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# BASIC LSR-FORTESSA TUTORIAL

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# BASIC LSRFORTESSA TUTORIAL

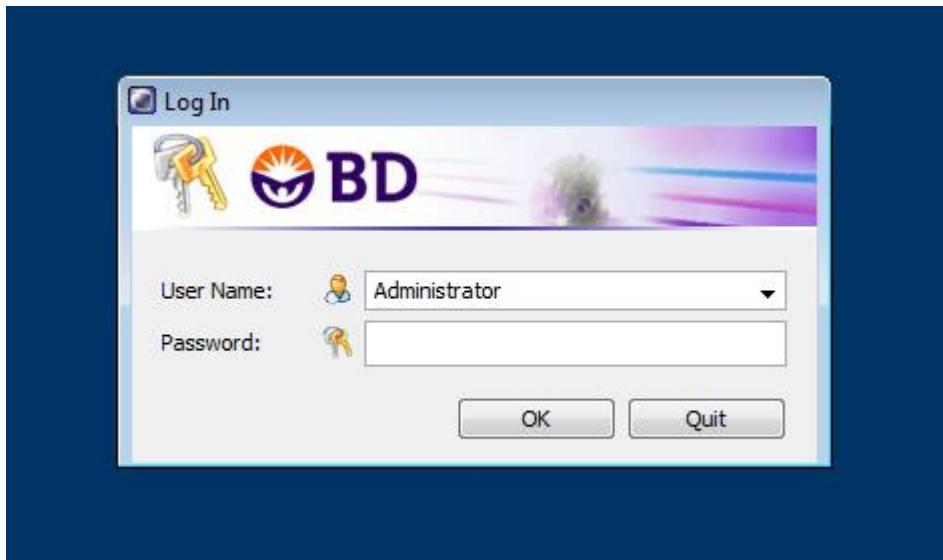
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This tutorial is to introduce you to the use of the LSRFortessa with the FACSDiva software.

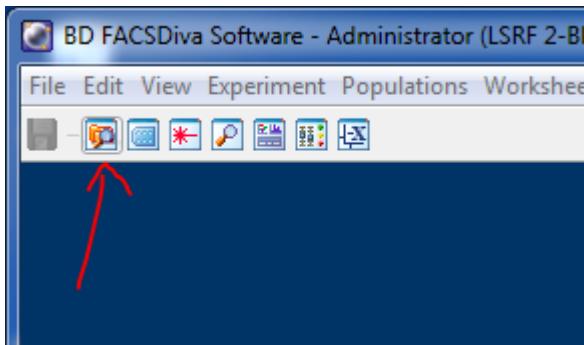
First, select the proper icon on the Desktop:



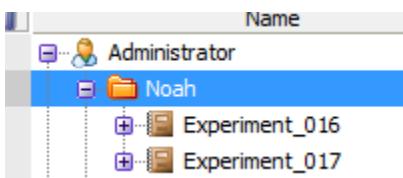
Next, enter the password enter the FACSDiva application.



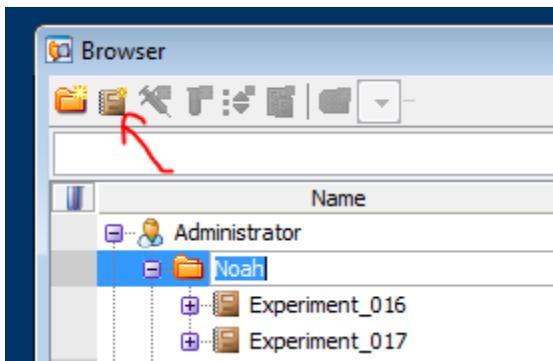
Once you have entered the application, open the browser by clicking on the following button:



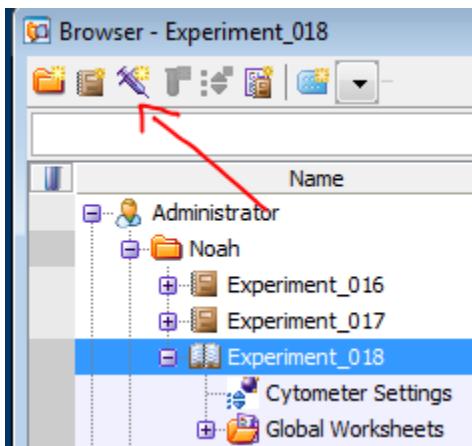
Next, highlight the folder you use to store your experiments. If you do not have a folder, click on the folder icon to create one.



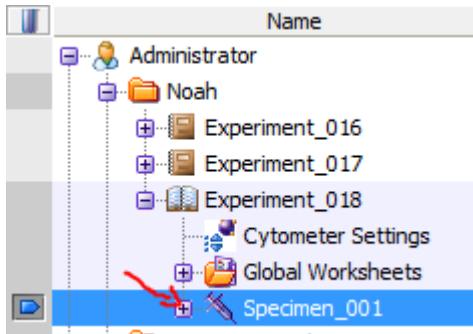
Next, select the “new experiment” button as follows:



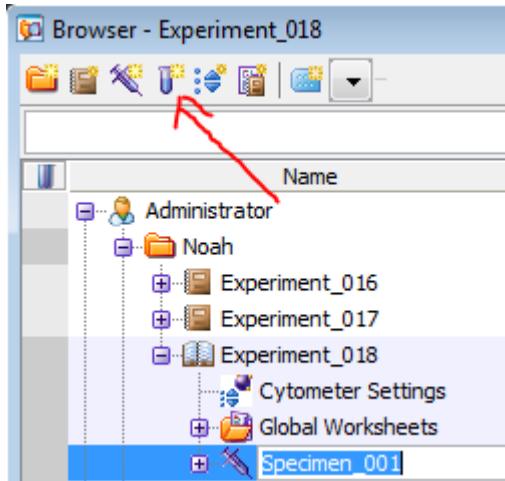
Select the new experiment you have just made. Then, select the “new specimen” button:



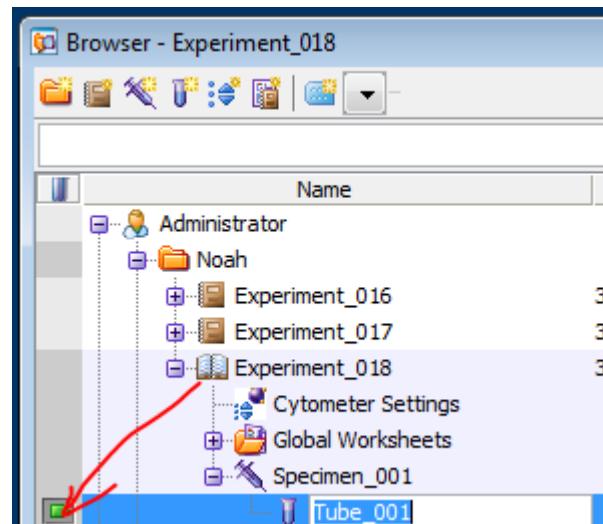
You should already have a tube within your new specimen. You can see your tube by expanding your specimen:



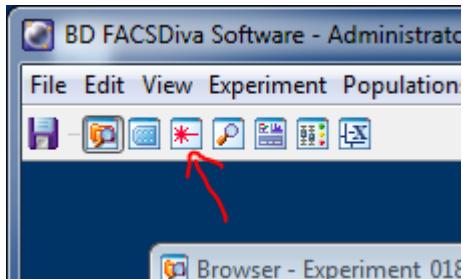
Alternatively, you can select your new specimen and select the “new tube” button:



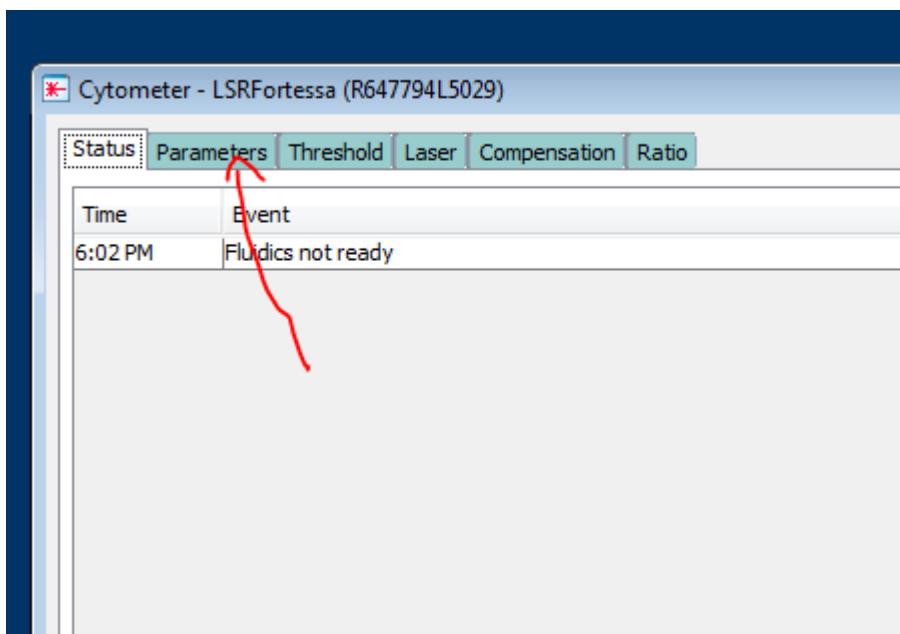
Now you can finally set up your workspace to collect data. Turn on the green arrow next to the first tube of your first specimen to begin work within the scope of the tube.



The first thing to do will be to set up your experiment with all of the proper lasers and filters. Open the cytometer window using the icon:



Switch to the parameters tab:



The list of parameters may be populated with a list of many different configurations. Notice that each of these configurations corresponds to a particular fluorochrome. When you choose a fluorochrome, the software automatically knows which detector to collect data from in the flow cytometer. These fluorochromes are common fluorochromes that are used for flow cytometry, but you can choose other fluorochromes with similar excitation and emission profiles to use with your panel. Compensation in the compensation section will allow you

We will first delete the default list so that you can create your own set. Click on the dot next to the first fluorochrome on the list. It should appear after the forward- and side-scatter channels:

| Cytometer - LSRFortessa (R647794L5029) |         |                                     |   |  |
|--|---------|-------------------------------------|---|--|
| Parameter                              | Voltage | Log                                 | A |  |
| • FSC                                  | 385     | <input type="checkbox"/>            |   |  |
| • SSC                                  | 309     | <input checked="" type="checkbox"/> |   |  |
| • Alexa Fluor 488                      | 430     | <input checked="" type="checkbox"/> |   |  |
| • PerCP-Cy5-5                          | 565     | <input checked="" type="checkbox"/> |   |  |
| • Alexa Fluor 647                      | 569     | <input checked="" type="checkbox"/> |   |  |
| • Alexa Fluor 700                      | 464     | <input checked="" type="checkbox"/> |   |  |
| • APC-Cy7                              | 483     | <input checked="" type="checkbox"/> |   |  |
| • Alexa Fluor 405                      | 421     | <input checked="" type="checkbox"/> |   |  |
| • Alexa Fluor 430                      | 443     | <input checked="" type="checkbox"/> |   |  |
| • BV605                                | 691     | <input checked="" type="checkbox"/> |   |  |
| • BV650                                | 641     | <input checked="" type="checkbox"/> |   |  |
| • BV711                                | 524     | <input checked="" type="checkbox"/> |   |  |
| • BV786                                | 570     | <input checked="" type="checkbox"/> |   |  |
| • DsRed                                | 492     | <input checked="" type="checkbox"/> |   |  |
| • PE-mCherry                           | 536     | <input checked="" type="checkbox"/> |   |  |
| • PE-Cy5-5                             | 536     | <input checked="" type="checkbox"/> |   |  |
| • 7-AAD                                | 484     | <input checked="" type="checkbox"/> |   |  |

Next, scroll down to the bottom and shift+click to highlight all of the parameters in the list except for FSC and SSC.

| Cytometer - LSRFortessa (R647794L5029) |         |                                     |                                     |                          |   |
|--|---------|-------------------------------------|-------------------------------------|--------------------------|---|
| Parameter                              | Voltage | Log                                 | A                                   | H                        | W |
| • SSC                                  |         | <input type="checkbox"/>            |                                     |                          |   |
| • Alexa Fluor 488                      | 430     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |                          |   |
| • PerCP-Cy5-5                          | 565     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • Alexa Fluor 647                      | 569     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • Alexa Fluor 700                      | 464     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • APC-Cy7                              | 483     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • Alexa Fluor 405                      | 421     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • Alexa Fluor 430                      | 443     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • BV605                                | 691     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • BV650                                | 641     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • BV711                                | 524     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • BV786                                | 570     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • DsRed                                | 492     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • PE-mCherry                           | 536     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • PE-Cy5-5                             | 536     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • 7-AAD                                | 484     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |
| • APC-Cy7                              | 548     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |   |

Now, select “delete”:

\* Cytometer - LSFRFortessa (R647794L5029)

Status Parameters Threshold Laser Compensation Ratio

| Parameter       | Voltage | Log                                 | A                                   | H                        | W                        |
|-----------------|---------|-------------------------------------|-------------------------------------|--------------------------|--------------------------|
| SSC             | 309     | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Alexa Fluor 488 | 430     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| PerCP-Cy5-5     | 565     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Alexa Fluor 647 | 569     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Alexa Fluor 700 | 464     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| APC-Cy7         | 483     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Alexa Fluor 405 | 421     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Alexa Fluor 430 | 443     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| BV605           | 691     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| BV650           | 641     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| BV711           | 524     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| BV786           | 570     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| DsRed           | 492     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| PE-mCherry      | 536     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| PE-Cy5-5        | 536     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 7-AAD           | 484     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| PE-Cy7          | 548     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Add Delete

Cytometer Connected

Now, you can add the parameters you need. Select the “add” button:

\* Cytometer - LSFRFortessa (R647794L5029)

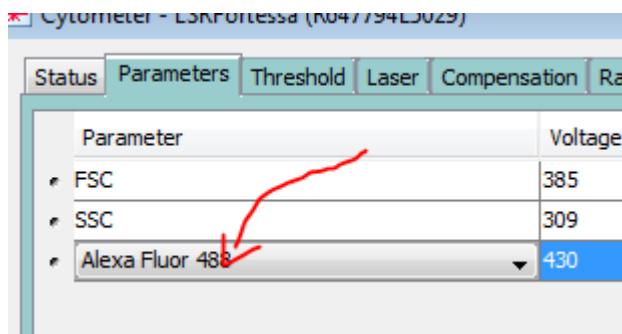
Status Parameters Threshold Laser Compensation Ratio

| Parameter | Voltage | Log                      | A                                   | H                        | W                        |
|-----------|---------|--------------------------|-------------------------------------|--------------------------|--------------------------|
| FSC       | 385     | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| SSC       | 309     | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Add Delete

Cytometer Connected

Click to change the absorption:

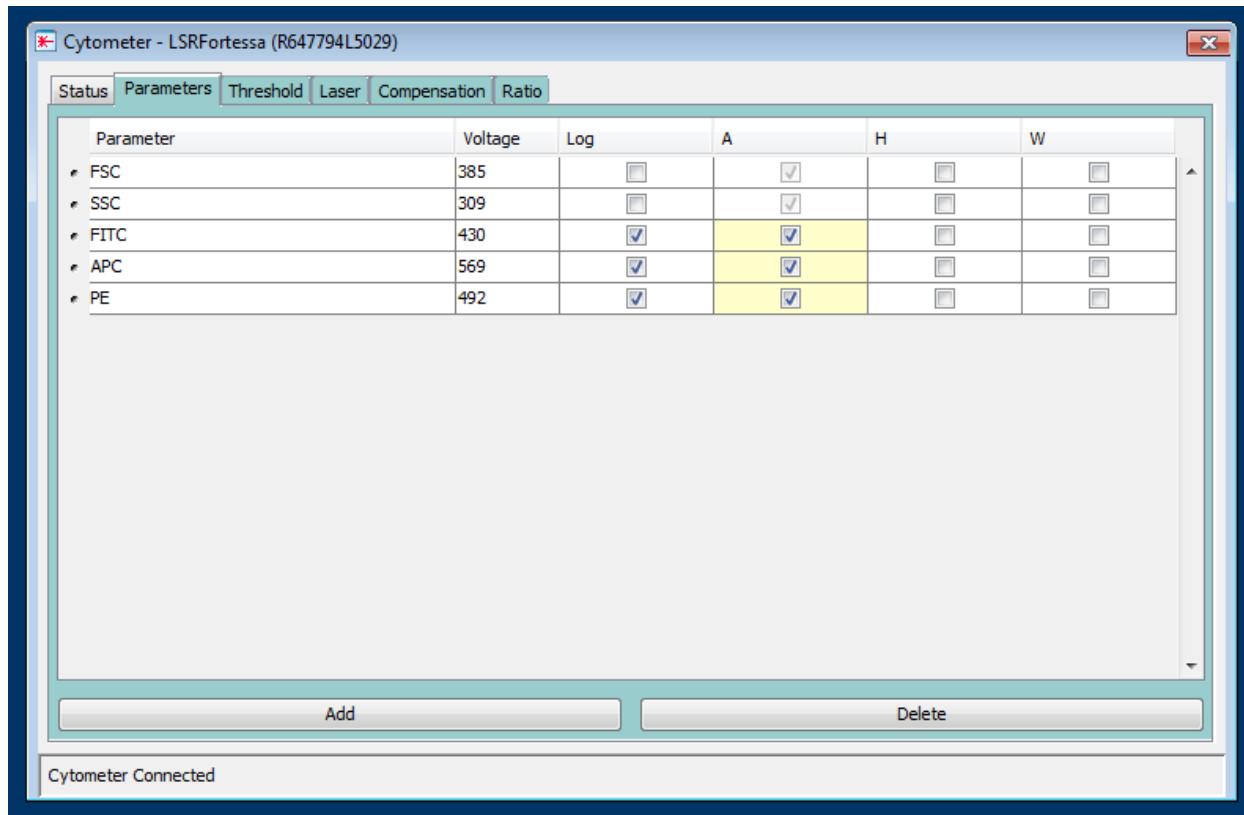


Cytometer - LSRFortessa (R047794L5029)

Status Parameters Threshold Laser Compensation Ratio

| Parameter       | Voltage |
|-----------------|---------|
| FSC             | 385     |
| SSC             | 309     |
| Alexa Fluor 488 | 430     |

Repeat to complete your panel.



Cytometer - LSRFortessa (R647794L5029)

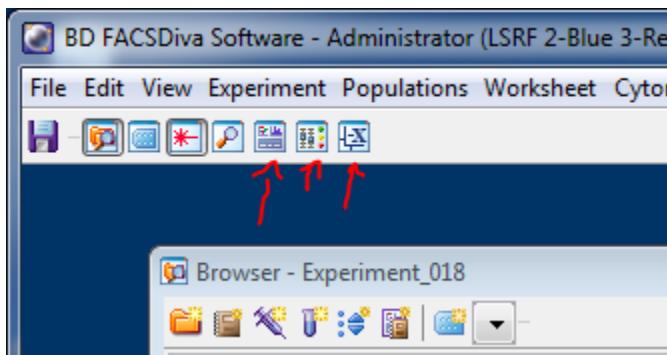
Status Parameters Threshold Laser Compensation Ratio

| Parameter | Voltage | Log                                 | A                                   | H                        | W                        |
|-----------|---------|-------------------------------------|-------------------------------------|--------------------------|--------------------------|
| FSC       | 385     | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| SSC       | 309     | <input type="checkbox"/>            | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| FITC      | 430     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| APC       | 569     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| PE        | 492     | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

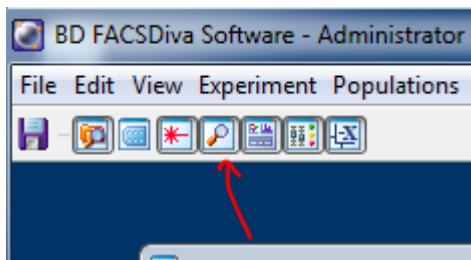
Add Delete

Cytometer Connected

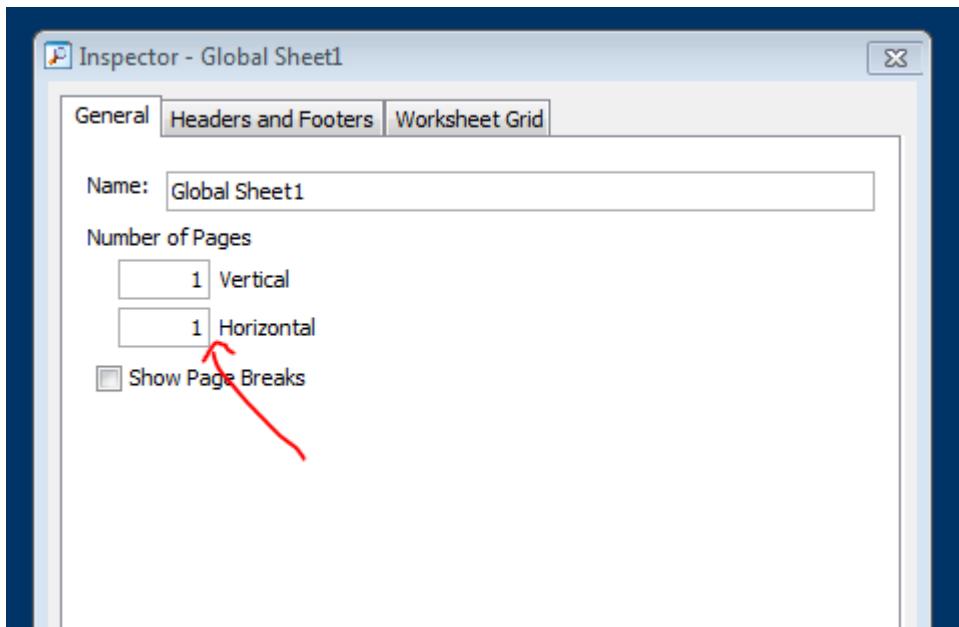
Great work. Now, you can open the worksheet, acquisition dashboard, and the biexponential editor:



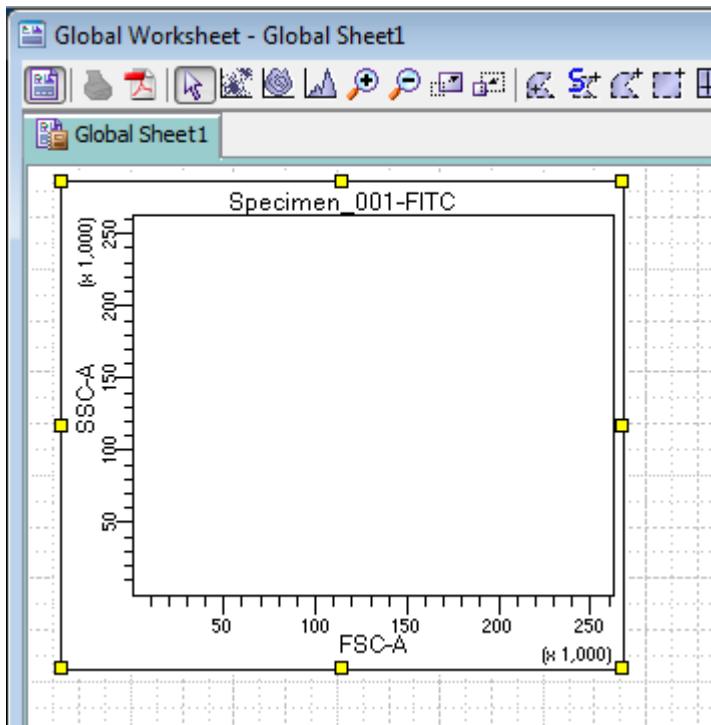
Several smaller windows should have appeared within the application window. One of them should contain what looks like a sheet of paper with a grid. You can make that larger by using the inspector to modify its properties. First open the inspector by selecting its icon in the toolbar:



Next, expand your worksheet by increasing the units of its width and/or height:

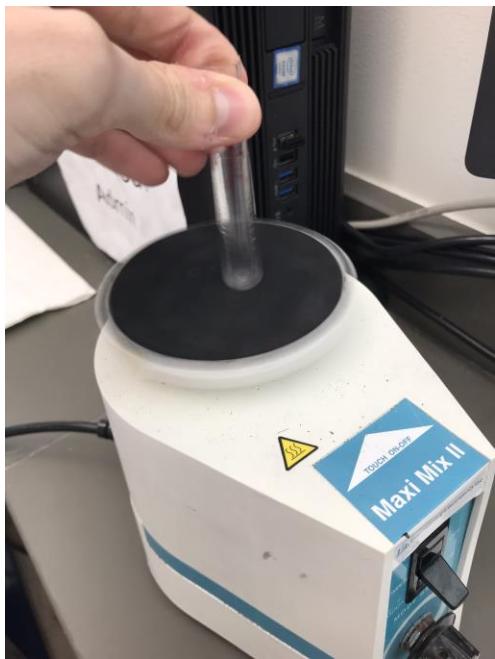


Next, you can create a new scatterplot by selecting the scatterplot button in the toolbar and then clicking and dragging within the worksheet:



Now you can begin to collect data in order to calibrate the lasers. Every fluorescent label you use must be calibrated against every other fluorescent label you use. In a real experiment, you should always have fluorescence-minus-one or "FMO" controls to use to calibrate your machine. However, in this tutorial we will be using beads each with only one fluorescent label.

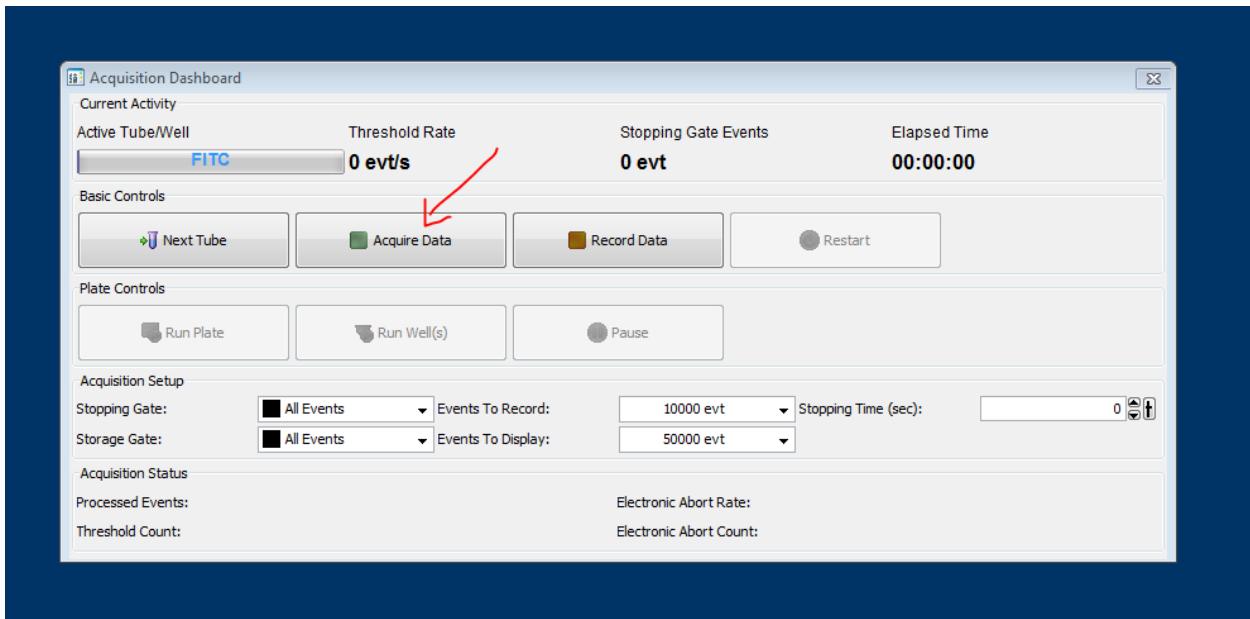
To acquire data from your sample, vortex your tube:



Then, swing out the arm of the machine, and replace the tube currently occupying the nozzle:



Select the “acquire data” button in the acquisition dashboard:



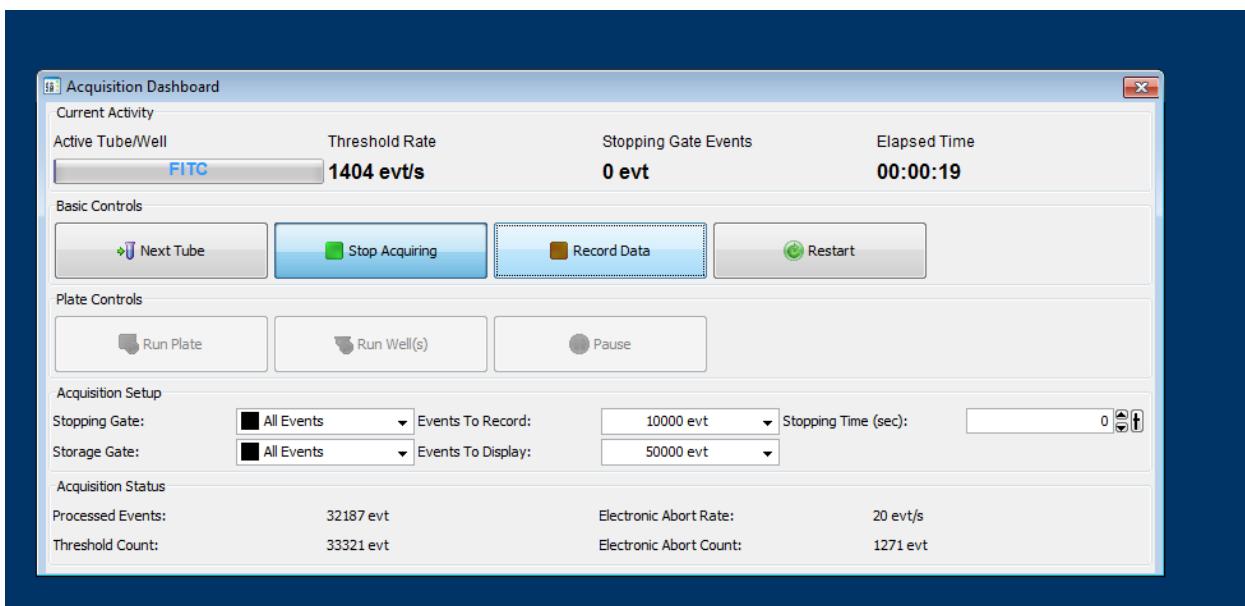
Next, switch the flow cytometer from “standby”...



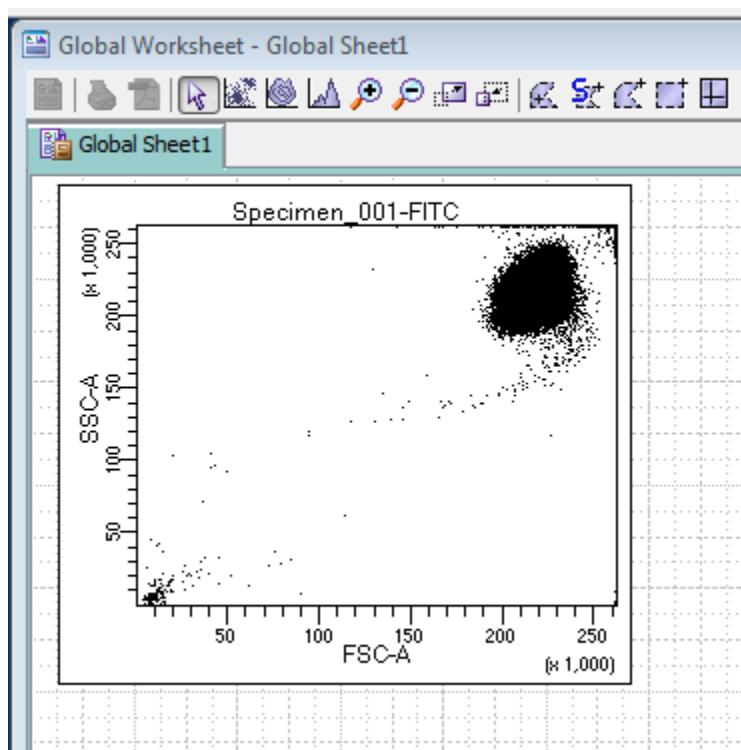
...to “run”:



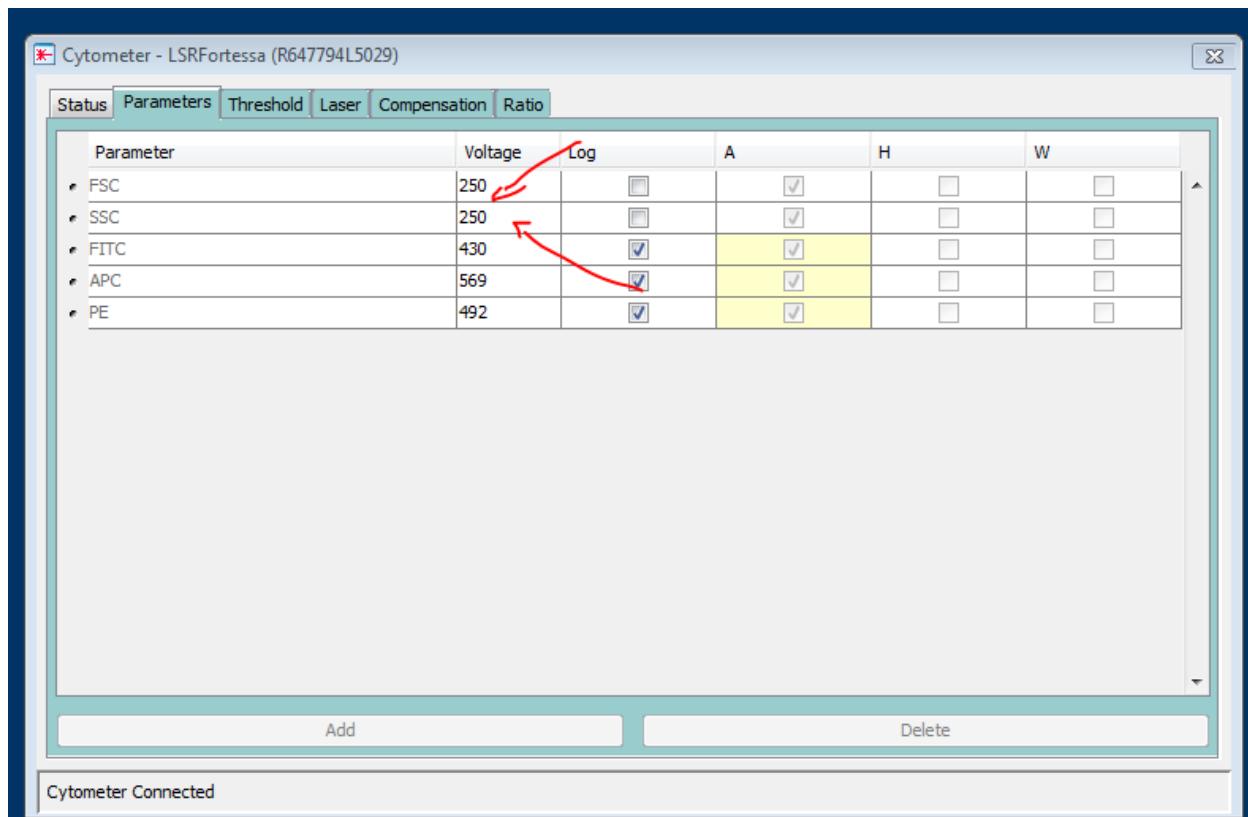
You should notice the event counter increasing in the acquisition dashboard:



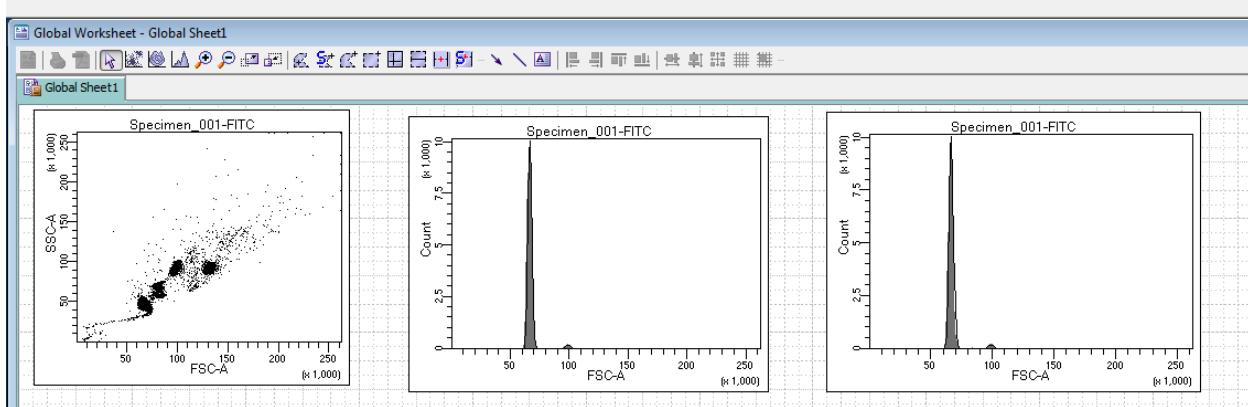
“FITC” is what I named the tube in the example, and in the above image, it should be covering a loading indicator that will fill up according to the “events to record” option under the “acquisition setup” region of the acquisition dashboard. However, we will not activate that now. We must first select calibrate our voltages. As you can see below, we should be seeing events being recorded in the worksheet:



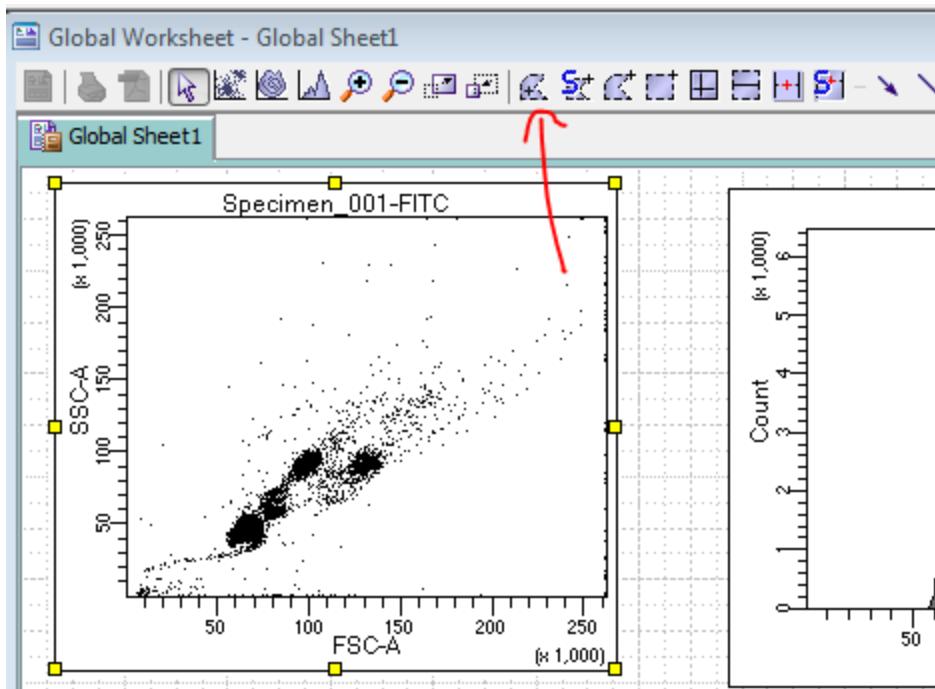
Choose the cytometer window and view the “FSC” and “SSC” voltages. These are the “forward scatter” and “side scatter” channels respectively:



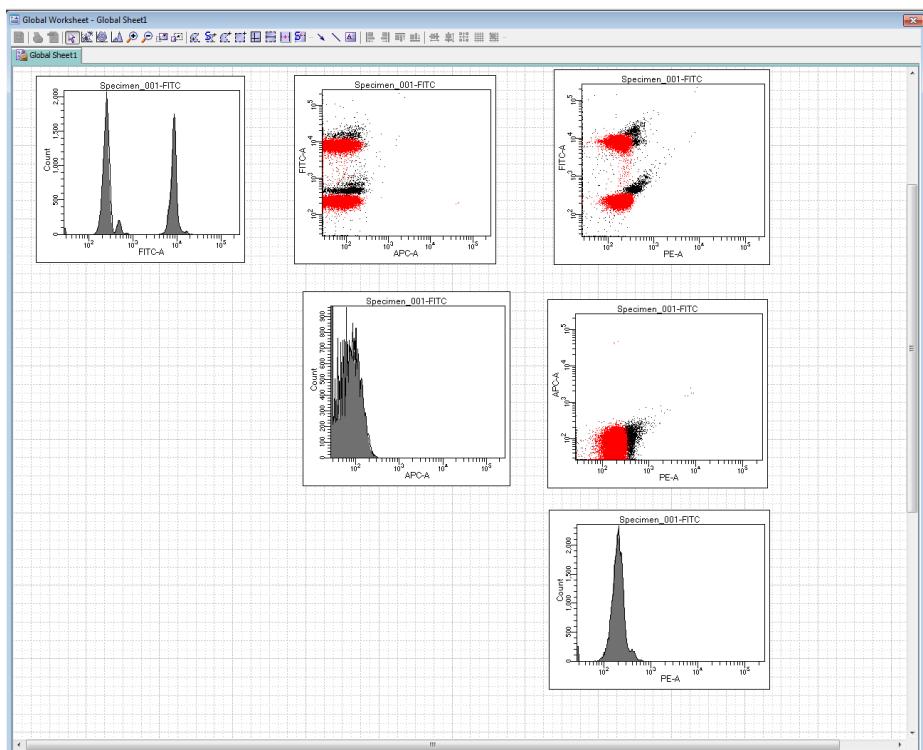
Adjust these voltages until they the cell population in the scatterplot falls close to the origin of the X- and Y-axes. Histograms can also be generated by selecting the histogram option in the toolbar and then clicking and dragging to size them within the worksheet.



Gate out the singlets. These will be highest concentration toward the bottom-left corner of your screen. Click on the gate icon to make a gate.



Now, create a matrix with each color opposed to every other color you are using. Also, include histograms along the trace of the matrix.



Now, select the first tube with a fluorescent tag. This may be the tube you are already using. The above matrix has already been compensated, but with that first fluorescent marker or FMO, you need to change the voltages so that you can clearly see both the negative and positive populations within the window. If the range that the detector can see is not large enough to accommodate both, focus on making sure that the positive populations are well within the positive threshold.

Once you have changed the voltages of all of your parameters so that the positive and negative populations align along their respective axes (it is very important that they are parallel), you may begin again with your first tube, focus on the acquisition dashboard, and select “acquire data” and then “record data.” Each time you complete your tube, select “next tube,” and repeat acquisition.

## Further Methods

TO BE EDITED

1. FSC-H vs FSC-W
2. SSC-H vs SSC-W