Imaging and Flow Cytometry Core

BD FACSymphony A5 SE Standard Operation Protocol

System Startup and Shutdown Procedures (For Experienced users only)

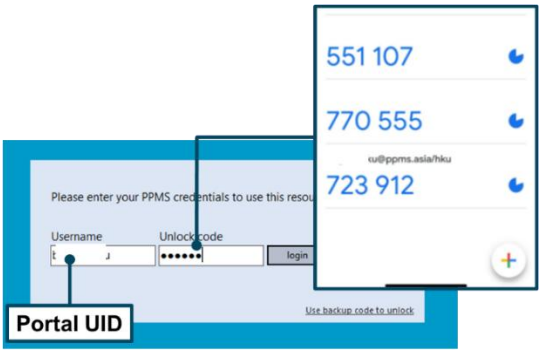
Machine Start up:

- Refill the sheath tank and empty waste tank if necessary, make sure all the tubings are connected properly.

- Turn on the computer power and log in using **User** account.

(Please find the password posted under the bottom of the computer screen)

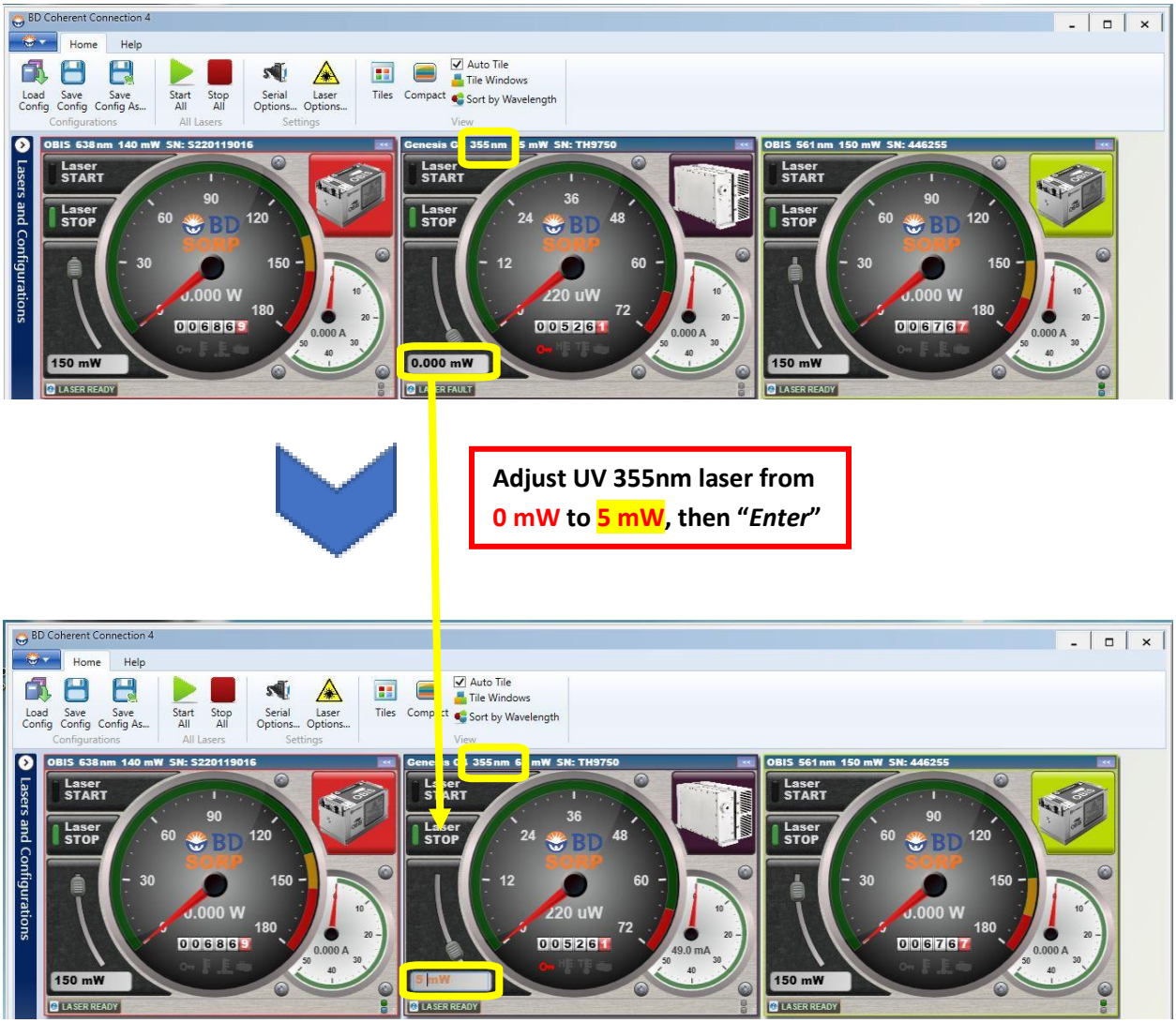
- Ensure to start **Tracker** before other software launch.



