



Gate it.
Plate it.
Graph it.
Print it.

Developed by scientists, for scientists.



FlowlogicTM
Flow Cytometry Analysis Software

Quickstart Guide

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Flowlogic™ Quickstart

Flow Cytometry Analysis Software

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 **Manual** found in the **Help** menu and online tutorials at www.inivai.com.

Workspace Overview

File Navigator

File Inspector

Switch between Analysis, Statistics and Report

Workspace

Overlay plot list

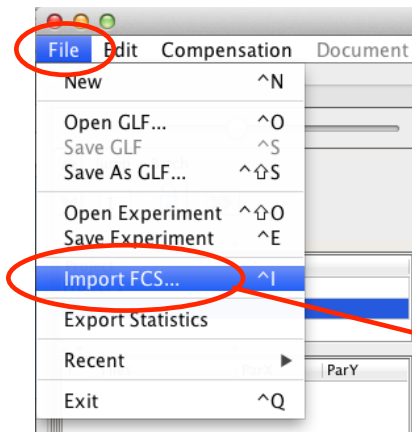
Advanced Functions Drawer

The screenshot shows the Flowlogic software interface. The menu bar at the top includes File, Edit, Compensation, Document, and Help. Below the menu bar is a toolbar with various icons. The main workspace is divided into several panes. On the left is the File Navigator, which shows a project tree with 'Project 1' and 'Experiment 1'. Below it is the File Inspector, which shows a list of files and their properties. The central plot area contains four plots: 'Sample 1' (a scatter plot of SSC-A vs FSC-A), 'Live cells' (a scatter plot of CD45 PE-Cy5-A vs TCR-B APC-A), 'R1' (a histogram of CD4 Pacific-Orange-A), and 'Overlay #1' (a histogram of CD8 Pacific-Blue-A). The bottom pane contains a Data Array table and an Advanced Functions Drawer. The Data Array table has columns for Population, Events, % Of Total, % Of Parent, Parameter, Mean, GeoMean, Median, StdDev, CoefVar, and RoCofVar. The Advanced Functions Drawer is located at the bottom right of the interface.

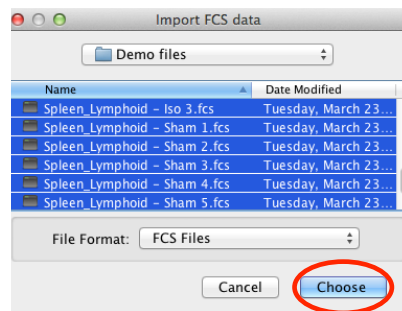
Population	Events	% Of Total	% Of Parent	Parameter	Mean	GeoMean	Median	StdDev	CoefVar	RoCofVar
Live cells	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	FSC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
R1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	FSC-H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	FSC-W	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	SSC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NK1-1 FITC-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	B220 PE-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	CD45 PE-Cy5-A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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**.

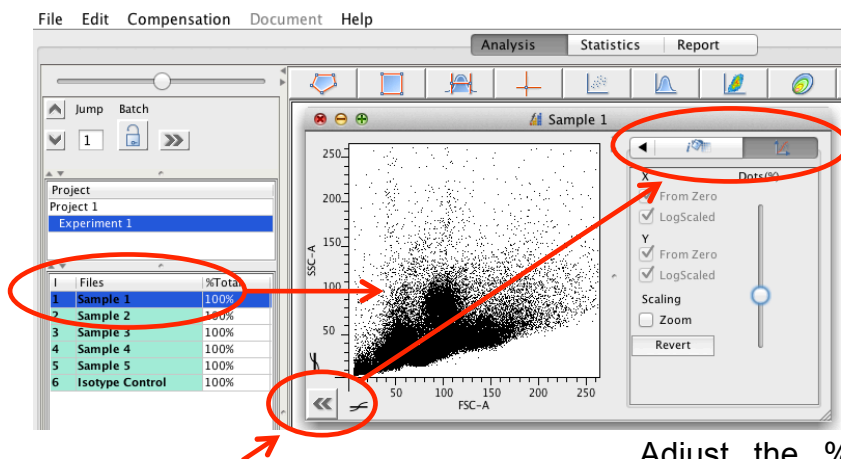


Alternatively, highlight and drag your FCS files directly into a project folder in the File Inspector.

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.

