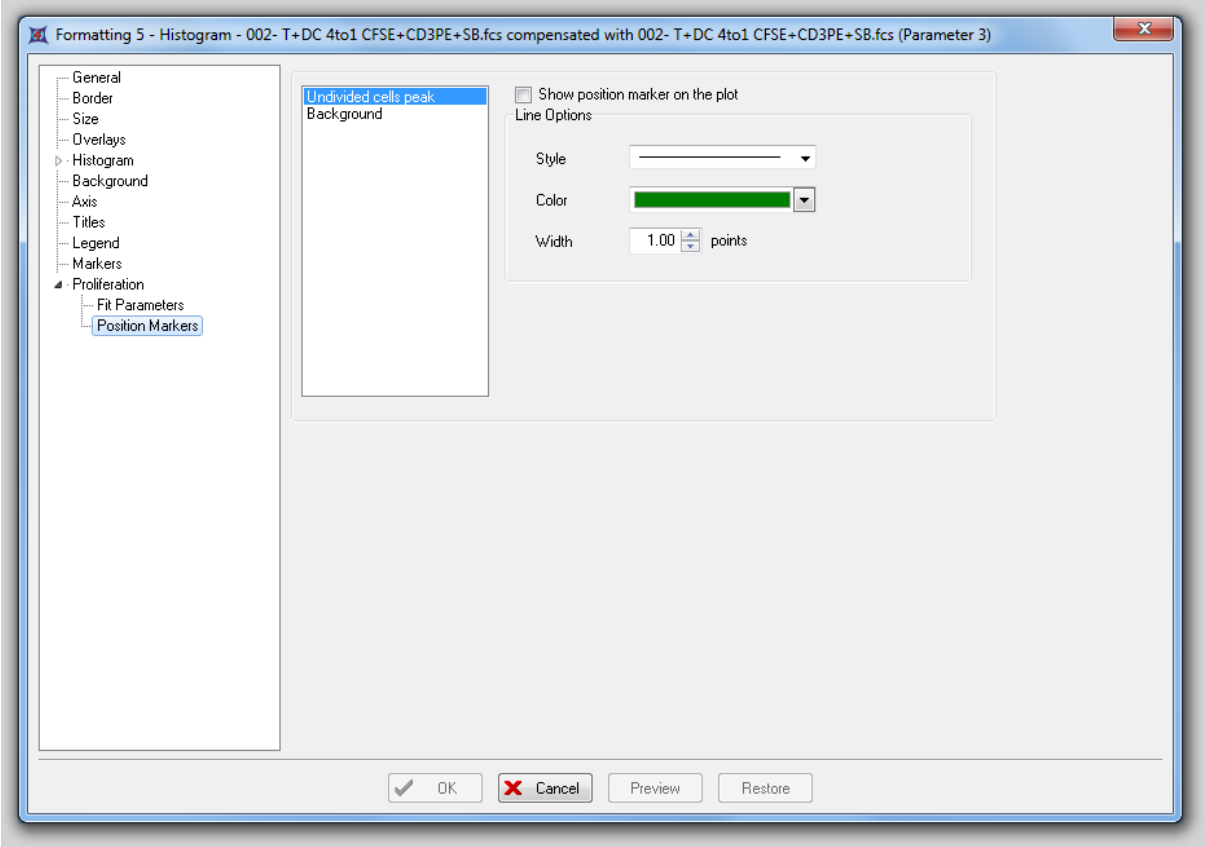

Figure 26.4 Formatting Proliferation Position Markers

25.4 Proliferation Statistics

When performing a Proliferation Analysis, FCS Express automatically calculates several proliferation fit[468] and proliferation population[468] statistics from your data. To change user preferences for which of these statistics are displayed, refer to the Proliferation Fit Statistics preferences[131] and Proliferation Population Statistics preferences[132] sections of the Statistic Preferences[126] subchapter of Formatting Objects[116].

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 26.5).

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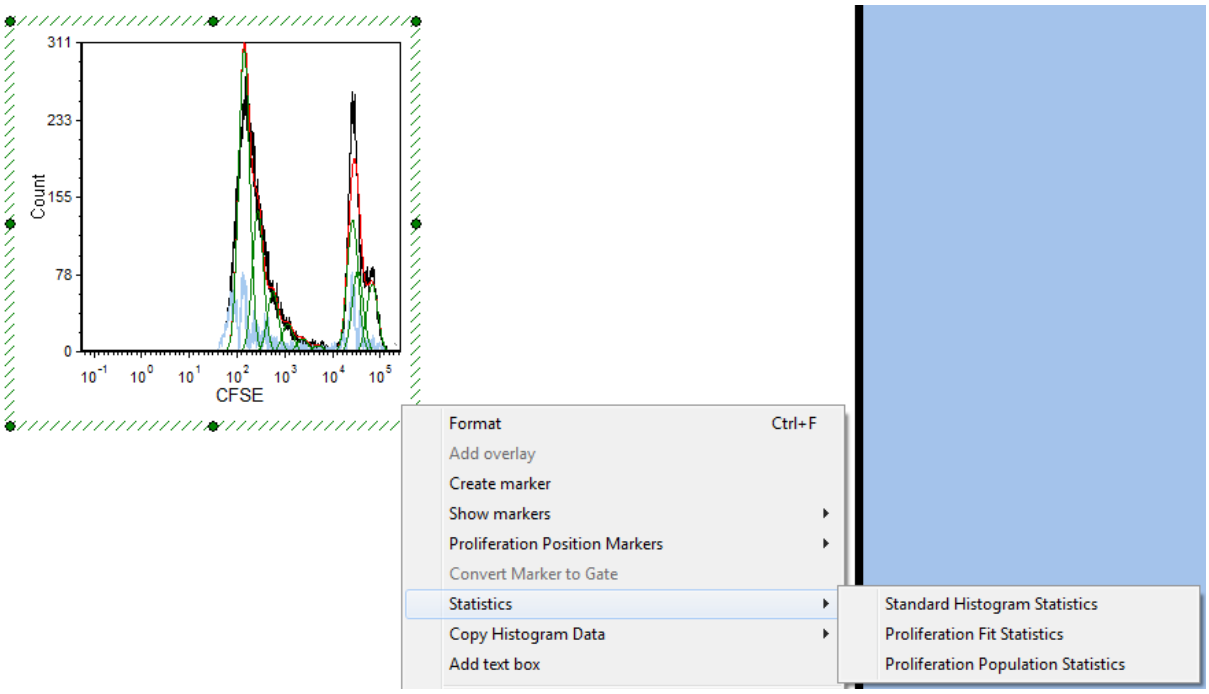

Figure 26.5 Choosing Proliferation Statistics

The first choice, **Standard Histogram Statistics**, represents the statistics available for non-Proliferation histograms[215].

Proliferation Fit Statistics[131] describe the fitted data. Figure 26.6 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
10.04	5.80	53.10	0.53	36882.00	6326.36	16.22	63.68	66049.97	0.00

Figure 26.6 Proliferation Fit Statistics

Proliferation Population Statistics[132] pertain to the multiple generations that have proliferated during the experiment (Figure 26.7). The statistic equations that are calculated are shown in the table below.

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Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells
Undivided Cells	66049.97	68.00	25.03	2.68	0.00	2967.00
Generation 1	32661.17	81.00	25.03	2.82	0.50	3560.00
Generation 2	25698.96	132.00	25.03	2.88	0.78	5827.00
Generation 3	0.00	0.00	0.00	0.00	0.00	0.00
Generation 4	4519.33	6.00	25.03	3.30	0.00	238.00
Generation 5	2136.54	13.00	25.03	3.53	0.48	562.00
Generation 6	1105.08	28.00	25.03	3.78	0.49	1216.00
Generation 7	546.45	60.00	25.03	4.08	0.49	2623.00
Generation 8	270.22	142.00	25.03	4.42	0.48	6254.00
Generation 9	139.76	304.00	25.03	4.80	0.51	13438.00

Figure 26.7 Proliferation Population Statistics

Proliferation Statistics Table. *P* is the total number of peaks found (where P_0 is the undivided generation). N is the number of cells in a generation. All statistics below assume that no cell death occurred. μ is the mean of a population.

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.
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.

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% Divided	$\frac{\sum_{i=1}^{P-1} \frac{N_i}{2^i}}{\text{\# original Cells}}$	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).
RMS Error	$\frac{\sum_{i=0}^{R-1} \left(O_{(i)} - F_{(i)}\right)^2}{\text{Degrees of Freedom}}$	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.

25.5 Proliferation-Specific Tokens

All of the Proliferation statistics can be used as tokens[326]. Proliferation tokens can be inserted into text boxes[328], used in Custom Token[348] or Alert formulas, and exported to reports during batch processing[412].

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**.

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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** page.
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 26.8. Choose a population from the **Select Population** drop-down list, a statistic from the list box, and click **OK**.

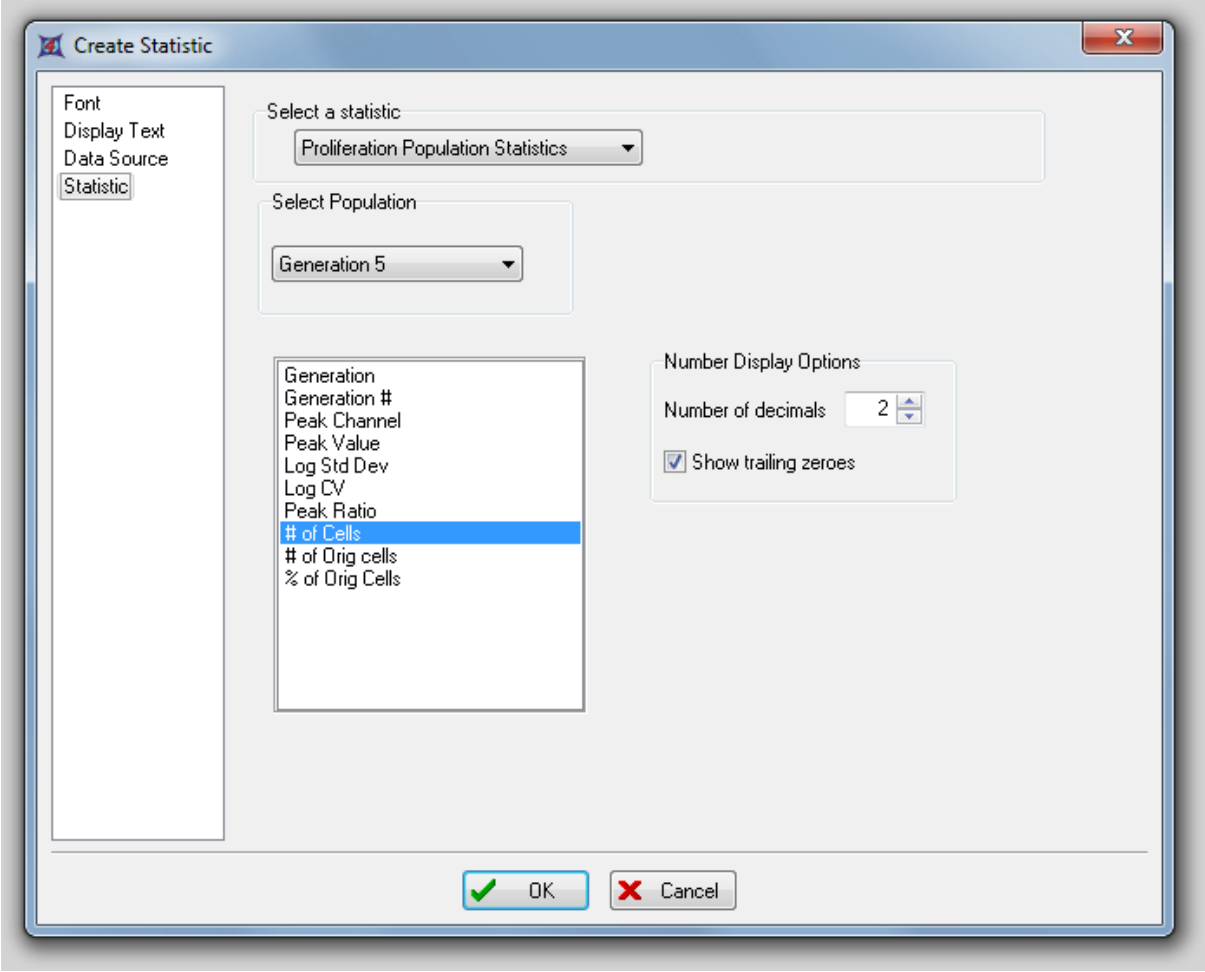

Figure 26.8 Choosing a Proliferation Population Statistic Token

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Chapter

26 Kinetics Analysis

Calcium ions play an important role in cell signalling. 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 27.2.

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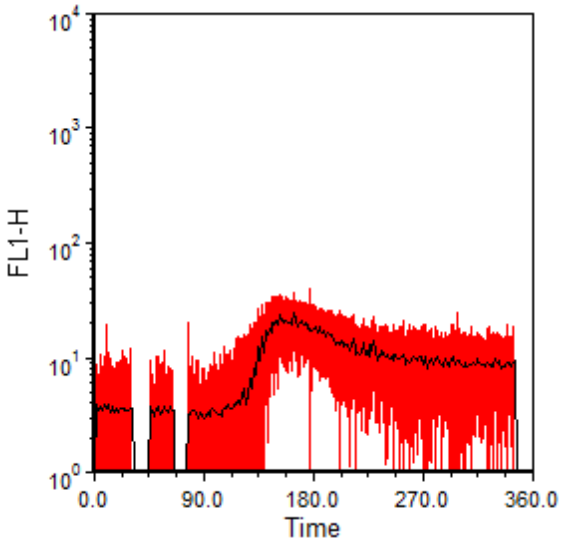

Figure 27.2 Kinetics Histogram (Median FL1-H vs Time). Standard Deviation shown in red.

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 27.3).

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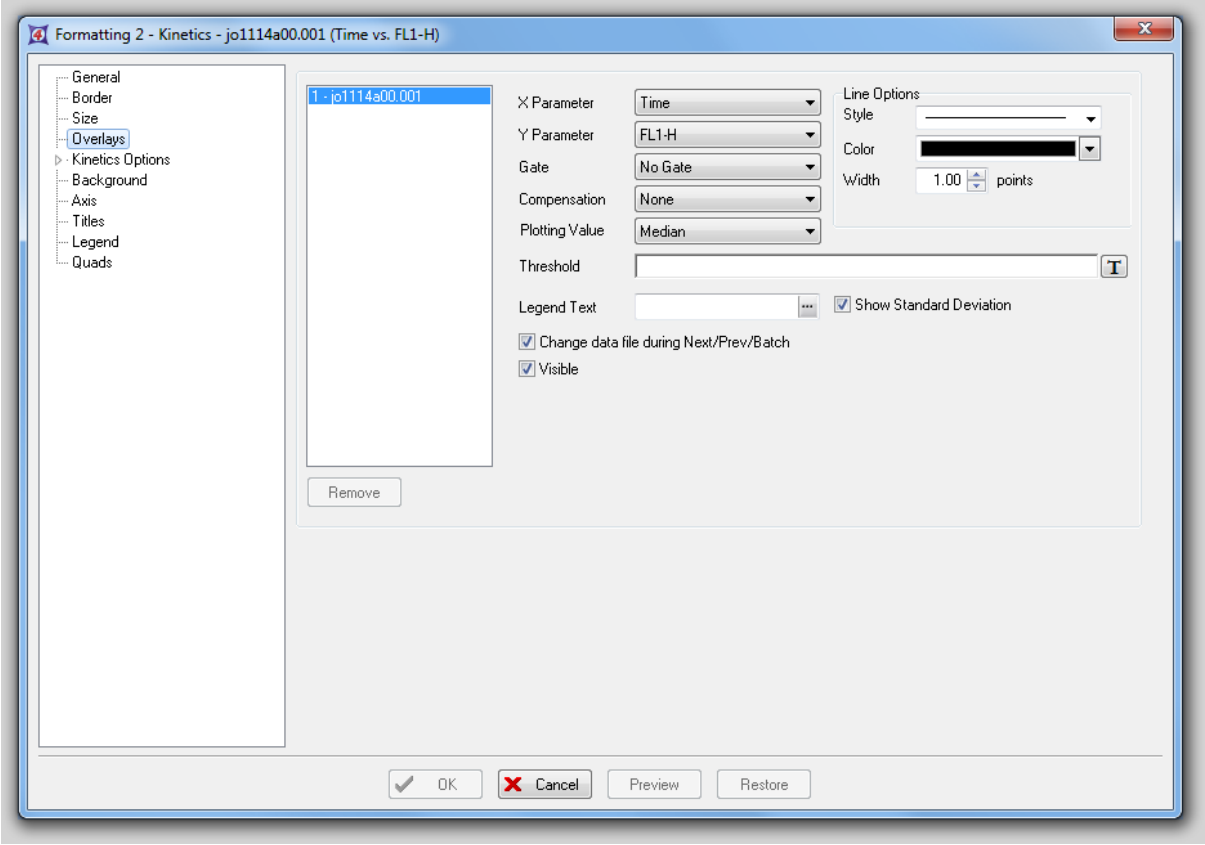

Figure 27.3 Formatting Kinetics Overlays

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 27.4) 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 340.

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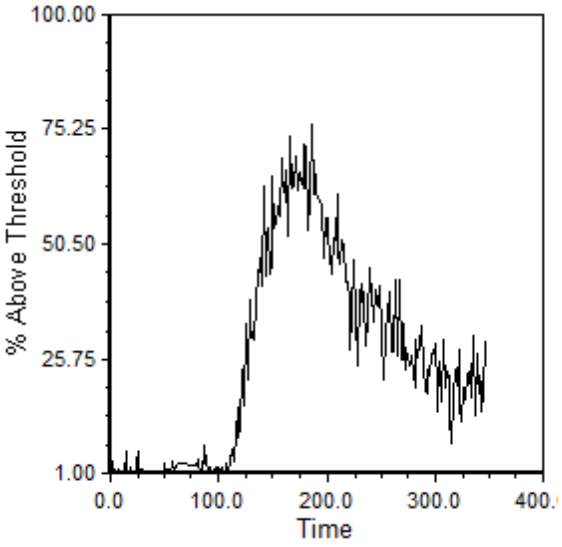

Figure 27.4 Plotting the % Above Threshold Value

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

In order to calculate and display Kinetics statistics, you must create a 1D gate on the Time parameter of the Kinetics plot. The 1D gate can be set in one of the following ways:

- Select the **Gating** (or **Home**)→**Create Gates**→**Create Marker** command.
- Right-click on the Kinetics plot and select **Create gate**→**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. Position the cursor where you want the right marker boundary to be, and release the mouse button.

After creating the 1D gate, to display statistics (Figure 27.5) right-click on the Kinetics plot and select **Kinetics Statistics** from the pop-up menu.

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Overlay Number	Overlay Legend	Gate	Start Time	End Time	Elapsed Time	Peak Value	Peak Time	Start to Peak Elapsed Time	Start to Peak Slope	Rising Phase baseline	Start Rising Phase	Rise to Peak Elapsed Time	Rise to Peak Slope
1.00	jo1114a00.001	Gate 2	79.12	153.33	74.21	7.02	149.79	70.67	0.04	112.69	112.69	37.10	0.06

Figure 27.5 Kinetics Statistics

The algorithm is designed to automatically detect the beginning of the rising phase, so the user does not always have to position the 1D gate right at the start of the rising phase. You can position the start marker after the point where the drug was added, and FCS Express will detect where the rising phase starts. The possible statistics to display are described in the table below.

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.
Start Time	The time at the left marker boundary.
End Time	The time at the right marker boundary.
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 start marker to the peak.
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.

See Formatting Statistics 218 to choose which statistics to display.

26.4 Kinetics-Specific Tokens

All of the Kinetics statistics can be used as tokens 326. Kinetics tokens can be inserted into text boxes 328, used in Custom Token 348 or Alert formulas, and exported to reports during batch processing 412.

To insert a Kinetics statistics token into a text box:

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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. Open the **Statistic** page.
6. Choose **Kinetics stats** from the **Select a Statistic** drop-down list (Figure 27.6). Choose a statistic from the list box, then click **OK**.

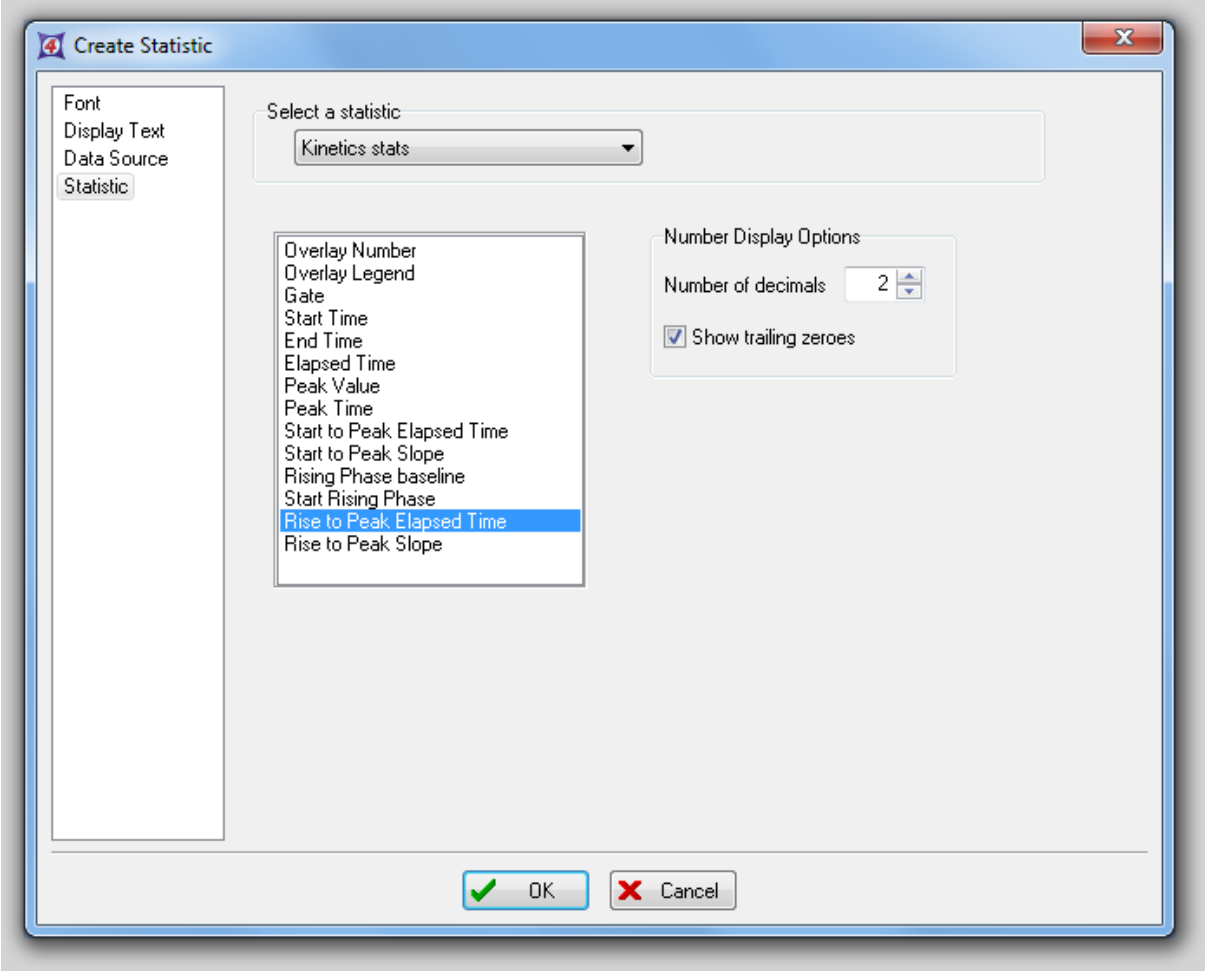

Figure 27.6 Creating a Kinetics Statistics Token

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Chapter

27 Heat Maps and Picture Plots

In high throughput laboratories, flow cytometry and cell imaging are often done in 96-well microtiter plates. The data from a 96-well microtiter plate normally contains 96 separate data files. FCS Express will automatically merge the files into one dataset and associate each "cell" with the particular well that it was located in.

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. By choosing an appropriate color scheme and statistic to display, you can get an overview of all of the wells in your experiment. This enables rapid visualization of outliers by comparing the statistical data in an individual well to the statistical data of the entire microtiter plate. Note that the heat map feature is an extra add-on that must be purchased. Please see our Purchasing Webpage for more details.

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.

27.1 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.

If you are loading a list of data files collected together in a microtiter plate, or a BD Accuri C6 file as a plate, choose **FCS Plate folder** from the **Files of type** drop-down list in the Select a Data File 43 dialog. Then choose a folder that contains the files. The folder should contain an extra keywords file 314 to indicate the size of the plate.

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 484 is shown as the title of the y-axis. Each well displays a color according to the chosen color scheme 486 (Figure 28.2).

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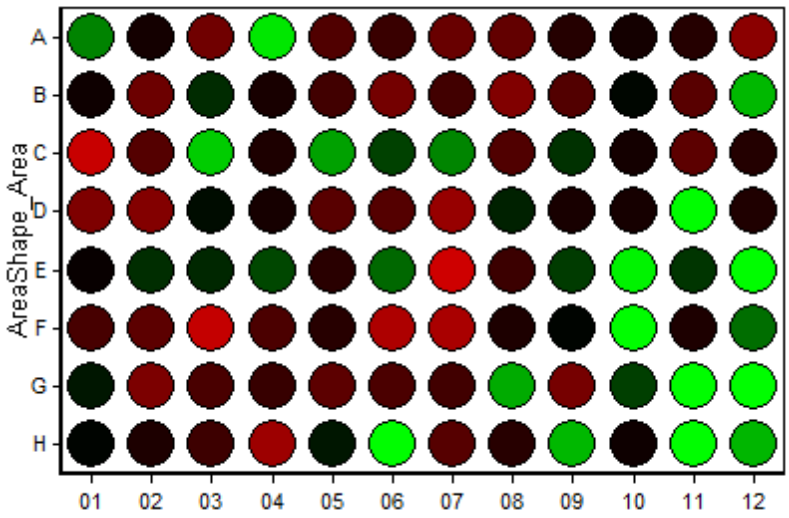

Figure 28.2 Heat Map

When a **Data Grid** is displayed for the **Heat Map**, a **Well** column will be present showing the well where each cell is located (Figure 28.3).

	Cell #	Gates	AreaShape_Area	AreaShape_	Outlines	rawDNA	rawGFP	Well
▶	1		106.00	0.68				A01
	2		170.00	0.76				A01
	3		216.00	0.69				A01
	4		344.00	0.66				A01
	5		223.00	0.66				A01
	6		266.00	0.71				A01
	7		364.00	0.56				A01
	8		139.00	0.63				A01
	9		305.00	0.85				A01
	10		169.00	0.70				A01
	11		149.00	0.41				A01
	12		212.00	0.63				A01
	13		207.00	0.66				A01
	14		140.00	0.56				A01
	15		223.00	0.71				A01
	16		153.00	0.74				A01

Figure 28.3 Data Grid with Well Column

If you have a **Text Box** on a layout containing a **Heat Map**, you can insert a table displaying the actual values from each well. For a 96-well microtiter plate, this will insert

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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 28.4).

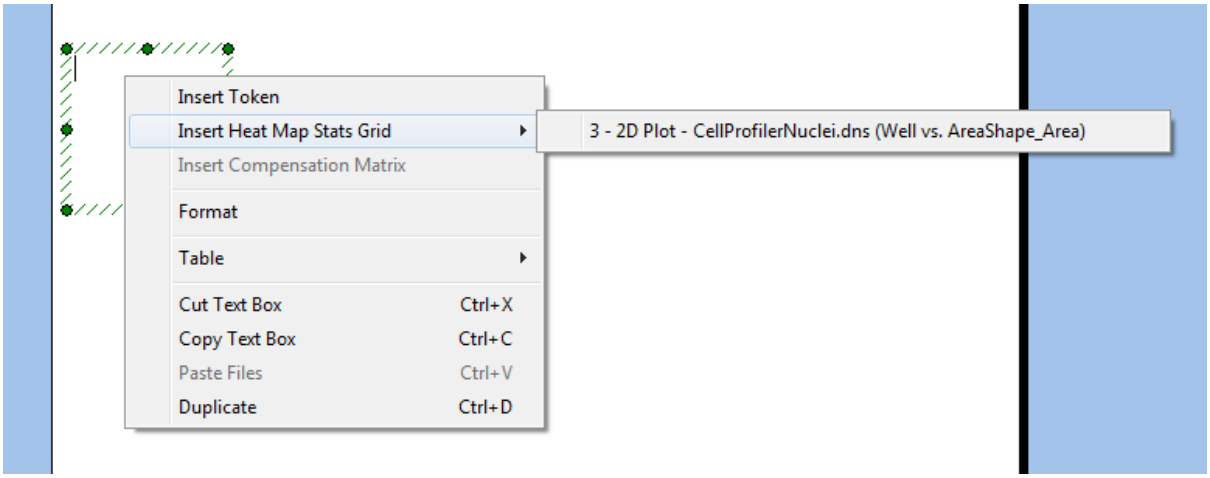

Figure 28.4 Insert Heat Map Data Grid into a Text Box

27.1.1 Setting the Parameter to Display

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→Plot Options→Overlays** command.
- Right-click on the **Heat Map**, select **Format** from the pop-up menu, then choose the **Overlays** category (Figure 28.5).
- 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.

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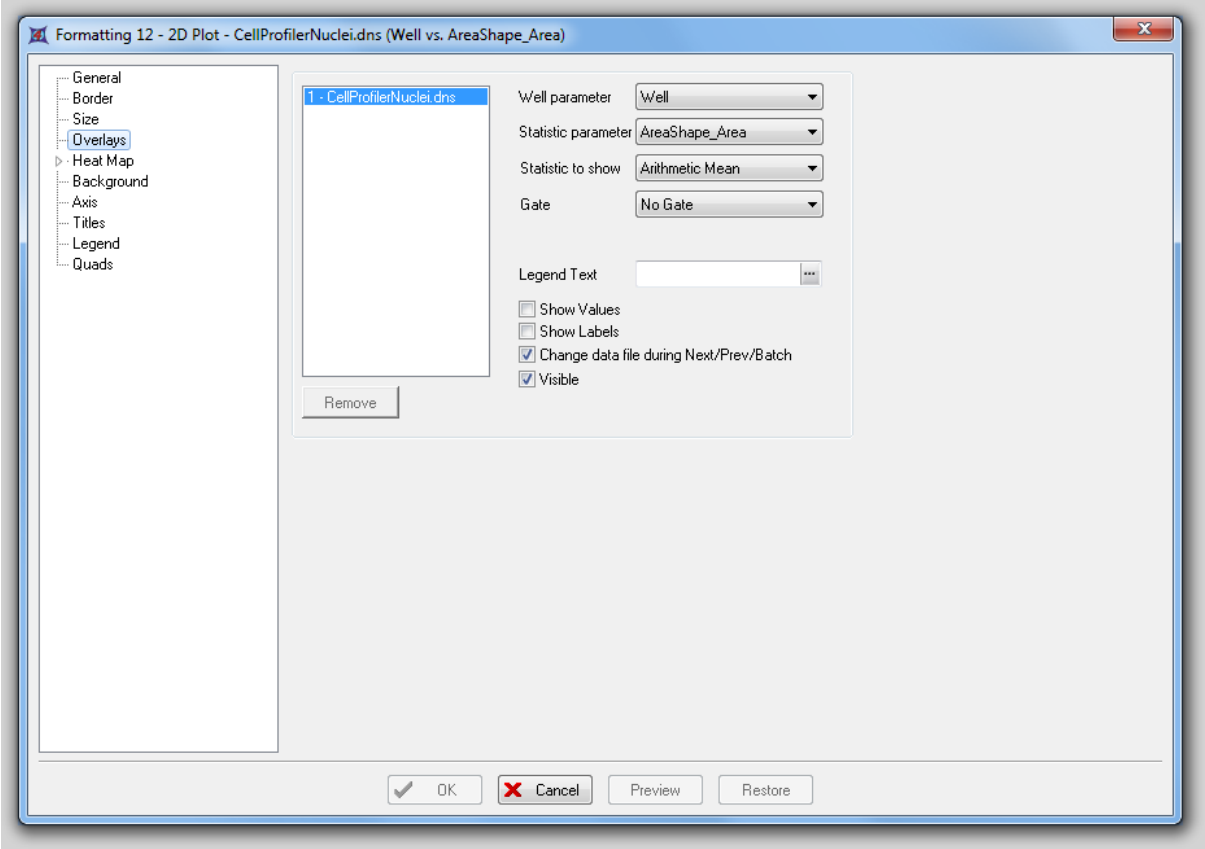

Figure 28.5 Heat Map - Setting the Statistic Parameter and Statistic to Show

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 in each well spot according to the color scheme property 486. 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/ calculated by your instrument. Select the **Statistic to show** from the predefined statistics in the corresponding drop-down list.

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.

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27.1.2 Choosing a Color Scheme

To choose the color scheme on a heat map, make sure the heat map is selected or in edit mode, then use one of the following methods:

- Select the **Format→2D Options→Color Levels** command (Figure 28.6).
- Right-click on the **Heat Map**, select **Format** from the pop-up menu, then choose **Heat Map→Color Levels**.

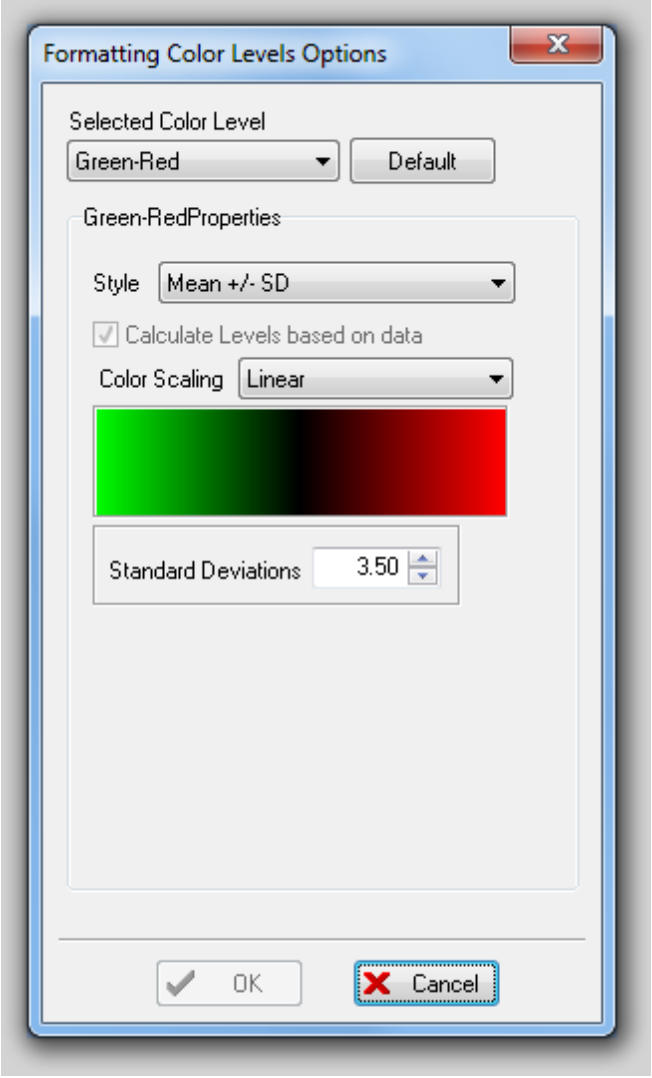

Figure 28.6 Formatting Color Level Options

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 that are close to the average for the statistic being displayed will be colored

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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. Another useful color scheme is the **Threshold** scheme (Figure 28.7).

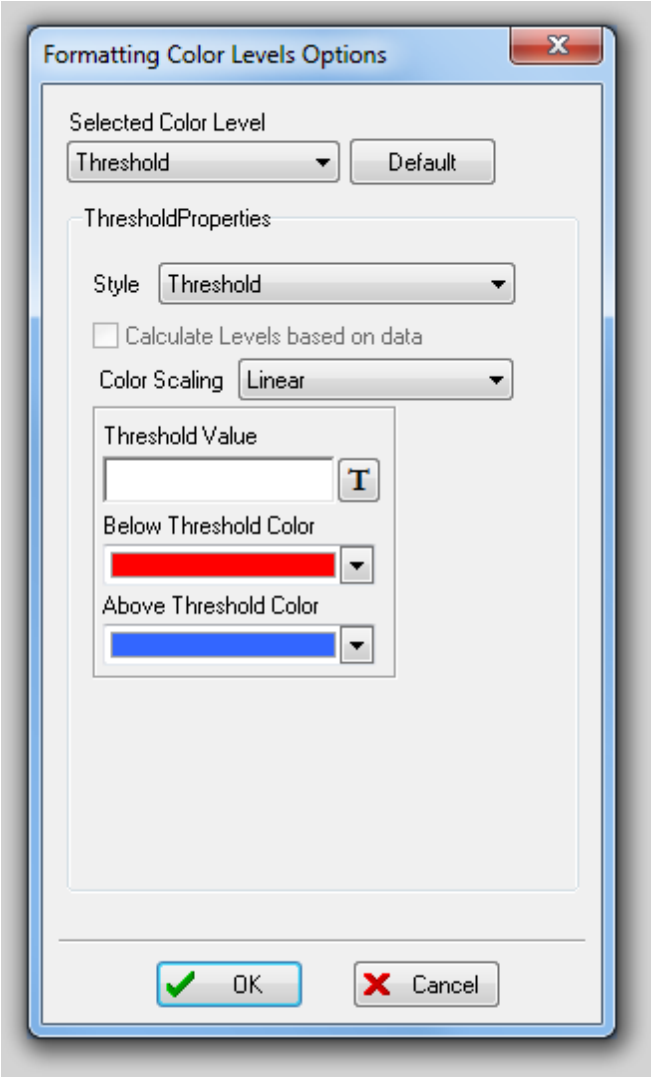

Figure 28.7 Threshold Color Scheme

When the **Selected Color Level** and **Style** are both 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. 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 one color, and wells with a value above the threshold value will be displayed in the other color.

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27.1.3 Well Gates

You can set a gate on one or more wells in a heat map. To create a **Well** gate:

1. Select the **Gating** (or **Home**)→**Create Gates**→**Well** command. (Alternatively, right-click on the heat map and choose **Create gate**→**Well**).
2. Click on a white area of the heat map. An **Editing** window (with instructions) for the well gate will appear above the heat map (Figure 28.8).

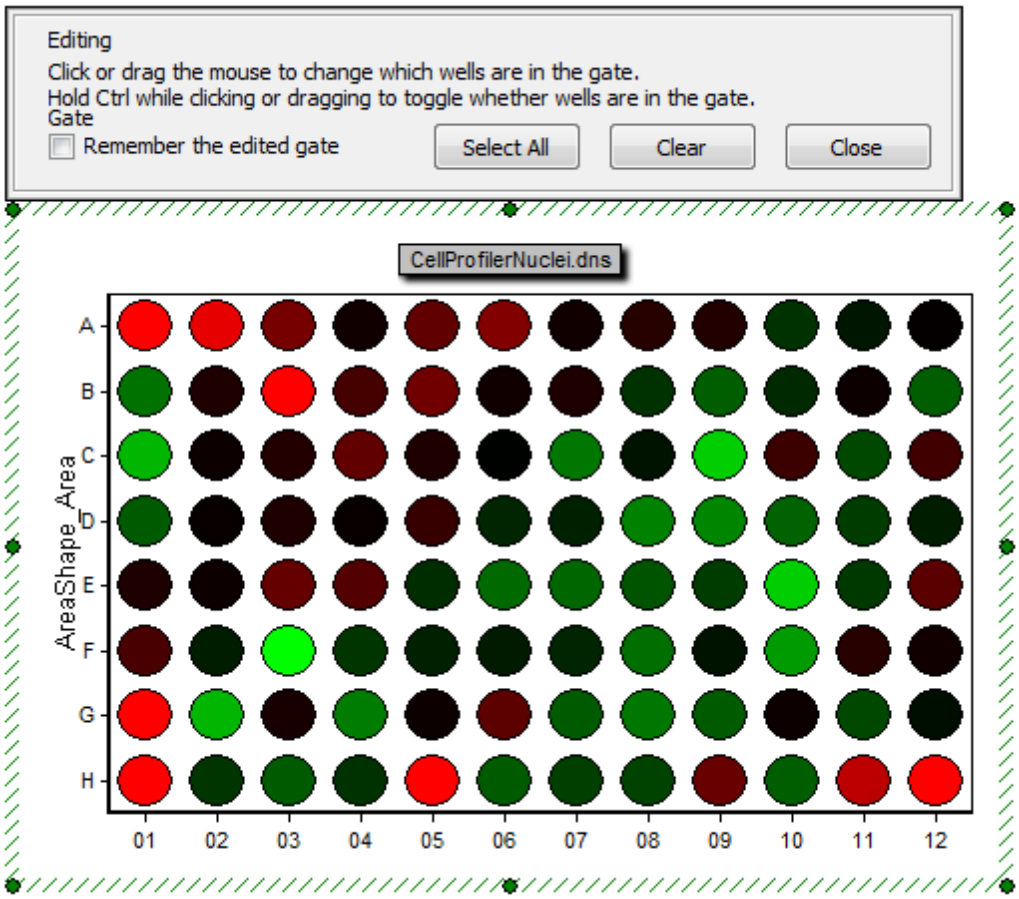

Figure 28.8 Setting a Well Gate

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.
- 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

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on **OK**.

The well(s) in the gate will be outlined by a circle that is the same as the gate color (Figure 28.9).

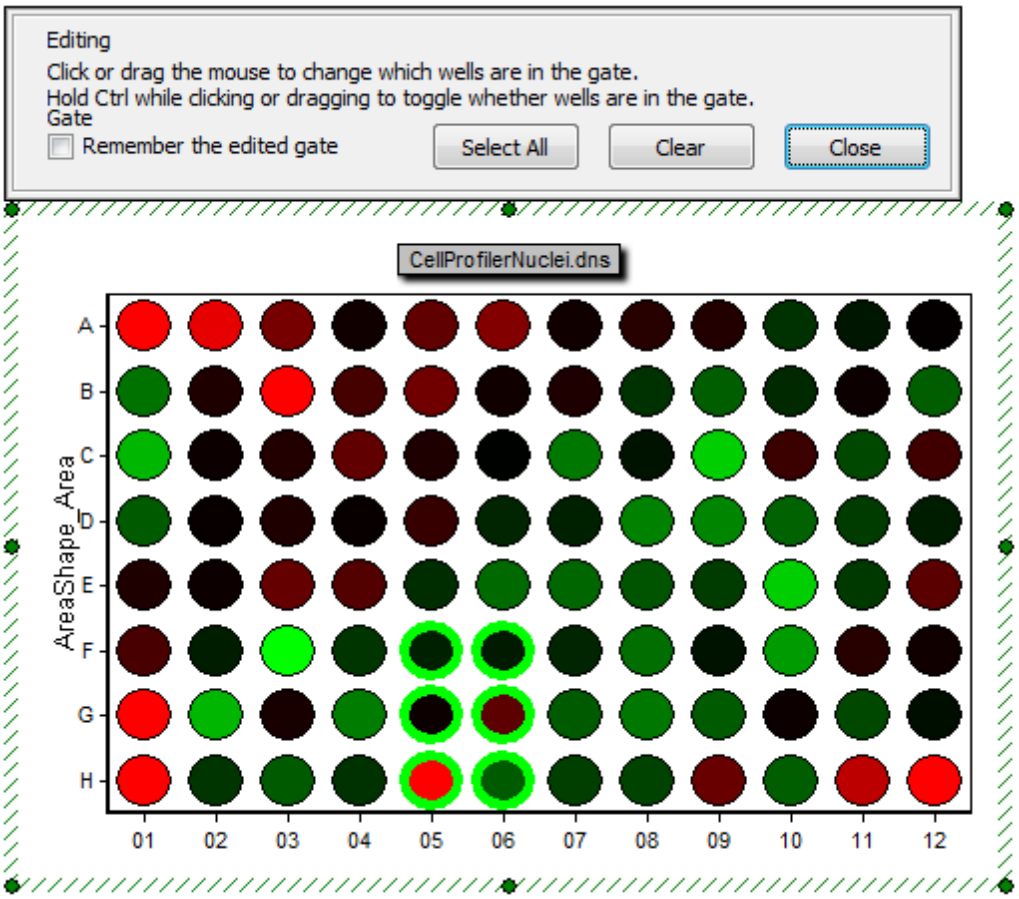

Figure 28.9 Well Gate. Six wells are outlined in green (rows F-H, columns 05-06)

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, 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 (Figure 28.10).

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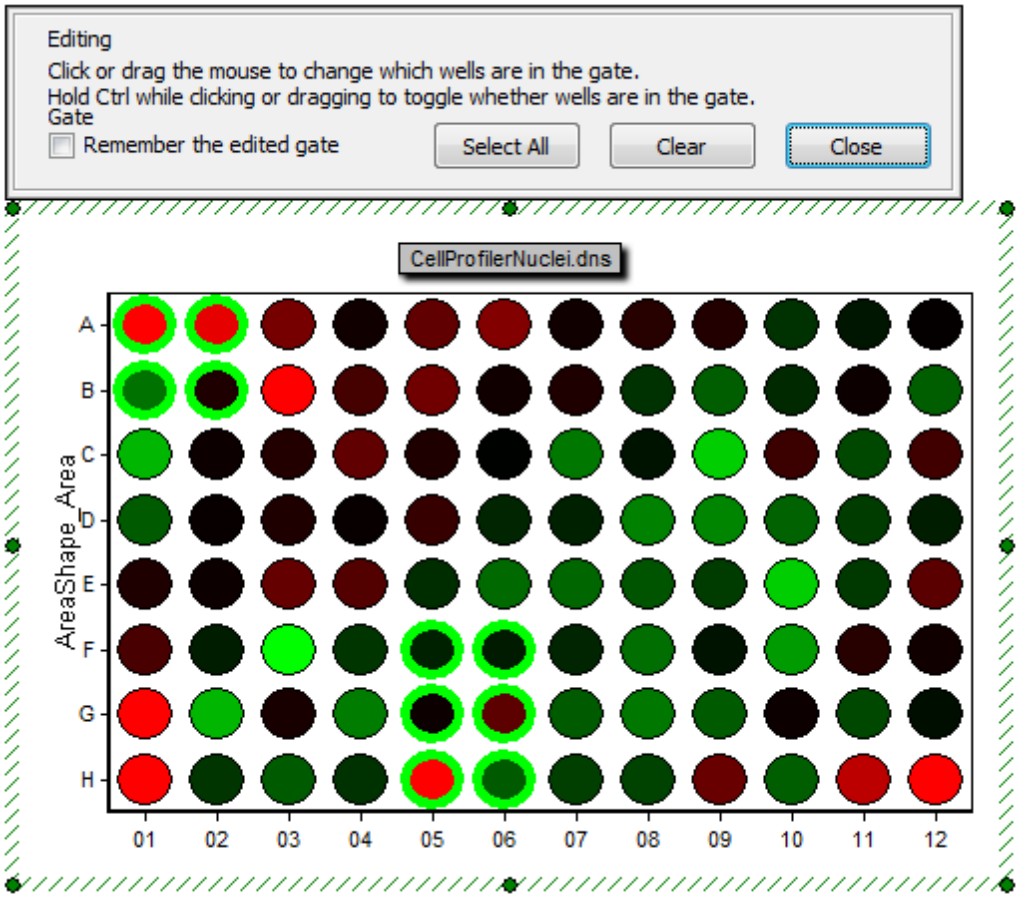

Figure 28.10 Well Gates. Four new wells (rows A-B, columns 01-02) have been added to the previous gate by holding down the Ctrl key when dragging across the new wells.

Click **Close** when you are finished editing.

To activate (and modify) an existing **Well** gate, right-click on the **Heat Map**, select **Activate gate**, and choose the gate to activate from the submenu.

27.2 Working with Picture Plots

To insert a **Picture Plot** on your layout, use the **Insert→2D Plots→Picture Plot** command.

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 an appropriate **Gate** (e.g., a single well gate that you set on the corresponding Heat Map[488]) using the drop-down lists (Figure 28.11). Alternatively, you can select the **Picture Plot** and use the **Format→Plot Options→Overlays** command.

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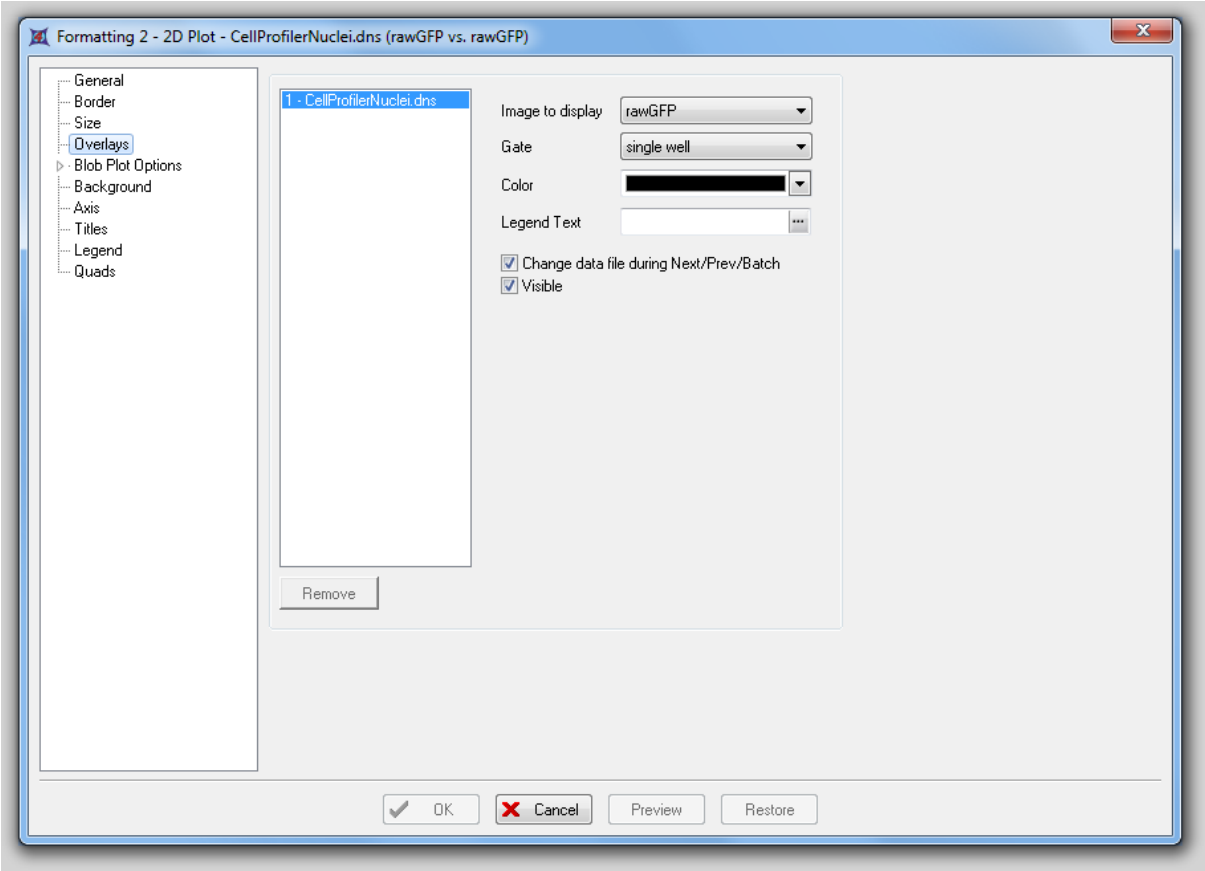

Figure 28.11 Formatting a Picture Plot - Overlays Category

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 28.12). The axes are in pixels with the origin (0,0) located in the upper left corner 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.

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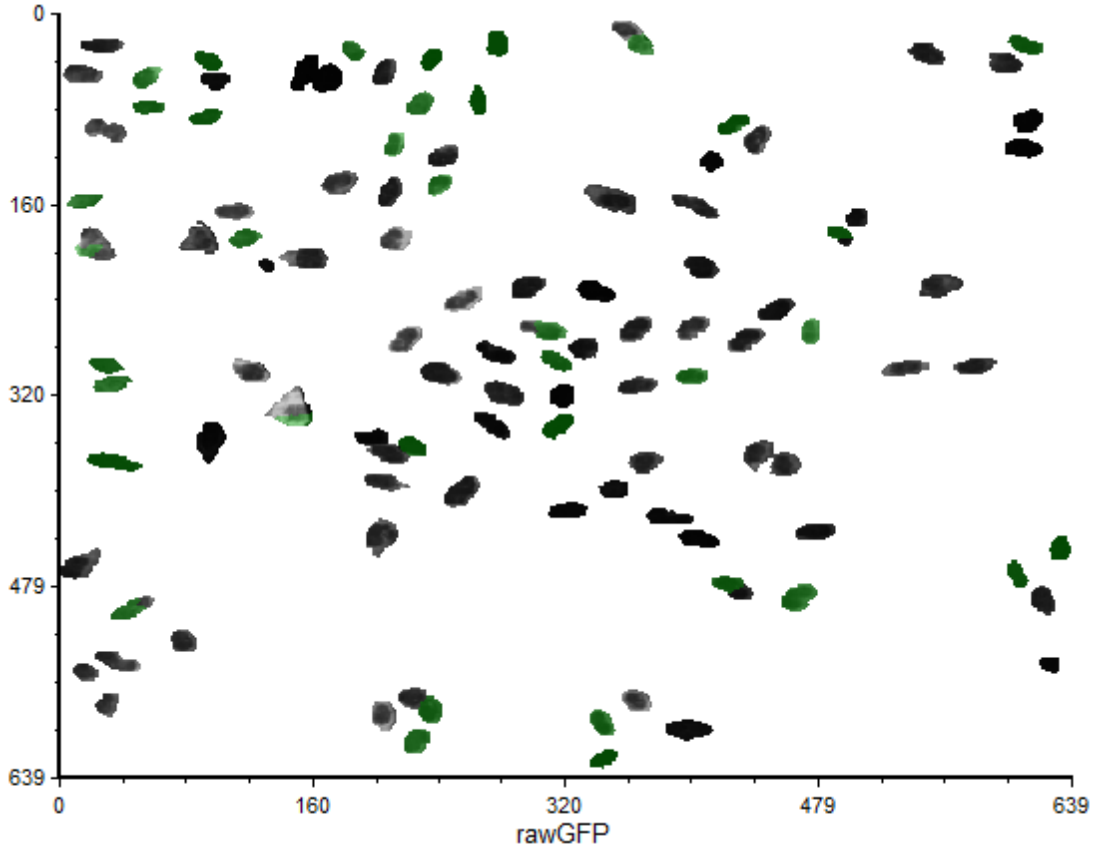

Figure 28.12 A Picture Plot

To display the images from a different well, modify the single well gate by right-clicking on the Heat Map, selecting Activate gate, choosing the appropriate gate from the submenu, and then selecting a different well488.

Picture Plots can be back-gated 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 back-gating:

1. Right-click on the **Picture Plot**.
2. Select **Format** from the pop-up menu.
3. Click on the triangle next to **Picture Plot Options**.
4. Choose the **Display Gates Options** category.
5. Set the gates to display according to your criteria (Figure 28.13).

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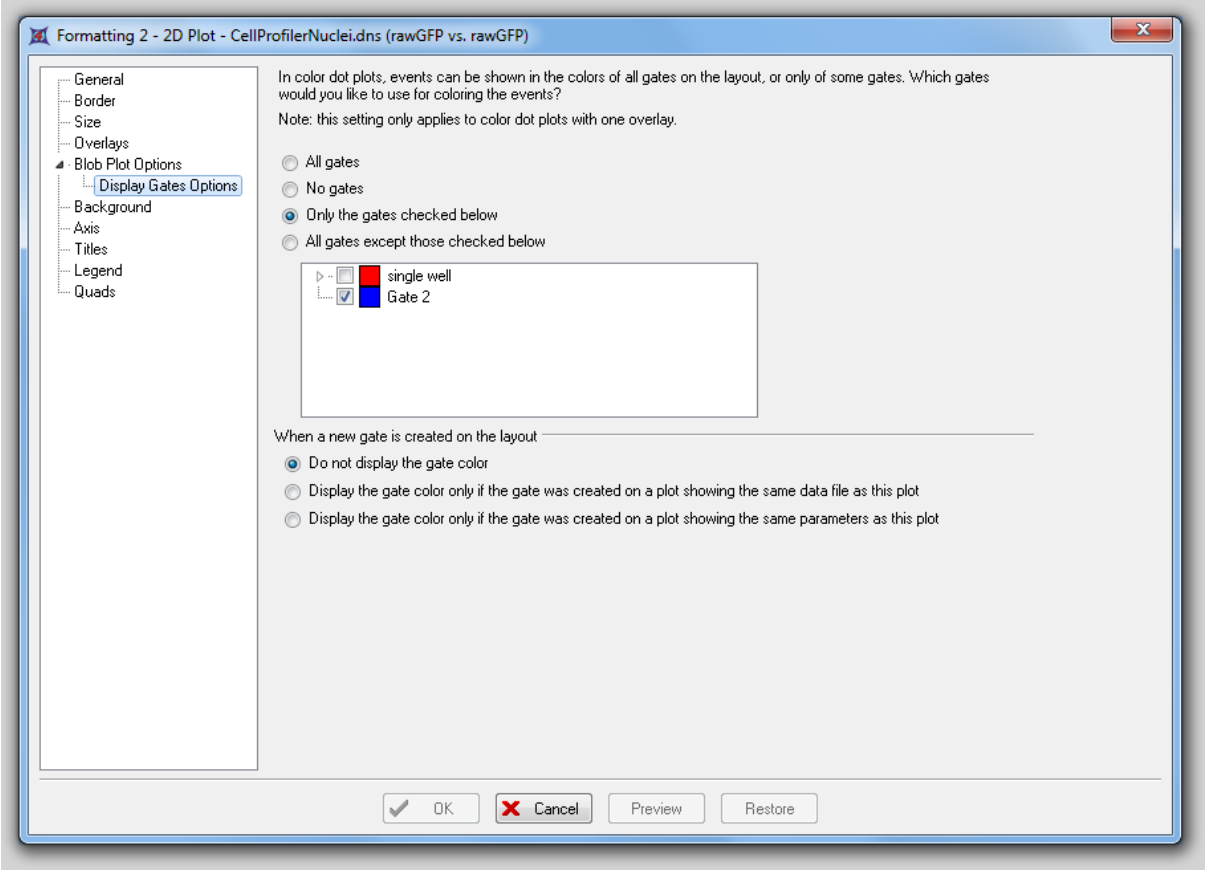

Figure 28.13 Picture Plot - Display Gates Options

In the example, Gate 2 (dark blue) has been chosen. When you click OK, the images in Gate 2 will be colored blue (Figure 28.14).

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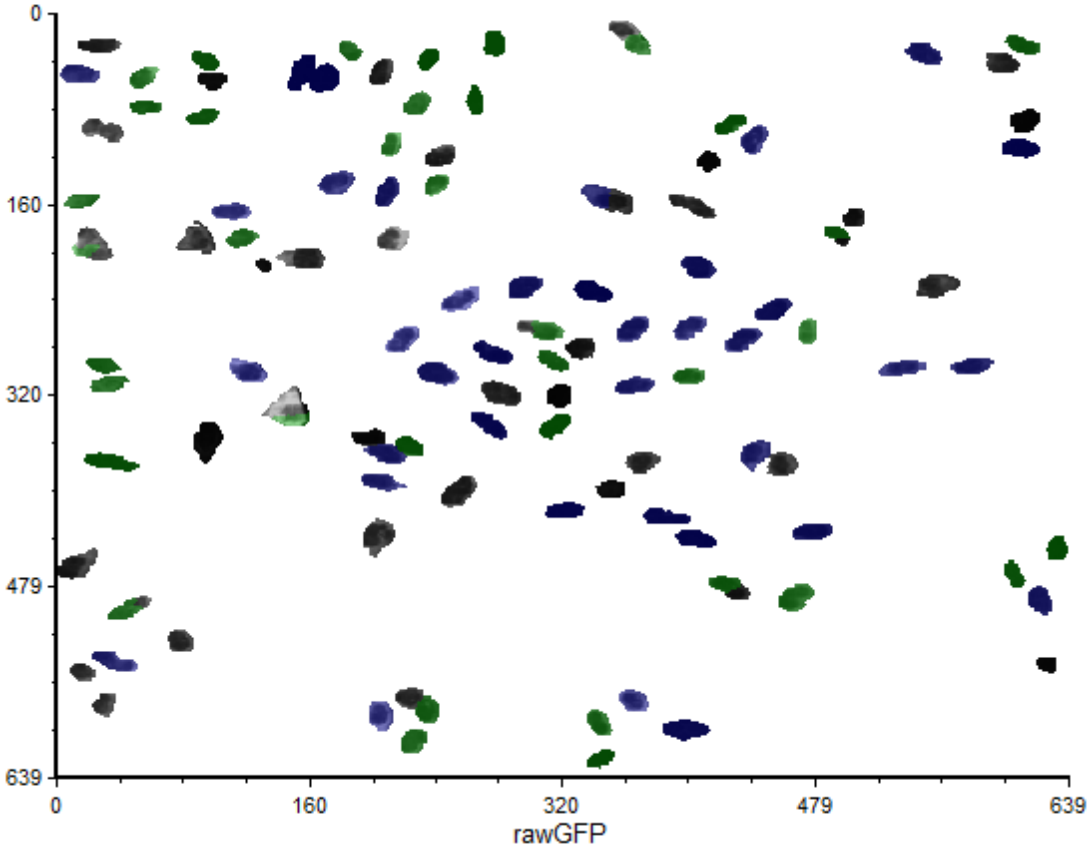

Figure 28.14 Back-Gated Picture Plot. Many of the cells display the dark blue color of Gate 2.

You can also gate on a single cell or group of cells on a **Picture Plot**. Choose a gate shape from the **Gating→Create Gates** group and apply it to the plot.

In Figure 28.15, a single cell gate (rectangle) has been drawn on the **Picture Plot** (see cell in the top center of the plot). You can use that gate just like any other gate in FCS Express. For example, you can back-gate a Data Grid 323 to show your single cell gate.

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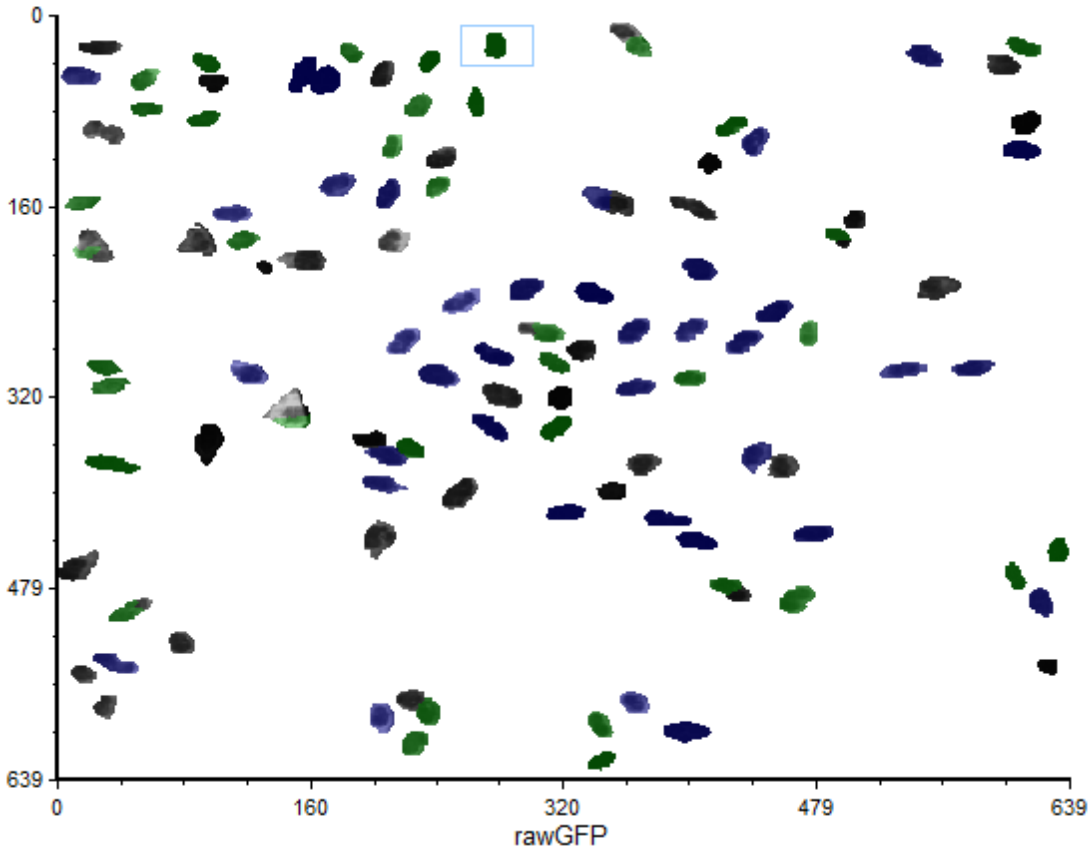

Figure 28.15 Single-cell Gate (rectangle) Drawn on a Picture Plot

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Chapter

28 Collaboration Tool

The Collaboration Tool is remote meeting and desktop sharing software that enables you to connect with other users in real-time meetings via the Internet right from FCS Express Version 4. A meeting organizer can open a session and others who join the session can view FCS Express 4 presentations. Participants can view the presenter's screen and chat with other attendees. In addition, participants can be given the privilege to remotely control another attendees' computer screen. Files can also be shared between the attendees.

28.1 Opening, Joining, and Reserving a Session

To organize a meeting and start a session, use the **Application Button→ Collaborate→Start Collaboration** command. This will bring up the Portal window.

There are four choices at the top of the screen:

- **Create a new meeting**
  - As a meeting organizer, choose this option to open a new, unreserved meeting (Figure 30.2). Enter a **Username** and **Password** which are only valid for this session. The Username will be seen by the other attendees. Click **Login**.

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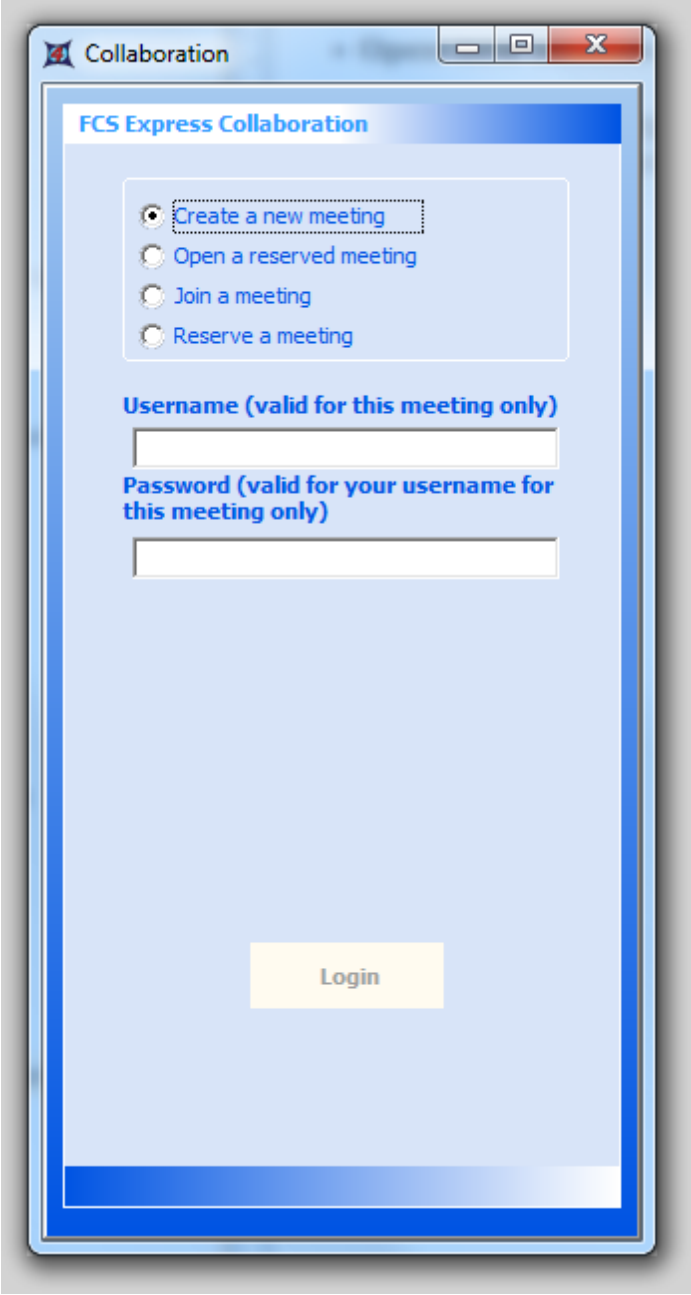

Figure 30.2 Collaboration Portal -- Create a New Meeting

- The meeting will open and you will be assigned a meeting ID number. In the example in Figure 30.3, the meeting ID number is **616913** and the meeting organizer (**TheOrganizer**) is listed as the first/only attendee of the meeting. Give the meeting ID to the other people that you want to attend your meeting.

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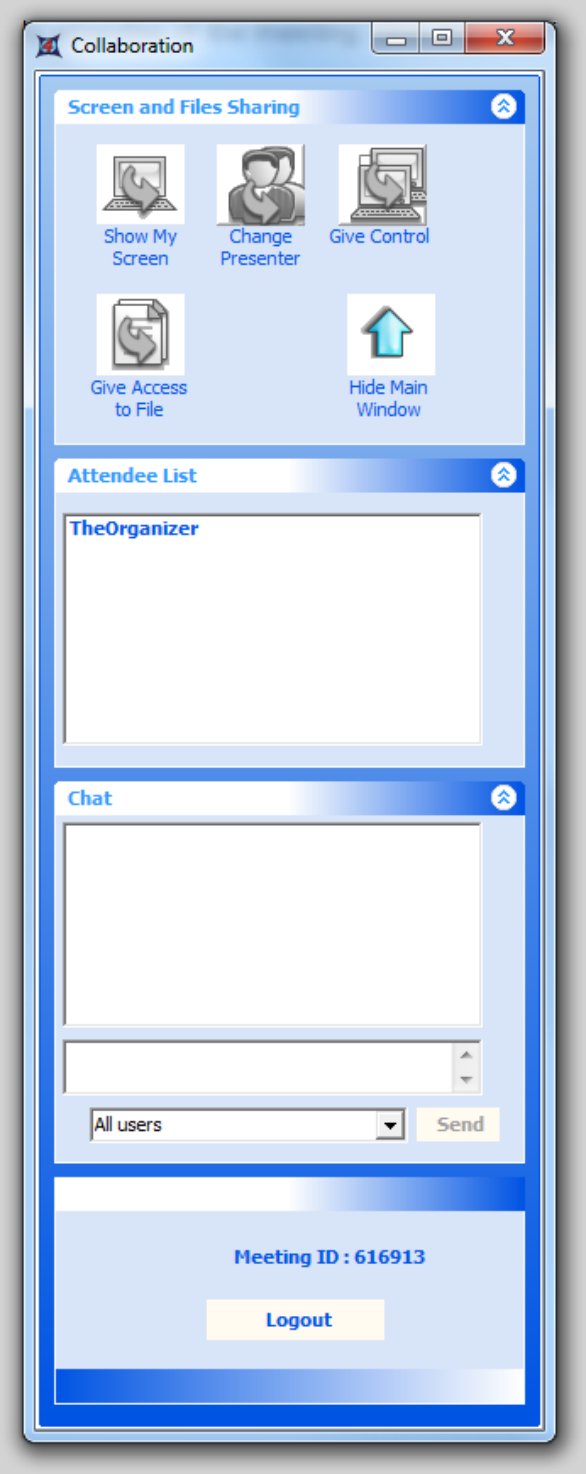

Figure 30.3 An Open Collaboration Session

- **Open a reserved meeting**

  To open a meeting that you have previously reserved, choose this radio

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button and enter the **Meeting ID** number and **Reservation code** (Figure 30.4) that you saved when you reserved the session. Click **Login**. Your session will open. Provide the meeting ID to the other attendees.

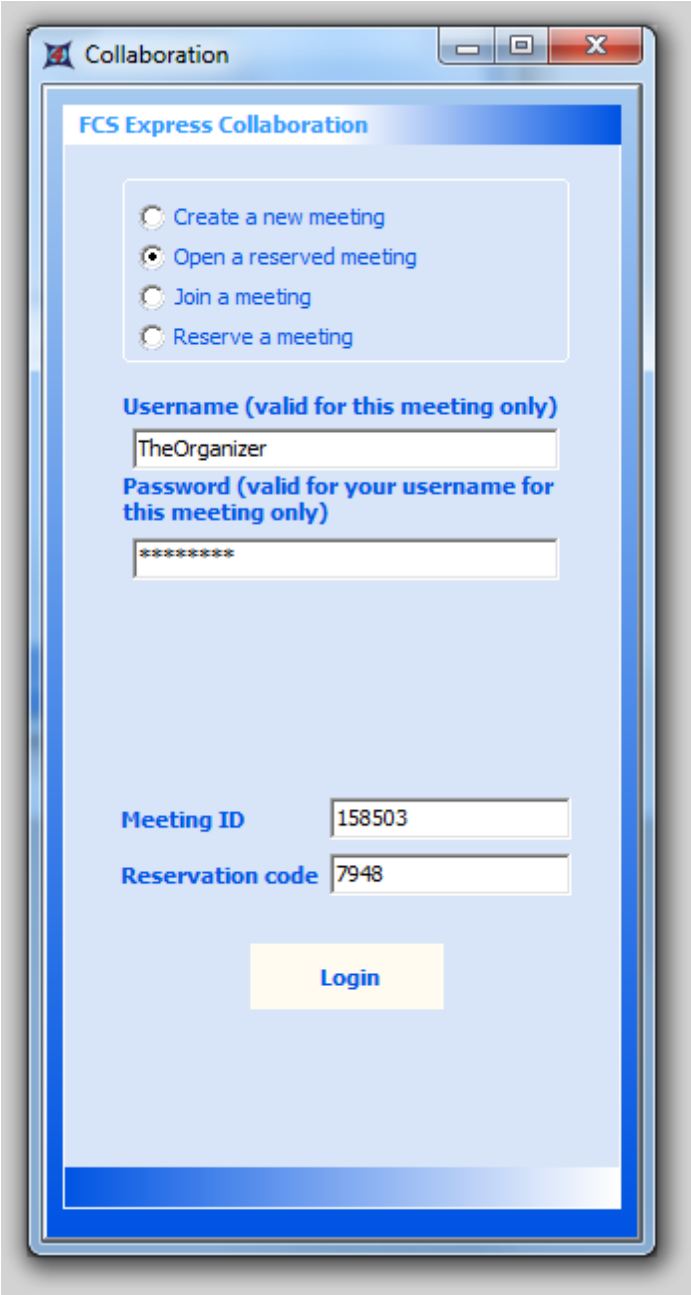

Figure 30.4 Opening a Reserved Meeting

- **Join an meeting**

  The default option. Choose this radio button if the organizer has provided you with a meeting ID number.

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- **Reserve a meeting**

  Use this option to reserve a meeting up to 10 days in advance. Chose this radio button (Figure 30.5), select a date using the drop-down calendar, and click the **Make reservation** button. You will be assigned a Meeting ID and a **Reservation code**. *Write down these numbers. You will need them to open the reserved meeting.* The meeting ID can be e-mailed to the other participants in advance of the meeting so that they can sign in at the designated time.

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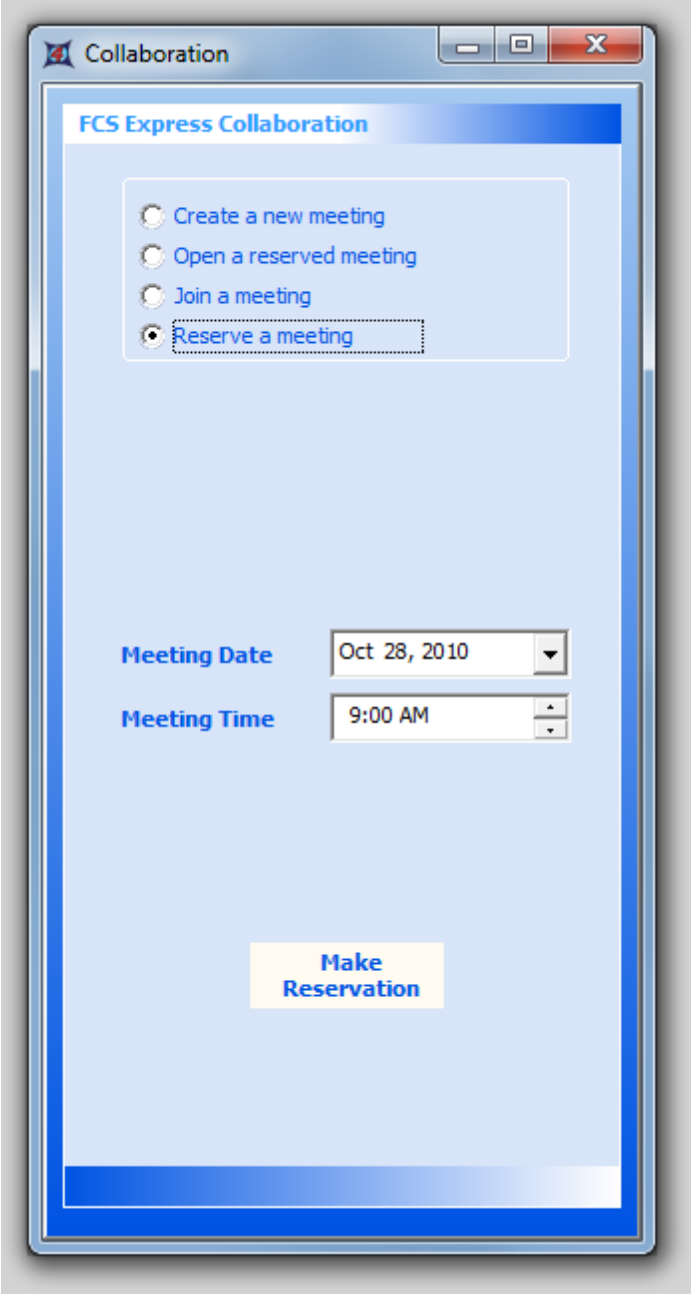

Figure 30.5 Reserving a Meeting

28.2 Conducting a Meeting

The organizer of the meeting is the default presenter, and initially controls the meeting. Once a Meeting ID has been issued, the **Collaboration** window will be divided into four sections (Figure 30.6):

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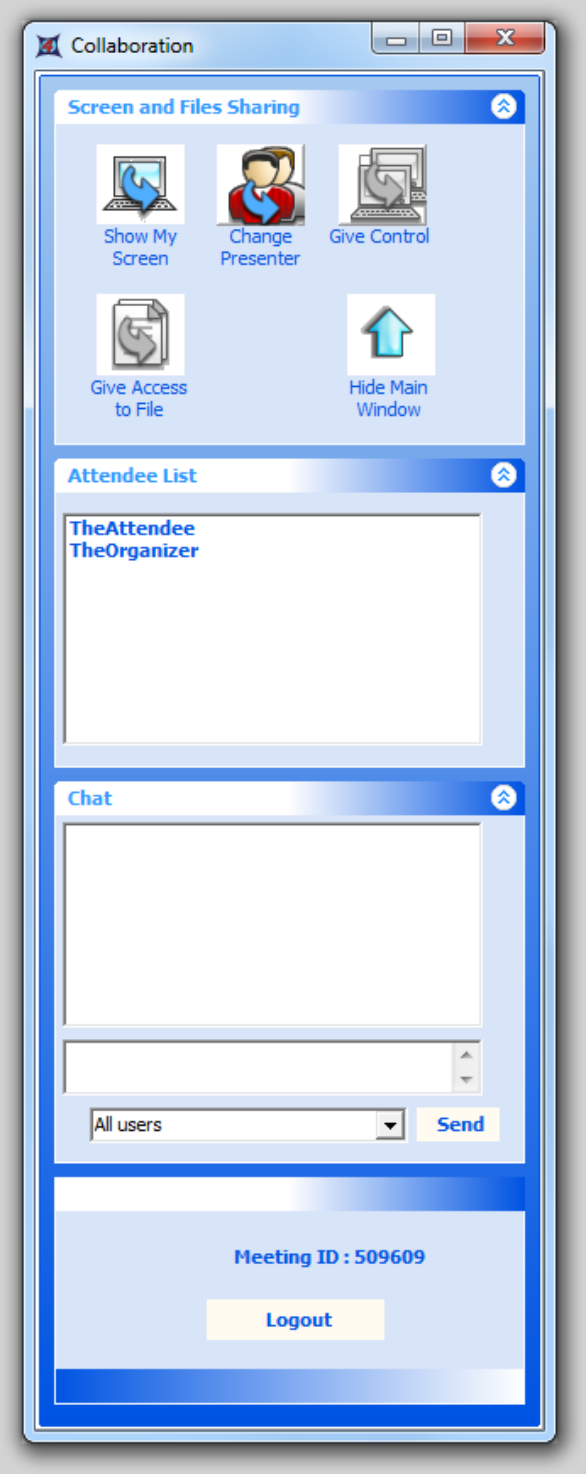

Figure 30.6 Collaboration Window with a Meeting in Progress

- **Screen and Files Sharing**

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This section contains five buttons which have the following functions:

- The **Show My Screen** button starts/stops the display of the presenter's desktop to the other attendees. Initially, the organizer is the presenter. When **Show My Screen** is clicked by the presenter, all of the meeting attendees will see the presenter's desktop on their computer screens, the button's label will change to **Stop Showing**, and the **Give Control** button will be activated. Clicking the button again (**Stop Showing**) stops the display of your desktop (the other users will see their own desktops again).

- The **Change Presenter** button transfers the presenter's role to another attendee. Click on the button and select the user to become the new presenter from the pop-up menu. They will be prompted to accept or decline the role as presenter. If they accept, they will be asked if they want to start showing their screen. You will be notified if they accept. As presenter, they are now in control of the meeting (i. e., they can now give control to other users and transfer the role as presenter to other attendees).

- The **Give Control** button allows the presenter to let another user control their desktop. Click on the button and select the user to give control to from the pop-up menu. That user will see the following message from the FCS Collaboration Portal *You can control the presenter's desktop*. When they click on **OK**, the user will be controlling the presenter's computer with their mouse. If the presenter clicks on the button again (the label has changed to **Change/Stop Control**), they will be presented with a pop-up menu allowing them to take back control or pass it to another attendee.

- The **Give Access to File** button is only activated once the presenter gives control to another attendee. Then, when the presenter clicks on this button, it activates the ability for the other user to access files on the presenter's computer. *Since access to file transfer means that the other user has complete privileges over browsing, creating, deleting, and moving files on your computer, you will be prompted as to whether or not you want to give that permission*.

  Once file access has been granted, both parties will be presented with a **Select the folder where the received files will be saved** dialog (Figure 30.7). *Note: Even if file transfer will be in only one direction, both parties must choose an "Inbox" using this dialog to proceed.*

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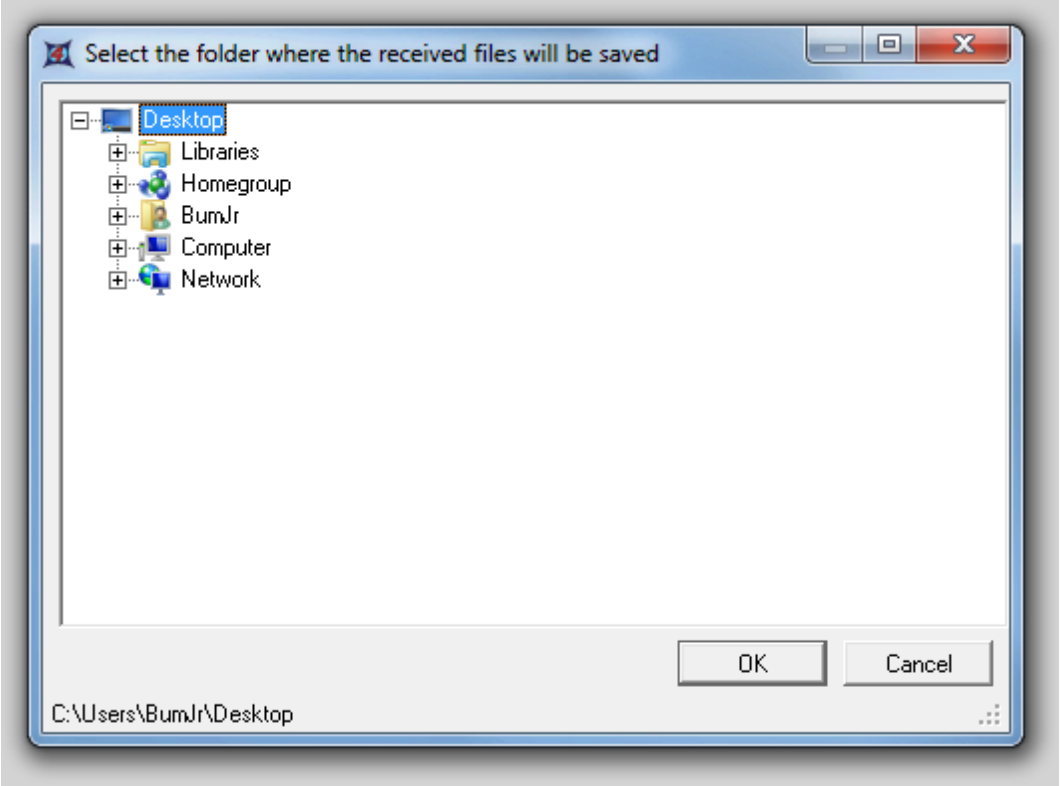

Figure 30.7 Choosing an Inbox for File Transfer

To transfer a file, the user who has been given permission, should perform the following steps:

1. Find the **Files** window labeled with the presenter's username (Figure 30.8). In the example, the presenter was *TheOrganizer*.

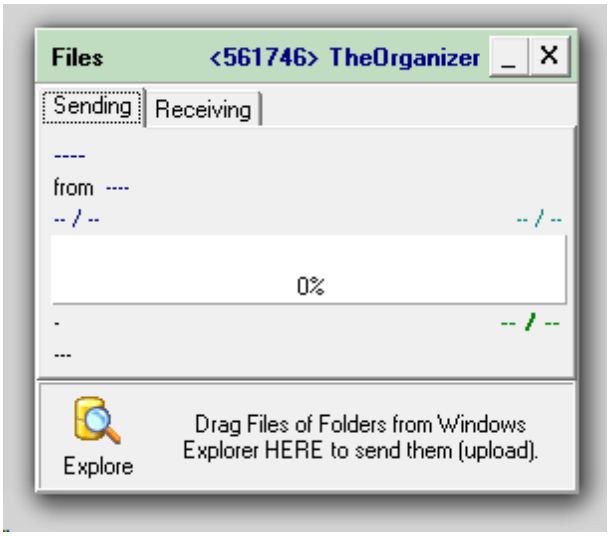

Figure 30.8 Files Window

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2. Click on the **Explore** icon (button) in the lower left of the presenter's Files window to bring up the presenter's File Explorer window (Figure 30.9).

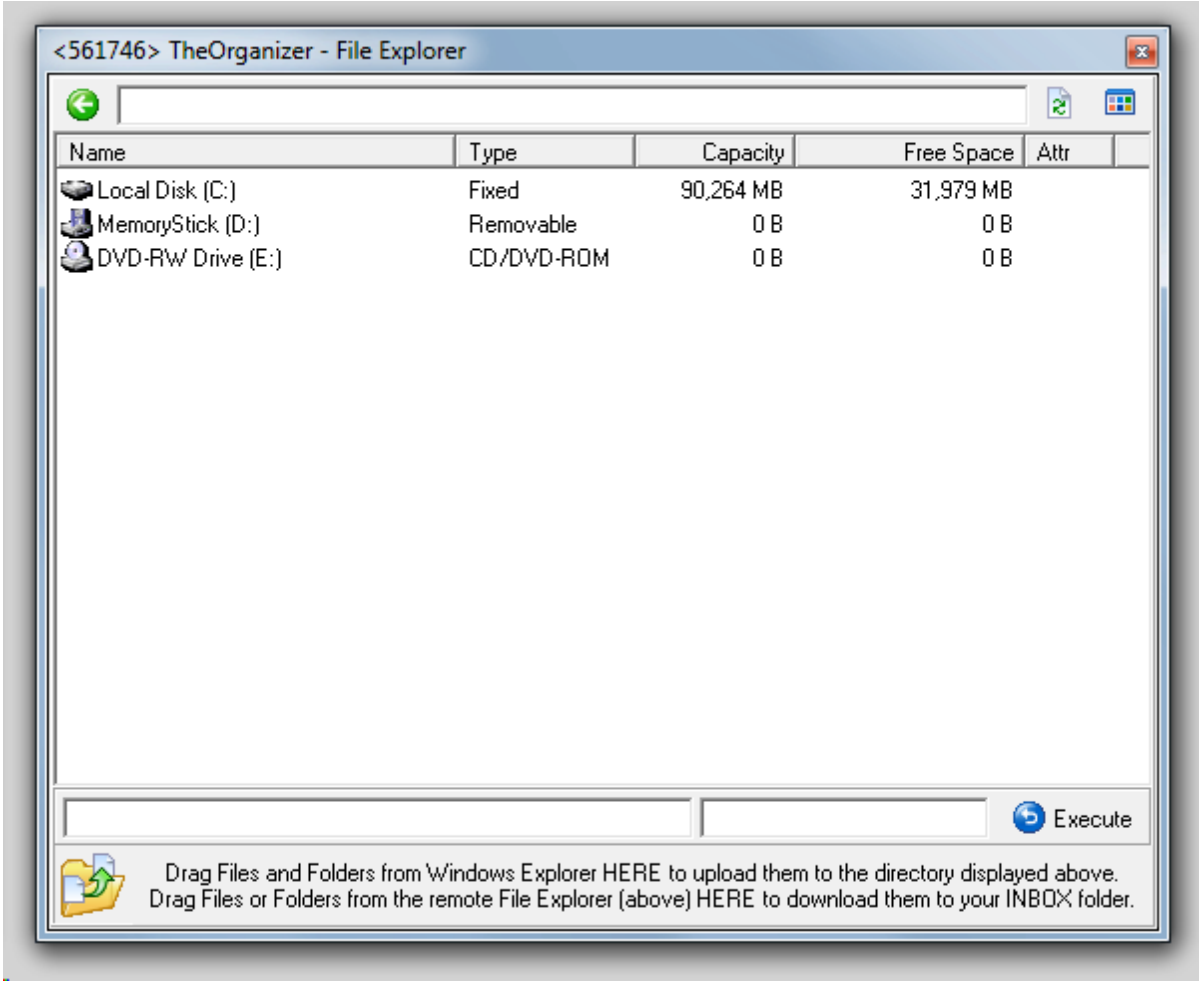

Figure 30.9 File Explorer Window

3. Browse through the window to find the file you want. Then, click and drag that file to the bottom of the window and release it in the region marked "**HERE**" at the bottom of the window (Figure 30.10). In the Figure, note that the cursor has changed to an *arrow with a document* as the selected file is dragged to the drop area.

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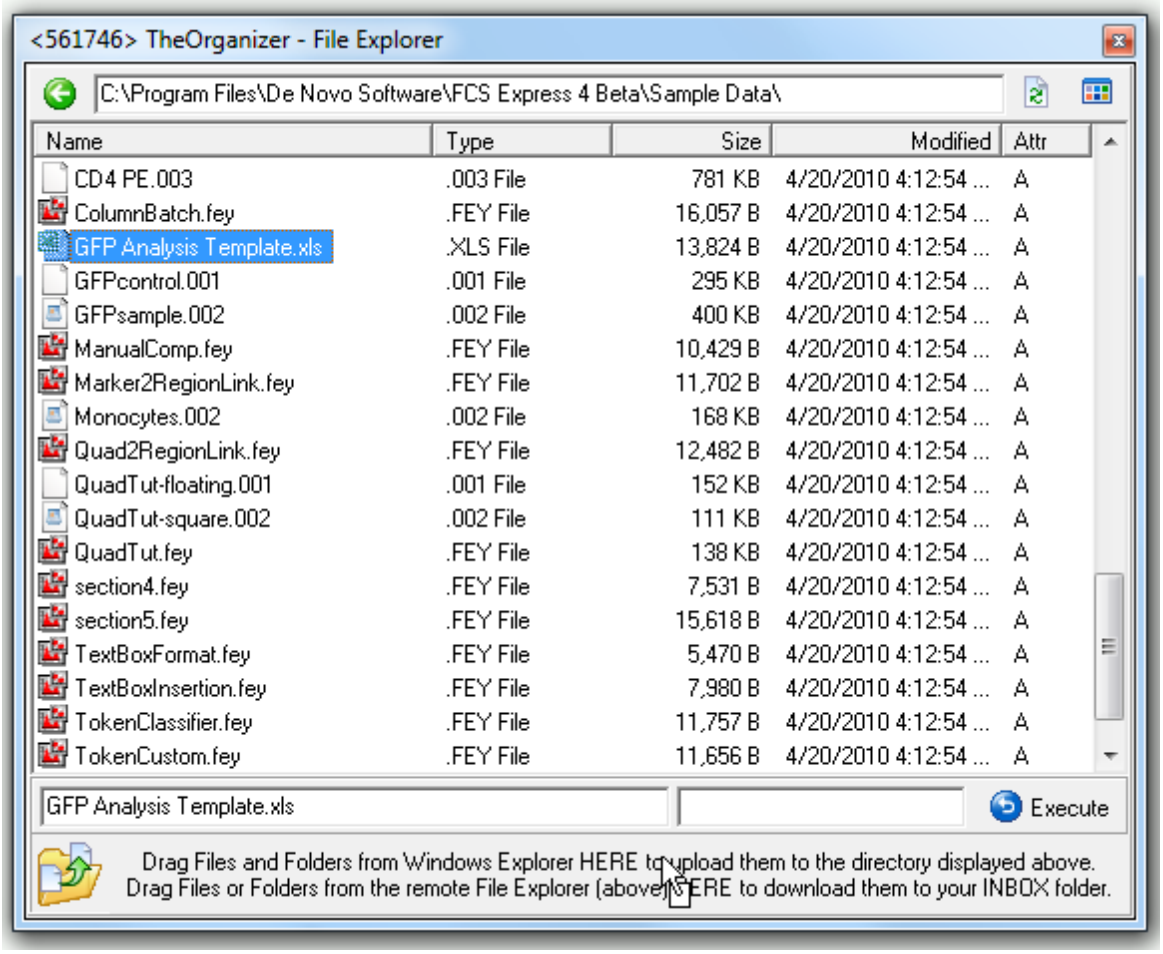

Figure 30.10 File Sharing - Drag & Drop

4. The file will be transferred to the "Inbox" (chosen above) of the user's computer. The progress can be monitored in the **Files** windows (Figure 30.11).

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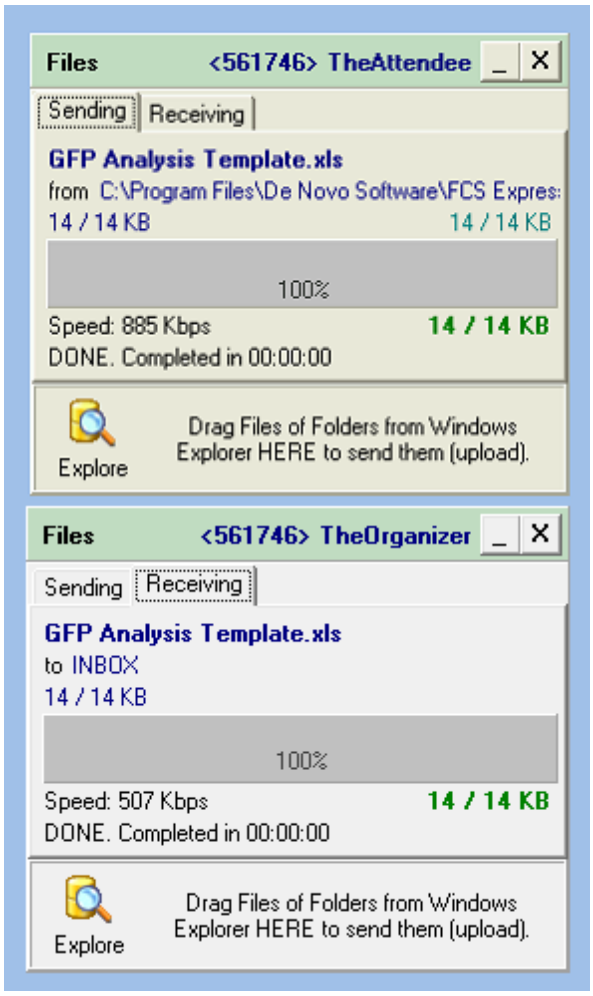

Figure 30.11 File Sharing Progress

- **Hide Main Window**

  Click this button to minimize the **Portal** window (Figure 30.12). To re-open the **Portal** window, click on the down-arrow icon (button) on the right of the minimized window.

Figure 30.12 Minimized Portal Window

- **Attendee List**

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This area shows the usernames for each person attending the meeting.

- **Chat**

  This section contains four controls (Figure 30.13):

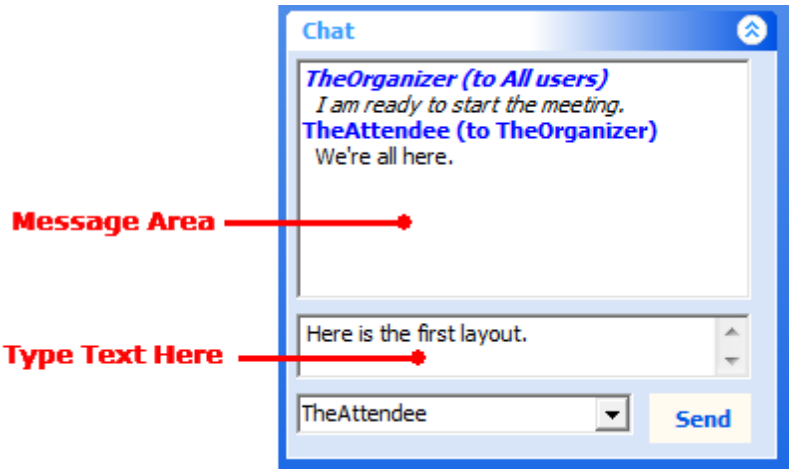

Figure 30.13 Chat Area

  - *Message area*. The chat messages will be displayed in this box, along with an indication of who sent each message.

  - *Enter chat message text*. This is the area where you type your message text.

  - *Drop-down list of users*. Use this control to choose which user(s) to send your chat message to (**All users** or an individual user listed by username).

  - **Send** button. When you have entered the text and chosen the user (s) to send the message to, click **Send**.

- **Meeting ID and Logout Button**

  To quit the meeting, click **Logout**.

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Chapter

29 Upgrading From Version 3

FCS Express 4 is the result of extensive customer feedback, giving you the features you need most. The new version continues our tradition of innovative, powerful software, designed to let you work in a modern PowerPoint™ style framework.

In addition, Version 4 has been expanded to bring the full power of flow-style analysis to the world of image cytometry. All of the standard FCS Express features that you've grown to know and trust are there, 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.

29.1 New Features

In addition to the new features described in the other sections of this chapter, FCS Express Version 4 has the following improvements:

- Speed - FCS Express now uses all the cores in your computer to dramatically speed up your analysis.
- Speed of saving layouts has been dramatically improved.
- New Collaboration tool 498 within FCS Express.
- Calculate regression models 245 - Linear Regression, Exponential, Sigmoidal, Michaelis Menten.

29.1.1 New Data Loading and Saving Features

FCS Express Version 4 has the following new data loading/saving features:

- When loading a layout with linked data, FCS Express will show you a list of any data files that can't be found, and give you the option of loading the files from a different folder 55.
- Load CSV 43 and tab-delimited files.
- Load from Isocyte 146.
- Load from Cell Profiler 146.
- Load FCS data as plates 314 (BD 430, Guava, Sony, Quanta, Accuri 437, Hypercyt).
- Load Time parameter ($FCS v3.0 TIMESTEP units) 144.
- Choose the starting value for parameters displayed in a log scale 144.
- Load extra keywords as you load the data file by creating an ExtraKeywords file 314.
- Saving data is now included in the Export and Batch Export windows 314.
- Save data in a variety of formats, including FCS 2.0 and 3.0 142.
- The number of parameters that you can export is unlimited.

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29.1.2 DNA Analysis

FCS Express Version 4 includes several new Multicycle DNA analysis features:

- Multicycle is now installed by default. No separate installation is required 444.
- Single cell cycle fitting (with no background correction) is included free 444.
- Multicycle warns you if single cycle fitting is not appropriate 444.
- Faster analysis.

29.1.3 New Plot Types and Enhancements

FCS Express Version 4 adds the following new plot types and enhancements:

- Plot statistical data with bar charts and scatter plots 241.
- Automatically create a new gated plot by dragging a gate to any open area in the layout 201.
- Dragging one plot onto another changes the target plot data file (either replacing the file or adding an overlay) 113.
- Scatter plots – precisely control the dot size and shape of 2D data 241.
- Visualize microtiter plate data using Heat Map 482 displays.
- New color palettes for Contour and Density plots 181.
- Show outliers on Density and Contour plots 175.
- Stacked histogram display 172.
- Data Grid – create your own custom data 234 sets. Create and apply gates to the grid.
- Change plot types from one plot to another from the Format Tab 40.
- Magnify directly on 2D plots 162.
- Magnify both the resolution and axis options 162.
- Kinetics Plots 474.
- Rounded border around footers and/or titles 154.
- Draw 1D gates directly on dot plots, in either direction 193.
- Gates can automatically be added to gates to display 183.
- Recreate marker, gate, or quad text boxes 105.
- New gate text box options for automatically displaying names and/or default statistics chosen by the user 134.
- Turn off the yellow wall along the Y-axis in 3D (surface) plots 160.

29.1.4 New User Interface Features

The new user interface has the following key features:

- New ribbon style menu system 30 with a modern Microsoft Office 2007/2010 look and feel.
- Multi-page views 64. You can now view up to four pages at once.
- Layouts now open in separate windows 65.
- Tabs on pages allow for easier organization and arrangement 58.
- Improved Drag and Drop. Seamlessly Drag & Drop plots, data files 76,

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objects[75], gates[77], tokens, and more.
- Docking[66] system.
- Page visibility can be based on a token[58].
- Each page can have a different background or none at all[58].
- Pages can now be named[58].
- Many windows that were modal (only one could be opened at a time, e.g., Alerts, Custom Tokens[348]) are now non-modal and can be used with other elements of the layout.
- Text boxes can be copied from one layout to another[36].
- Tokens can (optionally) change their font based on their value[359].
- Export to more graphics formats (PNG, GIF, TIFF, JPEG, EMF, BMP)[62].
- Insert pictures from more graphics formats (PNG, GIF, TIFF, JPEG, EMF, BMP).[92]
- Display measurement units in various formats (metric or imperial)[118].
- When you maximize a plot, you have the option to keep the aspect ratio locked[118].
- When you change page orientation, you have the option to keep the aspect ratio locked for each object on the page[61].
- Task bar has been removed[28].

29.1.5 New Gating Features

FCS Express 4 has the following new gating features:

- Linked Gates. Link gates to move them together, unlink to move gates separately[205].
- Gate labels can now show statistics (such as % positive) automatically[134].
- Resize function for polygon and freeform gates[193].
- Draw 1D gates in either direction on dot plots[193].
- Easier to create NOT gates[193], replace gates, and control gate colors.
- Recreate deleted gate text boxes from the right-click menu[105].
- Gate position can be locked to avoid accidental movements[202].
- Dragging and dropping a gate from a plot to an empty part of a page can either duplicate the existing plot and apply the gate, or delete the gate, depending on a user preference[134].
- Gate Navigator[77] gives you an overview of your gates, and what plots are gated on each one.
- Option to move the associated text box when a gate or marker is moved[134].
- New gate types - Cell Number gates[323] and Well Gates[488].
- Draw gates on picture coordinates for Picture Plots[490].
- Gate naming now restarts at *Gate 1*.
- Move gates outside the boundaries of the plot area.
- Gate calculations now use all the cores in your computer.

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29.1.6 New Batch Processing Features

These new features apply to Batch Processing:

- Batch processing window is now a dockable 66 window.
- Batch Actions activation state can be controlled via custom tokens 397.
- Save already processed data even if an error is encountered during the batch 420.
- Append exported data to an excel spreadsheet at a specific row, the first available row, or a worksheet 400.
- Name newly created Excel worksheets during the batch, based on a token 400.
- Changes to Batch Processing Actions are automatically applied. No need to press OK 386.
- Export the text of a text box 397 (particularly useful in conjunction with canned comments).
- Enhanced drag & drop 412. Drag plots, text boxes, tokens, and stats to create batch actions.
- Fully supports all versions of PowerPoint, Excel and Word for Batch reports 400.
- Export to Picture supports more graphics formats 394 (PNG, GIF, TIFF, JPEG, EMF, BMP).

29.1.7 New Custom Token Features

There are new features when working with custom tokens:

- New dockable 66 Custom Token 348 window – drag & drop tokens to create and insert them into text boxes.
- Create folders to organize tokens 349.
- Tokens can change fonts based on their value 359.
- Excel-style formula evaluation (formulas must start with "=") 348.
- Insert any available function into the formula using the right-click menu 358.
- Improved speed in calculating custom formulas.
- Custom tokens update in real time, as you are changing the formula.

29.1.8 New Parameter Math Features

Parameter Math 260 has been improved to include the following:

- No need to press the Perform button in order to use parameters that were created in previous parameter math sequences steps 263.
- Parameter math formulas can now contain tokens 260.
- When evaluating parameter math formulas containing tokens, the tokens can be preserved in the formula, so that the data will constantly update 260.
- Parameter math formulas can contain a variable representing the row number 260.

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29.1.9 Navigators

Navigators in FCS Express provide an overview of your layout. They are dockable windows[66] with drag and drop functionality to help you manipulate the objects in your layout[75], the data sources[76] connected to your objects, and the gates[77] on your plots.

FCS Express Version 4 adds the following three Navigators:

- Layout Navigator[75]
- Data Navigator[76]
- Gate Navigator[77]

29.1.10 New Data Grid Features

The following new Data Grid features have been added:

- Add[238] and delete[239] rows and columns (Custom Data only).
- Edit[235] individual cells (Custom Data only).
- Rename[240] columns (Custom Data only).
- Gate data grids[323].
- Show Gates to Display (a column that shows the color of each gate the cell is in)[323].
- Create gates based on rows in the grid (Cell Number Gates)[323].
- Show images[482].
- Show and Hide columns[323].

29.1.11 New Compensation Features

FCS Express 4 has new compensation features:

- Save/Export[309] and Load[309] compensation matrices to be applied to individual data files, or to all data files in a folder.
- Plate data can have individual compensations for each well[305].
- Calculating compensation uses all the cores on your computer.
- Read Partec compensation.
- Compensation is automatically unloaded when the data using the compensation is no longer used.

29.1.12 New Channel Calibration Features

A new Channel Calibration feature in FCS Express 4 is the ability to edit existing Channel Calibration items[284].

29.1.13 New Data List Features

The **Data List** (note new name, formerly the *File List*) has several new features in FCS Express Version 4:

- It is now a dockable[66] window which can be docked to either side, top or

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bottom.
- Sort by more than one keyword 377.
- Sorting and other Data List options are now saved with the layout 53.
- When selecting a file for a plot or to add to the list, you can select from the file system, from the Data List, or from the Active Files (the files currently open in the layout) 112.

29.1.14 Custom Data

With FCS Express Version 4, you can create Custom Data 234:

- Create 234 and plot 241 your own data sets.
- Link data values to statistics on other data and plots 235.
- Use custom tokens in your data 235.
- Use parameter math to create a new parameter on your custom data with tokens in it 260.
- Plot your results on bar charts and scatter plots 241 within FCS Express, without needing to export data to another program (like Excel).

29.1.15 New Licensing Options

There are new licensing options in FCS Express 4:

- Internet dongle
- Site license

29.1.16 FCS Express 4 Image Cytometry

FCS Express now gives you the ability to analyze your Image Cytometry data:

- Picture plots 490.
- Gate on pictures 490.
- Backgate 490.
- Show pictures in Data Grids 482.
- Read from several manufacturers' formats.
- Perform flow-style analysis on your images.

29.2 Security System

The Security System 520 in FCS Express 4 has new enhancements:

- Improved security infrastructure.
- Use SQL server to store security permissions.

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Chapter

30 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.

30.1 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 32.2).

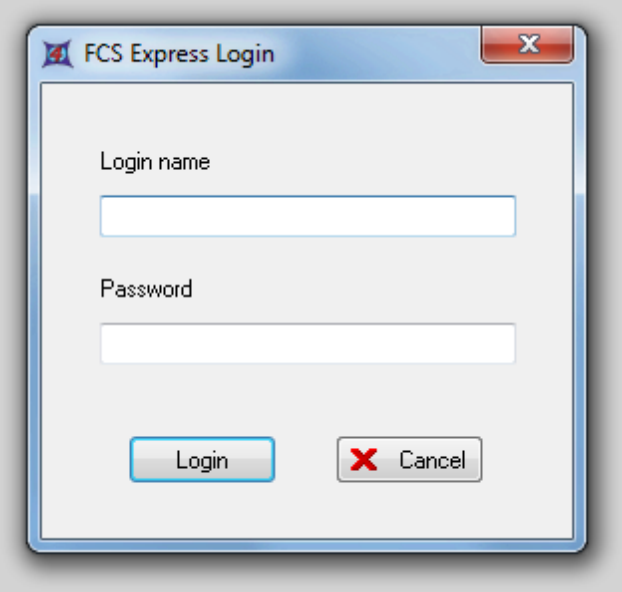

Figure 32.2 Security Login

Users are created and managed from the **Application Button→Security** gallery within FCS Express (Figure 32.3).

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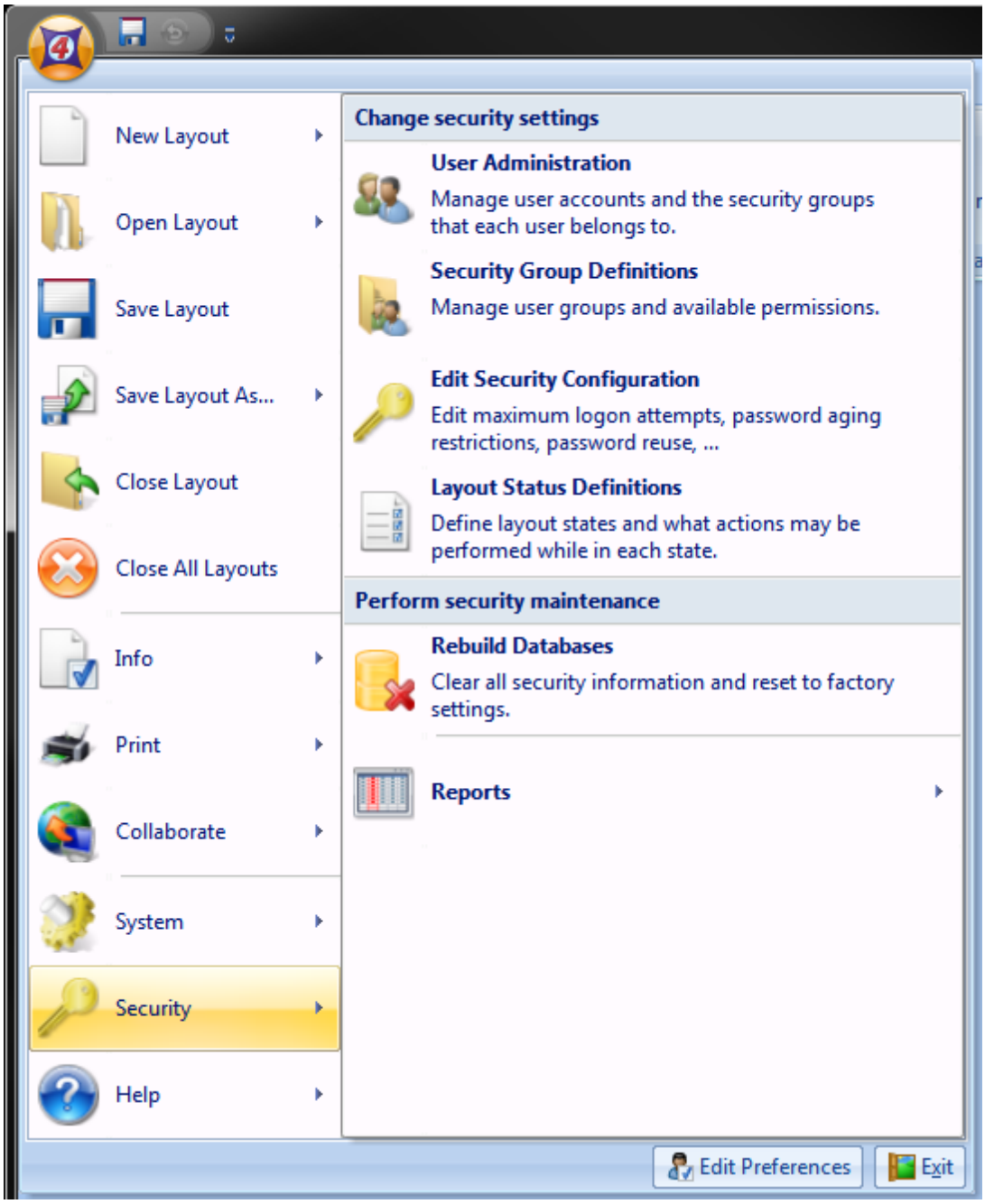

Figure 32.3 Security Gallery

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.

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30.2 Layout-Based Security

The second level of security is based on restricting functionality for particular layouts. Every layout has a Layout Security Group 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. 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 the password is lost, the only way to open the layout is to send it to De Novo Software.

30.3 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 525. The Administrator is part of the Administrator Security Group. For more information on Security Groups, see Working with Security Groups 532. By definition, the Administrator Security Group can perform every action and cannot be deleted.

To manage users, use the **Application Button→Security→User Administration** command. This will bring up the User Administration dialog (Figure 32.4). Users are listed in alphabetical order by last name.

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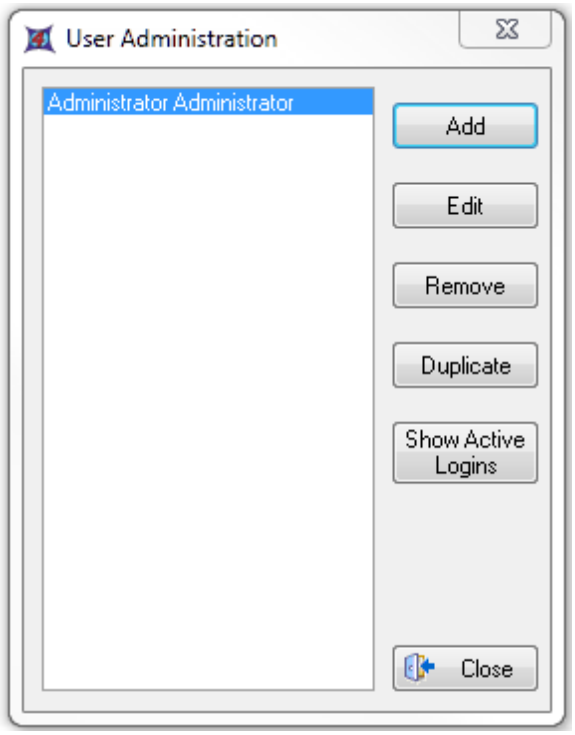

Figure 32.4 User Administration Dialog

Clicking on the **Show Active Logins** button will bring up the **Active Logins** dialog (Figure 32.5) 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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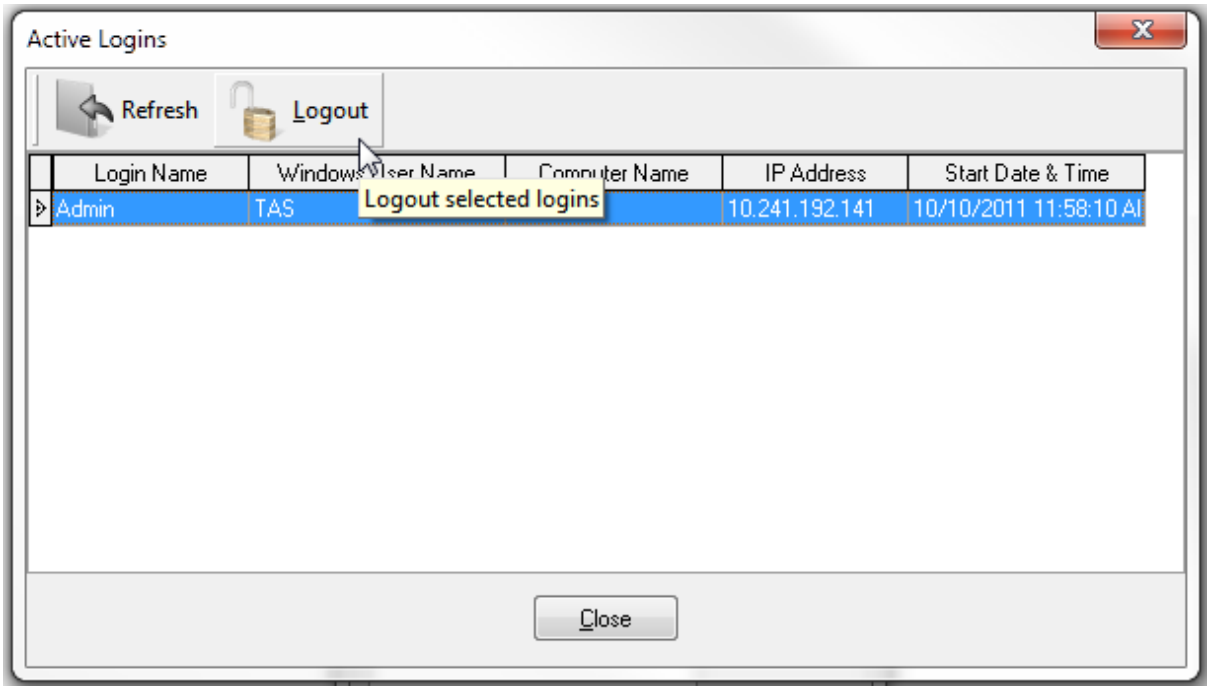

Figure 32.5 Active Logins Dialog

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.

30.3.1 Creating a User

To create a new user:

1. Use the **Application Button→Security→User Administration** command.
2. Click on the **Add** button in the **User Administration** dialog.

The Edit User Information dialog will appear (Figure 32.6).

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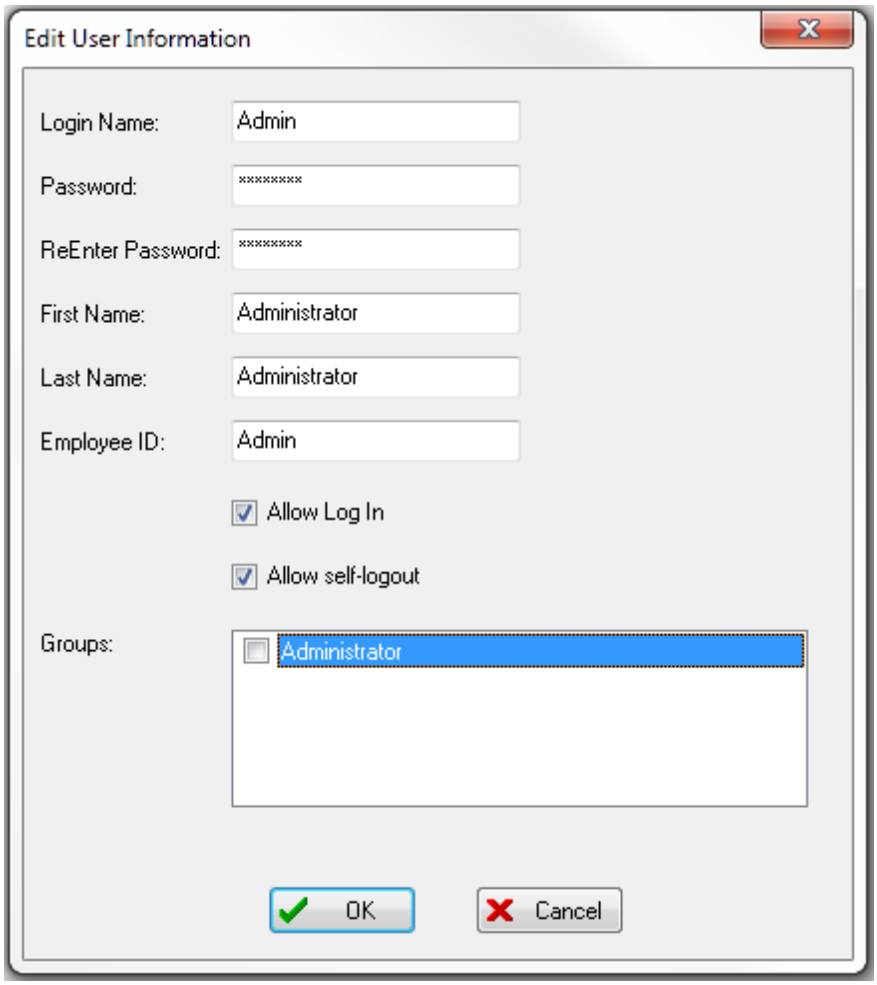

Figure 32.6 Edit User Information Dialog

All of the fields except for **Employee ID** are mandatory. The **Login Name** field must be unique (no two users in FCS Express can have the same Login Name). To manage the Security Groups, see Working with Security Groups 532. 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 remote location.

30.3.2 Editing a User

To edit an existing user:

1. Use the **Application Button→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 **Application Button→System→Edit Information for Current User** command. The **Edit User Information** dialog will appear, but only the password field will be

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enabled (Figure 32.7).

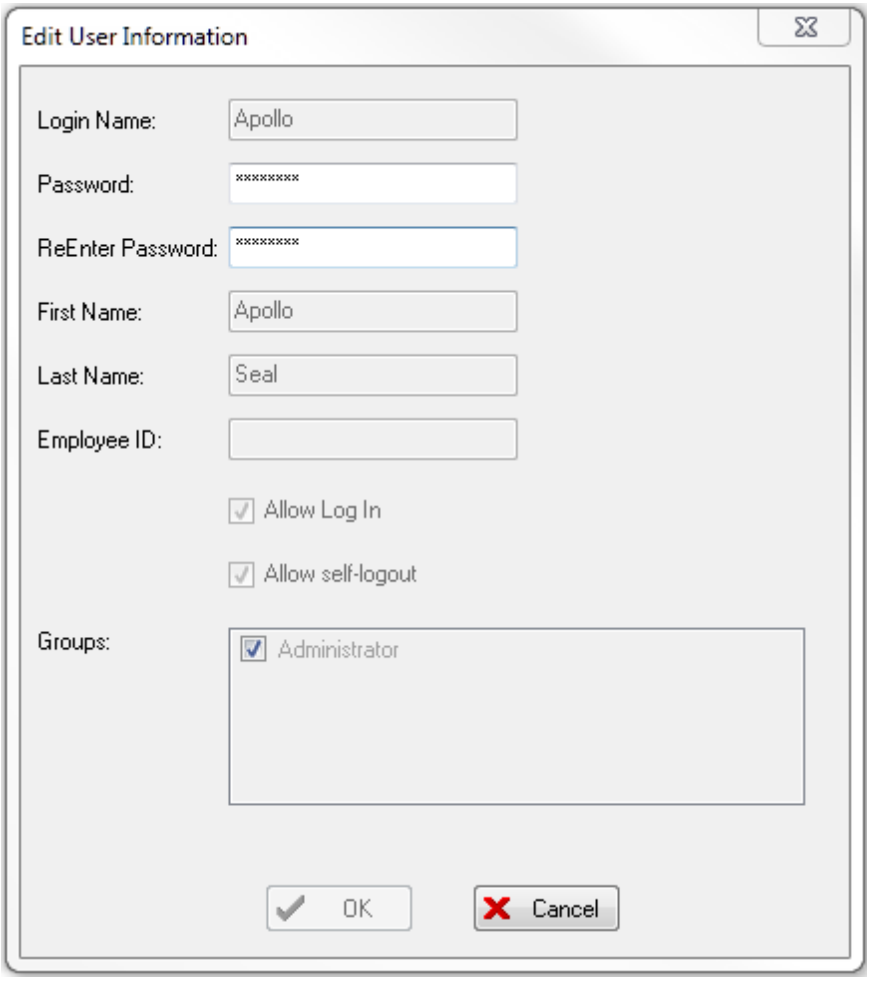

Figure 32.7 Change Current User Password

30.3.3 Deleting a User

To delete a user:

1. Use the **Application Button→Security→User Administration** command.
2. Select the user, and click on the **Remove** button in the **User Administration** dialog.

FCS Express will not allow you to delete the last administrator who can log in.

Note: You cannot undelete a user, so please use this feature with caution.

30.3.4 Editing Security Configurations

FCS Express uses the **Edit Security Configuration** dialog (Figure 32.8) which is accessed from the **Applications Button→Security→Edit Security**

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Configuration to manage Password Policies[528], SMTP Servers[531], and E-Mail Notifications[529].

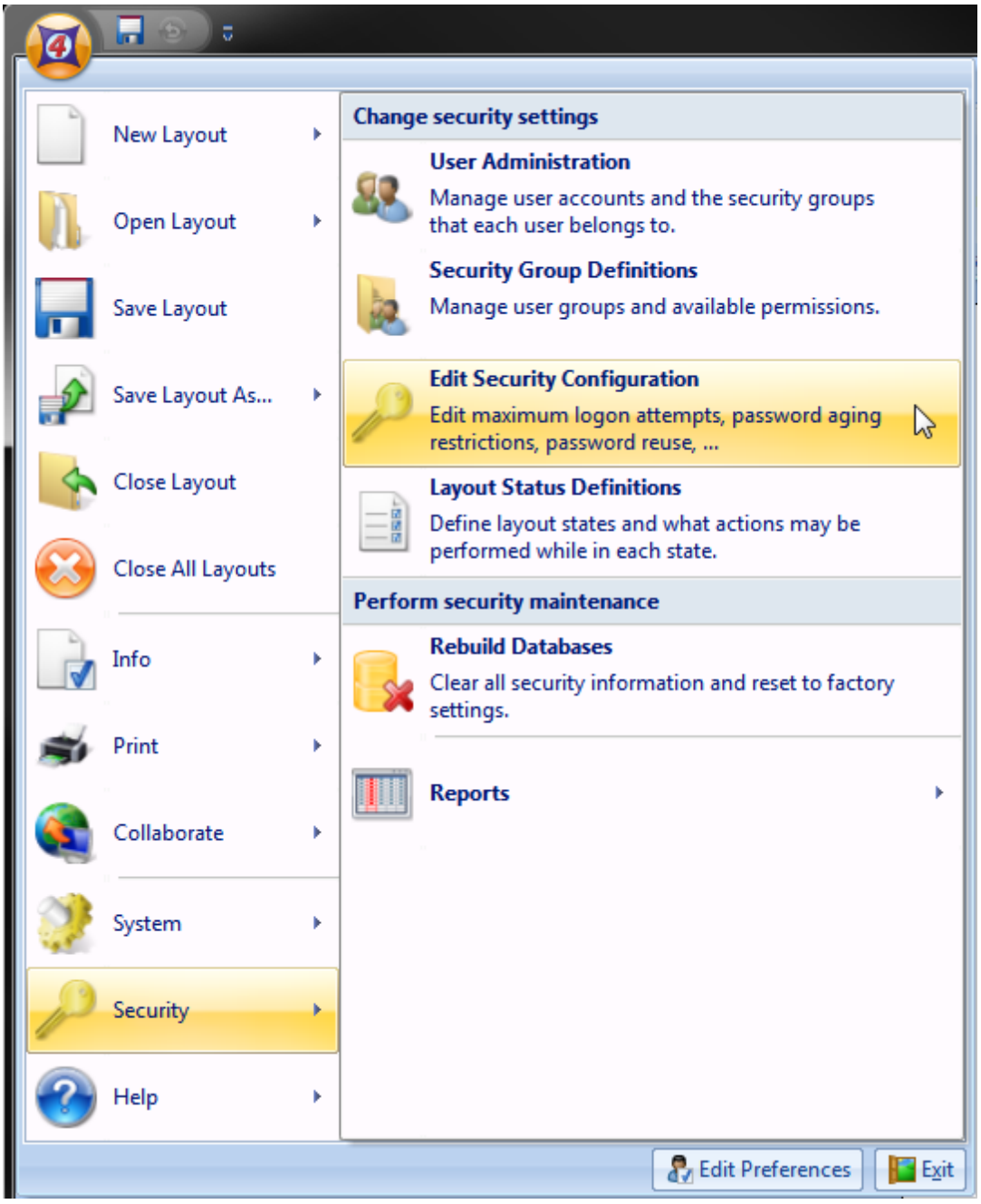

Figure 32.8 Navigating to the Edit Security Configuration dialog

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30.3.4.1 Managing Password Configurations

Note: In previous releases FCS Express used an XML file to manage password configurations. Upon updating and running 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 **Applications Button →Security→Edit Security Configuration**.

The **Edit Security Configuration** dialog will appear as in Figure 32.9 below.

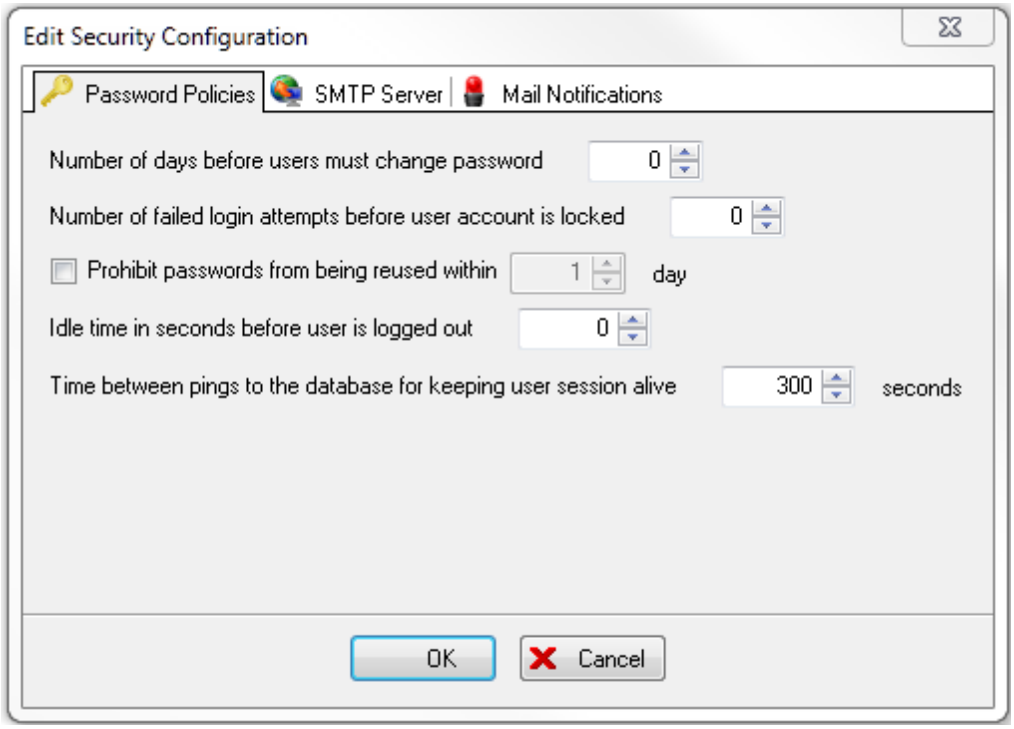

Figure 32.9 The Password Policies Dialog

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	A value of 0 (zero) indicates the user will never

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user is logged out	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 531 or Mail Notifications 529.

30.3.4.2 Mail Notifications

The **Mail Notification** dialog (Figure 32.10) can be accessed through the **Applications Button→Security→Edit Security Configuration→Mail Notifications** tab.

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.

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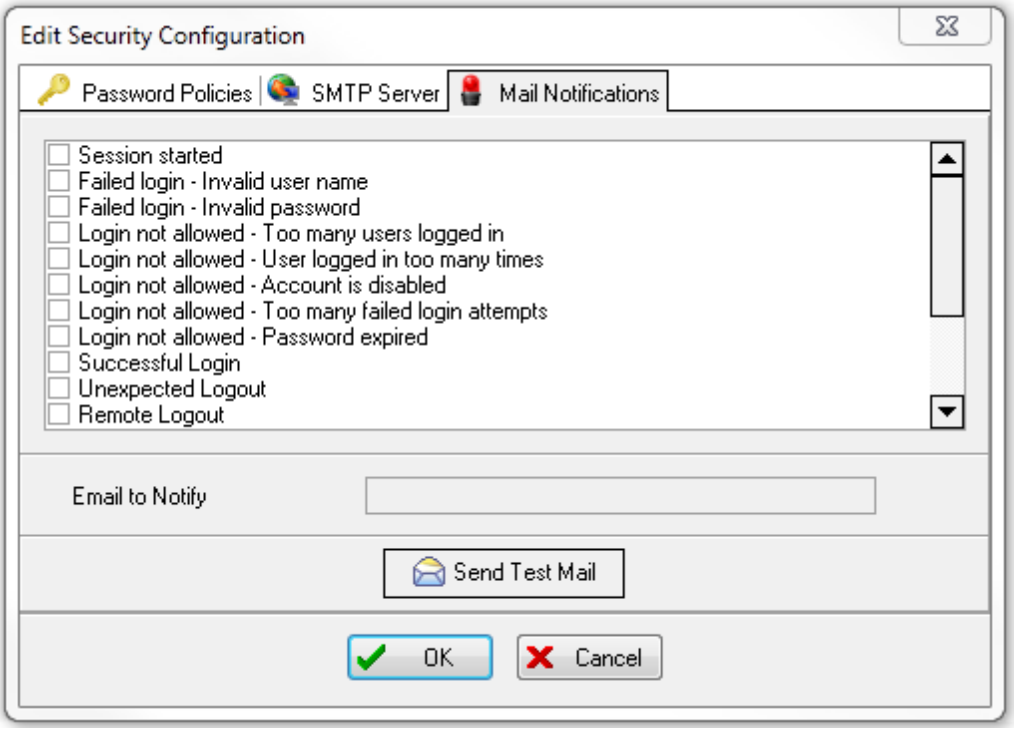

Figure 32.10 Mail Notifications Dialog

To setup a mail notification:

1. Configure your SMTP Server 531.
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
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 when a user tries to log in and and there are too many users are logged

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	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 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

30.3.4.3 SMTP Server

The **SMTP Server** dialog (Figure 32.11) can be accessed through the **Applications Button→Security→Edit Security Configuration→SMTP Server** tab.

Enter the SMTP Server information for your preferred email provider in this category to allow Mail Notifications 529 to be sent out.

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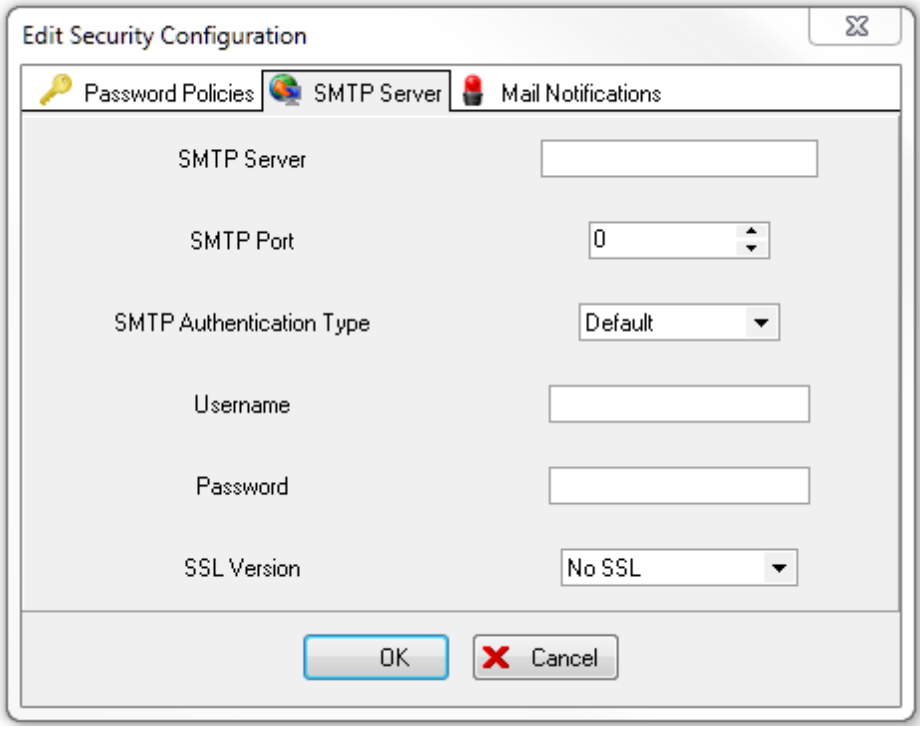

Figure 32.11 The SMTP Server Dialog

30.4 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 32.12.

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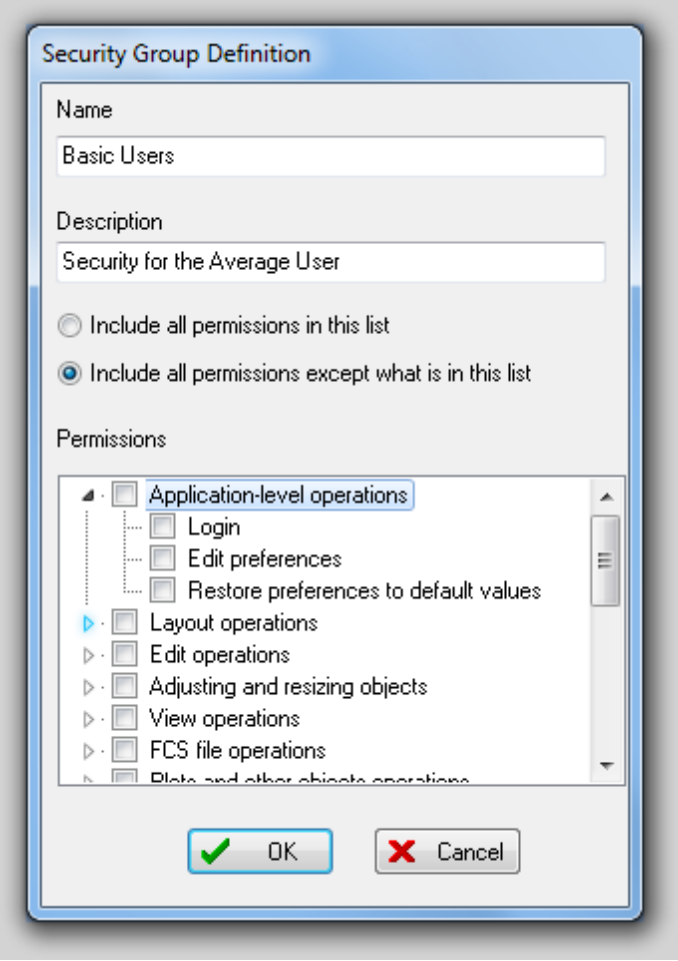

Figure 32.12 Security Group Definition Dialog

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[525]. 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 printing a layout. 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 print the layout, they will see the message shown in Figure 32.13. This notifies the user that they are unable to perform the action.

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Figure 32.13 Operation Not Allowed

30.4.1 Creating a User Security Group

To create a new Security Group, use the **Application Button→Security→ Security Group Definitions** command. The **Security Group Definitions** dialog will appear (Figure 32.14).

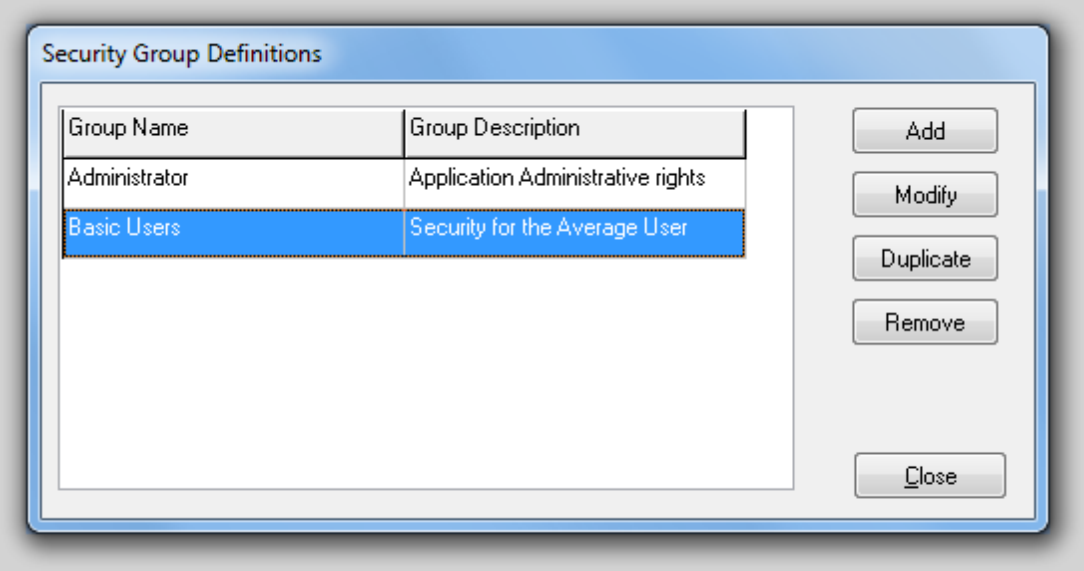

Figure 32.14 Security Group Definitions Dialog

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 32.15).

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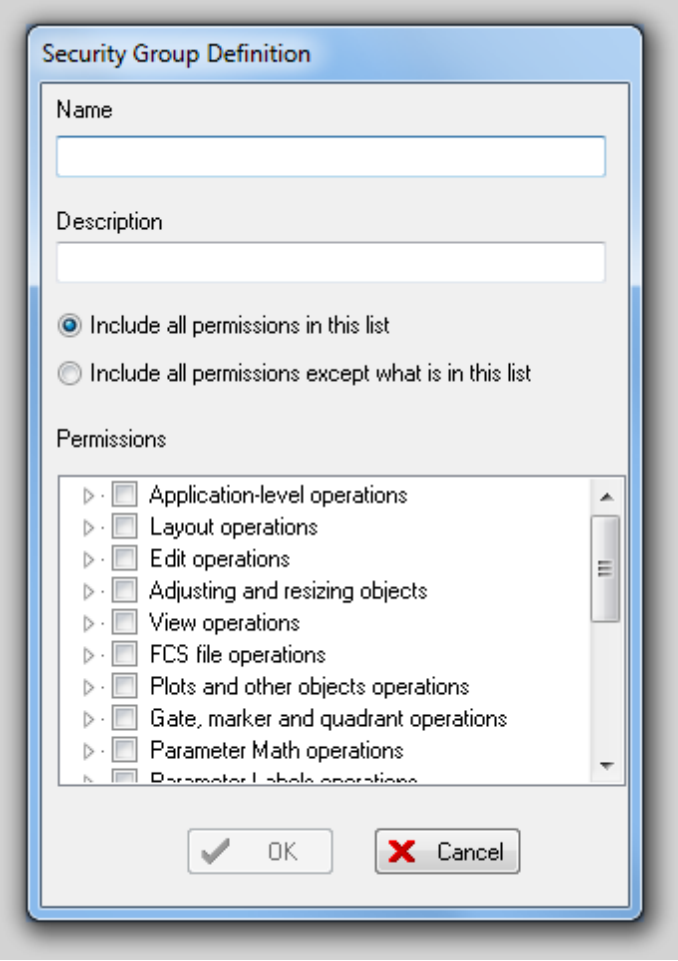

Figure 32.15 Adding a New Security Group

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**.

30.4.2 Editing a Security Group

To edit a Security Group, use the **Application Button→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 532, 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.

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30.4.3 Duplicating a Security Group

Duplicating a group allows you to easily create similar Security Groups. To duplicate a group, use the **Application Button→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, and set the exact permissions you need.

30.4.4 Deleting a Security Group

To delete a group, use the **Application Button→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.

30.5 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 **Application Button→ Security→Layout Status Definitions** command to bring up the **Layout Status Definitions** dialog (Figure (31.16).

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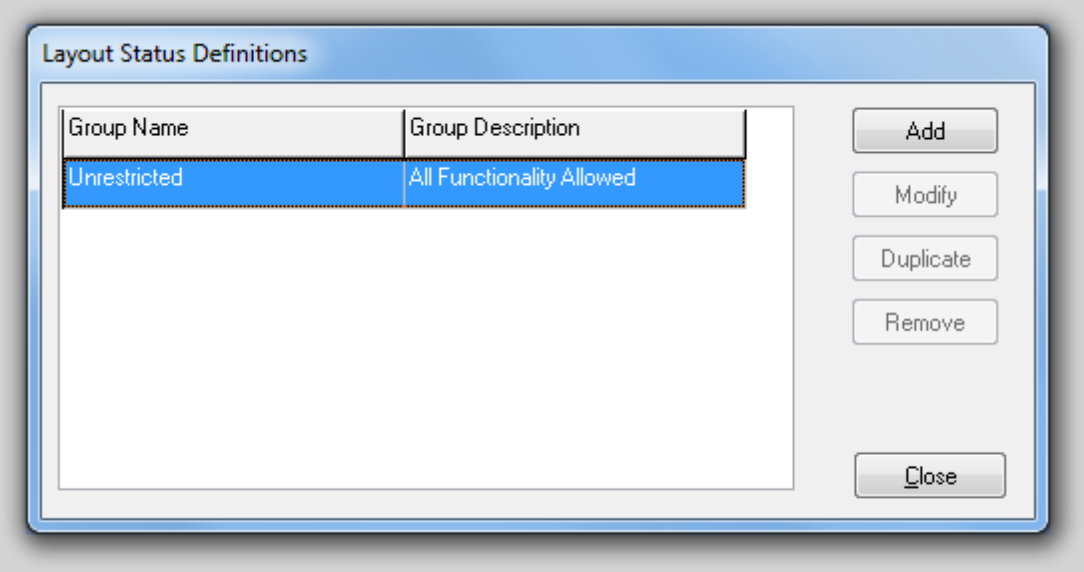

Figure 32.16 Layout Status Definitions Dialog

Layout security groups are modified535, added534, and deleted536 in the same manner as user security groups.

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 **Application Button→Info→Layout Properties** command (Figure 32.17). 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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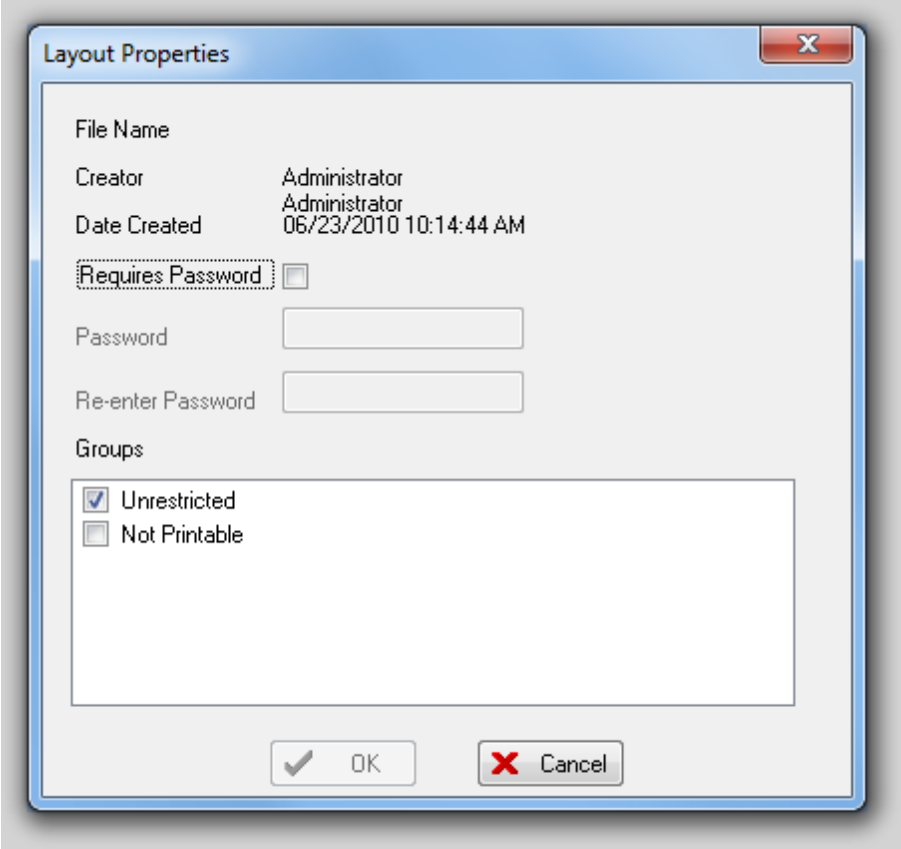

Figure 32.17 Assigning a Layout to a Security Group

The **Layout Properties** 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.

30.6 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.

In order to manage the databases, only a single user can be logged in. If the databases are deleted, they can be recreated by using the **Application Button→ Security→Rebuild Databases** command.

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30.7 Available Security Permissions

- Layout operations
  - Open layout
    - Open Layout
    - Open From FTP
    - Open From SFTP
    - Open From SFTP Connection File
  - Navigators and Other Dockable Windows
    - View Batch Actions
    - View Data List
    - View Alerts
    - View Custom Tokens
    - View Canned Comments
  - Save layout
  - Save layout as
  - Print layout
  - View Print Setup
  - Change Print 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
- Data file operations
  - Open a Data file
  - Modify the contents of a data file

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- 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 file list
- Search the file list
- Remove from the file list
- Format the File List Options
- Move a file Up in the file list
- Move a file Down in the file list
- Increment to the next file on selected objects
- Increment to the next file on all objects
- Increment to the previous file on selected objects
- Increment to the previous file on all objects
- Change file on selected objects
- Change file on 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 Heat Map
  - Insert AutoShapes
- Remove 2D plots
- Remove histograms
- Remove DNA histograms
- Remove Proliferation histograms
- Remove text boxes

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- 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
- 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
- Other plot operations
  - Send plot to BP
- 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
- Load gates
- Save gates
- Add a combination gate
- Change Gate Properties
- Create a new marker
- Modify a marker
- Remove a marker

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- 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

❖ 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
  - View batch processing options
  - Change batch processing options
  - Run 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
    - Save Data Files
      - Add Save Data File
  - Add batch folders
    - Add Folder
    - Add ZIP Folder
    - Add FTP Upload
    - Add Secure FTP Upload
    - Add Icon Lab Folder
    - Add Upload to Starlims

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- Add report batch actions
  - add Export To Excel (Column Mode)
  - add Export To Excel (Cell Mode)
  - add Word Report
  - add PDF Form
  - add XML Report
  - add Text Report
  - add Export To US Labs
  - add Export To EMMES
  - add Export To Data Innovations
  - add Export to Etelenext (Clarient)
  - add Export to Etelenext (Neogenomics)
  - add Export to Etelenext (CSI-Labs)
  - add Export To StarLims
  - add Export To GenPath/Flow
  - add Export To DDS
  - add Database Report
  - add RLD Report
  - add RLD-II Report
  - add Export To Vital Axis
  - add Export to Power
- 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
  - add Published Layout
  - add Published Layout (CSI)
- Rename batch actions
- Paste batch actions
- 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

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- 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
- View compensations
- Change compensation settings
- Load compensations
- Save compensations
- Create compensation
- Edit compensation
- Delete compensation
- Assign compensation
- Add a target
- Edit a target
- Delete a target
- Clear targets
- Add a source
- Edit a source
- Delete a source
- Run Automatic Compensation

❖ Other tools
- Modify Concentration calculator settings
- Concentration calculator calculate
- Histogram subtraction
- Batch export
- Diva Import
- C6 Import

❖ Security Operations
- Layout Status Definitions
  - View Layout Status List
  - Add new Layout Status
  - Modify a Layout Status
  - Duplicate a Layout Status
  - Remove a Layout Status
  - Press OK on the Layout Status Definition Screen
- Electronic Signature operations
  - View signature list
  - Change signature list
  - Sign
  - Unsign

❖ Help operations

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- 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
- Next Selected
- Font

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- 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

30.8 Security Database Options

If you purchase FCS Express with the Security add-on, you have the choice of three database options

- Microsoft SQL Server
- ElevateDB Local Server
- ElevateDB Client Server

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 547, and share the same security system. Note that to use FCS Express with SQL Server, you must purchase your own license for SQL Server.

ElevateDB local server

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The ElevateDB 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.

ElevateDB client server

The ElevateDB client server uses the same ElevateDB databases as the ElevateDB local server, however, the databases are accessed via a database server. This requires the installation of the ElevateDB database server on the computer which will host the database files. The ElevateDB server is a very low maintenance server that installs easily and offers the advantages of a client server achitecture. This option can be provided for free with the purchase of your security system.

30.9 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.

1. Create a database in the SQL server instance. This will be the database that FCS Express will use.
2. Create a file in the same folder where you have installed FCS Express and call it FCSEDB.ini. Place the following text in that file

If you will be using an ADO connection

[MSSQLConnection]
ConnectionString=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

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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:

[MSSQLConnection]
ConnectionString=Provider=SQLOLEDB.1;Initial Catalog=<database>;Data Source=<Database Host>\<Database Instance>;User ID=<user name>; Password=<password>

If you will be using an ODBC connection

[MSSQLConnection]

ConnectionString=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.

3. Save the modified FCSEDB.ini file into the folder where FCS Express is installed

When FCS Express starts, it will connect to your database and automatically create all the required tables and indices.

30.10 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.

ElevateDB Local Server

ElevateDB 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:

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1. Make sure everyone is logged out of FCS Express, and that the program is shut down on every computer.

2. Copy the folder ElevateDBData to your backup storage location.

To restore, simply copy the backed up folder back to the original location.

ElevateDB Client Server

When you set up ElevateDB Client Server, you will have a program called ElevatedDB Manager. ElevateDB Manager has Backup and Restore functionality built in. To back up a database, 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.

2. Run ElevateDB Manager.
   a. The first time you are backing up your database, create a Store:
      i. In the navigation tree, right-click on the **Stores** node.
      ii. Select Create New Store...
      iii. A dialog box will allow you to select the name of the store, and the location. Enter a name, and in the Local Store tab, select a folder location for your backups.
   b. In the navigation tree, expand the **Databases** node.
   c. Right-click on your database
   d. Select **Backup Database...**
   e. A dialog box will allow you to select the name for your database, and to select the Store.

To restore the database, right-click on your database as above, and select **Restore**. A dialog box will allow you to select the Store and the particular backup you want to restore from.

30.11 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 548 for details. To determine whether a specific release will update the database, please check the release notes on the download page.

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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.

30.12 Security Reports

FCS Express allows you to view and manage reports for user activity. Reports can be accessed from the **Applications Button→Security→Reports** gallery (Figure 32.18).

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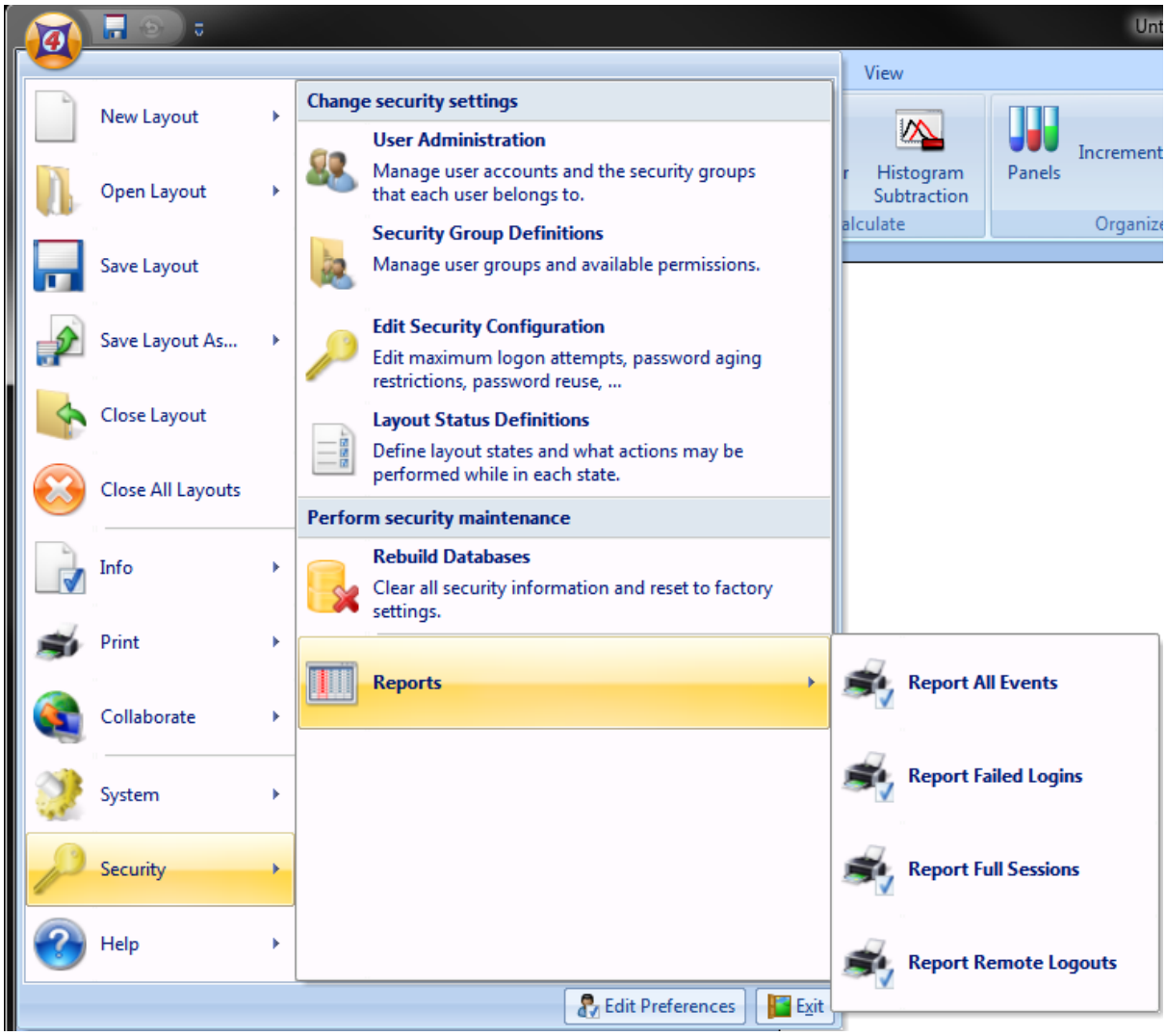

Figure 32.18 Navigating to the Security Reports Gallery

Four types of reports are available (click on each link for more details):

- **Report All Events** 552 **-** Lists a history of all logging events
- **Report Failed Logins** 553 **-** Reports a list of failed logins and the reason for failure
- **Report Full Sessions** 554 **-** Reports a list of all full sessions in FCS Express
- **Report Remote Logouts** 555 **-** Reports a list of all logouts from a remote location

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All security reports contain a list of columns that can be arranged 556 or grouped 556 as well as the option to define Filter Criteria 556 and **Refresh the Report** for the new filter (Figure 32.19). You also have the option to export the report 561 in the following formats:

- **TXT**
- **HTML**
- **XML + XSL**
- **XLS**

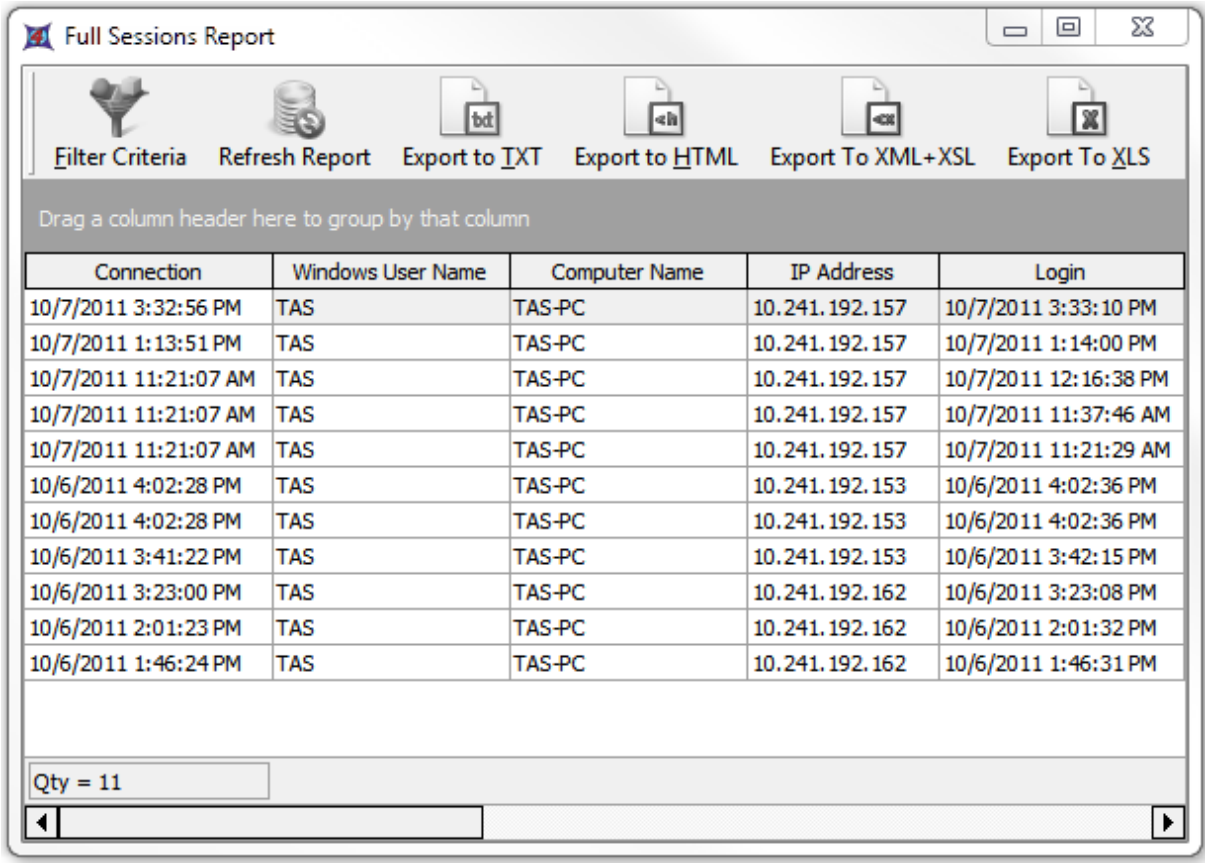

Figure 32. 19 Example of a Reports Dialog

30.12.1 Report All Events

Report All Events can be accessed from the **Applications Button →Security→Reports→Report All Events**.

Choosing an **All Events Report** (Figure 32.20) 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 556 according to your needs.

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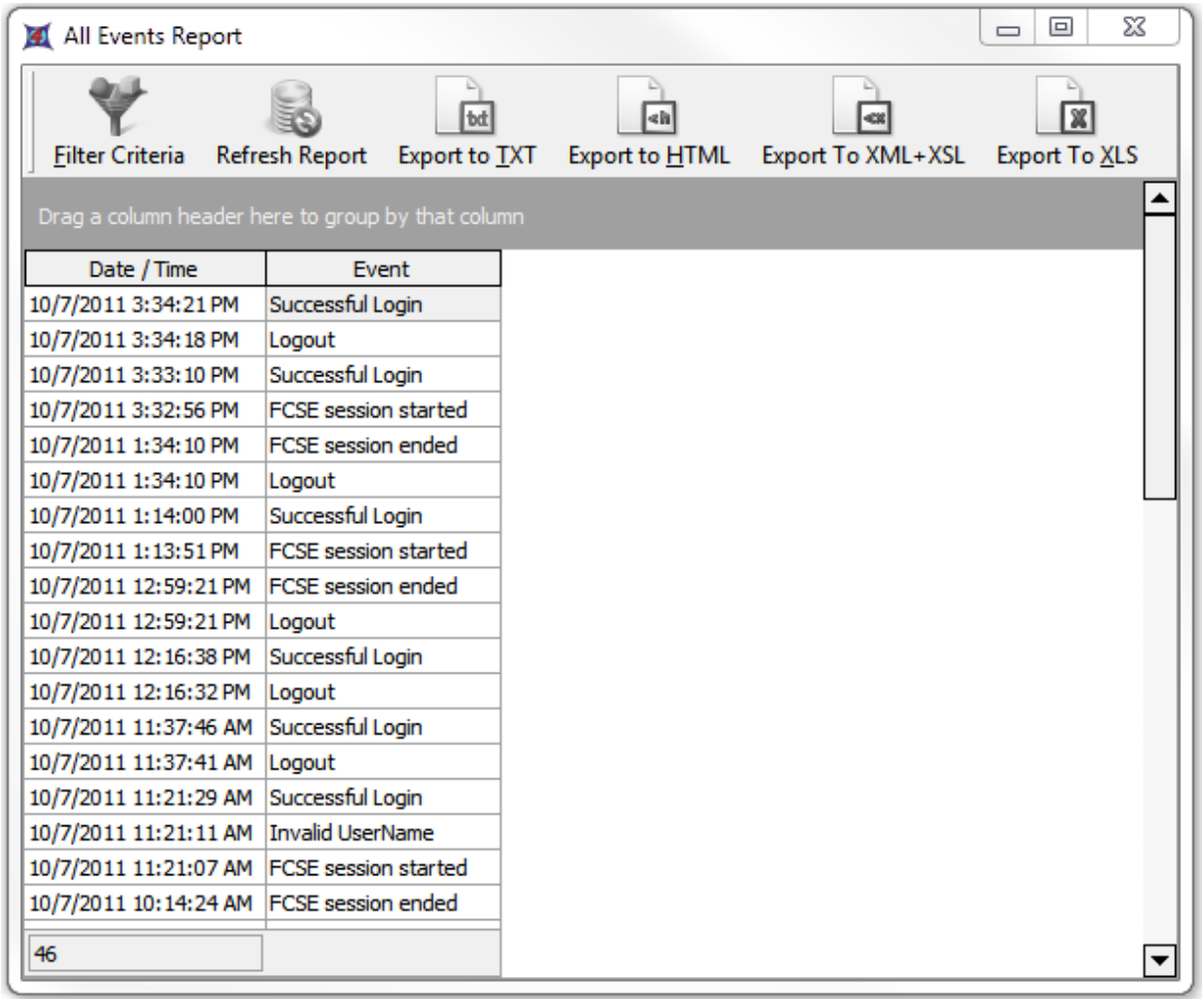

Figure 32.20 The Report All Events Report Dialog

30.12.2 Report Failed Logins

Report Failed Logins can be accessed from the **Applications Button →Security→Reports→Report Failed Logins**.

Being able to track failed logins in a requirement of the 21 CFR Part 11.

Choosing a **Failed Logins Report** (Figure 32.21) 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

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The report can be arranged, grouped, and filtered556 according to your needs.

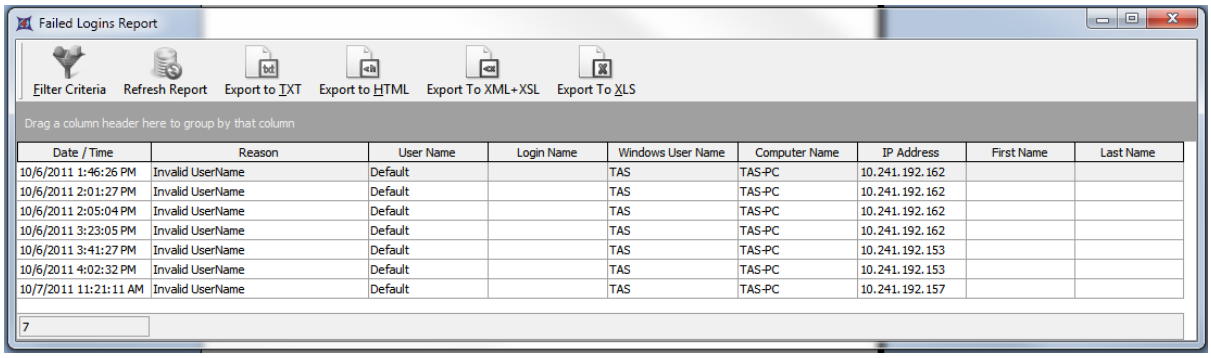

Figure 32.21 The Failed Logins Report Dialog

30.12.3 Report Full Sessions

Report Full Sessions can be accessed from the **Applications Button →Security→Reports→Report Full Sessions**.

Choosing a **Full Sessions Report** (Figure 32.22) 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 filtered556 according to your needs.

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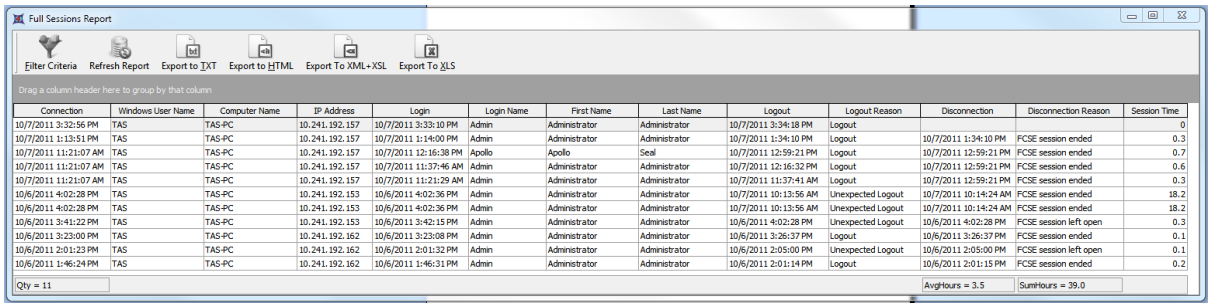

Figure 32.22 The Full Session Report Dialog

30.12.4 Report Remote Logouts

Report Remote Logouts can be accessed from the **Applications Button →Security→Reports→Report Remote Logouts**.

Choosing a **Remote Logouts Report** (Figure 32.23) 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 556 according to your needs.

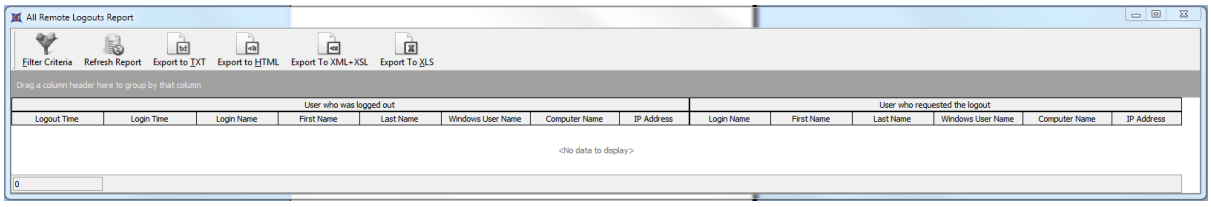

Figure 32.23 The Remote Logouts Report Dialog

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30.12.5 Arranging, Grouping, and Filtering Reports

All security reports contain a list of columns that can be arranged 556 or grouped 557 as well as the option to define Filter Criteria 560 (for a date range) and **Refresh the Report** for the new filter.

Please see the following topics for details:
- Arranging Columns 556
- Grouping Reports 557
- Filtering on a Date Range 560

30.12.5. 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 32.24).
   - 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 32.25).

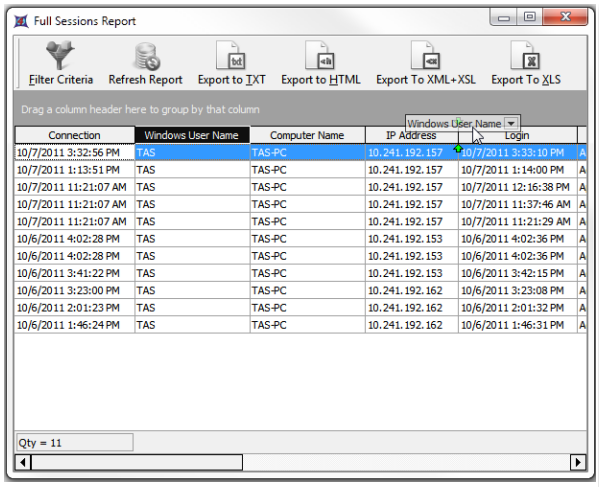

Figure 32.24 Dragging a column to a new location.

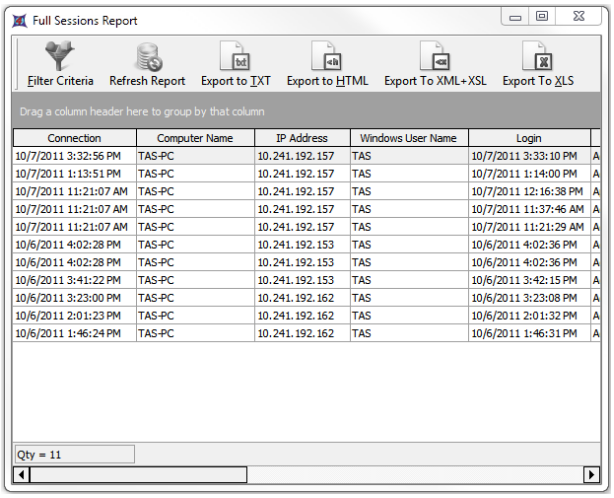

Figure 32.25 The report after the column has been moved.

Date range filtering may also be done on any column, group, or custom filter using the Filter Criteria 560 button.

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30.12.5. 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 32.26).
   - 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 32.27).

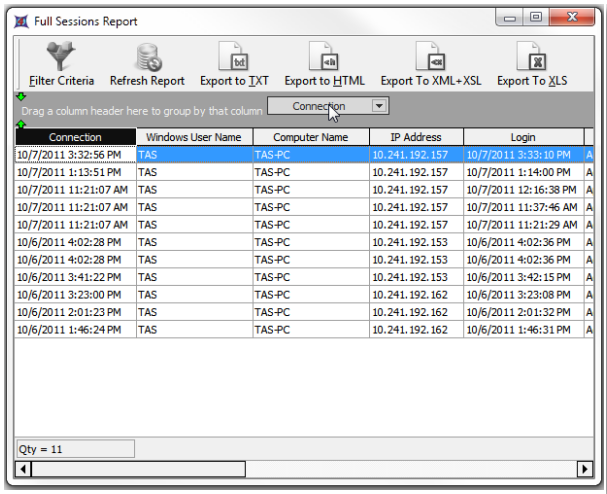

Figure 32.26 Dragging a column header into the grouping area

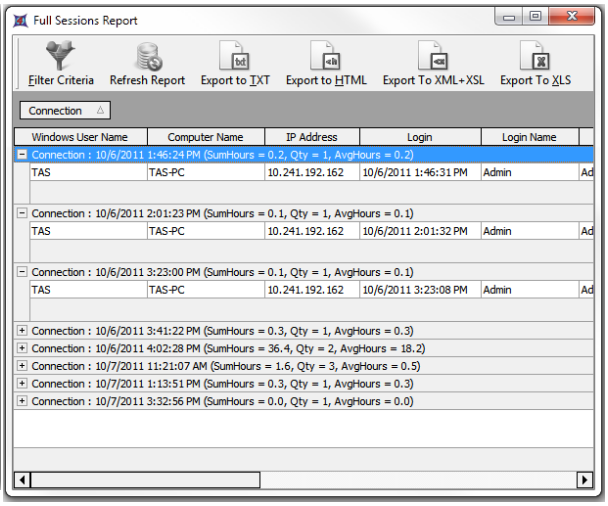

Figure 32.27 A report after being grouped by Connection

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 32.28).
   - In the example below the column header for Computer Name and IP Address were subsequently was dragged to the grouping area.

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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.

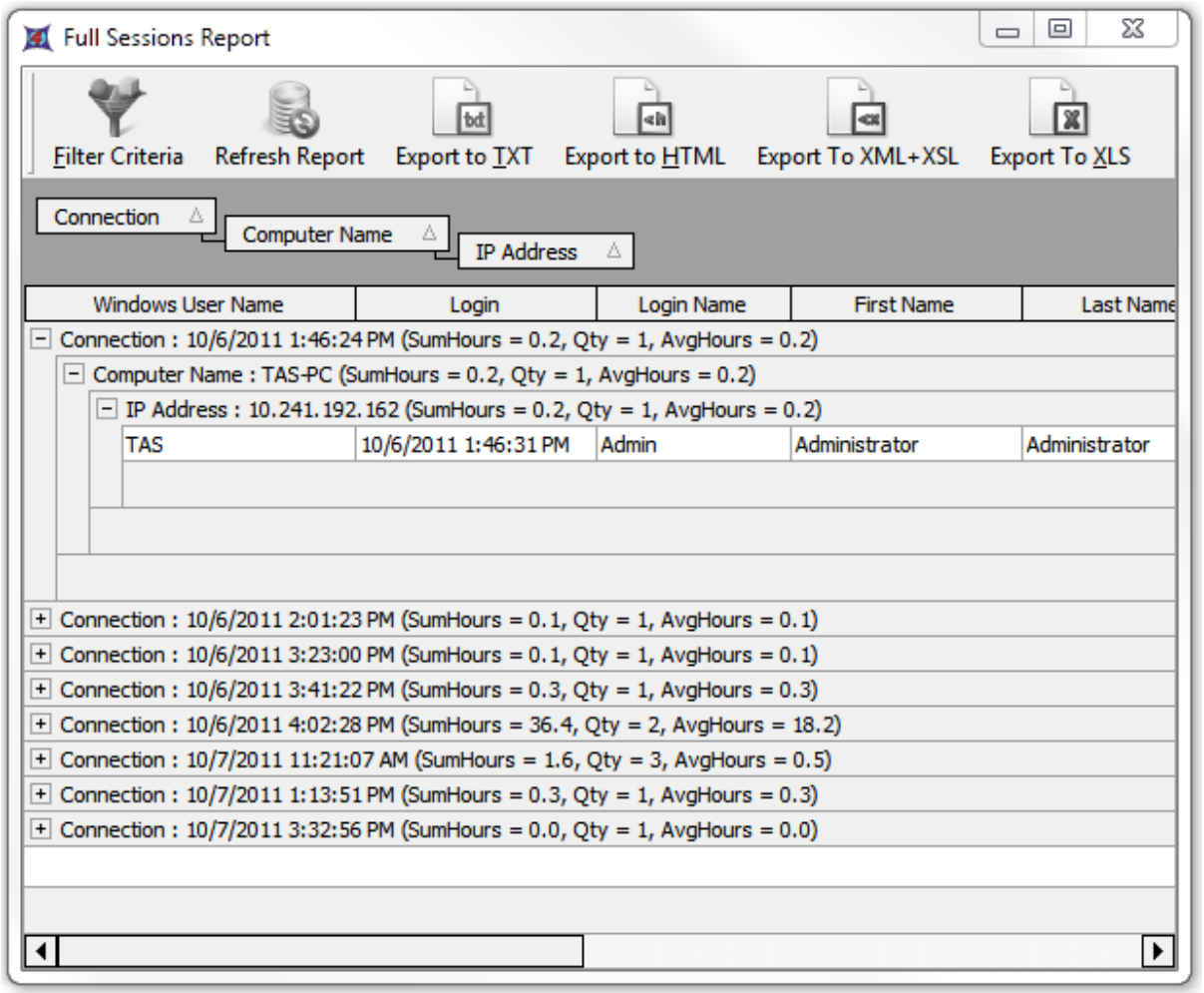

Figure 32.28 Using the grouping feature to create sub-groups

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 32.29).

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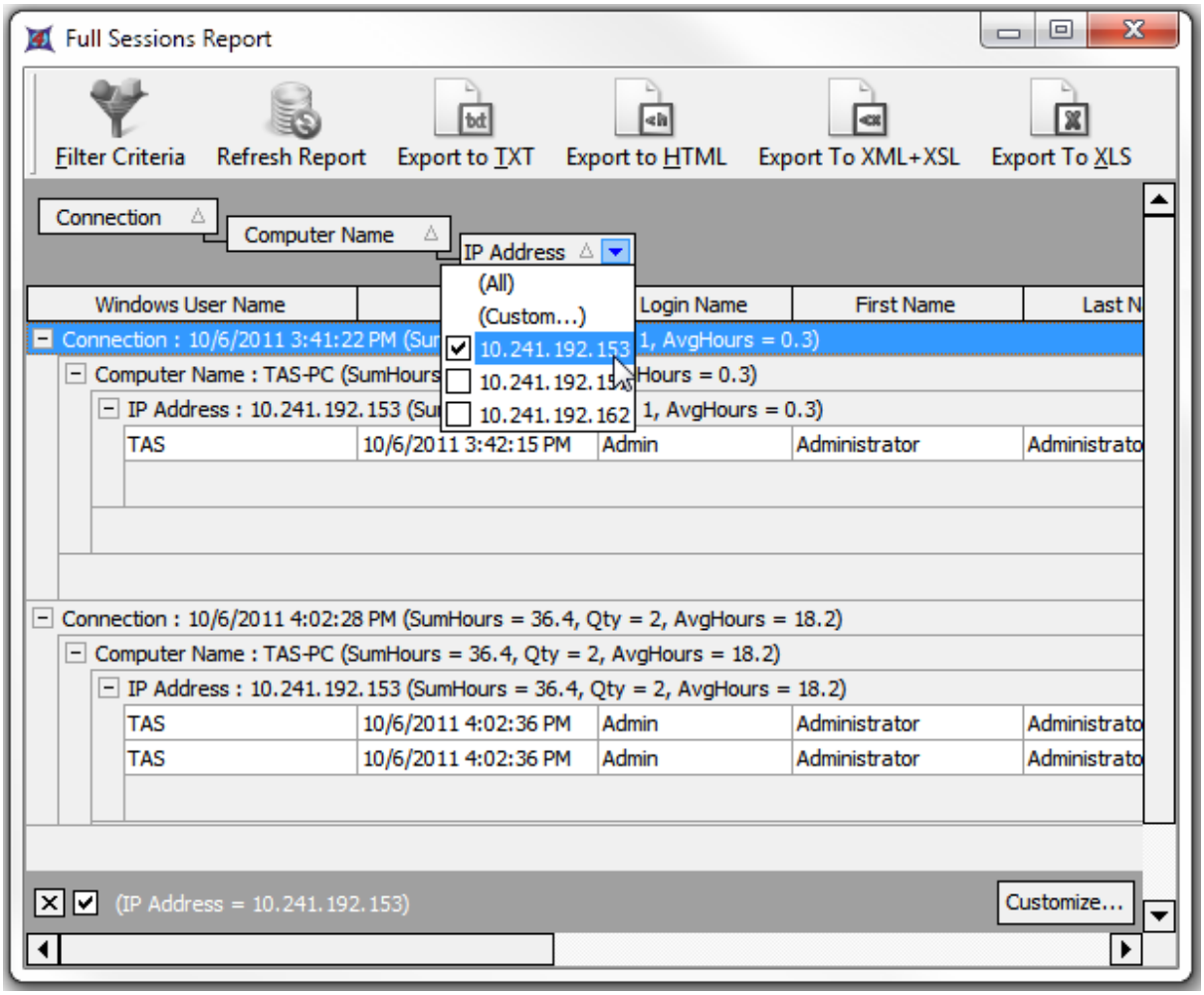

Figure 32.29 Using a check box to further classify a group or sub-group

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 32.30 and (Figure 32.31 below).

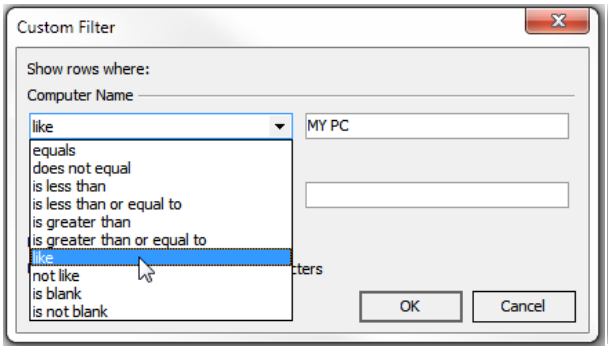

Figure 32.30 Choosing a custom classification for reports

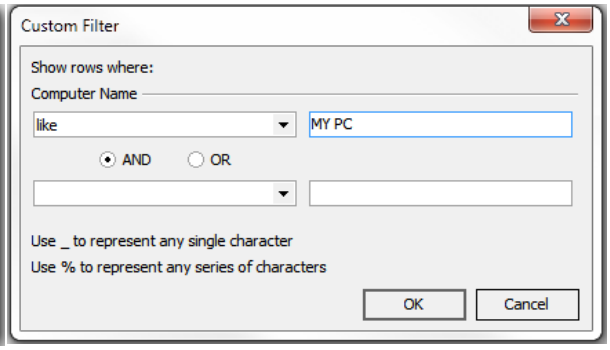

Figure 32.31 Using a custom classification for reports

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Date range filtering may also be done on any column, group, or custom filter using the Filter Criteria 560 button.

30.12.5. 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 32.32).
3. Click **OK**.

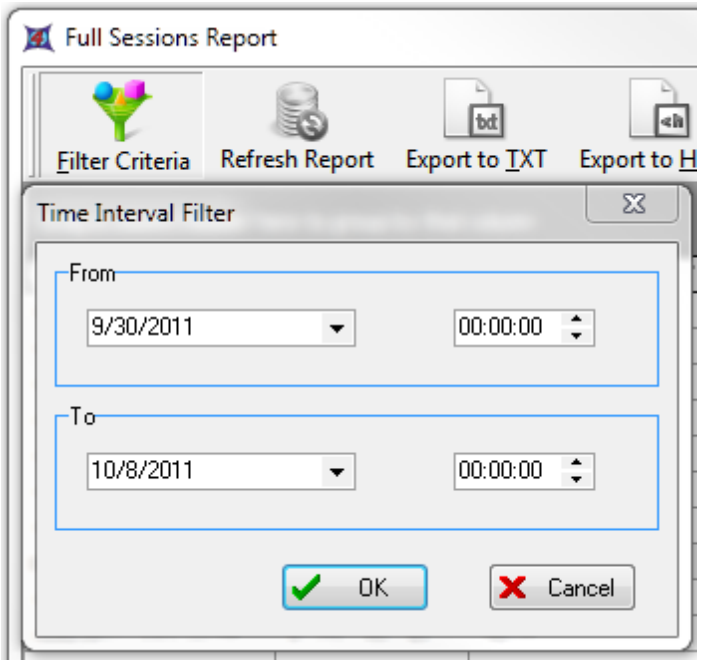

Figure 32.32 Choosing the date and time Filter Criteria

4. Click on the **Refresh Report** button to apply the new **Filter Criteria** to the report (Figure 32.33).

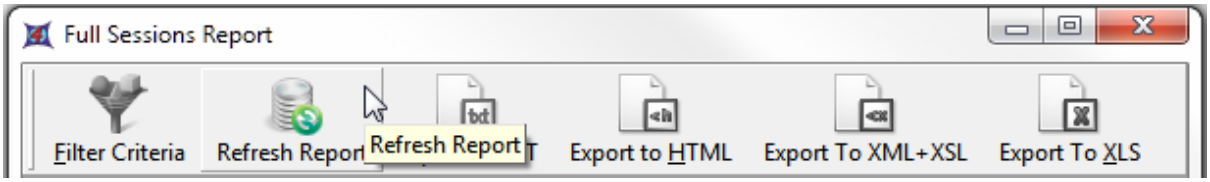

Figure 32.33 Choosing to refresh the report to the new Filter Criteria

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30.12.6 Exporting Reports

You have the option to export any report in the following formats:

- **TXT**
- **HTML**
- **XML + XSL**
- **XLS**

To export a report:

1. Click on the button for the export type you want (**Export to HTML** in this examples) (Figure 32.34).

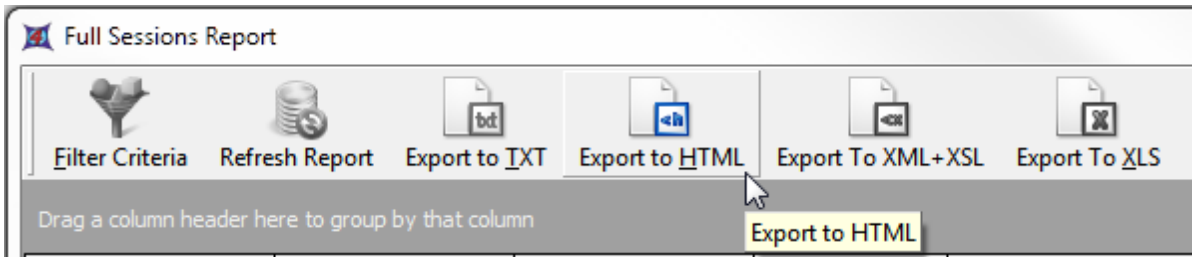

Figure 32.34 Choosing to export the report as an HTML file

2. Choose a location and name for the file on your drive (Figure 32.35).
3. Click **Save**.

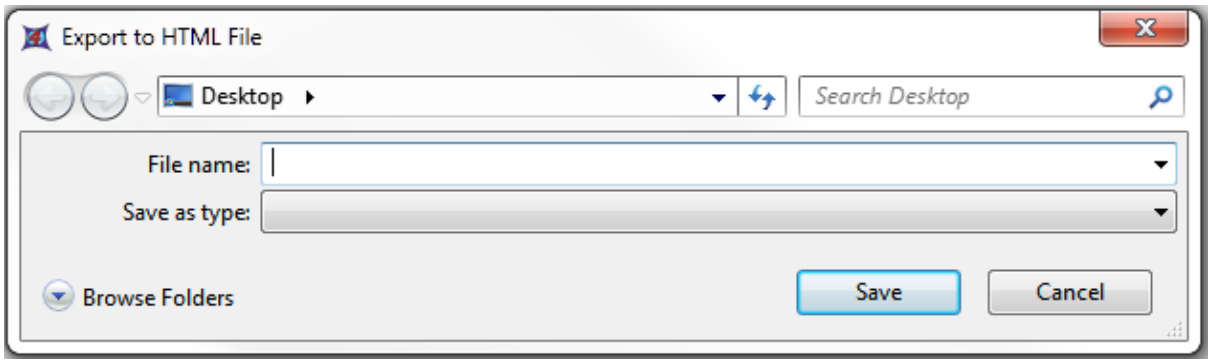

Figure 32.35 The export to HTML file dialog

30.13 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:

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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 analyses.

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

31 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 features520. With the security features activated, a new **Electronic Signatures** gallery will be present when you select **Application Button→Info** (Figure 33.2).

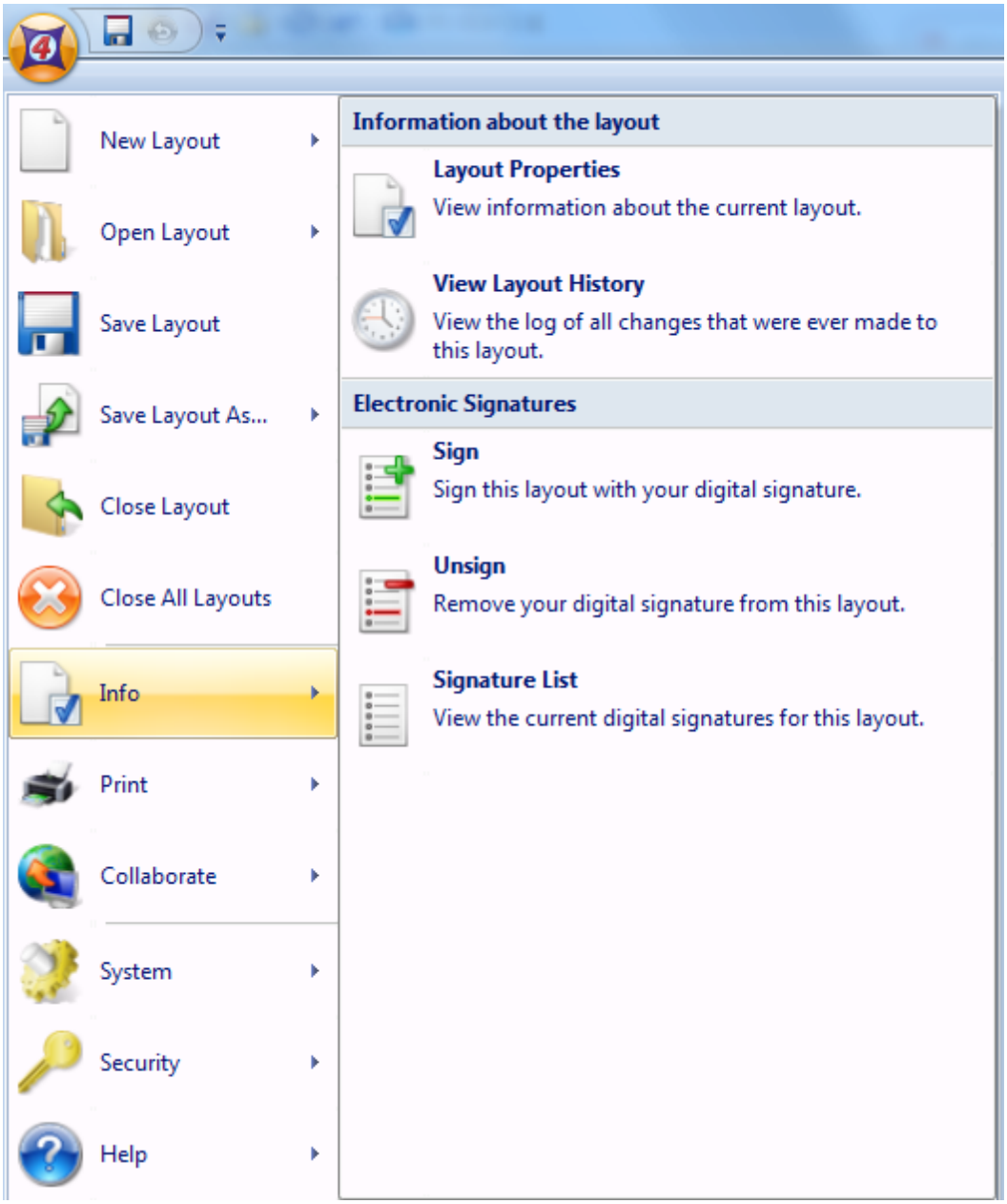

Figure 33.2 Electronic Signatures Gallery

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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.

31.1 Maintaining Signatures

Signatures can be maintained from the **Signature List** dialog (Figure 33.3) by selecting the **Application Button→Info→Signature List** command.

Figure 33.3 Signature List Dialog

When you are finished adding 565, modifying 566, or deleting 567 signatures, press **OK** to save your changes or **Cancel** to discard them.

31.1.1 Adding Signatures

To add a new signature:

1. Use the **Application Button→Info→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 33.4).

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Figure 33.4 Adding a New Signature

The signature properties are described in the section on Modifying Signatures 566.

31.1.2 Modifying Signatures

To modify an existing signature:

1. Use the **Application Button→Info→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 33.5).

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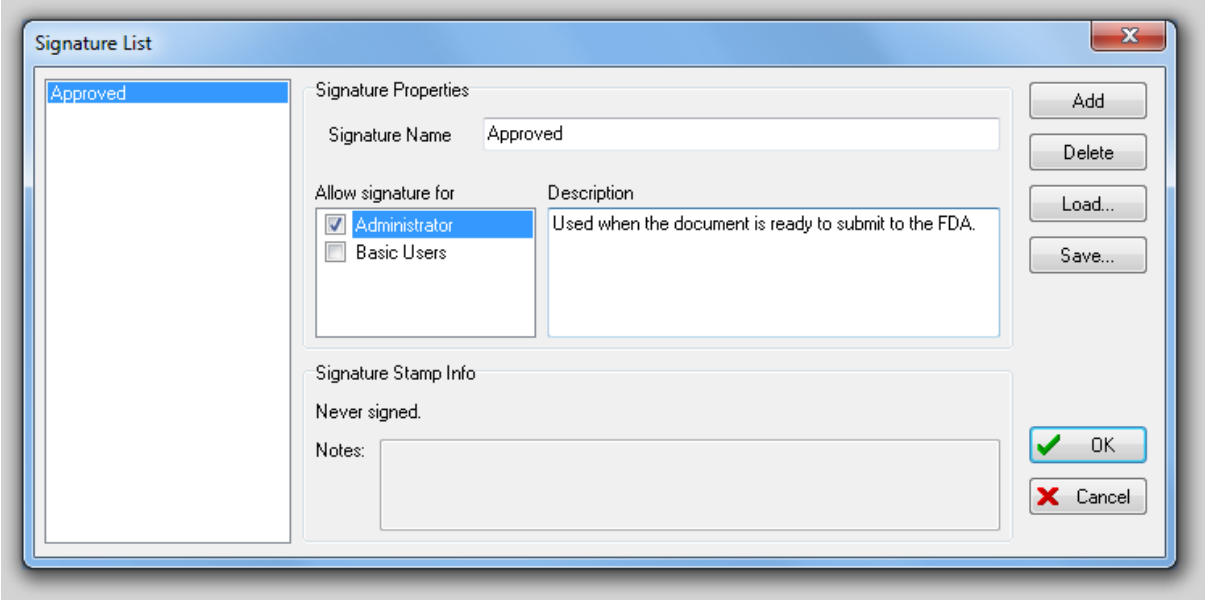

Figure 33.5 Modifying a Signature

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[532] 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.

31.1.3 Deleting Signatures

To delete a signature:

1. Use the **Application Button→Info→Signature List** command to bring up the **Signature List** dialog.
2. Select the signature from the list on the left and press the **Delete** button.

31.2 Signing Layouts

To sign a layout, use the **Application Button→Info→Sign** command to bring up the Sign dialog (Figure 33.6).

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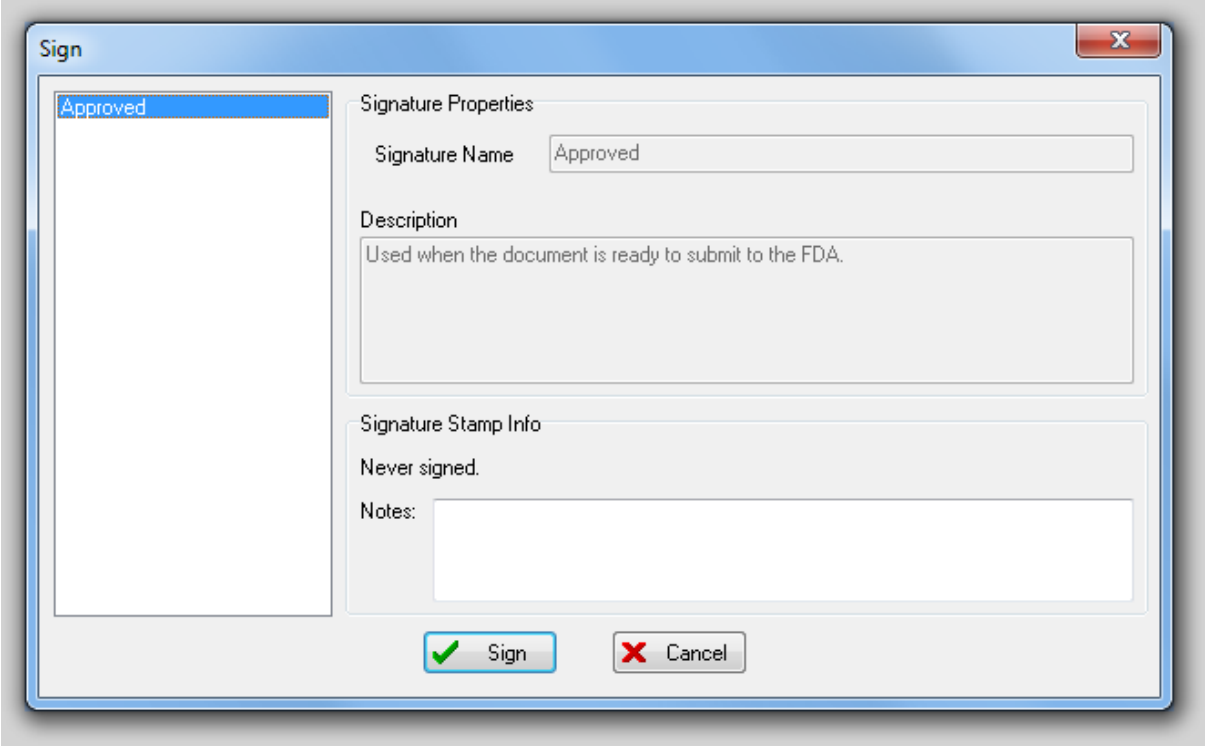

Figure 33.6 Signing a Layout

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 can be entered in the **Notes** field. After pressing **Sign**, the user will be required to confirm their password.

31.3 Unsigning Layouts

To unsign an already signed layout, use the **Application Button→Info→Unsign** command to bring up the **Unsign** dialog (Figure 33.7).

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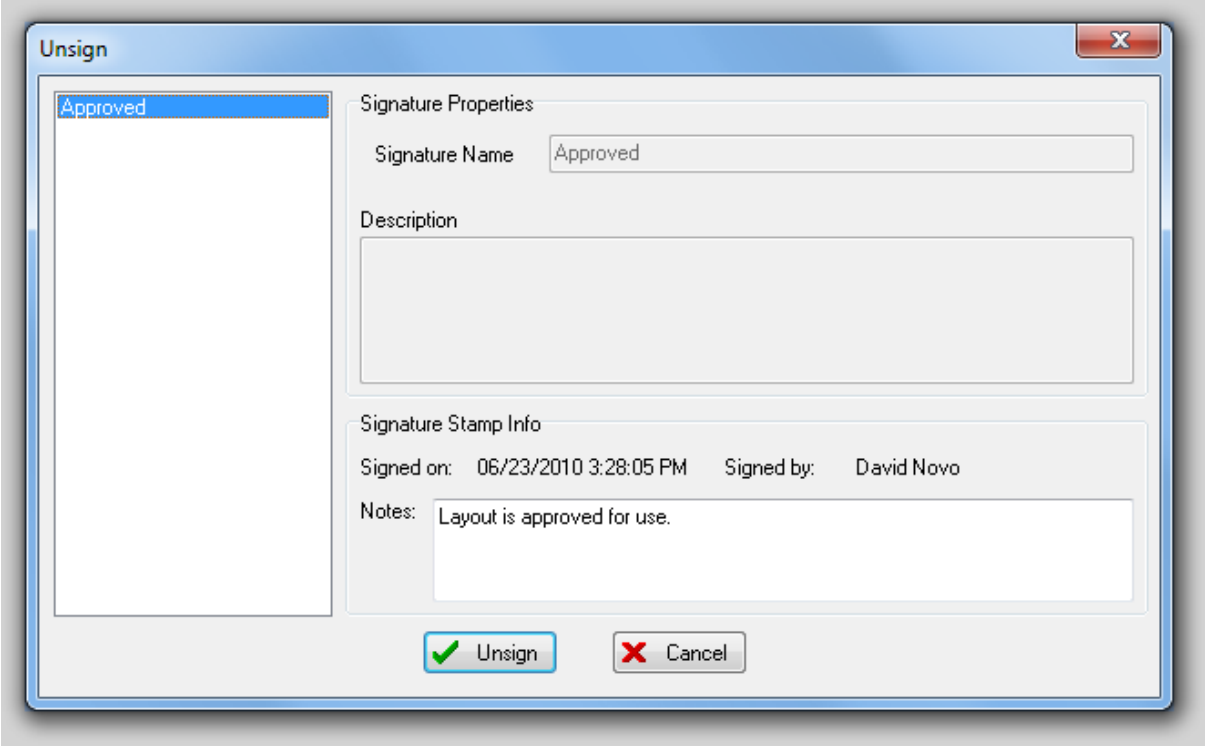

Figure 33.7 Unsigning a Layout

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.

31.4 Displaying Signature Information

Once you have created and set up your signatures 565 in FCS Express, you may want to display their details and signing status 574 on your layout pages.

By default, the signature 565 details and status do not appear on a layout page. However, you can easily display the information about the signatures 565 using signature token 328.

A signature token can display the following kinds of information:

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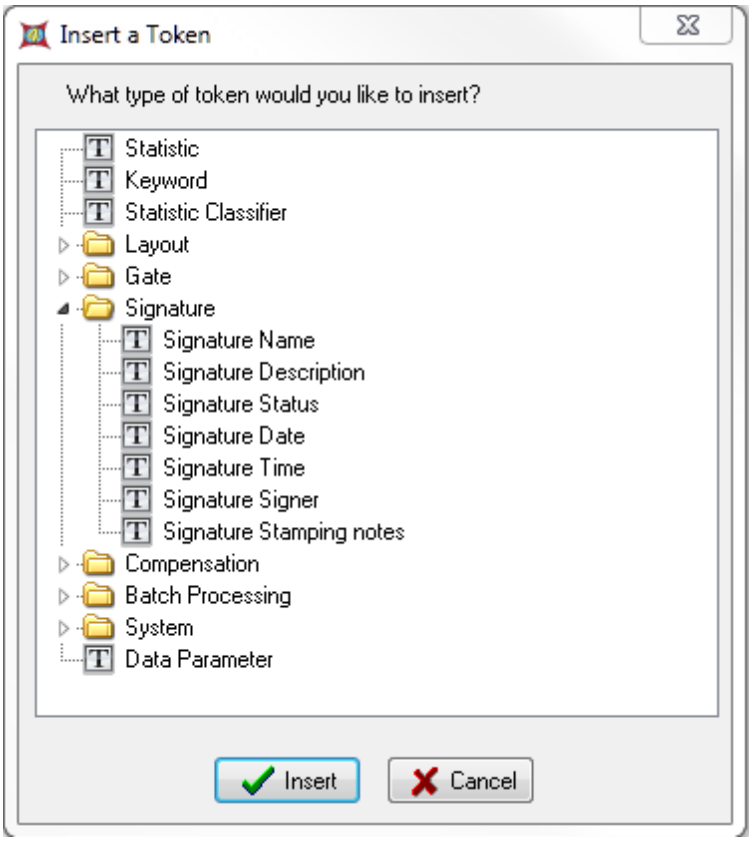
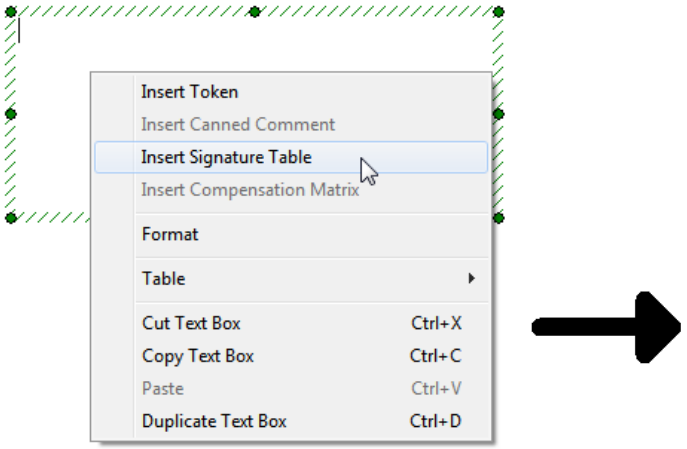
1. **Signature Name**
2. **Signature Description**
3. **Signature Status**574
   Never Signed|Signed|Unsigned
4. **Signature Date**
5. **Signature Time**
6. **Signature Signer**
   Never Signed *or Name*
7. **Signature Stamping Notes**



To display the signature tokens, you need to first create a text box675. Once you have the text box675, there are two ways to insert signature tokens into the text box675:

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.



Signature Name	Status	Signer	Date
Initial Analysis Complete	Signed	Administrator	07/21/2011 10:42:36 AM
Peer Reviewed	Unsigned	Administrator	07/21/2011 10:43:22 AM
Approved	Unsigned		07/21/2011 10:41:11 AM

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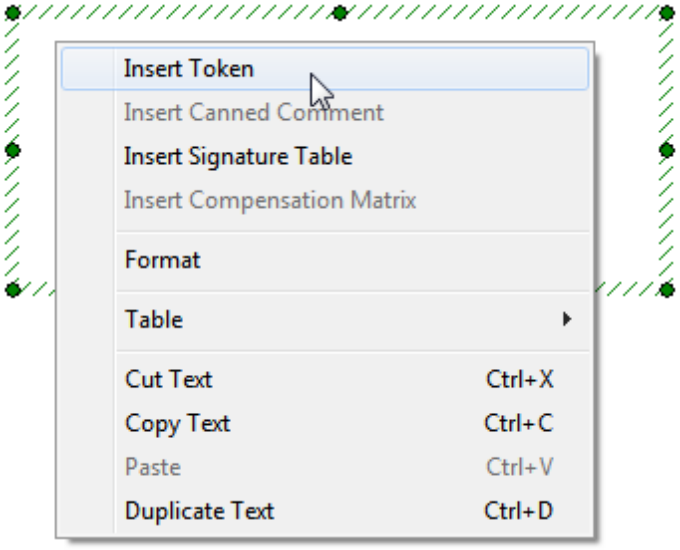
2. Using individual signature tokens

a. Right-click in the text box, and select **Insert Token**328. The **Insert a Token**328 window will appear.



b. In the **Insert a Token**328 window, expand the **Signature**565 node then select the type of signature565 information you want to display, and press **Insert** (in this example we will choose **Signature Name**). The token editor will appear.

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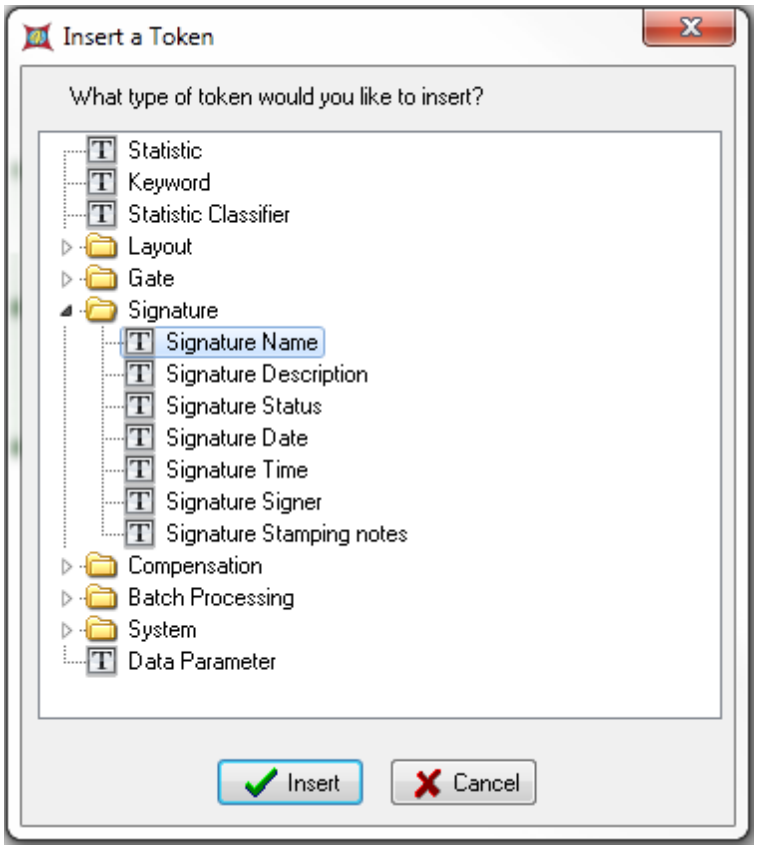

c. In the token editor click on the **Signature information** category to confirm your choice.

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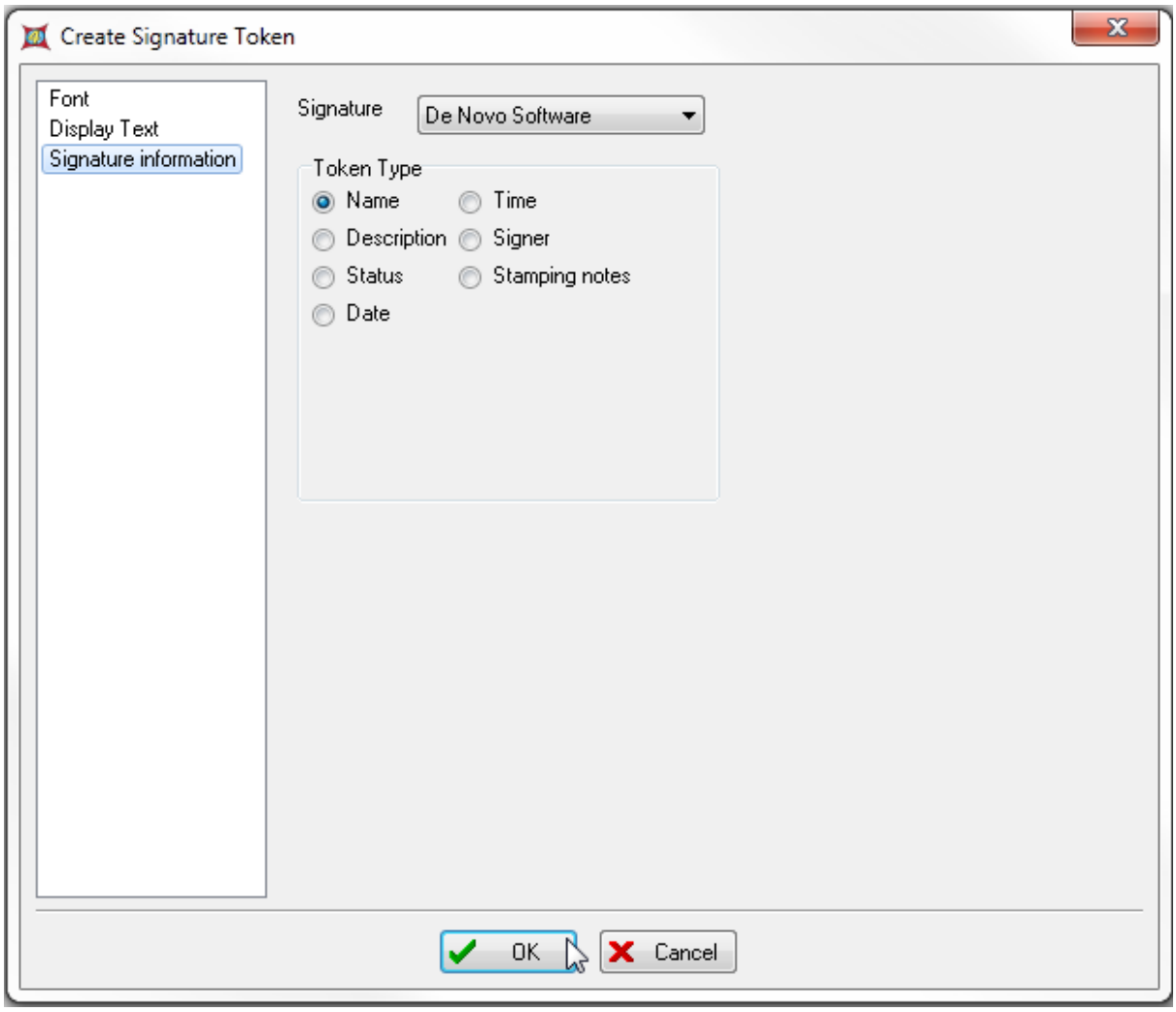
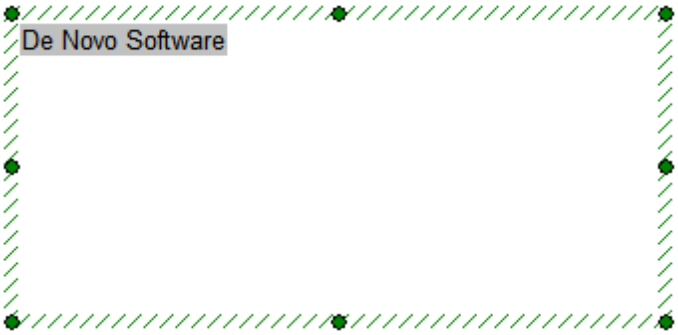


Note: You will notice in the **Signature information** category you have the option to choose any defined signature from the **Signature** drop down. You can choose which signature token you would like to insert from the **Token Type** radio buttons. This is useful if you would like to edit or change the token in the layout without deleting and inserting a new token.

d. Click **OK** to insert the token.



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The new signature token will appear in the text box as in the image above.

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.

31.4.1 Signature Status

Once you have created and set up your signatures 565 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 348 to hide the signature status if the layout has never been signed or has been unsigned. A custom token 348 of this type will therefore only display the signature status when the layout has been signed. Instructions for creating this type of custom token 348 can be found in the hiding signature tokens 356 section of the custom tokens 348 chapter.

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Chapter

32 Layout History

FCS Express tracks every action performed by a user on a layout. To view the history, use the **Application Button→Info→View Layout History** command to bring up the Layout History Viewer window (Figure 34.2).

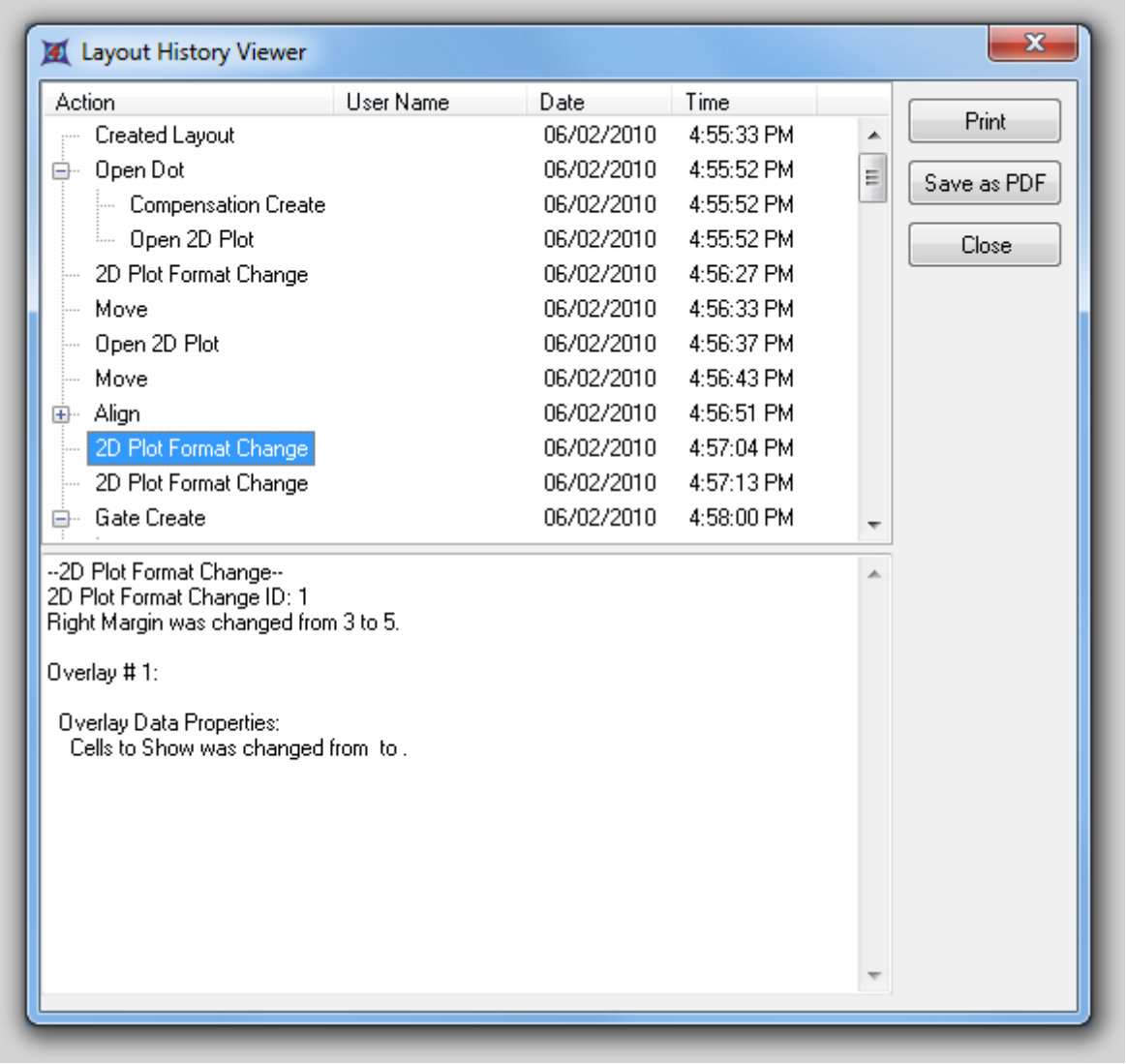

Figure 34.2 Layout History Viewer

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.
- The **Time** column contains the time the action was performed.

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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.

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Chapter

33 Publishing Layouts

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 587, 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 587.

De Novo Software also provides a free service to publish your layouts 580. 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 588. In addition, you can publish the layout to your own web site 583.

33.1 Publishing a Layout to the De Novo Software Publishing Site

To publish a layout to the De Novo Software web site, use the **Application Button→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 35.2).

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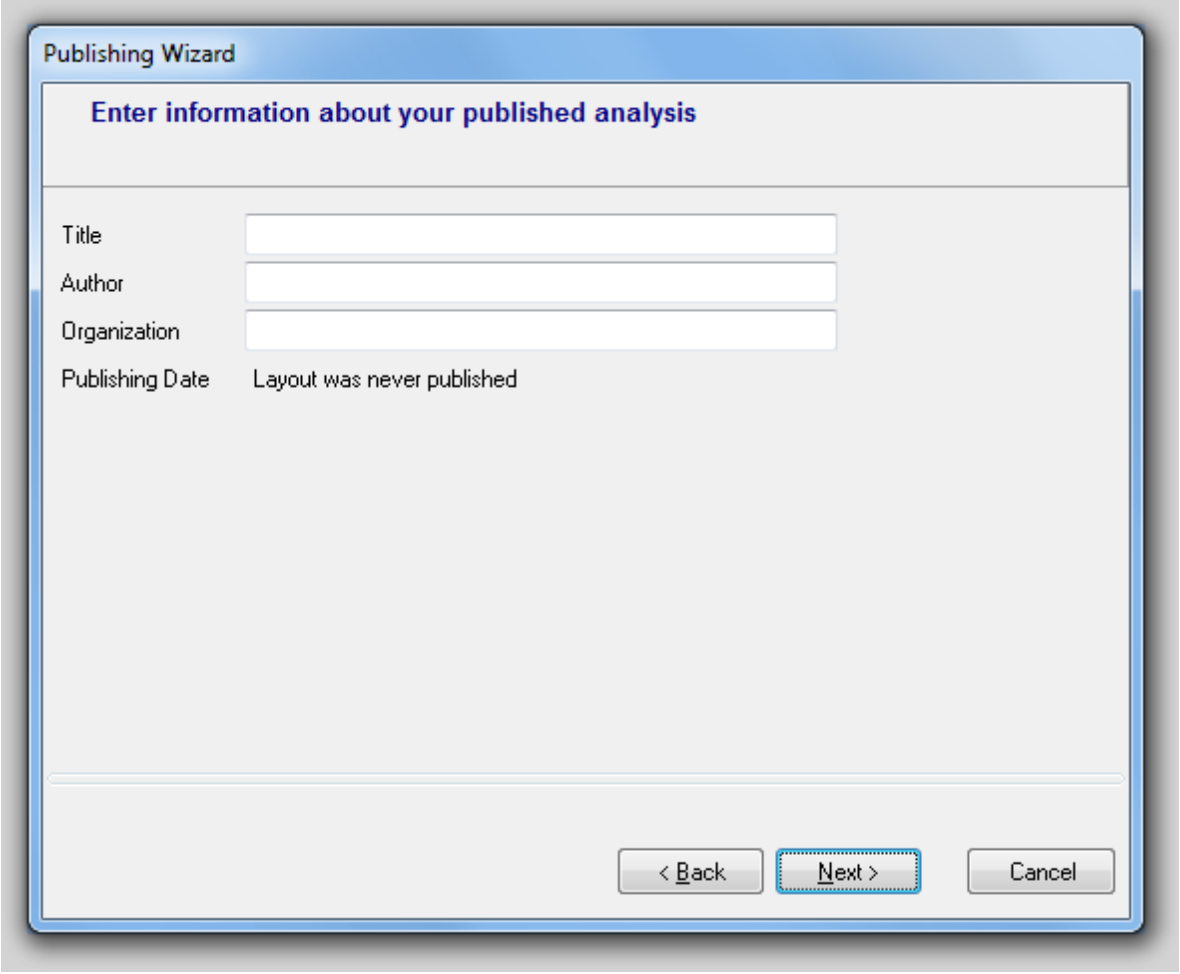

Figure 35.2 Publishing Information

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 35.3) 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 583. Press **Next** to publish your layout on the De Novo Software web site.

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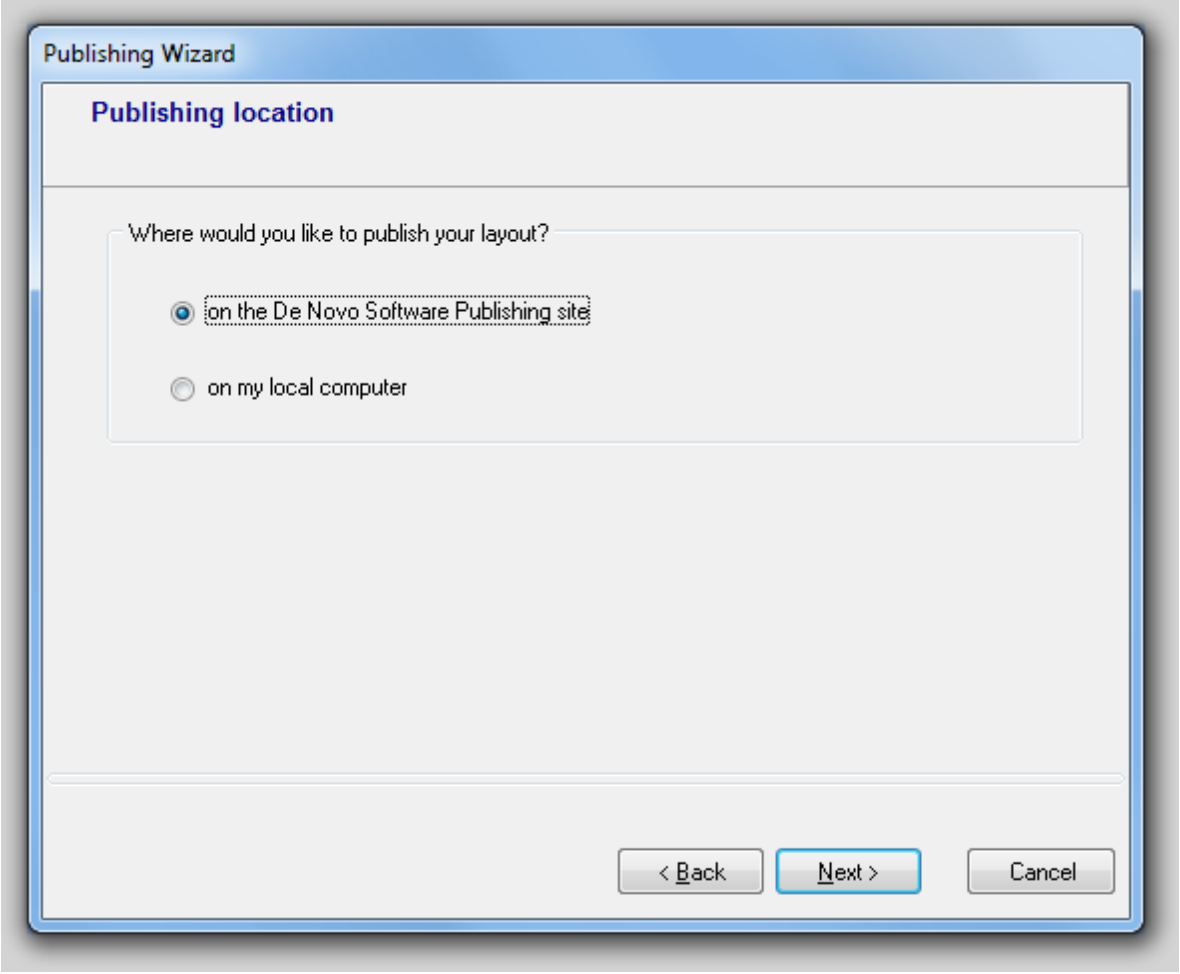

Figure 35.3 Publishing Location

The **Publishing Wizard** will ask you to log in using your email address and password (Figure 35.4). 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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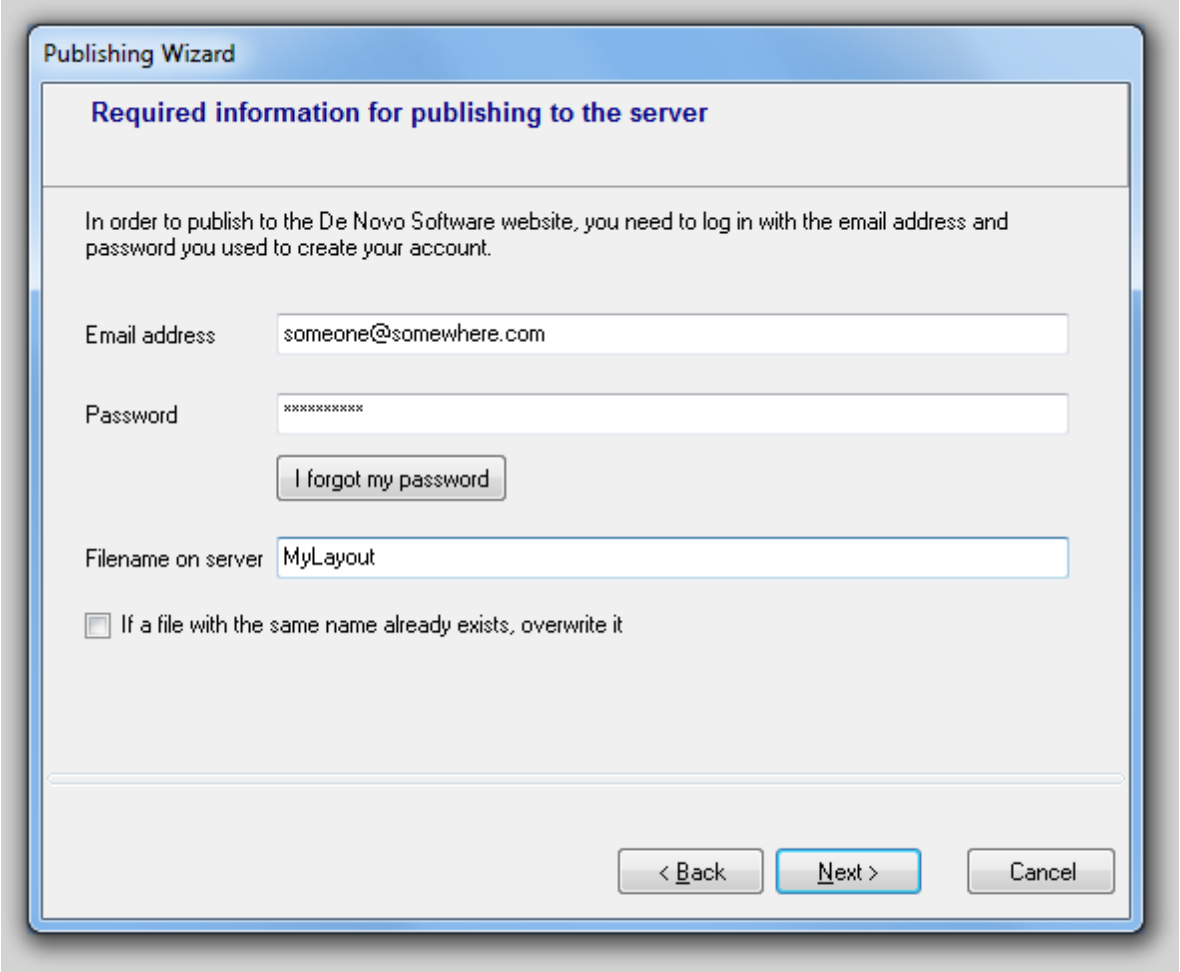

Figure 35.4 Logging in to the De Novo Software Server

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 585.

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 587 to view your data files.

33.2 Advanced Publishing

More advanced publishing capabilities are available if you have purchased the publishing add-on module. This 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.

If you are using the advanced publisher, the **on my local computer** option will be available on the Location page of the Publishing Wizard 580. If you select this

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radio button and press **Next**, the **Set permissions for the published layout** page (Figure 35.5) will appear.

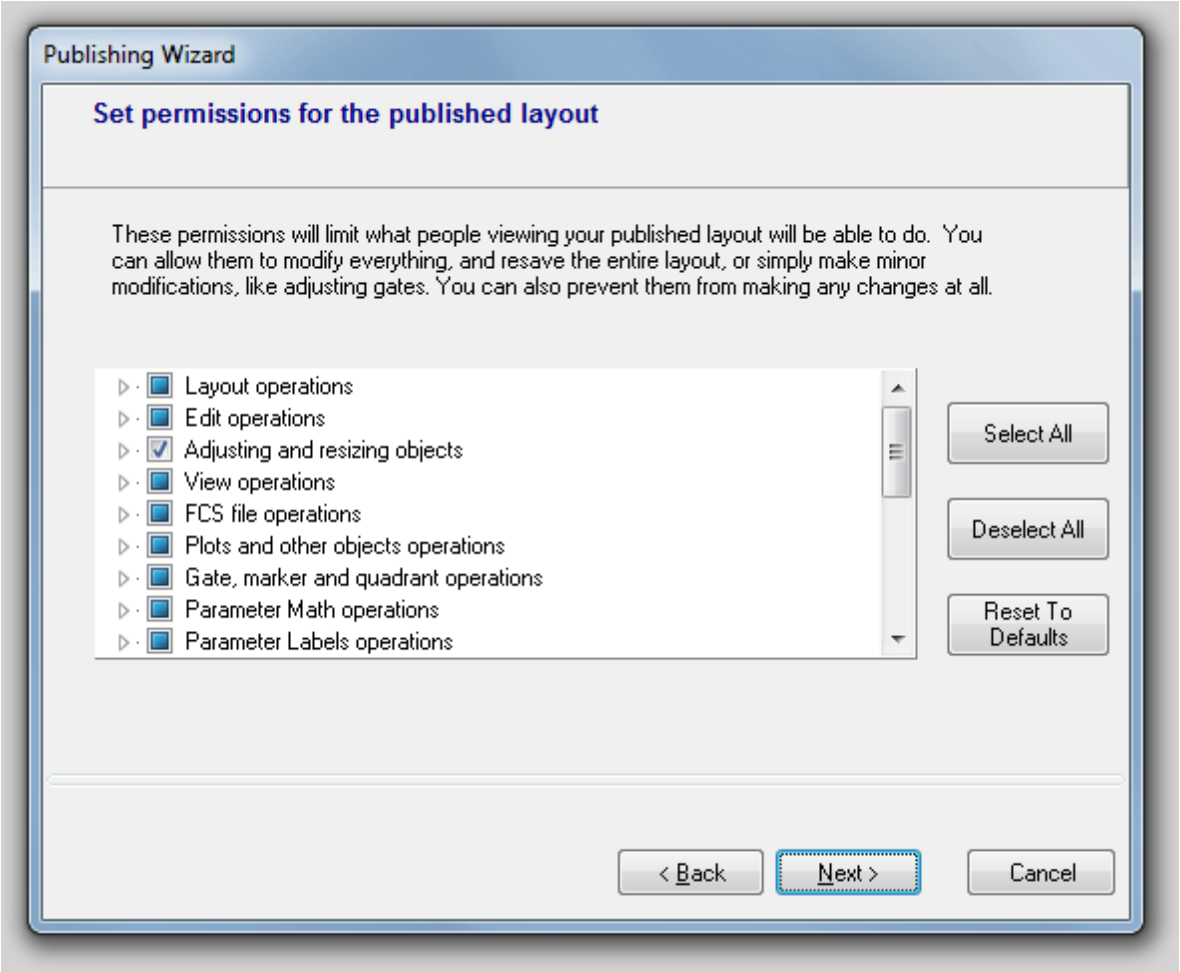

Figure 35.5 Publishing Security Permissions

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.

When publishing to your local computer, the next page requires you to select the

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file name. Your published file will have the same extension as a standard FCS Express layout (.fey) and the data will automatically be embedded. Any published layout can be opened in the FCS Express Reader.

33.3 Default Publishing Options

The **Default Permissions** and **Publishing Options** can be set in the Advanced Publishing category of the Edit Preferences dialog 140.

33.4 Maintaining 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 **Application Button→Collaborate→Publishing Maintenance** command to open the **Manage your Published Layouts** dialog (Figure 35.6).

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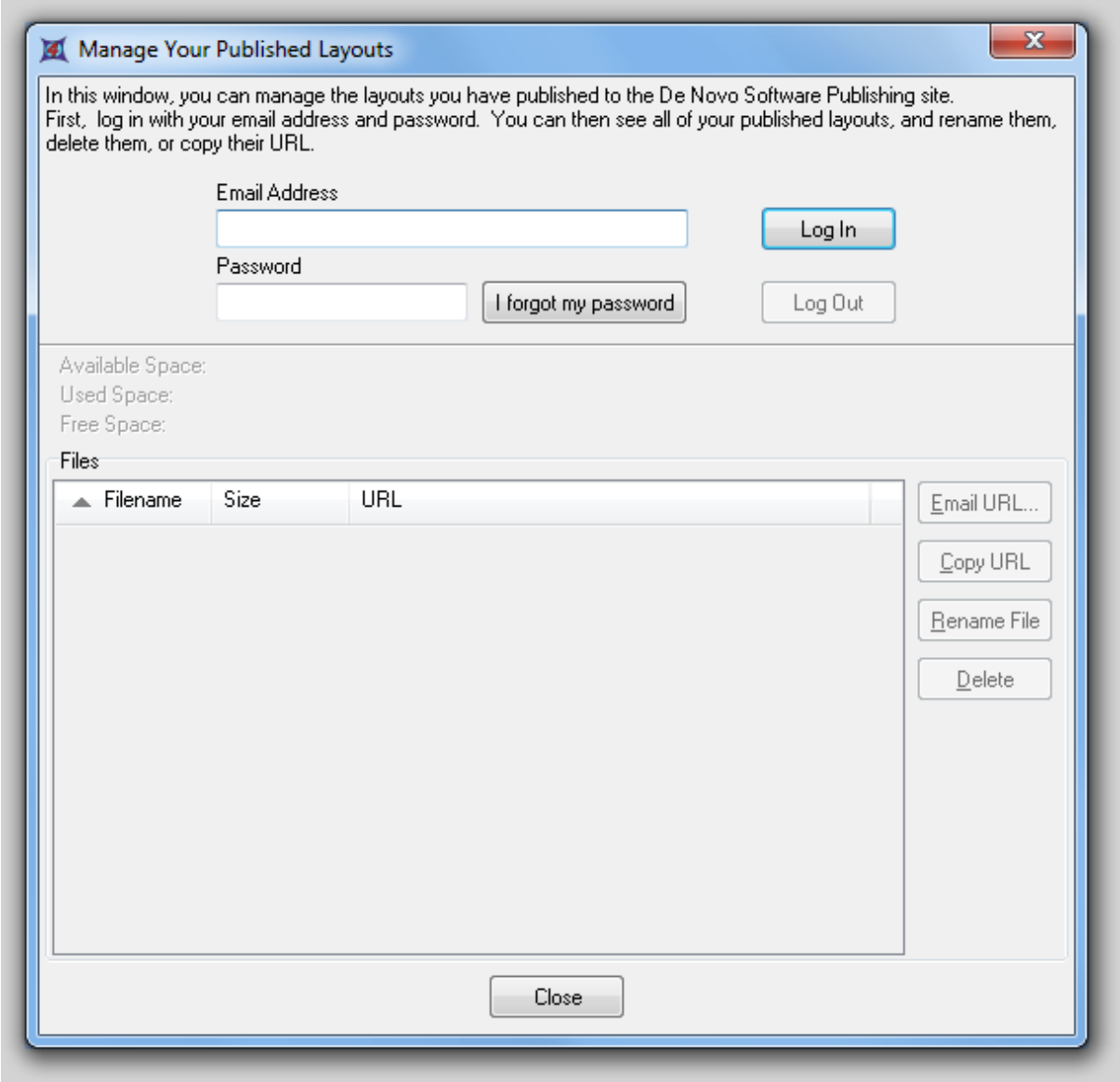

Figure 35.6 Managing Published Layouts

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 plan588.

33.5 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 580 (or that you chose when publishing to your own site). Paste the URL into any web browser (Figure 35.7) and press **Enter**.

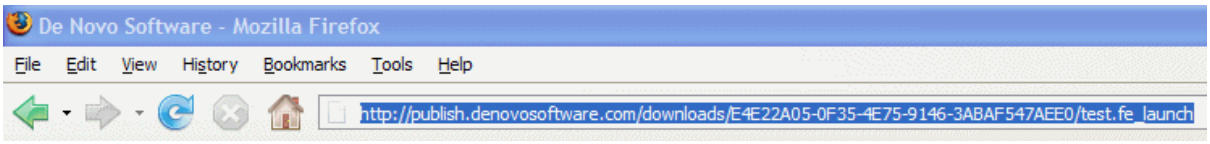

Figure 35.7 Pasting the URL of a Published Layout into a Web Browser

33.6 FCS Express Reader

With FCS Express Reader, you can:

- See published analyses 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.

33.6.1 Obtaining the FCS Express Reader

FCS Express Reader is available free at http://www.denovosoftware.com/site/reader.shtml.

33.6.2 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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33.6.3 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 **Help→Upgrade from Reader** in the main menu (Figure 35.8).

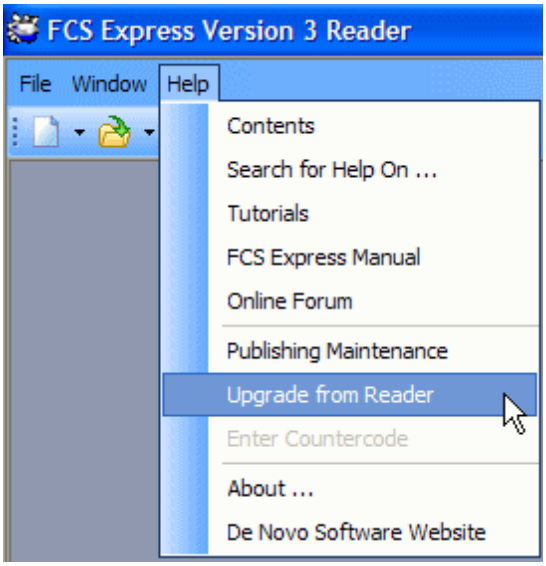

Figure 35.8 Upgrade FCS Express Reader

33.7 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

34 Tutorials

This chapter contains detailed tutorials of the functions of FCS Express Version 4 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:

http://www.denovosoftware.com/download/TutorialSampleData.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 591
- Inserting Plots 607
- Formatting Plots 620
- Gating 650
- Text Boxes and Tokens 675
- Quadrants 712
- Quadrant/Marker to Gate Linking 722
- Compensation 736
- Batch Processing 767
- Creating and Using Panels 821
- Docking 845
- Data Navigator 866
- Gate Navigator 889
- Layout Navigator 904
- Creating and Using Custom Data 924
- Bar and Scatter Plots 963
- Titration 986
- High Content Flow 1027
- DNA Analysis 1069
- Proliferation 1105
- Kinetics 1127
- Cell Concentration 1150
- Channel Calibration 1169
- CellProfiler Export 1183
- ImageJ Export 1275
- Exporting from IsoCyte 1300
- Picture Plots 1315
- Regression Analysis 1342
- Introduction to FCS Express 4 Image Cytometry 1373

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- Immunophenotyping 1463
- Changing Common Preferences 1513

34.1 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 591.

34.1.1 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.

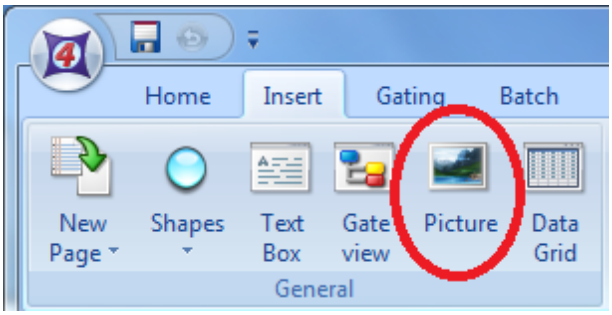

Figure T1.1 Insert Picture

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.

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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 592.

34.1.2 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 592.

34.1.3 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.

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Figure T1.3 Resizing an Object

We will now learn to move an object 593.

34.1.4 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.
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.

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Figure T1.4 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 594.

34.1.5 Deleting an Object

To delete an object:

1. Select the object as described above 592.
2. Press the **Delete** key on the keyboard

OR

3. Select the **Home→Editing→Delete** command (Figure T1.5).

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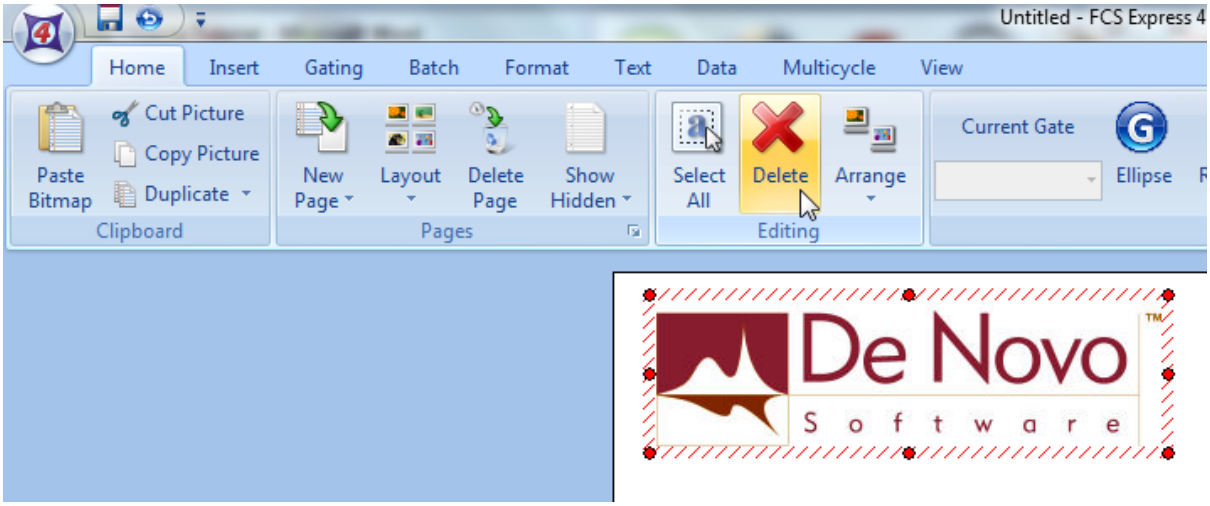

Figure T1.5 Deleting Objects

If you decide that you didn't want to delete the object, you can undo 595 your changes in the next section.

34.1.6 Undoing Changes

FCS Express Version 4 allows for any number of previous operations to be undone.

To undo 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 594, click the **Undo** button. You can also press **Ctrl+Z** to undo changes.

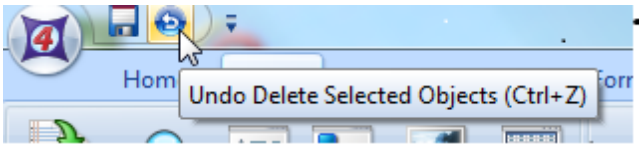

Figure T1.6 Undo

The next topic is duplicating objects 595.

34.1.7 Duplicating Objects

To duplicate an object:

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1. Select the object as described above 592.
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).

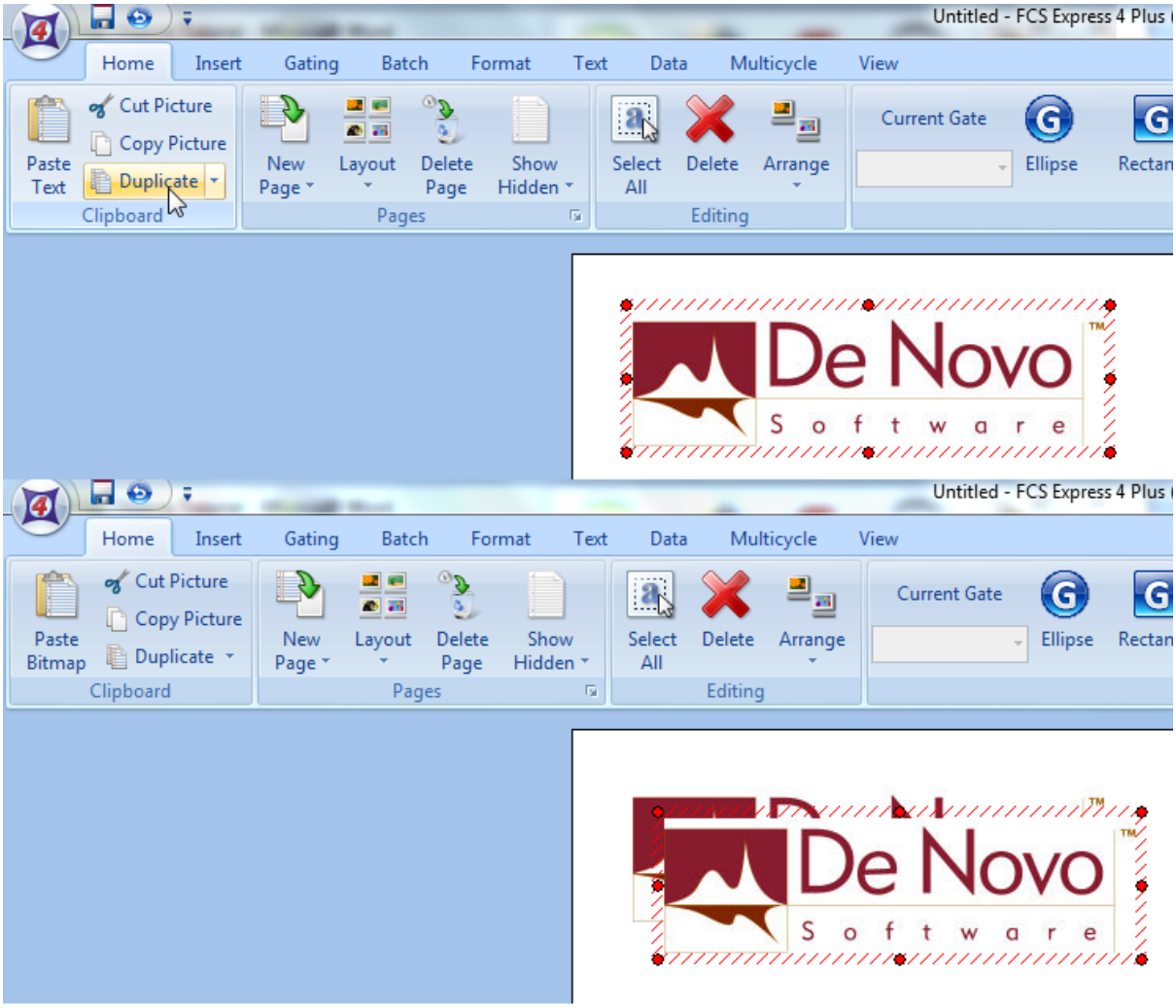

Figure T1.7 Duplicating Objects

In the next section, we will select multiple objects 597.

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34.1.8 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.
2. Press and hold the left mouse button.
3. Move the cursor. A blue highlighted box will appear as you move the cursor around the layout.
4. Size the blue 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

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are selected.

6. Release the **Shift** key. All the objects you have selected will have a red border.

The next section is copying objects 598.

34.1.9 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 592.
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.

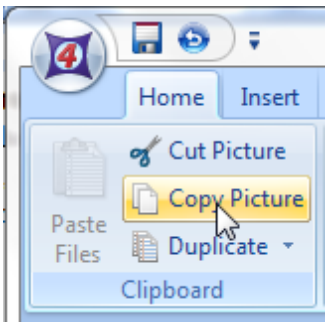

Figure T1.9 Copying Objects

We will now proceed to pasting objects 598.

34.1.10 Pasting Objects

To paste objects that are on the clipboard:

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1. Select the **Paste Picture** 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.

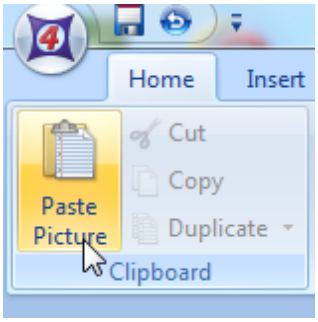

Figure T1.10 Pasting Objects

In the next step, we will learn to group objects 599.

34.1.11 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 592.
2. Select **Group** from the **Home→Editing→Arrange** command drop-down list (Figure T1.11).

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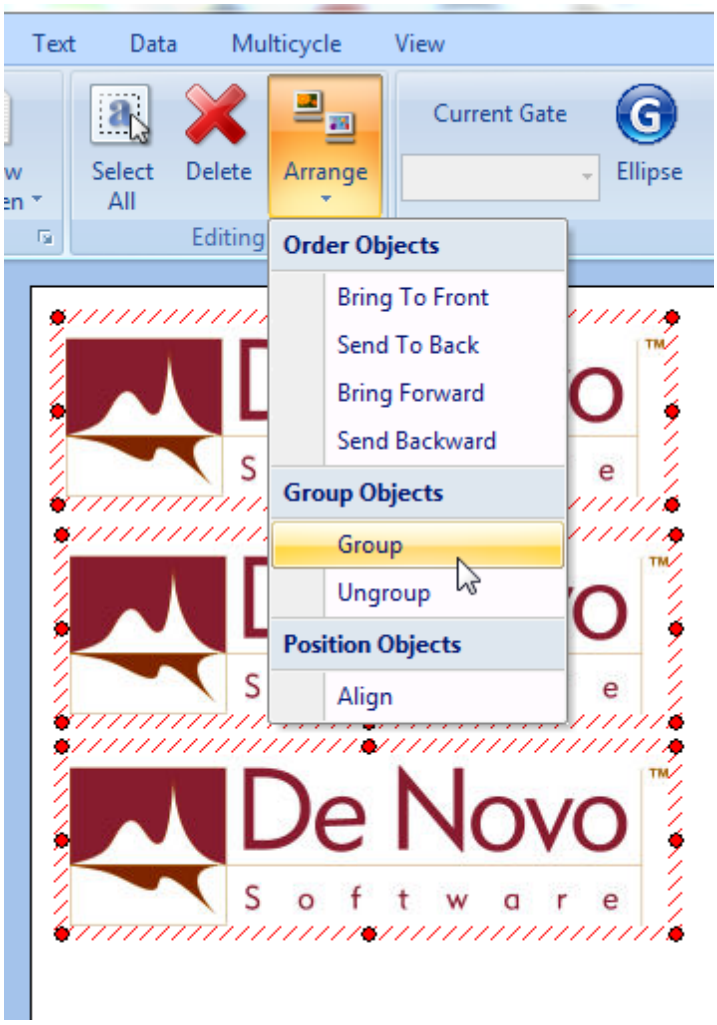

Figure T1.11 Grouping Objects

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.

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[601].

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34.1.12 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 592 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** dialog box will appear in the center of the layout.
3. Select **Centers** from the horizontal alignment list while leaving the vertical alignment at **No Change** (Figure T1.13).
4. Press the **OK** button.

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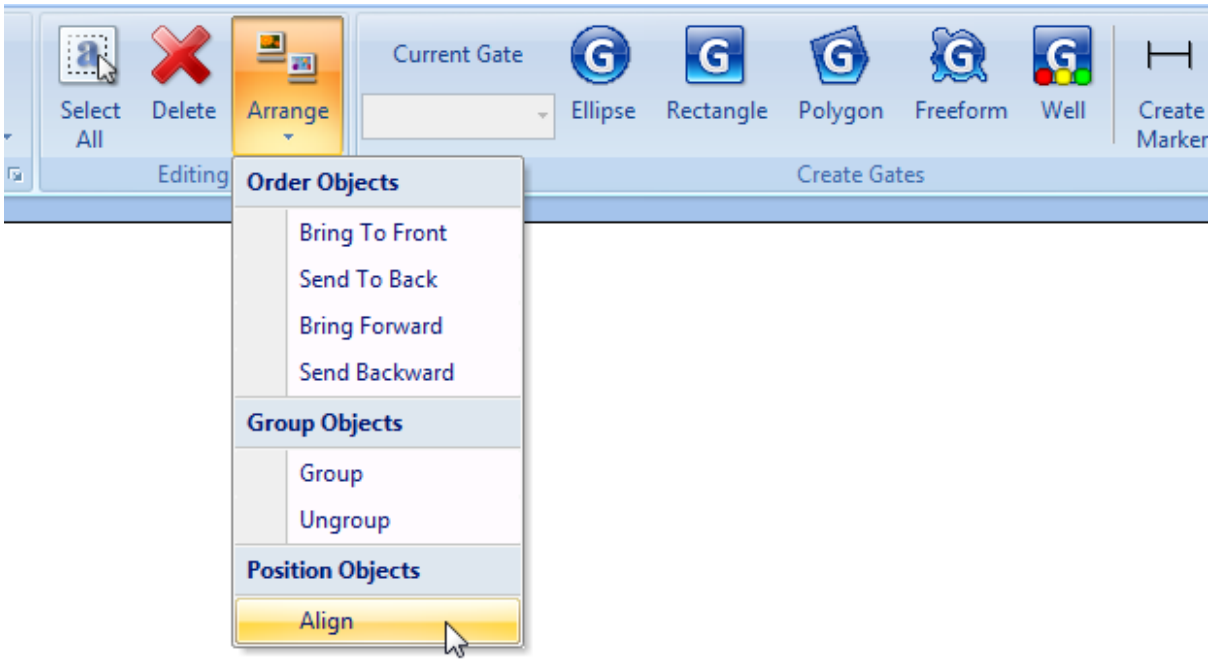

Figure T1.12 Aligning Objects (Step 2)

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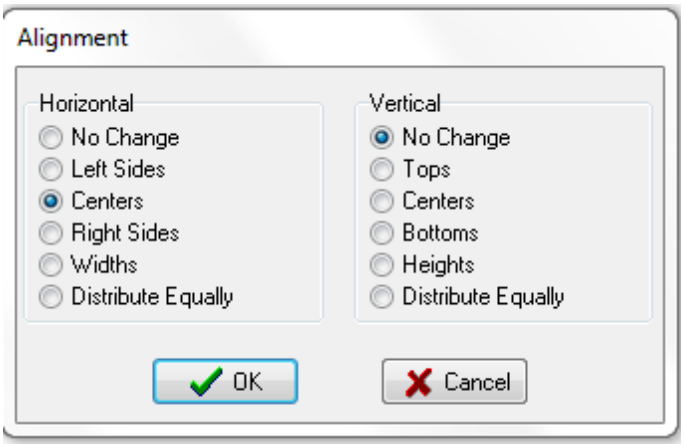

Figure T1.13 Aligning Objects (Step 3)

The selected objects are now horizontally aligned and the horizontal centers of the objects are identical (Figure T1.14).

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Figure T1.14 Aligning Objects (Final Result)

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** command drop-down list.

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4. Select **Widths** from the horizontal alignment list.
5. Select **Heights** from the vertical alignment list.
6. Press the **OK** button.

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 605.

34.1.13 Ordering Objects

FCS Express Version 4 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 592.
2. Select **Bring to Front** from the **Home→Editing→Arrange** command drop-down list (Figure T1.16).

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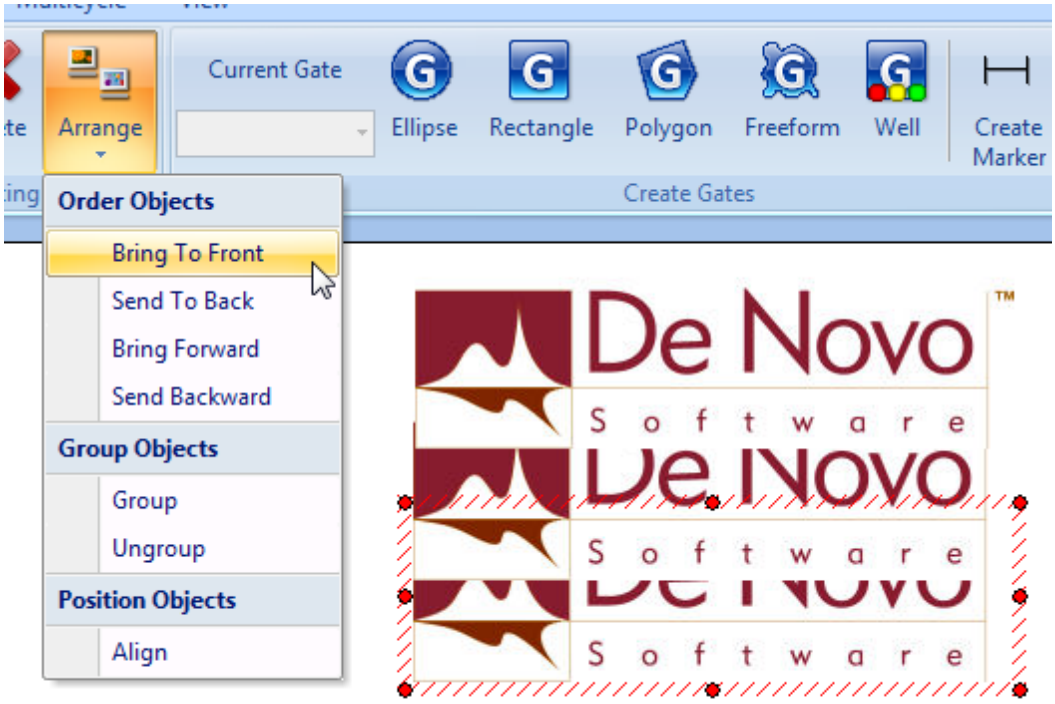

Figure T1.16 Ordering Objects (Step 2)

The selected object will be brought to the front (Figure T1.17).

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Figure T1.17 Ordering Objects (Final Result)

34.2 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 590. The files **GFPcontrol.001** and **GFPsample.002** are used here.

We will start by inserting a 2D plot 607.

34.2.1 Inserting a 2D Plot

To insert a 2D plot:

1. Select the **Insert→2D Plots→Dot** command (Figure T2.1).

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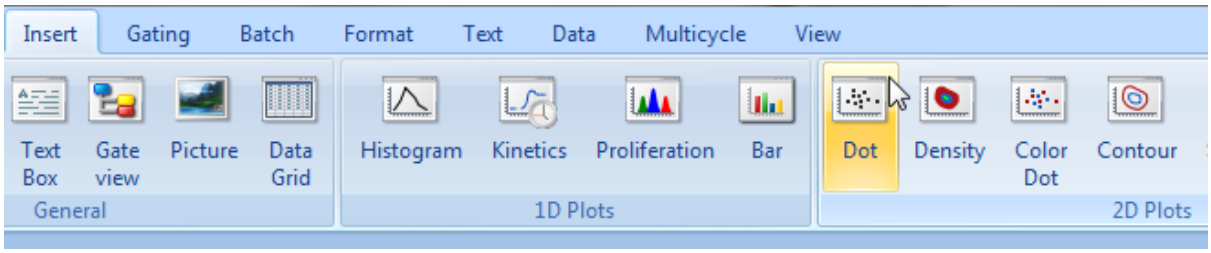

Figure T2.1 Inserting a Dot Plot

2. Move the mouse to a place on the layout where you would like the top-left corner of the plot to be located.

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).

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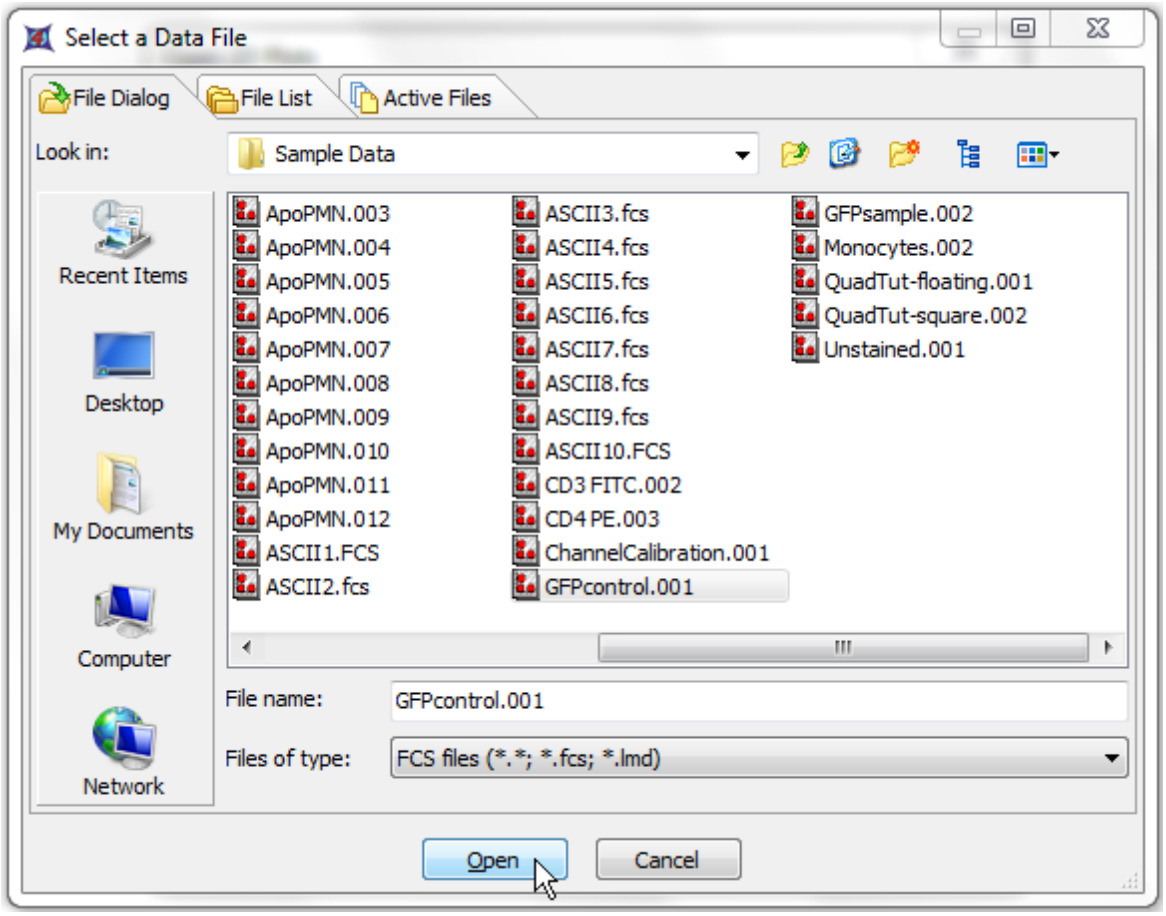

Figure T2.2 Select a Data File Dialog

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 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 592.

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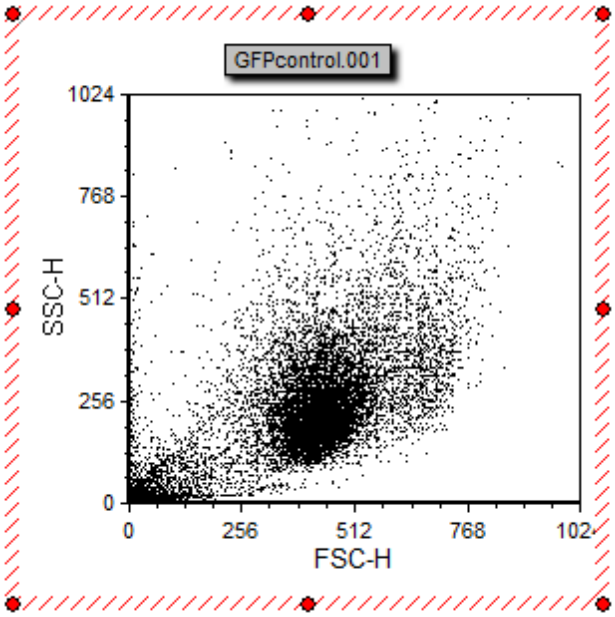

Figure T2.3 Inserting a 2D Plot

Next, we will insert a histogram 610.

34.2.2 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).

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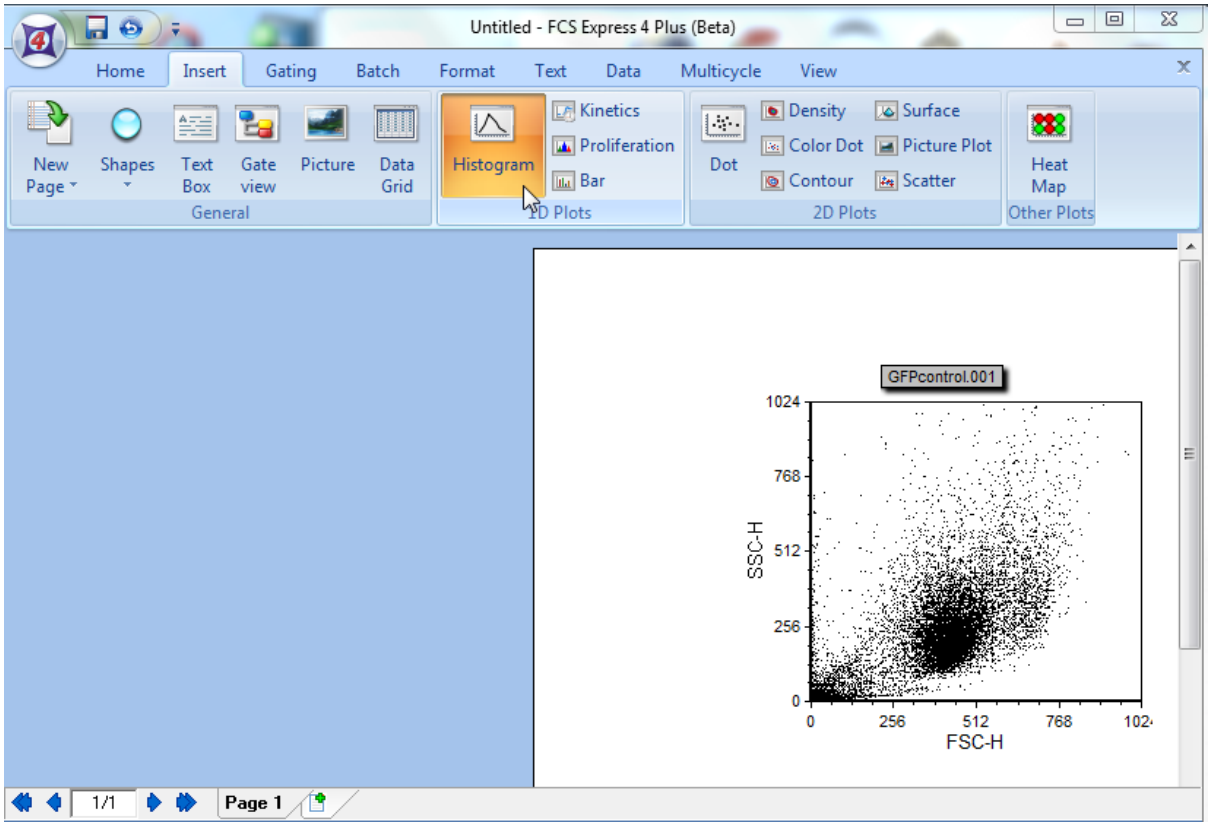

Figure T2.4 Insert Histogram Command

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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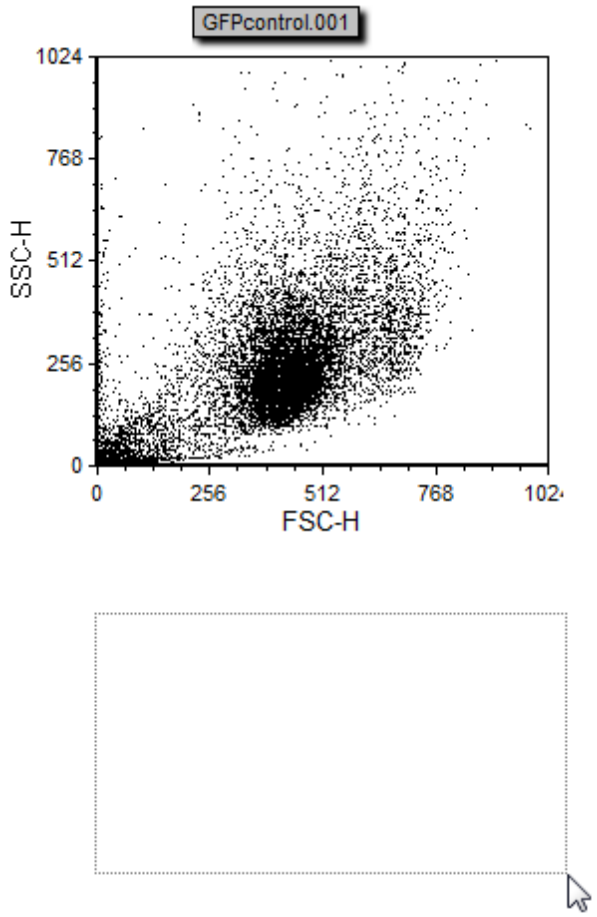

Figure T2.5 Insert Histogram (Step 4)

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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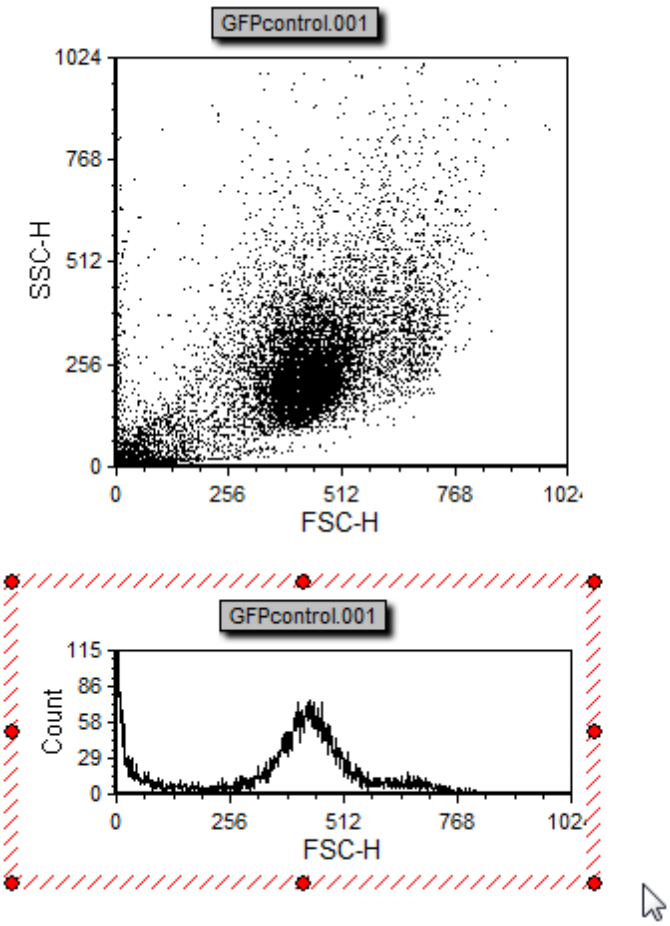

Figure T2.6 Insert Histogram (Step 5)

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 **Edit Preferences** (described below 615).

Plots can also be inserted using the Data→Save/Load→Open command 613.

34.2.3 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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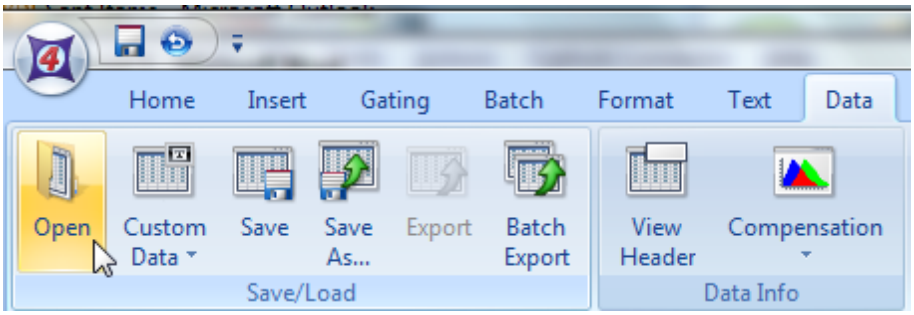

Figure T2.7 Open Command

A **Select a Data File** dialog will open as previously shown in Figure T2.2 607.

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).

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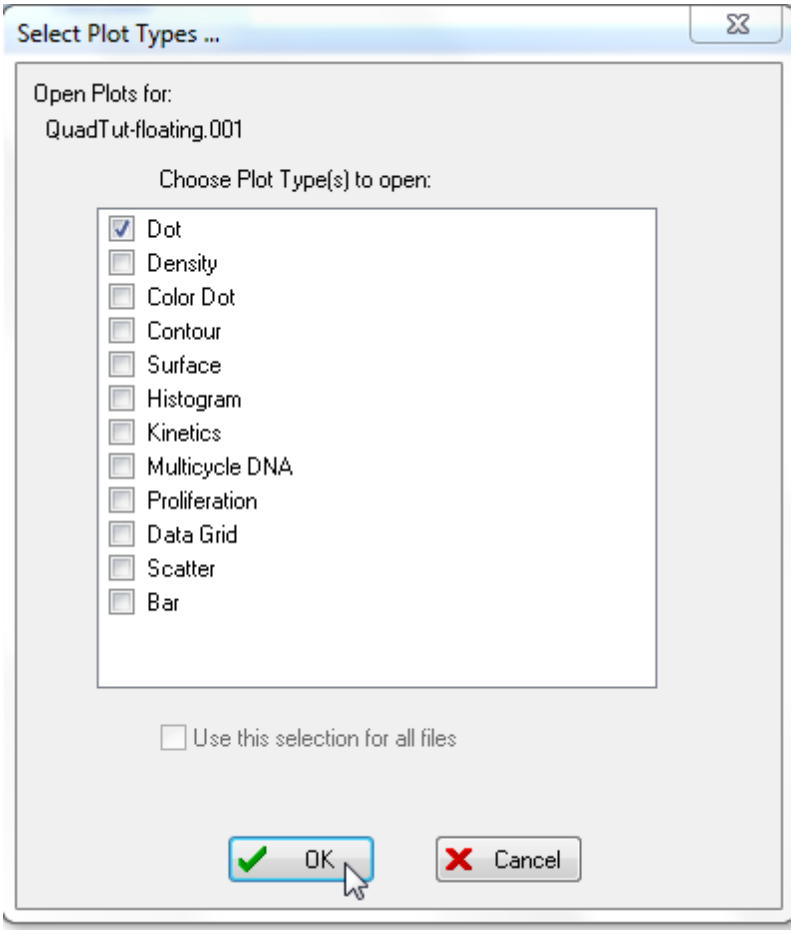

Figure T2.8 Select Plot Types... Dialog

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 T2.3 607.

You can force the display of the selection dialog 615 using by setting a user preference.

34.2.4 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 **Edit**

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Preferences.

1. Select the **Application Button** (circled in red in the upper left-hand corner of Figure T2.9).
2. Select **Edit Preference**s (shown in the lower right of Figure T2.9).

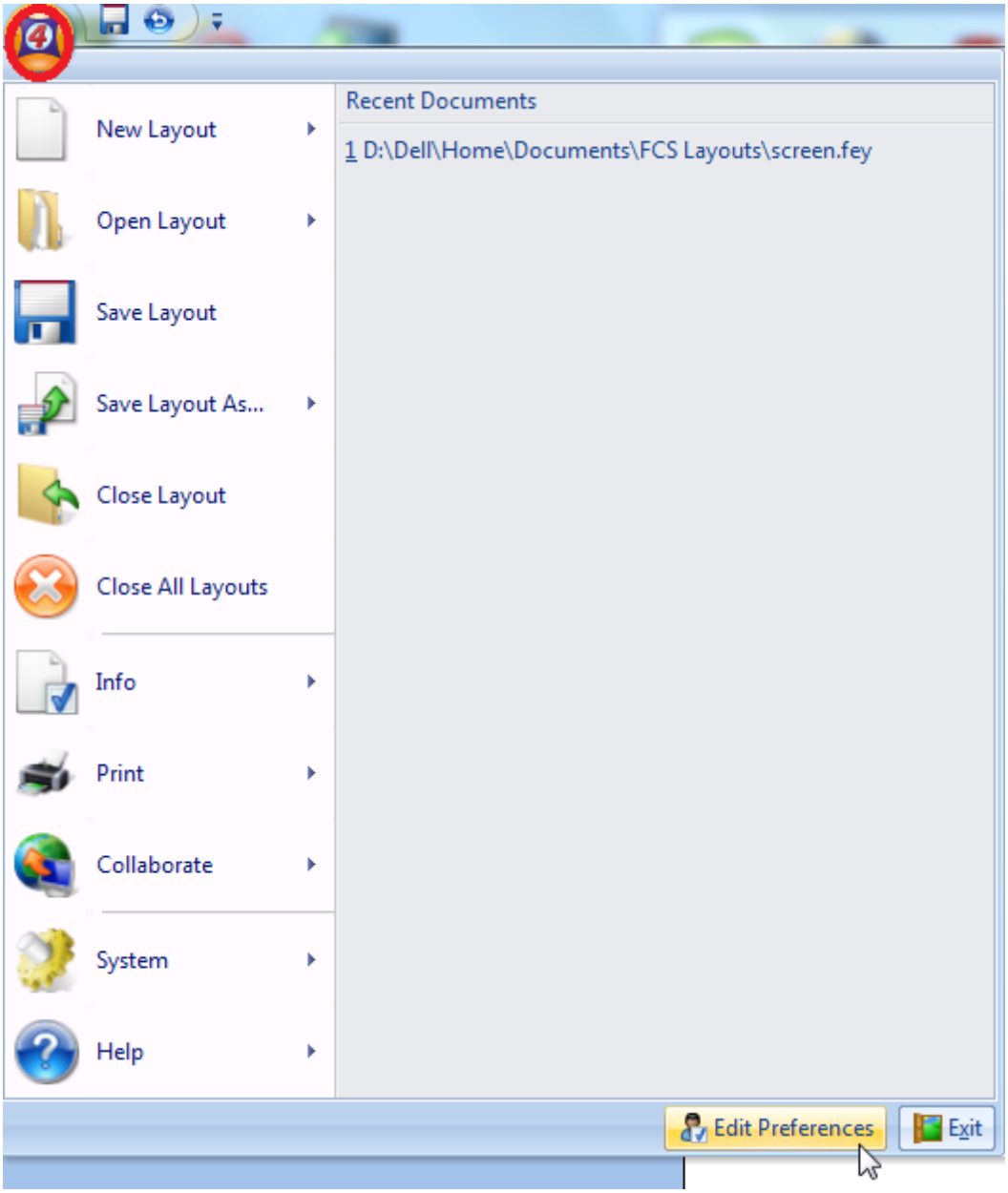

Figure T2.9 Edit Preferences Button

The **Edit Preferences** dialog will appear (Figure T2.10).

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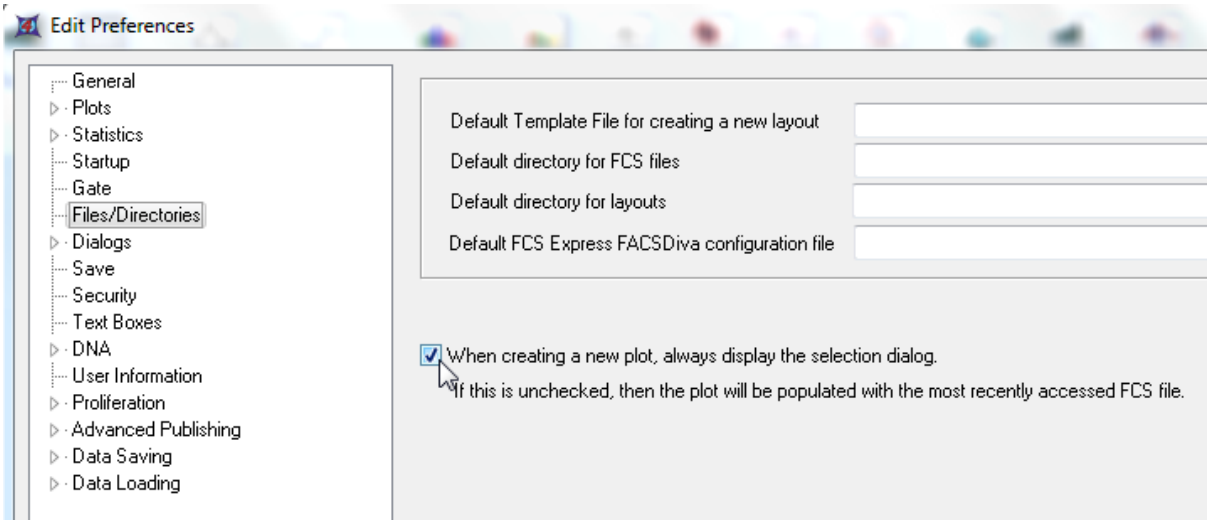

Figure T2.10 Files/Directories Page of Edit Preferences Dialog

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 T2.2 607.

7. Select the file **GFPsample.002** from the **Select a Data File** dialog box.
8. Select **Open**.

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.

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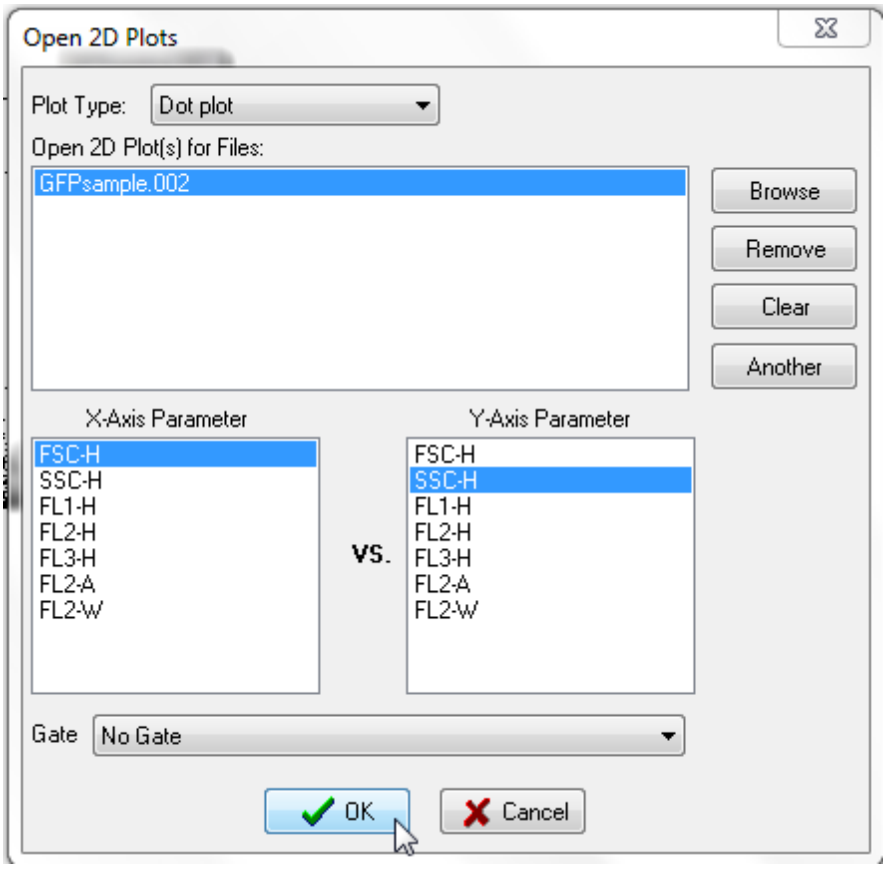

Figure T2.11 Open 2D Plots Dialog

The 2D dot plot of GFPsample.002 will now appear on the layout as shown in Figure T2.12.

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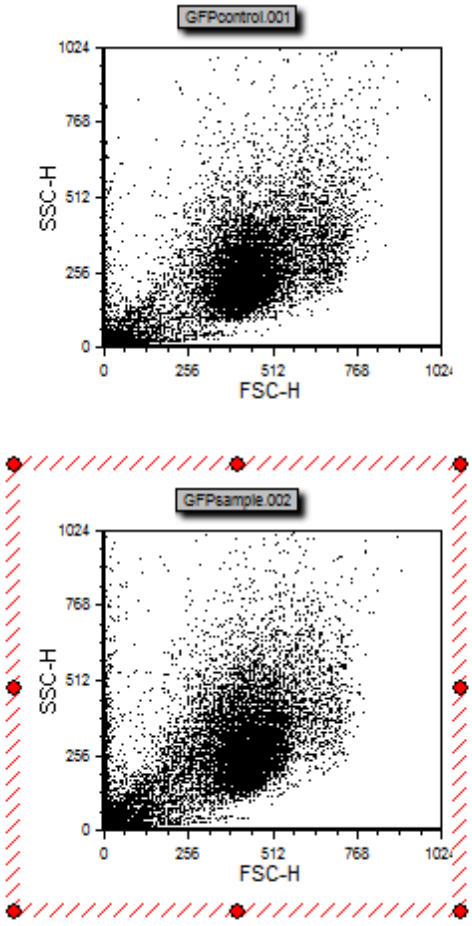

Figure T2.12 Inserted 2D Plot

Finally, we will insert plots from Windows Explorer[619].

34.2.5 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:

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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.
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 T2.8 613.

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.

Multiple files may be selected from Windows Explorer and added to the layout. The **Select Plot Types...** dialog will appear prior to adding each plot, if multiple plots are added simultaneously.

34.3 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 590. 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 620.

34.3.1 Formatting Plots Using the Pop-up Menu

In the course of this example, we will:

- Open a Layout file.
- Insert data into a plot.

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- Format a plot using the pop-up menu.

1. Select **Application Button→Open Layout** (Figure T3.1).

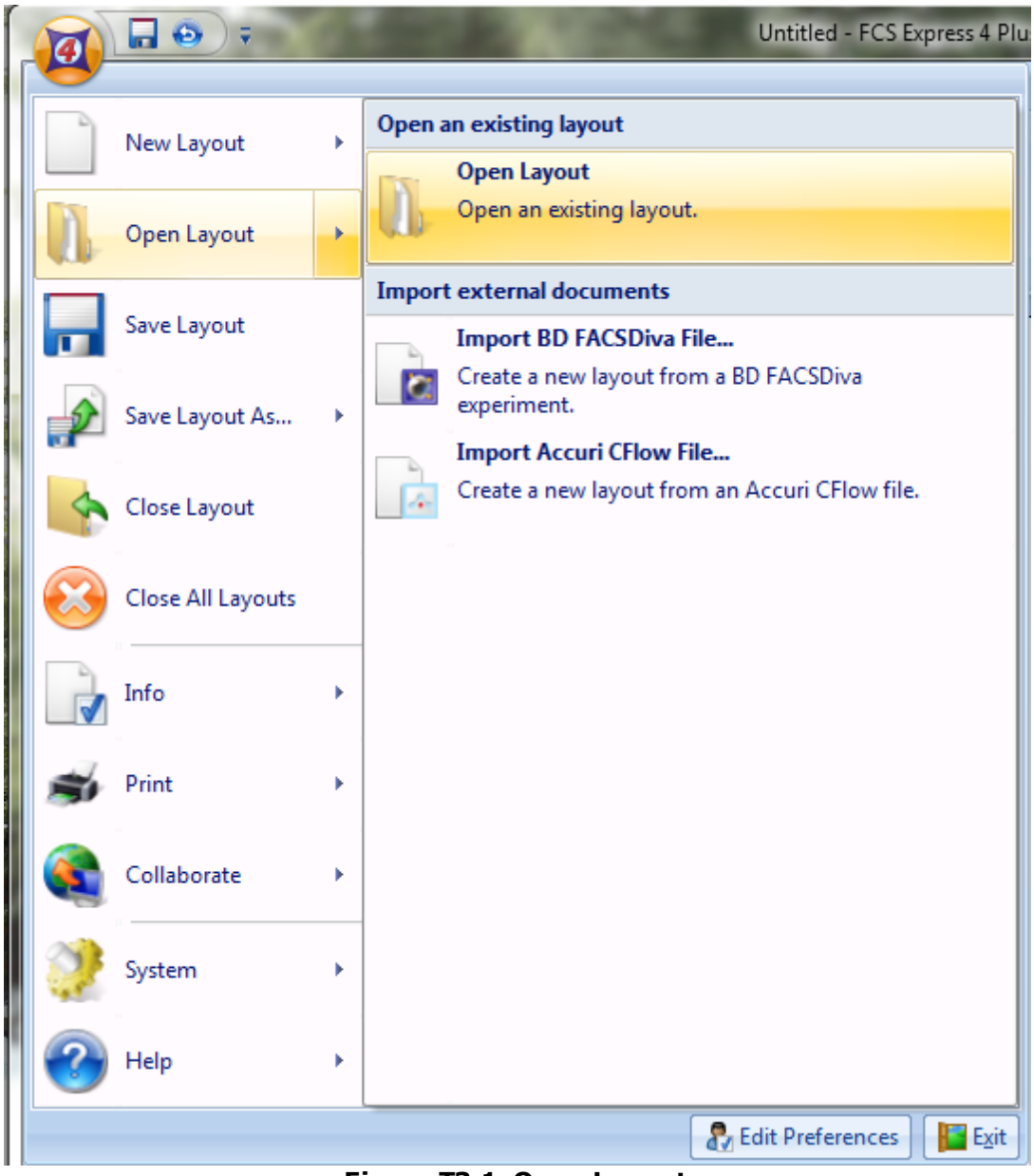

Figure T3.1 Open Layout

2. Select the **section4.fey** layout file located in the Tutorial Sample Data archive 590 from the **Load Layout** dialog (Figure T3.2).

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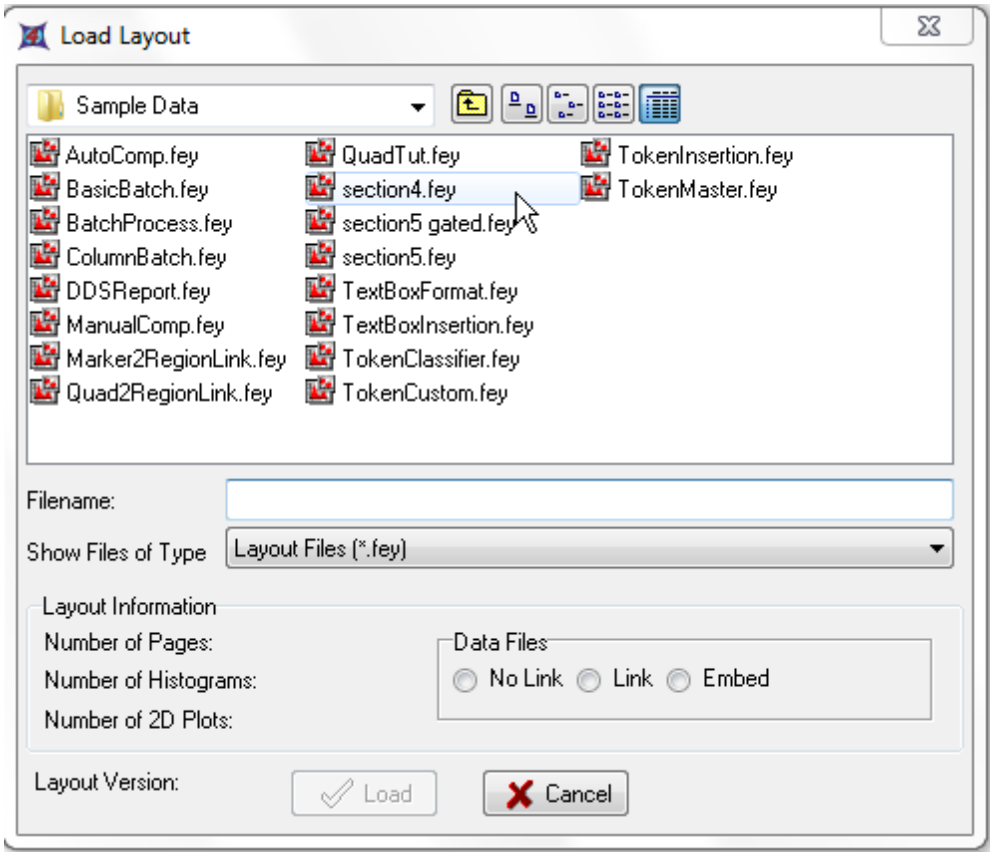

Figure T3.2 Load Layout Dialog

The Layout should have a single blank plot (Figure T3.3).

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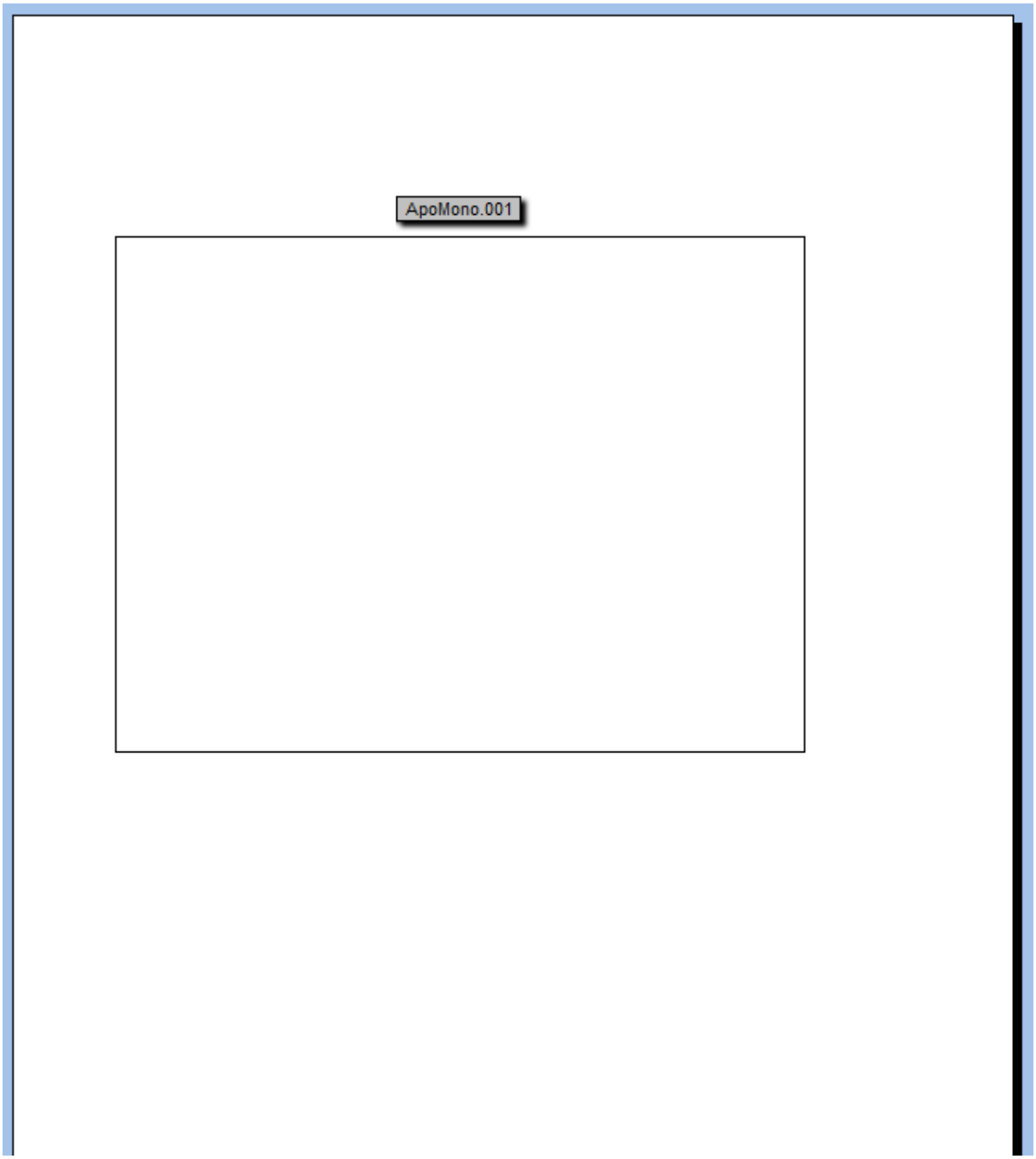

Figure T3.3 Section 4 Layout

3. Select the **Data→Change Data on All Objects→Select** command to insert a data file into the plot (denoted by the cursor in Figure T3.4).

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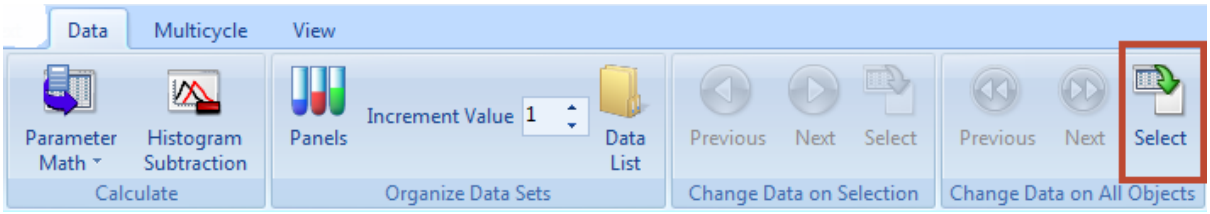

Figure T3.4 Select Command (Change Data on All Objects)

4. Select the **ApoMono.001** FCS file located in the Tutorial Sample Data archive 590.
5. Right-click on the plot so the pop-up menu appears (Figure T3.5).
6. Select **Format** from the pop-up menu.

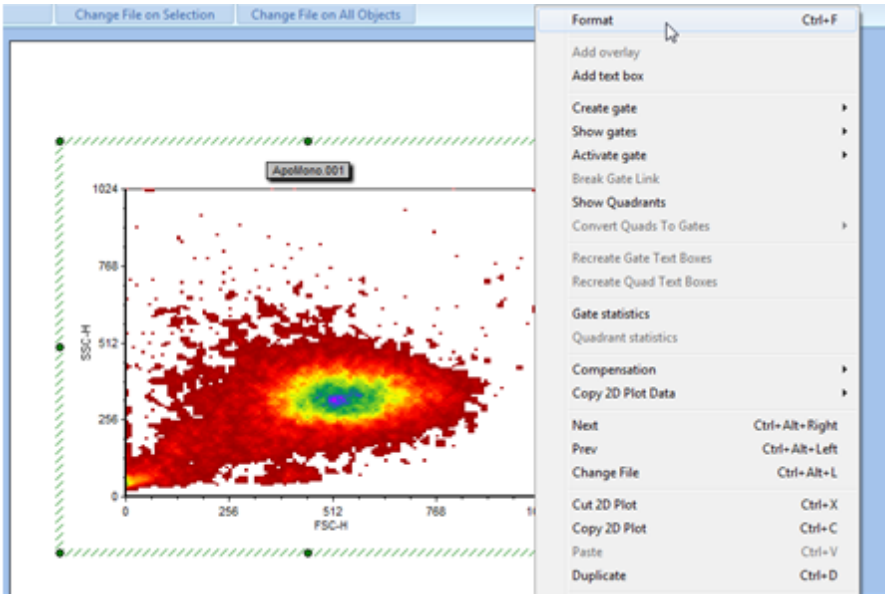

Figure T3.5 Selecting Format from the Pop-up Menu

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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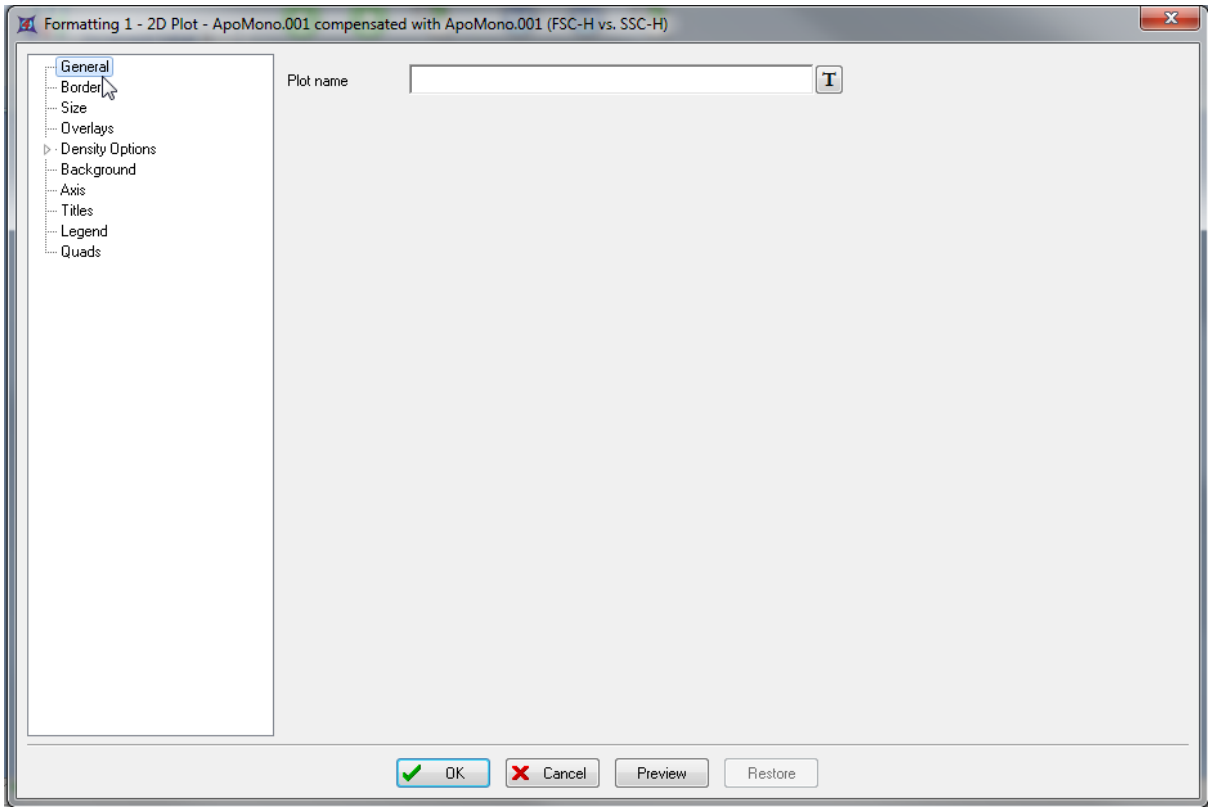

Figure T3.6 Formatting Dialog for 2D Plots

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 34.

In the next section, we will format multiple plots at the same time 625.

34.3.2 Formatting Multiple Plots

In the course of this example, we will:

- Duplicate a plot using the pop-up menu.

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- 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).

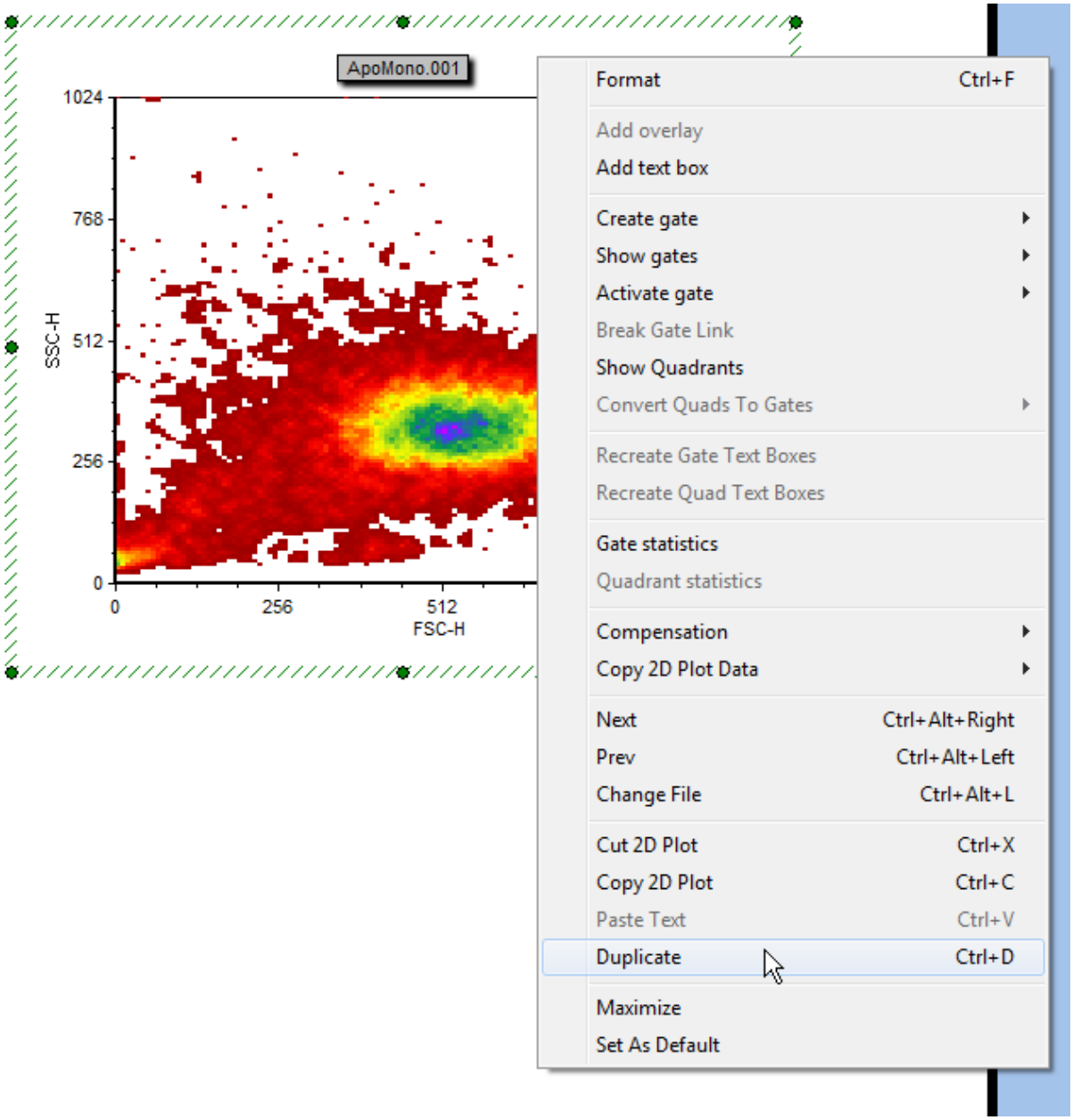

Figure T3.7 Duplicating a Plot

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A duplicate copy of the plot should appear on top of the original plot (Figure T3.8).

