

## BD FACSARIA III

### QUICK GUIDE: START UP, QUALITY CONTROL, CLEANING & SHUT DOWN

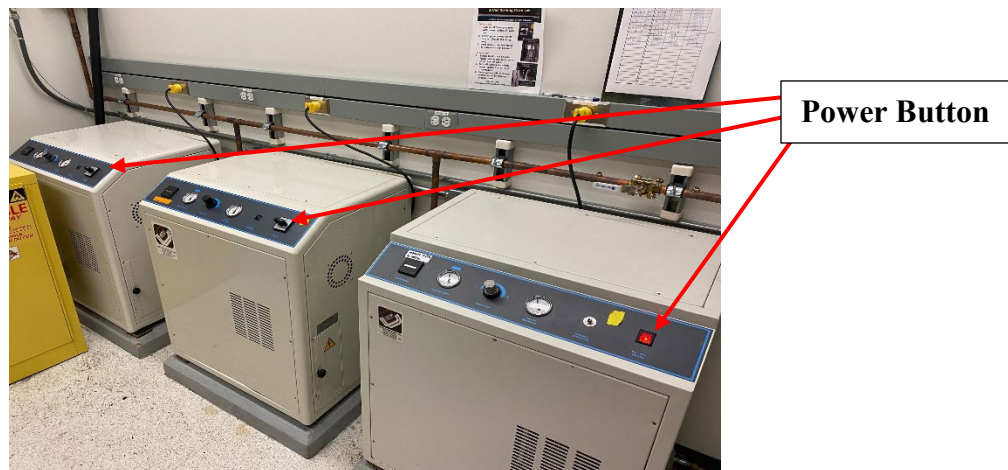
#### PROCEDURE

##### 1.0 Start Up

- 1.1. Check that the sheath tank is full, if not fill the tank with autoclaved 1X PBS. Check if waste tank is empty, if not empty the waste tank by dumping the waste in to the sink and running the tap water for few minute. Add 250 ml of bleach to the empty waste tank.



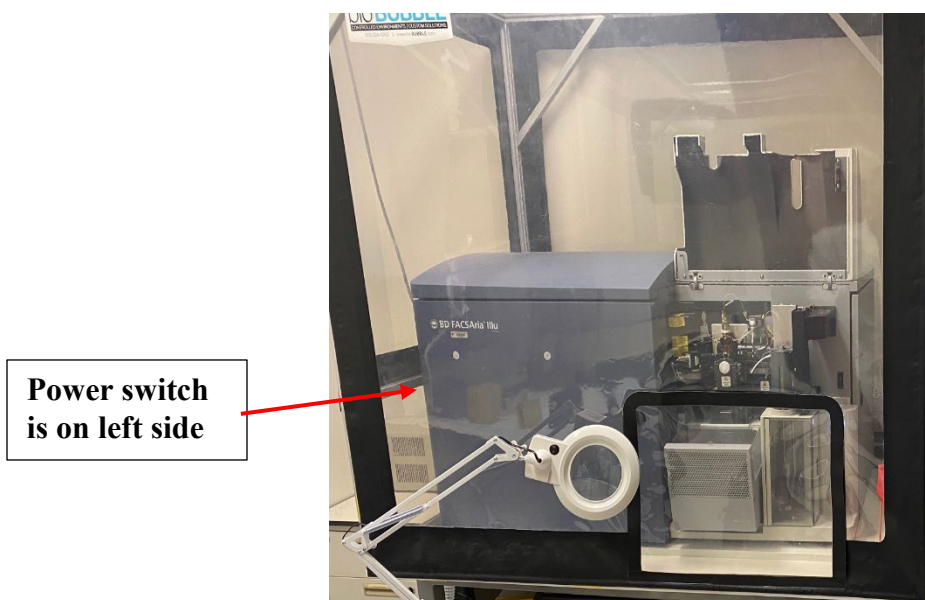
- 1.2. Check that the auxiliary compressors are on and the Cougar control panel is on (in the compressor room) and that the air valve on the wall in the sorter room is open.



