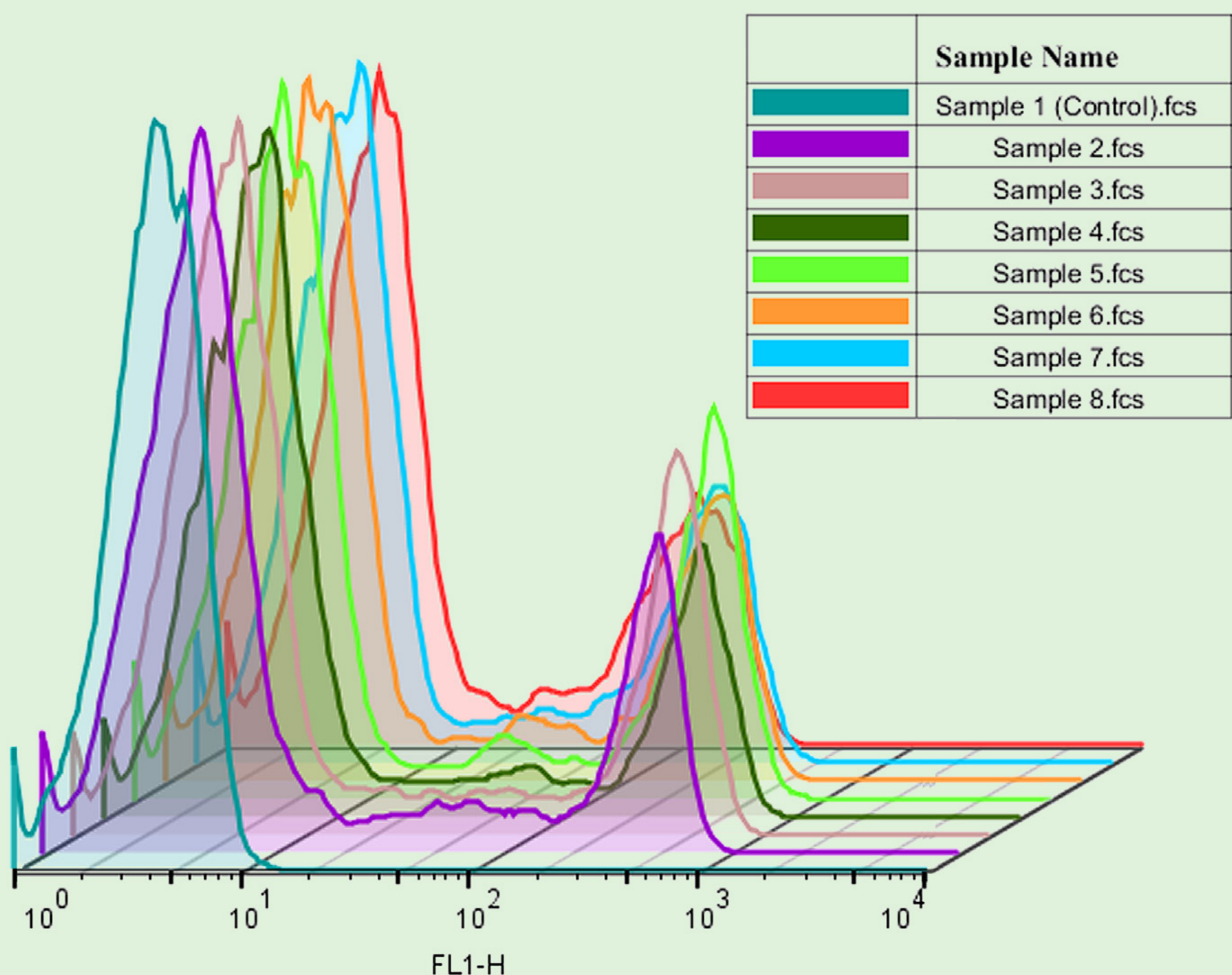


HACKING FLOWJO VX

42 Time-Saving FlowJo Shortcuts To Help You Get Your Data
Published No Matter What Flow Cytometer It Came From



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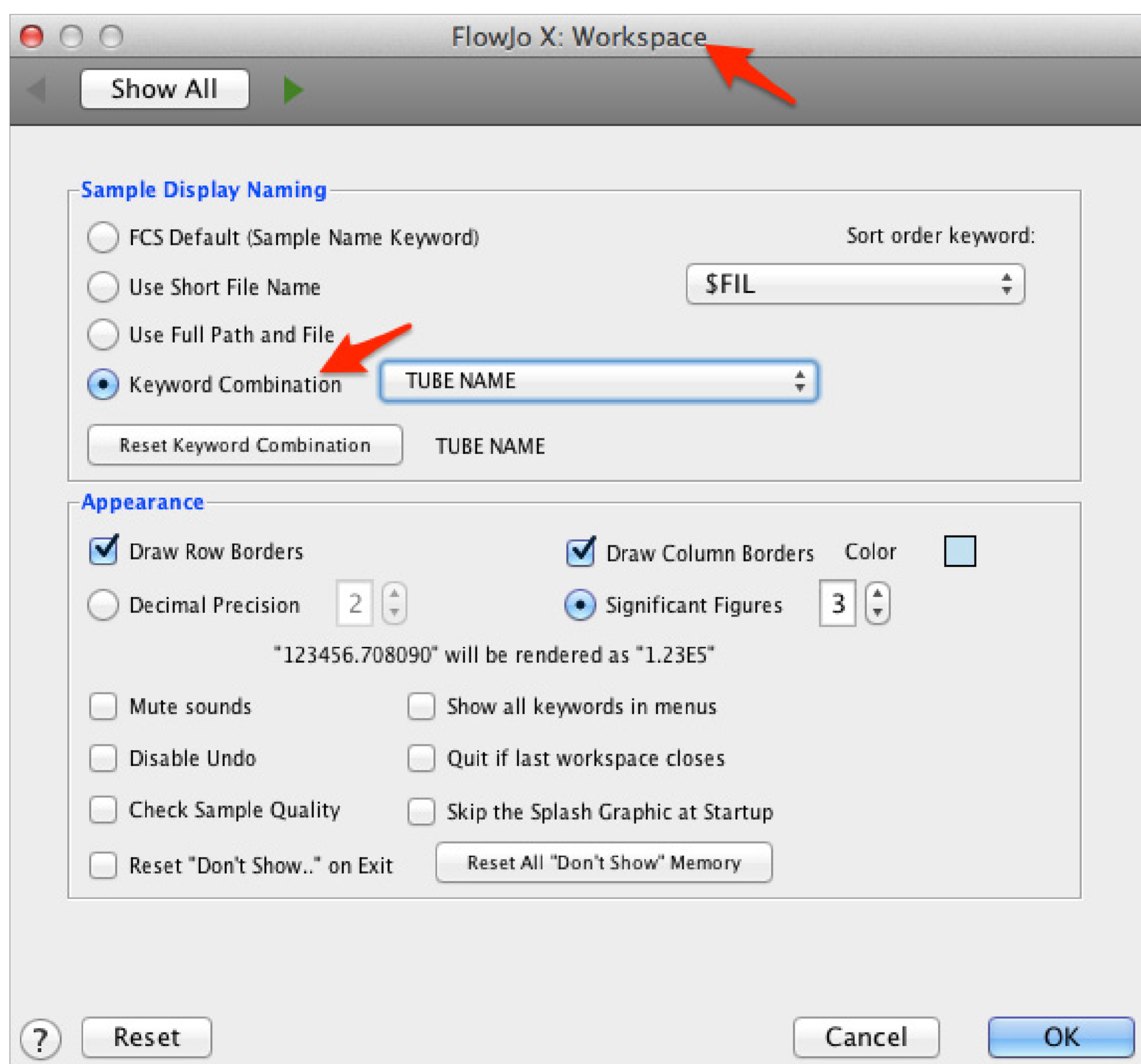
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HACKING FLOWJO VX

42 Time-Saving FlowJo Shortcuts To Help You Get Your Data Published No Matter What Flow Cytometer It Came From

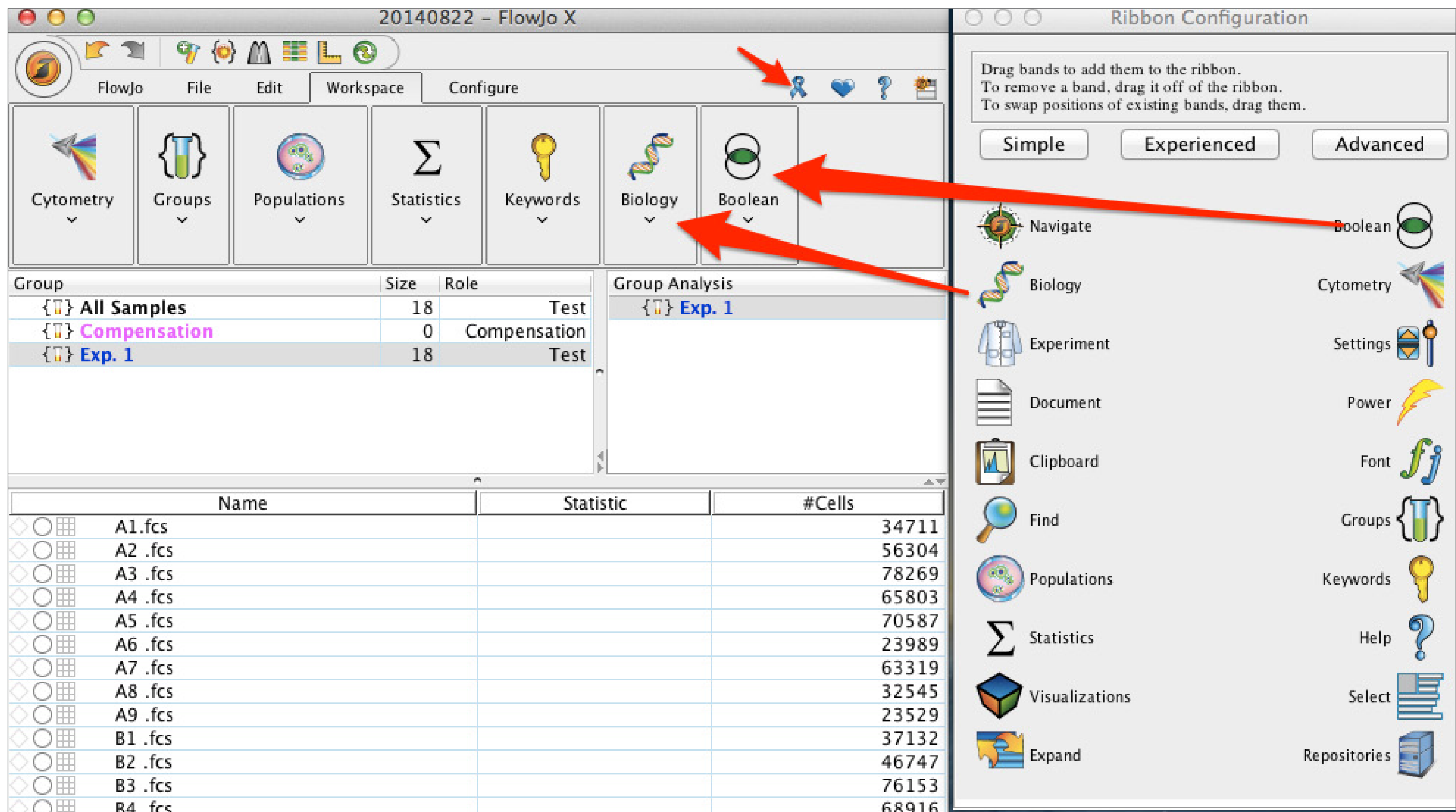
1. Change the default name of your files.

If your files had a different name when you viewed them on the cytometer, or if you simply want the name to be different now, you can change FlowJo's default naming convention in the preferences. If you are unsure what keyword you used for naming, see point five below. If you want to use your own keyword for naming, see point six below. Once you added a new keyword to your files, you can use this keyword as the name here in the naming preferences section.



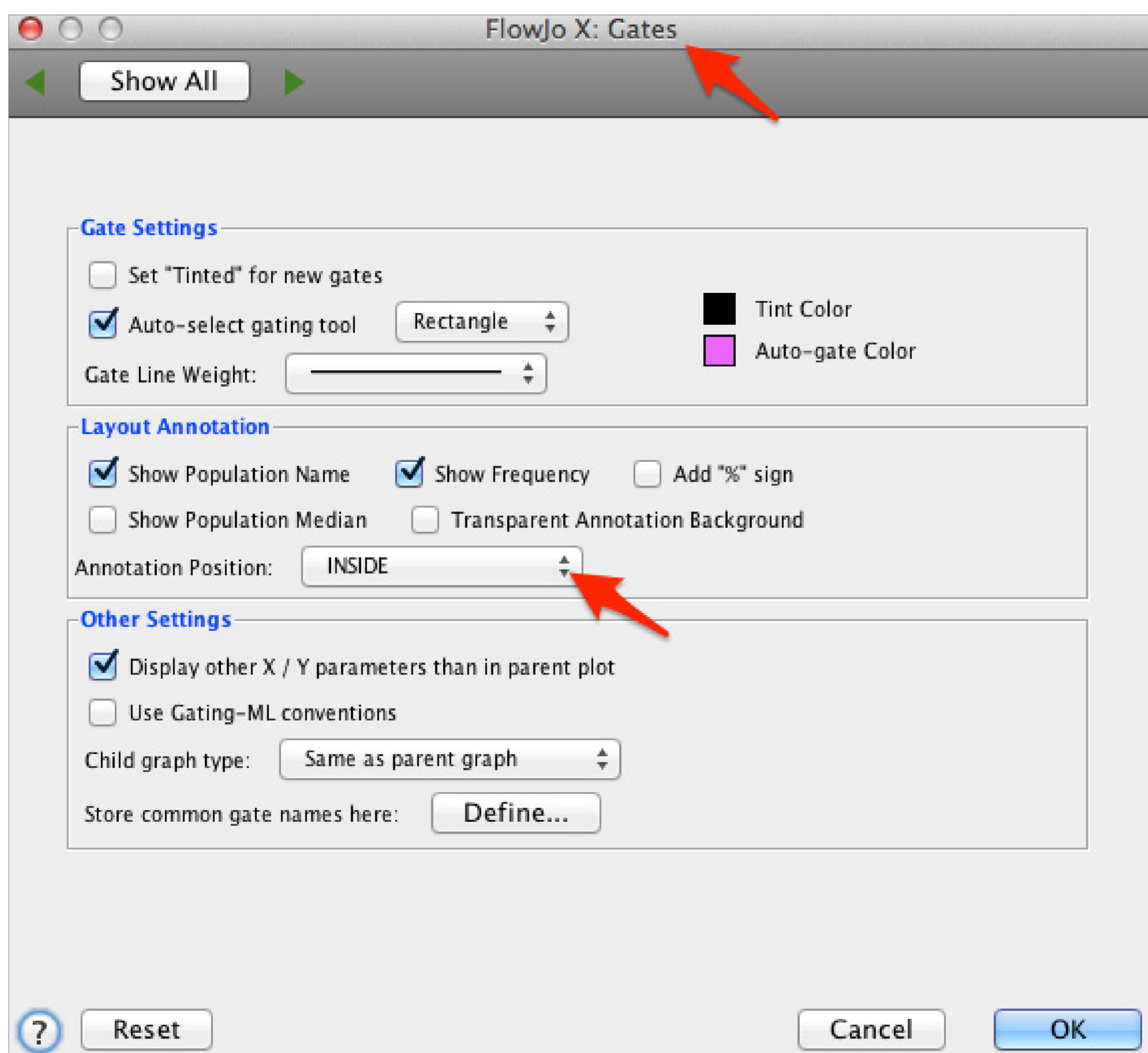
2. Edit your workspace band.

You can edit your workspace band with features you use most often. Put all the feature sets you use most often in the workspace band so you don't have to switch around to other bands all the time. This will allow you to simply stay in the workspace band. It may not seem like it, but this will save you a lot of time.



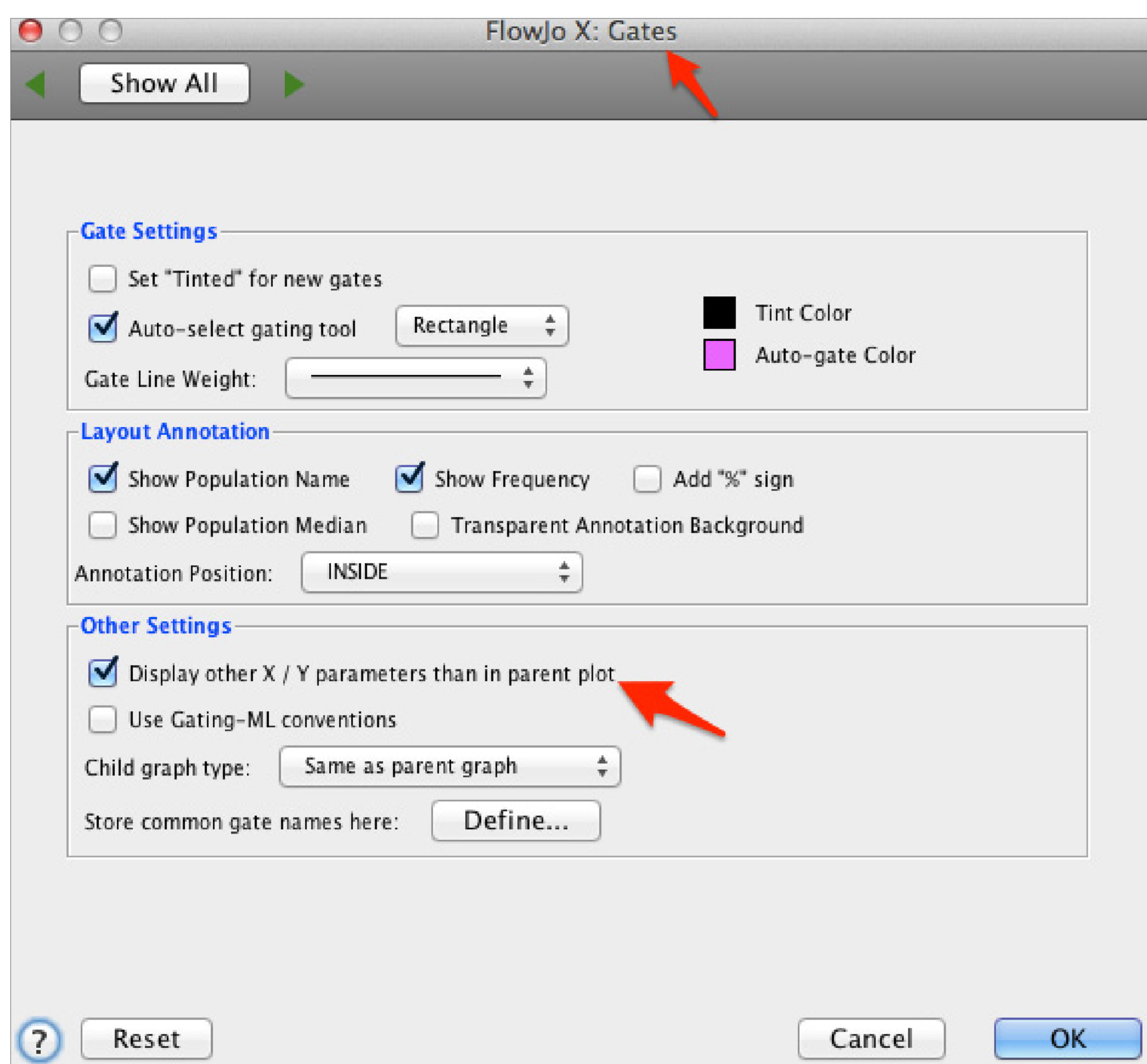
3. Get rid of annoying annotations.

The annotation popping up right in the middle of any gate I create is annoying. The good news is you can change the preferences to cause it to pop-up in some other location. You can also change the pop-up itself. You can even make the background transparent. It makes the text a little harder to see on some samples, especially dense populations, but it gets rid of the white box around your gate annotation. Bonus—you can edit the default settings here too, like if you want to get ride of the % sign.



