FACSAria Fusion Cell Sorter
Startup, QC, Cleaning & Shutdown SOP

Startup Procedure

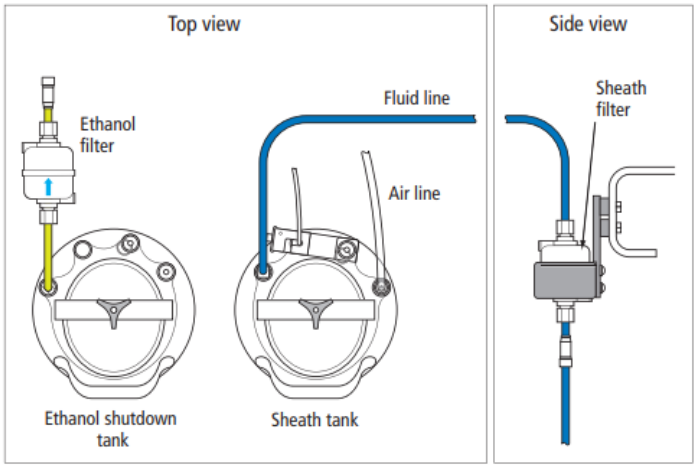
Pre-Startup Inspection

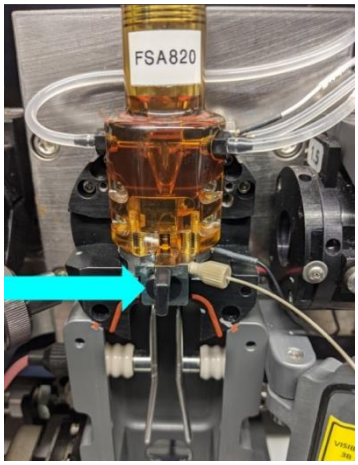
1. If you need to maintain the sorted cells at refrigerated temperatures (+4°C) turn on the waterbath *(Note: it will take about 20 minutes to cool the collection tube/plate holder)*.
2. Inspect the fluidics cart:
    a. Check that the Sheath Tank is filled to the mark (refill if needed);
    b. Check that the Ethanol Tank is filled to the mark (refill if needed);
    c. Check that the Waste Tank is empty (empty if needed);
    d. Connect the Sheath line to the bottom of the Sheath filter and the Air line to the top of the Sheath Tank.
3. Start up the biosafety cabinet by pulling up the sash to the mark, and switching on the light and fan.
4. Turn of the air pressure by turning the blue valve of the air pressure gauge. Set the air pressure to 100 psi.

Daily Startup

1. Swith ON the sorter at the main power button and check that the laser power buttons are ON.
2. After a few seconds, turn on the PC and log into Windows using the public credentials (user: BDOperator).
3. After 20-30 seconds, launch BD FACS Diva and log in with your personal account. Wait for DIVA to connect with the sorter.
    a. If the connection fails, quit DIVA, turn off sorter and PC, and repeat from step 1.
4. On the sorter open:
    a. Flow Cell Access Door
    b. Sample Collection Chamber Door
    c. Sort Block Door
5. Perform a **Fluidics Startup** (**Cytometer > Fluidics Startup**), following the wizard:
    a. Confirm that Sheath and Air lines are correctly connected to the Sheath Tank and NOT to the Ethanol Tank -> select **DONE**
    b. Confirm that the closed-loop nozzle is installed in the flow cell -> select **DONE**
    c. When the Sheath tank gets pressurized, tighten the lid of the tank.
    d. When prompted to remove the closed-loop nozzle and insert the nozzle, select **DONE** for both **WITHOUT PERFORMING THESE STEPS** (further cleaning needed).