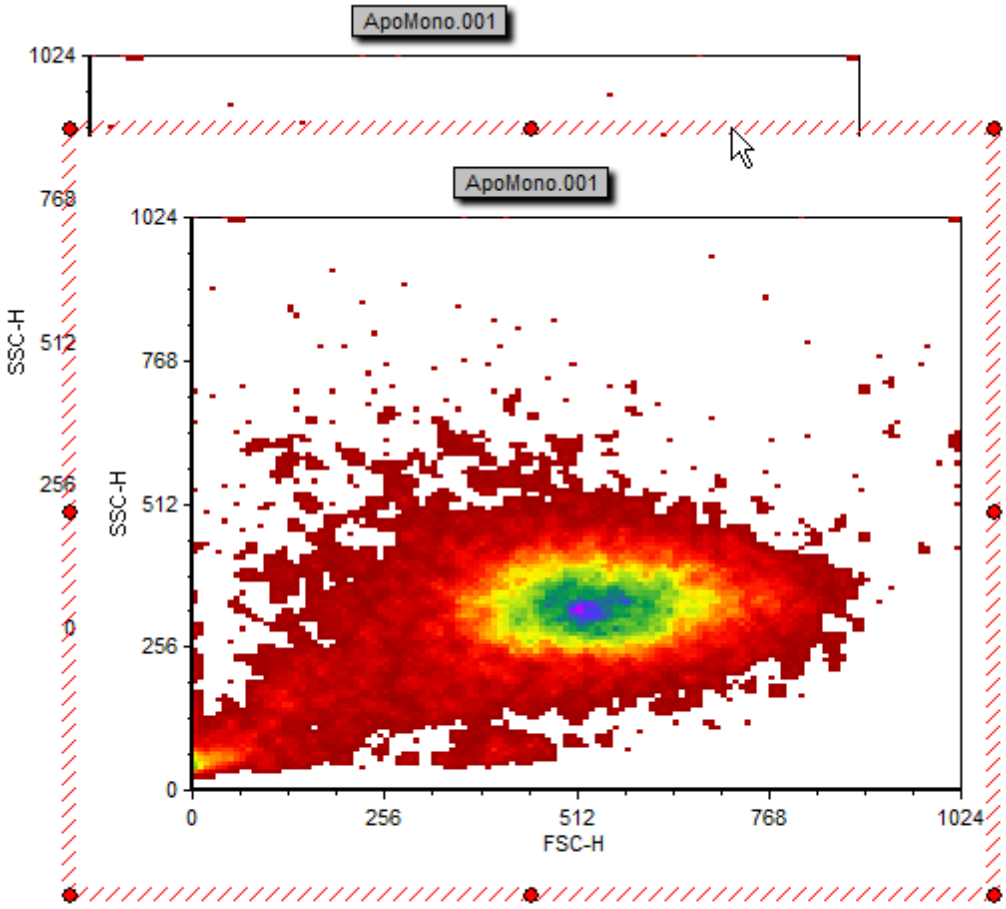

Figure T3.8 Duplicated Plot

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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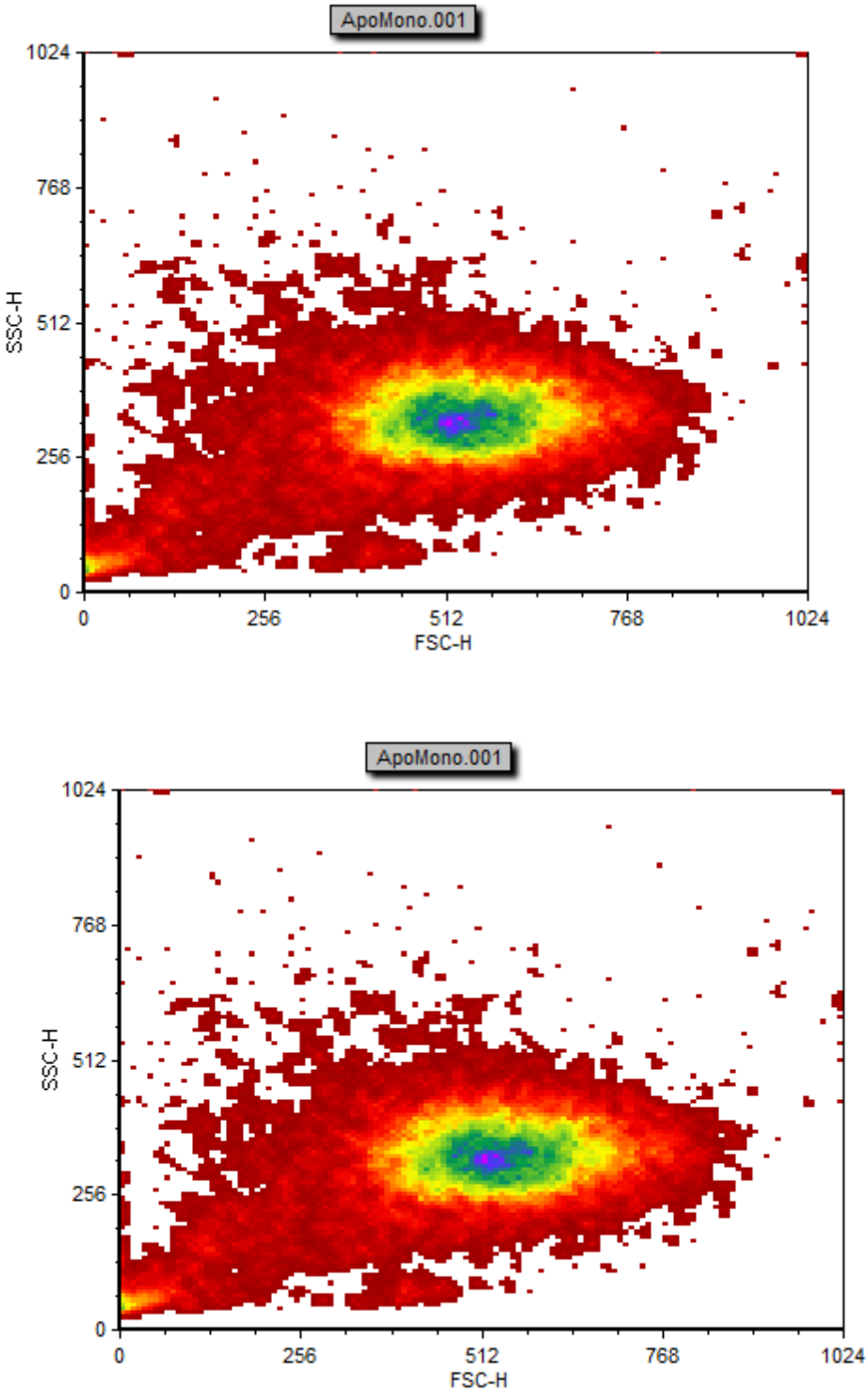

Figure T3.9 Duplicated Plots on a Layout Page

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6. Select both plots on the layout (see the tutorial on selecting multiple objects[597]).
7. Select **Format→Object Options→Border/Line** (Figure T3.10).

We will format the border/line of both plots at the same time.

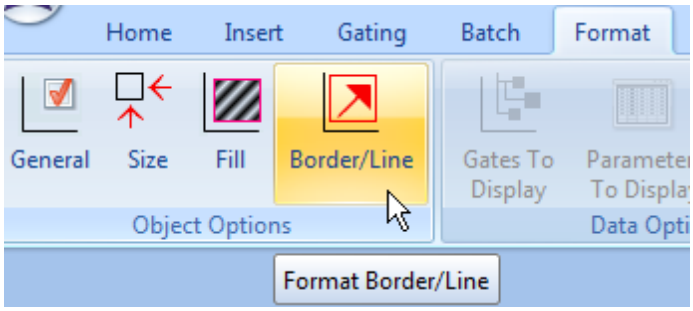

Figure T3.10 Format Border/Line Command

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.

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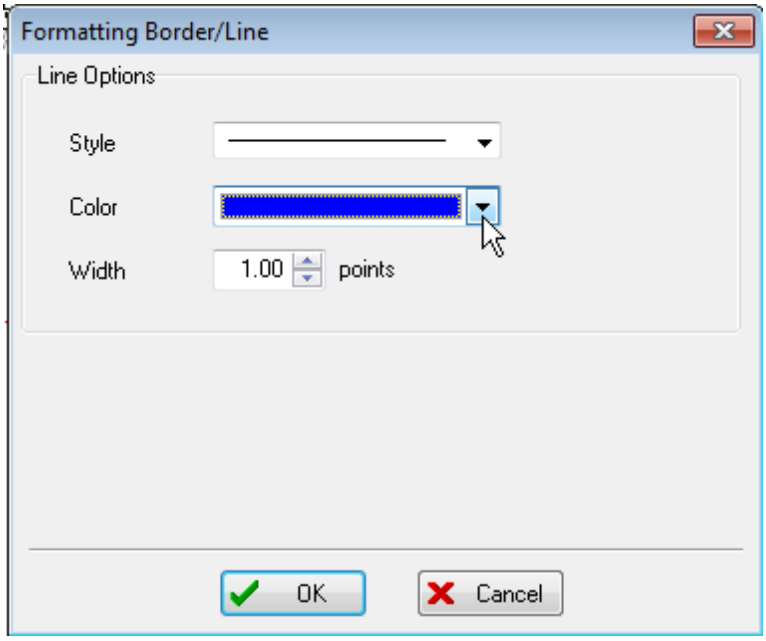

Figure T3.11 Formatting Border/Line Dialog

Both plots will now have a blue border, as shown in Figure T3.12.

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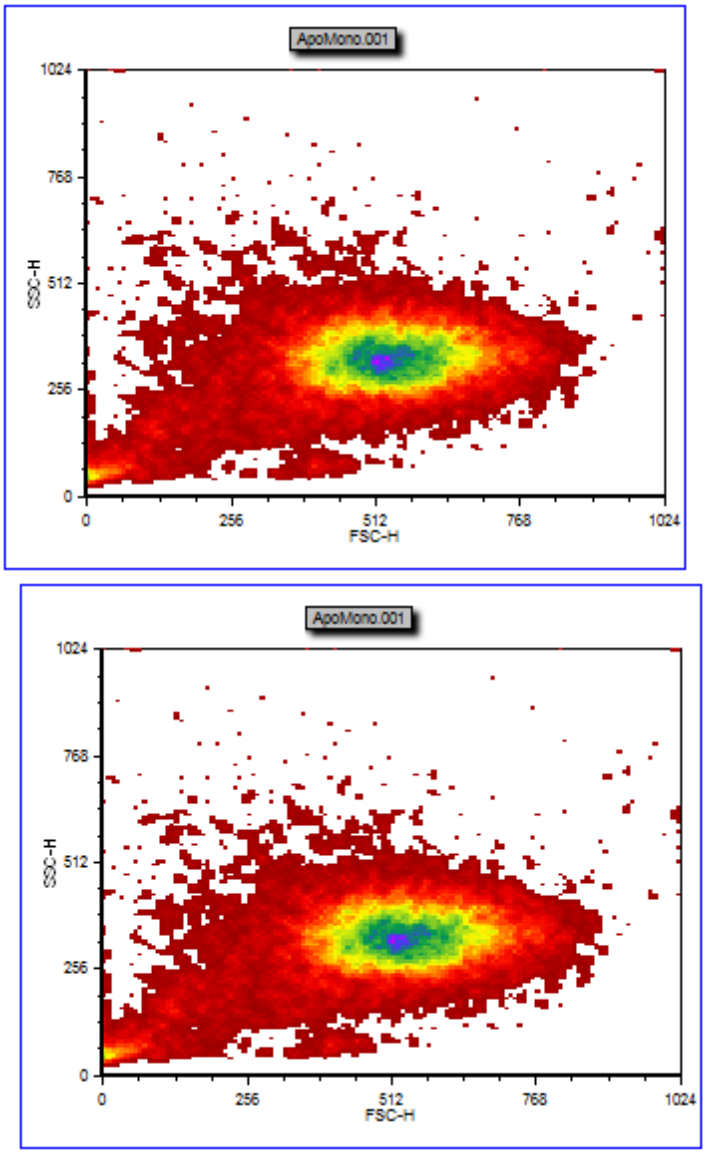

Figure T3.12 Plots with a Blue Border

12. Select **Undo** from the Quick Access Toolbar 34 to undo the formatting changes to the plots (Figure T3.13).

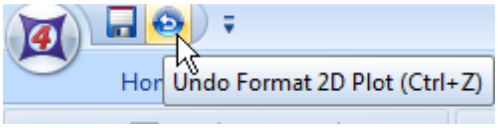

Figure T3.13 Undo

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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.

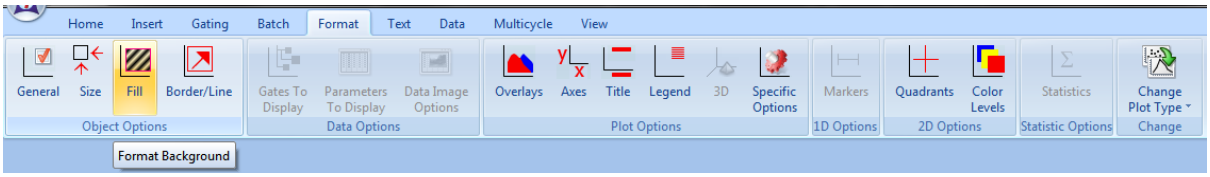

Figure T3.14 Format Tab

In the next section, we will format plot titles 632.

34.3.3 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):

1. Right-click on the top plot to open the pop-up menu.
2. Select **Format** from the pop-up menu (Figure T3.5 620).
3. Click on the **Titles** category from the **Plot Formatting** dialog (Figure T3.6

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620).

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. Select **Right** from the **Alignment** list to right align the title. ❹
9. Change the **Shadow Size** to **0** to remove the shadow from the background of the header. ❺

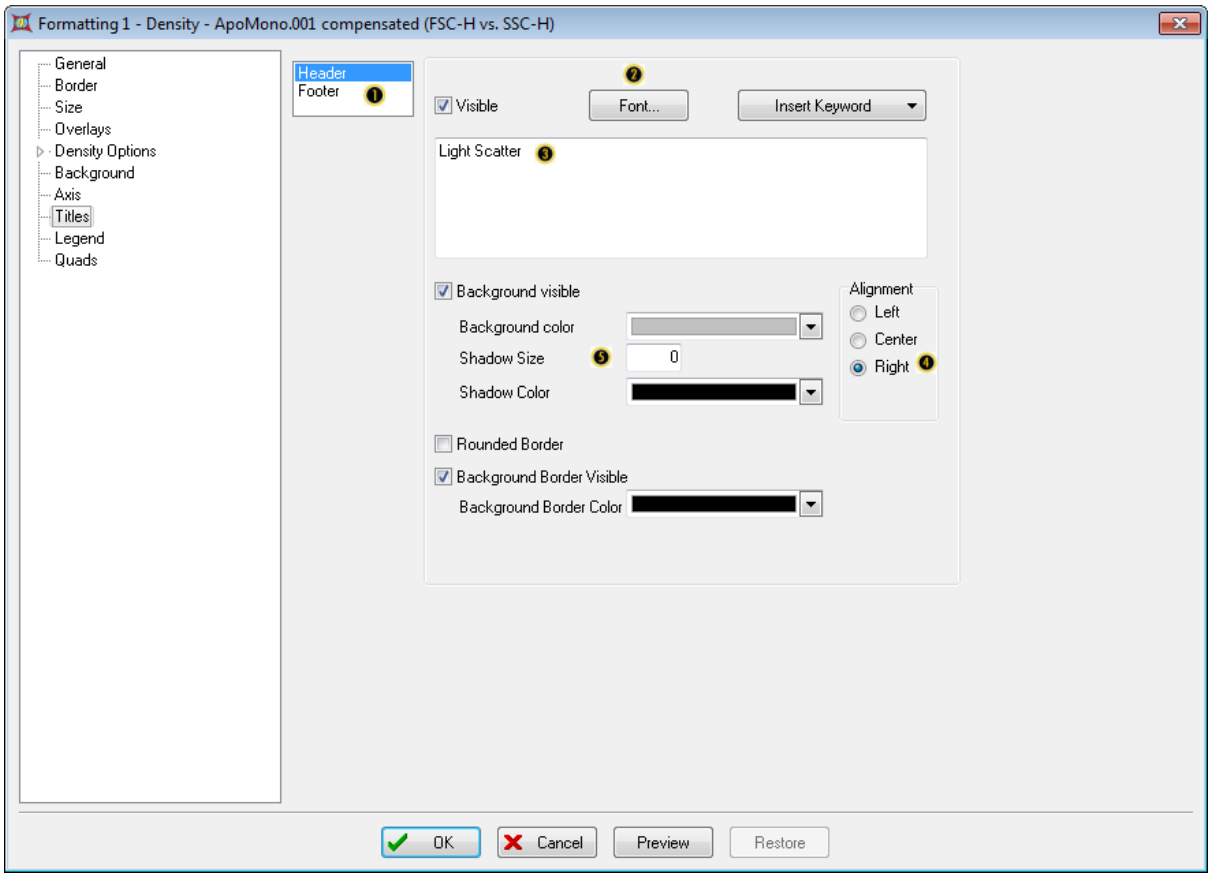

Figure T3.15 Formatting Titles Dialog

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

The plot should now look like the plot in Figure T3.16B.

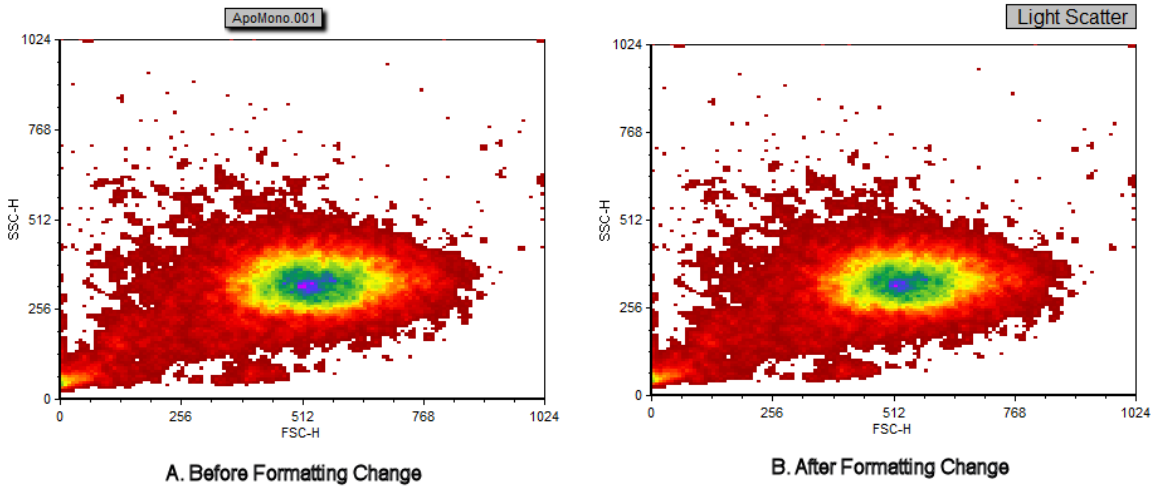

Figure T3.16 Changing the Header Properties

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 Keyword** button ❸ and select **Keyword from Data** from the drop down 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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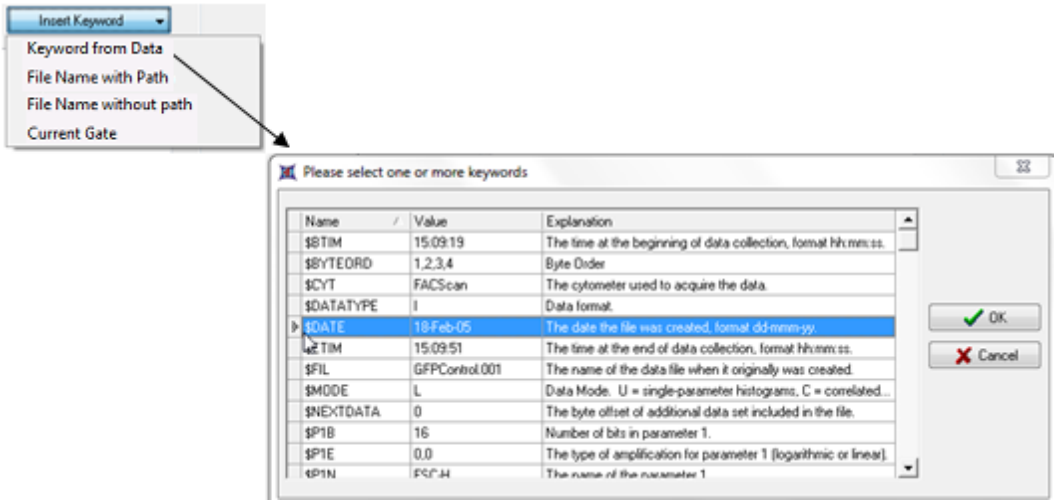

Figure T3.17 Select FCS Keyword

7. Select the **$Date** keyword. 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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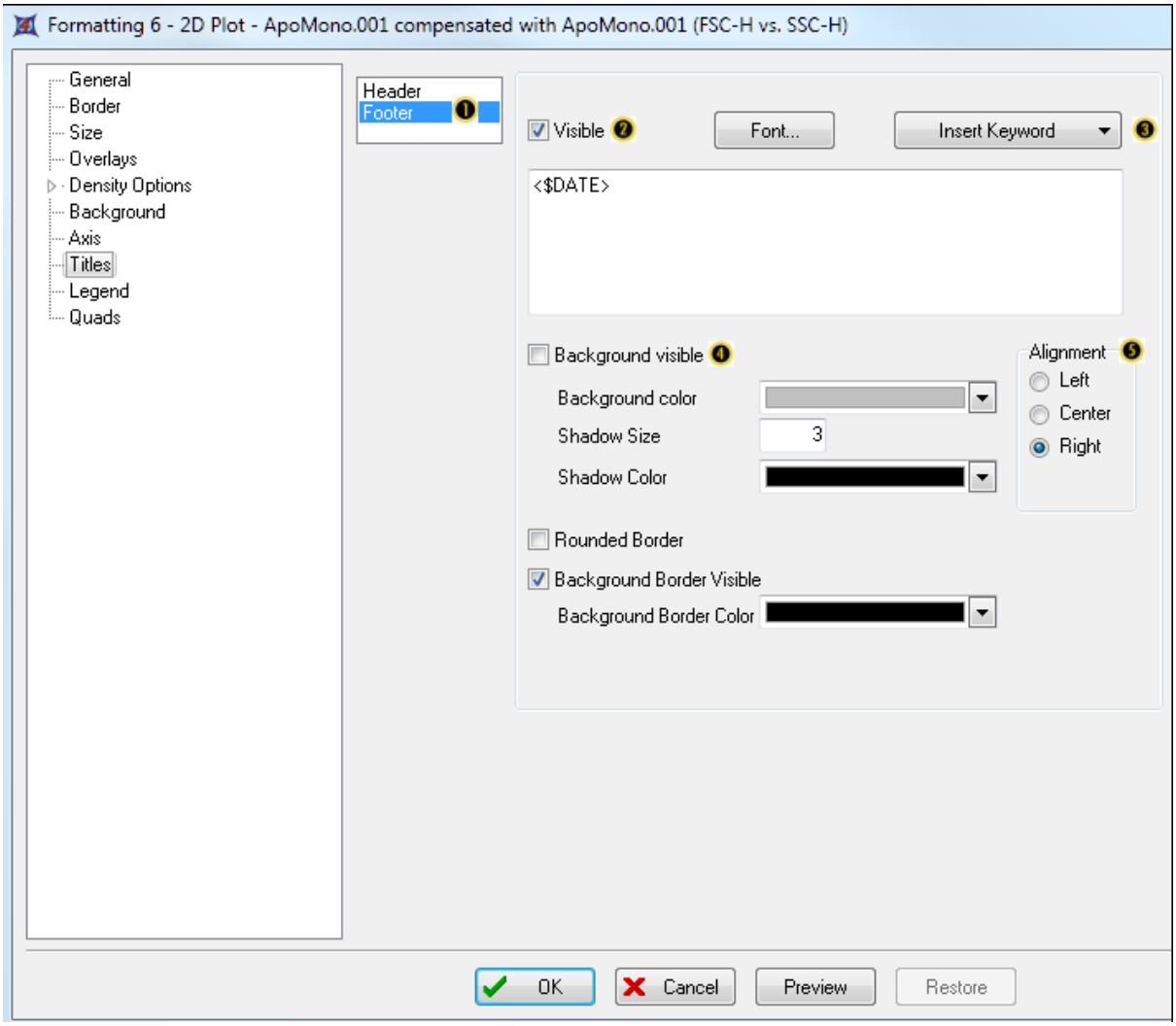

Figure T3.18 Formatting Titles Category (Footer)

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

The plot should now look like Figure T3.19B.

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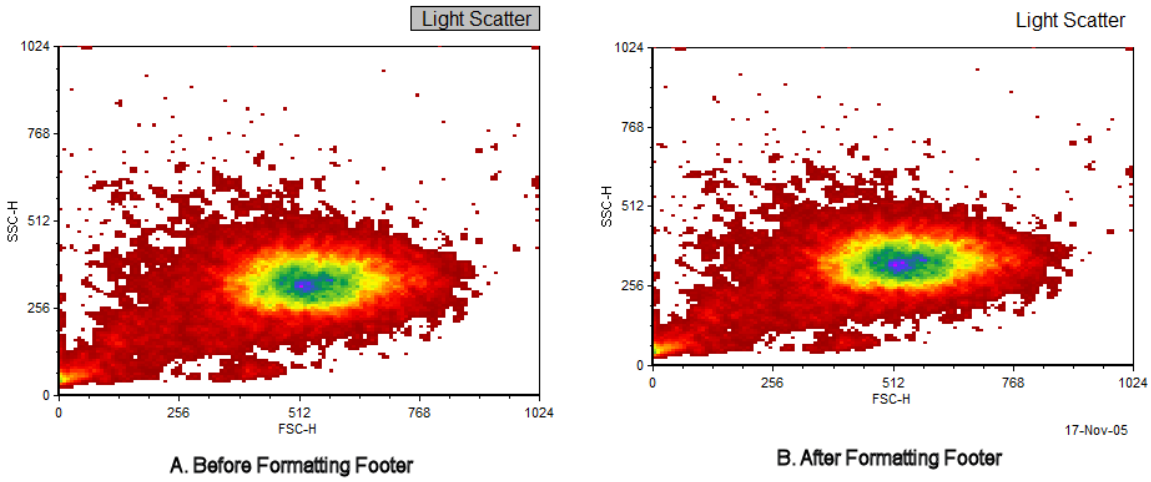

Figure T3.19 Changing the Footer Properties

Next, we will format the plot axes 637.

34.3.4 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.
3. Click on the **Y Axis** ❷ to set the properties for the Y axis.

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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 ❺ to turn it on.
7. Click on the **X Axis** ❷ 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** ❹ to 10.

Press **OK** to accept the changes.

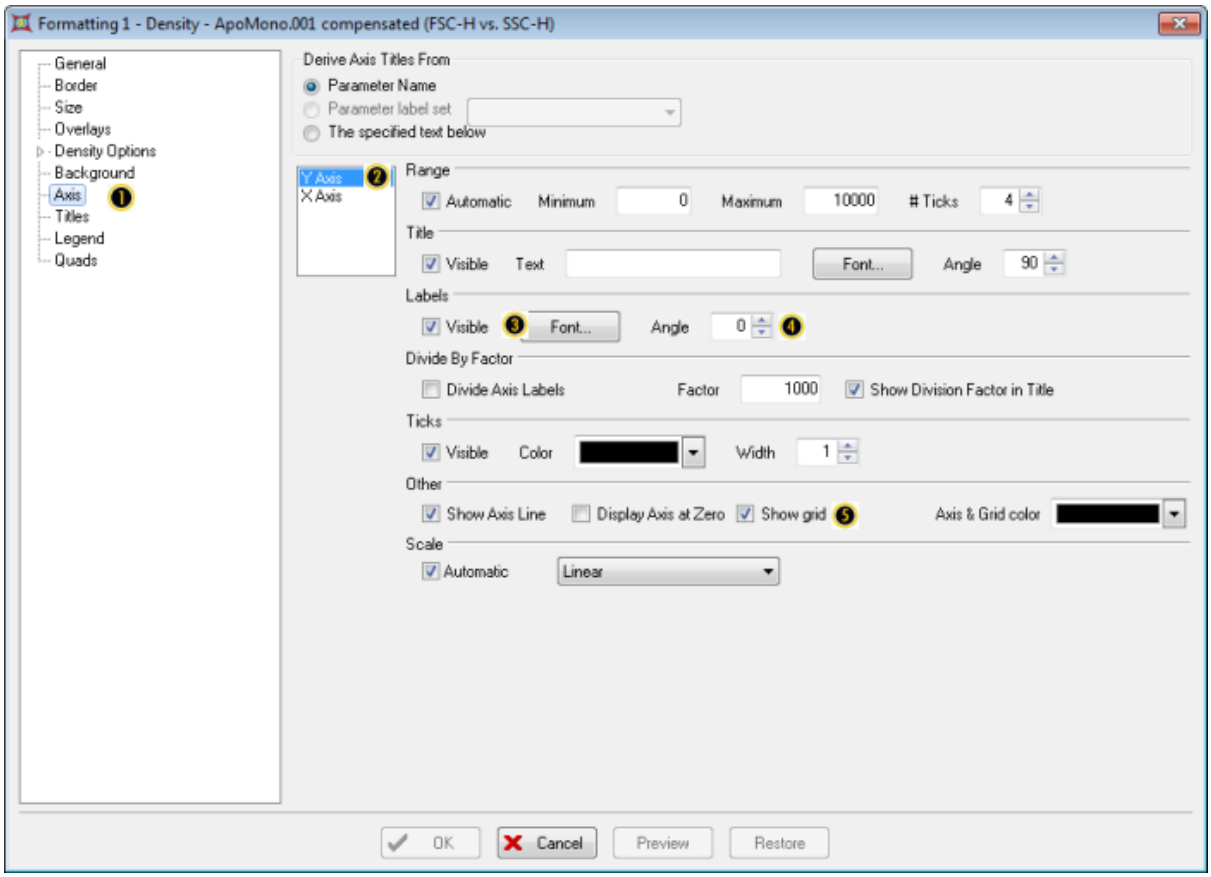

Figure T3.20 Axis Formatting

The axes of the plot have now been modified from those shown in Figure T3.21A to those seen in Figure T3.21B.

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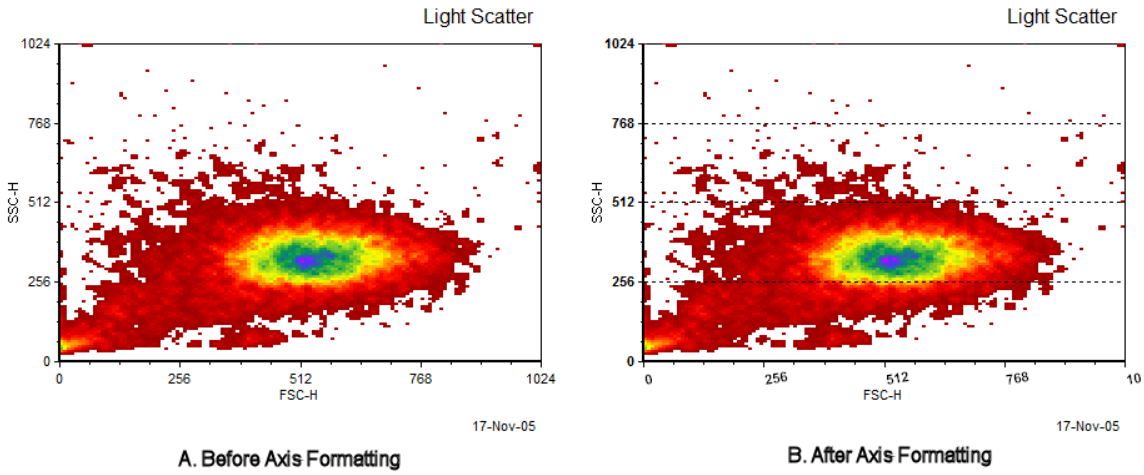

Figure T3.21 Formatting Axis Example

In the next section, we will format the plot background 639.

34.3.5 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):

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.

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4. Select turquoise as the gradient **Start Color**.
5. Change the gradient **End Color** to white.
6. 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 T3.21B 637.

Press **OK** to accept the changes.

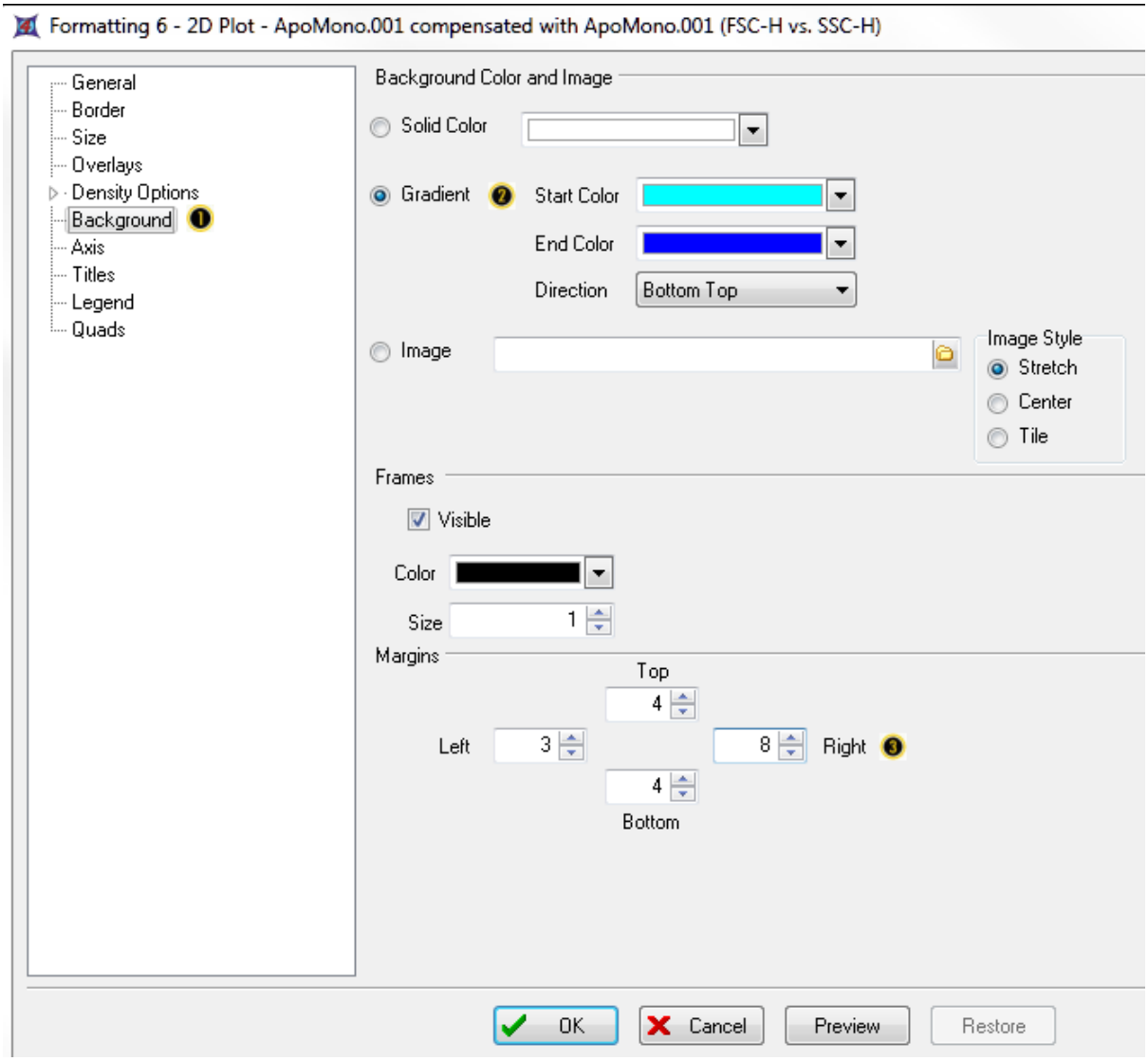

Figure T3.22 Background Formatting

The plot background has now been modified to display a gradient background as shown in Figure T3.23B.

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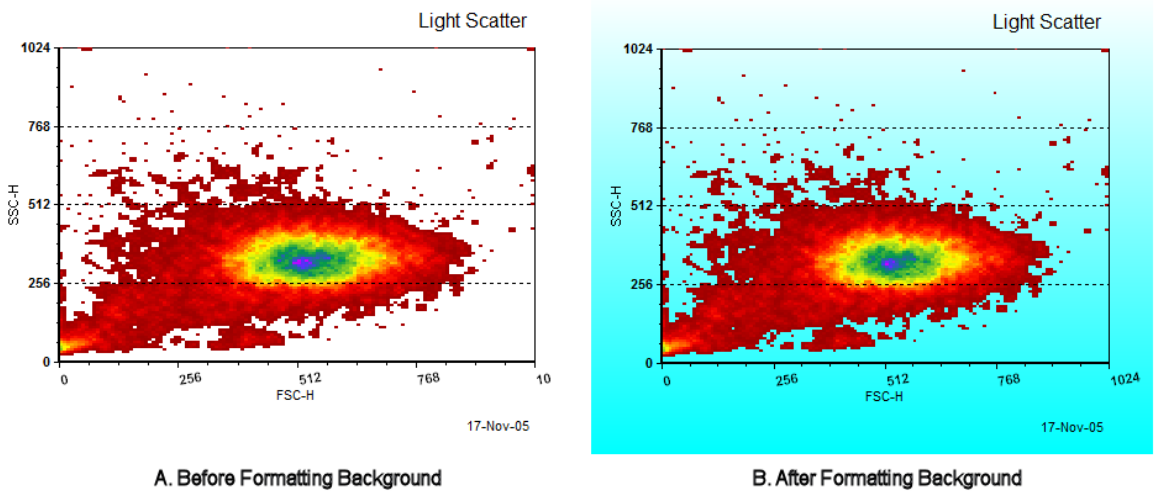

Figure T3.23 Formatting Background

Next, we will format the legend 641.

34.3.6 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 tutorial – Inserting Plots 607.
2. Right-click on the histogram.
3. Select **Add Overlay** from the pop-up menu.
4. Choose the **ApoMono.002** data file. Leave the rest of the properties on the add overlay window alone.

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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.*

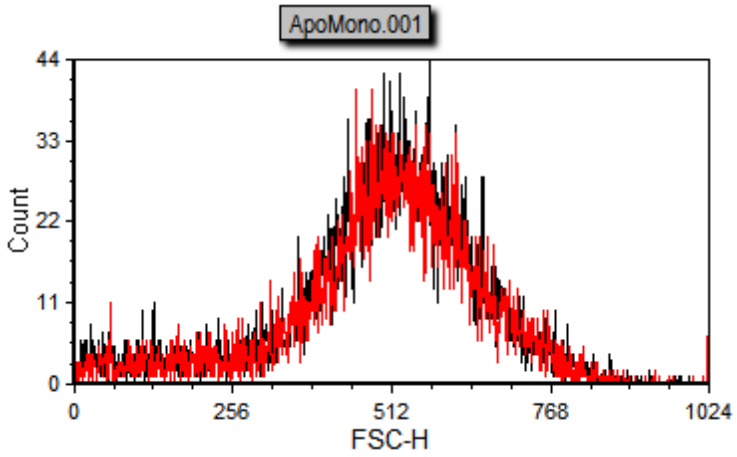

Figure T3.24 Histogram with Two Overlays

To format the legend (numbers refer to Figure T3.25):

1. Right-click on the histogram and select **Format** from the pop-up menu.
2. Select the Legend category 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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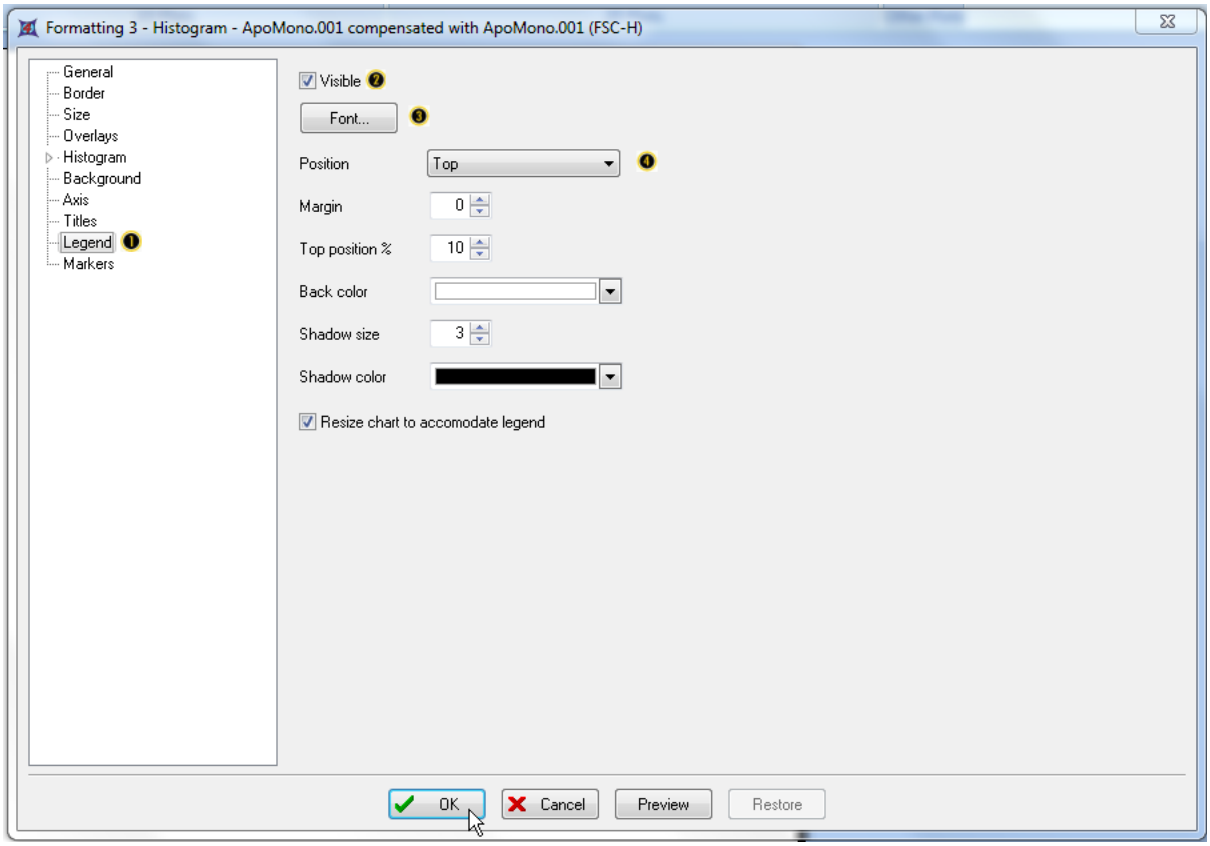

Figure T3.25 Formatting - Legend Category

The plot should now look like Figure T3.26.

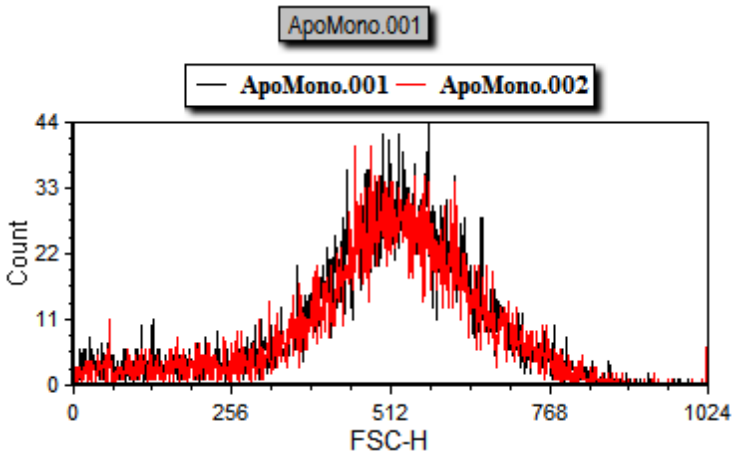

Figure T3.26 Histogram with Formatted Legend

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Overlays can be formatted either as stacked or overlaid644.

34.3.7 Changing Histogram Overlays

To change histogram overlays to stacked:

1. Select the histogram (see selecting objects592).
2. Select the **Format→Plot Options→Specific Options** command (Figure T3.27). A **Formatting Histogram Specific Options** dialog will appear (Figure T3.28).

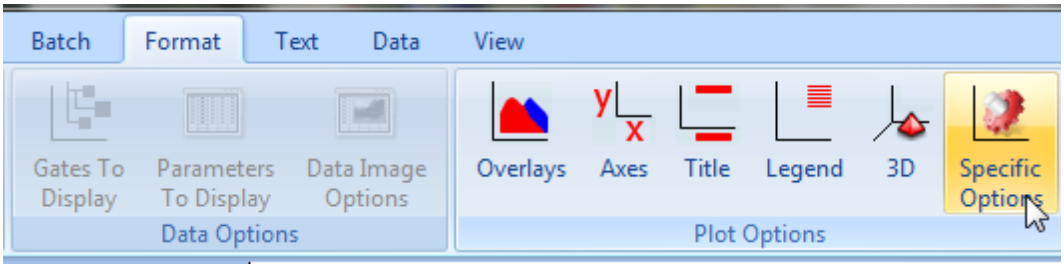

Figure T3.27 Format Specific Options Command

3. Check **Stacked** ❶ from the **Display multiple overlay data** section (Figure T3.28).
4. Click **OK**.

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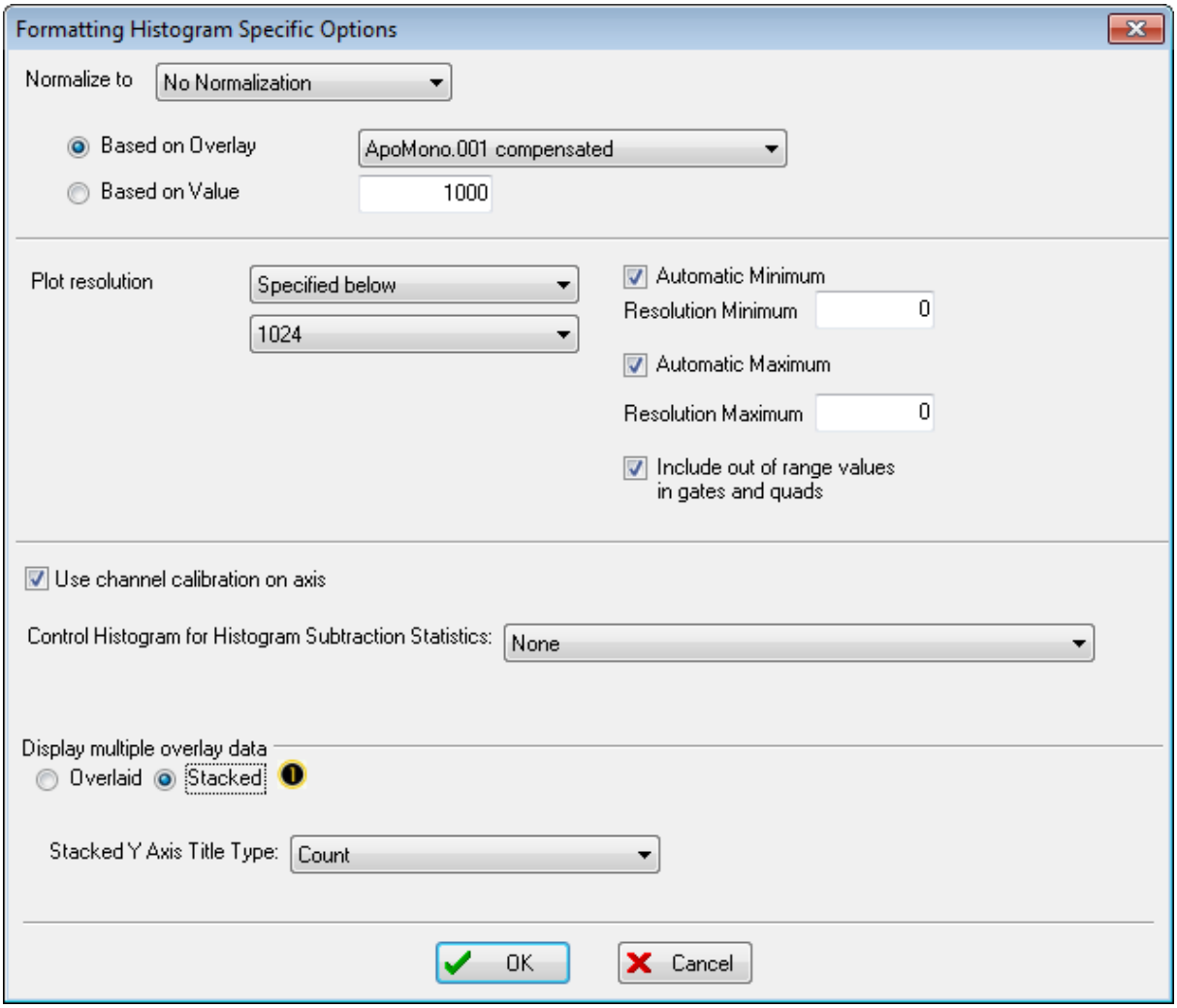

Figure T3.28 Format Histogram (Stacked Overlays)

The histogram will change to stacked (Figure T3.29).

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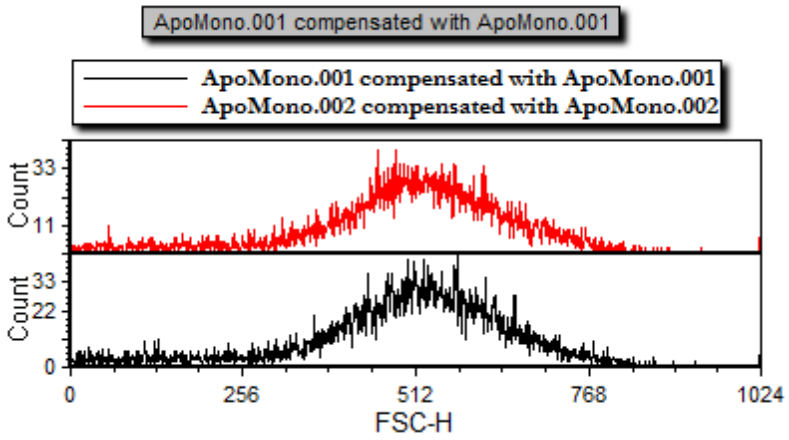

Figure T3.29 Stacked Histograms

To change the histogram overlays back to overlaid:

1. Select the **Format→Plot Options→Specific Options** command. A **Formatting Histogram Specific Options** dialog will appear (Figure T3.30).
2. Check **Overlaid** ❶ from the **Display multiple overlay data** section.
3. Click **OK**.

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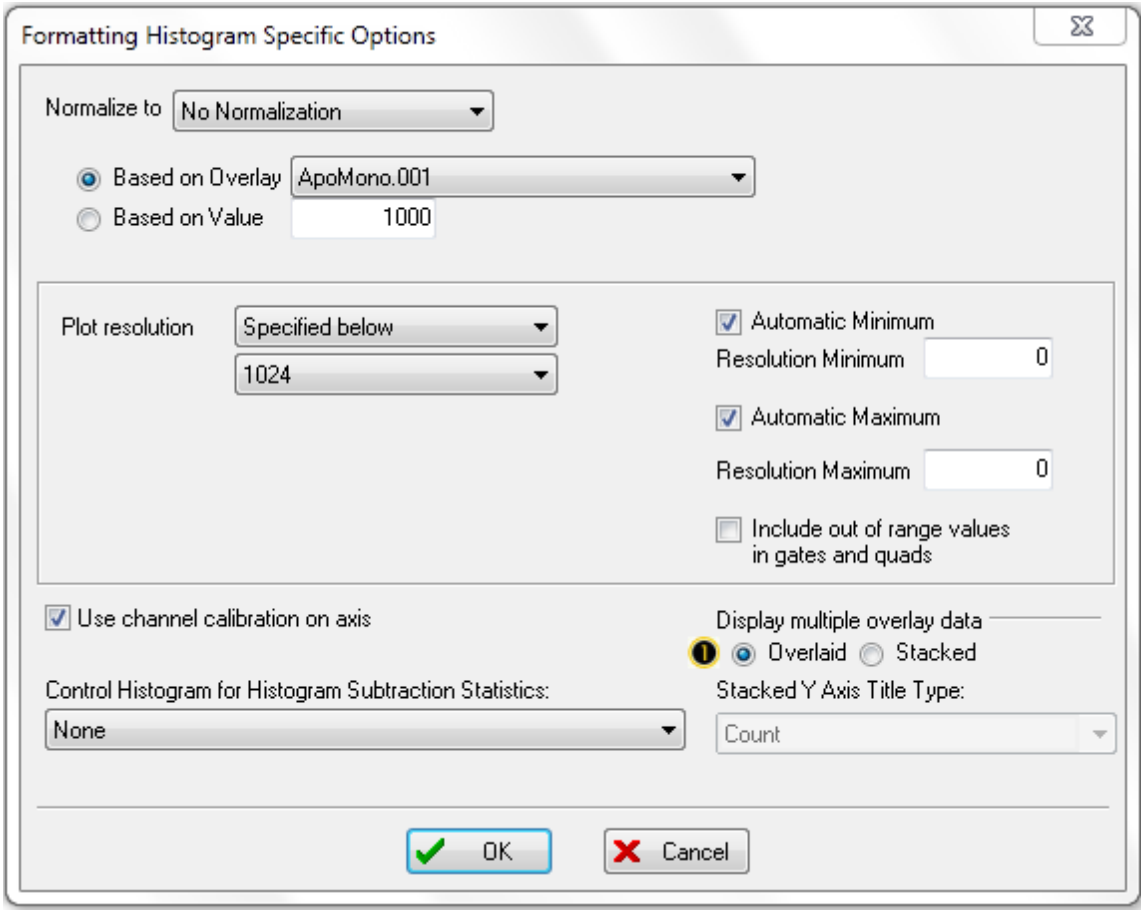

Figure T3.30 Format Histogram (Overlaid Overlays)

The histogram will change back to the overlaid style (Figure T3.26 641).

Certain plots can be formatted in 3D 648.

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34.3.8 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 T3.26 641. Numbering refers to Figure T3.31.

1. Right-click on the histogram and select **Format**.
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 %** ❸ to 100%.
5. Adjust the **Zoom** slider ❹ to 70%.
6. Adjust the **Vertical offset** slider ❺ to approximately 10%.

Press **OK** to accept the changes.

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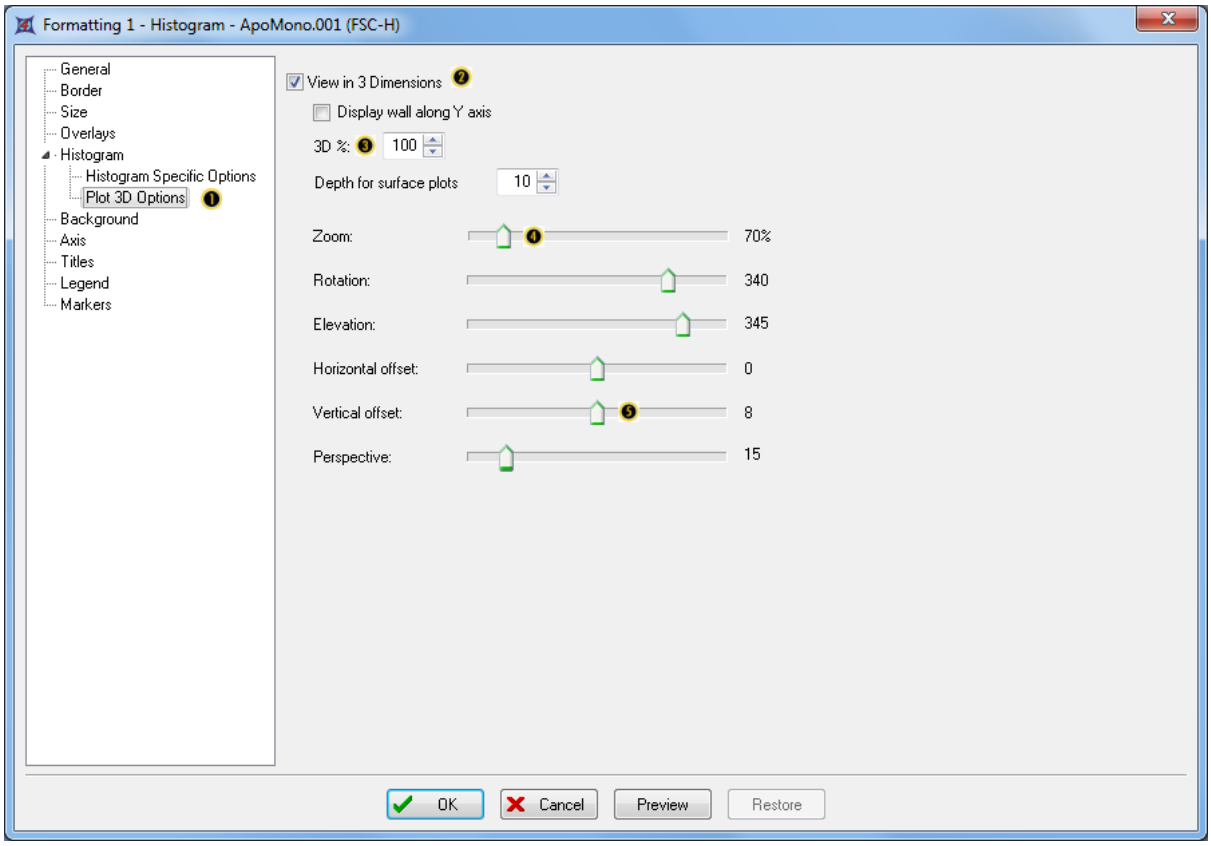

Figure T3.31 Histogram 3D Options

The plot should now look similar to Figure T3.32.

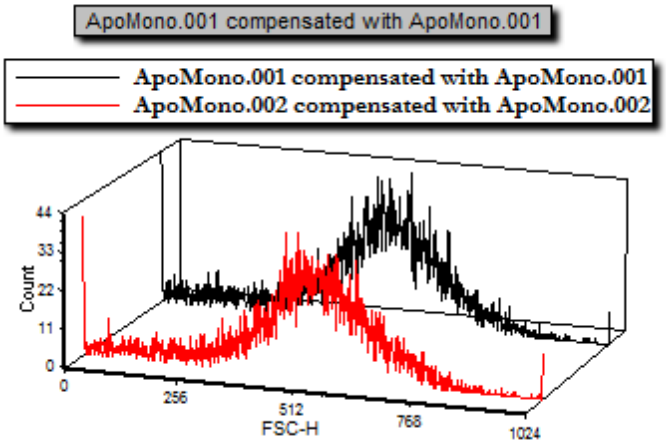

Figure T3.32 Histogram with 3D Overlays

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34.4 Gating

In FCS Express Version 4, 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 192.

The first section, will introduce creating gates 650.

34.4.1 Creating Gates

Using the commands from the **Create Gates** groups on the **Home** and **Gating** tabs 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).

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.

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 590. The **section5.fey** layout and the **ApoMono.001** and **ApoMono.002** data files will be used here.

To Open a Layout:

1. Select **Application Button→Open Layout**.
2. Open the **section5.fey** layout located in the Tutorial Sample Data archive 590.
3. Select the **Home→Editing→Select All** command.
4. Select the **Data→Change Data on Selection→Select** command.

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5. Select the file **ApoMono.001** located in the *FCS Express Sample Data* directory.

Gates are usually created using the **Gating** tab. The Gating commands are shown in Figure T4.1 and described in the table below. Gating commands are also present on the **Home** tab. Gating of data obtained from multiwell plates will be discussed in a subsequent tutorial.

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):

1. Move the mouse so that it is over the **Rectangle** gate button.
2. Pause for half of a second.

Figure T4.2 Gating Command Hint

We are going to insert four different kinds of gates on the different plots on the

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layout.

To create a Rectangular Gate:

1. Select the **Gating→Create Gates→Rectangle** command. You will know it is pressed because it will have a different color background

.

We will create the rectangular gate on the density plot (Figure T4.3) underneath where it says "Create the **rectangular gate** on this plot".

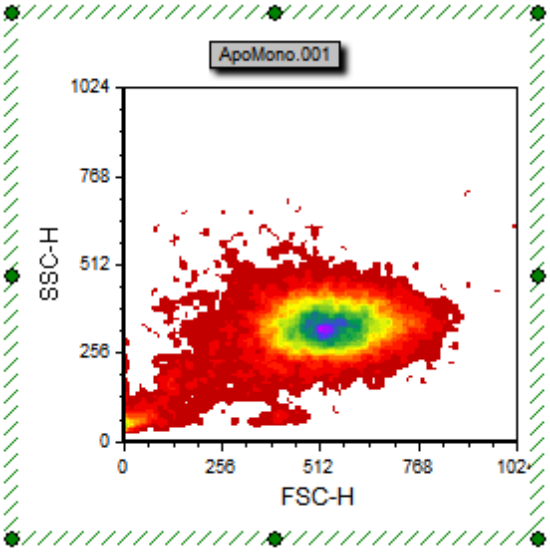

Figure T4.3 Density Plot for First Gating Excercise

2. Move the cursor to the top left corner of where you would like the gate to begin.

3. Press and hold the left mouse button.

4. Move the cursor to where you want the bottom right margin of the gate to be, while holding the left mouse button down (Figure T4.4).

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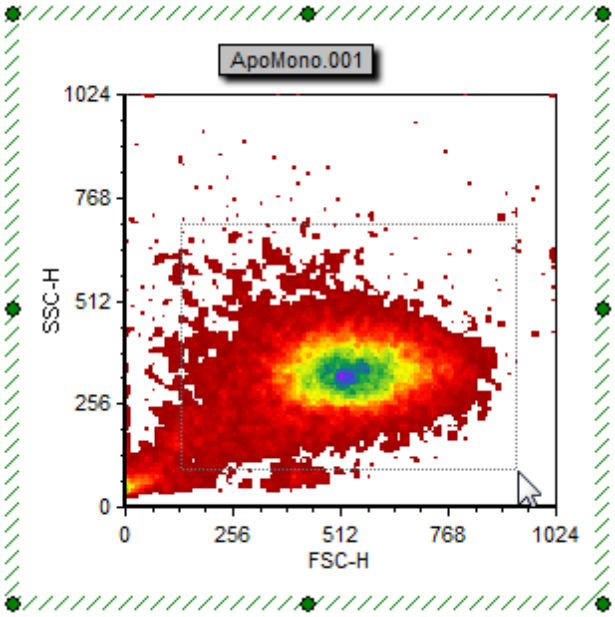

Figure T4.4 Creating Rectangular Gate

5. Release the mouse button.

The **Create New Gate** dialog will appear (Figure T4.5). We can now give the gate a name.

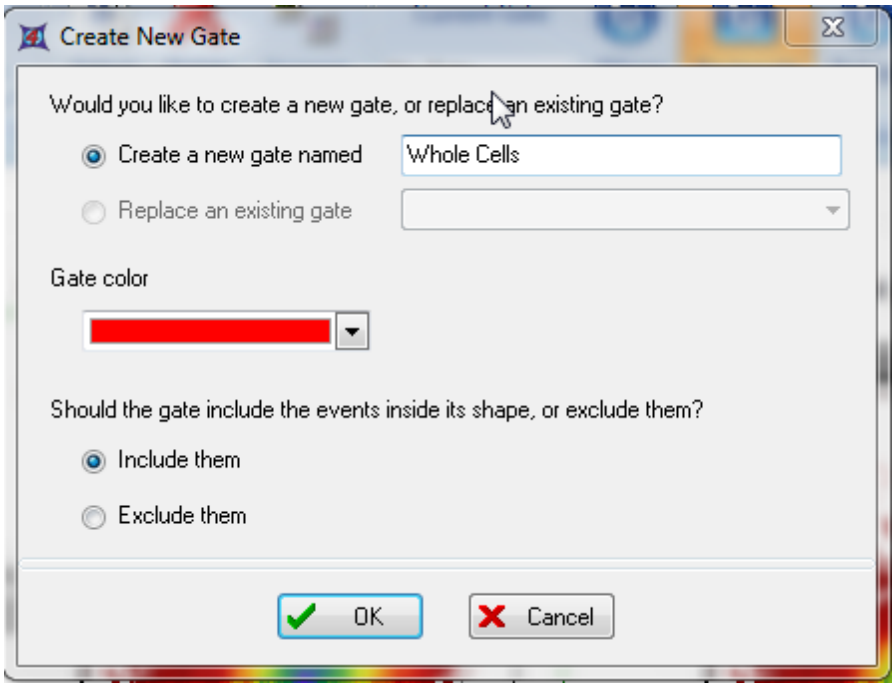

Figure T4.5 Create New Gate Dialog

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6. Enter "Whole Cells" in the **Create a new gate named** edit field.
7. Click **OK** to accept the name.

The gate and a text box displaying the gate name will appear in the plot (Figure T4.6).

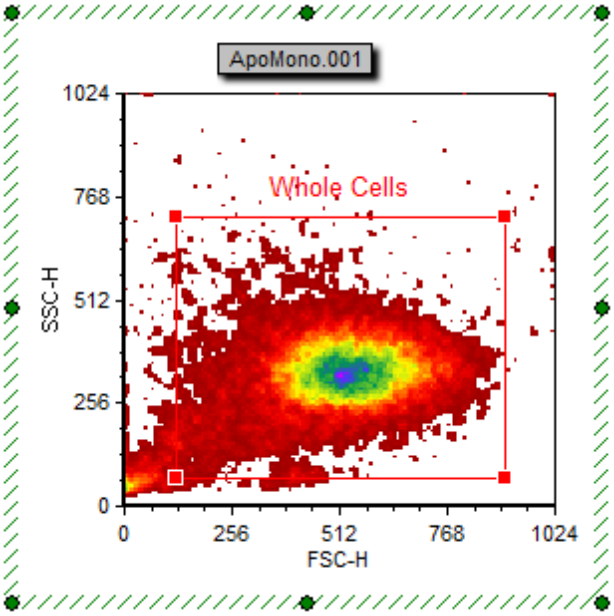

Figure T4.6 Newly Created Rectangular Gate

Note: You can cancel the creation of gates after you begin (i.e., anytime during step 2 to 5) by pressing ESC on the keyboard. This technique applies to all types of gates.

To create an Elliptical Gate:

1. Select the **Gating→Create Gates→Ellipse** command. You will know it is pressed because it will have a different color background

.

We will create the elliptical gate on the plot underneath where is says "Create the **elliptical gate** on this plot".

2. Follow steps 2-5 above to create the elliptical gate.

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3. Enter "Cells in ellipse" in the **Create a new gate named** edit field.
4. Press **OK** to accept the name.

To create a Polygon Gate:

We will create the polygon gate on the plot underneath where it says "Create the **polygon gate** on this plot".

1. Select the **Gating→Create Gates→Polygon** command. You will know it is pressed because it will have a different color background

.
2. Move the cursor to a point where you want to start your gate.
3. Click the mouse to start the polygon gate. A black square will appear to indicate where the first gate point was created.
4. Move the mouse to a point where you want to create another vertex of the gate. There will be a line following the cursor as you move.
5. Click the mouse where you want the new vertex to be.
6. Repeat steps 4-5 until you want to close the polygon.
7. Close the polygon gate (Figure T4.7) by clicking on the black square from your first point. Alternatively, you can press the space bar to close the polygon.

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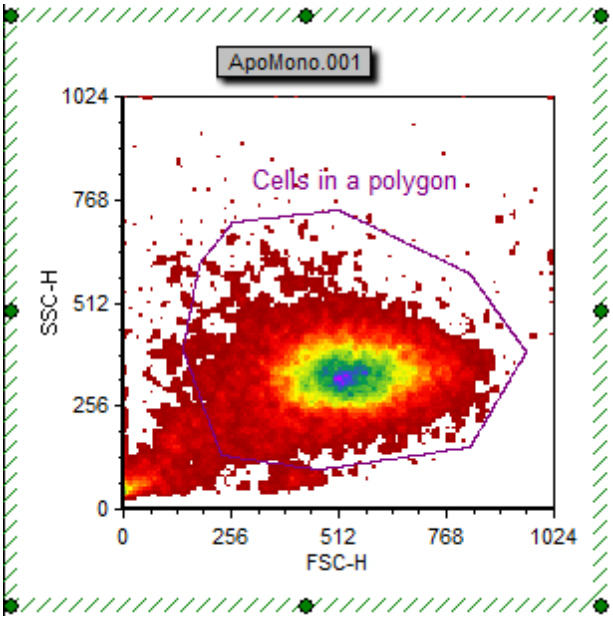

Figure T4.7 Polygon Gate

A **Create New Gate** dialog will appear, similar to Figure T4.5. We can now give the gate a name:

8. Enter "Cells in a polygon" in the **Create a new gate named** edit field.
9. Press **OK** to accept the name.

To create a Freeform Gate:

1. Select the **Gating→Create Gates→Freeform** command. You will know it is pressed because it will have a different color background

.
2. Move the cursor to the place where you want to start the gate.
3. Press and hold down the left mouse button.
4. 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.
5. Release the mouse to finish creating the gate (Figure T4.8).

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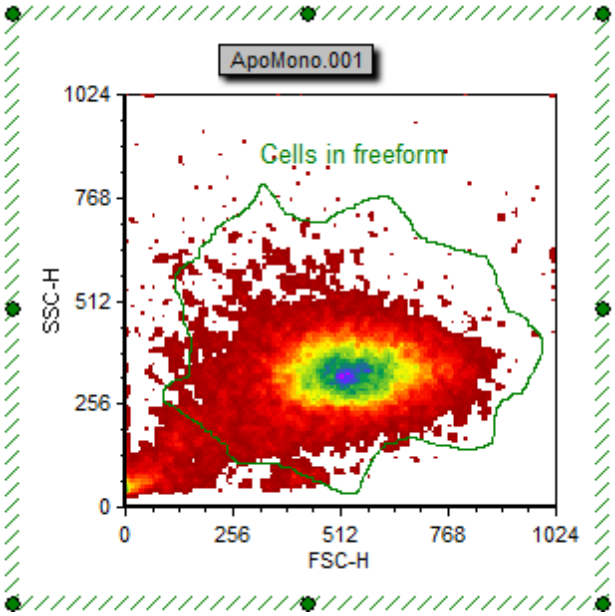

Figure T4.8 Freeform Gate

A **Create New Gate** dialog will appear, again similar to Figure T4.5. We can now give the gate a name:

6. Enter "Cells in freeform" in the **Create a new gate named** edit field.
7. Press **OK** to accept the name.

In the next section, we will create a 1D gate on a 2D plot 657.

34.4.2 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.

To create a 1D Gate on a 2D plot:

1. Select the **Gating→Create Gates→Create Marker** command.

You will know the **Create Marker** command is selected because it will have a different color background

.

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We will now create a 1D gate on the 2D plot underneath where it says "Create the **elliptical gate** on this plot" (Figure T4.9).

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.

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).

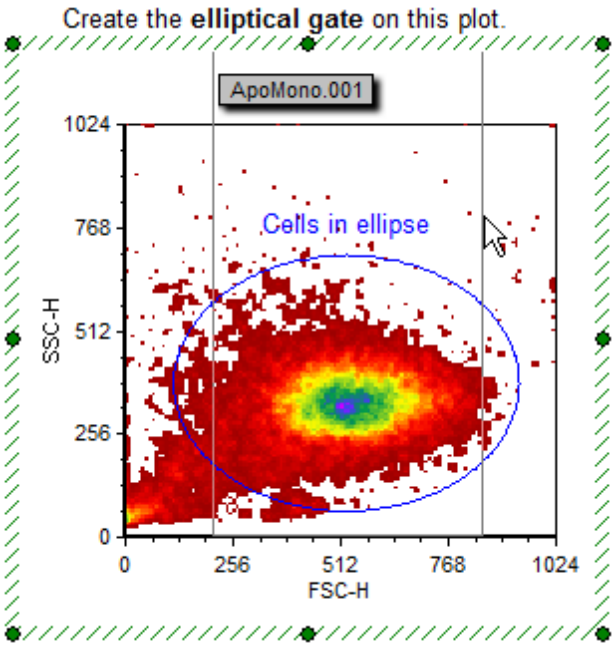

Figure T4.9 Creating a 1D Gate on a 2D Plot

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.

A 1D gate now appears on the 2D plot (Figure T4.10).

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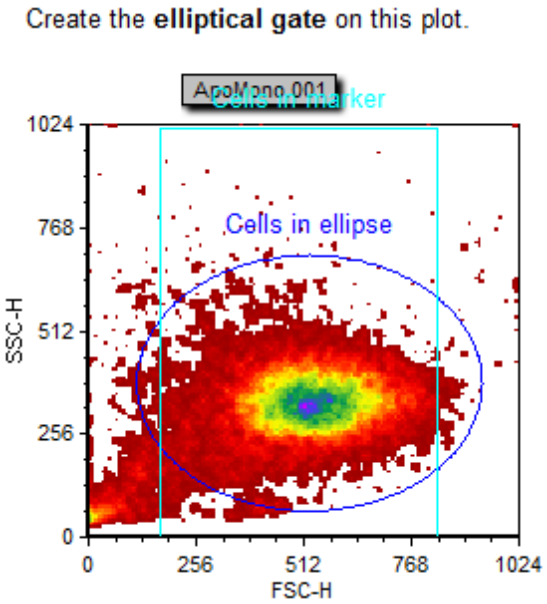

Figure T4.10 1D Gate on a 2D Plot

Next, we will create a gate from a marker 659.

34.4.3 Creating a Gate from a Marker

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 the **Home→Editing→Select All** command to select all plots.
3. Select the **Data→Change Data on Selection→Select** command.
4. Select the file **ApoMono.002** located in the Tutorial Sample Data archive 590.
5. Press **Open** on the file dialog to accept the file choice.
6. Click and hold down the mouse button on the X-axis label, (FSC-H), of the histogram.
7. Scroll the mouse to choose FL2-H as the new X-axis label.

The histogram should now look similar to Figure T4.11.

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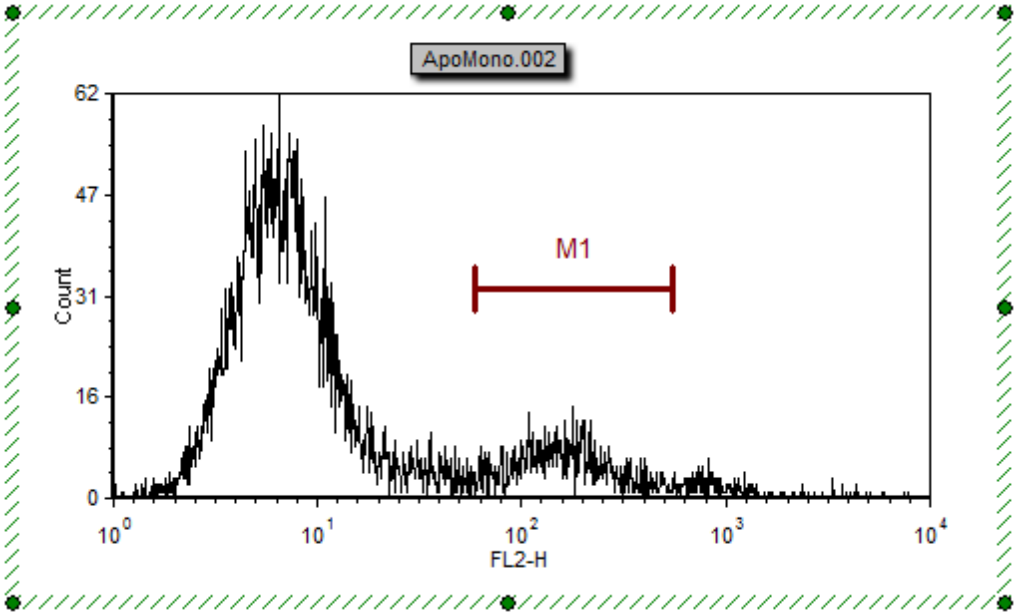

Figure T4.11 Histogram with Marker

8. Right-click on the histogram to bring up the pop-up menu.

9. Select **Convert Marker to Gate→Marker #1 (M1) →Convert and Link** from the pop-up menu (Figure T4.12).

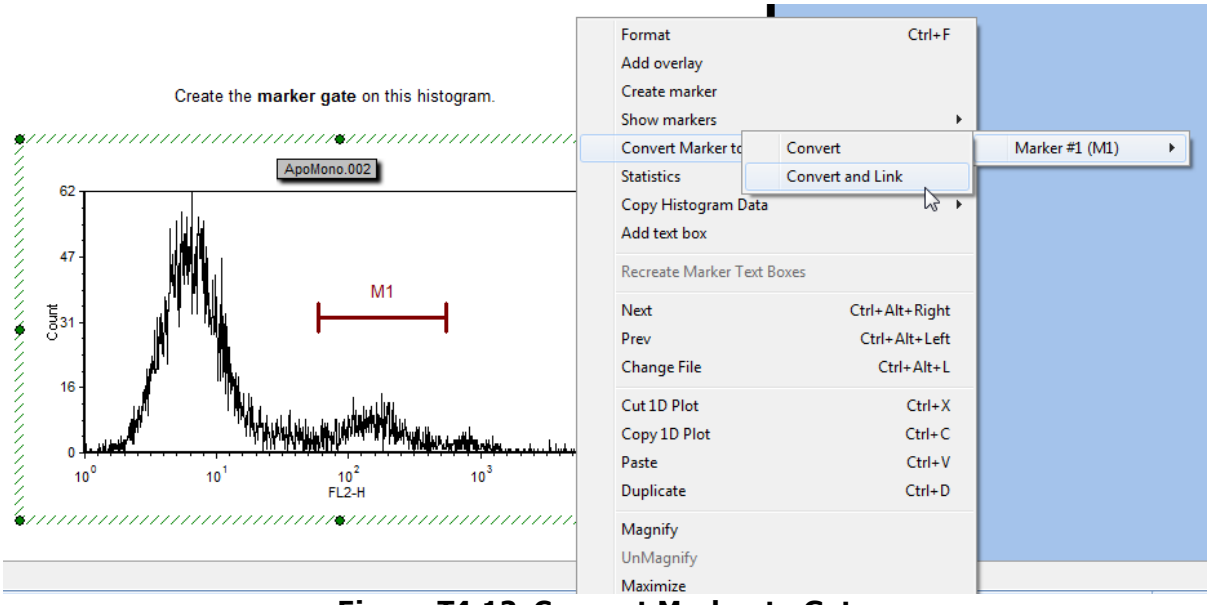

Figure T4.12 Convert Marker to Gate

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A dialog will appear asking you to name the gate.

10. Enter "Marker Gate" in the **Create a new gate named** edit field.

11. 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.

In the next section, we will create a gate from a quadrant 661.

34.4.4 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 210). Creating a quadrant gate is very similar to creating a marker gate.

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.

2. Select **Convert Quads to Gate→Lower Right→Convert and Link** from the pop-up menu (Figure T4.13).

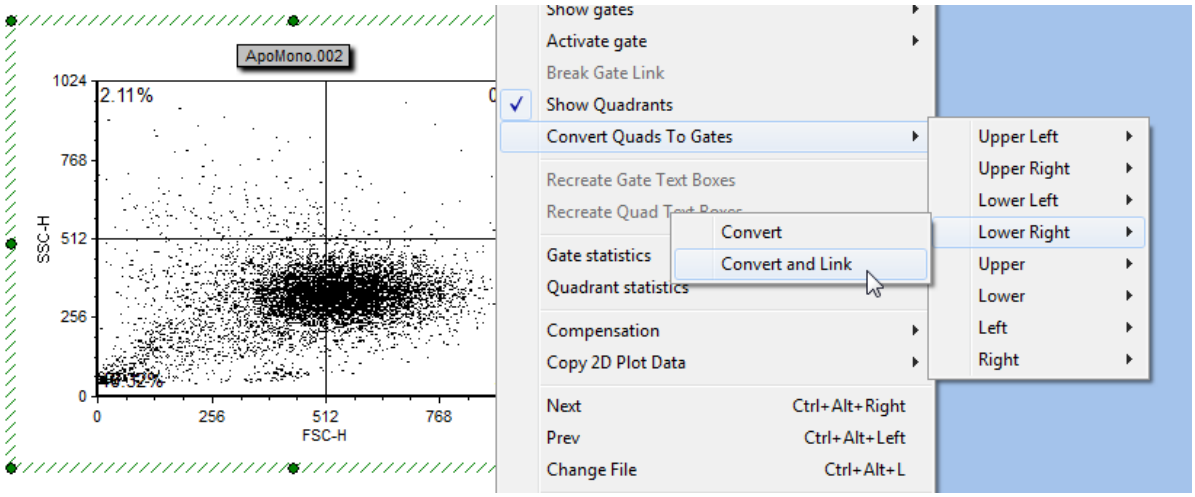

Figure T4.13 Converting a Quadrant to a Gate

A dialog will appear asking you to name the gate.

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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 662.

34.4.5 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.

In the course of this example we will:

- Learn about the Gate View feature.
- Create a combination gate.

We will continue using the same layout as we were using above.

To insert a Gate View:

1. Select the **Page 1** tab from the **Layout Navigation Toolbar**.
2. Select the **Insert→General→Gate View** command.
3. Click the left mouse button on the layout where you want the upper left of the **Gate View** to appear.
4. The **Gate View** (Figure T4.14) now appears on the layout.

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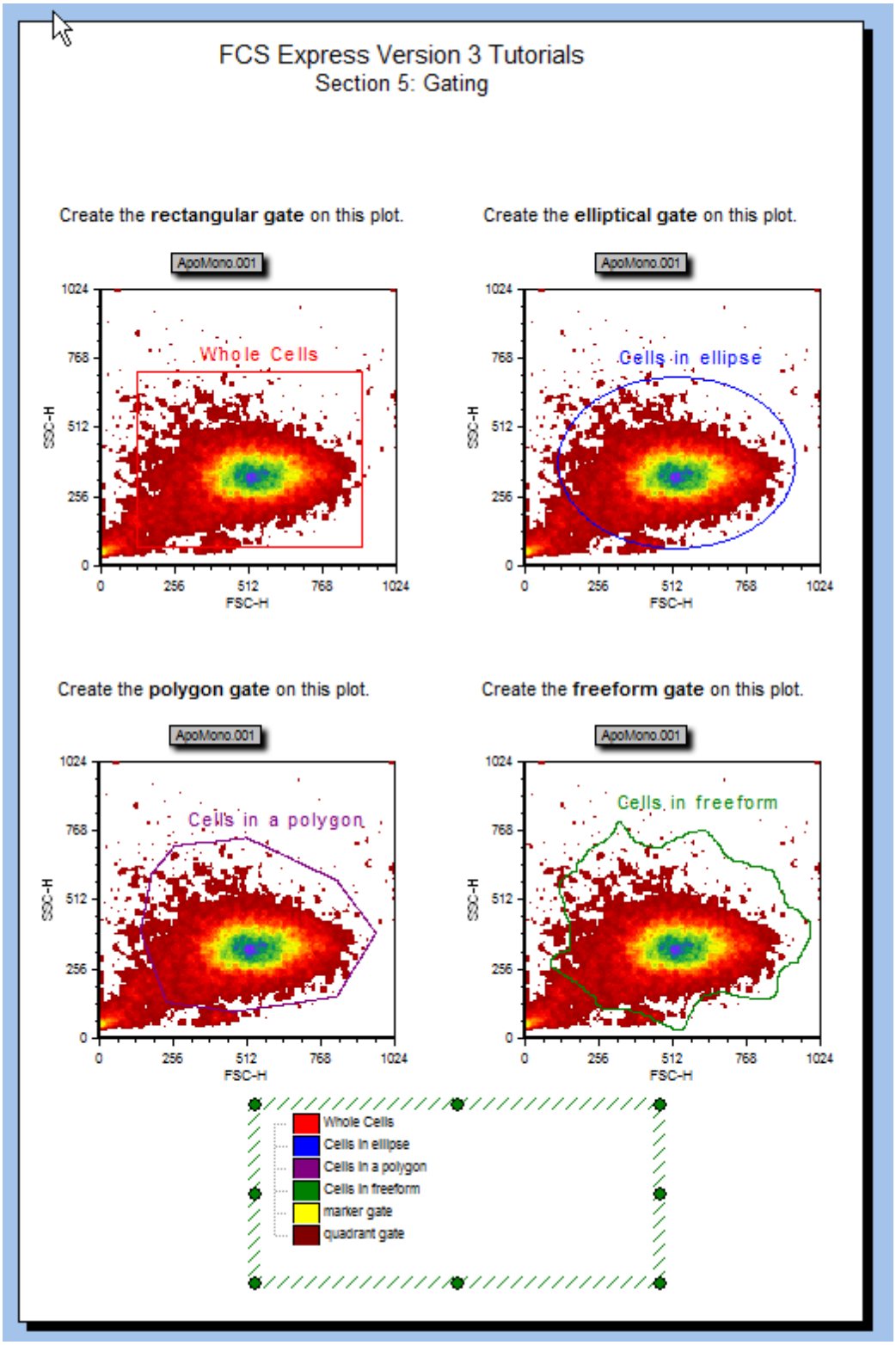

Figure T4.14 A Gate View on a Layout Page

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To create a Combination Gate:

1. Right-click on the **Gate View** to bring up the pop-up menu.
2. Select **Add Combination Gate** from the menu (Figure T4.15).

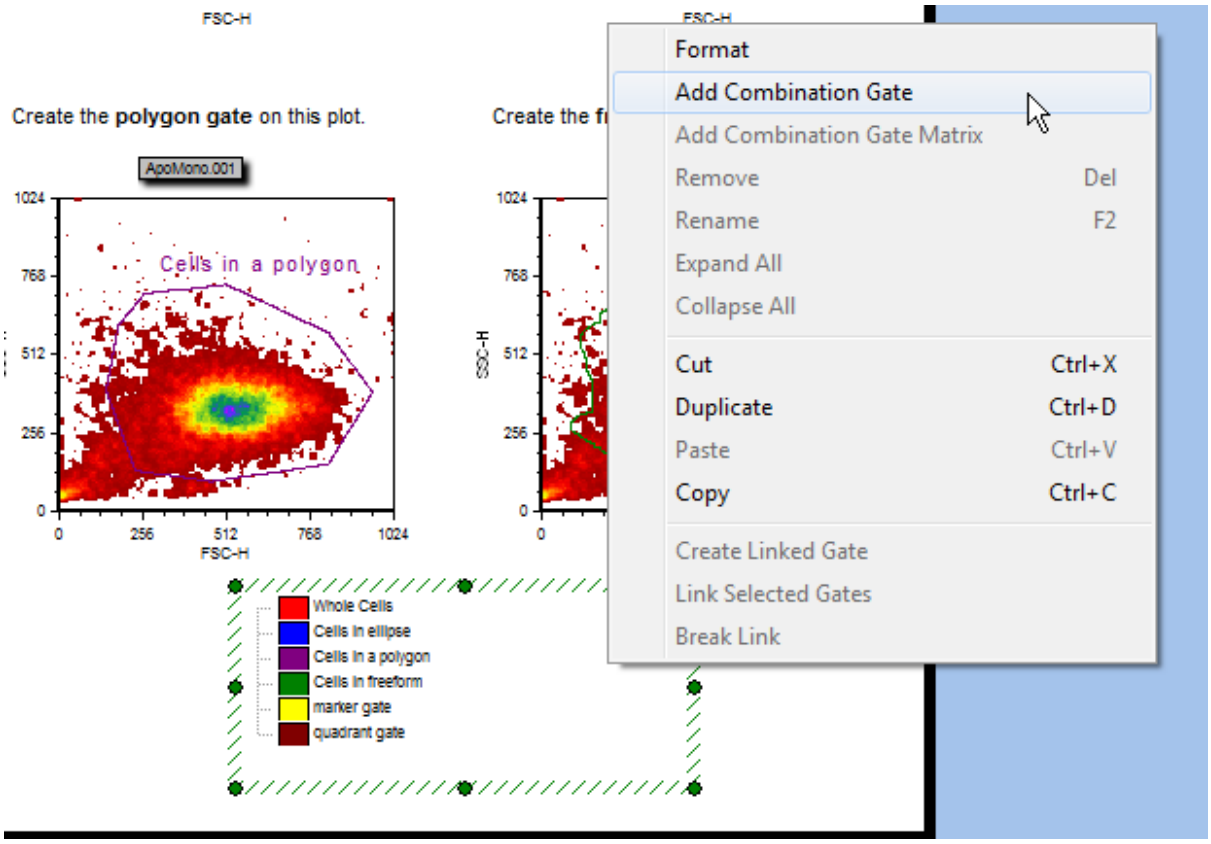

Figure T4.15 Add a Combination Gate

3. The **Create Gate** editor will appear (Figure T4.16).

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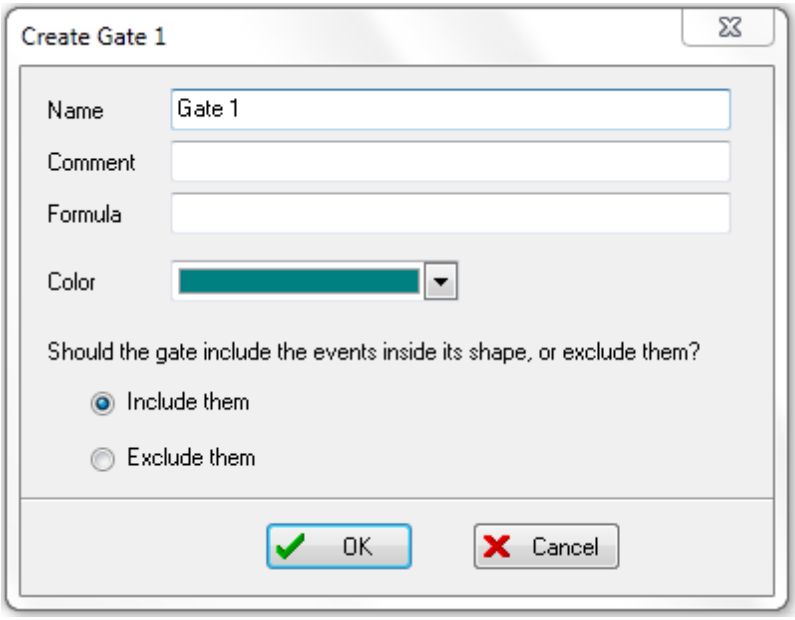

Figure T4.16 Create Gate Dialog

4. Type "My Combination Gate" in the **Name** field.
5. Click in the formula field.
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".
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". The screen should look similar Figure T4.17.
14. Press the **OK** button to create the combination gate.

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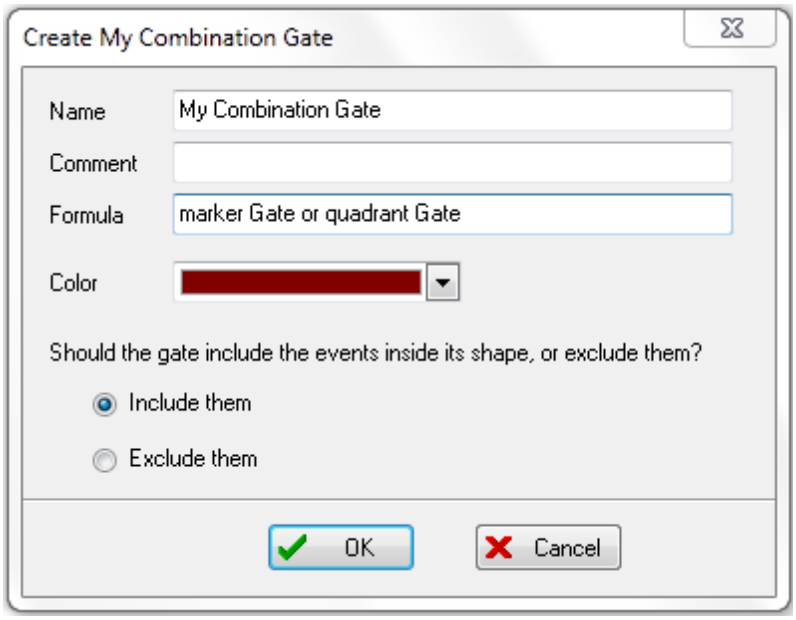

Figure T4.17 Create My Combination Gate Dialog

More information about creating combination gates and all the different possible operators can be found in the section on Creating Gates[193] in the main part of the manual.

Next, we will learn how to move a gate[666].

34.4.6 Moving Gates

In the course of this example we will:

- Activate a gate
- Move a gate

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, as previously shown in Figure T4.6[650].
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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The next topic is resizing a gate 667.

34.4.7 Resizing Gates

In the course of this example we will:

- Resize a gate.
- Resize a gate with multiple vertices proportionally.
- Resize a vertex of a gate with multiple vertices independently.

1. Activate the "Whole Cells" gate by clicking inside the gate.
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.

You can press **Esc** on the keyboard while moving or resizing a gate to cancel each respective 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.
2. Move the mouse to the top right large outer resizing handle of the gate (indicated by the cursor in Figure T4.18). You will know you are in the correct place when the mouse cursor changes to a two-headed arrow.

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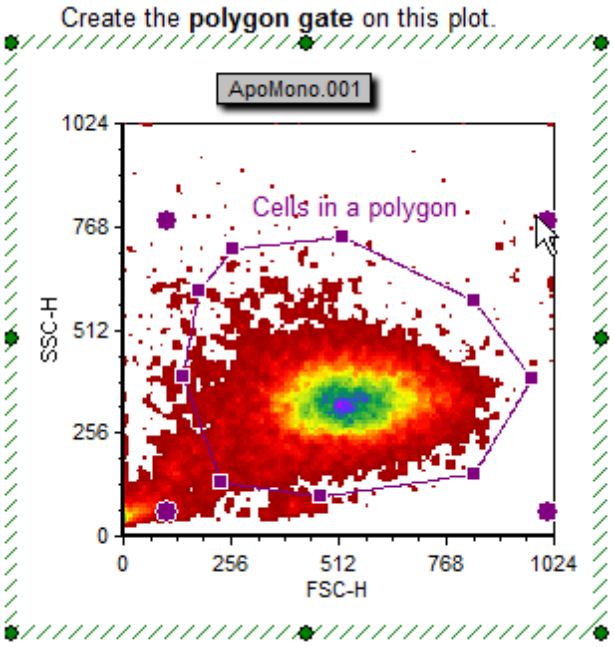

Figure T4.18 Proportional Resizing of a Polygon Gate

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 (indicated by the cursor in Figure T4.19A). 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 (indicated by the cursor in Figure T4.19B).

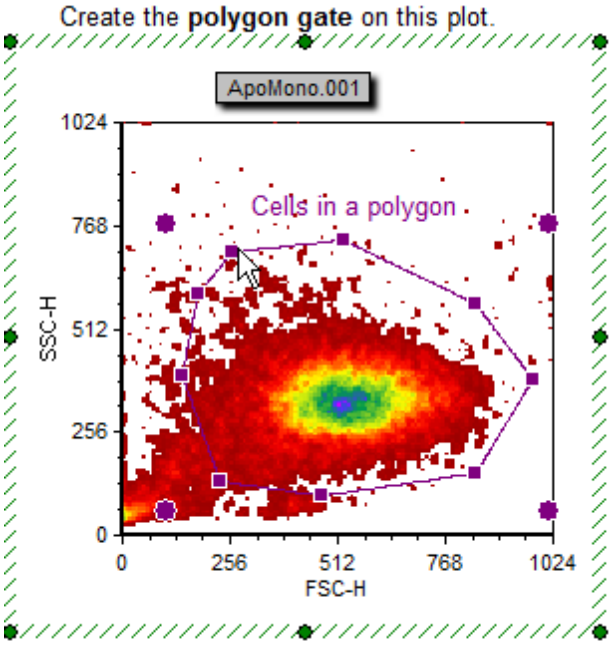

Figure T4.19A Starting to Reisze a Polygon Vertex

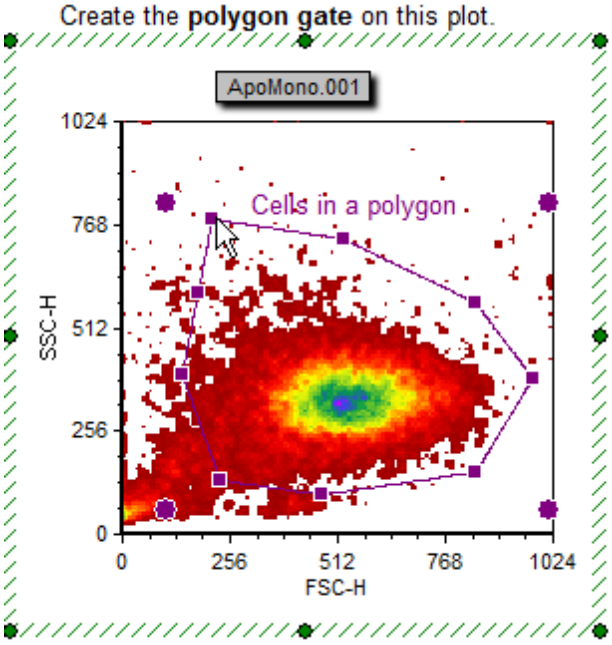

Figure T4.19B Moving a Polygon Vertex

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In the next example, we will delete a gate.

34.4.8 Deleting Gates

In the course of this example we will:

- Delete a gate

1. Activate the "Whole Cells" gate by clicking in the gate.
2. Press the **Delete** button on the keyboard.

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.

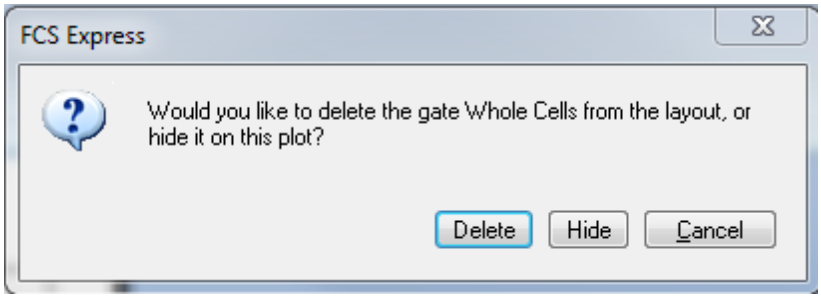

Figure T4.20 Deleting a Gate

The "Whole Cells" gate should now disappear from the plot.

Once gates are created, they can be applied to a plot.

34.4.9 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 popup menu.
3. Select the **Overlays** Category ❶.
4. Change the **Gate** ❷ to "Cells in ellipse".

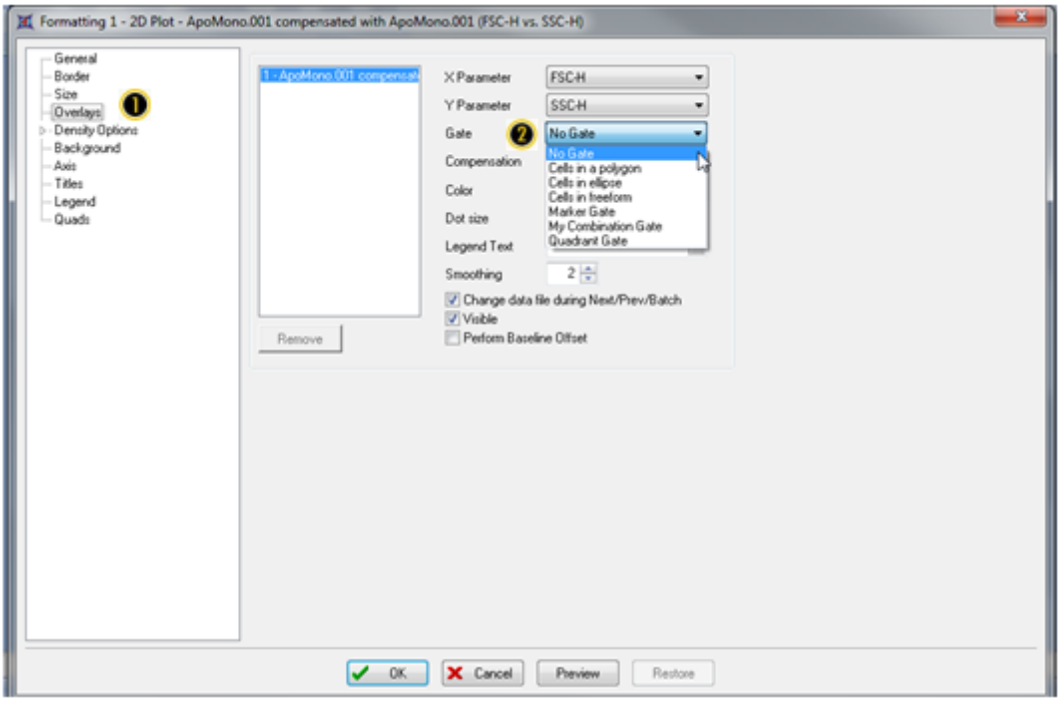

Figure T4.21 Formatting 2D Plots (Overlays Category)

5. Press the **OK** button to accept the change.

The plot should now look similar Figure T4.22. 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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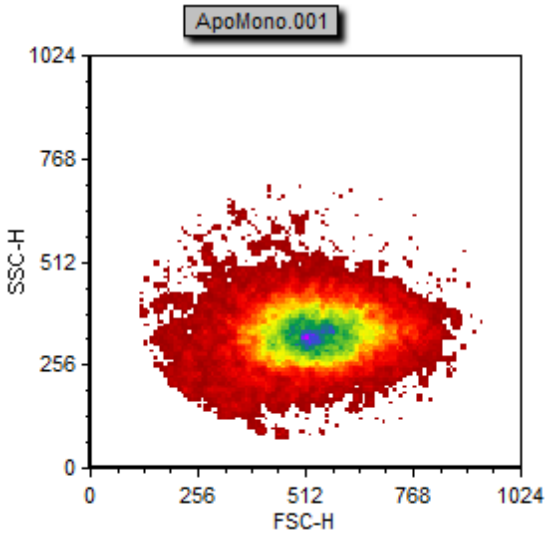

Figure T4.22 Plot Gated on "Cells in Ellipse"

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 edit 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".

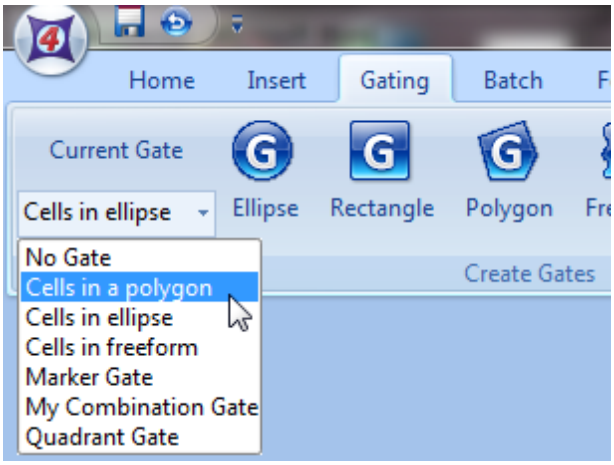

Figure T4.23 Selecting the "Cells in a Polygon" Gate

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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.

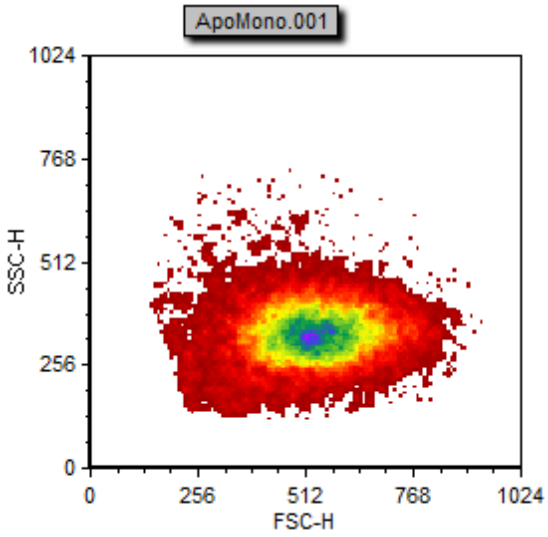

Figure T4.24 Plot Gated on "Cells in a Polygon"

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.

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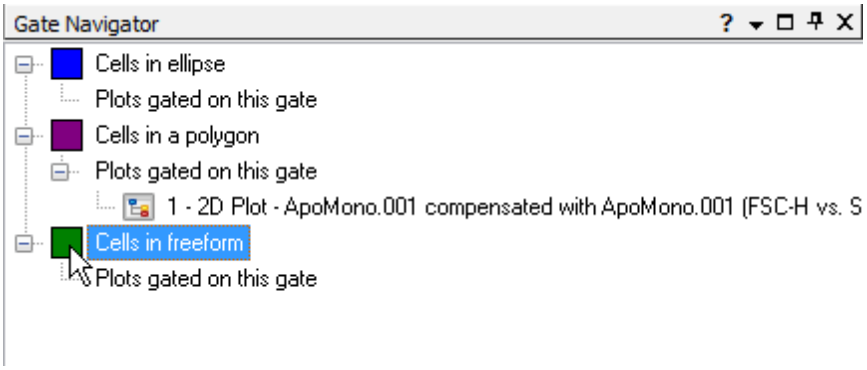

Figure T4.25 Gate Navigator

12. Move the **Gate Navigator** to the side of the layout.
13. Move the cursor over the **Cells in freeform** on the **Gate Navigator**.
14. Hold down the left mouse button to select the **Cells in freeform** gate. You will know the gate is selected because **Cells in freeform** will be highlighted (Figure T4.26).
15. Continue to hold down the mouse button and drag the gate onto the 2D plot labeled "Create the **rectangular gate** on this plot".
16. Release the mouse button.

The "Cells in freeform" gate is now applied to the plot as indicated on the updated **Gate Navigator** (Figure T4.26).

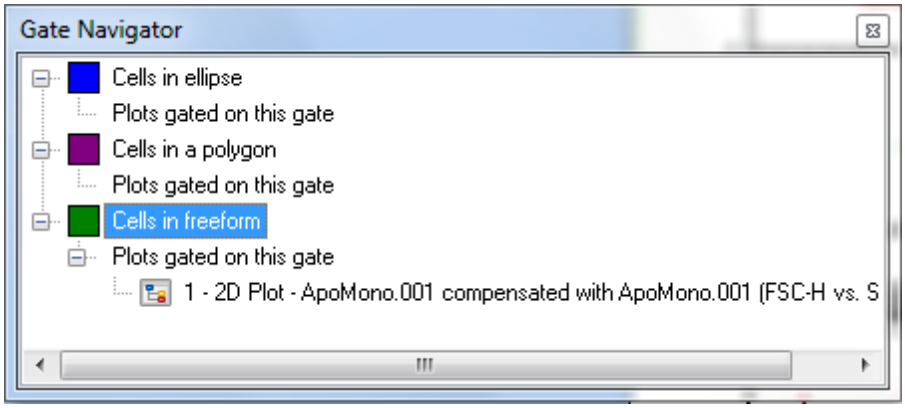

Figure T4.26 Gate Navigator Updated

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34.5 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 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 675.

34.5.1 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 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 **Application Button→New Layout** 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.

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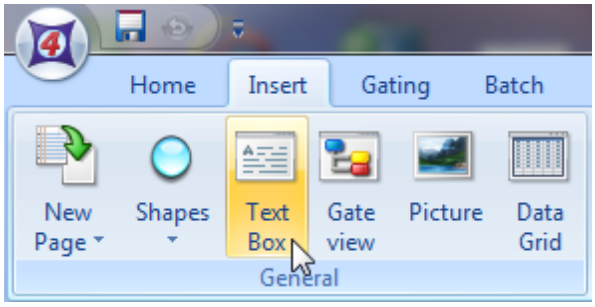

Figure T5.1 Selecting the Insert Text Box Command

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. To change this default size setting, select **Application Button→Edit Preferences→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™.

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.

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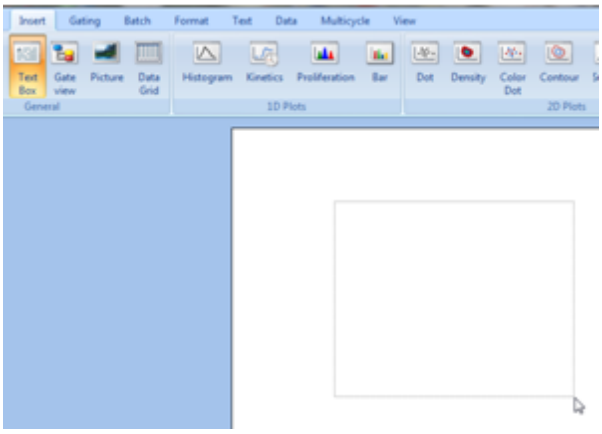

Figure T5.3 Inserting a Custom-sized Text Box

To insert a text box into a 2D plot or histogram:

1. **Select Application Button→Close Layout** to close the layout we were just working on.
2. Select **Application Button→Open Layout**.
3. Open the layout **TextBoxInsertion.fey** found in the *FCS Express Sample Data* folder.

This layout contains a density plot of FSC vs. SSC of file ApoMono.001.

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.

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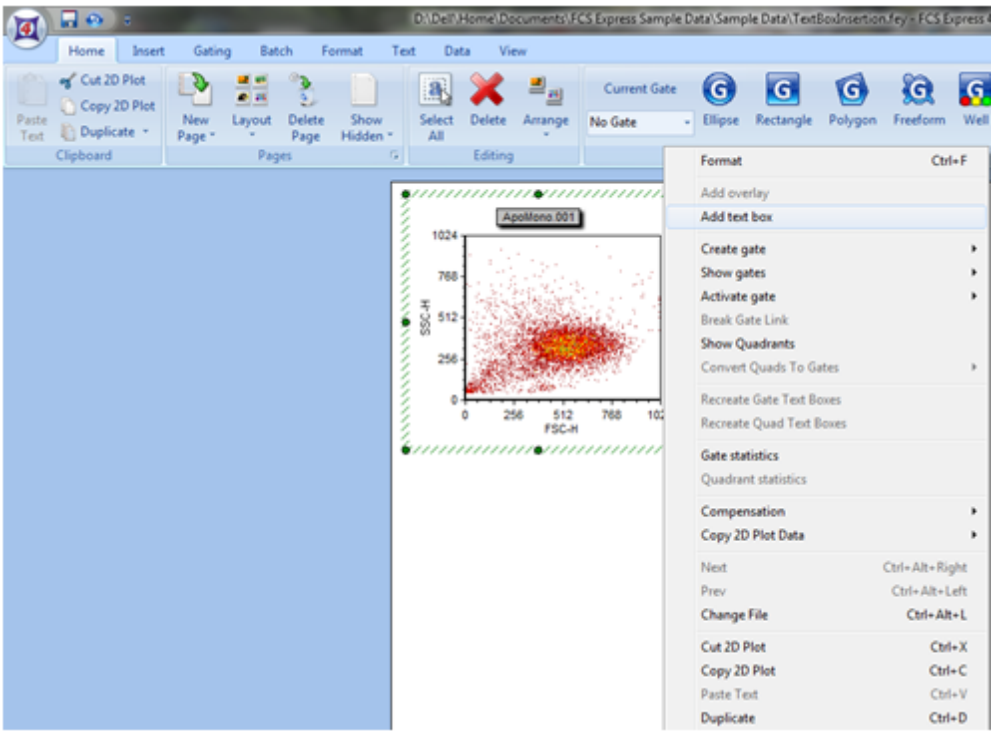

Figure T5.4 Adding a Text Box to a Plot

A small text box will appear in the upper left-hand corner of the plot (Figure T5.5). You can now begin entering text.

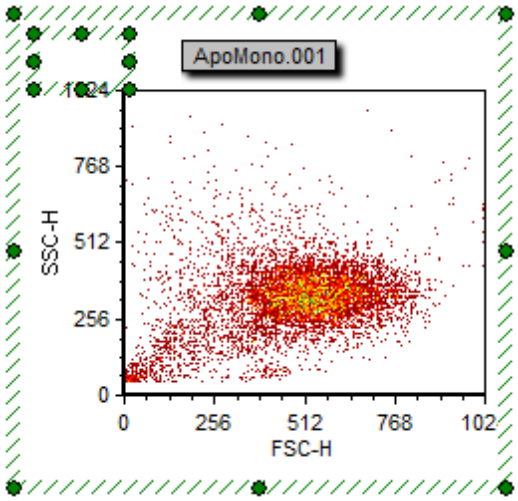

Figure T5.5 Text Box Added to a Plot

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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 593.

6. Select **Application Button→Close Layout** to close the layout we were just working on.

In the next section, we will format the text in a text box 679.

34.5.2 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 **Application Button→Open Layout**.
2. Open the layout **TextBoxFormat.fey** found in the *FCS Express Sample Data* folder.

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

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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™.

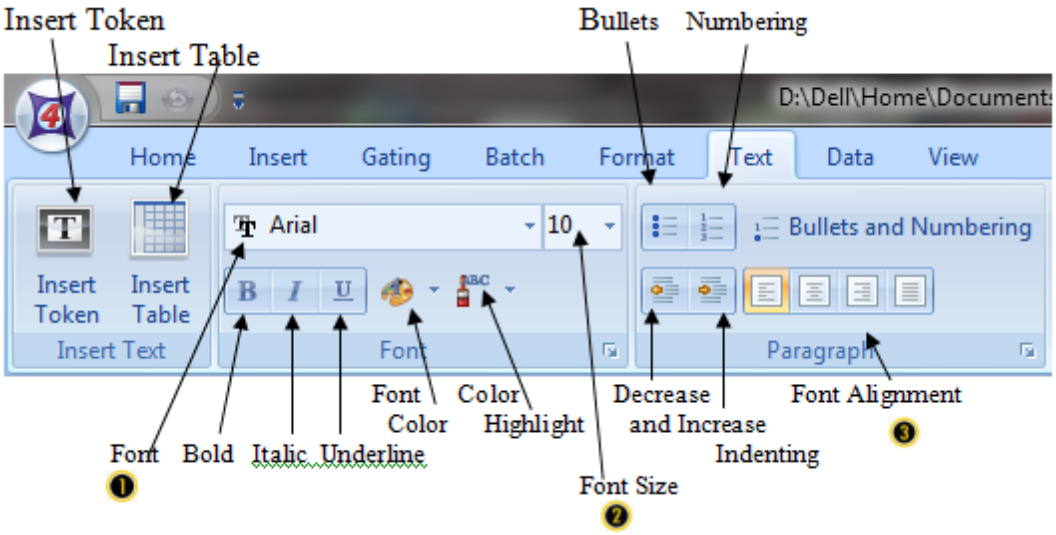

Figure T5.7 Text Formatting Commands

3. Highlight the text in the text box.
4. Change the Font ❶ to Courier Bold.
5. Increase the Font Size ❷ to 15pt.
6. Change the Font Alignment ❸ to Right.

The text should now look like the 'After' image on the right in Figure T5.8.

Figure T5.8 Formatting a Text Box

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Next, we will change the text box properties 681.

34.5.3 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.

To format the Text Box properties:

1. Right-click on the text box from Figure T5.6 679.
2. Select **Format** from the pop-up menu (Figure T5.9).

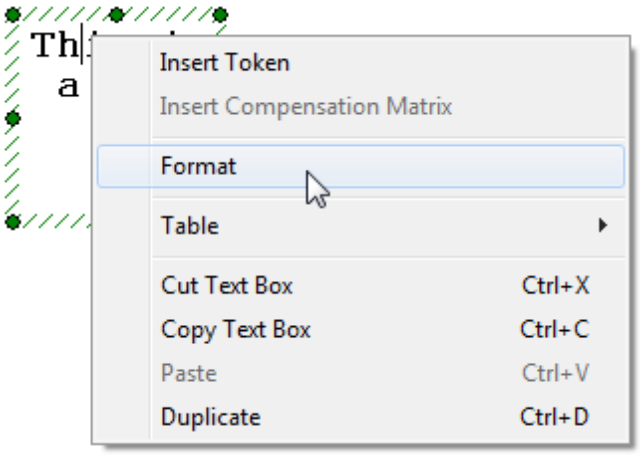

Figure T5.9 Selecting Format from the Pop-up Menu

The **Formatting Text box** dialog (Figure T5.10) will appear with four categories:

A. General – To change the name of a text box.

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B. Border – To change or add a border to a text box. ❷

C. Size – To change the size of a text box. ❸

D. Background – To change the background of the text box. ❹

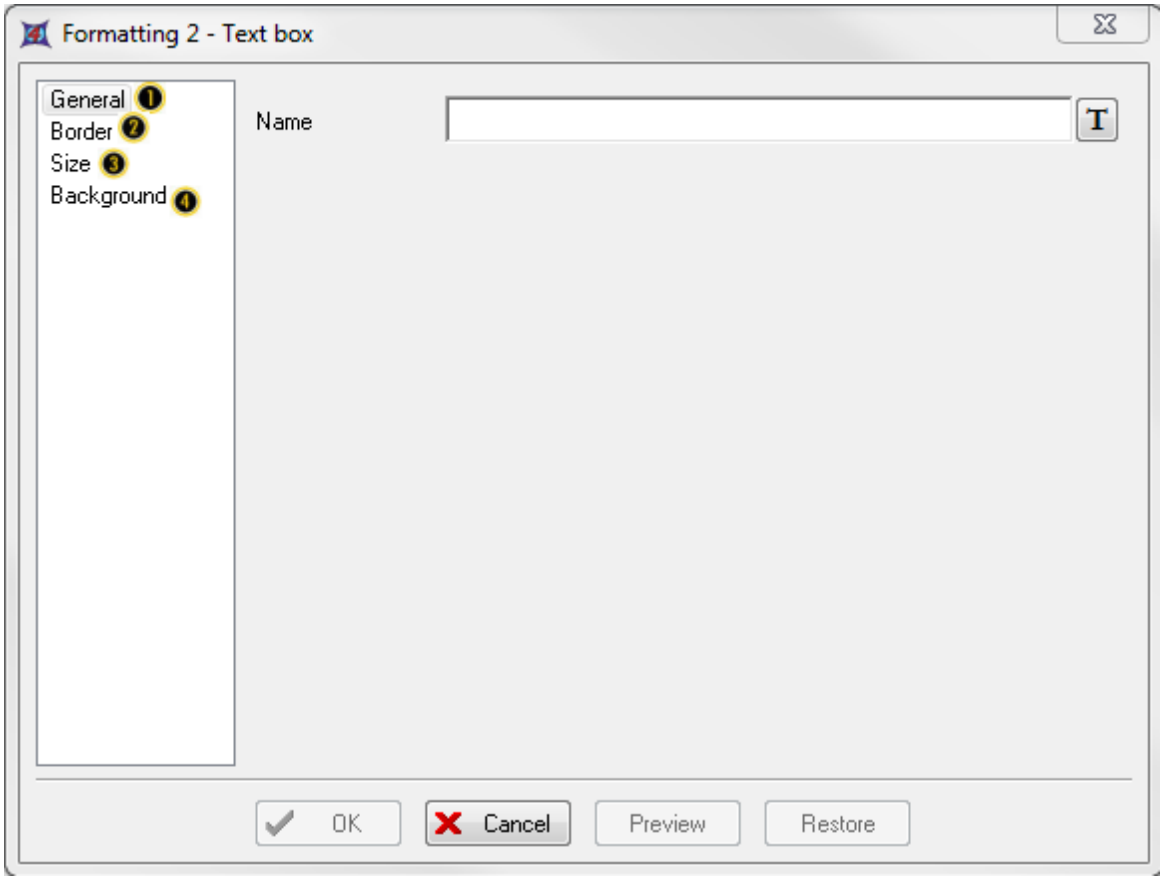

Figure T5.10 Formatting Text Box Dialog

To Modify the Border:

1. Click on the **Border** category ❷ on the **Formatting Text box** dialog (Figure T5.10).

The **Border** page will appear (Figure T5.11).

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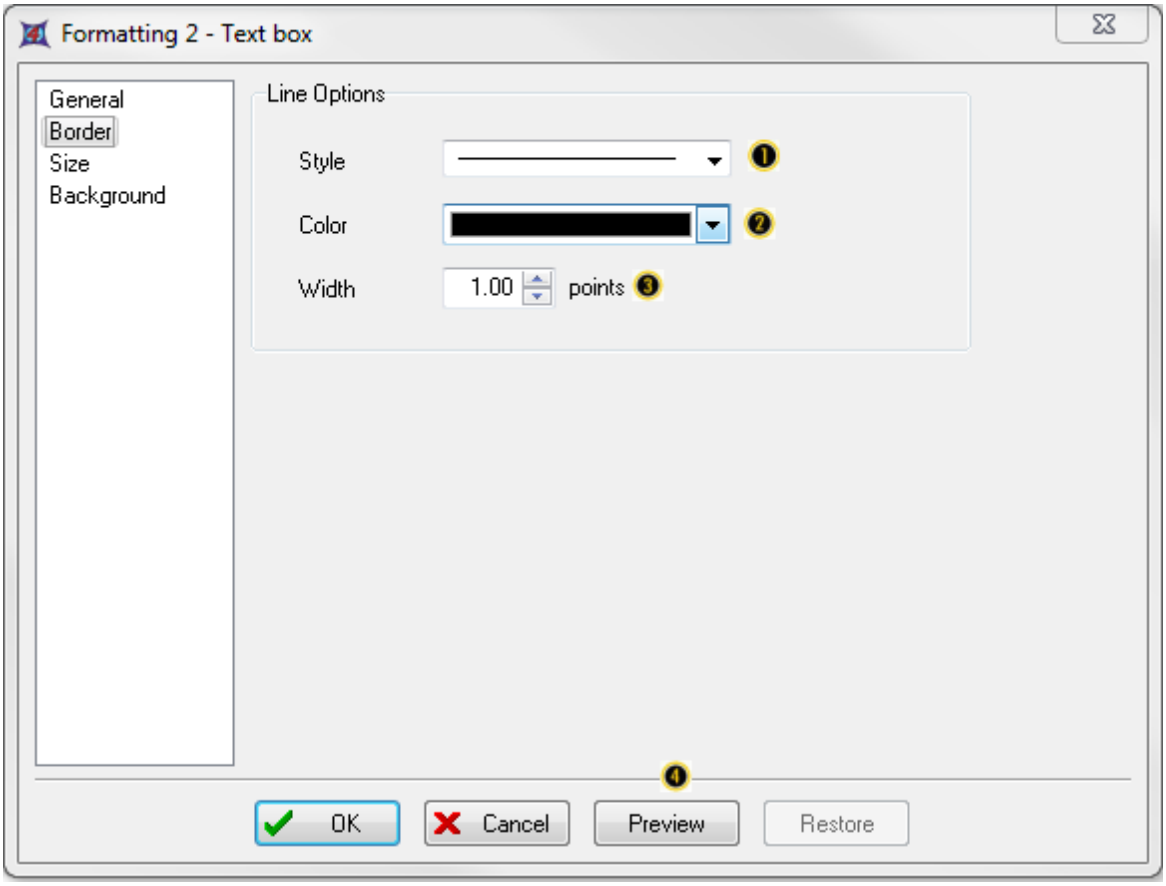

Figure T5.11 Formatting Text Box Dialog -- Border Category

From the **Formatting Text box Border** page, shown in Figure T5.11, you can change the line style, color, and width. The default setting is no line.

2. Select **Solid Line** from the **Style** ❶ drop-down list.
3. Select **Black** from the **Color** ❷ drop-down list.
4. Select **1.00** points from the **Width** ❸ spin control.
5. Select **Preview** ❹.

The text box should now look like 'After' image on the right of Figure T5.12.

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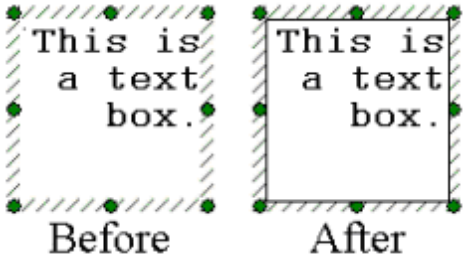

Figure T5.12 Text Box with Border

To Modify the Size:

1. Click on the **Size** category (❸ in Figure T5.10).

The **Formatting Text box Size** page will appear (Figure T5.13) where you can change the dimensions of the text box.

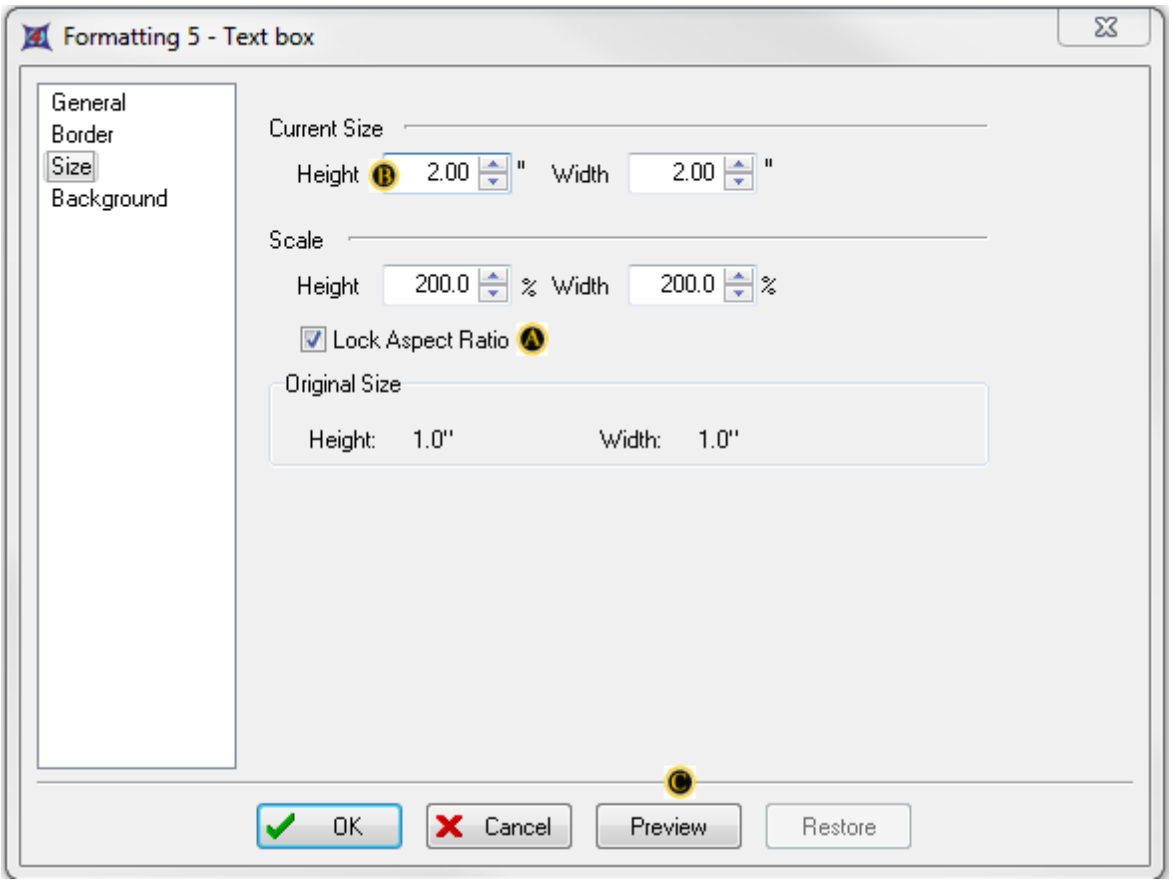

Figure T5.12 Formatting Text Box Dialog -- Size Category

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2. Click on **Lock Aspect Ratio** A.

This will maintain the ratio of the text box size relative to the original text box.

3. Change the **Height** to **2.00 inches** B. By changing the height to 2.00 inches, the width will automatically change to 2.00 inches.

4. Select **Preview** C.

The text box should now look like the 'After' image on the right of Figure T5.14.

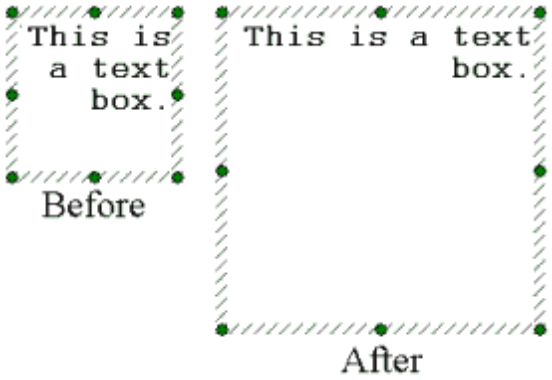

Figure T5.14 Modified Text Box Size

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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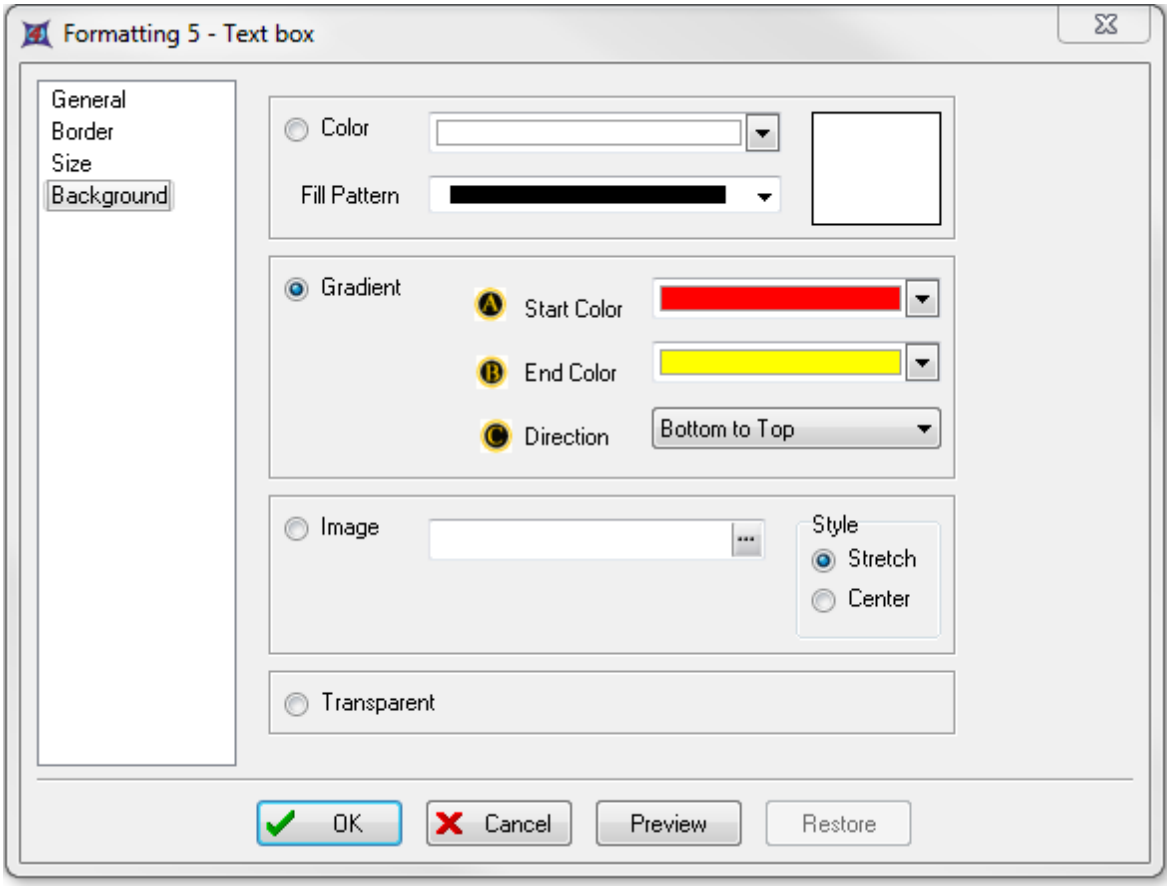

Figure T5.15 Formatting Text Box Dialog -- Background Category

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 **Red** (in Figure T5.15).
4. Change the **End Color** to **Yellow** (in Figure T5.15).
5. Change the **Direction** to **Bottom to Top** (in Figure T5.15).
6. Select **OK**.

The resulting image should now look like the image on the right of Figure T5.16.

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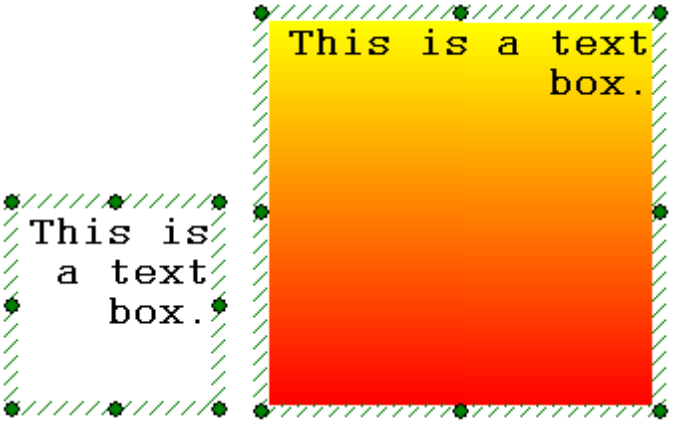

Figure T5.16 Text Box with New Background

7. Select **Application Button→Close Layout** to close the layout we were just working on.

Next, we will insert and modify a table 687.

34.5.4 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 **Application Button→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" 675.
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.)

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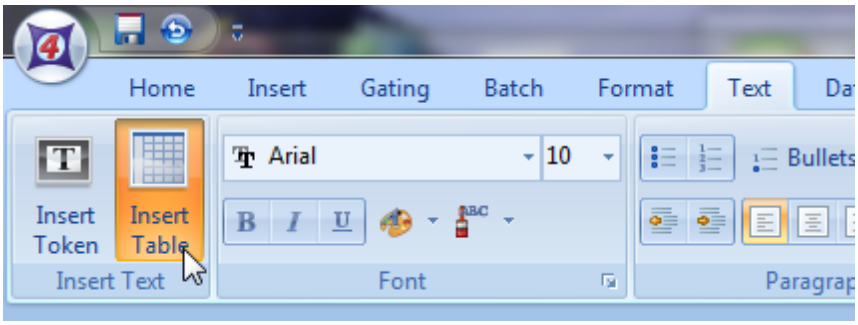

Figure T5.17 Insert Table Command

An **Insert Table** dialog will now appear (Figure T5.18).

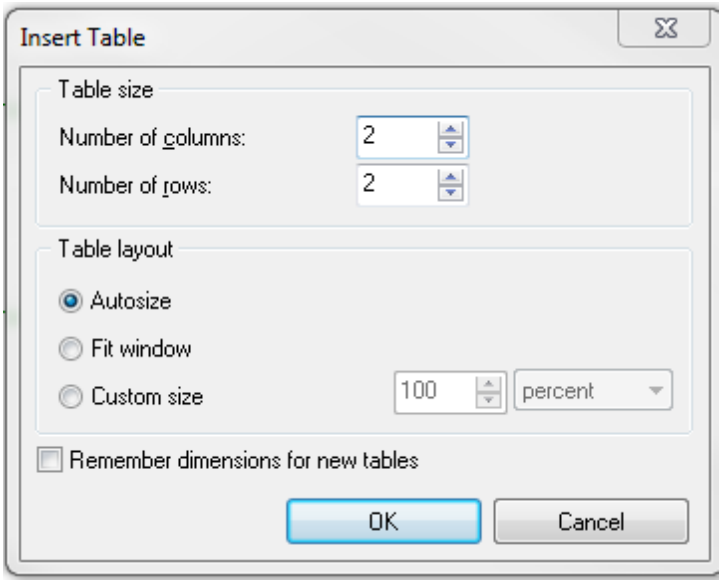

Figure T.18 Insert Table Dialog

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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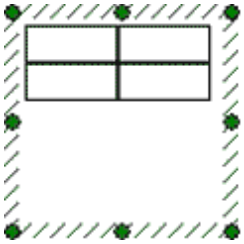

Figure T5.19
Table Inserted in a Text Box

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**.

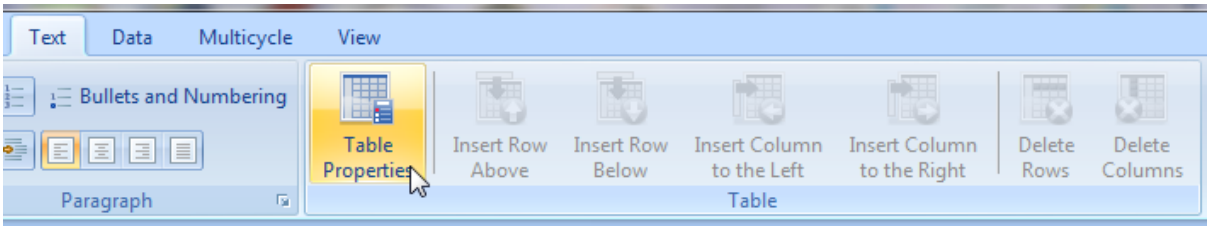

Figure T5.20 Table Properties Command

A Table **Properties** dialog will appear (left side of Figure T5.21).

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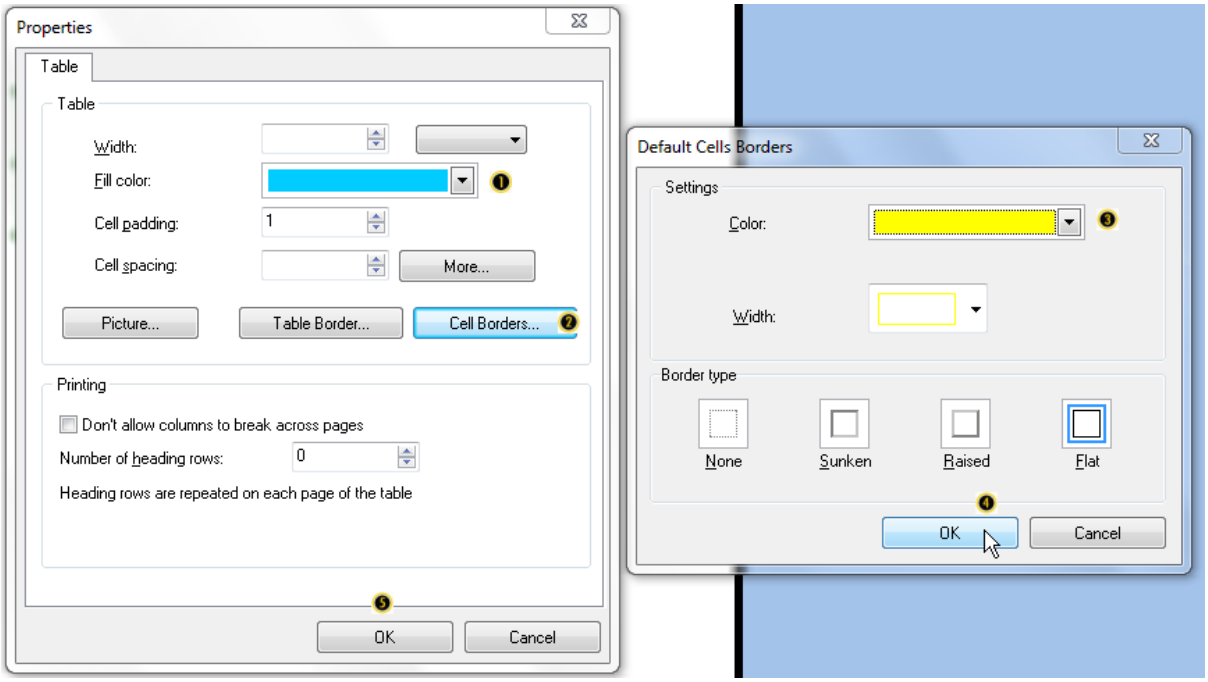

Figure T5.21 Table Properties and Default Cells Border Dialogs

3. Select the color **Sky Blue** from the **Fill Color** ❶ drop-down list.
4. Click on the **Cell Borders...** button ❷.

The **Default Cells Borders** dialog will appear (right side of Figure T5.21).

5. Change the cell border **Color** to **Yellow** ❸.
6. Press the **OK** button ❹ on the **Default Cells Borders** dialog.
7. Press the **OK** button ❺ on the Table **Properties** dialog to accept the changes.

The resulting table should now look like the one on the right of Figure T5.22.

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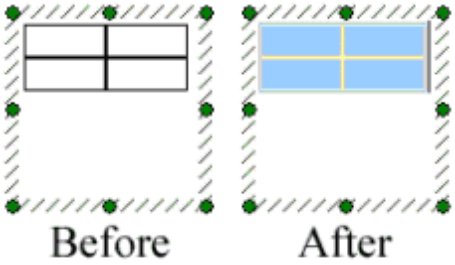

Figure T5.22 Formatted Table

8. Select **Application Button→Close Layout** to close the layout we were just working on.

We will now proceed to inserting and modifying a token 691.

34.5.5 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 590.

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 **Application Button→Open Layout**.
2. Open the layout **TokenInsertion.fey** found in the Tutorial Sample Data archive 590.
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'.

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4. Right-click inside the selected text box.
5. Select **Insert Token** from the pop-up menu (Figure T5.23).

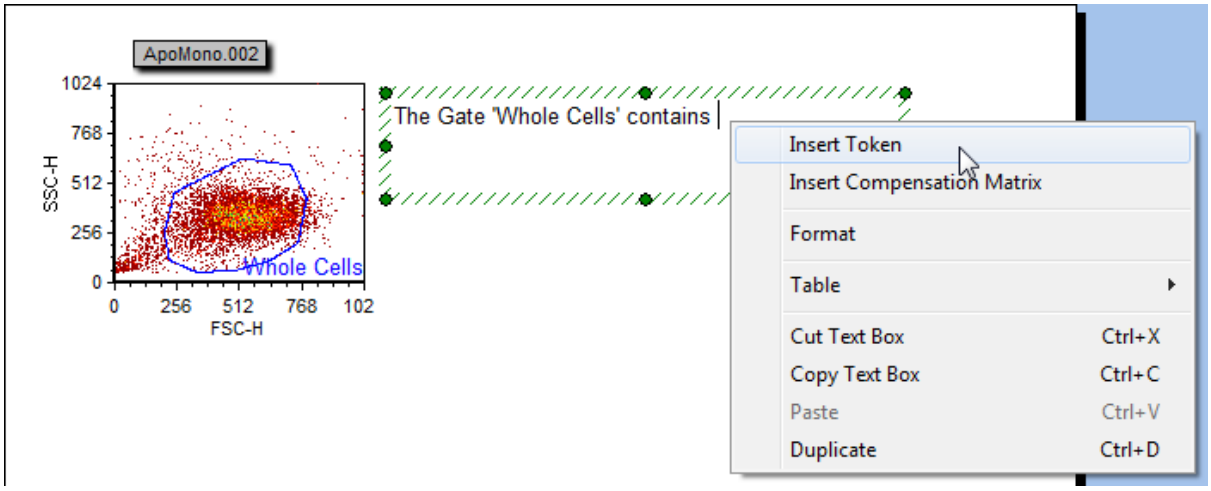

Figure T5.23 Inserting a Token

You can also insert a token using the **Text→Insert Text→Insert Token** command.

An **Insert a Token** dialog will appear (Figure T5.24).

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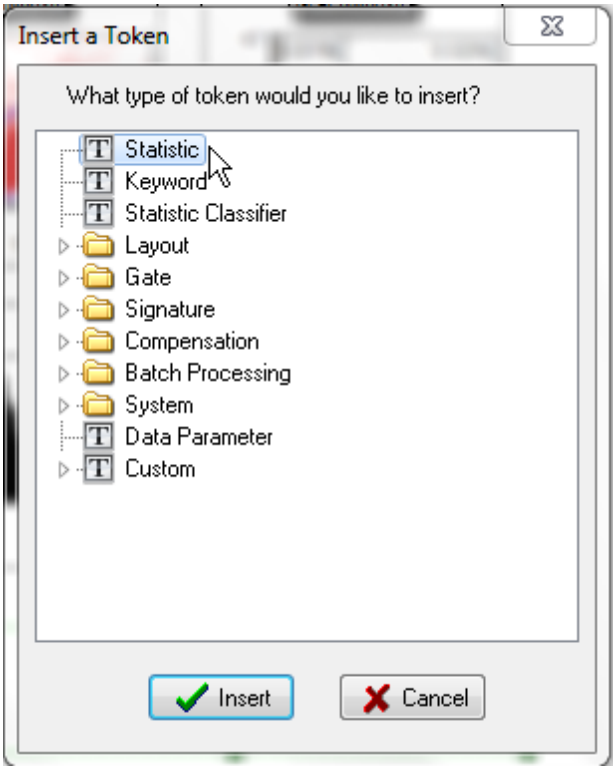

Figure T5.24 Insert a Token Dialog

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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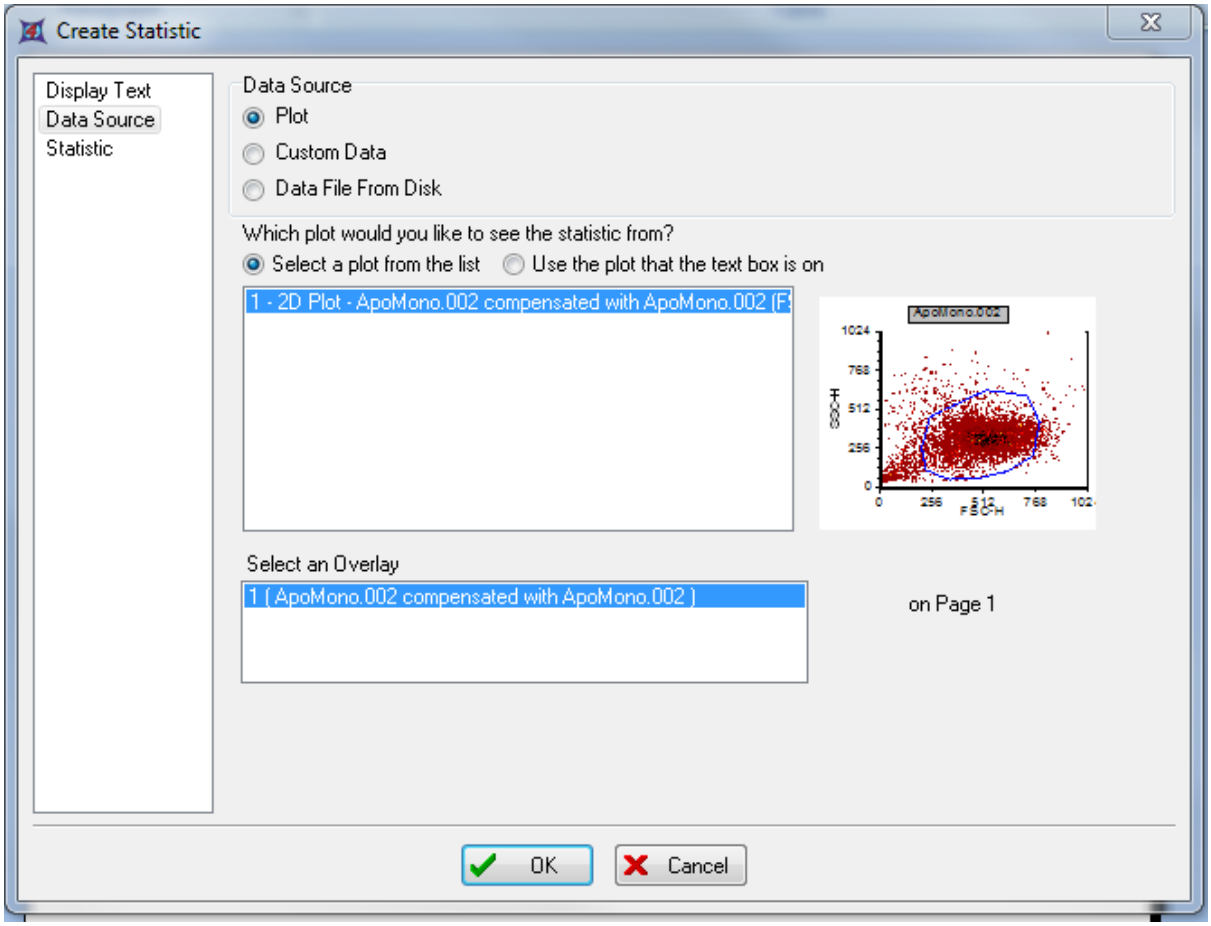

Figure T5.25 Create Statistic Dialog -- Data Source Category

8. Click on the **Statistic** Category (in Figure T5.26) of the **Create Statistic** dialog.

The **Statistic** page of the **Create Statistic** dialog will now appear (Figure T5.26).

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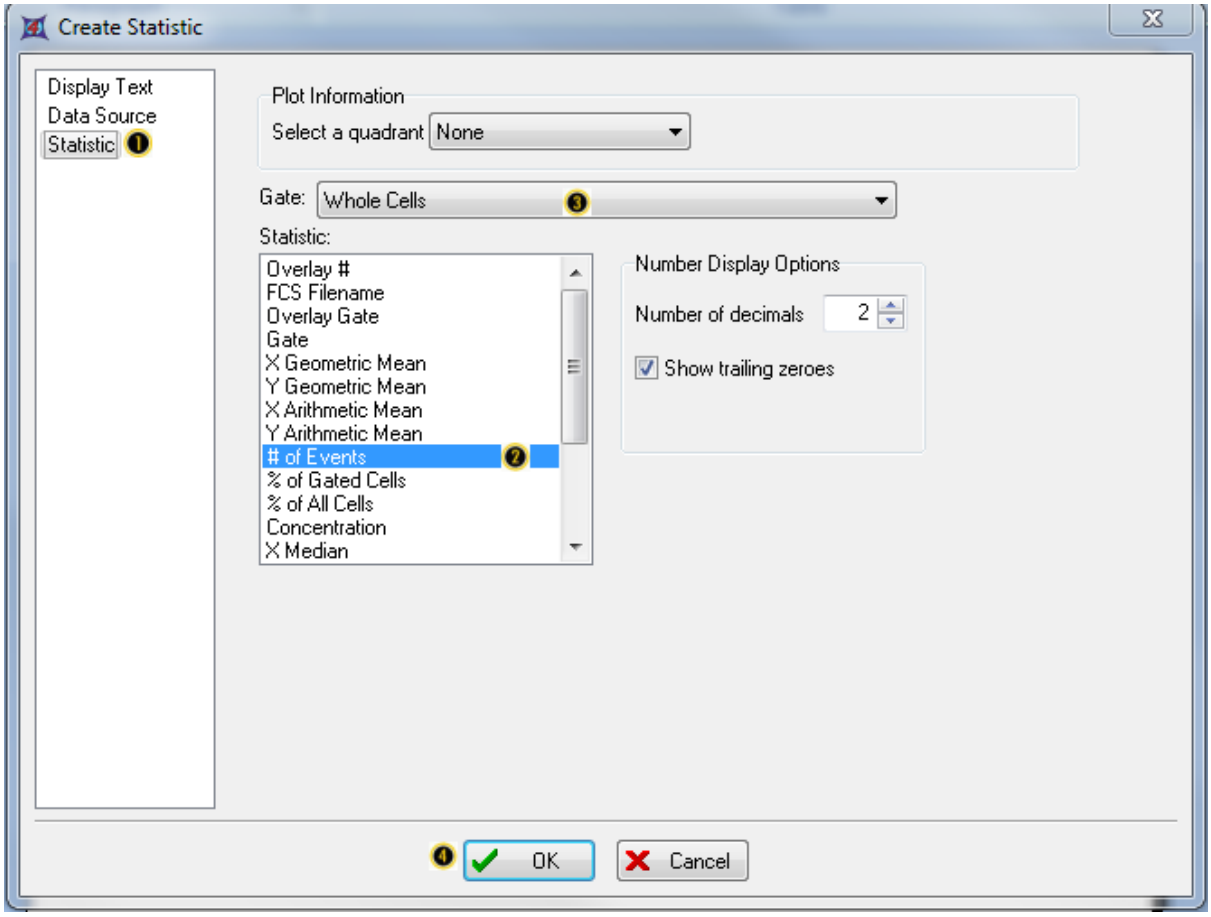

Figure T5.26 Create Statistic Dialog -- Statistic Category

We will select the statistic we want the token to display (Figure T5.26).

9. Select # **of Events** ❷ in the **Statistic** list box.
10. Select **Whole Cells** ❸ from the **Gate** drop-down list.
11. Select **OK** ❹.

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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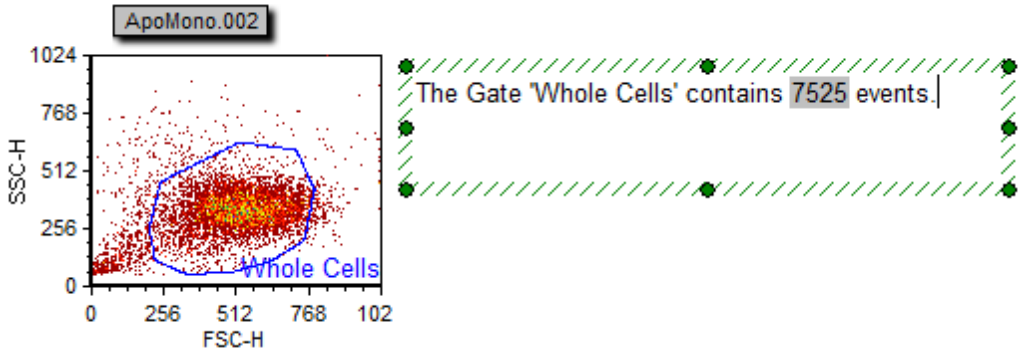

Figure T5.27 Text Box with Token

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, 15pt, and Bold. For more help on changing text in text boxes, see the section on Formatting Text in a Text Box 679.

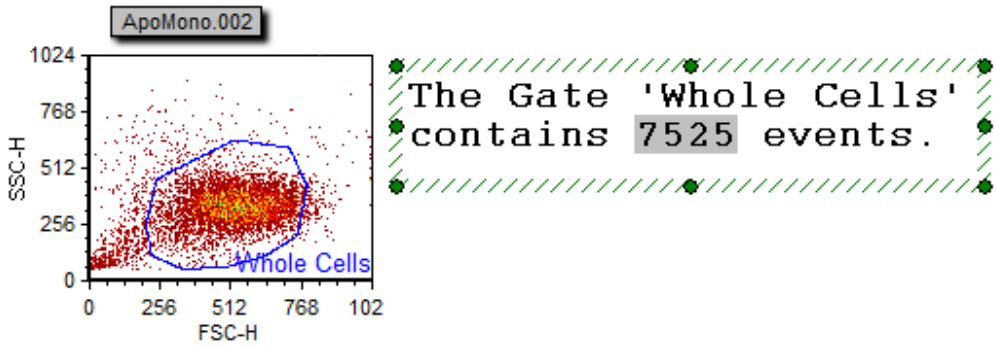

Figure T5.28 Formatted Token Font

Tokens can also be inserted by dragging and dropping the plot onto the text box.

To insert a Token by Drag and Drop:

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First, we need to delete the token in the text box.

1. Highlight "**7525 events**." in the text box.
2. Hit the **Delete** key to delete the token.
3. Left-click on the border of the **SSC-H v FSC-H ApoMono.002** plot. The border of the plot should turn red to indicate it is in selected mode.
4. While holding down the left mouse button, drag the cursor (Figure T5.28) to the space after the word "**contains**" in the text box (Figure T5.29).

Release the mouse button to drop the plot into the text box.

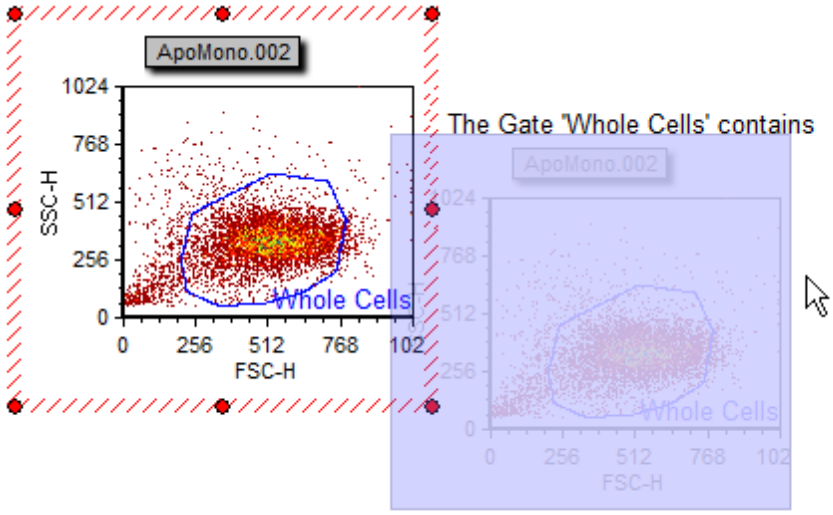

Figure T5.28 Dragging Token from Plot onto Text Box

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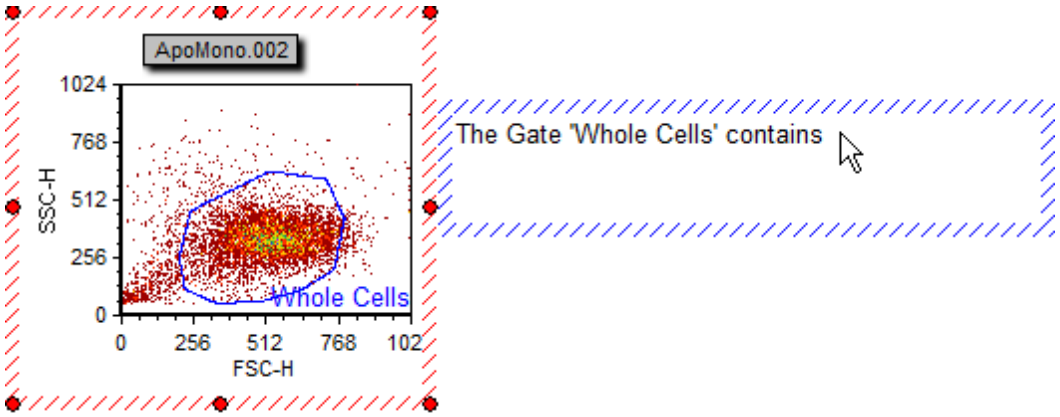

Figure T5.29 Dropping Token onto Text Box

The **Paste Special** dialog will appear (Figure T5.30).

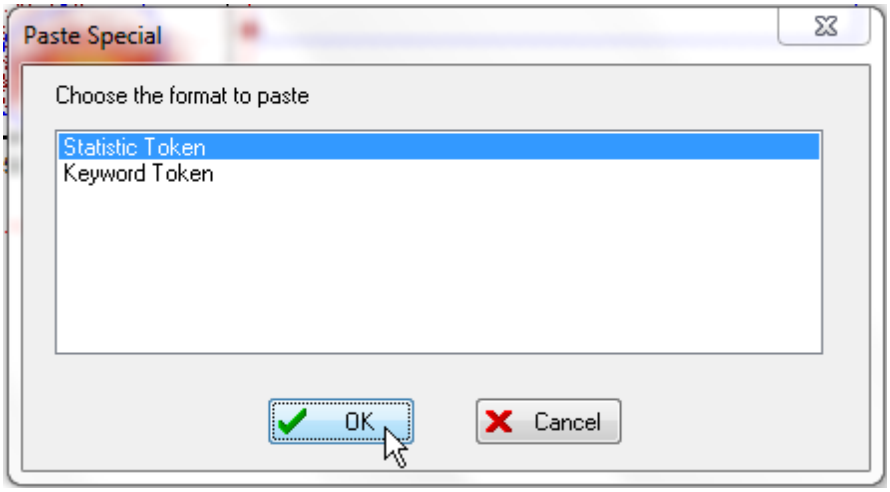

Figure T5.30 Paste Special Dialog

6. Select **Statistic Token** from the **Paste Special** dialog (Figure T5.30).
7. Click **OK**.

The **Edit Statistic** dialog will now appear (Figure T5.31)

8. Select **Whole Cells** ❶ in the **Gate** field (Figure T5.31).
9. Select # **of Events** ❷ from the **Statistic** box.
10. Select **OK**.

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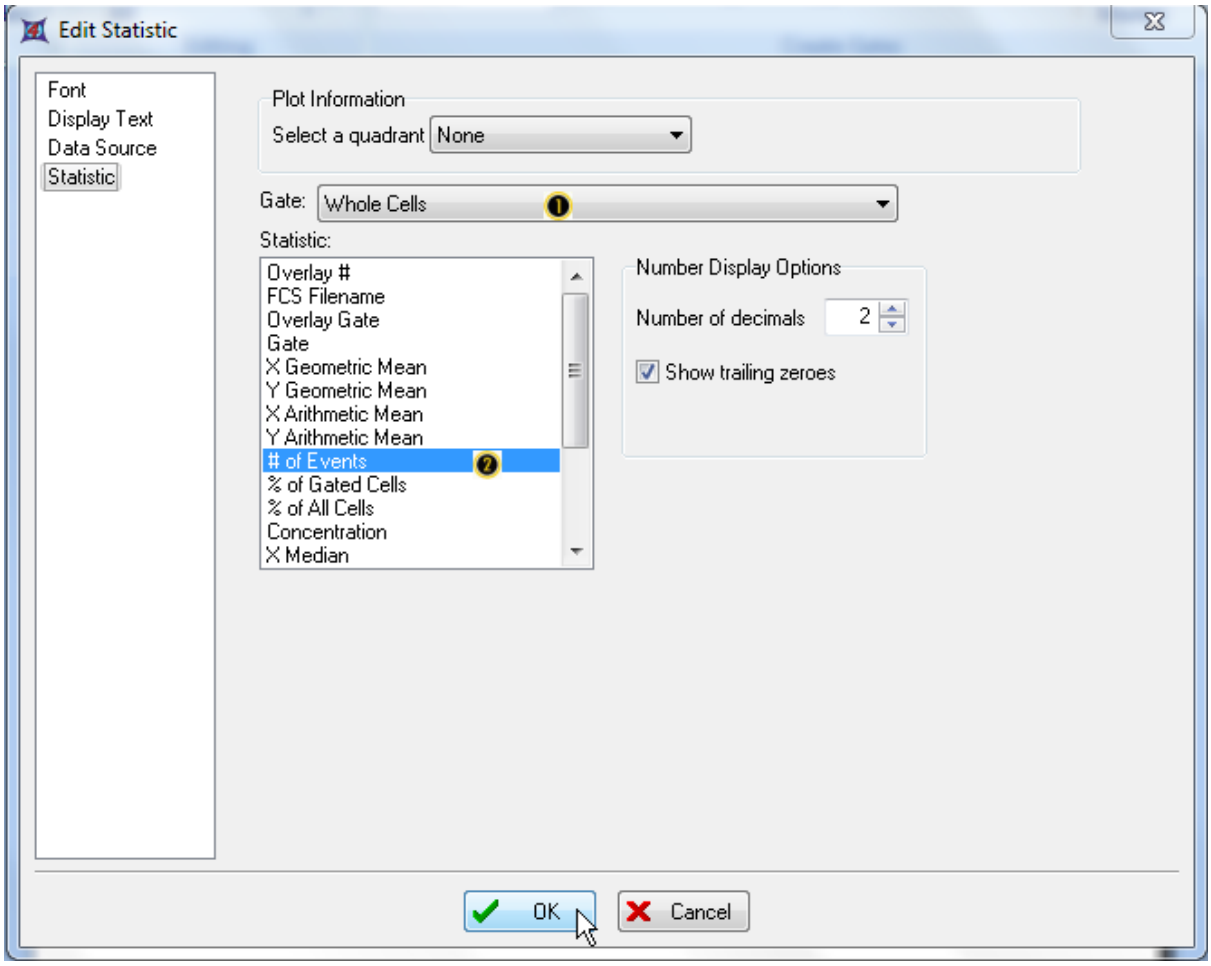

Figure T5.31 Edit Statistic Dialog -- Statistic Category

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).

11. Click after the number of events token (highlighted in grey) in the text box.

12. 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 T5.28.

13. Select **Application Button→Close Layout** to close the layout we were just working on.

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In the next example, we will creating a custom token700.

34.5.6 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 **Application Button→Open Layout**.
2. Open the layout **TokenCustom.fey** found in the Tutorial Sample Data archive590.

The layout, shown in Figure T5.43, contains the completed plot and text box from the section of this tutorial on inserting a token691. Below them is a new plot of FL1 vs. FL2 with quadrants and another text box with the text 'Apo(+)/Apo (-) ratio:'. We will be inserting the custom defined token statistic here (Figure T5.43).

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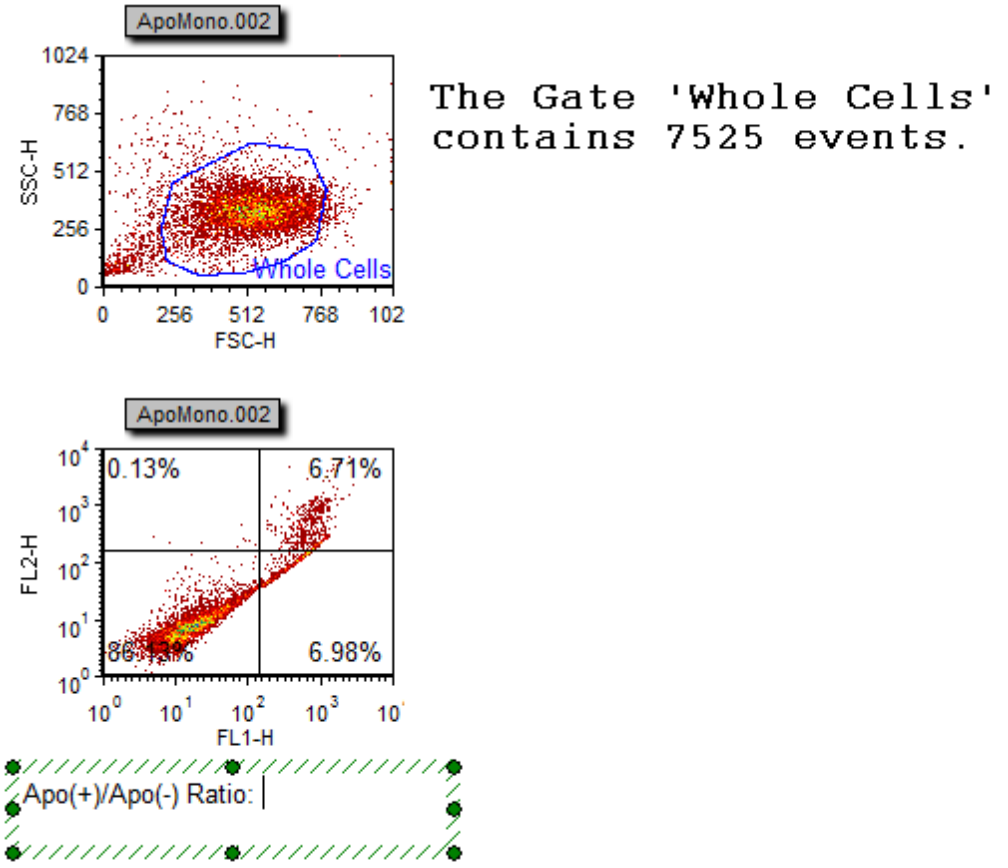

Figure T5.43 Custom Token Layout (TokenCustom.fey)

3. Click in the text box at te end of the incomplete text '**Apo(+)/Apo(-) Ratio:**'.

4. Select the **View→Tokens→Custom Tokens** command (Figure T5.44).

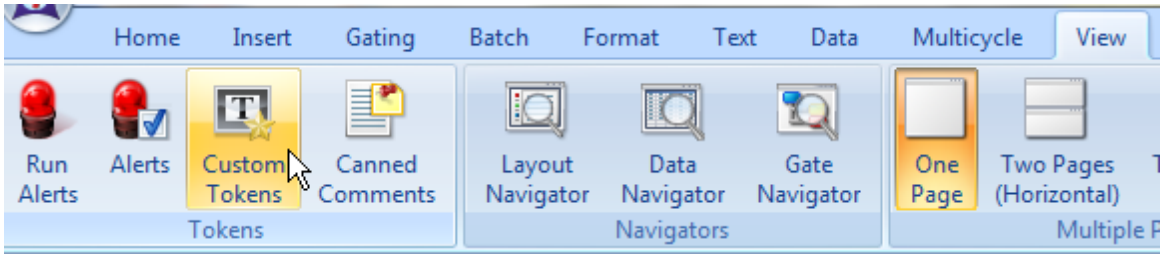

Figure T5.44 Selecting the Custom Tokens Command

The **Custom Tokens** window should now appear (Figure T5.45).

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5. Right-click inside the **Custom Tokens** dialog.
6. Select **New Custom Token** from the pop-up menu (Figure T5.45).

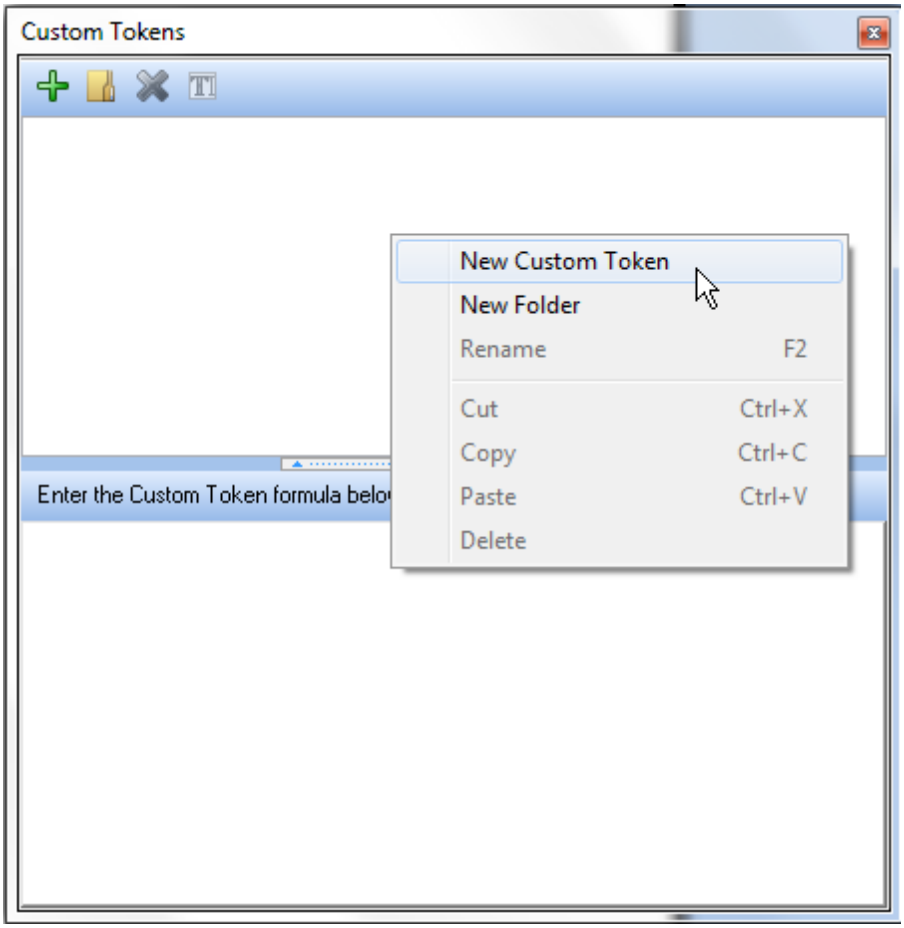

Figure T5.45 Creating a New Custom Token

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).

7. Type "**Apo(+)/Apo(-) Ratio:** " into the Name field of the New Custom Token (Figure T5.46).
8. Press **Enter**.

The Custom Token "Apo(+)/Apo(-) Ratio:" should now be highlighted (Figure T5.46).

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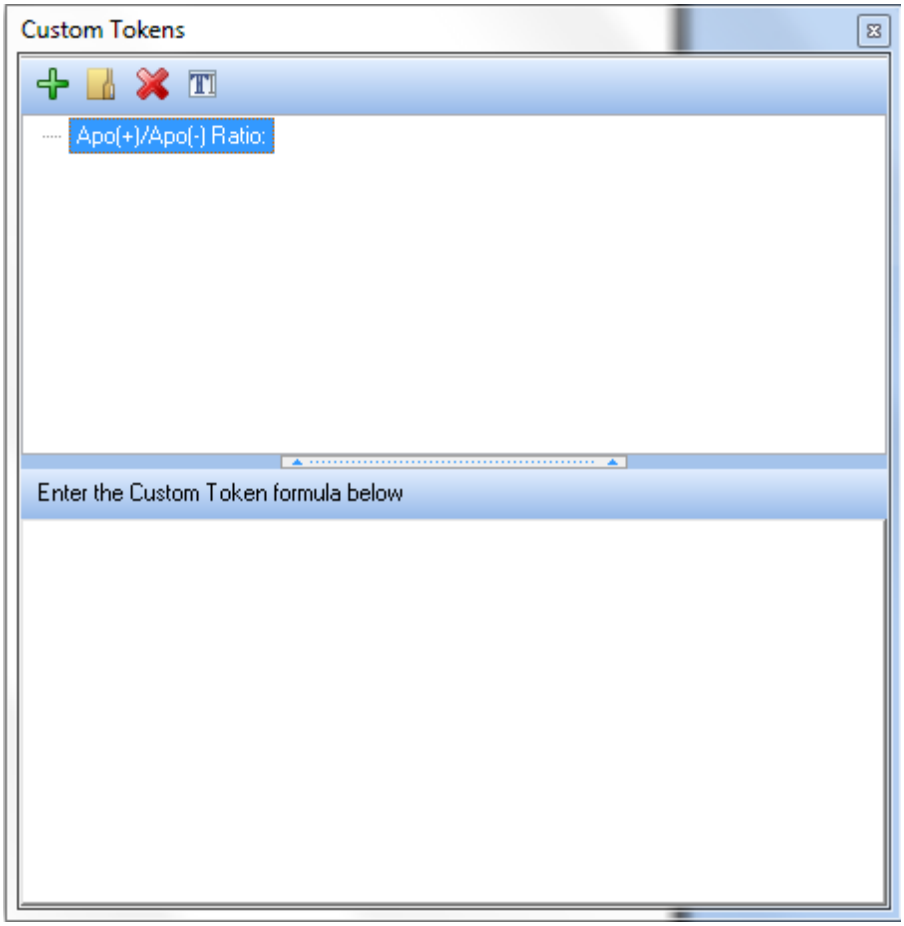

Figure T5.46 Custom Tokens Editor

9. Left-click in the lower half of the Custom Tokens window and enter an equals sign **" = "** ❶ (Figure T5.47).

10. Right-click on the lower half of the **Custom Tokens** window after the equals sign to bring up the associated pop-up menu.

11. Select **Insert Token** ❷ from the pop-up menu (Figure T5.47).

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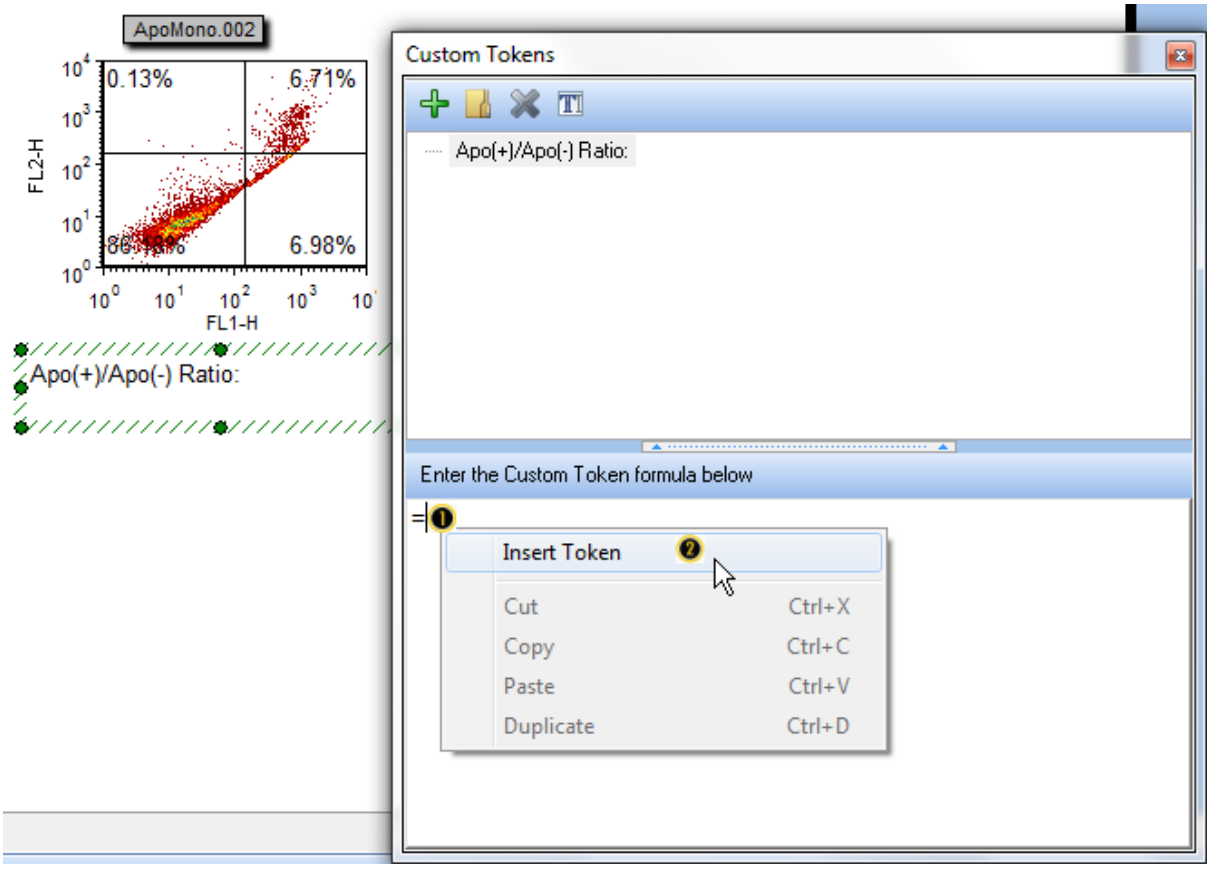

Figure T5.47 Entering a Custom Token Formula

An **Insert a Token** dialog will appear (Figure T5.24 691).

12. Click on **Statistic**.

13. Click **Insert**. (Figure T5.24 691).

A **Create Statistic** dialog will appear with the **Data Source** Category open (Figure T5.36).

14. Select **6 - 2D plot - ApoMono.002 (FL1 vs. FL2)** as the data source.

15. Click on the **Statistic** Category (❶ Figure T5.36).

The **Statistic** Category of the **Create Statistic** Window will appear (Figure T5.37). We will now select the statistic that will define the numerator of our custom token.

16. Select **Upper Right** ❶ in the **Select a quadrant** drop-down list, to select the numerator for our custom statistic token.

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17. Select **% of Gated Cells** ❷ in the **Statistic** list box.
18. Select **Whole Cells** ❸ in the **Gate** drop-down list.
19. Select **OK** (Figure T5.37).

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 "/" (❶Figure T5.48) after the quadrant statistic.
21. Right-click after the "/" symbol to bring up the pop-up menu.
22. Select **Insert Token** from the pop-up menu (❷Figure T5.48).

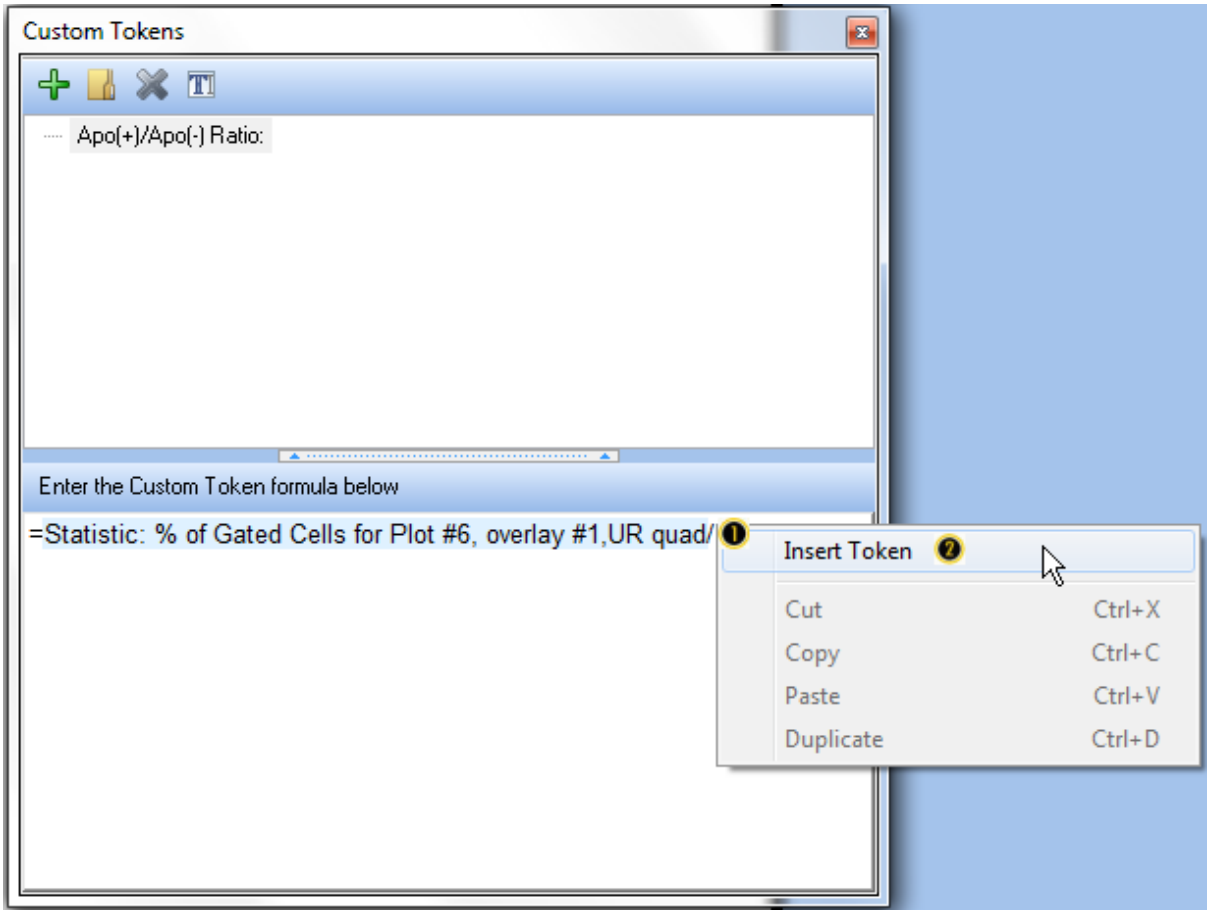

Figure T5.48 Numerator Definition for Custom Token

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An **Insert a Token** dialog box will appear (Figure T5.24 691).

23. Click **Statistic** as the token to be inserted.
24. Click **Insert** (Figure T5.24 691).

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 **6 - 2D plot - ApoMono.002 (FL1 vs. FL2)** as the data source .
26. Click on the **Statistic** Category (❶ Figure T5.49).
27. Select **Lower** ❷ in the **Select a quadrant** drop-down list to define the denominator of our custom statistic token.
28. Select **% of Gated Cells** ❸ in the Statistic list box.
29. Select **Whole Cells** ❹ in the Gate drop-down list.

The screen should now look like Figure T5.49.

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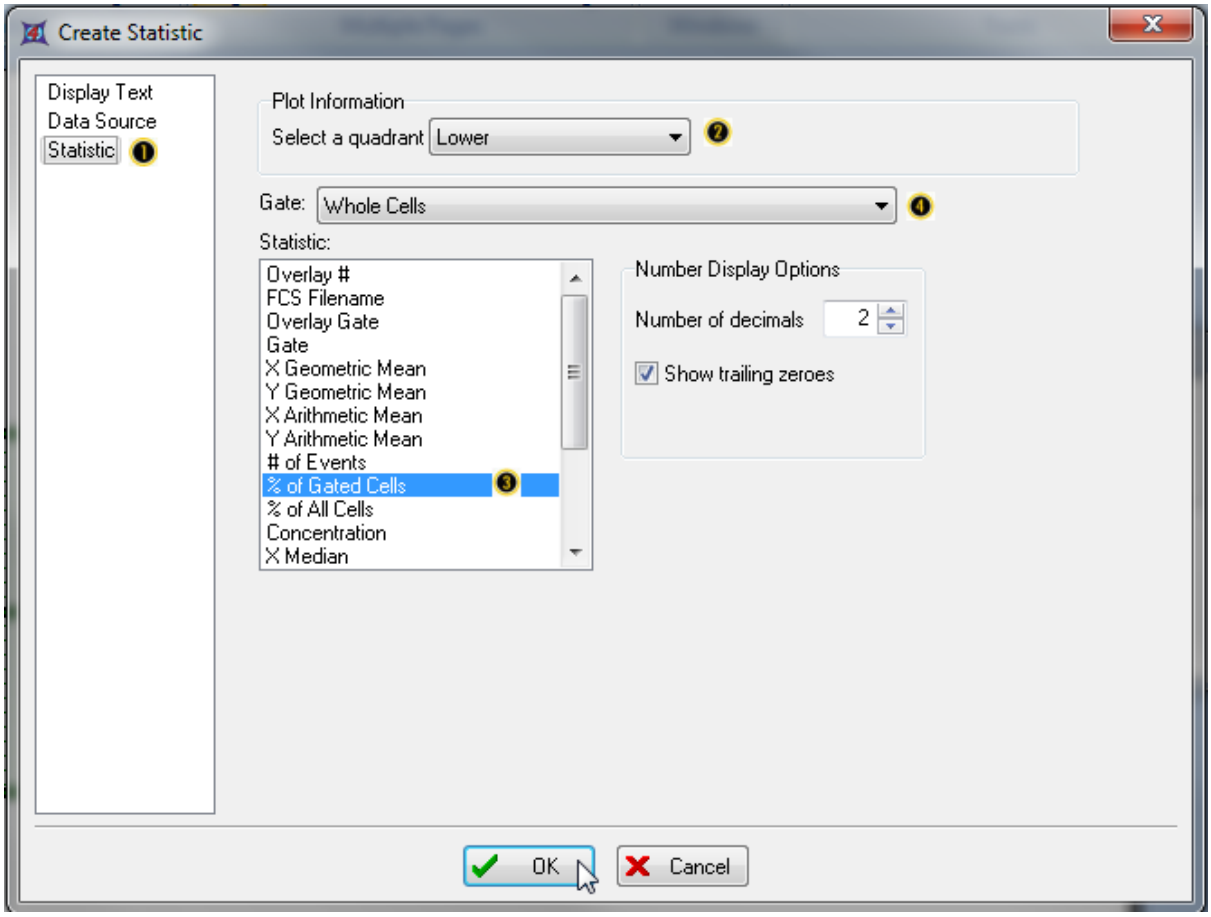

Figure T5.49 Defining the Denominator for the Custom Token

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 are highlighted in blue to indicate that they are being treated as numbers, as opposed to text.

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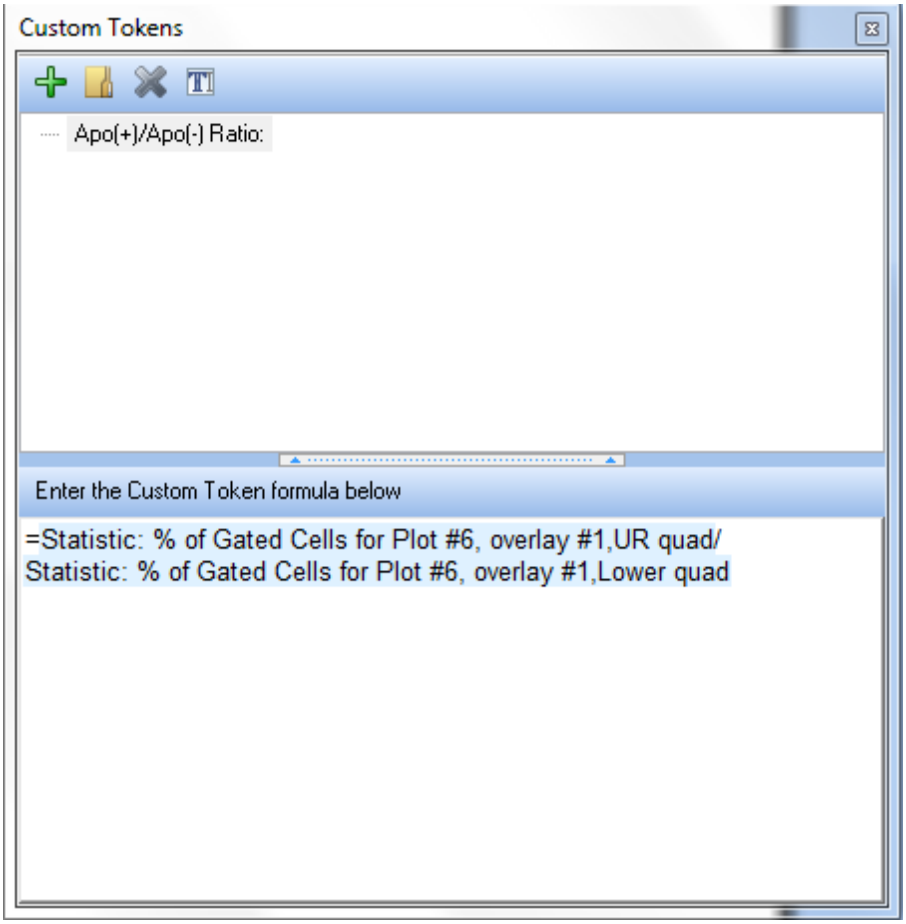

Figure T5.50 Final Custom Token Formula

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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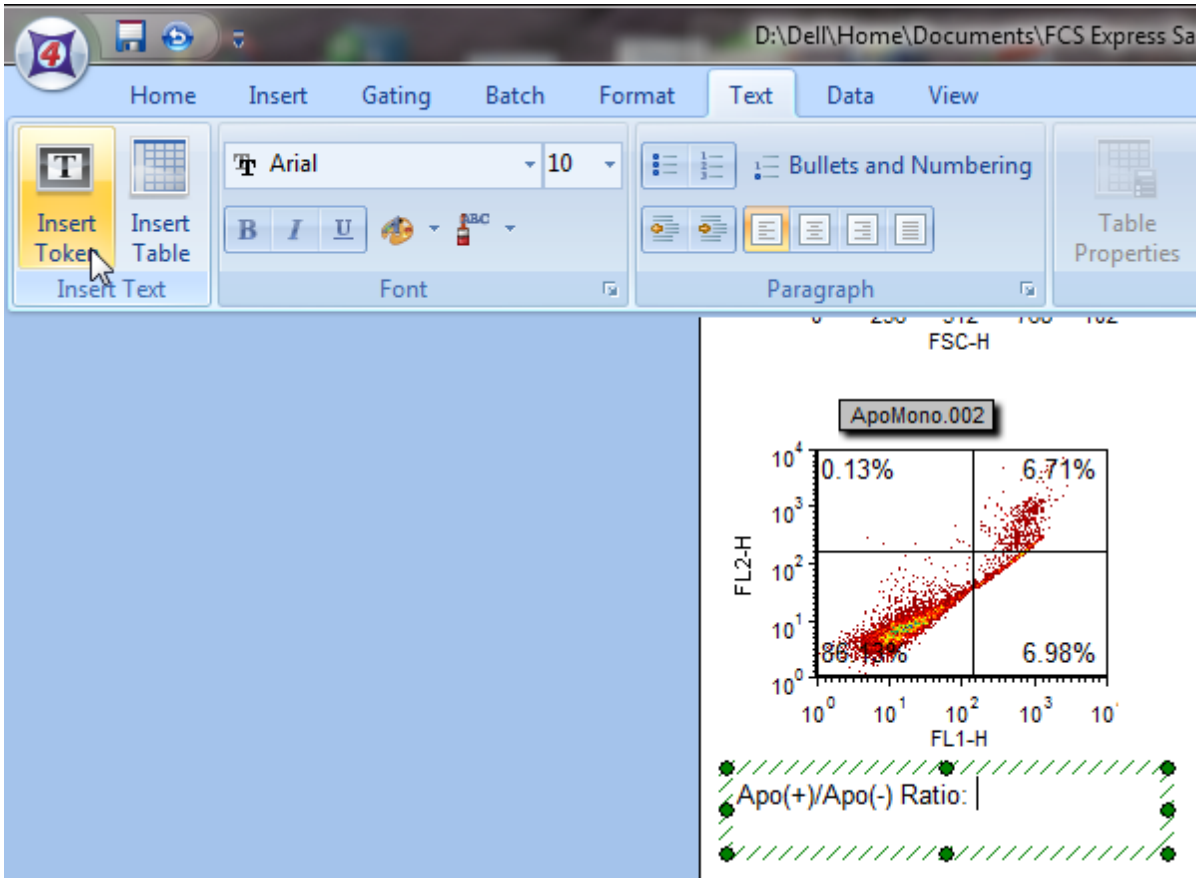

Figure T5.51 Insert Token Command

The **Insert a Token** dialog will appear with the new custom token **Apo(+)/Apo (-) Ratio:** listed (as shown in 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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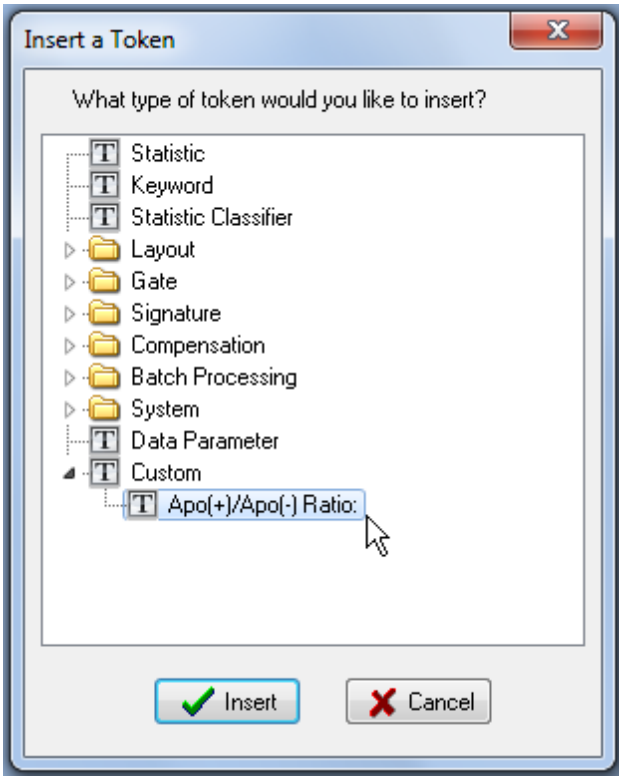

Figure T5.52 Selecting the Custom Token

The Apo(+)/Apo(-) Ratio: value of '0.07' will appear highlighted in grey (Figure T5.53).

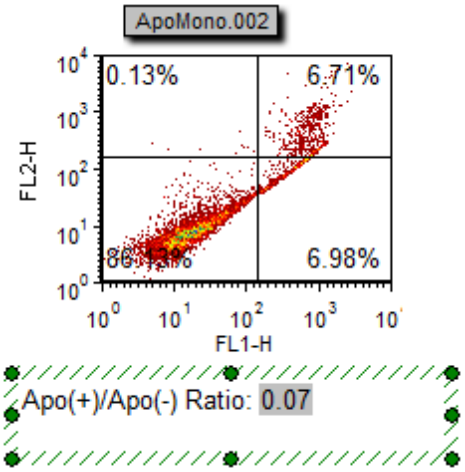

Figure T5.53 Displaying the Custom Token in the Text Box

We will now delete the Custom Token and then insert it by dragging it from the **Custom Tokens** window.

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35. Select the gray box after the "**Apo(+)/Apo(-) Ratio:**" text (Figure T5.53).

36. Press the **Backspace** or **Delete** key to remove the custom token.

The **Custom Tokens** window should still be on the layout as shown in Figure T5.54.

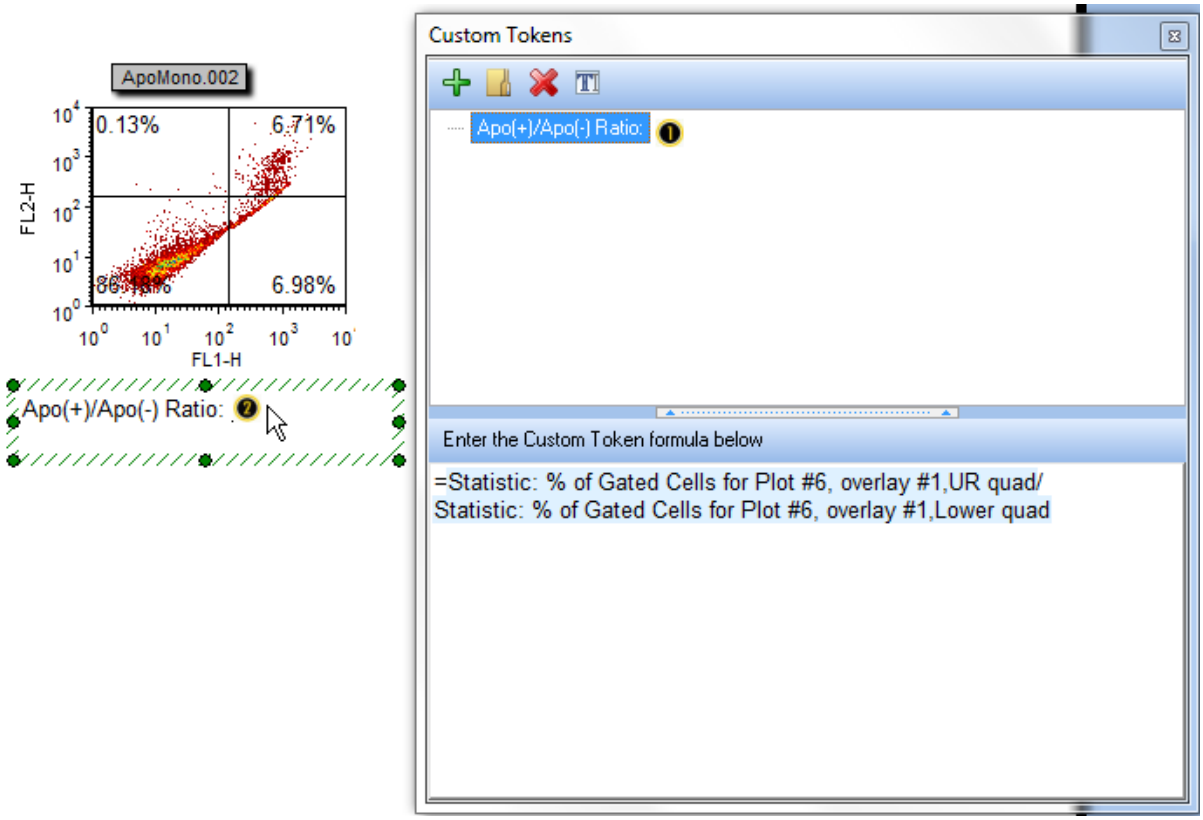

Figure T5.54 Dragging Custom Token onto Text Box

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.

39. Release the mouse button.

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The custom token is now reinserted in the text box as shown in Figure T5.53.

34.6 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 712.

34.6.1 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 **Application Button→Open Layout**.
2. Open the layout **QuadTut.fey** found in the Tutorial Sample Data archive 590.

Two density dot plots now appear on the layout.

3. Select the **Gating→Create Gates→Quadrants** command (Figure T6.1).

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

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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).

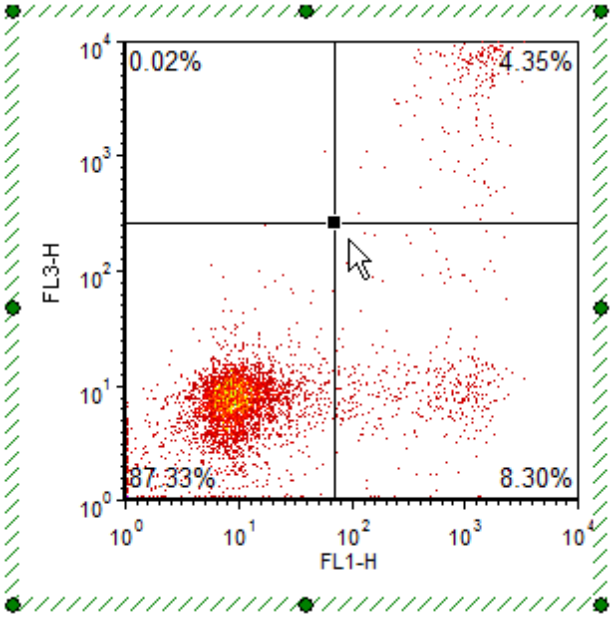

Figure T6.2 Plot with Quadrants

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).

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.

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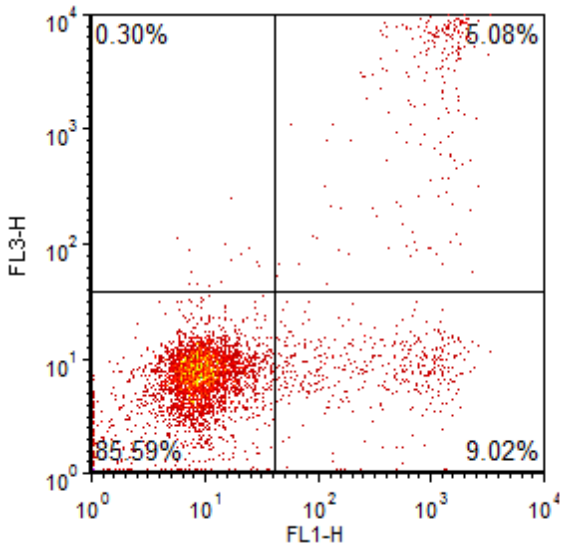

Figure T6.3 Redefined Quadrants

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.

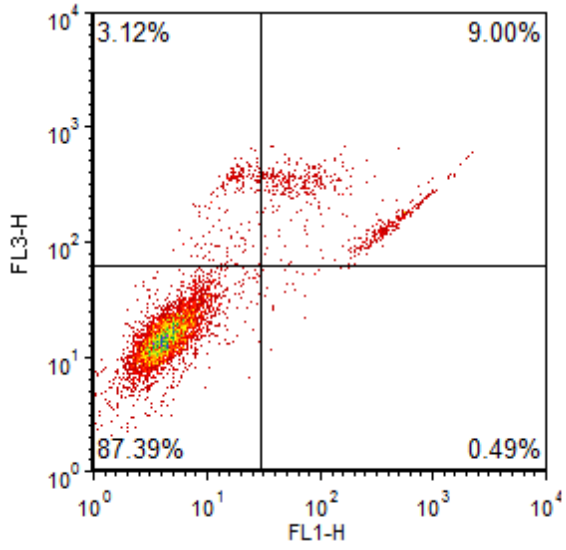

Figure T6.4 Improperly Defined Regions Using Standard Quadrants

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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).

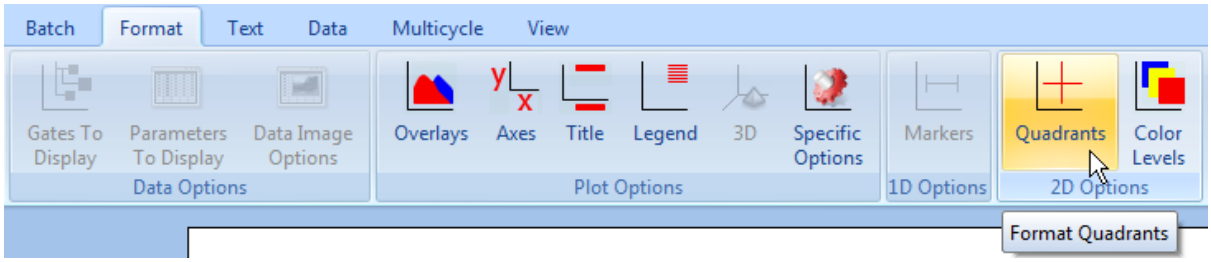

Figure T6.5 Selecting the Format Quadrants Command

The **Formatting Quadrants** dialog will now appear (Figure T6.6).

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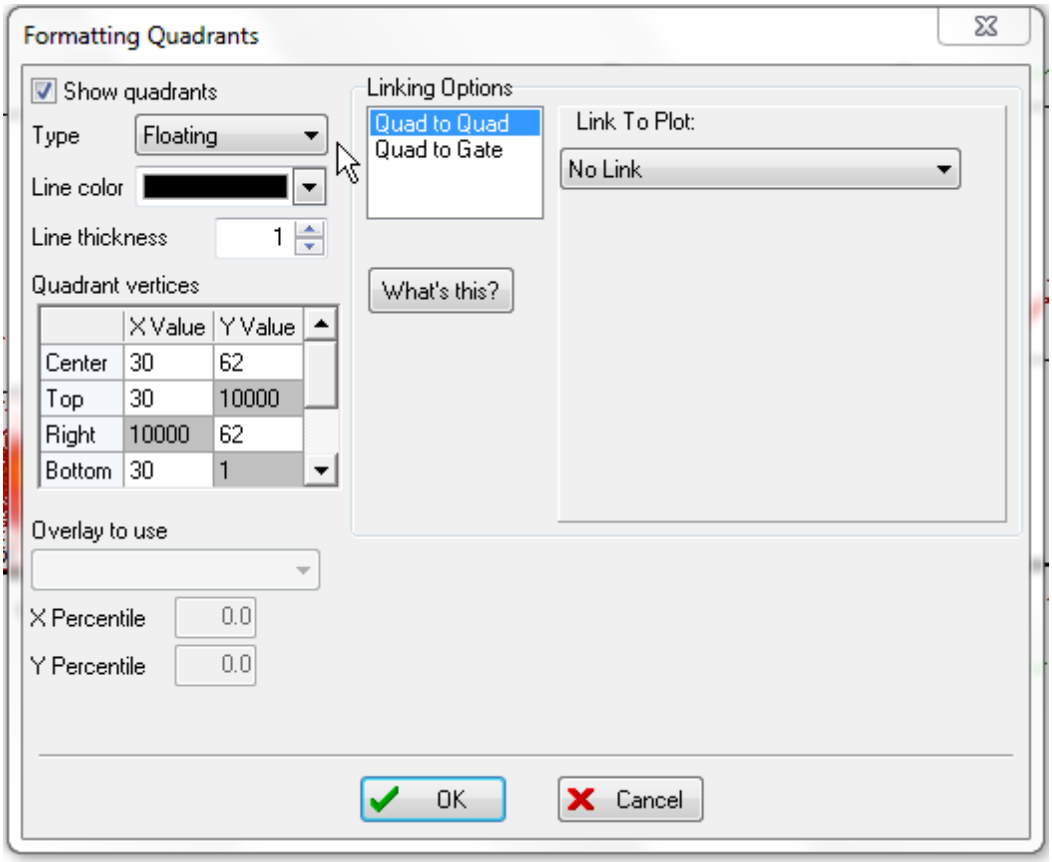

Figure T6.6 Formatting Quadrants Dialog

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**.

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.

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- Click **OK**.

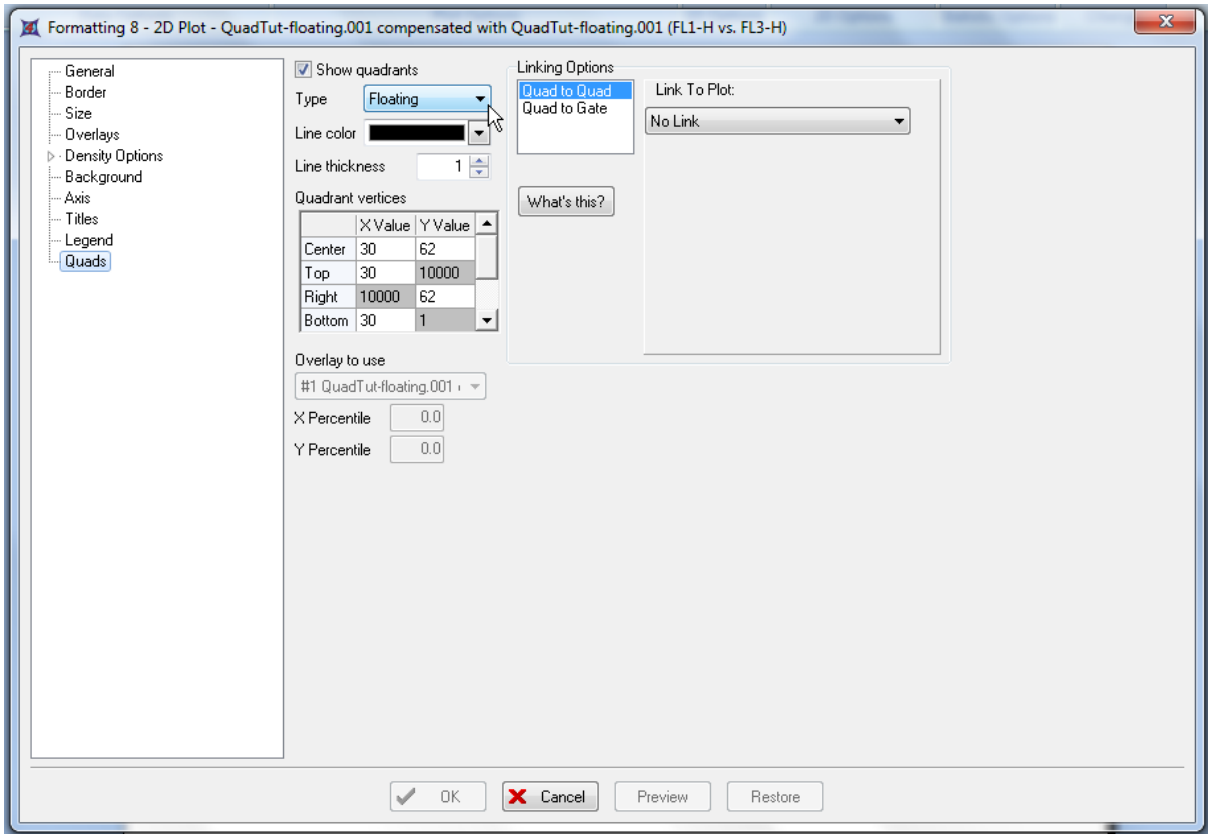

Figure T6.7 Quads Category of the Formatting Dialog

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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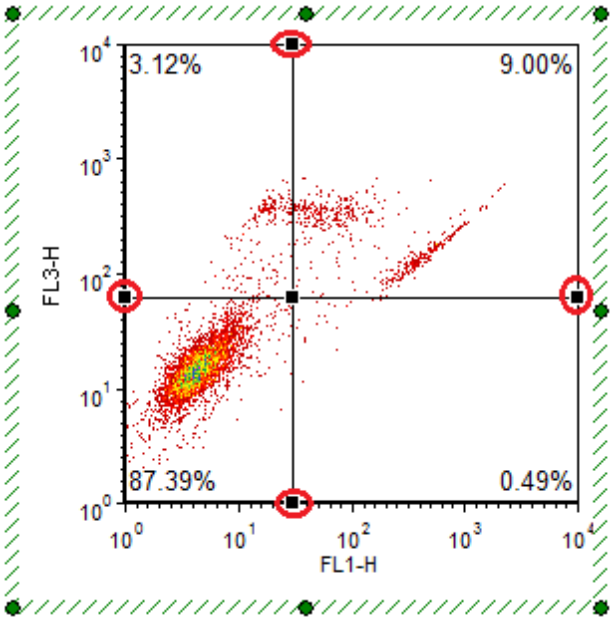

Figure T6.8 Floating Quadrant Selected for Editing

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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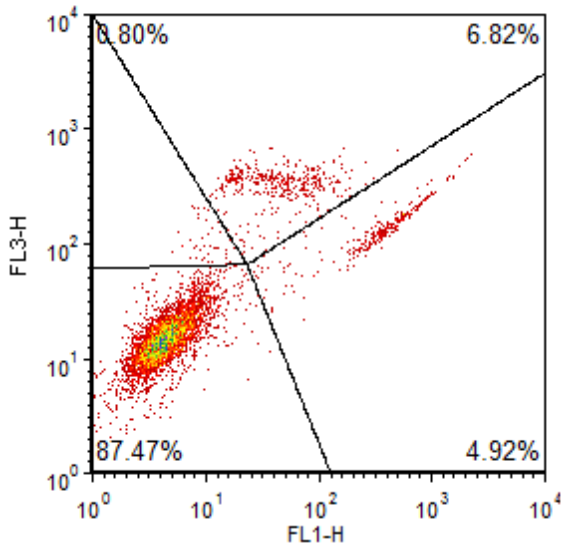

Figure T6.9 Floating Quadrants

Next, we will set up percentile quadrants 719.

34.6.2 Percentile Quadrants

In the course of this example, we will:

- Learn how to set rectangular quadrants using percentiles.

FCS Express allows you to set quadrants according to percentages instead of manual positioning.

To set a quadrant with percentages:

1. Click once on the left plot to select it.
2. Select the **Format→2D Options→Quadrants** command (Figure T6.5 712).

The **Formatting Quadrants** dialog will appear, similar to Figure T6.10.

3. 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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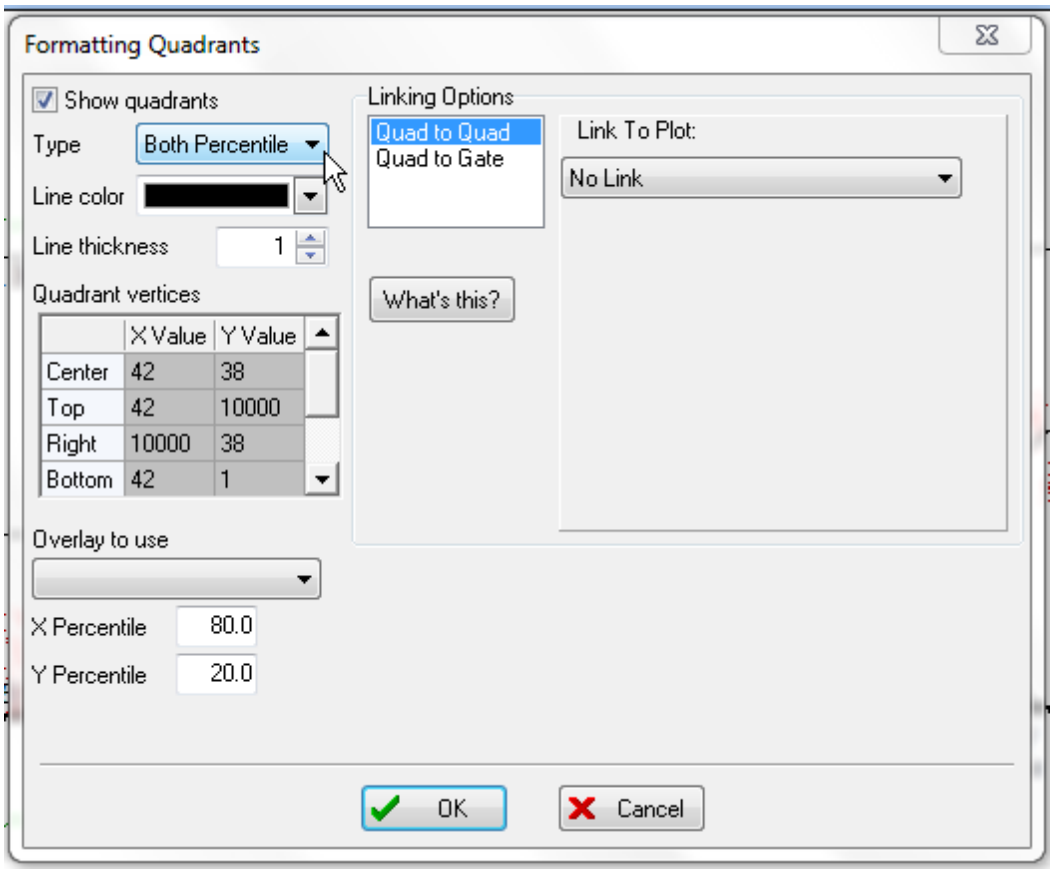

Figure T6.10 Formatting Quadrants Dialog -- Setting Type to Both Percentile

4. Enter "**80**" in **X Percentile** field (Figure T6.10)
5. Enter "20" in the **Y Percentile** field (Figure T6.10).
6. 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).

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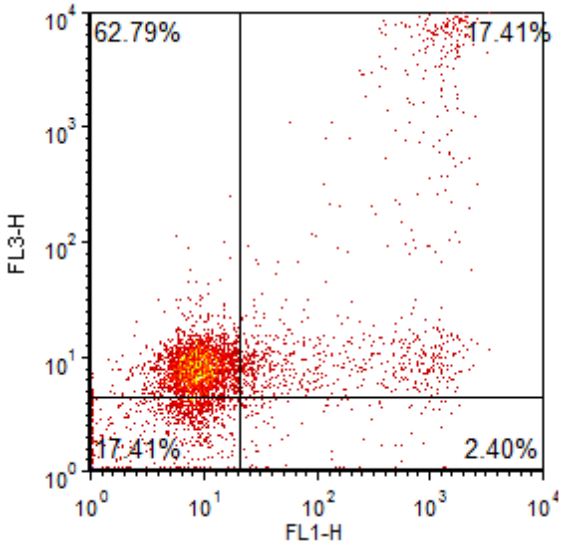

Figure T6.11 Percentile Quadrants of 80% and 20%

We will now use the plot pop-up menu to format the quadrant and change the X and Y percentile values.

7. Right-click on the left plot to bring up the associated pop-up menu.
8. Select **Format** from the pop-up menu.
9. Select the **Quads** category from the left of the **Formatting** dialog (Figure T6.12)
10. Change the **X and Y Percentiles** to **82 and 8** respectively (Figure T6.12).
11. Click **Preview**, indicated by the cursor in Figure T6.12.

Using **Preview** allows you to quickly manipulate the percentiles until you reach your desired result.

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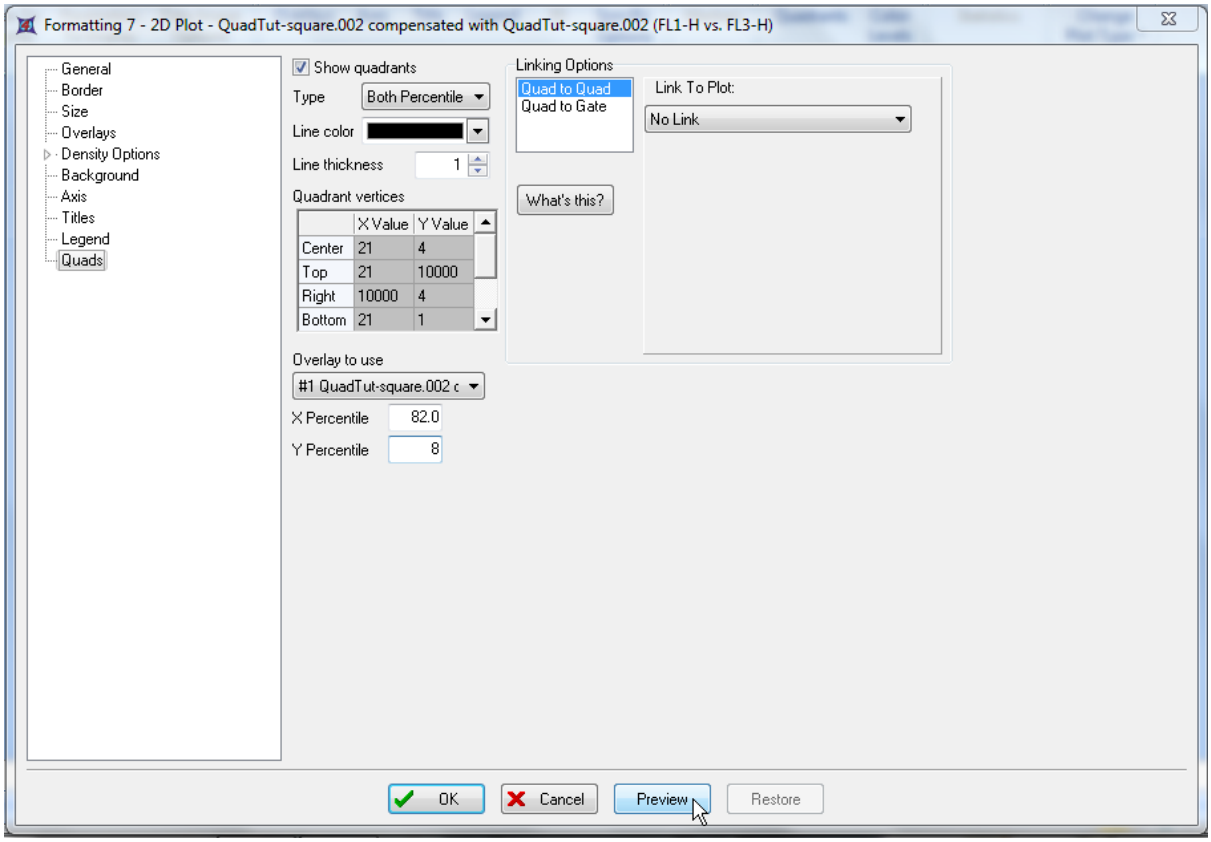

Figure T6.12 Using Preview while Setting Percentile Quadrant Values

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.

34.7 Quadrant/Marker to Gate Linking

One of the many timesaving 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 722.

34.7.1 Quadrant to Gate Linking

In the course of this example, we will:

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- 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 **Application Button→Open Layout**.
2. Open the layout **Quad2RegionLink.fey** found in the Tutorial Sample Data archive 590.

This layout contains three 2D density plots of the file **GFPsample.002** (Figure T7.1). The left plot is of FSC vs. SSC with a gate on **Whole Cells**. The upper plot is of FL1 vs. FL2 gated on **Whole Cells** with quadrants. The lower plot is a duplicate plot of FL1 vs. FL2 but without quadrants.

Marker to Gate Linking 729

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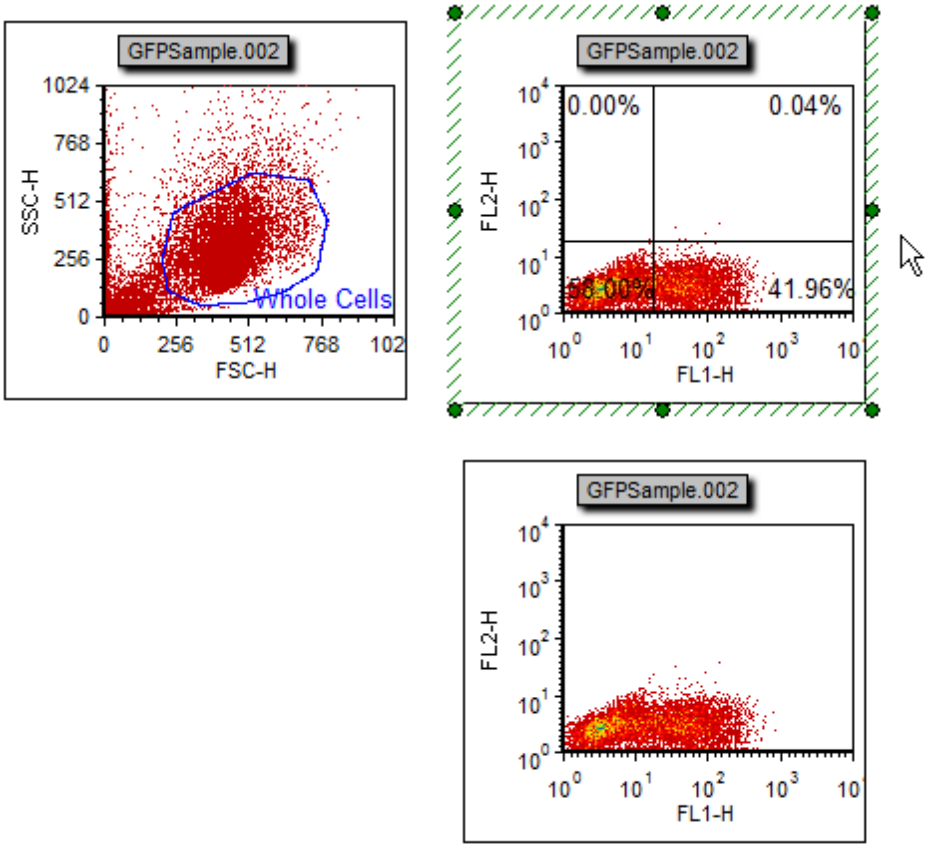

Figure T7.1 Quad2RegionLink.fey Layout

3. Right-click on the upper right 2D plot of FL1 vs. FL2 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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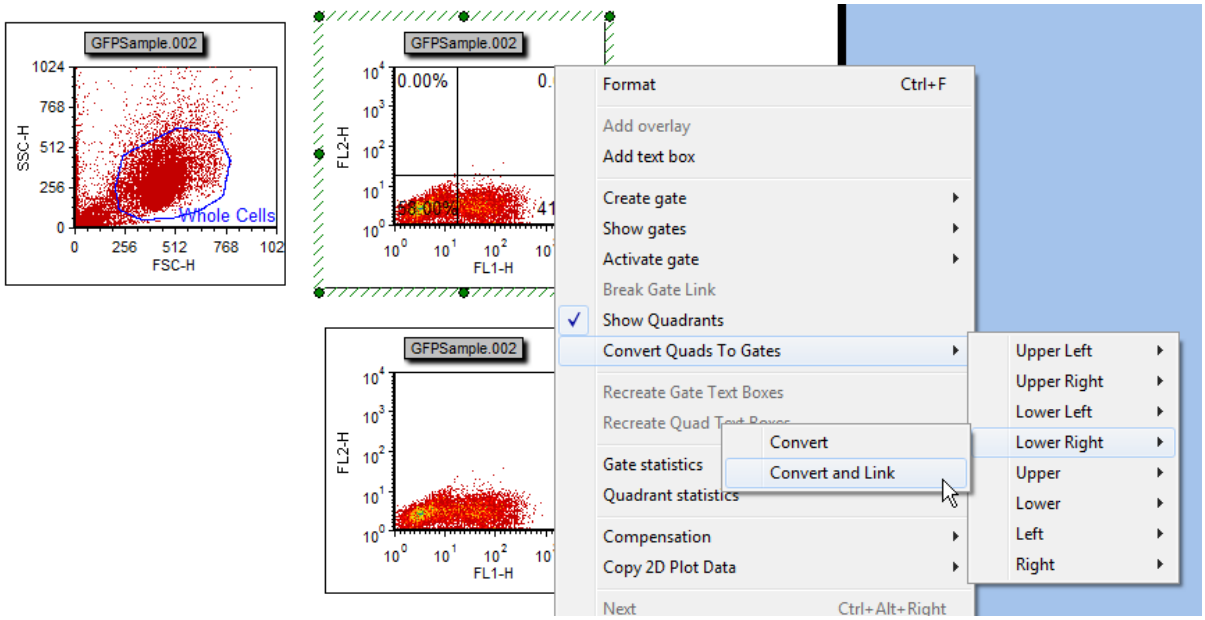

Figure T7.2 Converting and Linking the Lower Right Quadrant to a Gate

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.

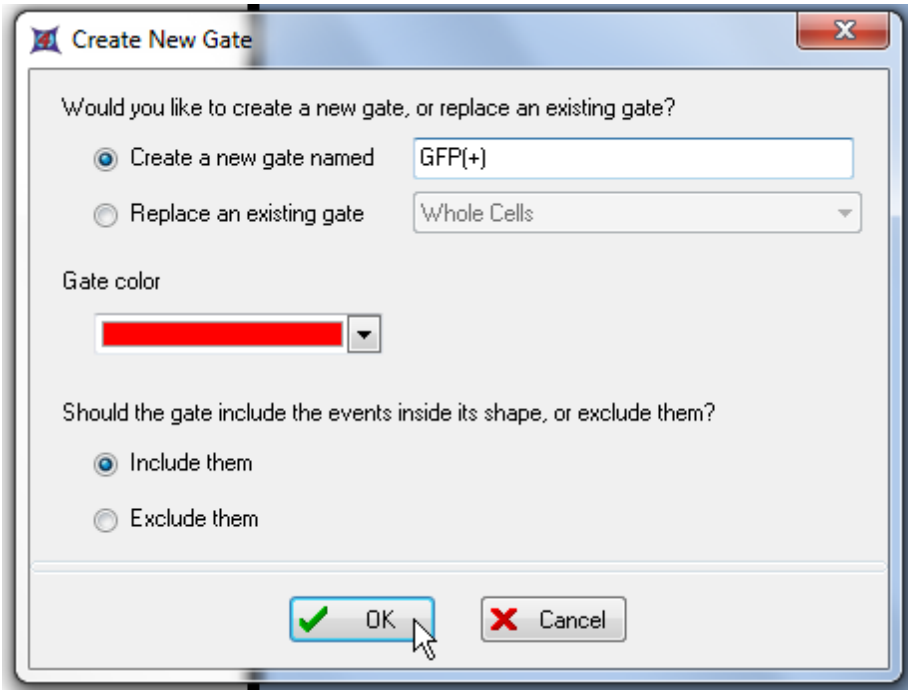

Figure T7.3 Create New Gate Dialog

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5. Enter "**GFP(+)**" in the **Create a new gate named** field.
6. Click **OK**.

We will now apply the newly created **GFP(+)** gate, that is linked to the lower right quadrant, to the lower right 2D plot.

7. Click inside the 2D plot of FL1 vs. FL2 on the lower right without the quadrants. The border will appear green (Figure T7.4).
8. Select the **Gating** tab (Figure T7.4).
9. Select **GFP(+)** from the **Current Gate** drop-down list (Figure T7.4).

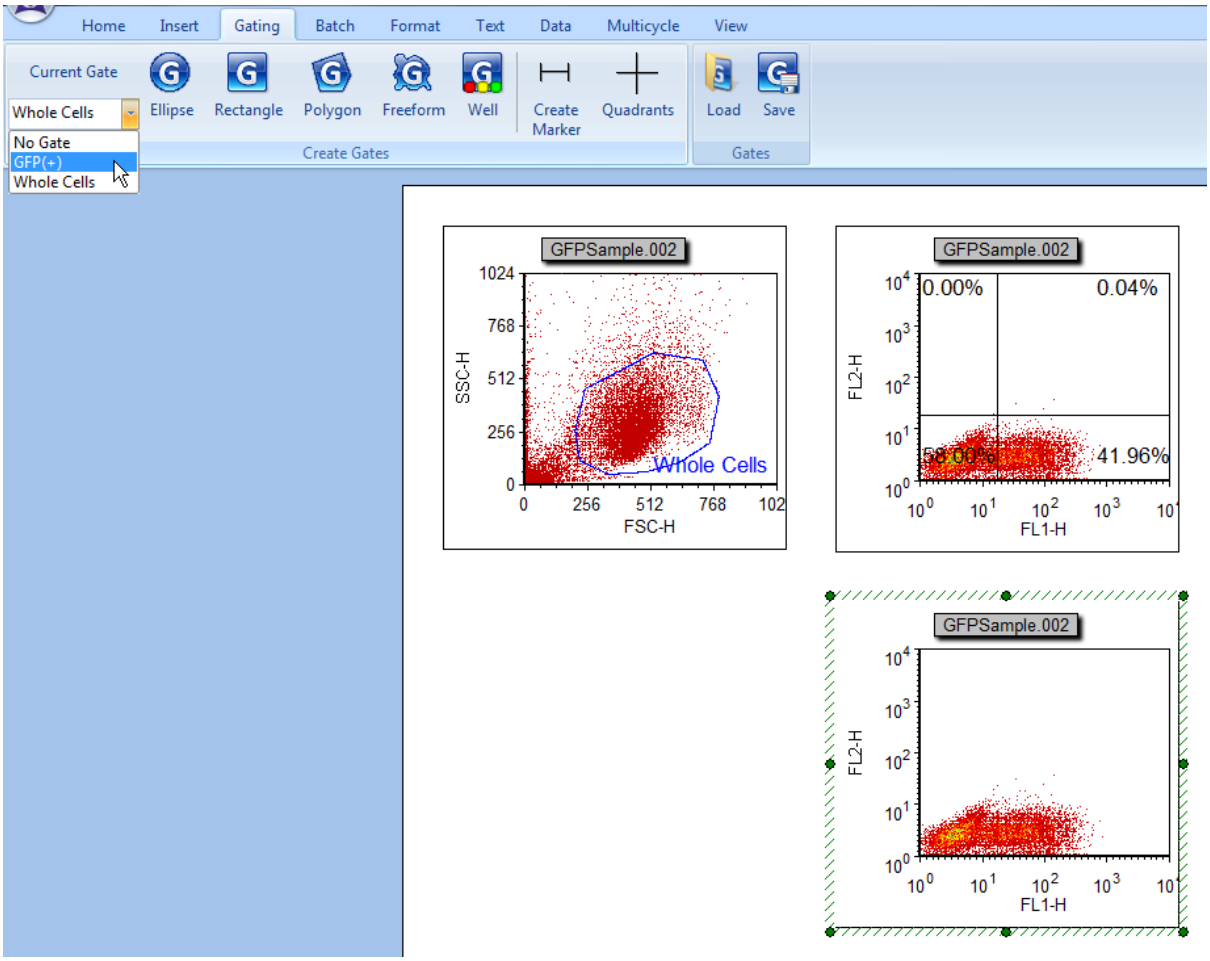

Figure T7.4 Selecting the GFP(+) Gate for the Lower Right 2D Plot

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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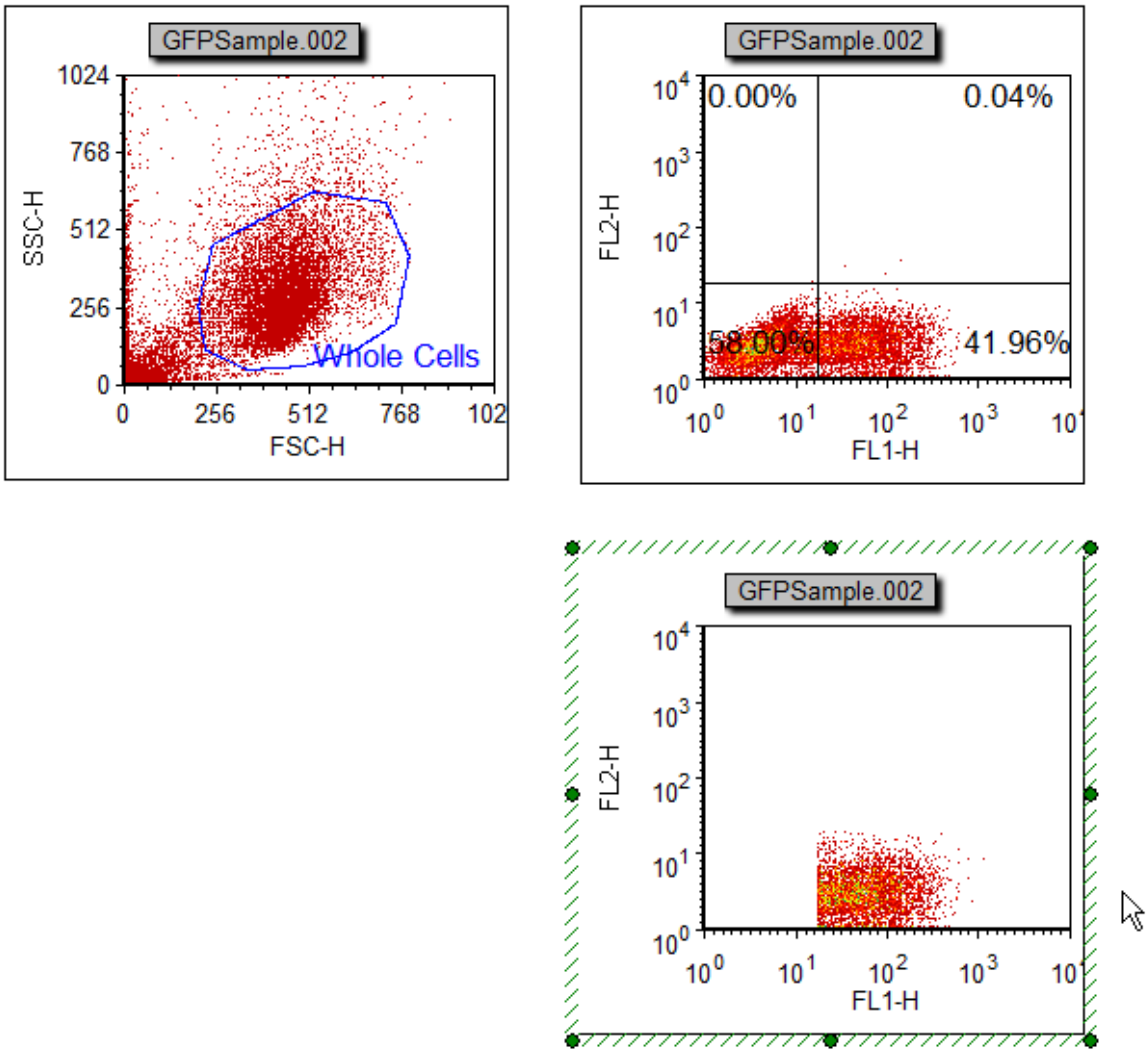

Figure T7.5 Lower Right Plot Gated on GFP(+)

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).

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

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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).

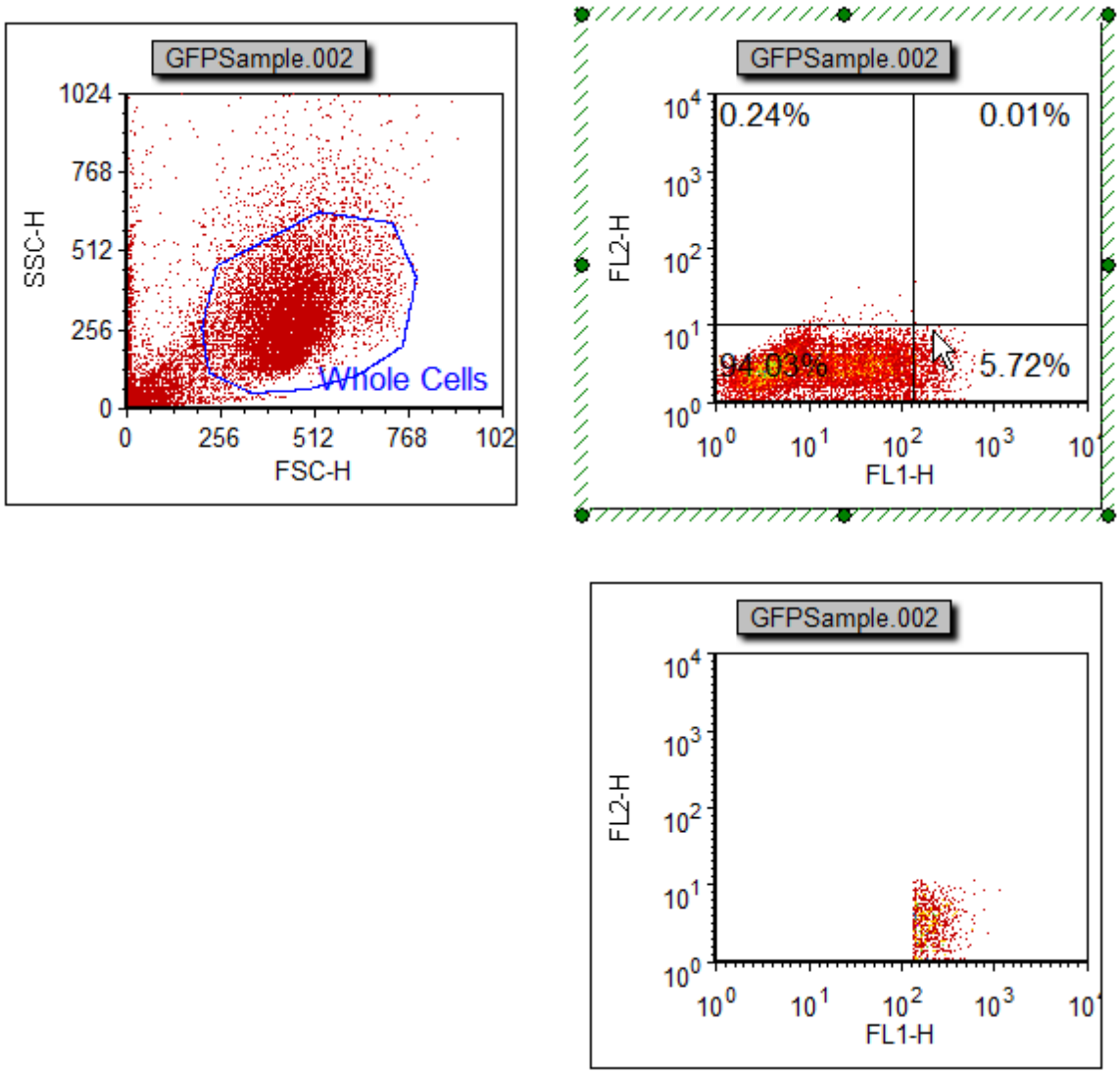

Figure T7.6 GFP(+) Gate Updated after Quadrant Move

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15. Select **Application Button→Close Layout**.

In the next section, we will link a marker to a gate 729.

34.7.2 Marker to Gate Linking

In the course of this example, we will:

- Link a marker to a gate.
- 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 657.

1. Select **Application Button→Open Layout**.
2. Open the layout **Marker2RegionLink.fey** found in the Tutorial Sample Data archive 590.

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.

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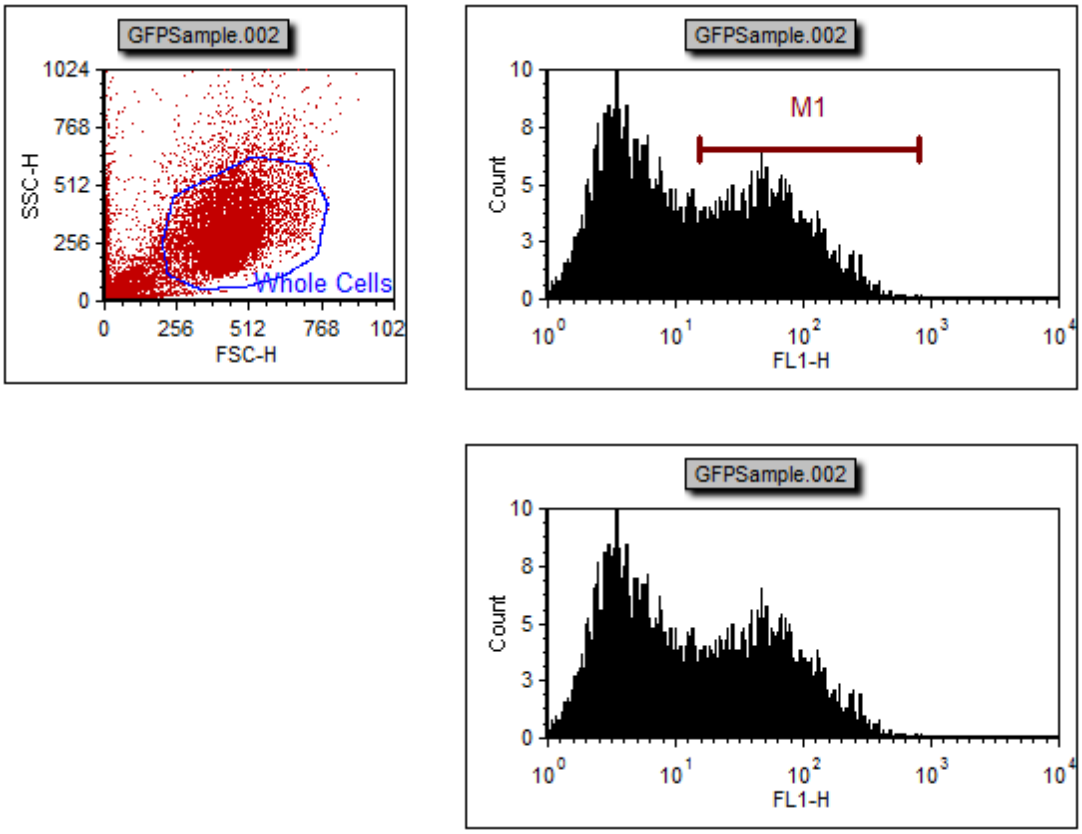

Figure T7.7 Marker2RegionLink.fey Layout

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).

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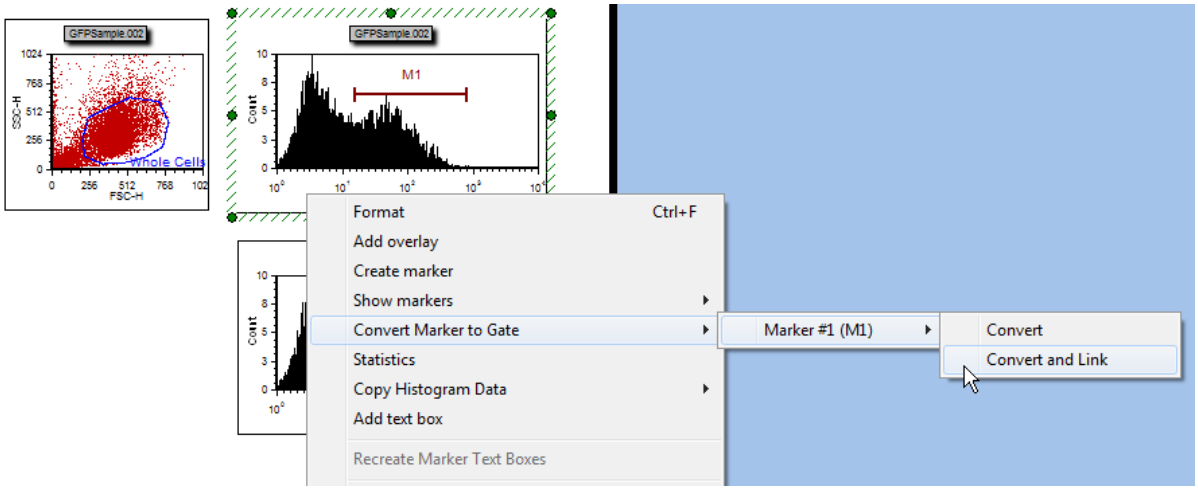

Figure T7.8 Converting the Marker M1 to a Gate

A **Create New Gate** dialog will appear as shown in Figure T7.3 722. 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 T7.3 722).
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 T7.4 722.
    - 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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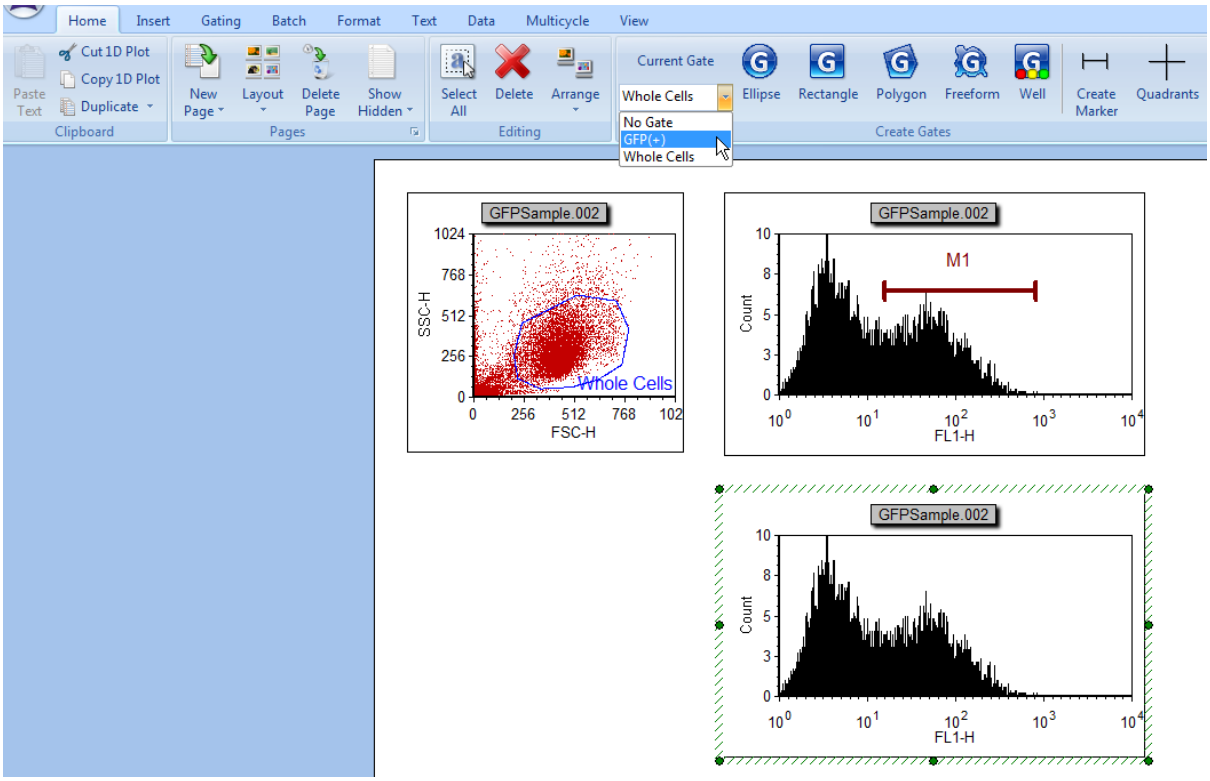

Figure T7.9 Applying the GFP(+) Gate to the Lower Right Histogram

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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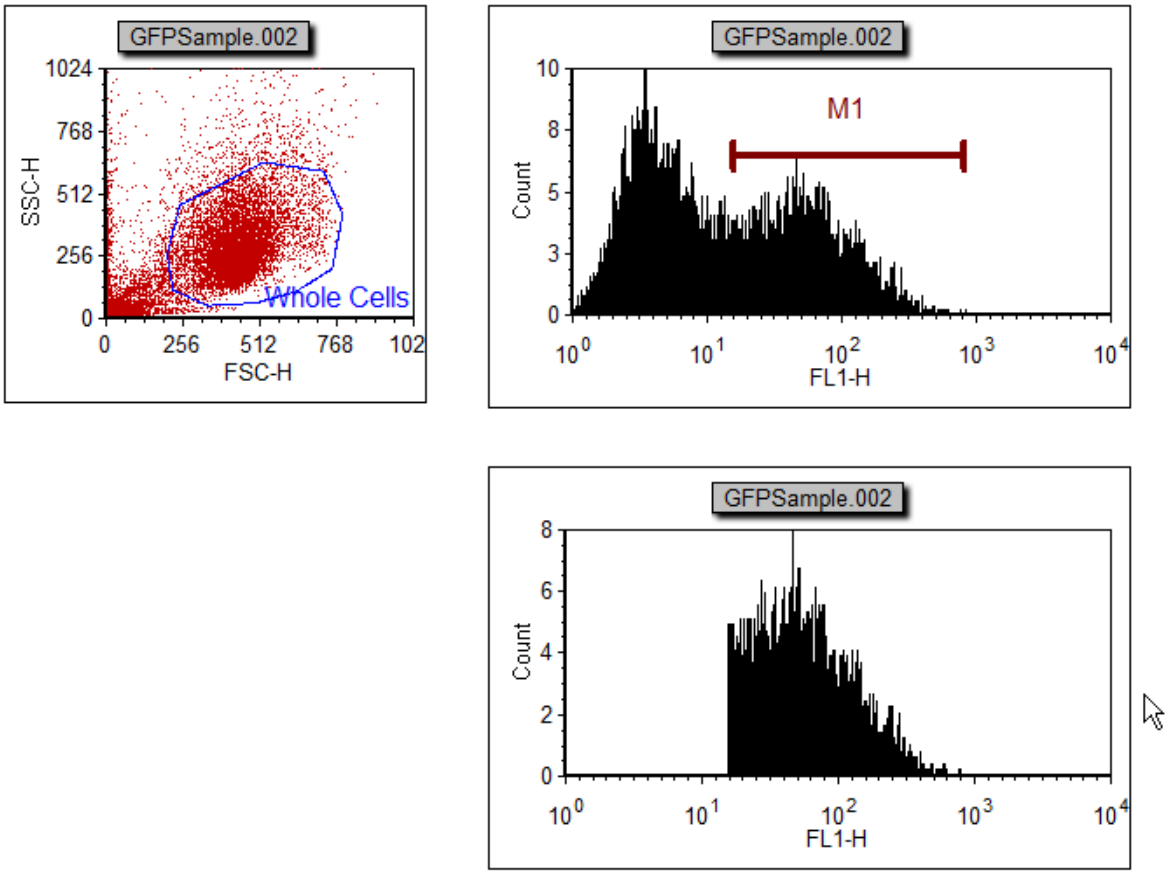

Figure T7.10 Lower Right Histogram Gated on GFP(+)

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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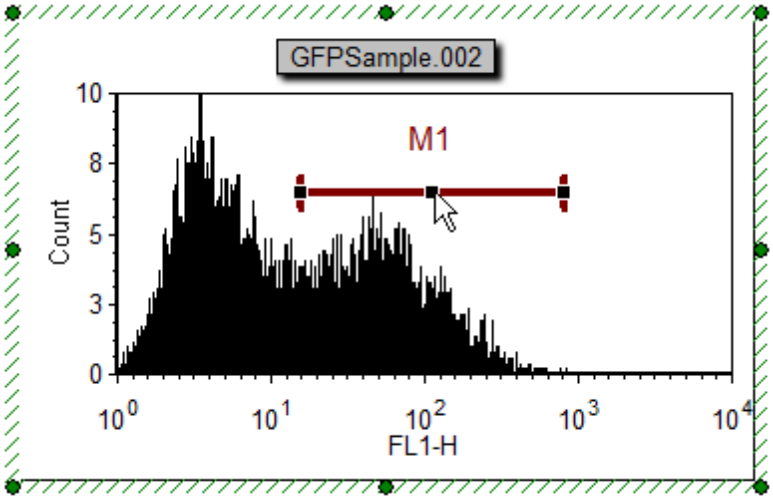

Figure T7.11 Active Marker M1 with Resizing Rectangles

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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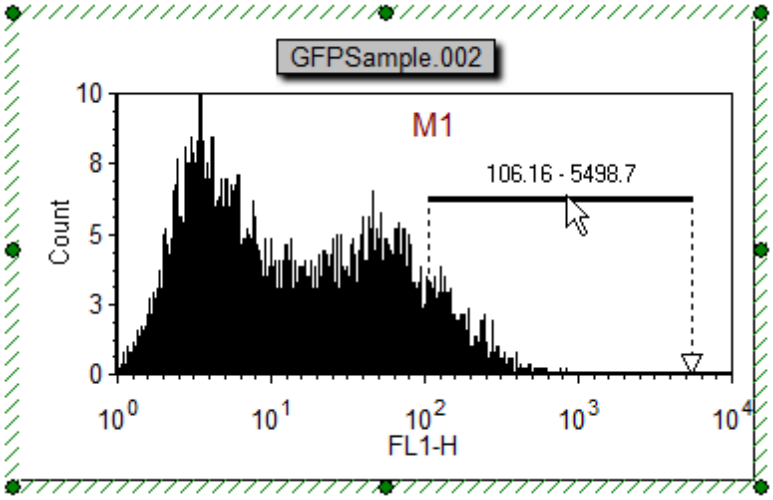

Figure T7.12 Moving Marker M1

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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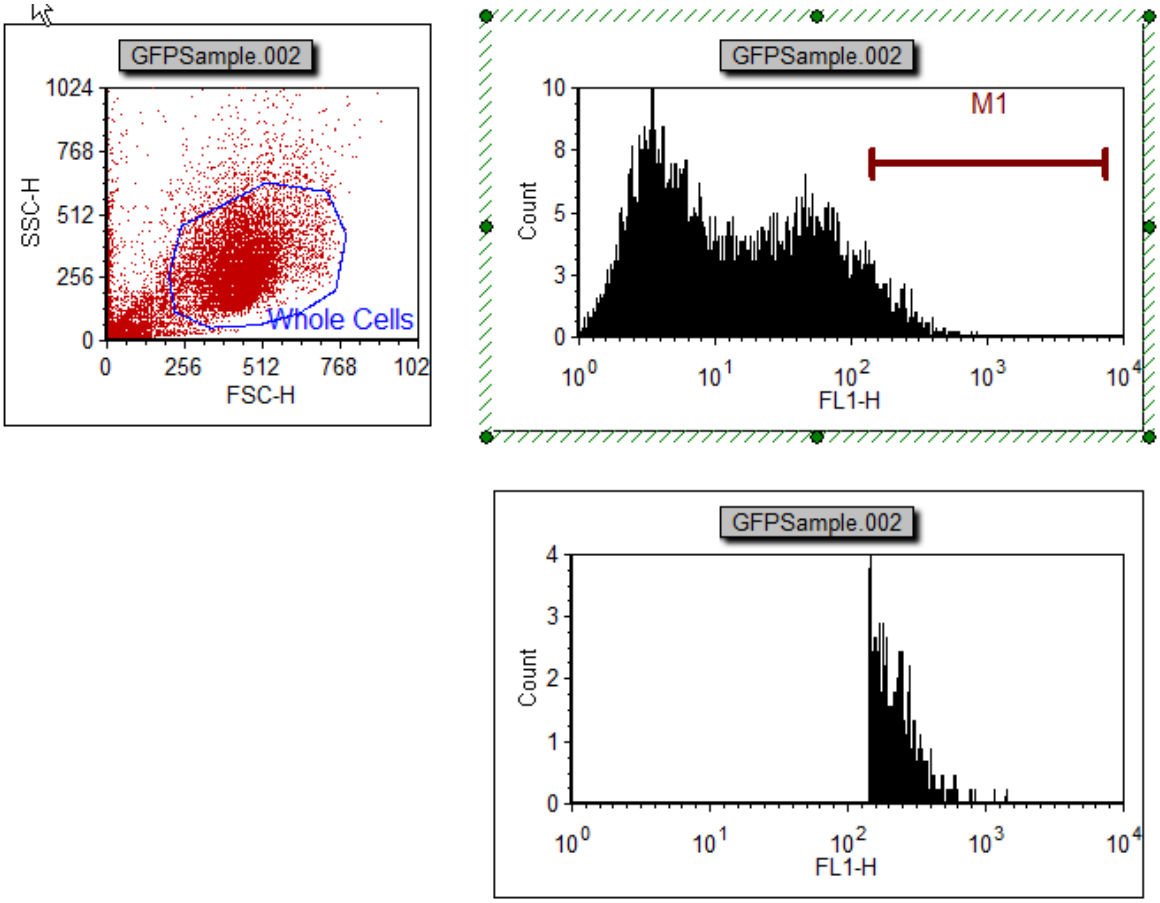

Figure T7.13 Histogram Gated on GFP(+) Updated to Reflect Changes made to Marker M1 Position

34.8 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 737 method allows you to adjust the compensation levels using sliders that allow you to adjust the amount of compensation. The Automatic 741 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 765 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.

The first example will show Manual Compensation 737.

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34.8.1 Manual Compensation

To adjust the compensation manually:

1. Select **Application Button→Open Layout**.
2. Open the layout **ManualComp.fey** found in the Tutorial Sample Data archive 590.

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).

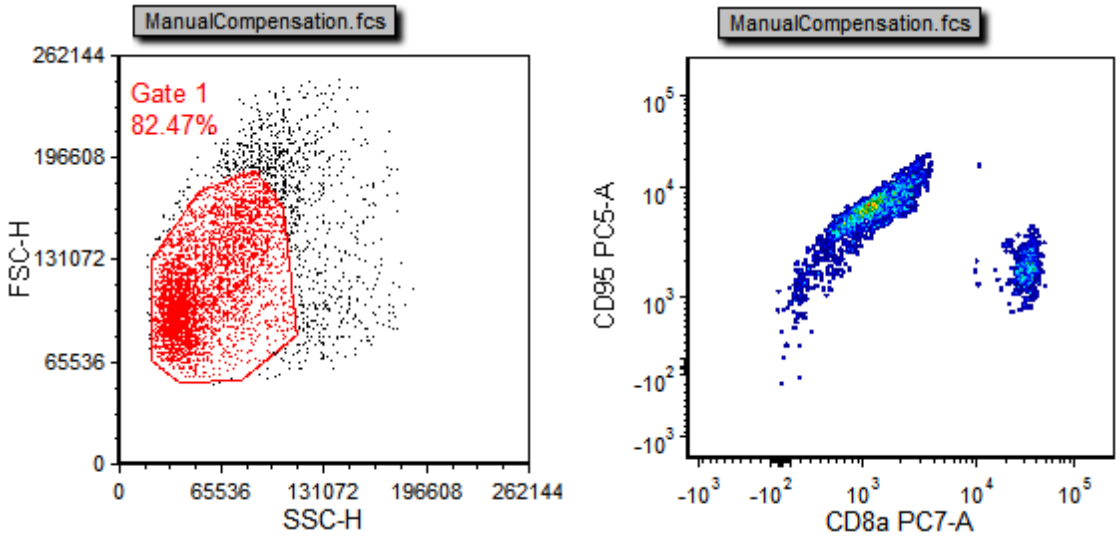

Figure T8.1 Uncompensated Plots

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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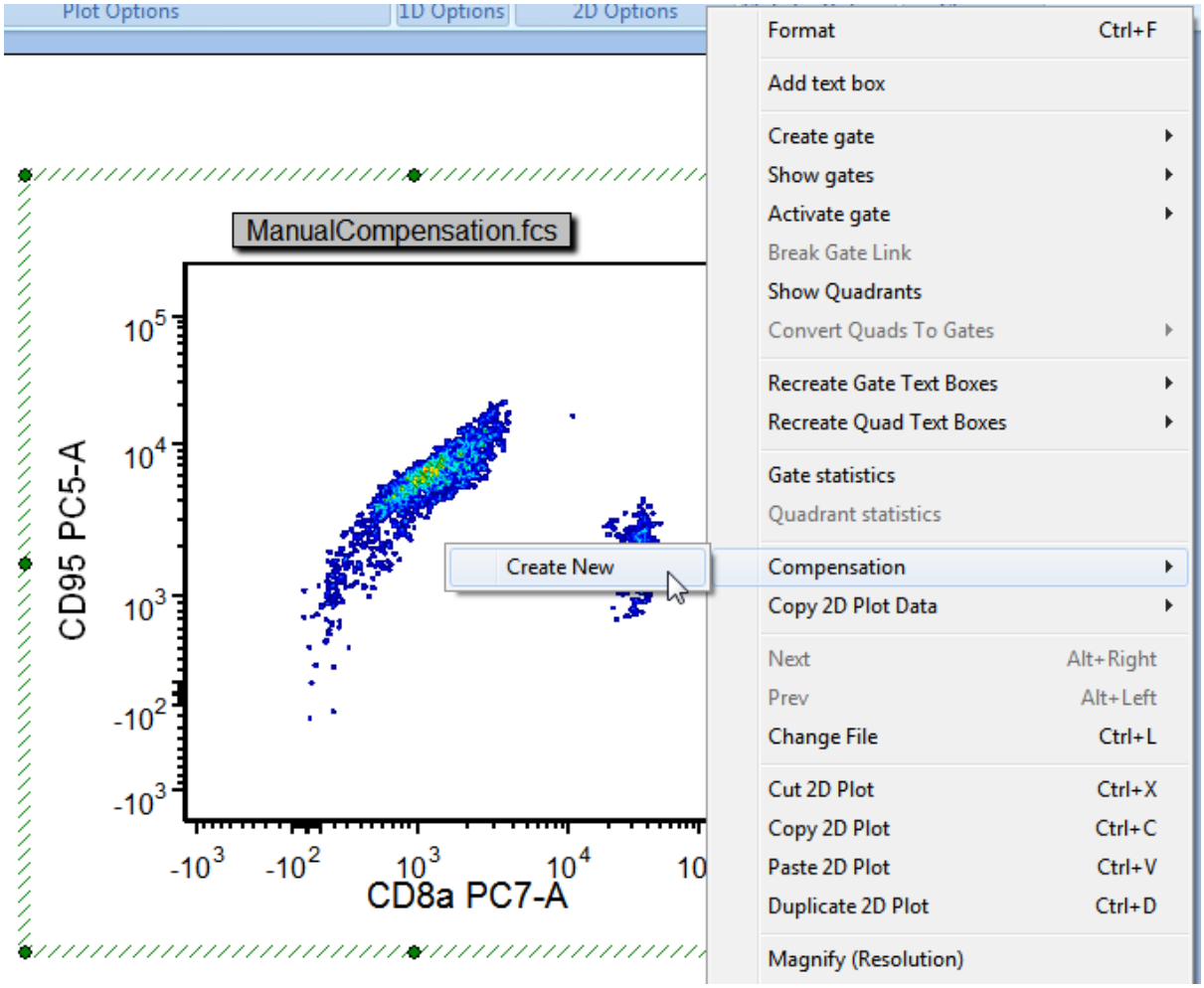

Figure T8.2 Selecting Create New Compensation

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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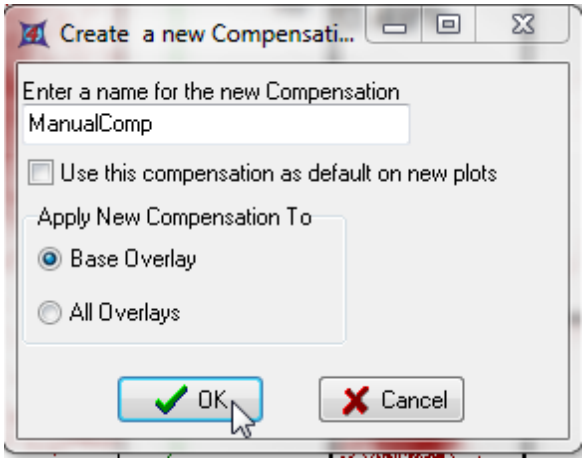

Figure T8.3 Create a new Compensation Dialog

An **Edit Compensation** dialog will now appear (Figure T8.4).

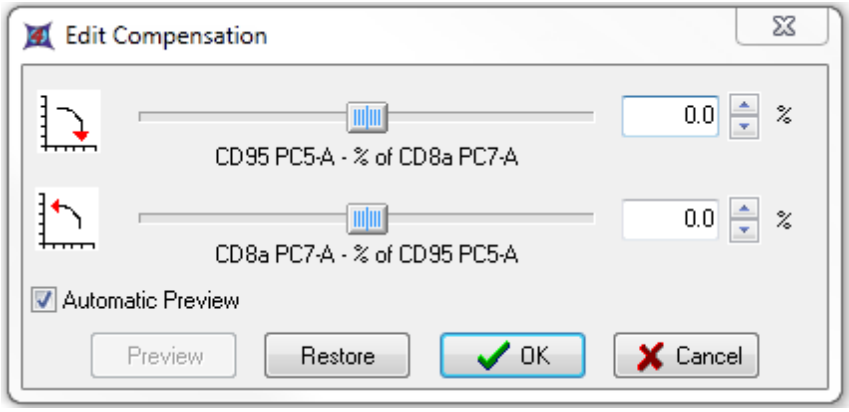

Figure T8.4 Edit Compensation Dialog

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.

   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.

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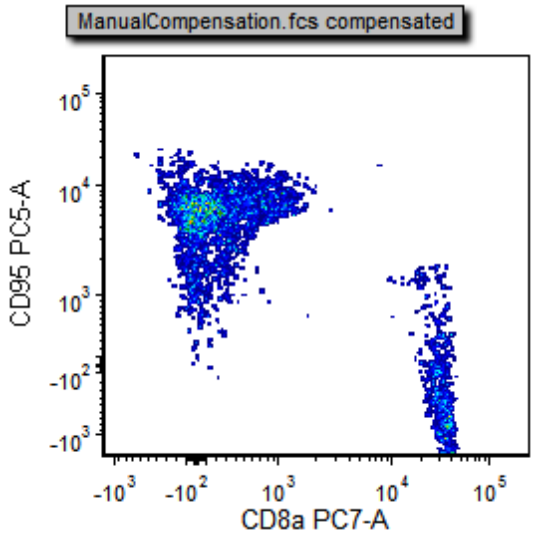

Figure T8.5 Manual Compensation using Edit Compensation Sliders

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 tutorial.*

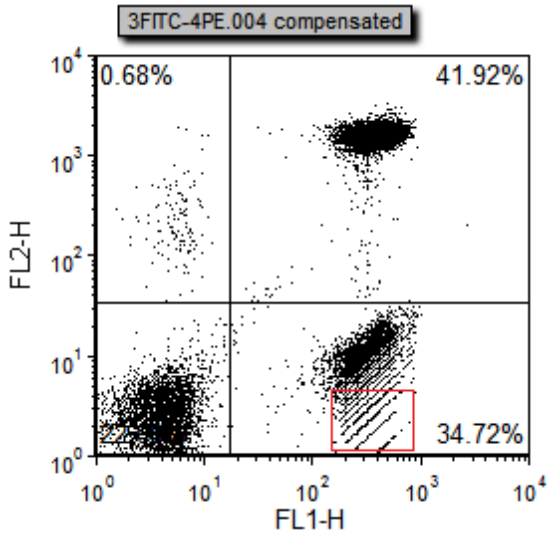

Figure T8.21 - Digitization errors after compensation can be seen in the red box. Please refer to the Compensation Digitization Errors tutorial to correct this type of error.

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In the next section, we will use Automatic Compensation 741.

34.8.2 Automatic Compensation

To create a Compensation Definition automatically from single stained controls:

1. Select **Application Button→Open Layout**.
2. Open the layout **AutoComp.fey** found in the Tutorial Sample Data archive 590.

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 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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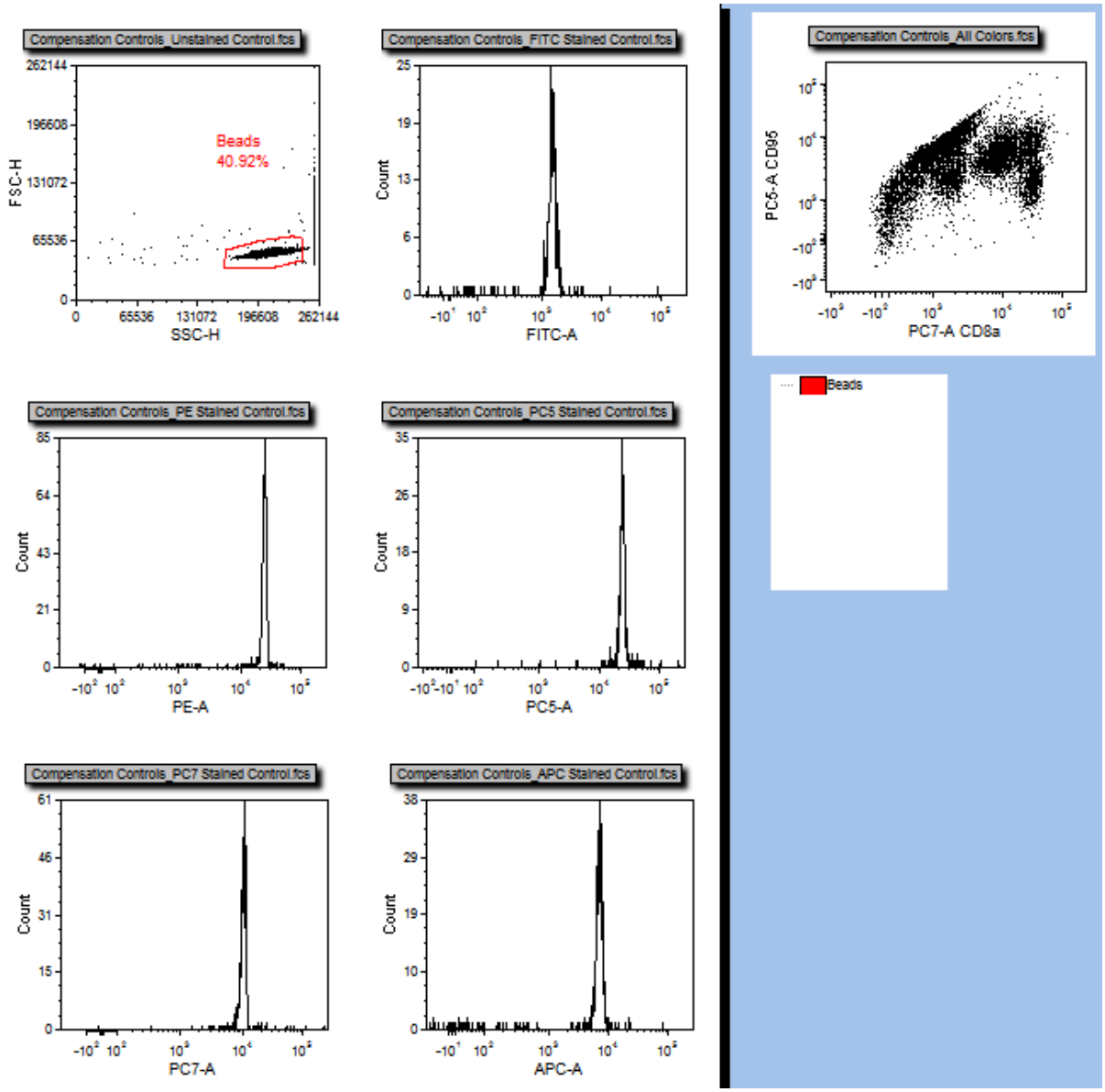

Figure T8.6 Autocomp.fey Layout

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 **Data** tab→**Data Info**→**Compensations** command to open the **Compensations** navigator (Figure T8.7).

The **Compensations** navigator can be moved any where on the screen and it is dockable and pinnable 66. Please feel free to move it around the screen as needed while working on this tutorial.

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4. Click on the green 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).

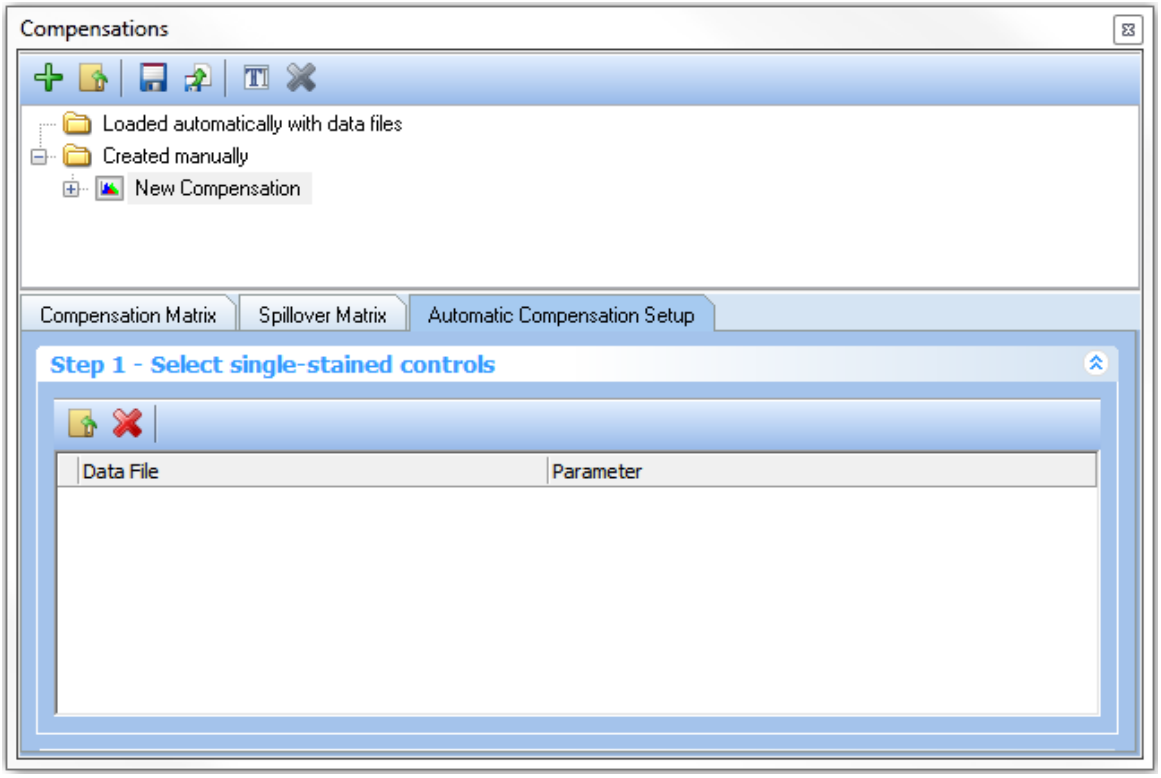

Figure T8.7 - The Compensations navigator. Use the navigator to add new compensations, edit definitions, and to create new Automatic Compensation Setups.

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. Click on the **Data List** tab (Figure T8.8).

7. Multiple select all of the files in the data list (Figure T8.8).

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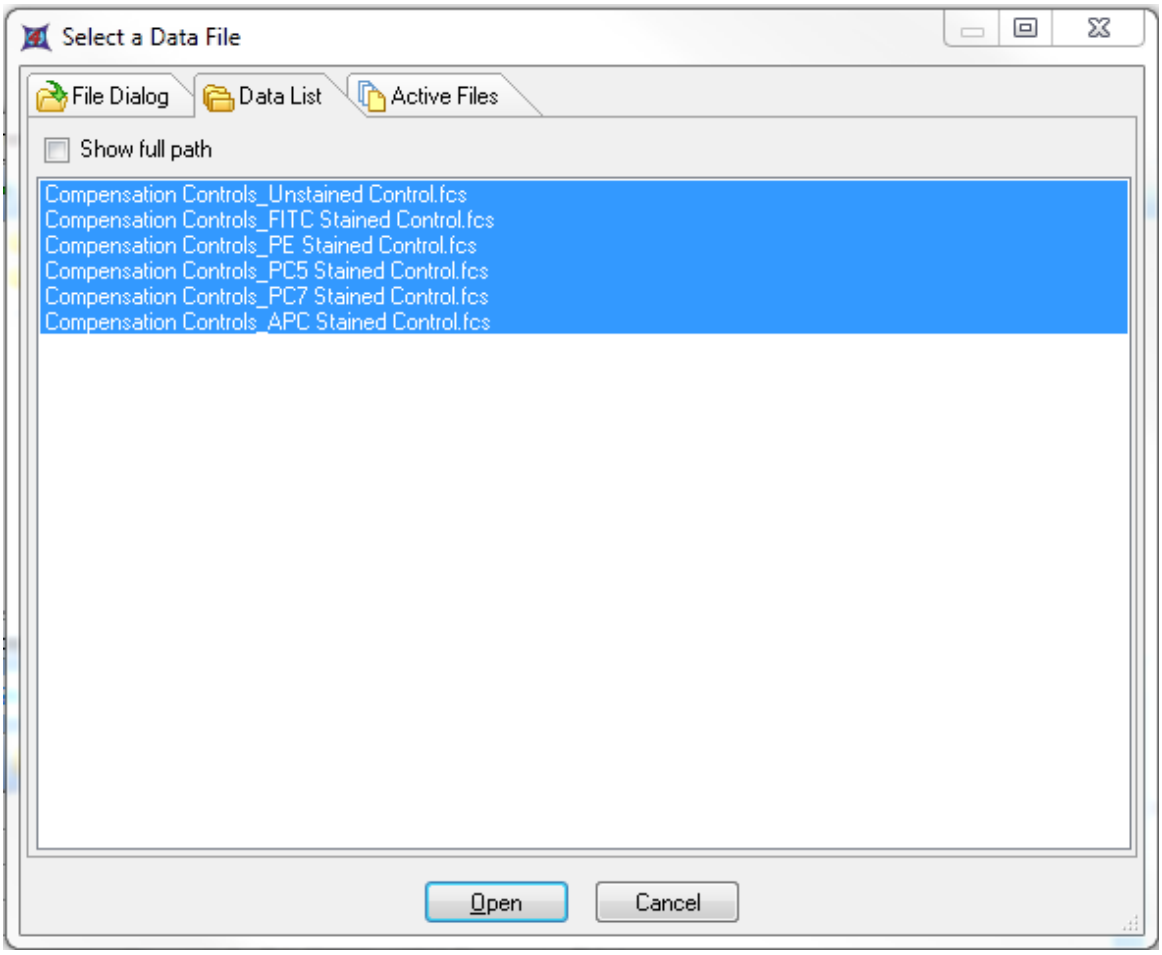

Figure T8.8 - Use the Select a Data File window and Data List tab to select all of the files for use in the Automatic Compensation Setup.

8. Click **Open**.

Notice that all of the single stain control files and the unstained control file have been brought into the **Select files and parameters** window (Figure T8.9). We will now assign the correct parameter for each individual file by matching the the single stain control name in the file name to the **Parameter** in the **-select parameter-** drop down list.

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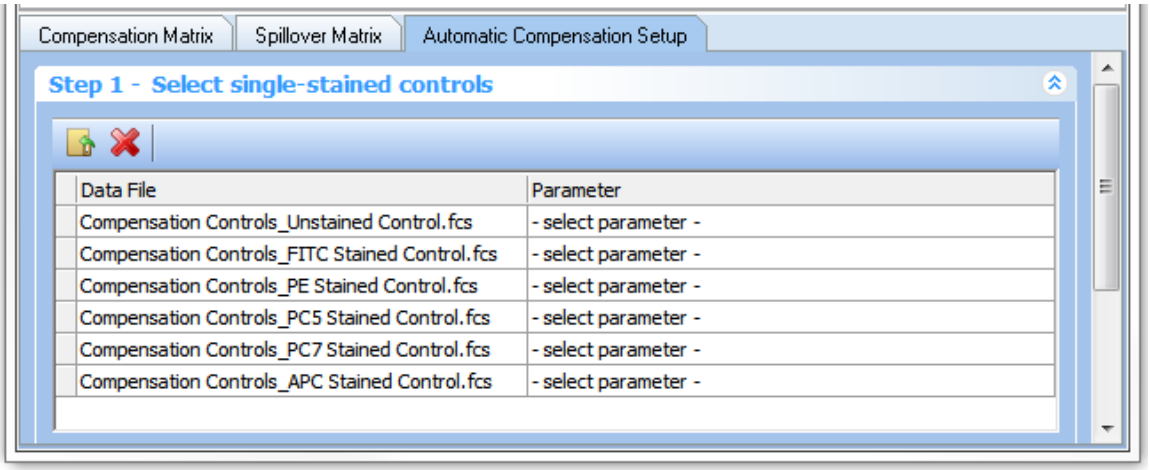

Figure T8.9 - Files have been added to Step 1 in the Automatic Compensation Setup. The Parameter column drop downs will be used to match the single stained sample data control files to the respective parameter for compensation.

9. Click on **-select parameter-** for the first file (Compensation Controls_Unstained Control.fcs) (Figure T8.10).

10. Select **Universal Negative** from the bottom of the **-select parameter-** drop down list (Figure T8.10).

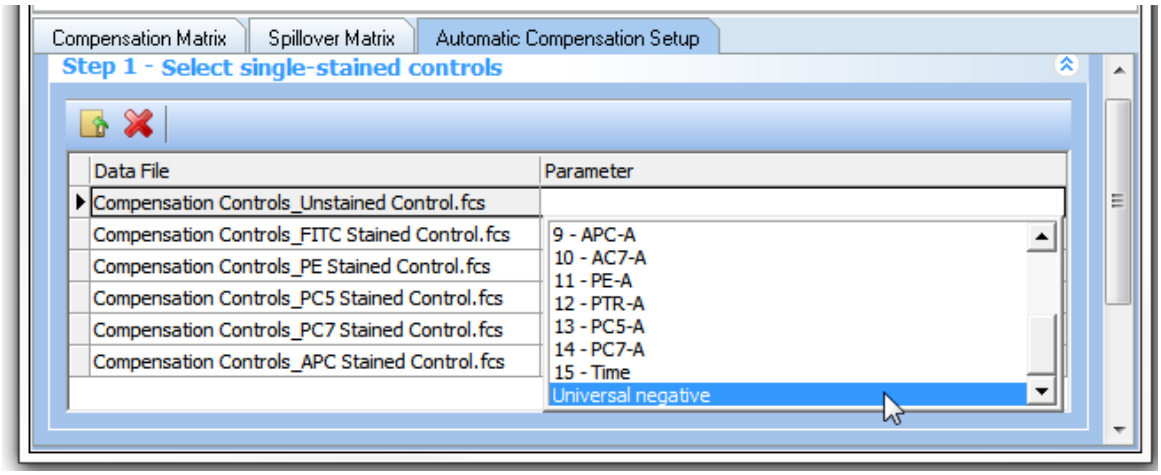

Figure T8.10 - Selecting the Universal negative parameter to match the Unstained Control.fcs file.

This will assign the Compensation Controls_Unstained Control.fcs file as the universal negative control for the rest of the single stained controls.

11. Click on **-select parameter-** for the second file (Compensation Controls_FITC Stained Control.fcs) (Figure T8.11).

12. Select **7- FITC-A** from the **-select parameter-** drop down list (Figure

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T8.11).

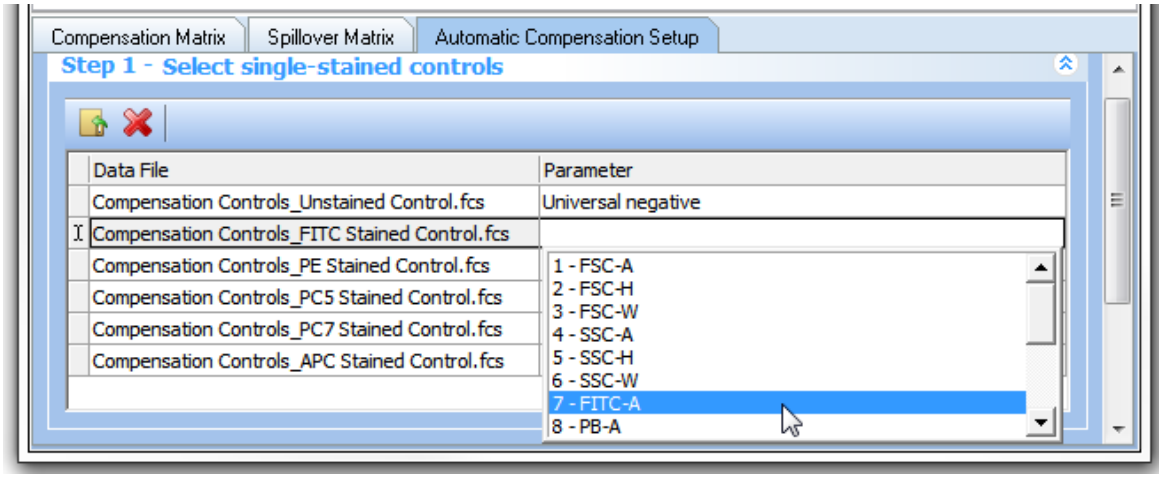

Figure T8.11 - Selecting the FITC-A parameter to match the FITC Stained Control.fcs file.

13. 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.

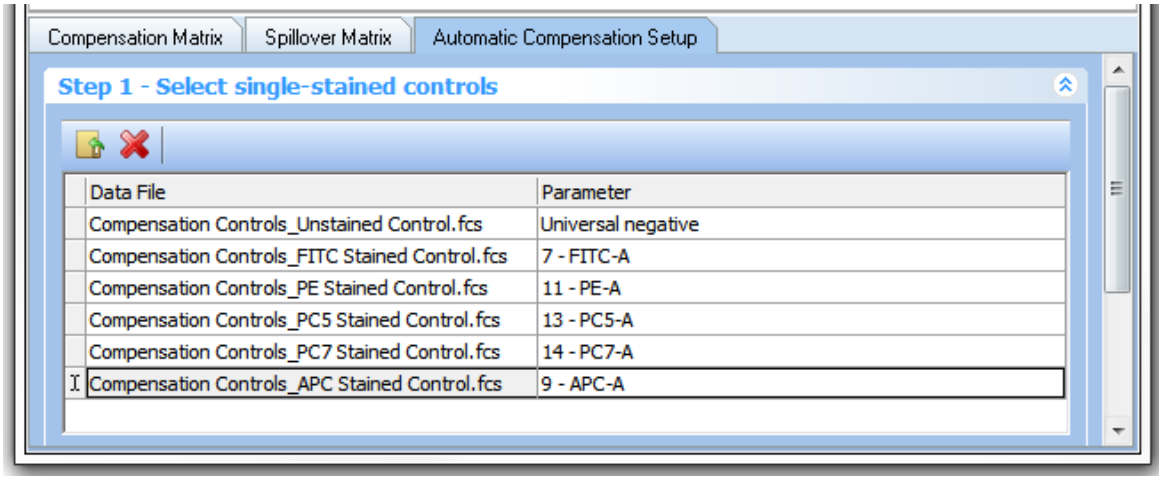

Figure T8.12 - All Parameters have been matched to their respective single stain control.fcs file.

14. Click on **Step 2 - Build histograms** to expand the build histograms window (T8.13).

15. Click on the **Build Histograms** button (T8.13).

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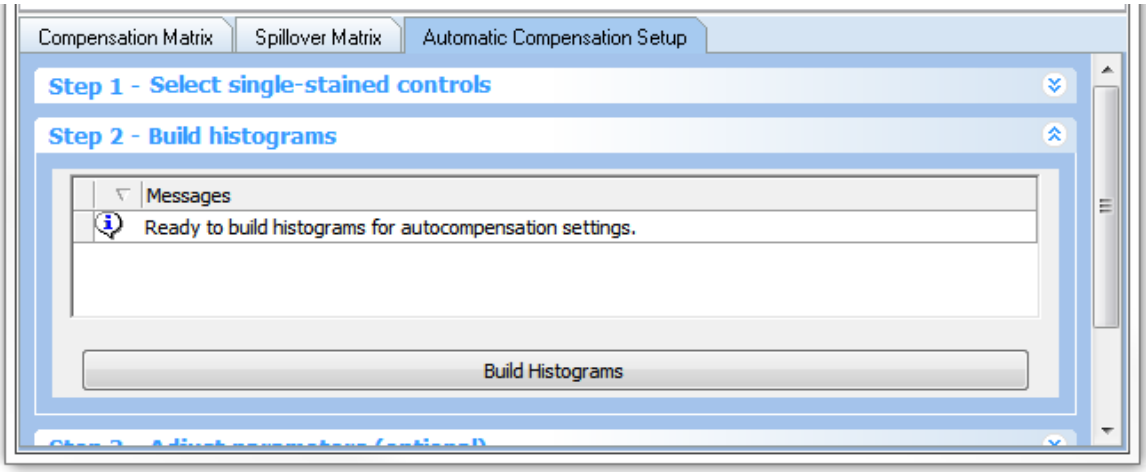

Figure T8.13 - The Build Histograms button will create a new page for scatter parameters and a new page containing a postive control and negative control histogram for each individual stain.

Six new pages will be created; **Scatter Plot, FITC-A control, PE-A control, PC5-A control, PC7-A control,** and the **APC-A control** page (Figure T8.14). 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.

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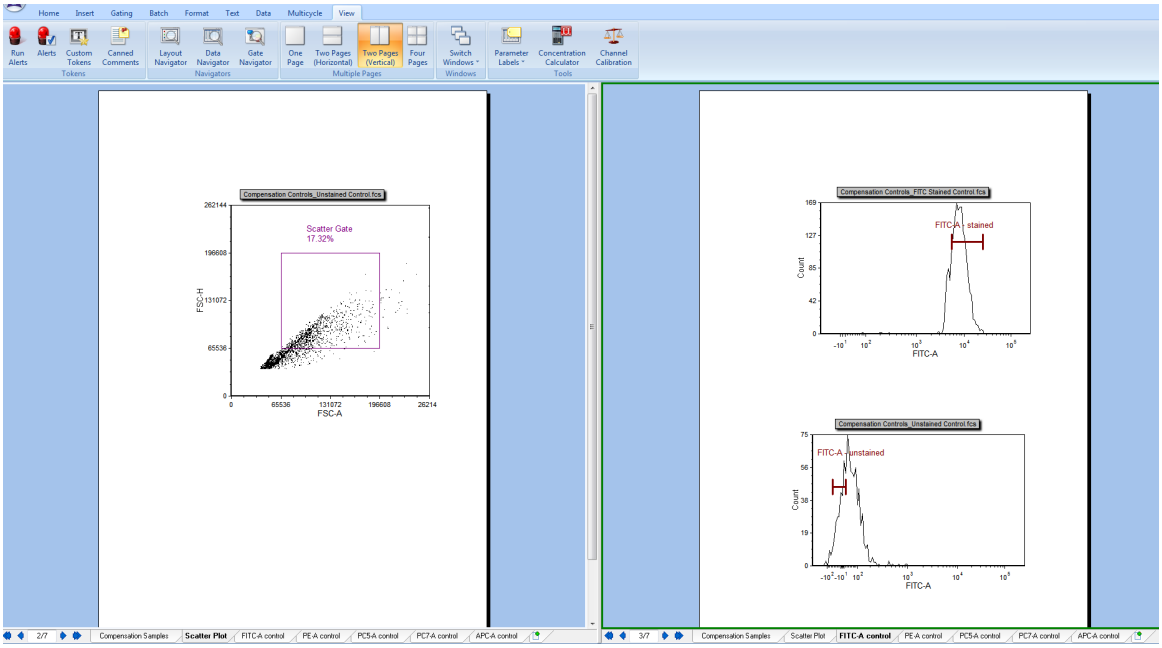

Figure T8.14 - Clicking on the build histograms button results in a Scatter Plot page and a new page with every single stained control.

In this instance FSC-H vs FSC-A are not the correct parameters for defining scatter so we will replace and move the scatter gate. First we will move to a two page view to make navigating the data easier.

16. Click on the **View** tab→**Multiple Page→Two Pages (Vertical)**.
17. Select the **Scatter Plot** page for the left page.
18. Select the **FITC-A control** page for the right page.
19. Change the X-Axis parameter on the Unstained Control dot plot (left page) to **SSC-H** (Figure T8.15)

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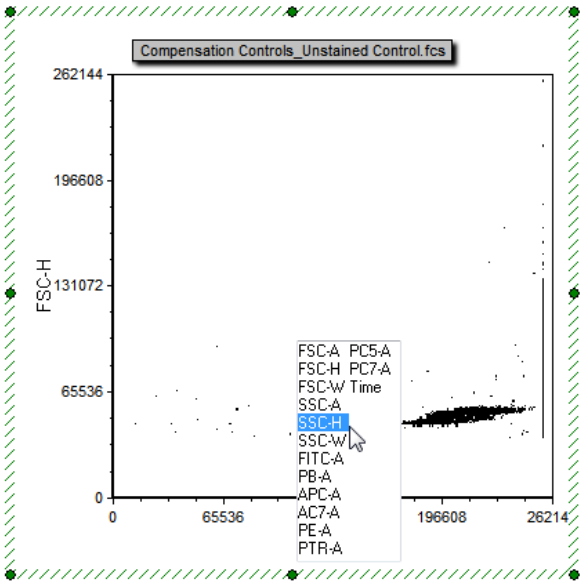

Figure T8.15 - Changing the X-Axis parameter to SSC-H.

20. Choose the **Gating** tab→**Create Gates**→**Rectangle**.

21. Draw a gate around the beads population like the one in Figure T8.16 below.

22. Chose the **Replace an existing gate** radio button in the Create New Gate dialog (Figure T8.16).

23. Choose **Scatter Gate** from the bottom of the drop down list (Figure T8.16).

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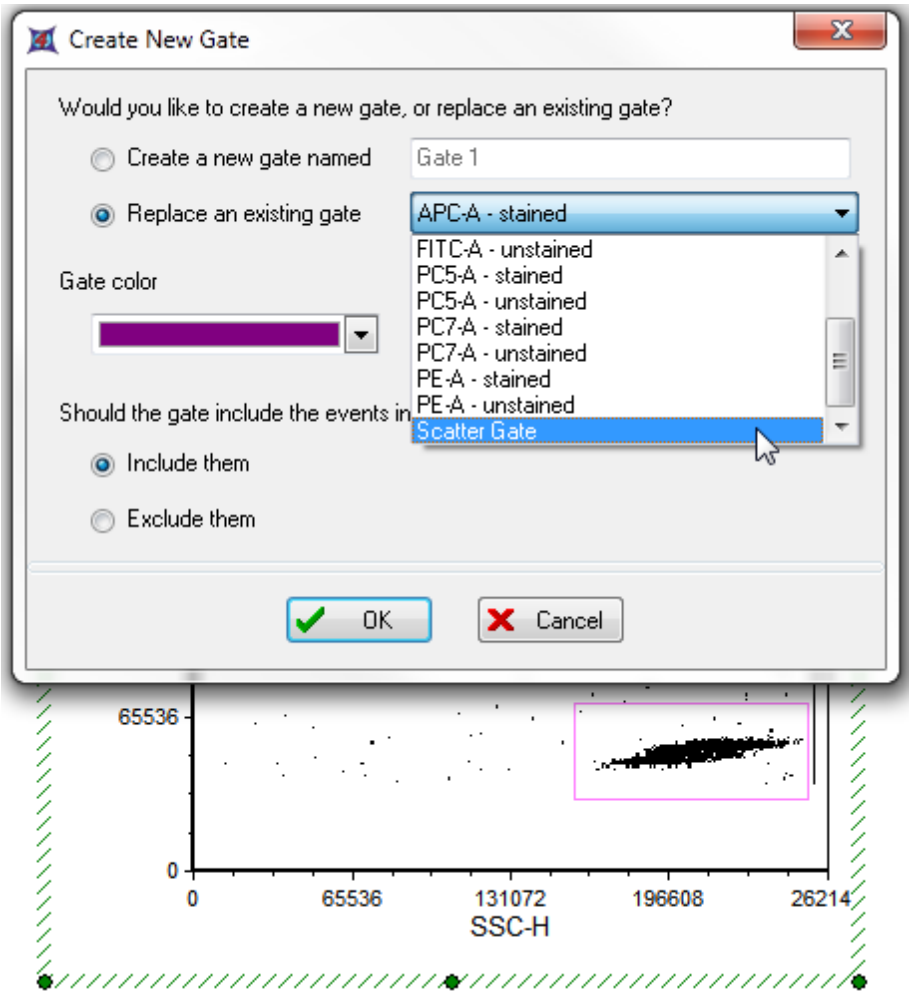

Figure T8.16 - Creating a gate around the bead population on the plot and choosing to replace the Scatter Gate with the newly created gate.

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. 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 754.

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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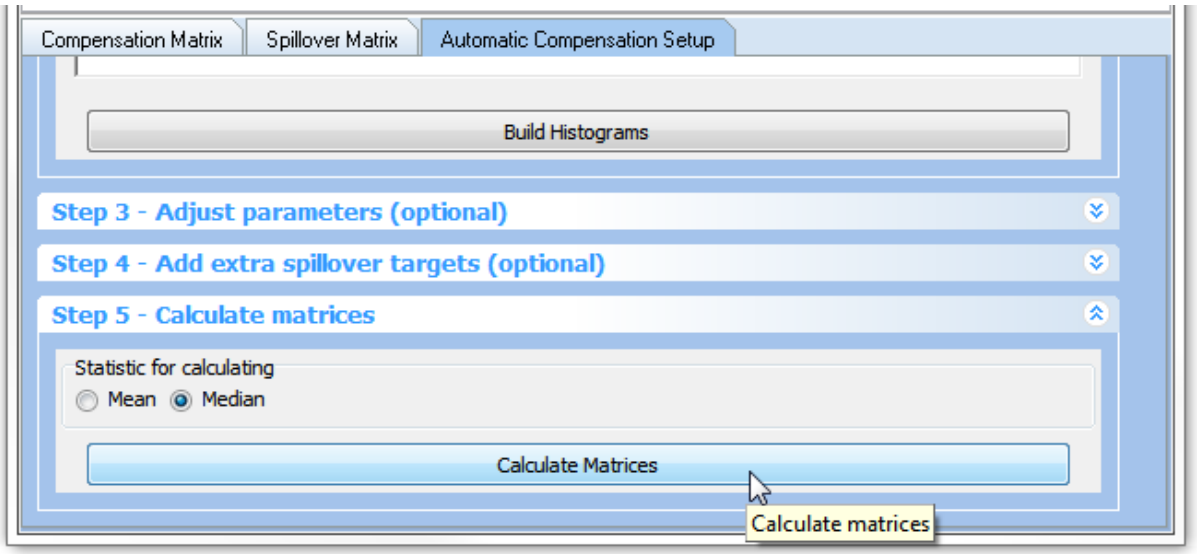

Figure T8.17 - Click the Calculate Matrices button to complete your automatic compensation setup.

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 20-24 of this tutorial.)

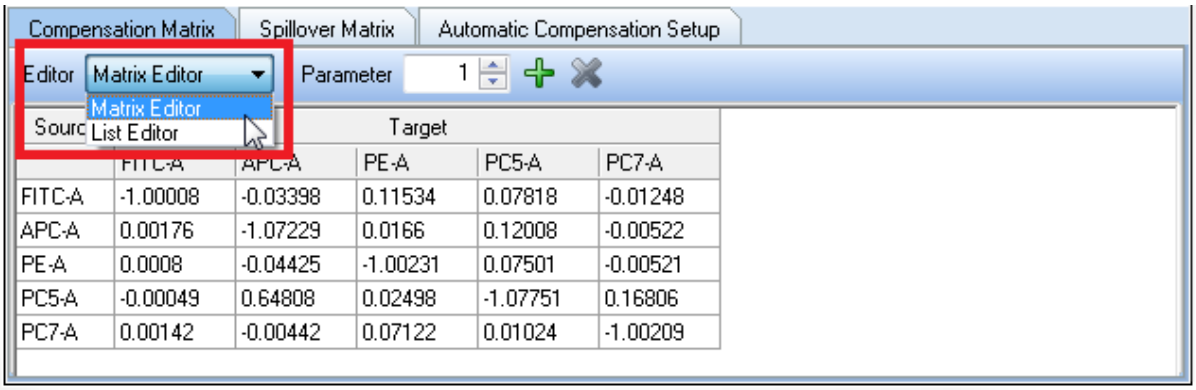

Figure T8.18 - The Compensation Matrix create from the Automatic Compensation Setup. The red box indicates you can change from Matrix Editor to List Editor view. The Spillover Matrix tab will display the spillover matrix for your compensation definition.

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.

29. Click on the **New Compensation** compensation definition in the **Compensations** navigator to select it (Figure T8.19).

30. While click and holding on the **New Compensation** compensation definition drag it on top of the Compensation Controls_All Colors.fcs plot in the upper right of the layout (Drag and drop the **New Compensation** definition onto the plot as indicated by the red arrow)(Figure T8.19).

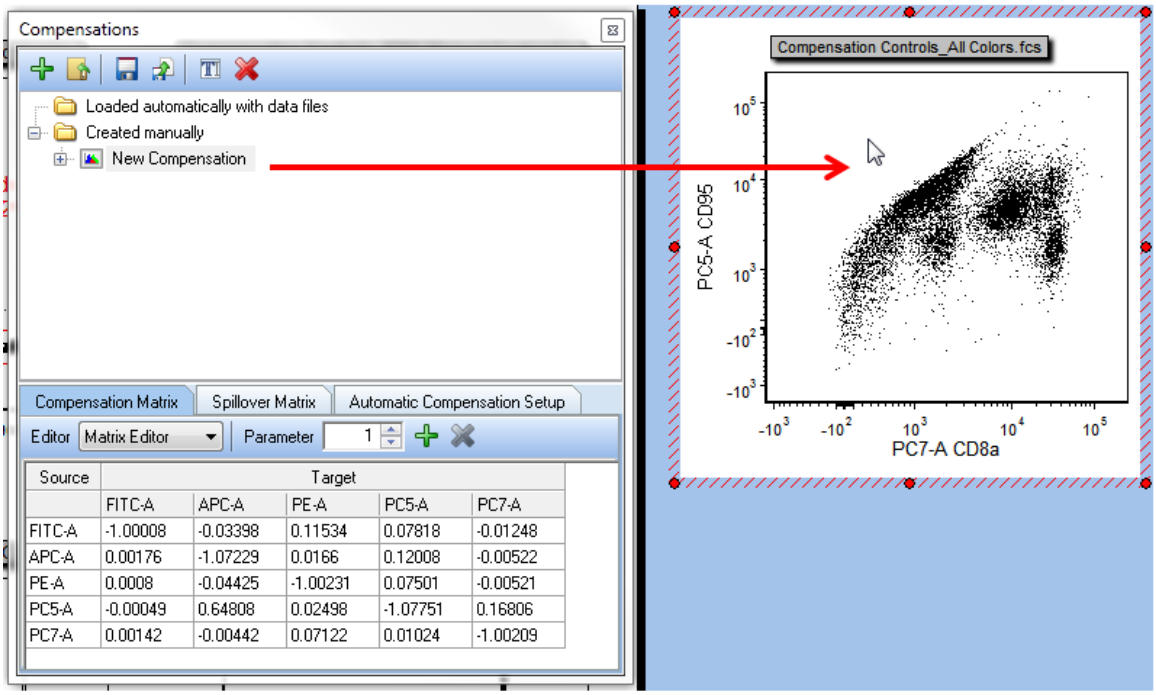

Figure T8.19 - Dragging the New Compensation definition from the Compensations navigator and dropping it on a plot will apply that compensation definition to the plot.

The plot will update and show the data properly compensated as in figure T8.20 below.

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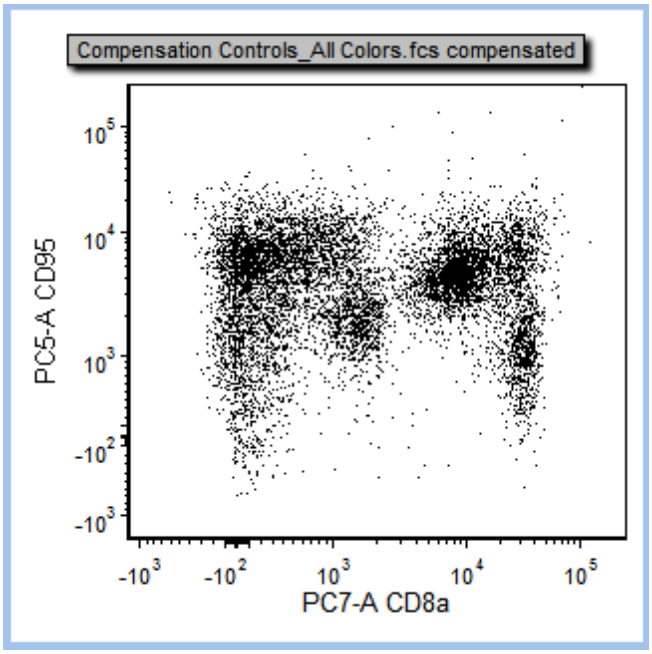

Figure T8.20 - The All Colors control plot with the compensation defined in the Automatic Compensation Setup feature applied.

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).

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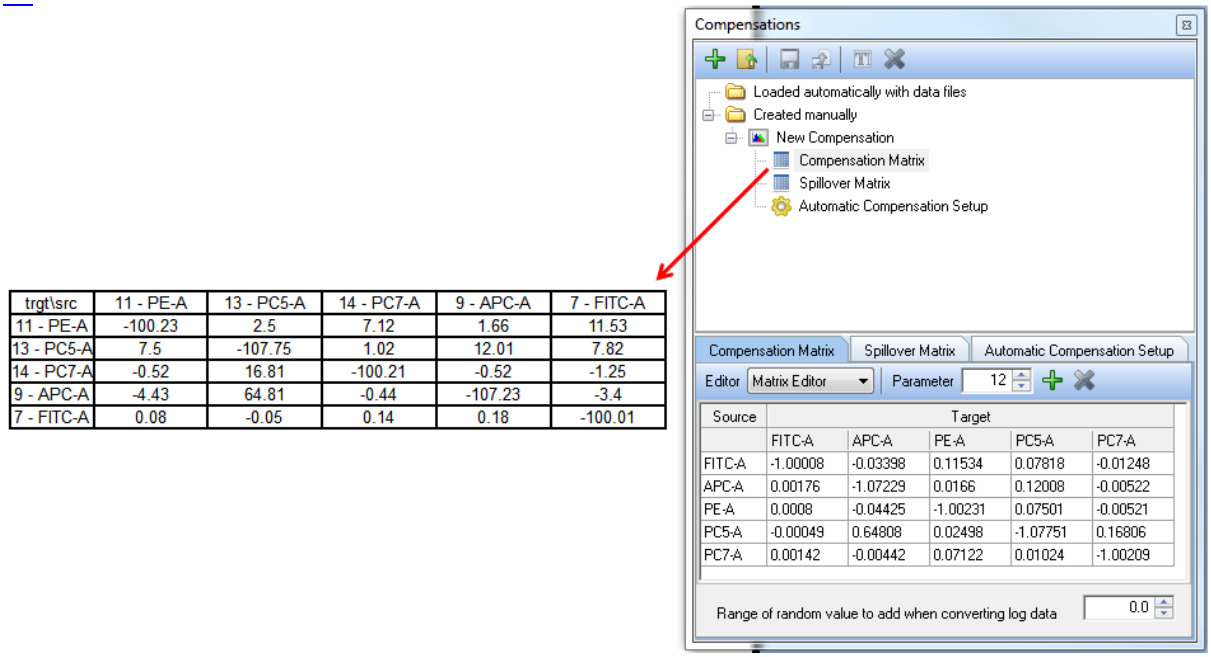

trgt\src	11 - PE-A	13 - PC5-A	14 - PC7-A	9 - APC-A	7 - FITC-A
11 - PE-A	-100.23	2.5	7.12	1.66	11.53
13 - PC5-A	7.5	-107.75	1.02	12.01	7.82
14 - PC7-A	-0.52	16.81	-100.21	-0.52	-1.25
9 - APC-A	-4.43	64.81	-0.44	-107.23	-3.4
7 - FITC-A	0.08	-0.05	0.14	0.18	-100.01

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.

The compensation matrix will now be displayed in a text box (FigureT8.21). Steps 31 and 32 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)** 300 and **Step 4 - Add extra spillover targets (optional)** 302 may also be used to refine your single stain populations (**Adjust parameters** 300) or to have other targets defined without defining a source (**Add extra spillover targets** 302). If you would like to learn more about these advanced features please see the **Automatic Compensation- Optional Steps 3 and 4** 755 tutorial.

Please see the Manual Compensation 737 tutorial or the Compensation 288 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* 765 *tutorial.*

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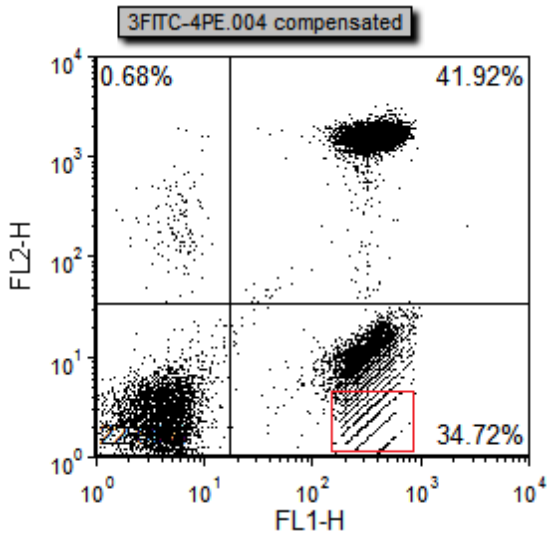

Figure T8.22 - Digitization errors after compensation can be seen in the red box. Please refer to the Compensation Digitization Errors tutorial to correct this type of error.

34.8.2.1 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 Step 3 and Step 4. If you are not yet familiar with automatic compensation in FCS Express please complete the **Automatic Compensation** 741 tutorial before starting this section.

- **Step 3 - Adjust parameters (optional)** 300 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** 300 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 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

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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 targets defined without defining a source.
  - **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 here. The layout ontains 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.

- **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.

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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 **Application Button→Open Layout**.
2. Open the layout **AutoCompAdditionalSteps.fey** found in the Tutorial Sample Data archive 590. (see above for a description of the layout 756).
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 **Step 3- Adjust parameters (optional)** (figure T8.22).

