

BD INFLUX**USE AND MAINTENANCE**

PURPOSE

The purpose of this procedure is to ensure proper and consistent use and maintenance of the BD Influx cell sorter including tasks such as startup, alignment, quality control, data acquisition, sorting, shutdown, and troubleshooting.

SCOPE

Compliance with this procedure is the responsibility of all employees and users of the Advance Cytometry and Sorting Facility at South Campus's BD Influx cell sorter.

DEFINITIONS

PBS: Phosphate Buffered Saline.

diH₂O: Deionized water.

Auxiliary Compressors: An in-house air compressor system located in room 2SCRB4.2204.

Sort Monitoring Rack: The three screens used for viewing the stream above the display monitor.

AMO Filter: Aerosol Management Option filter is used to evacuate aerosolized particles from the sort chamber.

Voltage Pulse: The electronic signal derived from fluorescent light emission that represents positive events. Voltage pulse signals can be visualized as peaks on the oscilloscope below the monitor.

Laser Delay Bucket: The range of voltage pulse signal that is recognized from a certain laser. Laser delay buckets can be visualized on the oscilloscope below the monitor.

MATERIALS AND REAGENTS

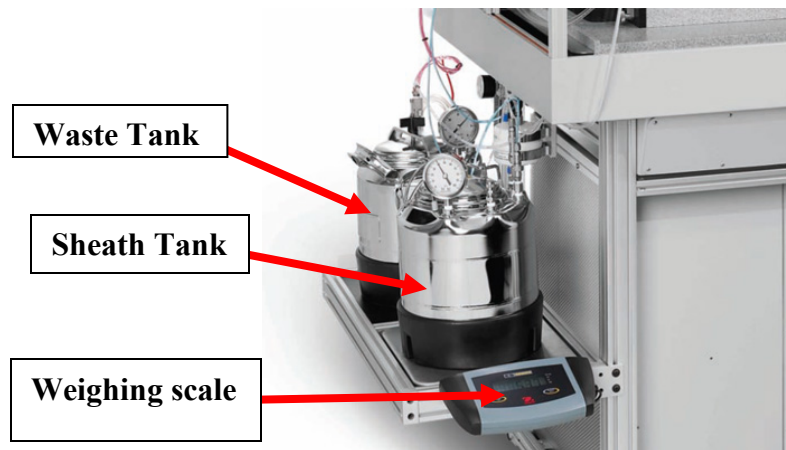
- BD Influx cell sorter.
- Sterile PBS.
- 10% bleach.
- diH₂O.
- 70% ethanol.

- 5 ml polypropylene round bottom tubes.
- Spherotech UltraRainbow calibration beads.
- BD FACS Accudrop beads.
- Kimwipes.
- Debubble reservoir.
- Q-Tips.

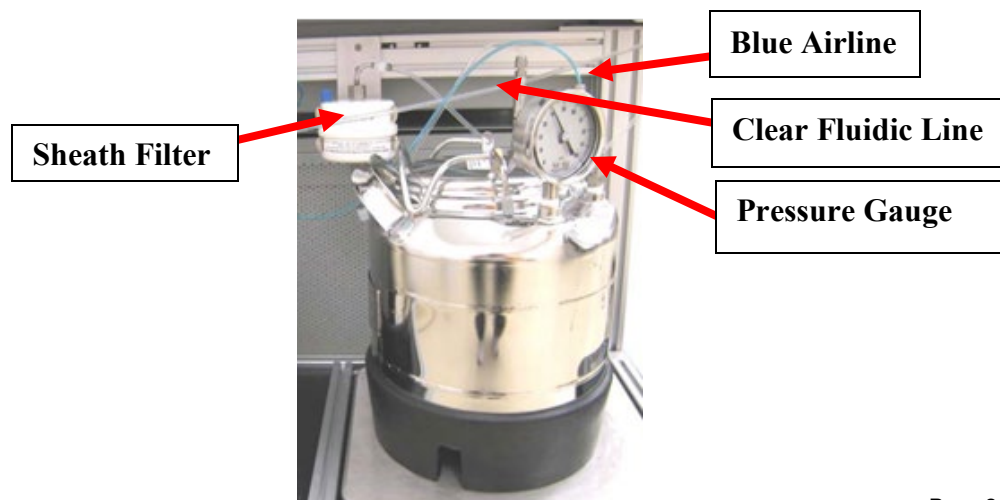
PROCEDURE

1.0 Start Up

- 1.1. Check that the sheath tank is full and the waste tank is empty. If the waste is full add 250 ml of bleach, wait 30 minutes, and empty the waste tank. (usually bleach is added to the waste tank previous evening while shutdown, if so empty the waste tank without adding bleach)



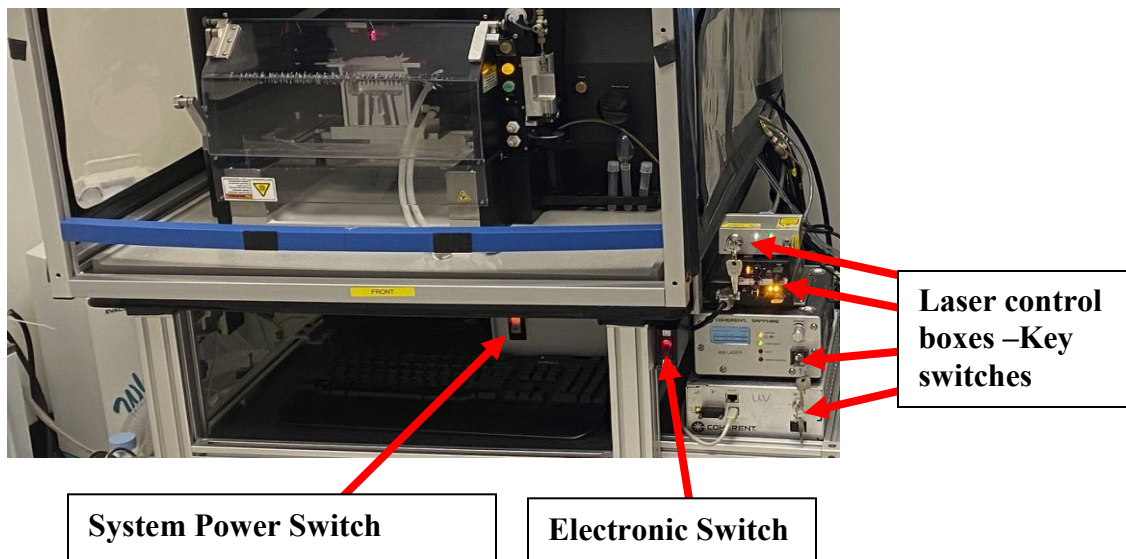
- 1.2. Check that the blue airline and the clear fluidics line is connected to the sheath tank, and that the pressure release valve is closed. Also check if the inlet and outlet lines from sheath tank are connected to the filter.



