FlowJo Basic Tutorial

FlowJo software is used for the analysis of flow cytometry data. This tutorial is designed to introduce you to FlowJo by demonstrating the analysis of a simple experiment. A FITC anti-CD8 reagent was titrated to determine the appropriate reagent concentration.

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FlowJo Basic Tutorial by Jennifer Wilshire.

FlowJo was written by Adam Treister and Mario Roederer, based on concepts developed with David Parks, Martin Bigos, and Wayne Moore.

We are indebted to the Herzenberg laboratory at Stanford and our active and enthusiastic users for their ideas, discussions, and tireless testing of new versions.

Basic Tutorial

Introduction

FlowJo software is used for the analysis of flow cytometry data. You can use FlowJo to analyze all of your flow cytometry data - regardless of the cytometer used to collect your data files. This tutorial is designed to introduce you to FlowJo and to the 6 steps involved in analyzing a basic immunophenotyping experiment.

1. Load samples into the workspace.
2. Analyze the control sample in detail (gate subsets and add statistics).
3. Copy gates and statistics to all samples.
4. Verify gates on all samples
5. Generate a graphical report including all samples.
6. Generate a table of statistics from all samples.

Learning to use FlowJo to efficiently analyze your flow experiments is not difficult, although it is worth noting that there are several differences between FlowJo and other flow cytometry analysis packages. The power of FlowJo and the ease with which it handles data analysis are a direct result of these differences.

FlowJo organizes and displays all the data files from an experiment in a Workspace window. The Workspace window not only lists all the sample files being analyzed, but also displays each gated subpopulation and statistic as a node under the sample name. Nodes are organized in a hierarchical manner so that it is easy to distinguish the relationship between each population. Being able to view the summary of an entire experiment in a Workspace window means that there is no need to keep track of gates or statistics and your data analysis is organized in a biologically meaningful way.

The repetitive tasks required to analyze flow data are greatly simplified by FlowJo. Batch processing is used to apply analyses (gated subpopulations and statistics) to many samples effortlessly. FlowJo also produces tables of statistics and graphical reports that summarize multiple data samples with a click of a button.

Instructions to guide you through the analysis of a basic immunophenotyping experiment are contained in this tutorial. Follow along with the provided data files to get a feel of how FlowJo works! If you wish to skip steps, we provide premade Workspace files that correspond to each analysis step. The demonstration data and premade Workspace files can be found in the "Basic Tutorial" folder on the FlowJo CD or at www.flowjo.com/tutorial.html.

Experimental Method
The set of data files used to illustrate this tutorial consists of an experiment titrating a newly made antibody reagent. This reagent was produced by conjugating purified anti-CD8 antibody to the fluorochrome Flourescein (FITC). A titration experiment was carried out to determine the appropriate reagent concentration for sample staining.

Human peripheral blood mononuclear cell preparation from a single healthy donor was divided into eight tubes. The first tube is the unstained control. The subsequent seven tubes were stained with a serial two-fold dilution of the anti-CD8-FITC reagent starting at 2 µg/ml. Cells were stained for 20 minutes, washed and resuspended in staining media. The data files were aquired with 10,000 events collected for each sample. This data can be found on the FlowJo CD in the Basic Tutorial folder or you can download it from www.flowjo.com/tutorial.html.
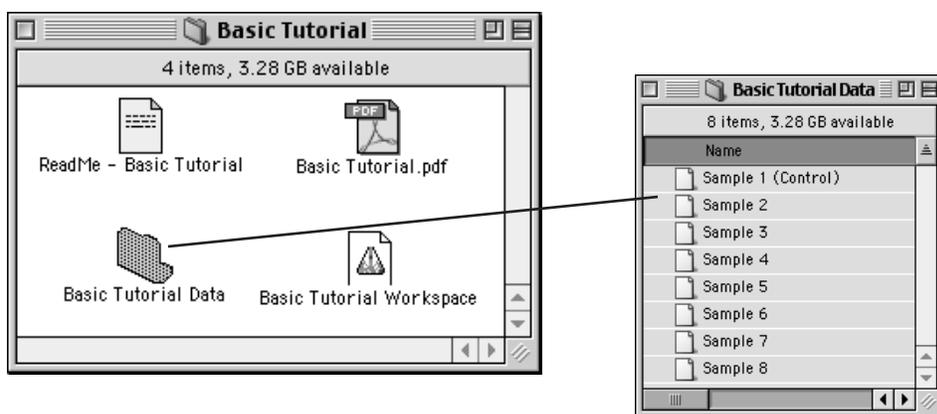
Experimental Goal
The goal of this titration experiment is to determine the concentration of the anti-CD8-FITC reagent that achieves saturating staining; i.e., maximal staining of the CD8 T cells under defined conditions (20 minutes at room temperature). The data analysis in this tutorial consists of identifying the subpopulation of cells that are stained with anti-CD8 antibody and calculating a median fluorescence intensity statistic on this subpopulation. The computed statistics will be used to determine the concentration of anti-CD8-FITC reagent to use in subsequent experiments.

> **Note:** Even if you do not produce your own antibody reagents, the recommended concentration of purchased antibody reagents is frequently in excess of the saturating titre. Therefore, a simple titration experiment can conserve your reagents and save money!
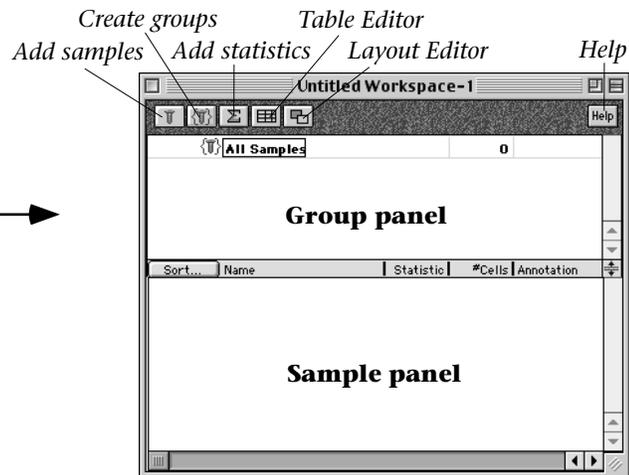
1. Load samples into the workspace.

