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Quickstart Manual

FlowLogic® Data Reduction Software

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Thank you for choosing FlowLogic. This quickstart guide will teach you the basics and have you analyzing your flow data within minutes. For optimal performance, customize the setup of FlowLogic by selecting Preferences, located under the Edit menu. For a more detailed explanation of the functions, view the User Manual found in the Help menu.

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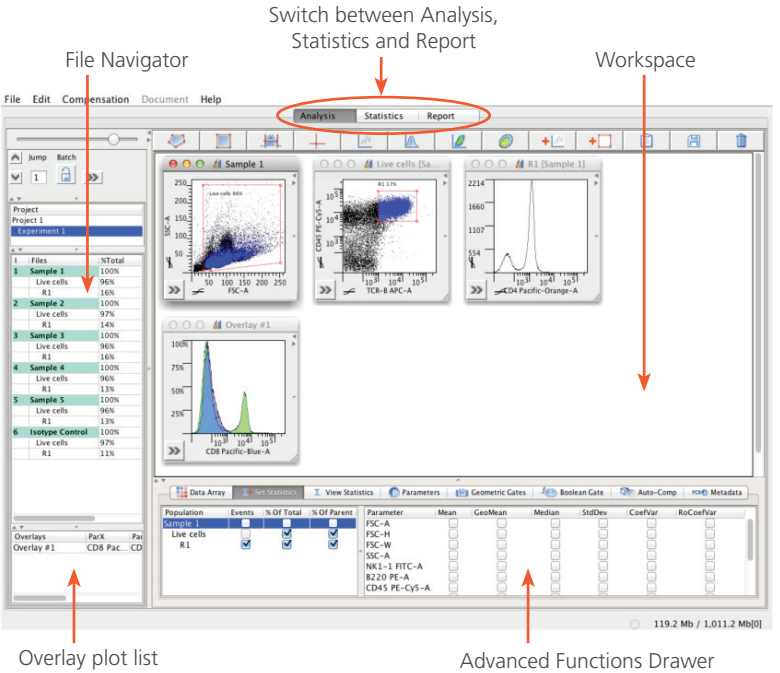
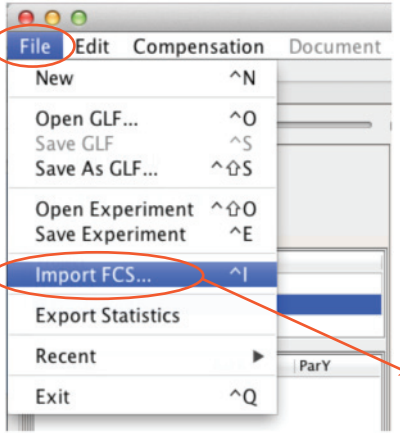
Workspace Overview

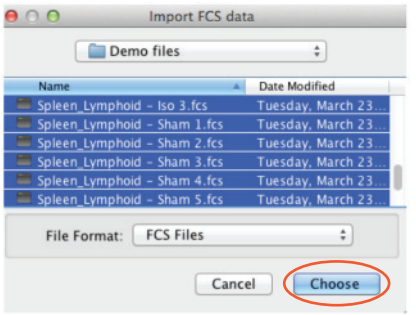


Analysis

1. Importing FCS files



To begin, click File on the menu bar and select Import FCS… from the drop down menu. Locate and highlight your files and click Choose.

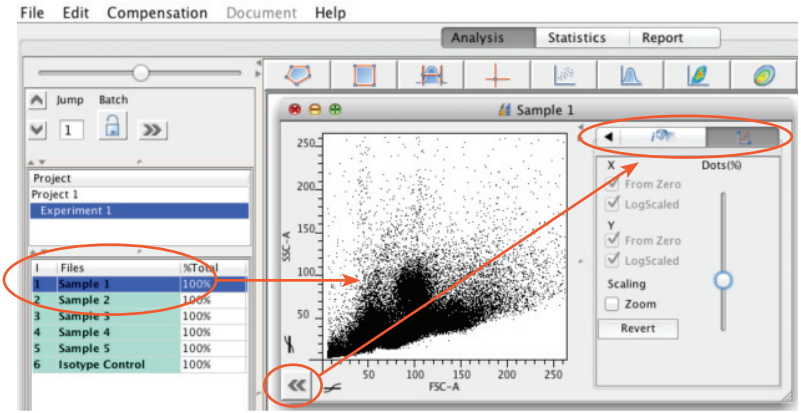


Alternatively, highlight and drag your FCS files directly into a project folder in the File Inspector.

