LSRFortessa Guide

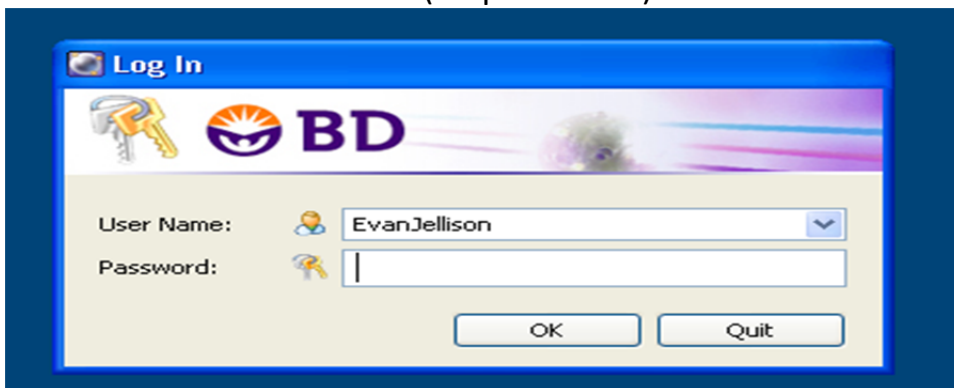Cell Evaluation and Therapy Core
HO324

Before Use:
Prime the instrument twice with no tube and arm to the side
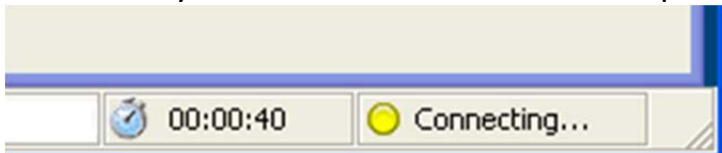Ensure that sheath is full and waste is empty

To Begin:

Log into iLabs tracker with your MUSC email and your iLabs password
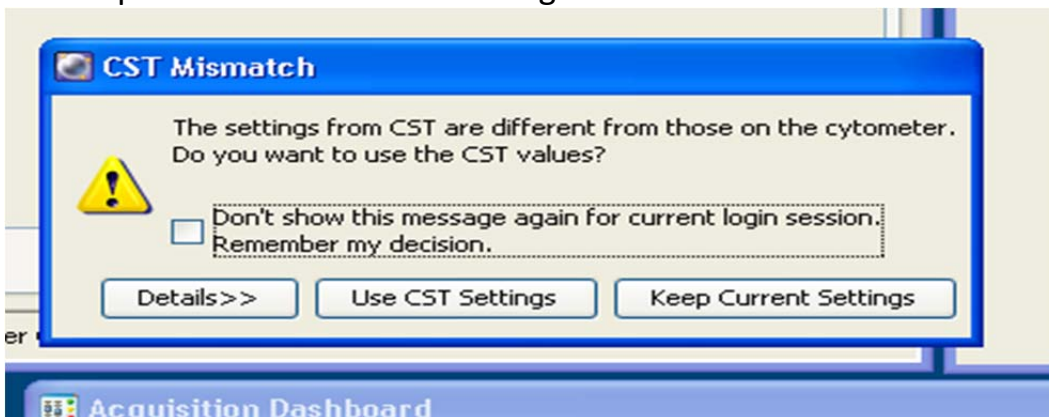
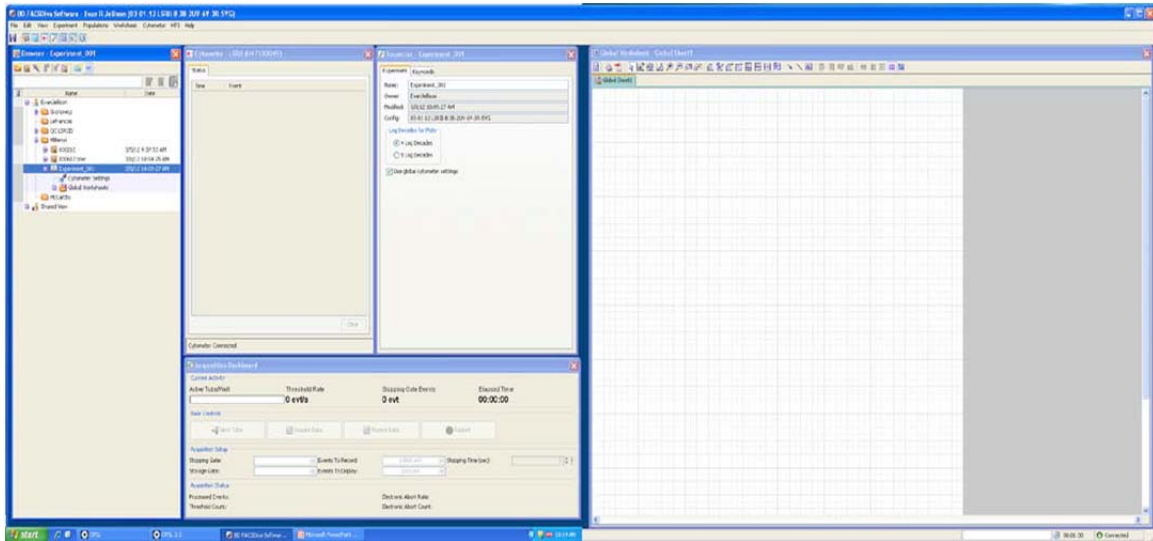Launch Diva from the desktop

Diva user name: Last name (no passwords)