4. Keep the parameters of your child gates the same as your parent gate.

I don't like how VX changes the parameters I'm looking at when I view a child population. I prefer to see the gated population first. Maybe it doesn't bother you as much but, if you want to change it, you can.



5. View the keywords of your files.

Keywords are important and can tell you a substantial amount of information about your files. Many of them are always recorded with your data, like the cytometer you used (\$CYT), the date the file was acquired (\$DATE), and the start time and end time (\$BTIM and \$ETIM, respectively).

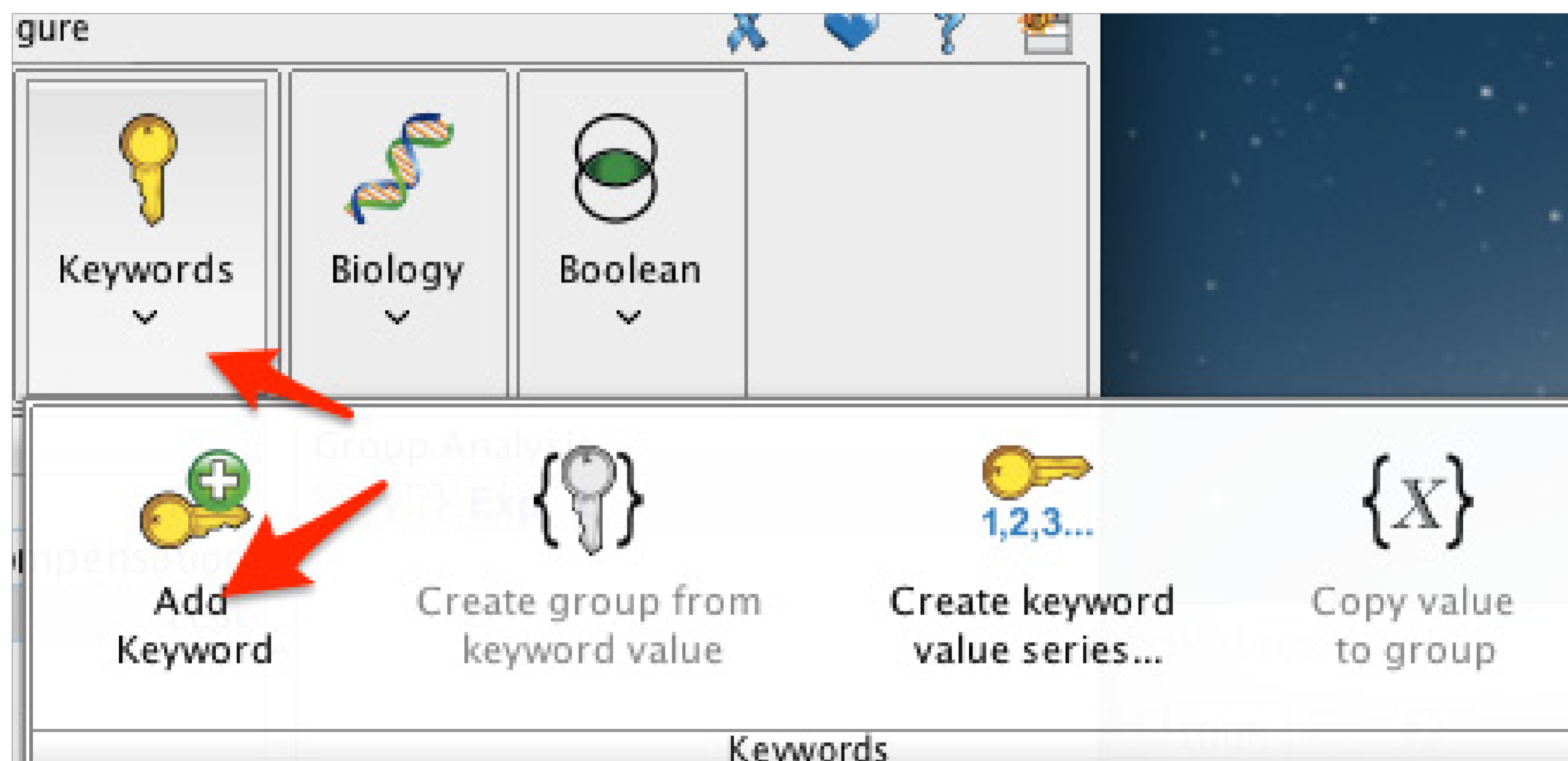
Name	Statistic	#Cells
A1.fcs		34711
A2.fcs		56304
A3.fcs		78269
A4.fcs		65803
A5.fcs		70587
A6.fcs		23989
A7.fcs		63319
A8.fcs		32545
A9.fcs		23529
B1.fcs		37132
B2.fcs		46747
B3.fcs		76153
B4.fcs		68916
B5.fcs		71737
B6.fcs		22701
B7.fcs		39218
B8.fcs		30457
B9.fcs		

You can also add your own keywords when you acquire, which will be displayed here. One of the best keywords to remember to use is the \$PnS keyword. Each parameter gets a set of keywords, \$PnN and

\$PnS. \$PnN is recorded by the machine, like \$P4N may equal FL1 or sometimes 525/50 or something. \$PnS is the stain name, so you can put the target like CD3 here or you can add an entire conjugate like CD3 FITC.

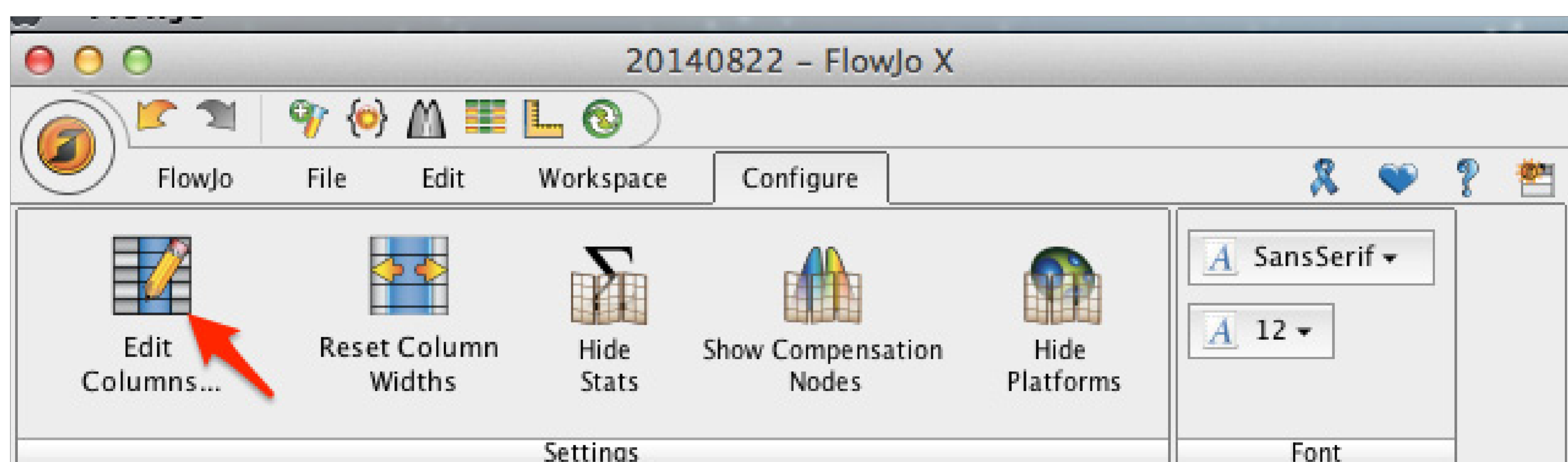
6. Add new keywords.

Maybe you forgot to set a keyword up during acquisition because you were in a hurry. Don't worry, you can still do it in FlowJo VX. There's a blank column in the workspace where you can enter in whatever values you want. Here, you can use some cool tools like "copy value to group" under "groups" and "create keyword value series under "keywords."



7. Edit existing keywords.

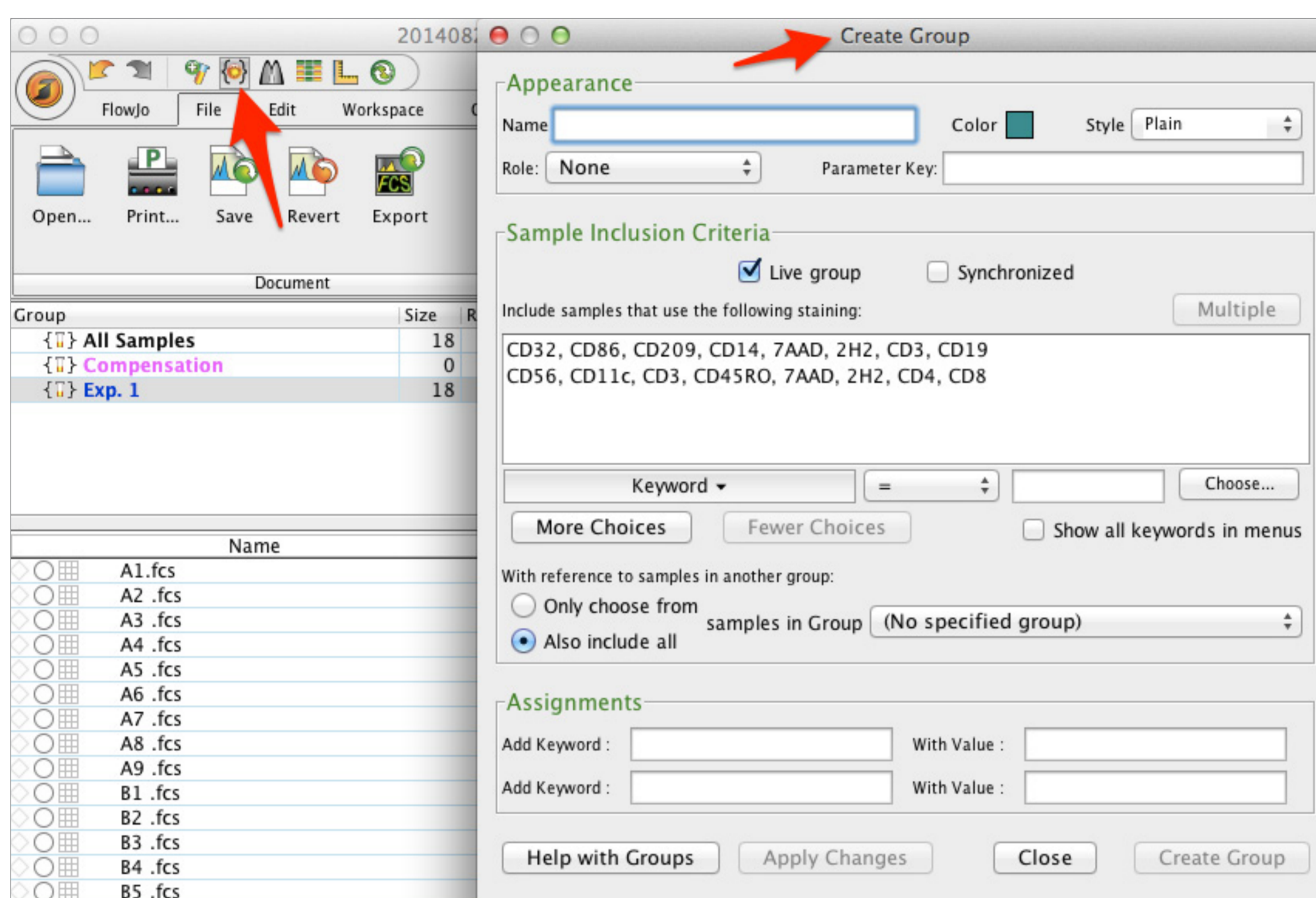
When you add a keyword to your workspace, you can edit it in the workspace. Just double-click in the cell to edit. Note: any edit in the workspace does not change the keyword within the file. Hence, if you take this file to another workspace, the keyword edits are not transferred.



Name	Statistic	#Cells	PE-Cy7-A reagent
B1 .fcs		37132	CD45RA
B2 .fcs		467	CD45RO
B3 .fcs		7157	CD45RO
B4 .fcs		68916	CD45RO
B5 .fcs		71737	CD45RO
B6 .fcs		22701	CD45RO
B7 .fcs		54464	CD45RO
B8 .fcs		39216	CD45RO
B9 .fcs		30457	CD45RO

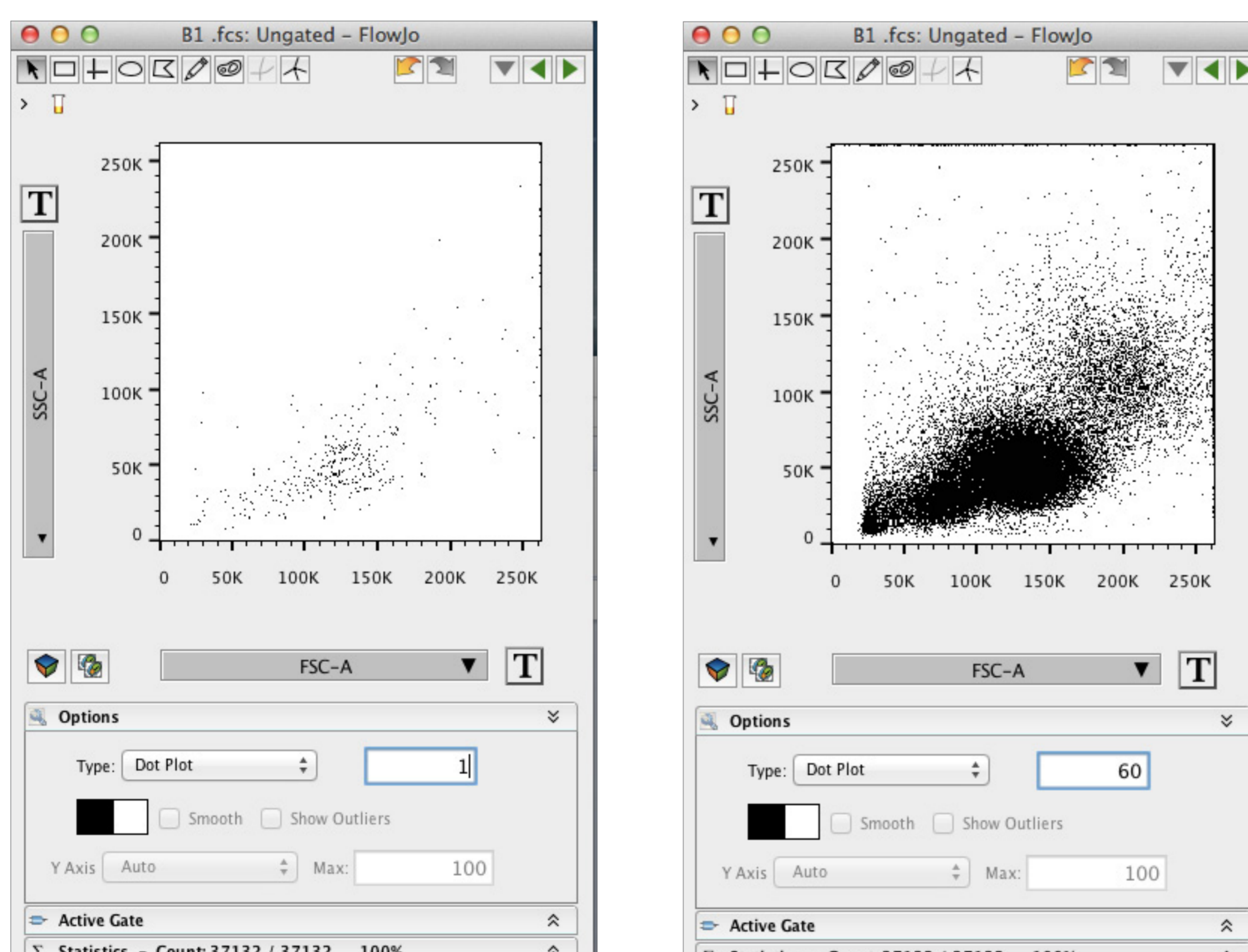
8. Use groups to organize samples.

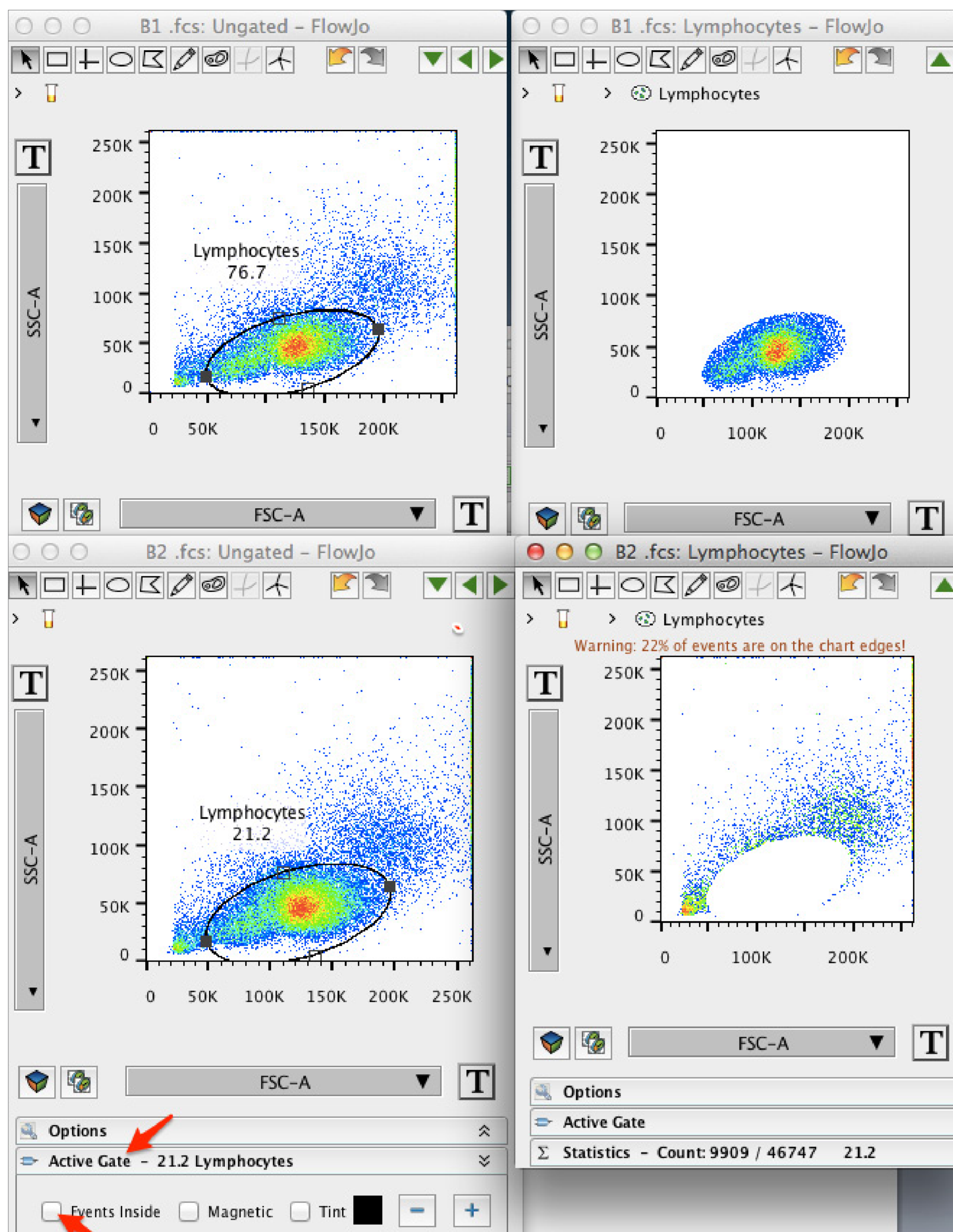
Groups are great for organizing samples for common analyses. When batching gates, you can separate samples into different groups and ensure that the gate settings you want are only applied to the samples within that group. Different patient samples, tissue types, drug treatments, or time points are all examples of how you might separate your samples into different groups.