Workspace Structure

The Workspace window is the main launching point for all analyses in FlowJo. It is composed of three main parts: a set of function buttons at the top, a "group" panel, and a "sample" panel. The sample panel contains a list of all of the sample files (tubes) which belong to the currently-selected group. The group panel contains the names of the groups of sample files that you have created. The "All Samples" group is created automatically and contains all the samples loaded into the Workspace. In this tutorial we will focus on a data set that does not need to be divided further into groups for analysis (see the Advanced Tutorial for a full appreciation of the power of groups and their usefulness in organizing complex data sets - www.flowjo.com/tutorial.html).

Open *FlowJo by double clicking the application icon.*

Create a **new workspace.**

Create groups — *Table Editor*
Add samples — *Add statistics* — *Layout Editor* — *Help*

Group panel

Sample panel

The left three function buttons at the top of the Workspace allow you to add components (samples, groups, statistics) to the Workspace. The right two function buttons open separate Table Editor and Layout Editor windows. These editors are saved with the workspace and are used for making tables of statistics and graphical reports of your data. When you move the mouse over any button in FlowJo, a short description of the button's function will show up in the window.

> The most important button in FlowJo is the **Help button**, which is found in all windows and dialog boxes. This button launches a network browser and takes you directly to the FlowJo online reference manual page relevant to the window you are working on. To view the entire reference manual, point your web browser to: www.flowjo.com/v4/reference.html.

Create a New Workspace

Launch FlowJo by clicking on the application Icon. Select New Workspace from the File menu to create a new Workspace.

Add Samples

There are two ways to add experimental data files to the FlowJo Workspace. Either click the Add Samples function button [🗁] or select the samples in the "Basic Tutorial Data" folder and drag them to the Workspace window. To select all the samples at once, hold the Shift key while clicking the sample names. Click on the selected files and hold the mouse button down while dragging over to the Workspace window. Release the mouse to drop the files in the Workspace window.

Drag *files or folders directly onto the workspace window.*
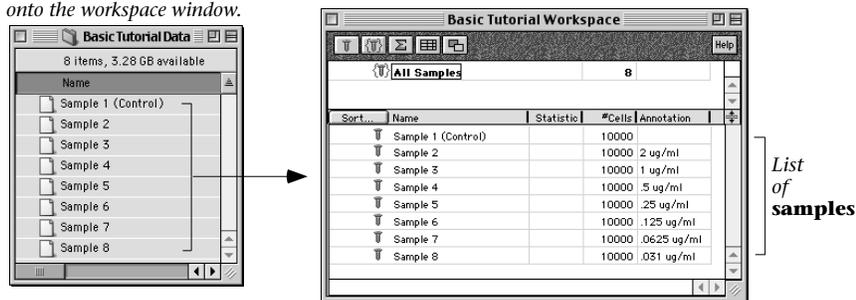
*List of* **samples**

Graph Options

Display a plot of Sample 1 (the unstained control) by double clicking on the name of the sample in the workspace window. As shown below, FlowJo opens a Graph window displaying a bivariate dot plot of the cells in Sample 1. The initial graph that FlowJo displays is always a Forward vs. Side Scatter plot because this view is commonly used to remove dead cells and to identify the leukocytes subpopulations of interest. The parameters that are displayed in the graph are easily changed by clicking on the X or Y axis labels and selecting another parameter from the pulldown menu. Try this yourself!

Open Graph tools box.

Sample name

Double click sample name to open graph window display.
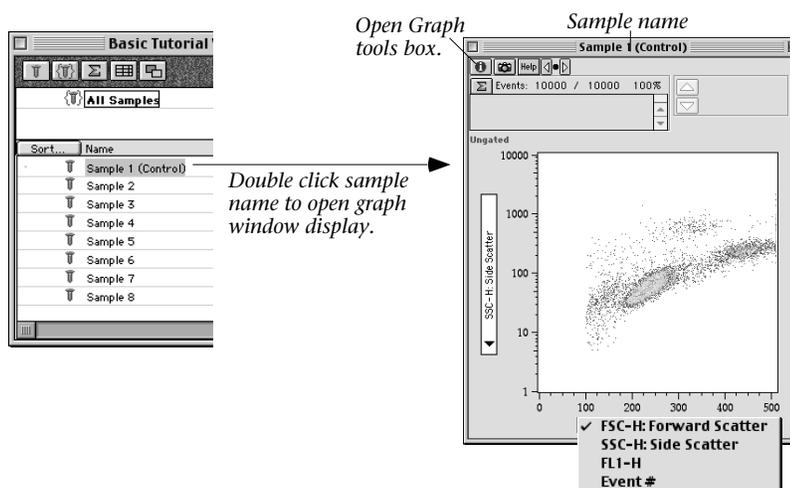
Several attributes of a Graph can be altered using the **Graph Tools** window. To open this window, click on the blue "i" button in the top left corner of the Graph window. From this window you can choose a gating tool or change the parameters displayed. In addition, you can choose the plot type used to display the cells in your sample.
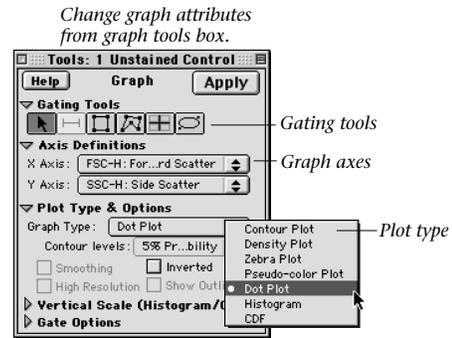
FlowJo supports several different ways to graphically display flow data. The full range of choices is shown in the Plot Type and Options pulldown in the Graph Tools window. Each plot type has additional display features for further customization of the graphical display. Try turning on Smoothing and Inverted. Explore your choices!

Contour Plot (with outliers) • Density Plot • Zebra Plot • Pseudocolor Plot • Dot Plot
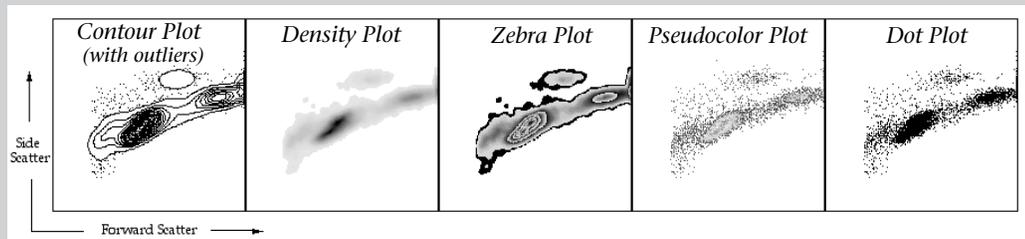
Dot Plots display rare events well; however, these plots frequently saturate in areas of high cell density. Visual information about the cell population in the saturated area of the plot is lost. (Dot plots are drawn showing only 8000 cells by default by FlowJo in order to offset this problem).

Contour lines drawn on **Contour Plots** are similar to altitude contours on geographical maps. Five percent of cells fall between each contour line in a 5% contour plot. One drawback of contour plots is that 5% of cells fall outside of the last contour line and information about rare populations is not visible. In order to not lose information about these populations, click on the "Show Outliers" box and click Apply. **Outlier Plots** combine the density estimation of contours with the rare event information of a Dot plot.

Density Plots provide density information (i.e., the number of events within an area) by using different shades of gray.

Zebra Plots are a mix of contour (20%) and density plots.
Pseudocolor Dot Plots provide benefits of a dot plot without the loss of information due to saturation. Saturating is avoided by drawing dots in different colors according to the cell density in that area. Turn on the smoothing option for the pseudocolor plot to display a **Color Density Plot.**
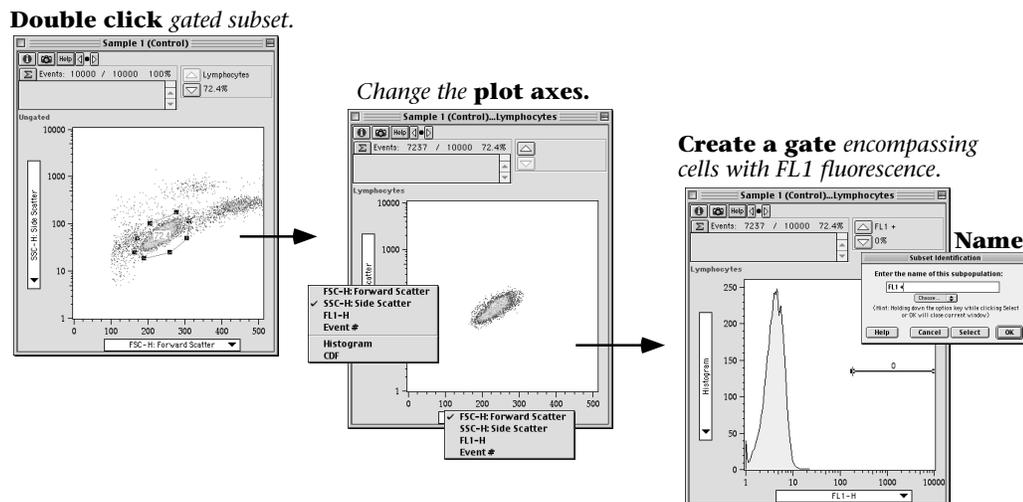
2. Analyze the control sample in detail.

Gating
Creating gates is very easy in FlowJo. Simply click anywhere in the Graph window to begin a polygon gate. Move the mouse and click again to add a side to the polygon gate. Repeat until you close the gate by clicking back on the starting point. (A polygon gate can also be closed by double clicking or typing the space bar.) Once you have a closed polygon, you will be asked to name this subpopulation of cells.

Draw a Gate *on the lymphocyte subset.*

Name *the subset.*
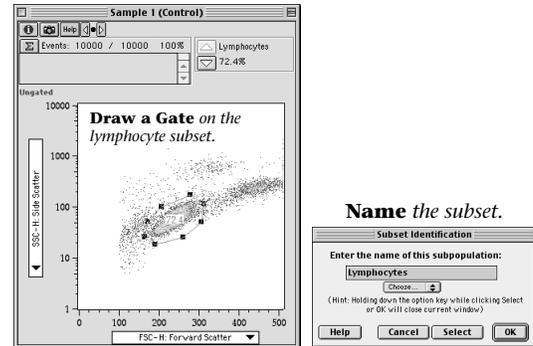
On a Forward Scatter vs. Side Scatter plot, draw a polygon gate encompassing the lymphocyte subpopulation as shown above. Name this subpopulation *Lymphocytes* and click OK. You can easily adjust a side of a gate, by clicking and dragging any of the "handles" of the polygon. Handles (small black boxes found at the intersection of each polygon side) are only displayed on selected gates. To move the entire gate, pass the mouse over the gate until a "hand" cursor is displayed. Click and drag the gate to another location. When the cursor becomes a percentage sign you can move the frequency number.

Double click on the *Lymphocytes* gate in the Graph window to open a new graph displaying only the subset of cells that falls within the Lymphocyte gate (middle graph below). Click on the X-axis label and choose FL-1 from the pulldown menu. Click on the Y-axis label and select Histogram from the menu. Click and drag to draw a gate on the Fl-1 histogram (below right) and name this subset *"FL1+"*.

Double click *gated subset.*

Change the **plot axes.**

Create a gate *encompassing cells with FL1 fluorescence.*

Name

Hint: The polygon is the default gating tool, however, there are several other tools that can be chosen from the Graph Tools window (opened by clicking the "i" button in the Graph window). A Rectangle, Quadrant or Oval gating tool can be selected from this window.

Hierarchical Description of Sample Subsets

The gating tree we created in order to divide Sample 1 into subpopulations is shown underneath the sample name. The names of the subpopulation gates are displayed as nodes in the Workspace in a hierarchical manner; each subpopulation node is shown indented and underneath the sample or subset that is its direct ancestor.

When you generated the *Lymphocyte* gate, you may have noticed a change in the Workspace window. FlowJo placed the name *Lymphocytes* in a new row underneath Sample 1. This new row represents the subpopulation of cells that fall inside the *Lymphocyte* gate. The *Lymphocytes* node is displayed in the Workspace window indented and underneath the Sample name because the Lymphocyte population is a subset of Sample 1. When you generated the *FL1+* gate, this gate was drawn only on the cells that fall within the *Lymphocyte* subpopulation. Thus, the *FL1+* gate name is written indented and underneath *Lymphocytes* as shown in the Workspace to the right.

		Basic Tutorial Workspace			Help
All Samples				8	

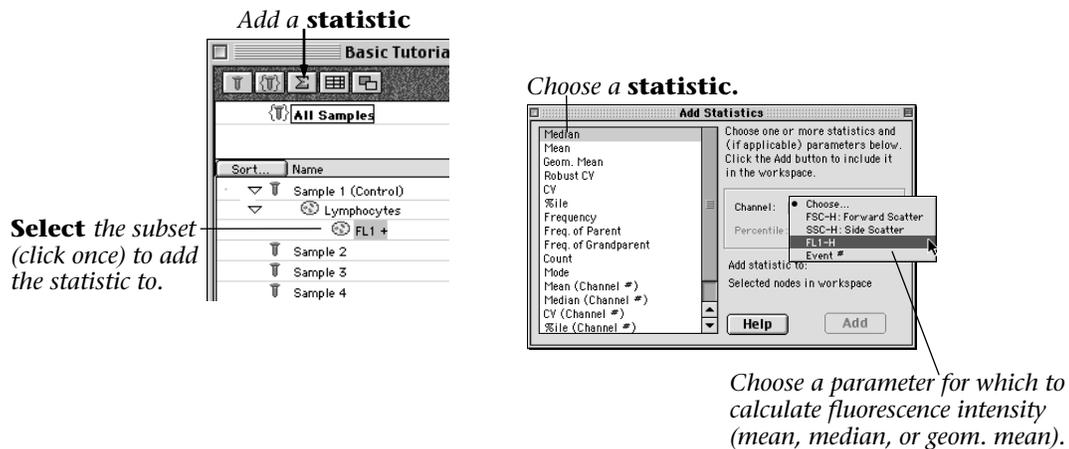| Sort...	Name	Statistic	#Cells	Annotation
▽	Sample 1 (Control)		10000	
▽	Lymphocytes	72.4	7237	
	FL1 +	0	0	
	Sample 2		10000	2 ug/ml
	Sample 3		10000	1 ug/ml
	Sample 4		10000	.5 ug/ml
	Sample 5		10000	.25 ug/ml
	Sample 6		10000	.125 ug/ml
	Sample 7		10000	.0625 ug/ml
	Sample 8		10000	.031 ug/ml

To easily describe the relationship between populations in the gating tree, we use a genealogical tree paradigm. For example, the *Lymphocytes* population is the "Parent" of the *FL1+* population. Likewise, Sample 1 is the "Parent" of the *Lymphocytes* subpopulation. Sample 1 is also the "Grandparent" of the *FL1+* subpopulation.

Sample 1 — Parent
Grandparent — Lymphocytes — Parent
FL1+
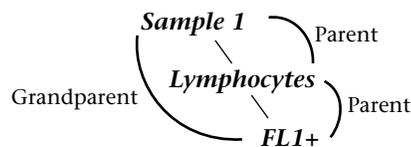
Adding Statistics

There are many statistics that can be computed and displayed by FlowJo. Except for the "Frequency of Parent" statistic, which is always displayed for each subpopulation, statistics must be added as separate nodes in the Workspace window. Each statistic node holds a single value (a single statistic), which is recomputed whenever a gate that affects that statistic changes. The full list of statistics includes such computations as fluorescence intensity, CV or frequency within a parent population.
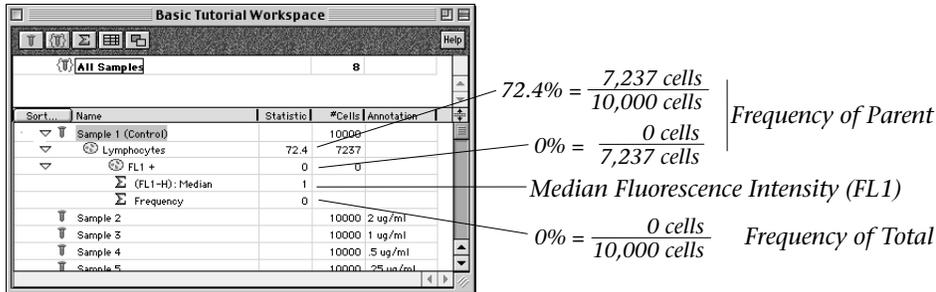
Statistics are added to any sample or subpopulation by selecting one or more population nodes in the Workspace and clicking on the "Add Statistic" function button; this brings up the Add Statistic dialog box. You can request any of the statistics listed in the dialog box and the chosen statistics are added to the Workspace window as nodes marked by a sigma sign.

Add a **statistic**

Select the subset (click once) to add the statistic to.

Choose a **statistic.**

Choose a parameter for which to calculate fluorescence intensity (mean, median, or geom. mean).

Select the *FL1+* subpopulation under Sample 1 in the Workspace window (click once to select - shading indicates selection). Click the middle function button in the Workspace window to open the Add Statistics dialog box. Choose the Median Fluorescent Intensity. Before clicking "Add", you need to choose a parameter to compute this statistic on. Use the pulldown menu on the right side of the Add Statistic Dialog window to choose FL-1 and click "Add". Repeat the process and add the "Frequency" statistic to the *FL1+* subset. Click "Add".

> **Hint:** If a statistic node in the workspace does not show its value, then you may request it to be computed. Select any sample and select "Recalculate" from the "Workspace" menu.

Notice that in the Workspace window, there are two new nodes (lines of text) under the *FL1+* subpopulation name. The "Median Fluorescence Intensity" (MFI) describes the brightness of the FITC parameter and the "Frequency" is the frequency of the entire sample (Frequency of Total).

$$72.4\% = \frac{7,237 \text{ cells}}{10,000 \text{ cells}}$$ Frequency of Parent

$$0\% = \frac{0 \text{ cells}}{7,237 \text{ cells}}$$

Median Fluorescence Intensity (FL1)

$$0\% = \frac{0 \text{ cells}}{10,000 \text{ cells}}$$ Frequency of Total

Statistics: The statistic "Frequency" refers to the frequency of the whole sample and can be thought of as Frequency of Total. The Frequency of Parent refers to the frequency of the direct ancestor population (referred to as the "Parent" population in the genealogical tree metaphor). The Frequency of Parent is calculated by dividing the number of cells in the subpopulation by the number of cells in its direct ancestor population. The Frequency of Parent statistic is automatically calculated for every population of cells in the Workspace.

This is a good time to save your Workspace. At this point, you should have added data files to a new Workspace, drawn gates to divide Sample 1 into subpopulations, and asked for statistics to be calculated for the subpopulations. If you wish to continue with the tutorial but have not made your own Workspace, premade Workspaces are provided that correspond to each analysis step. Premade Workspaces can be found in the "Basic Tutorial" folder on the FlowJo CD or at www.flowjo.com/tutorial.html.

3. Copy gates and statistics to all samples.

Drag and Drop Gates and Statistics.
In FlowJo, all analysis nodes (gated subpopulations and statistics) can be applied to any other sample using drag and drop. Dragging any node (population or statistic) onto the name of another population will create a new node. This is an easy way to build and expand analysis trees. This method allows flexibility as you can drag one or more node from any sample to build a new gating tree. The gating tree represents the subsets of a sample in the same way Boolean expressions, such as AND and NOT, do in other analysis programs.

Since you have already saved this Workspace, feel free to play with drag and drop in order to create analysis trees on other samples or expand the gating tree on Sample 1. When you wish to continue with Step 3 of the Tutorial choose "Revert" from the File menu to revert to the previously saved Workspace and eliminate the extra nodes you have created. You should now have a Workspace with a gating tree applied to Sample 1 only. If you wish you can also use the premade Workspace (Step 2) to continue with the Tutorial.

Apply the Gating Tree to Every Sample.
Analyses can be applied to every sample simultaneously by dragging the gating tree onto the **All Samples** group name in the upper panel of the workspace window. In Step 2 we analyzed the control sample in detail and now it is appropriate to apply the gating tree to the other seven samples in the experimental data set.

Select all the gates and statistics (Shift-click) and drag them onto the All Samples group in the group (upper) panel of the Workspace. Release the mouse when you roll over the All Samples name (it becomes shaded). Watch as the gating tree is applied to all eight samples.

Before FlowJo will apply the gates to each sample in the group, you will encounter a "Confirm Action" dialog box (shown on the next page). This box asks you to confirm that you want to apply the gating tree that we created for Sample 1 to the rest of the samples. FlowJo is warning us that we did not stain all of the samples in the same way (i.e., Sample 1 (unstained control) was not stained with FITC-CD8 antibody). The annotation of each sample during collection reflects this fact. Thus, FlowJo is reminding us that we are "dragging gates based on stains that are not in the destination sample". In this case, this action is perfectly reasonable, so click "Yes".

Gates and statistics are applied to all samples.

Drag and drop *gating tree onto the All Samples group.*

Bold *font style*

Click *yes.*
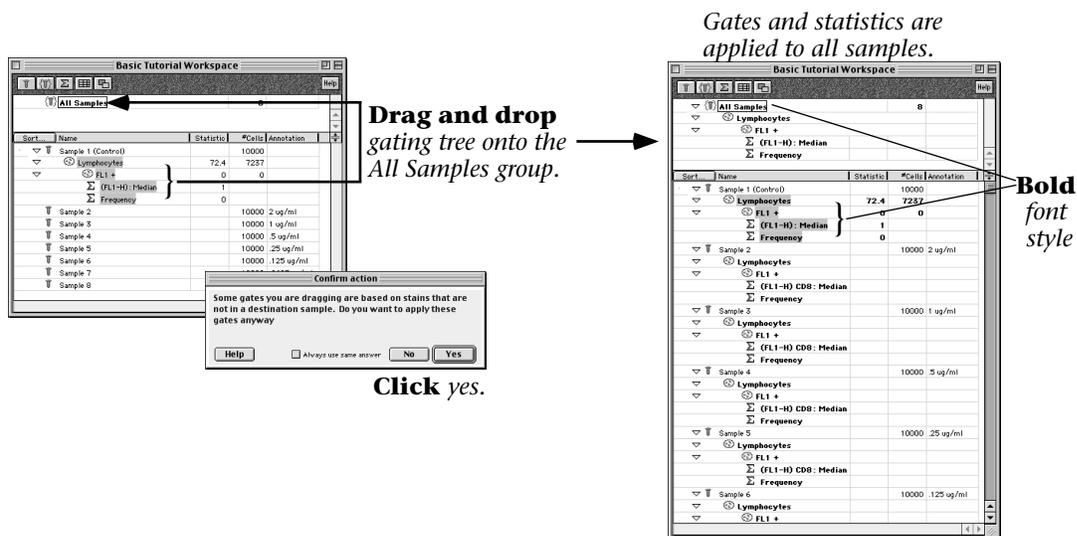
Notice that the font of the nodes in the gating tree becomes **bold**. The font of the All Samples group is also **bold**. FlowJo used colors and font types to help you keep track of gates and groups. The bold font of the gating tree on the samples is a visual clue that these gates are identical to those that were applied to the All Samples group. This feature becomes important when you have several different groups or when altering gates on individual samples (see Step 4).

Hint: The All Samples group serves well for the analysis of the titration data. However, more complex experiments may consist of samples with different characteristics making it inappropriate to apply one gating tree to every sample in the Workspace. In this case, samples can be grouped according to their similarities to enable batch processing of only those samples with similar characteristics. See the Advanced Tutorial for a full appreciation of the power of groups and their usefulness in organizing a more complex data set - www.flowjo.com/tutorial.html.

4. Verify gates on all samples.
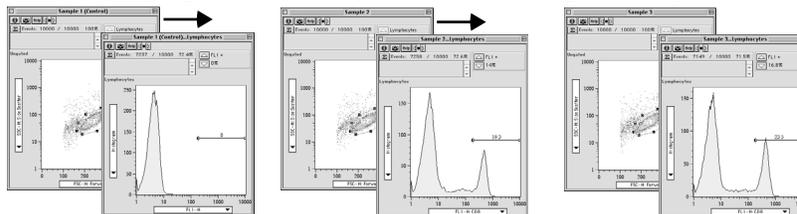
Checking Gates

Applying the gating tree to many samples at once is very easy; however, FlowJo does not check the gates on each sample for you! In order to check your gates, open the graph window of Sample 1 (double click on the sample name in the Workspace window) and adjust to display Forward vs. Side Scatter.

Instead of opening the graphs for each individual sample to check the gates, you can click on the "forward" Yellow Arrow at the top of the Graph window to cycle to the next sample. The black dot allows you to select a particular sample for display. Use the yellow arrows to check the *Lymphocytes* gate on each sample.

Yellow arrow **cycles** *the graph to the* **next sample.**
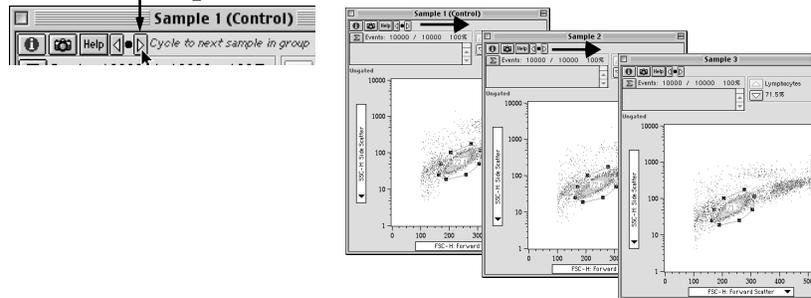
Cycle **both graphs** *to next sample by holding the* **option key.**

Open a graph displaying the *FL1+* gate (double click on the *Lymphocytes* node in the Workspace window) and display the two Graphs beside each other. In order to check the gates in both graphs at once, hold the Option Key while clicking on the Yellow Arrows. The **Option Key** causes all open graphs to follow the command to cycle to the next sample.

Altering Gates

While checking the gates on each sample it becomes clear that adjustments need to be made. Gates can be altered on each individual sample or the adjustments can be applied to all the samples.

Original FL1+ gate.
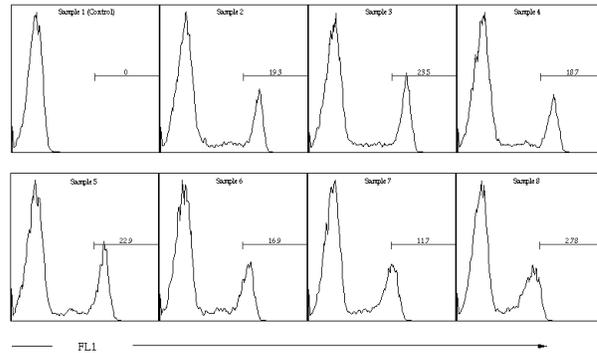
In our example data, the *FL1+* gate we initially drew does not include the entire FITC+ subset (the fluorescence cutoff is too high). Open the Graph window displaying the *FL1+* gate for Sample 1 and adjust the *FL1+* gate by clicking on the left handle of the histogram gate and dragging to the left. We want to adjust the *FL1+* gate to start at a lower fluorescence intensity and include a larger portion of the histogram (as shown in the figure below).
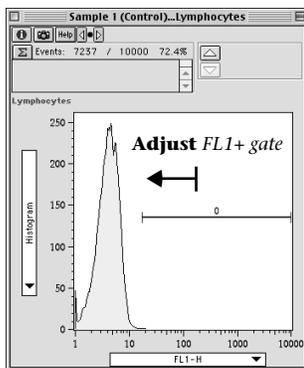
Adjusted FL1+ gate on Unstained Control*.
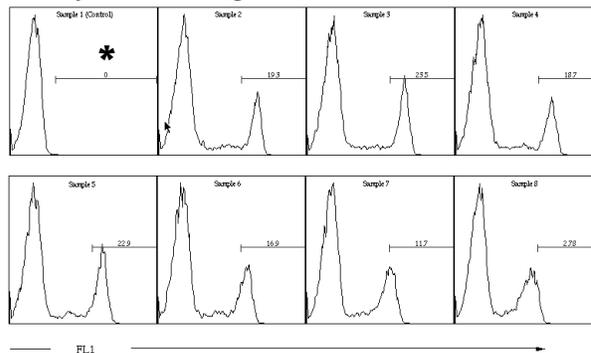
Notice in the Workspace window, that the font of the *FL1+* gate name is no longer bold. This indicates that the *FL1+* gate is no longer the same as the gate that was dragged onto the All Samples group. Changing the *FL1+* gate on one sample DOES NOT automatically change the *FL1+* gate on all samples. The *FL1+* gate on the other samples remains bold as these gates are the same as the gate that was initially dragged onto the group.

You now have two options. Either leave the new gate on Sample 1 and the original gate on the rest of the samples or apply the new gate to all the samples. Apply the new *FL1+* gate to all the samples by dragging the *FL1+* gate from Sample 1 up onto the All Samples group. Click once to select the *FL1+* gate under Sample 1 (the *FL1+* gate will become highlighted to indicate that it has been selected). Click and drag this node up to the All Samples group. Release the mouse when you are over the *Lymphocytes* node (it will become highlighted) in the All Samples group. Notice that all of the samples now have the new *FL1+* gate.

Drag *and* **drop** *FL1+*
gate onto the **group.**

Adjusted FL1+ gate applied to all samples.
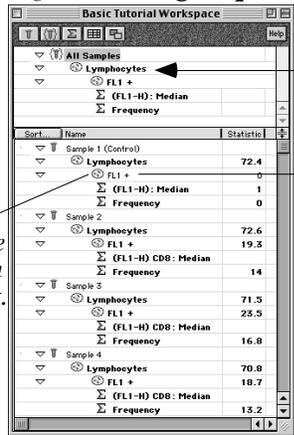
Adjusted
FL1+ gate
written in
plain text.
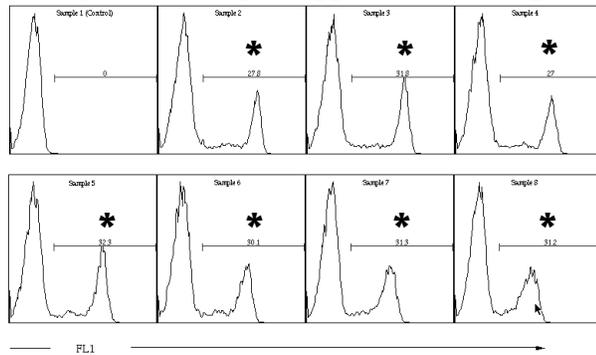
Hint: This exercise illustrates two of the powerful features of FlowJo. First, FlowJo gives you the ability to make sure that all of your samples are analyzed identically--i.e., have exactly the same gates, statistics, and other analyses, in order to limit subjectivity in the data analysis. Second, FlowJo gives you the ability to make sample-specific modifications. There are times when the *Lymphocyte* gate (or any other gate) must be moved slightly to accommodate a specific sample. FlowJo lets you move the gate or change the analysis for individual samples without affecting all the rest. The workspace provides feedback that this has occurred, but allows you to proceed with your batch analyses and report generation using the sample-specific modifications for those samples where it is necessary. With these two features, FlowJo provides an environment which can be as rigid or as flexible as you need!

5.Generate a graphical report of all samples.

Layout Editor

The Layout Editor and Table Editor are used for making graphical and statistical reports to summarize all the samples. Now that we have drawn gates to divide each sample into subpopulations, added statistics, and checked our gates, it is time to make a summary of this experiment. The Table and Layout Editors work in similarly; each editor opens a new window that is saved with the Workspace. In these editor windows, you define which statistics or graphs to display in your report and FlowJo compiles them into one document. The repetitive tasks are done for you!