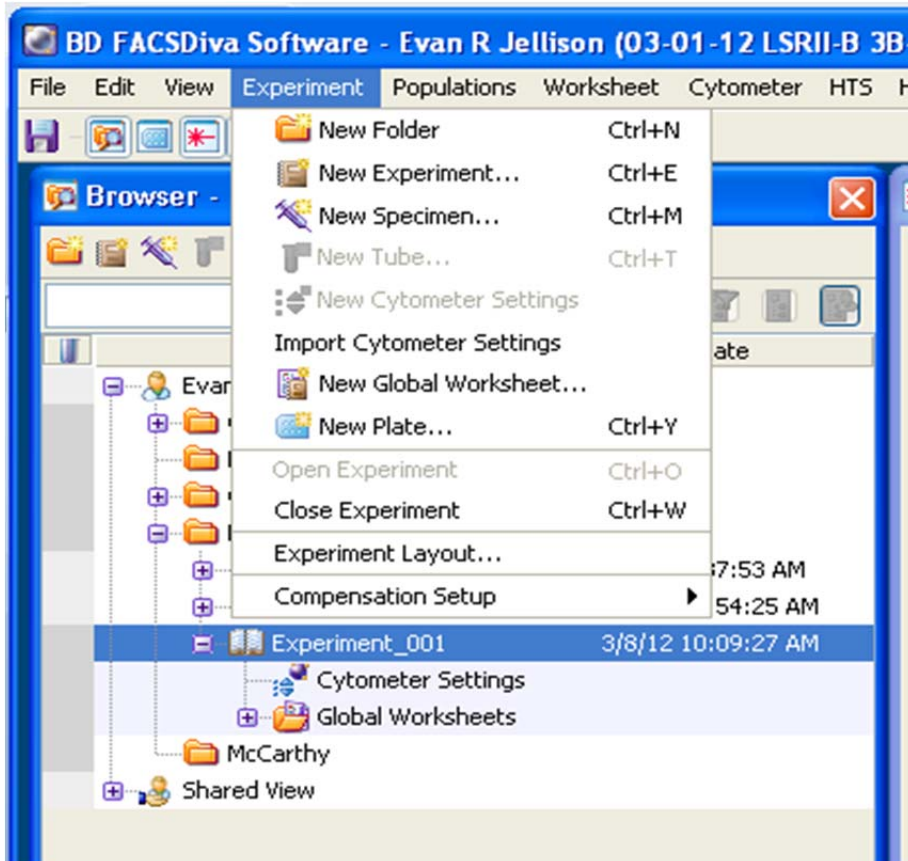Allow the cytometer to connect to the computer

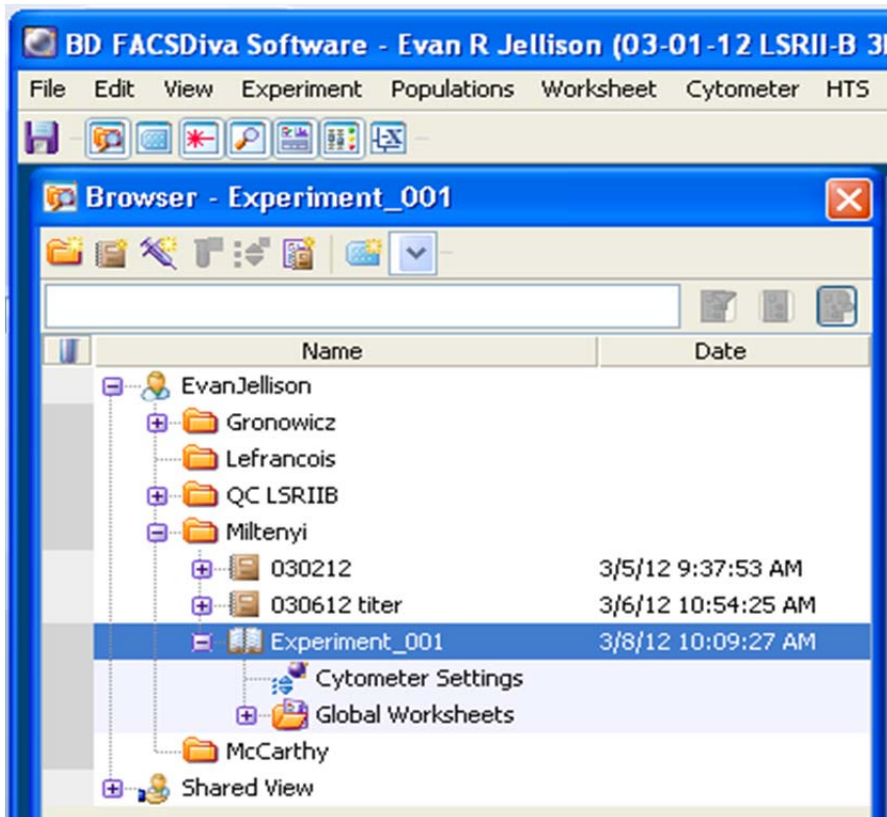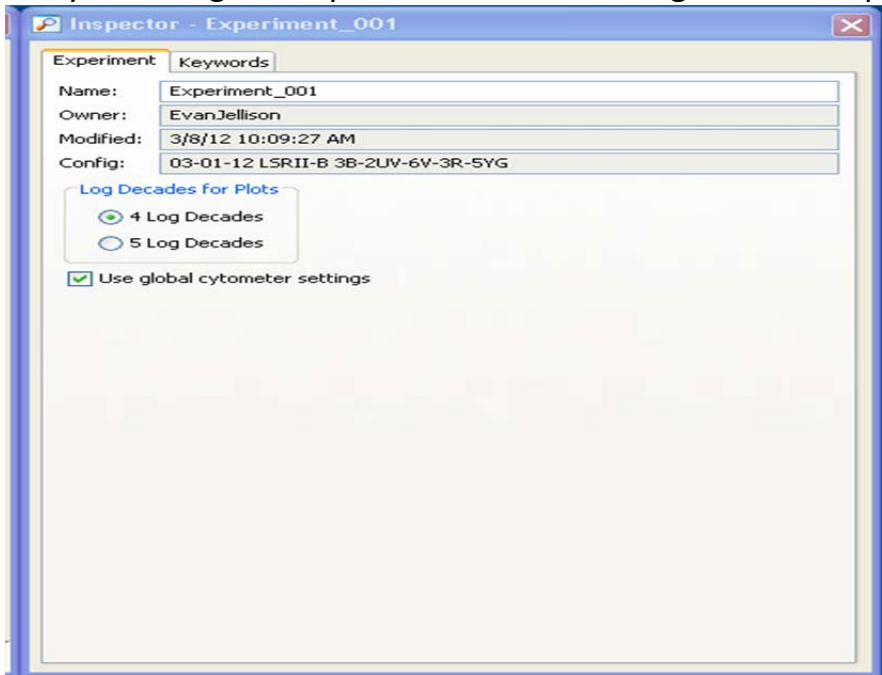If Prompted choose "use CST settings"

