BD FACSDiva Software Basic Experiment Guide

Please note, this guide is not intended to take the place of in-person training but is to be used as a reference.

GETTING STARTED

1. If you are the first user of the day: Boot up the computer and log on to Windows, user name User, password user. Wait 10 seconds, then start the cytometer.

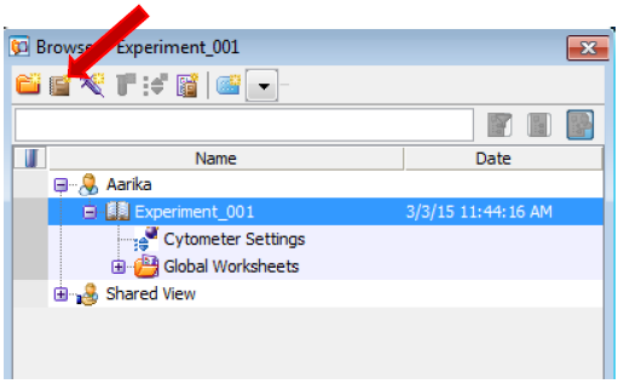
2. Check sheath fluid and waste levels. Empty waste and fill sheath fluid if appropriate. Check sheath tank is pressurized, waste lines are connected, and press run to be sure tube is pressurizing.

3. Prime the instrument. Remove tube from the instrument and press the PRIME button. When the instrument goes back to STANDBY, replace water tube and RUN on HI for 2-3 minutes.

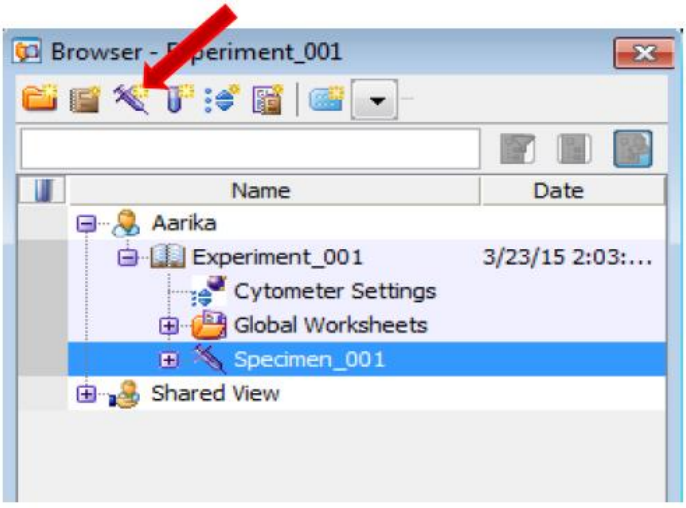
4. Open the BD FACSDiva software and log on. Allow the instrument to connect to the software if necessary.

Setting up an Experiment

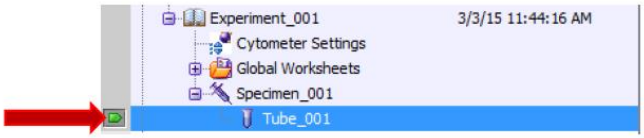
5. Click the New Experiment icon.



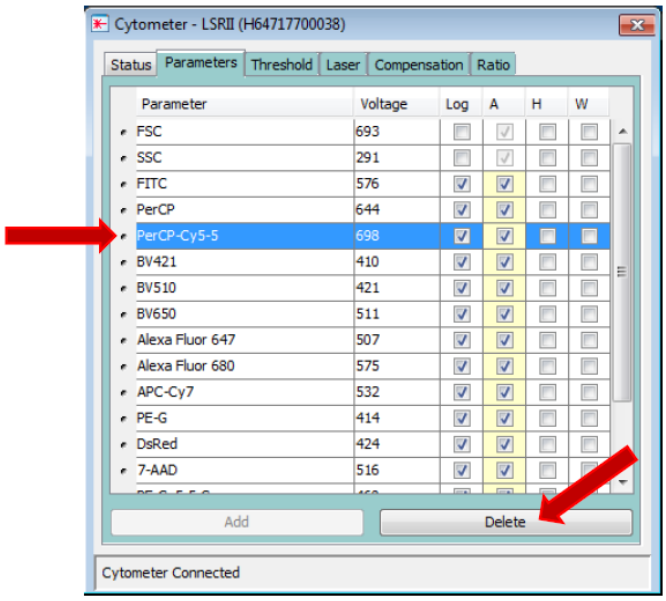
6. Click the New Specimen icon.