2. Opening dot plots

FCS files appear in the File Inspector. Double click or right click on a file to open it as a dot plot.

Right click the Files title to re-order your FCS files.

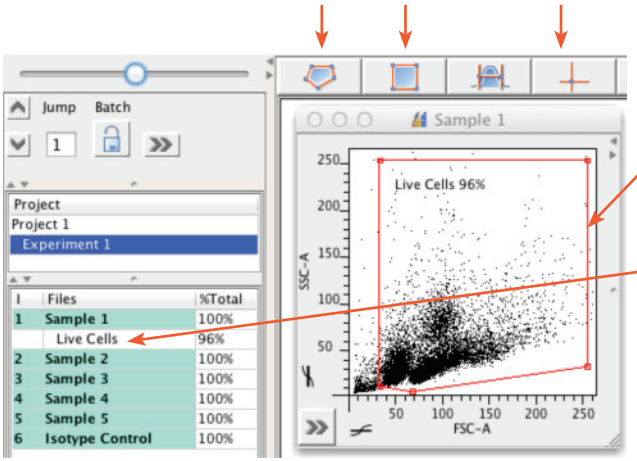


Adjust the % of dots to be displayed.

Click ≪ to open the plot side draw. Scroll through the menu options (Gate List, Boolean Gates, Statistics, Interactive Compensation Matrix, Scaling).

3. Drawing gates

Highlight your plot and select the gating tool you require (polygon, rectangle or quadrant).



Use your cursor to draw your gate. Right click or double click to finish the gate.

The gate appears in the File Inspector. Right click on the gate label to rename it.

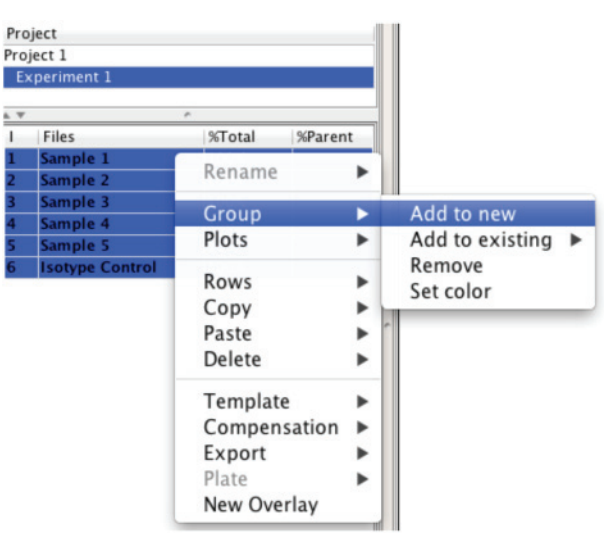
Double click or right click on the gate name to open a daughter plot.

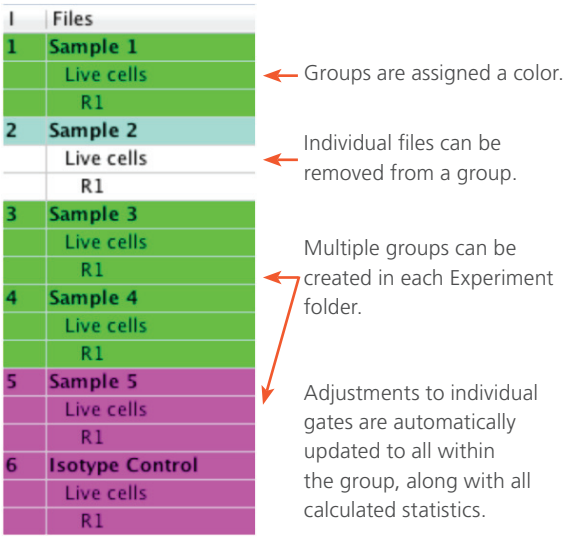
Tip: Renaming your gates appropriately will make creating graphs, performing array analysis and generating reports much easier.

4. Creating groups

Creating groups can make analysis of multiple samples very quick and easy. If a gate is applied to one file in a group it is automatically applied to the same parameters on all other files in the same group.