- 1.3. Turn on the computer. Log in to Windows as the Operator. The password is “BDIS”.
- 1.4. Double click the DIVA icon to open the instrument acquisition software. Log in to the software as the Administrator. The password is “loki”. Wait until the software indicates that the cytometer is not connected in the “Cytometer Control Panel”.

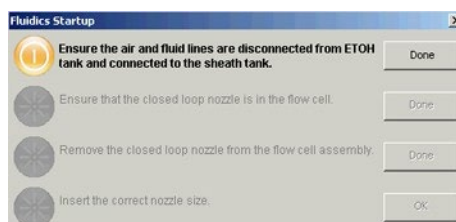


- 1.5. Turn on the instrument system power by pressing the green button on the left side of the instrument. Wait until the software connects to the cytometer (it might take about 3 to 5 min).

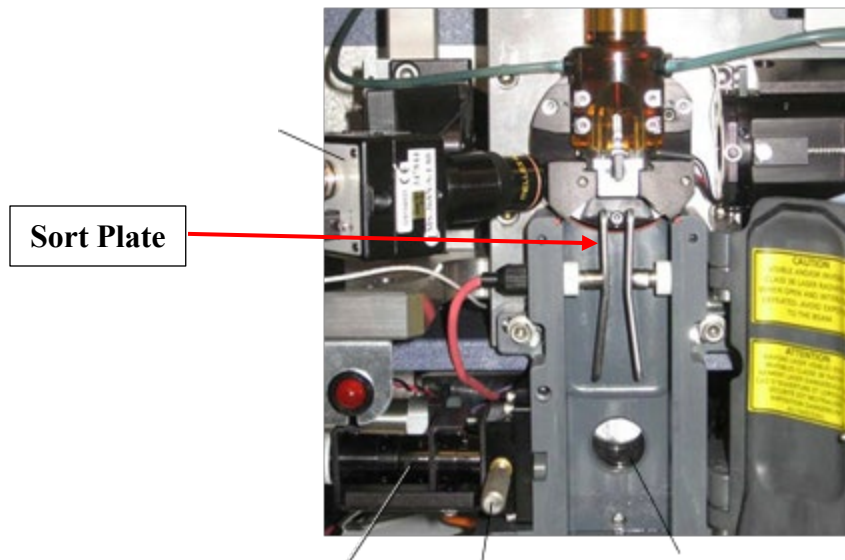


- 1.6. Ensure that the sheath line is connected to the sheath filter and the sheath filter and the air lines are connected to the sheath tank. On the main toolbar select Cytometer > Fluidics Startup and follow the software prompts.

*Note: The closed loop nozzle is not inserted during fluidics startup or shutdown and all steps referencing it can be ignored.*

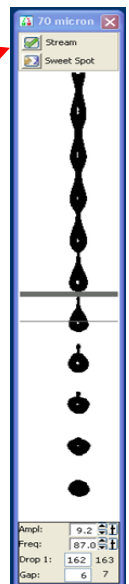
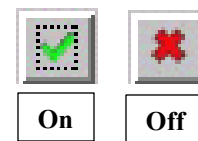
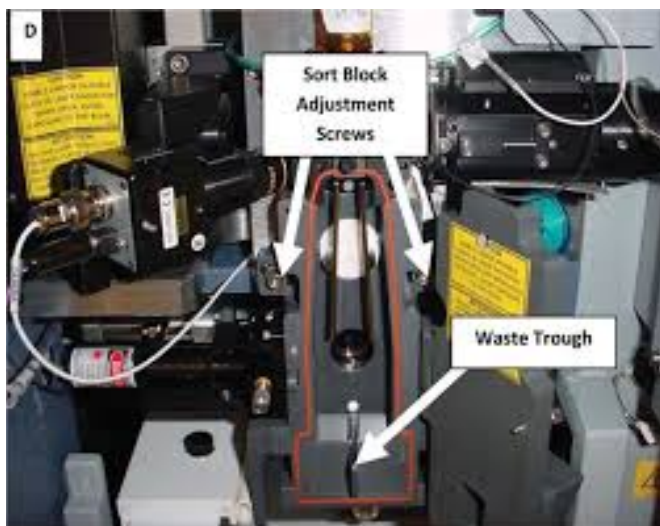