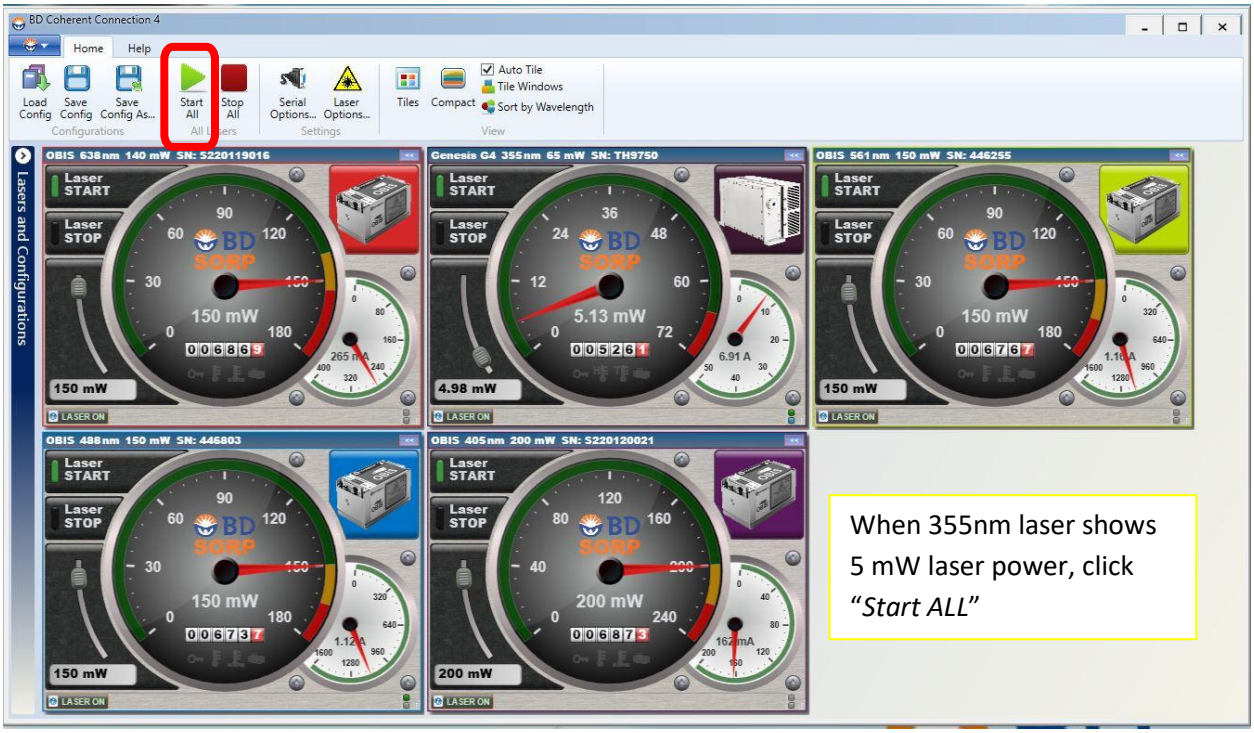
- Turn on the **Main Power** button at the upper right of Symphony A5.

- Double click the **BD Coherent Connection** icon on the desktop to start the program.