Figure 4-3 Air and fluid lines connected to the sheath tank





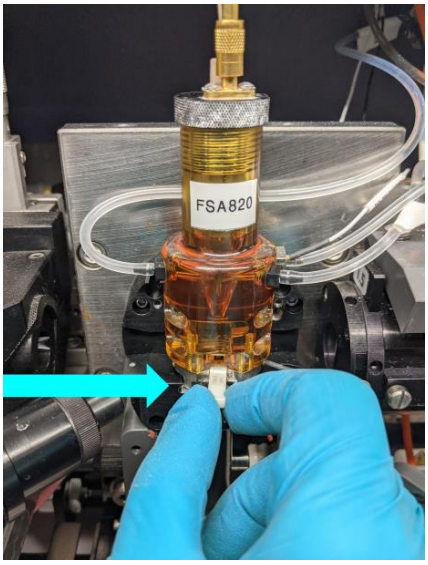
6. Cleaning of the flow cell:
    a. Prepare 2 **fresh** FACS tubes containing each 4 mL of 30% Contrad and MQ water, respectively. *(These tubes must be prepared fresh every day)*
    b. Insert the 30% Contrad tube in the tube holder in the sample chamber and select **Cytometer > Cleaning Modes > Clean Flow Cell** to perform cleaning of the flow cell;
    c. Inspect visually the sample chamber to check that the detergent is aspirated by the sample probe. If no fluid is aspirated, refer to the **Appendix - Troubleshooting A** for troubleshooting.
    d. Perform the Clean Flow Cell procedure for a total of 4 times with 30% Contrad and 4 times with MQ water.
7. Cleaning of the deflection plates and sort block:
    a. Remove the deflection plates using the appropriate tool and wipe them clean with KimTech wipes imbibed with MQ water, and 70% ethanol. Re-install the deflection plates.
    b. Wipe clean the sort block using KimTech wipes imbibed with MQ water, and 70% ethanol.

Selecting the instrument configuration and starting the stream

1. Check the instrument configuration installed by inspecting the **Breakoff Window** (see **Appendix**).
    a. To change configuration, select **Cytometer > View Configuration**. The CS&T module is launched.
    b. Select the instrument configuration you want to use and choose "**Set Configuration**". Confirm by selecting "**OK**" in the dialogue pop-up. Exit the CS&T module by selecting OK at the bottom right of the window.

c. After DIVA reconnects, when prompted choose "**Use CS&T settings**" to complete the configuration change. The new configuration in use is indicated in the Breakoff Window,

2. Remove the closed-loop nozzle from the flow cell and insert the nozzle.
3. Select the Stream button in the Breakoff Window to start the stream.
4. Check that the stream flows exactly at the centre of the waste drawer.
    a. If not, see **Appendix – Troubleshooting B** for how to adjust the sort block.
5. Rinse all 3 compartments of the waste drawer and check that they are draining.
    a. If they´re not, contact BFC staff.
6. Close the Sort Block Door and the Flow Cell Access Door.



QC check

1. The QC check is performed using **CS&T beads**.
    a. Check the Facility fridge to see if there is a FACS tube with resuspended CS&T beads (resuspended beads can be used for up to 5 days).
    b. If no tube with resuspended beads is available, prepare a new tube by adding 350 µL FACSFlow or PBS + 1 drop of vortexed CS&T beads to a FACS tube. Write the date of preparation on the tube.
2. Load the CS&T tube on the sample loader.
3. On DIVA, select **Cytometer > CST** and enter the CS&T module:
    a. Insert the current bead lot number
    b. Select **Run** and confirm to start the QC check.
    c. *On Fusion Sansa never enlarge/maximise the CS&T module window to full screen, as that can result in QC failure.*
4. Assess the QC results:
    a. **CS&T passed with no warnings -> instrument performance is OK**
    b. **CS&T passed with warnings -> instrument performance is acceptable BUT you must inform BFC Staff of the warnings**
    c. **CS&T failed -> instrument performance not acceptable. If CS&T beads is 4-5 days old, prepare a fresh tube and repeat from step 2. If QC keeps failing, contact BFC Staff.**
5. Unload the CS&T tube, exit the CS&T module and wait for DIVA to reconnect, accepting the CS&T settings.

Stabilising the stream

1. Perform a sample line backflush (**Cytometer > Cleaning Modes > Sample Line Backflush**) to remove residual CS&T beads from the sample line.

2. Open a Cleaning Experiment (**Experiment > New Experiment > General tab > Daily Cleaning**) and load a tube with 4 mL of MQ water. Run and record at flow rate = 11.0 for 20 min (1200 sec) to stabilize the stream.
3. After 20 min, unload the tube of MQ water and adjust the amplitude value in the Breakoff Window to achieve optimal Gap and Drop1 values. Consult the **Appendix** for guidance on optimal values for both sorters.
4. Select the **Sweet Spot** button to activate the automatic adjustment of the drop drive amplitude.

