

BD FACSAria® SORP Standard Operation Protocol

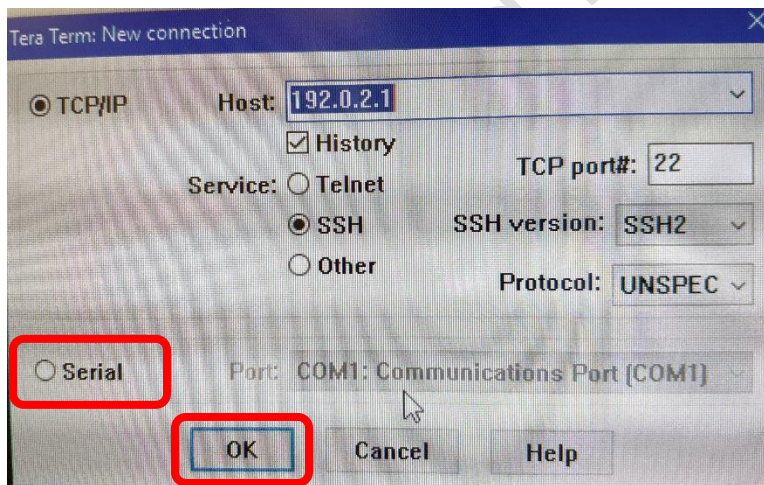
Instrument Startup and Shutdown Procedures (For Experienced users only)

Instrument Startup:

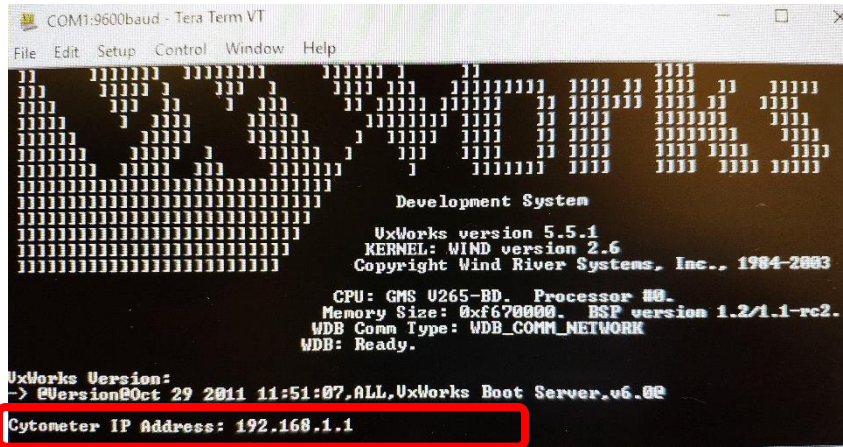
1. Refill the sheath tank and empty waste tank if necessary, make sure all the tubing is connected properly and insert the exhaustion drain tray into the cart.
2. Turn on the computer.
Account: **BDAdmin**;
Password: **please refer to the label attached on the screen**
3. Double click **Tera Term** icon on the desktop to start the program.



Select “Serial” and click “OK”.



4. Turn on the power switch on the left side of the sorter body.
5. Turn on the power of chiller.
6. Ensure that **Cytometer IP address** (192.168.1.1) displays on the Tera Term window before proceed to the next step.

