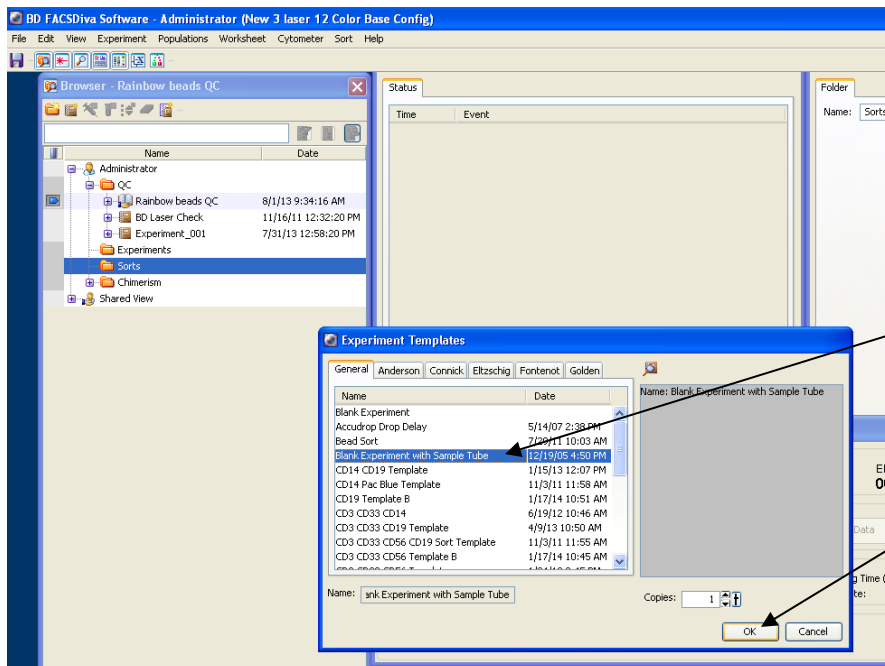
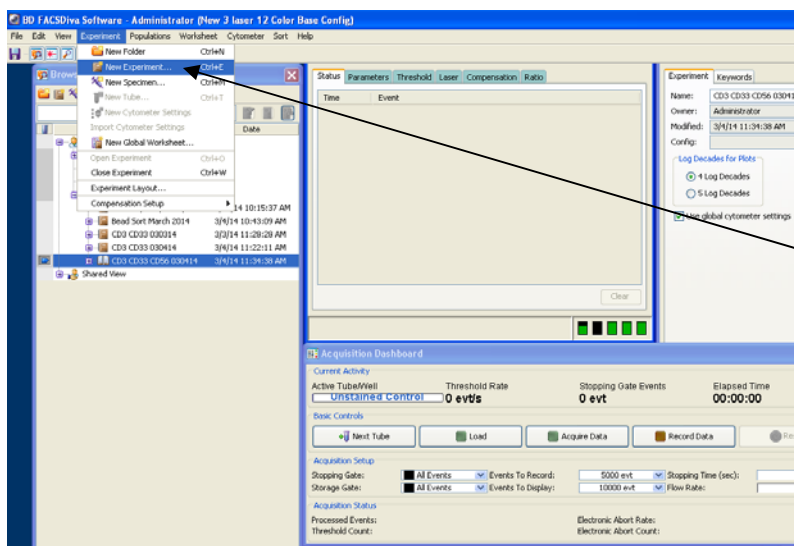
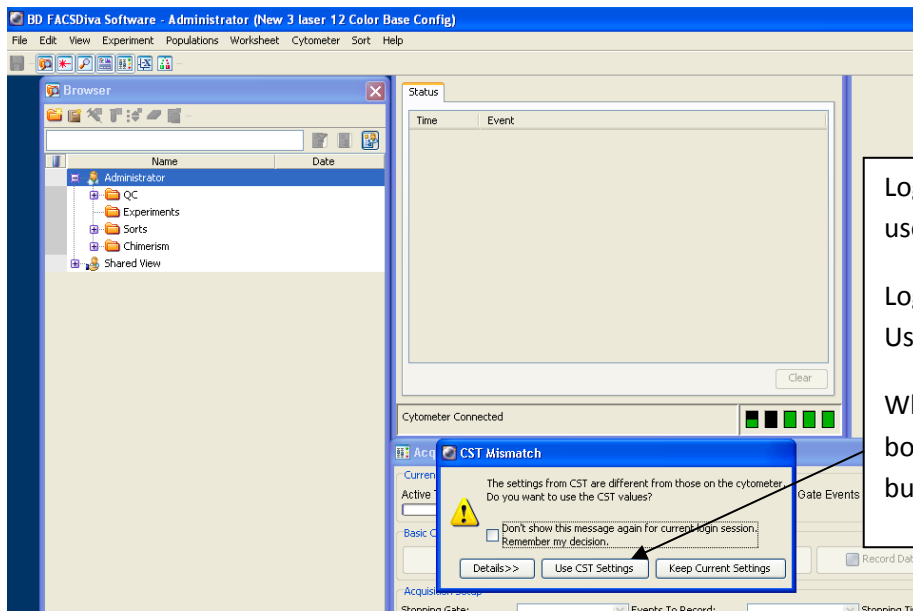
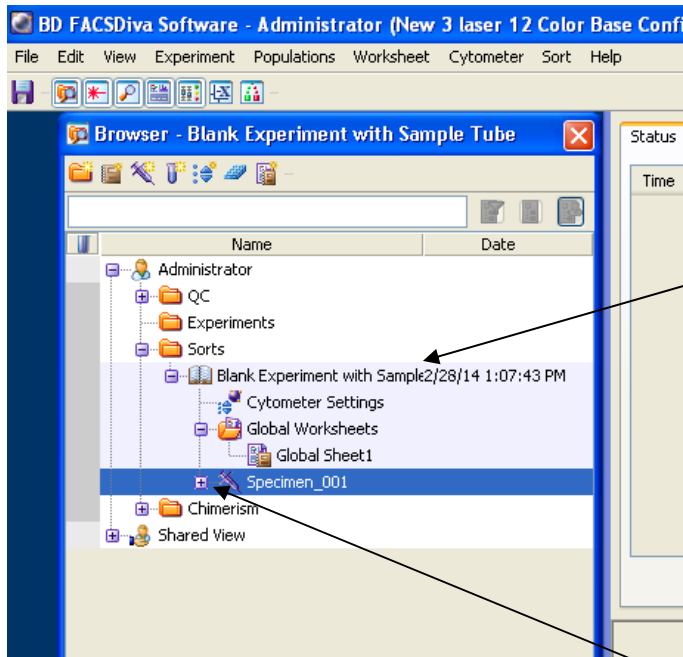
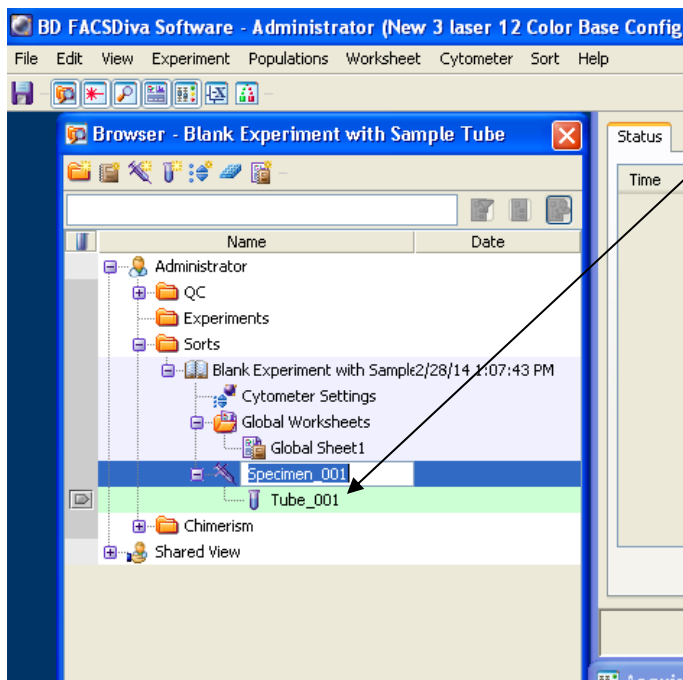