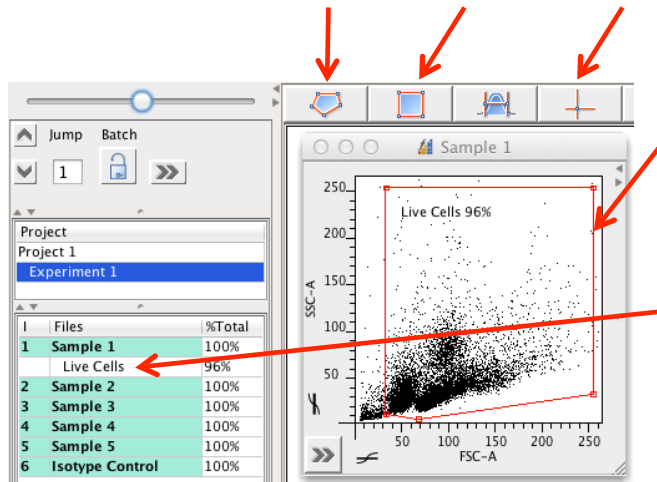


Click to open the plot side draw. Scroll through the menu options (**Gate List**, **Boolean Gates**, **Statistics**, **Interactive Compensation Matrix**, **Scaling**).

Adjust the % of dots to be displayed.

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.

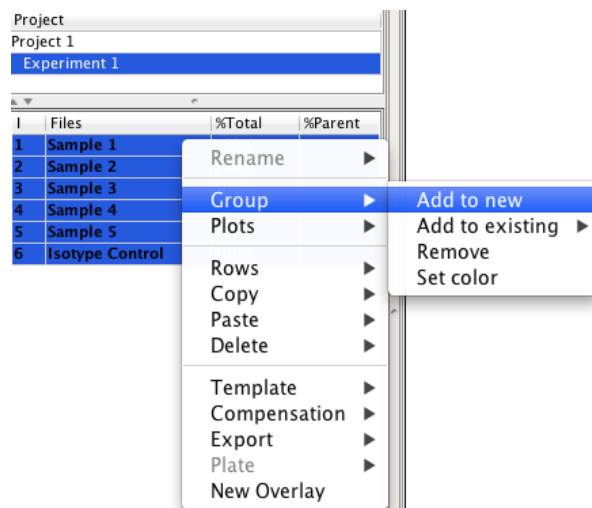
Double click or right click on the gate name to open a daughter plot.

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 to be grouped, right click and select **Group, Add to new**.



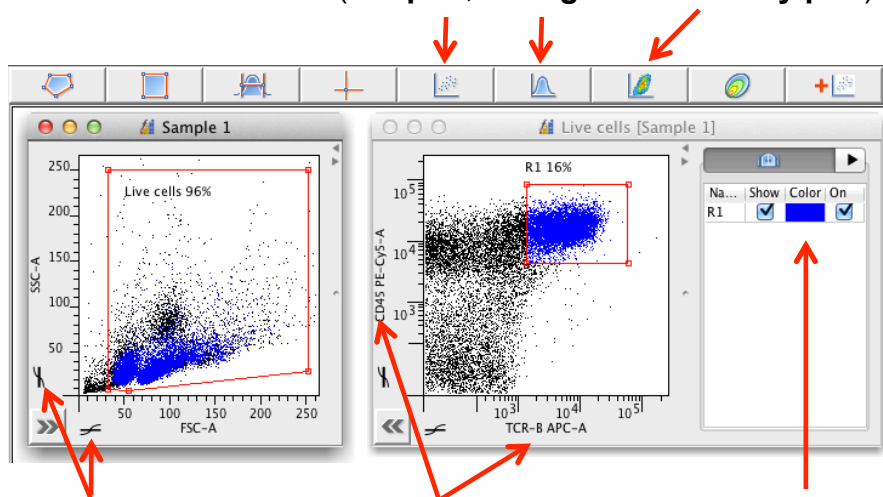
Creating groups continued...


- Groups are assigned a color.
- Individual files can be removed from a group.
- Multiple groups can be created in each Experiment folder.
- Adjustments to individual gates are automatically updated to all within the group, along with all calculated statistics.

1	Files
1	Sample 1
	Live cells
	R1
2	Sample 2
	Live cells
	R1
3	Sample 3
	Live cells
	R1
4	Sample 4
	Live cells
	R1
5	Sample 5
	Live cells
	R1
6	Isotype Control
	Live cells
	R1

