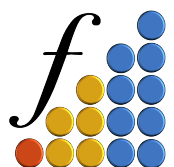
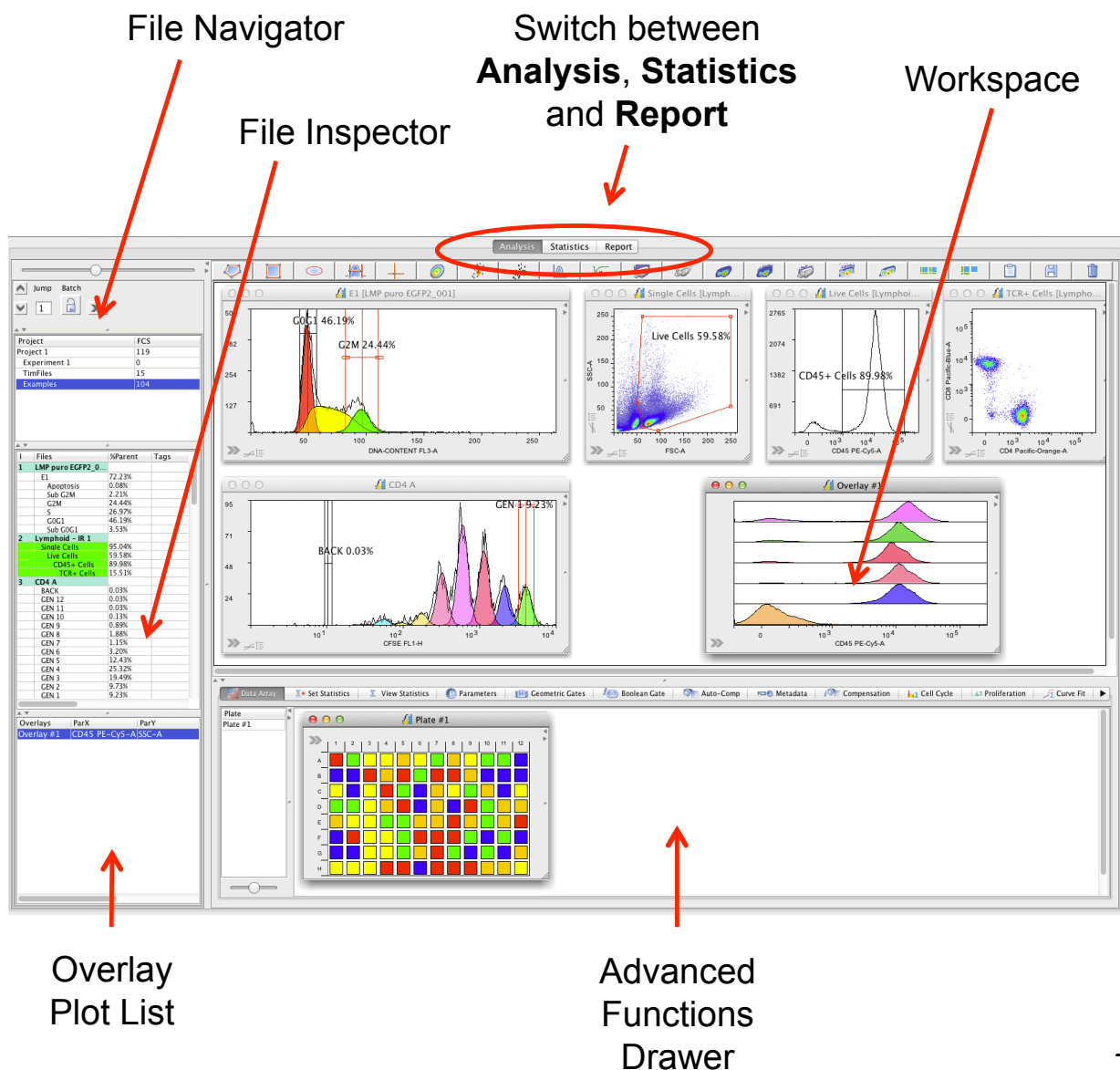


Flowlogic™ Quickstart

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 by clicking FlowLogic in the menu bar. For a more detailed explanation of the functions, view the **Manual** found in the **Help** menu.

Workspace Overview



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Statistics

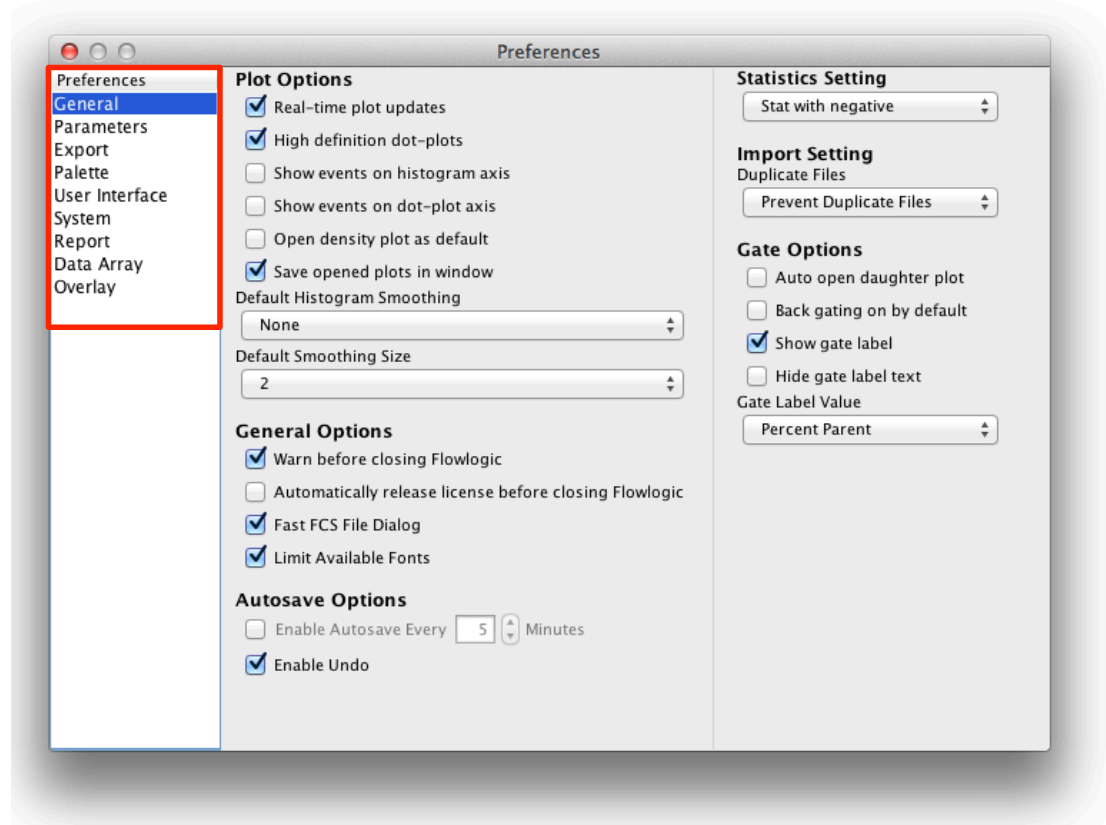
pg.18

Report

pg.20

Program Preferences

Located by clicking FlowLogic in the menu bar, options in the Preferences allow the user to customize **General** plot and gate options, **Parameters**, **Export Settings**, **Color Palettes**, the **User Interface**, **System**, **Reports**, **Data Arrays** and **Overlays**.





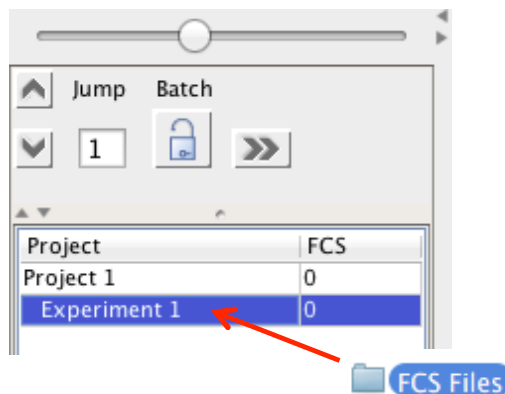
Analysis

1. Importing FCS files



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

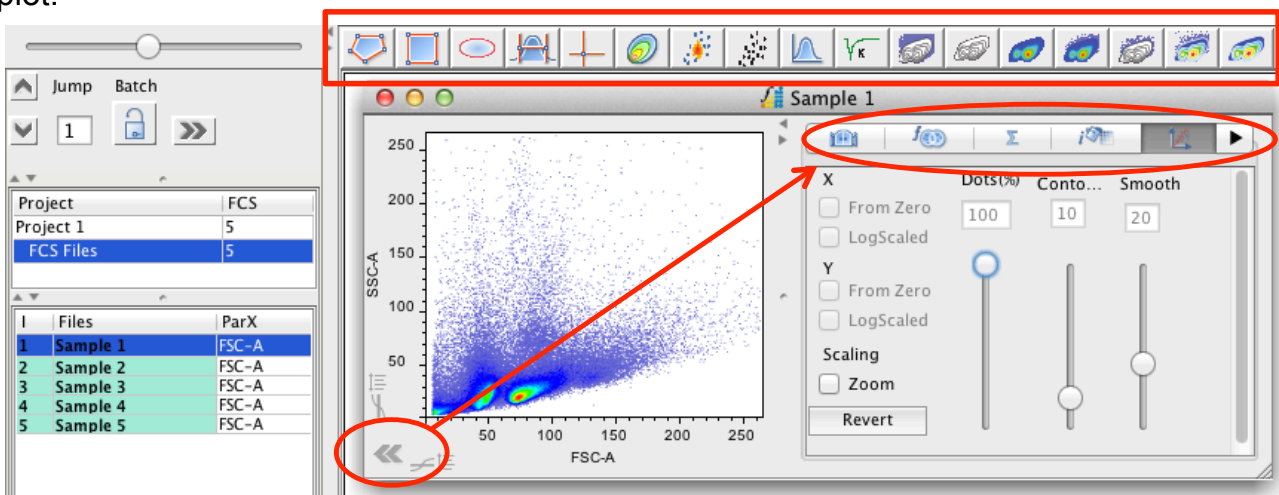
Alternatively, right click on an Experiment Folder or drag FCS files or folders containing FCS files directly into an Experiment Folder in the **File Navigator**.



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.