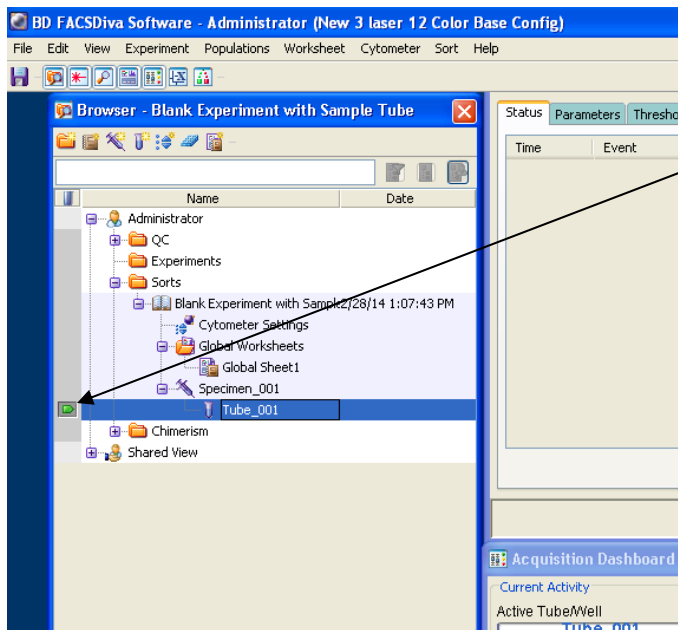


Your "Blank Experiment with Sample Tube" will appear in the Browser screen.