- 1.7. Ensure that the sort block and plates are clean and free of residue. To clean them wet a Kim wipe with diH<sub>2</sub>O or ethanol, wipe the plates and block thoroughly, then dry them with a new Kim wipe.



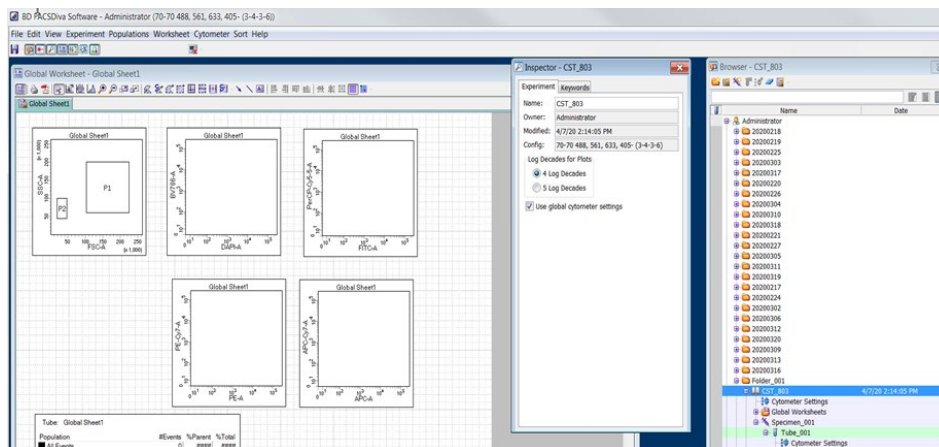
## 2.0 Quality Control

- 2.1. Turn on the stream by clicking the red "X" on the breakoff window. Ensure that the stream is centered in the waste catcher, close the sort block, and allow the stream to stabilize.



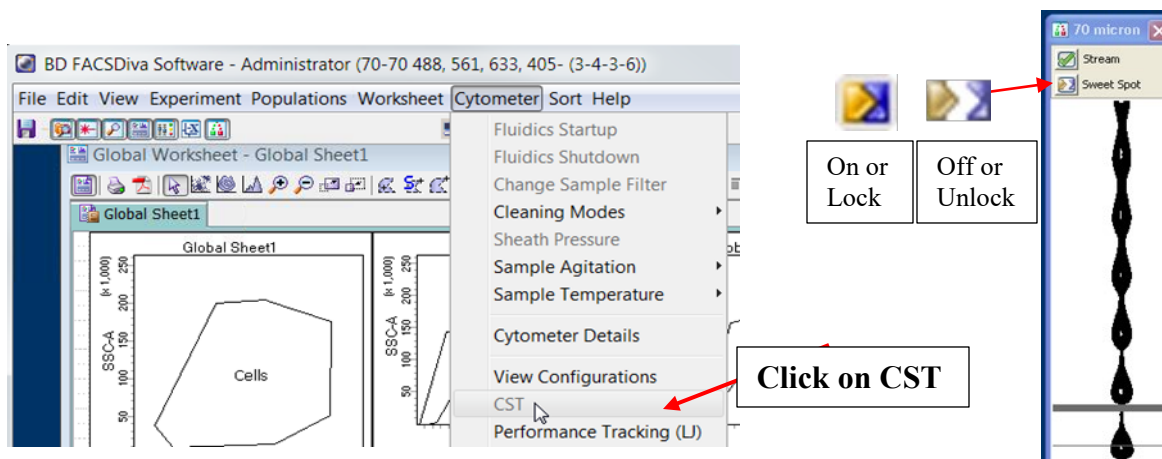
*Note: If the stream is not in the center, take the Allen wrench, loosen the flow chamber, adjust the chamber by moving it very gently with your hand so that the stream is centered in the waste catcher, and tighten the flow chamber using the Allen wrench.*