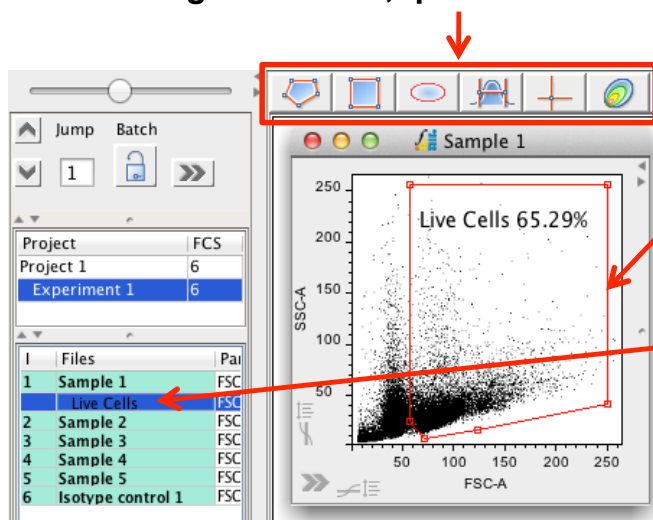
Gating tools and plot display options, including standard dot plots, density plots, histograms, contour plots and pseudo-color plots appear in the toolbar



Click here to open the plot side drawer for the **Gate List**, **Boolean Gates**, **Statistics**, **Interactive Compensation**, **Scaling**, **Cell Cycle Analysis**, **Proliferation Analysis**, **Curve Fitting** and **Kinetics**.

3. Drawing gates

Highlight your plot and select the gating tool you require (**polygon**, **rectangle**, **ellipse**, **histogram marker**, **quadrant** or **autogating**).



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 the daughter plot.

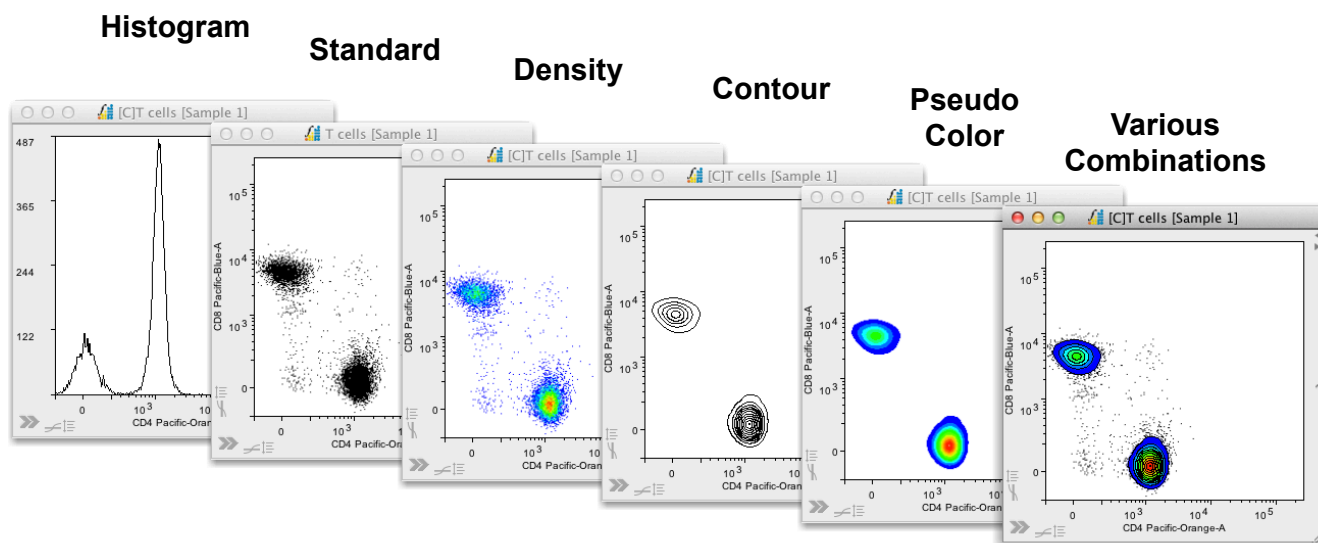
Tip: Renaming your gates appropriately will make creating graphs, performing array analysis and generating reports much easier.

4. Plot options

Different plot options can be selected directly from the toolbar located above the workspace.



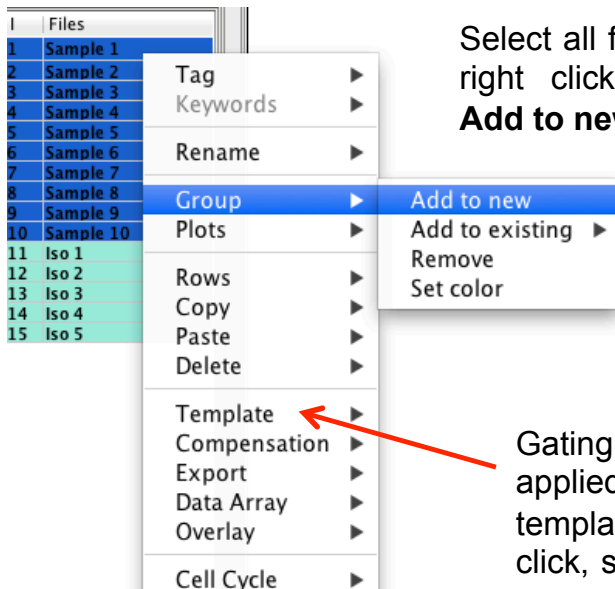
These include:



Open the plot side drawer and select the Scaling tab to change the **% dots displayed**, **number of contours** and **smoothness of contours**.

5. 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 group.



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

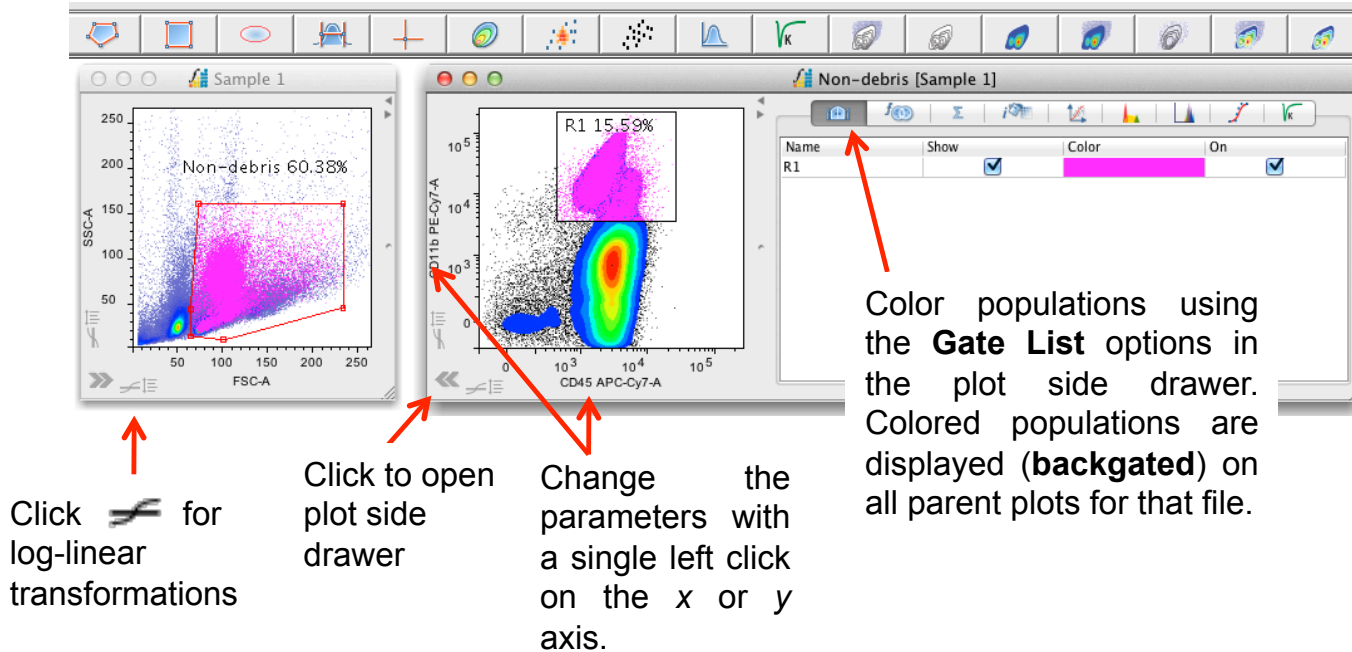
Gating templates can also be saved and applied to future experiments. To save a template, highlight the file and gates, right click, select **Template, Gate** and **Save Gate Template**.

- Groups are assigned a color.
- Individual files or even individual gates 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.**

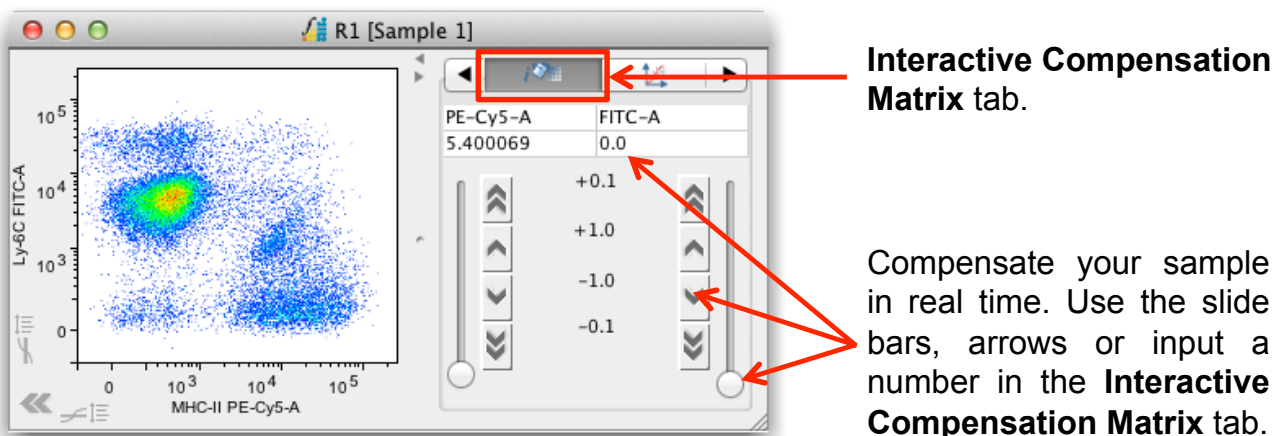
I	Files
1	Sample 1
	Live
	M1
2	Sample 2
	Live
	M1
3	Sample 3
	Live
	M1
4	Sample 4
	Live
	M1
5	Sample 5
	Live
	M1
6	Sample 6
	Live
	M1

6. Manipulating dot plots & backgating

Change the graph type
(dot plot, histogram, density plot, contour plot pseudo color plot and combinations of these types).