5. Manipulating dot plots

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

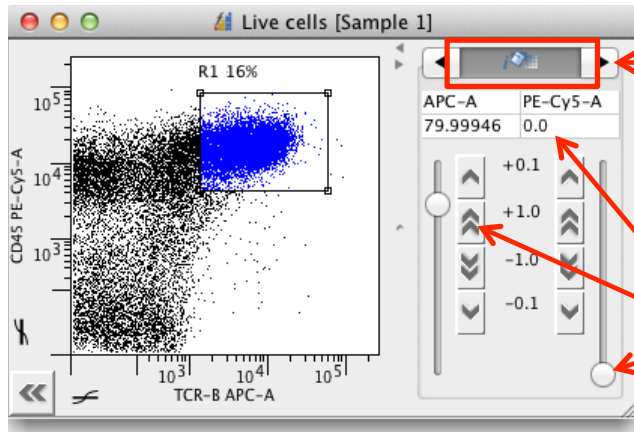


Click  for log-linear transformations

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

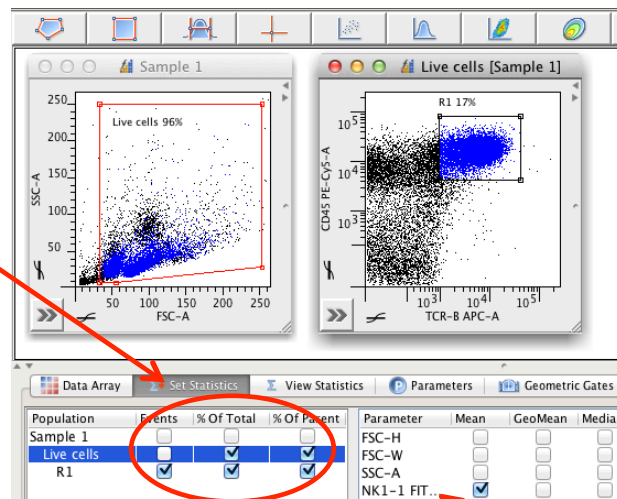


Interactive Compensation Matrix tab.

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

7. Calculating statistics

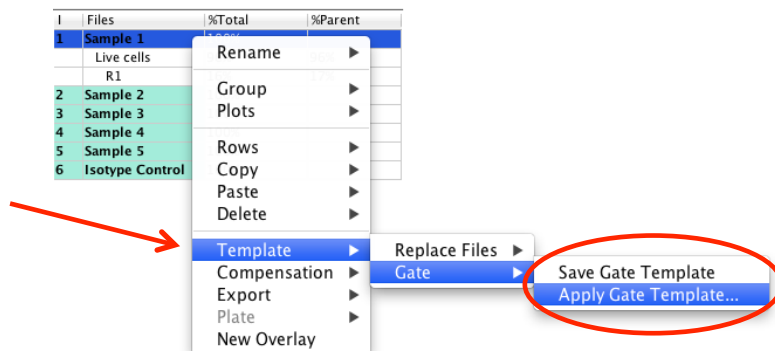
Pull up the **Advanced Functions** drawer and choose the **Set Statistics** tab.



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

8. Viewing & exporting statistics

The statistics and gating hierarchy linked to an 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.

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%		

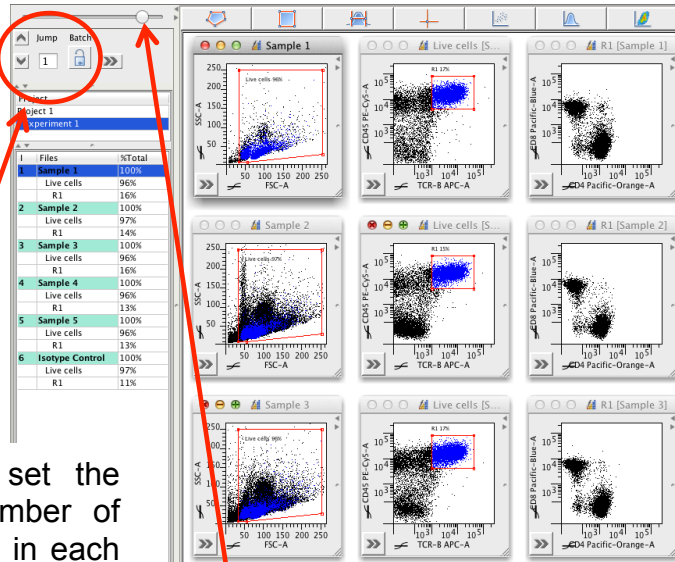
Right click on the statistics table and choose **Copy Statistics** to place them on the computer's clipboard.

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.

In the **Edit** menu, choose **Plot Arrangement** followed by **Number of Windows**. Select the number of plots to be displayed horizontally.

