FLUIDIGM

FCS Express Basics Demonstration

Length: Approximately 60 minutes

Goal:	To show basics of data analysis in FCS Express
Objectives:	By the end of this module, you will be able to: • Understand how to perform basic analysis of data acquired on a Helios mass cytomer using FCS Express
Materials:	• Computer • Data files acquired on day 3 of training or any other sample data files. Preferably same cells run at different cell concentrations
Prior to the Lesson:	If you are not using the data from day 3 of training, identify a data set to use for the demo.

Introduction/Hook

There are many different platforms that one can use to analyze mass cytometry data. We support FCS Express which is a fully featured flow cytometry data analysis package and has been designed to work seamlessly with mass cytometry data. New Helios instruments and Hyperion Imaging Systems (as of August 15, 2020) will include a one-year license of FCS Express Flow software.

Demonstration

NOTE A a copy of FCS Express Flow is distributed with new Helios and Hyperion instruments. You can claim your free copy of FCS Express via the license packet that was provided with the instrument. Additional licenses, support, and free training can be requested through De Novo Software any time at support@denovosoftware.com. Alternatively, you can download a one-month free trial from https://denovosoftware.com/demo-overview/.

NOTE While this is a brief overview, De Novo Software has a series of online tutorials, short videos and other resources at https://denovosoftware.com/full-access/.

NOTE To save time, access the 7C human kit analysis_SOP-template.fey, which is an SOP-based FCS Express layout available on the De Novo Software website (data is provided as a zip file and contains the layout and sample data). The layout includes most of the comprehensive, pre-gated cleanup strategy and in-layout step-by-step instructions for phenotypic marker subset gating, statistics, and batch processing to MS Office applications. The rest of this document will show how to create the template from scratch in a New Layout, which is a good exercise for learning how to work with your data in FCS Express.
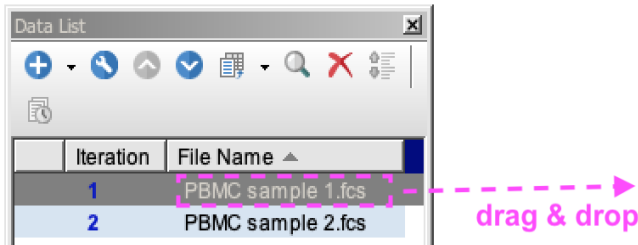
Create plots and gates

1. Launch FCS Express and click on **New Layout** in the Startup screen to start your analysis.
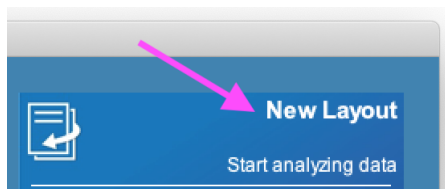
2. Click **Data** tab→**Data List,** and add the .fcs files by dragging and dropping them into the Data List window or clicking the blue plus button ⊕ .
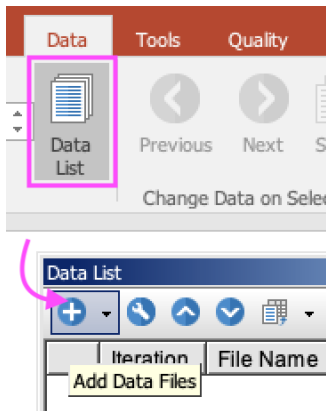

3. Drag & drop the filename from the **Data List** window to an empty spot on the layout page.

4. Choose a plot type (**Density** is recommended), and click **OK.**
5. Change the X-axis to **Time** and the Y-axis to **193Ir_DNA2**. Alternatively, follow the-full Cleanup strategy in Table 1 below, which culminates in this same *193Ir_DNA2* vs *Time* plot. (*see also original publication for clean-up strategy* Bagwell, C.B. et al. "Automated data cleanup for mass cytometry." Cytometry Part A 97 (2020): 184–198"). In either case, follow Steps 7-8 below to draw gates and create new plots gated on those populations.
    Hint: Click on an axis title and start typing the name of a parameter to narrow down the list.

