Canto II Standard Operation Protocol
Machine Startup and Shutdown Procedures (For Experienced users only)

**Machine Start up:**

- Refill the sheath tank and empty waste tank if necessary, make sure all the tubing are connected properly.
- Check the quantity of Shutdown Solution, replace with a new bottle or top up with the remaining shutdown solution located on the trolley if necessary.
- Turn on the computer power and log in to Administrator window (Password: BDIS).
- Double click the Cytometer Status and make sure a white log sheet appears on the monitor screen.

![Cytometer Status](image)

- Turn on the Power button at the left side of Canto II.
- When “Boot up completed” appears at the cytometer status log sheet, double click the BD FACS Diva Software → login to “0Cleaning” account, no password required.

![BD FACS Diva Software](image)

- Wait for about 1 minute → Go to “Cytometer” → “Fluidics Startup” → click “OK” to initiate the startup process.
- When the Fluidics startup completed → release bubbles from the filters.

![Fluidics Startup](image)

- Go to “Cytometer” → “CST”.
- Get the well-prepared CST beads from the fridge (prepare a new batch if needed: vortex the CST tube with red cap vigorously followed by adding 1 drop of the beads into 350ul PBS→ mix well before loading). The CST tube must be labeled with CST, lot number and date.
- Once entering CST mode, BD FACS Diva software will be disconnected automatically.
- Double check with the CST lot number and uncheck the “Load tube manually”.
- Click “Run” to initiate the CST test. When the CST test is completed → exit from the CST mode so that BD FACS Diva software can be connected.
- Log out from the 0Cleaning account and log in to your own account for sample acquisition.
- If some parameters could not pass CST test, cleaning flow cell is needed. To clean flow cell,
  o  Tube holder must be pushed to the left (see Figure 1).
  o  Load solution 2 (Rinse solution) onto the sample injection tube.
  o  Go to “Cytometer” → “cleaning modes” → “Clean Flow Cell” → Click “OK”.
  o  Repeat the cleaning process for around 5-6 times.
  o  Place the tube holder back to its original position (Figure 2), followed by running CST test again.

- Machine Shutdown:
  - After performing the 3-step cleaning process with Cleaning Solution 1,2 and 3 for 5 minutes each, go to “Cytometer” → “Fluidics Shutdown” → click “OK”
  - Quit the BD FACS Diva Software when fluidics shutdown is completed, followed by shutting down the computer → Turn off the Power button of Canto II.
  - Refill sheath fluid → Empty the waste tank right away if the waste is left than half a tank. Add in some bleach if the waste level is over half a tank.
  - Add in some bleach and make sure its volume covers the bottom layer of the tank.
  - Remove the sponge and dry it beside the sink.
  - Turn off the lights and lock all the doors before leaving.