Welcome to Diva!

Choose a new experiment

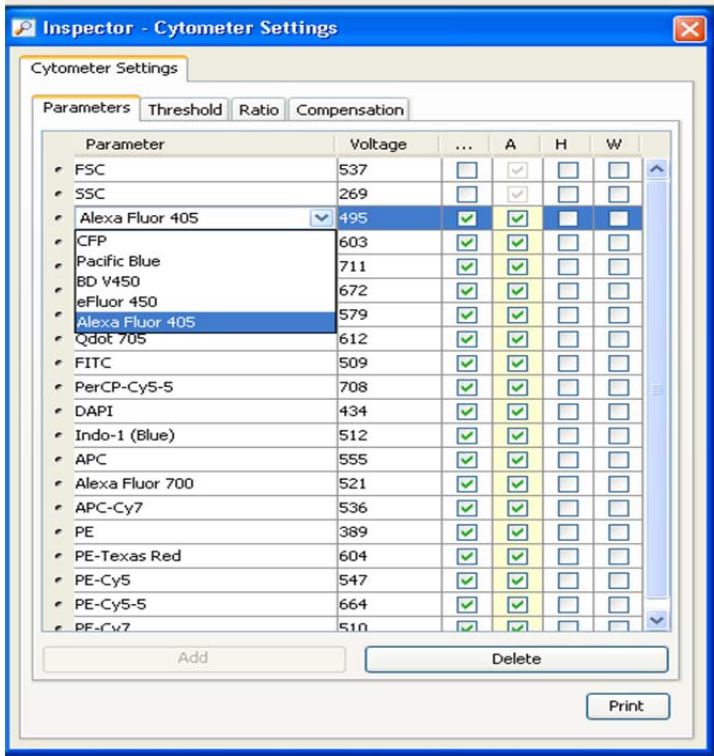You can rename your experiment by right clicking and selecting "rename" or by selecting the experiment and renaming it in the inspector.
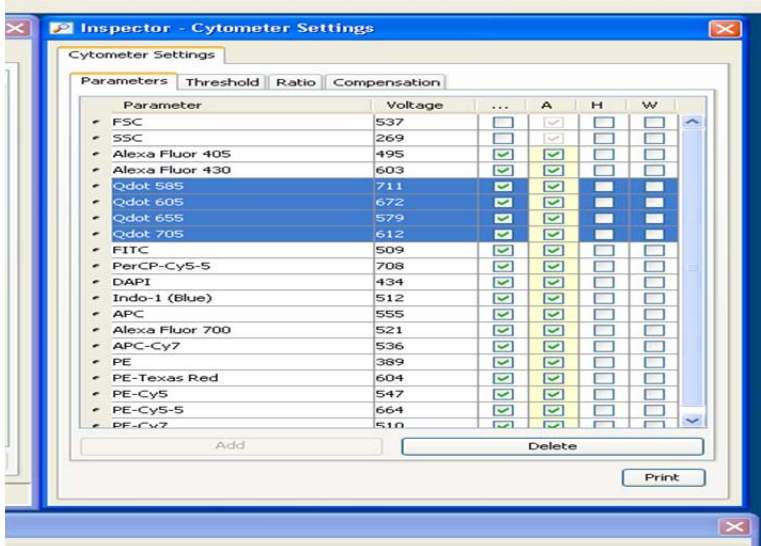
Cytometer Settings:

Select the appropriate flurophores for your experiment. You can select these from the drop down window in the inspector. The ones you do not need can be deleted.


Multiple parameters can be deleted at once by clicking the dot outside while holding down the Ctl key.

