BD FACSAria SORP Standard Operation Protocol

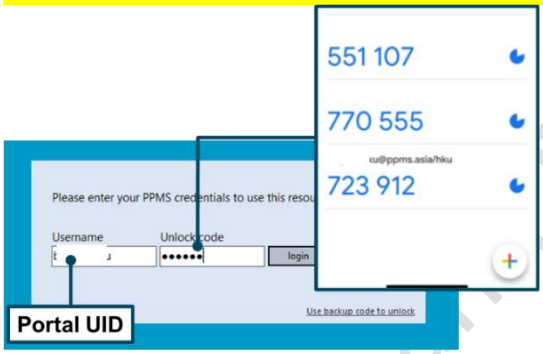
Instrument Startup and Shutdown Procedures (For Experienced users only)

Instrument Start up:

1. Refill the sheath tank and empty waste tank if necessary, make sure all the tubing is connected properly and insert the exhaustion drain tray into the cart.

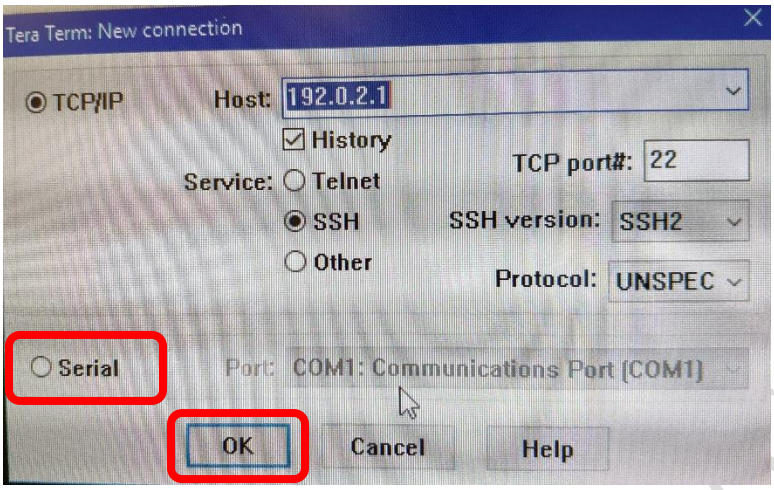
2. Turn on the computer.
   Account and password: **please refer to the label attached on the screen**

3. Ensure to start **Tracker** before Tera Term launch.



4. Double click **Tera Term** icon on the desktop to start the program.

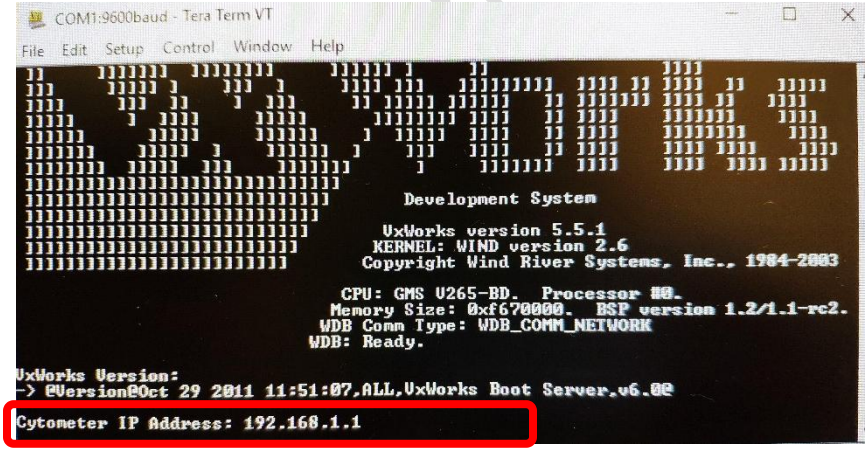
   Select “Serial” and click “OK”.



