BD FACSDiva UF Tutorial

UF
Flow Cytometry Core Facility

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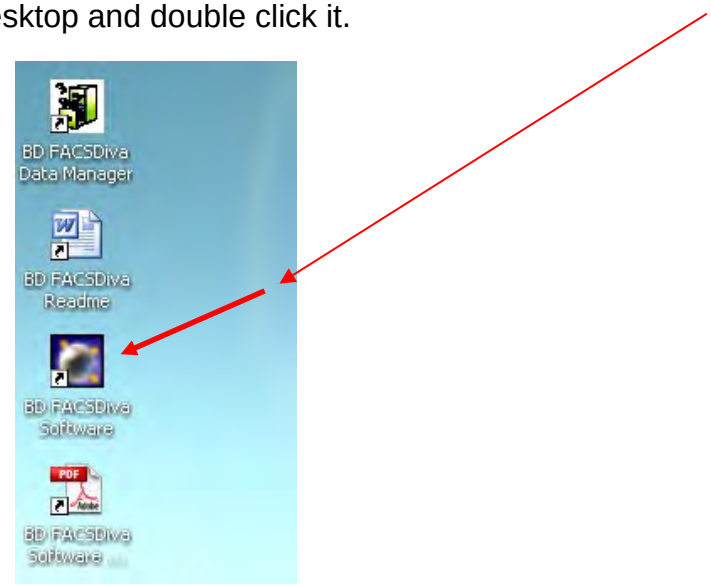
1. Computer and Software Login

To log into the computer, use your **Gatorlink** as the username and your password as the password. To log in to the **FACSDiva software** you need to locate the icon on the desktop and double click it.



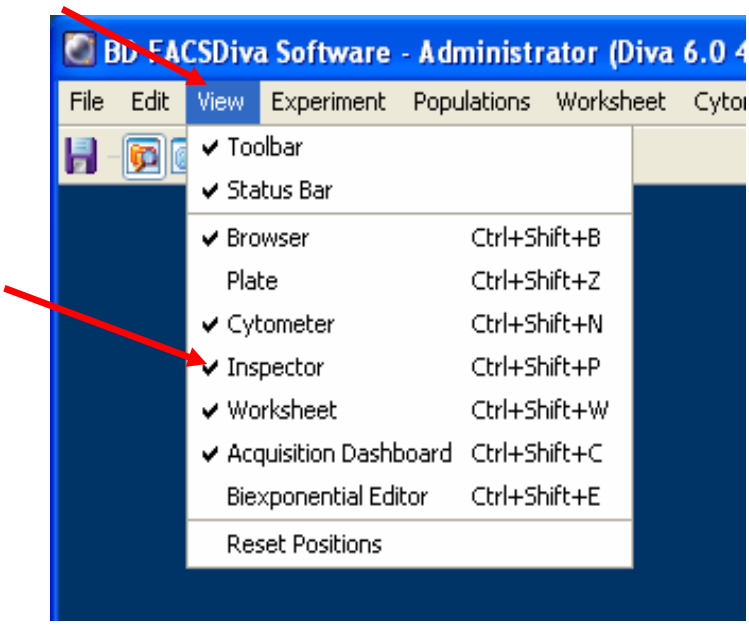
In the login window, scroll down through the list of user names to locate yours.

2. Setting Up Your Experiment

2.1. Setting Up the Workspace

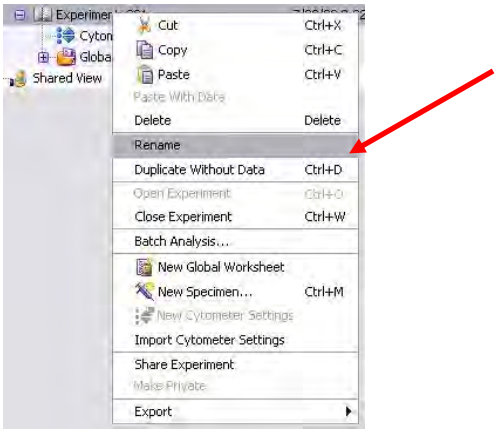
Before you start, make sure that you have all the necessary windows open. Go to the **"View"** menu and make sure the following boxes are checked:

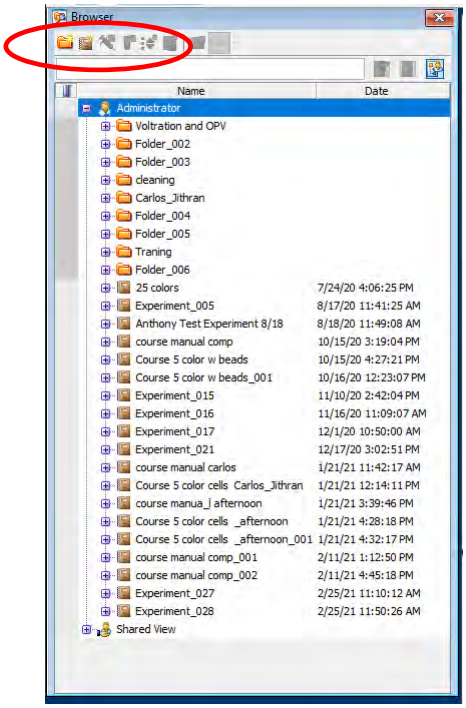
- ✓ **Browser**
- ✓ **Cytometer**
- ✓ **Inspector**
- ✓ **Worksheet**
- ✓ **Acquisition Dashboard**



2.2. Creating a New Experiment

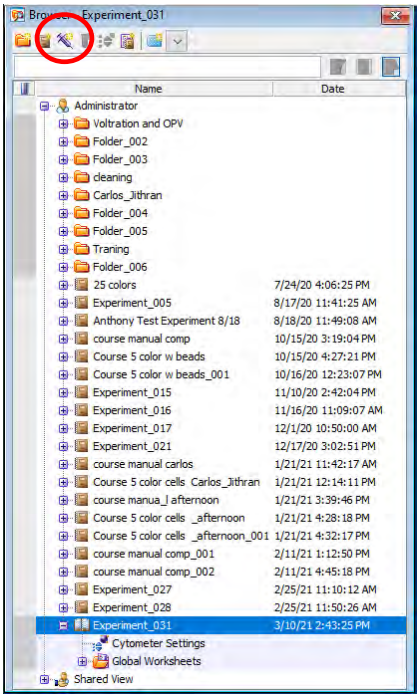
Click the **"New Experiment"** icon () in the **browser toolbar**. The new experiment will be created with a generic name. Right click on the name of the experiment. Select **"Rename"**. Rename it using the date and specific information of your experiment to make your name unique.