- Adjust 355 nm laser from 0 to **5 mW**



- When 355nm laser shows laser power of 5 mW, click "*Start ALL*"



- Then adjust the 355nm laser from 5 to **60 mW** and "*Enter*"

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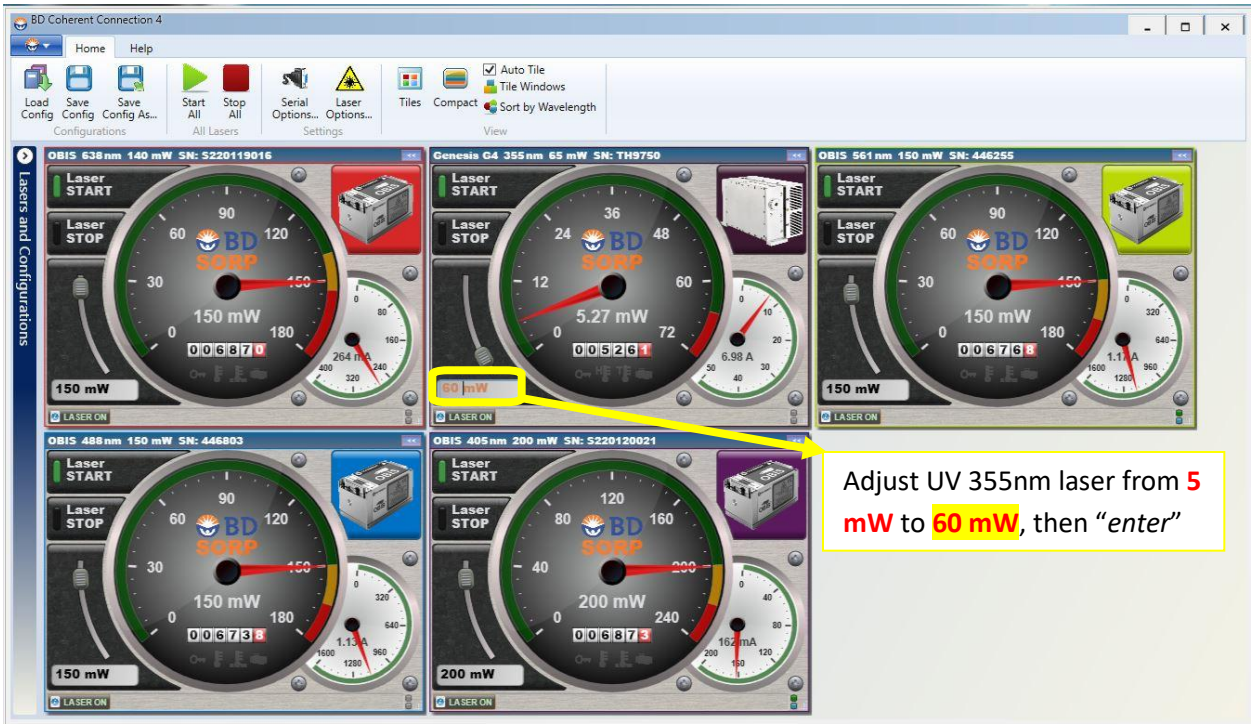
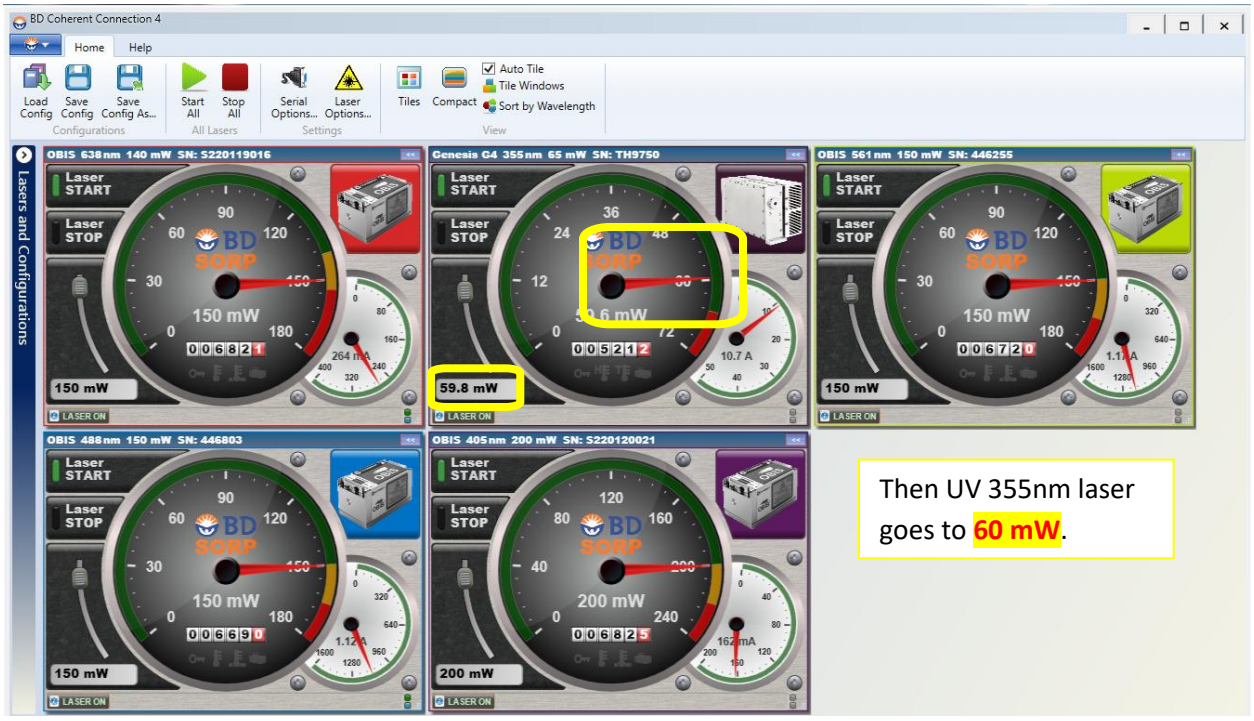