1.3. Check that the auxiliary compressors are on and open the air and vacuum valves on the wall.



1.4. Turn on the system power (main switch under machine) and wait until it starts beeping.



Oscilloscope

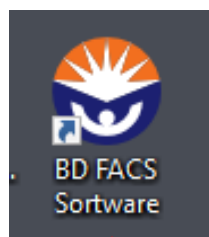


1.5. Once it starts beeping turn the electronic switch on to the left of lasers. Wait until the oscilloscope loads then turn the computer on and turn the key switches to the right of the instrument to fire the 488 nm, 640 nm, 405 nm, and 561 nm lasers.

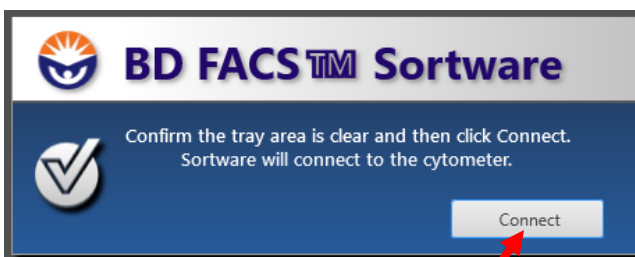
Note: The UV laser should only be fired if it will be in use that day. The UV laser should be used at least once per week usually Monday.

Note: How to turn ON the UV laser: UV laser is turned ON by pressing the power switch on the laser box and wait for 10 to 15min then turn the key to fire the laser. Also, you need to open Coherent (OPSL 3.7) software window on the desktop. Type 100 at set power and press ON button on the top right corner. Wait until Power (mW) reaches 100.

- 1.6. Double click the BD FACS Software icon to open the instrument acquisition software. Ensure that the tray area is clear and then click “Connect”.

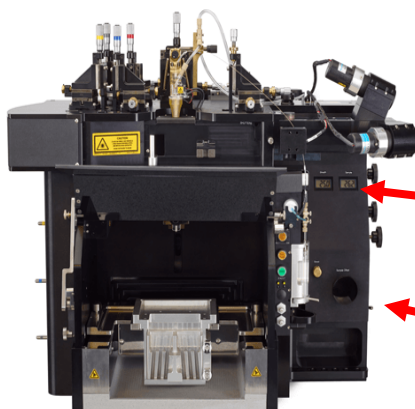


Software icon



Click to Connect software to the cytometer

- 1.7. Turn on the pressure switch on the right side of the instrument. Ensure that the sheath tank is pressurizing. If air is escaping from around the seal, re-seat the lid. You may have to moisten the large black o-ring inside the lid with diH₂O.



Sheath and Sample Pressure display

Pressure Switch

- 1.8. Purge any bubbles from the sheath filter by carefully opening the bleeder valve on the top of the filter until sheath flow begins.

Blue cap of the filter bleeder valve



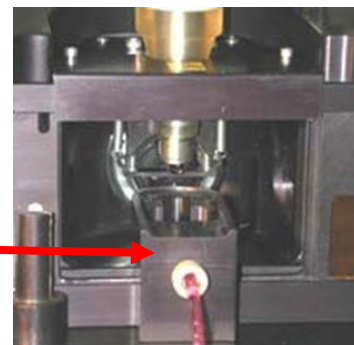
- 1.9. Remove bubbles from the sample line. Follow step A when starting from a dry shutdown. Follow step B when starting from a wet shutdown.

A. Removing Bubbles after a Dry Shutdown

- Sonicate the nozzle tip for three to five minutes in 10% bleach. Push a small amount of diH₂O from a syringe through the nozzle tip after sonication to ensure the nozzle tip is clear.

- Place the flush bucket beneath the nozzle. Press the yellow Rinse button on the instrument to allow any large air bubbles to flow out of the system, and quickly press Rinse again to stop the flow of sheath. Repeat this step a few times until sheath flows freely without any air.

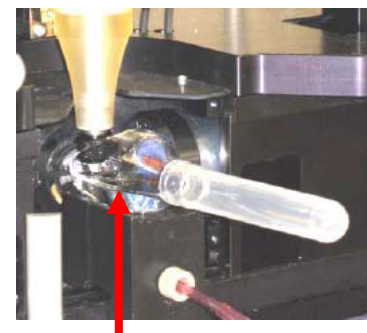
Flush Bucket



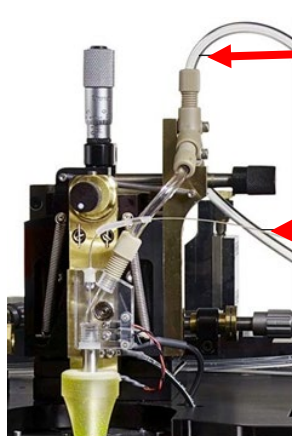
- Install the nozzle tip on the nozzle, ensuring that it forms a tight seal.



- Place the debubble reservoir beneath the nozzle. Press the green Run button on the instrument to fill the reservoir with sheath. Once the nozzle tip is submerged, press Run again to stop the flow of sheath.
- Press the yellow Purge button on the instrument to pull up sheath through the nozzle.



Debubble reservoir



**Y Shaped
Purge line**

**Sheath
Line**

- Once the air bubbles have passed the Y shaped purge valve press the yellow Pulse button on the instrument to free any additional loose bubbles.
- Repeat the previous three steps until no more bubbles are released from the nozzle.
- For additional help removing stubborn bubbles please see the Troubleshooting section of this SOP.

B. Removing Bubbles after a Wet Shutdown

- Ensure that the debubble reservoir is beneath the nozzle and has enough liquid to submerge the nozzle tip. If the nozzle tip is not submerged, press the green Run button on the instrument to fill the reservoir with sheath. Once the nozzle tip is submerged, press Run again to stop the flow of sheath.
- Press the yellow Purge button on the instrument to pull up sheath through the nozzle.
- Once the air bubbles have passed the Y shaped purge valve press the yellow Pulse button on the instrument to free any additional loose bubbles.
- Repeat the previous three steps until no more bubbles are released from the nozzle.

1.10. Press the Run button and remove the debubble reservoir. Gently dry the nozzle tip with a Q-Tip. Verify that the stream is flowing straight.