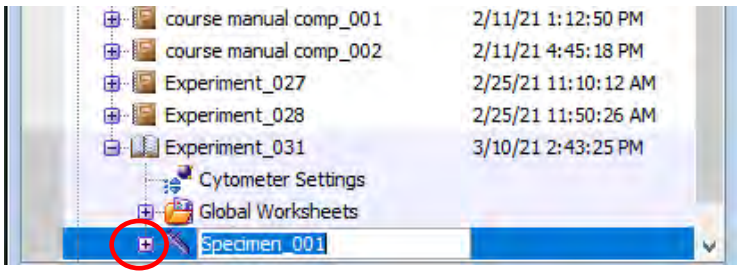




2.3. Creating a New Specimen

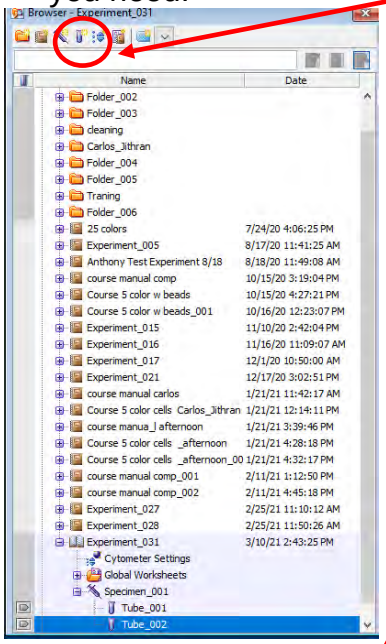
Click the **"New Specimen"** icon () to add a specimen and a tube to the experiment; click once on the plus sign (**+**) next to **"Specimen_001"** to expand it.

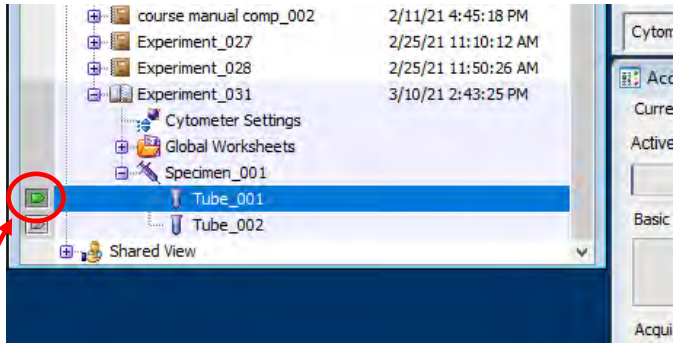




2.4. Creating a New Tube

Click the **“New Tube”** icon () to add second tube. Add as many tubes as you need.



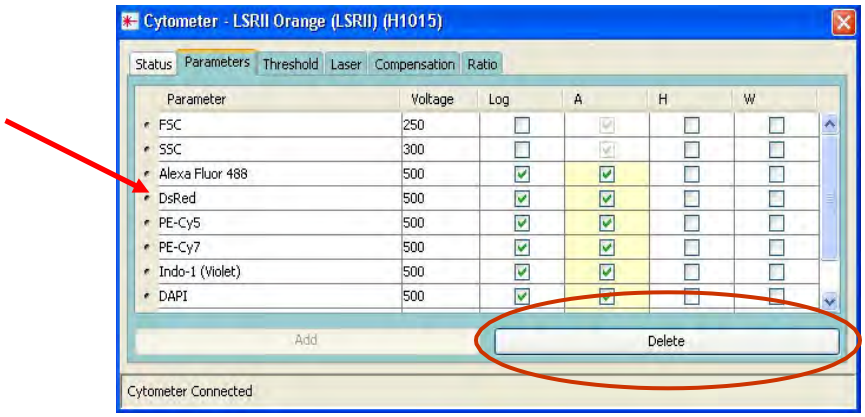


In the browser, click the icon to the far left of the tube named **“Tube_001”**. The pointer changes to green. This **MUST** be selected to change the parameters of your experiment.

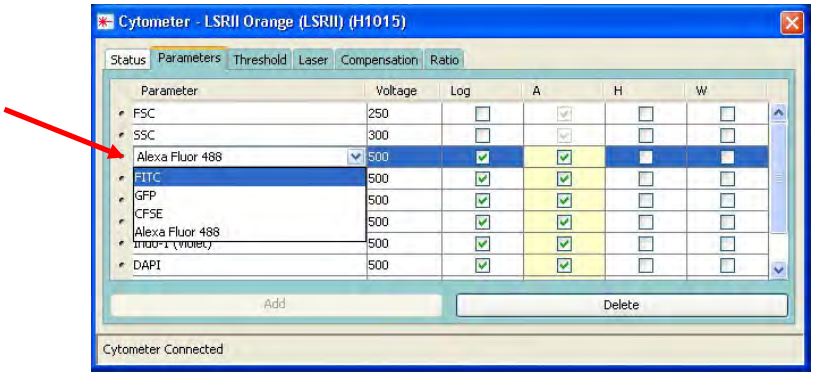
The current tube pointer indicates the tube to which instrument setting adjustments will apply and for which acquisition data will be shown.

2.5. Selecting Parameters

Click the **"Parameters"** tab in the **"Cytometer"** window and delete all the parameters that you don't need. To delete parameters, click the selection button next to each parameter that you want to delete. Hold down the Ctrl key to select more than one. After you are finished selecting, click the **"Delete"** button. You can add parameters by clicking the **"Add"** button.

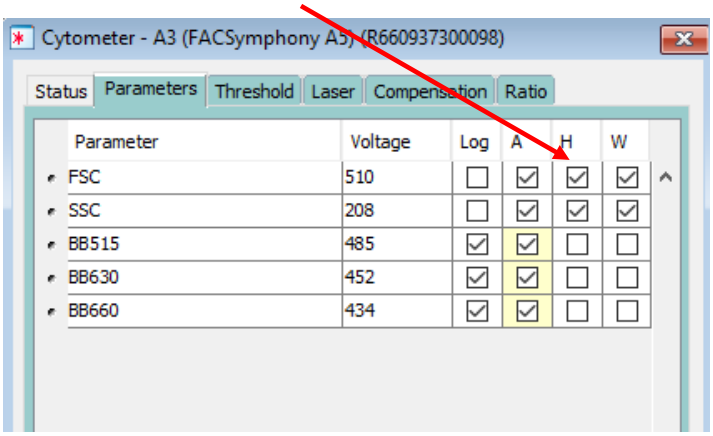


You can choose which parameters to use by using a scroll down menu:



2.6. Selecting H and W as a Parameter

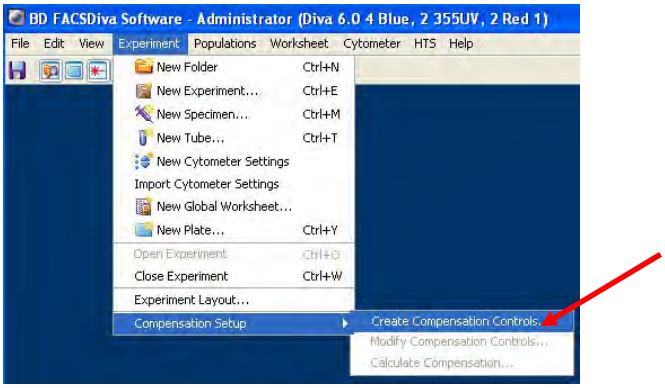
Check **"H"** and "**W**" for FSC and SSC Only.