9. Edit the number of dots displayed.

Most of FlowJo's plots are density based, which means they show you all of the data by default. However, dot plots can be used to control the exact number of dots displayed. Anything greater than 101 will be that exact number of (random) events. Anything entered between 1-100 will be a percent of (random) events.





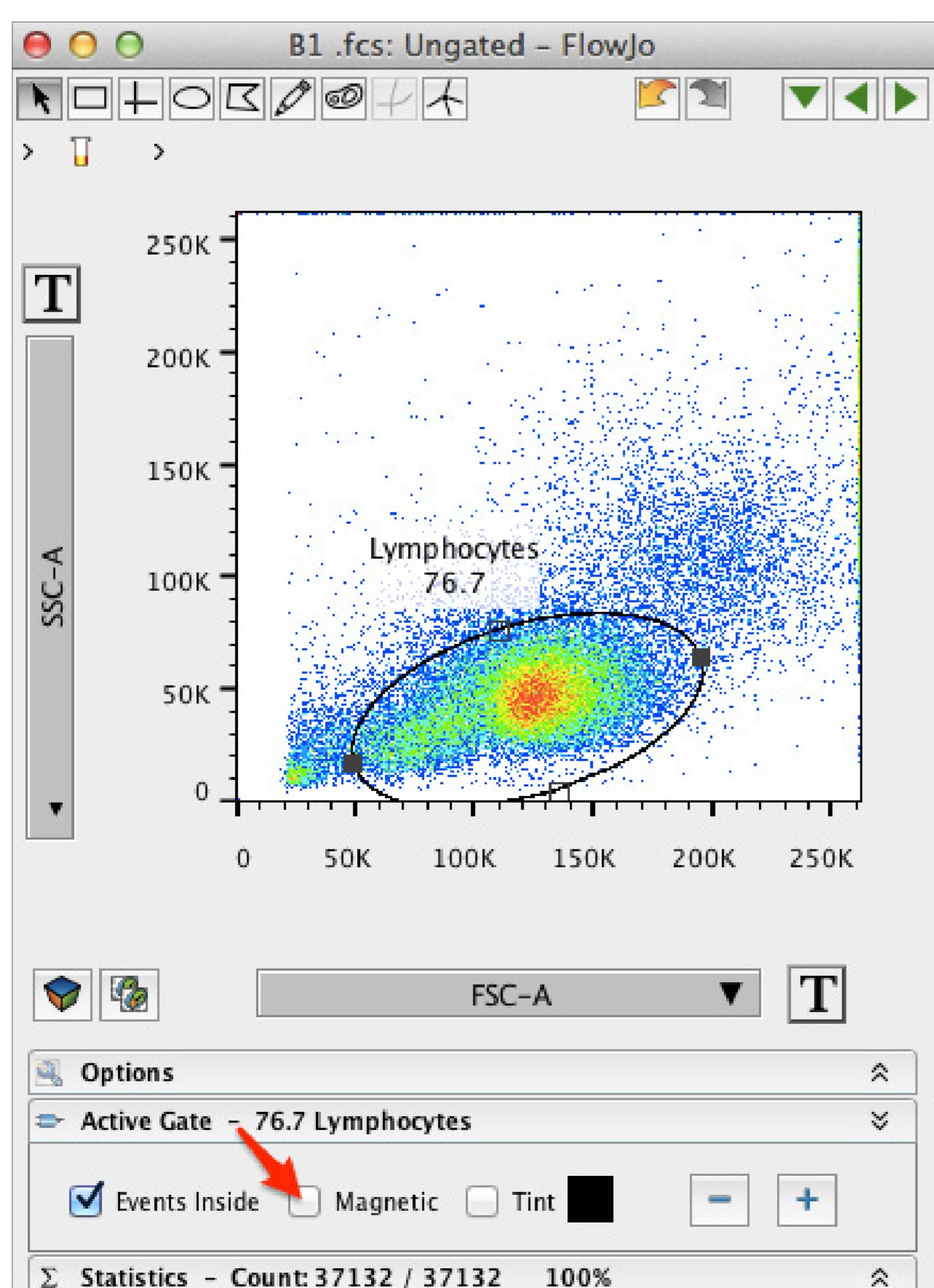
10. Make NOT gates in the graph window.

You can uncheck the 'gate events inside' box to make a NOT gate.

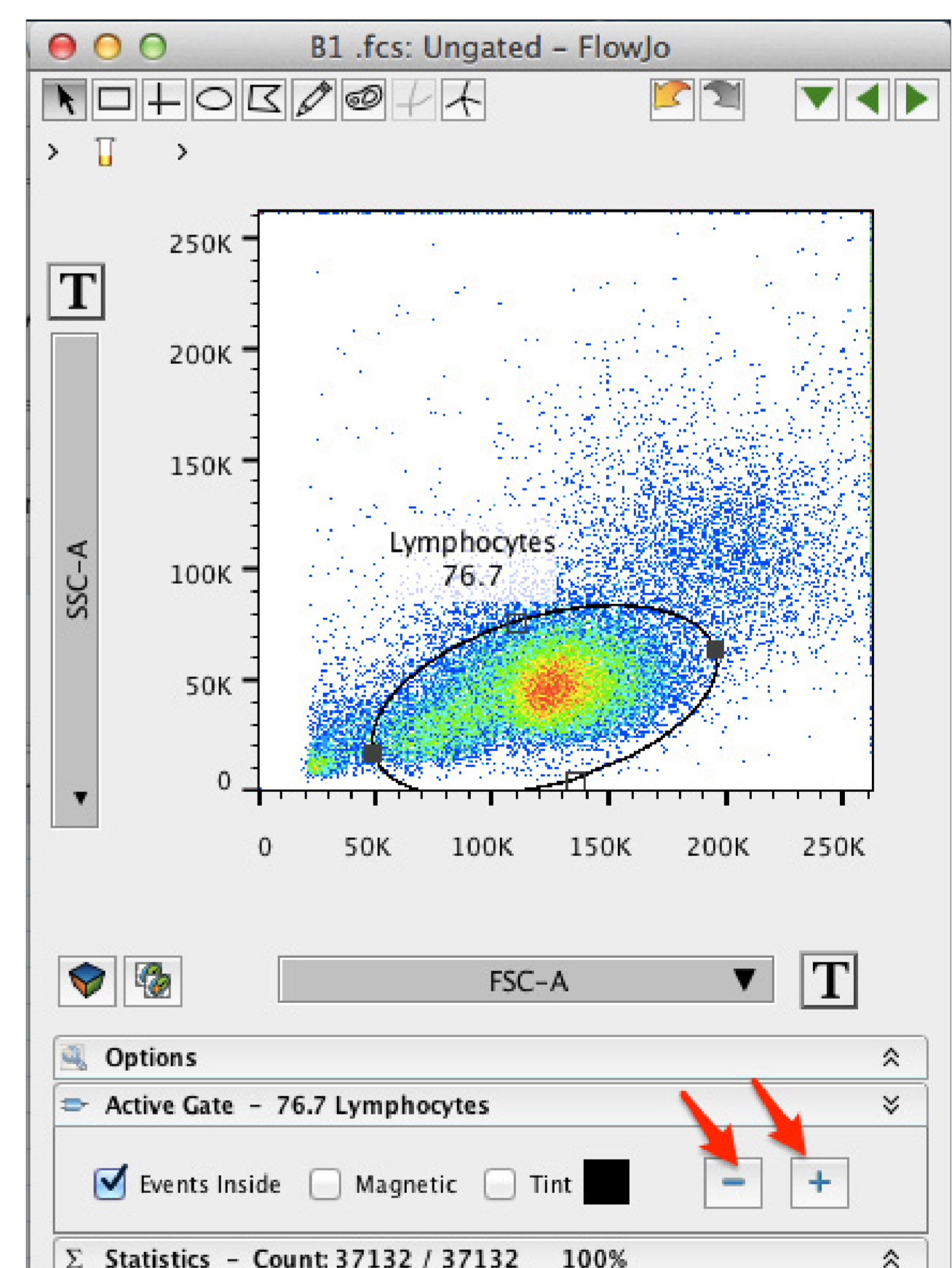
11. Use the plus and minus signs to quickly increase and decrease the gate size.

I love this new feature in VX; it's very useful.

12. Use magnetic gates.

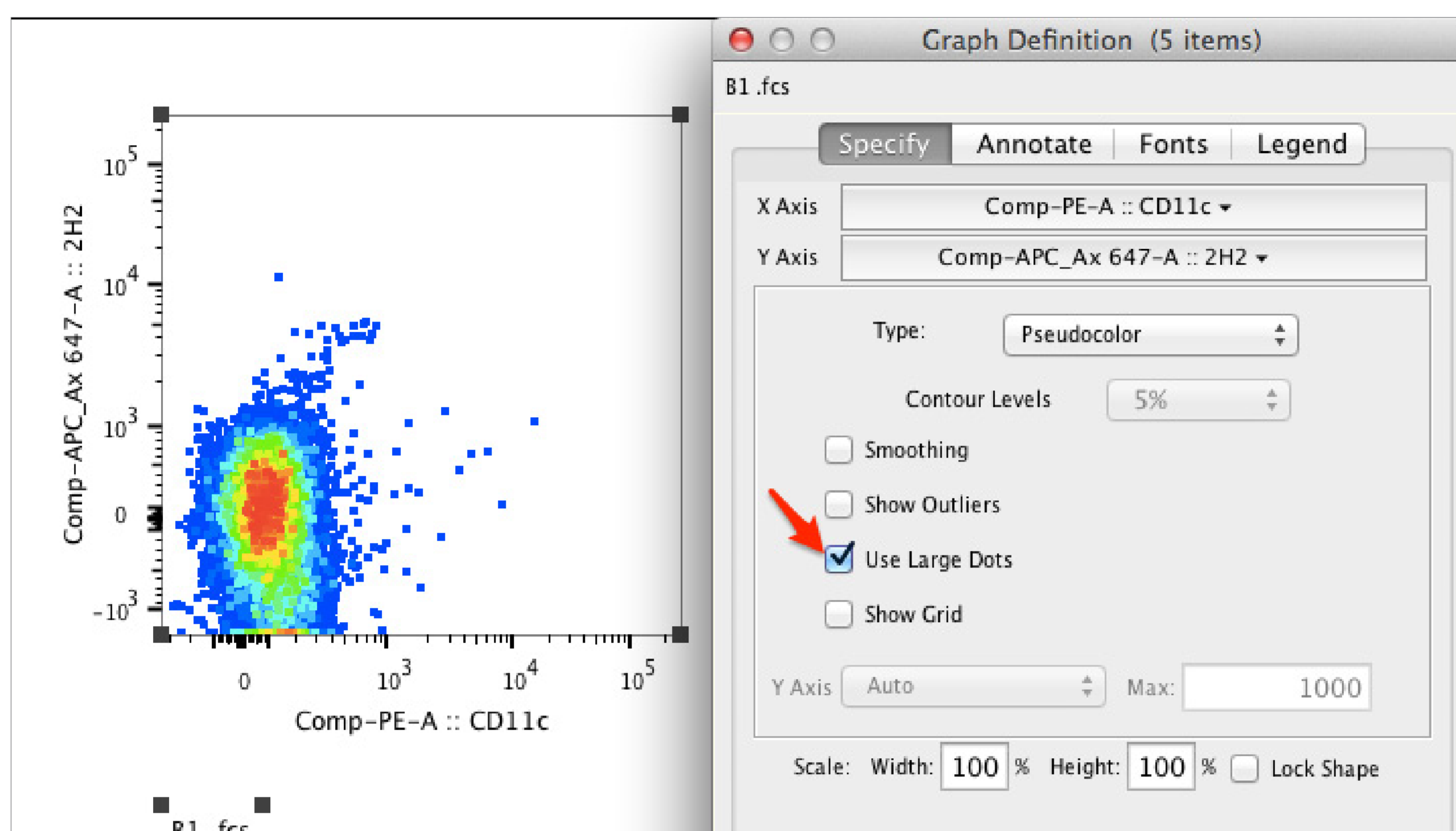


These are great for batch processing. If all your patient samples have slightly different profiles, you can set a magnetic gate on the first sample, batch it to the group, and the magnetic gate will move around for each sample to align the center point of the magnetic gate to the densest part of the population.



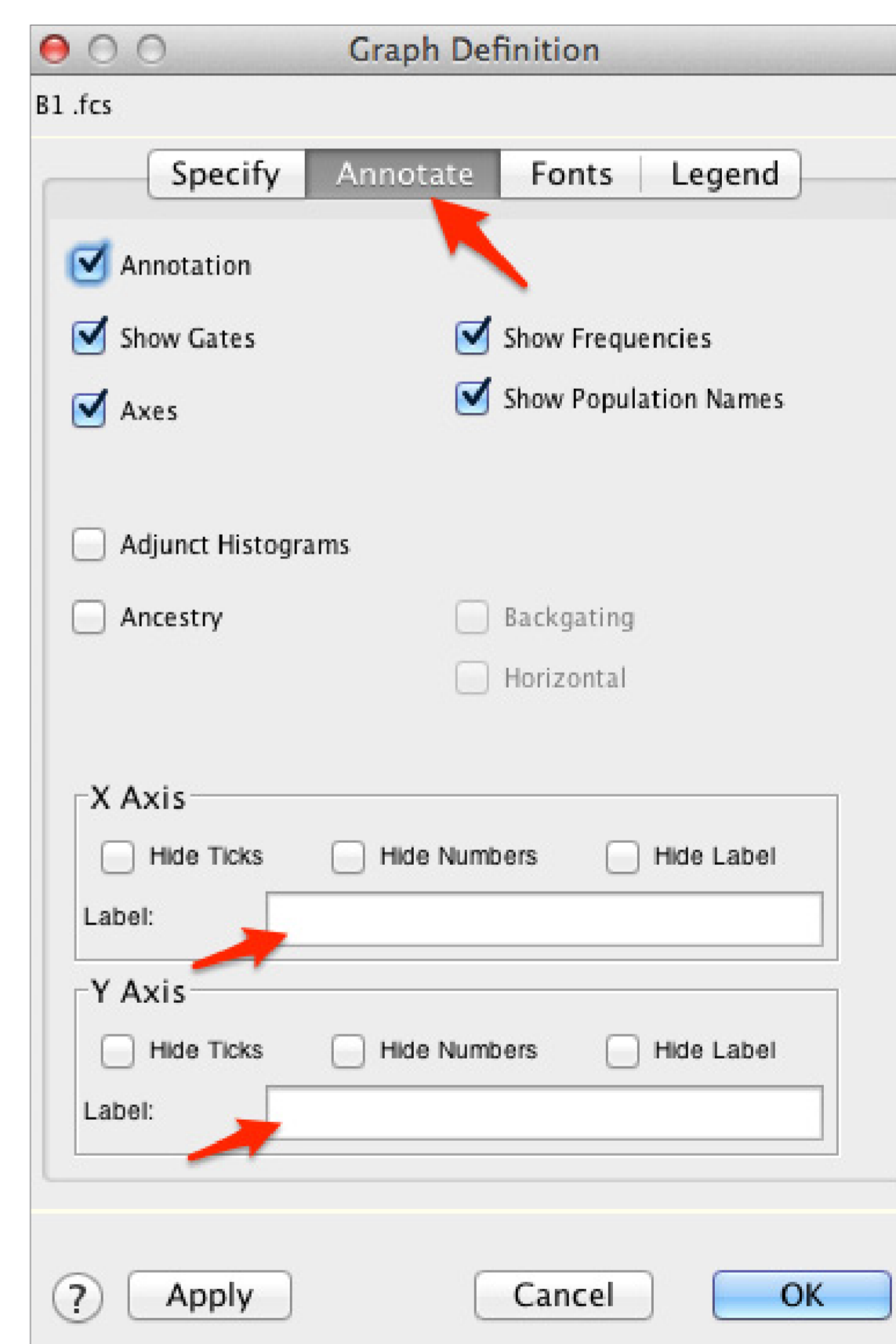
13. If you only have a few events, use the option to “show large dots”.

When you only have 4-5 events in a population, it can often be difficult to see. If you turn off high resolution, you can see the data better. All you have to do is double-click on a plot in the layout editor and under the specify tab, check the box for ‘Use Large Dots’.



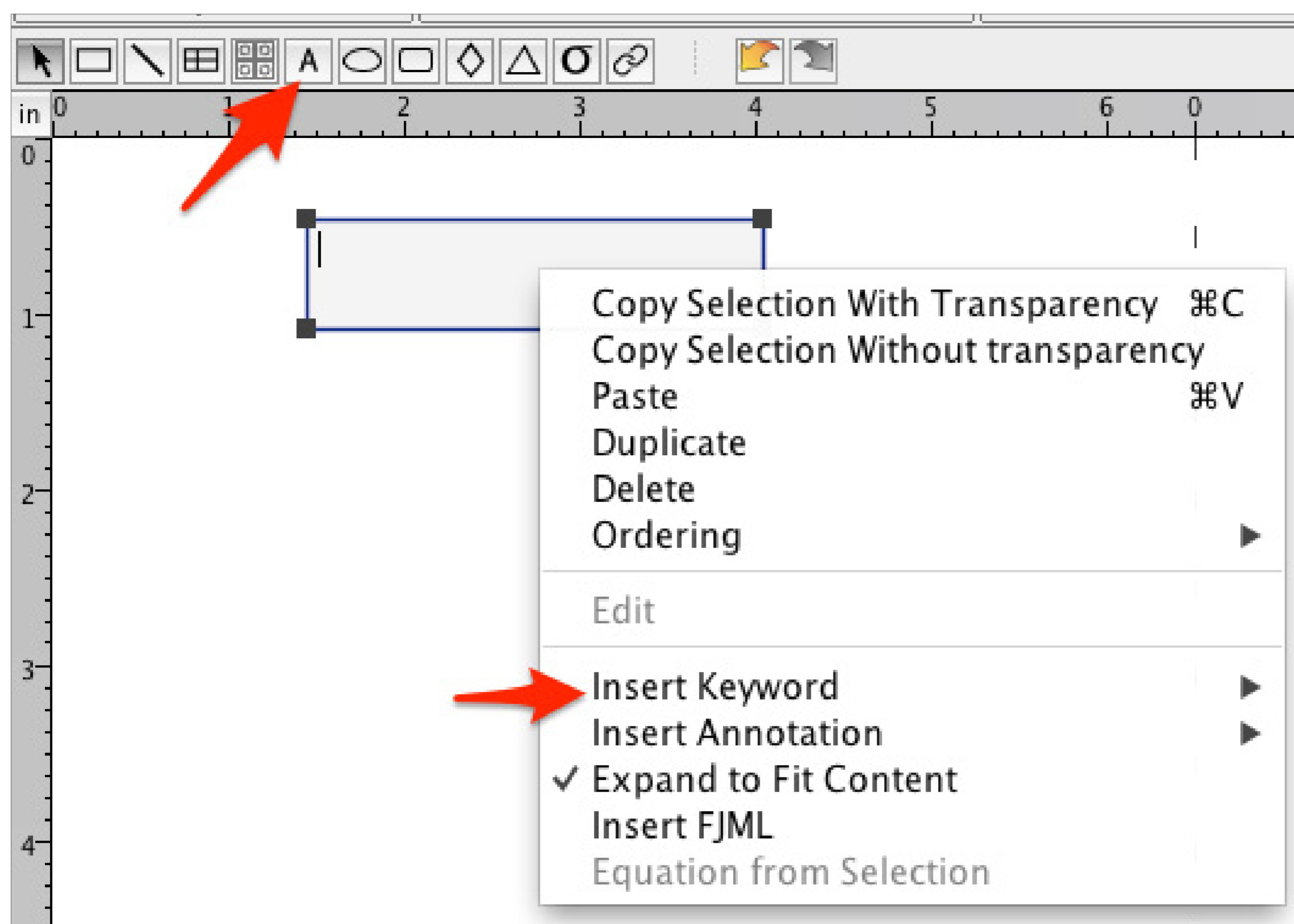
14. Make sure your axes are labeled properly and cleanly with something that makes sense.