1.11. Ensure that the plate voltage is off on the sort monitoring rack. Clean and dry the deflection plates in the open position using 70% ethanol and Kimwipes. Close the deflection plates.



1.12. Press the yellow Illumination and red Plates buttons on the sort monitoring rack to visualize the stream and charge the plates.

1.13. Remove the flush bucket from under the nozzle.

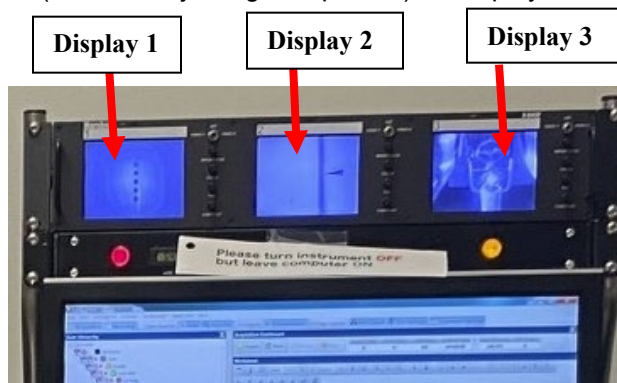
2.0 Alignment and Quality Control

2.1. In BD FACS Software on the main toolbar select File > Workspace > Restore and restore the most recent "QC Alignment" workspace for the appropriate nozzle size.

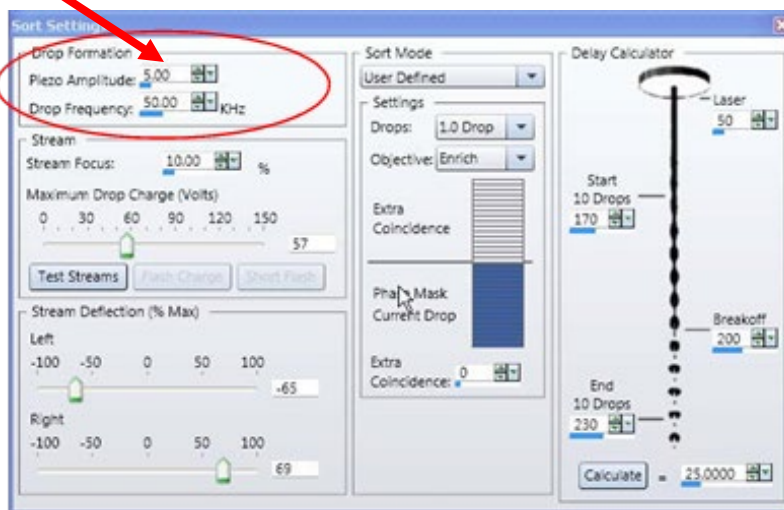
2.2. Allow the stream to stabilize and then adjust the Piezo Amplitude in the top Sort Settings window so that the last attached drop is centered (indicated by the green pointer) on display 2 on the sort monitoring rack.

Note: The height of the Drop Position Camera can also be adjusted if adjusting the Piezo Amplitude does not center the last attached drop.

Note: The Drop Position Camera should be adjusted when changing between nozzle sizes.



Adjust the Piezo Amplitude



- 2.3. With the analysis chamber door open, adjust the nozzle height using the **silver vertical adjustment knob** on top of the flow cell chamber so that the shadow of the nozzle is just out of view on display 1 of the sort monitoring rack.

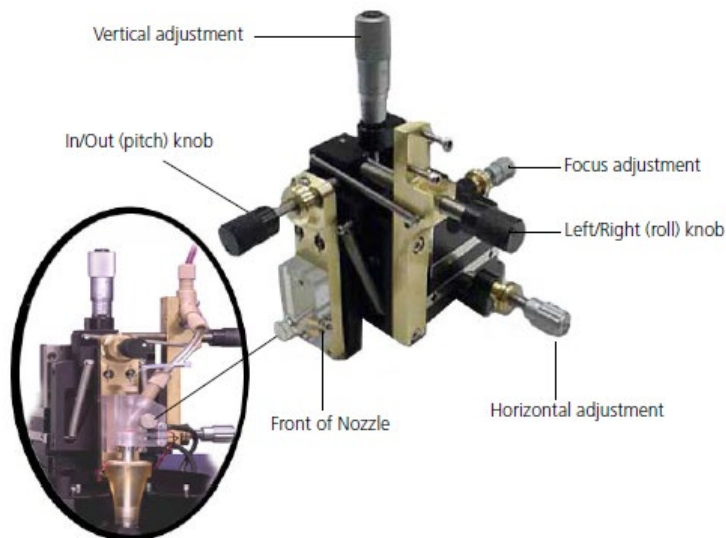
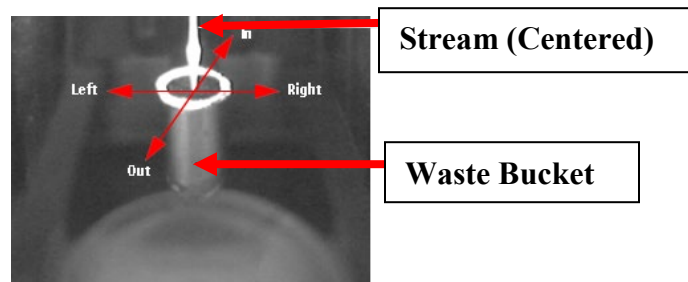
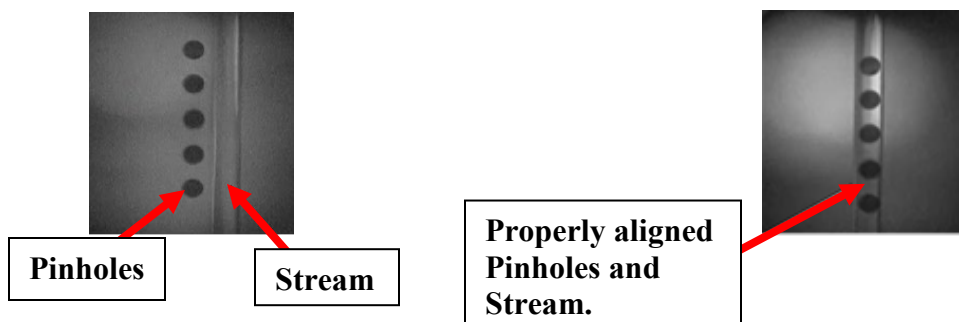


Figure1: Nozzle Assembly Adjustment Knobs. BD Biosciences. "BD Influx Cell Sorter User's Guide". 2011.

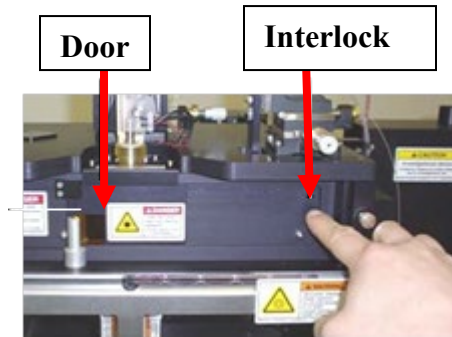
- 2.4. Looking at display 3 on the sort monitoring rack, adjust the black pitch and roll knobs on the flow cell chamber (see Figure 1) until the sort stream is as bright as possible and centered in the waste bucket.



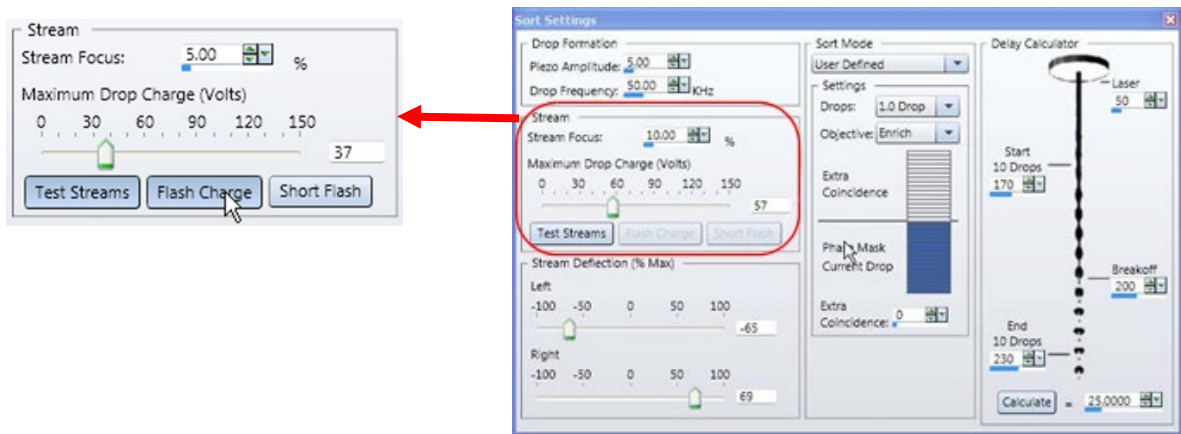
- 2.5. Adjust the silver horizontal adjustment knob on the right side of the flow cell chamber (see Figure 1) so that the stream is centered on the pinholes (display 1).



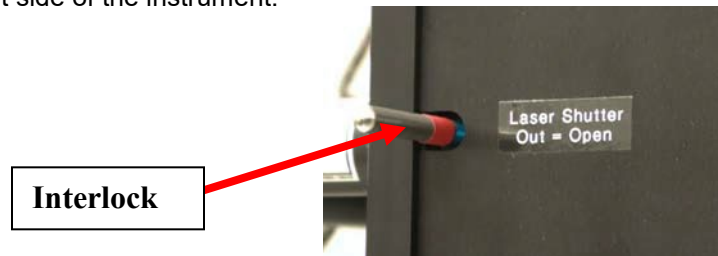
- 2.6. Ensure that the stream is in focus by adjusting the silver focus adjustment knob on the back of the flow cell chamber (see Figure 1).
- 2.7. Close the analysis chamber door and activate the interlock by covering the hole on the right side of the door.



- 2.8. Turn on the sort streams by clicking “Test Streams” in the Sort Settings window. Adjust the Piezo Amplitude and Drop Charge until the streams are as tight as possible in display 3 on the sort monitoring rack. You may have to readjust the “in/out black pitch knob to ensure that the left and right side streams are equally bright. Click “Flash Charge” and adjust the Piezo Amplitude again as needed. Finally, click “Short Flash” and adjust the Piezo Amplitude as needed. Click “Test Streams” again to turn off the side streams.

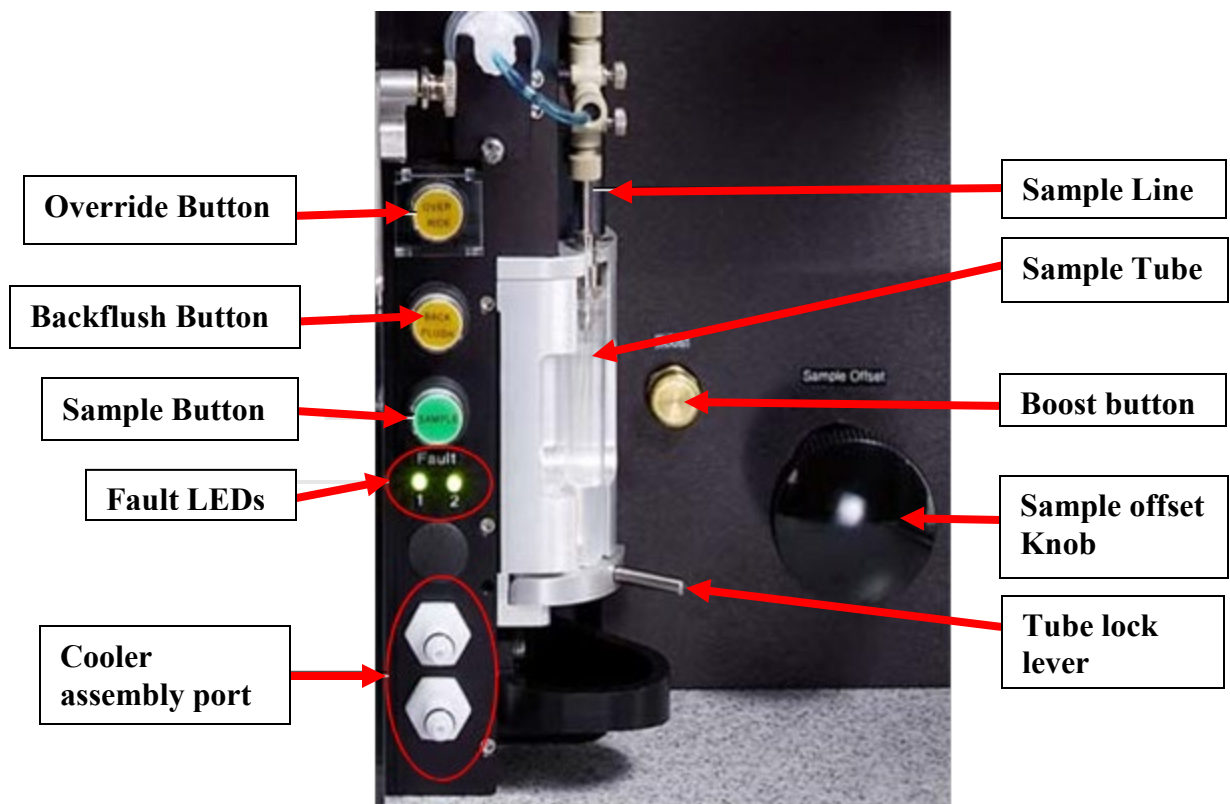


- 2.9. Ensure that the shutters are open for the 405 nm, 488 nm, 561, and 640 nm lasers by pulling the interlock pins out on the left side of the instrument.



Note: The UV laser shutter should only be open if it will be in use that day.

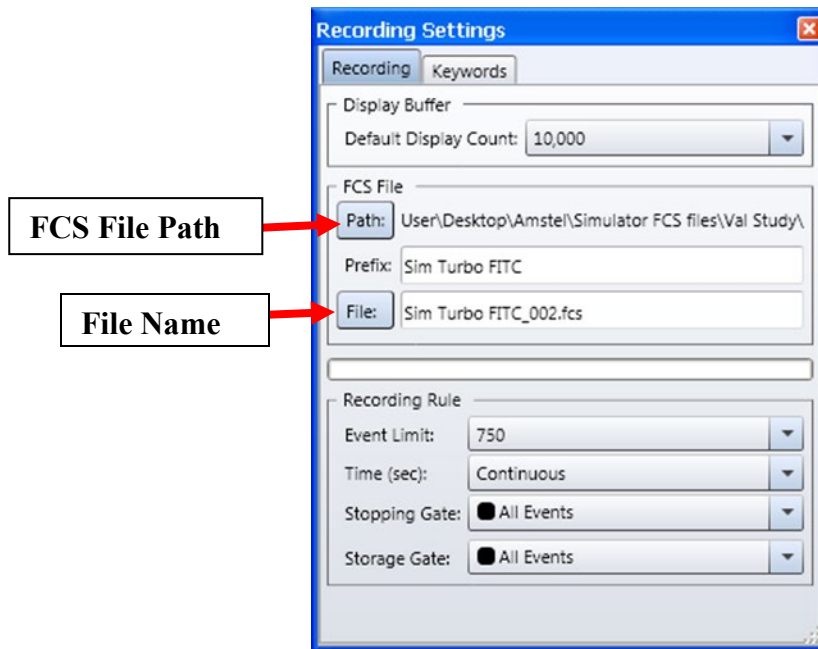
- 2.10. Ensure that the BioBubble containment unit is correctly sealed and that the AMO filter is attached and pulling less than 2 inches of water.
- 2.11. Press the yellow Backflush button on the front of the instrument to allow the sample line to flush for a few seconds. Load Ultra Rainbow calibration beads onto the sample station, close the arm, and press the green Sample button.



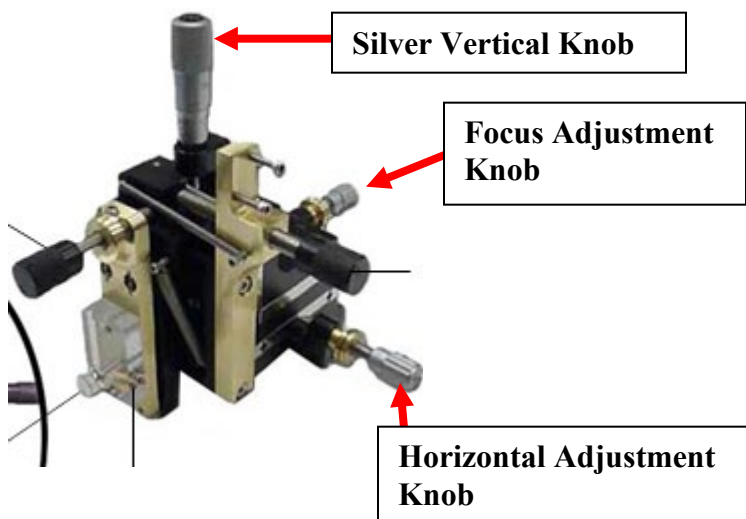
Note: All samples should be prepared in 5 ml polypropylene round-bottom tubes.

- 2.12. Click "Acquire" on the Software Acquisition Dashboard. Press and hold the brass Boost button on the instrument until events are accumulating on the Acquisition Dashboard and a flash is visible in the first pinhole in display 1 on the sort monitoring rack.

- 2.13. Adjust the sample flow rate using the black Sample Offset knob on the instrument until the event rate is between 300 and 500 events/second on the Acquisition Dashboard.
- 2.14. In the Recording Settings window set the Default Display Count to 500 and set the Recording Rule Event Limit to 2,000. For the FCS File Path select User > Desktop > QC files Ultra Rainbow > the current year > the current month. For the Prefix enter the date using the form YYYYMMDD.

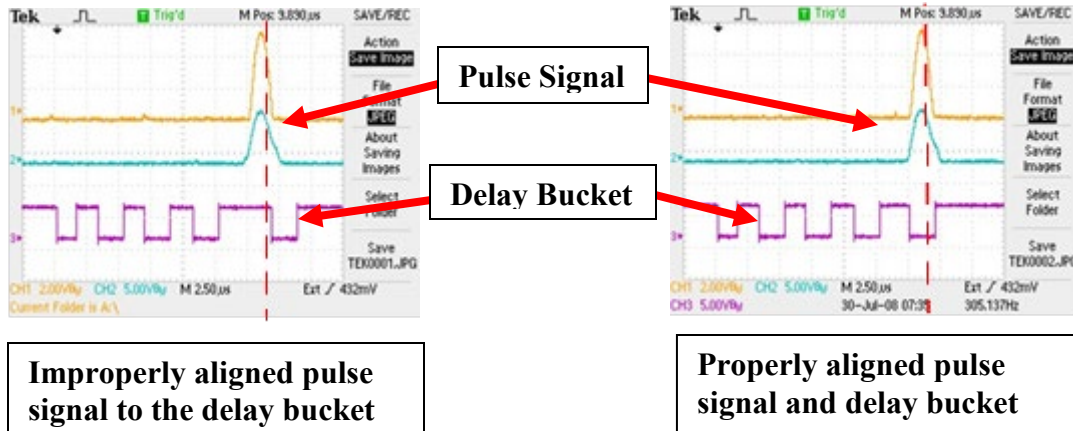


- 2.15. Click on the first plot for the 488 nm laser on the Worksheet. Adjust the silver vertical and focus adjustment knobs above the flow cell until the signal on the first 488 nm laser plot is as bright as possible. Minor adjustment of the silver horizontal knob might be necessary. Ensure that the shadow of the flow cell has not dropped too low into the image. Ensure that the voltage pulse signal is falling into the first laser delay bucket on the oscilloscope below the monitor.

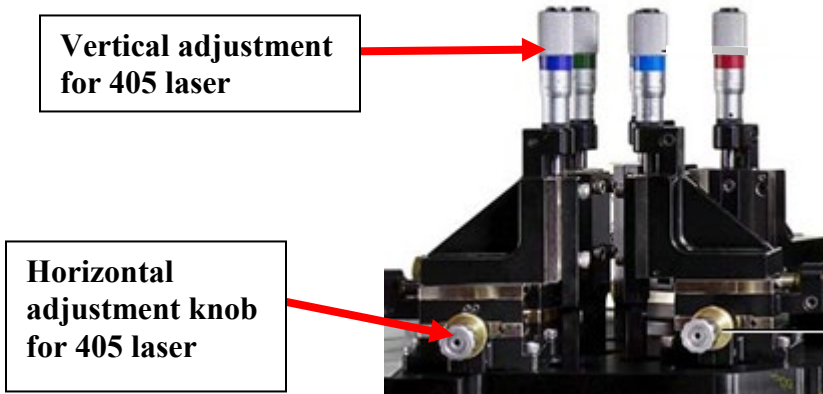


Note: The 488 nm laser delay should always be 0.00, and the laser itself should not be adjusted.

Images of oscilloscope display



- 2.16. Click on the first plot for the 405 nm laser on the Worksheet. Adjust the silver and violet vertical and horizontal adjustment knobs until the signal on the first 405 nm laser plot is as bright as possible. Ensure that the voltage pulse signal is falling into the appropriate laser delay bucket on the oscilloscope below the monitor. If the voltage pulse needs to be adjusted, change the Delay in the Cytometer Settings window.

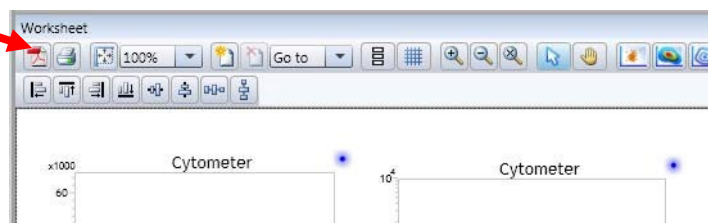


- 2.17. Continue optimizing laser delay and position for all lasers being used that day as described in the previous step.

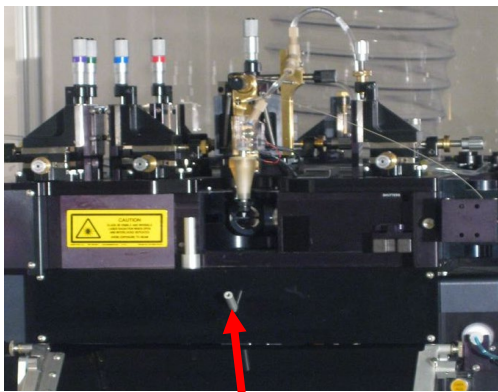
*Note: Laser adjustment knobs are color coded based on the wavelength. Only one laser should be adjusted at a time, and should not be changed once it is set. 405 nm corresponds to violet, 488 nm corresponds to blue, 561 nm corresponds to green, 640 nm corresponds to red, and UV corresponds to grey. **The 488 nm blue knobs should not be adjusted daily.***

- 2.18. Once all lasers have been optimized, click "Record" on the Acquisition Dashboard. Save a PDF of the Worksheet by selecting the PDF icon at the top of the Worksheet, and save it in User > Desktop > QC files Ultra Rainbow > the current year > the current month. The file name should be the date using the form YYYYMMDD.

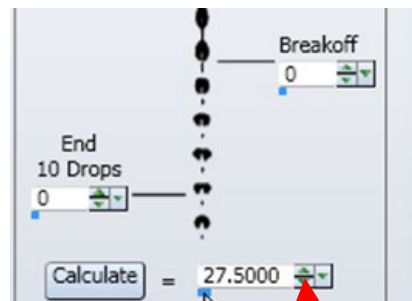
Click for PDF



- 2.19. Click “Stop” on the Acquisition Dashboard, press the green Sample button on the instrument, remove the Ultra Rainbow beads from the sample station, and press the yellow Backflush button to allow the flow cell to flush for a few seconds.
- 2.20. On the Influx Daily Settings log to the right of the computers, record the date, nozzle size, sheath pressure, drop frequency, appropriate laser delays, and Piezo Amplitude.
- 2.21. You can run Accudrop beads on the same QC acquisition templet, Load the Accudrop beads onto the sample station, close the arm, and press the green Sample button. Turn off the lights in the room.
- 2.22. Click “Acquire” on the Acquisition Dashboard. Press and hold the brass Boost button on the instrument until events are accumulating. Ensure that the bead population is within the sort region.
- 2.23. Adjust the sample flow rate using the black Sample Offset knob on the instrument until the event rate is between 1,000 and 3,000 events/second on the Acquisition Dashboard.
- 2.24. Turn the Accudrop filter knob clockwise and click “Sort” on the Sort Layout window. Adjust the drop delay next to the “Calculate” button in the Sort Settings window until the left side stream is as bright as possible, and the center waste stream is as dim as possible.



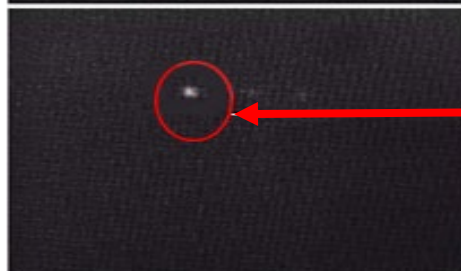
Accudrop filter knob



Adjust by clicking on the small up and down arrows



Incorrect drop delay with beads sorted to waste



Correct drop delay with beads sorted to the left

- 2.25. Click "Stop" on the Sort Layout window to stop sorting. Turn the Accudrop filter knob counterclockwise to return it to its original position. Click "Stop" on the Acquisition Dashboard, press the green Sample button, remove the Accudrop beads from the sample station, and press the yellow Backflush button to allow the flow cell to flush for a few seconds.
- 2.26. Record the settings, the camera position and drop delay in the Influx Daily Settings log.
- 2.27. Load a tube with approximately 3 ml of 10% bleach onto the sample station, close the arm, and press the green Sample button. Allow the bleach to run for approximately three minutes. Press the green Sample button, remove the bleach from the sample station, replace it with a tube of diH₂O, close the arm, press the green Sample button, and allow the water to run for approximately three minutes. Finally, press the green Sample button, and remove the water from the sample station and press the yellow backflush button.



Camera position
digital display

3.0 Acquiring Data and Sorting

- 3.1. Open an existing workspace in Software by going to File > Workspace > Restore and restore the desired workspace, ensuring that it is for the appropriate nozzle size. Uncheck "Restore Sort Settings" and "Restore Window Layout".
- 3.2. Ensure that the laser delays, particularly for the lasers being used in the experiment, match those determined during QC for the day by checking the Influx Daily Settings log. These laser delays may need to be adjusted depending upon the signals generated by the cells.
- 3.3. Make any needed adjustments to the Worksheet so that the appropriate populations can be detected. Add the populations to be sorted in the Sort Layout window.
- 3.4. In the Recording Settings window set the Default Display Count and the Recording Rule Event Limit to the desired values. For the FCS File Path select Desktop > "Influx User Data" the current month and year > appropriate PI, then create a folder with the user's name and date using the form YYYYMMDD. For the Prefix enter the user's name, the date using the form YYYYMMDD, and any additional information for the first sample.
- 3.5. In the Sort Layout window select the desired Sort Device and Sort Mode settings. Ensure that the sort stage is clear, and then click "Sort Ready". Place the appropriate sort collection device holder in the sort chamber, including empty or covered sort collection device(s). Click "Test Streams" in the

Cytometer Settings

Lasers / Detectors Status

✓ PMT Power

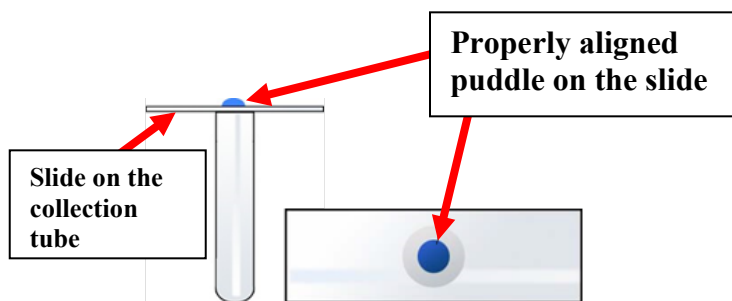
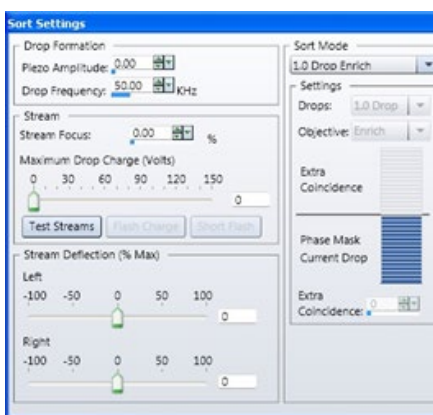
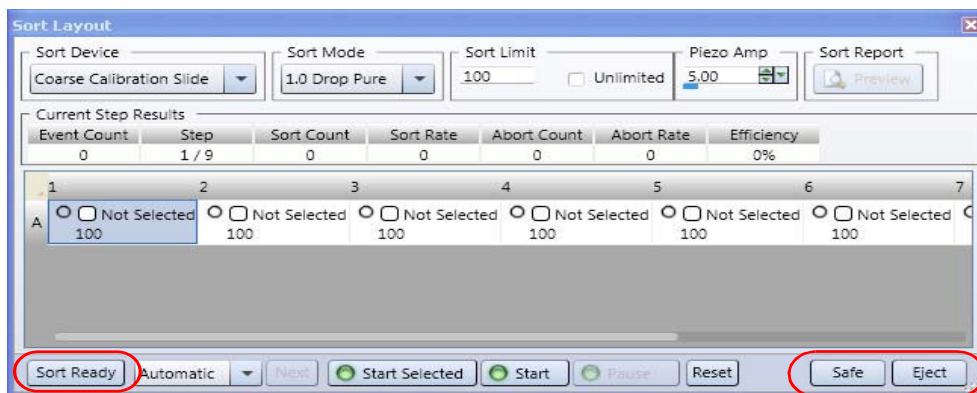
Trigger

Detector: FSC (Per) Threshold Level: 3341 Scope Channel 1: FSC (Per) Scope Channel 2: FSC (Par)

Detector	Voltage	Log	Name	Label
Laser 1 Delay: 0.00				
✓ PMT 1	31.81		FSC (Per)	
✓ PMT 2	23.69		FSC (Par)	
✓ PMT 3	25.64		SSC	
✓ PMT 4	43.38	✓	530/40 (488)	CD3 FITC
✓ PMT 5	39.33		580/30 (488)	
✓ PMT 6	71.68	✓	692/40 (488)	CD45 PerCP-C
Laser 2 Delay: 3.00				
✓ PMT 13	49.88	✓	585/29 (561)	CD16+56 PE
✓ PMT 14	54.86		610/20 (561)	
✓ PMT 15	56.35		670/30 (561)	
✓ PMT 16	54.83		750LP (561)	
Laser 3 Delay: 7.88				
✓ PMT 9	32.22		460/50 (355)	
✓ PMT 10	49.55		670/30 (355)	
Laser 4 Delay: 12.00				
✓ PMT 11	52.34	✓	460/50 (405)	CD8 V450
✓ PMT 12	52.21		550/50 (405)	
Laser 5 Delay: 17.55				
✓ PMT 7	60.27	✓	670/30 (640)	CD4 APC
✓ PMT 8	71.78	✓	750LP (640)	CD19 APC-H7

Sort Settings window, then adjust the Stream Deflection so that the side stream(s) are centered in the sort collection device(s).

Note: Steps 3.2 through 3.5 do not need to be done in order.



3.6. Load the first sample onto the sample station, close the arm, and press the green Sample button.

Note: All samples should be prepared in 40 μ m filter cap 5 ml polypropylene round-bottom tubes.

Note: Do not allow the sample tube to run dry.

3.7. Click “Acquire” on the Acquisition Dashboard. Press and hold the brass Boost button on the instrument until events are accumulating. Adjust voltages using the sliders on the axes as needed so that populations are on scale.

3.8. Ensure that the sort collection devices are installed properly, and then click “Sort” in the Sort Layout window to begin sorting.

3.9. To record a file, click “Record” on the Acquisition Dashboard. A PDF of the Worksheet can be saved by selecting the PDF icon at the top of the Worksheet. It should be saved in the file path Desktop > “Influx User Data” the current month and year> appropriate PI> current user and date folder. The file name should include the date using the form YYYYMMDD.