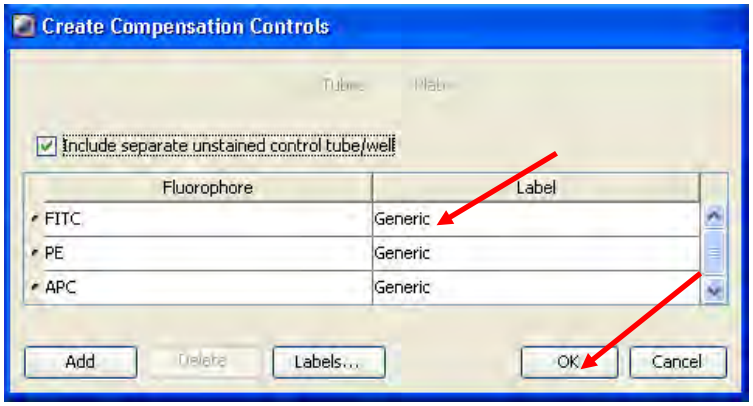
3. Acquiring Compensation Controls and Calculating Compensation

3.1. Creating a Compensation Control Specimen

Create Compensation Controls if you are going to use more than one fluorochrome. Select **"Compensation Setup"** from the **"Experiment"** menu; then select **"Create Compensation Controls"**.

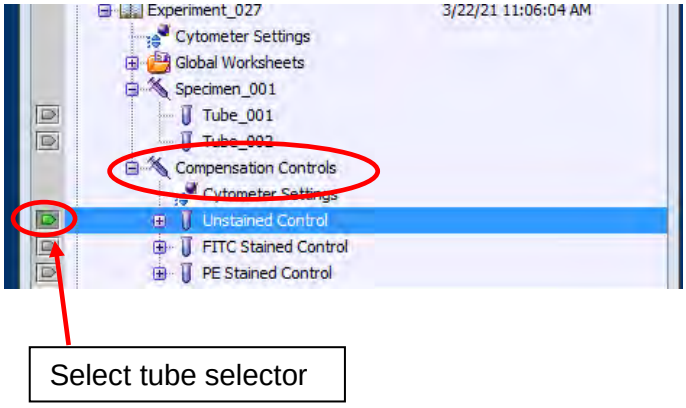


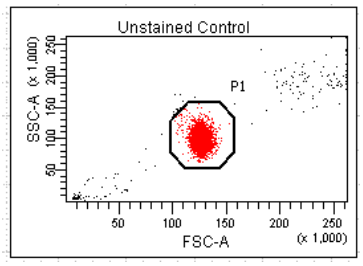
LEAVE THE LABEL AS "GENERIC", There is a BD Glitch where the compensation values revert to zero if you change it. Change the labels later the Experiment Layout.

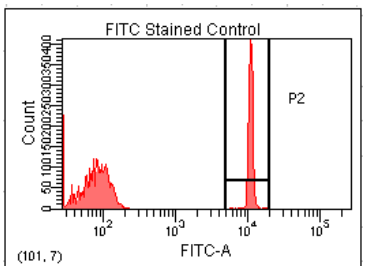


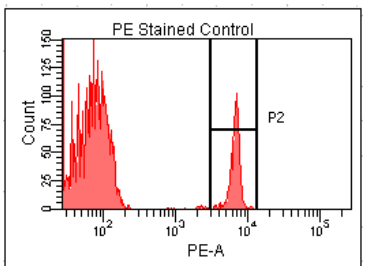
3.2. Recording Compensation Tube Data

"Global Worksheet" should change to **"Normal Worksheet"** automatically. Click once on the plus sign (+) next to the **Compensation Controls Specimen** to expand it. Select **"Unstained Control"** by clicking on the icon to the left of the tube name. Place the tube with the unstained sample on the **Sample Injection Port (SIP)**. Press the **"Low"** and the **"Run"** buttons on the **Fortessa/Symphony instrument (On Canto just press Acquire is software)**. Click the **"Acquire Data"** button, wait a few seconds until the threshold rate is stable, and then click the **"Record Data"** button on the **Acquisition Dashboard**. After you are done recording, adjust the P1 gate to include your population of interest. Then you may right click on the P1 gate and select **"Apply to All Compensation Controls"**. Record data for all tubes in the Common Control Specimen. Adjust **P1** and **P2** gates as n



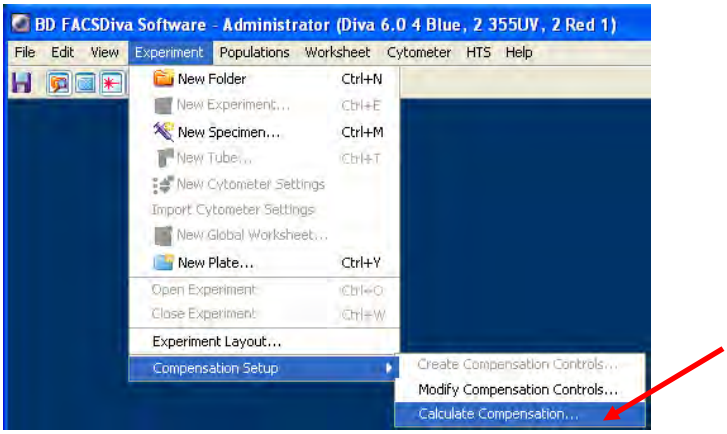




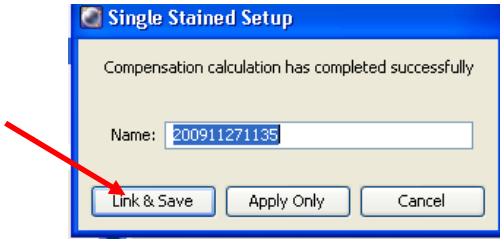


3.3. Calculating Compensation

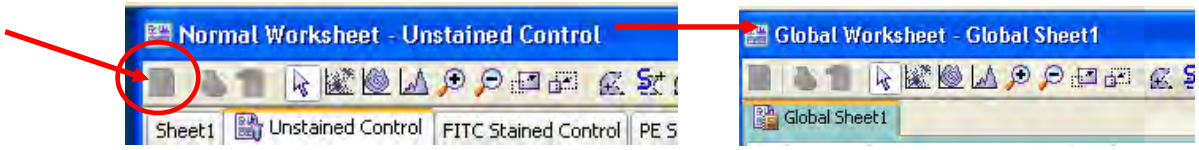
Navigate to **"Experiment"**, then **"Compensation Setup"**, and then select **"Calculate Compensation"**.



