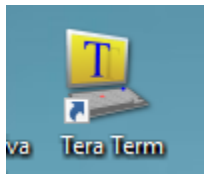


ARIA/SYMPHONY SORTER REFERENCE GUIDE

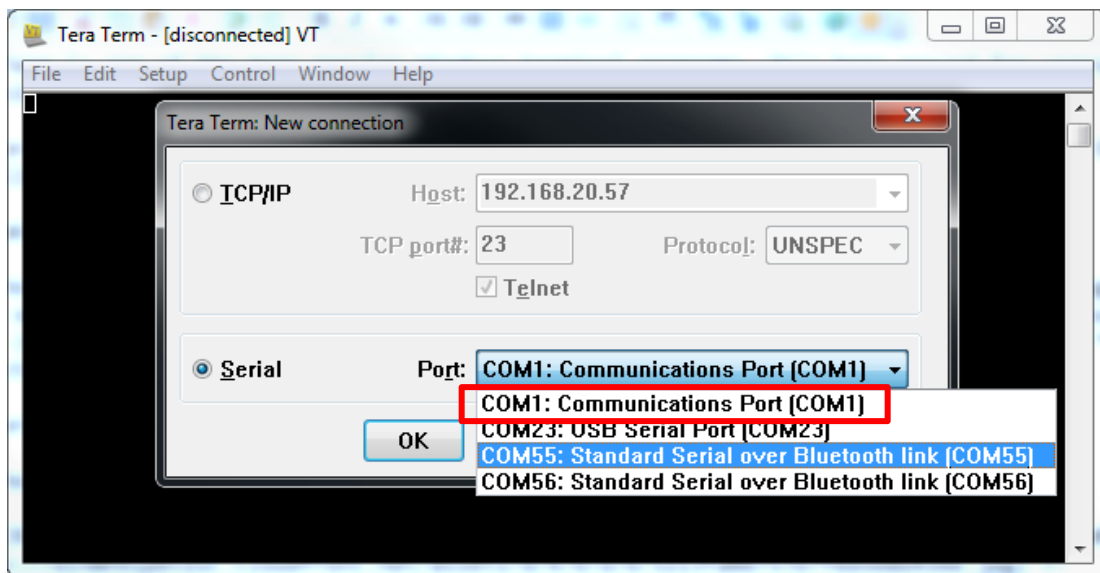
IF THE MACHINE IS OFF OR YOU ARE THE FIRST USER OF THE DAY

* Skip to page 12 if the machine is already on. *

1. The computer should be on.
 - a. To log into the PC: **Username:** BAdmin **Password:** BDIS
2. Unlock the screen with your PPMS Account.
3. Launch Tera Term.



4. Click on Serial, make sure the port is on COM1, and click OK.



5. Turn on the cytometer by pressing the green button on the side of the instrument or biosafety cabinet.

6. Open BD Coherent 4.



Make sure all the lasers are on and visible



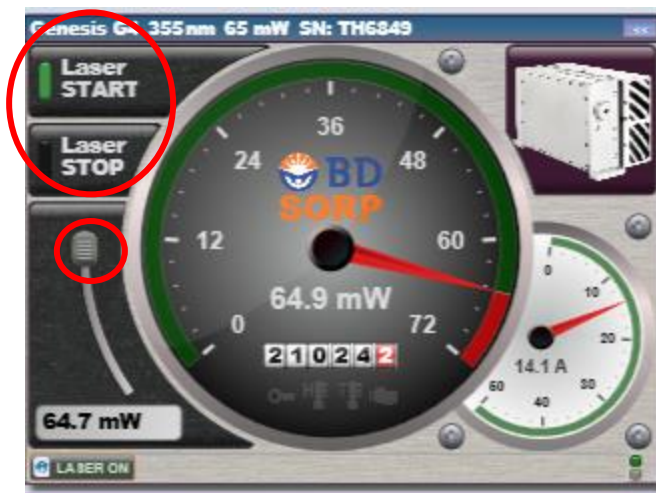
All lasers should have the green start button lit up.



Make sure the pointer is not past the yellow power band.



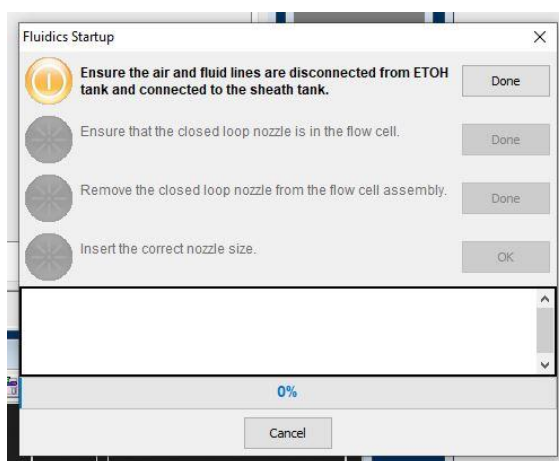
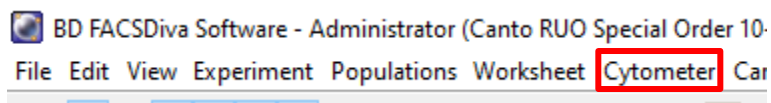
*Note about the UV laser: You will need to press STOP → START and then turn up the voltage to 60mW.



7. Turn on DIVA Software and log in using the credentials you received after training.



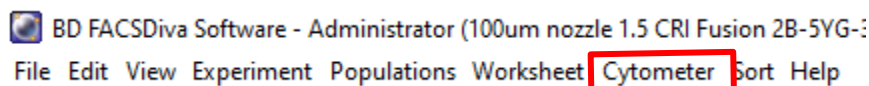
8. Once connected, select Cytometer -> Fluidics Startup.



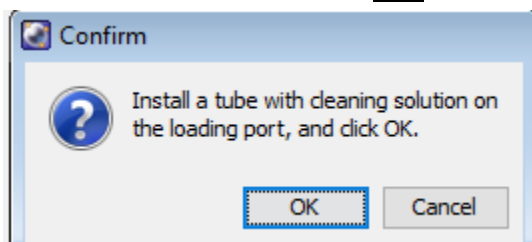
9. Follow the instructions presented on the screen, but don't insert your new nozzle.

10. Remove the closed-loop nozzle.

11. Select Cytometer → Cleaning Modes → Clean Flow Cell



12. Install a tube of bleach then hit OK.



13. Repeat step 11 two times.

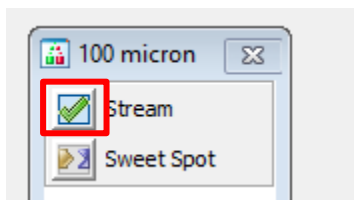


a.

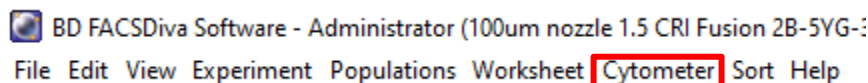
i. *This is the lowest volume you want in your tube. If needed, fill with relevant fluid.*

14. Let the bleach sit in the fluid line for five minutes.

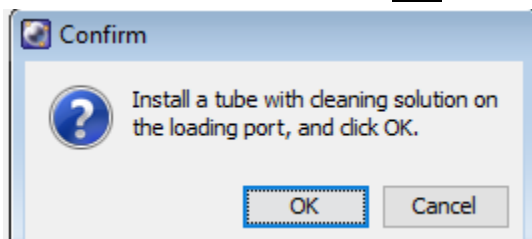
15. Turn on the stream and run for two minutes. Turn off stream when complete.



16. Select Cytometer → Cleaning Modes → Clean Flow Cell.



17. Install a tube of Contrad then hit OK.



18. Repeat step 16 two times.

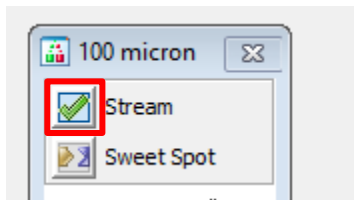


a.

- i. *This is the lowest volume you want in your tube. If needed, fill with relevant fluid.*

19. Let the Contrad set in the fluid line for five minutes.

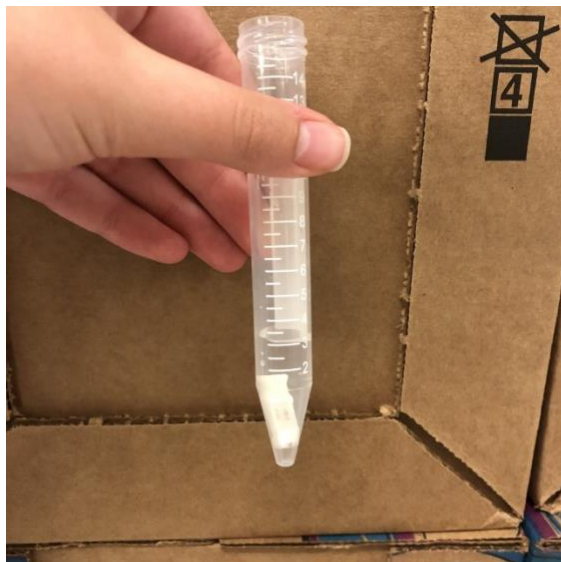
20. Turn on the stream and run for two minutes. Turn off stream when complete.



21. Open the flow cell access door and dry the deflection plates with Kimwipes.



22. Fill a tube with water and place the nozzle inside.

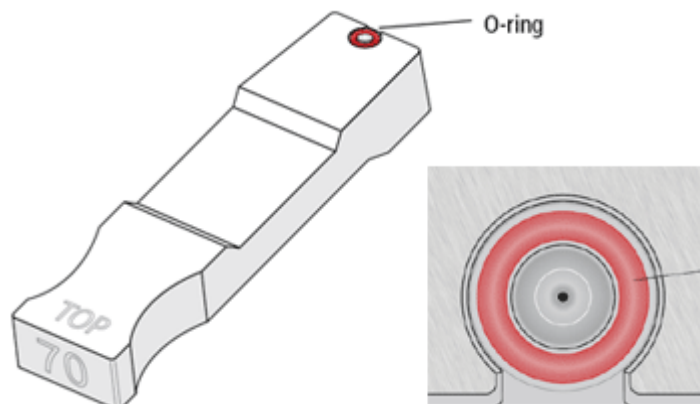


23. Sonicate the nozzle for about two minutes.



24. Dry the nozzle with a KIMWIPE (Do not use abrasive paper towel).

25. Look under the microscope for any debris or O-ring deterioration.



- a.
 - b. You should be able to see through the center hole of the nozzle.
 - c. The red O-ring should be in place. If it is not, please let a flow-core associate know.
26. Insert the nozzle.



27. When you change nozzles, select the correct instrument configuration in Cytometer → View Configuration → Set configuration → OK.

BD FACSDiva Software - Administrator (100um nozzle 1.5 CRI Fusion 2B-5YG-3R-5V-3UV)

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

Cytometer Configuration

Cytometer: BD FACSaria III
Cytometer Name: FACSariaIII
Serial Number: 1

Current Configuration: 100um nozzle 1.5 CRI Fusion 2B-5YG-3R-5V-3UV

Configurations Parameters Filters and Mirrors

Base Configurations

Configuration	Date
Fusion 2B-5YG-3R-5V-3UV	03/30/15 ...
CRI Configurations	
70um nozzle 1.0 CRI Fusion 2B-5YG-3R-5V-3UV	04/23/15 ...
70um nozzle 2.0 CRI Fusion 2B-5YG-3R-5V-3UV	04/23/15 ...
100um nozzle 2.0 CRI Fusion 2B-5YG-3R-5V-3UV	04/24/15 ...
100um nozzle 1.5 CRI Fusion 2B-5YG-3R-5V-3UV	01/17/17 ...
70um nozzle 1.5 CRI Fusion 2B-5YG-3R-5V-3UV	02/13/18 ...

100um nozzle 1.5 CRI Fusion 2B-5YG-3R-5V-3UV

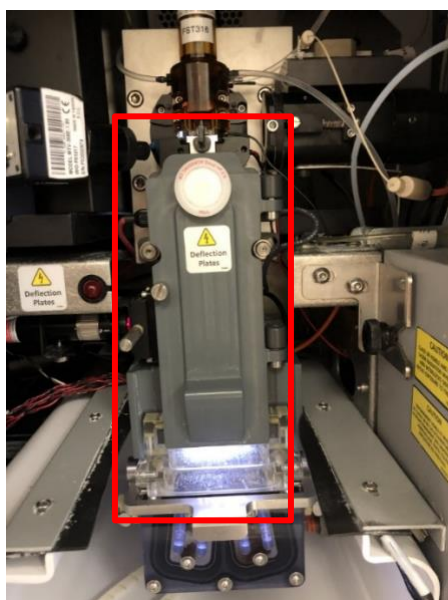
Blue Laser (488nm) FSC
Violet Laser (405nm)
Red Laser (637nm)
Yellow-Green Laser (561nm)
UV Laser (355nm)

Sheath Pressure (psi): 22.00 Window Extension (µs): 2.00 Comments:
Nozzle Size (µ): 100

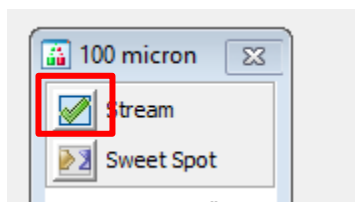
Set Configuration Print Export OK Cancel

Select the nozzle you are using.

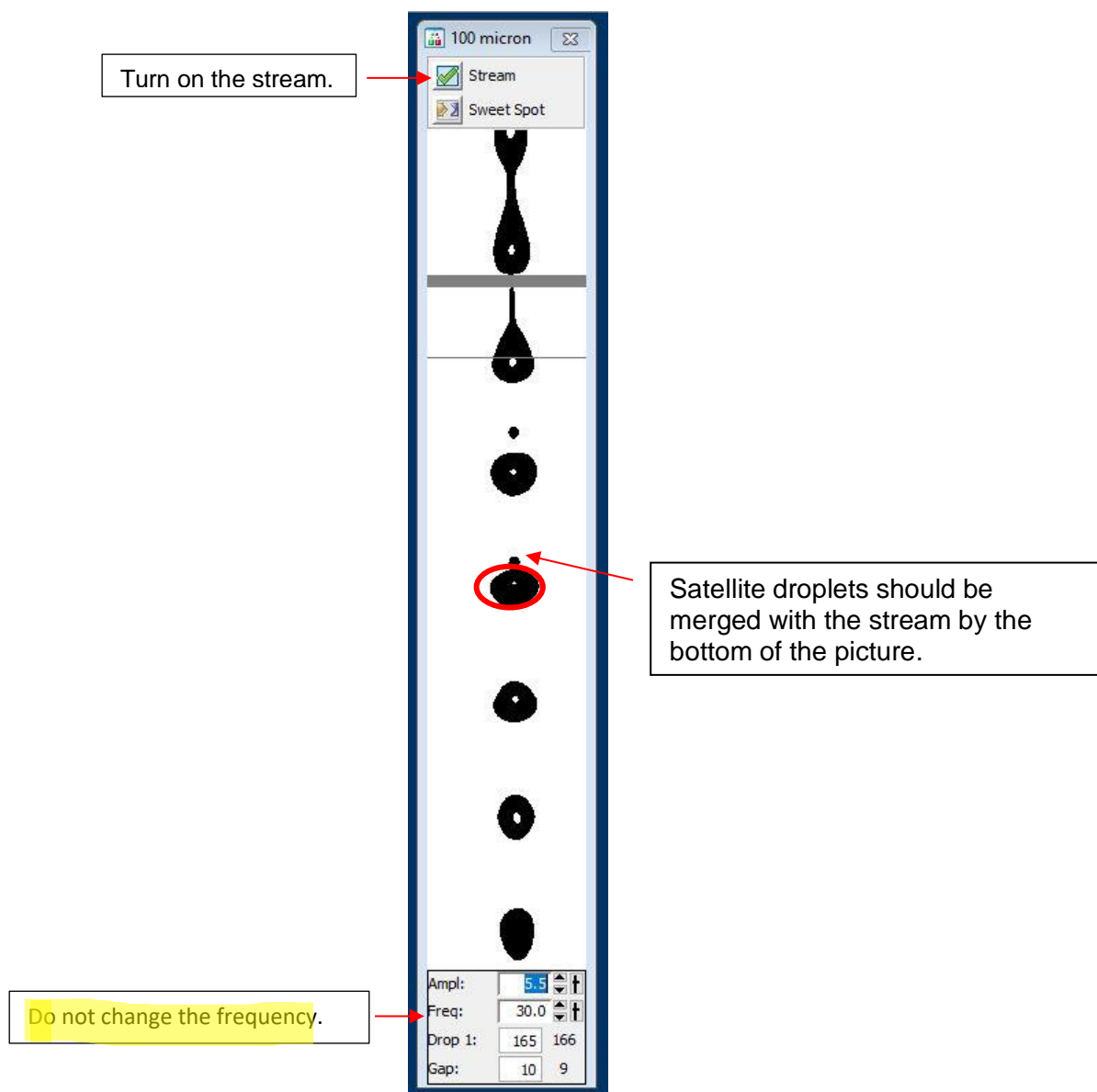
28. Close the flow cell access door.



29. Turn on the stream.

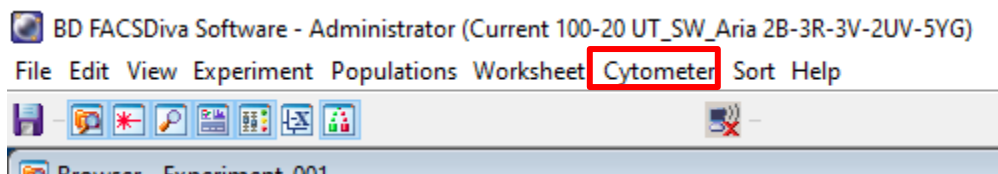


30. Check the stream quality (position, satellite, and drop resolution).



31. Increase/Decrease Amplitude to set connected stream height.

32. Set drop 1 values (=) to each other.
33. After optimizing, go to Cytometer → CST.

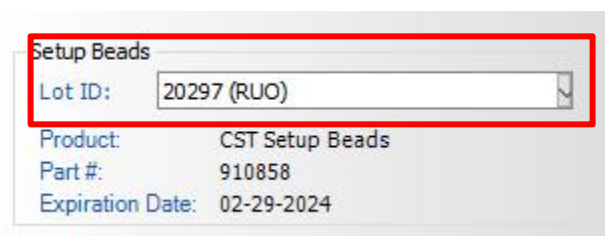


34. Take a clean FACS tube and add two drops of CST beads (Blue Bottle) with ~ 300uL of FACS flow. Note: Use Red Bottle if running CST on Symphony.

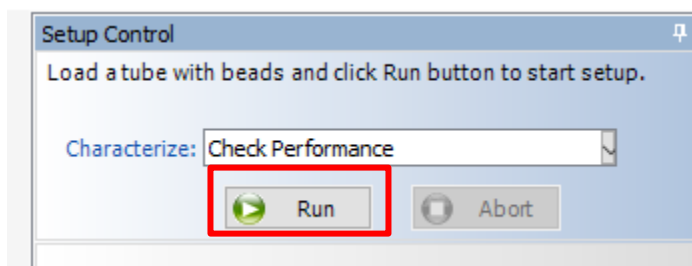


- i. The above photo should serve as reference for how much FACS flow to fill the tube with.

35. Verify that the lot number on the side of the bead's container matches the one on the screen.



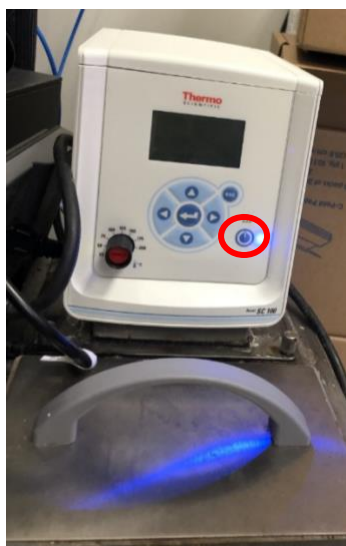
36. Load the tube and press Run.



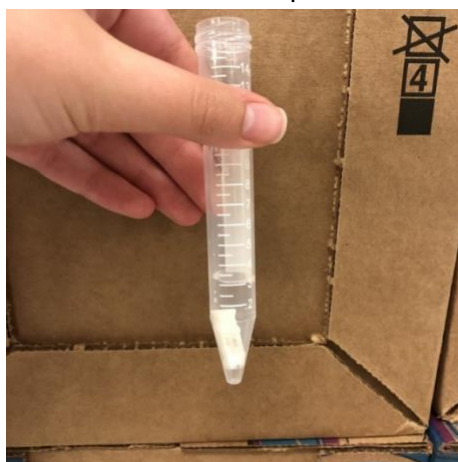
37. Wait for CST to PASS.
 - a. If it fails, try the following steps:
 - i. **Make sure CST window is NOT in full screen for the Symphony.**
 - ii. Re-run CST.
 - iii. Make new CST beads and re-run CST.
 - iv. Run another cleaning cycle, then re-run CST.
 - v. Shut down the cytometer, restart the computer, and run CST.

ANY USER OF THE DAY

1. Log into your PPMS account.
2. Log into DIVA.
3. Turn on the chiller if needed. This is recommended for better cell recovery.



4. Fill a tube with water and place the nozzle inside.

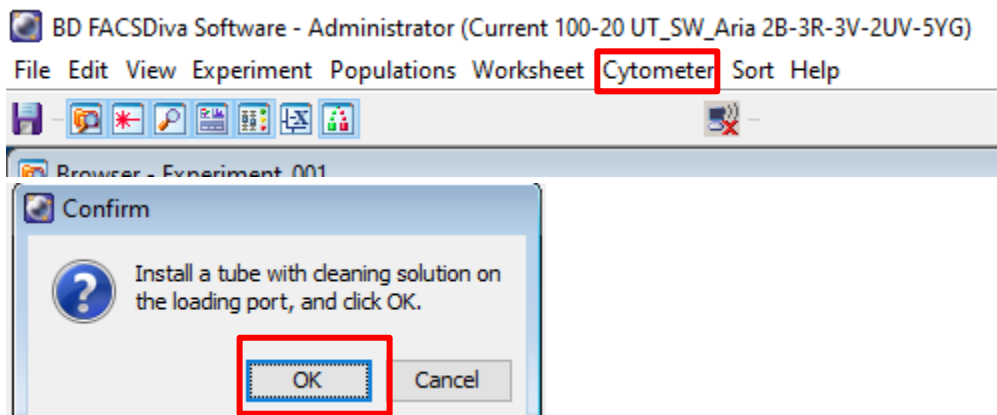


5. Sonicate the nozzle.



6. While the nozzle is sonicating, install a tube of Windex in the sample loader.

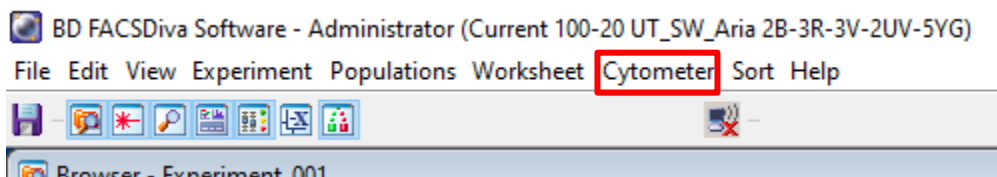
7. Select Cytometer → Cleaning Modes → Clean Flow Cell.

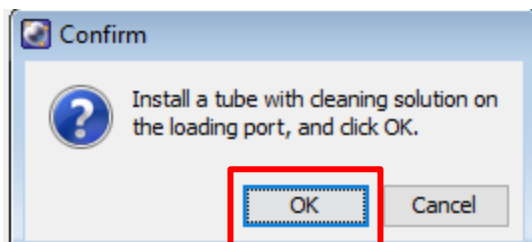


8. Repeat step 7 twice with the tube of Windex.

9. Install a tube of shutdown solution in the sample loader.

10. Select Cytometer → Cleaning Modes → Clean Flow Cell.





11. Repeat step 9 twice with the shutdown solution.

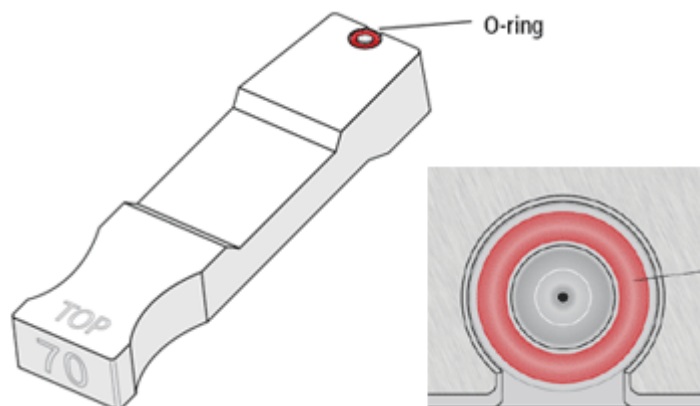
12. Clean the deflection plates with a Kimwipe.



13. Turn off the sonicator.

14. Dry the nozzle with a KIMWIPE (Do not use abrasive paper towels).

15. Look under the microscope for any debris or O-ring deterioration.

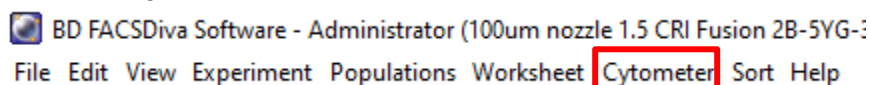


- a.
- b. You should be able to see through the center hole of the nozzle.
- c. The red O-ring should be in place, if it is not, please let a flow-core associate know.

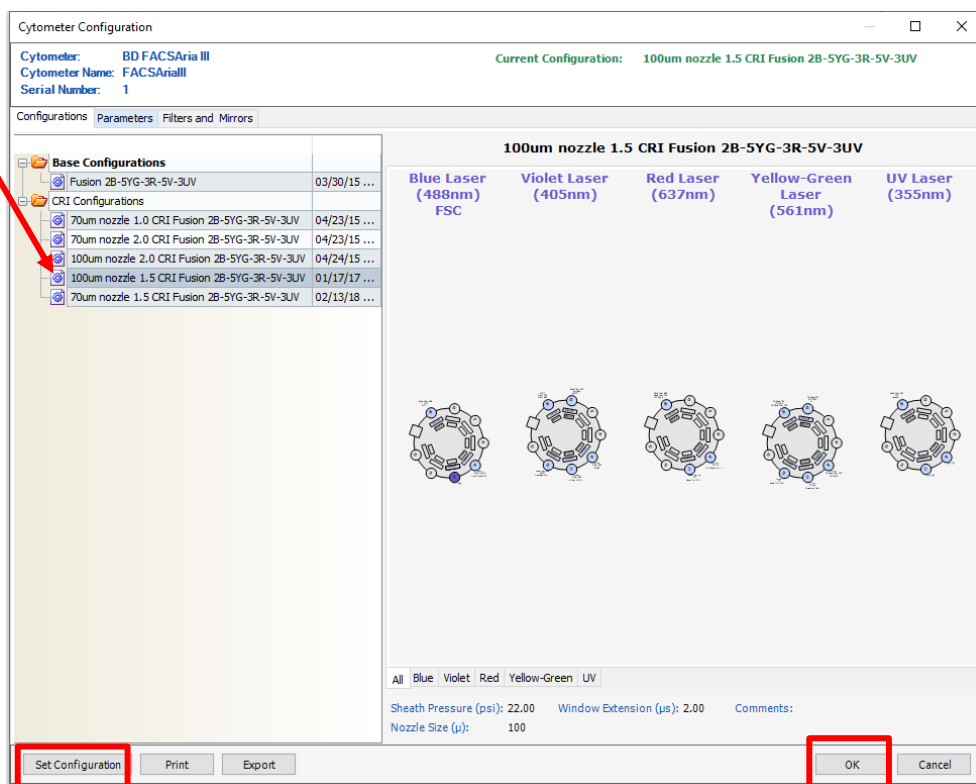
16. Insert the nozzle.



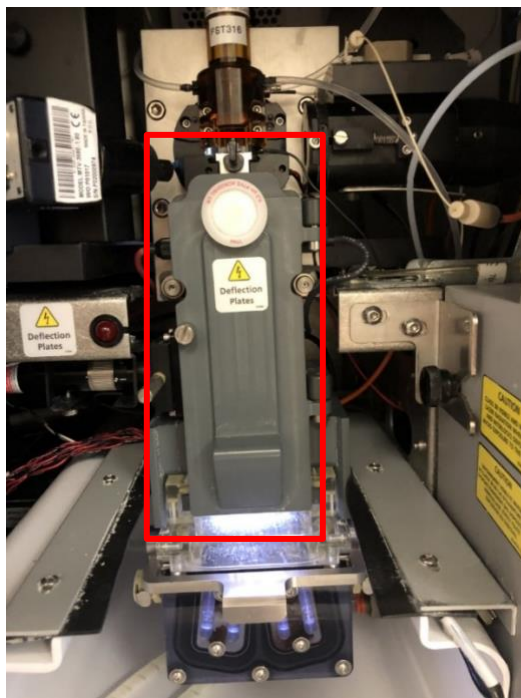
17. When you change nozzles select the correct instrument configuration in Cytometer → View Configuration.



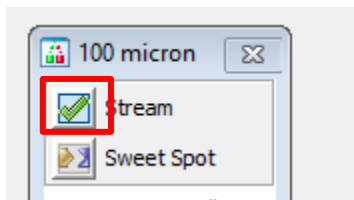
Select the nozzle you are using



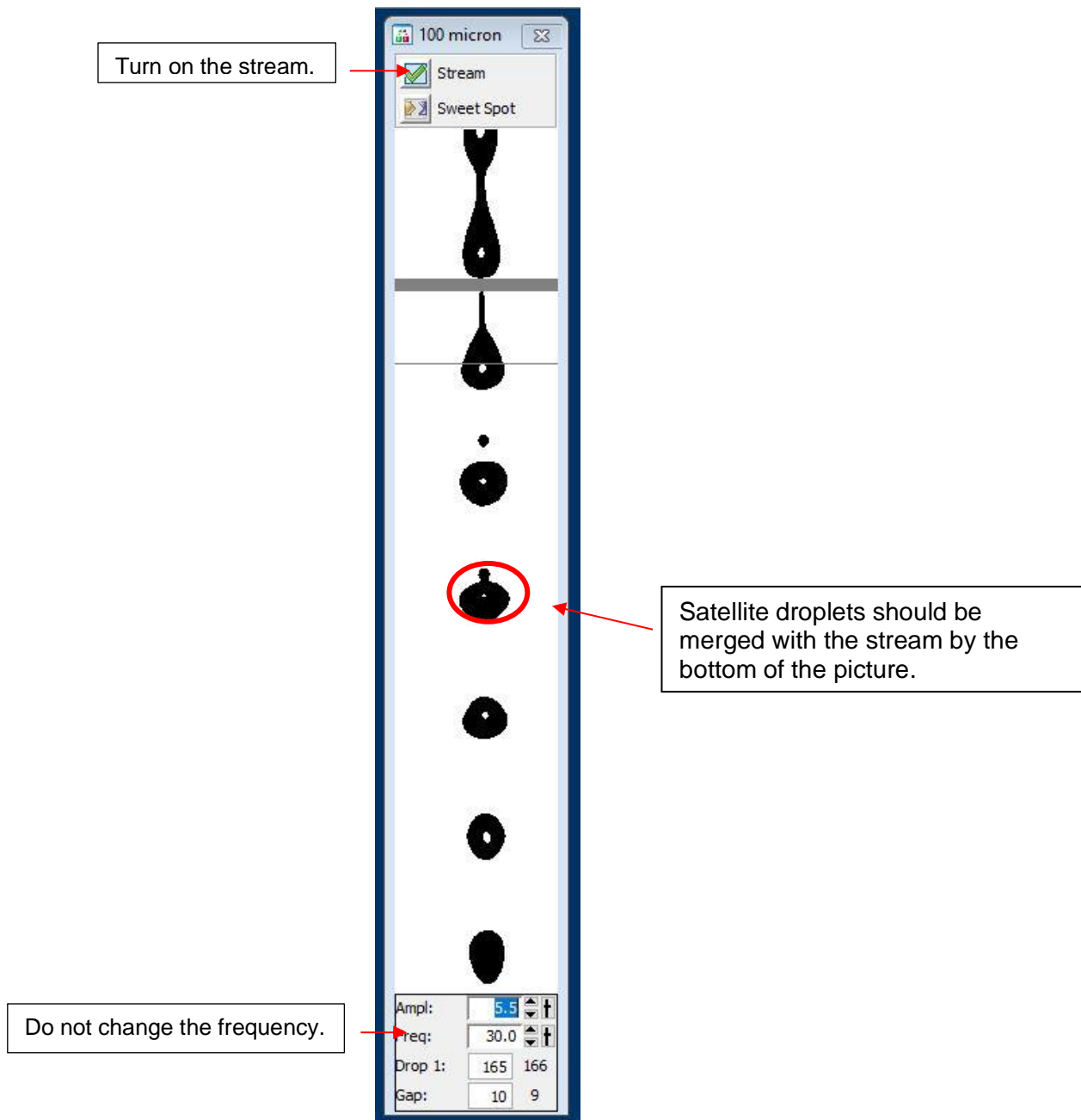
18. Close the flow cell access door.



19. Turn on the stream.



20. Check the stream quality (position, satellite, and drop resolution).

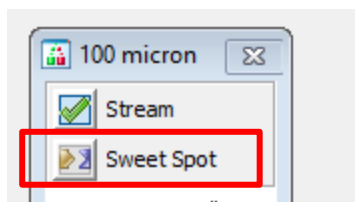


21. Increase/Decrease Amplitude to set connected stream height.

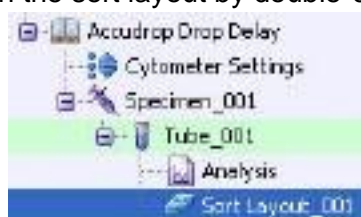
22. Set Drop 1 values (=) to each other.

ACCUDROP

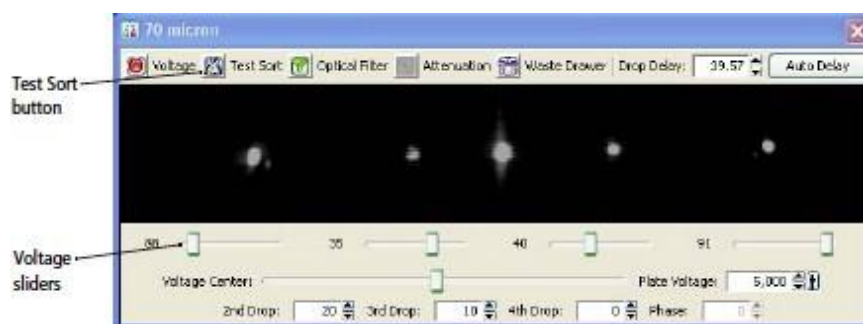
1. Turn on Sweet Spot.



2. Select Experiment → New experiment → Accudrop delay template.
3. Expand Specimen_001 and Expand Tube_001.
4. Set the current tube pointer to Tube_001.
5. Open the sort layout by double-clicking it.

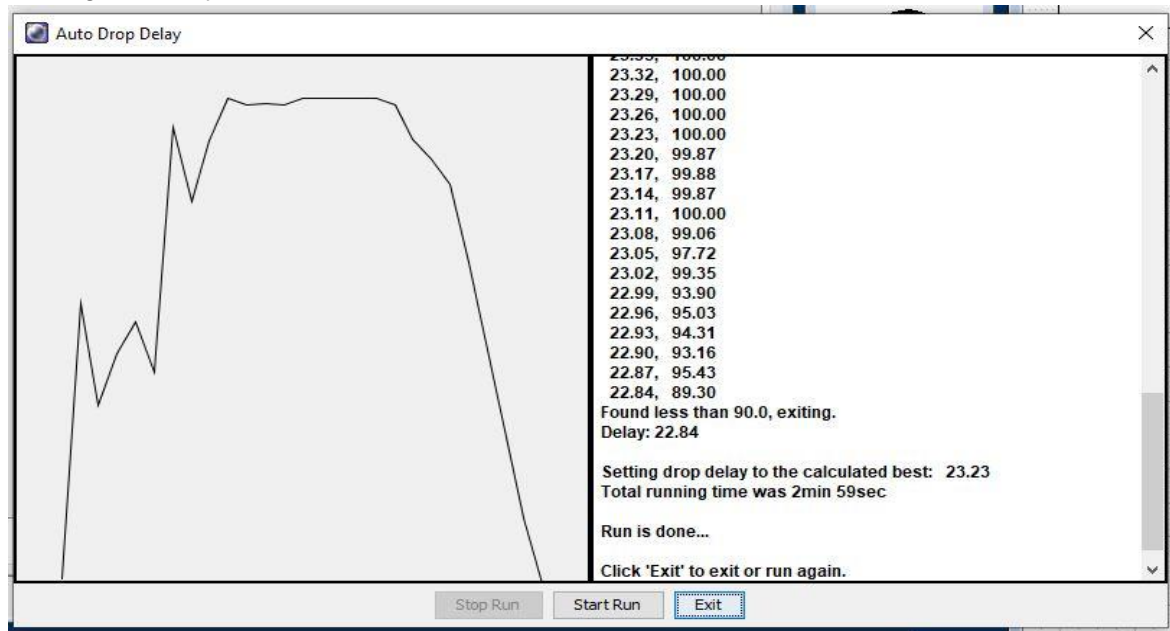


6. Open the Sort Device app and select your device using this ICON:
 - a. For Symphony: You can select your device from the drop-down menu on the side stream window.
7. Select the appropriate tube setting for its respective machine.
 - a. Symphony: Six-tube layout
 - b. Fusion, Aria (4 laser), Aria (5 laser): Four-tube layout
8. Turn on the Voltage (it will appear red) in the Side Stream window.
9. Select Test Sort in the Side Stream window.



10. Adjust the micrometer dial to obtain the brightest bead spots. *Minor adjustments only*
– dial can break if turned too tight.
11. Adjust the left voltage slider until the stream is in between the lines.
12. De-select Test Sort in the Side Stream window.
13. Prepare the Accudrop Bead Tube using 1 drop of beads and 0.5 ml FACS flow
(concentration should be a light **VIOLET** in color).
14. Vortex the tube.
15. Place the Accudrop Bead Tube in the sample chamber.
16. Close Chamber door (**Do not slam**): If door is not closed, there will be **0 events** per second.
17. Load the Accudrop Bead Tube.
18. Adjust the flow rate to increase or decrease events/sec.
 - a. $70\mu\text{m}$ = 1,000 to 3,000
 - b. $85\mu\text{m}$ = 800 to 2,000
 - c. $100\mu\text{m}$ = 600 to 1,500
 - d. $130\mu\text{m}$ = 400 to 1,200
19. Turn on the Voltage in the Side Stream window.
20. Turn on the Optical Filter in the Side Stream window.
21. Select Sort on the Sort Layout window (beads should not be sorting in left box).
22. Select Cancel to keep the tubes covered with the waste drawer.
23. Click on the Auto Delay button in the Side Stream window.
24. Select Start Run in the Auto Drop Delay dialog.

25. Monitor the Auto Drop Delay dialog for progress. A message appears when the process is completed. You should receive a curve; this signifies the instrument is sorting correctly.



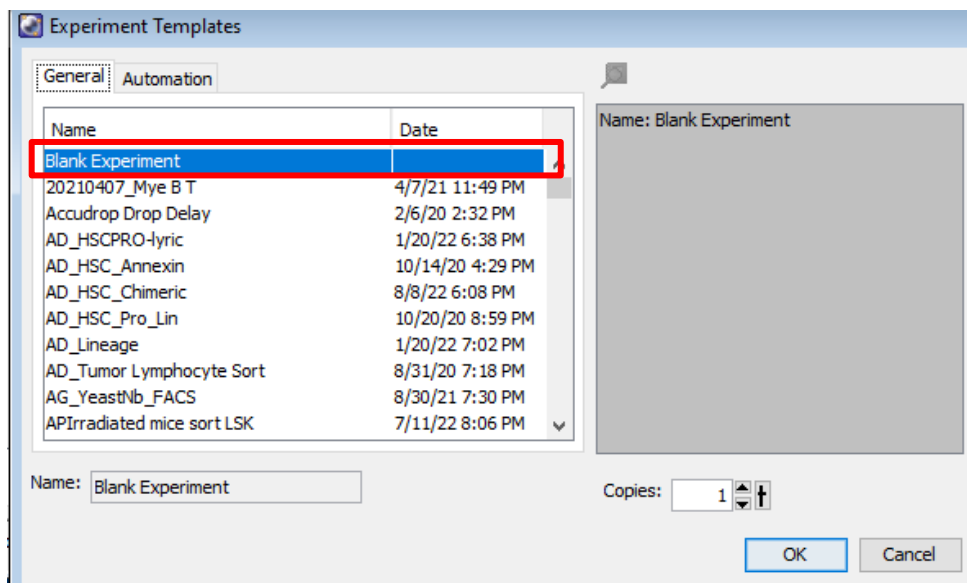
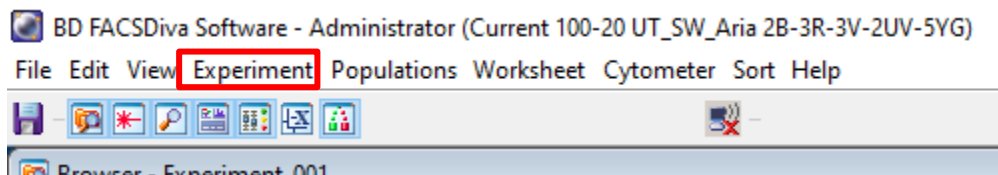
26. Select Exit.

27. Deselect Acquiring Data.

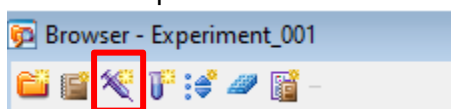
28. Unload the Accudrop Bead Tube.

FOR A NEW EXPERIMENT

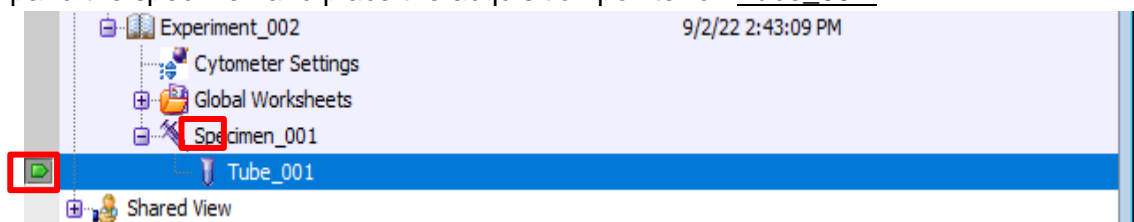
1. Select Experiment → New Experiment → Blank Experiment.



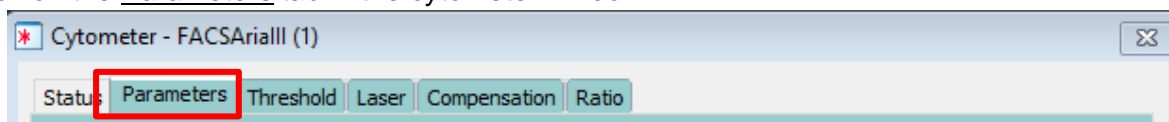
2. Create a new specimen.



3. Expand the specimen and place the acquisition pointer on Tube_001.



4. Click on the Parameters tab in the cytometer window.



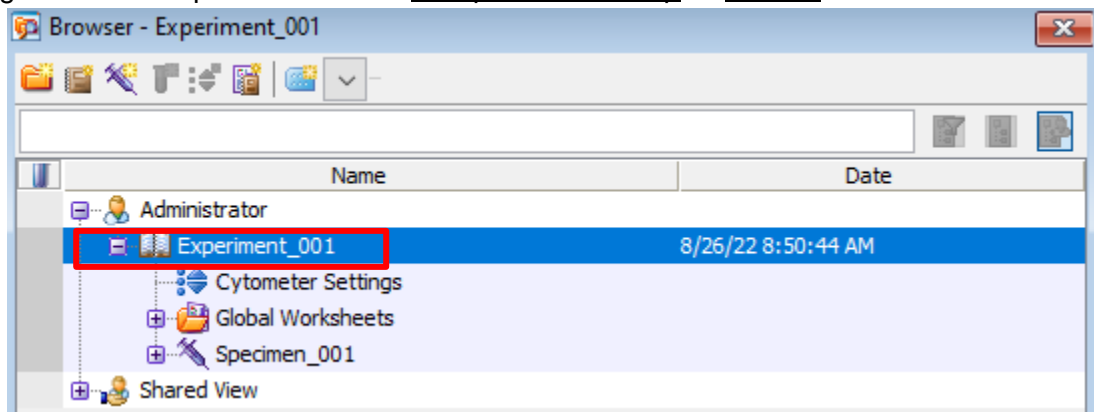
5. Delete all fluorochromes and add the fluorochromes you will be working with.



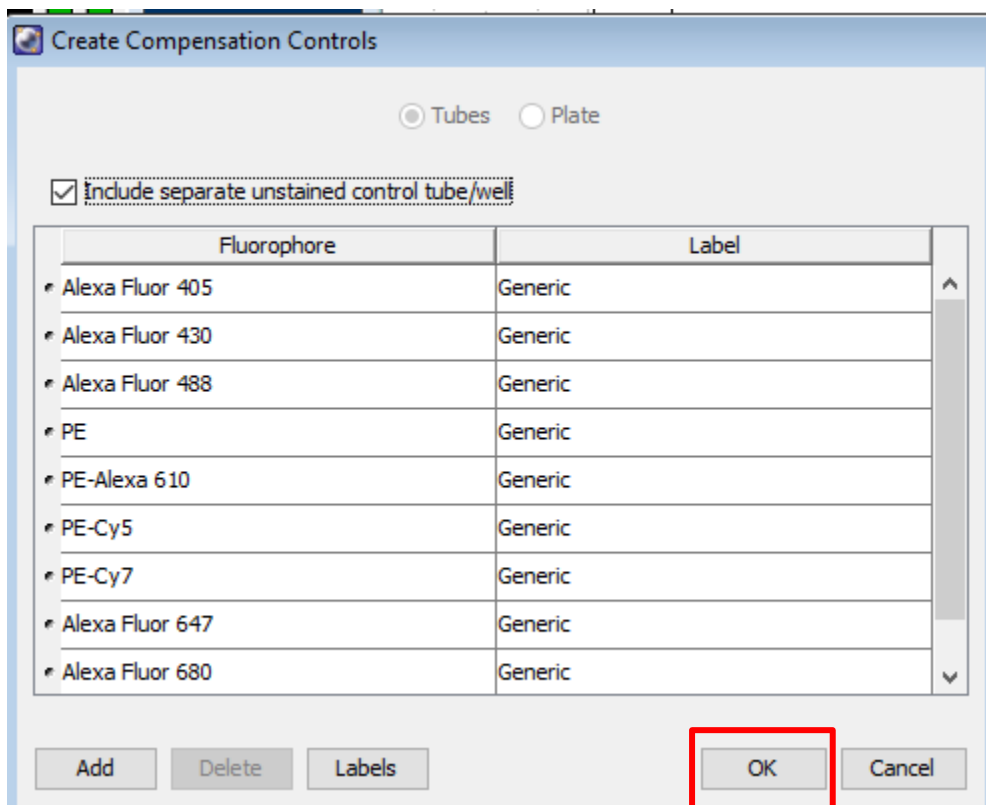
A screenshot of a software interface showing a list of items with 'Add' and 'Delete' buttons at the bottom.

CALCULATING COMPENSATION

1. Right click the experiment's title → Compensation setup → Create.



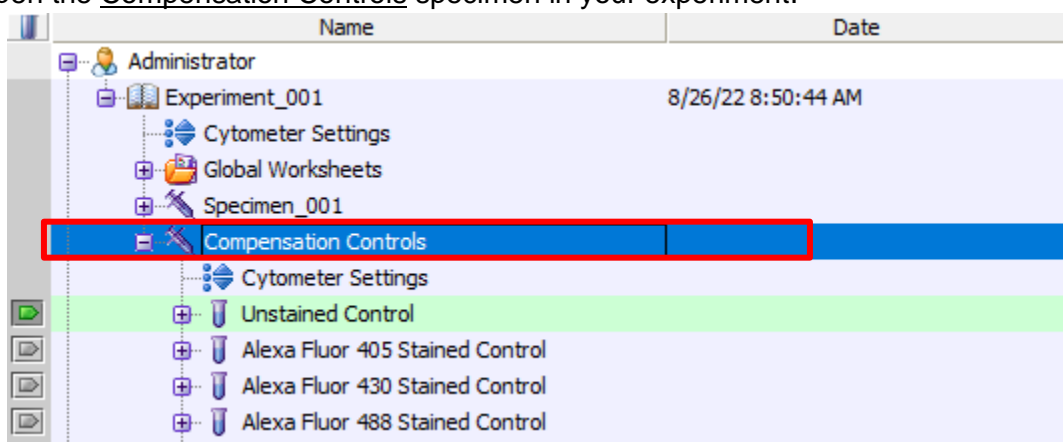
- a. Verify compensation controls coincide with your chosen fluorochromes, then press OK.



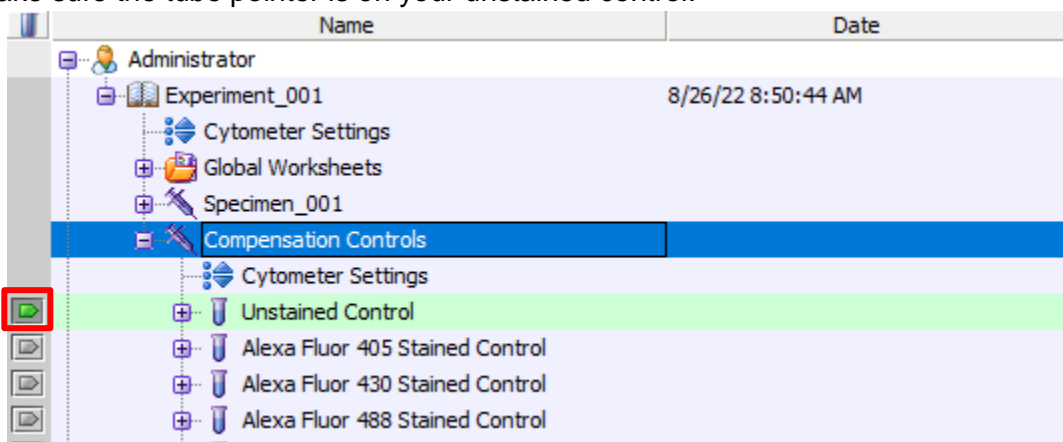
A screenshot of the 'Create Compensation Controls' dialog box. The 'Tubes' radio button is selected. The checkbox 'Include separate unstained control tube/well' is checked. A table lists fluorophores and their labels. The 'OK' button is highlighted with a red box.

Fluorophore	Label
Alexa Fluor 405	Generic
Alexa Fluor 430	Generic
Alexa Fluor 488	Generic
PE	Generic
PE-Alexa 610	Generic
PE-Cy5	Generic
PE-Cy7	Generic
Alexa Fluor 647	Generic
Alexa Fluor 680	Generic

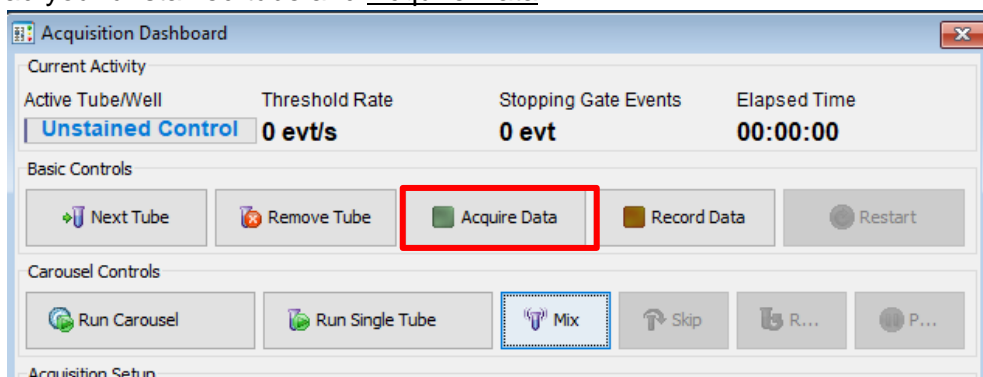
- Open the Compensation Controls specimen in your experiment.



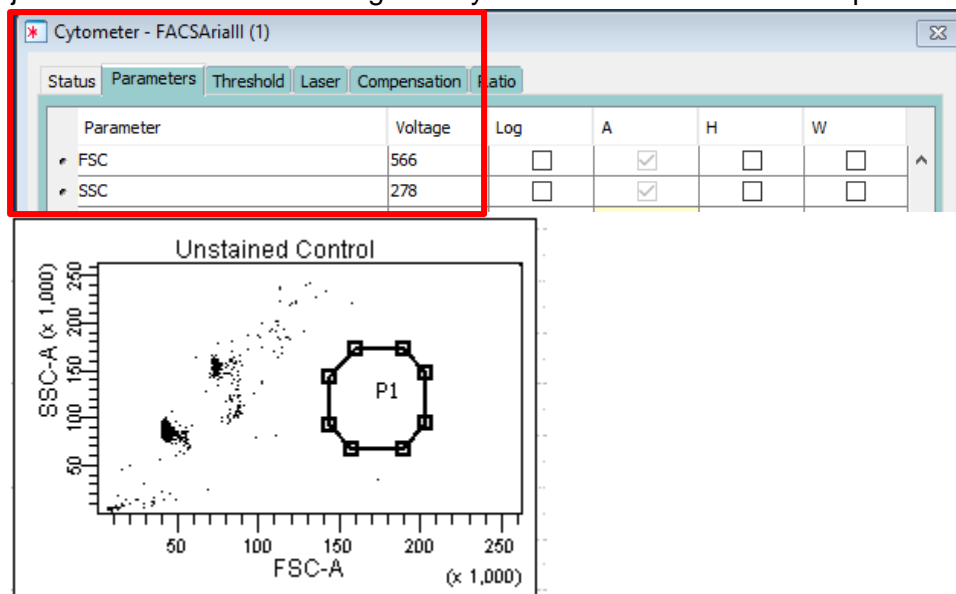
- Make sure the tube pointer is on your unstained control.



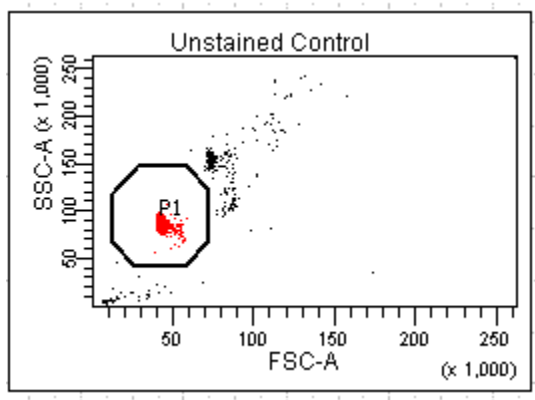
- Load your unstained tube and Acquire Data.



5. Adjust the FSC and SSC voltage until your cells are on-scale in the plot.



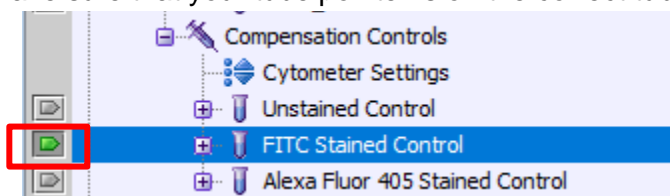
6. Move the P1 gate until it encompasses the population of interest.



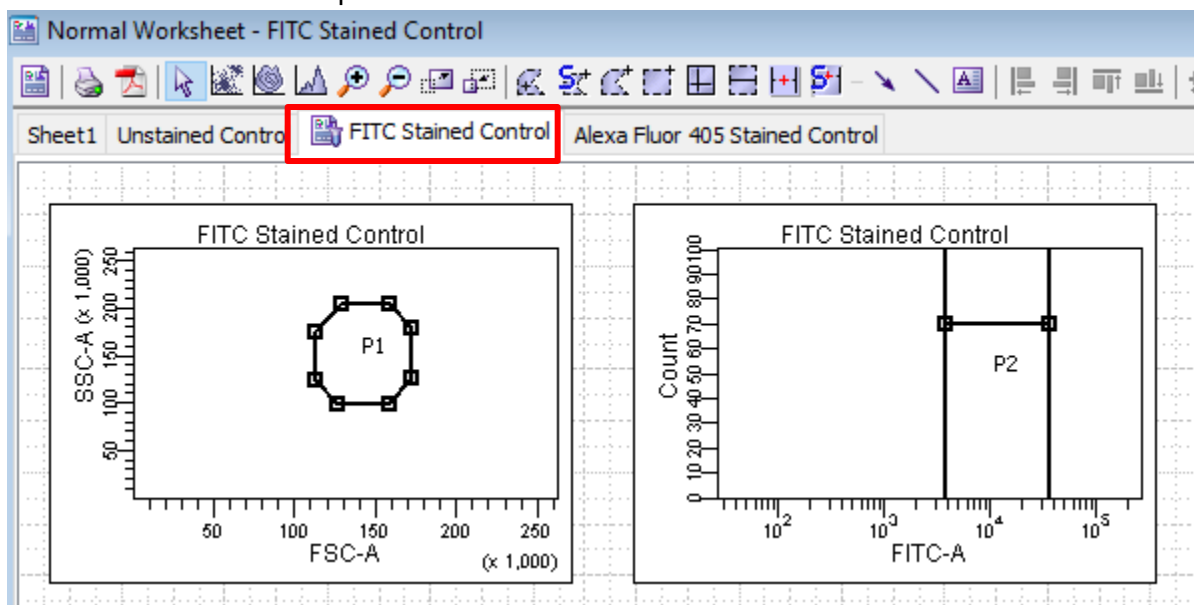
7. Right click the P1 gate → apply to all compensation controls.
8. Look at the histograms for the fluorochromes selected in your experiment.
9. Adjust the voltage of each fluorochrome until the negative population is close to the axis.
 - a. It's important to make sure you can see both ends of your population. If it bleeds into the side of the graph, increase the voltage until you can see the bottom left of the peak.
10. Acquire data again and Record.
11. Unload your unstained tube.

12. Load your first compensation tube.

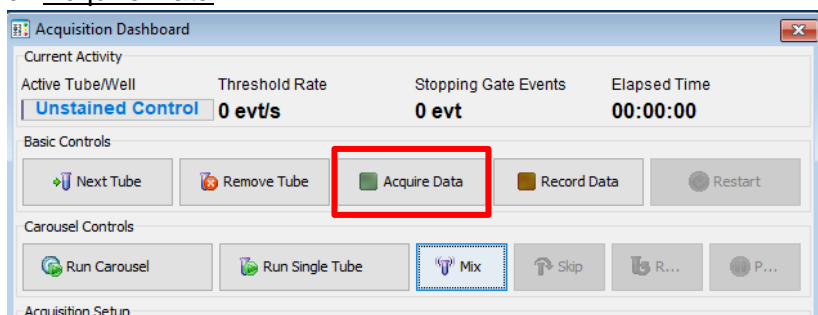
13. Make sure that your tube pointer is on the correct tube.



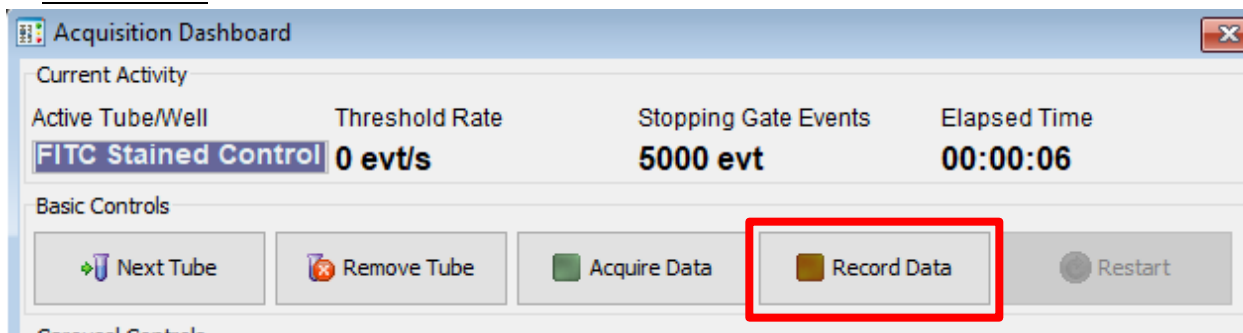
14. Click on the next worksheet panel.



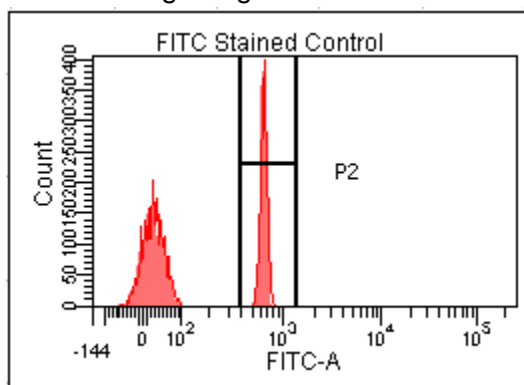
15. Click Acquire Data.



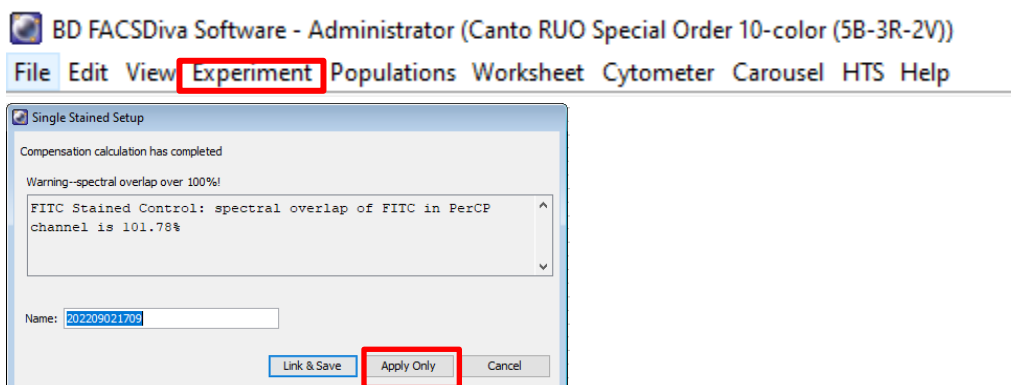
16. Click Record Data.