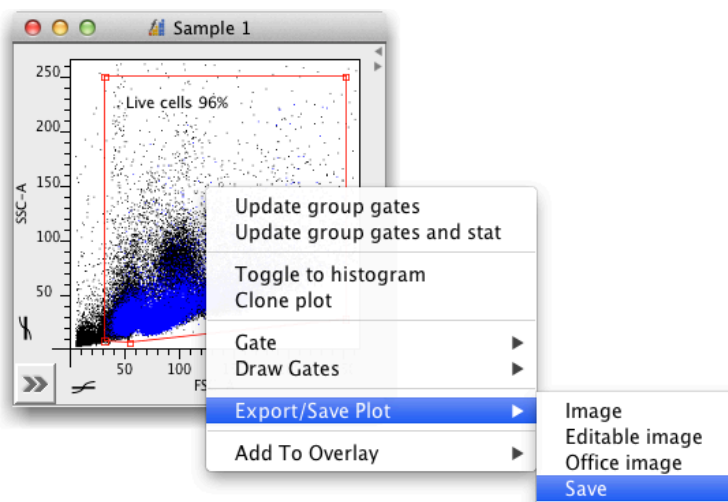


With the padlock open, set the **Jump** number to the number of files to be scrolled through in each step. Jump up and down with the arrows.

Use the slide bar to adjust the size of the graphs.

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.



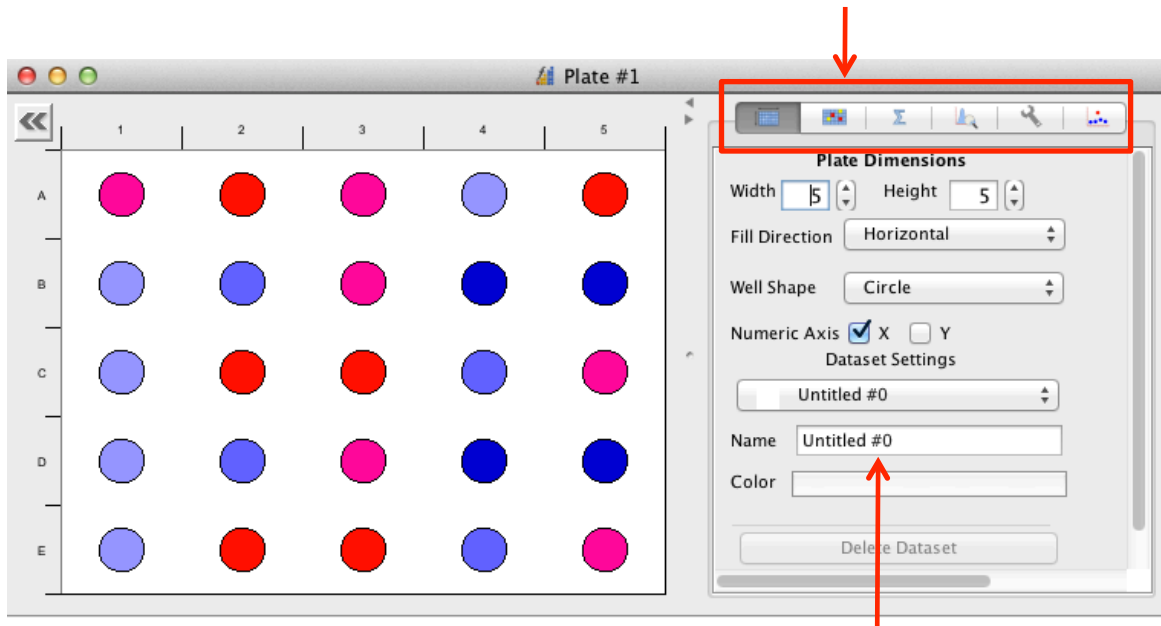
11. Data Array

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

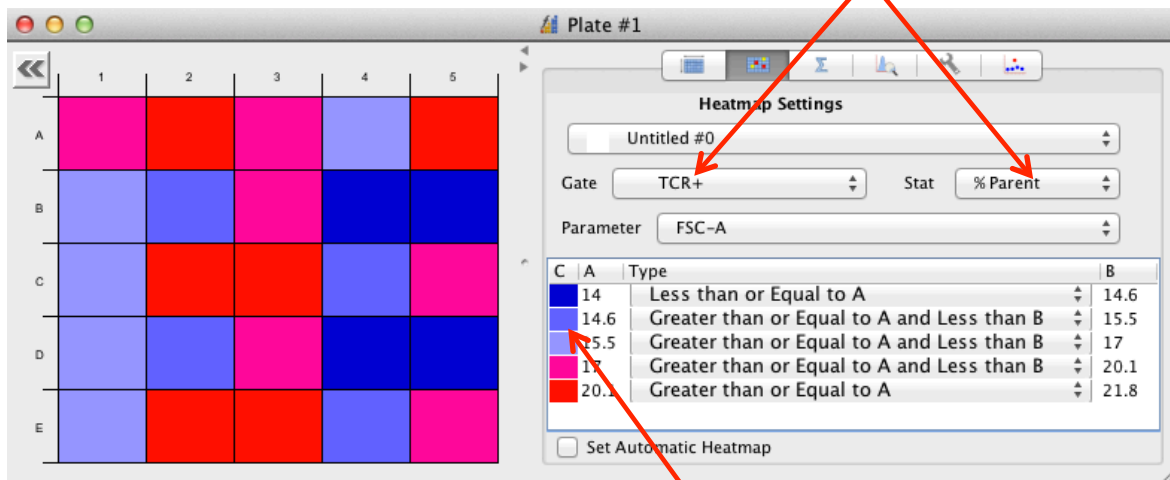
Plate Setup, Heatmap Settings, Statistics, Plot Viewer, Advanced Functions, Plate Outlier Detection.



