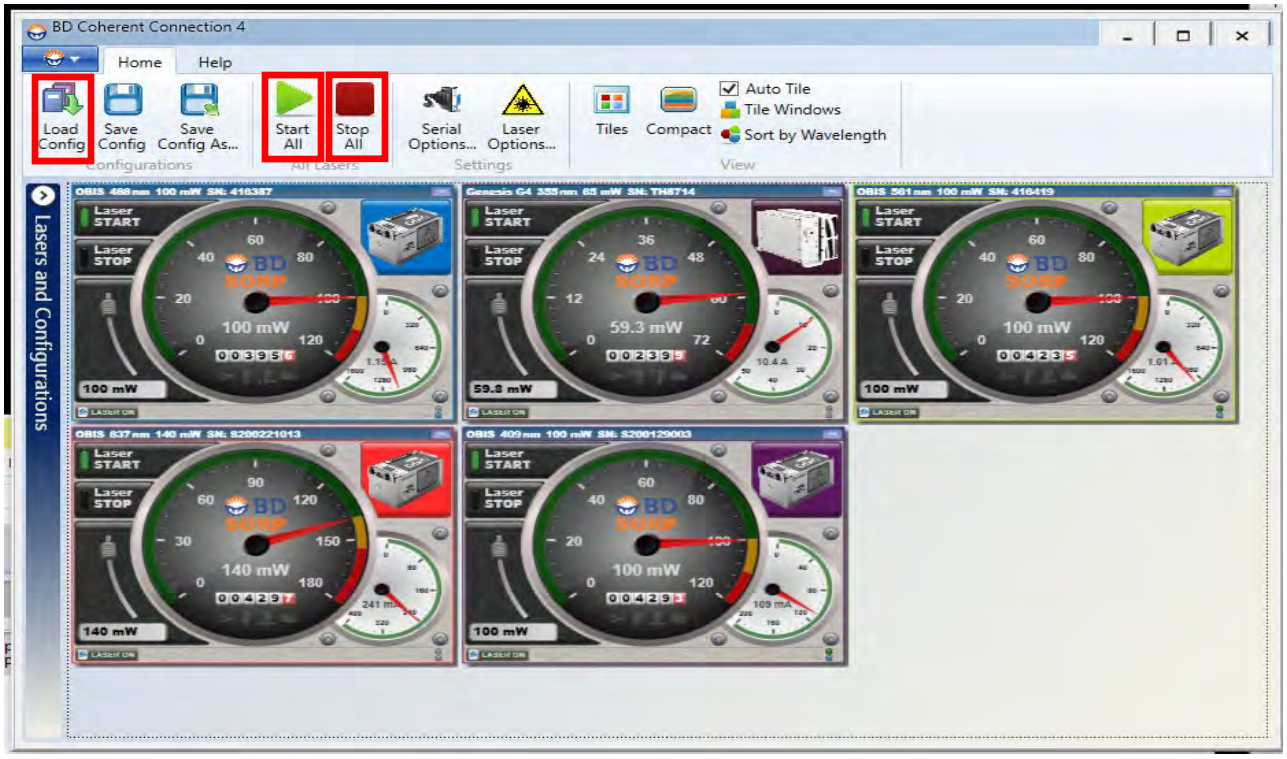
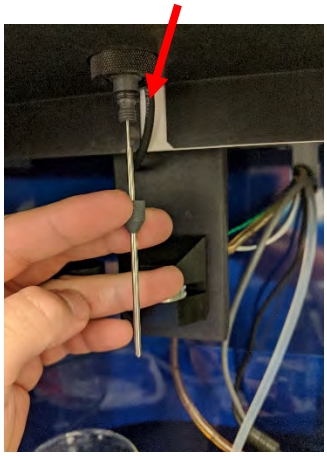
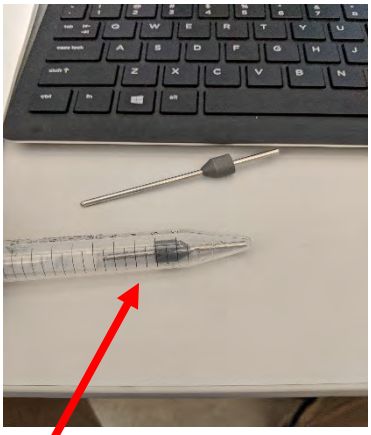
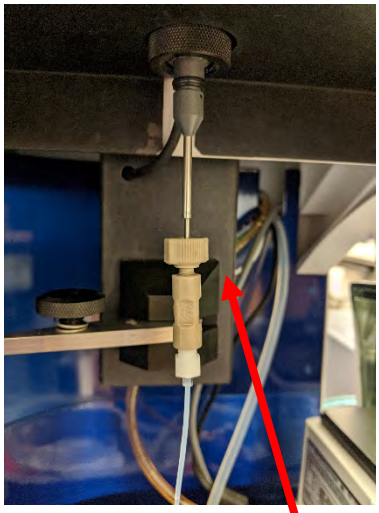
Step By Step Guide for Symphony A3 Cytometer

1. Ensure the Instrument is on
    a. Check the Power switch on the HTS System, located on the back of the HTS, Make sure it is **“ON”**
    b. Check the Power to the FacsFlow Supply System, Make sure it is **“ON”**
2. Log into computer.
3. Open BD Coherent Connection 4 Software
    a. Wait for all 5 lasers to load, then Click “**Load Config**”
        i. Open the “UV Laser” Configuration File
    b. Turn on all the lasers by pressing “**Start All**”
        i. Check that there are no laser faults or Laser ready signals.
        ii. If Present, Press Stop All, Wait ten seconds, Press Start All
    c. Minimize BD Coherent Connection 4 Software