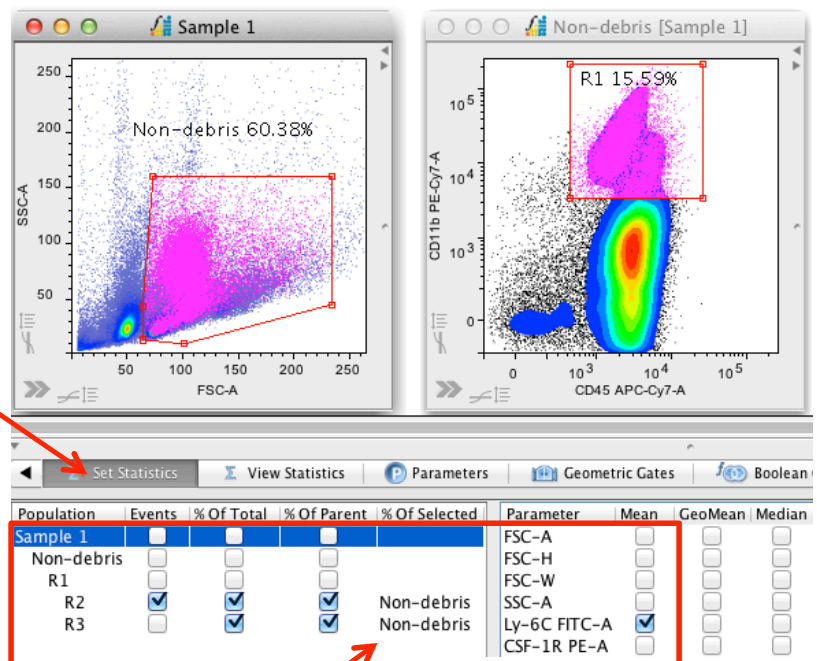
7. Live compensation



8. Calculating and viewing statistics

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

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



From the **% of Selected** column, click to reveal a drop down menu allowing the choice to select the percentage of any other population in the hierarchy.

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

File	R2 Events	R2 % Parent	R2 % Total	R2 % Non-debris	R3 % Parent	R3 % Total	R3 % Non-debris	R3 Ly-6C FITC-A Mean
Sample 1	3591	13.68	1.29	2.13	6.07	0.57	0.95	27060.37
Sample 2	3647	12.70	1.25	2.13	6.87			
Sample 3	4434	14.49	1.52	2.58	7.82			
Sample 4	3839	10.70	1.33	2.13	6.85			
Sample 5	2670	9.01	0.92	1.61	6.79			
Sample 6	2565	6.75	0.88	1.69	8.10			

Copy Statistics
Export Statistics
✓ Display Horizontal
Display Split
Table Setup

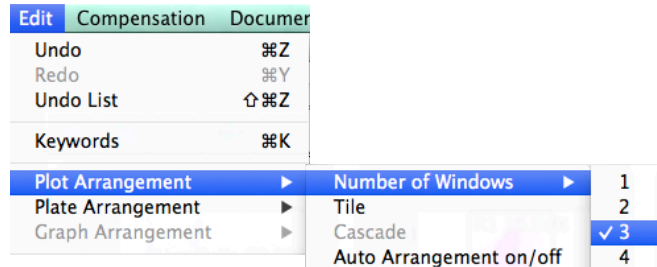
Right click on the statistics table and choose **Copy Statistics** to place them on the clipboard.

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

9. Navigating through your plots

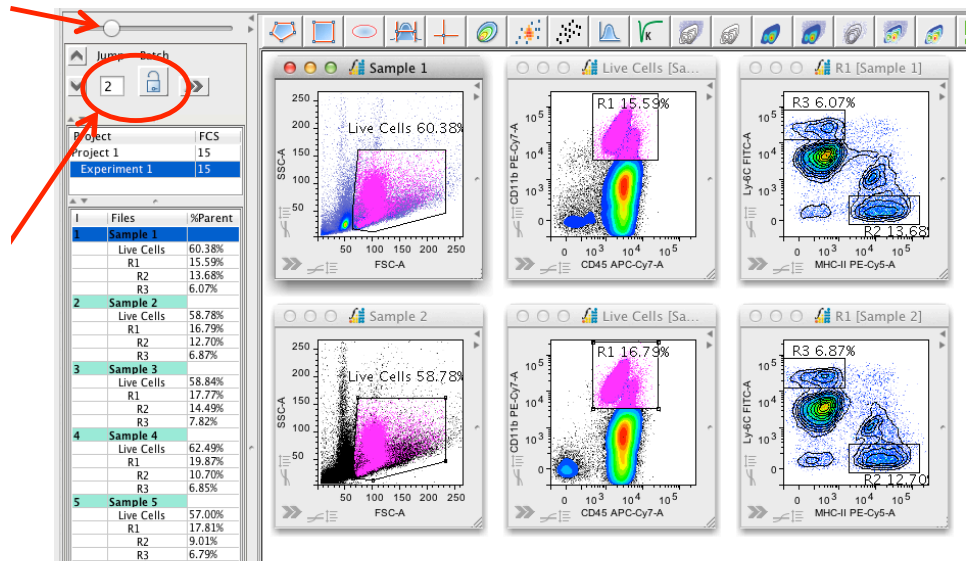
It is easy to effectively view multiple plots from many samples by defining how you would like the workspace to be set up.

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



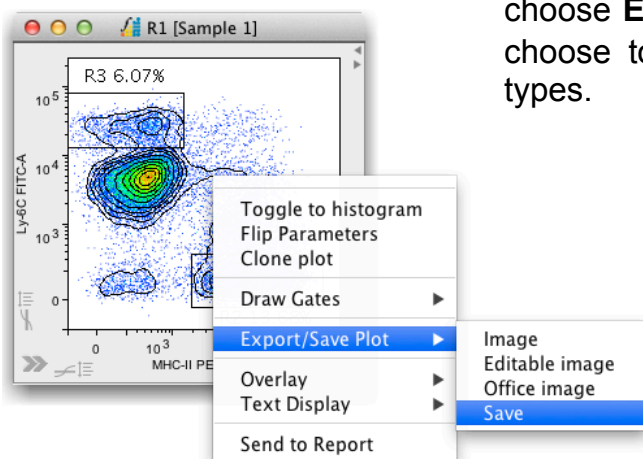
Use the slide bar to adjust the size of the plots.

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.



10. Exporting plots

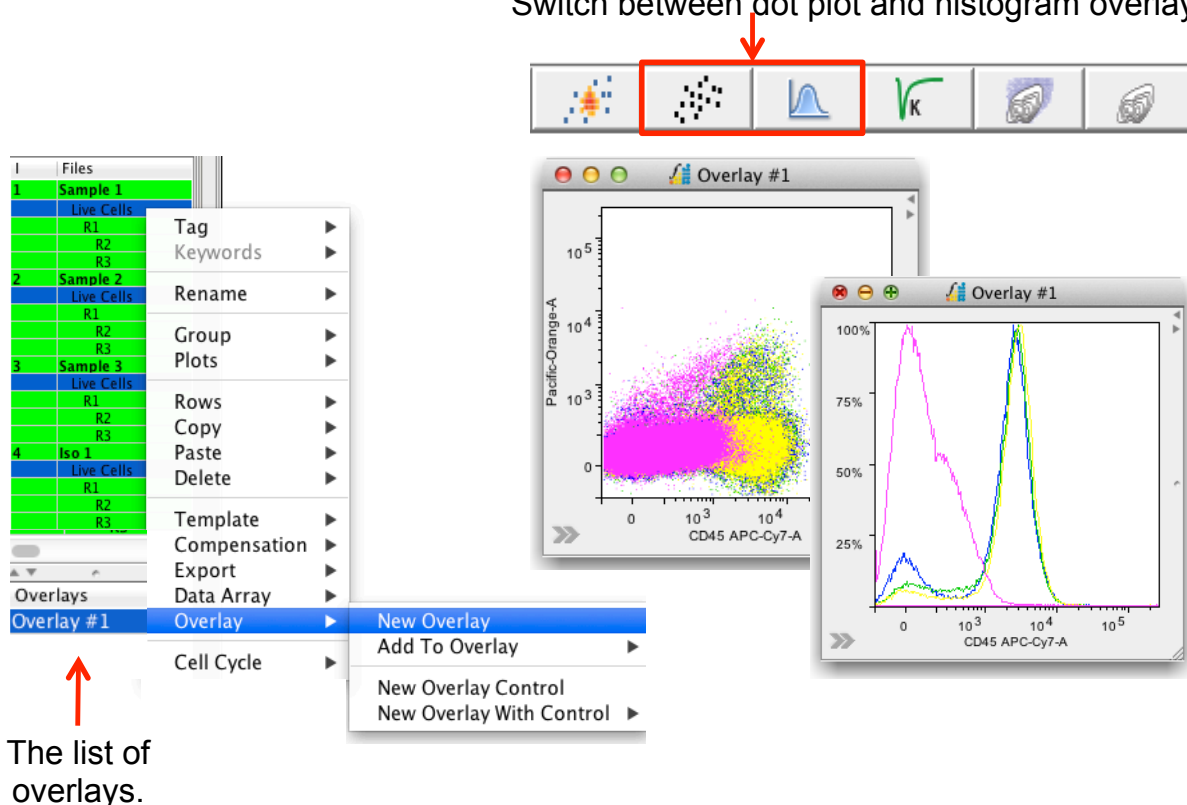
To export a plot, right click on it and choose **Export/Save Plot**. You can then choose to save the plot as 1 of 6 file types.



