



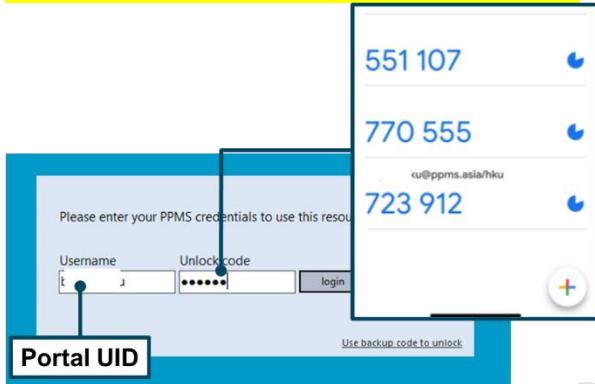
Imaging and Flow Cytometry Core

BD FACSaria® Fusion 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.
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.**



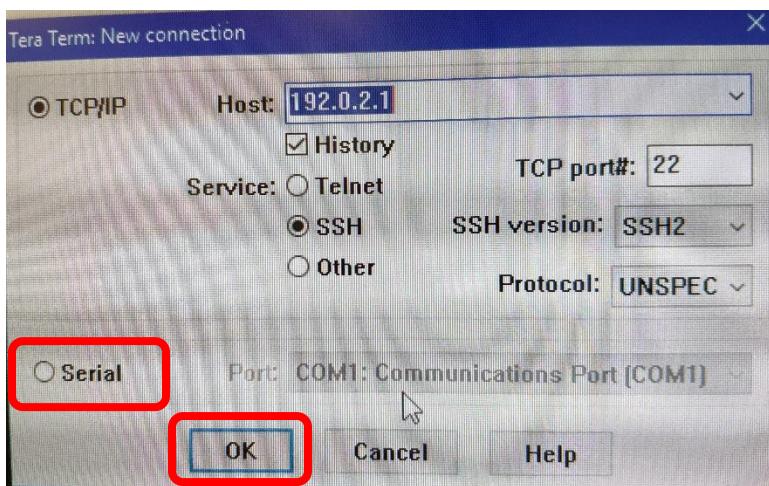
4. Double click **Tera Term** icon on the desktop to start the program.



Select "Serial" and click "OK".



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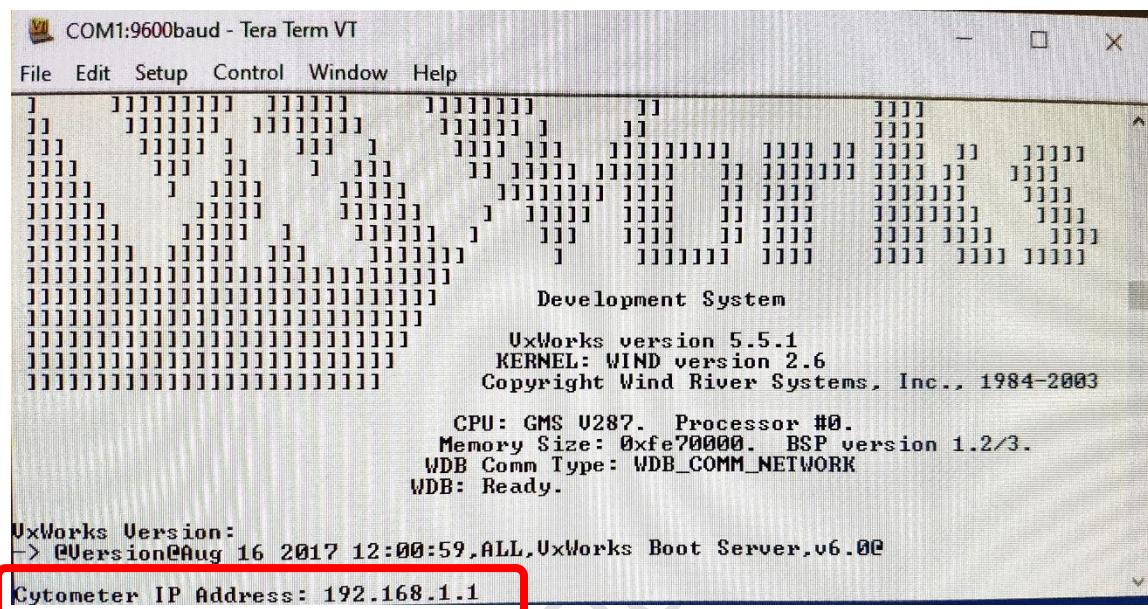
5. Turn on the power switch on the right side of the sorter body.
6. Turn **ON** the **4** lasers equipped (405nm, 488nm, 561nm and 640nm).