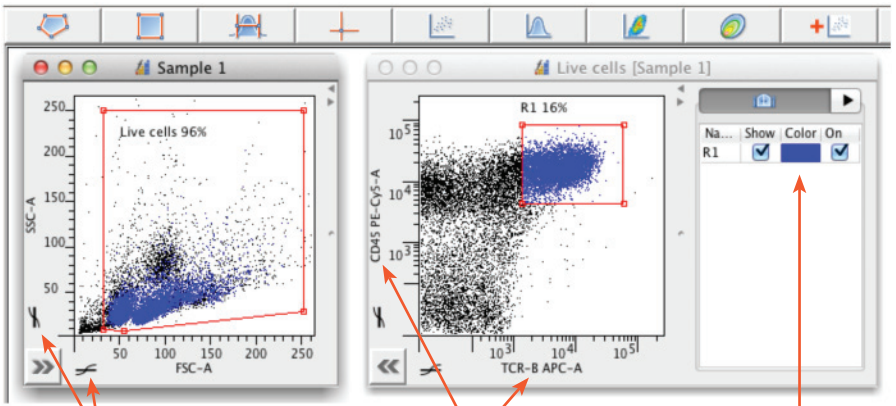
Select all files to be added and grouped, right click and select Group, Add to new.





5. Manipulating dot plots

Change the graph type
(dot plot, histogram or density plot).

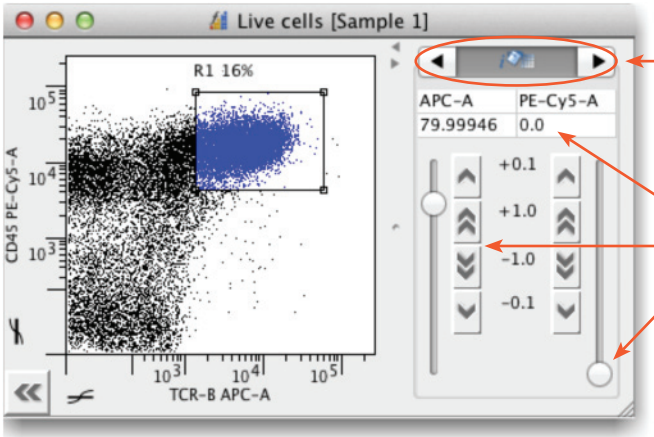


Click for log-linear transformations

Change the parameters with a single left click on the **x** or **y** axis.

Color populations using the Gate List options in the plot side drawer. Colored populations are displayed on all parent plots for that sample.

6. Live compensation

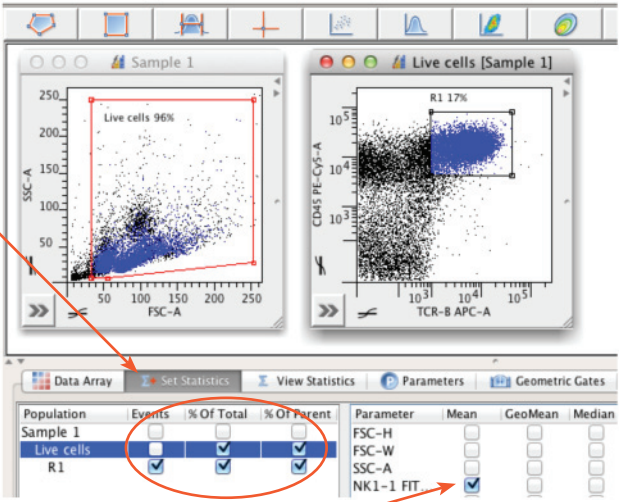


Interactive Compensation Matrix tab.

Compensate your sample in real time. Use the slide bars, arrows or input a number in the Interactive Compensation Matrix tab.

7. Calculating statistics

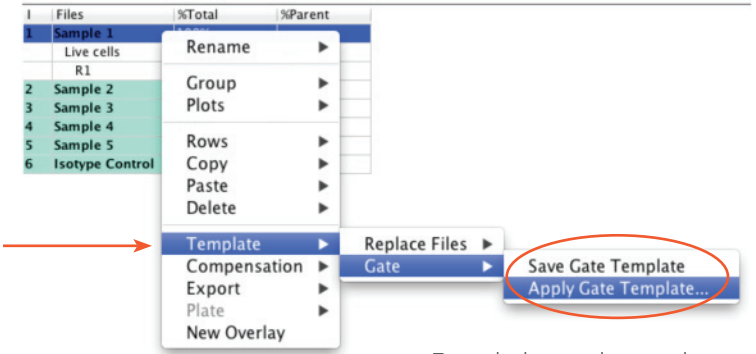
Pull up the Advanced Functions drawer and choose the Set Statistics Tab.