At a minimum, select the Area **(A)**, Height **(H)** and Width **(W)** parameters for **FSC** in order to eliminate doublets from your analysis. FSC should be collected using linear scale. SSC can be collected in either linear or log scale, depending on the needs of the experiment or preference of the lab. All fluorescence parameters should be collected in log scale unless DNA cell cycle analysis is to be performed. Area should be selected for fluorescence parameters on LSR II instruments.
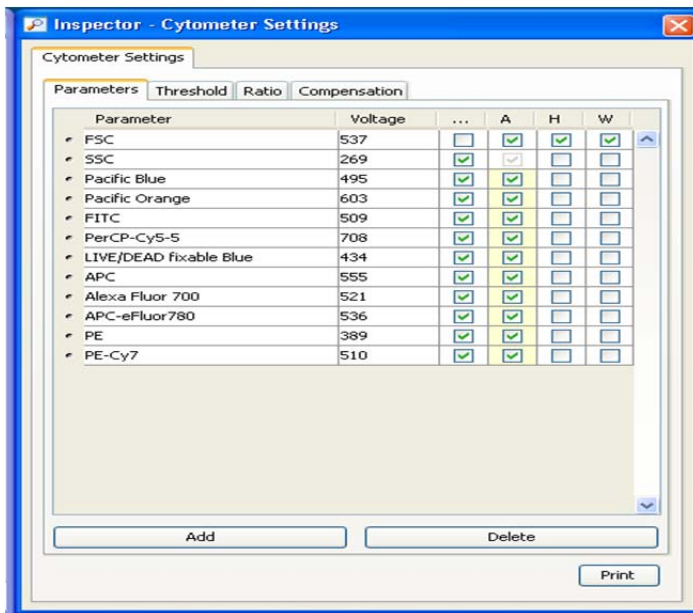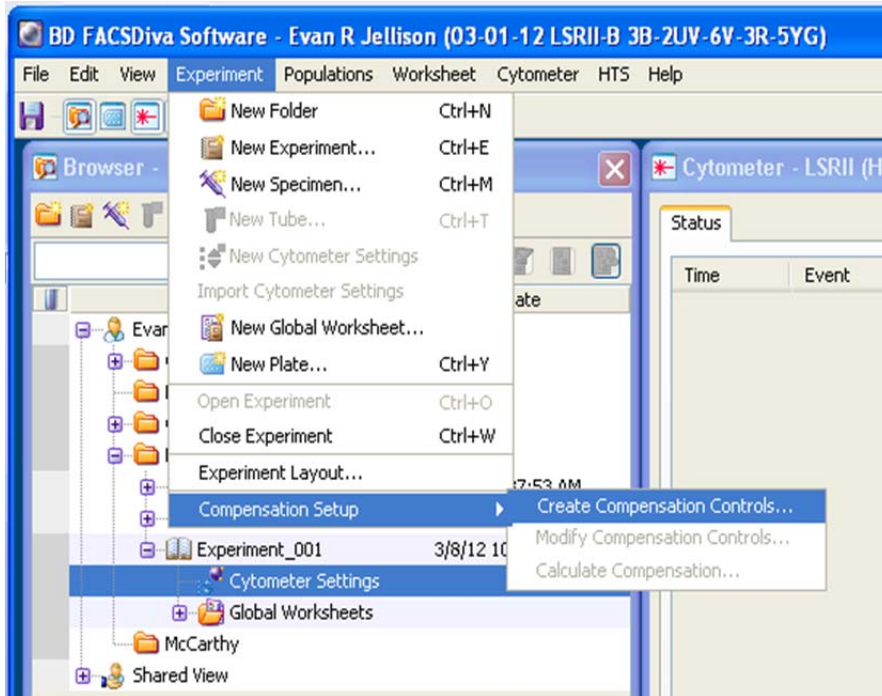

Example of the same data collected in SSC-Linear and SSC-Log:
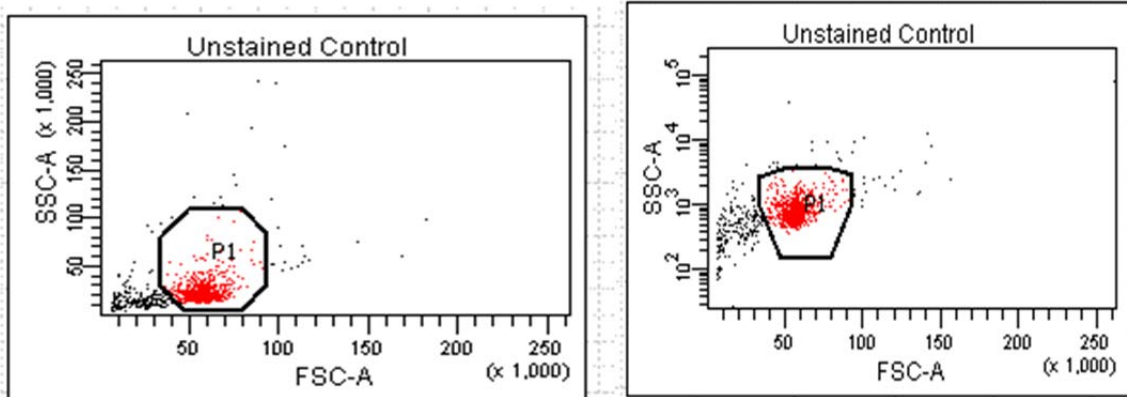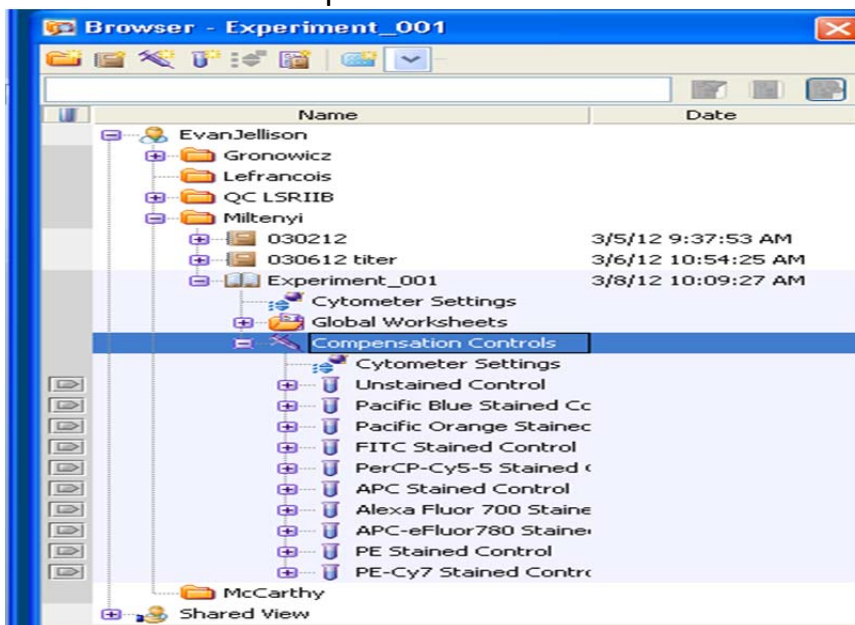
Automatic Compensation:
Create compensation controls by selecting the Experiment dropdown>
Compensation Setup> Create Compensation Controls

Select the OK button. You will see a specimen appear in the Browser
window labeled Compensation Controls.

