Global Worksheet - Global Sheet1

Specimen_001-Tube_001

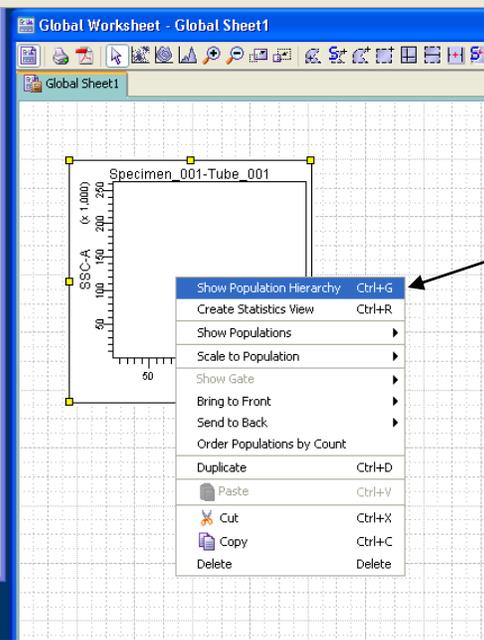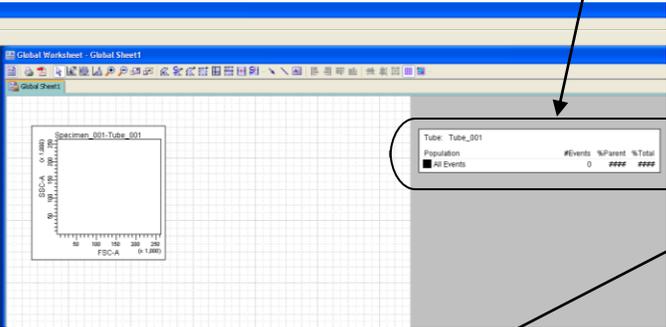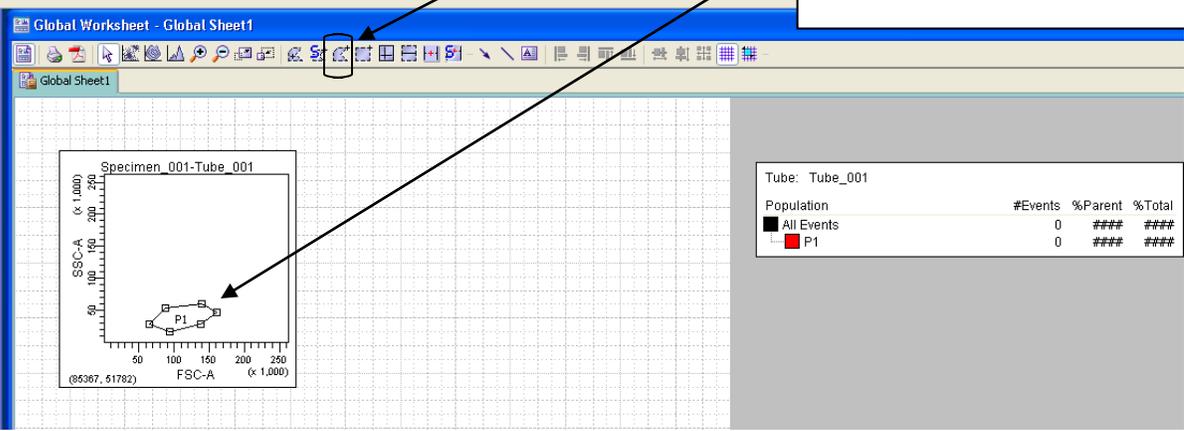SSC-A (x 1,000)

FSC-A (x 1,000)

To add a Dot Plot:

Click on the "Dot Plot" icon THEN click anywhere on the worksheet

A dot plot will appear (defaulted to FSC-A vs SSC-A)