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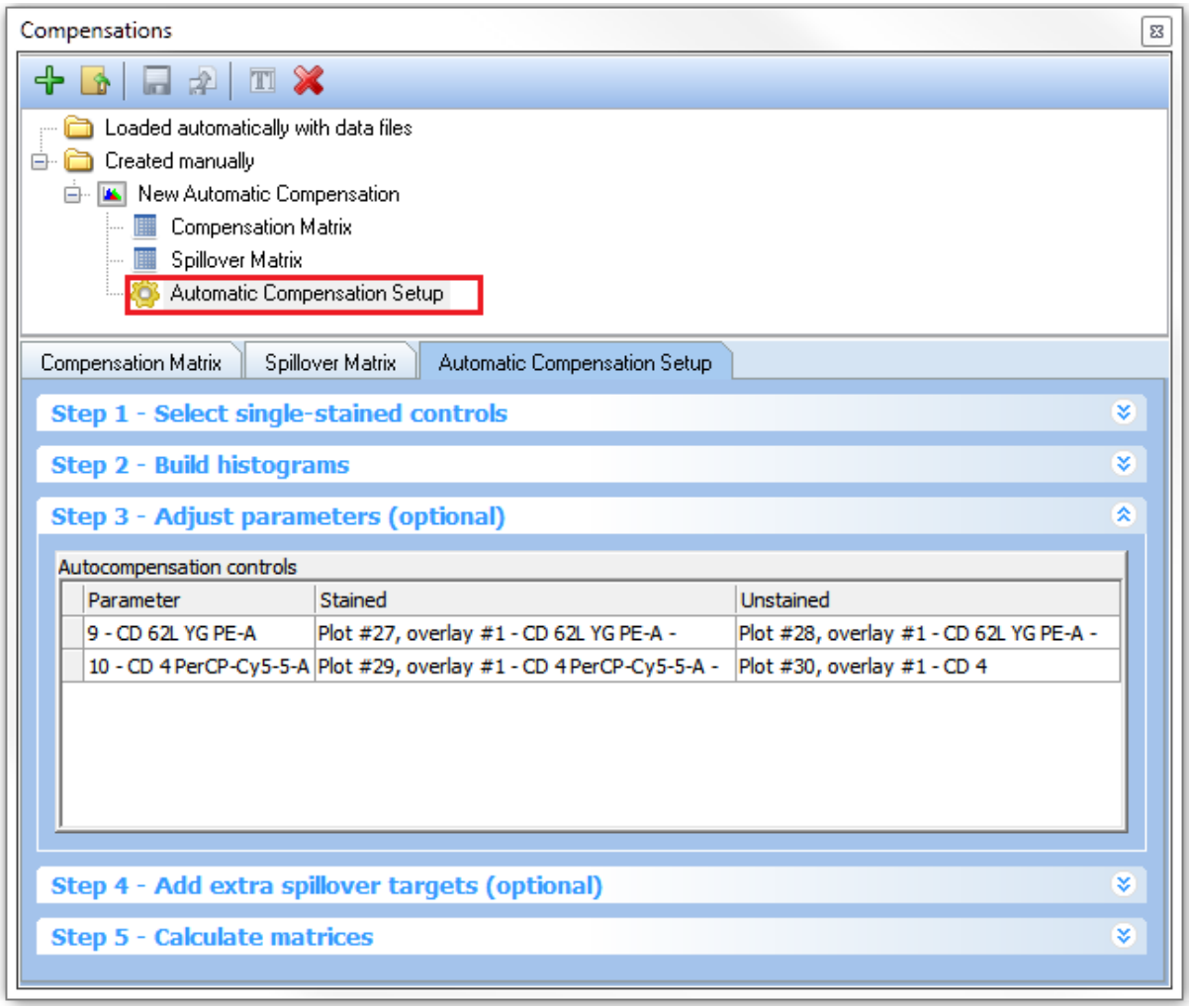

Figure T8.22 - Selecting the Automatic Compensation setup and Step 3 for the New Automatic Compensation definition.

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.

5. Click and hold on the **Negative Control** gate in the gate view.

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6. Drag the **Negative Control** gate onto the **Unstained** column in the **CD62L YG PE-A** row and drop it here.
7. Click and hold on the **Negative Control gate** in the gate view (Figure T8.23).
8. Drag the **Negative Control gate** onto the **Unstained** column in the **CD4 PerCP-Cy5-5-A** row and drop it here(Figure T8.23).

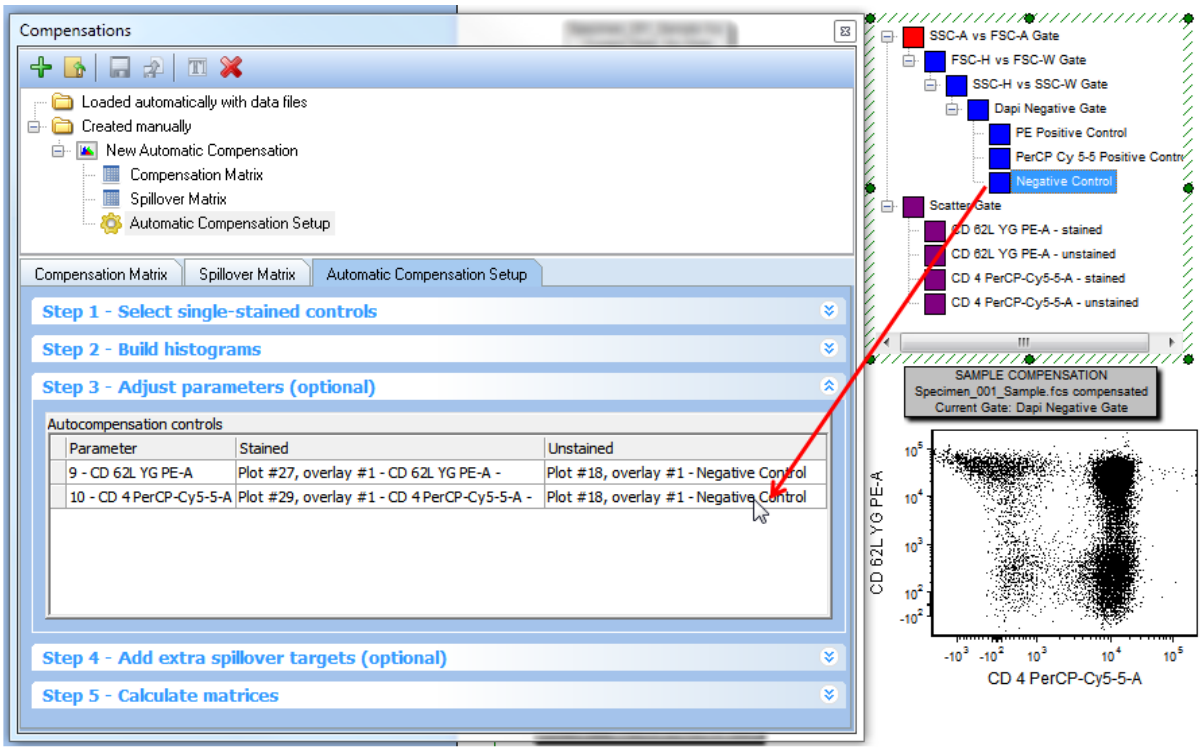

Figure T8.23 - Dragging the Negative Control gate from the gate view to the Unstained column for both parameters.

9. Click and hold on the **PE Positive Control** gate in the **Data for New Compensation Gates plot** (off the right hand side of page).
10. Drag the **PE Positive Control** gate onto the **Stained** column in the **CD62L YG PE-A** row and drop it here.
11. 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).
12. Drag the **PerCPCY5-5** gate onto the **Stained** column in the **CD4 PerCP-Cy5-5-A** row and drop it here(Figure T8.24).

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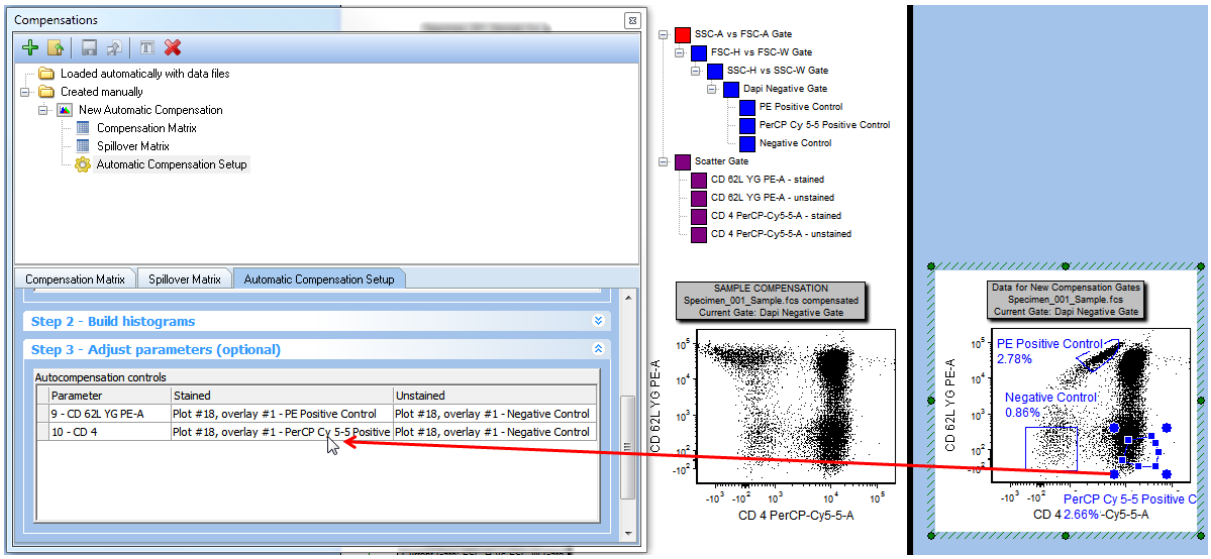

Figure T8.24 - Dragging and dropping gates from a plot to the Stained column in step 3 for each parameter.

Step 3 - Adjust parameters (optional) will look like figure T8.25 below when you are done.

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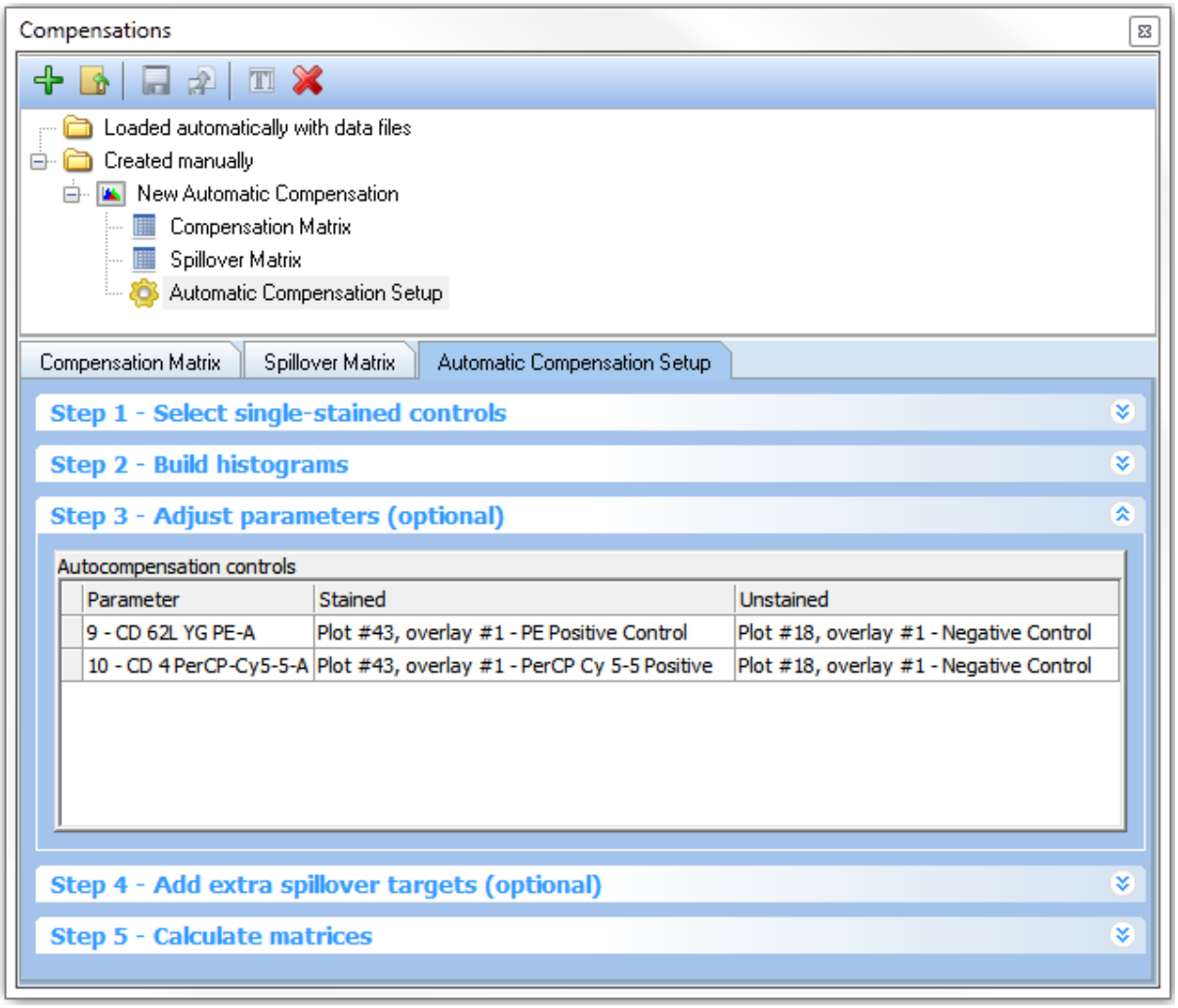

Figure T8.25 - Step 3 - Adjust parameters (optional) after dragging and dropping gates from the gate view and plots onto the stained and unstained population columns.

13. Click on **Step 5 - Calculate matrices**.
14. Click the **Calculate Matrices** button.

Notice that the compensation 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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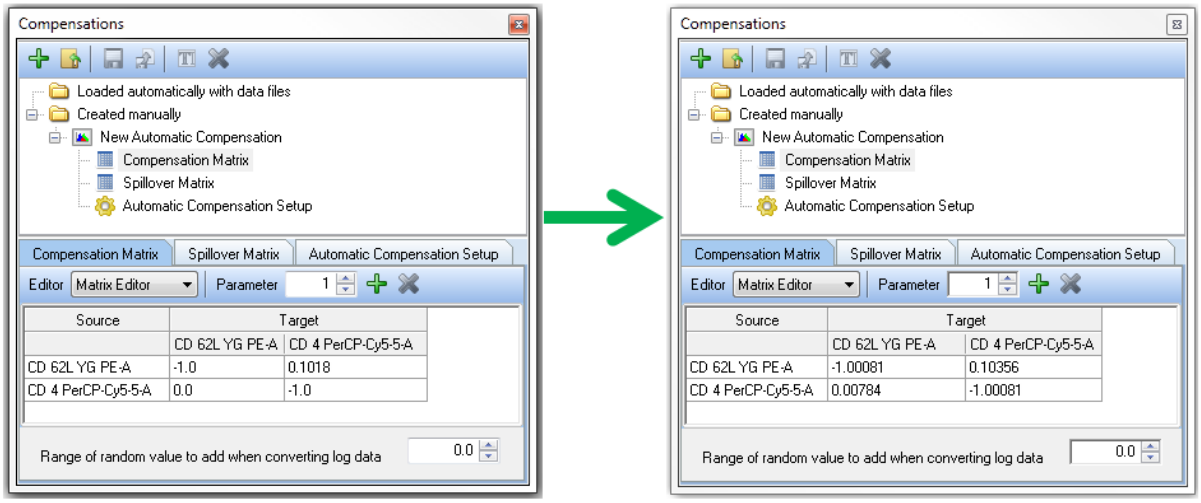

Figure T8.26 - The original Automatic Compensation Setup definition (left) compared to the Automatic Compensation Setup definition (right) that is based on a gating hierarchy and manually defined positive and negative controls.

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.

15. Select **Application Button→Close Layout**. (Do not save any changes)
16. Select **Application Button→Open Layout**.
17. Open the layout **AutoCompAdditionalSteps.fey** found in the Tutorial Sample Data archive 590.
18. Click on the **Created manually** folder→**New Automatic Compensation→Automatic Compensation Setup** in the **Compensations** navigator (figure T8.22 red box).
19. Click and hold on the **Scatter Gate** in the Gate View.
20. Drag the **Scatter Gate** on top of the **Dapi Negative Gate** and drop it here (Figure T8.27).

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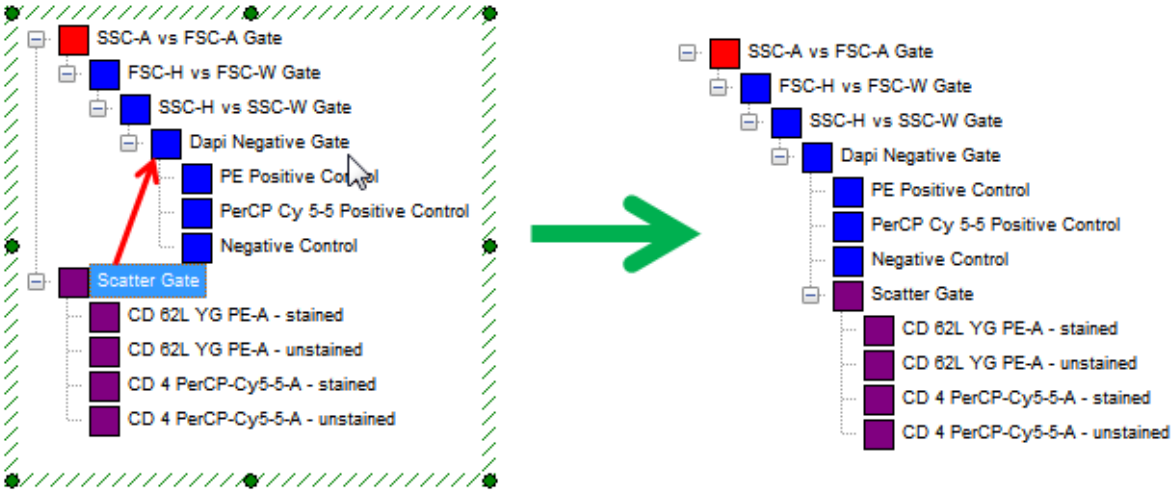

Figure T8.27 - Dragging and dropping the Scatter Gate onto the Dapi Negative Gate to create a new gating hierarchy

21. Click on **Step 5 - Calculate matrices**.
22. Click the **Calculate Matrices** button.

Notice that the compensation 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.

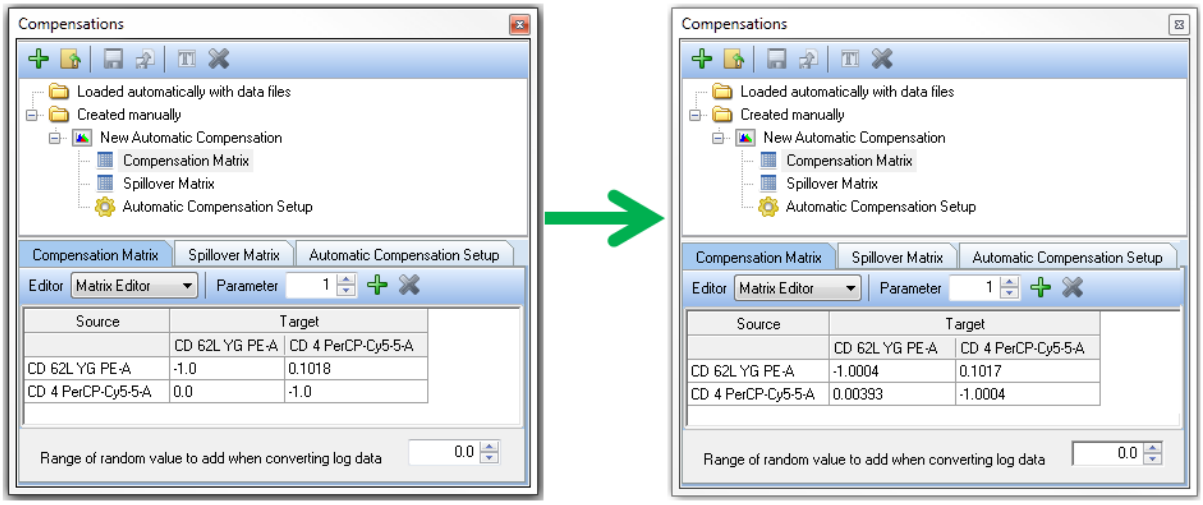

Figure T8.28 - The original Automatic Compensation Setup definition (left) compared to the Automatic Compensation Setup definition (right) that is based on the new gating hierarchy of the Scatter Gate.

We will now use **Step 4 - Add extra spillover targets (optional)** to determine the theoretical spillover for the FITC parameter.

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23. Click on **Step 4 - Add extra spillover targets (optional)** to expand the window.

24. Check the **CD 44 FITC-A** check box (Figure T8.28).

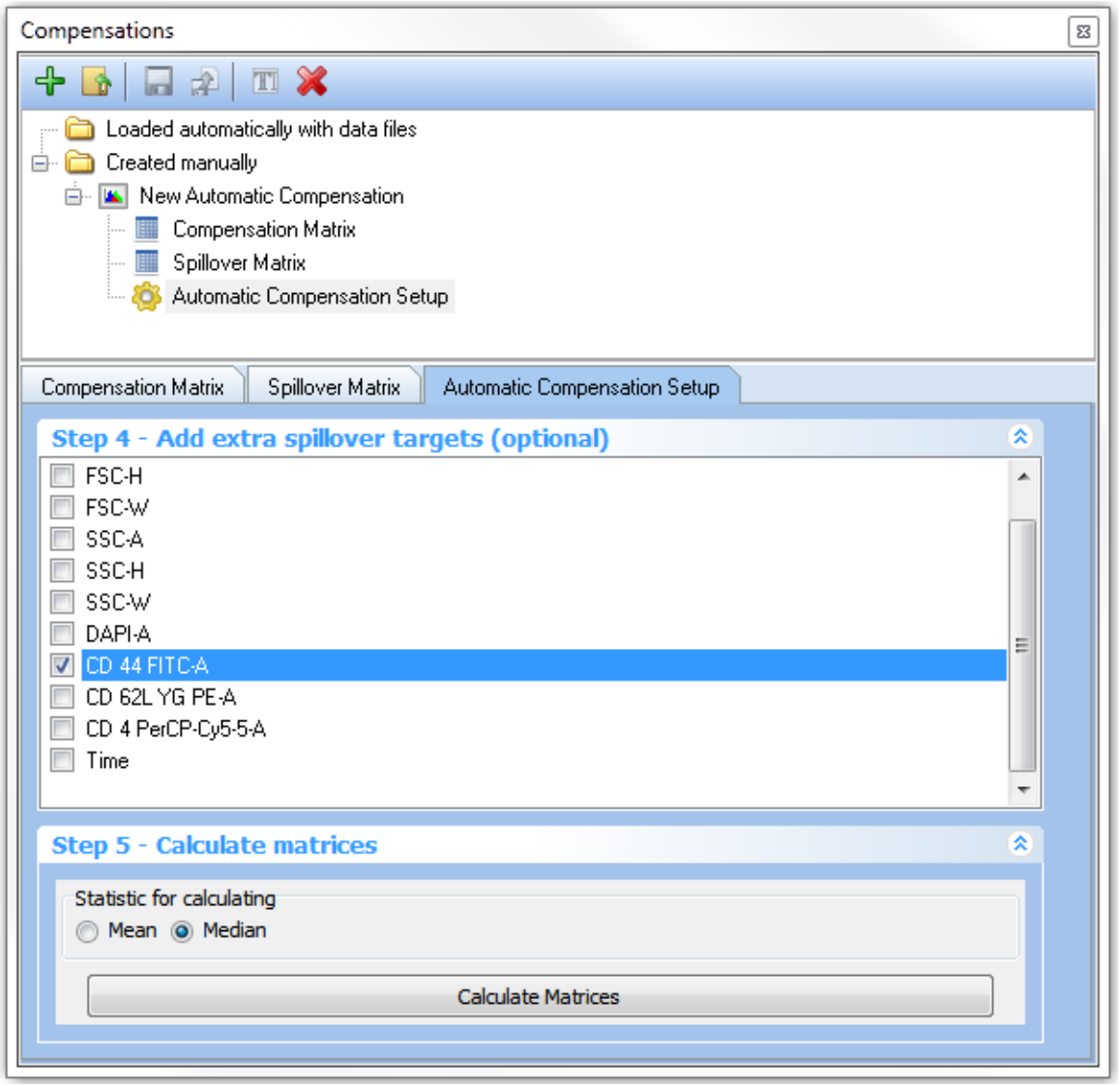

Figure T8.28 - Adding an extra spillover target in Step 4.

25. Click the **Calculate Matrices** button in **Step 5 - Calculate matrices**.

The **Compensation Matrix** tab will now display all of your original parameters along with the **CD 44 FITC-A** parameter that you selected in **Step 4- Add extra spillover targets (optional)** (Figure T8.29). The column for **CD 44 FITC-A** in the matrix is displaying the theoretical compensation needed against all other targets.

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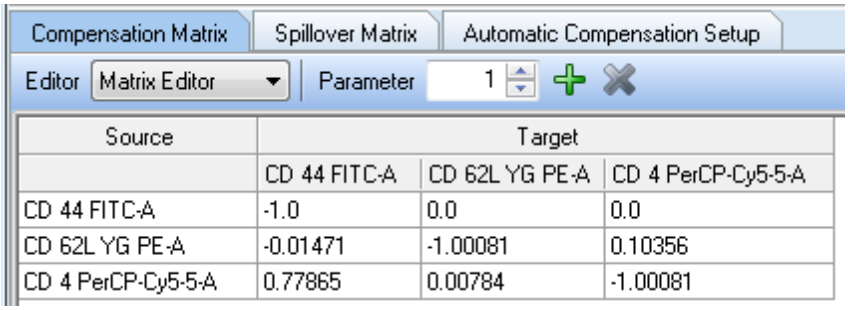

Source	Target		
	CD 44 FITC-A	CD 62L YG PE-A	CD 4 PerCP-Cy5-5-A
CD 44 FITC-A	-1.0	0.0	0.0
CD 62L YG PE-A	-0.01471	-1.00081	0.10356
CD 4 PerCP-Cy5-5-A	0.77865	0.00784	-1.00081

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 737 tutorial or the Compensation 288 section of the manual for more details on compensation in FCS Express.

34.8.3 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 293 topic. Please follow the steps below to learn how to correct for digitization errors.

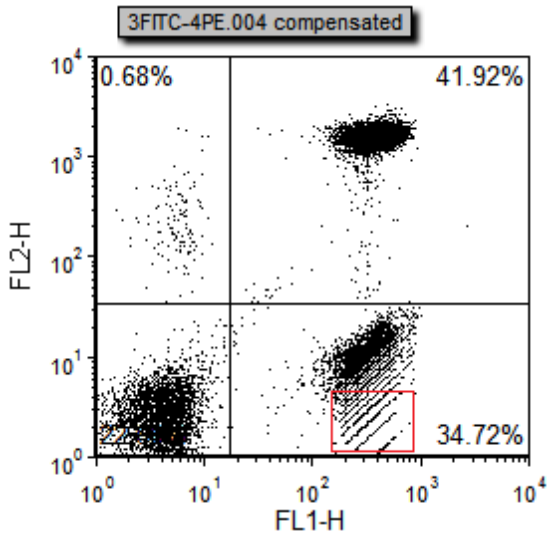

Figure T8.30 - Digitization errors can be seen highlighted in the red box.

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1. Select **Application Button→Open Layout**.
2. Open the layout **ManualComp.fey** found in the Tutorial Sample Data archive 590.
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 **Data→Data Info→Compensations** command
5. Select the **Create Manually** folder→**DigitizationError** compensation definition (figure T8.31)

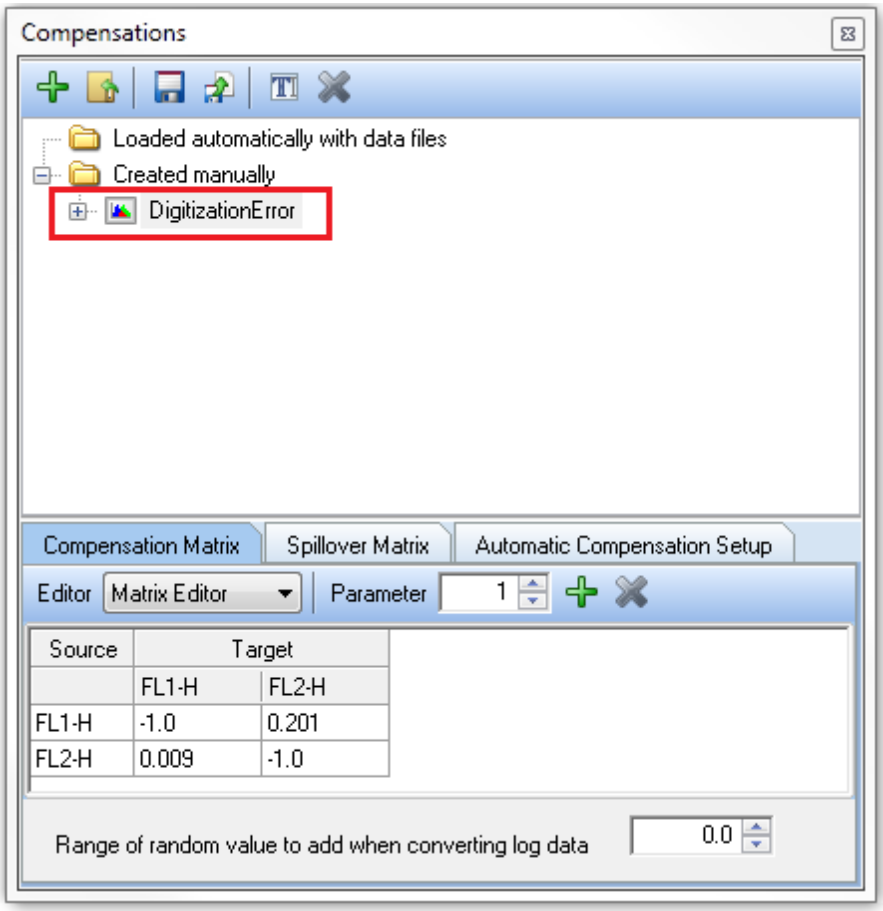

Figure T8.31 - Selecting the DigitizationError compensation definition for editing.

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

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random value entered above was added to visually correct the data displayed in the 2D plot.

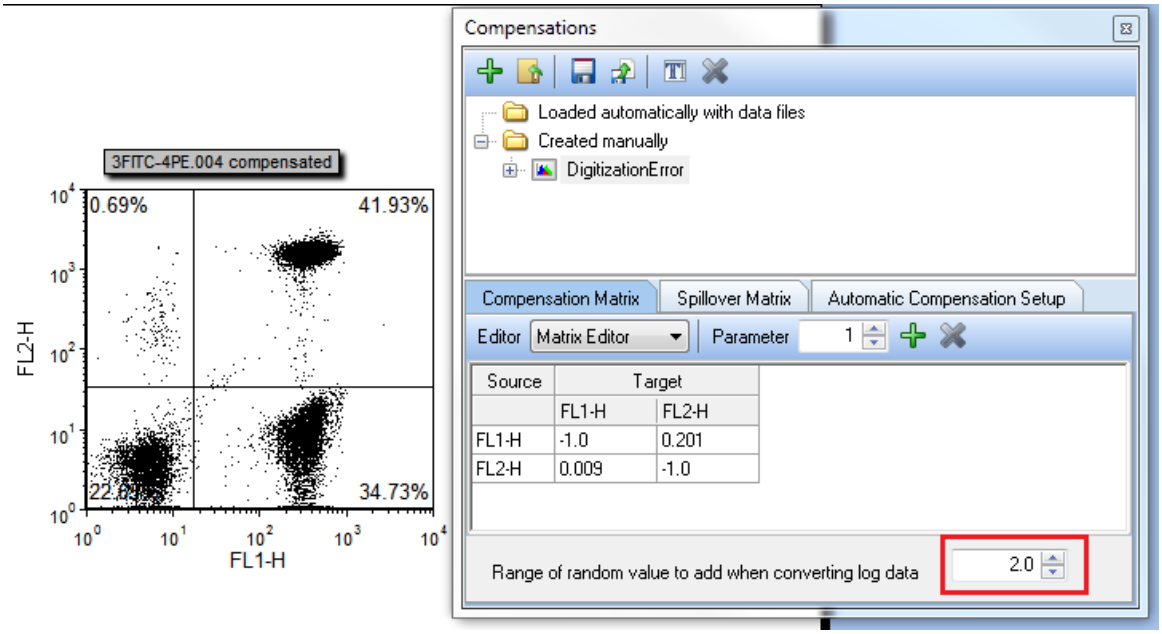

Figure T8.32 - Changing the Range of random value to add when converting log data to 2.0.

34.9 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 Server808.

In the first example, we will export plots to a PowerPoint file767.

34.9.1 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.

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To Run a Batch Processing Action:

1. Select **Application Button→Open Layout**.
2. Open the layout **BatchProcess.fey** found in the Tutorial Sample Data archive 590.

This layout contains two 2D density plots and a histogram of the file **ApoMono.001** (Figure T9.1). The left plot is of FSC vs. SSC with a gate on **Whole Cells**. The right plot is of FL1 vs. FL2 gated on **Whole Cells** with quadrants. The histogram of FL2 is also gated on **Whole Cells**.

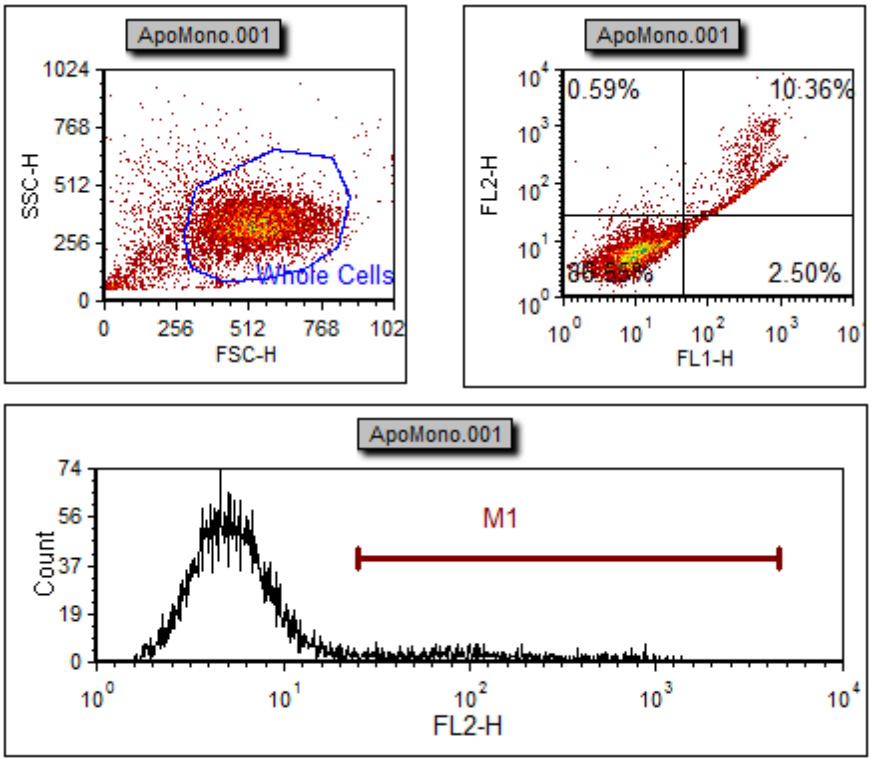

Figure T9.1 Batch Layout (BatchProcess.fey)

In order to run a batch process, you need to put files in the **Data List**.

1. Select the **Batch→Organize Data Sets→Data List** command (Figure T9.2).

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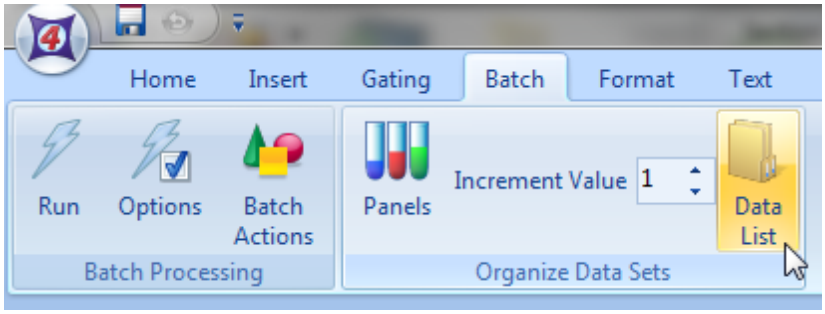

Figure T9.2 Selecting the Data List Command

A **Data List** window will appear (Figure T9.3).

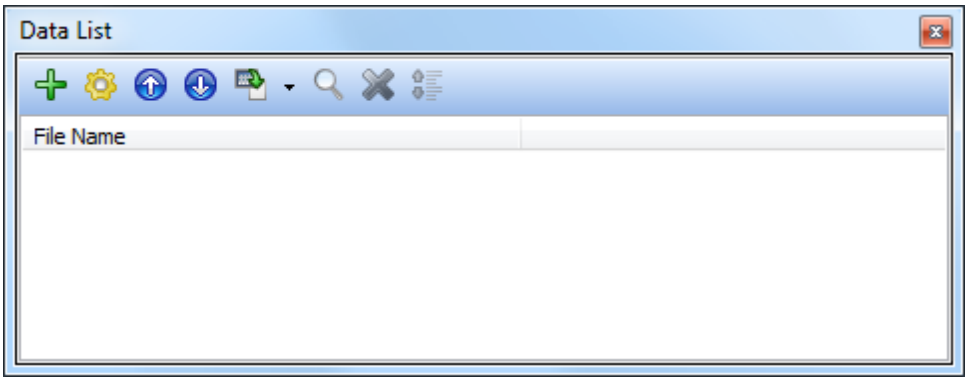

Figure T9.3 Data List Window

4. Click the green plus to add files .

A **Select a Data File** dialog will appear (Figure T9.4).

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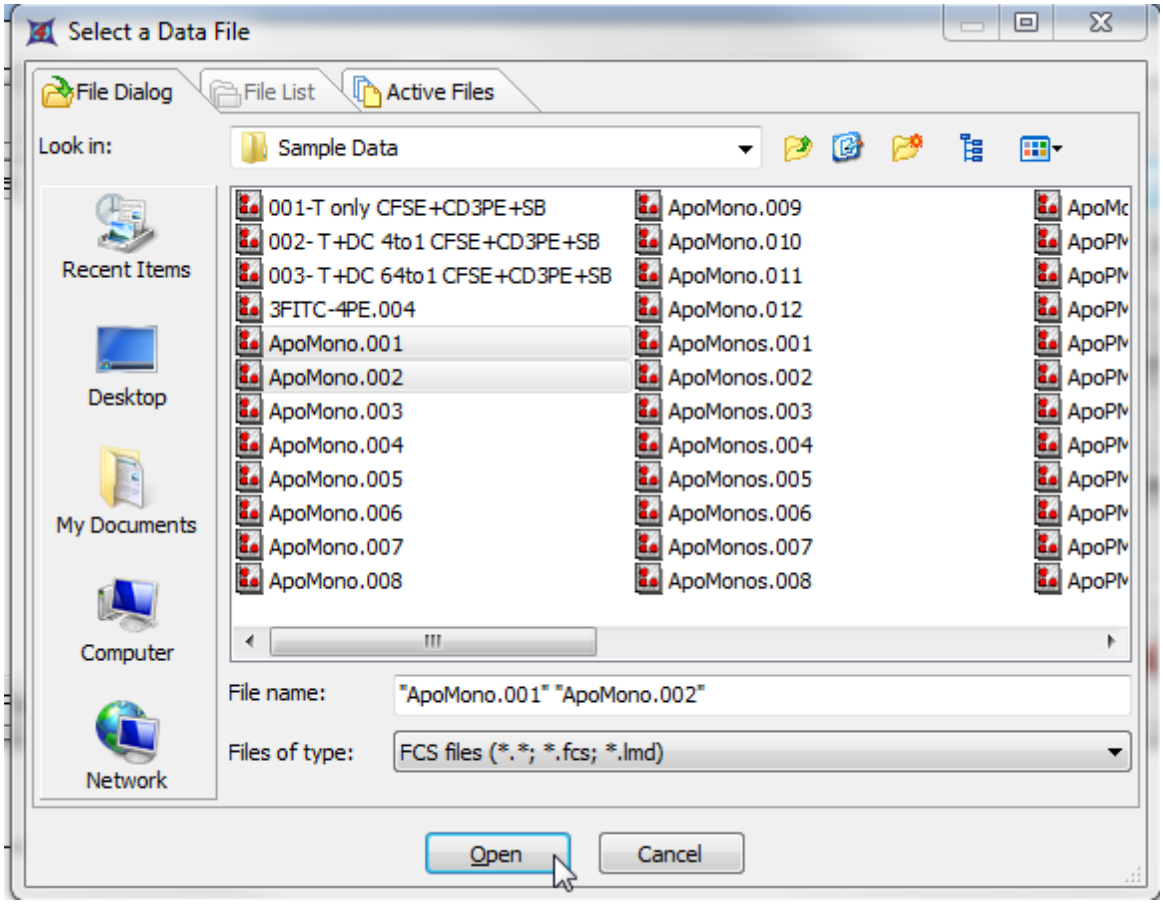

Figure T9.4 Select a Data File Dialog

5. Select the two files **ApoMono.001** and **ApoMono.002**.
6. Click **Open**.

The **Data List** window will show the two files (Figure T9.5).

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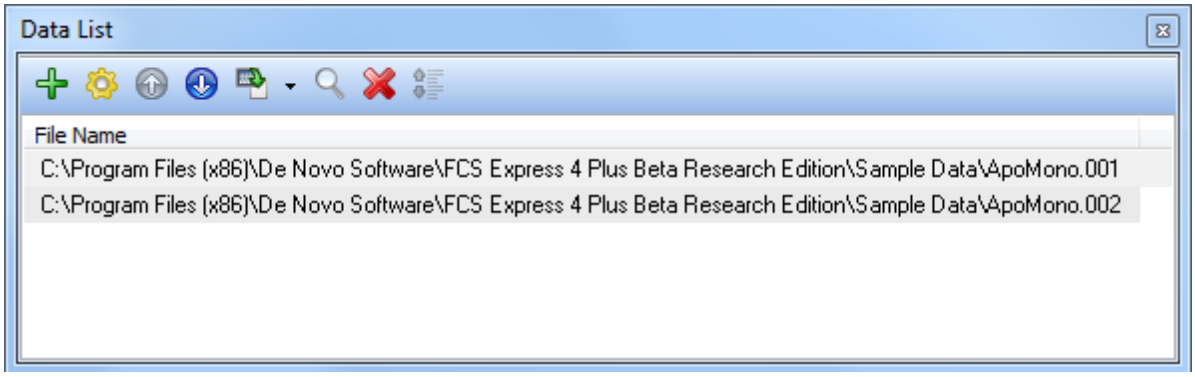

Figure T9.5 Data List with Two Files

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: ApoMono.001 and ApoMono.002. 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).

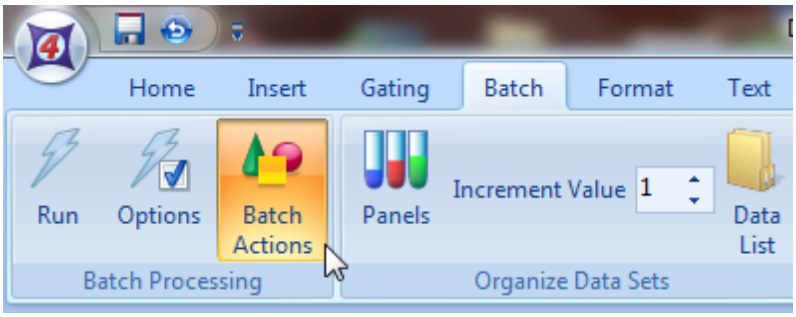

Figure T9.6 Selecting the Batch Actions Command

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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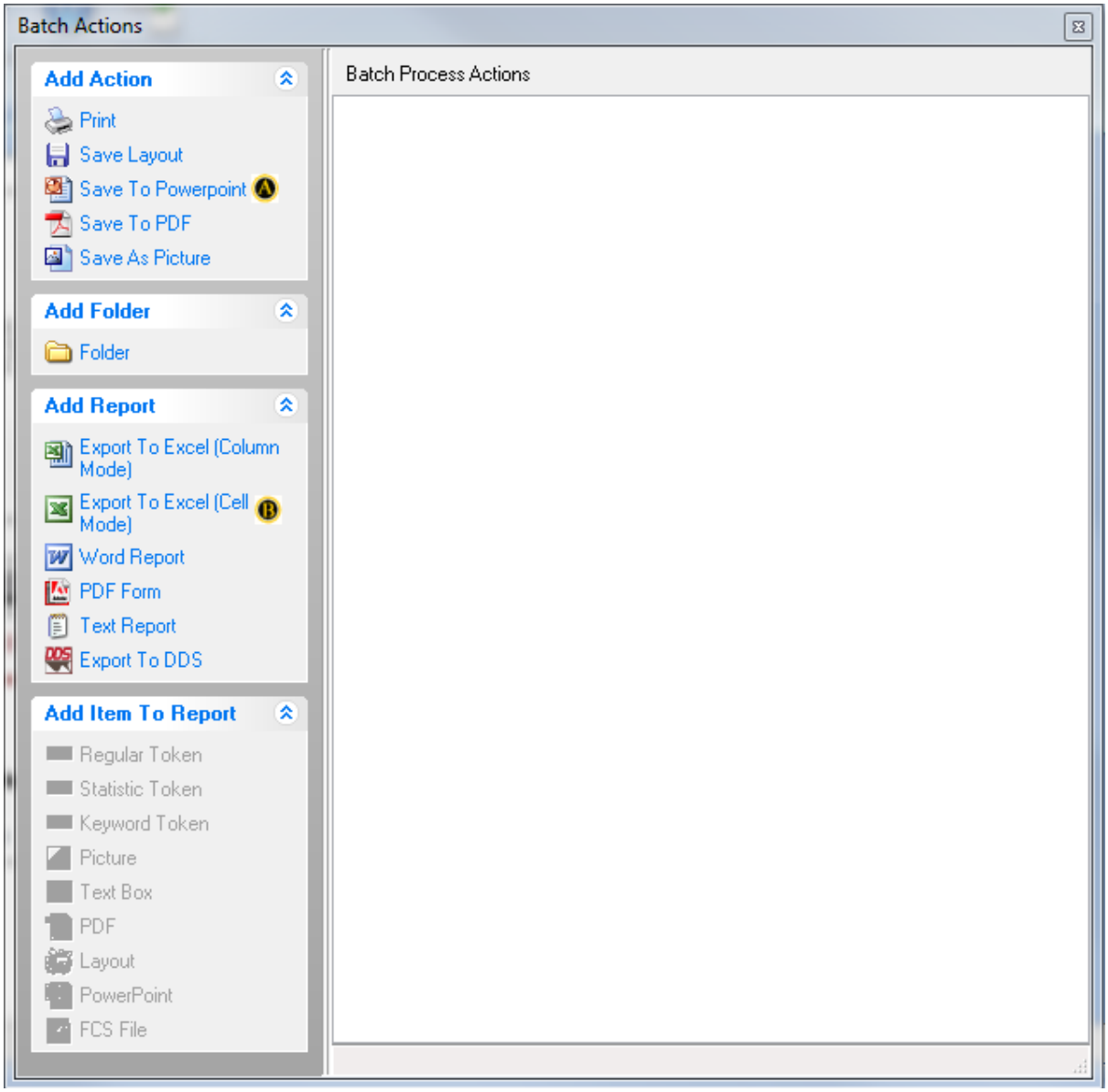

Figure T9.7 Batch Actions Window

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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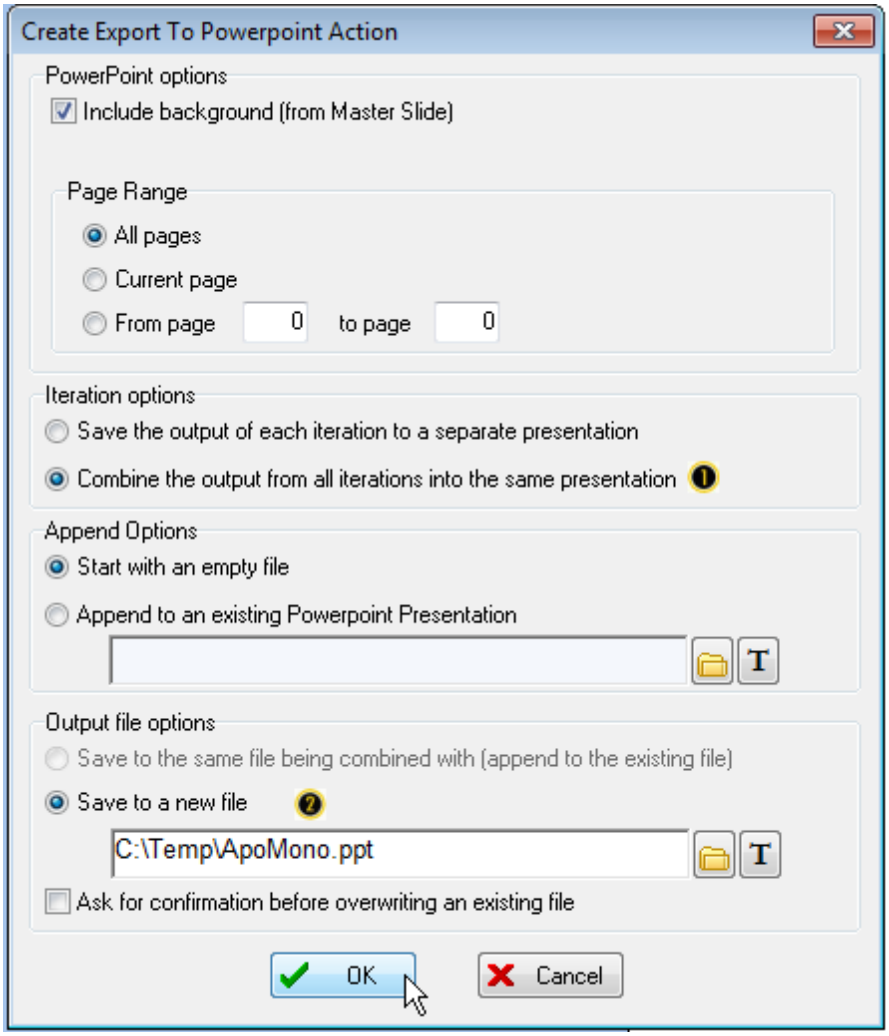

Figure T9.8 Create Export to PowerPoint Action Dialog

9. Select **Combine the output from all iterations into the same presentation** from the **Iteration options** section ❶.
10. Select **Save to a new file** from the **Output file options** section ❷.
11. Type **C:\Temp\ApoMono.ppt** as the **Save to a new file** location of your PowerPoint presentation. (If you do not have a C:\Temp directory, please choose any directory).
12. Click **OK**.

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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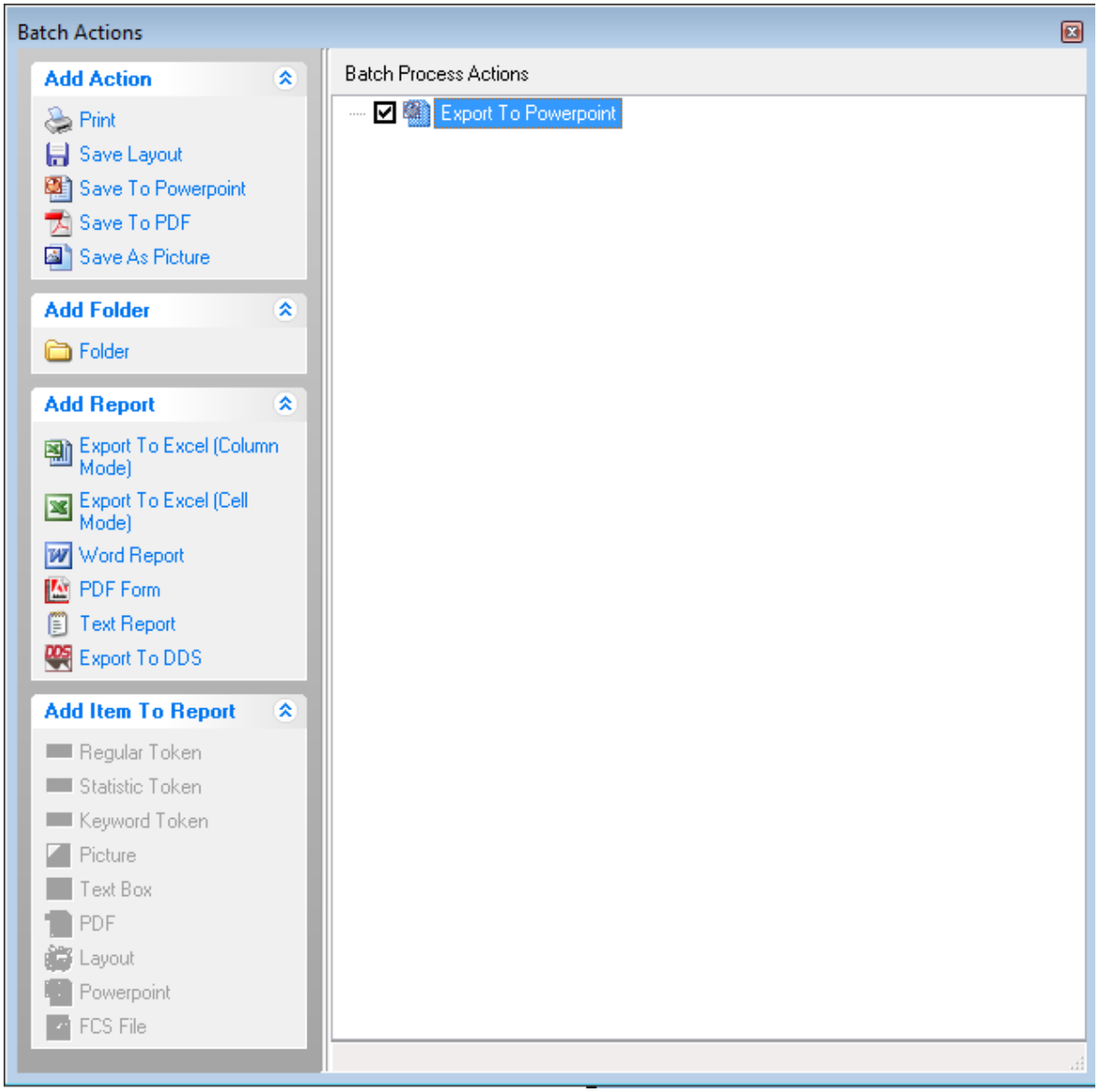

Figure T9.9 Batch Actions Window Updated with Export to PowerPoint Action

13. Select the **Batch→Batch Processing→Run** command (Figure T9.10).

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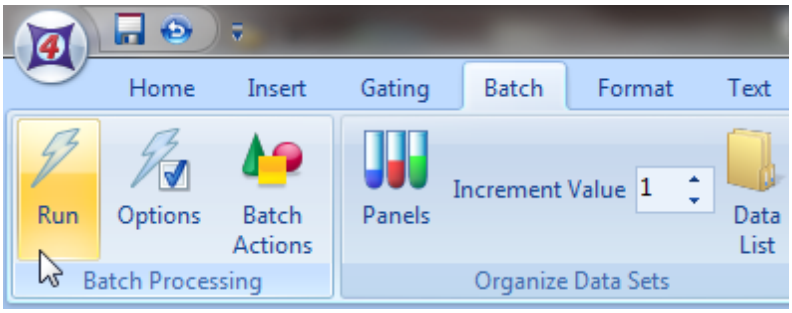

Figure T9.10 Selecting the Run Batch Process Command

FCS Express will now run the batch process. After a series of automated steps, PowerPoint will launch and open the file **ApoMono.ppt**. The file will consist of two pages: the first will be a slide showing the layout with the data file **ApoMono.001** (Figure T9.11) and the second of **ApoMono.002** (Figure T9.12).

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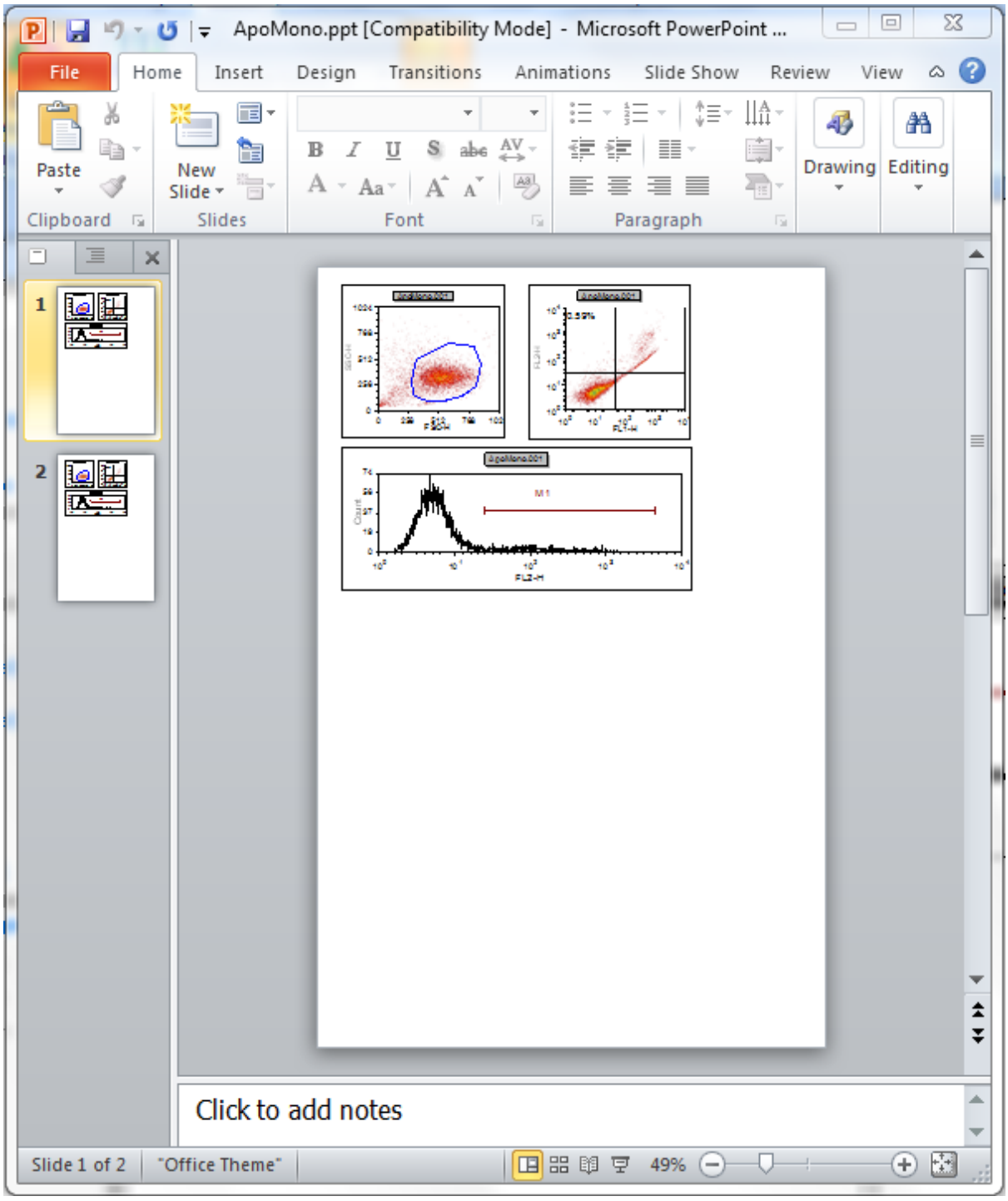

Figure T9.11 Slide 1 in PowerPoint

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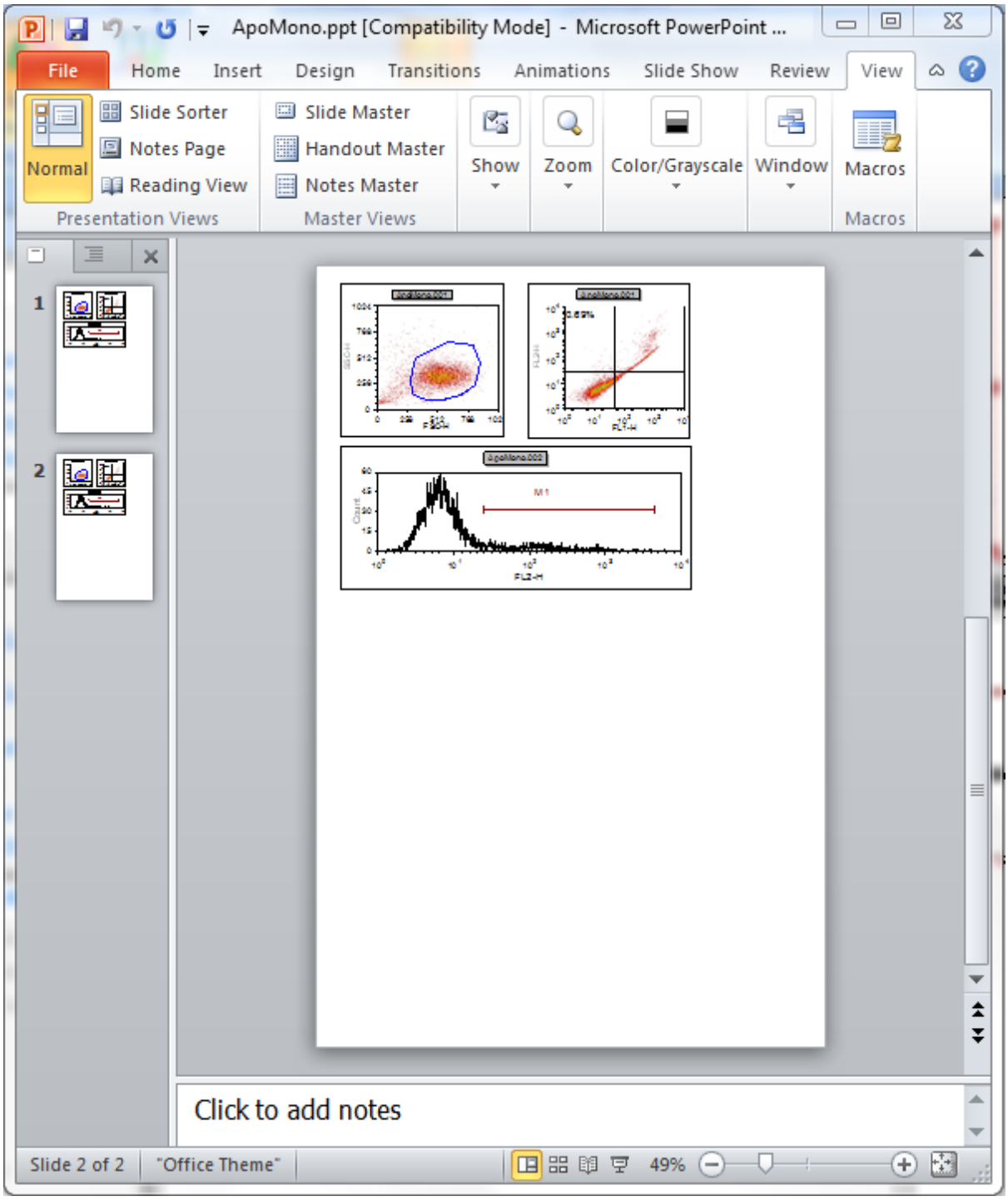

Figure T9.12 Slide 2 in PowerPoint

In the next section, we will export to an Excel report (Cell Mode 778).

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34.9.2 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 sake, we are going to start with the same layout as before. However, you can add reports to layouts that already have actions defined.

1. Select **Application Button→Close Layout**.
2. Select **Application Button→Open Layout**.
3. Open the layout **BatchProcess.fey** found in the Tutorial Sample Data archive 590.

To Perform an Excel Report Batch Process:

4. Create a **Data List** with files **ApoMono.001** and **ApoMono.002** (see the previous section 767).
5. Select the **Batch→Batch Processing→Batch Actions** command (Figure T9.6 767).

A **Batch Actions** window will appear (Figure T9.7).

6. Select **Export to Excel (Cell Mode)** (Figure T9.7 767) 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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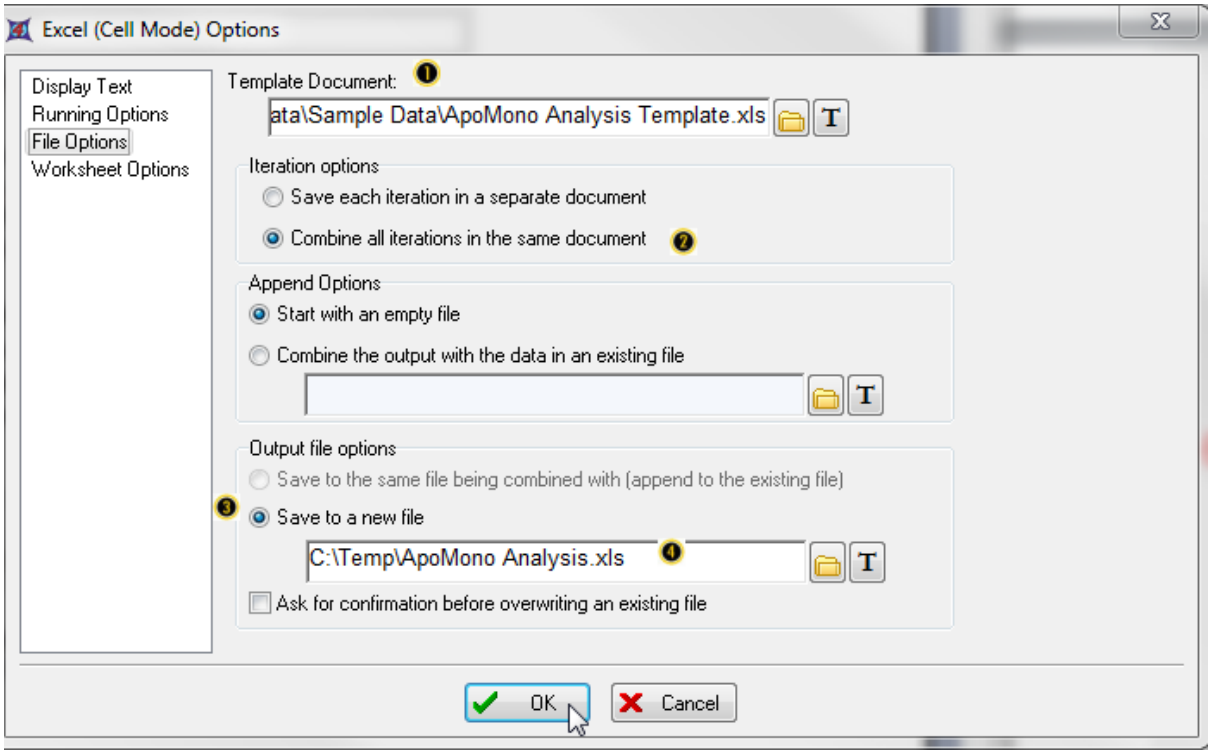

Figure T9.13 Excel (Cell Mode) Options Dialog

7. Click on the Open File Icon in the **Template Document:** field ❶ (Numbers refer to Figure T9.13).

8. Select **ApoMono Analysis Template.xls** from *FCS Express Sample Data* folder.

ApoMono Analysis Template.xls is an Excel file that contains the background for your report. The Excel template we are using is shown in Figure T9.14.

9. Click on **Combine all iterations in the same document** in the **Iteration options** section ❷.

10. Click on **Save to a new file** from the **Output file options** section ❸.

11. Type **C:\Temp\ApoMono Analysis.xls** in the **Save to a new file** field to specify the output location of your Excel Report ❹. (If you do not have a C:\Temp directory, please choose any directory).

12. Click **OK**.

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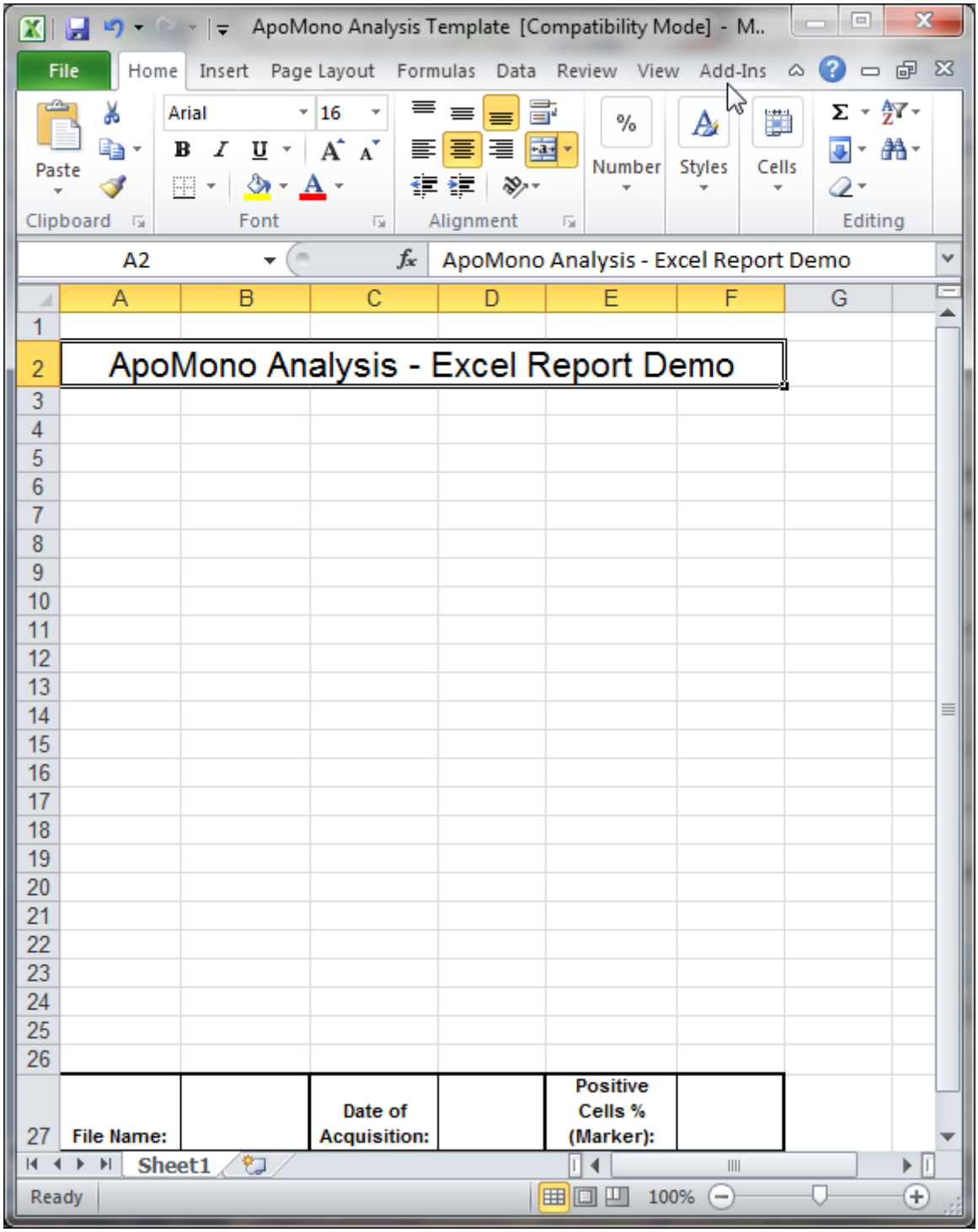

Figure T9.14 ApoMono Analysis Excel Template

The **Batch Actions** window will show the export to **Excel (Cell Mode)** action listed (Figure T9.15).

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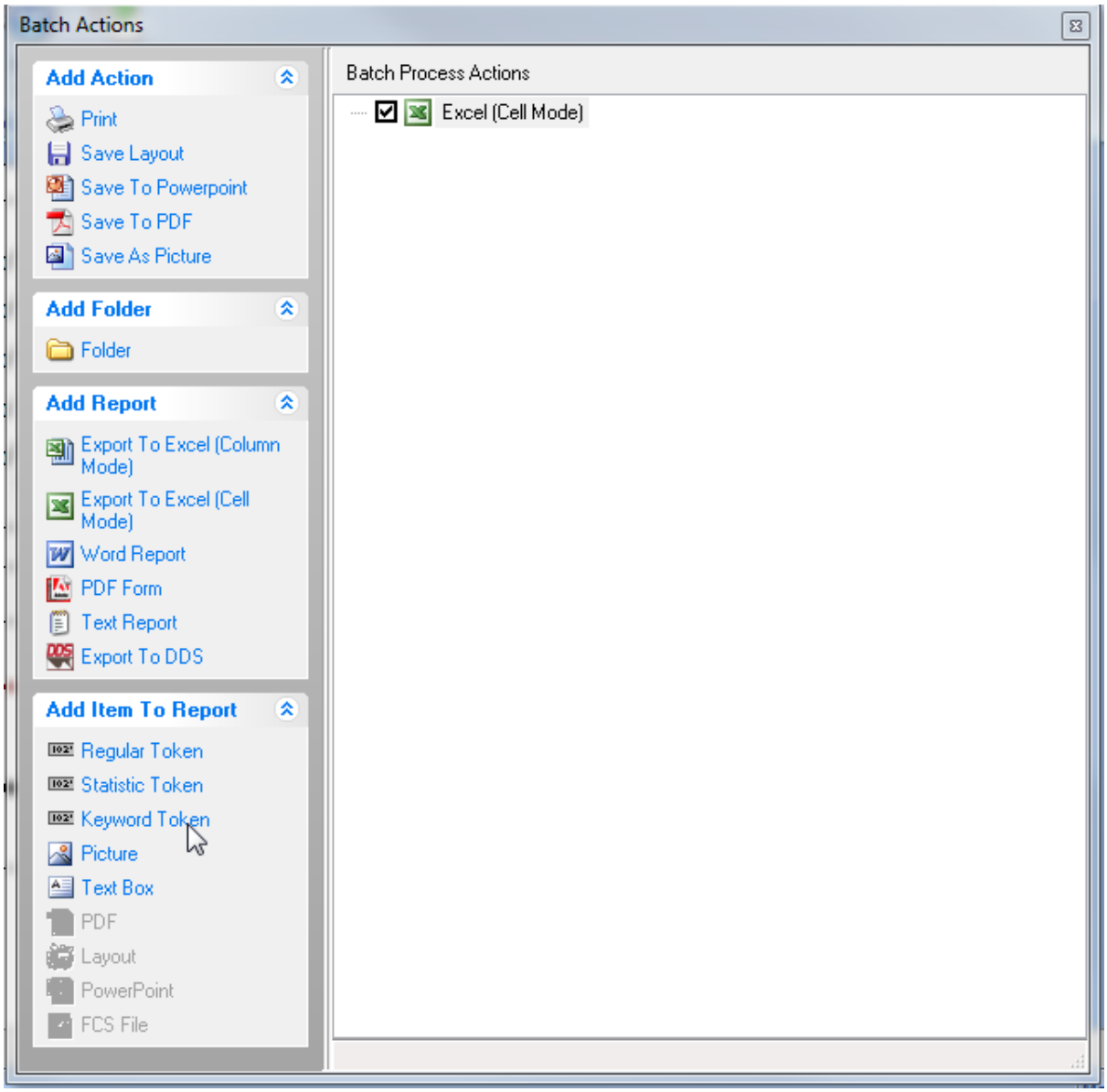

Figure T9.15 Batch Actions Window with the Export To Excel Action

13. 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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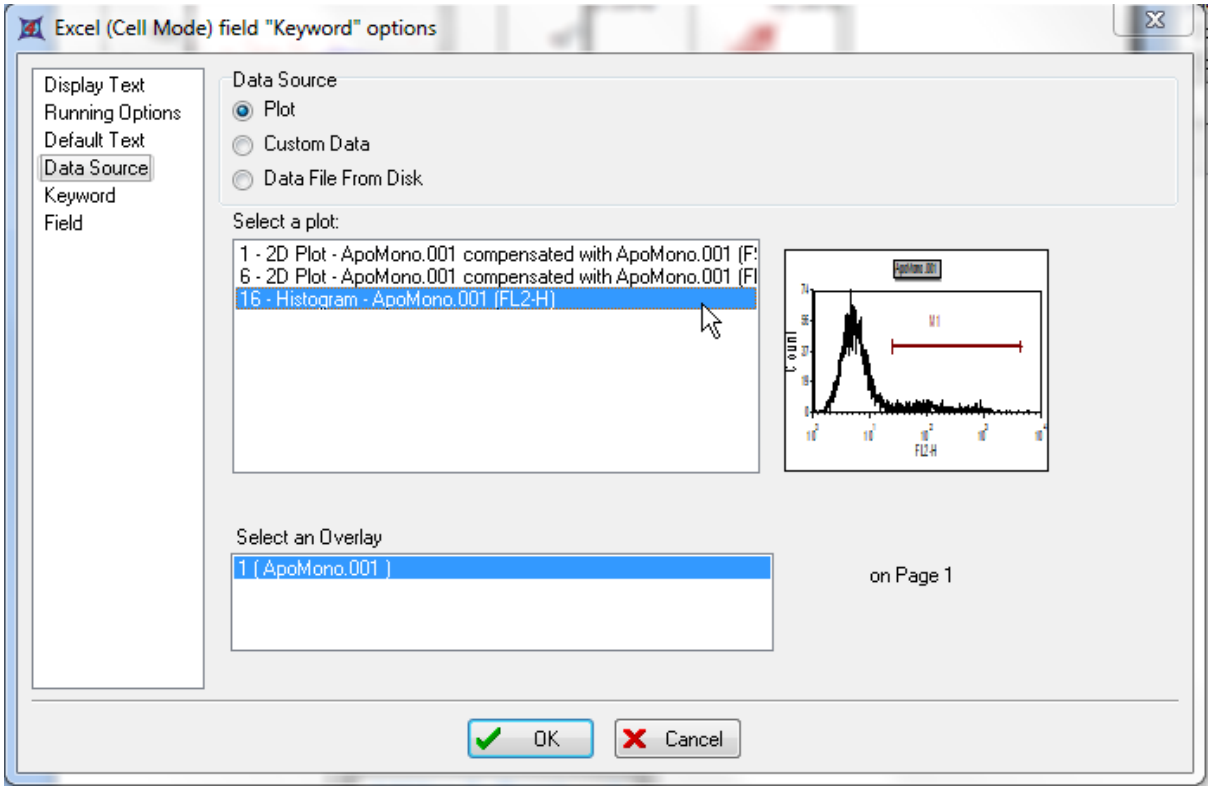

Figure T9.16 Excel (Cell Mode) field "Keyword" options Dialog

14. Select the **Keyword** category (Figure T9.16).

The **Keyword** category page will appear (Figure T9.17).

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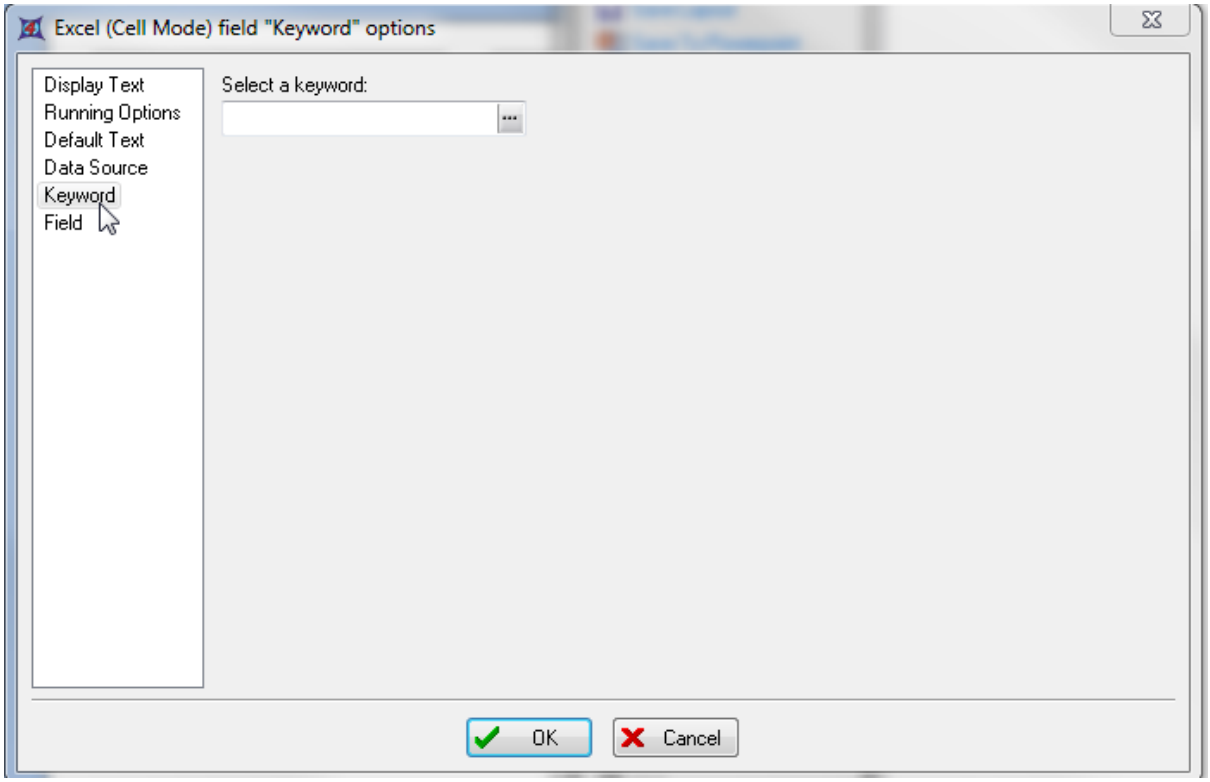

Figure T9.17 Excel (Cell Mode) field "Keyword" options Dialog -- Keyword Category

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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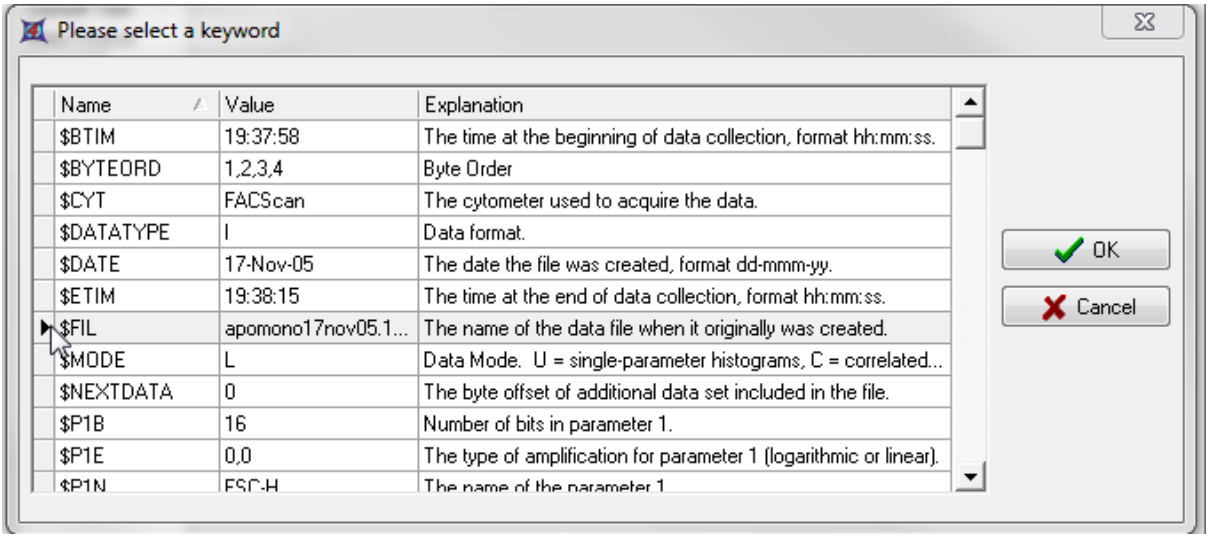

Figure T9.18 Dialog Displaying Keywords Found in the FCS File

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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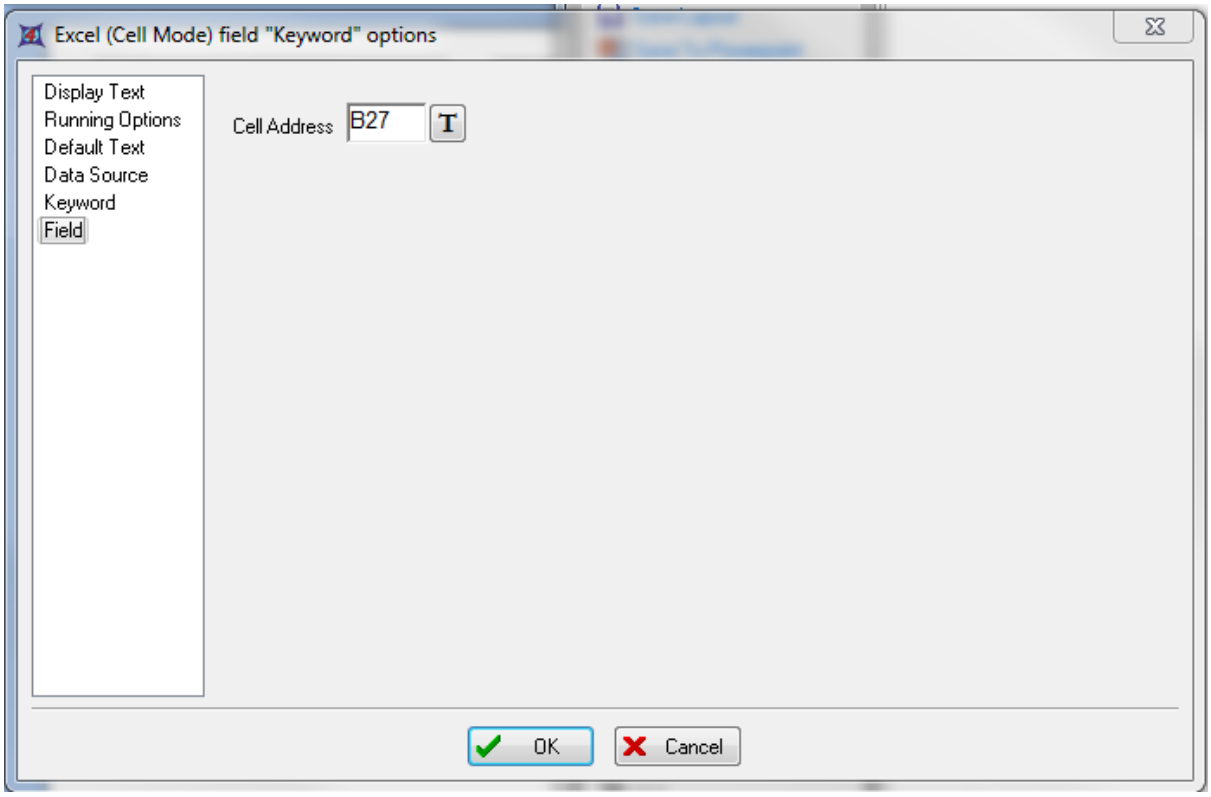

Figure T9.19 Setting the Excel Report Cell Address

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 of interest 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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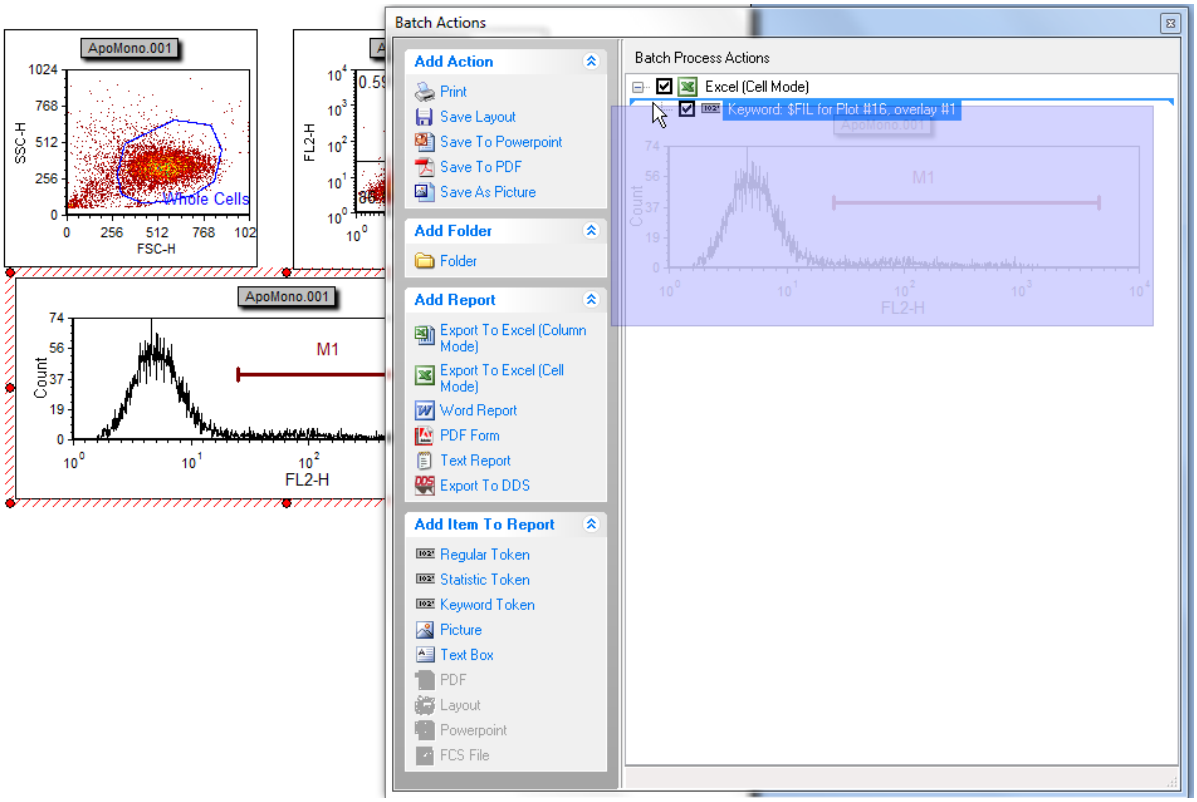

Figure T9.20A Dragging and Dropping a Histogram onto the Batch Actions Window

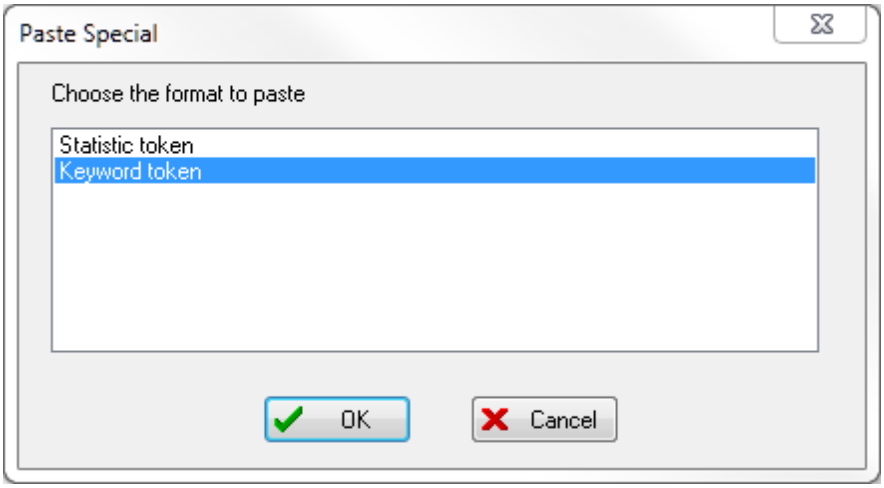

Figure T9.20B Paste Special Dialog

24. Click on the **Keyword token** item (Figure T9.20B).

25. Click **OK**.

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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).

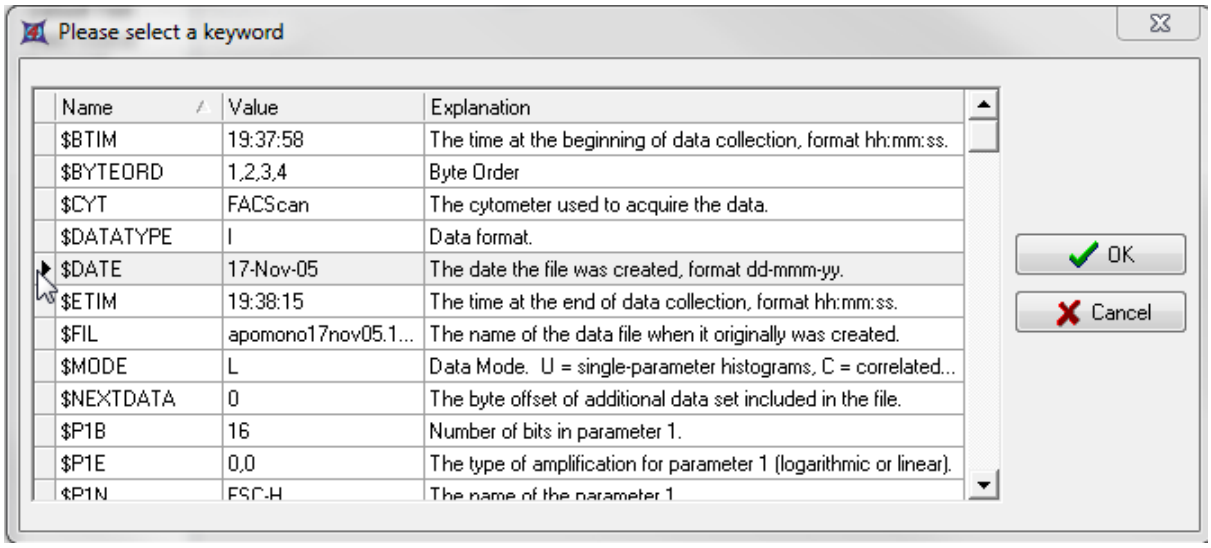

Figure T9.21 Inserting the $DATE Keyword

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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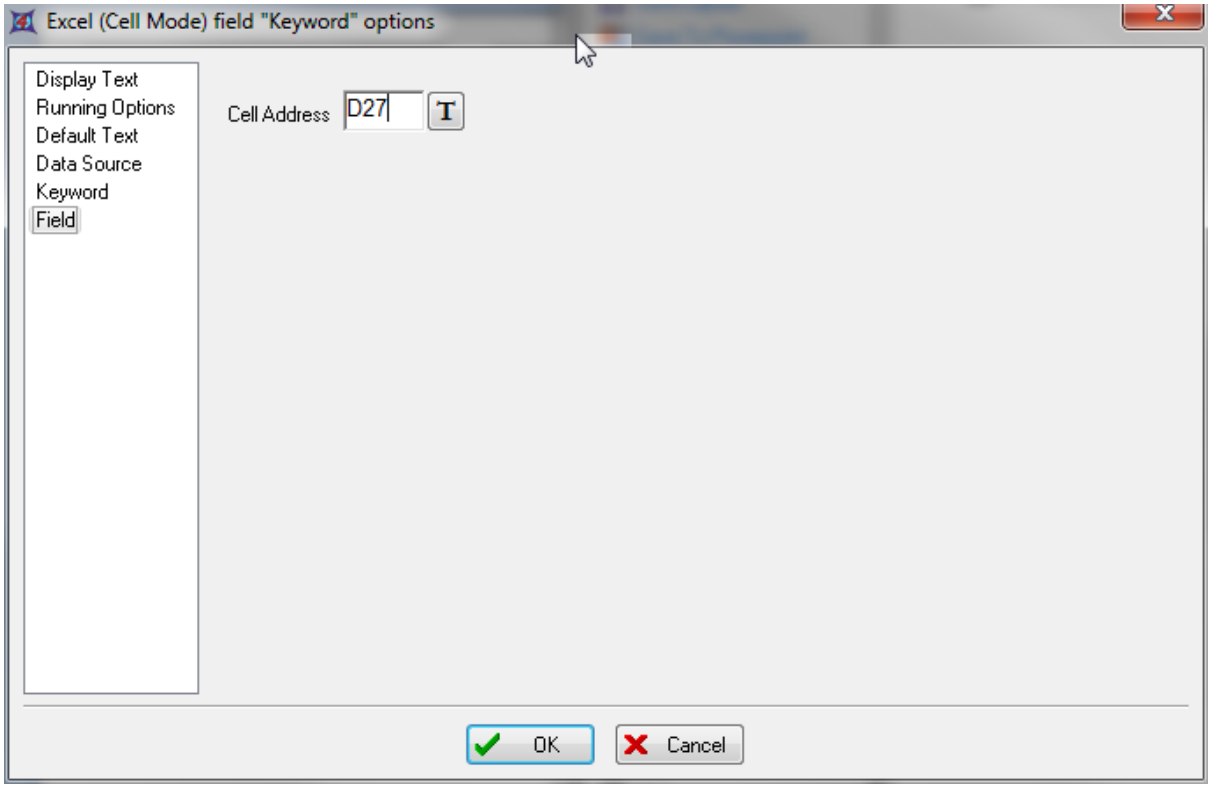

Figure T9.22 Cell Address Field Defined for Batch Export of $DATE Keyword to Excel

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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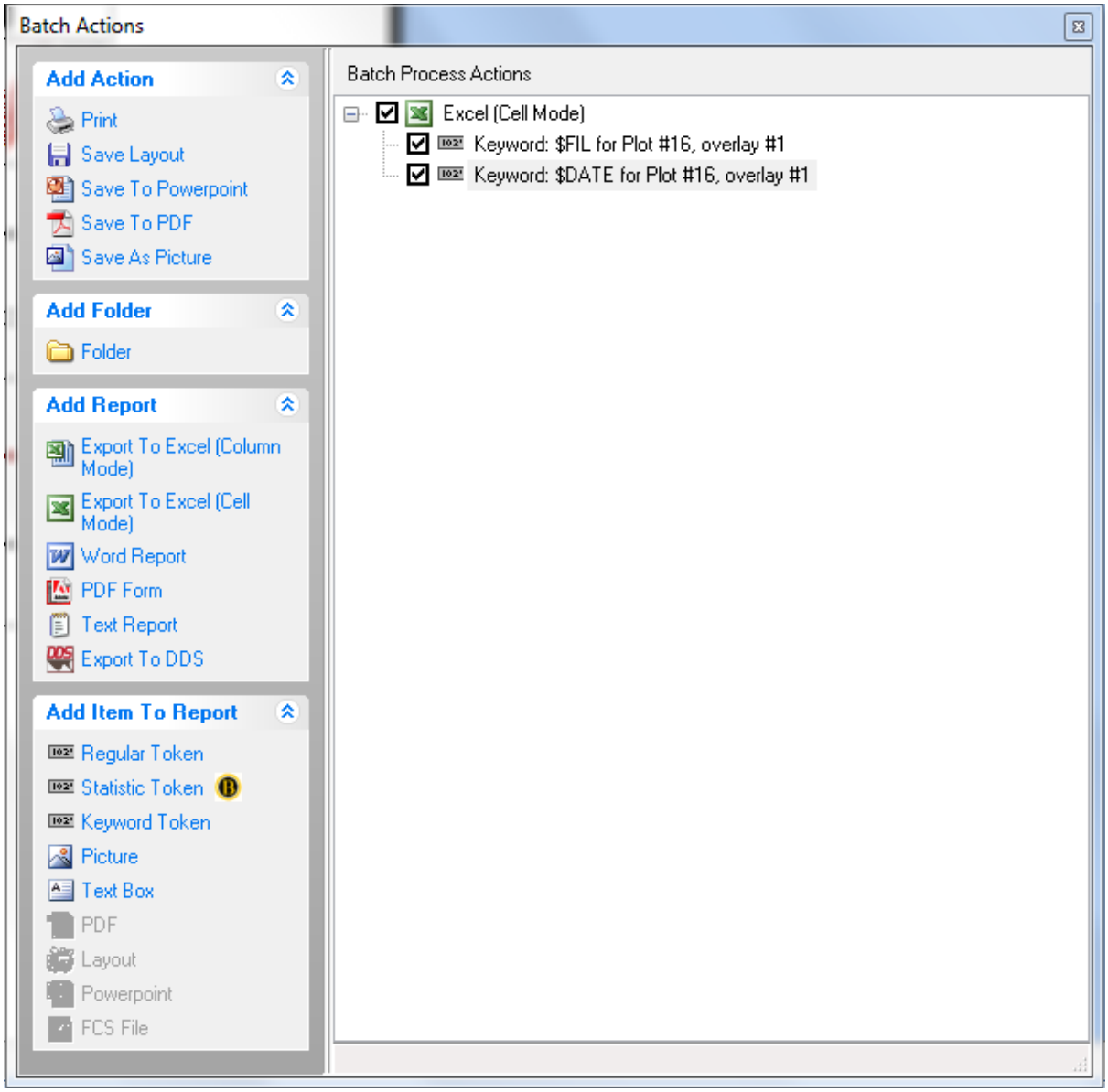

Figure T9.23 Batch Actions Window with $DATE Keyword Exported

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.

34. Click on the **Data Source** category (Figure T9.16).

35. Select **Histogram – ApoMono.001** from the **Select a plot**: field (Figure T9.16).

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36. Click on the **Statistic** Category (Figure T9.24).

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.

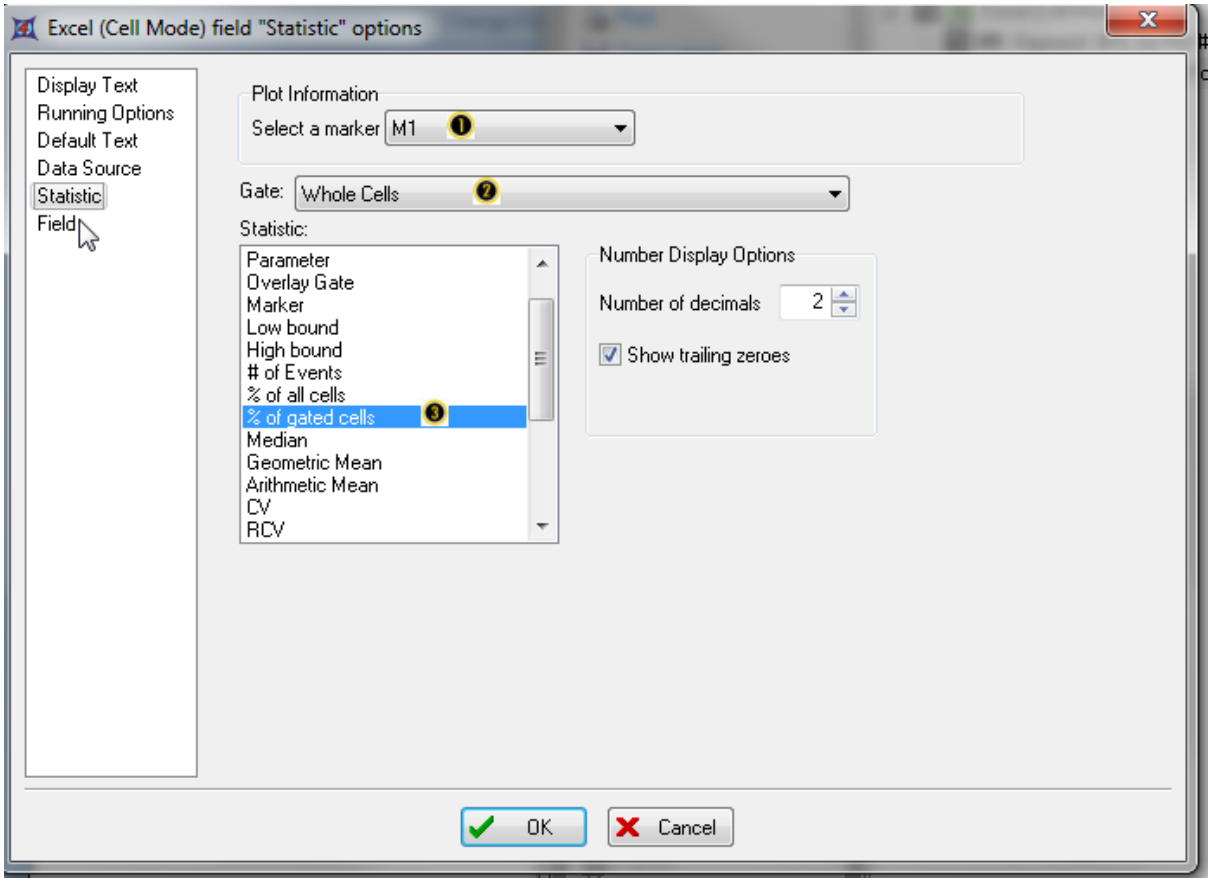

Figure T9.24 Selecting a Statistic to Export

37. Select the marker **M1** from the **Plot Information** drop-down list ❶.

38. Select the Gate **Whole Cells** from the **Gate:** drop-down list ❷.

39. Select the Statistic **% of gated cells** from the **Statistic**: list box ❸.

40. Click on the **Field** category (Figure T9.24).

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.

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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).

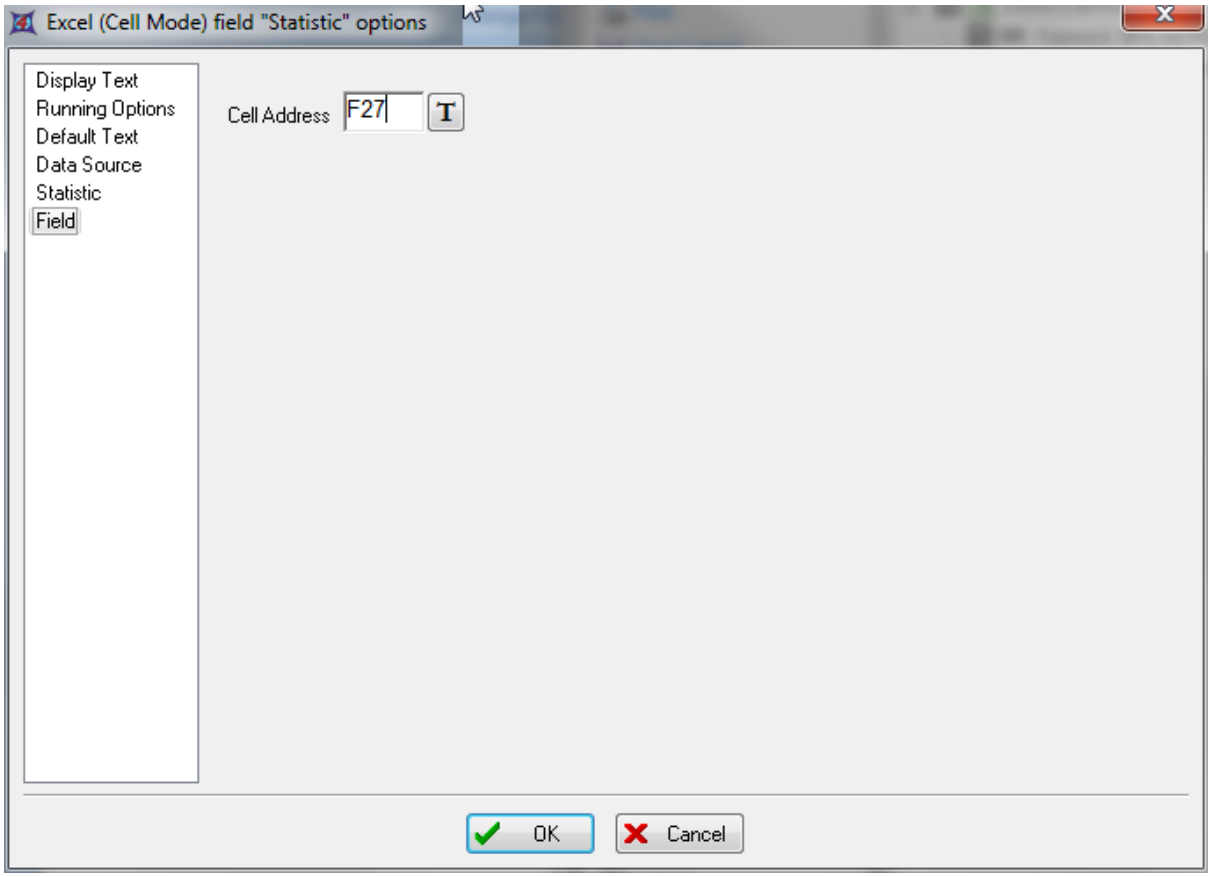

Figure T9.25 Setting the Excel Cell Address

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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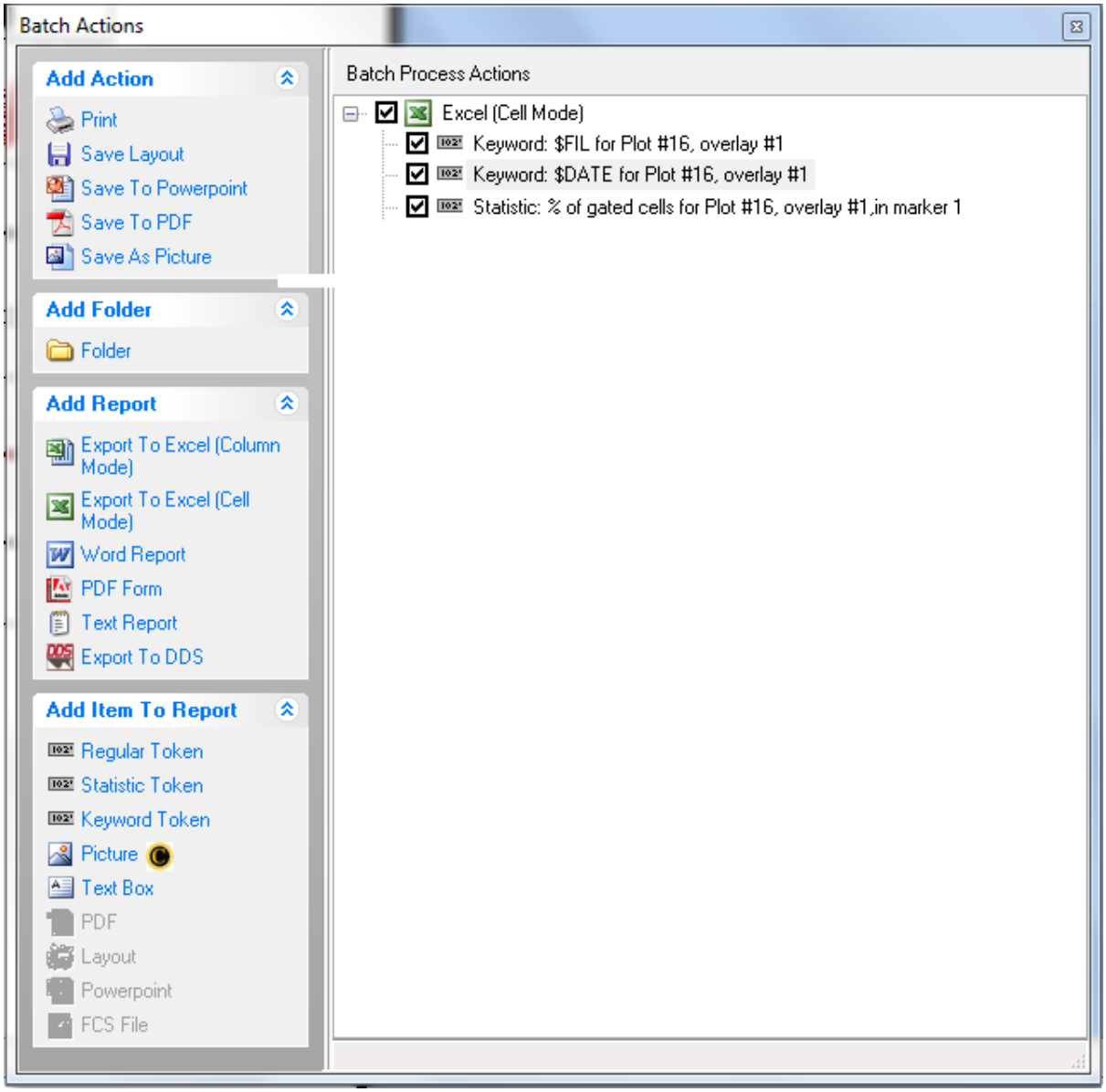

Figure T9.26 Batch Actions with Statistic % of Gated Cells to be Exported to Excel

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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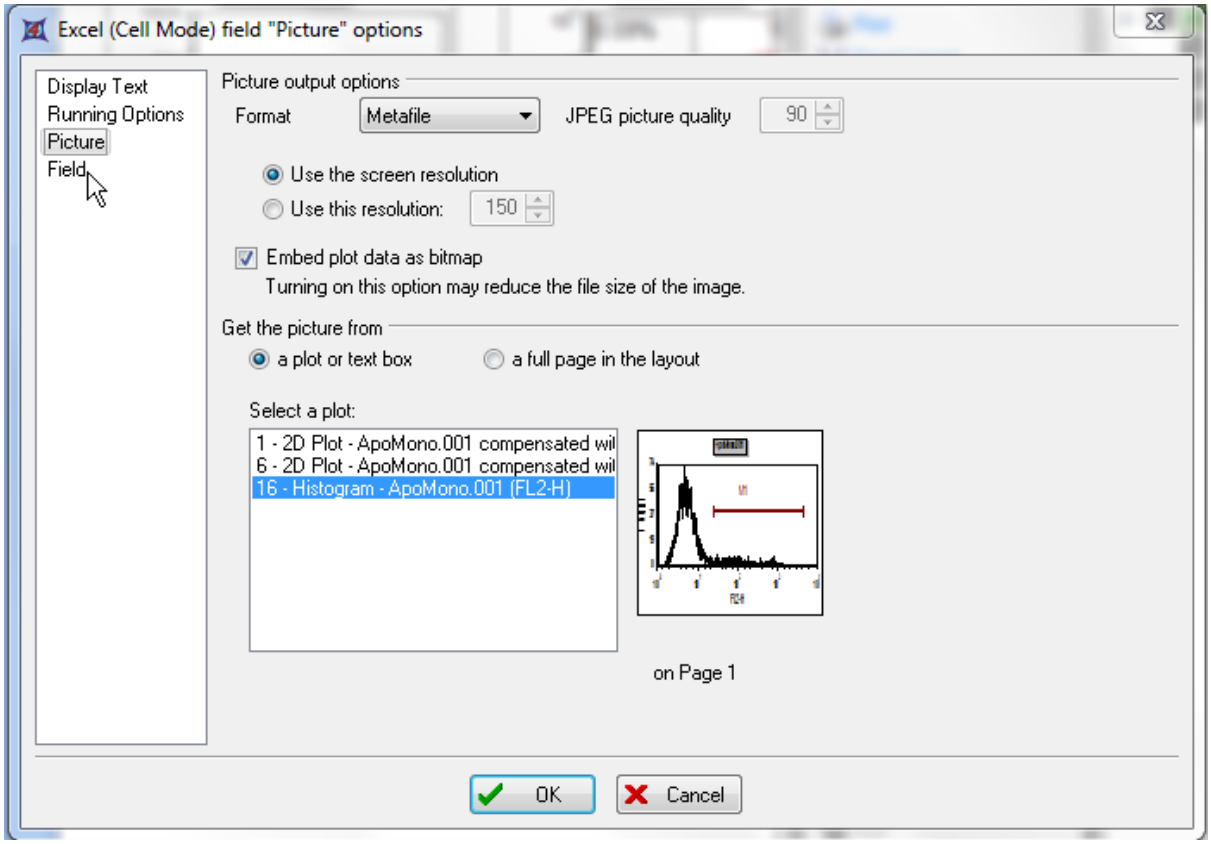

Figure T9.27 Excel (Cell Mode) field "Picture" options Dialog

43. Select **Histogram – ApoMono.001** 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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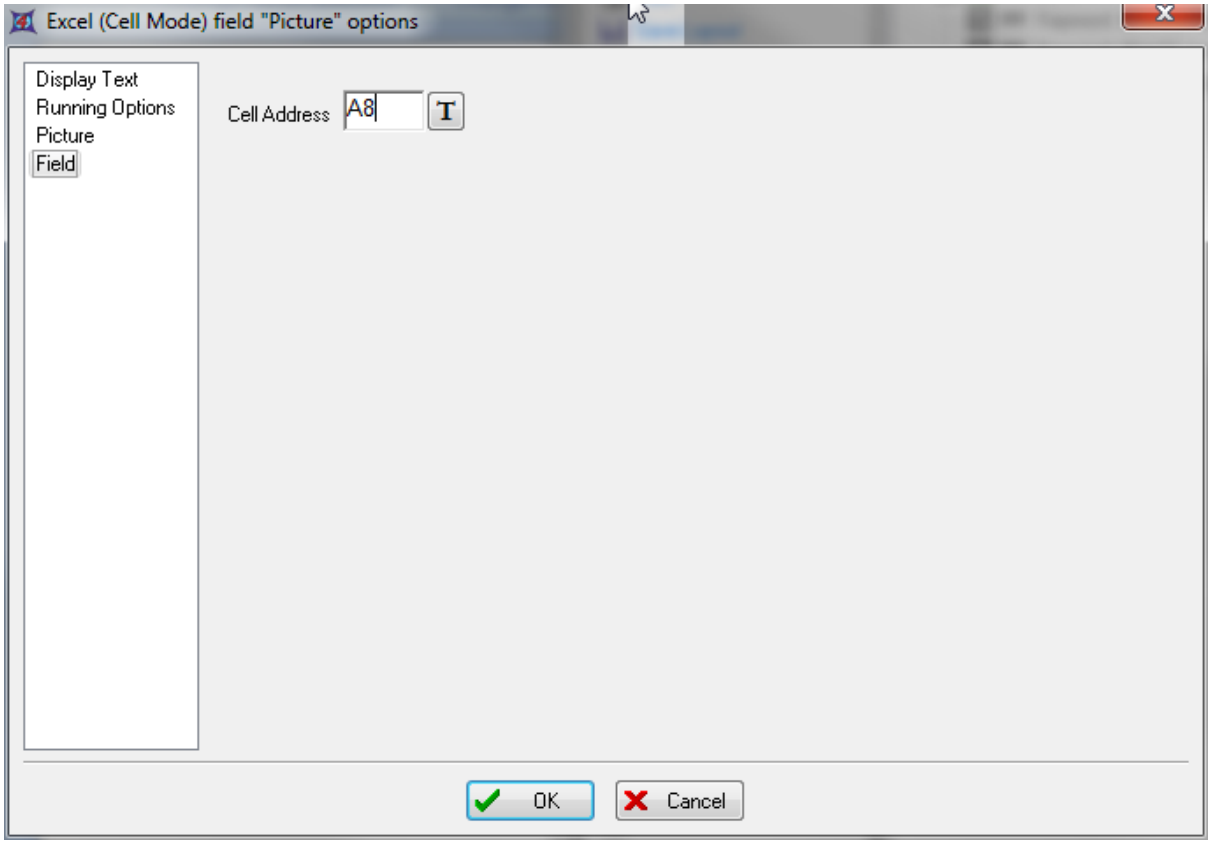

Figure T9.28 Setting the Excel Cell Address for the Picture Field

45. Type **A8** in the **Cell Address** field (Figure 28).

46. Click **OK**.

We have now told FCS Express to export a picture of **Histogram – ApoMono.001** 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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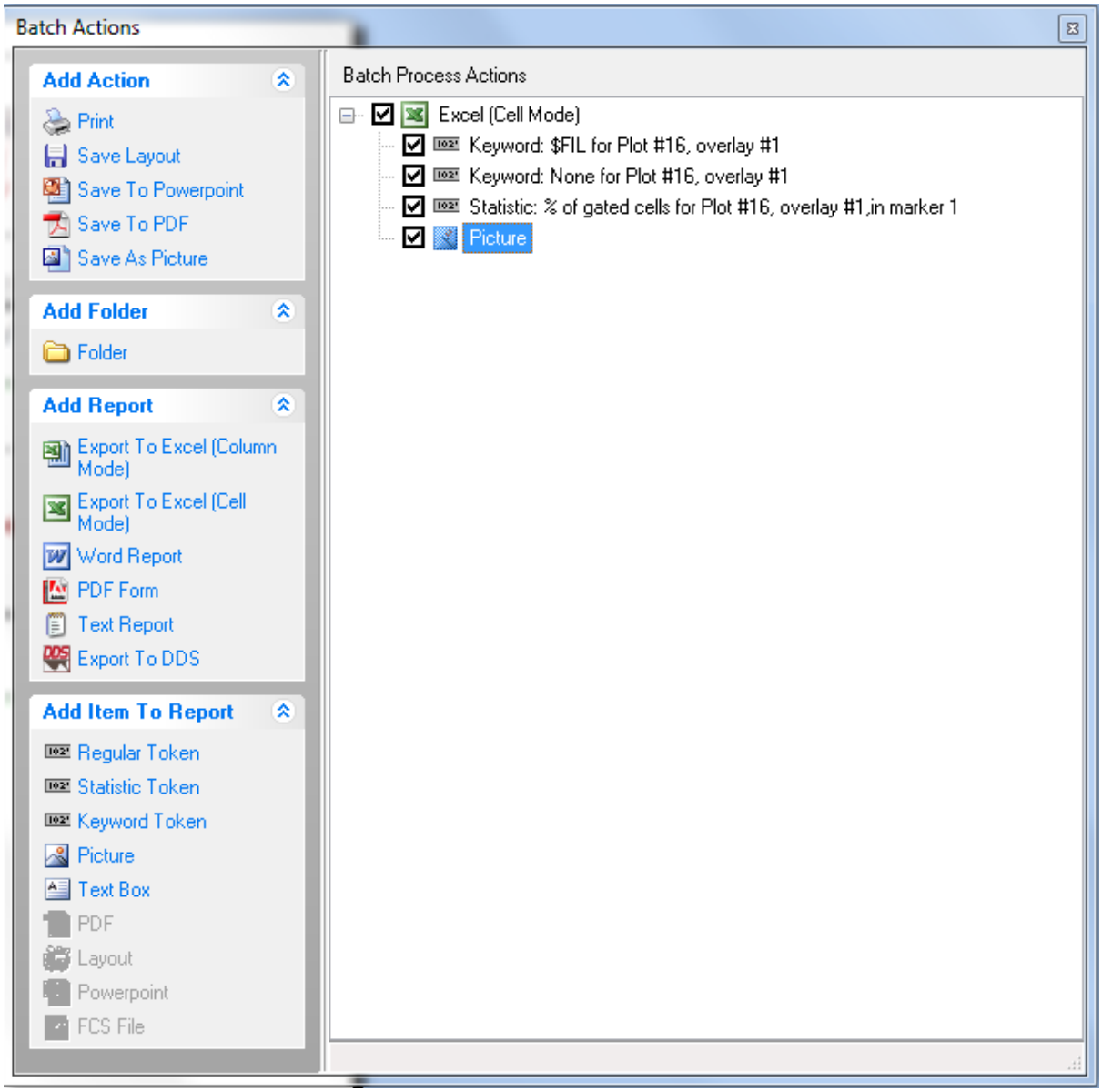

Figure T9.29 Batch Actions with Picture to be Exported to Excel

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** command (Figure T9.10 767).

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 **ApoMono.001** and

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ApoMono.002.

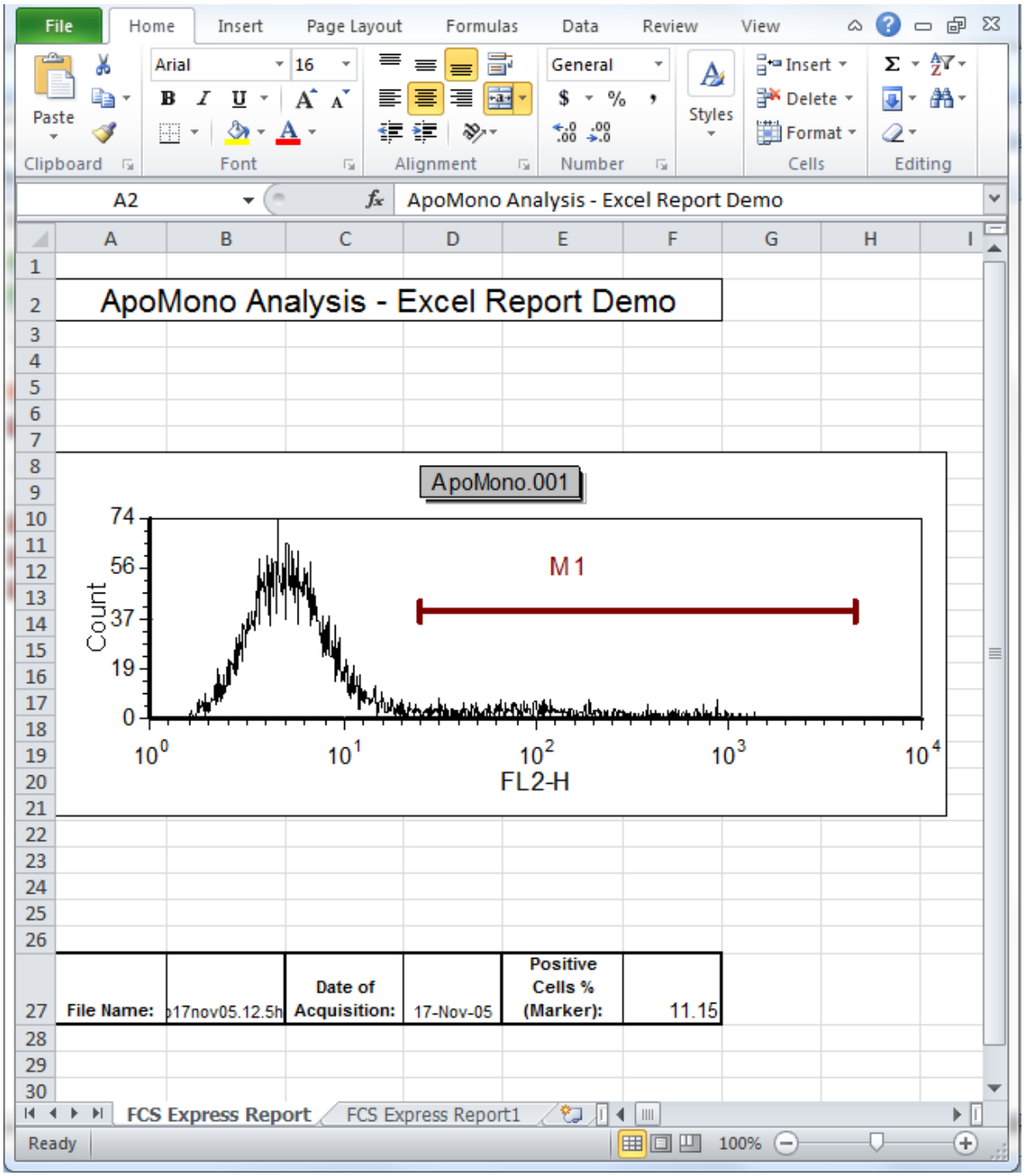

Figure T9.30 First Sheet of the Final Excel Report from the Batch Processing

Next, we will batch process an Excel report in column mode 797.

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34.9.3 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.
- Move the items from the Excel Cell Mode report to the Column Mode report.
- Insert column headers into the Excel batch analysis report.
- Insert a picture into the Excel Column Mode batch analysis report.

1. Select the **Batch→Batch Processing→Batch Actions** command. The Batch Actions window appears as previously shown in Figure T9.29 778.
2. Select **Export To Excel (Column Mode)** from the **Add Report** section.

An **Excel (Column Mode) Options** dialog appears (Figure T9.31).

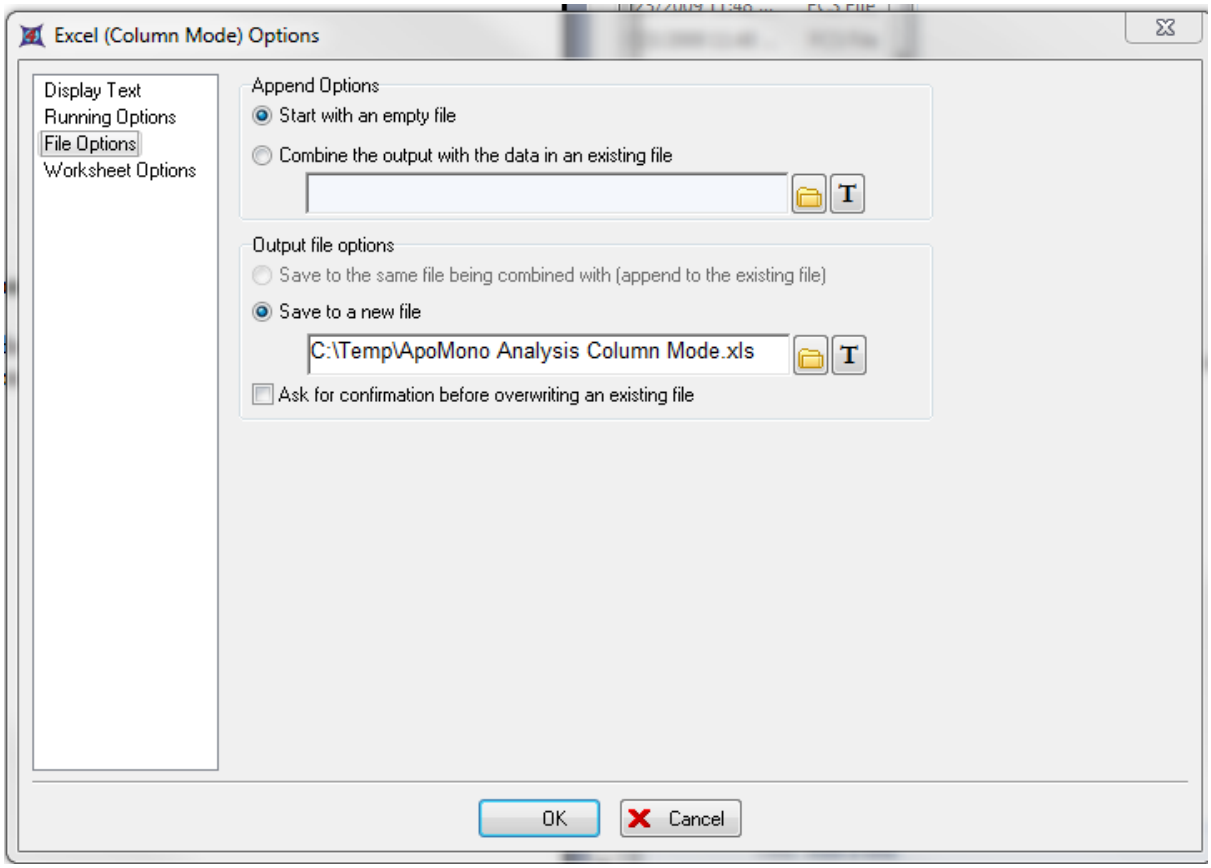

Figure T9.31 Excel (Column Mode) Options Dialog

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3. Type **C:\Temp\ApoMono Analysis Column Mode.xls** in the **Save to a new file** field (Figure T9.31). (If you do not have a C:\Temp directory, please choose any directory.)

4. Click **OK**.

An **Excel (Column Mode)** process will now appear in the **Batch Process Actions** section of the window (Figure T9.32). We are now going to move the Batch Process Actions from **Excel (Cell Mode)** to **Excel (Column Mode)** by copying and pasting the items (Figure T9.32).

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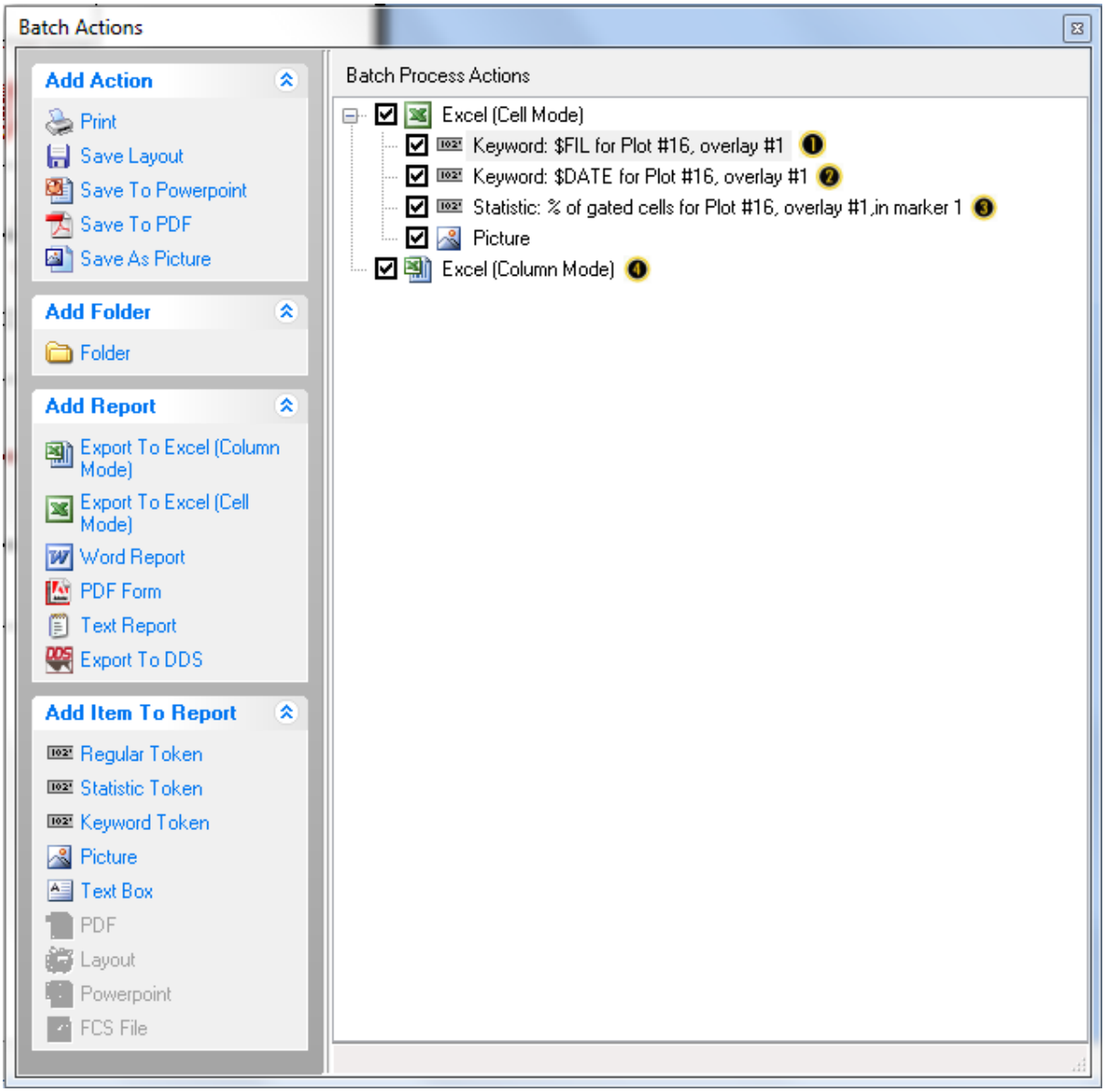

Figure T9.32 Excel (Column Mode) Process in the Batch Process Actions

5. Left-click to select the **Keyword: $FIL** ❶ from the **Excel (Cell mode)** batch process (Figure T9.32).

6. While holding down the **Ctrl** key, left-click to also select the **Keyword: $DATE** ❷ and then **Statistic: % of gated cells** ❸ from the **Excel (Cell Mode)** batch process (Figure T9.32).

7. Simultaneously hold down the **Ctrl** and **"C"** keys to copy the selected items.

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- Alternatively, right-click to bring up the Batch Process Actions pop-up menu (Figure T9.33).
- Select **Copy BP Actions** (A) (Figure T9.33).

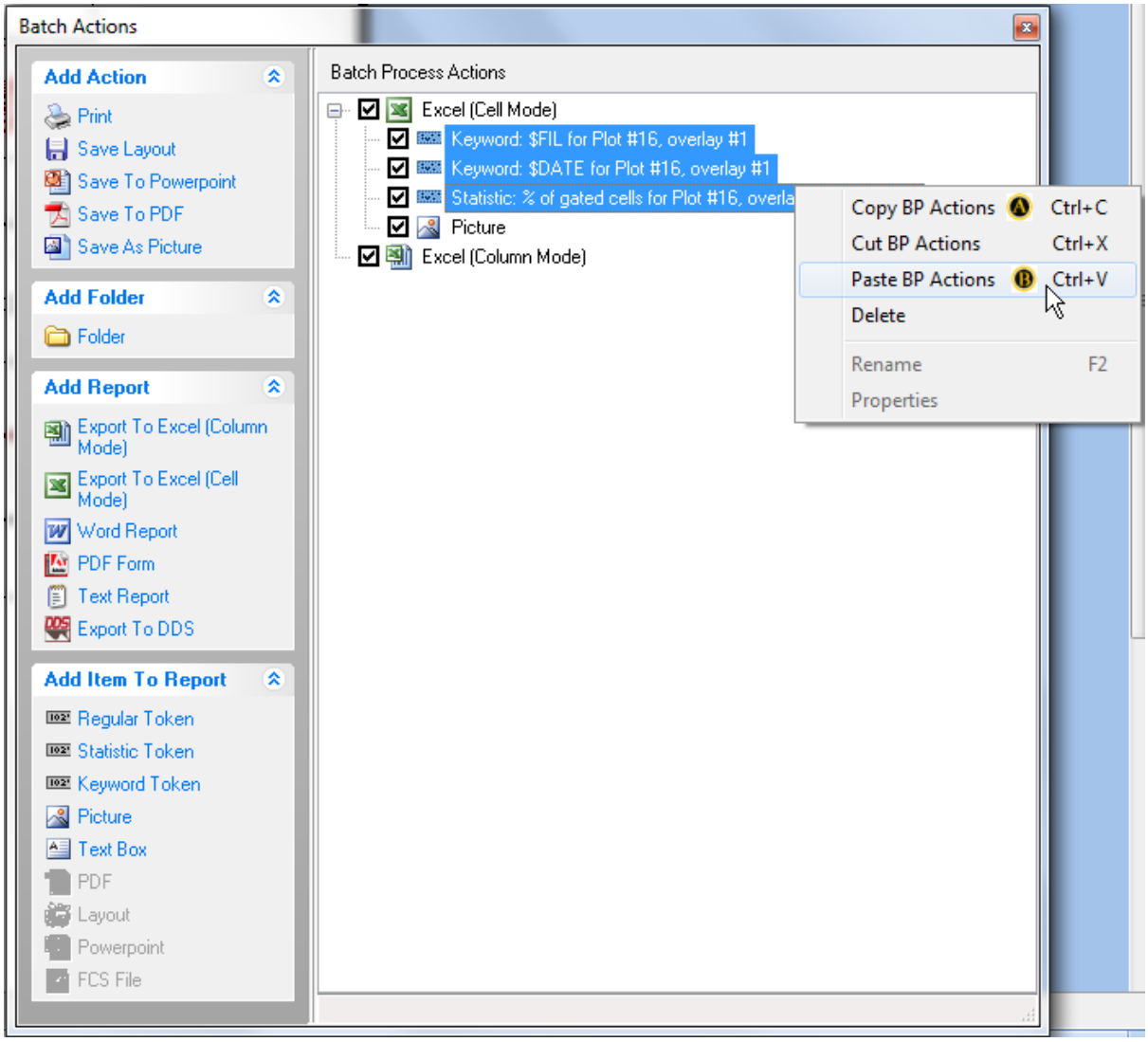

Figure T9.33 Batch Process Actions Pop-up Menu to Copy and Paste Actions

8. Left-click to select the **Excel (Column Mode)** (B) batch process in the **Batch Actions** window (Figure T9.32).
9. Simultaneously hold down the **Ctrl and "V"** keys to paste the selected items to the **Excel (Column Mode)** Batch Process Actions (Figure T9.32).

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- Alternatively, right-click to bring up the Batch Process Actions pop-up menu (Figure T9.33).
- Select **Paste BP Actions** (Figure T9.33).

The Batch Process Actions are now copied from **Excel (Cell Mode)** to **Excel (Column Mode)** as shown in Figure T9.34.

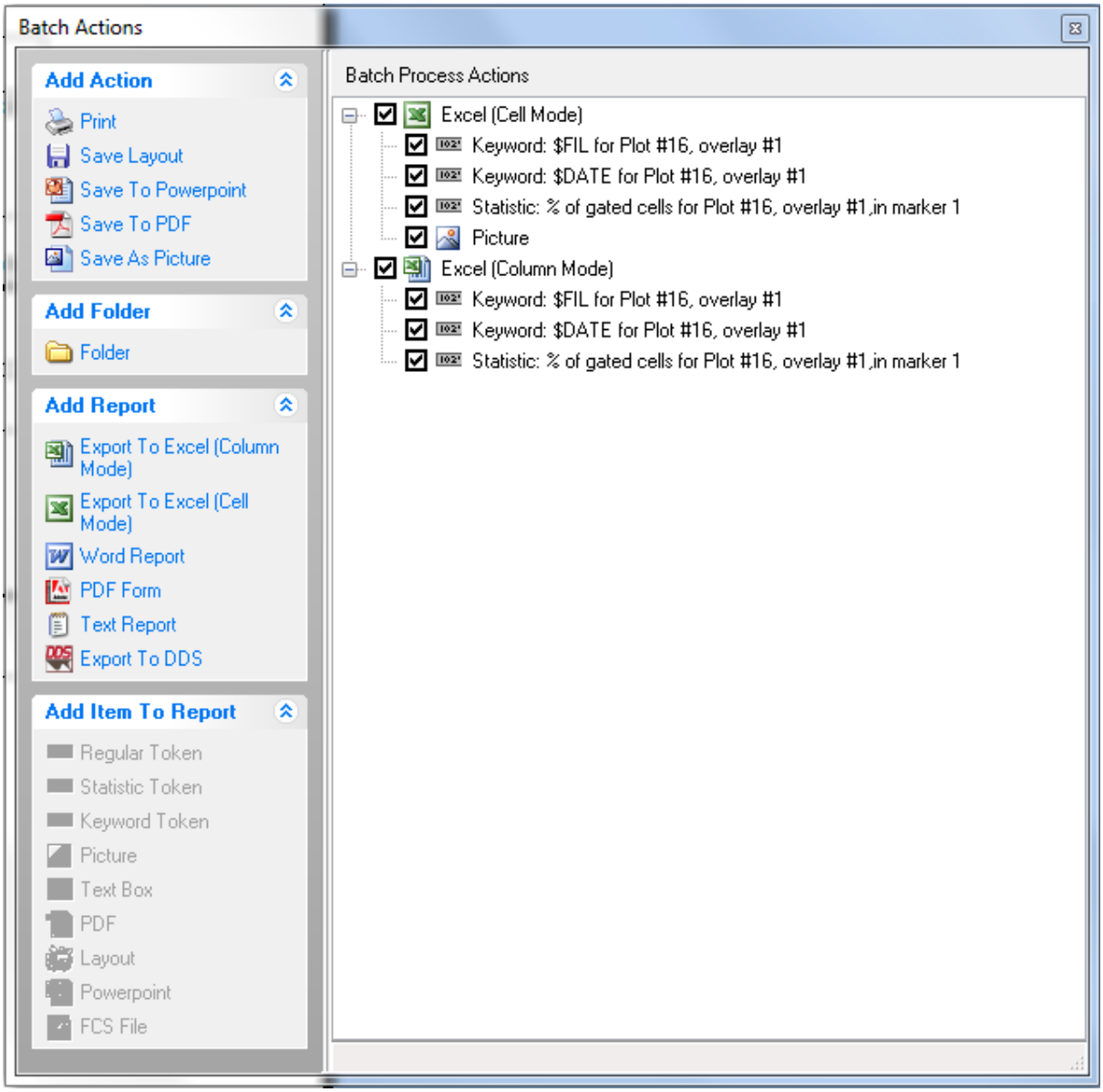

Figure T9.34 Batch Actions Dialog with Actions Pasted to Excel (Column Mode)

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10. Double-click on the **Keyword: $FIL** token under the **Excel (Column Mode)** batch action.

The **Excel field "Keyword: $FIL" options** dialog appears, similar to Figure T9.16 778 but for **Column Mode** rather than **Cell Mode**.

11. Click on the **Field** category (Figure T9.16 778) to bring up the Field page (Figure T9.35).

12. Type **File Name** in the **Column Name** field (Figure T9.35).

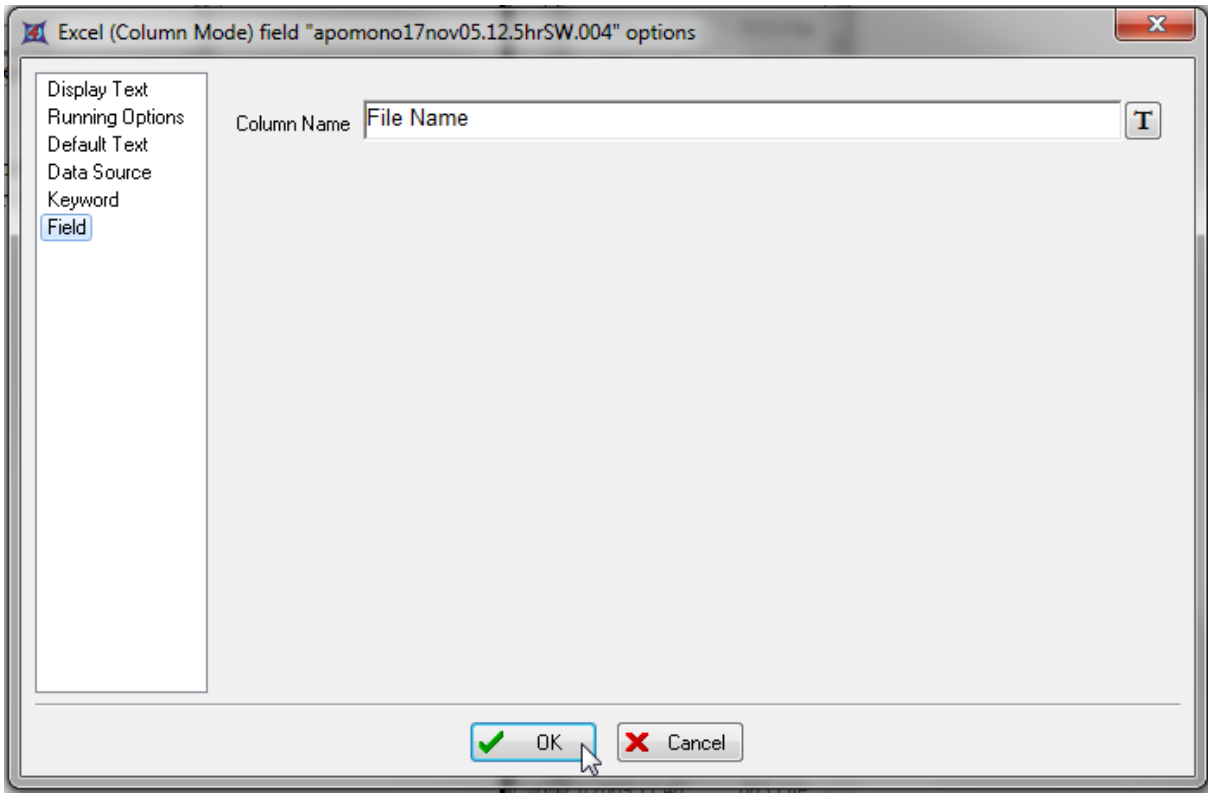

Figure T9.35 Setting Column Name for Excel (Column Mode) Batch Export

13. Press **OK** to accept the Column Name and close the window.

We have now defined the Column Name for the batch Excel (Column Mode) export of the Keyword token $FIL.

14. Double-click on the **Keyword: $DATE** token under the **Excel (Column Mode)** batch action.

Again, the **Excel field "Keyword: $DATE" options** dialog appears, similar to Figure T9.16 778 but for **Column Mode** rather than **Cell Mode**.

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15. Click on the **Field** category (Figure T9.16 778) to bring up the Field page (Figure T9.35).

16. Type **Date** in the Column Name field.

17. Press **OK**.

18. Repeat this process by double-clicking on the **Statistic: % of gated cells** token under the **Excel (Column Mode)** batch action.

Again the **Excel field "Statistic: % of gated cells" options** dialog box appears, similar to Figure T9.16 778 but for **Column Mode** rather than **Cell Mode**.

19. Click on the **Field** category (Figure T9.16 778) to bring up the Field page (Figure T9.35).

20. Type **Positive Cells % (Marker)** in the Column Name field.

21. Press **OK**.

We have now defined all the Column Names for the Excel (Column Mode) batch export (Figure T9.36).

22. Deselect the checkbox next to the **Excel (Cell Mode)** batch action (Figure T9.36).

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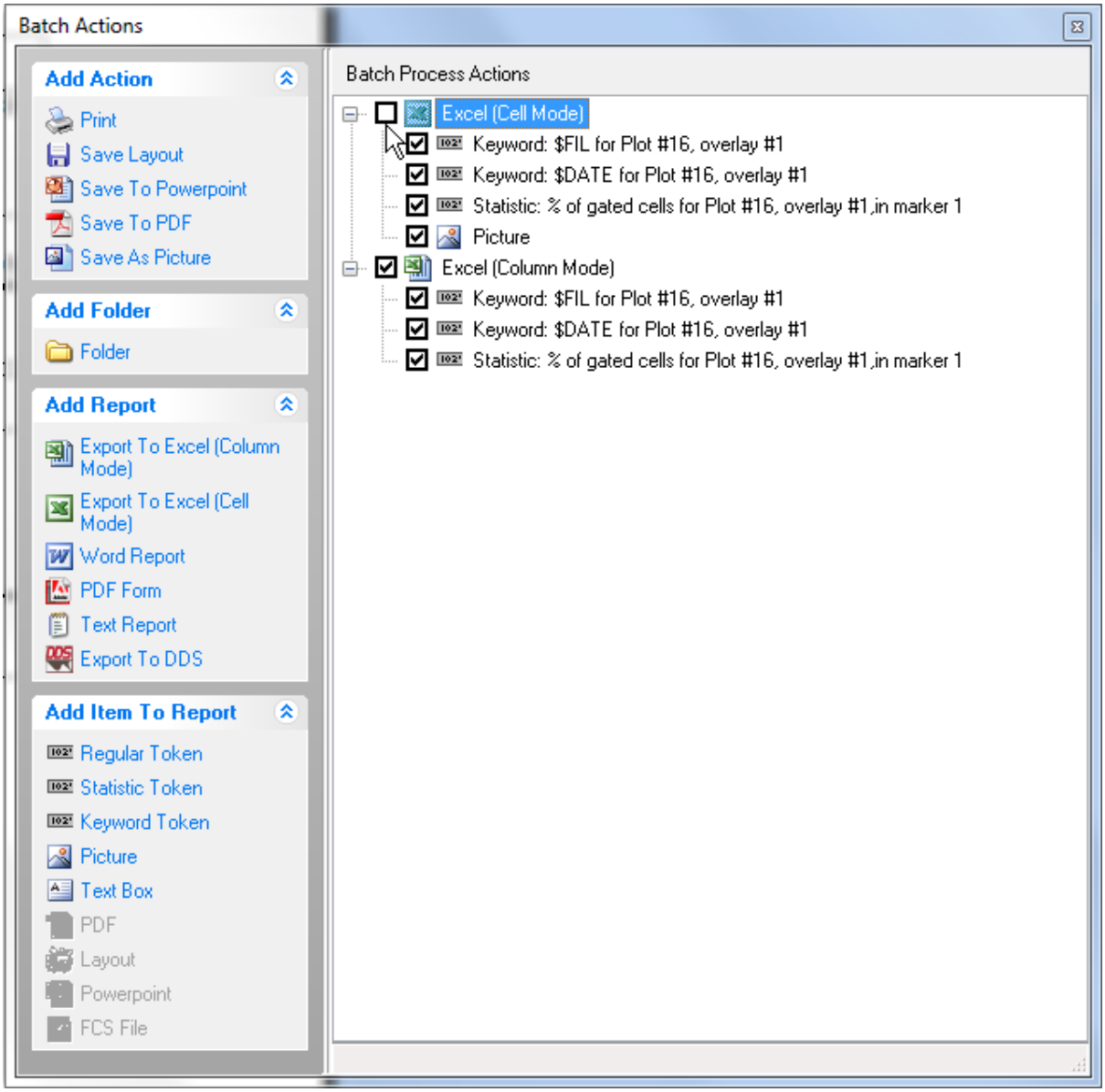

Figure T9.36 Deselect Excel (Cell Mode) to Batch Process Only Excel (Column Mode)

Now we are ready to run the Batch Processing to export to **Excel (Column Mode)**.

23. Select the **Batch→Batch Processing→Run** command (Figure T9.10 767).

An Excel (Column Mode) report will appear with the results of the batch process (Figure T9.37).

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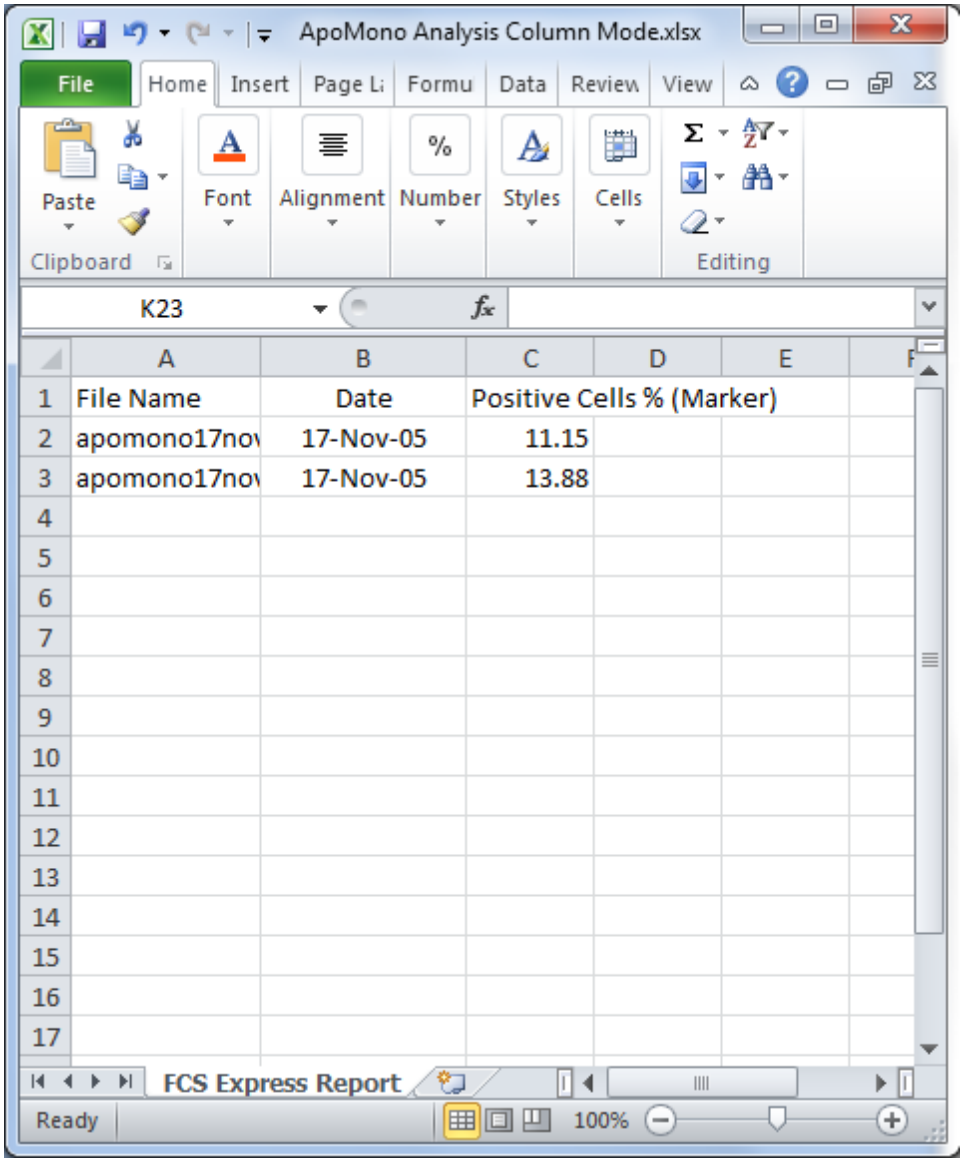

Figure T9.37 Excel (Column Mode) Report

We will now insert the image of a plot from FCS Express onto the Excel spreadsheet using drag and drop.

24. Select the **ApoMono.001** histogram from the **BatchProcess.fey** FCS Express layout. The histogram border will turn red upon selection.

25. Hover the mouse pointer on the border of the histogram until the cursor changes to a four-headed arrow cursor.

26. Press the left mouse button when the cursor changes to the four-headed arrow cursor.

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27. Continue to hold down the left mouse button and drag the plot from the FCS layout to the Excel (Column Mode) report spreadsheet (Figure T9.38).

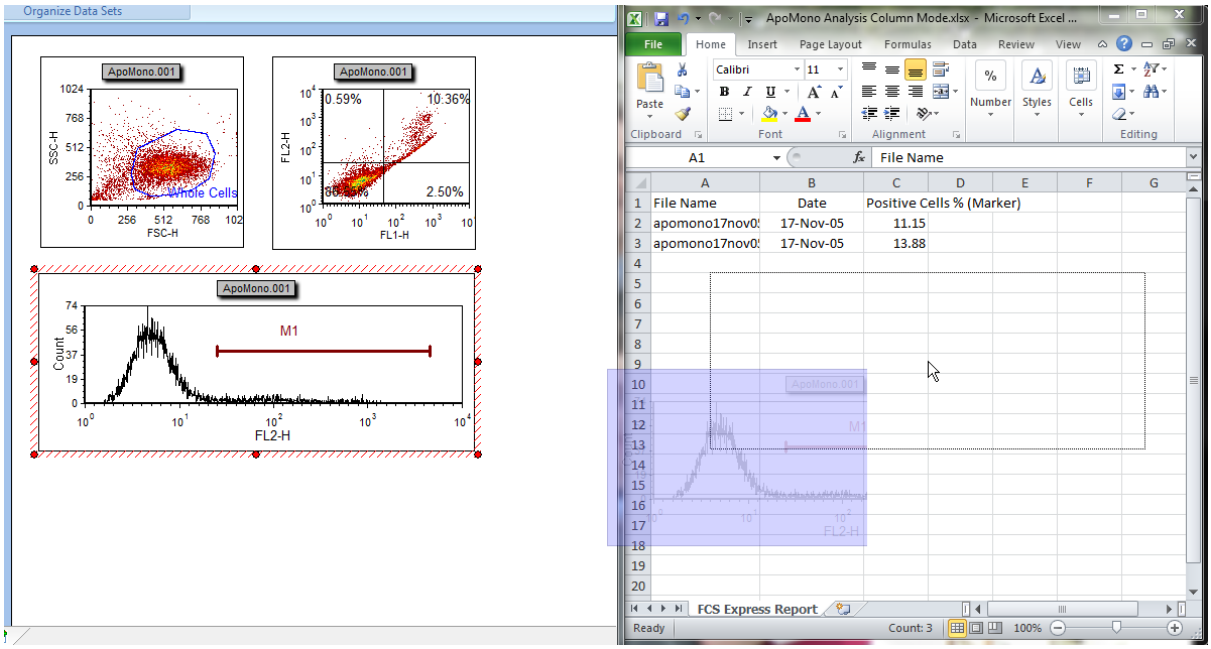

Figure T9.38 Dragging and Dropping a Plot Image from FCS Express to an Excel Spreadsheet

28. Release the mouse when the histogram is under the statistics in the **Excel (Column Mode)** batch report (Figure T9.38).

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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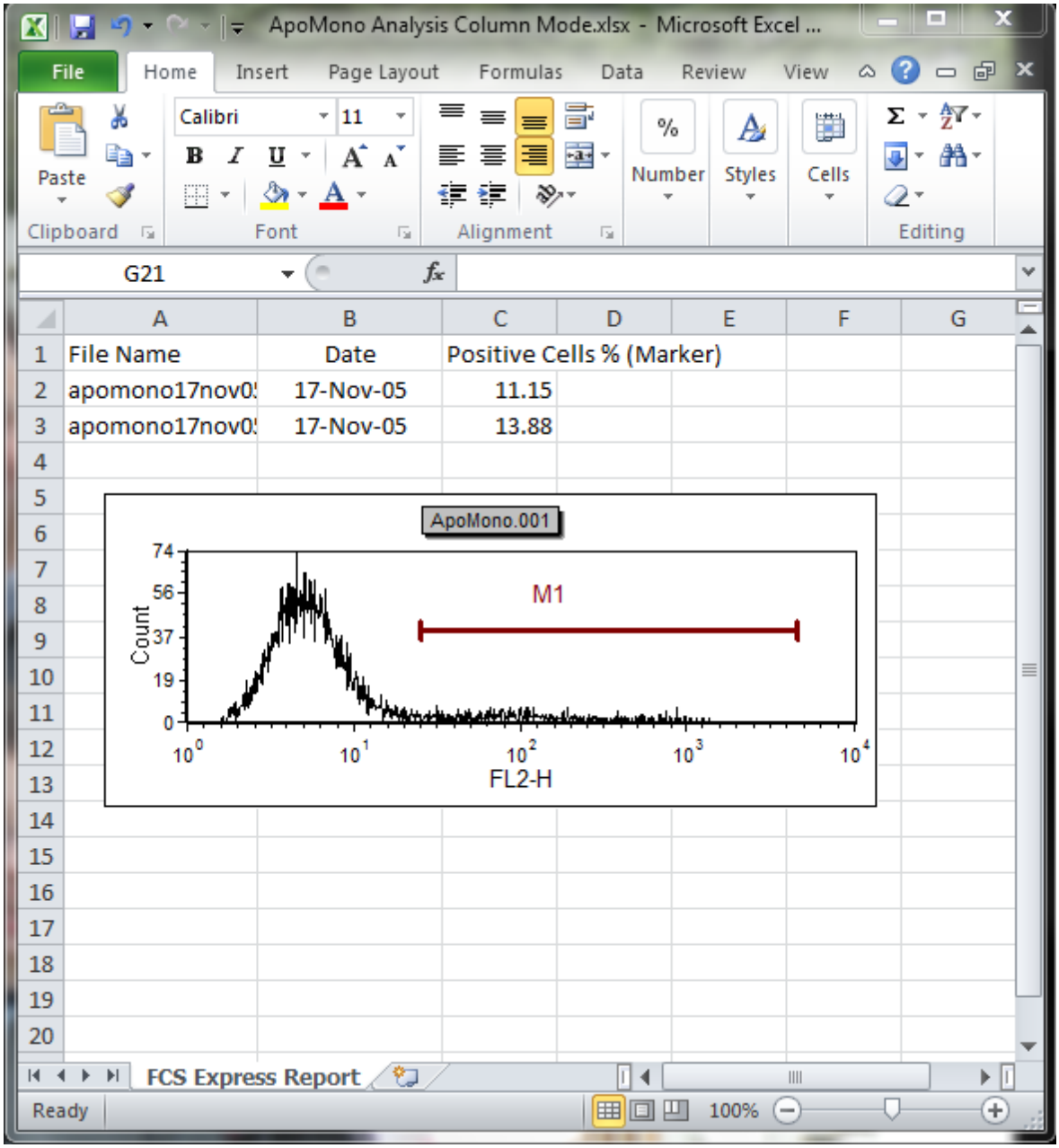

Figure T9.39 Excel (Column Mode) Report with a Plot Image Added

It is very useful to dock the **Batch Actions** window to the side of the layout when defining the actions of a batch process. Docking the **Batch Actions** dialog makes it easier to drag and drop plots and tokens onto it, allowing you to set up the batch process actions very quickly. An example of a **Batch Actions** window docked to the left side of the layout is shown in Figure T9.40. Please refer to the Docking Tutorial 845 for more details on using the docking feature in FCS Express.

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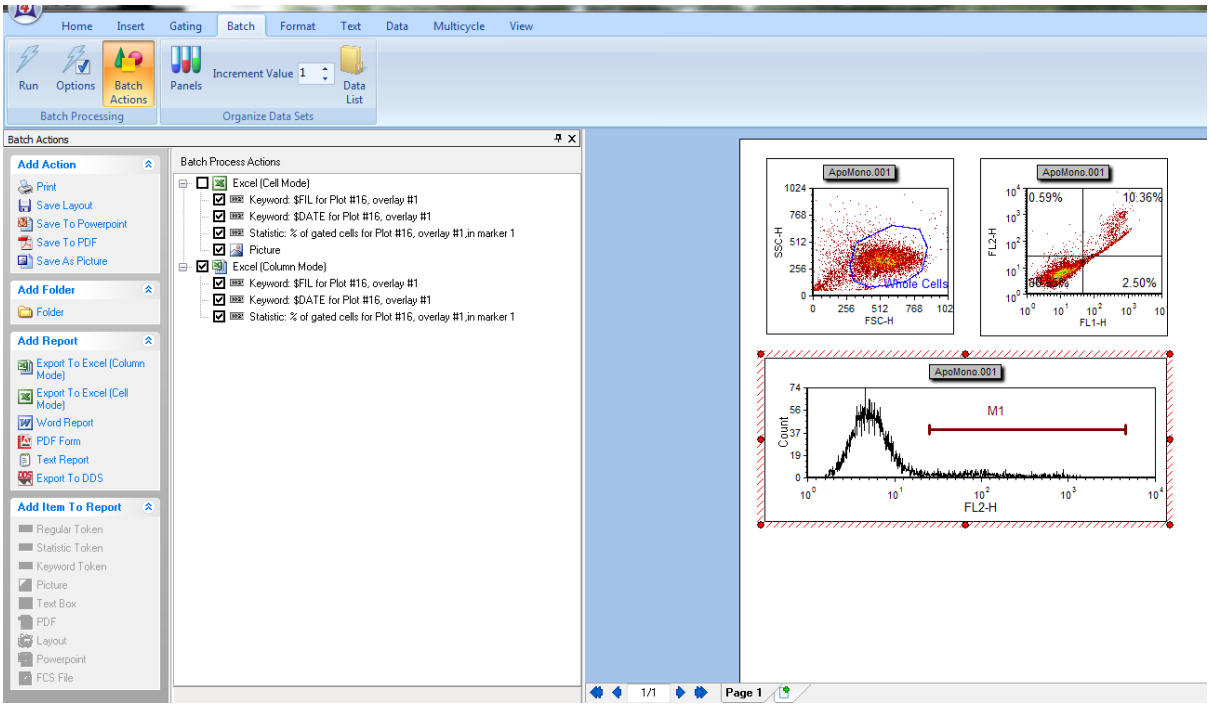

Figure T9.40 Batch Actions Window Docked to the Left Side of the Layout

34.9.4 Exporting to LabKey

LabKey Software and De Novo Software have worked together to provide direct export capabilities from FCS Express 4 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 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 386.
- Add the following items to the LabKey Server Report Batch Actions 386:
  - Keyword Tokens 342
  - Statistic Tokens 337
  - FCS Express Layout (.FEY) file
  - .FCS files
  - Picture 414 of a 2D plot (JPEG fomat)

To export to LabKey Server:

The first step in exporting to LabKey Server is to register and install

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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.

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 **Application Button→Open Layout**.

4. Open the layout **LabKeyServerExportTutorial.fey** found in the "LabKey Server Export" folder within the Tutorial Sample Data archive 590.

   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 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.

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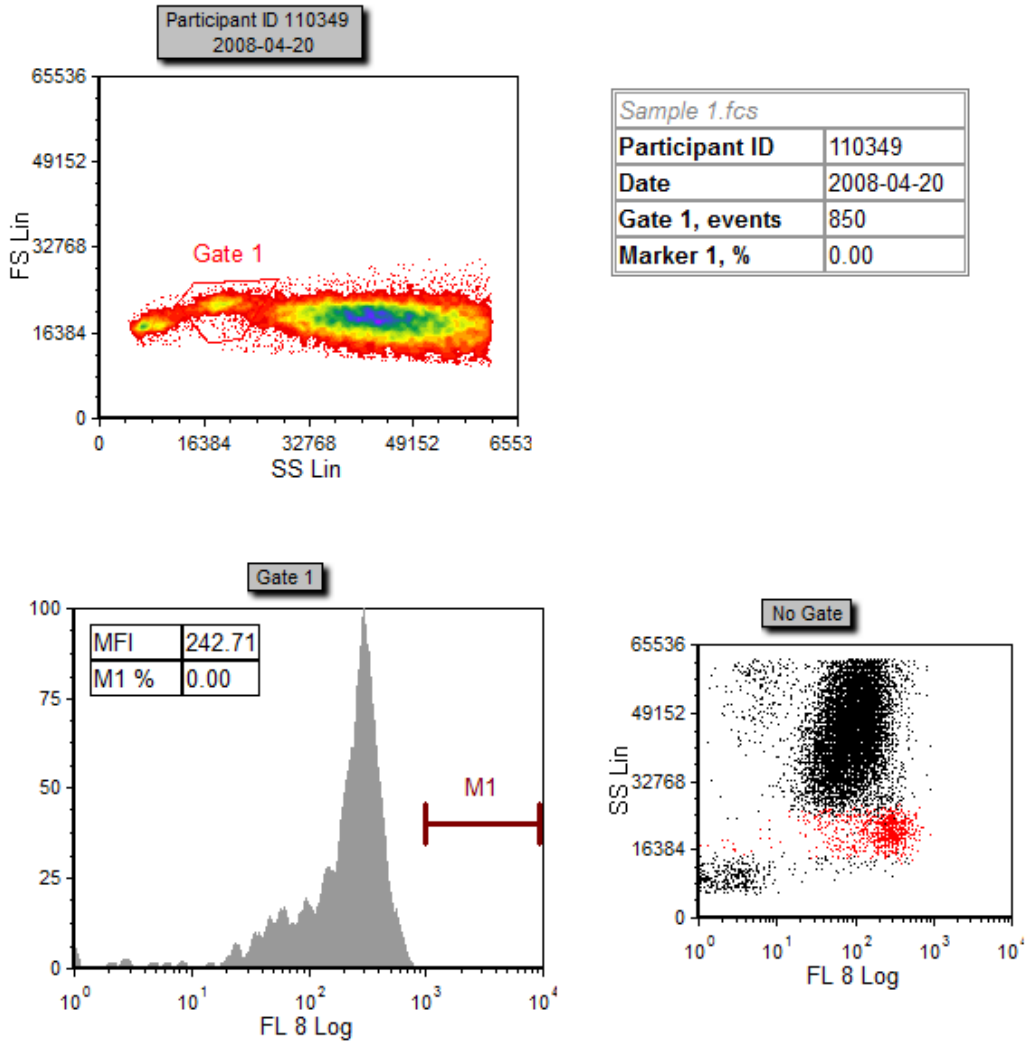

Figure T9.40 Example of the LabKeyServerExportTutorial.fey layout

5. Select the **Batch→Batch Processing→Batch Actions** command (Figure T9.41).

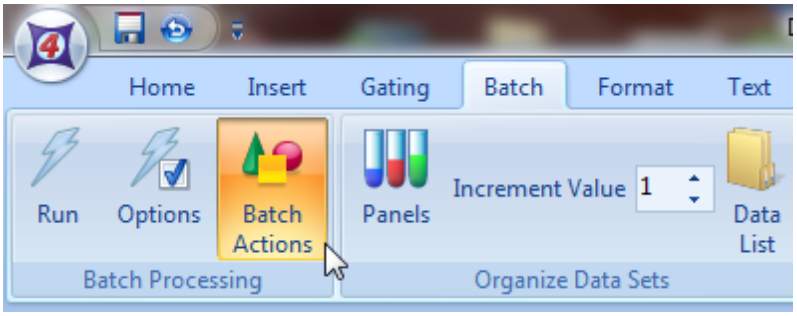

Figure T9.41 Selecting the Batch Actions command

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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).

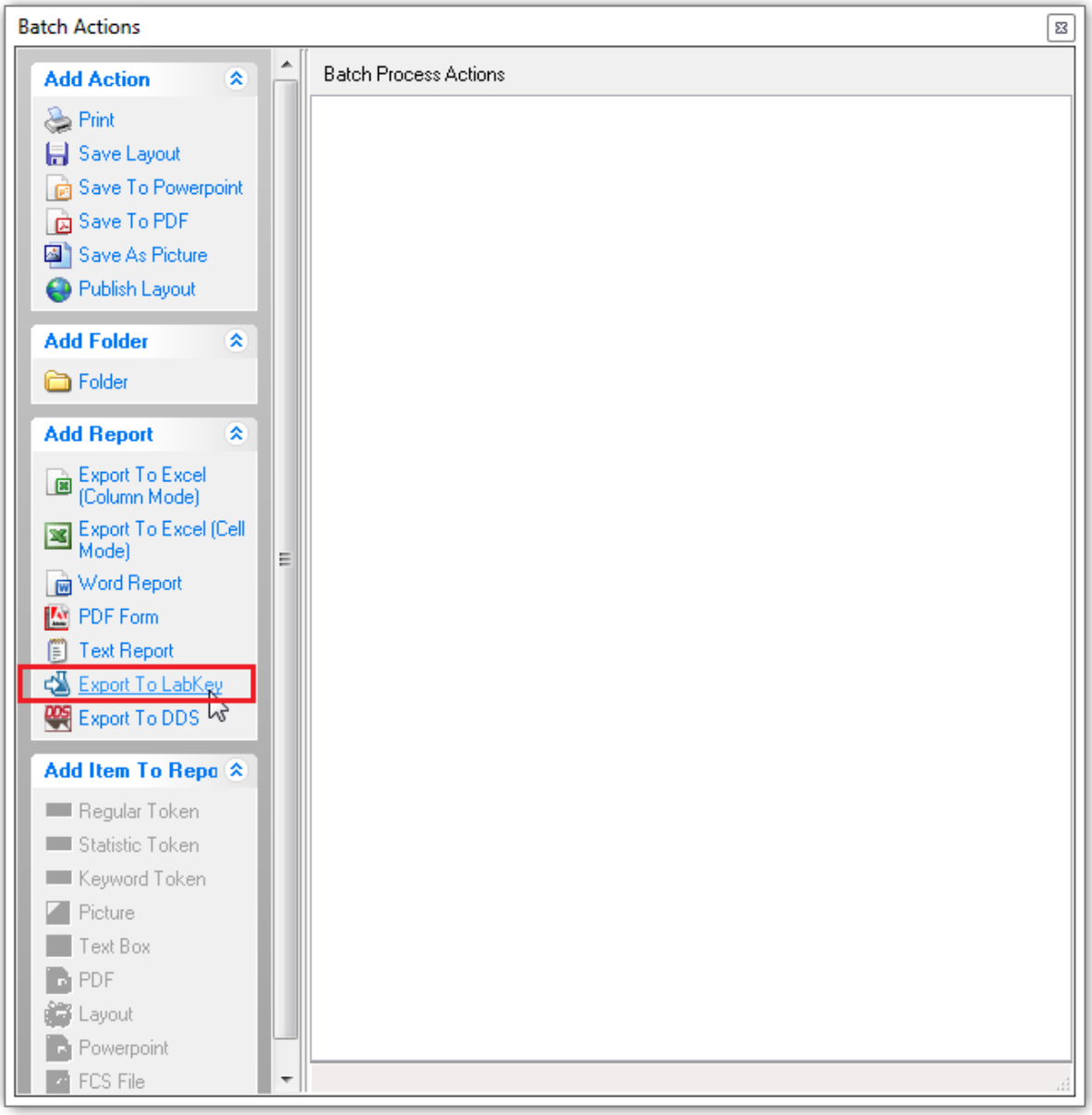

Figure T9.42 Adding a LabKey Report

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

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LabKey tutorial.

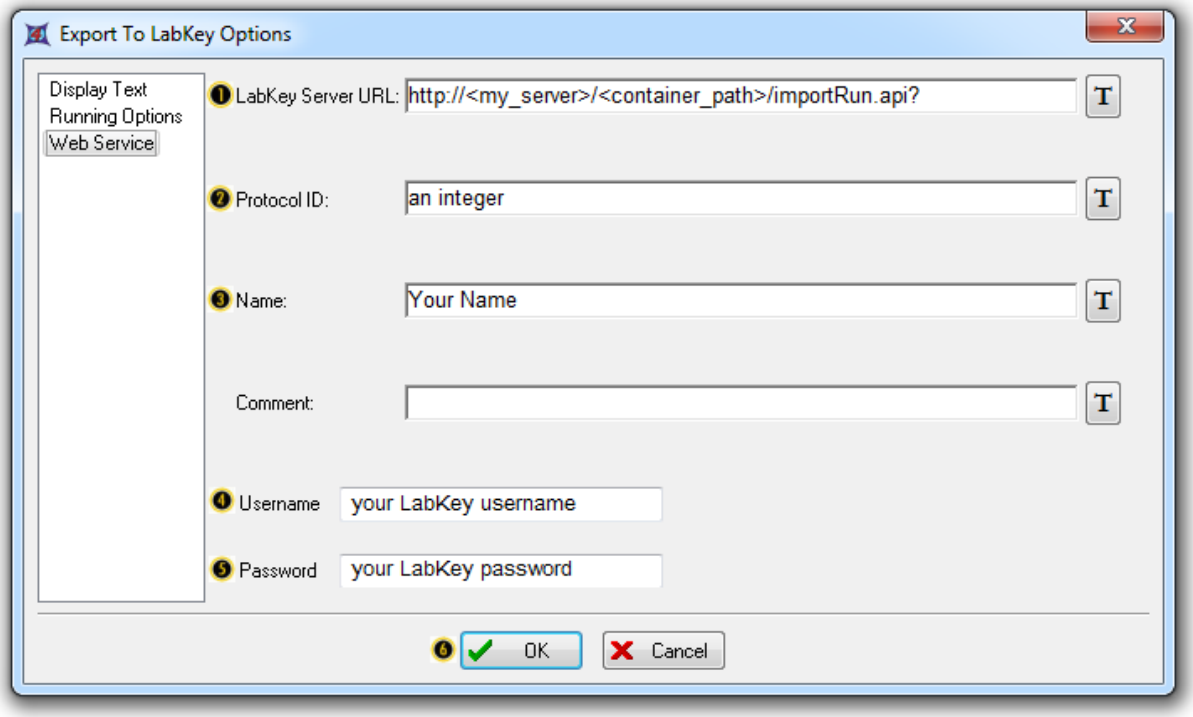

Figure T9.43 Export to LabKey Options dialog box

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).

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**

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action which has just appeared in the **Batch Actions** window (Figure T9.44, red arrow).

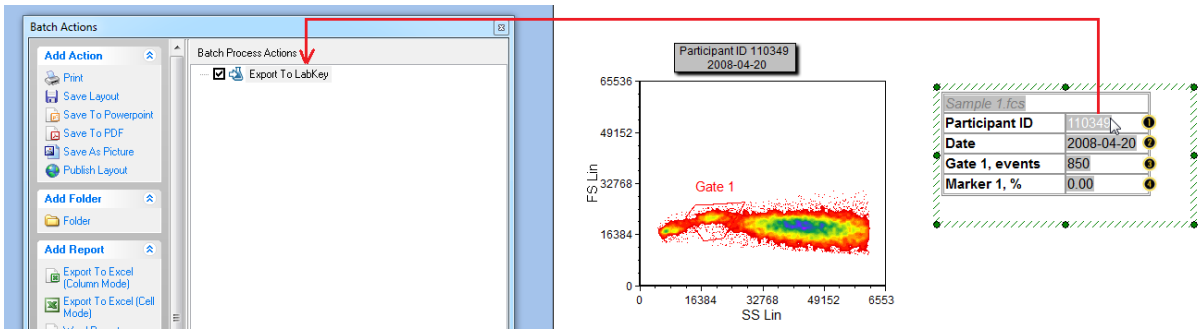

Figure T9.44 Dragging and dropping Tokens from the Table into the Batch Actions Window

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).

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).

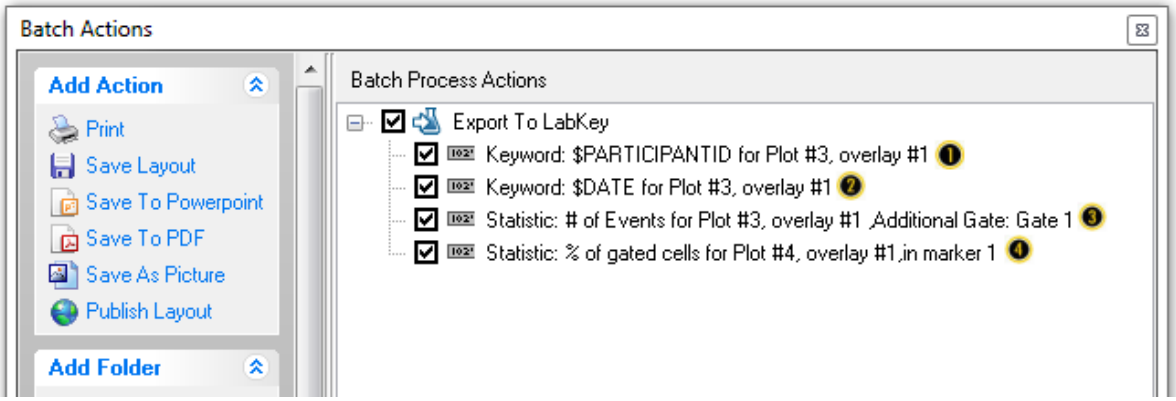

Figure T9.45 Export to LabKey Batch Action with Tokens added to Report

17. Click on the **Field** category on the left (Figure T9.46 ❶).

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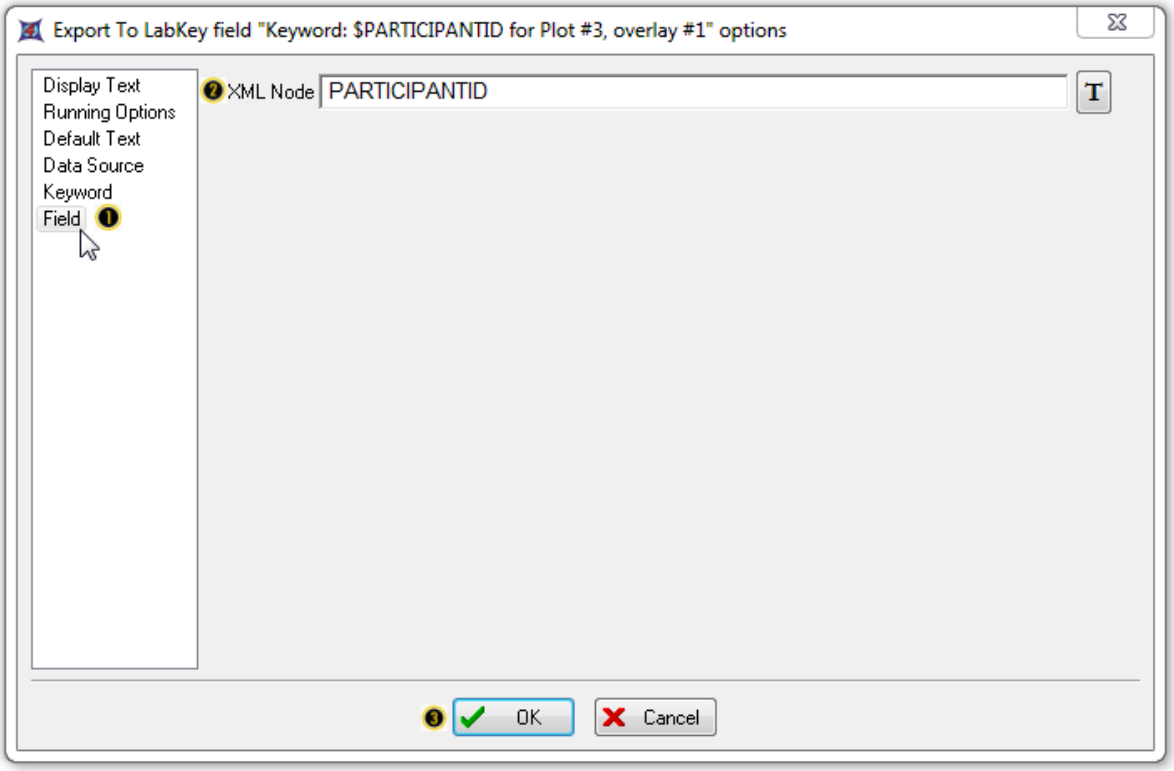

Figure T9.46 Entering text into the XML node field

18. Type the text "PARTICIPANTID" in the **XML Node** field (Figure T9.46 ❷). The **XML Node** field ensures that this data is exported to the correct Data Field in LabKey Server.

19. Click **OK** ❸.

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

21. Click on the **Export to LabKey** action in the **Batch Actions** window (Figure T9.47 ❶) to highlight it.

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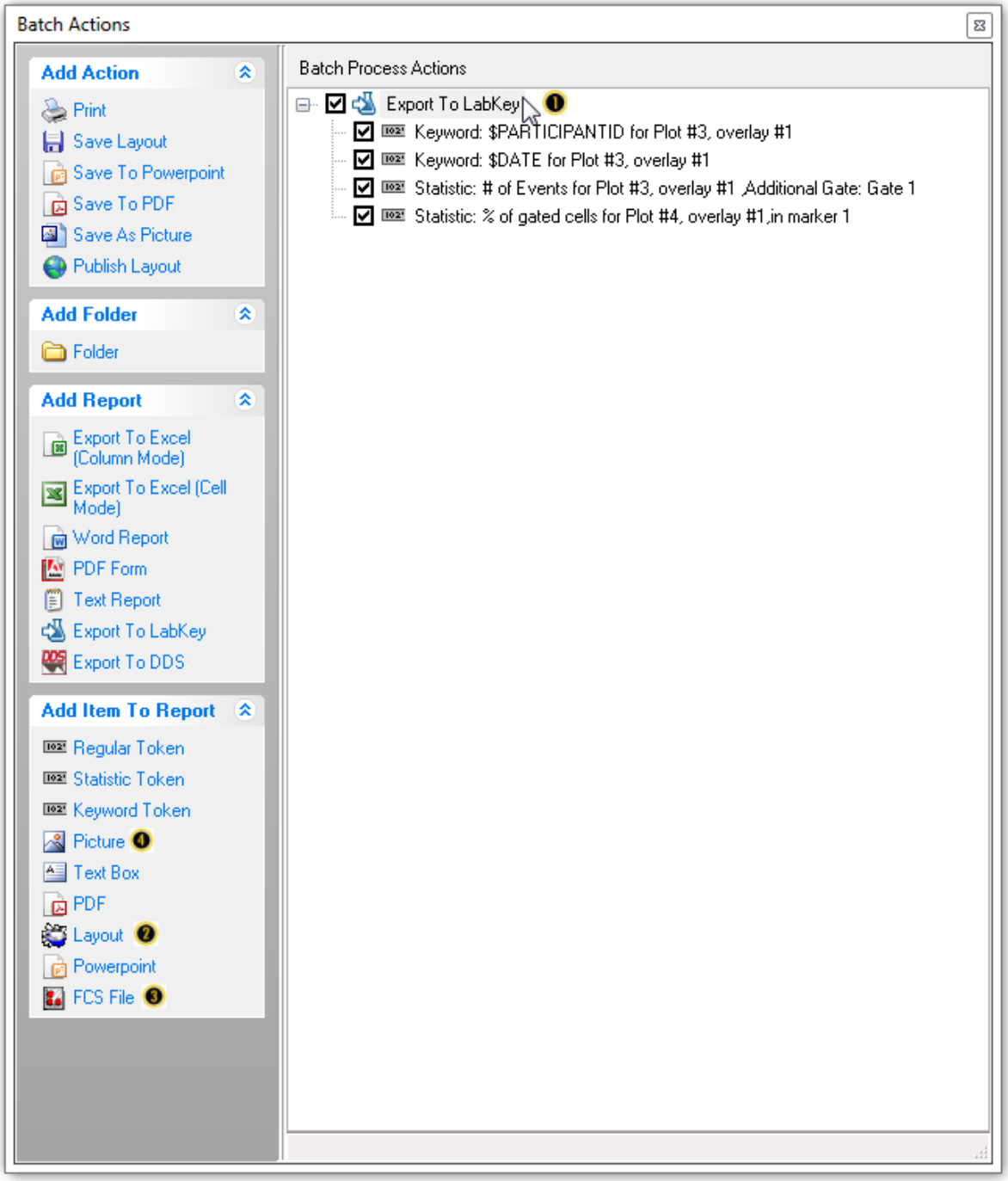

Figure T9.47 Adding items to the LabKey Report

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[53].

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23. Click on the **Field** category on the left of the dialog window which opens (Figure T9.48 ❶).

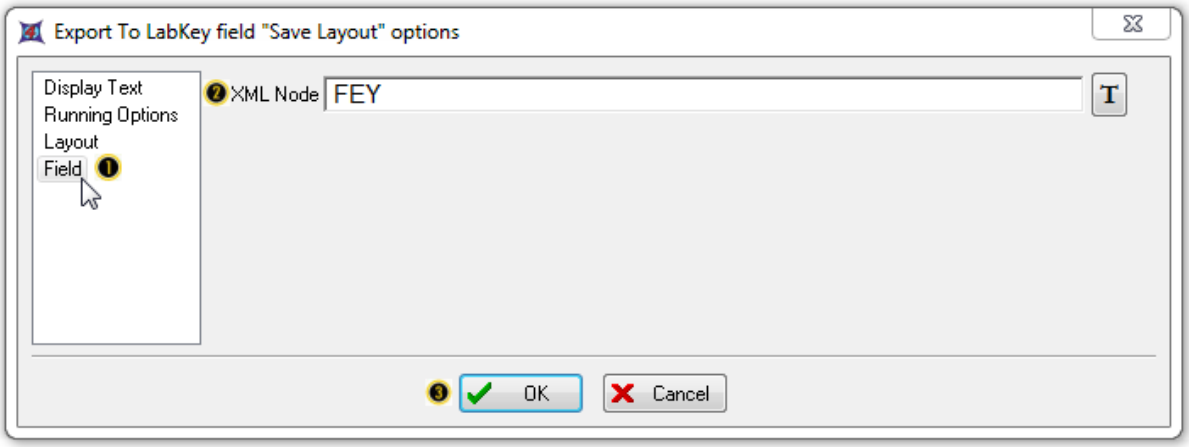

Figure T9.48 Entering text into the XML Node field

24. Type "FEY" in the **XML Node** field (Figure T9.48 ❷).

25. Click **OK** to close the dialog window (Figure T9.48 ❸).

26. Click on the **FCS File** icon under the **Add Item to Report** category of the **Batch Actions** window (Figure T9.47 ❸).

27. 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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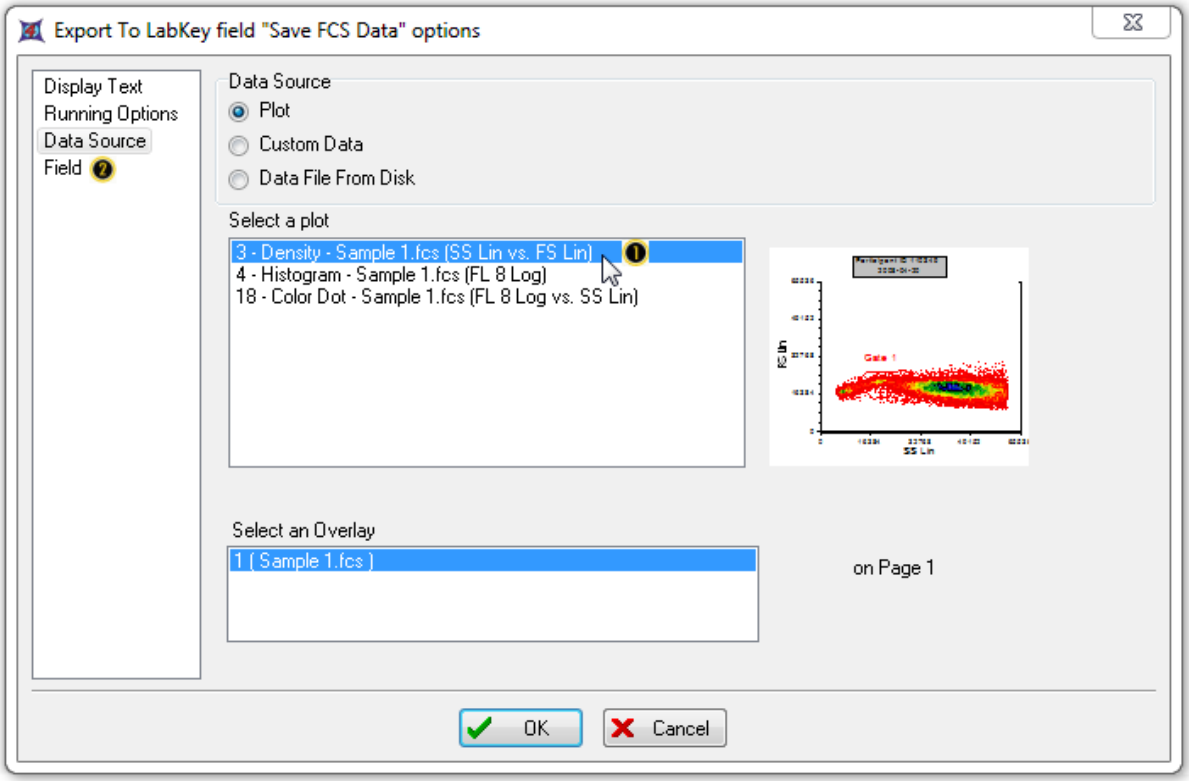

Figure T9.49 Data Source options for exported FCS files

28. Click on the **Field** category on the left of the dialog window (Figure T9.49 ❷).

29. Type "FCS" in the **XML Node** field (Figure T9.50 ❶).

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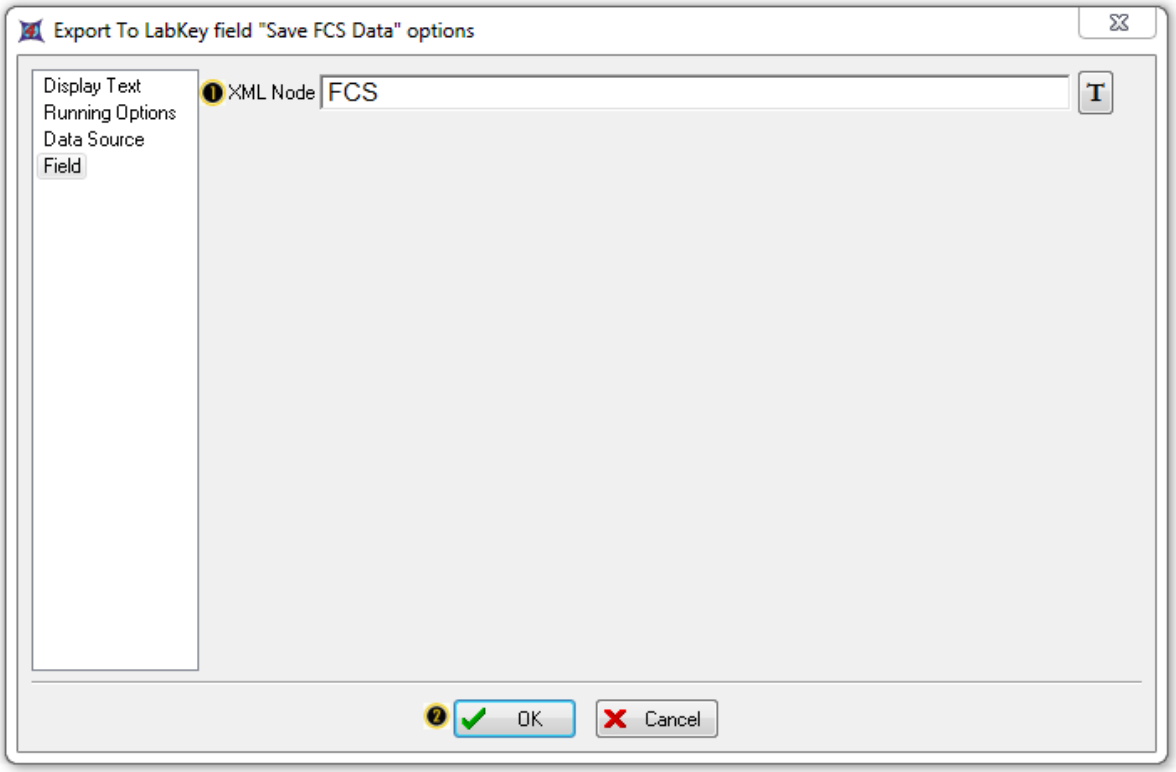

Figure T9.50 Entering text into the XML Node field

30. Click **OK** to close the dialog window (Figure T9.50 ❷).

31. Click on the **Picture** icon under the **Add Item to Report** category of the **Batch Actions** window (Figure T9.47 ❹).

32. Select **JPEG** from the **Format** drop-down menu at right, in the window that opens (Figure T9.51 ❶).

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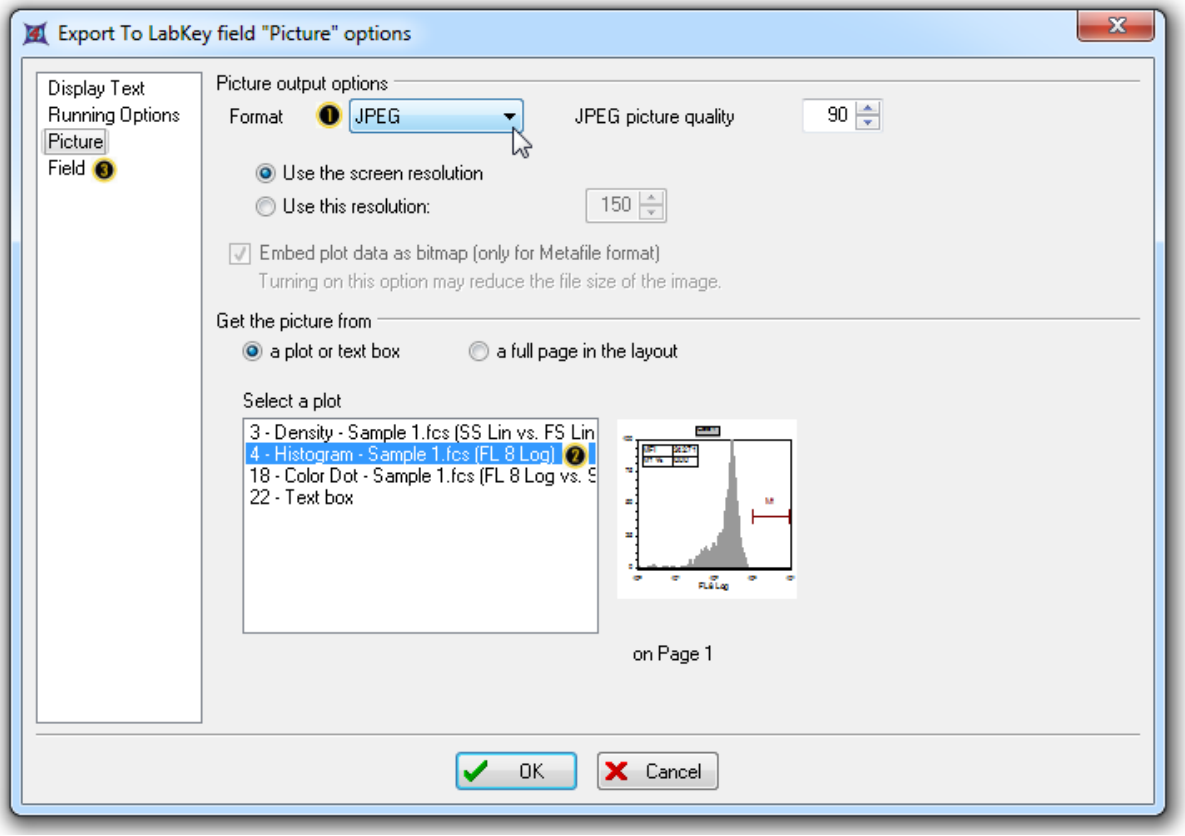

Figure T9.51 Format and data source options for exported pictures

33. 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).

34. Click on the **Field** category on the left of the dialog window (Figure T9.51 ❸).

35. Type "JPEG" in the **XML Node** field (Figure T9.52 ❶)

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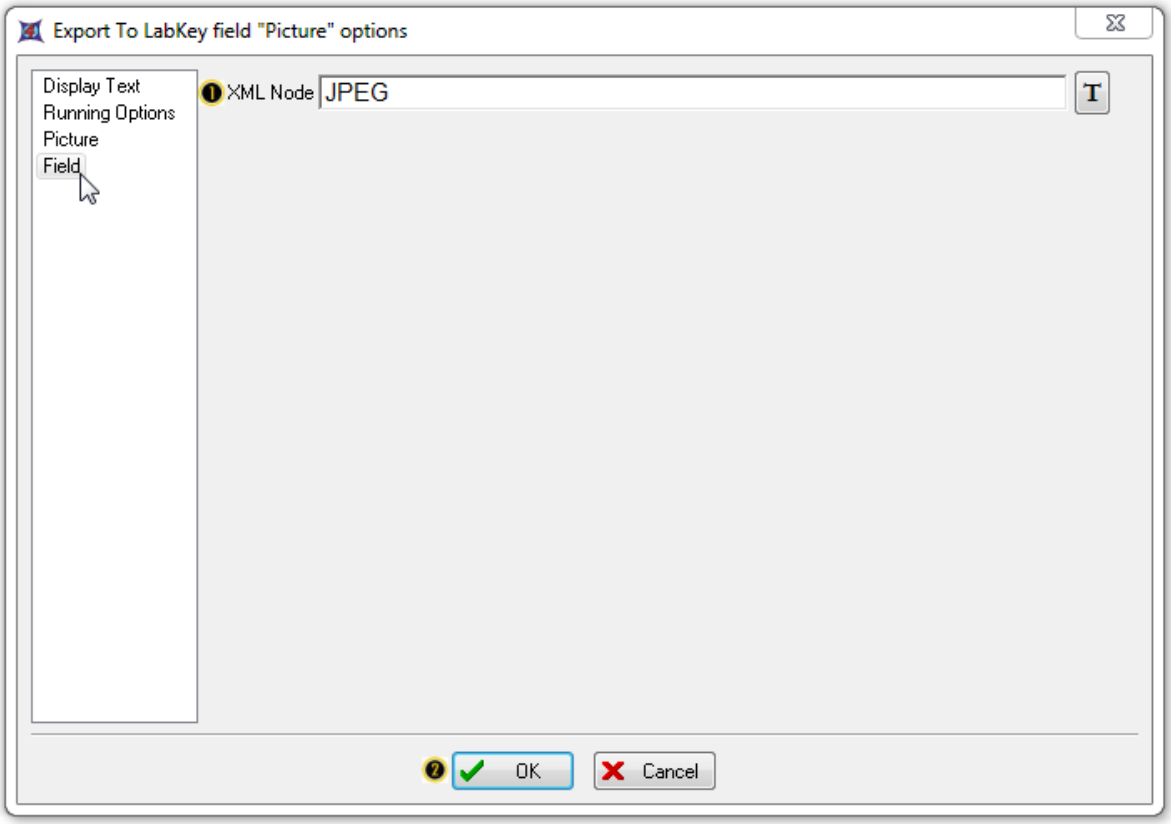

Figure T9.52 Entering text into the XML Node field

36. Click **OK** to close the dialog window (Figure T9.52 ❷).

37. Select the **Batch→Batch Processing→Run** command (Figure T9.53). FCS Express will now export the items to the LabKey Server.

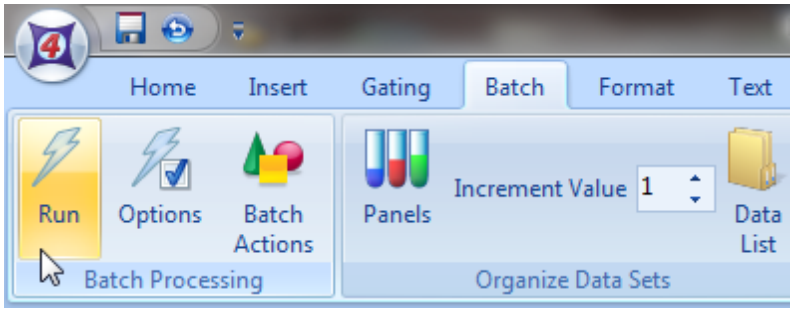

Figure T9.53 Running the Batch Process

38. 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.

You have now set up a **LabKey Report** in the **Batch Actions** window from

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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.

34.10 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 422 after the layout has already been created.

In the first section, we will set up the panel 821.

34.10.1 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 Annexin V – PI staining of polymorphonuclear cells from human peripheral blood. The panel consists of four different calcium concentrations that comprise the "set" that will repeat itself. The analysis will be repeated for three different treatments.

We will begin by defining the panel and then setting up the file 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**

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command.

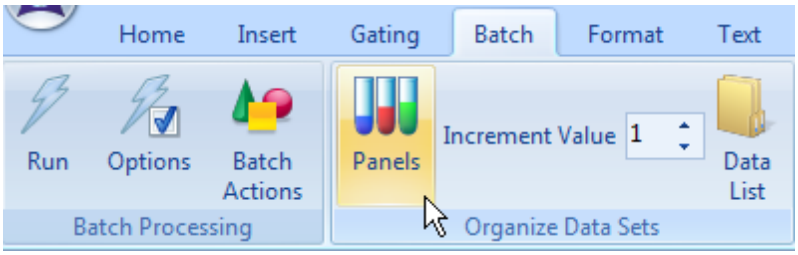

Figure T10.1 Selecting the Panels Command

The **Panel Information** dialog appears as shown in Figure T10.2.

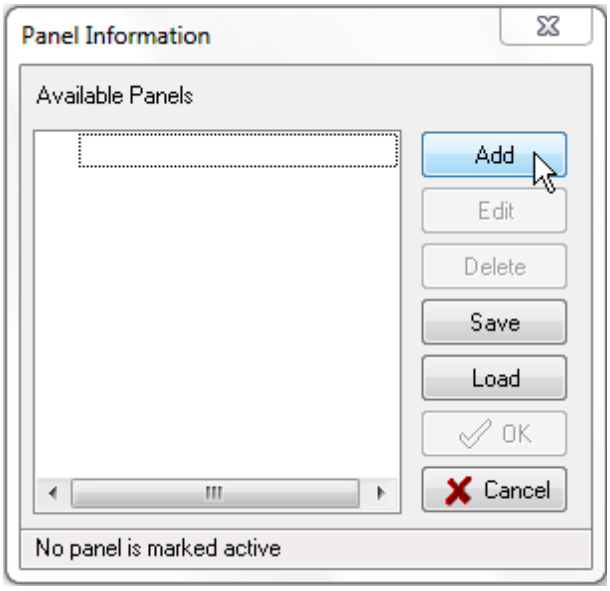

Figure T10.2 Panel Information Dialog

3. Click the **Add** button (Figure T10.2).

The **Add Panel** dialog will appear as shown in Figure T10.3A.

4. Enter "**Calcium Concentrations**" in the **Panel Name** field.
5. Click on the upward triangle in the **Number of Tubes** spin control until it reaches **4**. The dialog should now look like Figure T10.3B.
6. Enter the following text for each tube in the **Name** column, in order from

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1 to 4: "**1 mM Ca**", "**1.75 mM Ca**", "**2.5 mM Ca**" and"**5 mM Ca**". The dialog should now look like Figure T10.3C.

7. Click **OK** to close the **Add Panel** dialog.

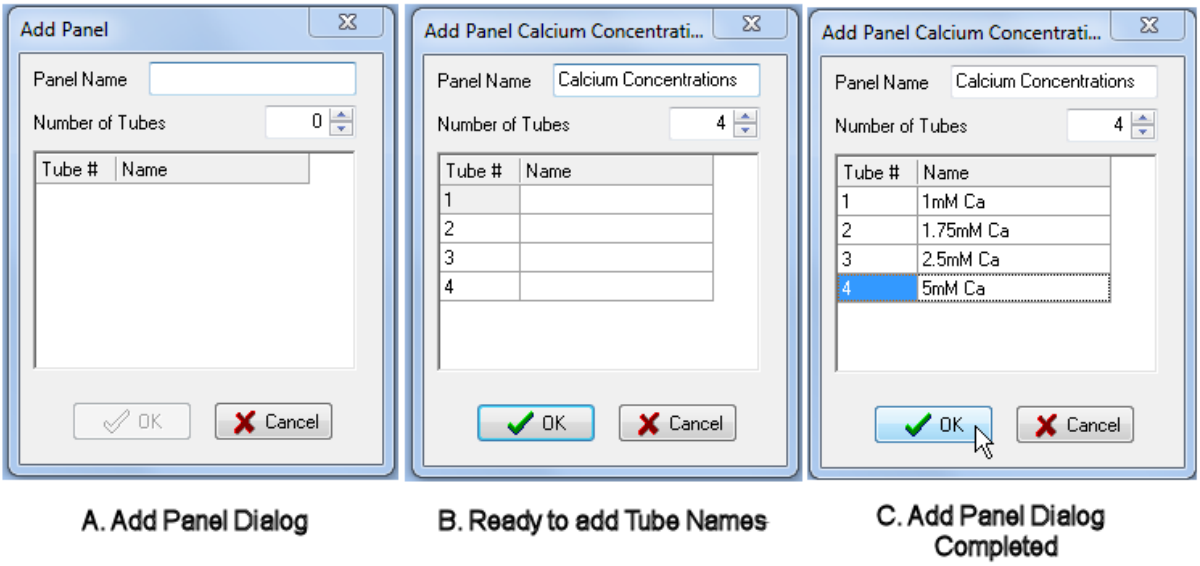

Figure T10.3 Setting up a Panel using the Add Panel Dialog

The **Panel Information** dialog will now appear updated with the newly added panel information, as shown in Figure T10.4.

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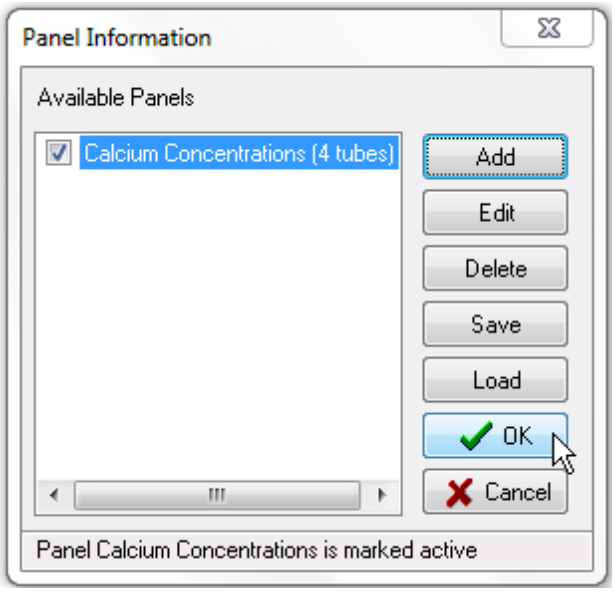

Figure T10.4 Updated Panel Information Dialog

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.

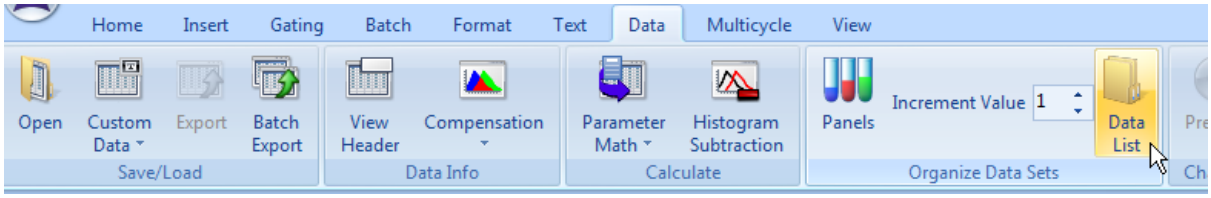

Figure T10.5 Selecting the Data List Command

A **Data List** window opens as shown in Figure T10.6.

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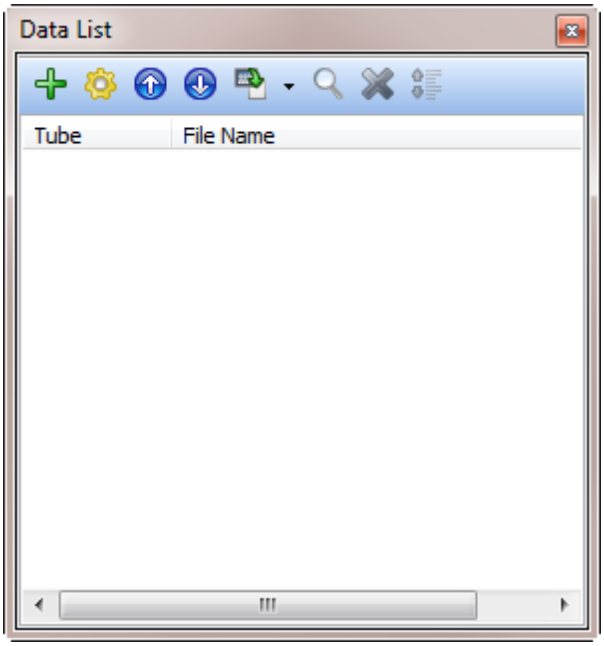

Figure T10.6 Data List Window

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 12 files named **ApoPMN.001** through **ApoPMN.012** as shown in Figure T10.7. (To select multiple files at once, click on the first file, **ApoPMN.001**, press and hold the **Shift** key on the keyboard, and then click on the last file, **ApoPMN.012**.)

13. Click **Open** (Figure T10.7).

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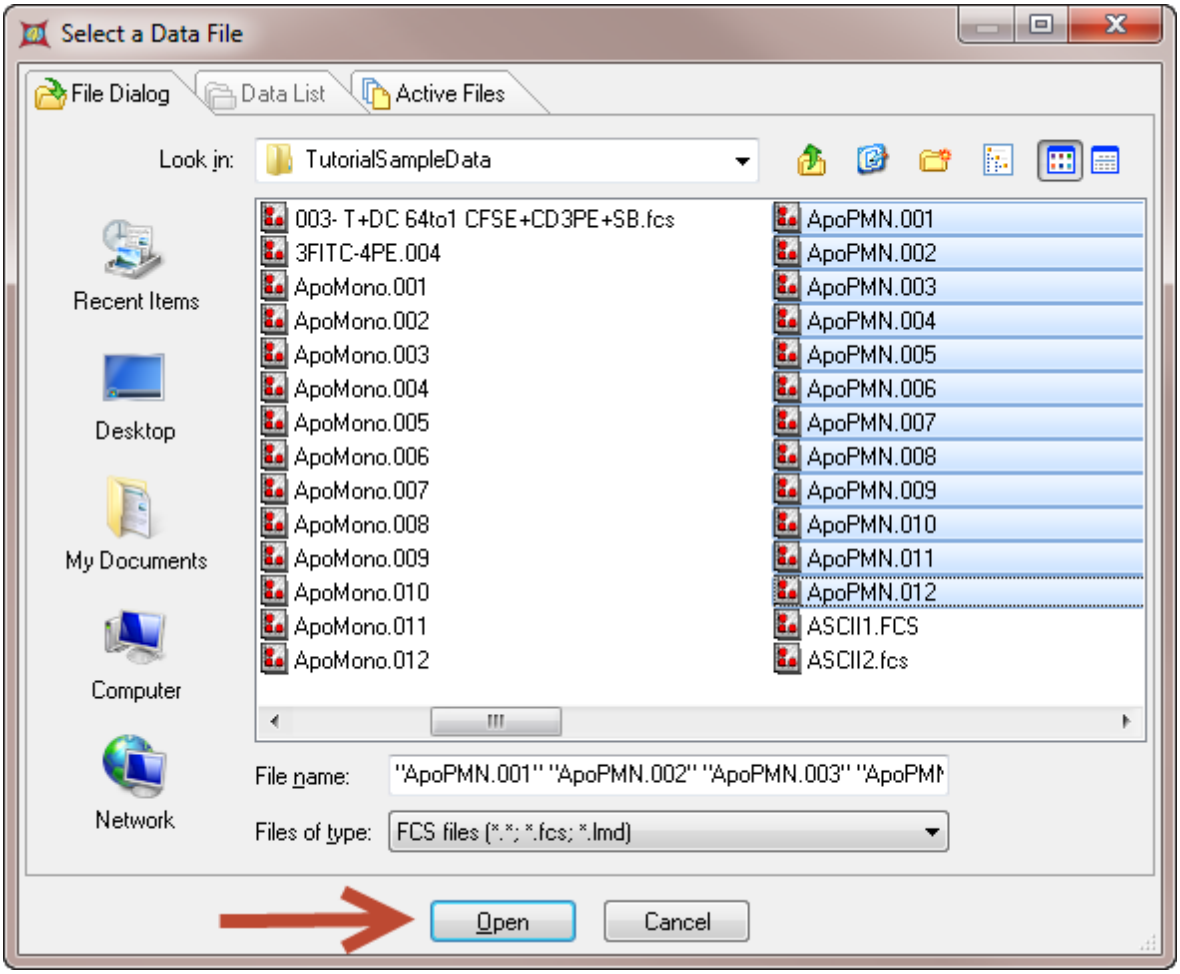

Figure T10.7 Selecting ApoPMN Files

The **Data List** window is now updated with the list of files, and the associated pathnames, for the files just selected. The **Data List** should appear as shown in Figure T10.8, although different pathnames maybe displayed.

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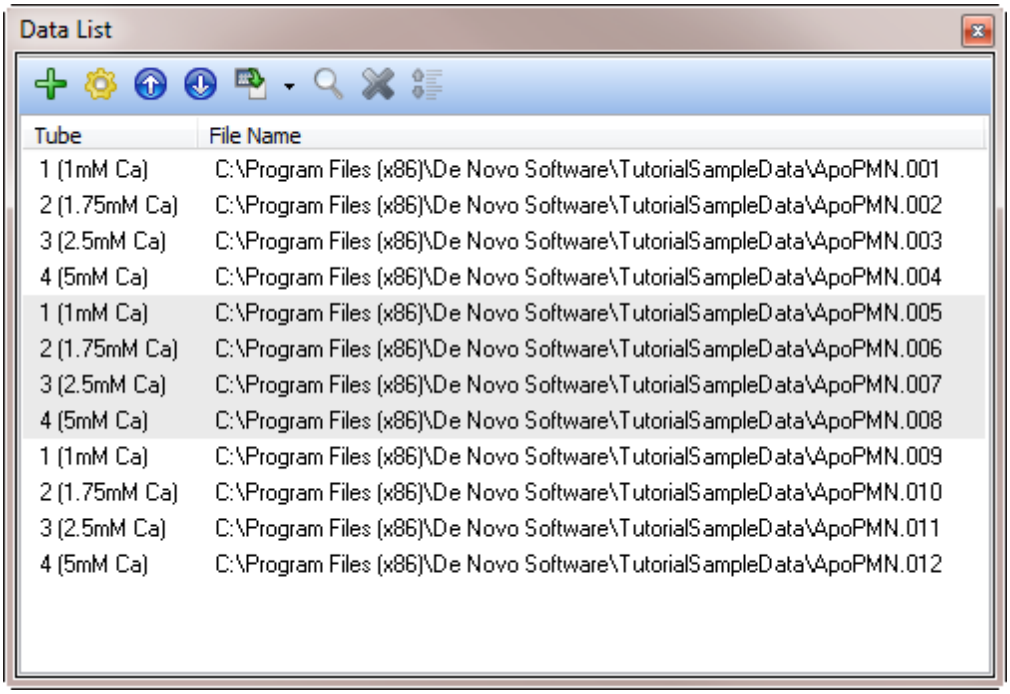

Figure T10.8 Updated Data List with Corresponding Tubes for the Panel

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 file list. We will now proceed to add the **Sample ID**, from the FCS keyword, to the file list.

14. Click on a sample in the file 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 File List** dialog.

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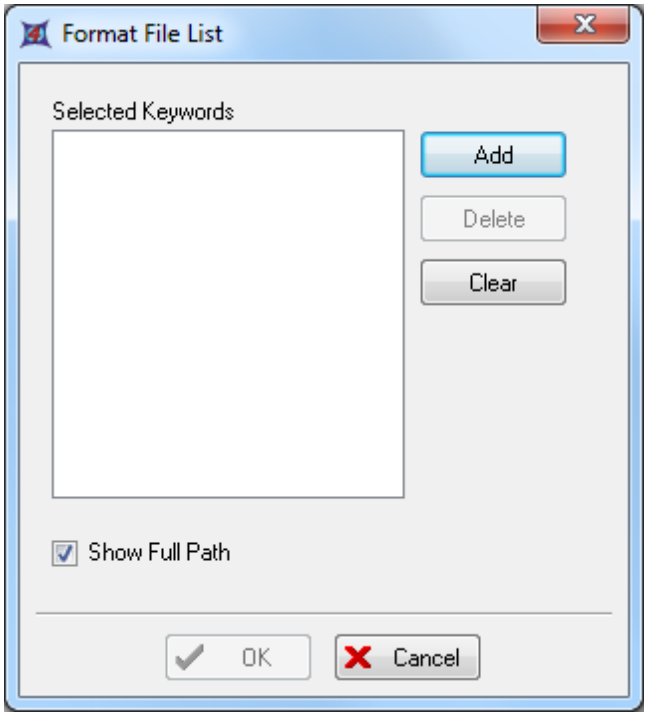

Figure T10.9 Format File List Dialog

The **Please select one or more keywords** dialog appears, as shown in Figure T10.10. This is where we will select the keyword **Sample ID** to display in the **Data List**.

17. Click on the down arrow to scroll down to the bottom of the list.

18. Click on **SAMPLE ID**, highlighted in Figure T10.10. If you want to display more than one keyword, you can select multiple keywords by holding down the **Ctrl** key on the keyboard while clicking on each keyword. For this example, we will only select Sample ID.

19. Click **OK** (Figure T10.10).

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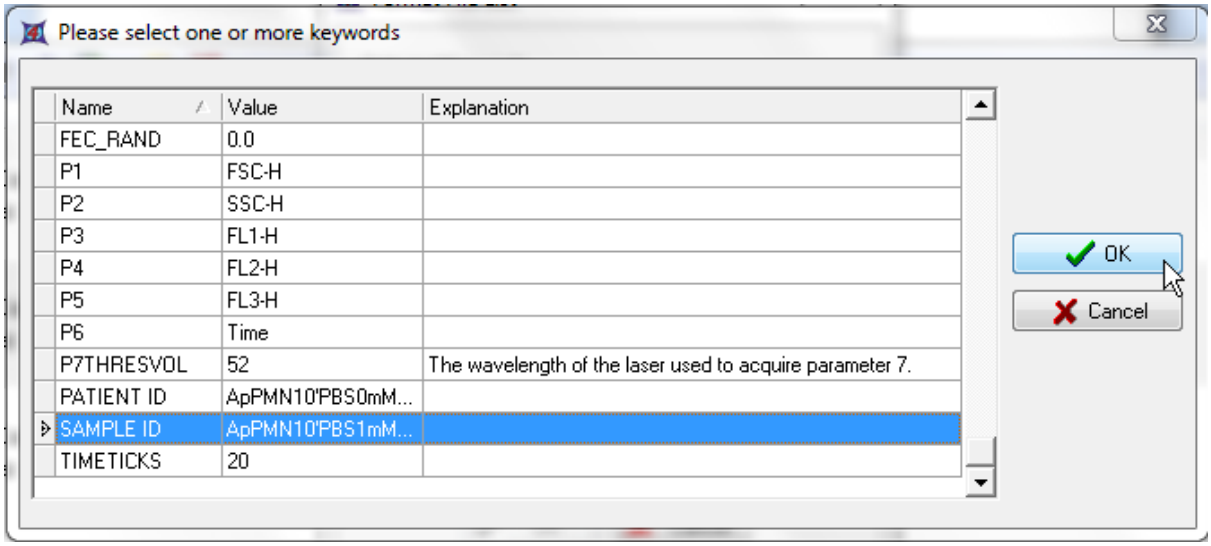

Figure T10.10 Selecting an FCS Keyword to use in the Data List

The **Format File List** dialog will be updated with **SAMPLE ID** in the **Selected Keywords** field, as shown in Figure T10.12.

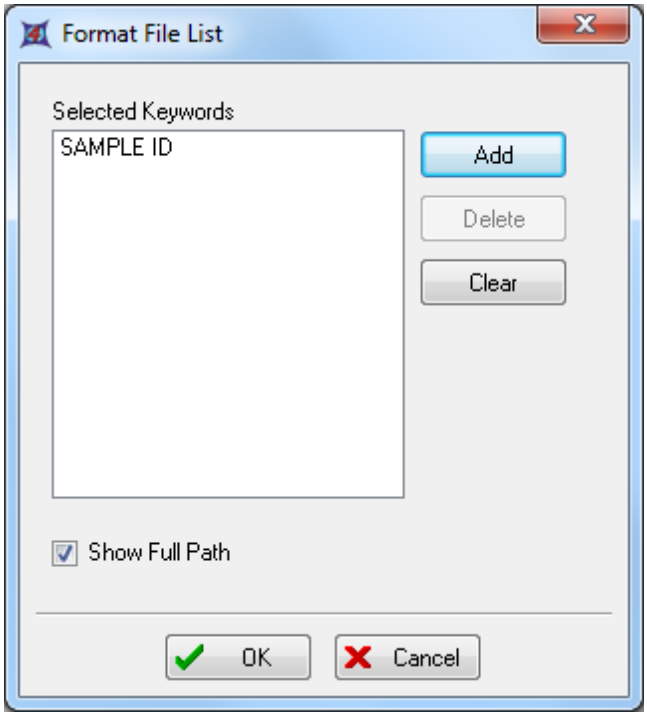

Figure T10.12 Updated Format File List Dialog with SAMPLE ID as the Selected Keyword

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20. Select **OK** on the **Format File List** dialog (Figure T10.12).

The **Data List** dialog will now appear updated with the column **SAMPLE ID**, as shown in Figure T10.13.

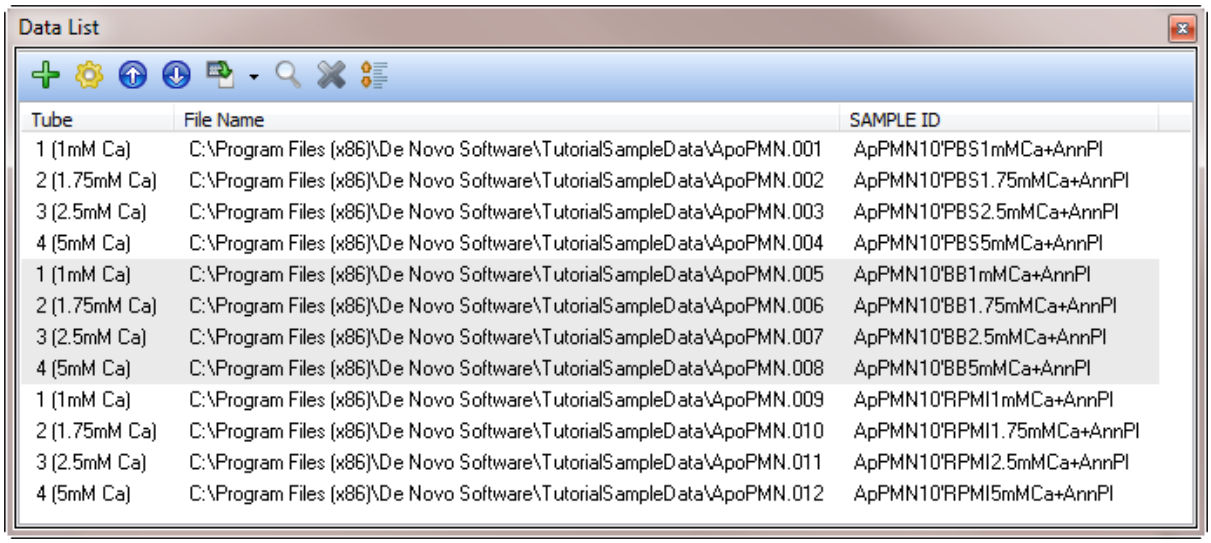

Figure T10.13 Data List Window Updated with the SAMPLE ID Column

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 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.

We will now proceed to creating the layout 830.

34.10.2 Creating the Layout

In the course of this example, we will:

- Create a layout to use for our panel.

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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[607], formatting plots[620], and using gates[650].

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 four files from the list (click on the first one, hold down the **Shift** key and click on the fourth one).
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 **Density Plot** option and uncheck all other plot types.
6. Check the **Use this selection for all files** option.
7. Click **OK** (Figure T10.14).

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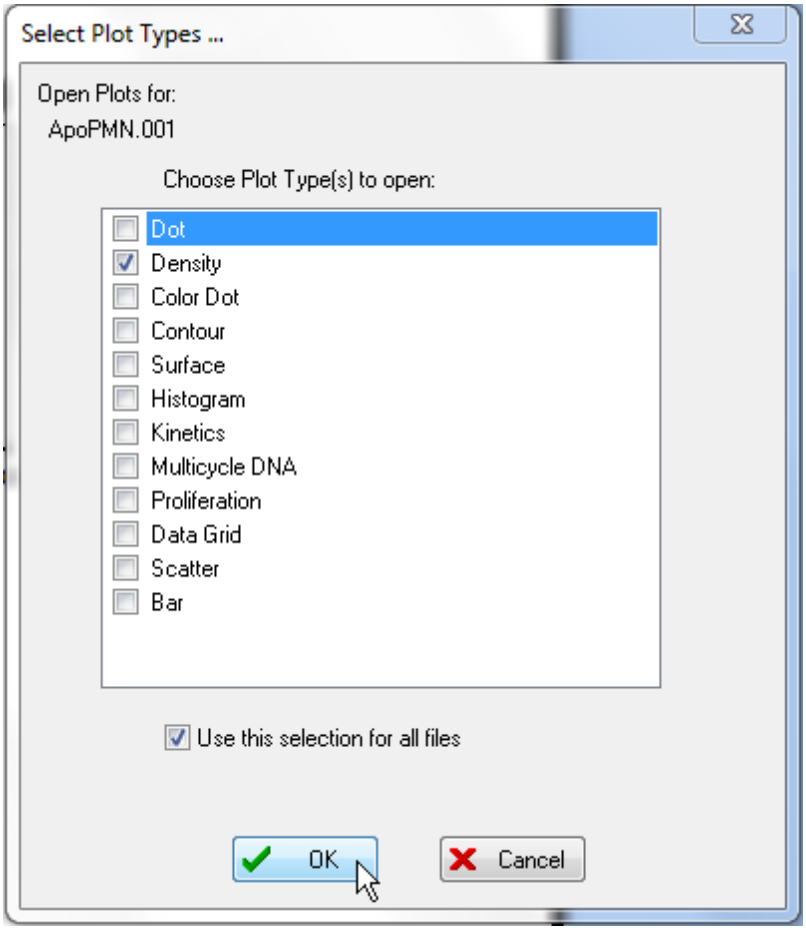

Figure T10.14 Select Plot Types... Dialog

Four density plots, using the first four data files selected in the **Data List**, are now created on the layout. We now need to resize the density plots so that we can arrange them in a column on the layout.

8. Select the four density plots by holding down **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. Select the **Format→Object Options→Size** command (Figure T10.15).

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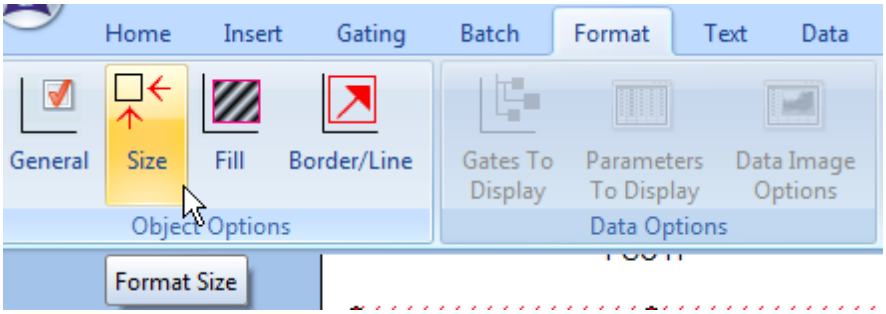

Figure T10.15 Selecting the Format Size Command

A **Formatting Size** dialog similar to Figure T10.16 will appear.

10. Enter "**1.9**" in both the **Height** and **Width** fields, as shown in Figure T10.16. Alternatively, click on the down arrow button next to the **Height** and **Width** field to lower the value in the fields to "**1.9**".

11. Click **OK**.

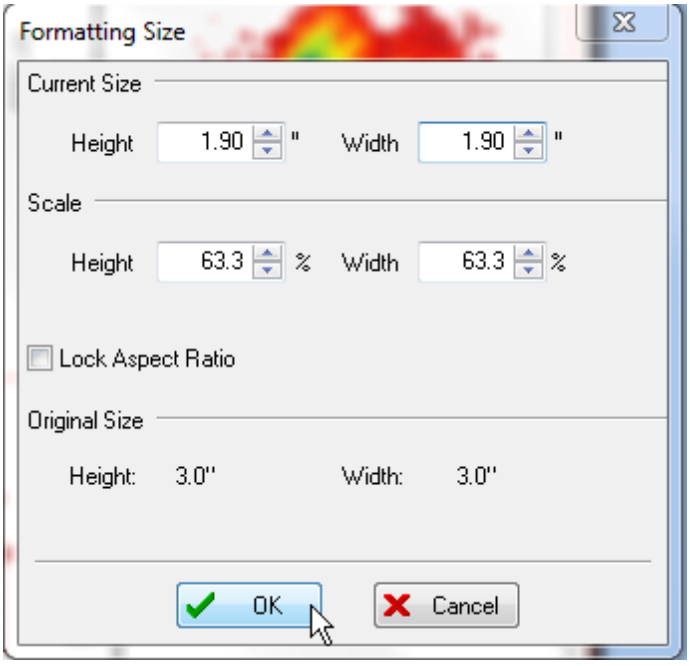

Figure T10.16 Formatting Size Dialog

We will now set the Zoom to be able to view the whole layout on the screen so that we can then more easily arrange the four density plots on the layout.

12. Select Zoom from the Zoom Toolbar (Figure T10.17).

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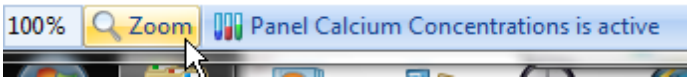

Figure T10.17 Zoom Toolbar

The **Zoom** dialog will appear (Figure T10.18).

13. Select the **Full Page** radio button.

14. Click **OK** (Figure T10.18).

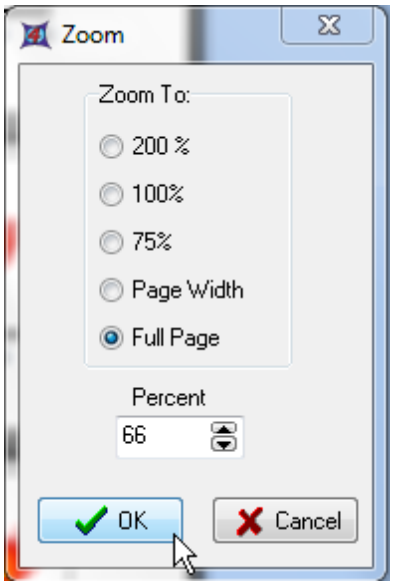

Figure T10.18 Zoom Dialog

The full page of the layout should now be displayed on the screen.

15. Move the density plots and arrange them in a column, in order.

Your layout should look similar to Figure T10.19, although the colors might be different. For more information on manipulating objects in FCS Express please refer to the tutorial on Using Objects 593.

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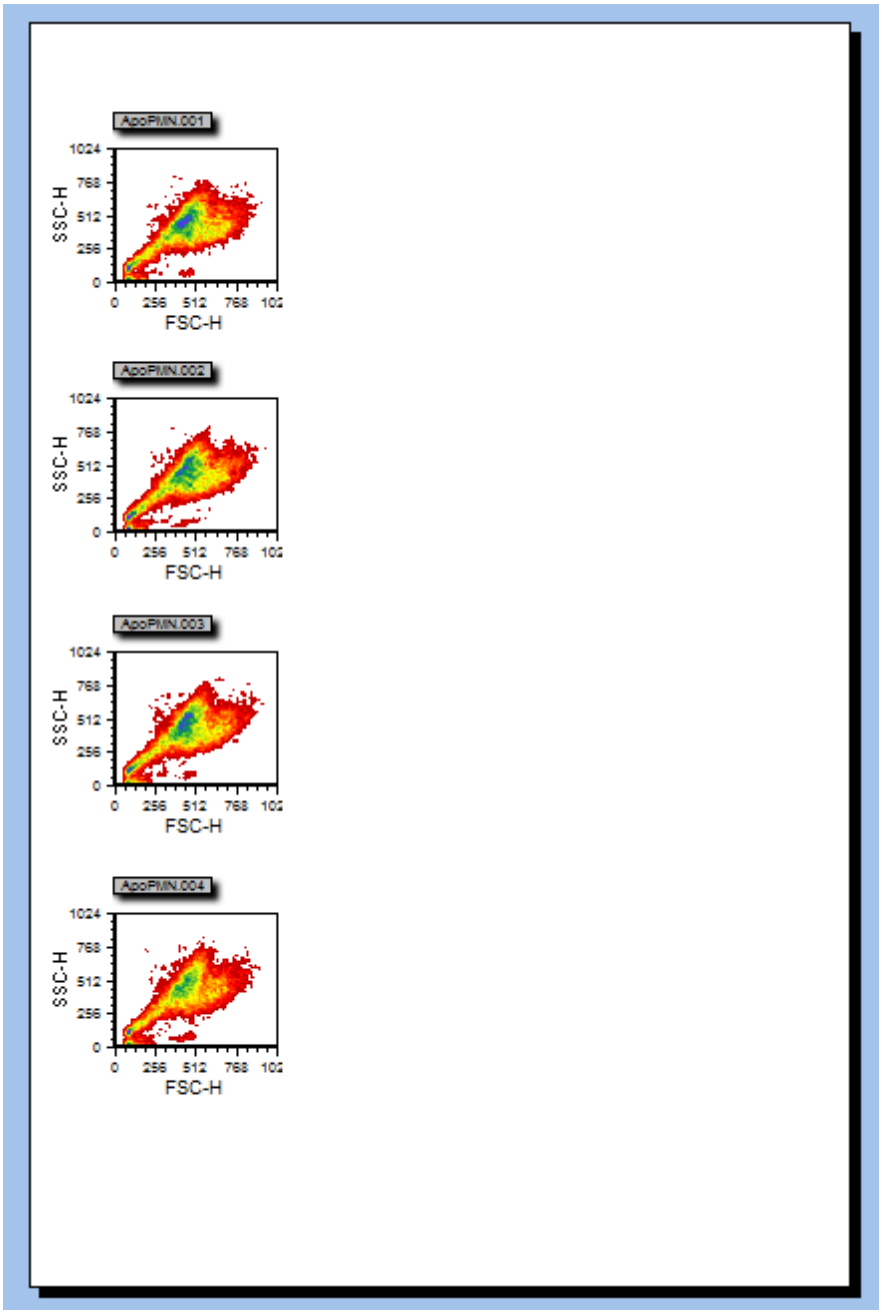

Figure T10.19 Plots Arranged in Order

We will now set the titles of the plots to display the **File Name** and **Sample ID**, just like the **Data List**, in order to better identify them.

16. Select the **Home→Editing→Select All** command, to select all plots.

17. Select the **Format→Plot Options→Title** command.

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The **Formatting Titles** dialog will appear similar to Figure T10.20. Please refer to this Figure for the next steps.

18. Click the **Header** ❶ category on the left panel.
19. Delete the contents of the text box ❷ in the middle of the options area (if there were any contents).
20. Uncheck the **Background visible** ❸ option.
21. Select the **Center** ❹ option in the **Alignment** section.
22. Click the **Insert Keyword** button ❺ (Figure T10.20).

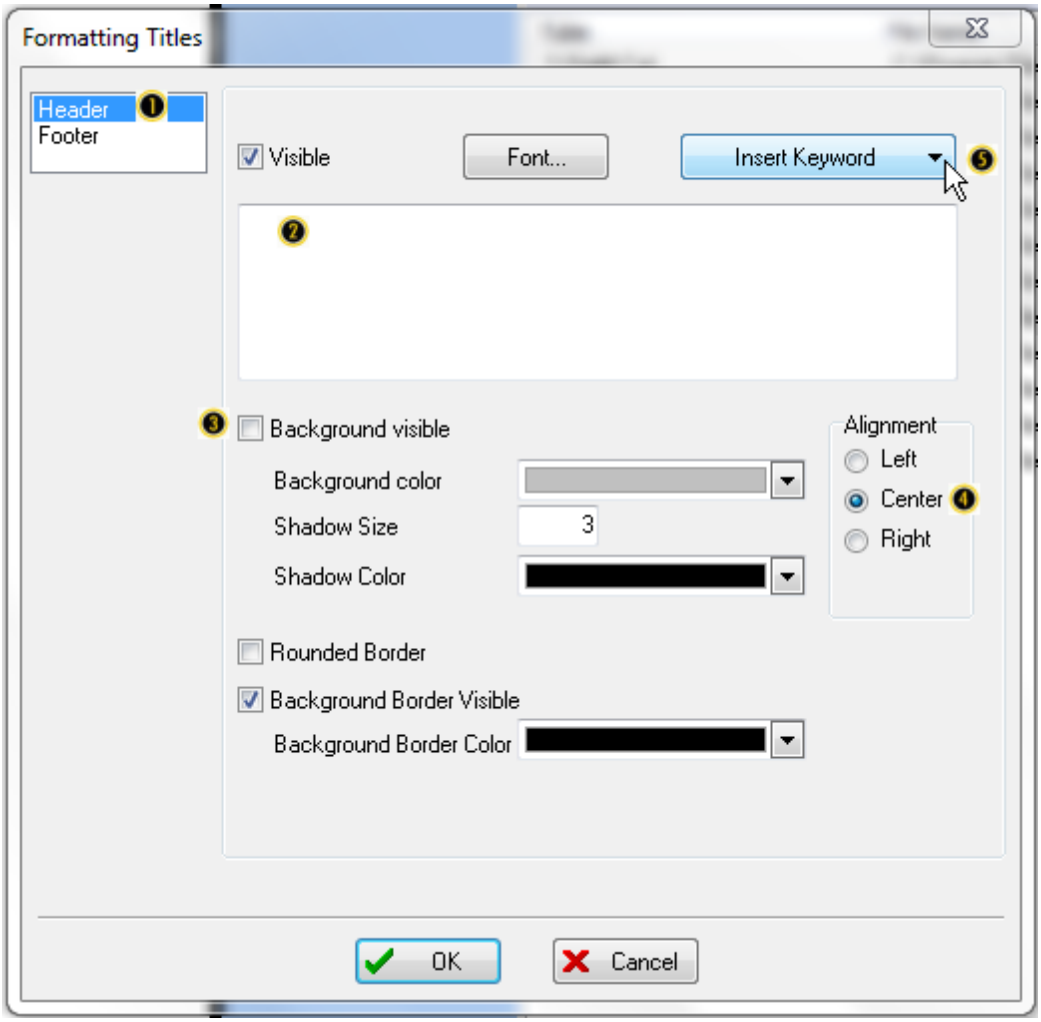

Figure T10.20 Formatting Titles Dialog

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23. Select **File Name without path** from the drop-down list that appears, indicated by the cursor in Figure T10.21.

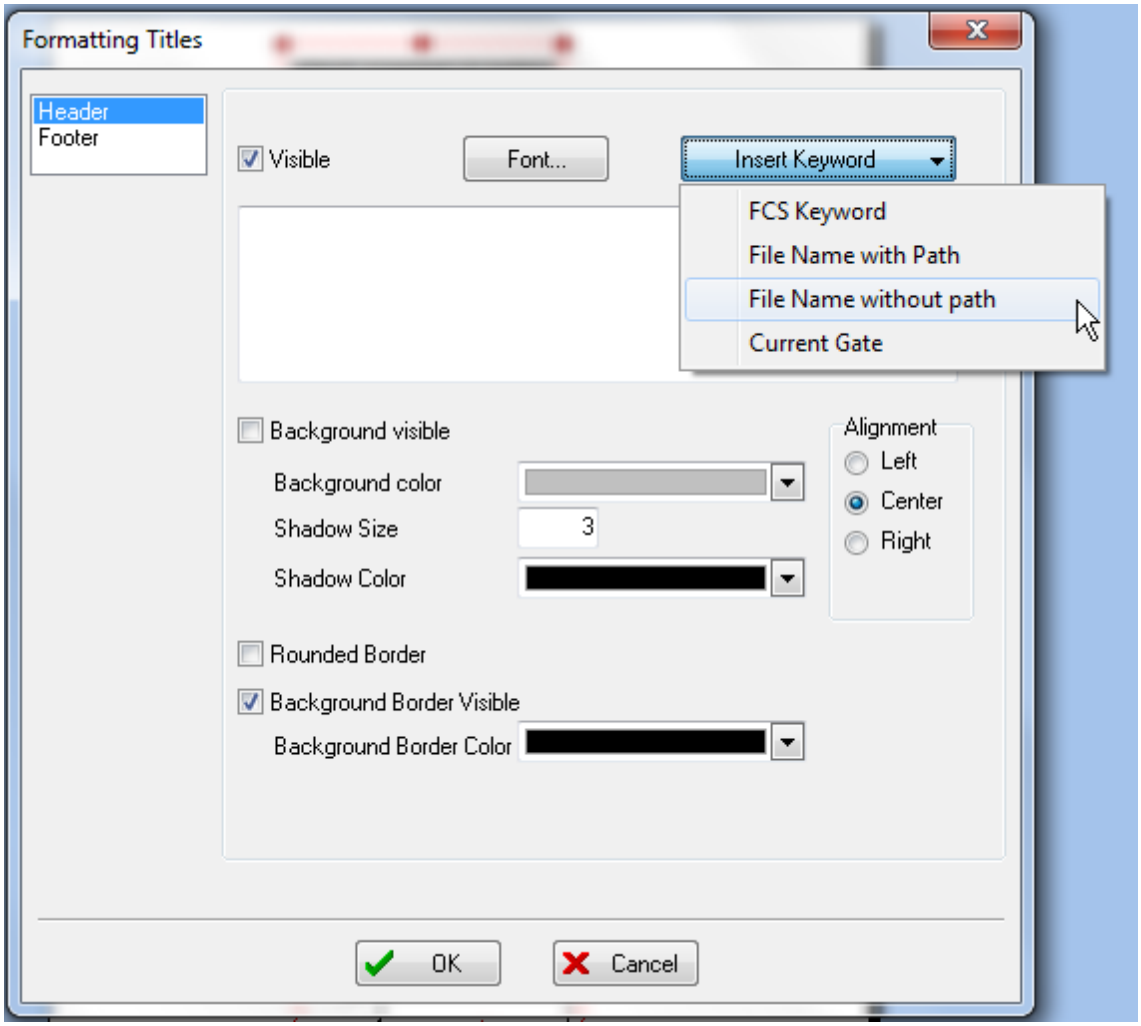

Figure T10.21 Selecting File Name without path from the Insert Keyword drop-down list on the Formatting Titles Dialog

The text string "<$FCSE_FILENAMENOPATH>" now appears in the text box in the middle of the **Formatting Titles** dialog.

24. Click at the end of the text string and insert a blank space with the **space bar**.

25. Click the **Insert Keyword** button.

26. Select **Keyword from data** from the drop-down list that appears.

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The **Please select one or more keywords...** dialog appears, as previously shown in Figure T10.10 821.

27. Scroll to the end of the available keywords list.
28. Click on **SAMPLE ID** (Figure T10.10 821).
29. Click **OK** (Figure T10.10 821).

A second text string will be added to the text box in the **Formatting Titles** dialog, and it should now show "<$FCSE_FILENAMENOPATH> <SAMPLE ID>" and look like Figure T10.22.

30. Click **OK** to close the **Formatting Titles** dialog.

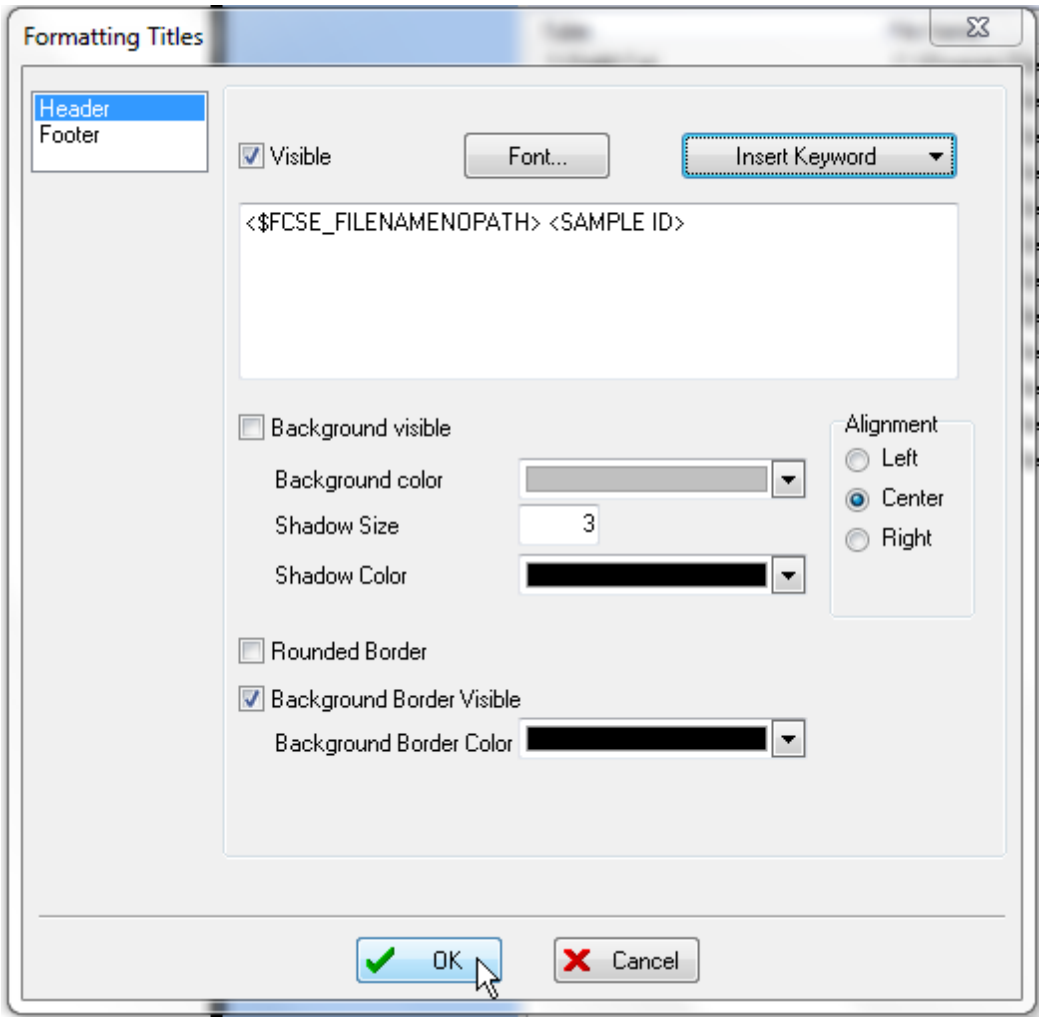

Figure T10.22 Final Selection of Options on the Formatting Titles Dialog

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The plots will now show the title, as shown in Figure T10.23. However, because the plots have been reduced in size some of the title information is cut-off. If you wish to view all the title information for the plots you can use the **Formatting Titles** dialog and select **Font** to reduce the size of the font used in the titles.

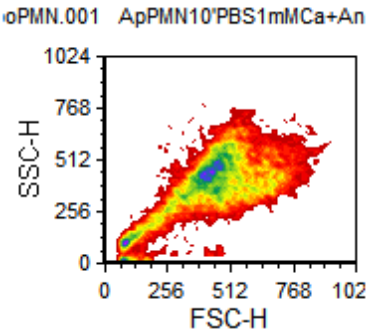

Figure T10.23 Density Plot with FCS Filename and Sample ID in the Title

In the next section, we will use the panel 839.

34.10.3 Using the Panel

In the course of this example, we will:

- Use the panel to analyze the rest of the samples.
- Extend the layout easily using the panel set up in the previous steps.
- Load new data into the existing layout.

We will now proceed to use the panel on the layout we created to easily analyze the rest of the samples.

1. Select the four density plots by holding down the **Ctrl** key on the keyboard and clicking on each plot. Alternatively, select the **Home→Editing→Select All** command, or click and hold the mouse button down and drag a box that encompasses all four plots.
2. Select the **Data→Change Data on Selection→Next** command (Figure T10.24).

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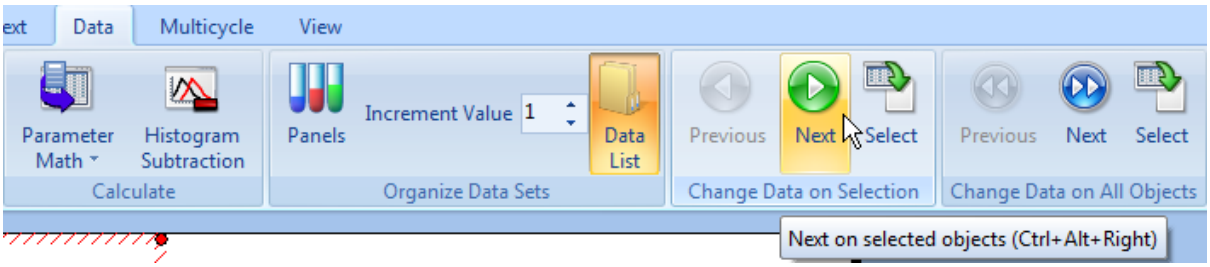

Figure T10.24 Selecting the Next Command

Notice that after clicking the **Next** button, the four selected plots changed, advancing to the next set of data in the panel listed in the **Data List**; files 1 through 4 changed to files 5 through 8.

3. Select **Data→Change Data on Selection→Next** again, to advance to the third and last set of data in the panel in the **Data List**.

Alternatively, without selecting any plots, you can select **Data→Change Data on All Objects→Next** from the ribbon (shown in the far right on Figure T10.24) to advance to the next set of data.

To move back through the data set in the panel, again select all the data plots, then select the **Data→Change Data on Selection→Previous** command. Alternatively, without selecting any plots, select the **Data→Change Data on All Objects→Previous** command to move back through the data set.

If there were any statistics or tokens associated with the plots, they would have advanced in accordance, as well. This is a powerful feature to quickly evaluate experiments or sets of experiments.

Prior to performing the next set of steps, we need to edit the system preferences to ensure we have the desired settings for the next set of operations.

4. Select **Application Button→Edit Preferences**.

The **Edit Preferences** dialog appears similar to Figure T10.25; refer to this figure for the following steps.

5. Select the **General** ❶ category in the left side list.

6. Select **to the right of** ❷ from the **position of the duplicate object** drop-down list in the **Duplicate object options** section.

7. Set **0.18** ❸ inches in the **distance between original and duplicated**

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object(s) field.

8. Click **OK** (Figure T10.25).

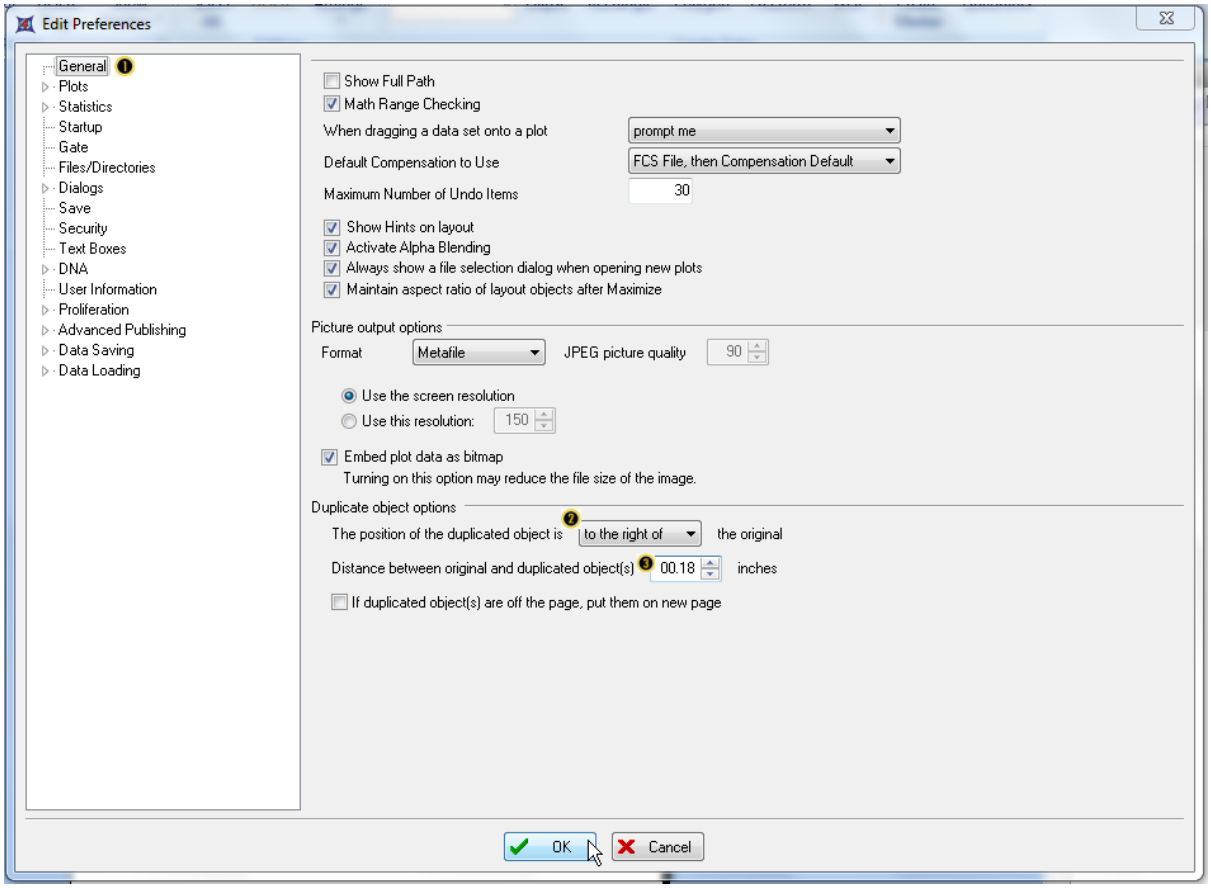

Figure T10.25 Selecting Preferences in the General Category of the Edit Preferences Dialog

Setting these options in the **Edit Preferences** dialog ensures that our plots will be placed 0.18 inches to the right of the original plots when they are duplicated. We will now proceed to expand the layout using the panel.

9. Select the four density plots.

10. Select the **Home→Clipboard→Duplicate** command.

Four new density plots now appear; duplicates of the original four. The new plots are 0.18 inches to the right of the original plots, and they are currently selected.

11. Select the **Data→Change Data on Selection→Next** command.

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The four original plots now show files 1-4, and the second column of plots show files 5-8.

12. Select the four density plots in the second column.

Repeat steps 10 and 11 to duplicate the second column of plots and change the last column of plots to the last set of data in the panel.

Alternatively, you can select the four original density plots in the first column, and then select the **Home→Clipboard→Duplicate→Duplicate and Next** command from the drop-down list associated with **Duplicate** item, as shown in Figure T10.26. This is a handy shortcut that will both duplicate the four plots, and also perform a **Next** operation to display the next data set in the newly duplicated plots.

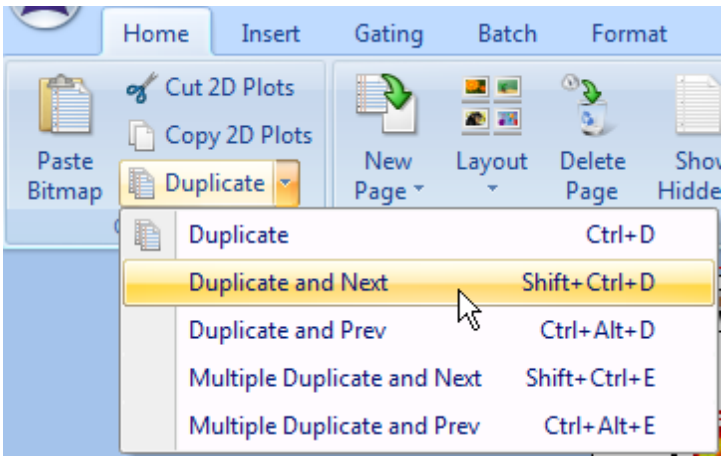

Figure T10.26 Selecting Duplicate and Next from Duplicate

The resulting Calcium Concentrations panel layout, using either method to duplicate and display the data sets, should look similar to Figure T10.27.

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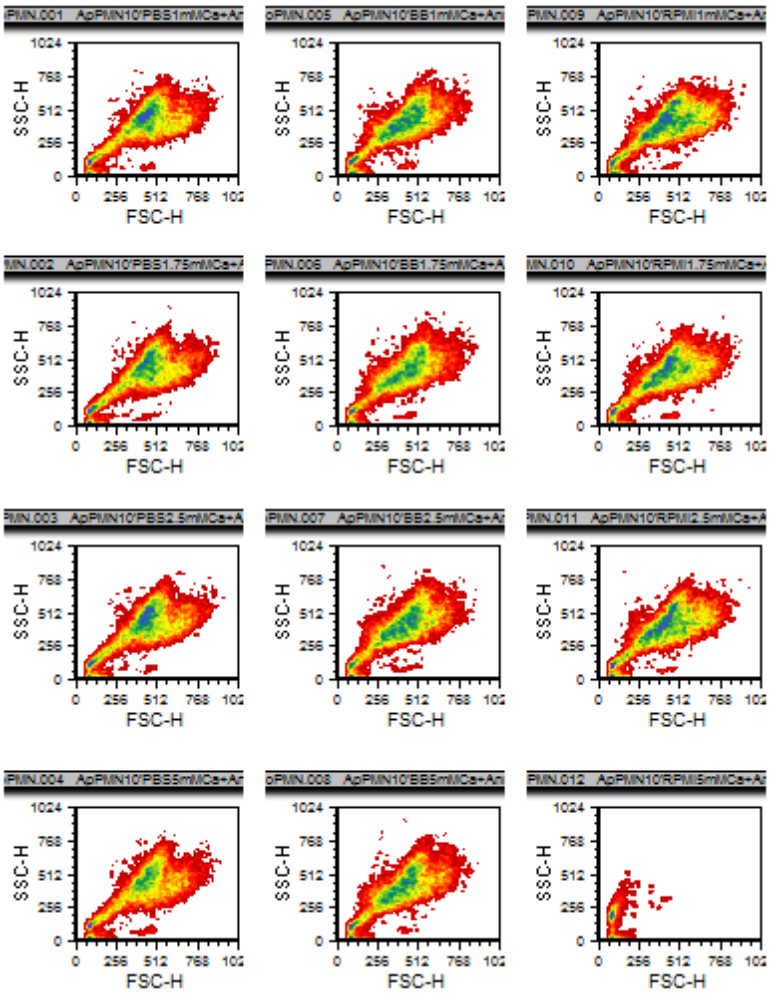

Figure T10.27 Full Panel Layout

As you can see, once a panel is created, you can easily expand your layout to show all your data.

We will now proceed to load new data into the layout. If the **Data List** window is already open, then skip to step 15.

13. Select **Data→Organize Data Sets→Data List** from the ribbon to open the **Data List** window.

14. Select all the files in the **Data List**.

15. Click on the Delete button on the top of the **Data List** dialog, to remove all the files.

All the density plots on the layout will now appear blank because we have

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deleted on the data files from the **Data List**.

16. Click on the **Add** button at the top of the **Data List** window.

The **Select a Data File** dialog will appear, as previously shown in Figure T10.7 821.

17. Navigate to where the **Sample Data** folder is located.

18. Select the files named **ApoMono.001** through **ApoMono.012**.

19. Click **Open** on the **Select a Data File** dialog.

All the previous files in the layout are now replaced by the files just selected (**ApoMono.001** – **ApoMono.0012**). Note how their Sample ID matches the Panel tube in the **Data List**. Remember that if some of them do not match you can reorder the files in the list.

Note that all the density plots have been replaced by the new files – in the correct place and order! This is a powerful way to recycle and reuse your standard layouts. You can repeat the same procedure for any new experiment, reusing the analysis layout, by just opening the old layout, loading the new data and then saving the layout with a new name.

Finally, we will see examples of saving and loading panels 844.

34.10.4 Saving and Loading Panels

In the course of this example, we will:

- 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 the Calcium Concentrations panel listed and marked as active, as previously shown in Figure T10.4 821.

2. Click the **Save** button on the **Panel Information** dialog.

The **Save As** dialog appears.

3. Navigate to the **Sample Data** folder and enter "**PanelDemo**" in the File Name field.

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4. Click **Save** on the **Save As** dialog.

We have now saved the Calcium Concentrations panel. We will now save the current layout, and then load the saved panel into a new layout.

5. Click on **Cancel** to close the **Panel Information** dialog.

6. Select **Application Button→Save Layout**, to save the current layout. Alternatively, select the **Save** icon located in the Quick Access Toolbar 34.

If you have not previously saved the layout then then the **Save Layout** dialog appears.

7. Navigate to the **Sample Data** folder and enter "**PanelDemoLayout**" in the File Name field.

8. Click **Save** on the **Save Layout** dialog.

9. Select **Application Button→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, as previously shown in Figure T10.2 821.

11. Click the **Load** button on the **Panel Information** dialog.

The **Open** dialog will now appear.

12. Navigate to the **Sample Data** folder and select the "**PanelDemo. PanelInfo**" file.

13. Click **Open**.

The **Panel Information** dialog will now show the Calcium Concentrations panel you saved, previously shown in Figure T10.4 821. 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 the Calcium Concentrations panel and close the dialog.

The Calcium Concentrations panel is now active on this layout and you could choose to create a new layout of plots to analyze with this panel.

34.11 Docking

FCS Express V4 has the useful feature of docking windows on the layout. The

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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 904, Data Navigator 866, and Gate Navigator 889.

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 **Application Button→Open Layout**.
2. Open the layout **Docking.fey** found in the *FCS Express Sample Data* folder.

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.

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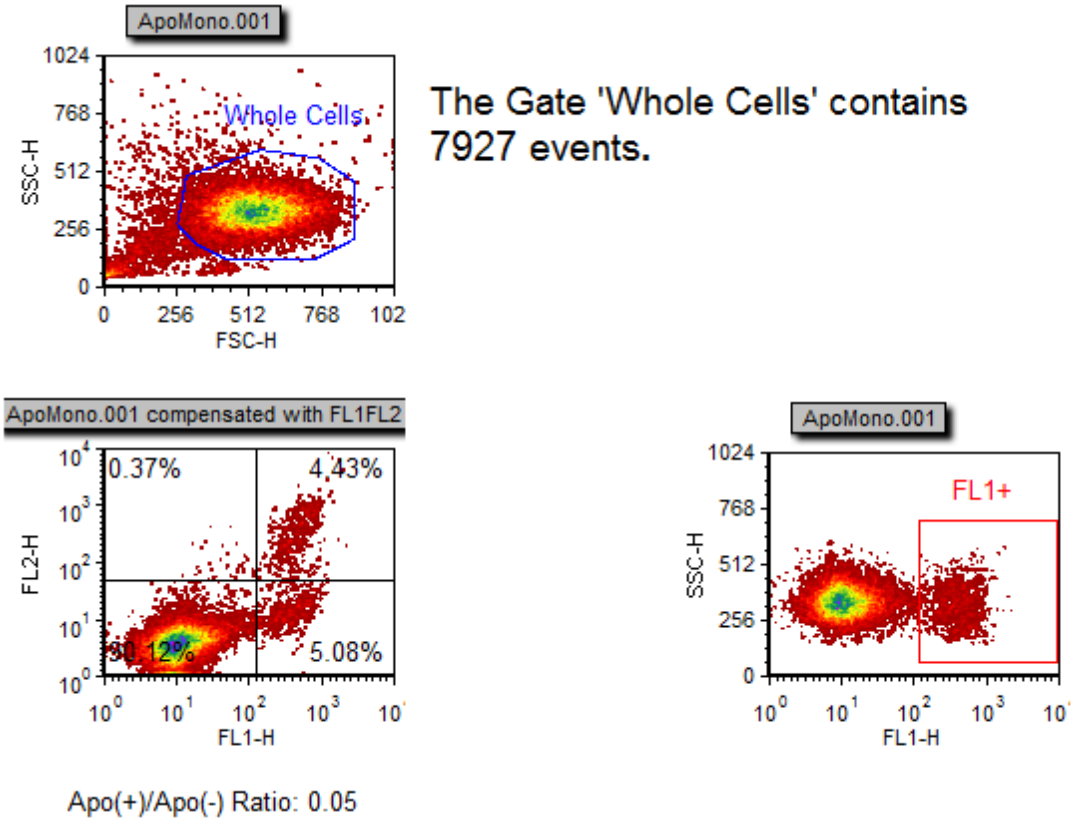

Figure T11.1 Docking Layout

3. Select the **View→Navigators→Gate Navigator** command (Figure T11.2).

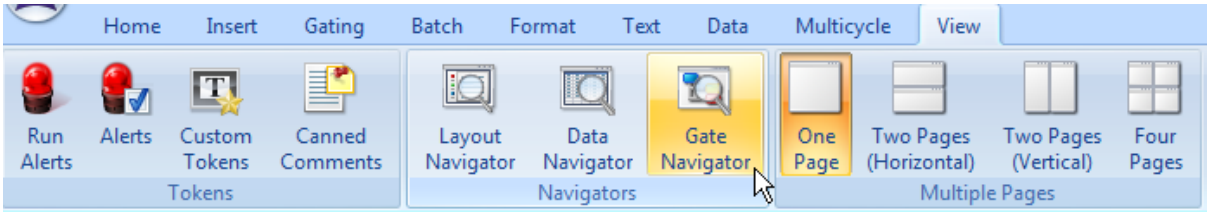

Figure T11.2 Selecting the Gate Navigator Command

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.

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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.

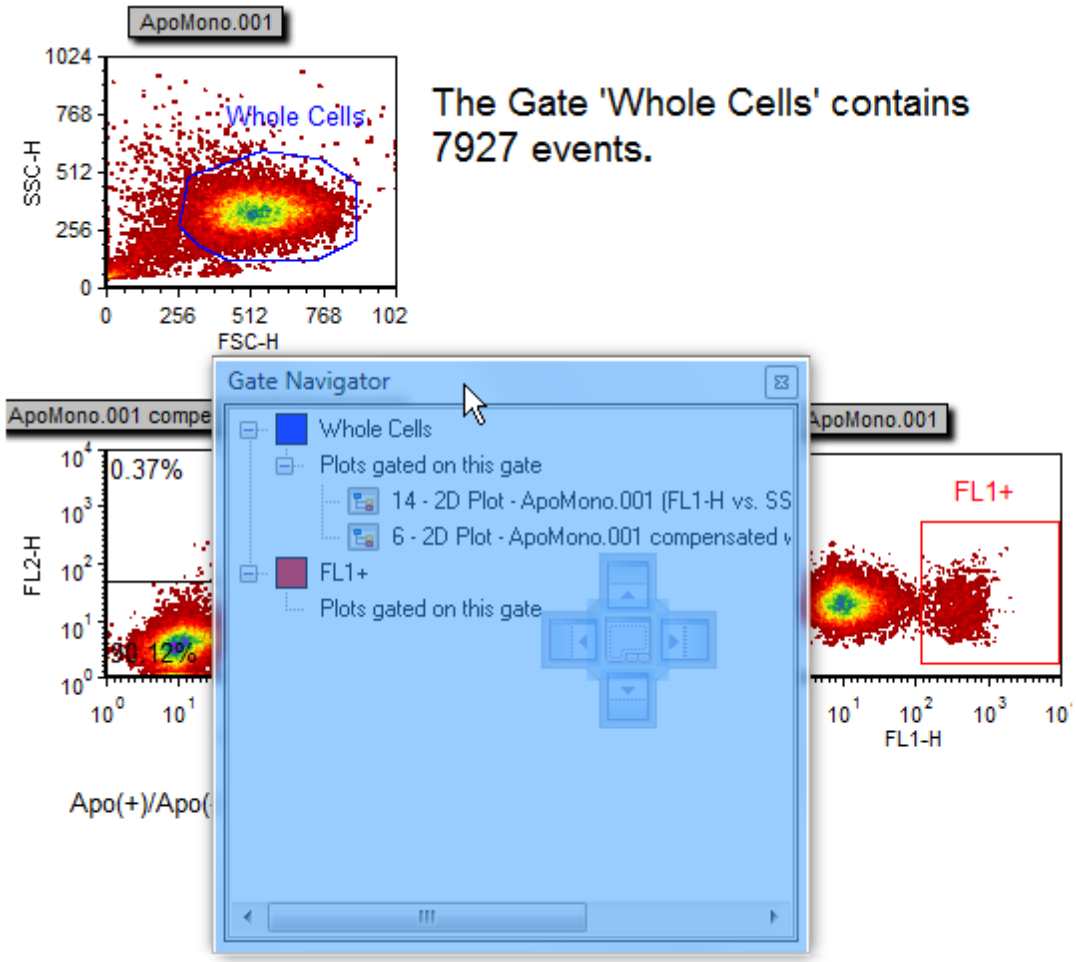

Figure T11.3 Docking Icon in the Center of the Layout

Continue to hold down the mouse button and drag the **Gate Navigator** towards

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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.

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).

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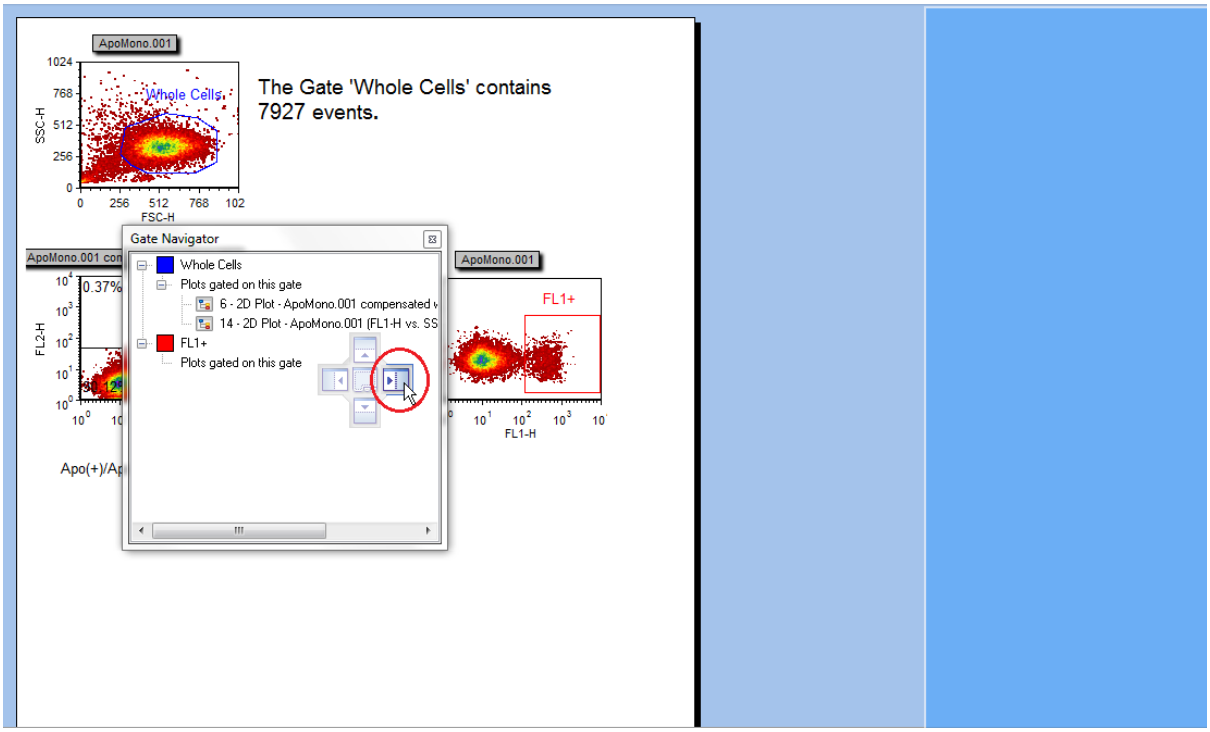

Figure T11.4A Gate Navigator -- Cursor on the Right Arrow of the Docking Icon

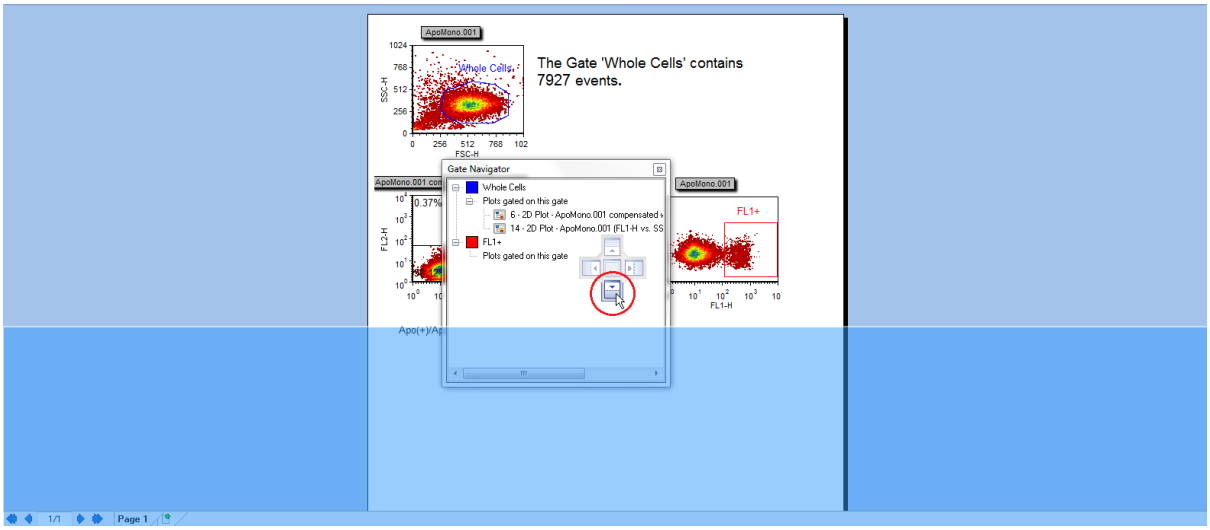

Figure T11.4B Gate Navigator -- Cursor on the Bottom Arrow of the Docking Icon

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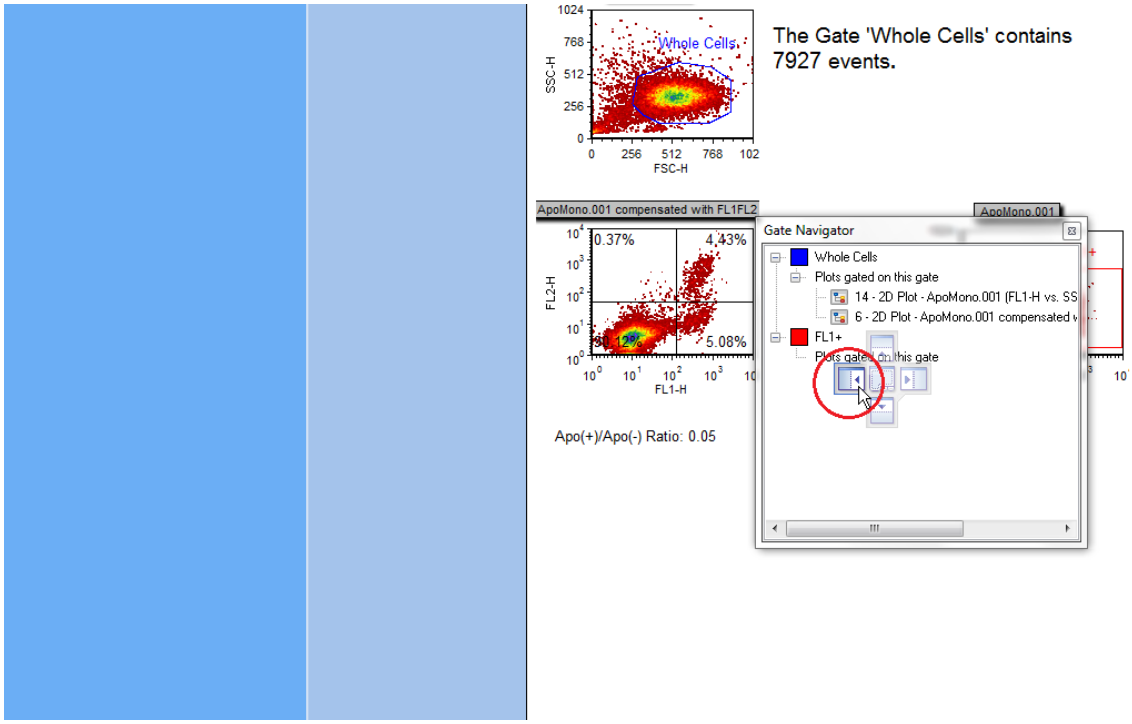

Figure T11.4C Gate Navigator -- Cursor on the Left Arrow of the Docking Icon

The **Gate Navigator** is now docked on the left side of the layout as shown in Figure T11.5.

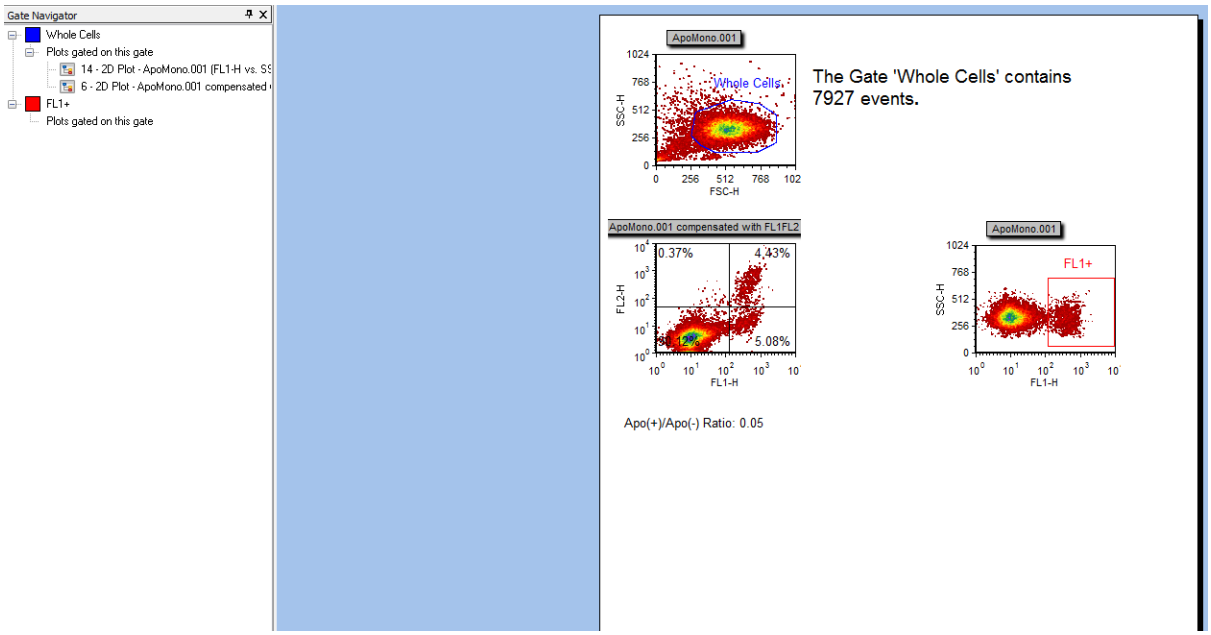

Figure T11.5 Gate Navigator Docked on the Left Side of the Layout

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In the next section, we will learn the features of a docked window 852.

34.11.1 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 T11.3 845.

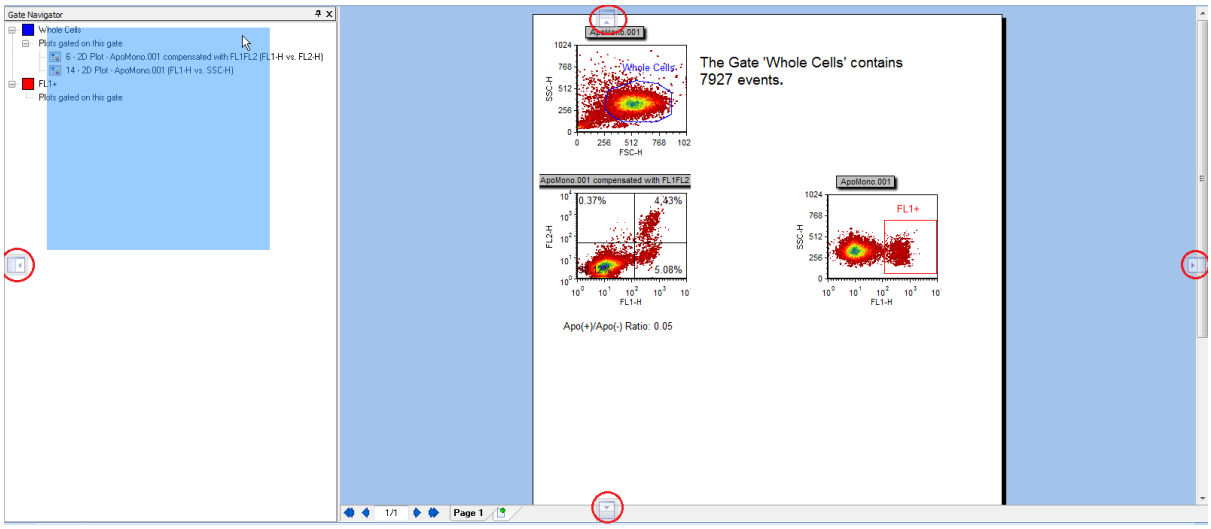

Figure T11.6 Four Outer Docking Icon Arrows Circled in Red

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.

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Notice that a blue rectangle appears at the bottom of the layout, again to indicate the proposed docking location for the window.

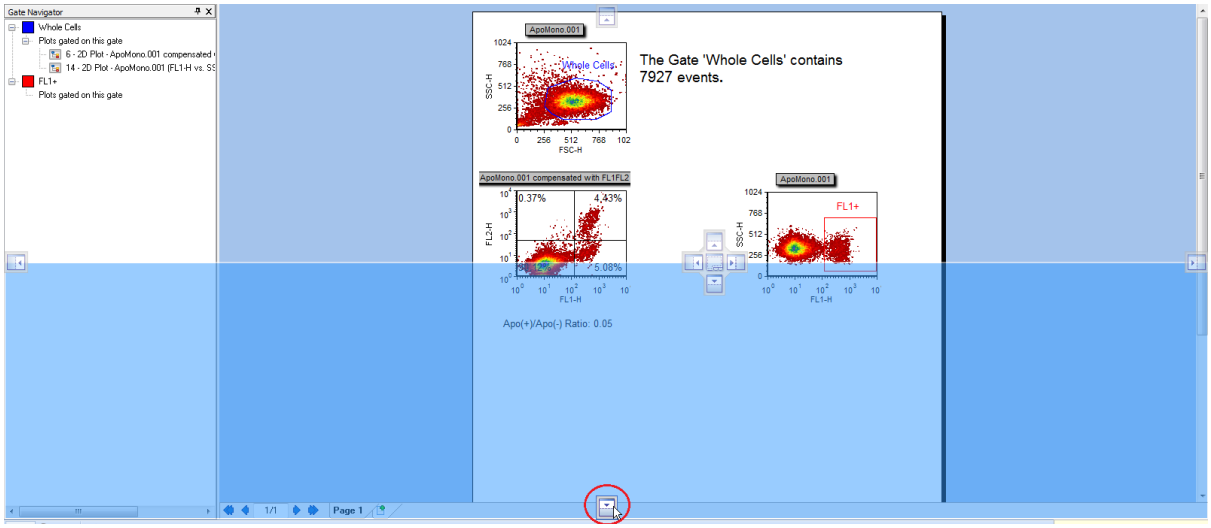

Figure T11.7 Bottom Docking Icon Arrow

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.

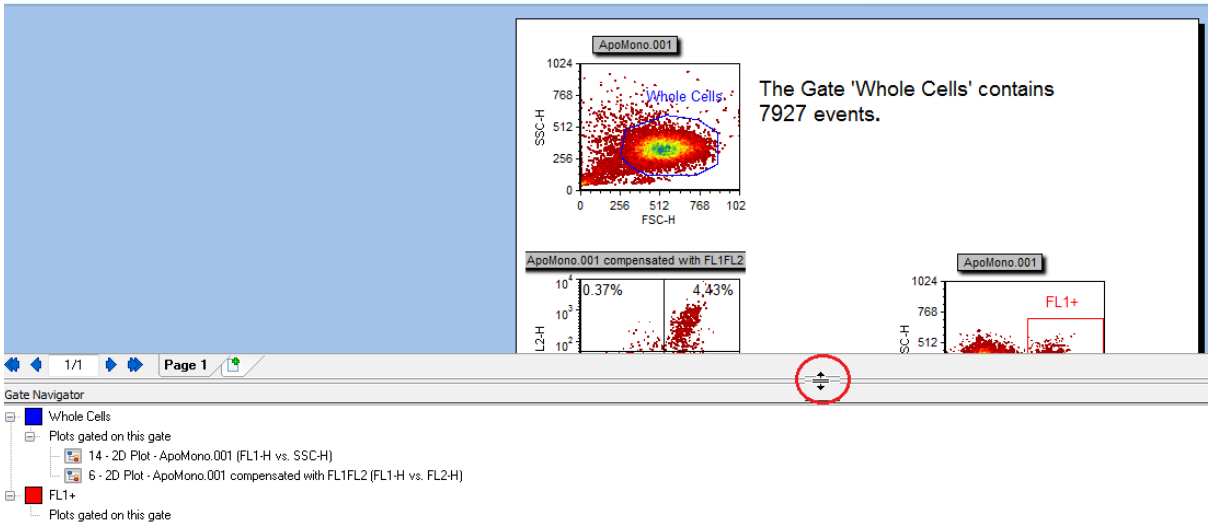

Figure T11.8 Gate Navigator Docked at the Bottom of the Layout

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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.

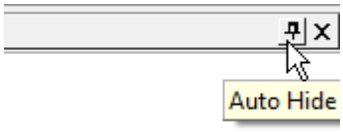

Figure T11.9 Auto Hide Icon

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.

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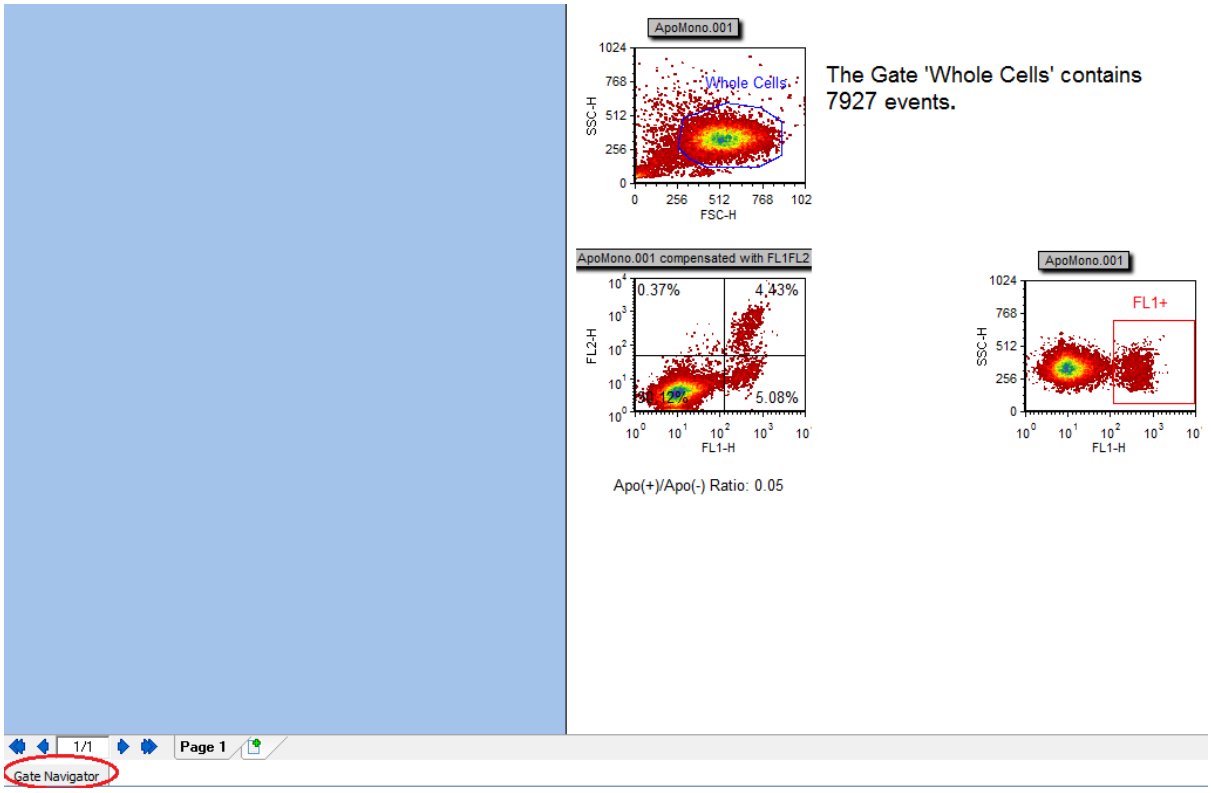

Figure T11.10 Gate Navigator Hidden using the Auto Hide Feature

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 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.

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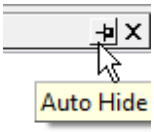

Figure T11.11 Removing Auto Hide

In the next section, we will cover docking features with multiple windows docked 856.

34.11.2 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.

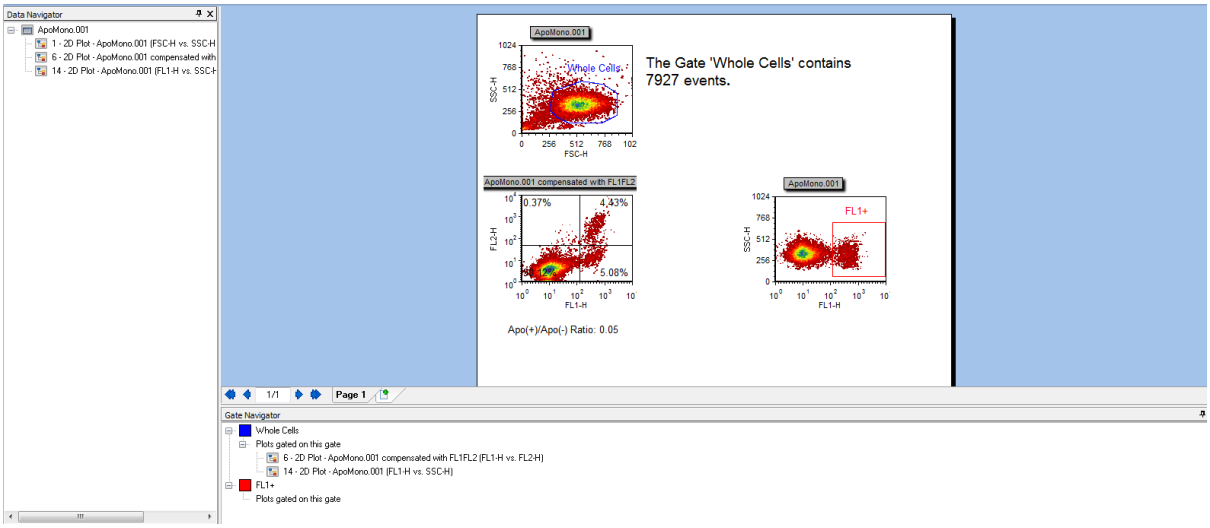

Figure T11.12 Layout with Gate Navigator and Data Navigator Docked

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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.

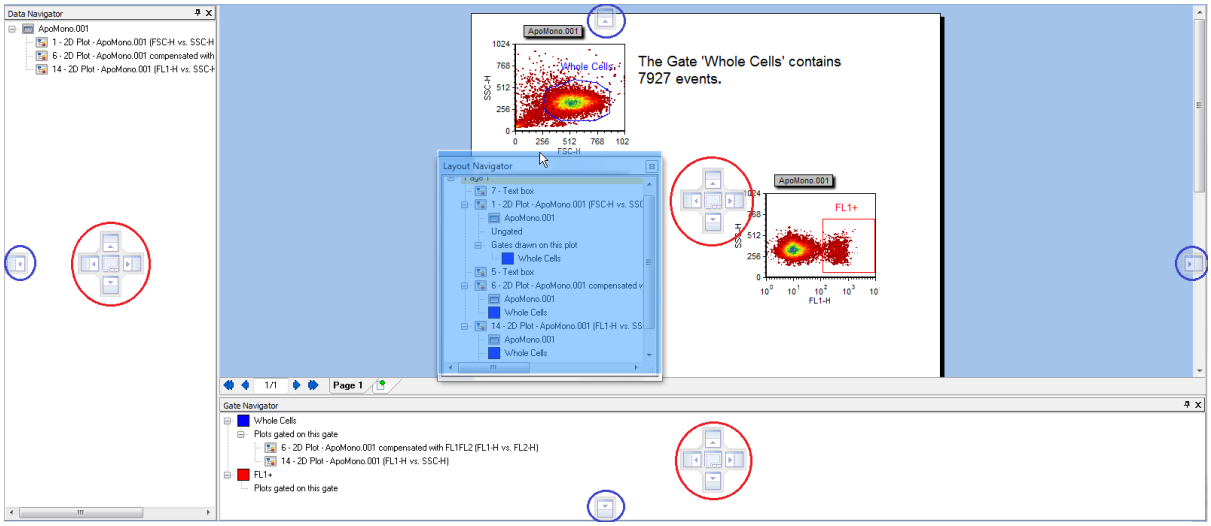

Figure T11.13 Initial Docking Icons

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.

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6. Release the mouse button when the **Layout Navigator** is over the right arrow, indicated by the blue circle in Figure T11.14.

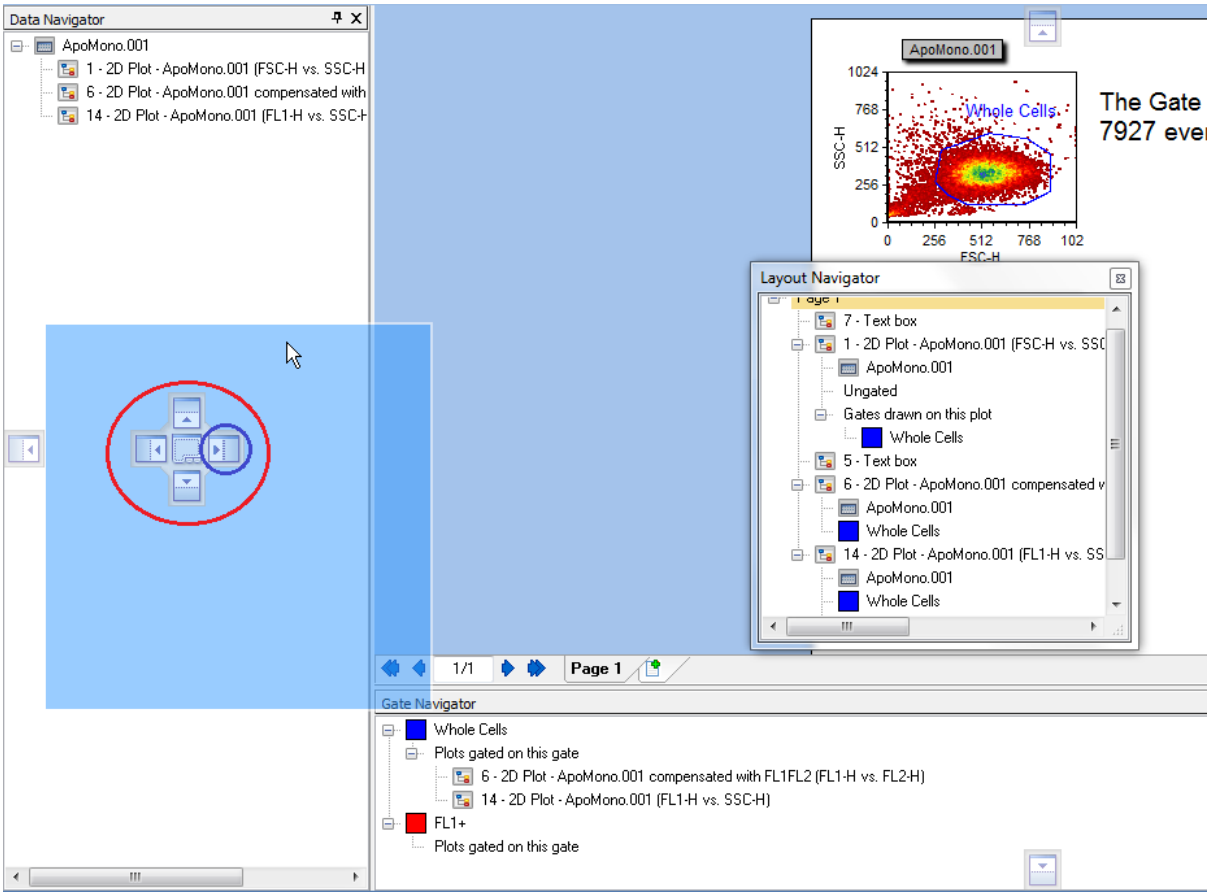

Figure T11.14 Docking Layout Navigator

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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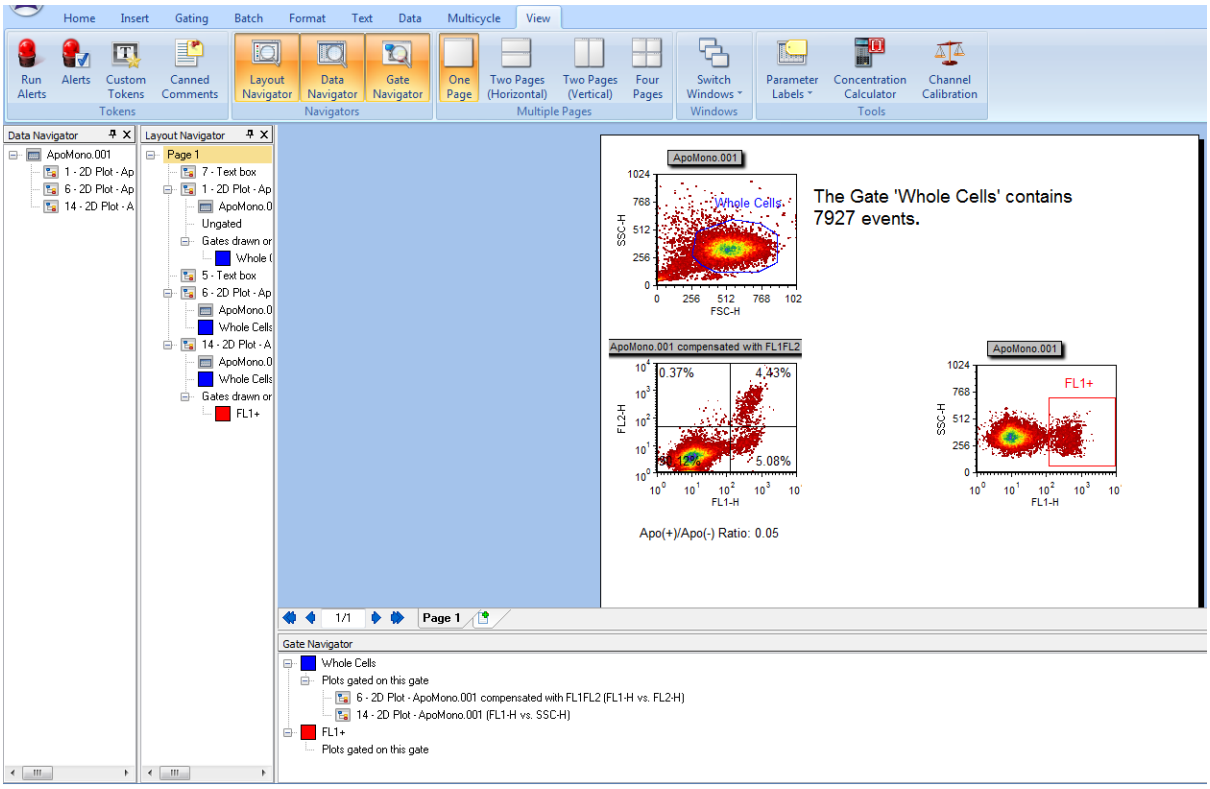

Figure T11.15 Layout Navigator Docked to Right of the Data Navigator

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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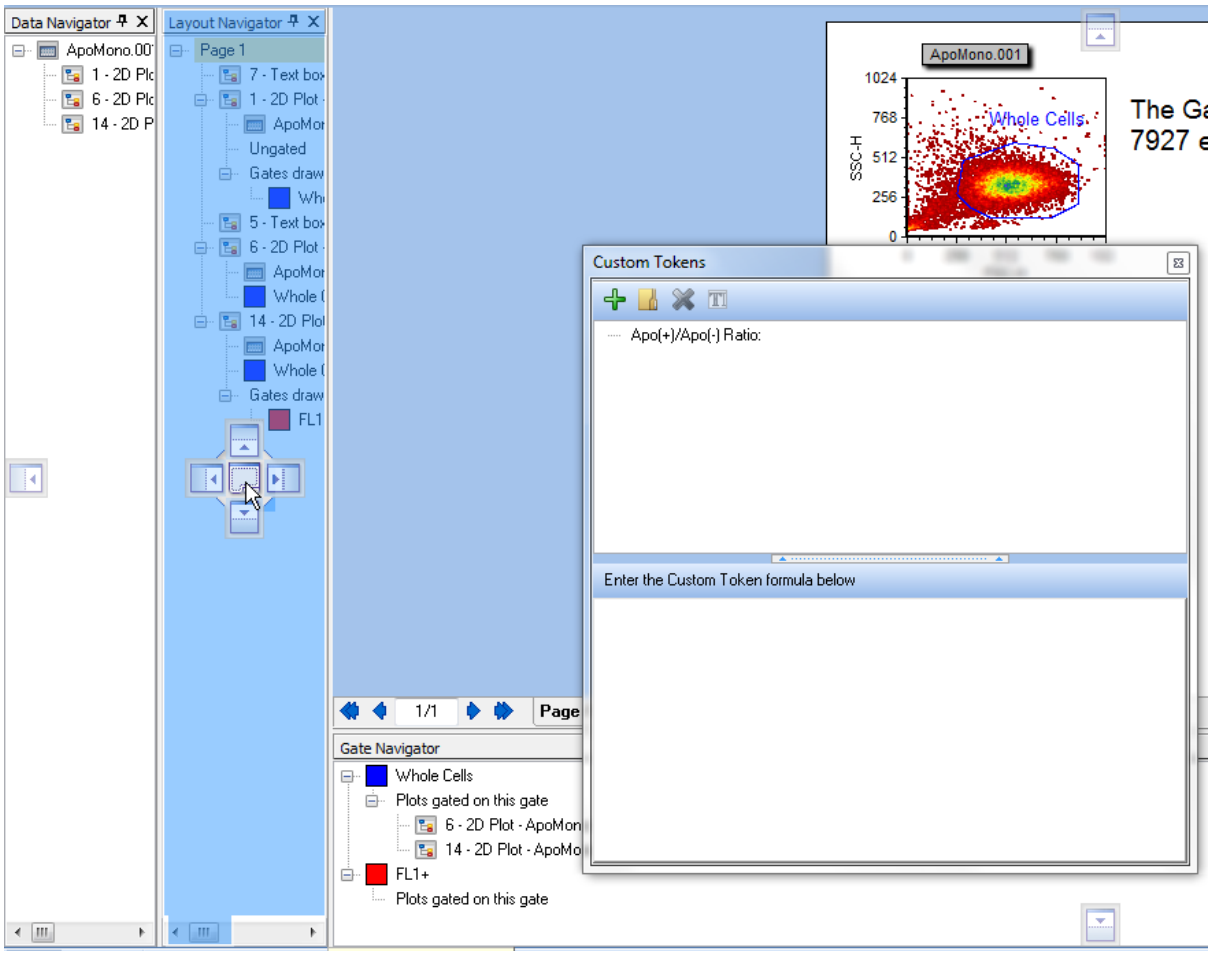

Figure T11.16 Docking Custom Tokens on top of the Layout Navigator

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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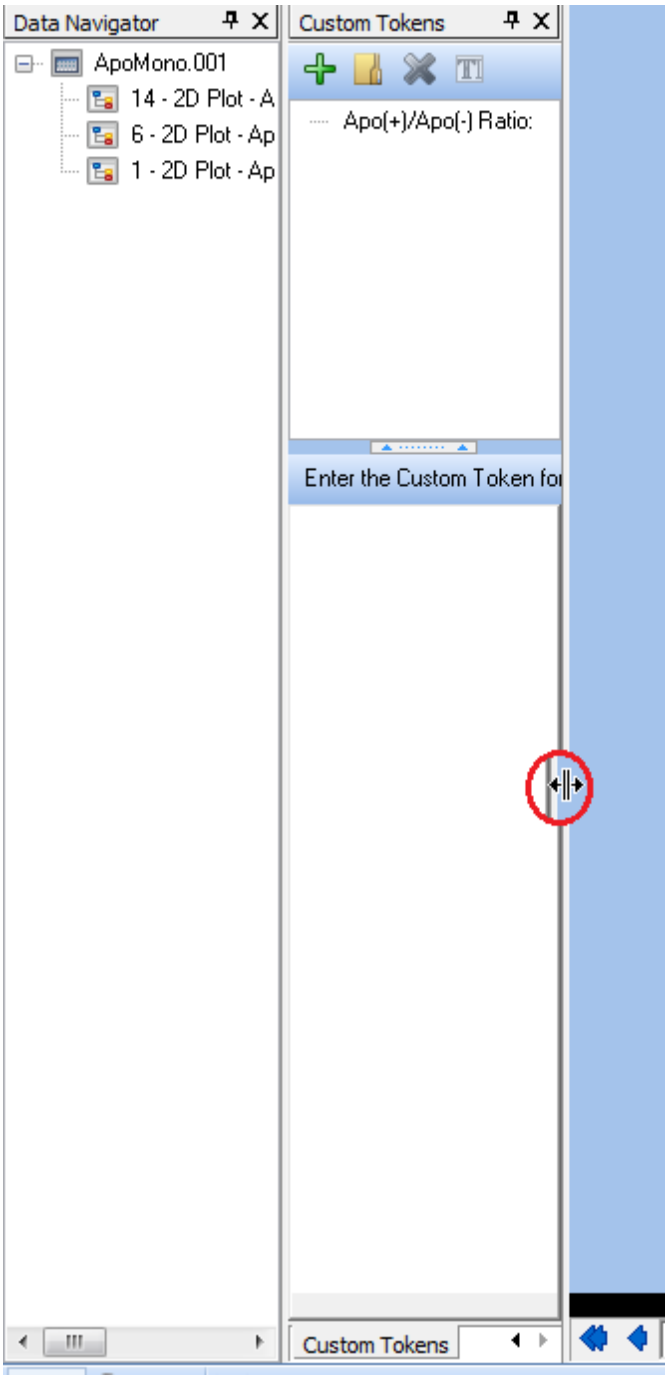

Figure T11.17 Custom Tokens Docked on top of the Layout Navigator

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,

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indicated by the red circle 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).

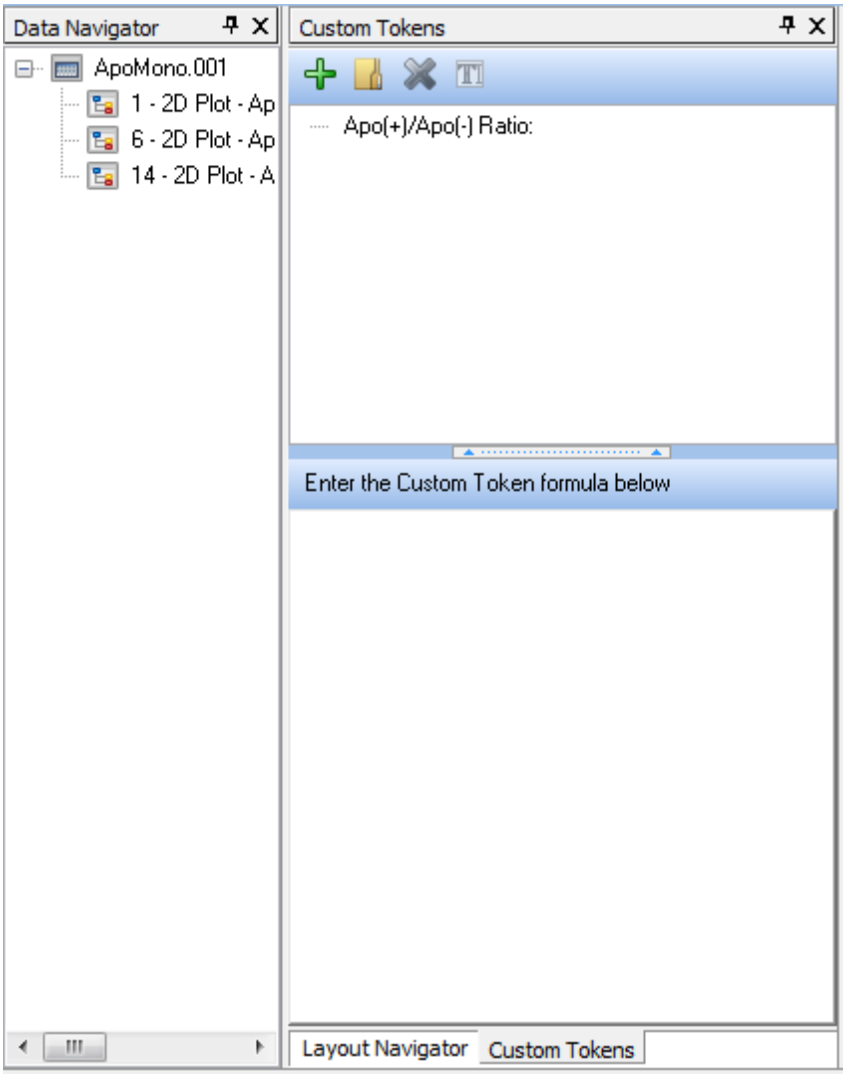

Figure T11.18 Custom Tokens and Layout Navigator Tabs

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.