Open the Layout Editor window by clicking the right most function button in the Workspace window. The left panel of the Layout Editor window shows the names of the layouts and the right panel is a drawing board. In the drawing board, you will create a graphical display for a single sample. FlowJo will then create a batch report by iterating through all the samples in the group and retrieving the matching graphs for each sample.
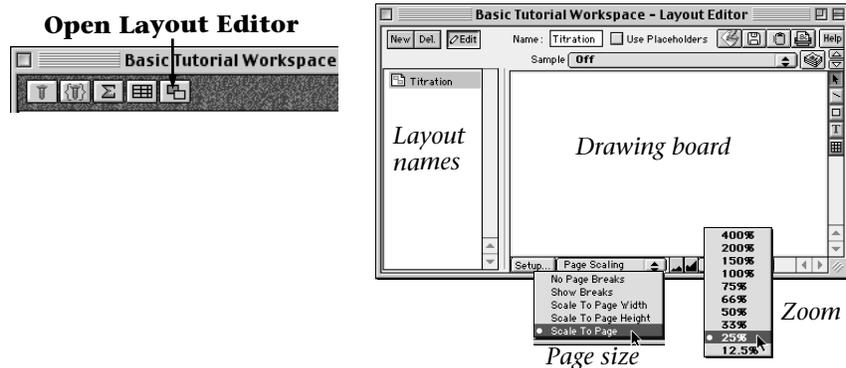
This tutorial will demonstrate three methods of creating a graphical report using the Layout Editor. 1) Display graphs from every sample in a Batch layout. 2) Overlay histograms from every sample. 3) Combine the overlay and batch report to overlay each sample onto the control histogram.

1) Batch Report

Drag the *Lymphocytes* subpopulation node from Sample 1 in the Workspace window to the right panel of the Layout Editor. A histogram of the FL-1 parameter should appear in the window. (Double click on the graph in the Layout Editor to change the parameters displayed). Shift-click to select the two statistics, Median and Frequency, under Sample 1 and drag them to the right panel in the Layout Editor.

Drag *Lymphocyte subset and statistic nodes to the Layout Editor.*

Click to create a **batch report.**

Make a batch report by clicking on the "Stack of Papers" button in the upper right corner of the Layout Editor. Click "Create" in the dialog window as the default values are appropriate for this report. A new layout is created, named "Layout-batch", and listed on the left panel of the Layout Editor. To create this layout, FlowJo "batched" through each sample and retrieved an FL-1 histogram and the requested statistics for each sample.
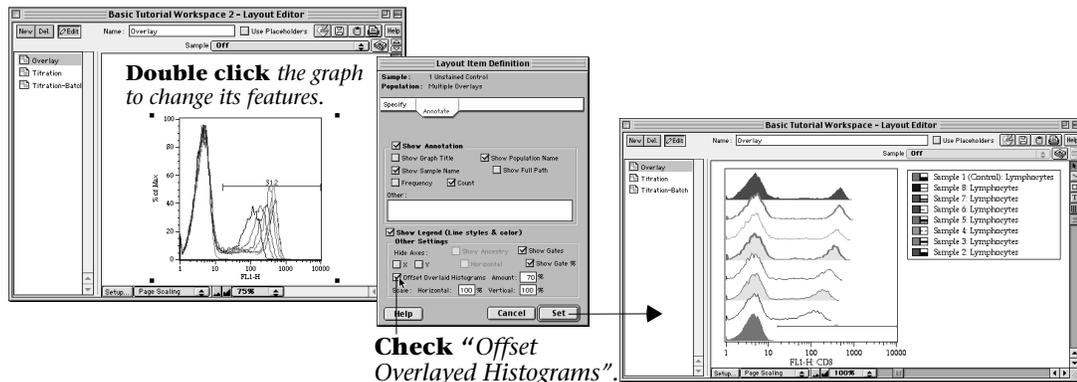
2) Histogram Overlay

Create a new layout by clicking the New button in the Layout Editor. In this layout, you will overlay a histogram of the *Lymphocytes* subpopulation from every sample. First drag the *Lymphocytes* node from Sample 1 to the Layout Editor (adjust if necessary to display a histogram of the FL-1 parameter). Shift-click the *Lymphocytes* nodes from Samples 2-8 and drag them all on top of the graph already in the Layout Editor. When you release the mouse, the *Lymphocytes* subpopulation from every sample will be overlayed in one plot.

Create a **New Layout.**
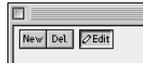
Drag *and* **drop** *subset onto existing graphs in the Layout Editor to* **overlay**.

Double click *the graph to change its features.*

Check "*Offset Overlayed Histograms*".

When a large number of histograms are overlayed, viewing the individual histograms becomes difficult. To address this problem, you need to offset the histograms. Double-click on the Graph to open the dialog box that adjusts the graph's features. Click on the "Annotate" tab within the dialog window and check the box marked "Offset Overlayed Histograms". Click "Set". Your graph should look like the one at the right above. To change the color or line style of a histogram, click on the color box or line box beside the subset name in the legend.

3) Setting a Control Sample

Many experiments, including this one, are made up of one control and several test samples. Our final report style will overlay each individual sample onto the unstained control sample for direct comparison. To do this you will overlay Sample 2 onto Sample 1 (unstained control), define which graph is the control, and then create a batch report showing each sample overlayed on the control.

Create a new Layout and drag the *Lymphocytes* node from Sample 1 to the Layout Editor. Drag the *Lymphocytes* node from Sample 2 onto the graph in the Layout Editor to create an overlay. Click on the line box in the legend to make the Sample 1 histogram "solid".

Step 5   *21*

Create *a New Layout.*

Overlay *the Lymphocyte subset from Sample 2 onto Sample 1 (control).*

Double click *on the graph to change its features.*

Choose *the control sample.*
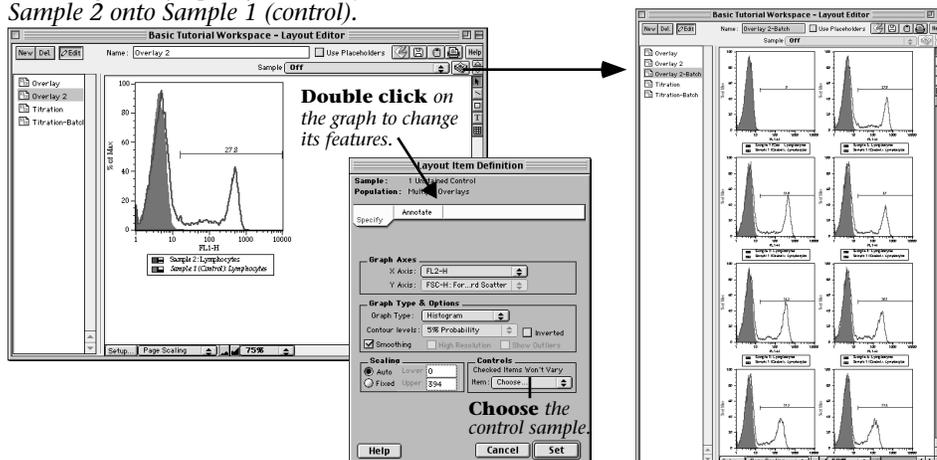
Define which sample is the control and will not vary. Double click on the graph in the Layout Editor and click on the Specify folder in the dialog window that pops up. In the Specify folder, there is an area marked "Controls". Choose Sample 1 and click "Set" - now Sample 1 will not vary when you create a batch report of the histogram overlay.