Global Worksheet - Global Sheet1

Specimen_001-Tube_001

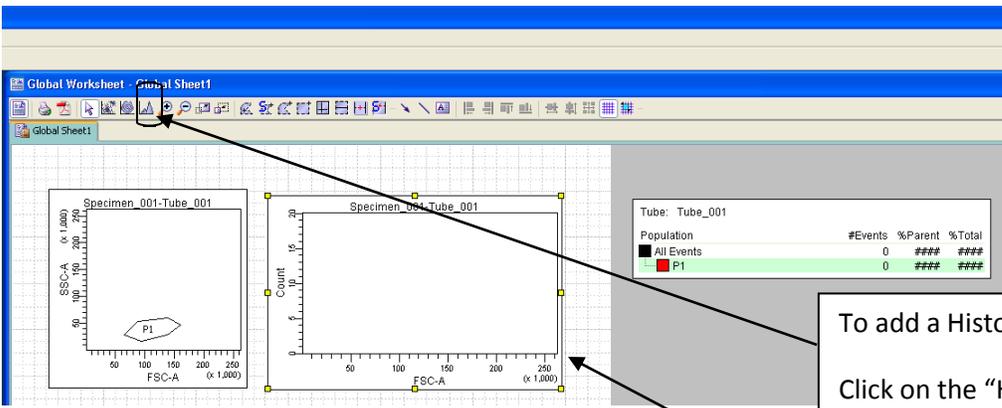| Show Population Hierarchy | Ctrl+G |
Create Statistics View	Ctrl+R
Show Populations	▶
Scale to Population	▶
Show Gate	▶
Bring to Front	▶
Send to Back	▶
Order Populations by Count	
Duplicate	Ctrl+D
Paste	Ctrl+V
Cut	Ctrl+X
Copy	Ctrl+C
Delete	Delete

Show the experiment's population hierarchy by right clicking on the dot plot and selecting "Show Population Hierarchy"

The Population Hierarchy box will appear

The Population Hierarchy box is very important. It will show you if you have properly set up the flow of cells through the gates.

Global Worksheet - Global Sheet1

Specimen_001-Tube_001

Tube: Tube_001

| Population | #Events | %Parent | %Total |
| All Events | 0 | #### | #### |

To add a polygon gate:

Click on the "Polygon Gate" icon and click on the dot plot. Each additional click will add an additional vertice to the polygon gate.

Creating a hexagon or octagon polygon gate works well.

Global Worksheet - Global Sheet1

Specimen_001-Tube_001

SSC-A (x 1,000)

P1

(85367, 51782)

FSC-A (x 1,000)

Tube: Tube_001

| Population | #Events | %Parent | %Total |
| All Events | 0 | #### | #### |
| P1 | 0 | #### | #### |

To add a Histogram:

Click on the "Histogram" icon Then click anywhere on the worksheet

A histogram plot will appear (defaulted to FSC-A)
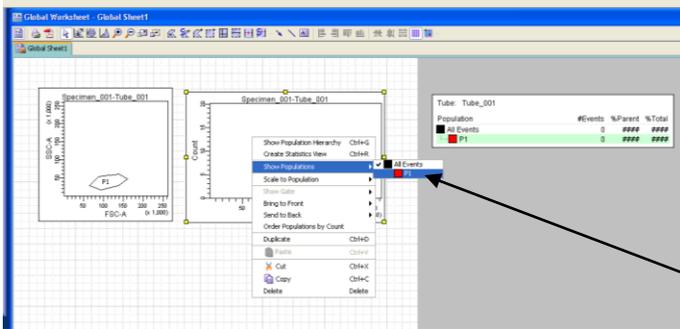
To tell the software where the cells are coming from for this histogram:

Right click on the plot and select "Show Population" then click on which gate you want the cells to come from. Now, any event in polygon gate "P1" will go to the histogram.