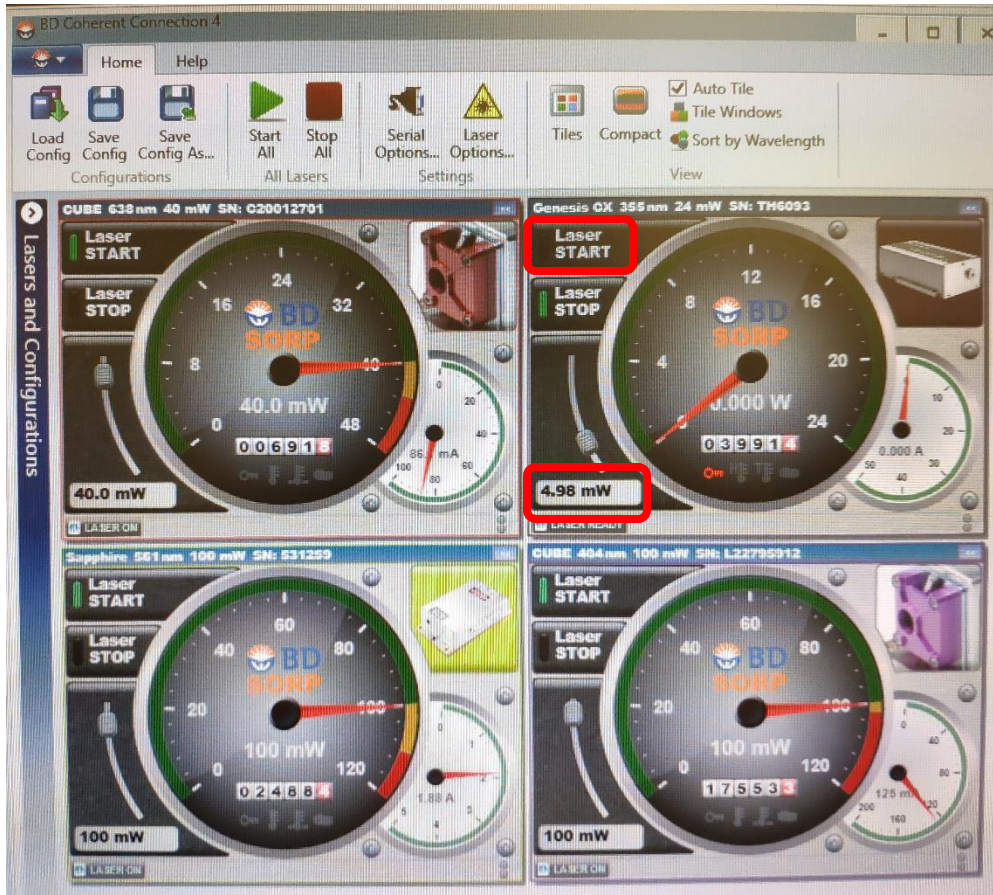


```
COM1:9600baud - Tera Term VT
File Edit Setup Control Window Help
Development System
UxWorks version 5.5.1
KERNEL: WIND version 2.6
Copyright Wind River Systems, Inc., 1984-2003
CPU: GMS U265-BD, Processor #0.
Memory Size: 0xf670000, BSP version 1.2/1.1-rc2.
WDB Comm Type: WDB_COMM_NETWORK
WDB: Ready.
UxWorks Version:
-> @Version@Oct 29 2011 11:51:07,ALL,UxWorks Boot Server,v6.0@
Cytometer IP Address: 192.168.1.1
```

7. Double click **BD Coherent Connection4** icon on the desktop to start the program.



8. 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 mM, adjust to **20 mM** and "**Enter**", then check whether it goes to nearly 20 mM.
(Lasers need at least 30 minutes to warm up)



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

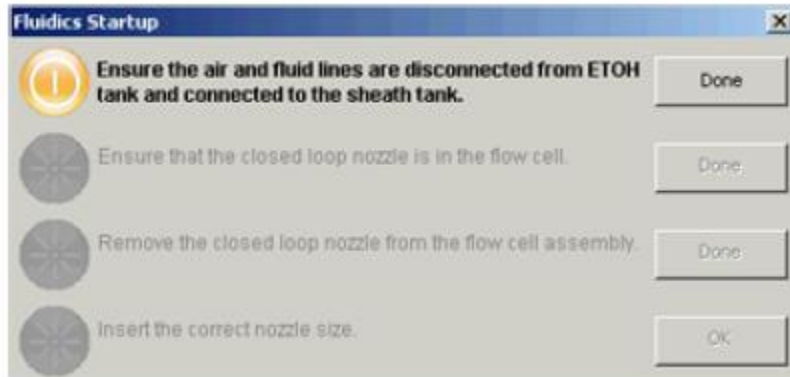


- Log into FACSDiva software with **Ocleaning** user name. No password is needed. Click “OK” to login.

- Once the system has connected, click “Use CST Settings”.



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



- Check the gas and fluid lines are properly connected to the sheath tank. Click “Done”.
- Check the closed loop nozzle is in the flow cell. Click “Done”. Fluidic Startup will begin.
- Once Fluidic Startup is completed, remove the closed loop nozzle from the flow cell. Click “Done”
- Insert the 100um nozzle. Click “OK” to complete the procedure.

13. (Optional) Clean Flow Cell for two times after Fluidics Startup.

- Select **Cytometer > Cleaning Modes > Clean Flow Cell**.
- When prompted, install a tube containing approximately 3 mL of MilliQ water, then click **OK**.
- The cytometer loads the tube and fills the flow cell with the DI water.
- Click **OK** when the completion dialog appears.

14. Turn ON the stream as described in FACSaria® SORP - Basic Operation - Stream setup for cell sorting (100um nozzle) section.