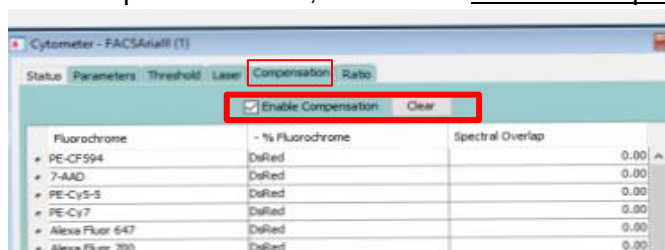
17. Move the histogram gate until it surrounds your positive population.



18. Repeat steps 10-17 until all your compensation tubes have been completed.
19. Select Experiment→Compensation Setup→Calculate Compensation → Apply Only.

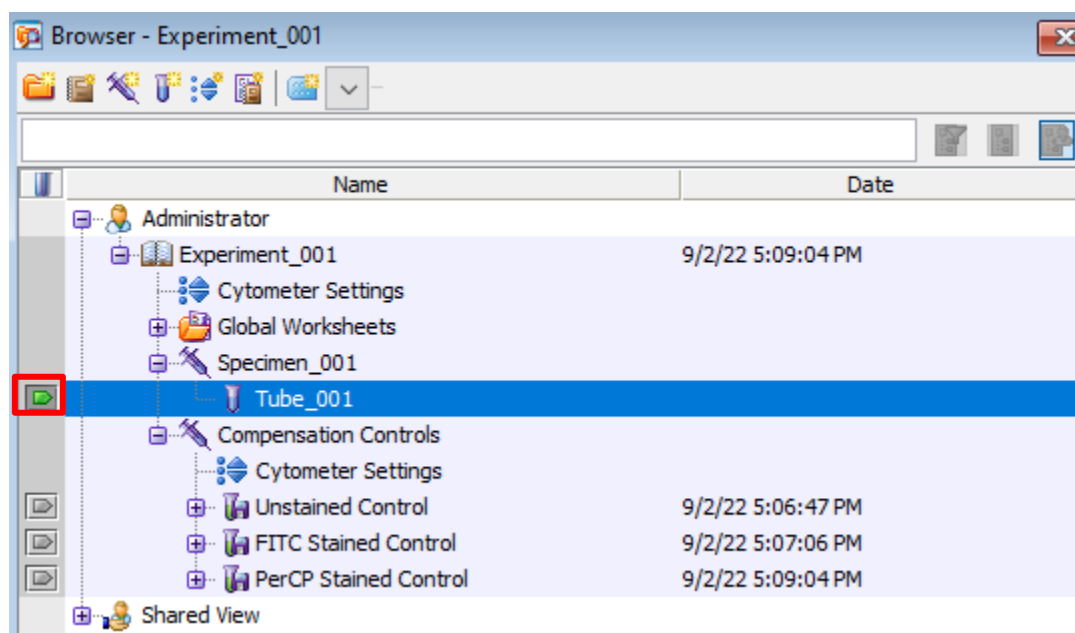


20. In the compensation Tab, make sure Enable Compensation is selected.

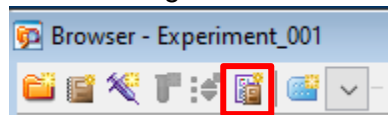


ACQUISITION

1. Place the Acquisition Pointer on Tube_001.



2. Create a new global worksheet.



3. Create your graphs, dot plots, and gates to run your experiment.



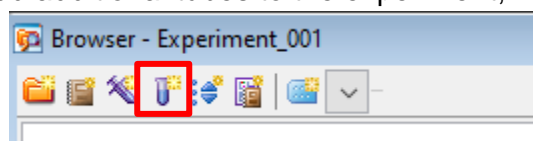
*Hover over each item for more information.

4. Once you have your plots set up, right-click a plot and select Show Population Hierarchy.

Tube: Tube_001

Population	#Events	%Parent	%Total
■ All Events	0	####	####
■ P1	0	####	####
■ P2	0	####	####
■ P3	0	####	####
■ P4	0	####	####

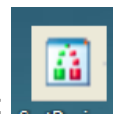
5. Add additional tubes to the experiment, if needed.



6. Run a fully stained sample and set up gates.
 - i. Double check that the gates are collecting the populations of interest.

TUBE SORTING

1. Create a new sort layout by clicking New Sort Layout.



2. Open the Sort Device app and select your device. ICON:

3. If on the Symphony, you can select the device from the drop-down menu on the side stream window.

4. In the Sort layout window select the collection device.

5. Change the precision mode:

- a. Purity: two tubes
- b. 4-way purity: four tubes
- c. Single Cell: plate

6. Specify the number of cells you want in each tube or leave as continuous.

7. Select a save report option.

8. Assign the sorting gates to each tube by clicking in the blank space.
9. Turn on voltage and test sort in the side stream window. The number of streams to test depends on how many populations you are sorting (2 populations = 2 side streams).
10. Adjust side stream sliders until the streams are aligned in the collection tubes you are using.
11. Insert a collection device with prepared tubes.
 - a. Make sure to have a layer of buffer at the bottom of your tubes so the cells don't dry out—this will aid in recovery.
12. Attach the chiller extensions to the device if needed—this will aid in recovery.
13. Insert your sample tube into the sample chamber.
14. Select Load → Acquire Data → Sort.
15. Click OK.

PLATE SORTING

1. Create a new sort layout by clicking New Sort Layout.
2. Put the ACDU into the designated position on the sort block.
3. Open the Sort Device app and select your device (ACDU).
4. In the Sort layout window select the collection device.
5. Change the precision mode:
 - a. Purity: two tubes
 - b. 4-way purity: four tubes
 - c. Single Cell: plate
6. Specify the number of cells you want in each well.

7. Select a save report option.
8. Assign the sorting gates to each well.
9. Turn on the voltage and adjust the far-left stream appropriately.
10. In the Sort layout window, press the Eject button so that the platform comes all the way forward.
11. Place your plate **with the lid on** onto the platform.
 - a. Make sure that A1 is positioned on the tray correctly.
12. On the menu bar, select Sort → Home Device.
13. Select the correct plate you are using.
14. Press Go Home.
15. Turn on the voltage and double click test sort.
16. Open waste drawer by pressing waste drawer icon.
17. Make sure stream lands on the A1 well.
 - a. If the droplet is off center, make adjustments by manipulating the arrow buttons.
18. Repeat steps 15-16 until the droplet is in the center of the A1 well.
19. When adjustments have been made, select Set Home.
20. Bring plate forward using the eject button and take off lid.
21. Select Sort → Home Device.
22. Select Go Home.
23. Select Acquire Data → Sort → Ok.

EXPORTING DATA

1. BioHPC

2. Cytobank

***NOTE:** The flow core deletes experiments off DIVA on the first of every month. They are stored in a cloud backup. Please make sure that you are saving your experiments after every session.*

CLEANING BETWEEN USERS

1. Turn off the stream.
2. Open the flow cell access door.
3. Remove the nozzle.
4. Insert closed loop nozzle.
5. Fill a tube with water and place the nozzle inside.
6. Sonicate the nozzle.
7. While the nozzle is sonicating, install a tube of Windex.
8. Select Cytometer → Cleaning Modes → Clean Flow Cell.
9. Repeat step 8 twice with a tube of Windex.
10. Install a tube of shutdown solution.
11. Select Cytometer → Cleaning Modes → Clean Flow Cell.
12. Repeat step 11 twice with the shutdown solution.
13. Turn off the sonicator.
14. Dry the nozzle with a Kimwipe.
15. Place the nozzle back in its box for the next user.
16. Dry the deflection plates with a Kimwipe.

17. Wipe down the workspace.
18. Check the sheath and waste levels.
19. Log out of DIVA and PPMS.

DAILY SHUTDOWN FOR THE LAST USER OF THE DAY

1. Turn off the stream.
2. Open the flow cell access door.
3. Remove the nozzle.
4. Insert closed loop nozzle.
5. Fill a tube with water and place the nozzle inside.
6. Sonicate the nozzle.
7. While the nozzle is sonicating, install a tube of Windex.
8. Select Cytometer → Cleaning Modes → Clean Flow Cell.
9. Repeat step 8 twice with the tube of Windex.
10. Install a tube of shutdown solution.
11. Select Cytometer → Cleaning Modes → Clean Flow Cell.
12. Repeat step 11 twice with the shutdown solution.
13. Turn off the sonicator.
14. Dry the nozzle with a Kimwipe.
15. Place the nozzle back in its box for the next user.
16. Dry the deflection plates with a Kimwipe.
17. Wipe down the workspace.

18. Check the sheath and waste levels.
19. Log out of DIVA.
20. Log out of BD Coherent.
21. Log out of Tera Term.
22. Shut down the cytometer by pressing the green button on the side of the instrument or bio safety cabinet.
23. Vent the air pressure from the sheath tank by pulling up on the vent ring.