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

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

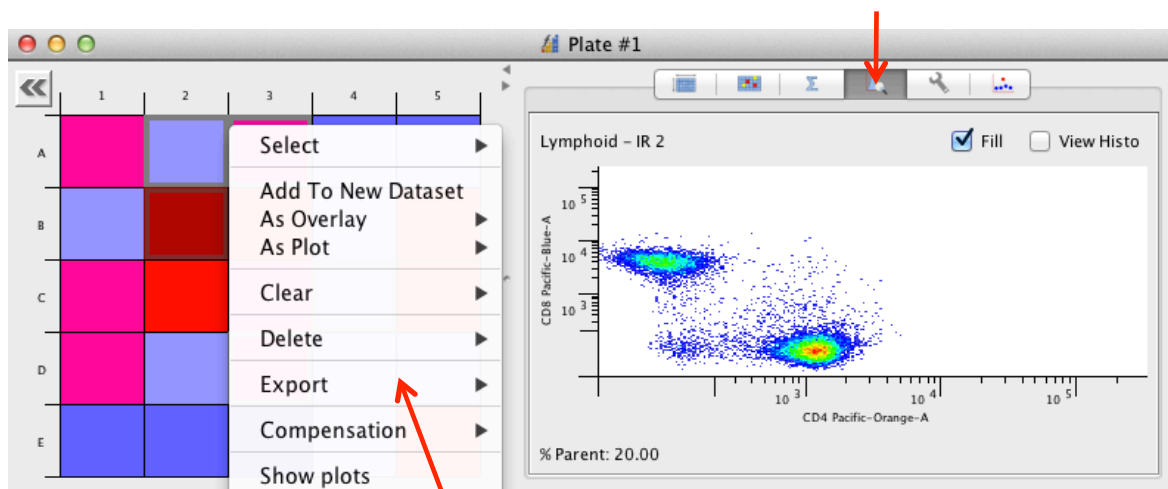
Choose the population and the statistic to be displayed in the wells.



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

Click on the colors to change it for a particular range.

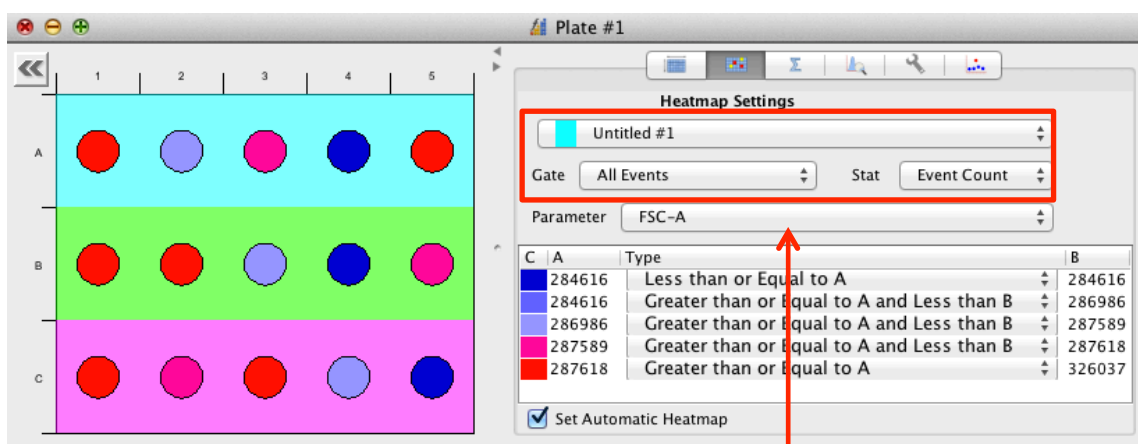
Individual dot plots and histograms are displayed in the Plot Viewer tab when you hover the mouse over the wells.