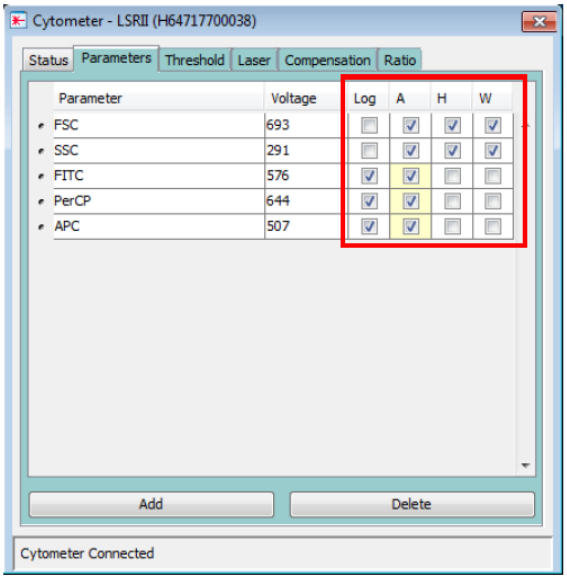
7. Expand the specimen and make the tube active by clicking the arrow next to the tube. The arrow will turn green.



8. In the Cytometer window under the Parameters tab, delete the colors you are NOT using in your experiment by clicking the dot to the left of the color name, then clicking Delete.



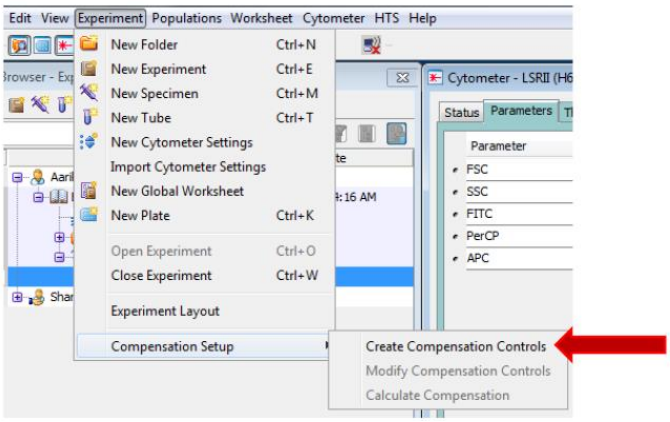
9. In this example, the panel includes FITC, PerCP and APC; your panel may vary. Make the checkboxes match the example below (all color channels should be the same).



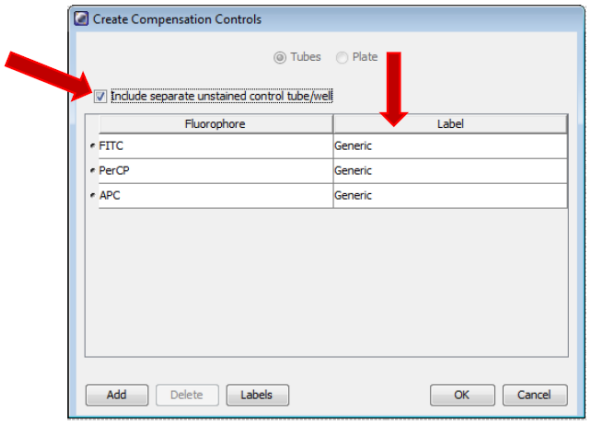
COMPENSATION

Compensation is not necessary for single-color experiments.

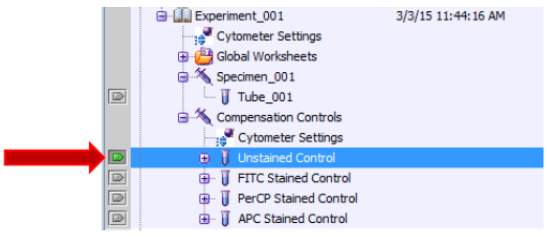
10. Create compensation controls for multi-color experiments. Select Experiment > Compensation Setup > Create Compensation Controls.

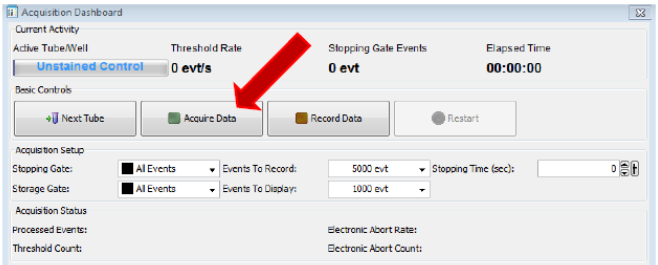


11. In the Create Compensation Controls window, ensure that all colors are labeled “Generic” and that the separate unstained control box is checked. Then select “OK.”

