Imaging and Flow Cytometry Core

BD FACSDiscover S8 Cell Sorter Startup and Shutdown Standard Operation Protocol

(For experienced user only)

Startup procedure

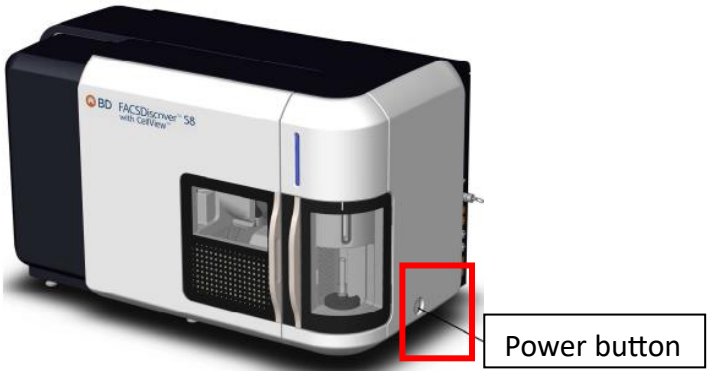
1. Press the Power button on the PC workstation.

2. Log into Microsoft Window OS on the workstation.

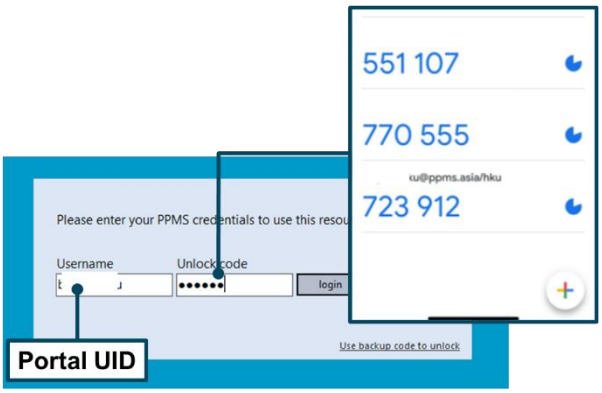
- Account: User
- Please find the password posted under the bottom of the computer screen

3. Turn on the compressor.

4. Press the Power on/off button on the side of the instrument.



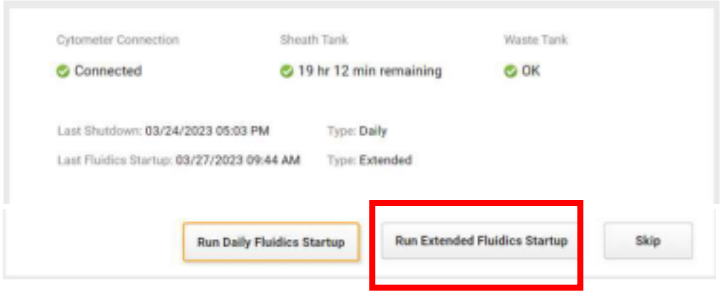
5. log in Tracker



6. From the desktop, open the BD FACSChorus software and log in your account. The software connects with the sorter within 5 minutes. Ater the connection is established successfully; the **Connected** status turns green.

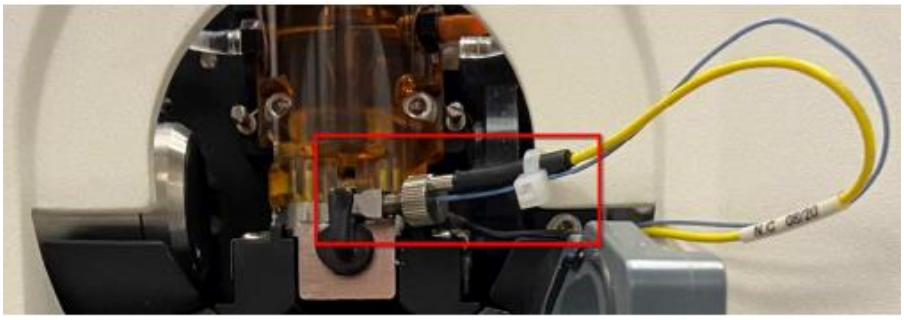
7. Fluidics startup

Click **Run Extended Fluidics Startup**



7-1 Perform step 1 (in the dialog) by clicking **Start** to initiate extended fluidics startup.