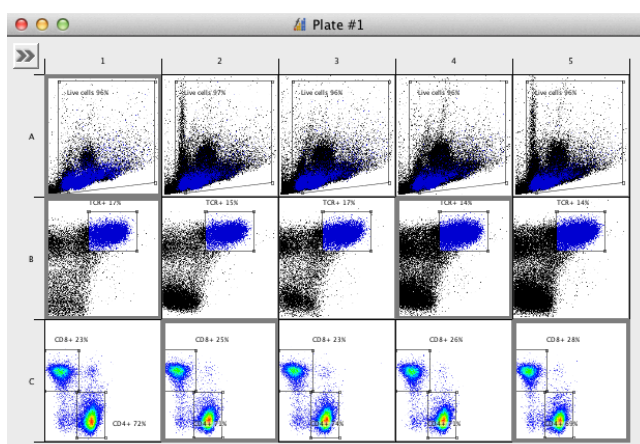
Right click on the plate to bring up menu options to export the plate, clear contents and delete rows/columns.

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 5 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 (statistics) that you wish to view.



Right click on the plate and select **Plots**. Select **Show Gates**. The wells are replaced with dot plots of the specific file. 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. A great advantage is that if you need to adjust a gate on a dot plot, all graphs and statistical analyses are updated automatically!

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

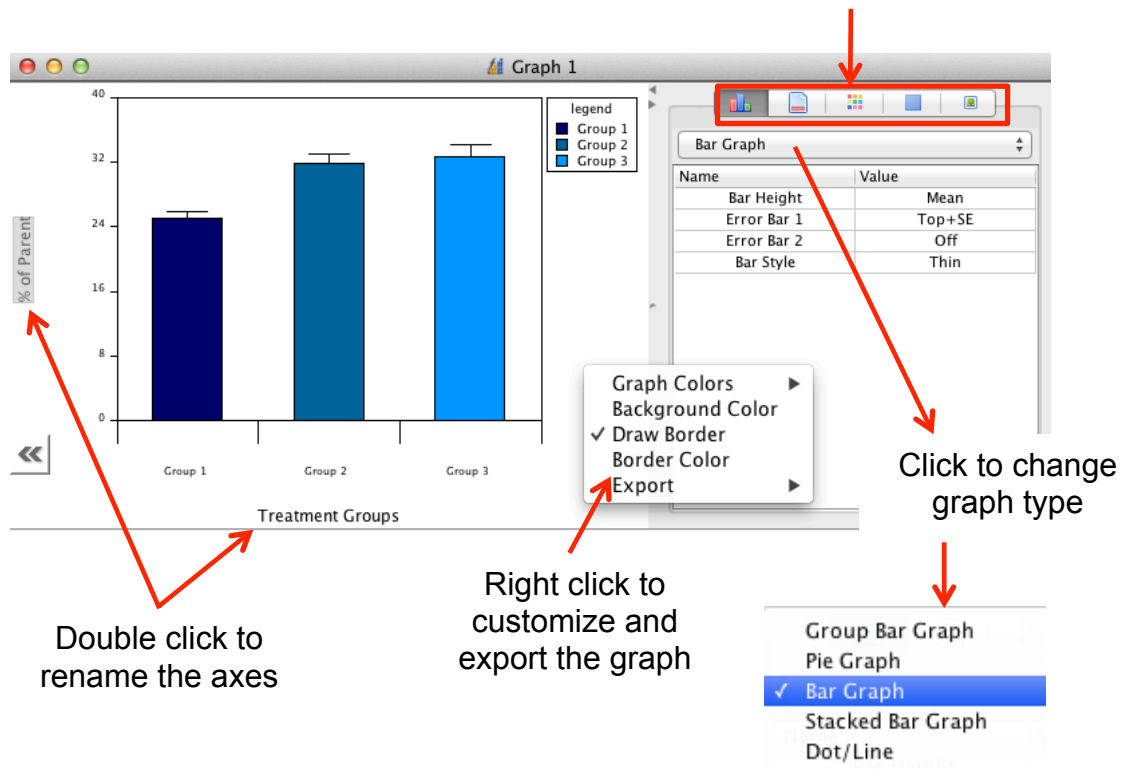
Double click text to rename

Gate		Statistic		Parameter		Replicate	
CD8+		% Parent				1	
Gate	Data	1	2	3	4	5	6
CD8+	Group 1	23	25	23	26	28	
CD8+	Group 2	36	29	30	31	33	
CD8+	Group 3	32	32	33	38	28	
All Events	Untitled # 3						