Choose the statistics you wish to calculate by ticking the boxes.

8. Viewing & exporting statistics

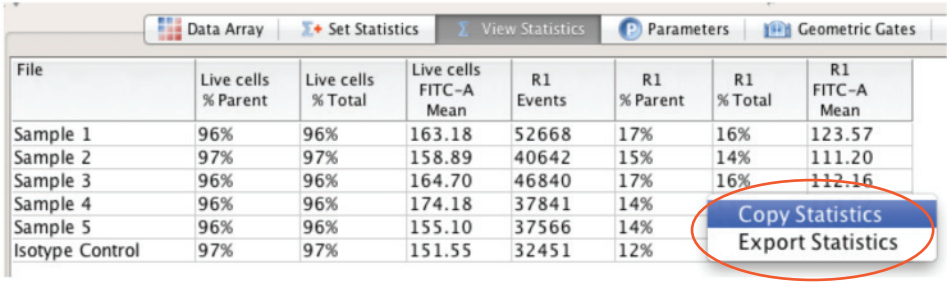
The statistics and gating hierarchy linked to a FCS file can be saved as a template and applied to other samples. To do so, right click on the file and select Template, Gate. Save Gate Template.



To apply the template to other files, highlight them in the File Inspector, right click and choose Apply Gate Template… This will result in the statistics being calculated for all FCS files having the template applied.

8. Viewing & exporting statistics (cont.)

Statistics are viewable in the View Statistics tab in the Advanced Functions drawer.



File	Live cells % Parent	Live cells % Total	Live cells FITC-A Mean	R1 Events	R1 % Parent	R1 % Total	R1 FITC-A Mean
Sample 1	96%	96%	163.18	52668	17%	16%	123.57
Sample 2	97%	97%	158.89	40642	15%	14%	111.20
Sample 3	96%	96%	164.70	46840	17%	16%	112.16
Sample 4	96%	96%	174.18	37841	14%		
Sample 5	96%	96%	155.10	37566	14%		
Isotype Control	97%	97%	151.55	32451	12%		

Right click on the statistics table and choose Copy Statistics to place them on the computer's clipboard.

Alternatively, statistics can be exported by selecting File, Export Statistics.

9. Navigating through your plots

It is easy to effectively view multiple plots from many samples by defining how you would like the workspace to be set up.

To apply the template to other files, highlight them in the File Inspector, right click and choose Apply Gate Template. This will result in the statistics being calculated for all FCS files having the template applied.

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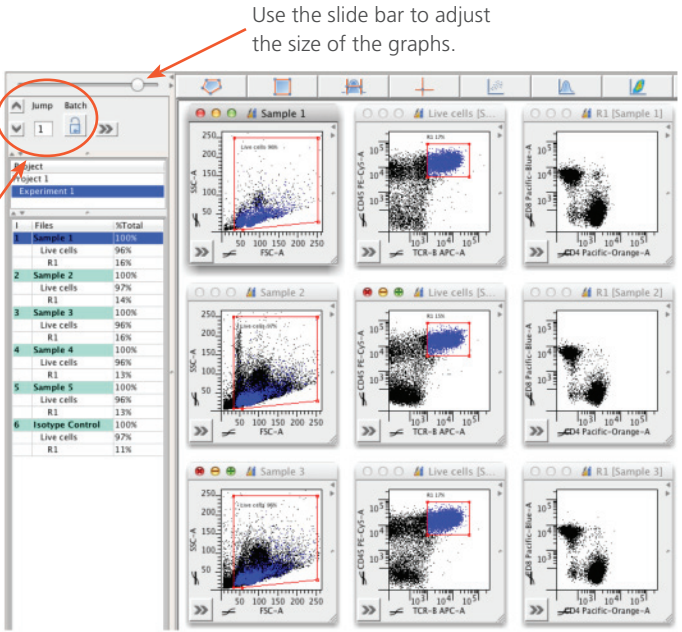
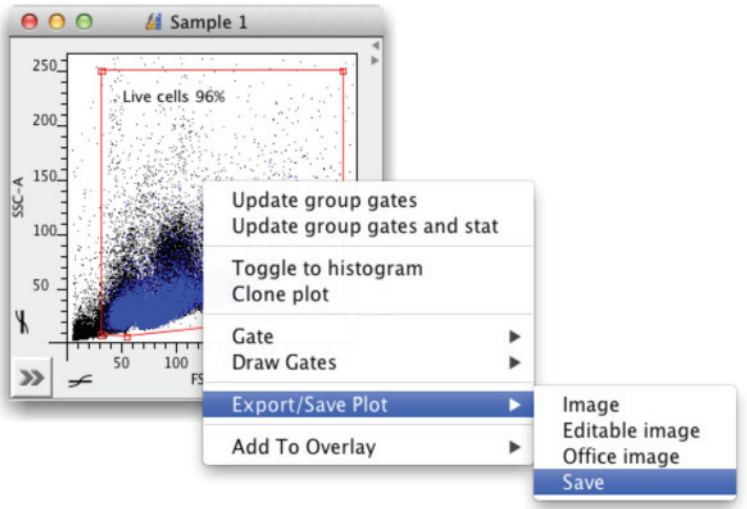