6. To add the current gate to the plot title, double-click on the plot to open the plot's Formatting window. Choose **Title** from the top dropdown menu (a), click to place your mouse cursor in the next available line of the Title Options text field (b), and choose "**Current Gate**" from the **Insert Token** dropdown menu (c).
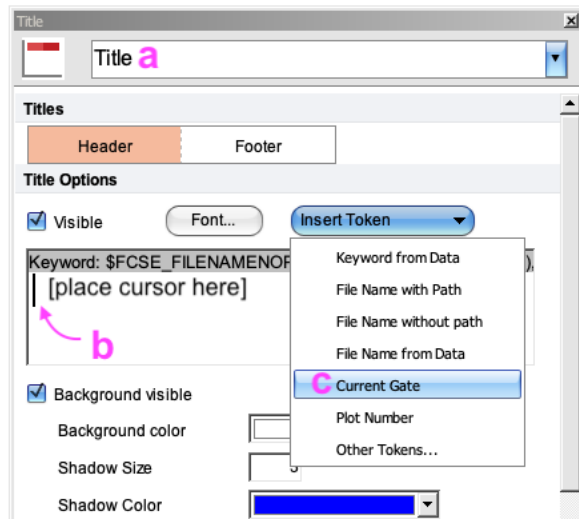
7. Click on the **Gating** tab→**Rectangle**, and draw a new gate on the intended population. Name the gate as suggested in Table 1 below (according to where you start in the Cleanup strategy), choose a gate color, and click **OK**.

8. Drag and drop the gate out to a new spot on the layout, by placing your mouse cursor in the middle of the gate, while keeping the mouse button pressed and releasing it when your cursor is outside of the original plot. A new plot gated on that gate will be inserted, and can be moved anywhere on the layout.
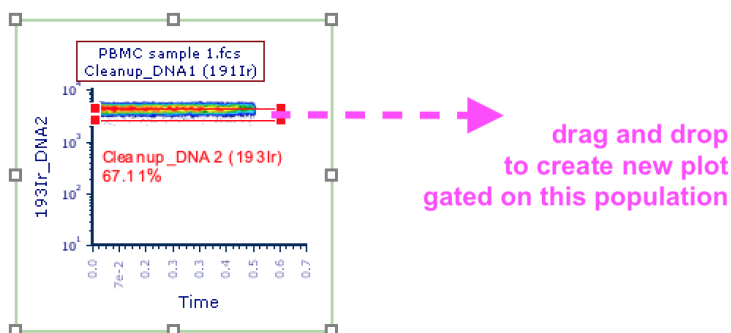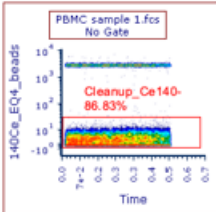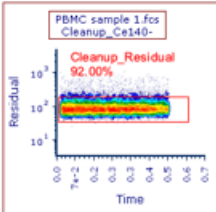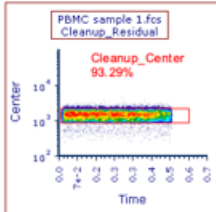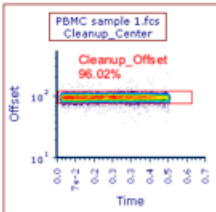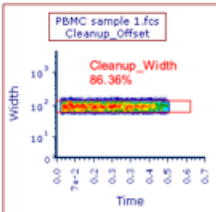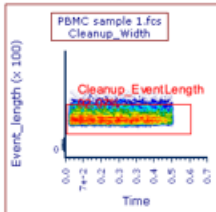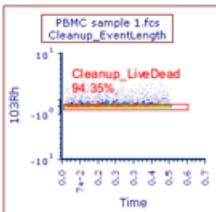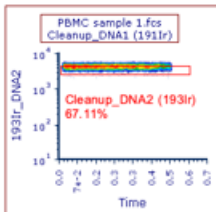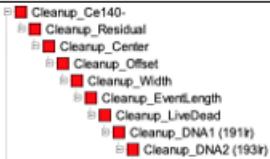
Table 1 complete Cleanup gating strategy

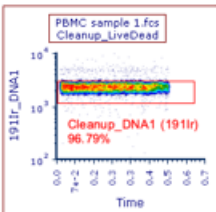| 1. Bead vs. Time	2. Residual vs. Time	3. Center vs. Time
Beads removal Adjust the Cleanup_Ce140- gate to select the low intensity events.	**Gaussian discrimination, Residual** Adjust the Cleanup_Residual gate to select the largest band of events (mid-range intensity).	**Gaussian discrimination, Center** Adjust the Cleanup_Center gate to select the largest band of events (mid-to-high range intensity).
4. Offset vs. Time	5. Width vs. Time	6. Event Length vs. Time
Gaussian discrimination, Offset Adjust the Cleanup_Offset gate to select the largest band of events (mid-range intensity).	**Gaussian discrimination, Width** Adjust the Cleanup_Width gate to select the largest band of events (mid-range intensity).	**Event Length** Adjust the Cleanup_EventLength gate to select the largest band of events (low range intensity).
7. Live Dead vs. Time	8. DNA1 vs. Time	9. DNA2 vs. Time
Cell viability Adjust the Cleanup_LiveDead gate to select the largest band of events (low range intensity). Note: 103Rh or 195Pt, depending on whether 103Rh intercalator or cisplatin is used in the experiment.	**Nucleated (DNA-containing) cells, 191Ir** Adjust the Cleanup_DNA1 (191Ir) gate to select the largest band of events (mid-to-high range intensity).	**Nucleated (DNA-containing) cells, 193Ir** Adjust the Cleanup_DNA2 (193Ir) gate to select the largest band of events (mid-to-high range intensity).