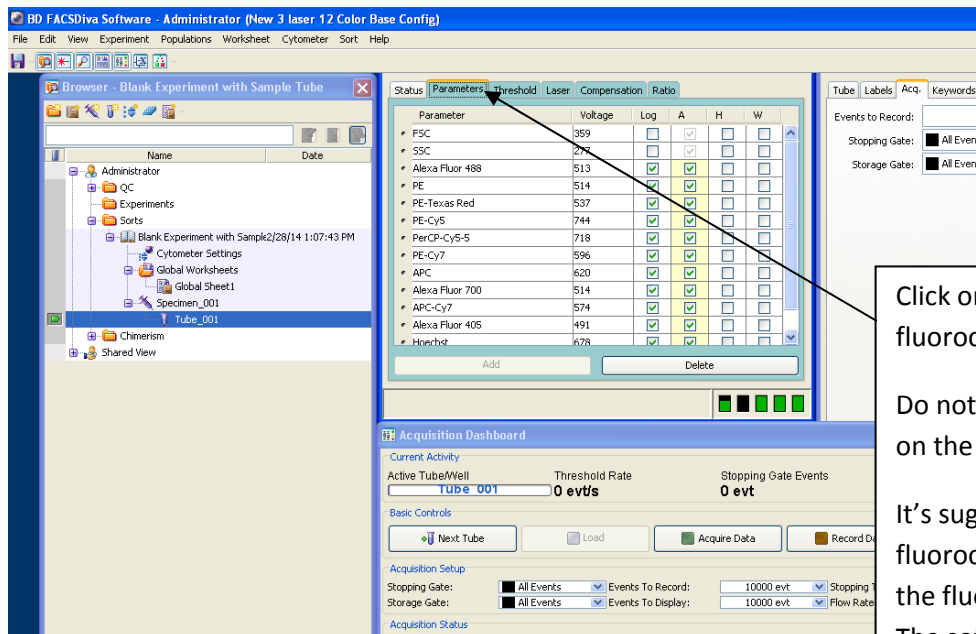
To rename the experiment, right click on the experiment and click on "Rename"



Click on the "+" icon to the left of "Specimen_001" to expand so the sample tube can be seen



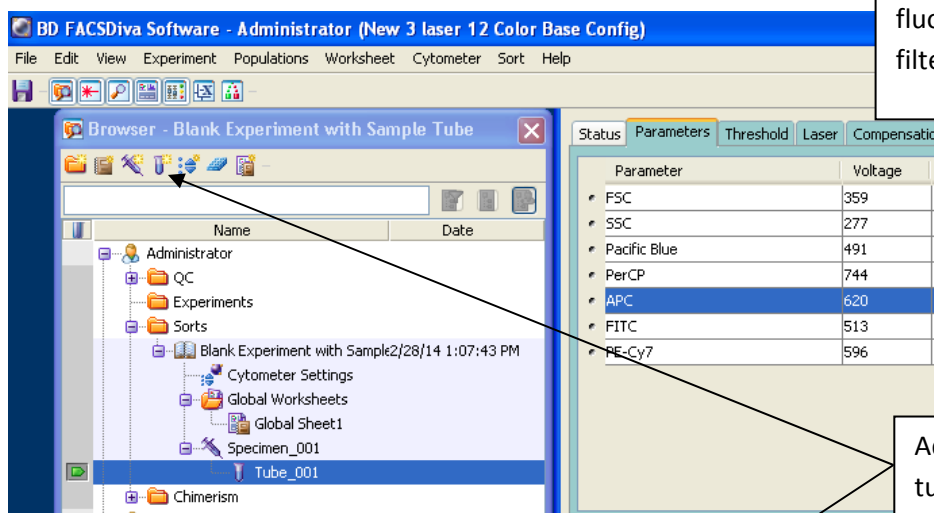
Click on the arrow to the left of the tube so the tube is highlighted. Now you will be able to add or delete fluorochromes for your experiment (see next page)



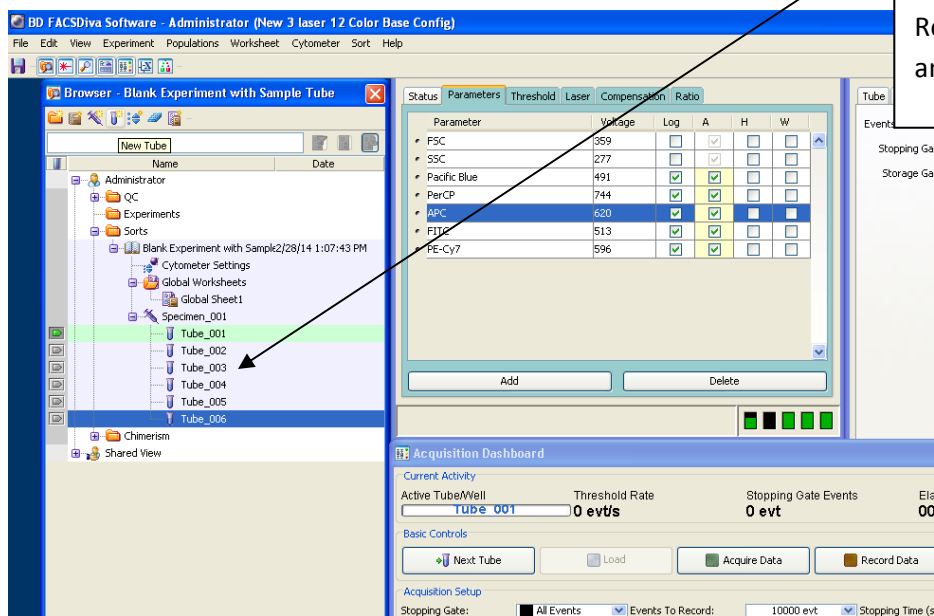
Click on the “Parameters” tab to add and delete fluorochromes.

Do not delete FSC and SSC! They must remain on the panel!