The computer will calculate compensation for all parameters. In the window that appears, select **"Link & Save"**



Switch back to **Global Worksheet** by clicking on the top left icon. Now you are ready to collect data for your samples.



The pointer on the far left of the tube for which you are about to record the data should be selected.

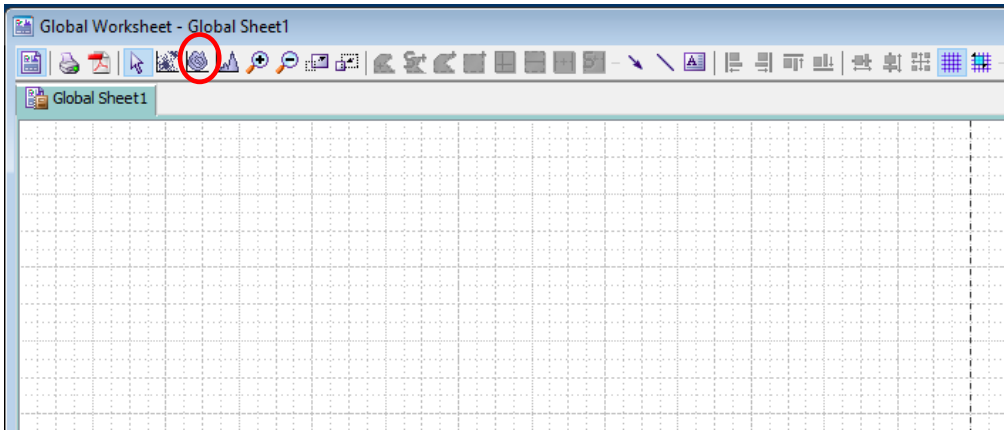
4. Setting up a Worksheet

4.1. a Dot Plot And Contour Plot

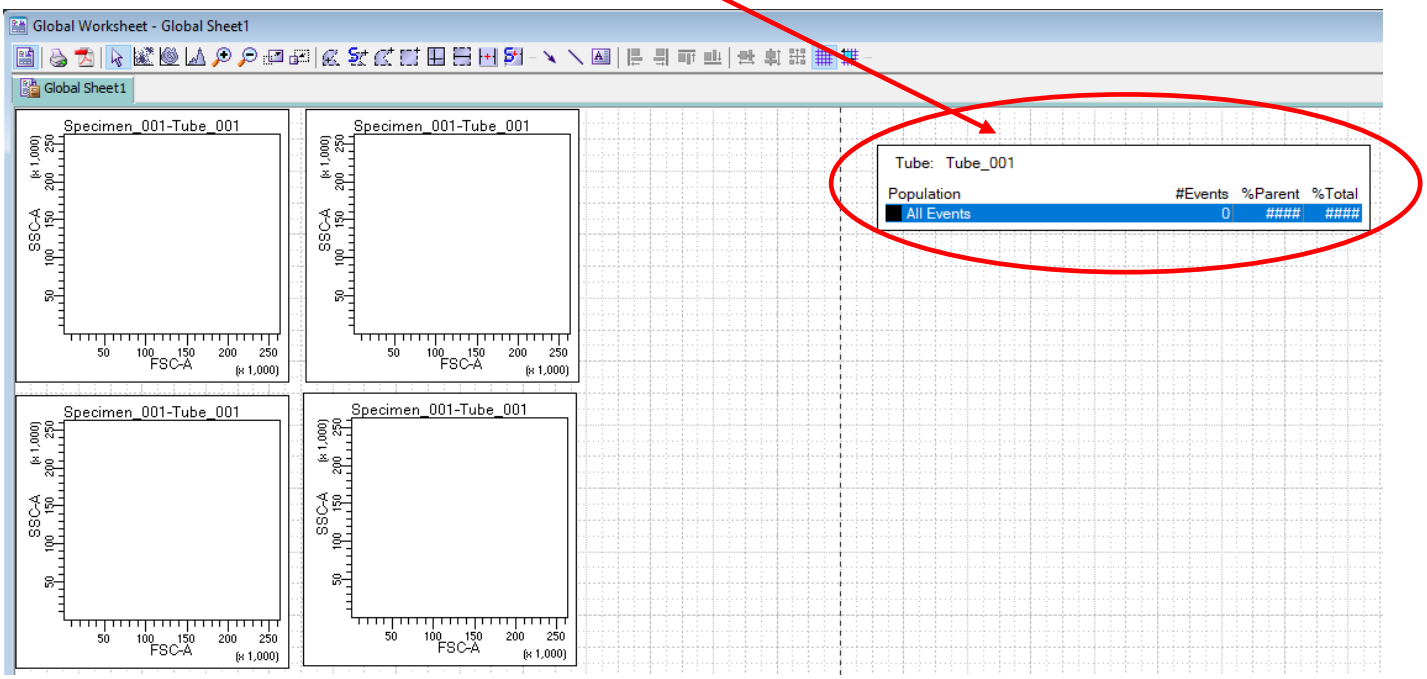
Create a FSC-A vs. SSC-A plot on the global worksheet. Select the **"Dot Plot"** icon

( ) on the Worksheet toolbar and click once in the upper left corner of the worksheet field. A default size plot is drawn.