- Remove air bubbles from the sheath filter.
- Load a tube of 3 mL MilliQ Water (Solution 3) and run at **HI** speed for about 30 minutes.
- Double click the BD FACS Diva Software → login to your account

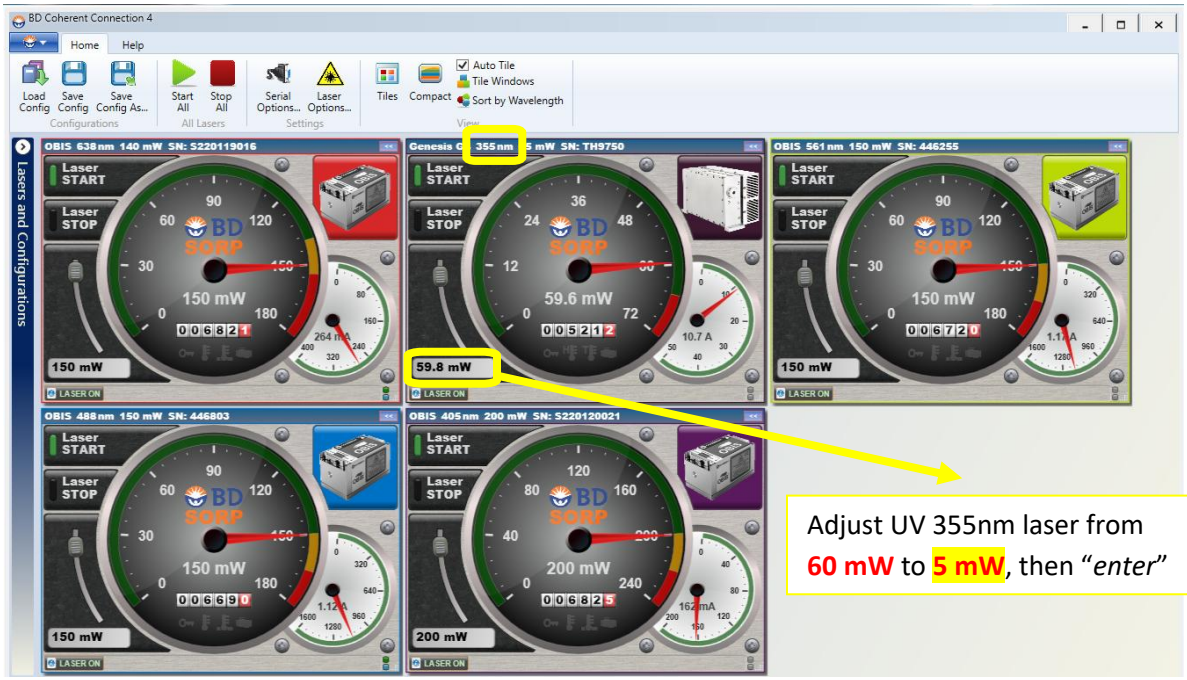
- The system is ready to use when it shows “Cytometer connected”.
- After 30 minutes of cleaning, adjust the flow rate to “**LOW**”.
- Go to “Cytometer” → “**CST**”.
- Get the well-prepared CST beads from the fridge (prepare a new batch if needed: vortex the CST tube with **red** cap vigorously followed by adding **1 drop** of the beads into **350 µl** 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 before loading.
- 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.
- Begin to run your samples.