Determination of the Drop Delay

1. The drop delay determination is done using **Accudrop Beads**.
    a. Check the Facility fridge to see if there is a FACS tube with resuspended Accudrop Beads (resuspended beads are stable up to a month).
    b. If no tube with resuspended beads is available, prepare a new tube by adding 2.5 mL of FACSFlow or PBS and 3 drops of vortexed Accudrop beads.
2. On DIVA open an Accudrop Drop Delay experiment (**Experiment > New Experiment > General tab > Accudrop Drop Delay**). Expand Specimen_001 and Tube_001 and open the Sort Layout.
3. Load the tube with Accudrop beads and wait for the event to be displayed on the histogram.
4. In the **Side Stream Window** (see **Appendix**) turn on the **Voltage** on the deflection plates (voltage indicator becomes red).
5. Use the micrometer dial of the sorter to obtain the brightest bead spot on the central stream.
6. In the Side Stream Window apply the **Optical Filter** to detect the Accudrop Beads.
7. In the Sort Layout press Sort to start sorting Accudrop beads. Select **Cancel** in the Confirm dialog (*we don't collect sorted accudrop beads, so we leave the waste drawer closed*).
8. In the Side Stream Window adjust the left voltage slider so that the deflected beads are at the center of the squared box of the left stream.
9. In the Acquisition Dashboard, adjust the flow rate so that you achieve the following threshold rate:
    a. 70 µm nozzle: 1000 – 3000 events/s
    b. 85 µm nozzle: 800 – 2000 events/s
    c. 100 µm nozzle: 600 – 1500 events/s
10. In the Side Stream Window, select **Auto Delay** and then **Start Run**. Monitor the run until the process is completed.
11. Check that you obtain a % of deflected Accudrop beads equal or higher than 95%. If that is not achieved, repeat the Auto Drop Delay run.
12. Exit the Auto Drop Delay window, stop the sort and acquisition and unload the beads.
13. Perform a sample line backflush (**Cytometer > Cleaning Modes > Sample Line Backflush**) to remove residual beads in the sample line.

Note: do not change the Precision mode in the Sort Layout of the Accudrop Exp, because that will corrupt the template and lead to incorrect determinations of the drop delay.

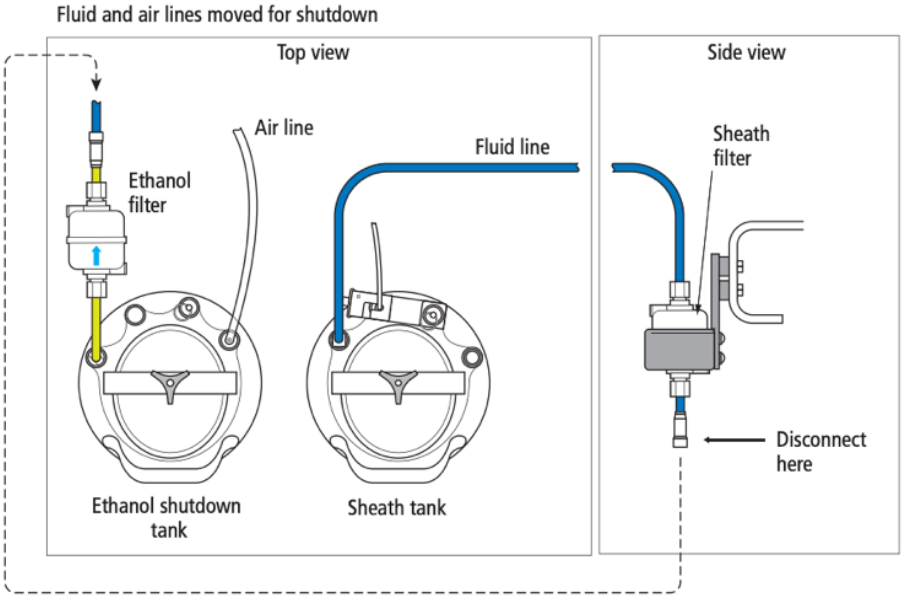
Cleaning Procedure after every sort

1. Open the cleaning experiment and load a tube of 4 mL of 30% Contrad. Set the flow rate to 11.0 and the stopping condition to 1200 s (20 minutes). When the acquisition starts, select **Record Data** to record the cleaning.
2. After the contrad cleaning, load a tube with 4 mL of MQ water. Set the flow rate to 11.0 and the stopping condition to 600 s (10 minutes). When the acquisition starts, select **Record Data** to record the cleaning.
3. Unload the tube.
    a. If you are the last user of the day, start the Shutdown Procedure.
    b. If another user is booked after you, log out from DIVA software (**File > Log Out**).