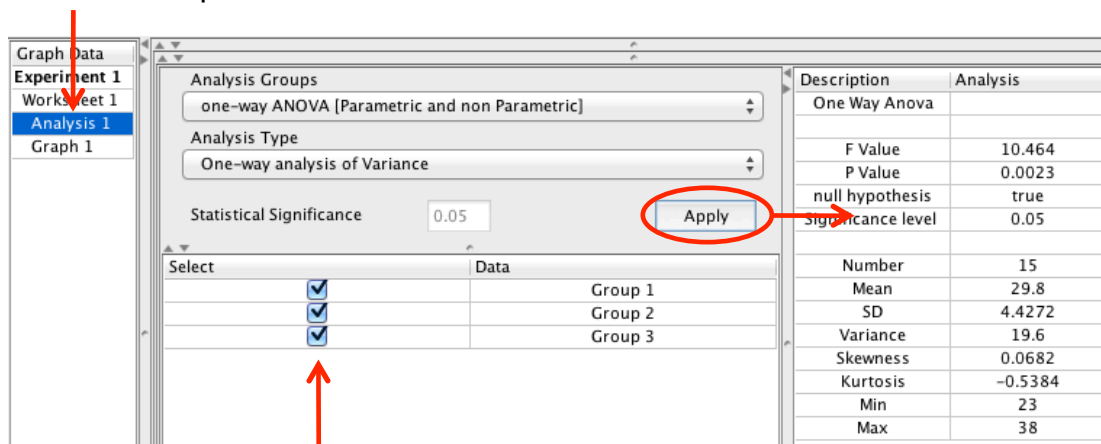
Drag selected files to the next empty row

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 the **Graph Settings, Color Settings, Legend Settings and Border & Background Settings** to customize your graph.



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 from the drop down menus.



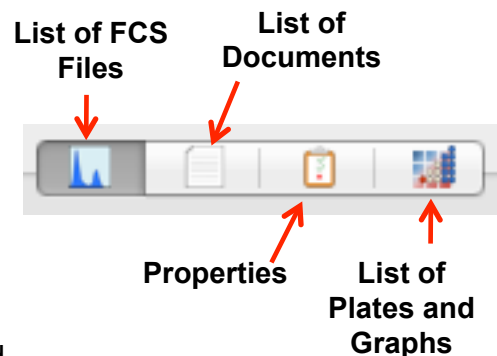
Report

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.

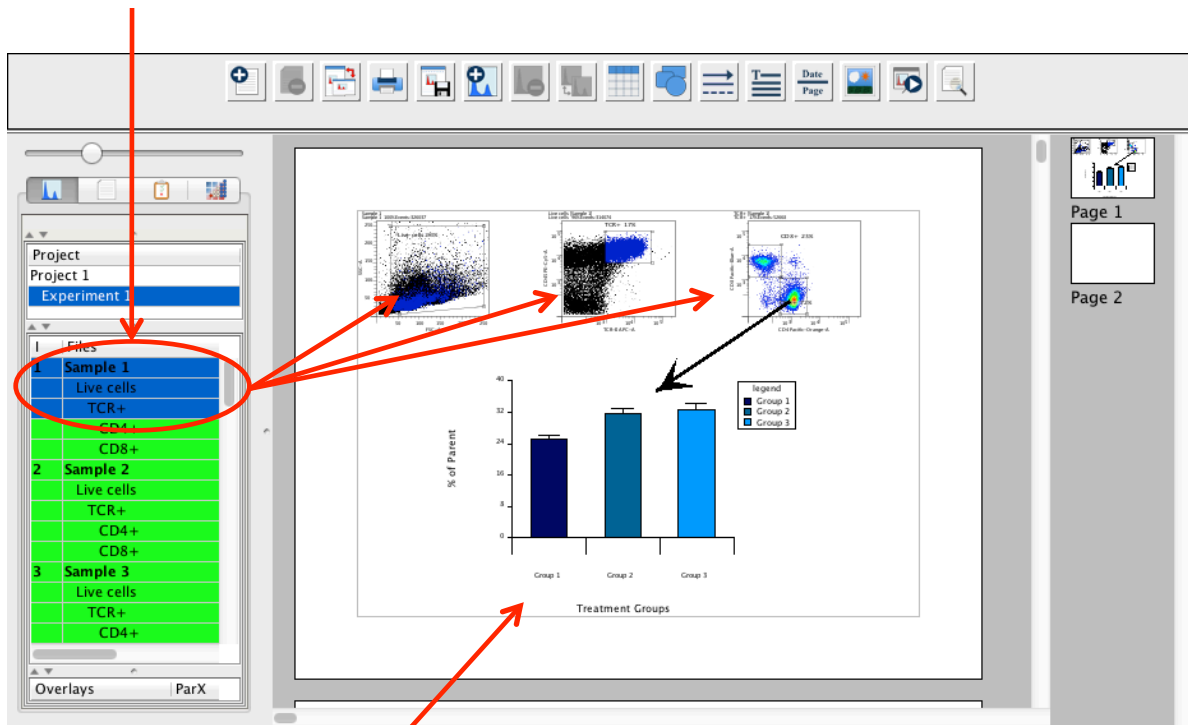
Of course, any changes made to your analysis will be updated in your report automatically.