7-2 Perform step 2 (in the dialog) by inserting the closed-loop nozzle with the O-ring facing up. Then click **Continue**.

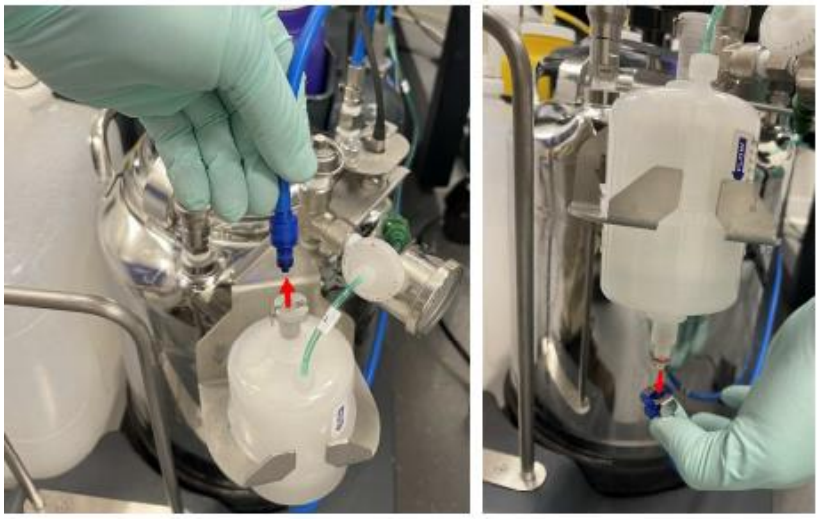


7-3 Perform step 3 (in the dialog) to prepare the tanks and tubing connections for the extended cleaning cycle by performing the following steps:

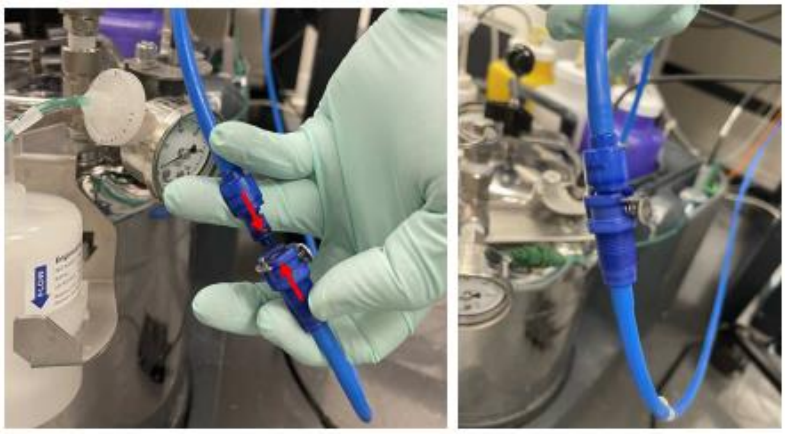
a. Fill the DI water cleaning fluid bottle with 1.2 L of **sterile DI** water.

b. Perform the following steps to bypass the sheath filter:

- Disconnect the sheath line connectors (top and bottom) from the sheath filter.

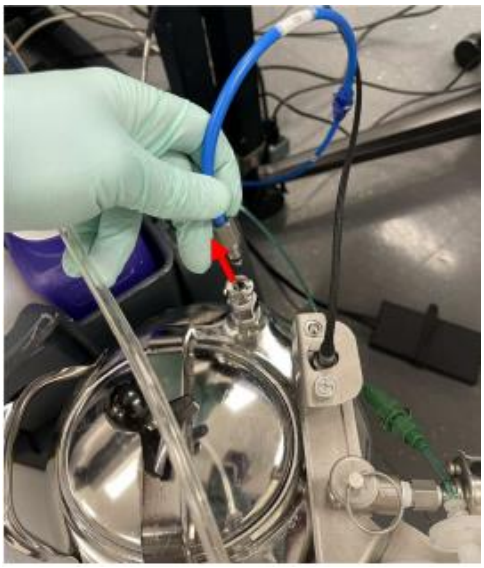


- Connect the sheath line connectors together.



c. Perform the following steps to disconnect the sheath line from sheath tank and connect it to the cleaning port on the fluid storage cart.