If you right click the plot and go to "Show Population" again, then the gate will look like a button that's pushed in (This version of DIVA had a check mark)
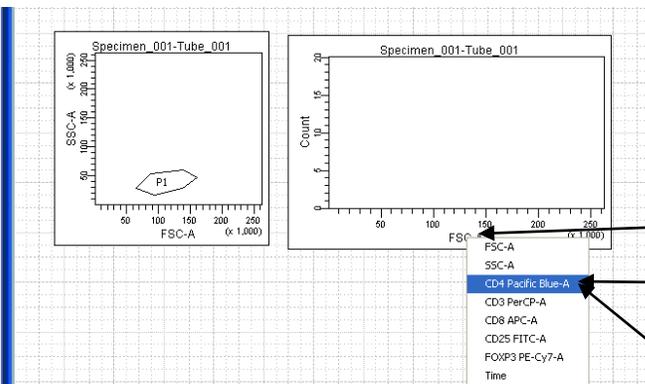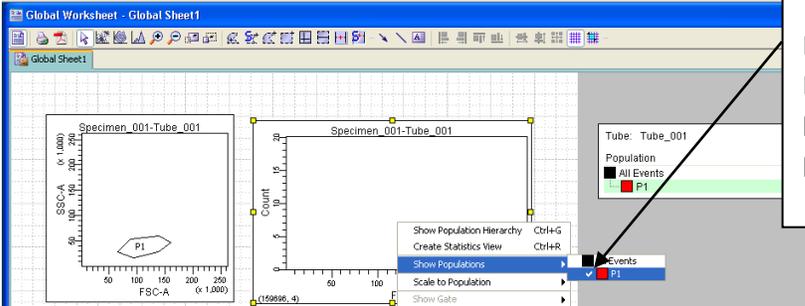
To tell the histogram which fluorochrome to be detecting:

Right click on the x-axis parameter (FSC-A)→

Select which fluorochrome you would like this plot to detect→

The histogram will now only detect the fluorochrome you have selected

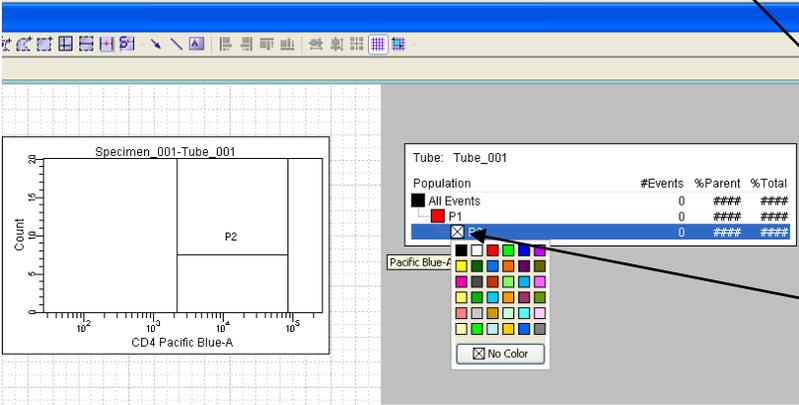**Global Worksheet - Global Sheet1**

Global Sheet1
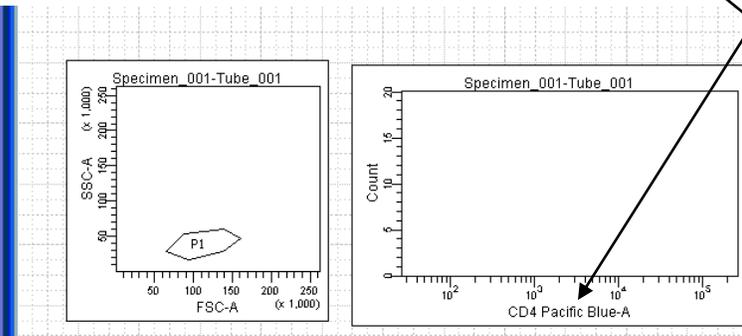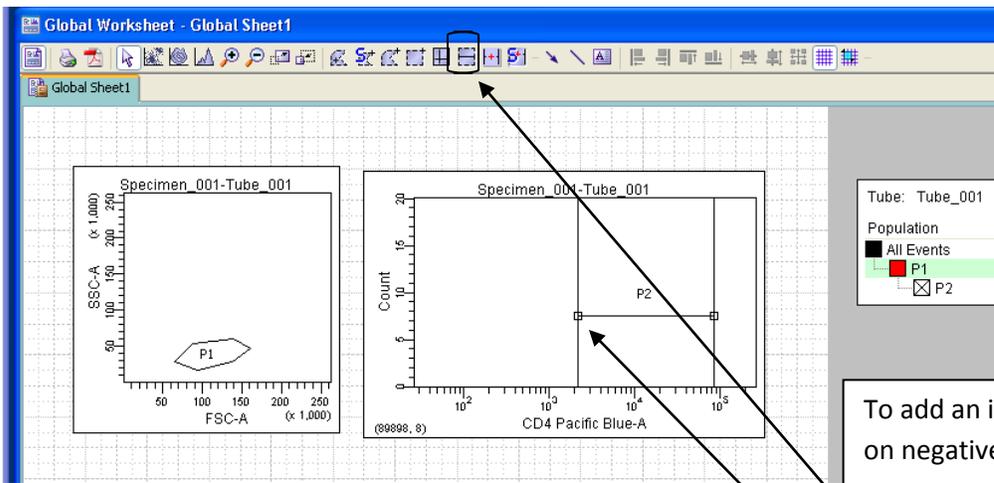
To add an interval gate in the histogram to gate on negative or positive populations:

Click on the "Interval Gate" icon then

Click on the histogram

To add a color to a gate:

Double click on the box with an "X" in it and a pop up will appear with colors to choose from. Click on the color you would like to use. (I find it best to move from light colors to dark colors on the Population Hierarchy. This allows the important, further downstream population to be noticeable on the earlier dot plots.)
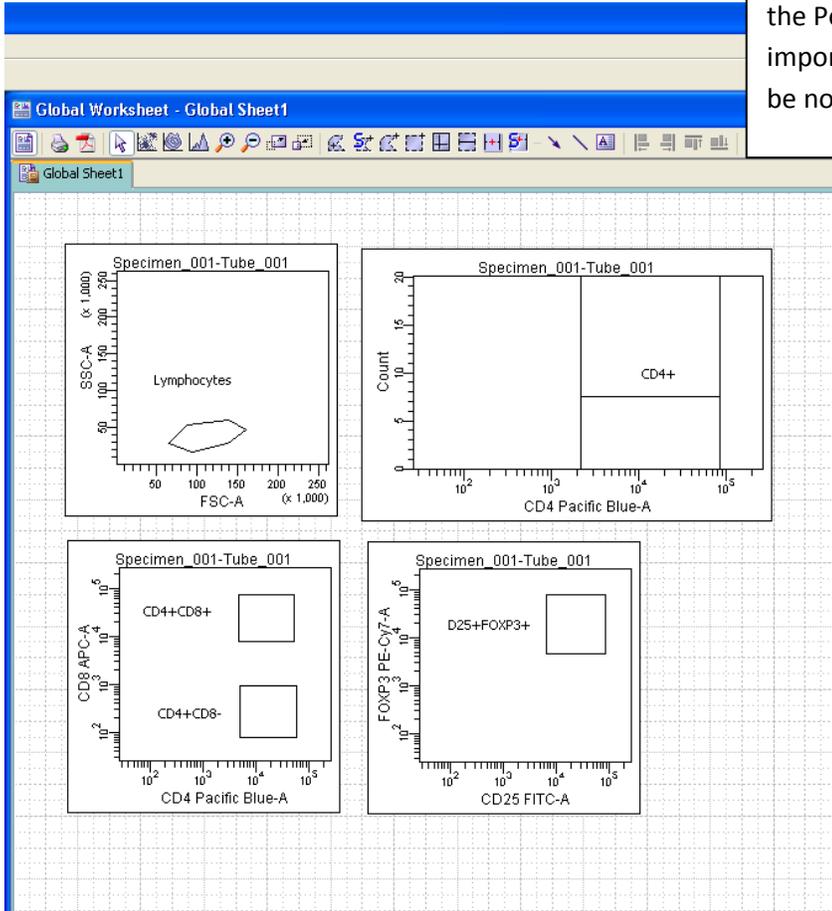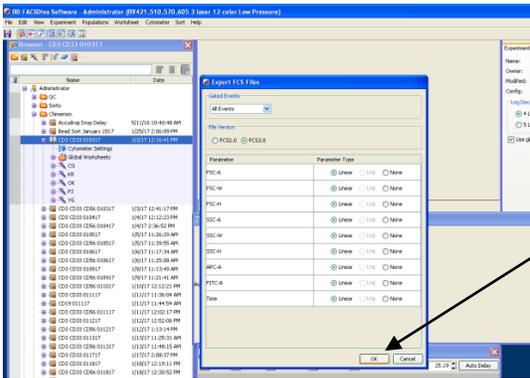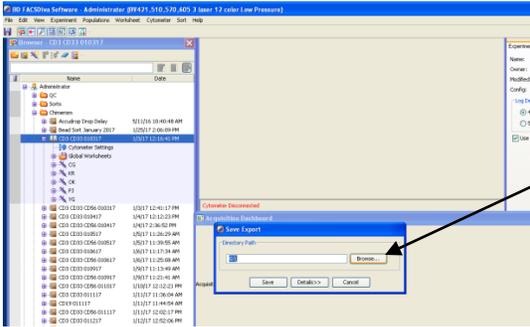
Repeat these steps with other plots and gates to create your experiment (See last page for what each icon creates).
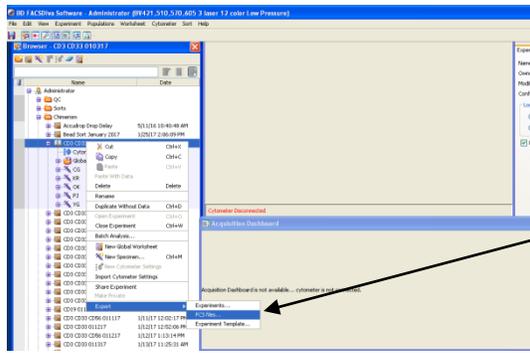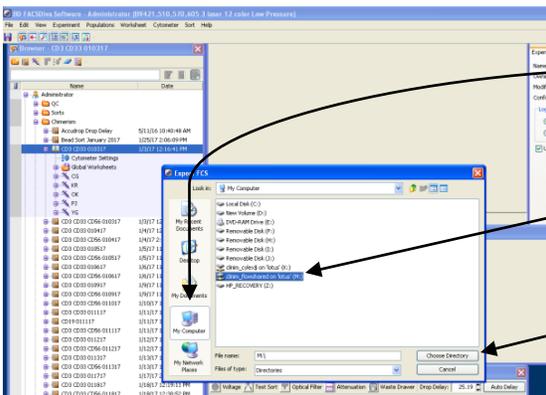
To export your data:

Right click on your experiment→select Export→click on FCS Files

An "Export FCS Files" pop up box appears. Click the "OK" button

A "Save Export" pop up box will appear. Click "Browse" button to select where you want to save your FCS files

An "Export FCS" pop up box will appear. Click on "My Computer" icon→

Select the network drive you want to save to→ find the folder you want to save to→select the folder→

Click on the "Choose Directory" button
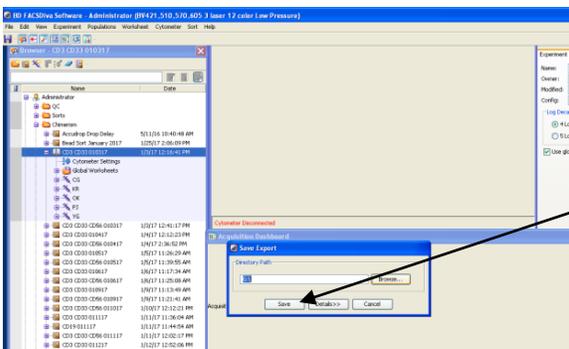
The "Save Export" pop up box will appear again, but this time click on the "Save" button.

