



## Imaging and Flow Cytometry Core

### BD LSR Fortessa 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 tubings are connected properly.
- Turn on the computer and log in to “**User**” account  
(Please find the password posted under the bottom of the computer screen).
- **Wait until Tracker is initiated, login onto Tracker.**
- Double click the “Tera Term” icon on the computer desktop and select “Serial” followed by clicking “OK”, make sure a black log sheet appears on the monitor screen.
- Turn on the Power button at the upper right of Fortessa.
- Remove air bubbles from the sheath filter.
- Load a tube of rinse solution (Solution 2), adjust the “Sample Fine ADJ” on the control panel to the maximum 10<sup>th</sup> round followed by running the solution at HI speed for about 30 minutes to clean the flow cell.
- Once the **IP address** appears in the Tera Term log sheet, double click the BD FACS Diva Software → login to “OCleaning” account, no password required.
- The system is ready to use when it shows “Cytometer connected”.
- After 30 minutes of cleaning, adjust the “**Sample Fine ADJ**” to the 5<sup>th</sup> round, change the flow rate to “LOW” and set the machine at “Standby” mode.
- Go to “Cytometer” → “**CST**”.
- Get the well-prepared CST beads from the fridge (prepare a new batch if needed: vortex the CST tube 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 check the box “Load tube manually”.
- Click on the “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 OCleaning account and log in to your own account for sample acquisition.
- If some parameters could not pass CST test, cleaning flow cell for longer period is needed.
  - a) Prepare one tube of 2ml FACS Rinse
  - b) Run 10 min at “HI” flow rate
  - c) Prime 2 times with FACS Rinse
  - d) Run CST again



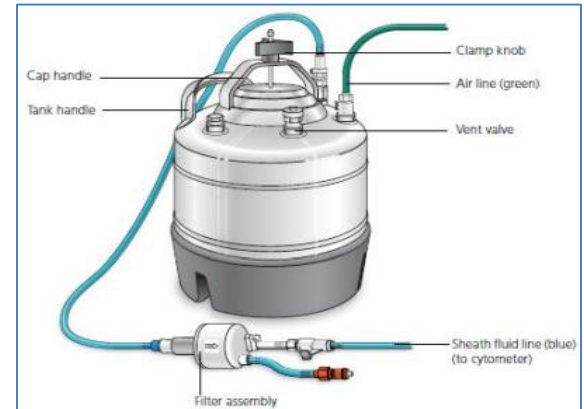
#### Machine Shutdown:

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- After running through the whole process of machine cleaning, turn off the POWER button at the upper right of Fortessa.
- Exit BD FACSDiva software and Tera Term, then shutdown the computer. (No need to log out Tracker)
- Refill the sheath and discard the waste.

### Refill Sheath Container

1. Make sure the flow cytometer is in STANDBY mode.
2. Disconnect the air line (green) and fluid line (Blue) from the sheath container.
3. Depressurize the sheath container by pulling up on the vent valve.
4. Remove the sheath container lid.
5. Unscrew the clamp knob and push down to loosen, if necessary.
6. Tilt the cap to the side to remove it from the tank.
7. Add sheath fluid (on the trolley) to the sheath container.
8. Fix the sheath lid and tighten the clamp knob to finger tight.
9. Reconnect the airline (green) and fluid line (Blue).
10. Please pay attention to fluid line (Blue) to make sure it is not twisted or stretched.
11. Remove air bubbles from the sheath filter.



### Empty Waste Tank

1. Make sure the flow cytometer is in STANDBY mode.
2. Disconnect the orange waste tubing and the black level sensor line from the waste container.
3. Take the waste tank to the sink.
4. Remove the lid from the waste tank.
5. Empty the waste container.
6. Add approximately 0.5L of bleach to the waste in which it could cover the bottom layer of the container and tighten the lid.



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7. Reconnect the orange waste tubing and level sensor line.