3.10. Once sorting and/or recording has completed click “Stop” on the Sort Layout window to stop sorting, and click “Stop” on the Acquisition Dashboard. A PDF of the Sort Report can be saved by selecting the “Sort Report Preview” button on the Sort Layout window. It should be saved in the file path Desktop > “Influx User Data” the current month and year> appropriate PI> current user and date folder. The file name should include the date using the form YYYYMMDD.

- 3.11. Press the green Sample button, remove the sample from the sample station, and press the yellow Backflush button to allow the flow cell to flush for a few seconds.
- 3.12. Repeat acquisition and sorting steps for as many samples as necessary.
- 3.13. When all sorting and recording is completed load a tube with approximately 3 ml of 10% bleach onto the sample station, close the arm, and press the green Sample button. Allow the bleach to run for approximately three minutes.
- Note: All samples should be prepared in 5 ml polypropylene round-bottom tubes.*
- Note: Do not allow the sample tube to run dry.*
- 3.14. Press the green Sample button, remove the bleach from the sample station, replace it with a tube of diH₂O, close the arm, press the green Sample button, and allow the water to run for approximately three minutes. Finally, press the green Sample button, and remove the water from the sample station.

4.0 Shutdown

- 4.1. Load a tube with approximately 3 ml of 10% bleach onto the sample station, close the arm, and press the green Sample button. Allow the bleach to run for approximately five minutes.
- 4.2. Press the green Sample button, remove the bleach from the sample station, replace it with a tube of diH₂O, close the arm, press the green Sample button, and allow the water to run for approximately five minutes, then remove the water from the sample station.
- 4.3. Turn the key switches to the right of the instrument to turn off all of the lasers.
- 4.4. Press the red Plates and yellow Illumination buttons on the sort monitoring rack to turn off plate voltage and stream illumination.
- 4.5. In the Software click Cytometer > Shutdown Cytometer. Wait for the Dell computer to turn off, then click File > Exit. Shut down the Alienware computer. Turn off the instrument electronics switch below the front right corner of the instrument. Place the flush bucket beneath the nozzle.
- 4.6. Shut down fluidics. Follow step A for a dry shutdown, which should be performed approximately once per week, usually Friday afternoon. Follow step B for a wet shutdown, which should usually be performed for weekday shutdowns.

A. Fluidics Dry Shutdown

- Turn off the stream by pressing the green Run button. Depressurize the system by flipping the silver switch on the right side of the instrument.
- Bypass the sheath filter to the left of the instrument by connecting the fluid in and out lines to each other.
- Fill a tank with approximately 1 L of 10% bleach, and switch the air and fluid lines to the bleach tank. Repressurize the system, press the yellow Rinse and yellow Backflush buttons, and allow the bleach to run until it is empty.

Note: The tank must be tilted toward the fluid line to allow all of the fluid to drain out.

- Press the Rinse and Backflush buttons to turn them off. Depressurize the system. Fill the bleach tank with approximately 1 L of diH₂O. Repressurize the system, press Rinse and Backflush, and allow the diH₂O to run until it is empty.
- Press the Rinse and Backflush buttons to turn them off. Depressurize the system. Empty the sheath tank, then fill it with approximately 1 L of 70% ethanol. Repressurize the system, press Rinse and Backflush, and allow the ethanol to run until it is empty.
- Press the Rinse and Backflush buttons to turn them off. Depressurize the system. Disconnect the air and fluid lines from the sheath tank and reconnect them to each other. Press the Rinse button and allow air to run through the system for approximately 15 minutes.
- Press the Rinse button to turn it off. Depressurize the system. Reconnect the air and fluid lines to the sheath tank. Reattach the sheath filter to the fluid line.

B. Fluidics Wet Shutdown

- Rest the debubble reservoir on the flush bucket and allow it to fill with sheath fluid so that the nozzle tip is submerged
- Turn off the stream by pressing the green Run button. Depressurize the system by flipping the silver switch on the right side of the instrument.

4.7. Ensure that the plate voltage is off on the sort monitoring rack. Clean and dry the deflection plates in the open position using 70% ethanol and Kimwipes.

4.8. Clean any salt crystals around the flow cell, black interlock doors, and sort chamber.

4.9. Close the air and vacuum valves on the wall, and turn off the auxiliary compressors. Turn off the main power switch under the instrument.

Note: The auxiliary compressors should NOT be turned off if other instruments that use them are still operating.

4.10. Disconnect the waste tank, add 250 ml of bleach, wait 30 minutes, empty the waste tank, and rinse it with 70% ethanol.

5.0 Troubleshooting

5.1. Pressurizing the Sheath Tank

- If the sheath tank does not pressurize ensure that the pressure release valve on the sheath tank is closed.
- Reinstall the sheath tank cover, feel and listen to be sure it is seated properly.
- The large black gasket can be wet with sterile diH₂O to help form a seal.

5.2. Removing Bubbles from the System Lines

- If small bubbles persist place the debubbler beneath the nozzle press the green Run button on the instrument to fill the reservoir with sheath. Once the nozzle tip is submerged, press Run again to stop the flow of sheath.
- Press the yellow Purge button on the instrument to pull up Sheath through the nozzle.

- C. Lower the debubbler enough to expose the nozzle tip to air very briefly, and then submerge the nozzle tip again.
- D. Allow the large bubble that forms to entirely pass the Y shaped purge valve, then press the yellow Pulse button on the instrument to free any additional loose bubbles.
- E. If small bubbles persist in the fluid lines fill the debubbler with 70% ethanol until the nozzle tip is submerged.
- F. Press the yellow Purge button on the instrument to pull up ethanol through the nozzle.
- G. Once the air bubbles have passed the Y shaped purge valve press the yellow Pulse button on the instrument to free any additional loose bubbles.
- H. Repeat the previous three steps until no more bubbles are released from the nozzle. If the bubbles have cleared press the green Run button, remove the debubbler, and allow the stream to stabilize for at least ten minutes.

5.3. Clearing a Clog

See SOP QM001: Clearing a Sorter Clog.

RELATED PROCEDURES

QM001: Clearing a Sorter Clog.

DM001: User Data Management.

REFERENCES

BD Biosciences. "BD Influx Cell Sorter User's Guide". 2011.

REVISION HISTORY

VERSION	ACTION	DATE	INITIALS
1.0	Initial release	08-08-2016	KMR
1.1	Review	06-07-2019	KC/VP
2.0	Version Update	04-16-2020	VP/KR