Expand the compensation control specimen by clicking the **+** symbol.

Click on the Tube pointer next to the unstained control, it should turn green.
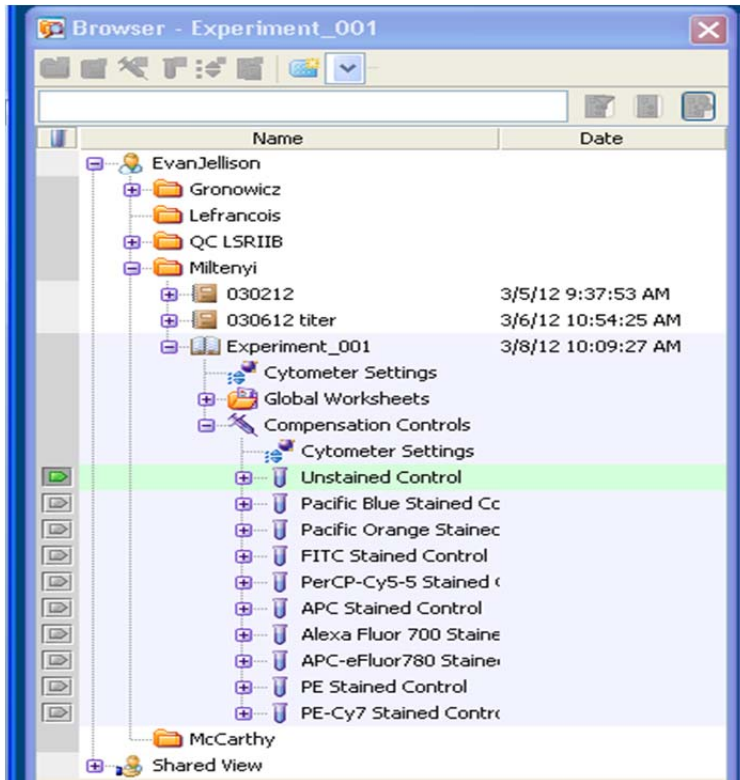
The tube pointer must be green on the tube you wish to be acquiring/recording. Simply highlighting the tube will not suffice.

      Green point- active

      Yellow point- acquiring

      Orange point- recording

Place your unstained cells on the SIP tube and select Acquire in the Acquisition Dashboard window.

DiVa sets the events to record at 5000. You may elect to increase this number by selecting the dropdown or typing the number of events you want to record in the box.

Highlight your adjusted P1 gate, right click, and select Apply to All Compensation Controls.

In the Acquisition Dashboard select Next Tube or select the Tube Pointer next to the first single color control sample
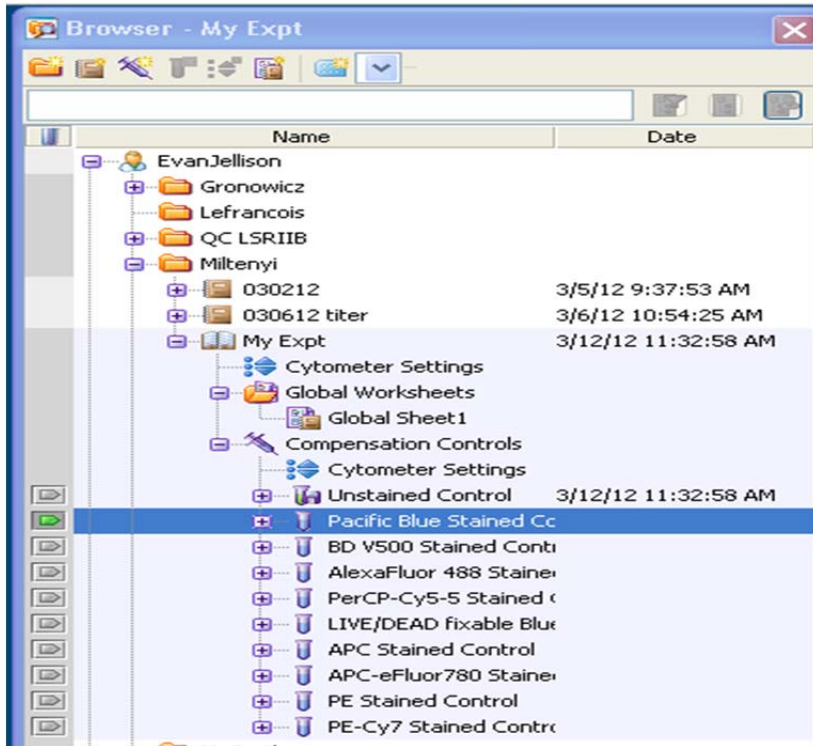
Compensate calculation values by selecting Experiment dropdown > compensation setup > calculate compensation…