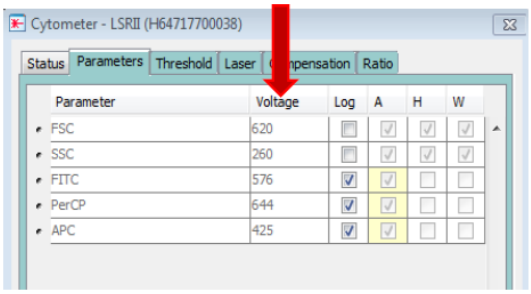


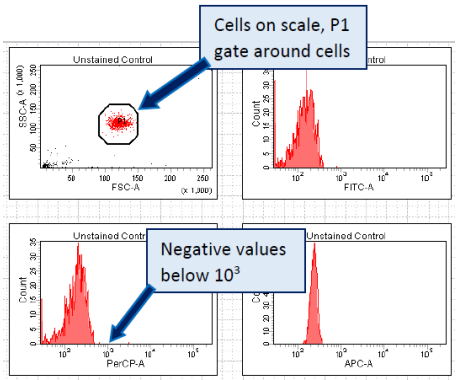
12. Diva will create a new specimen called “Compensation Controls.” Expand the specimen and make the Unstained Control tube active. Load your unstained specimen on to the instrument and press the RUN button. Then select “Acquire Data” on the Acquisition Dashboard.



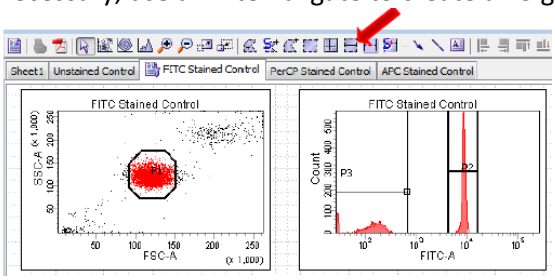


13. Adjust your FSC and SSC voltages in the Cytometer window to ensure your cells are all on scale. Move the P1 gate so that it contains the majority of your cells.

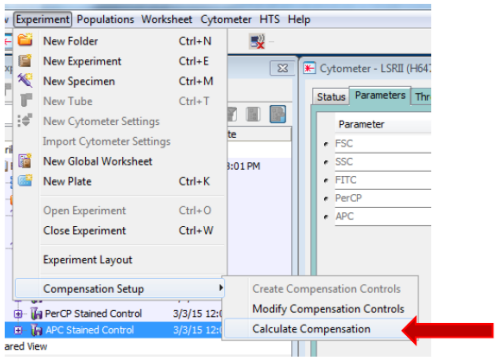


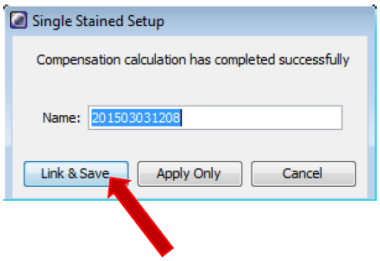


14. Run each of your single-stained controls. Use the P2 gate to select only your positive peak. If necessary, use an interval gate to create a P3 gate around your negative peak.

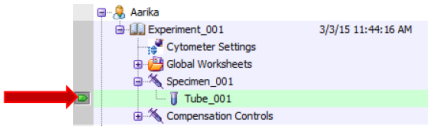


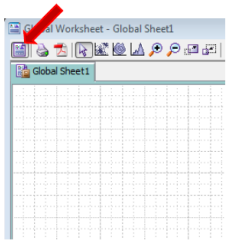
15. Select Experiment > Compensation Setup > Calculate Compensation. When the Single Stained Setup box appears, select “Link & Save” to apply the calculated values to your experiment. Load a tube of water on to the instrument and place it in STANDBY.





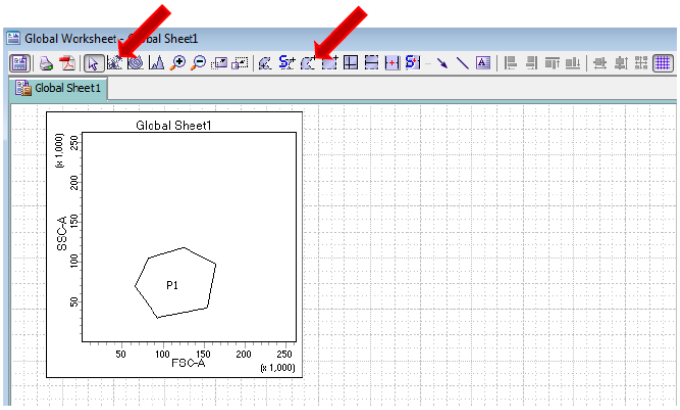
16. Return to your original sample tube (“Tube_001”) in the Browser. Return to the blank Global Worksheet by clicking on the Global Worksheet icon in the Worksheet window.