Machine Shutdown:

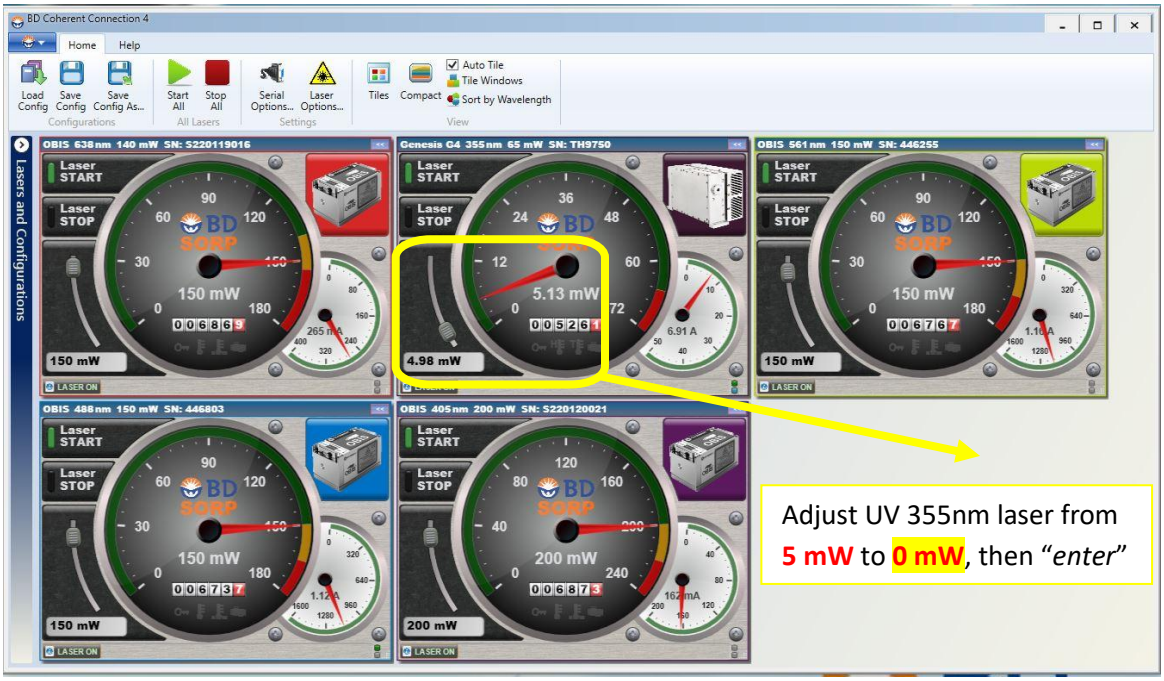
Shutdown lasers:

1. Click the **BD Coherent Connection** icon to show the lasers

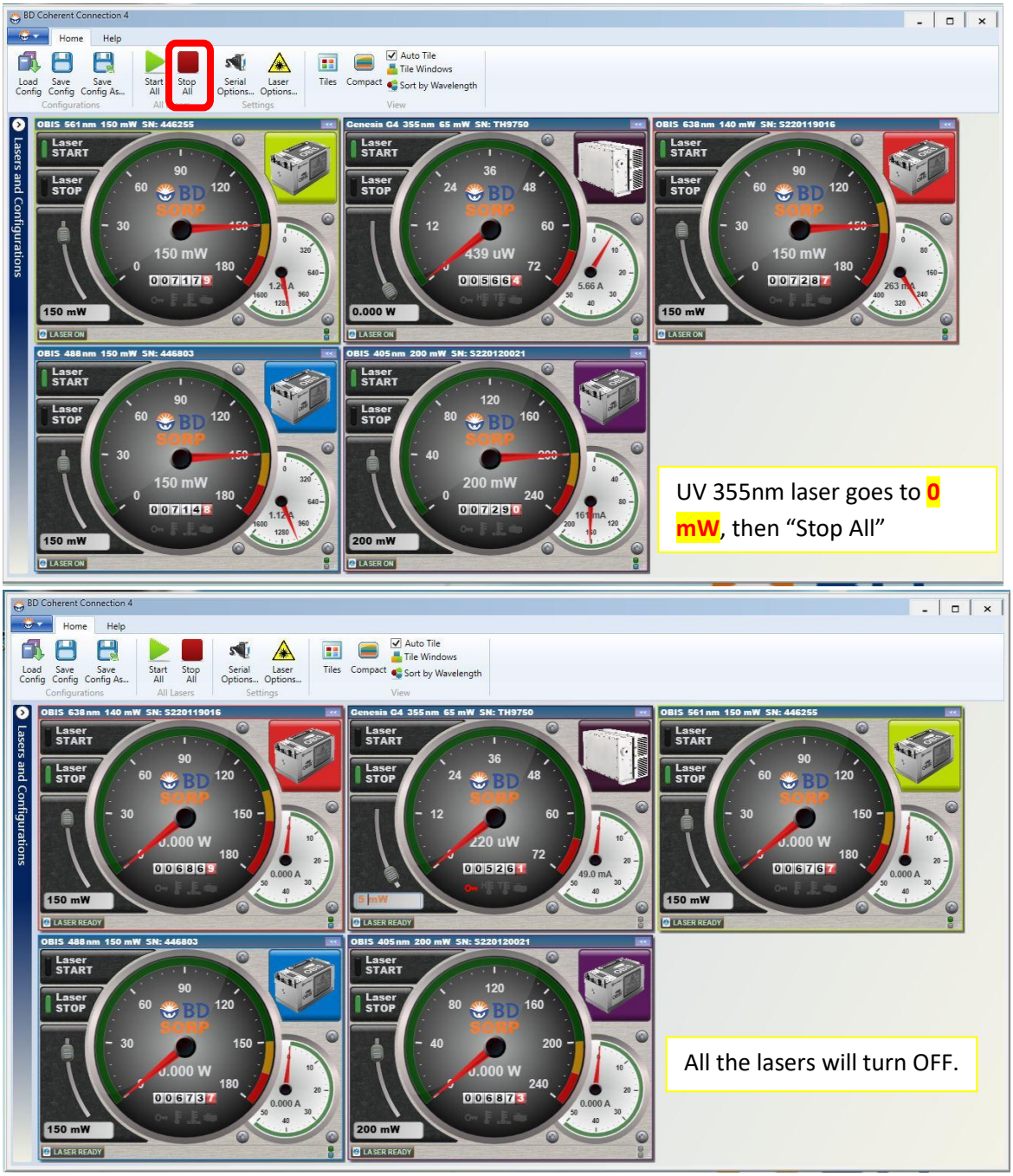
2. Adjust the power of 355 nm UV laser from 60 mW to 5 mW, then “enter”.