5. Turn on the power switch on the left side of the sorter body.

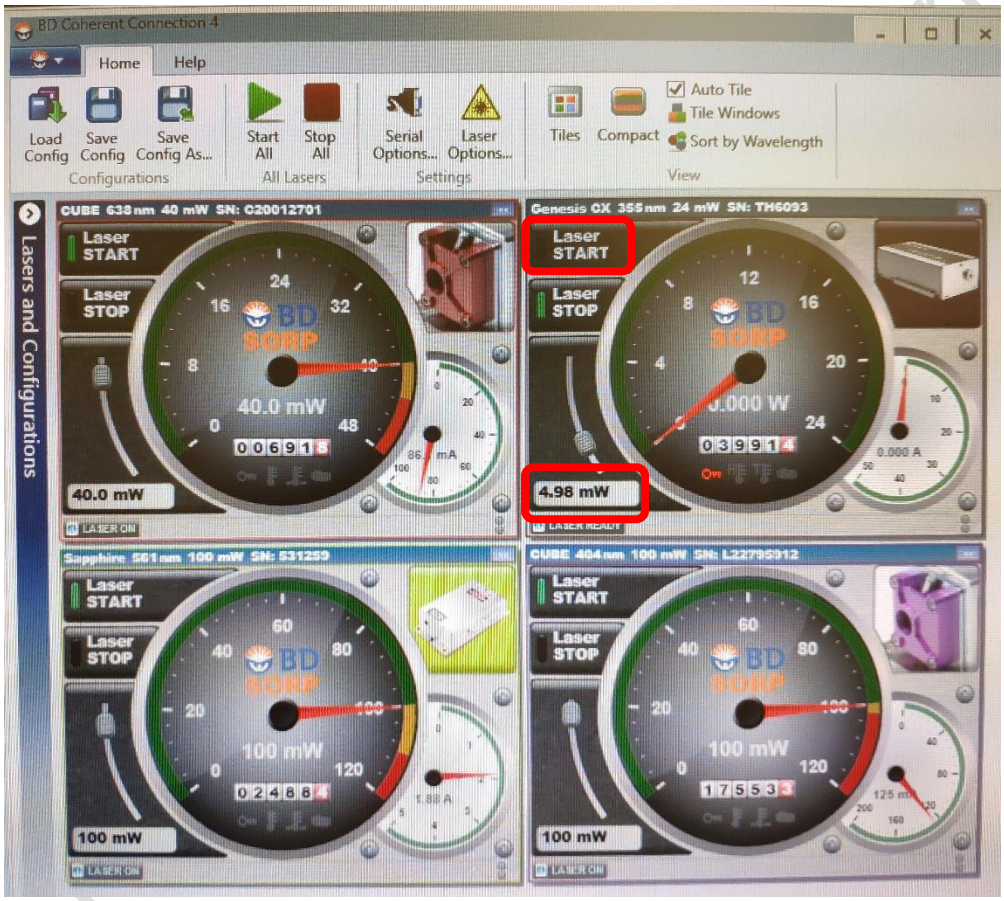
6. Turn on the power of chiller.

7. Ensure that **Cytometer IP address** (192.168.1.1) displays on the Cytometer Status window before proceed to the next step.



8. Double click **<u>BD Coherent Connection4</u>** icon on the desktop to start the program.

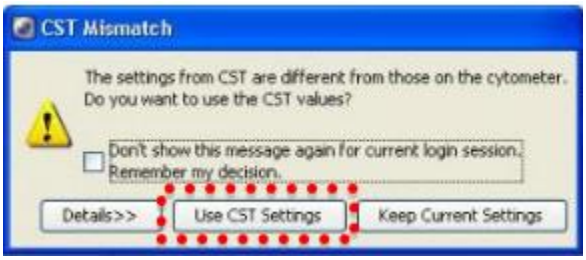
9. Ensure ALL the lasers have been turned on successfully except UV355 nm laser. Manually startup the UV355nm laser by click "**Laser START**", when it increases to 5 mW, adjust to **20 mW** and "**Enter**", then check whether it goes to nearly 20 mW.
   (Lasers need at least 30 minutes to warm up)



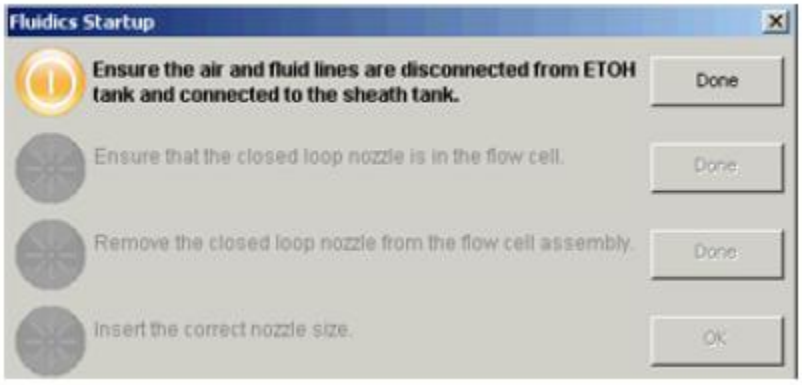
10. Double click **BD FACSDiva Software** icon on the desktop to start the program.