Shutdown Procedure

1. Press the Stream button in the Breakoff window to stop the stream.
2. Remove the nozzle from the flow cell and insert the closed loop nozzle.
3. Put the used nozzle in a new clean FACS tube with about 1 mL of MQ water. Transfer in the rack in the waterbath sonicator and sonicate for 2 minutes. Retrieve the nozzle, gently pat dry without touching the O ring and store in the nozzle box (Fusion Jon) or in the nozzle rack (Fusion Sansa).
4. Cleaning of the flow cell:
    a. Prepare 2 FACS tubes containing each 4 mL of 30% Contrad and MQ water, respectively.
    b. Insert the 30% Contrad tube in the tube holder in the sample chamber and select **Cytometer > Cleaning Modes > Clean Flow Cell** to perform cleaning of the flow cell;
    c. Perform the Clean Flow Cell procedure for a total of 4 times with 30% Contrad and 4 times with MQ water.
5. Cleaning of the deflection plates and sort block:
    a. Remove the deflection plates using the appropriate tool and wipe them clean with KimTech wipes imbibed with MQ water, and 70% ethanol. Re-install the deflection plates.
    b. Wipe clean the sort block using KimTech wipes imbibed with MQ water, and 70% ethanol.
6. Prepare for the Fluidics Shutdown:
    a. Disconnect the Sheath Line after the Sheath filter and connect the 2nd part of Sheath Line to the bottom of Ethanol Filter.
    b. Disconnect the Air Line from the Sheath Tank and connect the Air Line to the Ethanol Tank.
    c. Prepare a tube with 2 mL of MQ water and place it in the tube holder.
7. Perform the **Fluidics Shutdown** (**Cytometer > Fluidics Shutdown**), following the wizard and selecting Done at every task performed.
8. Once the Fluidics Shutdown is complete, exit the wizard, quit DIVA, shutdown the PC the sorter and the biosafety hood, and turn off the air pressure at the pressure outlet.
9. Disconnect the Sheath Line and Air Line from the Ethanol Tank.
10. De-pressurize and re-fill the Sheath Tank with BD FACSFlow Sheath Fluid up to the internal mark.

11. De-pressurize and re-fill the Ethanol Tank with 70% Ethanol up to the internal mark.
12. Disconnect the Waste Tank and empty the waste in one of the cubitainer boxes for the sorter waste available in the lab red trolley. Connect back the Waste Tank to the fluidics cart.

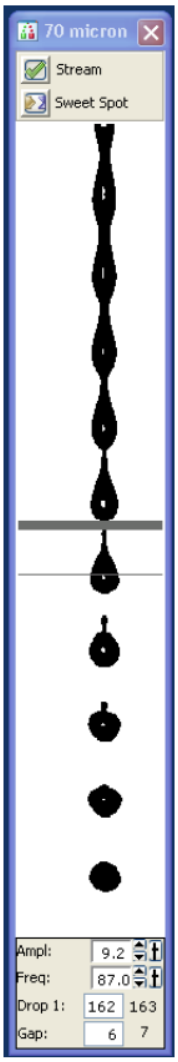


APPENDIX

Breakoff Window

Table 2-1 Breakoff window controls

Control	Description
Stream button  On Off	Turns the stream on and off.
Sweet Spot button  On Off	Enables automatic adjustment of the drop drive amplitude to maintain the stability of the breakoff point. When the Sweet Spot is on, the Amplitude and Frequency fields are disabled. The amplitude is automatically adjusted by the software. To enable the fields, turn off the Sweet Spot.
Amplitude field	Adjusts the amplitude or intensity of the drop drive, from 1.0–80.0 volts. The drop drive amplitude determines the breakoff point. A higher amplitude value results in a shorter stream breakoff. A lower amplitude results in a longer stream breakoff. Typically, the amplitude is set once, at the beginning of a sorting experiment, and then maintained via the Sweet Spot.
Frequency field	Determines the number of drops formed per second and the size of the drops. (Drop size is also influenced by the nozzle size.) The drop drive frequency can be adjusted from 1.0–102.0 kHz. The higher the frequency, the more drops are generated per second and the smaller the drops. The lower the frequency, the fewer drops generated per second and the larger the drops.