- Disconnect the sheath line from the sheath tank.



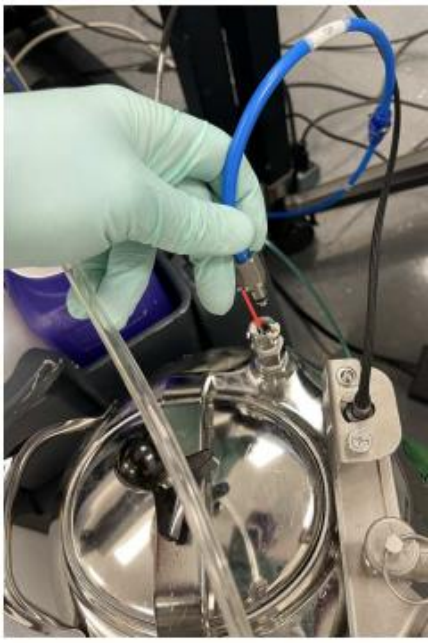
- Connect the sheath line to the cleaning port on the fluid storage cart.

d. Empty the waste tank. Add 200 mL bleach to the waste tank if needed.

e. In the step 3 of the dialog, click **Continue**.

8. Perform step 4 (in the dialog) to prepare the tanks and tubing connections for operation by performing by the following steps:
   a. Fill the sheath tank with PBS.
   b. Disconnect the sheath line from the cleaning port.

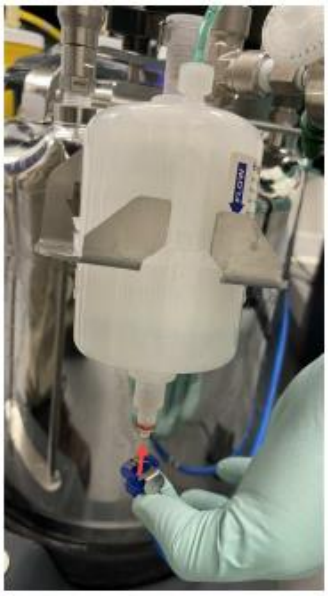
c. Reconnect the sheath line to the sheath tank.



d. Reinstall the sheath filter into the sheath line by performing the following steps:

- Disconnect the sheath line connectors

- Reconnect the sheath line connectors to the top and bottom connector of the sheath filter.



e. In the step 4 of the dialog, click **Continue**.

9. Perform step 5 (in the dialog) by clicking **Continue** to purge the sheath filter and to prime the fluidics.

10. Click **Close** in the Extended Fluidics Startup dialog.

11. Flow Cell Clean

a. Follow the prompts to complete the tasks on the screen.
b. Use **DI water or 1.5% BD Detergent Solution Concentrate** to clean the flow cell.
c. Click **Continue** to proceed to the next step.

12. Insert sort nozzle

a. Remove the closed-loop nozzle.
b. Insert the sort nozzle.
c. Click **Continue**.
d. (Optional) To see the development of the stream, click the **Stream** status indicator in the Navigation bar.

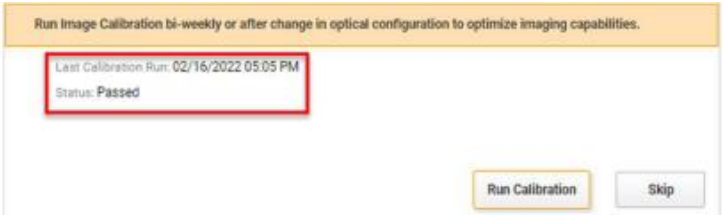
13. Setup and QC

a. Select a bead lot file by expanding the dropdown on the right and selecting the bead lot number.

b. Load a tube with BD FACSDiscover Setup Beads (vortex the stock tube before adding 2 drops into 0.5 mL PBS, vortex the ready-for-use beads, and label “S8 setup” and date on the tube). Close the sample input door. Make sure that the sort collection chamber door is closed as well.
c. Select **Daily** performance and click **Run**.
The system unloads the tube after the steps are successfully completed.
d. Click **Continue** to proceed to the next step.