Then create 3 "**Contour Plots**"



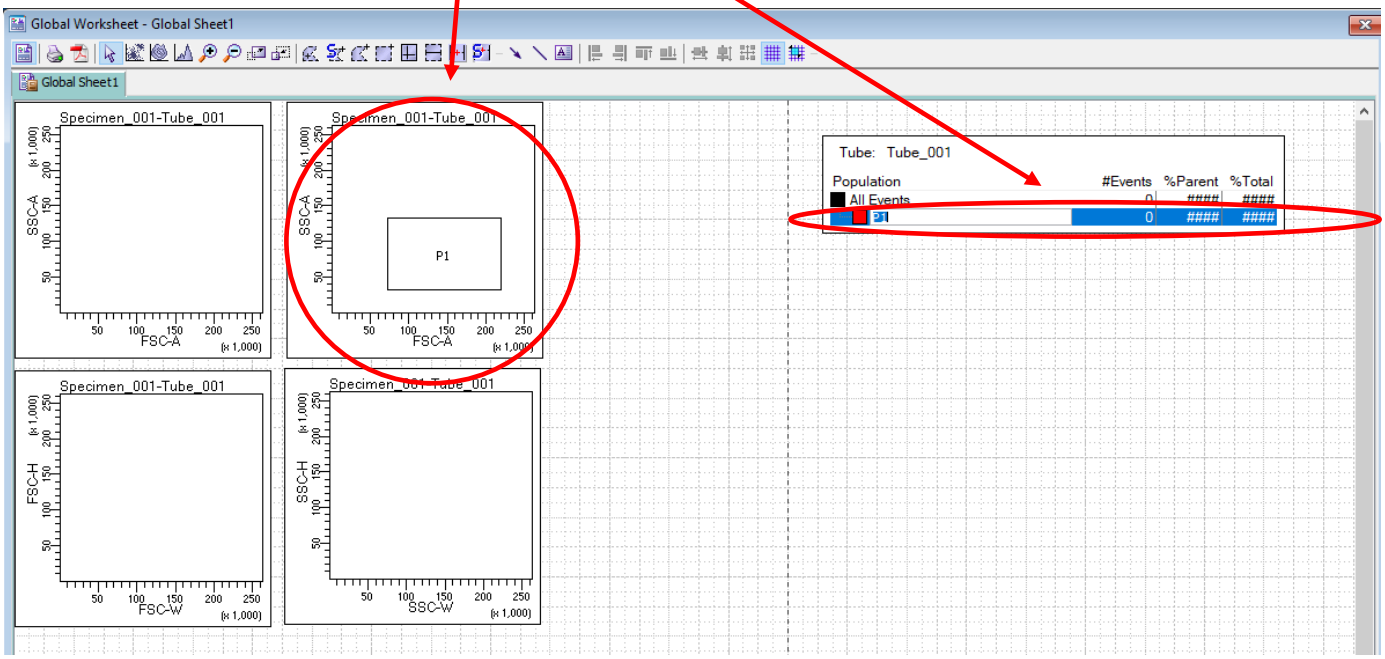
Then Press Control+"G" to create a population hierarchy.



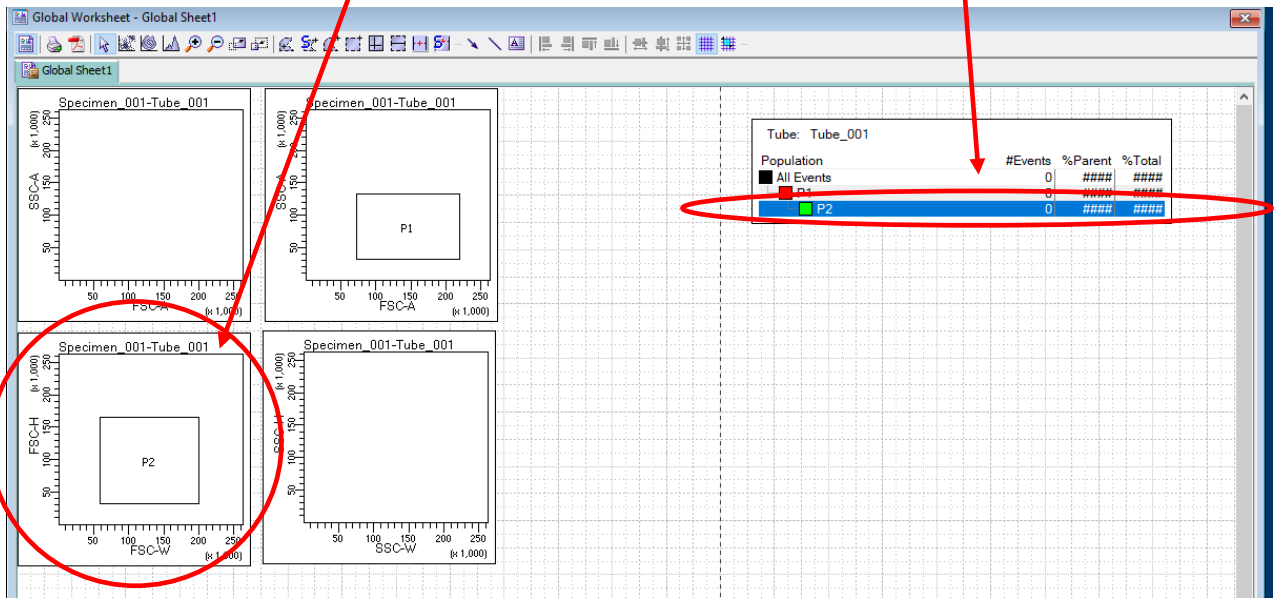
4.2. Creating Your First Gates

Create a gate by selecting the **"Polygonal Gate"** button or the "**Square Gate Button**"  and drawing it in the dot plot. Be sure you finish your gate at the same point you started drawing it. This gate will let you gate on a single cell population and exclude aggregates. This gating strategy may not be appropriate for your cell type. For example, If you are working with whole blood, bone marrow, or with samples that may contain more than one cell type, you may use a different gating type.