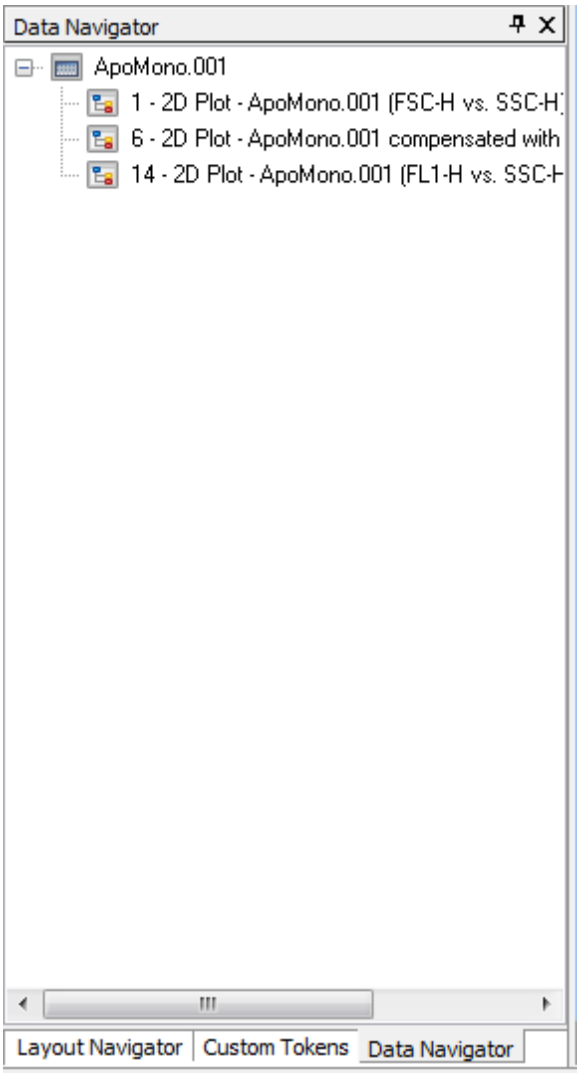

Figure T11.19 Data Navigator Docked on top of Custom Tokens and Layout Navigator

17. Click on the **Auto Hide** icon in the upper right corner of **Data Navigator**, previously shown in Figure T11.9 852.

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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.

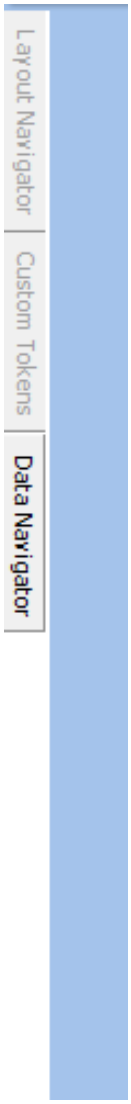

Figure T11.20 Docked Windows using Auto Hide Feature

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In the last section, we will undock a window 865.

34.11.3 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.
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 Navigato**r visible.
5. Click on the **Auto Hide** icon in the upper right corner of the docked **Data Navigator**, as previously shown in Figure T11.11 852, 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.

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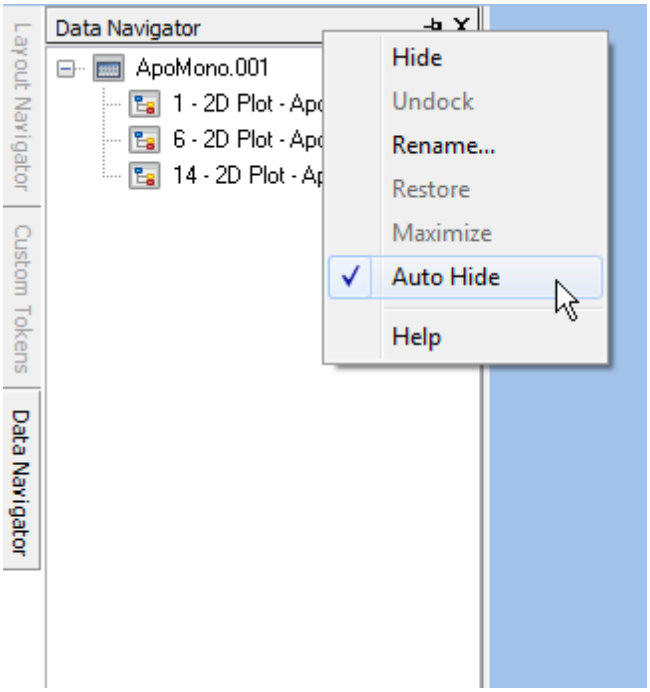

Figure T11.21 Removing Auto Hide using the Pop-up Menu

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.

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.

34.12 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**.

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- 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 867.

34.12.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on two pages.

1. Select **Application Button→Open Layout**.
2. Open the layout **DataNavigator.fey** found in the Tutorial Sample Data archive 590.

The first page of the layout appears, as shown in Figure T12.1.

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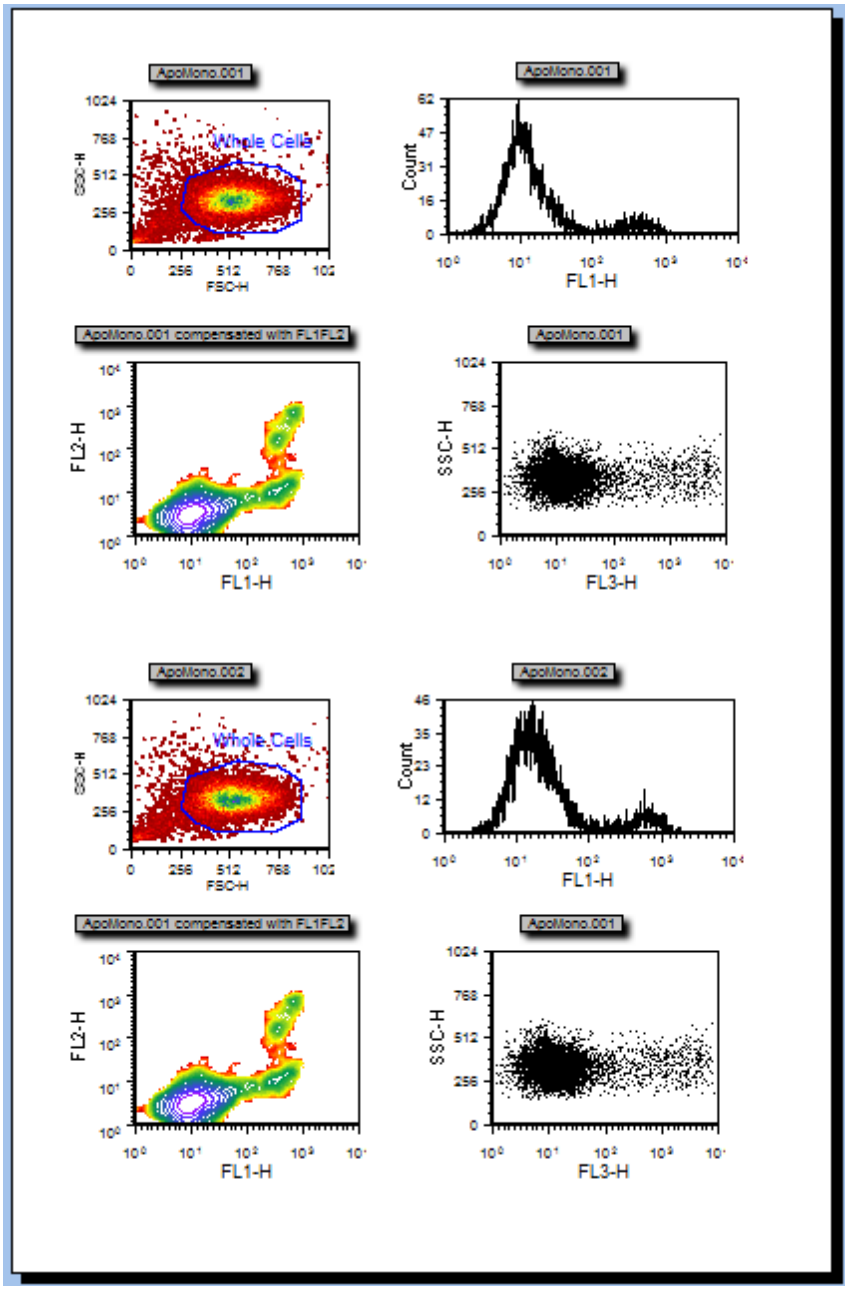

Figure T12.1 Page 1 of the Data Navigator Layout (DataNavigator.fey)

We will now open the **Data Navigator.**

3. Select the **View→Navigators→Data Navigator** command (Figure T12.2).

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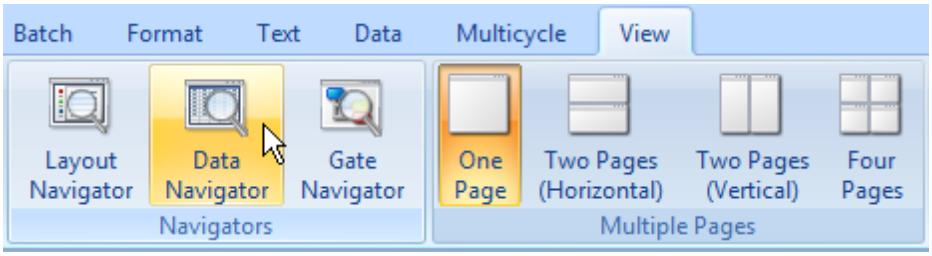

Figure T12.2 Selecting the Data Navigator Command

The **Data Navigator** appears, as shown in Figure T12.3. We will now dock it to the left side of the layout. For more information on docking, please refer to the Docking tutorial 845.

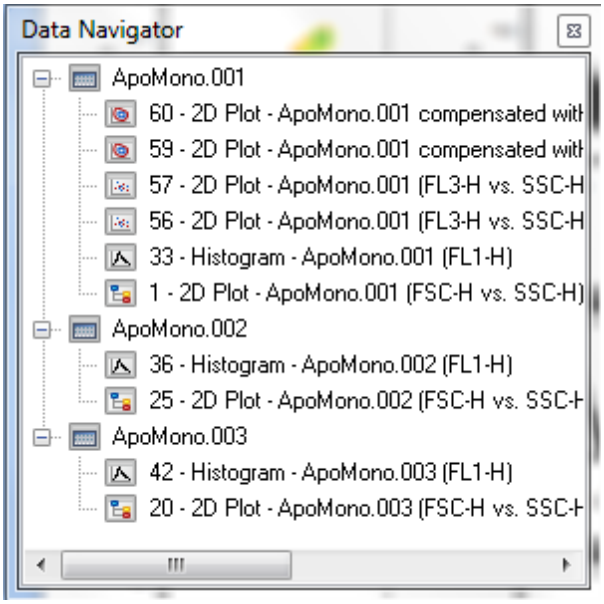

Figure T12.3 Data Navigator

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.

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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.

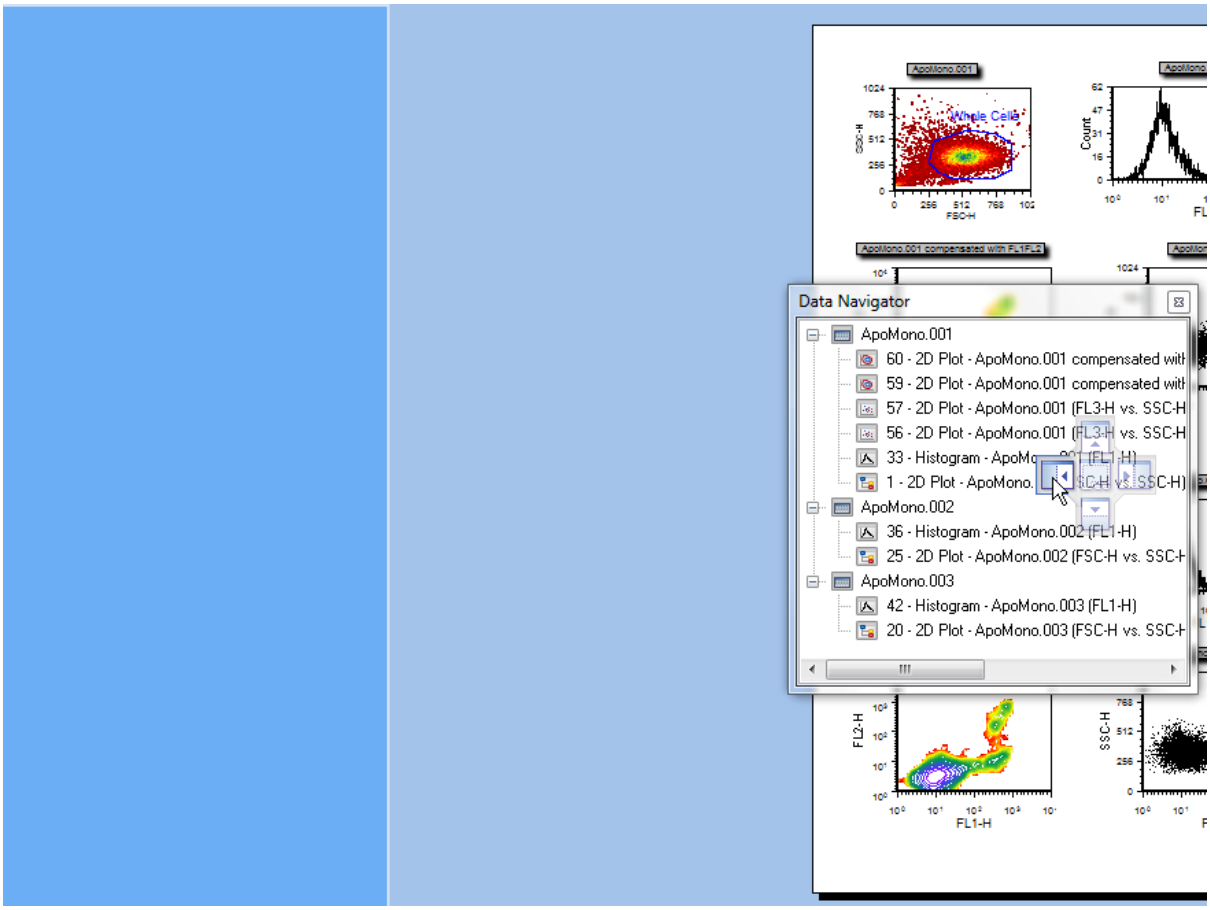

Figure T12.4 Docking the Data Navigator to the Left Side

The **Data Navigator** is now docked on the left side of the layout. However, not all the text is 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 circle 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.

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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.

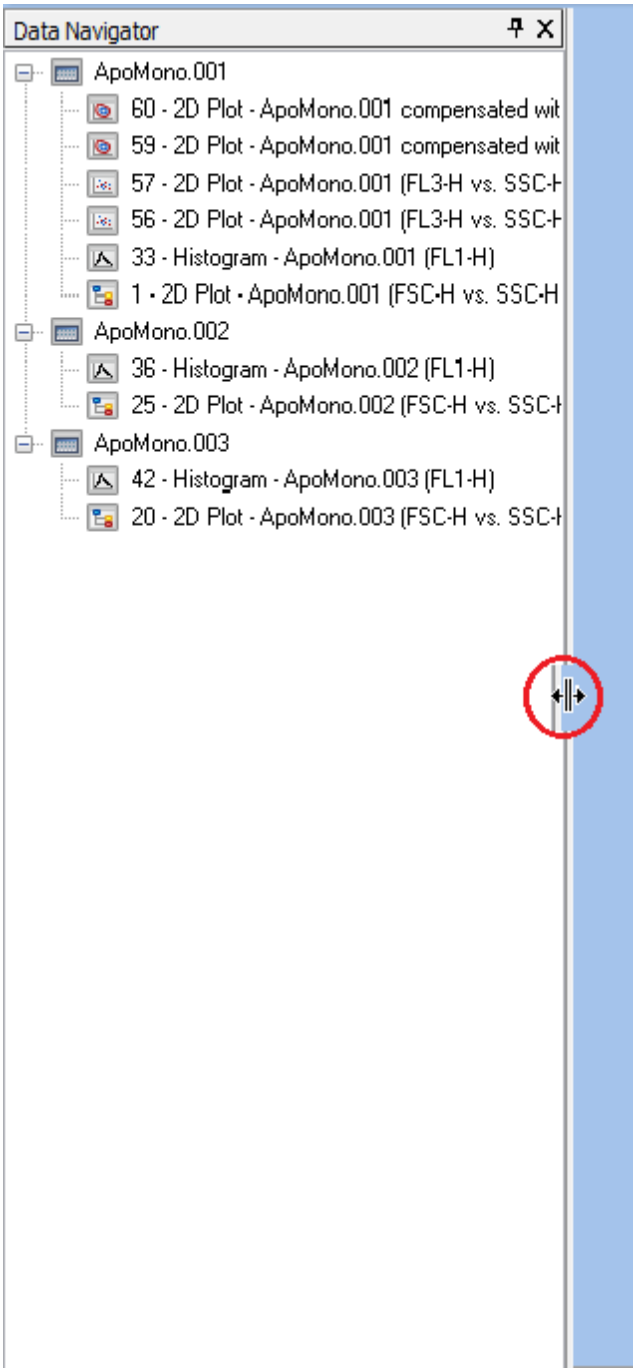

Figure T12.5 Resizing the Data Navigator

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The docked **Data Navigator** should appear similar to Figure T12.6.

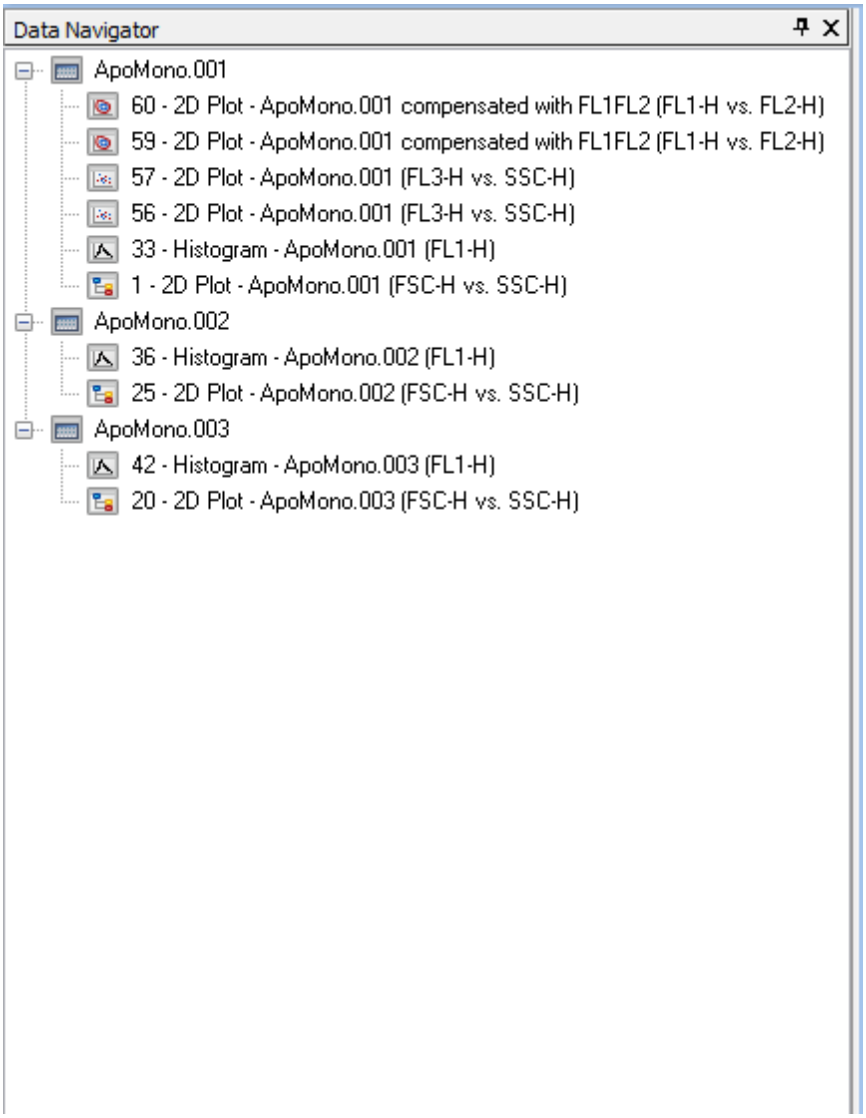

Figure T12.6 Docked Data Navigator

In the next section, we will explore the features of the Data Navigator872.

34.12.2 Data Navigator Features

As shown in Figure T12.6867, 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 **2D Plot – ApoMono.002 (FSC-H vs. SSC-**

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H), under the data file listing for **ApoMono.002**, on 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**.

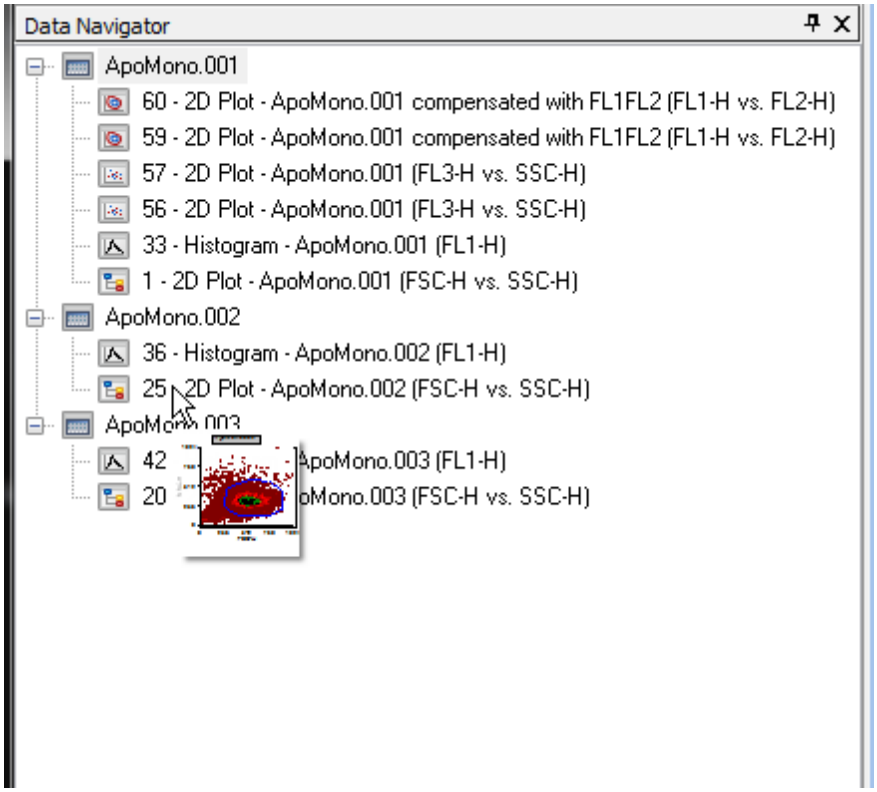

Figure T12.7 Thumbnail Image of a 2D Plot on the Data Navigator

We will now use the **Data Navigator** to change the data displayed in a 2D plot. We will replace the ApoMono.001 data in the 2D plot in the lower right of the layout with the ApoMono.002 data using just the **Data Navigator**.

2. Place the cursor over data file **ApoMono.002** in the **Data Navigator**, highlighted in blue in Figure T12.8.

3. Press and hold the left mouse button.

4. Drag the **ApoMono.002** data file up to the plot listing **57 – 2D Plot – ApoMono.001 (FL2-H vs. SSC-H)**, indicated by the cursor and highlighted in blue in Figure T12.8.

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5. Release the mouse button when the **ApoMono.002** data file is on top of the listing **57 – 2D Plot – ApoMono.001 (FL2-H vs. SSC-H)**.

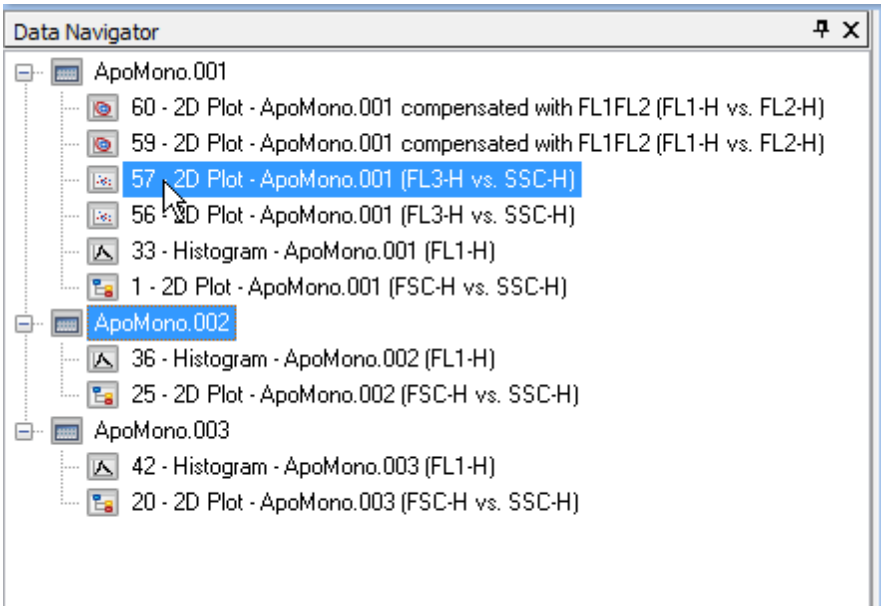

Figure T12.8 Dragging the ApoMono.002 data file onto a 2D plot in the Data Navigator

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**.

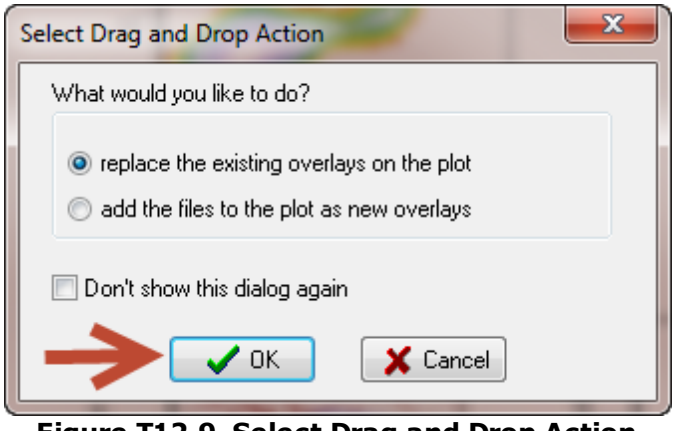

Figure T12.9 Select Drag and Drop Action Dialog

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The 2D plot in the lower right of the layout is now selected and updates to reflect the ApoMono.002 data, outlined in green in Figure T12.10.

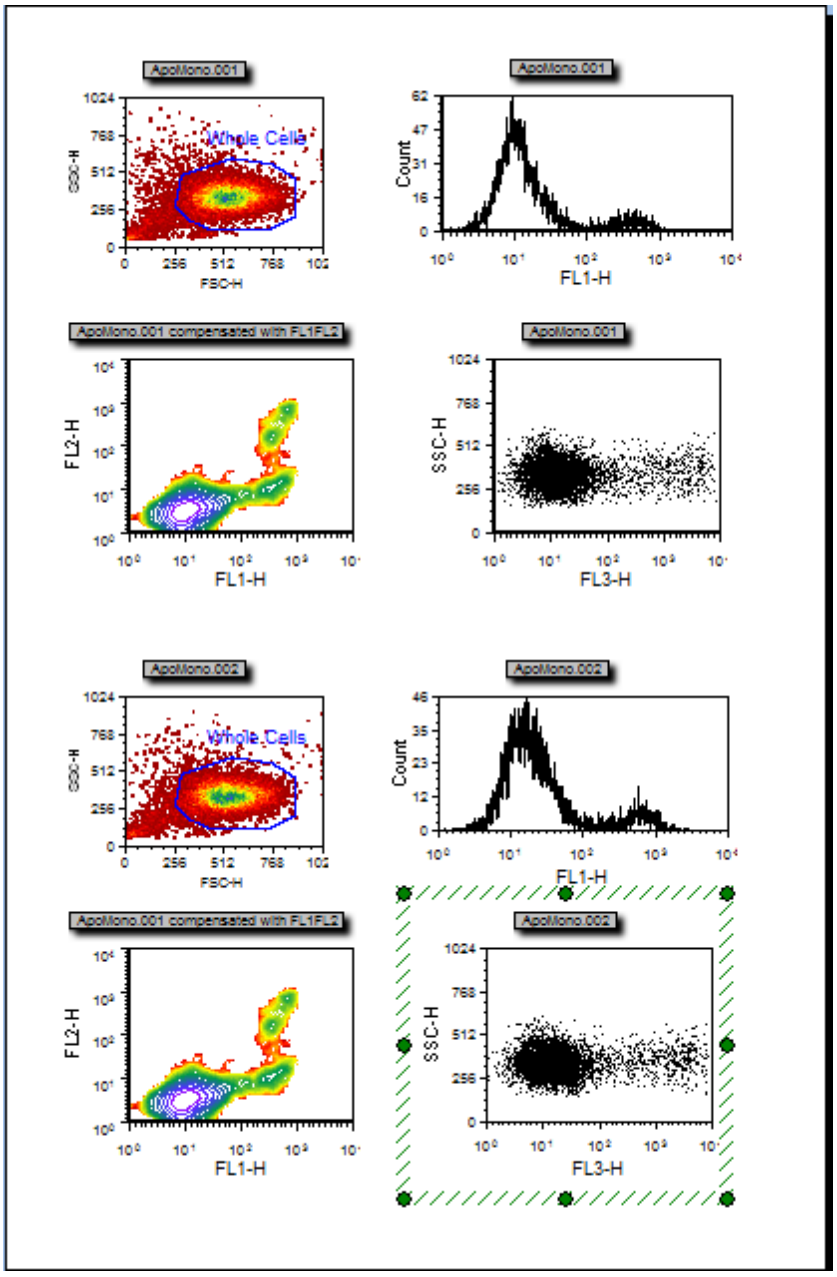

Figure T12.10 Selected and Updated 2D Plot with ApoMono.2 Data

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The **Data Navigator** is also updated to reflect the changes, as shown in Figure T12.11. The plot **57 – 2D Plot – ApoMono.002 (FL2-H vs. SSC-H)** appears under the **ApoMono.002** data file listing, as indicated by the cursor and the thumbnail image in Figure T12.11.

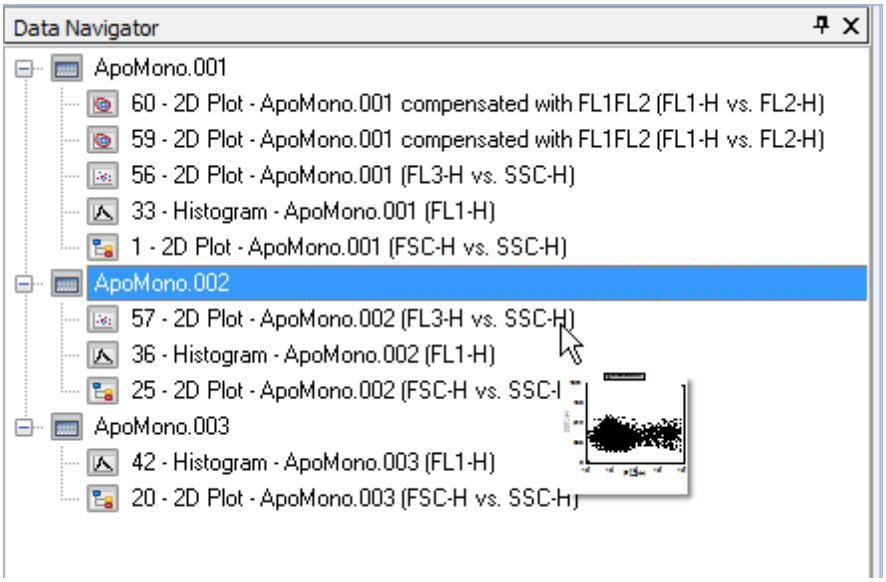

Figure T12.11 Data Navigator Updated to Reflect ApoMono.002 Plot Changes

We will now use drag and drop in the **Data Navigator** to overlay data on a histogram.

8. Place the cursor over data file **ApoMono.002** in the **Data Navigator**, highlighted in blue in Figure T12.12.

9. Press and hold the left mouse button.

10. Drag the **ApoMono.002** data file up to the histogram plot listing **33 – Histogram – ApoMono.001 (FL1)**, indicated by the cursor and highlighted in blue in Figure T12.12.

11. Release the mouse button when the **ApoMono.002** data file is on top of the histogram listing.

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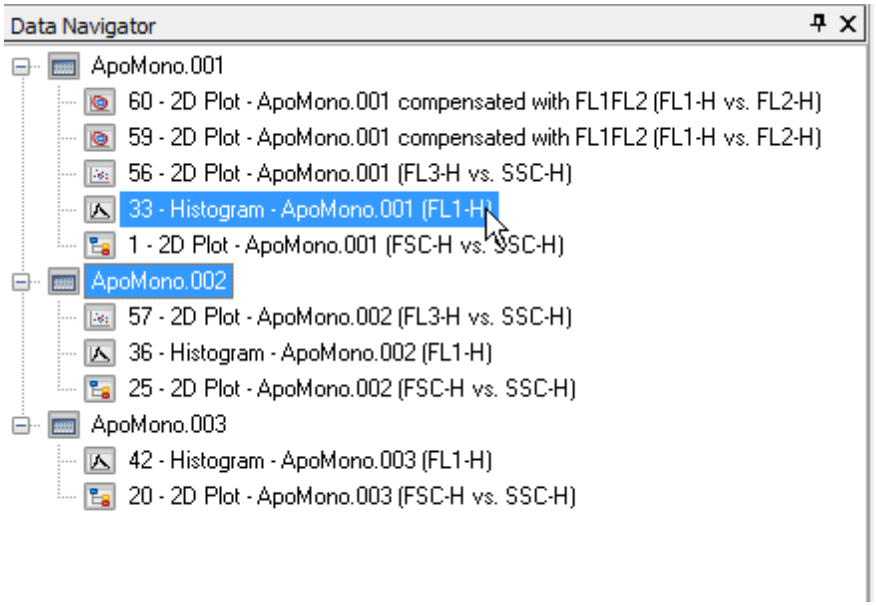

Figure T12.12 Dragging ApoMono.002 onto a Histogram Plot in the Data Navigator

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 ApoMono.002 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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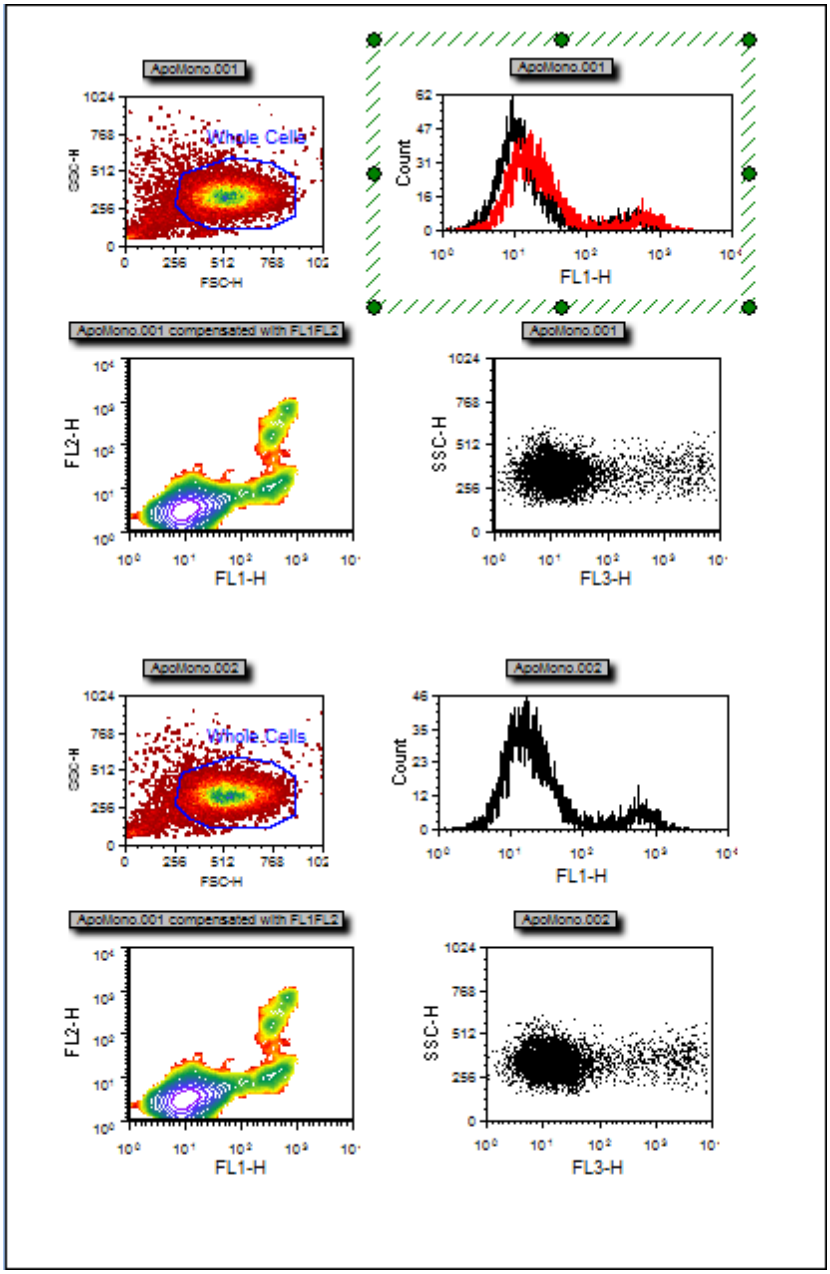

Figure T12.13 Histogram Updated to Show the Overlay of ApoMono.002 Data

Again, the **Data Navigator** also updates to reflect the changes as shown in Figure T12.14. Under the data file listing for **ApoMono.002**, a listing now appears for **33 – Histogram – ApoMono.001 (FL1-H)**, 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 **ApoMono.002** is the second overlay on the **ApoMono.001** histogram. Note that under the **ApoMono.001** data file listing, the histogram **33 – Histogram –**

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ApoMono.001 (FL1-H) listing has also been updated with the sub-listing **Overlay 1** to indicate that the data ApoMono.001 is the base data.

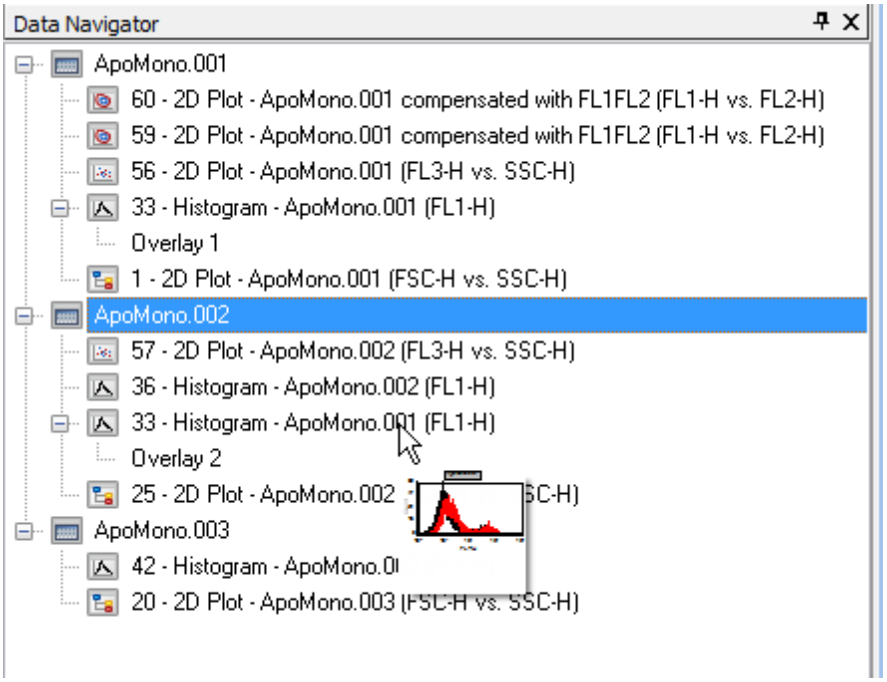

Figure T12.14 Updated Data Navigator with Overlay of ApoMono.002 Data

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 **ApoMono.003** 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 object** from the pop-up menu, indicated by the cursor in Figure T12.15.

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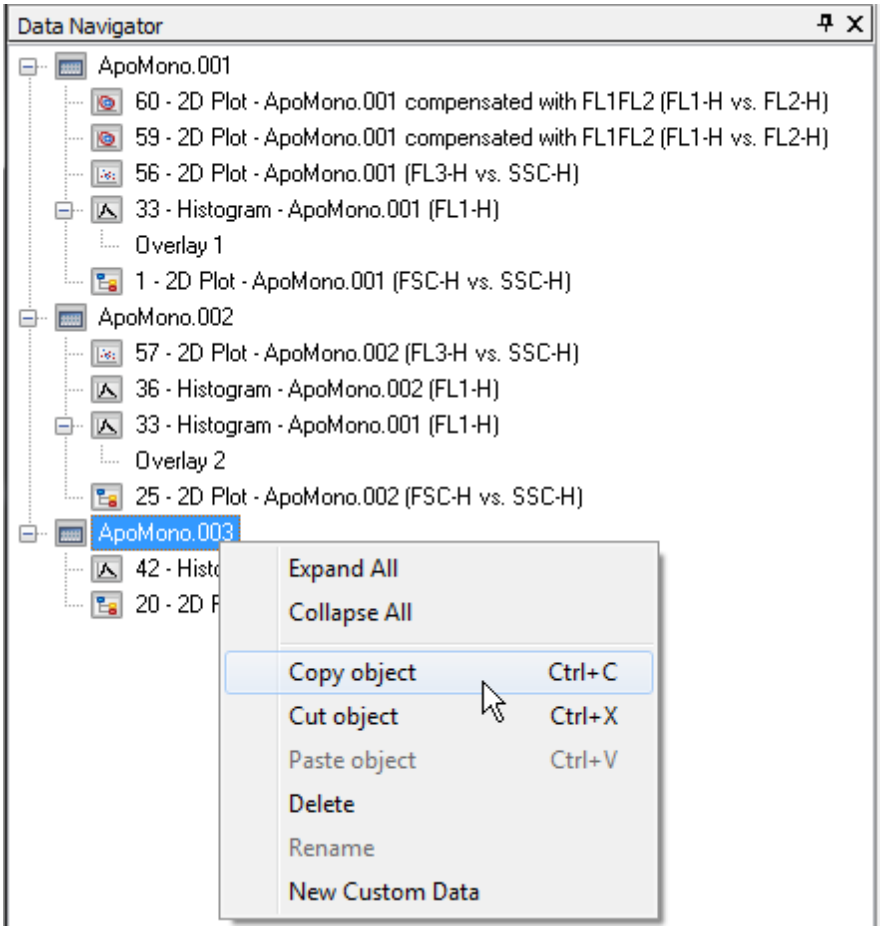

Figure T12.15 Copy Object Using Data Navigator Pop-up Menu

17. Place the cursor over the listing **33 – Histogram – ApoMono.001 (Fl1-H)** listed under **ApoMono.001** 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 object** from the pop-up menu, indicated by the cursor in Figure T12.16.

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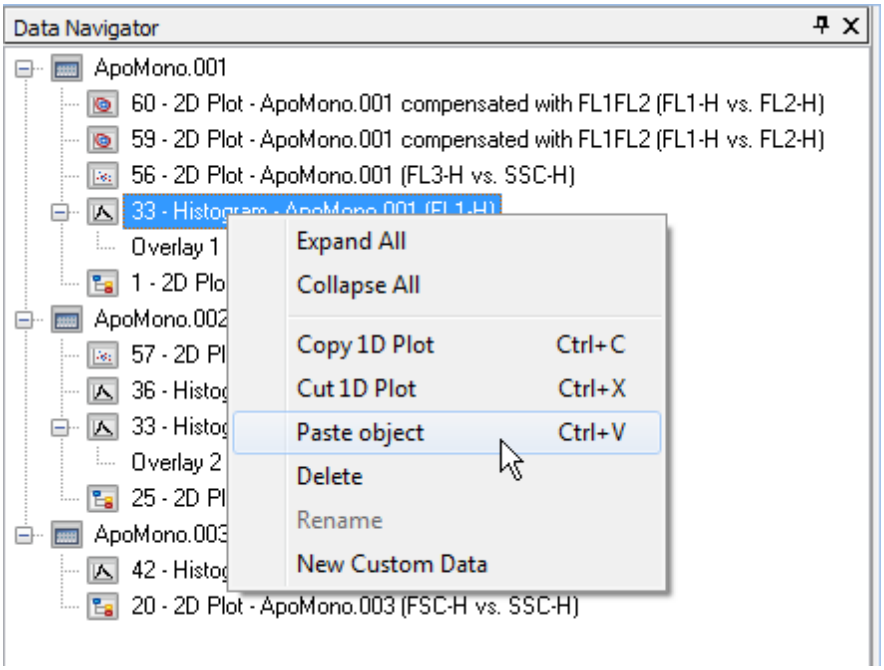

Figure T12.16 Paste Object Using Data Navigator Pop-up Menu

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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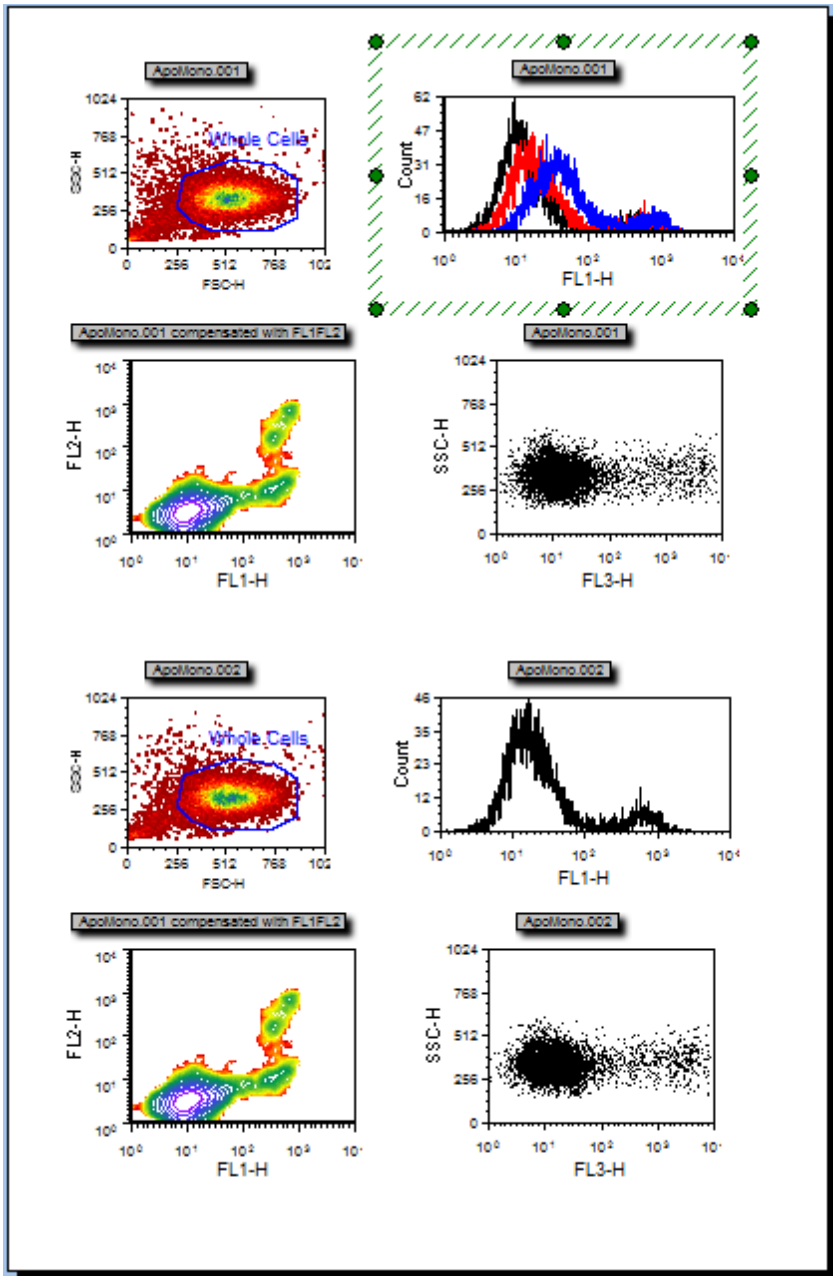

Figure T12.17 Histogram Updated to Show the Overlay of ApoMono.003 Data

Again, the **Data Navigator** updates to reflect the changes as shown in Figure T12.18. Under the data file listing for **ApoMono.003**, a listing now appears for **33 – Histogram – ApoMono.001 (FL1-H)**, 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 **ApoMono.003** is the third overlay on the **ApoMono.001** histogram.

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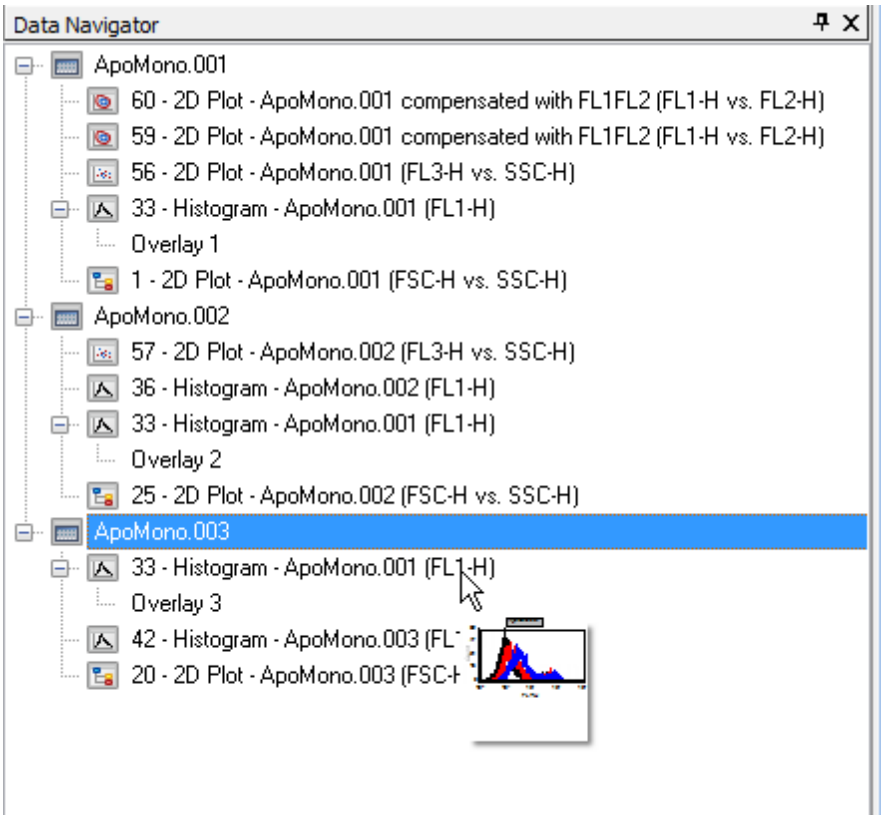

Figure T12.18 Updated Data Navigator with Overlay of ApoMono.003 Data

We will now drag a data file from the **Data Navigator** onto a plot on the layout. On the layout, the contour plot of FL1-H vs. FL2-H, in the lower left corner, is using the data ApoMono.001. We will replace that with ApoMono.002 data.

22. Place the cursor over data file **ApoMono.002** in the **Data Navigator**, highlighted in blue in Figure T12.19.

23. Press and hold the left mouse button.

24. Drag the data file **ApoMono.002** onto the layout.

25. Release the mouse button when **ApoMono.002** is on top of the contour plot of FL1-H vs. FL2-H, in the lower left corner, indicated by the cursor and outlined in blue in Figure T12.19.

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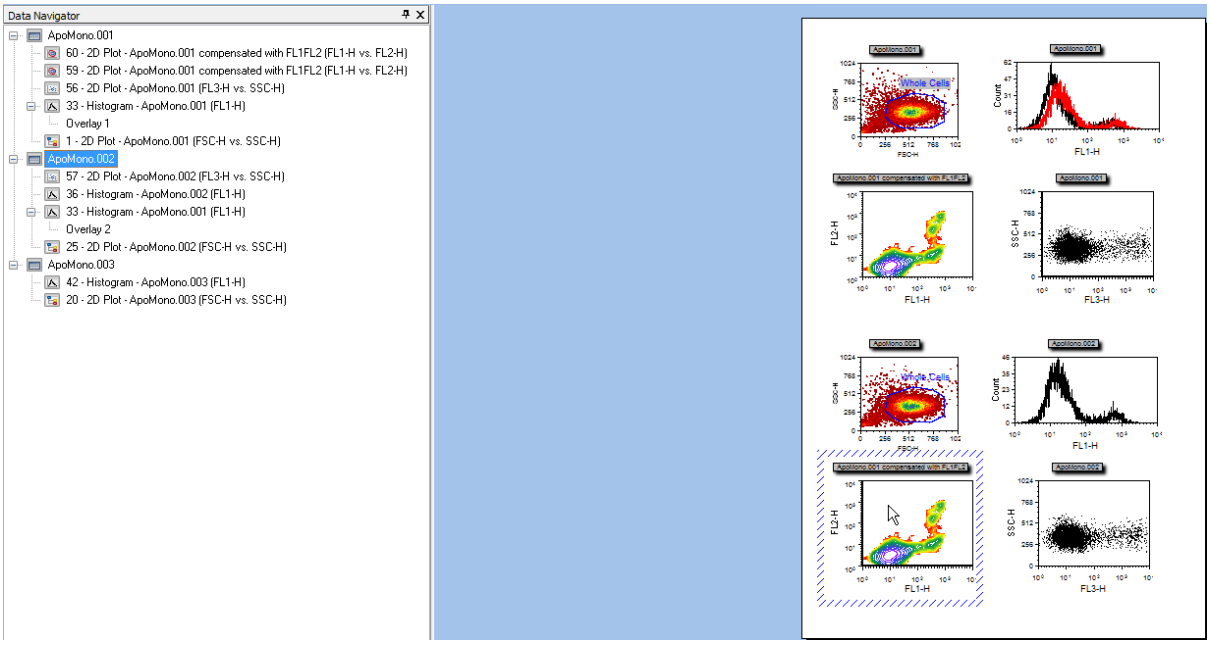

Figure T12.19 Dragging and Dropping ApoMono.002 from the Data Navigator onto the Contour Plot on the Layout

Both the contour plot on the layout, in the lower left, and the **Data Navigator** have updated to reflect the ApoMono.002 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**.

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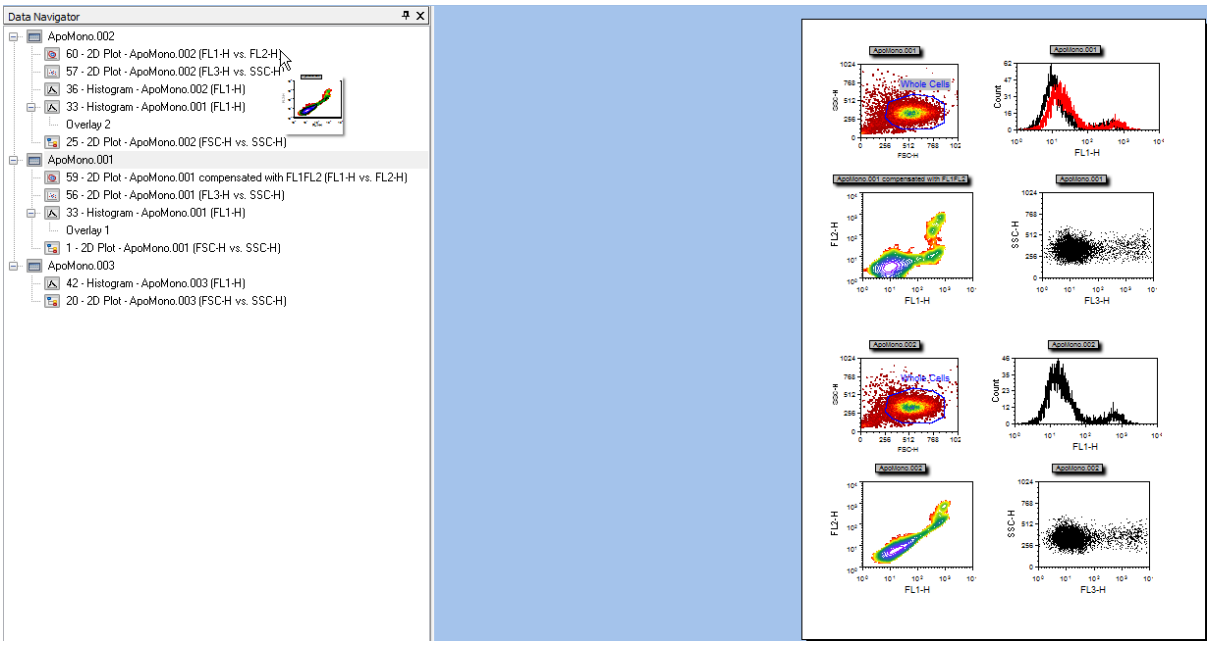

Figure T12.20 Updated Layout and Data Navigator with ApoMono.002 data on the Contour Plot

Instead of dragging the data file from the **Data Navigator** and dropping it onto the contour 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 contour 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 **ApoMono.001** on the **Data Navigator**.

27. Right-click to bring up the associated pop-up menu.

28. Select **Copy object** 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

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Page 2 of the layout will now appear as shown in 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 object** from the pop-up menu.

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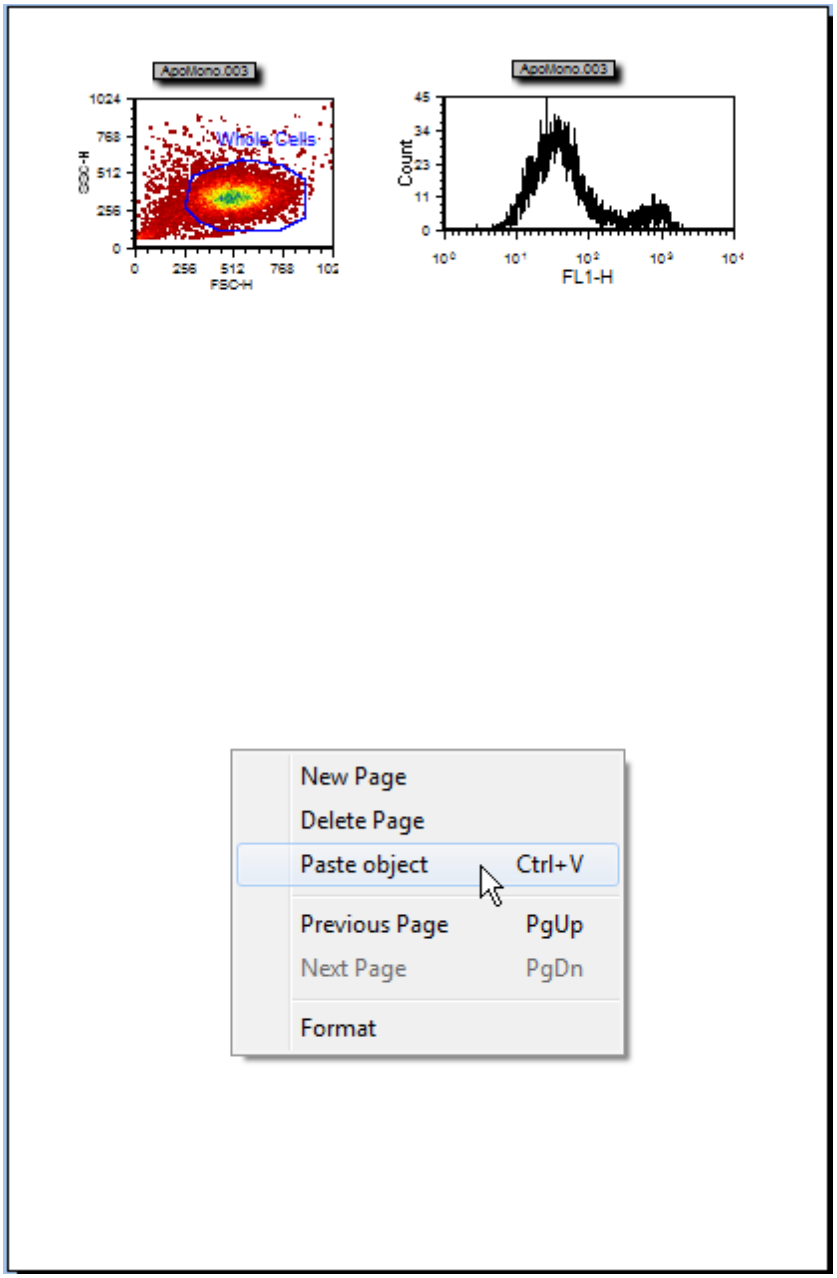

Figure T12.22 Page 2 of the Layout with the Pop-up Menu

The **Select Plot Types...** dialog appears, as shown in Figure T12.23.

33. Select **Density** and **Contour** as the types of plots to open (Figure T12.23).

34. Click **OK**.

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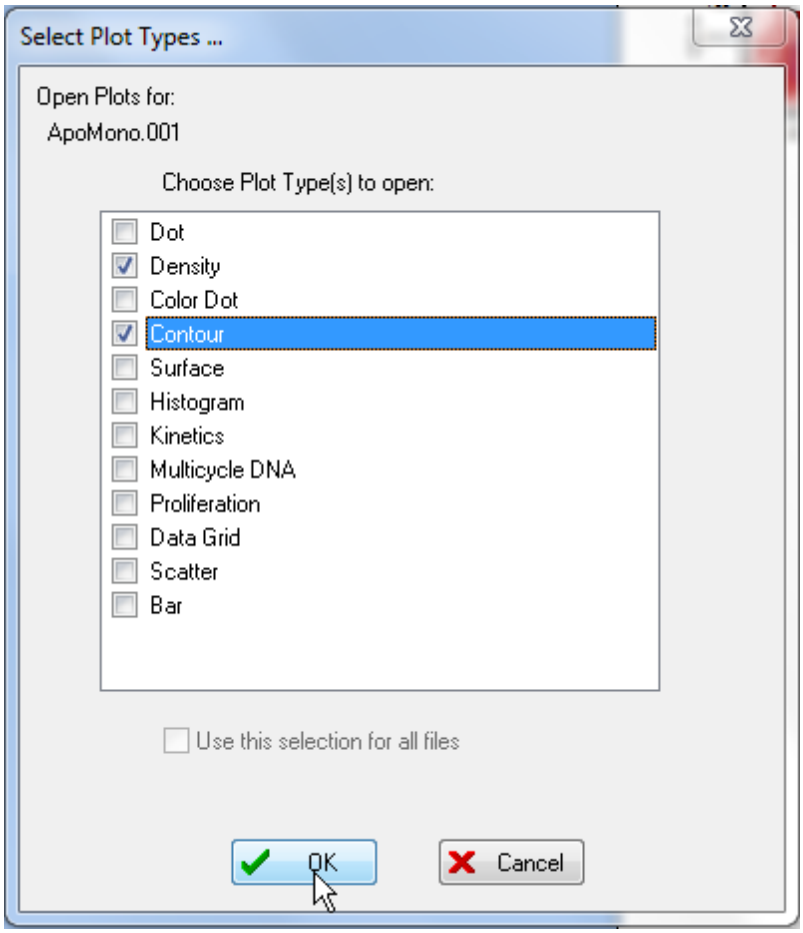

Figure T12.23 Select Plot Types... Dialog

A contour and density plot of ApoMono.001 appear, using the default parameters of FSC vs. SSC, as shown in Figure T12.24.

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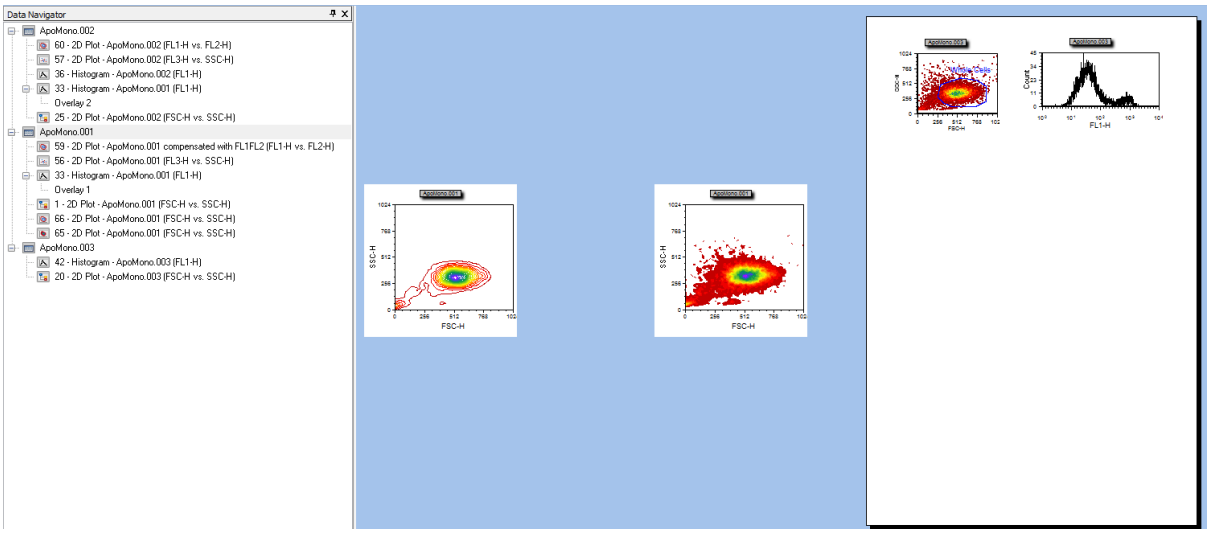

Figure T12.24 Contour and Density Plots of ApoMono.001 Data

34.13 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 889.

34.13.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on two pages.

1. Select **Application Button→Open Layout**.
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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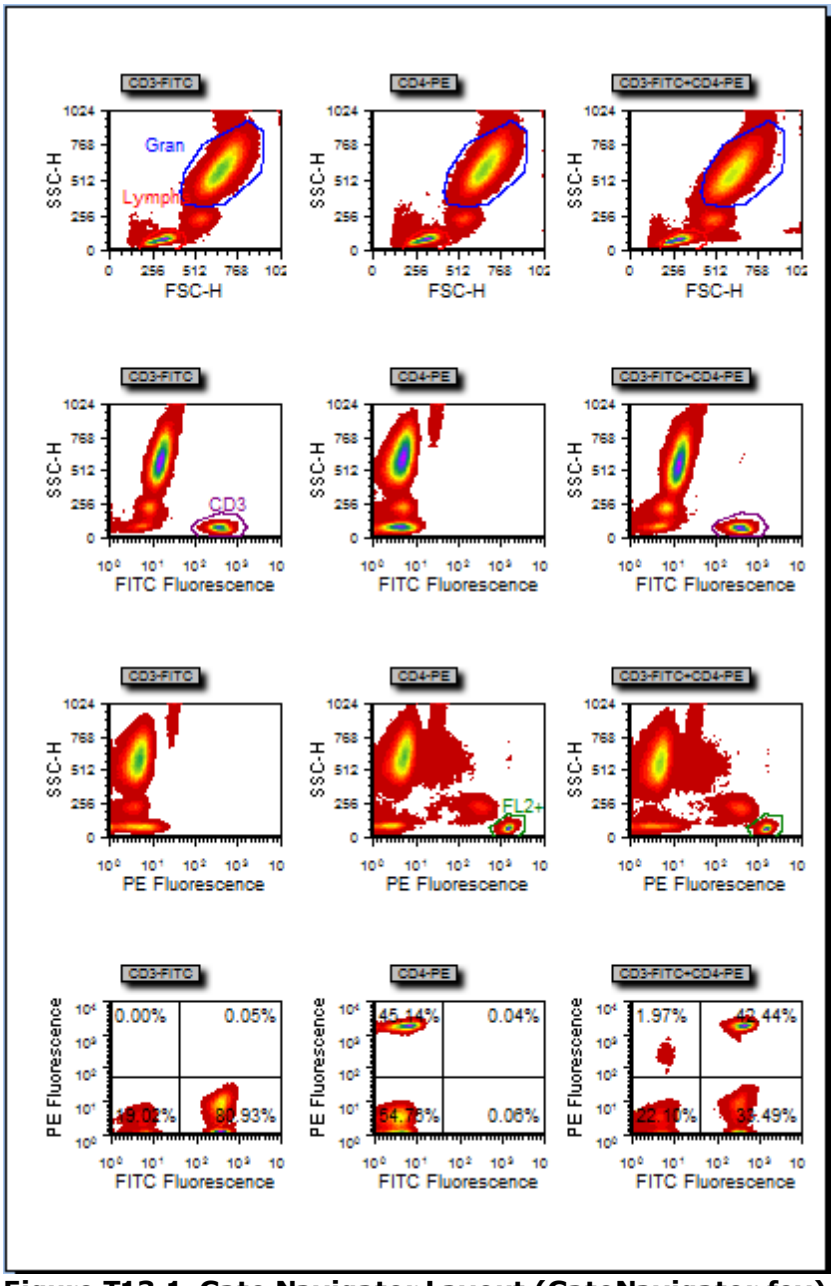

Figure T13.1 Gate Navigator Layout (GateNavigator.fey)

We will now open the **Gate Navigator.**

3. Select the **View→Navigators→Gate Navigator** command (Figure T13.2).

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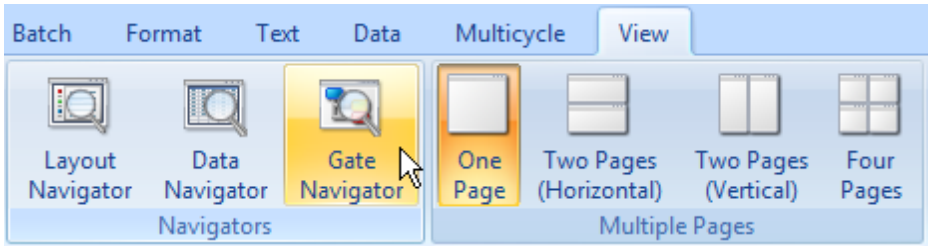

Figure T13.2 Selecting the Gate Navigator Command

The **Gate Navigator** appears, as shown in Figure T13.3. We will now expand the menu to view all the plots using these gates.

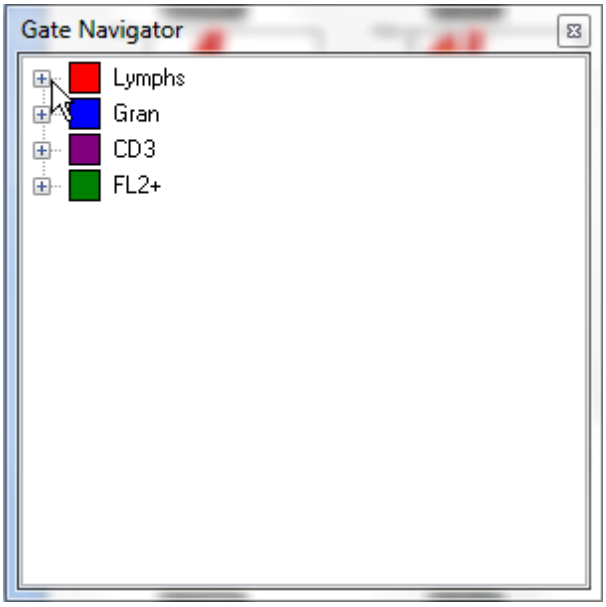

Figure T13.3 Gate Navigator Window

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.

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.

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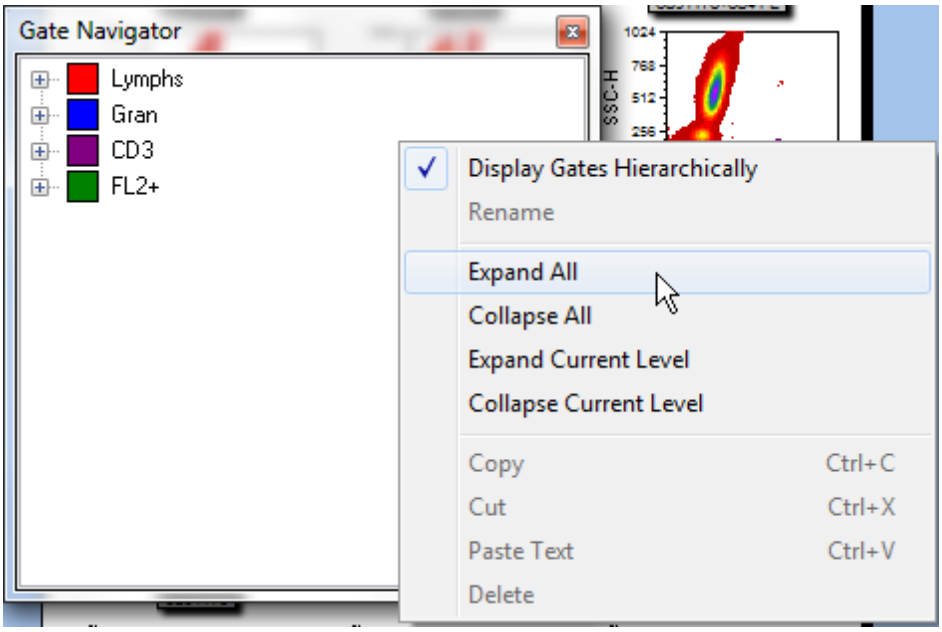

Figure T13.4 Gate Navigator Pop-up Menu

The **Gate Navigator** should now appear similar to Figure T13.5.

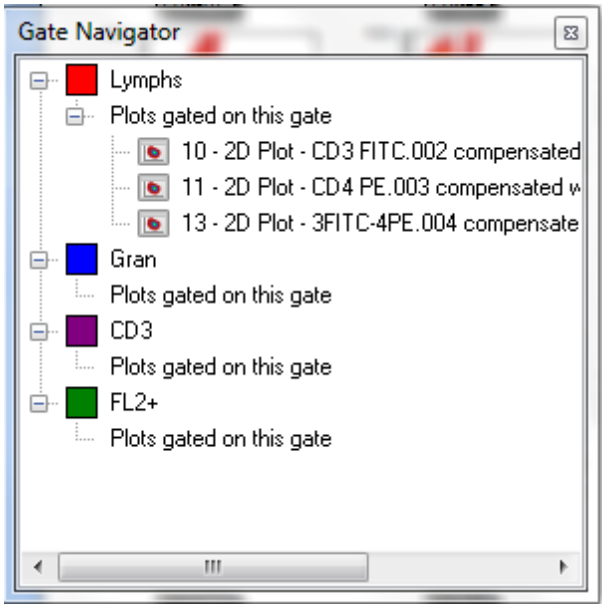

Figure T13.5 Gate Navigator with Listings Expanded

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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 845.

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.

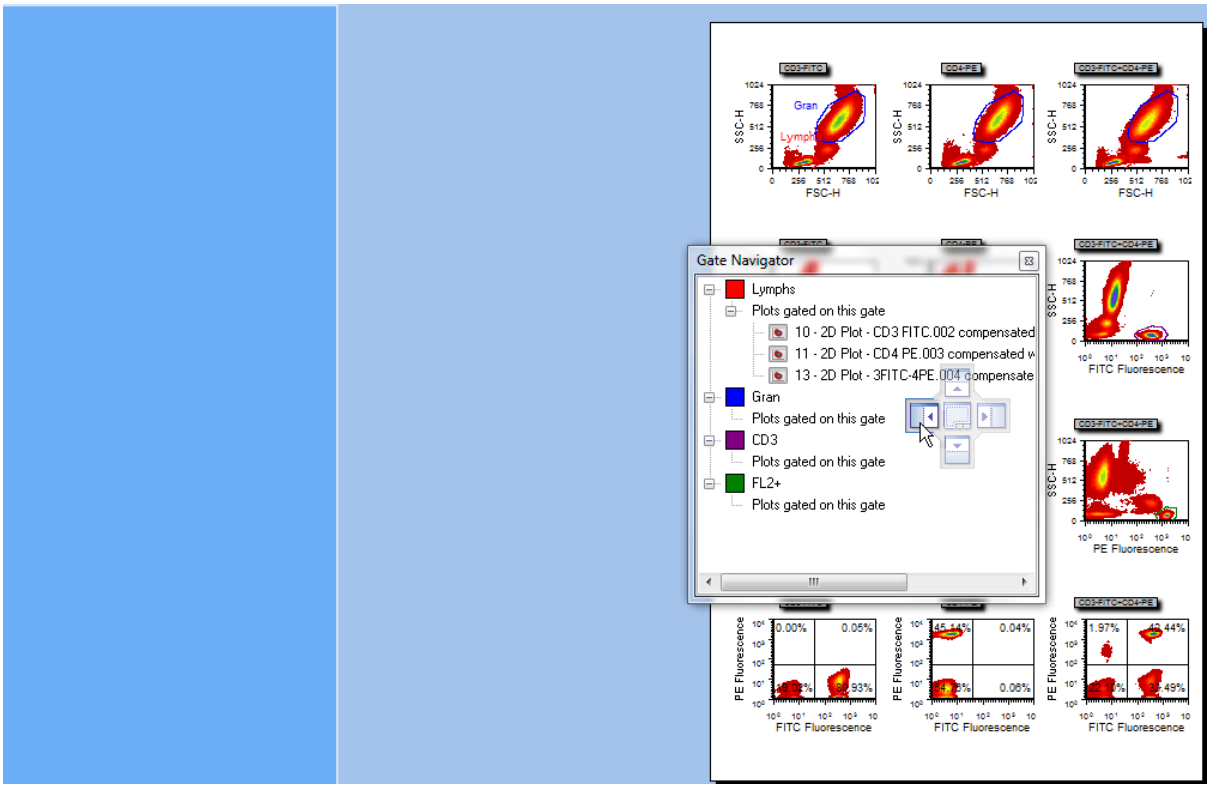

Figure T13.6 Docking the Gate Navigator to the Left Side of the Layout

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

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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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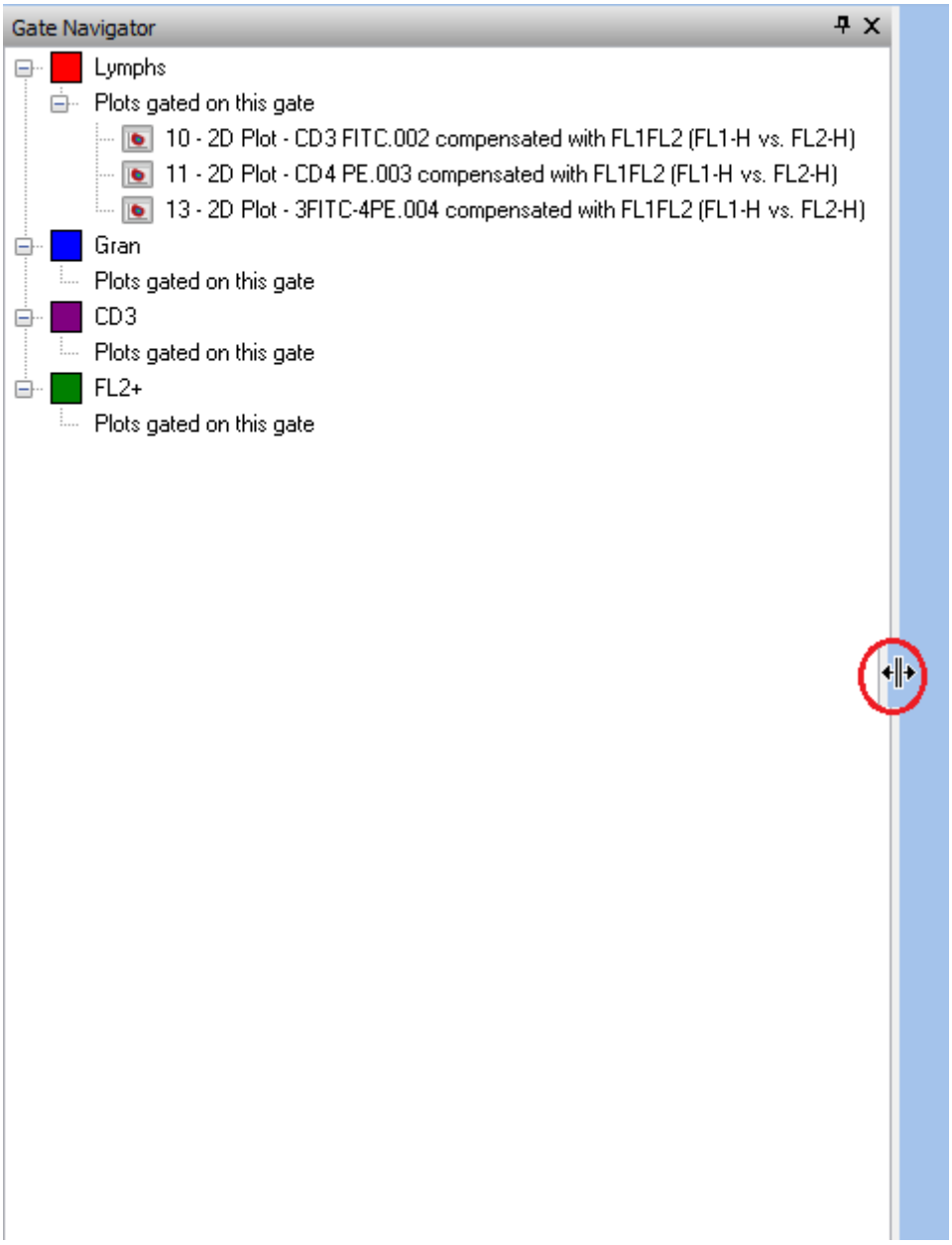

Figure T13.7 Resizing the Gate Navigator

In the next section, we will explore the features of the Gate Navigator 895.

34.13.2 Gate Navigator Features

As shown in Figure T13.7 889, 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

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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.
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.

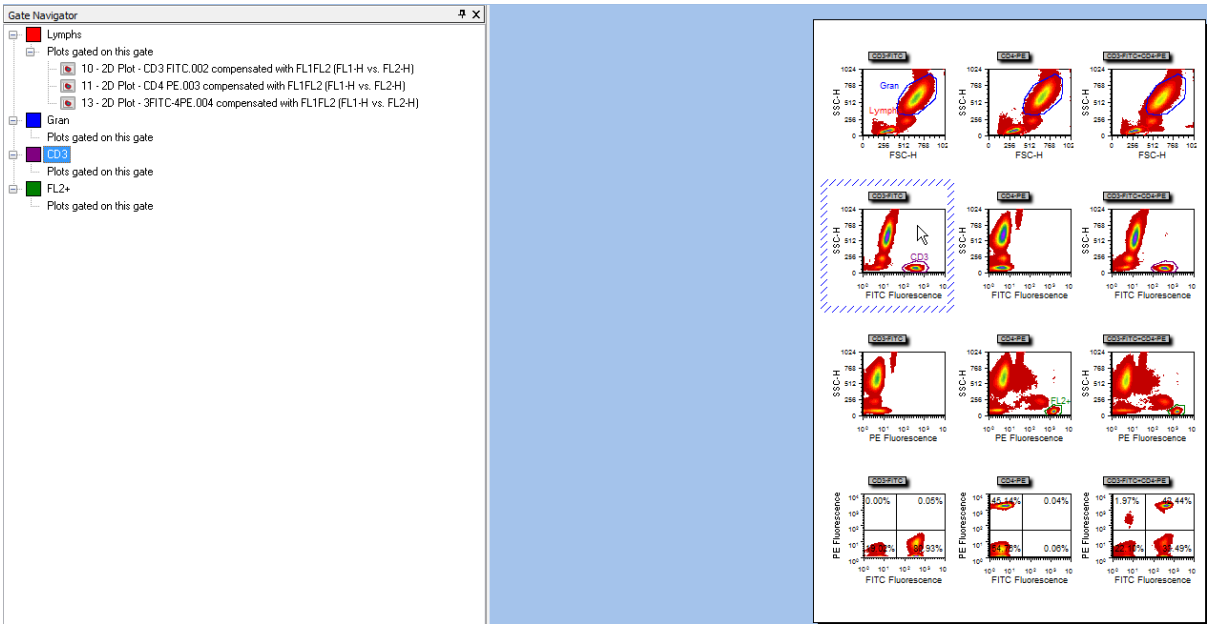

Figure T13.8 Dragging and Dropping the CD3 Gate from the Gate Navigator onto a Density Plot on the Layout

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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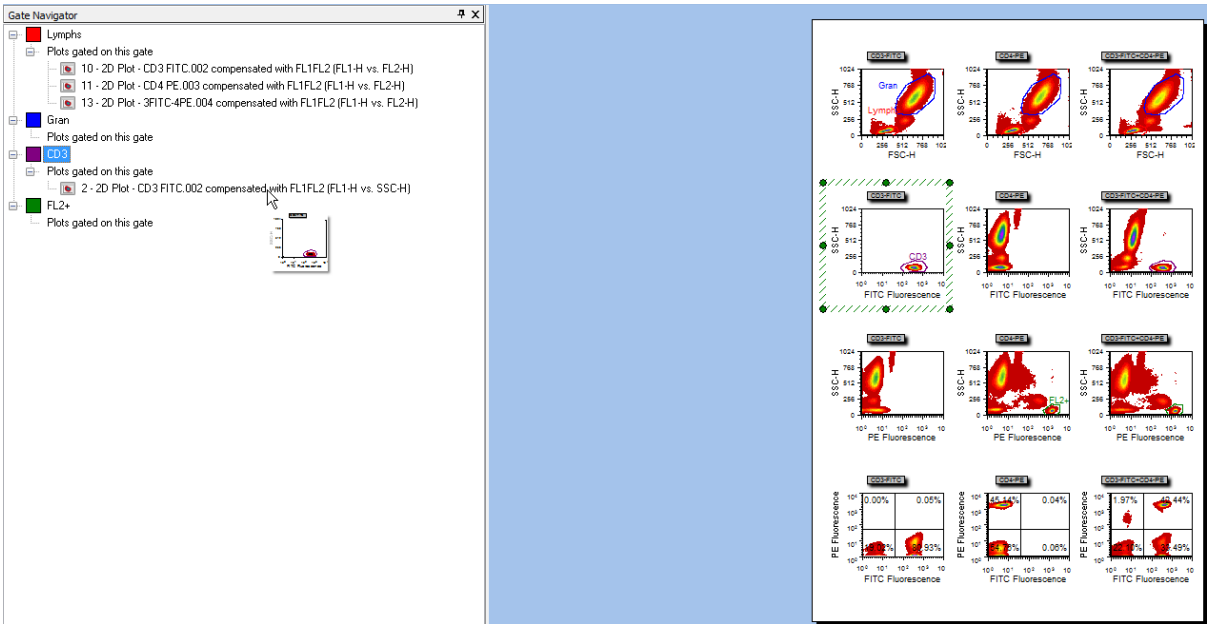

Figure T13.9 Application of the CD3 Gate to the 2D Density Plot of CD3-FITC (FL1-H vs. SSC-H)

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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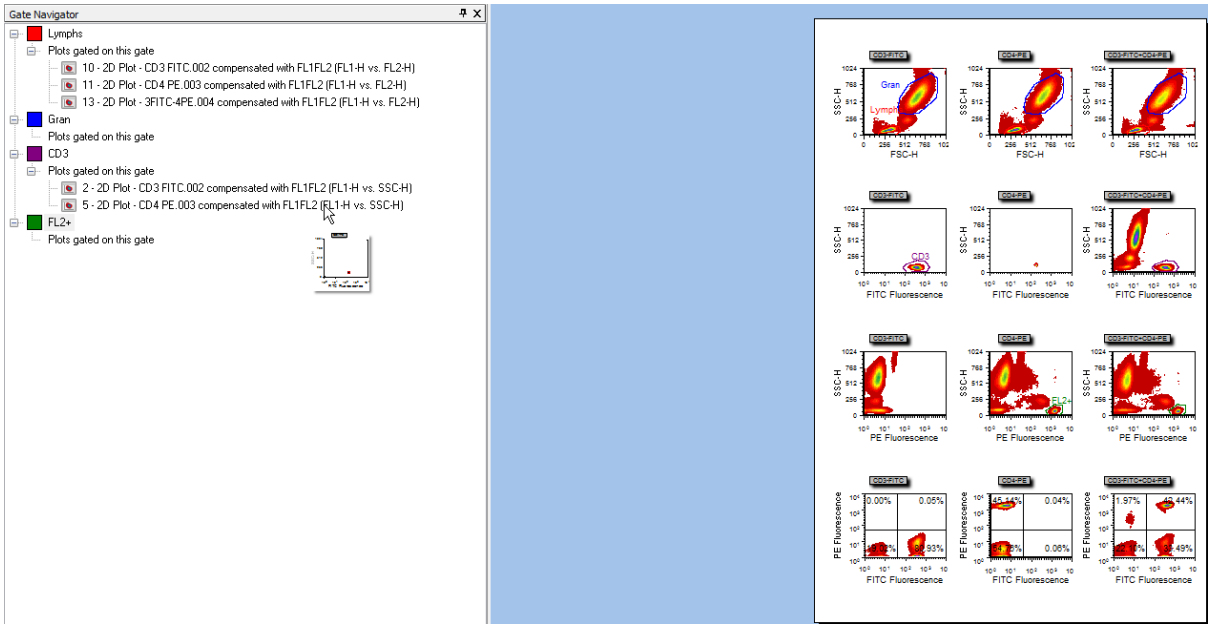

Figure T13.10 Application of the CD3 Gate to the 2D Density Plot of CD4-PE (FL1-H vs. SSC-H)

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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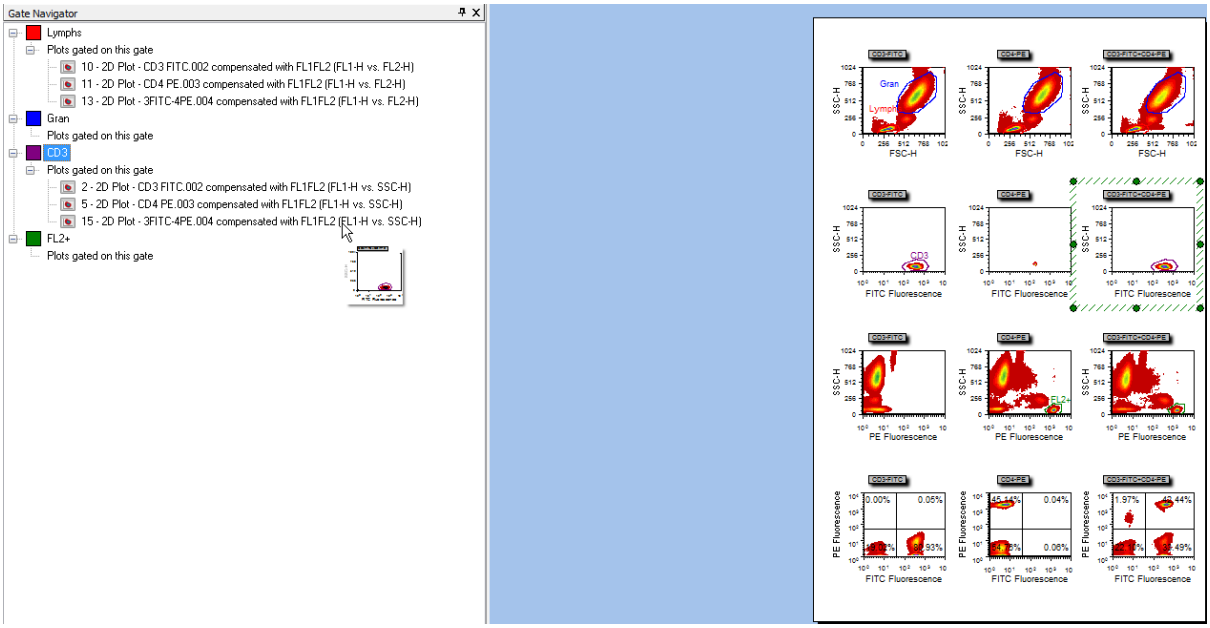

Figure T13.11 Application of the CD3 Gate to the 2D Density Plot of CD3-FITC+CD4-PE (FL1-H vs. SSC-H)

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

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the third row of the right column of the layout.

The 2D plots on the layout and **Gate Navigator** should now look similar to Figure T13.12.

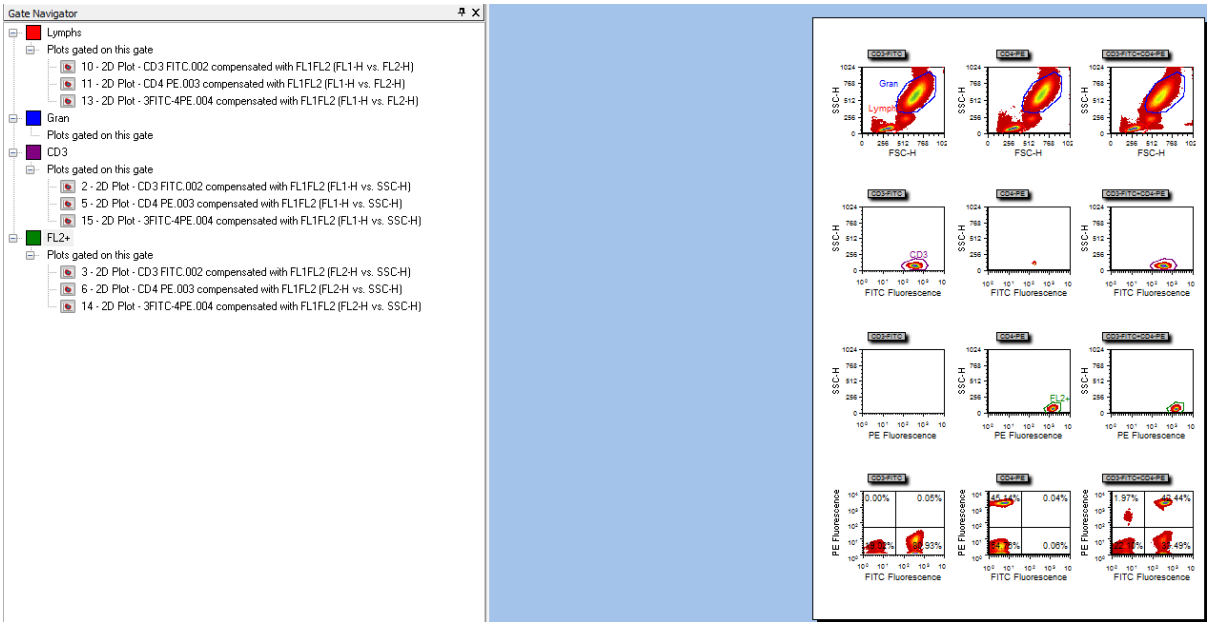

Figure T13.12 2D Plots and Gate Navigator Updated to Reflect the Application of Gates

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 **2D plot – CD3 FITC.002 (FL1-H vs. SSC-H)**, indicated by the cursor and highlighted in blue in Figure T13.13.

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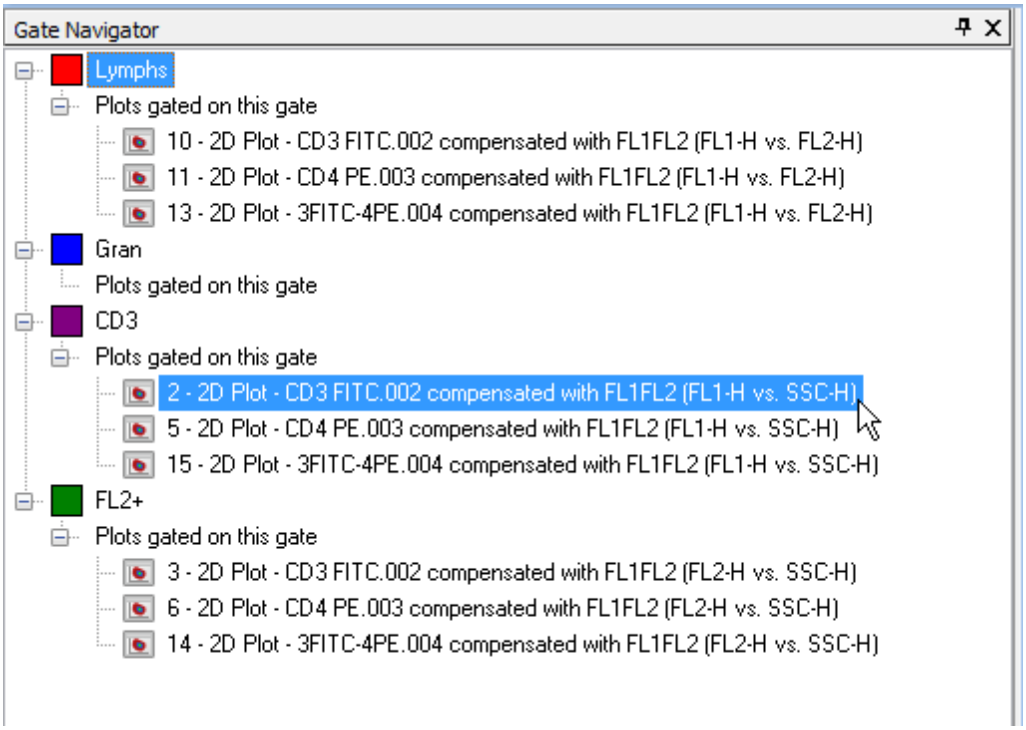

Figure T13.13 Dragging and Dropping the Lymphs Gate on the Gate Navigator

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 and the thumbnail image in Figure T13.14.

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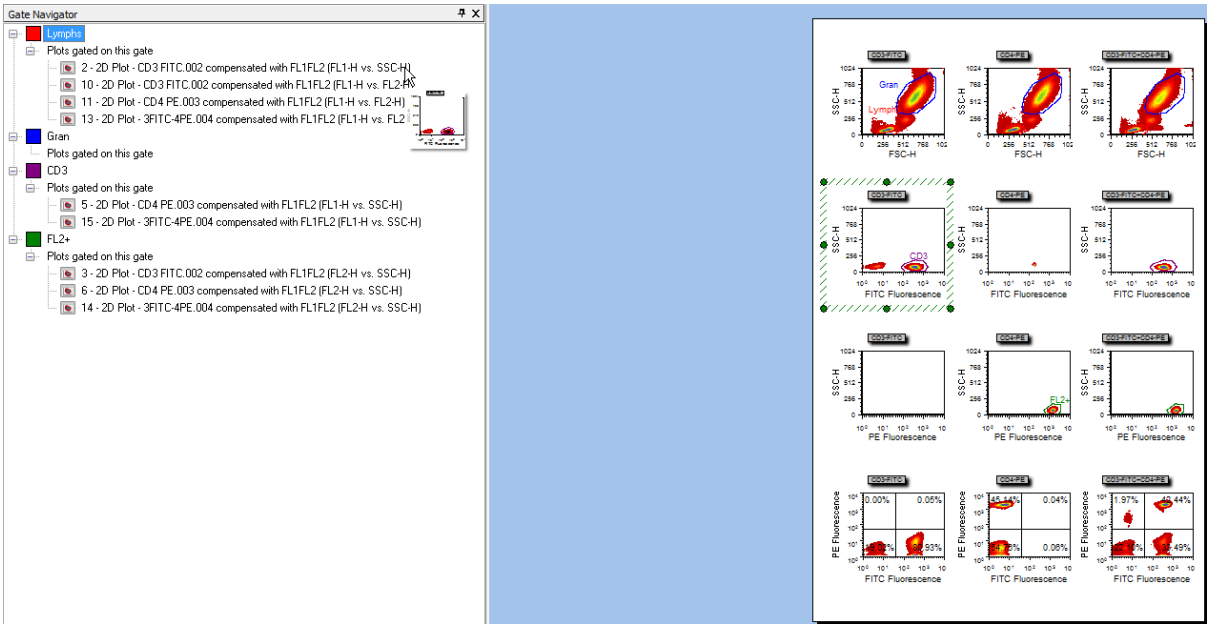

Figure T13.14 Updated Gate Navigator and Layout Reflect the Application of the Lymphs Gate

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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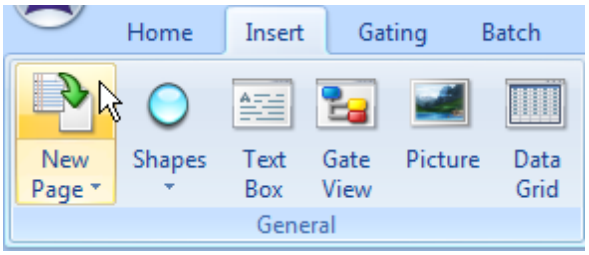

Figure T13.16 Selecting the Insert New Page Command

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.

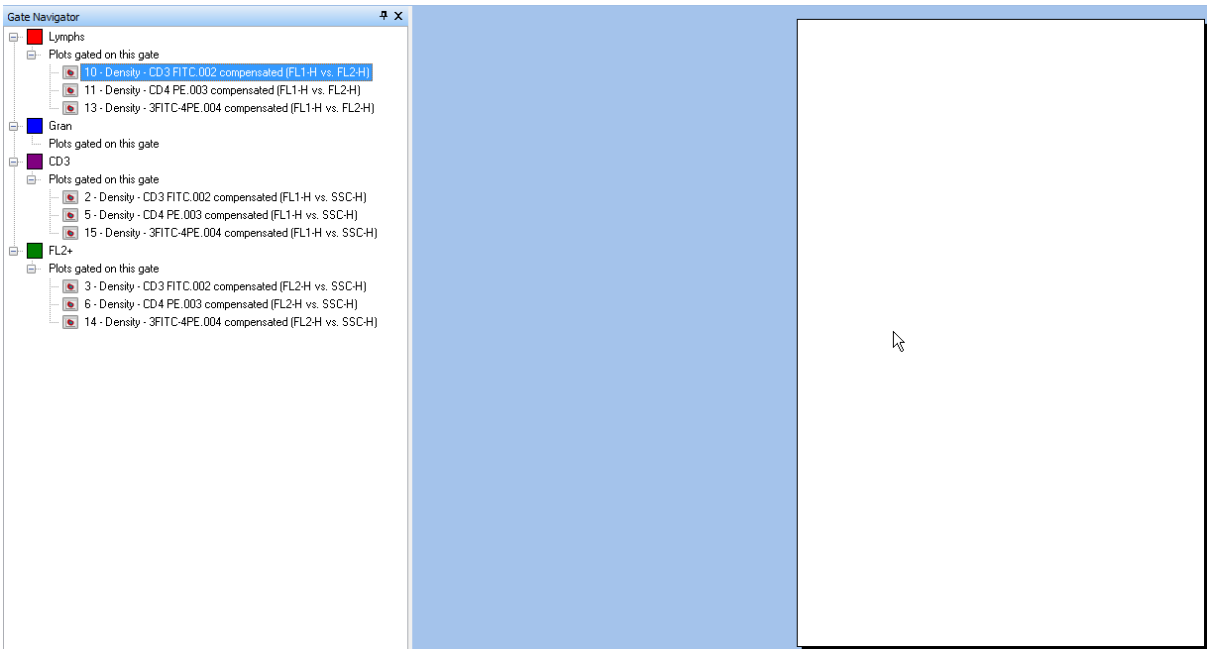

Figure T13.17 Dragging and Dropping a 2D Density Plot Listing onto Page 2 of the Layout

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The 2D density plot of CD3FITC.002 (FL1-H vs. FL2-H), gated with the **Lymphs** gate, now appears on page 2 of the layout, similar to Figure T13.18.

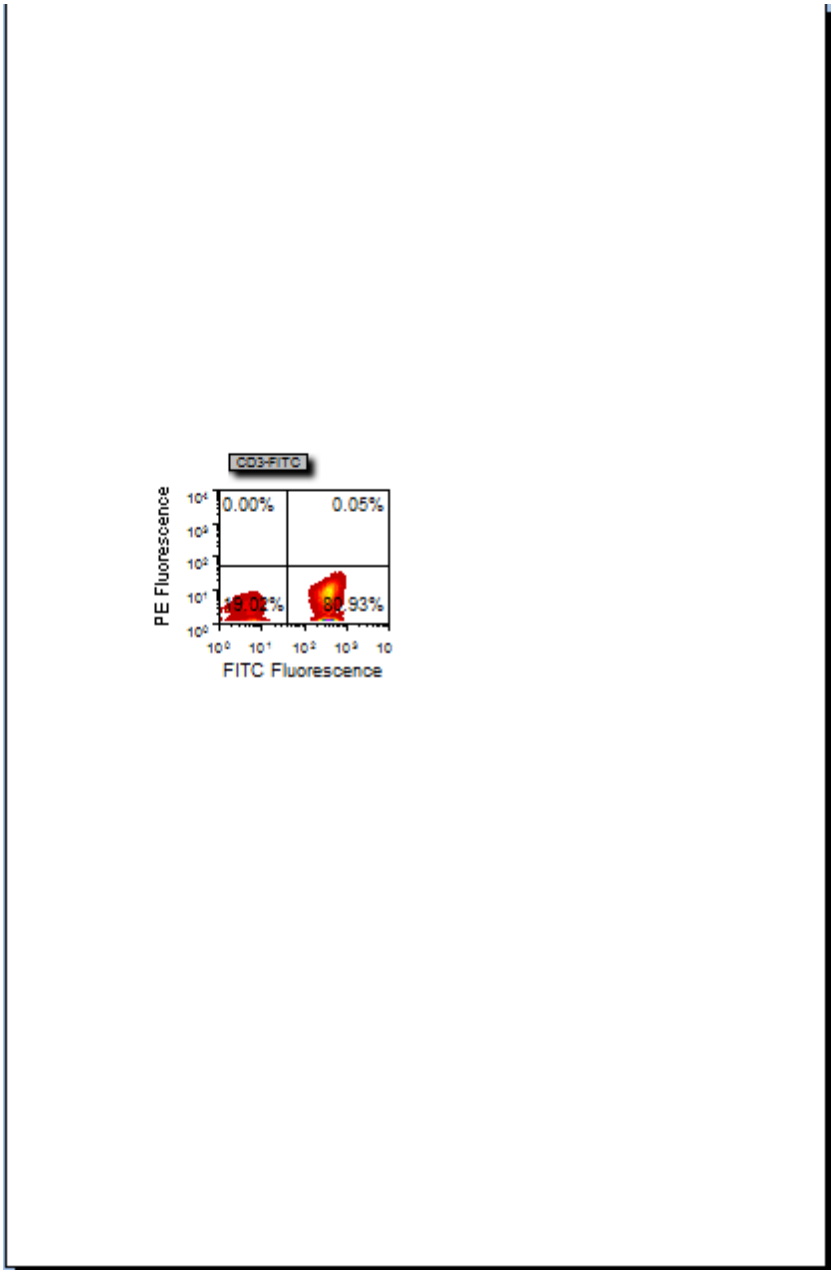

Figure T13.18 2D Plot on Page 2 of the Layout

34.14 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

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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.
  - Move a plot from one page to another.

In the first exercise, we will open and dock the Layout Navigator 905.

34.14.1 Opening and Docking

To begin, we will open a saved layout that already has plots and gates on multiple pages.

1. Select **Application Button→Open Layout**.
2. Open the layout **LayoutNavigator.fey** found in the Tutorial Sample Data archive 590.

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

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The **Zoom** dialog appears, as shown in Figure T14.2.

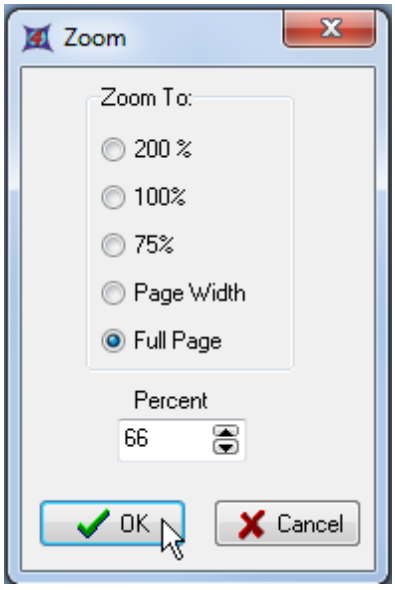

Figure T14.2 Zoom Dialog

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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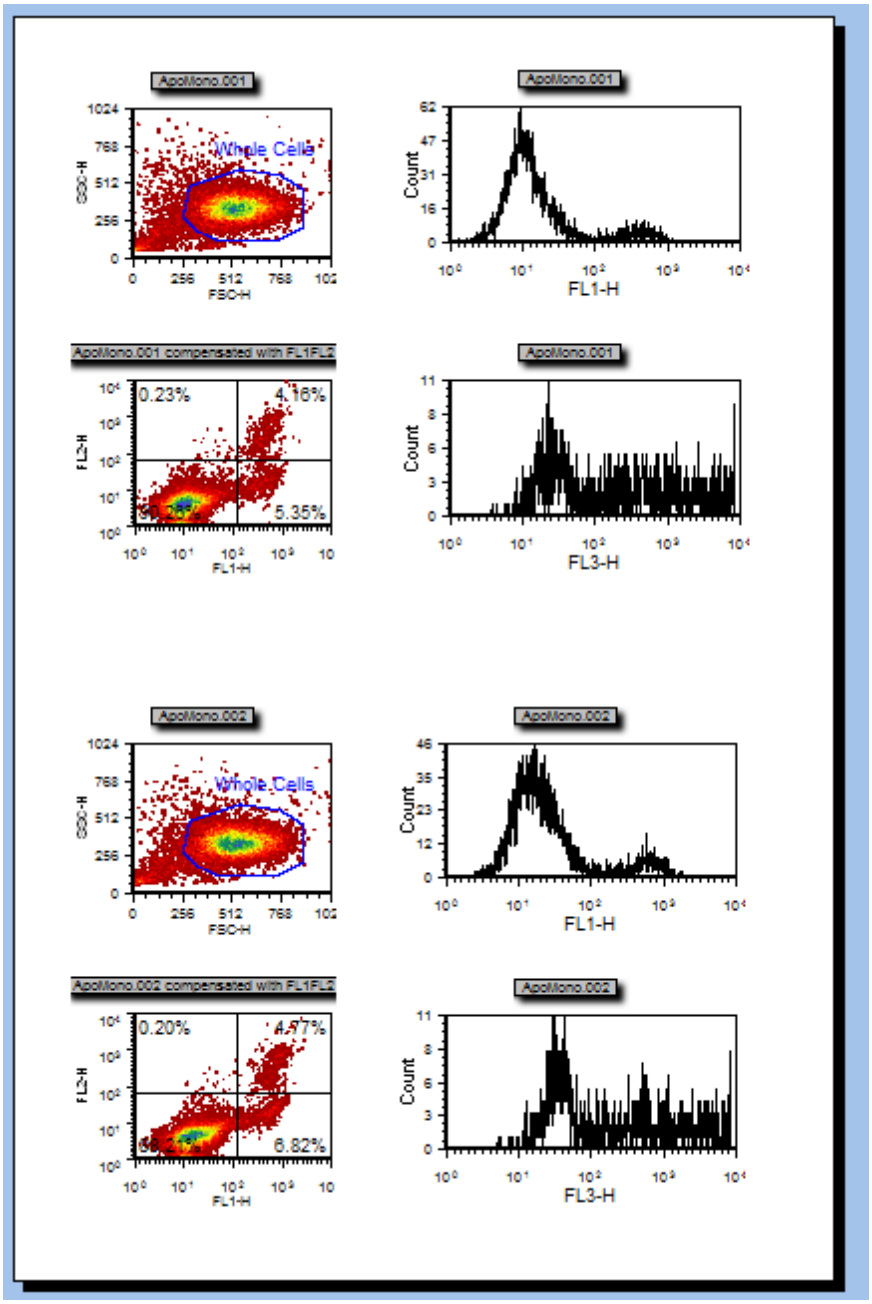

Figure T14.3 Layout Navigator Layout (LayoutNavigator.fey)

We will now open the **Layout Navigator.**

6. Select the **View→Navigators→Layout Navigator** command (Figure T14.4).

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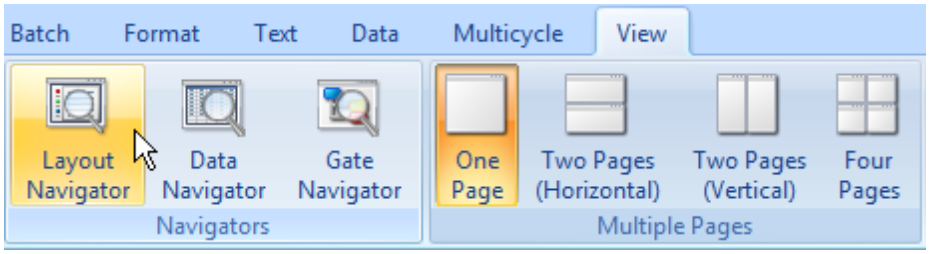

Figure T14.4 Selecting the Layout Navigator Command

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 845.

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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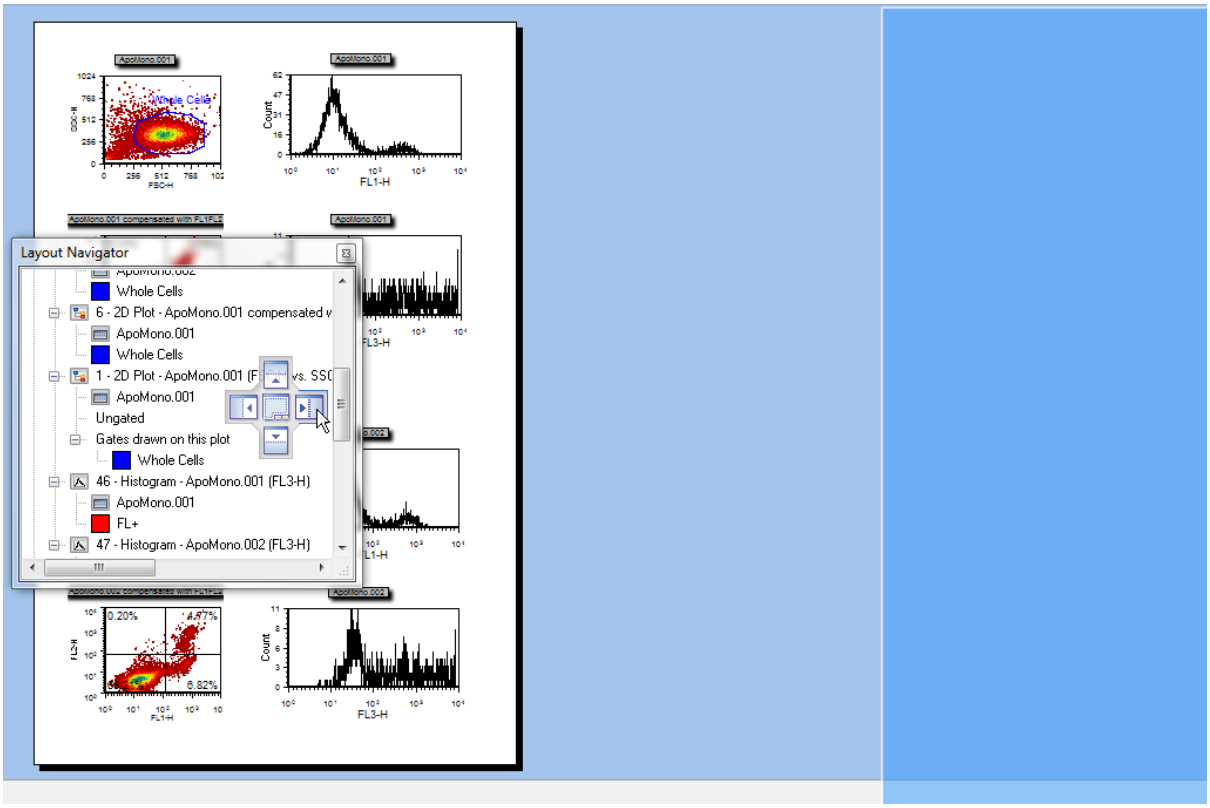

Figure T14.5 Docking the Layout Navigator

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. Place the mouse over the outer left border of the docked **Layout Navigator**.

12. Move the mouse slightly over the border until the resizing icon appears.

13. Press and hold down the left mouse button and continue to hold it while dragging the border to the left.

14. 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.

15. Use the scroll bar on the right side of the docked window to scroll to the top of the **Layout Navigator**.

The docked **Layout Navigator** should appear similar to Figure T14.6.

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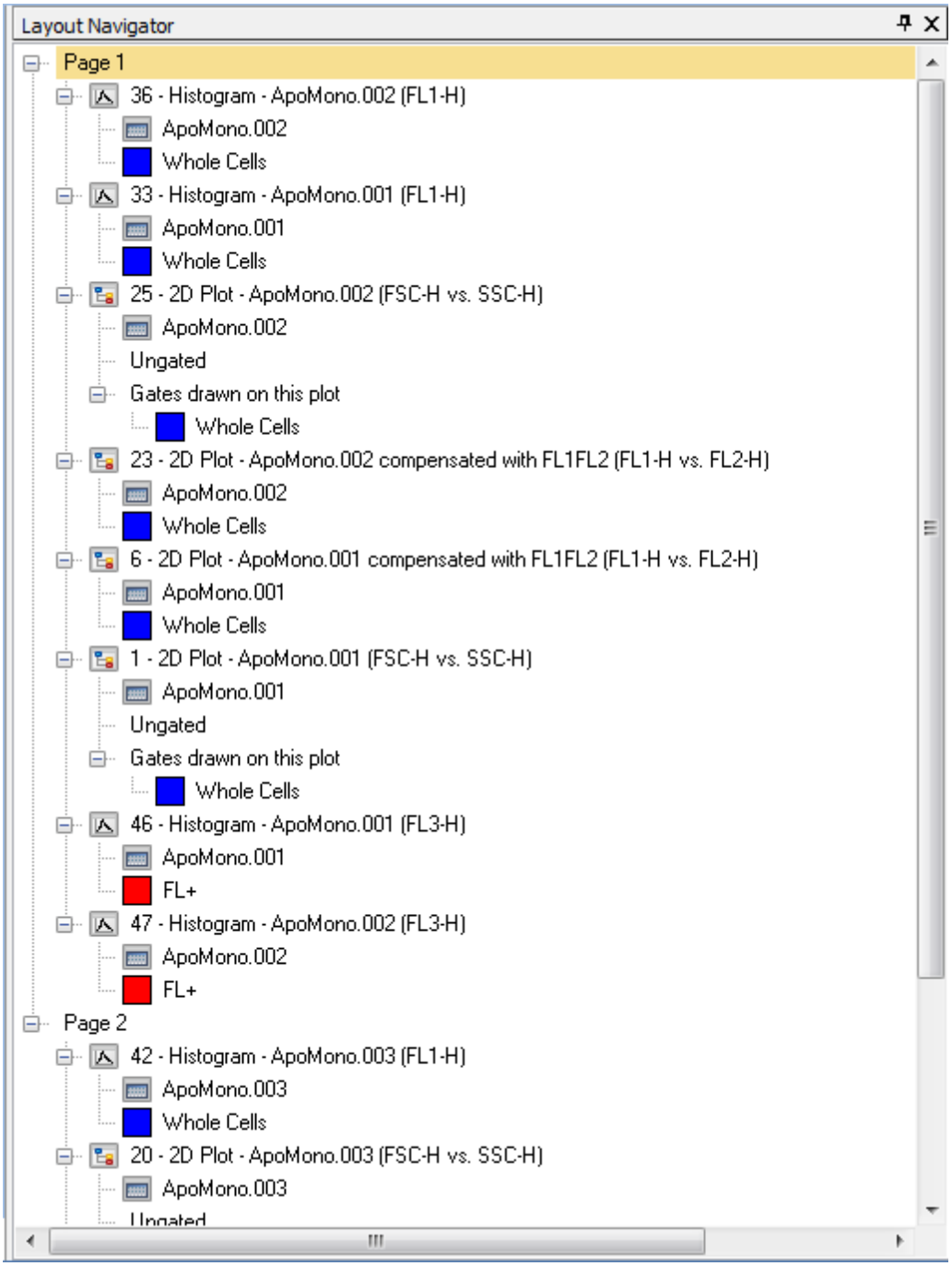

Figure T14.6 Docked Layout Navigator

In the next section, we will learn the features of the Layout Navigator 910.

34.14.2 Layout Navigator Features

The **Layout Navigator** is organized by the Pages of the layout. Notice that the

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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.

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.

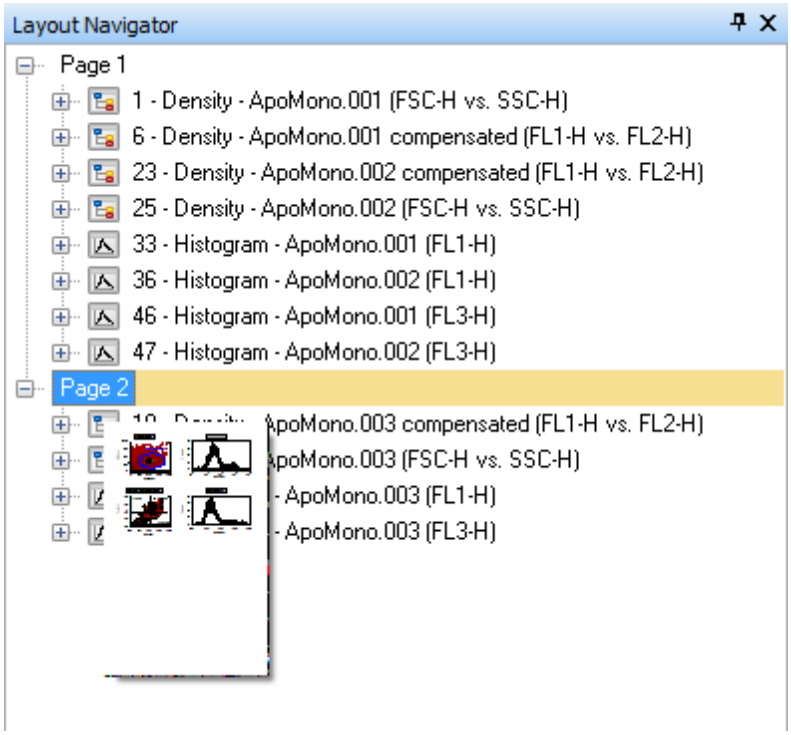

Figure T14.7 Image of Page 2 on the Layout Navigator

2. Click on **Page 2** on the **Layout Navigator**.

Page 2 of the layout now appears with four 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**, 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.

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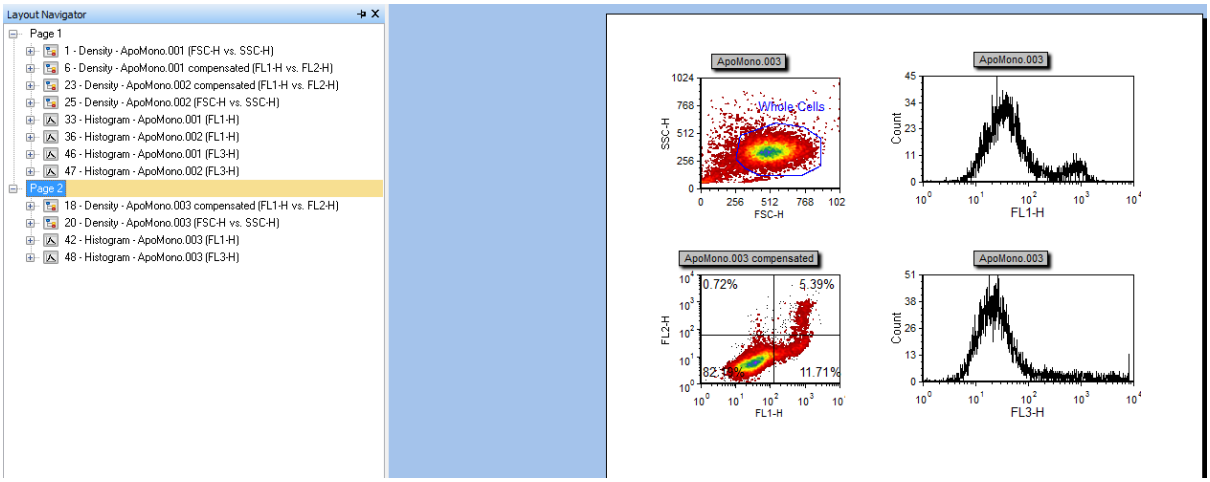

Figure T14.8 Page 2 of the Layout

3. Select the ApoMono.003 FL3-H histogram plot, located in the lower right of the layout (Figure T14.9).

Notice that the listing for the ApoMono.003 (FL3-H) histogram is now highlighted in the **Layout Navigator**, as shown in Figure T14.9.

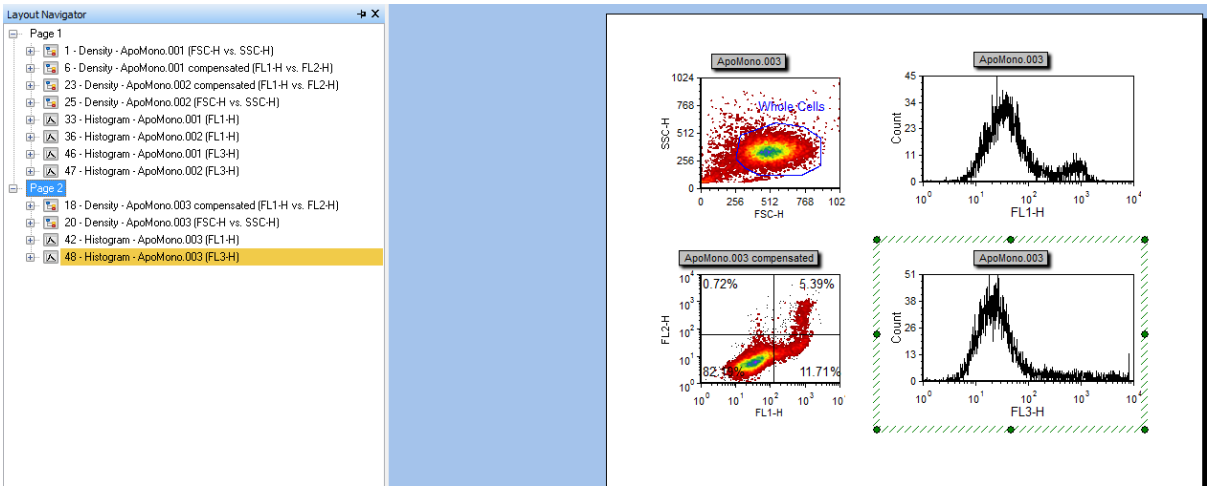

Figure T14.9 ApoMono.003 (FL3-H) Histogram Selected

Unlike the other histograms of FL3-H, this one is not gated using the **FL+** gate. We will now use the **Layout Navigator** to apply the **FL+** gate to the ApoMono.003 (FL3-H) histogram.

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4. Place the cursor over the **FL+** gate, listed under **Histogram – ApoMono.002 (FL3-H)** 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 **FL+** gate down towards the listing for **Histogram – ApoMono.003 (FL3-H)**, on Page 2 of the **Layout Navigator**.
7. Release the mouse button when the **FL+** gate is over the **Histogram – ApoMono.003 (FL3-H)** 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 histogram plot on the layout.

A. Click on **Page 2** in the **Layout Navigator** to display that layout page.

B. Place the cursor over the **FL+** gate, listed under **Histogram – ApoMono.002 (FL3-H)** on Page 1 of the **Layout Navigator**, highlighted in blue in Figure T14.10.

C. Press and hold the left mouse button.

D. Continue to hold the mouse button and drag the **FL+** gate onto the histogram plot of **ApoMono.003 FL3-H** that is visible on Page 2.

E. Release the mouse button when the FL+ gate is on the histogram plot of **ApoMono.003 FL3-H**.

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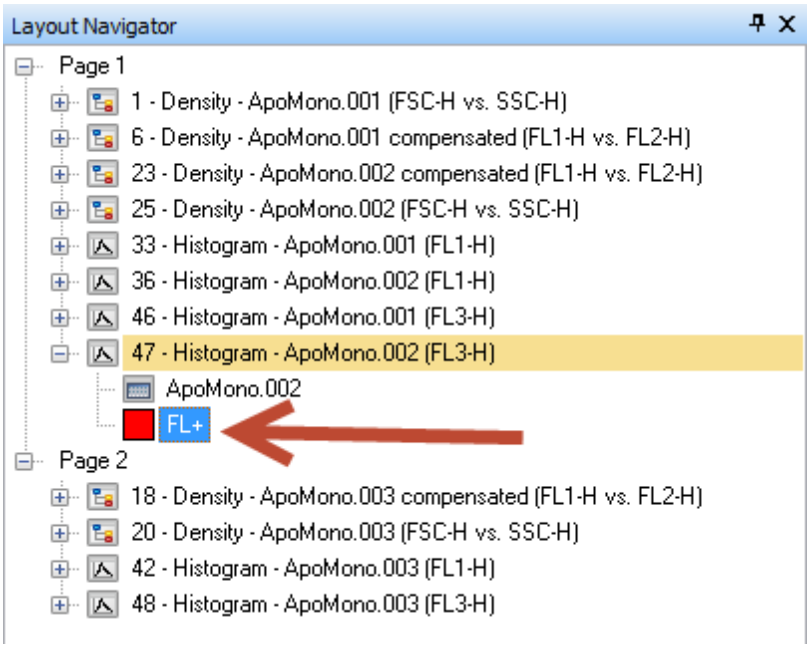

Figure T14.10 Dragging and Dropping the FL+ Gate onto ApoMono.003 (FL3-H) with the Layout Navigator

The ApoMono.003 (FL3-H) histogram updates to reflect the application of the **FL+** gate, as shown in Figure T14.11. The **Layout Navigator** also updates to reflect the **FL+** gate on the ApoMono.003 (FL3-H) histogram.

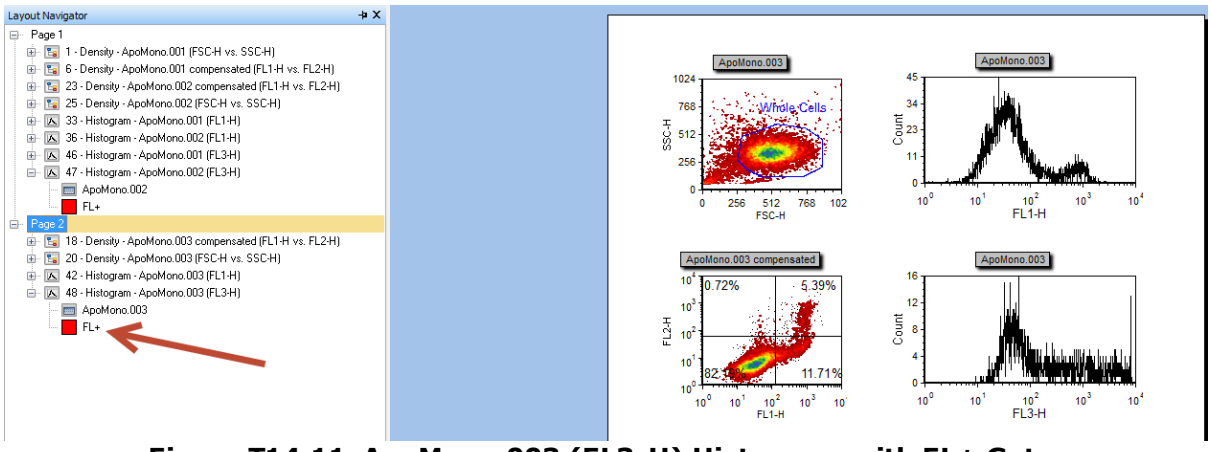

Figure T14.11 ApoMono.003 (FL3-H) Histogram with FL+ Gate

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**

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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 one of the listings for the **ApoMono.002** data in the **Layout Navigator** (it doesn't matter which plot it's listed under) indicated by the cursor in Figure T14.12.

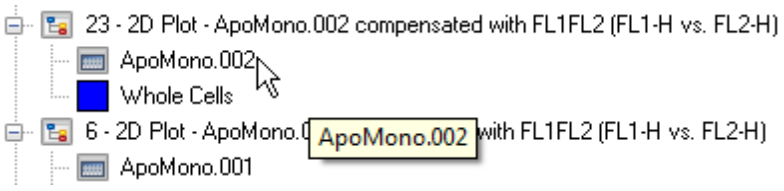

Figure T14.12 ApoMono.002 Data Listing in the Layout Navigator

11. Press and hold the left mouse button.

12. Continue to hold down the mouse button and drag the ApoMono.002 data onto the layout.

13. Release the mouse button when the cursor is over the histogram of ApoMono.003 FL1-H.

Alternatively, you can drag and drop the data within the **Layout Navigator**.

A. Place the cursor over one of the listings for the ApoMono.002 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 ApoMono.002 data down the **Navigator Layout** towards the **Histogram – ApoMono.003 (FL1-H)** plot listing.

D. Release the mouse button when the cursor is over the **Histogram – ApoMono.003 (FL1-H)** plot listing, highlighted and indicated by the cursor in Figure T14.13.

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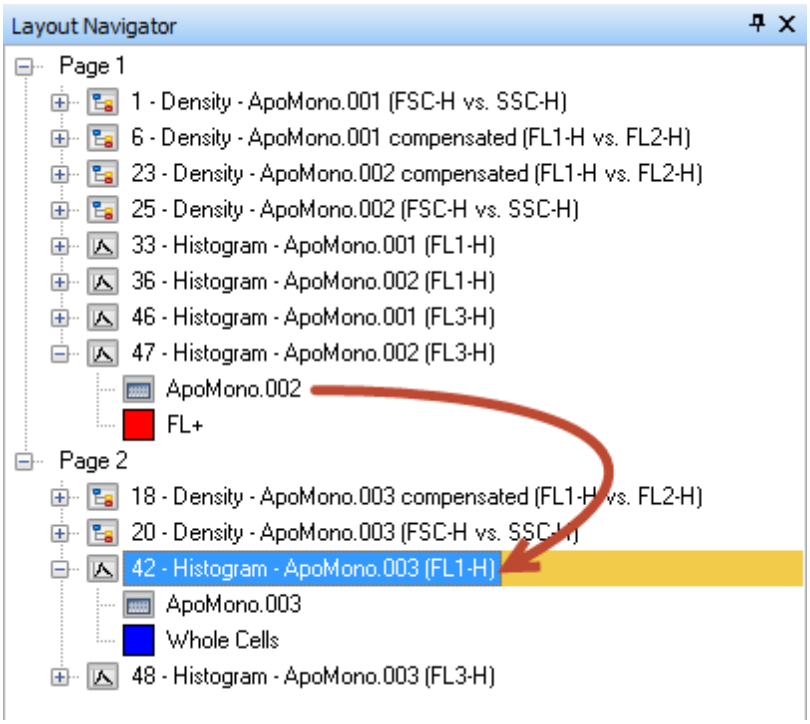

Figure T14.13 Dragging and Dropping ApoMono.002 Data within the Layout Navigator

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**.

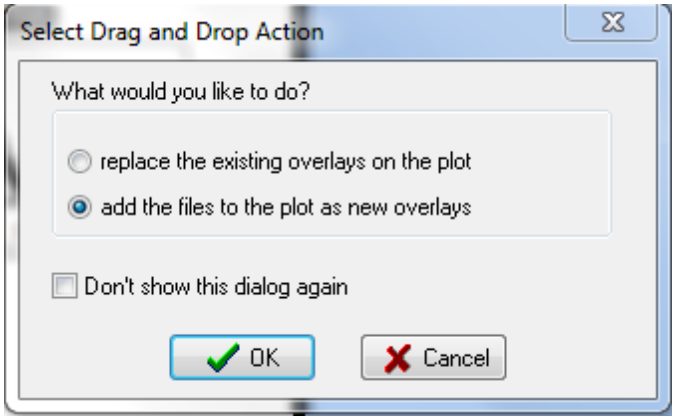

Figure T14.14 Select Drag and Drop Action Dialog

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The ApoMono.003 FL1-H histogram now updates to reflect the overlay of the ApoMono.002 data as shown in Figure T14.15. The **Layout Navigator** also updates to show that there is now overlay data on the ApoMono.003 (FL1-H) histogram, highlighted in Figure T14.15.

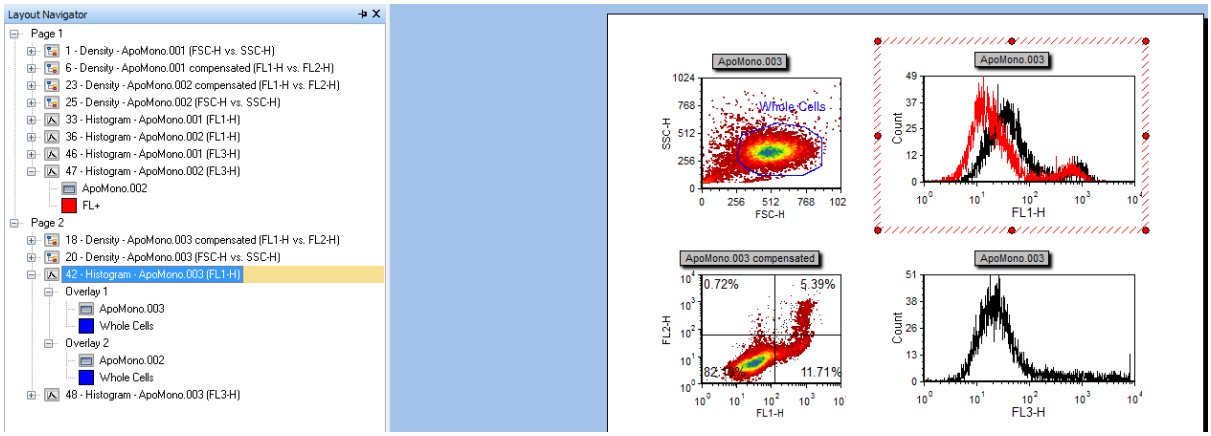

Figure T14.15 ApoMono.003 (FL1-H) Histogram with Overlay of ApoMono.002 Data

We will now undo the overlay on the histogram.

16. Click on the **Undo** icon on the **Quick Access Toolbar** (Figure T14.16).

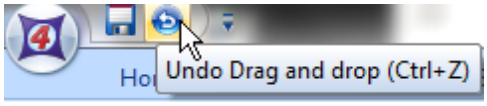

Figure T14.16 Undo Drag and Drop

The ApoMono.003 FL1-H histogram 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 **ApoMono.002** data in the **Layout Navigator** (it doesn't matter which plot it's listed under)

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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 ApoMono.002 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.

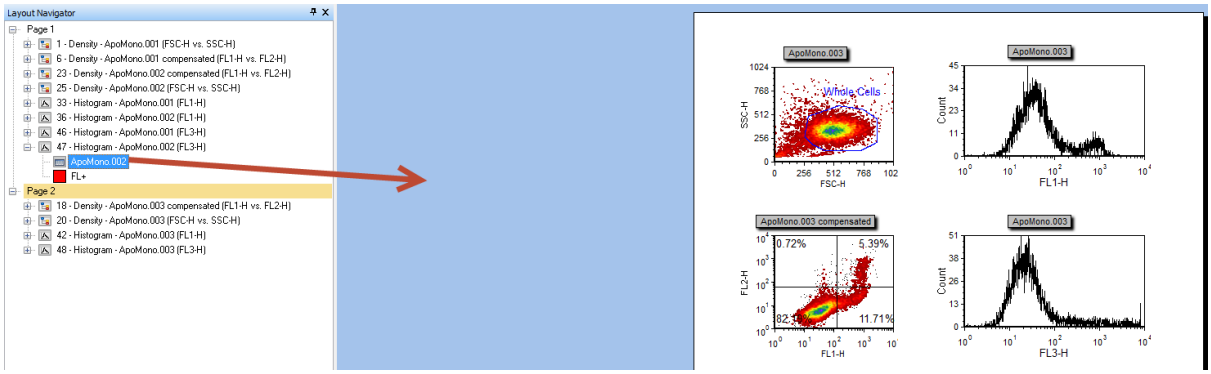

Figure T14.17 Dragging and Dropping ApoMono.002 Data onto the Layout

The **Select Plot Types...** dialog appears, as shown in Figure T14.18.

22. Select **Density** as the type of plot to open for the ApoMono.002 data (Figure T14.18).

23. Click **OK**.

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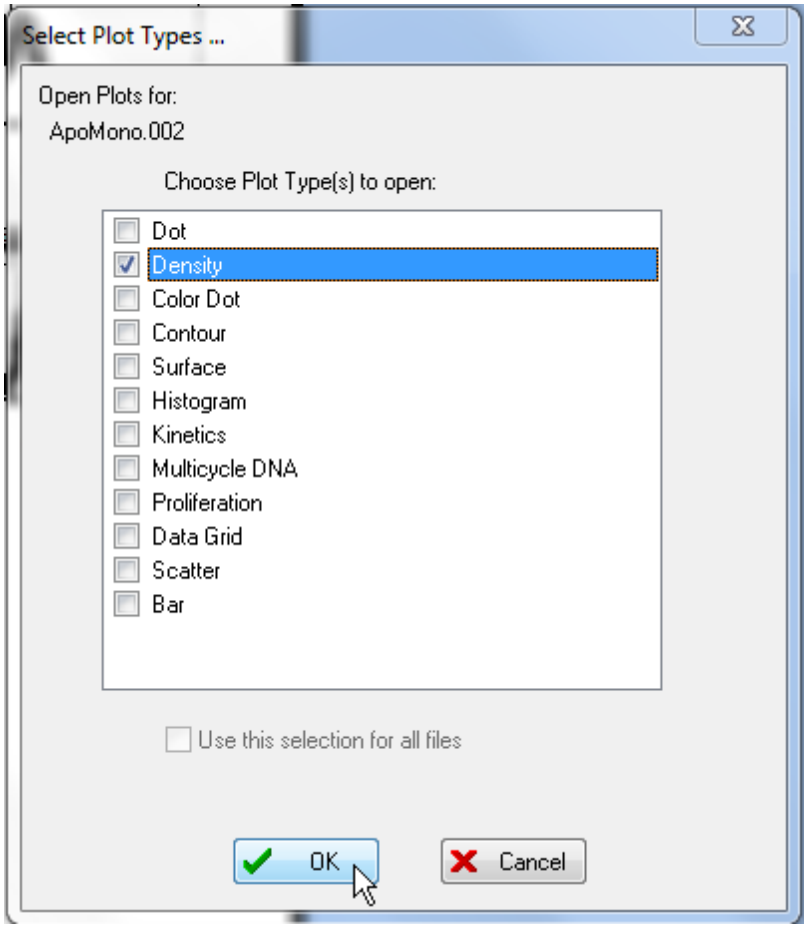

Figure T14.18 Select Plot Types... Dialog

A density plot of the ApoMono.002 data now appears on the layout, with FSC vs. SSC 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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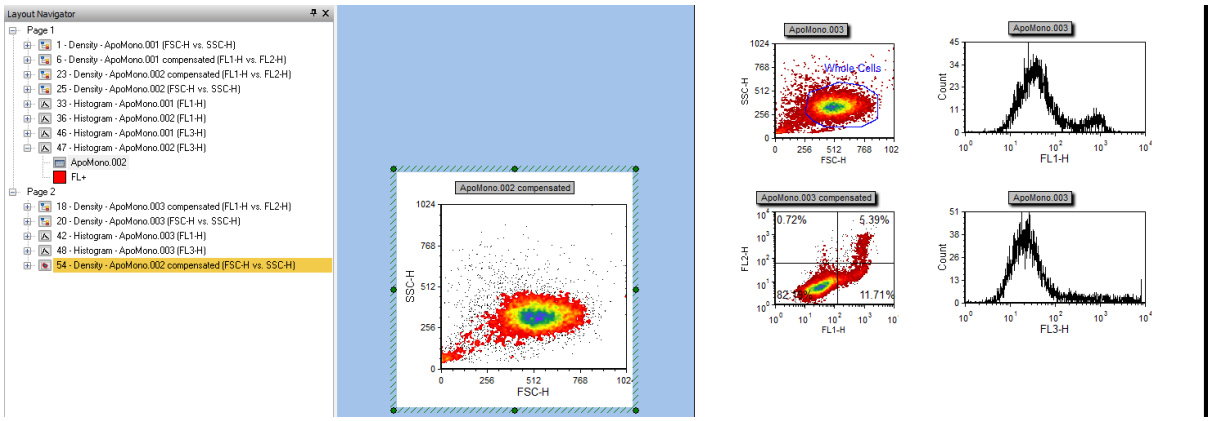

Figure T14.19 Density plot of ApoMono.002 with Updated Layout Navigator

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 **23 - Density – ApoMono.002 compensated (FL1-H vs. FL2-H)**, 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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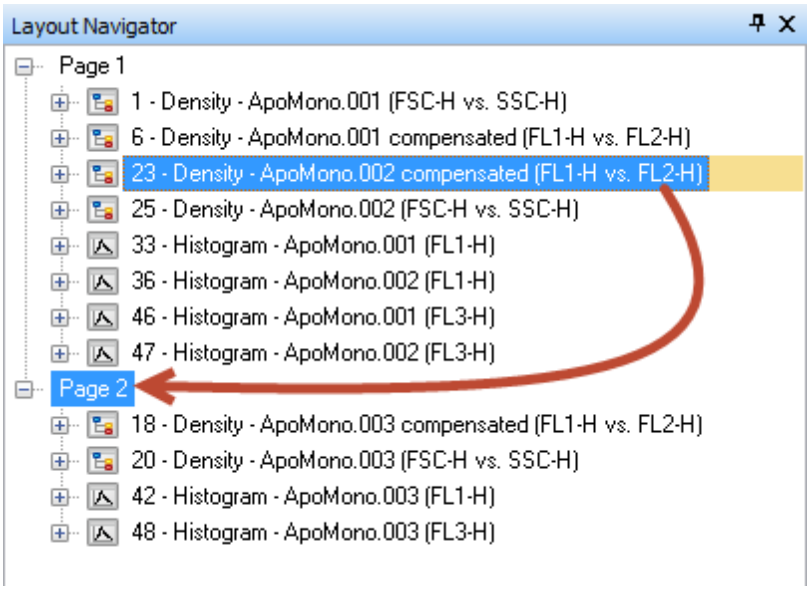

Figure T14.20 Moving a Plot to Page 2 in the Layout Navigator

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 the ApoMono.002 2D plot of FL1 versus FL2 is now missing, as it was moved to page 2 of the layout.

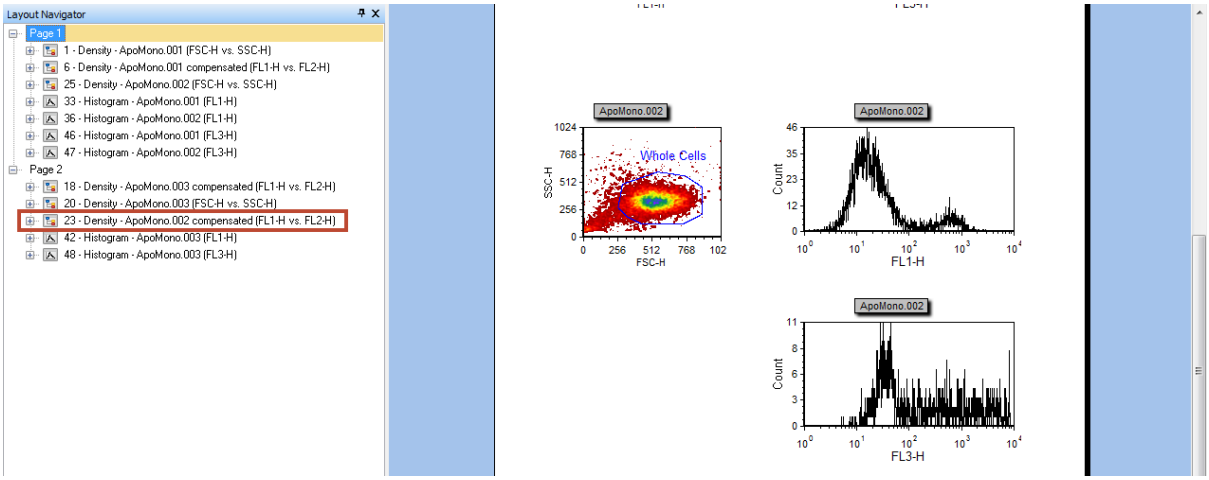

Figure T14.21 Page 1 of the Layout without the ApoMono.002 2D Plot

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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 the ApoMono.002 density plot of FL1 versus FL2 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.

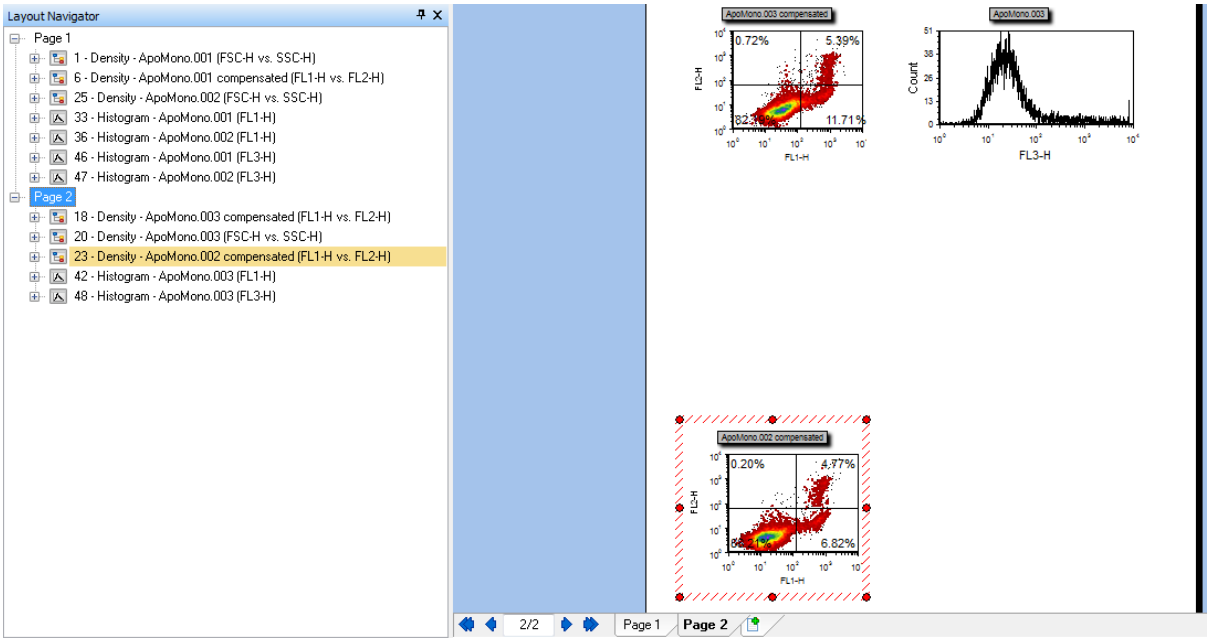

Figure T14.22 Page 2 of the Layout with the Addition of the ApoMono.002 2D Plot

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 **23 - Density Plot – ApoMono.002 compensated (FL1-H vs. FL2-H)** listed on Page 2 of the **Layout Navigator**.

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32. Right-click on the density plot on Page 2 of the layout to bring up the menu, shown in Figure T14.23.

33. Select **Cut 2D Plot** from the menu (Figure T14.23).

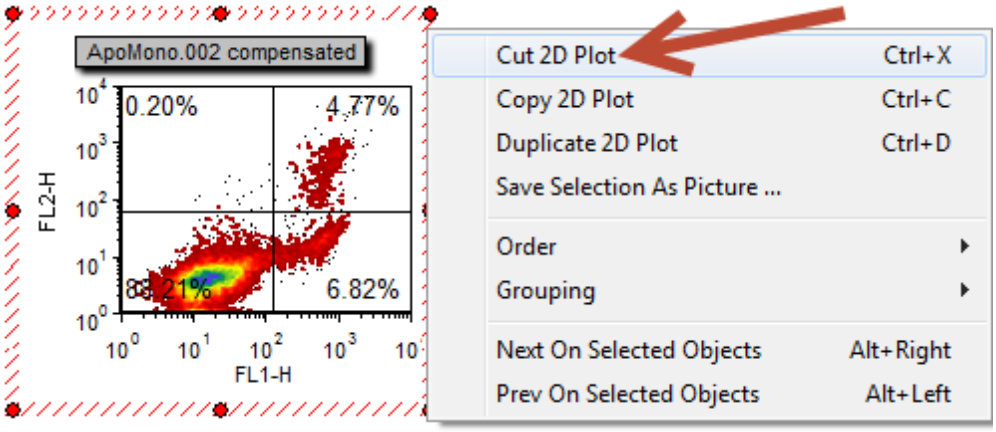

Figure T14.23 Layout Navigator 2D Plot Pop-up Menu

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 2D Plot** from the menu (Figure T14.24).

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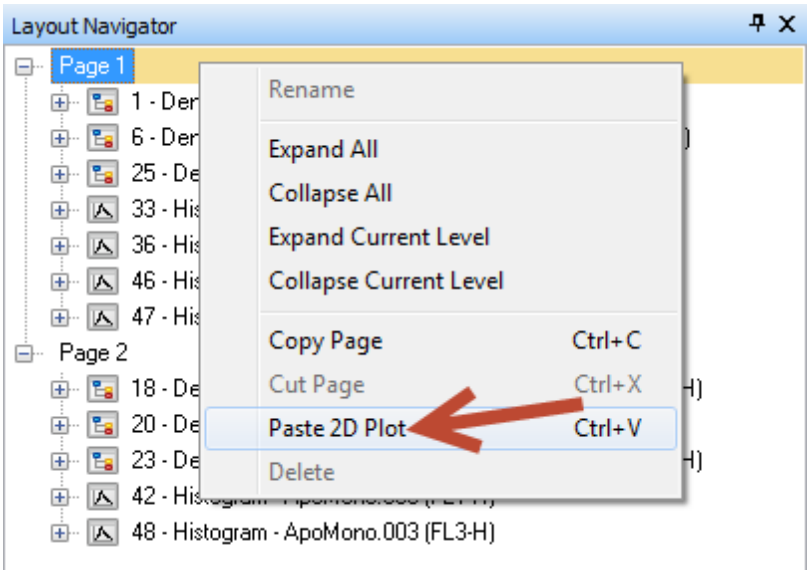

Figure T14.24 Paste 2D Plot Menu Item

Alternatively, after following steps 31 through 33 to cut the density 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 density plot.

The density plot is now pasted back onto page 1 of the layout and the **Layout Navigator** updates to reflect this change.

34.15 Creating and Using Custom Data

FCS Express provides a number of tools that allow you to create and graph your own data sets. Your data sets (called custom datasets) can contain data from many sources including, but not limited to, tokens such as keyword, statistics, custom tokens, and token classifiers. The advantage of creating a custom data set containing tokens is that when your layout changes (e.g., by making a gate or displaying a new set of data) your custom data set will change as well. The use of a custom data set allows you to easily demonstrate the important results from your experiments using a data table or by graphing the results using a bar chart or scatter plot. In this tutorial, we will focus on creating and using custom data sets.

We will create a custom dataset 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

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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 Data Grids with custom labels.
- Create and insert various kinds of tokens and data into a Data Grid.
- Explore various ways of entering information into a Data Grid.
- Insert new columns into a Data Grid.
- Insert token classifiers into a Data Grid.

First, we will load a layout in preparation for creation of a custom dataset925.

34.15.1 Loading a Layout for Custom Data

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 **Application Button→Open Layout**.
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.

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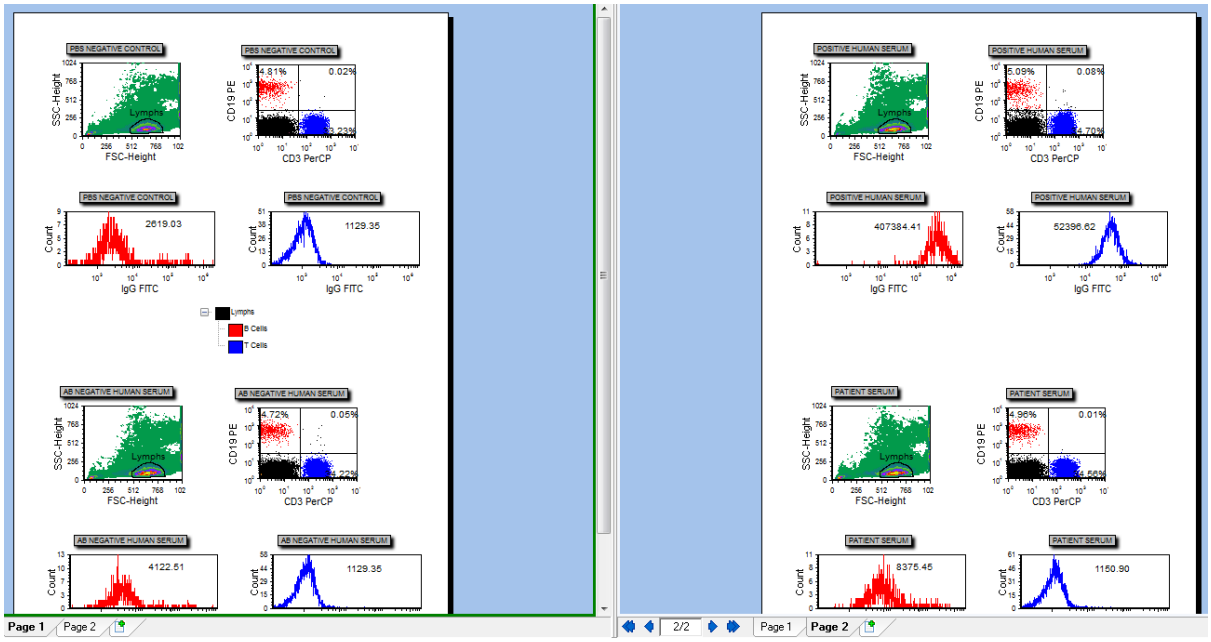

Figure T15.1 HLA Custom Data.fey

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 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.

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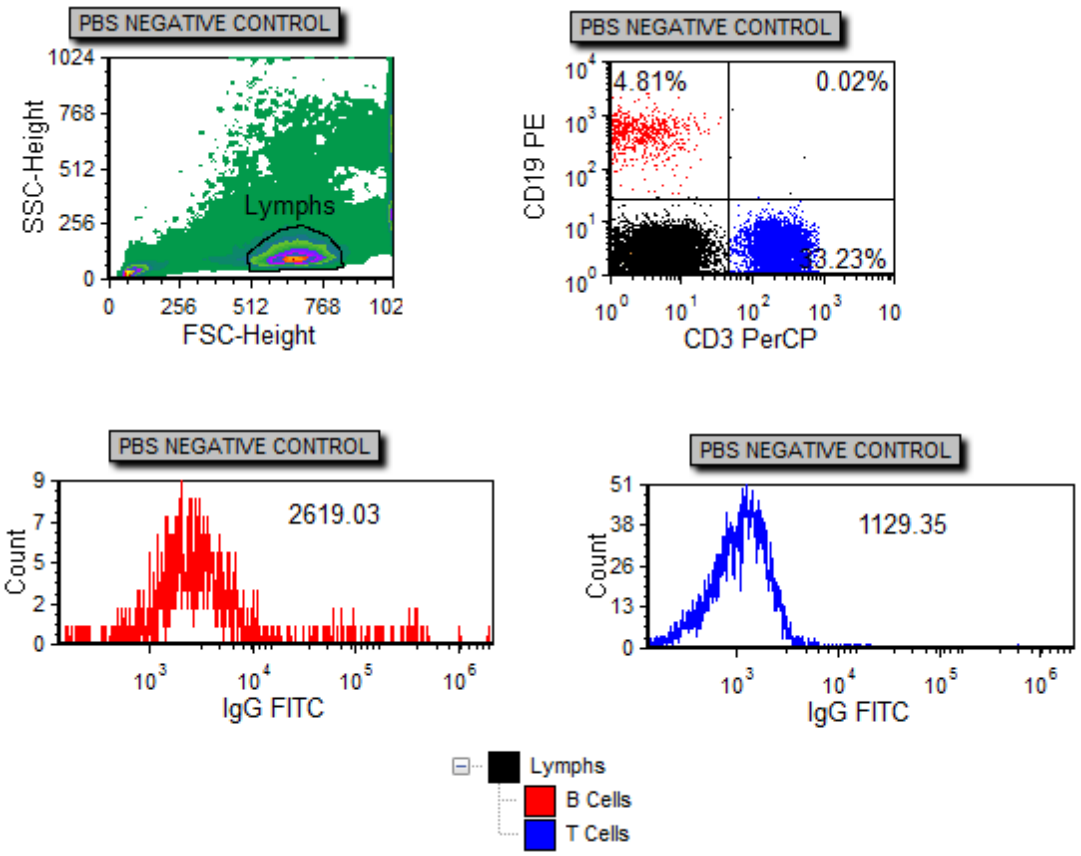

Figure T15.2 Layout of PBS NEGATVE CONTROL Plots and Gate View

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[1169]. 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 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 insert a data grid[927].

34.15.2 Inserting a Data Grid

In this section, we will:

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- Create a Data Grid with custom labels.
- Create and insert tokens and data into the Data Grid.

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 layout and then inserting a custom data table.

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.

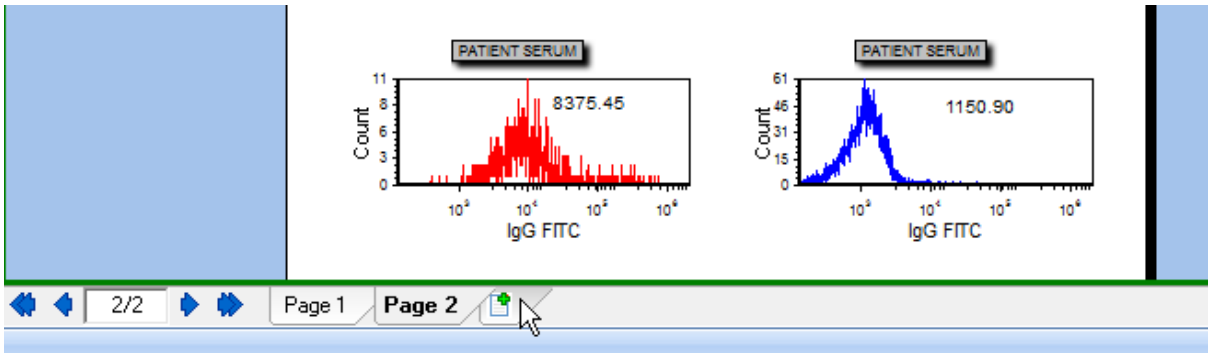

Figure T15.3 New Page Tab/Icon

A new blank page appears.

2. Select the **Data→Save/Load→Custom Data→New Custom Data** command (Figure T15.4).

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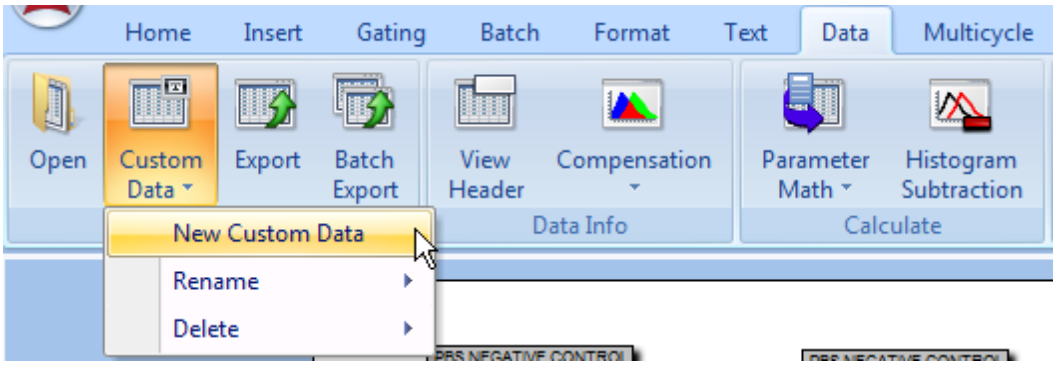

Figure T15.4 New Custom Data Command

The **Create new custom data** dialog appears. Please refer to Figure T15.5 for the following steps.

3. Type 'HLA Cross-match' in the **Name** field.
4. Change the **Columns** value to '3'.
5. Leave the **Rows** value at '4'.
6. Select the **Open Data Grid** checkbox.
7. Click **OK**.

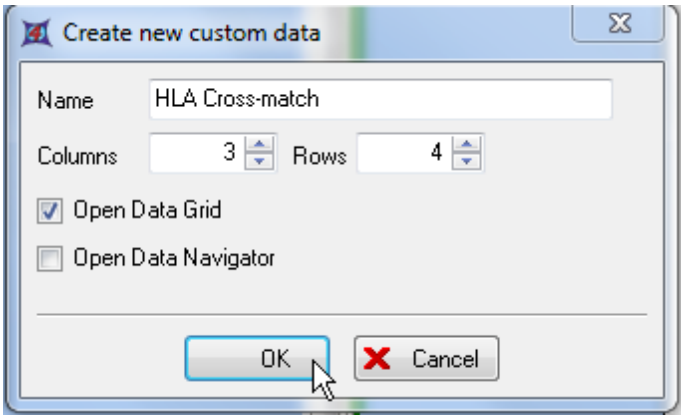

Figure T15.5 Create New Custom Data Dialog

An empty data grid should appear on Page 3 of the layout, as shown in Figure T15.6. If the data grid appears on another page, you can cut and paste it onto Page 3 of the layout.

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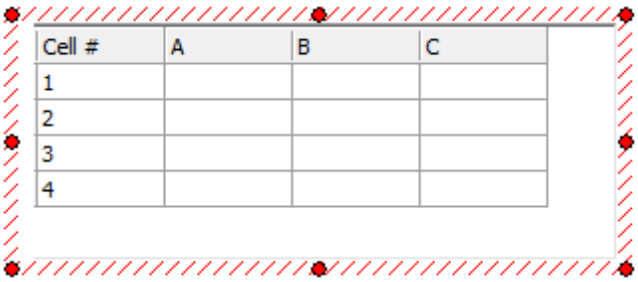

Figure T15.6 Empty Data Grid

We will now name the columns. Please refer to Figure T15.7 for the following steps.

8. Right-click inside the cell labeled 'A' for column A, to bring up the pop-up menu.

9. Select **Rename Selected Column** from the pop-up menu, indicated by the cursor.

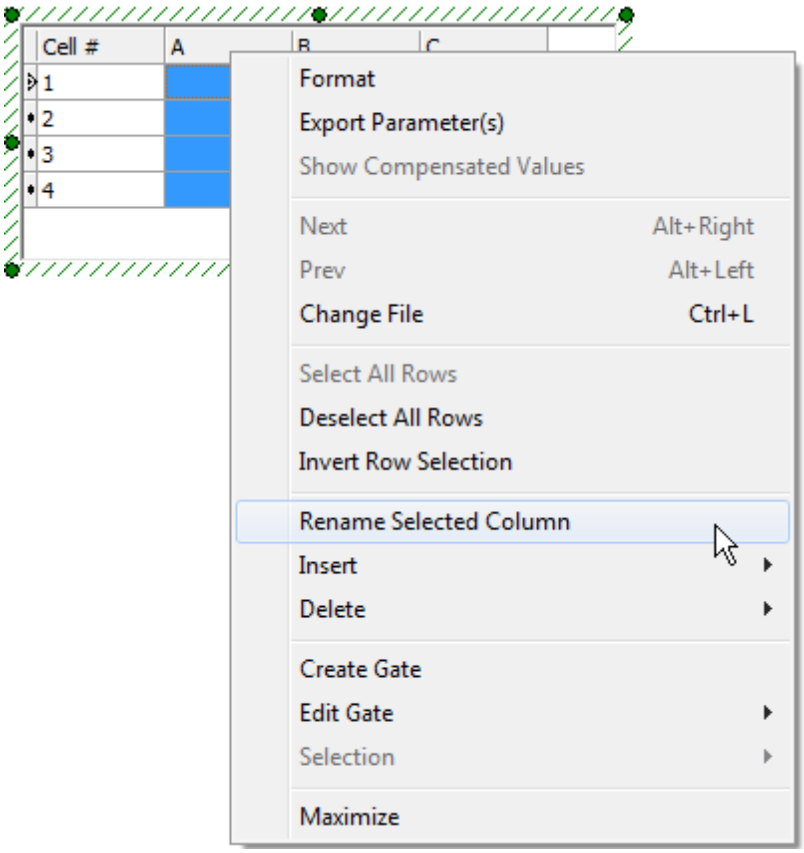

Figure T15.7 Rename Selected Column Command

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The **New Column Name** dialog appears, similar to Figure T15.8.

10. Type 'Sample' in the text field.
11. Click **OK**.

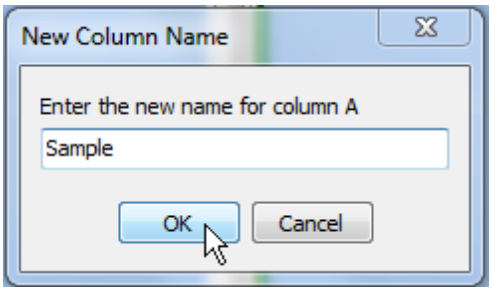

Figure T15.8 New Column Name Dialog

The first column, previously labeled 'A', will now change to 'Sample'.

12. Repeat Steps 8 and 9 for the column labeled 'B'.
13. Type 'Bcell Median' as the new column name.
14. Click **OK**.
15. Repeat Steps 8 and 9 for the column labeled 'C'.
16. Type 'Tcell Median' as the new column name.
17. Click **OK**.

The columns of the Data Grid are now appropriately labeled, as shown in Figure T15.9.

Cell #	Sample	Bcell Median	Tcell Median
1			
2			
3			
4			

Figure T15.9 Data Grid with Column Labels

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In the next exercise, we will create custom HLA Data 932.

34.15.3 Creating Custom Data

In this section, we will:

- Insert keyword and statistic tokens into the Data Grid.
- Explore various ways of entering information into the Data Grid.

We will insert a keyword token in the cells of 'Sample' column. The FCS 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 'Sample' column, shown highlighted in blue in Figure T15.10.

The Data Grid editor window appears on top, as shown in Figure T15.10. We are now going to insert the keyword token into the cell.

2. Click on the Token icon, indicated by the cursor in Figure T15.10.

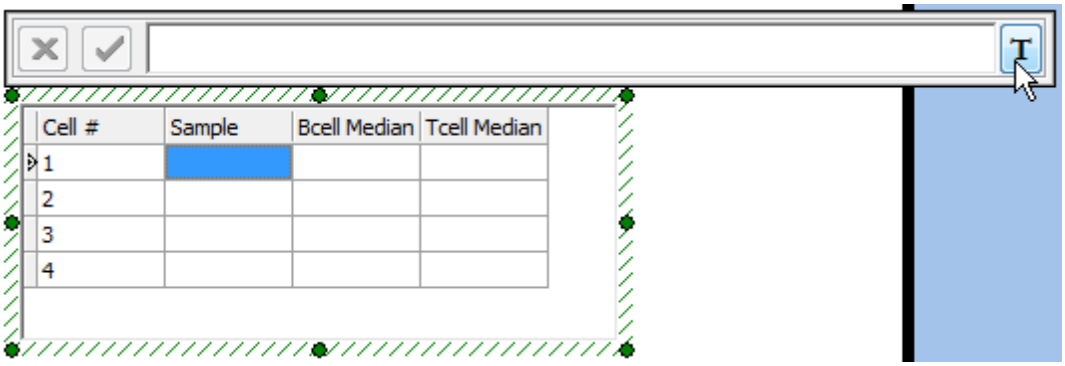

Figure T15.10 Data Grid with Editor

The **Insert a Token** dialog appears, as shown in Figure T15.11.

3. Select **Keyword**.
4. Click **OK**.

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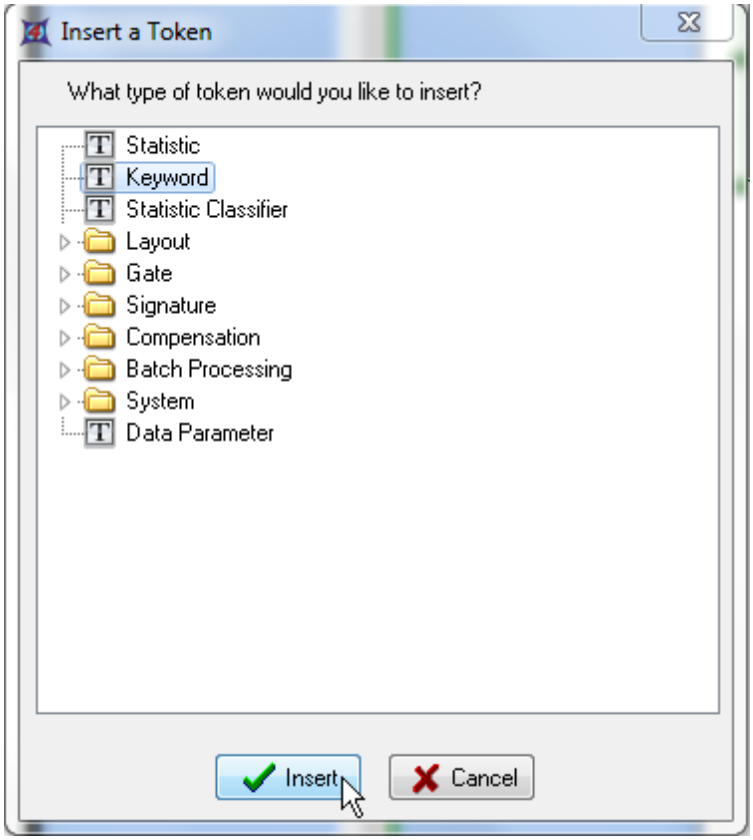

Figure T15.11 Insert a Token Dialog

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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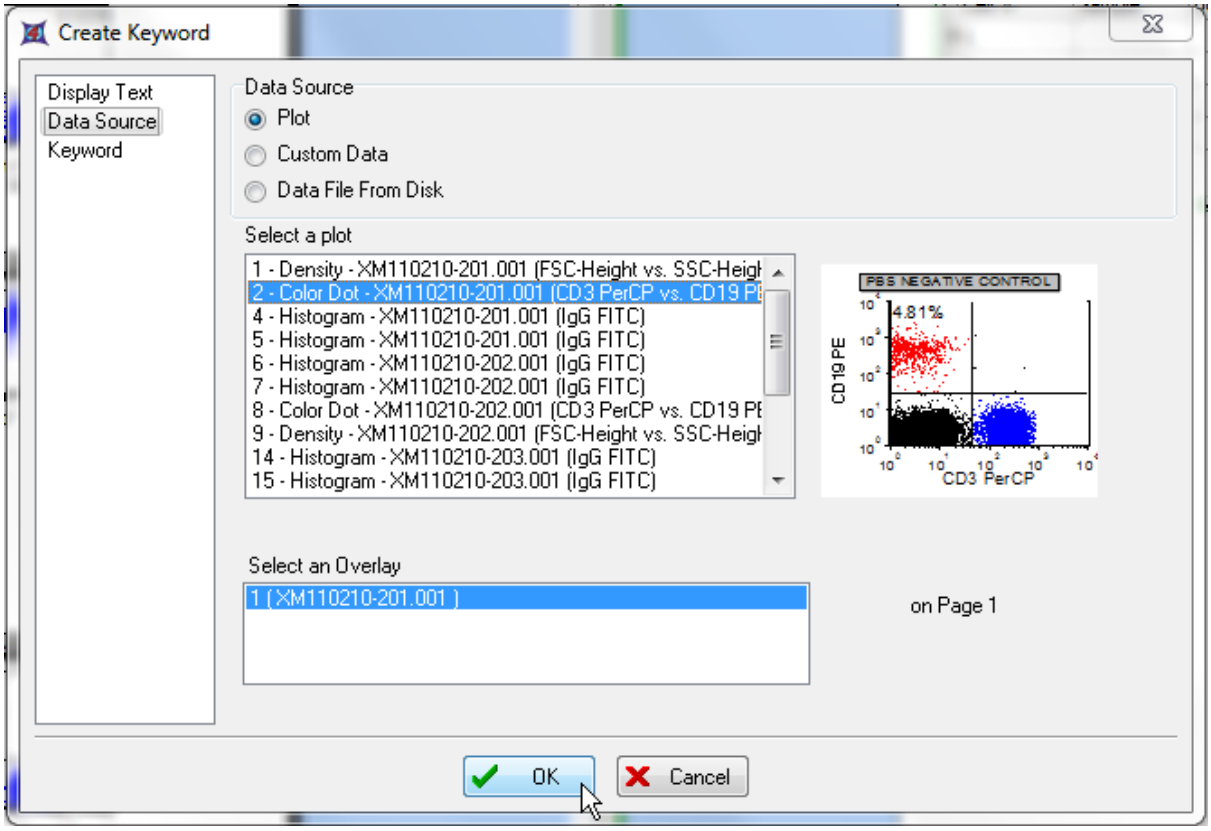

Figure T15.12 Create Keyword Dialog

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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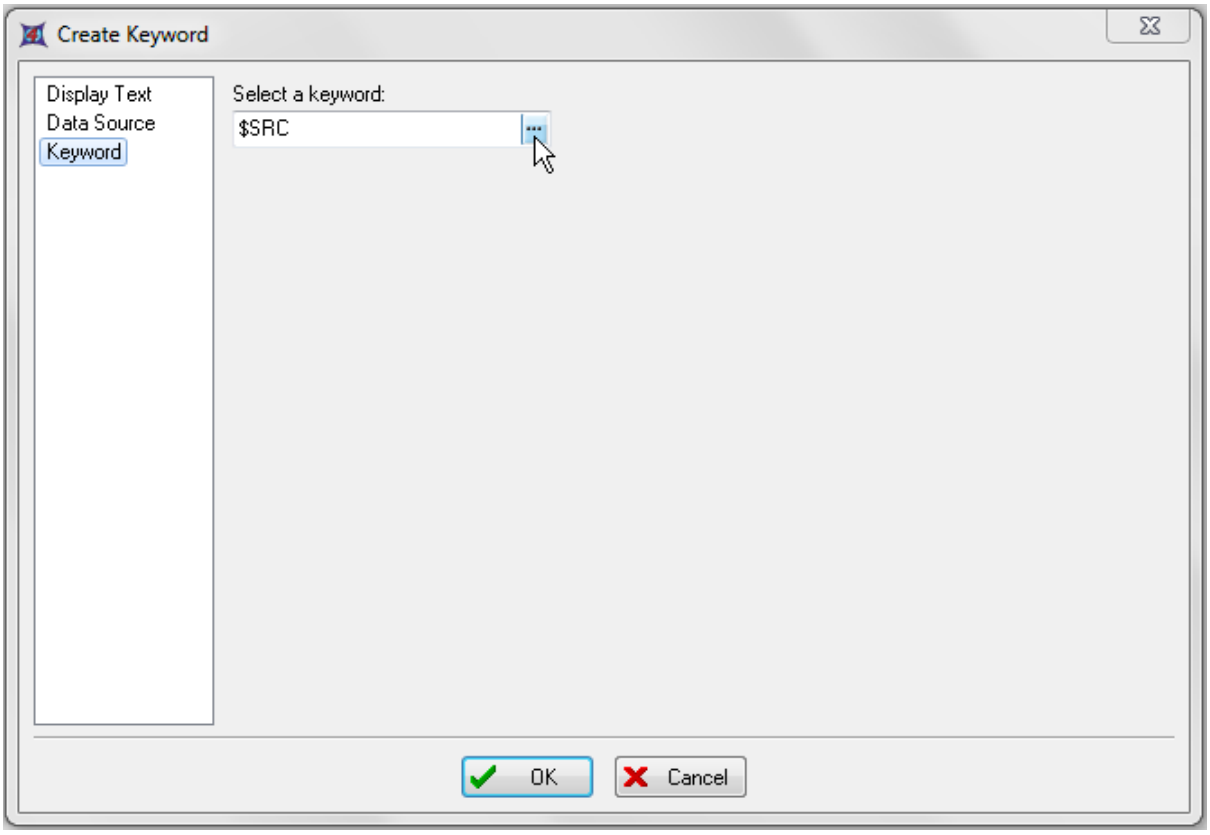

Figure T15.13 Create Keyword Dialog -- Keyword Category

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.

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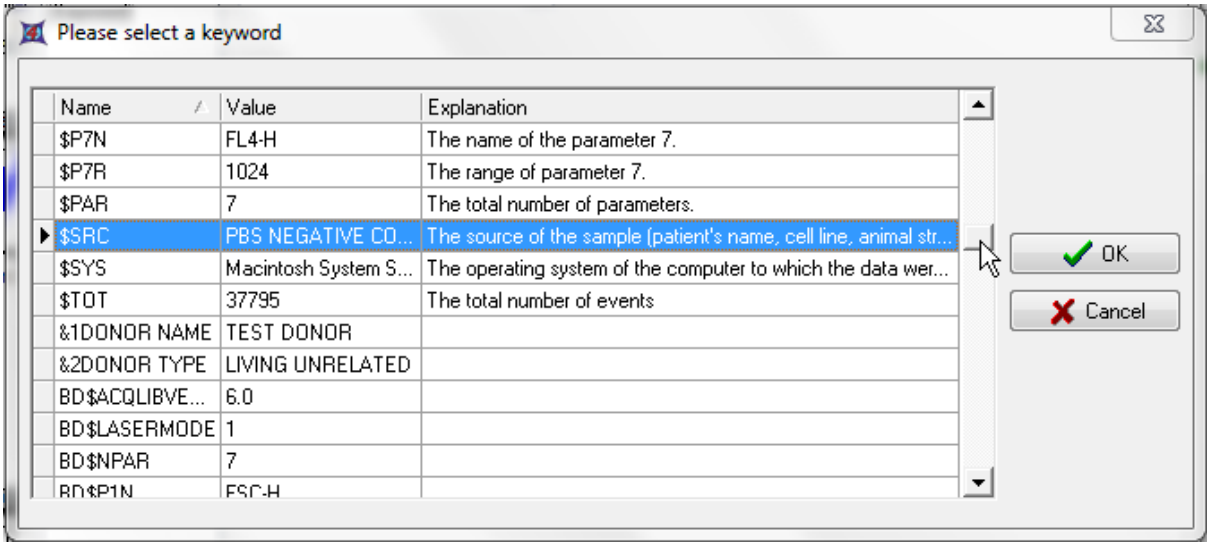

Figure T15.14 Please Select a Keyword Dialog

The Data Grid updates to reflect the insertion of the keyword, with the editor window filled in, as shown in Figure T15.15.

9. Hit ENTER on the keyboard or click on the green check icon, indicated by the cursor in Figure T15.15, to accept the entry.

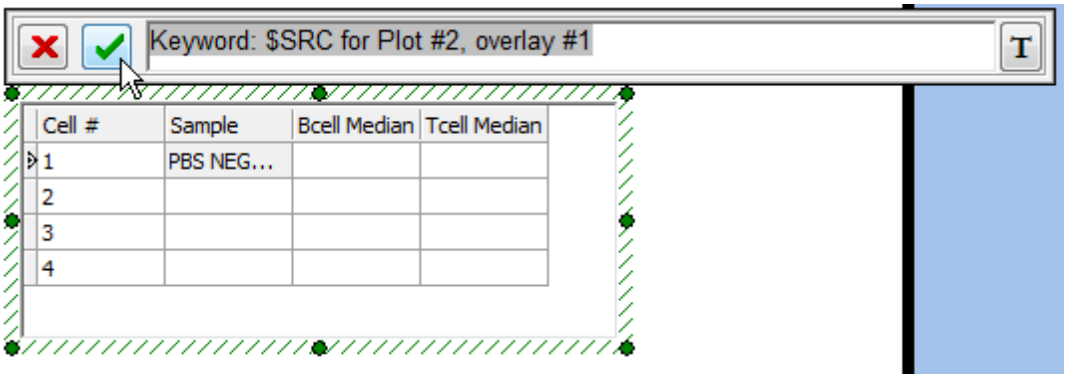

Figure T15.15 Accept Custom Data Entry

We will now fill in the rest of the 'Sample' 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 NEGATIVE HUMAN SERUM' 2D plots, shown highlighted in red in Figure T15.16.

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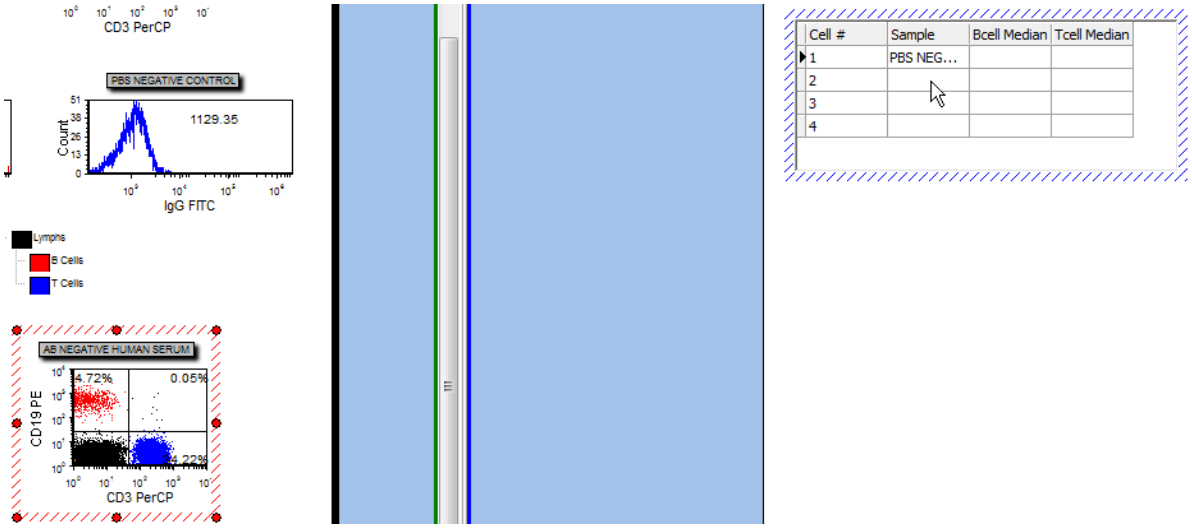
11. Press and hold the left mouse button and drag the plot towards the Data Grid, as shown in Figure T15.16.

12. Release the left button when the plot is over the second cell of the Sample column, indicated by the cursor in Figure T15.16.



Figre T15.16 Dragging and Dropping the AB NEGATIVE HUMAN SERUM Plot onto the Data Grid

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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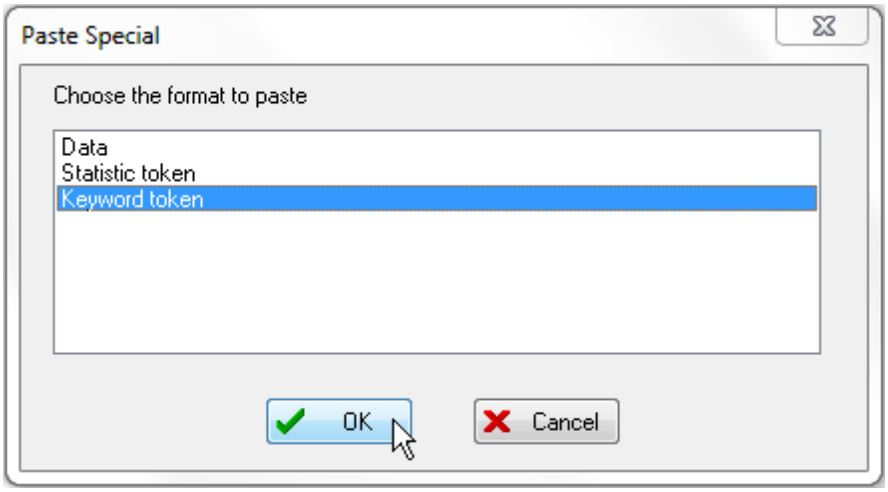

Figure T15.17 Paste Special Dialog

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 **Sample** 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 **Sample** column.

17. Repeat steps 10 through 16 for the third cell in the **Sample** column using an AB POSITIVE SERUM plot.

For the last cell in the Sample column, we will manually type in the sample information to demonstrate how to use the Data Grid editor.

18. Click inside the fourth cell of the Sample column, highlighted in blue in Figure T15.18.

The Custom Data editor appears on top of the data grid as shown in Figure T15.18.

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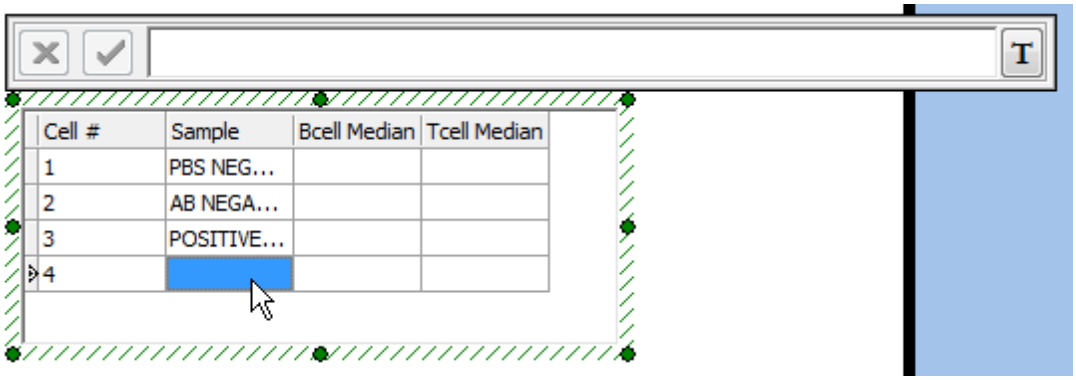

Figure T15.18 Selecting a Cell in the Data Grid

18. Type 'PATIENT SERUM'.

Once you begin typing the text will automatically appear in the Custom Data editor, as shown in Figure T15.19. Also notice in Figure T15.19 that the Accept icon, shown as the green check mark, and Delete icon, shown as the red X, are now in color, indicating they are active. We will now accept the information typed in the editor.

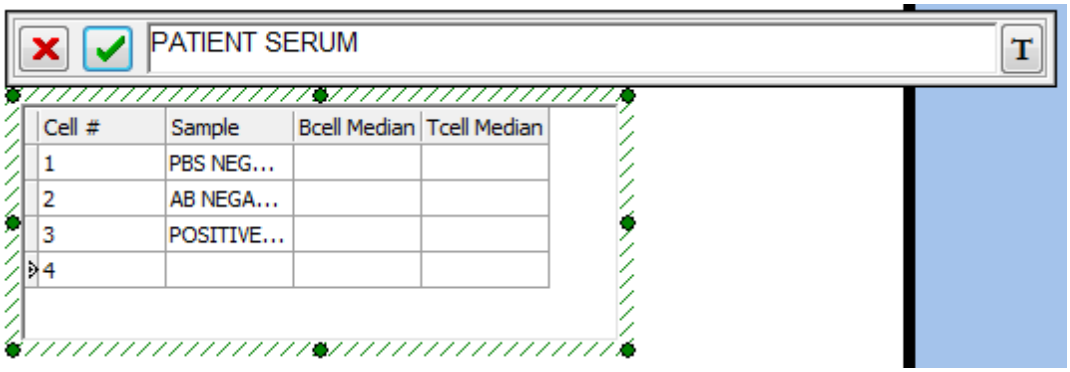

Figure T15.19 Manual Entry of Data in the Data Grid Editor

19. Hit ENTER on the keyboard, or Click the Accept icon in the editor.

Alternatively, you can click anywhere outside the Custom Data grid to accept the entry in the Custom Data editor. 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 cancelled.

The Sample column of the Data Grid is now filled in and should appear similar to Figure T15.20.

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Cell #	Sample	Bcell Median	Tcell Median
1	PBS NEG...		
2	AB NEGA...		
3	POSITIVE...		
4	PATIENT ...		

Figure T15.20 Sample Column Completed

We will now insert the 'Bcell Median' and '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 'Bcell Median' and 'Tcell Median' columns.

20. Place the cursor at the beginning of the B cell median token value in the PBS NEGATIVE CONTROL histogram, shown in red on the layout.

21. Select the B-cell median token text. You will know it is selected because the text will be white, indicated by the cursor in Figure T15.21.

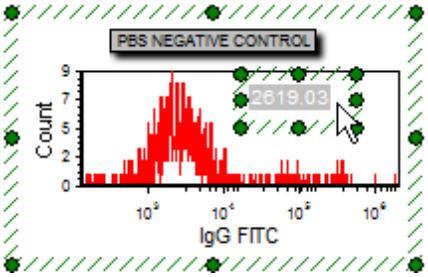

Figure T15.21 Token Value Selected

22. Copy the text using **Ctrl+C** on the keyboard.

Alternatively, after Step 22, the B-cell median token text can be copied using the pop-up menu.

- Right click to bring up the pop-up menu.
- Select **Copy Text** from the pop-up menu, shown highlighted and indicated by the cursor in Figure T15.22.

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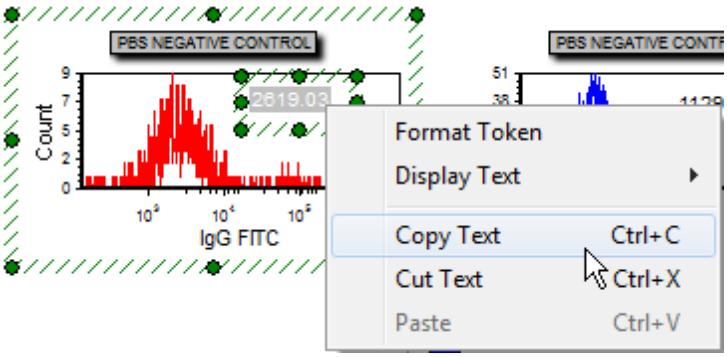

Figure T15.22 Copying a Token Using the Pop-up Menu

We will now paste the B-cell median token into the appropriate cell of the 'Bcell Median' column of the Data Grid.

23. Click inside the first cell of the 'Bcell Median' column to select it.

The Custom Data editor will appear on top the data grid.

24. Click inside the Custom Data editor text window.

25. Paste the text using **Ctrl+V** on the keyboard.

26. Click anywhere outside the Data Grid to accept the entry.

Alternatively, after Step 25, the token text can be pasted using the pop-up menu.

- Right-click inside the first cell of the 'Bcell Median' column to bring up the pop-up menu.
- Select **Paste Text** from the pop-up menu.
- Click anywhere outside the Data Grid to accept the entry.

The rest of the 'Bcell Median' 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, POSTIVE SERUM, and PATIENT SERUM. The 'Tcell Median' 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 median tokens for the B-cell tokens. All the cells of the 'Bcell Median' and 'Tcell Median' columns of the Data Grid should be filled in and appear similar to Figure T15.23.

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Cell #	Sample	Bcell Median	Tcell Median
1	PBS NEG...	2619.03	1129.35
2	AB NEGA...	4122.51	1129.35
3	POSITIVE...	407384.41	52396.62
4	PATIENT ...	8375.45	1150.90

Figure T15.23 Data Grid with B- and T-Cell Median Values

In the next section, we will create and use custom tokens in the data grid 942.

34.15.4 Create Custom Tokens

In this section, we will:

- Create custom tokens.
- Insert new columns into the Data Grid.
- Insert custom tokens into the Data Grid.
- Create token classifiers to use in the Data Grid.

We now have all the essential data in our Data Grid; however, we still need to determine if the patient serum tested is a 'positive', 'negative', or 'weak' cross-match to the donor cells.

To begin, we must first create custom tokens that normalize the 'sample' median fluorescence values based on a 'negative' value. We will use the median MESF FITC values from the B- and T-cells of the PBS NEGATIVE CONTROL as the 'negative' value and subtract this value from the median MESF FITC value of the samples. We will begin by inserting the **Custom Tokens** window.

1. Select the **View→Tokens→Custom Tokens** command.

The **Custom Tokens** window appears on the layout. We will now dock the window to the right side of the layout. For more information on docking, please see the Docking tutorial 845.

2. Place the cursor over the top border of the **Custom Tokens** window.
3. Press and hold the left mouse button and drag the **Custom Tokens** window towards the right arrow of the four-headed Docking icon.

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4. Release the button when the **Custom Tokens** window is over the right arrow of the four-headed Docking icon.

The **Custom Tokens** window is now docked on the right side of the layout. Now, we will create the custom tokens that represent the MESF channel shift of the samples relative to the PBS NEGATIVE CONTROL sample. For more information on creating custom tokens, refer to the Custom Token Tutorial 700.

5. Click on the + symbol on the top border of the **Custom Tokens** window, indicated by the cursor in Figure T15.24.

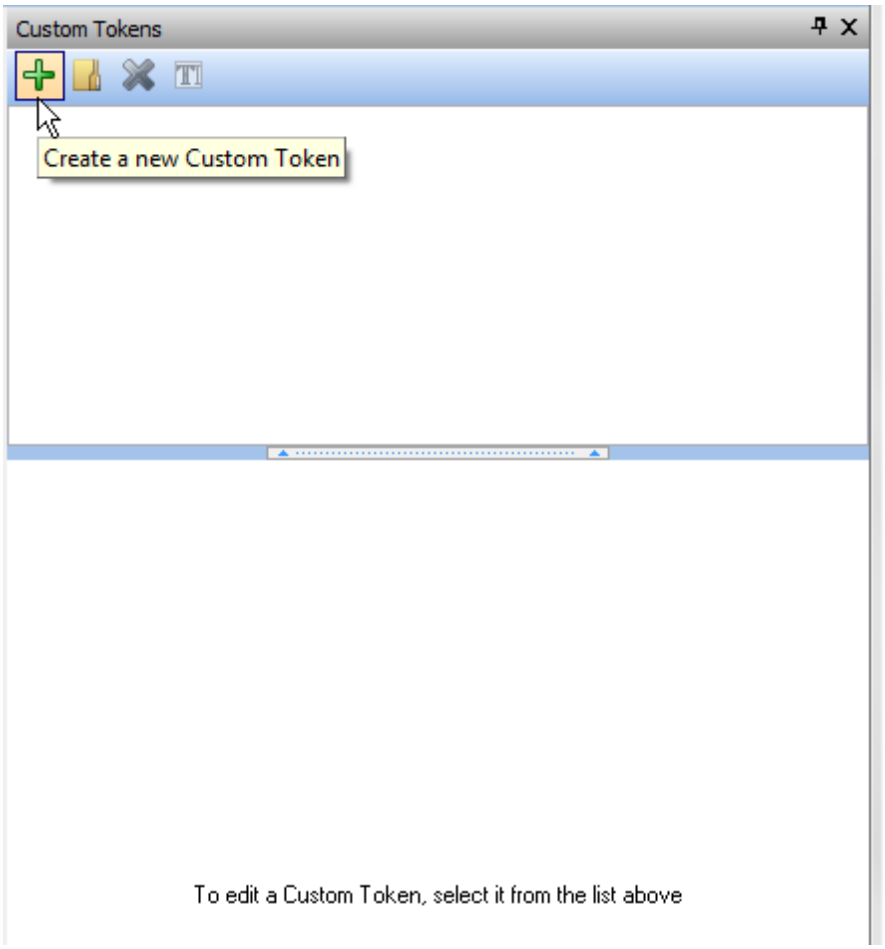

Figure T15.24 Creating a New Custom Token

6. Type 'BShift PBS' for the Custom Token name.

7. Click in the bottom of the Custom Token window to start entering a formula.

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8. Type '=' in the Custom Token definition area.

We will now copy the appropriate median token value in the Data Grid into the custom token definition. Refer to Figure T15.25 for the following steps.

9. Click inside the first cell in the 'Bcell Median' column of the Data Grid, indicated by the cursor and highlighted in grey.

The Custom Data editor window appears on top with the statistic token definition in the editor window.

10. Select the statistic token in the editor window. You will know it is selected because the text will be white.

11. Copy the text using **Ctrl+C** on the keyboard.

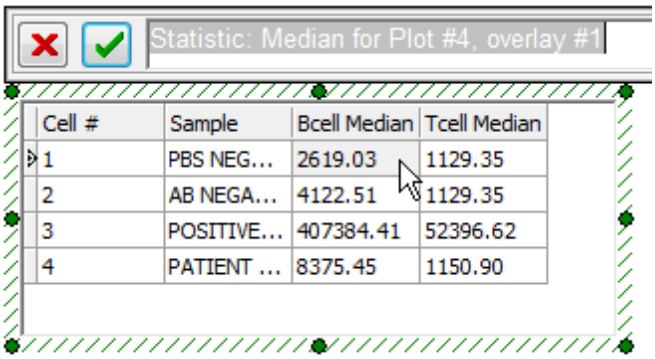

Figure T15.25 Copying the 'BCell Median' Token for the PBS NEGATIVE CONTROL Sample

Please refer to Figure T15.26 for the following steps.

12. Select the 'BShift PBS' Custom token.

13. Click to the right of the '=' inside the Custom Token definition area.

14. Paste the 'Bcell Median' token definition using **Ctrl+V** on the keyboard.

15. Type '-' next to the 'Bcell Median' token definition.

16. Paste the 'Bcell Median' token definition using **Ctrl+V** on the keyboard.

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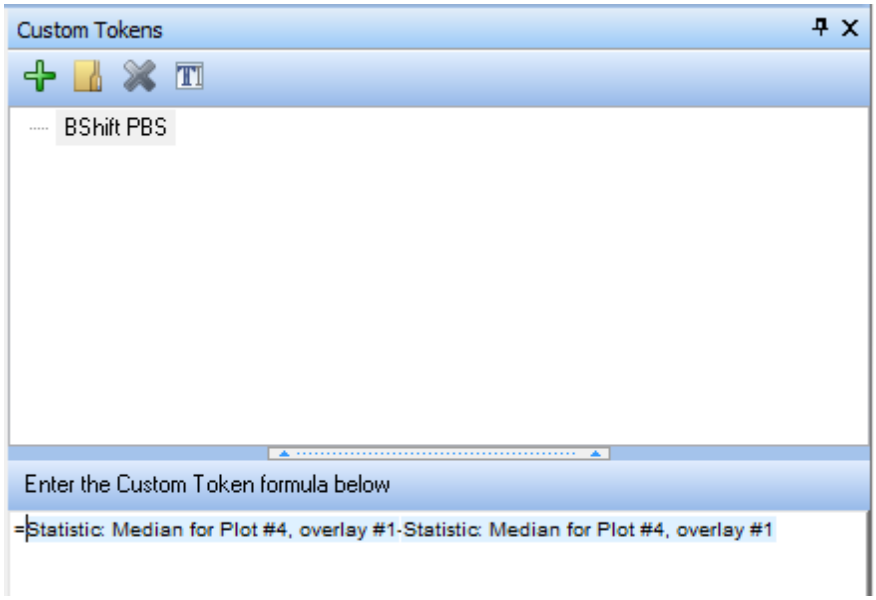

Figure T15.26 Creating the B-cell Median Shift Custom Token Definition

We have now created the Custom Token definition for the B-cell median MESF shift of the PBS NEGATIVE CONTROL sample, called 'BShift PBS'. By definition, this shift should equal zero. We will now create the Custom Token for B-cell median shift of the AB NEGATIVE HUMAN SERUM sample, calling it 'BShift NegSerum', by copying and pasting the definition of the 'BShift PBS' Custom Token. We will then drag and drop the 'Bcell Median' statistic token (of the AB NEGATIVE SERUM sample) from the Data Grid into the appropriate place in the Custom Token definition of 'BShift NegSerum'.

17. If it is not already selected, then select the 'BShift PBS' Custom token.

18. Place the cursor to the left of the '=' in the Custom Token definition window.

19. Select the definition of the 'BShift PBS' Custom Token. You will know it is selected because the text will be white.

20. Copy the 'BShift PBS' Custom Token definition using **Ctrl+C** on the keyboard.

21. Left-click on the symbol on the top border of the **Custom Tokens** window, indicated by the cursor in Figure T15.24.

22. Type 'BShift NegSerum' for the Custom Token name of the newly created custom token.

23. Click inside the Custom Token definition area.

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24. Paste the 'Bcell Median' token definition using **Ctrl+V** on the keyboard.

Refer to Figure 28 for the following steps.

25. Delete the text just after the '=' and before the '-'.

26. Select the cell in second row of the 'Bcell Median' column of the Data Grid, shown highlighted in Figure T15.27.

27. Select the statistic token in the editor window. You will know it is selected because the text will be white.

28. Press and hold the left mouse button and drag the token toward the Custom Token definition window.

29. Release the button when the token is after the '=' and before the '-' in the Custom Token definition window.

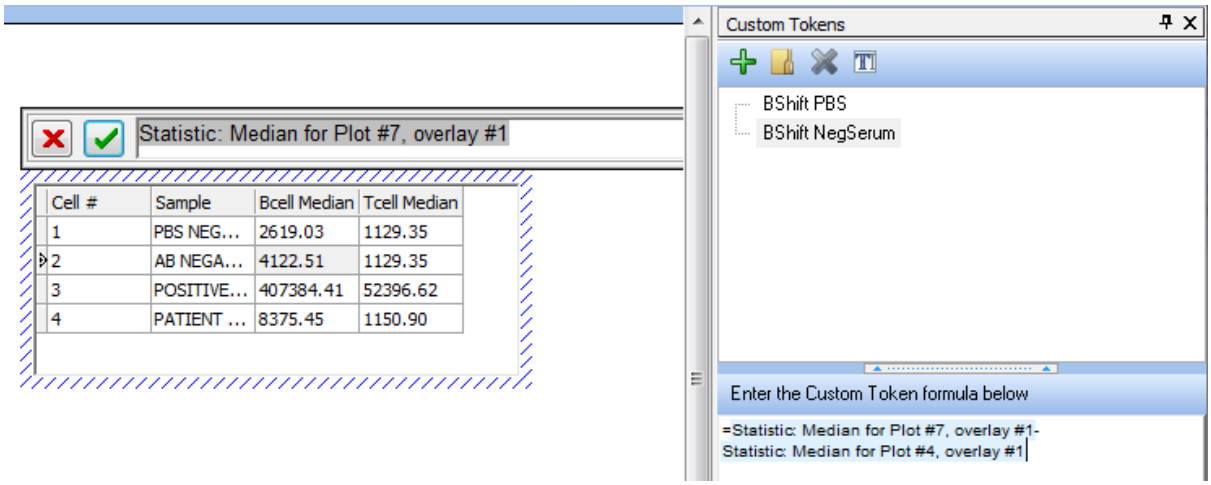

Figure T15.27 Dragging and Dropping the 'Bcell Median' Token for the AB NEGATIVE HUMAN SERUM Sample into the Custom Token Definition

The 'BShift NegSerum' Custom Token is now defined as the B-cell median of the AB NEGATIVE HUMAN SERUM – the B-cell median of the PBS NEGATIVE CONTROL, as shown in Figure T15.27.

To define the rest of the B shift Custom Tokens, repeat Steps 16 through 28 using the appropriate B-cell median tokens from the Data Grid for the POSITIVE HUMAN SERUM and PATIENT SERUM samples. Use the Custom Token names 'BShift PosSerum' and 'BShift Patient', respectively.

Again, repeat Steps 16 through 28 to define the T shift Custom Tokens using the appropriate T-cell median tokens from the Data Grid for all the samples.

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Remember to subtract the T-cell median of the PBS NEGATIVE CONTROL from all the samples and substitute 'T' for 'B' in the Custom Token names.

When you are done, the **Custom Tokens** window should look similar to Figure T15.28, which shows the definition for the 'TShift Patient' Custom Token.

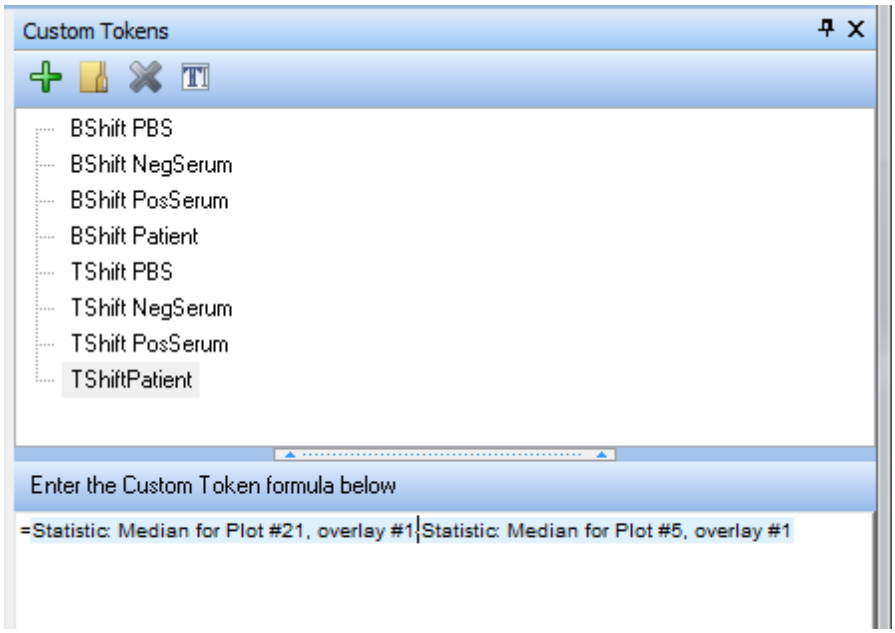

Figure T15.28 Custom Tokens Window

We will now add the B and T Shift Custom Tokens to the Data Grid, so we need to add two columns to the Data Grid. Refer to Figure T15.29 for the following steps.

30. Right-click on the 'Tcell Median' column heading to bring up the pop-up menu.

31. Select **Insert→Column to the Right** from the pop-up menu.

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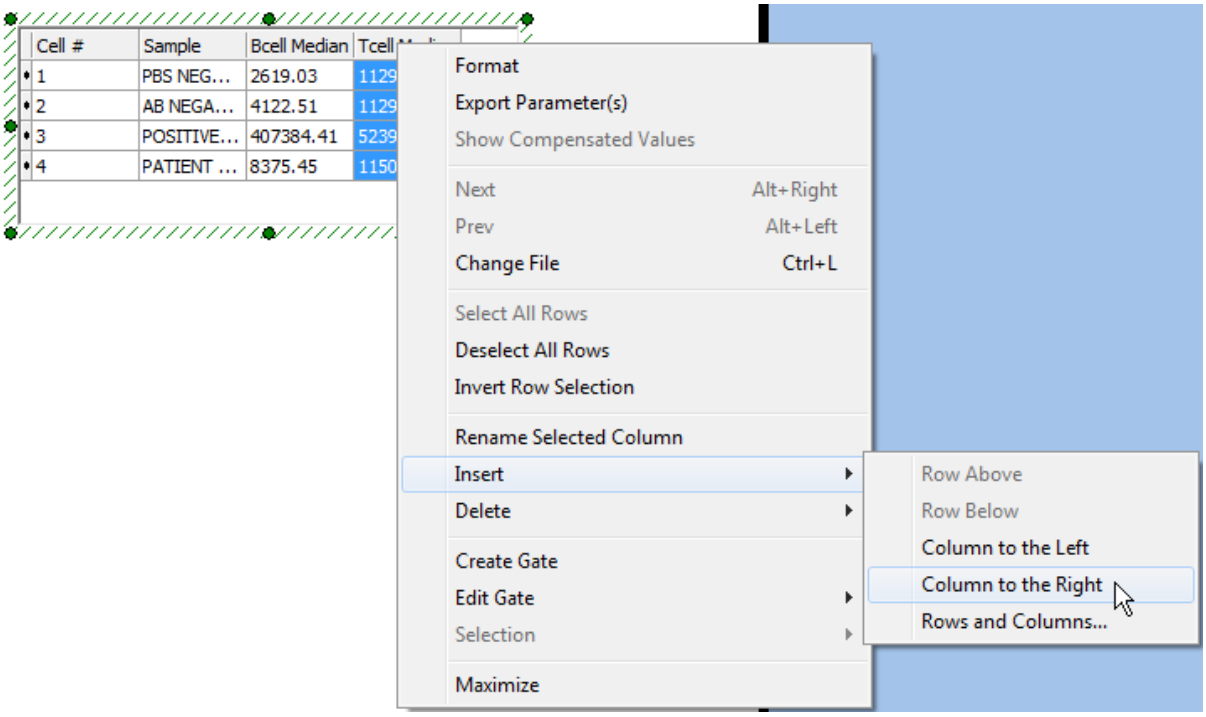

Figure T15.29 Inserting a New Column into the Data Grid

A **New Column Name** dialog appears, similar to previously shown Figure T15.8 927.

32. Type 'BShift' in the New Column Name text field.

33. Click **OK**.

A new column labeled 'BShift' appears on the Data Grid. Using steps 29 through 32, insert a new column labeled 'TShift' to the right of the newly created 'BShift' column. When you are done the Data Grid should appear similar to Figure T15.30.

Cell #	Sample	Bcell Median	Tcell Median	BShift	TShift
1	PBS NEG...	2619.03	1129.35		
2	AB NEGA...	4122.51	1129.35		
3	POSITIVE...	407384.41	52396.62		
4	PATIENT ...	8375.45	1150.90		

Figure T15.30 Data Grid with New 'BShift' and 'TShift' Columns

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We will now drag and drop the Custom Tokens into the cell of the new BShift and TShift columns. Please refer to Figure T15.31 for the following steps.

34. Place the cursor over the 'BShift PBS' Custom Token, shown highlighted in blue.

35. Press and hold the left mouse button.

36. Drag the Custom Token toward the 'BShift' column of the Data Grid.

37. Release the button when the Custom Token is over the first cell of the 'BShift' column of the Data Grid, indicated by the cursor.

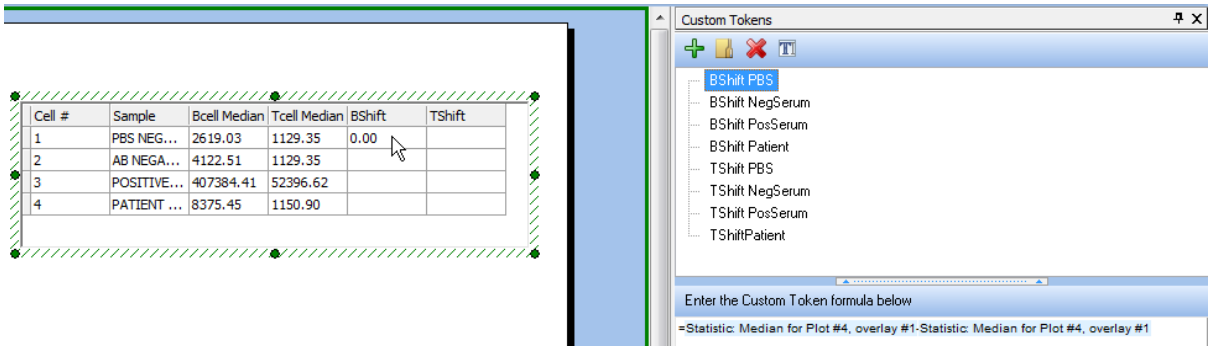

Figure T15.31 Dragging and Dropping the 'BShift PBS' Custom Token into the Data Grid

The Data Grid updates to reflect the insertion of the 'BShift PBS' Custom Token value, which is shown as '0.00' in Figure T15.31. This makes sense because the B-cell median MESF fluorescence value of the PBS NEGATIVE CONTROL sample is being used to define the negative. Continue to fill in the Data Grid by dragging and dropping each Custom Token into the appropriate cell of the BShift or TShift columns. When you are done, the Data Grid should look like Figure T15.32.

Cell #	Sample	Bcell Median	Tcell Median	BShift	TShift
1	PBS NEG...	2619.03	1129.35	0.00	0.00
2	AB NEGA...	4122.51	1129.35	1503.48	0.00
3	POSITIVE...	407384.41	52396.62	404765.38	51267.27
4	PATIENT ...	8375.45	1150.90	5756.42	21.55

Figure T15.32 Data Grid with 'BShift' and 'TShift' Columns Completed

We can now close the docked **Custom Tokens** window.

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38. Select the 'X' in the upper right corner of the docked **Custom Tokens** window.

Next, we will create and use token classifiers in the data grid950.

34.15.5 Create Token Classifiers

In this section, we will:

- Create token classifiers using the custom tokens.
- Create a new Data Grid with custom labels.
- Insert token classifiers into the new Data Grid.

Using the 'BShift' and 'TShift' Custom Token data, we will now define token classifiers ('NEG', 'POS', and 'WEAK') in the Data Grid. We will use the token classifiers to classify our samples and ultimately determine the status of the patient serum cross-match. To begin, we will insert a new Data Grid with three columns and four rows in which we will put our final results.

1. If it is not already active, make Page 3 of the layout active.
2. Repeat Steps 1 through 7 in the Inserting a Data Grid section927, except name the Data Grid 'HLA Results'.

A Data Grid should appear on Page 3 of the layout. Move it under the 'HLA cross-match' Data Grid to a clear area on the page. We will now rename the columns of the newly created 'HLA Results' Data Grid.

3. Repeat Steps 8 through 17 in the Inserting a Data Grid section927, except name the columns 'Sample', 'B Result', and 'T Result'.

We will now insert the Keyword Token *$SRC* into the 'Sample' column by copying the Keyword Tokens from the Sample column of the 'HLA cross-match' Data Grid. Please refer to Figure T15.33 for the following steps.

4. Click inside the first cell in the 'Sample' column of the 'HLA cross-match' Data Grid, shown highlighted in grey.
5. Select the Keyword Token from the 'HLA cross-match' Data Grid editor window. The selected the text is shown in white.
6. Copy the text using **Ctrl+C** on the keyboard.
7. Click inside the first cell in the 'Sample' column of the newly created 'HLA

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Results' Data Grid, indicated by the cursor.

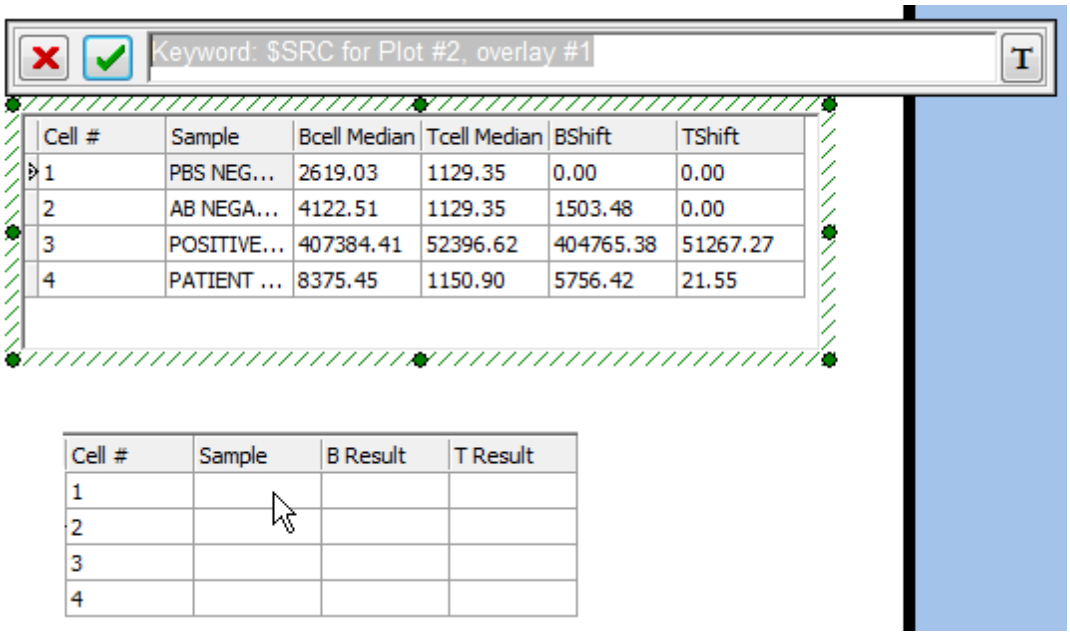

Figure T15.33 Copying the Keyword Token from the 'HLA cross-match' Data Grid

We will now paste the Keyword Token into the 'Sample' column of the newly created "HLA Results" Data Grid. Please refer to Figure T15.34 for the following steps.

8. Click inside the first cell in the 'Sample' column of the 'HLA Results' Data Grid, shown highlighted in grey.

9. Paste the Keyword Token text inside the 'HLA Results' Data Grid editor using **Ctrl+V** on the keyboard.

10. Click on the green check mark to accept the entry, indicated by the cursor.

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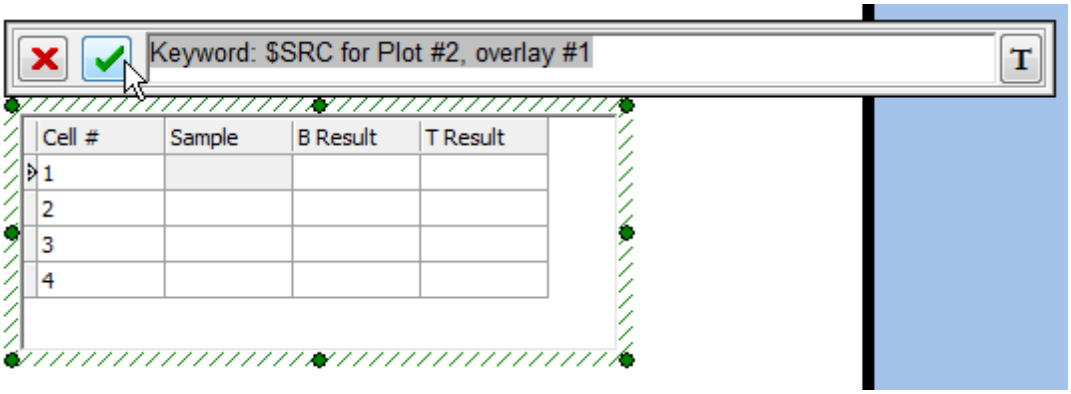

Figure T15.34 Pasting the Keyword Token into the 'Sample' Column of the 'HLA Results' Data Grid

We will now fill in the rest of the Sample column using copy and paste.

11. Repeat steps 4 through 10 for the second, third, and fourth cells of the 'Sample' column of the Data Grids until the 'Sample' column in the new grid is filled, as shown in Figure T15.35.

When you are done the HLA Results Data Grid should appear similar to Figure T15.35.

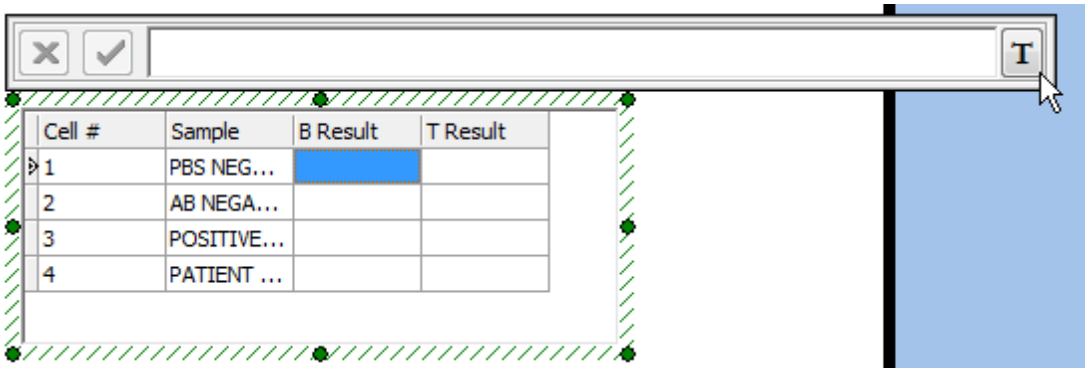

Figure T15.35 New Data Grid with 'Sample', 'B Result', and T Result' Columns

We will now begin defining the Token Classifier to classify the results for each sample. For more information on creating Token Classifiers, see the

.

12. Click inside the first cell in the 'B Result' column, shown highlighted in blue in Figure T15.35.

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13. Click on the Token icon in the Custom Data editor window, indicated by the cursor in Figure T15.35.

The **Insert a Token** dialog appears, similar to the previously shown Figure T15.11 932. However, you will notice that there are now new listings under the **Custom** category.

14. Select **Statistic Classifier** from the **Insert a Token** dialog.

15. Click **Insert**.

The **Classification** category of the **Create Statistic Classifier** dialog appears, as shown in Figure T15.36. This is where we will begin to define the Statistic Classifier to classify our results as either 'NEG', 'WEAK', or 'POS'. However, first we need to tell the Classifier what we want it to classify.

16. Click on the Token icon next to the 'Enter a value to classify:' text field, indicated by the cursor in Figure T15.36.

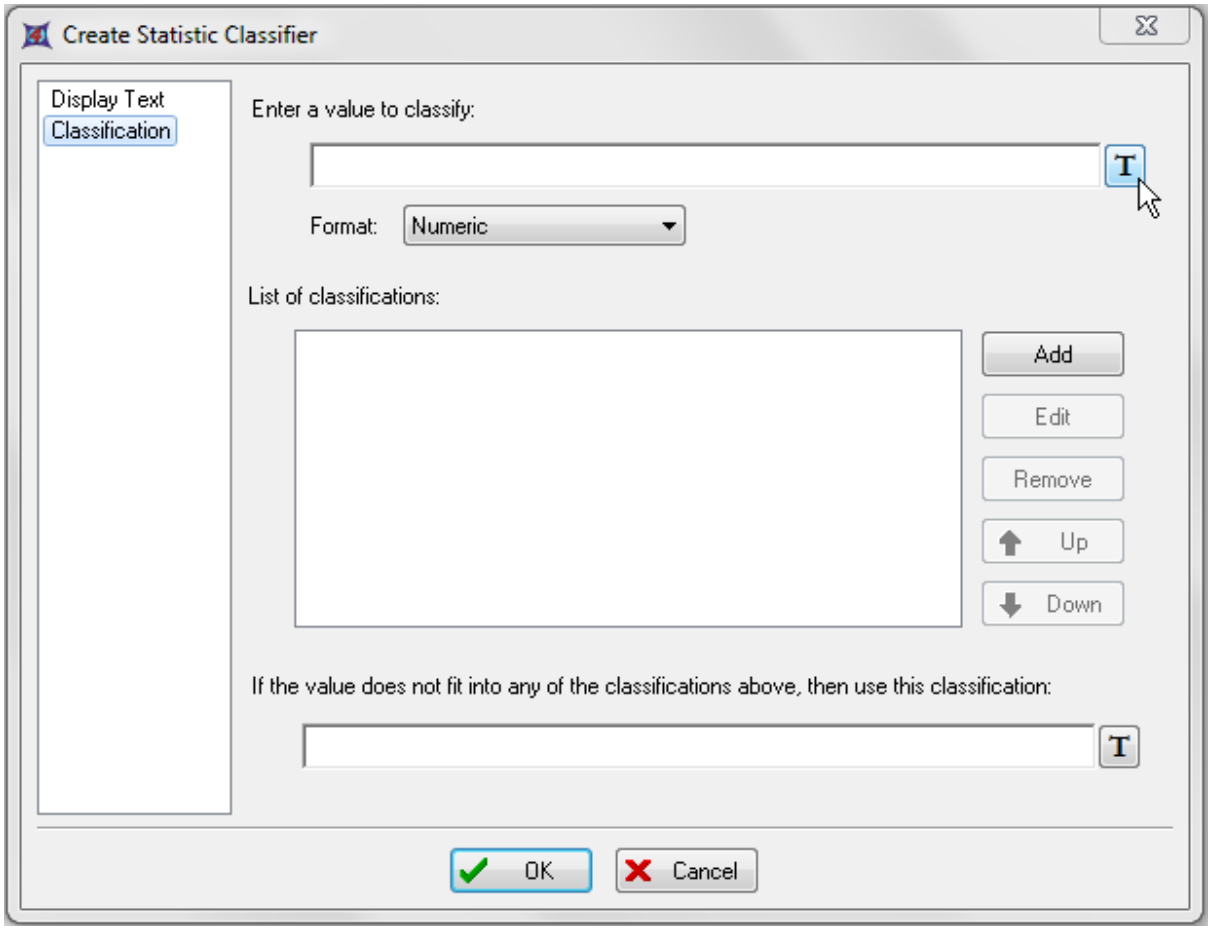

Figure T15.36 Create Statistic Classifier Dialog -- Classification Category

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The **Insert a Token** dialog reappears, as shown in Figure T15.37. This is where we will choose the Custom Token for our Token Classifier to classify.

17. Select **BShift PBS** from the **Custom** category of the **Insert a Token** dialog, shown highlighted in blue in Figure T15.37.

18. Click **Insert**.

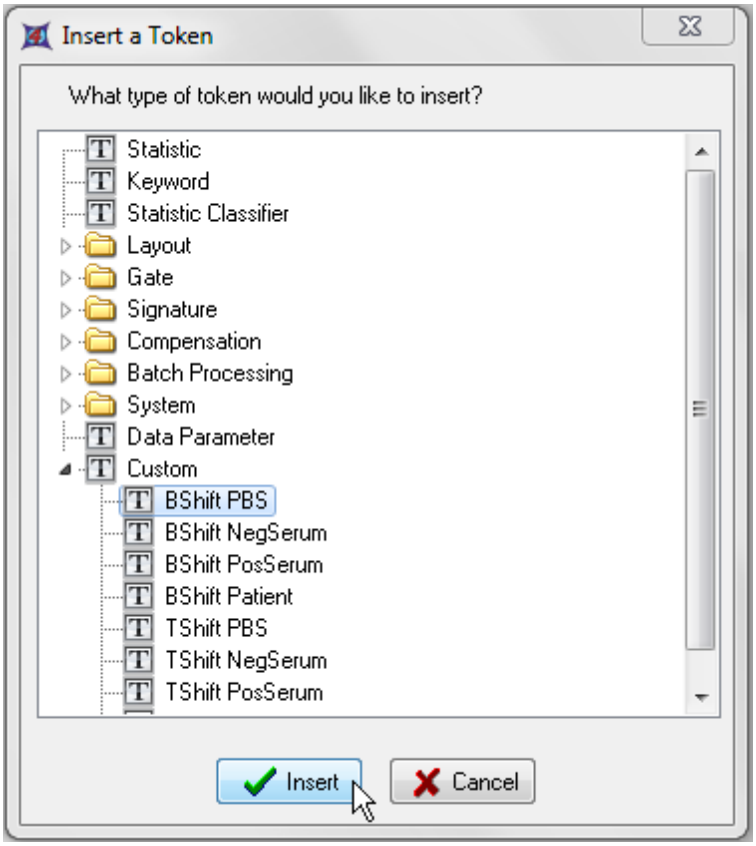

Figure T15.37 Insert a Token Dialog with Custom Tokens to Classify

The **Classification** category of the **Create Statistic Classifier** dialog reappears. However, now the 'Enter a value to classify:' field has the Custom Token 'BShift PBS' entry, as shown in Figure T15.38. We will now enter the specific list of classifiers to use.

19. Click the **Add** button, indicated by the cursor and highlighted in blue in Figure T15.38.

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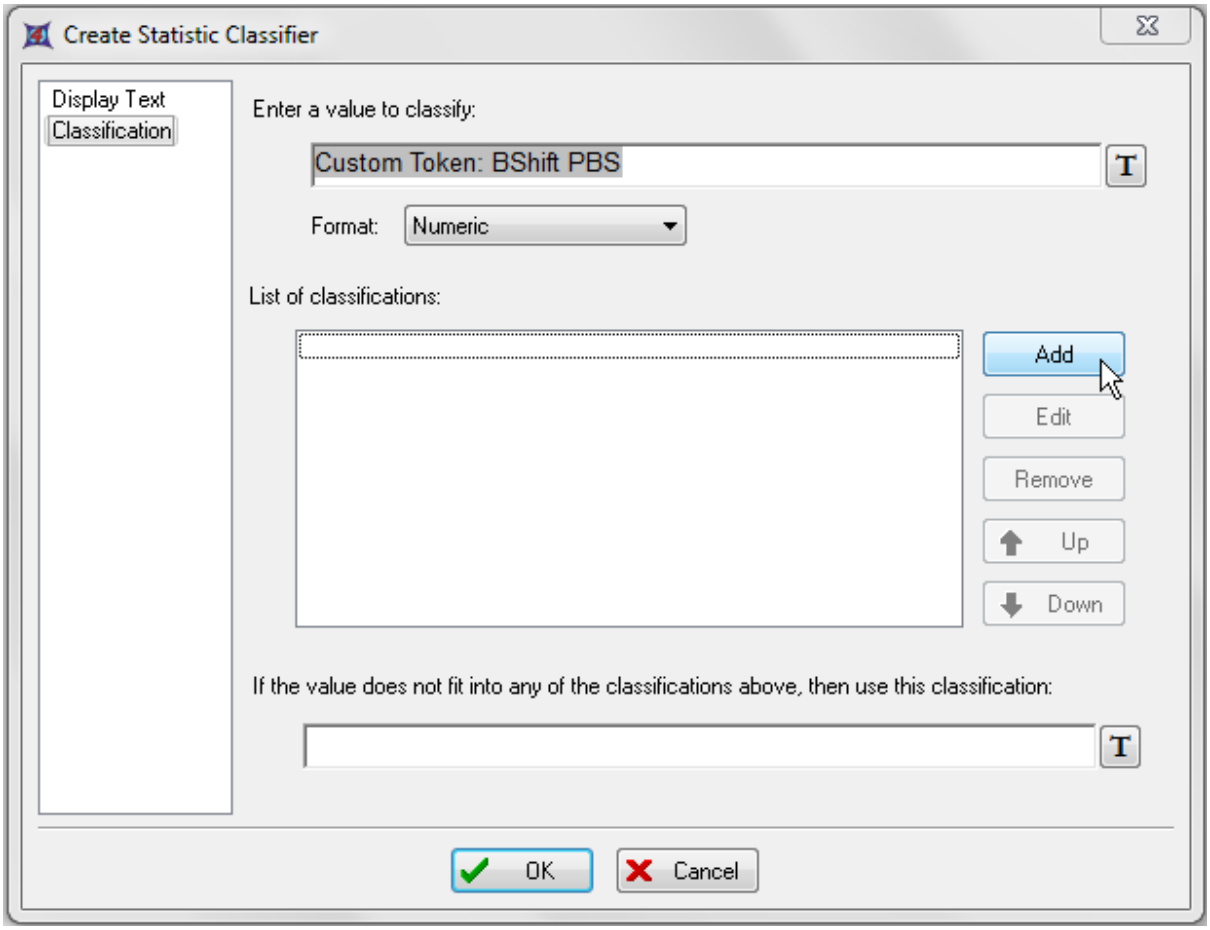

Figure T15.38 Adding the List of Classifiers in the Create Statistic Classifier Dialog

The **Edit Classification** dialog appears. Please refer to Figure T15.39 for the following steps.

20. Type 'NEG' in the **Classify the token as** text field.
21. Type '0' in the **if its value is between** text field.
22. Type '19999' in the **and** text field.
23. Click **OK**.

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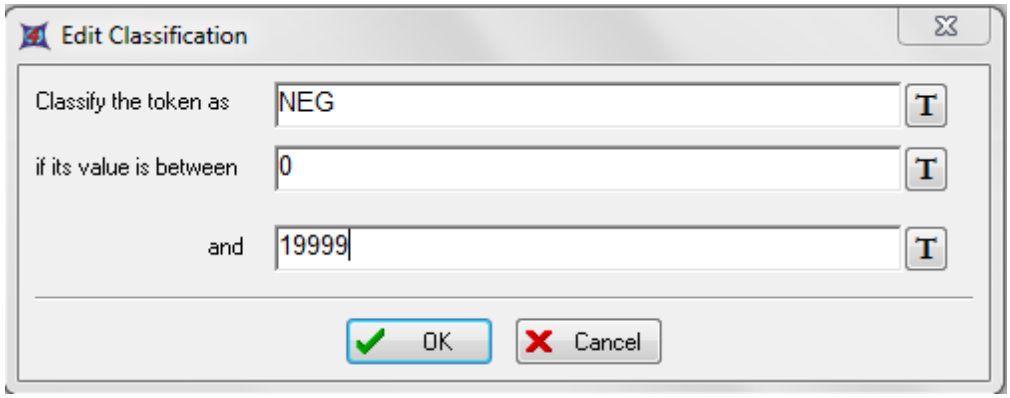

Figure T15.39 Edit Classification Dialog

The **Classification** category of the **Edit Statistic Classifier** dialog appears. However, now the **List of classifications:** field has the 'NEG' definition listed. We have just defined the 'NEG' classification for the 'BShift' Custom Token to be between 0 and 19,999. This means that if the sample's MESF FITC median fluorescence value of the B-cells, adjusted by the PBS NEGATIVE CONTROL, is less than 20,000 it is classified as negative or 'NEG'.

We will now continue to define the two other classification categories of 'WEAK' and 'POS'.

24. Click the **Add** button, indicated by the cursor and highlighted in blue in Figure T15.38.
25. Type 'WEAK' in the **Classify the token as** text field of the **Create Classification** dialog.
26. Type '20000' in the **if its value is between** text field.
27. Type '49999' in the **and** text field.
28. Click **OK**.
29. Click the **Add** button, indicated by the cursor and highlighted in blue in Figure T15.38.
30. Type 'POS' in the **Classify the token as** text field.
31. Type '50000' in the **if its value is between** text field.
32. Type '1200000' in the **and** text field.
33. Click **OK**.

The **Edit Statistic Classifier** dialog should now appear similar to Figure T15.40 with the complete classifier definition for the 'BShift' Custom Tokens.

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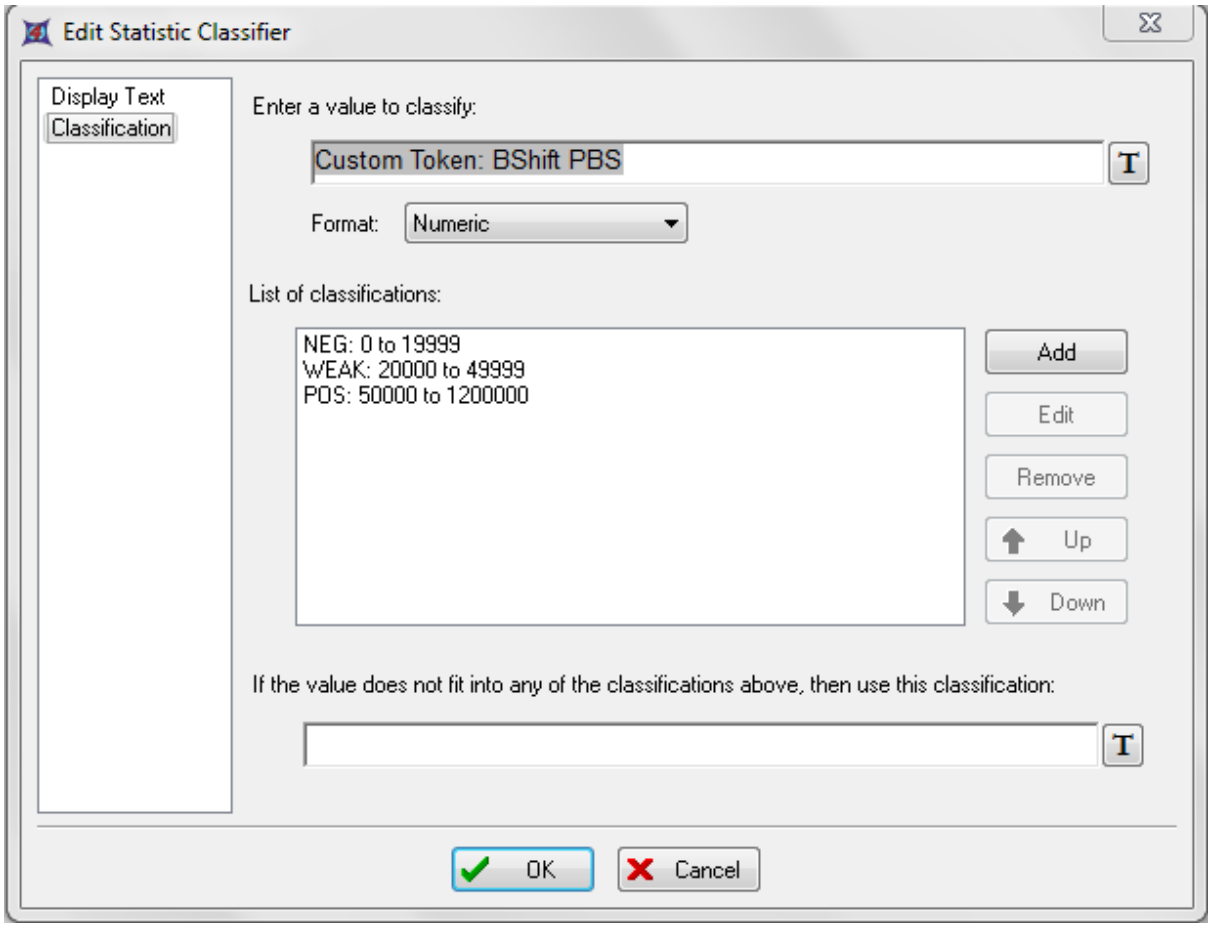

Figure T15.40 The 'B Result' Token Classifier Definition

34. Click **OK** on the **Edit Statistic Classifier** dialog to accept the definition.

The 'HLA Results' Data Grid now updates to reflect the addition of the Token Classifier to the first cell of the 'B Result' column, as shown in Figure T15.41.

Cell #	Sample	B Result	T Result
1	PBS NEG...	NEG	
2	AB NEGA...		
3	POSITIVE...		
4	PATIENT ...		

Figure T15.41 Token Classifier for the 'B Result' Column

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Now that we have created the Token Classifier to use for the 'B Result' column, we can copy and paste the Classifier into the other cells of the column. However, we will need to edit the Token Classifier to use the appropriate sample data.

35. Copy the 'BShift PBS' Custom Token Classifier definition we just created in the previous steps.

36. Paste the 'BShift PBS' Custom Token Classifier into the 'HLA Results' Data Grid editor for the second cell.

We now need to format the Token Classifier definition so that it uses BShift Custom Token from the AB NEGATIVE HUMAN SERUM sample.

37. Double-click on 'Classification of: 0.00' in the Data Grid editor.

The **Display Text** category of the **Edit Statistic Classifier** dialog appears.

38. Select the **Classification** category of the **Edit Statistic Classifier** dialog (similar to Figure T15. 40).

We will now edit the value to classify.

39. Delete the entry 'Custom Token: BShift PBS' in the **Enter a value to classify:** text field.

40. Click on the Token icon next to the **Enter a value to classify:** text field, indicated by the cursor in Figure T15.36.

The **Insert a Token** dialog appears, as previously shown in Figure T15.37. This is where we will choose a different Custom Token to classify.

41. Select **BShift NegSerum** from the **Custom** category of the **Insert a Token** dialog.

42. Click **Insert**.

The **Classification** category of the **Edit Statistic Classifier** dialog reappears. However, now the **Enter a value to classify:** text field has the Custom Token 'BShift NegSerum'entry. We will now fill in the rest of the "B Result' column repeating the above steps.

43. Repeat Steps 36 through 42 for the third cell in the 'B Result' column. However, on Step 41 choose **BShift PosSerum**.

44. Repeat Steps 36 through 42 for the fourth cell in the 'B Result' column. However, on Step 41 choose **BShift Patient**.

The 'B Result' column of the 'HLA Results' Data Grid is now complete and should appear similar to Figure T15.42.

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Cell #	Sample	B Result	T Result
1	PBS NEG...	NEG	
2	AB NEGA...	NEG	
3	POSITIVE...	POS	
4	PATIENT ...	NEG	

Figure T15.42 Completed 'B Result' Column

Now, we will define the Token Classifier for the 'T Result' column using the 'TShift' Custom Tokens.

45. Select the first cell of the 'B Result' column.

46. Copy the Token Classifier 'Classification of: 0.00'.

47. Select the first cell of the 'T Result' column

48. Paste the Token Classifier into the 'T Result' grid editor for the first cell of the 'T Result' column.

49. Double-click on 'Classifier 'Classification of: 0.00', to edit it.

The **Display Text** category of the **Edit Statistic Classifier** dialog appears.

50. Select the **Classification** category of the **Edit Statistic Classifier** dialog.

We will edit both the value to classify and the list of classifications. Refer to Figure T15.43 for the following steps.

51. Select **NEG: 0 to 19999** in the **List of classifications:** list box, shown highlighted in blue.

52. Click **Edit**.

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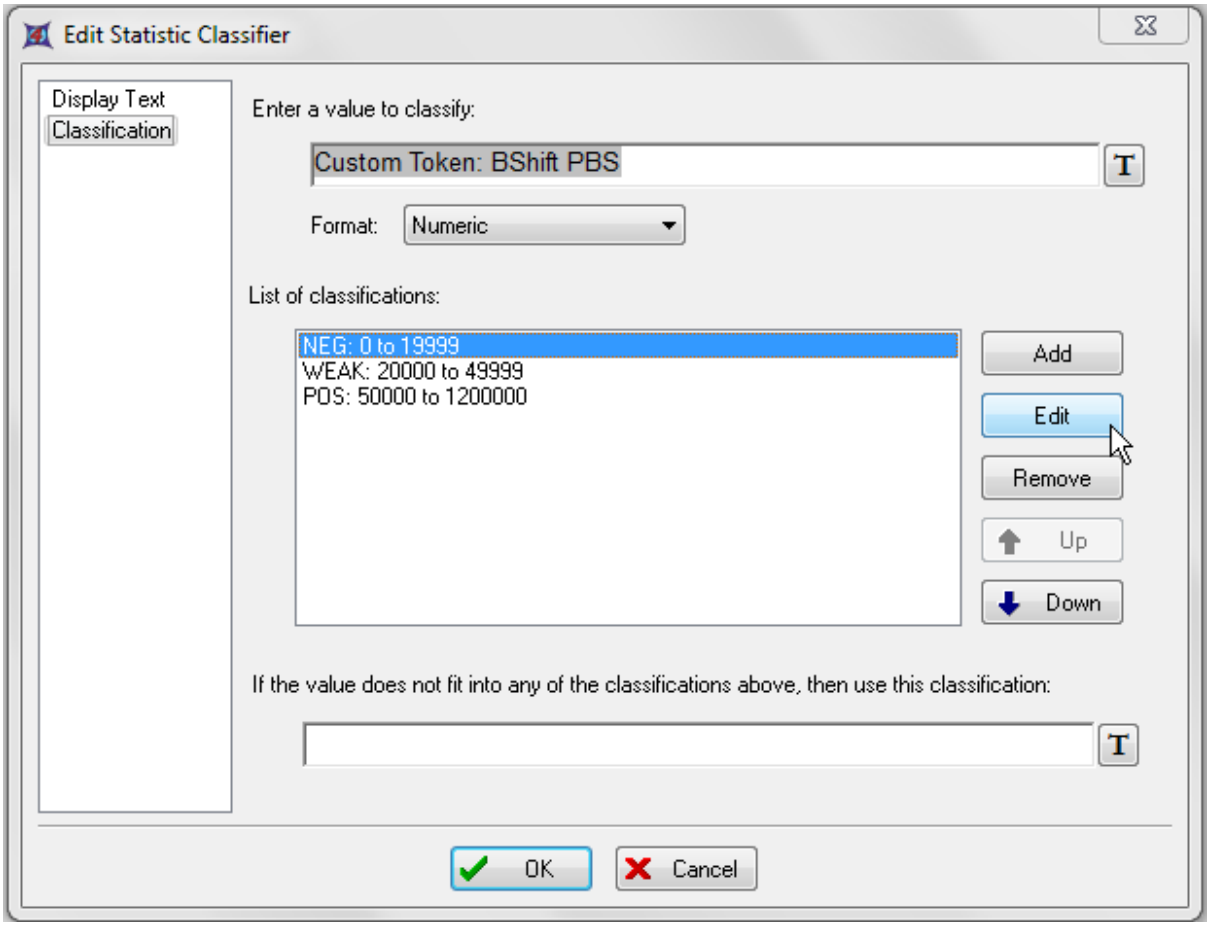

Figure T15.43 Editing the 'T Result' Token Classifier

The **Edit Classification** dialog appears, as previously shown in Figure T15.39. Here we will change the upper limit defining 'NEG'.

53. Change '19999' to '1999' in the **and** text field.

54. Click **OK**.

The **Classification** category of the Edit **Statistic Classifier** dialog reappears with the 'NEG' classification redefined to be between 0 and 1,999. We will now continue to edit the 'WEAK' and 'POS' classification categories.

55. Select **WEAK: 20000 to 49999** in the **List of classifications:** list box.

56. Click **Edit**.

57. Change '20000' to '2000' in the **if its value is between** text field.

58. Change '49999' to '7999' in the **and** text field.

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59. Click **OK**.

60. Select **POS: 50000 to 1200000** in the **List of classifications:** list box.

61. Click **Edit**.

62. Change '49999' to '8000' in the **if its value is between** text field.

63. Click **OK**.

Now we need to change the value to classify from the 'BShift PBS' Custom Token to the 'TShift PBS' Custom Token.

64. Delete the entry 'Custom Token: BShift PBS' in the **Enter a value to classify:** text field.

65. Click on the Token icon next to the **Enter a value to classify:** text window, indicated by the cursor in Figure T15.36.

The **Insert a Token** dialog appears, as previously shown in Figure T15.37.

66. Select **TShift PBS** from the **Custom** category of the **Insert a Token** dialog.

67. Click **Insert**.

The **Edit Statistic Classifier** dialog will reappear and should look similar to Figure T15.44 with the complete classifier definition for the 'TShift PBS' Custom Token.

68. Click **OK**.

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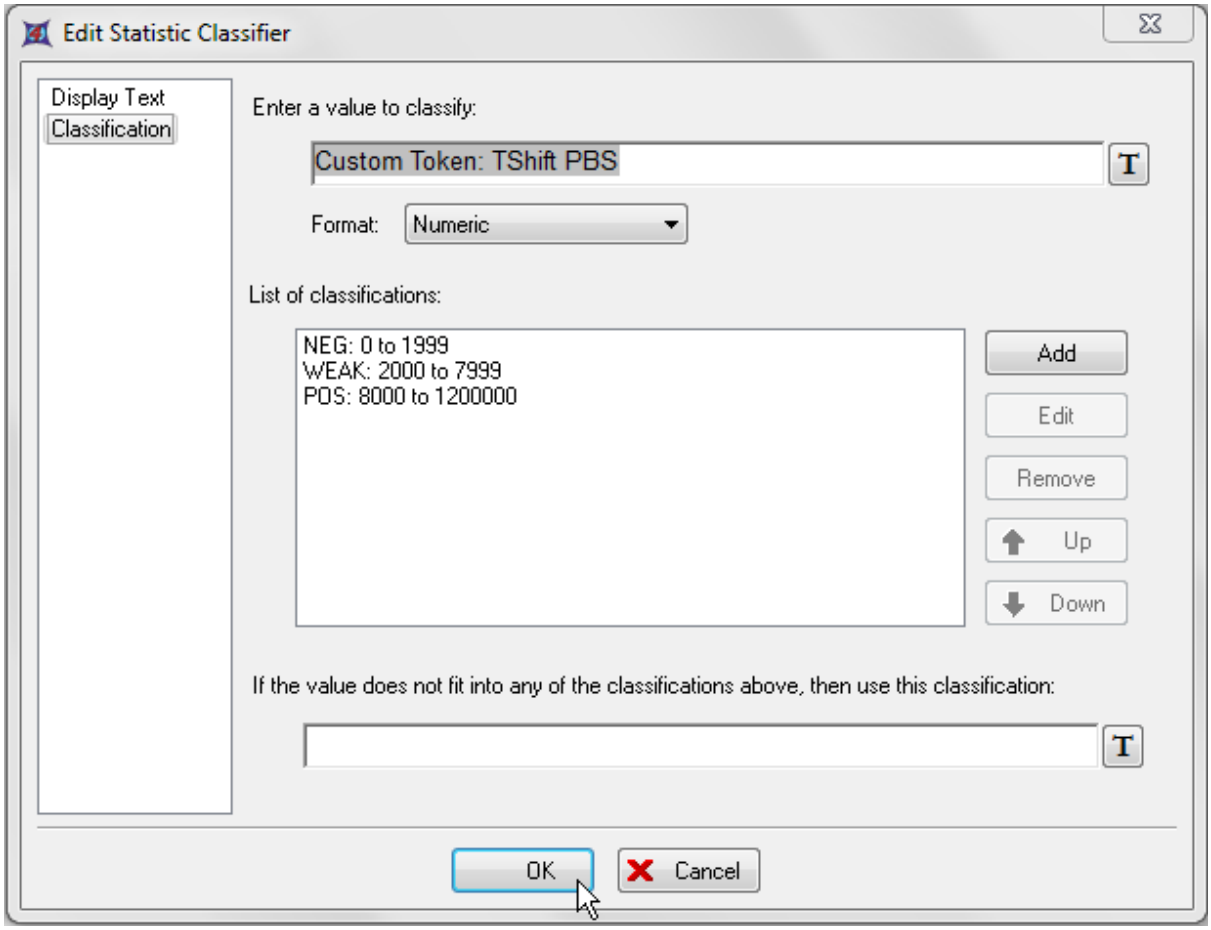

Figure T15.44 Classifier Definitions of the 'TShift PBS' Custom Token for the 'T Result' Column

We have defined the list of classifications for the 'TShift PBS' Custom Token. Now, we need to apply this definition to the other 'TShift' Custom Token to fill in the rest of the 'T Result' column. Again, the easiest way to do this is to copy the 'TShift PBS' Custom Token definition from the first cell of the 'T Result' column into the other cells in the column.

69. Repeat Steps 35 through 44 for the cells of the 'T Result' column and substitute 'TShift' Custom Tokens for 'BShift' Custom Tokens.

When you are done copying, pasting, and editing the Token Classifiers, the 'HLA Results' Data Grid will be filled in and should look similar to Figure T15.45.

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Cell #	Sample	B Result	T Result
1	PBS NEG...	NEG	NEG
2	AB NEGA...	NEG	NEG
3	POSITIVE...	POS	POS
4	PATIENT ...	NEG	NEG

Figure T15.45 Completed 'HLA Results' Data Grid

Now that we have created all the Custom Token and Classifiers in the custom data table, we can save this layout and re-use it with a different set of data. Because most of the Data Grids use only tokens, the data values will automatically update to the data plot used.

34.16 Bar and Scatter Plots

FCS Express now allows the user to display bar and scatter plots directly on their layouts. Bar and scatter plots created in FCS Express automatically update in real time as your gates are adjusted. By using bar and scatter plots, there is no longer a need for intermediate steps such as creating tables, exporting data, reassembling data in Excel, and exporting newly generated graphs for further image processing. Bar and scatter plots can be formatted and exported to PowerPoint and PDF, just like any other plot in FCS Express, making them easy to use. Bar and scatter plots allow you to summarize your results and generate publication quality graphics in record time.

In the course of this tutorial you will learn how to:

- Use a custom data grid for plotting bar and scatter data
- Create tokens for use in bar and scatter plots
- Manually enter values for bar and scatter plots
- Use tokens for creating error bars on your plots
- Format Bar and Scatter Plots
- Create conditional formulas to highlight interesting data sets
- Overlay multiple bar plots and scatter plots

Bar and scatter plots rely on custom data grids to display the data of interest. Custom Data grids can be thought of as spreadsheets, where the values in the cells of the spreadsheet are statistical values based on your plotted data. Please

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familiarize yourself with the custom data feature prior to beginning this tutorial by visiting the Custom Data tutorial 924.

In the first exercise, we will create bar and scatter plots 964.

34.16.1 Create Bar and Scatter Plots

In the course of this section, you will learn how to:

- Use a custom data grid for plotting bar and scatter data
- Create tokens for use in bar and scatter plots
- Manually enter values for bar and scatter plots
- Use tokens for creating error bars on your plots

In this example, we will be displaying data in a Bar Plot. Please note that the steps for creating a custom data grid for bar plots also apply for creating a scatter plot.

We will be using the **CreatingBarandScatter.fey** layout located in the Tutorial Sample Data archive 590. The layout contains samples from four conditions run in replicate. The conditions are as follows:

Condition	Sample Name	Marker
HSA Untreated	Sample 1	HSA-APC
HSA Treated	Sample 2	HSA-APC
GFP Untreated	Sample 3	GFP
GFP Treated	Sample 4	GFP

1. Open the **CreatingBarandScatter.fey** layout from the *FCS Express Sample Data* directory.

The layout contains four pages: HSA-APC Staining, GFP Expression, Bar Plot, and Scatter Plot.

2. Choose the **View→Multiple Pages→Two Pages (Vertical)** command.

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3. Click on the Bar Plot page tab for the left side view.
4. Click on the HSA-APC Staining page tab for the right side view.

The layout will now look like Figure T16.1.

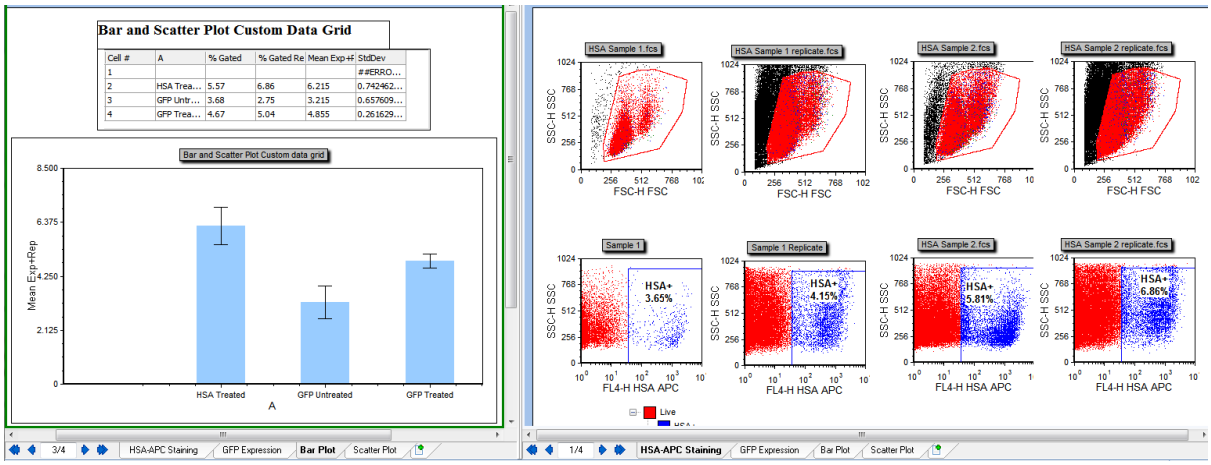

Figure T16.1 Create Bar and Scatter Layout Viewed with Multiple Pages

A Custom Data Grid named **Bar and Scatter Plot Custom Data Grid** is visible on the **Bar Plot** page. For convenience, three of the rows have already been filled out (Figure T16.2). We will complete the top row just as the other three were completed.

Bar and Scatter Plot Custom Data Grid

Cell #	A	% Gated	% Gated Re	Mean Exp+F	StdDev
1					##ERRO...
2	HSA Trea...	5.81	6.86	6.335	0.742462...
3	GFP Untr...	3.68	2.75	3.215	0.657609...
4	GFP Trea...	4.67	5.04	4.855	0.261629...

Figure T16.2 Custom Data Grid for Bar and Scatter Plot Tutorial

5. Click on the Column A row header to highlight it.
6. Right-click on the column header.

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7. Choose **Rename Selected Column** from the pop-up menu.
8. Enter "Sample" in the **New Column Name** dialog.
9. Click **OK**.
10. Click on Row 1 in the **Sample** column.
11. Enter "HSA Untreated" as the sample name.
12. Select the plot in the **HSA-APC Staining** page tab named **Sample 1** (Figure T16.3).
13. Shrink the **HSA+** gate to expose the text box containing the gate name and the statistic.

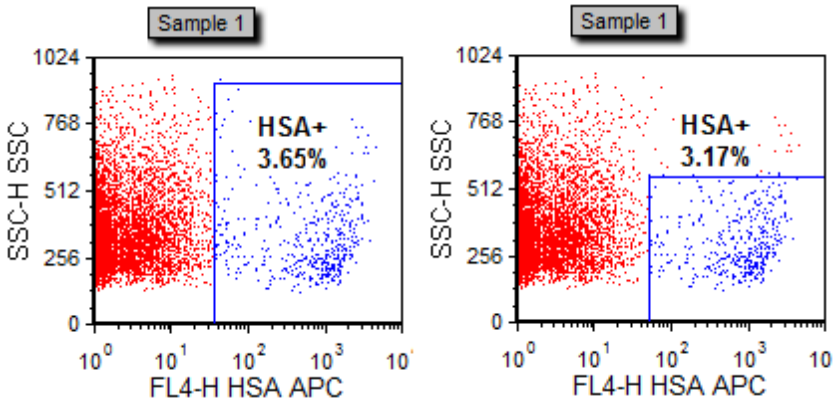

Figure T16.3 Selecting Sample 1 Before and After Shrinking Gate

13. After shrinking the gate, click in the gate statistics text box on the plot (the border of the text box should turn green to indicate the text box is in edit mode) .
14. Select the % gated token (the token text will turn white when selected, see Figure T16.4).

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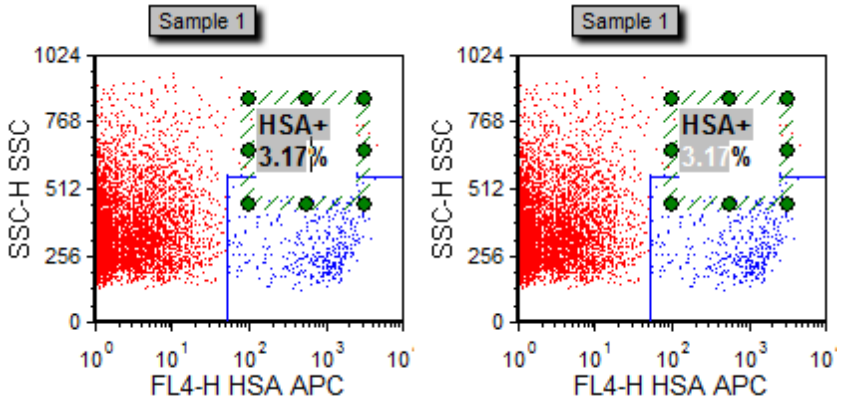

Figure T16.4 Left: Token Ready for Selection. Right: Token Selected.

15. While pointing to the selected token, hold down the **Ctrl** key and drag the token to Row 1 in the **% Gated** column.

16. Click **OK** in the **Paste Special** dialog (Figure T16.5).

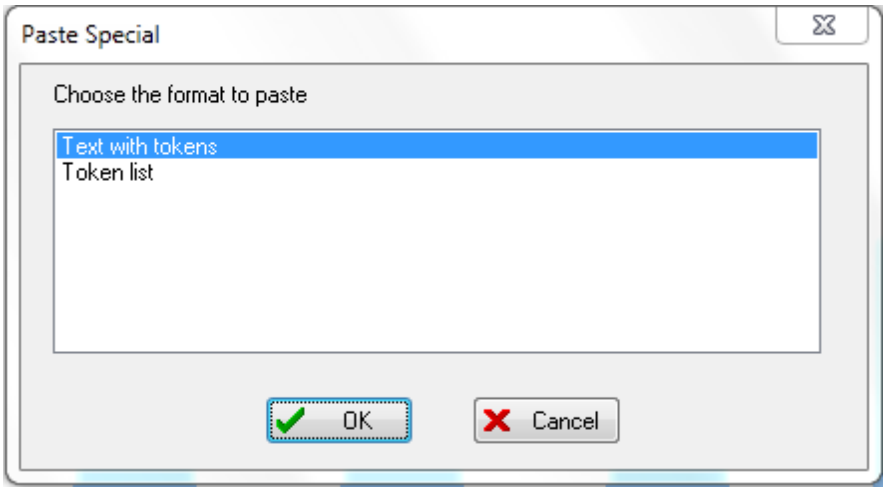

Figure T16.5 Paste Special Dialog

17. As an alternative, you can also right click on the selected % gated statistic and choose **Copy Text**. Then, you can select the cell in Row 1 under the **% Gated** column of the custom data grid to bring up the formula field. Right click in the formula field and choose **Paste**.

18. Repeat steps 14-16 for the **Sample 1 Replicate** plot, dragging into row 1 of the **% Gated Rep** column.

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19. Click on Row 1 of the **% Gated** column.
20. Select the token description in the dialog.
21. Hit **Ctrl+C** to copy the token.
22. Click Row 1 in the **Mean Exp+Rep** column. A dialog box will appear to enter the cell text.
23. Right-click in the dialog box.
24. Highlight **Insert Function** in the pop-up menu.
25. Choose **Mean([number1,number2,...])** from the list of functions (Figure T16.6).

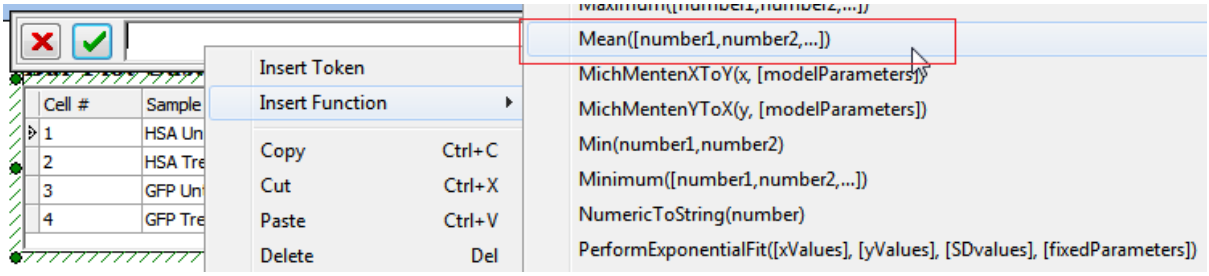

Figure T16.6 Selecting the Mean Function

26. Select **number 1** in the dialog (Figure T16.7).
27. Hit **Ctrl+V** to paste the token copied in step 21 (Figure T16.7).

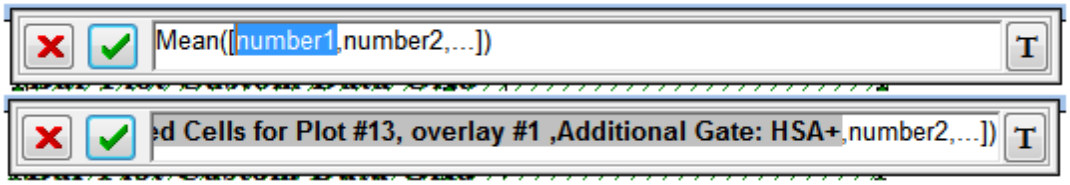

Figure T16.7 Top: Select number 1 for Replacement with a Token. Bottom: Pasting the Copied Token to Replace the Function Reference.

28. Repeat steps 20-22 with row 1 of the **% Gated Rep** column.
29. Highlight **number 2,...** in the **Mean Exp+Rep** Column.
30. Hit **Ctrl+V** to paste the token copied in step 28.

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31. Insert an "=" sign before the word **Mean** in the function formula (Figure T16.8).

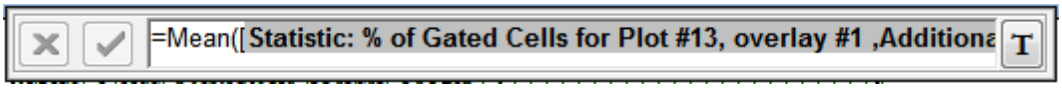

Figure T16.8 Inserting an Equal Sign Before Mean Completes the Function Formula

32. Hit **Enter**.

The first bar in the plot for HSA Untreated will now appear since it is based on the mean value of the two replicate samples. We will now use the standard deviation function to create an error bar for the HSA Untreated bar. For this example, the StdDev function has been chosen just as the mean function was chosen in steps 24 and 25. The statistic token for Sample 1 replicate has already been entered and we will now enter the other statistic token for Sample 1.

33. Click on Row 1 in the **StdDev** column.

34. Select the text "**INSERT SAMPLE 1 STAIN STAT HERE**" (Figure T16.9).

Figure T16.9 Selecting Text to Insert a Token

35. Right-click on the highlighted text.

36. Choose **Insert Token** from the pop-up menu.

37. Double-click on **Statistic** from the **Insert a Token** dialog.

38. Choose **HSA Sample 1 Stain** from the **Select a Plot** list box (Figure T16.10).

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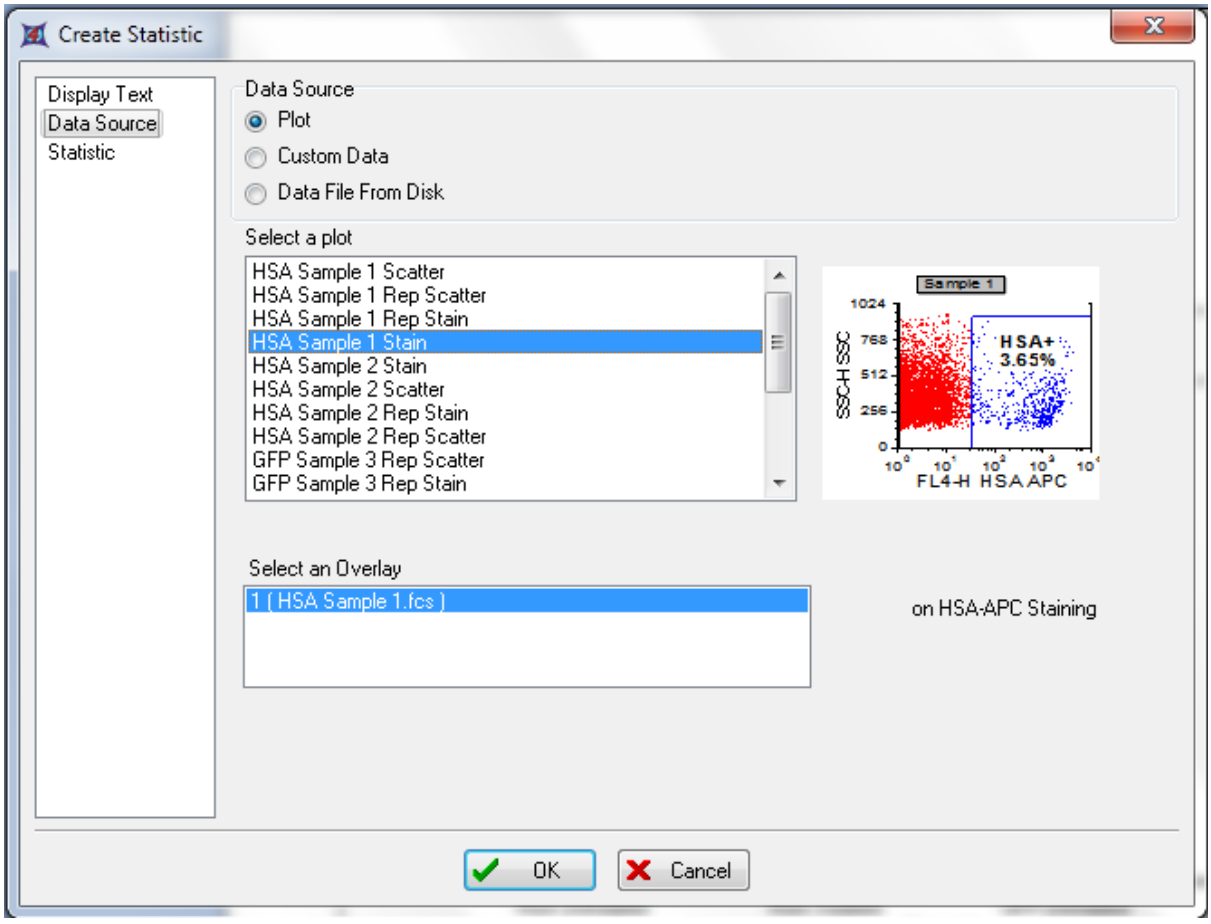

Figure T16.10 Choosing the Sample 1 Stain Plot from the List

39. Choose the **Statistic** category on the left.
40. Choose **HSA+** from the **Gate** drop-down list (Figure T16.11).
41. Choose **% of Gated Cells** from the **Statistic** list box (Figure T16.11).
42. Click **OK**.

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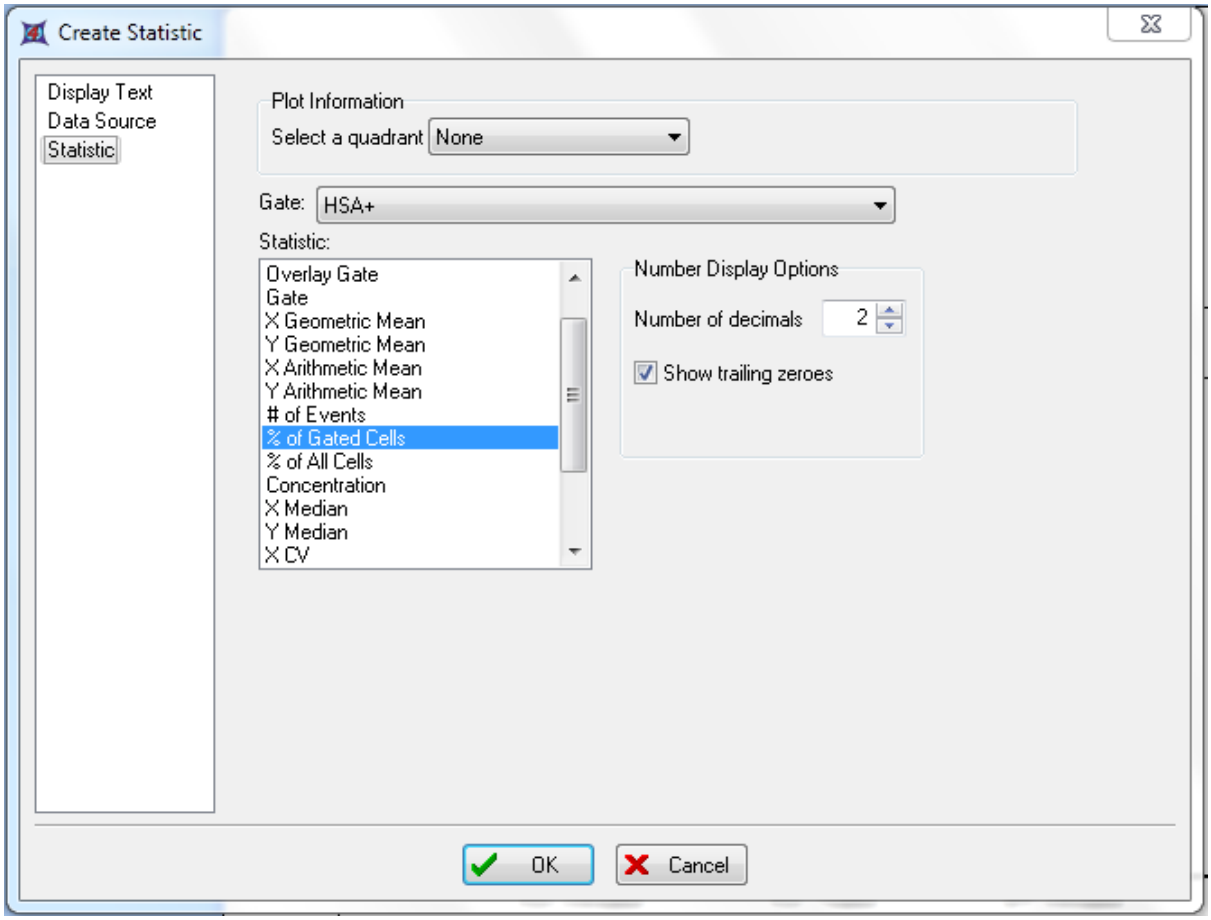

Figure T16.11 Choosing the Gate and Token for the Standard Deviation Function

43. Enter an "=" sign before the text **StdDev** in the custom data formula editor.

44. Click on the layout to exit the custom data formula editor.

The Bar Plot will now look like Figure T16.12. The **HSA Untreated** bar is now displaying an error bar based on the standard deviation of sample 1 and its replicate. Try moving any HSA+ gate on the HSA-APC Staining page to watch as the bars and error bars update automatically.

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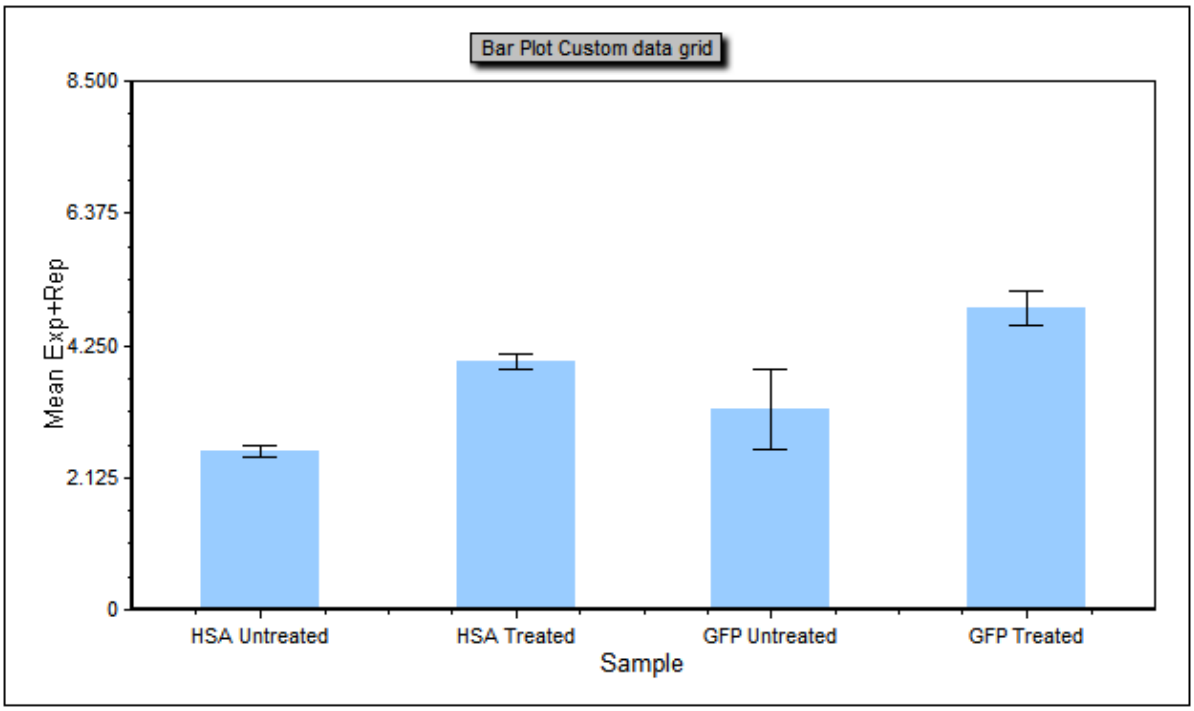

Figure T16.12 Completed Bar Plot

Data that is entered in a custom data grid, like the one that was just set up, can also be graphed in a scatter plot. As an example, we will now plot the data we just created in a scatter plot that has already been inserted and predefined.

45. Click on the **Scatter Plot** page tab.

46. Right-click on the **StdDev** column in the Bar and Scatter Plot custom data grid.

47. Choose **Insert→Column to the Right** (Figure T16.13).

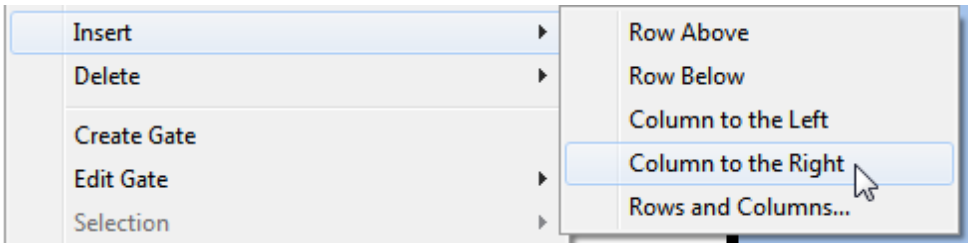

Figure T16.13 Inserting a Column to the Right of the Last Column

48. Name the column "Sample #" (Figure T16.14).

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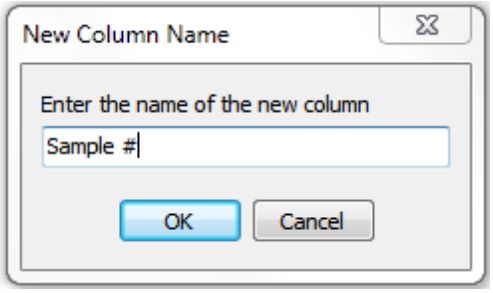

Figure T16.14 New Column Name Dialog

49. Choose **Sample #** as the X-Axis parameter in the Scatter plot.
50. Click on Row 1 of the **Sample #** column in the Bar and Scatter Plot custom data grid.
51. Enter the number "1" in the cell.
52. Repeat steps 51-52 with the numbers 2-4 in rows 2-4 of the **Sample #** column.

Notice how the scatter plot automatically updates as values are entered into the Bar and Scatter Plot custom data grid.

In the next section, we will format the bar and scatter plots 973.

34.16.2 Formatting Bar and Scatter Plots

While creating bar and scatter plots is a useful way to review data in real time, most users will eventually want to present data for publication or review. All plots can be fully formatted in FCS Express, which means creating publication quality figures can be achieved directly in the software without exporting to intermediate editing programs. In this tutorial, we will focus on formatting options unique to Bar and Scatter Plots. For information on general formatting, please refer to the Formatting Plots tutorial 620. We will use the **FormattingBarandScatterPlots.fey** layout which contains sample data files, a bar plot, and a scatter plot, that we will:

- Format Bar and Scatter Plots to:
  - Adjust bar/point colors and fill

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- Change error bar style and color
- Apply highlighting to bars and points
- Show data values on the plot
- Connect lines with Scatter plots

- Create conditional formulas to highlight interesting data sets
- Overlay multiple bar plots or scatter plots

Formatting Bar Plots

1. Open the **FormattingBarandScatterPlots.fey** layout from the Tutorial Sample Data archive 590.
2. Choose the **View→Multiple Pages→Two Pages (Vertical)** command.
3. Choose the **Bar Plot** page tab for the left side view.
4. Choose the **Scatter Plot** page tab for the right side view.

We will now overlay the data that is being shown in the scatter plot on the bar plot to view data from the **% Gated** column, along with the data from the **Mean Exp+ Rep** Column. We will also format the bars of the new overlay to change the color, fill, and border.

5. Select and drag the scatter plot on top of the bar plot (Figure T16.15).

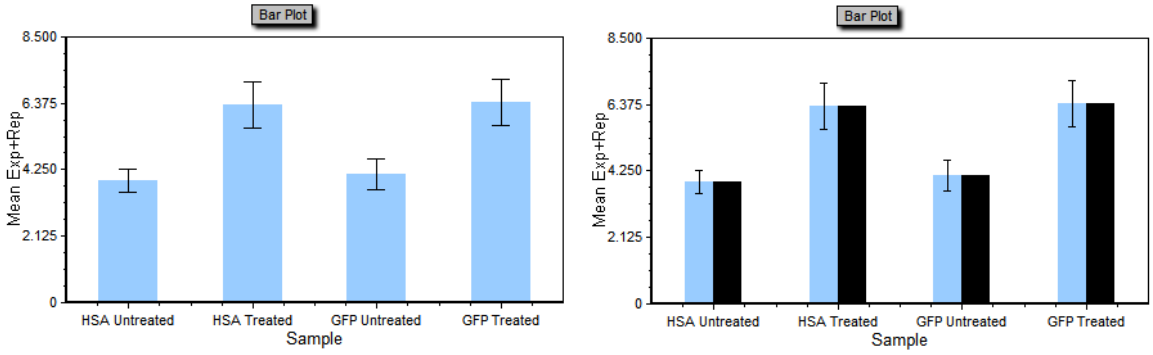

Figure T16.15 Left: Bar Plot Before Dragging and Dropping Scatter Plot as Overlay. Right: Bar Plot After Dragging and Dropping Scatter Plot as Overlay

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6. Right-click on the bar plot.
7. Choose **Format** from the pop-up menu.
8. Choose the **Overlays** category.
9. Click on overlay "2" to select it.

The following steps can be seen in Figure T16.16.

10. Choose % Gated from the **Y Parameter** drop-down list ❶.
11. Change the **Bar Color** to Red ❷.
12. Change the **Bar Fill Pattern** to **Backward Diagonal** ❸.
13. Change the **Line Options for:** to Bar Border.
14. Choose the first line **Style** (solid) from the **Bar Border Properties** box ❹.
15. Change the **Width** to 1.5 ❺.
16. Check the **Show Marks** check box ❻.
17. Click **Preview**.

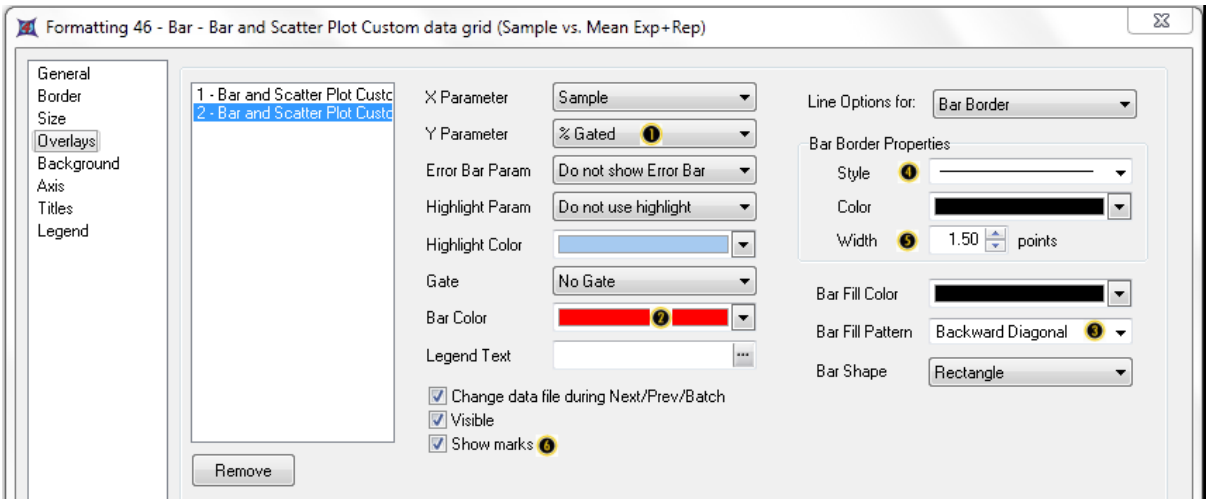

Figure T16.16 Formatting Bar Plot Overlay 2

The Bar Plot will now look like Figure T16.17. It is displaying bars from the % Gated and Mean Exp+Rep columns. The Show Marks option has created boxes on the plot which represent the value displayed by the bar.

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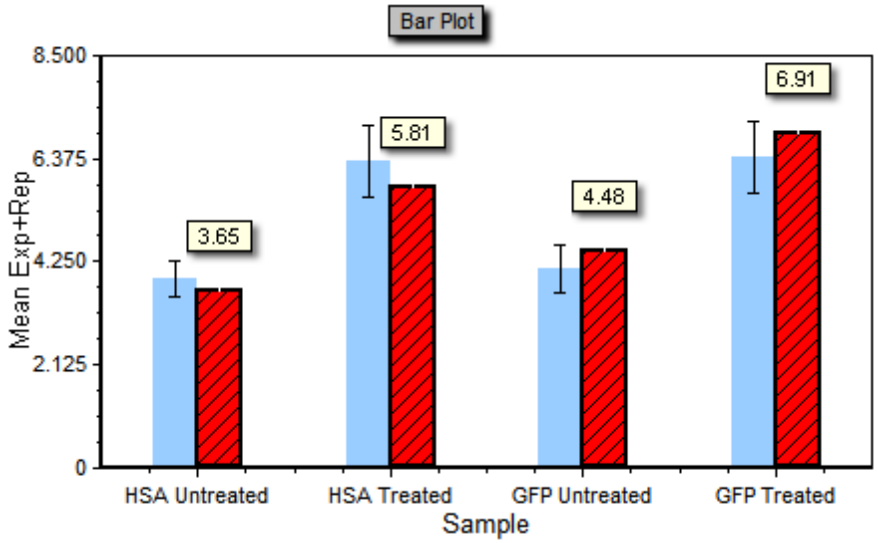

Figure T16.17 Bar Plot After Formatting was Applied

We will now format the error bars (Figure T16.18).

18. Click on overlay "1" to select it.
19. Change the **Line Options for:** to **Error Bar Pen** ❶.
20. Change the **Style** under Error bar properties to a dashed line ❷.
21. Change the **Color** to blue ❸.
22. Change the **Width** to 1.5 ❹.
23. Click **Preview**.

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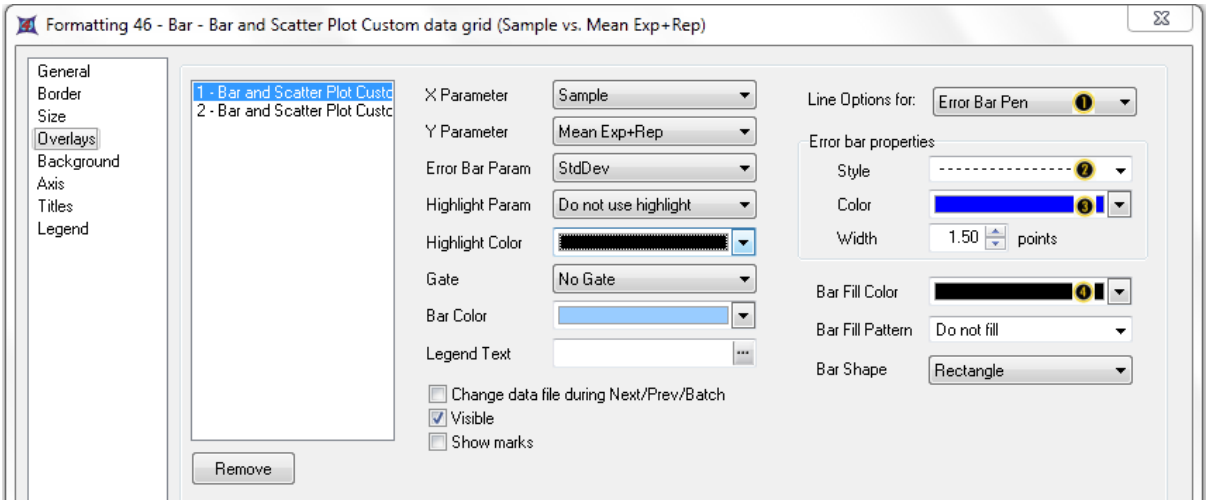

Figure T16.18 Formatting Error Bars

The Bar Plot will now look like Figure T16.19. The error bars have changed to a blue dash style with a thicker width.

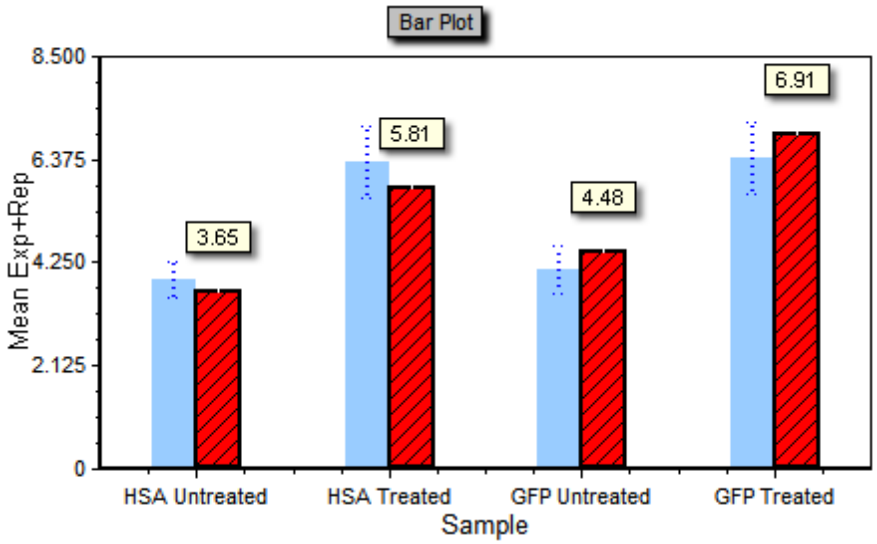

Figure T16.19 Error Bars Formatted on the Bar Plot

We will now use the highlighting feature to highlight bars of interest. A highlighted bar will not be drawn in the color of the overlay, but in a special highlight color that you can select. A column labeled highlighting is located in the Bar and Scatter Plot Custom Data Grid, where each cell contains the following formula:

=if (Token for % Gated cells in each sample) < 4 then "true" else "false"

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This formula will assign a true or false value based on the result of the referenced token (double-click on the token in the custom data grid to view its origin). When using the highlighting feature, a true value will indicate to highlight the bar and a false value will not highlight the bar. We will now apply the highlighting parameter to the original overlay in the bar plot to highlight any bars where the % Gated value is less than 4%.

24. Highlight the base overlay "1" .
25. Choose Highlighting from the **Highlight Param** drop-down list.
26. Choose Orange for the **Highlight Color**.
27. Click **OK**.

The bar plot will now look like Figure T16.20. The bars in which the % Gated column is less than 4% will be highlighted in orange. We will now change the GFP+ gate to watch the highlighting change in response to regating.

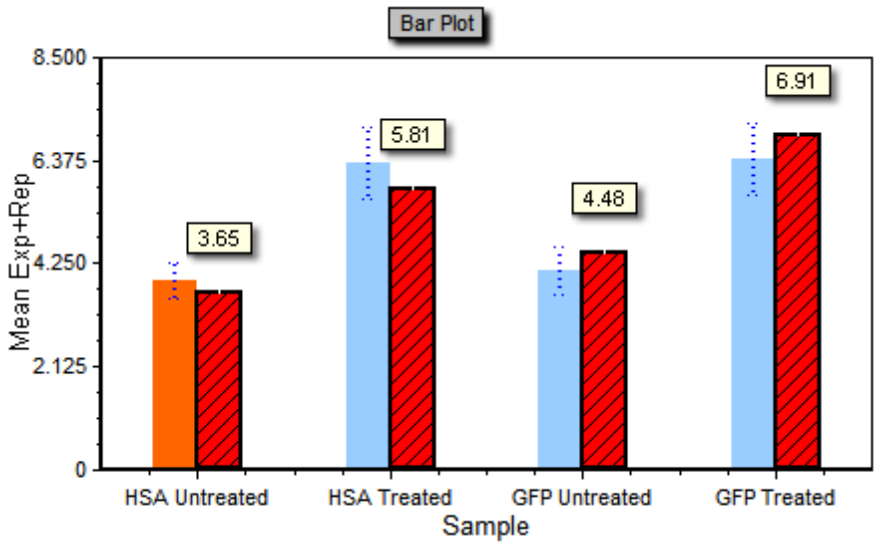

Figure T16.20 Highlighting Applied to Bar Plot

28. Change the right side page tab to **GFP Expression**.
29. Move the left side of the GFP+ gate to approximately 10^3 as in Figure T16.21.

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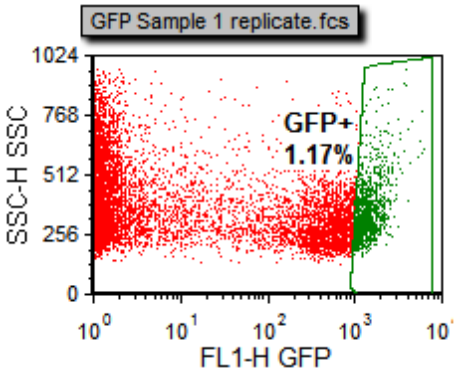

Figure T16.21 Adjusting the GFP+ Gate

Notice how the value for % Gated for GFP Untreated and Treated in the custom data grid changes to less than 4%. In addition, the highlighting value changes to true. Therefore, highlighting is added to the bars (Figure T16.22).

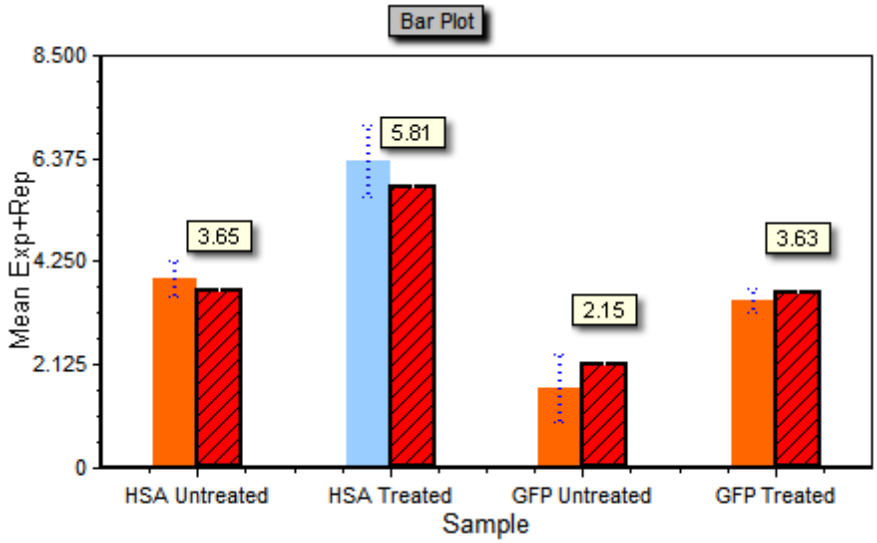

Figure T16.22 Bar Plot Colors Automatically Updating in Response to Moving a Gate

We also have the ability to choose which overlay to view in the bar plot.

30. Right-click on the bar plot.
31. Choose **Format** from the pop-up menu.

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32. Choose the **Overlays** category.
33. Click on Overlay "2" to select it.
34. Uncheck the **Visible** check box.
35. Click **OK**.

The bar plot will now be displaying only the data from the first overlay in which the Y Parameter is set to the Mean Exp+Rep Column.

Formatting Scatter Plots

1. Open the **FormattingBarandScatterPlots.fey** layout from the *FCS Express Sample Data* directory.
2. Choose the **View→Multiple Pages→Two Pages (Vertical)** command.
3. Choose the **Scatter Plot** page tab for the left side view.
4. Choose the **Bar Plot** page tab for the right side view.

We will now overlay the data that is being shown in the bar plot on the scatter plot to view data from the % Gated column along with data from the Mean Exp+ Rep column.

5. Select and drag the bar plot on top of the scatter plot.
6. Right-click on the scatter plot.
7. Choose **Format** from the pop-up menu.
8. Choose the **Overlays** category.
9. Click on Overlay "2" to select it.

The following steps can be seen in Figure T16.23.

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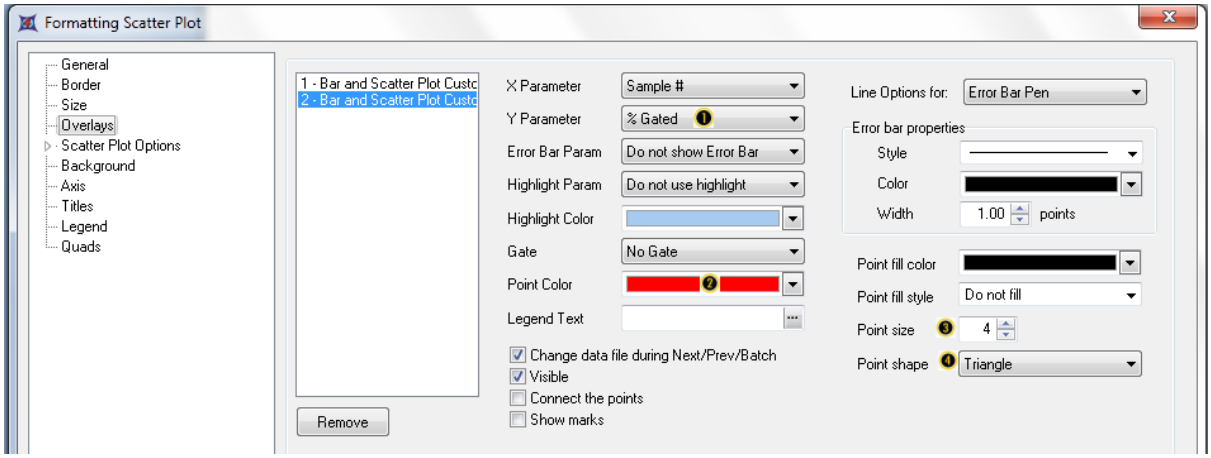

Figure T16.23 Formatting Scatter Plot Points

10. Choose **% Gated** from the **Y Parameter** drop-down list ❶.
11. Change the **Point Color** to Red ❷.
12. Change the **Point size** to 4 ❸.
13. Change the **Point shape** to **triangle** ❹.
14. Click **Preview**.

The Scatter Plot will now look like Figure T16.24. It is displaying points from the % Gated and Mean Exp+Rep columns with black squares and % Gated with red triangles.

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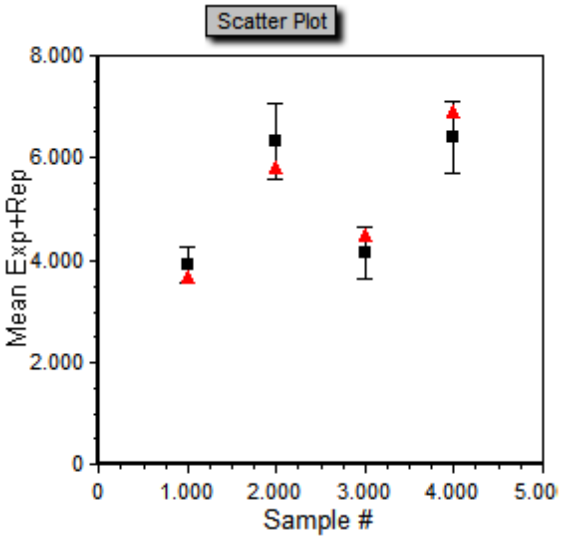

Figure T16.24 Scatter Plot After Formatting Points

We will now connect the points with a line (steps in Figure T16.25).

15. Check the **Connect the points** check box ❶.
16. Change the **Line Options for:** to **Connecting Lines** ❷.
17. Change the **Style** under **Connecting Lines Options** to the third option (dash) ❸.
18. Change the **Color** to blue ❹.
19. Change the **Width** to 1.5 ❺ .
20. Click **Preview**.

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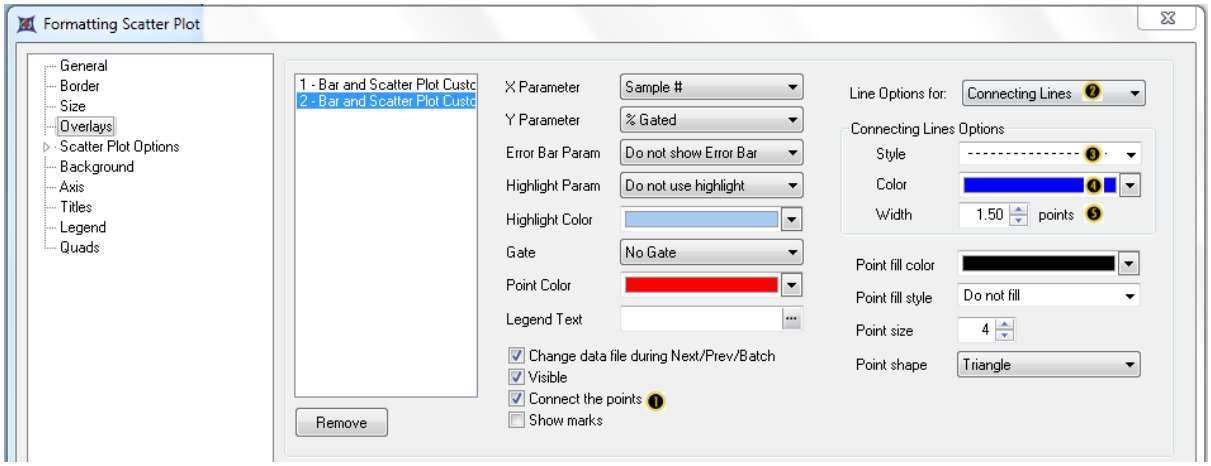

Figure T16.25 Formatting Scatter Plot Dialog

The connecting line for overlay 2 is now displayed as a blue dashed line (Figure T16.26).

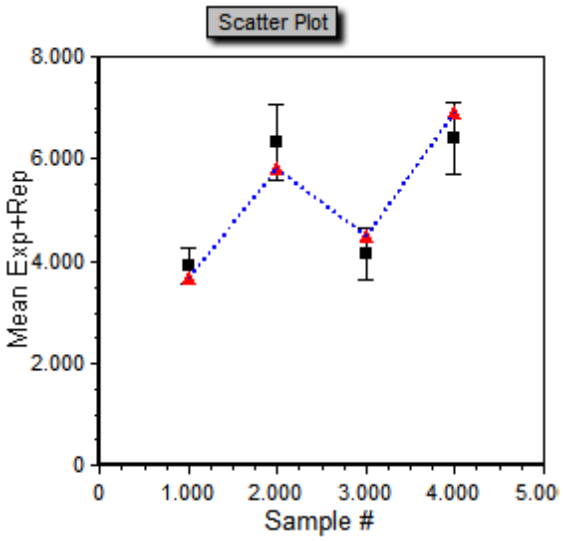

Figure T16.26 Points in the Scatter Plot Connected by Lines

We will now use the Highlighting feature to highlight points of interest. A column labeled highlighting is located in the Bar and Scatter Plot Custom Data Grid in which each cell contains the following formula:

=if (Token for % Gated cells in each sample) < 4 then "true" else "false"

This formula will assign a true or false value based on the result of the referenced token (double-click on the token in the custom data grid to view its origin). When using the highlighting feature a true value will indicate to highlight the point

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and a false value will not highlight the point. We will now apply the highlighting parameter to the original overlay in the scatter plot to highlight any points where the % Gated value is less than 4%.

21. Highlight the base overlay "1" .
22. Choose Highlighting from the **Highlight Param** drop-down list.
23. Choose Orange for the **Highlight Color**.
24. Click **OK**.

The scatter plot will now look like Figure T16.27 and the points in which the % Gated column is less than 4% will be highlighted in orange. We will now change the GFP+ gate to watch the highlighting change in response to regating.

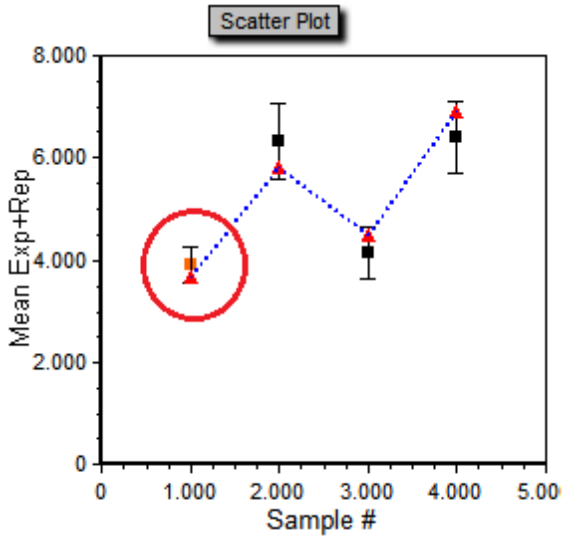

Figure T16.27 Highlighting Applied to the Scatter Plot. The First Square Point has Change to Orange.

25. Click on the **GFP Expression** page tab.
26. Move the left side of the GFP+ gate to approximately 10^3 as in Figure T16.28.

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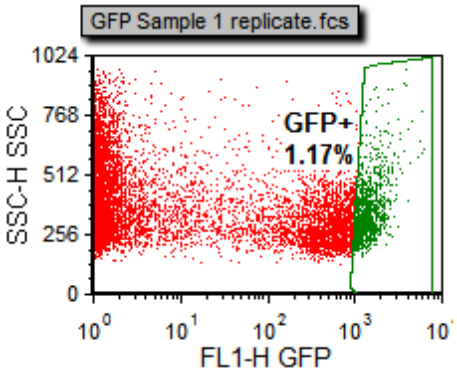

Figure T16.28 Adjusting the GFP+ Gate

Notice how the values for % Gated for GFP Untreated and Treated in the custom data grid changes to less than 4%. The Highlighting value changes to true and highlighting is added to new points (Figure T16.29).

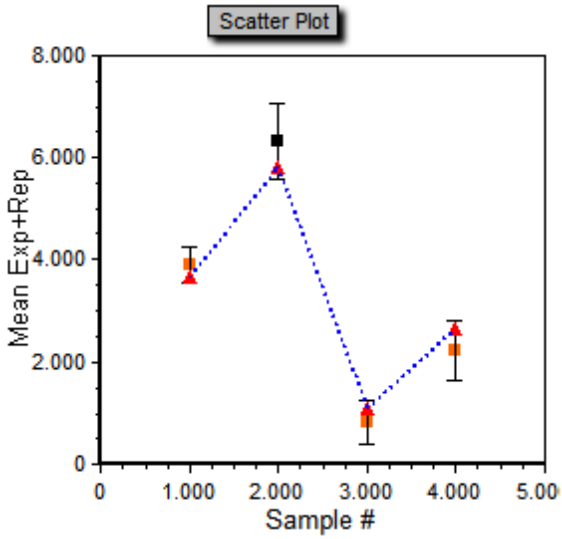

Figure T16.29 Scatter PLot Highlighting Updated After Changing Gate

We also have the ability to choose which overlay to view in the scatter plot.

27. Right-click on the scatter plot.
28. Choose **Format** from the pop-up menu.
29. Choose the **Overlays** category.

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30. Click on Overlay "2" to select it.
31. Uncheck the **Visible** check box.
32. Click **OK**.

The scatter plot will now be displaying only the data from the first overlay in which the Y Parameter is set to the Mean Exp+Rep column.

34.17 Titration

FCS Express provides a number of tools that allow you to create and graph your own data sets. Your data sets (called custom datasets) can contain data from your gates. When your gates change, so will your custom datasets and any plot derived from them. This allows you to easily create summary plots that show your entire experimental results.

In this tutorial, we will use custom datasets and plots to aid in the visualization of data from antibody titration experiments, to demonstrate selecting the ideal antibody concentration, so that the amount of antibody used for staining cells is the concentration that yields the best separation between positive and negative cells.

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 found 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:

- Define custom tokens to automatically calculate a CD64 signal to noise ratio.
- Create custom data table using the custom tokens.
- Insert a scatter plot displaying the custom data.

In the first exercise, we will define custom tokens 986.

34.17.1 Define Custom Tokens

For this tutorial, we will be using a layout file that has 2D density plots with defined gates from seven data files, each having different amounts of CD64 antibody.

1. Select **Application Button→Open Layout**.

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2. Open the layout **Titration.fey** found in the *FCS Express Sample Data* folder.

The following layout should appear as shown in Figure T17.1.