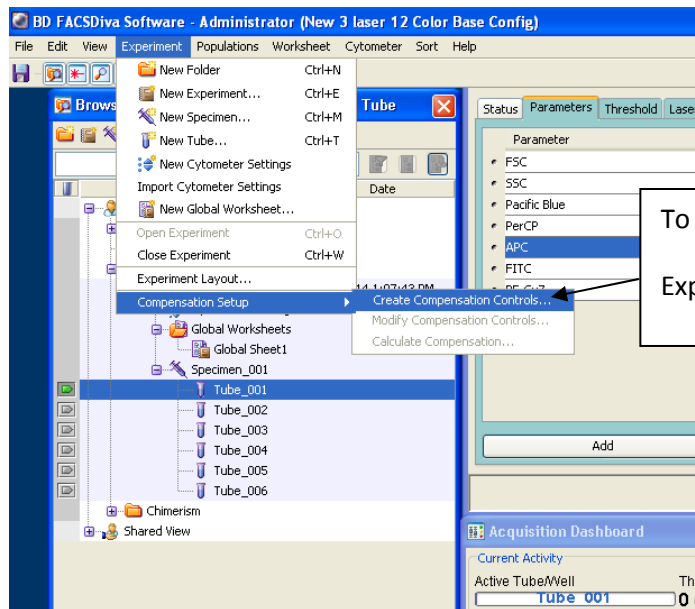
It’s suggested to delete all of the fluorochromes, **EXCEPT** FSC and SSC, and add the fluorochromes in your panel one at a time. The software will not allow you to use fluorochromes that are detected in the same filter.



Add tubes to your experiment by clicking the tube icon in the Browser screen.



Rename the tubes by right clicking on a tube and clicking on “Rename”

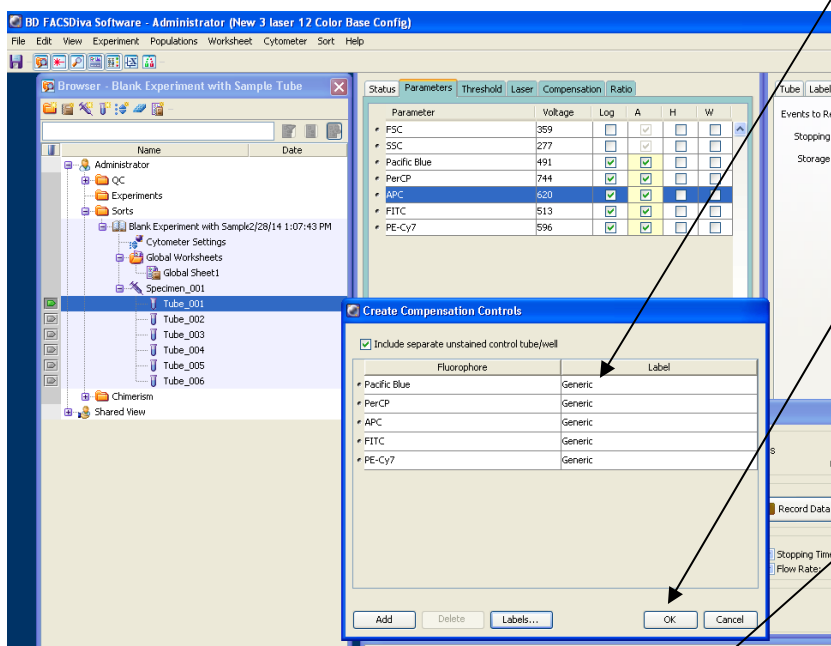


To create compensation controls:

Experiment → Compensation Setup → Create Compensation Controls

Only fluorochromes with the label "Generic" should be used. If there are fluorochromes that are not labeled "Generic" then delete them.