10. Exporting plots

To export a plot, right click on it and choose Export/Save Plot. If you then select Save you will have the option of saving the plot as a .jpg, .png, .svg, .eps, .pdf or a .ps file type.



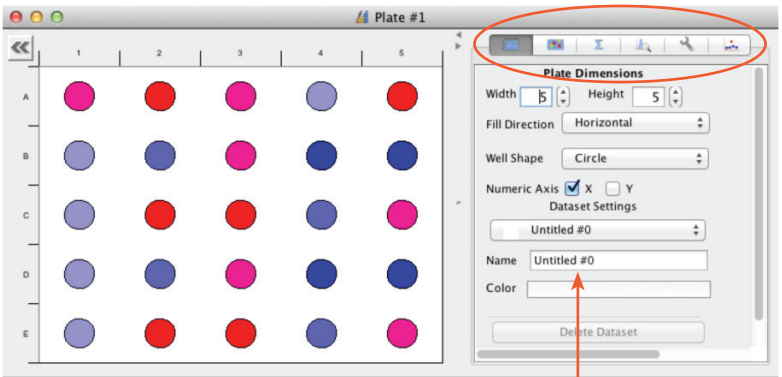
11. Data Array

FlowLogic offers a data array analysis function visualized as a plate. This is useful for fast analysis of large data sets. It also provides unique display options for all types of analysis and the easy generation of heat maps.

Select Data Array from the Advanced Functions Drawer. Right click in the plate window and select New Data Array. Drag files directly from the File Inspector into the plate.

Side Drawer Tabs

Plate Setup, Heatmap Settings, Statistics, Plot Viewer, Advanced Functions, Plate Outlier Detection.

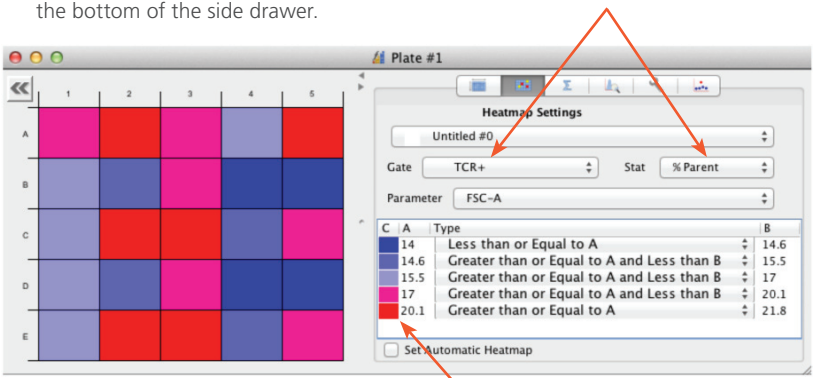


In the Plate Side Drawer under the Plate Setup tab, modify the plate to suit your experiment.

11. Data Array (cont.)

Heatmaps can be generated automatically by ticking the box at the bottom of the side drawer.

Choose the population and the statistic to be displayed in the wells.