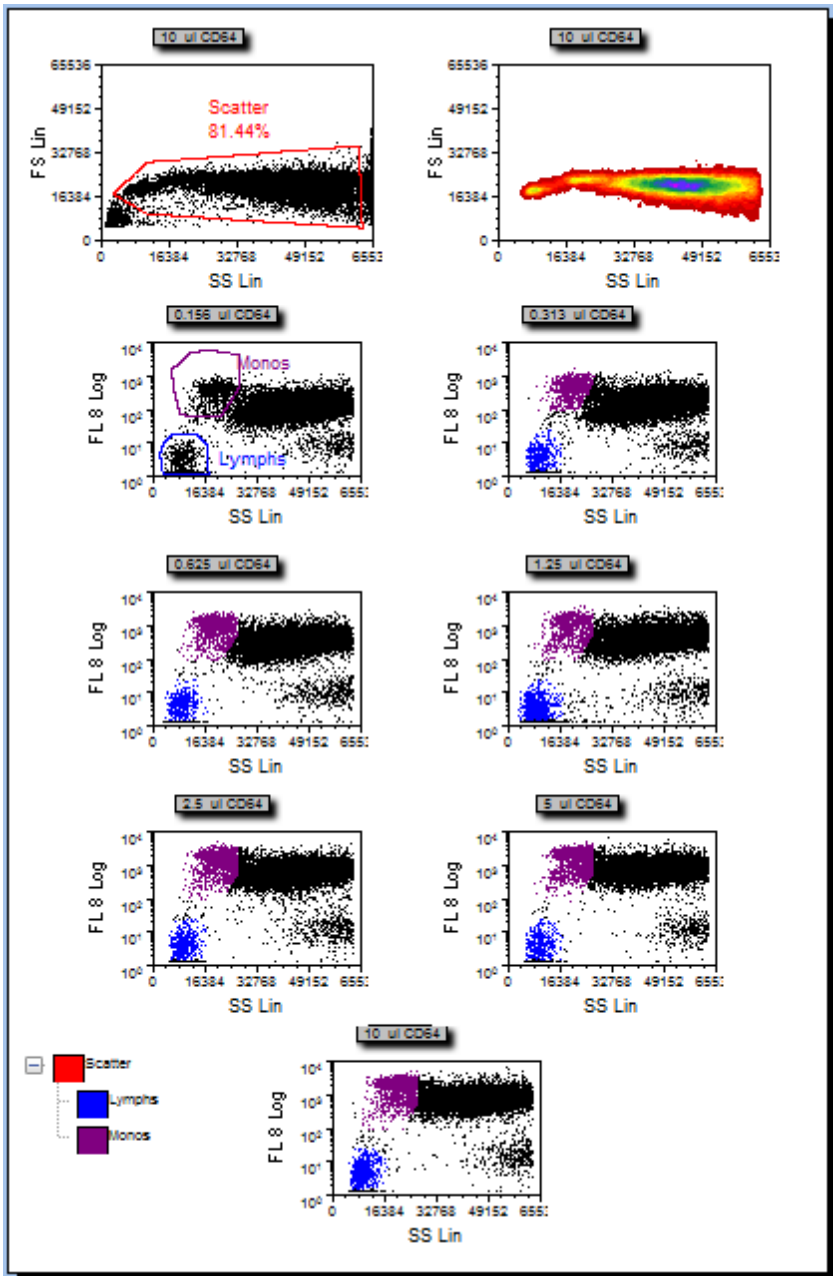

Figure T17.1 Titration Layout

In the upper left corner of the layout is a dot plot of Side Scatter versus Forward

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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 now define the custom tokens for each of the seven volumes of CD64 used in order to calculate a CD64 signal to noise ratio. The custom tokens 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 **View→Tokens→Custom Tokens** command (Figure T17.2).

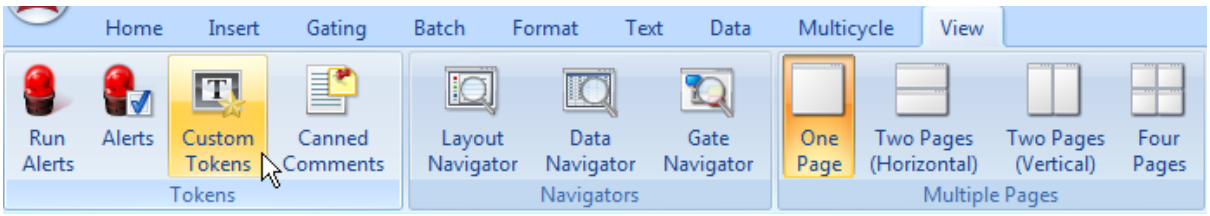

Figure T17.2 Selecting the Custom Tokens Command

The **Custom Tokens** window now appears on the layout. We will now dock the window to the left side of the layout. For more information on docking, please refer to the Docking tutorial 845.

4. Place the cursor on the top border of the **Custom Tokens** window.
5. Press and hold the left mouse button.
6. Continue to hold the left button while dragging the **Custom Tokens** window towards the left arrow of the Docking arrows icon.
7. Release the button when the cursor is over the left arrow of the Docking arrows icon, as indicated by the cursor in Figure T17.3.

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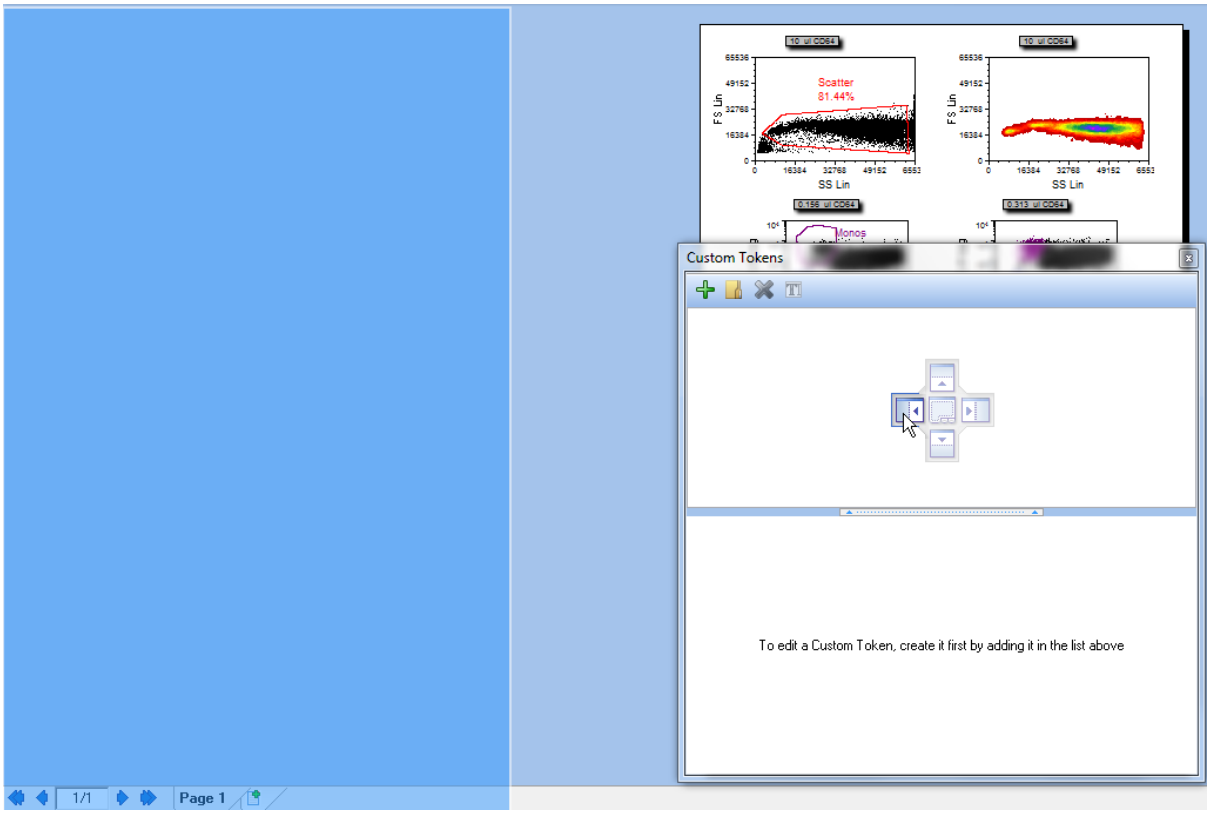

Figure T17.3 Docking the Custom Tokens Window

The **Custom Tokens** window is now docked on the left side of the layout. We will now begin defining the custom tokens. For more information on creating custom tokens, please refer to the Using Text Boxes and Tokens tutorial 675.

8. Left-click on the "+" symbol on the top border of the **Custom Tokens** window, indicated by the cursor in Figure T17.4.

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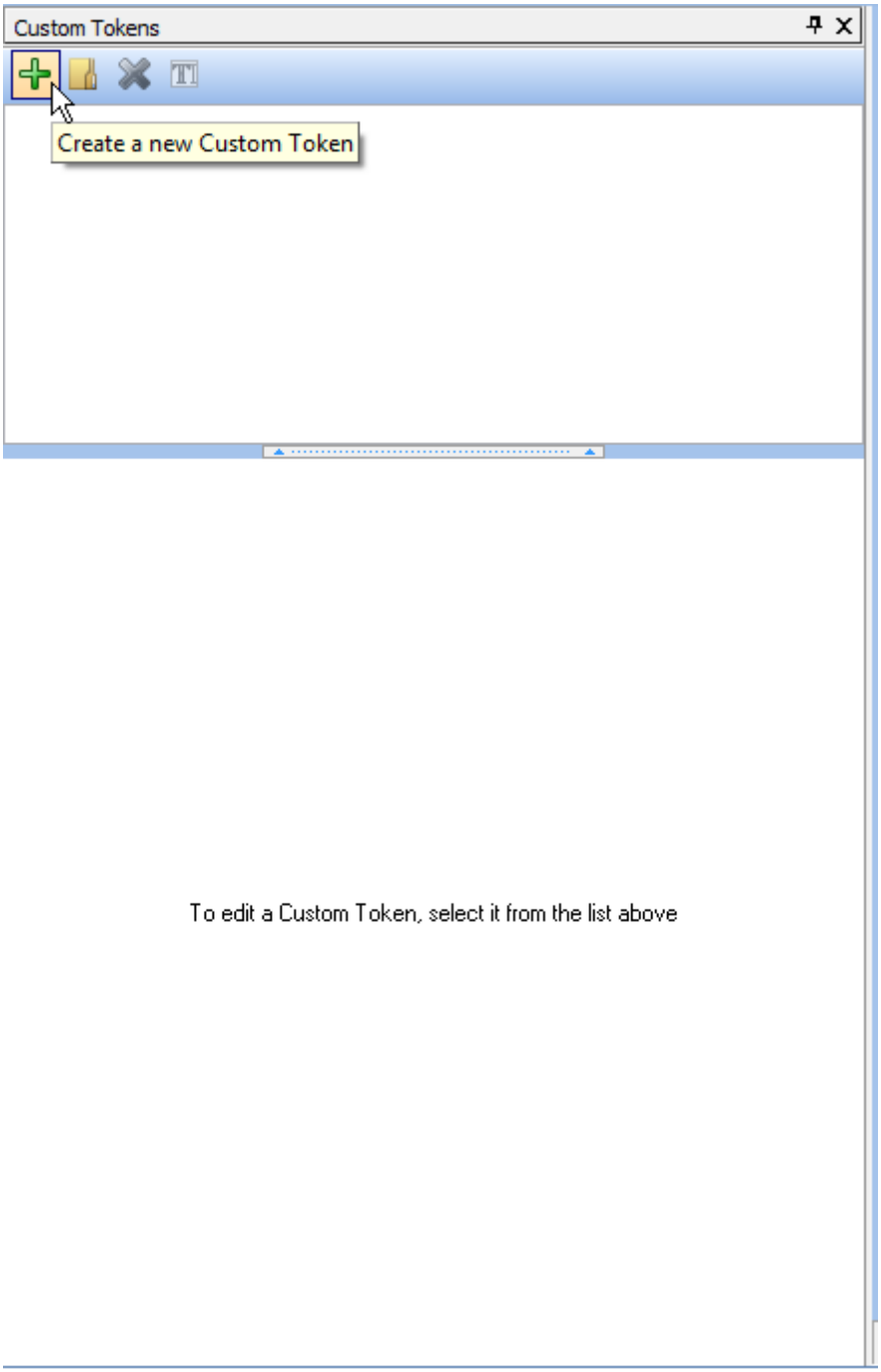

Figure T17.4 Creating a New Custom Token

A new Custom Token[348] appears in the top half of the **Custom Tokens** window, shown highlighted in blue in Figure T17.5.

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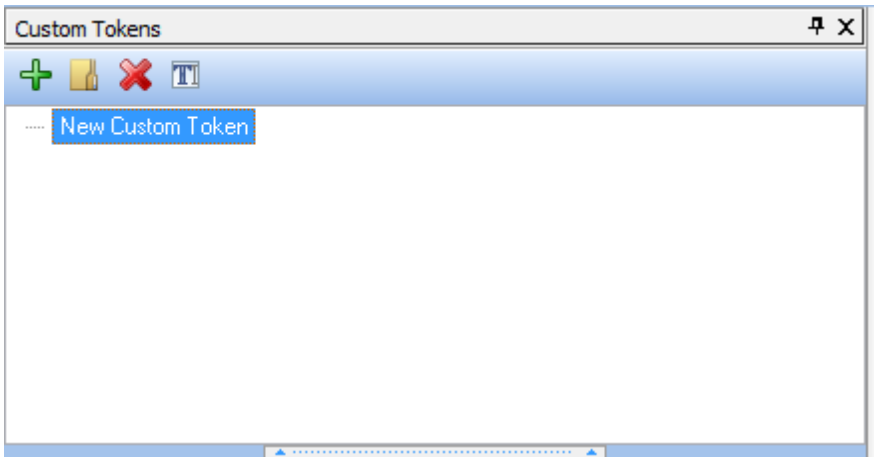

Figure T17.5 New Custom Token

9. Left-click on the **New Custom Token** to edit it.
10. Type '0.156 ratio'.
11. Hit Enter; **0.156 ratio** will be highlighted in blue.
12. Left-click inside the lower half of the Custom Tokens Window, under **Enter Custom Token formula below**.
13. Type '=' (Figure T17.6).

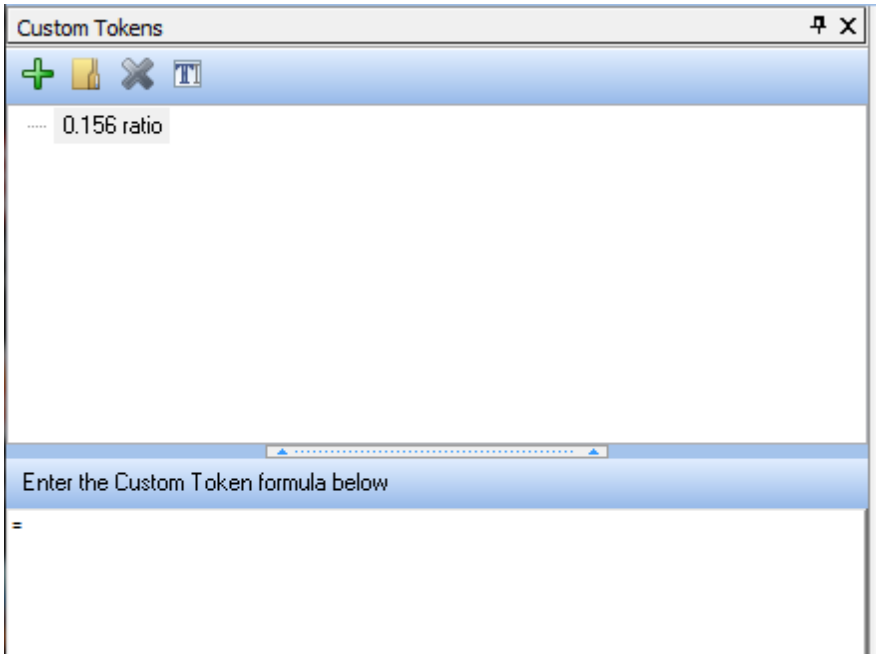

Figure T17.6 Beginning the Custom Token Formula

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14. Right-click next to the' **=**' to bring up the pop-up menu.

15. Select **Insert Token** from the pop-up menu, indicated by the cursor in Figure T17.7.

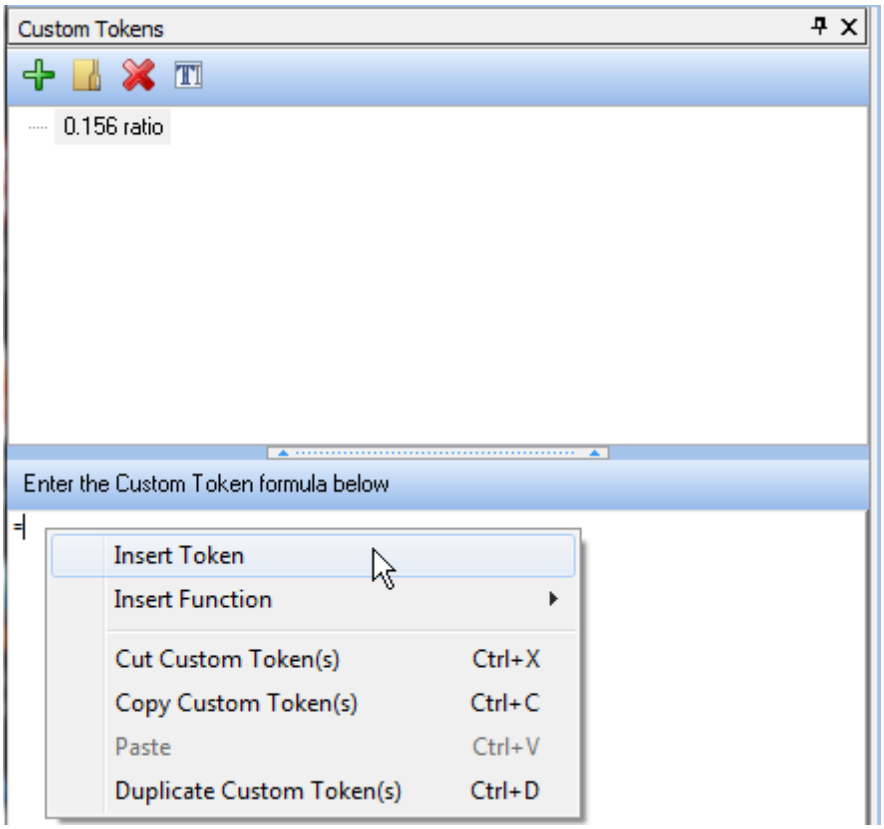

Figure T17.7 Insert Token Item from the Pop-up Menu

The **Insert a Token** dialog appears (Figure T17.8).

16. Select **Statistic**, shown highlighted in blue in Figure T17.8.

17. Click **Insert**.

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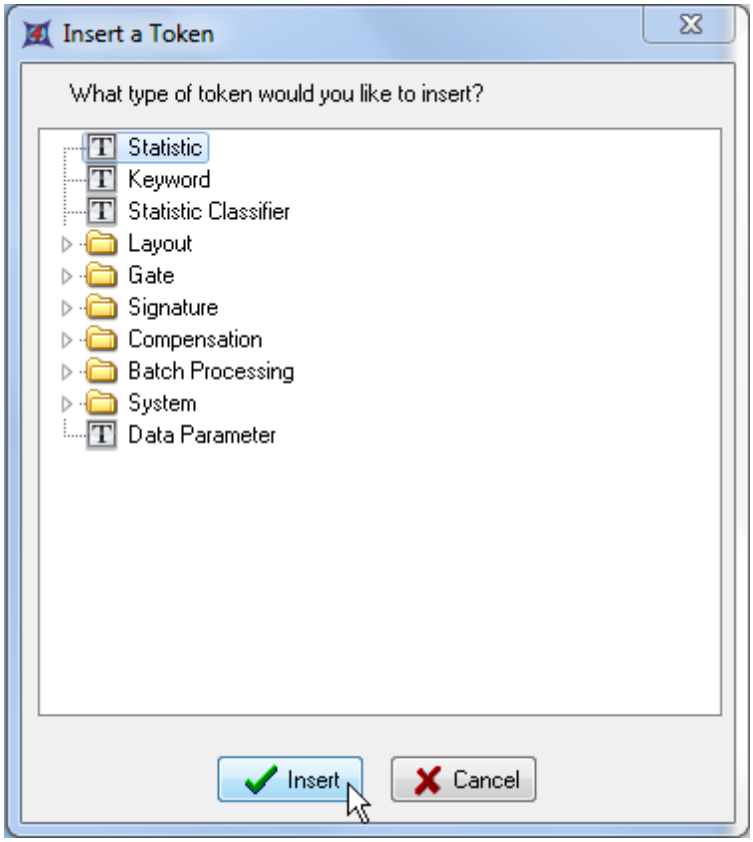

Figure T17.8 Insert a Token Dialog

The **Create Statistic** dialog, with the **Data Source** category, appears, similar to Figure T17.9. This is where we will select the data source to use in the custom token definition.

18. Select **Color Dot - .156ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**, shown highlighted in blue in Figure T17.9.

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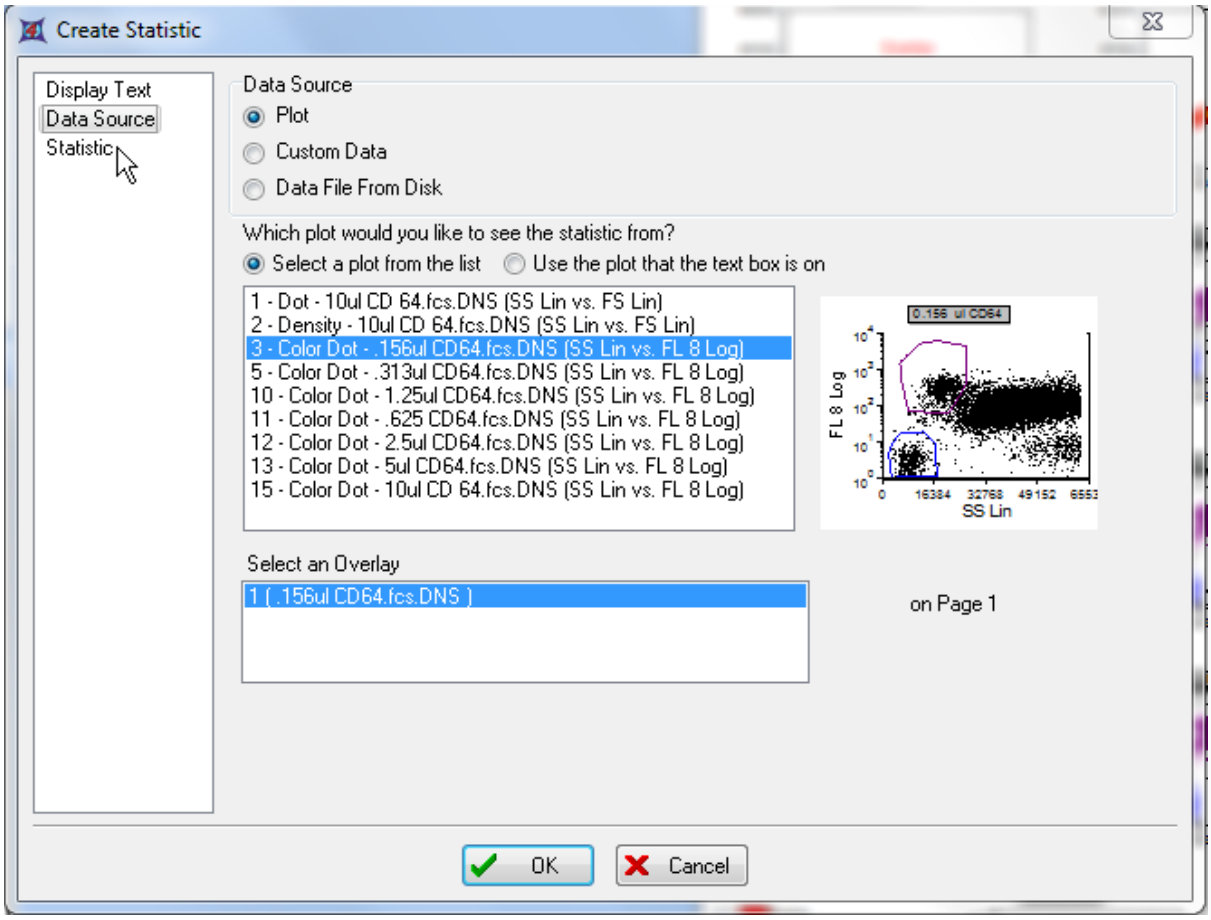

Figure T17.9 Create Statistic Dialog -- Data Source

19. Select the **Statistic** category on the left, indicated by the cursor in Figure T17.9.

The **Statistic** page of the **Create Statistic** dialog now appears, similar to Figure T17.10.

20. Select **Monos** from the **Gate:** drop-down list A.

21. Select **Y-median** from the **Statistics:** list box B.

22. Select **OK**.

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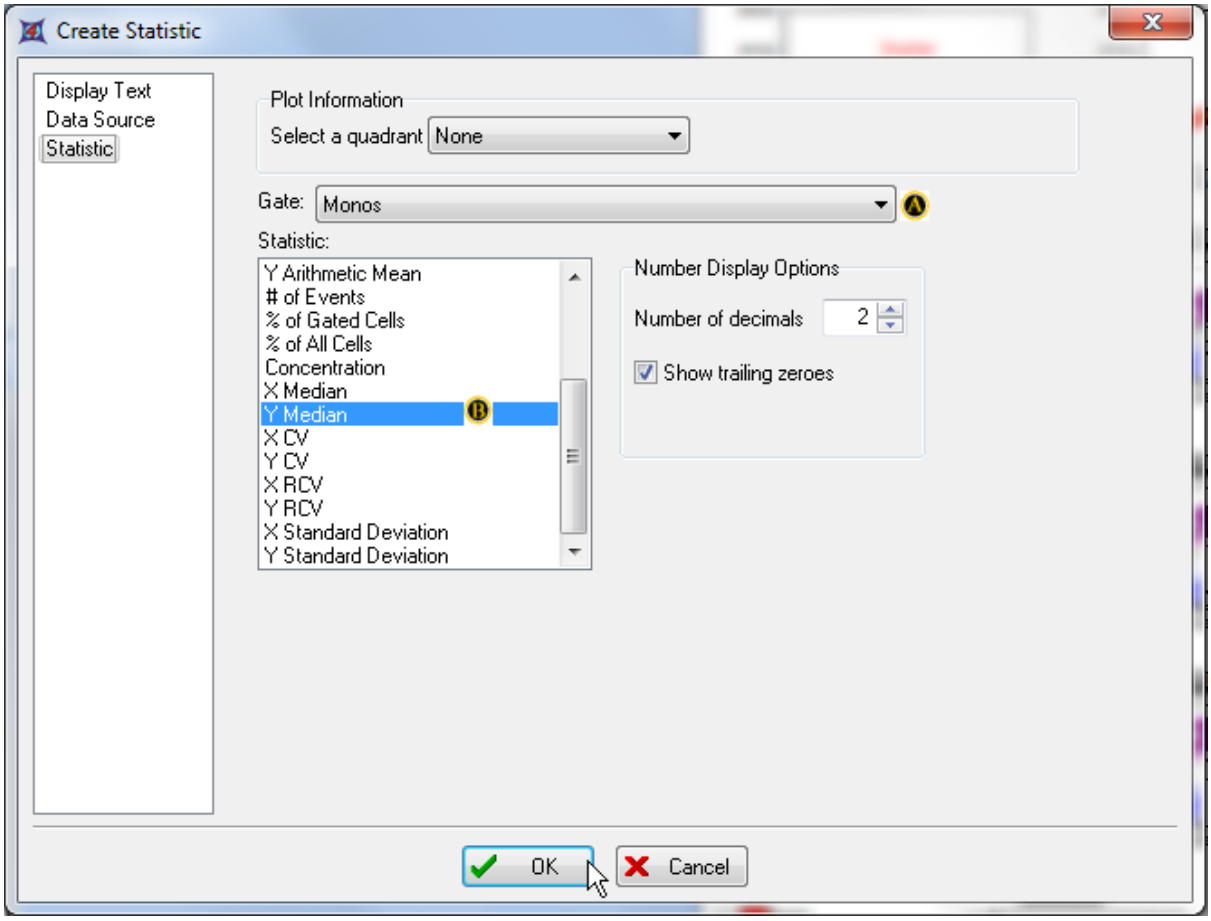

Figure T17.10 Create Statistic Dialog -- Statistic Category

The Custom Tokens definition of '0.156 ratio' updates to reflect the changes made, shown in Figure T17.11.

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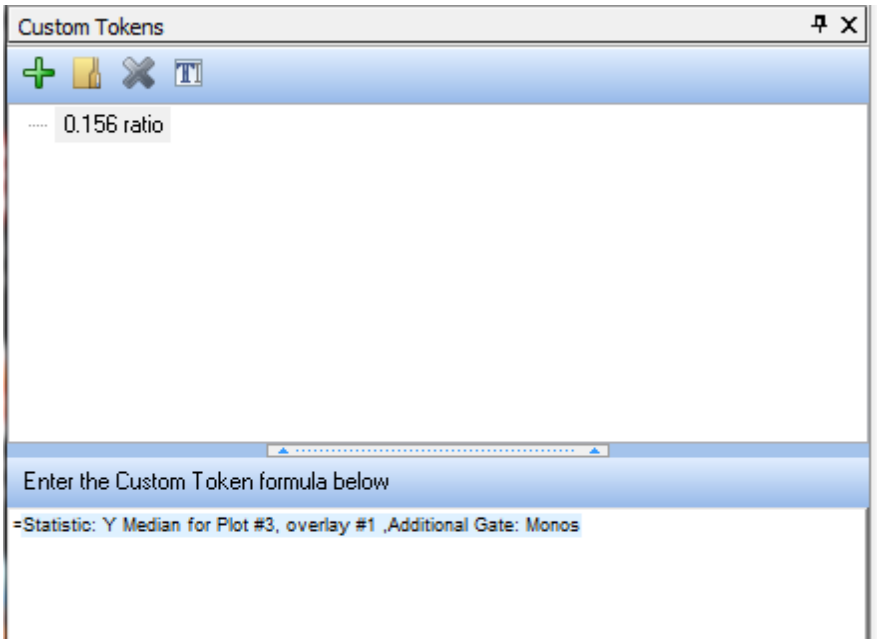

Figure T17.11 Numerator Definition of Custom Token '0.156 Ratio'

We have now defined the numerator, the positive population, of the **0.156 ratio** custom token. Now we will define the denominator, the negative population, of the custom token.

23. Type '/' after the text '**Statistic: Y Median for Plot #3, overlay #1, Additional Gate: Monos**' in the Custom Token formula window. *Note:* '/' is the division symbol, just like in Microsoft Excel.

24. Select the text '**Statistic: Y Median for Plot #3, overlay #1, Additional Gate: Monos**'.

25. Copy the text using Ctrl+C on the keyboard.

Alternatively the text can be copied using the pop-up menu.

- Right-click after selecting the token to bring up the pop-up menu.
- Select **Copy Text** from the pop-up menu.

26. Place the cursor after the '/' symbol and left click.

27. Paste the custom token text using Ctrl+V on the keyboard.

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Alternatively the text can be pasted using the pop-up menu.

- Right-click after the '/' symbol to bring up the pop-up menu.
- Select **Paste Text** from the pop-up menu.

The Custom Token formula definition updates to reflect the copying and pasting of the numerator text statistic to the denominator. We will now edit the denominator definition.

28. Double-click on the newly pasted denominator token, in the **Custom Token** definition window.

The **Edit Statistic** dialog appears, similar to the **Create Statistic** dialog previously shown in Figure T17.9. We will now modify the definition to use the *Lymphs* gate; we do not need to edit the **Data Source** page because we are still using the same plot.

29. Select **Statistic** from the category section of the **Edit Statistic** dialog.

The **Statistic** page of the **Edit Statistic** dialog appears, again similar to the **Create Statistic** dialog previously shown in Figure T17.10; however, this time we will select the *Lymphs* gate.

30. Select **Lymphs** from the **Gate:** drop-down list.
31. Select **OK**.

The Custom Token formula for **0.156 ratio** updates to reflect the changes made, as shown in Figure T17.12.

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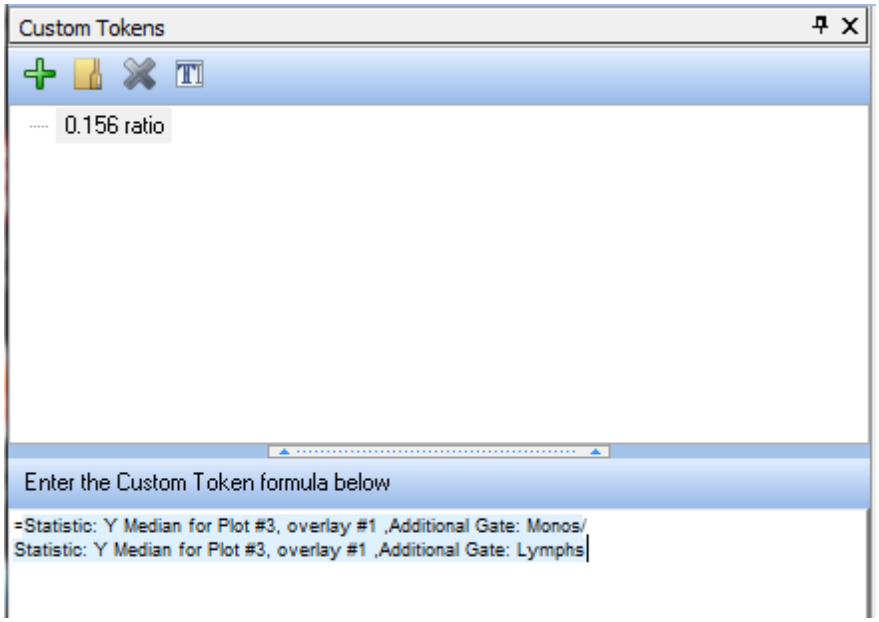

Figure T17.12 Custom Token Formula for 0.156 ratio

We will now create a Custom Token formula for the 0.313µl CD64 data. However, instead of using copy and paste to define the Custom Token, we will drag and drop the color dot plot of 0.313µl CD64 onto the lower half of the **Custom Tokens** window.

32. Left-click on the "+" symbol on the top border of the **Custom Tokens** window, indicated by the cursor in Figure T17.4.

33. Type '0.313 ratio' inside the **New Custom Token** text.

34. Press Enter; **0.313 ratio** will be highlighted in blue.

35. Left-click inside the lower half of the **Custom Tokens** window, under **Enter Custom Token formula below**.

36. Type ' =' (Figure T17.6).

37. Place the cursor over the plot of **0.313ul CD64,** shown with a red border in Figure T17.13.

38. Press and hold the left mouse button.

39. Continue to hold the left button and drag the plot onto the lower half of the **Custom Tokens** window.

40. Release the left button when the plot is to the right of the '=', indicated by the cursor in Figure T17.13.

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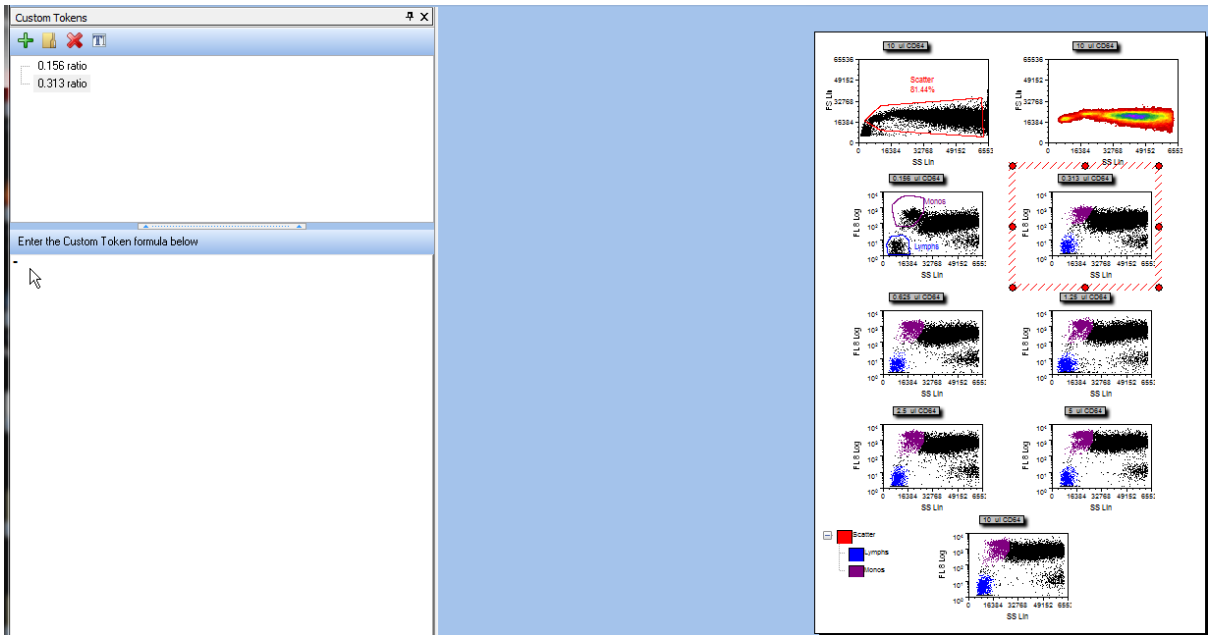

Figure T17.13 Dragging and Dropping the 0.313µl CD64 Plot onto the Custom Token Formula

The **Paste Special** dialog will appear as shown in Figure T17.14.

41. Select **Statistic token**, shown highlighted in blue in Figure T17.14.
42. Click **OK**.

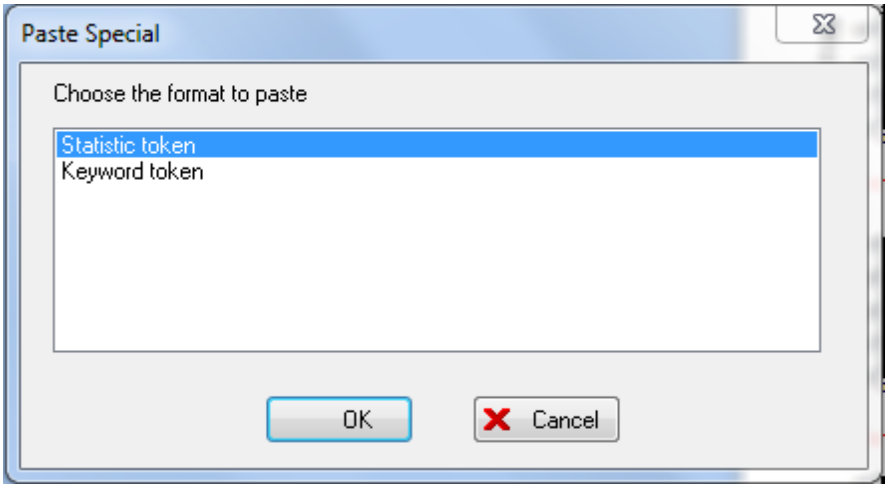

Figure T17.14 Paste Special Dialog

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The **Statistic** page of the **Edit Statistic** dialog appears, again similar to the **Create Statistic** dialog previously shown in Figure T17.10. Agai,n we will use the same statistic settings shown in Figure T17.10 to define the numerator of **0.313 ratio** Custom Token. The Data source has already been set to the 0.313 µl CD64 plot when we dragged and dropped the plot onto the **Custom Tokens** definition window. Refer to Figure T17.10 for the following steps

43. Select **Monos** from the **Gate:** drop-down list .
44. Select **Y-median** from the **Statistics:** list box .
45. Select **OK**.

We have defined the numerated for the **0.313 ratio** Custom Token. Now, we will define the denominator, again dragging and dropping the plot of **0.313ul CD64**.

46. Type '/' after the text '**Statistic: Y Median for Plot #5, overlay #1, Additional Gate: Monos**' in the Custom Token formula window.
47. Place the cursor over the plot of **0.313ul CD64,** shown with red border in Figure T17.13.
48. Press and hold the left mouse button.
49. Continue to hold the left button and drag the plot onto lower half of the **Custom Tokens** window.
50. Release the left button when the plot is to the right of the '/'.

The **Paste Special** dialog will appear again, as previously shown in Figure T17.14.

51. Select **Statistic token**, shown highlighted in blue in Figure T17.14.
52. Click **OK**.

The **Statistic** page of the **Edit Statistic** dialog appears, similar to the **Create Statistic** dialog previously shown in Figure T17.10. Again, we will use the **Y-median** as the statistics option. However, now we will select the *Lymphs* gate to define the denominator.

53. Select **Lymphs** from the **Gate:** drop-down list.
54. Select **Y-median** from the **Statistics:** list box.
55. Select **OK**.

The **Custom Tokens** window should now appear similar to Figure T17.15, with the definitions for Custom Tokens for **0.156 ratio** and **0.313 ratio**.

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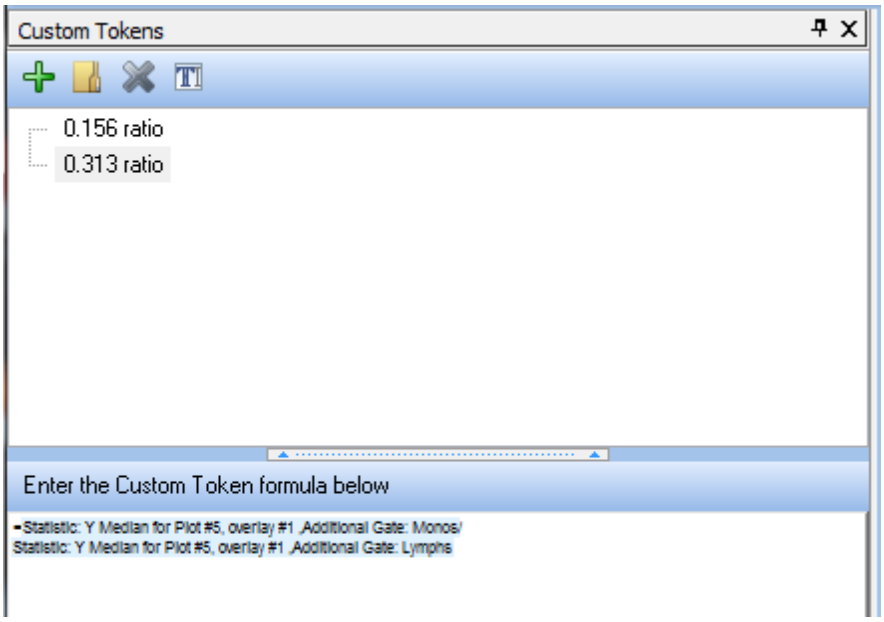

Figure T17.15 Custom Token Definitions for the First Two CD64 Concentrations

Now we will define the Custom Token for the 0.625µl CD64 data. Instead of defining the Custom Token from the beginning, we will copy and paste the definition of the **0.313 ratio** Custom Token.

56. Left-click on the "+" symbol on the top border of the **Custom Tokens** window.
57. Type '0.625 ratio' in the **New Custom Token** text.
58. Hit Enter; **0.625 ratio** will be highlighted in blue.
59. Select **0.313 ratio** in the **Custom Tokens** upper window.
60. Place the cursor in the lower half of the **Custom Tokens** window before the '='.
61. Press and hold the left mouse button and select the text definition of the **0.313 ratio** Custom Token.
62. Copy the text using **Ctrl+C** on the keyboard.
63. Select the **0.625 ratio** in the **Custom Tokens** upper window.
64. Place the cursor in the lower half of **the Custom Tokens** window.

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65. Paste the text using **Ctrl+V** on the keyboard.

66. Place the cursor over the text defining the numerator '**Statistic: Y Median for Plot #5, overlay #1, Additional Gate: Monos**'.

67. Double-click to bring up the **Edit Statistic** dialog, **Data Source** category.

68. Select the **Color Dot - 0.625ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**.

69. Select **OK**.

70. Place the cursor over the text defining the denominator '**Statistic: Y Median for Plot #5, overlay #1, Additional Gate: Lymphs**'.

71. Double-click to bring up the **Edit Statistic** dialog, **Data Source** category, similar to the **Create Statistic** dialog previously shown in Figure T17.9.

72. Select the **Color Dot - 0.625ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**.

73. Select **OK**.

The **0.625 ratio** Custom Token has now been defined to use the 0.625µl CD64 data. We will now use copy and paste in the pop-up menu to define the 1.25 ratio token using the definition of the **0.625 ratio** Custom Token.

74. Place the cursor over the **0.625 ratio** text in the **Custom Tokens** window.

75. Right-click to bring up the pop-up menu.

76. Select **Copy Custom Token(s)** from the pop-up menu (Figure T17.16).

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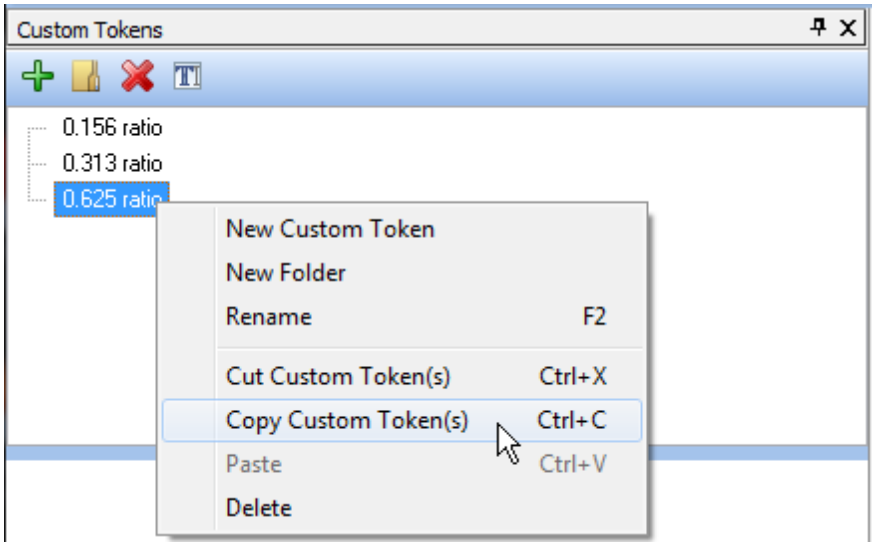

Figure T17.16 Copy Custom Token(s) Item in the Pop-up Menu

77. Place the cursor under the **0.625 ratio** text in the **Custom Tokens** window.

78. Right-click to bring up the pop-up menu.

79. Select **Paste Custom Token(s)** from the pop-up menu (Figure T17.17).

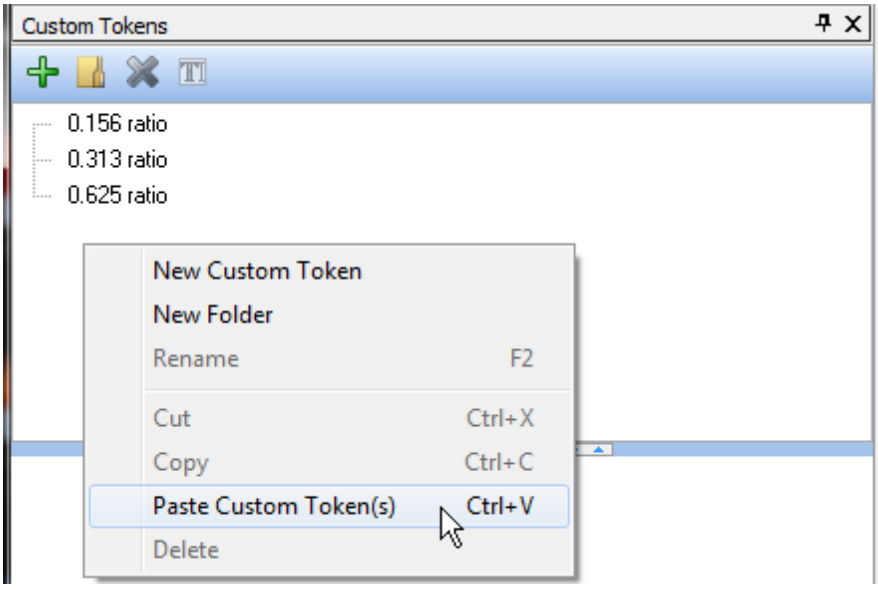

Figure T17.17 Paste Custom Token(s) Item in the Pop-up Menu

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The 0.625 ratio custom token now appears twice. We now need to edit both the name of this custom token and the definition to use the 1.25µl CD64 plot data.

80. Place the cursor over the text **0.625 ratio** in the **Custom Tokens** window, shown highlighted in Figure T17.18.

81. Right-click to bring up the pop-menu.

82. Select **Rename** from the pop-up menu.

83. Type '1.25 ratio'.

84. Place the cursor in the lower half of the **Custom Tokens** window over the token defining the numerator '**Statistic: Y Median for Plot #11, overlay #1, Additional Gate: Monos**', indicated by the cursor in Figure T17.18.

85. Double-click to bring up the **Edit Statistic** dialog, **Data Source** category.

86. Select the **Color Dot - 1.25ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**.

87. Select **OK**.

88. Place the cursor over the token defining the denominator '**Statistic: Y Median for Plot #11, overlay #1, Additional Gate: Lymphs**'.

89. Double-click to bring up the **Edit Statistic** dialog, **Data Source** category, similar to the **Create Statistic** dialog previously shown in Figure T17.9.

90. Select the **Color Dot - 1.25ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**.

91. Select **OK**.

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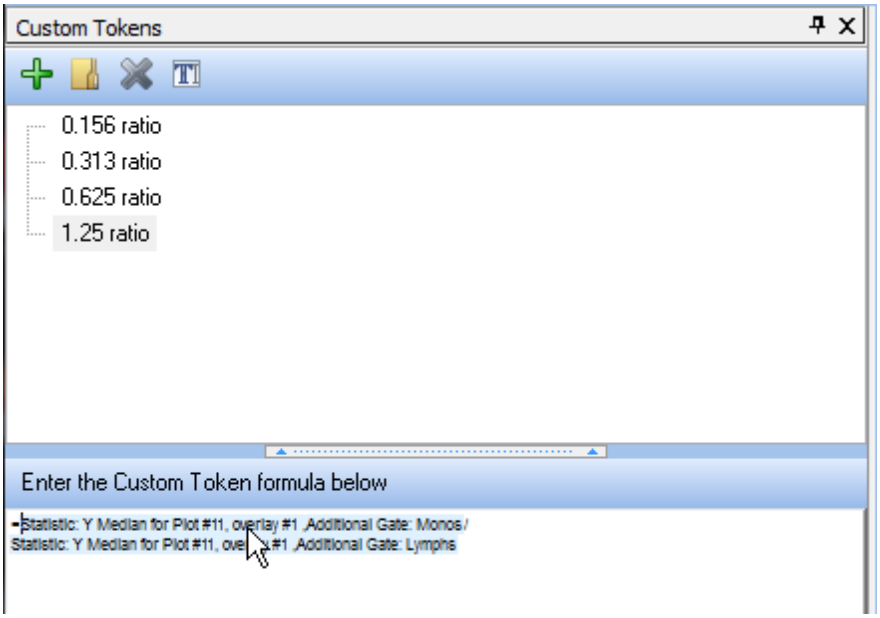

Figure T17.18 Editing Custom Token 1.25 Ratio

Continue to define the Custom Token ratios for the 2.5µl, 5µl, and 10µl CD64 plots using the previous copy and paste instructions. Repeat instructions 75 through 92 substituting 2.5µl for the 1.25µl data, and then the 5µl and 10µl data, until all the Custom Tokens have been defined. The final Custom Token window should look similar to Figure T17.19.

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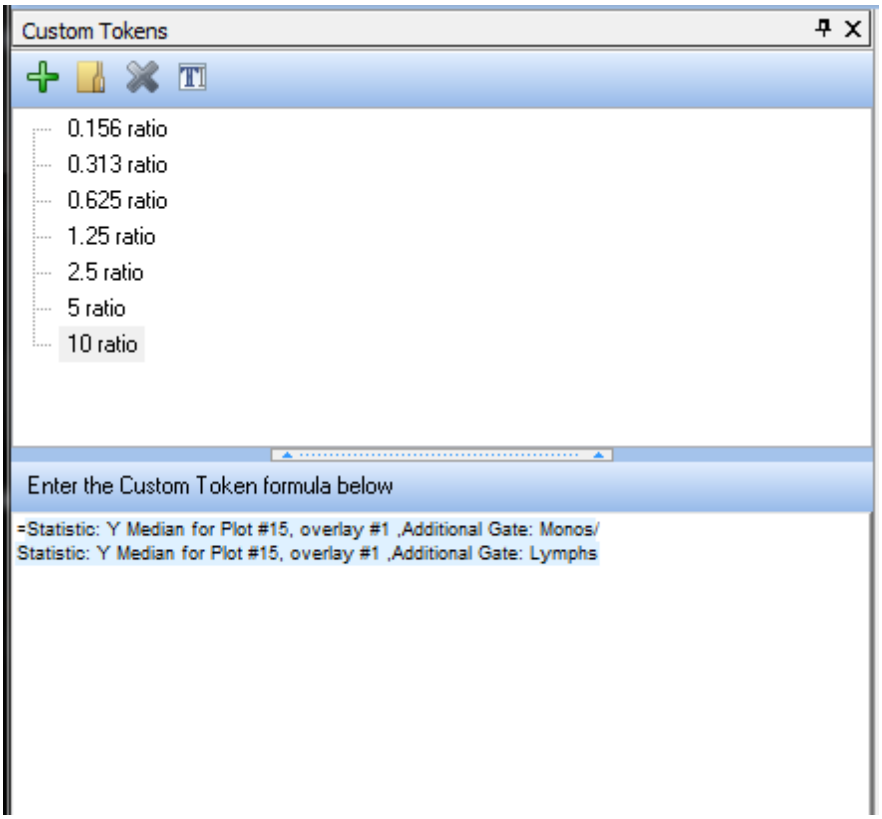

Figure T17.19 Final Custom Token Definitions

In the next section, we will create a custom data table 1006.

34.17.2 Create Custom Data Table

We will now use the Custom Tokens of the titration ratios to create a custom data table of the CD64 concentration (volume) versus the CD64 staining of the Monocyte/Lymphocyte population. A custom data table is similar to a spreadsheet, where you can create and edit cells. We will begin by inserting a new blank page on the layout.

1. Select the **Insert→General→New Page** command (Figure T17.20).

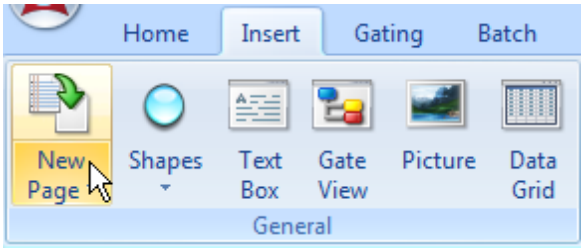

Figure T17.20 Insert New Page

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A blank new page will appear. We will now change the view so that we can view both pages at the same time.

2. Select the **View→Multiple Pages→Two Pages (Vertical)** command (Figure T17.21).

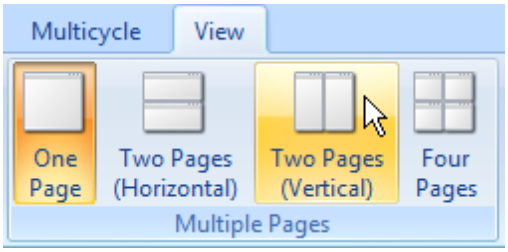

Figure T17.21 View Two Pages

Both layout pages now appear as shown in Figure T17.22 with Page 2 to the left of Page 1.

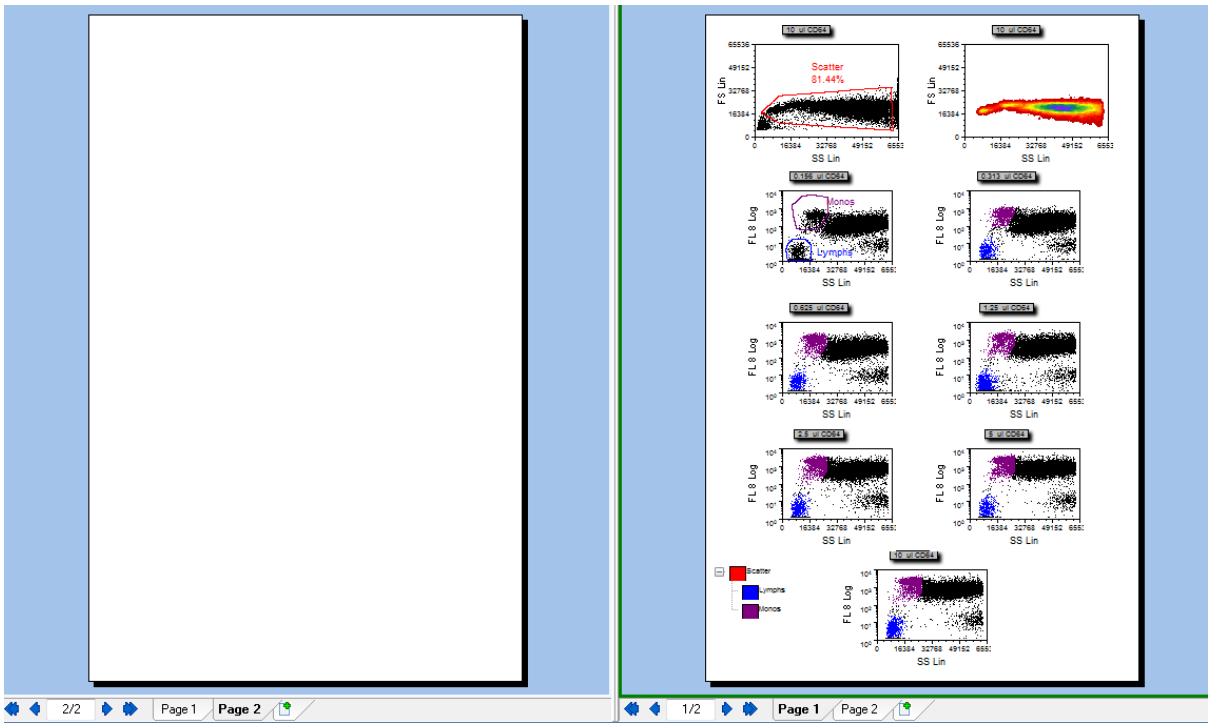

Figure T17.22 Both Layout Pages

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We will now insert a custom data table onto Page 2 of the layout.

3. Select the **Data→Save/Load→Custom Data→New Custom Data** command (Figure T17.23).

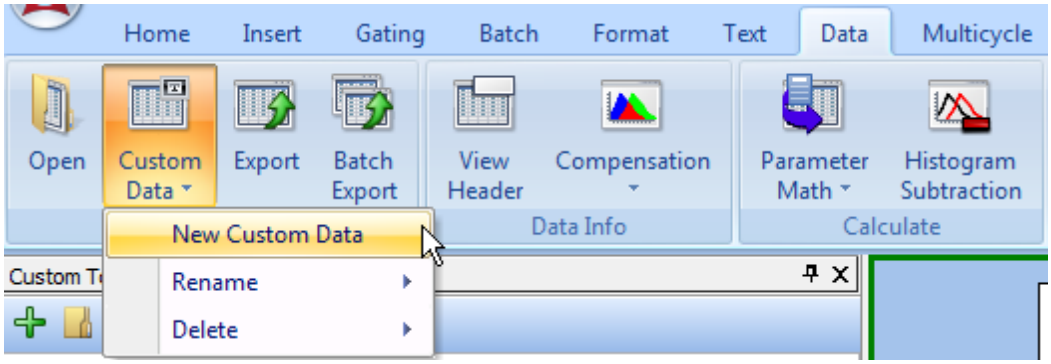

Figure T17.23 Selecting the New Custom Data Command

The **Create new custom data** dialog appears, similar to Figure T17.24.

4. Type 'Titration CD64' in the title window.
5. Change the **Columns** value to '2'.
6. Change the **Rows** value to '7'.
7. Select the **Open Data Grid** checkbox.
8. Click **OK**.

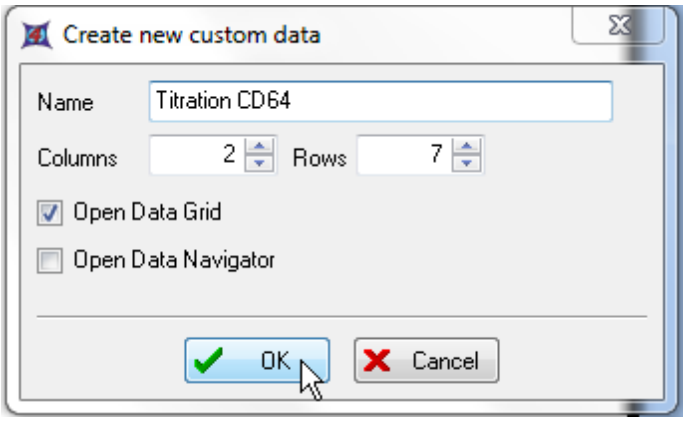

Figure T17.24 Create New Custom Data Dialog

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A custom data table appears on Page 2 of the layout. We will now format the table to put in appropriate column names.

9. Click on the column labeled 'A', shown highlighted in blue in Figure T17.25.
10. Right-click to bring up the pop-up menu.
11. Select **Rename Selected Column** from the pop-up menu.

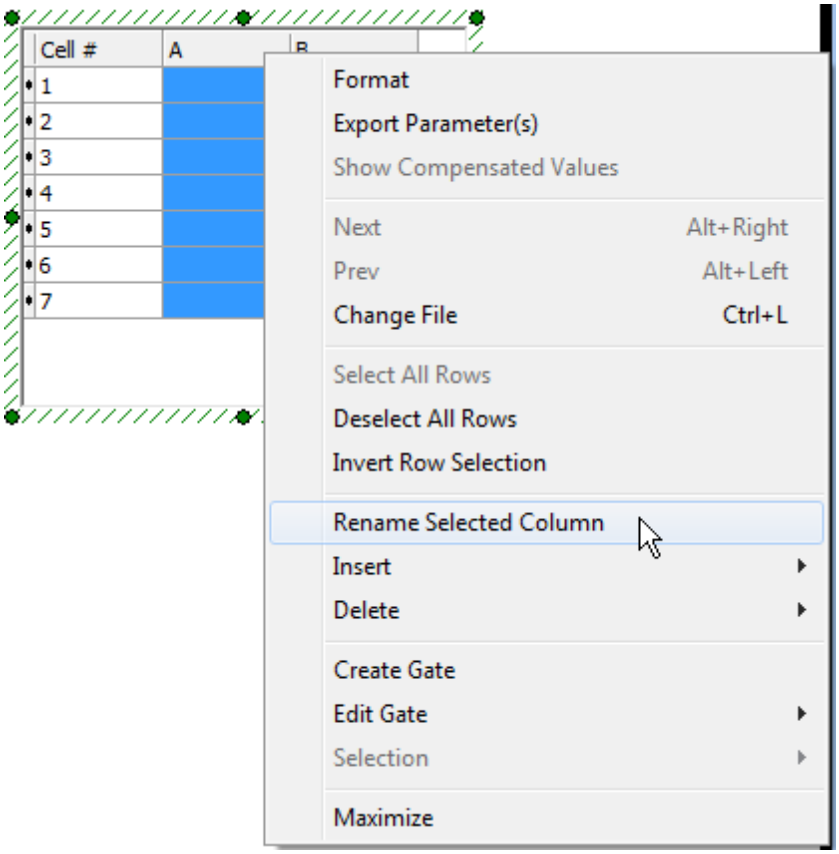

Figure T17.25 Rename Selected Column Menu Item

The **New Column Name** dialog appears, similar to Figure T17.26.

12. Type 'volume [ul]' in the **Enter the new name for column A** window.
13. Click **OK**.

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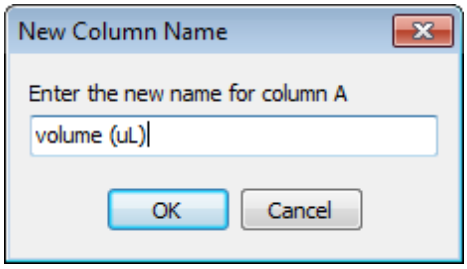

Figure T17.26 New Column Name Dialog

The custom data table updates to reflect the new column name. We will now repeat steps 9 – 11 for column B.

14. Click on the column labeled 'B'.
15. Right-click to bring up the pop-up menu.
16. Select **Rename Selected Column**.
17. Type 'M/L ratio' in the **Enter the new name for column B** window of the **New Column Name** dialog. This is the Monos/Lymphs ratio we defined in the custom tokens.
18. Click **OK**.

The columns of the custom data table are now appropriately labeled as shown in Figure T17.27.

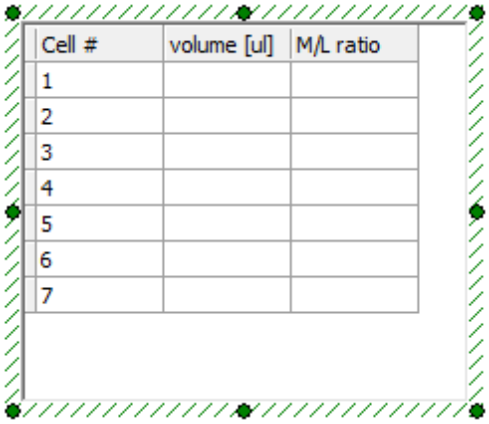

Figure T17.27 Custom Data Table with Column Names

We will now fill the custom data table with the appropriate values, by dragging

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and dropping the appropriate plot onto the custom data table. The **volume** column of the custom data table will be filled using the keyword *conc*, found in the data file, which refers to the volume of CD64 used. We could directly type the volume into the custom data table. However, using the keyword token *conc* provides more flexibility for analysis, yielding a generic template that can be used for future titration experiments. If you have another titration experiment with seven concentrations, which uses the monocyte:lymphocyte staining ratio and a *conc* keyword, you will be able to load the data files into this layout and directly use the custom token and custom table definitions.

19. Place the cursor over the plot of **0.156 ul CD64,** shown with red border in Figure T17.28.

20. Press and hold the left mouse button and drag the plot onto the custom data table.

21. Release the mouse when the cursor is on the top cell of the **volume [ul]** column, indicated by the cursor in Figure T17.28.

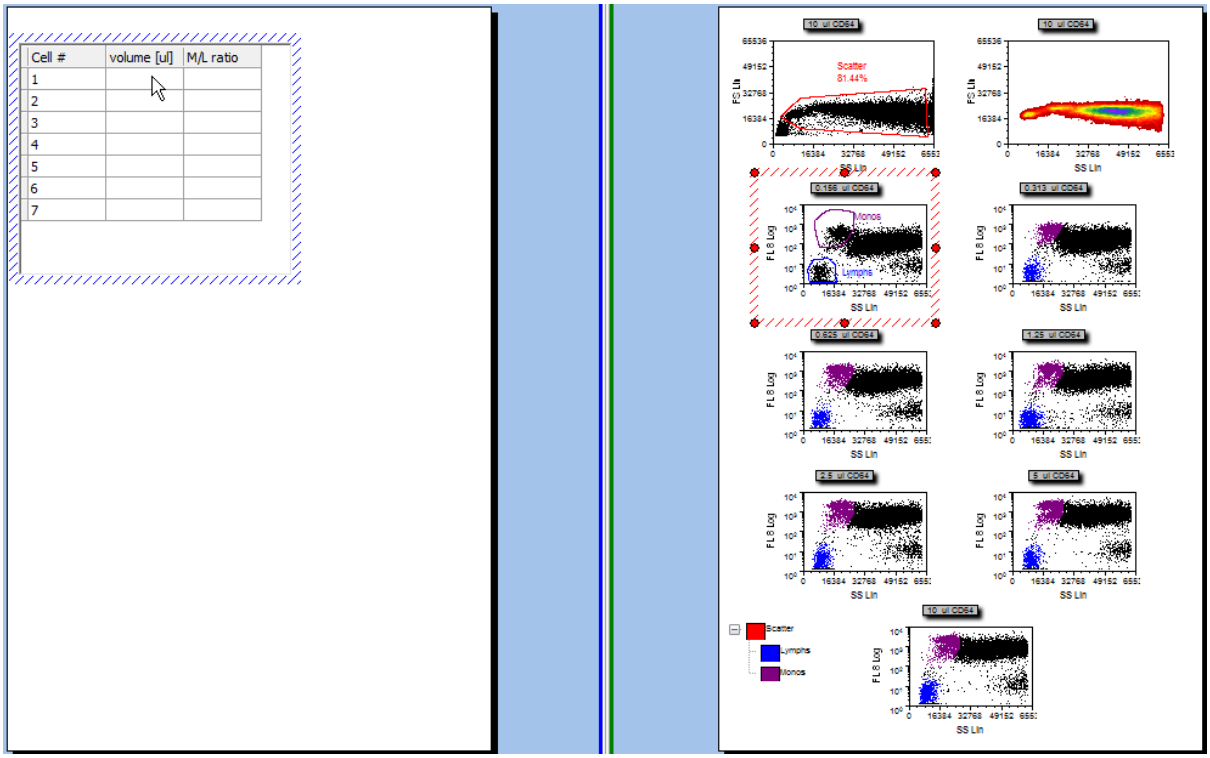

Figure T17.28 Dragging and Dropping the 0.156 ul CD64 Plot onto the Custom Data Table

The **Paste Special** dialog appears, shown in Figure T17.29. All the data files

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have a keyword 'conc' that specifies the volume of CD64 used for staining.

22. Select **keyword token**, shown highlighted in Figure T17.29.

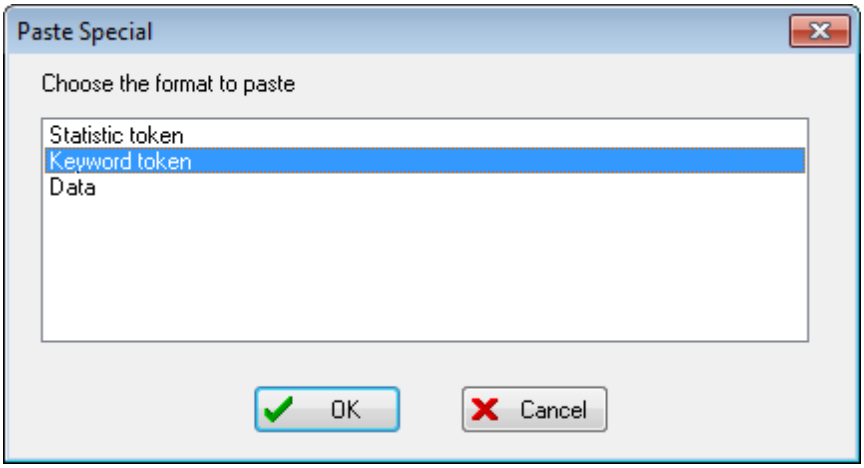

Figure T17.29 Keyword Token in the Paste Special Dialog

The **Edit Keyword** dialog appears, similar to Figure T17.30.

23. Type 'conc' in the **Select a keyword:** field, indicated by the cursor in Figure T17.30.

24. Click **OK**.

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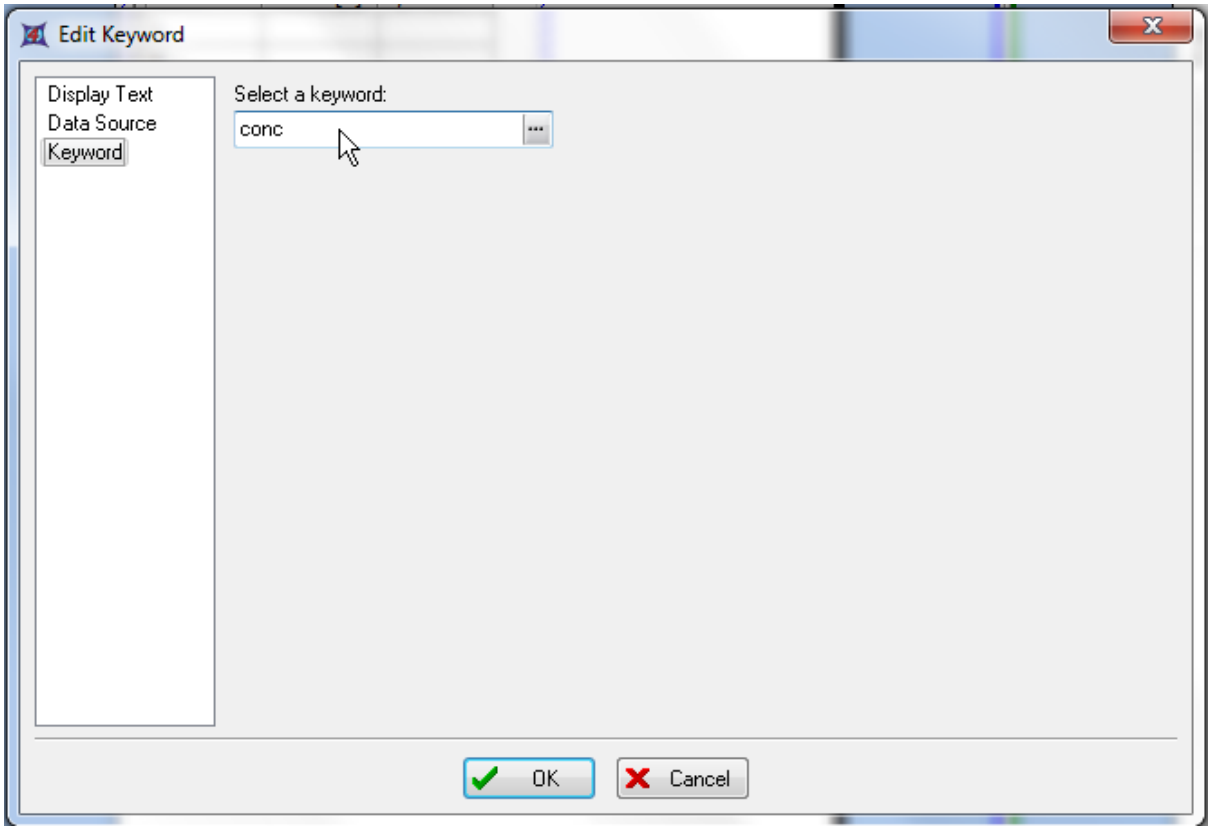

Figure T17.30 Edit Keyword Dialog

The first cell of the **volume** column, in the custom data table, updates to reflect the entry of the keyword *conc*, which contains the value of 0.156. We will now copy the keyword token entry and paste it into the next cell in the **volume** column.

25. Click on **0.156**, shown highlighted in blue in Figure T17.31.

26. Select the text 'Keyword: conc for Plot #3, overlay #1', shown highlighted in gray in the cell editor (Figure T17.31). *Note:* whenever you click on a cell, the cell editor appears allowing you to edit the current contents of the cells.

27. Copy the text using **Ctrl+C** on the keyboard.

28. Click inside the next cell in the column, indicated by the cursor in Figure T17.31.

29. Place the cursor in the editor window (it should be empty).

30. Paste the text using **Ctrl+V** on the keyboard.

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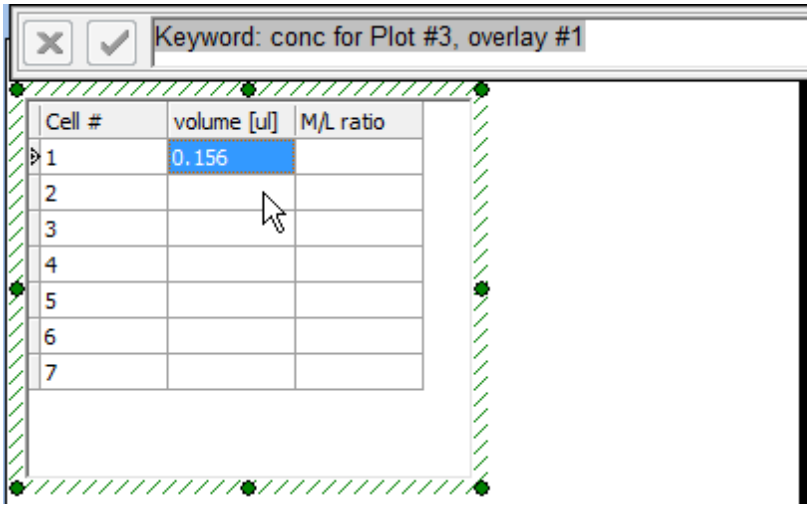

Figure T17.31 Copying and Pasting the Conc Keyword

We will now edit the keyword token to use the 0.313 µl CD64 plot.

31. Double-click on the text 'Keyword: conc for Plot #3, overlay #1'.

The **Edit Statistic** page **Data Source** category appears, similar to the **Create Statistic** dialog previously shown in Figure T17.9 986.

32. Select **Color Dot - 0.313 ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**.

33. Select **OK**.

Repeat steps 28 through 33 for the other entries of the volume column using the other five remaining data plots, 0.625 µl CD64 through 10 µl CD64. The custom data table should look similar to Figure T17.32.

Cell #	volume [ul]	M/L ratio
1	0.156	
2	0.313	
3	0.625	
4	1.25	
5	2.5	
6	5	
7	10	

Figure T17.32 Custom Data Table with Volumn Values

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We will now enter the values for **the M/L ratio** column, which represent the Monos/Lymphs ratio, by dragging and dropping the custom ratio tokens we previously defined onto the **M/L ratio** column of the custom data table.

34. Place the cursor on the **0.156 ratio** in the **Custom Tokens** window, highlighted in blue in Figure T17.33.

35. Press and hold the left mouse button while dragging the **0.156 ratio** over to the custom data table.

36. Release the button when the cursor is over the top cell of the **M/L ratio** column, indicated by the cursor in Figure T17.33.

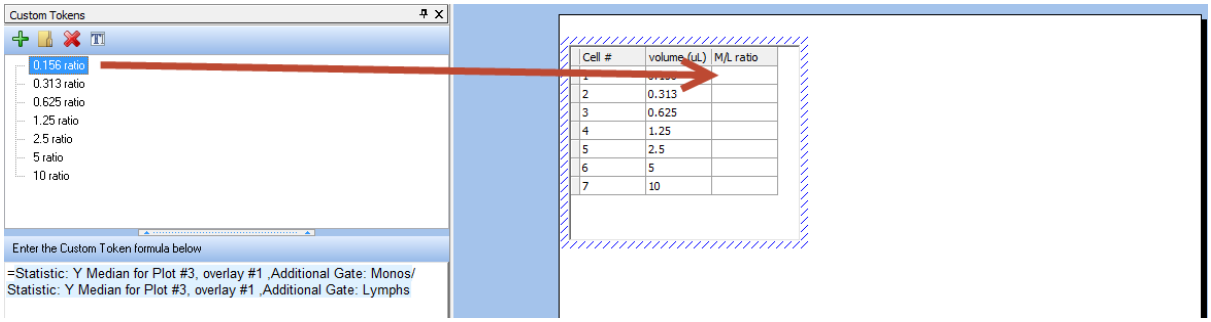

Figure T17.33 Dragging and Dropping the Custom Token onto the Custom Data Table

37. When the mouse is released, a Paste Special dialog box will appear, as shown in Figure T17.34.

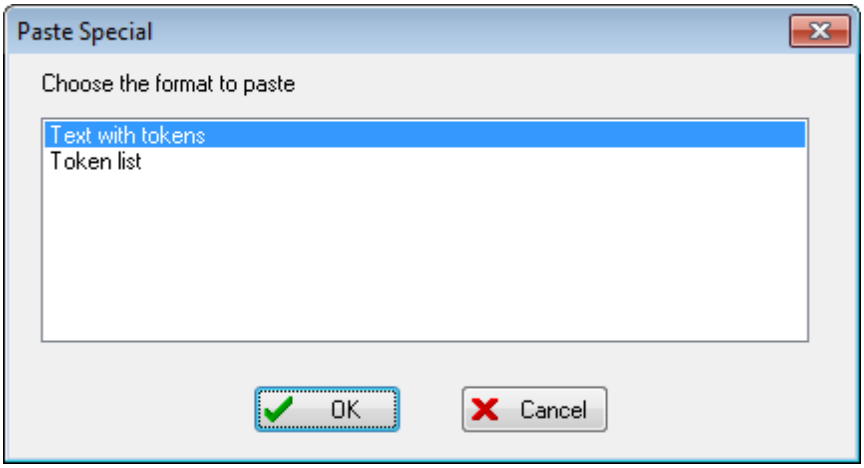

Figure T17.34 Paste Special box - Text with Tokens

The custom data table is now updated to show the ratio of Monocytes to Lymphocytes for the 0.156µl concentration of CD64, as shown in Figure

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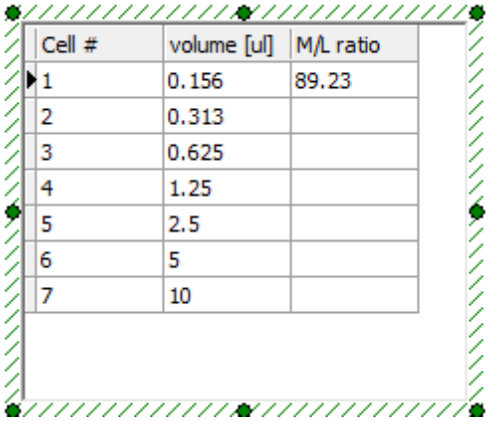
T17.35.



Cell #	volume [ul]	M/L ratio
1	0.156	89.23
2	0.313	
3	0.625	
4	1.25	
5	2.5	
6	5	
7	10	

Figure T17.35 Custom Data Table Updated with 0.156µl Data

Repeat steps 34 through 36 for each of the custom token ratios, 0.313 through 10, to fill in the **M/L ratio** column on the custom data table. The custom data table should now look similar to Figure T17.36.

Cell #	volume [ul]	M/L ratio
1	0.156	90.39
2	0.313	128.74
3	0.625	245.42
4	1.25	260.02
5	2.5	364.16
6	5	394.73
7	10	325.67

Figure T17.36 Final Custom Data Table

In examining the custom data table, one can see that the largest Monos/Lymphs ratio (394.73) is obtained using 5µl of CD64. In the next section, we will insert a scatter plot of this data so that we can better display the titration curve.

34.17.3 Insert a Scatter Plot

1. Select the custom data table, it should now have a green border.
2. Select the **Insert→2D Plots→Scatter** command (Figure T17.37).

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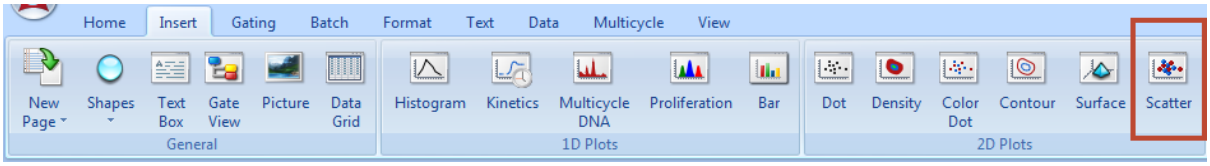

Figure T17.37 Insert Scatter Plot Command

3. Click on a blank area of Page 2 of the layout.

A 2D scatter plot using the titration data in the custom data table appears, shown in Figure T17.38.

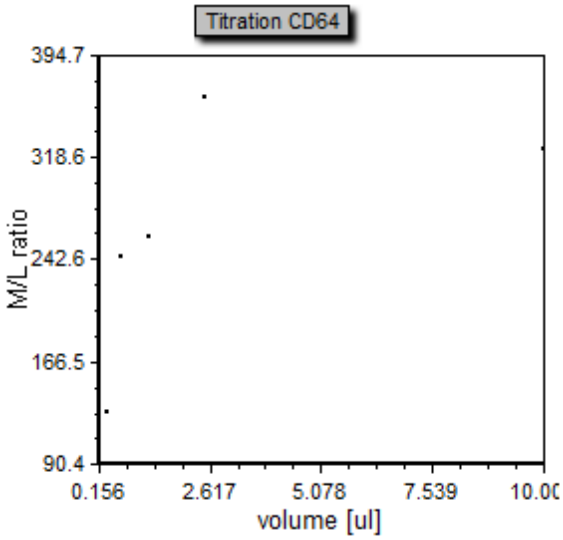

Figure T17.38 Scatter Plot of Titration Data

However, because the data points in the scatter plot of the titration data are so small, it is hard to properly visualize the data. We will now format the plot to increase the size of the data points and connect them. We will also change the scale of the x and y-axes to better see the maximum data points.

4. Right-click inside the scatter plot to bring up the pop-up menu.

5. Select **Format** from the pop-up menu.

The **Formatting** dialog for the scatter plot appears. Refer to Figure T17.39 for the following steps.

6. Select the **Overlays** category, shown highlighted.

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7. Change the point size to '3' (A).
8. Click **Connect the points** (B).
9. Select the **Axis** (C) category.

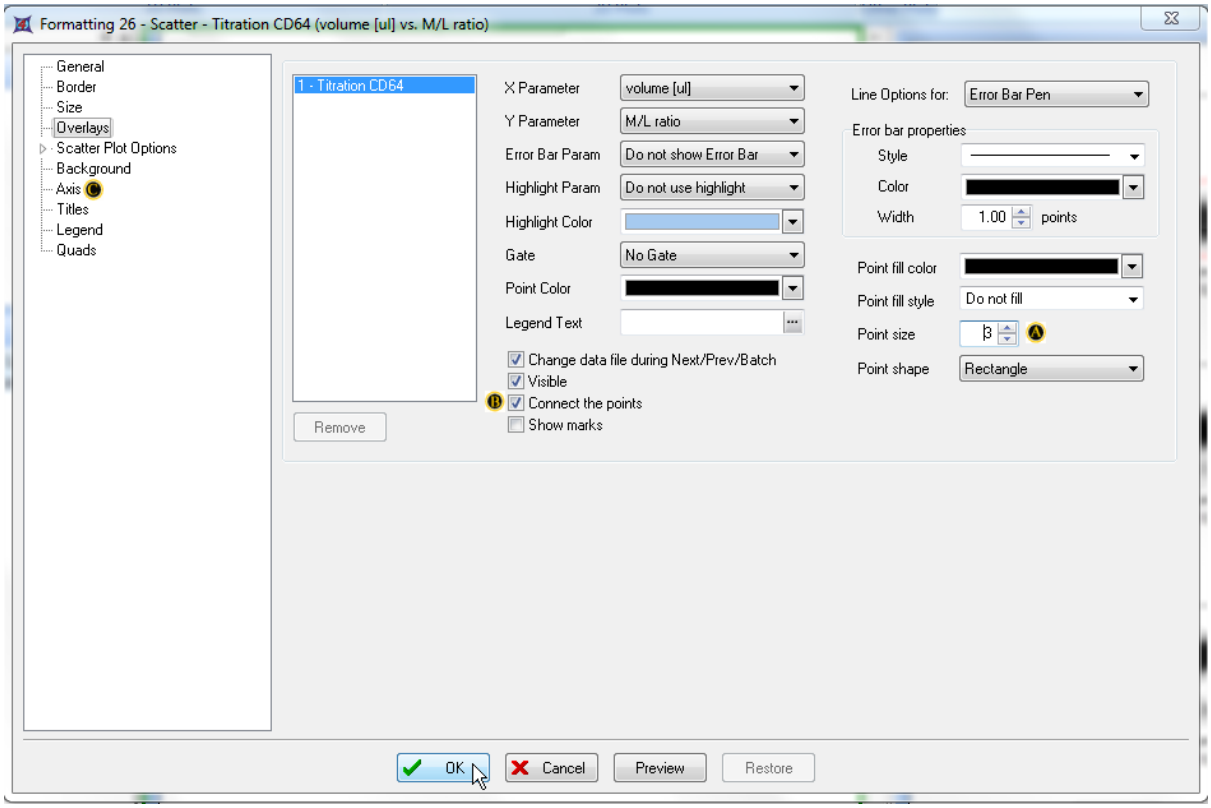

Figure T17.39 Formatting Scatter Plot Options

The **Axis** page of the **Formatting** scatter plot dialog appears. Please refer to Figures T17.39A and T17.39B for the following steps.

10. Select **Y axis**, shown highlighted in blue in Figure T17.40A.
11. Deselect the **Automatic Range** box, indicated by the cursor.
12. Enter '**500**' in the **Maximum Range** window, shown highlighted in blue.
13. Select **X axis**, shown highlighted in blue in Figure T17.40B.
14. Deselect the **Automatic Range** box, indicated by the cursor.
15. Enter '**12**' in the **Maximum Range** window, shown highlighted in blue.

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16. Click **OK**.

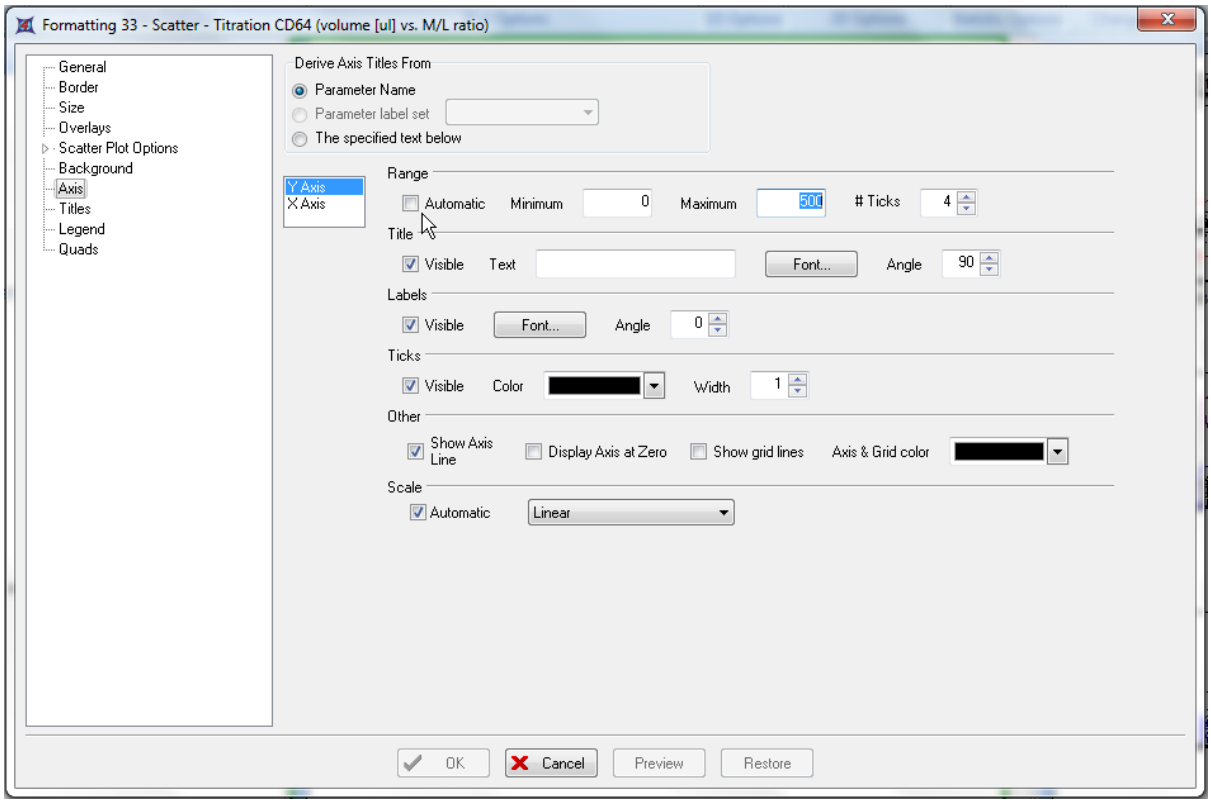

Figure T17.40A Formatting the Y-axis of the Scatter Plot

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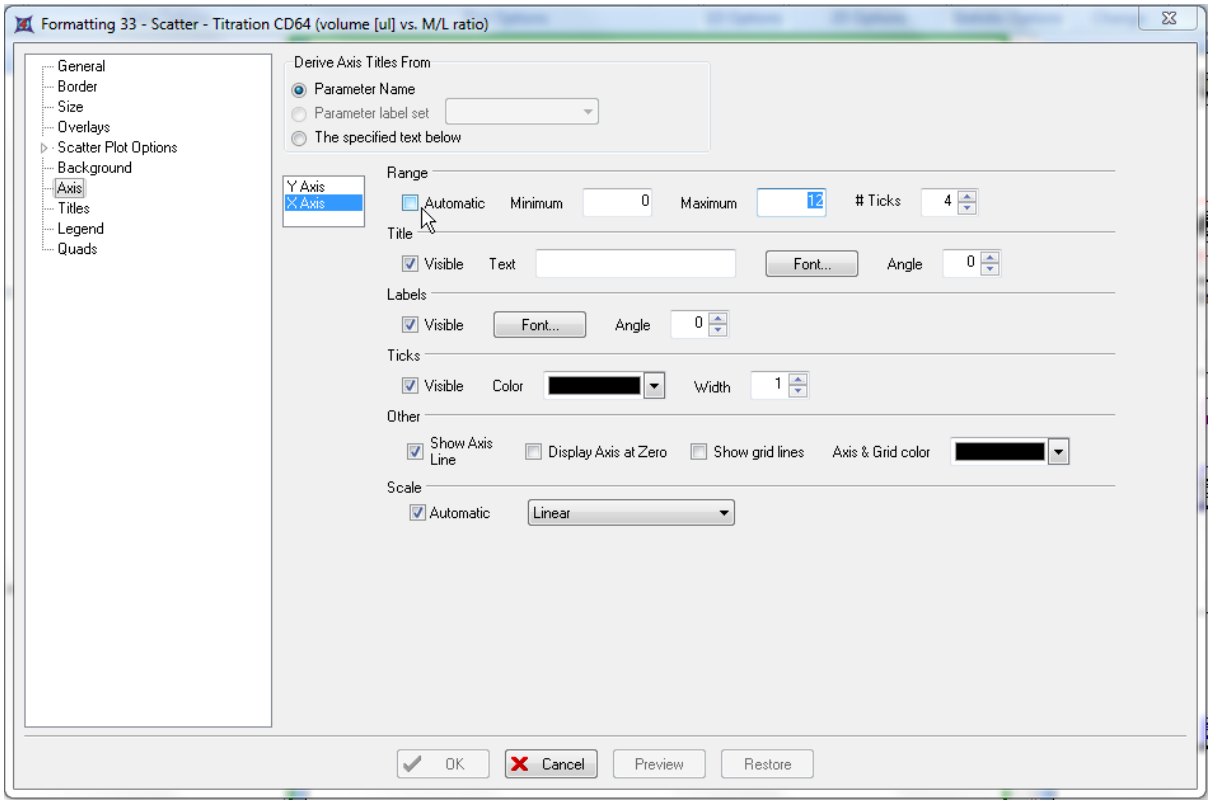

Figure T17.40B Formatting the X-axis of the Scatter Plot

The titration scatter plot now appears with larger connected data points and re-scaled axes as shown in Figure T17.40.

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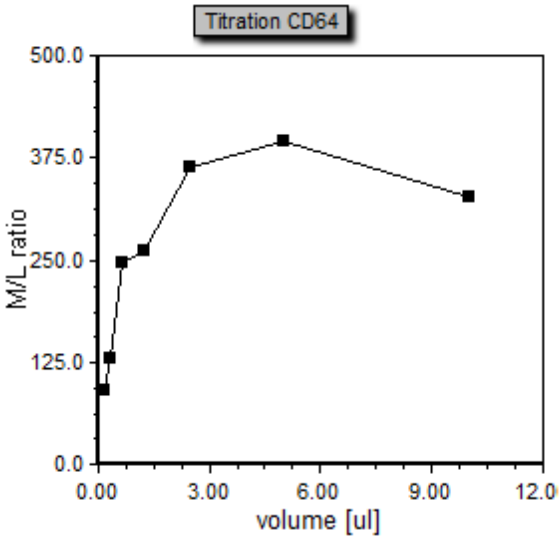

Figure T17.41 Formatted Titration Scatter Plot

We will now add more data to the titration plot to reflect the Coefficient of Variance (CV) of the Monocyte population. We will enter this data as an error bar on the scatter plot. To begin, we will add a new column, for the monocyte CV data, to the titration custom data table.

17. Right-click inside the **M/L ratio** column of the titration custom data table to bring up the pop-up menu.

18. Select **Insert→Column to the Right** from the pop-up menu, highlighted in blue and indicated by the cursor in Figure T17.42.

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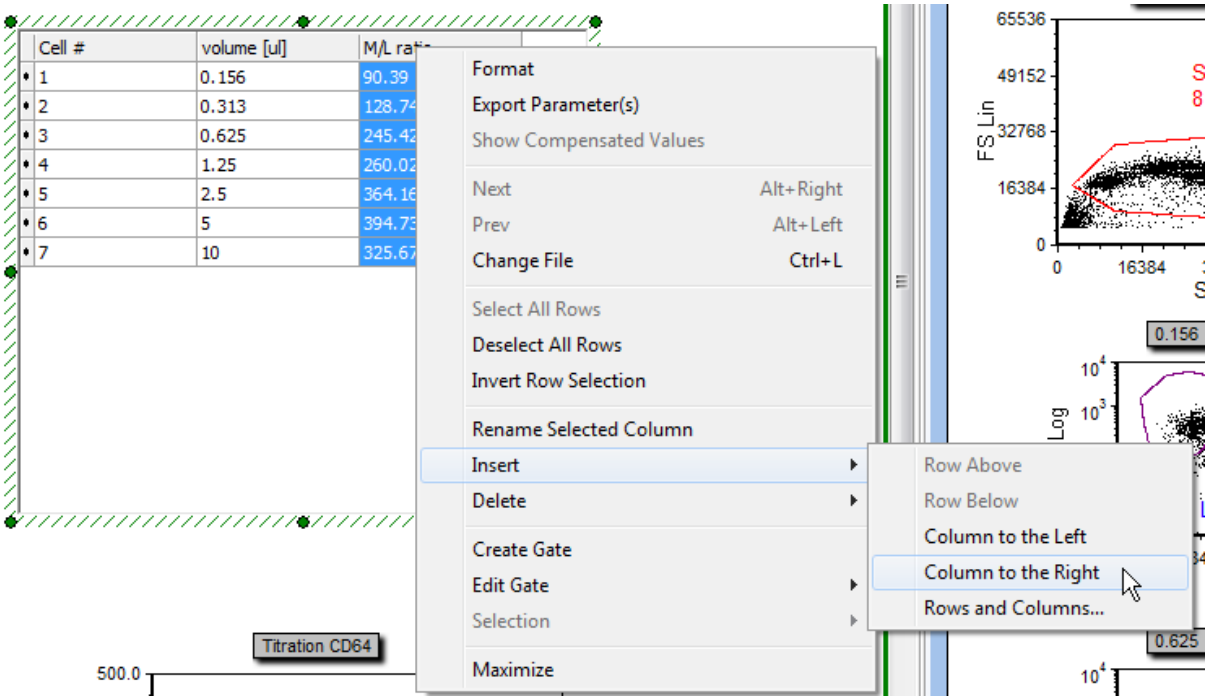

Figure T17.42 Inserting a New Column in the Custom Data Table

The **New Colum Name** dialog appears.

19. Enter '**Monos CV**' in the new column name field.

20. Click **OK**.

A new blank column appears to the right of the **M/L ratio** column labeled **Monos CV**. We will now insert the token representing the CV of the CD64 fluorescence of the monocyte population into the new column. Please refer to Figure T17.43 for the following steps.

21. Click inside the top cell of the **Monos CV** column, shown highlighted in blue.

22. Click on the token icon, indicated by the cursor and highlighted in blue.

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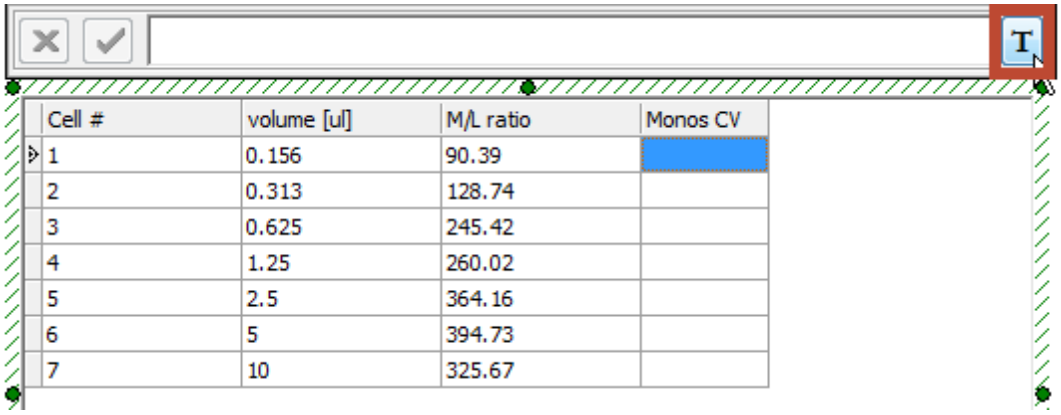

Cell #	volume [ul]	M/L ratio	Monos CV
1	0.156	90.39	
2	0.313	128.74	
3	0.625	245.42	
4	1.25	260.02	
5	2.5	364.16	
6	5	394.73	
7	10	325.67	

Figure T17.43 Inserting Token Data into a Cell of the New Column

The **Insert a Token** dialog appears, as previously shown in Figure T17.8 986.

23. Select the **Statistic** token.
24. Click **Insert**.

The **Create Statistic** dialog appears, as previously shown in Figure T17.9 986.

25. Select **Color Dot - .156ul CD64.fcs.DNS (SS Lin vs. FL 8 Log)**, shown highlighted in blue in Figure T17.9 986.
26. Select the **Statistic** category on the left, indicated by the cursor in Figure T17.9 986.

The **Statistic** page of the **Create Statistic** dialog now appears; here, we will select the Y CV of the Monos gate as the statistic used for the token. Please refer to Figure T17.44 for the following steps.

27. Select **Y CV** from the **Statistic:** list box, shown highlighted in blue.
28. Select **Monos** from the **Gate:** drop-down list, indicated by the cursor and highlighted in blue.
29. Click **OK**.

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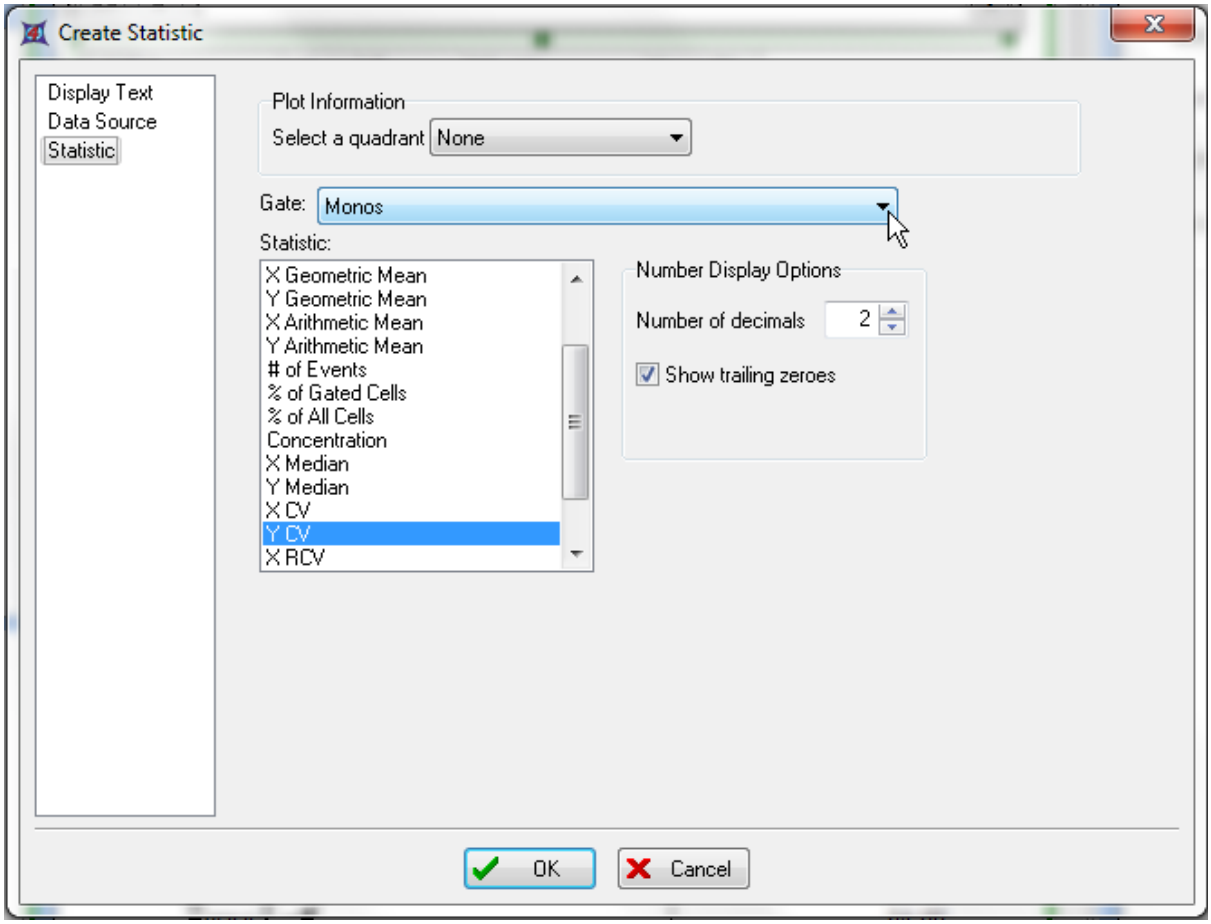
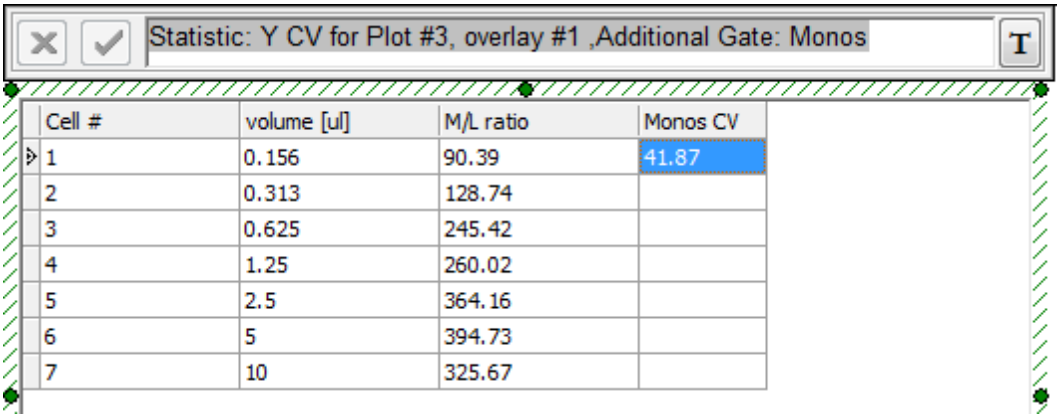


Figure T17.44 Create Statistic Dialog for Monos CV

The titration custom data table updates to reflect the insertion of the **Monos CV** statistic token for the 0.156µl data, as shown highlighted in blue in Figure T17.45.



Cell #	volume [ul]	M/L ratio	Monos CV
1	0.156	90.39	41.87
2	0.313	128.74	
3	0.625	245.42	
4	1.25	260.02	
5	2.5	364.16	
6	5	394.73	
7	10	325.67	

Figure T17.45 Monos CV Token Data

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Continue to fill in the **Monos CV** column for the other CD64 volumes, repeating steps 21 through 29. Alternatively, you can use a combination of copy and paste, as previously demonstrated, being sure to update the token to use the appropriate data plot. When the **Monos CV** column is filled in it should look similar to Figure T17.46.

Cell #	volume [ul]	M/L ratio	Monos CV
1	0.156	90.39	41.87
2	0.313	128.74	45.12
3	0.625	245.42	46.92
4	1.25	260.02	59.94
5	2.5	364.16	55.13
6	5	394.73	54.00
7	10	325.67	56.65

Figure T17.46 Titration Custom Data Table with Monos CV Column Completed

We will now use the Monos CV data as the error bar data for the titration scatter plot.

30. Click inside the titration scatter plot to select it.

31. Select the **Format→Plot Options→Overlays** command.

The Formatting Overlays dialog appears. Please refer to Figure T17.47 for the following steps.

32. Select **Monos CV** from the **Error Bar Param** drop-down list.

33. Select the color blue from the **Error bar properties Color** drop-down list.

34. Click **OK**.

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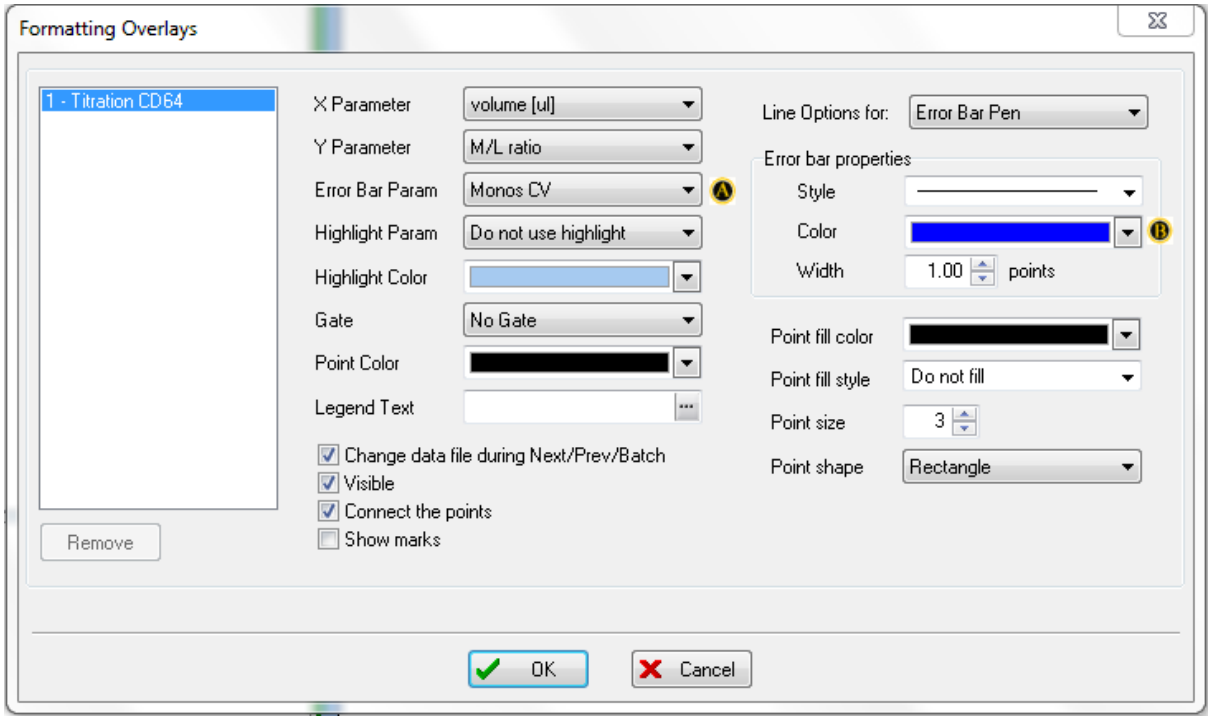

Figure T17.47 Formatting Overlays Dialog for the Titration Scatter Plot

The titration scatter plot updates to reflect the addition of blue error bars using the Monos CV data, as shown in Figure T17.48.

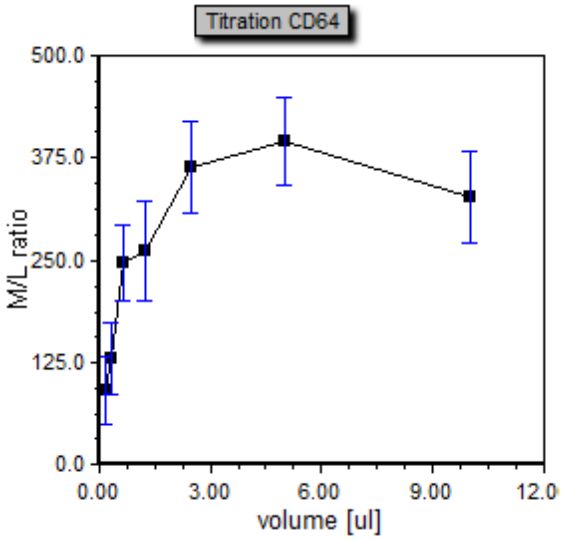

Figure T17.48 Titration Scatter Plot with Error Bars

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34.18 High Content Flow

FCS Express Version 4 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
- 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 1027.

34.18.1 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 590. This layout contains one 2D Plot

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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 590. 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.

1. Load the **HighContentFormatting.fey** layout from the Tutorial Sample Data archive 590.
2. Select the **Data→Organize Data Sets→Data List** command.
3. Click the + symbol to add data files to the data list.
4. Choose **FCS Plate folder** from the **Files of type:** drop-down list (Figure T18.1).

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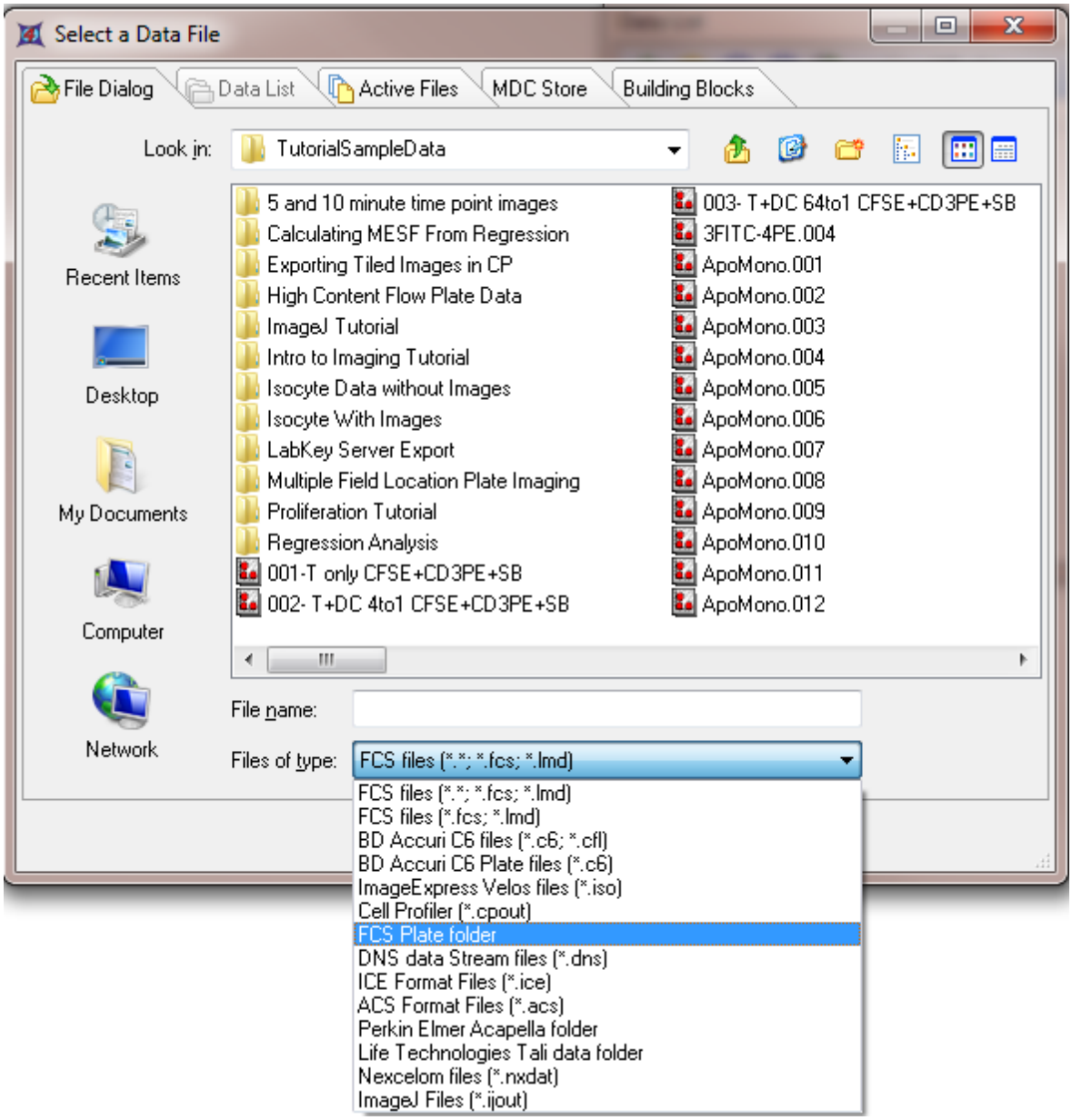

Figure T18.1 Choosing FCS Plate Folder from the Files of Type: Drop-down List

5. Navigate to the **High Content Flow Plate Data** folder in the Tutorial Sample Data archive 590 (Figure T18.2).

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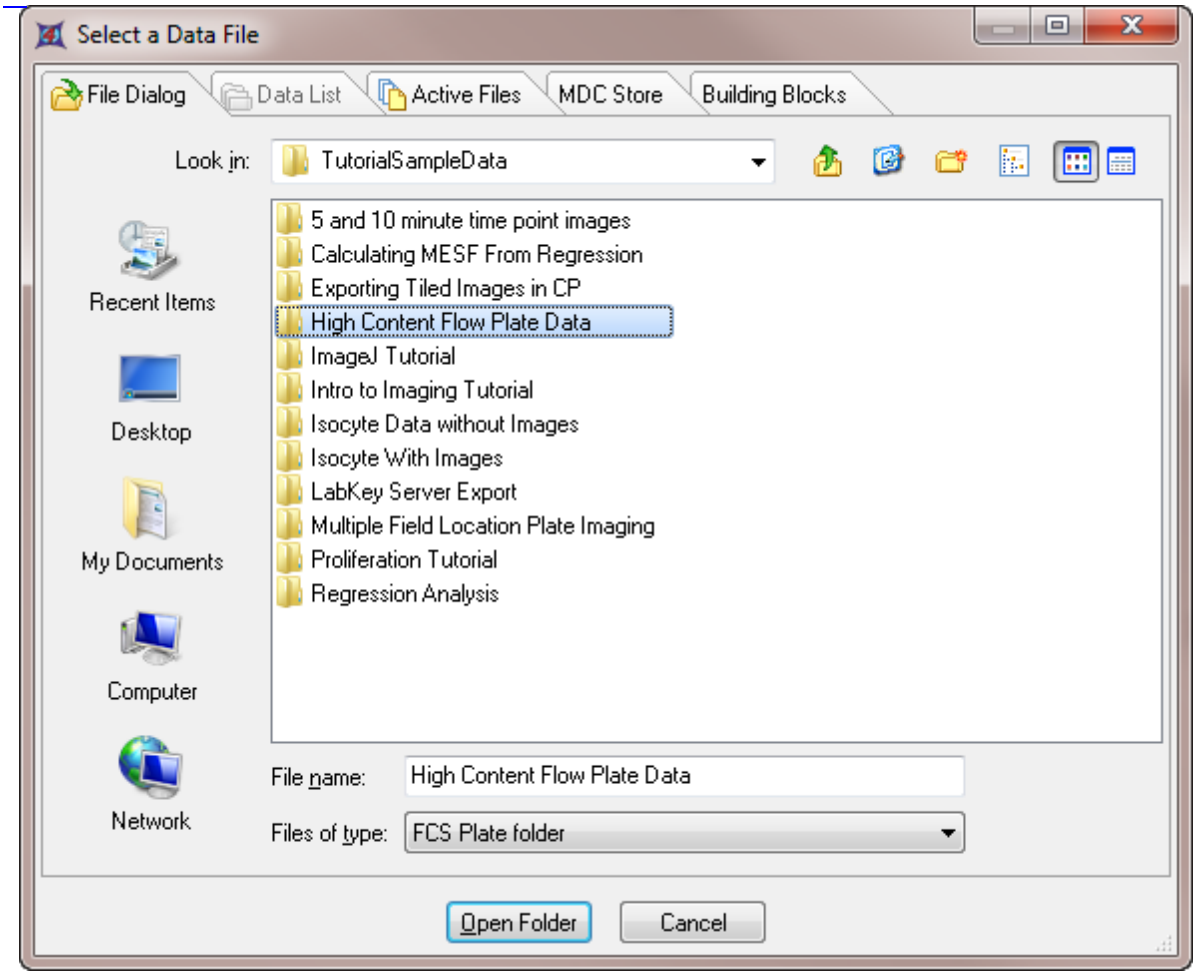

Figure T18.2 - Choosing the High Content Flow Plate Data Folder

6. Click once on the folder. Do not navigate into the folder as we want to select the entire contents.

7. Click **Open**.

The folder will now appear in the data list as a file. This 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 sample folder and is named "**plate.AllExtraKeywords.txt**". 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.

8. Choose **Insert→Other Plots→Heat Map**.

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9. Click in the place on the layout where you wish to insert the Heat Map.

10. Click on the **Data List** tab in the **Select a Data File** dialog (Figure T18.3).

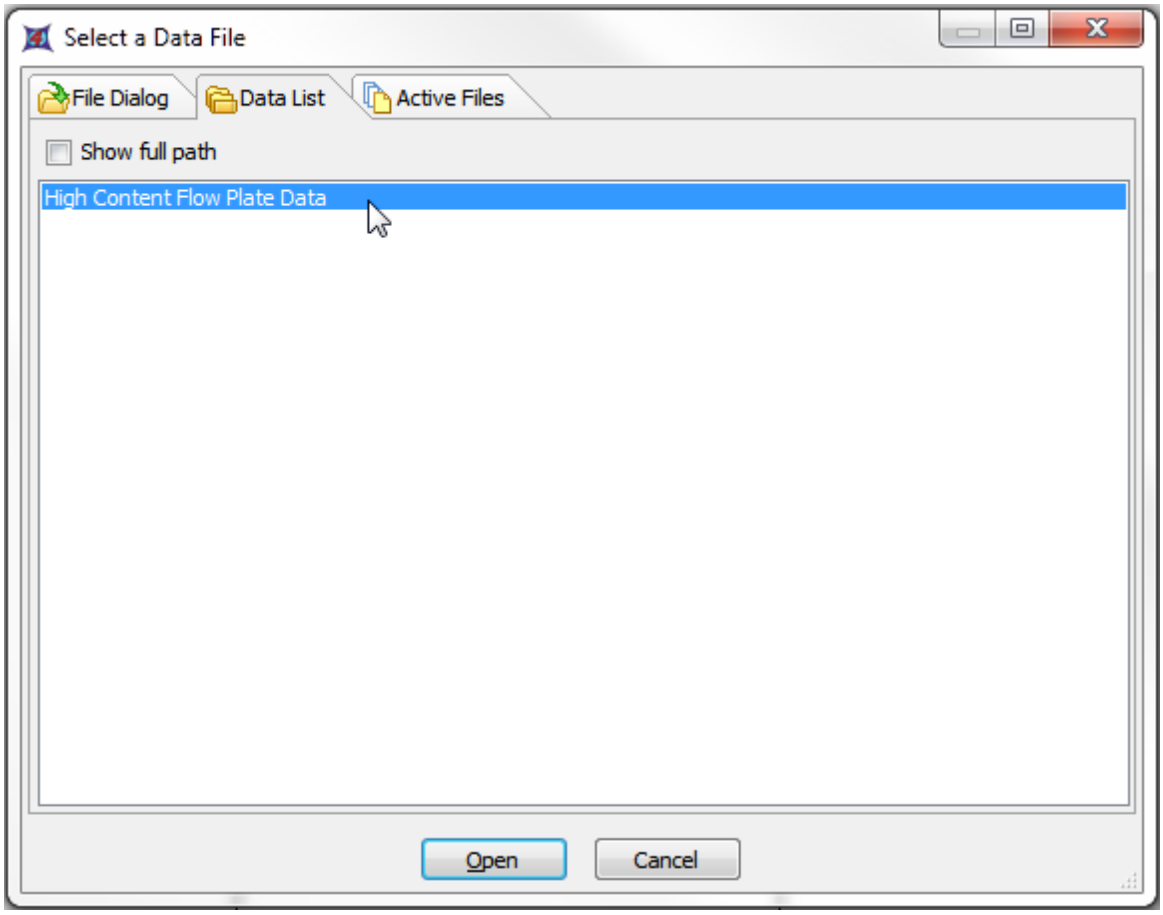

Figure T18.3 Choosing a Data File from the Data List Tab

11. Double-click on **High Content Flow Plate Data** to choose the data folder.

12. Click **OK**.

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.

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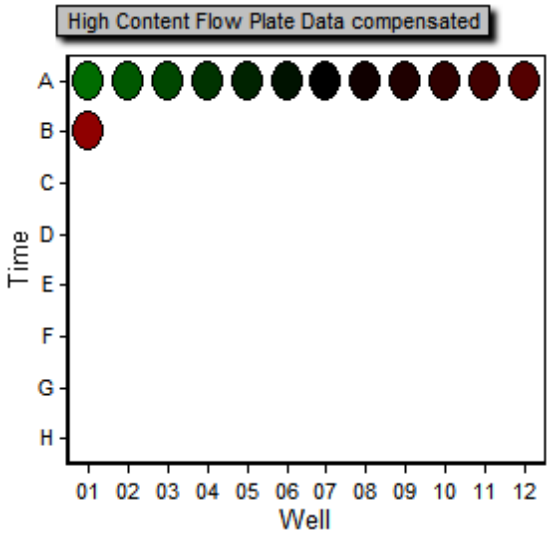

Figure T18.4 Inserted Heat Map

13. Click on the axis parameter along the row labels (Figure T18.5).

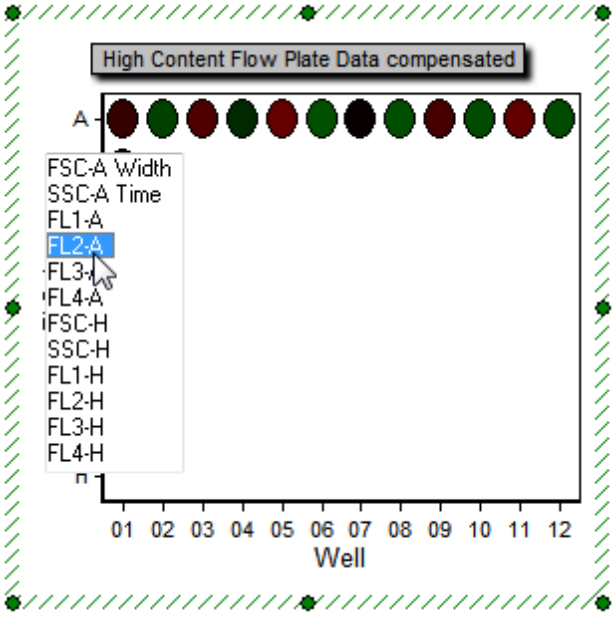

Figure T18.5 Changing the Heat Map Parameter

14. Choose **FL2-A** from the pop-up menu.

Note: The colors in the plot above now reflect the Arithmetic mean of the FL2-A

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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.

15. Select the Heat Map.
16. Choose **Home→Clipboard→Duplicate 2D Plot**. (Alternatively, use the copy and paste commands). A copy of the original heat map will appear on the layout. Arrange the plots so they look like Figure T18.6.

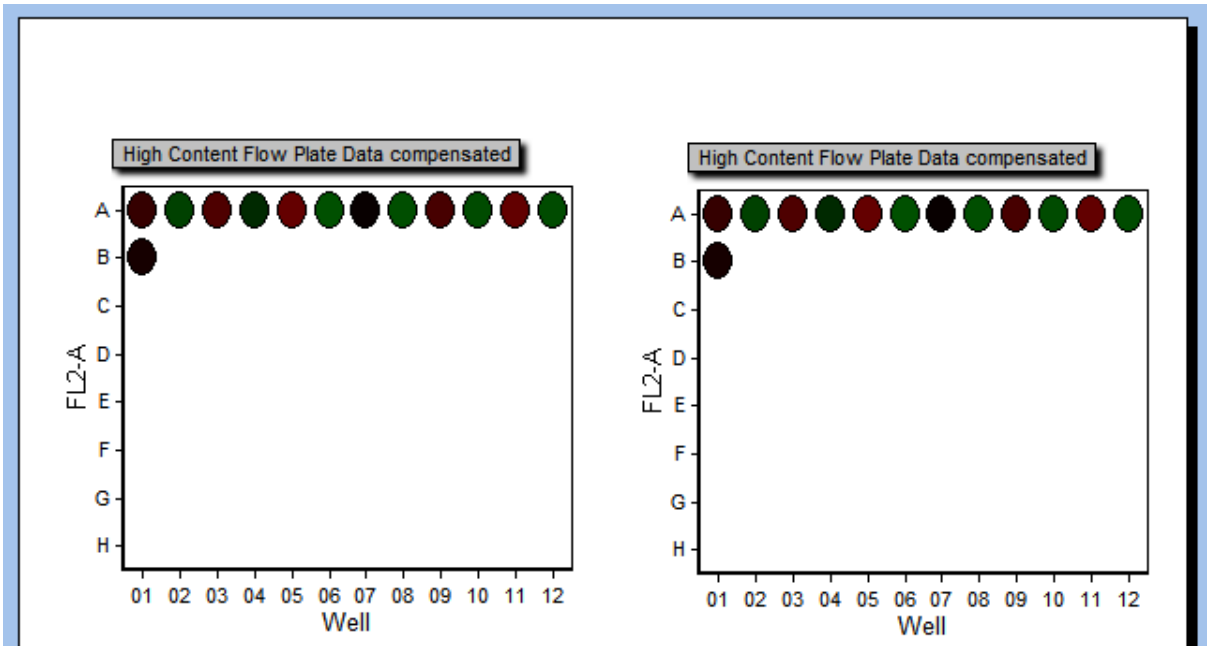

Figure T18.6 Duplicate Heat Maps

17. Right-click on the newly created Heat Map on the right side of the layout.
18. Choose **Format** from the pop-up menu.
19. Choose the **Heat Map→Heat Map Specific Options** category.
20. Change the Orientation from **Landscape** to **Portrait** (Figure T18.7).

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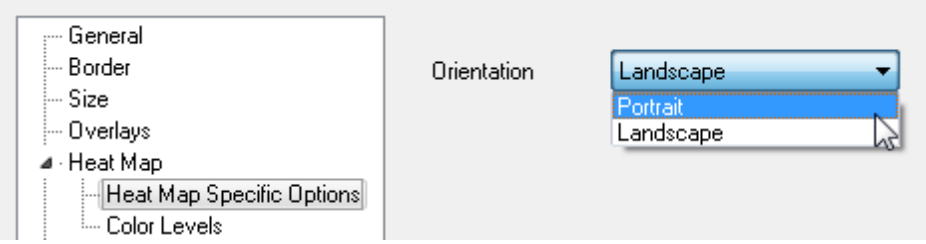

Figure T18.7 Changing Orientation from Landscape to Portrait

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.

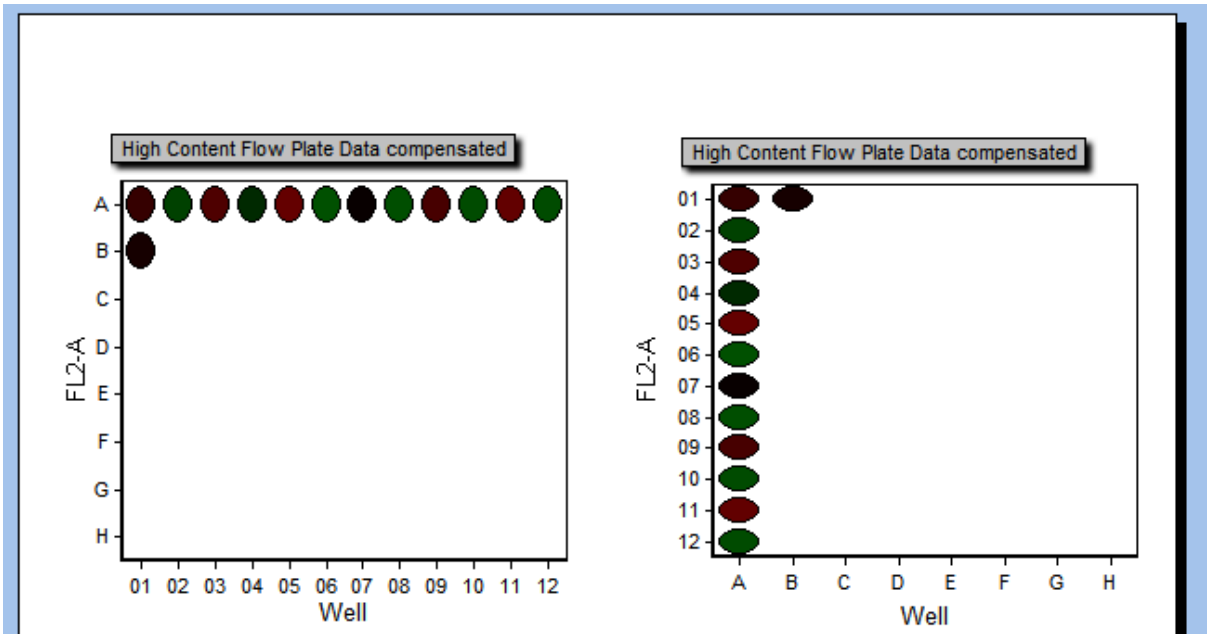

Figure T18.8 Heat Maps Displayed in Landscape and Portrait Modes

We will now change the statistic that is being represented by colors in the heat map.

21. Hit **Ctrl+Z** to undo the orientation change.
22. Right-click on the left plot.
23. Choose **Format** from the pop-up menu.
24. Choose the **Overlays** category.

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The heat map is currently displaying the Arithmetic Mean of all the events in the well as indicated in the Statistic to show field.

25. Change the **Statistic to show** to **Median** from the drop-down list (Figure T18.9).

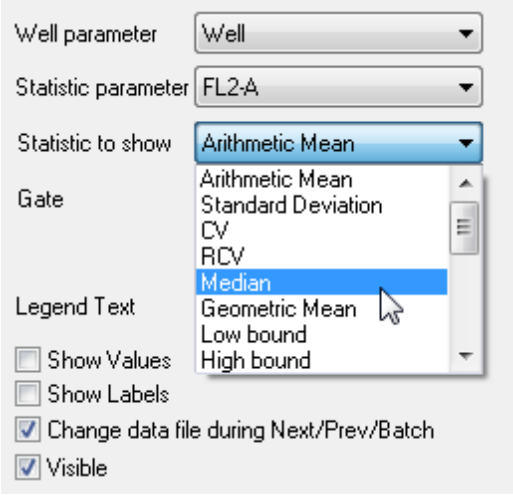

Figure T18.9 Changing the Statistic to Show in the Heat Map

26. Click **OK**.

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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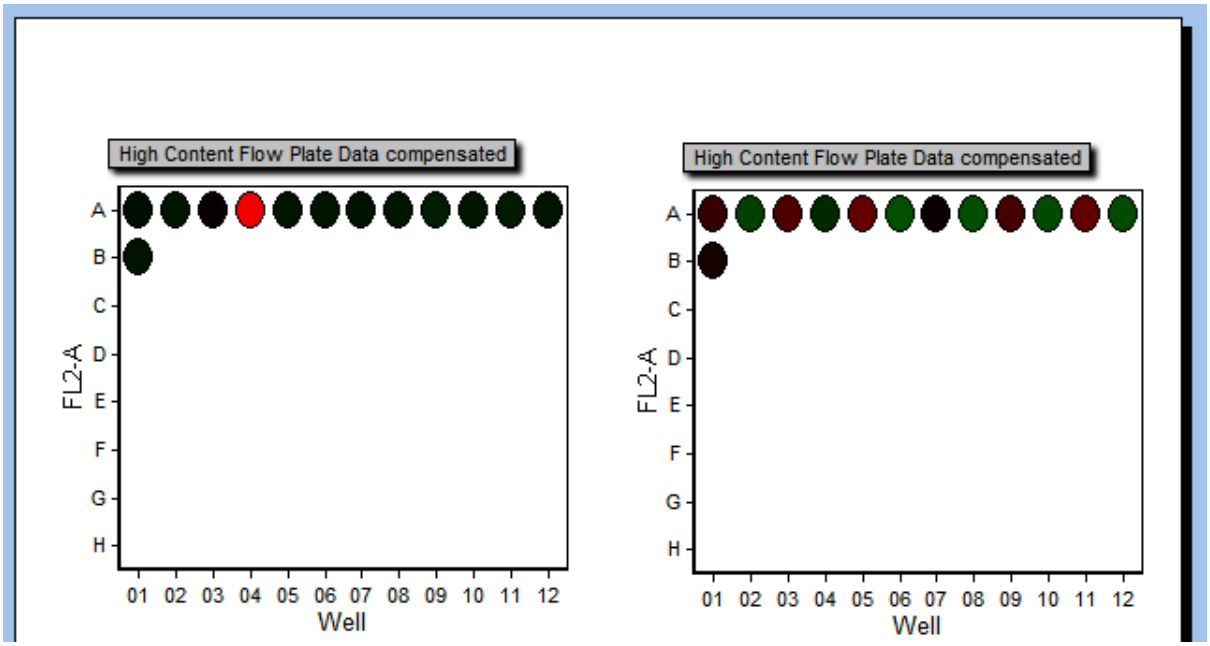

Figure T18.10 Heat Maps Displaying Statistics for Median (Left) and Arithmetic Mean (Right).

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.

27. Right-click on the left plot.
28. Choose **Format** from the pop-up menu.
29. Choose the **Heat Map→Color Levels** category.
30. Change the **Selected Color Level** from the drop-down list to **Rainbow** (Figure T18.11).

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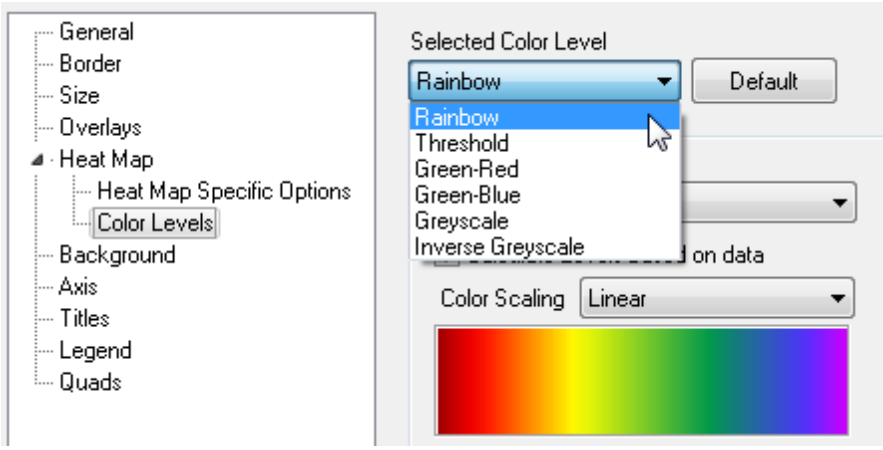

Figure T18.11 Changing the Color Level of a Heat Map

31. Click **Preview**.

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.

32. Change the **Selected Color Level** from the drop-down list to **Threshold**.

33. Enter "1000" in the **Threshold Value** field.

34. Change the **Below Threshold Color** to Yellow.

35. Change the **Above Threshold Color** to Orange.

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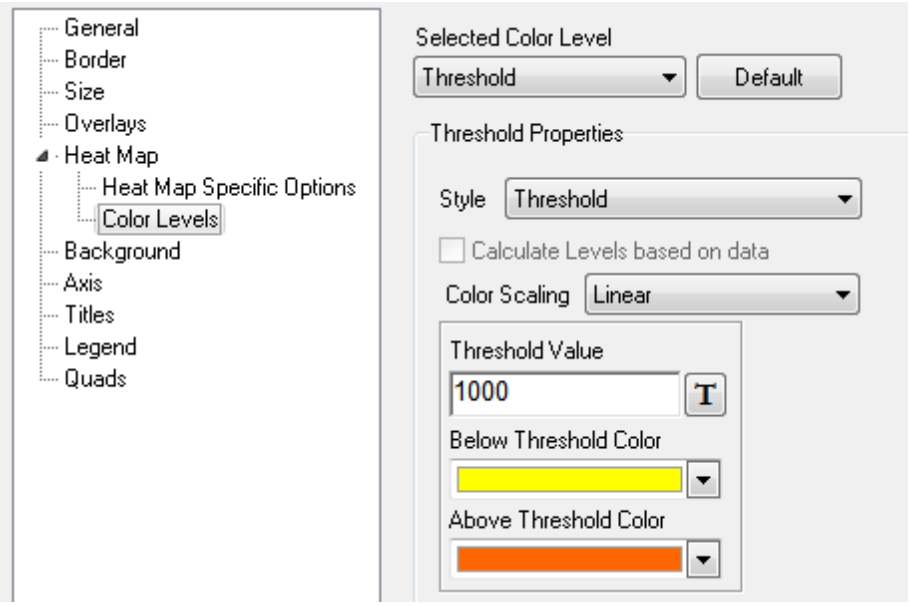

Figure T18.12 Defining a Threshold to Display as a Color Level in a Heat Map

36. 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.

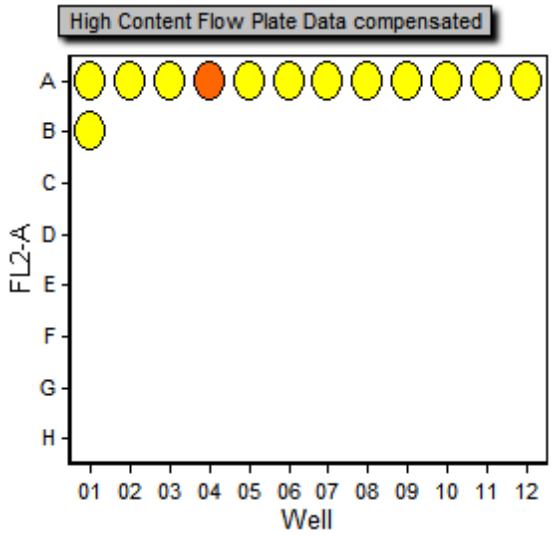

Figure T18.13 Heat Map with Threshold Color Level

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Note: See Choosing a Color Scheme 486 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.

37. Select both heat maps on the layout.
38. Choose the **Format→Plot Options→Overlays** command.
39. Change the **Statistic to show** to **Median**.
40. Click **OK**.
41. Right-click on the left plot.
42. Choose **Format** from the pop-up menu.
43. Choose the **Heat Map→Color Levels** category.
44. Change the **Selected Color Level** to **Green-Red**.
45. Change the **Style** to **Percentile** from the drop-down list (Figure T18.14).

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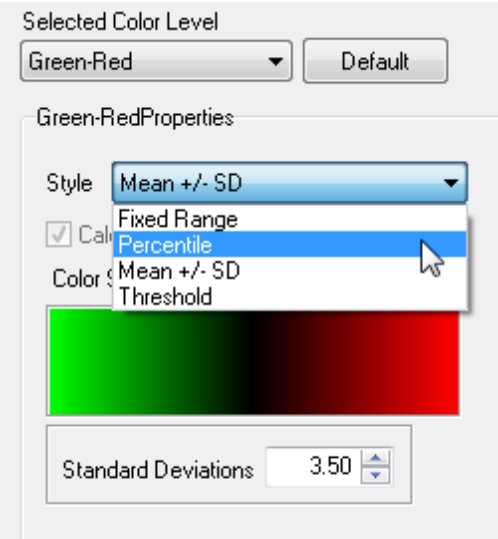

Figure T18.14 Changing the Style of the Heat Map Color Levels

45. Click **OK**.
46. Repeat steps 40-43 on the right plot.
47. Change the **Style** to **Fixed Range** (on the right plot).
48. Change the **Color Scaling** to **Logarithmic** from the drop-down list (on the right plot).
49. 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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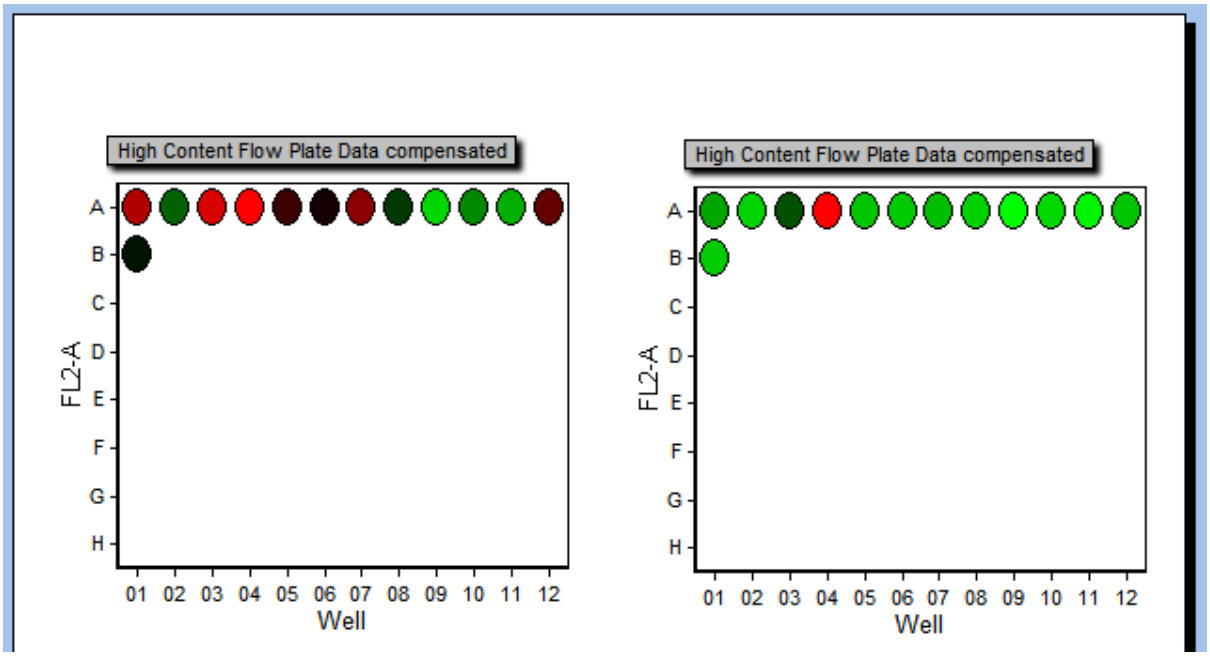

Figure T18.15 Adjusting Color Styles

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.

50. Select the plot labeled "**2D Plot for Gating**".
51. Choose the **Data→Change Data on Selection→Select** command.
52. Choose the **Data List** tab.
53. Select **High Content Flow Plate Data**.
54. Click **Open**.
55. Apply two gates to the 2D plot as in Figure T18.16 below.

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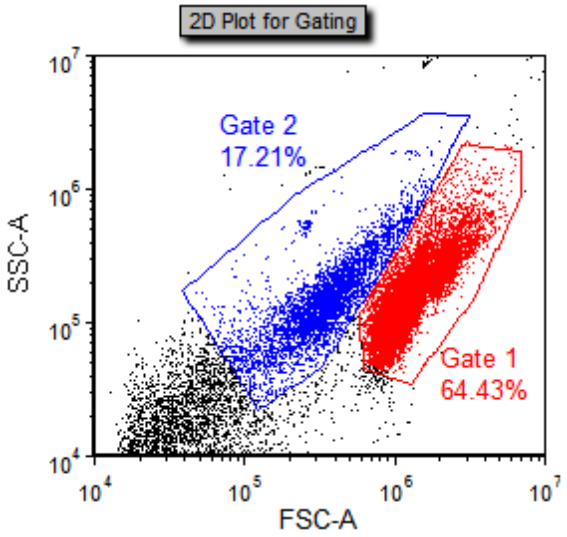

Figure T18.16 A Gated 2D Plot Created from the High Content Flow Plate Data

Note: Since the plot above is ungated, it is showing all the events from all the wells in the data file.

56. Select the Heat Map on the left.

57. Choose **Gate 1** from the **Gating→Create Gates→Current Gate** drop-down list.

58. Select the Heat Map on the right.

59. Choose **Gate 2** from the **Gating→Create Gates→Current Gate** drop-down list.

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 1042.

34.18.2 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

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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). 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→Organize Data Sets→Data List** command.
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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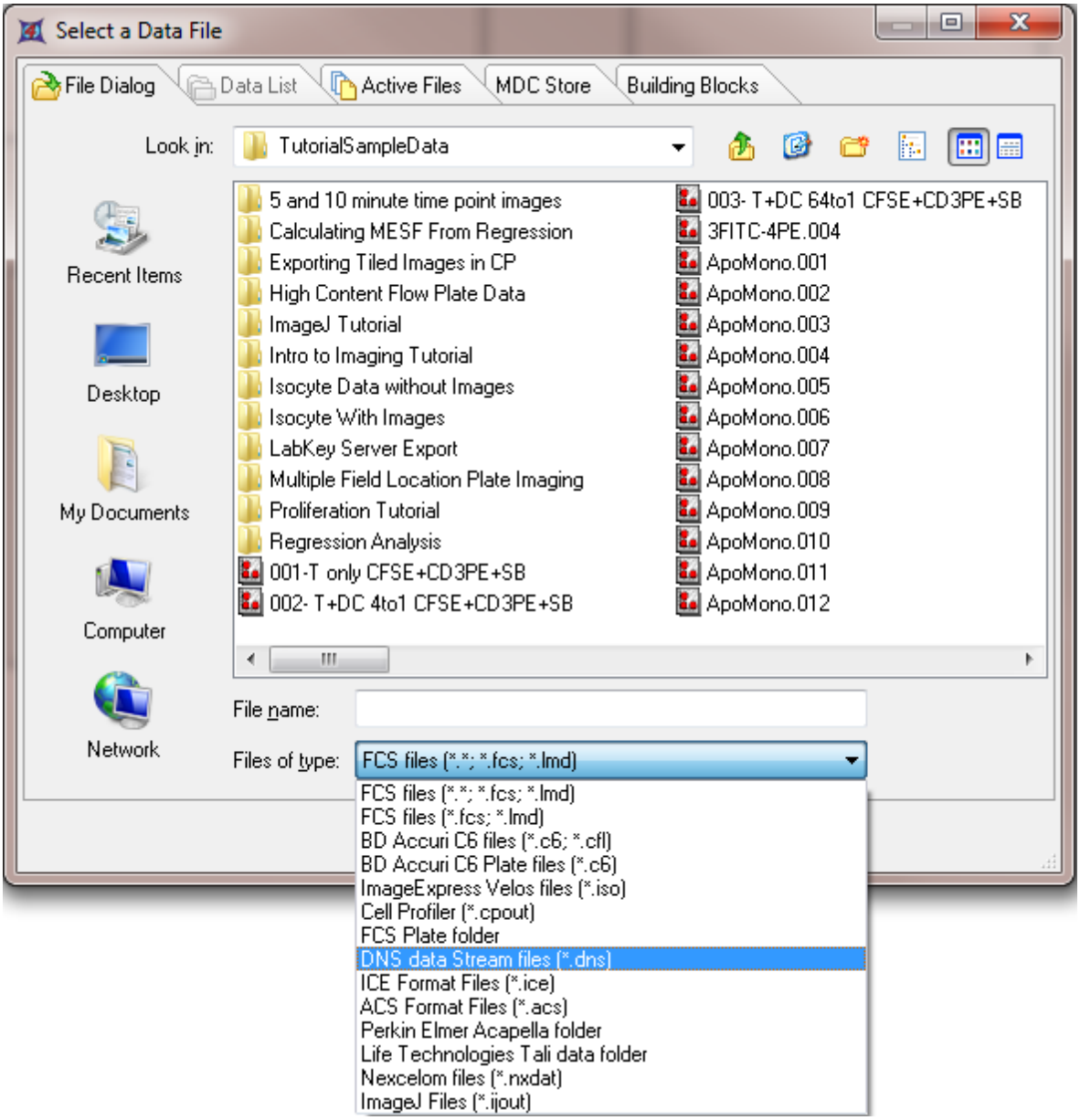

Figure T18.17 Choosing DNS Data Stream Files

5. Navigate to the FCS Express *Tutorial Sample Data* directory (Figure T18.18).

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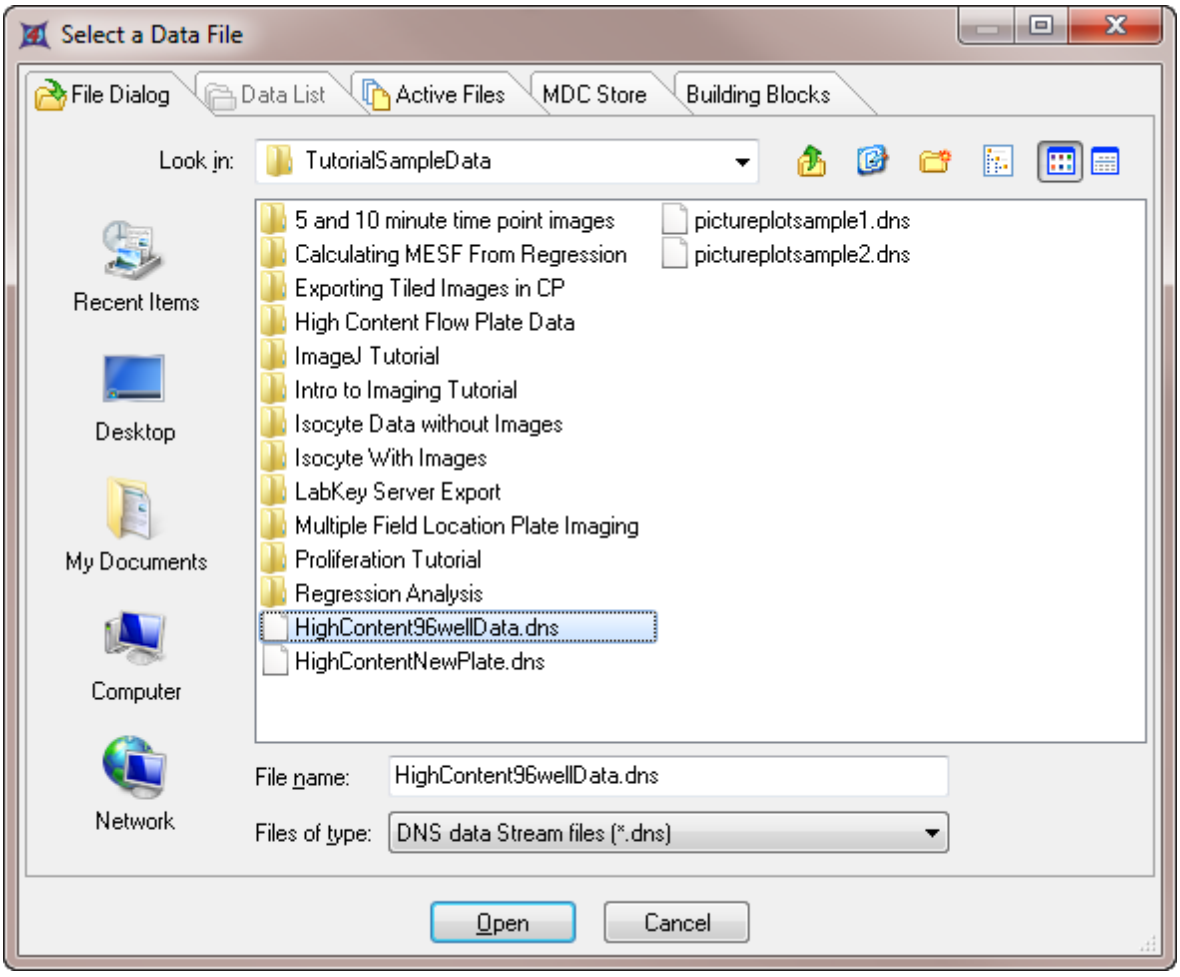

Figure T18.18 Choosing the HighContent96wellData.dns File

6. Choose the file "**HighContent96wellData.dns**" (Figure T18.18).
7. Click **Open**.

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→Change Data on All Objects→Select** command.
9. Choose the **Data List** tab.
10. Highlight the **HighContent96wellData.dns** file.
11. Click **Open**.

The layout will now look like Figure T18.19.

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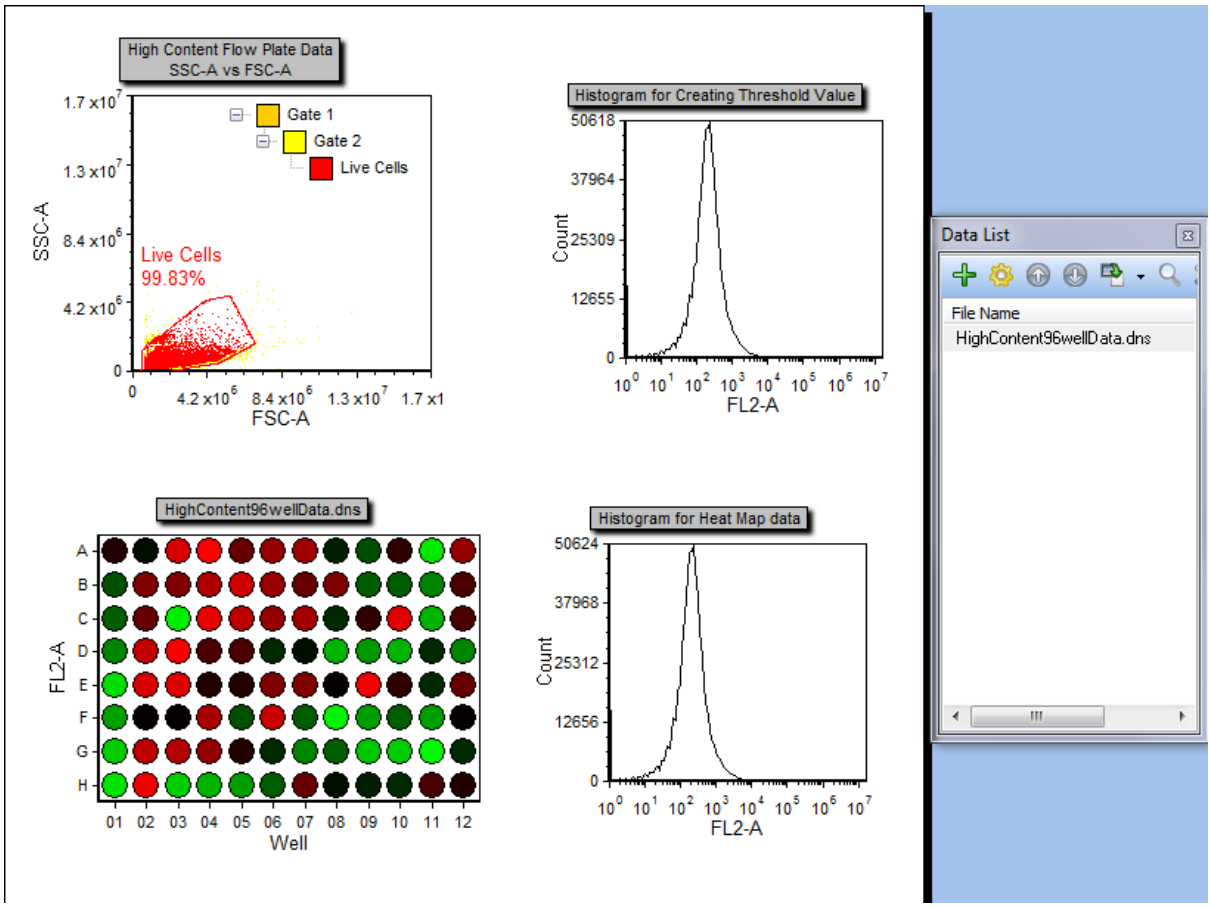

Figure T18.19 High Content Analysis Layout after Loading HighContent96wellData.dns File

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 apply gates to 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

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select the well for gating).

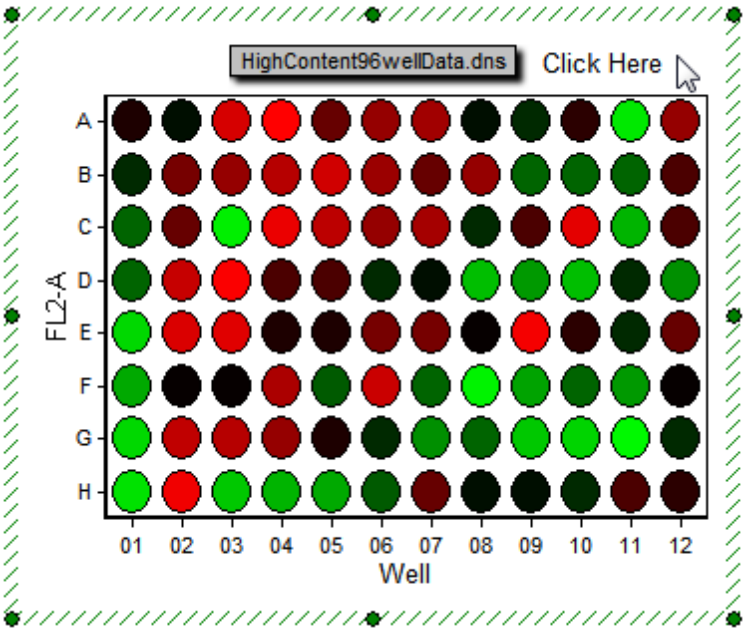

Figure T18.20 Creating a Well Gate

The Editing New Well Gate window will appear (Figure T18.21).

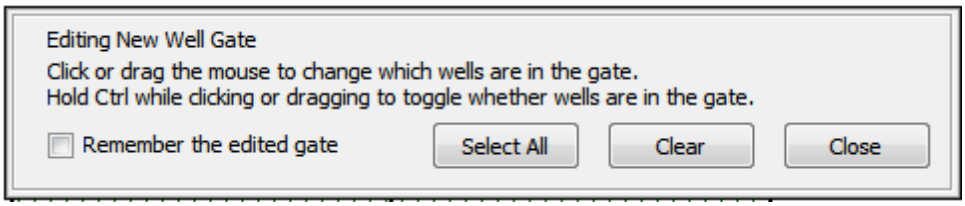

Figure T18.21 Editing a New Well Gate

15. Click on Well H12. The **Create New Gate** dialog will appear.
16. Enter "Negative Control" for the gate name.
17. Change the gate color to Green.
18. Click **OK**.
19. Choose the **Gating→Create Gates→Well** command (Figure T18.22).

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Figure T18.22 Selecting the Create Well Gate Command

20. Click directly on Well A4. The **Create New Gate** dialog will appear.
21. Enter "Experimental" for the gate name.
22. Change the gate color to Blue**.**
23. Drag the Negative Control and Experimental gates onto the Live Cells gate to create a hierarchical gate in the gate view.

The layout and gate view should now look like Figure T18.23.

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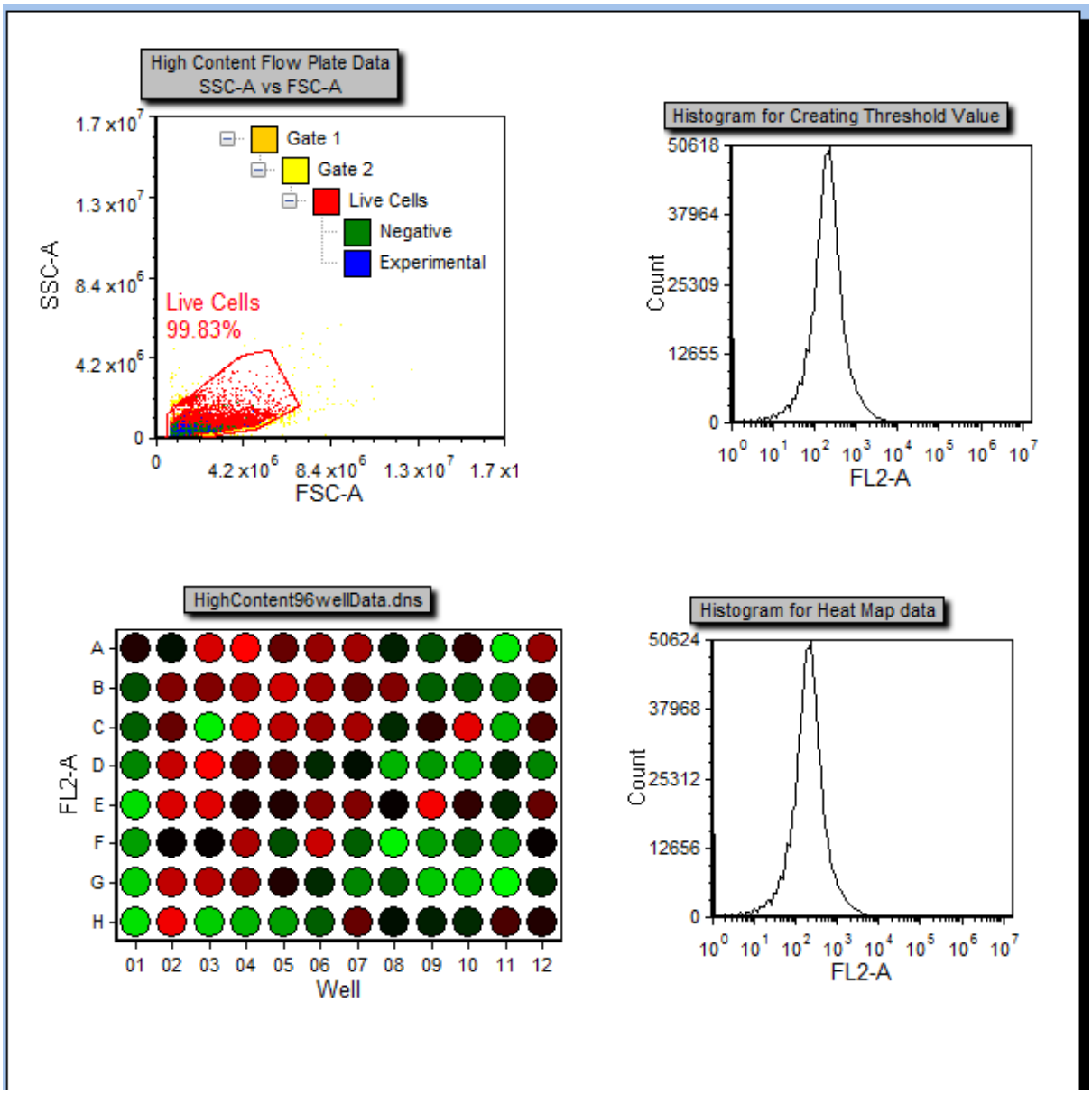

Figure T18.23 Layout with Gate View Indicating Hierarchical Gating of the Negative Control and Experimental Gates

We will now create a histogram overlay to view data from separate wells.

24. Drag and drop the Heat Map to the plot labeled **Histogram for Heat Map data** on the layout. This will automatically populate the histogram with the same data set that is showing on the heat map.

25. Select the option to **Add the files to the plot as new overlays** and click **OK.**

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26. Right-click on the histogram.
27. Choose **Format** from the pop-up menu.
28. Choose the **Overlays** category.

Two overlays for the same FCS Plate folder file will be displayed in the overlay list.

28. Highlight the first overlay in the list.
29. Change the **Gate** to "Negative Control".
30. Change the **Color** under **Line Options** to Green.
31. Highlight the second overlay in the list.
32. Change the **Gate** to "Experimental".
33. Change the **Color** under **Line Options** to Blue.
34. 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.

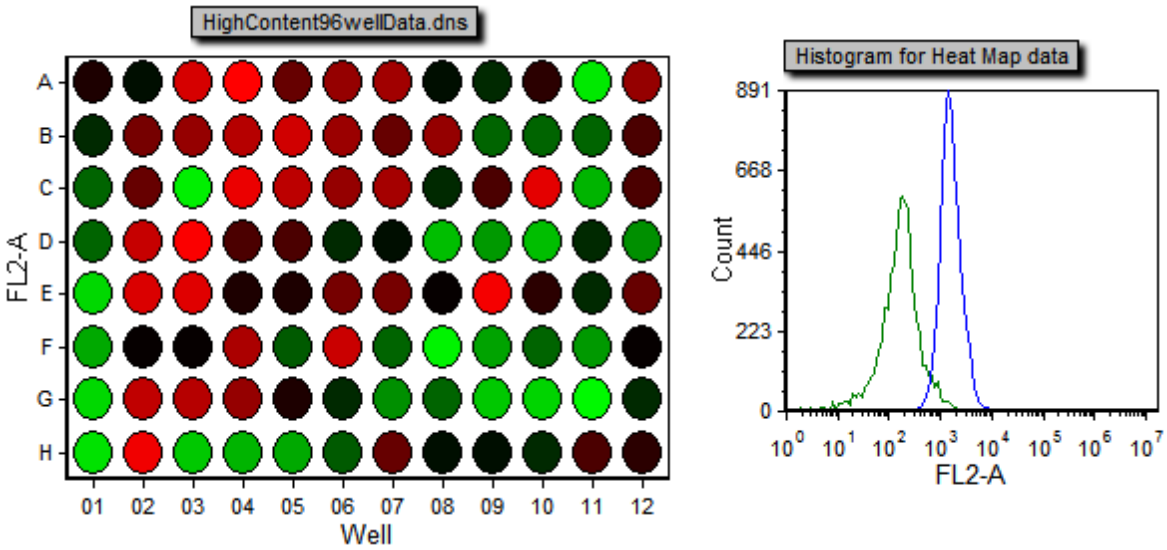

Figure T18.24 Histogram Displaying Events from Well Gates

We will now edit the well gates to select different wells.

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35. Right-click on the Heat Map.

36. 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).

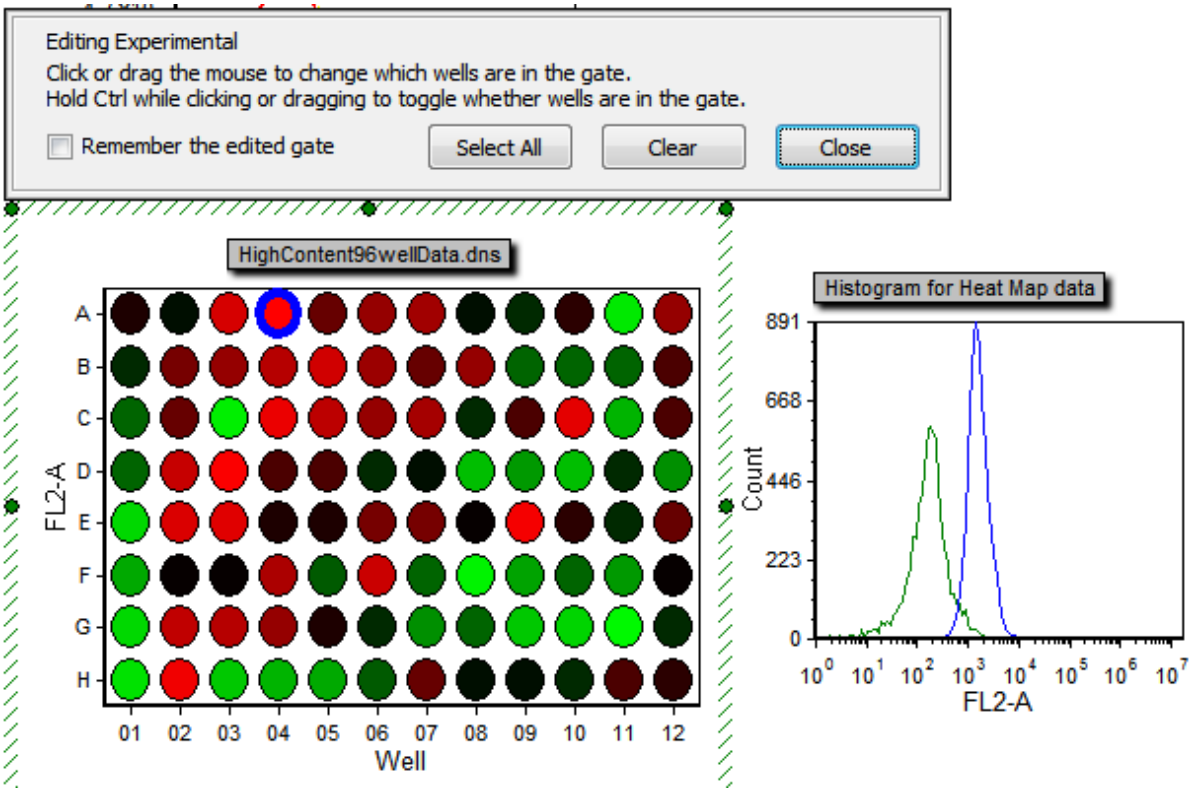

Figure T18.25 Editing the Experimental Well Gate (A4)

37. 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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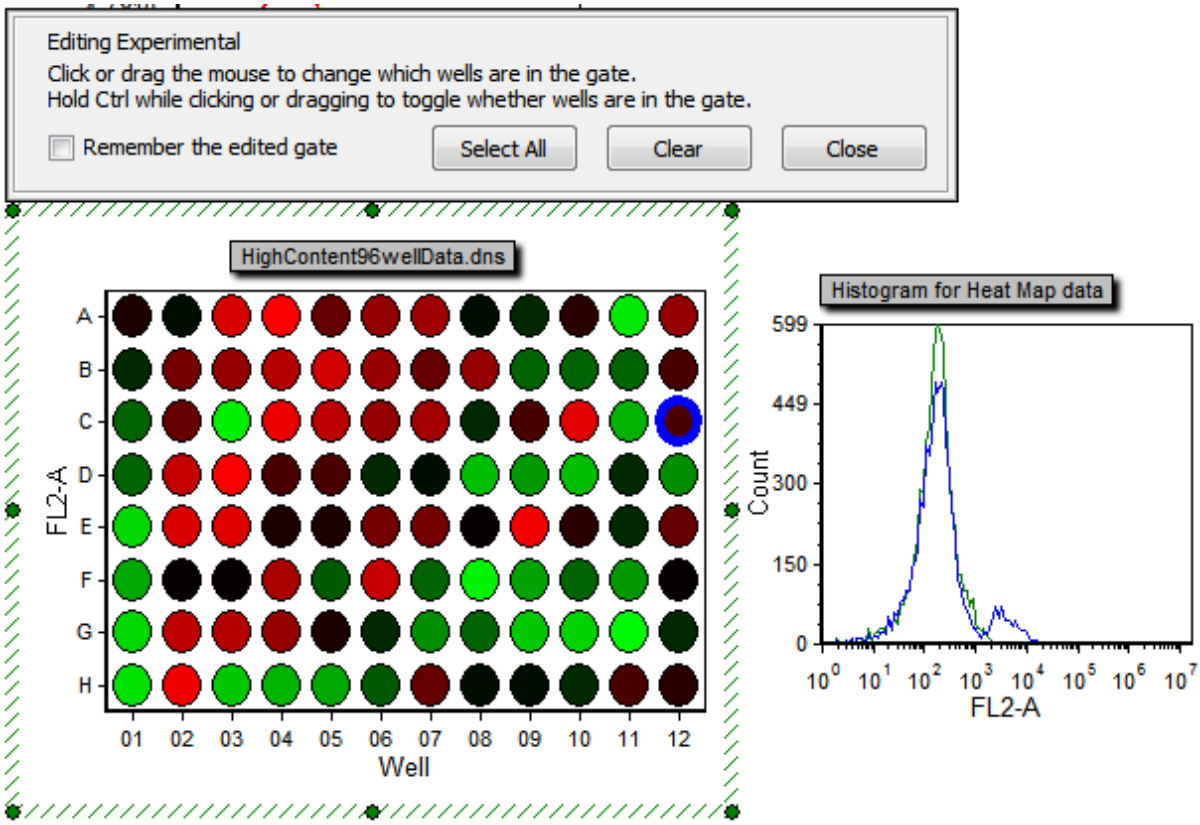

Figure T18.26 Experimental Well Gate Changed to C12

38. Click **Close** in the well gate **Editing** window.
39. Hit **Ctrl+Z** to undo the gate change.
40. Right-click on the Heat Map**.**
41. Choose **Edit Gate→Negative Control** from the pop-up menu.
42. Press and hold down the **Ctrl** button.
43. Click on wells C1 and F1 (Figure T18.27).
44. Release the **Ctrl** button.

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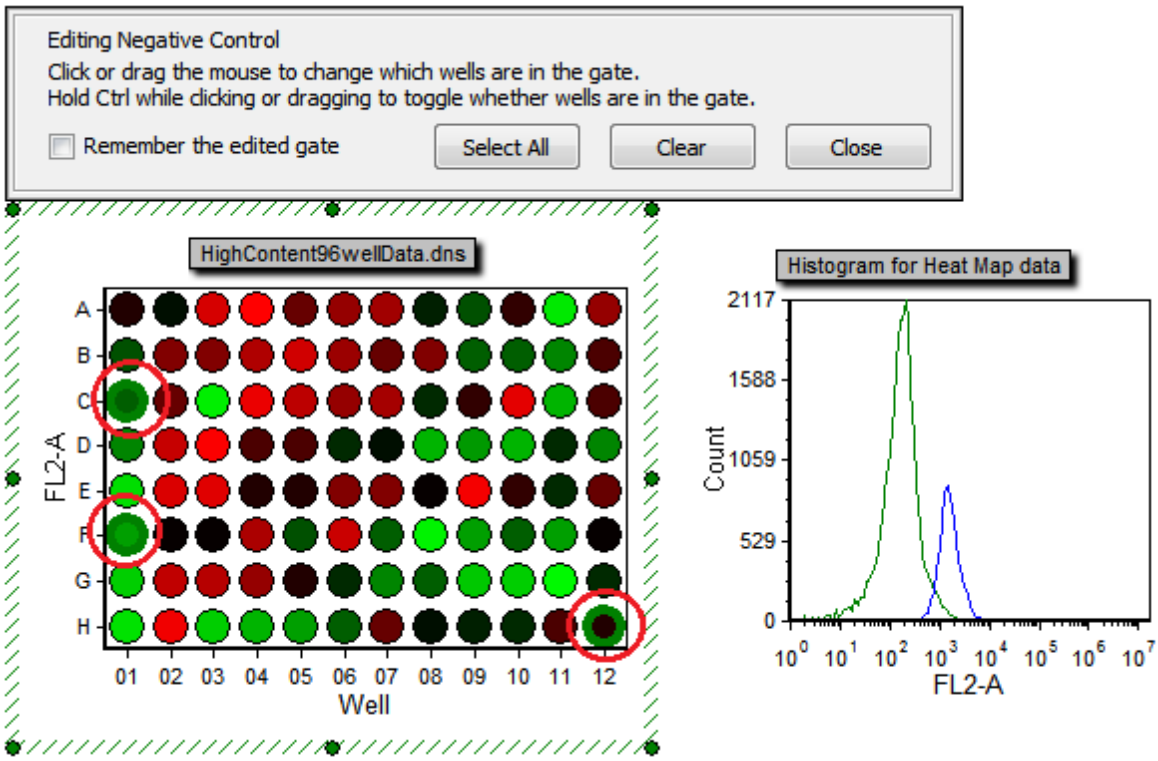

Figure T18.27 Selecting Multiple Non-consecutive Wells (C1, F1, and H12)

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.

45. Left-click and hold the mouse button down just above and to the right of well B1.

46. 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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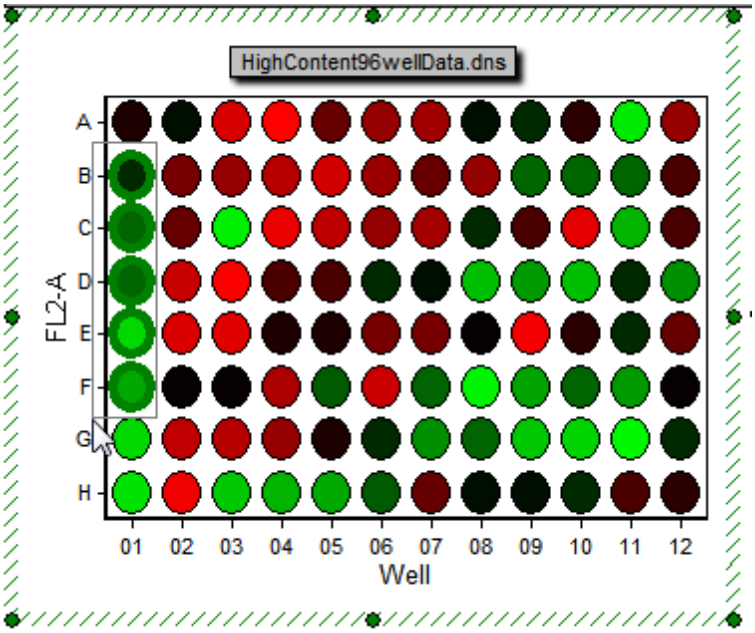

Figure T18.28 Selecting Multiple Continuous Wells

47. 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.

48. Hold down the **Ctrl** key.

49. Select wells B1-F1.

50. Click on well G9 to select it (continue holding down the **Ctrl** key).

51. Create a new selection box (with the **Ctrl** key still being held down) to encompass wells A8-A11.

52. Release the **Ctrl** key.

53. 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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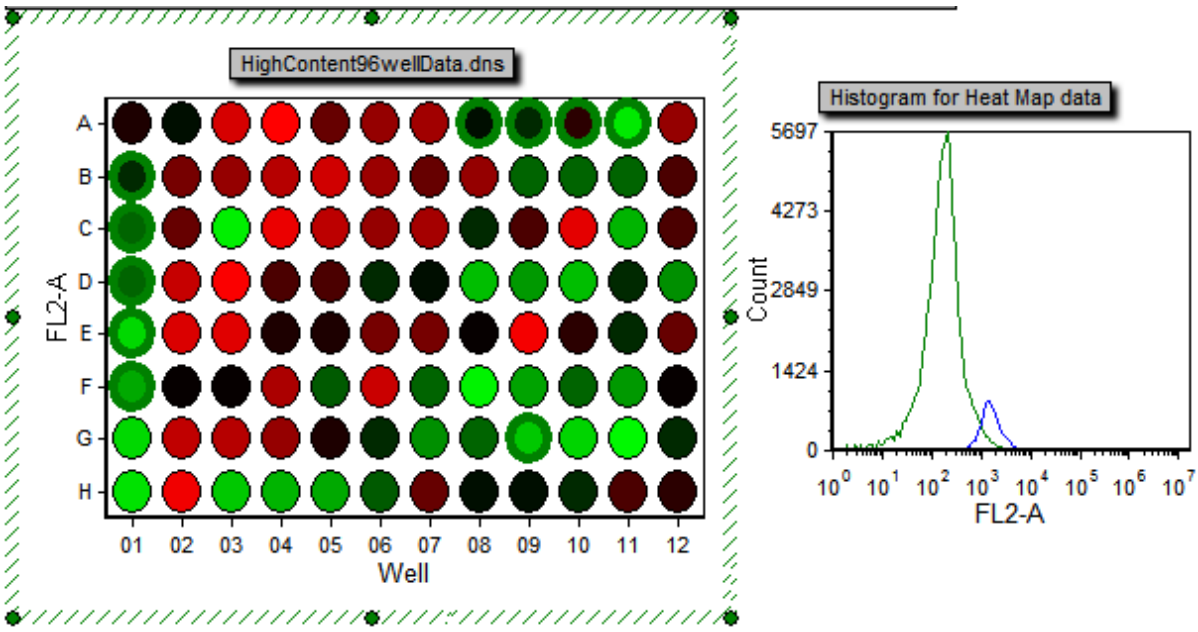

Figure T18.29 Multiple Well Selection

Gates may also be deselected from the well gating dialog in a similar manner.

54. Right-click on the Heat Map.
55. Choose **Edit Gate→Negative Control** from the pop-up menu.
56. **Ctrl+Click** on well A8. Notice how the well gate is removed.
57. **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.

58. Select the plot labeled "Histogram for Creating Threshold Value".
59. Choose the **Data→Change Data on Selection→Select** command.
60. Choose the **Data List** tab.
61. Choose **HighContent96wellData.dns**.
62. Click **Open**.
63. Right-click on the **Heat Map.**

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64. Choose **Format** from the pop-up menu.
65. Choose the **Heat Map→Colors Levels** category.
66. Change the **Style** to **Threshold** from the drop down window.
67. Click on the symbol in the **Threshold Value** field to insert a token.
68. Double-click on **Statistic** from the **Insert a Token** dialog.
69. Choose the "**Histogram for Creating Threshold Value**" in the **Select a plot** list box.
70. Choose the **Statistic** category on the left of the **Create Statistic** dialog.
71. Set the **Gate** to **Live Cells** from the drop-down list.
72. Highlight **Median** in the **Statistic** list box.
73. Click **OK**.
74. Change the **Below Threshold Color** to Black.
75. Change the **Above Threshold Color** to Red.
76. 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.

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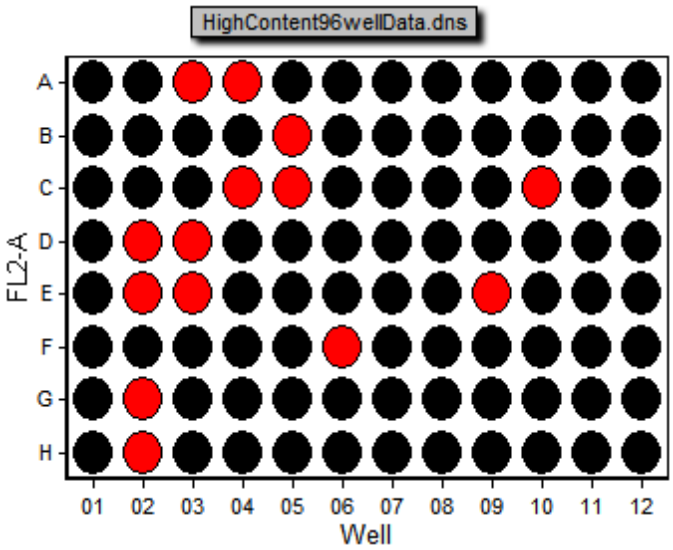

Figure T18.30 Threshold Defined as Median Value

In the next section, we will use batch analysis to analyze multiple plates[1057].

34.18.3 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[767] 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.
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.
5. Choose the files **HighContent96wellData.dns** and

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HighContentNewPlate.dns.

6. Click **Open**.
7. Choose the **Data→Change Data on All Objects→Select** command.
8. Click the **Data List** tab.
9. Choose **HighContent96wellData.dns**.
10. Click **Open**.

The layout will now look like Figure T18.31. 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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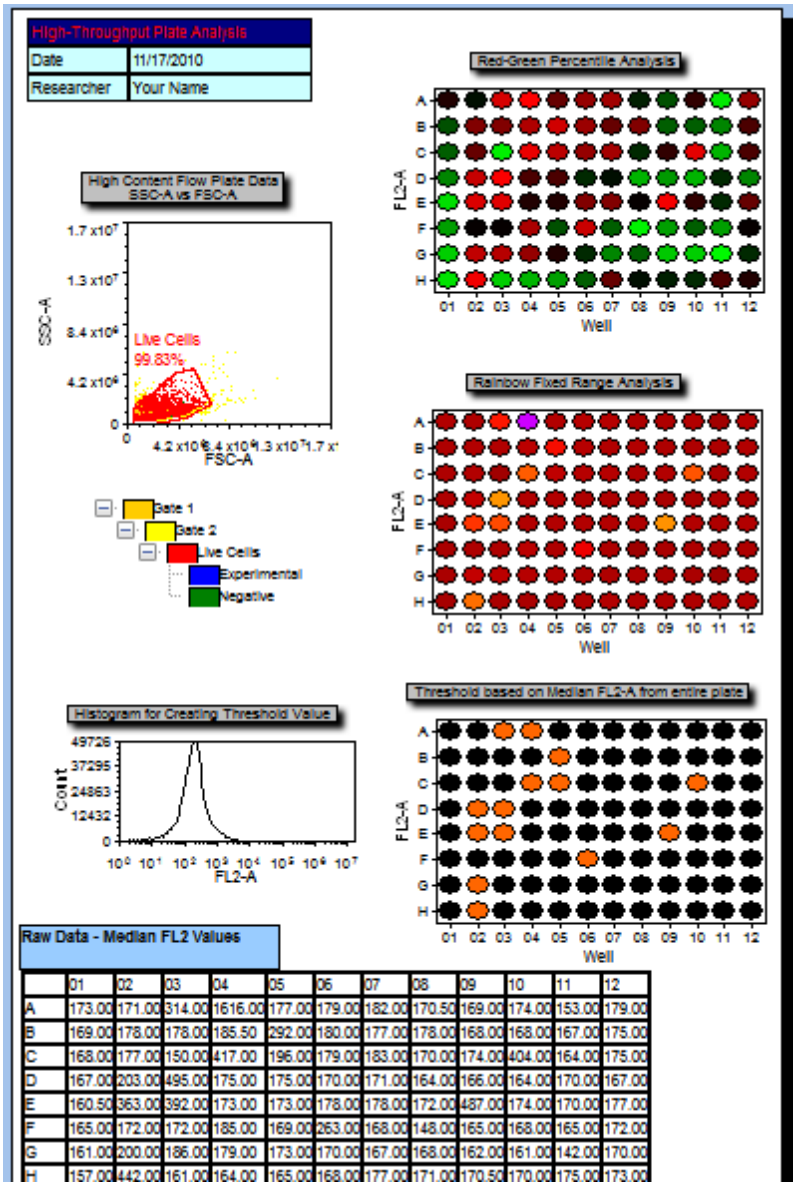

	01	02	03	04	05	06	07	08	09	10	11	12
A	173.00	171.00	314.00	1616.00	177.00	179.00	182.00	170.50	169.00	174.00	153.00	179.00
B	169.00	178.00	178.00	185.50	292.00	180.00	177.00	178.00	168.00	168.00	167.00	175.00
C	168.00	177.00	150.00	417.00	196.00	179.00	183.00	170.00	174.00	404.00	164.00	175.00
D	167.00	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 T18.31 High Content Batch Layout with Data

11. Choose the **Batch→Batch Processing→Batch Actions** command.
12. Choose **Save to PDF** from the **Add Action** area.
13. Click the **Combine the output from all iterations into a single file** radio button under **Iteration options**.
14. Choose a file name and location for saving the pdf in the **Save to a new file** field.
15. Click directly after the file name and before the extension.

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16. Click on the T symbol to insert a token.
17. Choose **Batch Processing**? **Current iteration** in the **Insert a Token** dialog.
18. Click **Insert**.

The **Create Export To PDF Action** dialog should look similar to Figure T18.32.

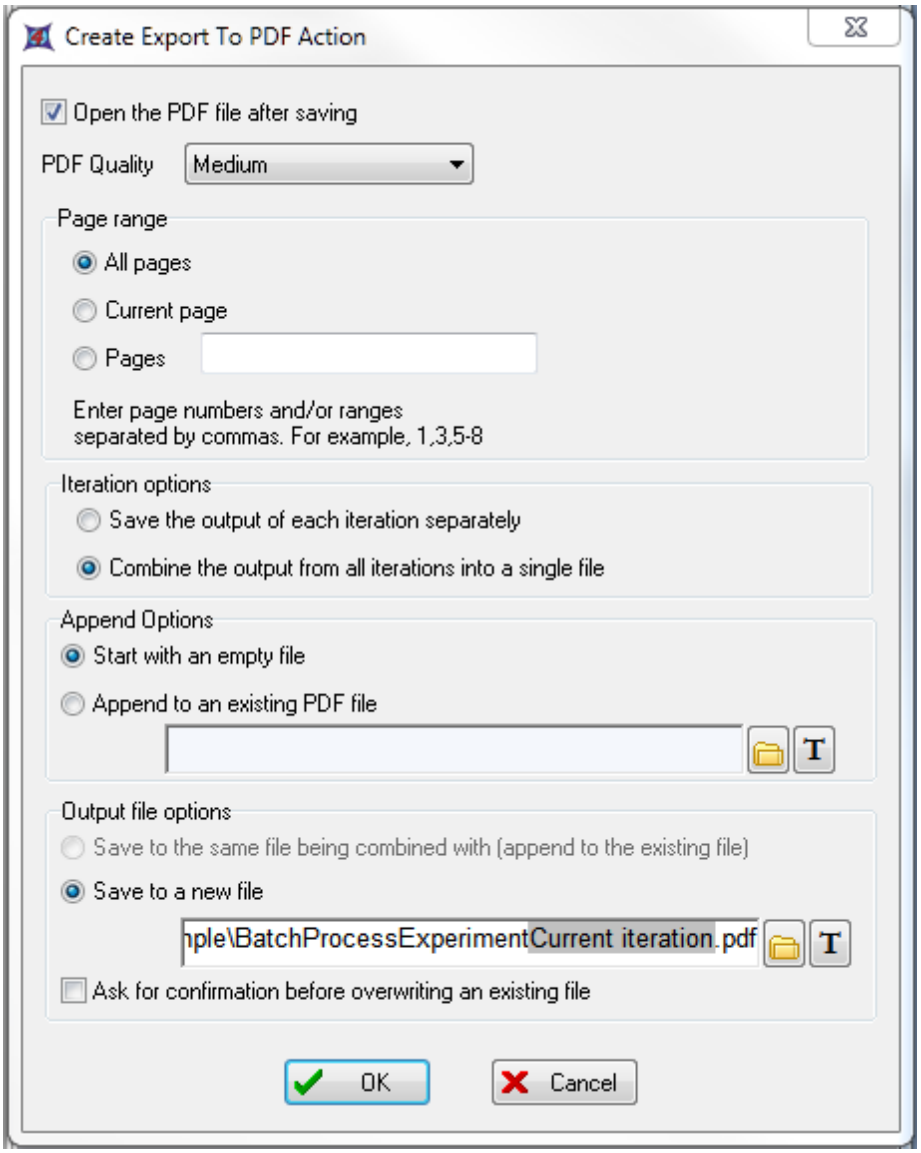

Figure T18.32 Create Export To PDF Action Dialog

19. Click **OK**.

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20. Choose **Save to Powerpoint** from the **Add Action** area.
21. Choose a file name and location for saving the Powerpoint file in the **Save to a new file** field.
22. Click directly after the file name and before the extension.
23. Click on the T symbol to insert a token.
24. Choose **Batch Processing→Current iteration** in the **Insert a Token** dialog.
25. Click **Insert**.

The **Create Export To Powerpoint Action** dialog should look similar to Figure T18.33.

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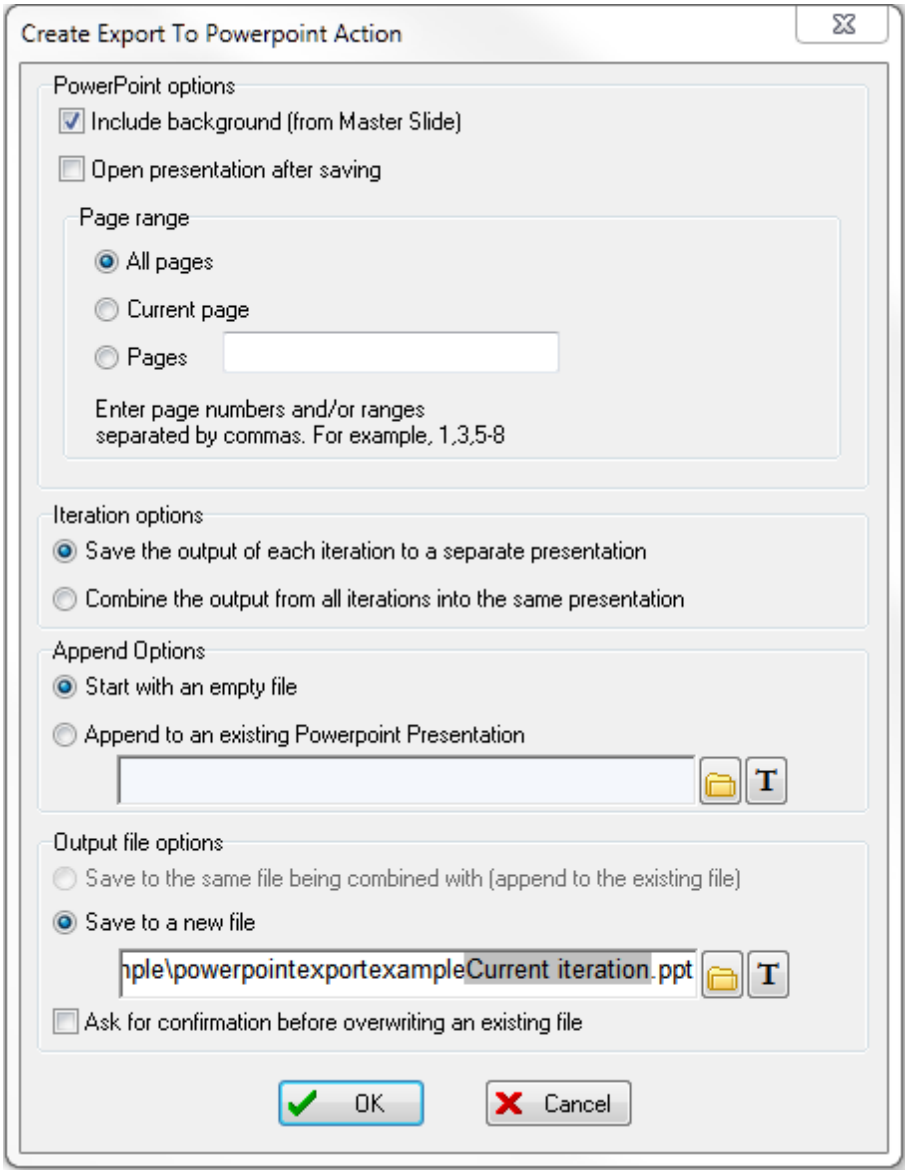

Figure T18.33 Create Export To Powerpoint Action Dialog

26. 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.

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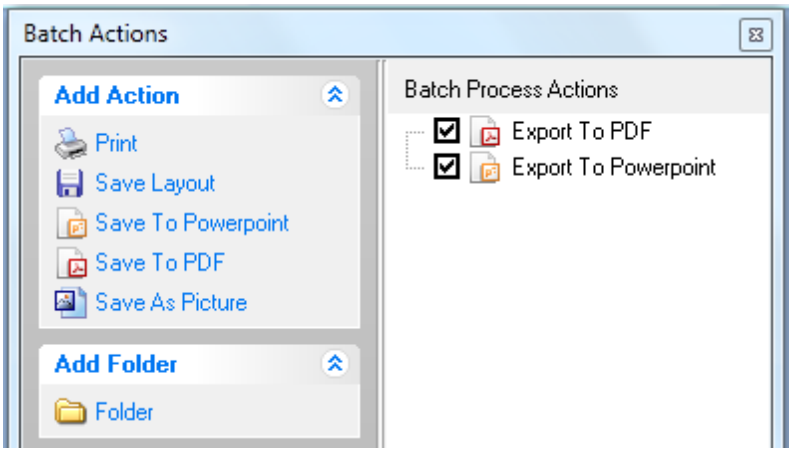

Figure T18.34 Batch Actions Window

27. 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 pages in the PDF and two pages in the PowerPoint presentation.

In the last exercise, we will use the Z-Prime function 1063.

34.18.4 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 675.

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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.

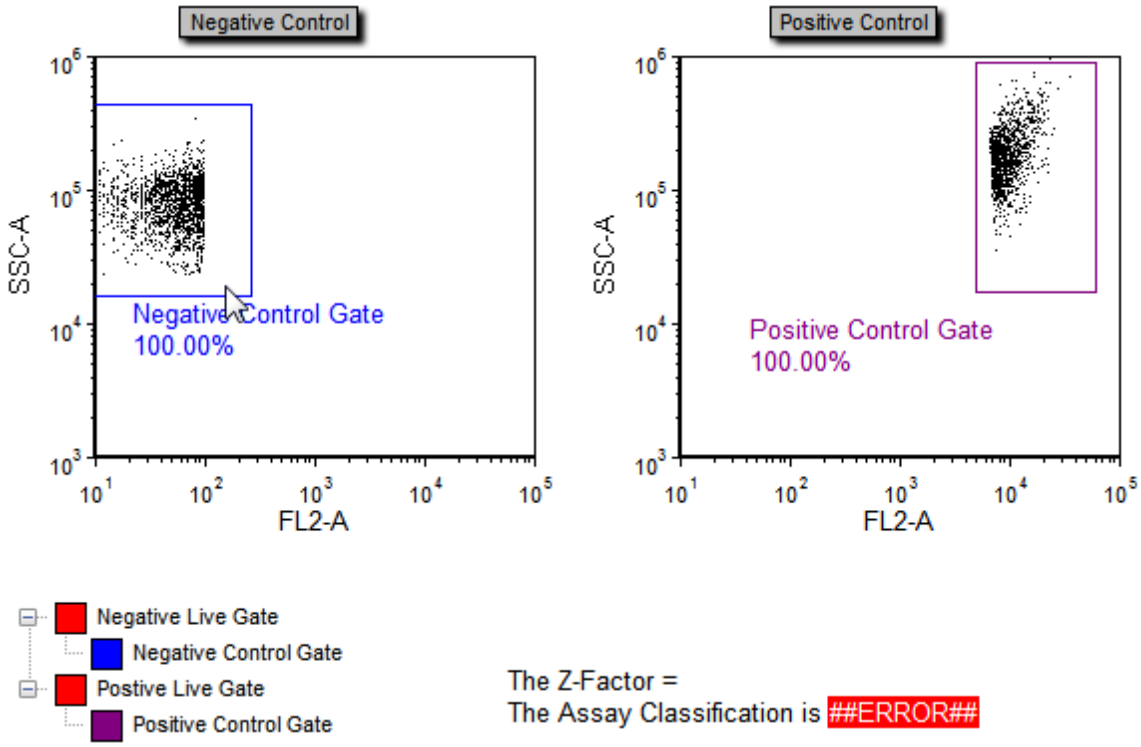

Figure T18.35 High Content Calculating Z-Prime Layout

2. Choose the **View→Tokens→Custom Tokens** command.
3. Click the ✚ symbol in the **Custom Tokens** window.
4. Name the new custom token "Z Factor".
5. Click in the **Enter the Custom Token formula below** field.
6. Enter an equals sign ("=").
7. Right-click after the equals sign.
8. Choose **Insert Function** from the pop-up menu.
9. Choose **ZPrime([mean negative, SD negative, mean positive, sd**

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positive]) from the list (Figure T18.36). (Alternatively, press Z with the list highlighted to automatically insert the function).

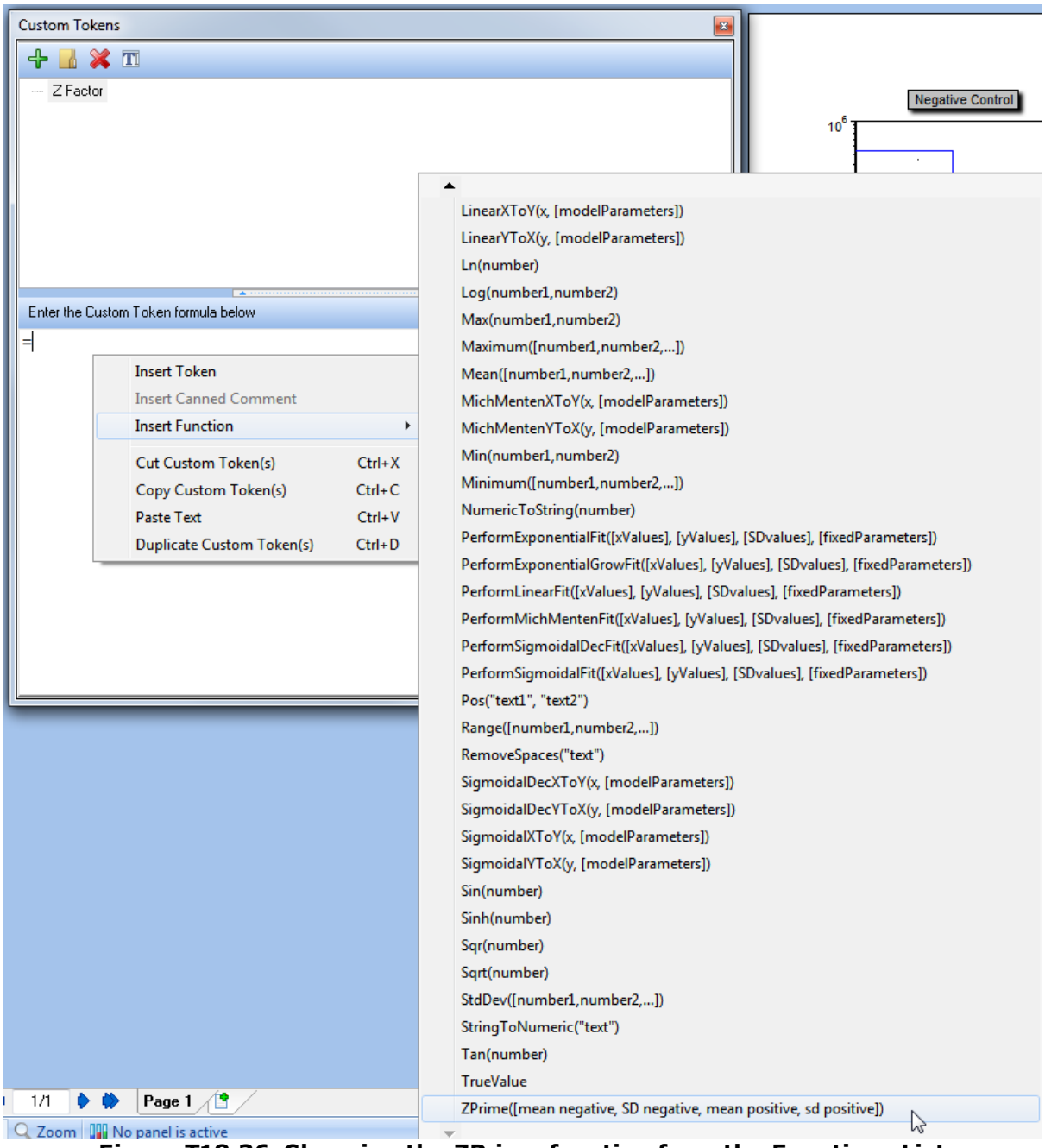

Figure T18.36 Choosing the ZPrime function from the Functions List

10. Highlight **[mean negative** in the formula field (Figure T18.37).

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11. Right-click with the text highlighted and choose **Insert Token**.
12. Double-click on **Statistic**.
13. Choose the **Negative Control** plot as the data source.
14. Click on **Statistic** on the left.
15. Choose Negative Control Gate from the drop-down list.
16. Choose **X Arithmetic Mean** from the **Statistic** list box.
17. Click **OK**.

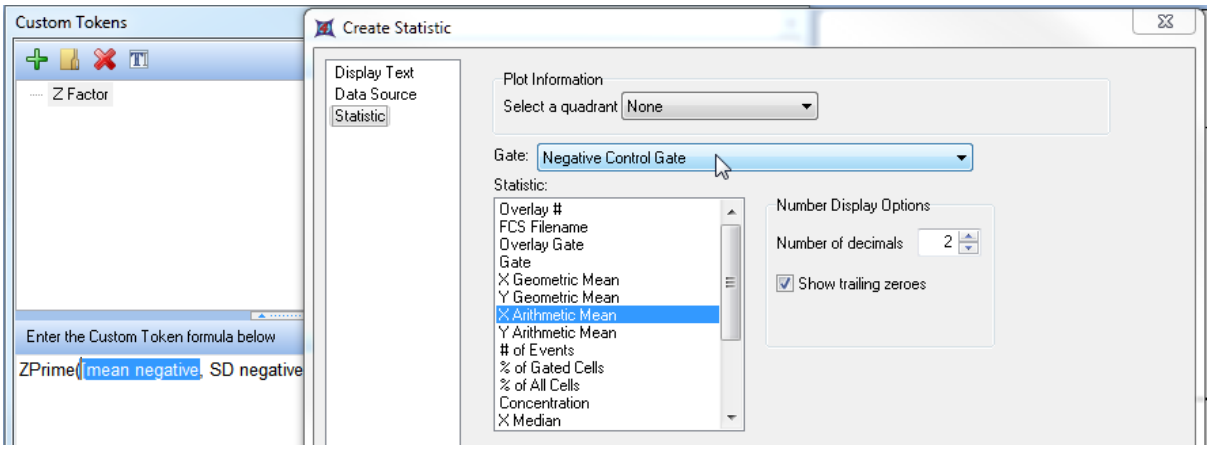

Figure T18.37 Replacing Text in a function with a Token

18. Highlight **SD negative** in the formula field.
19. Right-click with the text highlighted and choose **Insert Token**.
20. Double-click on **Statistic**.
21. Choose the **Negative Control** plot as the data source.
22. Click on **Statistic** on the left.
23. Choose the **Negative Control Gate** from the drop-down gate list
24. Choose **X Standard Deviation** from the **Statistic** list box.
25. Click **OK**.
26. Repeat steps 11-25 with "**mean positive**" and "**SD positive]**" using the **Positive Control** plot and 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 place holders have been replaced with the representative token value.

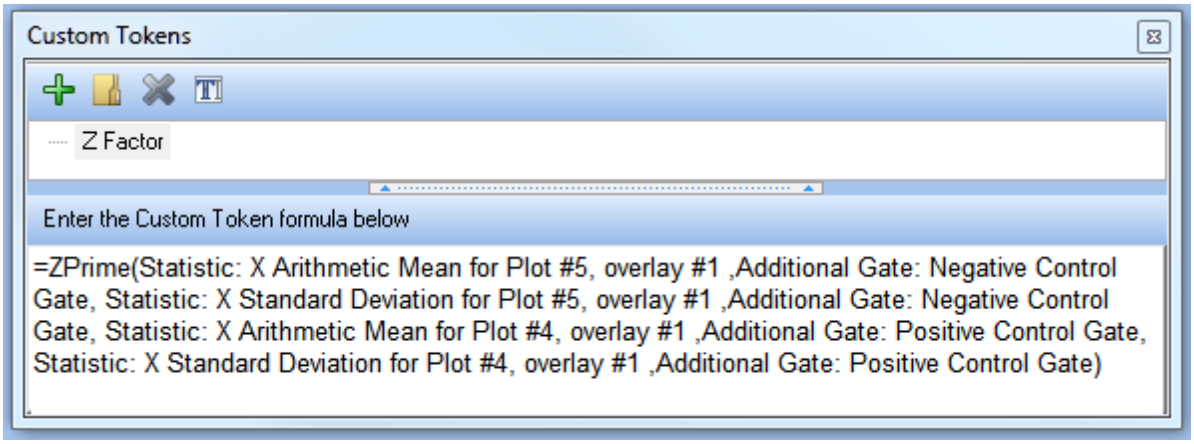

Figure T18.38 Z Factor Represented by the Z-Prime Function in FCS Express

27. Right-click after "**The Z-Factor =**" in the text box on the layout.
28. Choose **Insert Token** from the pop-up menu.
29. 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.
- 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

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the Z-Factor. To enable the classification token:

30. Right-click on **##ERROR##** next to "**The Assay Classification is**".
31. Choose **Format Token** from the pop-up menu.
32. Choose the **Classification** category.
33. Delete "**4 does not exist**" from the **Enter a value to classify** field.
34. Click on the T symbol in the **Enter a value to classify** field.
35. Double-click on **Z Factor** from the **Insert a Token** dialog.

The **Edit Statistic Classifier** dialog should look like Figure T18.39.

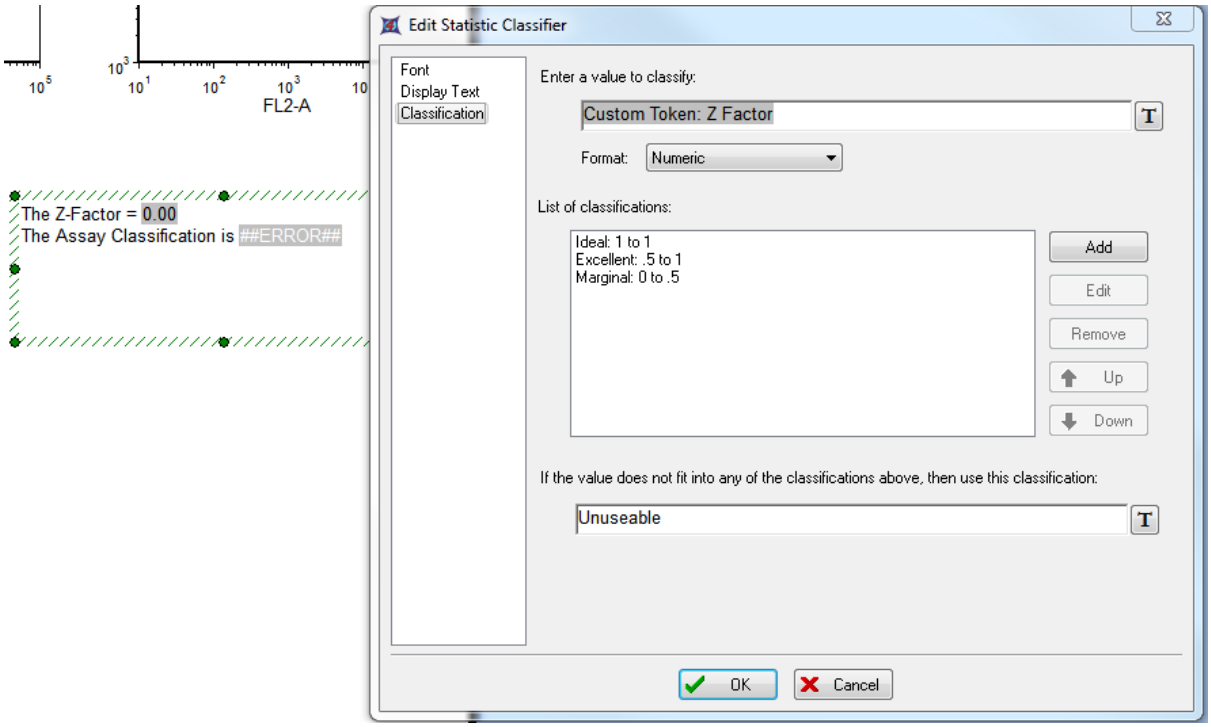

Figure T18.39 Changing the Classification Token to Z Factor

36. Click **OK**.

The Assay Classification will read **Marginal** since the value is 0.00. 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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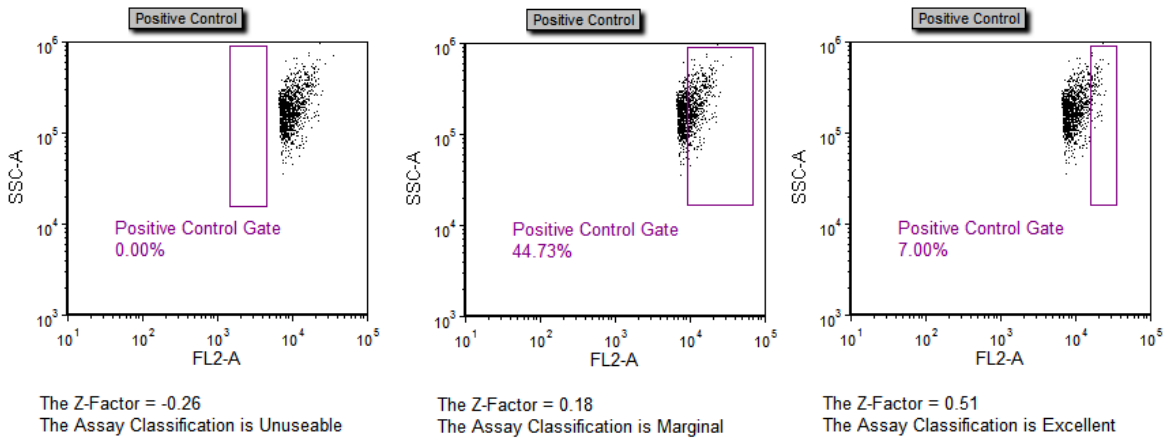

Figure T18.40 Gating on the Sample Data Causes the Z Factor and Classification to Update in Real Time

34.19 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 Version 4 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 modelled. 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.
- 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 1070.

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34.19.1 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 **Application Button→New Layout**.
2. Select the **Insert→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 *TutorialSampleData* 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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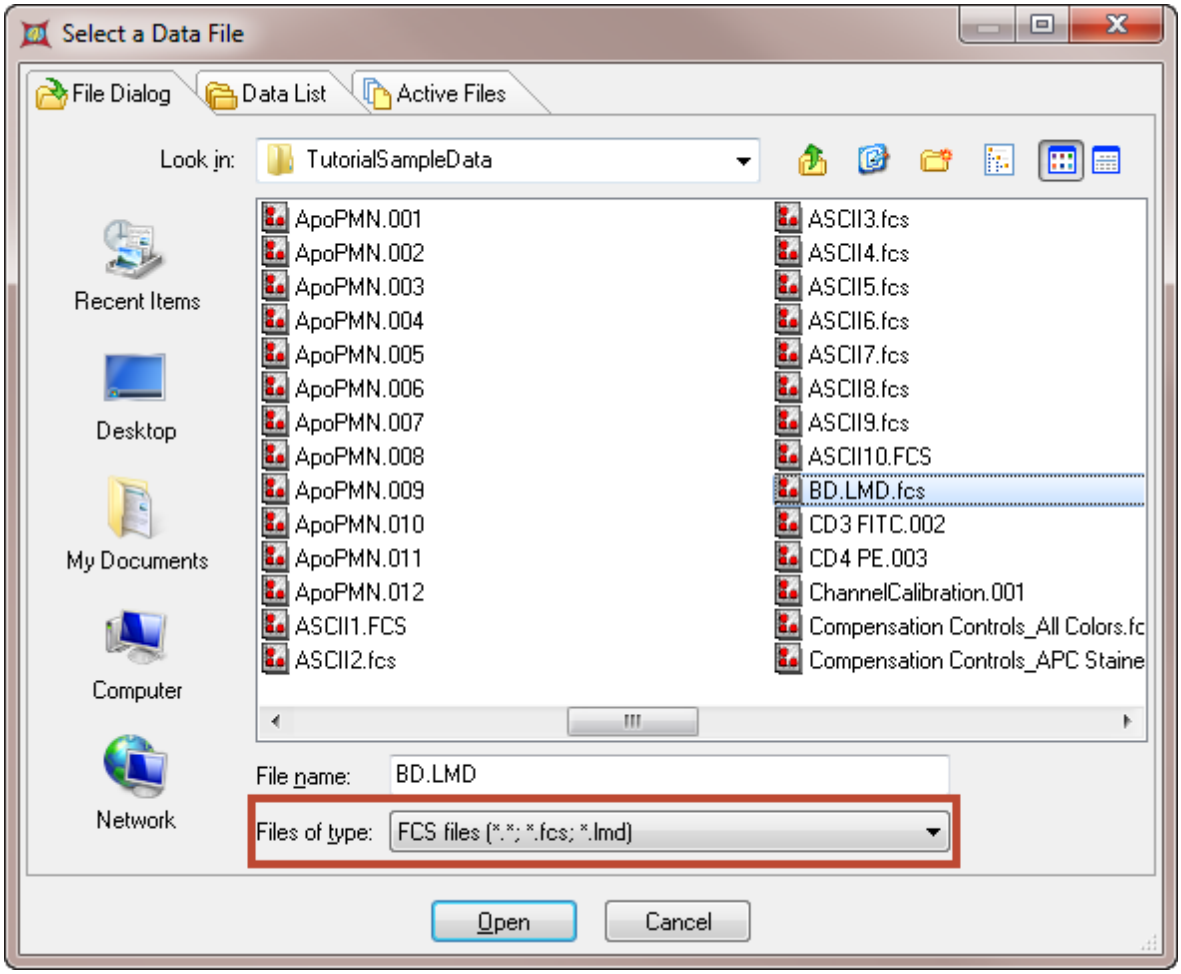

Figure T19.1 Select a Data File Dialog

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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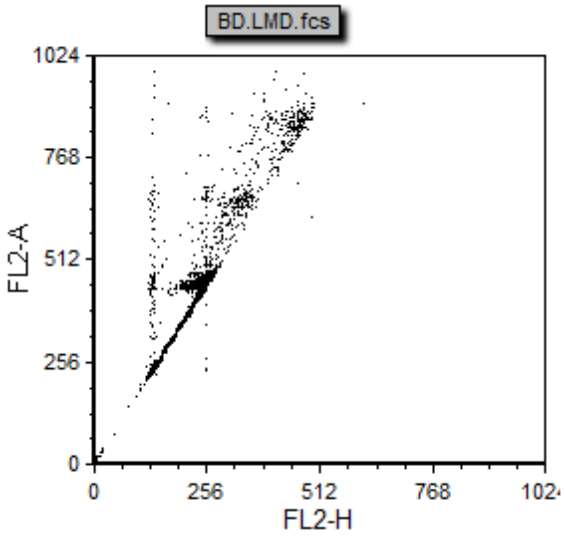

Figure T19.2 Dot Plot of FL2-H vs FL2-A

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 tutorial650.

9. Select the FL2-H versus FL2-A dot plot.
10. Select the **Gating→Create Gates→Polygon** command.
11. 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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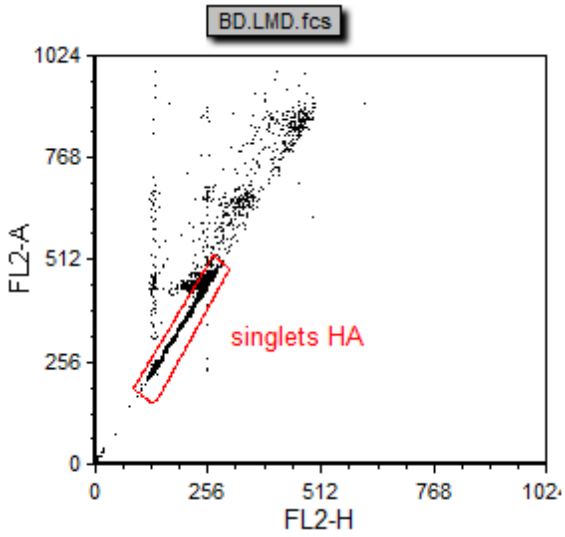

Figure T19.3 Gate to Exclude Doublets and Cells iwth High DNA Content

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→1D Plots→Multicycle DNA** command (Figure T19.4).
14. Click on a blank area of the layout.

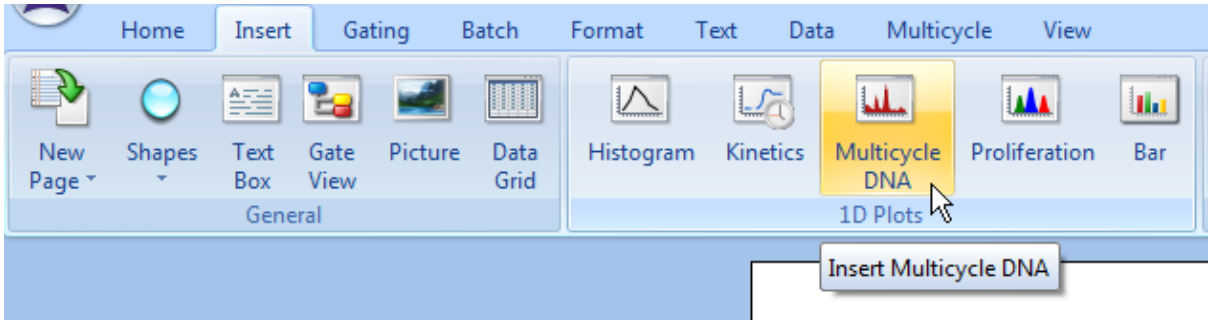

Figure T19.4 Selecting the Insert Muticycle DNA Command

The **Select a Data File** dialog appears, as previously shown in Figure T19.1.

15. Navigate to the *FCS Express Sample Data* folder.
16. Select the file **BD.LMD.FCS** (Figure T19.1).

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17. Click **Open**.

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. Select 'singlets HA' from the **Gate** drop-down list, indicated by the cursor.
20. Click **OK**.

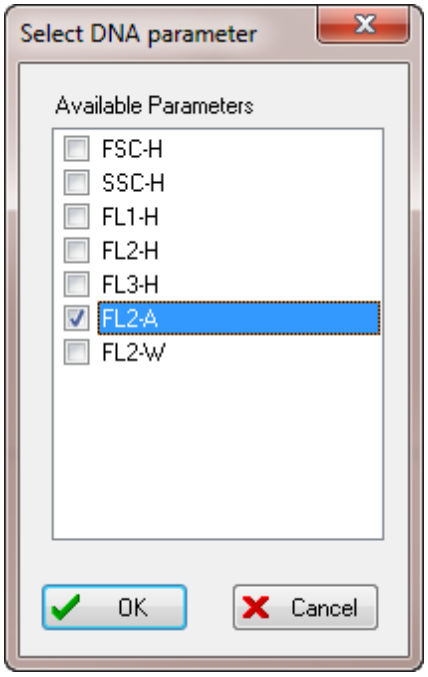

Figure T19.5 Select DNA Parameter Dialog

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).

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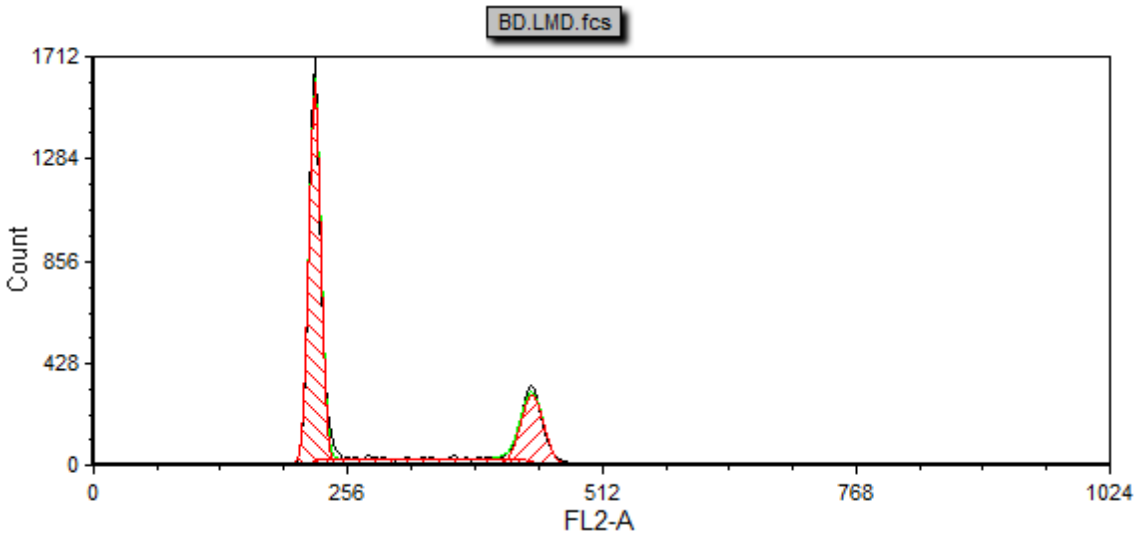

Figure T19.6 MultiCycle Analysis of FL2-A Using Singlets HA Gate

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 T19.7).
24. Click on the **Visible** checkbox.
25. Click **OK** (Figure T19.7).

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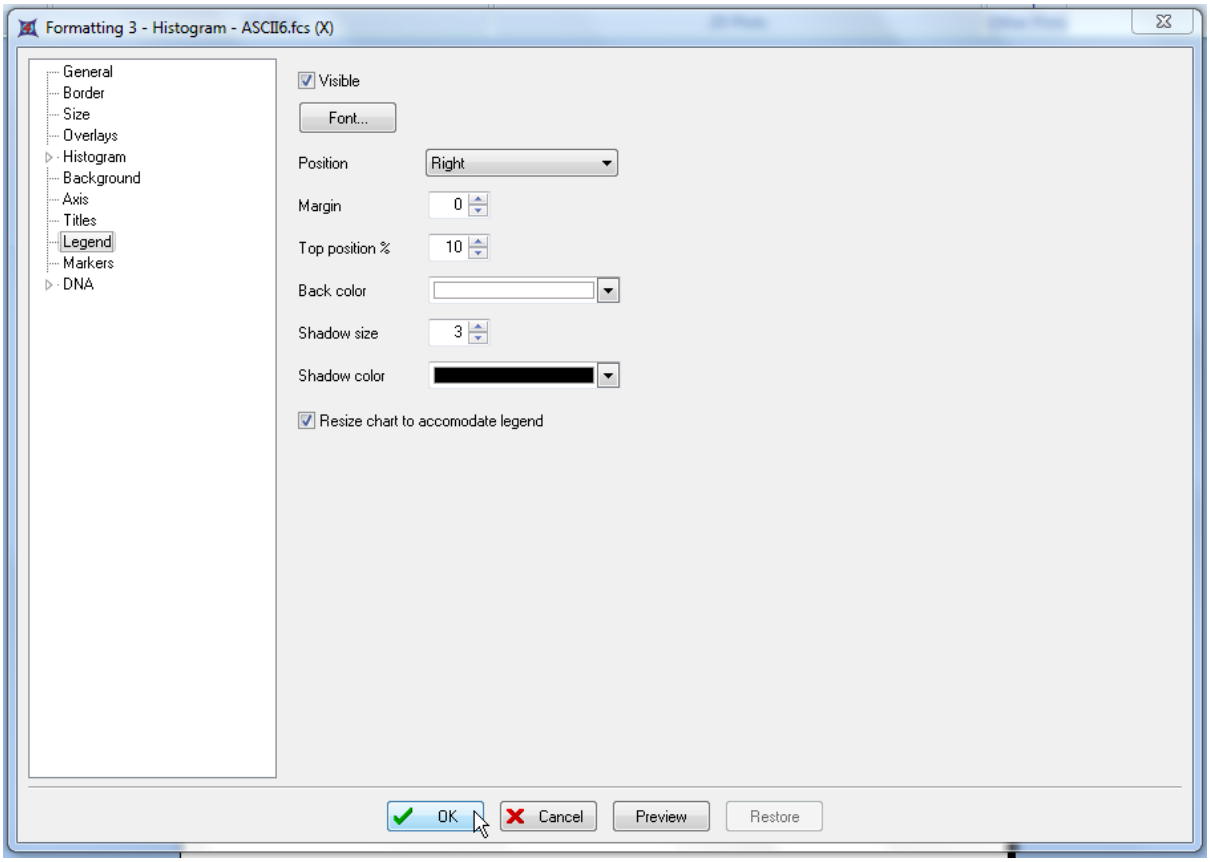

Figure T19.7 Formatting the DNA histogram to Display the Legend Associated with MultiCycle Analysis

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 T19.8).

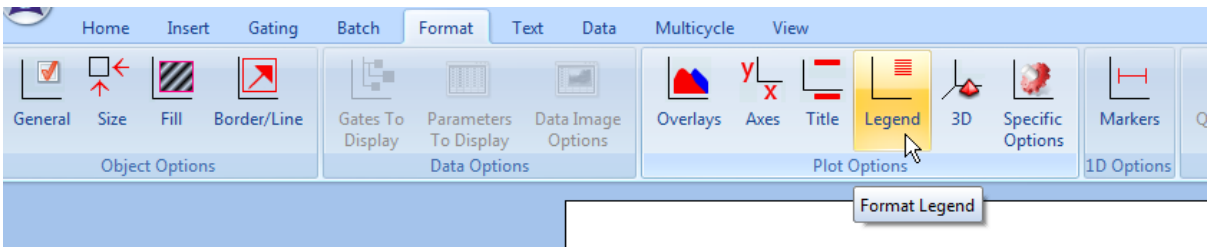

Figure T19.8 Selecting the Format Legend Command

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The **Formatting Legend** dialog appears (Figure T19.9).

- Click on the **Visible** checkbox in the **Formatting Legend** dialog.
- Click **OK**.

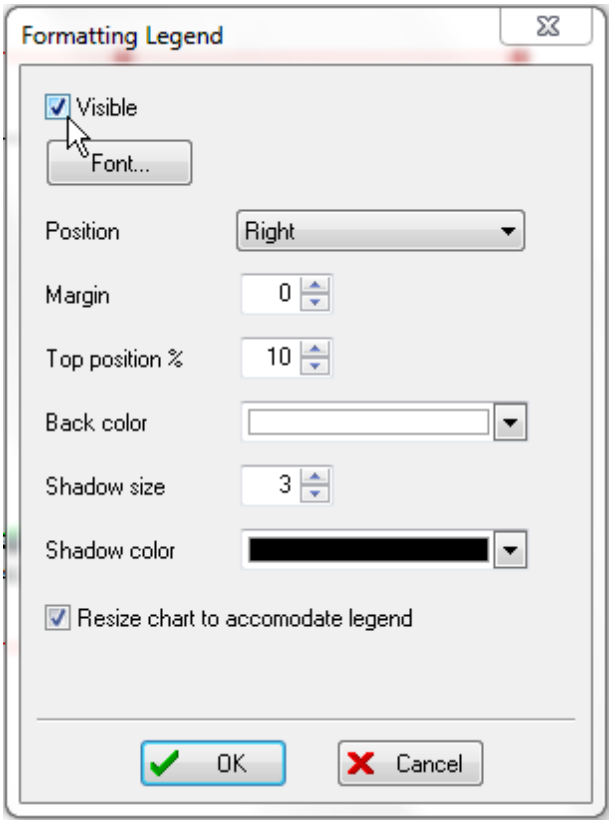

Figure T19.9 Formatting Legend Dialog

The legend associated with the MultiCycle DNA analysis will appear to the right of the histogram (Figure T19.10).

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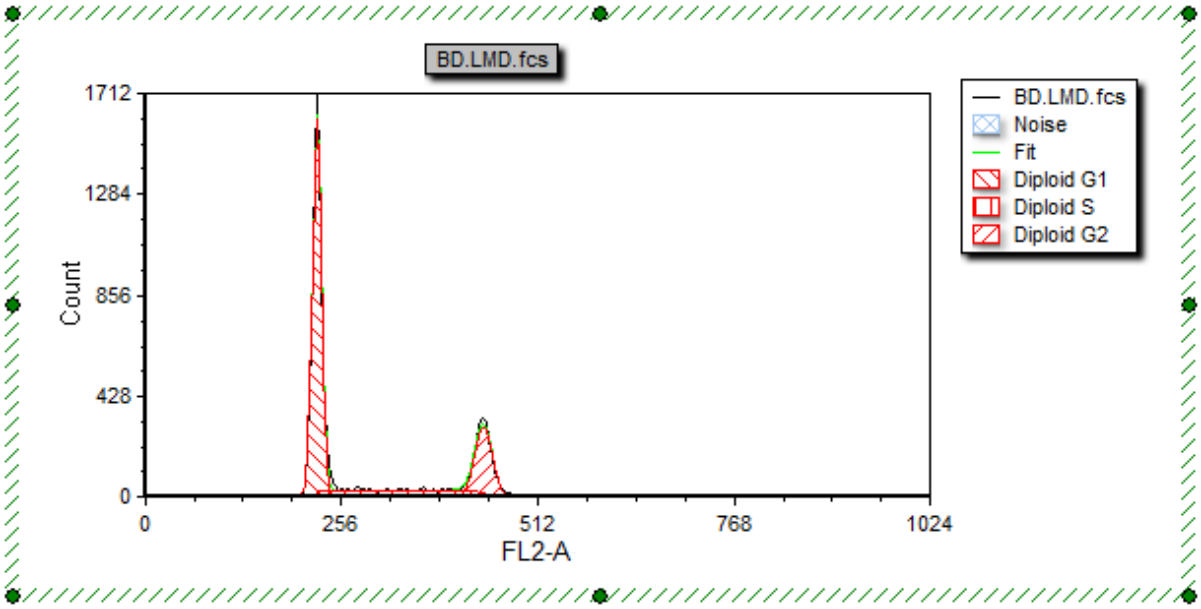

Figure T19.10 MultiCycle DNA Histogram Cell Cycle Analysis with Associated Legend

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 T19.10).

27. Select the **Multicycle** tab (Figure T19.11).

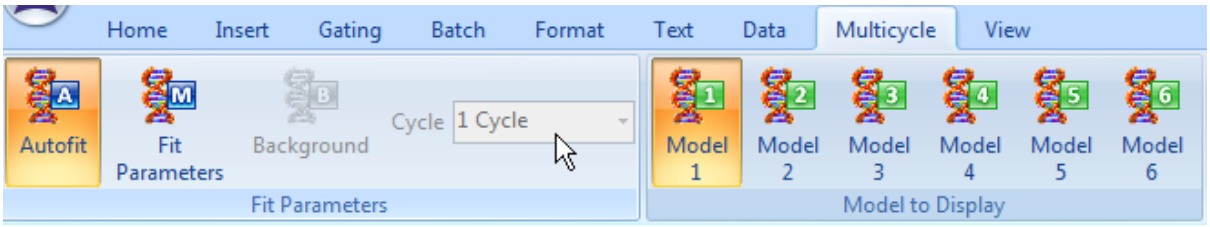

Figure T19.11 The Multicycle Tab

Notice that **Autofit** and **Model 1** are highlighted in orange (Figure T19.11). 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.

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Note: For an explanation of the differences between all the models, please see the MultiCycle Manual.

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 T19.12).

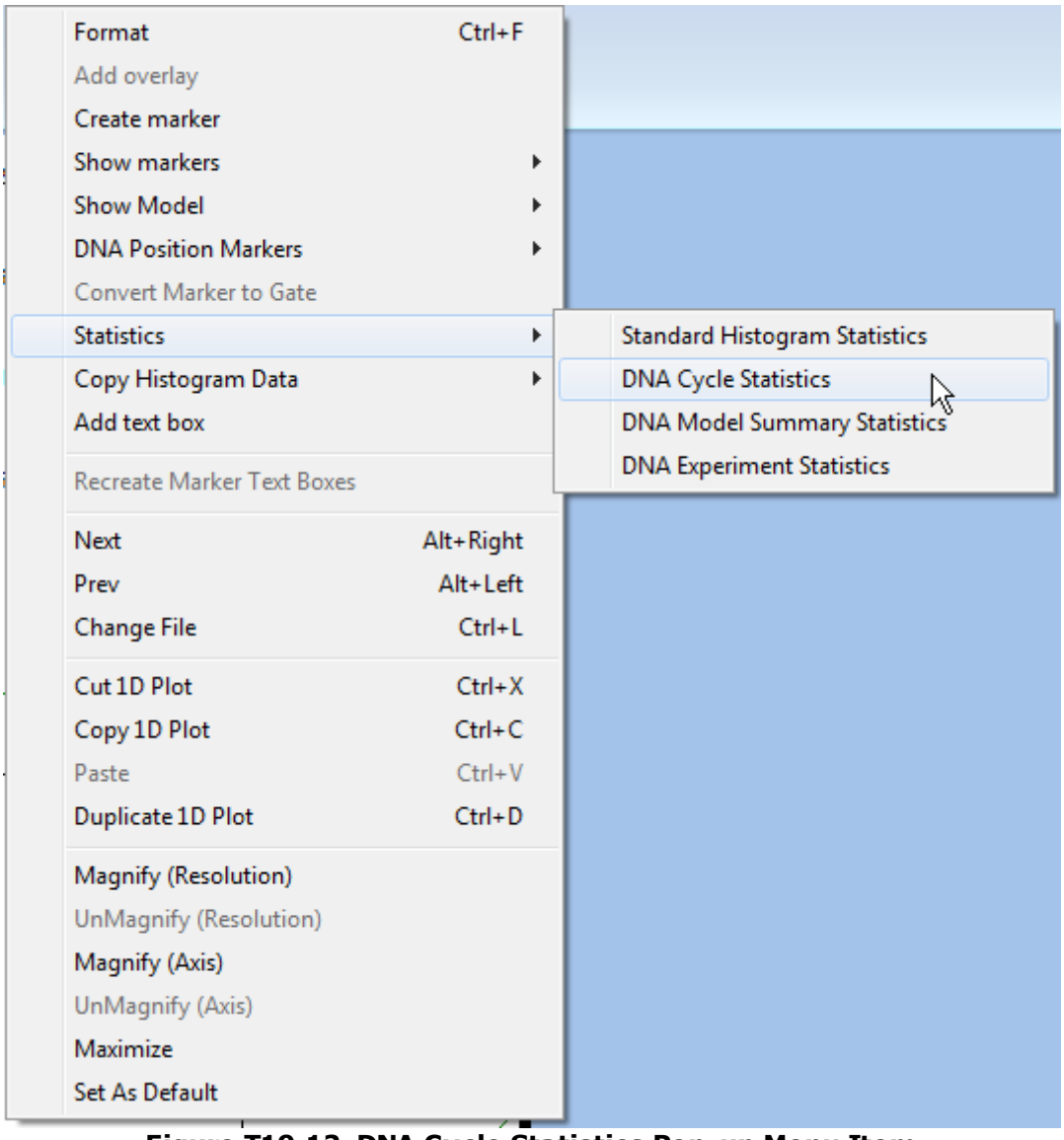

Figure T19.12 DNA Cycle Statistics Pop-up Menu Item

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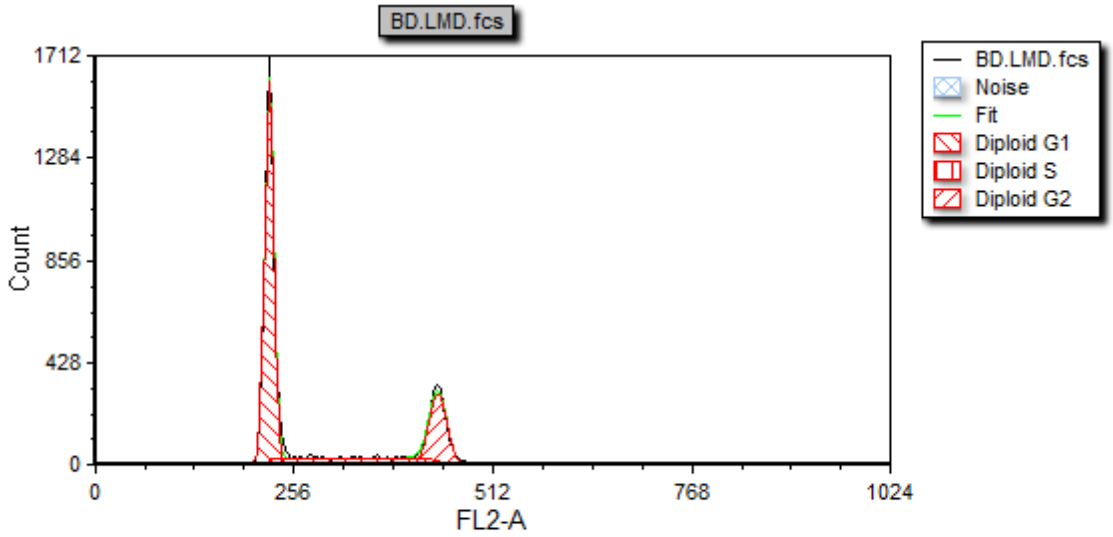
The **DNA Cycle Statistics** window of the current model (**Model 1**) will now appear (Figure T19.13). Note that you will have slightly different values for the statistics depending on how your singlet gate was drawn.

30. Move the **DNA Cycle Statistics** window beneath the plot (Figure T19.13).



Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	227.49	2.54	61.72	445.10	2.53	21.94	16.34	1.96	100.00	0.18

Figure T19.13 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 T19.12.

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 T19.11). 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**

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Cycle Statistics window (Figure T19.14).

34. Right-click on the DNA Histogram to bring up the associated pop-up menu.

35. Select **Statistics→DNA Experiment Statistics** from th**e** pop-up menu, previously shown in Figure T19.12.

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 T19.14).

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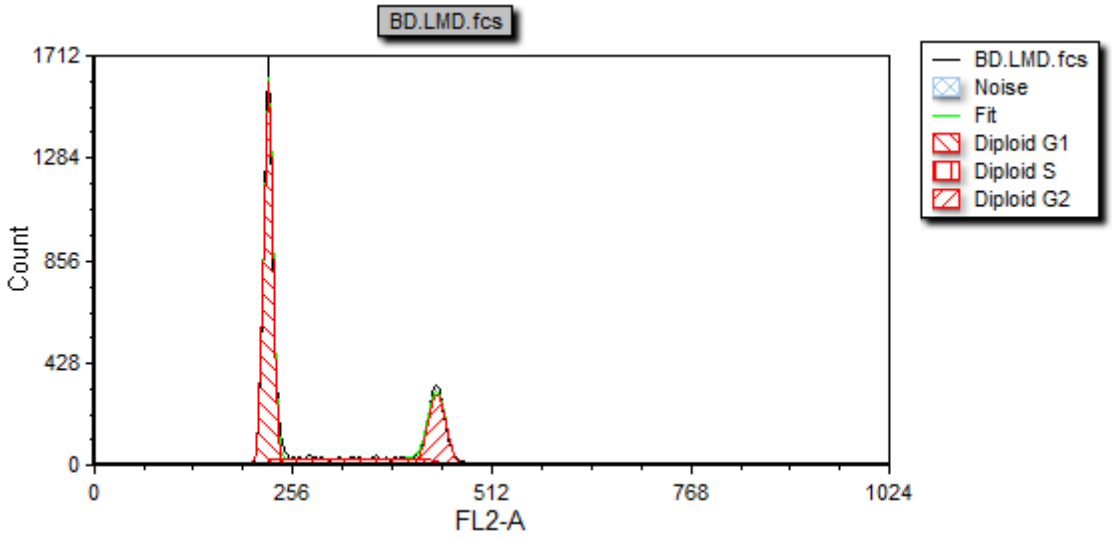

Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	B.A.D.
Diploid	227.49	2.54	61.72	445.10	2.53	21.94	16.34	1.96	100.00	0.18

Model	Dip %G2	Dip %S	Chis
SL S0	21.94	16.34	2.65
SL CL S0	18.32	17.03	2.39
+G2/G1 Fixed	20.14	17.46	4.18
+Aggregates	24.22	16.32	6.04
+S Order = 1	22.31	16.20	2.56
+CVs Fixed	22.28	16.23	2.53

Interpretation

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=16.3, %G2=21.9
The S Phase confidence is good

Experiment Statistics

Chi sq: 2.65
BAD: 0.18
Number of cells: 9647.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

Figure T19.14 DNA Histogram with All of the MultiCycle Statistics Windows

In the next section, we will perform an expanded analysis with Autofit.

34.19.2 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.

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Please refer to Figure T19.15 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 T19.15).
5. Repeat steps 2 through 4 for the top upper left corner of the gate.

The gate should now appear similar to Figure T19.15, modified to include cells with higher content DNA, but still excluding most of the doublets.

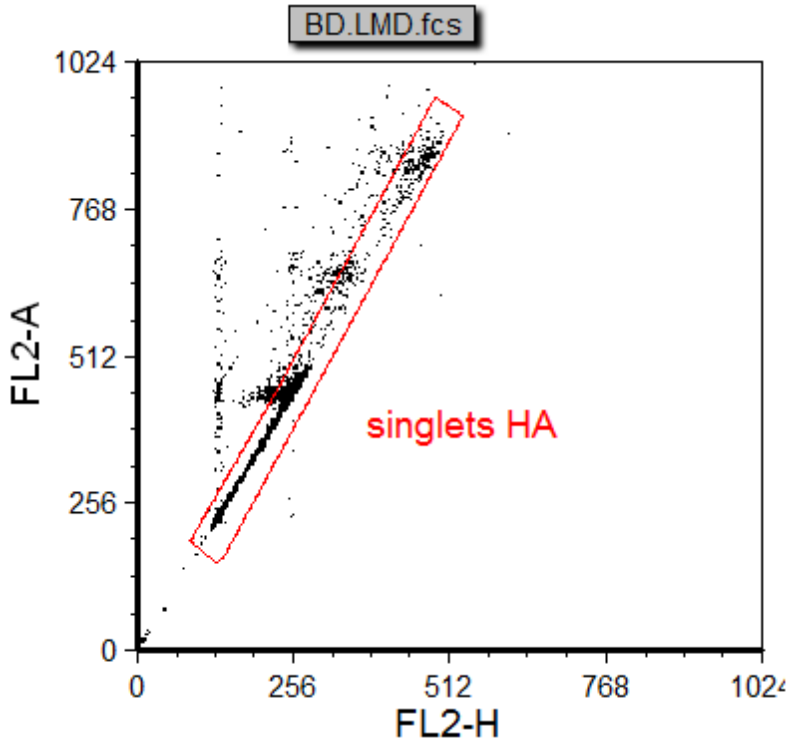

Figure T19.15 Gate Modified to Include Cells with Higher DNA Content

The Autofit function of MultiCycle updates the cell cycle analysis, as shown in Figure T19.16. *Note:* Figure T19.16 shows the MultiCycle analysis using the

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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 T19.16. 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.

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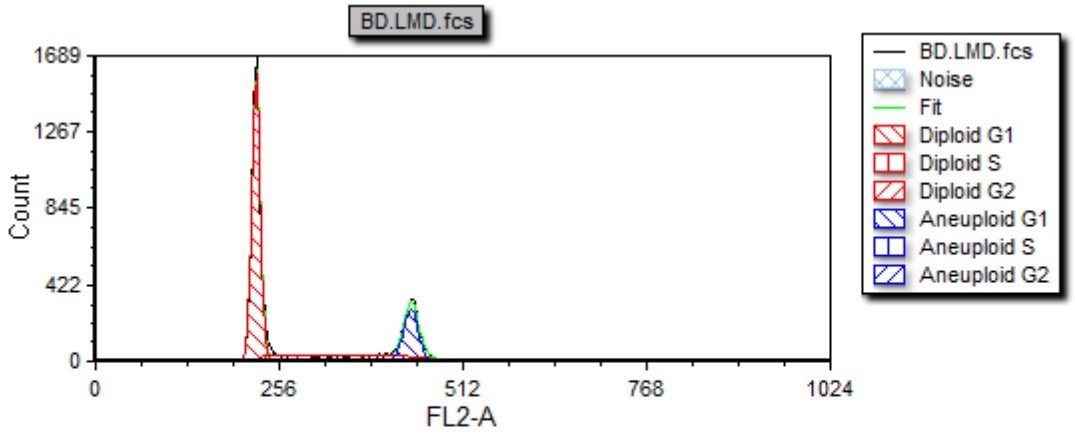

Cycle	G1 Mean	G1 CV	%G1	G2 Mean	G2 CV	%G2	%S	G2/G1	%Total	D.I.	B.A.D.
Diploid	227.48	2.56	76.01	460.58	2.56	2.46	21.53	2.03	78.23	n/a	0.17
Aneuploid A	443.67	2.23	84.49	853.52	2.23	5.96	9.55	1.92	21.77	1.95	0.11

Model	Dip %G2	Dip %S	A %G2	A %S	Chis
SL S0	2.46	21.53	5.96	9.55	1.72
SL CL S0	2.50	21.53	6.16	7.36	1.64
+G2/G1 Fixed	9.57	19.14	4.06	16.02	2.16
+Aggregates	0.00	20.21	4.98	7.09	1.81
+S Order = 1	2.31	21.38	5.94	9.29	1.71
+CVs Fixed	0.02	22.10	5.76	8.56	1.75

Interpretation

MultiCycle suggestions (a guideline only):
A near-tetraploid aneuploid DNA content is observed.
The tetraploid %S=9.55, %G2=5.96
The S Phase confidence is fair
Note: % cells, inter-model error,

Experiment Statistics

Chi sq: 1.72
Average %S: 18.92
Average %S aneuploid: 9.55
BAD: 0.18
Number of cells: 10068.00
Number of cycles: 2.00
Cycle fit model: 2 Cycle

Figure T19.16 Autofit MultiCycle Analysis with Modified gate

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 T19.12 1070. For more information on formatting, please see the tutorial on Formatting Plots 620. First, we will close the **DNA Experiment Statistics** window so that we have room to expand the **DNA Model Summary Statistics** window.

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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 T19.17).

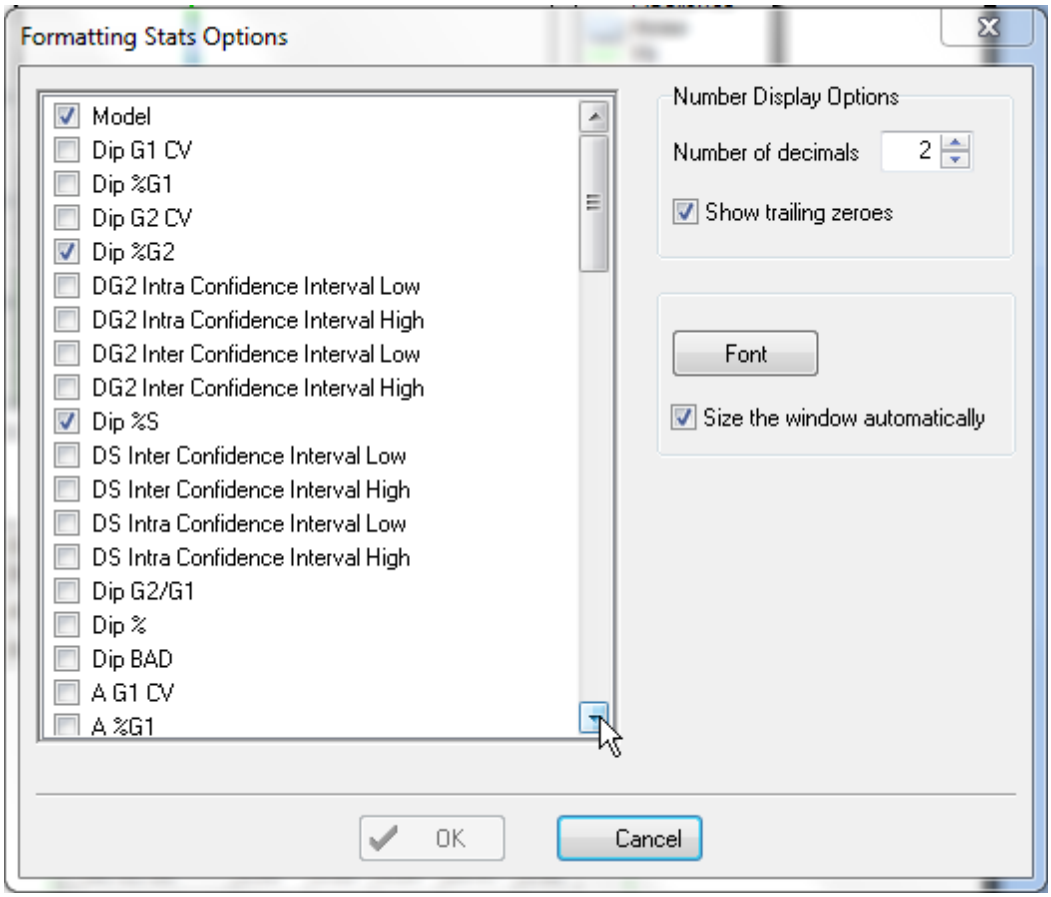

Figure T19.17 Formatting Stats Options Dialog for DNA Model Summary Statistics

11. Select the down arrow, shown by the cursor on Figure T19.17.

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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 T19.18.
14. Click **OK**.

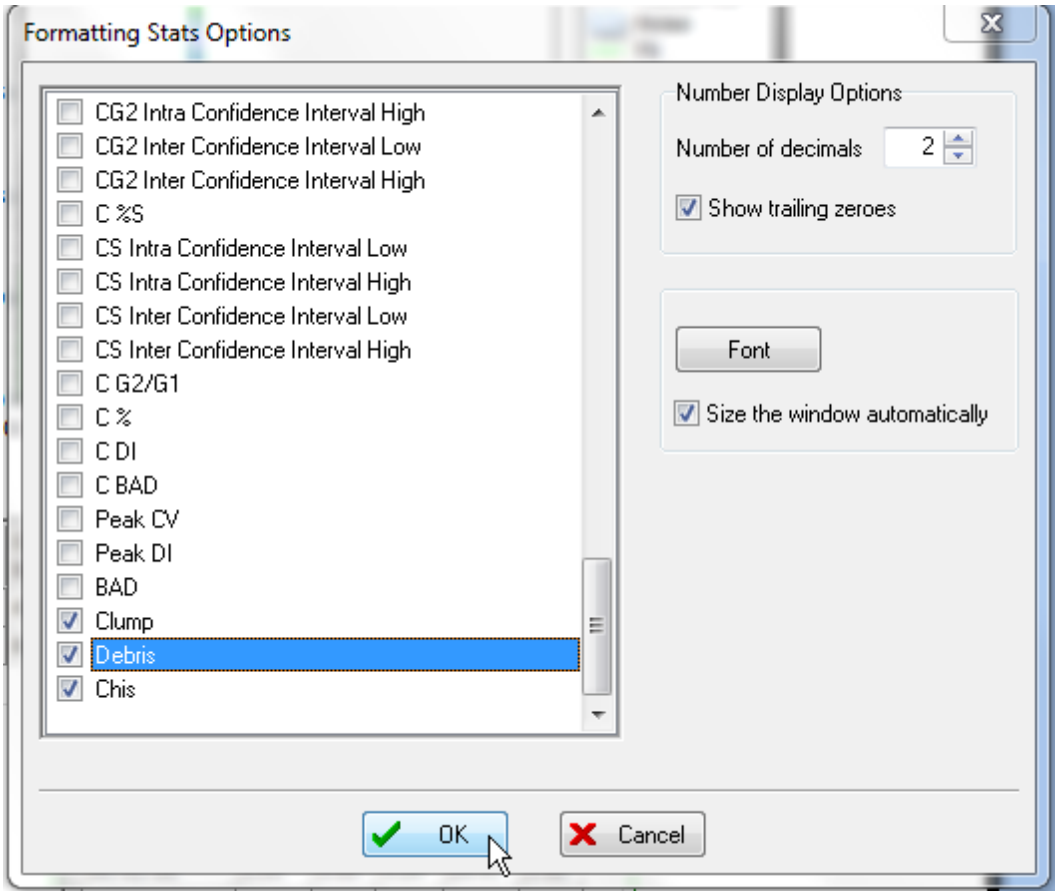

Figure T19.18 Selecting Clump and Debris from the Formatting Stats Options Dialog

The **DNA Model Summary Statistics** table will now update to show the selected statistics as shown in Figure T19.19.

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Model	Dip %G2	Dip %S	A %G2	A %S	Clump	Debris	Chis
SL S0	1.12	22.21	5.89	8.01	-1.00	0.27	1.63
SL CL S0	1.19	22.18	6.06	6.40	0.75	0.27	1.60
+G2/G1 Fixed	9.59	19.16	3.89	14.43	-1.00	0.27	2.05
+Aggregates	0.00	20.21	5.10	6.26	0.60	0.28	1.74
+S Order = 1	1.14	21.97	5.83	7.82	-1.00	0.28	1.62
+CVs Fixed	0.00	22.47	5.74	7.34	-1.00	0.30	1.70

Figure T19.19 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 T19.20).

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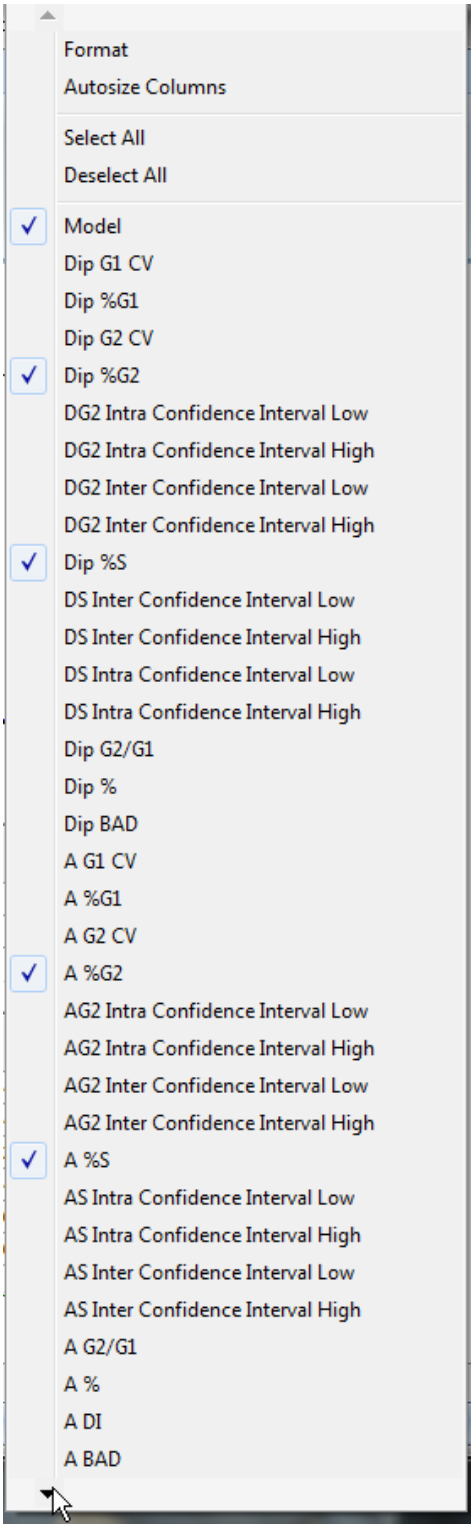

Figure T19.20 DNA Model Summary Statistics Pop-up Menu

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- Select the arrow in the lower left corner of the menu to scroll down, indicated by the cursor in Figure T19.20.
- Continue to hold down the left mouse button until the menu scrolls all the way down (Figure T19.21).
- Left-click next to **Clump**, as indicated by the cursor on Figure T19.21, 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 T19.21.

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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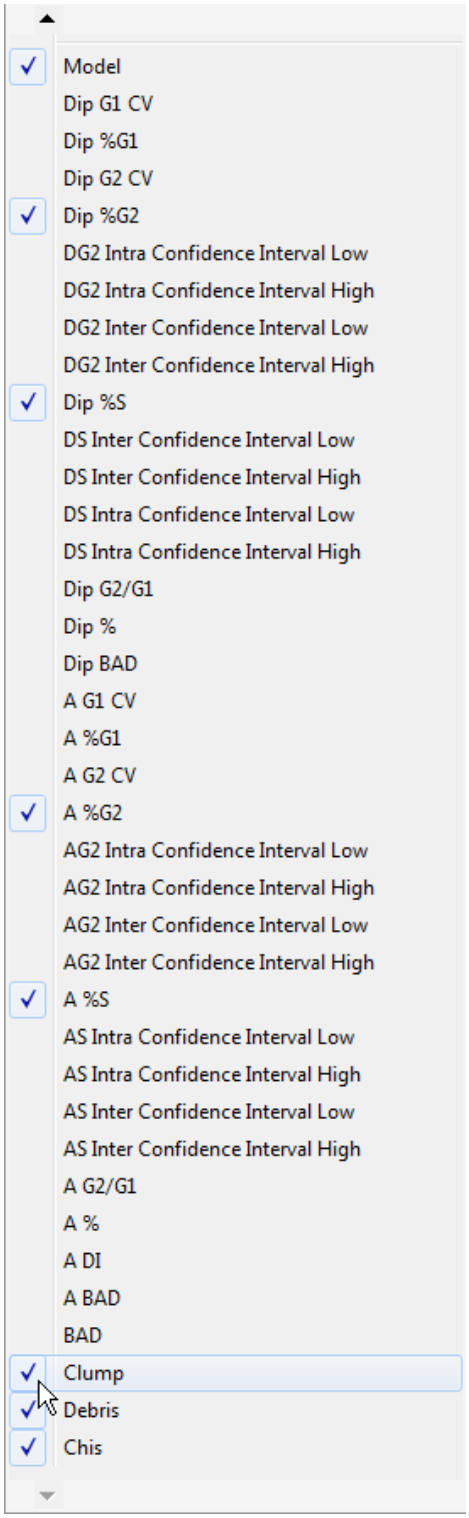

Figure T19.21 Deselecting Clump from the DNA Model Summary Statistics Pop-up Menu

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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 T19.22. 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	1.12	22.21	5.89	8.01	1.63
SL CL S0	1.19	22.18	6.06	6.40	1.60
+G2/G1 Fixed	9.59	19.16	3.89	14.43	2.05
+Aggregates	0.00	20.21	5.10	6.26	1.74
+S Order = 1	1.14	21.97	5.83	7.82	1.62
+CVs Fixed	0.00	22.47	5.74	7.34	1.70

Figure T19.22 DNA Cell Cycle Model with the Lowest Chis (Chi-square) Value

In the next section, we will format a DNA histogram 1092.

34.19.3 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.

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1. Click inside the MultiCycle histogram to select it.
2. Select the **Format→Plot Options→Overlays** command.

The **Formatting Overlays** dialog window appears, shown in Figure T19.23. Please refer to Figure T19.23 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**.

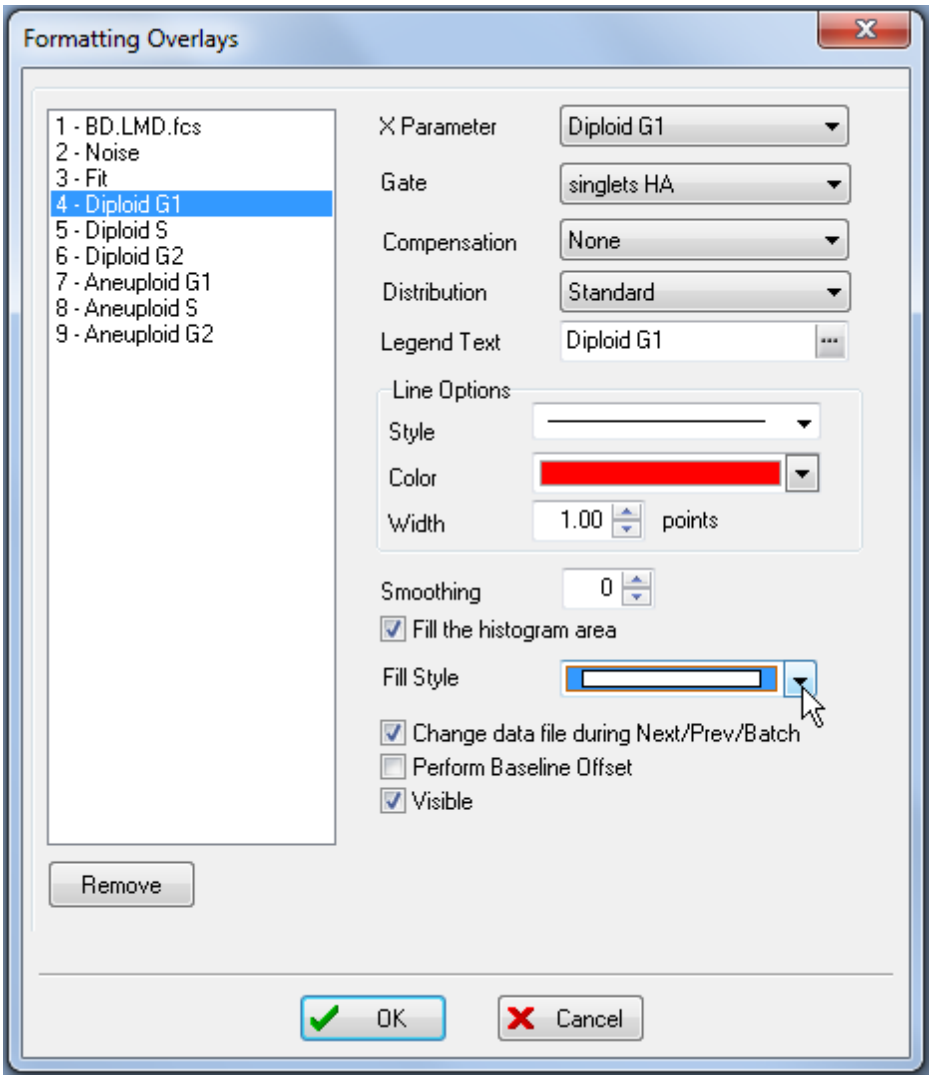

Figure T19.23 Formatting Overlays Dialog for a MultiCycle DNA Histogram

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The MultiCycle histogram will now update to reflect the changes as shown in Figure T19.24. Alternatively, we could have accessed the histogram Overlay menu by right-clicking on the DNA histogram to bring up the pop-up menu and selecting **Format** and then **Overlays**.

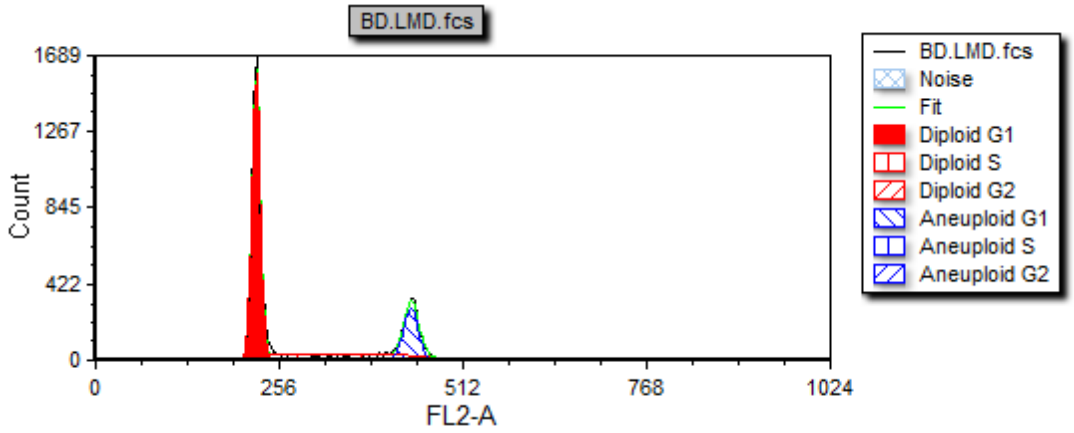

Figure T19.24 MultiCycle DNA Histogram Updated with Solid Fill on the Diploid Peak

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 T19.25.

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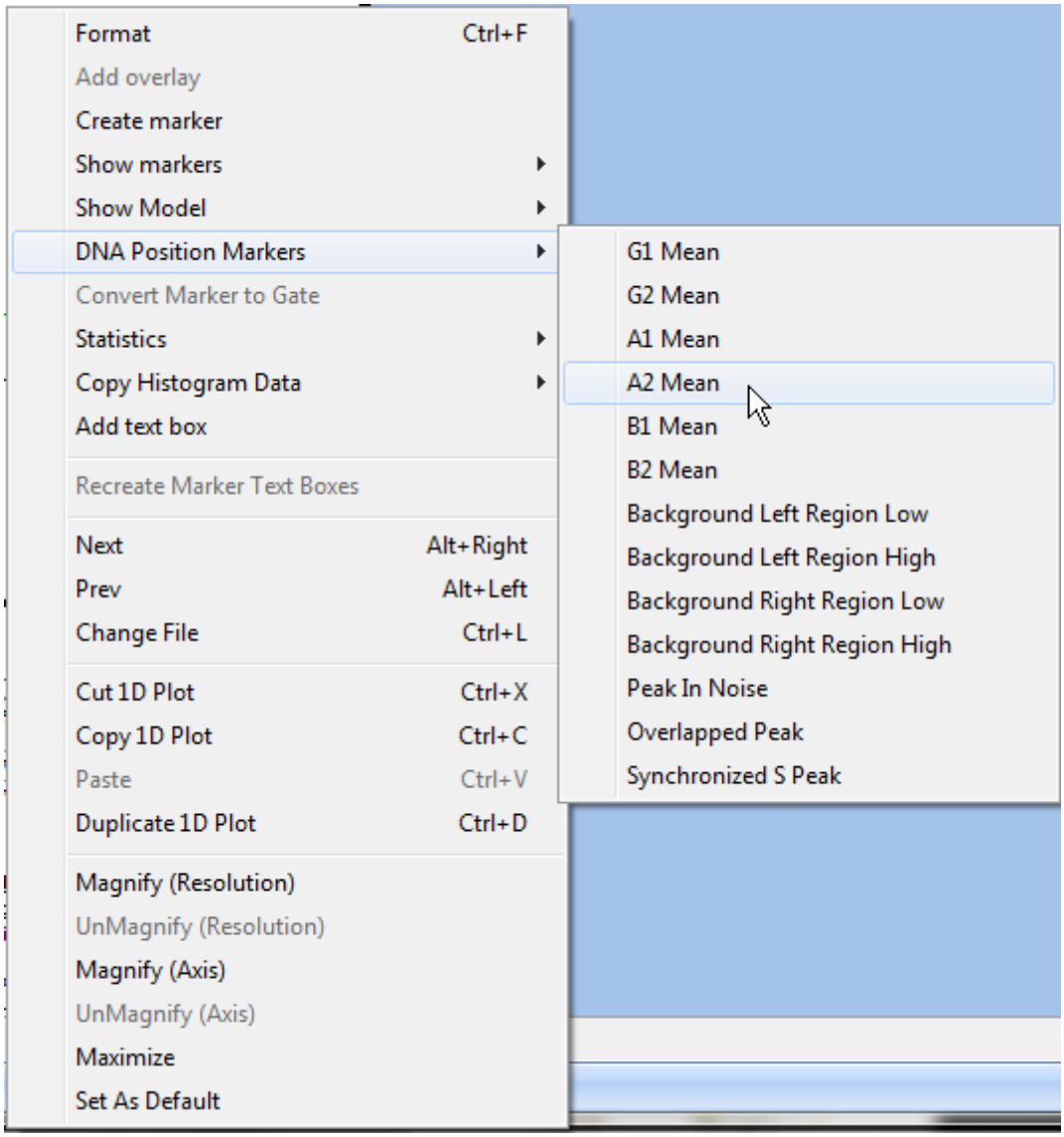

Figure T19.25 Selecting DNA Position Markers on the Pop-up Menu

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 T19.26 for the following steps.

- Select **DNA→Position Markers** from the category on the left, shown outlined in blue.

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- Select the box **Show position marker on the plot**, indicated by the cursor.
- Select **A2 Mean**, shown highlighted in blue.
- Click **OK**.

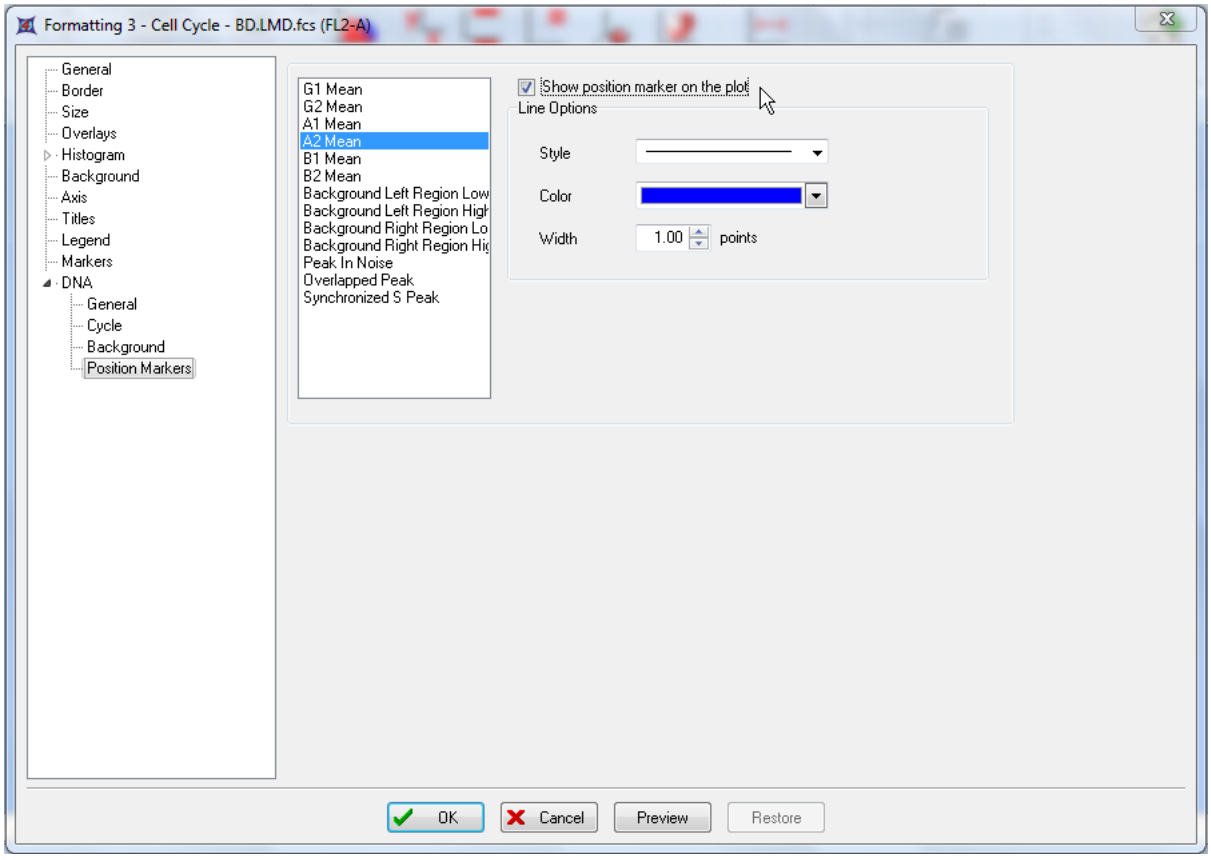

Figure T19.26 Formatting DNA Histogram Position Markers

A vertical line marking the location of the mean of the aneuploid G2 peak appears on the Multicycle DNA histogram (Figure T19.27).

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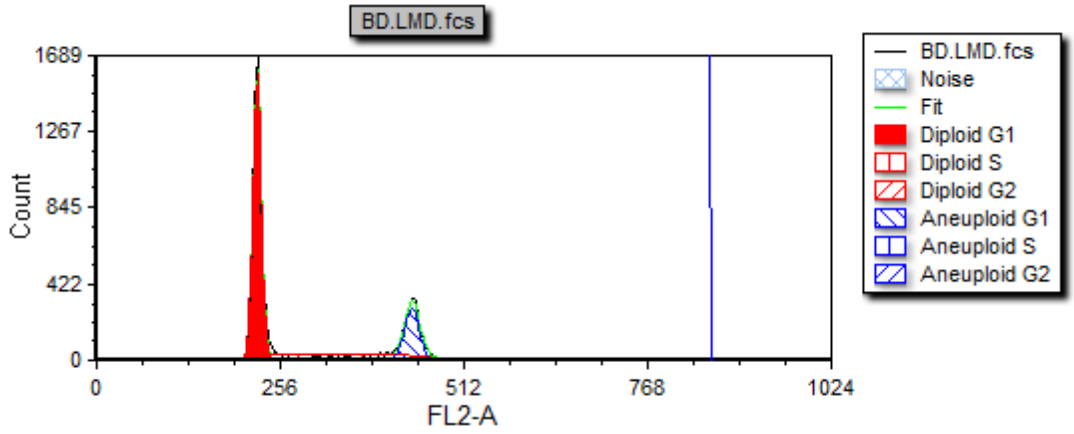

Figure T19.27 MultiCycle Histogram Updated with a DNA Position Marker on the Aneuploid G2 Mean

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 T19.25).

Please refer to Figure T19.28 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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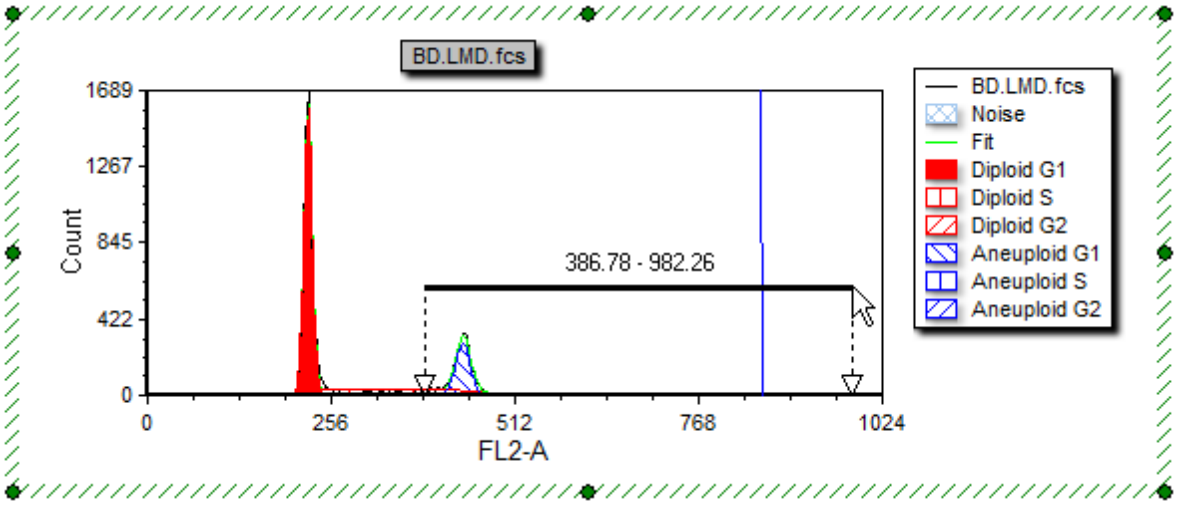

Figure T19.28 Selecting an Area on the X-axis to Zoom

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 T19.29.

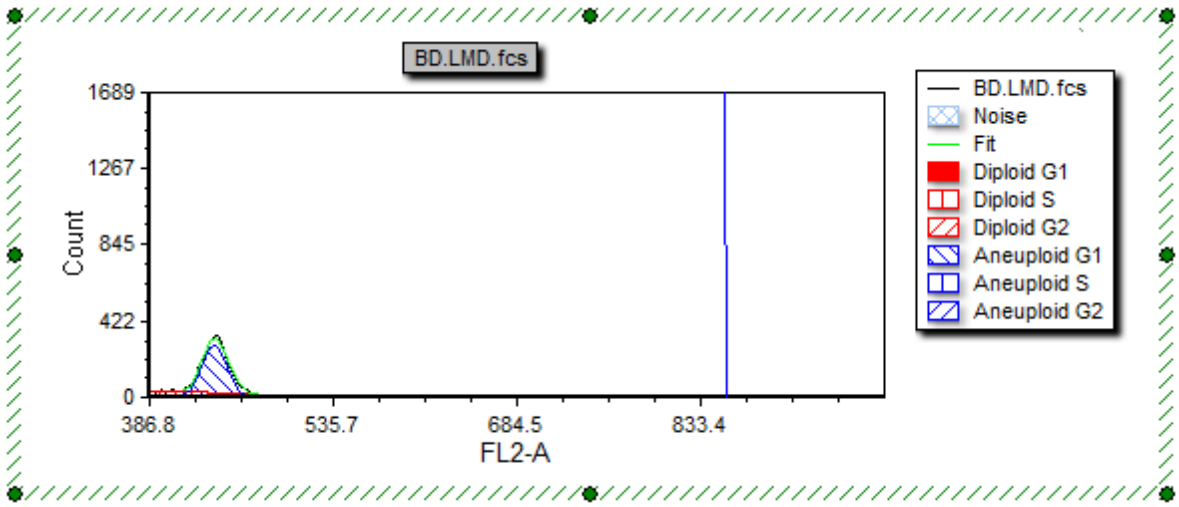

Figure T19.29 Histogram Zoomed

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 T19.30 for the

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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**.

Figure T19.30 Formatting Axes Dialog

The MultiCycle histogram updates, scaling the y-axis from 0 to 300 as shown in

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Figure T19.31.

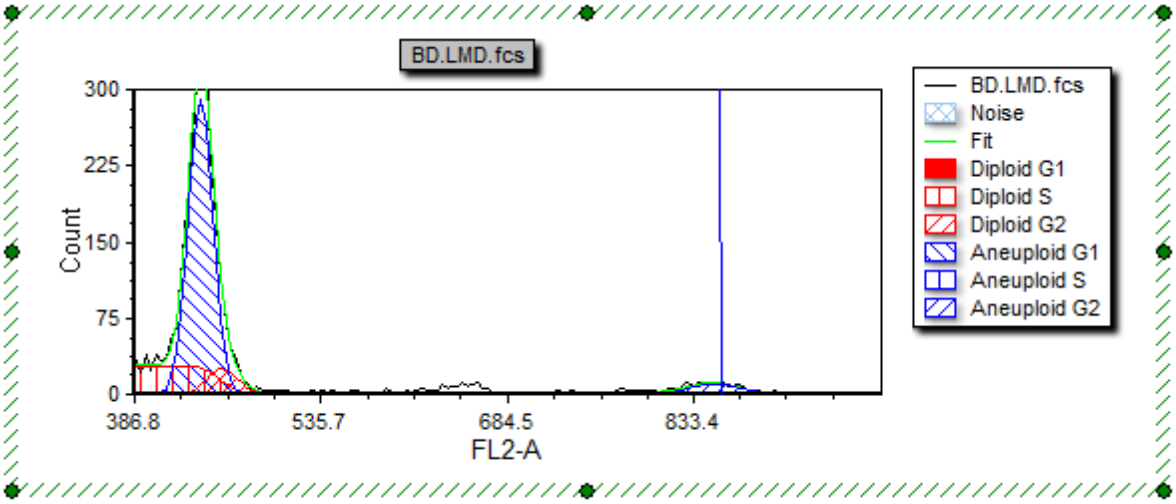

Figure T19.31 MultiCycle Histogram with Rescaled Y-axis

Next, we will perform MultiCycle analysis of 3 cycle data.

34.19.4 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 **Application Button→New Layout**.
2. Select the **Insert→1D Plots→Multicycle DNA** command (Figure T19.4).
3. Click on a blank area of the layout.

The **Select a Data File** dialog appears, as previously shown in Figure T19.1.

4. Navigate to the *FCS Express Sample Data* folder.
5. Select the file **ASCII4.fcs**.
6. Click **Open**.

The MultiCycle DNA histogram now appears as shown in Figure T19.32. Note the two aneuploid populations, shown in blue and green, that make up a majority of this histogram.

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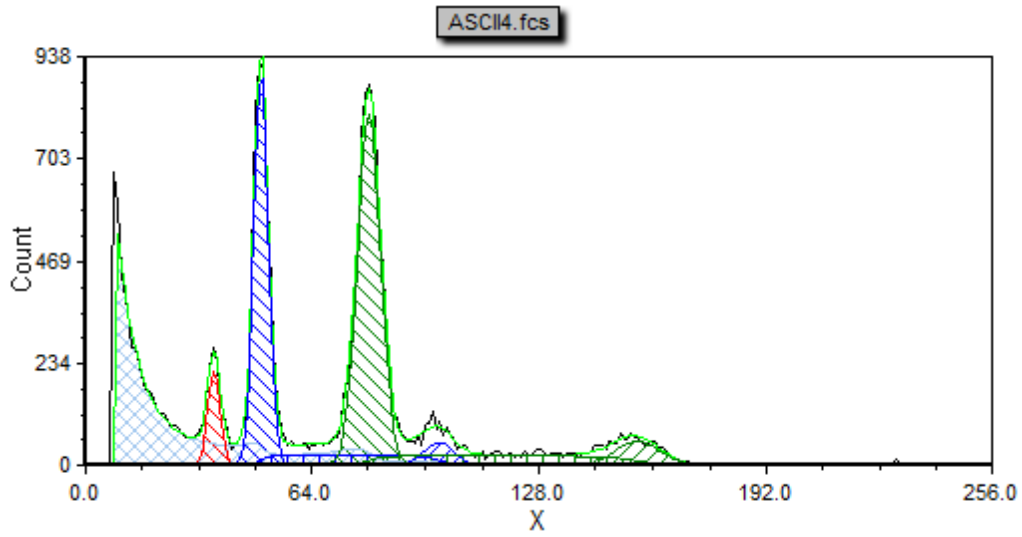

Figure T19.32 MultiCycle DNA Histogram with Two Aneuploid Populations

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→1D Plots→Histogram** command.
8. Click on a blank area of the layout.

The **Select a Data File** dialog appears, as previously shown in Figure T19.1 1070.

9. Navigate to the *FCS Express Sample Data* folder.
10. Select the file **ASCII8.fcs**.
11. Click **Open**.

The histogram of ASCII8.fcs appears, as shown in Figure T19.33. 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

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this case.

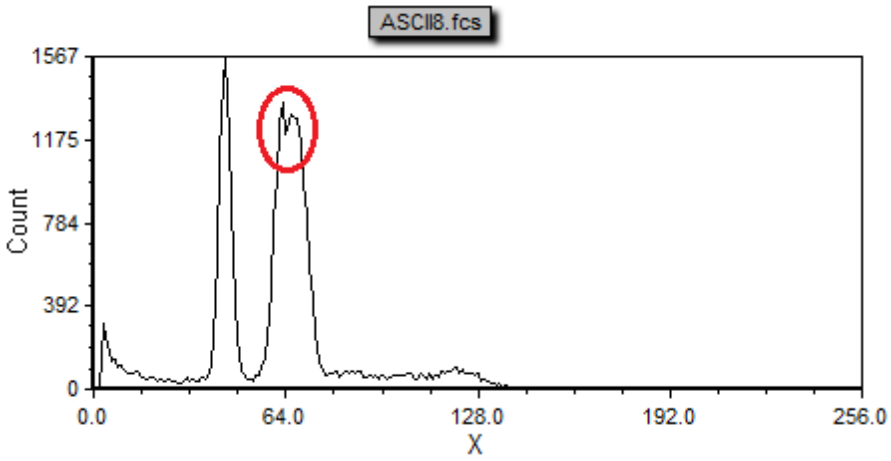

Figure T19.33 ASCII8.fcs Histogram

12. Select the **Insert→1D Plots→Multicycle DNA** command (Figure T19.4 1070).

13. Click on a blank area of the layout.

The **Select a Data File** dialog appears, as previously shown in Figure T19.1 1070.

14. Navigate to the *FCS Express Sample Data* folder.

15. Select the file **ASCII8.fcs**.

16. Click **Open**.

The MultiCycle DNA histogram now appears as shown in Figure T19.34. 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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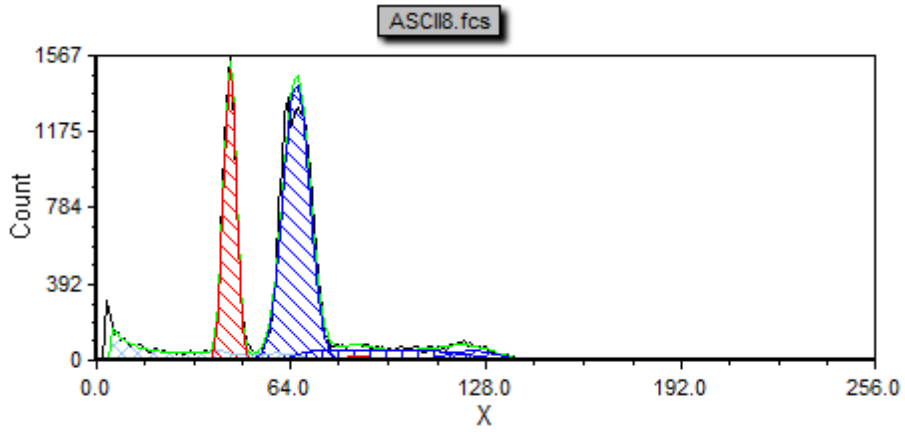

Figure T19.34 Autofit MultiCycle Analysis of ASCII8.fcs

17. Click inside the MultiCycle histogram of ASCII8.fcs to select it.
18. Select the **Multicycle→Fit Parameters→Fit Parameters** command.

The **Formatting Fit Model Options** dialog appears. Please refer to Figure T19.35 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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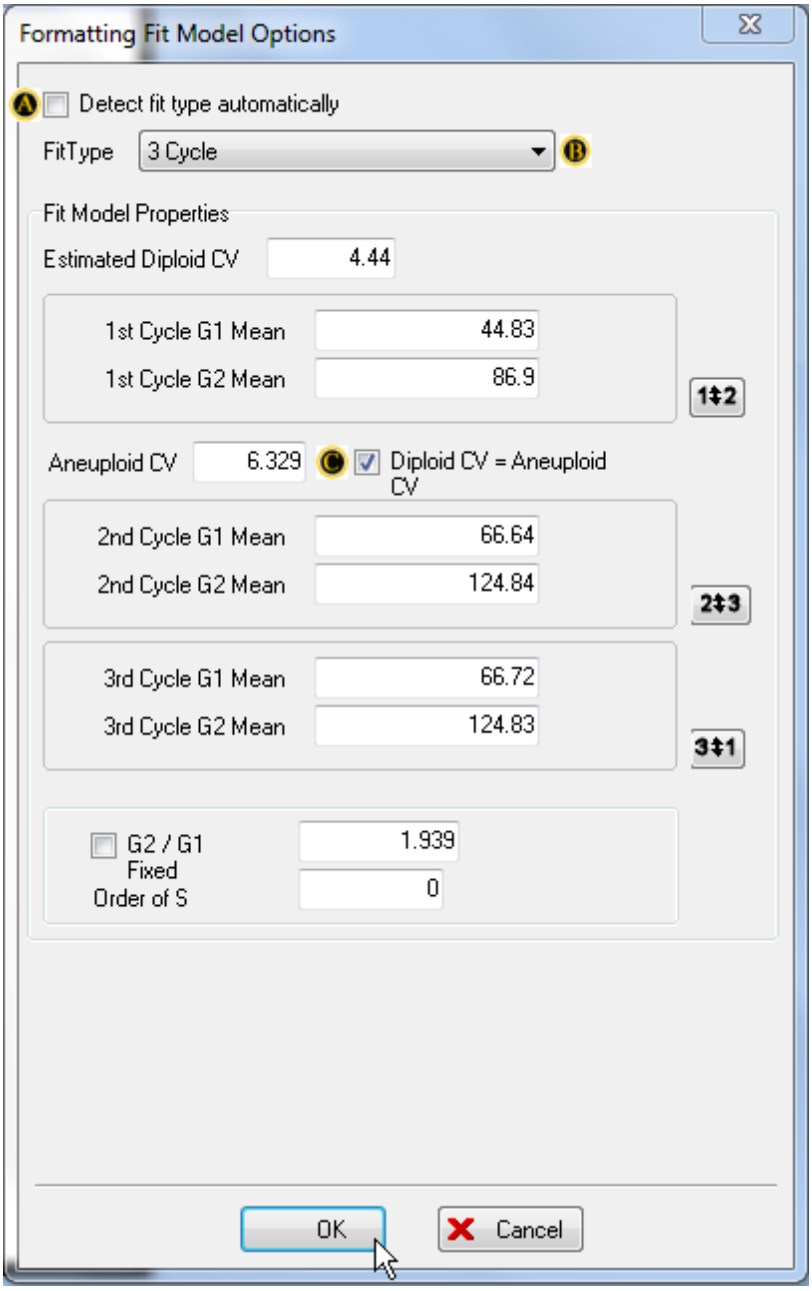

Figure T19.35 Formatting Fit Model Options Dialog

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 T19.36.

Alternatively, you can turn off Autofit by deselecting it on the **Multicycle** tab and

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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 T19.35.

- Select the box next to **Diploid CV = Aneuploid CV** (Figure T19.35).
- Click **OK**.

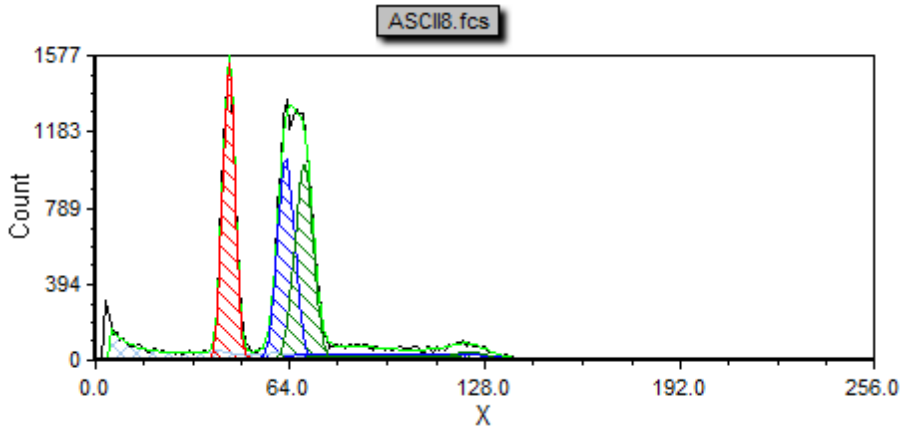

Figure T19.36 Manual MultiCycle Fit

34.20 Proliferation

One of the most common methods used to study cell proliferation[462] 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.

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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 462 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 contain a marker that is linked to the Non Proliferating Control gate (M1) and is labeled Proliferation Histogram in the title. The other histogram is labeled Background Noise Histogram and 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 58). 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.

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We will begin by learning how to insert a proliferation plot and how to convert a histogram to a proliferation plot1107.

34.20.1 Inserting Proliferation Plots

A proliferation plot can be created just like any other plot in FCS Express and regular histograms610 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** icon (Figure T20.1).

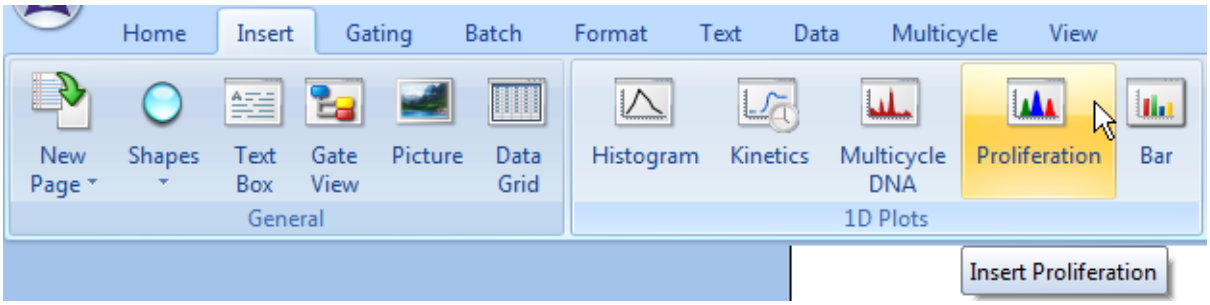

Figure T20.1 Inserting a Proliferation Plot

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.
5. Choose the **Format Tab** from the ribbon→**Plot Options→Overlays**.
6. Change the **X Parameter** to **CellTrace Violet-H**.
7. Change the **Gate** to **Sytox Live Cells Gate**.
8. Click **OK**.

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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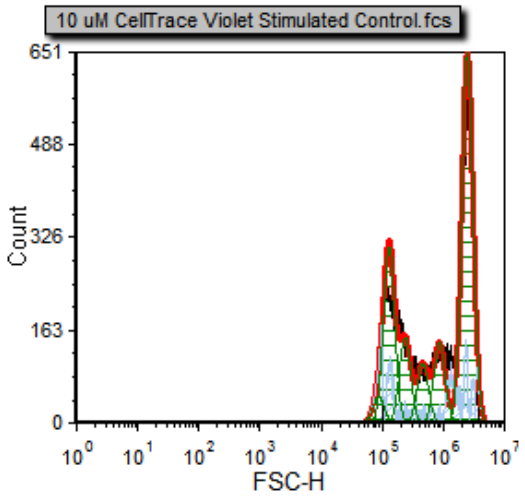

Figure T20.2a Proliferation plot before formatting the X Axis parameter and applying the Sytox gate.

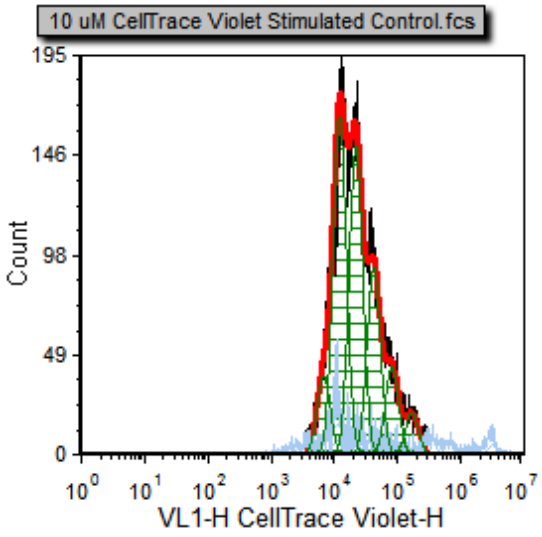

Figure T20.2b Proliferation plot after formatting the X Axis parameter and applying the Sytox gate.

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** icon.
12. Select **Proliferation** from the drop down (Figure T20.3).

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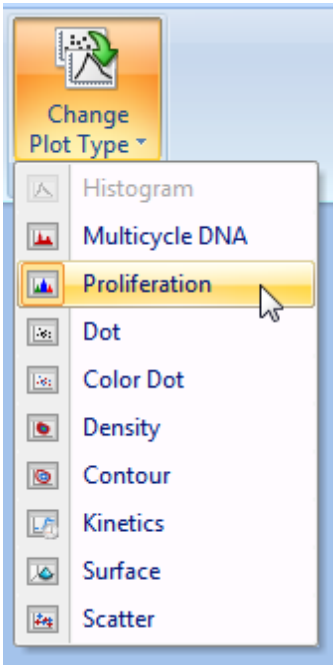

Figure T20.3 Choosing to change to plot type from a histogram to a proliferation plot.

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 1109 we will learn how to further modify the default fit for use with the data.

34.20.2 Fitting Proliferating Cells

In the course of this section we will:

- Learn how the curve fitting algorithm and parameters are fitted to a histogram.
- Use tokens to define a Fixed Starting Generation and Background.
- Insert proliferation position markers.

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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 T20.4a). 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 Objects→Previous** command to decrease one file previous to the file 10 uM CellTrace Violet Control.fcs (Figure T20.4b).

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 Objects→Next** command to advance two files to 10 uM CellTrace Violet Stimulated Sample.fcs (Figure T20.4c).

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.

5. Select the **Data Tab→Change Data on All Objects→Next** command to the end of the Data List to Unstained Control.fcs (Figure T20.4d).

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 fitting the background peak as it it was a

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population of proliferating cells. We will modify the fitting parameters later on to tell FCS Express to treat these as the background.

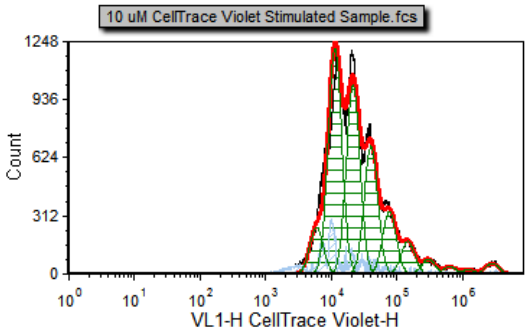

Figure T20.4a Proliferation positive control

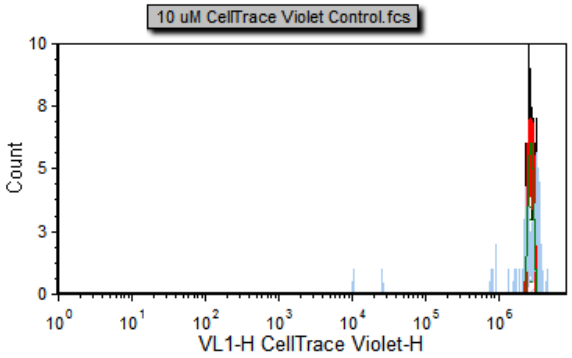

Figure T20.4b Proliferation negative control

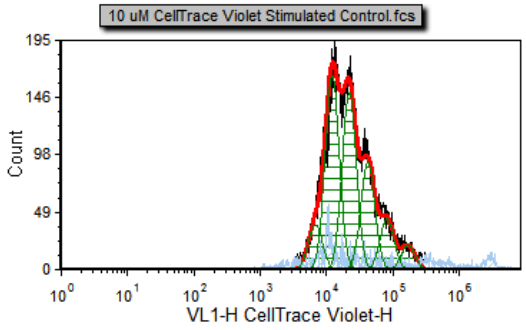

Figure T20.4c Proliferation stimulated sample

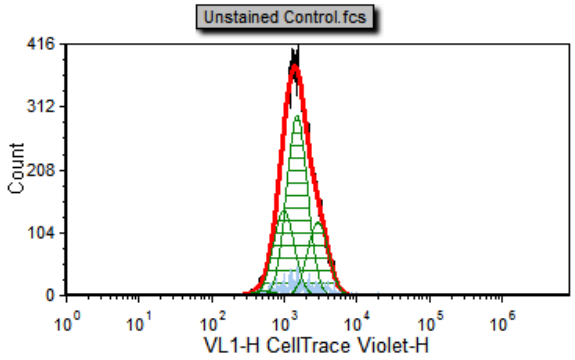

Figure T20.4d Proliferation unstained control

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.

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.

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Note that there are cells in the proliferation stimulated sample (Figure T20.4c) 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 Objects→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 T20.5).

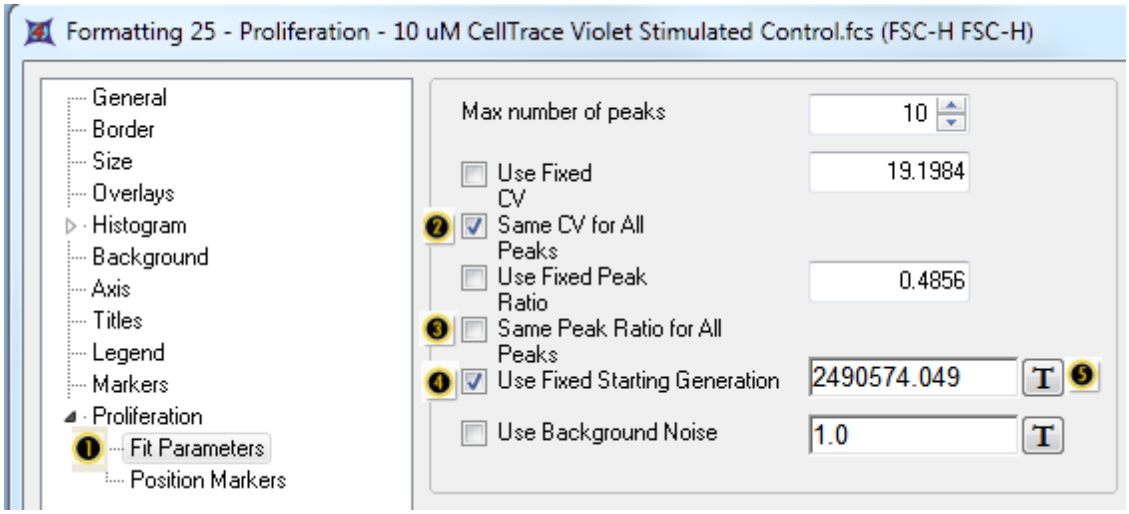

Figure T20.5 Adjusting fit parameters in the formatting dialog.

9. Navigate to the **Proliferation→Fit Parameters** ❶ category (Figure T20.5).

10. Check the **Use Fixed Starting Generation** check box ❹ (Figure T20.5).

11. Delete the numbers in the white text box next to the **Use Fixed Starting Generation** option (Figure T20.5).

12. Click on the T ❺ token symbol next to the text box (Figure T20.5).

    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

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in blue below (Figure T20.6).

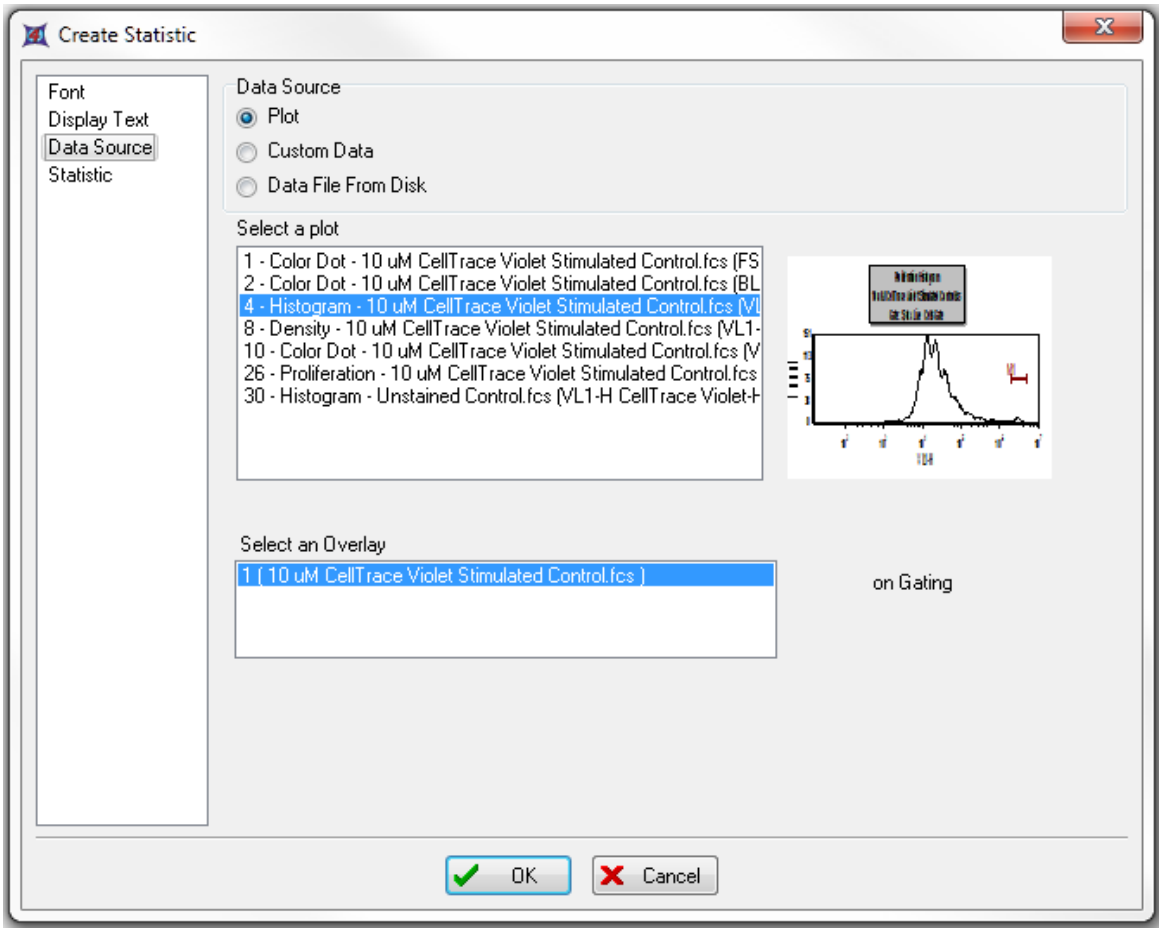

Figure T20.6 Select the histogram, plot 4, as the data source for the token.

15. Click on the **Statistic** category on the left.
16. Set the **marker** to M1.
17. Select **Geometric Mean** from the **Statistic** list.
18. Click **OK**.
19. Click on the **Proliferation→Position Markers** category.
20. Check the box for **Show position marker on the plot** with **Undivided cells peak** highlighted in blue. (Figure T20.7).

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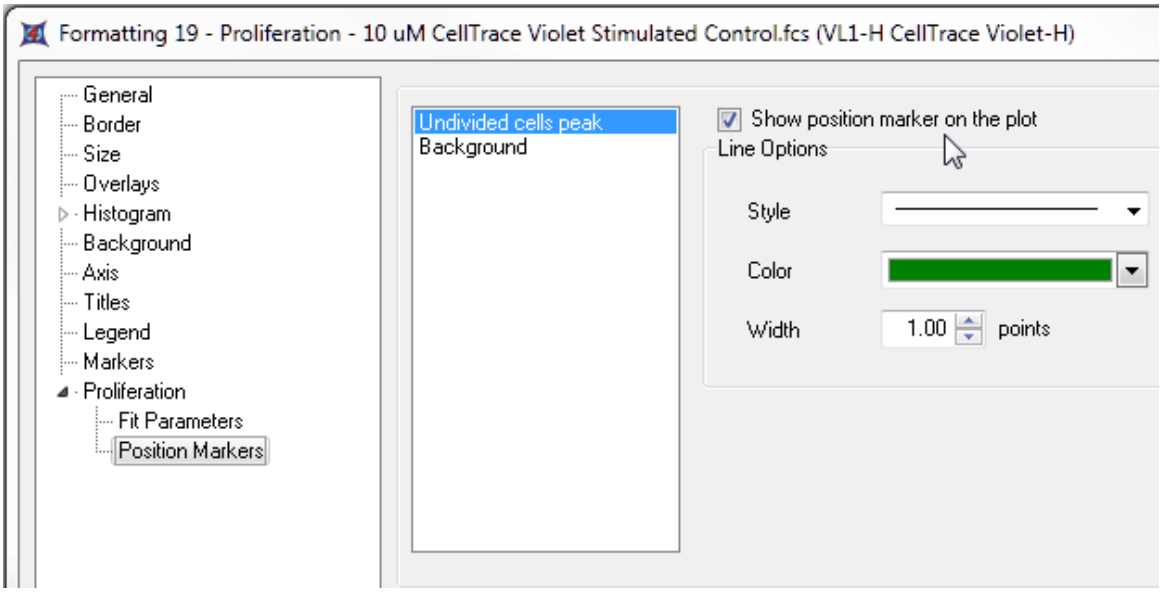

Figure T20.7 Checking the Show position marker on the plot box for the Undivided cells peak.