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.

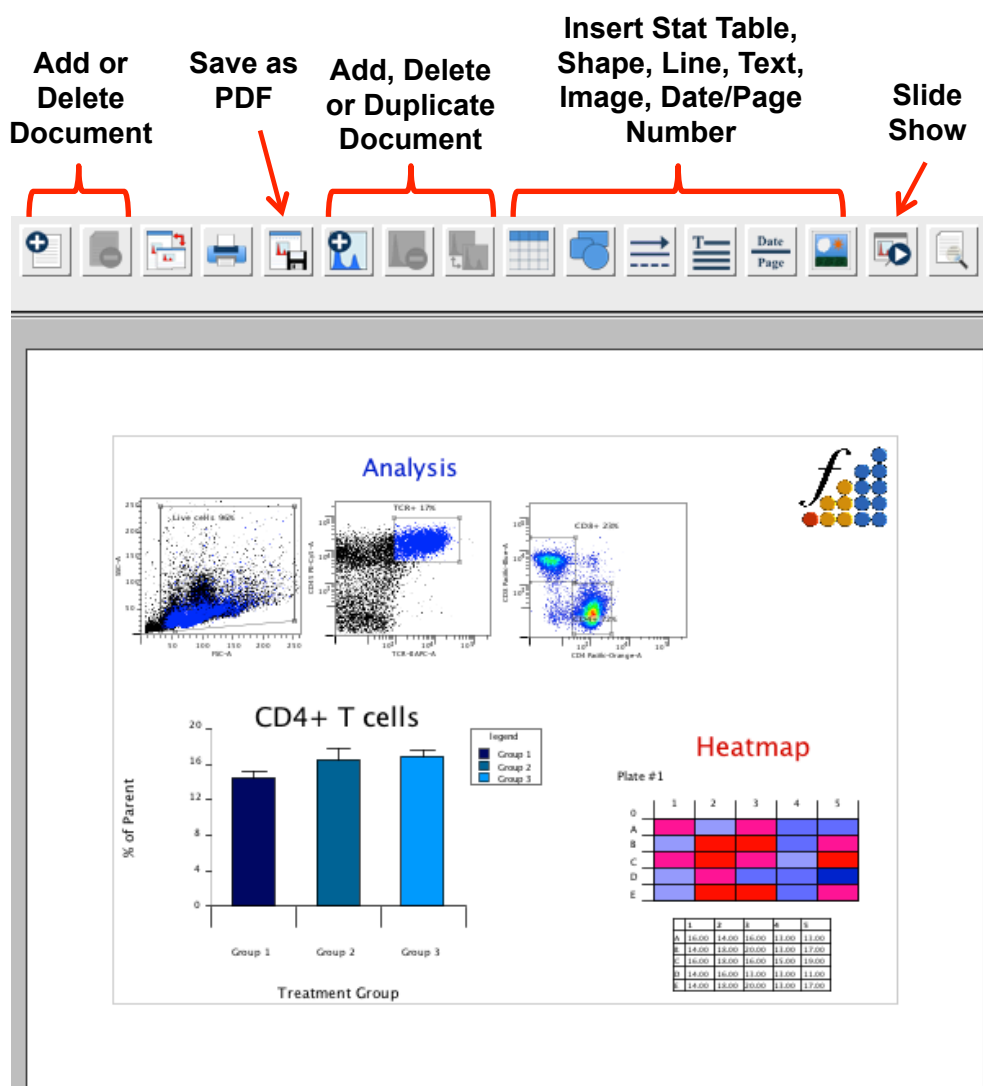


To add dot plots to your report, highlight and drag one or more FCS files from **the List of FCS Files**. The plots will appear as they were last viewed when analyzing.



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

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, heatmaps 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**.



Technical Specifications

Fully tested (using Java SE ver. 5–7) on:

- Linux (Fedora 5–8)
- Suse 10.x
- Ubuntu 6.04 to 11.10 (x64, x86)
- Mac OS X (10.4–10.7)
- Solaris 10
- Windows 2000, XP, Vista, 7 (x64, x86)

Min. RAM required: 1GB

CPU: 1.5GHz Dual Core



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