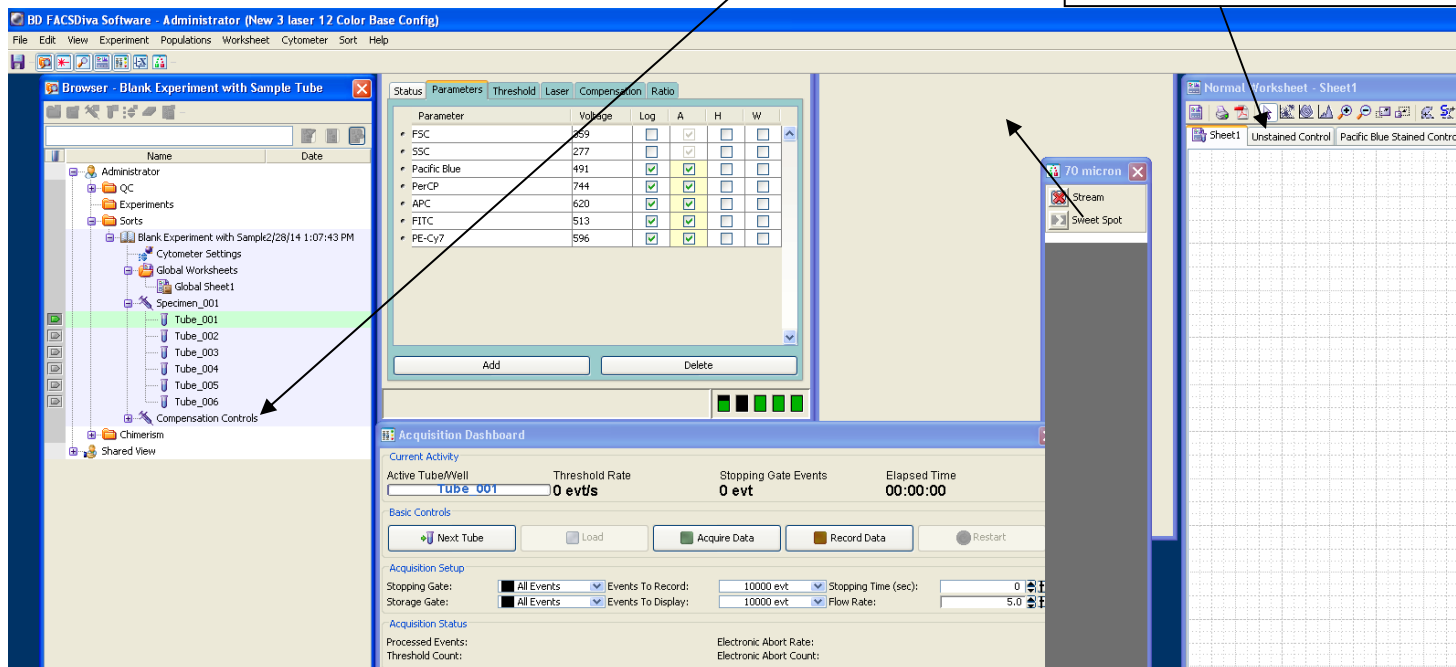
Click "OK" button

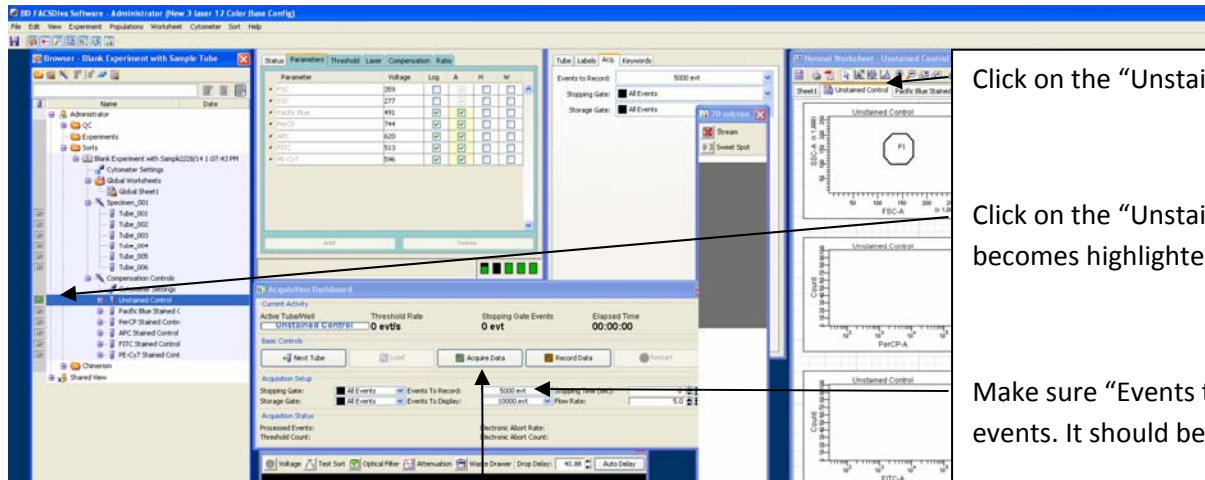


The compensation controls will be automatically created under your experiment

AND

the software will automatically toggle to the compensation control graphs





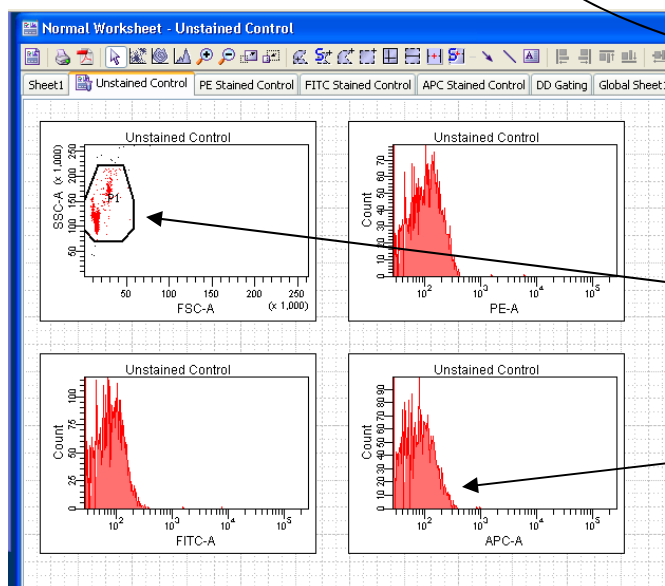
Click on the “Unstained Control” tab

Click on the “Unstained Control” tube so it becomes highlighted

Make sure “Events to Record” is at 5,000 events. It should be defaulted to 5,000

Load the unstained control tube onto the SIP → close the arm → Push the “Run” and “Low” buttons on the machine