21. Click **OK**.

The proliferation histogram should now look like Figure T20.8.

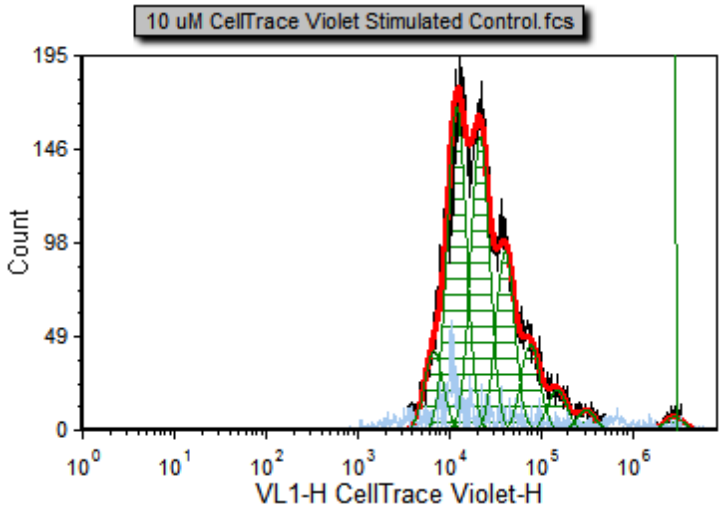

Figure T20.8 The proliferation plot after adjusting the starting generation and choosing to display the Undivided cells peak marker.

Notice on the Gating page there is a histogram labeled Background Noise with a marker labeled Background noise. 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

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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 T20.5.

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 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**.
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 T20.9a with the Starting Generation and Background noise based on the markers from the histogram in Figure T20.9b.

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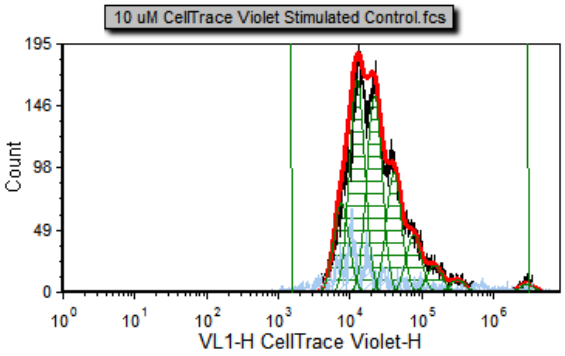

Figure T20.9a Proliferation plot with Background Noise Position and Starting Generation Markers defined.

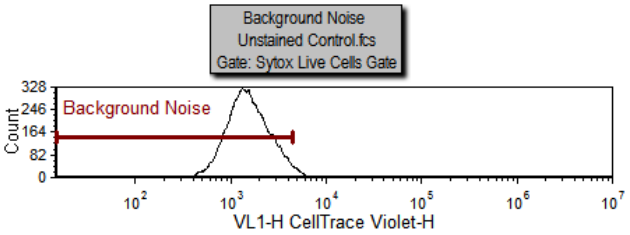

Figure T20.9b Histogram with markers that define the starting generation and background noise for proliferation analysis.

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. 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 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** ❸ option (Figure T20.5).
- 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** ❷ option, as shown above Figure T20.5.
- 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 1117.

34.20.3 Formatting a Proliferation Histogram

In the course of this section we will :

- Learn how to adjust the proliferation overlays to better visualize the populations of interest.

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- 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 from the Tutorial Sample Data folder.
2. Click once on the proliferation histogram to select it.
3. Select the **Format Tab→Plot Options→Overlays** command.

   The **Formatting Overlays** dialog will appear (Figure T20.10).
4. Select the **Noise** overlay from the left (Figure T20.10a).
5. Uncheck the **Visible** box.
6. Select the **Generation 6** overlay from the left (Figure T20.10b).
7. Change the **Color** to Blue.
8. In the **Fill Style** drop down select the 1^{st} option for a completely filled rectangle.
9. Select the **Generation 8** overlay from the left (Figure T20.10c).
10. In the **Fill Style** drop down Select the 1^{st} option for a completely filled rectangle.
11. Click **OK**.

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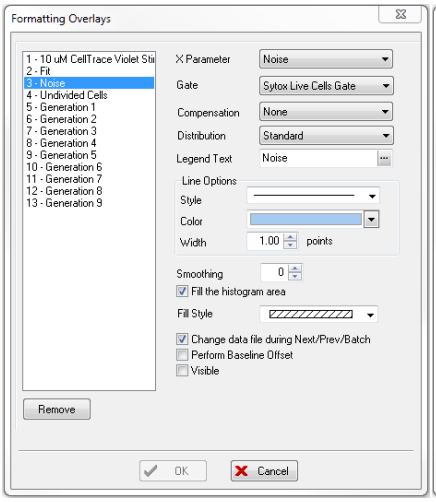

Figure T20.10a Removing the noise overlay from the proliferation plot.

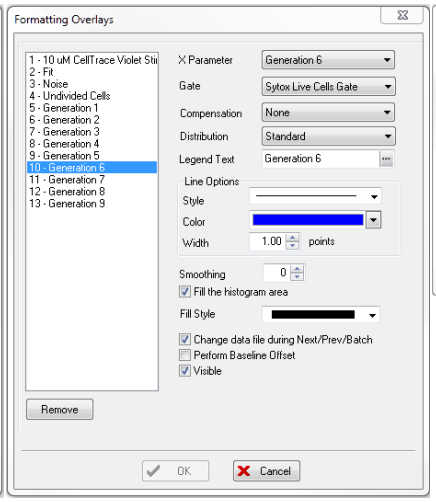

Figure T20.10b Coloring generation 6 in the proliferation plot blue.

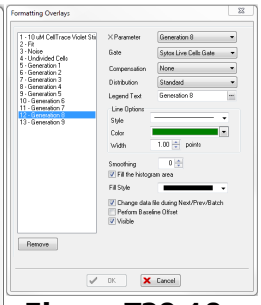

Figure T20.10c Coloring generation 8 in the proliferation plot green.

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 (Figure T20.11). 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.

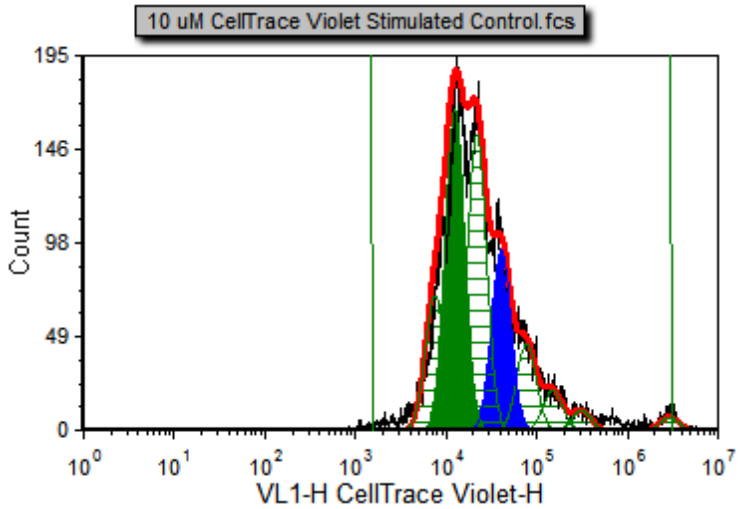

Figure T20.11 The formatted proliferation plot displaying different generations in different colors with the noise overlay removed.

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

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changing these parameters affect these two statistics. The combination of settings that provides the least **RMS error** and **R-Chi Square** is mathematically 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 Figure T20.12.

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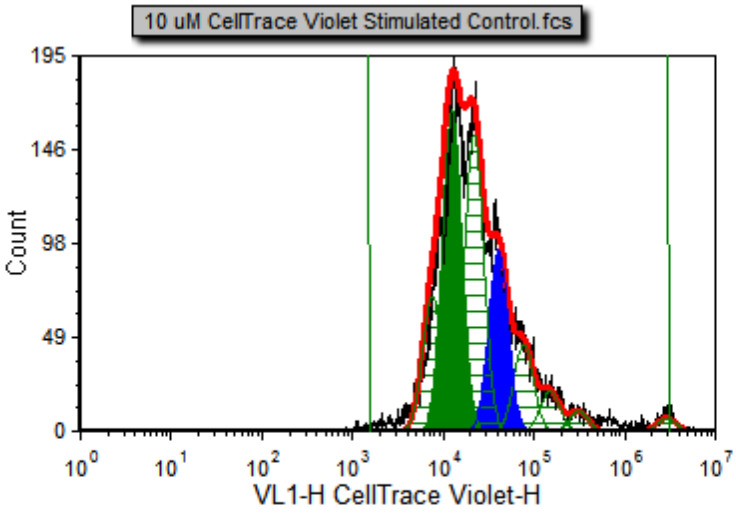

Division Index	Proliferation Index	% Divided	Peak Ratio	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square	Starting Generation Mean	Background Noise
76.58	43.11	55.72	0.52	22241.00	512.60	8.58	4.93	2913319.40	1489.57

Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells
Undivided Cells	2569344.21	6.00	22.23	2.33	0.00	227.00
Generation 1	0.00	0.00	0.00	0.00	0.00	0.00
Generation 2	0.00	0.00	0.00	0.00	0.00	0.00
Generation 3	295111.05	10.00	22.23	2.74	0.00	363.00
Generation 4	141970.77	21.00	22.23	2.90	0.48	808.00
Generation 5	73827.72	45.00	22.23	3.08	0.51	1749.00
Generation 6	40858.80	94.00	22.23	3.26	0.52	3650.00
Generation 7	21580.80	154.00	22.23	3.46	0.52	6033.00
Generation 8	12321.30	167.00	22.23	3.67	0.54	6552.00
Generation 9	7371.06	70.00	22.23	3.88	0.55	2717.00

Figure T20.12 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.

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The **Formatting Overlays** dialog will appear, similar to Figure T20.10.

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 T20.13). 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.

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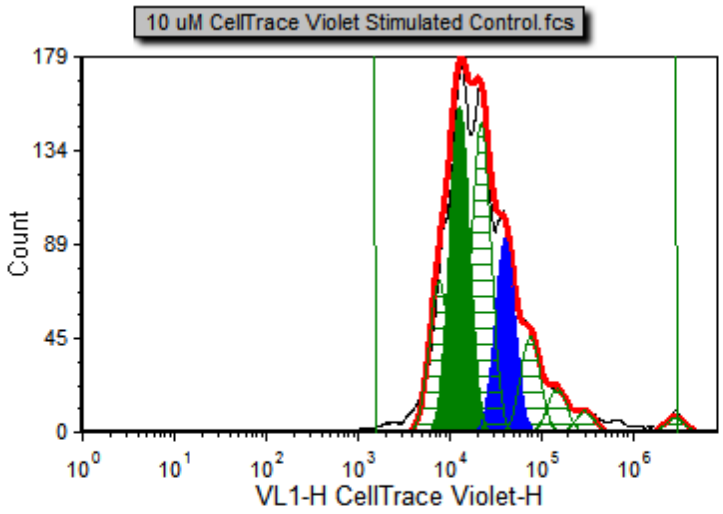

Division Index	Proliferation Index	% Divided	Peak Ratio	# of Fitted Cells	# of Original Cells	RMS Error	R-Chi Square	Starting Generation Mean	Background Noise
75.66	40.11	52.39	0.53	21716.00	537.70	5.45	14.92	2913319.40	1489.57

Generation	Peak Channel	Peak Value	Log Std Dev	Log CV	Peak Ratio	# of Cells
Undivided Cells	2650605.67	7.00	22.44	2.35	0.00	256.00
Generation 1	0.00	0.00	0.00	0.00	0.00	0.00
Generation 2	0.00	0.00	0.00	0.00	0.00	0.00
Generation 3	286063.63	10.00	22.44	2.77	0.00	363.00
Generation 4	148758.98	21.00	22.44	2.93	0.50	790.00
Generation 5	76162.69	46.00	22.44	3.11	0.51	1782.00
Generation 6	40227.61	92.00	22.44	3.29	0.52	3612.00
Generation 7	22263.35	147.00	22.44	3.49	0.53	5797.00
Generation 8	12710.99	155.00	22.44	3.69	0.55	6151.00
Generation 9	7723.50	72.00	22.44	3.90	0.55	2818.00

Figure T20.13 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[372] in the next section[1123] to summarize the three sample experiment.

34.20.4 Batch Processing Proliferation Data

In the previous sections we have inserted proliferation plots[1107], optimized the proliferation fit[1109], formatted the proliferation plot[1117], and inserted proliferation fit

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and population statistics. 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 (Figure T20.14).
4. Check the **Open presentation after saving box** ❶(Figure T20.14).
5. Check the **Combine the output from all iterations in to the same presentation** radio button ❷(Figure T20.14).
6. Click on the folder icon under the **Output file options category→Save to a new file** ❸(Figure T20.14).

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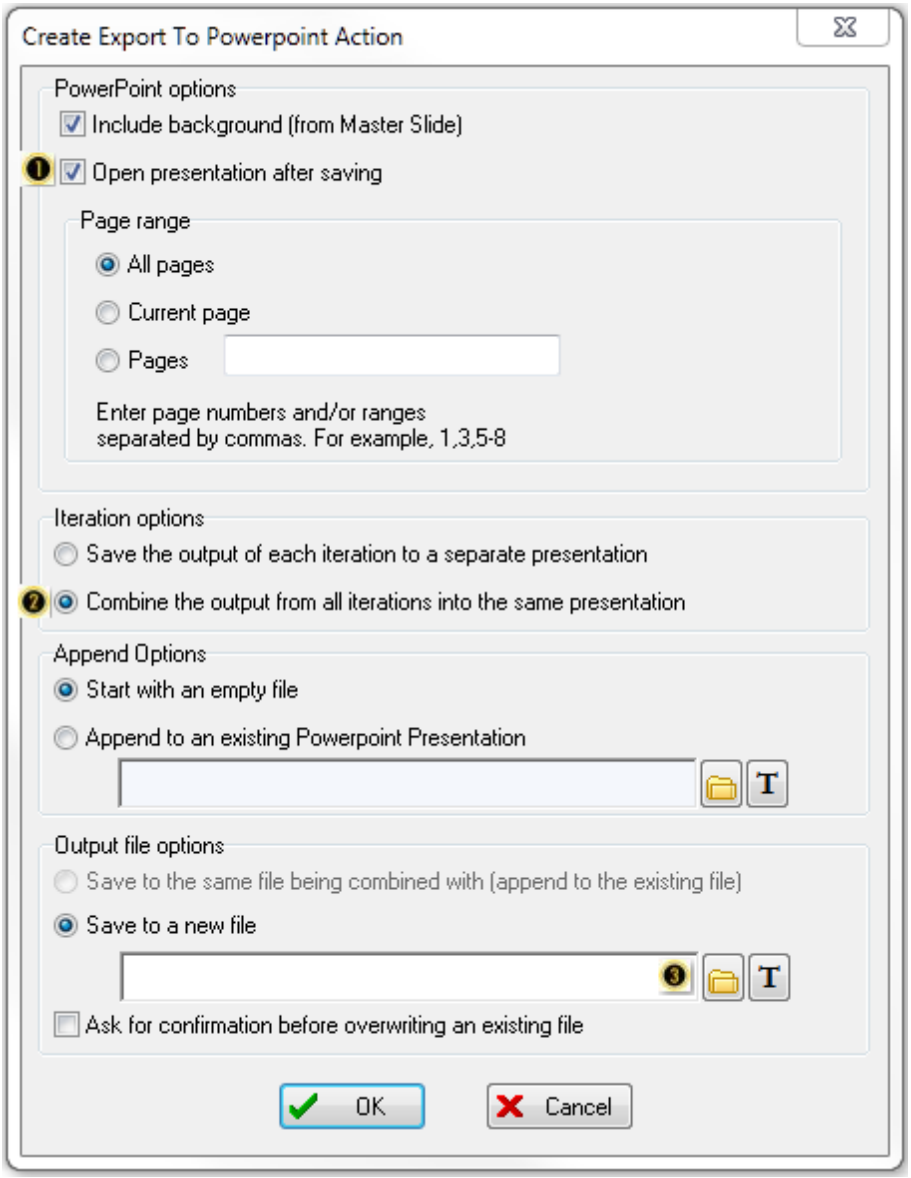

Figure T20.14 Editing the Create Export To Powerpoint Action options.

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 Exel (Column Mode)** in the **Add Report** category.
    The **Excel (Column Mode) Options** window will open.
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.

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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 (Figure T20.15).

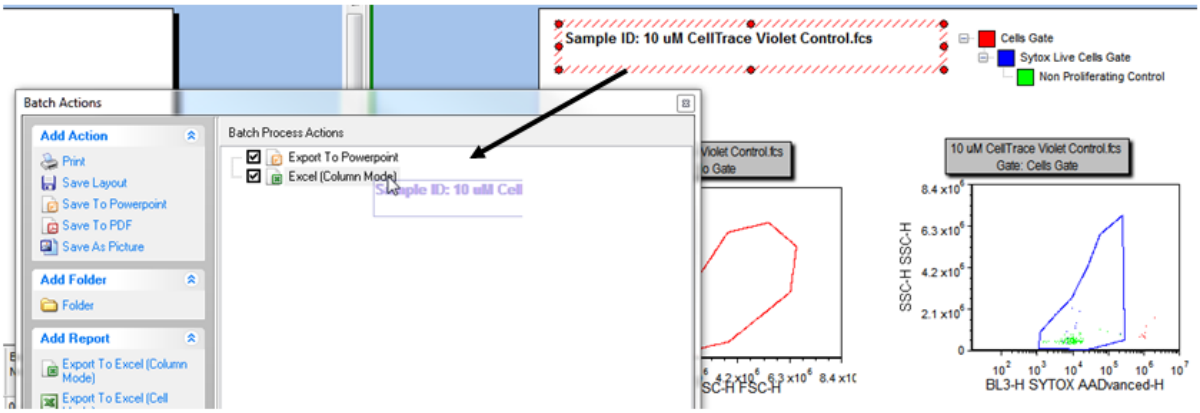

Figure T20.15 Dragging and dropping the sample ID text box to the Excel (Column Mode) batch action.

16. Highlight all of the statistics in the Proliferation Fit Statistics Table.
17. Drag the selection on top of the **Excel (Column Mode)** icon in the **Batch Process Actions** window (Figure T20.16).

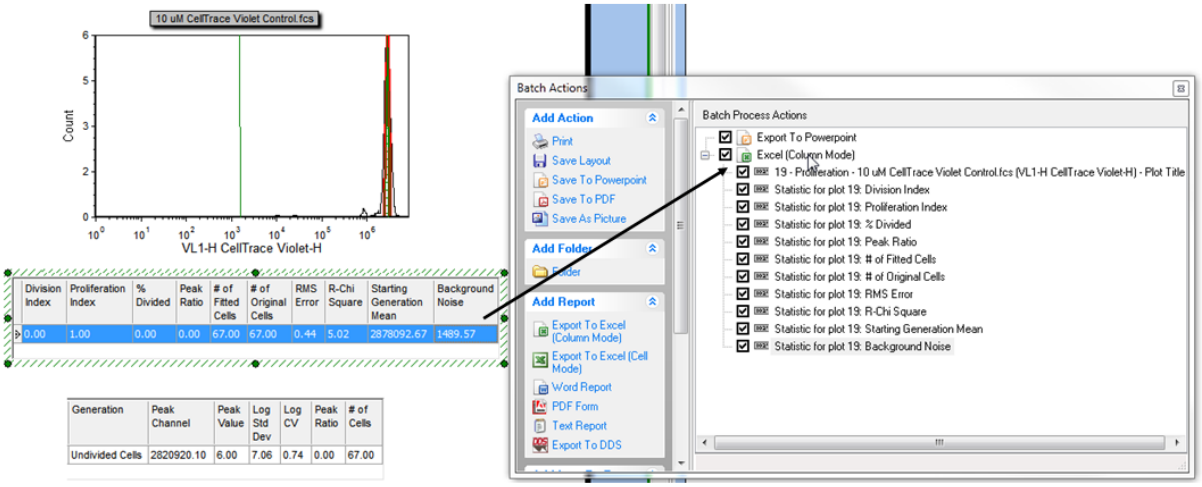

Figure T20.16 Dragging and dropping the Proliferation Fit Statistics table to the Excel (Column Mode) batch action.

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

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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.

34.21 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.

In the course of this tutorial we will show how to:

- Create parameter math formulas to view ratios.
- 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 1127.

34.21.1 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

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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 590. The **KineticsTutorial.fey**, **kinetics10.010**, and **kinetics25.025** data files have been embedded and will be used here.

1. Select **Application Button→Open Layout**.
2. Select the **KineticsTutorial.fey** layout file located in the *FCS Express Sample Data* directory from the **Load Layout** dialog.
3. Choose **Data→Calculate→Parameter Math→Sequence Setup** (Figure T21.1).

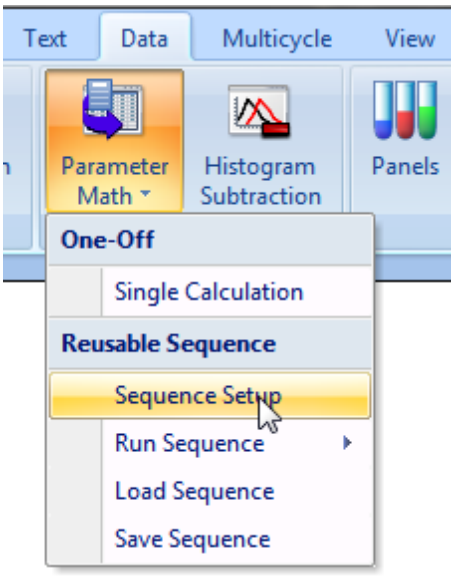

Figure T21.1 Selecting the Parameter Math Sequence Setup Command

4. Choose **New** in the **Parameter Math Sequence** dialog (Figure T21.2) to define a new formula.

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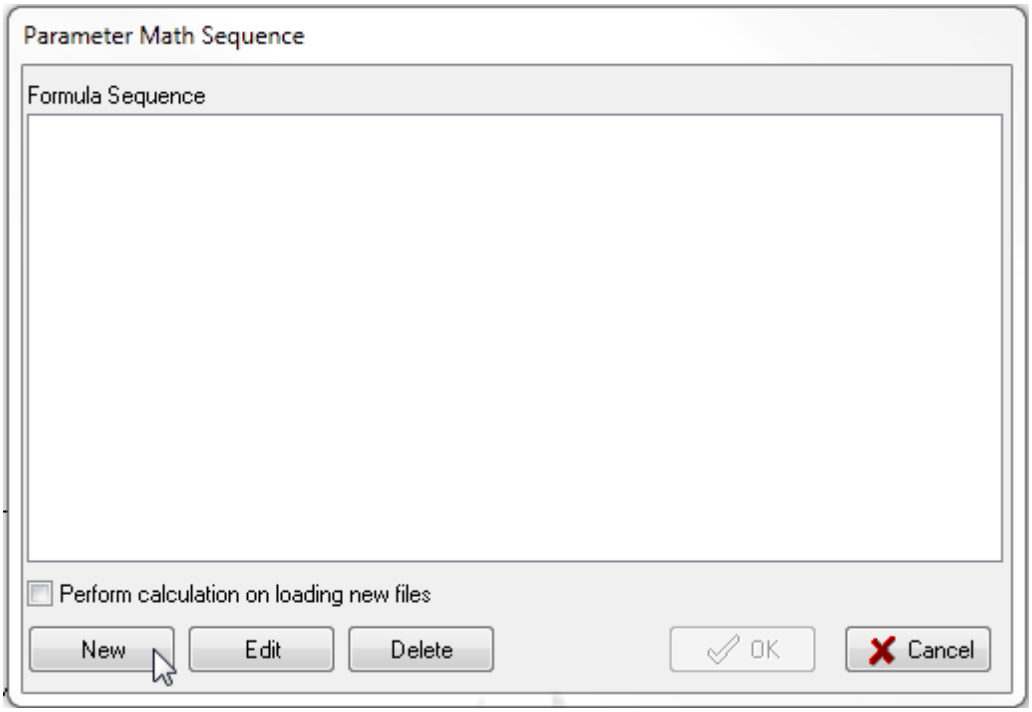

Figure T21.2 Choosing to Define a New Parameter Math Formula

The following instructions refer to Figure T21.3.

5. Enter **Ca++ Ratio** in the **Output Parameter Name** box ❶.
6. Change the mathematics operator to "**/**" for division ❷.
7. Change the **Second Input Parameter** to **FL3-H** ❸.
8. Click **OK** ❹.

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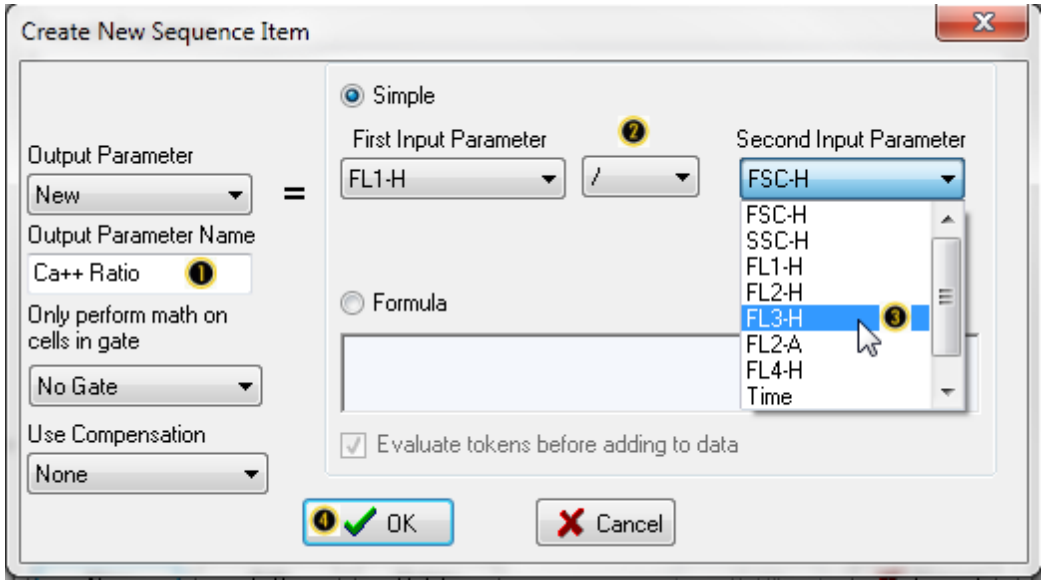

Figure T21.3 Defining a Parameter Math Sequence Item Formula

The **Parameter Math Sequence** dialog will now display the formula sequence we defined (Figure T21.4).

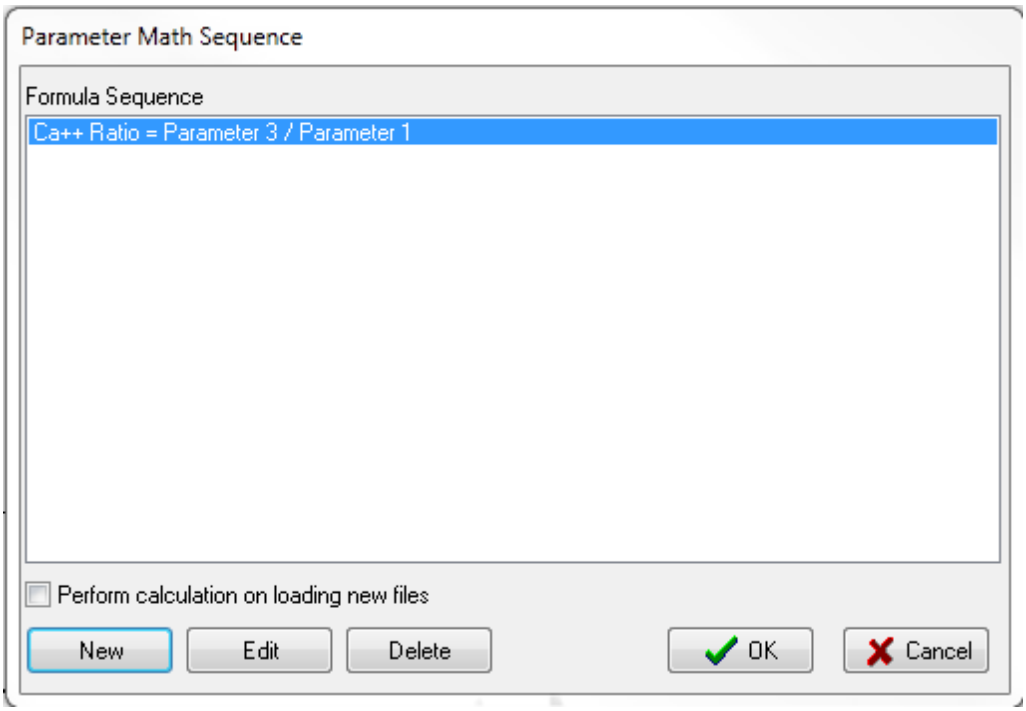

Figure T21.4 Parameter Math Sequence Dialog with Formula Displayed

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9. Click **OK**.

10. Choose the **Data→Calculate→Parameter Math→Run Sequence→On All Files** command to apply the parameter math formula (Figure T21.5).

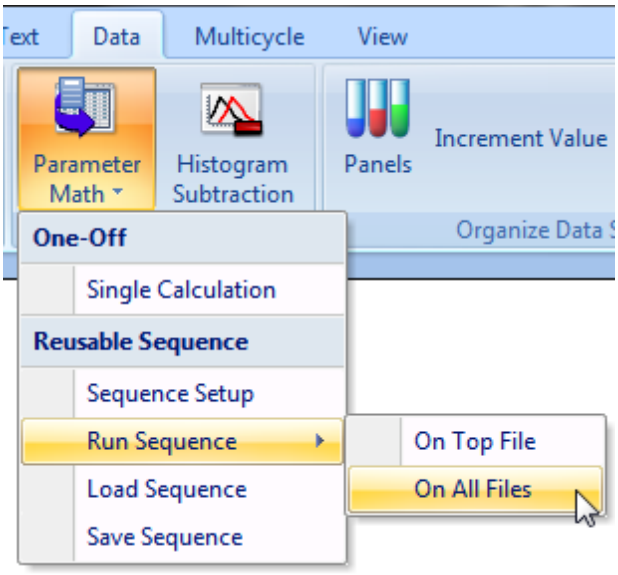

Figure T21.5 Applying the Parameter Math Formula to All Files

11. Hit **Ctrl+A** to select all plots.

12. Choose the **Format→Plot Options→Overlays** command.

13. Change the **Y-Parameter** to **Ca++ Ratio** (i.e., the formula defined by the parameter math) from the drop-down list (Figure T21.6).

14. Click **OK**.

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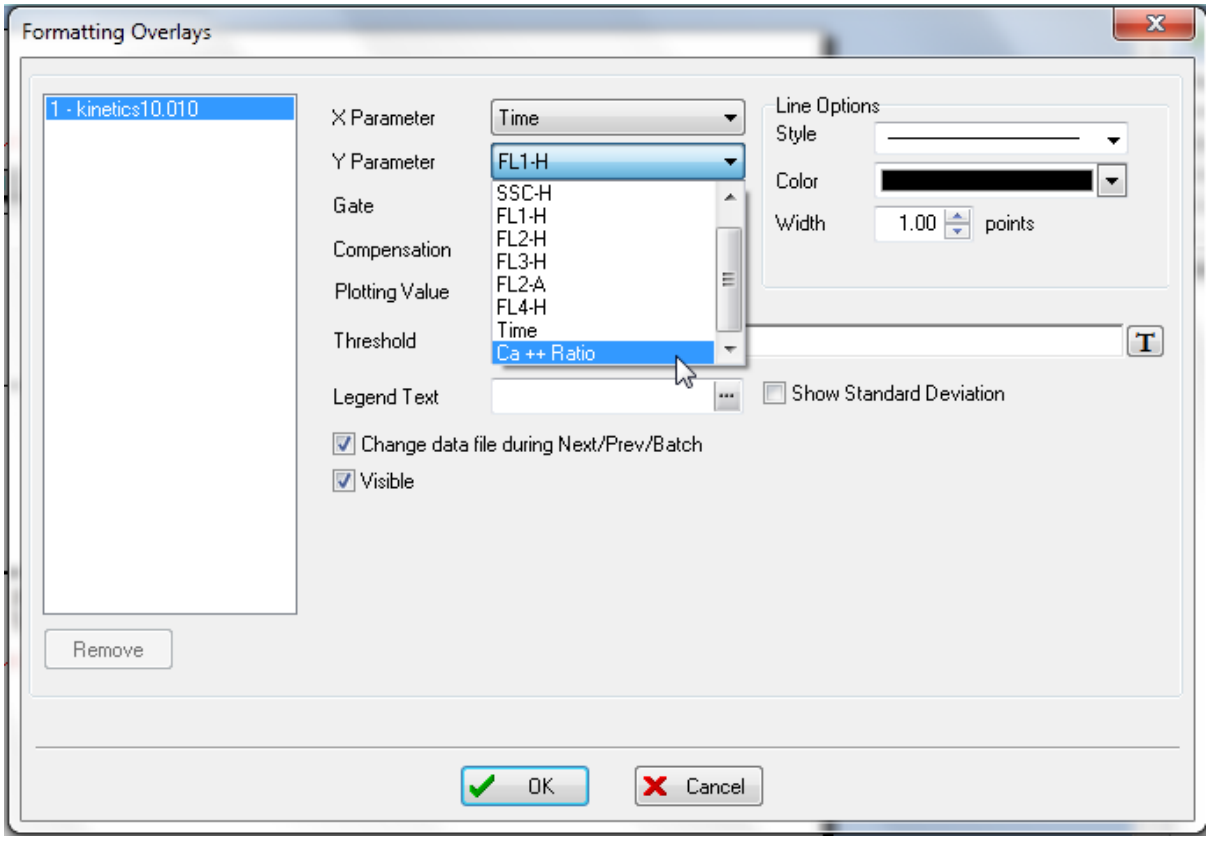

Figure T21.6 Changing the Y Axis to the Ratio Defined by the Parameter Math

The plots on the layout should now look like Figure 22.7. 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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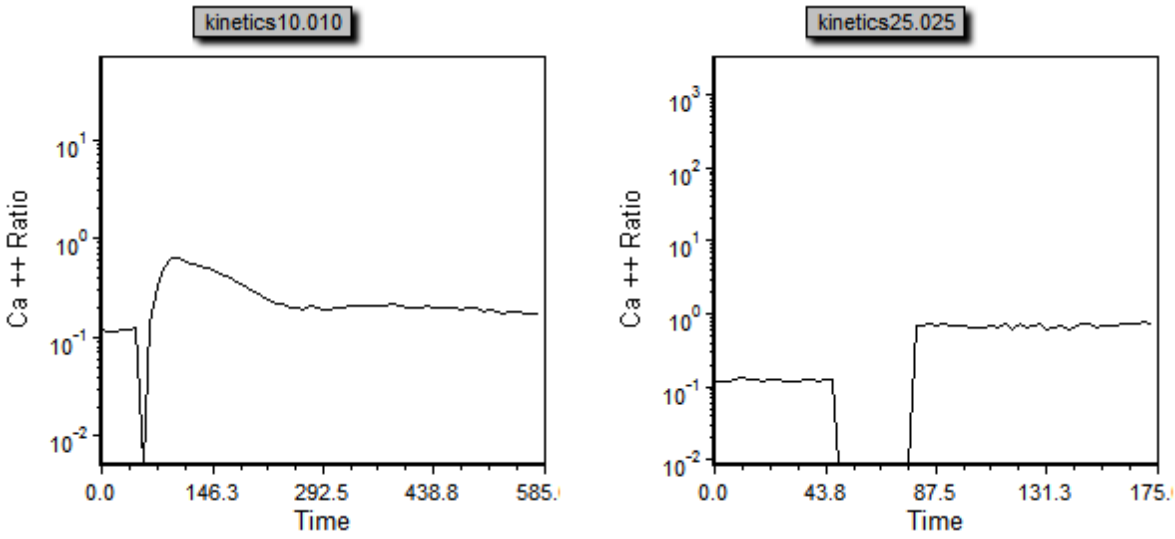

Figure T21.7 Ca++ Ratio vs Time

15. Hit **Ctrl+A** to select all plots.
16. Choose the **Format→Plot Options→Axes** command.

Refer to Figure T21.8 for the following steps.

17. Select **Y-Axis** from the list box on the left ❶.
18. Uncheck the **Automatic** check box in **Range** ❷**.**
19. Change the range **Minimum** to 0 ❸**.**
20. Change the range **Maximum** to 1 ❹**.**
21. Uncheck the **Automatic** check box in **Scale** ❺.
22. Choose **Linear** from the drop-down list ❻.
23. Click **OK**.

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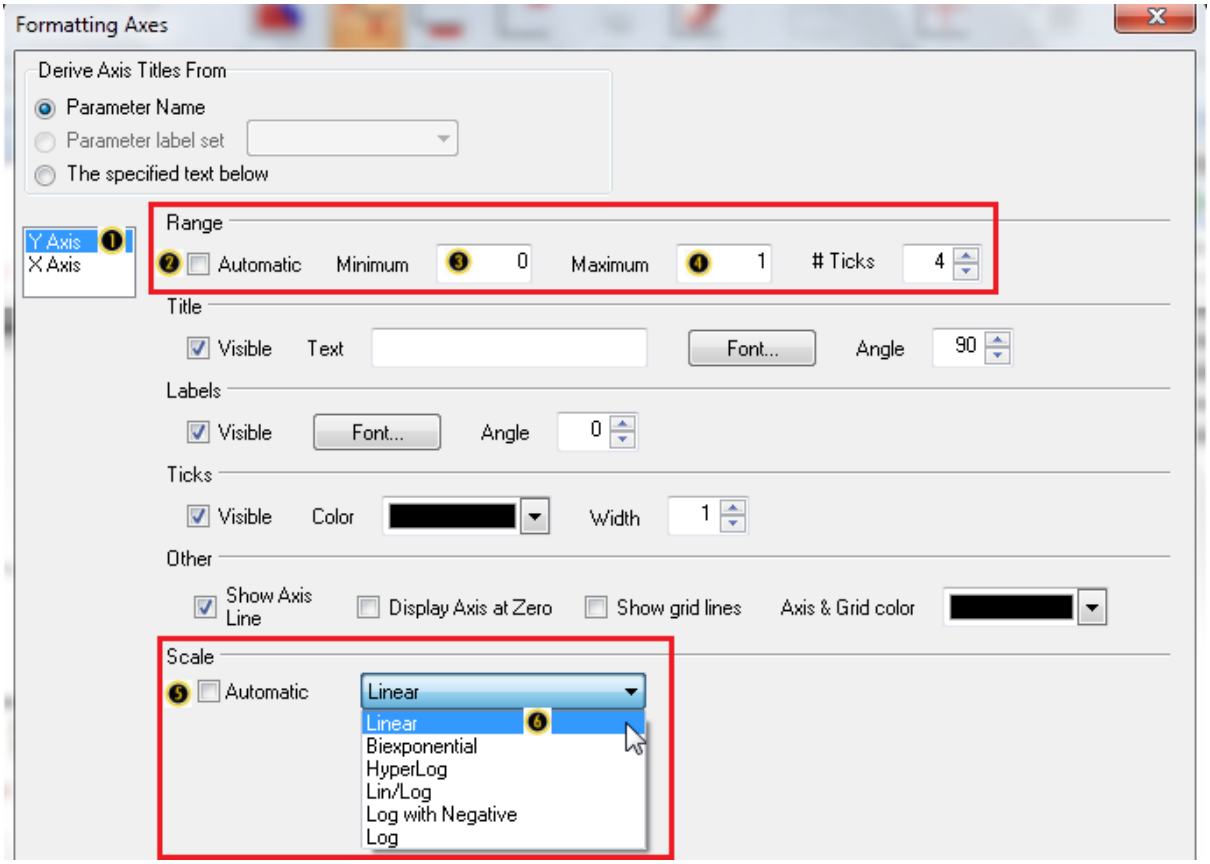

Figure T21.8 Formatting Axes Dialog

The plots in the layout should now look like Figure T21.9.

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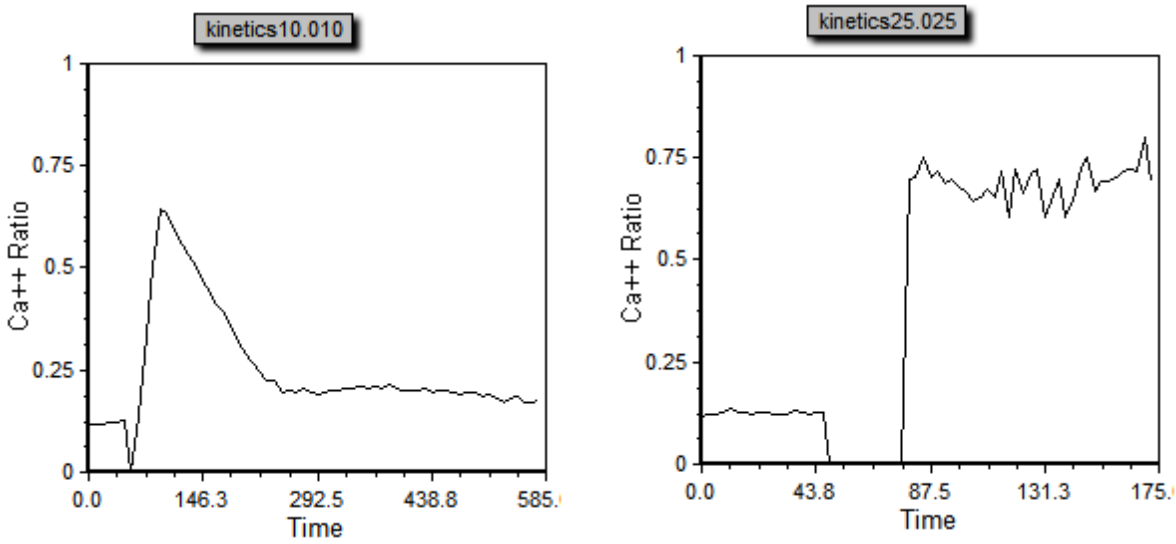

Figure T21.9 Plots Scaled Correctly to Display the Ca++ Ratio

In the next section, we will apply gating and statistics to kinetics plots[1135].

34.21.2 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[590]. The **KineticsTutorial.fey**, **kinetics10.010**, and **kinetics25.025** data files will be used here. Please complete the steps in the Using Parameter Math[1127] section before beginning.

Note: In order to obtain statistics from kinetics plots, you have to create 1D gates on the Time axis. You can create as many 1D gates as you like and FCS Express will calculate a set of statistics for each gate.

1. Choose the **Gating→Create Gates→Create Marker** (Figure T21.10).

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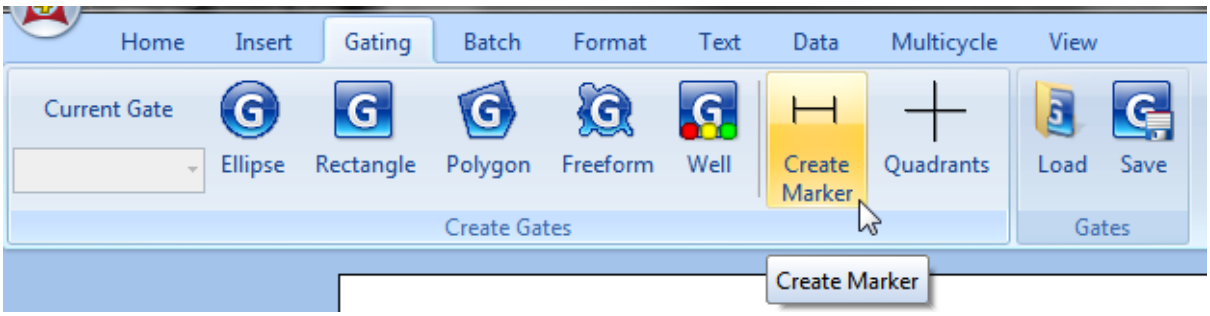

Figure T21.10 Creating a Marker

2. Create a 1D (marker) gate on sample **kinetics25.025** similar to the one in Figure T21.11.
3. Name the gate "Marker Gate 1".
4. Choose the **Gating→Create Gates→Create Marker** command.
5. Create a 1D (marker) gate on sample **kinetis10.010** similar to the one in Figure T21.11.
6. Name the gate "Marker Gate 2".

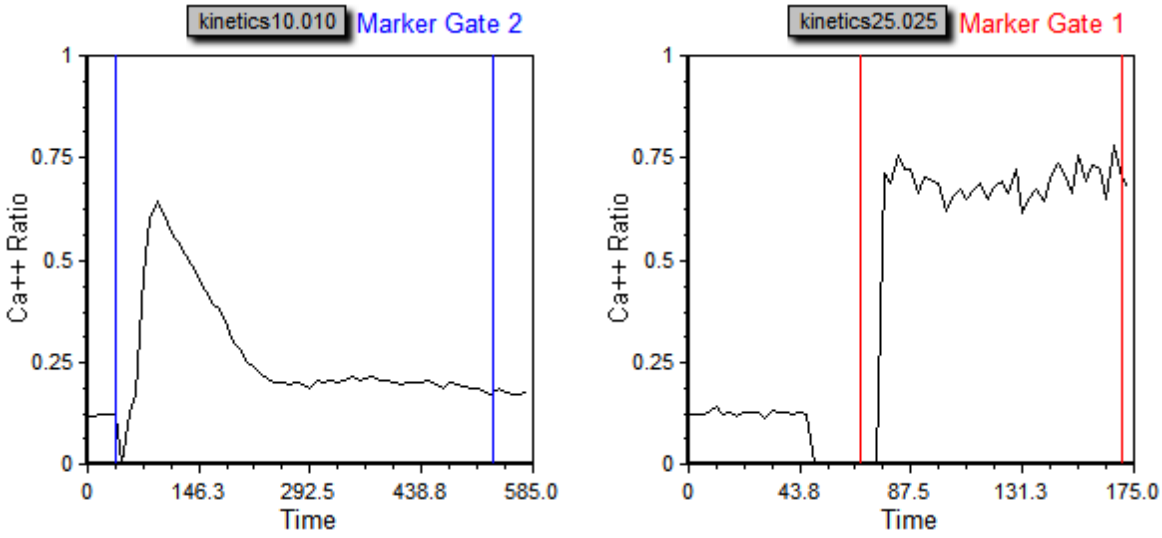

Figure T21.11 Kinetics Plots with 1D Gates Applied

7. Right-click on the **kinetics25.025** plot.
8. Choose **Kinetics Statistics** from the pop-up menu. A **Kinetics**

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Statistics window will appear on the layout.

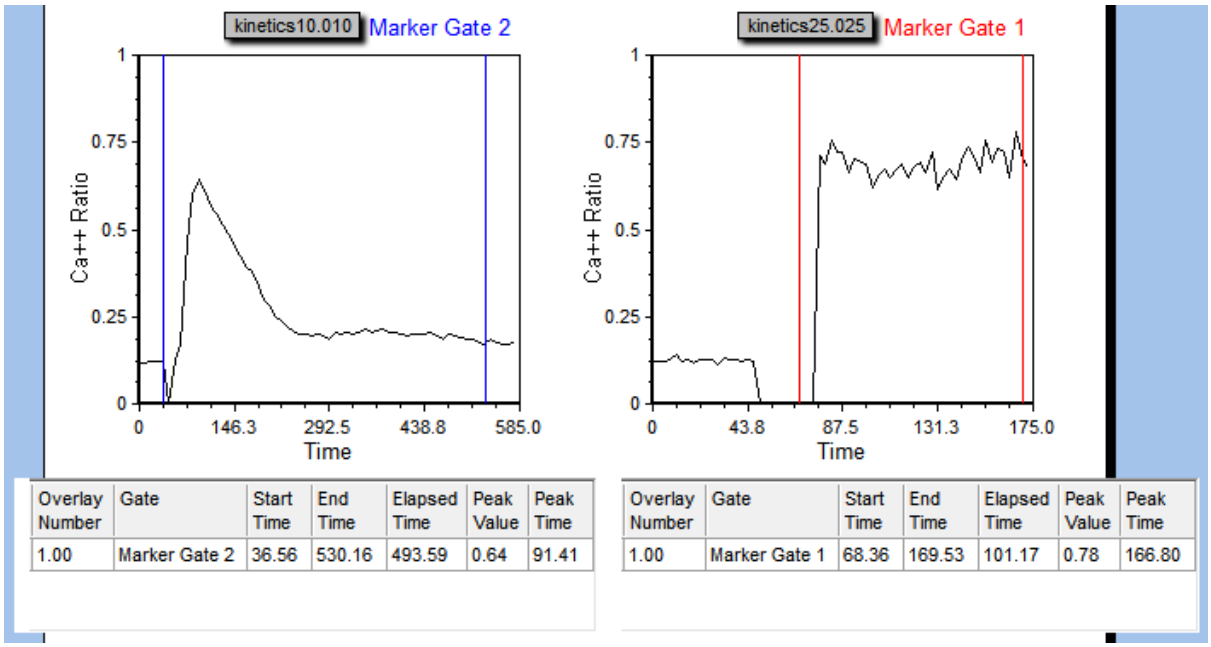
9. Move the **Kinetics Statistics** window to a convenient position on the layout.

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
1.00	Marker Gate 2	36.56	530.16	493.59	0.64	91.41

Overlay Number	Gate	Start Time	End Time	Elapsed Time	Peak Value	Peak Time
1.00	Marker Gate 1	68.36	169.53	101.17	0.78	166.80

Figure T21.12 Plots with Kinetics Statistics Windows

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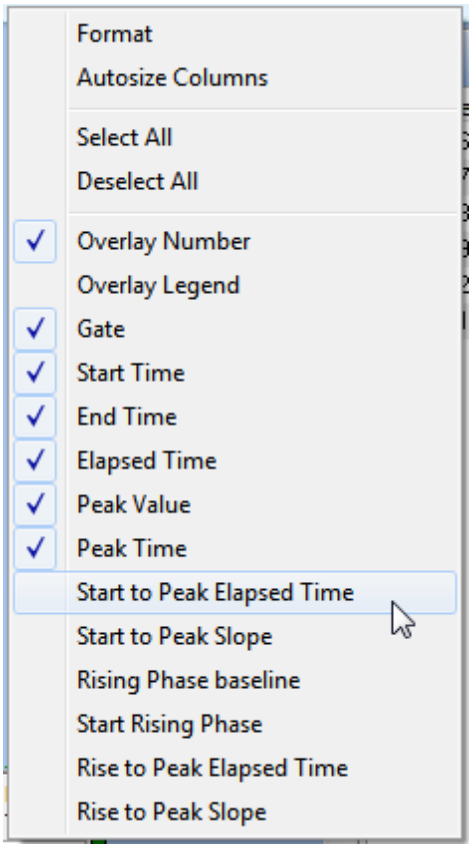

Figure T21.13 Kinetics Statistics Pop-up Menu

Next, we will explore different plotting values 1138.

34.21.3 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 590. 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.

1. Click on the **kinetics10.010** plot to select it.
2. Right-click on the plot and choose **Format** from the pop-up menu.

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3. Select the **Overlays** category.
4. Change the **Plotting Value** from **Median** to **Mean** (Figure T21.14).

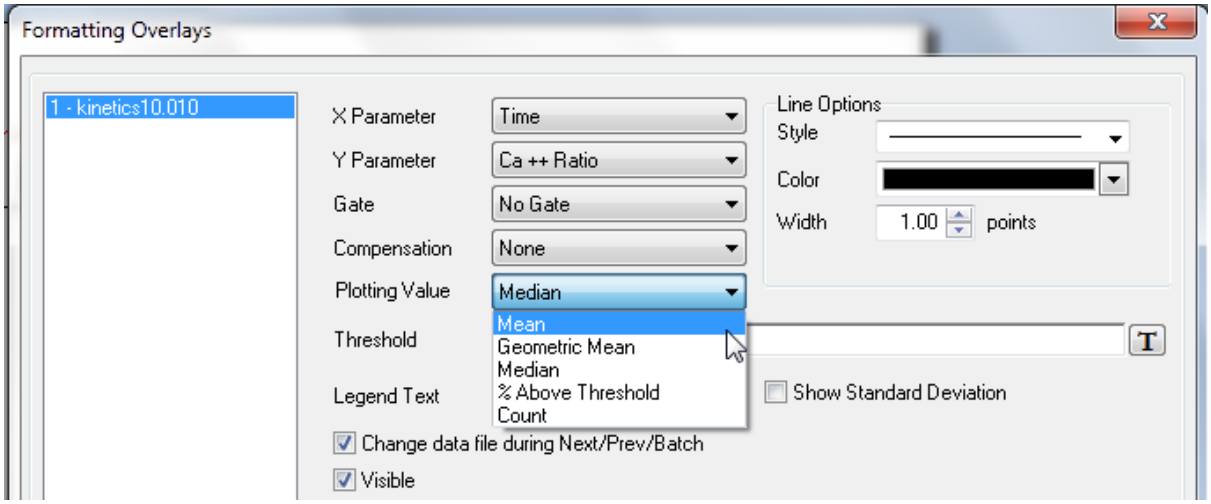

Figure T21.14 Changing the Plotting Value

5. Move the **Formatting Overlays** dialog 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**.
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**.

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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, and the spike at the end of acquisition where the flow rate increased because of cells becoming concentrated as they settled throughout the acquisition.

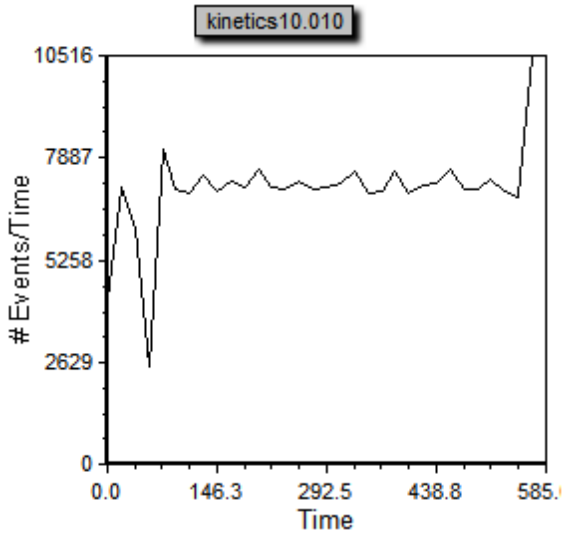

Figure T21.15 Kinetics Plot Displaying Count vs. Time

We will now assign a threshold to view **% Above Threshold** as a plotting value on a new page and plot.

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.

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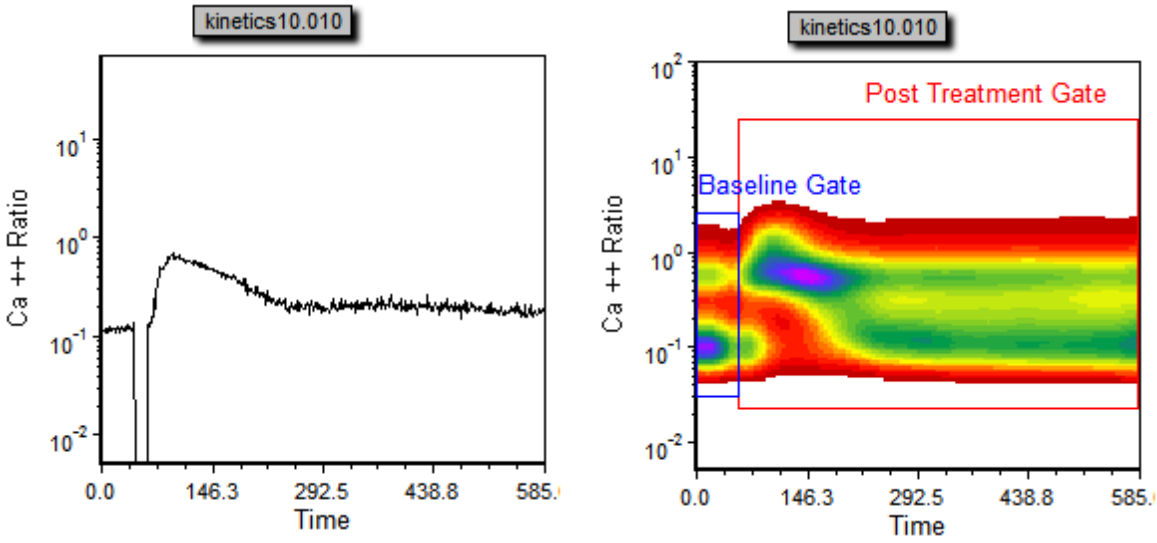

Figure T21.16 Kinetics and Density Plots with Thresholding Gates Applied

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 the Y median of the baseline gate plus two standard deviations of the Y median in the baseline gate.)*

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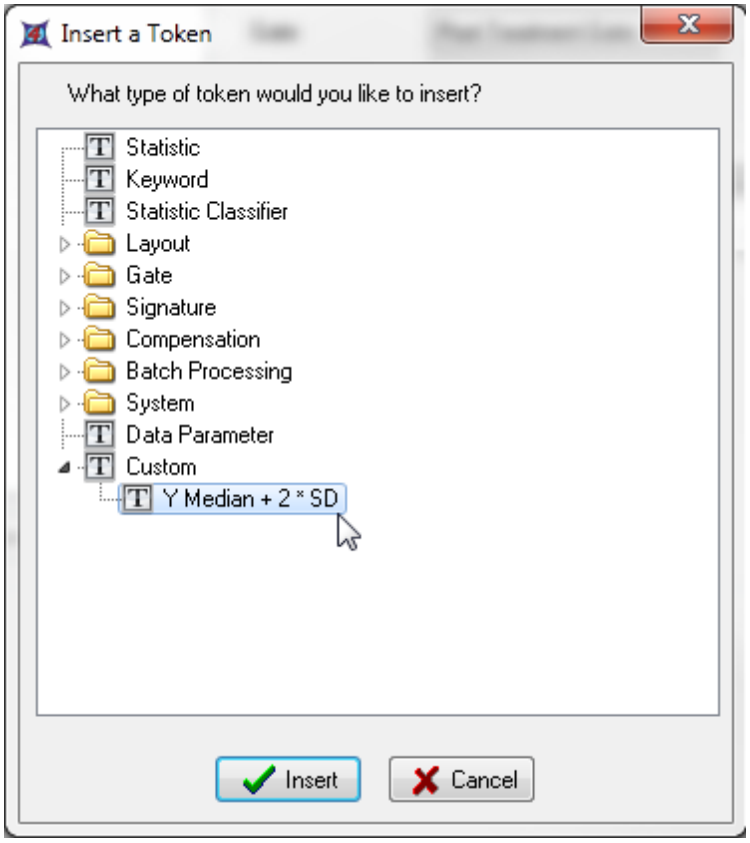

Figure T21.17 Choosing a Custom Token as a Threshold Value

21. Click **OK**.

The plots in the layout should now look like Figure T21.18. The plot 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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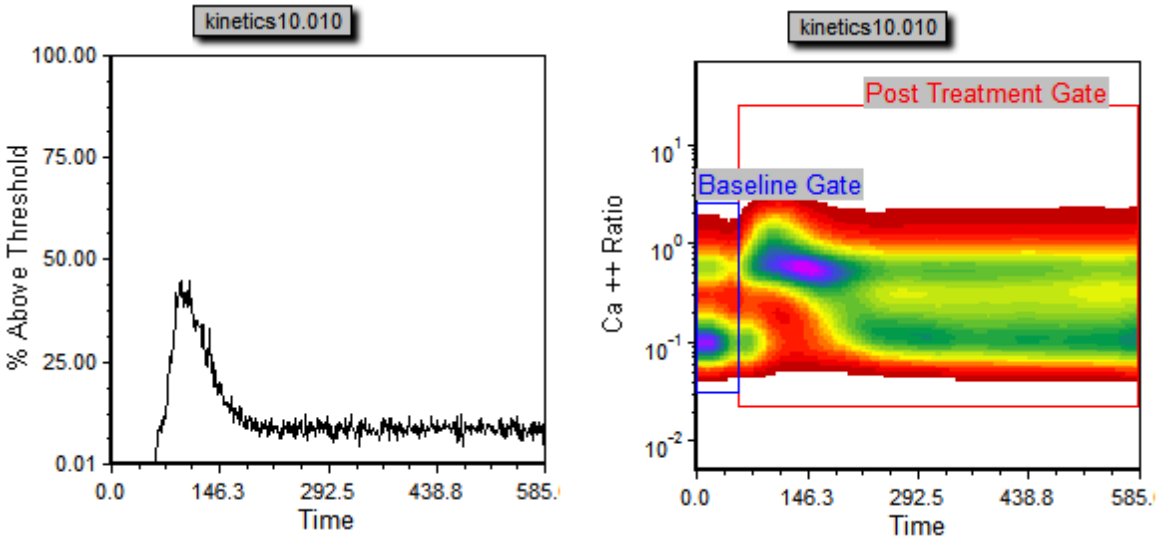

Figure T21.18 Kinetics Plot Displayed as % Above Threshold

In the next exercise, we will overlay and format kinetics plots[1143].

34.21.4 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[590]. The **KineticsTutorialFormatting.fey**, **kinetics8.008**, **kinetics9.009, kinetics10.010**, and **kinetics25.025** data files will be used here.

1. Select **Application Button→Open Layout**.
2. Select the **KineticsTutorialFormatting.fey** layout file located in the *FCS Express Sample Data* directory from the **Load Layout** dialog.
3. Right-click on the **Kinetics Overlay** plot.
4. Choose **Add overlay** from the pop-up menu.
5. Click the **Data List** tab in the **Select a Data File** dialog.
6. **Ctrl+Click** on samples **kinetics8.008**, **kinetics9.009**, and

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kinetics10.010 to select them from the data list.

7. Click **Open**.
8. Click **OK**.
9. Change the Y-Axis parameter on the **Kinetics Overlay** plot to **Ca++ Ratio**.
10. Select the **Live Cells** gate from **Gating→Create Gates→Current Gate**.

The plot now contains overlays for each sample and should look like Figure T21.19.

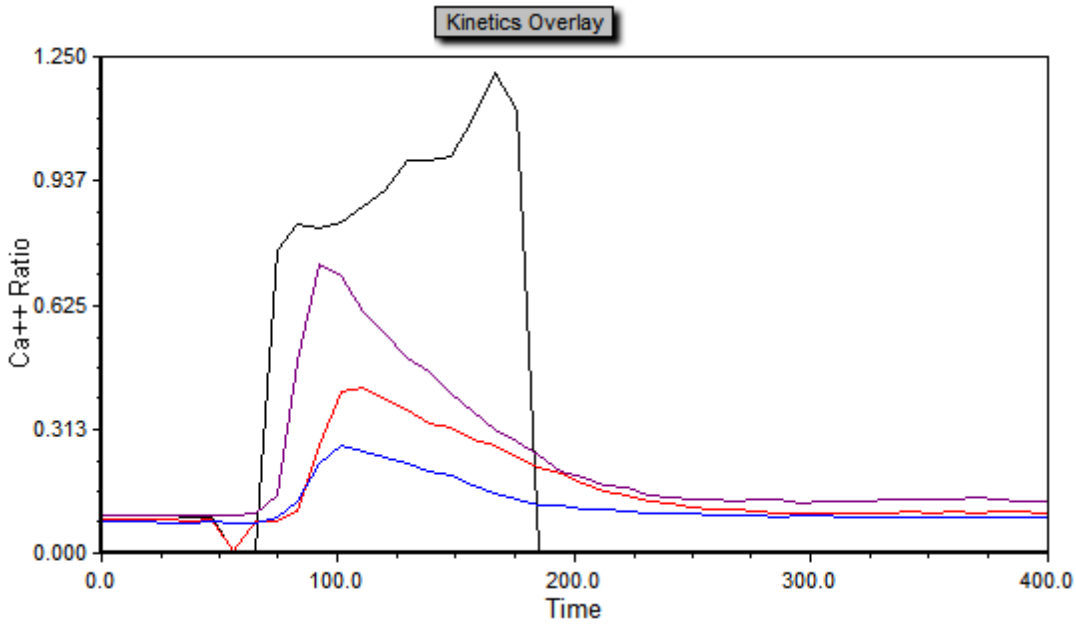

Figure T21.19 Multiple Kinetics Overlays

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**.

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23. Choose the **Legend** category.
24. Click on the **Visible** check box to select it.
25. Click **OK**.

The plot should now look like the Figure T21.20.

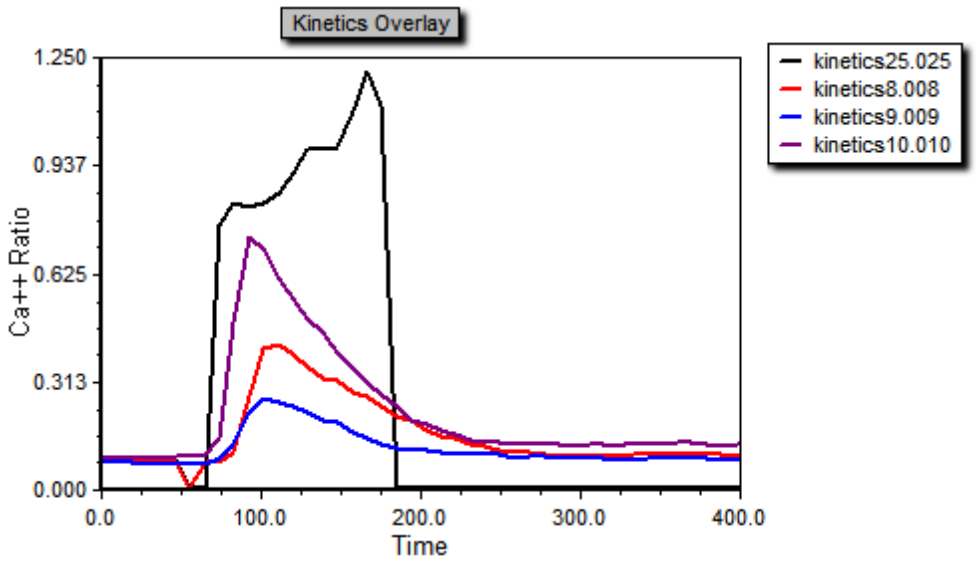

Figure T21.20 Kinetics Plots Formatted

Finally, we will change the resolution of a kinetics plot[1145].

34.21.5 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[590]. The **KineticsTutorialScaling.fey**, **kinetics10.010**, and **kinetics25.025** data files will be used here.

1. Select **Application Button→Open Layout**.
2. Select the **KineticsTutorialScaling.fey** layout file located in the *FCS*

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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 of Fl-1. 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.

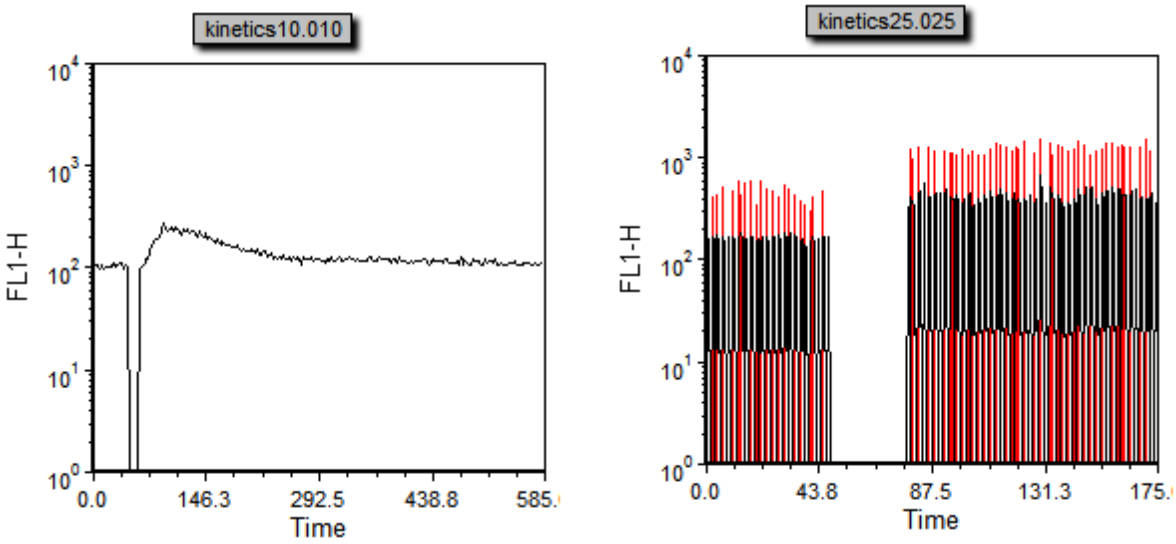

Figure T21.21 Kinetics Tutorial Layout After Adding Sample Data

3. Click on the **kinetics25.025** plot to select it.
4. Choose the **Format→Plot Options→Overlays** command.
5. Uncheck the **Show Standard Deviation** check box (Figure T21.22).

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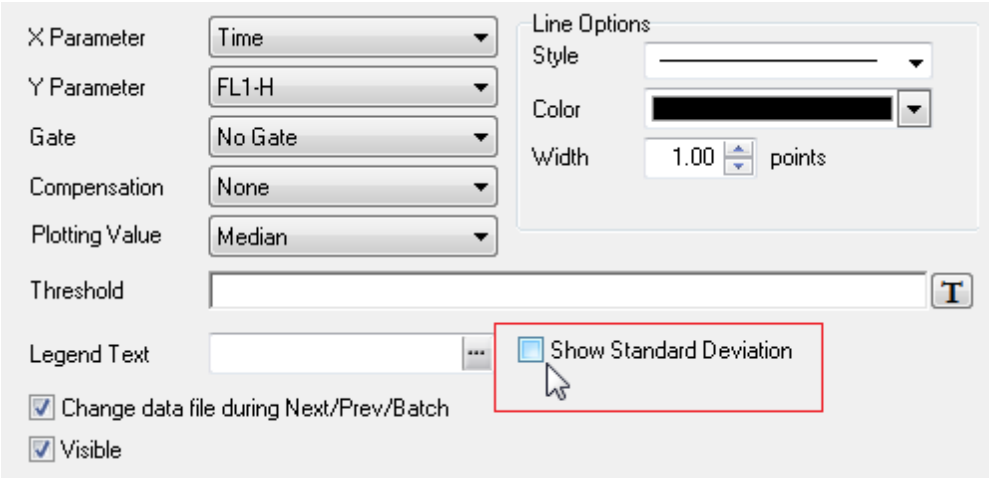

Figure T21.22 Unchecking Show Standard Deviation

6. Click **OK**.

The red standard deviation highlighting will now be removed as in Figure T21.23.

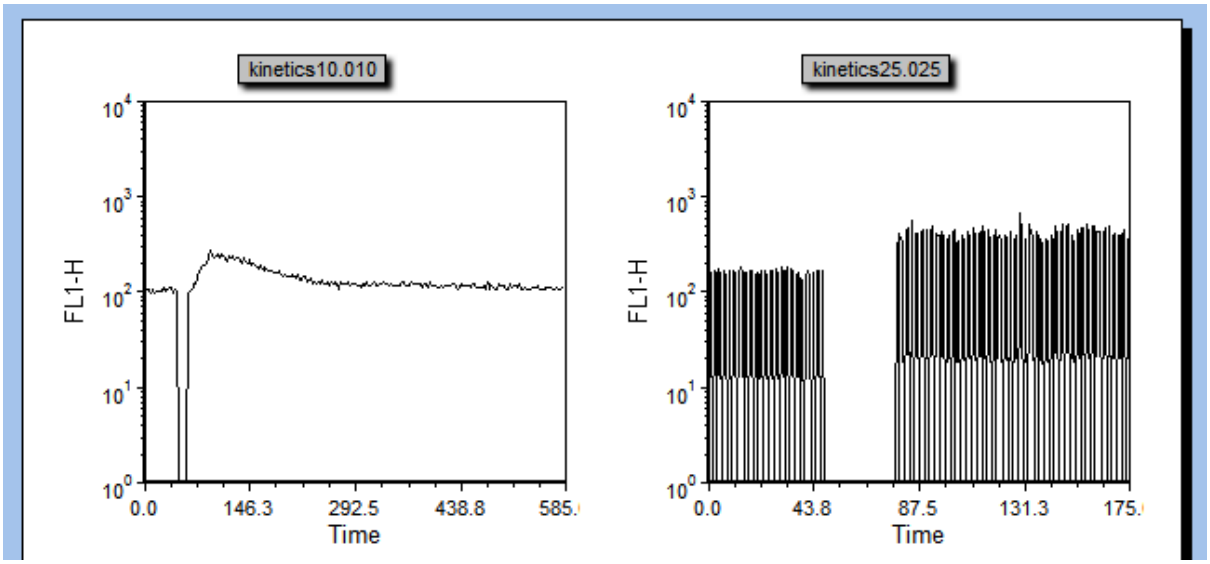

Figure T21.23 Sample Plots with Standard Deviation Highlighting Removed

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.

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9. Choose the **Kinetics Option→Binned Plot Specific Options** category.

10. Click on the **Resolution** drop-down list under **Resolution Options** (Figure T21.24).

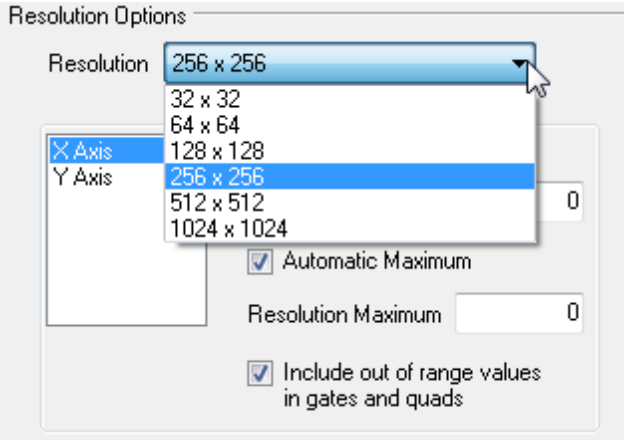

Figure T21.24 Changing the Resolution on a Plot

11. Highlight the **64x64** resolution option.

12. Click **OK.**

The plot will now change and be scaled properly as seen in Figure T21.25.

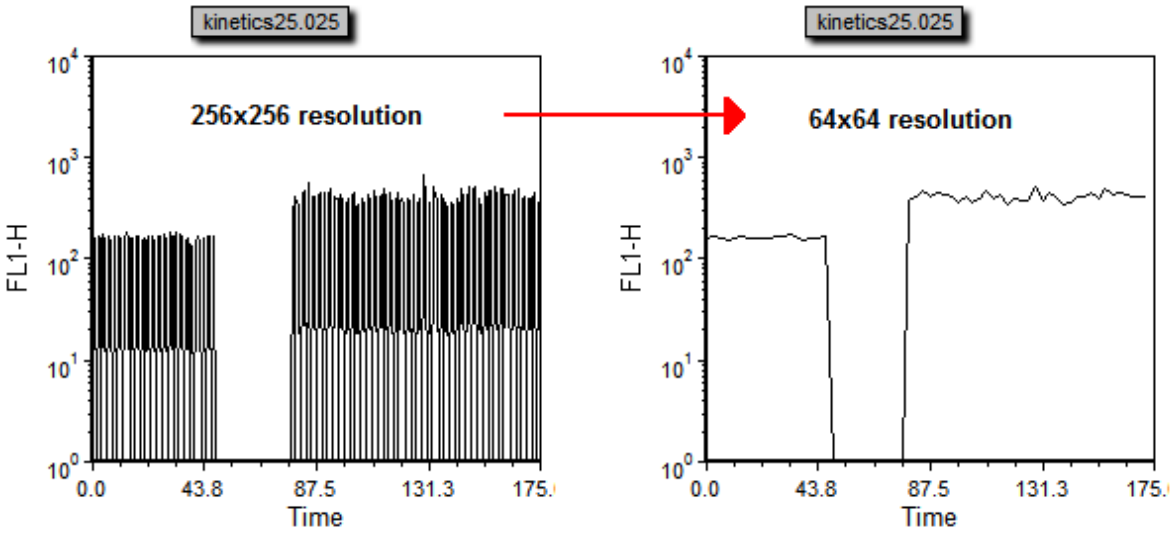

Figure T21.25 Changing the Resolution: 256x256 (Left) to 64x64 (Right)

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After following the instruction above, the layout should look like Figure T21.26.

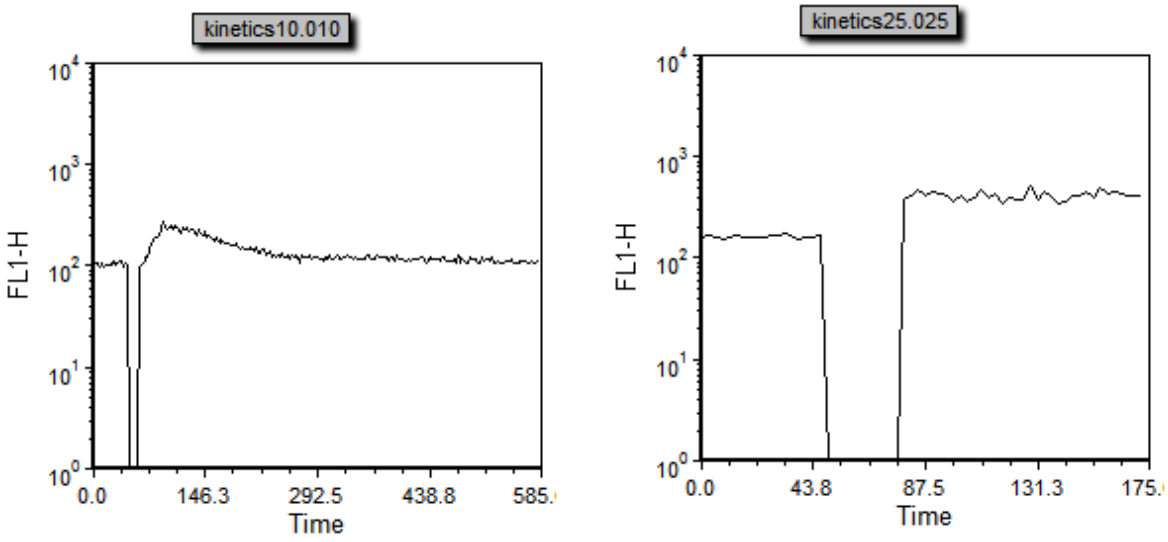

Figure T21.26 Kinetics Plots Scaled Properly

We will now apply standard deviation highlighting to both of the plots.

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.

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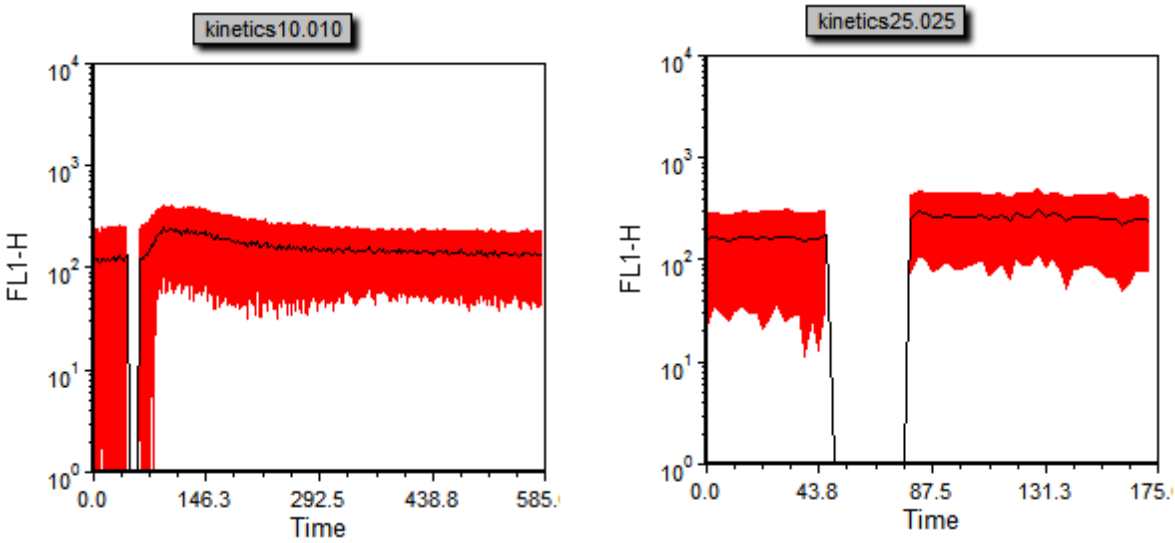

Figure T21.27 Kinetics Plots with Standard Deviation Highlighting Applied

34.22 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, or b) using the sample flow rate and acquisition time. 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 1150.

34.22.1 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 607, 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 of the file **Monocytes.002** found in the *FCS Express Sample 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).

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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 tutorial650.

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 tutorial650.

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.

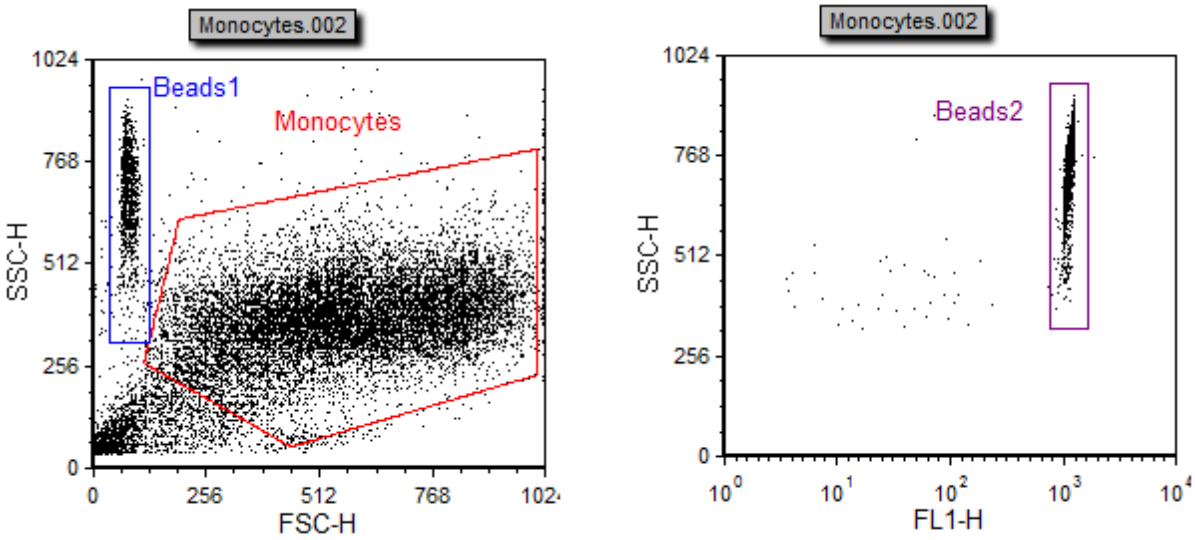

Figure T22.1 Gates for Monocytes and Beads

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.

We will now set the **Beads2** gate as a child of the **Beads1** gate. This will cause the events in the **Beads2** gate to be selected only from the events in the **Beads1** gate. This is analogous to a combination gate: "**Beads1 AND Beads2**".

8. Select the **Insert→General→Gate View** command (Figure T22.2).

9. Left-click on the layout where you would like to insert the **Gate View** window.

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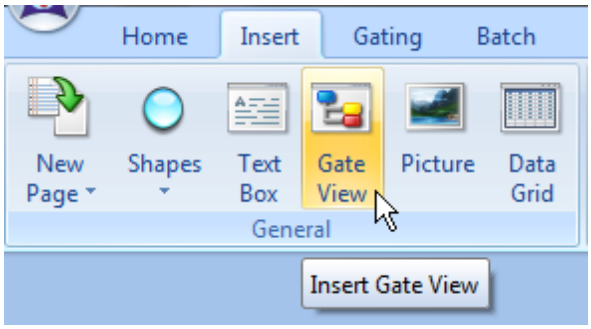

Figure T22.2 Gate View Command

The **Gate View** window will appear as shown in Figure T22.3.

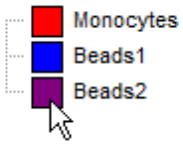

Figure T22.3 Gate View

10. Place the mouse over **Beads2** gate in the **Gate View** window (Figure T22.3).
11. Hold down the left mouse button and drag **Beads2** over the top of the **Beads1** gate.
12. Release the mouse button to drop **Beads2** gate onto **Beads 1** gate.

The **Gate View** should now look like Figure T22.4.

Figure T22.4 Gate View with Child Gate

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The **Beads2** gate is now a child of the **Beads1** gate as shown in Figure T22.4.

In the next section, we will use the Concentration Calculator 1153 tool.

34.22.2 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.

1. Select the **View→Tools→Concentration Calculator** command (Figure T22.5).

Figure T22.5 Concentration Calculator Command

The **Concentration Calculator** dialog now appears as shown in Figure T22.6. The following steps all refer to Figure T22.6.

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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 T22.6).

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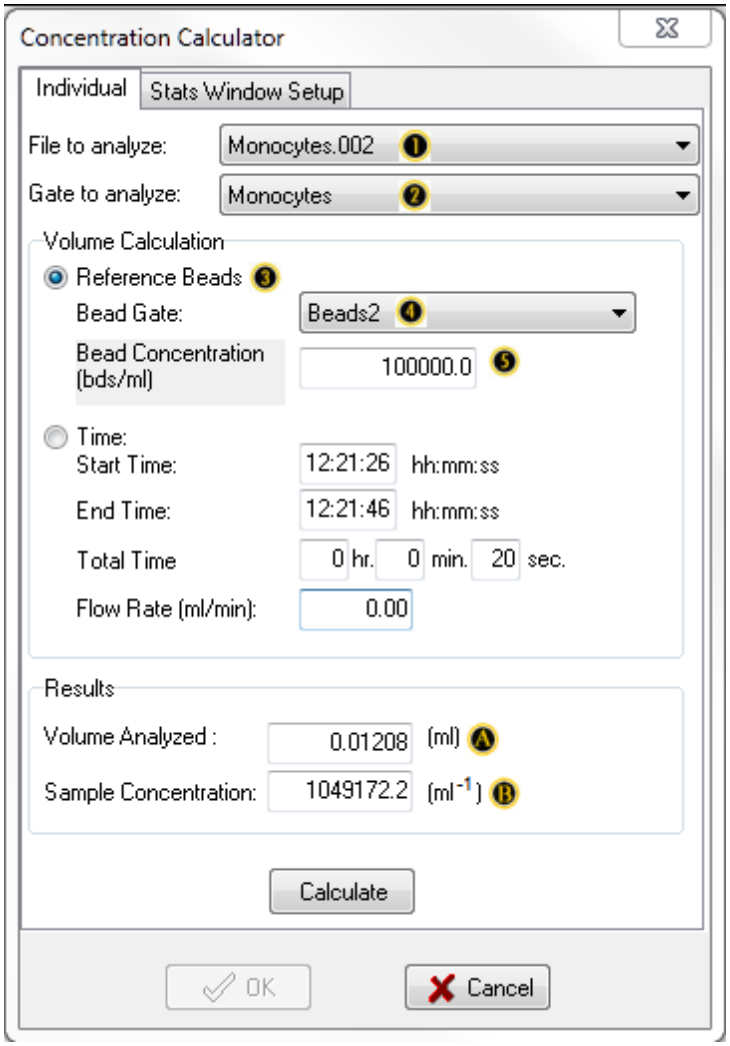

Figure T22.6 Concentration Calculator Dialog Using Beads

8. Click **Cancel** to close the **Concentration Calculator** dialog.

In the next example, we will calculate the concentration using time and flow rate 1155.

34.22.3 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.

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- Obtain a one time result.

1. Select the **View→Tools→Concentration Calculator** command (Figure T22.5 1153).

The **Concentration Calculator** dialog appears again as shown in Figure T22.7. The following steps all refer to Figure T22.7.

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 **Time** radio button ❸ 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 we will use a value of **0.06** mL/min ❹.
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 T22.7).

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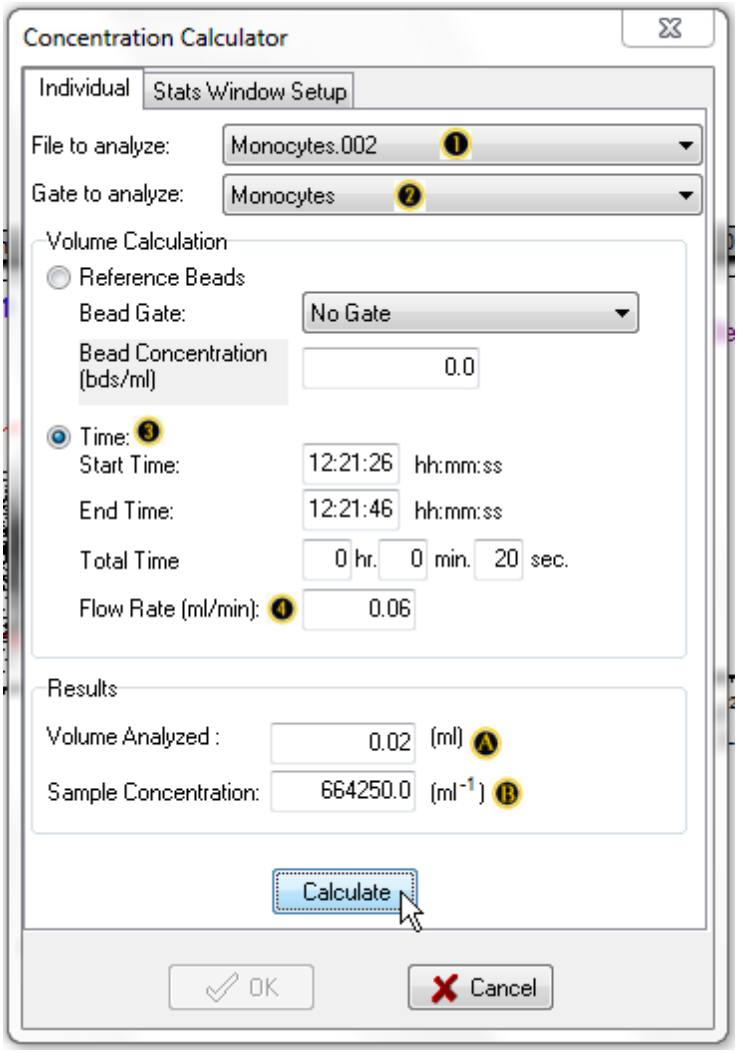

Figure T22.7 Concentration Calculator Dialog Using Time and Flow Rate

7. Click **Cancel** to close the **Concentration Calculator** dialog.

Next, we will show the calculated concentration in a statistics window.

34.22.4 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**.

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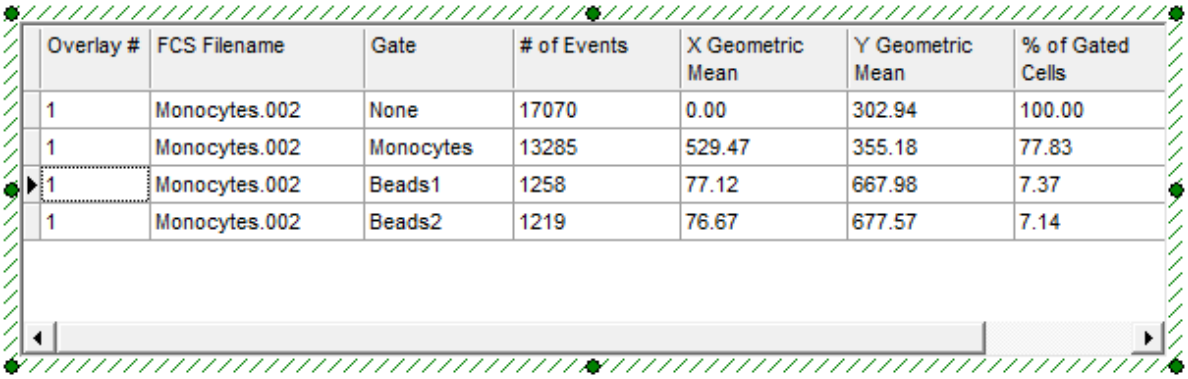
We have learned how to obtain one-time calculations of the cell concentration in a sample. We will now learn how to present these results in the layout using the **statistics window**.

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 **Gate Statistics** from the pop-up menu.

The **statistics window** should appear (Figure T22.8). Move it to a convenient place in the layout.



Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells
1	Monocytes.002	None	17070	0.00	302.94	100.00
1	Monocytes.002	Monocytes	13285	529.47	355.18	77.83
1	Monocytes.002	Beads1	1258	77.12	667.98	7.37
1	Monocytes.002	Beads2	1219	76.67	677.57	7.14

Figure T22.8 Statistics Window

3. Click once on the **statistics window** to select it; it should have a green border (Figure T22.8).

4. Select the **Format→Statistics Options→Statistics** command (Figure T22.9).

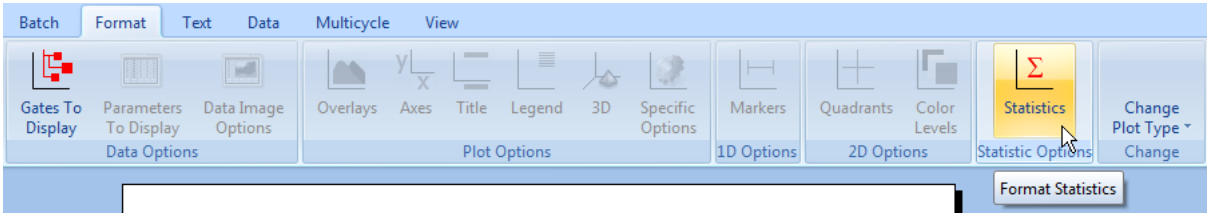


Figure T22.9 Selecting Format Statistics Command

The **Formatting Statistics** dialog now appears (Figure T22.10). We are now

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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 T22.10):
    - **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.

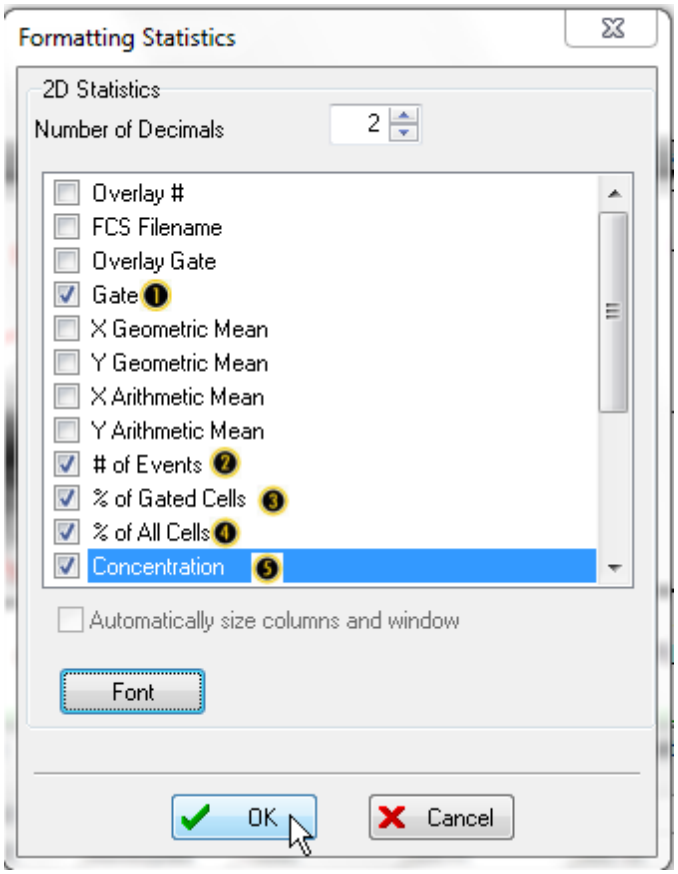

Figure T22.10 Formatting Statistics Dialog

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The **statistics window** will now appear as shown in Figure T22.11 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	0.00
Monocytes	13231	77.51	77.51	0.00
Beads1	1261	7.39	7.39	0.00
Beads2	1220	7.15	7.15	0.00

Figure T22.11 Statistics Window after Formatting

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 T22.12).

  B. Left-click on the individual statistics you want displayed.

  C. Left-click on the items you want removed (Figure T22.12).

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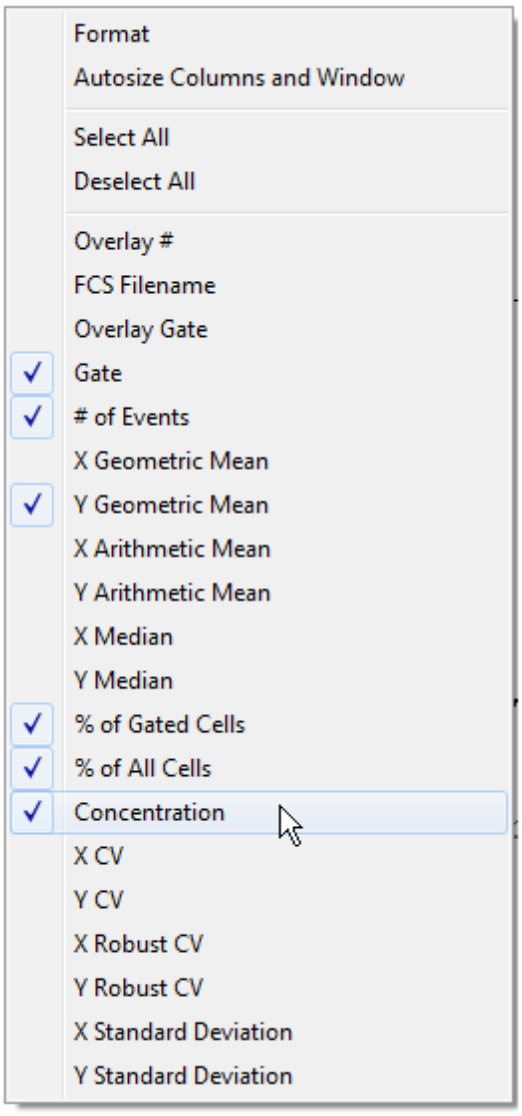

Figure T22.12 Statistics Window Pop-up Menu

- Choosing the statistics to be displayed using the **statistics window Format** dialog:

  A. Right-click on the **statistics window** to bring up the associated pop-up menu (Figure T22.12).

  B. Select **Format** from the pop-up menu.

  C. Select the **Stats** category from the left panel of the **Format** dialog (Figure T22.13).

  D. Set the statistics you want displayed and then click **OK** (Figure

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T22.13).

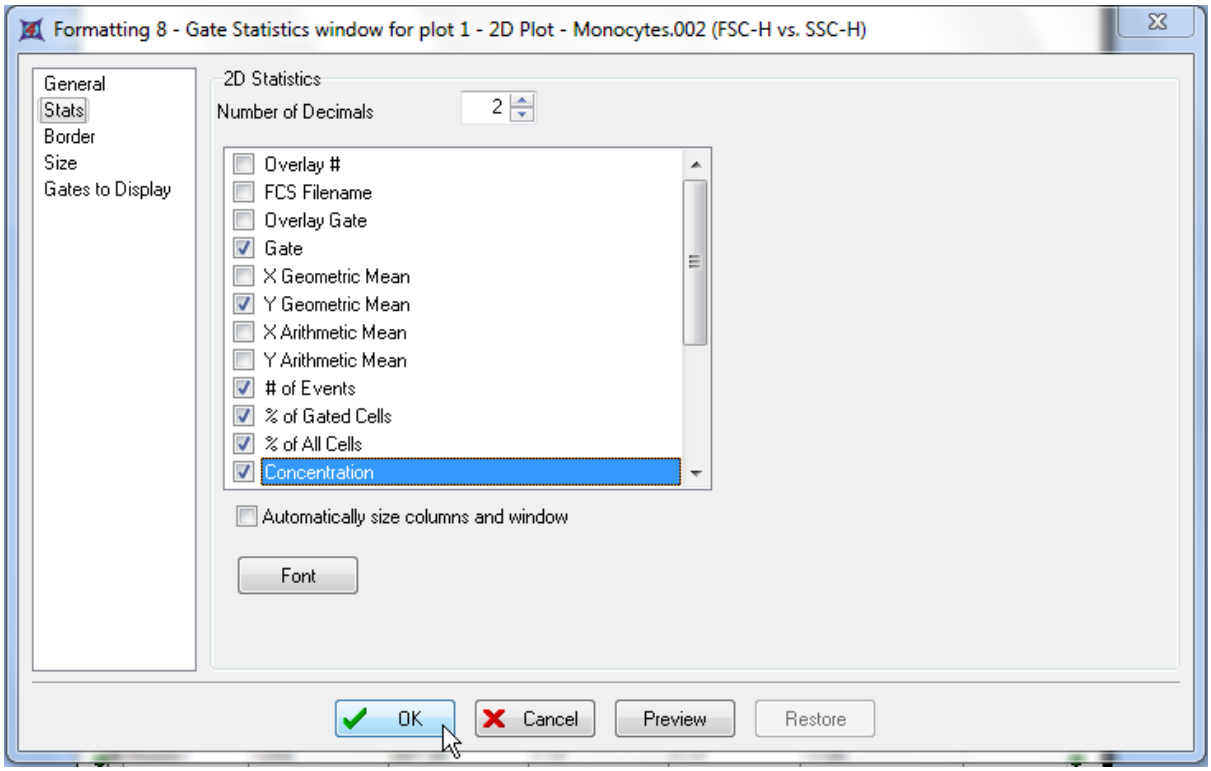

Figure T22.13 Stats Category of the Statistics Window Format Dialog

As you can see in Figure T22.11, the **statistics window** has a **Concentration** column, but it is undefined and shows only zeros. We will now setup FCS Express to calculate these concentrations.

7. Select the **View→Tools→Concentration Calculator** command (Figure T22.5 1153).

The **Concentration Calculator** dialog appears as previously shown in Figure T22.7 1155.

8. Click on the second tab titled **Stats Window Setup** (Figure T22.14).

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 T22.14.

9. Select the **Reference Beads** radio button.

10. Select **Beads2** in the Bead Gate drop-down list.

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11. Enter **100000** in the **Bead Concentration** field.
12. Click **OK** (Figure T22.14).

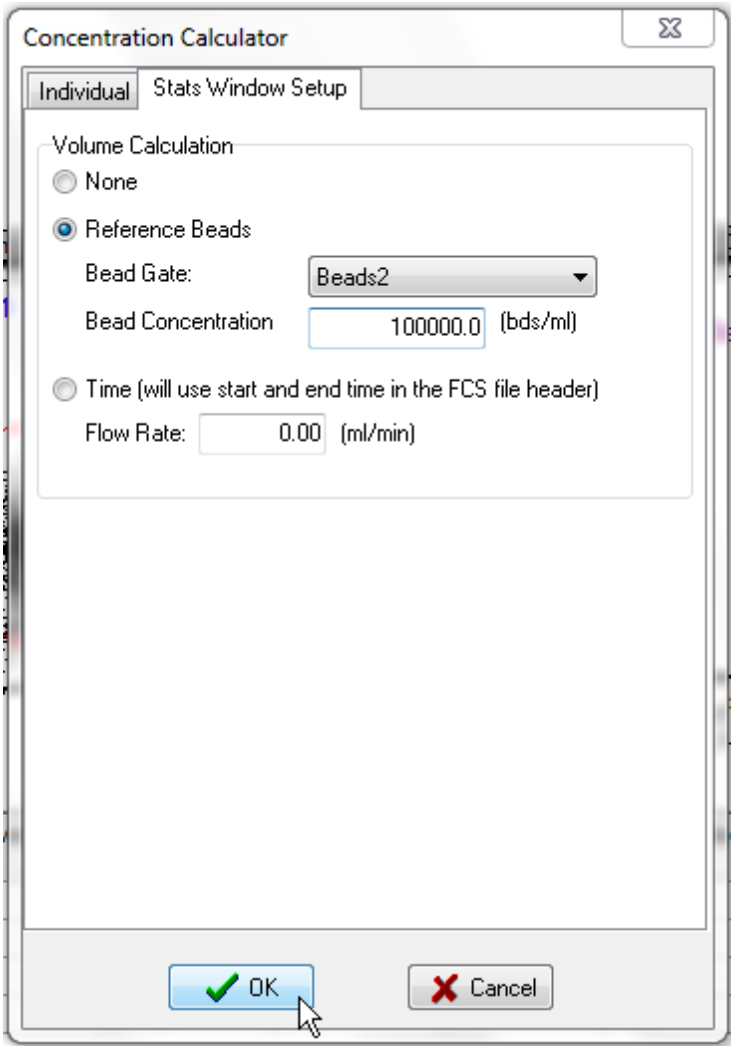

Figure T22.14 Concentration Calculator Dialog -- Stats Window Setup

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 T22.15). 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	Y Geometric Mean	% of Gated Cells	% of All Cells	Concentration
None	17070	302.94	100.00	100.00	1400328.14
Monocytes	13285	355.18	77.83	77.83	1089827.73
Beads1	1258	667.98	7.37	7.37	103199.34
Beads2	1219	677.57	7.14	7.14	100000.00

Figure T22.15 Statistics Window with Concentration Results by Gate

Now, we will show the concentration as a token1164.

34.22.5 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 tokens675. In case you haven't setup the concentration calculation, please do so now in the previous section of this tutorial1157.

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 T22.16).
4. Select **Insert Token** from the pop-up menu (Figure T22.16).

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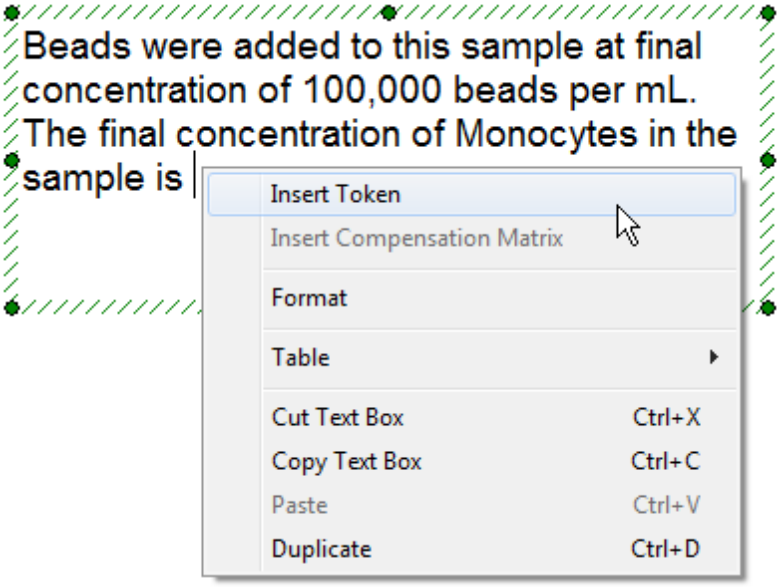

Figure T22.16 Inserting a Token in a Text Box

The **Insert a Token** dialog now appears (Figure T22.17).

5. Select **Statistic** from the **Insert a Token** dialog.
6. Click **Insert** (Figure T22.17).

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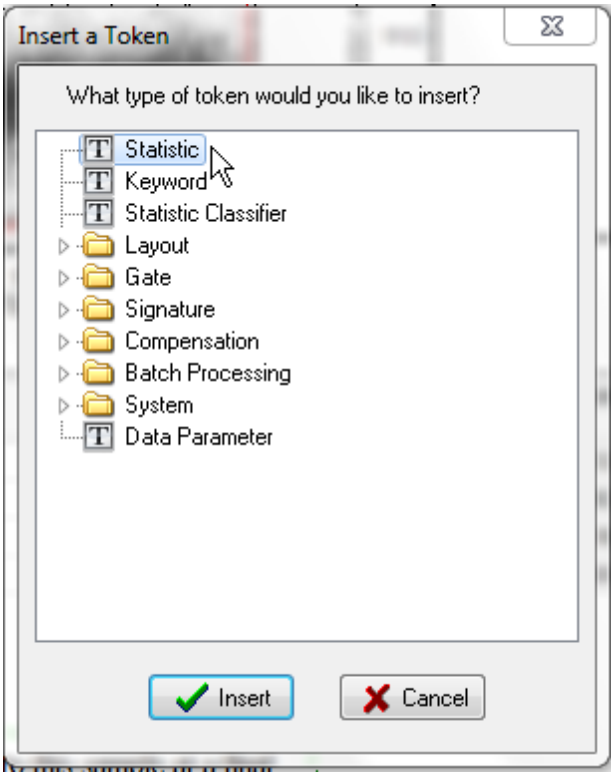

Figure T22.17 Insert a Token Dialog

The **Create Statistic** dialog now appears with the **Data Source** category displayed (Figure T22.18). 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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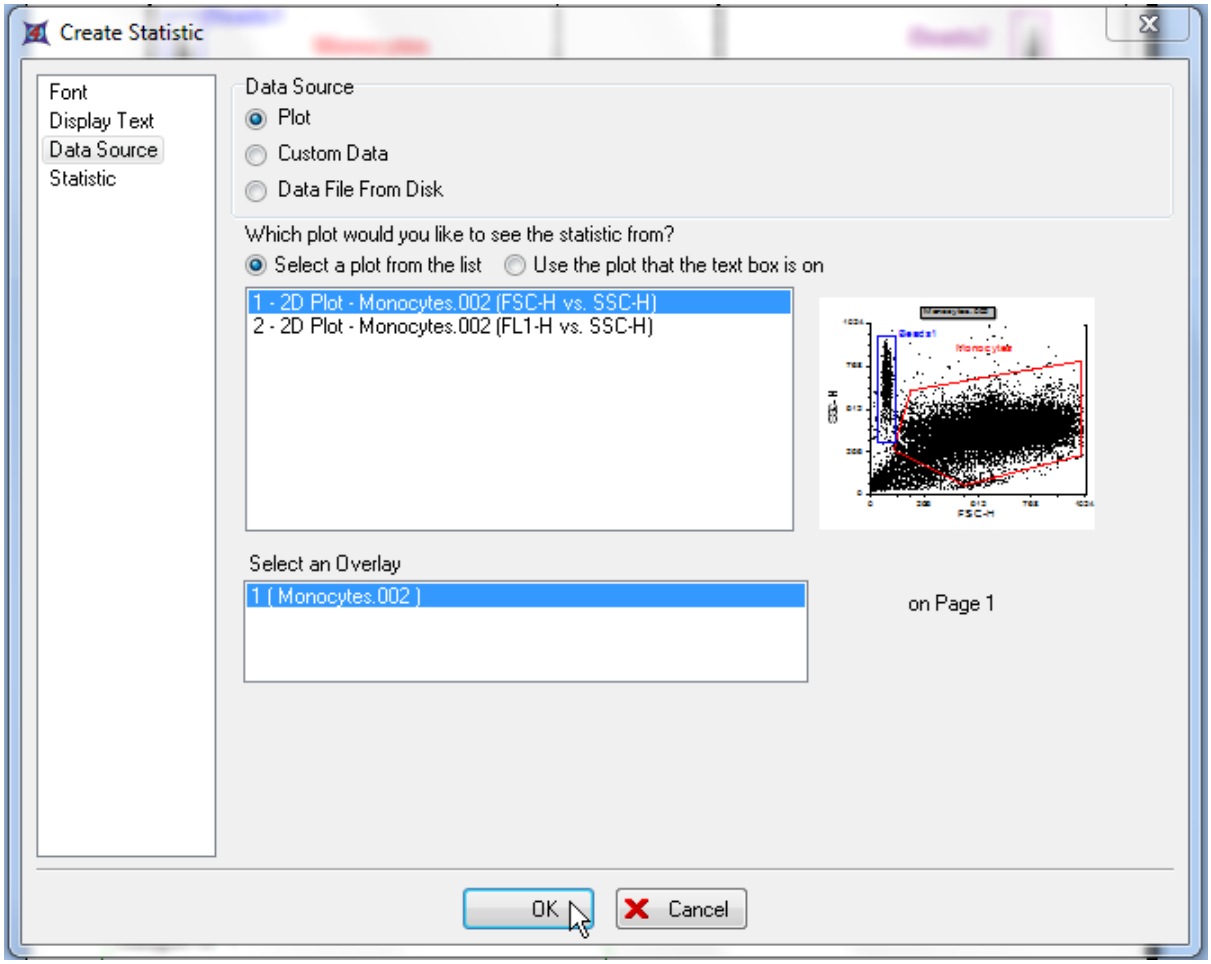

Figure T22.18 Create Statistic Dialog -- Data Source

7. Click on the **Statistic** category in the left panel (Figure T22.19).
8. Select **Monocytes** from the **Gate** drop-down list.
9. Select **Concentration** from the **Statistic** scrolling list.
10. Click **OK** (Figure T22.19).

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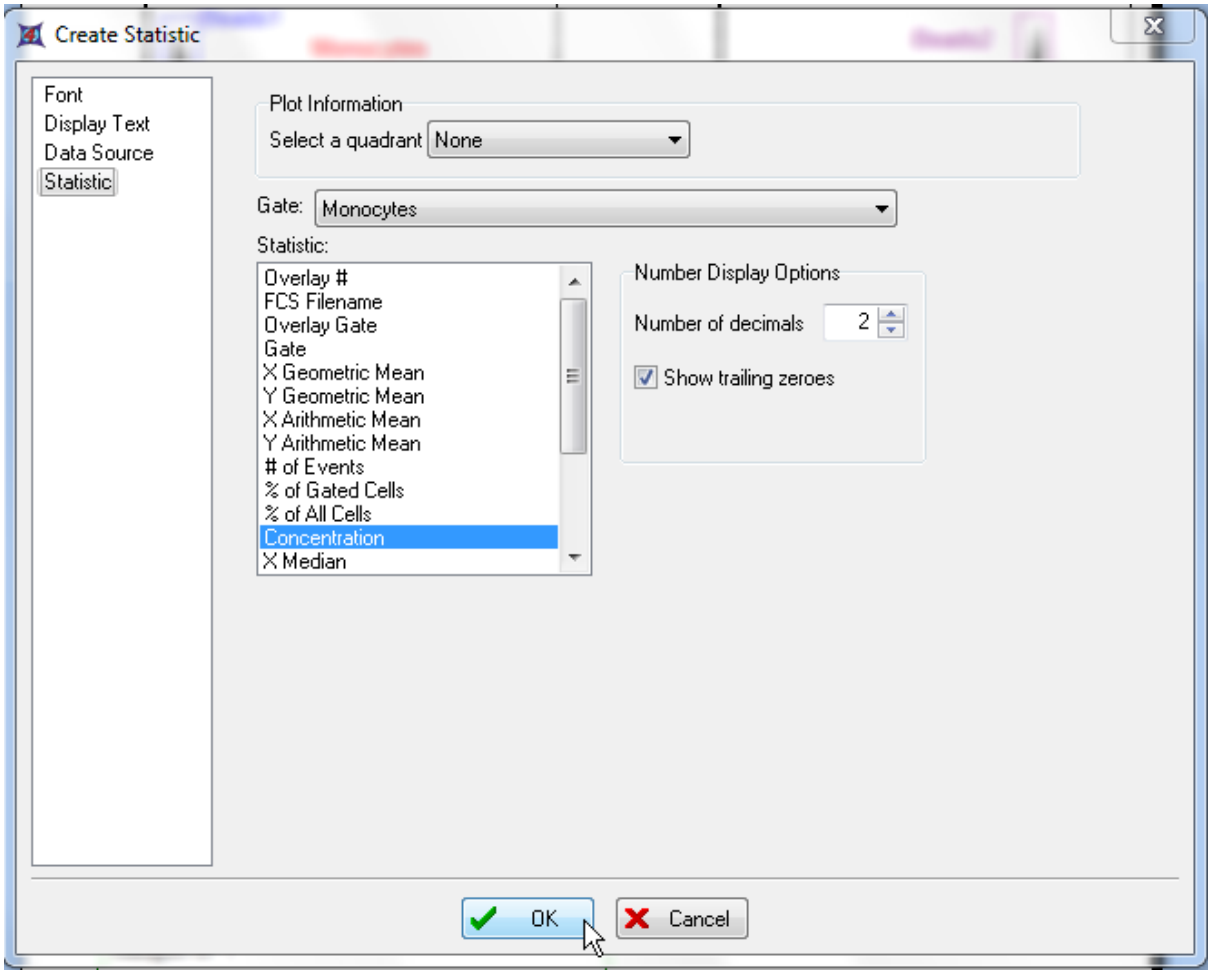

Figure T22.19 Selecting Concentration as the Statistic Token

The text box is now updated with the concentration of the monocytes using the token we just defined, shown highlighted in grey in Figure T22.20.

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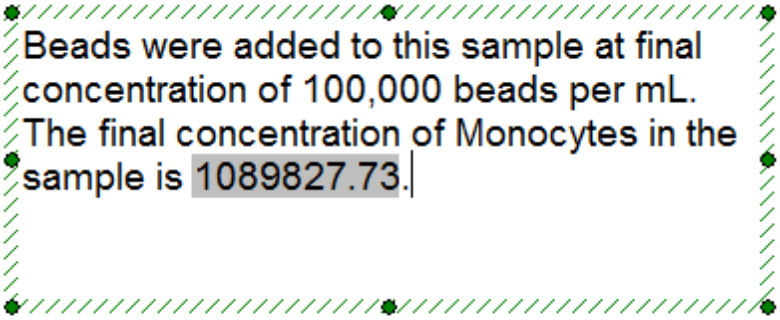

Figure T22.20 Text Box with the Concentration Token Value

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 1153, 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.

34.23 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 1169.

34.23.1 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

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ChannelCalibration.001 located in the Tutorial Sample Data archive 590. For information on how to do this, please see the tutorial on Inserting Plots 607.

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 650.

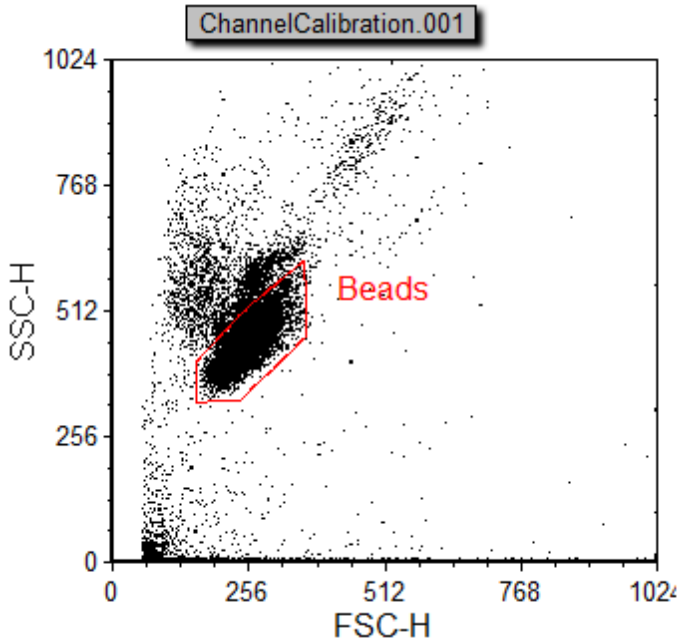

Figure T23.1 Polygonal Gate

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.

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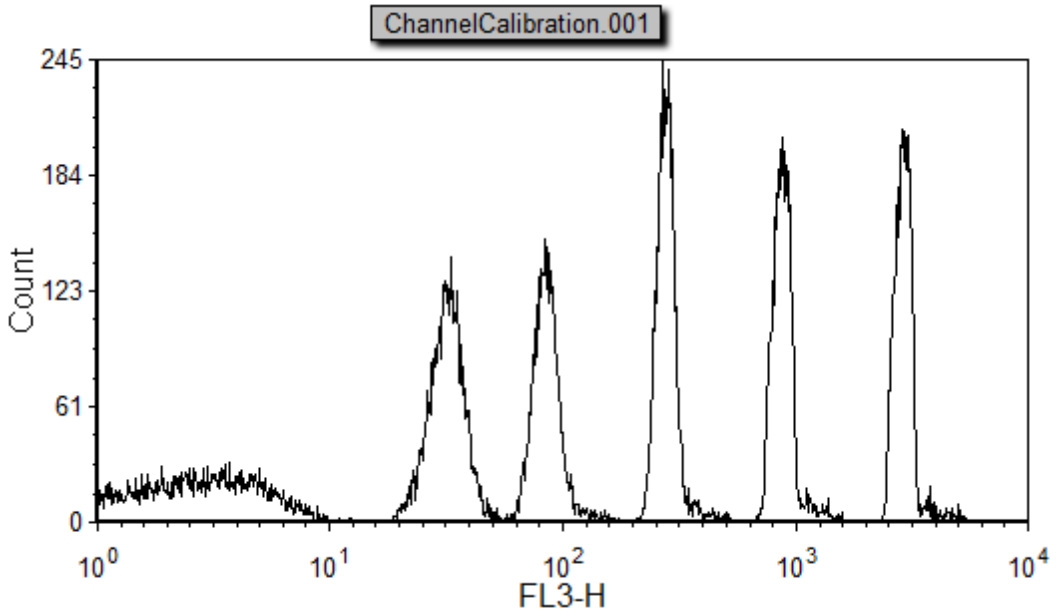

Figure T23.2 Histogram of FL3-H with Beads Gate Applied

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 729.

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.

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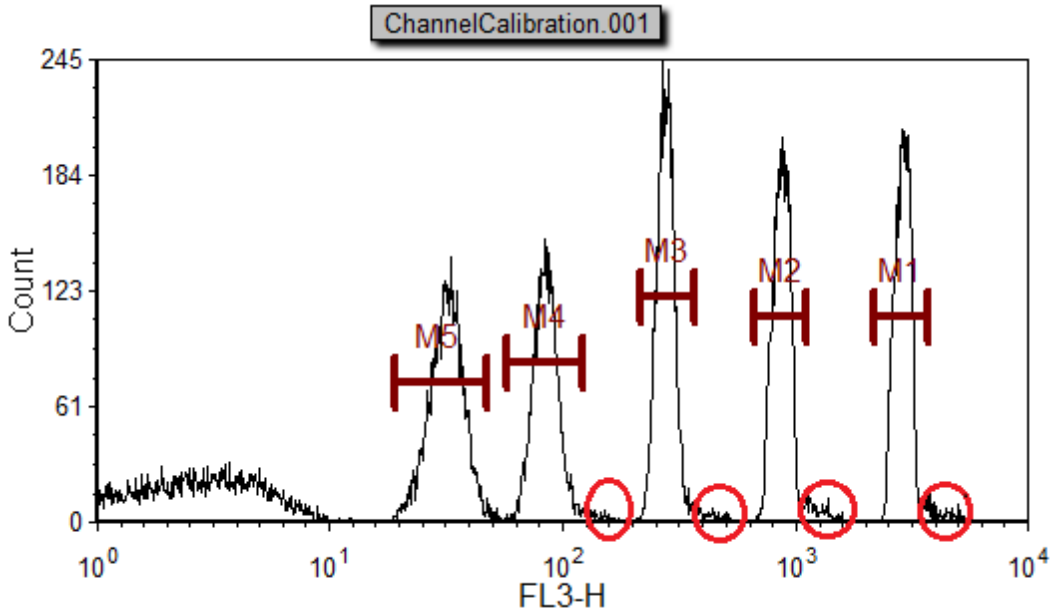

Figure T23.3 Markers M1 through M5 for the five Fluorescent Peaks (red circles indicate doublets)

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 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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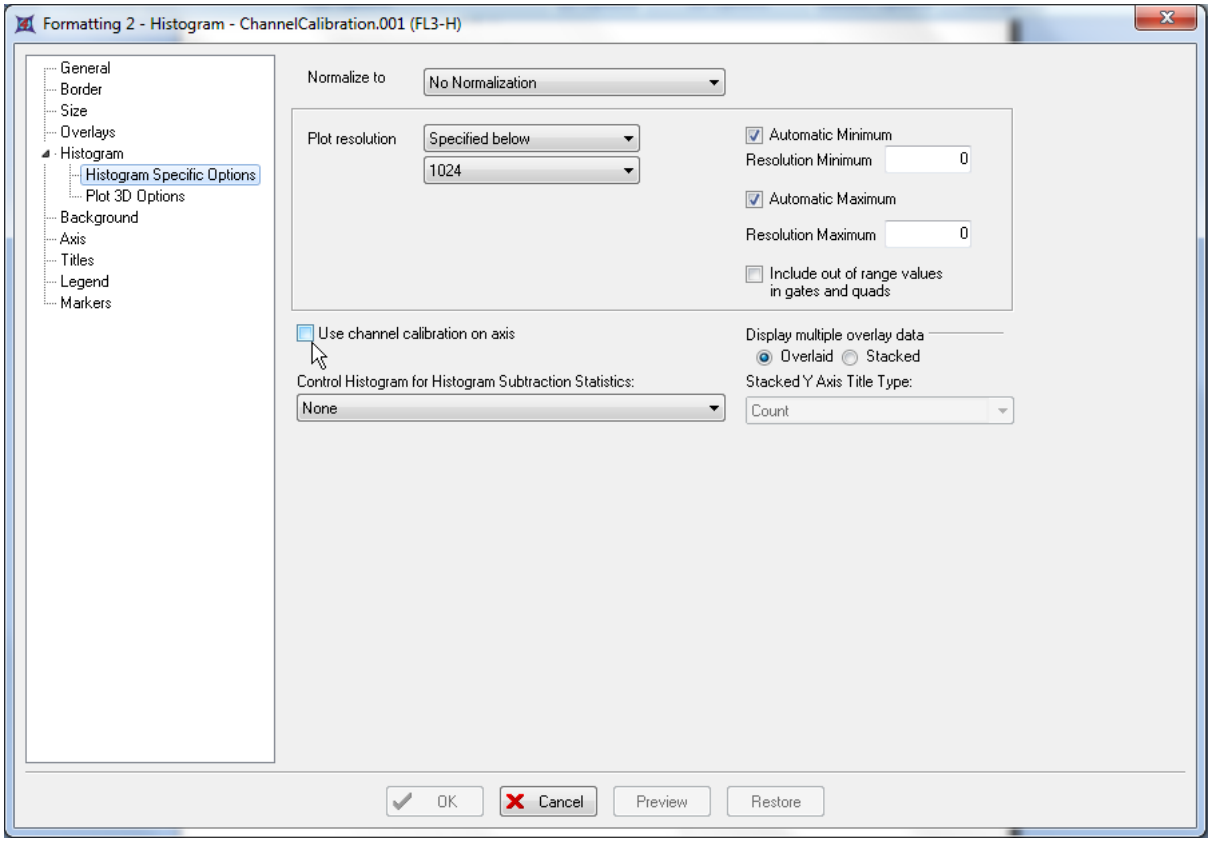

Figure T23.4 Formatting Dialog -- Unselecting Use channel calibration on axis

In the next section, we will set up the channel calibration tool[1173].

34.23.2 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 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

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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 **View→Tools →Channel Calibration** command (Figure T23.5).

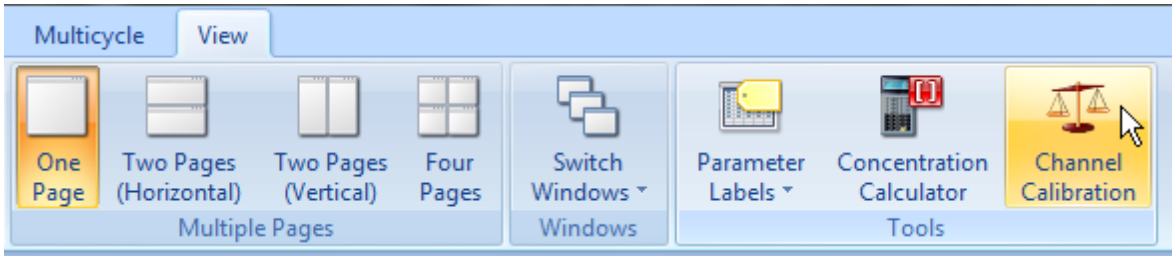

Figure T23.5 Selecting the Channel Calibration Tool Command

The **Channel Calibration** dialog appears (Figure T23.6).

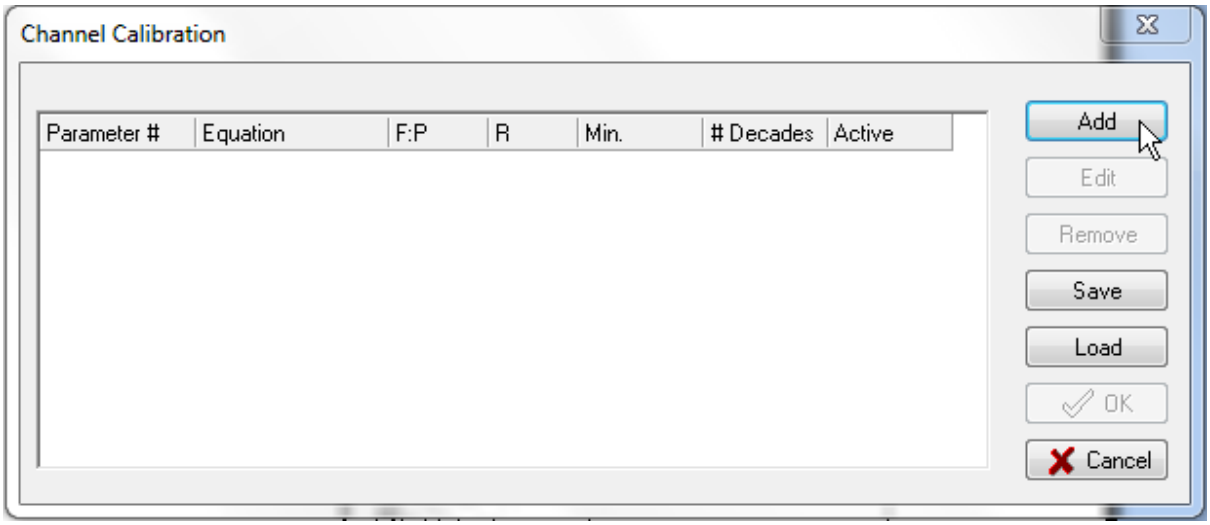

Figure T23.6 Channel Calibration Dialog

2. Click the **Add** button on the **Channel Calibration** dialog (Figure T23.6).

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A **New Calibration** dialog now appears, similar to Figure T23.7.

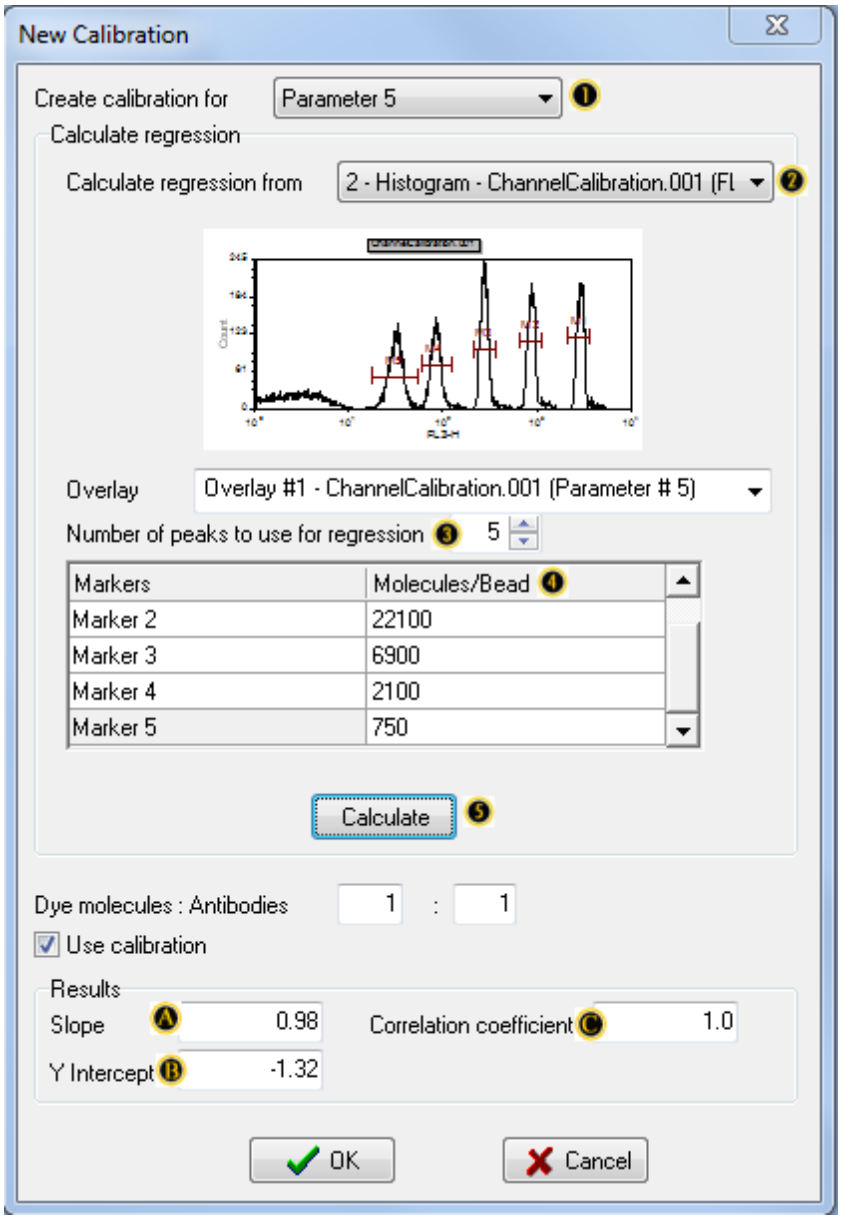

Figure T23.7 New Calibration Dialog

3. Select **Parameter 5** 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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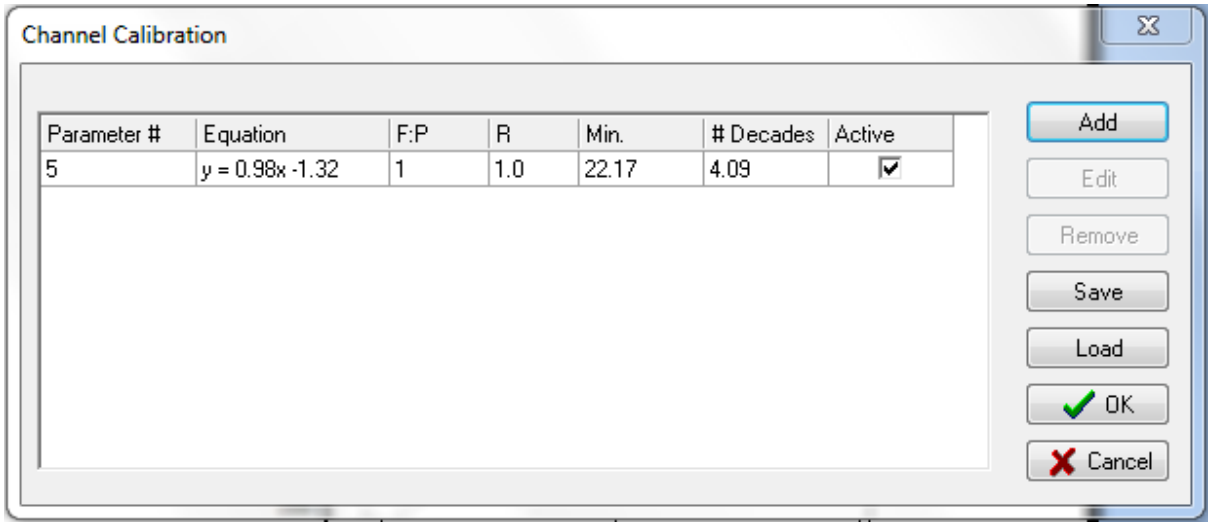

Figure T23.8 Updated Channel Calibration Dialog

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 1177.

34.23.3 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 590.
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 the mean equivalent number of soluble PE-Cy5 molecules. This is

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because the default setting for all plots is to use calibrated axes.

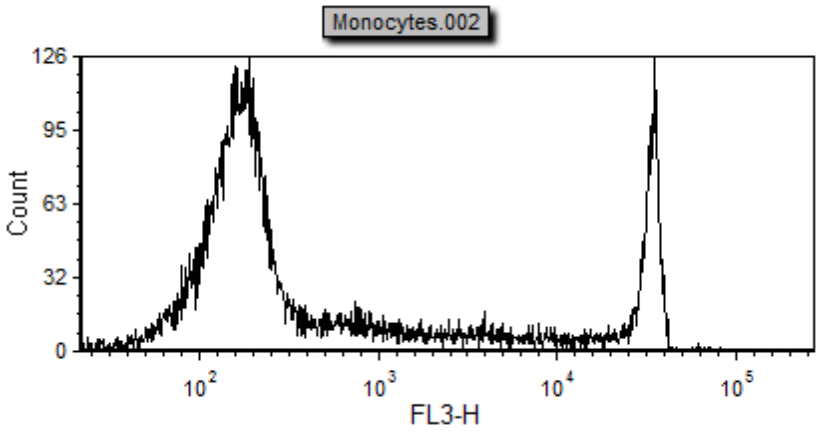

Figure T23.9 Histogram with Calibrated Axis

4. Right-click on the histogram to bring up the associated pop-up menu.
5. Select **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).

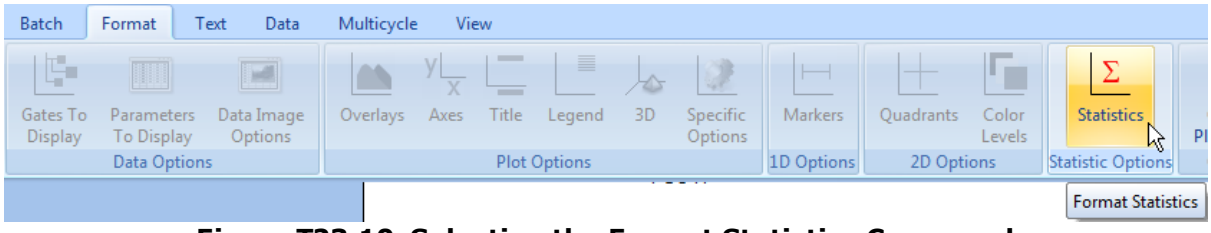

Figure T23.10 Selecting the Format Statistics Command

The **Formatting Statistics** dialog now appears.

9. Select the following items on the **Formatting Statistics** dialog: **Median**, **Geometric Mean**, **Arithmetic Mean**, **CV**, **Peak Value**, **Peak Channel**.

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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	Peak Value	Peak Channel
208.85	585.87	6075.46	204.76	126.00	190.51

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 1169.

Notice that the **Use channel calibration on axis** is checked.

16. Deselect the **Use channel calibration on axis** option (Figure T23.4 1169).
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.

In the next section, we will cover saving and loading channel calibrations 1180.

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34.23.4 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 **View→Tools→Channel Calibration** command (Figure T23.5 1173).

The **Channel Calibration** dialog will appear as previously shown in Figure T23.8 1173.

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 **View→Tools→Channel Calibration** command.

The **Channel Calibration** dialog appears empty, with no calibrations defined, as previously shown in Figure T23.6 1173.

10. Click the **Load** button.

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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 1173, 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 1181.

34.23.5 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 **Application Button→Edit Preferences**.

The **Edit Preferences** dialog now appears (Figure T23.12).

2. Select the **Plots→Dot Options→Binned Dots Specific Options** subcategory on the left side of the **Edit Preference**s 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 **Edit Preferences** dialog.

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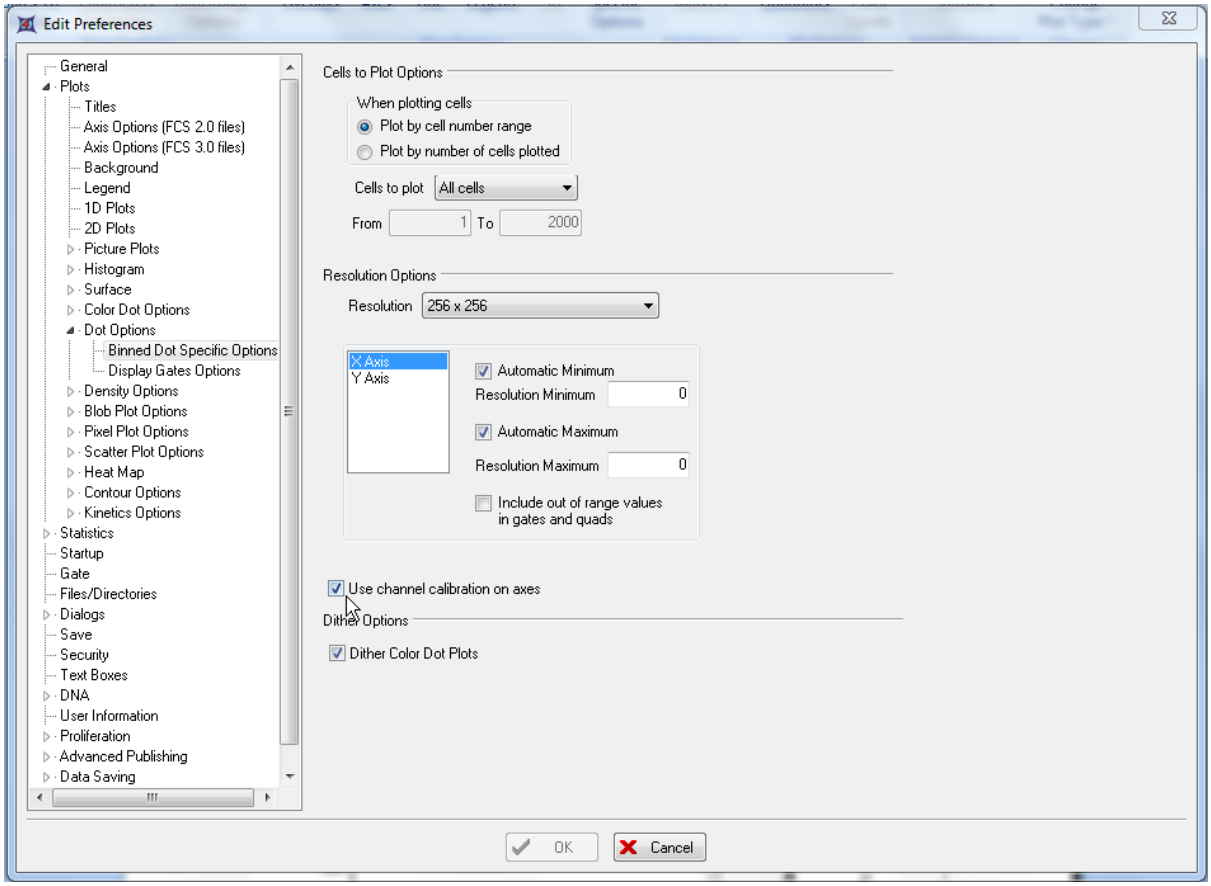

Figure T23.12 Edit Preferences Dialog -- Unselecting Use channel calibration on axes for 2D Dot Plots

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 **Edit Preferences** 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.

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.

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34.24 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 Version 4. 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 V4.

Note: This tutorial will not focus on how to create and use pipelines in CellProfiler. The focus of this tutorial will be on how to modify an existing pipeline to export the data in a format that FCS Express can read.

First, we will setup to export 96 well data from CellProfiler 1188.

34.24.1 CellProfiler for FCS Express Knowledge Base

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 4 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 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

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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 if you choose to **Load images** (figure T24.67 below).

- The original image files should be stored in the same folder where that contains your .cptoc, cpout, and image mask files.

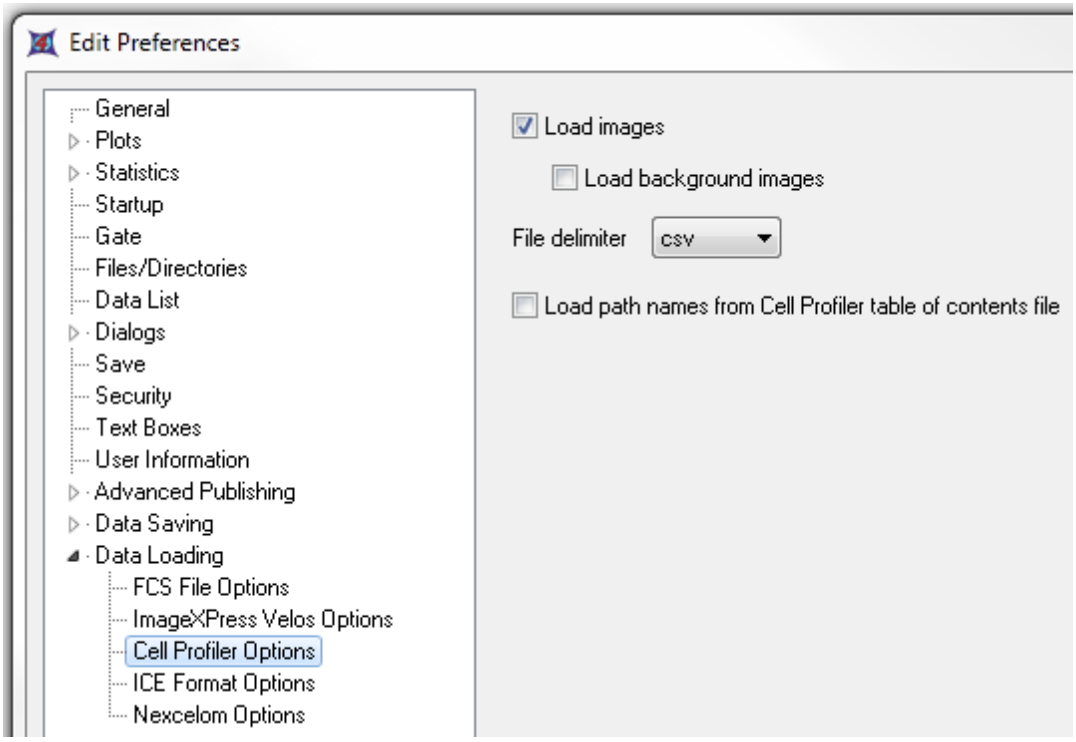

Figure T24.67 CellProfiler Data Loading Options

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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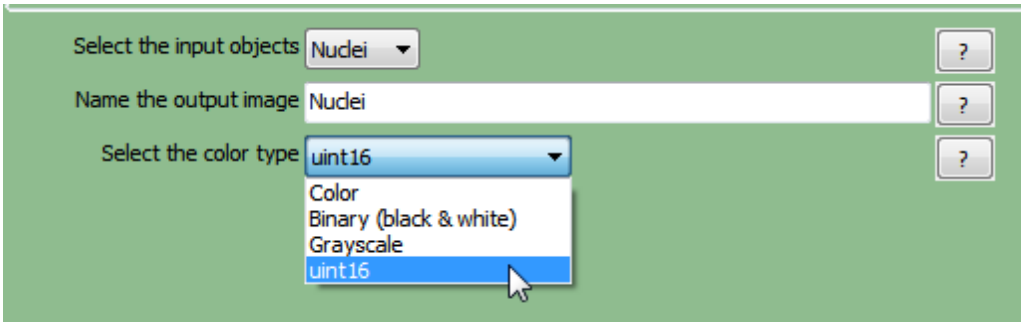

Figure T24.68 ConvertObjectsToImage Module: Naming the output image the same as the input object.

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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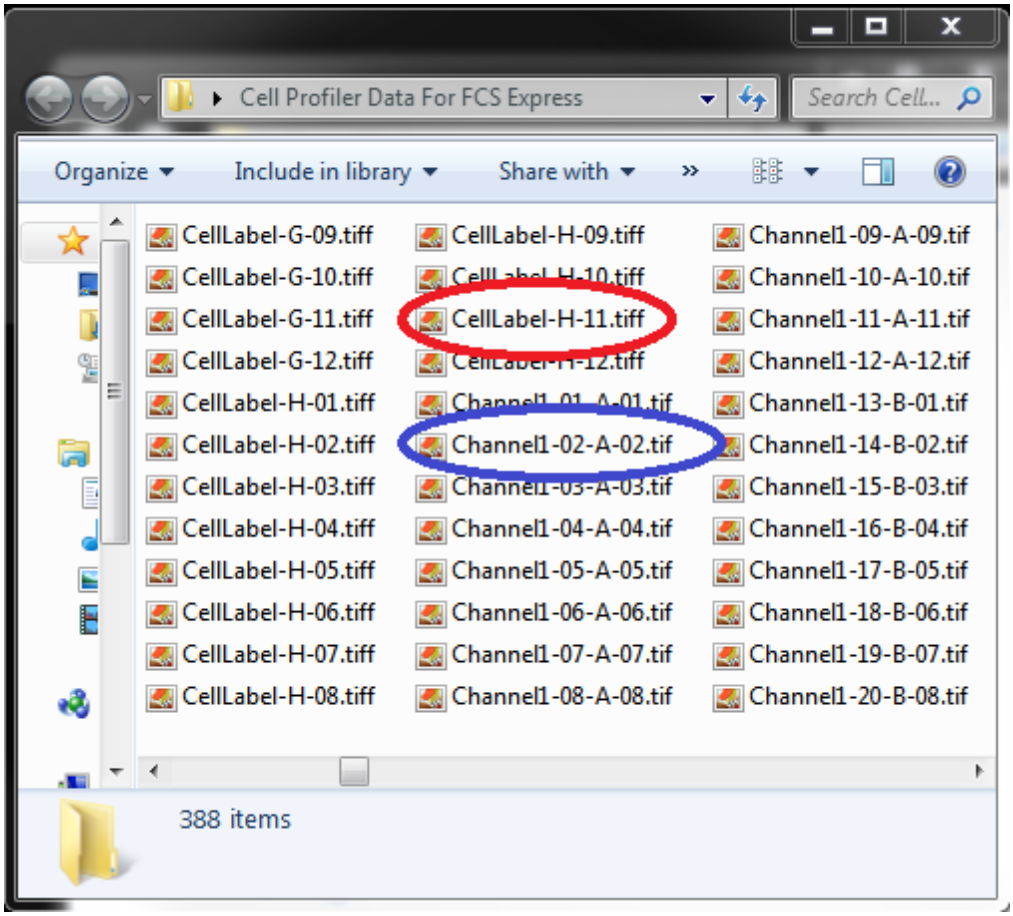

Figure T24.69 NucleiLabel and CellLabel image mask .tiff files have been created for every well of the plate. The file names reflect the Row and Column numbers for the CellLabels (Red Oval) based on the original images (Blue Oval).

- **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

    mytiledimage_row00_col02.tif

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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.
  -

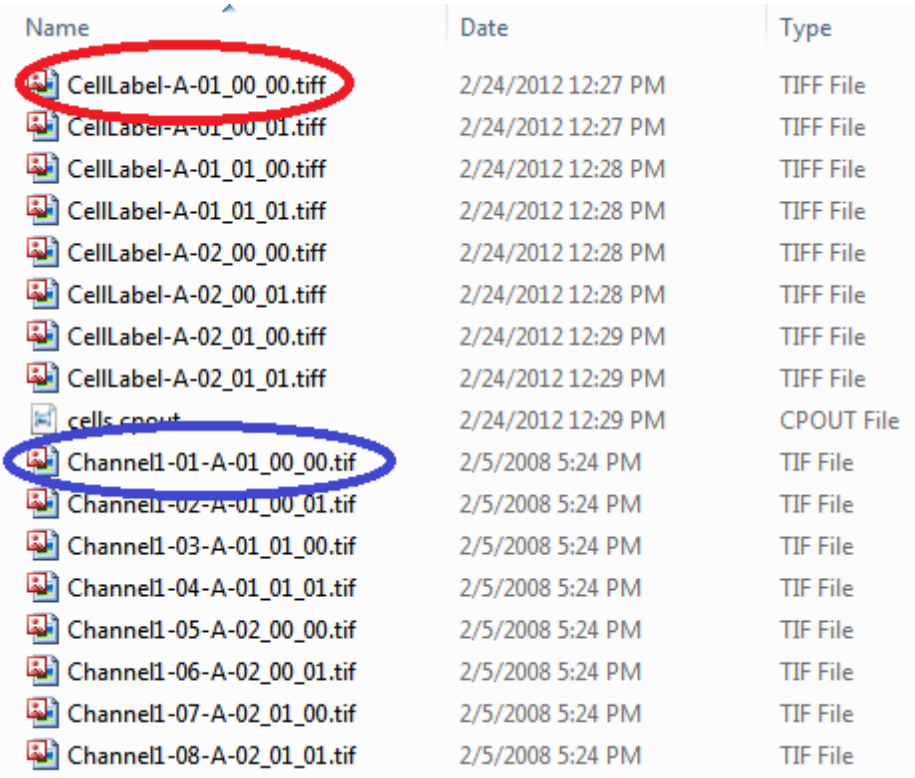

Figure T24.70 CellLabel image mask .tiff files have been created for every well of the plate. The file names reflect the Row and Column letters and numbers as well as the image location Row and Col numbers for the CellLabels (Red Oval) based on the original images (Blue Oval).

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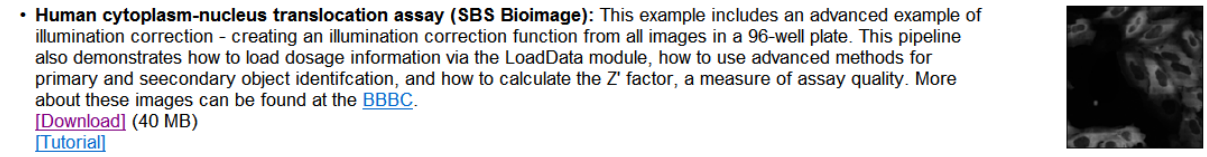
34.24.2 Exporting 96 Well Format Images

The CellProfiler modules that are needed or must be amended for export to FCS Express 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 590 and is named **Section1pipelineCOMPLETED.cp**. 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 (SBS Bioimage)"**.



CellProfiler Example Link

The steps for exporting data from CellProfiler have been broken down by module according to the Section1pipeline.cp. After loading the Section1pipeline.cp, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window (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, we recommend deleting it to conserve disk space.

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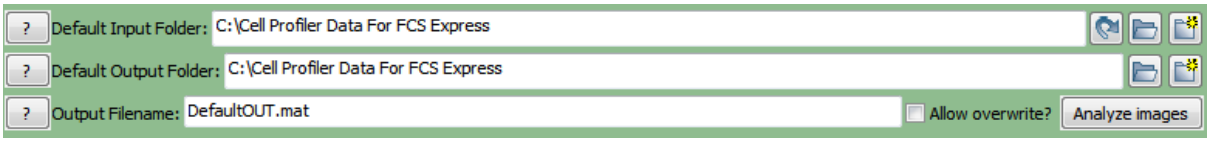

Figure T24.1 Set the Default Input and Output Folders to the Same Location

LoadImages Module Setup

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 (Figure T24.2):

1. Click on the **Channel2-** drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example we will use "Channel2-[0-9]{2}-(?P<Row>[A-H])-(?P<Column>[0-9]{2}).tif" which defines the plate location of the image by Row Letter and Column Number found in the image name.

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.

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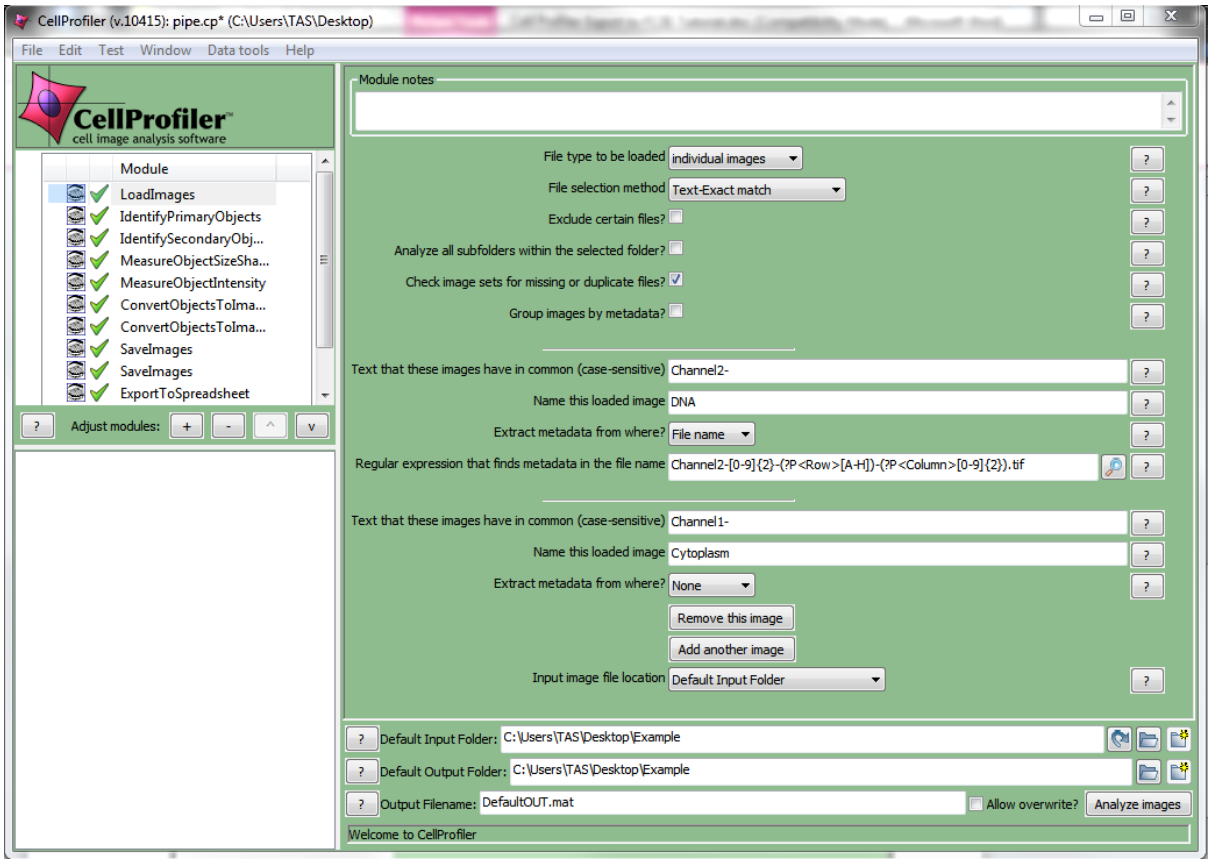

Figure T24.2 Setting up a Regular Expression in the LoadImages Module for a 96 Well Plate Experiment

ConvertObjectsToImage Modules

In this module, 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.

4. Select the first **ConvertObjectsToImage** module in the pipeline.

5. Choose **Select the input objects** as **Nuclei** from the drop-down list.

6. **Name the output image** with any name you prefer. For this example, use "Nuclei".

7. **Select the color type** from the drop-down list and choose **uint16** (Figure T24.3).

8. Select the second **ConvertObjectsToImage** module in the pipeline.

9. Choose **Select the input object** as **Cells** from the drop-down list.

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10. **Name the output image** with any name you prefer. For this example, use "Cells".

11. **Select the color type** from the drop-down list and choose **uint16**.

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)*

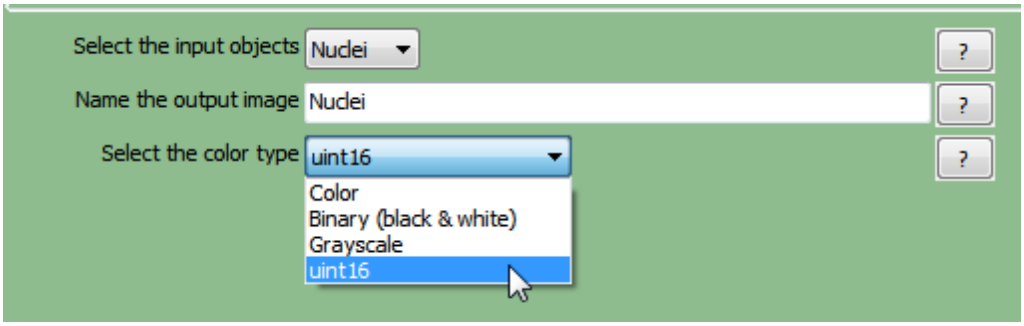

Figure T24.3 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** module must now be saved and related to the original images/data.

12. Select the first **SaveImages** module in the pipeline.

13. **Select the type of image to save** as **Image** from the drop-down list.

14. **Select the image to save** as **Cells**.

Note: When defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.

15. **Select method for constructing file names** as **single name** from the drop-down list.

16. **Enter single file name** as any name you prefer followed by a hyphen **"-"**. For this example use **CellLabel-**.

17. Right-click after **CellLabel-**, choose **Row,** and enter a hyphen **"-"**after it.

18. Right-click after **Row-** and choose **Column**.

19. Select the second **SaveImages** module in the pipeline**.**

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20. **Select the type of image to save** as **Image** from the drop-down list.
21. **Select the image to save** as **Nuclei**.
22. **Select method for constructing file names** as **Single name** from the drop-down list.
23. **Enter single file name** as any name you prefer followed by a hyphen **"-"**. In this example we have chosen **NucleiLabel-**.
24. Right-click after **NucleiLabel-**, choose **Row,** and enter a hyphen **"-"**after it.
25. Right-click after **Row-** and choose **Column.**

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.

Perform steps 26-32 on both **SaveImages** modules set up for **CellLabel** and **NucleiLabel**.

26. **Select the format to use** as **tif** from the drop-down list.
27. Set **Image bit depth** at **16** from the drop-down list.
28. Choose **Default Output Folder** from **Output file location.**
29. Check the **Overwrite existing files without warning?** box.
30. Set **Select how often to save** as **Every cycle** from the drop-down list.
31. **Select colormap** as **gray** from the drop-down list.
32. Check the **Update file names within CellProfiler?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.4a

*(Note: in CellProfiler Version 2 the **Update names within CellProfiler?** check box has been renamed **Store file and path information to the saved image?** Figure T24.4b)*

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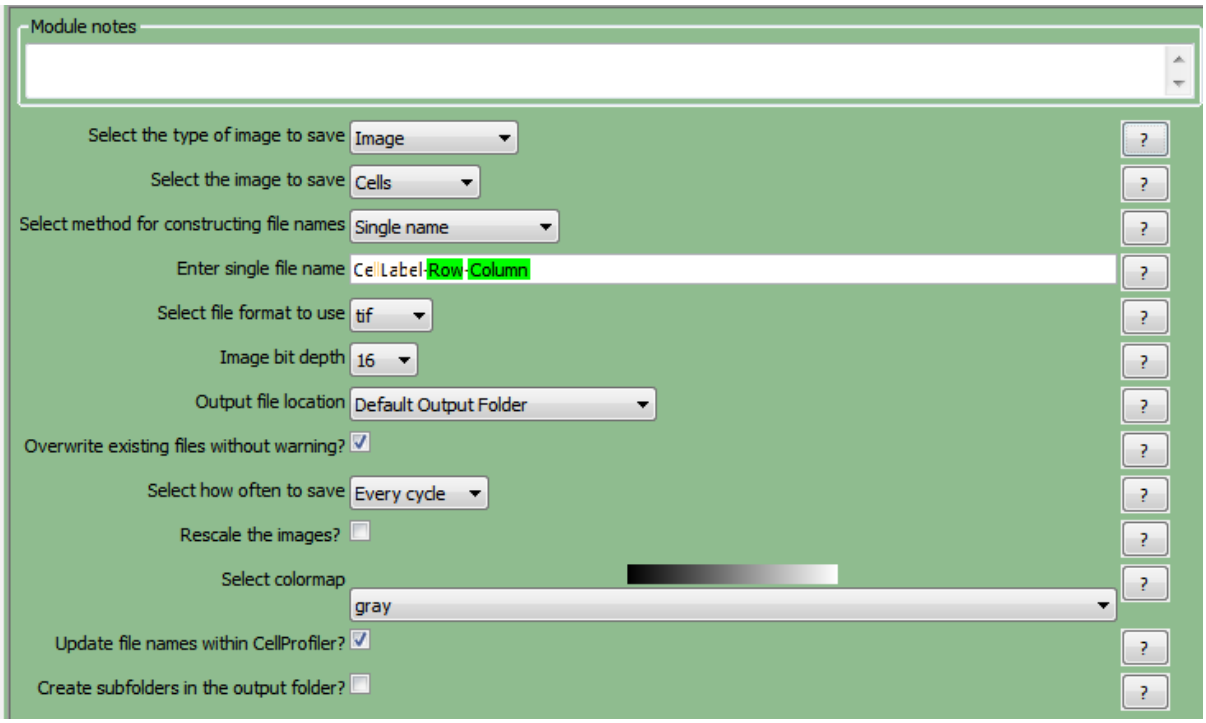

Figure T24.4a Defining the SaveImages Module

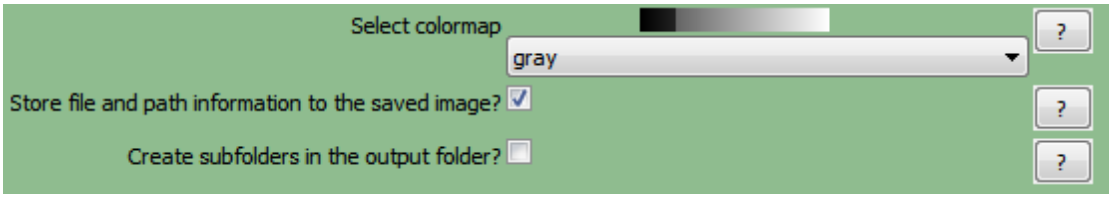

Figure T24.4b Defining the SaveImages Module in CellProfiler V2

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 section)

33. Select the **ExportToSpreadsheet** module.

34. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.

35. Uncheck the **Prepend the output file name to the data file names?** box.

36. Choose **Output file location** as **Default Output Folder** from the drop-

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down list.

37. Uncheck **Export all measurements?**.
38. Choose **Image** from the first **Data to export** drop-down list.
39. Uncheck **Use the object name for the file name?**.
40. Set the file name as **Image.cptoc.**
41. Choose **Add another data set**.
42. Choose **Nuclei** from the second **Data to export** drop-down list.
43. Uncheck **Use the object name for the file name**.
44. Set the file name as **nuclei.cpout**.
45. Repeat steps 41-44 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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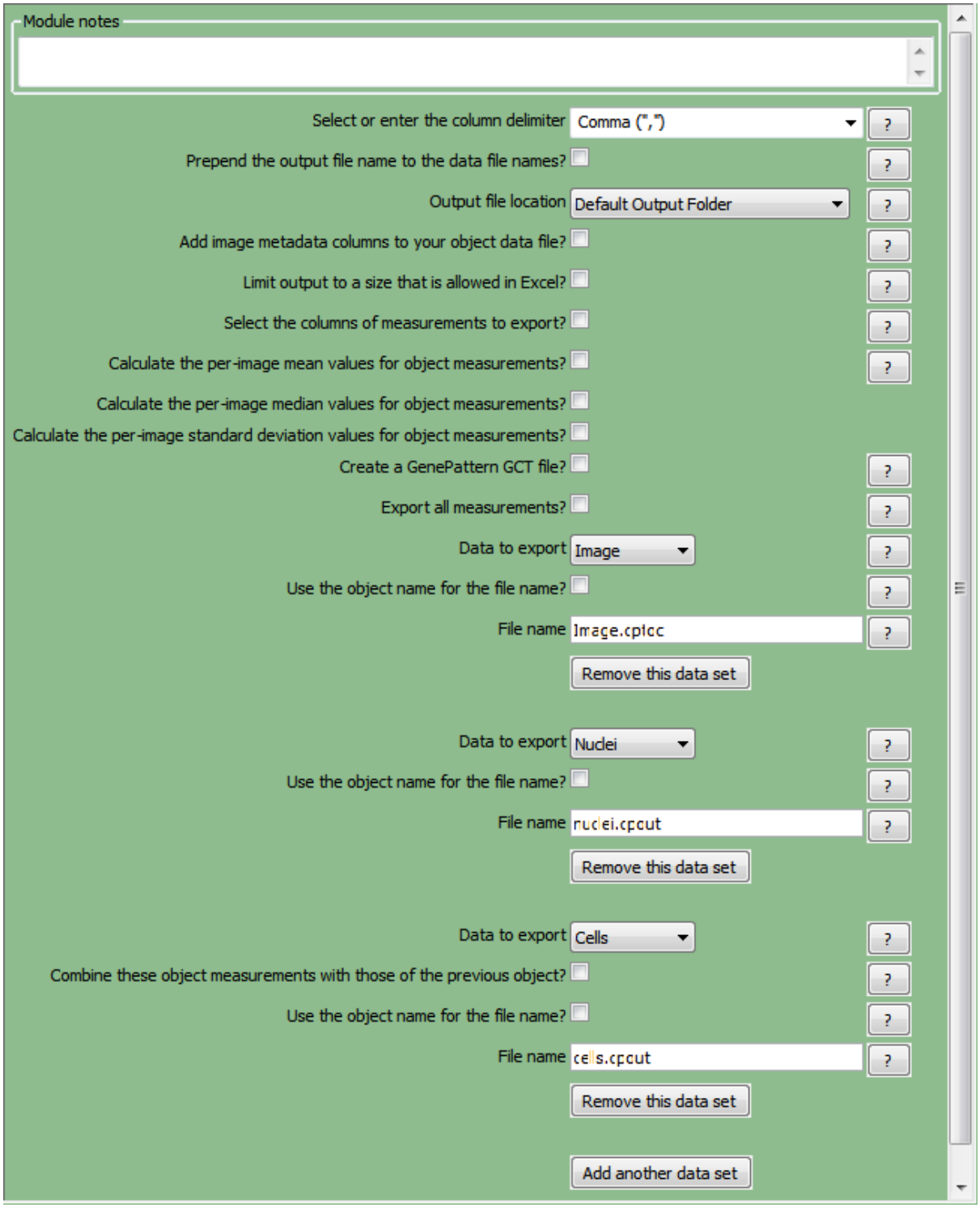

Figure T24.5 ExportToSpreadsheet Module Set Up to Export Image.cptoc, nuclei.cpout, and cells.cpout

46. Select **Analyze images** to run the pipeline. The folder where your images

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are stored will now contain the cells.cpout, nuclei.cpout and Image.cptoc files (Figure T24.6A). There will also be a CellLabel and NucleiLabel image associated with every original image/well from the plate (Figure T24.6B).

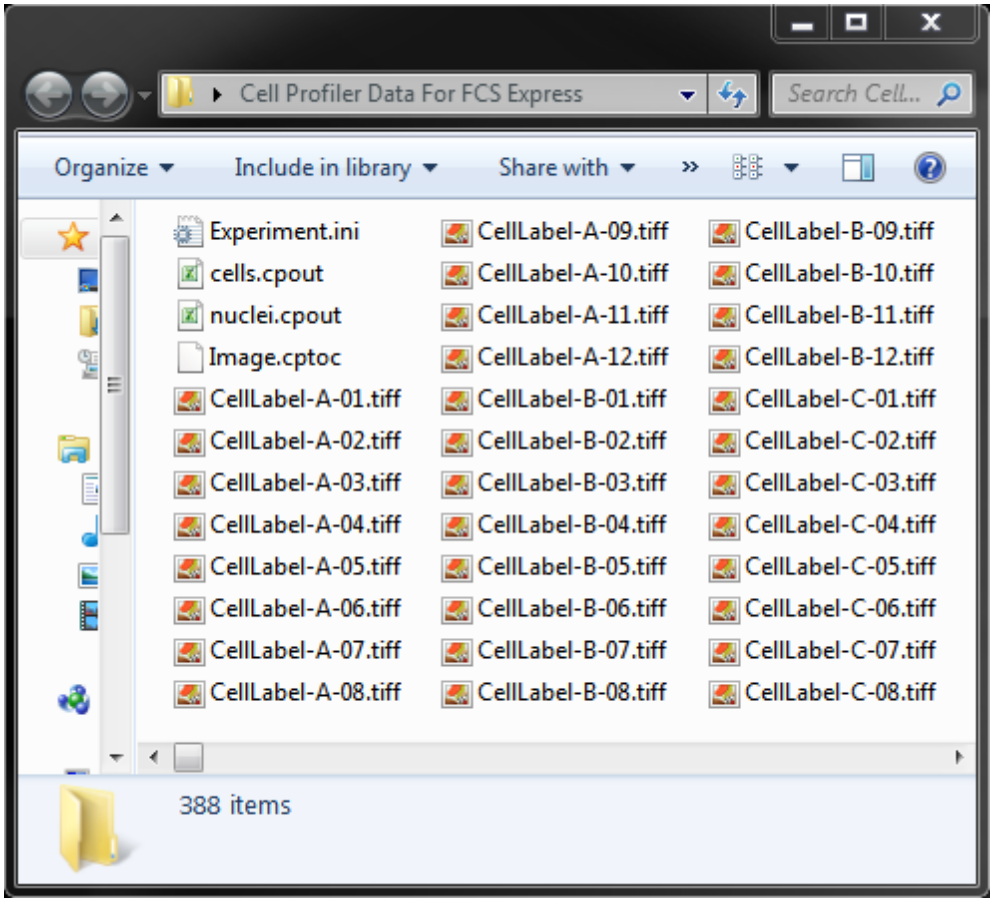

Figure T24.6A cells.cpout, nuclei.cpout, and Image.cptoc Files were Created. Experiment.ini was Downloaded.

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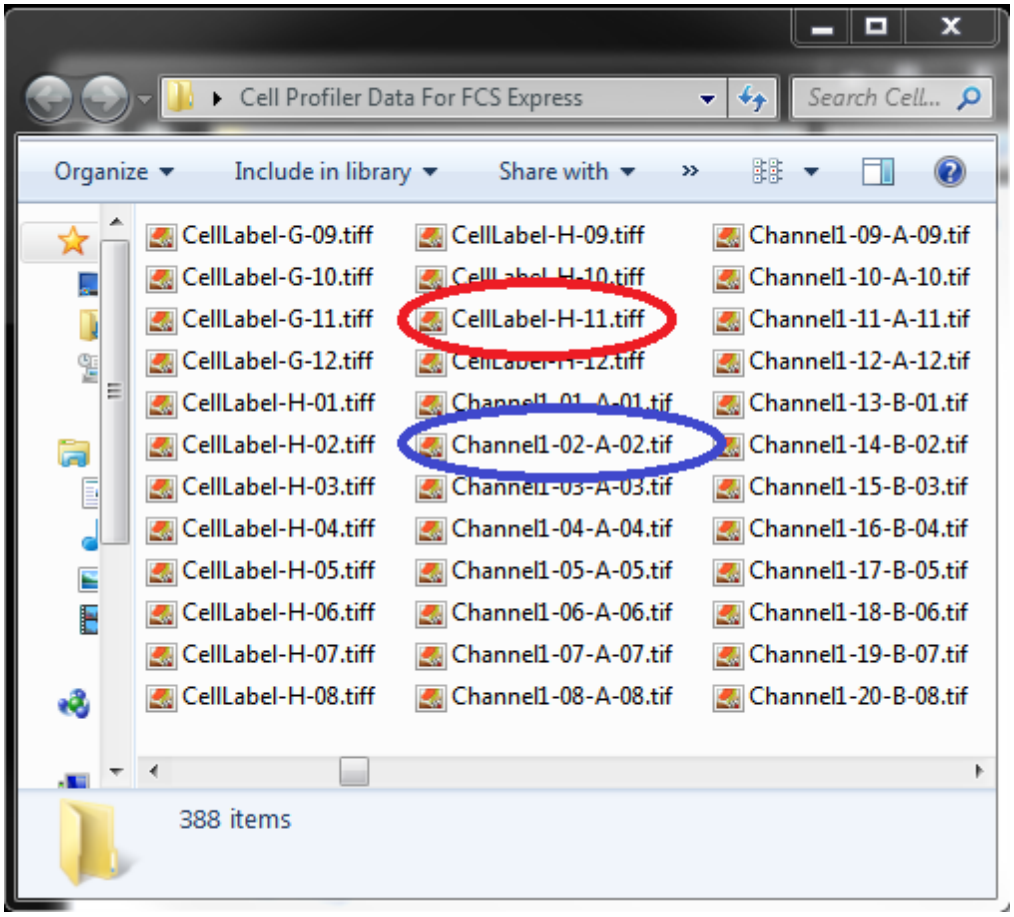

Figure T24.6B NucleiLabel and CellLabel image mask .tiff Have Been Created for Every Well of the Plate. The File Names Reflect the Row and Column Numbers for the CellLabels (Red Oval) and Channels (Blue Oval).

In the next section, we will import and analyze the 96 well image data1197.

34.24.3 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 V4. 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 archive590 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 (Figure T24.6A1188).

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1. Download the example data from the CellProfiler Examples website.
2. Load the **Section1pipelineCOMPLETED.cp** pipeline into CellProfiler.
3. Select the default input and output folders in CellProfiler to load and store the data as shown in Figure T24.1 1188.
4. Analyze the images in CellProfiler.

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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

5. Open FCS Express V4.
6. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.7).
7. Select **csv** from the **delimiter** drop-down list.
8. Check the **Load Images** check box.
9. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
10. Click **OK** to close the **Edit Preferences** dialog.

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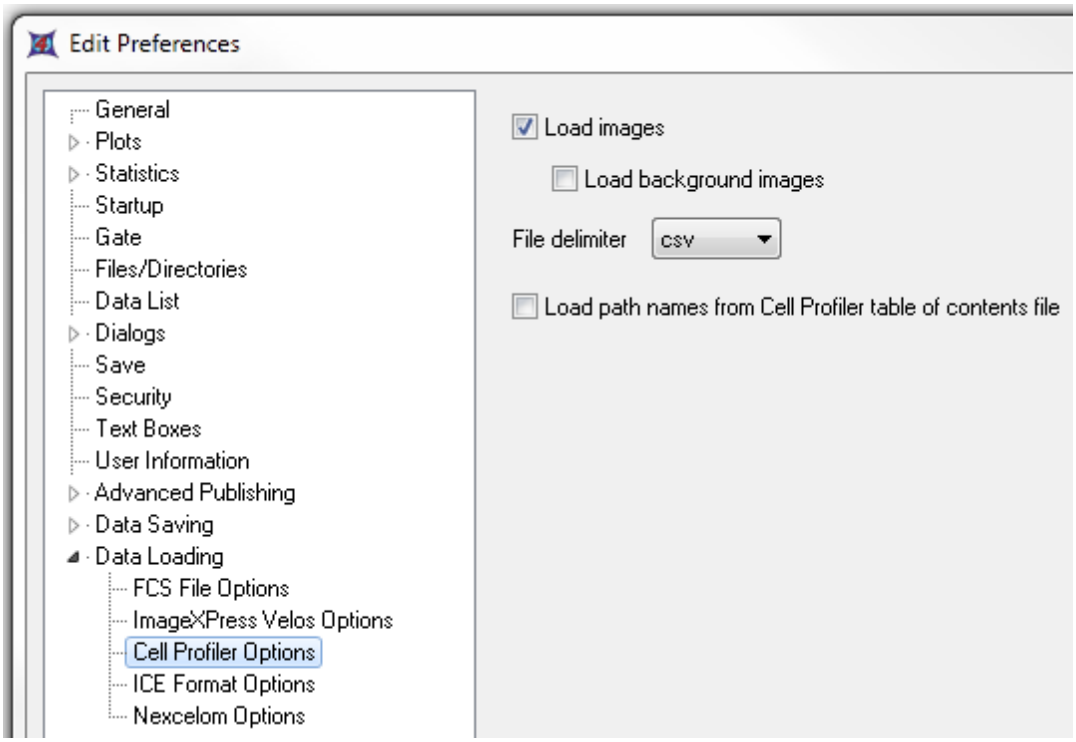

Figure T24.7 CellProfiler Data Loading Options for a 96 Well Plate Experiment with Images

11. Select the **Insert→Other Plots→Heat Map** command (Figure T24.8).

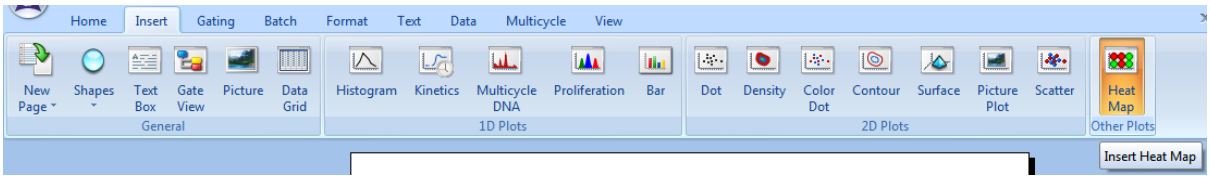

Figure T24.8 Choosing to Insert a Heat Map

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 T24.9). 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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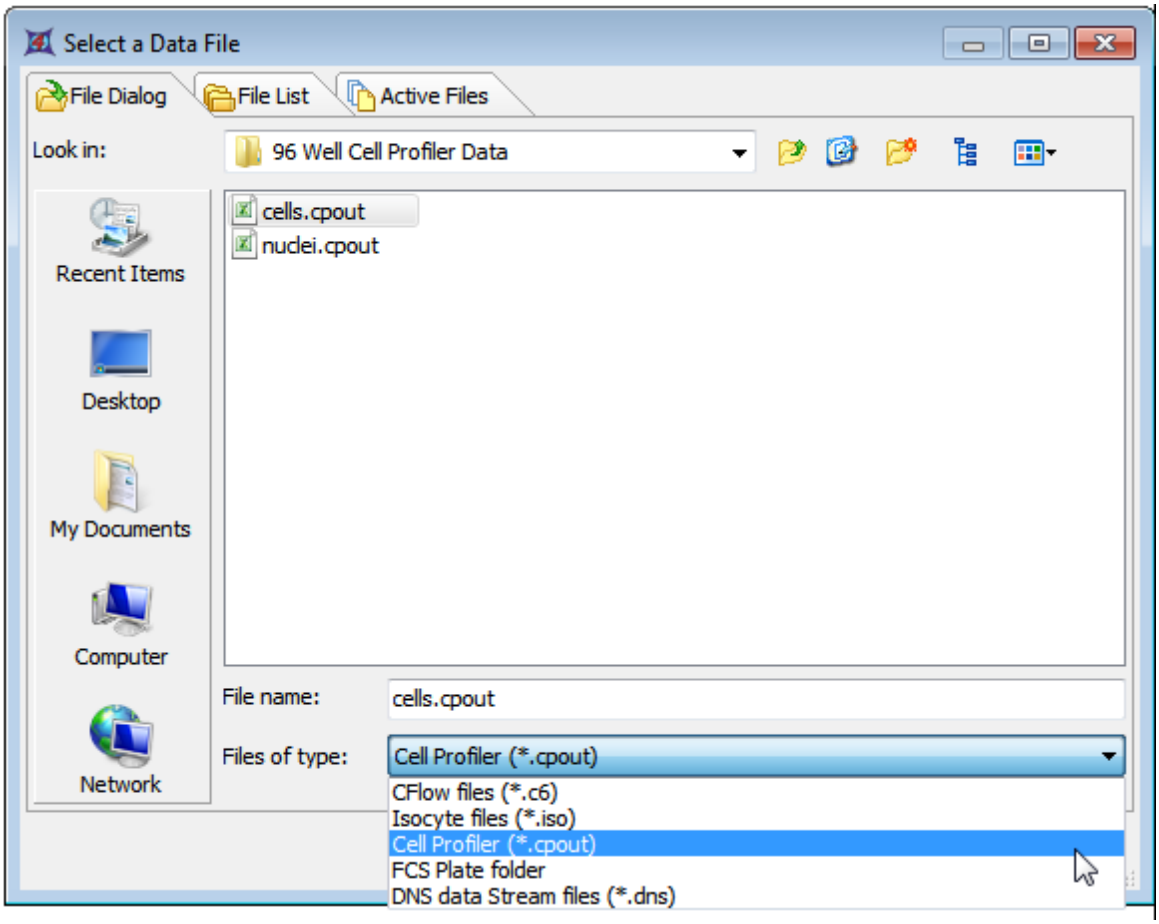

Figure T24.9 Choosing CellProfiler Files

15. Highlight **cells.cpout**.

16. 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.

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 T24.10).

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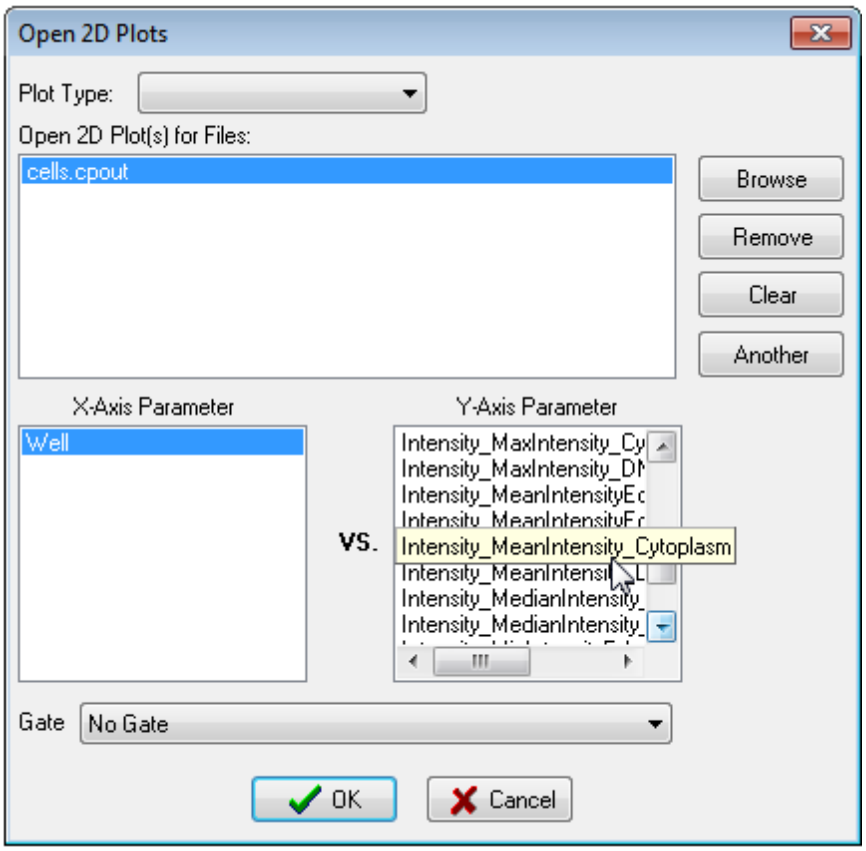

Figure T24.10 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

18. 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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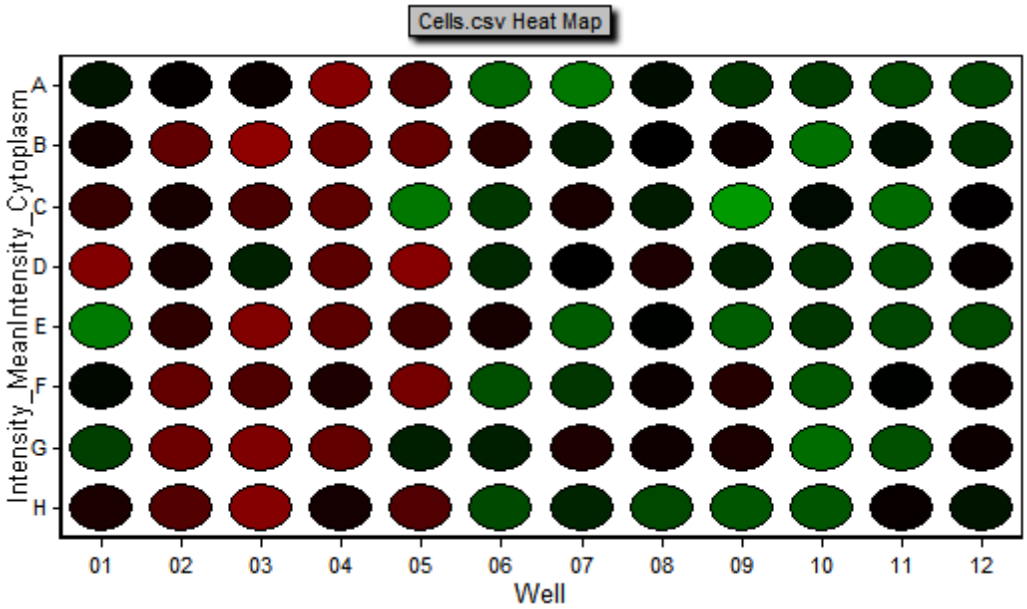

Figure T24.11 Example of a Heat Map Generated from CellProfiler Data

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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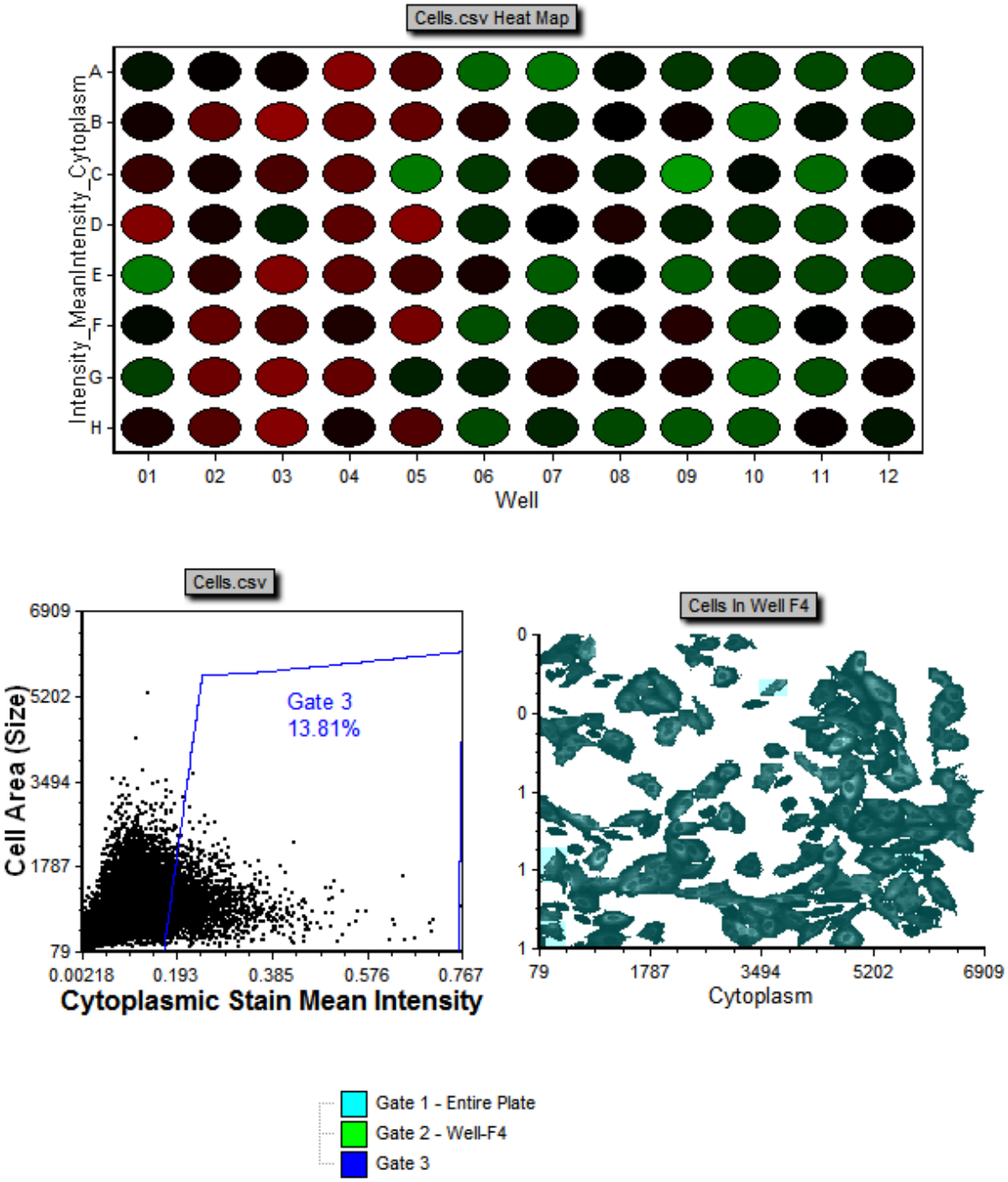

Figure T24.12 A Heat Map from CellProfiler Data with Corresponding Dot and Picture Plots

Next, we will export single images from CellProfiler1204.

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34.24.4 Exporting Single Images

In the next two sections, you will learn:

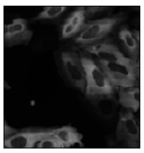
- How to export CellProfiler data from a set of single images that were not obtained from a 96 well plate.
- How to import the data into FCS Express V4.

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.

The CellProfiler modules that are needed or must be amended for export to FCS Express in this section are LoadImages, ConvertObjectsTo Images, SaveImages, and ExportToSpreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive and is named **Section2pipelineCOMPLETED.cp**. 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 labeled "**5 and 10 minute time point images"**. These images are based on the "**Human cytoplasm-nucleus translocation assay (SBS Bioimage)"** sample experiment found at CellProfiler Examples.

- **Human cytoplasm-nucleus translocation assay (SBS Bioimage):** This example includes an advanced example of illumination correction - creating an illumination correction function from all images in a 96-well plate. This pipeline also demonstrates how to load dosage information via the LoadData module, how to use advanced methods for primary and seecondary object identifcation, and how to calculate the Z' factor, a measure of assay quality. More about these images can be found at the BBBC.
  [Download] (40 MB)
  [Tutorial]



The steps for exporting data from CellProfiler have been broken down by module. After loading the **Section2pipeline.cp**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window (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, we recommend deleting

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it to conserve disk space.

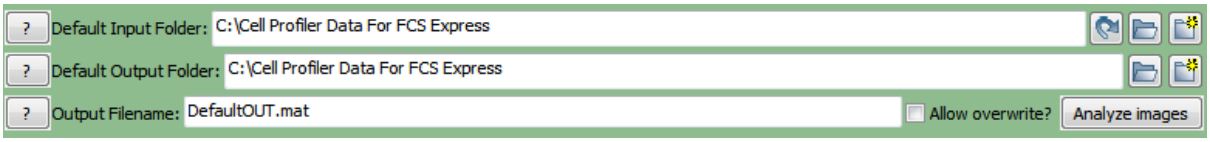

Figure T24.13 Set the Default Input and Output Folders to the Same Location

LoadImages Module Setup

In order for FCS Express to recognize your data throughout the pipeline, 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 (Figure T24.14):

1. Click on the **Channel2-** drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example we will use "Channel2-[0-9]{2}-(?P<Row>[A-H])-(?P<Column>[0-9]{2}).tif" which defines the plate location of the image by Row Letter and Column Number found in the image name.

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.

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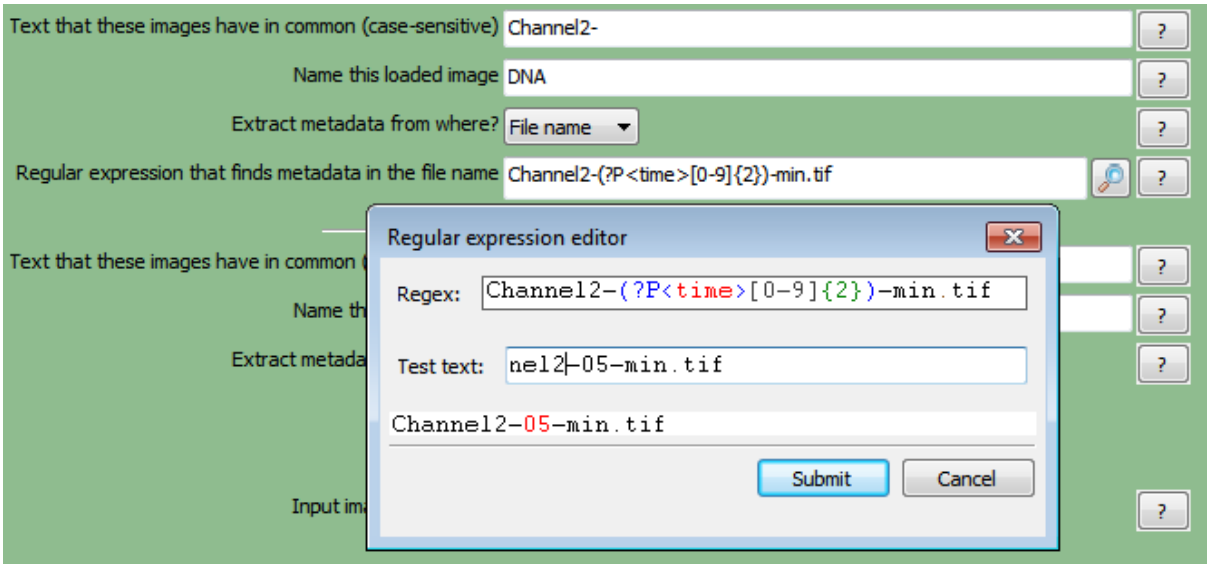

Figure T24.14 Setting Up a Regular Expression in the LoadImages Module

ConvertObjectsToImage Modules

In this module, 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.

4. Select the first **ConvertObjectsToImage** module in the pipeline.
5. Choose **Nuclei** from the **Select the input objects** drop-down list.
6. **Name the output image** with any name you prefer. For this example, use "Nuclei".
7. **Select the color type** from the drop-down list and choose **uint16** (Figure T24.15).
8. Select the second **ConvertObjectsToImage** module in the pipeline.
9. Choose **Select the input object** as **Cells** from the drop-down list.
10. **Name the output image** with any name you prefer. For this example, use "Cells".
11. **Select the color type** from the drop-down list and choose **uint16**.

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***

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output image *name must be the same as the* ***Select the input objects*** *name and is case sensitive)*

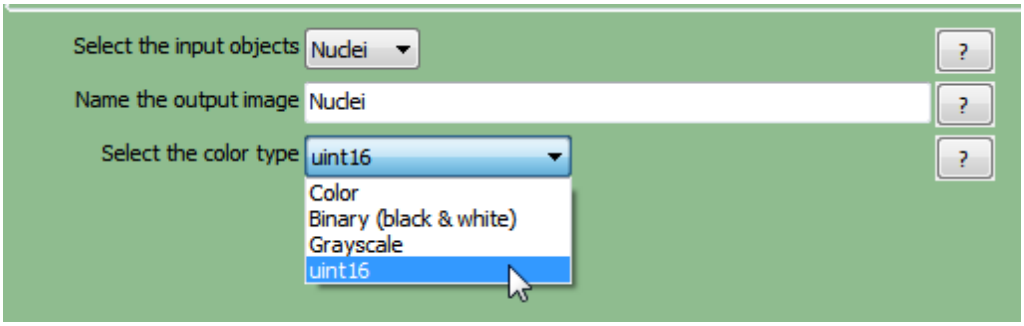

Figure T24.15 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** module must now be saved and related to the original images/data.

12. Select the first **SaveImages** module in the pipeline.
13. **Select the type of image to save** as **Image** from the drop-down list.
14. **Select the image to save** as **Cells**.

    Note: When defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.

15. **Select method for constructing file names** as **single name** from the drop-down list.
16. **Enter single file name** as any name you prefer followed by a hyphen **"-"**. For this example use **CellLabel-**.
17. Right-click after **CellLabel-**, choose **time**.
18. Select the second **SaveImages** module in the pipeline**.**
19. **Select the type of image to save** as **Image** from the drop-down list.
20. **Select the image to save** as **Nuclei**.
21. **Select method for constructing file names** as **Single name** from the drop-down list.
22. **Enter single file name** as any name you prefer followed by a hyphen

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"-". In this example we have chosen **NucleiLabel-**.

23. Right-click after **NucleiLabel-**, choose **time**.

Note: "time" value 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.

Perform steps 24-30 on both **SaveImages** modules set up for **CellLabel** and **NucleiLabel**.

24. **Select the format to use** as **tif** from the drop-down list.
25. Set **Image bit depth** at **16** from the drop-down list.
26. Choose **Default Output Folder** from **Output file location.**
27. Check the **Overwrite existing files without warning?** box.
28. Set **Select how often to save** as **Every cycle** from the drop-down list.
29. **Select colormap** as **gray** from the drop-down list.
30. Check the **Update file names within CellProfiler?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.16a.

*(Note: in CellProfiler Version 2 the **Update names within CellProfiler?** check box has been renamed **Store file and path information to the saved image?** Figure T24.4b)*

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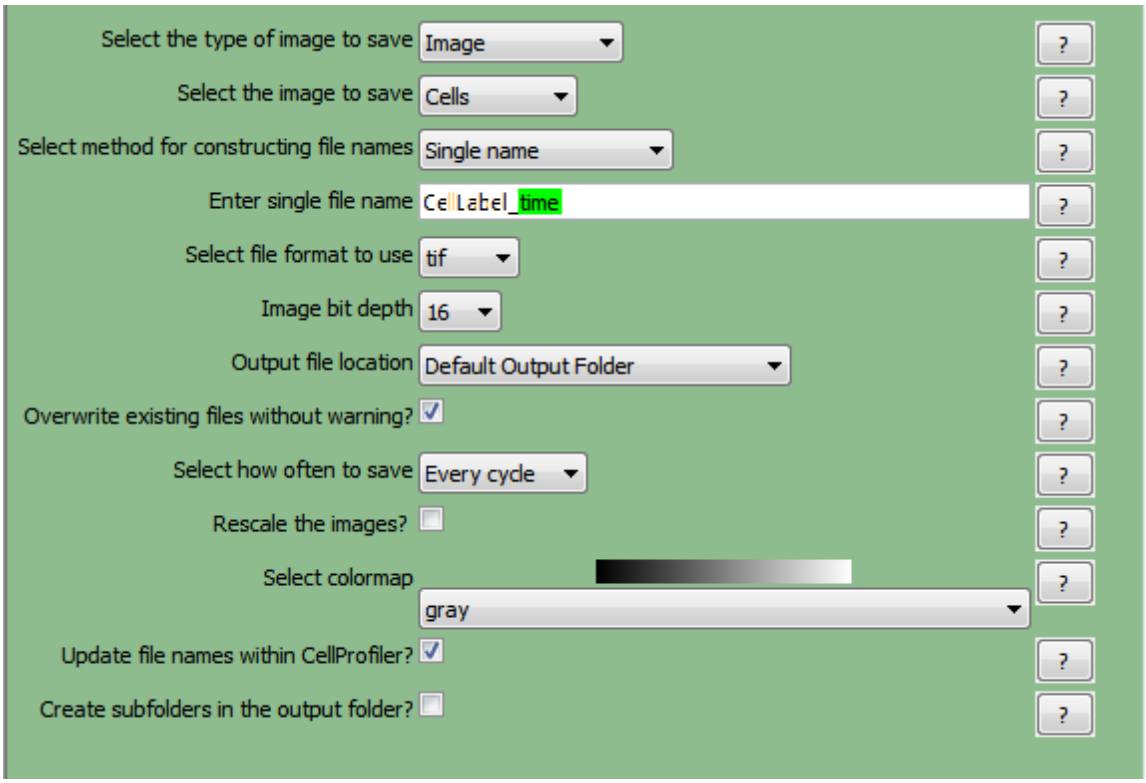

Figure T24.16a Defining the SaveImages Module

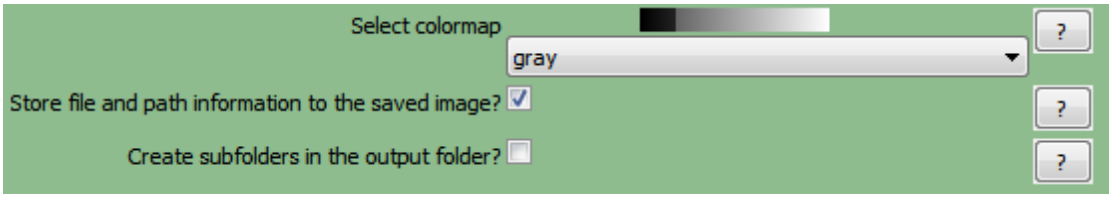

Figure T24.16b Defining the SaveImages Module in CellProfiler V2

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 1238section)

31. Select the **ExportToSpreadsheet** module.
32. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.
33. Uncheck the **Prepend the output file name to the data file names?** box.

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34. Choose **Output file location** as **Default Output Folder** from the drop-down list.
35. Uncheck **Export all measurements?**.
36. Choose **Image** from the first **Data to export** drop-down list.
37. Uncheck **Use the object name for the file name?**.
38. Set the file name as **Image.cptoc.**
39. Choose **Add another data set**.
40. Choose **Nuclei** from the second **Data to export** drop-down list.
41. Uncheck **Use the object name for the file name**.
42. Set the file name as **nuclei.cpout**.
43. Repeat steps 39-42 for **"Cells"** and set the file name as **cells.cpout**.

The **ExportToSpreadsheet** module options should look like Figure T24.17 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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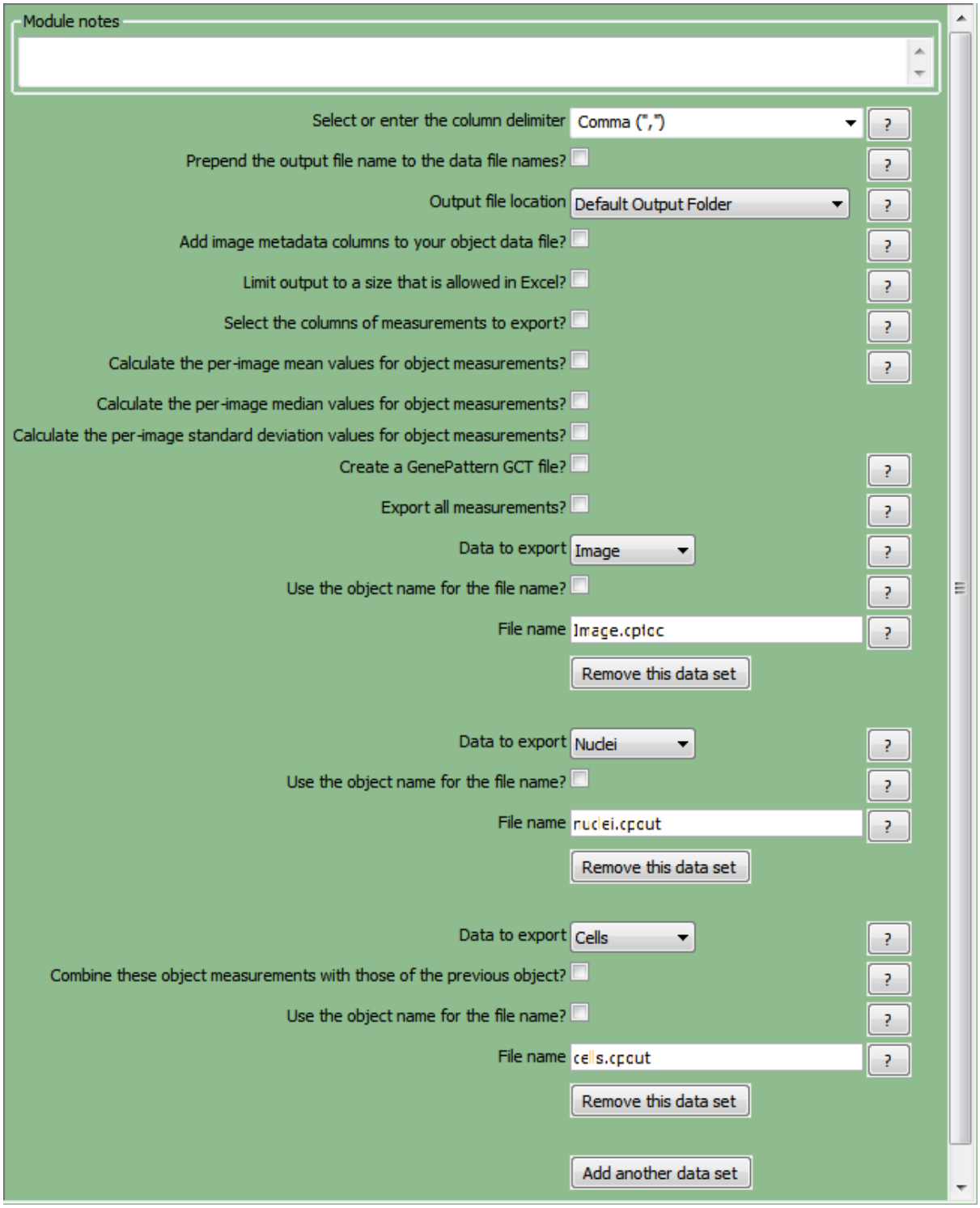

Figure T24.17 ExportToSpreadsheet Module

44. **Select Analyze images** to run the pipeline. The folder where your images are stored will now contain the cells.cpout, nuclei.cpout and

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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 T24.18).

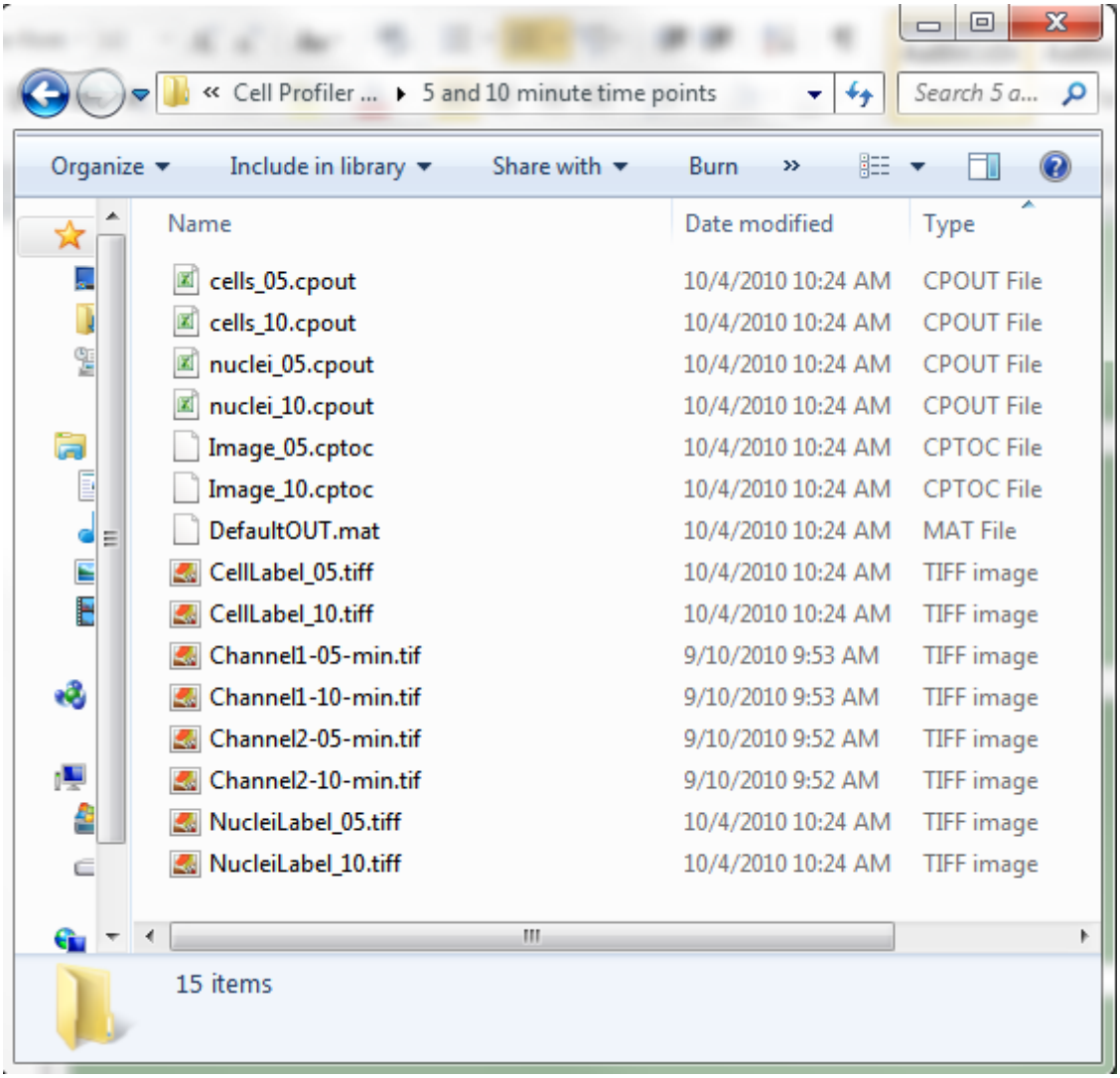

Figure T24.18 Example of Output Folder once CellProfiler Analysis is Complete

In the next section, we will import the single image experiment into FCS Express.

34.24.5 Importing Single Image Data

This section pertains to data from a set of single images that were not obtained

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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 V4.

1. Load the Section2pipelineCOMPLETED.cp pipeline in CellProfiler.
2. Select the default input and output folders in CellProfiler to load and store the data as in Figure T24.13 1204.
3. Analyze the images in CellProfiler.

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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

4. Open FCS Express V4.
5. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.19).
6. Select **csv** from the **delimiter** drop-down list.
7. Check the **Load Images** check box.
8. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
9. Click **OK** to close the **Edit Preferences** dialog.

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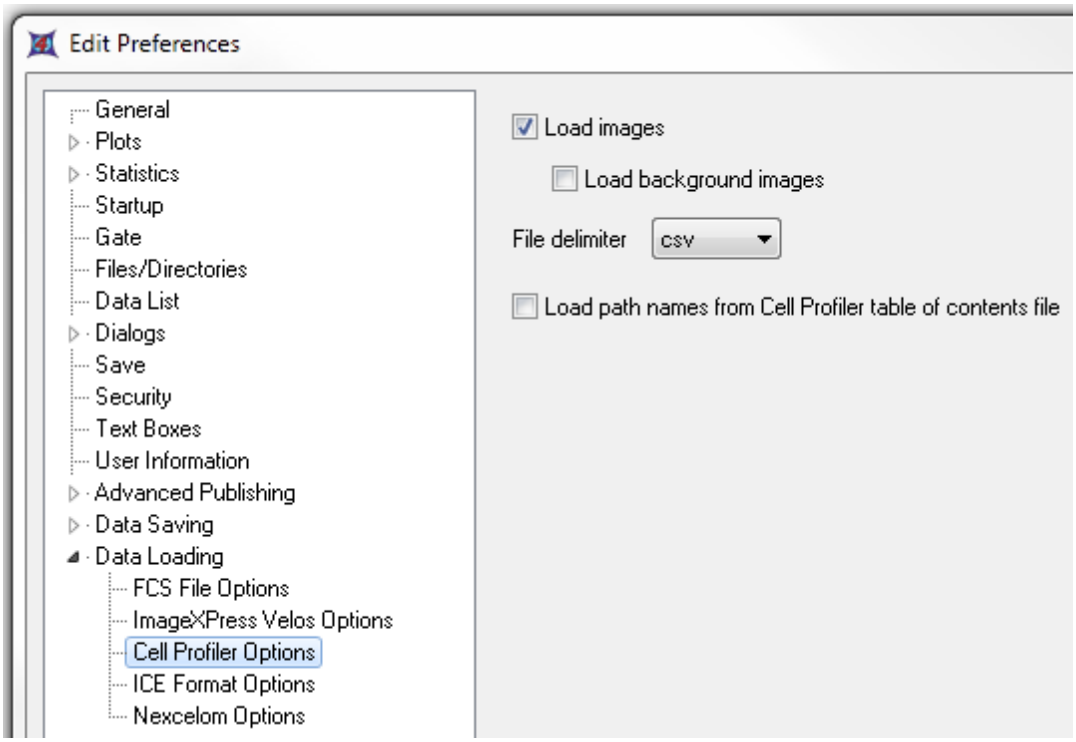

Figure T24.19 CellProfiler Data Loading Options

10. Select the **Insert→2D Plots →Picture Plots** command (Figure T24.20).

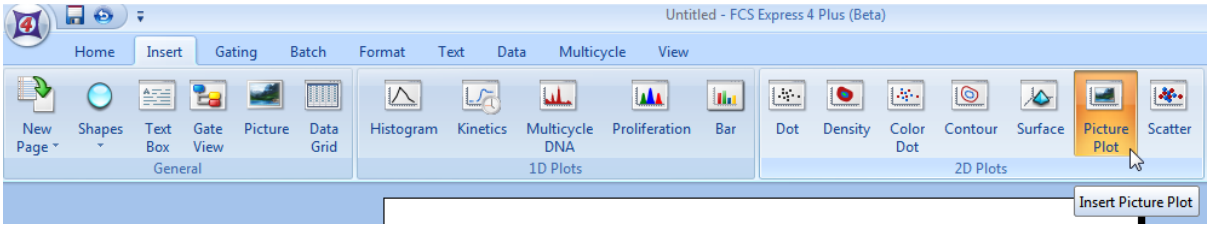

Figure T24.20 Choosing a Picture Plot to Insert

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)** (Figure T24.21)**.** Icons for the documents outputted by CellProfiler will

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appear. For this example, they will be labeled cells_05.cpout and nuclei_05.cpout.

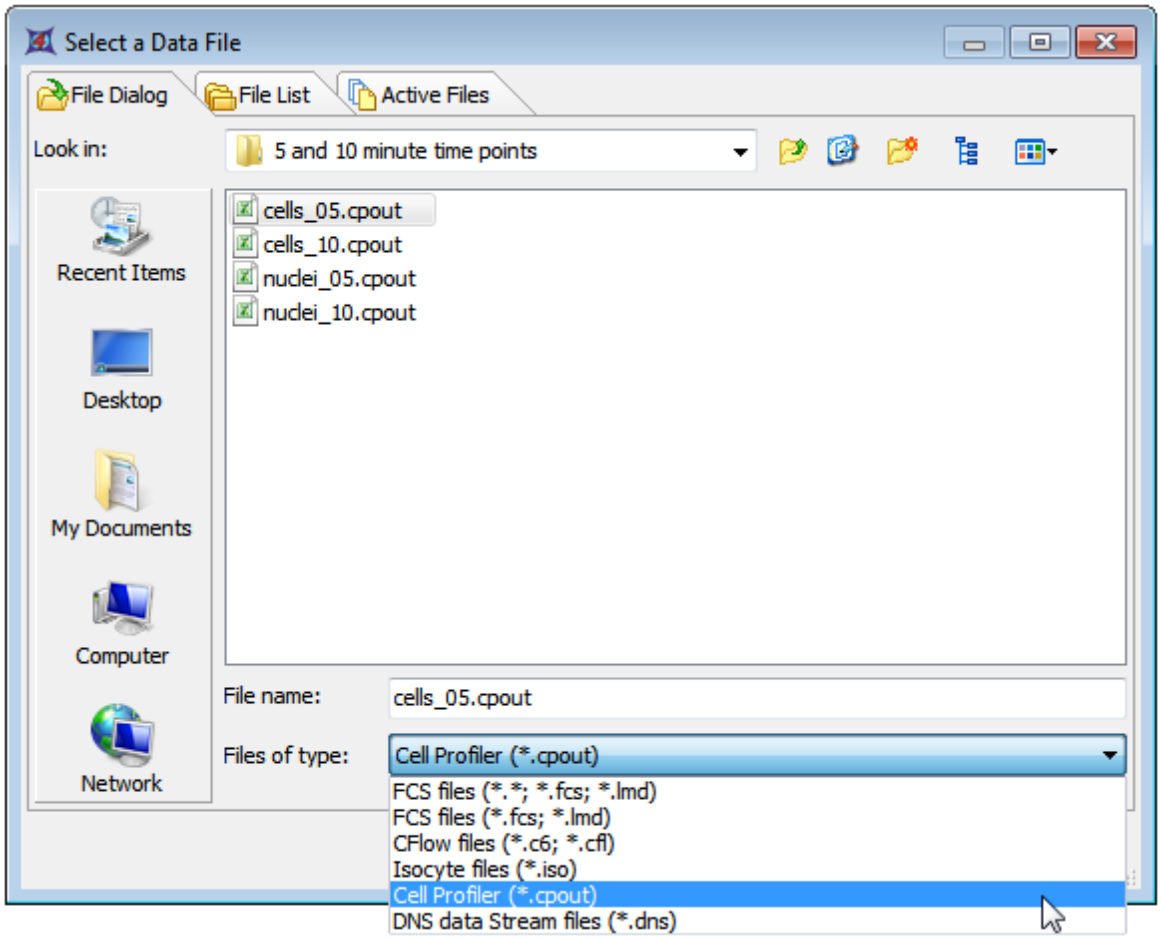

Figure T24.21 Select a Data File Dialog

14. Highlight **cells_05.cpout** and 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. **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.

15. Highlight **Cytoplasm** from the **Image Parameter** list box of the **Open 2D Plots** dialog (Figure T24.22) to view the cytoplasmic image.

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16. Click **OK**.

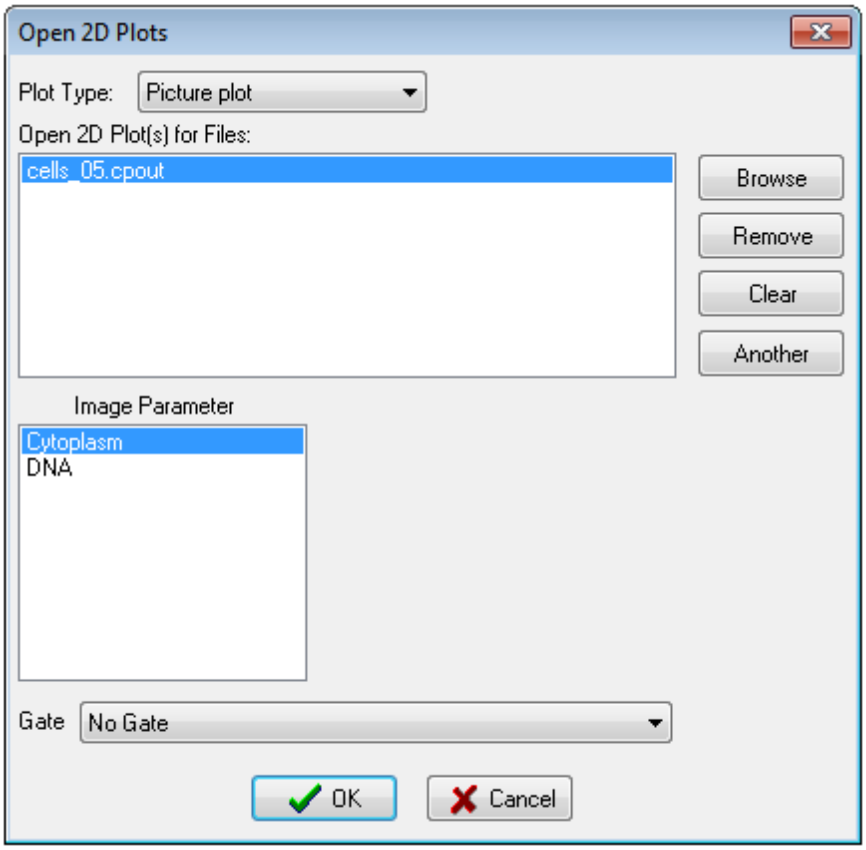

Figure T24.22 Choosing Image Parameters from the Open 2D Plots Dialog

A **Picture Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.23).

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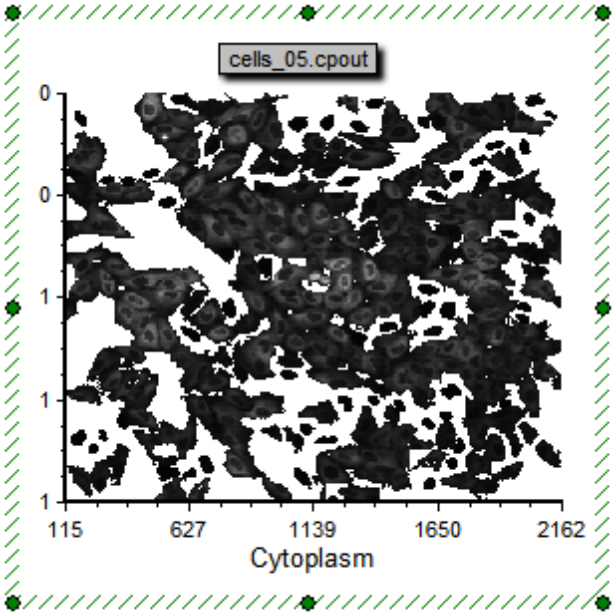

Figure T24.23 Example of the Cells (5 Minute Time Point) Picture Plot

Any 2D gate can now be created on the **Picture Plots**. 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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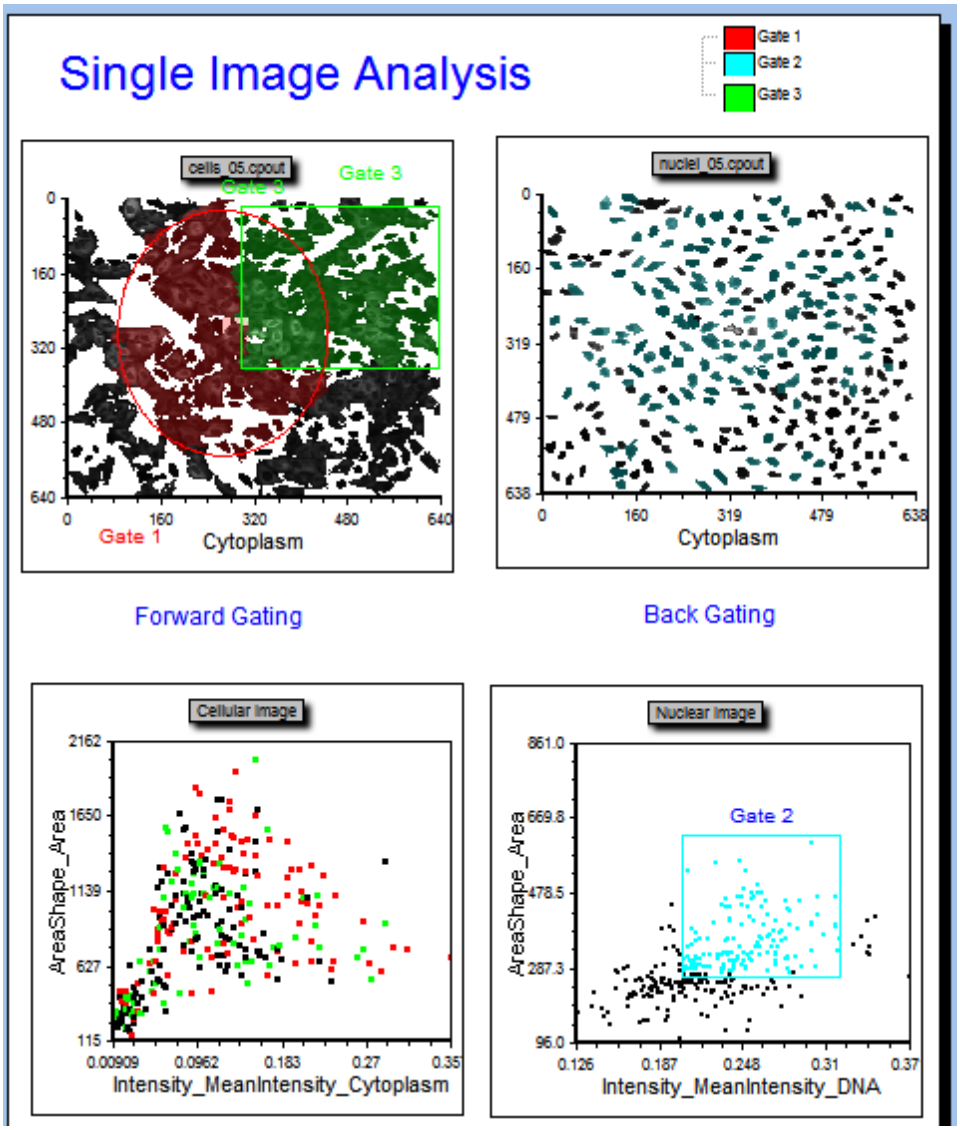

Figure T24.24 Example of Gating on a Picture Plot and Backgating from a Dot Plot to a Picture Plot

In the next exercise, we will export numeric data only from a 96 well plate.

34.24.6 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.

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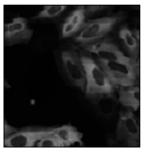
The CellProfiler modules that are needed or must be amended for export to FCS Express in this section are LoadImages and ExportToSpreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive 590 and is named **Section3pipelineCOMPLETED.cp**. The completed pipeline 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 (SBS Bioimage)"**.

- **Human cytoplasm-nucleus translocation assay (SBS Bioimage):** This example includes an advanced example of illumination correction - creating an illumination correction function from all images in a 96-well plate. This pipeline also demonstrates how to load dosage information via the LoadData module, how to use advanced methods for primary and seecondary object identifcation, and how to calculate the Z' factor, a measure of assay quality. More about these images can be found at the BBBC.
  [Download] (40 MB)
  [Tutorial]



CellProfiler Example Link

The steps for exporting data from CellProfiler have been broken down by module according to the Section3pipeline.cp. After loading the Section3pipeline.cp, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window.

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.

LoadImages Module Setup

In order for FCS Express to recognize your data throughout the pipeline, 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:

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1. Click on the drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example we will use "Channel2-[0-9]{2}-(?P<Row>[A-H])-(?P<Column>[0-9]{2}).tif" which defines the plate location of the image by Row Letter and Column Number found in the image name.

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

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 section)

4. Select the **ExportToSpreadsheet** module.
5. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.
6. Uncheck the **Prepend the output file name to the data file names?** box.
7. Choose **Output file location** as **Default Output Folder** from the drop-down list.
8. Uncheck **Export all measurements?**.

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9. Choose **Image** from the first **Data to export** drop-down list.
10. Uncheck **Use the object name for the file name?**.
11. Set the file name as **Image.cptoc.**
12. Choose **Add another data set**.
13. Choose **Nuclei** from the second **Data to export** drop-down list.
14. Uncheck **Use the object name for the file name**.
15. Set the file name as **nuclei.cpout**.
16. Repeat steps 12-15 for **"Cells"** and set the file name as **cells.cpout**.

The **ExportToSpreadsheet** module options should look like Figure T24.25 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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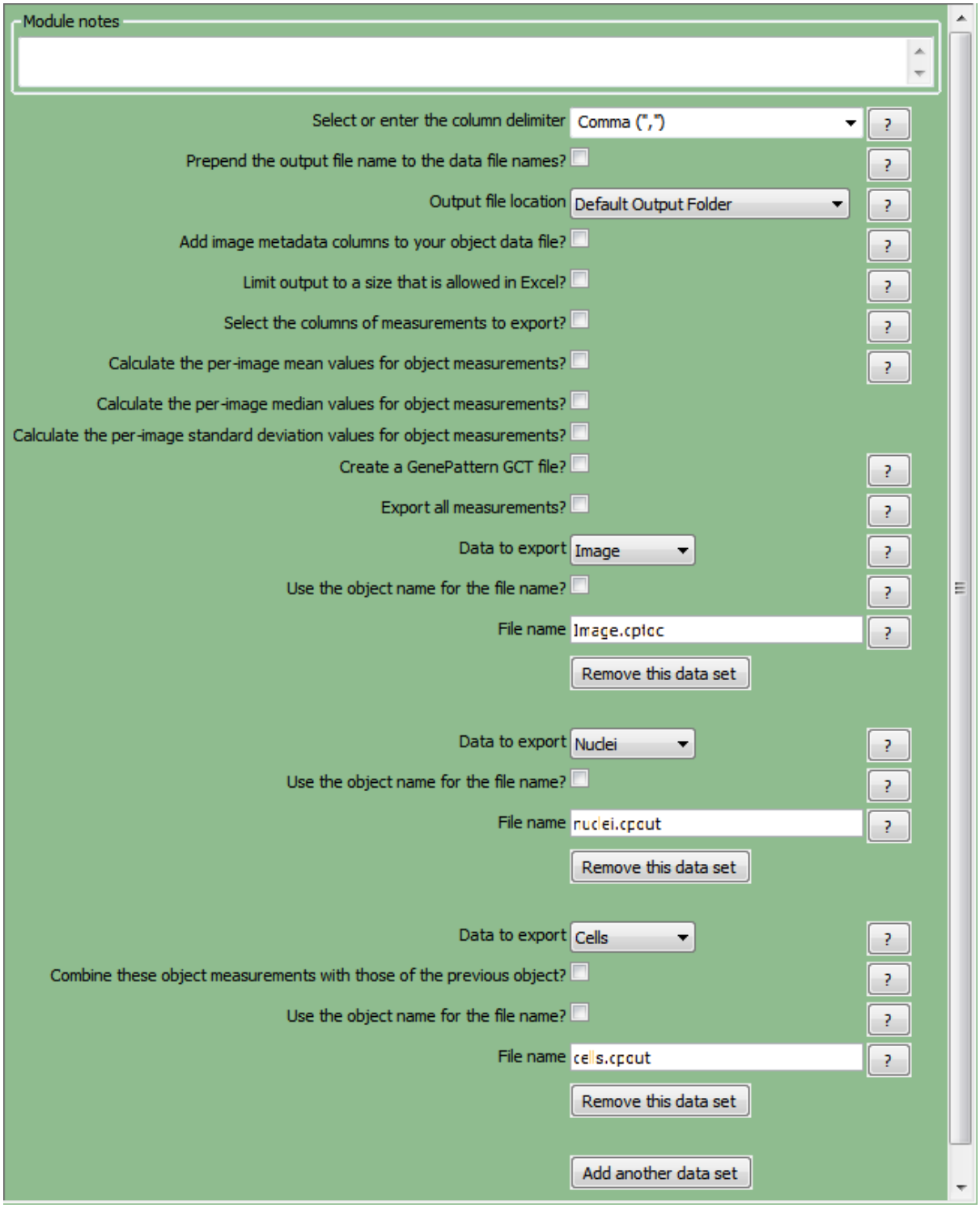

Figure T24.25 ExportToSpreadsheet Module

17. 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

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files (Figure T24.26).

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.

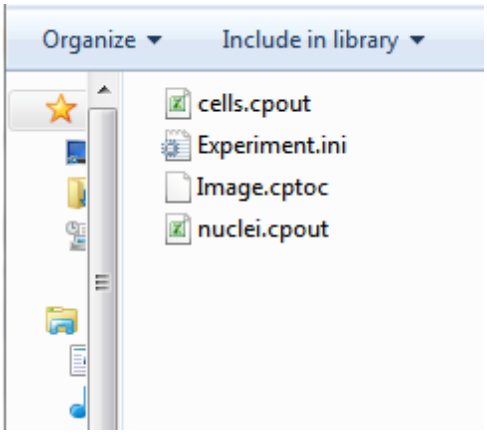

Figure T24.26 Data Only Output Files

Next, we will import and analyze the numeric data 1223.

34.24.7 Importing Numeric Data (96 Well)

In this section you will learn:

- How to import the data into FCS Express V4 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 V4. 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 590 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 found in theprevious section 1218.
2. Load the **Section3pipelineCOMPLETED.cp** pipeline in CellProfiler.

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3. Select the default input and output folders in CellProfiler to load and store the data.
4. Analyze the images in CellProfiler.

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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

5. Open FCS Express V4.
6. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.27).
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 **Edit Preferences** 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.

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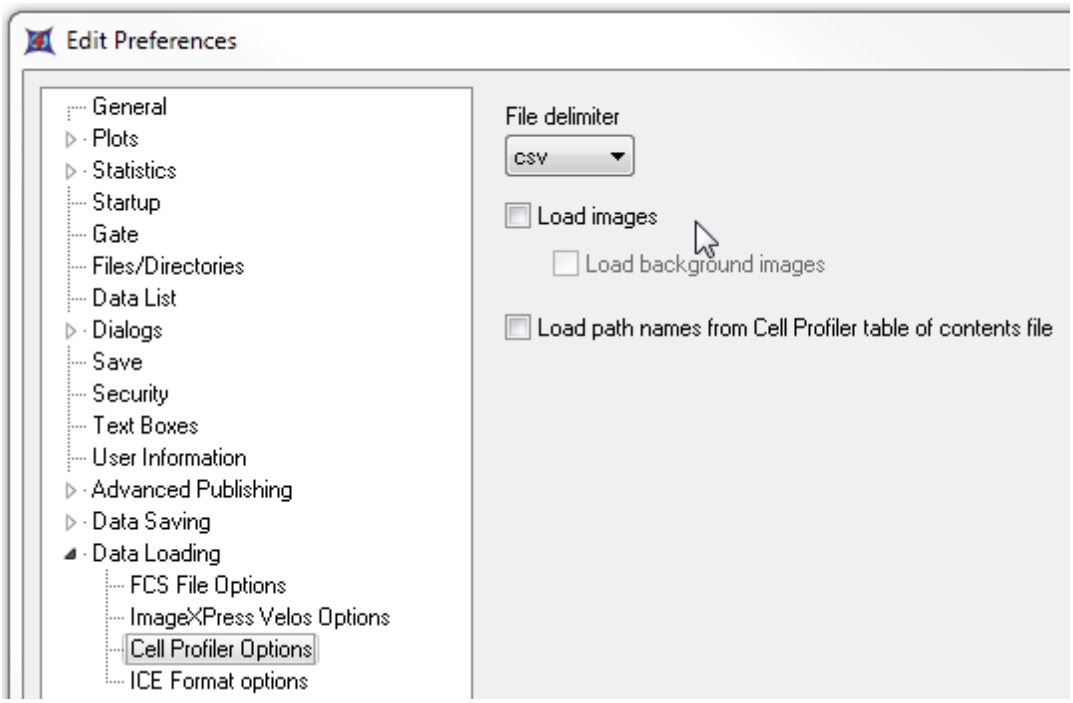

Figure T24.27 CellProfiler Data Loading Options for Data Only

10. Select the **Insert→Other Plots→Heat Map** command (Figure T24.28).

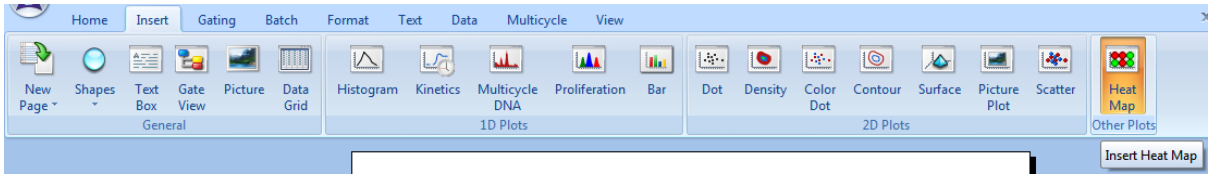

Figure T24.28 Choosing to Insert a Heat Map

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 **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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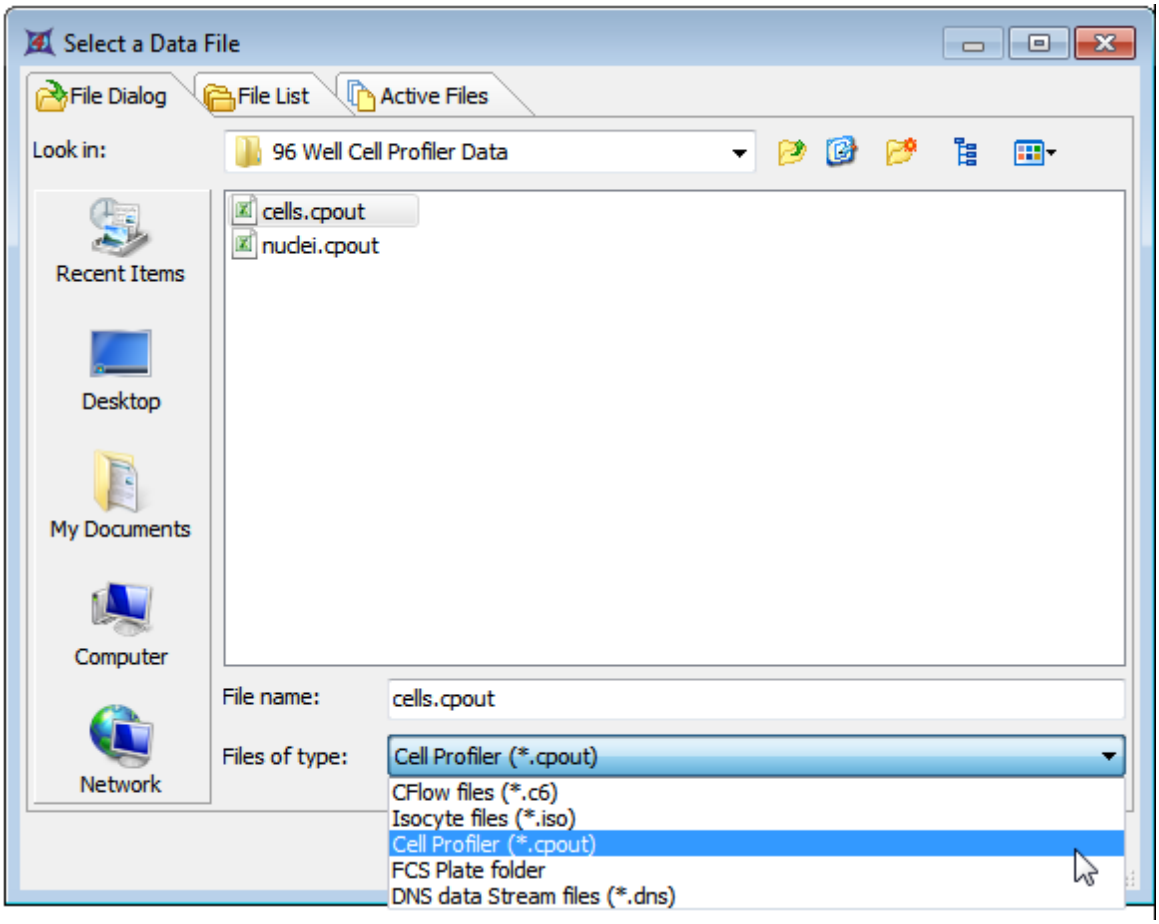

Figure T24.29 Choosing CellProfiler Files

14. 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.

15. 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).

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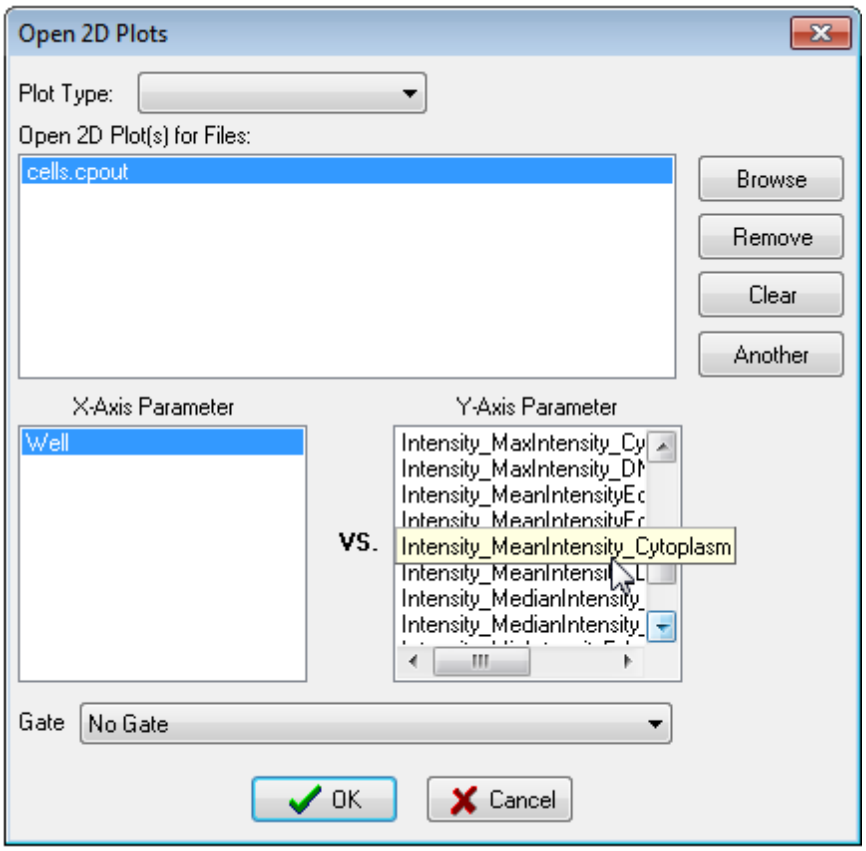

Figure T24.30 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

16. 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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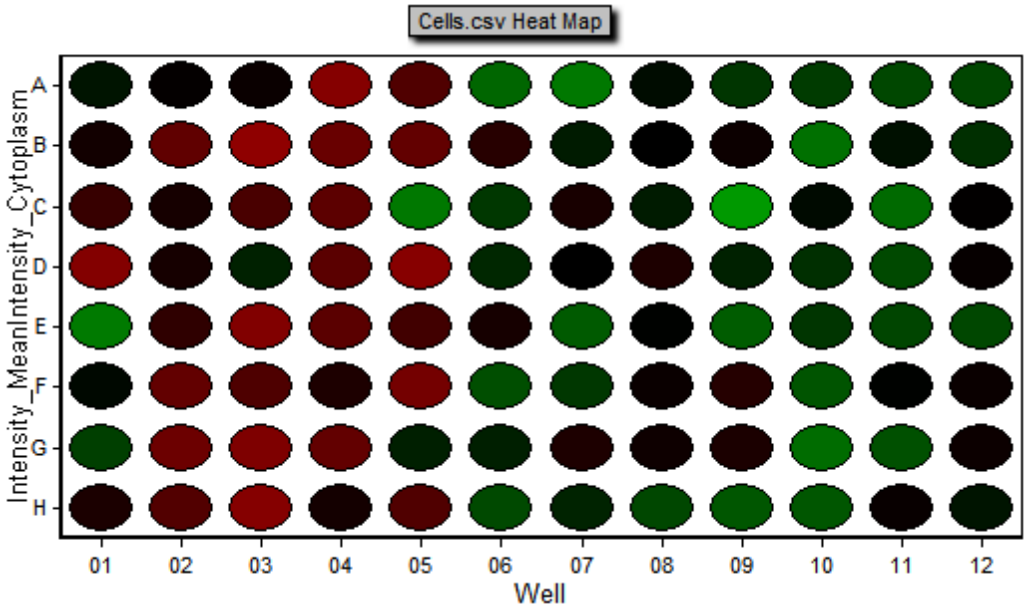

Figure T24.31 Example of a Heat Map Generated from CellProfiler Data

In the next exercise, we will export single image numeric data only[1228].

34.24.8 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.

The CellProfiler modules that are needed or must be amended for export to FCS Express in this section are LoadImages and ExportToSpreadsheet. The completed pipeline for this example can be found in the Tutorial Sample Data archive[590] and is named **Section4pipelineCOMPLETED.cp**. 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[590] and are labeled "**5 and 10 minute time point images**". These images are based on the "**Human cytoplasm-nucleus translocation assay (SBS Bioimage)**" sample experiment found at CellProfiler Examples.

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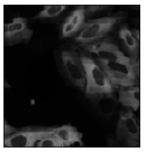
- **Human cytoplasm-nucleus translocation assay (SBS Bioimage):** This example includes an advanced example of illumination correction - creating an illumination correction function from all images in a 96-well plate. This pipeline also demonstrates how to load dosage information via the LoadData module, how to use advanced methods for primary and seecondary object identifcation, and how to calculate the Z' factor, a measure of assay quality. More about these images can be found at the BBBC.
  [Download] (40 MB)
  [Tutorial]



The steps for exporting data from CellProfiler have been broken down by module. After loading the **Section4pipeline.cp**, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window.

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.

LoadImages Module Setup

In order for FCS Express to recognize your data throughout the pipeline, 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:

1. Click on the **Channel2-** drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example, use "Channel2-(?P<time>[0-9]{2})-min.tif" which assigns a "time" label to the images in the data set based on the file name.

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

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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

Now that all of the images have been loaded and assigned to metadata, 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 section)

4. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.
5. Uncheck **Prepend the output file name to the data file names?**.
6. Choose **Output file location** as **Default Output Folder** from the drop-down list.
7. Uncheck **Export all measurements**.
8. Choose **Image** from the **Data to Export** drop-down list.
9. Uncheck **Use the object name for the file name**.
10. Type Image_ .
11. Right-click after "Image_" .
12. Select **time** from the regular expression pop-up menu.
13. Type .cptoc after "time".

The file name should now look like Figure T24.32.

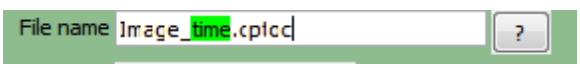

Figure T24.32 Inserting the "time" Variable into the File Name

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Note: **"time"** is a value you defined from a regular expression in the LoadImages 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 file.

14. Choose **Add another data set**.
15. Choose **Nuclei** from the **Data to Export** drop-down list.
16. Uncheck **Use the object name for the file name**.
17. Type nuclei_ .
18. Right-click after "nuclei_"
19. Select **time** from the regular expression pop-up menu.
20. Type .cpout after "time".
21. Choose **Add another data set**.
22. Choose **Cells** from the **Data to Export** drop-down list.
23. Uncheck **Use the object name for the file name**.
24. Type cells**_ .**
25. Right-click after "cells_" .
26. Select **time** from the regular expression pop-up menu.
27. Type .cpout after time.

The **Export to Spreadsheet** module options should look like Figure T24.33 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***

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express>.cpout *will result in a proper export while* ***dataset1nuclei.cpout*** *or* ***<regularexpression>_nuclei.cpout*** *will resulting in a non-working export.*

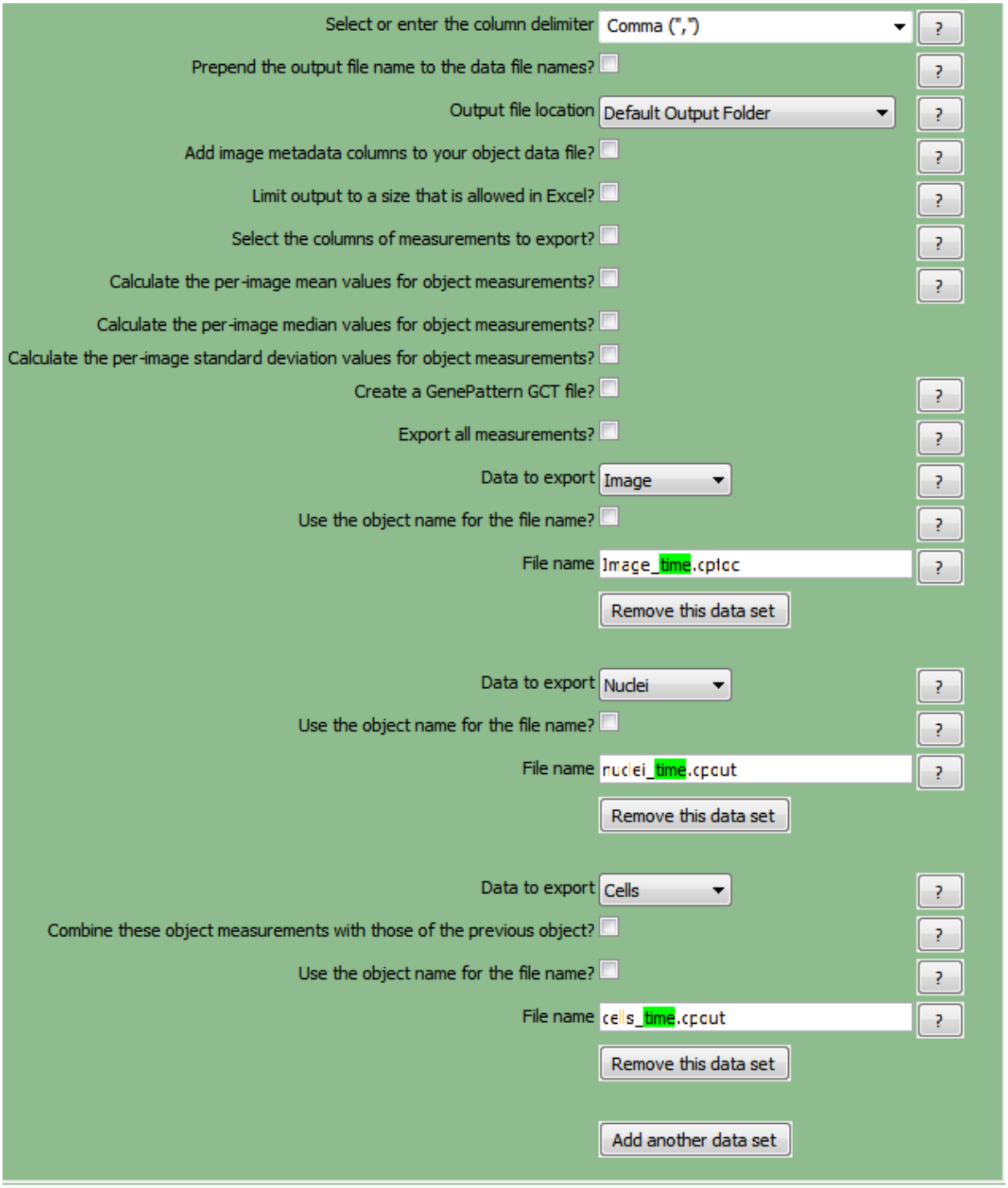

Figure T24.33 ExportToSpreadsheet Module

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28. 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 for each time point (Figure T24.34).

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.

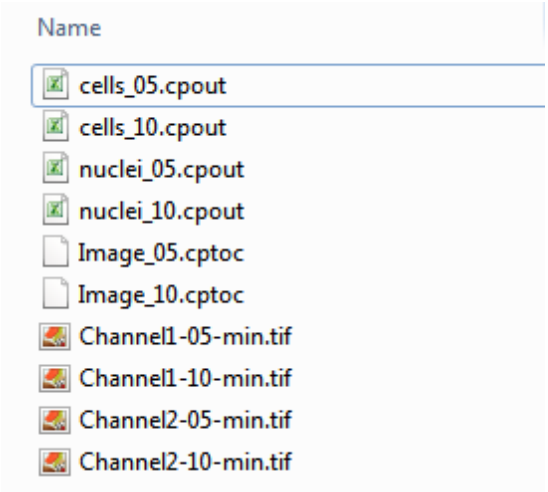

Figure T24.34 Example of CellProfiler Output Folder When Analysis is Complete

In the next exercise, we will import and analyze single image numeric data.

34.24.9 Import Single Image Numeric Data

In this section, you will learn:

- How to import the data into FCS Express V4 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 V4.

1. Load the **Section4pipelineCOMPLETED.cp** pipeline in CellProfiler.

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2. Select the default input and output folders in CellProfiler to load and store the data.

3. Analyze the images in CellProfiler.

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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

4. Open FCS Express V4.

5. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.27 1223).

6. Select **csv** from the **delimiter** drop-down list.

7. Uncheck the **Load path names from Cell Profiler table of contents file** check box.

Note: In this example there are no images associated with the data so the **Load Images** check box must remain unchecked.

8. Click **OK** to close the **Edit Preferences** dialog.

The dialog should look like Figure T24.35 when you are done.

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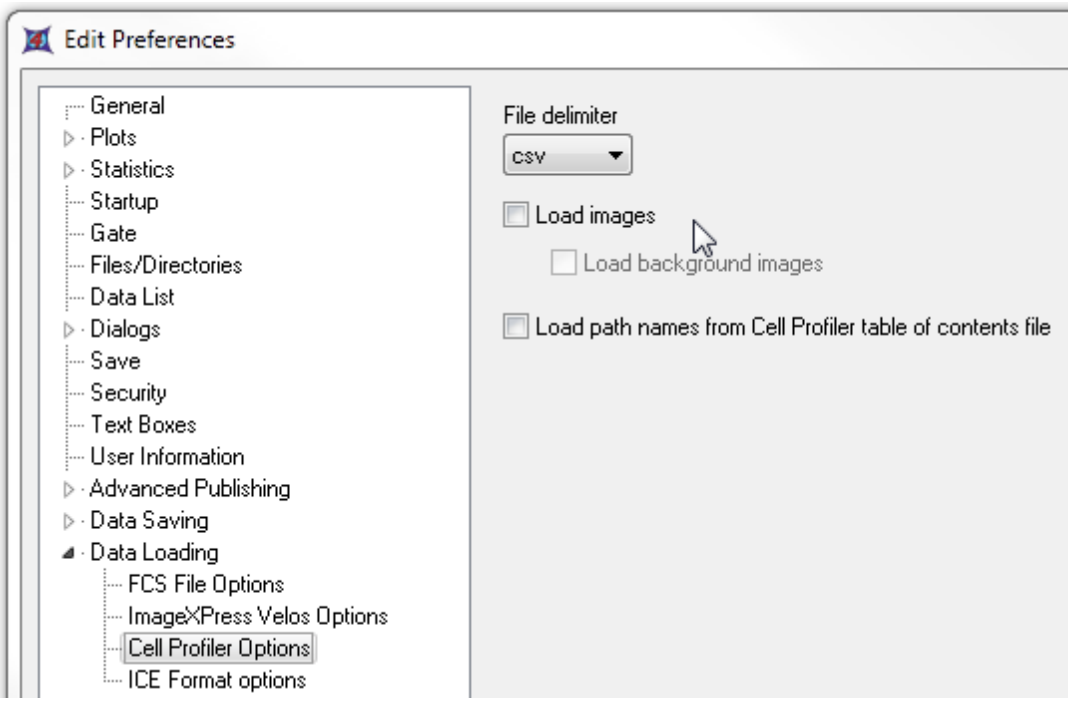

Figure T24.35 Data Loading Options for Data Only Importing

9. Select the **Insert→2D Plots→Dot** (Figure T24.36) command.

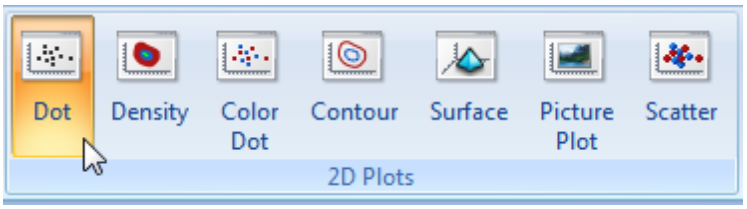

Figure T24.36 Inserting a Dot Plot

10. Click anywhere on the layout and the **Select a Data File** dialog will appear.

11. Browse to the *Default Output Folder* where you stored your output data from CellProfiler.

12. 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 T24.37).

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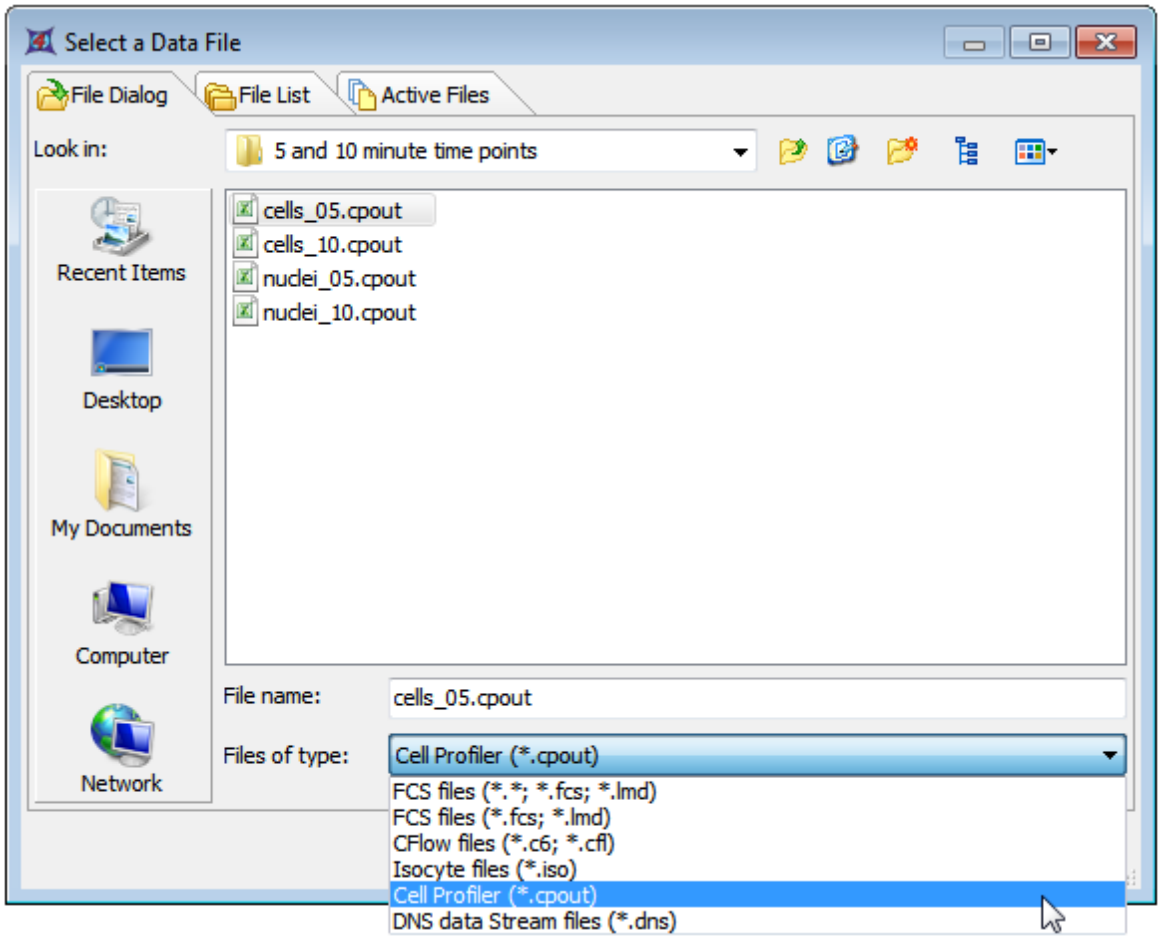

Figure T24.37 Select a Data File Dialog

13. Highlight **cells_05.cpout**.
14. 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 in the IdentifyPrimaryObjects module of CellProfiler which corresponds only to the nuclear area of the cells.

15. Select **AreaShape_Area** for the **X-Axis parameter** (Figure T24.38).
16. Select **Intensity_MeanIntensity_Cytoplasm** for the **Y-Axis parameter** (Figure T24.38).
17. Click **OK**.

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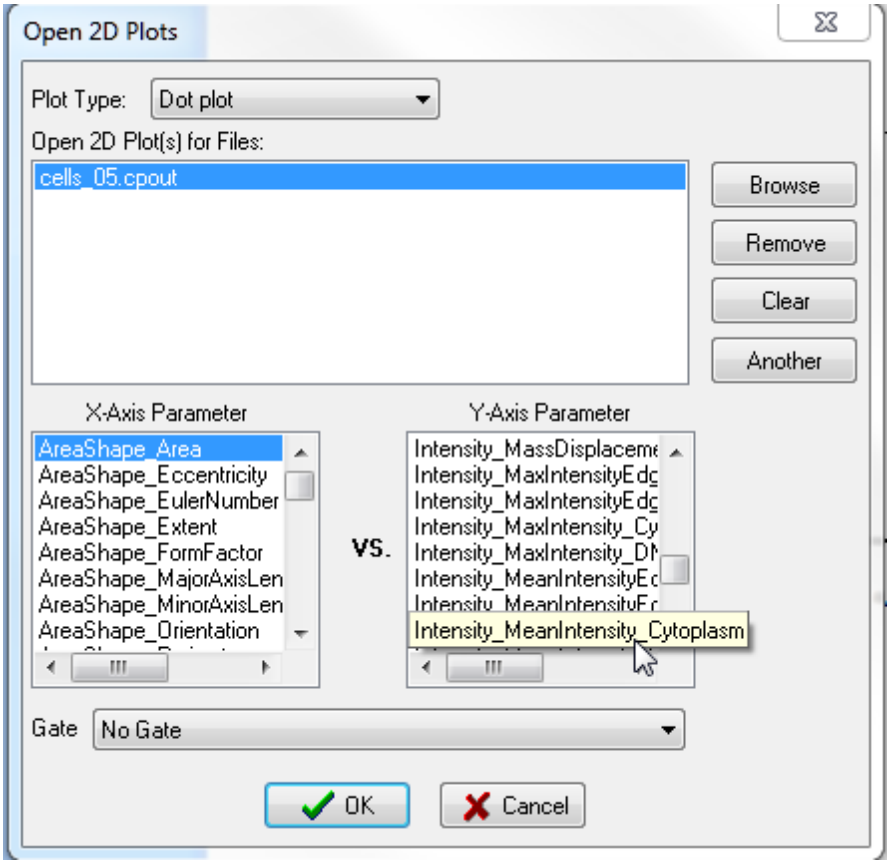

Figure T24.38 Open 2D Plots Dialog

A **Dot Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.39).

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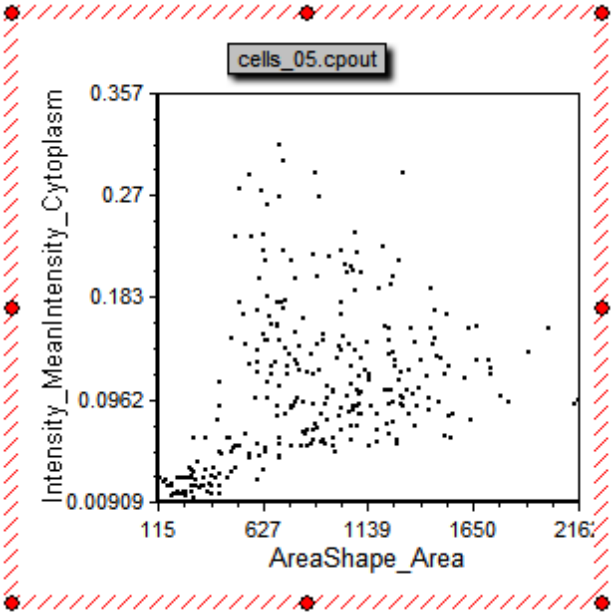

Figure T24.39 Dot Plot Created for cells_05.cpout

In the next exercise, we will Select Individual Parameters for CellProlifer Export 1238 .

34.24.1 Selecting Individual Parameters for CellProfiler Export 0

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 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 **Section2pipelineCOMPLETED.cp** pipeline located in the Tutorial Sample Data archive 590. The pipeline as been designed for use with the Exporting Single Images 1204 tutorial which should be completed before beginning this tutorial.

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1. Load the Section2pipelineComplete.cp pipeline in CellProfiler from the FCS Express 4 Tutorial Sample Data folder.
2. Right click on **ExportToSpreadsheet** module.
3. Choose **Duplicate** from the dialog.
4. Click on the first **ExportToSpreadsheet** module to select it.
5. Click on the **Remove this data set** button in the **Data to export Nuclei** (nuclei_time.cpout) data set.
6. 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 T24.40)

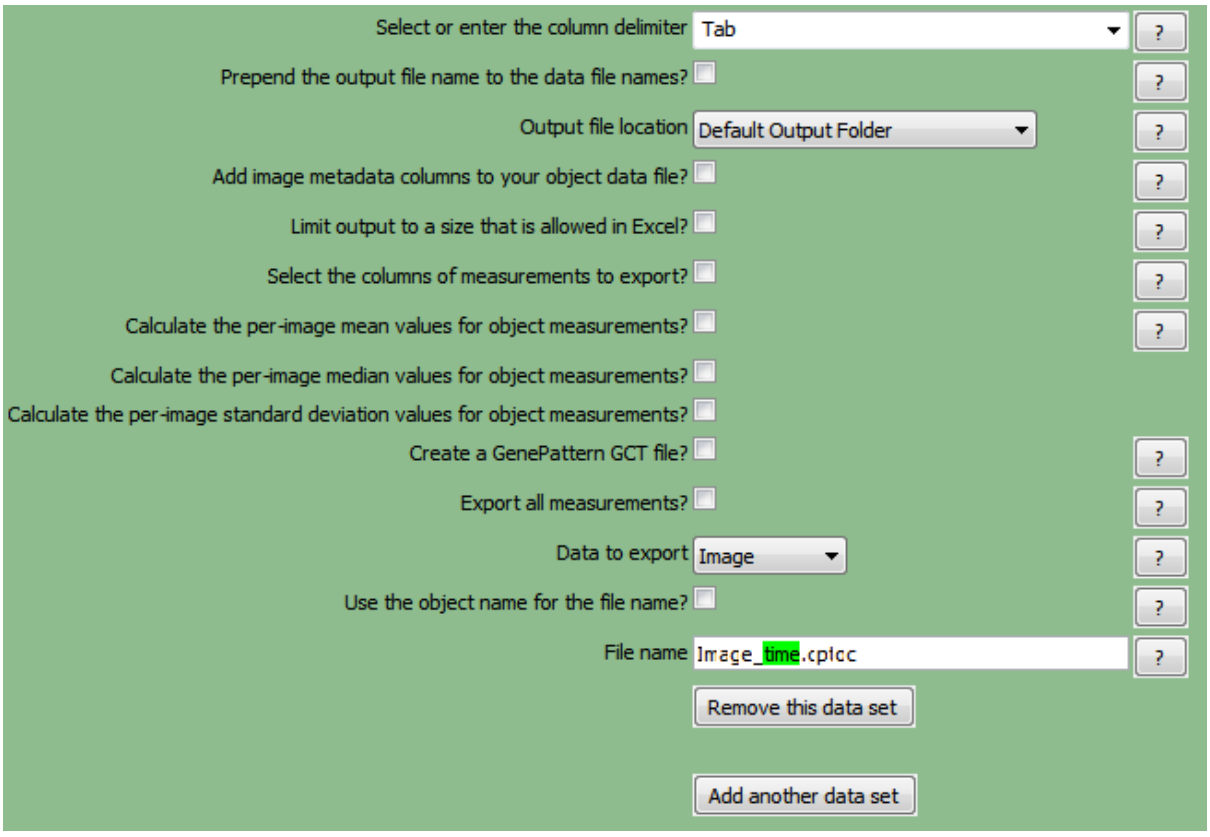

Figure T24.40 The first ExportToSpreadsheet module after the Data to export Nuceli and Cells has been removed.

7. Click on the second **ExportToSpreadsheet** module to select it.
8. Click on the **Remove this data set** button in the **Data to export Image** (Image_time.cpout) data set.

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(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 T24.41)

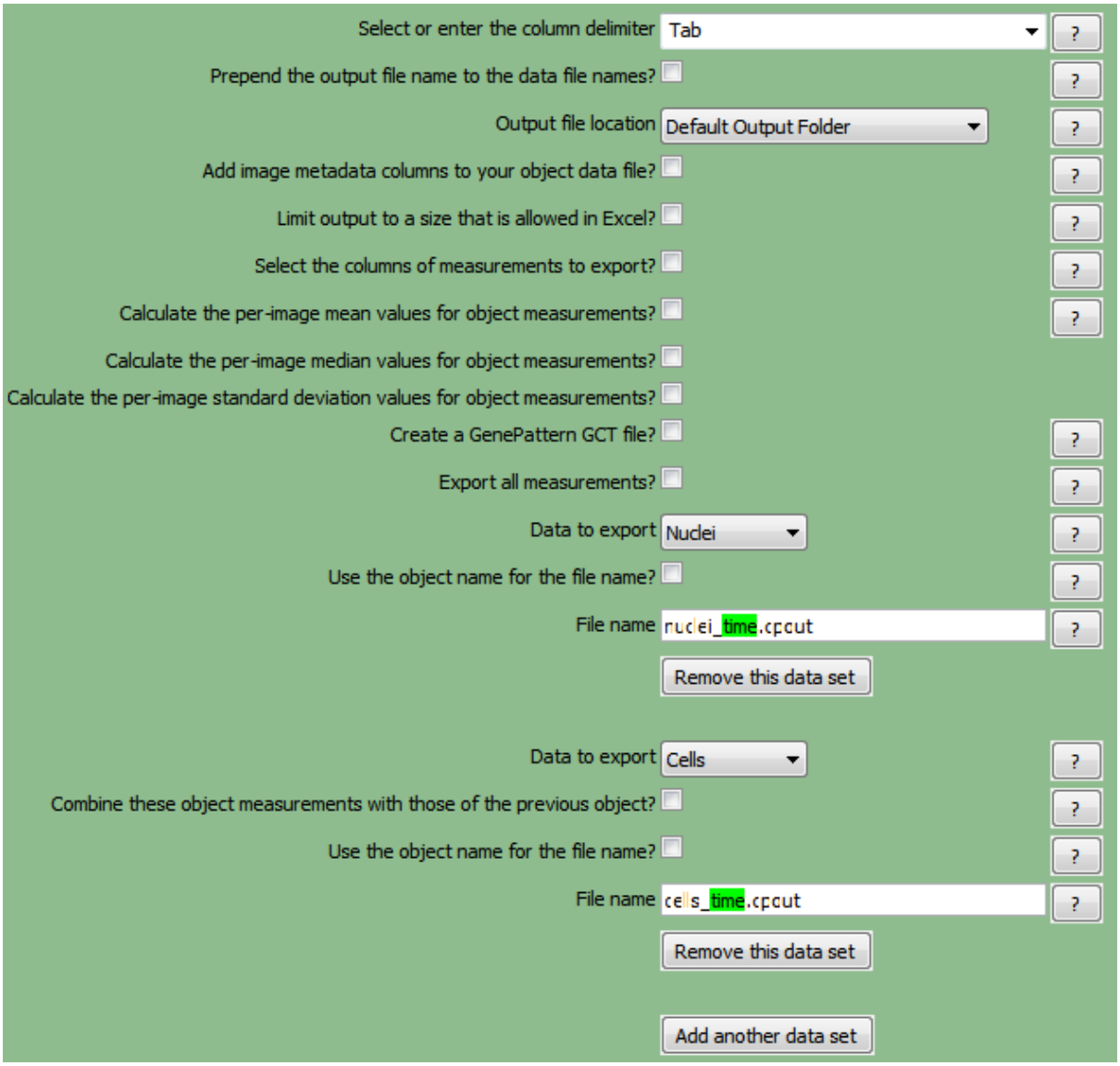

Figure T24.41 The second ExportToSpreadsheet module after the Data to export Image has been removed.

9. Check the box for **Select the columns of measurements to export** (Figure T24.42).
10. Click the **Press to select measurements** button (Figure T24.42).
11. Click on the **All** box to uncheck all items.
12. Expand the **Cells→AreaShape** categories (Figure T24.42).

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13. Check the box for **Area** (Figure T24.42).
14. Expand the **Cells→Intensity→MeanIntensity** category (Figure T24.42).
15. Check the box for **Cytoplasm** (Figure T24.42).
16. Check the box for **DNA** (Figure T24.42).

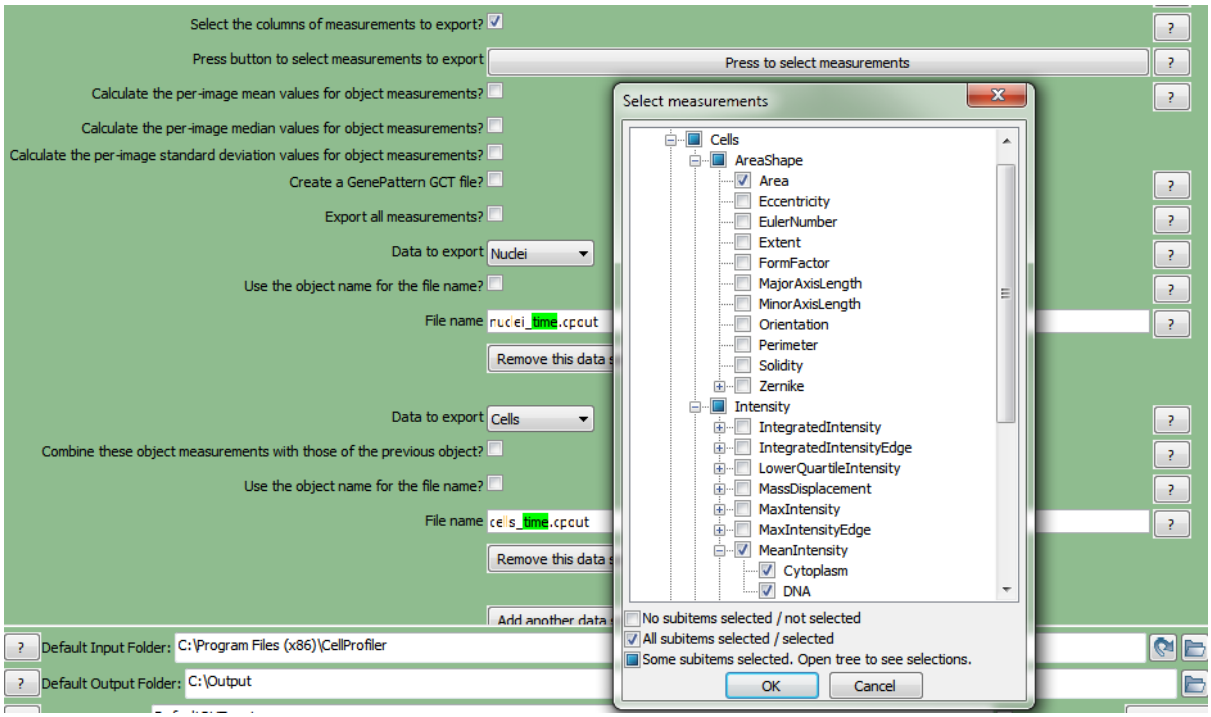

Figure T24.42 Choosing the Select the columns of measurements to export check box, clicking on the Press to select measurements button, and selecting the parameters to export for the Cells objects.

17. Expand the **Nuclei→AreaShape** categories
18. Check the box for **Area**.
19. Expand the **Nuclei→Intensity→MeanIntensity** category.
20. Check the box for **Cytoplasm**.
21. Check the box for **DNA.**
22. Click **OK**.
23. 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

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Express 4 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 cells.cpout and nuclei.cpout files in FCS Express 4 to view the new data set as you did in the Importing Single Image Data section 1212.

34.24.1 Exporting Tiled Images From CellProfiler 1

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

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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 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 590 within the **Exporting Tiled Images in CP** folder is named **CellProfilerTiledExportCOMPLETED.cp** 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 590 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

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according to the CellProfilerTiledExport.cp pipeline. After loading the CellProfilerTiledExport.cp pipeline in CellProfiler, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window (Figure T24.43).

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.

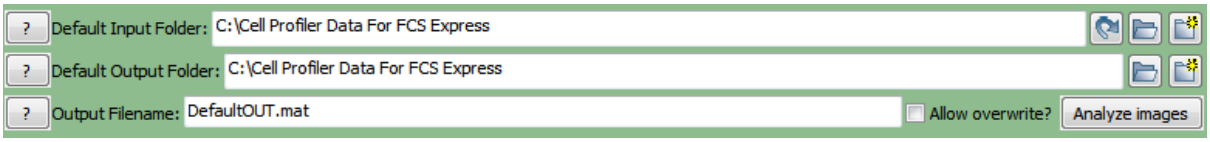

Figure T24.43 Set the Default Input and Output Folders to the Same Location

LoadImages Module Setup

In order for FCS Express to recognize your image data is in a row and column format for a tiled image, 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 (Figure T24.44):

1. Click on the **DAPI-** drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example we will use "**_0000_350_DAPI_row(?P<rowtile>[0-9]{1,3})_col(?P<coltile>[0-9]{1,3}).tiff**" which defines the row and column information of each tile as found in the image name.

Notes:

- Follow these links for a tutorial on "Regular Expressions" and how to translate regular expressions.

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- 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.

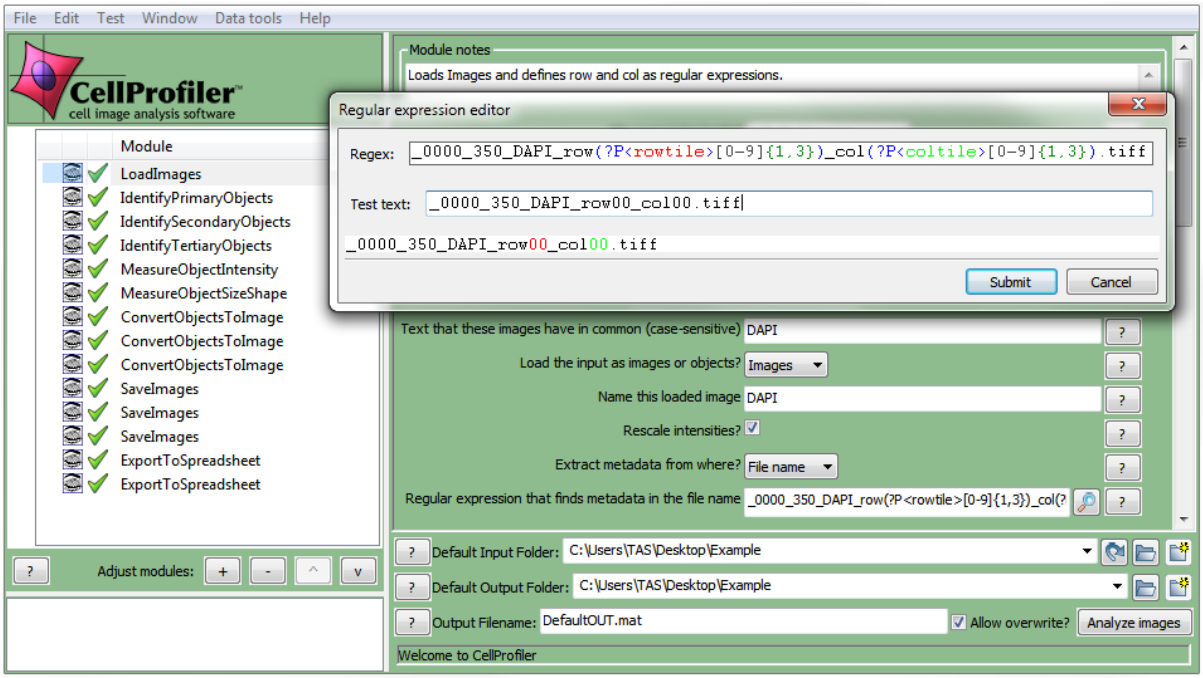

Figure T24.44 Setting up a Regular Expression in the LoadImages Module for a tiled image experiment.

ConvertObjectsToImage Modules

In this module, 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 T24.45 for an example of the module window.

4. Select the first **ConvertObjectsToImage** module in the pipeline.

5. Choose **Select the input objects** as **Nuclei** from the drop-down list.

6. **Name the output image** with any name you prefer. For this example, use "Nuclei" (case sensitive).

7. **Select the color type** from the drop-down list and choose **uint16** (Figure T24.3).

8. Select the second **ConvertObjectsToImage** module in the pipeline.

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9. Choose **Select the input object** as **Cells** from the drop-down list.
10. **Name the output image** with any name you prefer. For this example, use "Cells" (case sensitive).
11. **Select the color type** from the drop-down list and choose **unit16**.
12. Select the Third **ConvertObjectsToImage** module in the pipeline.
13. Choose **Select the input object** as **Cytoplasm** from the drop-down list.
14. **Name the output image** with any name you prefer. For this example, use "Cytoplasm" (case sensitive).
15. **Select the color type** from the drop-down list and choose **unit16**.

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)*

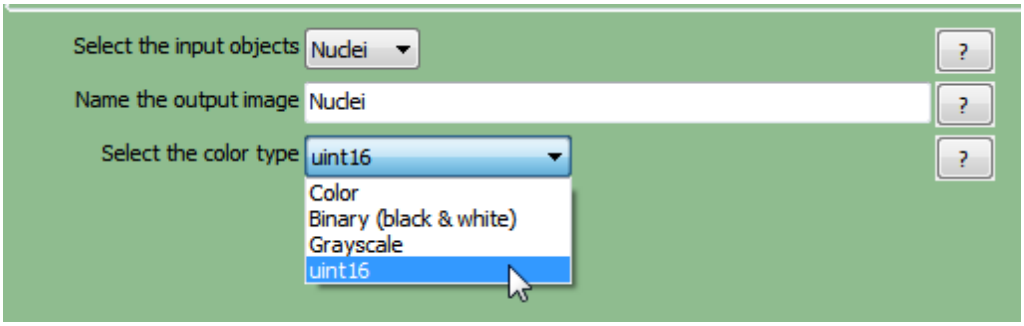

Figure T24.45 ConvertObjectsToImage Module: Defining Nuclei and Cells Image Masks

SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** module must now be saved and related to the original images/data.

12. Select the first **SaveImages** module in the pipeline.
13. **Select the type of image to save** as **Image** from the drop-down list.
14. **Select the image to save** as **Cells**.

    Note: When defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
15. **Select method for constructing file names** as **single name** from the

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drop-down list.

16. **Enter single file name** as any name you prefer followed by a hyphen **"_rowtile_columntile"**. For this example use **CellLabel_rowtile_columntile** .

17. Right-click after **CellLabel_rowtile** (but before the underscore "_"), choose **rowtile**.

18. Right-click after **columntile** and choose **coltile**.

19. Select the second **SaveImages** module in the pipeline**.**

20. **Select the type of image to save** as **Image** from the drop-down list.

21. **Select the image to save** as **Nuclei**.

22. **Select method for constructing file names** as **Single name** from the drop-down list.

23. **Enter single file name** as any name you prefer followed by a hyphen **"_rowtile_columntile"**. For this example use **NucleiLabel_rowtile_columntile** .

24. Right-click after **NucleiLabel_rowtile** (but before the underscore "_"), choose **rowtile**.

25. Right-click after **columntile** and choose **coltile**.

26. Select the third **SaveImages** module in the pipeline.

27. **Select the type of image to save** as **Image** from the drop-down list.

28. **Select the image to save** as **Cytoplasm**.

29. **Select method for constructing file names** as **Single name** from the drop-down list.

30. **Enter single file name** as any name you prefer followed by a hyphen **"_rowtile_columntile"**. For this example use **CytoplasmLabel_rowtile_columntile** .

31. Right-click after **CytoplasmLabel_rowtile** (but before the underscore "_"), choose **rowtile**.

32. Right-click after **columntile** and choose **coltile**.

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

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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 33-39 on all the **SaveImages** modules set up for **CellLabel, NucleiLabel,** and **CytoplasmLabel**.

33. **Select the format to use** as **tif** from the drop-down list.
34. Set **Image bit depth** at **16** from the drop-down list.
35. Choose **Default Output Folder** from **Output file location.**
36. Check the **Overwrite existing files without warning?** box.
37. Set **Select how often to save** as **Every cycle** from the drop-down list.
38. **Select colormap** as **gray** from the drop-down list.
39. Check the **Update file names within CellProfiler?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.46

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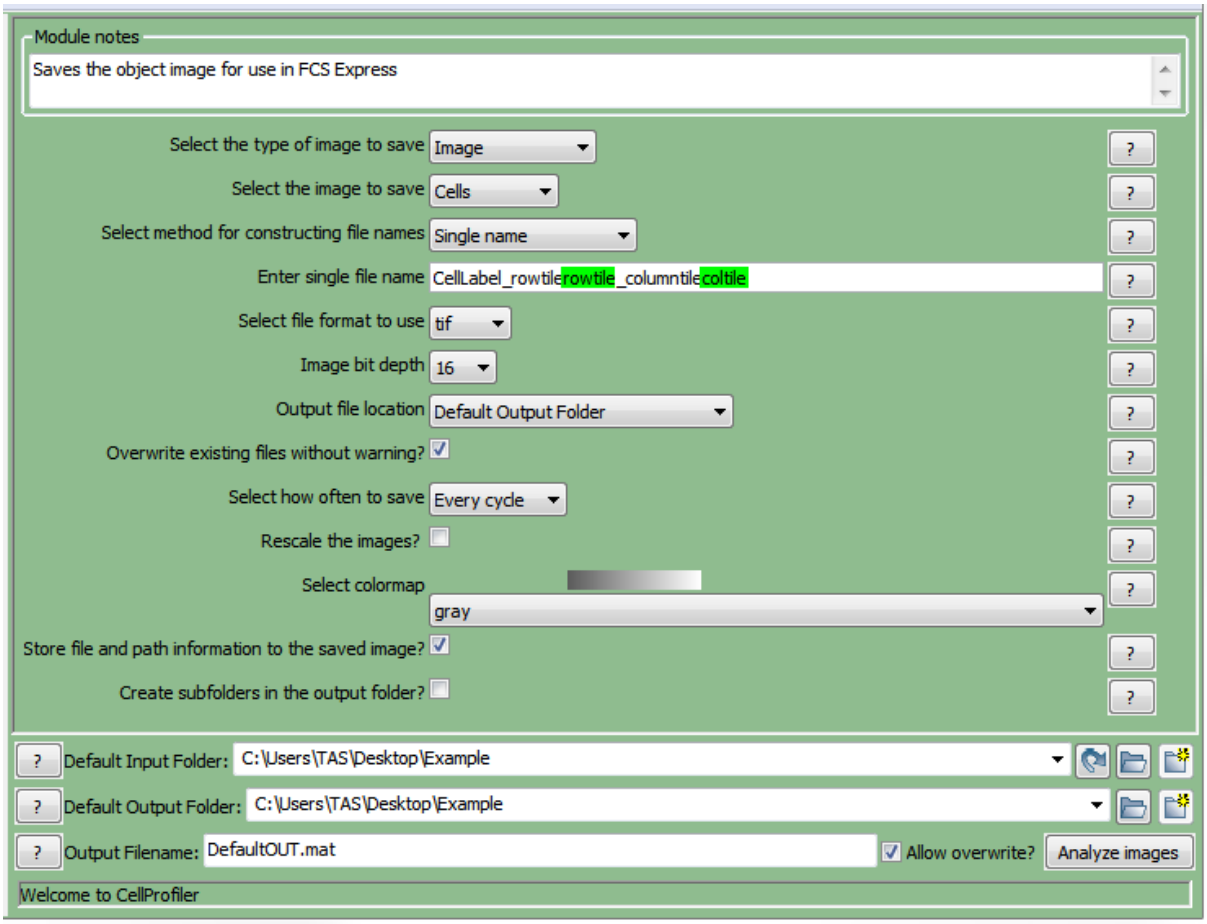

Figure T24.46 Defining the SaveImages Module

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 selected measurements.

(Note: For more details on only exporting certain parameters please see the Selecting Individual Parameters for CellProlifer Export section)

33. Select the first **ExportToSpreadsheet** module.

34. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.

35. Uncheck the **Prepend the output file name to the data file names?** box.

36. Choose **Output file location** as **Default Output Folder** from the drop-

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down list.

37. Uncheck **Export all measurements, using default file names?**.
38. Choose **Image** from the first **Data to export** drop-down list.
39. Uncheck **Use the object name for the file name?**.
40. Set the file name as **Image.cptoc.**
41. Select the second **ExportToSpreadsheet** module.
42. **Select or enter the column delimiter** by choosing **Comma (",")** from the drop-down list.
43. Uncheck the **Prepend the output file name to the data file names?** box.
44. Choose **Output file location** as **Default Output Folder** from the drop-down list.
45. Check the box for **Select the columns of measurements to export?**.

    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.

46. Choose **Add another data set**.
47. Choose **Cells** from the second **Data to export** drop-down list.
48. Uncheck **Use the object name for the file name**.
49. Set the file name as **cells.cpout**.
50. Repeat steps 47-50 for **"Cytoplasm"** and set the file name as **cytoplasm.cpout**.

The second **ExportToSpreadsheet** module options should look like Figure T24.47 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.

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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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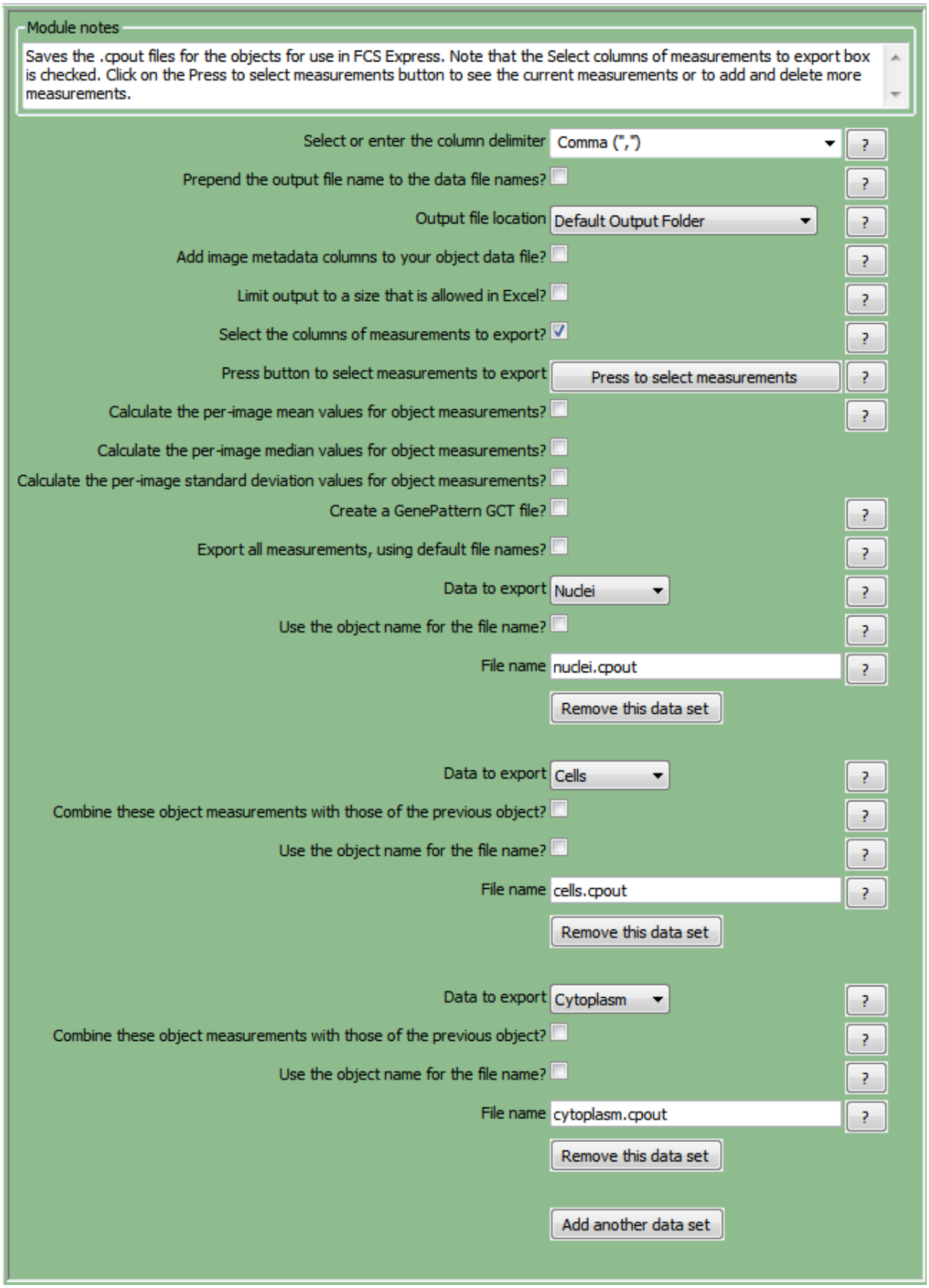

Figure T24.47 The second ExportToSpreadsheet Module Set Up to Export nuclei.cpout, cells.cpout, and cytoplasm.cpout.

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46. 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.

In the next section, we will import and analyze the Tiled or Montage Image 1253.

34.24.1 Importing Tiled Images 2

This section pertains to importing data that was exported from CellProfiler and generated from a set of tiled images. Please see Exporting Tiled Images 1242 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 V4.

1. Load the **CellProfilerTiledExportCOMPLETED.cp** 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 1242 tutorial.
3. Analyze the images in CellProfiler.

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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

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4. Open FCS Express V4.
5. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.48).
6. Select **csv** from the **delimiter** drop-down list.
7. Check the **Load Images** check box.
8. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**
9. Click **OK** to close the **Edit Preferences** dialog.

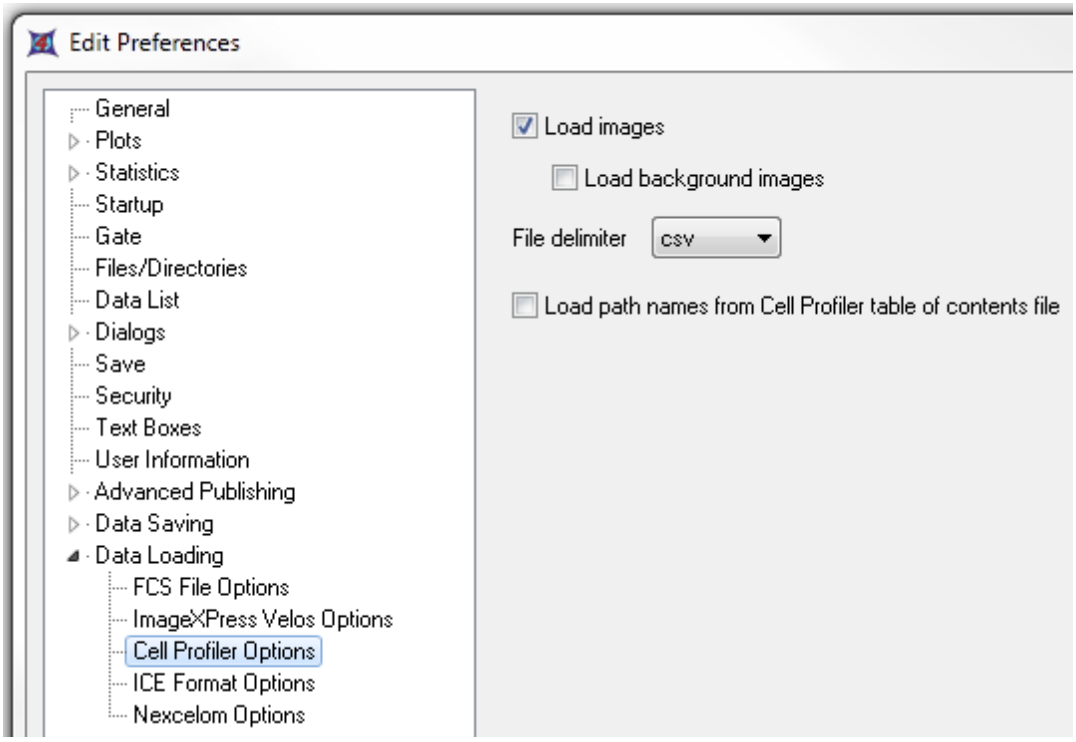

Figure T24.48 CellProfiler Data Loading Options

10. Select the **Insert→2D Plots →Picture Plots** command (Figure T24.49).

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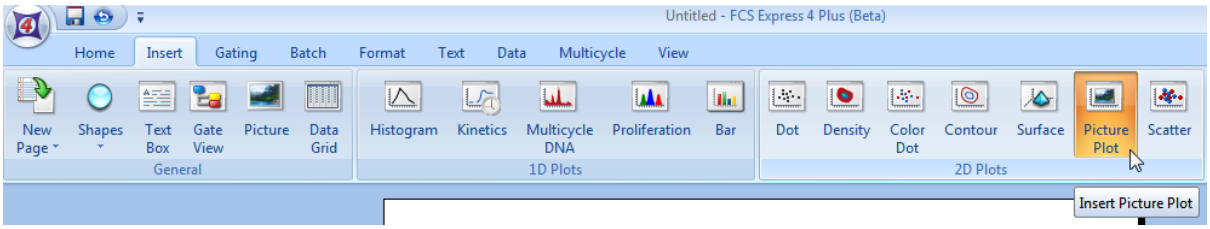

Figure T24.49 Choosing a Picture Plot to Insert

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)** (Figure T24.50)**.** 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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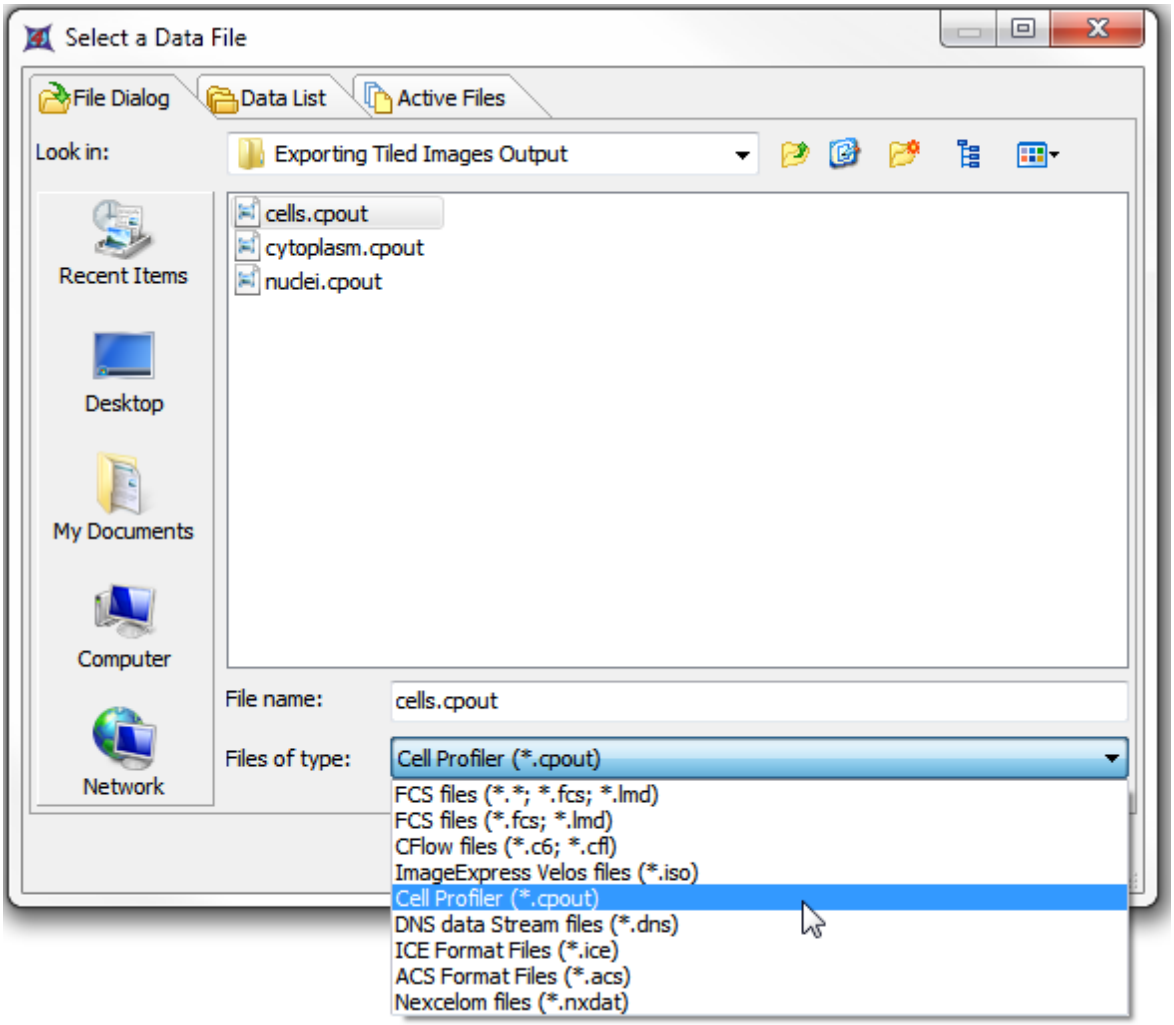

Figure T24.50 Select a Data File Dialog

14. 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 IdentifyTertiaryObjects module of CellProfiler which corresponds to the area between the nuclear object and the cell boundary.

15. Highlight CellMask from the **Image Parameter** list box of the **Open 2D Plots** dialog (Figure T24.51) to view the cellular image.

16. Click **OK**.

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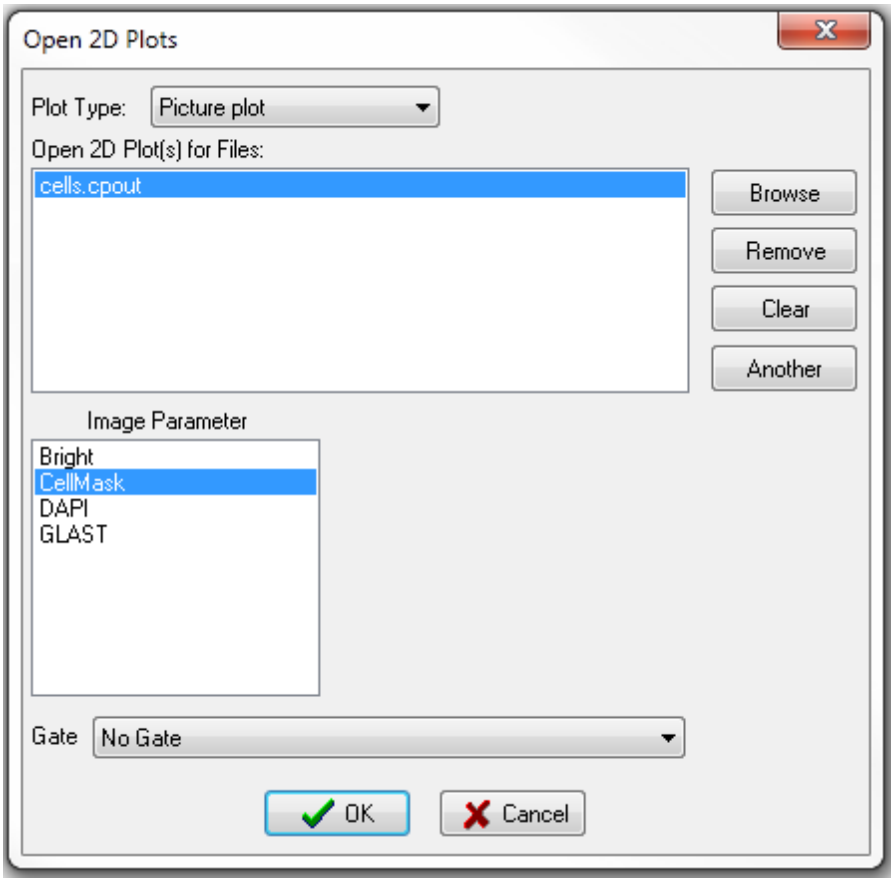

Figure T24.51 Choosing Image Parameters from the Open 2D Plots Dialog

A **Picture Plot** will now appear on your layout indicating that the data was successfully imported (Figure T24.52).

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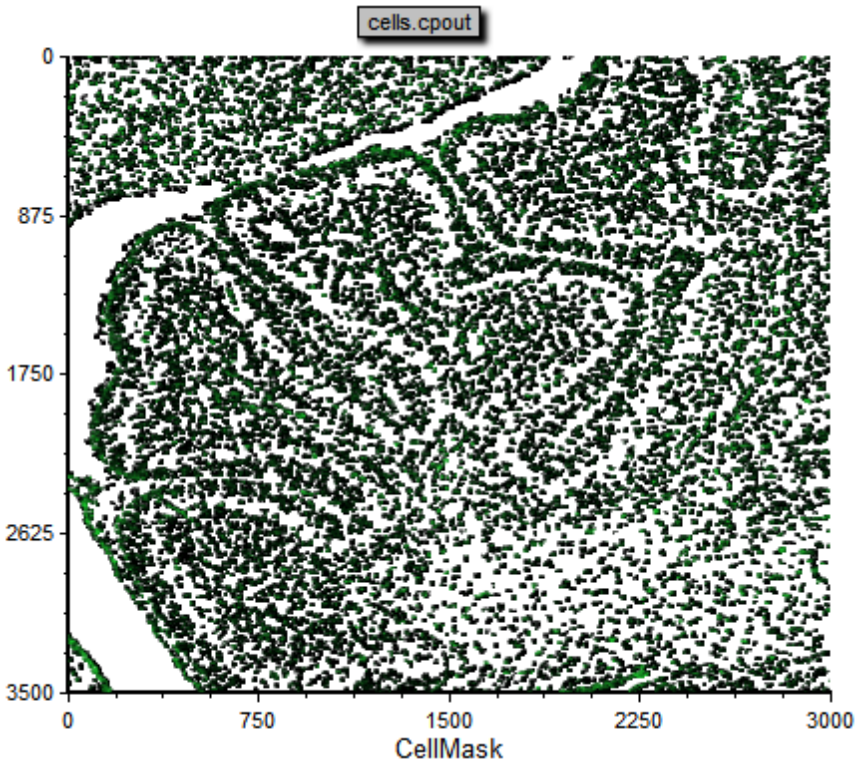

Figure T24.52 Example of the Cells.cpout Picture Plot

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.

17. Right click on the picture plot.
18. Choose **Format** from the drop down list.
19. Choose the **Background** category in the formatting dialog (Figure T24.53).
20. Click on the **Solid Color** drop down box (Figure T24.53).
21. Choose **Black** as the background color (Figure T24.53).
22. Click **OK**.

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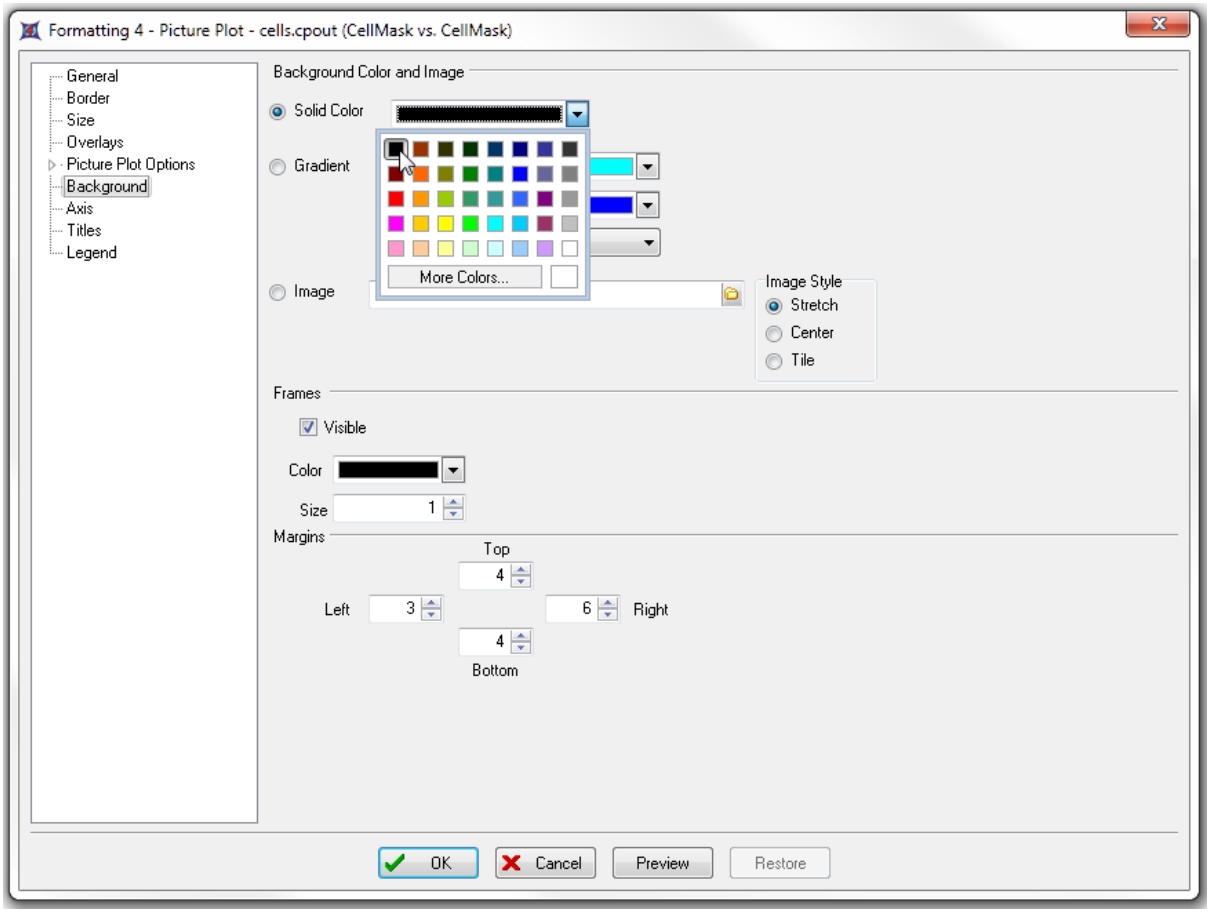

Figure T24.53 The Background formatting options dialog.