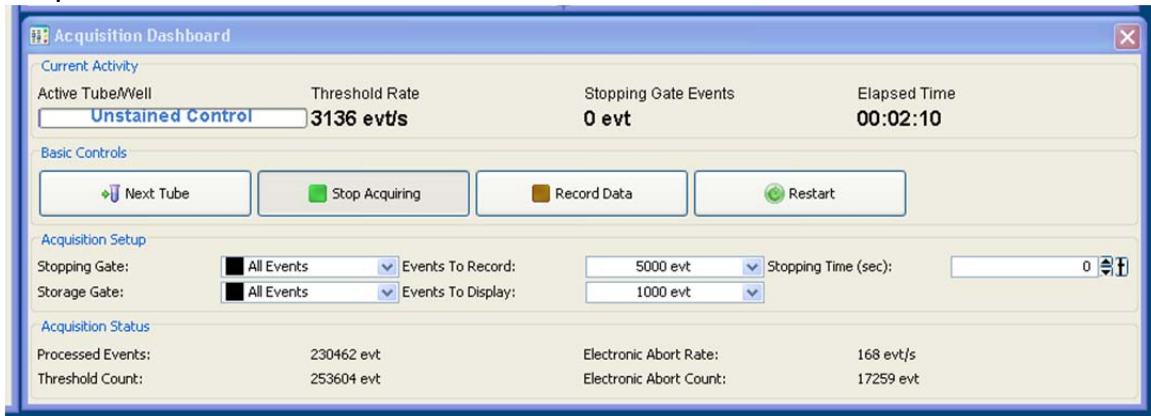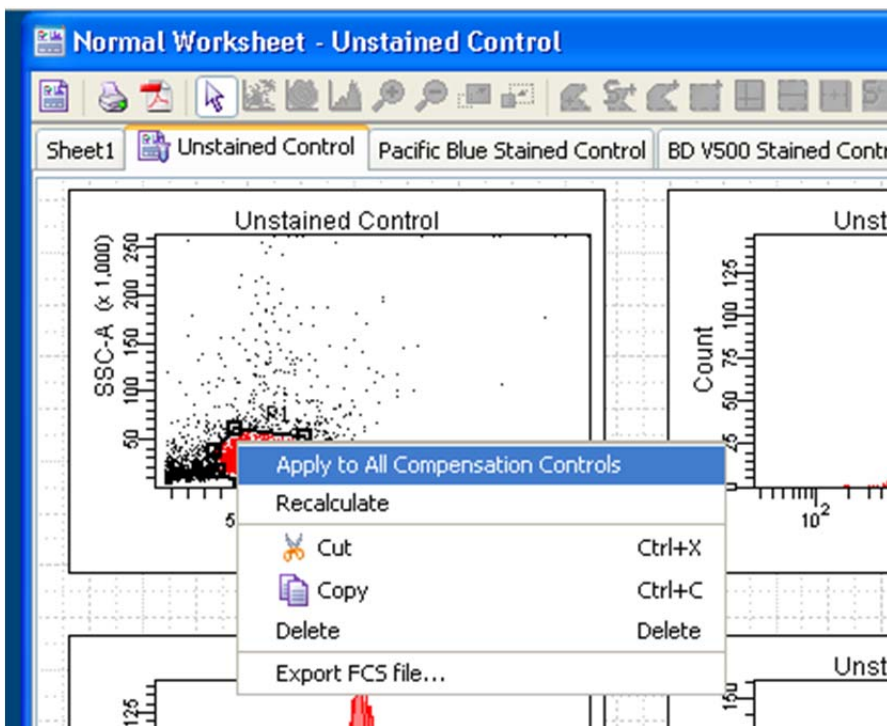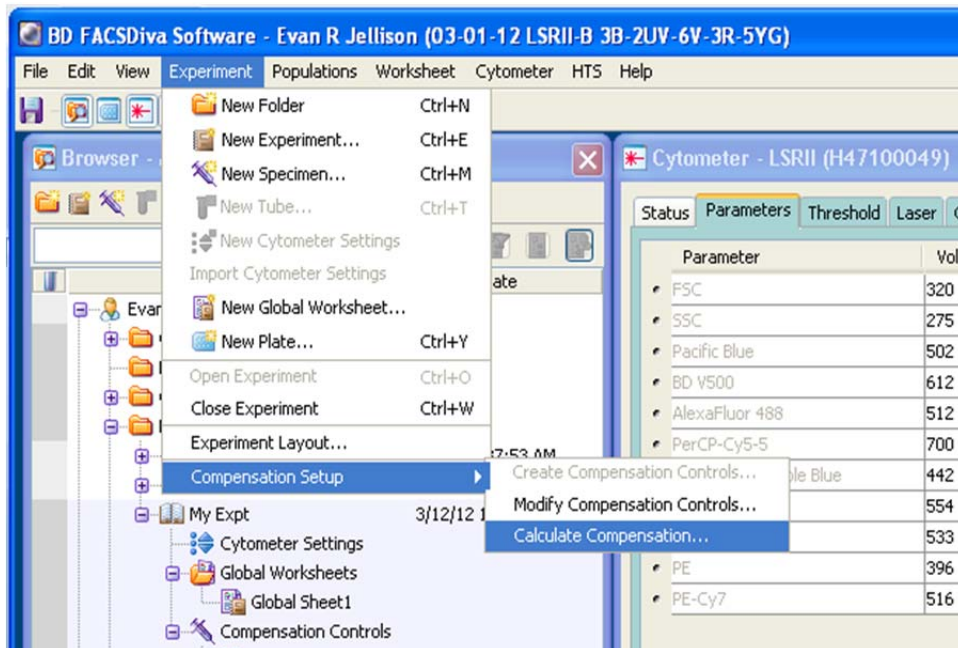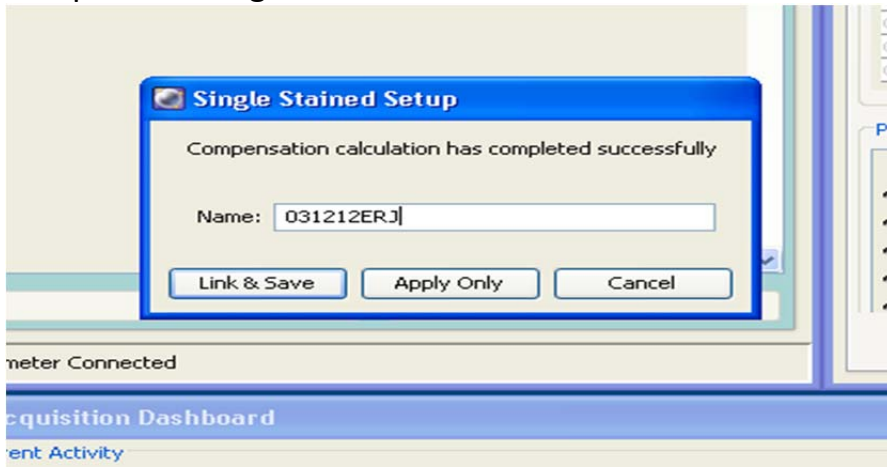If compensation is calculated successfully you will be asked if you want to "apply" or "link and save". You may save this if you want to access this compensation again.