Click on “Acquire Data” button

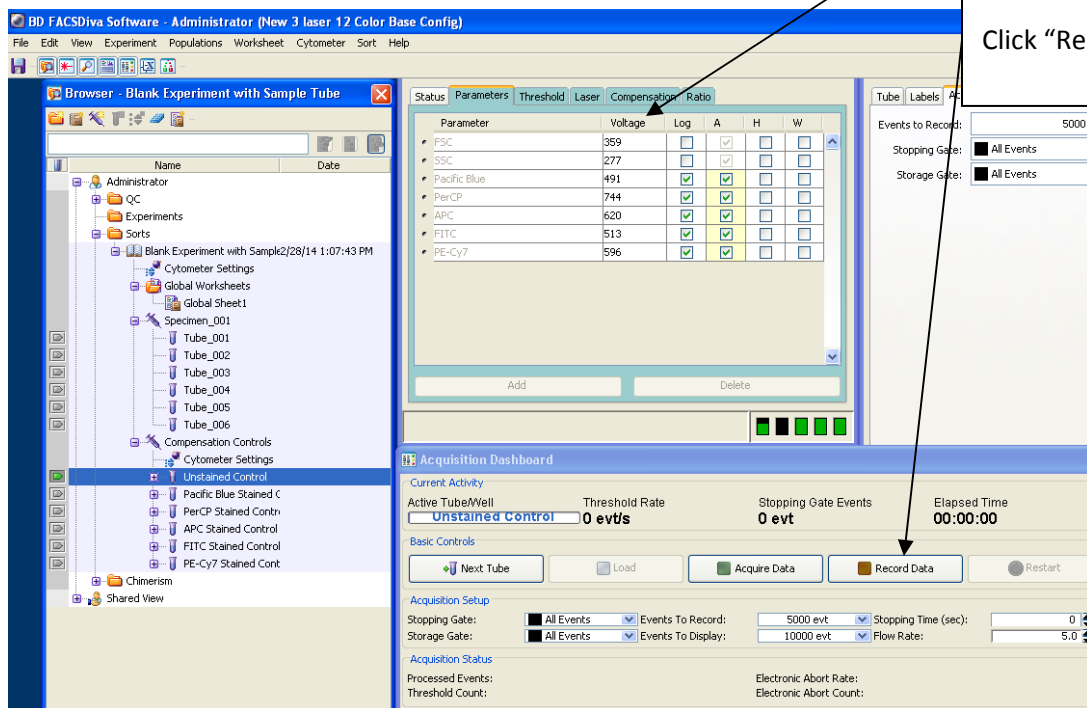


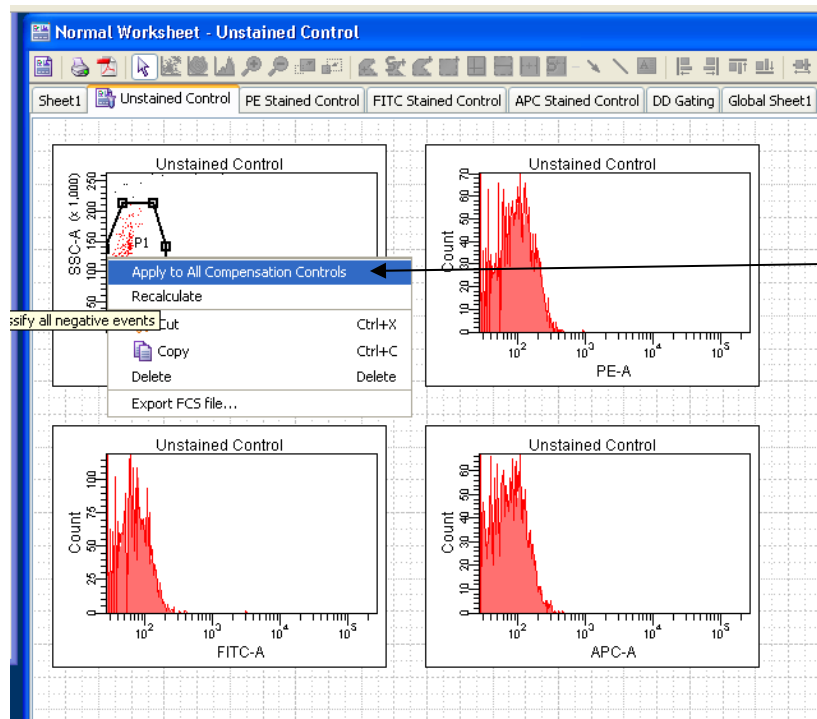
Make sure the FCS vs SSC has all the bead populations in the gate

Make sure the entire unstained control peaks are visible in the graph.

If necessary Change the voltages for a fluorochrome in order to get the entire unstained control peak visible in the graph.