11. Log into FACSDiva software with **0cleaning** user name. No password is needed. Click ''OK'' to login.

12. Once the system has connected, click **Use CST Settings**.



13. Click **Cytometer > Fluidics Startup**. Follow the prompts on the screen to complete startup procedure.



---- Check the gas and fluid line is properly connected to the sheath tank. Click “Done”.
---- Check the closed loop nozzle is in the flow cell. Click “Done”. Fluidic Startup will begin.
---- When it goes to the third step “Remove the closed loop nozzle from the flow cell assembly”, click “Done”. (Please don’t remove the closed loop nozzle at this moment because we suggest you do step 14 to clean the flow cell twice.)
---- When it goes to the fourth step “Insert the correct nozzle size”, click “OK”. (Please don’t insert the correct nozzle at this moment because we suggest you do step 14 to clean the flow cell twice.)

14. Clean Flow Cell twice after Fluidics Startup before removing closed-loop nozzle.
---- Select **Cytometer** > **Cleaning Modes** > **Clean Flow Cell**.
---- When prompted, install a tube containing approximately 3 mL of solution 2 (BD Rinse), then click **OK**.
---- The cytometer loads the tube and fills the flow cell with the BD Rinse.
---- Click **OK** when the completion dialog appears.
---- Repeat the flow cell cleaning with BD Rinse buffer.

15. After Flow Cell cleaning is complete,
---- Remove the closed loop nozzle from the flow cell assembly
---- Insert the correct nozzle size (100 um nozzle)

---- start the stream

16. Prepare a tube BD CS&T Beads (**350 uL PBS + 1 drop of beads**, vortex beads bottle before use and return the stock back to fridge as soon as possible).

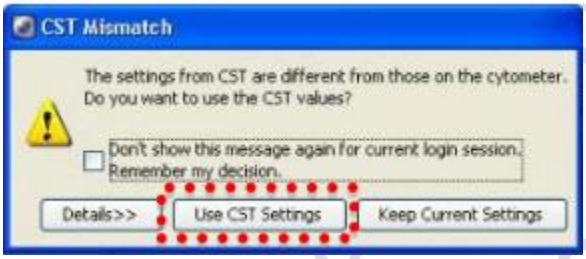
17. Click **Cytometer > CST**. (**Please confirm whether the ND filter is 1.0**)

18. Load the tube.

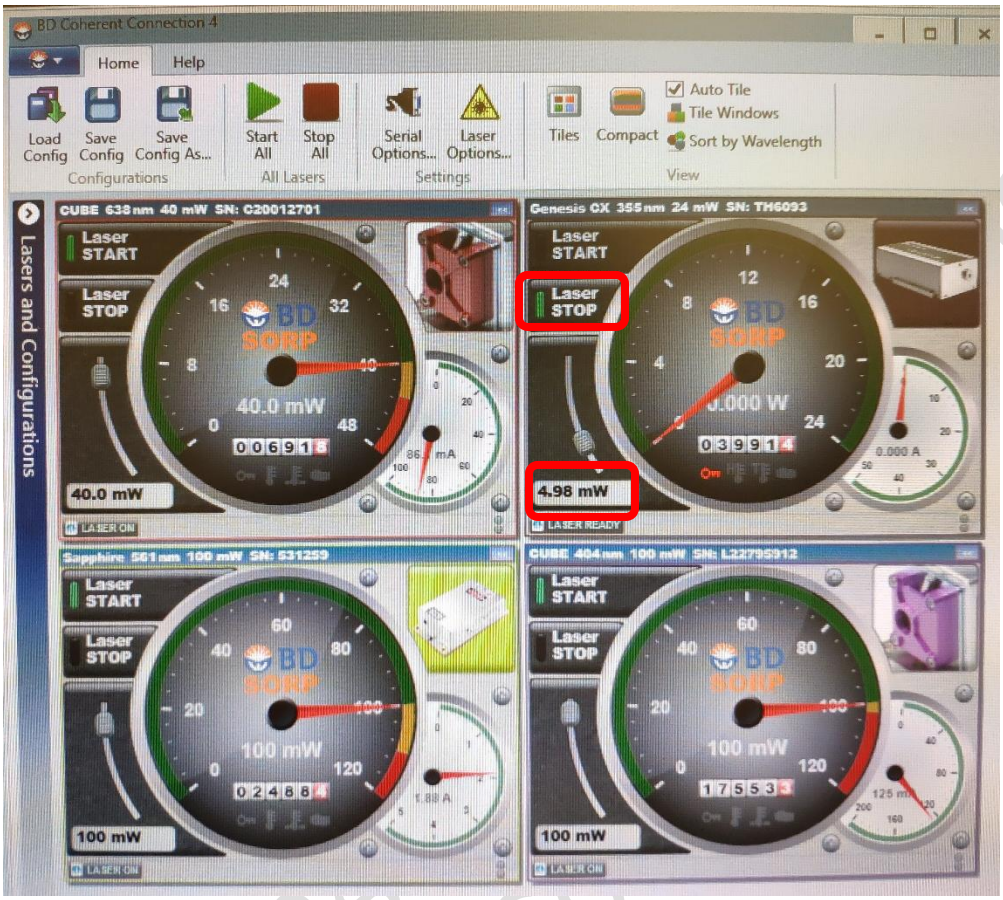
19. Verify the Bead Lot Number and click "**Run"**.