Side Stream Window

Figure 2-15 Side Stream window

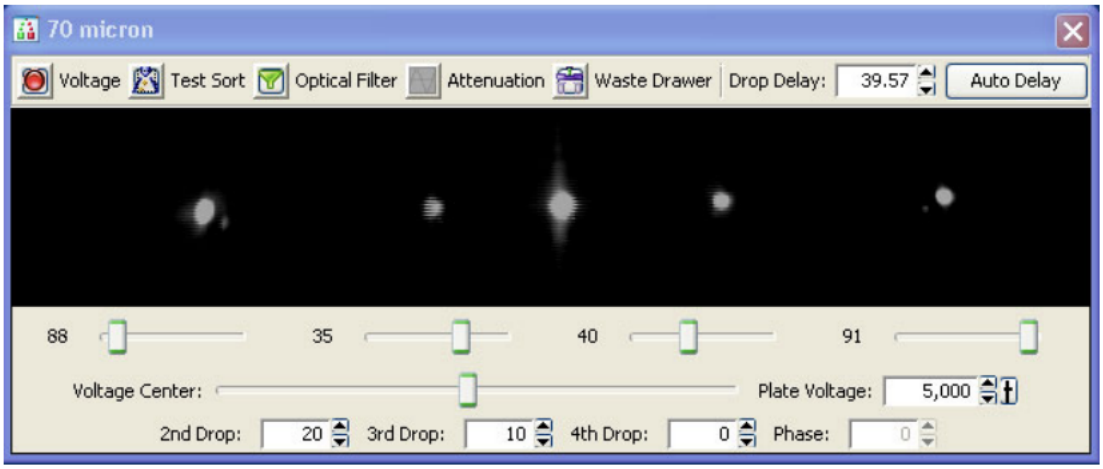


Table 2-2 Side Stream window controls

Control	Description
Voltage button  On Off	Turns the plate voltage on and off.
Test Sort button  On Off	Generates test side streams based on test sort pulses.
Optical Filter button  In Out	Controls the position of the optical filter in front of the lower (Accudrop) camera.
Attenuation button  Off On	Decreases the amplitude of the drop drive. At lower pressures, you may need to turn on attenuation to dampen the amplitude.
Waste Drawer button  Closed Open	Opens or closes the aspirator drawer depending on its current state. The default state is closed. For more information, see Aspirator Drawer on page 32.
Drop Delay field	Sets the amount of time between when an event is measured and the breakoff point, from 10–140 drops. The drop delay value determines which drop will be deflected. The drop delay value is set experimentally using BD FACS Accudrop technology.
Auto Delay	Opens the Auto Drop Delay dialog.
Voltage sliders (far left, left, right, far right)	Set the percentage of charge to be applied to the corresponding stream (as a percentage of maximum).

Recommended values for Stream Setup

Table 3-2 Typical sort setup values

Setting	70 micron	85 micron	100 micron	130 micron
Sheath Pressure	70	45	20	AVAILABLE ONLY FOR ASSISTED USE
Amplitude	60	32	12	
Frequency	87	47	30	
Drop 1	150	150	150	
Gap (upper limit)	6 (14)	7 (17)	10 (21)	
Attenuation	Off	Off	Off	
Drop Delay	47.00	30.00	27.00	
Far left voltage	100	100	80	
Left voltage	40	35	30	
Right voltage	40	35	30	
Far right voltage	100	100	80	
Plate voltage	4,500	4,000	2,500	
2nd Drop	20	20	10	
3rd Drop	10	10	5	
4th Drop	0	0	0	
Laser Delay (blue)	0.00	0.00	0.00	

Amplitude: may vary day-to-day, in our sorters is generally always below 50.0.

Frequency: always use the BD setup value.

Drop1: set as close as possible to 150. As a general rule, never set it below 140, nor above 220.