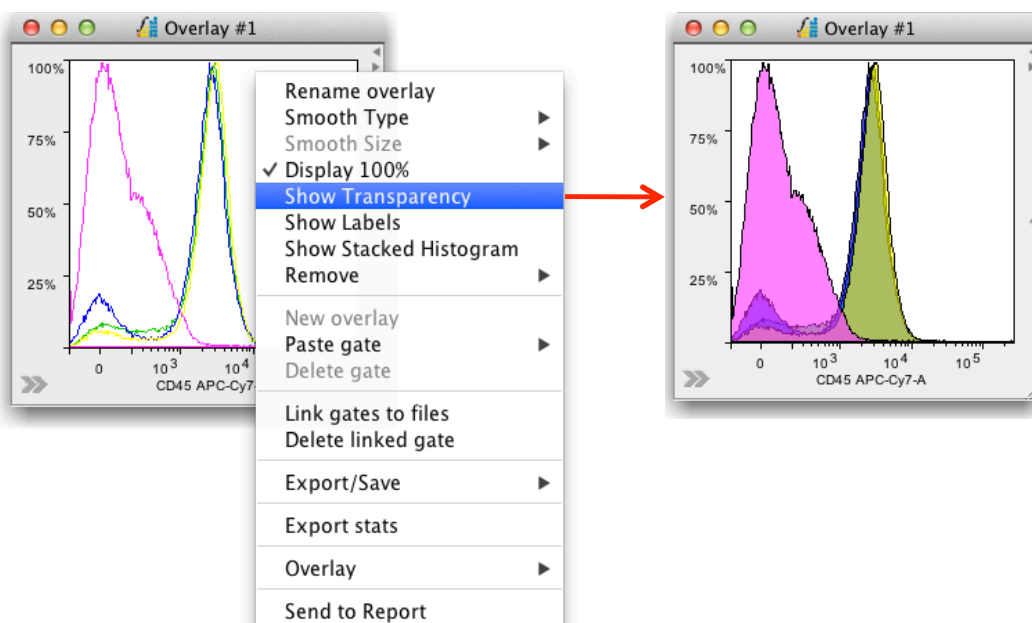
11. Creating overlays

To generate an overlay, select the required populations from the File Inspector, right click and from the menu choose **Overlay** and **New Overlay**. The overlay will then be displayed in the Workspace and listed in the pull up drawer below the File Inspector.

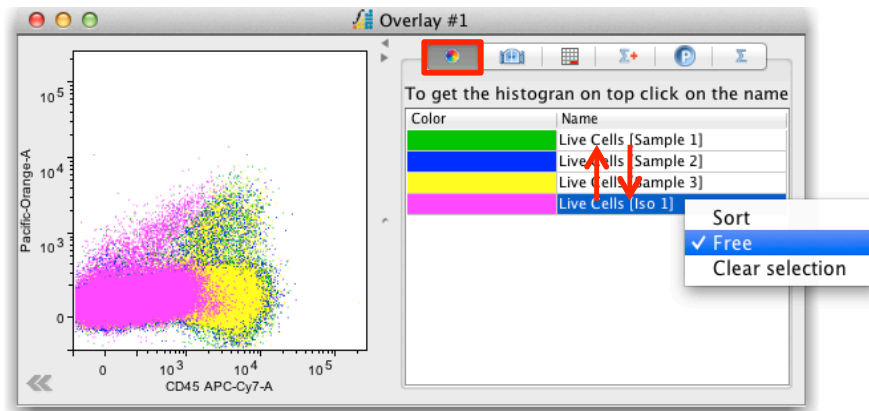
Switch between dot plot and histogram overlays.



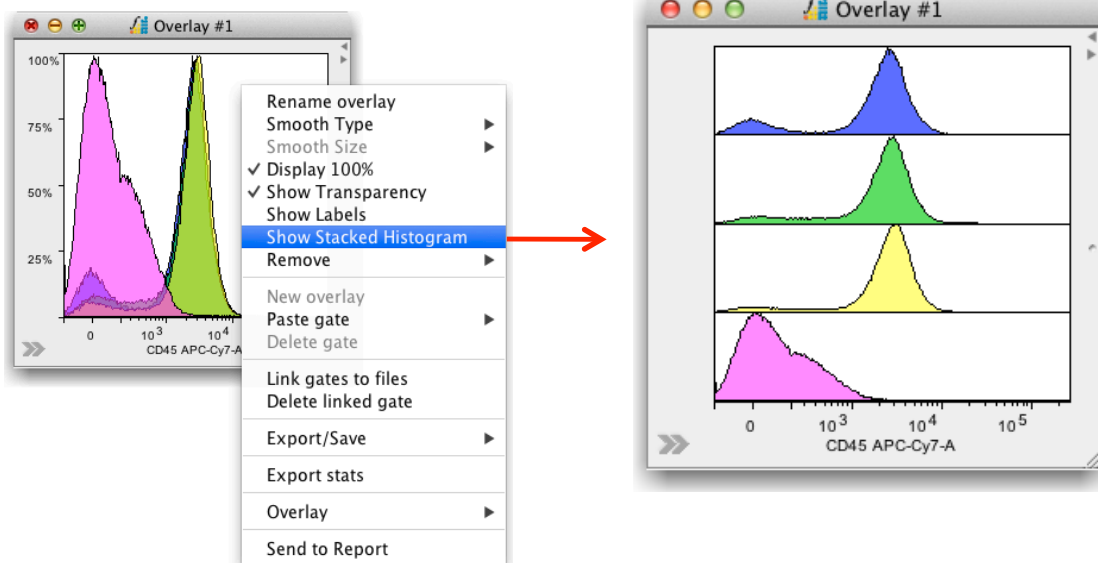
Right click in the overlay window for a range of display and export options, such as turning transparency on and off.



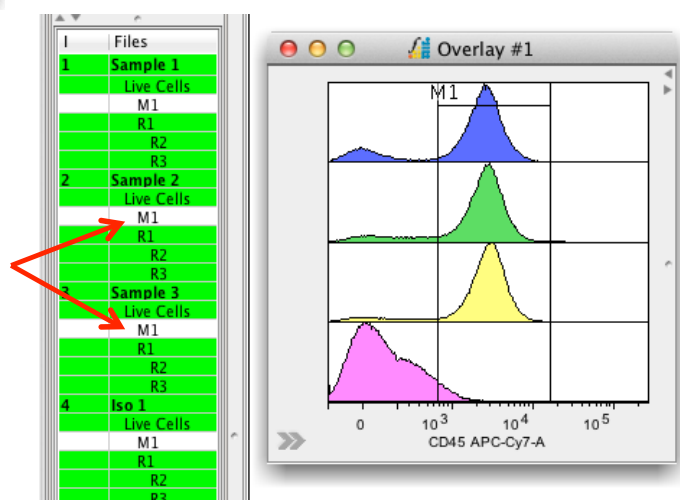
The order that populations are displayed can be easily changed. In the Overlay Colors tab, right click and set the samples to **Free**. Then, click and drag the samples in the list to re-order them.



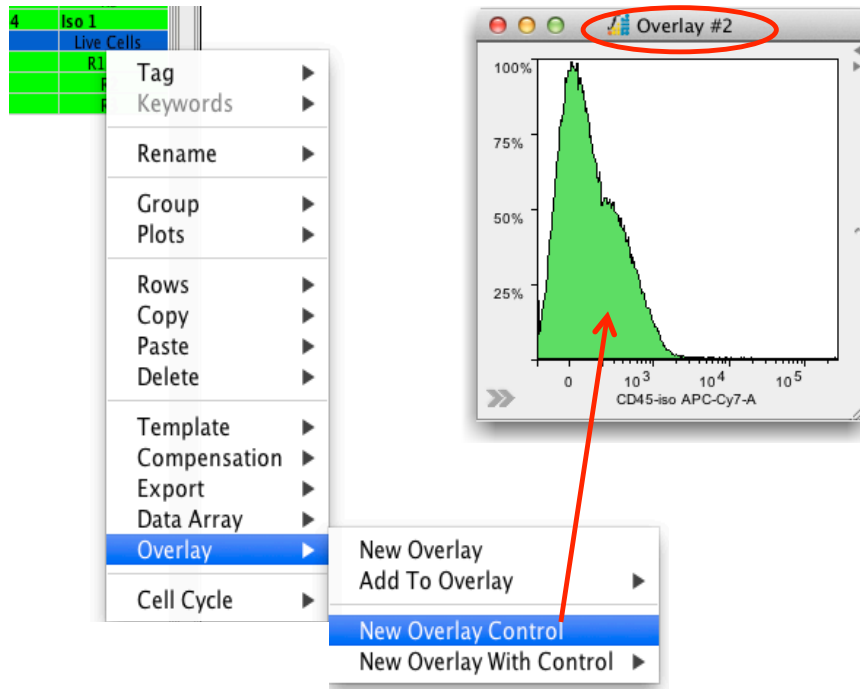
Histogram overlays can also be separated into **Stacked Histograms**.



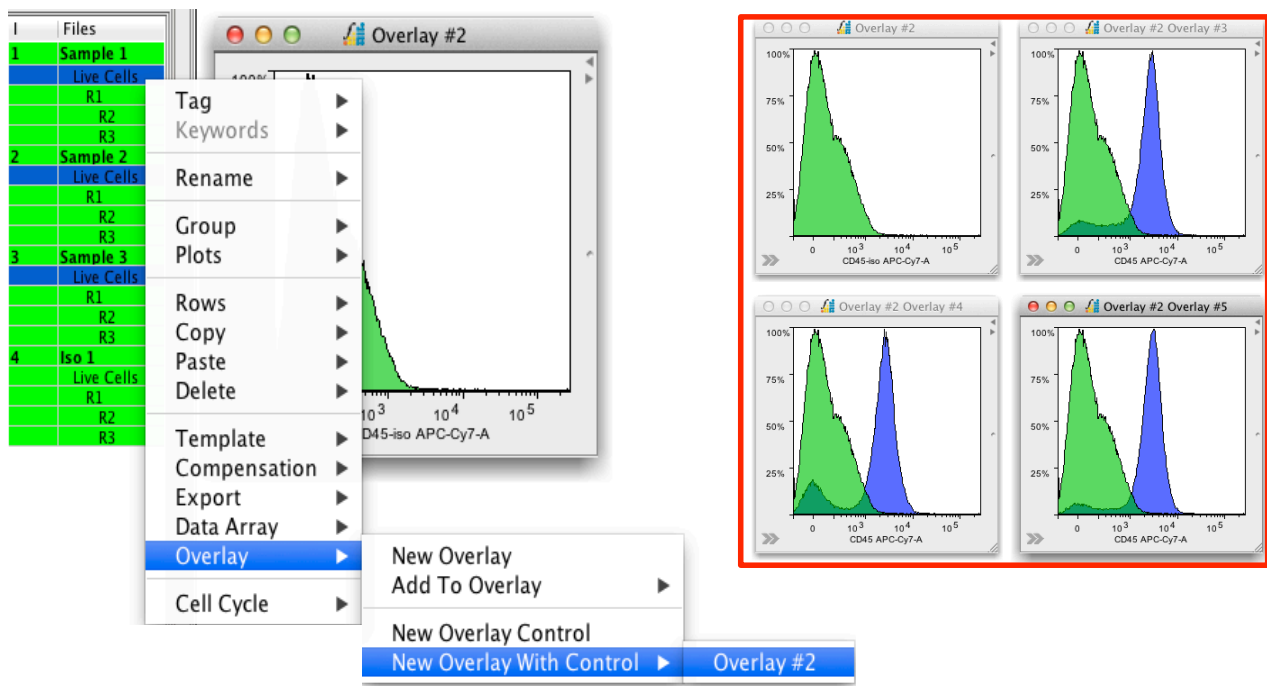
Gates can also be drawn on overlays. The gate is then added to each of the samples and displayed in the File Inspector.