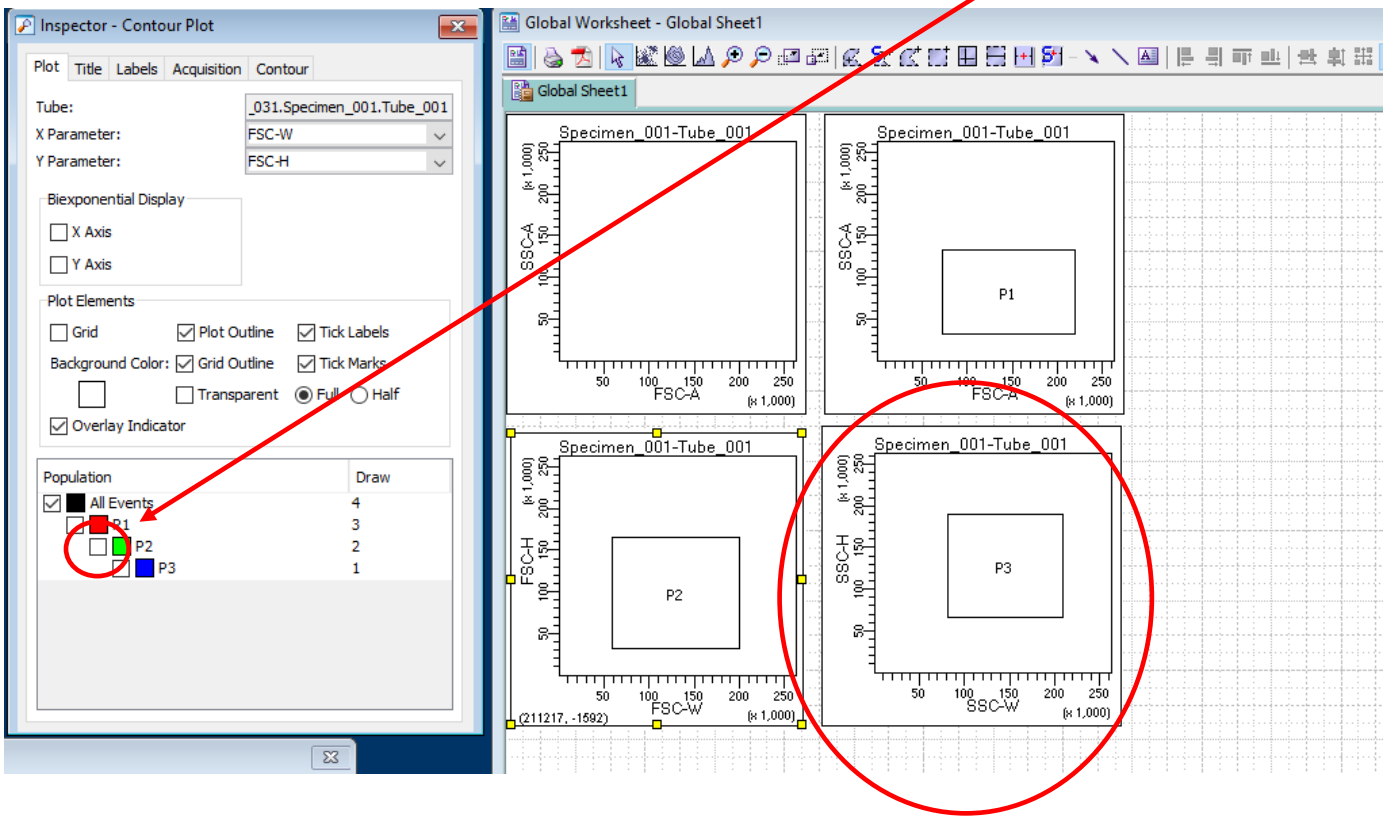
Once you create your first gate in your first Contour Plot, gate "**P1**" will appear in the Population Hierarchy window. Be Sure to click on "**P1**" inside the Population hierarchy window once created.



Create your Second gate in your Second Contour Plot, gate "**P2**" will appear in the Population Hierarchy window. Click "**P2**" in the population hierarchy window once created.

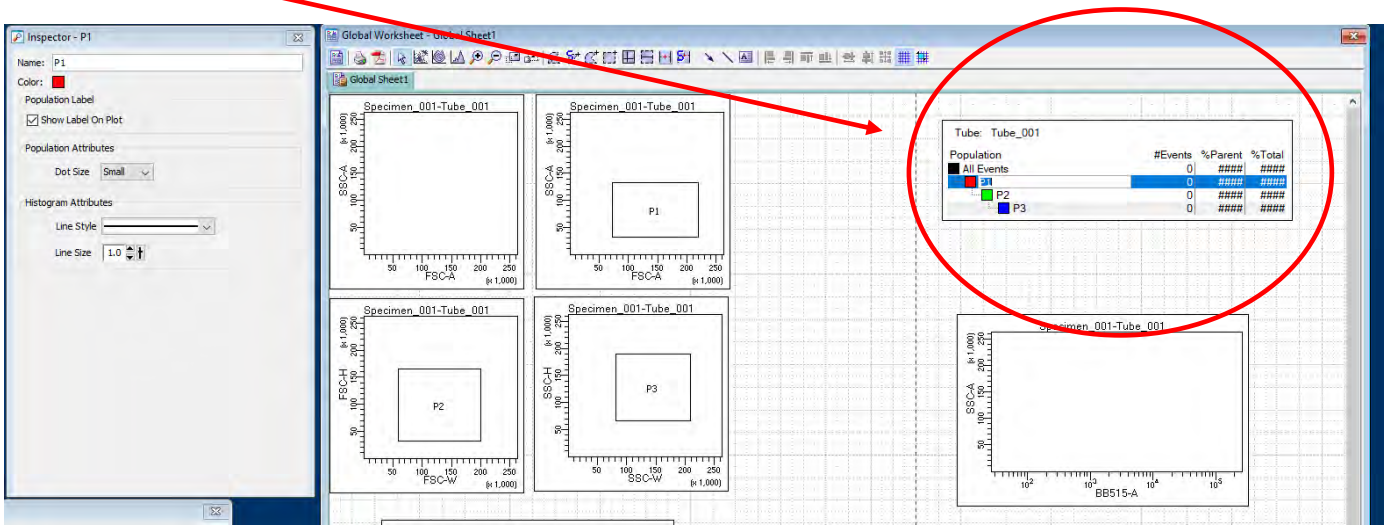


Click the **Third contour plot**. And in the inspector window, Plot Tab Check the P2 Box. Or Right Click the **Second Contour Plot>Show Populations>P2**.



Now you can relabel the Plots through the Population Hierarchy.

P1>Cells
P2>FSC Single Cells
P3>SSC Singles

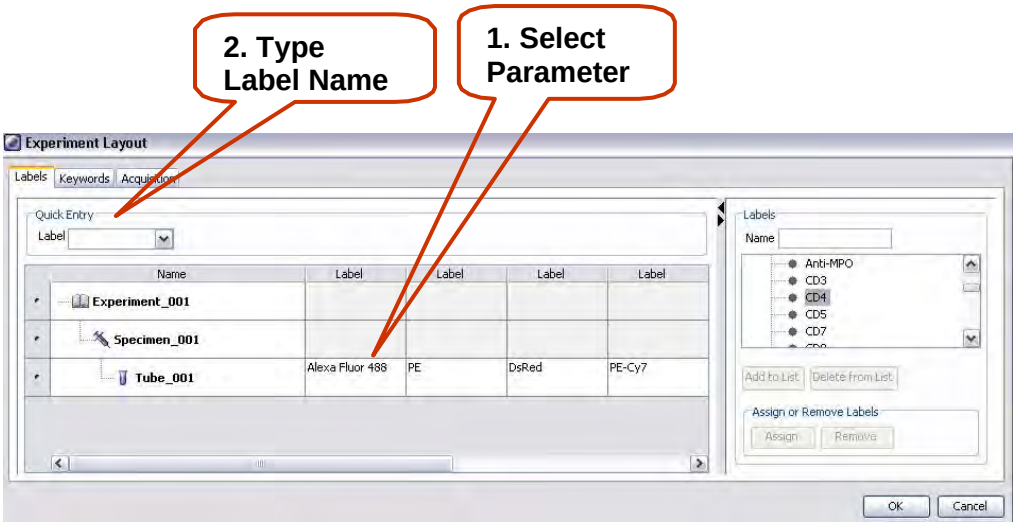


4.3. Opening an Experiment Layout

Choose "Experiment" > **"Experiment Layout"**.