Collecting Experimental Samples:

Add a new specimen by clicking the syringe icon or by navigating to the Experiment dropdown> New specimen and select Blank Panel.

Note: If the tube pointer is still pointed at your last single color control, the plots you create will show the events from the single color control
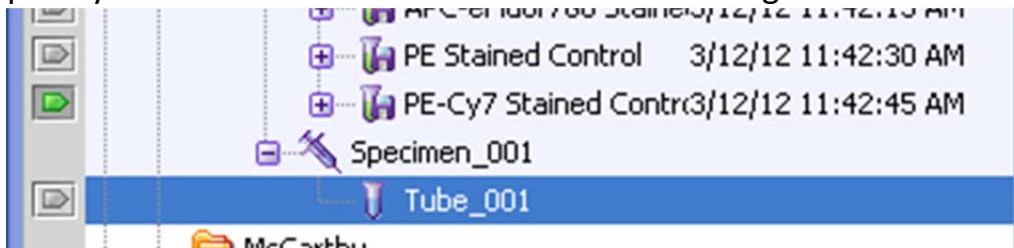
Create gates using the gate buttons on the Worksheet toolbar

Change the parameters on the plots by right clicking the label on the plot and selecting the desired parameter.
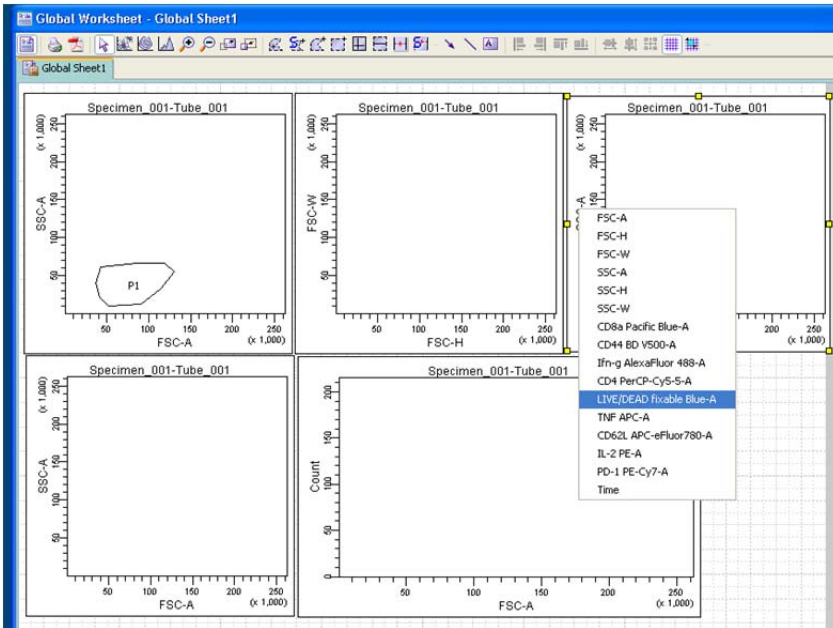
Some parameters may not be available until you move the Tube Pointer to the first experimental tube.

To make a plot showing only gated events either right click the plot and go to show populations…

Or double click parent gate and select "drill down"

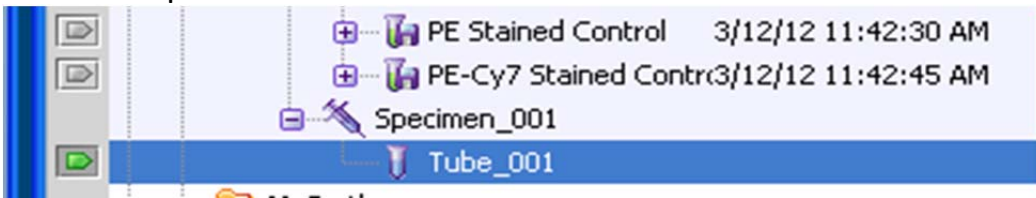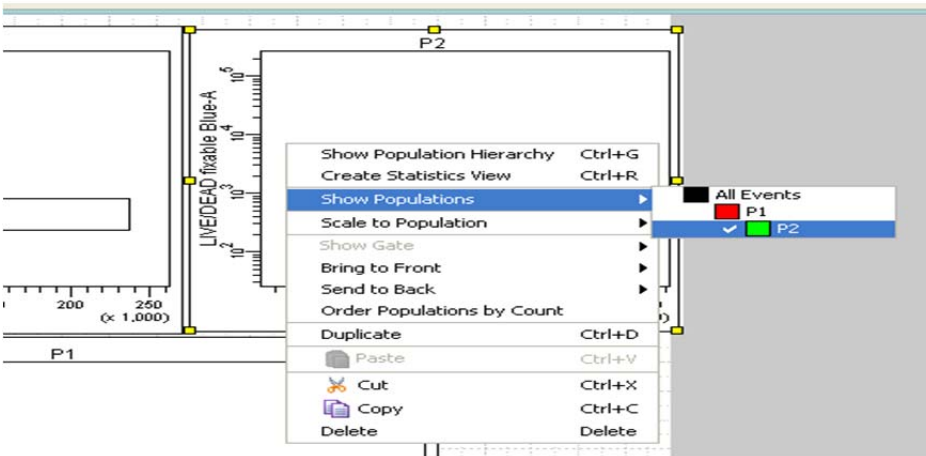**The Acquisition Dashboard:**