15. Prepare a tube BD CS&T **Research Beads** (350ul PBS + 1 drops of beads, vortex beads bottle before use).

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

17. Load the tube.

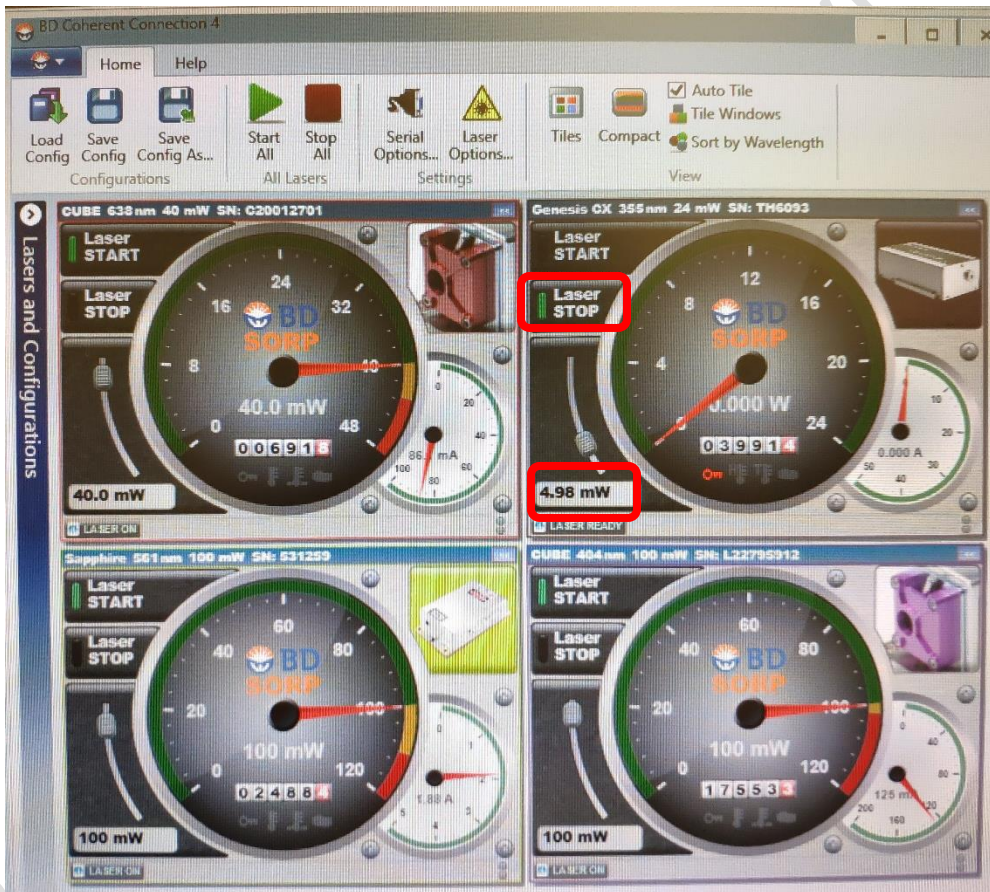
18. Verify the Bead Lot Number and click “**Run**”.

19. After CS&T has completed successfully, click “**Finish**” and quit the CS&T software.

20. Once the system has connected again, click “**Use CST Settings**”.



21. 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.



22. Click **File > Log out**

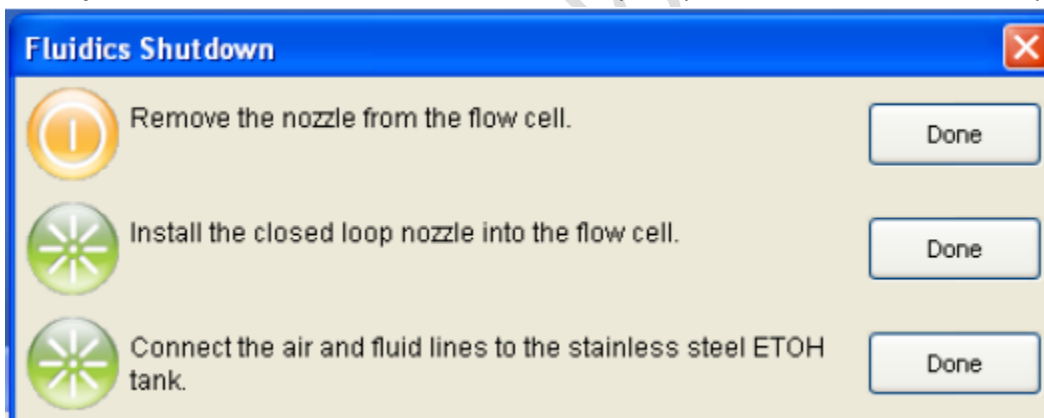
23. Login to your own account to perform Drop Delay Assay, test sort and your experiments.