3. When it decreases to 5 mW, adjust it from 5 mW to 0 mW, then “enter”.



4. When 355 nm laser goes to 0 mW, click “Stop All” to turn off all the lasers.

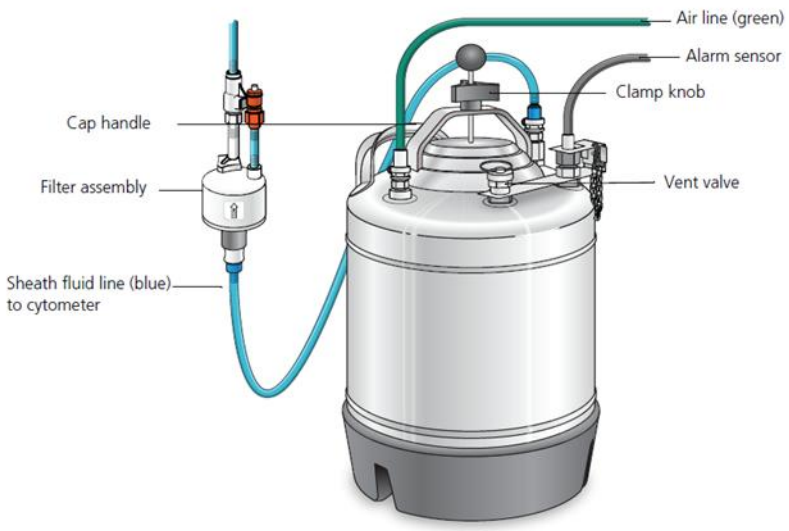


5. After running through the whole process of machine cleaning, turn off the POWER button at the upper right of Symphony.
6. Exit the BD FACS Diva software and Cytometer Status → Shutdown computer.
   *Note that experienced users don't need to log out the tracker otherwise you couldn't shut down the computer.

7. Refill sheath with sheath fluid from bottom bottles on the trolley.
8. Discard the waste to the sink and add around 0.5 L bleach to the waste tank.
9. Turn off the lights and lock all the doors before leaving.

Refill Sheath Container

1. Make sure the flow cytometer is in STANDBY mode or have already shutdown.



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 (bottom bottles on the trolley) to the sheath container.
8. Fix the sheath lid and tighten the clamp knob to finger-tight.
9. Reconnect the air line (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 if continue use it.

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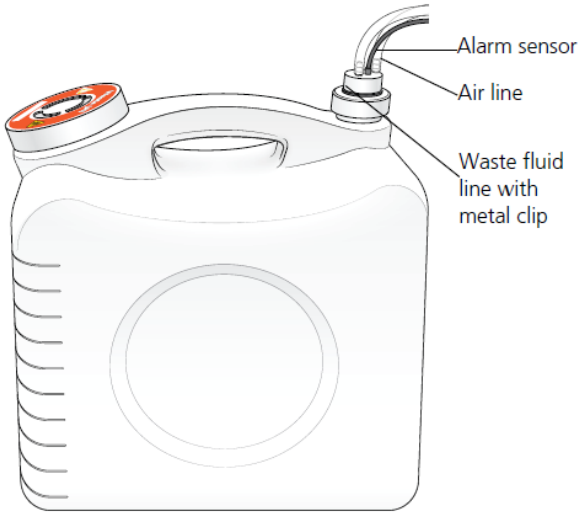
Empty Waste Tank

1. Make sure the flow cytometer is in STANDBY mode or have already shutdown.
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 of waste tank.
5. Empty the waste container.
6. Add approximately 0.5 L of bleach to the waste in which it could cover the bottom layer of the container and tighten the lid.
7. Reconnect the orange waste tubing and level sensor line.



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I. Troubleshooting

High CV after CST	a) Run Solution 3 for 10 min at high flow rate > Re-run CST b) Prime 2 times, then run MilliQ water for 15 min at "HI" 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 > De-bubble sheath filter
Waste tank is full	Press Standby > Empty waste tank > Add bleach > Connect the tank
Software Hang	Task manager > Kill app (java)> Close cmd > Re-start DIVA software
Sample clogged	Prime 2 times > Run Solution 1 at max flow rate for 5 min (record change in solution level) > Run solution 3 afterwards if solution level goes down If solution level not going down> Notify staff for wire cleaning