7. Turn on the power of chiller and press enter button.
8. Turn on the power of compressor.
9. Ensure that **Cytometer IP address (192.168.1.1)** displays on the Tera Term window before proceeding to the next step.



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```
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 U287. Processor #0.
Memory Size: 0xfe70000. BSP version 1.2/3.
WDB Comm Type: WDB_COMM_NETWORK
WDB: Ready.

UxWorks Version:
-> @Version@Aug 16 2017 12:00:59,ALL,UxWorks Boot Server,v6.0@

Cytometer IP Address: 192.168.1.1
```

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



11. Log into FACSDiva software with either “Ocleaning” account or your own account.

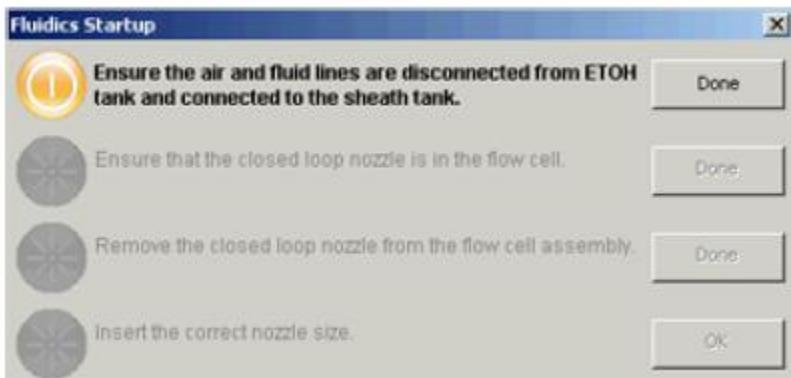
12. Once the system has connected, click “**Use CST Settings**”.



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



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- 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 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 FACSDiva CS&T Research Beads (**350 uL PBS + 1 drop of beads**, vortex beads before use, return stock beads back to fridge as soon as possible).
17. Click **Cytometer > CST**. (**Please confirm the ND filter is 1.5**)
18. Load the tube.



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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 ready-for-use beads back to fridge as soon as possible.
21. Once the system has connected again, click “Use CST Settings”.



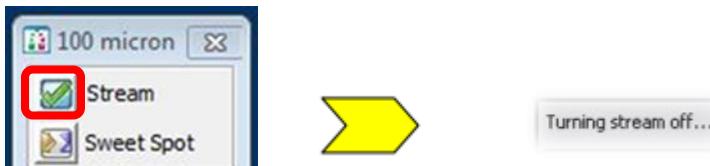
22. Click **File > Log out** if you use “Ocleaning” account for system startup.
23. Login to your own account to perform Drop Delay Assay, test sort and your experiments.



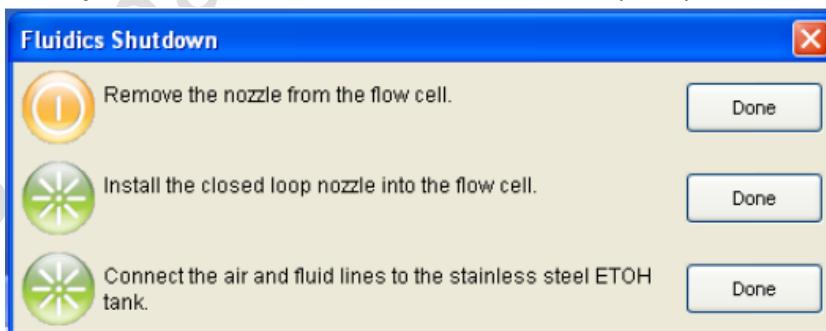
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Machine Shutdown:

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”.



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- 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. Quit the BD FACSDiva Software when fluidics shutdown is completed, quit Tera Term 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.*
 8. Turn off the Power button on sorter body.
 9. Turn off chiller and compressor 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. Turn off the BSC hood.
 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



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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