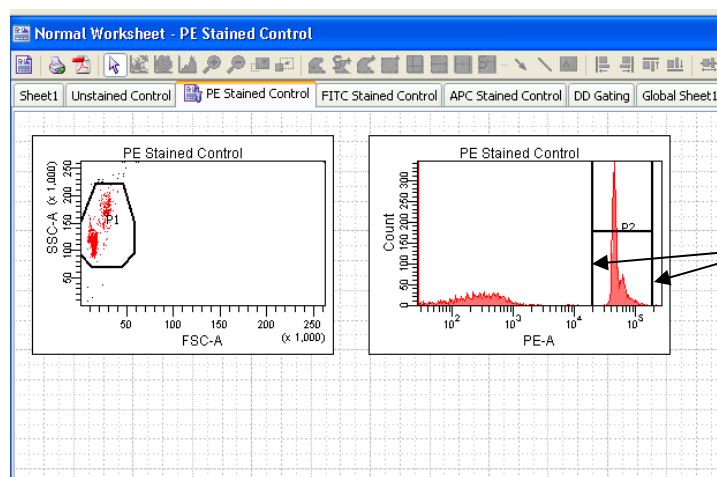
Click “Record Data” button





When recording of the data is finished:

right click on the gate surrounding the bead populations and click on "Apply to all Compensation Controls"



Run all of the positive controls

For the positive control beads, make sure the interval gate contains the entire positive peak, and the entire positive peak is visible on the graph.

If the entire peak is not visible in the graph, then change to voltage so it is. Once a voltage has been changed, all of the controls must be run and recorded again with the new voltage. The new voltage will have to be manually entered in for each control.

BD FACS Diva Software - Administrator (New 3 laser 12 Color Base Config)

File | Edit | View | Experiment | Populations | Worksheet | Cytometer | Sort | Help

Tube

Parameters

Parameter	Voltage	Log	A	H	W
FSC	359	☐	☐	☐	☐
SSC	277	☐	☐	☐	☐
Pacific Blue	491	☐	☐	☐	☐
PerCP	744	☐	☐	☐	☐
FITC	513	☐	☐	☐	☐
APC	620	☐	☐	☐	☐
APC	596	☐	☐	☐	☐

Calculate Compensation...

Acquisition Dashboard

Current Activity

Active Tube/Well: Unstained Control

Threshold Rate: 0 evts

Basic Controls

Next Tube

Load

Acquire Data

Record Data

Restart

Acquisition Setup

Stopping Gate: All Events

Storage Gate: All Events

Events to Record: 5000 evt

Events to Display: 10000 evt

Flow Rate: 5.0

Acquisition Status

Processed Events:

Threshold Count:

Electronic Abort Rate:

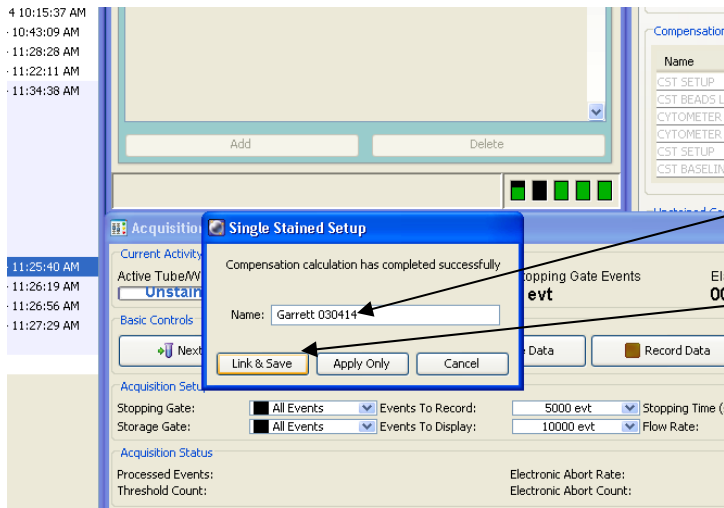
Electronic Abort Count:

Once all of the compensation controls have been run:

Experiment → Compensation Setup → Calculate Compensation

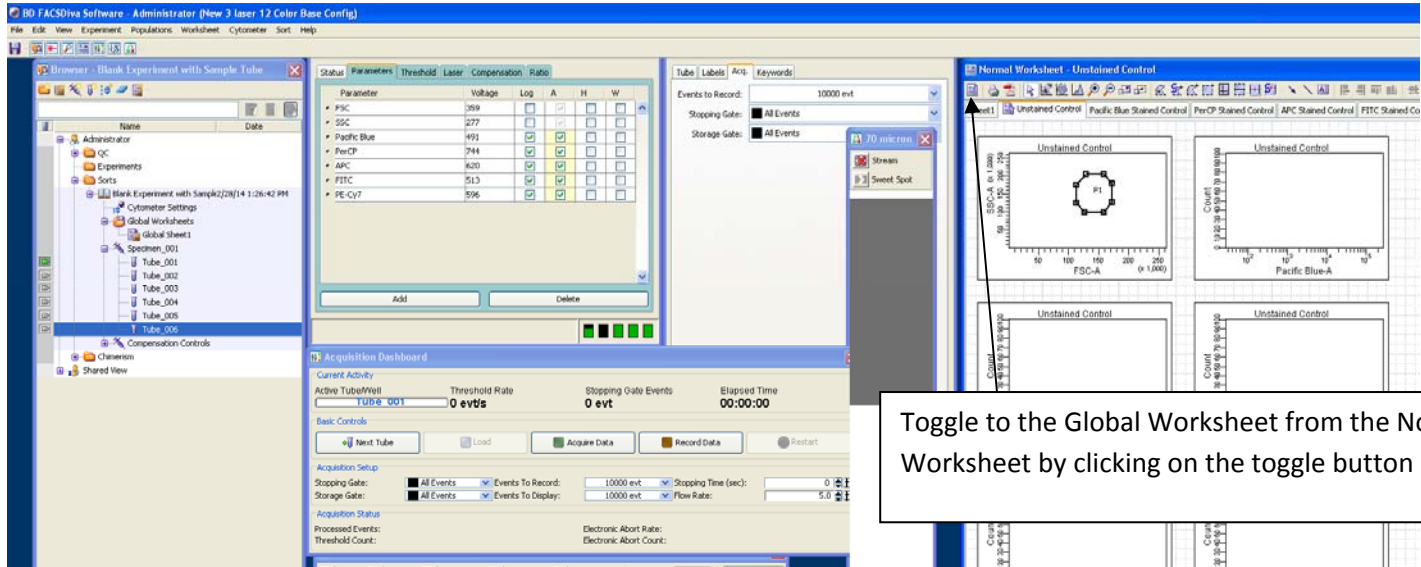
If there are any overlap problems the software will warn you about them