Many cytometers have a default axis label like FL1, or 525/50, as two examples. Some scientists doing flow are savvy enough to use the \$PnS keywords to enter in their own axis labels during acquisition. However, if you don't want to or don't have time, you can edit the labels right in FlowJo. Simply double-click a plot and use the annotate tab to edit the text, and then the fonts tab to edit the font style. In the annotate tab, you can enter the text you'd like for each label. In the fonts tab, you can make the text bigger, bold, or even change the color.



15. Add a text box to embed keywords, and even statistics, right into your figure.

To add a text box to your layout, right-click on it and “Insert Keyword”. Or, drag a statistic from the workspace into the layout editor. Embedding keywords and statistics right into the layout when generating your figures

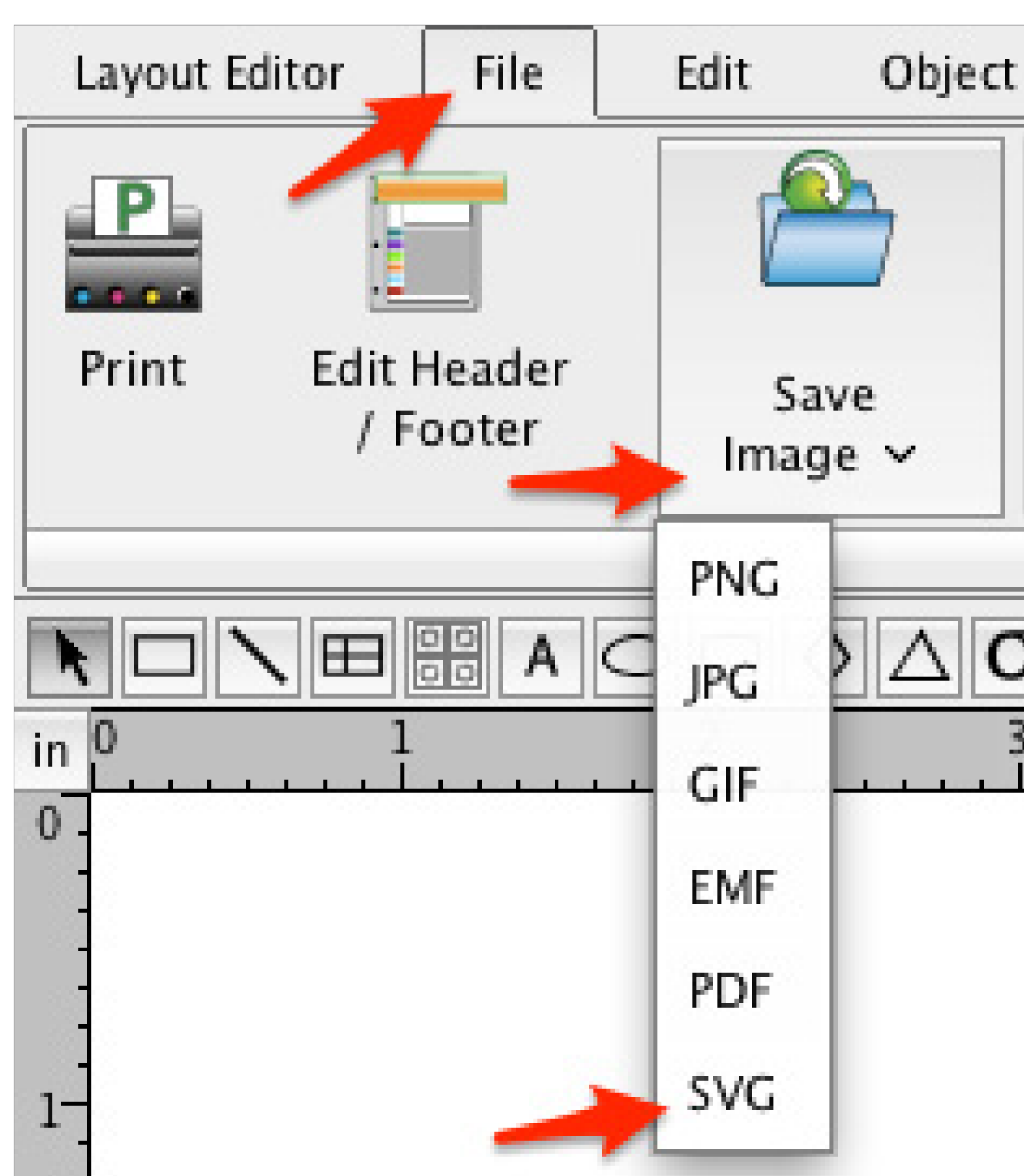


will ensure your figure is annotated properly with useful information. You can then double-click on the text box, and with the slider on the right, you can edit the text size and style. You can even create equations from

statistics by selecting the statistics, right-clicking, and selecting equation.

16. Use SVG for export.

Scalable Vector Graphics (SVG) are high resolution, high compression images that can be ungrouped in Adobe Illustrator, Photoshop, Intaglio, and Canvas. If you want to manipulate your graphics, you should use a program like the ones mentioned above. They are designed for graphics manipulation and management. PowerPoint (PPT) is great for presenting, but not for graphic editing. If you insist on using PPT for graphic editing, export as EMF. While not as good quality as SVG, EMF will allow

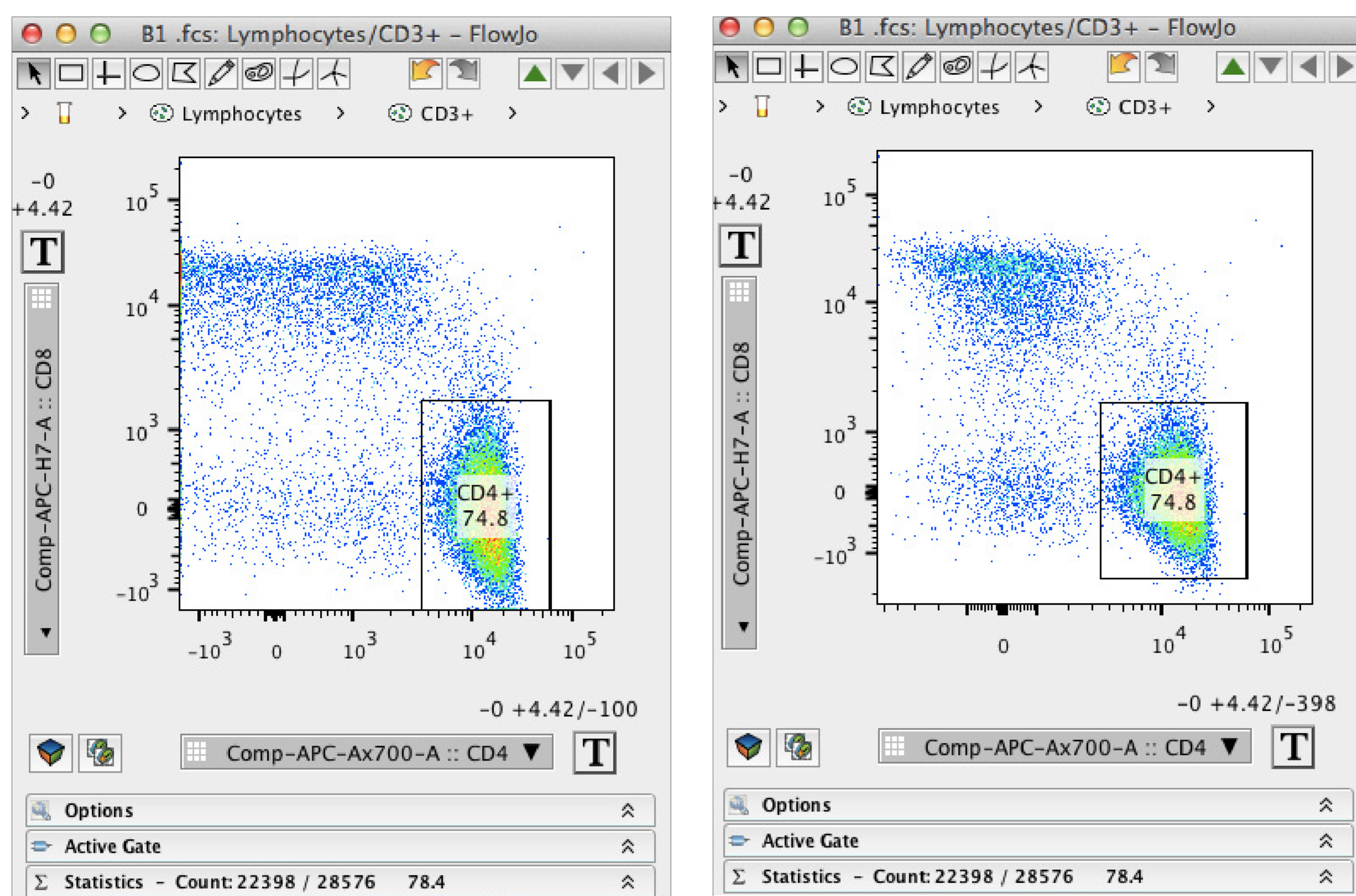


editing in PPT. PDF provides the best resolution out of FlowJo (300 dpi), but cannot be ungrouped. (For comparison, all other formats are at 70 dpi.) So, it may be best (easiest) to try to do all your graphics manipulations in FlowJo's layout editor and then save right as a PDF. If FlowJo doesn't provide all the tools you need, then use Illustrator or Canvas, but you'll have to export as a SVG to ungroup in those programs.

17. Change the width basis.

Use the T button in the graph window to adjust the width basis if you have any events on the axis, or if your negative population is very

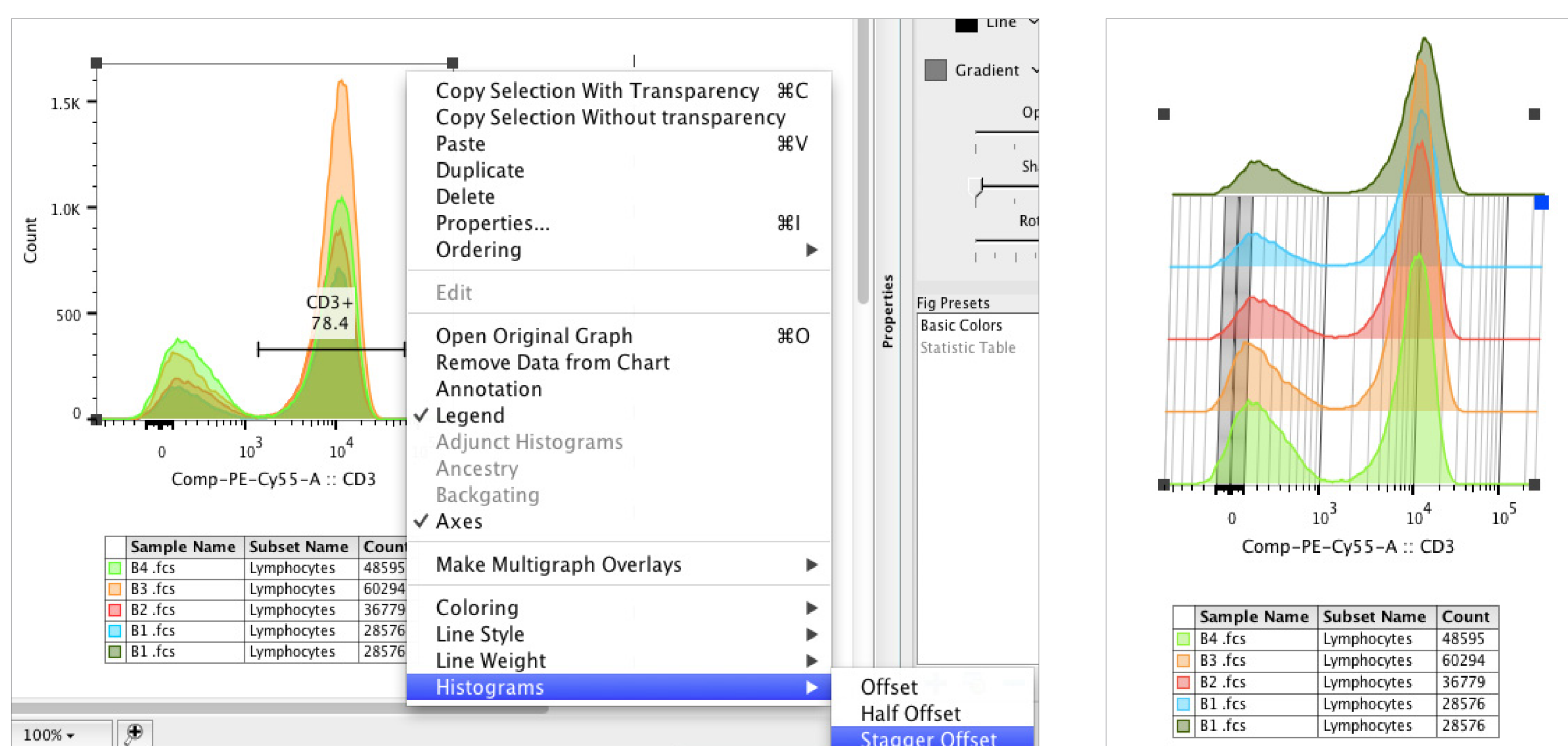
dispersed. Increasing the width basis (making it more negative) will tighten



the data up and give it a cleaner appearance. But, be careful to not increase the width basis too much—you don't want to add a lot of white space below the data, as this is lost space.

18. Offset your overlays.

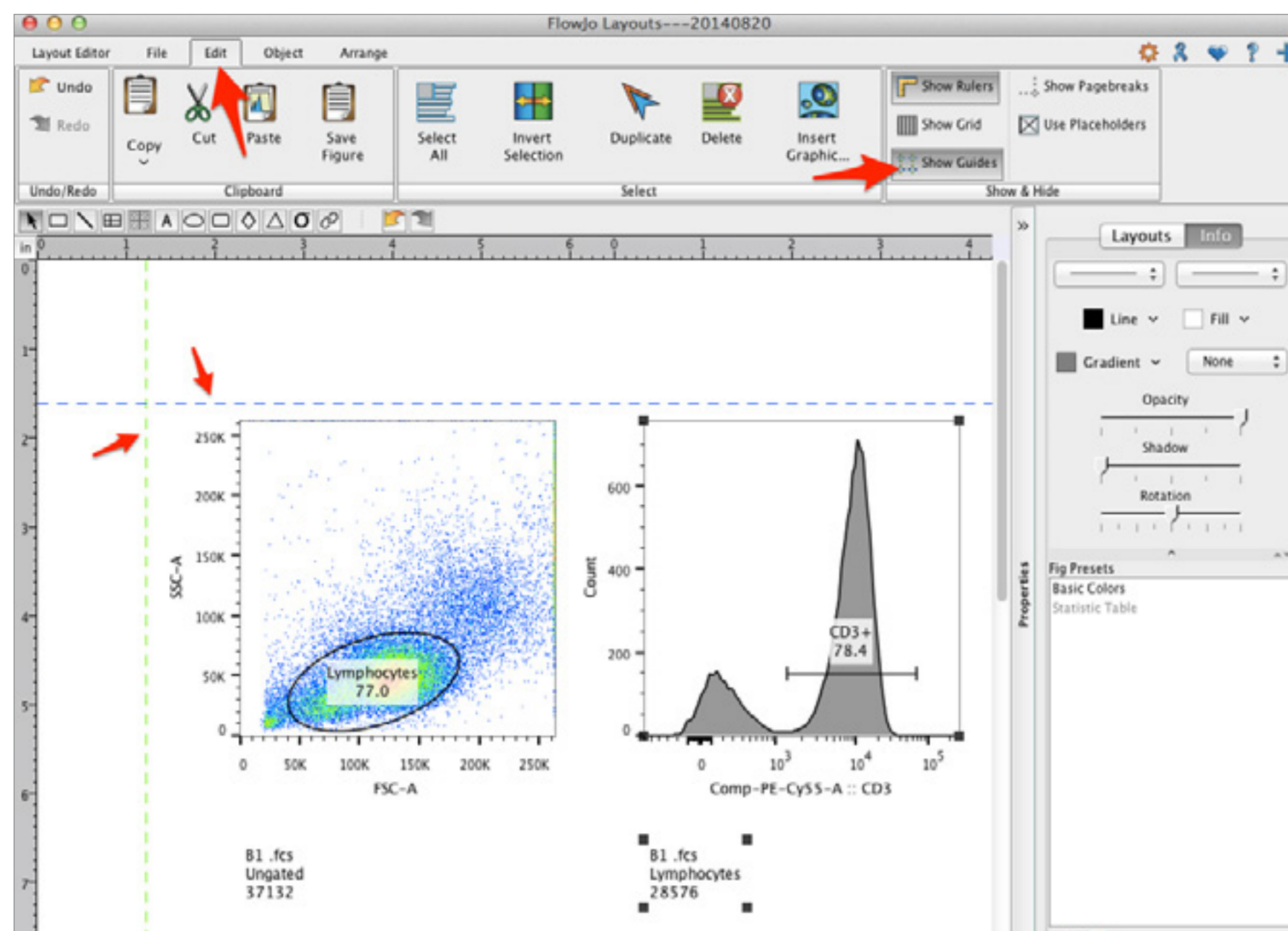
Histogram overlays are a great way to show changes in expression profiles between samples. However, it can be difficult to visualize differences, especially when the plots are right on top of each other. To view these differences, right-click on an overlay, scroll down to histogram, and click stagger offset. Then, in back right corner will allow you to edit the perspective to make a “3D” view of the data.



19. Use “guides” to align your plots.

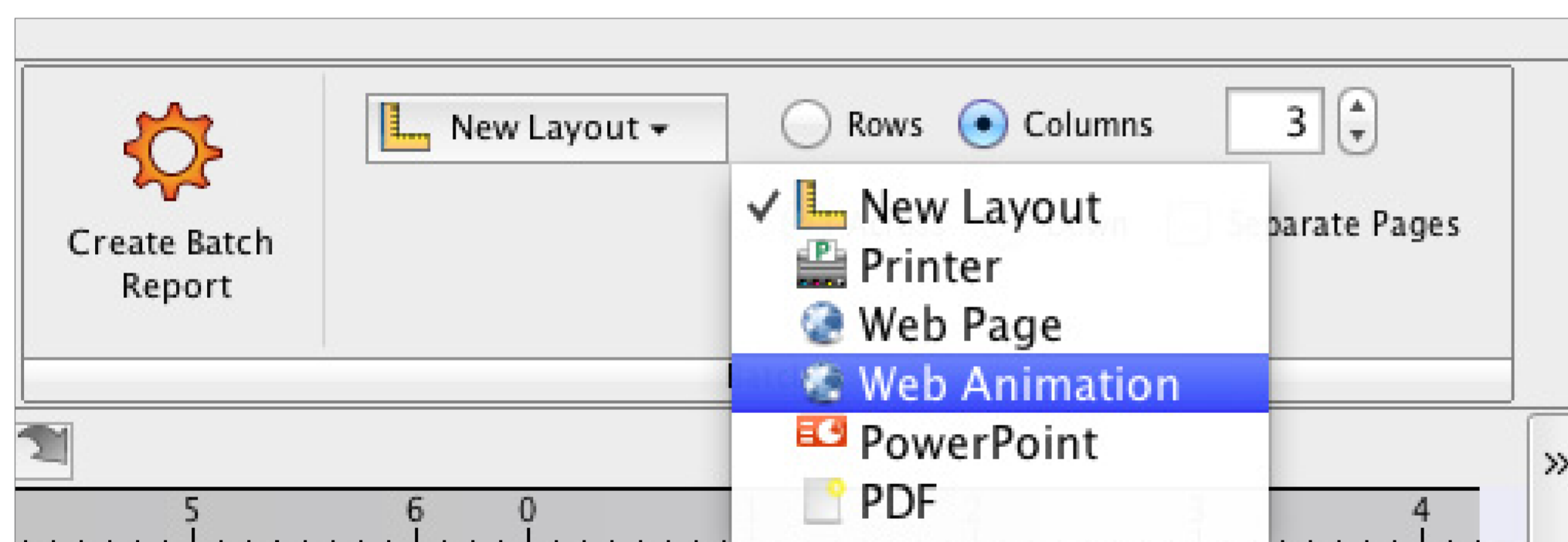
There are “snap-to” guides in FlowJo VX's layout that will allow you to quickly align your plots. You can also use the align features, which are under the Edit tab in the layout editor. This is where you can turn the

guides on. Finally, the arrange tab in the layout editor allows you to edit and align the tops, bottoms, centers, of your graphs and objects.