20. After CS&T has completed successfully, Click "**Finish",** and **quit** the CS&T software, return the ready-for use beads back to fridge as soon as possible.

21. Once the system has connected again, click "**Use CST Settings"**.



22. Turn OFF the UV laser if you don't use it, otherwise it will destroy the DNA. Manually adjust the UV355nm laser from 20 mW to **5 mW** and **"Enter"**, then check whether it goes to nearly 5 mW. Then click "**Laser STOP**" to turn off the UV laser.

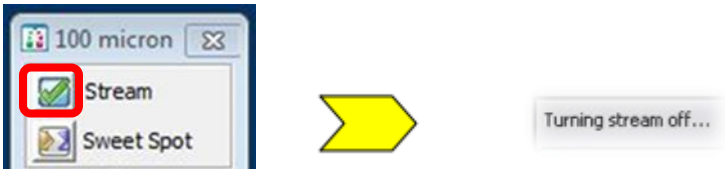


23. Click **File > Log out** if you use “0cleaning” account for system startup.

24. Login to your own account to perform Drop Delay Assay, test sort and your experiments.

Machine Shutdown:

1. After performing the 3-step cleaning process with Cleaning Solution 1, 2 and 3 at flow rate 11 for 5 minutes each (if you used PI, Rinse might be run for 10 mins), turn off the stream and wait until the *Turning stream off* message under Cytometer window disappeared.



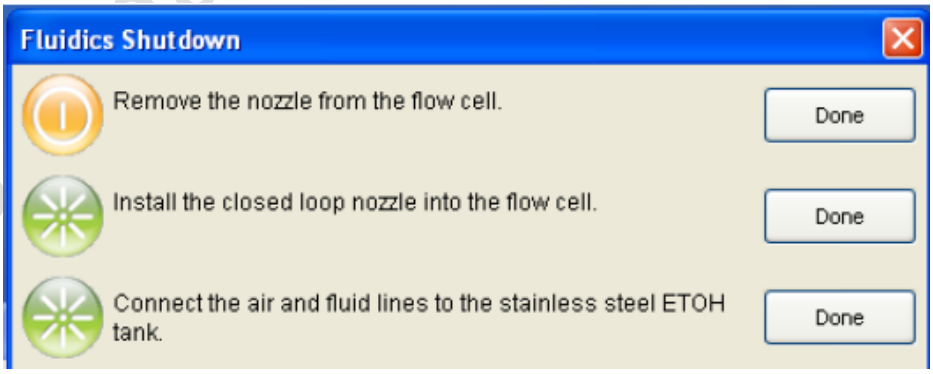
2. Remove the 100 um nozzle from the flow cell and sonicate for less than 1 min with O-ring facing up, then wrap with Kimwipe and put it to the collection box.

3. Insert the closed loop nozzle into the flow cell.

4. Try to clean Flow Cell twice before Fluidics Shutdown.
   ---- Select **Cytometer** > **Cleaning Modes** > **Clean Flow Cell**.
   ---- When prompted, install a tube containing approximately 3 mL of solution 2 (BD Rinse), then click **OK**.
   ---- The cytometer loads the tube and fills the flow cell with the BD Rinse.
   ---- Click **OK** when the completion dialog appears.
   ---- Repeat the flow cell cleaning with BD Rinse buffer.