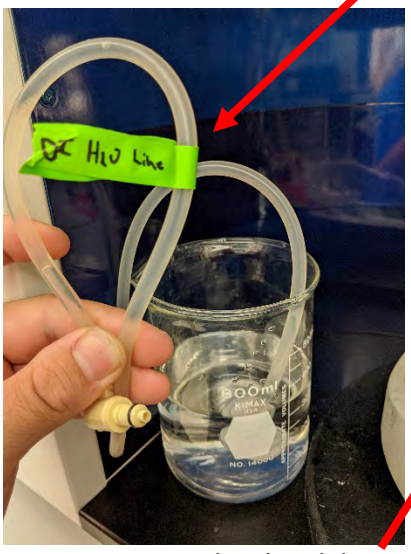


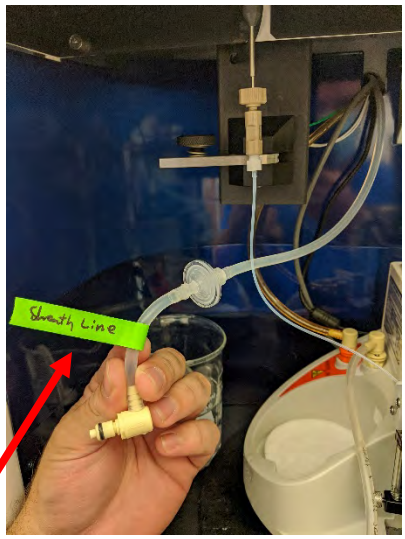
4. Open BD FacsDiva Software
5. From this step forward, run through your compensation in tubes as taught during training.
6. Once Compensation is complete, Ensure the HTS System is ON.
    a. The Power button is located on the back side of the HTS system and a Green Light will be Lit when system is “**ON**”.

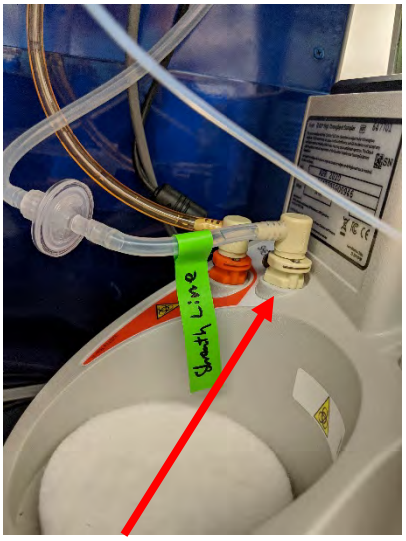
7. Disconnect (Un Screw) the Sample Probe (Long Probe).

8. Connect the HTS Probe (Short Probe).
    a. It is Located in the sliding drawer beneath the Symphony.
    b. Store Long Probe in the 15 ML Conical Tube.
9. Connect the Tan Probe Attachment From the HTS system, to the exposed thin metal tube.
    a. The probe should be inserted inside the tan attachment and then tighten attachment onto the probe.
10. Disconnect the DI water probe from the sheath port on the HTS.

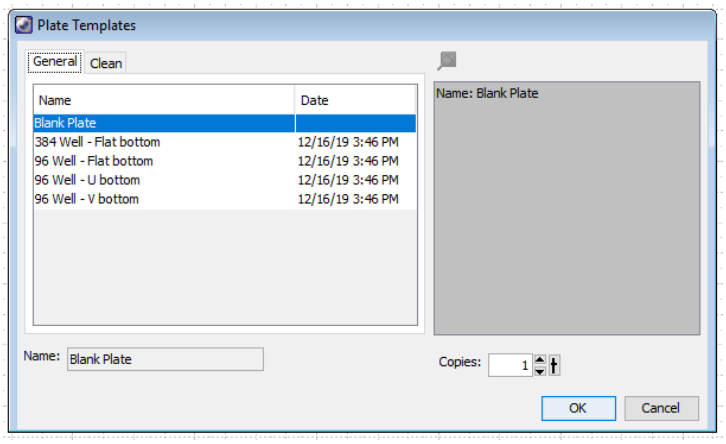




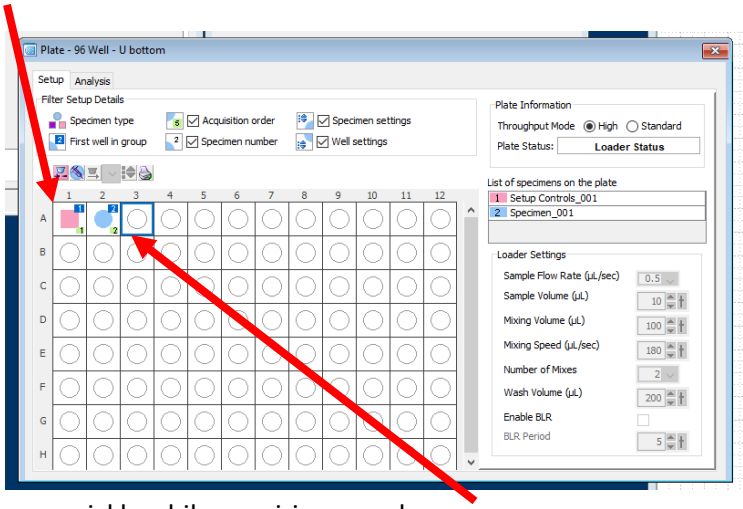


11. