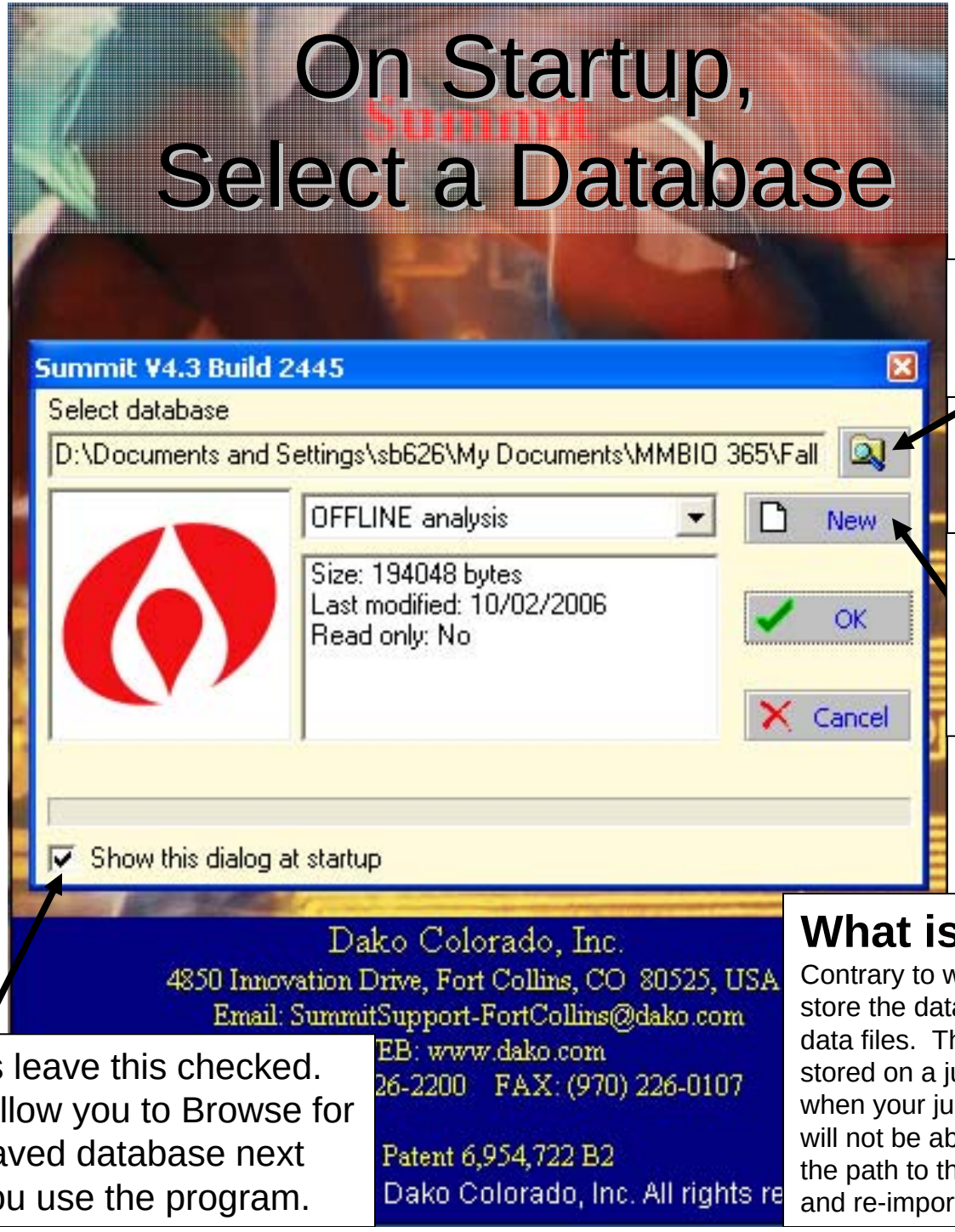
On Startup, Select a Database



The next time:
Browse to find the Database you saved already.

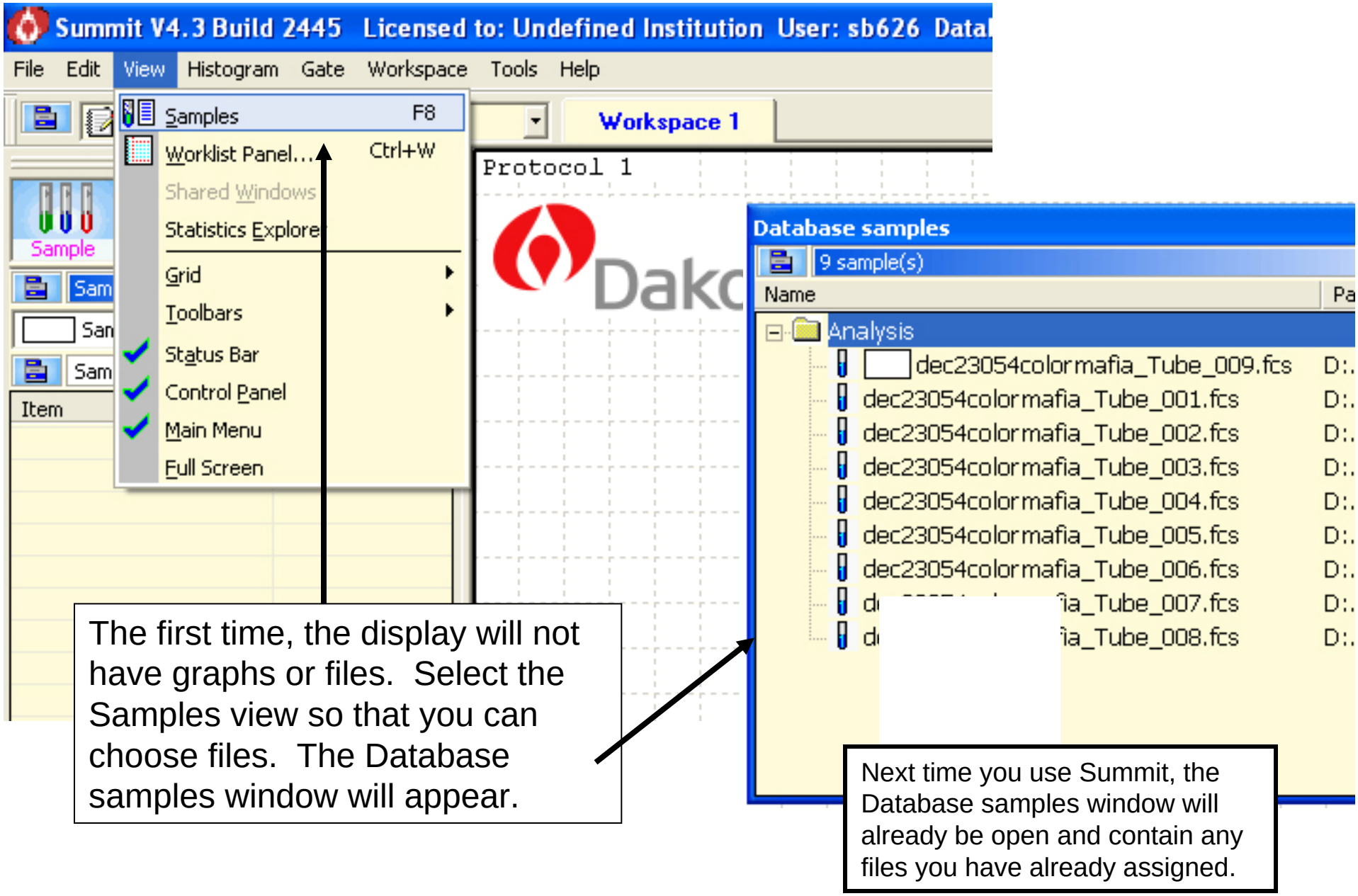
THE FIRST TIME: You must first click on "New" to name your Database. Save it in the folder where you can find it again. Once you Click OK, the database path will be set for what you saved.

Always leave this checked. It will allow you to Browse for your saved database next time you use the program.

What is stored in the database?

Contrary to what you might think, the database does not store the data files. Instead, it stores the pathway to the data files. This means that if you analyze samples stored on a jumpdrive and you later open your database when your jumpdrive is not in the computer port, Summit will not be able to find your data files and will inactivate the path to the file (meaning that you will have to delete and re-import the files to analyze them).

Open the Database sample window

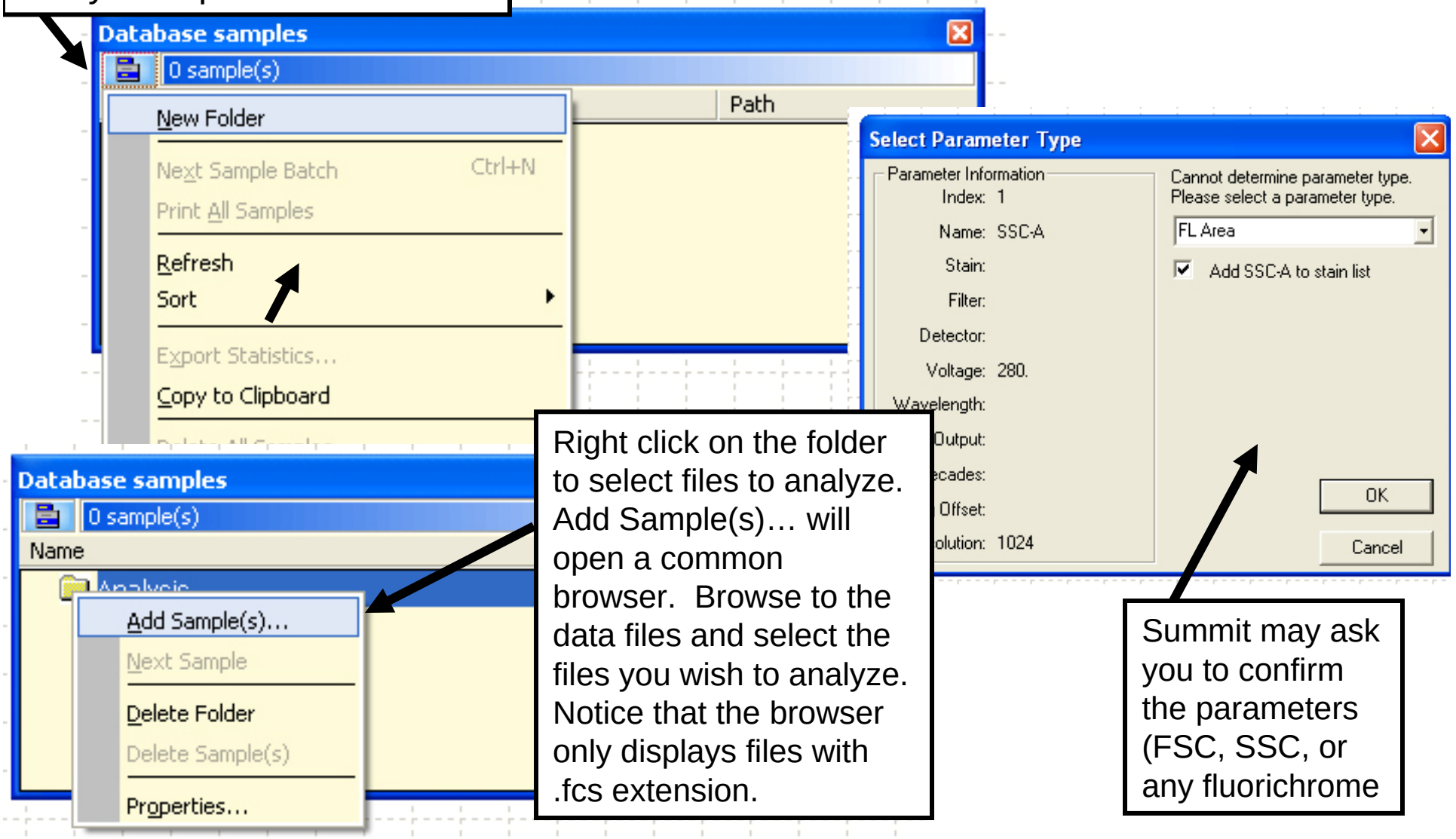


The first time, the display will not have graphs or files. Select the Samples view so that you can choose files. The Database samples window will appear.

Next time you use Summit, the Database samples window will already be open and contain any files you have already assigned.

Assigning Samples to Analyze

Right click on the top corner to make a Folder for a new analysis experiment.



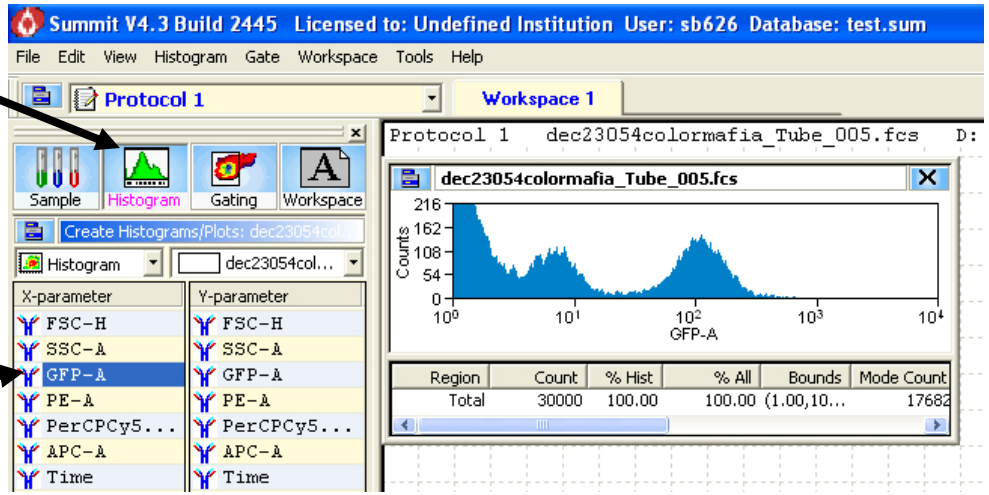
Right click on the folder to select files to analyze. Add Sample(s)… will open a common browser. Browse to the data files and select the files you wish to analyze. Notice that the browser only displays files with .fcs extension.

Summit may ask you to confirm the parameters (FSC, SSC, or any fluorichrome

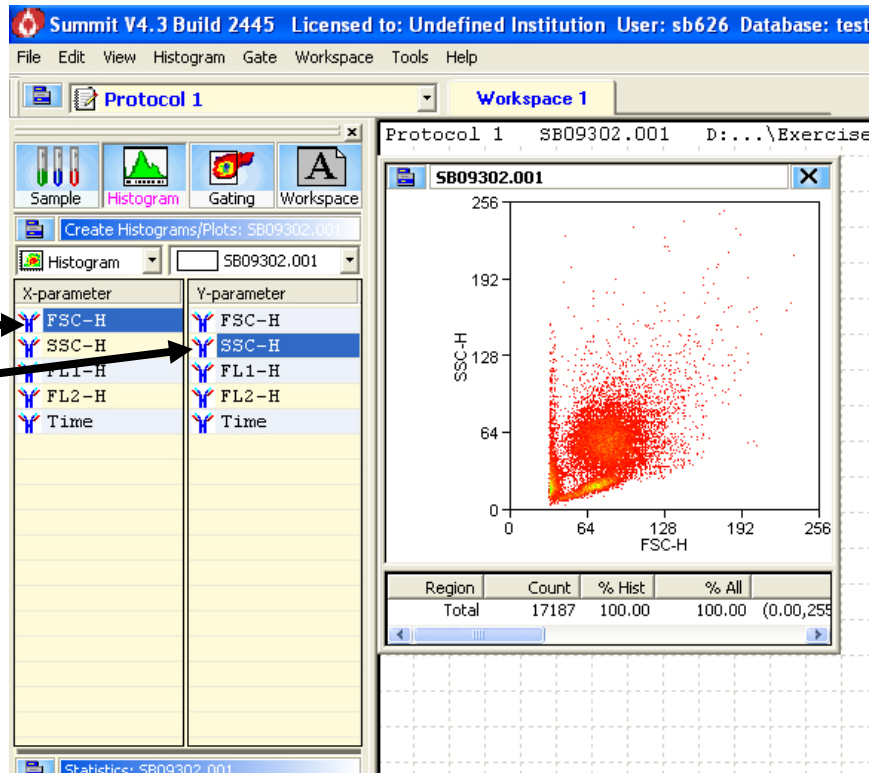
Create Graphs

Select the Histogram Toolbox.

In the Histogram Creation Toolbox, double click on one x-parameter to generate a one parameter histogram.



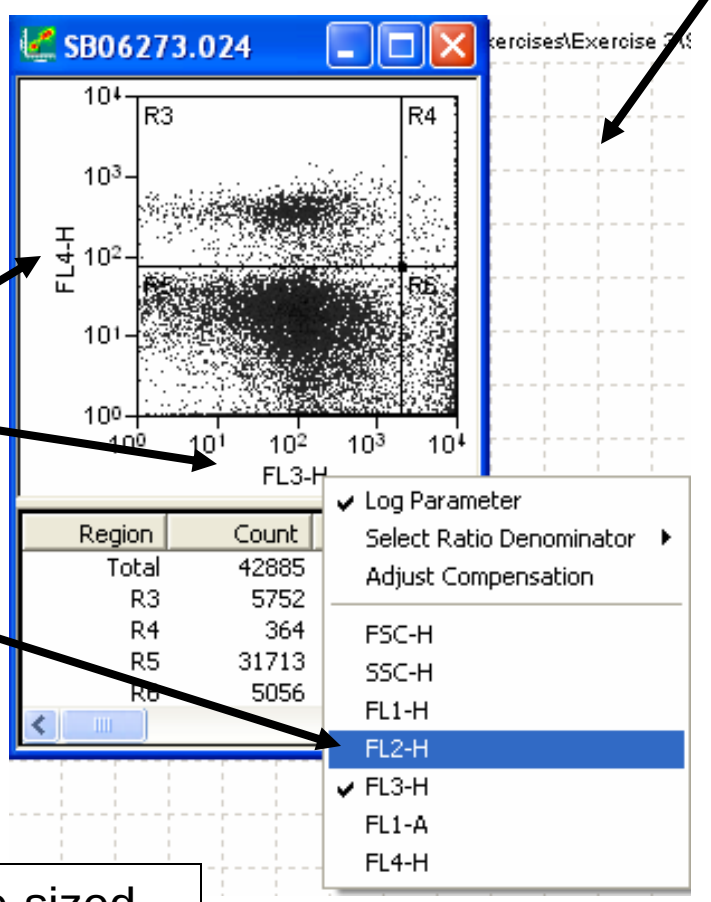
Choose an x-parameter, double click on the y-parameter to generate a two-parameter scatterplot.



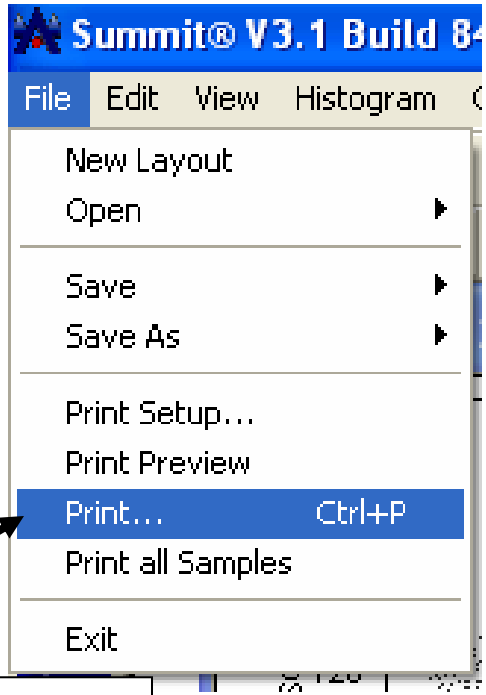
Altering Parameters on Graphs and Printing

The white grid background is the boundary of page that will be printed when you print. Be sure your graphs all fit on the white grid space. The Data Navigator will not be printed, even if it is overlapping on the white grid. Likewise, the histogram creation toolbar will not be printed.

To change the parameters on a graph, right click the label of either axis and choose a different parameter.

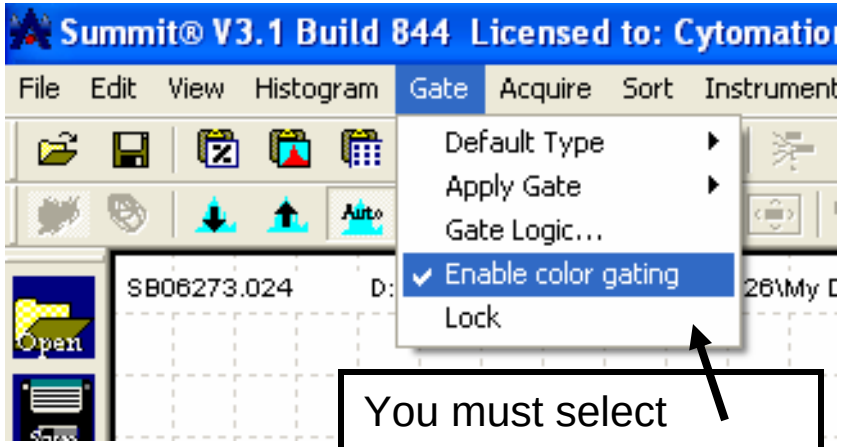


All graphs can be re-sized and repositioned on the page in the same manner as any standard window.



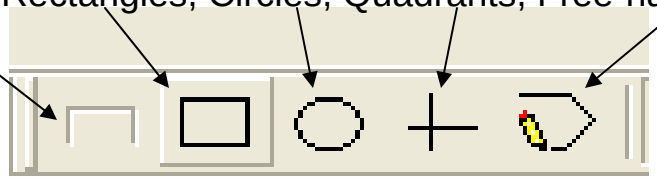
To print, select 'Print' from the Windows drop-down list.

Defining Regions & Setting up Color Gates



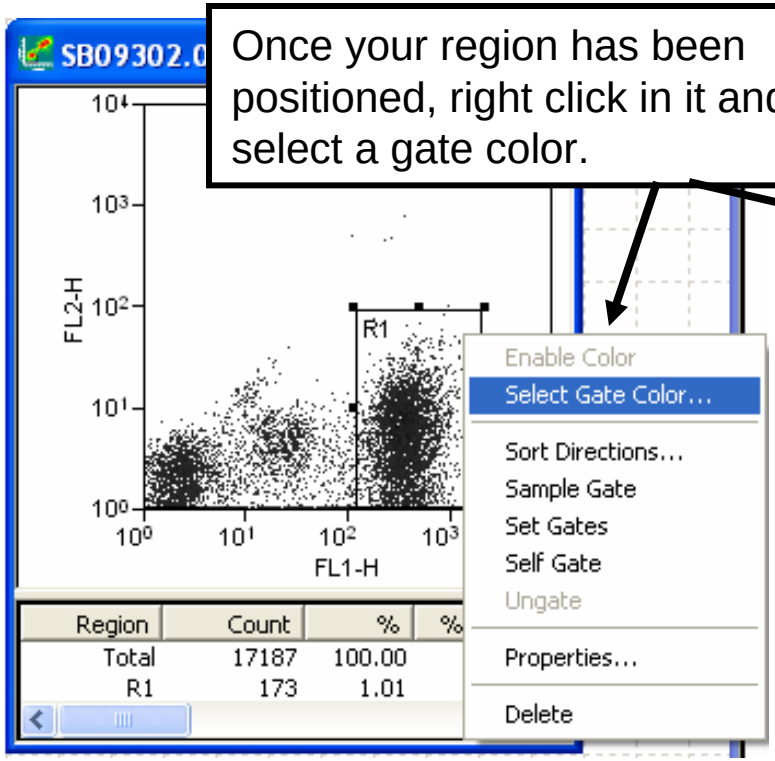
You must select "Enable color gating"

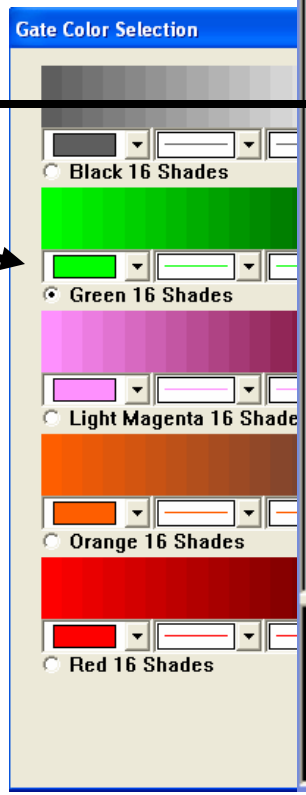
Bracket, Rectangles, Circles, Quadrants, Free-hand Draw



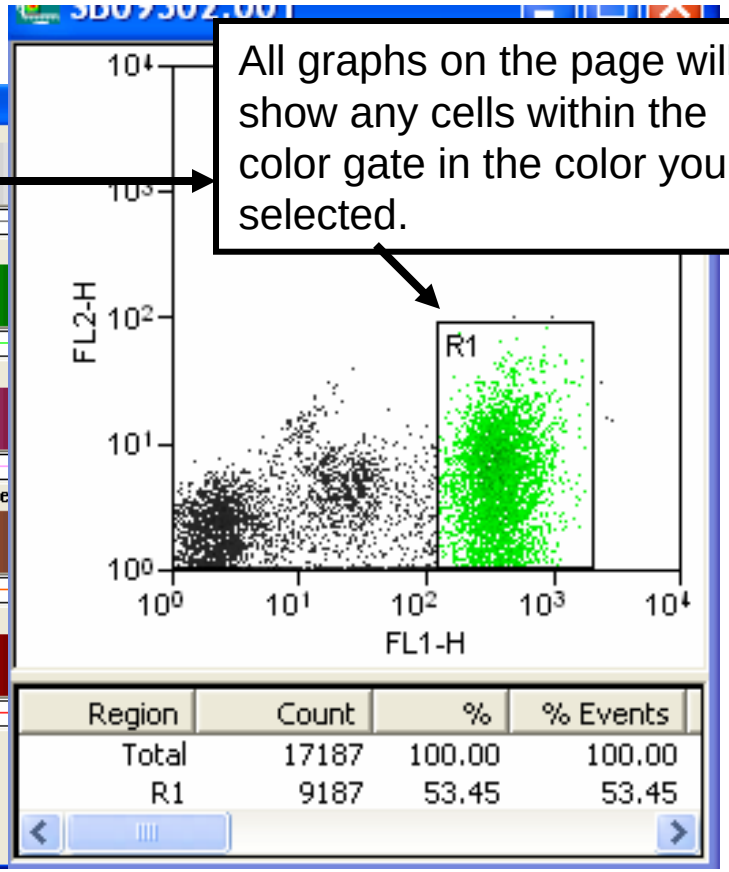
Be sure that a graph has been selected. The toolbar at the top of the screen allows you to draw regions on your graphs.

Once your region has been positioned, right click in it and select a gate color.



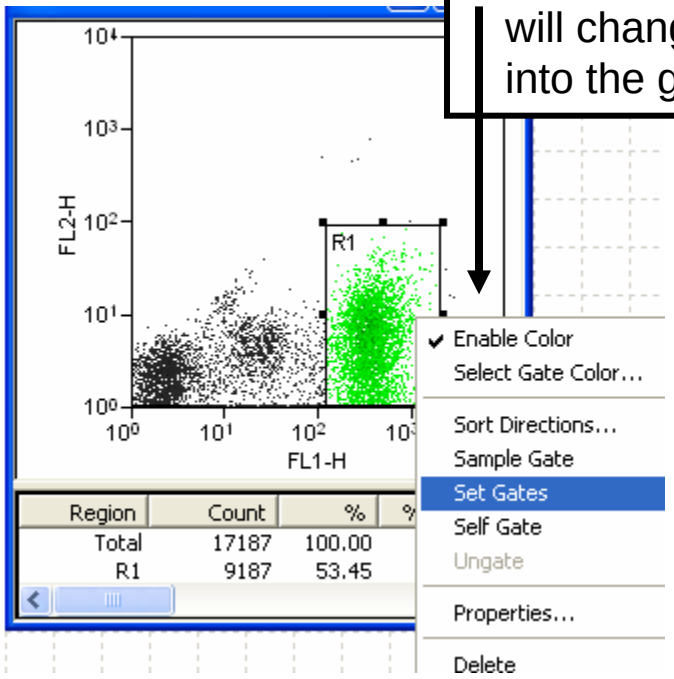


All graphs on the page will show any cells within the color gate in the color you selected.



Apply a gate to a graph

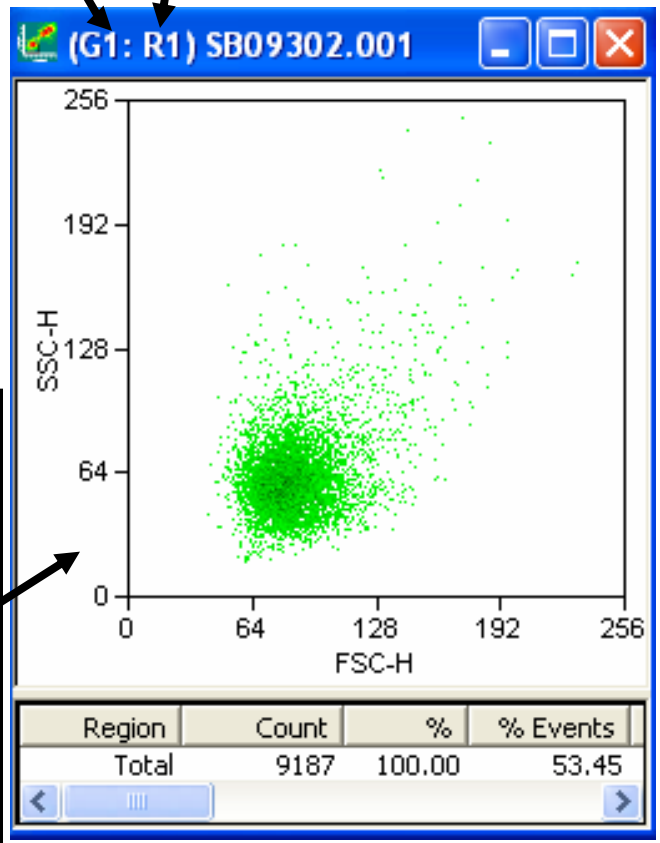
Once your region has been positioned, right click in it and select ‘Set Gates’. The arrow will change shape into the gating arrow.



Move the gating arrow on top of a graph and double click to apply the gate.

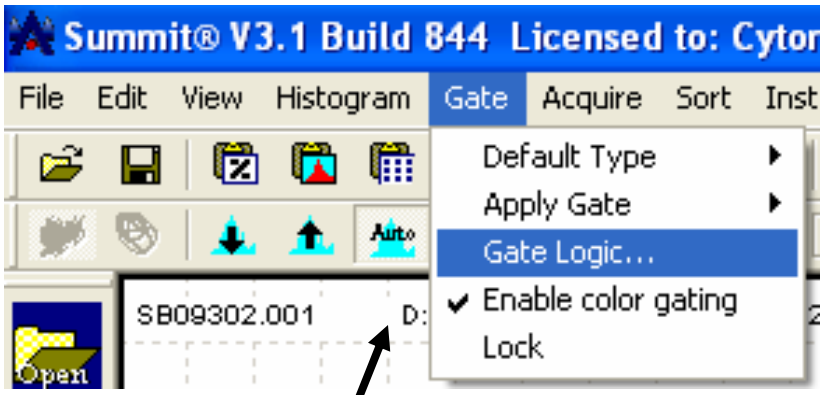
The graph will update, showing only the readings from cells that are within the region

The graph title will display which gate has been applied, and what region the gate includes.

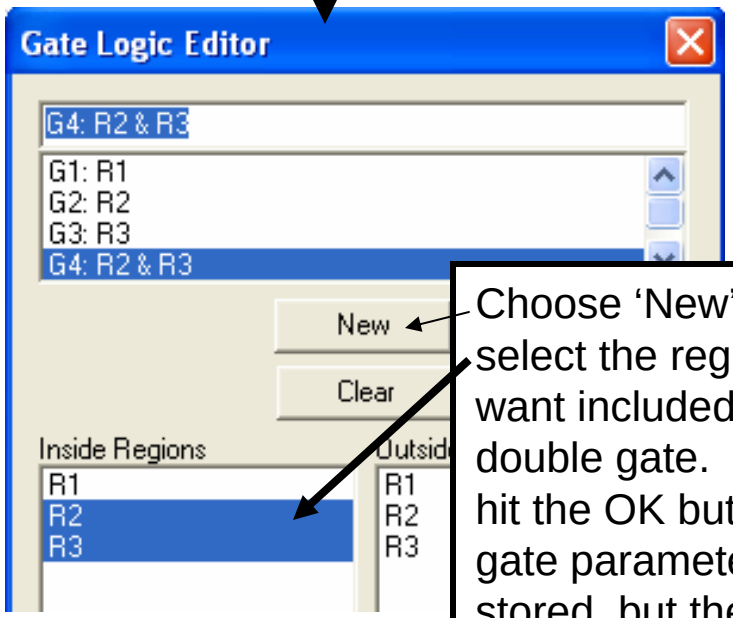


To remove an applied gate, right click on the graph and select ‘Ungate’.

Setting and Applying a Double Gate

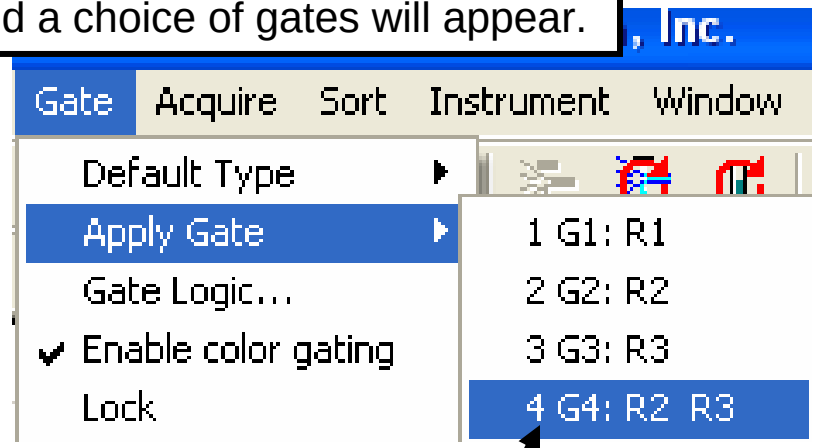


From the Windows drop-down list, select “Gate Logic”, and the Gate Logic Editor will appear.

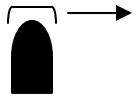


Choose ‘New’ and select the regions you want included in the double gate. When you hit the OK button, the gate parameter has been stored, but the gate has not been applied.

To apply a double gate (be sure that the double gate has been defined in the Gate Logic Editor), select “Apply Gate” from the Windows drop-down list and a choice of gates will appear.



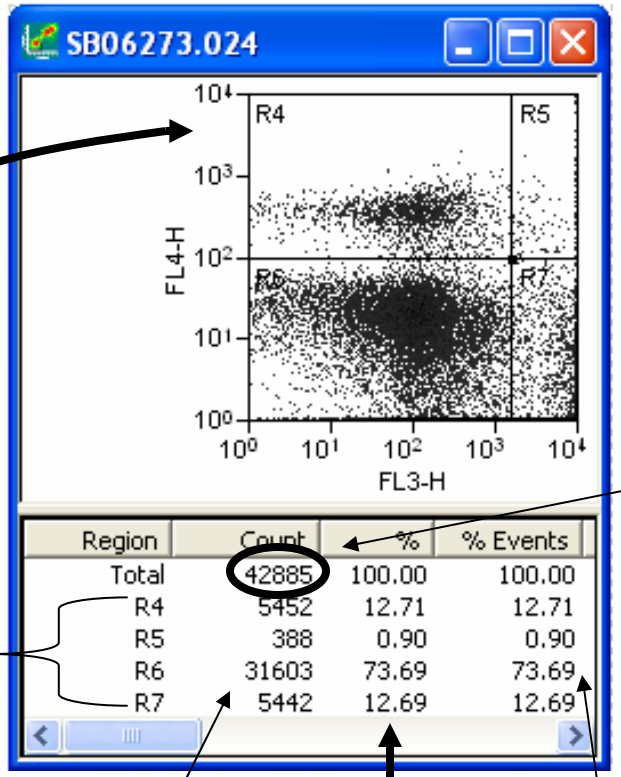
Click once on the double gate you wish to apply and the arrow will become the gating arrow. Move the gating arrow on top of a graph and double click to apply the gate.



Note: Cytomation has some programming flaws. Sometimes the Gate Logic Editor and the Apply Gate list don’t talk to each other very well. If you have problems, close the program and start it up and it should work.

Reading Statistics on Graphs

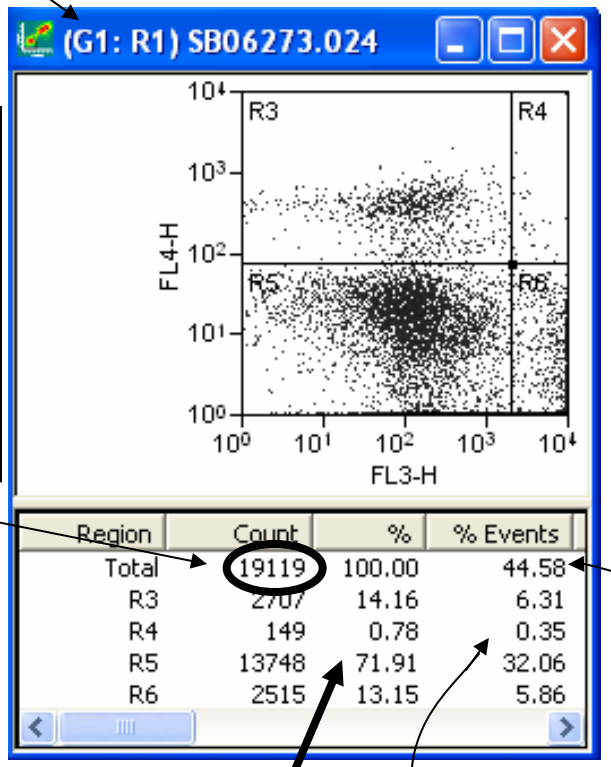
An ungated sample with Quadrants.



Region	Count	%	% Events
Total	42885	100.00	100.00
R4	5452	12.71	12.71
R5	388	0.90	0.90
R6	31603	73.69	73.69
R7	5442	12.69	12.69

The region 1 gate was applied to this graph.

The same sample with a Gate Applied.



Region	Count	%	% Events
Total	19119	100.00	44.58
R3	2707	14.16	6.31
R4	149	0.78	0.35
R5	13748	71.91	32.06
R6	2515	13.15	5.86

This is the same sample; but, once gated, the total cell count is lower, because the data only includes the gated population of cells.

Each region corresponds to regions on the graph.

Number of cells in each region.

Percent of cells in each region. **These are the numbers you will use.**

The % Events is the same as the % if the sample is not gated.

Percent of cells in each region for the gated population of cells. **These are the numbers you will use.**

The gate includes only this percent of the total population.

The % events breaks down the gated percent.