- 2.2. Create a new folder and name it with the date using the form YYYYMMDD. Open the CST/AccuDrop experiment template from the Flow Core folder.

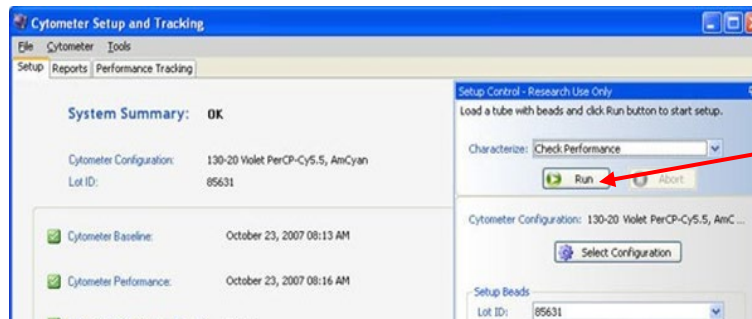


- 2.3. Place the CST beads (CST beads should be filtered before loading) into the cytometer loading port. Set the flow rate to 2.0 on the Acquisition Dashboard, click “Load” and verify that two scatter populations and three fluorescent populations are discernable. Click “Unload”.

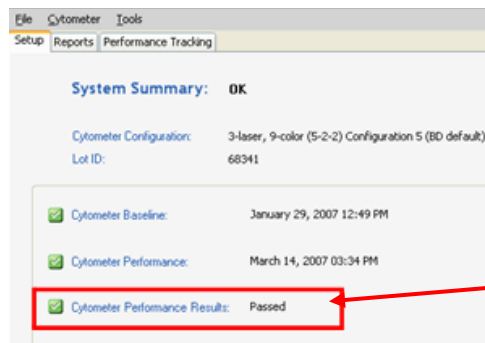
- 2.4. Ensure that Sweet Spot is turned off in the breakoff window. On the main toolbar select Cytometer > CST and wait until the cytometer connects to the CST software.



- 2.5. Ensure that the correct CST bead lot number is selected then click “Run”. Review the Performance Tracking Report, and make a note of any problems or errors on the whiteboard. Pay special attention to the  $\Delta$  PMT value and the laser delays as compared to previous runs.

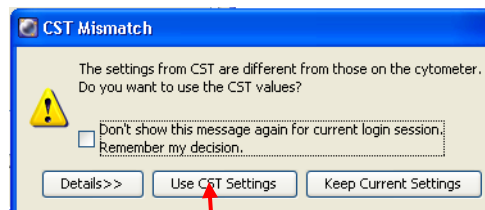


2.6.



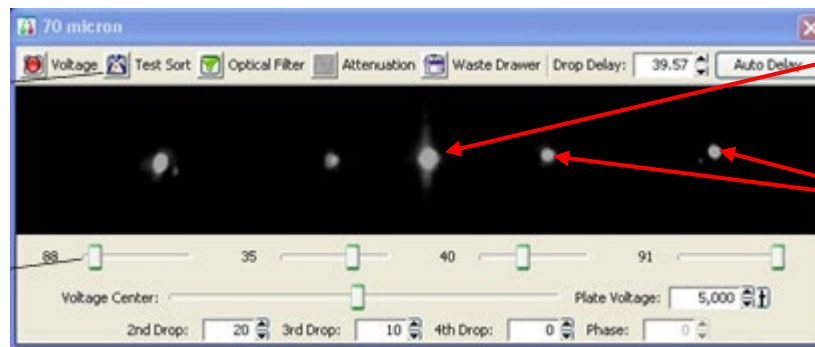
**Check if CST  
has PASSED**

2.7. Remove the CST beads from the loading port, exit the CST software, and allow the instrument to reconnect to DIVA. And click on "Use CST Settings" on the popup window.