Another option is to create multiple overlays involving different samples all against a single control. To set the control, right click on the population and select **Overlay** followed by **New Overlay Control**. The control sample will be displayed by itself.



Then, highlight the samples that you wish to overlay with the control, select **Overlay**, **New Overlay With Control** and select the control overlay. In this example, three different samples have been overlaid against the one control.



12. Data array

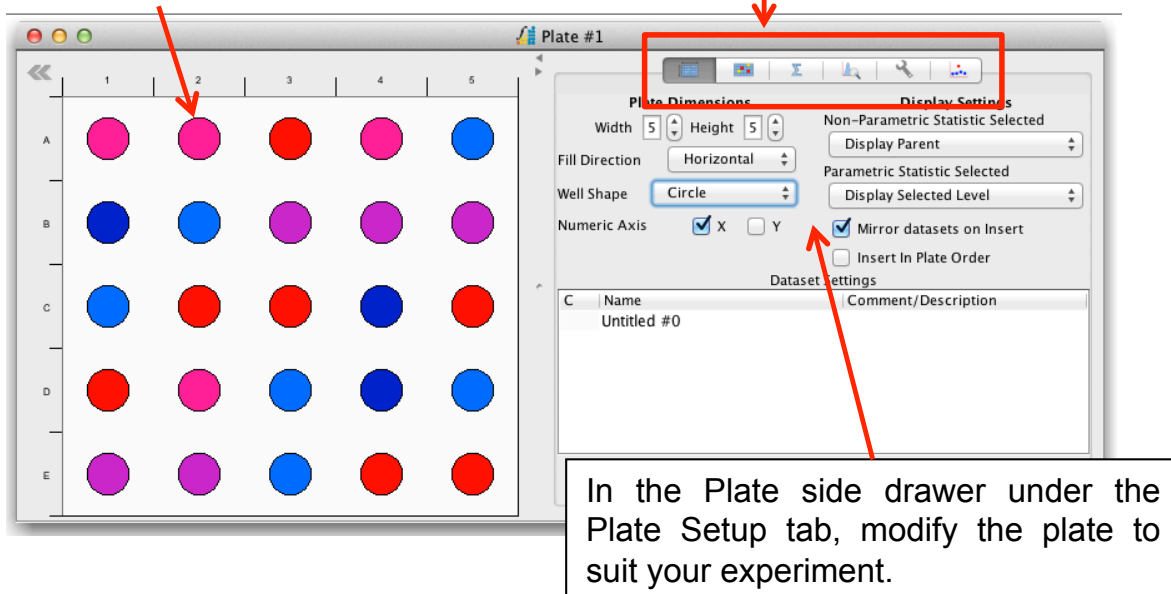
Flowlogic offers a data array analysis function visualized as a plate. This is useful for fast analysis of large data sets. It also provides unique display options for all types of analyses and the easy generation of heat maps.

Select **Data Array** from the Advanced Functions drawer. Right click in the plate window and select **New Data Array**.

Side Drawer Tabs

Drag files directly from the File Inspector into the plate.

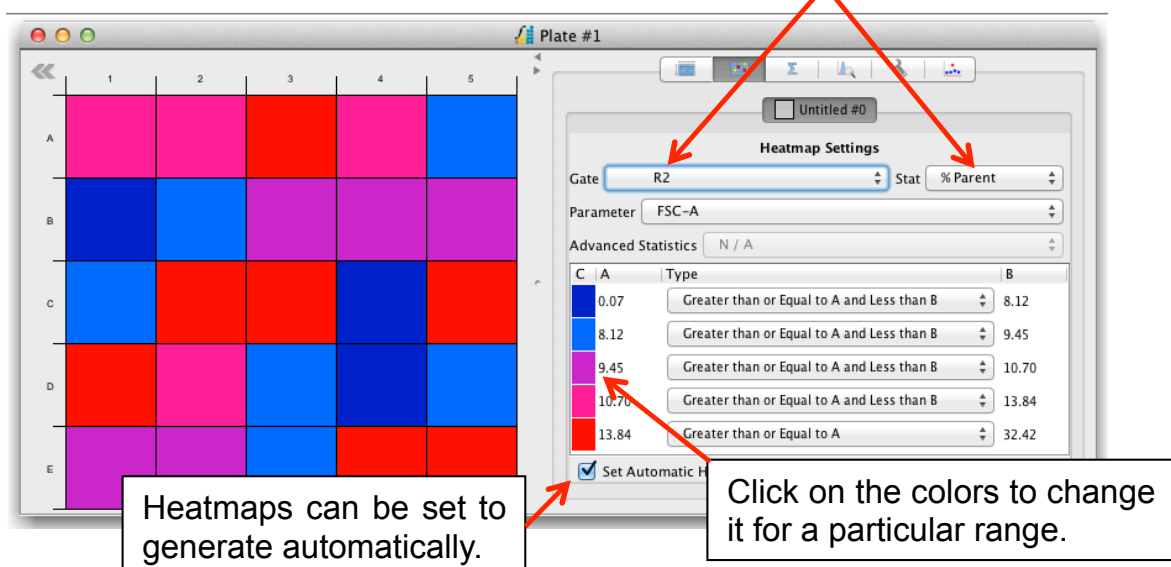
Plate Setup, Heatmap Settings, Statistics, Plot Viewer, Advanced Functions, Plate Outlier Detection.



In the Plate side drawer under the Plate Setup tab, modify the plate to suit your experiment.

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

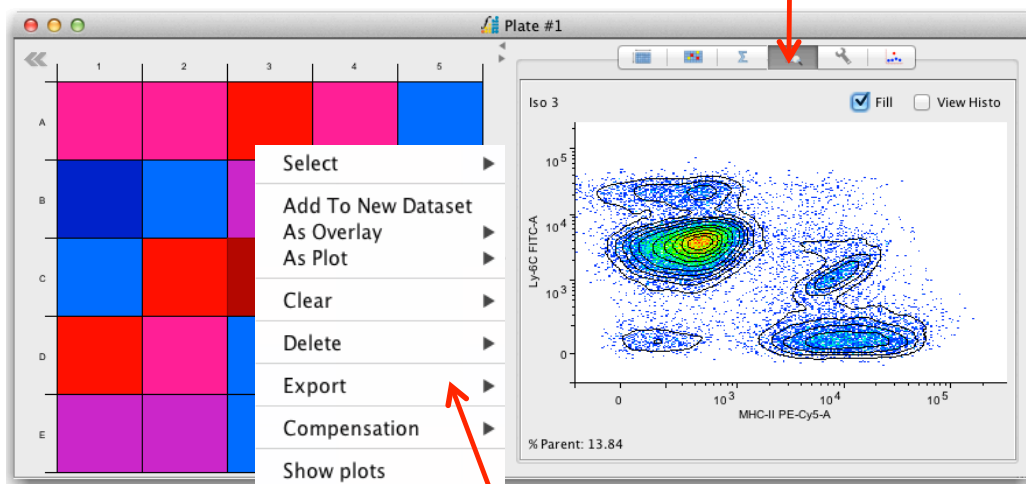
Choose the population and the statistic to be displayed in the wells.



Heatmaps can be set to generate automatically.

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

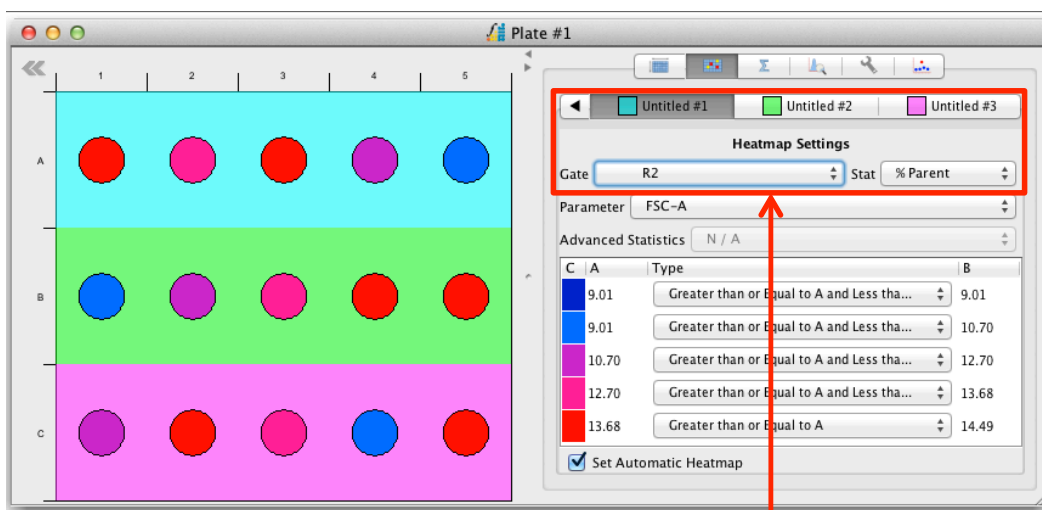
Individual dot plots and histograms are displayed in the Plot Viewer tab when the mouse hovers over the wells.



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

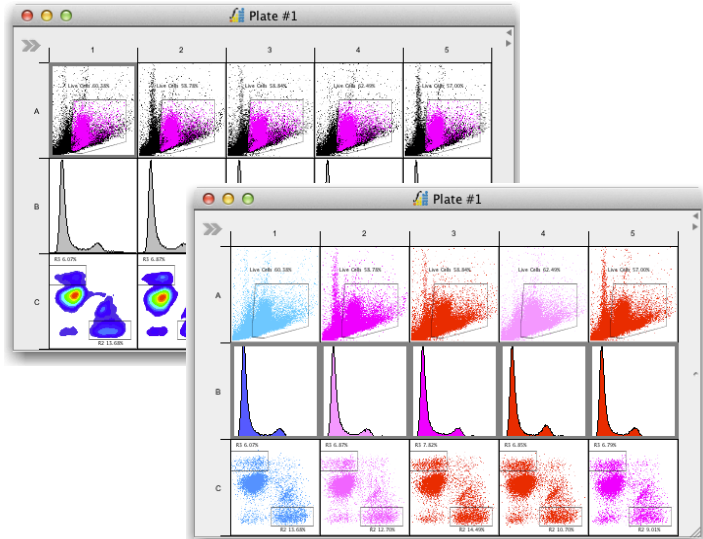
Create datasets to display and analyze different gates from the same samples. To create a new data set, highlight the wells to be included, right click and select **Add to new dataset**.