Full Gating Hierarchy Diagram (inserted in Step 12 below)

9. Insert a new layout page using the new page tab in the bottom-left corner of the layout.

10. Go back to **Page 1** in the layout. Copy and paste the *193Ir_DNA2* vs *Time* plot to the new page by clicking on the plot border (it will be highlighted in red when selected) and using Ctrl [PC] /Cmd [Mac]**-C** and Ctrl/Cmd**-V** keyboard shortcuts, respectively.

11. Repeat Steps 7-8 for each additional gate shown in the 7-color immunophenotypic subset gating (dragging and dropping gates out to create new plots as indicated by the dashed arrows in the figure below), changing axis parameters as needed on each new plot and naming each gate appropriately.
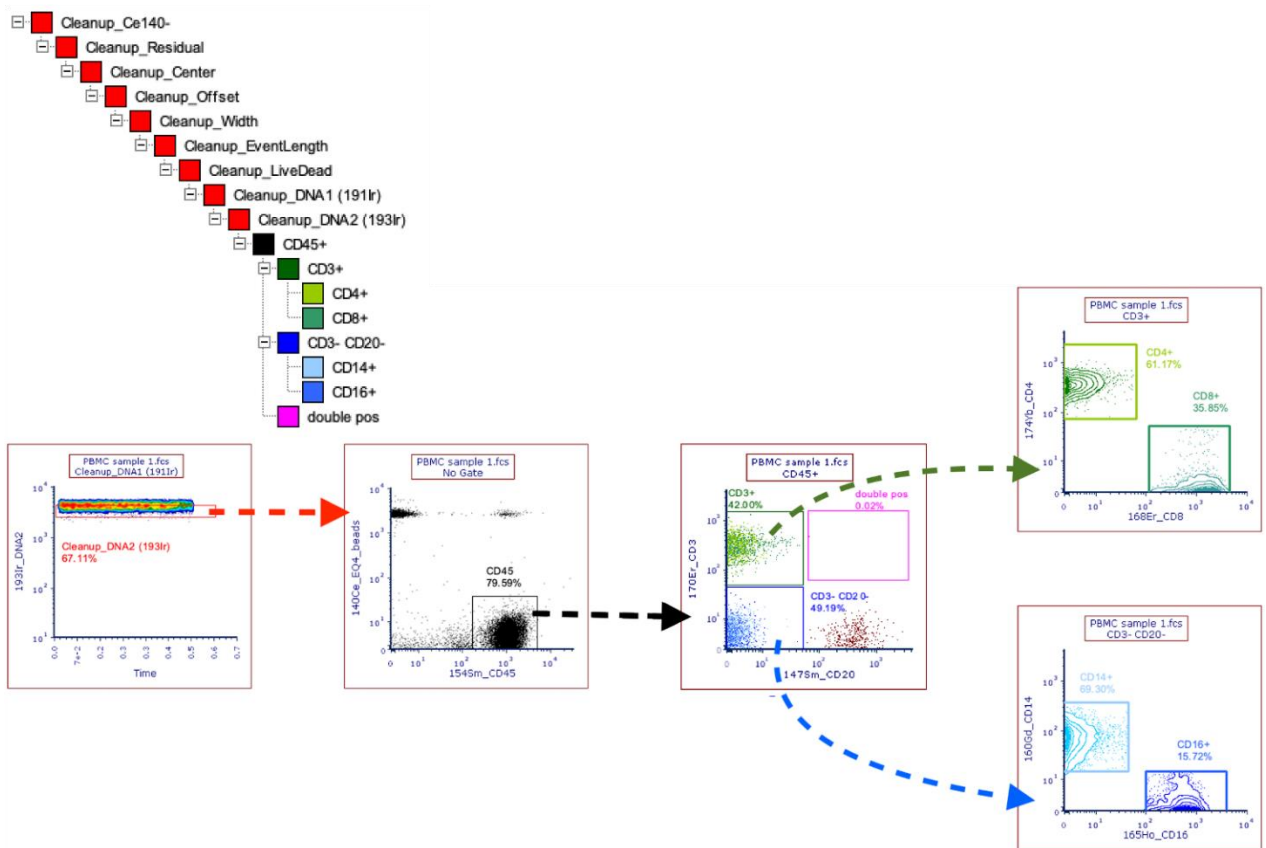

NOTE This is the best time to consider optimal cell concentrations. If you have collected data files from samples at multiple cell concentrations, you can look through different files to see that the 3+/19+ cell doublet population increases with higher than optimal cell concentration even though the plot is gated on singlets.