**Click this button**

2.8. Turn on Sweet Spot in the breakoff window. Ensure that the center stream is bright, tight, and centered in the side stream window. Check the side streams using "Sort Test"



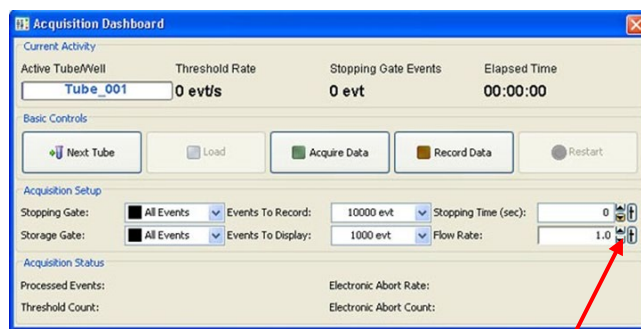
**Center Stream**

**Side Stream**



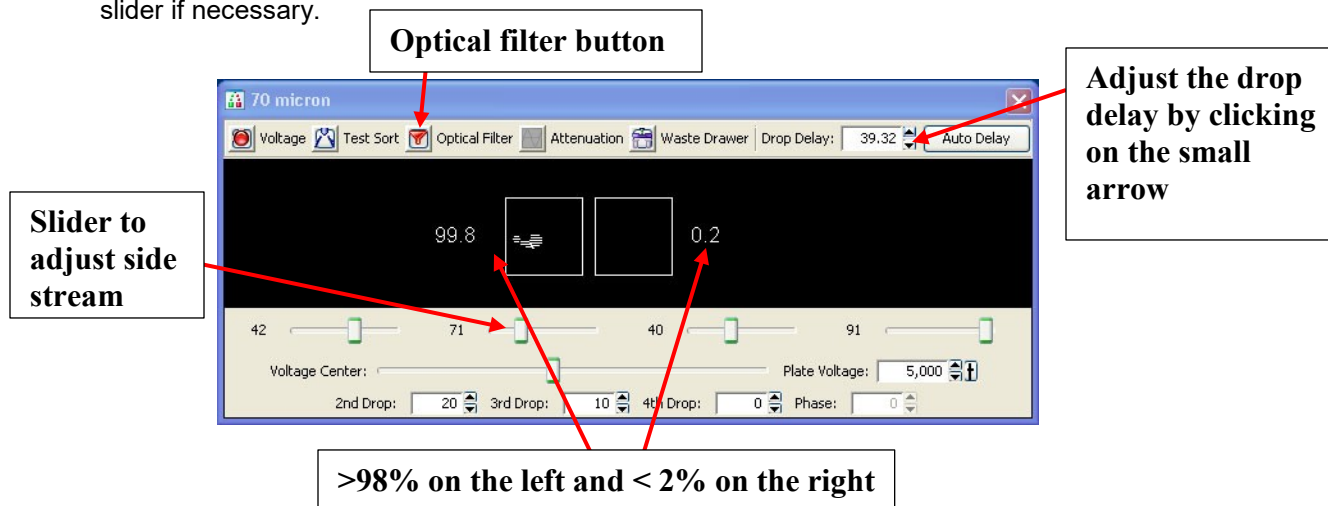
*Note: Please check point 2.5 for pictures to “turn on the sweet spot.”*

- 2.9. Accudrop experiment template is combined with CST template, no need to open a new template for Accudrop. Place the Accudrop beads into the cytometer loading port, and click “Load”. Adjust the flow rate so that the event rate is between 1,000 and 3,000 events per second.