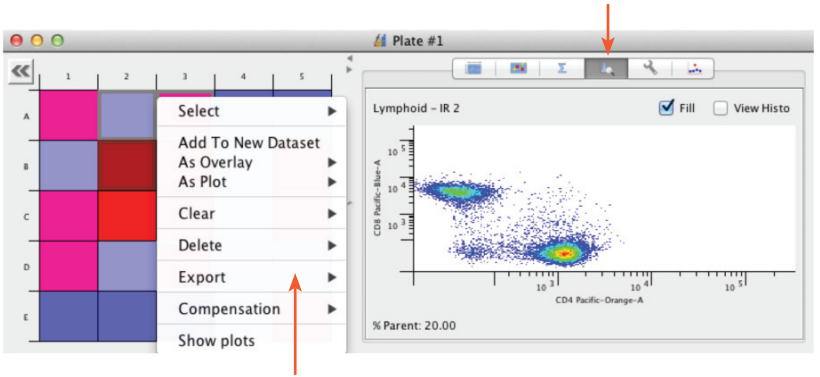


The well shape has been set to Fill in the Plate Setup tab.

Click on a color to change it for a particular range.

11. Data Array (cont.)

Individual dot plots and histograms are displayed in the Plot Viewer tab when you hover the mouse over the wells.

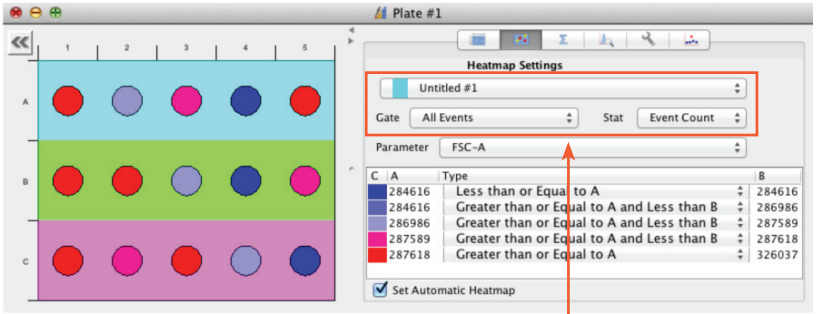


Right Click on the plate to bring up menu options to export the plate, clear contents and delete rows/columns.

11. Data Array (cont.)

Create datasets to display and analyze different gates from the same samples. To create a new data set, highlight the wells to be included, right click and select Add to New Dataset.

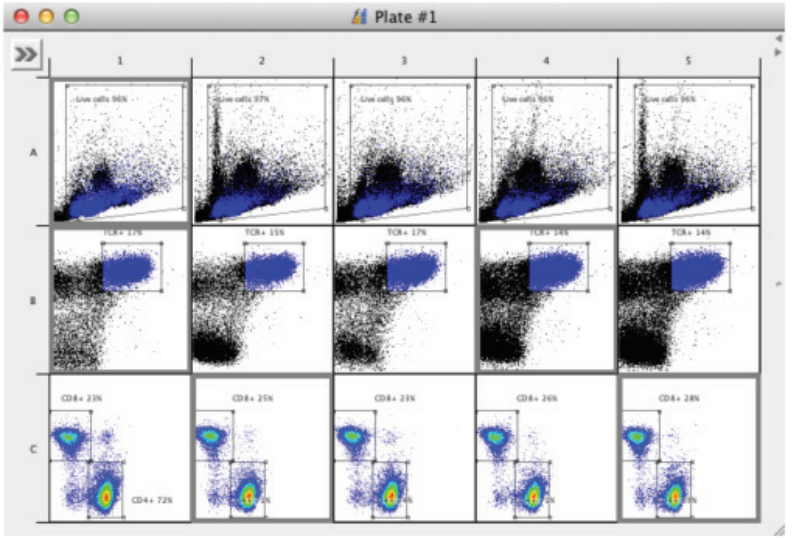
In the example below, the same five samples have been loaded into each row before being defined as a new dataset as indicated by the well background color.



In the Heatmap Settings tab, select the data set from the drop down menu followed by the Gate and the Stat that you wish to view.