Events to record: How many stopping events are going to be recorded?
Events to display: The number of events that appear in the worksheet window

Sample acquisition:
Acquire your first experimental tube. Adjust the flow rate using Low, Med, and High on the cytometer. When the event rate stabilizes (keep below 10,000 evt/s), click Record in the Acquisition Dashboard.

Recording will stop automatically when the Events To Record have been collected (or the Stopping Time has passed) but your sample will continue to be drawn as long as it remains on the SIP. If the Events to Record has not been achieved and there is no sample left to run, promptly remove your tube and move on. **Never leave an empty tube on the SIP with the arm engaged.** This will force bubbles into the flow cell.

Select Next Tube in the Acquisition Dashboard to move to the next sample or use the Tube Pointer to select the tube and record as above.
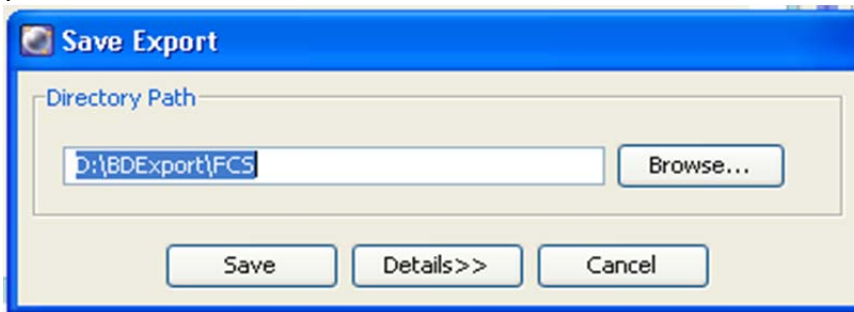
Once all of your tubes have been collected, export your FCS files. Highlight the experiment name in browser, right click, select Export > FCS files.
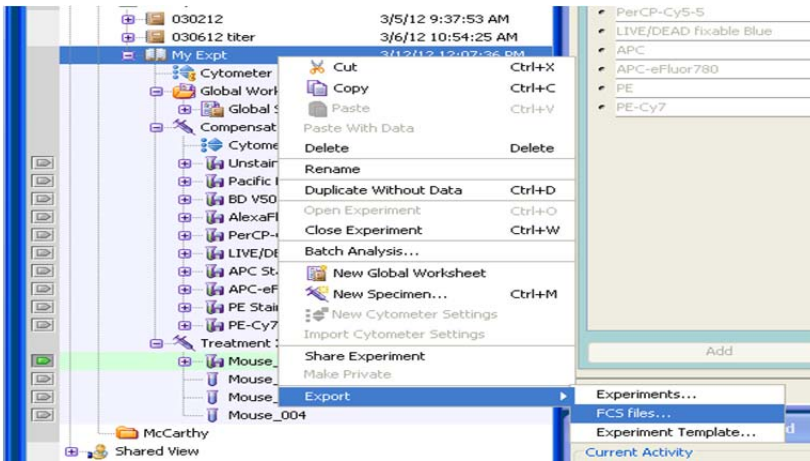
Select the type of FCS file required (generally this should be 3.0) and click the OK button.
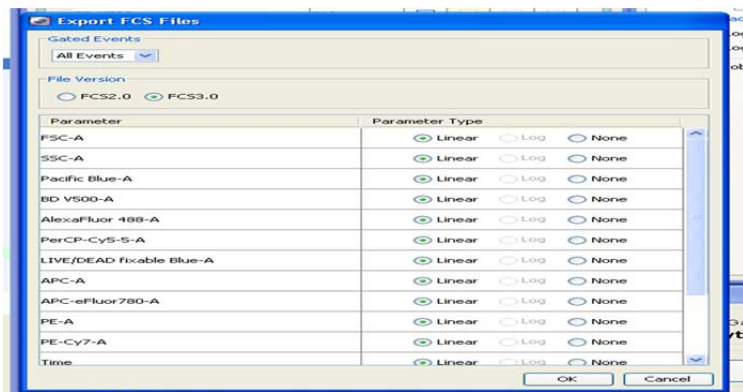
Export the FCS files to the D:/BDExport/FCS folder or to your attached portable drive.

When the Export FCS File(s) progress bar reaches 100%, copy the FCS files to your own personal drive. We encourage you to delete your own data from our computers, however we will **universally** delete the data after 2 weeks.

Clean and Prepare for the next user:
Put a tube with ~3ml (~half tube) BLEACH (squeeze bottle) on the SIP and run in HIGH for 5min. This step is crucial to decontaminate the instrument. Put a tube with ~3ml water on the SIP and run on high for approximately 3min.
Leave ~1mL of water on the SIP and hit STANDBY.

Check and fill sheath, empty waste and add approximately 300ml bleach.