**Adjust the flow rate by pressing the small arrow**

- 2.10. Turn on the voltage in the side stream window. Click “Sort” on the Sort Layout window, then click “Cancel” on the dialog box that comes up. Turn on the Optical Filter on the side stream window and ensure that the sorting stream is inside the filter box. Adjust the side stream voltage slider if necessary.



- 2.11. Optimize the drop delay until 98% of beads or more are being sorted. Unload the beads and record the date, nozzle size, drop delay, sort efficiency, 1st drop, and amplitude in the “Aria Sort Settings” log on the clipboard next to the sorter.
- 2.12. After the bead run, wash the sample line by running bleach as a sample followed by fresh water for a sample for 3 minutes each. Instrument is ready to run the experiment.

### 3.0 Cleaning

- 3.1. Place a tube with Contrad in the sample loading port, set the flow rate to 11.0 on the Acquisition Dashboard, and click “Load”. Allow Contrad to run for at least one minute, then click “Unload”. You will see a large number of scatter events in the plot due to the high viscosity of 100% Contrad.
- 3.2. Replace the Contrad tube with a tube of diH<sub>2</sub>O, and click “Load”. Allow diH<sub>2</sub>O to run for at least one minute. Click “Unload”.



## 4.0 Shut Down

4.1. Place shutdown solution on the sample loading port.

**Loading Port**

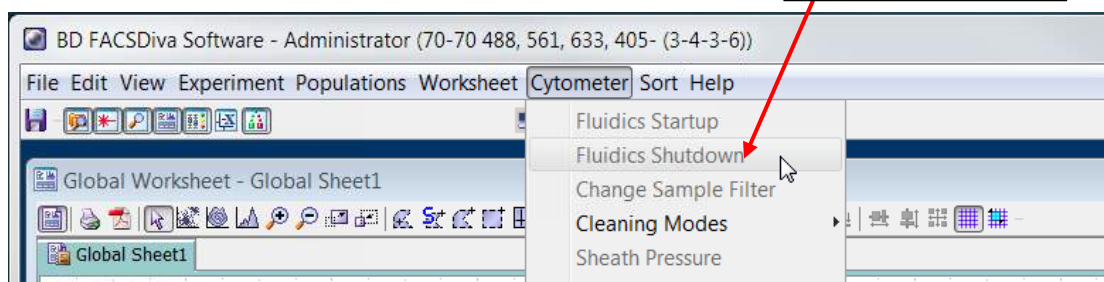
4.2. Turn off the stream by clicking the green check mark on the breakoff window. Ensure that the stainless steel ethanol tank is full. Connect the air line and the blue fluidics line to the ethanol tank.



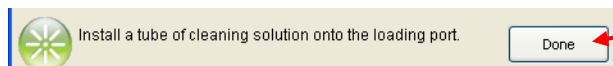
**Ethanol Tank**

4.3. On the main toolbar select Cytometer > Fluidics Shutdown and follow the software prompts. The final step cleans the flow cell with Shutdown Solution.

**Fluidic Shutdown**



*Note: The closed loop nozzle is not inserted during fluidics startup or shutdown and all steps referencing it can be ignored*



**Press done and  
Follow the prompts**

4.4. Ensure that the sort block and plates are clean and free of residue. To clean them wet a Kim wipe with diH<sub>2</sub>O or ethanol, wipe the plates and block thoroughly, then dry them with a new Kim wipe.

4.5. Empty the waste tank and add 250 ml of bleach to the empty waste tank. Reconnect the air line and the blue fluidics line to the sheath tank.

4.6. Exit DIVA and shut down the computer. Turn off the instrument system power by pressing the green button on the left side of the instrument.

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## RELATED PROCEDURES

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This handout is related to ACSF SOP IN005. Please see the full SOP for further information.