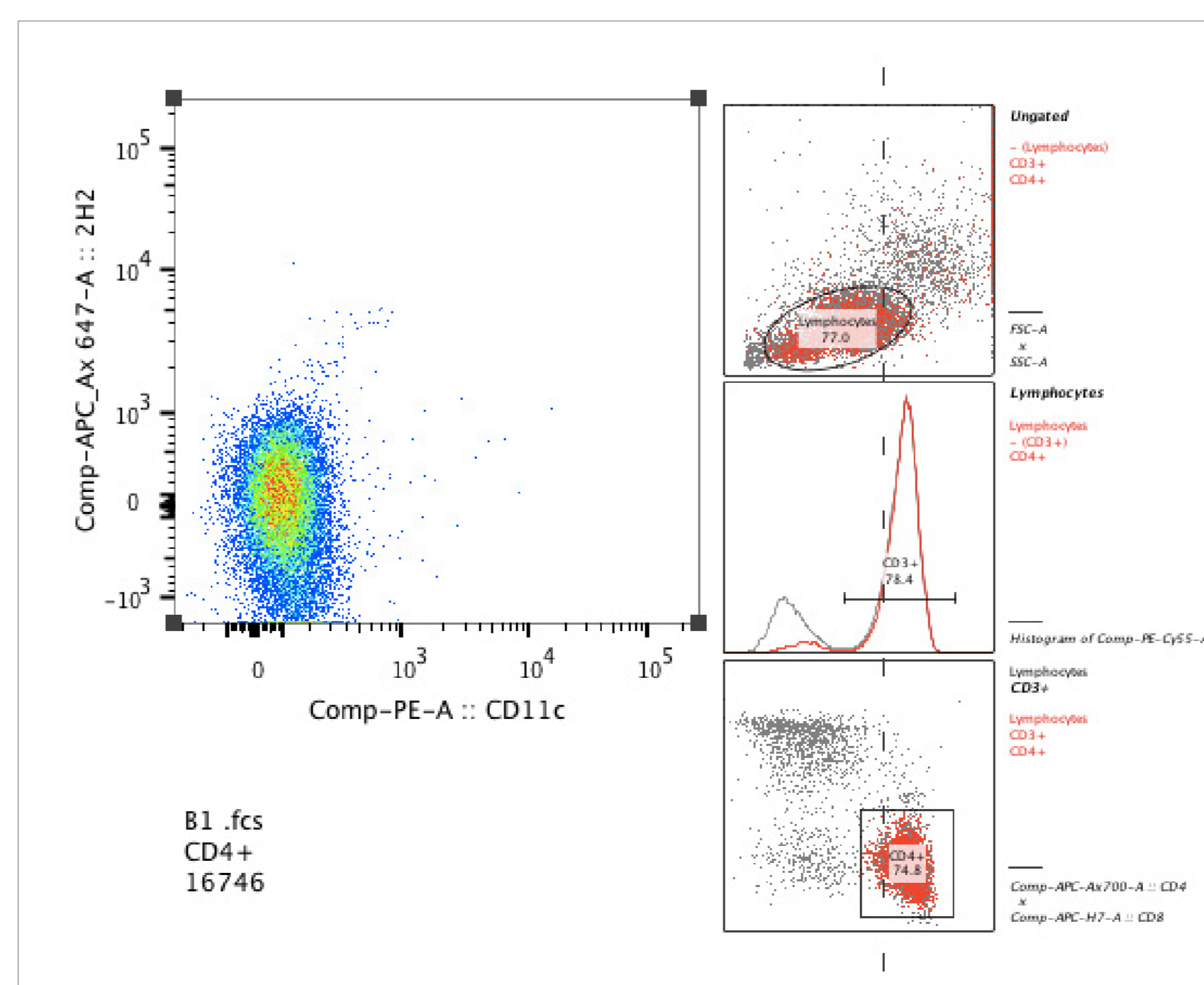
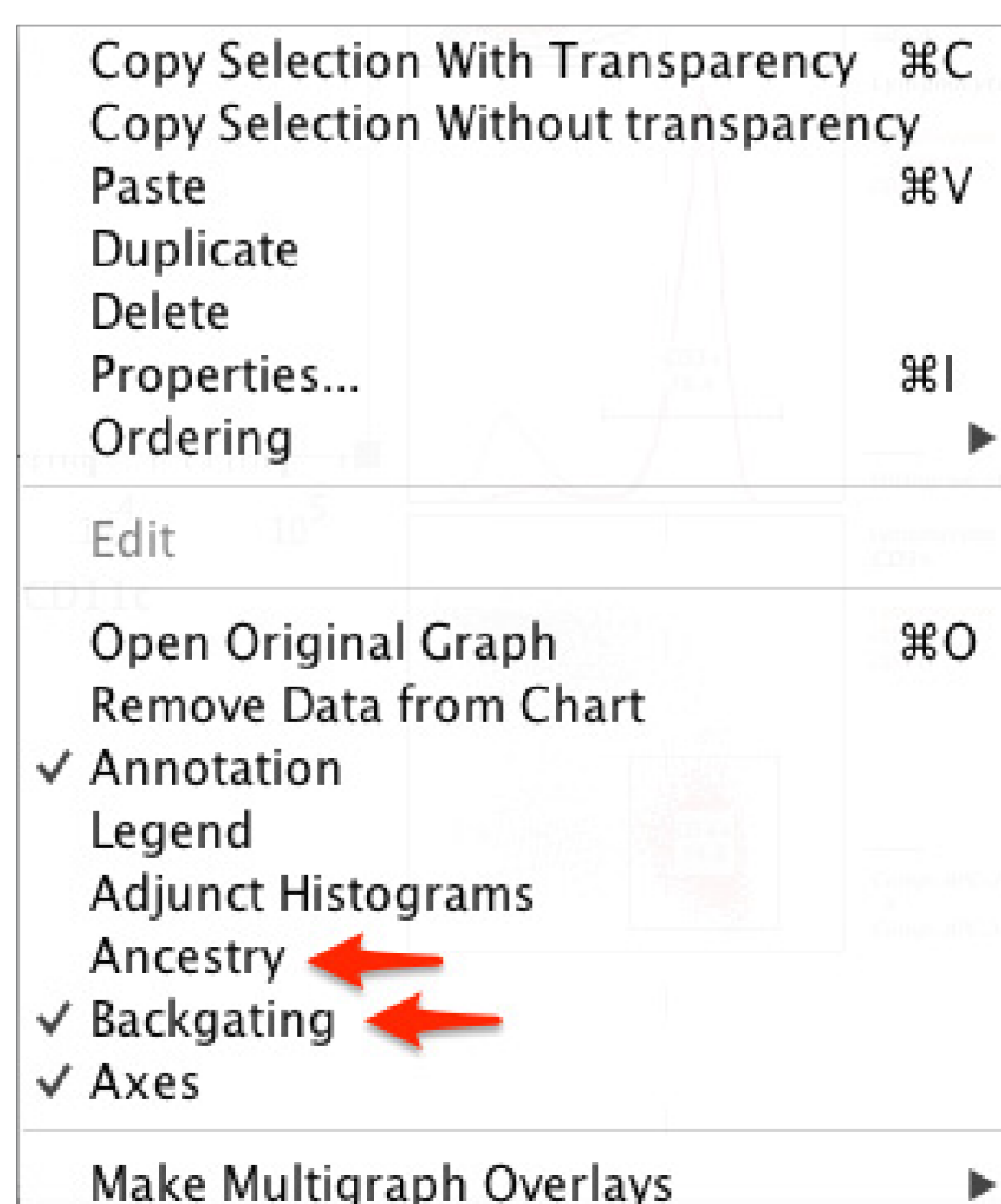
20. Try the batch to “web animation” feature.

This is an especially useful feature when you are trying to visualize changes in the fluorescence profile over a time series or when titrating a treatment with your samples.



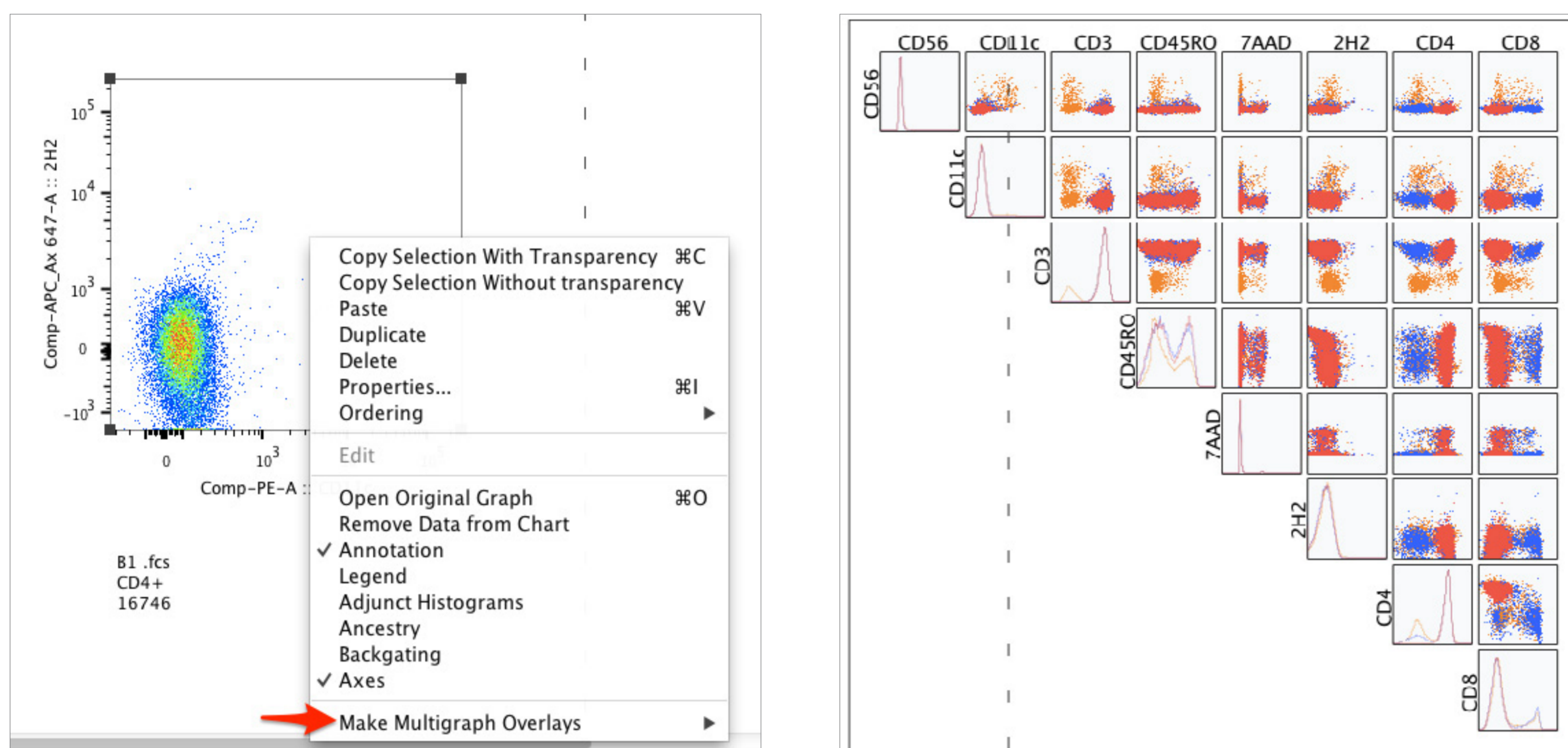
21. Use ancestry and back gating to get your point across quickly.

Right-click on a plot and select options for ancestry and back gating. Ancestry will show the derivation/gating strategy you used to isolate the population that is being viewed. The back gating feature will help indicate how each gate in your hierarchy contributed to the final population.



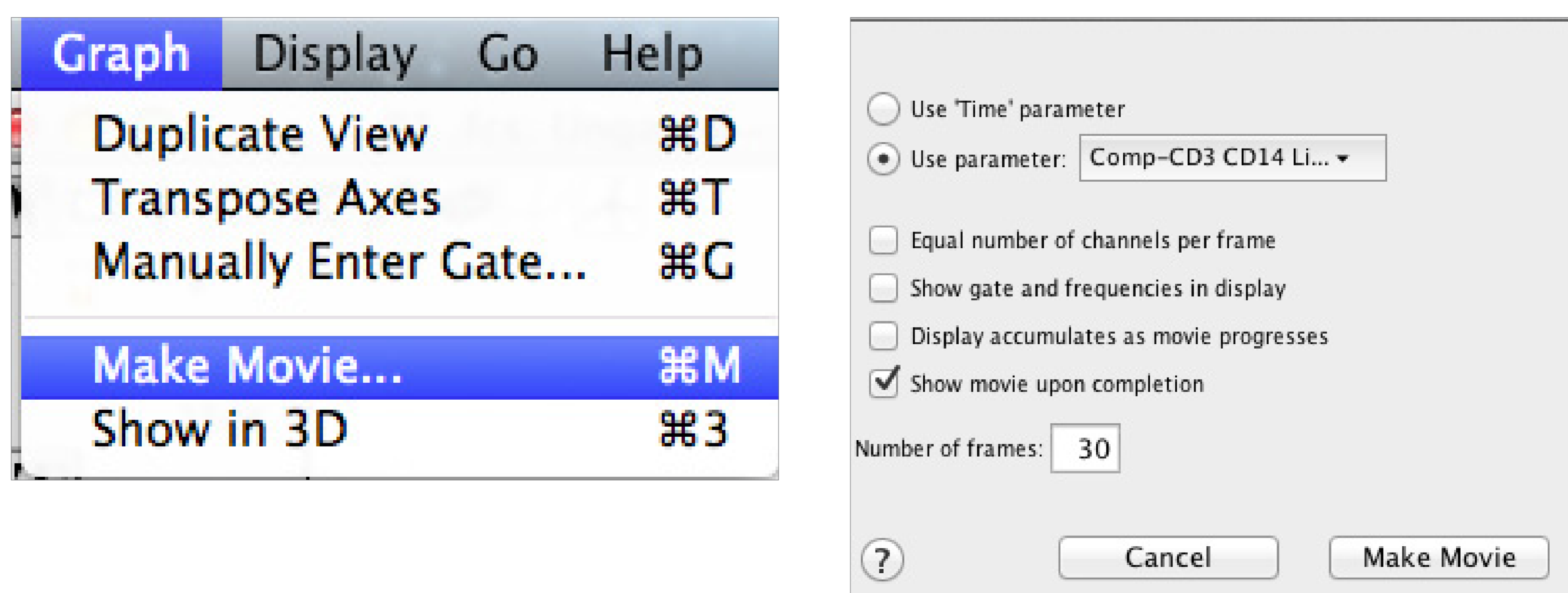
22. Explore your data with multigraph overlays.

The multigraph view can help you visualize patterns in your data that you might otherwise miss. To make a multigraph overlay view, right-click on a plot in the layout, then scroll down and select “make multigraph overlays”. From here, you can choose to view all the histograms, all parameters by Y, or NxN. By exploring these features, you’ll quickly see their utility.



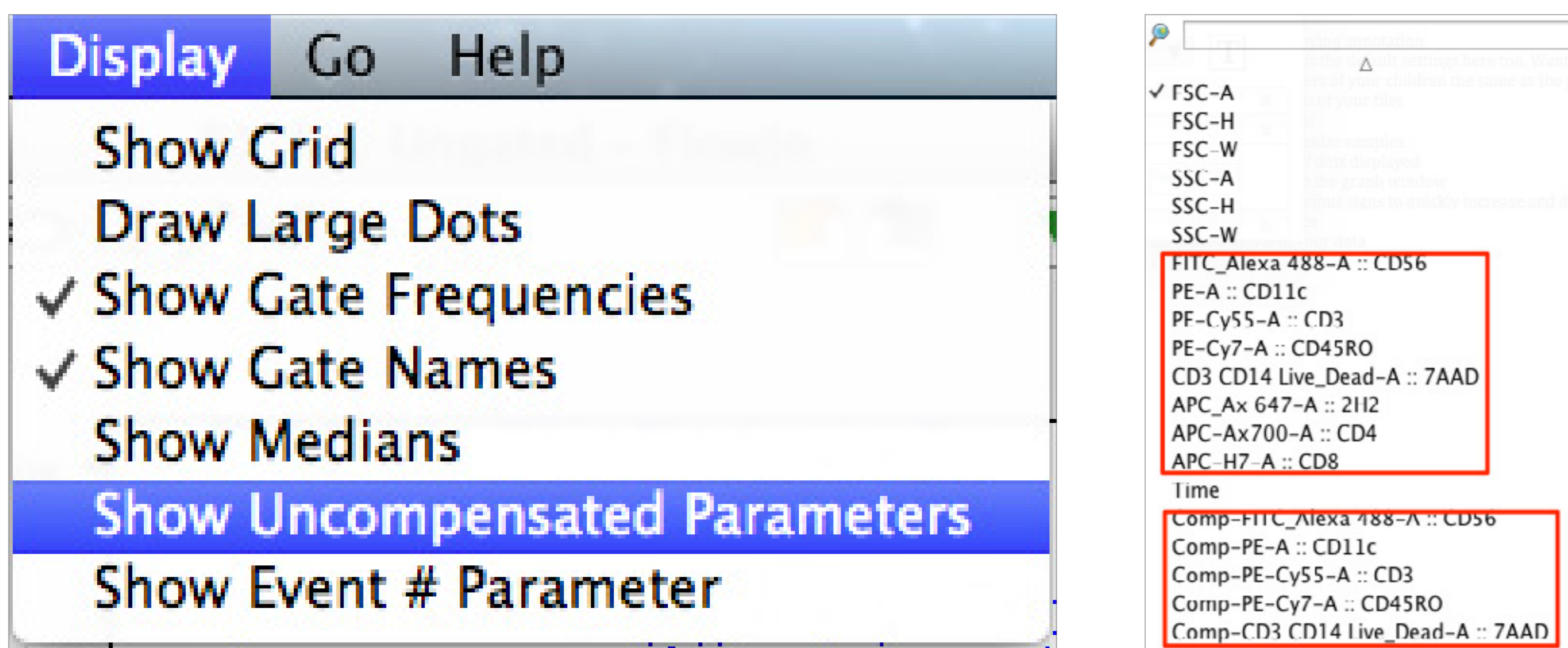
23. Make a movie of your data.

This is a really interesting feature that very few people use. Making a movie allows you to display the expression of two parameters using a third parameter as a control. For example, it is an excellent way to show expression profile of two parameters over the course of time (a third parameter). As another example, lets say you want to view the expression of two activation markers over the expression of all CD4+ cells. You could make a graph of the two activation markers, make a movie and set CD4 as the parameter to control the movie. The movie will make slices over the entire CD4 profile and show you the expression of the two activation markers at each slice.