The picture plot will now have a black background as the original image did (Figure T24.54)

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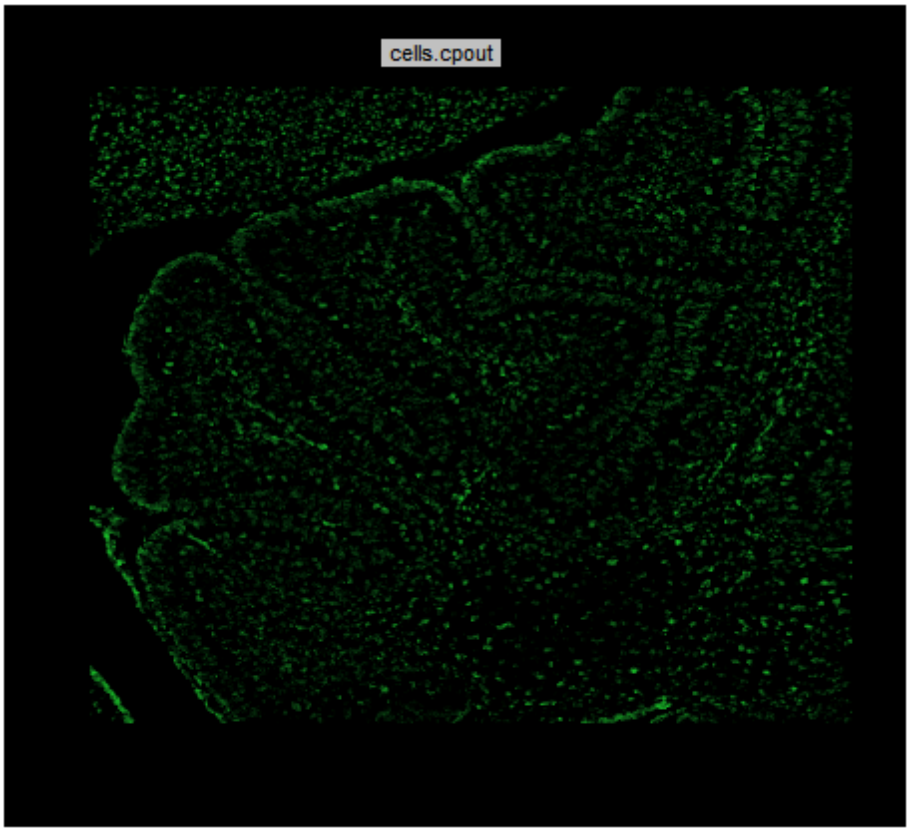

Figure T24.54 The Picture Plot has been formatted to use a black background.

To continue your analysis of the data please visit the Picture Plots 1315 or Introduction to FCS Express 4 Image Cytometry 1373 tutorials which describe working with your data in FCS Express 4 Image Cytometry.

34.24.13 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,

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multi field, imaging data processed by CellProfiler.

- Exporting Multiple Well, Multiple Field Location, Imaging data in CellProfiler for use in FCS Express.

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 T24.55 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).

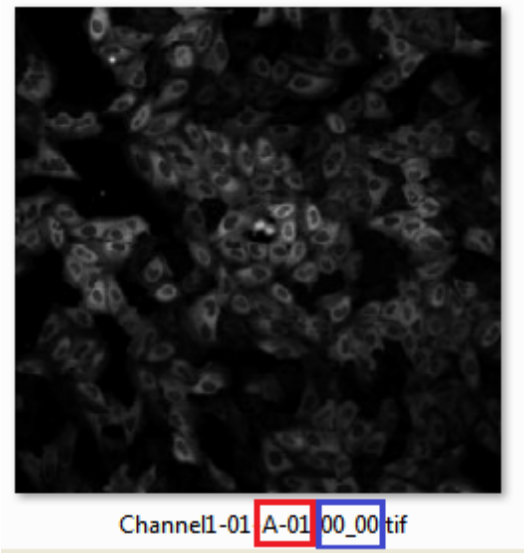

Figure T24.55 The image name is: Channel1-01-A-01_00_00. The red box highlights the well Row and Column information (well A01), and the blue box highlights the field location (position 00, 00).

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Exporting Multiple Field Location Plate Imaging Data in CellProfiler for use in FCS Express

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 590 within the **Multiple Field Location Plate Imaging** folder is named **MultipleFieldLocationPipelineCOMPLETED.cp**. 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 590 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.cp**. pipeline. After loading the **MultipleFieldLocationPipeline.cp**. pipeline in CellProfiler, follow the steps below to amend the pipeline to prepare for export to FCS Express.

Selecting Default Input and Output Folders

To organize your data correctly for FCS Express, first ensure that the default input (where your images are stored) and output folders in CellProfiler are set to the same folder in the Input/Output Folder window (Figure T24.56).

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.

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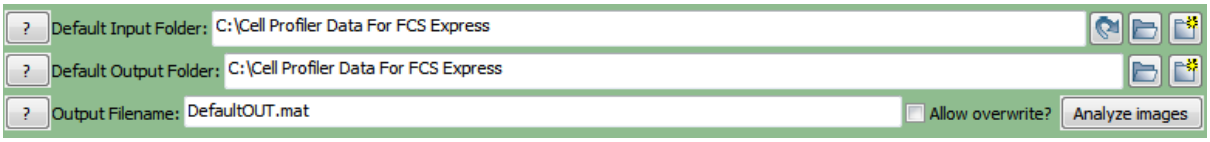

Figure T24.56 Set the Default Input and Output Folders to the Same Location

LoadImages Module Setup

In order for FCS Express to recognize your image data is in a well row and column and field location rowtile and coltile format for a multiple field location plate based assay, 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 (Figure T24.57):

1. Click on the **Channel2-** drop-down list labeled **Extract metadata from where?**.
2. Select which metadata you wish to use. For this example, choose **File name**.
3. Enter the "Regular Expression" you wish to use in the field labeled **Regular expression that finds metadata in the file name.** For this example we will use "**Channel2-[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**" which defines the well row and column (**Row** and **Column**) information and the field location (**rowtile** and **coltile**) information as found in the image name.

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.

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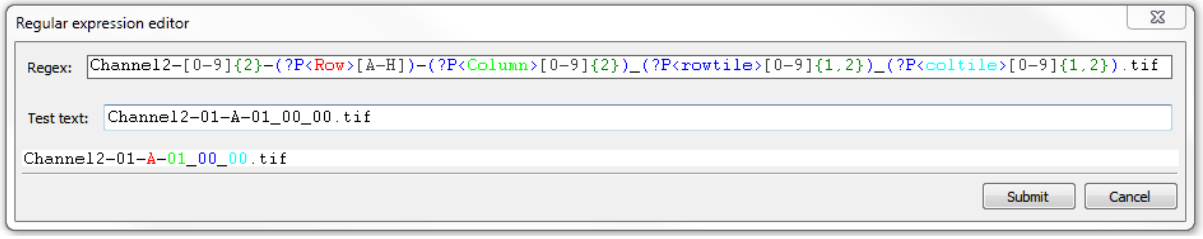

Figure T24.57 Setting up a Regular Expression in the LoadImages Module for a multiple field plate based experiment.

SaveImages Module

The image masks that were defined in the **ConvertObjectsToImage** module must now be saved and related to the original images/data.

12. Select the first **SaveImages** module in the pipeline.
13. **Select the type of image to save** as **Image** from the drop-down list.
14. **Select the image to save** as **Cells**.

    Note: When defining your own pipeline, select the appropriate image you defined in the **ConvertObjectsToImage** module.
15. **Select method for constructing file names** as **single name** from the drop-down list.
16. **Enter single file name** as any name you prefer followed by a hyphen. For this example use **CellLabel-**
17. Right-click after **CellLabel-** , choose **Row.**
18. Type a **-** (hyphen symbol) next to **Row**.
19. Right-click after **Row-** and choose **Column.**
20. Type a **_** (underscore symbol) next to **Column.**
21. Right-click after **Column_** and choose **rowtile**.
22. Type a **_** (underscore symbol) next to **rowtile.**
23. Right-click after **rowtile_** and choose **coltile**.

The completed **Enter single file name** field should now look like figure T24.58 below.

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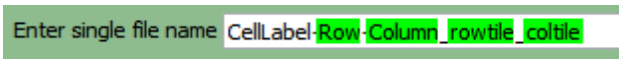

Figure T24.58 The completed Enter single file name field.

24. Select the second **SaveImages** module in the pipeline**.**
25. **Select the type of image to save** as **Image** from the drop-down list.
26. **Select the image to save** as **Nuclei**.
27. **Select method for constructing file names** as **Single name** from the drop-down list.
28. **Enter single file name** as any name you prefer followed by a hyphen. For this example use **NucleiLabel-**
29. Right-click after **Nuclei- ,** choose **Row.**
30. Repeat steps 18-23 above with the second **SaveImages** module.

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 33-36 on both the **SaveImages** modules set up for **CellLabel and NucleiLabel**

33. **Select the format to use** as **tif** from the drop-down list.
34. Set **Image bit depth** at **16** from the drop-down list.
35. Check the **Overwrite existing files without warning?** box.
36. Check the **Store file and path information to the saved image?** box.

The **SaveImages** module for **CellLabel** should now look like Figure T24.59

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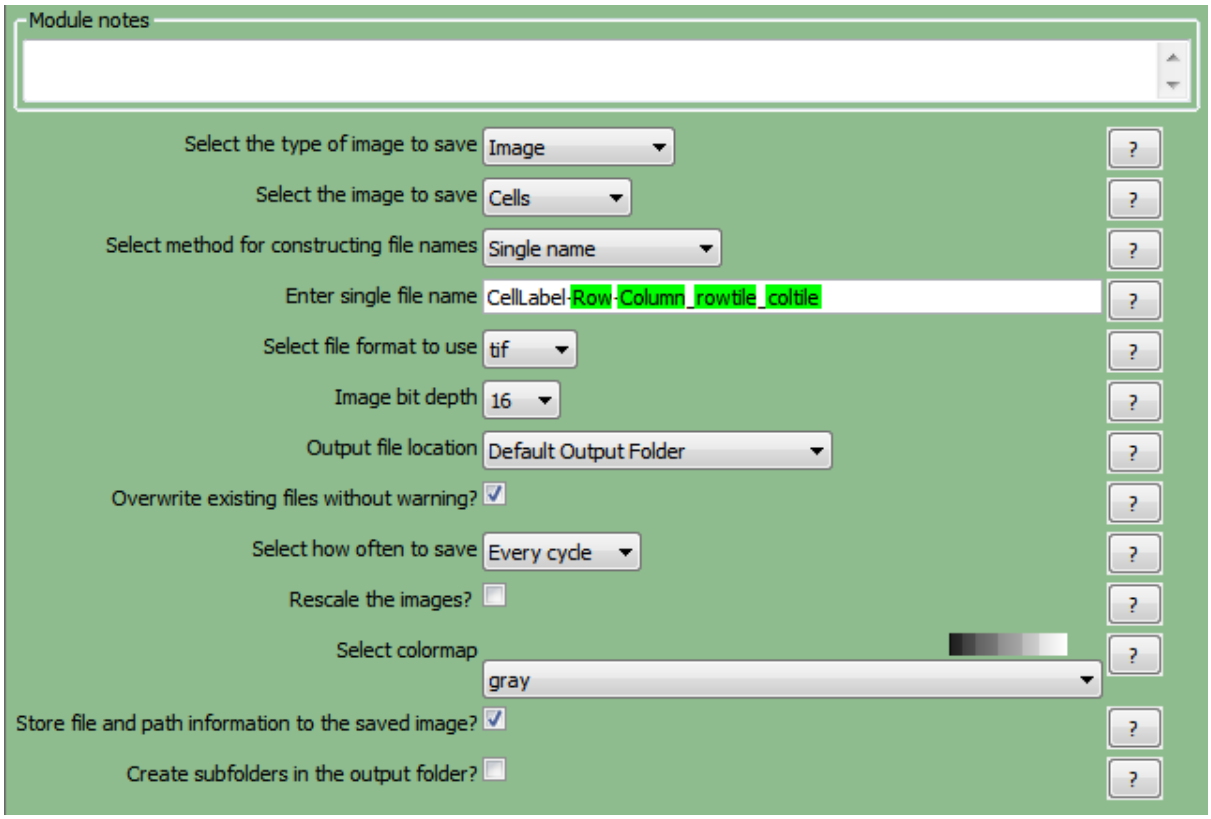

Figure T24.59 Defining the SaveImages Module

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. If you would like to export only certain parameters please see the export selected measurements section of the CellProfiler Export tutorials.

37. Select the **ExportToSpreadsheet** module.
38. Uncheck the **Prepend the output file name to the data file names?** box.
39. Uncheck **Export all measurements, using default file names?**.
40. Choose **Image** from the **Data to export** drop-down list.
41. Uncheck **Use the object name for the file name?** box.
42. Set the file name as **Image.cptoc.**

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43. Click on the **Add another data set** button.
44. Uncheck the **Use the object name for the file name?** box.
45. Choose **Cells** from the second **Data to export** drop-down list.
46. Uncheck the **Use the object name for the file name?** box.
47. Set the file name as **cells.cpout**.
48. Repeat steps 43-47 for **"nuclei"** and set the file name as **nuclei.cpout**.

The **ExportToSpreadsheet** module options should look like Figure T24.60 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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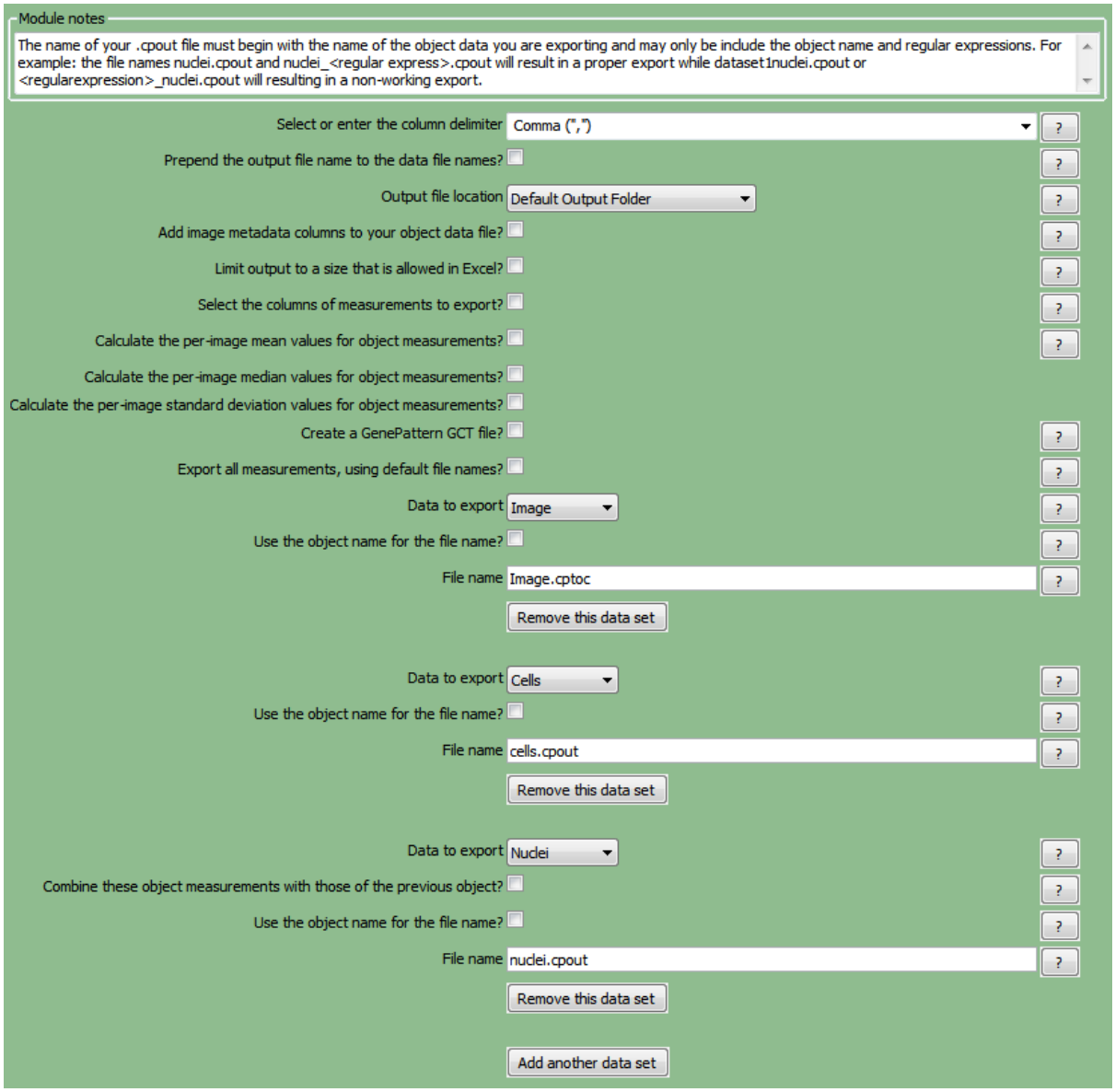

Figure T24.60 The ExportToSpreadsheet Module Set Up to Export image.cptoc, nuclei.cpout and cells.cpout.

49. 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 1269

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34.24.14 Importing Plate Based Multiple Field Location Imaging Data

After adjusting the export settings in CellProfiler and running your pipeline, you may proceed to import the data into FCS Express V4. 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 590 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 590 within the Multiple Field Location Plate Imaging folder.
2. Load the **MultipleFieldLocationPipelineCOMPLETED.cp** 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 1263.
4. Analyze the images in CellProfiler.
5. Ensure the **Experiment.ini file**, located in the Tutorial Sample Data archive 590 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 by:

A. Navigating to **Application Button→Edit Preferences→General**.

B. Check the **Always show a file selection dialog when opening new plots or showing a window that uses a data file** check box.

(Enabling this feature is not a requirement of FCS Express, it has been selected for convenience in this tutorial)

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6. Open FCS Express V4.

5. Open the **Edit Preferences** dialog and select **Cell Profiler Options** from the **Data Loading** category (Figure T24.61).

6. Select **csv** from the **delimiter** drop-down list.

7. Check the **Load Images** check box.

8. Check the **Load background images** check box.

9. Uncheck the **Load path names from Cell Profiler table of contents file** check box**.**

10. Click **OK** to close the **Edit Preferences** dialog.

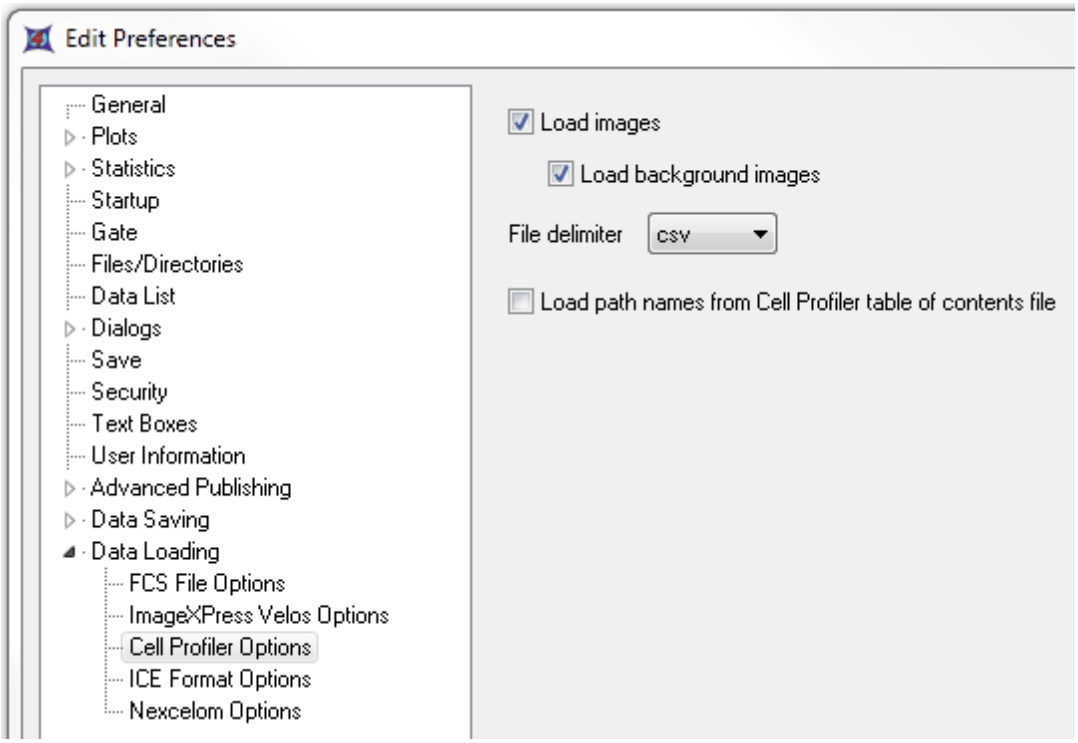

Figure T24.61 CellProfiler Data Loading Options for Importing Plate Based Multiple Field Location Imaging Data.

11. Select the **Insert→Other Plots→Heat Map** command (Figure T24.62).

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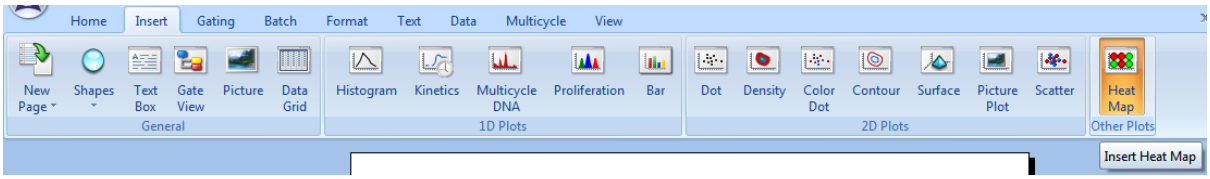

Figure T24.62 Choosing to Insert a Heat Map

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 T24.63). Only documents output by CellProfiler will appear. For this example, they will be labeled **cells.cpout** and **nuclei.cpout**.

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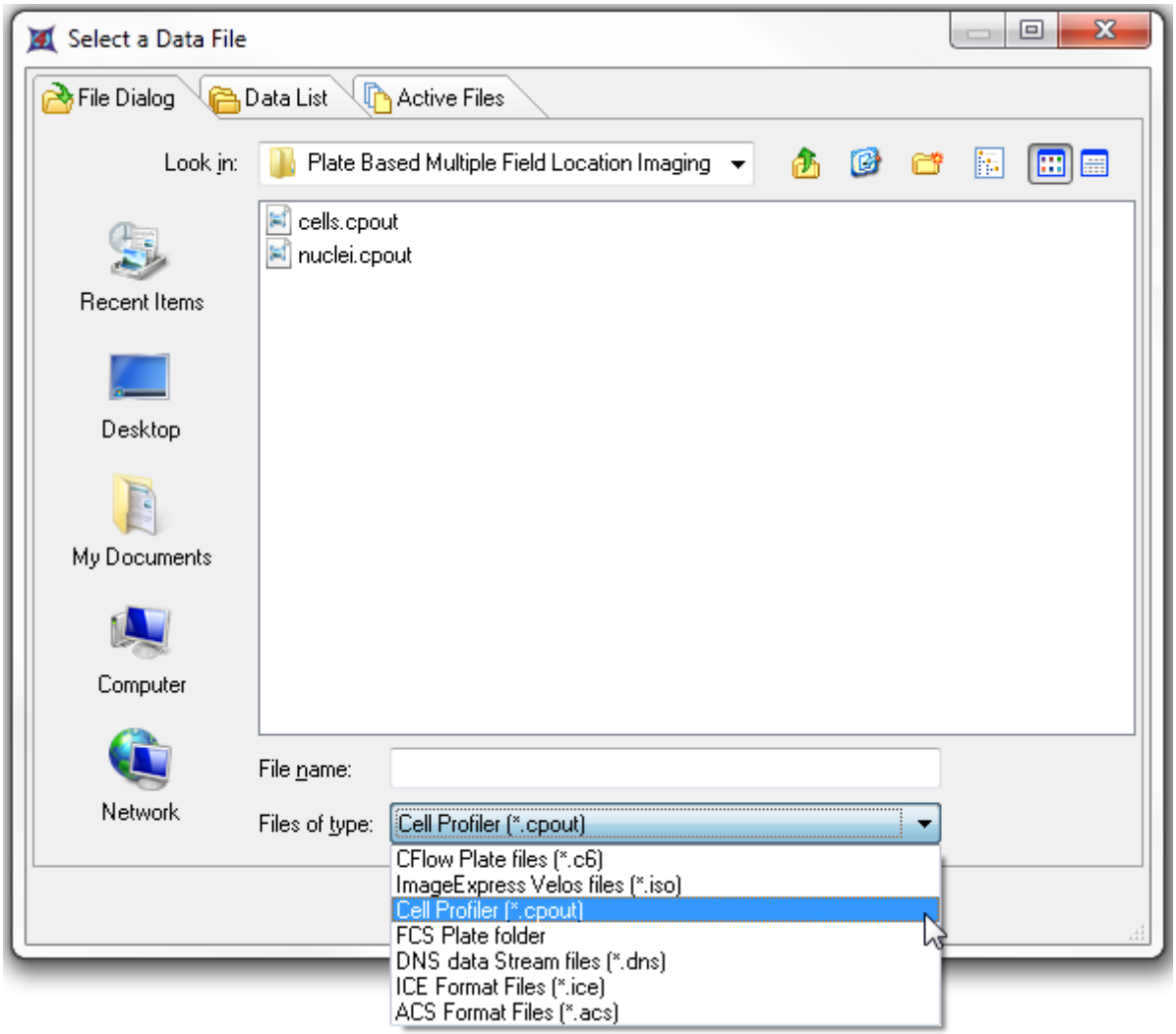

Figure T24.63 Choosing CellProfiler Files

15. Highlight **cells.cpout**.

16. 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.

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 T24.64).

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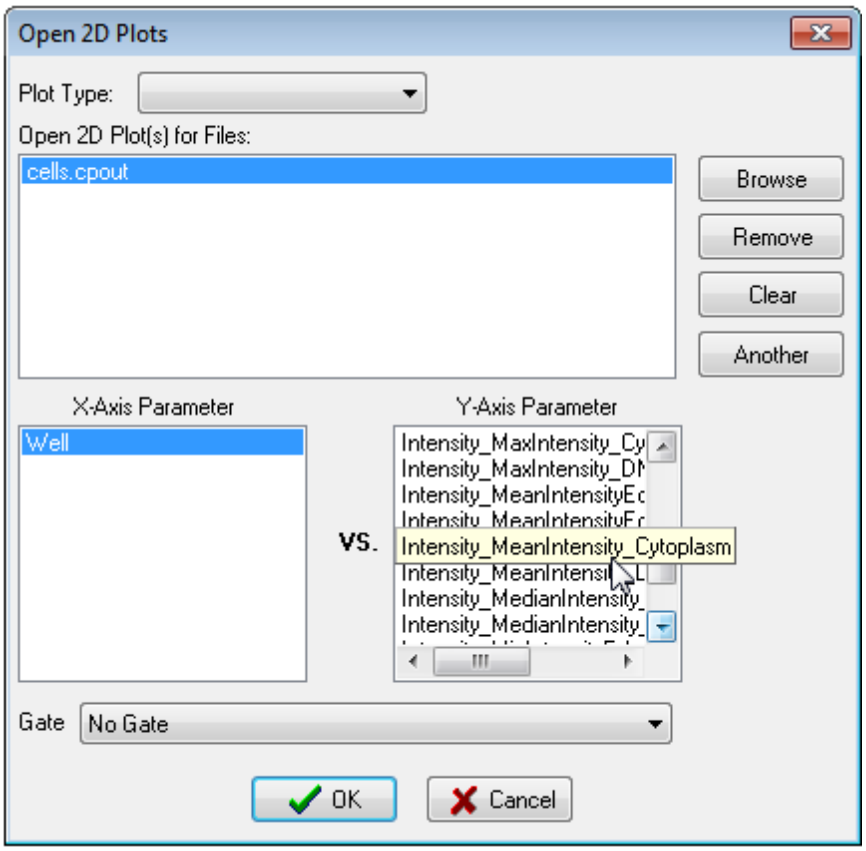

Figure T24.64 Opening a Heat Map and Choosing the Y-Axis Parameter from the Open 2D Plots Dialog

18. Click **OK**.

A Heat Map will now appear on your layout indicating that the data was successfully imported (Figure T24.65).

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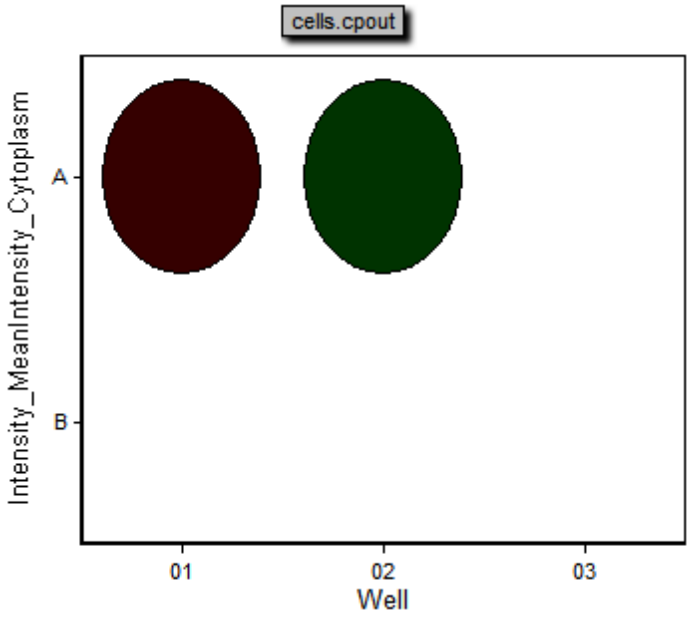

Figure T24.65 Example of a Heat Map Generated from a 6 Well Plate Based Multiple Field Location Imaging Data.

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.
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 T24.66).

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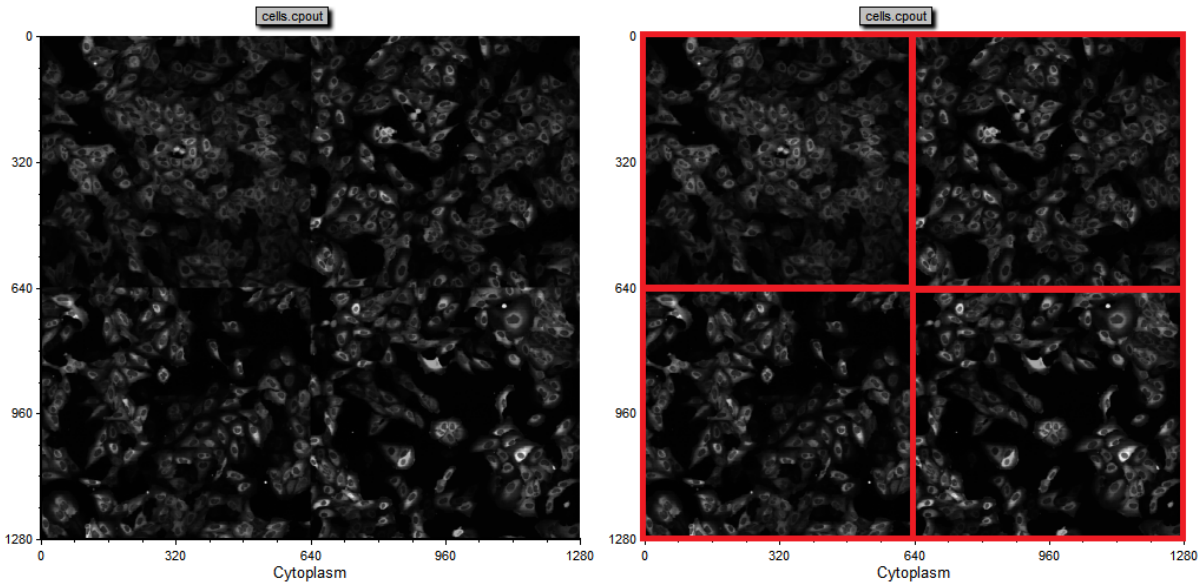

Figure T24.66 The picture plot has been gated on Gate 1, well A01, of the heat map. Four image fields may be observed in the image (left) or as marked by the red boxes (right).

Next, we will discuss frequently asked questions in the CellProfiler FCS Express Knowledge Base[1183].

34.25 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 4 (see our Example Webpage for specific examples). Once your data is properly exported from ImageJ, FCS Express 4 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[1373] such as picture plots and heat maps.

The tutorial will use sample data to:

- Export a single image from ImageJ to FCS Express[1289]
- Create a .ijx Analysis Configuration file[1281]
- Use a .ijx Analysis Configuration file to export a group or set of images from ImageJ to FCS Express[1293]

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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 1296
- ImageJ Macros for FCS Express General Overview 1276
- Overview for the DNSMacroExample.ijm macro used to export data from ImageJ to FCS Express 1277
- Overview for the DNSAnalysisCreator.ijm macro to create an .ijx Analysis Configuration File 1279
- ImageJ for FCS Express Knowledge Base 1296

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.

34.25.1 ImageJ Macros Overview

De Novo Software has provided two ImageJ macros that will help you export your image analysis from ImageJ to FCS Express 4 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 1277 will process your images and export your segmentation and single cell image analysis results in a way that FCS Express 4 Image Cytometry can read. The macro will process images and generate:

    1. Object by object numerical results
    2. Mask images of the segmented objects
    3. An ***.ijout** file that FCS Express 4 Image Cytometry will load to examine

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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)1277

- 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)1279

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 1277

34.25.1. 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 4 Image Cytometry can read. The macro can process and export images from ImageJ in the five use cases listed below.

A. A set of single standalone images:

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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.

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

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be shown in the toolbar (figure T28.92).

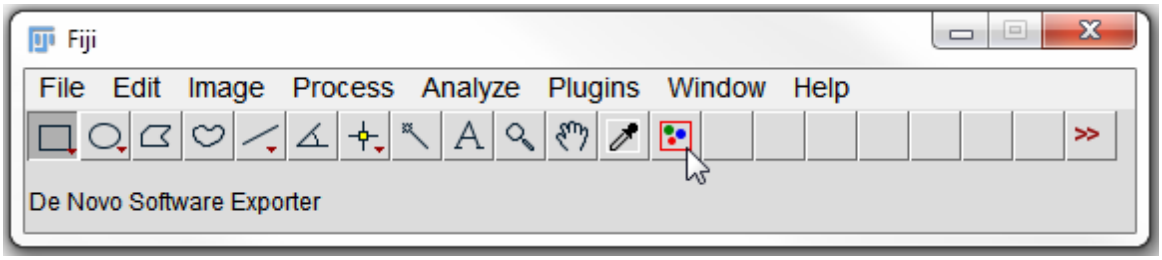

Figure T28.92 The ImageJ tool bar with the DnsExampleMacro.ijm (De Novo Software Exporter) installed.

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 1279

34.25.1. 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 1277.

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..

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

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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 1282
- Manually editing the .IJX file in ImageJ 1286 or Excel 1287

In the next section, we will complete the Creating a .ijx Analysis Configuration File section 1281 of the tutorial

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34.25.2 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 1277.

We will be using the macros and images from the Tutorial Sample Data archive 590 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 1282 *(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 1284

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.

*DNS-IJXwriter.xlsm Excel macro written by Peter Haub

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In the next section 1282, we will use the DNS-IJXwriter.xlsm macro-enabled Excel file to automatically generate an .ijx Analysis Configuration file.

34.25.2. 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)

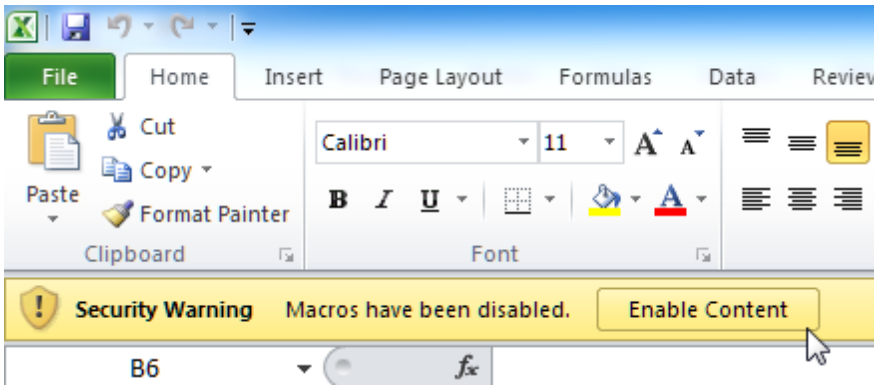

Figure T28.102 Enabling macros in a Microsoft Excel file.

3. Click on the **Read File Names** button (Fig. T28.103)

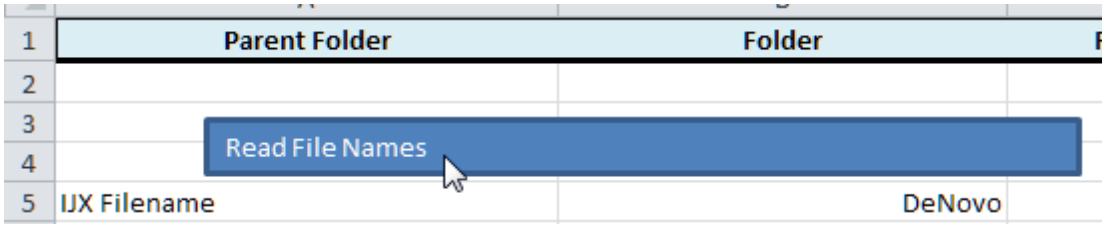

Figure T28.103 Clicking on the Read File Names macro button

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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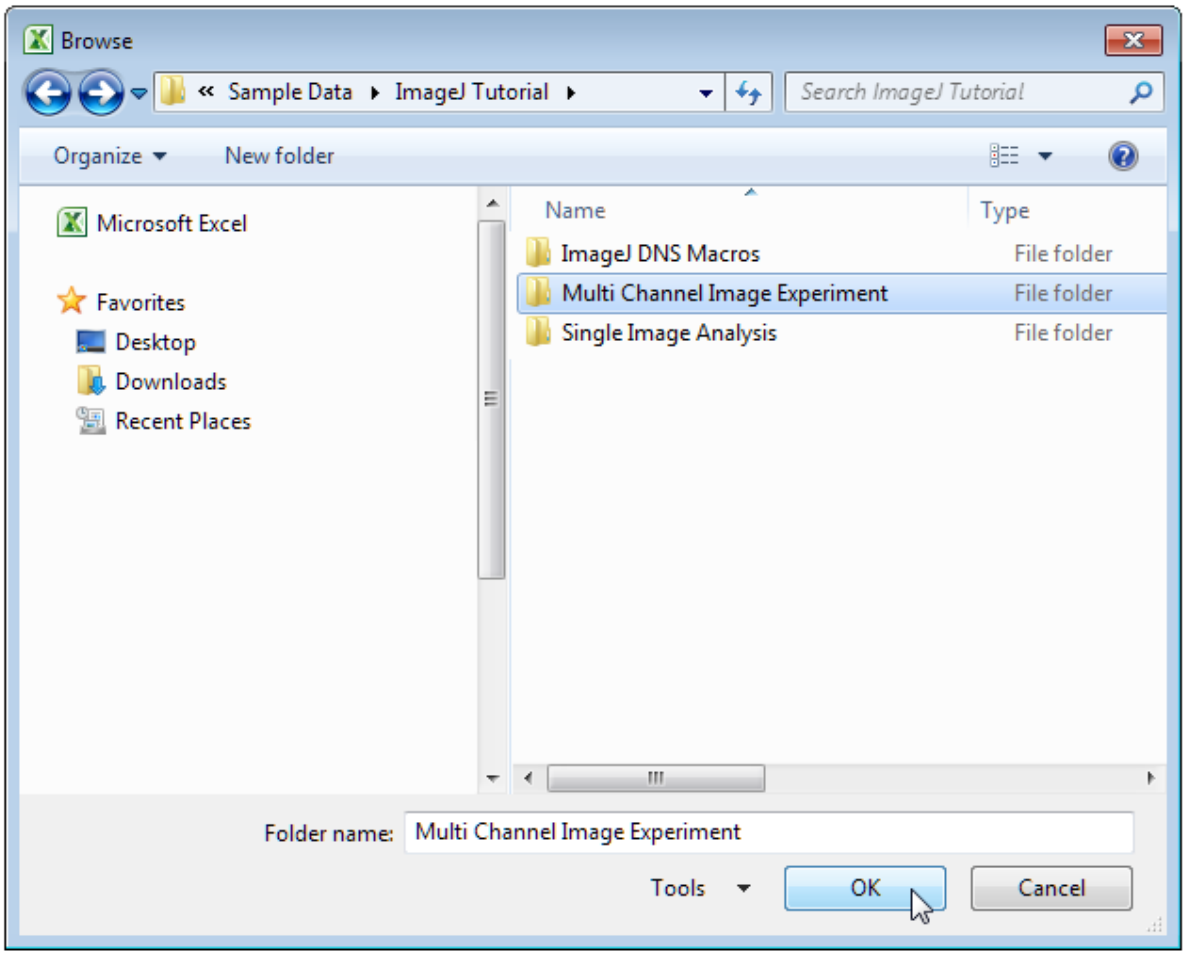

Figure T28.104 Navigating to the ImageJ Tutorial folder within the Tutorial Sample Data Archive

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 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

34.25.2. 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.

1. Open ImageJ.
2. Install the DNSAnalysisCreator.ijm macro in ImageJ.

   When this macro file is installed in ImageJ (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).

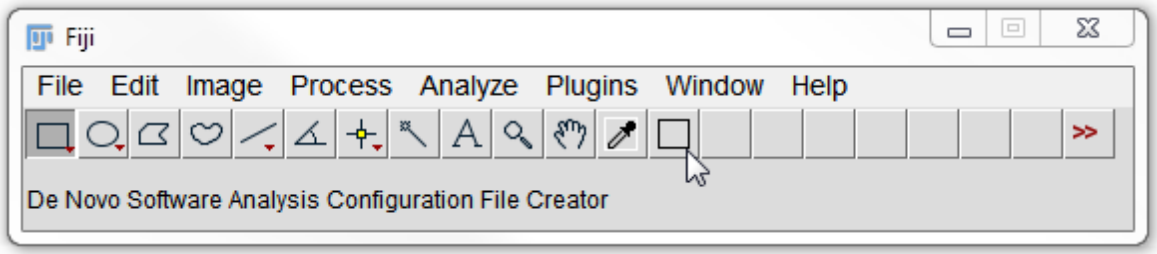

Figure T28.106. The ImageJ tool bar with the DNSAnalysisCreator.ijm macro installed. Notice the blue rectangle button that indicates the De Novo Software Analysis Configuration File Creator.

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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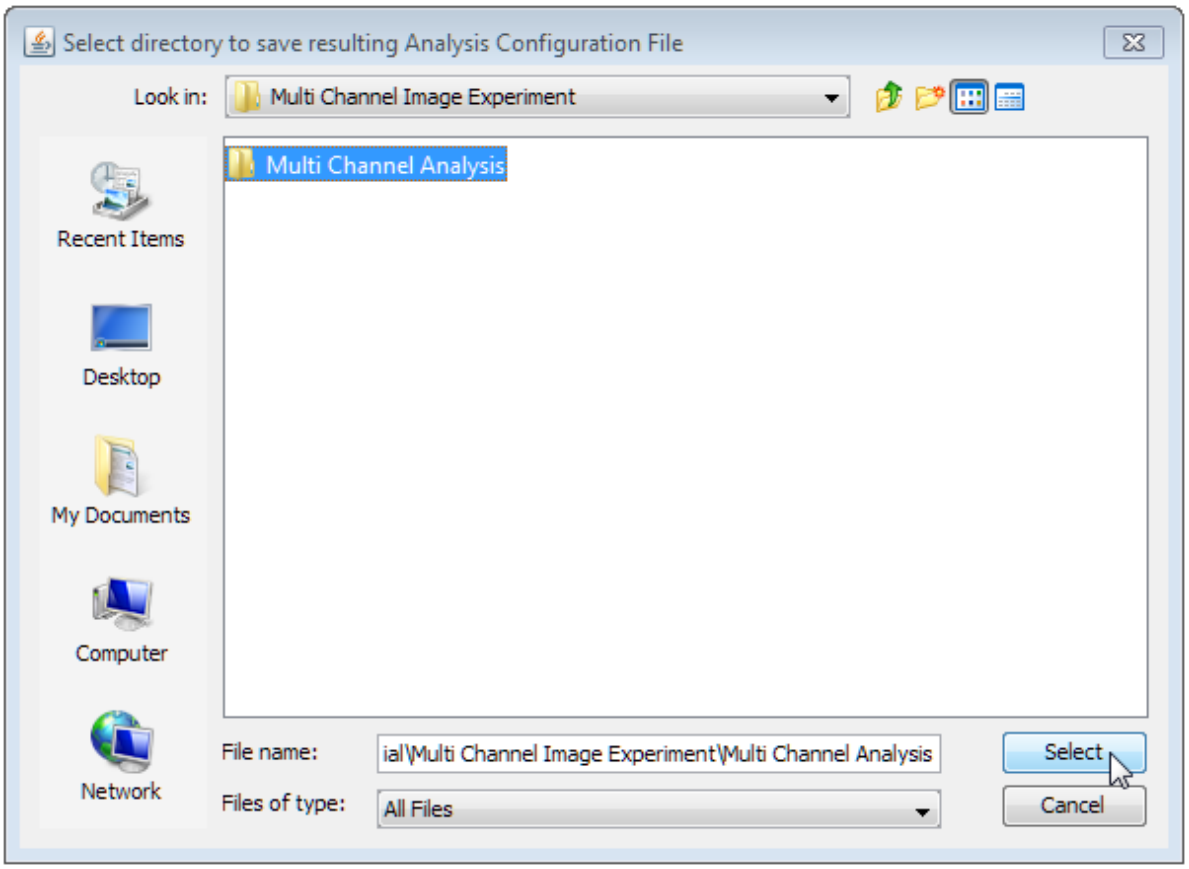

Figure T28.107 Selecting the Multi Channel Analysis folder directory which contains our images for the Analysis Configuration file.

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 Creator1279.

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 1286
- Editing in Microsoft Excel 1287

34.25.2.2.1 Editing an .IJX file in ImageJ

ImageJ can be used to directly edit a .IJX file created by the DNS Analysis Creator Macro 1279.

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.

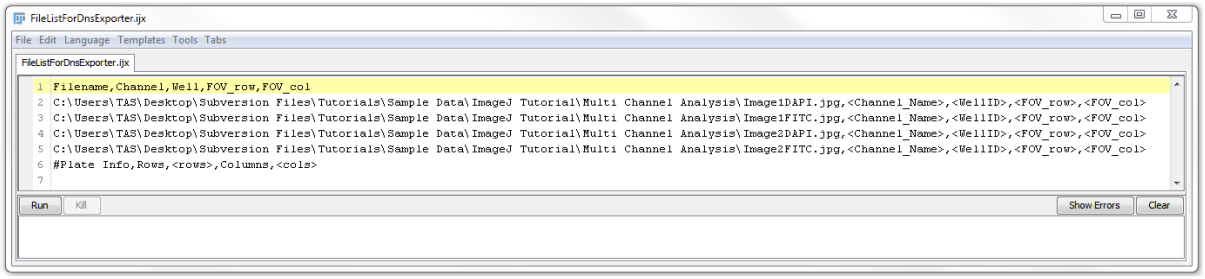

Figure T28.108 The FileListForDnsExporter.ijx file opened in ImageJ that was created in the previous steps.

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.

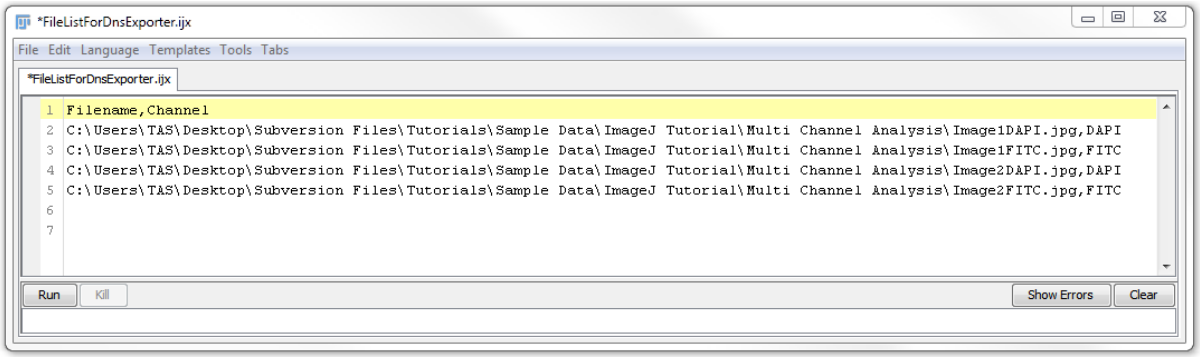

Figure T28.109 The FilelistForDnsExpoter.ijx file in ImageJ after editing to remove extra column headers, plate information, and adding the channel information.

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 1293

34.25.2.2.2 Editing an .IJX file in Excel

Microsoft Excel can be used to directly edit a .IJX file created by the DNS Analysis Creator Macro 1279.

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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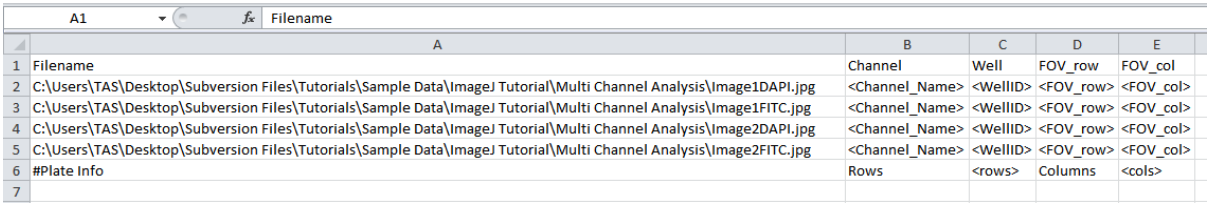
2. Open the **FileListforDnsExporter.csv** file with Microsoft Excel.

The File will open and the excel document will look like figure T28.110 below:

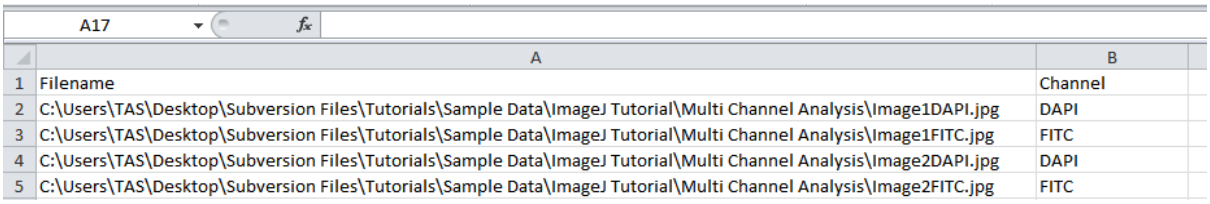


	A	B	C	D	E
1	Filename	Channel	Well	FOV_row	FOV_col
2	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel Analysis\Image1DAPI.jpg	<Channel_Name>	<WellID>	<FOV_row>	<FOV_col>
3	C:\Users\TAS\Desktop\Subversion Files\Tutorials\Sample Data\ImageJ Tutorial\Multi Channel 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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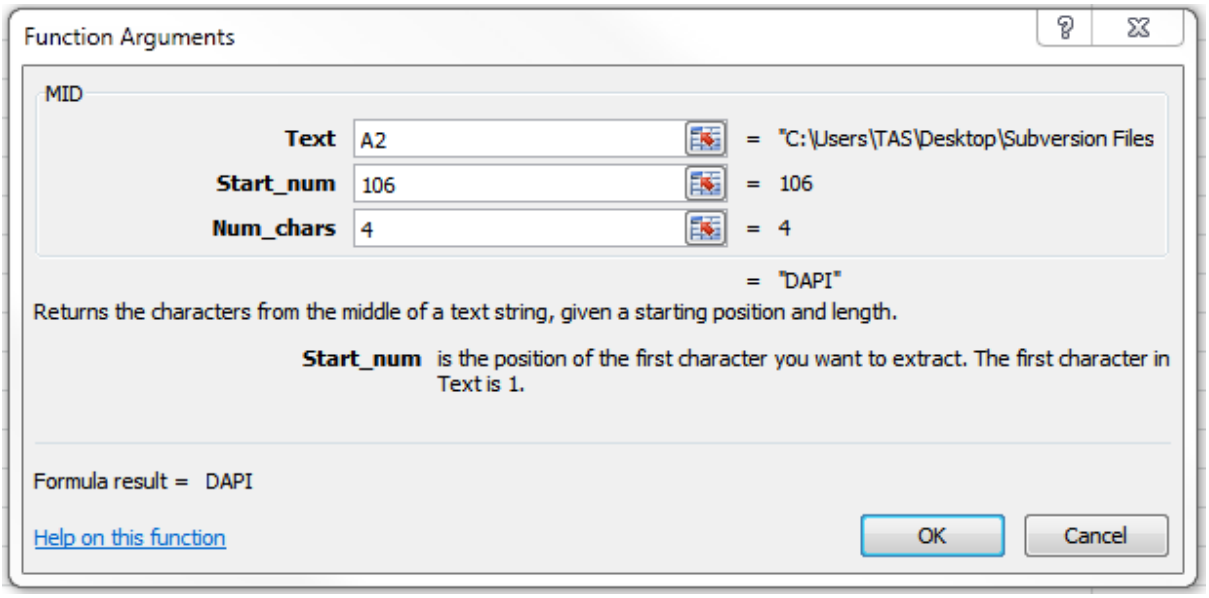

Figure T28.112 An example of using functions or formulas in Excel to automatically fill columns in the Analysis Configuration file.

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 1276 help topic.

In the next section, we will use the newly created .ijx file to analyze and export results from ImageJ to FCS Express 1293

34.25.3 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 1277 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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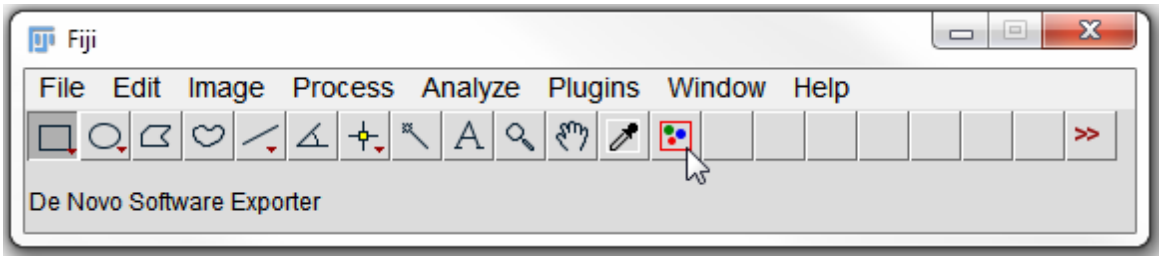
1. Open ImageJ.
2. Install the DNSMacroExample in ImageJ.

   When this macro file is installed in ImageJ[1297] (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).

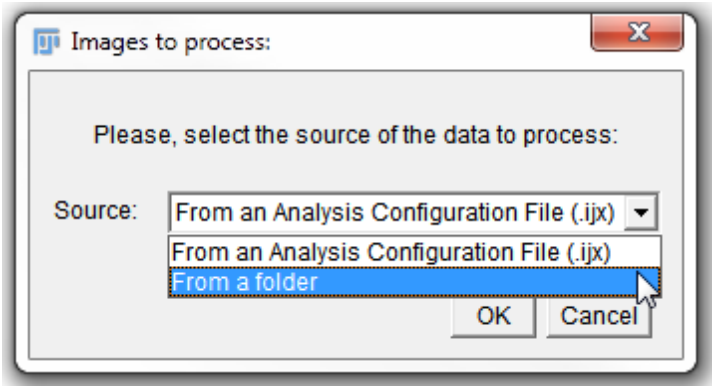


Figure T28.98 Selecting Images to process from a folder in ImageJ.

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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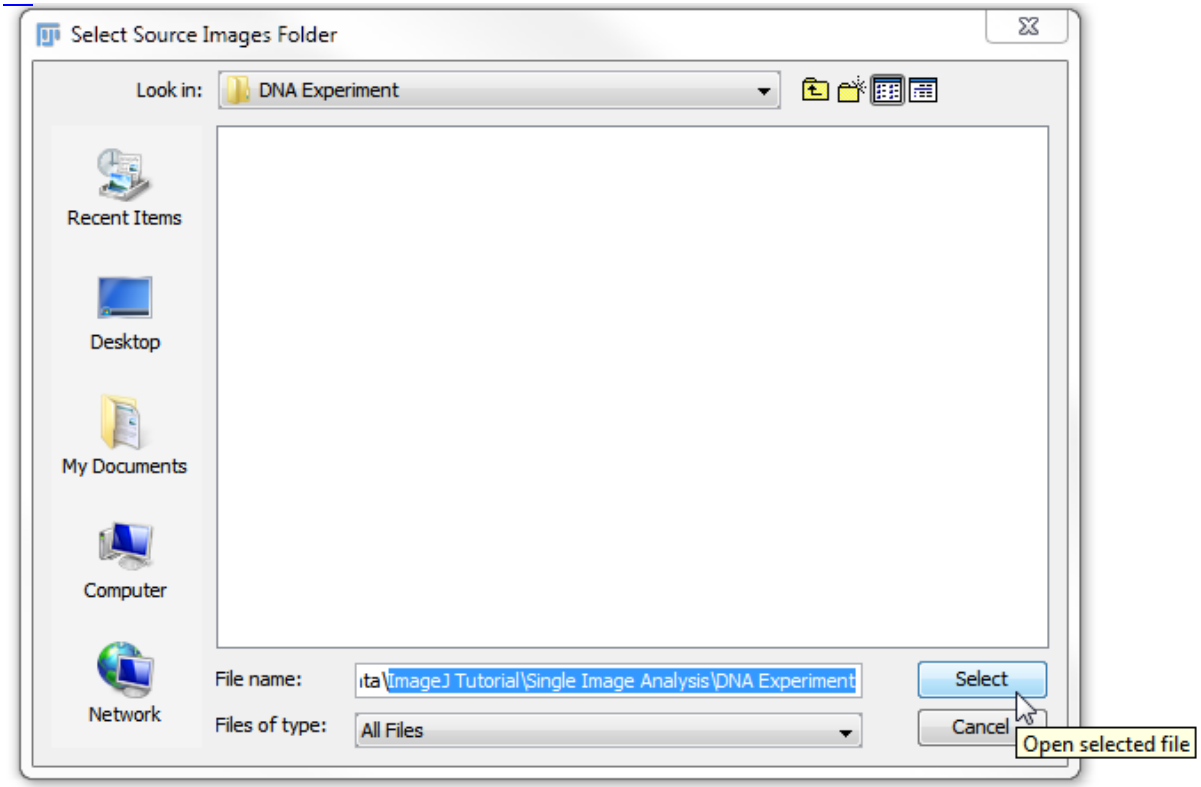

Figure T28.99 Selecting the DNA Experiment folder which contains our images of interest and pressing select.

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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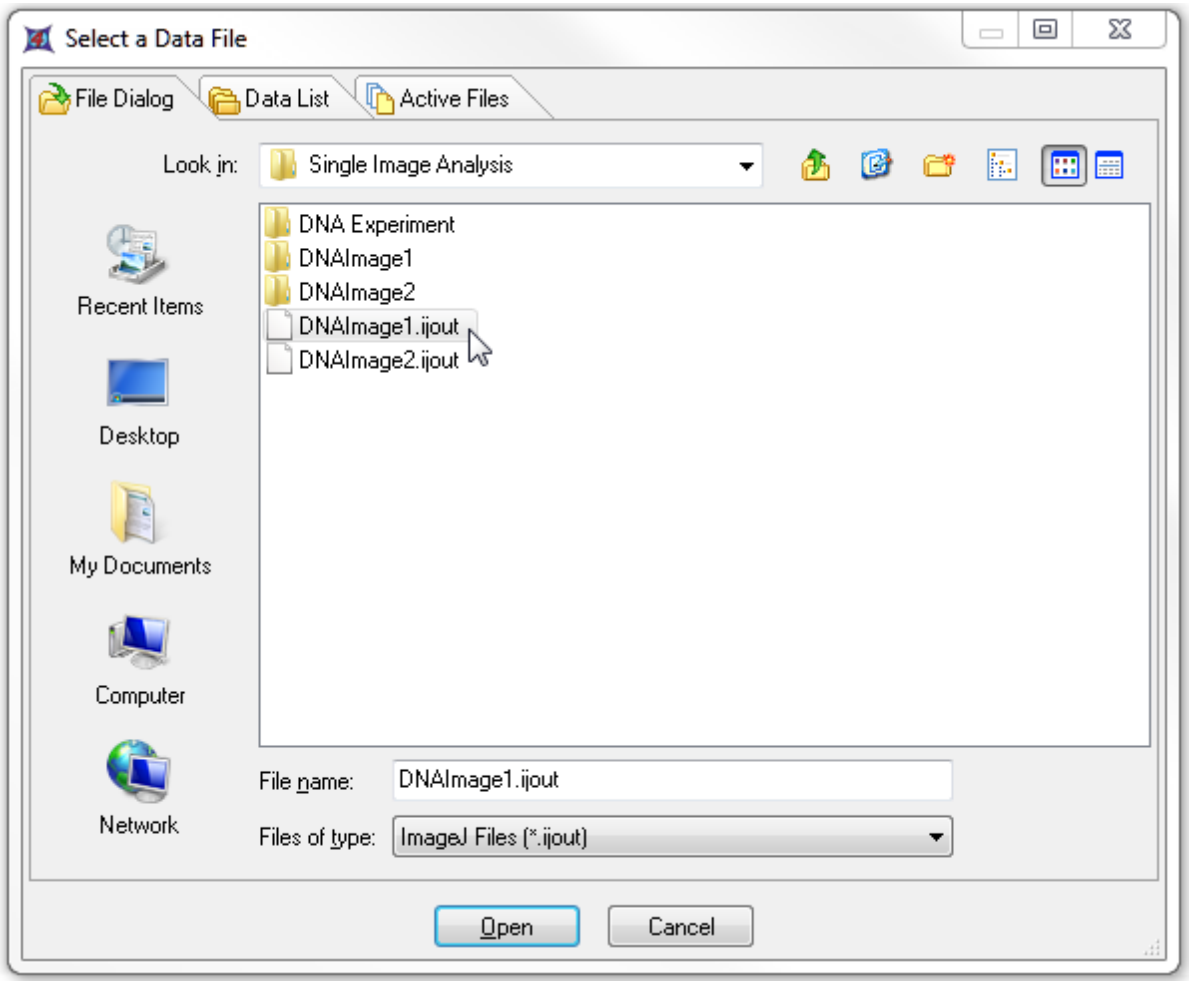

Figure T28.100 The Single Image Analysis folder after processing the images. There will be separate .ijout files for each image and folders that contain the results and masking files.

We will now open the exported data with FCS Express.

9. Open FCS Express 4 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

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from the first and second DNA images that were processed. Please see the further resources section below for more information about working with plots and imaging data in FCS Express.

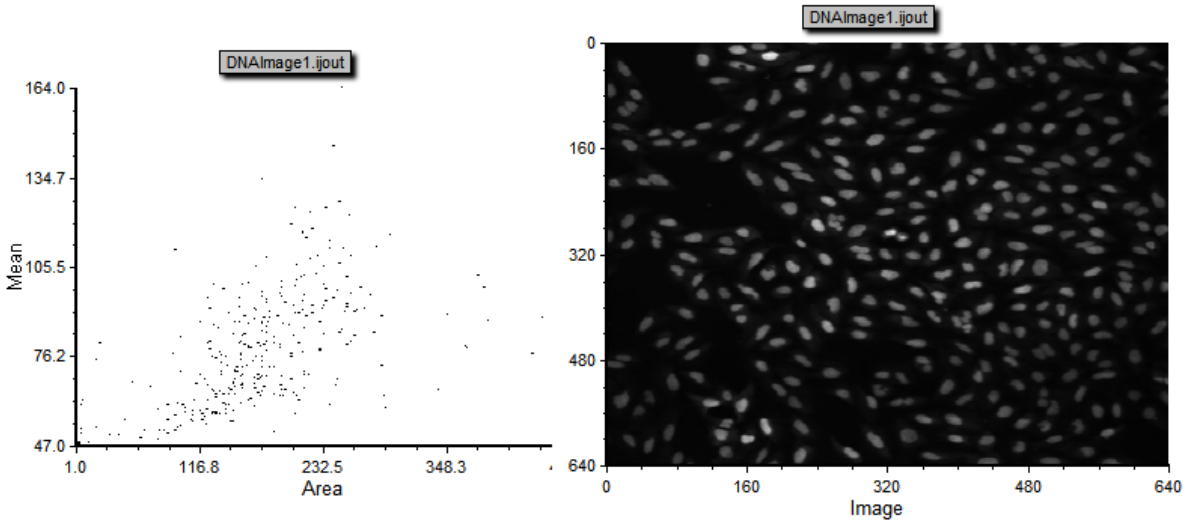

Figure T28.101 A dot plot and picture plot that were create from the DNAImage1.ijout file.

Further Resources:

- **Introduction to FCS Express 4 Image Cytometry Tutorial**[1373]
- **Picture Plots Tutorial**[1315]
- **ImageJ for FCS Express Knowledge Base**[1296]

34.25.4 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[1281] with the macro-enabled Excel worksheet[1282]* or manually edited in ImageJ[1286] or Excel[1287].

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 1297(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).

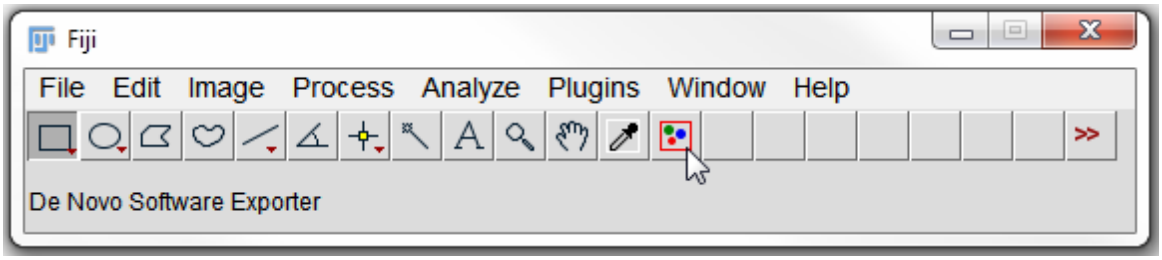

Figure T28.113. The ImageJ tool bar with the DNSMacroExample.ijm macro installed. Notice the red rectangle with three color blobs button that indicates the De Novo Software Exporter.

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).

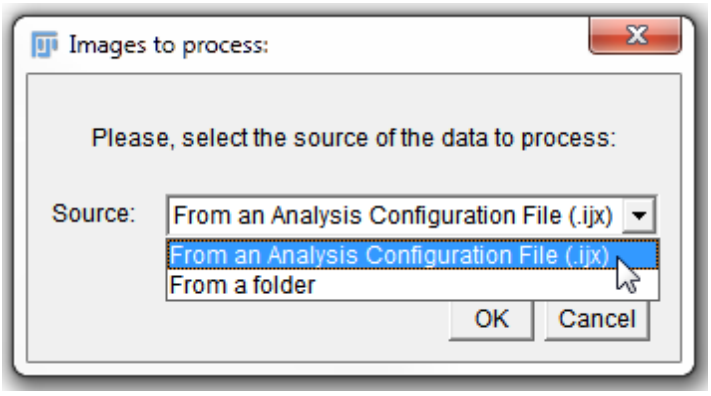

Figure T28.114 Choosing an .ijx file to process images.

5. Click **OK**.
6. Choose either **DeNovo.ijx** (if your .ijx file was created with the macro-enabled Excel worksheet 1282) or **FileListForDnsExporter.ijx** (if your .ijx file was manually edited in ImageJ 1286 or Excel 1287) 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 4 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→Multi Channel Analysis** 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.

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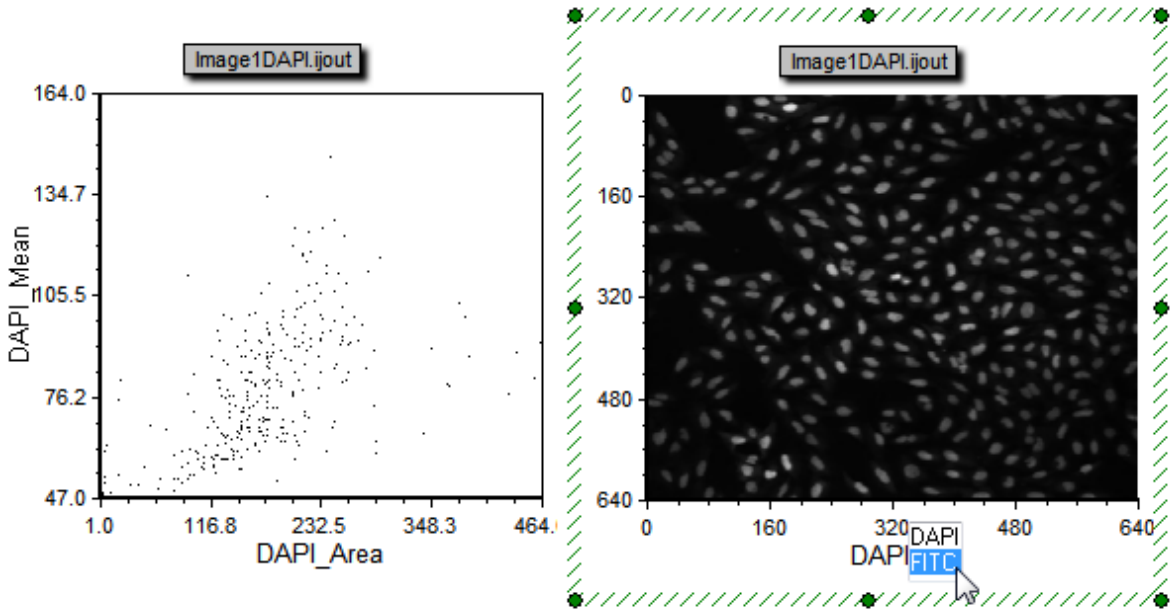

Figure T28.115 A dot plot and picture plot created from the .ijout files that were exported using the .ijx analysis configuration file. Since there are multiple channels you can change the axis of the picture plot to choose which channel to view.

Further Resources:

- **Introduction to FCS Express 4 Image Cytometry Tutorial** 1373
- **Picture Plots Tutorial** 1315
- **ImageJ for FCS Express Knowledge Base** 1296

*macro written by Peter Haub

34.25.5 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.

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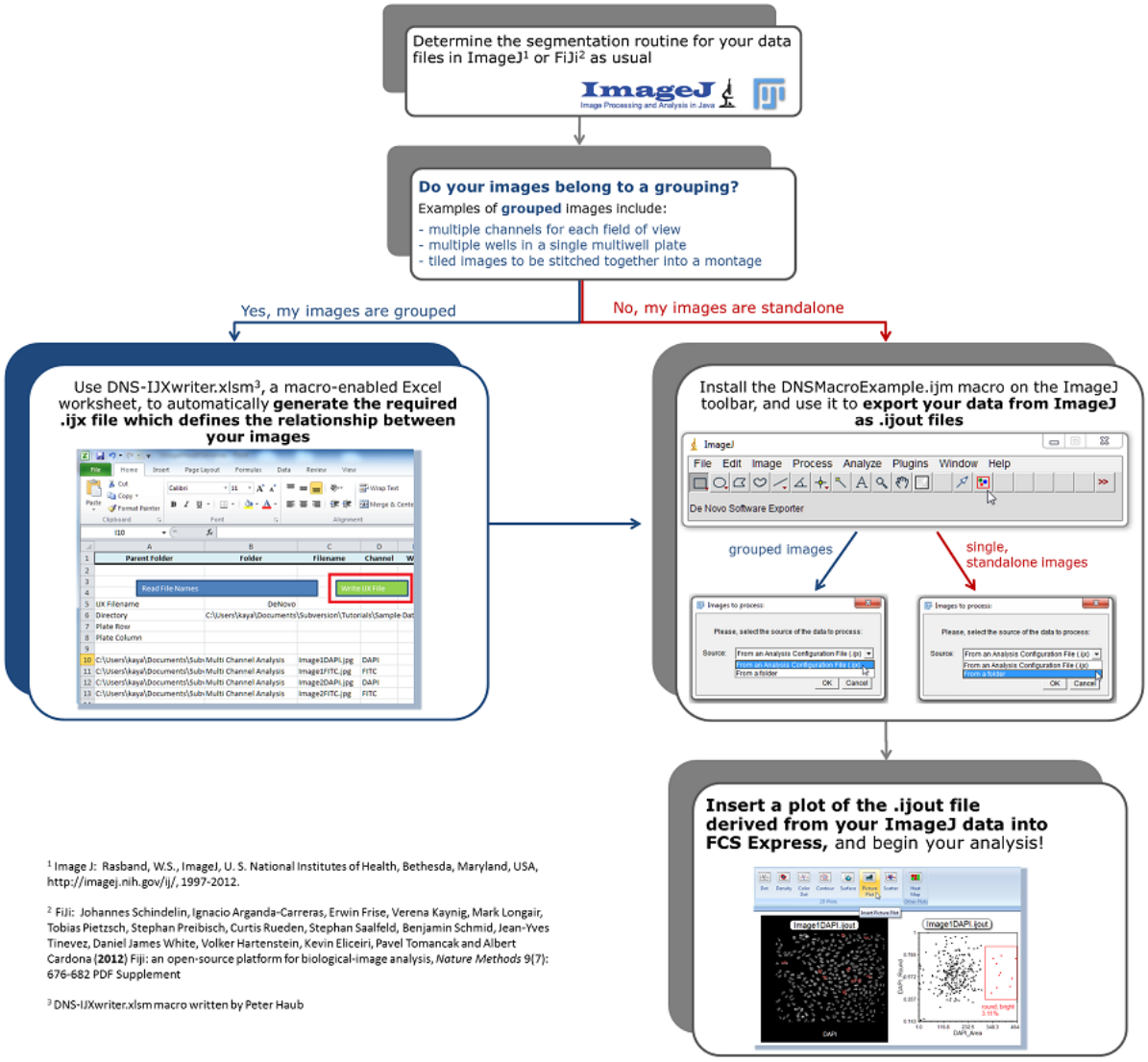

Figure T28.116 Workflow for analyzing single or grouped images segmented in ImageJ within FCS Express

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.*

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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 **Edit Preferences→Data Loading→ImageJ Options** for choosing how to load your data (figure T28.117).

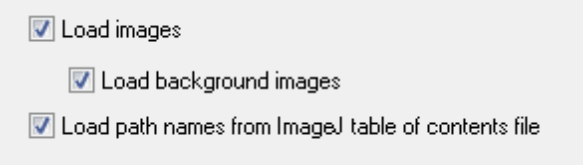

Figure T28.117 The ImageJ Data Loading options available from Edit Preferences in FCS Express 4 Image Cytometry.

Choosing **Load images** or **Load background images** will determine if you want to load just raw data (**Load images** unchecked), or raw data plus the original images (**Load images** checked), and/or background masking (**Load background images checked** to load masks/background or unchecked to not load masks/background).

"**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)

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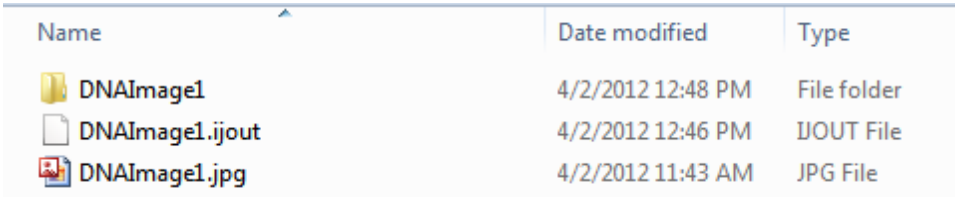

Figure T28.118 When moving .ijout files to another location on your computer the .ijout file, original image file, and folder containing the .results.txt and .masks.tif files must be at the same level.

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.

	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.

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```
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).

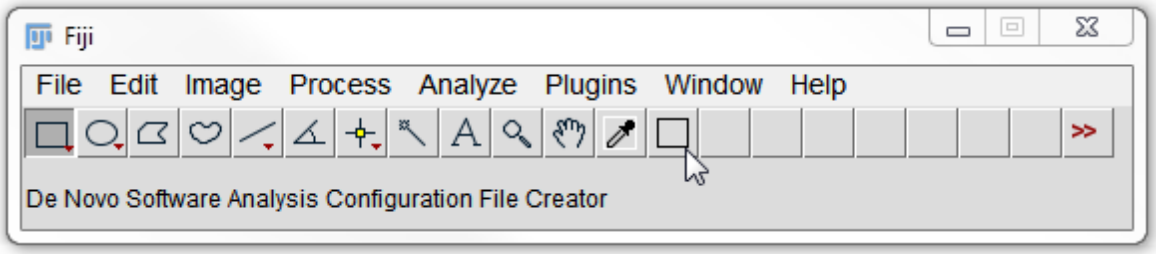

Figure T28.120 The ImageJ tool bar with the DnsAnalysisCreator.ijm macro installed. It is represented by a black box with the hint "De Novo Software Analysis Configuration File Creator".

34.26 Exporting from IsoCyte

The **IsoCyte**™ Imaging Cytometer enables robust real-time assays for microarray, bead, cell, and colony classification and enumeration in user-defined or commercially available formats. The **IsoCyte** 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 processing abilities of **FCS Express**. De Novo Software has teamed up with Molecular Devices to allow analysis of high throughput imaging in FCS Express Version 4. Once your data is properly saved within the **IsoCyte** 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 IsoCyte 1300.

34.26.1 Export an IsoCyte Experiment

In the course of this tutorial you will learn:

- How to save **IsoCyte** data with images from an experiment.
- How to import the data into FCS Express V4.

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Isocyte 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.

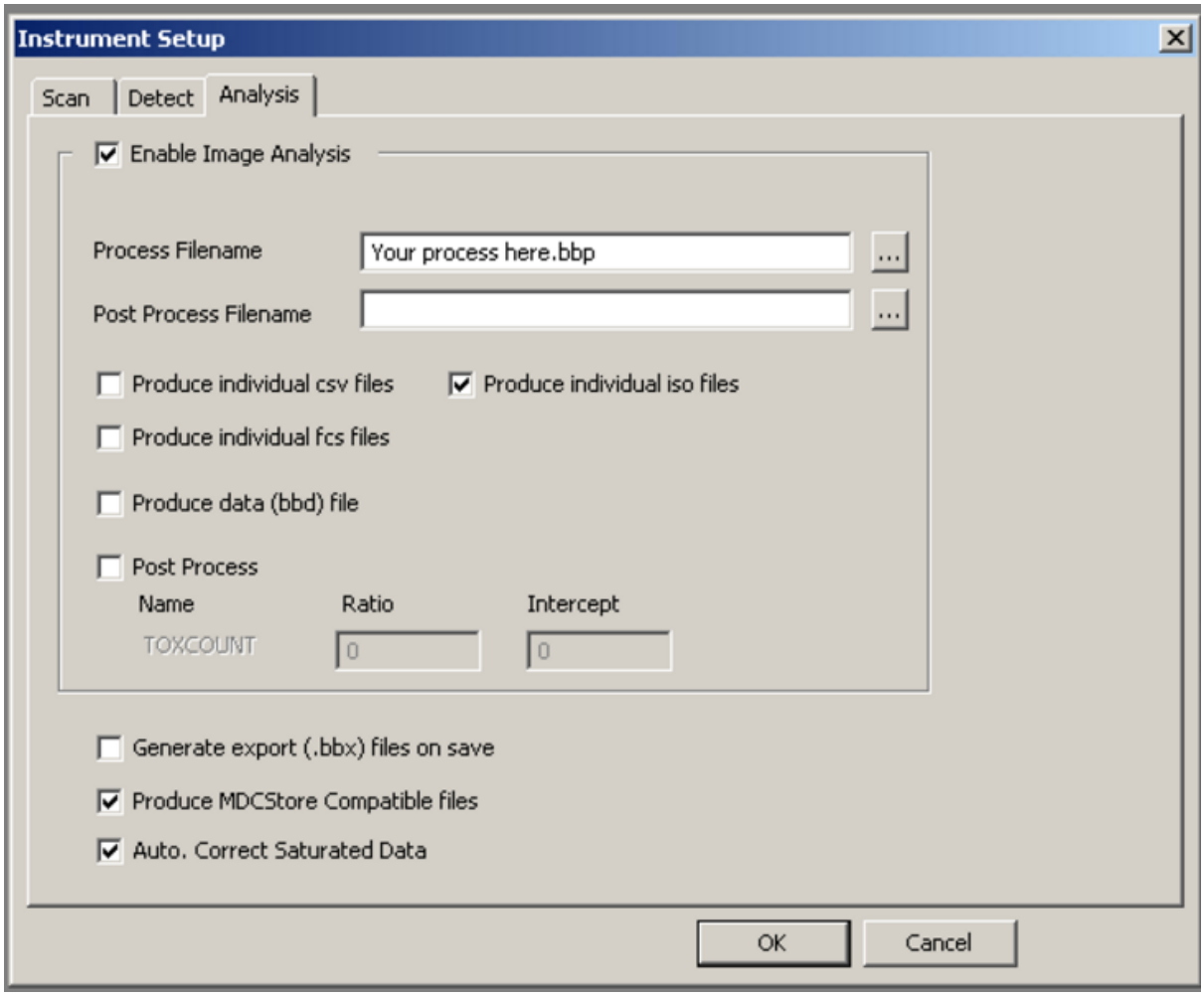

Figure T25.1 Instrument Setup for IsoCyte

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7. Analyze your experiment in IsoCyte.
8. Save your experiment in IsoCyte.

In the next section, we will import IsoCyte Data with images into FCS Express 1302.

34.26.2 Import IsoCyte Data with Images

After adjusting the export settings and analyzing your data in IsoCyte, you may proceed to import the data into FCS Express V4.

The IsoCyte files used for this section are located in the Tutorial Sample Data archive 590.

1. Select **Application Button→Edit Preferences→Data Loading→IsoCyte Options**.
2. Check the **Load cell images** check box.
3. Check the **Load as Plate** check box.
4. Click OK.

The **IsoCyte options** page of the **Data Loading** preferences should look like Figure T25.2.

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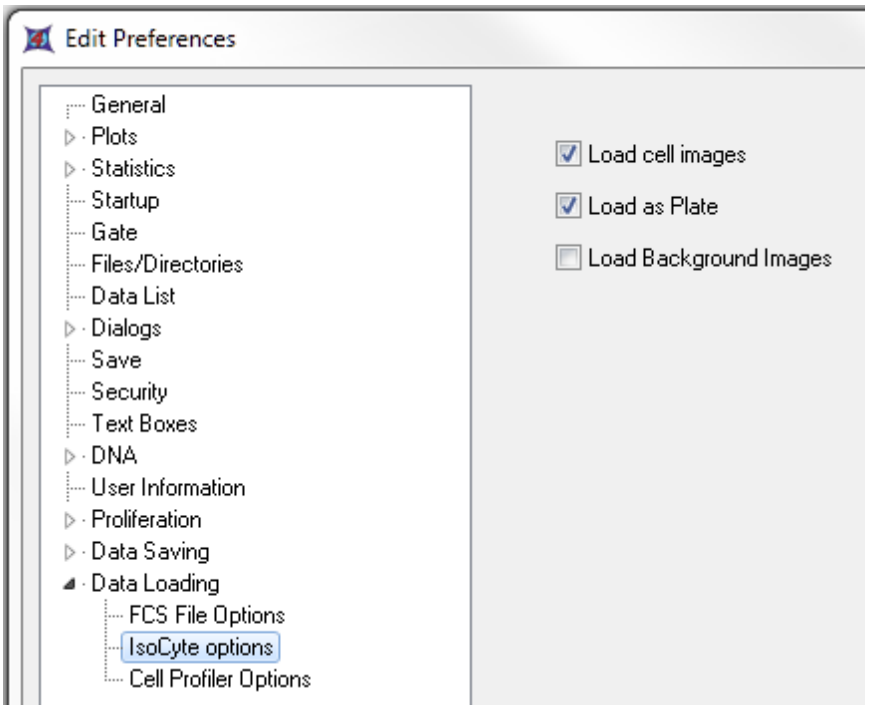

Figure T25.2 Setting up IsoCyte Data Loading Preferences in FCS Express

5. Select the **Insert→Other Plots→Heat Map** command (Figure T25.3).

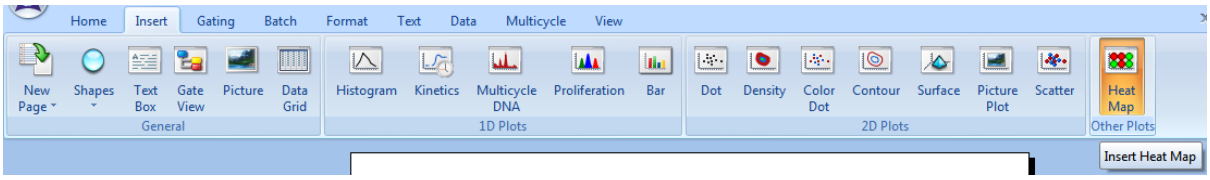

Figure T25.3 Inserting a Heat Map

6. Click anywhere on the layout.
7. Choose **Isocyte files (*.iso)** from the **files of type:** drop-down list in the **Select a Data File** dialog (Figure T25.4).

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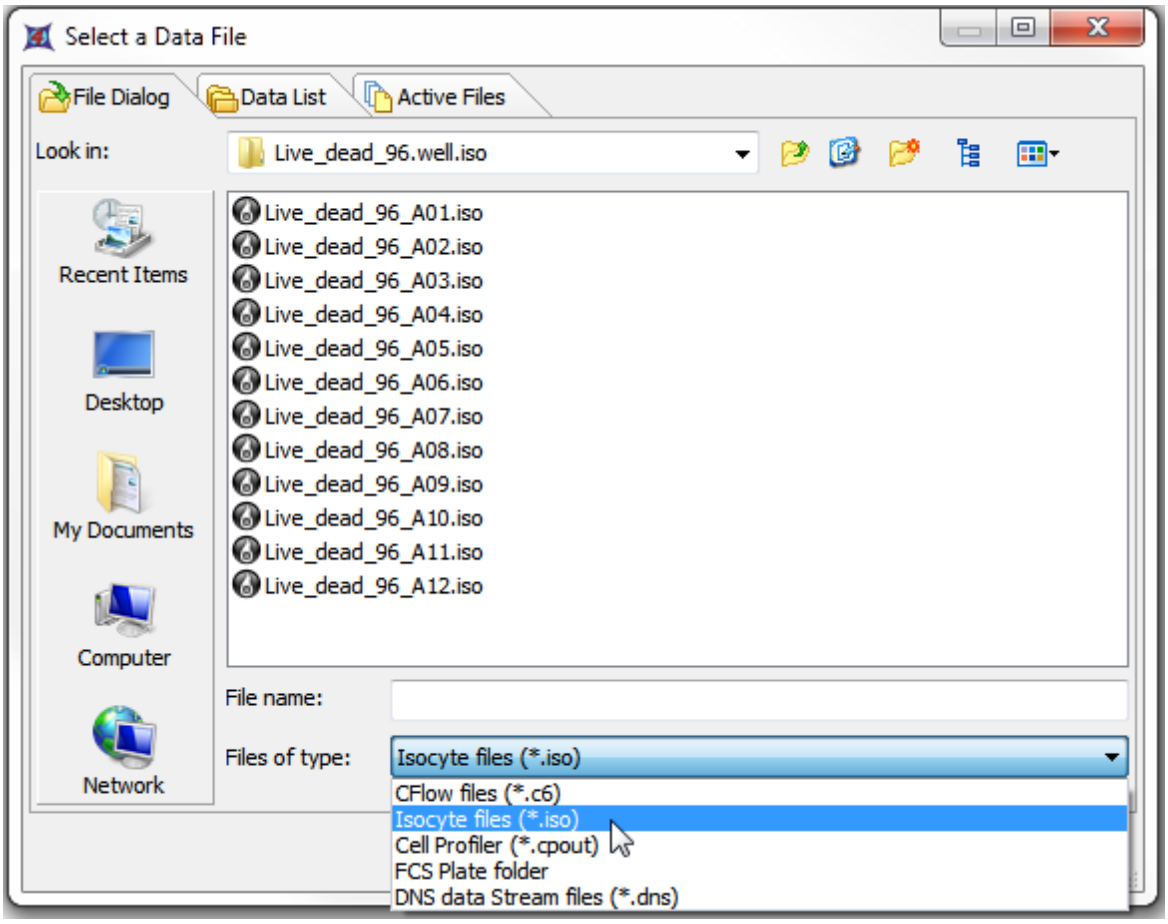

Figure T25.4 Choosing an IsoCyte file for a Heat Map

8. Navigate to the **Sample Data→Isocyte With Images→Live_dead_96.well.iso** folder.
9. Highlight the **Live_dead_96_A01.iso** file in the list.
10. Click **Open**.

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.

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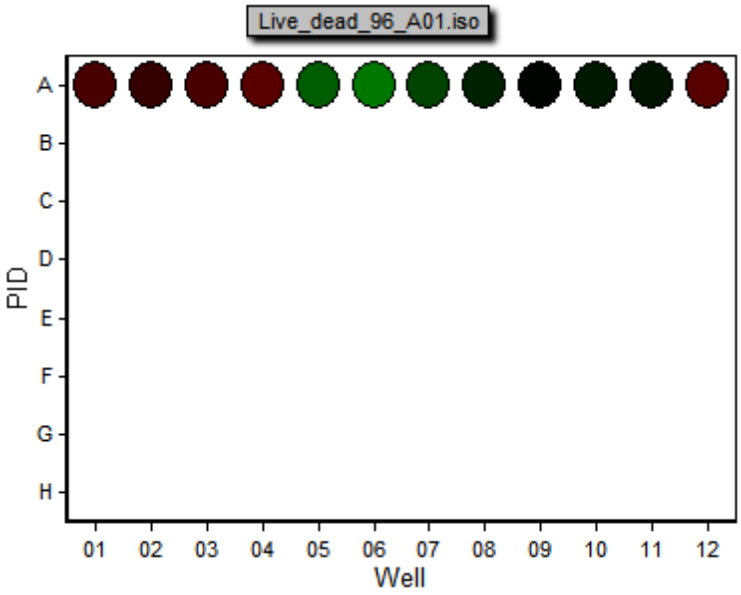

Figure T25.5 A Heat Map Generated from IsoCyte Data

To view the images associated with each well

11. Insert a **Picture Plot** by choosing **Insert→2D Plots→Picture Plot** (Figure T25.6).

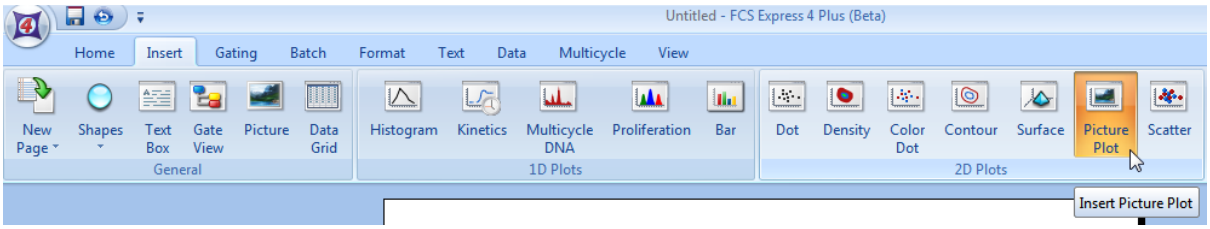

Figure T25.6 Insert a Picture Plot

12. Click anywhere on the layout.

A Picture Plot will appear on the layout (Figure T25.7). The picture plot is now displaying all of the images from the entire plate as an overlay. We will now create a well gate and apply it to the picture plot to display only the image associated with the well.

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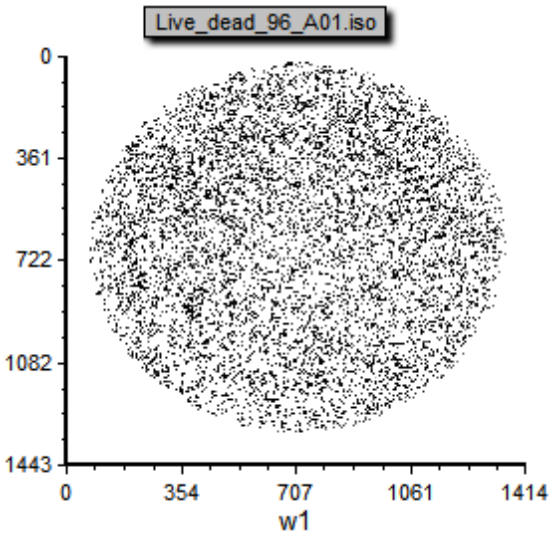

Figure T25.7 A Picture Plot with All Images/Cells from All Wells on the Same Plot

13. Create a Well Gate for well A6 on your **Heat Map** (Figure T25.8).

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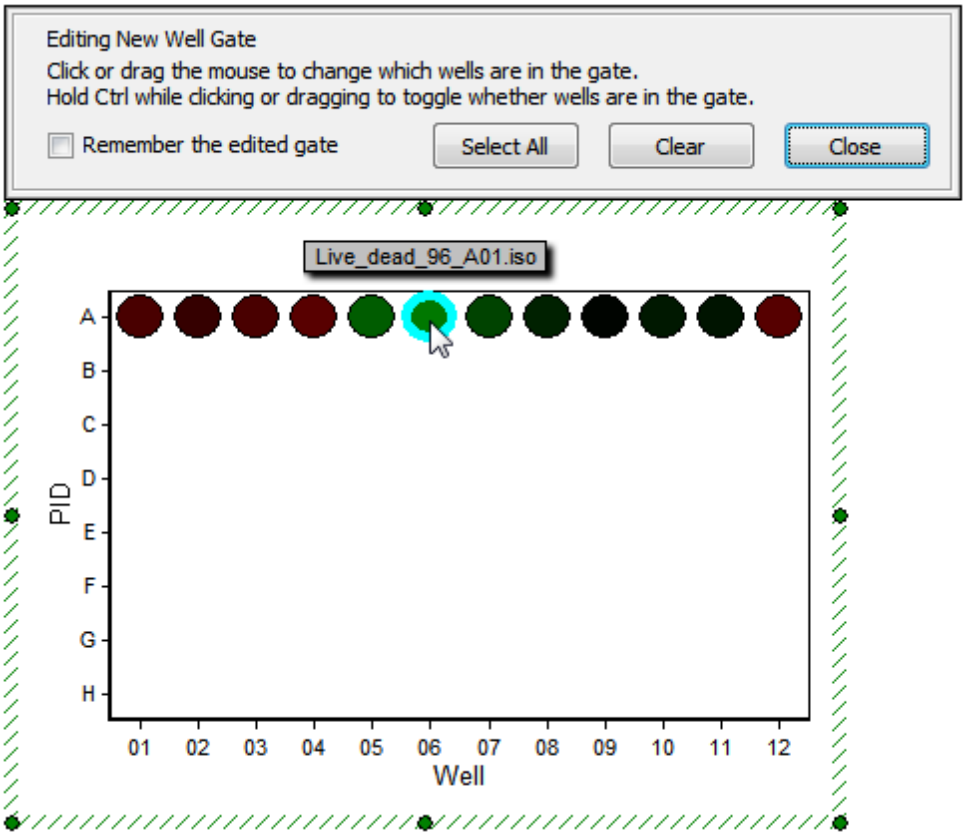

Figure T25.8 Gating a Heat Map

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 670.

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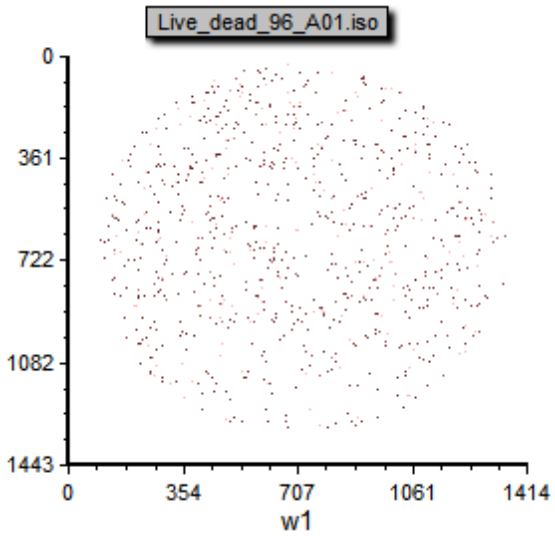

Figure T25.9 Picture Plot with Well Gate Applied

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 IsoCyte to export an experiment in numeric form (data only).

34.26.3 Export in Numeric Form (Data Only)

IsoCyte Setup – Saving Object Data

1. Select **Instrument Setup** from the main menu in the IsoCyte 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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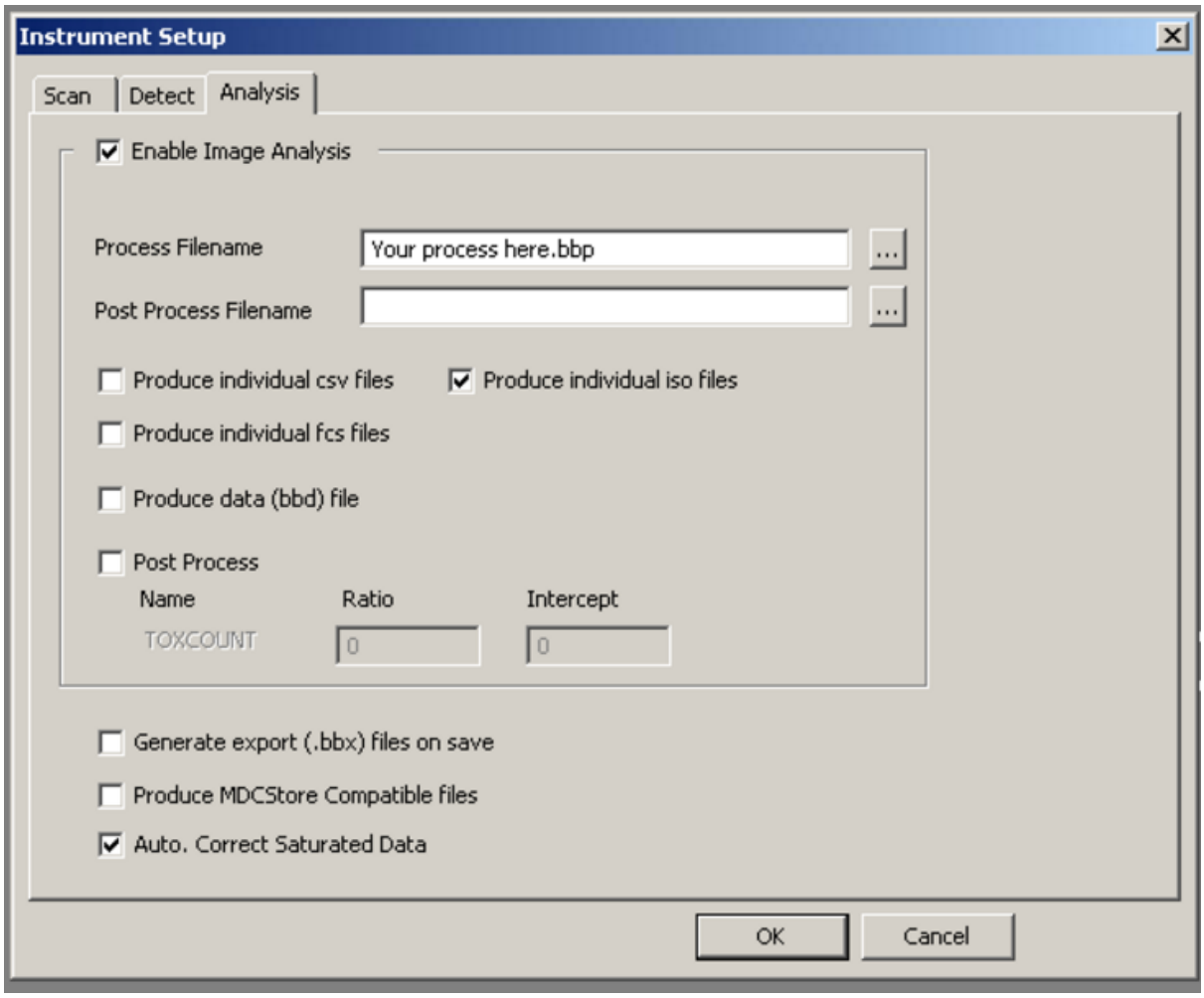

Figure T25.10 Instrument Setup for Data Only Exporting

5. Click **OK**.
6. Analyze your experiment in IsoCyte.
7. Save your experiment in IsoCyte.

Next, we will import IsoCyte data in numeric form into FCS Express 1309.

34.26.4 Import Numeric Form Data

After adjusting the export settings and analyzing in **IsoCyte** you may proceed to import the data into FCS Express V4.

The IsoCyte files used for this section are located in the Tutorial Sample Data

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archive 590.

1. Select **Application Button→Edit Preferences→Data Loading→IsoCyte Options**.
2. Unchecked the **Load cell images** check box.
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 IsoCyte data loading options should look like Figure T25.11 when you are done.

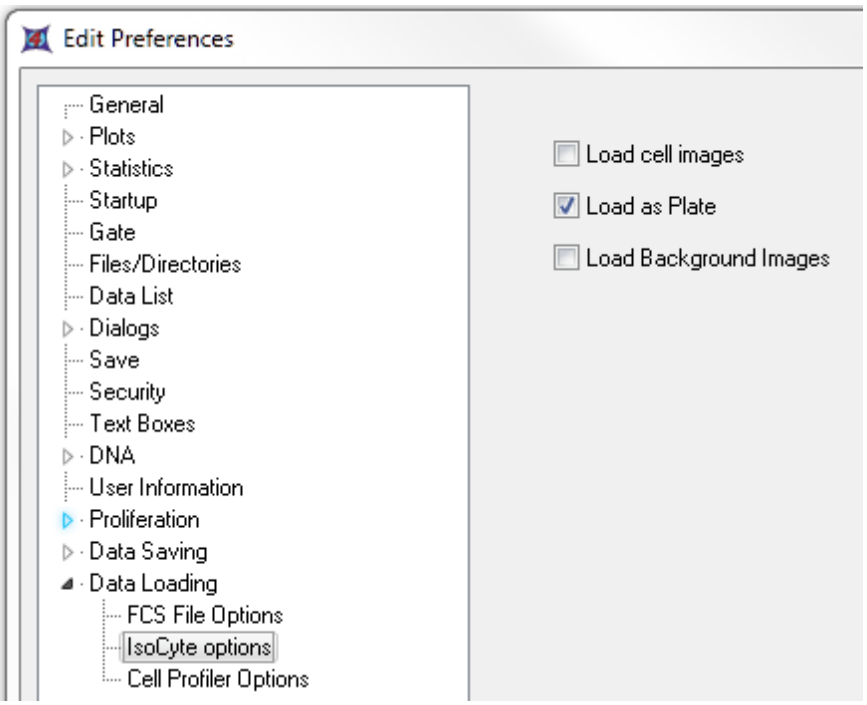

Figure T25.11 IsoCyte Data Loading Options for Numeric Data Only

4. Select the **Insert→Other Plots→Heat Map** command (Figure T25.12).

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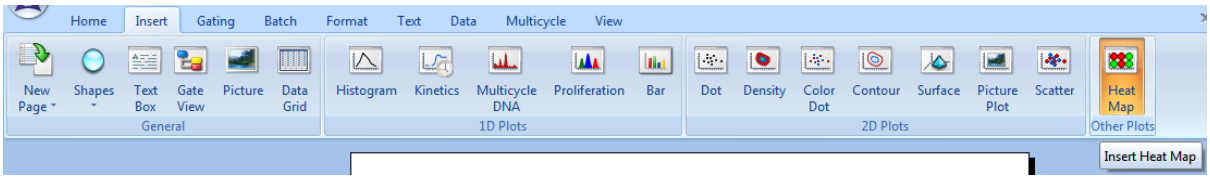

Figure T25.12 Insert a Heat Map

5. Click anywhere on the layout.
6. Choose **Isocyte files (*.iso)** from the **files of type:** drop-down list within the **Select a Data File** dialog (Figure T25.13).

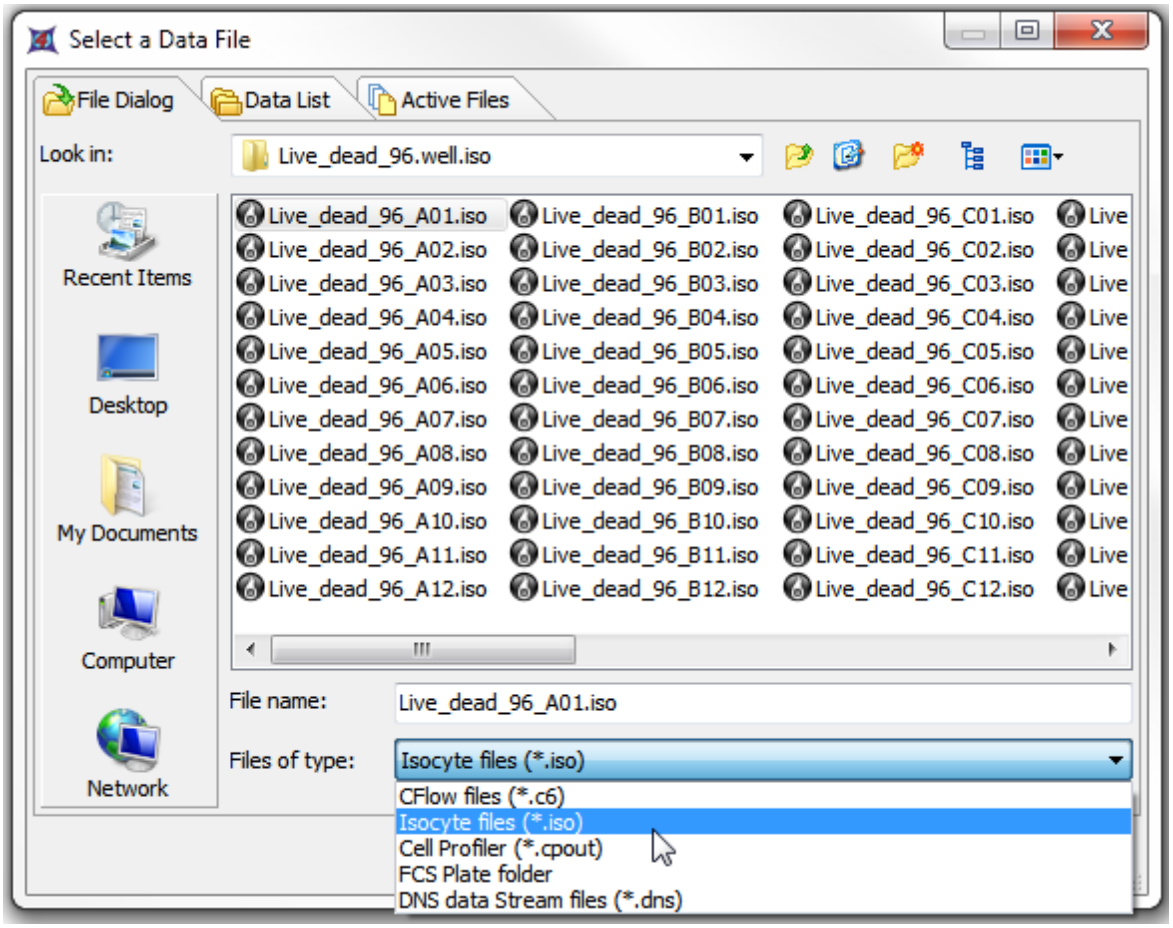

Figure T25.13 Choosing an IsoCyte File for a Heat Map

7. Navigate to the **Sample Data→Isocyte Data Without Images→Live_dead_96.well.iso** folder.
8. Highlight the **Live_dead_96_A01.iso file** in the list.

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9. Click **Open**.

A heat map containing your data set will appear indicating your import was successful.

To view the data associated with each well

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.

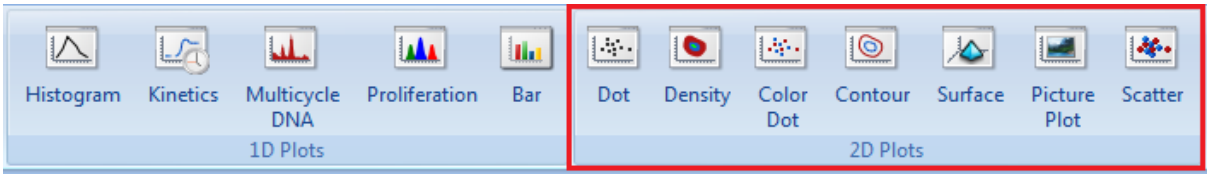

Figure T25.14 Insert a 2D Plot

11. Click anywhere on the layout.

A plot containing all of the events in the data set/**Heat Map** will appear (Figure T25.15).

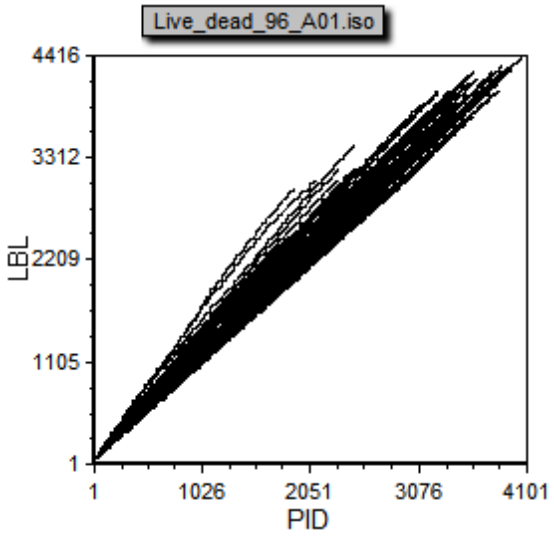

Figure T25.15 Dot Plot created from IsoCyte Data

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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.

13. Change the X-Axis parameter to IMEAN-3.

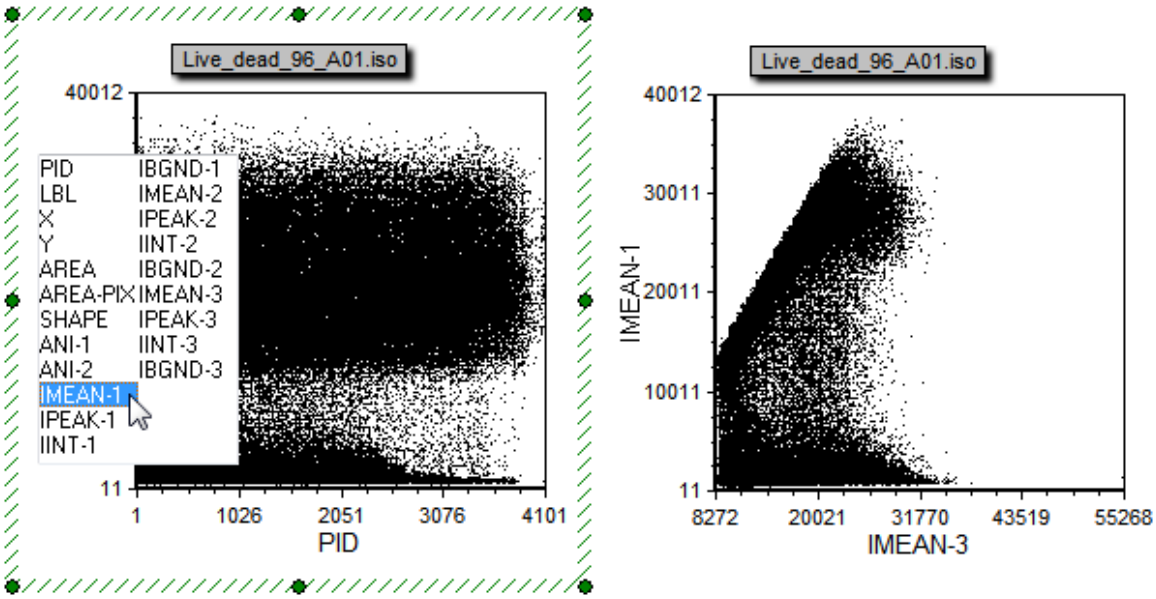

Figure T25.16 Changing the Plot Axes

14. Create a Well Gate for well C3 on your **Heat Map** (Figure T25.17).

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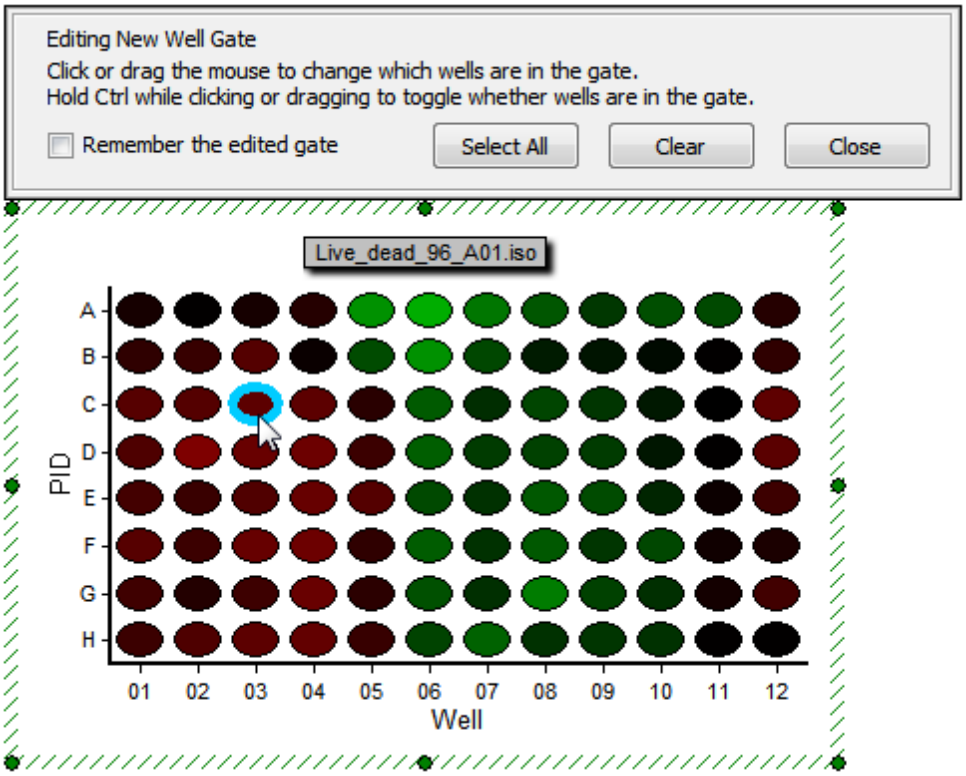

Figure T25.17 Create a Well Gate on the Heat Map

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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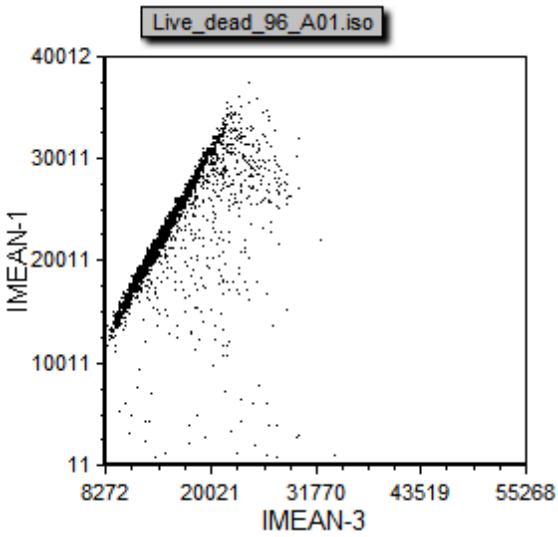

Figure T25.18 Dot Plot Gated by Well

34.27 Picture Plots

FCS Express now supports picture plots, which allow the user to import images for analysis. Picture plots display the imported image and allow the user to gate directly on the image to perform any downstream analysis available in FCS Express. Individual cells identified in a plot can further be isolated in a cell gallery, allowing the user to perform cell by 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.

34.27.1 Insert and Format Picture Plots

In the course of this section, you will learn how to:

- Define settings for viewing picture plots

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- 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.
3. Add pictureplotsample1.dns and pictureplotsample2.dns to the **Data List** from the Tutorial Sample Data archive 590.
4. Drag and drop pictureplotsample1.dns from the **Data List** to the layout.
5. Check the box for **Picture Plot** and ensure no other boxes are checked (Figure T26.1).

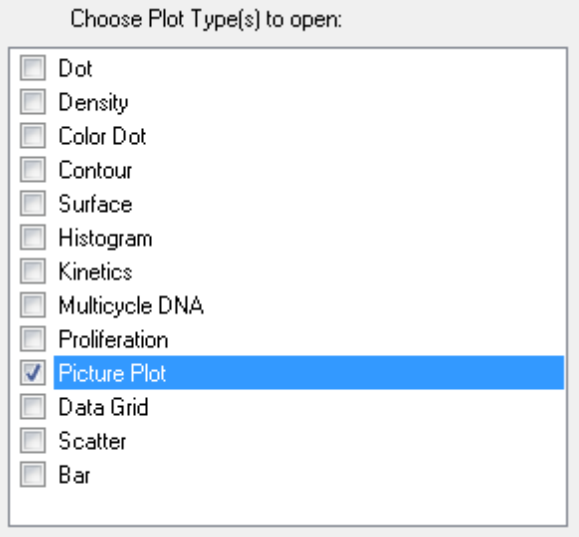

Figure T26.1 Choosing to Insert a Picture Plot

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

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not the background.

Note: The labels on the axes reflect the pixel coordinates. You can turn these off by changing the axis formatting options.

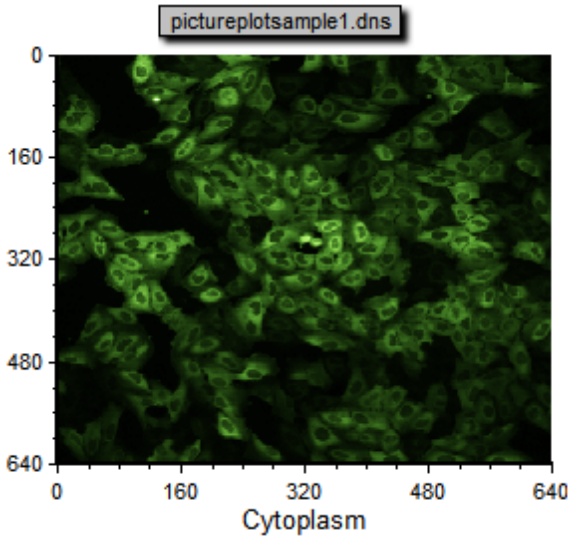

Figure T26.2 Picture Plot for pictureplotsample1.dns Inserted in the Layout

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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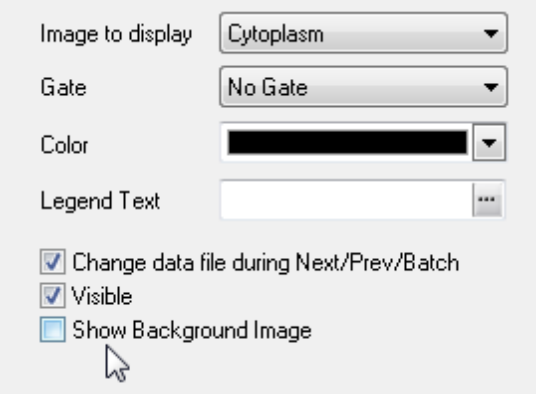

Figure T26.3 Deselecting the Show Background Images Option

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).

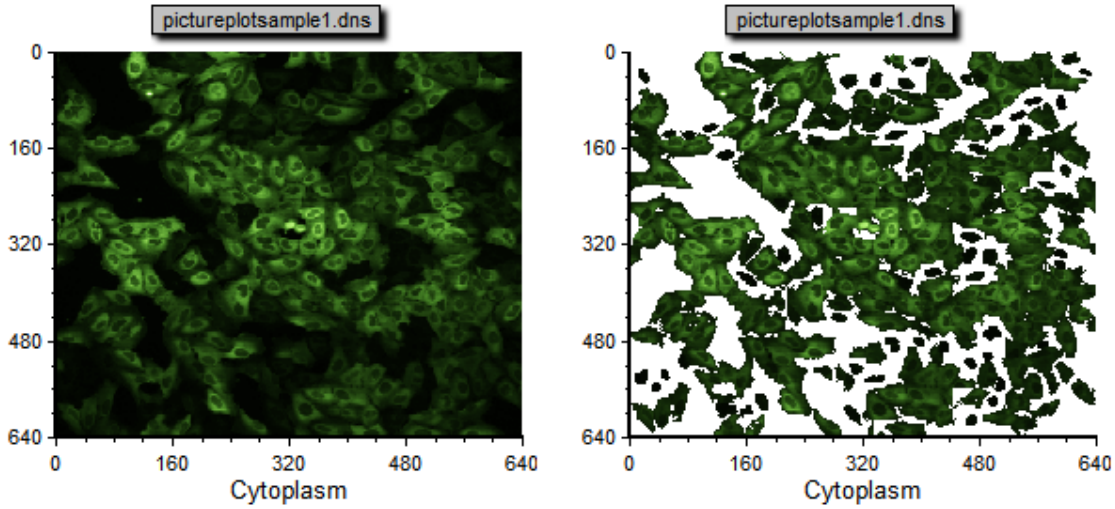

Figure T26.4 Before (Left) and After (Right) Removing the Background Image

We will now look at the other channel associated with the image and create a two channel merged image.

12. Click **Cancel** to revert back to the image with background.

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13. Copy and paste the picture plot anywhere in the layout.

14. Click and hold on the X-Axis label of the new plot (Figure T26.5).

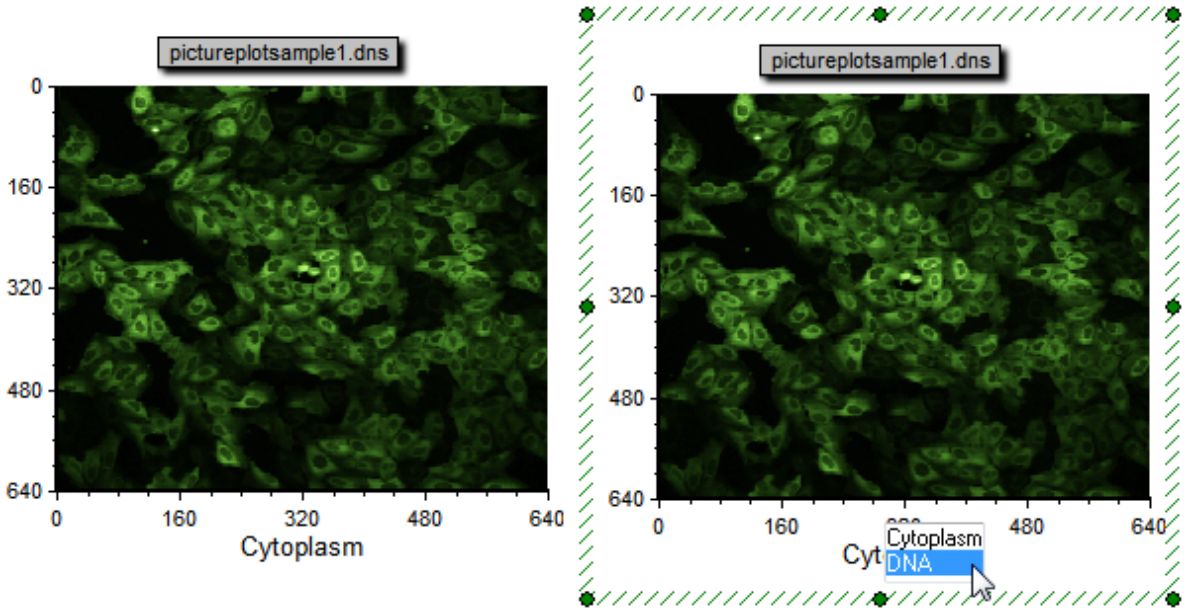

Figure T26.5 Choosing the DNA Imaging Channel

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.

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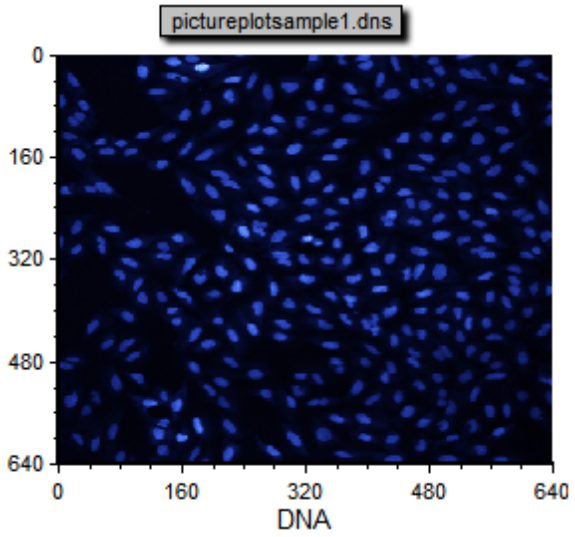

Figure T26.6 The DNA Image Channel Associated with pictureplotsample1.dns

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.

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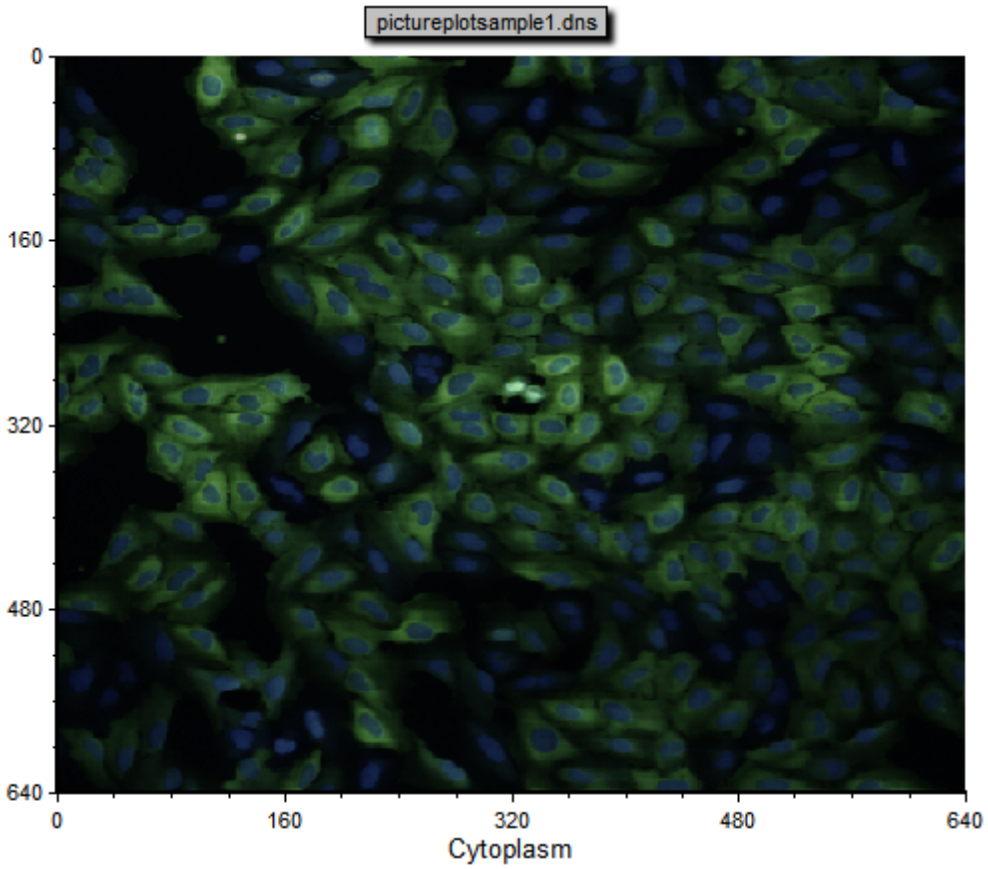

Figure T26.7 Picture Plot Overlay Displaying the Cytoplasmic and DNA Channels as a Merged Image

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.

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.

34.27.2 Gating with Picture Plots

In the course of this section, you will learn how to:

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- 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 650.

1. Open a new layout.
2. Choose **Data→Organize Data Sets→Data List**.
3. Add pictureplotsample1.dns to the **Data List** from the *FCS Express Sample Data* directory.
4. Drag and drop pictureplotsample1.dns from the **Data List** to the layout.
5. Check the boxes for **Picture Plot** and **Color Dot** and ensure no other boxes are checked (Figure T26.8).

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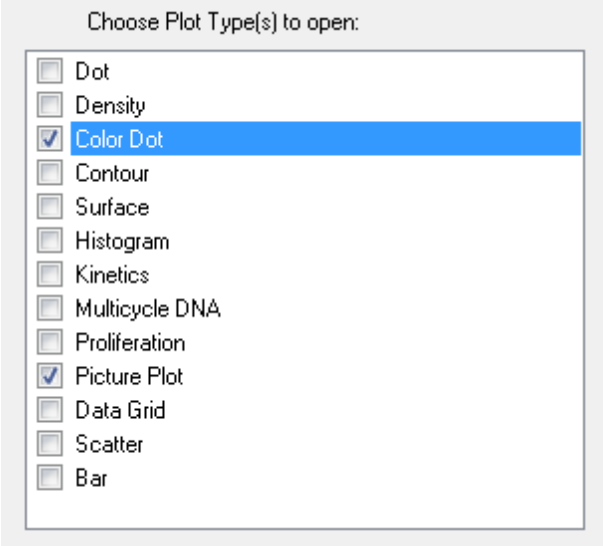

Figure T26.8 Choosing to Insert a Picture Plot and Color Dot Plot

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.

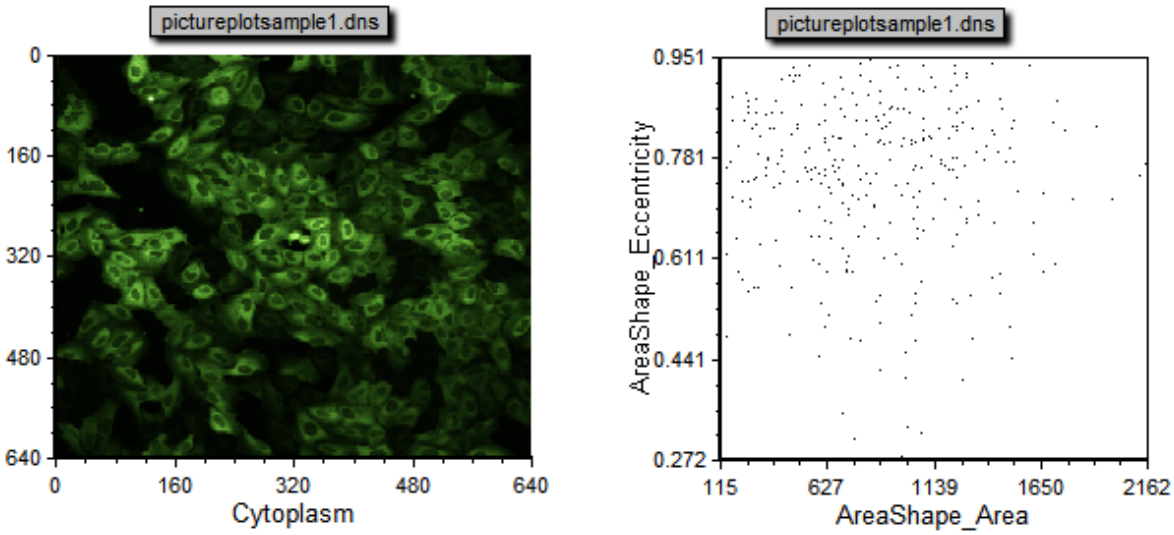

Figure T26.9 Arrangement of the Picture Plot and Color Dot Plot on the Layout

7. Create a rectangle gate on the picture plot similar to the one in Figure T26.10 and name it Picture Plot Gate 1.

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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.

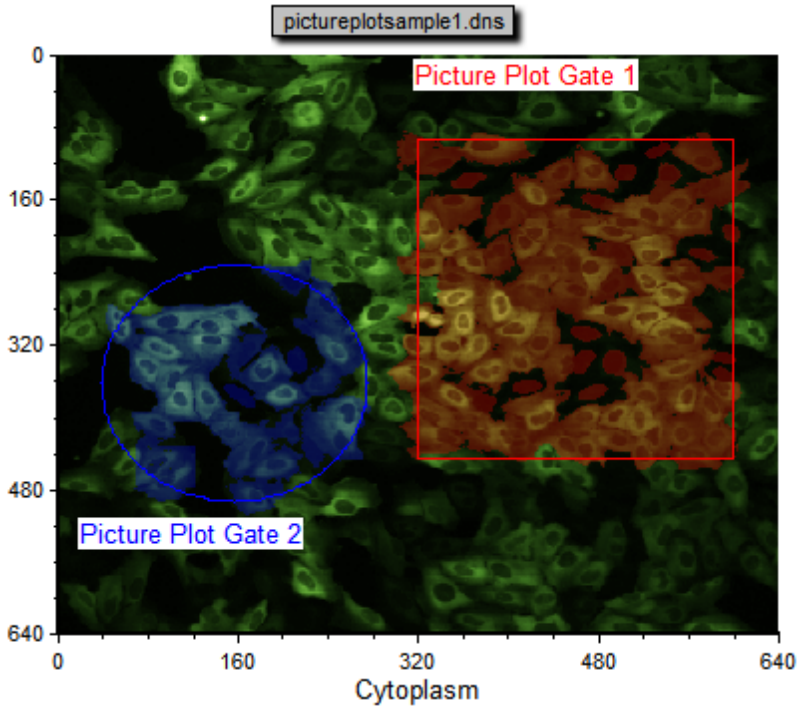

Figure T26.10 Creating Gates on the Picture Plot

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 10.
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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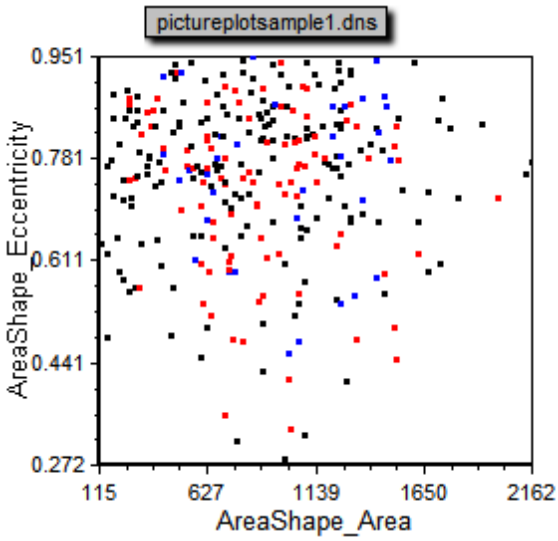

Figure T26.11 Color Dot Plot with Dots Enlarged

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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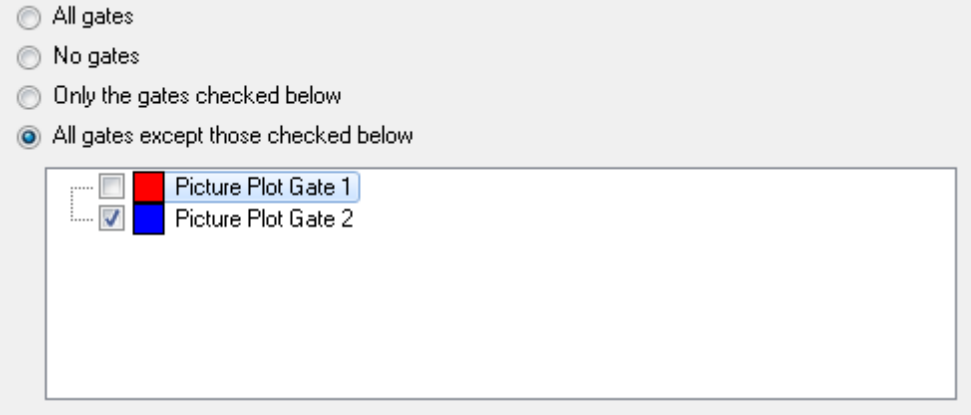

Figure T26.12 Choosing to Display All Gates Except Picture Plot Gate 2

19. Click **OK**.

The picture plot will now look like Figure T26.13, only highlighting the cells found within Picture Plot Gate 1.

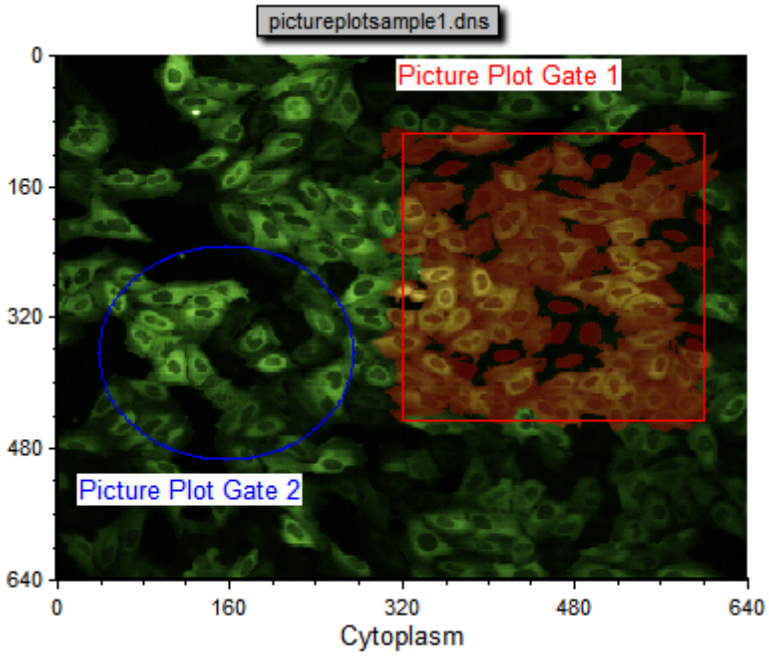

Figure T26.13 Picture Plot Highlighting Only Cells Found Within Picture Plot Gate 1

We will now gate the color dot plot based on the picture plot gates.

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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.

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).

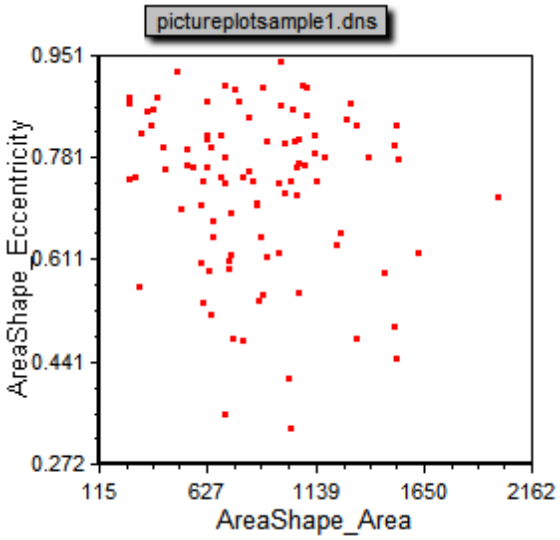

Figure 27.14 Color Dot Plot Displaying Cells from Picture Plot Gate 1

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.

23. Create a rectangle gate on the color dot plot similar to Figure T26.15 and name it Dot Plot Gate.

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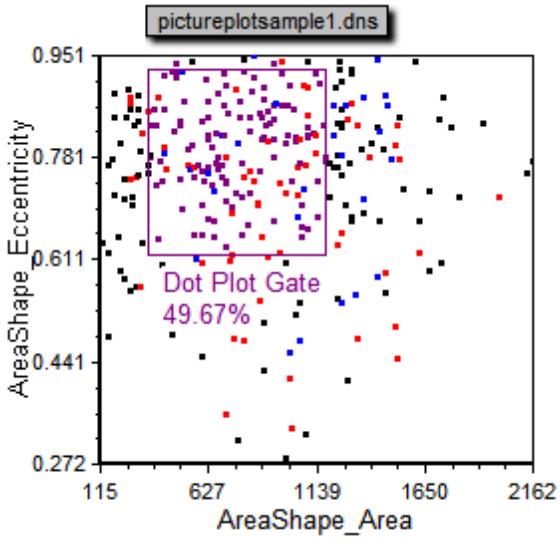

Figure T26.15 Applying a Gate to the Dot Plot

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.

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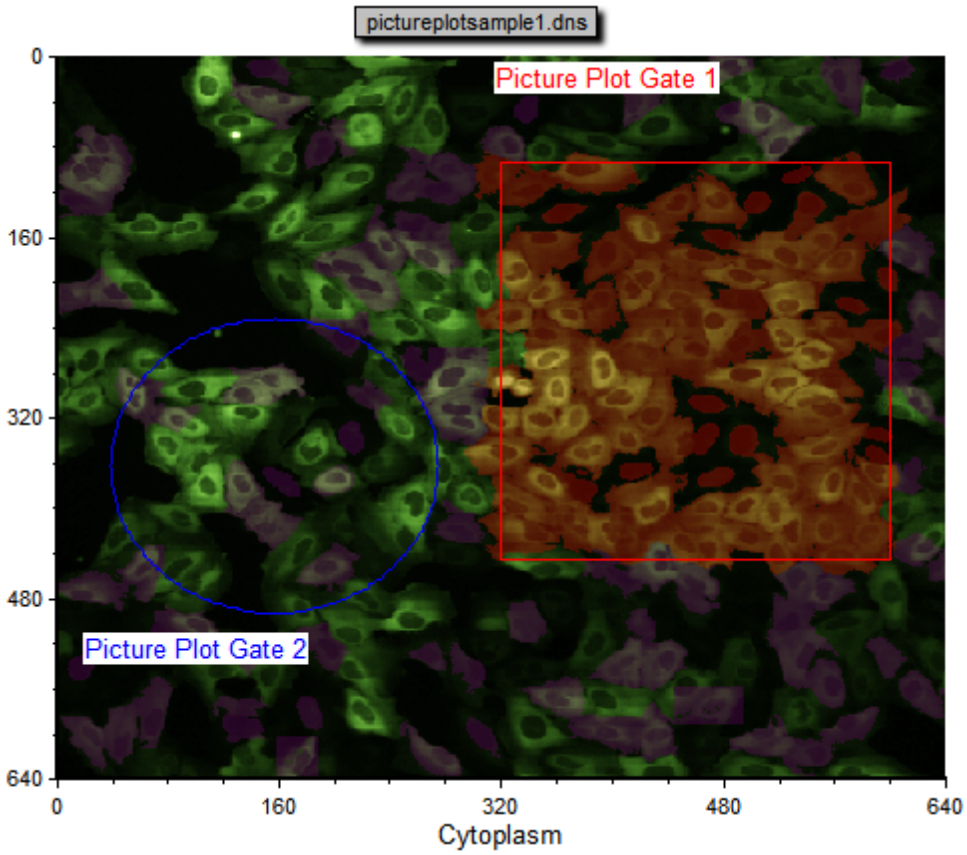

Figure T26.16 Picture Plot Displaying the Dot Plot Gated Cells in Purple

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**.
28. Click **OK**.
29. Navigate to **Gating→Create Gates→Current Gate** and choose **Dot Plot Gate** from the **Current Gate** drop-down list.

The picture plot will now look like 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.

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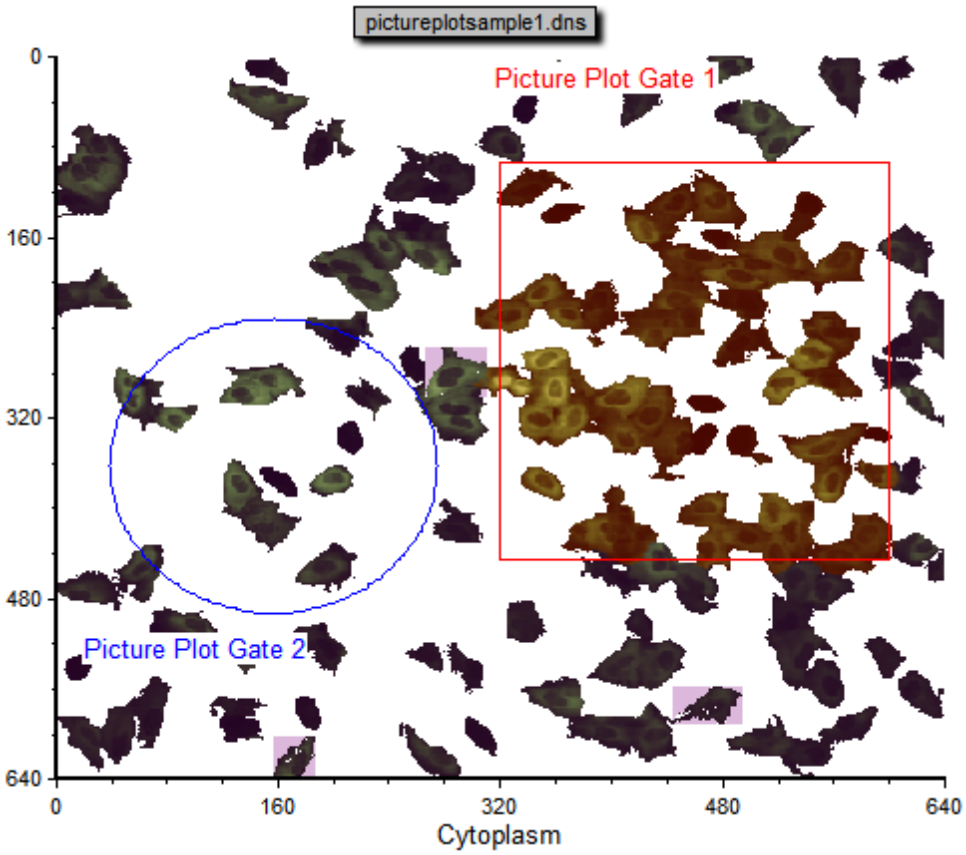

Figure T26.17 Picture Plot with Dot Plot Gate Applied

Next, we will use data grids for image analysis 1330.

34.27.3 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

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- Create gates in a data grid/cell gallery

1. Open a new layout.
2. Choose the **Data→Organize Data Sets→Data List** command.
3. Add pictureplotsample1.dns file to the **Data List** from the *FCS Express Sample Data* directory.
4. Drag and drop pictureplotsample1.dns from the **Data List** to the layout.
5. Check the boxes for **Data Grid**, **Picture Plot,** and **Color Dot** and ensure no other boxes are checked (Figure T26.18).

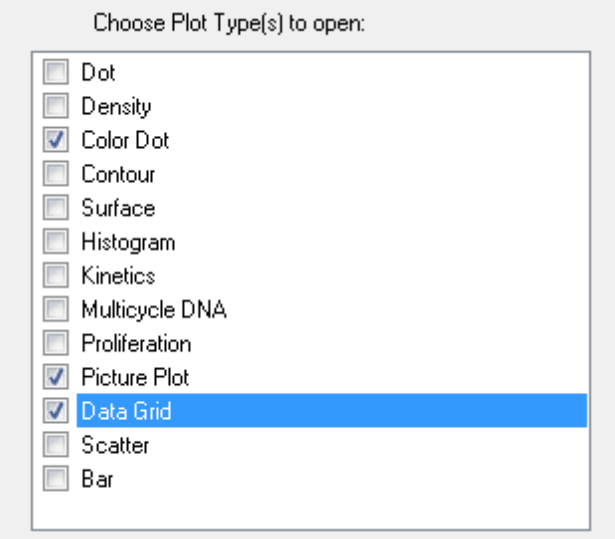

Figure T26.18 Inserting a Data Grid and Two Plots

6. 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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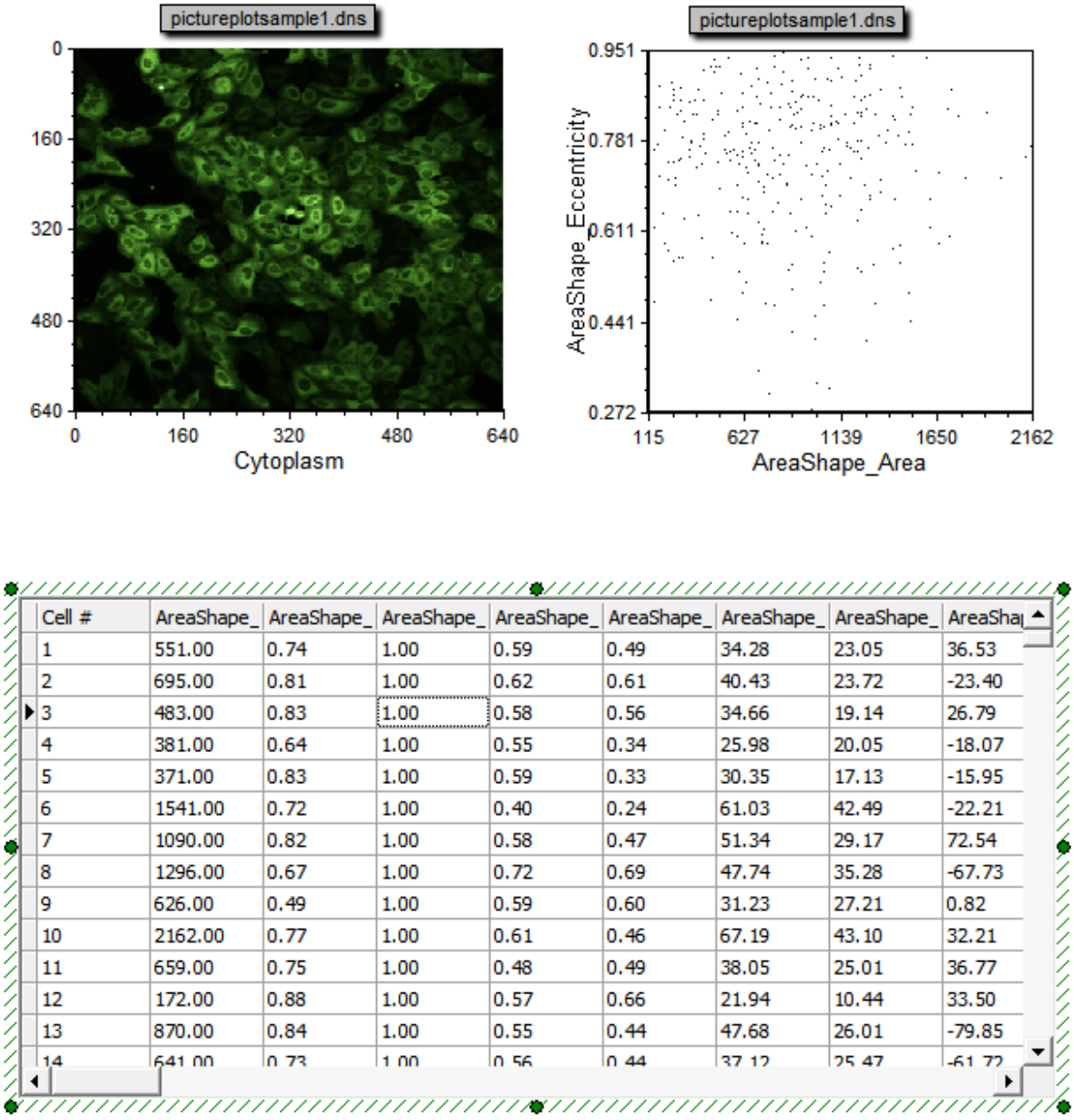

Cell #	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaShape_	AreaSha
1	551.00	0.74	1.00	0.59	0.49	34.28	23.05	36.53
2	695.00	0.81	1.00	0.62	0.61	40.43	23.72	-23.40
3	483.00	0.83	1.00	0.58	0.56	34.66	19.14	26.79
4	381.00	0.64	1.00	0.55	0.34	25.98	20.05	-18.07
5	371.00	0.83	1.00	0.59	0.33	30.35	17.13	-15.95
6	1541.00	0.72	1.00	0.40	0.24	61.03	42.49	-22.21
7	1090.00	0.82	1.00	0.58	0.47	51.34	29.17	72.54
8	1296.00	0.67	1.00	0.72	0.69	47.74	35.28	-67.73
9	626.00	0.49	1.00	0.59	0.60	31.23	27.21	0.82
10	2162.00	0.77	1.00	0.61	0.46	67.19	43.10	32.21
11	659.00	0.75	1.00	0.48	0.49	38.05	25.01	36.77
12	172.00	0.88	1.00	0.57	0.66	21.94	10.44	33.50
13	870.00	0.84	1.00	0.55	0.44	47.68	26.01	-79.85
14	641.00	0.73	1.00	0.56	0.44	37.12	25.47	-61.72

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.

7. Right-click on the data grid.
8. Choose **Format** from the pop-up menu.

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9. Choose the **Parameters to Display** category.
10. Click the **Only the items check below** radio button (Figure T26.20).
11. 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

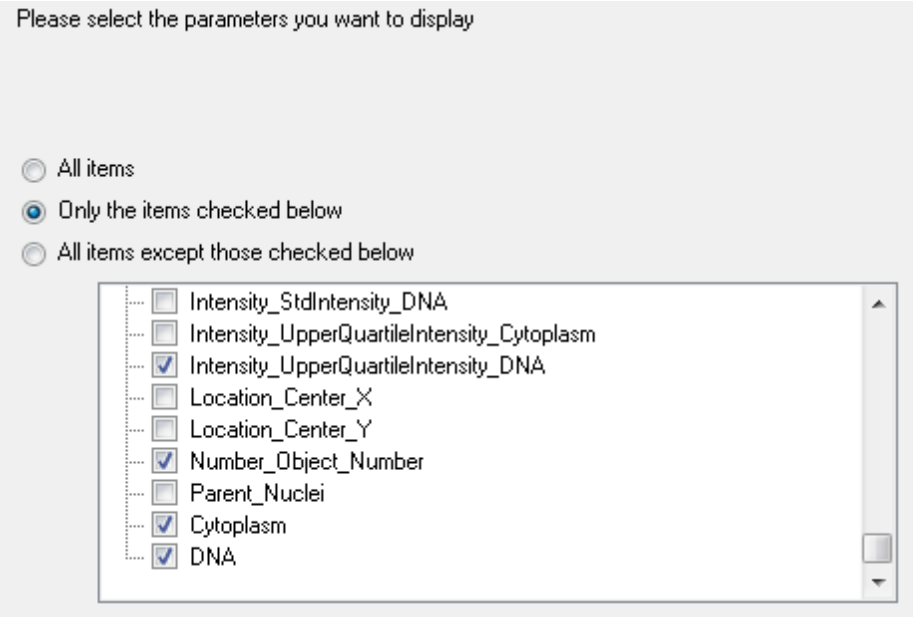

Figure T26.20 Formatting the Parameters to Display for the Data Grid

12. Choose the **Gates to Display Category**.
13. Click on the **All gates** radio button.
14. 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.

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Cell #	Gates	Intensity_Ur	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		0.06	7.00		
8		0.10	8.00		
9		0.18	9.00		
10		0.07	10.00		
11		0.14	11.00		
12		0.24	12.00		
13		0.14	13.00		
14		0.10	14.00		
15		0.09	15.00		

Figure T26.21 Data Grid Displaying the Chosen Parameters, Individual Cell Images, and a Column for Gates

15. Create a gate on the dot plot similar to the one in Figure T26.22 and name it "Dot Plot Gate".

16. Create a gate on the picture plot similar to the one in Figure T26.22 and name it "Picture Plot Gate".

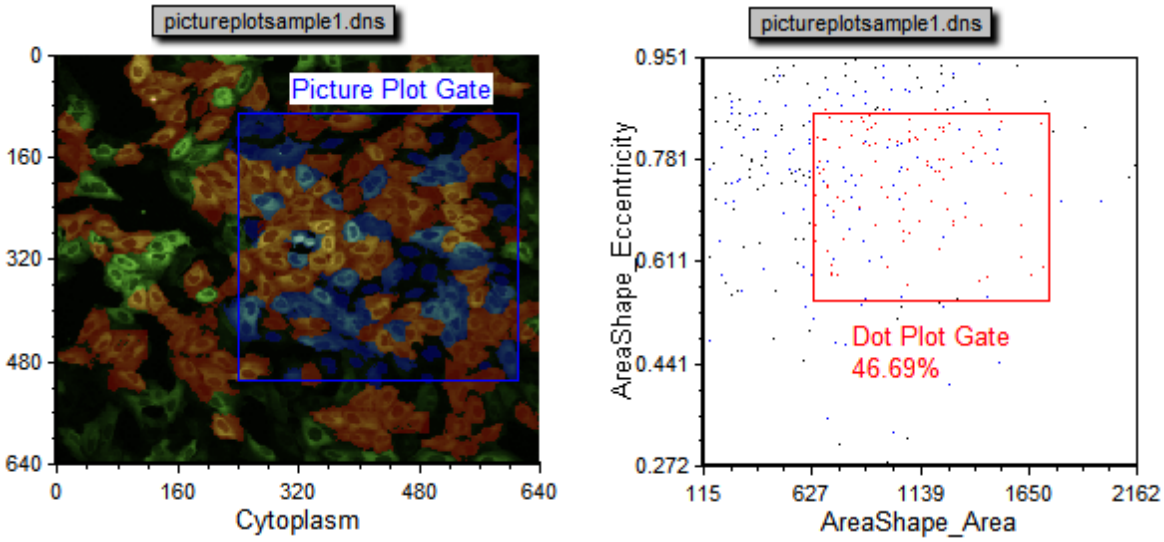

Figure T26.22 Examples of Gates to Create on the Picture and Dot Plots

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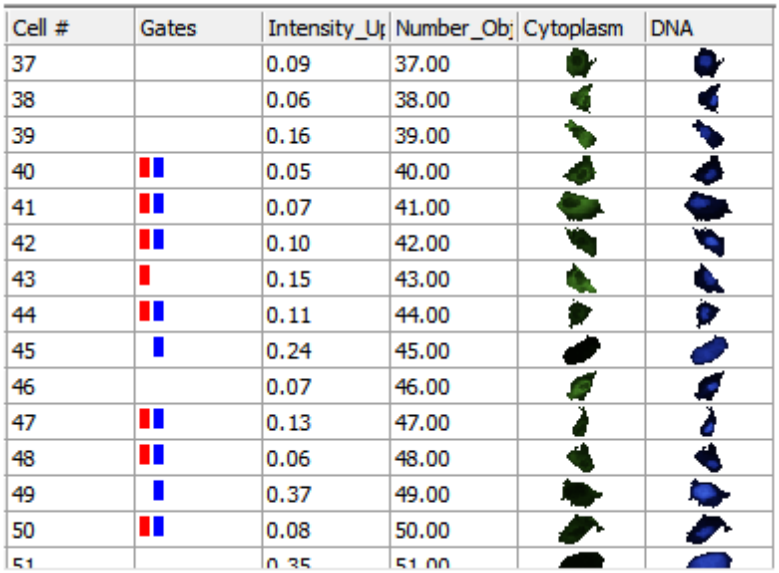
Scroll down through the data grid and notice that it is now displaying colored rectangular boxes in the gates column (Figure T26.23). These boxes represent which gates the individual cells fall within. If a cell falls into multiple gates, then multiple colored bars will appear next to the cells in the "Gates" column. If the "Gates" column is empty, then that cell does not fall into any gates that are currently being displayed.



Cell #	Gates	Intensity_Ur	Number_Ob	Cytoplasm	DNA
37		0.09	37.00		
38		0.06	38.00		
39		0.16	39.00		
40		0.05	40.00		
41		0.07	41.00		
42		0.10	42.00		
43		0.15	43.00		
44		0.11	44.00		
45		0.24	45.00		
46		0.07	46.00		
47		0.13	47.00		
48		0.06	48.00		
49		0.37	49.00		
50		0.08	50.00		
51		0.35	51.00		

Figure T26.23 Data Grid Displaying Dot Plot and Picture Plot Gates as Colored Rectangular Boxes

We will now apply the dot plot and picture plot gates to the data grid to view the individual cells that fall within these gates.

17. Click on the data grid to select it.

18. Navigate to **Gating→Create Gates→Current Gate** and choose **Dot Plot Gate** from the **Current Gate** drop-down list.

19. Adjust the dot plot gate to select fewer cells as in Figure T26.24.

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.

20. Click on the data grid to select it.

21. Navigate to **Gating→Create Gates→Current Gate** and choose **Picture Plot Gate** from the **Current Gate** drop-down list.

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22. Adjust the picture plot gate to select fewer cells as in Figure T26.24.

Notice how the cell gallery updates to display only the events found within the chosen picture plot gate. This is an example of "drilling down" to cells of interest.

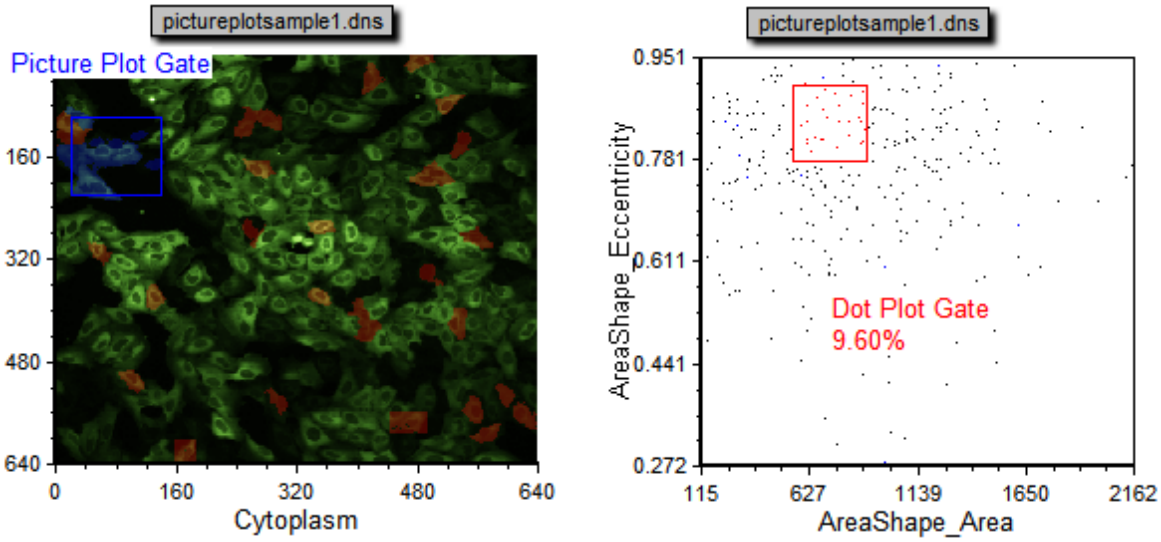

Figure T26.24 Adjusting the Dot Plot and Picture Plot Gates to Select Fewer Events

We will now create a gate directly on the data grid to display those events in the picture plot and scatter plot.

23. Click on the data grid to select it.

24. Navigate to **Gating→Create Gates→Current Gate** and choose **No Gate** from the **Current Gate** drop-down list.

25. Right-click on the data grid.

26. Choose **Create Gate** from the pop-up menu. A Range Gate selection area will appear as in Figure T26.25.

27. Click on **Range**.

28. Select **Add Range to Gate...** from the drop-down list.

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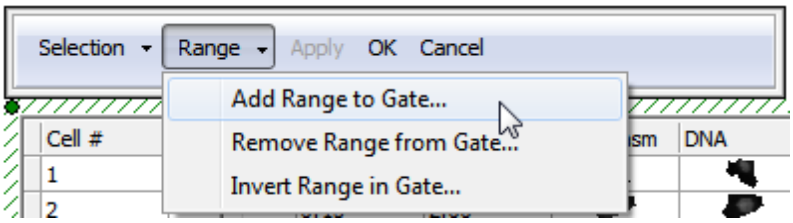

Figure T26.25 Range Gate Selection

29. Enter the range as **From** 1 **To** 100 (Figure T26.26).

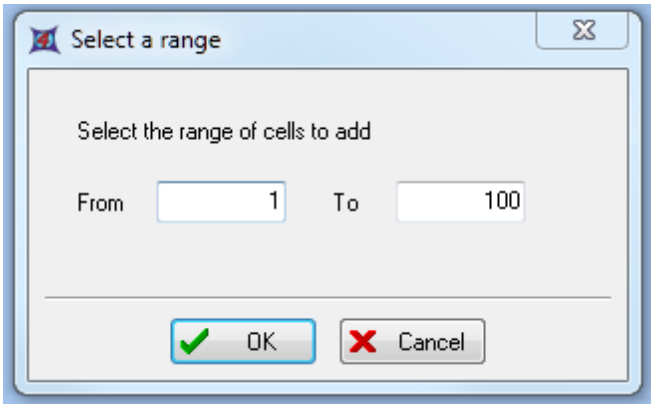

Figure T26.26 Select a Range Dialog

30. Click **OK**.
31. Click on **Range**.
32. Select **Add Range to Gate...** from the drop-down list.
33. Enter the range as **From** 180 **To** 250.
34. Click **OK**.
35. Click **OK** in the Range Gate selection window.
36. Name the new gate Range Gate.
37. Click **OK**.

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 T26.27).

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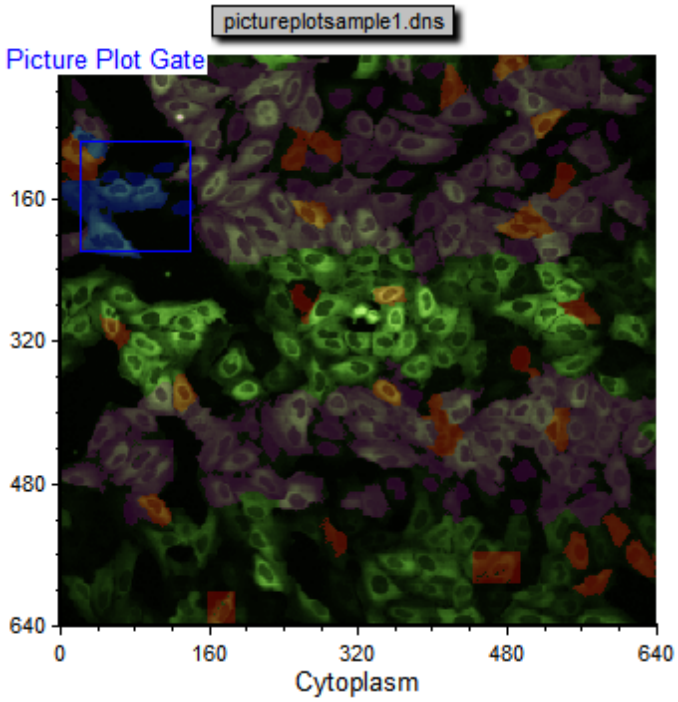

Figure T26.27 Picture Plot Displaying the Eevents in the Range Gate as Purple Highlighted Cells

Individual cells may also be selected in a range.

38. Right-click on the data grid.
39. Choose **Create Gate** from the pop-up menu.
40. Select cells 100 through 110 in the data grid by checking the boxes in the **New Gate** column (Figure T26.28).

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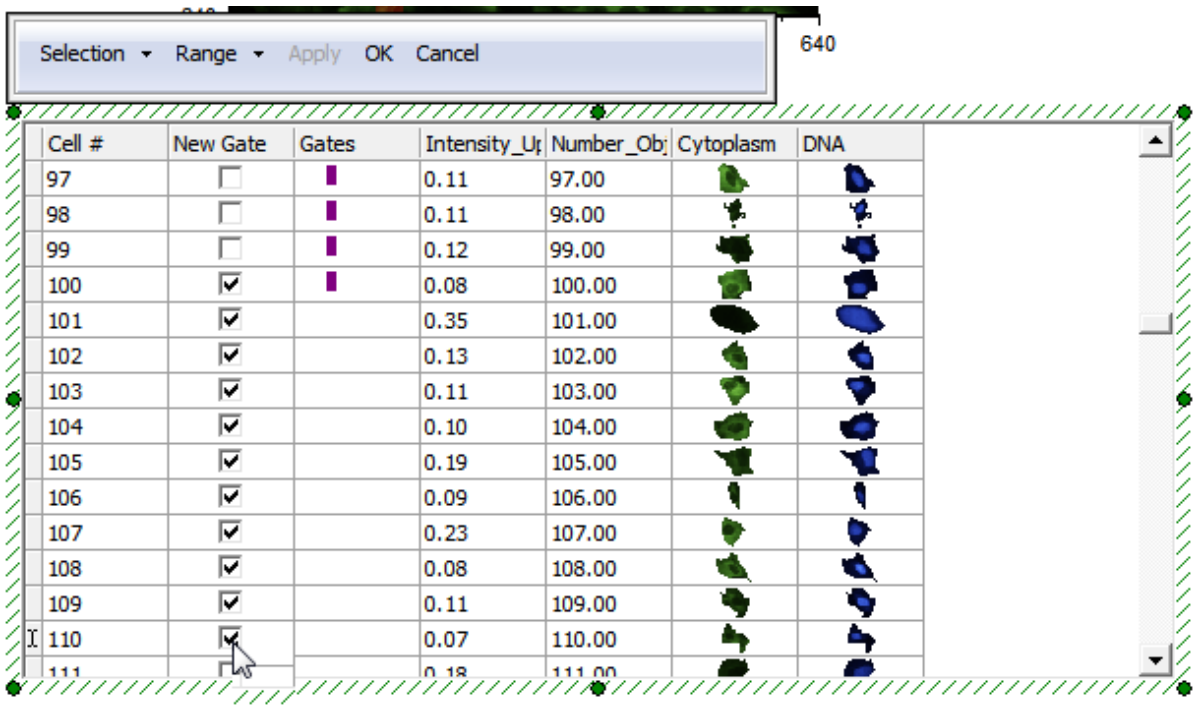

Figure T26.28 Selecting Individual Cells for Gating by Checking the Boxes in the New Gate Column

41. Click **OK** in the Range Gate selection area.
42. Name the gate "Selected Cells".
43. Click **OK**.

A new range gate will be created for these cells (Figure T26.29).

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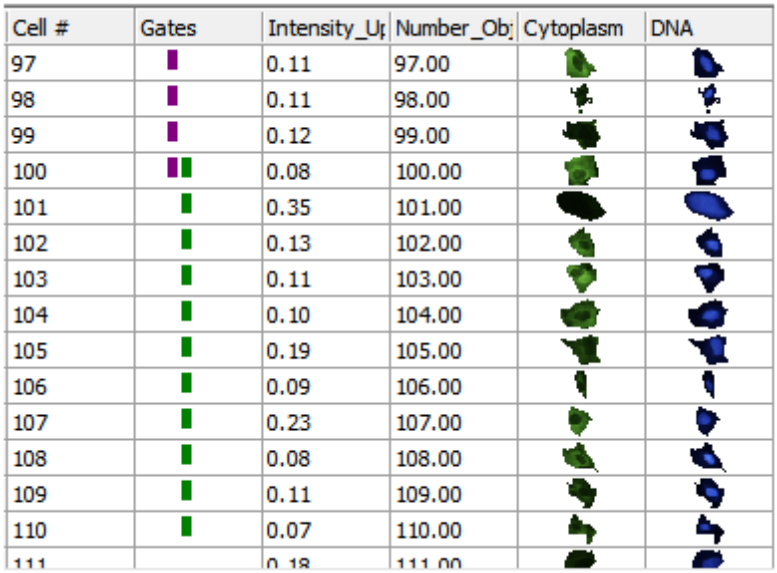

Cell #	Gates	Intensity_U	Number_Ob	Cytoplasm	DNA
97		0.11	97.00		
98		0.11	98.00		
99		0.12	99.00		
100		0.08	100.00		
101		0.35	101.00		
102		0.13	102.00		
103		0.11	103.00		
104		0.10	104.00		
105		0.19	105.00		
106		0.09	106.00		
107		0.23	107.00		
108		0.08	108.00		
109		0.11	109.00		
110		0.07	110.00		
111		0.18	111.00		

Figure T26.29 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.

44. Right-click on the picture plot.
45. Choose **Format** from the pop-up menu.
46. Choose the **Overlays** category.
47. Uncheck the **Show Background Image** box.
48. Click **OK**.
49. Right-click on the color dot plot.
50. C hoose **Format** from the pop-up menu.
51. Choose the **Overlays** category.
52. Change the **Dot size** to 10.
53. Click **OK**.

We will now gate both plots on the Selected Cells gate to view only these events in each plot.

54. Select the dot plot and picture plot.

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55. Navigate to **Gating→Create Gates→Current Gate** and choose **Selected Cells** from the **Current Gate** drop-down list.

The dot plot and picture plot will now display only cells that fall within the manually checked range boxes (Figure T26.30). A new range or individual cells may be added to the gate at any time.

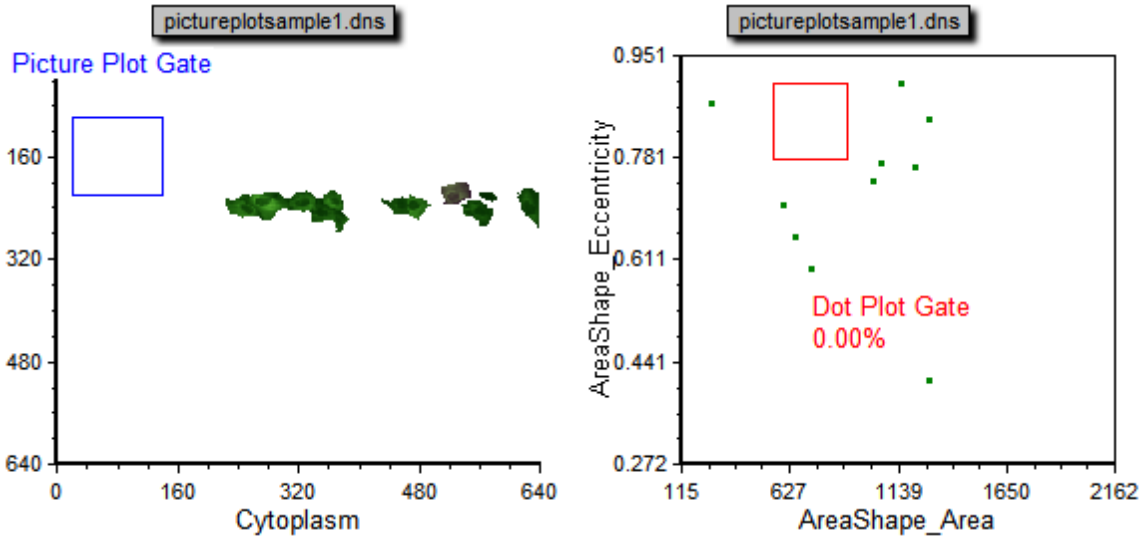

Figure T26.30 Plots Gated on the Range Gate

56. Right-click on the data grid.

57. Choose **Edit Gate→Selected Cells** from the pop-up menu.

58. Click on **Range**.

59. Select **Add Range to Gate** from the drop-down list.

60. Enter the range as **From** 1 **To** 50.

61. Click **OK**.

62. Click **Apply** in the Range Gate selection area to see the changes to the gate in real time.

63. 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 T26.31). Also notice that since events are now falling within the Dot Plot Gate, the percent gate statistic automatically updates.

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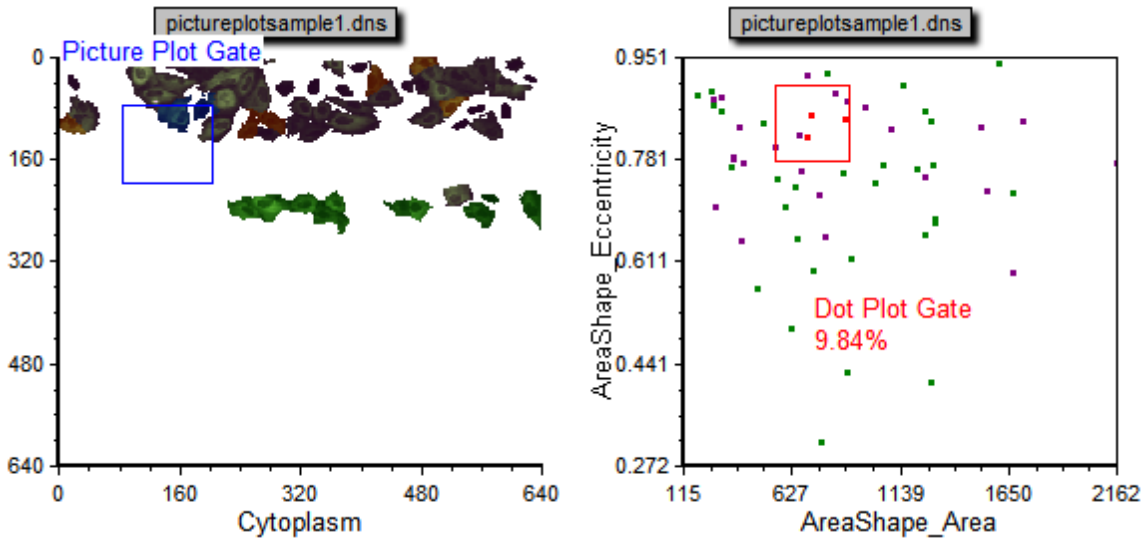

Figure T26.31 Picture Plot and Dot Plot After Adding a New Range to the Selected Cells Gate

34.28 Regression Analysis

FCS Express provides the user with many mathematical functions to create custom tokens and custom data sets. These functions operate similar to functions within Microsoft Excel™. In this tutorial we will perform a linear regression analysis on FITC MESF bead data 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 280 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 and a Data Grid.
- Insert a scatter plot of the Data Grid values.
- Create a linear regression custom token using the MESF bead data.
- Overlay the regression on to the scatter plot of the Data Grid.
- Save the linear regression formula in a text file usable by other FCS data files.

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- Apply the linear regression to an FCS data file to obtain MESF data.

First, we will complete the custom data grid[1343].

34.28.1 Complete a Custom Data Grid

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 Tutorial Sample Data archive[590].

The following layout should appear as shown in Figure T27.1.

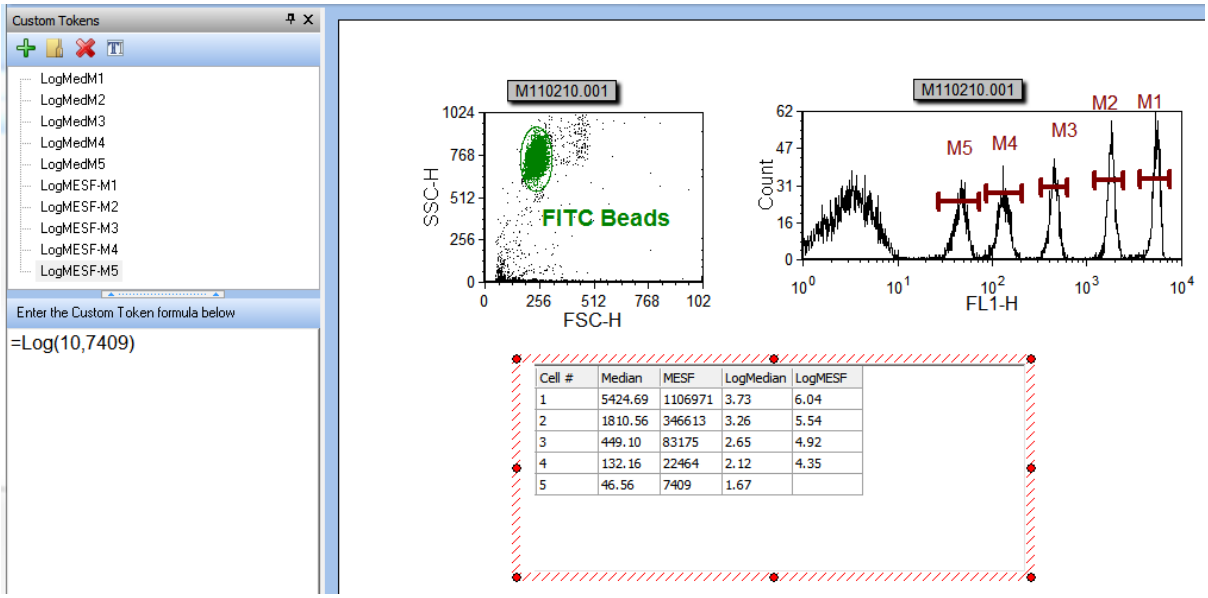

Figure T27.1 The FITC MESF Beads.fey Layout

In the upper left corner of the layout, is a scatter plot of the FITC beads with a gate around the single bead events. To the right of the scatter 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 Custom Data Grid, named 'FITC MESF'. Please take a moment and click on an entry in each column of the Data Grid and examine the data entry in the Data Grid editor. The first column is the median value for each of the defined markers on the histogram, and is defined using a statistics token. The second column is the MESF FITC value assigned to each of the bead

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populations, and is a manually entered data value. The third and fourth columns are the Log values of the median and MESF bead populations, and are defined by custom tokens. For more details on how to create a custom data grid, please see our Custom Data tutorial924.

On the left side of the screen is the docked **Custom Tokens** window. All the custom tokens use the Log function to convert linear values to log base 10 values. If you click on one of the custom tokens, the definition of that token appears in the lower half of the docked window. The Log function has two variables: the first variable indicates the Log base, in this case 10, and the second variable is the value you wish to find the logarithm of.

Notice that the bottom right cell of the Data Grid, under the 'LogMESF' column, is empty. We will now drag the custom token 'LogMESF-M5' from the docked **Custom Tokens** window into the empty cell to complete the custom data set.

2. Place the cursor over the custom token 'LogMESF-M5', shown highlighted in blue in Figure T27.2.

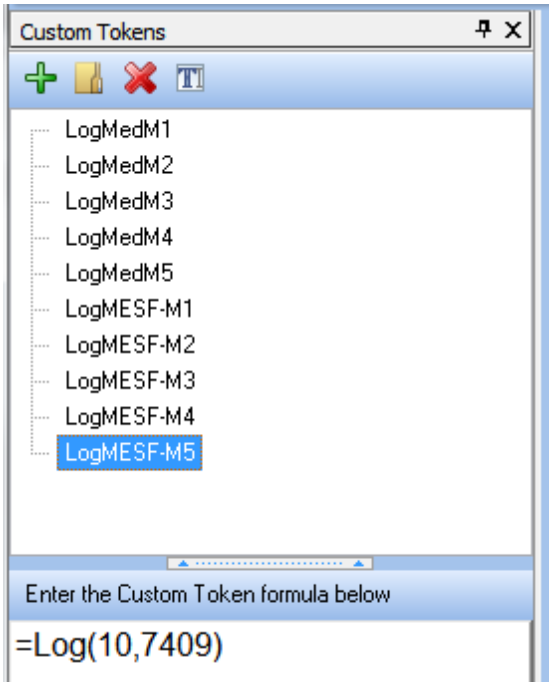

Figure T27.2 Selecting the LogMESF-M5 Custom Token

3. Drag the LogMESF-M5 custom token from the **Custom Tokens** window and drop it in Row 5 of the LogMESF column.

We have now completed the custom Data Grid as shown in Figure T27.3.

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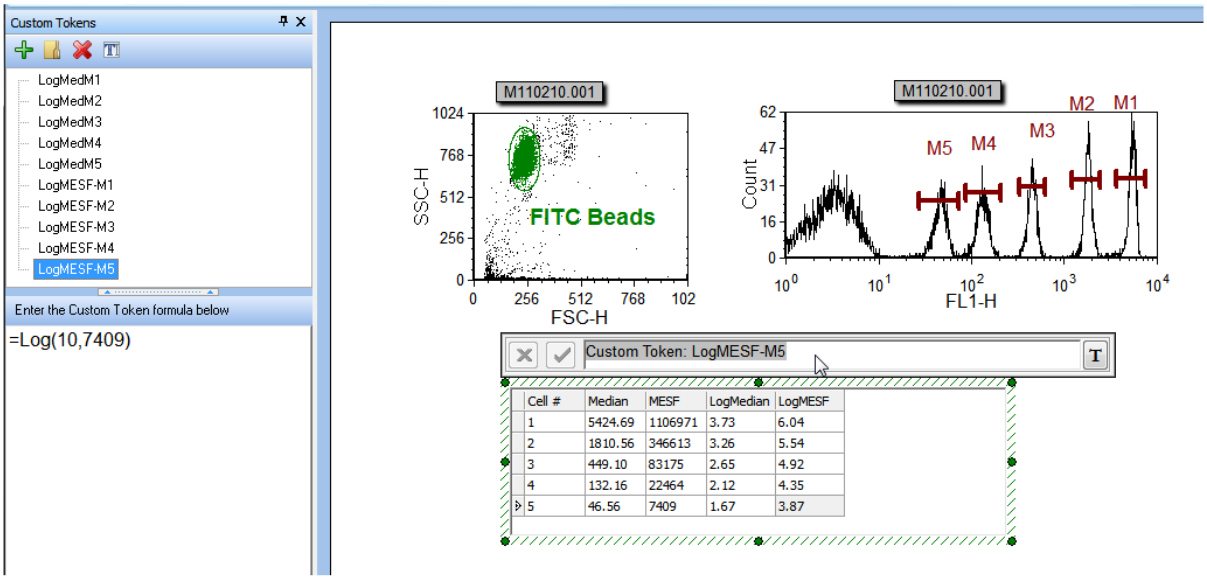

Figure T27.3 Custom Token Successfully Placed in the Custom Data Grid

Now, we will insert a scatter plot using the FITC bead data in the custom Data Grid.

4. Select the custom data table; it should now have a green border.
5. Select the **Insert→2D Plots→Scatter** command.
6. Click on a blank area of the layout below the custom data grid.

A 2D scatter plot using the data from the first two columns, 'Median' and 'MESF', of the custom Data Grid appears, shown in Figure T27.4.

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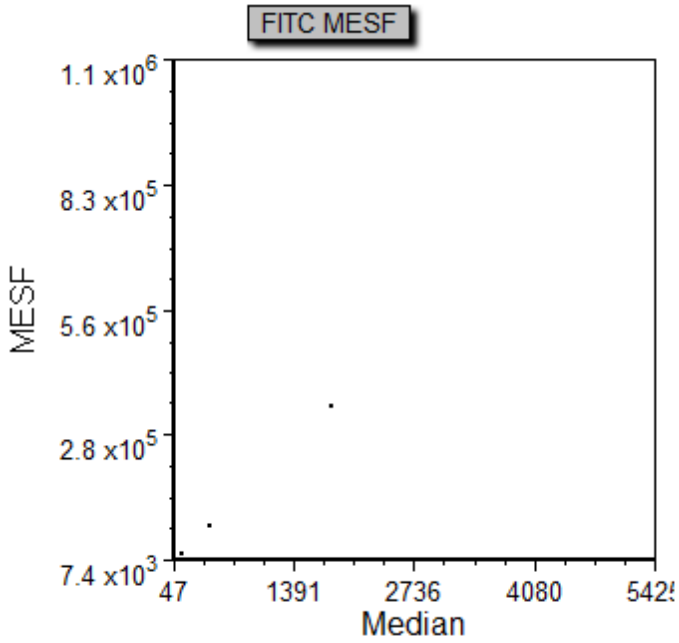

Figure T27.4 Scatter Plot of Median and MESF Data from the Data Grid

We will format the scatter plot to display the log Median and MESF data from the Data Grid.

7. Place the cursor over the y-axis 'MESF' label.
8. Press and hold the left mouse button and the list of parameters from the Data Grid appears, as shown in Figure T27.5.
9. Release the button when the cursor is over 'LogMESF', shown highlighted in blue and indicated by the cursor in Figure T27.5.

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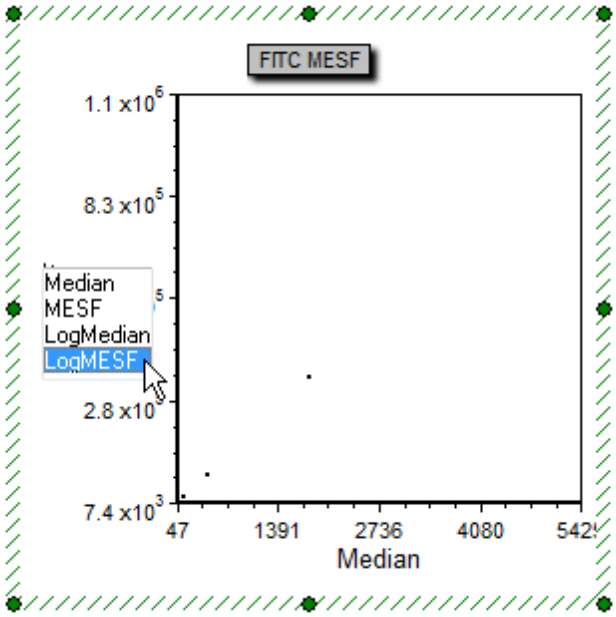

Figure T27.5 Selecting 'LogMESF' Data for the Y-axis

The scatter plot now updates to reflect the selection of the 'LogMESF' data for the Y-axis. We will now change the X-axis parameter to the 'LogMedian" data.

10. Place the cursor over the x-axis 'Median' label.

11. Press and hold the left mouse button and the list of parameters from the Data Grid appears.

12. Release the button when the cursor is over 'LogMedian'.

The scatter plot now updates to reflect the selection of the 'LogMedian' data for the X-axis and the scatter plot appears as shown in Figure T27.6.

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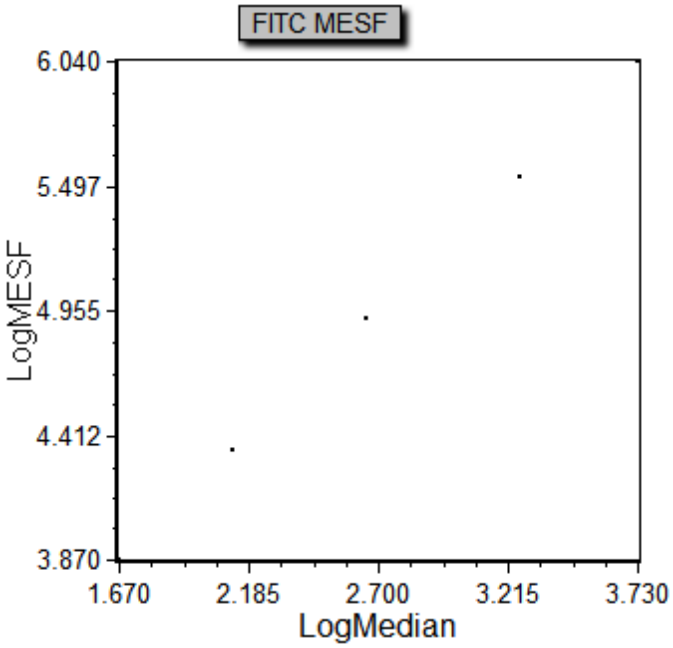

Figure T27.6 Scatter Plot with Log Median and MESF Values from the Custom Data Grid

We will now format the scatter plot to better visualize the five data points.

13. Right-click on the Scatter Plot.
14. Choose **Format** from the pop-up menu.
15. Choose the **Axis** category.
16. Click on **Y Axis** to the left of **Range**.
17. Change the **Y Axis Range Minimum** to 3.
18. Change the **Y Axis Range Maximum** to 7.
19. Click on **X Axis** to the left of **Range**.
20. Change the **X Axis Range Minimum** to 1.
21. Change the **X Axis Range Maximum** to 4.
22. Choose the **Overlays** category.
23. Change the **Point Size** to 2.
24. Click **OK**.

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The scatter plot will now look like Figure T27.7. It has been formatted to bring all of the data points into range and to display the points larger.

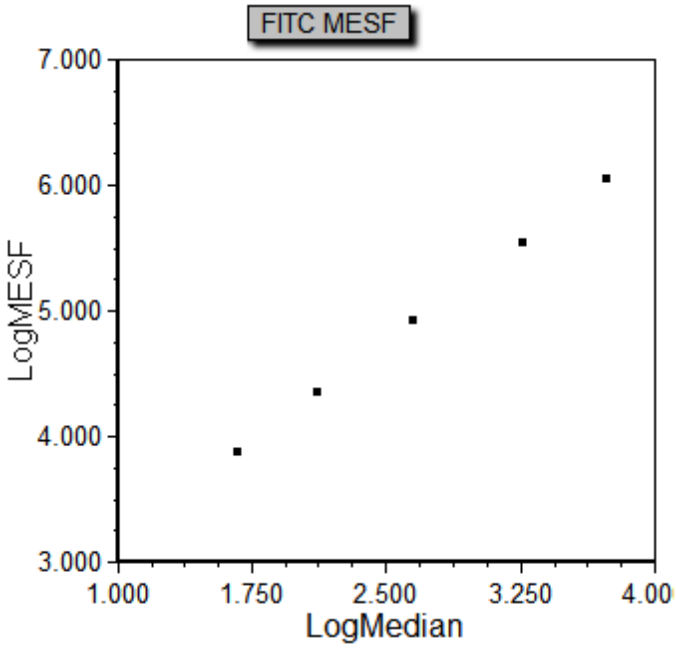

Figure T27.7 Scatter Plot Formatted to Display the Data Points Larger and on a Set Scale

Next, we will create a linear regression token[1349].

34.28.2 Create a Regression Token

We will now use the Log values (from the Data Grid) of the five FITC bead populations to perform a linear regression, resulting in a linear equation of the form $y = mx + b$. This linear fit will be accomplished by creating and using a custom token.

1. Click on the green plus sign in the top border of the **Custom Tokens** window.

A 'New Custom Token' label appears highlighted in blue.

2. Select the 'New Custom Token' label.
3. Type 'LinFitLogMedMESF'.
4. Right-click inside the Custom Tokens formula window.
5. Select **Insert Function→PerformLinearFit([xValues], [yValues],**

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[SDvalues], [fixedParameters]), as shown in Figure T27.8. (If necessary, click on the down arrow until 'PerformLinearFit' is visible.)

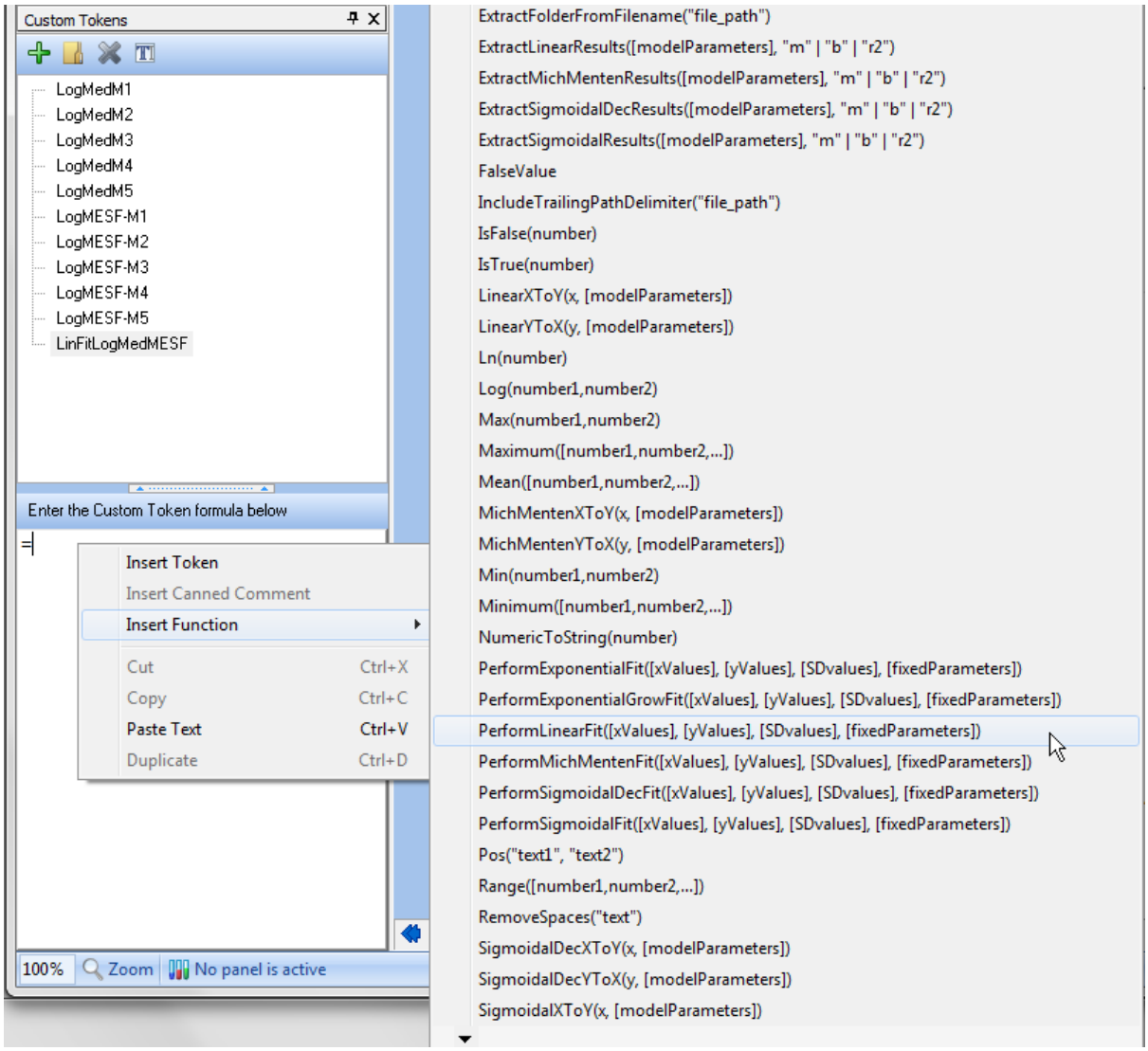

Figure T27.8 Inserting the PerformLinearFit Function as a Custom Token

The custom token formula for 'LinFitLogMedMESF' is updated to reflect the insertion of the PerformLinearFit function, as shown in Figure T27.9. However, we need to specify the x and y data values to use for the linear fit.

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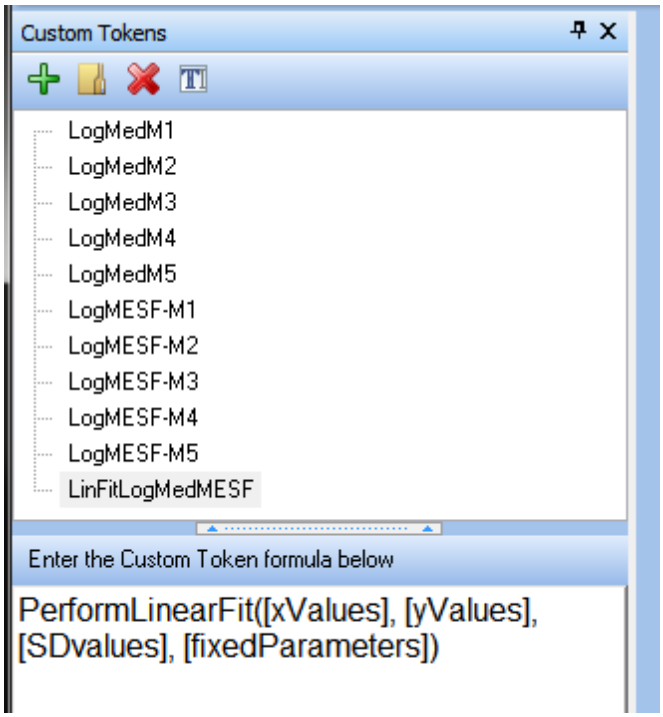

Figure T27.9 The Perform Linear Fit Function Displayed in the Custom Tokens Window

6. Highlight the text **xValues**.
7. Right-click on the highlighted text to bring up the pop-up menu.
8. Select **Insert Token** from the pop-up menu.

The **Insert a Token** dialog appears, similar to Figure T27.10

9. Select **Data Parameter**, shown highlighted in blue in Figure T27.10.
10. Click **Insert**.

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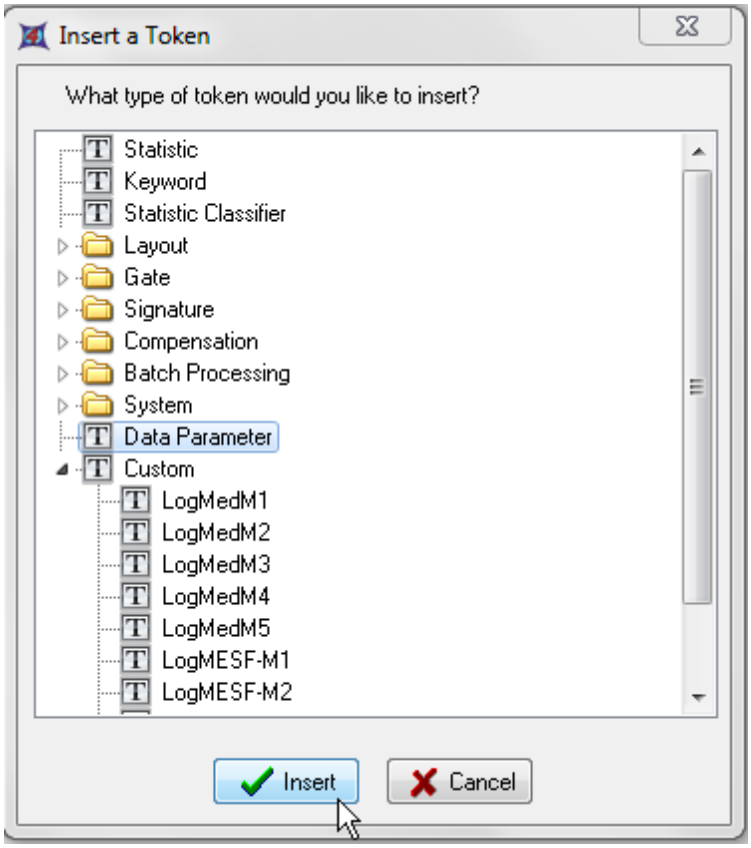

Figure T27.10 The Insert a Token Dialog with Data Parameter Selected

The **Create Data Parameter** dialog appears.

We will now use the Log Median data, from the FITC MESF Data Grid, as the X value in the Linear Fit function.

11. Select the **Data Source** category, shown highlighted in Figure T27.11.

12. Select the **Custom Data** radio button as the Data Source, as shown in Figure T27.11.

FITC MESF should appear, as shown highlighted in blue in Figure T27.11. Now, we will use the data from the 'FITC MESF' custom data. *Note:* The Custom Data was named 'FITC MESF' when it was originally created.

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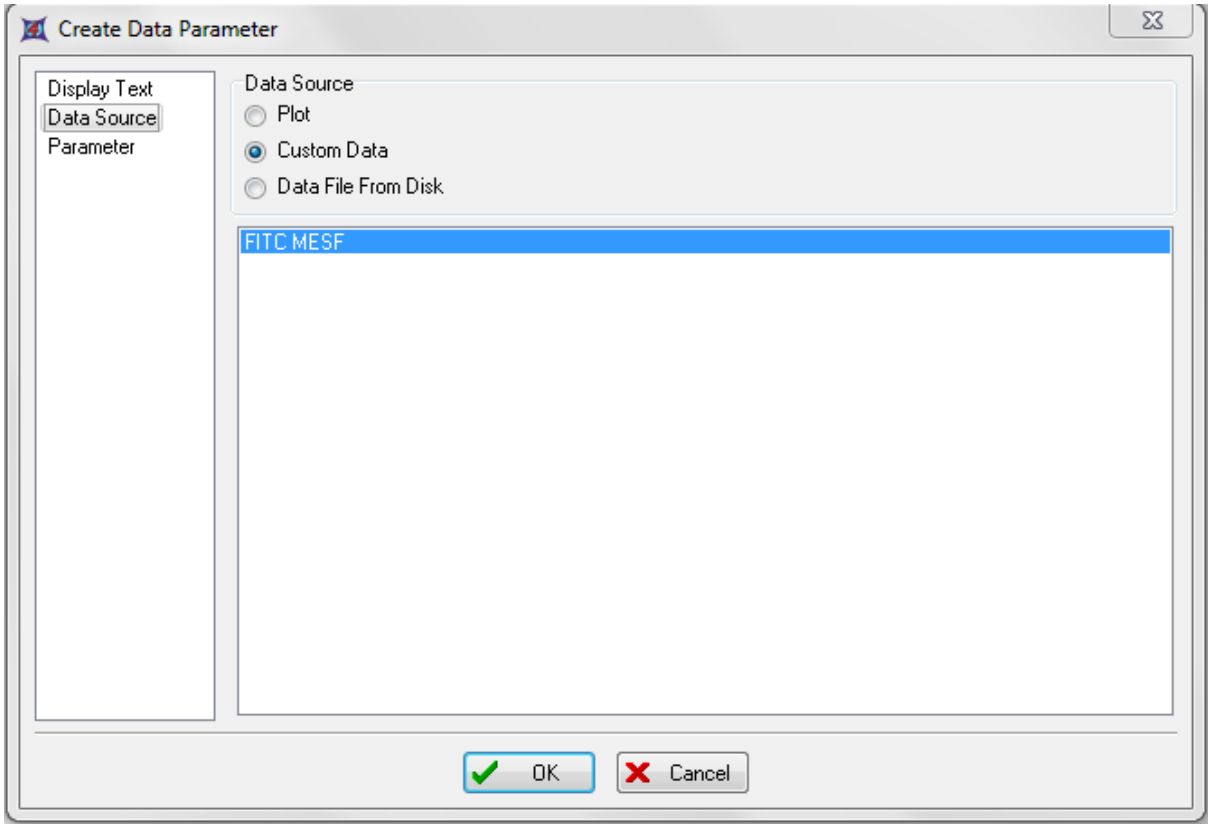

Figure T27.11 Choosing the Custom Data, FITC MESF, as the Data Source

13. Select the **Parameter** category, shown highlighted in Figure T27.12.

14. Select **LogMedian** from the **Parameter** drop-down list, shown highlighted in blue and indicated by the cursor in Figure T27.12.

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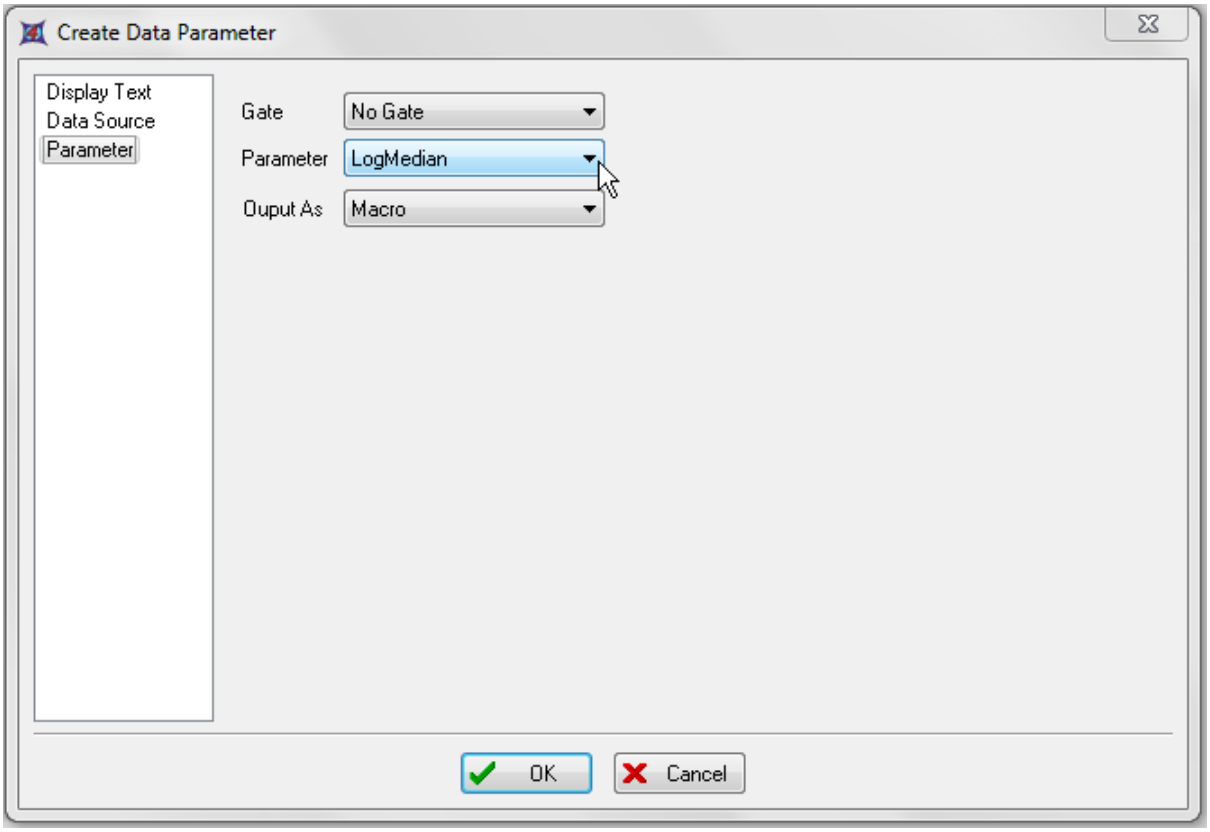

Figure T27.12 Choosing LogMedian as the X Value Parameter

15. Select the **Display Text** category, shown highlighted in Figure T27.13.

16. Select **Numeric** from the **Format** drop-down list, shown highlighted in blue and indicated by the cursor in Figure T27.13.

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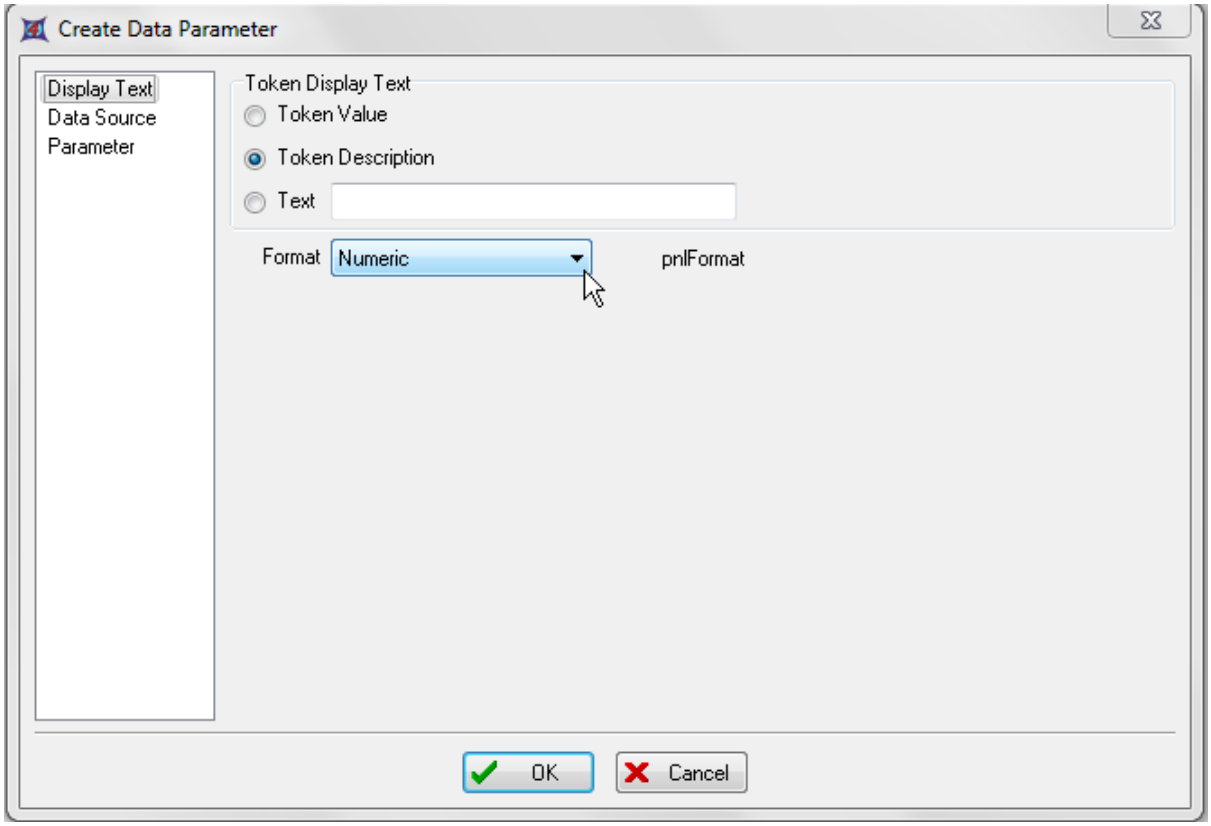

Figure T27.13 Choosing Numeric as the Format for the Display Text

17. Click **OK**.

The PerformLinearFit function definition updates to reflect the selection of the xValues, shown highlighted in Figure T27.14.

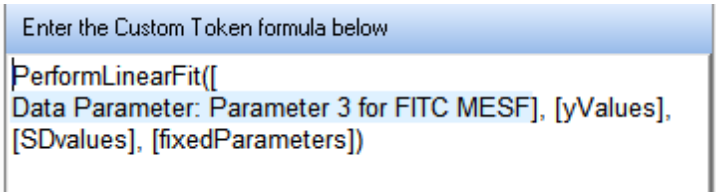

Figure T27.14 The Perform Linear Fit Function with the xValues Parameter Defined as a Token

Now, repeat steps 6 through 17 for the yValues, specifically highlighting and replacing the text **yValues** in step 6 and selecting **LogMESF** in step 14.

18. Delete the text **SDvalues** in the PerformLinearFit formula, being careful not to delete the surrounding brackets.

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19. Delete the text **fixedParameters**, again being careful not to delete the surrounding brackets.

20. Enter an '=' at the beginning of the formula as in Figure T27.15.

If we had wanted to force the y-intercept to be zero we could have entered 'b=0' in brackets where the text 'fixedParameters' had been. Also, if we had wanted to apply a standard deviation to the values as part of the linear fit, we would have entered that information in between the brackets where the 'SDvalues' text had been.

The PerformLinearFit function definition should now look like Figure T27.15.

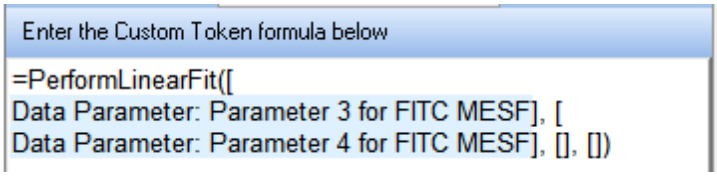

Figure T27.15 Completed PerformLinearFit Custom Token Formula

We will now insert the formula in the layout to view the data.

21. Drag the LinFitLogMedMESF custom token from the custom token navigator onto a blank area of the layout. A new textbox will be automatically created and the linear fit token will be inserted in it. The linear fit token will be displayed as in Figure T27.16.

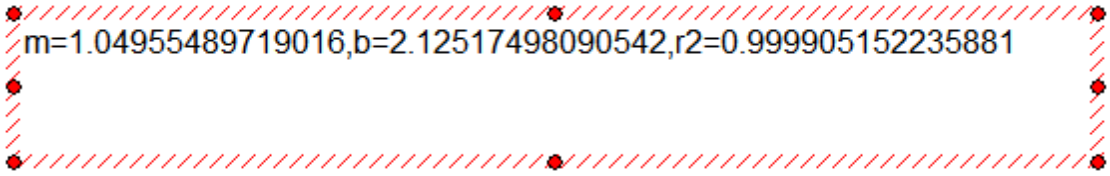

Figure T27.16 The LinFitLogMedMESF Custom Token Formula Output Displayed in a Text Box

In the next section, we will create a linear regression overlay.

34.28.3 Create a Regression Overlay

While defining a formula for a regression is useful it is often desirable to display the line fit with your data points. In this section we will use a line fit created for

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linear values and overlay it on the scatter plot.

1. Load the FITC MESF Beads Completed.fey layout located in the **Regression Analysis** folder within the Tutorial Sample Data archive 590.
2. Navigate to page 2 of the layout.
3. Choose the **Data→Save/Load→Custom Data→New Custom Data** command.
4. Name the new custom data Regression Line Fit (Figure T27.17).
5. Change the number of **Columns** to 2 (Figure T27.17).
6. Change the number of **Rows** to 10 (Figure T27.17).
7. Check the **Open Data Grid** box (Figure T27.17).

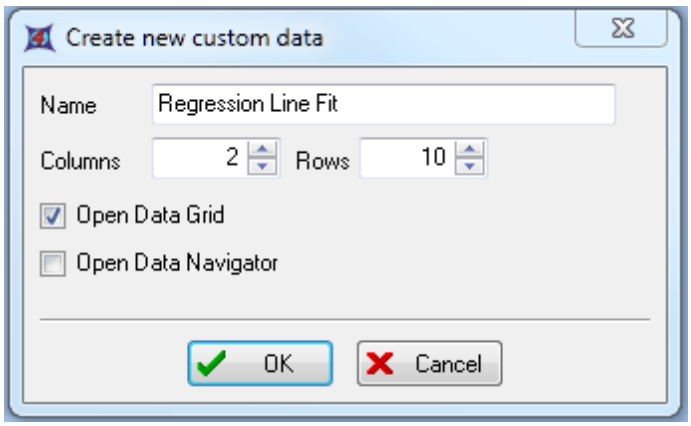

Figure T27.17 Creeating a New Custom Data Grid with 2 Columns and 10 Rows

8. Click **OK**.

We will use this custom data grid to calculate values for the line fit based the equation for the line which is defined by the formula above the scatter plot in the layout. We will first calculate a range of X values based on the data which will later be used to calculate Y values from the equation of the line.

The range of X values must be divided into bins based on your data. In this data set, the X values for Median range from about 40 to 5500 (see Median column in the custom data grid). For this example, we will put these values into 10 bins which results in an addition of 546 from one bin to the next [(5500-40)/10 = 546]. We will round 546 to 600 which will give us a final range of values from 40 to 5440. The range you define here will give the upper and lower limits of the

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line. We will perform this calculation for each of the 10 rows in the data grid using parameter math.

9. Click on the Regression Line Fit custom data grid to select it.
10. Choose the **Data→Calculate→Parameter Math→Single Calculation** command.
11. Change the **Output Parameter** to **A** (Figure T27.18).
12. Click on the **Formula** radio button (Figure T27.18).
13. Enter the following formula: '=40+(Row-1)*600' (Figure T27.18).
14. Click **Calculate** (Figure T27.18).

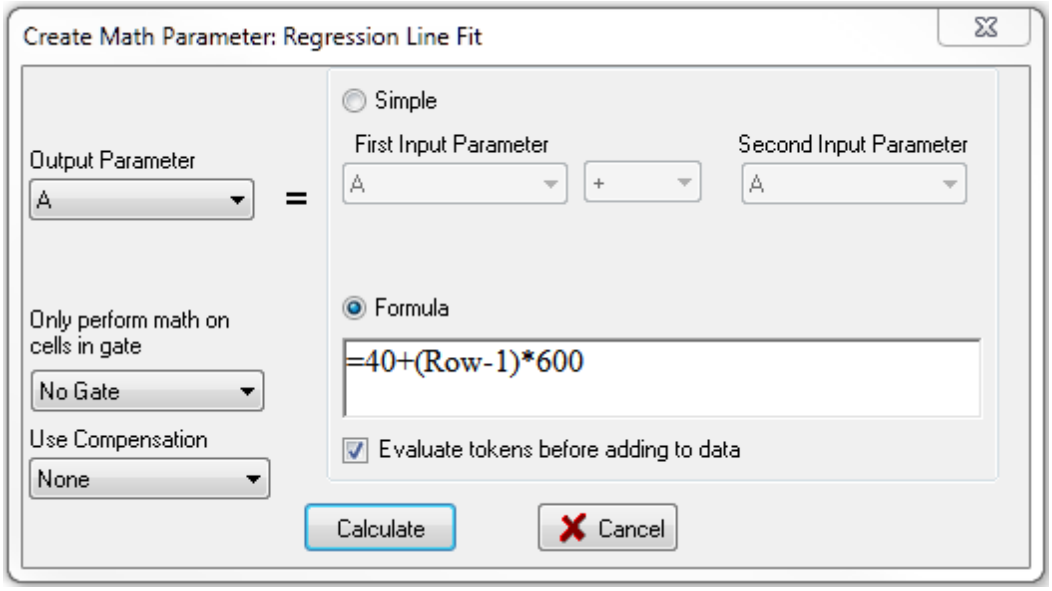

Figure T27.18 Defining Parameter Math for Column A in the Regression Line Fit Custom Data

Column A in the data grid will fill with all of the parameter math calculated values. The formula we used sets 40 as the minimum value and uses the current row number minus one as the multiplier for each bin. The result is the table of values in the custom data grid seen in Figure T27.19.

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Cell #	A	B
1	40	
2	640	
3	1240	
4	1840	
5	2440	
6	3040	
7	3640	
8	4240	
9	4840	
10	5440	

Figure 28.19 Column A Calculated by Parameter Math

We will now calculate the Y values for the line fit based on the equation for the line defined by the custom token *Linear Fit for Overlays* which was defined through steps similar to the Creating a Linear Regression Token 1349 section.

15. Click on the Regression Line Fit custom data grid to select it.
16. Choose the **Data→Calculate→Parameter Math→Single Calculation** command.
17. Change the **Output Parameter** to **B** (Figure T27.20).
18. Click on the **Formula** radio button (Figure T27.20).
19. Enter the following text in the formula box: =LinearXtoY(P1, [
20. Right-click after the bracket in the formula.
21. Choose **Insert Token.**
22. Insert the **Linear Fit for Overlays** token located in the **Custom** category.
23. Click **Insert**.
24. Enter a close bracket and close parenthesis after the token.

Uncheck the **Evaluate tokens before adding to data** box (Figure T27.20).

The final formula should look like Figure T27.20.

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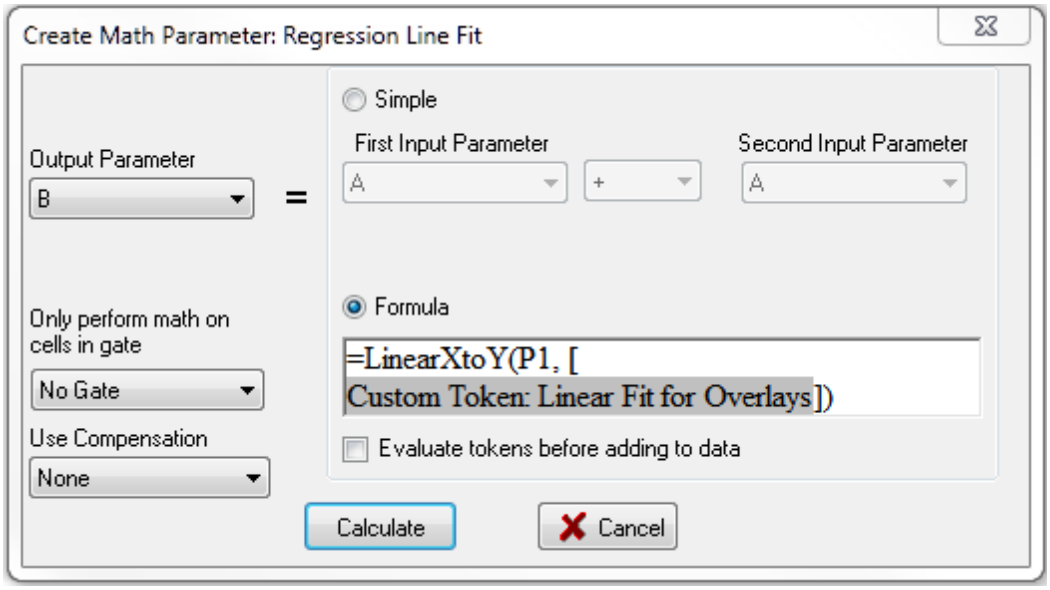

Figure T27.20 Defining the LinearXtoY Function

25. Click **Calculate**.

The data grid will update to display the Y values calculated from the equation of the line and the X values in the data grid (X values in the LinearXtoY formula were defined by P1=Parameter 1=Column A) as as seen in Figure T27.21.

Cell #	A	B
1	40	-446.873...
2	640	122391.5...
3	1240	245230.0...
4	1840	368068.5...
5	2440	490907.0...
6	3040	613745.4...
7	3640	736583.9...
8	4240	859422.4...
9	4840	982260.9...
10	5440	1105099....

Figure T27.21 Completed Custom Data Grid for Linear Regression

We will now overlay the data grid on the scatter plot to display the line fit.

26. Right-click on the scatter plot.

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27. Choose **Add overlay** from the pop-up menu.
28. Choose the **Active Files** tab (Figure T27.22).
29. Double-click on **Regression Line Fit** (Figure T27.22).

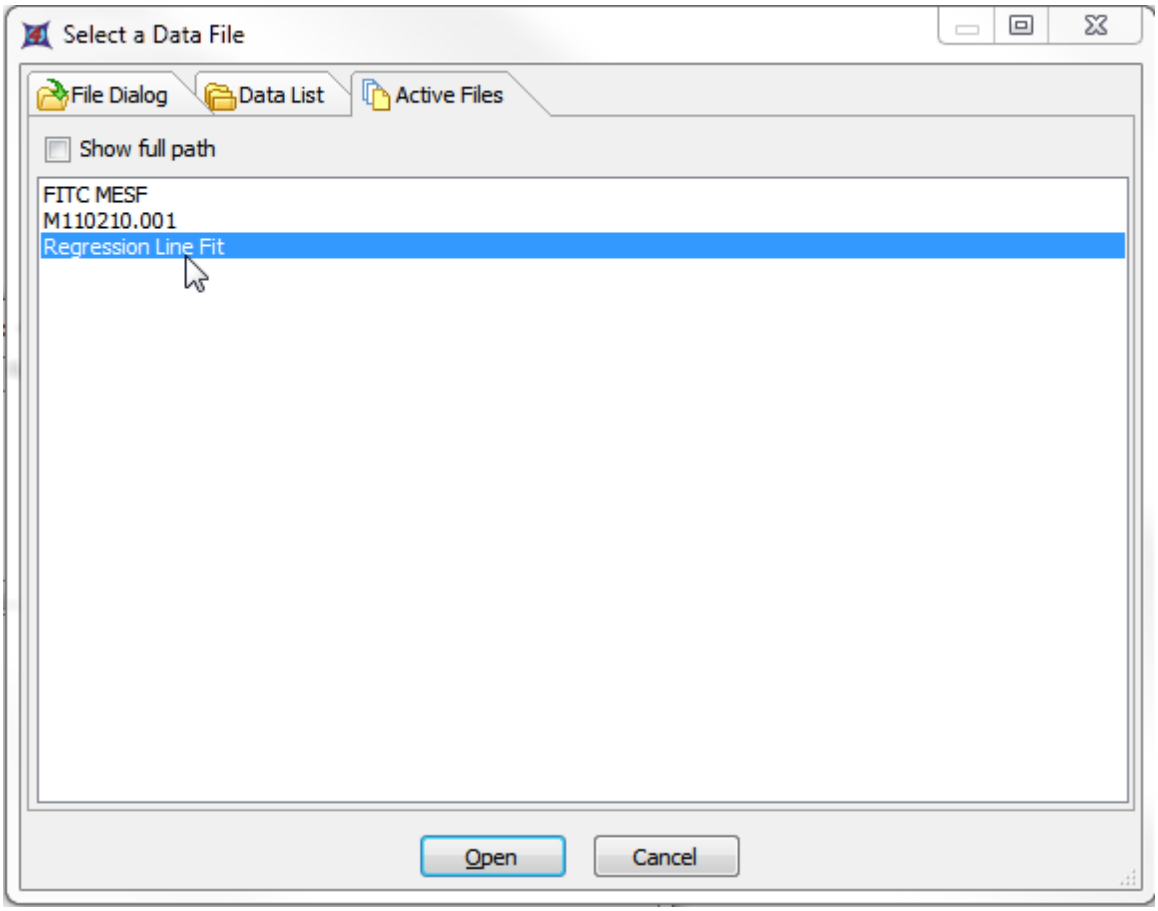

Figure T27.22 Choosing Regression Line Fit as an Overlay

30. Click **OK** in the **Add overlay(s) for:** dialog.

The regression line will now appear on the scatter plot as red dots (Figure T27.23). We will format the scatter plot to view the regression as a simple line.

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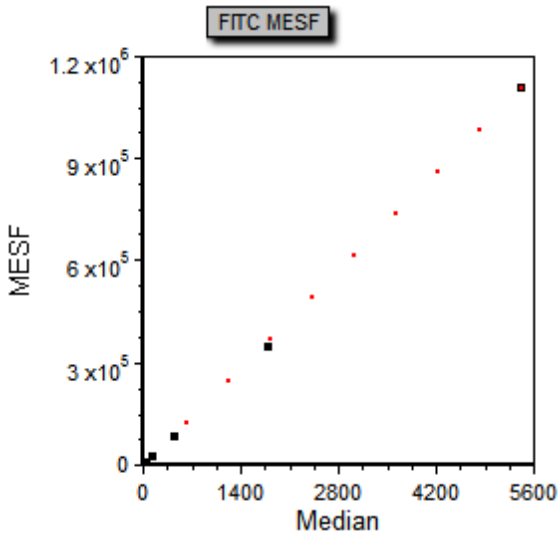

Figure T27.23 Regression Line Fit Data Overlayed on the Scatter Plot

31. Right-click on the scatter plot.
32. Choose **Format** from the pop-up menu.
33. Choose the **Overlays** category.
34. Choose the **Regression Line Fit** overlay.
35. Change the **Point Shape** to **Do Not Show**.
36. Check the **Connect the points** box.
37. Click **OK**.

The scatter plot will now look like Figure T27.24, displaying the raw data as dots and the linear regression line fit as a black line.

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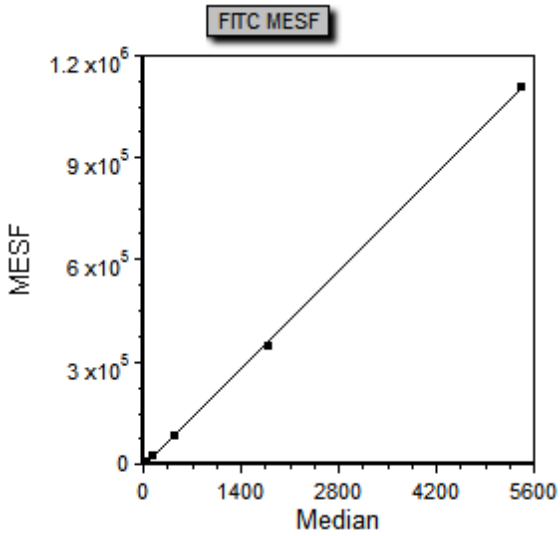

Figure T27.24 Scatter Plot Displaying Raw Data as Dots and the Regression as a Black Line

Next, we will apply a linear regression to an FCS data file to obtain MESF data1363.

34.28.4 Apply Regression to an FCS File

We will now use a linear regression formula to calculate MESF data. In the following example, the linear regression has been saved as a custom token. Ithas also been added to the data files as a keyword as described in the Creating a Linear Regression Token1349 section. We will complete a table for calculating MESF values using the custom token and keyword version of the regression formula, as well as create a scatter plot based on these values.

The layout we will be using contains four pages. The first two pages 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 archive590.
2. Navigate to the **MESF Calculations** page of the layout.

We will now create custom tokens 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. We will do this is two steps: First, the formula will convert Bcell Median values from Table 1 to log base 10 values; and then this log value will be used to calculate the Y, or MESF, values for our line based on the FITCMESF LinReg

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Custom token. The FITCMESF LinReg custom token contains a regression formula that has been determined from the Creating a Linear Regression Token 1349 section of this tutorial.

For convenience, custom tokens to convert the linear median values to log median values have been predefined as seen in Table 1. We will now proceed to create custom tokens for use in Table 2 for the FITC MESF values.

3. Click on the plus symbol in the docked **Custom Tokens** window to create a new custom token.
4. Name the new custom token 'Bcell LogMESF Patient'.
5. Right-click in the **Enter the Custom Token formula below** area of the **Custom Tokens** window.
6. Choose **Insert Function→LinearXtoY(x, [modelParameters])** from the pop-up menu.
7. Highlight the text 'x' in the formula brackets as in Figure T27.25.

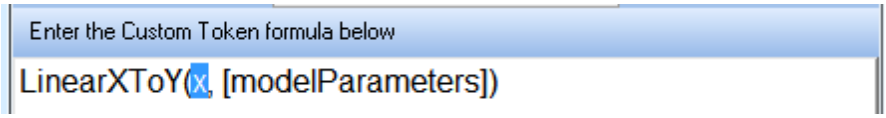

Figure T27.25 Highlighting the x Parameter for Replacement by a Token Value

8. Right-click on the highlighted text.
9. Choose **Insert Token** from the pop-up menu.
10. Insert the **Bcell LogPatient** token from the **Custom** category.
11. Highlight the text **modelParameters** in the formula.
12. Right click on the highlighted text.
13. Choose **Insert Token** from the pop-up menu.
14. Insert the **FITCMESF LinReg** token from the **Custom** category.
15. Enter an = sign before the formula.

The formula should now look like Figure T27.26.

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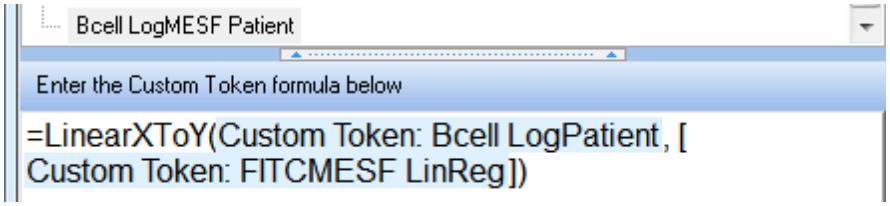

Figure T27.26 Formula for Calculating the Log MESF Value for the Patient Sample

16. Drag and drop the BCell LogMESF Patient token from the **Custom Tokens** window to Row 4 of the **Bcell Log MESF** column in Table 2.

The table will now display the B-Cell Log MESF value created from the custom token formula we just created. We will now create the same formula for the Tcell LogMESF.

17. Click on the plus symbol in the **Custom Tokens** window to create a new custom token.
18. Name the new custom token Tcell LogMESF Patient.
19. Right-click in **Enter the Custom Token formula below** area of the **Custom Tokens** window.
20. Choose **Insert Function→LinearXtoY(x, [modelParameters])** from the pop-up menu.
21. Highlight the text 'x' in the formula.
22. Right-click on the highlighted text.
23. Choose **Insert Token** from the pop-up menu.
24. Insert the **Tcell LogPatient** token from the **Custom** category.
25. Highlight the text **modelParameters** in the formula.
26. Right-click on the highlighted text.
27. Choose **Insert Token** from the pop-up menu.
28. Insert the **FITCMESF LinReg** token from the **Custom** category.
29. Enter an = sign before the formula.

The formula will now look like Figure T27.27.

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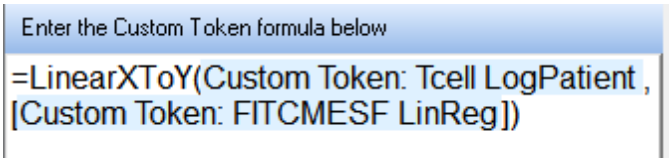

Figure T27.27 The Completed Custom Token Formula for Tcell LogMESF Patient

30. Drag and drop the TCell LogMESF Patient token from the **Custom Tokens** window to Row 4 of the **Tcell Log MESF** column in Table 2.

The table now displays the T-Cell Log MESF value created from the custom token formula we just created.

We will now create a custom token to calculate the linear MESF values for the Bcell MESF and Tcell MESF columns of Table 2 (the regression was calculated in log space, and we need to report the linear values).

31. Click on Row 4 of the **Bcell MESF** column in Table 2.
32. Enter the formula '= 10^' in the data grid editor.
33. Right-click after 10^.
34. Choose **Insert Token** from the pop-up menu.
35. Insert the **Bcell LogMESF Patient** token from the **Custom** category.

The formula will look like Figure T27.28.

Figure T27.28 Formula for Converting Log MESF Values to Linear MESF Values

36. Repeat steps 31 through 35 in Row 4 of the **Tcell MESF Column** in Table 2 with the **Tcell LogMESF Patient** token from the **Custom** category.

The table will now be complete and look like Figure T27.29.

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Table 2

HLA Cross-Match Data Set - FITC MESF Values

Cell #	Sample	Bcell LogMESF	Tcell LogMESF	Bcell MESF	Tcell MESF
1	PBS NEGATI...	3.42	3.05	2630.2679...	1122.0184...
2	AB NEGATIV...	3.62	3.05	4168.6938...	1122.0184...
3	POSITIVE H...	5.61	4.72	407380.27...	52480.746...
4	PATIENT SE...	3.92	3.06	8317.6377...	1148.1536...

Figure T27.29 The Completed HLA Cross Match FITC MESF Data Table

To see the results of the table expressed in a bar chart (Figure T27.30), please click on the **Bar Plots** page tab (for instructions on creating bar plots, please visit the Bar and Scatter Plots tutorial963). 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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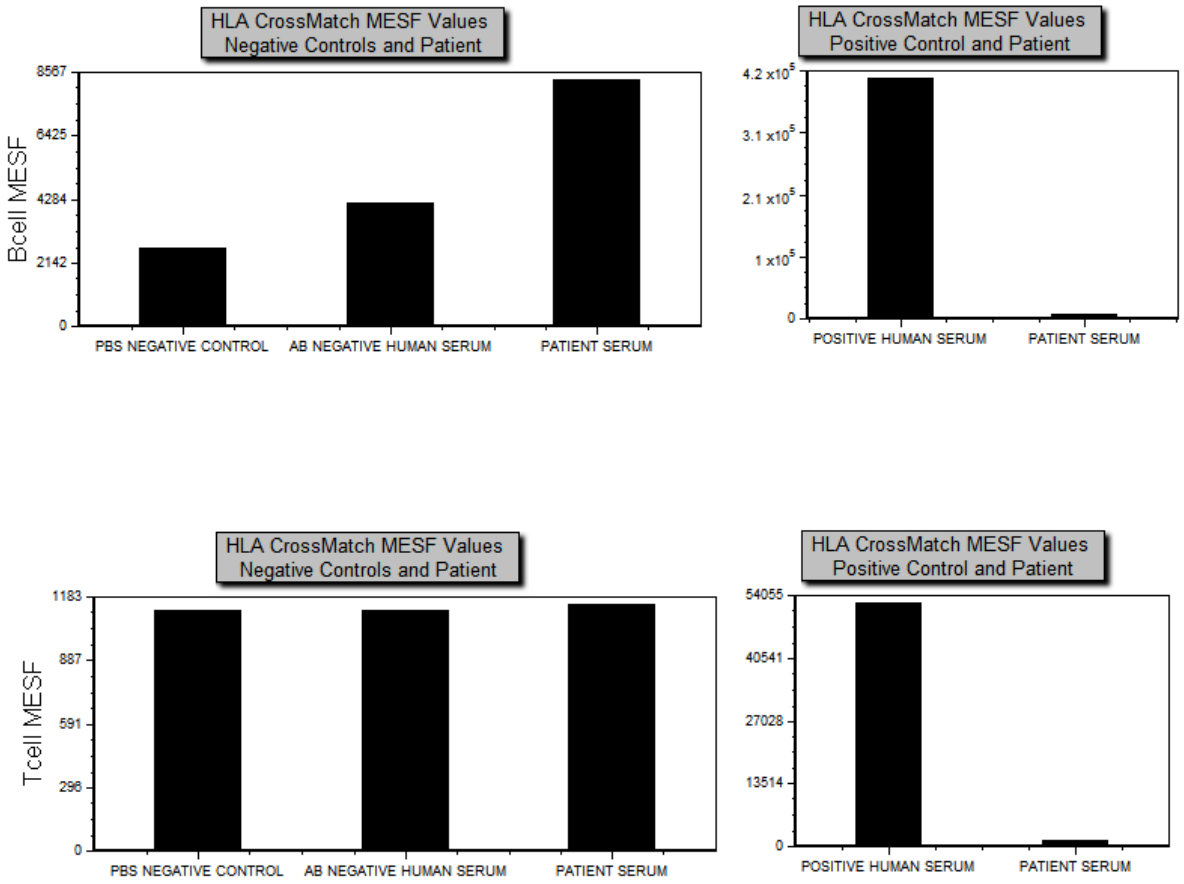

Figure T27.30 Bar Charts Displaying the FITC MESF HLA Cross Match Data from B- and T-cells

Next, we will save a previously created regression for use in another layout 1368.

34.28.5 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 **Linear Regression Analysis** folder within the Tutorial Sample Data archive 590.
2. Drag the **LinFitLogMedMESF** custom token from the **Custom Tokens** window onto a blank area of the layout.

A new textbox will be automatically created and the linear fit token will be

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inserted in it. The linear fit token will be displayed as in Figure T27.31.

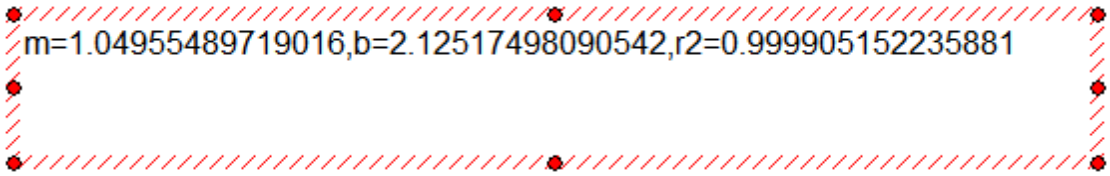

Figure T27.31 LinFitLogMedMESF Custom Token Formula Output Displayed in a Text Box

3. Right-click on the text box.
4. Choose **Copy Text Box** 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 '$' symbol followed by the name you would like the keyword displayed as before the formula followed by an equals sign (Figure T27.32). In this case, use: '$FITCMESFCalib='

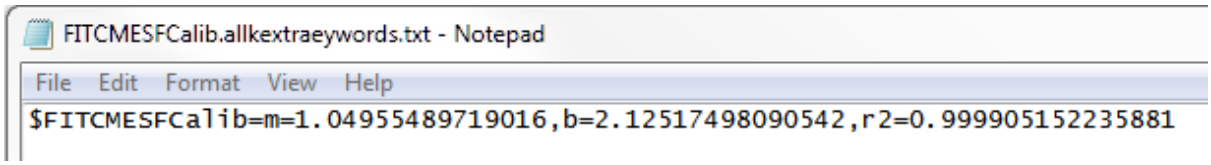

Figure T27.32 Creating an Extra Keyword for the Regression Formula in Notepad

8. Save the text document as FITCMESFCalib.AllExtraKeywords.txt (Figure T27.33) in a location that will be easy to access such as your Desktop.

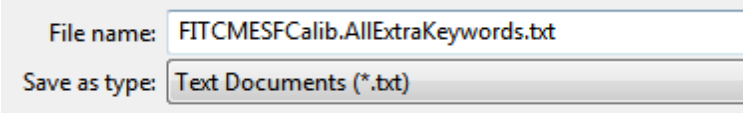

Figure T27.33 Saving the Keyword Text Document

FCS Express has the capability to load keywords from external files as part of loading a data file[314]. 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

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keyword available for use in another layout in the following steps.

9. Copy the FITCMESFCalib.AllExtraKeywords.txt file you just created and paste it into the **Linear Regression Analysis** folder within the *FCS Express Sample Data* directory (Figure T27.34).

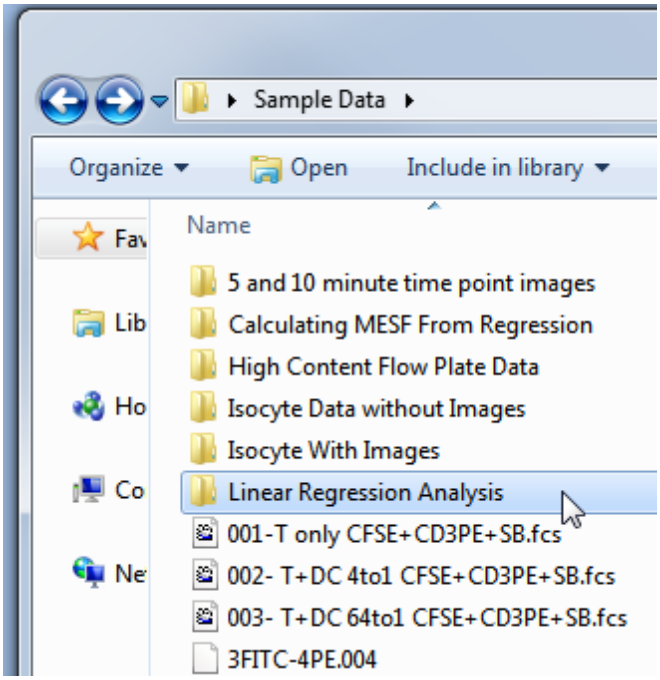

Figure T27.34 Paste the FITCMESFCalib.AllExtraKeywords.txt File into the Linear Regression Analysis Folder

10. Load the **HLA CrossMatch Keyword Regression.fey** layout located in the **Linear 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→LinearXtoY(x, [modelParameters])** from the pop-up menu.

14. Highlight the text 'x' in the formula brackets.

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15. Right-click on the highlighted text.
16. Chose **Insert Token** from the pop-up menu.
17. Insert 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**.
22. Choose the **Keyword** category in the **Create Keyword** dialog (Figure T27.35).
23. Click on the ellipsis.
24. Scroll down to locate the **$FITCMESFCALIB** keyword (Figure T27.35).
25. Click on the **$FITCMESFCALIB** keyword to select it (Figure T27.35).

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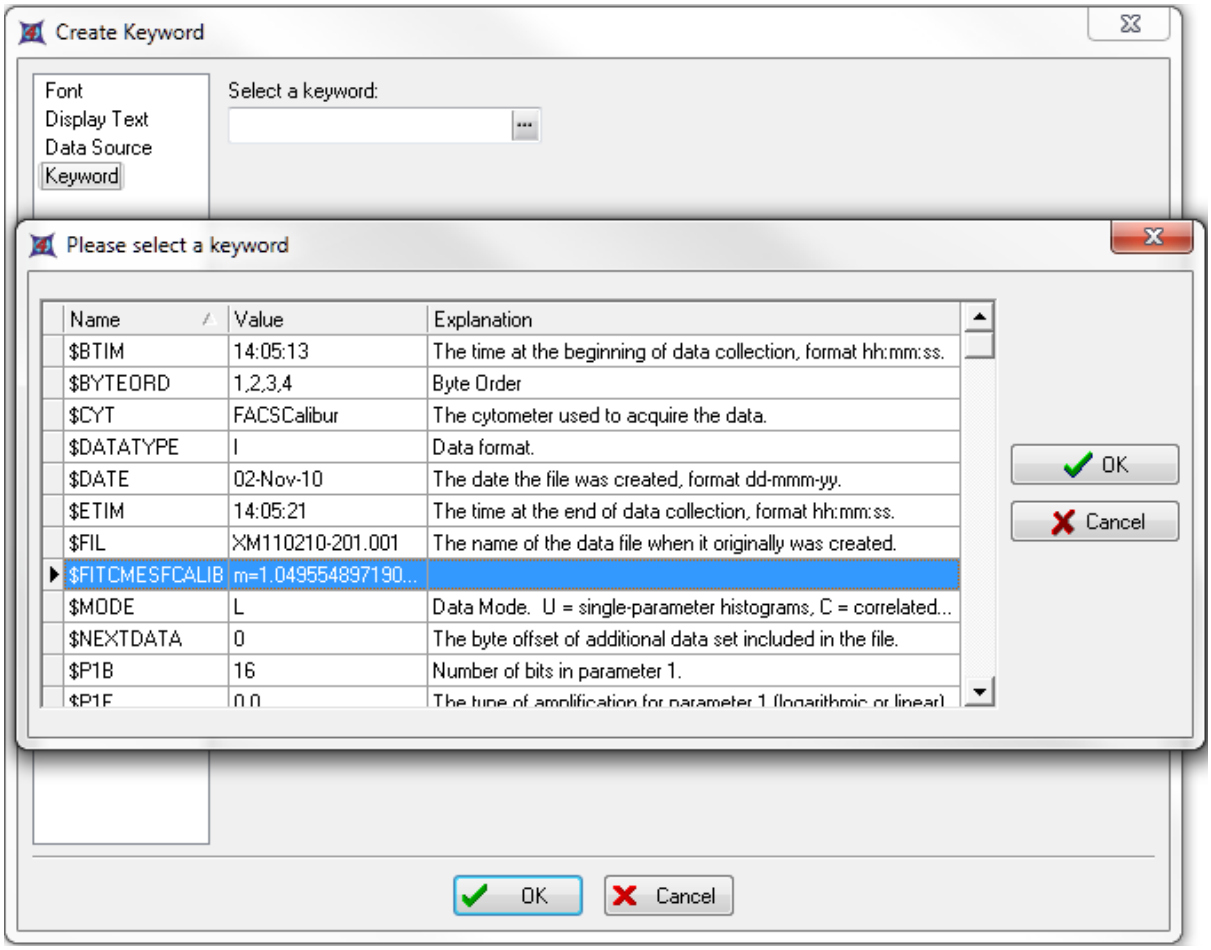

Figure T27.35 Choosing the $FITCMESFCALIB Keyword Token

26. Click **OK**.
27. Click **OK** again.
28. Enter an = sign before the formula.

The formula will now look like Figure T27.36.

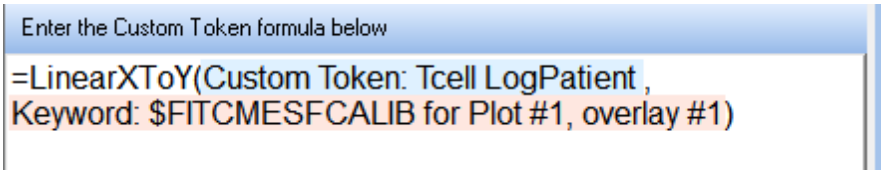

Figure T27.36 Completed Custom Token Formula for Tcell LogMESF Patient

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29. Drag and drop the **TCell LogMESF Patient** token from the **Custom Tokens** window to Row 4 of the **Tcell Log MESF** column in Table 2.

The table is now completed and displays the T-Cell Log MESF value created from the custom token formula we just created. The formula uses 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.

34.29 Introduction to FCS Express 4 Image Cytometry

A Flow Cytometry environment for Image Cytomtery Data

FCS Express 4 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 4 Image Cytometry.

- **Getting Started with Image Cytometry** 1374
- **Using the High Content Module with Image Data** 1406
- **Batch Processing and Reporting for Image Data** 1430

This tutorial closely follows the "Introduction to FCS Express 4 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 Content Add-on 482 to optimize and view a 96-well plate nuclear translocation assay. The High Content Add on enables review of multi-parametric results and

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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 4 for which there are existing detailed tutorials590. 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 Tutorial1183.

Next Topic1374

34.29.1 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
- Modify settings and gating for the data grid displaying a cell gallery

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Understanding Image Data Import with FCS Express 4 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 per-object multi-parametric results. Ideally, the data you will work with has all of these components available to FCS Express 4. 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 4 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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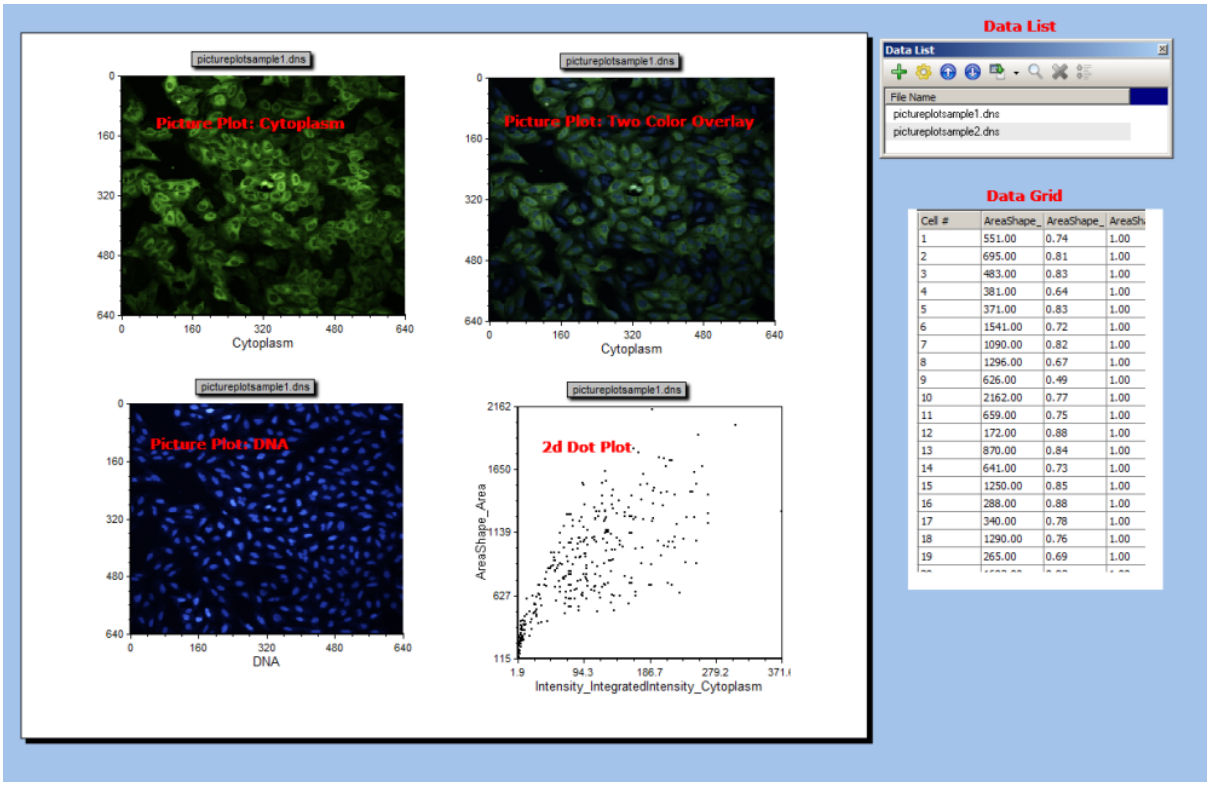

Figure T28.1 Overview of the IntroToImage1.fey layout

The Dot Plot[607] 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 Dot Plot[607] easily. We will proceed by changing the X-Axis parameter.

2. Press and hold the left mouse button down over the X-Axis label of the Dot Plot . You will see a menu of parameters appear (Figure T28.2).

3. Scroll through the list of parameters left or right by moving the mouse to the left or right of the list box, while holding down the mouse button.

4. Move the mouse until the parameter you wish to change to his highlighted.

5. Release the mouse button.

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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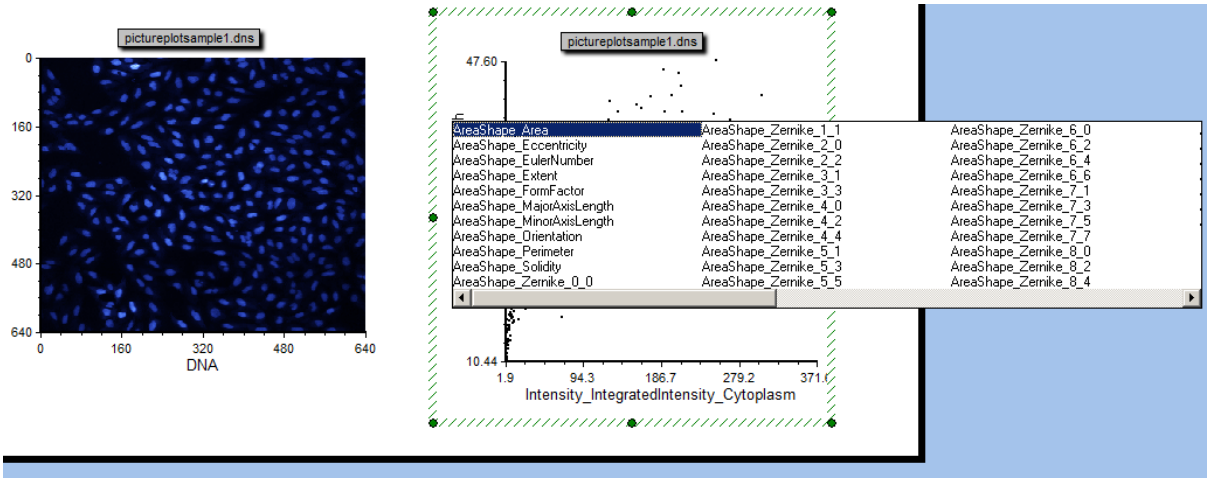

Figure T28.2 Selecting a New Parameter on a 2d Plot.

Undo is a powerful feature in FCS Express 4. Note that once you have made and change you can use CTRL+Z to undo your changes any time you like. FCS Express 4 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 Dot Plot 607.

8. Select **Format** from the pop-up menu (Figure T28.3).

This is the general format screen for the plot. All plots and many other items in FCS Express have the option to be formatted similarly.

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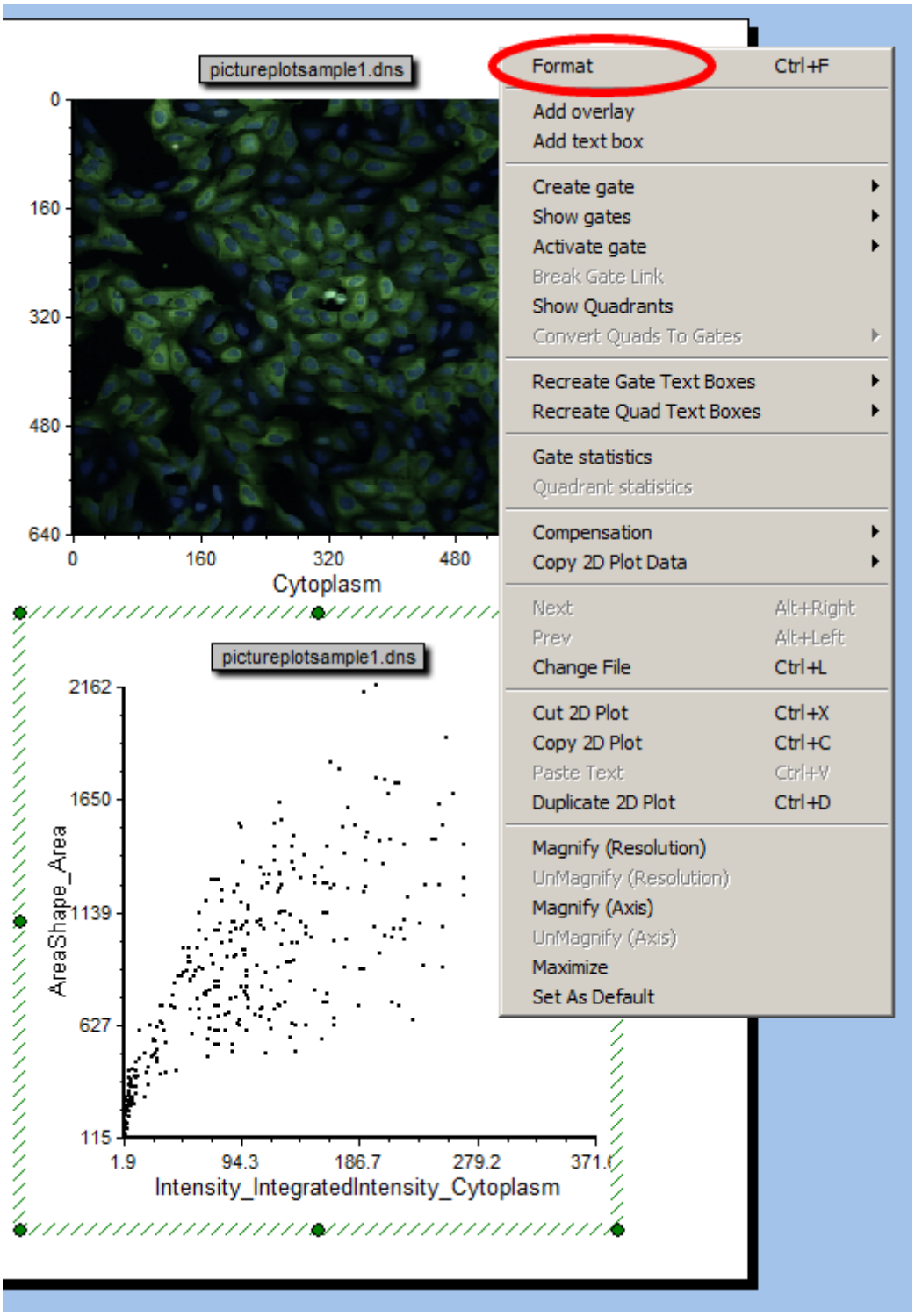

Figure T28.3 Launching the Format Menu for a Plot

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**

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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).

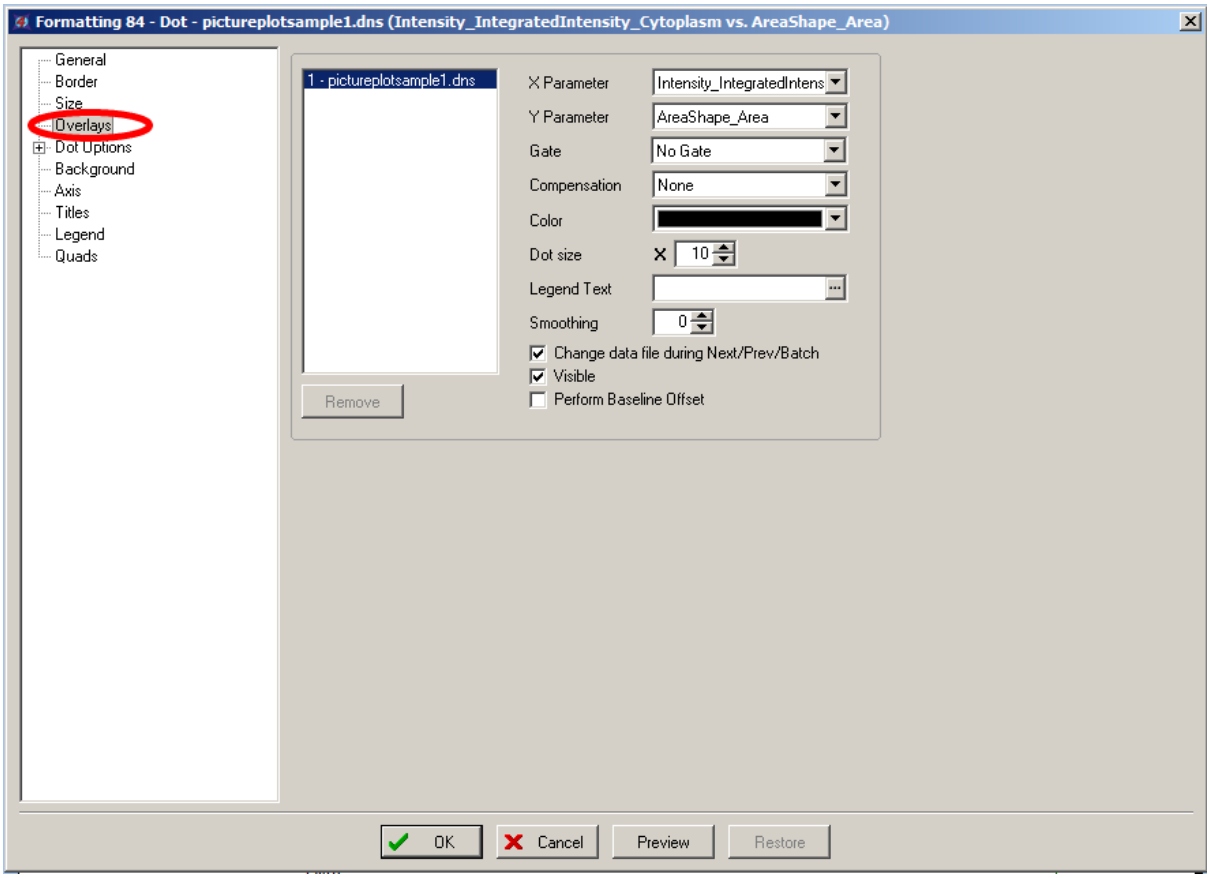

Figure T28.4 The Format Sub Menus

10. Confirm that the Dot Size is 10. If not, change it to 10. Click **OK** to close the format window.

11. Select the **Gating tab→Create Gates→Rectangle**. (Figure T28.5)

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Figure T28.5 Selection of Gate Type

12. Press and hold the left mouse button down on the center of the Dot Plot[607].

13. While holding the left mouse button down, drag to the right and down to draw a rectangular gate on the dot plot (Figure T28.6).

14. Release the mouse button when the gate is the desired size.

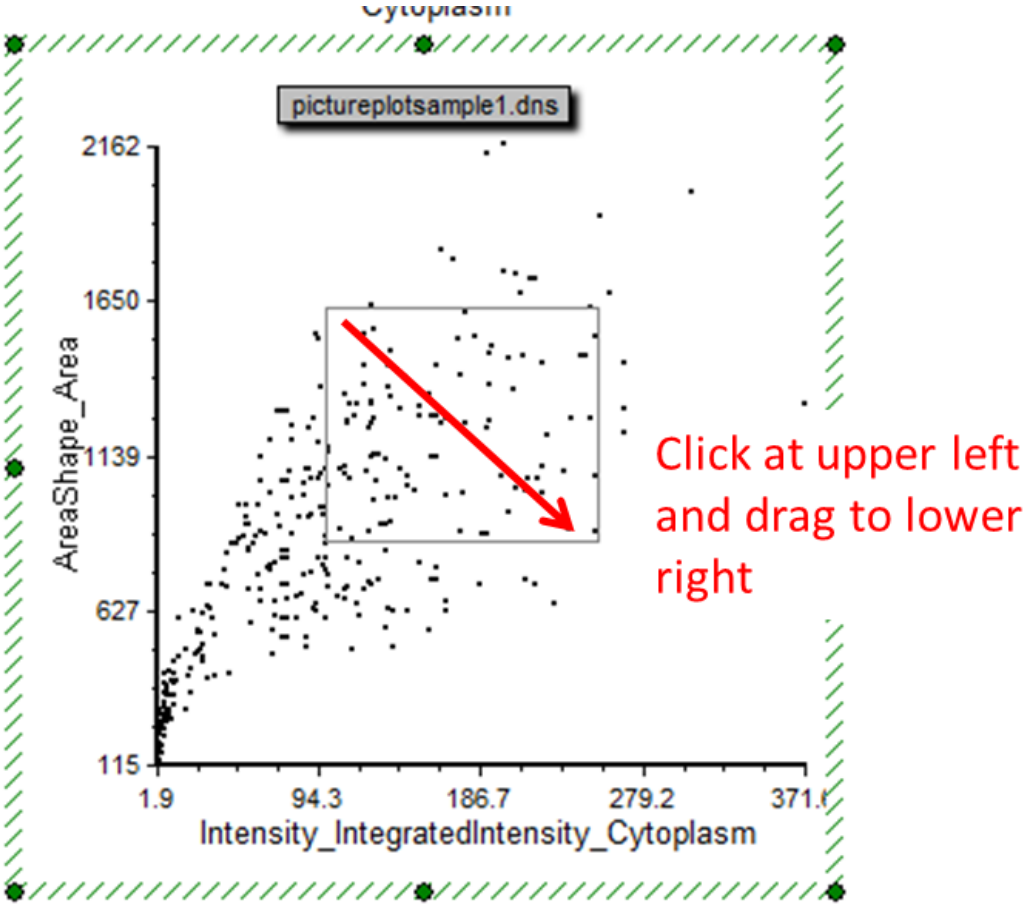

Figure T28.6 Drawing a Rectangular Gate

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15. Enter the name "**Cells Gate 1**" when the **Create New Gate** dialog appears (Figure T28.7).

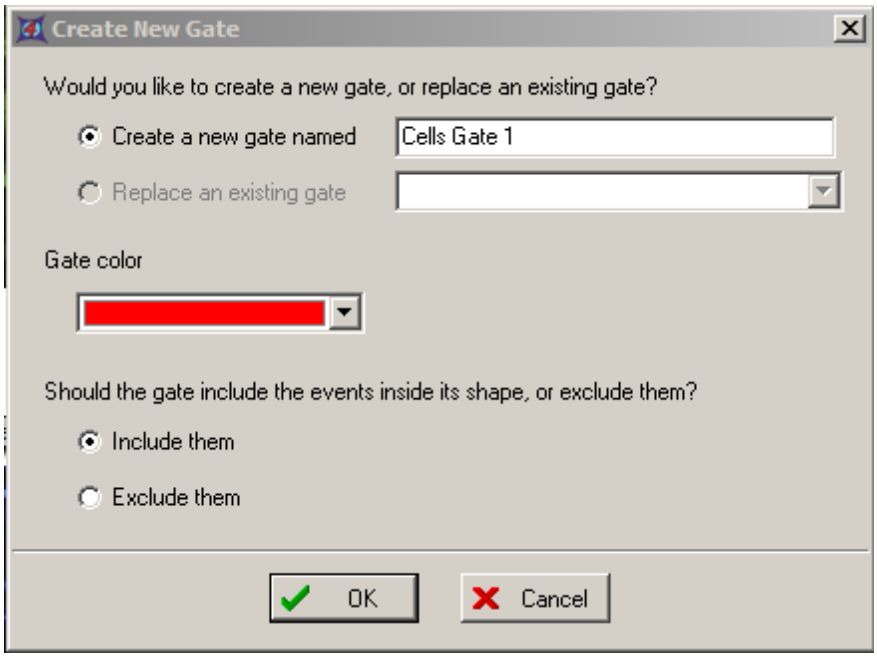

Figure T28.7 Create New Gate

16. Click **OK.** Notice that the cells included in the gate change to appear in red in both picture plots (Figure T28.8).

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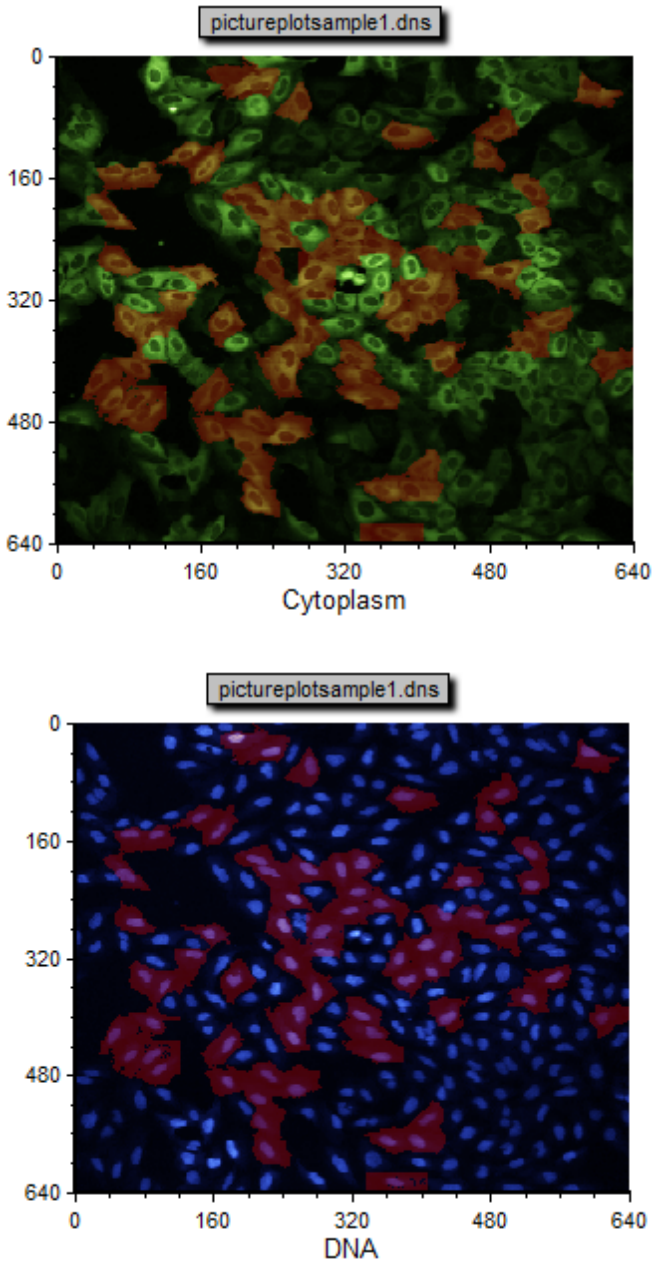

Figure T28.8 Highlighting of Cells Included in Cells Gate 1

Recall that **Unlimited Undo** is a powerful feature in FCS Express 4. Note that once you have made changes you can use CTRL+Z to undo your changes if you like.

17. Type CTRL+Z to undo the addition of **Cells Gate 1.**

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18. Repeat Steps 10-14 to recreate **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 from right to the left and down, in the direction of the red arrow in Figure T28.9 until the gate is similar to the size shown in Figure T28.9 below.

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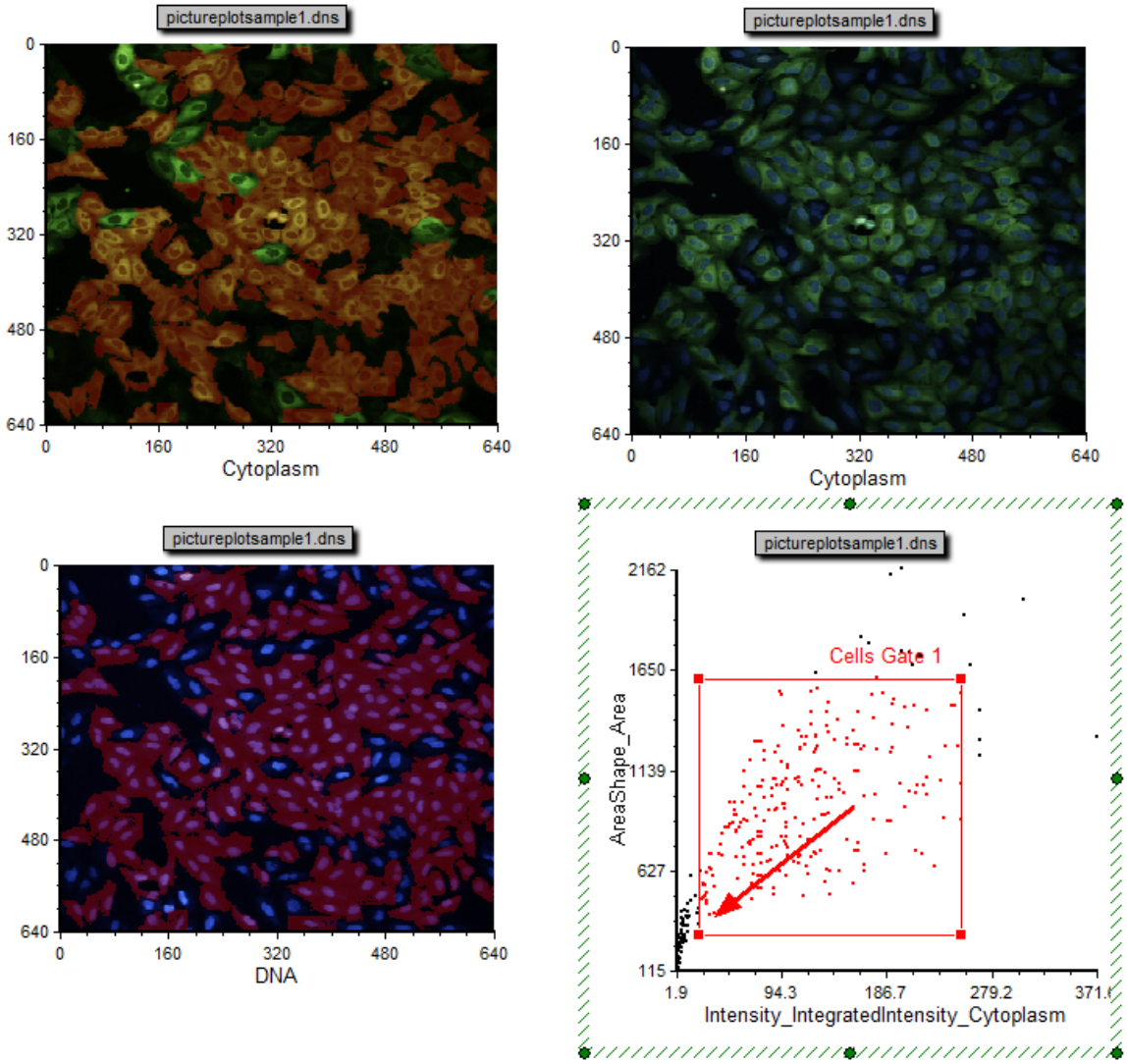

Figure T28.9 Increasing the Size of Cells Gate 1

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.

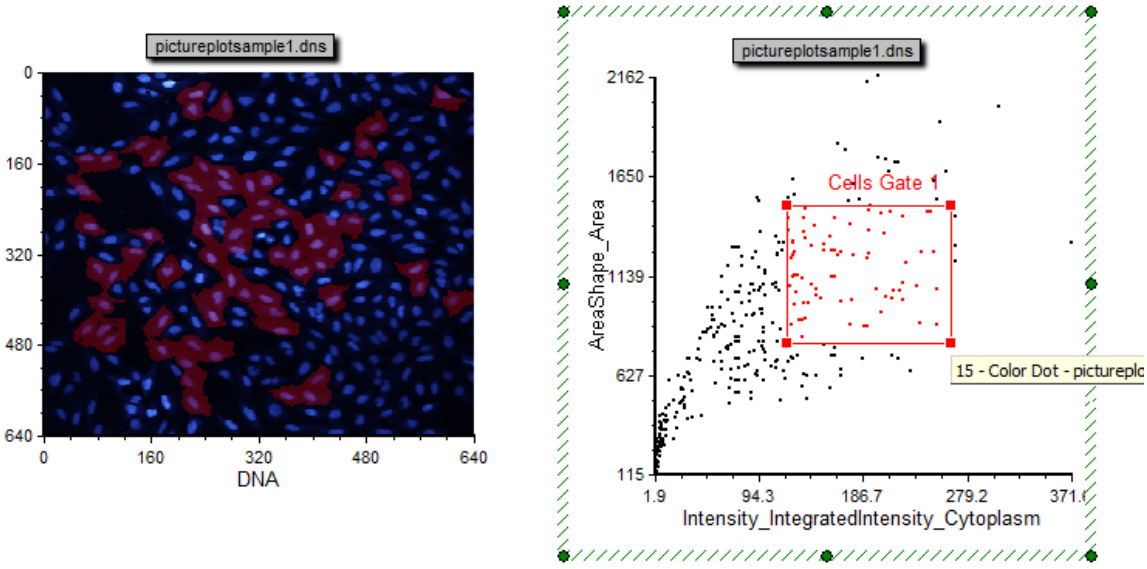

Figure T28.10 Moving Cells Gate 1

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)

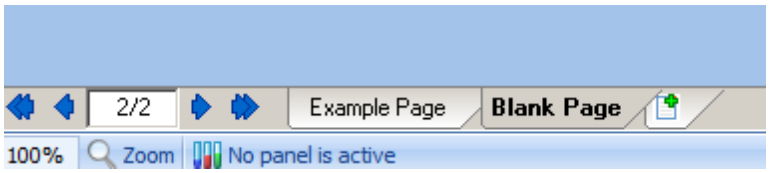

Figure T28.11 Selecting the Blank Page Tab

29. Click **Insert tab→Insert 2D plots→Picture Plot**

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30. Press and hold down the left mouse key 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).

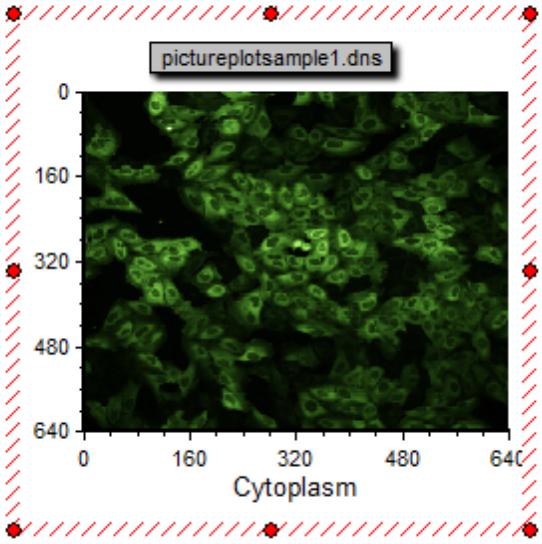

Figure T28.12 The Inserted Picture Plot

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)

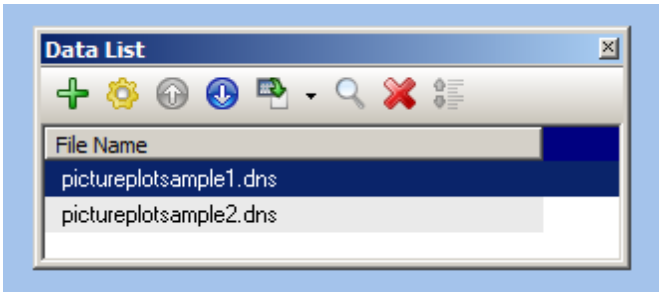

Figure T28.13 The Data List

31. Select the **pictureplotsample1.dns** Picture Plot by clicking on it.

32. Copy (CTRL+C) the **pictureplotsample1.dns** Picture Plot

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33. Paste (CTRL+V) **pictureplotsample1.dns** just below the original on the same page.

You will have two identical plots one above the other as shown in Figure T28.14.

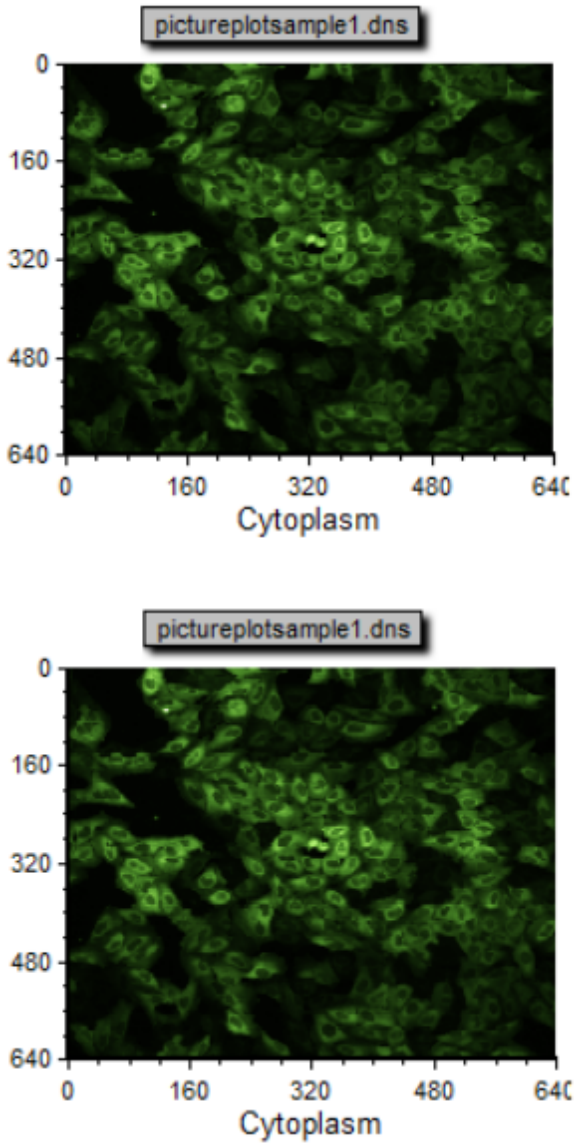

Figure T28.14 Copy and Paste a Picture Plot

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

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Figure T28.15 below.

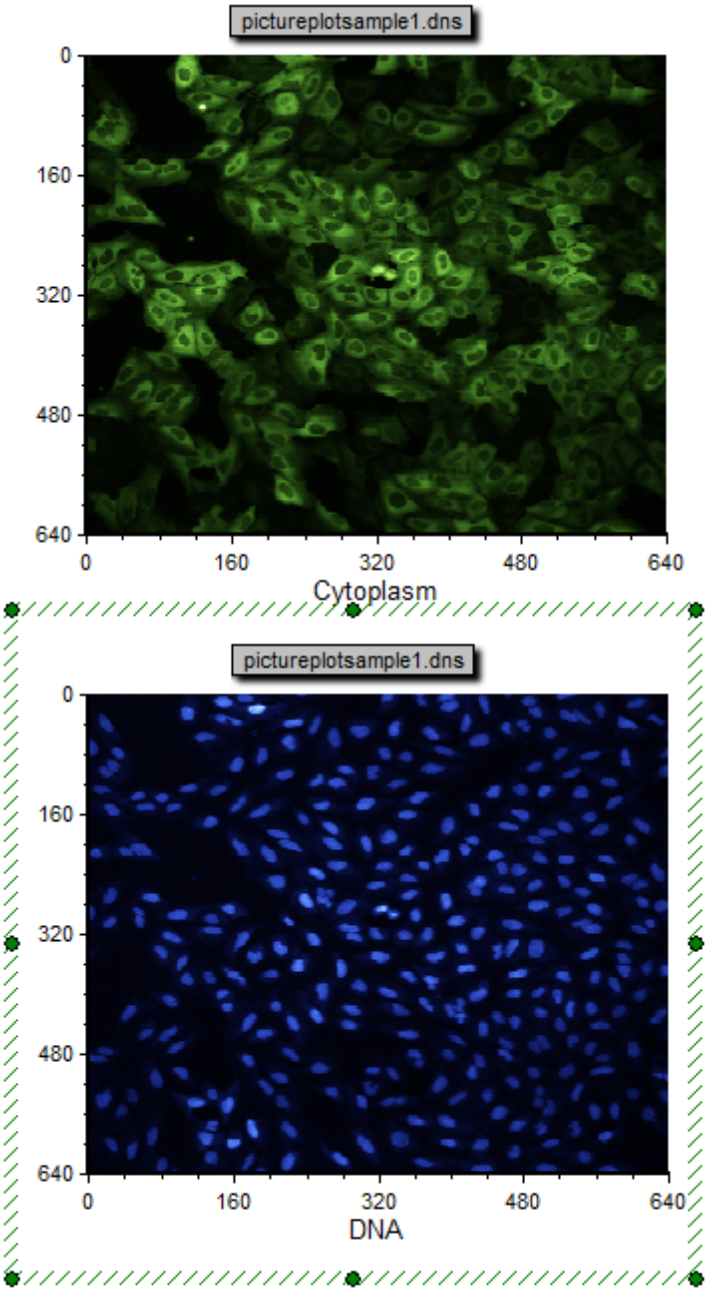

Figure T28.15 Changing the Image Parameter of a Picture Plot

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. 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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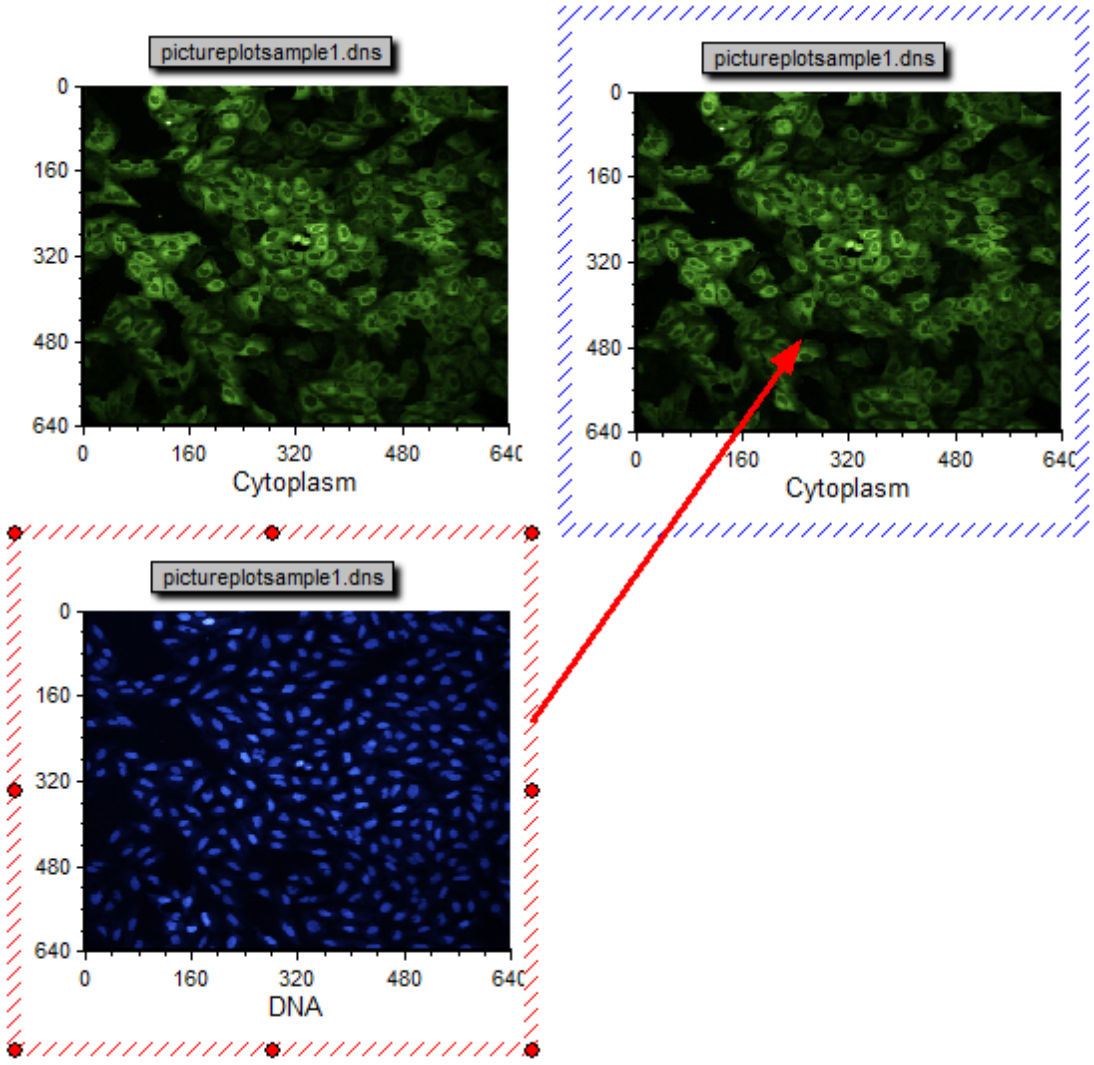

Figure T28.16 Drag and Drop a Picture Plot onto Another Picture Plot to Create a 2d Overlay

A **Paste Special** dialog will appear as see in in Figure T28.17 below.

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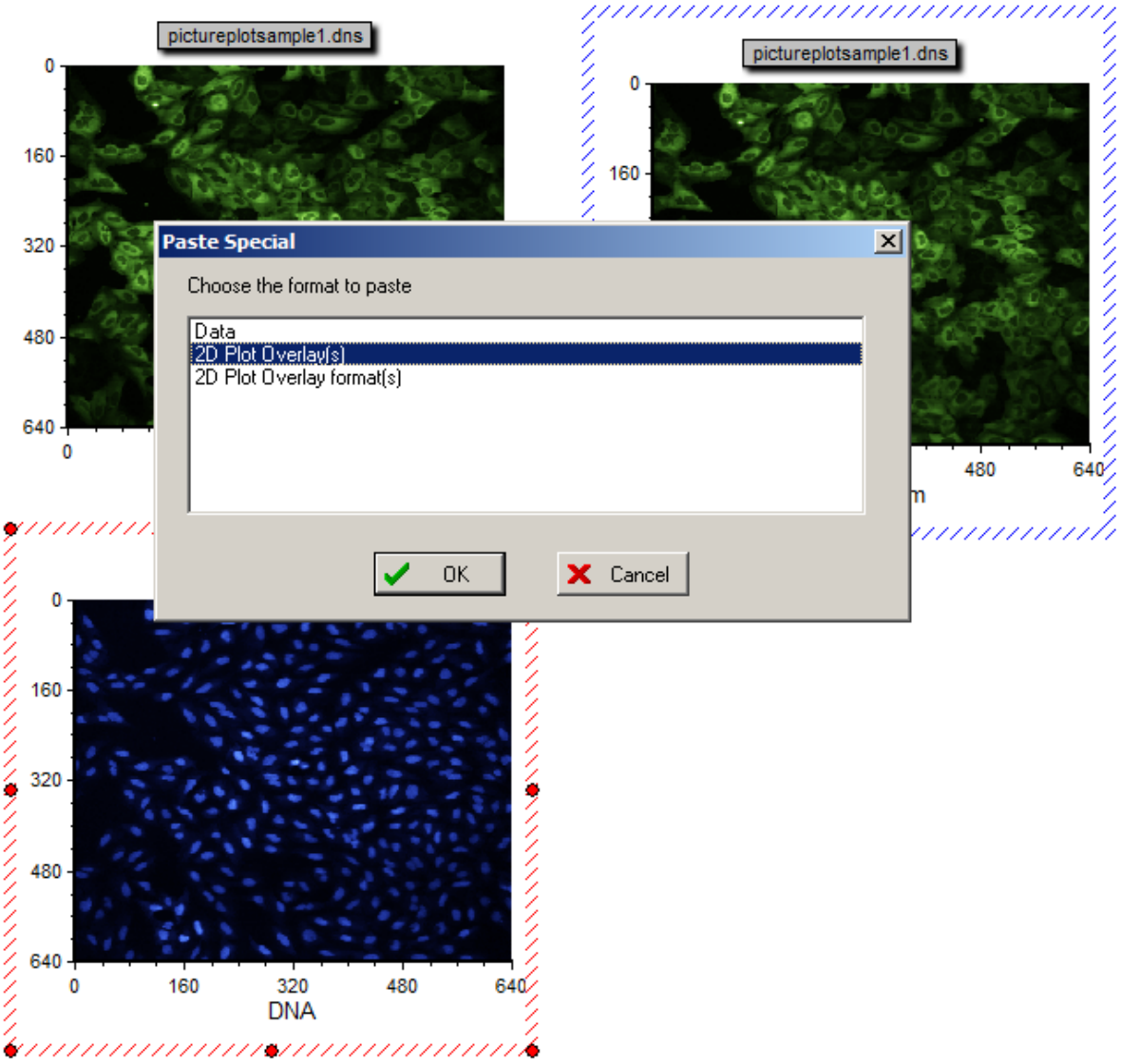

Figure T28.17 Selecting 2D Plot Overlay(s)

40. Select "**2D Plot Overlay(s)**".

41. Click **OK**.

This will create a two channel overlay as shown in Figure T28.18.

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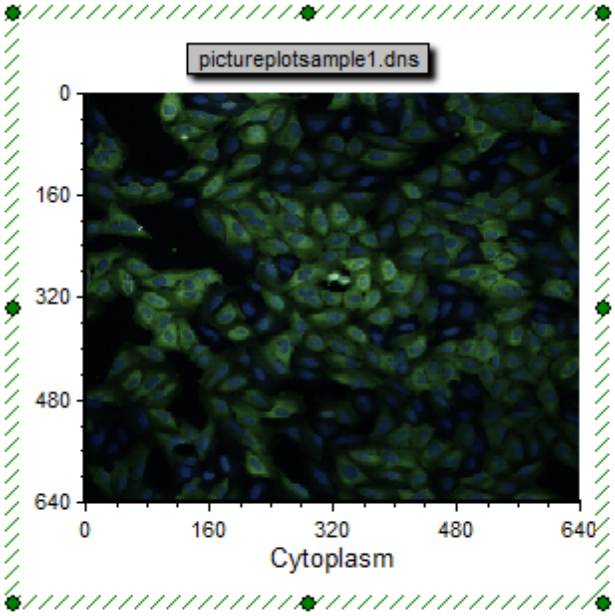

Figure T28.18 Completed 2D Overlay

Notes:

To learn other ways to load a new image data set and insert image plots see the Picture Plots 1315 tutorial.

To learn more ways to modify the data and image plots see the Formatting Plots 620 tutorial.

42. Insert a Dot Plot 607 from the **Insert Tab→Insert 2D Plots→Dot** (Figure T28.19).

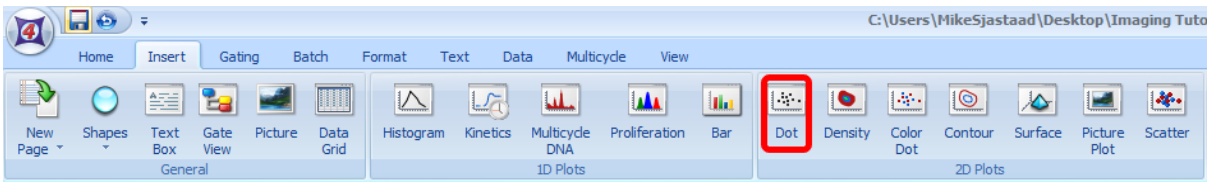

Figure T28.19 Selecting the Dot Plot Icon

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43. Right click on the Dot Plot[607] to open the **Format** Window in order to change from the default display (Figure T28.20).

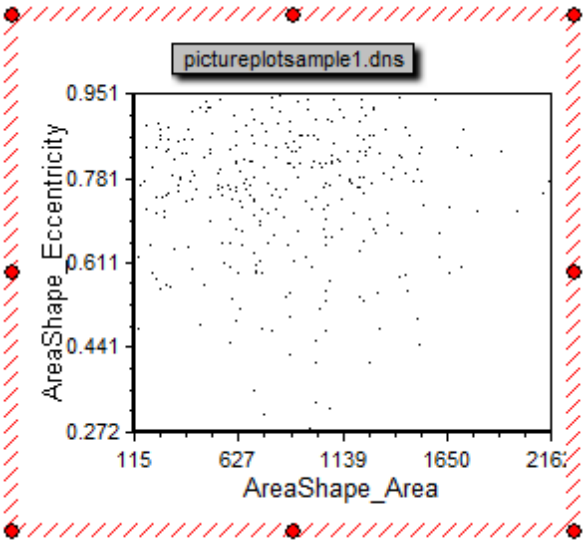

Figure T28.20 Default Configuration of Inserted Dot Plot

44. Select **Format**.

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 shown below in Figure T28.21.

48. Increase the **Dot size** from **1.0** to **10** (Figure T28.21).

49. Click **OK**.

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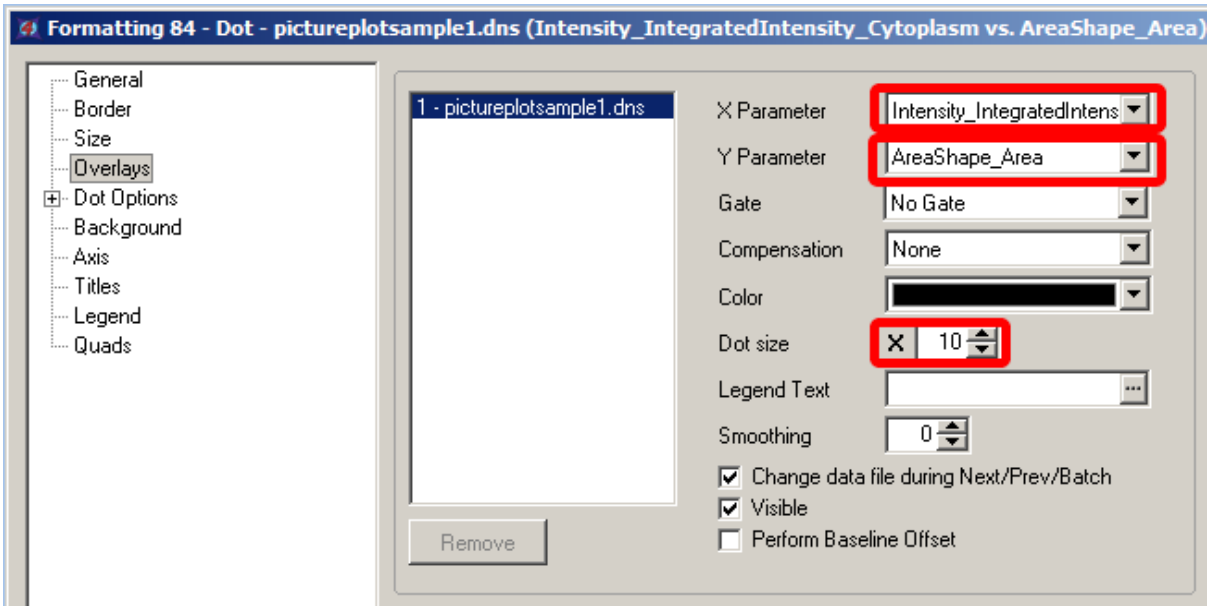

Figure T28.21 Format Settings for New Dot Plot

The Dot plot will now appear as in Figure T28.22.

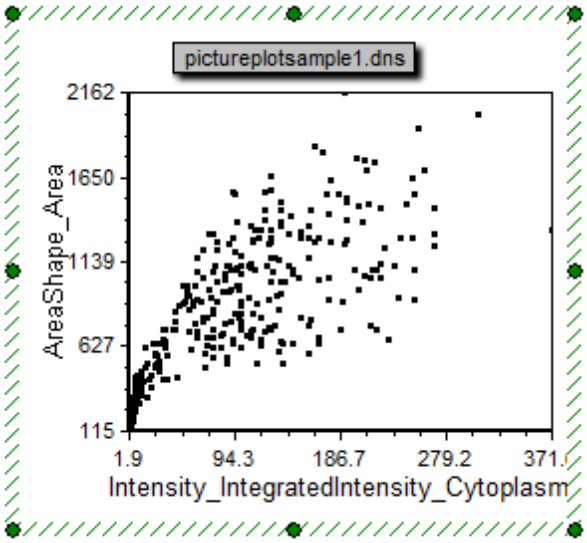

Figure T28.22 Formatted Dot Plot

50. Insert a gate[224] by selecting **Gating tab→Create Gates→Rectangle** as you have done previously in the tutorial (Steps 11-14).

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51. Name the gate[224] **Cells Gate 1**.

52. Click **OK**.

Your **Blank page** will now look the same as the Example Page as as in Figure T28.23.

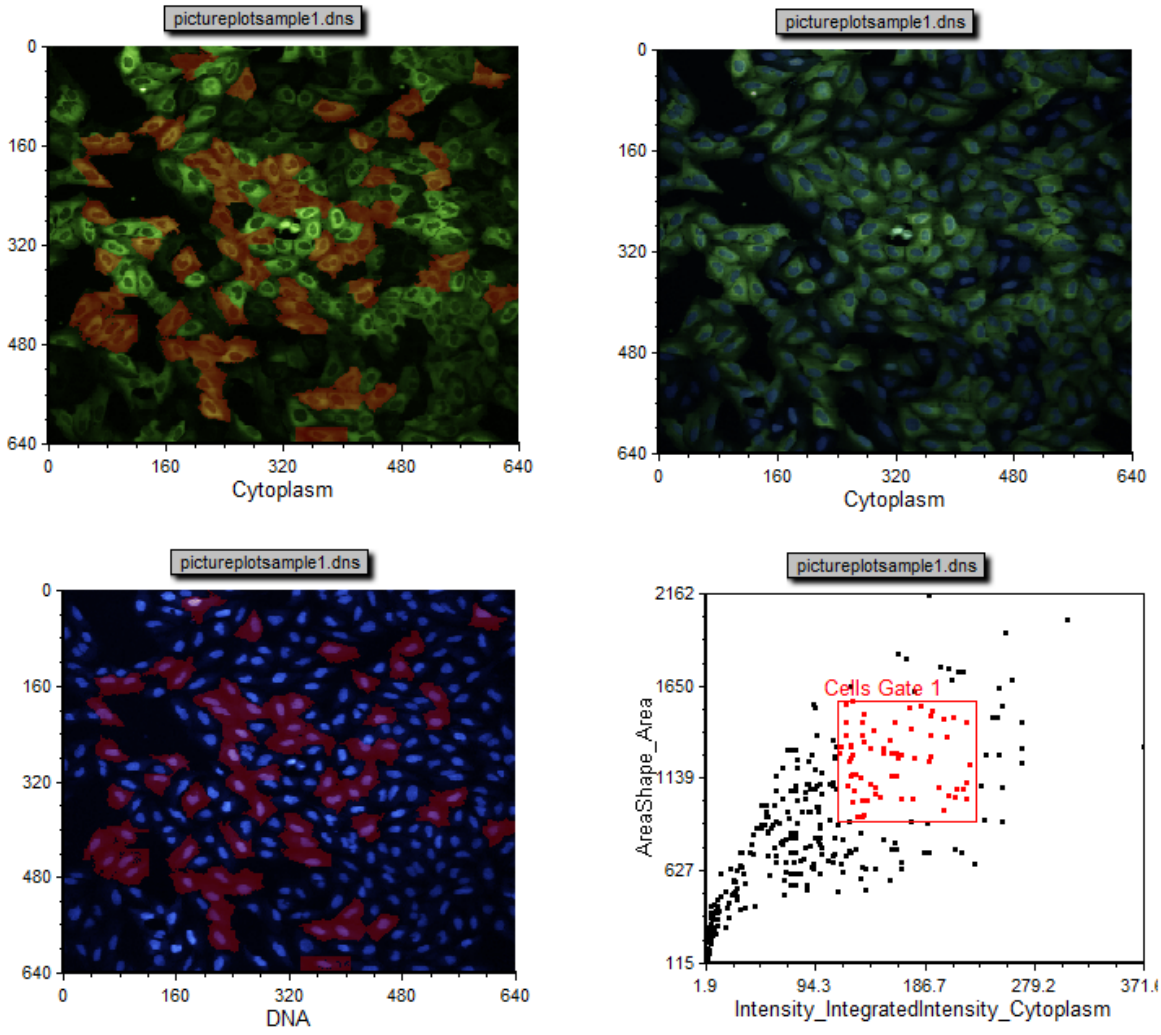

Figure T28.23 Completed Example Page

53. Close the layout, and **do not save** changes.

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Section 3: Image Gating Strategies

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 590→**Intro to Imaging Tutorial** folder.

This layout has a two Picture Plots 1315 and two color Dot Plots 607 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 1315. Note that we have also included a Gate View 202 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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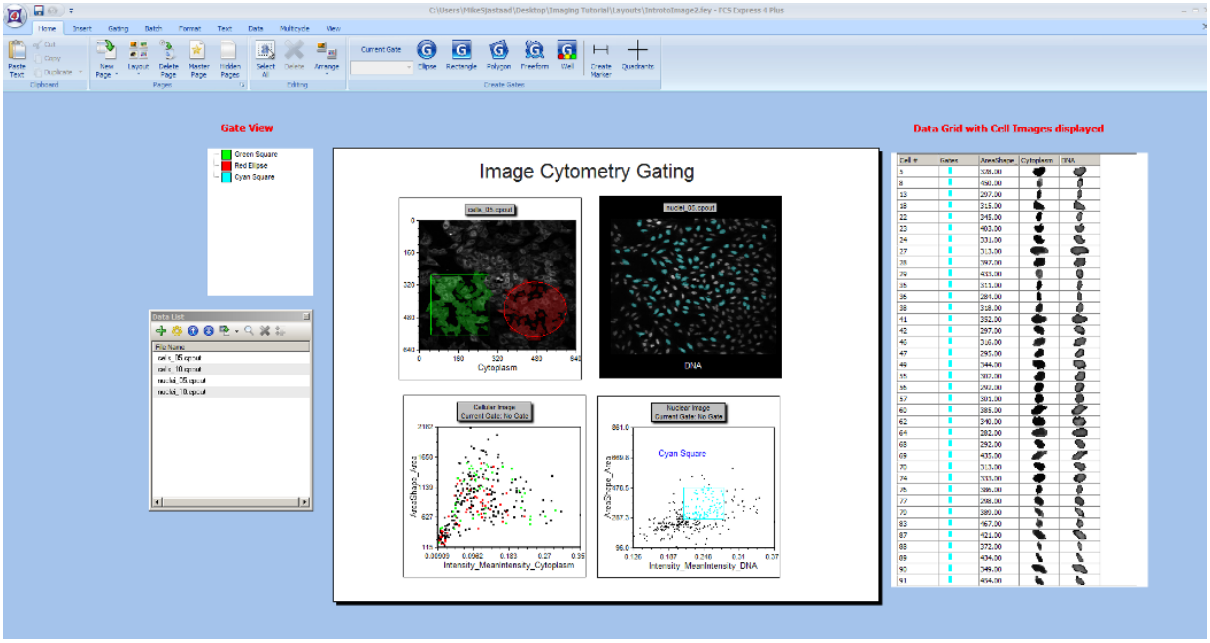
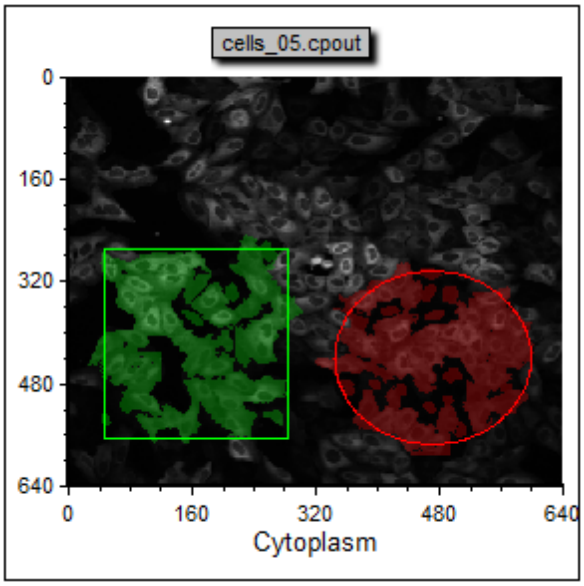


Figure T28.24 Complete Layout view for IntroToImage2.fey

55. Select and move the green square and red circle gates shown in Figure T28.25. Observe the red and green dots updating on the Dot Plot[607] below the image to indicate which cells are in the gates[224].



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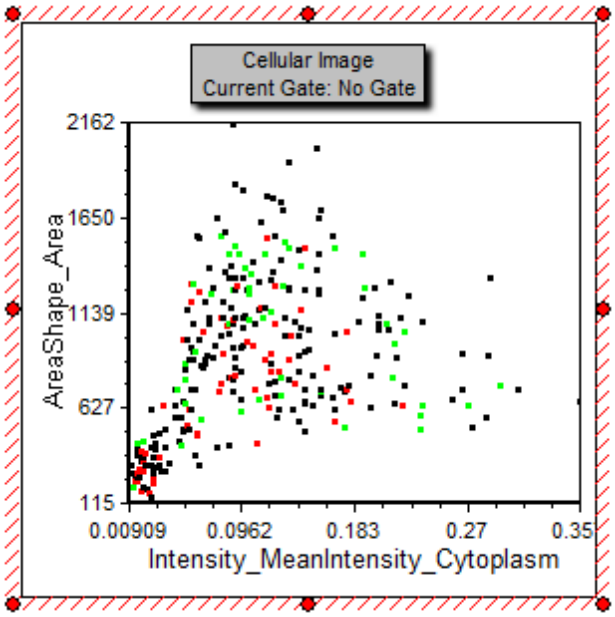
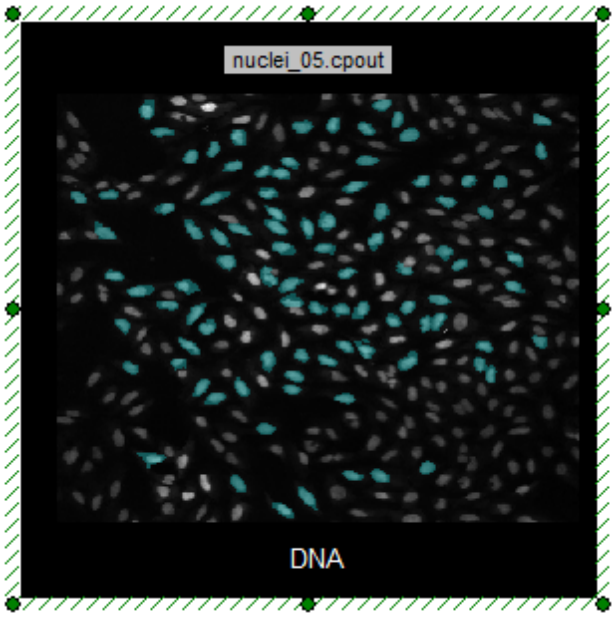


Figure T28.25 Dot Plot Displaying Gated Cells in Color

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.