11. Data Array (cont.)

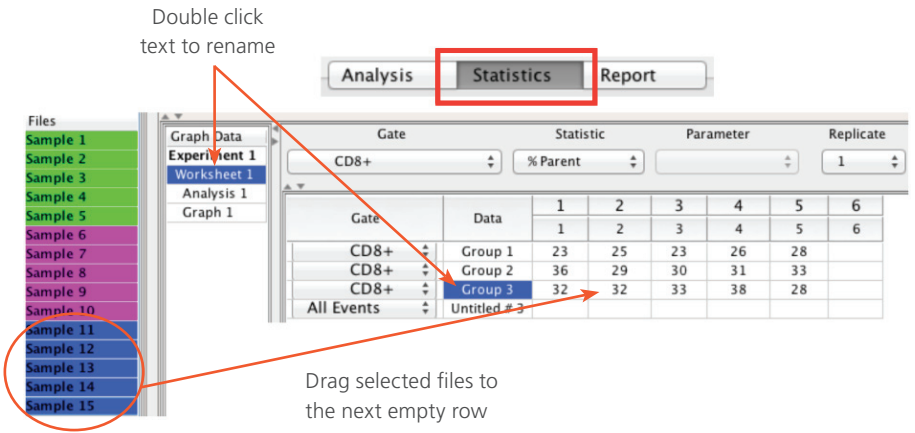
Right click on the plate and select Plots. Select Show Gates. The wells are replaced with dot plots of the specific sample. You can also choose to color the background to highlight the different datasets or color the dot plots to represent the heatmap colors.



Statistics

FlowLogic allows you to create graphs from your data and perform statistical analyses in a few easy steps. The best part is if you need to adjust a gate on a dot plot, all graphs and statistical analyses are updated automatically!

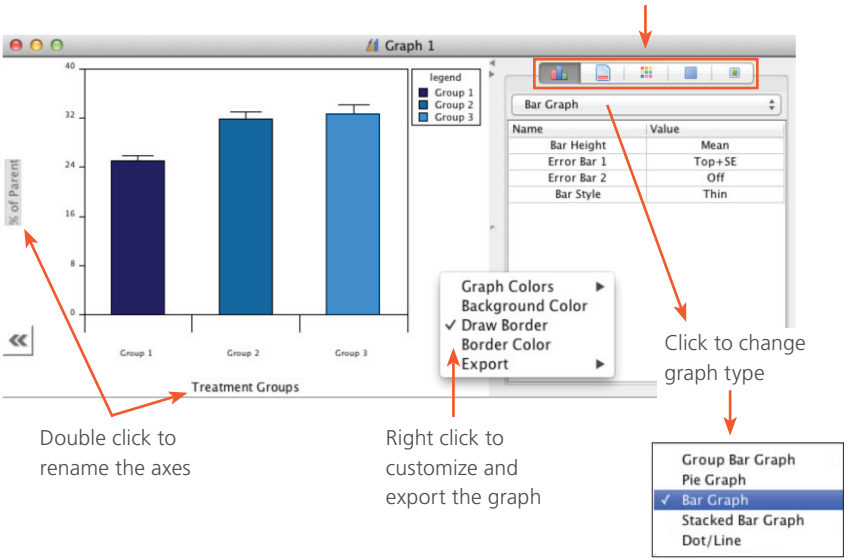
To graph your data, first select the Statistics window at the the top of the program and select Worksheet 1 In the Graph Data window. Then, select one group of samples from the File Inspector and drag them into the cell in column 1.



Statistics (cont.)

Double click Graph 1 In the Graph Data window to draw a graph of the data in the spreadsheet. The graph appears in the workspace.

Use functions in Graph Settings, Color Settings, Legend Settings and Border & Background Settings to customize your graph.



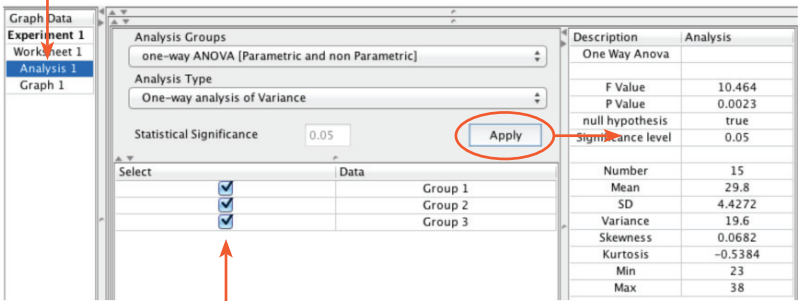
Double click to rename the axes

Right click to customize and export the graph

Click to change graph type

Statistics (cont.)

To perform a statistical analysis, click on Analysis In the Graph Data window. In the Analysis window, choose the statistical test you wish to use form the drop down menus.