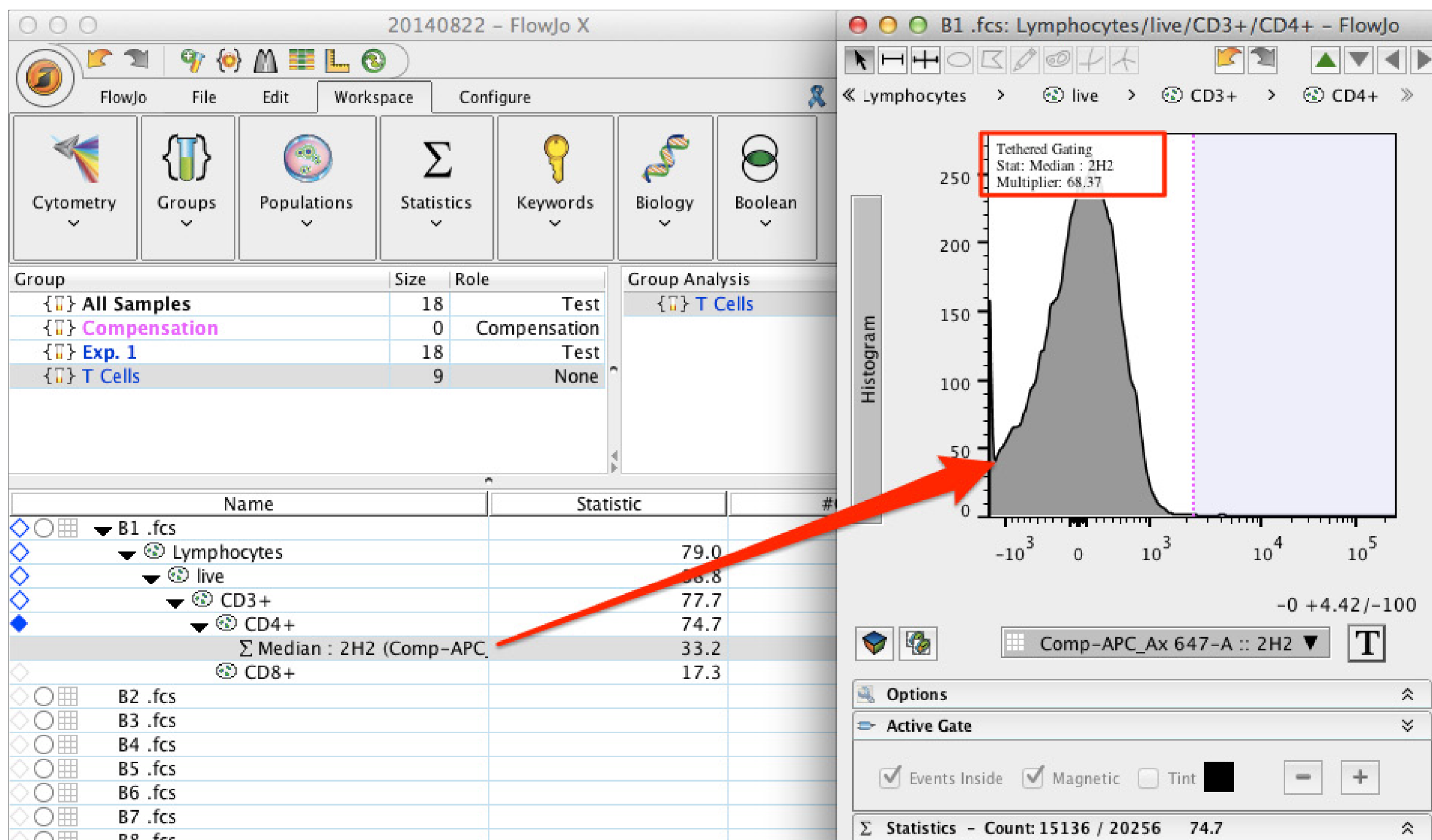
24. Show uncompensated parameters.

If you need to compare compensated data to uncompensated data, this is how you do it.



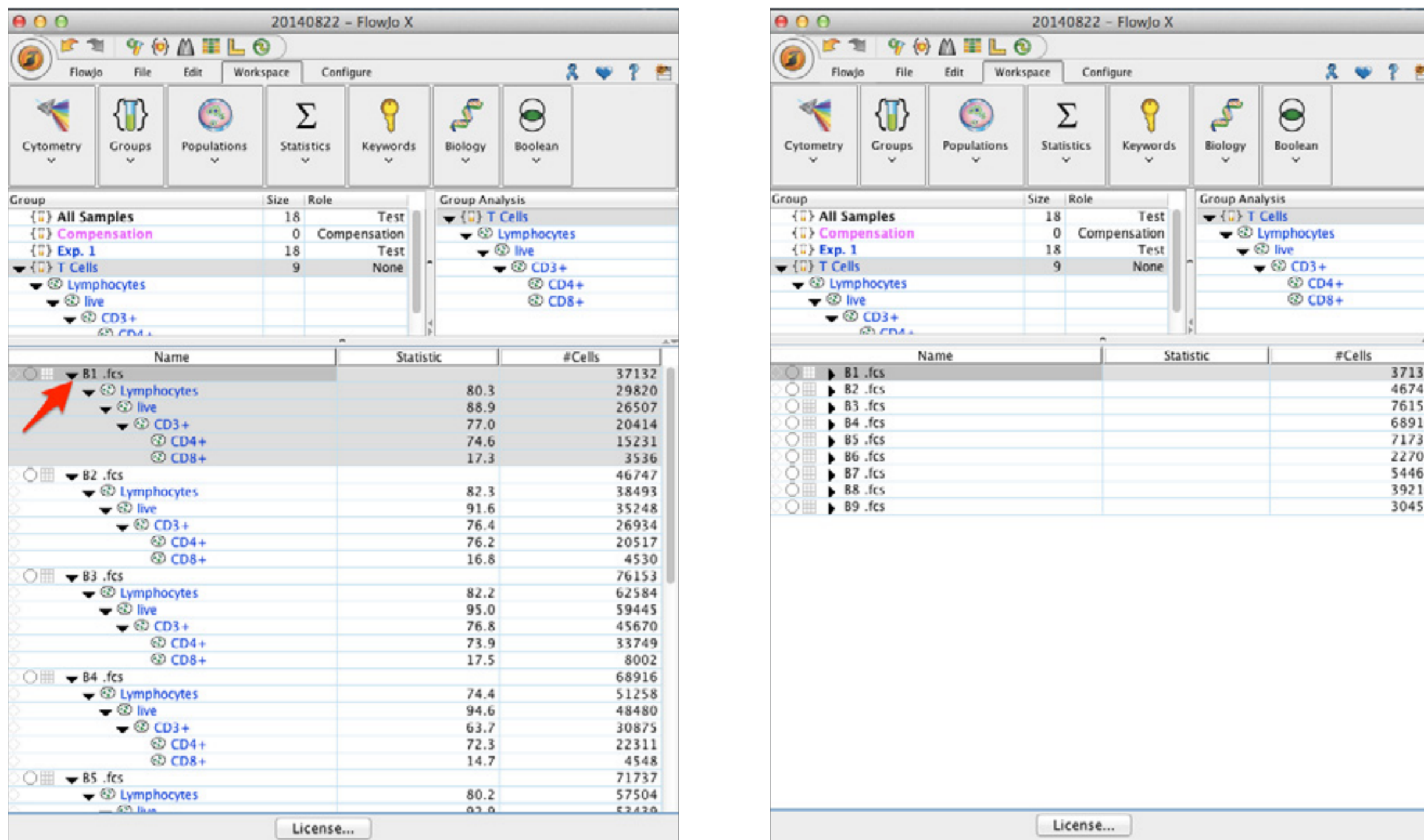
25. Make a gate with a statistic.

If you want to make a gate based on the multiplier of a statistic, simply drag any statistic onto a histogram and it will make an automatic gate based on the statistic. Note: the multiplier will change as you move the initial gate.



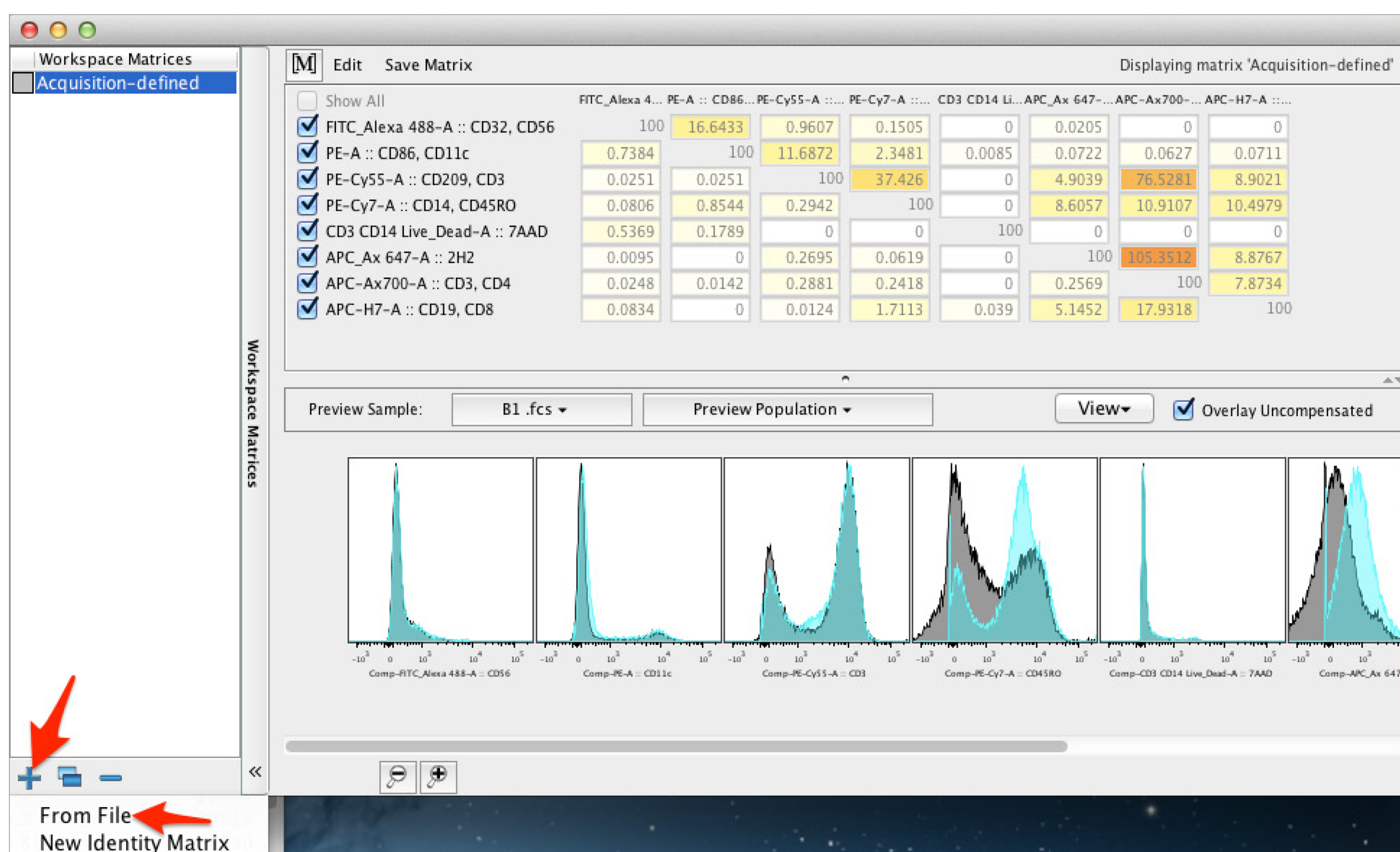
26. Hold down Alt when you click to collapse—it will collapse everything.

If your gating is getting out of control and you're having a hard time finding your samples, just hold down the Alt key while you click on the arrow of a sample. This will make all of your samples collapse.



27. Load an old matrix.

Never use a compensation matrix from a previous experiment... Unless you have to. Lets say you dropped a compensation control, or added bleach to it by accident—whatever—you can still visualize your data (even if you can't publish it). Just go to an old workspace, save the matrix, and load the old matrix for these new files. The compensation won't be correct, but it "should" be close enough to visualize your data. Note: all your fluors have to be the same to do this.

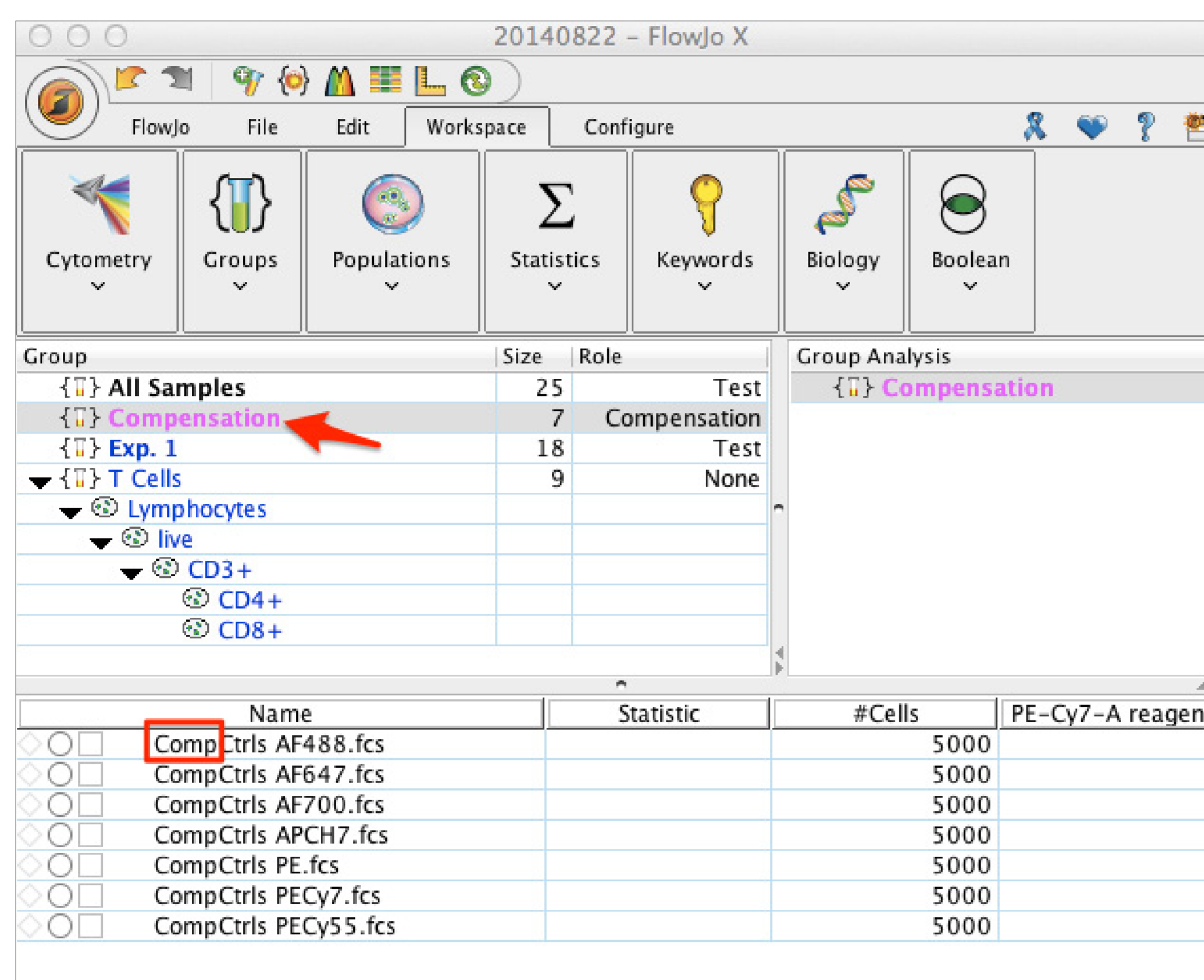


28. Create an entirely new matrix.

You should never have to do this but, if you do, here's how. When you create an entirely new matrix, you'll get a blank matrix to subjectively add compensation values to. Of course, you should never try to publish data that uses a completely subjective compensation matrix.

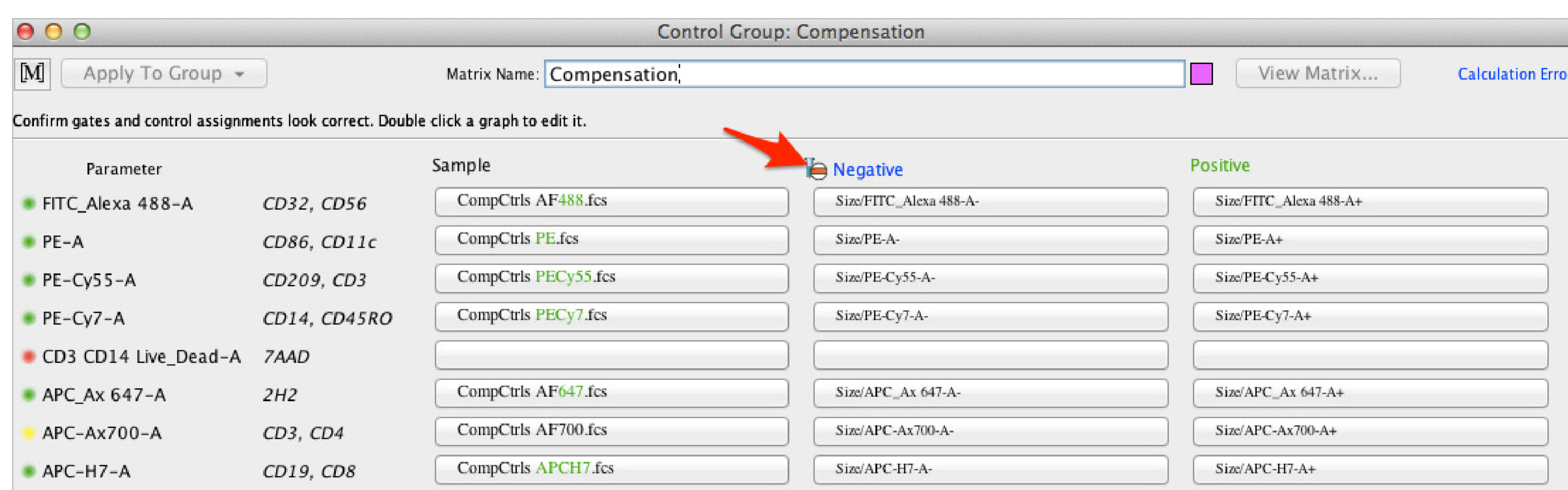
29. Use the phrase “comp” in your compensation controls.

This will cause the compensation controls to immediately go into the compensation group.



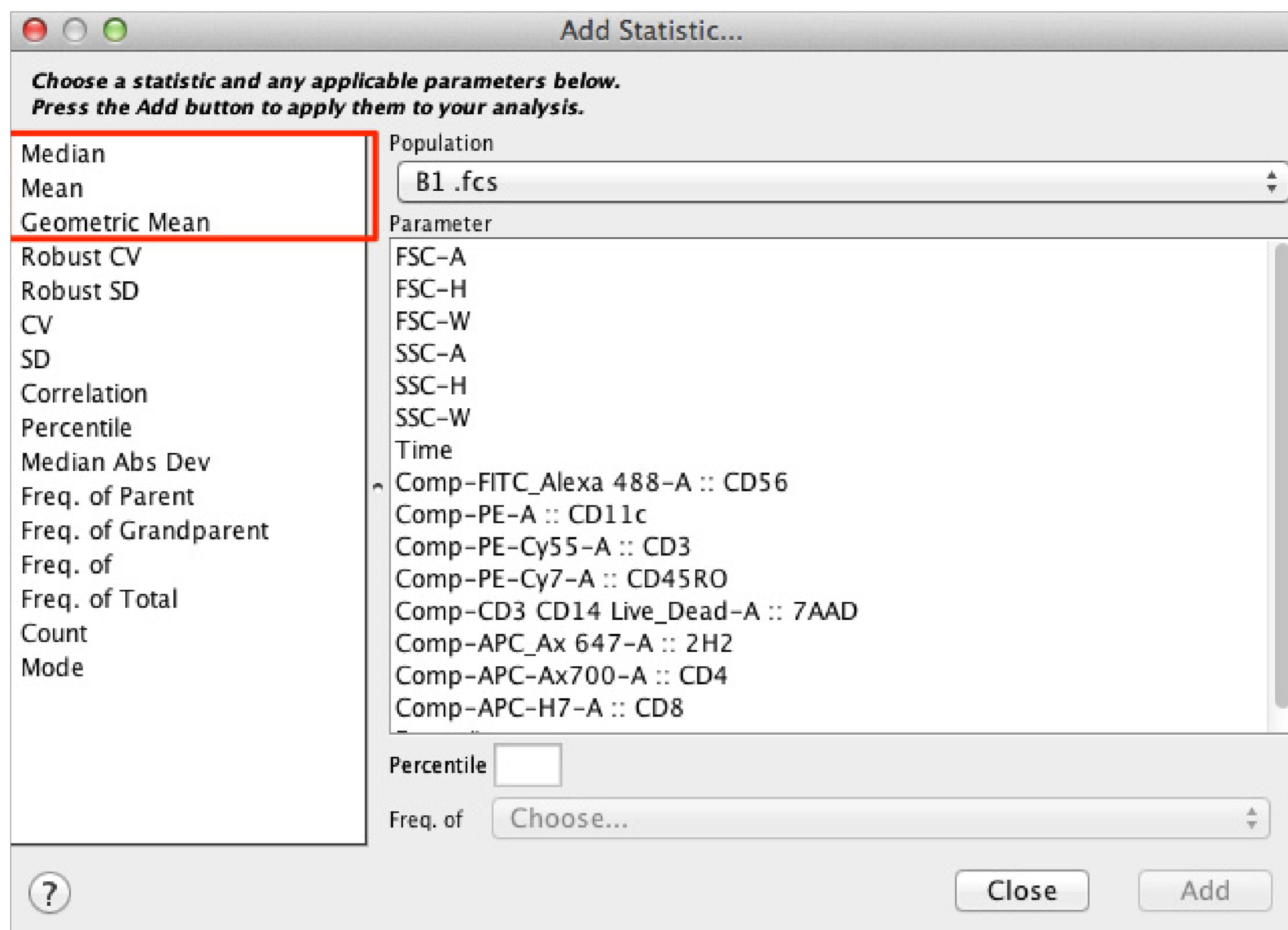
30. Drag an unstained control to use as a universal negative in compensation.