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.



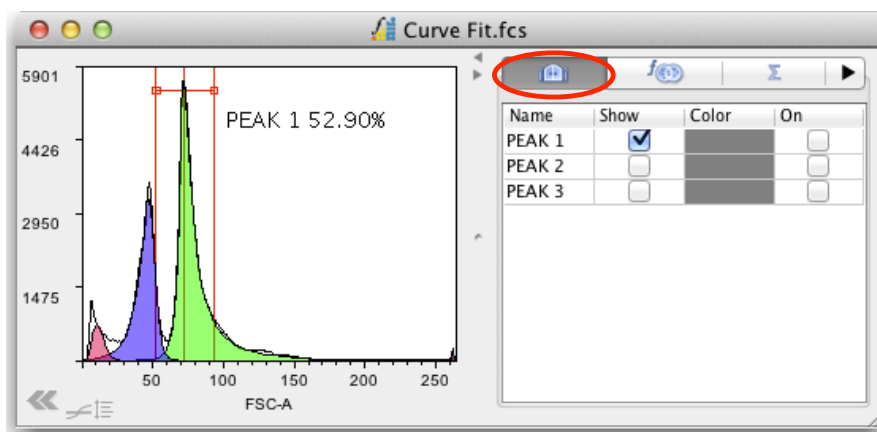
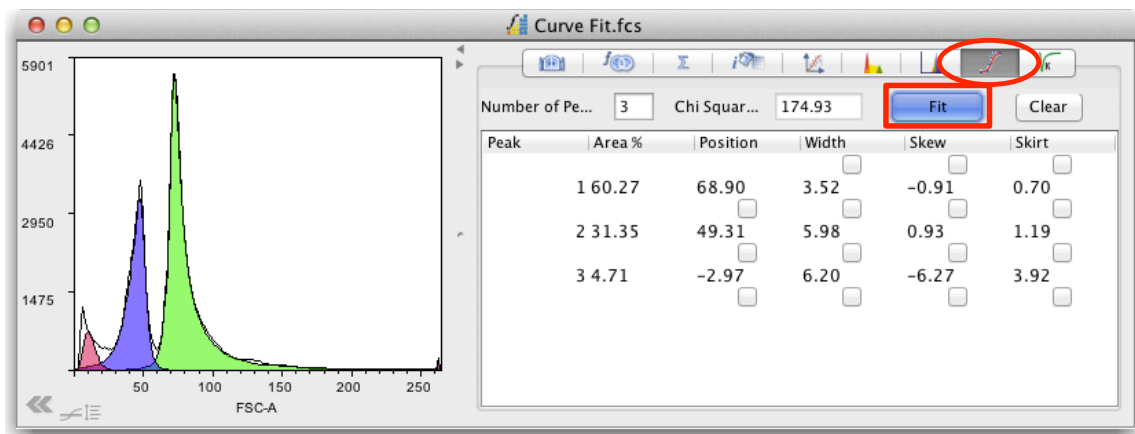
In the Heatmap Settings tab, select the data set from the drop down menu followed by the Gate and the Stat that you wish to view.

Right click on the plate and select **Plots**. Select **Show Plots**. 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 by again right clicking and choosing the options under **Plots**.



13. Curve fitting

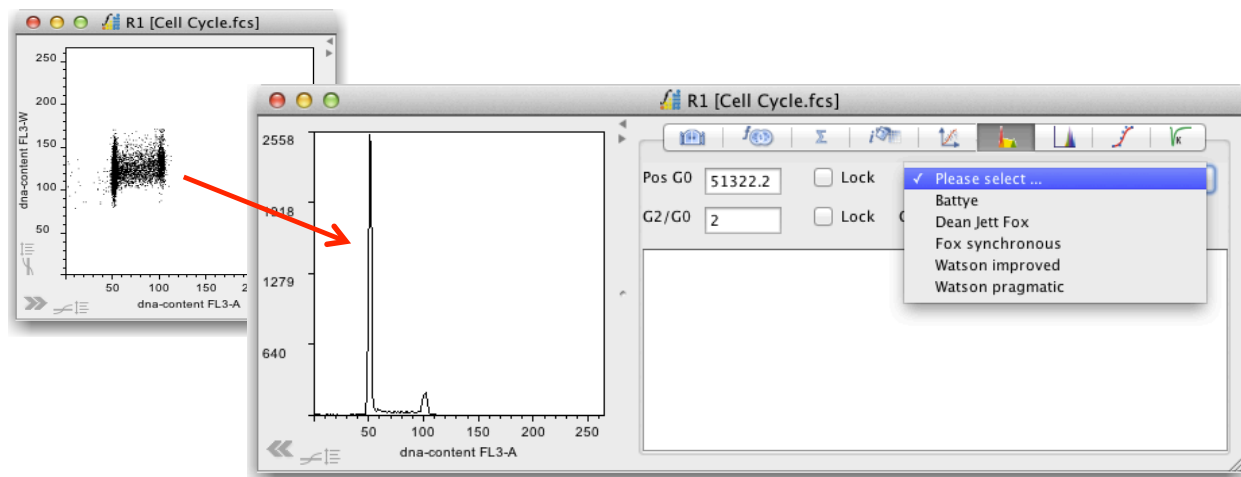
Curve fitting analysis can be performed on suitable histograms displaying a number of peaks. In the plot side drawer, select the **Curve Fit** tab and click **Fit**. The different peaks will be colored and the statistics relating to each peak, such as the area under the curve, will be displayed in the side drawer.



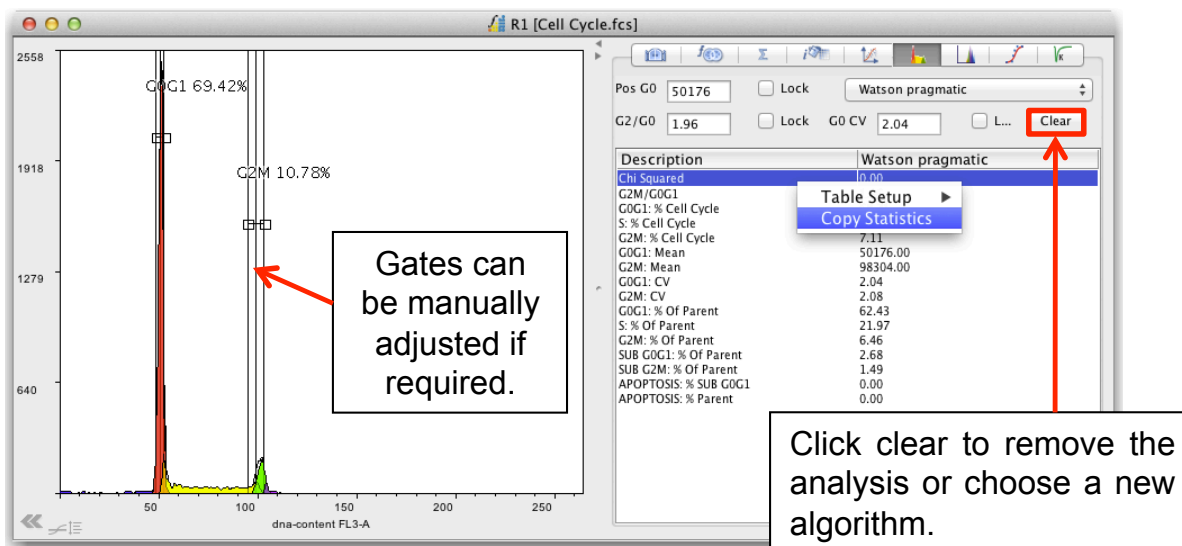
Gates relating to each peak can be turned on and off in the Gate List tab.

14. Cell cycle analysis

To perform cell cycle analysis, perform any pre-gating required on your DNA-content parameters before displaying it as a histogram. Then, in the Cell Cycle tab in the side drawer, choose from one of the 5 algorithms: **Battye**, **Dean Jett Fox**, **Fox synchronous**, **Watson improved** and **Watson pragmatic**.



The histogram is colored to represent the different phases of the cell cycle and the data relating to each phase is displayed in the plot side drawer. This data can be exported by right clicking and selecting **Copy Statistics**.

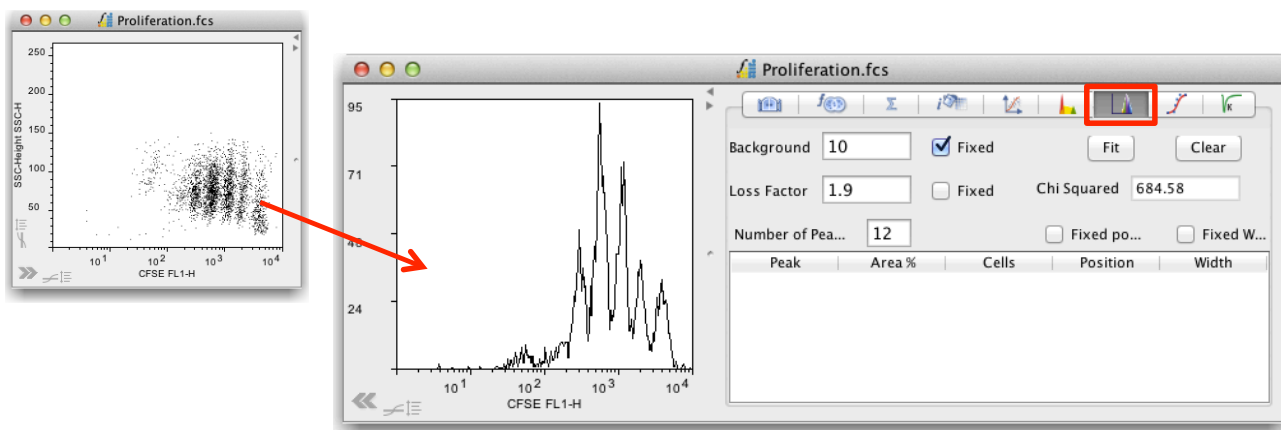


In the Gate List tab, extra gates, such as for apoptosis, can be turned on and colors can be changed.