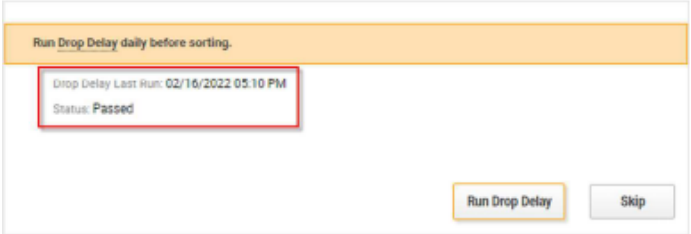
14. Image calibration

BD CellView Calibration Beads are used to optimize the imaging capability of the BD S8 cell sorter. It is recommended to run Image Calibration every 2 weeks.



Click **Run Calibration** and load the beads (vortex the stock tube before adding 2 drops into 0.5 mL PBS, vortex the ready-for-use beads, and label “S8 CellView” and date on the tube) if needed.

15. Drop delay



- If sorting is needed, click **Run Drop Delay** and load a tube with Accudrop Beads (vortex the stock tube before adding one drop into 0.5 mL PBS, vortex the ready-for-use beads, and label “S8 Accudrop” and date on the tube) and click **Continue**.

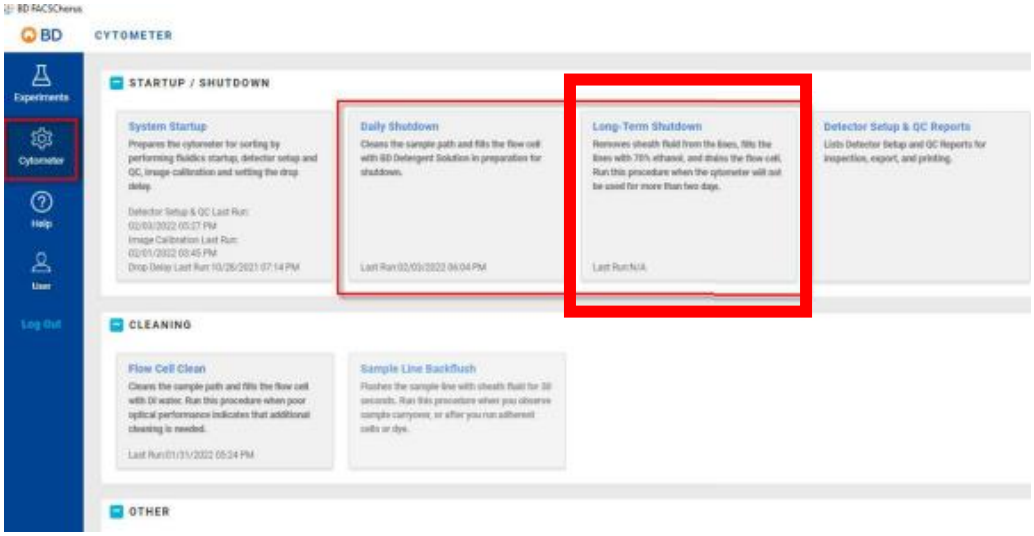
- When drop delay is successfully completed, a completion dialog is displayed.

- Click **Continue** to proceed to the Experiment page.