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, turn off the stream and wait until the “Turning stream off” message under Cytometer window disappeared.



2. **Fill the stainless-steel ethanol tank with 70% ethanol to the mark.**
3. **Remove the nozzle and install the closed loop nozzle into the flow cell. Try to clean the flow cell with Solution 2 (Rinse buffer) twice.**
4. 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”.

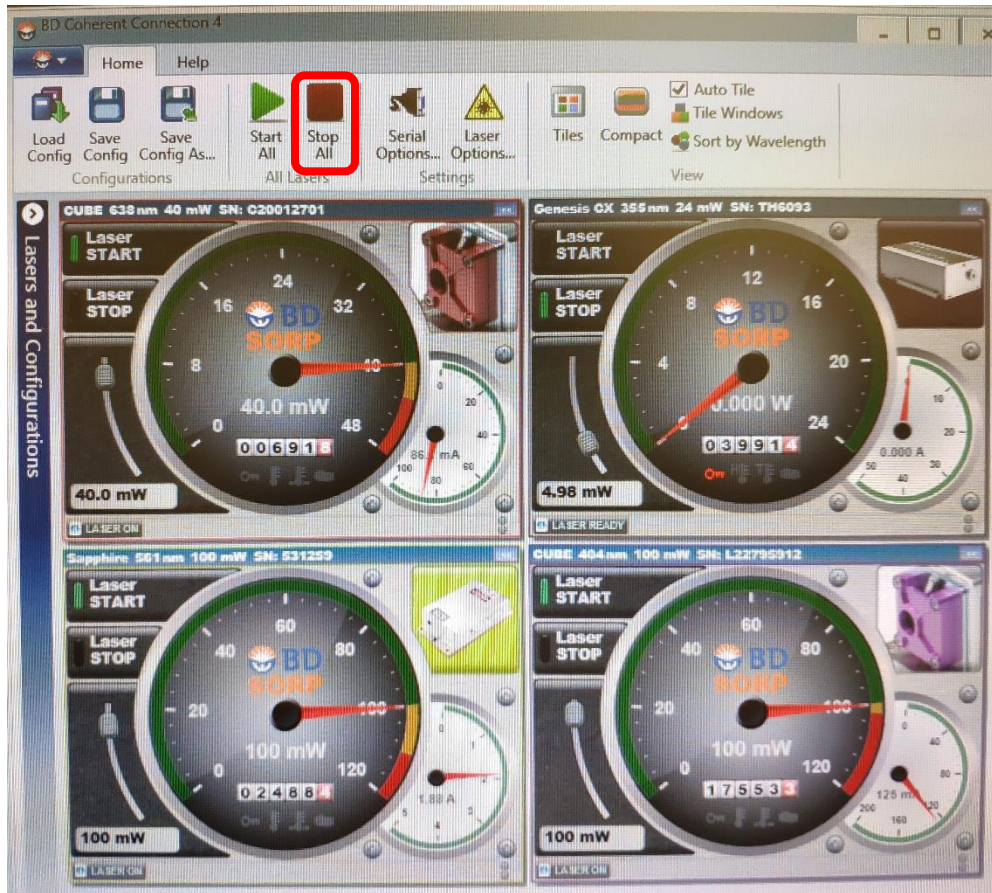
---- Insert the integrated closed-loop nozzle into the flow cell assembly and click “Done”.

---- 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.

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



6. Sonicate the nozzle in a beaker containing DI water for approximately 1 min, then dry with Kimwipe and put it back to the collection box.
7. Quit the BD FACSDiva Software when fluidics shutdown is completed, quit BD Coherent Connections and Tera Term software, followed by shutting down the computer.
8. Turn off the Power button on machine body.
9. Turn off chiller power.
10. Wipe the deflection plate and sort chamber with DI water.
11. Reconnect the fluid line and gas line from ethanol tank to sheath tank.
12. Disconnect the waste tank from the system. Empty the waste tank.
*If the waste tank is more than half filled, add some extra bleach and wait for 5 minutes before discarding the waste.

13. Add bleach and make sure its volume covers the bottom layer of the tank.
14. Connect the waste tank back to the system.
15. Take out the exhaustion drain tray from the wet cart and air dry near the sink.
16. Turn off the lights and lock all the doors before leaving.

CPoS - Imaging and Flow Cytometry
Core