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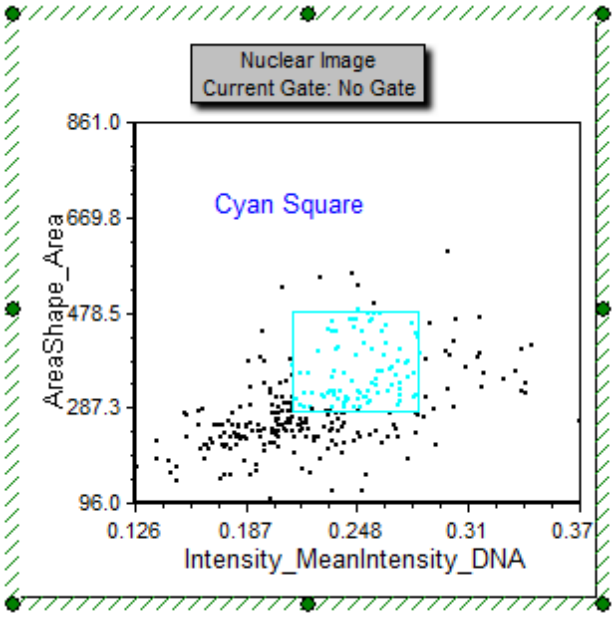

Figure T28.26 Cell Within Cyan Square Gate are Higlighted

56. Select, move, and increase the size of the **Cyan Square Gate** on the plot as demonstrated in previous steps of this tutorial (Steps 23-25).

Notice the cyan cells updating in the **DNA** image.

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.

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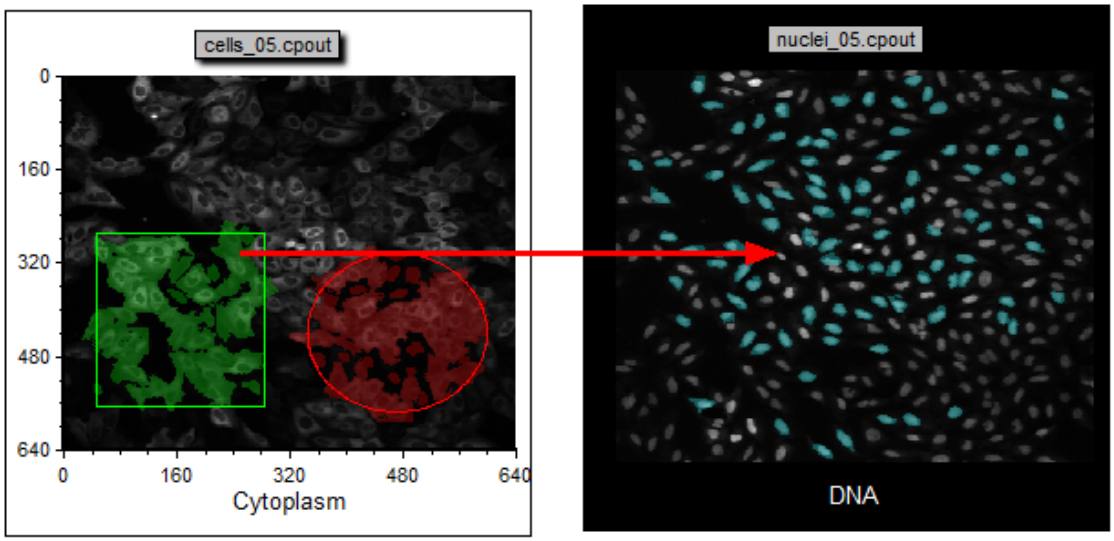

Figure T28.27 Drag and Drop a Gate to Another Plot

Notice that the only cells that appear cyan are those that fall in the **Green Square** gate. 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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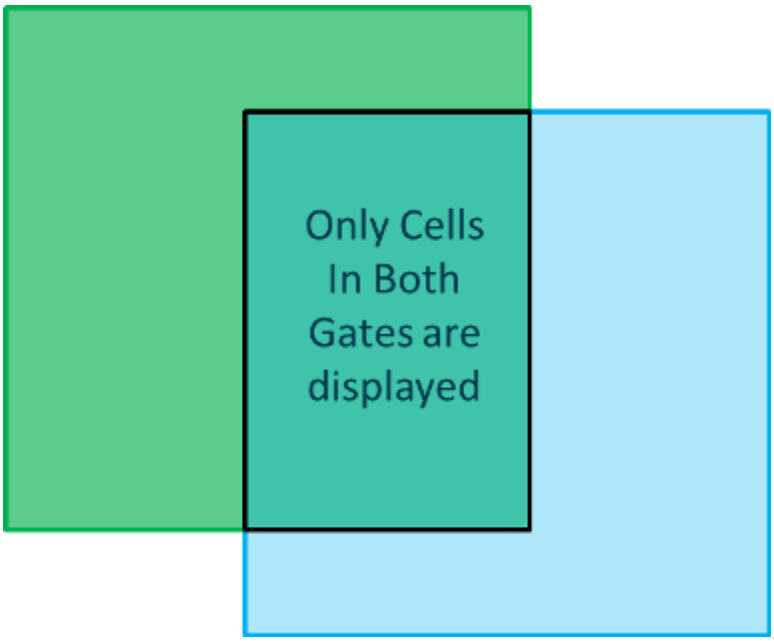

Figure T28.28a Venn Diagram of Combined Green Square and Cyan Gates

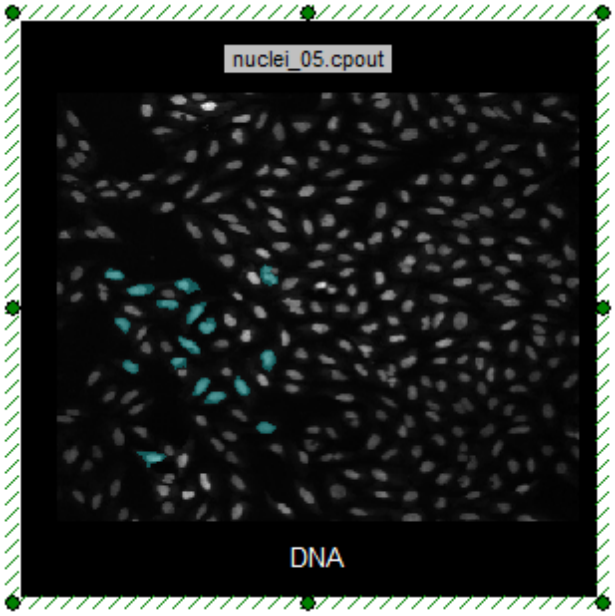

Figure T28.28b Highlighted Cells Are Within Combined Gates

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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 aate 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)

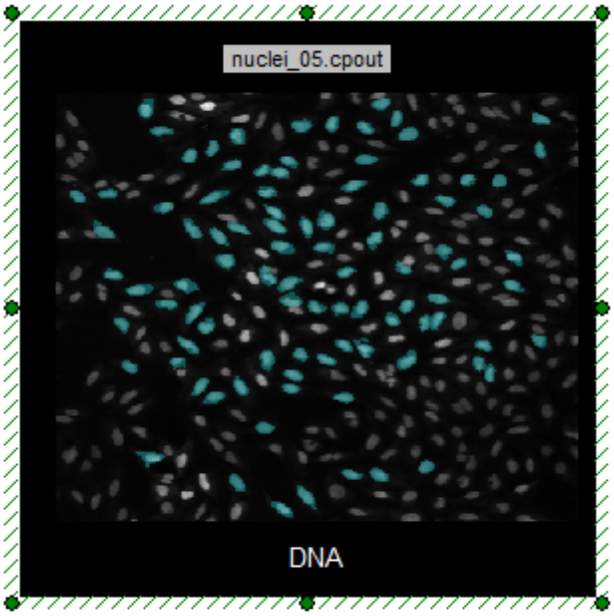

Figure T28.29 Cells within Cyan Gate Highlighted on Picture Plot

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 Grid927.

Notice the cell gallery component of the Data Grid927 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 Grid927 may not appear exactly as in Figure T28.30.

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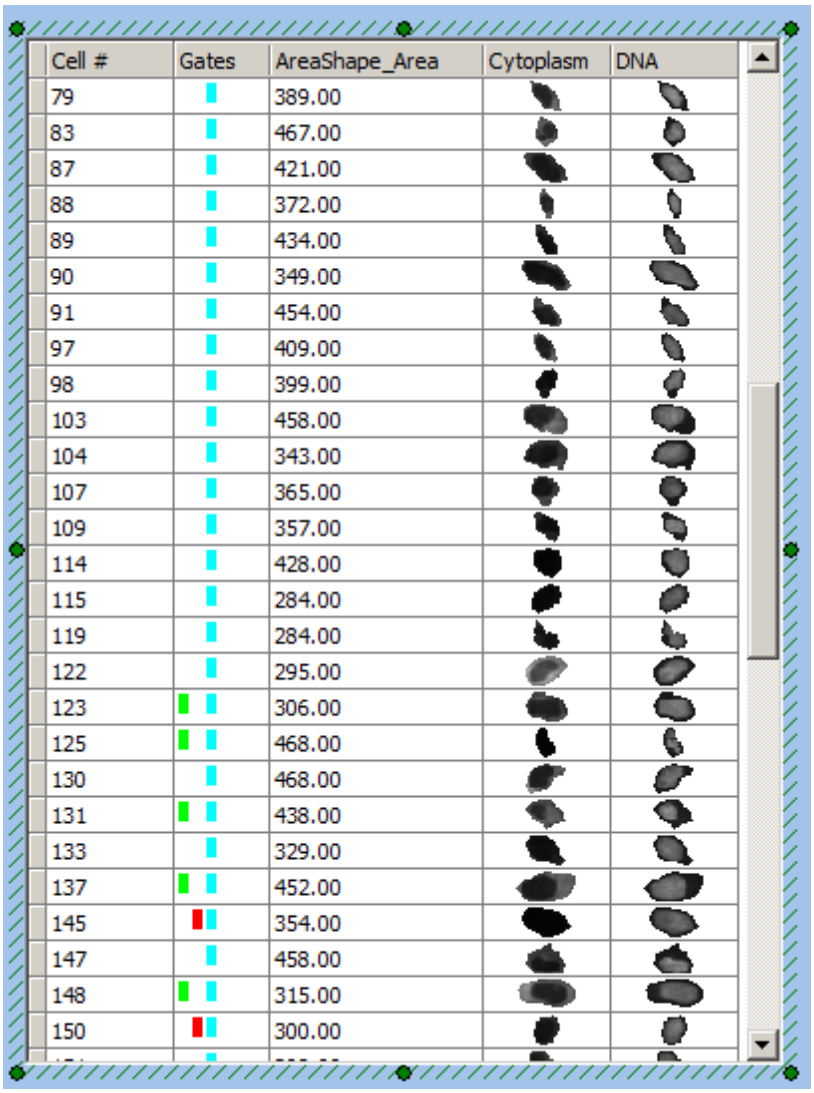

Cell #	Gates	AreaShape_Area	Cytoplasm	DNA
79		389.00		
83		467.00		
87		421.00		
88		372.00		
89		434.00		
90		349.00		
91		454.00		
97		409.00		
98		399.00		
103		458.00		
104		343.00		
107		365.00		
109		357.00		
114		428.00		
115		284.00		
119		284.00		
122		295.00		
123		306.00		
125		468.00		
130		468.00		
131		438.00		
133		329.00		
137		452.00		
145		354.00		
147		458.00		
148		315.00		
150		300.00		

Figure T28.30 Example of Data Grid with Cyan Gate Applied

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 927 displaying the cell gallery to select it.
63. Right click on the Data Grid 927 and select **Format** to open the format window for the cell gallery.

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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).

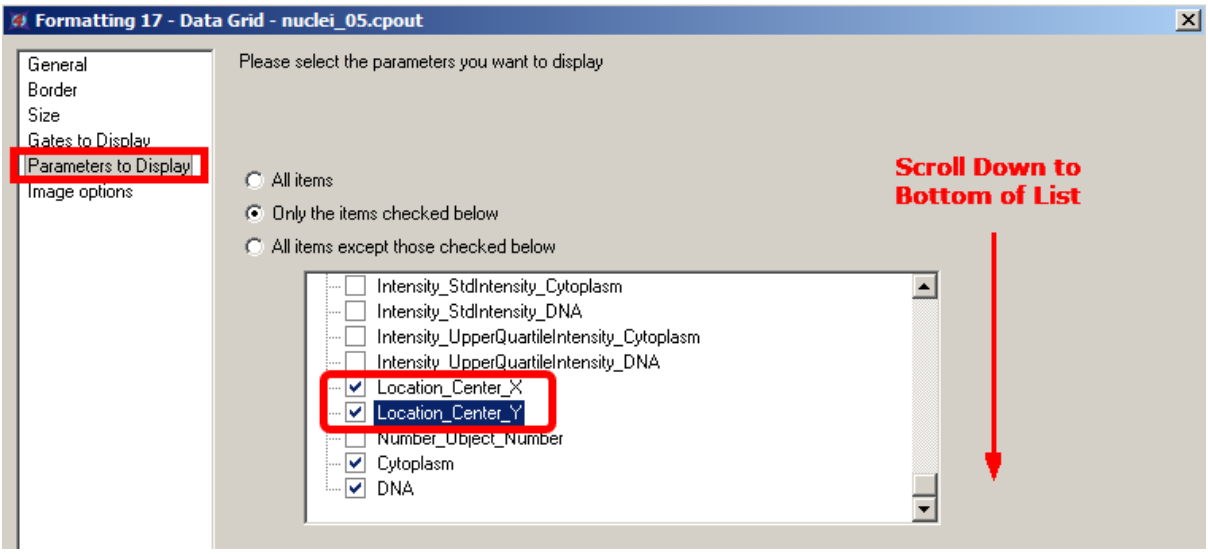

Figure T28.31 Selecting Parameters to Display in Data Grid

66. Click **OK**.

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.

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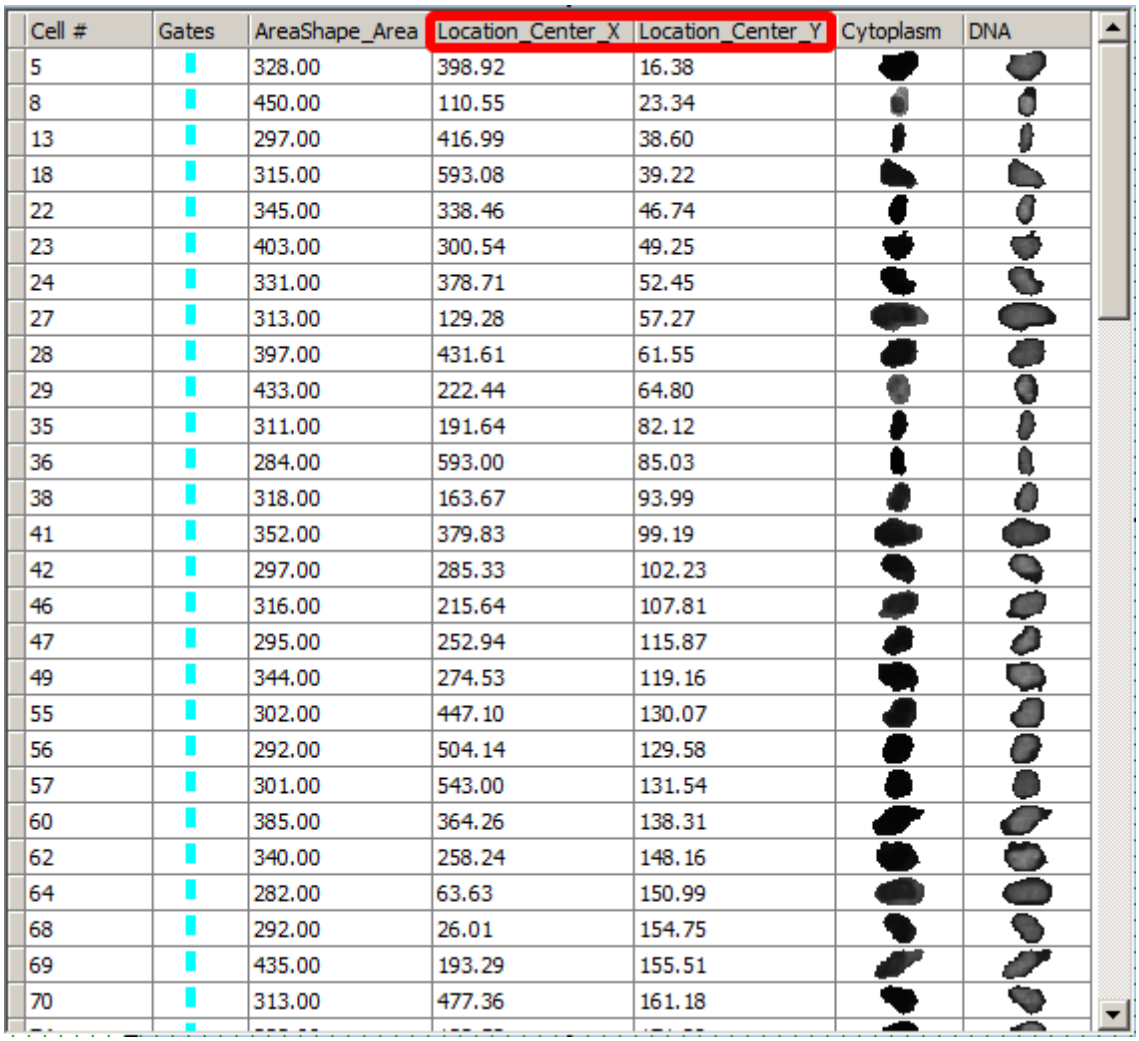

Cell #	Gates	AreaShape_Area	Location_Center_X	Location_Center_Y	Cytoplasm	DNA
5		328.00	398.92	16.38		
8		450.00	110.55	23.34		
13		297.00	416.99	38.60		
18		315.00	593.08	39.22		
22		345.00	338.46	46.74		
23		403.00	300.54	49.25		
24		331.00	378.71	52.45		
27		313.00	129.28	57.27		
28		397.00	431.61	61.55		
29		433.00	222.44	64.80		
35		311.00	191.64	82.12		
36		284.00	593.00	85.03		
38		318.00	163.67	93.99		
41		352.00	379.83	99.19		
42		297.00	285.33	102.23		
46		316.00	215.64	107.81		
47		295.00	252.94	115.87		
49		344.00	274.53	119.16		
55		302.00	447.10	130.07		
56		292.00	504.14	129.58		
57		301.00	543.00	131.54		
60		385.00	364.26	138.31		
62		340.00	258.24	148.16		
64		282.00	63.63	150.99		
68		292.00	26.01	154.75		
69		435.00	193.29	155.51		
70		313.00	477.36	161.18		

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 193".

Next Topic 1406

34.29.2 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

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- 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
- Optimize gate settings for the nuclear translocation assay
- See how FCS Express 4 "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 590 →**Intro to Imaging Tutorial** folder. This layout contains a detailed pre-configured template. Please complete the Getting started with Image Cytometry 1374 section of this Tutorial before beginning if you are not familiar with FCS Express 4 Image Cytometry.

Section 1: Image Gating Strategies for Data Analysis with the High Content Module

Image cytometry is often carried out on many images aquired 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 482. 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 1188 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 4 Image Cytometry.

1. Load the **IntroToImage3.fey** layout from the Tutorial Sample Data archive

   590 →**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 482 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

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green (low) to red (high) color scale.

Below the Heat Map[482] 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[1315] in the upper right displays an image of the cytoplasmic stain from well A-01. and the Dot Plot[607] below it displays the integrated cytoplasmic intensity plotted against area for the cells in that image. On the far left a "Gate View[202]" 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.

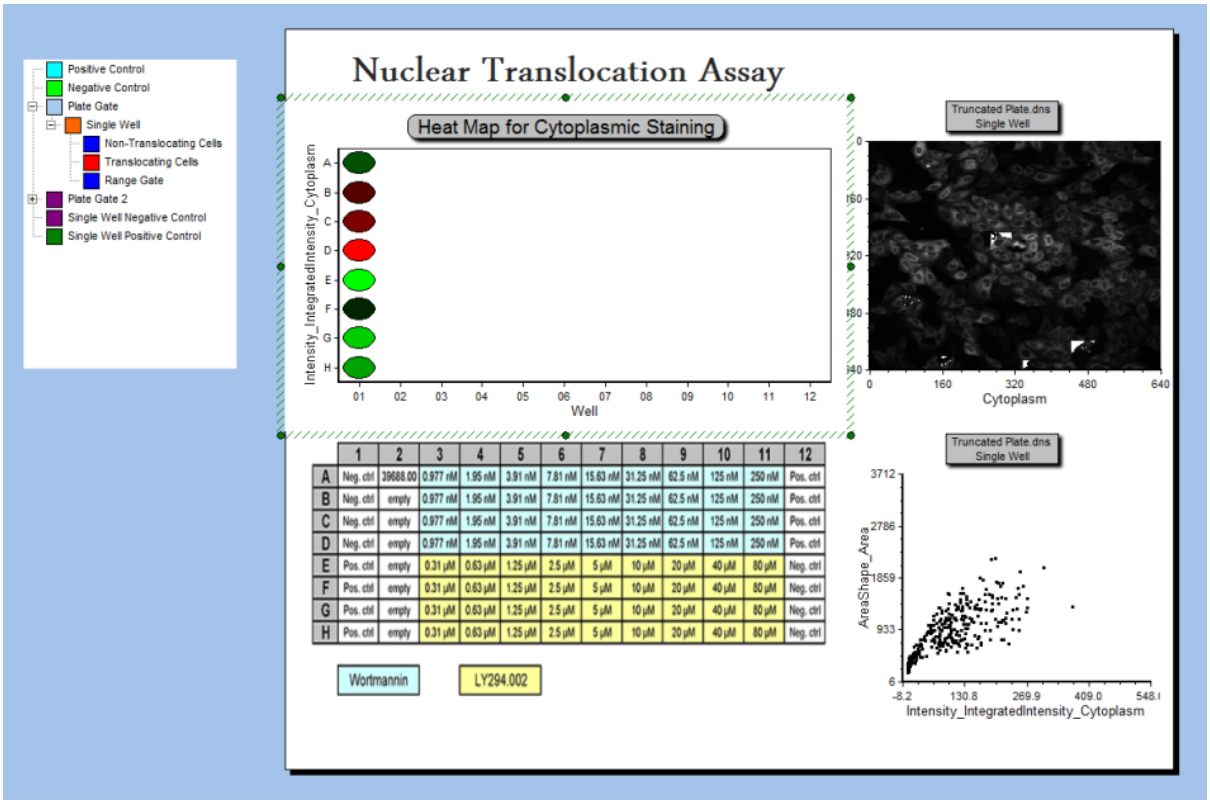

Figure T28.33 The Experiment Design Layout Page

2. Right click on the Heat Map[482].

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

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by changing the **Statistic parameter** drop down and you can choose the displayed 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**.

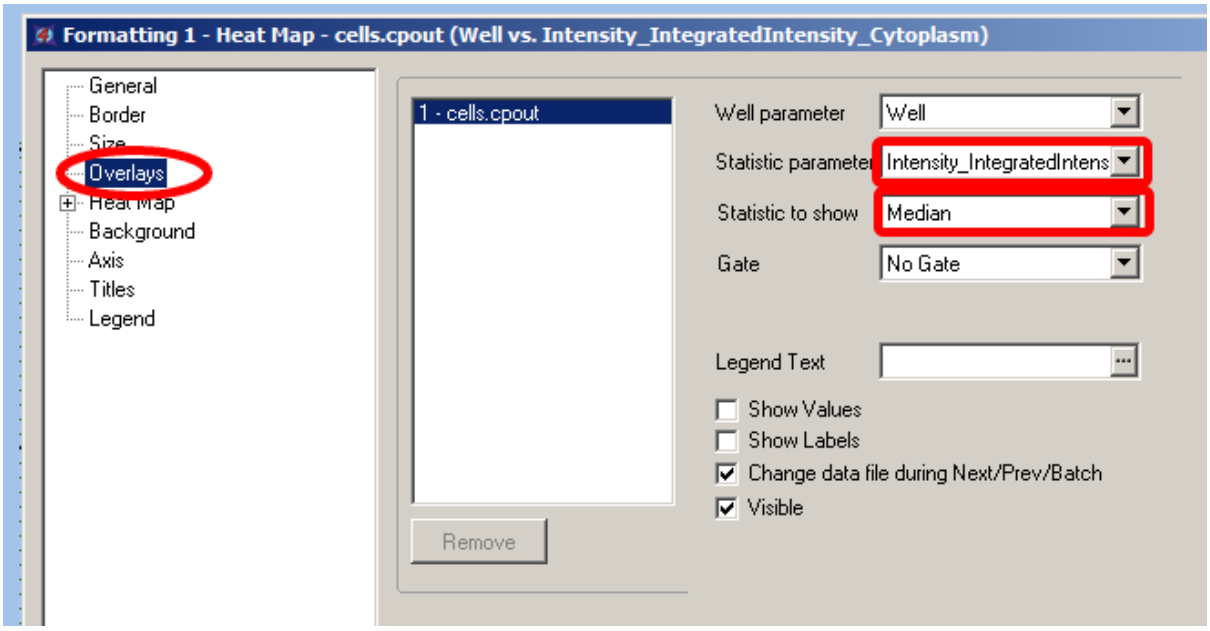

Figure T28.34 Format Options for Heat Map

5. Left Click on the "**+**" sign next to Heat Map[482] category to expand options for the heat map display (Figure T28.35).

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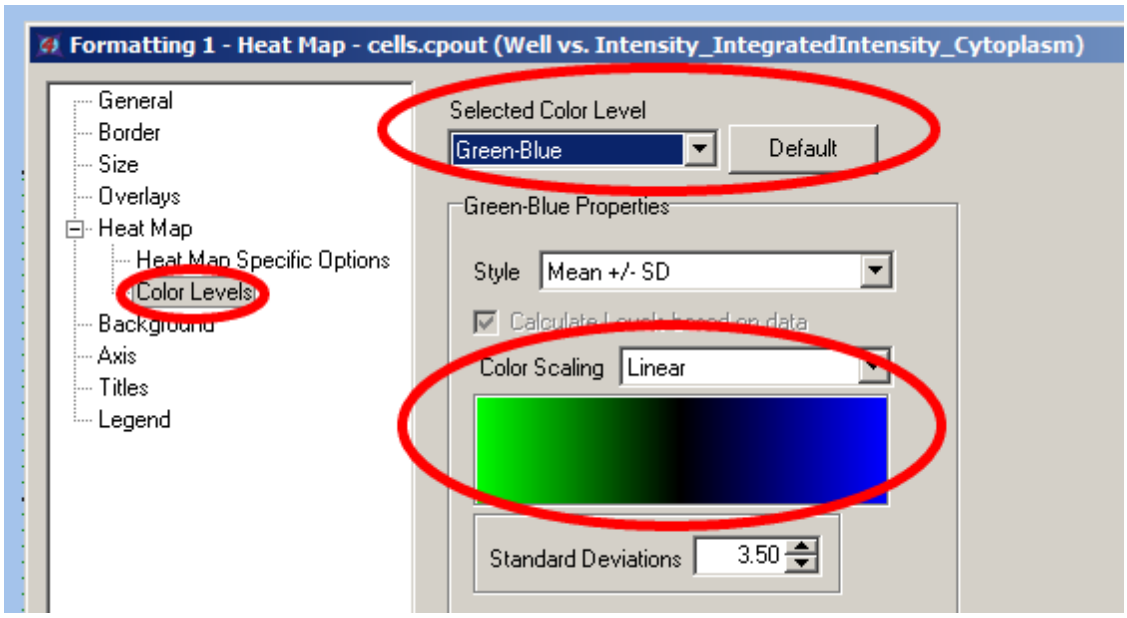

Figure T28.35 Color Formatting Option for Heat Maps

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.

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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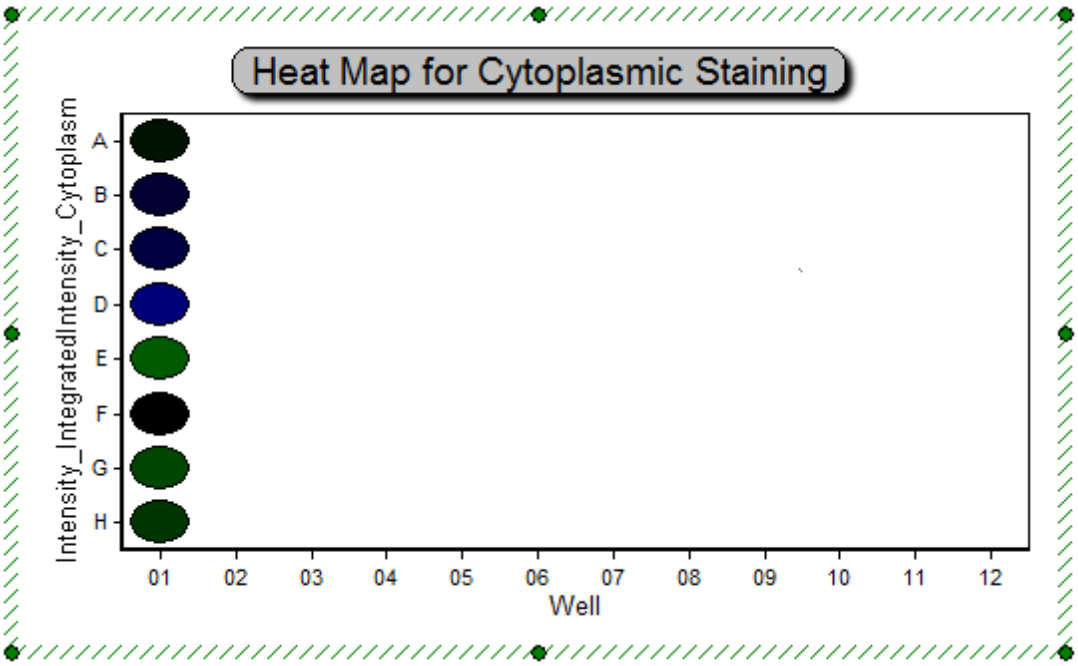

Figure T28.36 Green-Blue Scaled Heat Map

9. Right click on the Heat Map[482].

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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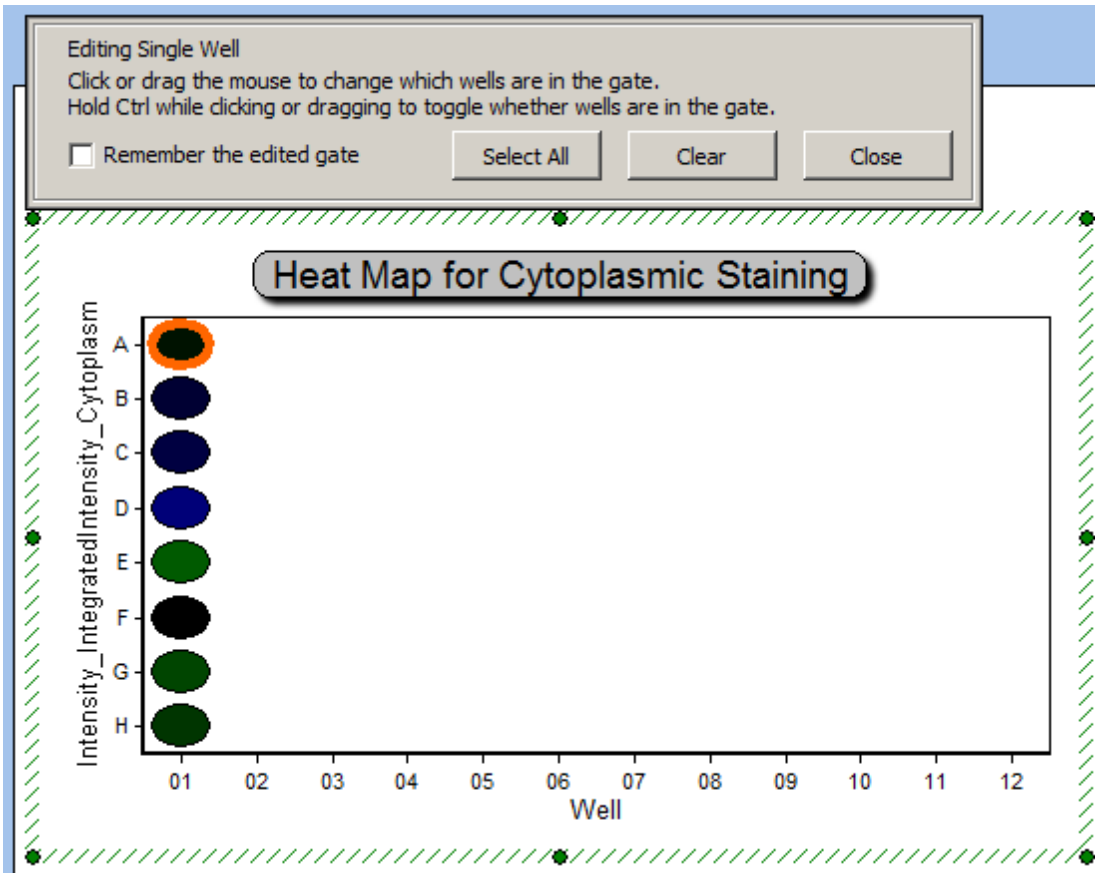

Figure T28.37a Activating Single Well Gate on Heat Map

	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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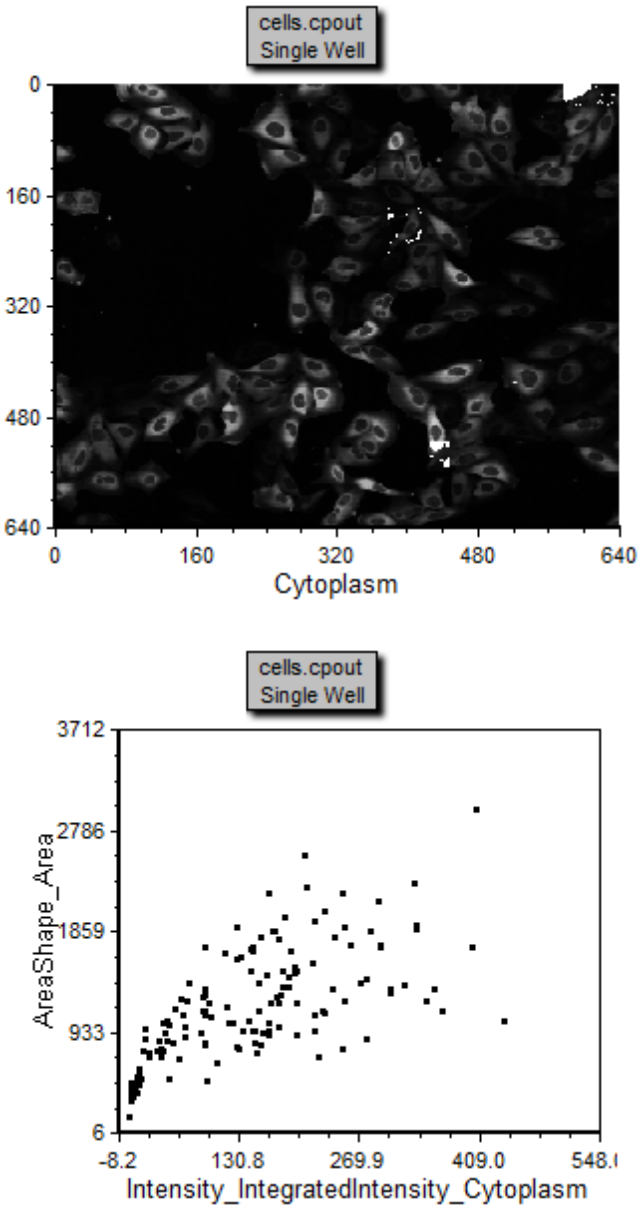

Figure T28.38a Well B-01

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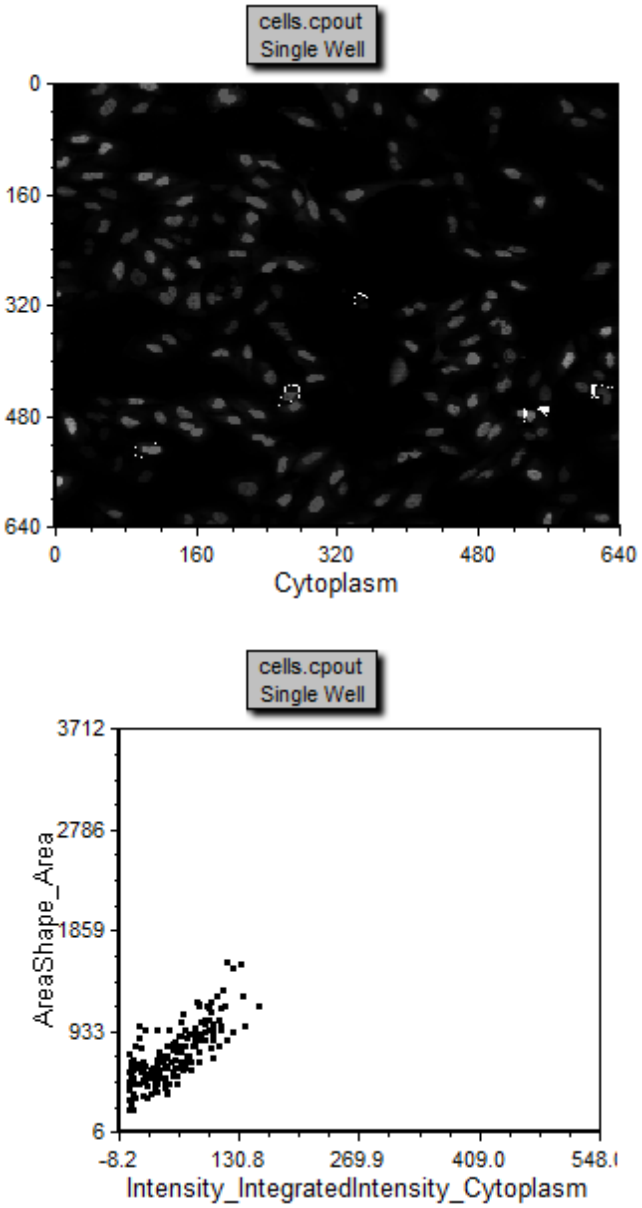

Figure T28.38b Well E-01

Choosing Optimal Parameters

13.Select the **Choosing Parameters** page.

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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 **Intensity_IntegratedIntensity_Cytoplasm** against **Intensity_IntegratedIntensity_DNA**. On this plot cells tend to be a loosely clustered distribution (Figure T28.39).

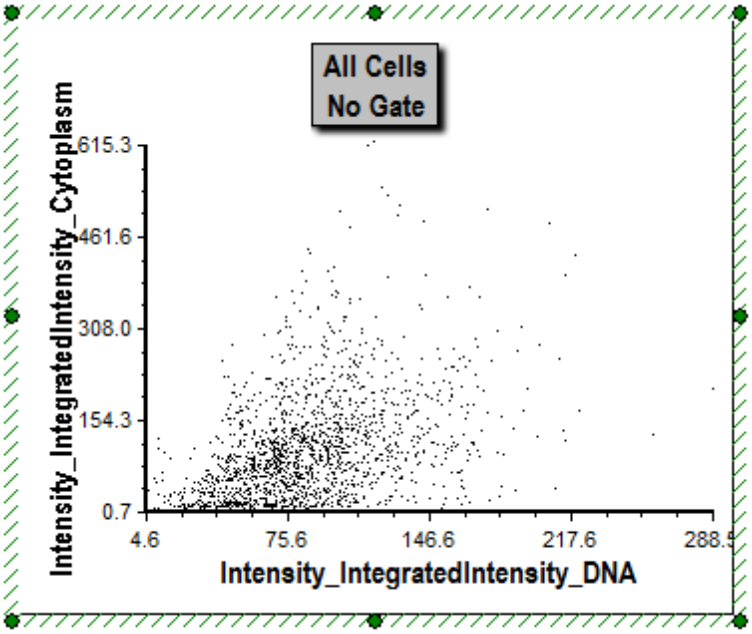

Figure T28.39 Choosing Parameters Using a Dot Plot

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.**

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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 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.

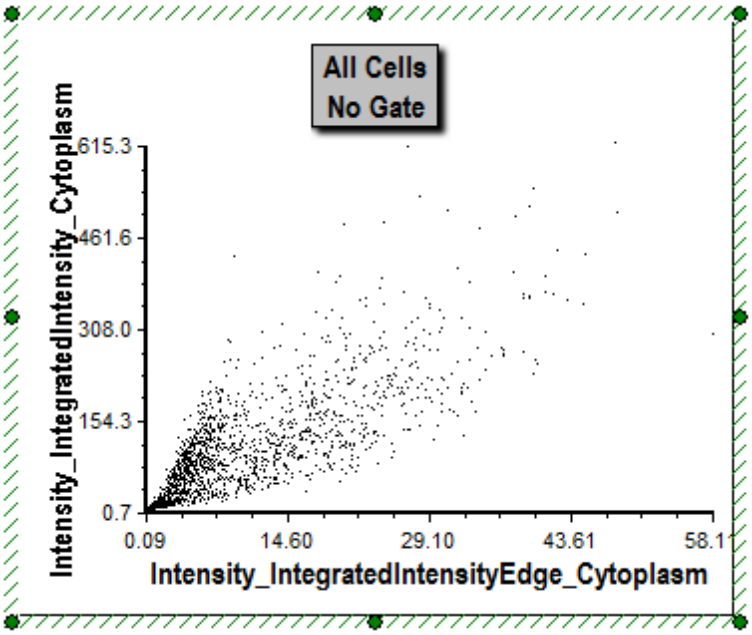

Figure T28.40 Choosing Alternate Parameters

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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 different population distributions. These have been used previously to help define gates for the tutorial.

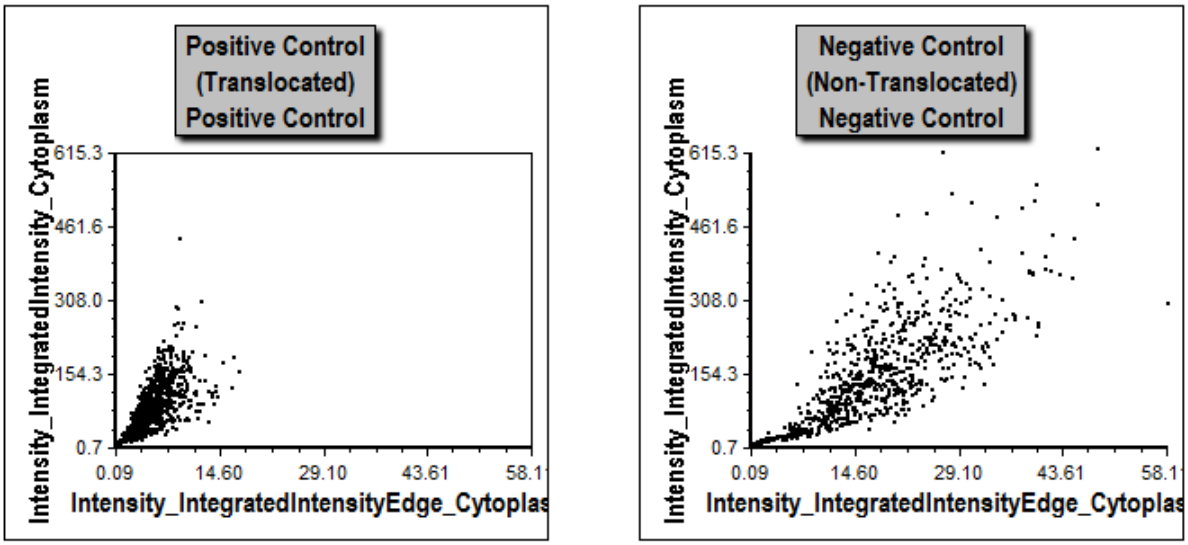

Figure T28.41 Comparison of Distributions of Positive and Negative Control Cells

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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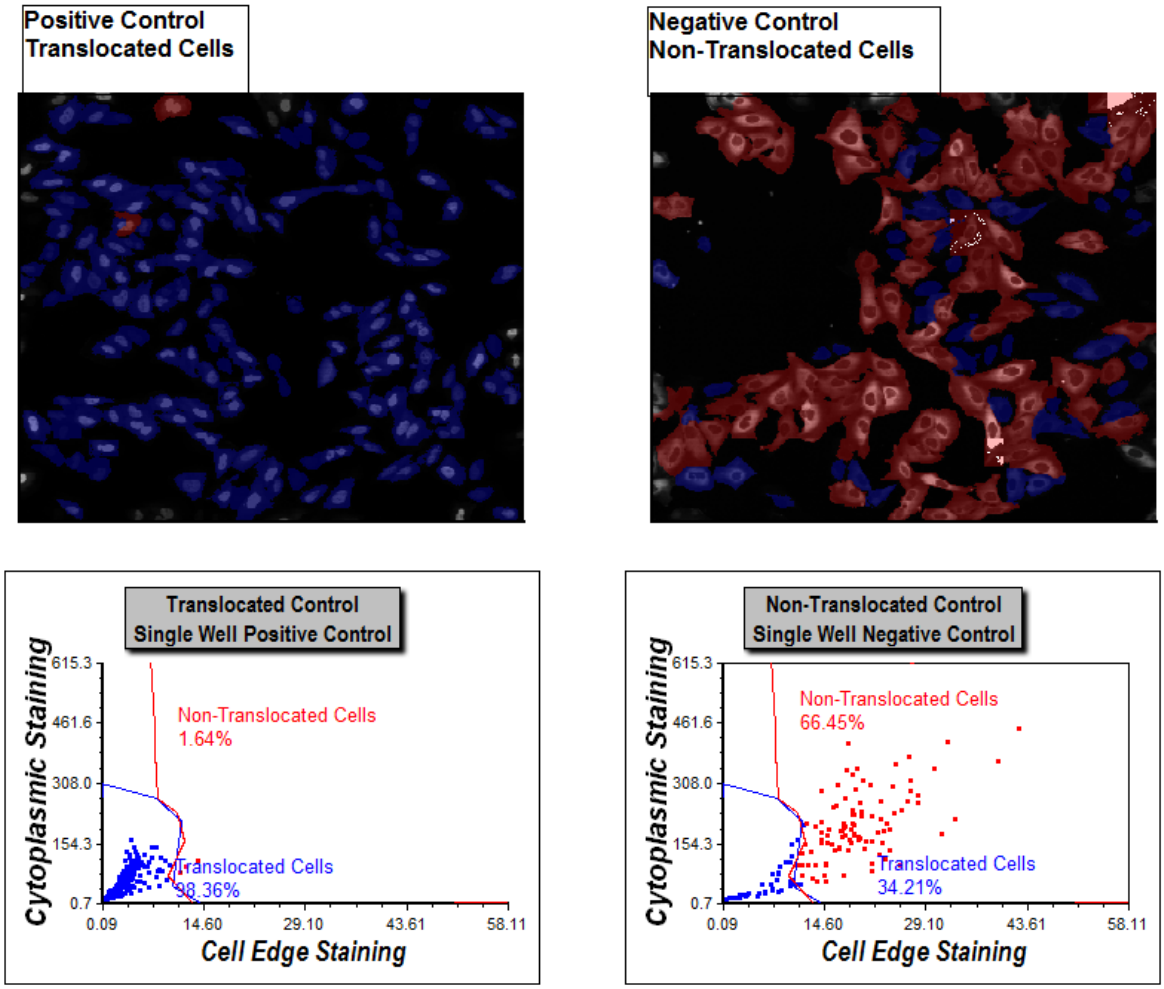

Figure T28.42 Display of Gates on Dot Plots and Gated Cell in Picture Plots

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. 98.36% of cells are **Translocated Cells** (blue) in the Positive Control picture plot on the left and only 1.64% 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 66.45% of the cells reside in the **Non-Translocated** gate and in the image they primarily exhibit cytoplasmic staining with an absence of nuclear staining. 34.21% 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 "Tokens691" in FCS Express. Tokens691 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[691] can be used in many locations in a layout to display information from other locations in the layout. The values or text of Tokens[691] update automatically as gates are repositioned or new data sets are loaded. We will not discuss Tokens[691] in detail here. For more information on creating and using "Tokens[691]" see the tutorial " Text Boxes and Tokens[328]" or review the Manual section entitled "Using Tokens[326]".

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.

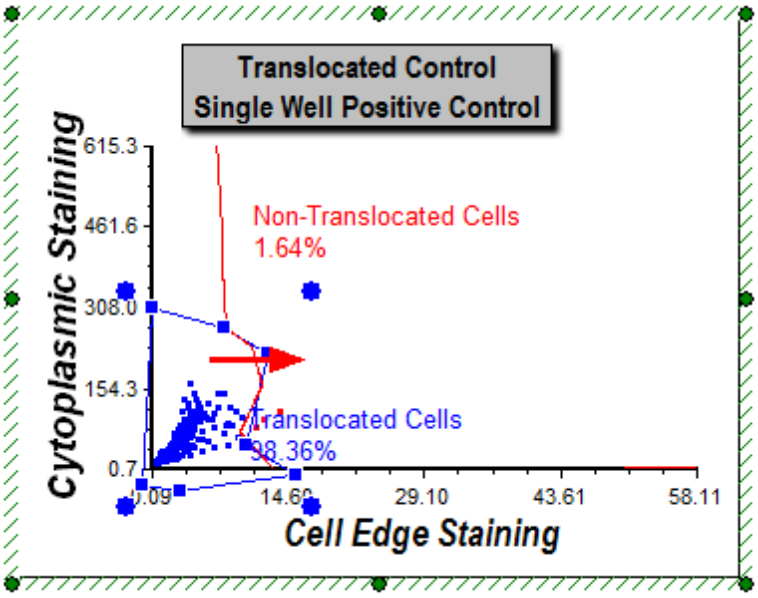

Figure T28.43 Moving the Translocated Cells Gate

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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.

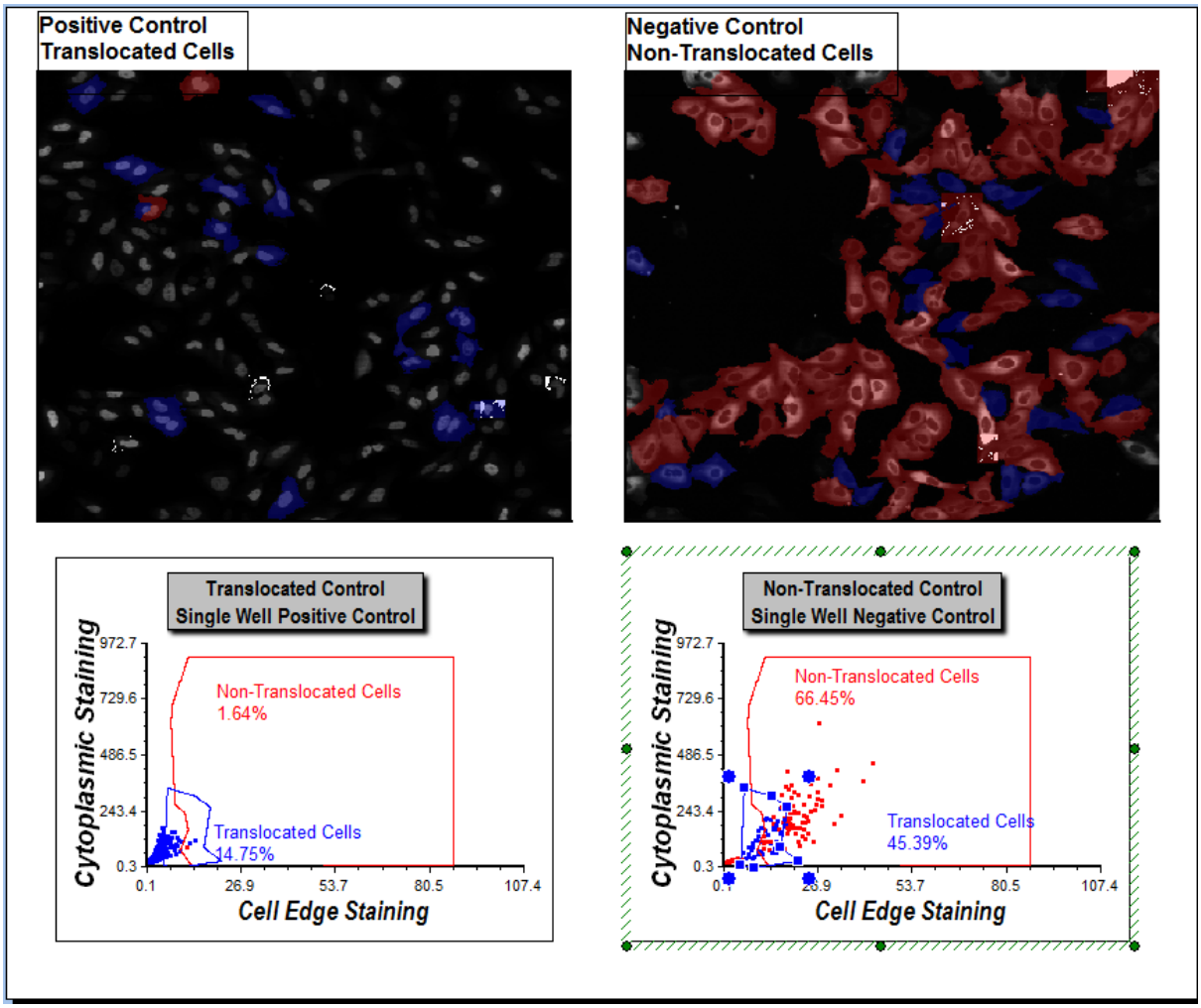

Figure T28.44 All Plots Update Following Repositioning of Translocated Cells Gate

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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.

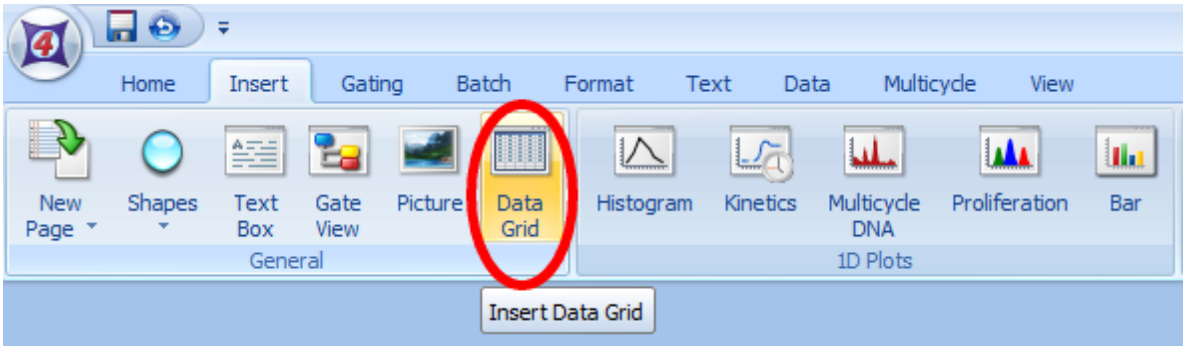

Figure T28.45 Inserting a Data Grid

26.Right click on the **Data Grid**.

27.Select **Format**. The **Formatting** dialog will appear (Figure T28.46).

28.Select the **Parameters to Display** category.

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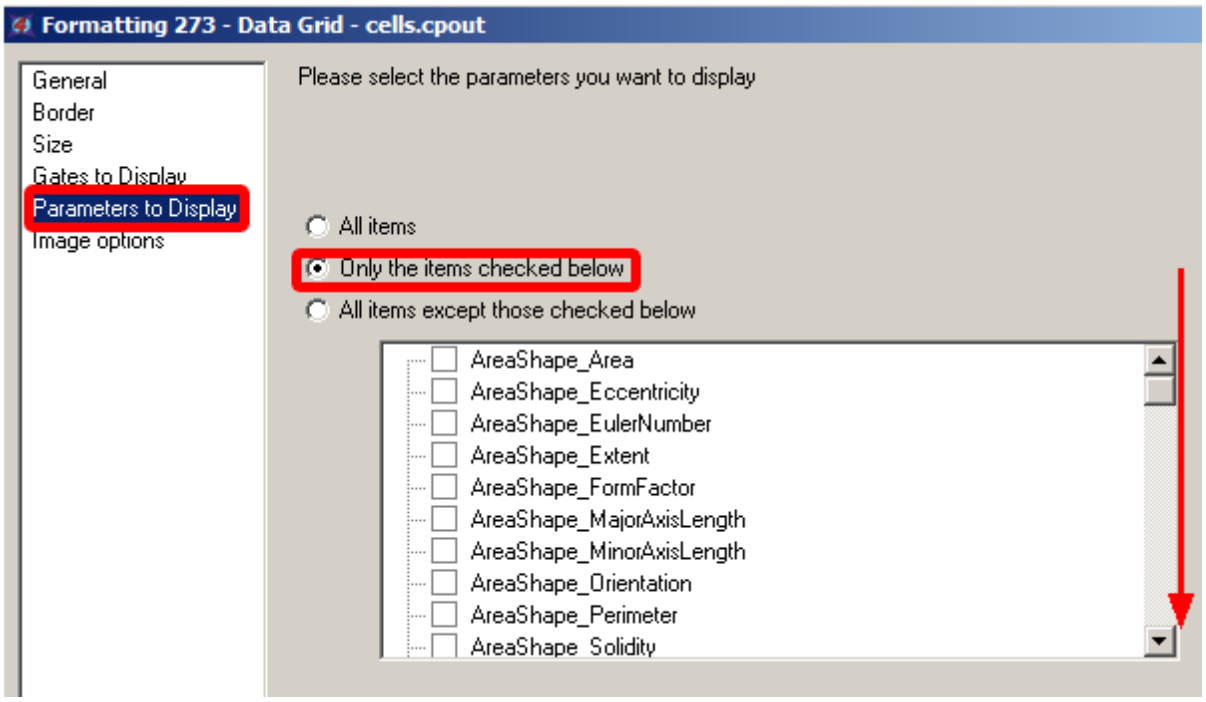

Figure T28.46 Selecting Parameters to Display in Data Grid

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).

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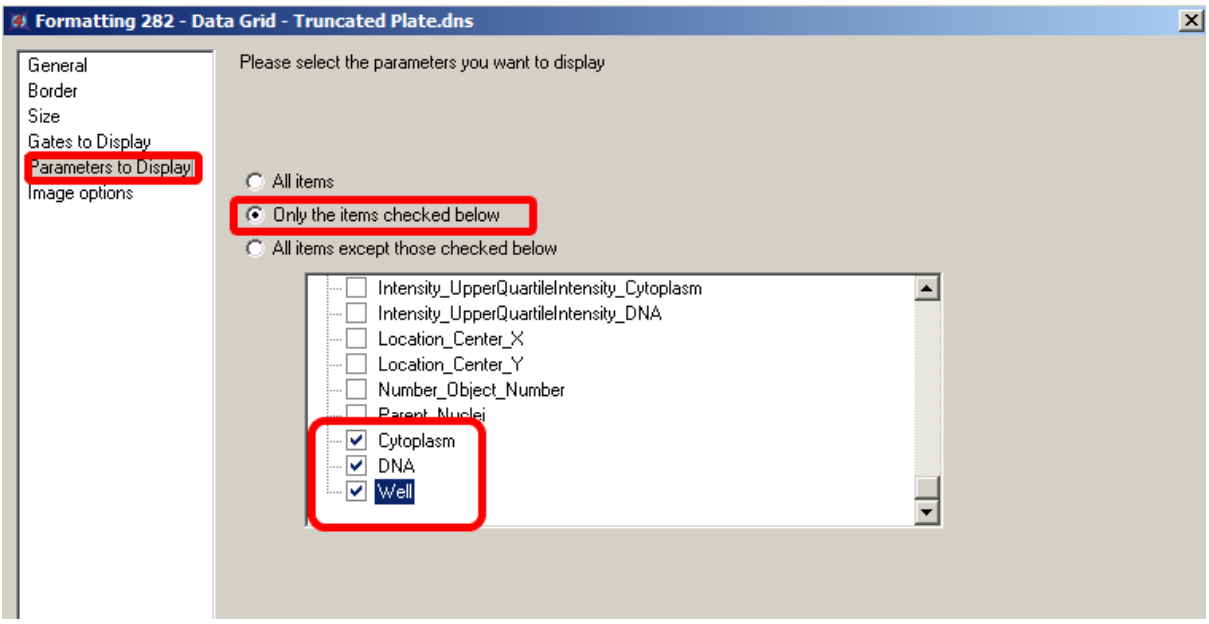

Figure T28.47 Configuring Parameters to Display

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).

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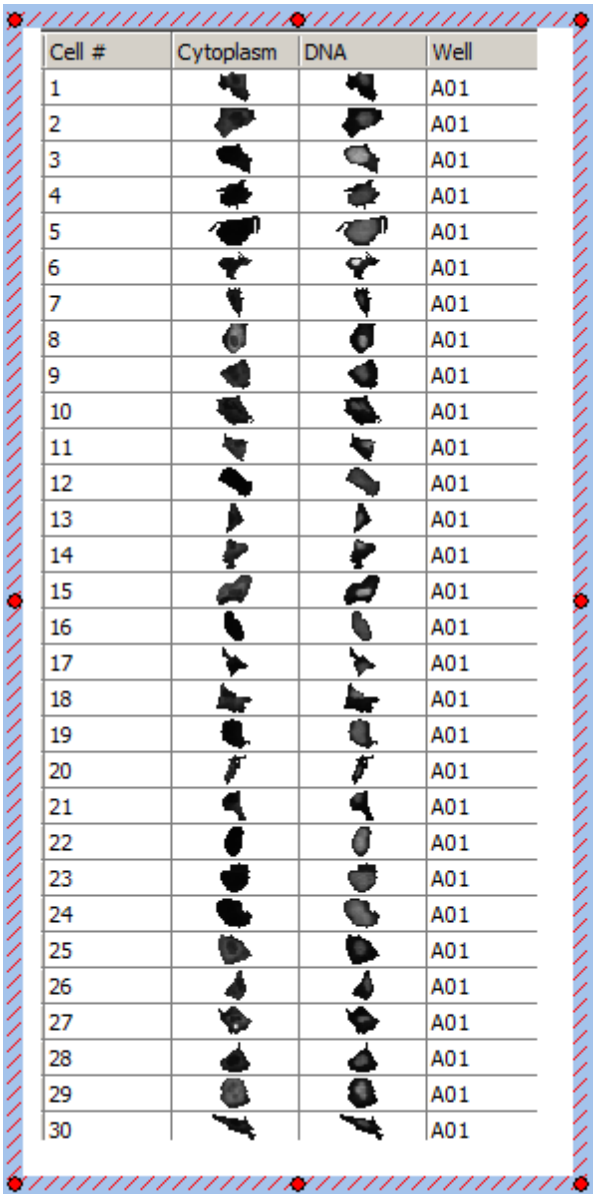

Cell #	Cytoplasm	DNA	Well
1			A01
2			A01
3			A01
4			A01
5			A01
6			A01
7			A01
8			A01
9			A01
10			A01
11			A01
12			A01
13			A01
14			A01
15			A01
16			A01
17			A01
18			A01
19			A01
20			A01
21			A01
22			A01
23			A01
24			A01
25			A01
26			A01
27			A01
28			A01
29			A01
30			A01

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.
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

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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 590.

40.Click on the "**Overall Review**" page.

Notice that 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 202 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 202 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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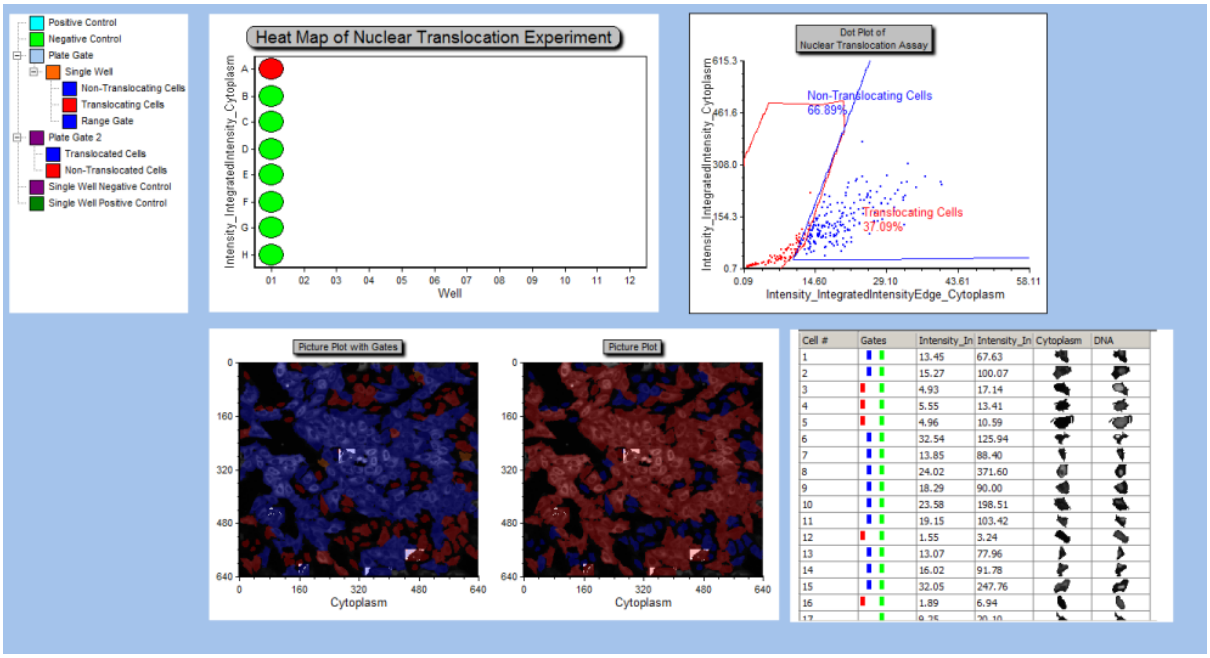

Figure T28.50 Review Page of IntroToImage3.fey layout

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 .

Next Topic 1430

34.29.3 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 the "IsoCyte". We will first open FCS Express 4 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 now fully compatible with FCS Express 4 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 4** from the Microsoft **Start Menu** or using your **FCS Express 4 desktop icon** to open a blank layout.

2. Select **Default** in the user selection menu (Figure T28.51).

3. Click **OK** .

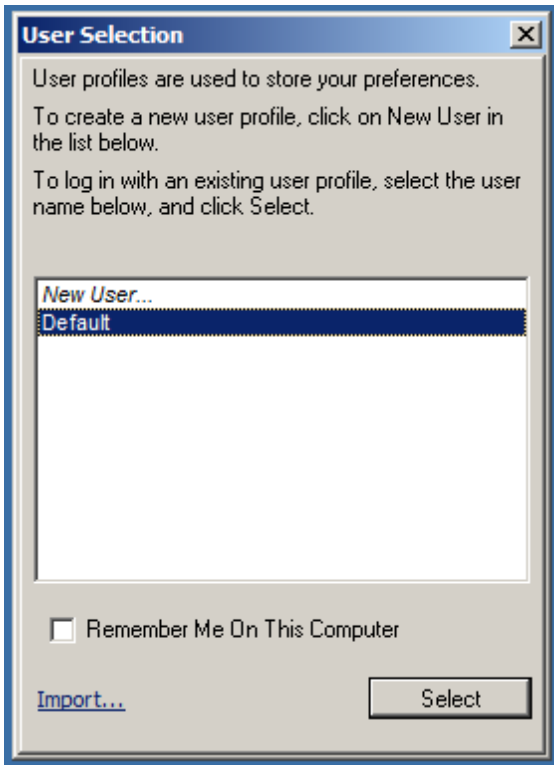

Figure T28.51 Selecting the Default User

FCS Express 4 will open with a single blank page and the **Home** tab selected. We will now confirm that the data loading preferences are set correctly.

4. Click on the **Application Button** with the left mouse button.

5. Click on **Edit Preferences** button on the lower right hand side of the menu that appears (Figure T28.52).

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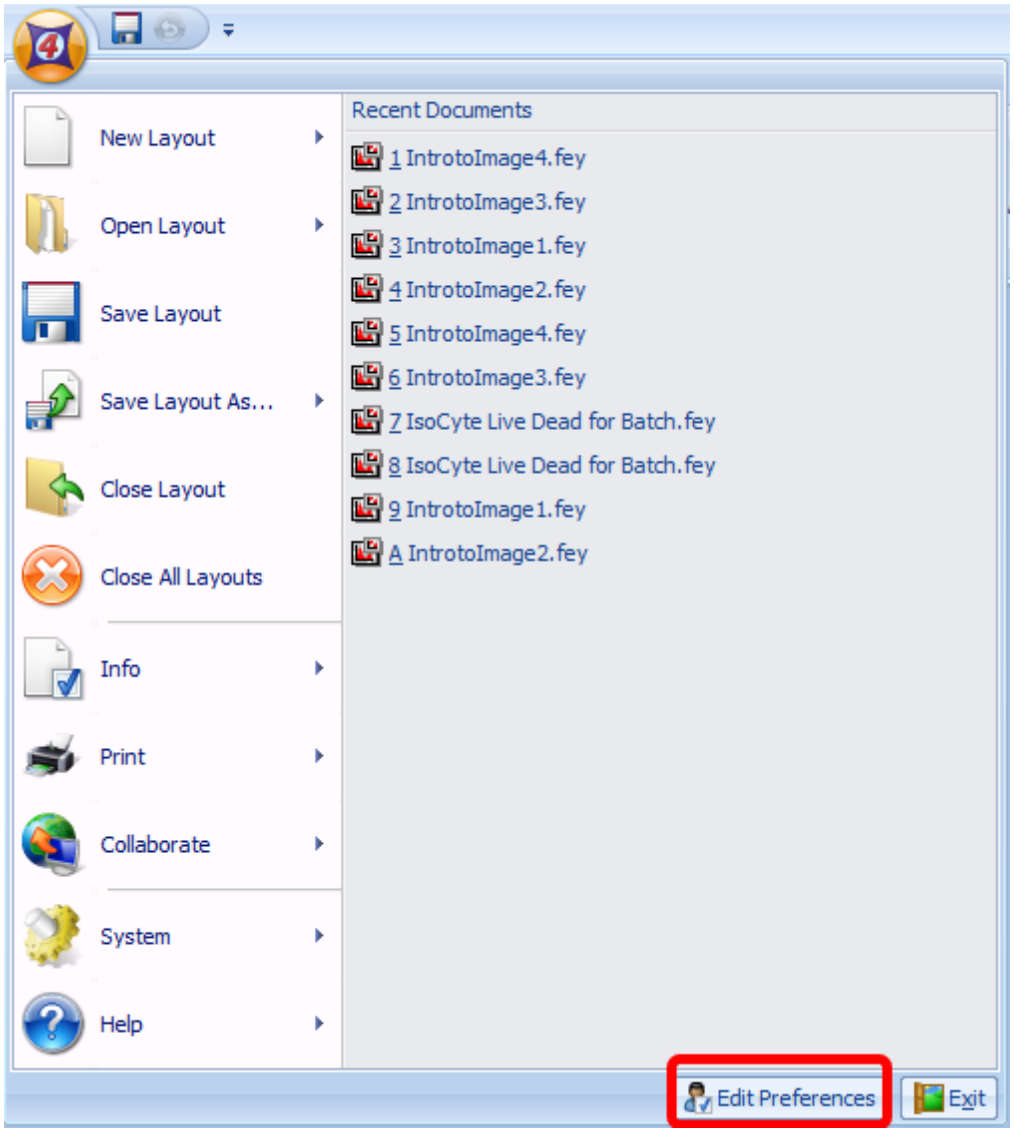

Figure T28.52 Edit Preference Button in Application Menu

5. Select the "**+**" sign on the **Data Loading** category in the left hand menu and a sub directory will open (Figure T28.53).

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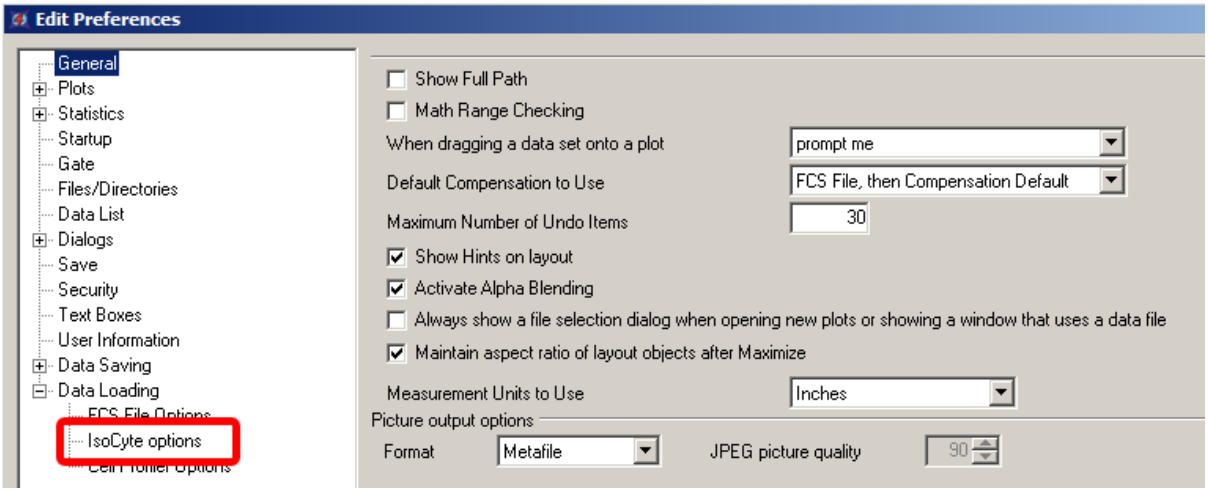

Figure T28.53 Data Loading Options

6. Click on **IsoCyte options** to view preference options (this is the previous name of the ImageXpress Velos). If the option indicates ImageXpress velos instead of IsoCyte this is the correct selection. The Isocyte Options category options will appear on the right (Figure T28.54).

7. 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 482. 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. We do not need background images in this case and they consume a lot of CPU/Memory resources.

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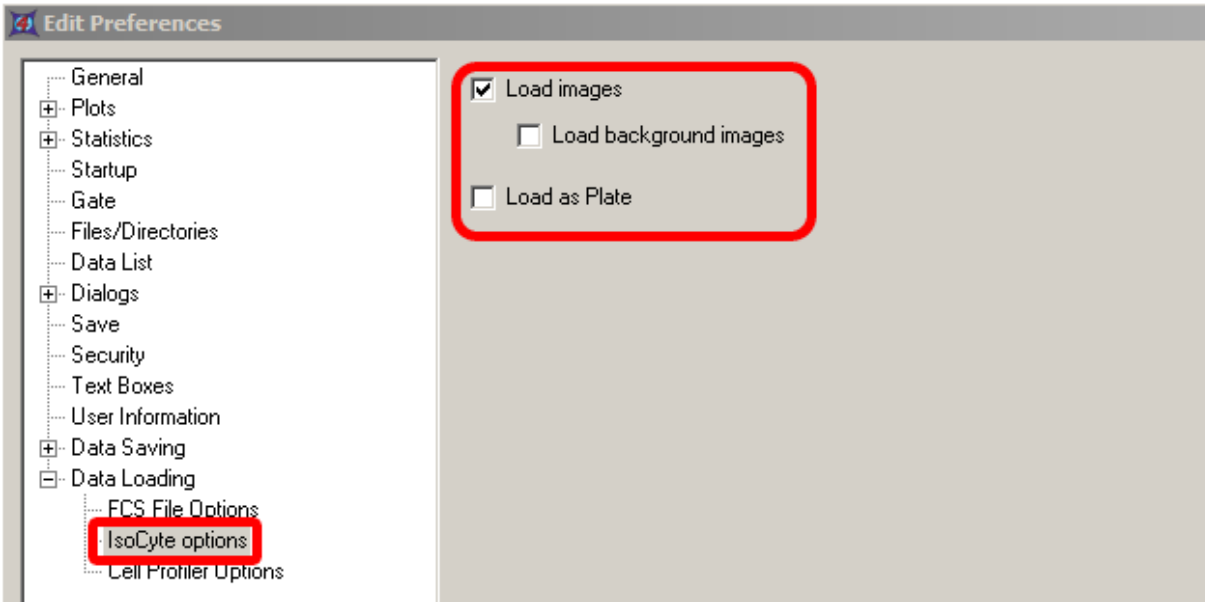

Figure T28.54 Correct Settings for Data Loading

8. Click **OK**.

9. Load the **IntroToImage4.fey** layout from the Tutorial Sample Data archive 590 →**Intro to Imaging Tutorial** folder.

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 202.(Figure T28.55). We will now import the data from the Tutorial Sample Data archive 590.

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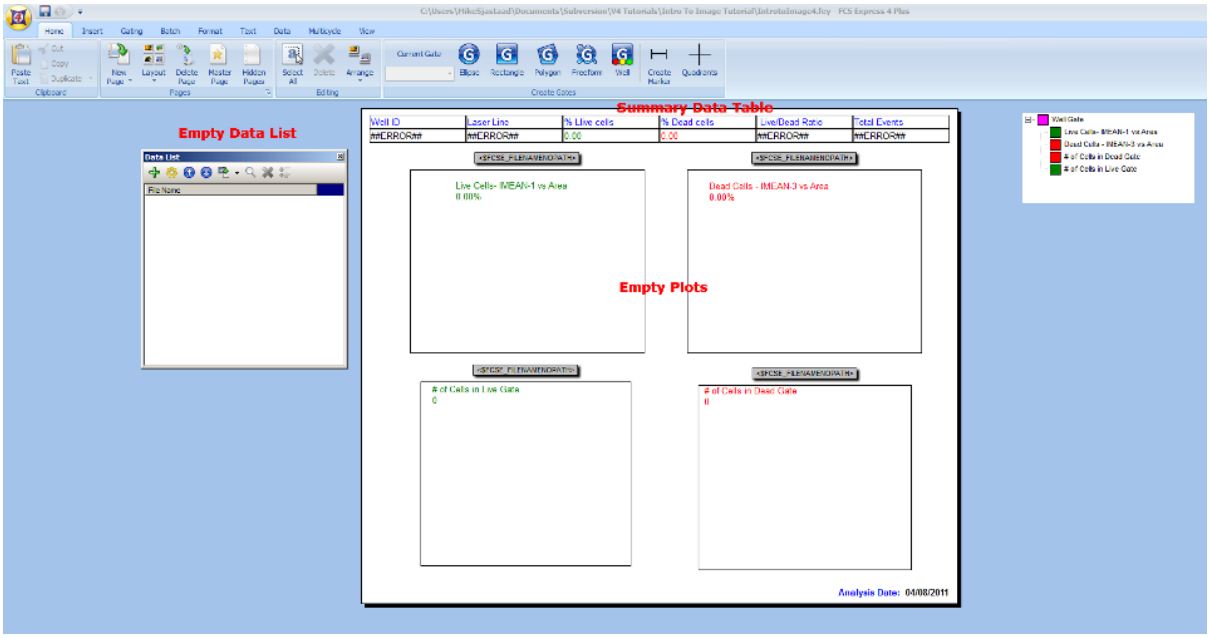

Figure T28.55 The Empty Layout Template

10. Click the green "**+**" on the empty data list to add data to the template (Figure T28.56).

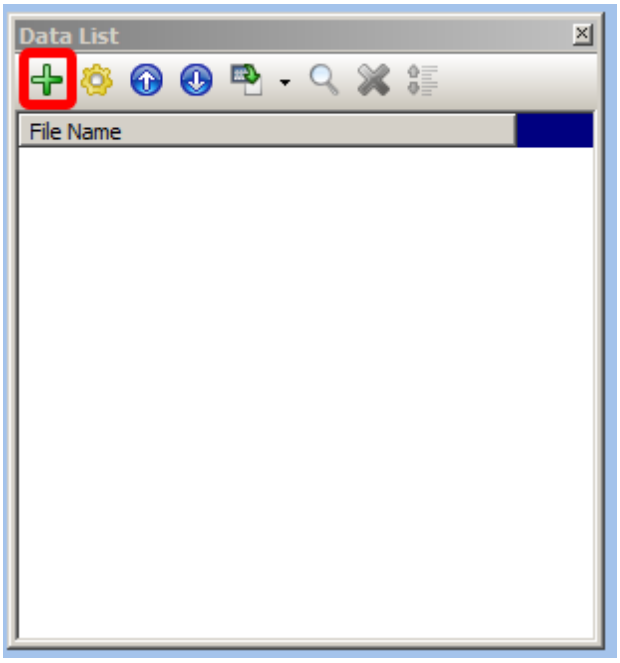

Figure T28.56 Empty Data List

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11. Navigate to the Sample Data > IsoCyte With Images > Live_dead_96.well.iso folder (Figure T28.57)

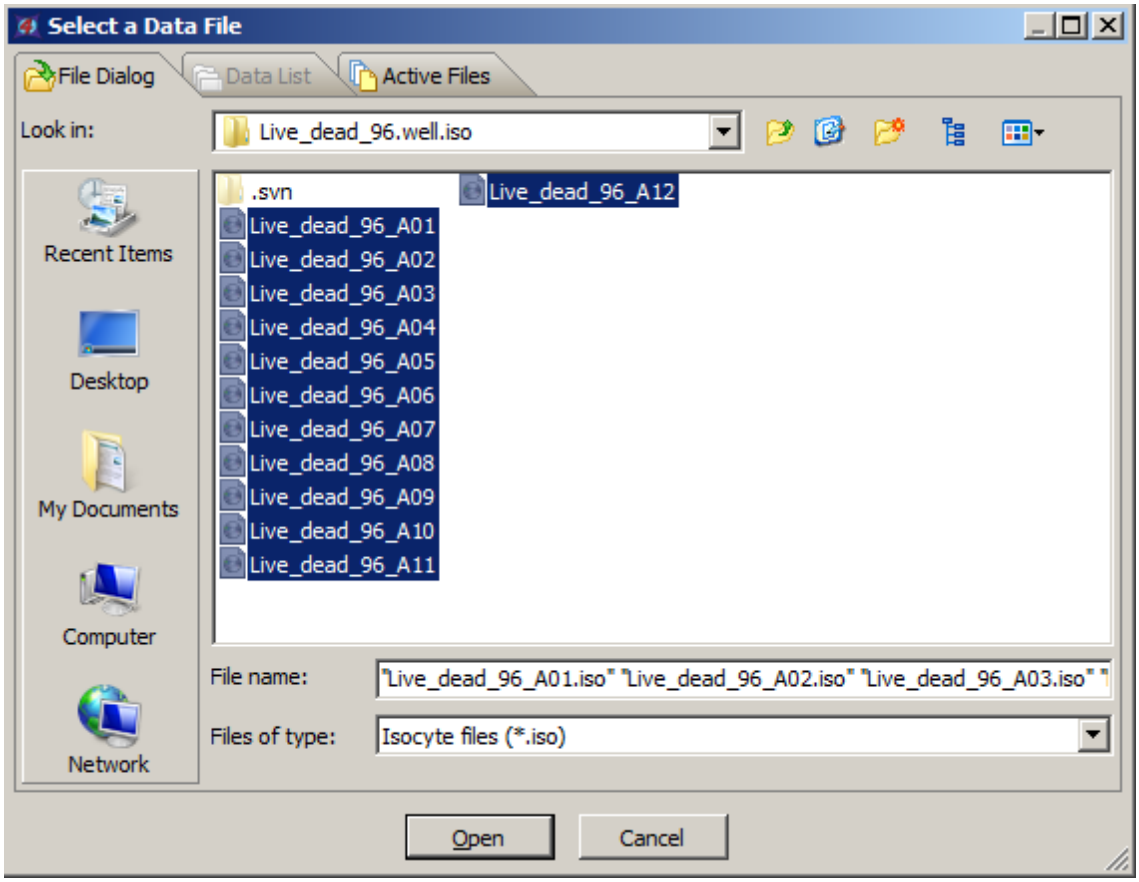

Figure T28.57 Selecting the Data Files to Import

12. Select all the **Live_dead_96...** files

13. 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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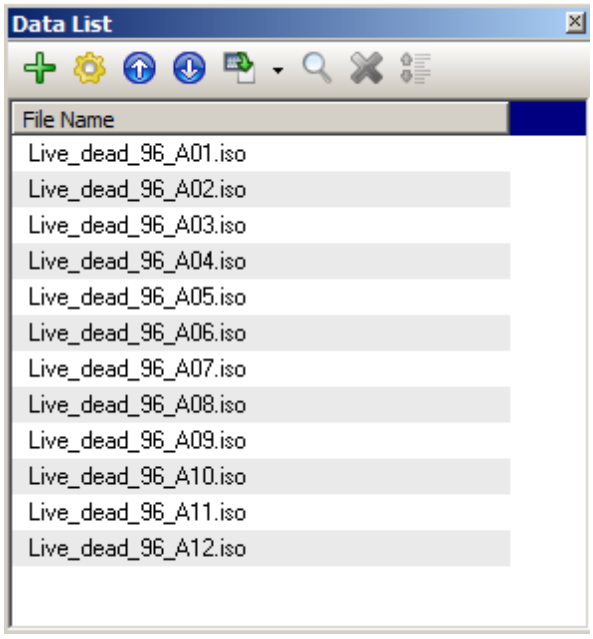

Figure T28.58 Data List showing Data Files

14. Click to select the first live-dead file and (it will highlight in dark blue) (Figure T28.59 below).

15. Click on the **Change data icon** in the Data List menu and select **Change Data on All Plots** in the drop down menu that appears (Figure T28.59).

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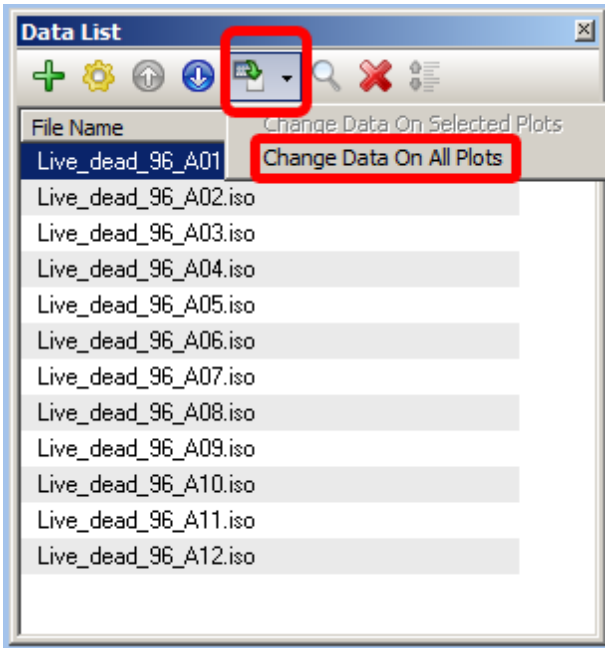

Figure T28.59 Selecting the Data File to Load Into Layout

All plots and tables in the layout page will populate with data from **well A01** (Figure T28.60).

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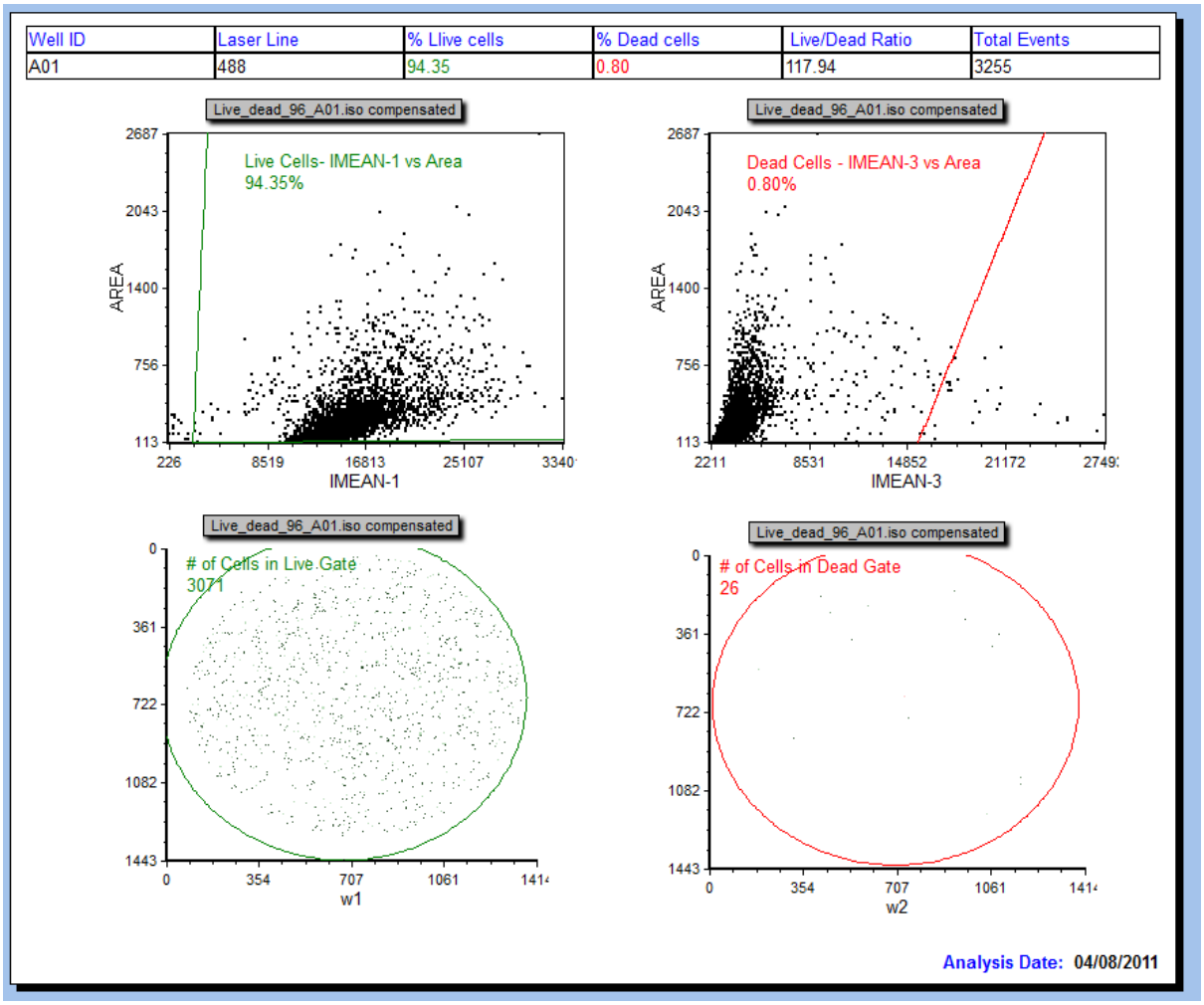

Figure T28.60 Batch Setup Page of IntroToImage4.fey Showing Data from Well A01

16. Click on the **Data List** tab in the ribbon (Figure T28.61).

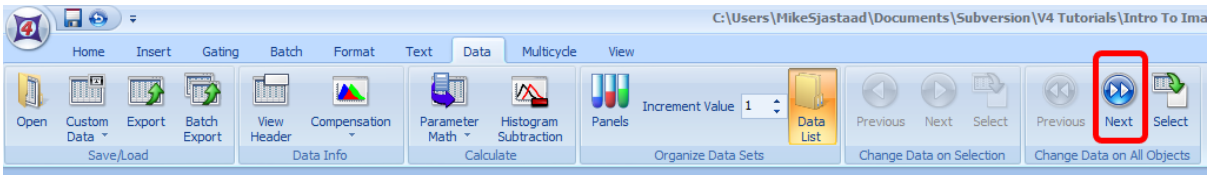

Figure T28.61 The Data List Tab

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Note: You can move to subsequent wells by clicking the Next button in the **Change Data on All Objects** menu. (Figure T28.61). Once you are further into the list you can move backwards with the **Previous** button. Please return to **well A01** if you have moved to another well.

17. Observe the **Data list** in the layout (Figure T28.62). Notice that data from **well A-01** through **well A-12** are in the list.

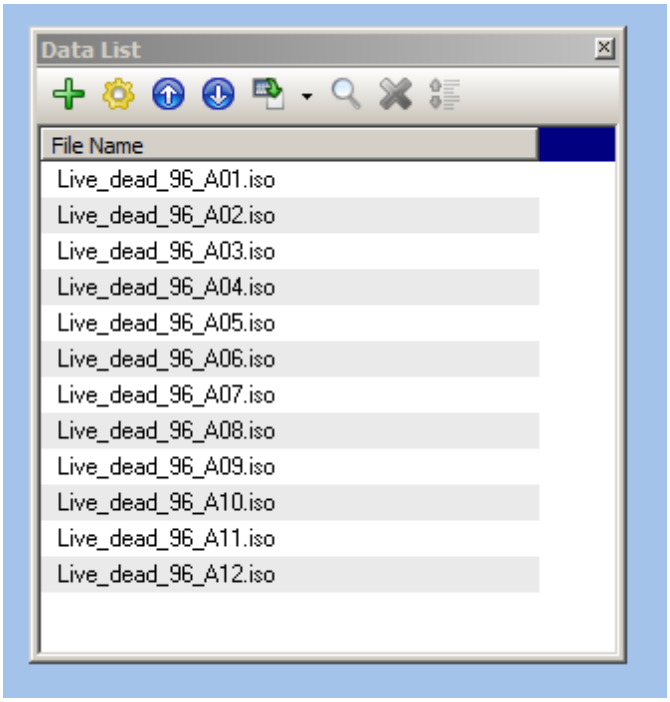

Figure T28.62 Data List

18. Close the **Data List** by clicking the **"x**" in the upper right hand corner of the data list window.

19. Reopen the **Data List** by clicking the **Data Tab→Organize Data Set→Data List** icon on the ribbon. This demonstrates how to open a data list.

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 T28.63). Two dot plots 380 on the top indicate the Live and Dead cell channels, respectively for the given well. The picture plots 73 on the bottom of the page are the live and dead images respectively. **Live Cells** and **Dead Cells** gates

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have been set in advance. Tokens[328] 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.

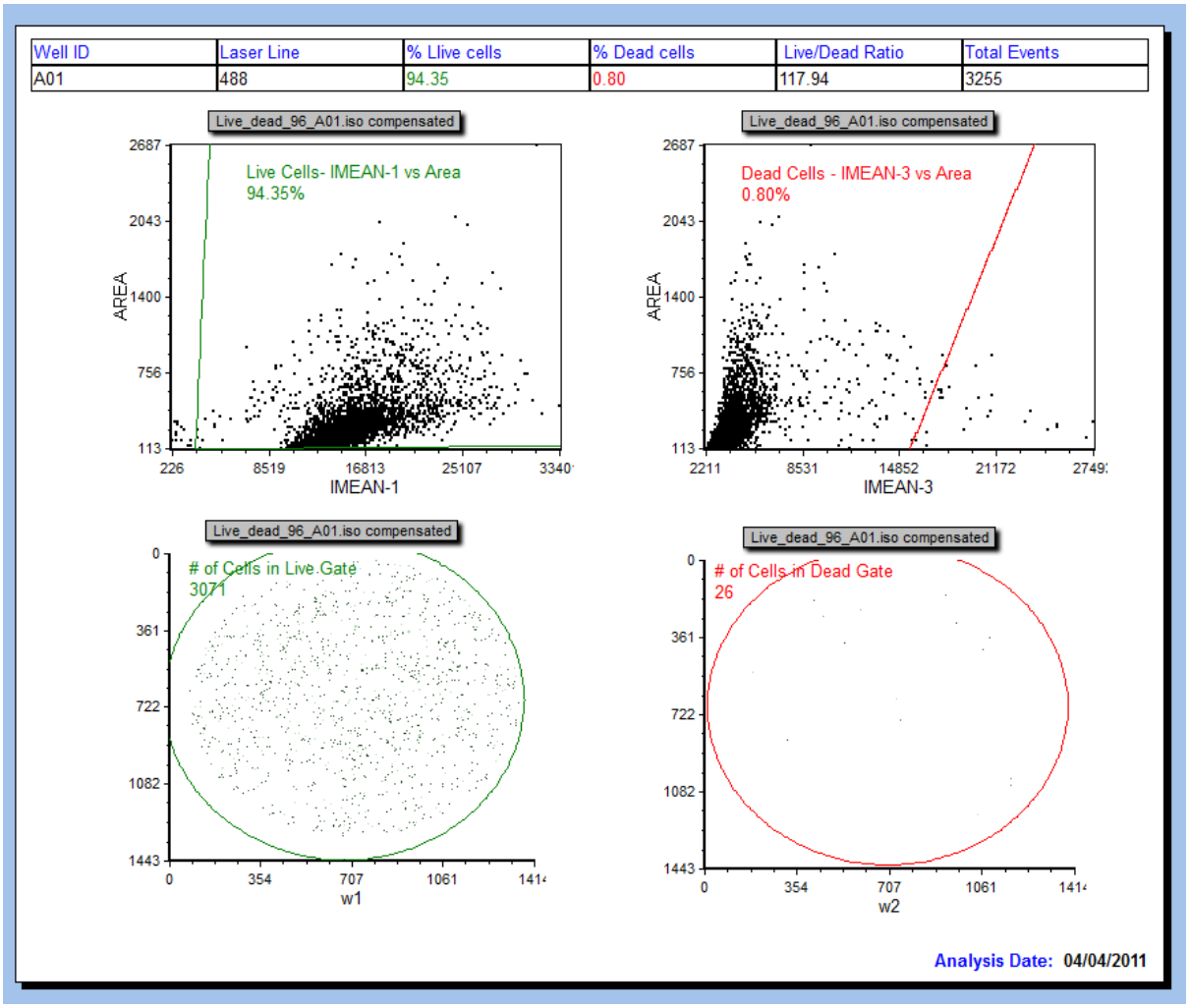

Figure T28.63 Batch Setup Page of IntroToImage4.fey

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 T28.64).

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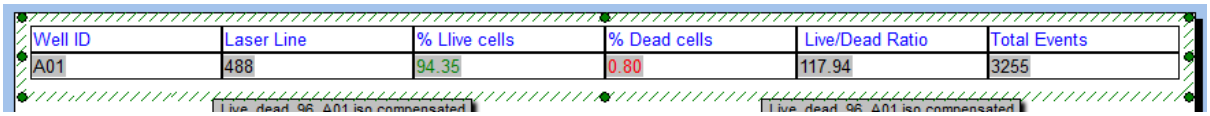

Figure T28.64 The Data Summary Table

21. Click on the **Data Tab→Next** button in the **Change Data on All Objects** menu. Notice that all of the four plots update to show data and images from **well A-02** (Figure T28.65).

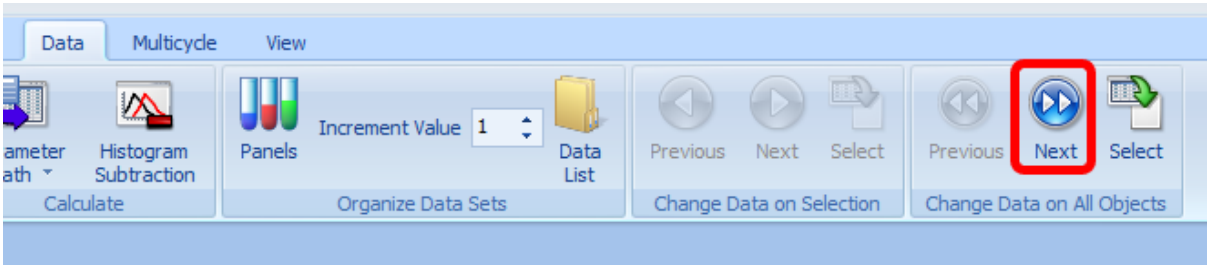

Figure T28.65 The Data Tab

22. Step through the data for each of the wells using the **Next** button 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.

23. Return to **well A-01** by pressing the **Previous** button in the **Change Data on All Objects** menu repeatedly.

Modifying the Summary Table

24. Click on the **Insert tab→General→Text Box** in the ribbon.

25. Insert at new text box on anywhere on the layout (Figure T28.66).

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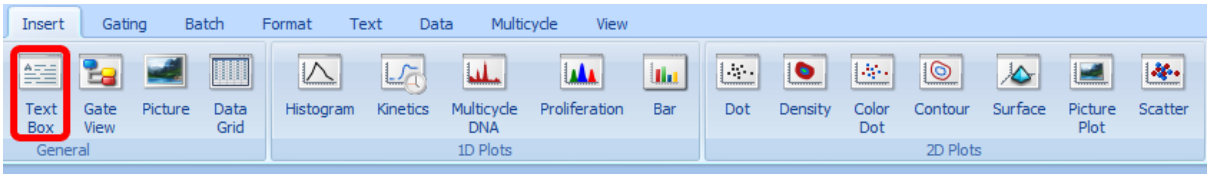

Figure T28.66 The Insert Tab

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).

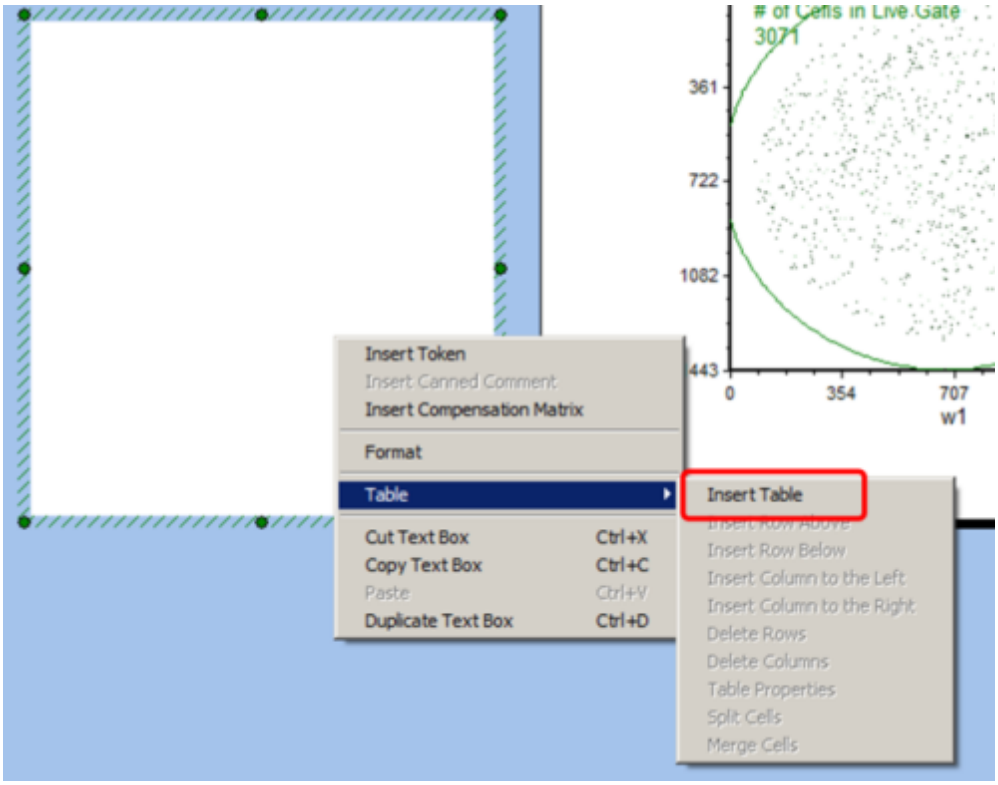

Figure T28.67 Inserting a Table

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**".

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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.

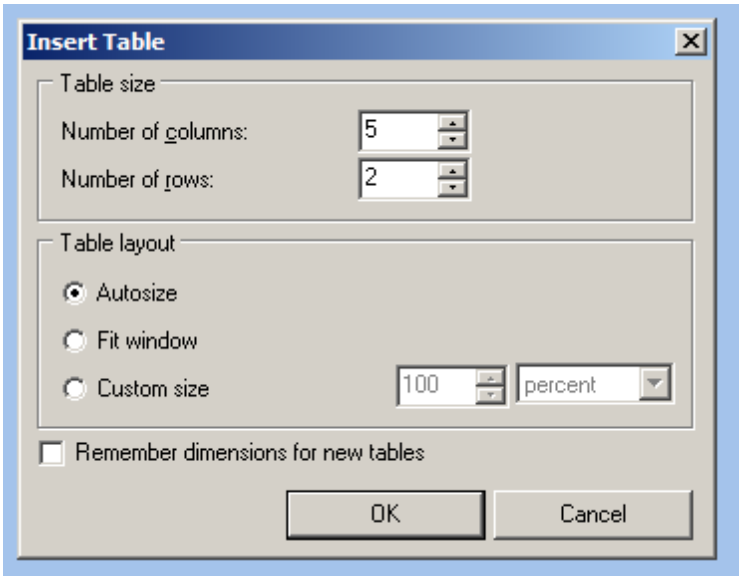

Figure T28.68 Insert Table Configuration

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).

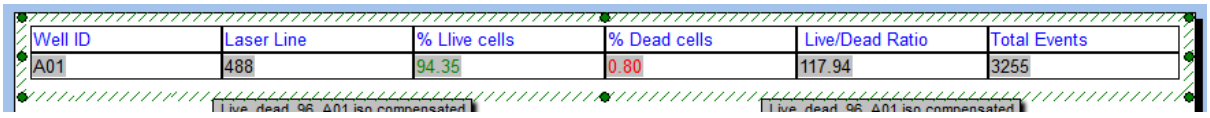

Figure T28.69 Summary Data Table

31. Edit the "**Well ID**"text as you would in Word, Excel or PowerPoint. Undo your edits with as many CTRL+Z commands as required.

32. 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

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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 denotes that it is a Token691. Notice the text turns white when selected (Figure T28.70).

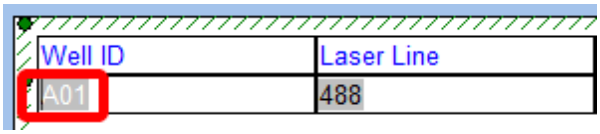

Figure T28.70 Selecting the Token

33. Double click on the A01 Token691. A formatting window will appear enabling selection and editing the token (Figure T28.71).

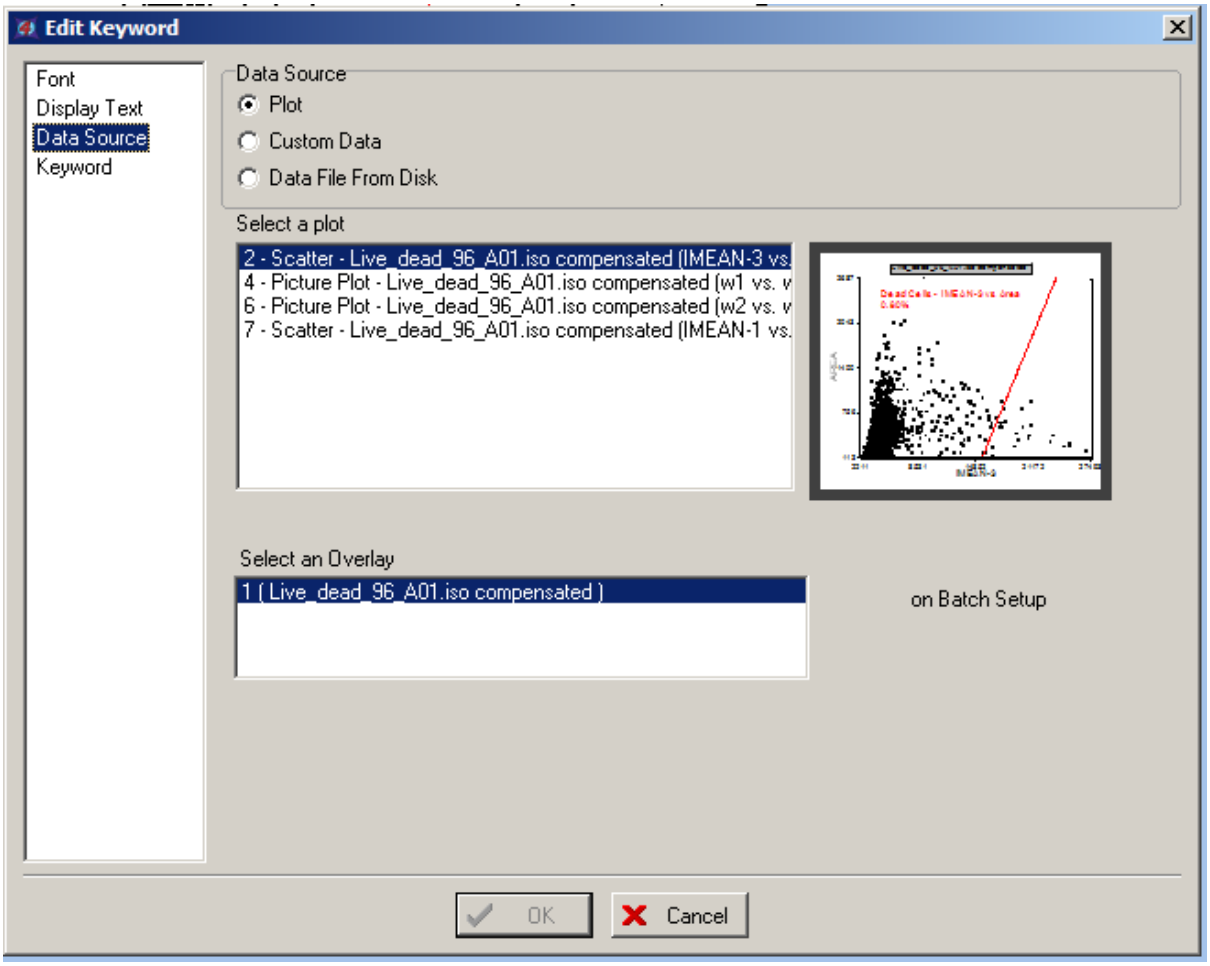

Figure T28.71 Edit Token Dialog Box

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34. Select the **Keyword** category to choose a token displaying a Keyword from the data set (Figure T28.72).

35. Click on the ellipsis (**...**).

36. Select **Well Name** from the **Please select a keyword** dialog.

37. Click **OK**. You have just replaced the **Well ID** with the **Well Name**.

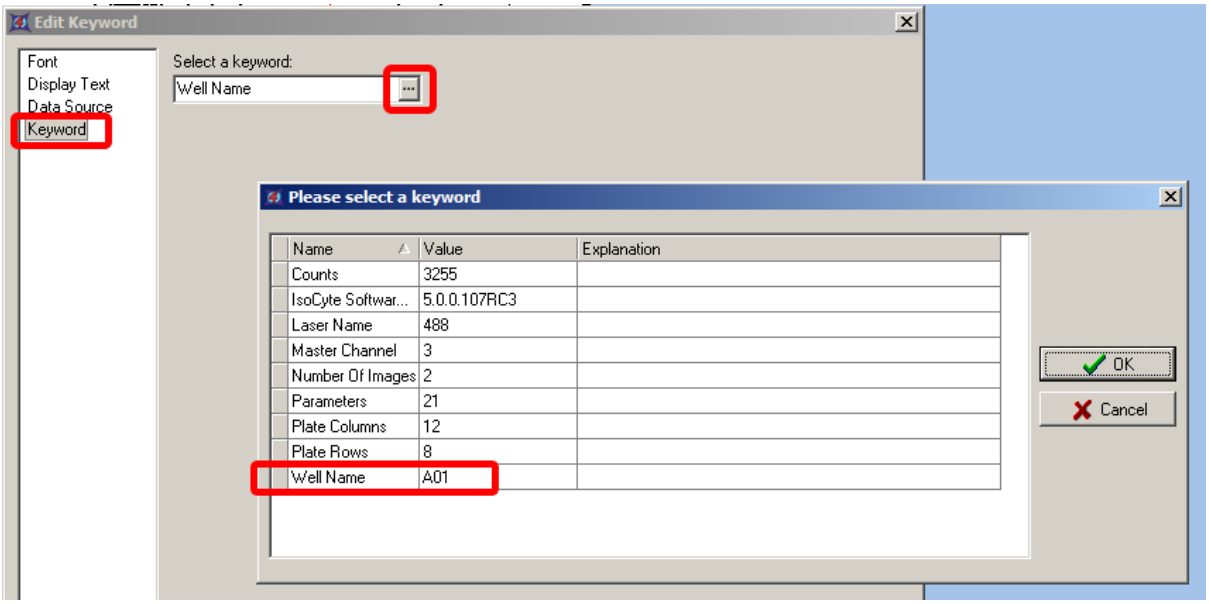

Figure T28.72 Selecting a Token To Display Well Name in the Summary Data Table

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 IsoCyte / ImageXpress Velos data into the first 2 cells.

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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.

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).

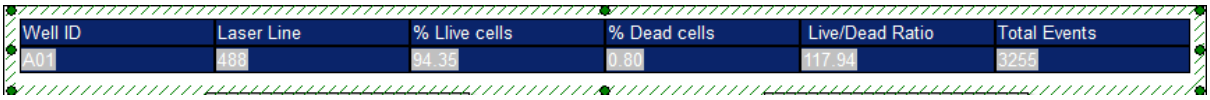

Figure T28.73 Selecting all Text in the Summary Data Table

39.Select the **Text** tab as shown in Figure T28.74.

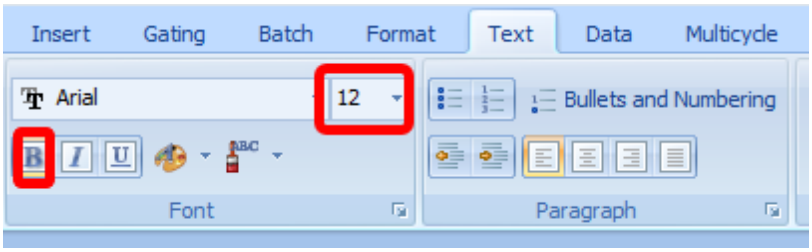

Figure T28.74 Text Tab Font and Bold Selection

40.Change the size of the font in the Table from **10pt** to **12pt** as shown in

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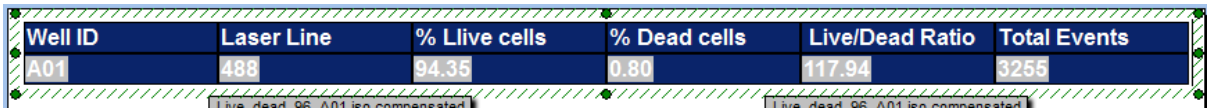
Figure T28.74 above.

41. Click on the "**B**" (bold) button to change all to **Bold** as shown in Figure T28.74 above.

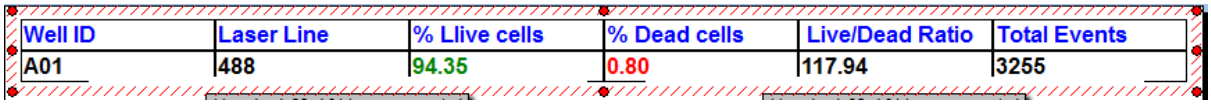
Notice you have similar options for editing text in a layout page as you would expect in any Microsoft product. This allows layouts to be customized in any way you require for custom reporting. The Summary Data Table will now appear as in Figure T28.75a and Figure T28.75b below.



Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	0.80	117.94	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.



Well ID	Laser Line	% Llive cells	% Dead cells	Live/Dead Ratio	Total Events
A01	488	94.35	0.80	117.94	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 4. 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 onBatch Processing 372 for detailed instructions on configuring the advanced batch and reporting functions.

43. Click on the **Batch tab→Batch Processing→Batch Actions** (Figure T28.76) . The Batch Actions window will appear as shown in Figure T28.77.

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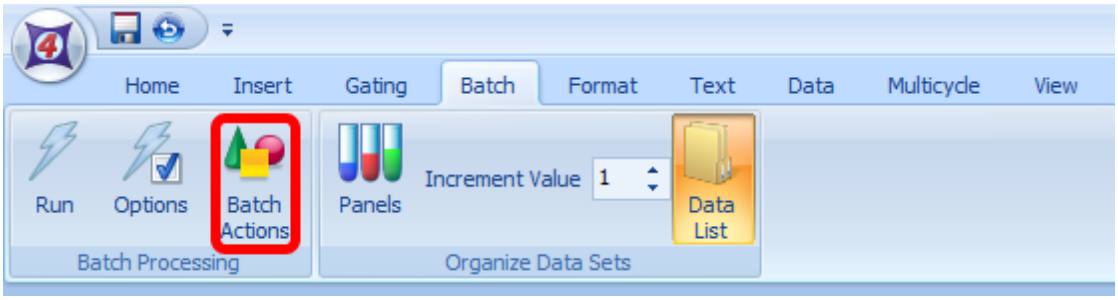

Figure T28.76 Opening the Batch Actions Window

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 372 tutorial.

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.

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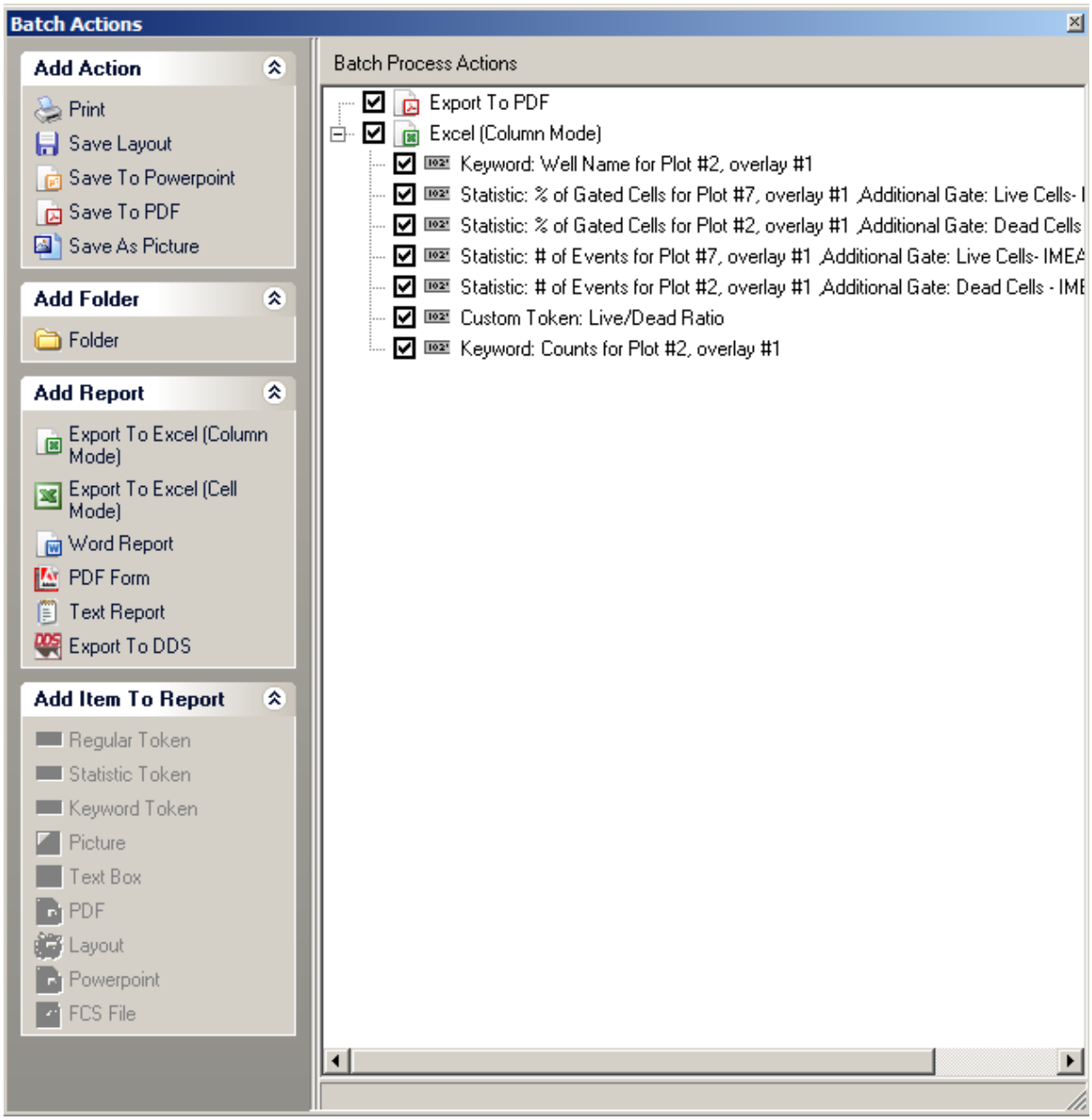

Figure T28.77 Batch Analysis Options and Configuration for IntroToImage4.fey

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**

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Setup page of the layout. Recall from that the Token will appear in white when selected properly (Figure T28.78).

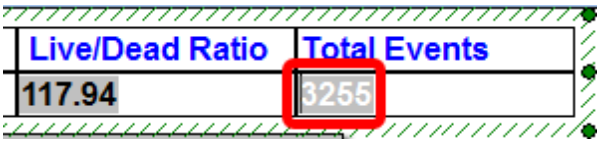

Figure T28.78 Selecting the Token for Total Events

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.

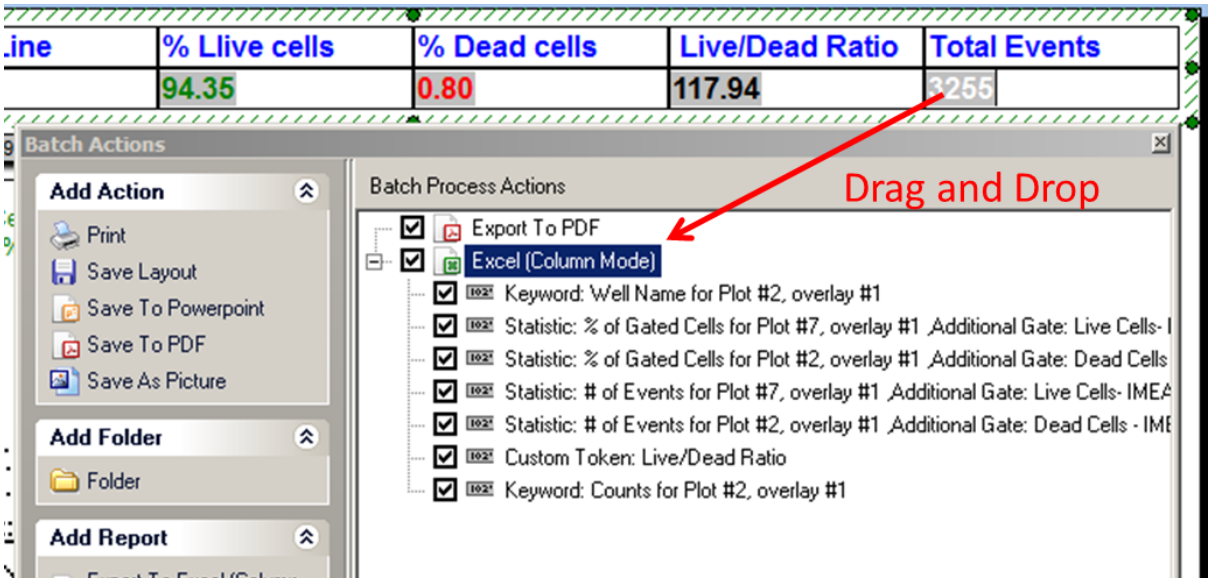

Figure T28.79 Drag and Drop Onto the Batch Actions List

46. Double click on the last item in the list, **Keyword: Counts for Plot #2, overlay #1**. A new window will appear (Figure T28.80).

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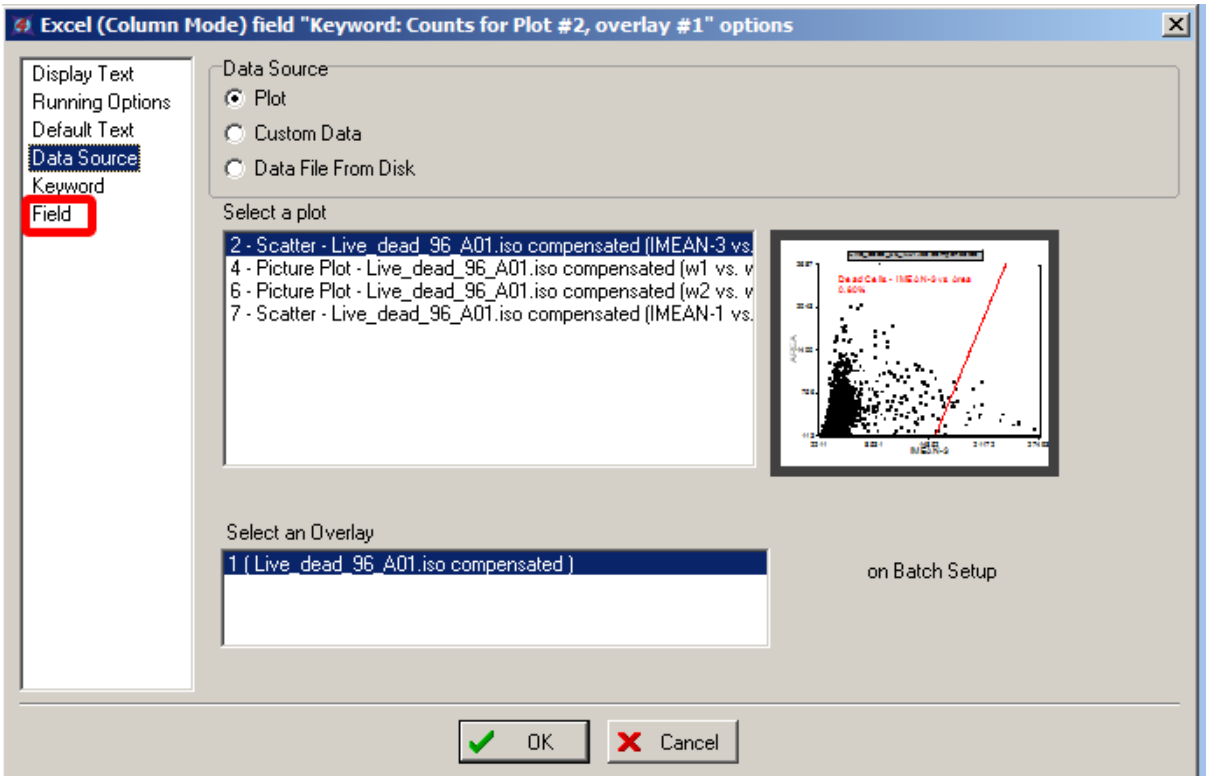

Figure T28.80 Modification of a Batch Export Command

47. Select the **Field** category. The **Column Name** Edit box will be empty and we will edit it. See Figure T28.81 below.

48. In the **Column Name** box enter "**Total Events**".

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

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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 the tutorial titled Batch Processing 767. There is also a detailed section in the Manual on Batch Processing 372.

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).

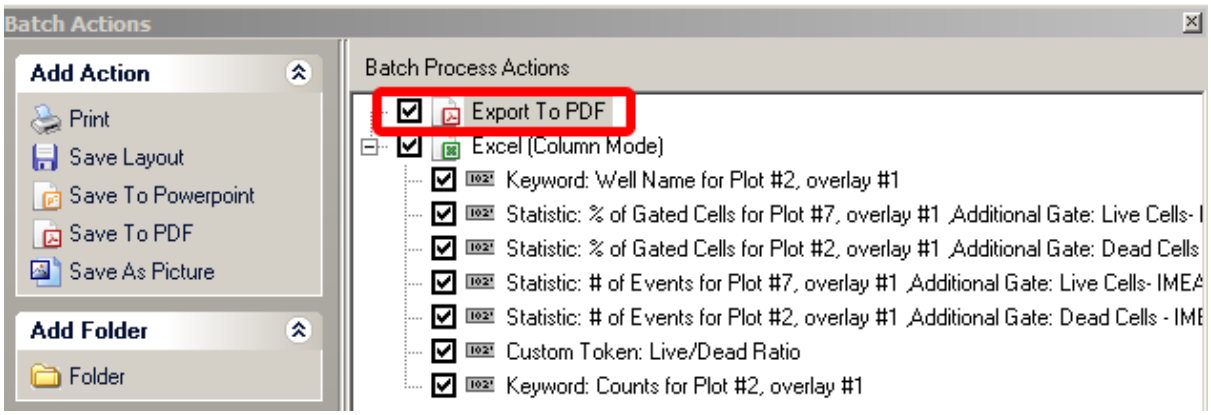

Figure T28.82 Editing the PDF Export Settings

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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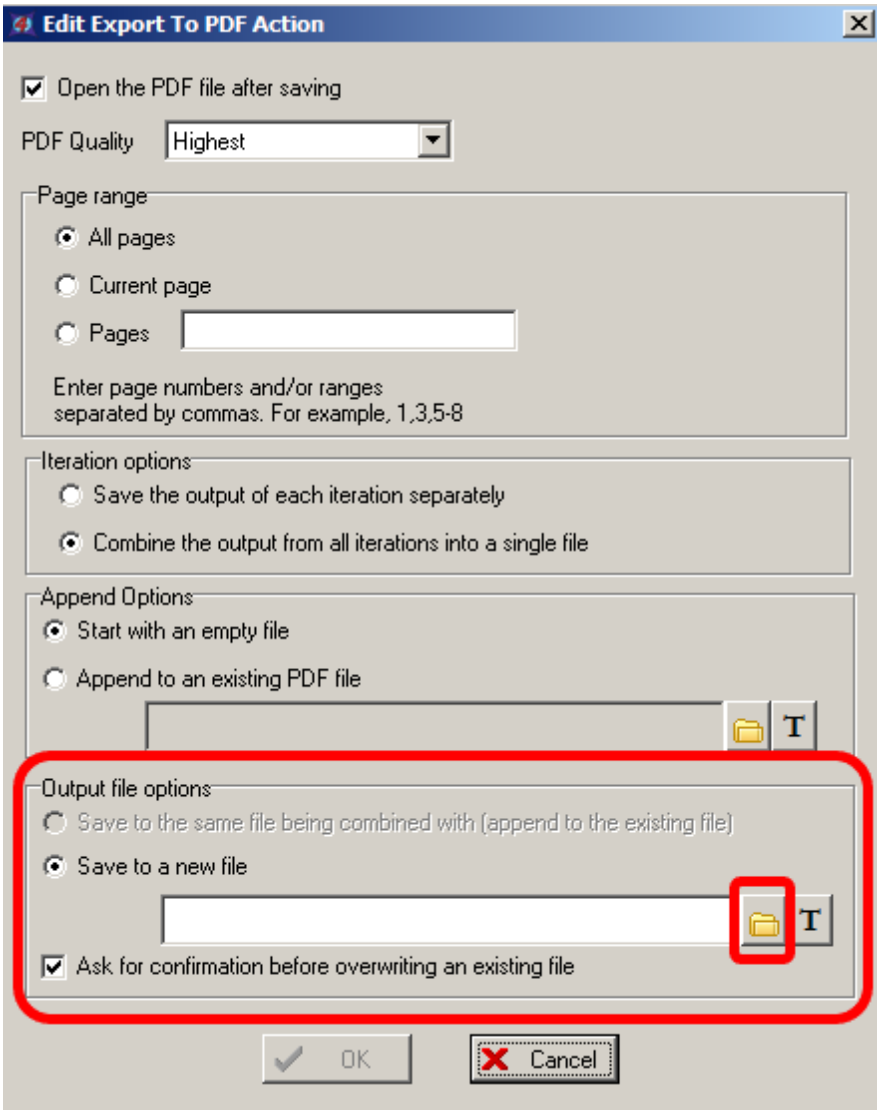

Figure T28.83 Editing the PDF Export Saving Path

52. Select your desktop and name your PDF "**MyBatchReport**".

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).

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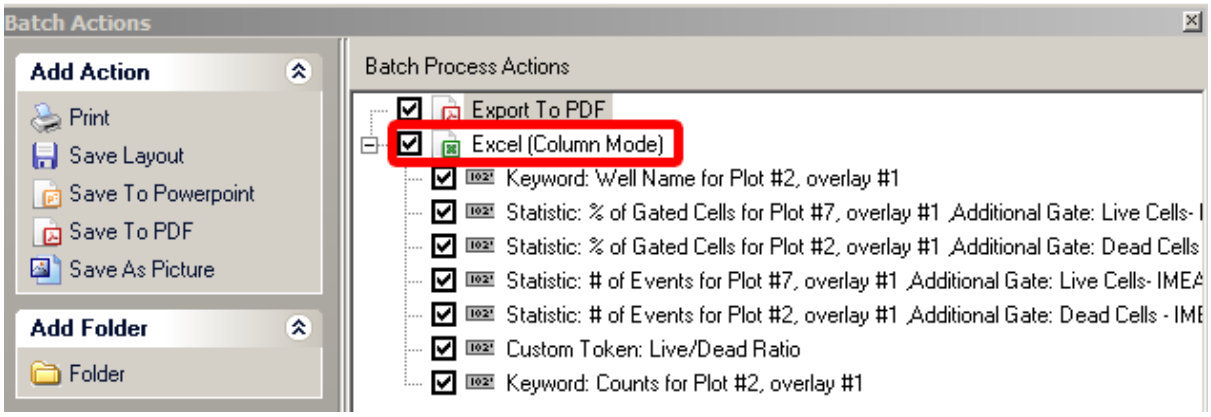

Figure T28.84 Editing the Excel Export Settings

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).

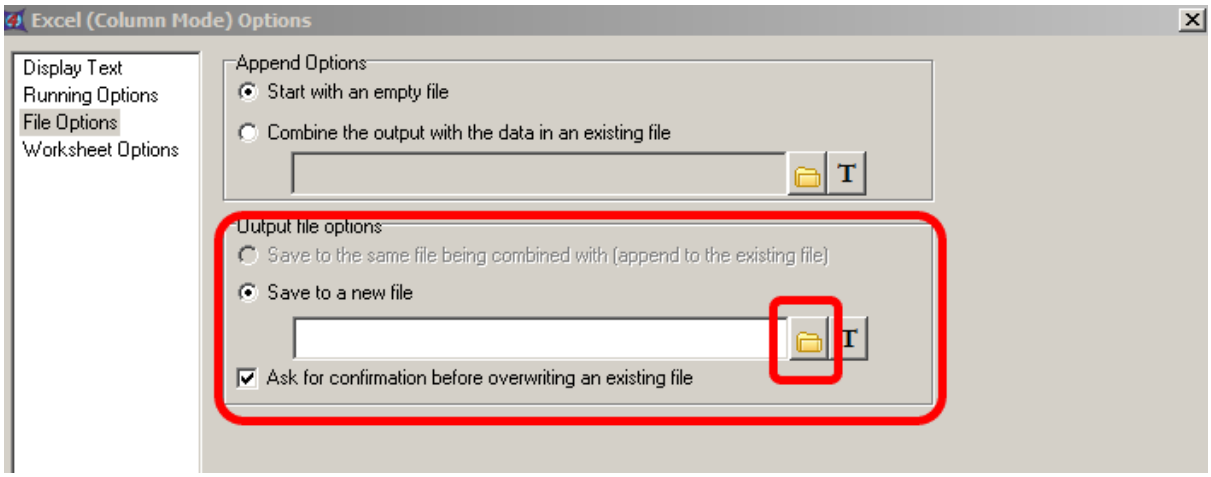

Figure T28.85 Editing the Excel Export Saving Path

Section 3: Running the Batch and Reviewing Reports

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57. Click on the **Batch tab→Batch Processing→Options**. A configuration window will appear (Figure T28.86).

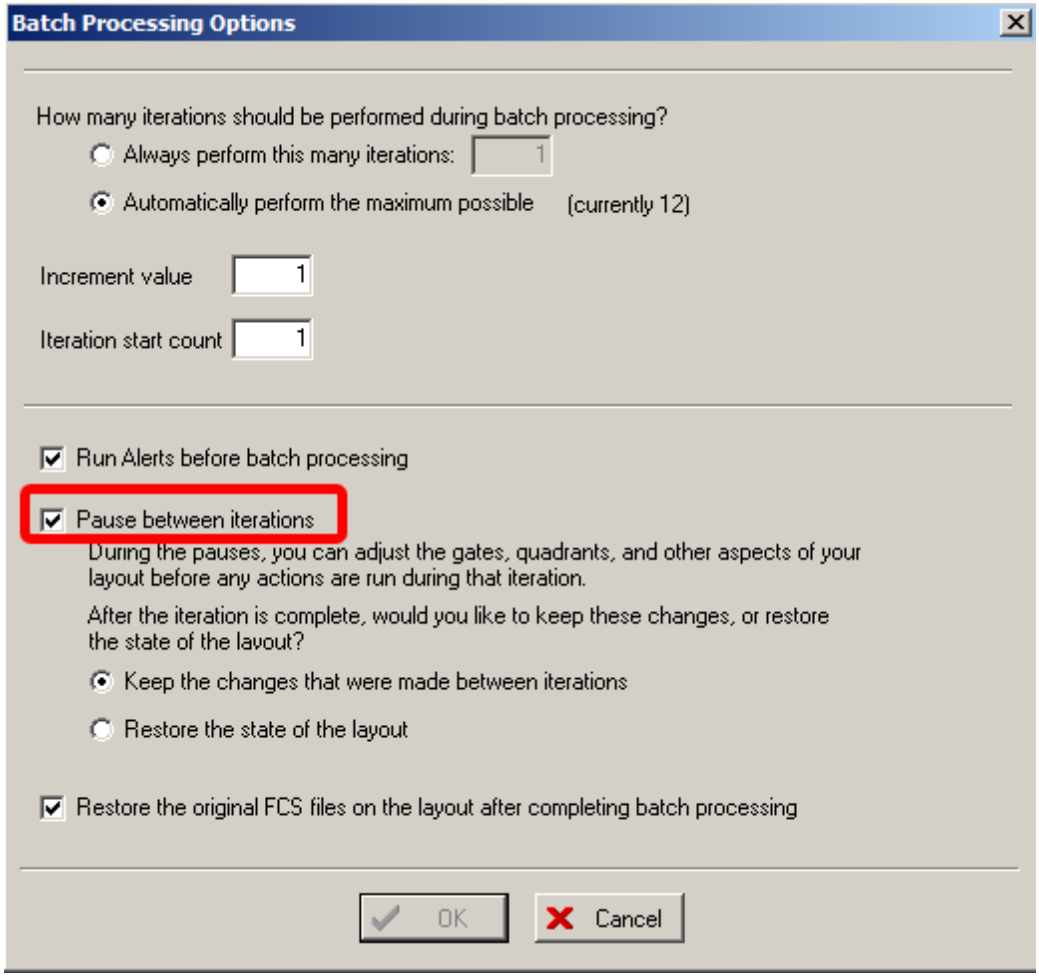

Figure T28.86 Configuring Batch Processing Options

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 **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.

58. Uncheck the box for **Pause between iterations**.

59. Click **OK**.

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60.Click on **Batch tab→Batch Processing→Run**.

The batch process will run for roughly one minute. A status window will appear during the batch process showing how far the batch has progressed (Figure T28.87).

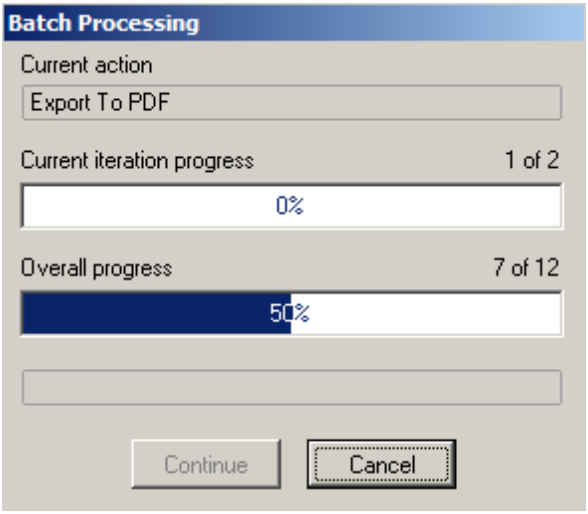

Figure T28.87 Batch Processing Status Window

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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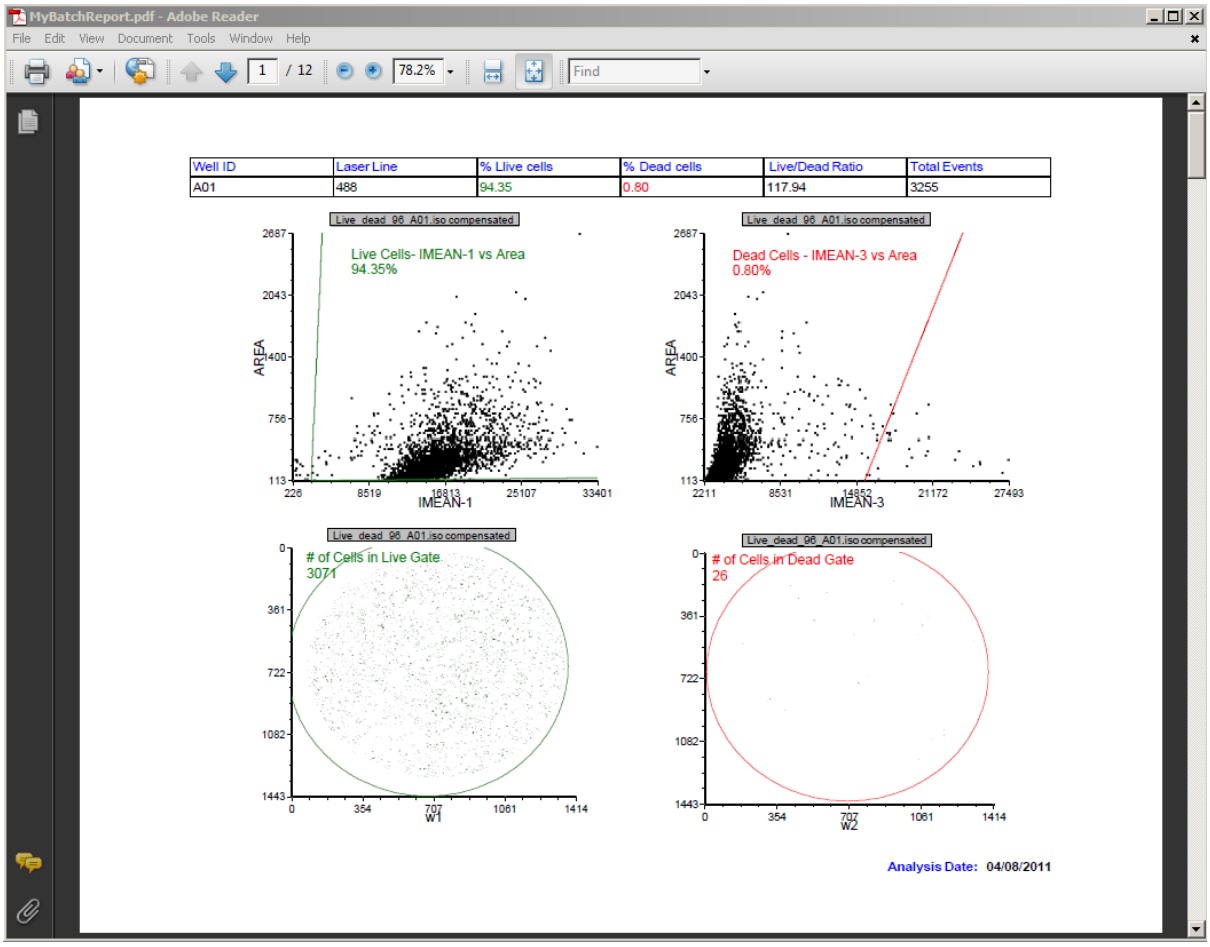

Figure T28.88 "MyBatchReport" PDF Report from Batch Actions

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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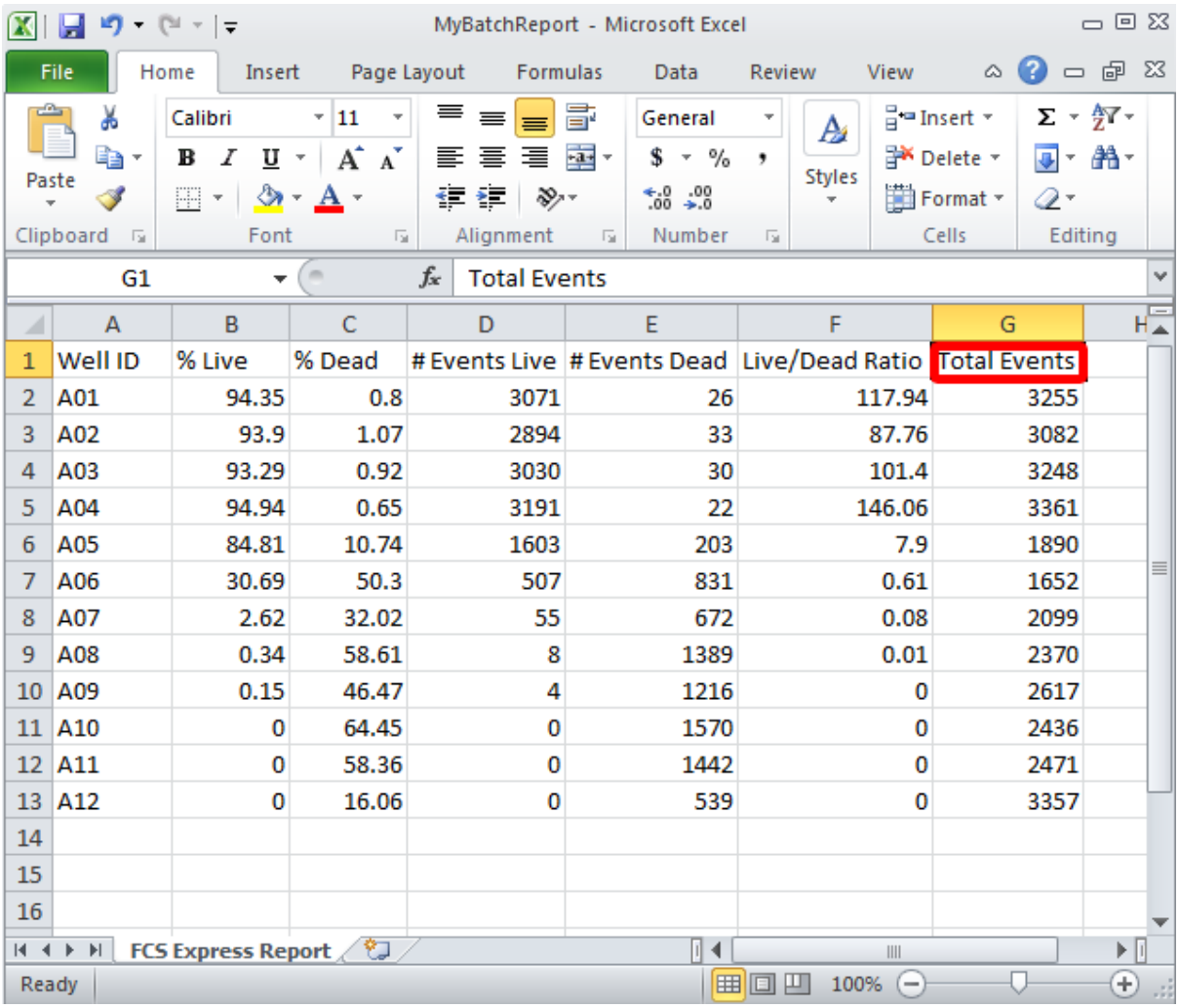

	A	B	C	D	E	F	G
1	Well ID	% Live	% Dead	# Events Live	# Events Dead	Live/Dead Ratio	Total Events
2	A01	94.35	0.8	3071	26	117.94	3255
3	A02	93.9	1.07	2894	33	87.76	3082
4	A03	93.29	0.92	3030	30	101.4	3248
5	A04	94.94	0.65	3191	22	146.06	3361
6	A05	84.81	10.74	1603	203	7.9	1890
7	A06	30.69	50.3	507	831	0.61	1652
8	A07	2.62	32.02	55	672	0.08	2099
9	A08	0.34	58.61	8	1389	0.01	2370
10	A09	0.15	46.47	4	1216	0	2617
11	A10	0	64.45	0	1570	0	2436
12	A11	0	58.36	0	1442	0	2471
13	A12	0	16.06	0	539	0	3357
14							
15							
16							

Figure T28.89 "MyBatchReport" Excel Spread Sheet Report from Batch Actions

62. Close 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.

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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.

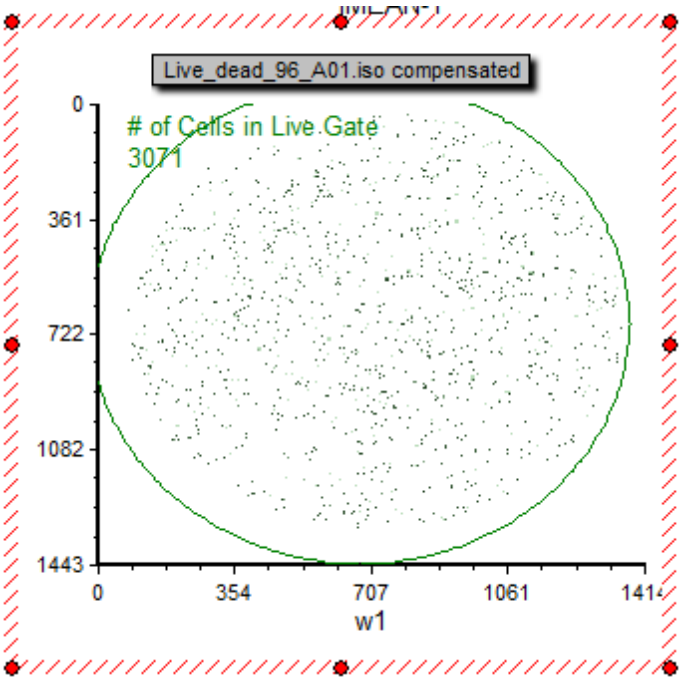

Figure T28.90 The Live_dead_96_A01.iso compensated" Picture plot

64. Click on the **Application button** **→Save Layout As → Save selection As Picture.** (Figure T28.91)

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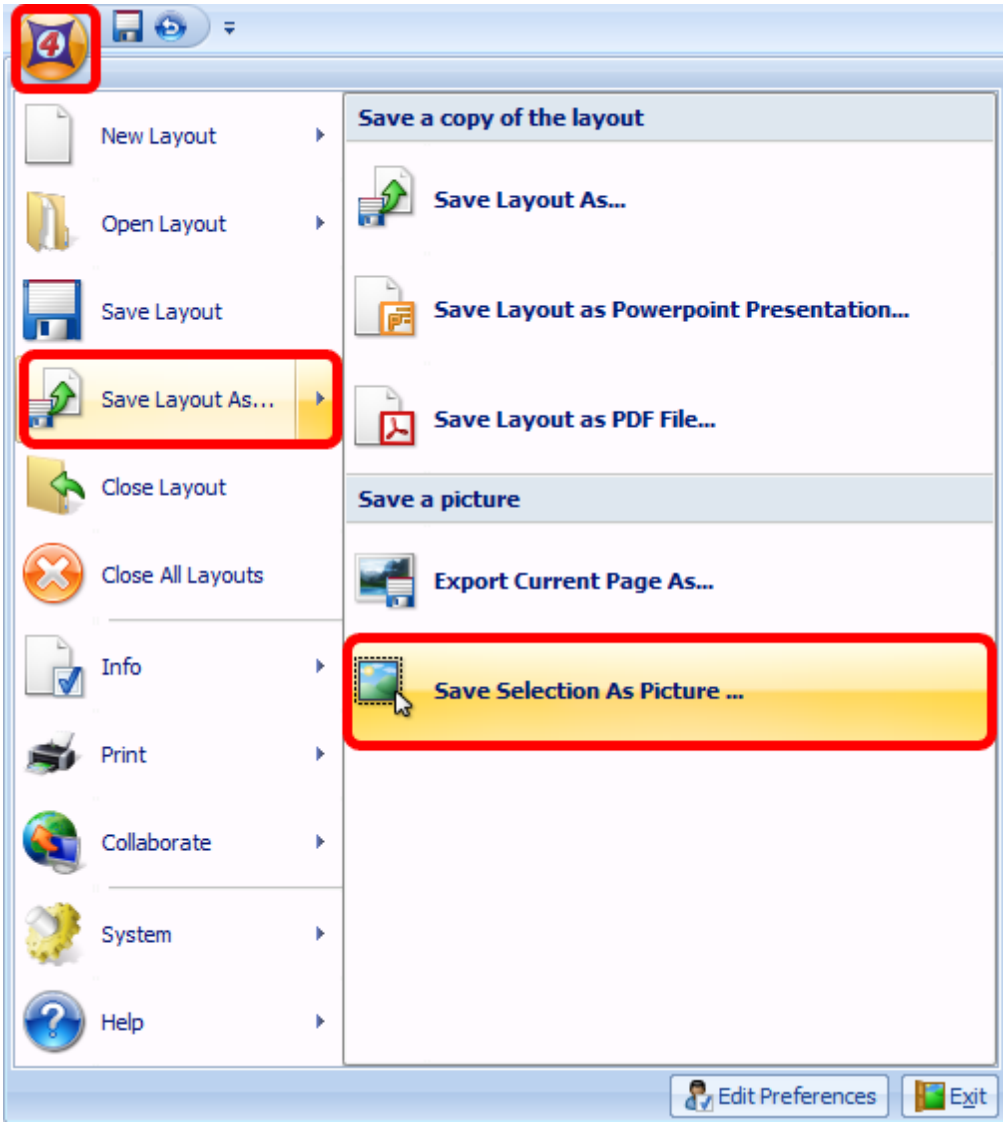

Figure T28.91 Saving a Selected Plot as a Picture

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.

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Congratulations! You have completed the **Introduction to FCS Express 4 Image Cytometry Tutorial.**

34.30 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 607 and format plots 620; create gates 192, markers 208, and quadrants 210; add default 691 and custom statistics 700 to your layout; and, batch export 372 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 1463 with plots and other analysis elements (e.g., gates, markers, quadrants), on the next page.
- Section 2: Add default and custom statistics 1490 to your layout, or to the provided layout.
- Section 3: Batch process your results to PowerPoint and Excel 1501, using your own layout or the provided layout.

You may also view the Immunophenotyping Analysis recorded webinar which accompanies this tutorial.

34.30.1 Building your layout

In this section, we will:

- Load an .fcs file 113 into the plots on the layout.
- Insert 607 and format plots 620.
- Create 193 and apply gates 670, markers 208, and quads 210.
- Change our Preferences 117 to commonly used settings 1513.

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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 26)*
- *Save Point #2 (Step 48)*
- *Save Point #3 (Step 78, end of this section)*

1. Select **Application Button→Open Layout**.
2. Open the layout **Immunophenotyping Tutorial.fey** found in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive.

   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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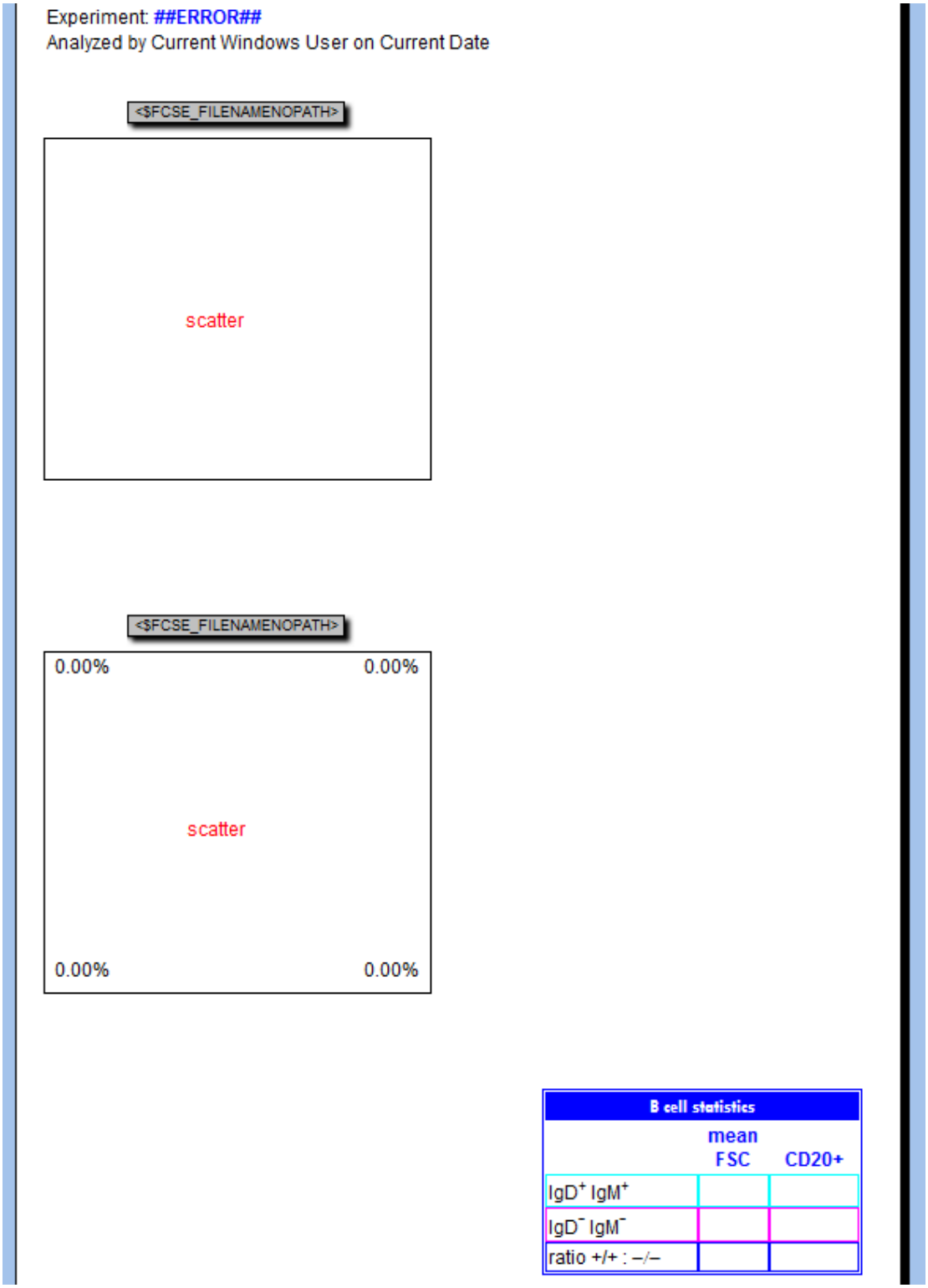

Figure T29.1 Immunophenotyping Tutorial.fey. Note that plots appear empty because the layout is saved as "unlinked".

We will first change our Preferences[117] so that our .fcs file-derived Stain and

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Name keywords[342] appear on our plot axes[158].

3. Follow the directions in the Changing Axis Labels section[1516] of the Changing Common Preferences tutorial[1513].

   Now, your plots' axes[158] for .fcs files subsequently loaded into this layout will be labeled with these descriptive keywords[342] that were saved with your .fcs files at the time of acquisition.

We will now load an .fcs file into the plots[113].

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[590], in the **Select a Data File** dialog box which appears (Fig. T29.2, ❷).

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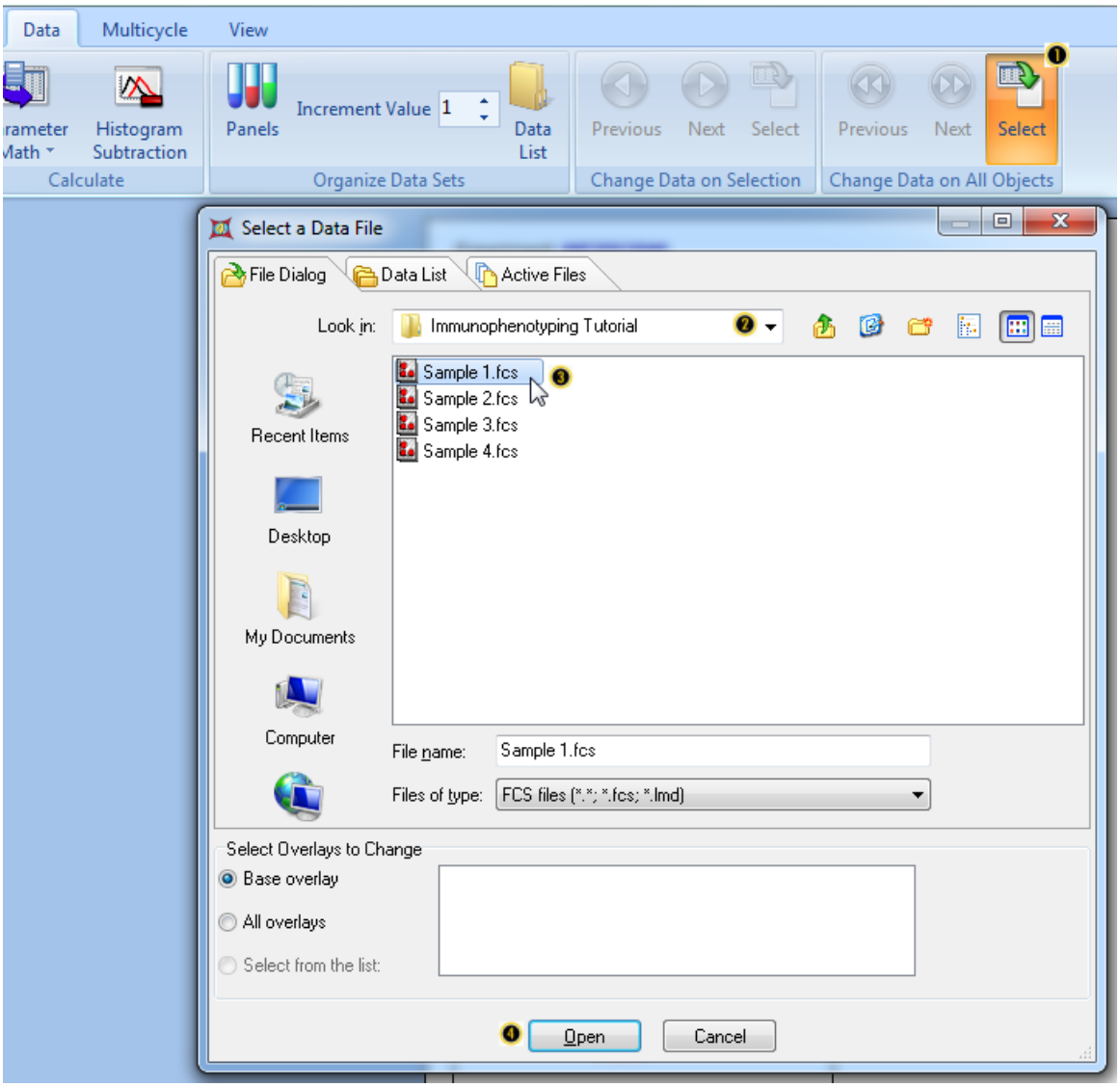

Figure T29.2 Loading .fcs files into the plots

6. Select "Sample 1.fcs" in the **Select a Data File** dialog (Fig. T29.2, ❸).

7. Click **Open** (Fig. T29.2, ❹).

The plots now display the data from the Sample 1.fcs file with a predefined "scatter" gate[193] and quadrants[210] (Fig. T29.3).

Note that the *Experiment* keyword[342] derived from the .fcs file is now displaying in the text box[96] at the top of the Layout, as are the Current Windows User and Current Date. All of these were inserted into a text box[96] as live-updating tokens[675].

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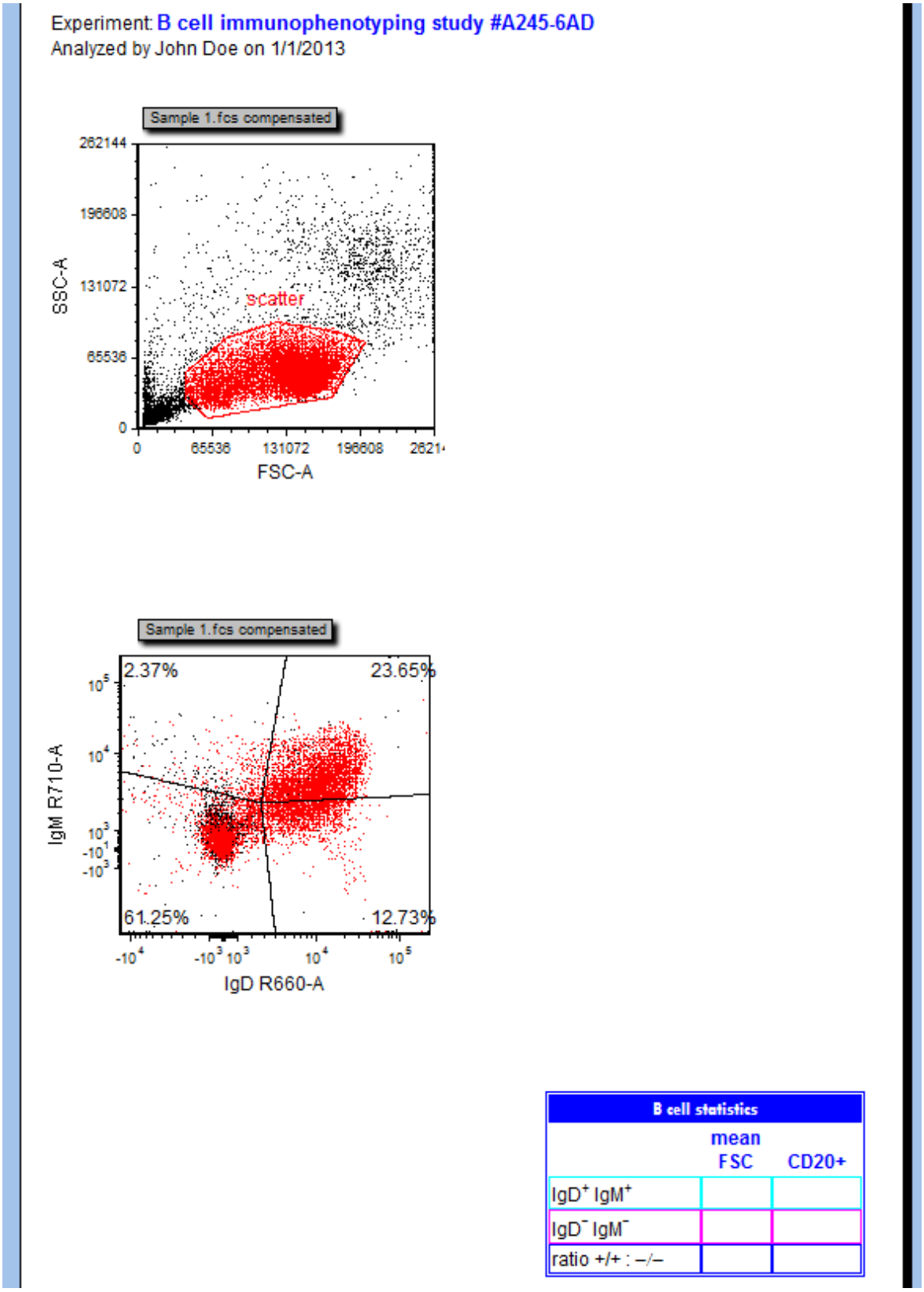

Figure T29.3 2D plots after changing file

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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.

   While dragging, you will start to see a ghost of the gate outline move along the path of the dotted arrow within the original plot.

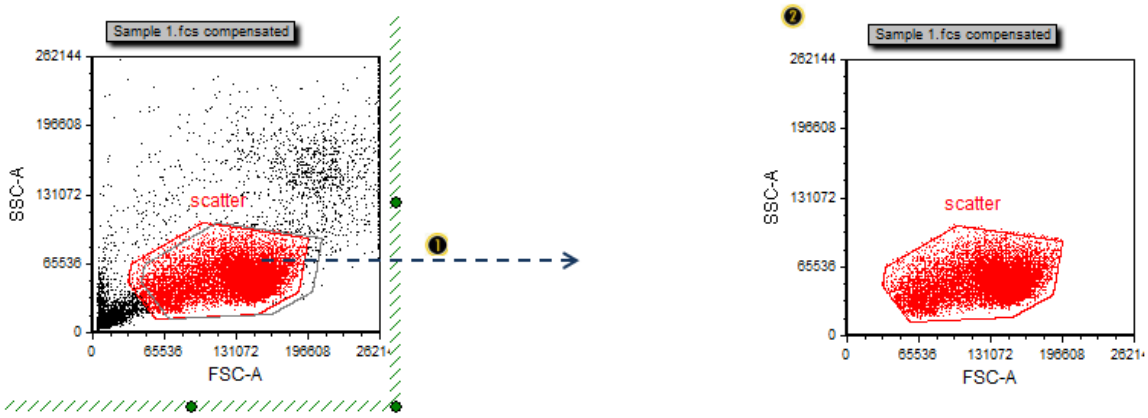

Figure T29.4 Dragging and dropping the "scatter" gate out of a plot to make a new plot gated on "scatter"

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. Press and hold on the X-axis of the new plot.

12. Select "**CD45 V800-A**" as the parameter by highlighting it with the mouse pointer (Fig. T29.5).

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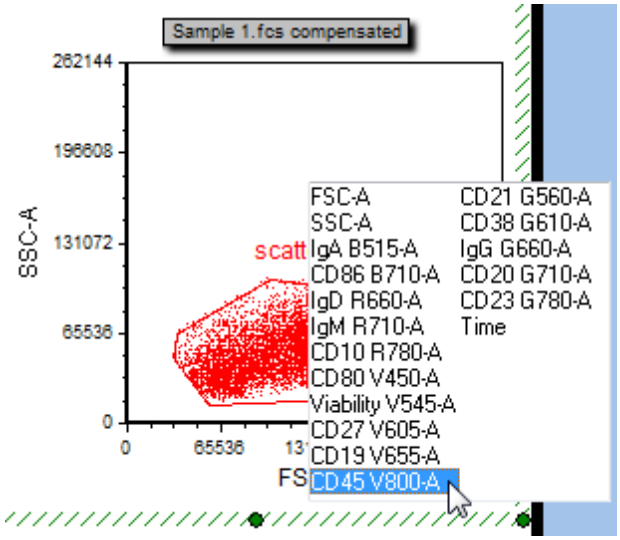

Figure T29.5 Changing the X-parameter of a plot

13. Release the mouse button.

    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[342] to appear on the axis. This change in your **Preferences** will affect all future layouts made under the current FCS Express User Profile[28].

14. Repeat steps 11-13 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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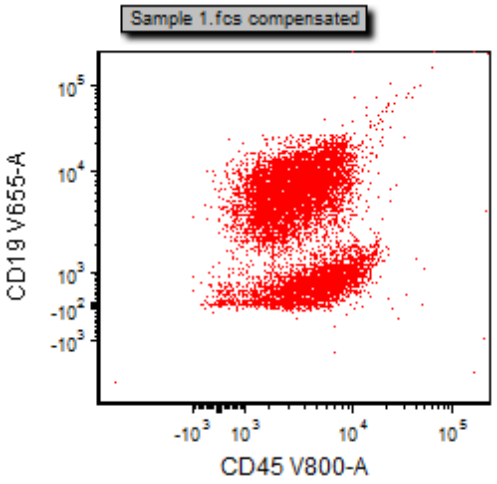

Figure T29.6 Plot with X- and Y-parameters changed to CD45 V800-A vs CD19 V655-A

15. Select the **Gating** tab→**Create Gates**→**Polygon** from the Ribbon (Fig. T29.7, outlined in red).

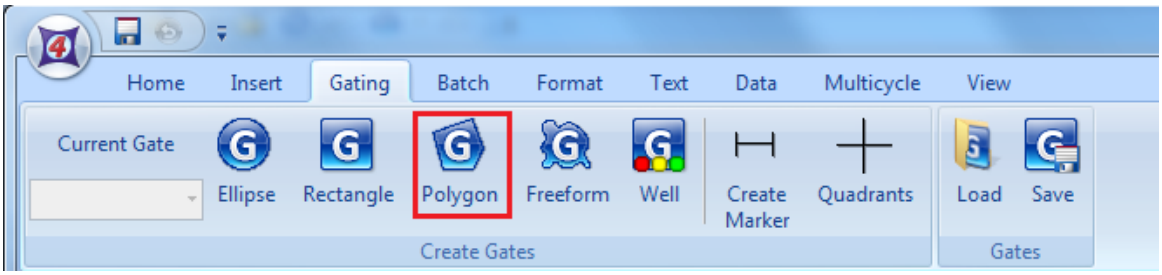

Figure T29.7 Selecting a gate shape from the Gating tab of the ribbon

16. 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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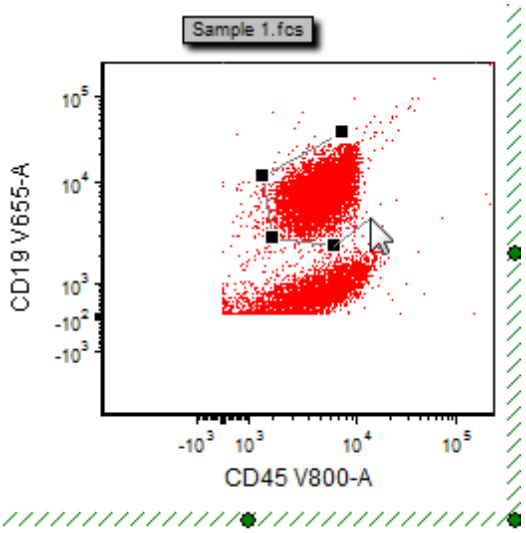

Figure T29.8 Drawing a polygon gate

17. Close the gate by clicking on the first vertex you defined (Fig. T29.9, ❶).

A dialog box[193] appears in which the gate name, color, and inclusion/ exclusion property may be defined.

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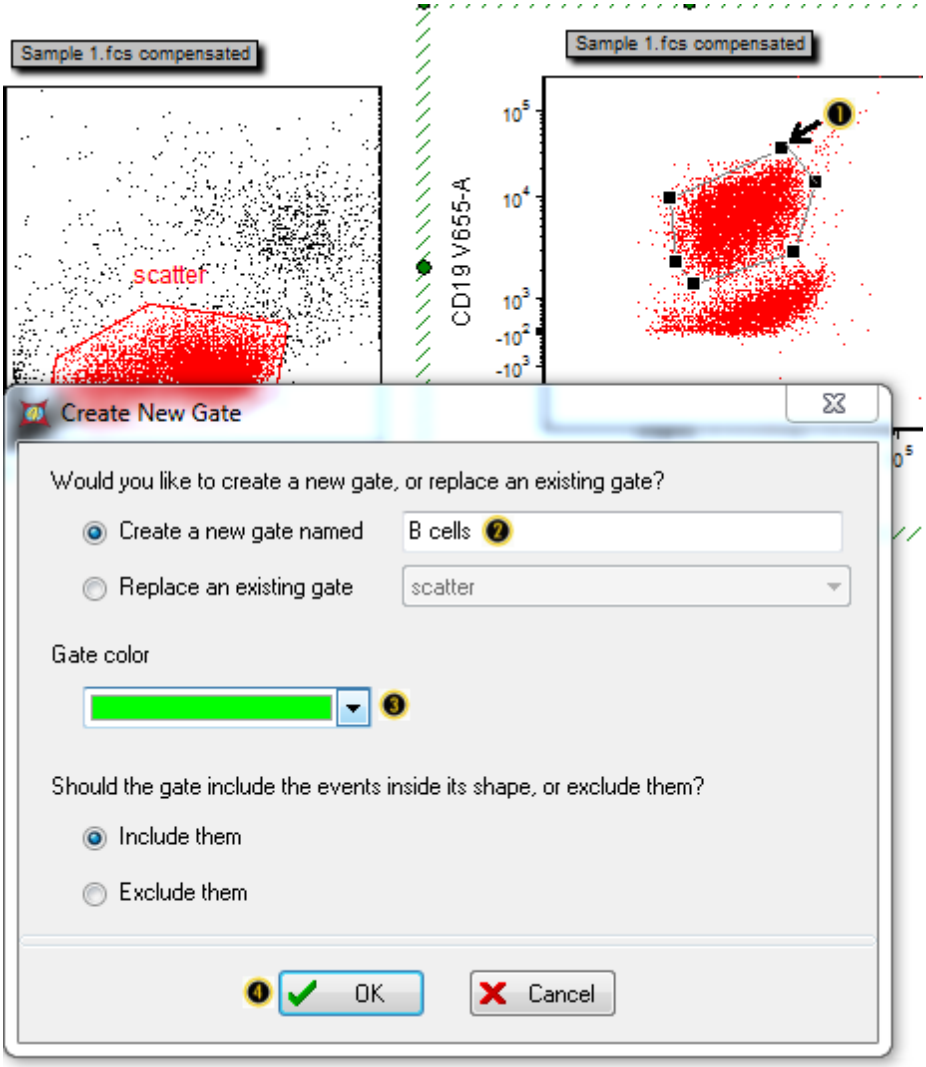

Figure T29.9 Closing and naming a polygonal gate

18. Enter "B cells" in the **Create a new gate name** field (Fig. T29.9, ❷)

19. Select *bright green* from the **Gate color** drop down menu. (Fig. T29.9, ❸).

20. Click **OK** (Fig. T29.9, ❹).

Go on to Step 21, or return to Steps 44-46 1481 if you were referred here from a more advanced point in this tutorial.

The plots now appears similar to Fig. T29.10. Note that the green events in the "B cells" gate are automatically backgated onto the original plot at left, and that some of them fall outside of the original "scatter" gate (Fig. T29.10, ❶: green arrows); also, the colors inside the boundary of the "B cells"

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gate in the second plot are mixed (Fig. T29.10, ❷). This is because in FCS Express, the default User Preference 117 is to not create gates hierarchically by default 134, so the "B cells" gate is not currently a child of the "scatter" gate. We will now adjust our gating logic so that "B cells" becomes a child gate of "scatter".

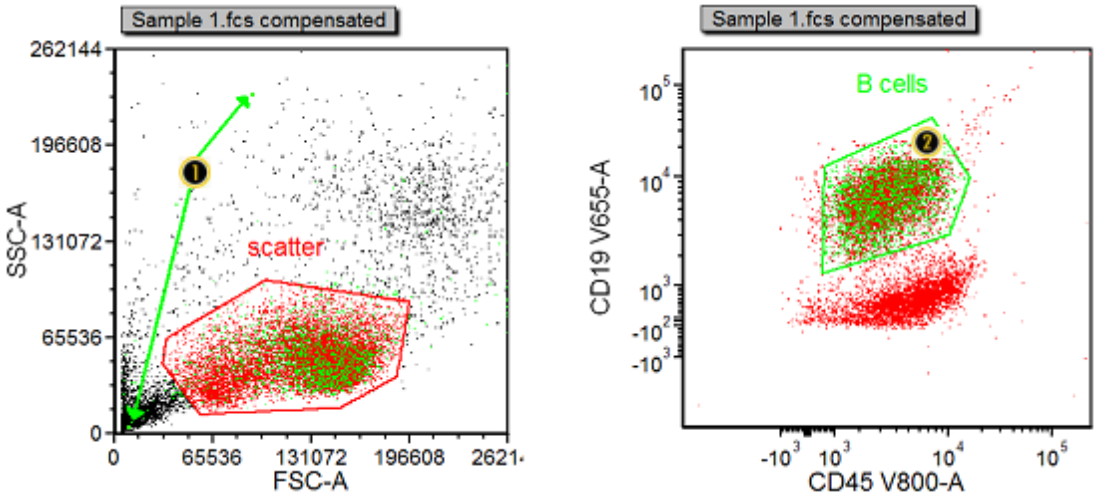

Figure T29.10 B cells gate with backgating onto original color dot plot at left

21. Select the **Insert** tab→**General**→**Gate View** 202 from the Ribbon (Fig. T29.11).

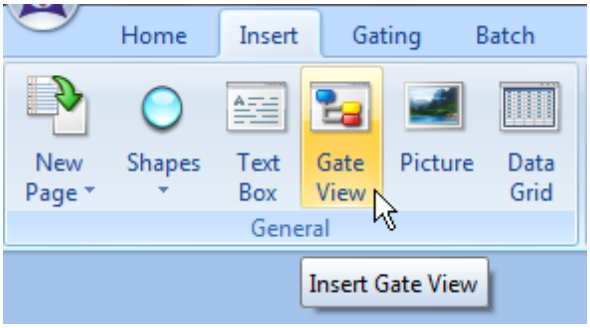

Figure T29.11 Inserting Gate View

22. Click once on the blue background of the Layout.

The Gate View 202 now appears to the side of our page (Fig. T29.12).

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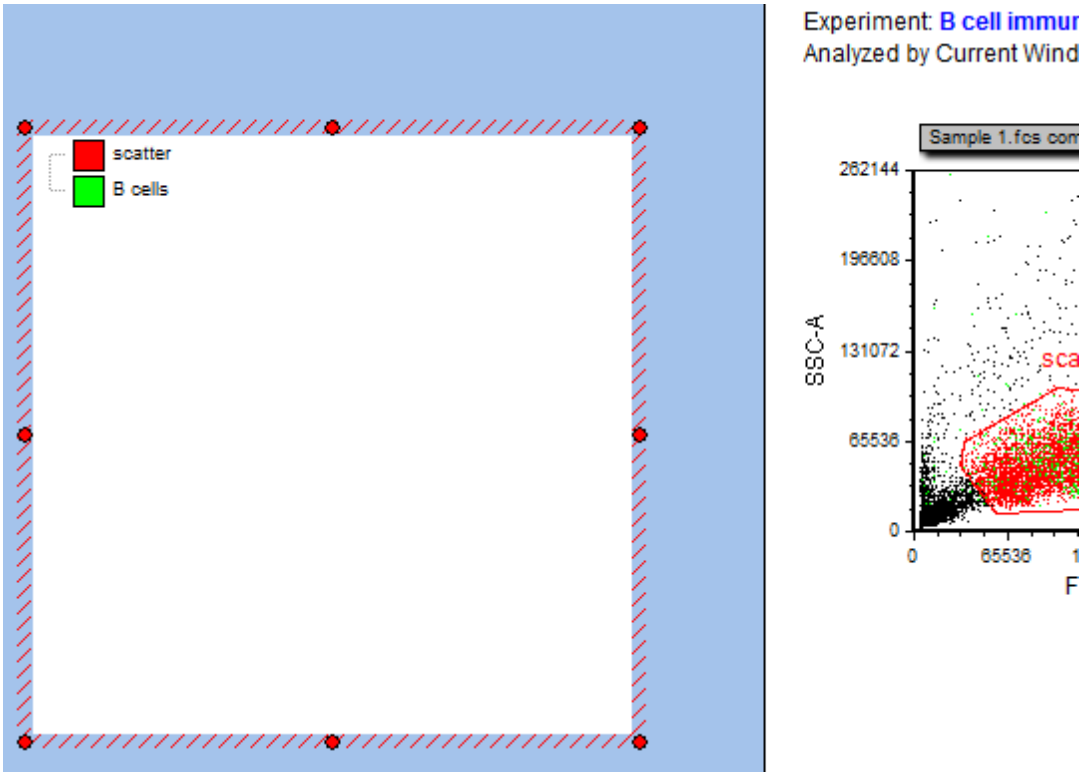

Figure T29.12 Gate View displaying an interactive legend of the existing gates

23. Press and hold on the "B cells" gate in the Gate View 202 (Fig. T29.13, ❶).

24. Drag the "B cells" gate onto the "scatter" gate (Fig. T29.13, ❷).

25. Release the mouse button. "B cells" is now a child of "scatter" (Fig. T29.13, ❸) and the coloring of the events on the Color Dot Plots 110 updates in accordance, resembling Fig. T29.14.

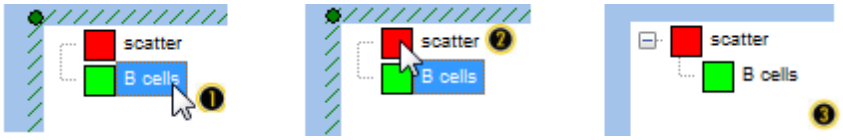

Figure T29.13 Adjusting gate logic in Gate View

26. Select **Application Button→Save Layout As→Save Layout As linked** 53 (second radio button as shown in Fig. 4.2) 53. 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.

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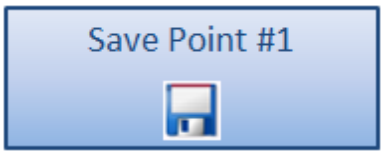

Save Point #1 (Step 26)

We will now adjust our gate preferences so that all subsequent gates are created hierarchically[134], and we will also add a "% gated" statistic to appear automatically in the gate text box on plots[134].

27. Follow the directions in the Creating Gates Hierarchically and Adding Statistics to Gate Text Box section[1517] of the Changing Common Preferences[1513] tutorial.

Note: These preferences[117] will take effect for all gate text boxes subsequently displayed on plots. To show the default statistic in an existing gate text box, we must recreate that text box[105] under the new conditions which we have just specified in preferences in Step 27 .

28. Right-click on the plot with the "B cells" gate visible (Fig. T29.14).

29. Select **Recreate Gate Text Boxes→ All** on the associated pop-up menu (Fig. T29.14, ❶). The desired statistic will now appear within the gate text box (Fig. T29.14, ❷). *Note: you may move the text box[96] to a more convenient location within the plot.*

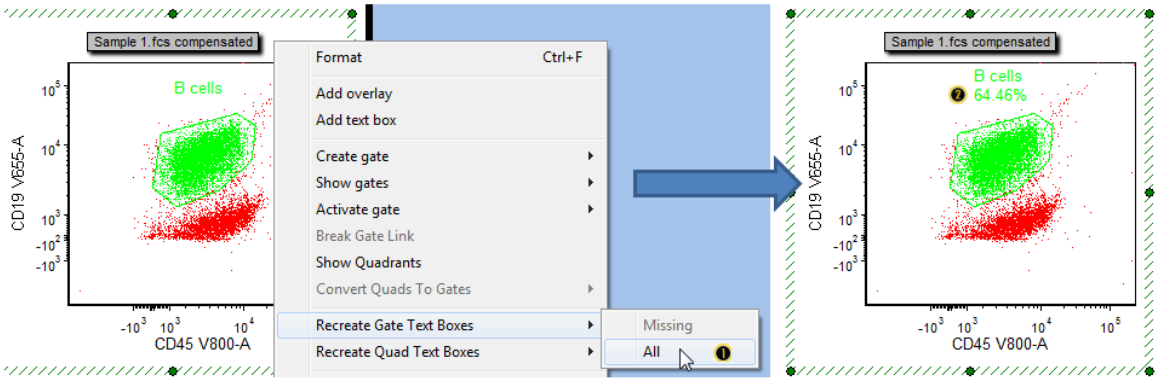

Figure T29.14 Recreating the "B cells" gate text box

We will now examine the phenotype of the B-cell population in more detail by gating the plot[670] in the lower-left corner on the "B cells" gate.

30. Press and hold anywhere inside the boundary of the green "B cells" gate

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(Fig. T29.15).

31. 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. T29.15, ❶).

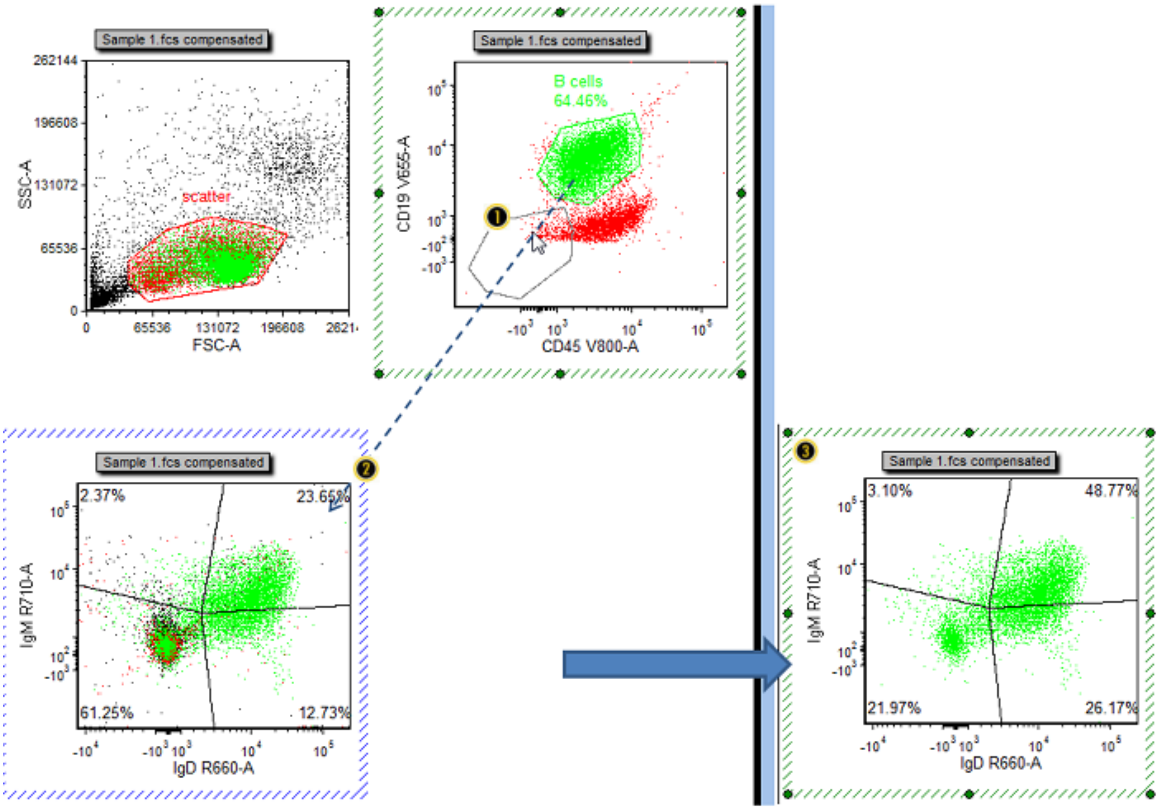

Figure T29.15 Gating a plot by dragging and dropping a gate from another plot

32. Release the mouse button when the recipient plot outline turns blue (Fig. T29.15, ❷).

    The recipient plot is now gated on "B cells" and appears similar to Fig. T29.15, ❸.

    Note: Dragging and dropping the "B cells" gate from the Gate View (pictured in Fig. T29.13) to the recipient plot would achieve the same action.

33. Select **Format** tab→**Change**→**Change Plot Type** from the Ribbon (Fig. T29.16, ❶) while the lower-left plot is still selected.

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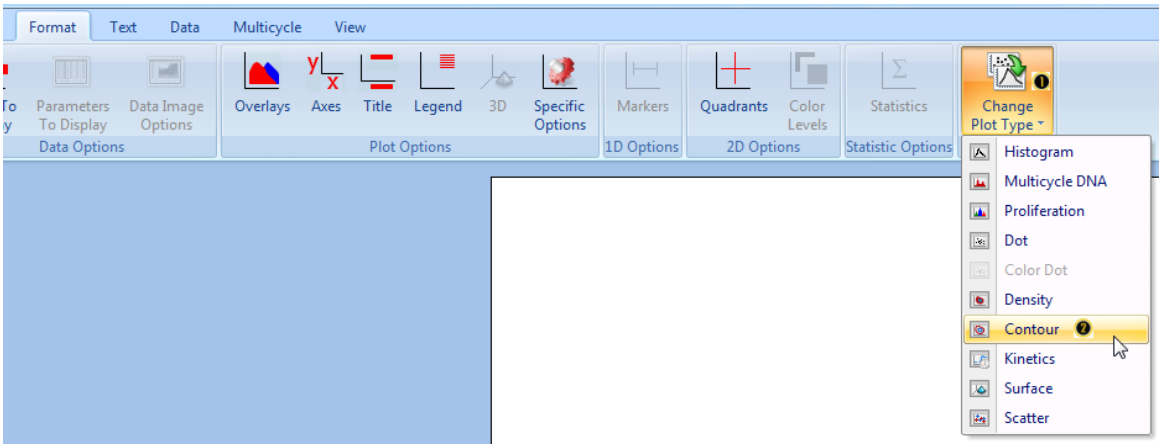

Fig. T29.16 Changing plot type of the selected plot

34. Click on **Contour** (Fig. T29.16, ❷).

    This plot now appears similar to the highlighted plot in Fig. T29.17.

Optional: Add outlier dots[175] to the Contour Plot, in Steps 35 to 40. Otherwise, proceed to Step 41[1480].

35. Right-click on the **Contour Plot**.

36. Select **Format** from the associated pop-up menu (Fig. T29.17, mouse pointer).

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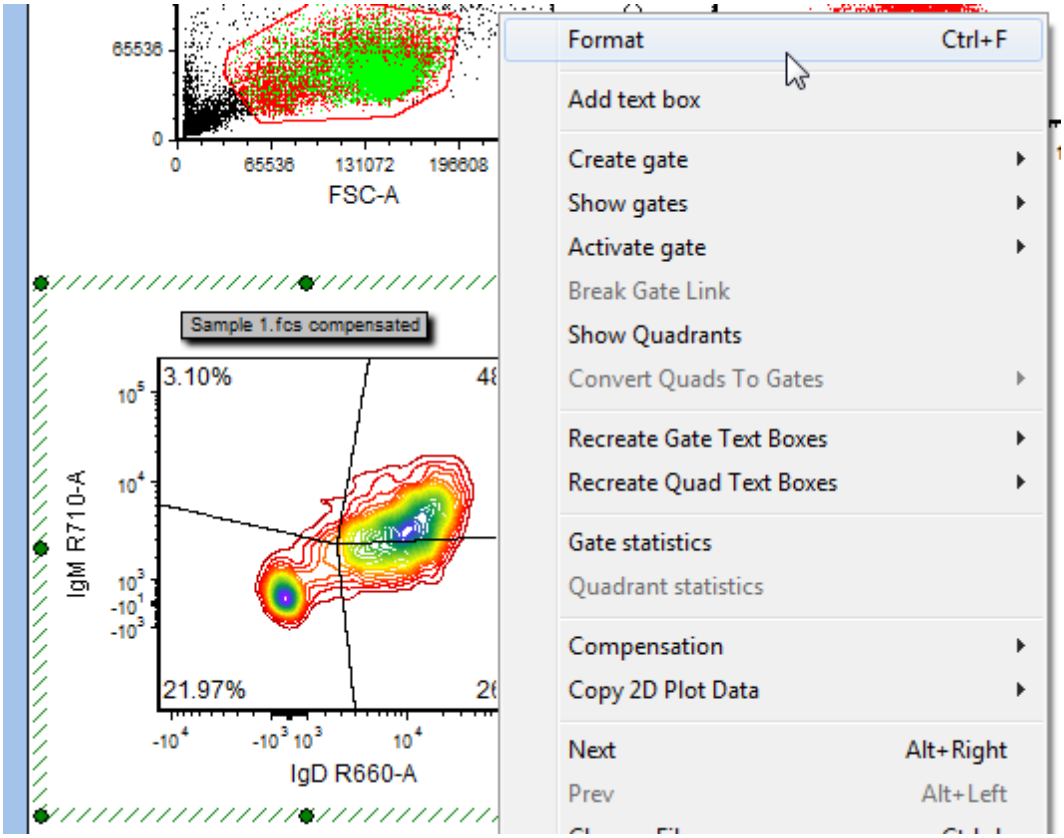

Figure T29.17 Right-clicking on a plot to reformat its appearance

37. Click on the **Overlays** category at the left (Fig. T29.18, ❶).

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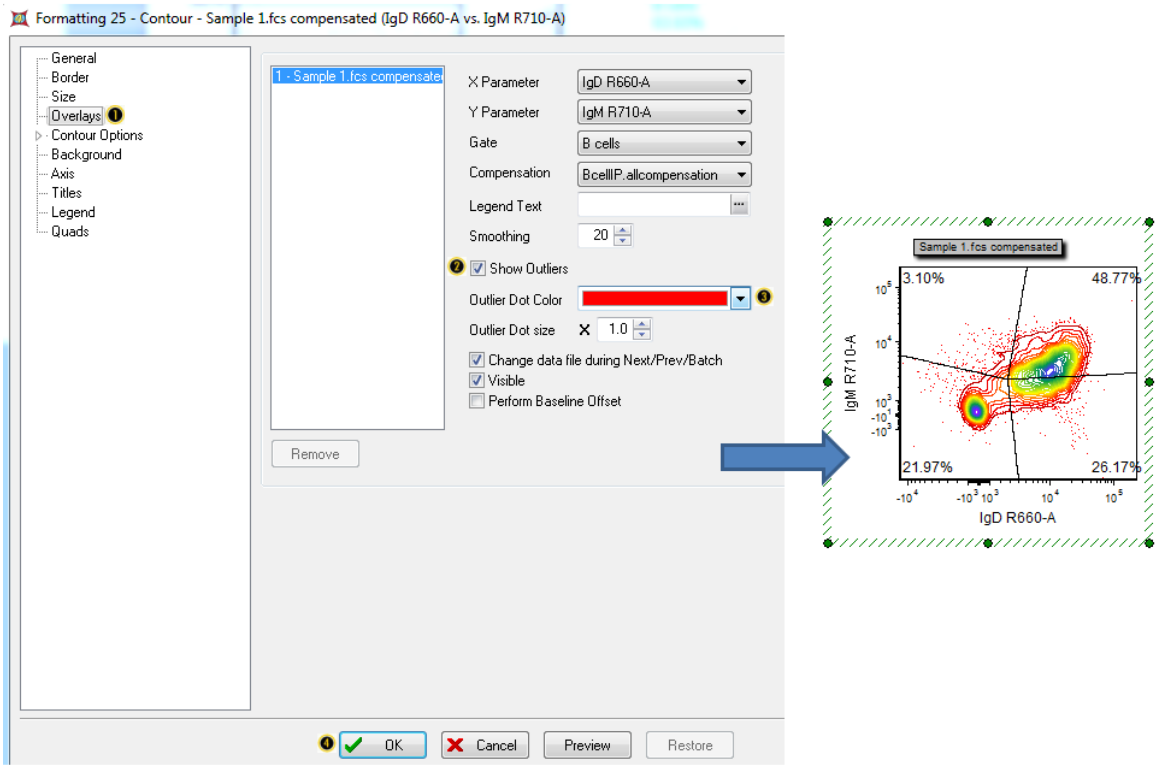

Figure T29.18 Adding outlier dots to a contour plot

38. Check the **Show Outliers** box (Figure T29.18, ❷).

39. Change the **Outlier Dot Color** to red (Figure T29.18, ❸).

40. Click **OK** (Figure T29.18, ❹). The plot now appears similar to Fig. T29.18, *right*.

We will now examine the phenotype of the IgD/IgM-double-negative and -double-positive populations of B-cells in more detail.

41. Press and drag the central Quadrant vertex (e.g., as shown in Fig. T29.19) to adjust its position so that the double-positive and double-negative populations are better divided.

    Note: Outer vertices of these Floating Quadrants[712] may also be adjusted, if desired.

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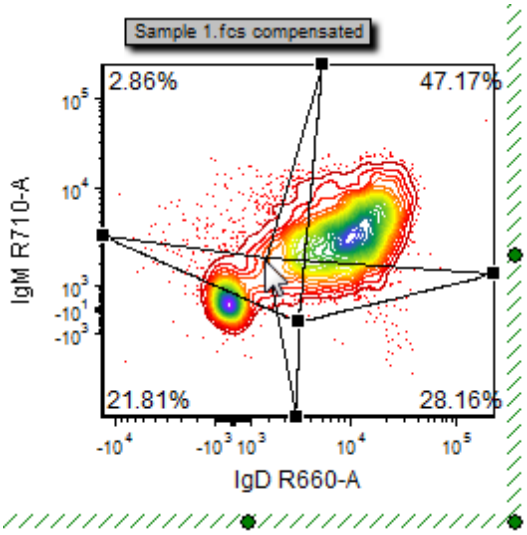

Figure T29.19 Adjusting the central quadrant vertex

42. Right-click on the contour plot (Fig. T29.20).

43. Select **Convert Quads to Gates→Upper Right→Convert and Link** (Fig. T29.20, mouse pointer).

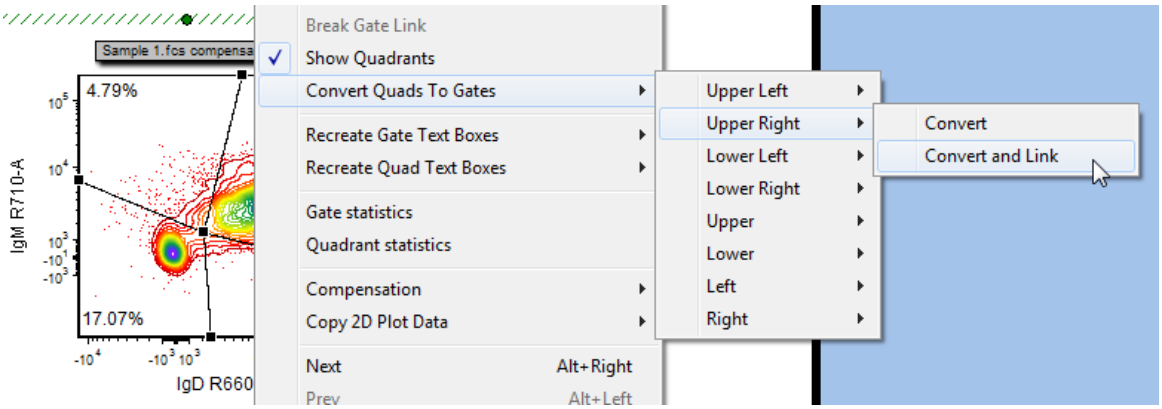

Fig. T29.20 Converting and linking the upper right quadrant to a gate

A dialog box similar to that in Fig. T29.9 1473 appears.

44. Repeat steps 18-20 1473 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.

45. Repeat steps 42-44 in order to **Convert and Link** the **Lower Left**

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Quadrant to a Gate.

46. Repeat steps 18-20 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.

47. Click on the "B cell" node in the **Gate View** to expand it (Fig. T29.21, ❶).

    Note that these new gates have been automatically defined as children of "B cells" (Fig. T29.21, ❷) as a result of adjusting our Gate Preferences in Step 27.

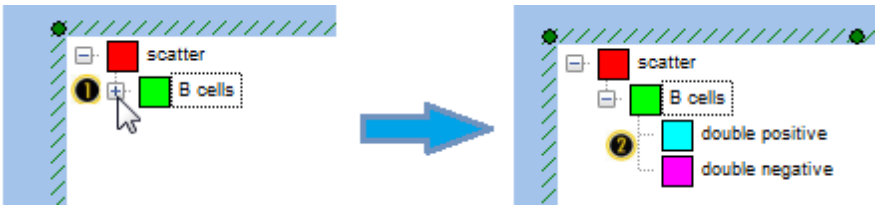
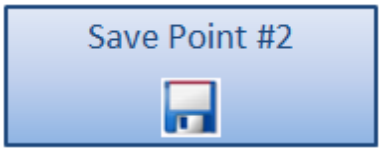


Figure T29.21 Expanding a node in Gate View, revealing the new gates automatically defined as children of B-cells gate

48. Select **Application Button→Save Layout As→Save Layout As 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. 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 48)

49. Select **Insert** tab→**1D Plots→Histogram** from the Ribbon (Fig. T29.22, outlined in red).

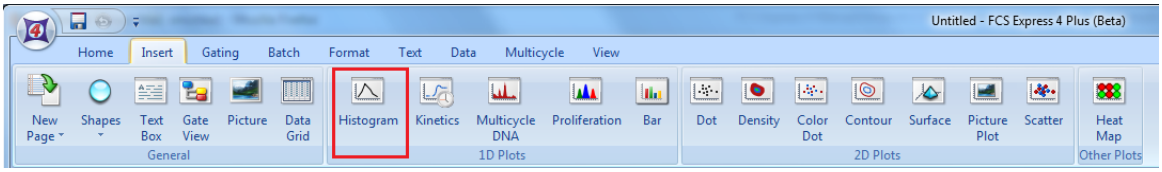


Figure T29.22 Selecting histogram from the Insert tab of the Ribbon

50. Press and hold on the blank space of the layout above the pre-formatted

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table (Fig. T29.23).

51. Drag the cursor until the histogram is of the desired size, while continuing to hold the mouse button (Fig. T29.23, ❶).

Note: Any plot, text box, or other object 147 on your layout may be resized 592 at any time.

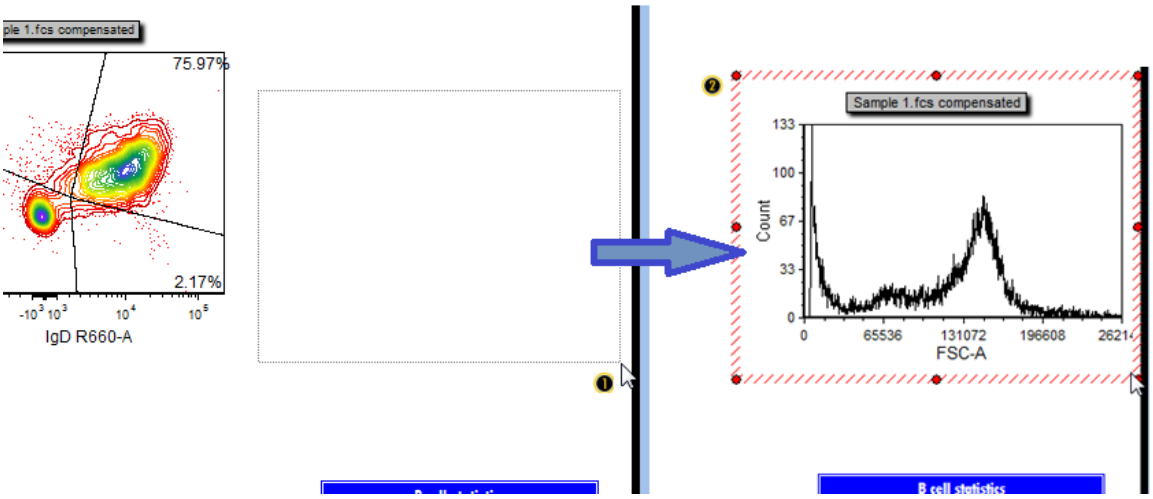

Fig. T29.23 Inserting a histogram onto the layout

52. Release the mouse button. A new histogram 167 appears, similar to Fig. T29.23, ❷.

We will now overlay 167 the "double-positive" and "double-negative" populations onto the histogram to compare their CD20-expression.

53. Right-click on the histogram 167 (Fig. T29.24).

54. Select **Add overlay** in the associated pop-up menu (Fig. T29.24).

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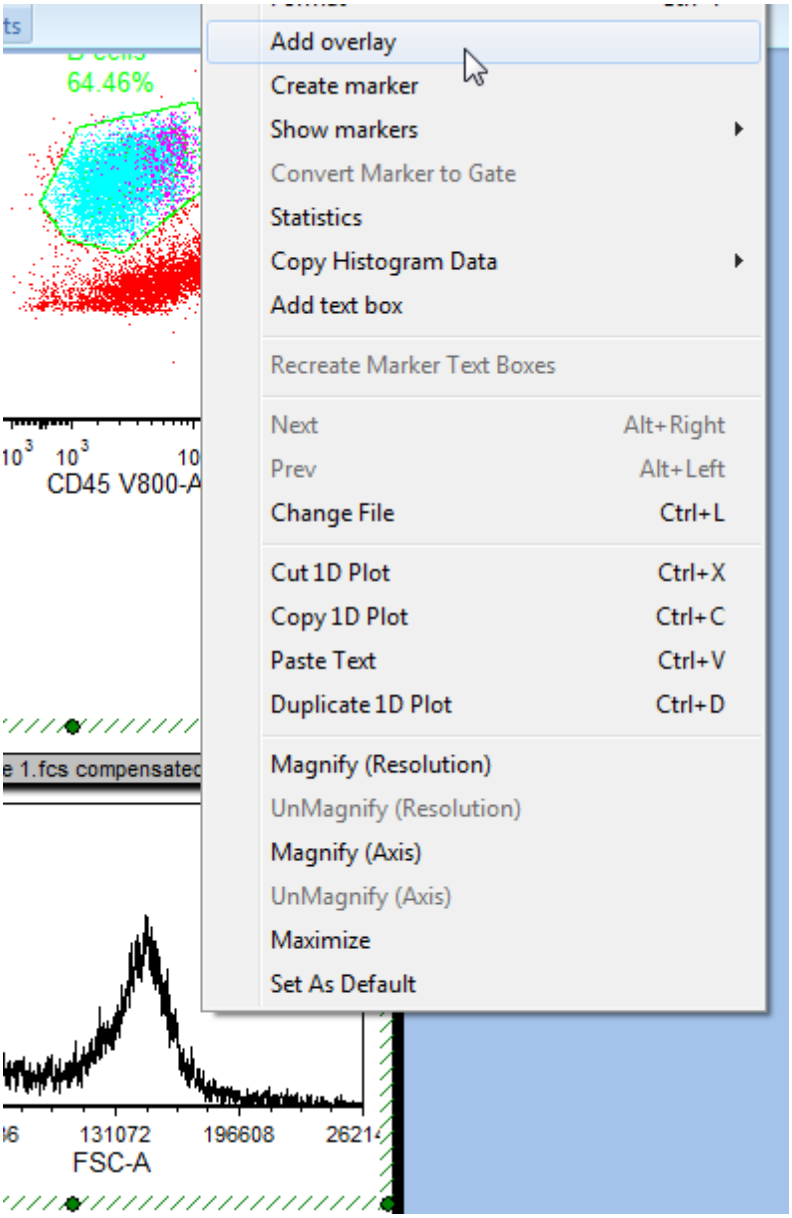

Figure T29.24 Adding an overlay to the histogram by right clicking on the plot and choosing "Add Overlay"

55. Repeat Steps 5-7 as in Fig. T29.2 to add "Sample 1.fcs" as a second overlay to the histogram.

56. Click **OK** on the additional dialog box that appears (Fig. T29.25).

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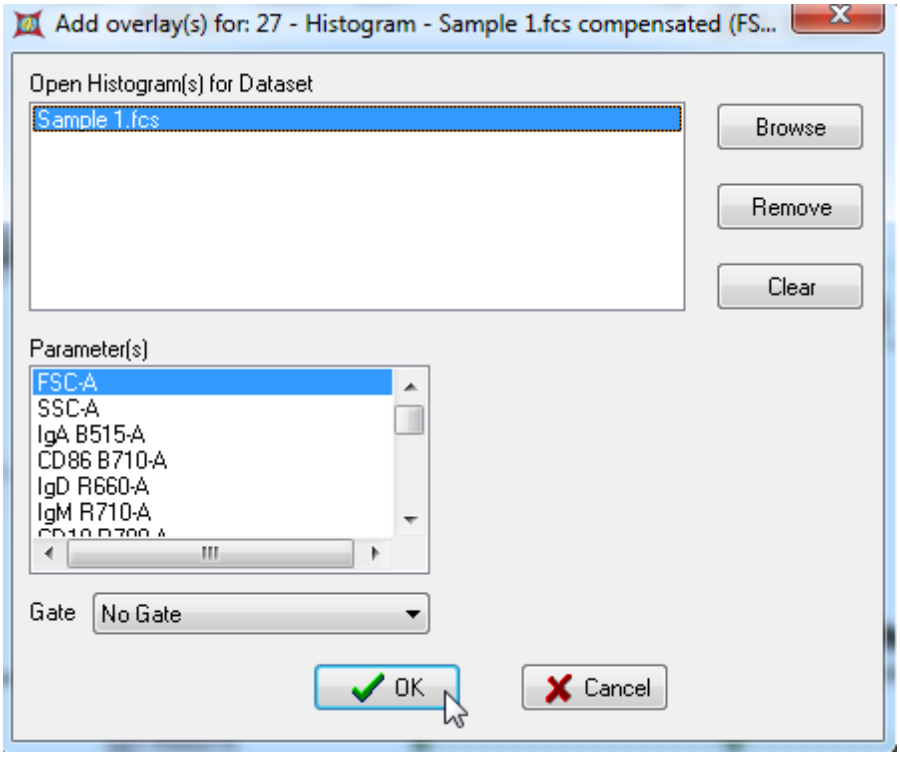

Fig. T29.25 Additional dialog box for adding overlays

57. Right-click on the histogram again (Fig. T29.26).

58. Select **Format** on the associated pop-up menu (Fig. T29.26).

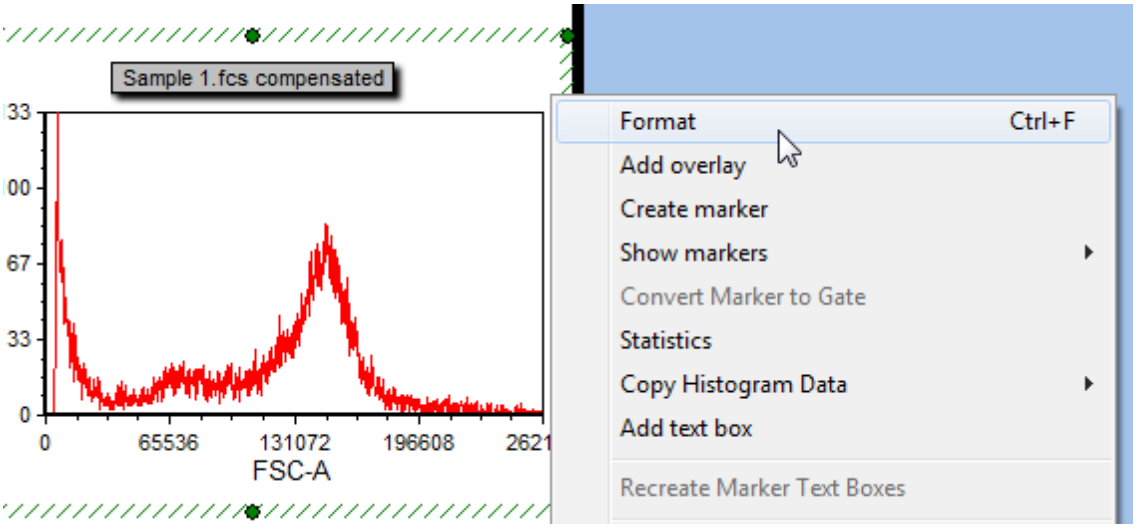

Figure T29.26 Right-clicking on the 1D-plot to access Format options

59. Select the **Overlays** category at the left (Fig. 29.27, ❶).

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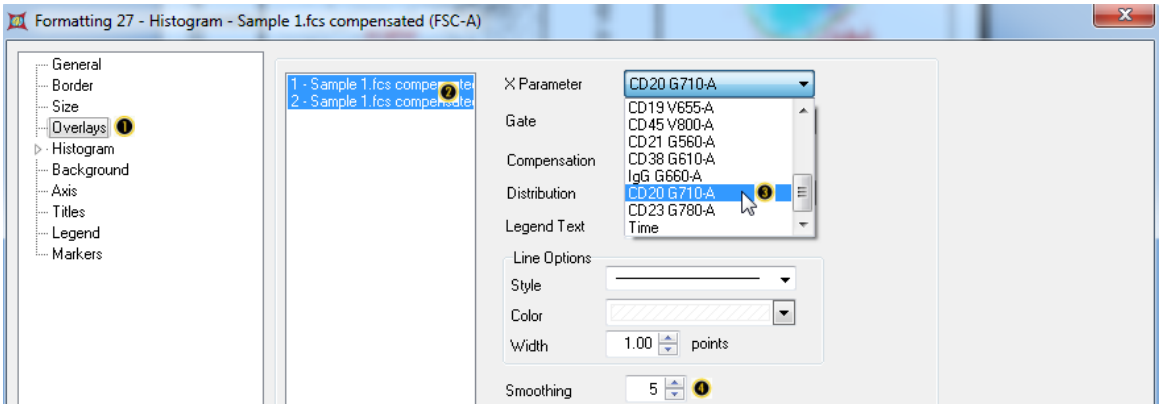

Figure T29.27 Formatting histogram overlays

60. Select both overlays by clicking on each while holding down *Ctrl* (Control) on the keyboard (Fig. 29.27, ❷).

61. Select "**CD20 G710-A**" from the **X Parameter** drop-down menu (Fig. 29.27, ❸).

62. Increase **Smoothing** 167 to "**5**" (Fig. 29.27, ❹).

63. Click on the first overlay ("**1-Sample 1.fcs compensate...**") so it alone is selected (Fig. 29.28, ❶).

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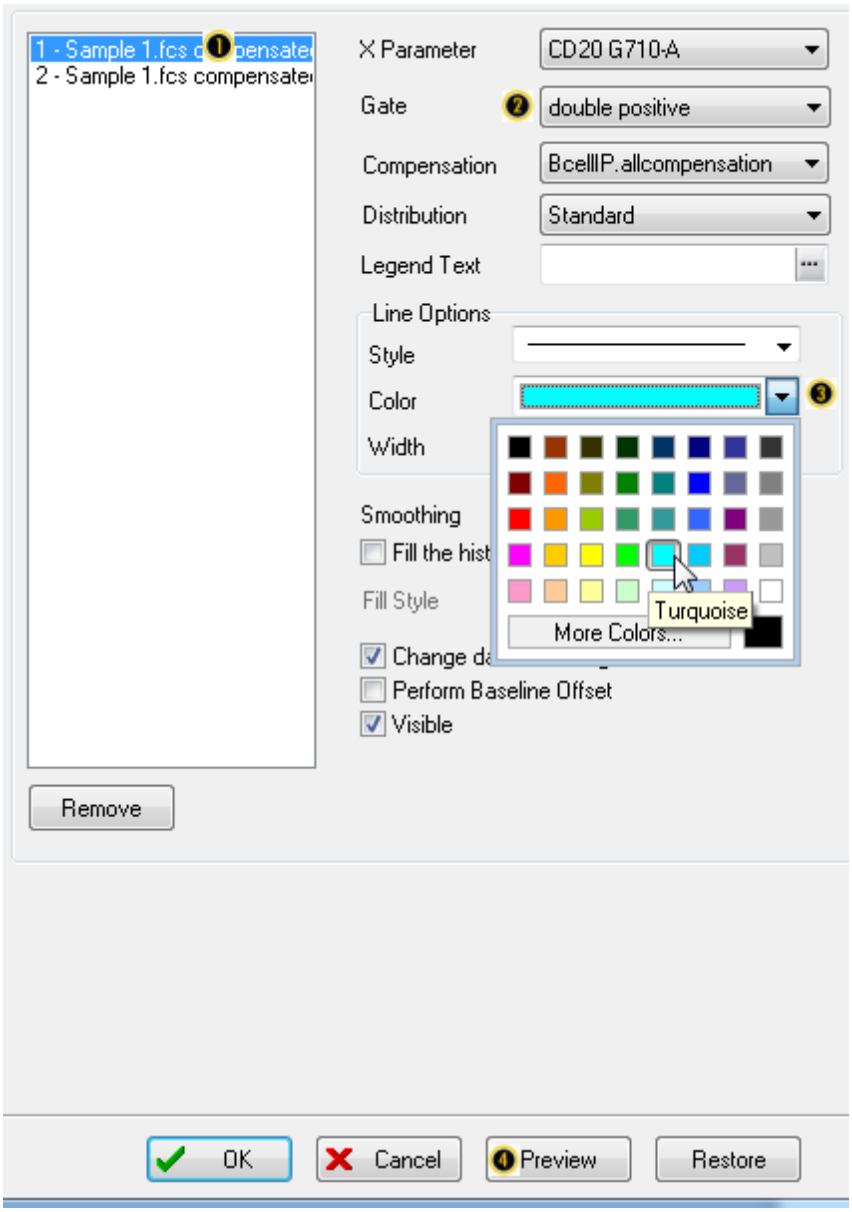

Figure T29.28 Formatting histogram overlays, continued

64. Select "**double positive**" from the **Gate** drop-down menu (Fig. 29.28, ❷).

65. Select turquoise as the **Color** under **Line Options** (Fig. 29.28, ❸).

66. Repeat Steps 63-65, 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.

67. Click **Preview** (Fig. 29.28, ❹). *(Note: Move the formatting window to the side to preview the formatting changes)*

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The histogram now appears similar to Fig. T29.29.

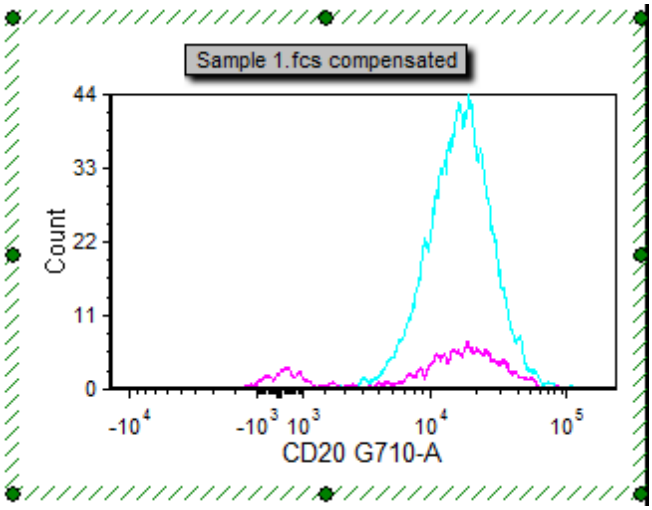

Figure T29.29 Preview of gates, colors, and smoothing applied to both histogram overlays

Optional: Normalize the histogram, in Steps 68-71. Otherwise, proceed to Step 72 1488**.**

68. Expand the Histogram, on the category at left (Fig. T29.30, ❶).

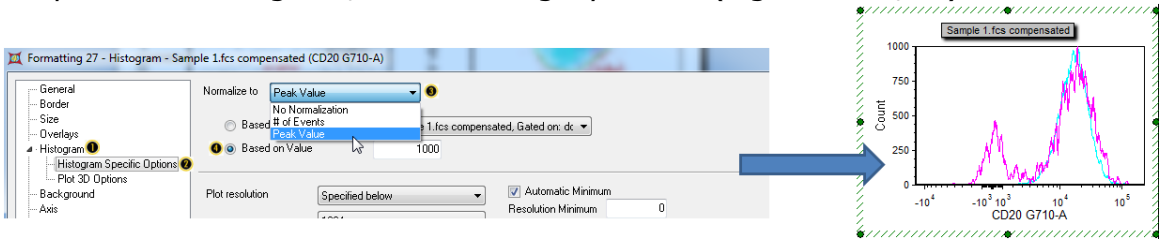

Figure T29.30 Normalizing a histogram

69. Click on **Histogram Specific Options** (Fig. T29.30, ❷).

70. Select **Peak Value** from the **Normalize to** drop-down menu (Fig. T29.30, ❸).

71. Select the **Based on Value** radio button (Fig. T29.30, ❹).

72. Click **OK** to close the **Formatting** pop-up window (Fig. T29.31). 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.

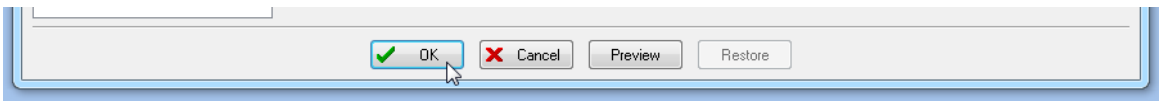

Figure T29.31 Clicking OK to accept the formatting changes.

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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.

73. Right-click on the histogram (Fig. T29.32).

74. Select **Create marker** on the the associated pop-up menu (Fig. T29.32).

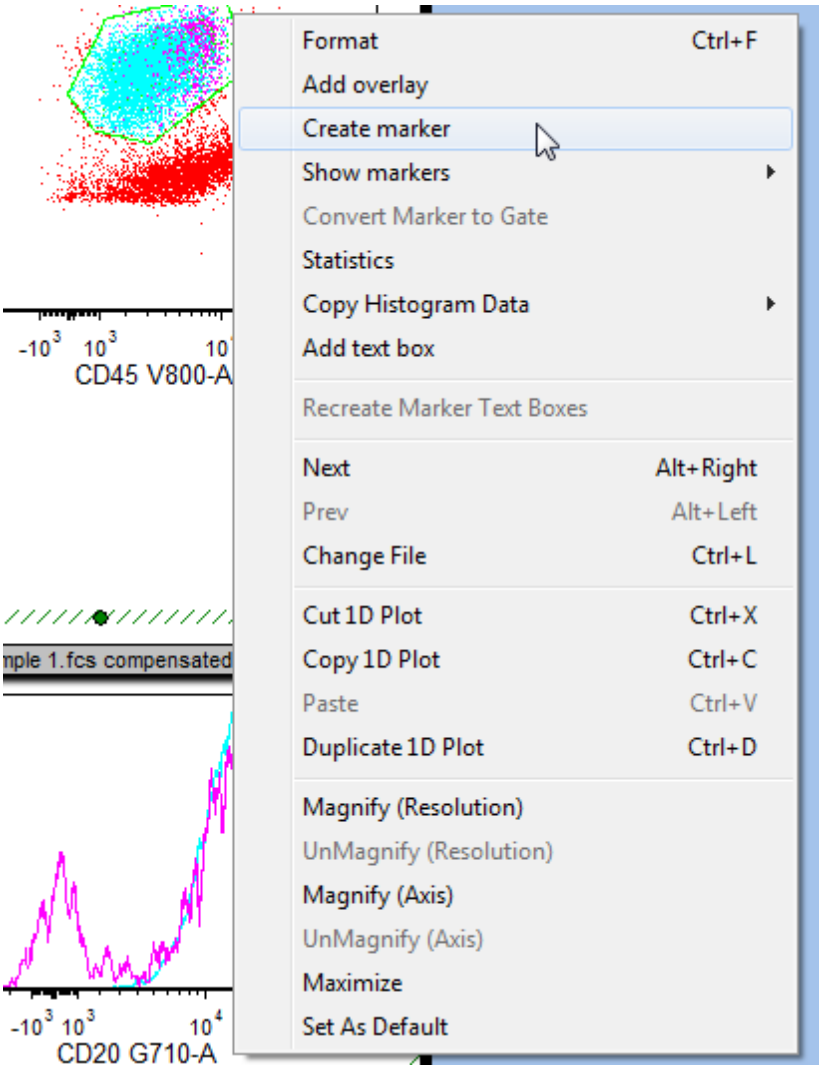

Figure T29.32 Right-clicking on the histogram to add a marker

75. Press and hold on the histogram to the left of the positive population peak (Fig. T29.33).

76. Drag the mouse pointer to the right until the marker spans the peak (Fig.

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T29.33, ❶).

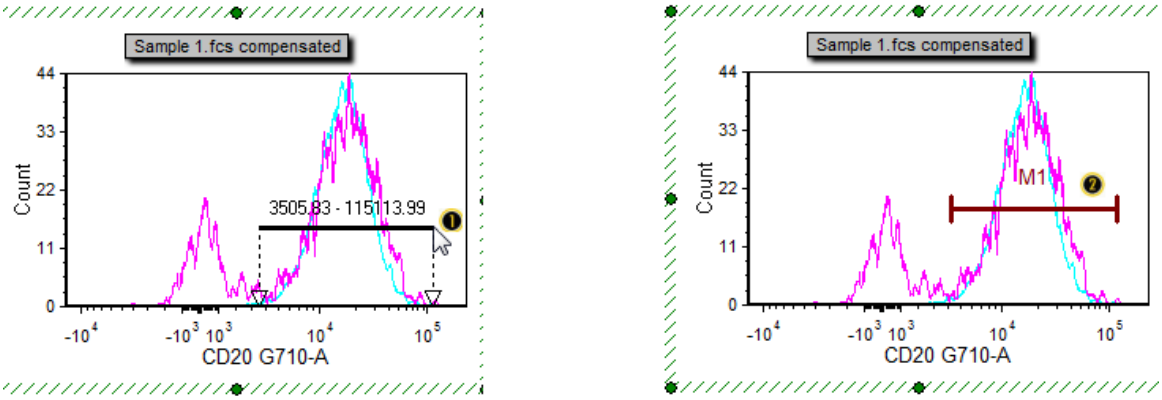
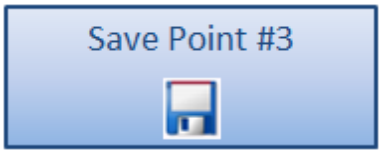


Figure T29.33 Creating a marker

77. Release the mouse. The marker[208] ("**M1**") has now been added to the plot (Fig. T29.33, ❷).

78. Select **Application Button→Save Layout As→Save Layout As** linked[53] (second radio button as shown in Fig. 4.2[53])[53], or simply press *Ctrl + S* on your keyboard if you already saved this layout linked[53] 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 78, 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[1490].

34.30.2 Adding statistics to your layout

In this section, we will:

- Add a default statistics table to our layout.
- Fill a custom text-table[687] with selected statistics[214].
- Define a custom ratio using a Custom Token[700].

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

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to this Immunophenotyping Tutorial Layout.

1. Select **Application Button→Open Layout**.
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 53 to the .fcs files in the same folder, and should resemble your Layout after completing the previous section of the tutorial 1463. 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. T29.34; *note: the plot pictured is from the provided layout for this section and shows red outlier dots added as in optional Steps 35-40 from Section 1* 1478*. 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** on the associated pop-up menu (Fig. T29.34).

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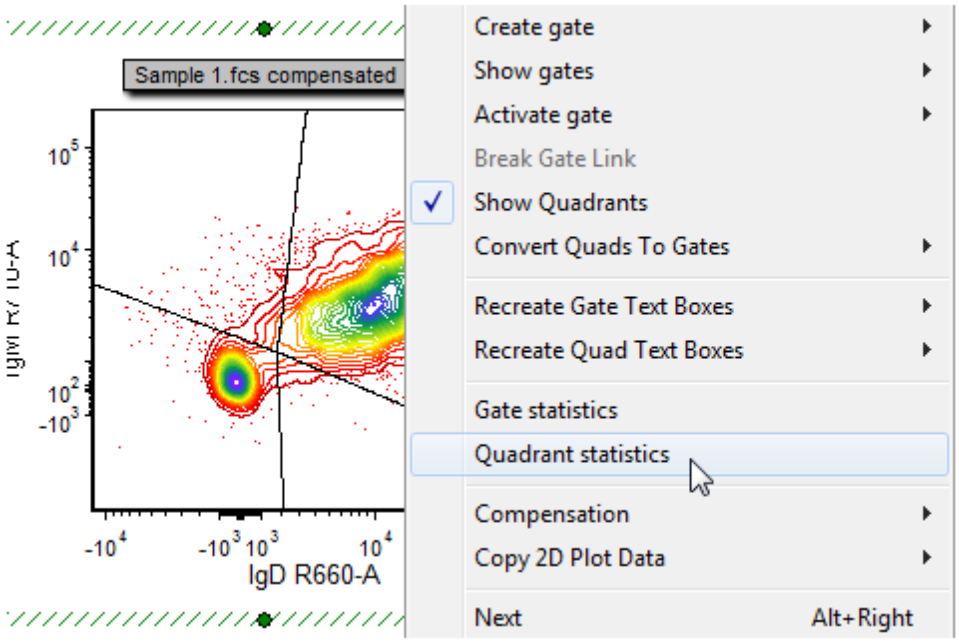

Overlay #	FCS Filename	Quadrant	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Sample 1.fcs compensated	UL	337	0.00	3540.36	4.06	1.88
1	Sample 1.fcs compensated	UR	6425	9118.03	0.00	77.46	35.81
1	Sample 1.fcs compensated	LL	1388	0.00	0.00	16.73	7.74
1	Sample 1.fcs compensated	LR	145	3850.31	0.00	1.75	0.81

Figure T29.34 Adding a default quadrant statistics window to the layout

A quadrant statistics window appears, similar to Fig. T29.34. *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.

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A Default statistics window is a convenient way to quickly display summary statistics[214] 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[328] to custom-made tables in a text box. We will now add statistical tokens to the text table[687] in the lower-right corner of the layout, which was custom designed within a text box[675]. The chief advantages of these custom tables are: statistics[214] from disparate plots may be displayed; an endless variety of statistics[214] including user-defined statistics[700] may be defined; and, the appearance of the table itself is fully formattable[687].

5. Click on the marker in the histogram to select it (Fig. T29.35)

   ***Note**: the histogram pictured is from the provided layout for this section and shows a normalized histogram as formatted in optional Steps 68-71 from the previous section[1488]. If you are using your own layout from the previous section and skipped those steps, your histogram will instead resemble Fig. T29.29[1488].)*

6. Drag the marker toward the table by hovering over the marker until a four-headed arrow appears, and pressing and holding the mouse button while dragging (Fig. T29.35,).

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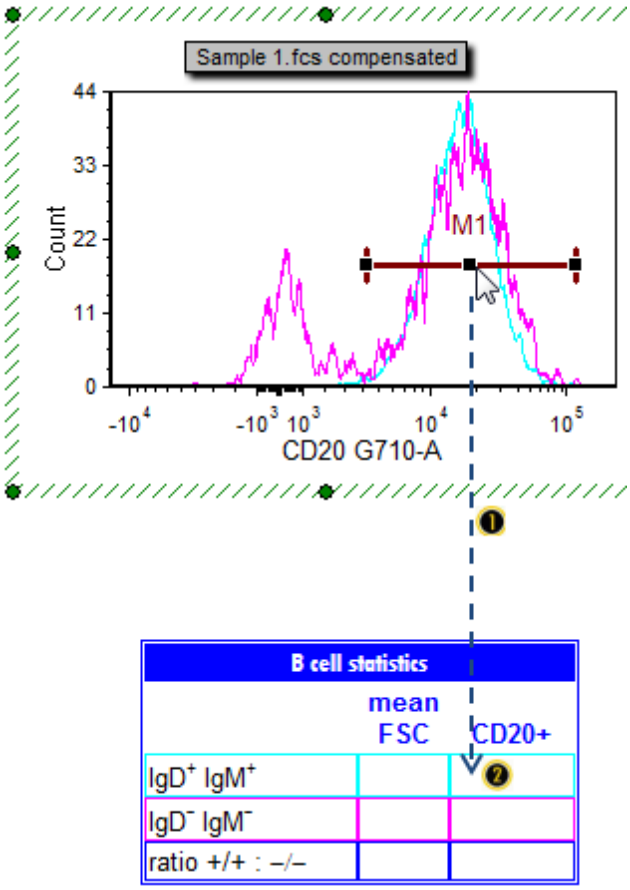

Figure T29.35 Dragging and dropping a marker-derived statistical token into a text table

7. Release the mouse when the cursor is visible within the upper-right, turquoise cell in the table (Fig. T29.35, ❷). Go on to Step 8 or to Step 11 1495 if you were referred here from a more advanced point in this tutorial.

8. Select "double positive" in the **Gate** drop-down menu of the **Edit Statistic** dialog box that appears (Fig. T29.36, ❶).

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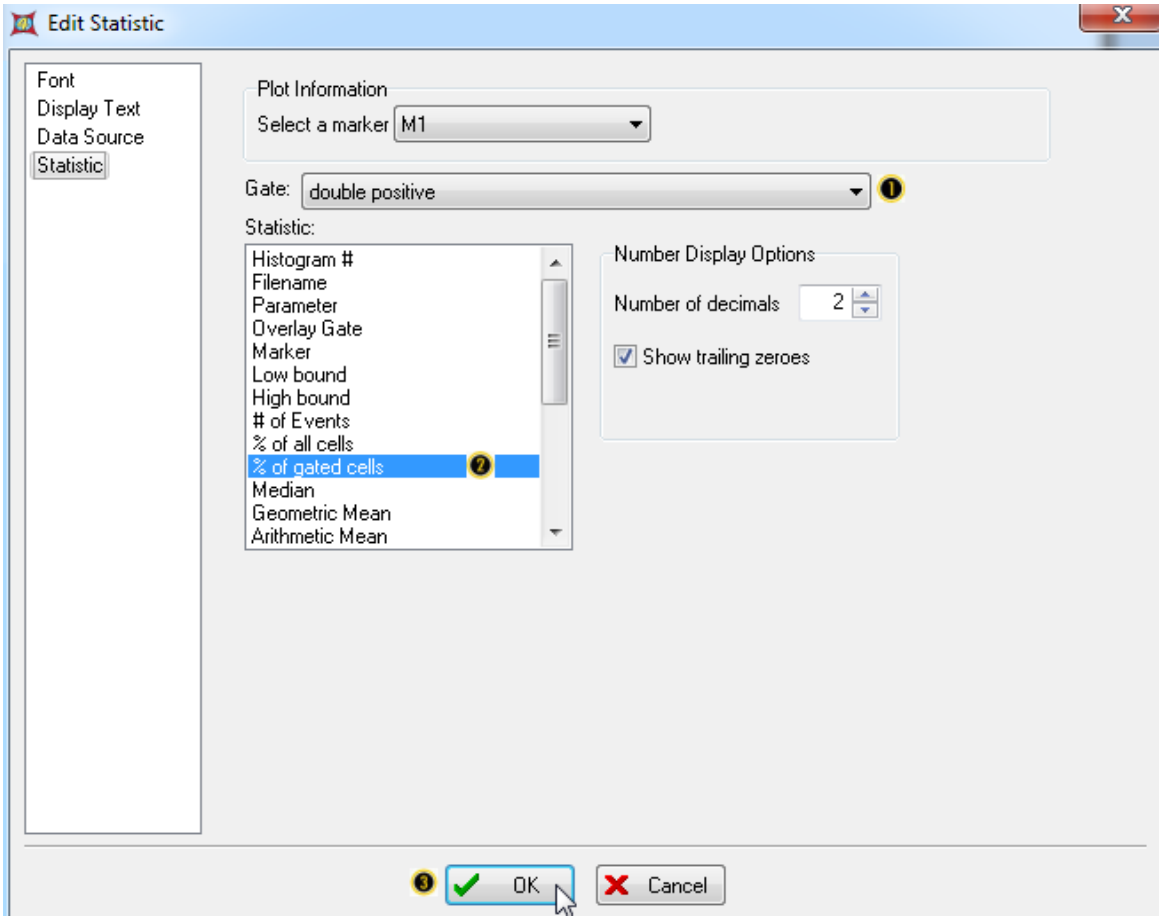

Figure T29.36 Selecting a marker-derived statistical token

9. Select **% of gated cells** in the **Statistic** list (Fig. T29.36, ❷).

10. Click **OK** (Fig. T29.36, ❸).

11. Repeat Steps 5-7 1493, but drag the marker into the pink cell reserved for the double-negative, CD20-positive population (immediately below the cell indicated in Fig. T29.35).

12. Repeat Steps 8-9, but select "double negative" in the **Gate** drop-down menu.

13. Click on **Data Source** in the category at left (Fig. T29.37, ❶).

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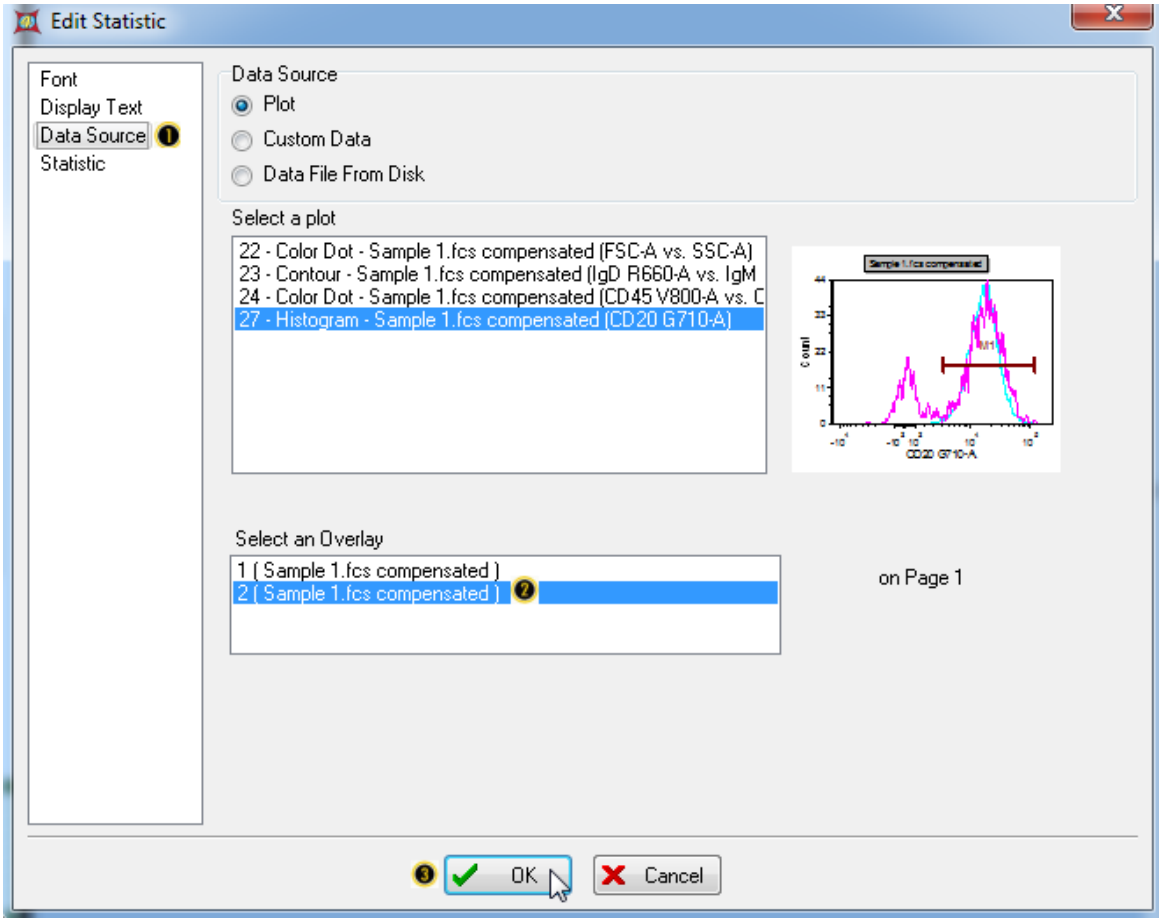

Figure T29.37 Defining the second overlay in the histogram as the data source for a statistical token

14. Click on the second overlay to select it as the **Data Source** for this statistic (Fig. T29.37, ❷).

15. Click **OK** (Fig. T29.37, ❸). The two tokens now appear in the text table 687 as shown in Fig. T29.38. *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.*

16. Press and hold the mouse button inside the boundaries of the red "scatter" gate in the upper-left color dot plot (Fig. T29.38).

17. Drag it toward the table. While dragging, you will start to see a ghost of the gate outline move along the path of the dotted arrow within the original plot (Fig. T29.38, ❶).

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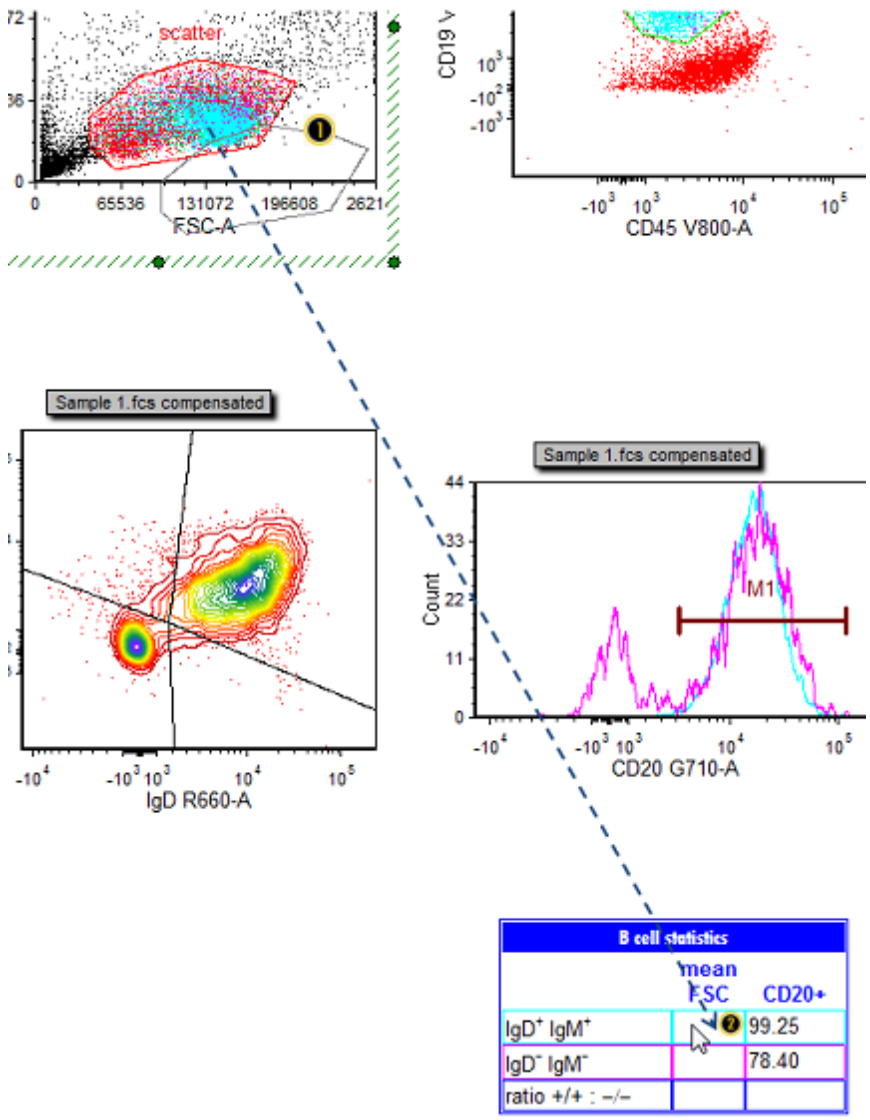

Figure T29.38 Dragging and dropping a gate-derived statistical token into a text table

18. Release the mouse when the cursor is visible inside the turquoise cell pictured in Fig. T29.38, ❷.

19. Select "double positive" from the **Gate** drop-down menu of the **Edit Statistic** dialog box that appears (Fig. T29.39, ❶).

20. Select **X Arithmetic Mean** in the **Statistic** category (Fig. T29.39, ❷).

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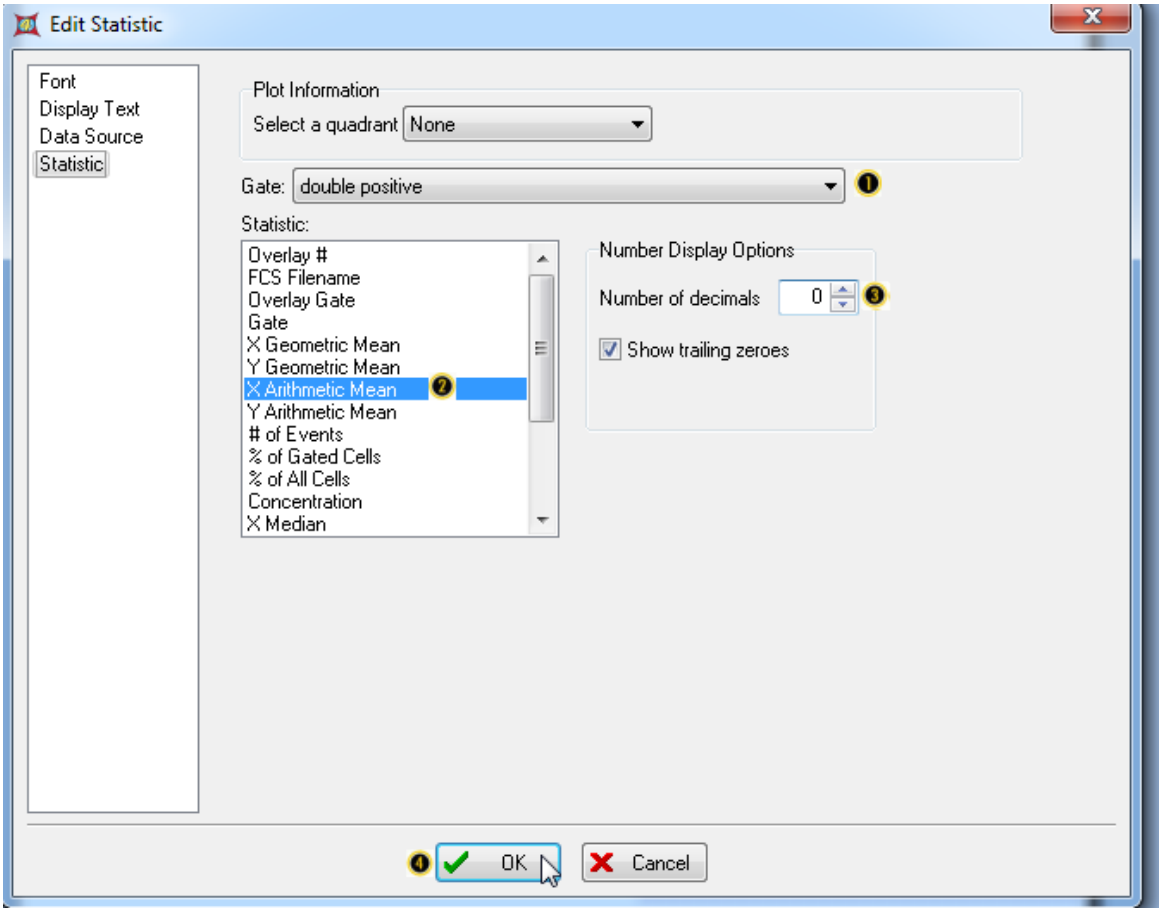

Figure T29.39 Selecting a gate-derived statistical token

21. Reduce the **Number of decimals** to "0" (Fig. T29.39, ❸).

22. Click **OK** (Fig. T29.39, ❹).

23. Repeat Steps 16-22 1496, but drag the "scatter" gate into the pink cell reserved for the double-negative population (immediately below the cell indicated in Fig T29.38), 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 700, which we will subsequently enter into the lower-right blue cell in the table reserved for "ratio +/+ : –/–".

24. Select the **View** tab →**Tokens**→**Custom Tokens** (Fig. T29.40).

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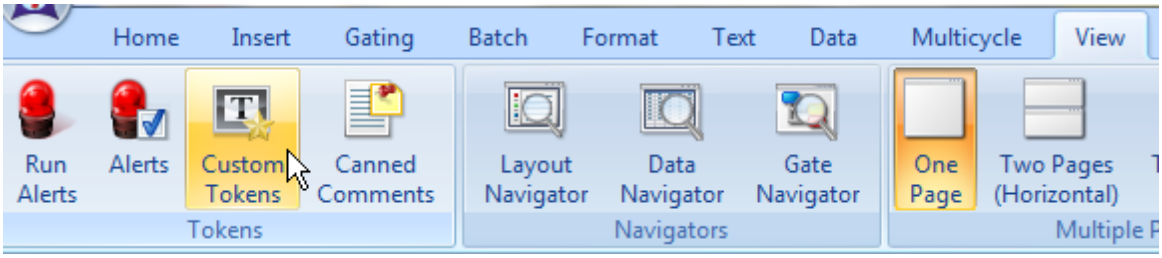

Figure T29.40 Opening the Custom Tokens window

25. Move the floating **Custom Tokens** window to the side of the page so the plots are visible.

26. Click the green Plus button (+) for (**Create a new Custom Token**) (Fig. T29.41, ❶).

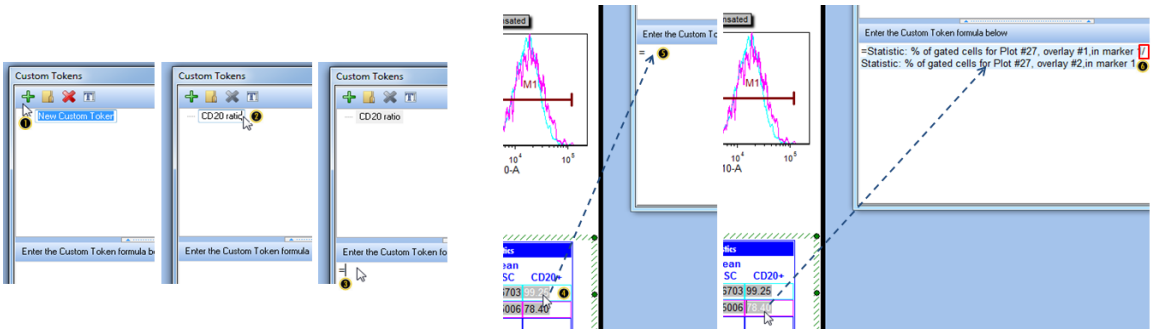

Figure T29.41 Defining a new custom token as a ratio between two regular statistical tokens

27. Type "CD20 ratio" as a descriptive name for the Custom Token (Fig. T29.41, 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[687] on the layout. Thus, as the values of the tokens[691] change among different samples in our experiment, the value of the "CD20 ratio" Custom Token[349] will update accordingly.

28. Type an equals sign ("=") in the **Enter the Custom Token formula below** pane of the **Custom Tokens** window (Fig. T29.41, ❸).

29. Highlight the Token in the upper right, turquoise cell of the Layout (Fig. T29.41, ❹).

30. Drag it toward the **Enter the Custom Token formula below** pane, while keeping the mouse button pressed.

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31. Release the mouse when the cursor appears after the equals sign ("=") (Fig. T29.41, ❺). The Token Description will appear there, highlighted in blue.

32. Type a division sign ("/") in the **Enter the Custom Token formula below** pane of the **Custom Tokens** window (Fig. T29.41, ❻).

33. 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. T29.41, *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.

34. Press and hold on the "CD20 ratio" Custom Token in the **Custom Tokens Window** (Fig. T29.42, ❶).

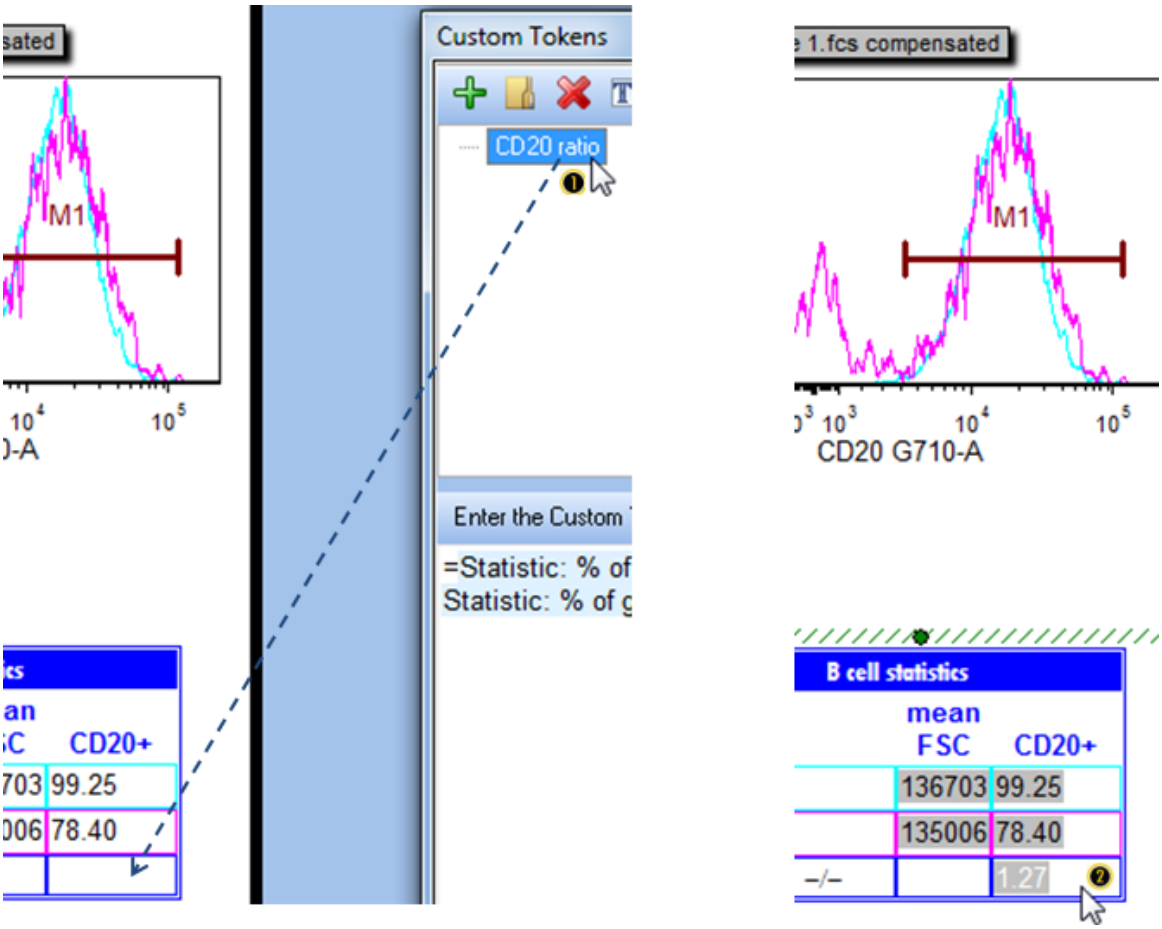

Figure T29.42 Dragging and dropping a custom token from the custom tokens window into the summary text table on the layout

35. Drag it toward the lower-right cell in the text table.

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36. 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. T29.42,). *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.*

You have now added a default statistics table to your layout, and have filled a custom text table687 with selected statistics214, including a custom ratio between two statistics700.

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 tutorial675 dedicated to this topic.

In the final section of this tutorial, we will batch process our data1501 to generate Microsoft PowerPoint™ and Excel™ reports.

34.30.3 Batch processing your analysis

In this final section of the tutorial, we will create Microsoft PowerPoint™ and Excel ™ exports using the powerful Batch Processing372 function of FCS Express.

1. Select **Application Button→Open Layout**.
2. Open the layout **Immunophenotyping Tutorial Ready for Batch Processing.fey** found in the "Immunophenotyping Tutorial" folder within the Tutorial Sample Data archive590.

   The layout has been saved linked53 to the .fcs files in the same folder, and should resemble your own layout after completing the previous section of the tutorial1490. You may use this provided layout or your own layout to proceed to Step 3.
3. Select the **Batch** tab→**Batch Processing→Batch Actions** (Fig. T29.43).

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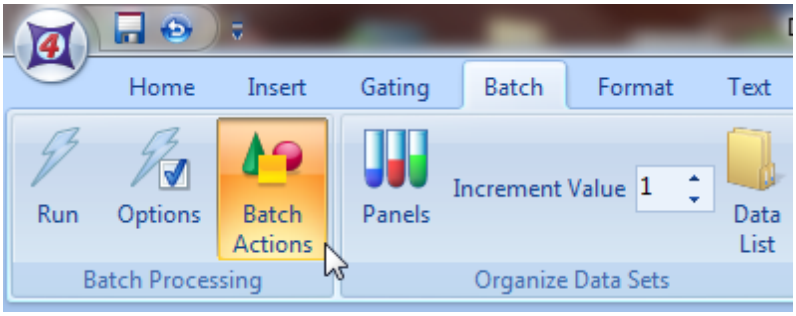

Figure T29.43 Opening the Batch Actions window

4. Move the **Batch Actions** window to the side of the page so that your plots are visible.

5. Click **Save to Powerpoint** in the **Add Action** category of the **Batch Actions** window (Fig. T29.44, ❶).

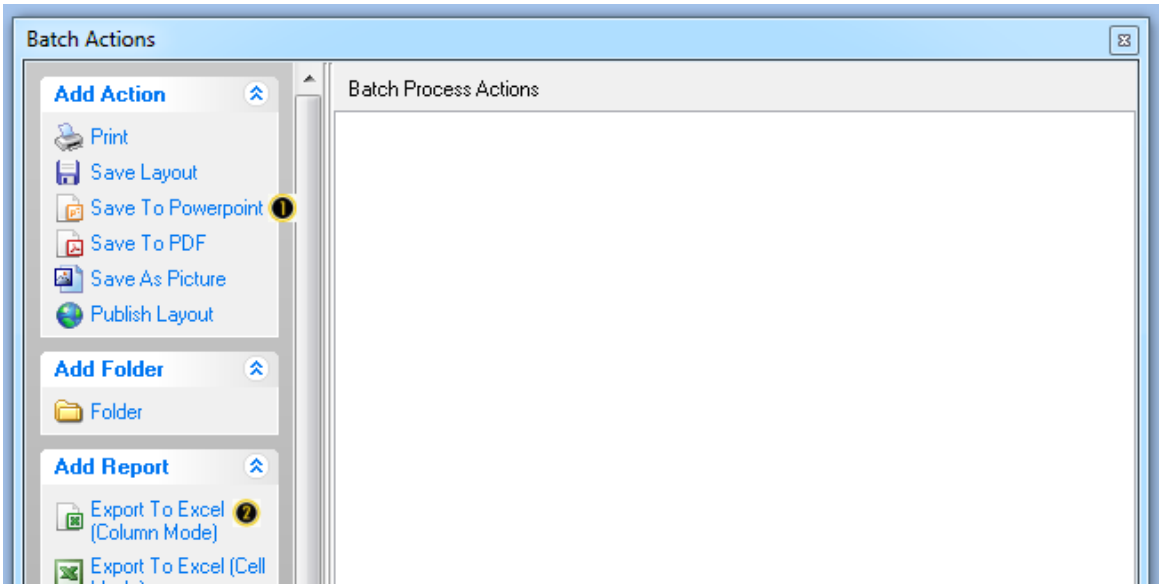

Figure T29.44 Batch actions window

6. Check the **Open presentation after saving** box in the **Create Export to Powerpoint Action** dialog box which opens automatically (Fig. T29.45, ❶).

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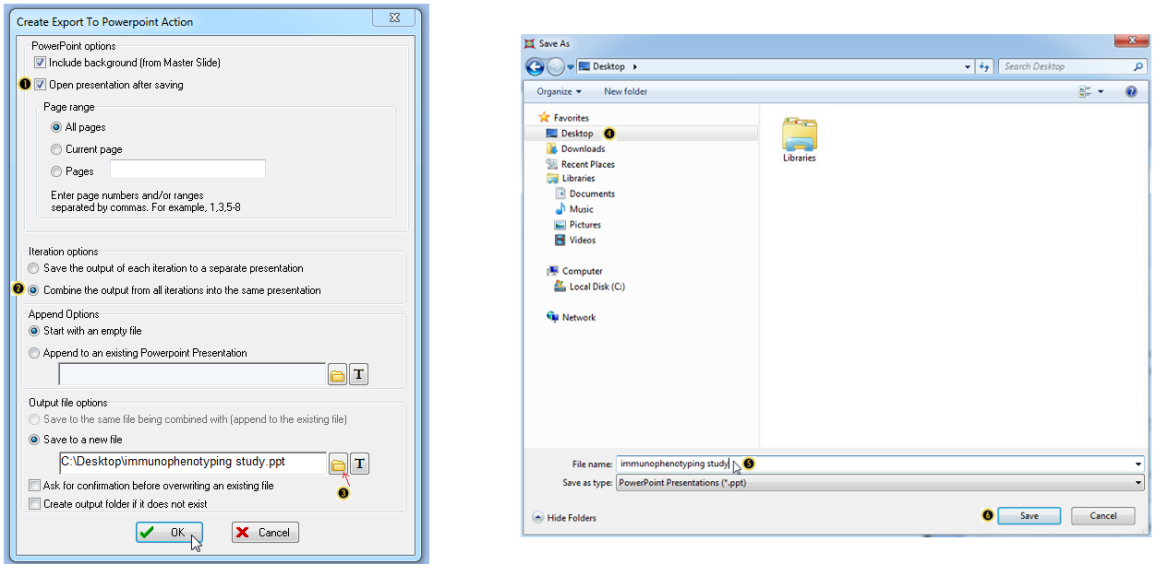

Figure T29.45 Adding a PowerPoint action to Batch Process Actions

7. Click the **Combine the output from all iterations into the same presentation** radio button in the **Iteration options** section (Fig. T29.45, 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. T29.45, 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. T29.45, ❹).

10. Type "immunophenotyping study" in the **File name** field (Fig. T29.45, ❺).

11. Click **Save** (Fig. T29.45, ❻).

12. Click **OK** in the **Create Export to Powerpoint Action** dialog box (Fig T29.45, mouse pointer).

13. Click on **Export to Excel (Column Mode)** in the **Add Report** category of the **Batch Actions** window (Fig T29.44, ❼).

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. T29.46, ❶).

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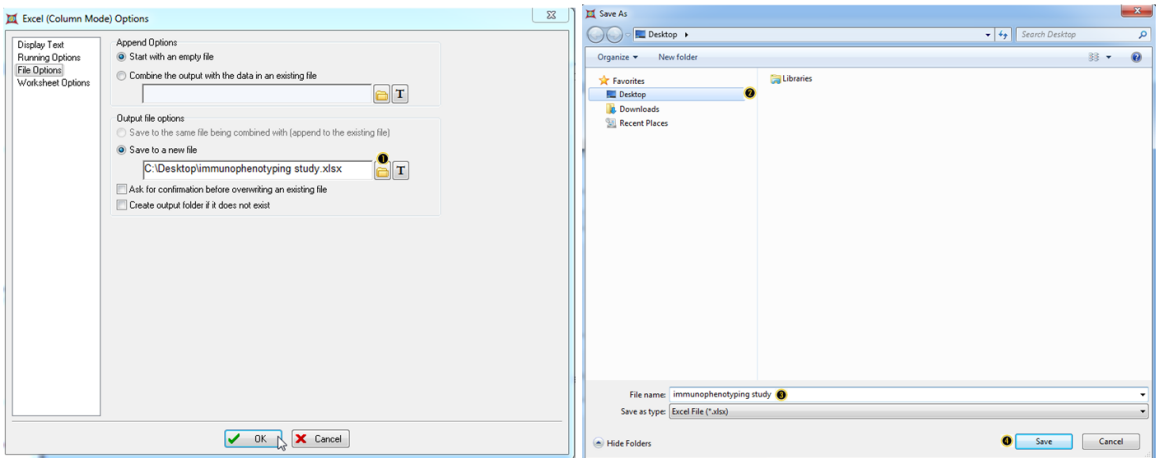

Figure T29.46 Adding an Excel (Column Mode) report to Batch Process Actions

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. T29.46, ❷).

16. Type "immunophenotyping study" in the **File name** field (Fig. T29.46, ❸).

17. Click **Save** (Fig. T29.46, ❹).

18. Click **OK** in the **Excel (Column Mode) Options** dialog box (Fig T29.46, mouse pointer).

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. T29.47, ❶).

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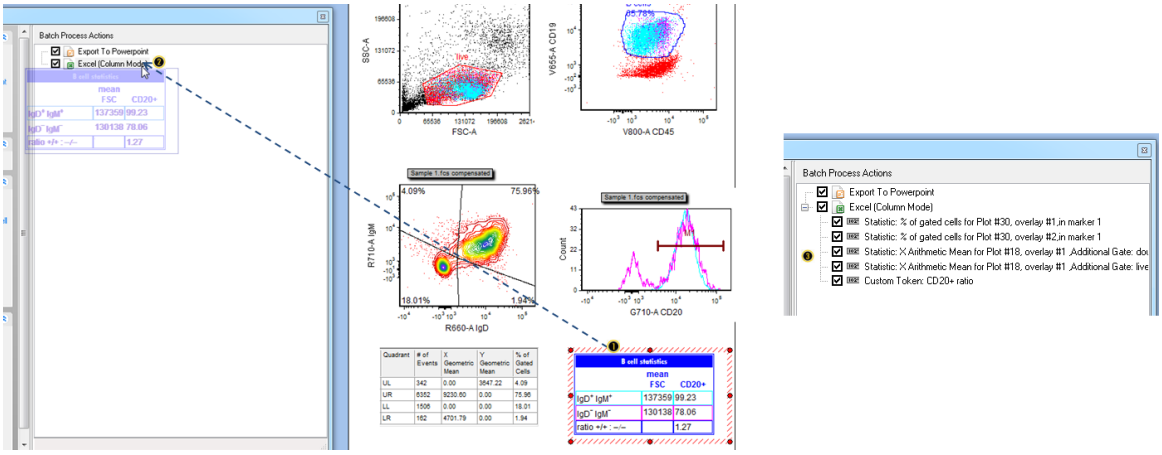

Figure T29.47 Dragging and dropping the tokens within a text table into the Excel export in the Batch Process Actions window.
(Note: the layout pictured has had the optional steps completed for adding red outlier dots to the contour plot, and for normalizing the histogram. If you are using your own layout 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. T29.47, 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. T29.47, ❸).

Note: You may also add tokens to your Excel exports[778] by dragging and dropping plot elements, including gates, markers, 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. T29.48, ❶).

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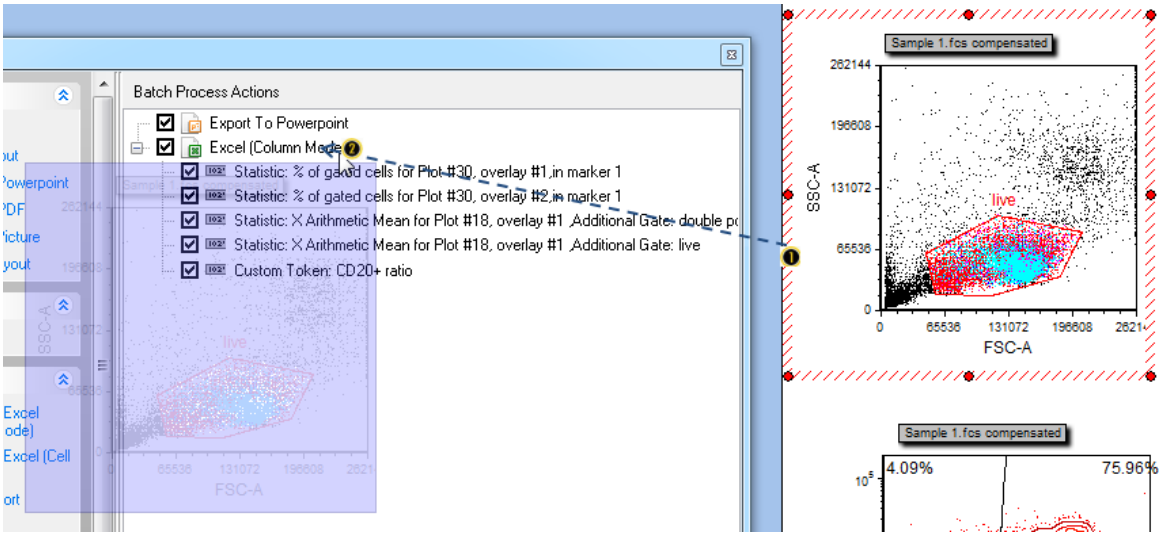

Figure T29.48 Dragging and dropping a plot-derived element into the Excel export of the Batch Process Actions window

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. T29.48, 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. T29.49, ❶).

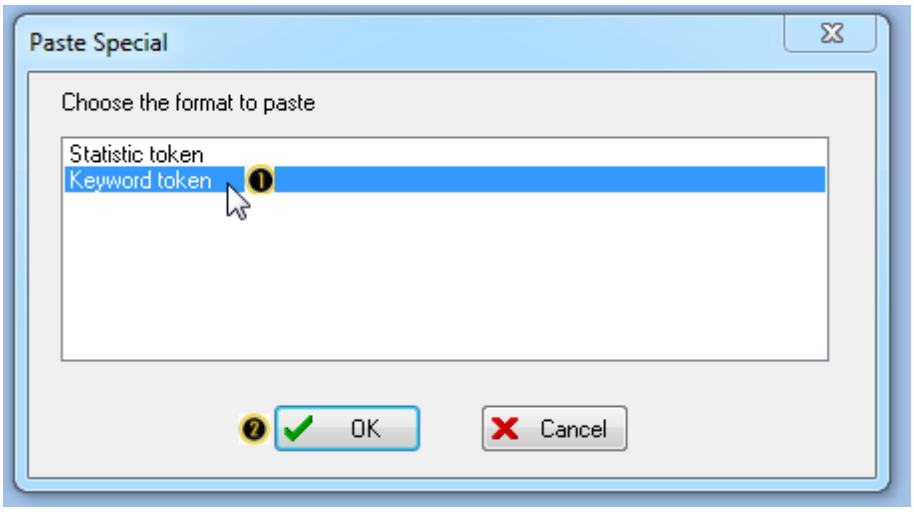

Figure T29.49 Selecting a keyword token

26. Click **OK** (Fig. T29.49, ❷).

27. Click on the ellipsis in the **Select a keyword** dialog box (Fig. T29.50, ❶).

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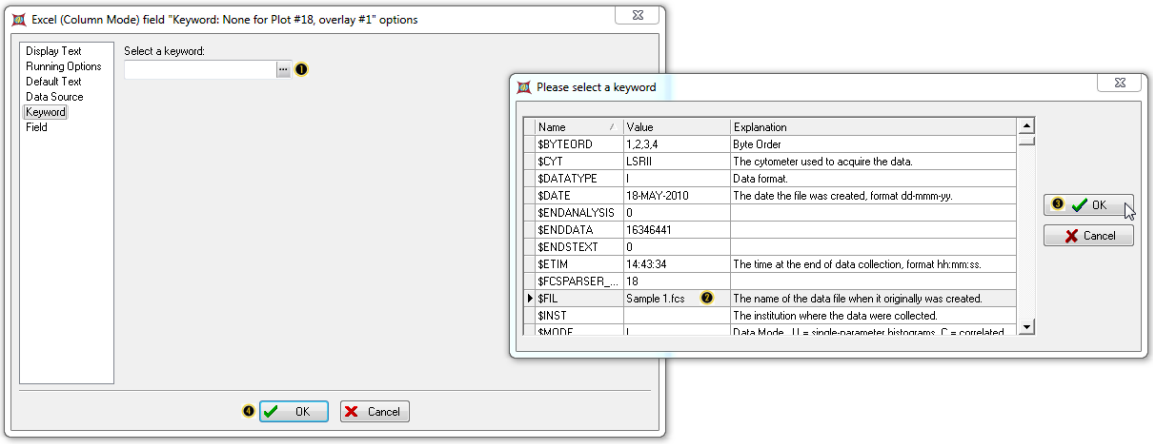

Figure T29.50 Selecting a specific keyword token

28. Scroll to and select **$FIL** in the **Please select a keyword** dialog box (Fig. T29.50, ❷).

29. Click **OK** in the **Please select a keyword** dialog box (Fig. T29.50, ❸).

30. Click **OK** in the **Excel (Column Mode) field "Keyword..."** dialog box (Fig. T29.50, ❹).

Several statistical and keyword tokens 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. T29.51, ❶).

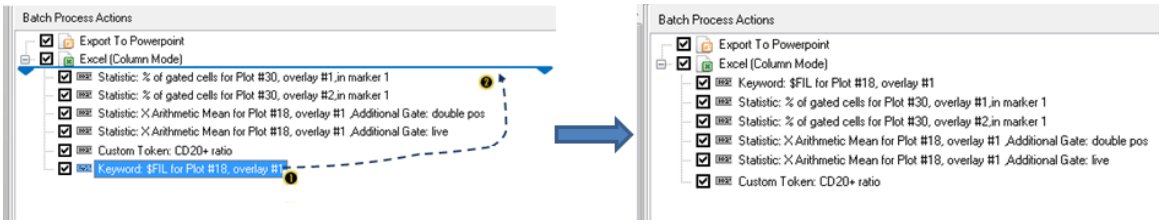

Figure T29.51 Reordering the tokens in the list to be exported to Excel

32. Drag this token to be directly below the **Excel (Column Mode)** icon (Fig. T29.51, ❷).

33. Release the mouse when the blue bar spanning the window width is at the position shown in Fig. T29.51, ❷. *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) and try again.*

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We will now assign a column name to one of the tokens so that an appropriate column title is exported to our Excel worksheet.

34. Double-click on the "**Custom Token: CD20+ ratio**" Custom Token in the **Batch Process Actions** window (Fig. T29.51, *right*, last item in list).

35. Click on **Field** in the category at left (Fig. T29.52, ❶).

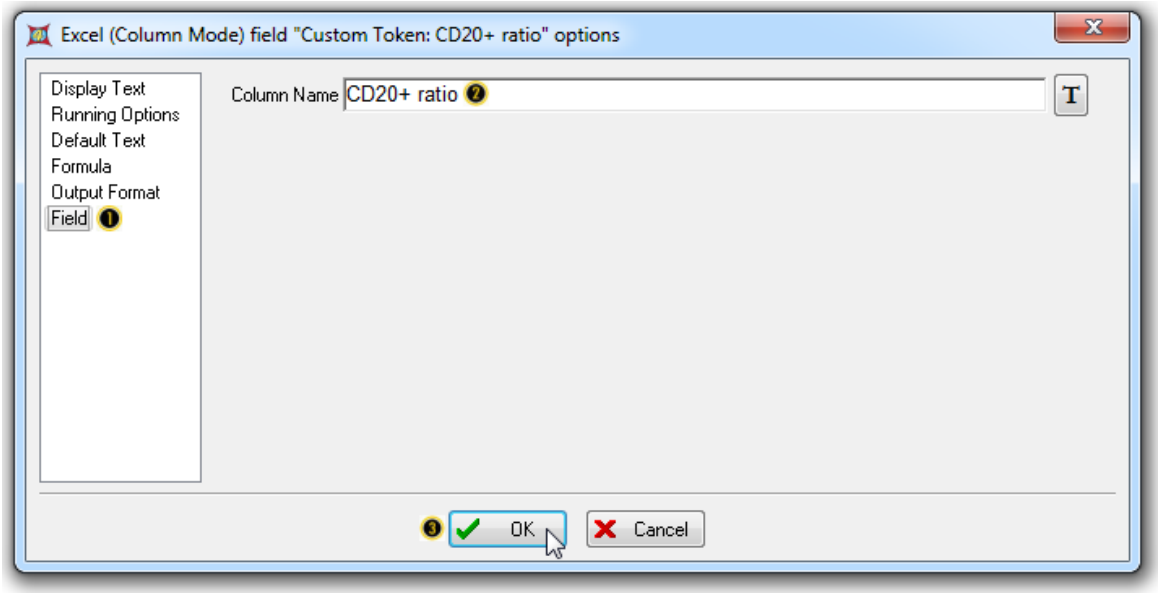

Figure T29.52 Assigning a column title to a token to be exported to Excel

36. Type "CD20+ ratio" in the **Column Name** (Fig. T29.52, ❷).

37. Click **OK** (Fig. T29.52, ❸).

Optional: You may assign appropriate column names to the other tokens in the Excel - Column Mode export list (Fig. T29.51,* right *) as well, using the same procedure outlined in Steps 34-37.

Although we are now ready to Batch Process 372 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 61 now or add a Print action 386 to the list of Batch Process Actions, and the printout would appear exactly as the layout does on the screen.

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38. Select the **Data** tab→**Organize Data Sets**→**Data List** (Fig T29.53, *left*, outlined in red). The Data List[373] appears (Fig. T29.53, *right*), which contains the four .fcs 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[374] 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.*

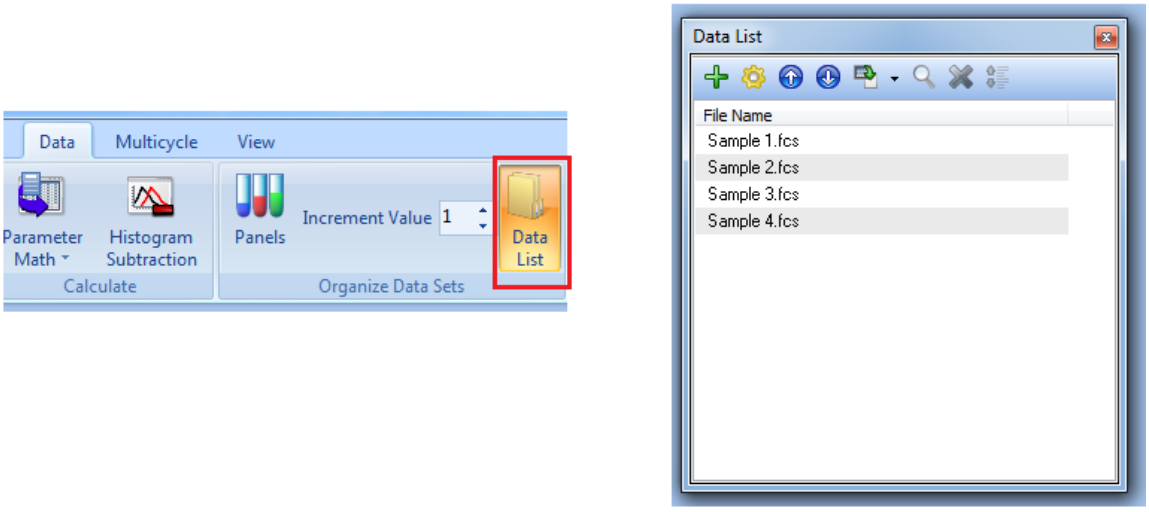

Figure T29.53 Opening the data list

39. Select the **Data** tab→ **Change Data on All Objects**→ **Next** (Fig. T29.54, outlined in red). 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.

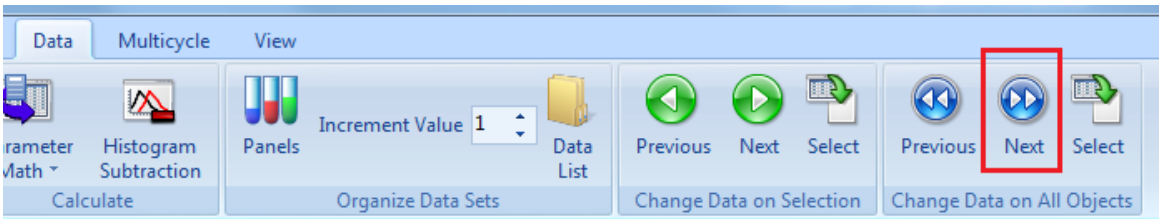

Figure T29.54 Changing data on all objects to the next iteration in the data list

40. Click on the **Data** tab→**Change Data on All Objects**→**Previous** (Fig. T29.54, to the left of **Next**) until "Sample 1.fcs" is displaying on the layout again.

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As you click **Next** to advance through your Data List, 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 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. *Note: you need not access Batch Options (as described in Steps 41-44 and 46-48) for batch processing FCS Express layouts in which no adjustment between iterations is required.*

41. Select the **Batch** tab→**Batch Processing**→**Options** (Fig. T29.55).

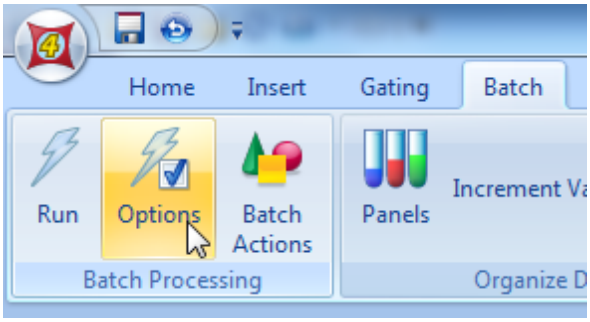

Figure T29.55 Accessing batch options

42. Check the **Pause between iterations** box in the **Batch Processing Options** dialog (Fig. T29.56, ❶).

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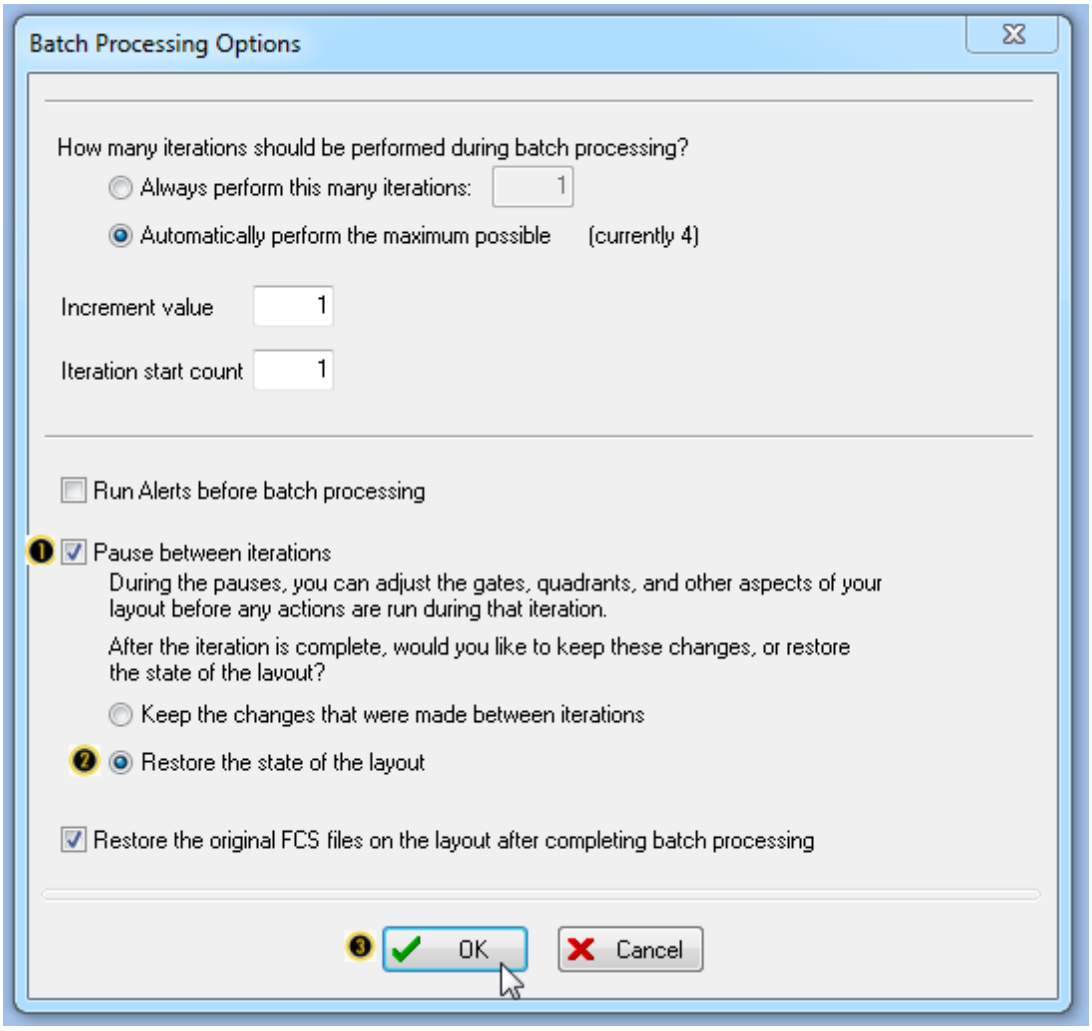

Figure T29.56 Adding a pause between iterations to our batch process

43. Click the **Restore the state of the layout** radio button (Fig. T29.56, ❷).

44. Click **OK** (Fig. T29.56, ❸).

45. Select the **Batch** tab→**Batch Processing**→**Run** (Fig. T29.57).

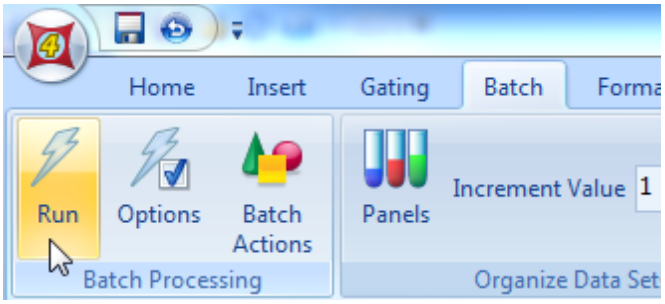

Figure T29.57 Running the batch process

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The **Batch Processing** status window appears (Fig. T29.58), indicating that 2 of 2 actions (i.e., one PowerPoint slide + one Excel row of statistics) have been completed for 1 of 4 iterations in the Data List.

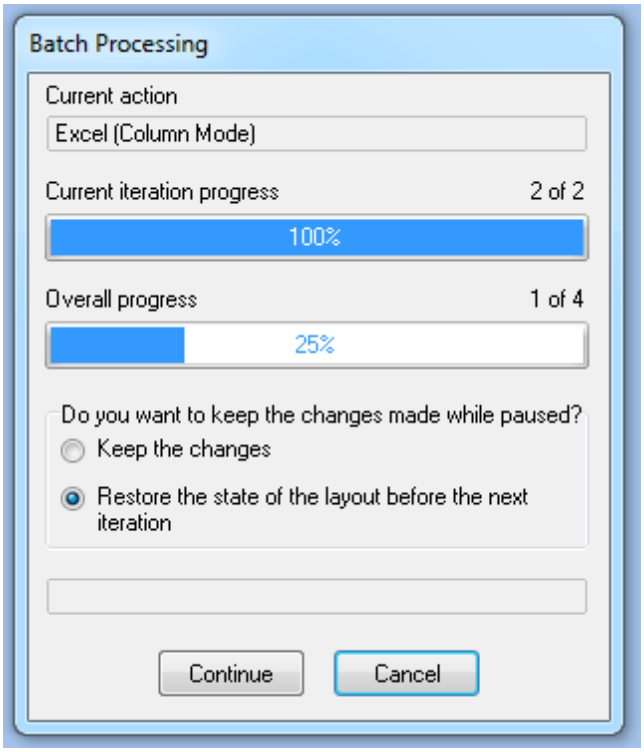

Figure T29.58 Status of the batch process (currently in progress)

46. 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.

47. Click **Continue** (Fig. T29.59).

48. Repeat Steps 46-47 until the end of the Batch Process.

49. 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: an example Powerpoint export of this layout is saved in the "Immunophenotyping Tutorial" folder within the Tutorial*

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Sample Data archive.

50. 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.

34.31 Changing Common Preferences

FCS Express provides many options that allow you to completely customize your working environment. The settings for these options are called Preferences 117. You can save time by creating a new user name 28 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 1513
- Changing axis labels to stain and name 1516
- Creating gates hierarchically and adding a %-gated statistic to gate text boxes 1517

34.31.1 Displaying Filenames Without Path in Data List

Here we will change our Preferences 117 so that only the .fcs filenames will appear by default in our Data List 373.

1. Select **Application Button→Edit Preferences** (Fig. T30.1, outlined in red).

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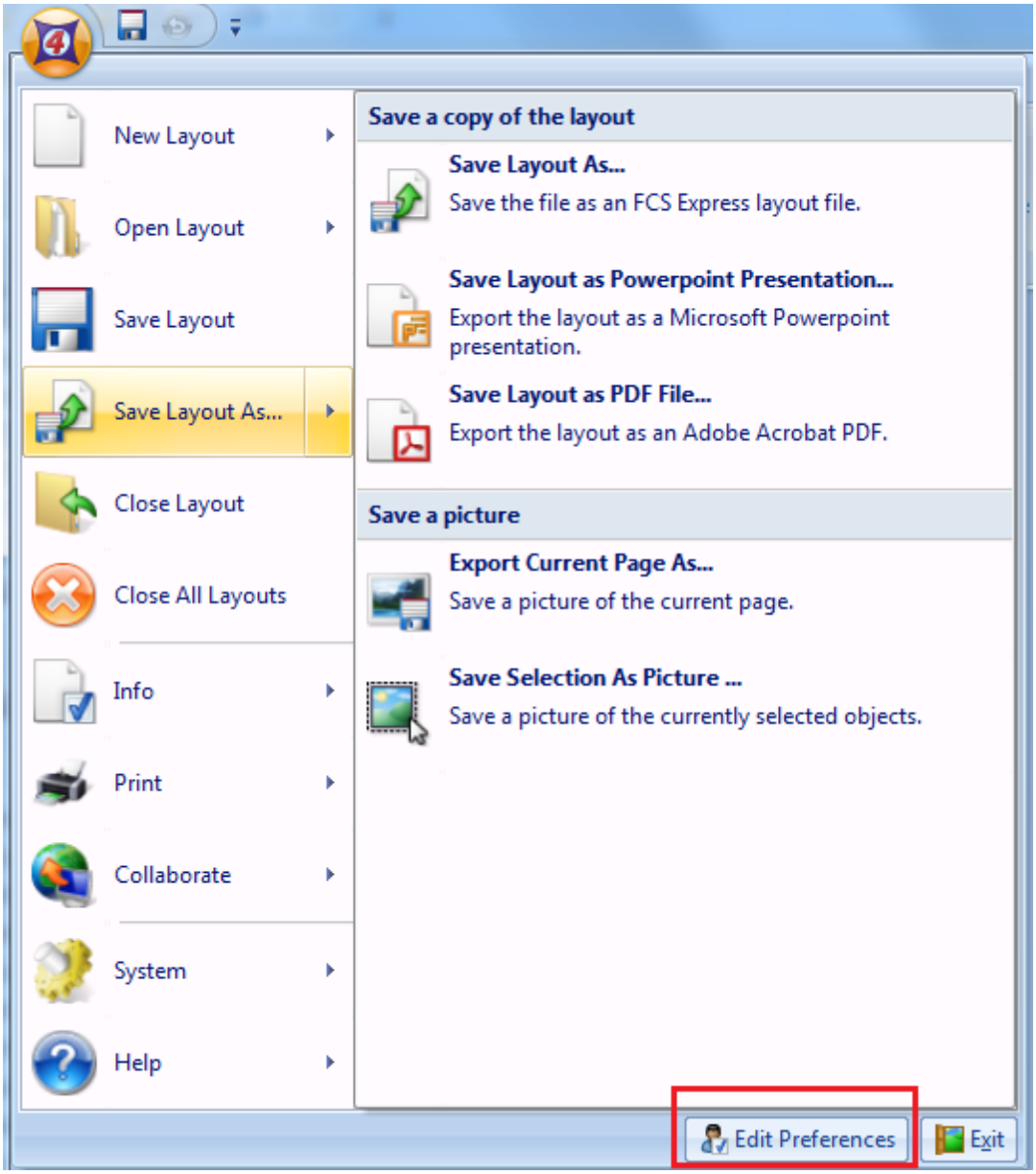

Figure T30.1 Accessing Preferences from the Application Button

2. Click on **Data List** in the category at left (Fig. T30.2, ❶).

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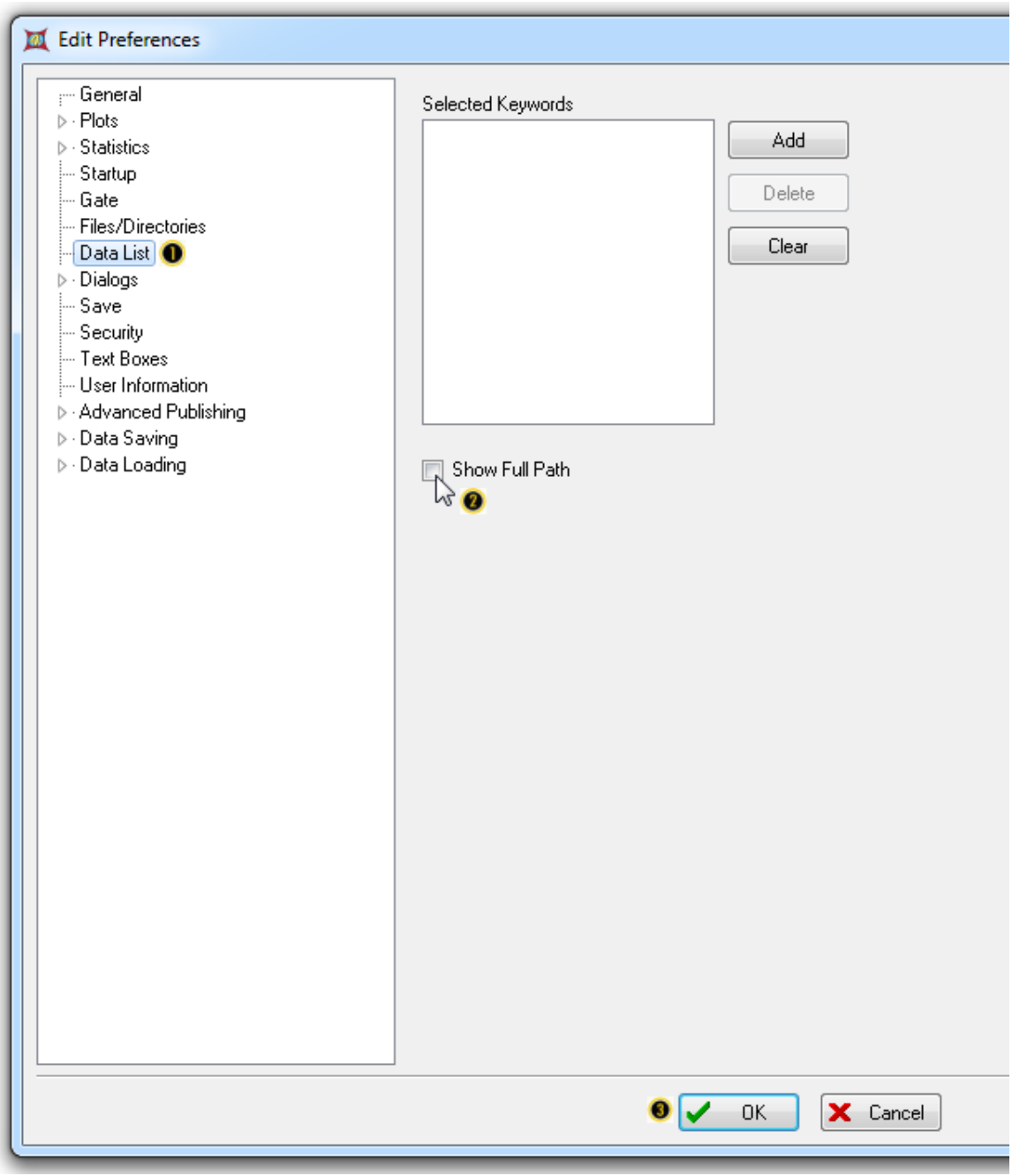

Figure T30.2 Data List preferences

3. Uncheck **Show Full Path** (Fig. T30.2, ❷).

4. Click **OK** (Fig. T30.2, ❸).

*Note: the current Preference for showing or hiding filepaths 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*377*.*

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34.31.2 Changing Axis Labels

Here we will change our Preferences[117] so that .fcs file-derived Stain and Name keywords appear on our plot axes[637].

1. Select **Application Button→Edit Preferences** (Fig. T30.3, outlined in red).

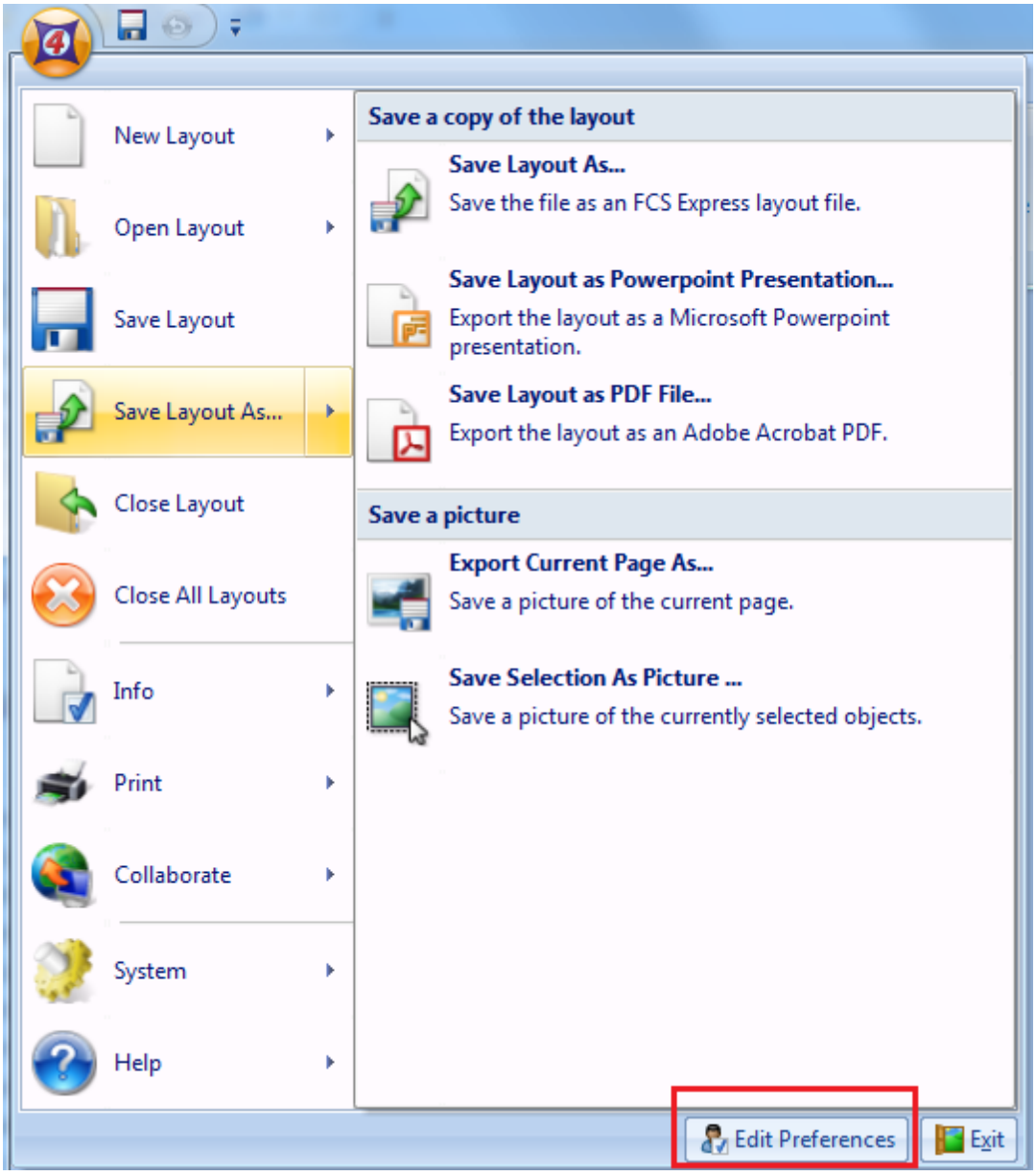

Figure T30.3 Accessing Preferences from the Application Button

2. Click on the **Data Loading** category → **FCS File Options** at the left (Fig.

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T30.4, ❶).

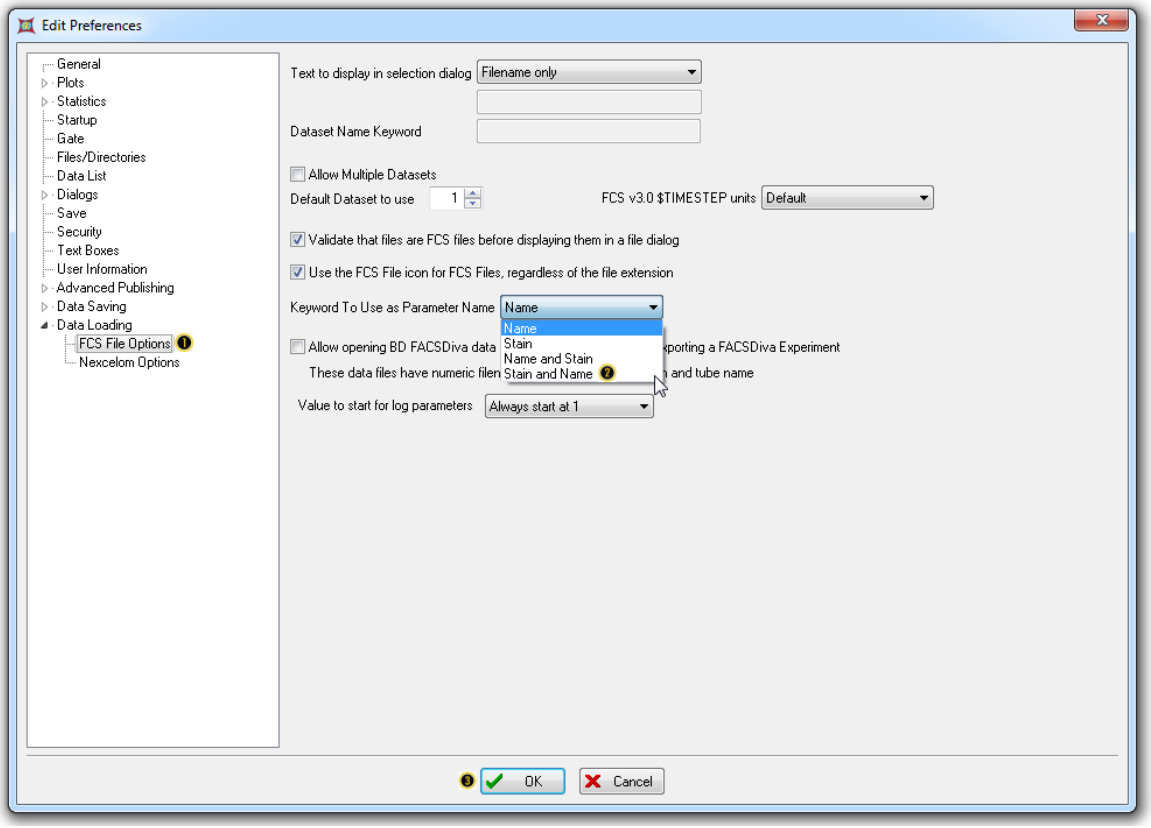

Figure T30.4 Selecting Stain and Name as the Parameter Name for plot axes

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, your plot axes[158] for .fcs files subsequently loaded in FCS Express will be labeled with these descriptive keywords that were saved with your .fcs files at the time of acquisition.

Go back to Immunophenotyping Tutorial.[1466]

34.31.3 Creating Gates Hierarchically and Adding Statistics to Gate Text Boxes

Here we will change our Preferences[117] so that gates[193] are created hierarchically by default, and a "% gated" statistic[326] is automatically added to gate text boxes[118].

1. Select **Application Button→Edit Preferences** (Fig. T30.5, outlined in red

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).

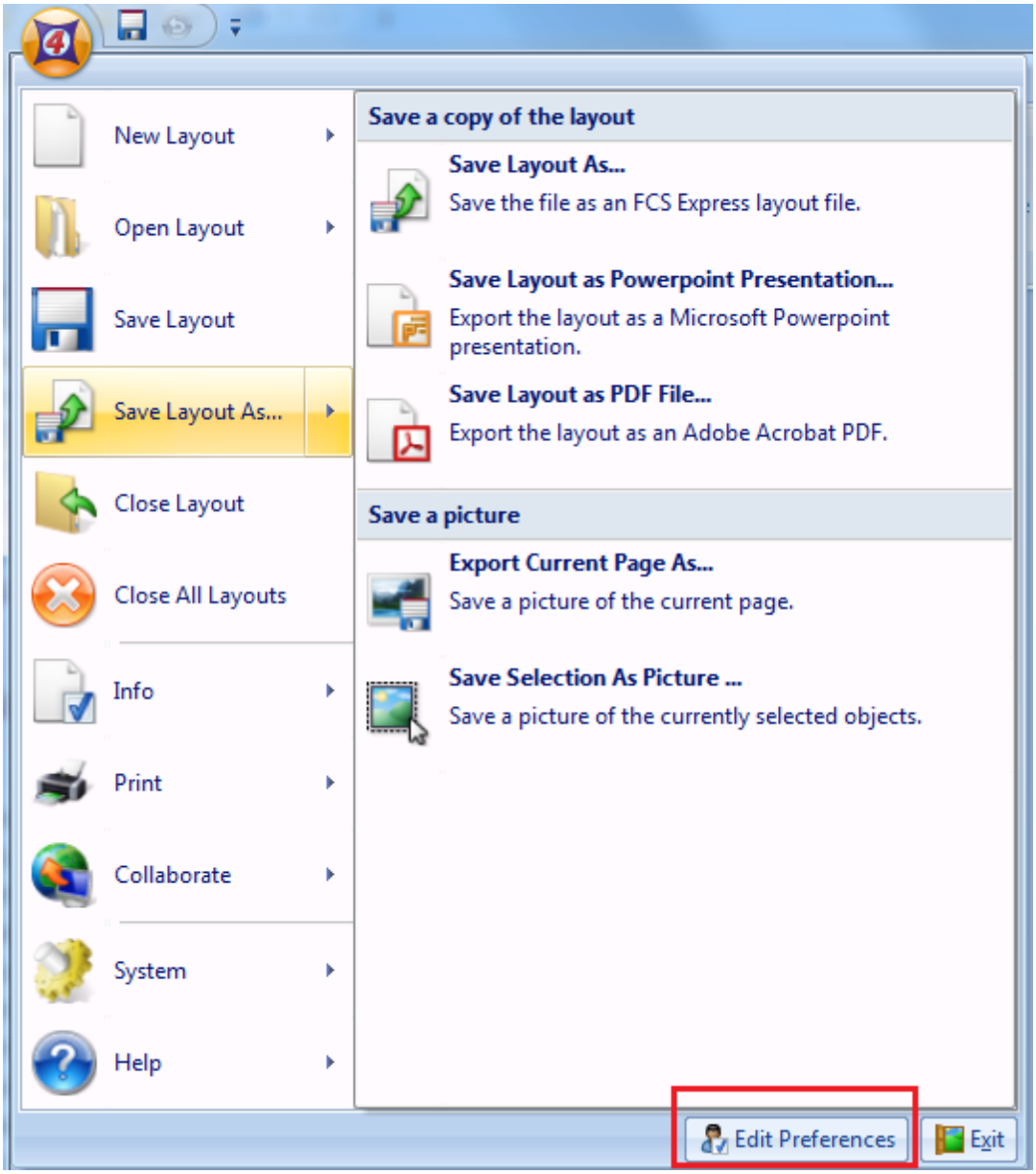

Figure T30.5 Accessing Preferences from the Application Button

2. Click on the **Gate** category at the left (Fig. T30.6, ❶).

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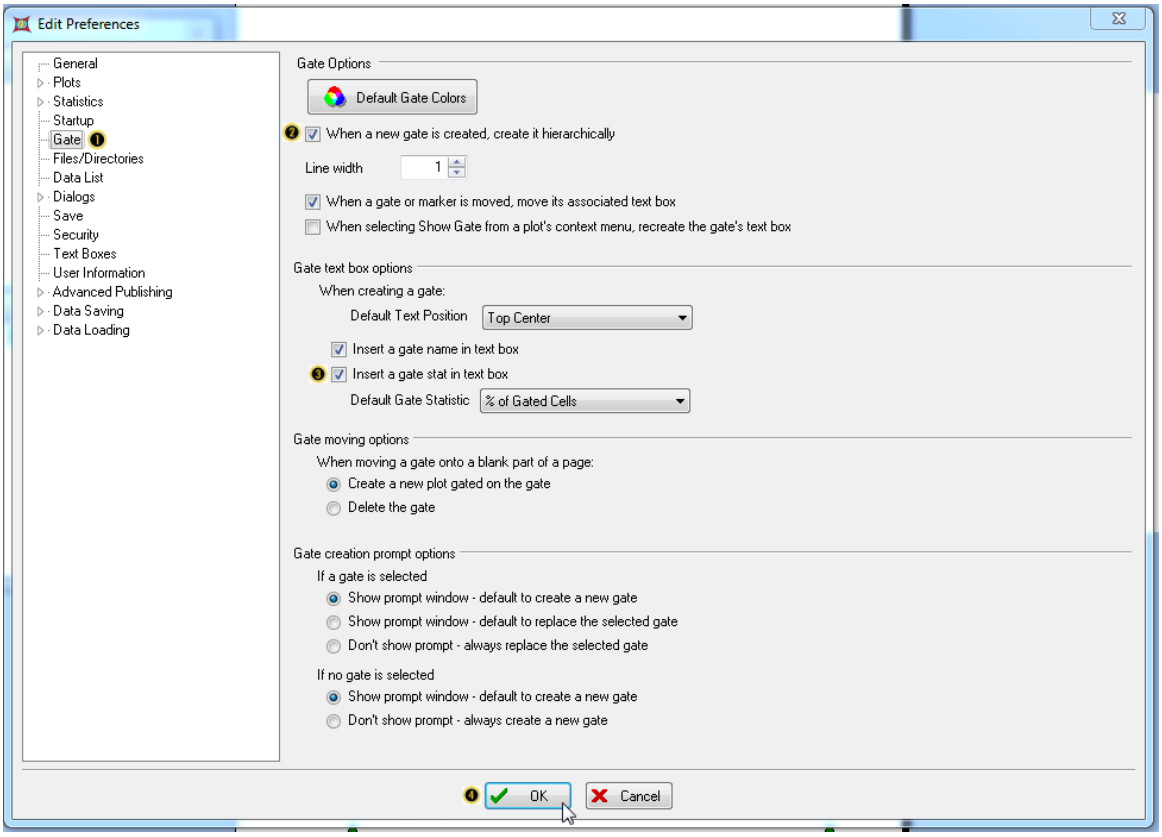

Figure T30.6 Adjusting gate preferences

3. Check 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 preferences for more details.*

5. Click **OK** (Fig. T30.6, ❹).

Go back to Immunophenotyping Tutorial.

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