While I prefer to always have samples with internal controls, many users like to use unstained controls. If you want to use an unstained control in FlowJo VX, make sure you gate on the unstained compensation control first, and then drag the gated population here.



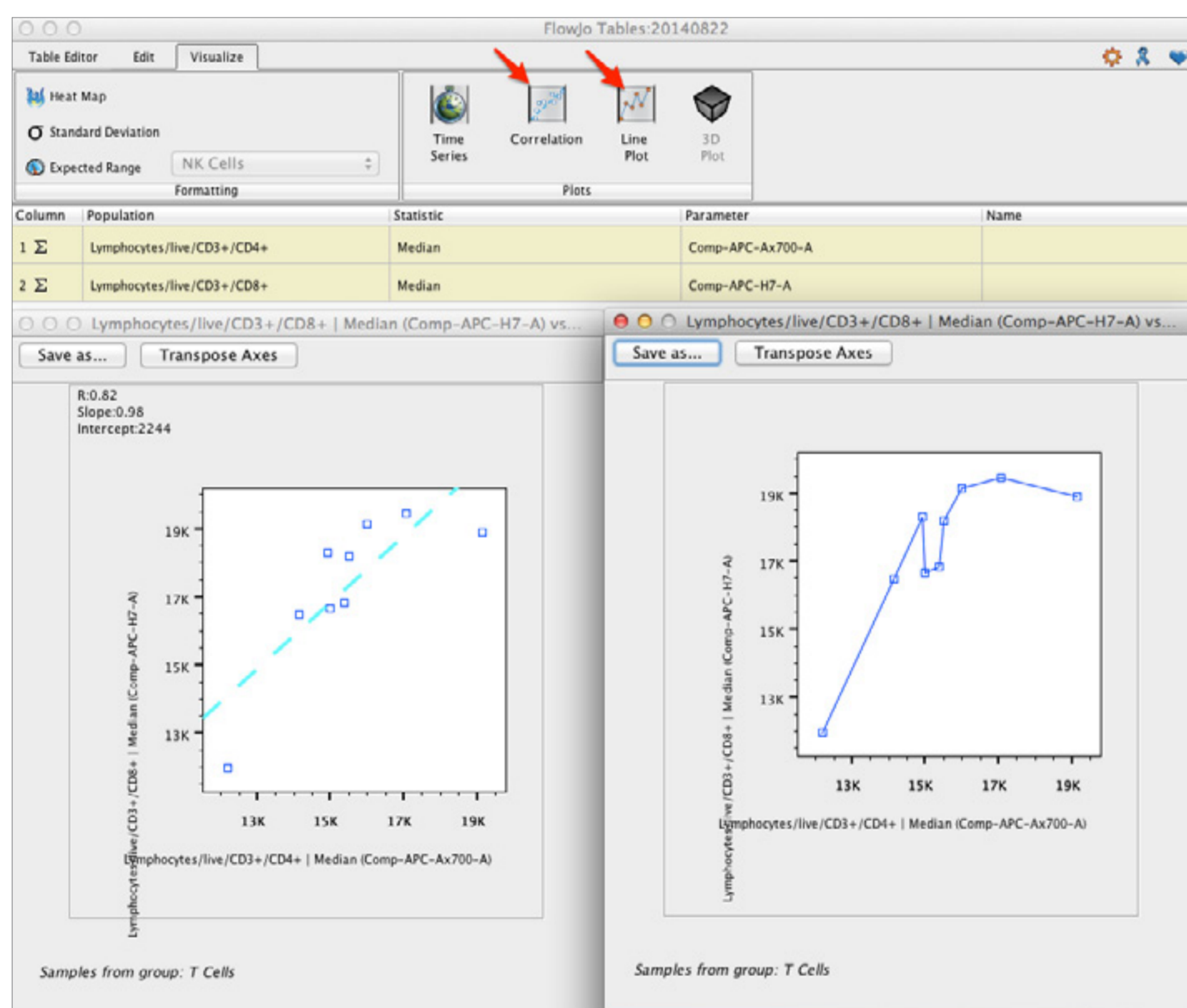
31. Know when to use mean, geometric mean, or median.

Mean is for linear data, geometric mean is for log data. Means assume a normal distribution. It's best to use medians for flow cytometry data because it's normally distributed regardless of whether or not your data is linear or log and it is not easily influenced by outliers.



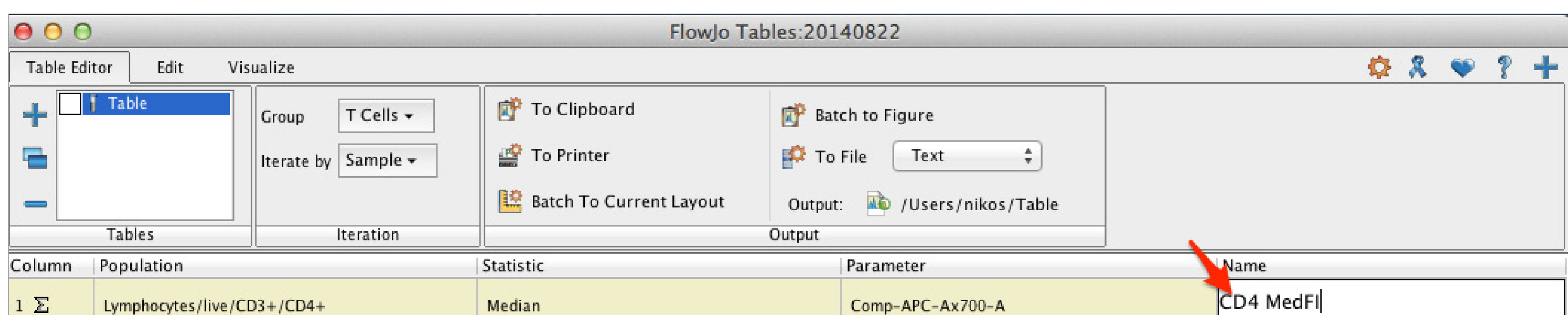
32. Create correlation and line plots in the table editor.

These look a lot better in Excel, but you can take a peak in VX using this feature to see if you even want to take the time to do it in Excel.



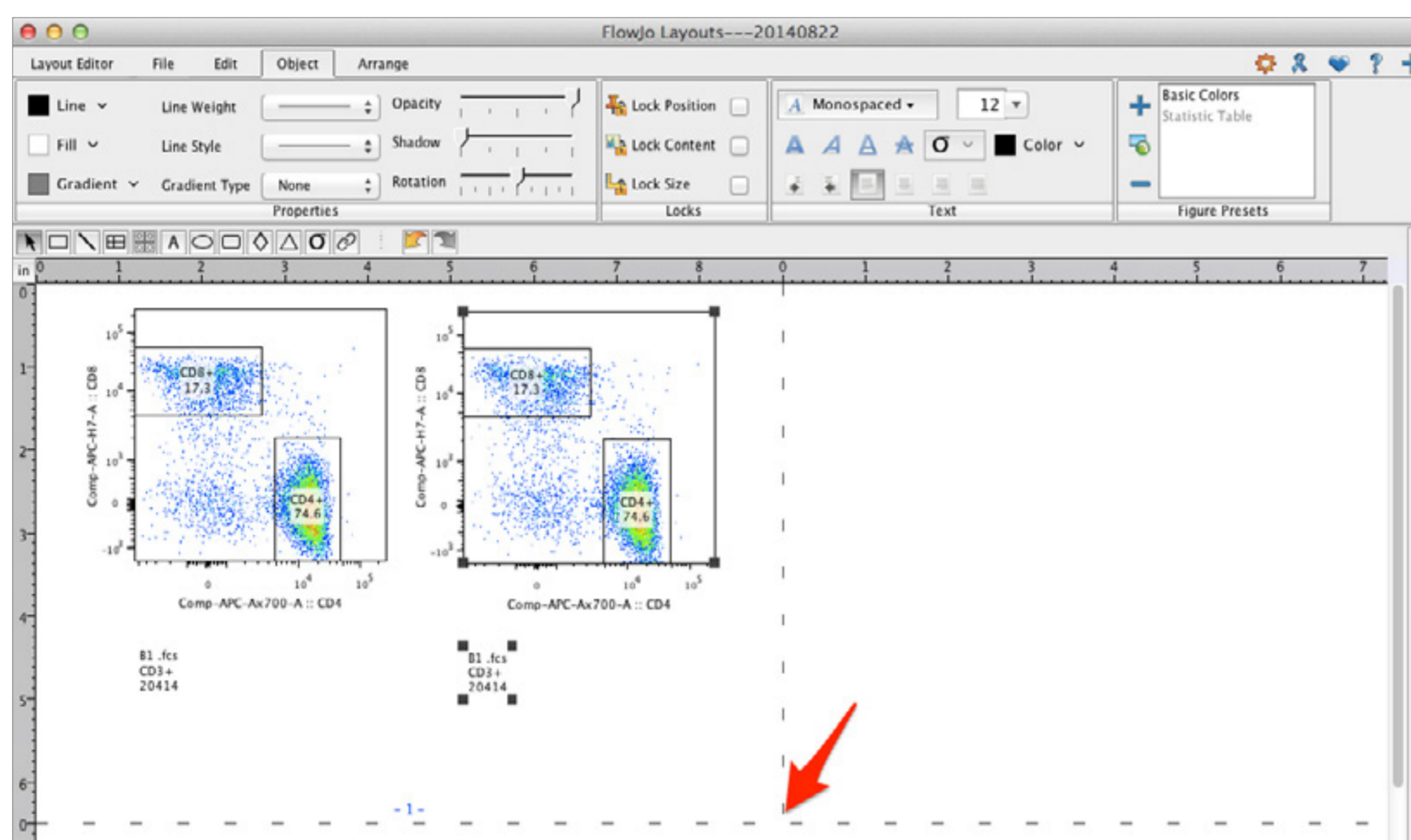
33. Type in the name field for any statistics in the table editor to give that column a custom name.

Your column headers will be complex if you don't do this. This is how you can edit the column header names to something that makes more sense to me.



34. Click on the page break intersections to change page size.

Rather than changing the graph size relative to the page, just change the page size. This will make all your plots smaller or bigger at the same time so you don't have to do it over and over for each plot.

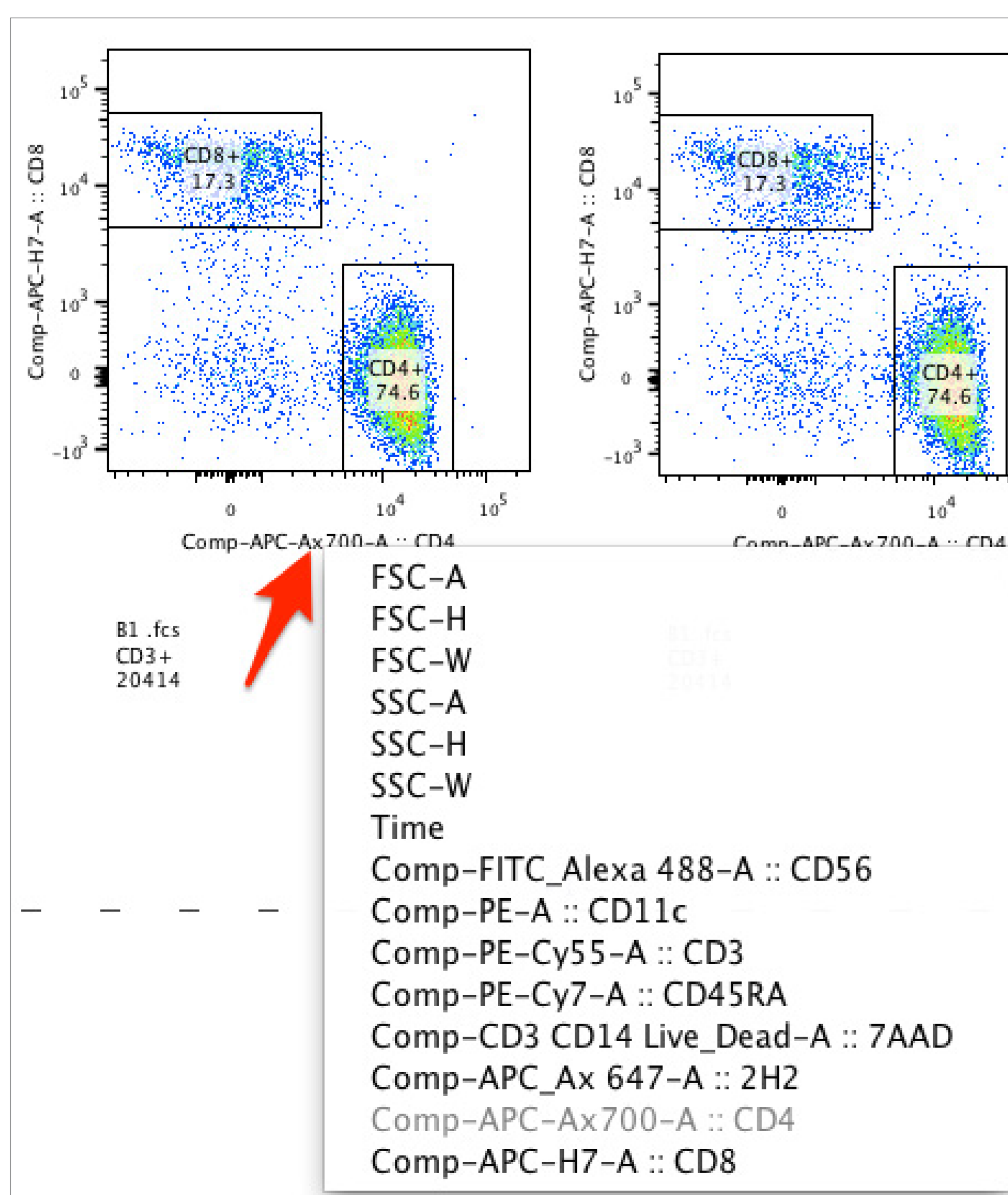


35. Hold Alt and click a graph in the layout editor to duplicate it.

If you ever need to make two or more plots of the same sample, drag the first one in the layout editor and then hold down the Alt key and click to make a copy.

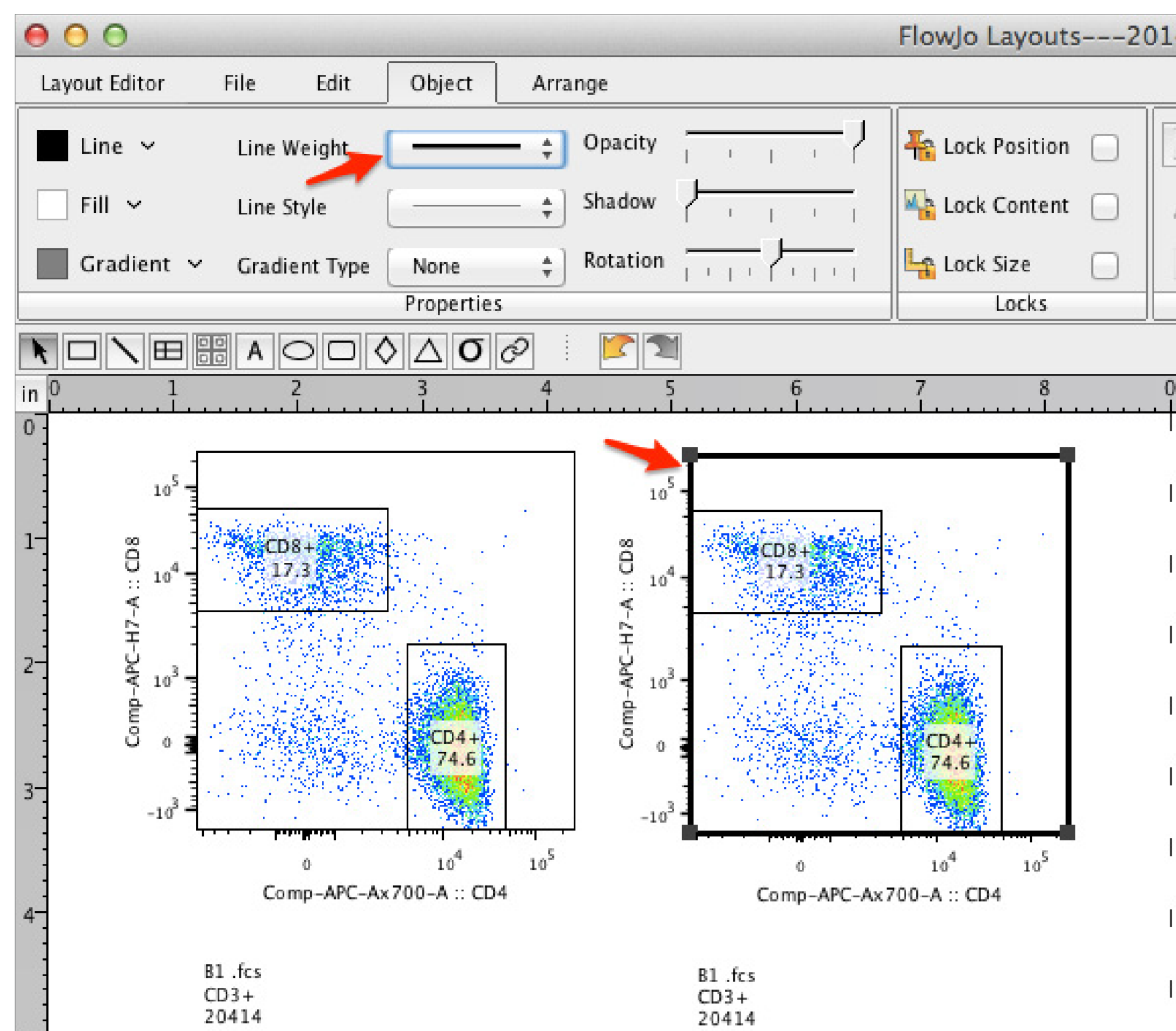
36. Right-click on a parameter in the layout editor to change the axis quickly.

You can right-click on the parameters of a plot in VX to change the axis.



