

FACSVantage-SE

Quick Guide

Starting Up Procedure:

1. Switch ON, Main Power & Computer Power in console.
2. Turn ON computer and wait for boot-up (Password = BDIS).
3. Launch DIVA (no password) and wait for cytometer to Connect.
4. Open stream access doors on fluidics bench.
5. Check Sheath Tank and fill to seam with Blood Bank Saline. While tank is open, disconnect air pressure hose from Ethanol Tank and connect it to the Sheath Tank. While air is flowing, insert the lid for Sheath Tank and let the internal pressure seat the lid, then secure it with lever.
6. Disconnect the fluidics supply line from the Ethanol Tank and connect it to the Sheath Tank.
7. Disconnect the Waste Tank, empty contents down drain and reconnect Waste Tank to cytometer.
8. On Main control panel, turn ON the Drop Strobe and Stream Lamps.
9. Remove tube of FACS-Clean from SIP port and leave support arm to the side.
10. On Fluidics Control Panel, switch to RUN. Check on the Stream View Screen that Sheath Pressure is set to 11 PSI. Allow the Ethanol in the fluidics lines to clear by letting the fluid run through the system for at least 5 minutes. Fluid should be dripping from the SIP (Sample Insertion Port).
11. Droplet Stream should be hitting the Waste Catcher close to the center and the stream should be in-between the High Voltage Deflection Plates (Caution voltage = 4,000 Volts).
12. Close the right hand door of droplet drawer. Observe stream in Stream View Screen.
13. In DIVA screen check that the Drop Drive Frequency is set for 20.0 kHz in the Drop Breakoff Window. Check that Drop Drive is ON, and Test Sort is OFF. Adjust Drop Drive Amplitude and Phase to bring the Last Connected Drop, Gap, and Drop 1 position to the marks set on the Stream View Screen.
14. Using the Stream View Screen and the Sample Differential on fluidics panel, set Differential Pressure to approximately 1.5 PSI.
15. Set Fluidics Control to STANDBY.
16. Switch Buffalo Filter unit ON.

Lasers Alignment and Optimization:

1. In DIVA, open CST experiment (Cytometer Setup & Tracking). Make a new sample tube and begin Acquisition. Place tube of CST beads on SIP, turn fluidics to RUN and wait for events to show up on Acquisition Dashboard. Adjust Sample Differential until your Events Per Second is approximately 1,000.
2. In Instrument Panel, click on Laser tab, set Window Extension to 0.00
3. Watching the FSC-H vs FITC-H plot in the Global Worksheet, adjust the Y knob of the Stream Positioner until you have peaked the signal.

4. Make small adjustments to the Beam Focus to peak all signals.
5. Using the FITC-H vs PE-Cy5-H plot, close the 488nm laser iris. If the brightest group of beads in the plot changes by more than 100 channels, adjust the X-positioner, Y-positioner, and Fluorescence Channel positioner until the bright group is optimized. Open iris full open. The 488nm channels are now aligned.
6. Open the Red Laser (644nm) access door, using the FITC-H vs APC-Cy7-H plot make small adjustments to the back most X and Y translator controls to optimize the APC-Cy7 signal. The Red 644nm laser is now aligned. Close access door.
7. Open the Violet 405nm laser access door, using the FITC-H vs QDot530-H plot, make small adjustments to the back most X and Y translator controls to optimize the QDot530 signal. The Violet 405nm laser is now aligned. Close access door.
8. In Instrument Panel, click on Laser tab, set Window Extension to 8.00
9. Close CST experiment and open the CRBC (Chicken Red Blood Cell) experiment. We will use this to adjust the Forward Scatter Obscuration Bar. Place sample of CRBC's on SIP and begin acquisition. Adjust Sample Differential until you have an event rate of approximately 3,000/second.
10. Adjust the Obscuration Bar Adjuster Knob until there is minimum separation between the small and large CRBC populations on the FSC histogram.
11. Set up a sort-layout to sort the small population from the large population and begin sort.
12. Observe the side streams and make any adjustments necessary to the Droplet Amplitude to fine tune the Last Connected Drop, Gap, and Drop1 to make side streams as tight as possible.

OPTIONAL – Test Sort:

1. Open CST Experiment and begin acquisition. Draw sort gates around the Bright population and Mid-Level population using FITC histogram.
2. Set up a sort-layout using Device = 2-Tube, Precision = Purity. Place 2-tube holder in collection drawer and steer side streams into position using Streams Tab and test sort.
3. Place collection tubes into tube holder and sort 10,000 beads into each collection tube.
4. Reanalyze the sorted populations. They should be at least 90% pure. If not, a new sort delay will have to be performed.

The FACSVantage is now prepared for your experiment.

Shutdown Procedure:

1. Turn Deflection Plates to OFF.
2. Turn Drop Drive to OFF.
3. Prepare a tube $\frac{3}{4}$ full of FACS-Clean (Bleach) solution. Place it on SIP and set fluidics to RUN, adjust Sample Differential to 2.0 PSI.
4. Run until half empty then set fluidics control to STANDBY.
5. Prepare a tube $\frac{3}{4}$ full of DeIonized Water. Place it on SIP and set fluidics to RUN, adjust Sample Differential to 2.0 PSI.
6. Run until half empty then set fluidics control to STANDBY

7. Turn fluidics control to OFF.
8. Disconnect air Pressure line from Sheath Tank and connect to Ethanol Tank.
Disconnect Sheath Fluid supply line from Sheath Tank and connect to Ethanol Tank.
9. Set Fluidics Control Knob to RUN.
10. Allow Ethanol to run through system for 5 minutes.
11. Rotate Fluidics Control Knob to OFF.
12. Leave tube of DIW on the SIP.
13. Switch Main Power Off
14. Log-off computer, shut computer off.
15. Switch computer power to OFF.
16. Turn Buffalo Filter Unit to OFF.