Your data is now saved to the folder you selected.

Make sure the FCS file transferred correctly before you delete the experiment from the Browser window. Any FCS files >4 months old are deleted.
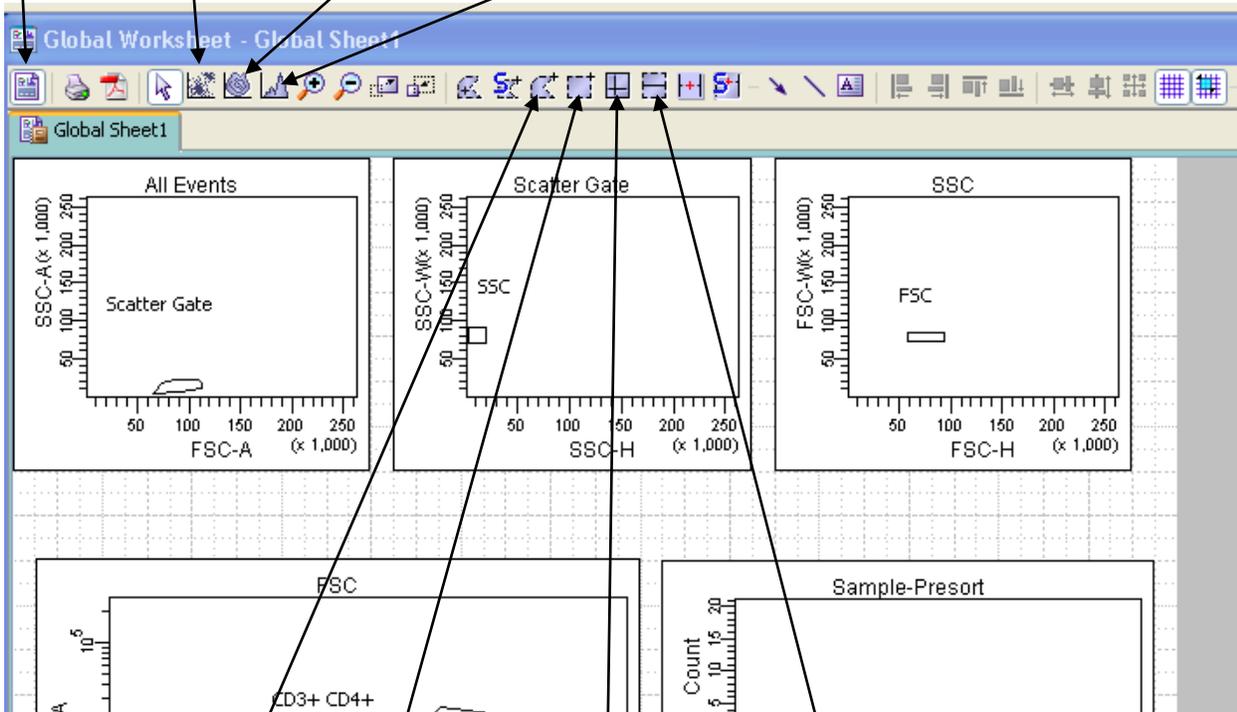
toggle:

toggle between compensation control graphs (Normal worksheet) and your experiment graphs (Global worksheets).

Dot plot

Contour (density) plot

Histogram plot

Global Worksheet - Global Sheet1

Global Sheet1

All Events

Scatter Gate

SSC

Scatter Gate

SSC

FSC

FSC

Sample-Presort

CD3+ CD4+

polygon gate:
Click on the "Polygon Gate" icon → click on the graph of interest → each addition click of the mouse will add a vertice to your polygon gate

Square gate

Quadrant gate

Interval gate