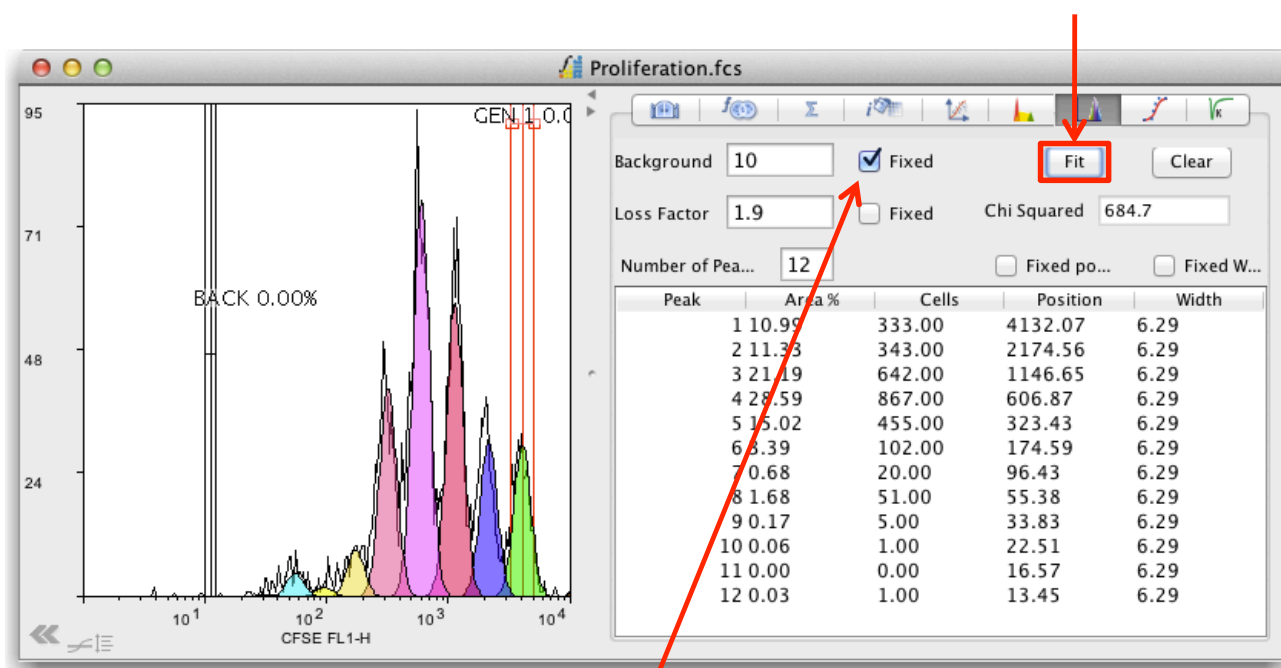
Name	Show	Color	On
Sub G0G1	<input type="checkbox"/>		<input checked="" type="checkbox"/>
G0G1	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>
S	<input type="checkbox"/>		<input checked="" type="checkbox"/>
G2M	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>
Sub G2M	<input type="checkbox"/>		<input checked="" type="checkbox"/>
Apoptosis	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>

15. Proliferation analysis

To assess proliferation, convert the data to a histogram displaying the appropriate parameter (in this case CFSE labeling) and select the Proliferation tab from the plot side drawer.



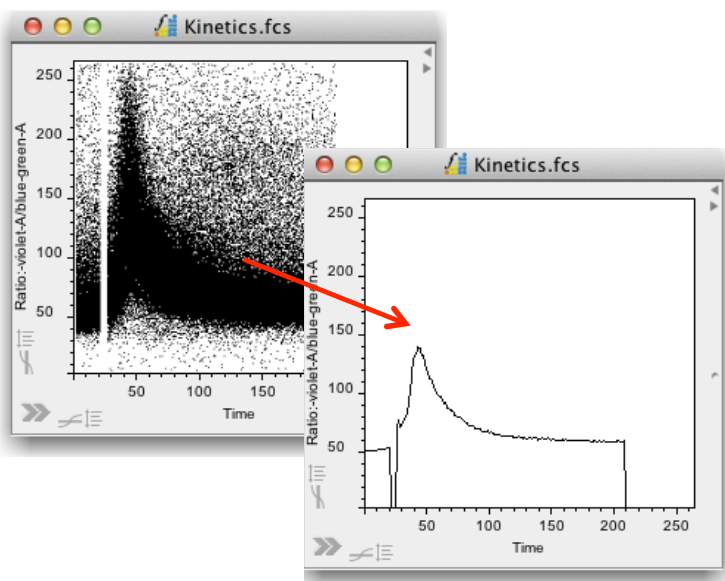
Click **Fit** to perform the curve fitting. The curves created for each peak will be colored on the plot and the related statistics will be displayed in the side drawer.



The background and loss factor can both be fixed and the gate adjusted manually within the constraints. The statistics can be exported by right clicking and choosing to copy or export.

16. Kinetics analysis

To assess cell cycle kinetics, display the data as a ratiometric parameter versus time. Then toggle to a kinetics plot using the button in the tool bar.



Parameters

Virtual Parameters

Input Parameter: Please Select

Operation: Please Select

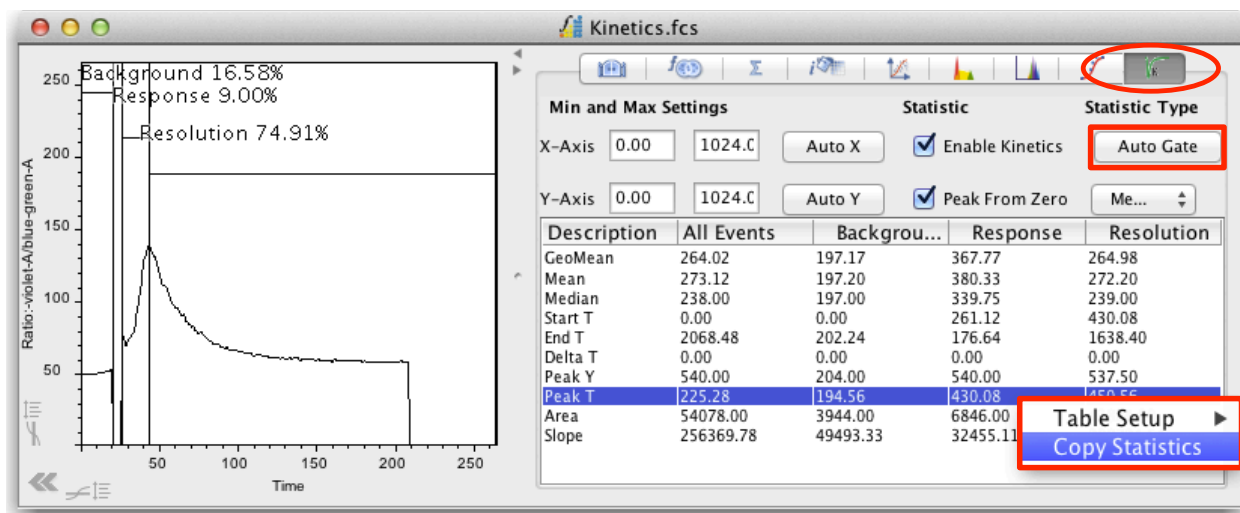
Parameter Name: Untitled Virtual Parameter

Parameter: Create New

Min: 0.0 Max: 0.0 Linear/Log: Linear

Add Delete

Ratiometric parameters can be created in the Parameters tab in the Advanced Functions drawer if they were not created at the time of acquisition.



Select the Kinetics tab in the plot side drawer and click **Auto Gate**. This will create Background, Response and Resolution gates. The data will be displayed in the side drawer and can be exported by right clicking and selecting **Copy Statistics**.



Statistics

Flow**logic** 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, 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 samples from the File Inspector and drag them into the cell in column 1.

Double click
text to rename

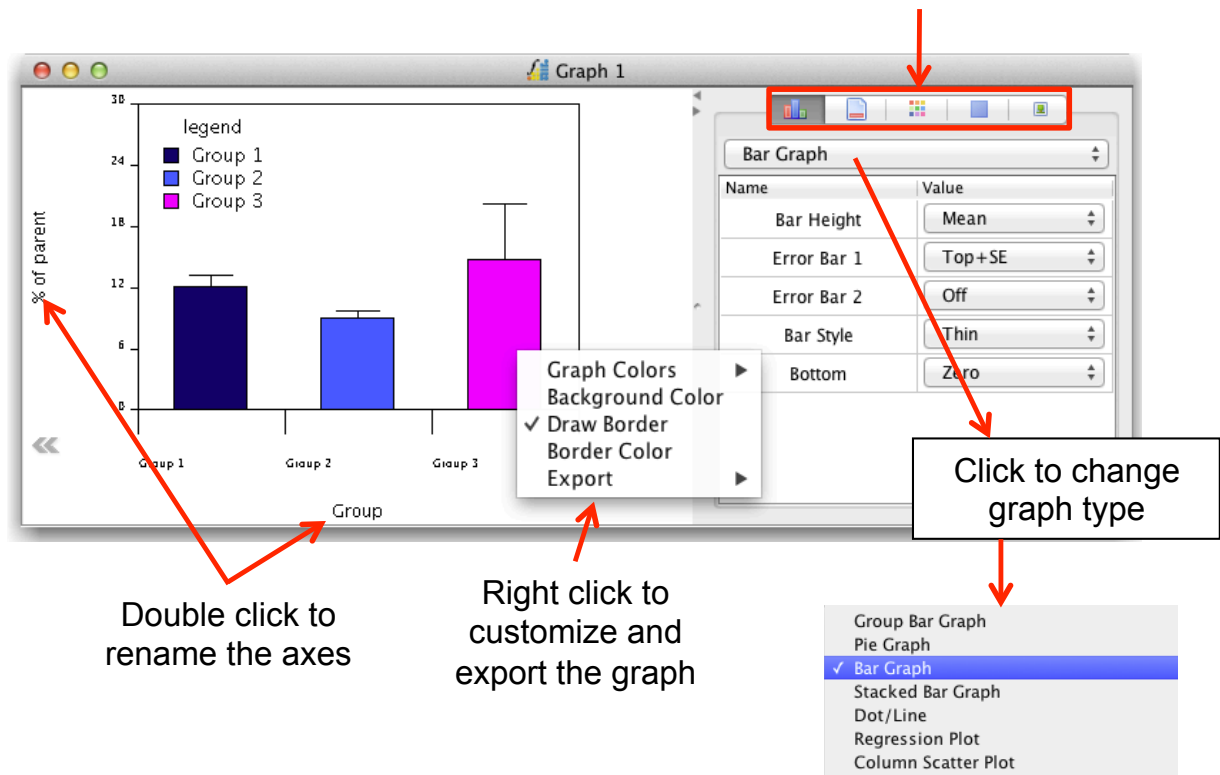
The screenshot shows the Flowlogic software interface. On the left is the 'Files' list with samples 1-10 and iso 1-5. In the center is the 'CS Data' window with a 'Graph Data' tab showing 'Experiment 1', 'Worksheet 1', 'Analysis 1', and 'Graph 1'. On the right is a data table with columns for Gate, Statistics, Data, and six numbered columns. The 'Data' column contains 'Group 1', 'Group 2', and 'Group 3'. A red circle highlights 'Iso 1' in the Files list. A red arrow points from 'Worksheet 1' in the CS Data window to the 'Data' column. Another red arrow points from 'Iso 1' to the 'Data' column. A third red arrow points from 'Group 3' in the Data column to the 'Data' column.