4 10:15:37 AM
 10:43:09 AM
 11:28:28 AM
 11:22:11 AM
 11:34:38 AM

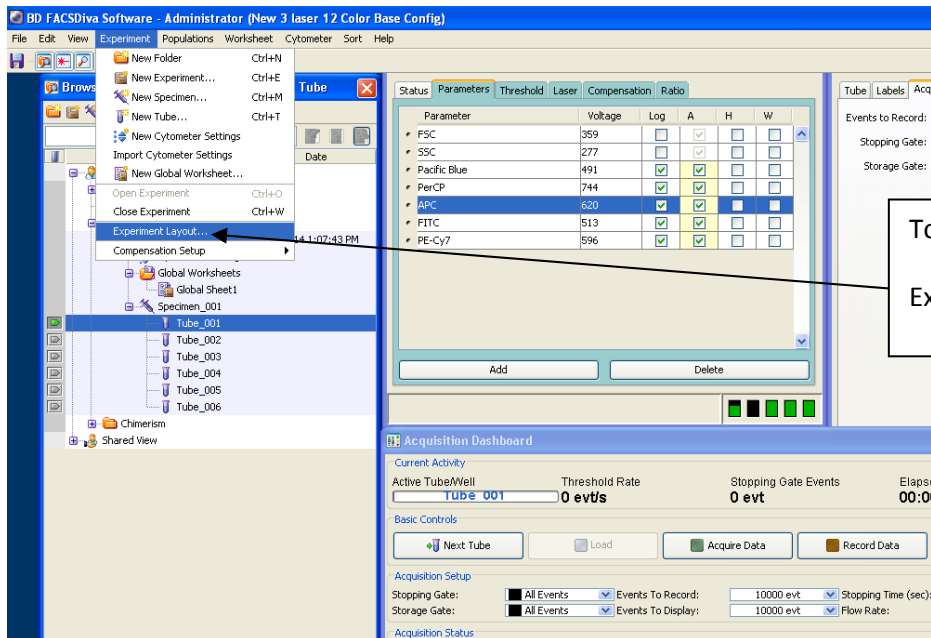


Name the compensation with your name and the date.

Click on "Link & Save" button

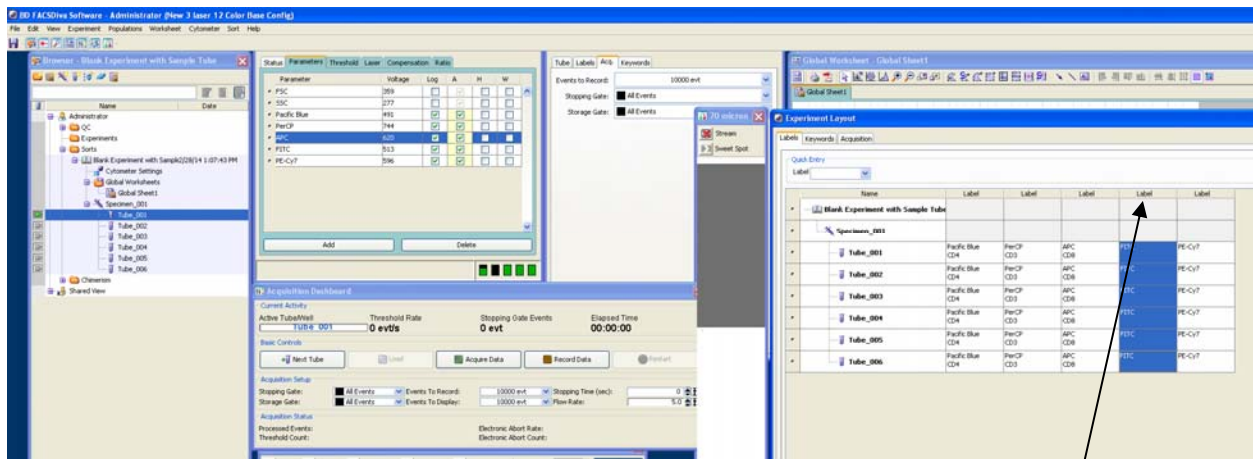


Toggle to the Global Worksheet from the Normal Worksheet by clicking on the toggle button



To link what each fluorochrome binds to:

Experiment → Experiment Layout



In the “Experiment Layout” pop up, click on the “Label” header above a fluorochrome so the column becomes highlighted. Type in what the fluorochrome binds to. Do this for each fluorochrome.

Click “OK” button when finished.

You are ready to create your experiment!