4.4. Labeling Parameters

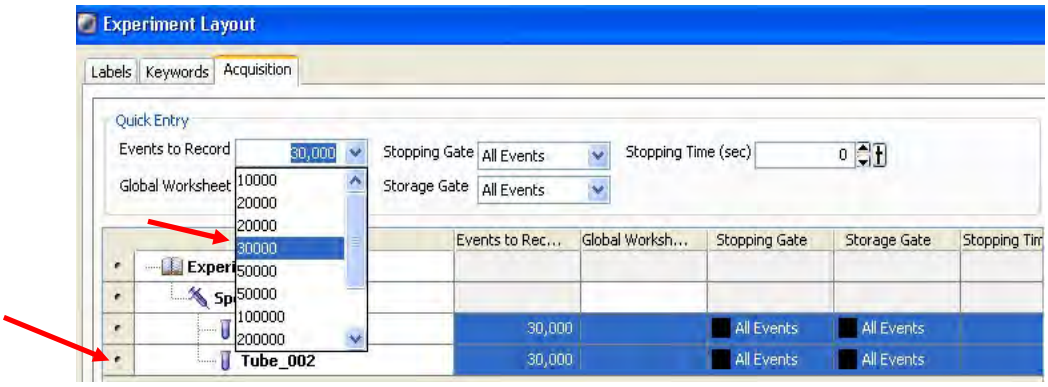
Define labels for each parameter.



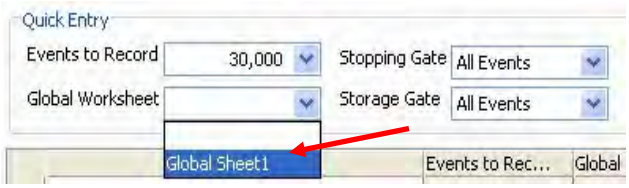
As an alternative, you may apply existed labels from the list on the right.

4.5. Setting Up the Number of Events to Record

In the "**Experiment Layout**" dialog box, click the "**Acquisition**" tab. Here you can set the amount of events to record for each tube. If you don't have specific preferences, we recommend that you record the same number of events in every tube. Select all tubes by clicking on the selecting dots located to the far left of your tube names while holding Shift. Then specify the number of events by using the "**Events to Record**" scroll down menu.



After that, select **Global Worksheet**.



Then, select **Stopping Gate**.

Leave "**Storage Gate**" value equal to "**All Events**"

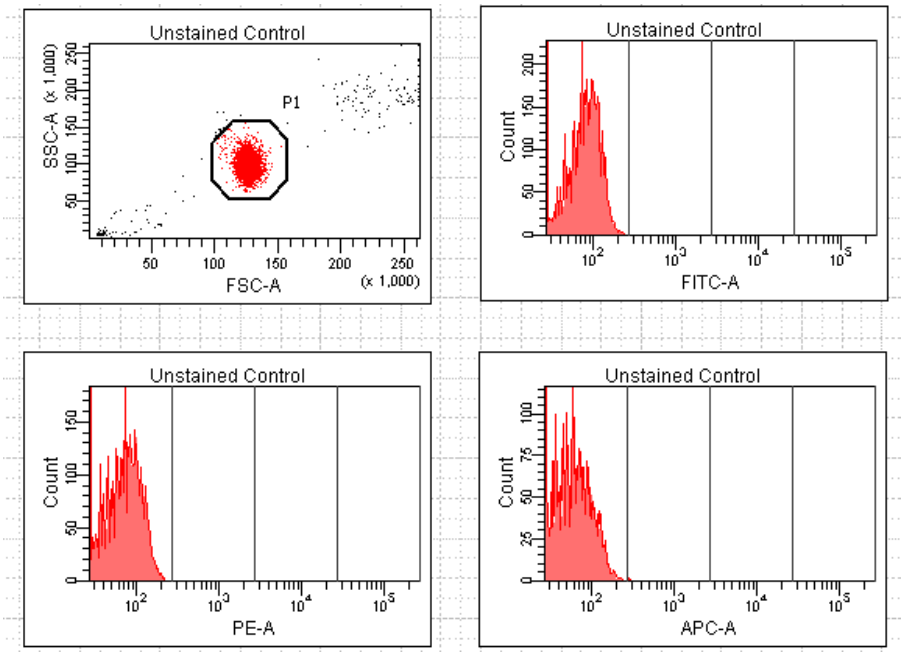
After everything is set, click the **"OK"** button in the right lower corner of the **"Experiment Layout"** window.

4.6. Setting Up Voltages Based on an Unstained/Negative Control

Now you are ready to acquire your first tube. Your first tube should be an unstained and untreated control. Make sure that the pointer on the far left from the tube name is activated.

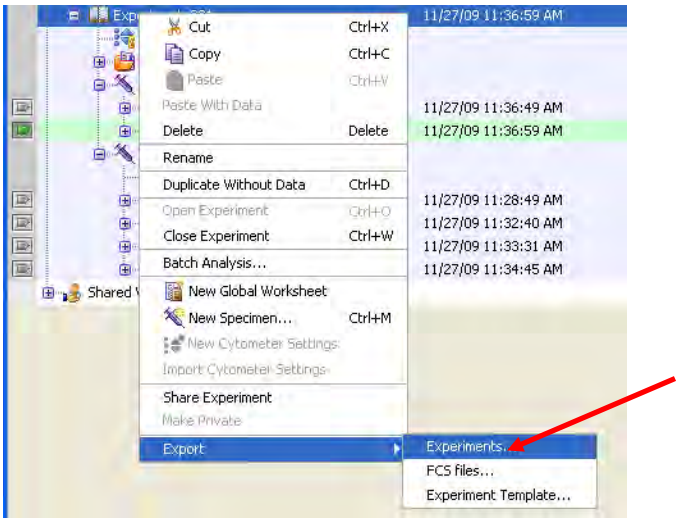
Place the tube on the Sample Injection Port (SIP). Press the **"Low"** and the **"Run"** buttons on the **Fortessa/Symphony instrument**. The **Canto** has the speed controls in the Acquisition dashboard menu. Click the **"Acquire Data"** button on the **"Acquisition Dashboard"**.