To generate a batch report click the "Stack of Papers" button and click "Create" in the dialog. You should now see a new layout displaying each individual sample overlayed on the Sample 1 histogram.

Drawing Tools
Note that there are several tools along the right edge of the Layout Editor window. These allow you to add lines, arrows, boxes, text boxes, and grids to your layouts. The layouts can be saved to disk (the default is in the PICT file format) or copied and pasted into any drawing program. Graphics saved as PICT files can be easily ungrouped into individual objects in order to adjust any part of the graph.

FlowJo Basic Tutorial

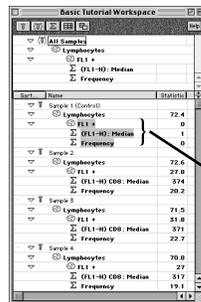# 6. Generate a table of statistics from all samples.

Table Editor

The Table Editor works in a similar manner as the Layout Editor. Define which statistics you wish to have calculated. FlowJo compiles these statistics from each sample and displays the resulting table.
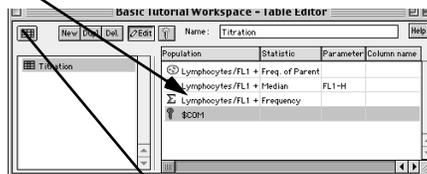
Open Table Editor

Open the Table Editor by clicking the fourth function button from the right. This opens a new window. The left panel shows the names of the table definitions and the right panel is where you define the statistics that will be compiled into a table.

Table names
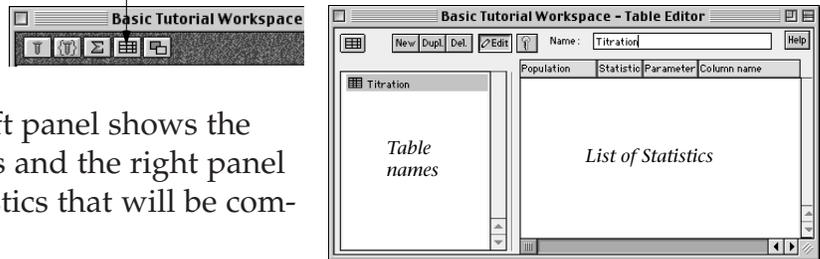
List of Statistics

Drag the *FL1+* subpopulation node and the Frequency and Median statistic nodes from Sample 1 to the right panel of the Table Editor (as shown below). Three statistics are now listed on the right side of the Table Editor. All three statistics are calculated for the *FL1+* population. When you dragged the subpopulation node itself (i.e., *FL1+*) to the Table Editor, the "Frequency of Parent" statistic was listed.

Drag *subset and statistic nodes to Table Editor.*

Click to create a **table.**

Each **row** *is a sample.*

Each **column** *is a statistic*

In order to add information stored in an FCS keyword, click the "Key" button ![Key button] at the top of the Table Editor. In this experiment, the Comments field ($COM keyword) was used to note the concentration of antibody each sample was stained with. To add this information to the table definition, choose $COM from the keyword list and click "Include".

Create the table by clicking the "grid" button ![grid button] in the upper left corner of the Table Editor. Each statistic listed in the Table Editor will become a column in the table and each sample will become a row. This table will not be saved with the workspace and must be saved, copied to the clipboard (for easy pasting into another program), or printed using the buttons along the top left of the table to perform these functions.
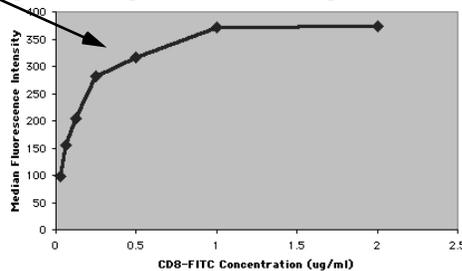
The fourth button at the top of the Table is the "Save and Launch Application" button. ![Save and Launch button] When you click this button, FlowJo saves the table and then launches a spreadsheet program (you will be asked to choose this application the first time you use this feature) and pastes the table into your chosen spreadsheet program. With a single click you can export any FlowJo table into a spreadsheet program for further analysis!
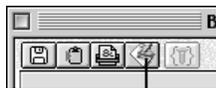
Click *button to:*
-save the table
-launch your favorite spreadsheet program
-paste table into spreadsheet program

Continue data analysis with spreadsheet program (create graph).

To finish analyzing this titration experiment, we will graph the Median Fluorescence Intensity of the FL1+ subpopulation versus the concentration of CD8-FITC antibody used to stain each sample. A staining concentration that reaches maximal fluorescence (i.e., is saturating) but does not use excess reagent will be chosen for subsequent laboratory use.

Click the fourth button in the upper left corner of the table. This saves the Table and transfers the data to the spreadsheet program of your choice. Use the spreadsheet program to produce a graph of Concentration of CD8-FITC reagent versus Median Fluorescence Intensity (as shown below). The saturating concentration of this reagent is 1 µg/ml. Therefore this CD8-FITC antibody reagent should be used at 1 µg/ml in further experiments as long as the same staining conditions are used.

Getting Help

Clicking on the **HELP** button in any FlowJo window launches a web browser to access a web page describing that topic, giving you "context-sensitive help".

Visit the FlowJo web page to view the entire **Reference Manual**:
www.flowjo.com/v4/reference.html

For a more in depth tutorial, download the **Advanced Tutorial** at:
www.flowjo.com/tutorial.html

Tech Notes - short step-by-step guides to FlowJo applications are available at:
 www.flowjo.com/tutorial.html

We encourage feedback! Please contact us with your comments about FlowJo, this Tutorial, or any of our Help files at:
flowjo@treestar.com

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