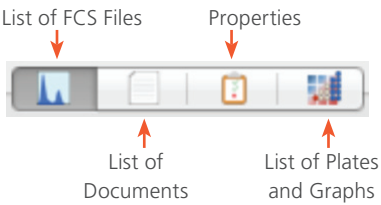
Tick to select the groups to analyze and press Apply. The result is displayed in the window to the right.

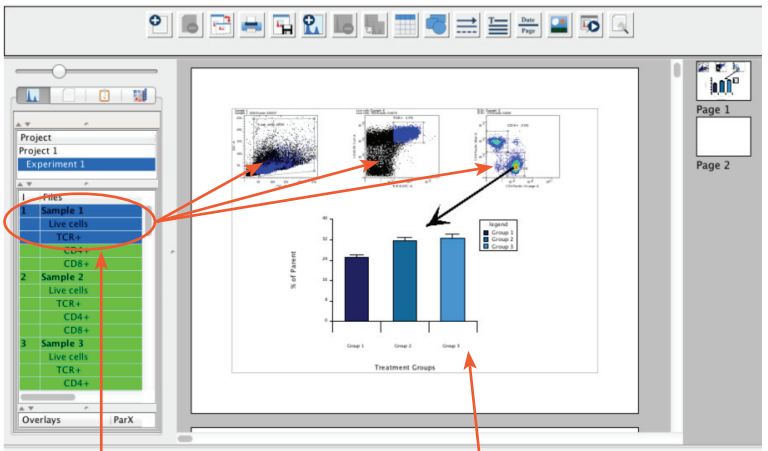
Report

The Report function in Flowlogic allows you to display and annotate plots, graphs, plates, heatmaps and tables. These can then be printed, displayed as a slideshow or saved as a PDF.

Any changes made to your analysis will be updated in your report automatically.

Select the Report Window from the top of the workspace.
Use the tabs at the top of the File Inspector to view your list of FCS files, list of documents, modify the properties of your report and view a list of all plates and graphs.





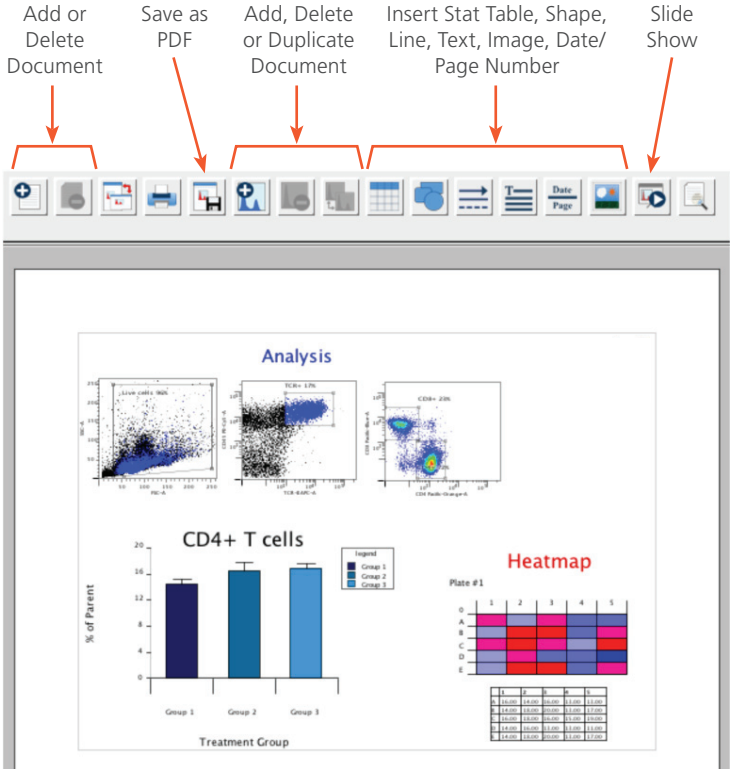
To add dot plots to your report, highlight and drag one or more FCS files from the list of FCS Files. The plots will appear as they were last viewed when analyzing.

Likewise, from the List of Plates and Graphs, highlight and drag your selection onto the report.

Report (cont.)

Use the elements in the toolbar to annotate the document, add or delete plots, print save and more. Hover over the buttons to display their function.

Documents with a combination of dot plots, graphs, heat maps and plates can be displayed as a slide show, saved as a PDF or printed as a report. Plots and graphs will be displayed as they exist in the Analysis or Statistics windows. To change their appearance, modify them in their original location. Smaller changes, such as removing titles, axes and labels can be done in Report.



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