16. Turn on the BSC and AMS if needed.

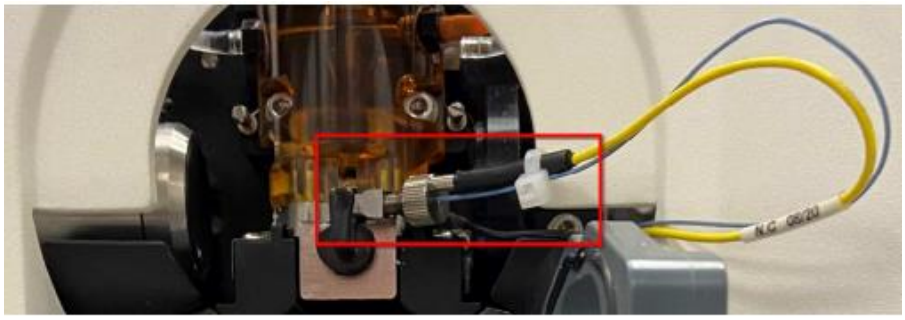
Shutdown procedure

1. Go to the **Cytometer** page, and select **Long-Term Shutdown**, the Long-Term Shutdown dialog displays.



2. Perform step 1 (in the dialog) by clicking **Start** to initiate the procedure.
   Note: if the stream is on, after clicking Start the system takes about 10 seconds to stop the stream. Do not proceed to the next step until the software prompts you in the Long-term Shutdown dialog.

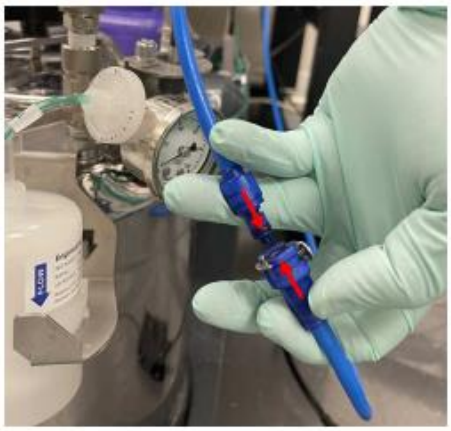
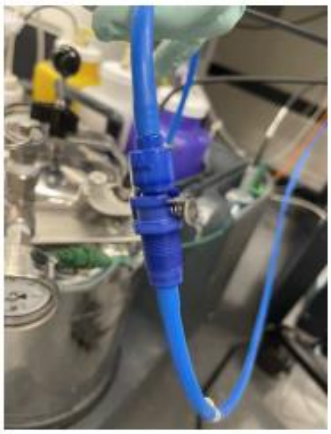
3. Perform step 2 (in the dialog) by inserting the closed-loop nozzle with the O-ring facing up. Then click **Continue**.



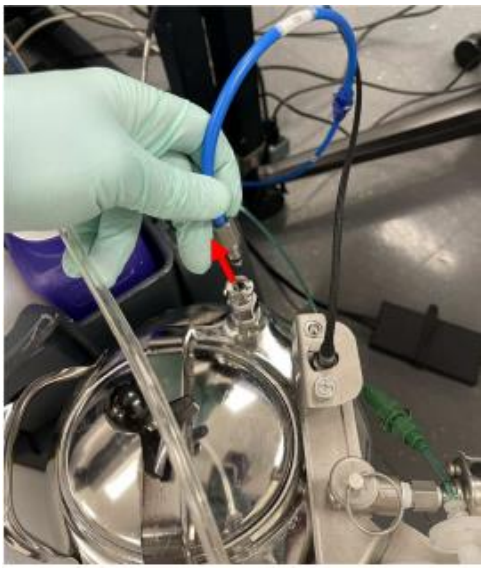
4. Perform step 3 (in the dialog) to prepare the bottles, tanks and tubing connections for the extended cleaning cycle by performing the following steps:

a. Fill the ethanol bottle with 1.1 L of 70% ethanol.
b. Perform the following steps to bypass the sheath filter:
   - Disconnect the sheath line connectors (top and bottom) from the sheath filter.


- Connect the sheath line connectors together.

c. Perform the following steps to disconnect the sheath line from sheath tank and connect it to the cleaning port on the fluid storage cart.
   - Disconnect the sheath line from the sheath tank.



   - Connect the sheath line to the cleaning port on the fluid storage cart.

d. In the step 3 of the dialog, click **Continue**.

5. Perform step 4 (in the dialog) by loading a clean tube with 3 mL of DI water and then click **Continue**. When the step is successfully completed, a confirmation message displays.

6. In the Long-term Shutdown dialog, click Close.

7. Click Log out in the Navigation bar to log out of BD FACSChorus software.

8. Power off the sorter.

9. Tun off the PC workstation.

10. Refill the sheath tank with PBS.

11. Empty the waste tank. Add 200 mL bleach to the waste tank.

12. Turn off the BSC and/or AMS.