Adjust Voltages so you can see the population of interest in the center of the FSC-A vs. SSC-A dot plot. Adjust the voltages so the autofluorescent signals on each parameter form peaks within the first decade. You need to be able to see both the ascending and descending slopes of each peak.

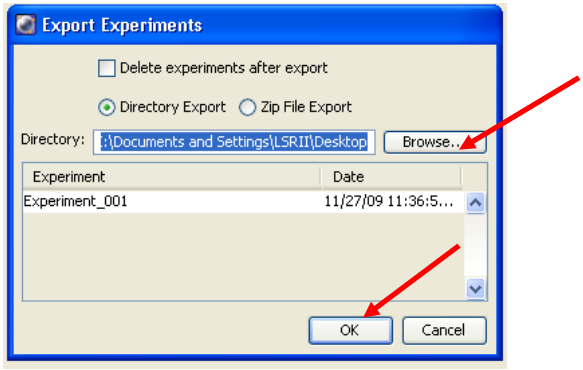


5. Exporting Recorded Data

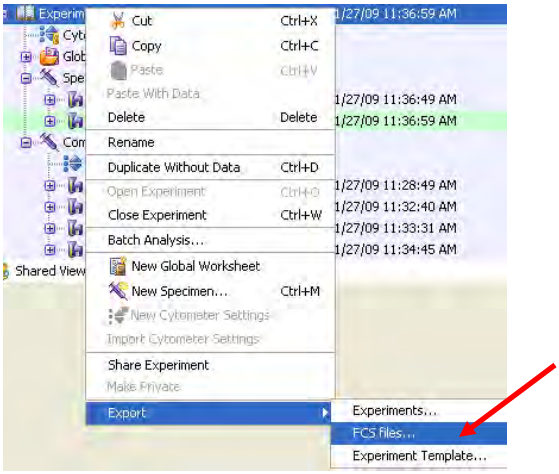
After you finish recording your last sample, export your Experiment and FCS files. Right click on the name of **your experiment** and in the menu select **"Export"**, then **"Experiments"**.



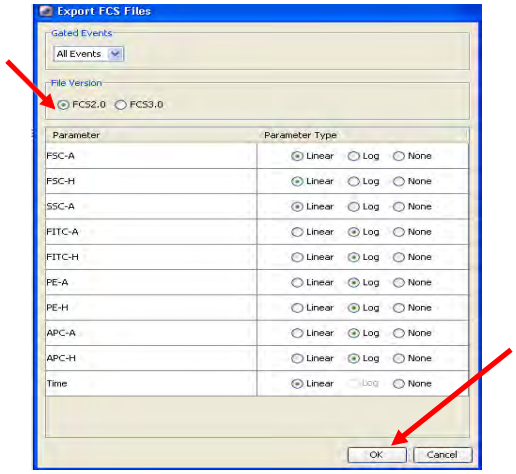
In the window that appears, click the **"Browse"** button, then select **"Desktop"** as the destination folder and click **"Export"** to save your files there.



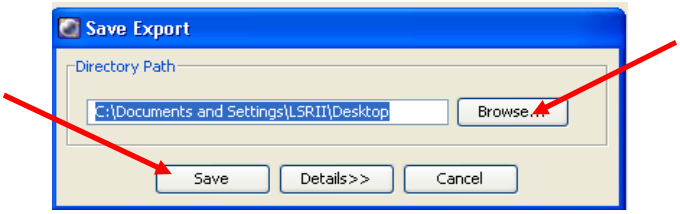
You also need to save the FCS files for analysis with third party software. Right click on the **Experiment name** again, select **Export**, but this time select **FCS files** to export.



Make sure you are exporting **"FCS 2.0 files"**; then press **"OK".**



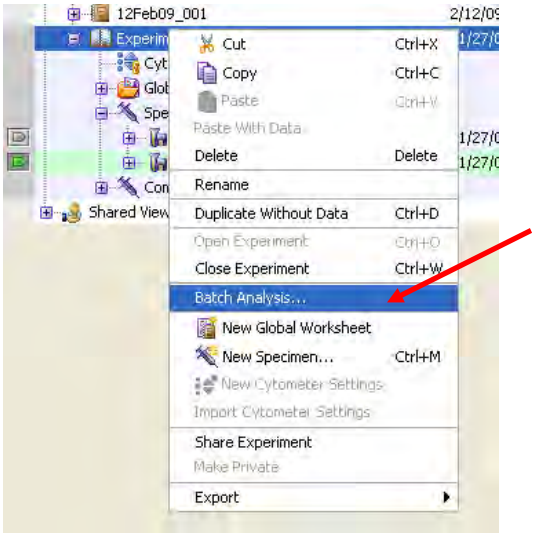
In the next window, click **"Browse"** to specify the directory path for the files you are exporting. We recommend saving them on the Desktop first or in the same folder as the Experiment.



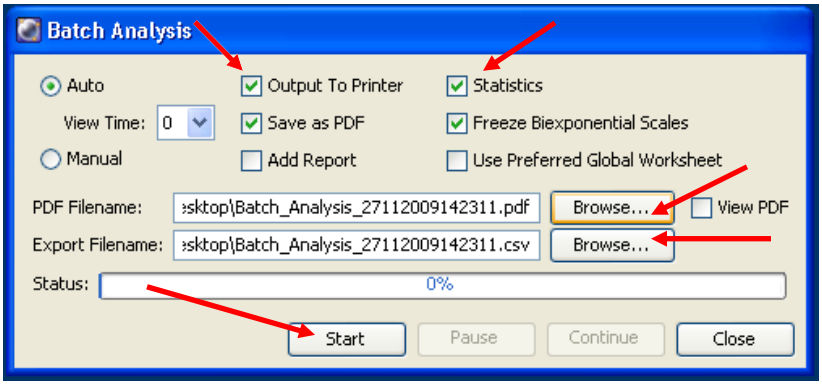
6. Batch Analysis

Now you may choose to perform a batch analysis. It will give you the option to print multiple worksheets or save them as a PDF file. It will also allow you to export the data from the "Statistics View" to a CSV file that can be read in Excel.

To start a batch analysis you need to right click on your **Experiment** name (or Specimen, or several selected tubes) and select **Batch Analysis**.



Then select the actions you want the software to perform. Also, it is important to specify the destination where your PDF and CSV files are going to be saved. Click the **"Browse"** button and select the same folder as the experiment on the desktop in both cases. Copy your folders to a storage device and proceed with the cleaning procedure.



7. Logging Out from the Software

To log out from the software, select **"Log Out"** from the **File** menu.