Gate	Statistics	Data	1	2	3	4	5	6
			1	2	3	4	5	6
R2		Group 1	13.68	12.70	14.49	10.70	9.01	
R2		Group 2	6.75	8.52	9.45	9.85	10.65	
R2		Group 3	8.12	32.42	13.84	0.07	19.21	

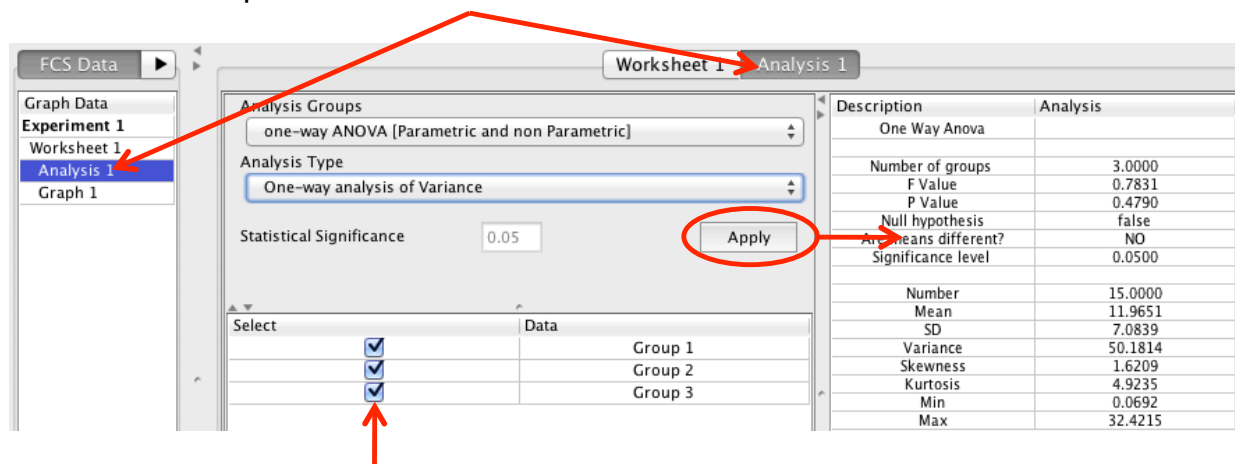
Drag selected files to
the next empty row

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.



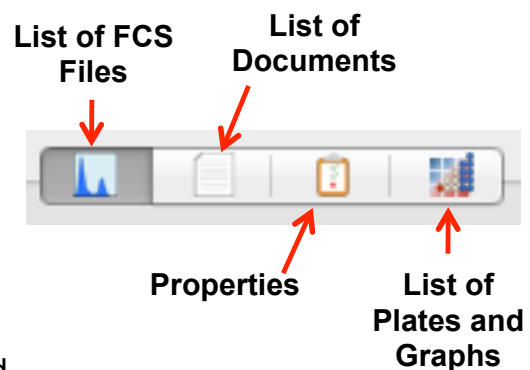


Report

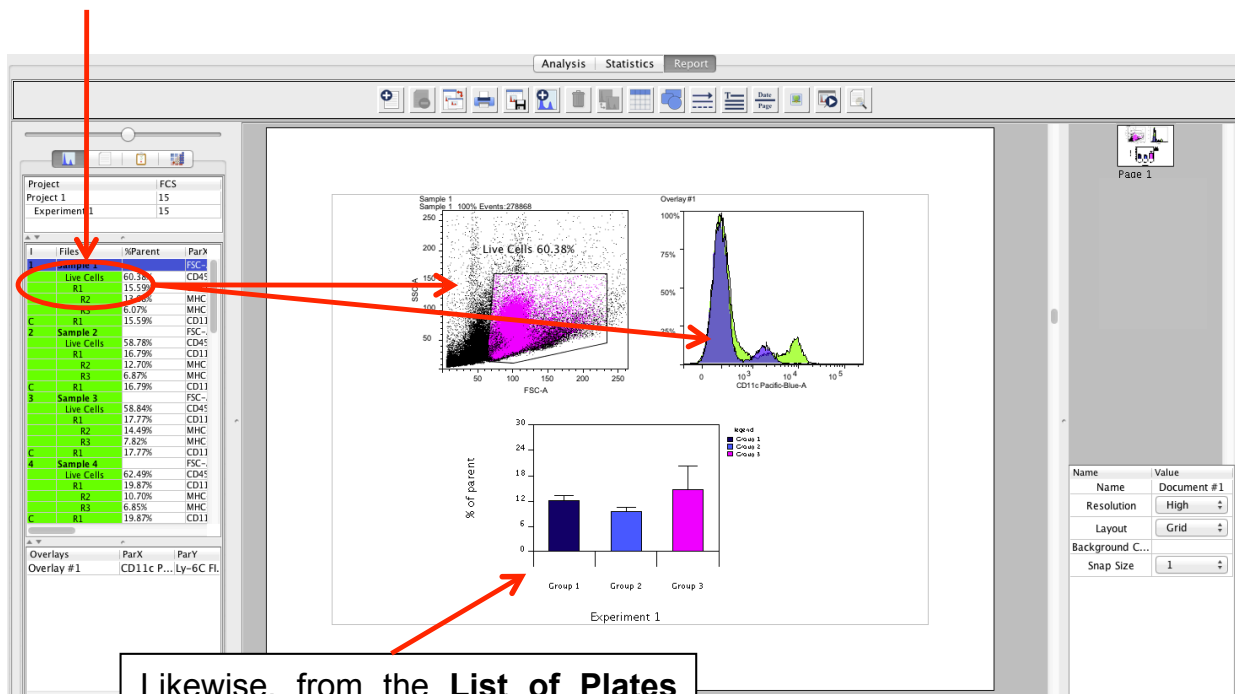
The **Report** function in Flowlogic allows you to display and annotate plots, graphs, plates, heatmaps and tables. These can then be printed, displayed as a slideshow or saved as a PDF.

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.

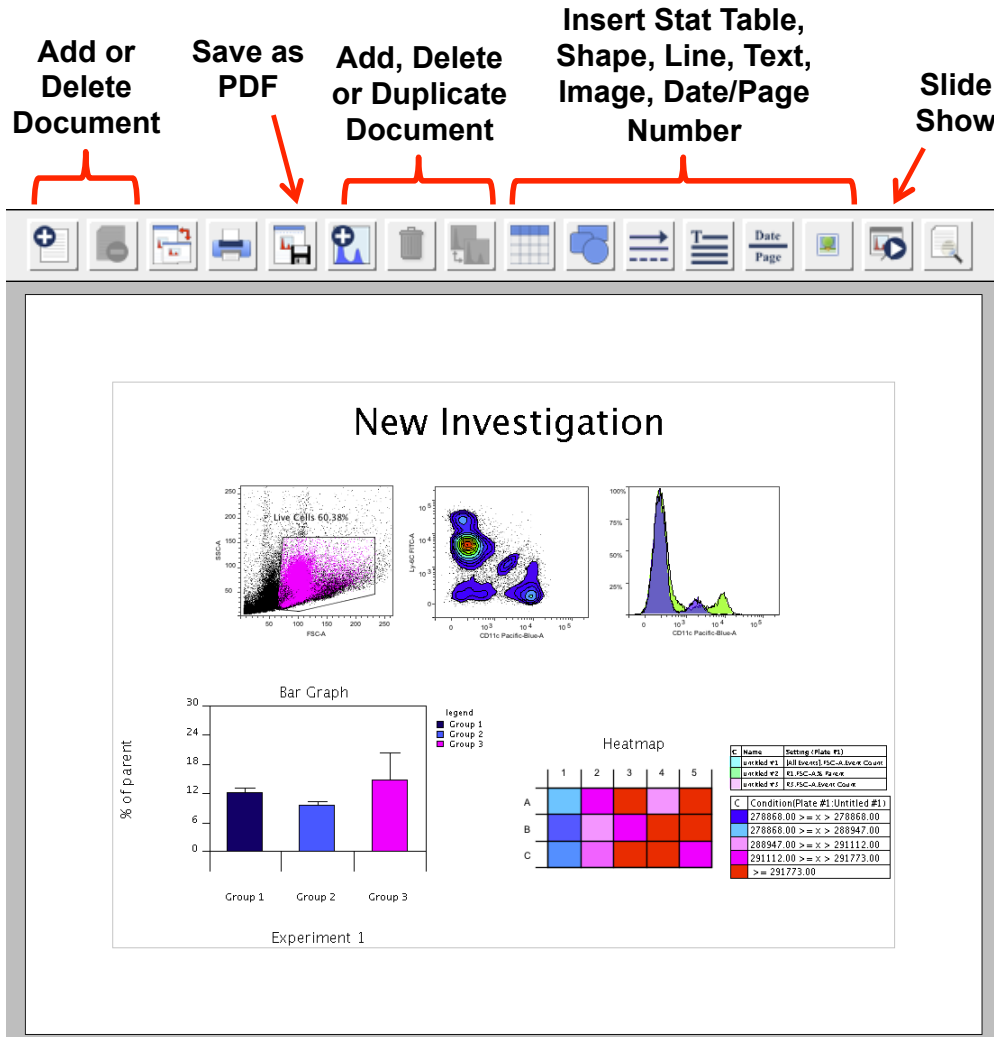


To add dot plots to your report, highlight and drag one or more FCS files from **the List of FCS Files**. The plots will appear as they were last viewed when analyzing.



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**.