5. **Fill the stainless steel ethanol tank with 70% ethanol to the mark.**

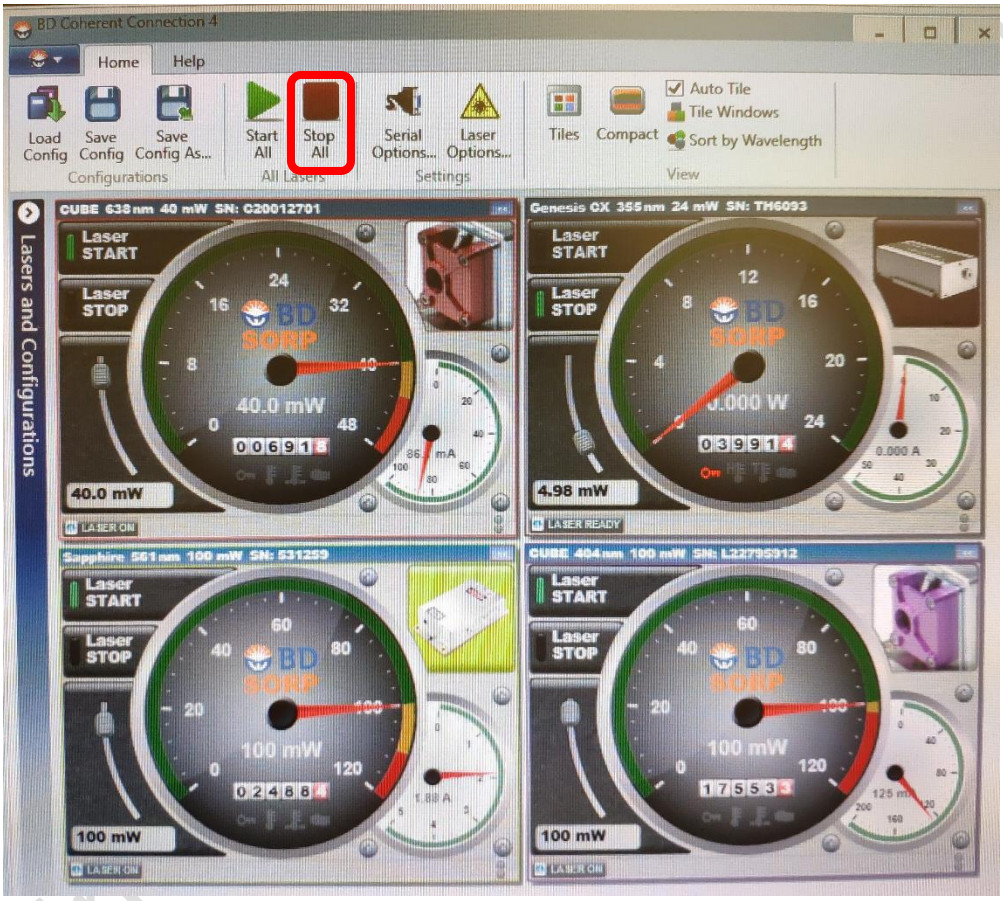
6. Click **Cytometer > Fluidics Shutdown** and follow the prompts on the screen to finish the procedure.



   ---- Remove the nozzle from the flow cell assembly and click “Done”.
   ---- Insert the integrated closed-loop nozzle into the flow cell assembly and click “Done”.
   ---- Connect the air and fluid lines to the stainless steel ethanol (ETOH) shutdown tank. Click “Done”.

---- Vent the air pressure from the sheath tank by pulling up on the ring on the pressure relief valve. Refill the tank with sheath buffer **PBS**.
---- Use 2ml of Cleaning Solution 3 to complete step 5.

7. Turn off all the lasers by click “Stop All”



8. Quit the BD FACSDiva Software when fluidics shutdown is completed, quit BD Coherent Connections and Cytometer Status software, followed by shutting down the computer.
*Note that experienced users don’t need to log out the tracker otherwise you couldn’t shut down the computer.

9. Turn off the Power button on machine body.

10. Turn off chiller power.

11. Wipe the deflection plate and sort chamber with DI water.

12. Reconnect the fluid line and gas line from ethanol tank to sheath tank.

13. Empty the waste tank right away if the waste is less than half a tank. Add in some bleach if the waste level is over half a tank.

14. Add in some bleach and make sure its volume covers the bottom layer of the tank.

15. Take out the exhaustion drain tray from the wet cart and air dry on the chiller.

16. Turn off the lights and lock all the doors before leaving.

Troubleshooting:

High CV after CST	Run Solution 1 and 3 for 10 min each at high flow rate > Re-run CST
High CV after re-run	Notify Staff
CST fail	Prepare fresh CST beads > CST
CST fail after re-run	Notify Staff
Low level of Sheath	Press Standby > De-gas the tank > Refill sheath > connect
Waste tank is full	Press Standby > Empty waste tank > Add bleach > Connect the tank > connect
Software Hang	Task manager > Kill app (java)> Close cmd > Re-start DIVA software
Nozzle clogged	Turn off the stream > sonicate the nozzle for less than 1 min > install > turn on the stream
Sample line clogged (event/s = 0)	Run Solution 1 at max flow rate for 5-10 mins (check the event/s) > Run solution 3 afterwards if more events could be detected If solution 1 couldn't fix the problem > Notify staff