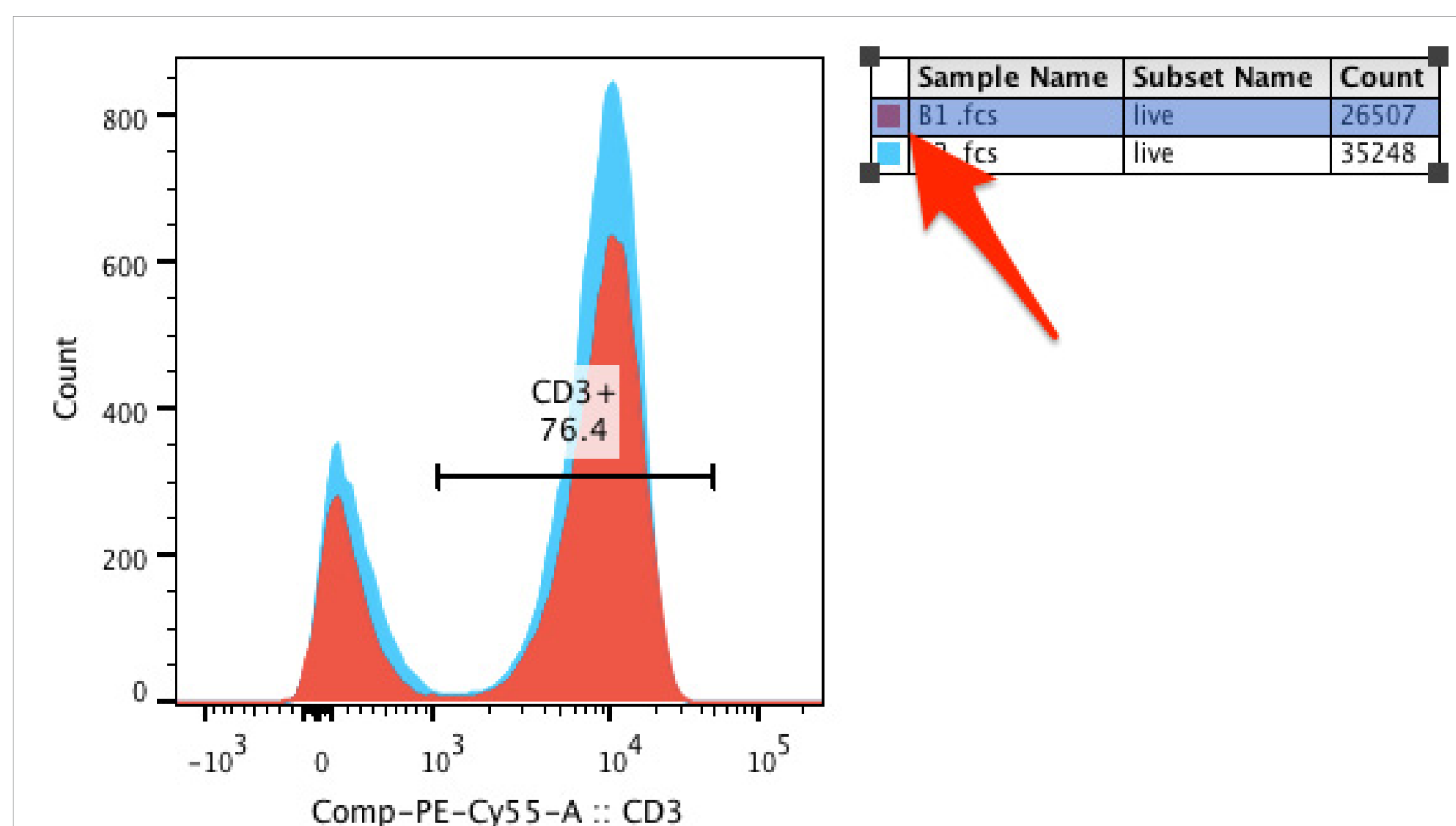
37. Use line weight to make graph outlines thicker.

You'd be surprised how often I'm asked this question. Here's your answer.



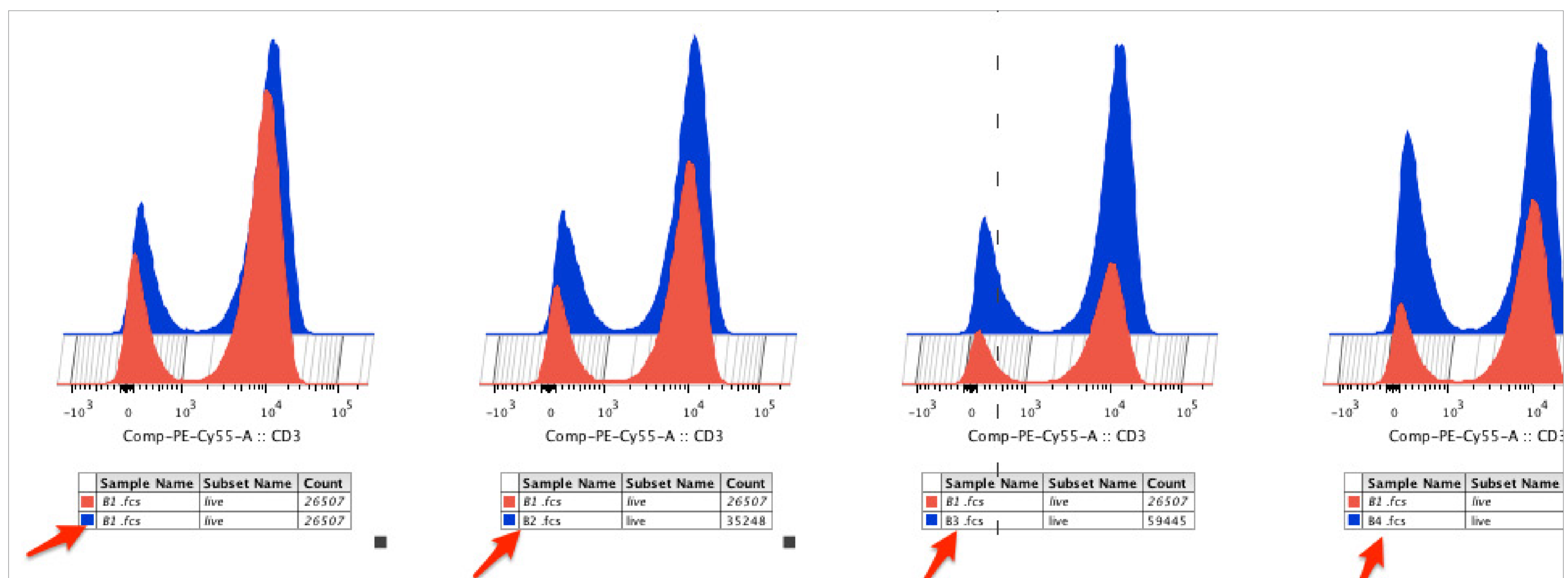
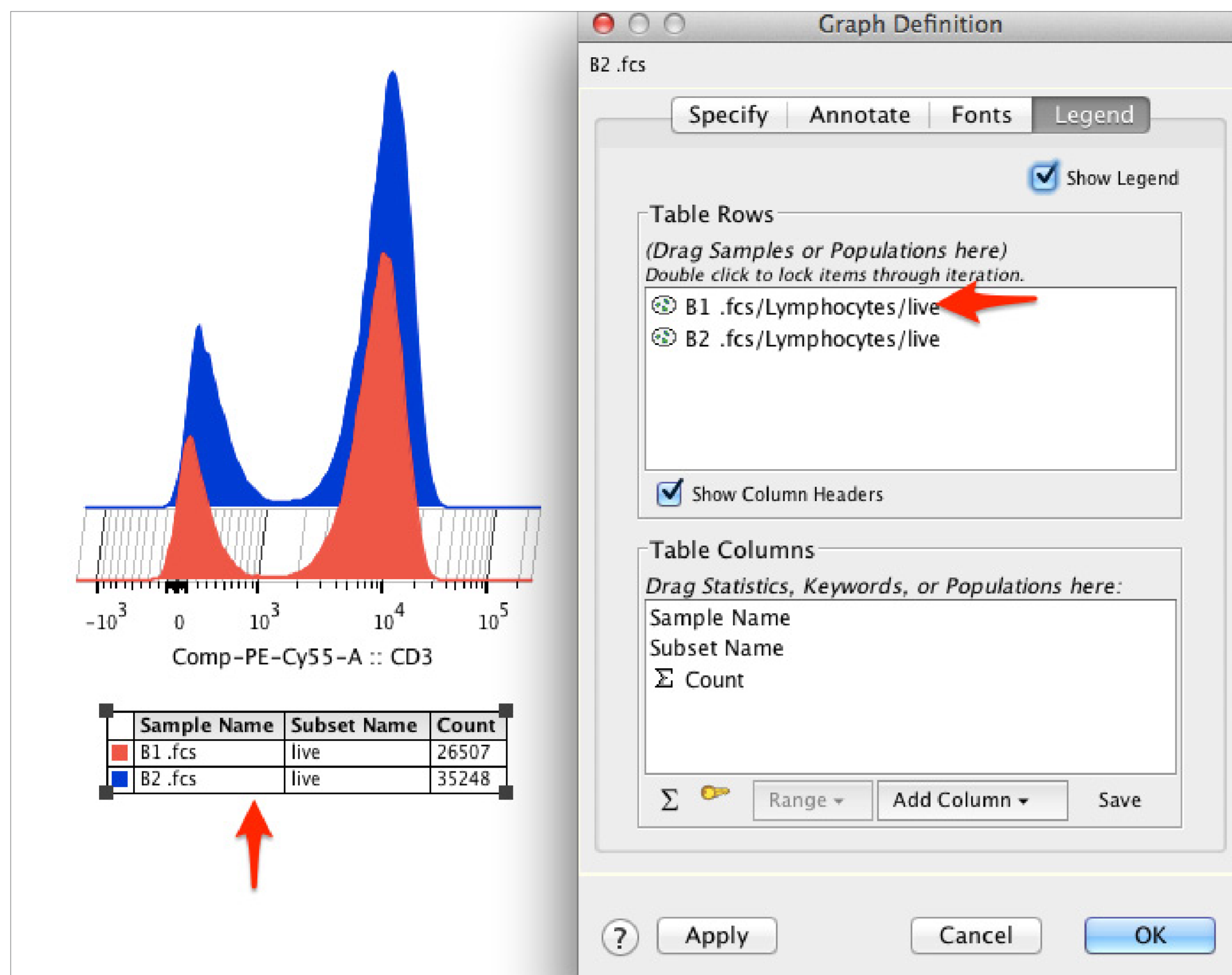
38. Hold down Alt when you right-click on the color box of a legend to change all layers at once.

This is a big time saving measure when you need to change all the layers of an overlay at once.



39. Lock a sample as a control for batching.

You can lock a sample or samples as controls when you batch, so every sample in the group is compared to that sample(s).



40. Export samples.

Maybe you want to export a sample or samples to have all the same number of events. Maybe you made a bunch of keyword edits and you want to write those into a new FCS file. There are a number of reasons why you may want to export a new FCS file. This is where you do it. All you have to do is right-click on a file and select export. You can export as a FCS file or other file type. CSV files, for example, will give you the fluorescence of every event for every parameter.

Population: /Users/nikos/exported.FCS3

Output: /Users/nikos/exported.FCS3

Parameter:

- FSC-A
- FSC-H
- FSC-W
- SSC-A
- SSC-H
- SSC-W
- FITC_Alexa 488-A
- PE-A
- PE-Cy55-A
- PE-Cy7-A
- CD3 CD14 Live_Dead-A
- APC_Ax 647-A
- APC-Ax700-A
- APC-H7-A
- Time
- Comp-FITC_Alexa 488-A
- Comp-PE-A
- Comp-PE-Cy55-A
- Comp-PE-Cy7-A
- Comp-CD3 CD14 Live_Dead-A
- Comp-APC_Ax 647-A
- Comp-APC-Ax700-A
- Comp-APC-H7-A

Event Count: 37132

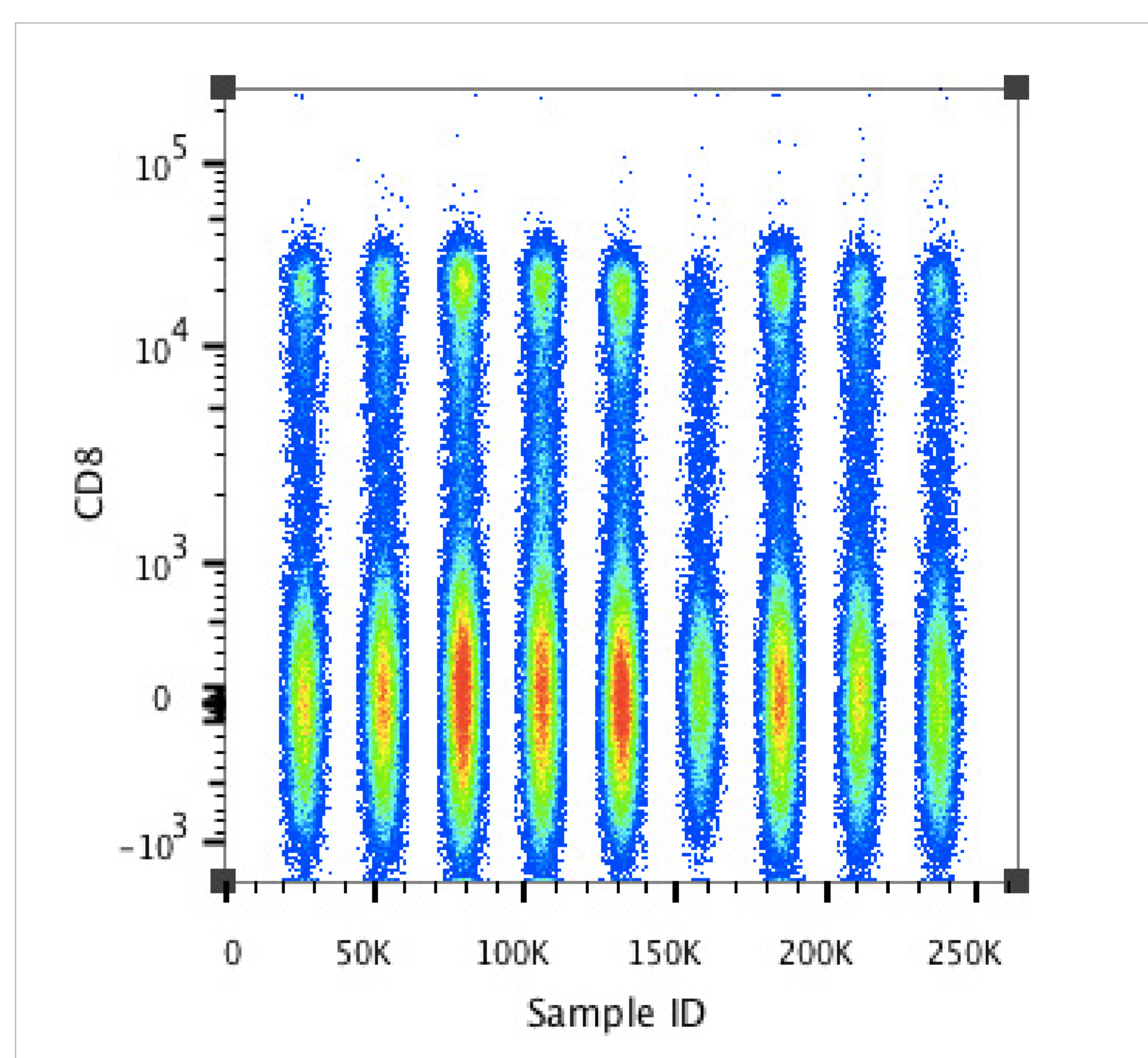
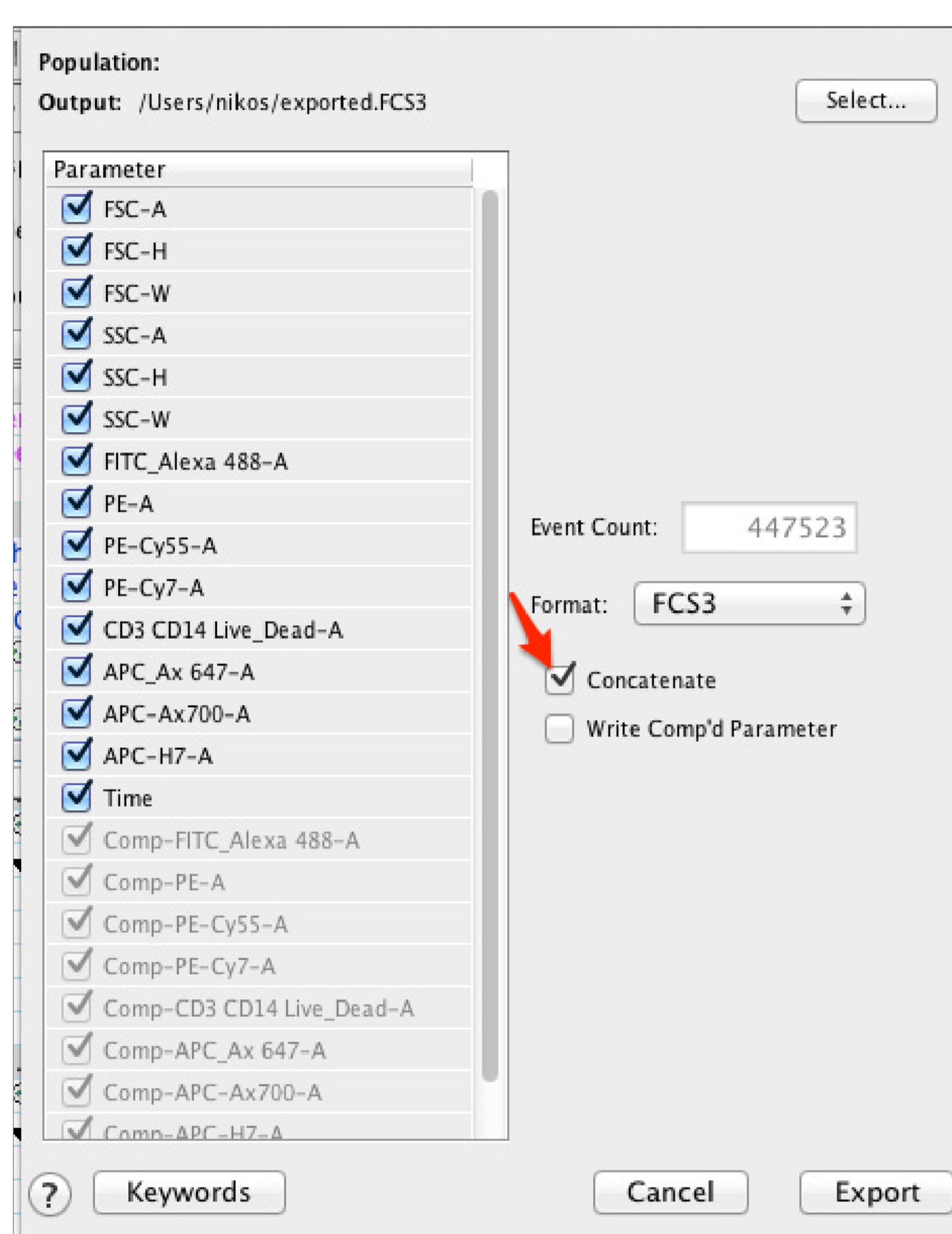
Format: FCS3

Write Comp'd Parameter

Buttons: Cancel, Export

41. Concatenate samples.

If you want to compile 1 or 100 samples into a single FCS files, use concatenate. Concatenate will add a new sample identifier parameter to the new file, so you can plot any parameter vs. the sample identifier parameter. Select all the samples you want to combine and then right-click > export. When you export the file, you can load the new file into a workspace and view the sample.



42. Use templates.

Use templates. Again, use templates. They will save you more time than anything else on this list. Templates preserve all of your analyses without having to save and store all of your data files. Once you have a template, simply do the same experiment again, open up the template, drag in your new FCS files from the second experiment, and—poof—everything will be gated for you. You may have to adjust some gates but, overall, you will save a significant amount of time.

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