NOTE The "double pos" gate is there to assess doublets. In this example, the data cleanup strategy was very effective and virtually no events fall into the gate. If a significant number of events fall in the gate (>~2%), the position of the Cleanup gates should be checked again.

NOTE If desired, adjust axis range (e.g., to zoom in the plot's range to populations of interest) via right-clicking on plot→**Magnify Resolution**. Changing the scaling (e.g., to Linear or Arcsinh) is usually not necessary as FCS Express automatically defaults to the appropriate scaling, but these settings can be accessed simultaneously for multiple plots via **Format** tab→**Axis.**

12. View the gating hierarchy, which may be changed at any time, via the **Insert** tab→**Gate View** object (click once on the layout to insert the object).
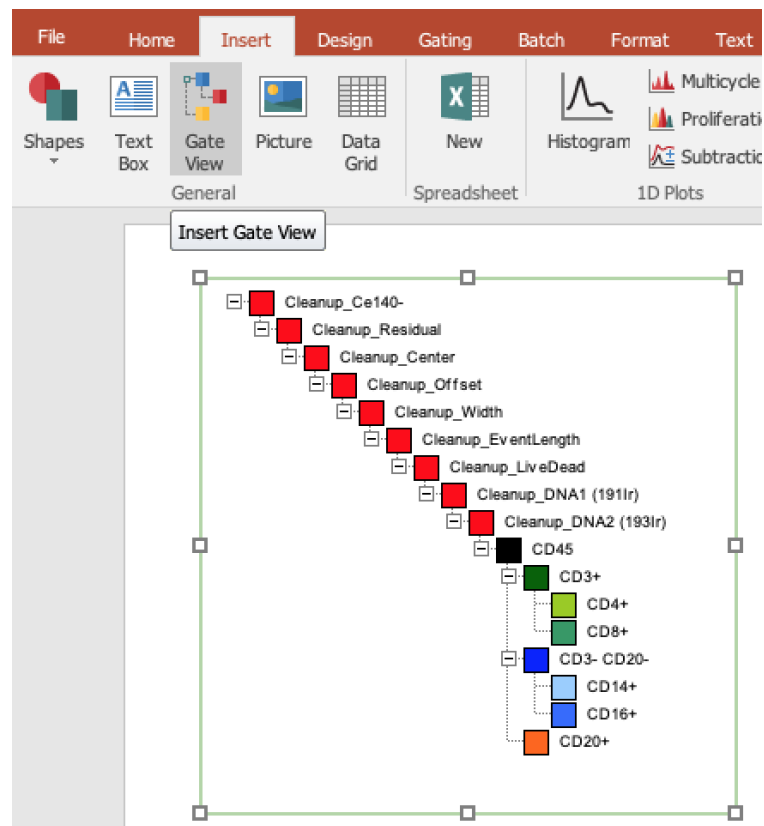

♦ **Add summary statistics**

13. Right-click on the plot gated on CD45.
14. Select **Statistics**→**Gate Statistics** from the pop-up menu.
15. Move the statistics window to a convenient spot on the layout where it is not overlapping any plots.
16. Right-click on the statistics window to toggle on or off desired statistics.

♦ *(Optional, for layouts with multiple datafiles)* **Summarize result of all datafiles via a Batch Process to PowerPoint**

17. Preview the results of your datafiles using the **Data** tab→**Change Data on All Objects**→**Next** command. Click **Previous** as many times as necessary to return to the first datafile (or use the **Iteration** dropdown menu at right).
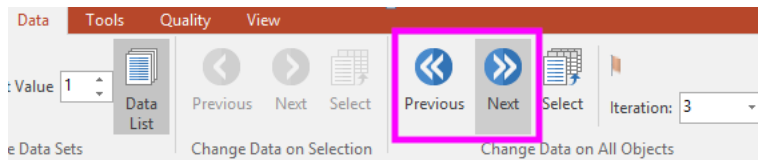
18. Select the **Batch** tab→**Batch Processing**→**Batch Actions** command.