Gap: use the following values:

- 70 µm: 6-7
- 85 µm: 8-9
- 100 µm: 10-11

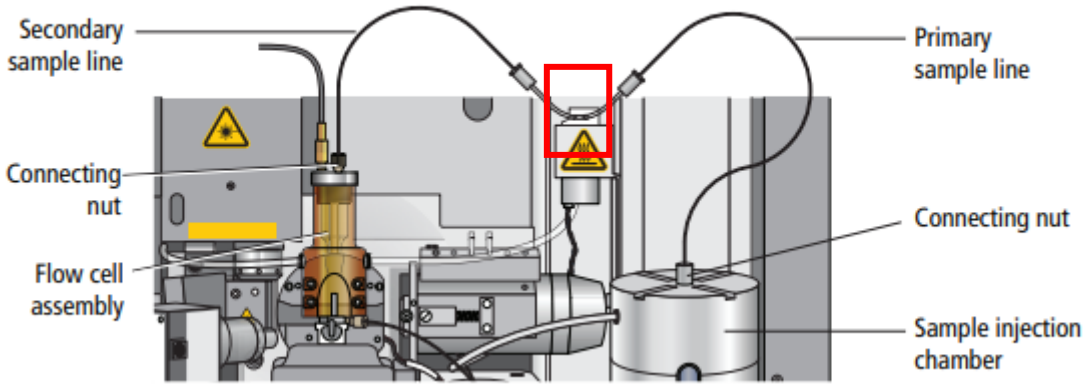
Higher gap values will result in unstable side stream(s) and sorting failures. **If unsure about the stream setup, always contact the Facility Staff.**

Troubleshooting

A - Sample Line not aspirating any volume from the tube (usually noticed at the Clean Flow Cell Procedure).

1. Take out the silicone tubing of the sample line from the pinch valve (see picture).
2. Gently massage and stretch with your fingers the silicone tubing.
3. Reinsert the silicone tubing in the bubble detector and the pinch valve.
4. Check that the sample line is inserted correctly: if you see the sample line leaking in the sample chamber, it means the silicone tubing is not inserted correctly in the bubble detector/pinch valve.
5. Repeat the Clean Flow Cell procedure to check that the issue is solved.
6. **If the issue persists, contact the Facility Staff.**

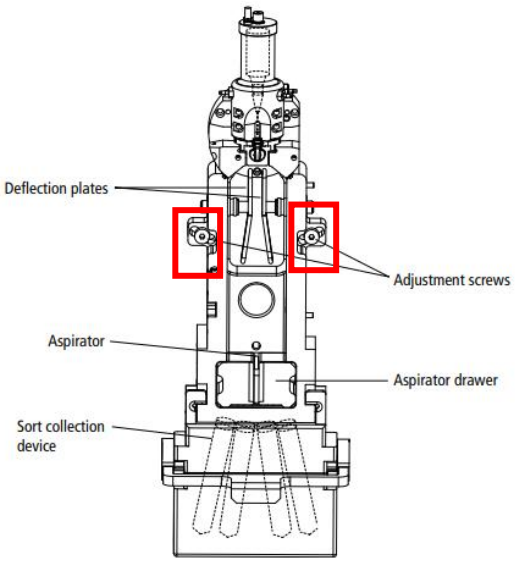
Figure 6-11 Primary and secondary sample lines



B - Stream not centered in the waste drawer – Adjusting the sort block

1. Prepare the hex key. (*Note: Fusion Sansa and Fusion Jon requires different hex key)*
2. Start the stream.
3. Place one hand underneath the sort block, to offer support.
4. With the other hand, loosen the two adjustment screws at both sides of the sort block (see picture) and adjust the position of the sort block so that the stream falls exactly at the center of the waste drawer.
5. Re-tighten the adjustment screws to fix the position of the sort block.

Figure 1-11 Sort block with door open



C - Voltage slider not working / Test Sort button not working.

1. Stop the stream.
2. Log out from DIVA.
3. Re-Launch DIVA and re-start the Stream.

D - No event visible when sample is loaded. Sample contains cells at 5 M cells/ml concentration.

1. Unload the sample, vortex and re-load.
2. Increase the flow rate: do you start seeing events at higher flow rates?
    a. Unload sample and apply lubricant around the base of the tube holder.
    b. Inform Facility Staff.