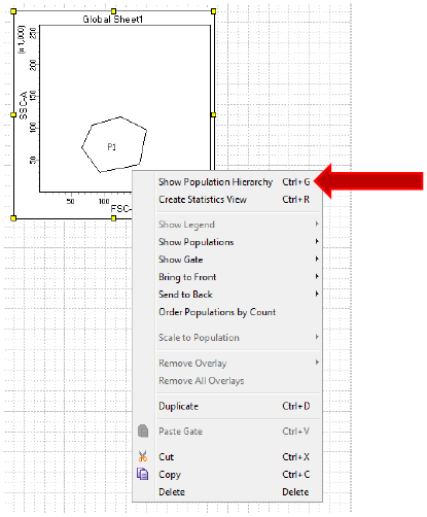


RUNNING SAMPLES

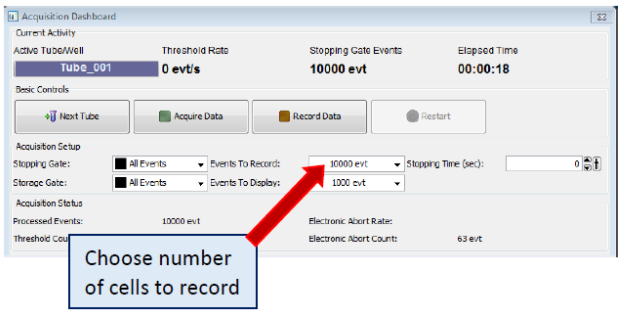
17. Create a FSC-A by SSC-A plot on the global worksheet and create a polygon gate to select your cells.



18. Create a population hierarchy by right-clicking on any dot plot and selecting “Show Population Hierarchy.” Create other dot plots or histograms as needed for your experiment.



19. Load your first sample tube onto the instrument and press RUN. Click “Acquire Data”. Adjust your FSC and SSC voltages as needed. Select the number of cells to record in the Stopping Gate settings on the Dashboard and click “Record Data.” After recording all samples, place the instrument in STANDBY with a tube of water.



Don’t forget to RECORD your data!!

FINISHING UP

20. Clean the instrument before leaving the lab. Load a tube with 3mL of 10% Bleach solution on to the instrument and select RUN and HI. Run the tube with the support arm to the side for 1 minute (liquid will be drained quickly) and then with the support arm under the tube for an additional 5 minutes.

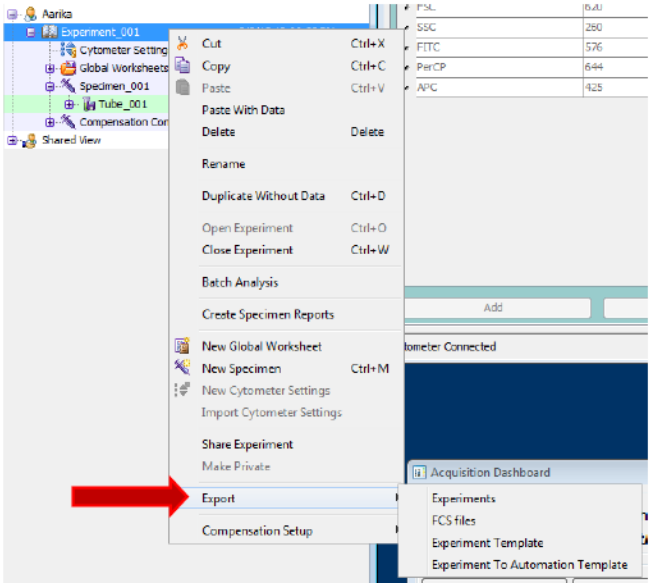
21. Repeat cleaning with a tube of DI H_2O. Run 1 minute with the support arm to the side and 5 minutes with the support arm under the tube.

22. After cleaning is complete, place the instrument in STANDBY (leave water tube in place) and log off the Diva software. Always leave a tube of water on the instrument when not in use.

23. If you are the last user of the day (check the online calendar to be sure), log off of the computer and shut down the instrument (press the big green button on the bottom right of the front of the LSRII or on the upper right side of the Fortessa), and pull up on the valve to vent the sheath tank.

EXPORTING DATA

24. To export your data, right-click on your experiment name in the browser, select Export then either Experiments or FCS Files. The experiment folder will be given the same name as your experiment in the browser, so be sure to name it so you can easily find it again. Names, dates, and brief experiment titles are recommended.



a. When exporting experiments, choose your external storage device or the computer hard drive location D:\Experiment Backup and click "OK."

b. When exporting FCS files, be sure to select FCS 3.0 files (should be default) and click "OK." Then select your location to export and select "Save."

25. Once your experiment is backed up, delete it from the browser to prevent data corruption or slowing of the software.