19. Move the **Batch Actions** window to the side of the page so that your plots are visible.

20. Click **Save to PowerPoint** in the **Add Action** category of the **Batch Actions** window.
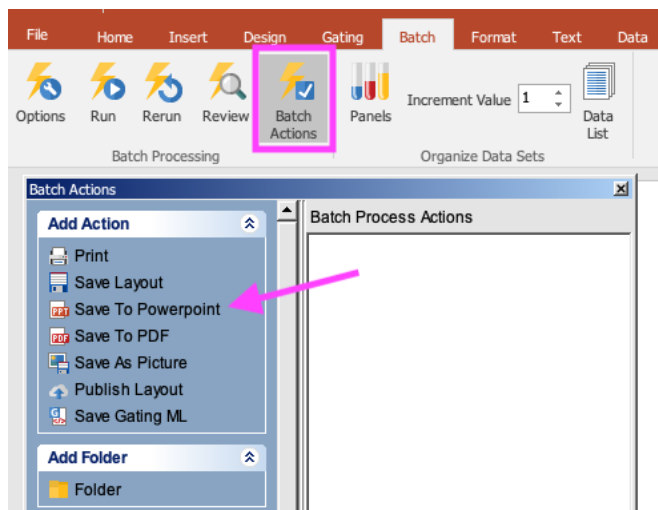
21. Check the **Open presentation after saving** box (a) and define the filename and filepath in the "Save to a new file" field (b) within **Output file options** section of the Create Export to Powerpoint Action pop-up dialog window.
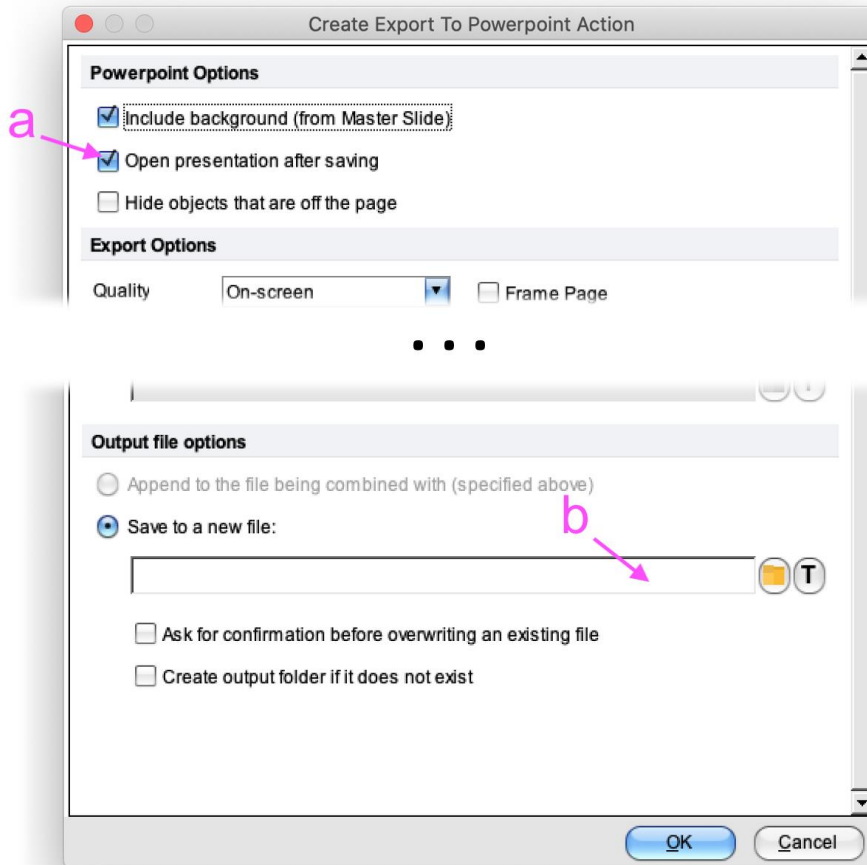
22. Select the **Batch** tab→**Batch Processing**→**Run**. View the PowerPoint file which contains a visual summary of the datafiles in this experiment.

Summary / Transition

Now that you know the basics of analysis in FCS Express, you will be able to analyze data that you generate on the Helios mass cytometer. For additional practice, you can find tutorials on the De Novo Software website. For a more in-depth training on FCS Express, your Fluidigm field applications specialist can put you in contact with your De Novo Software FAS or you can reach out directly to support@denovosoftware.com.

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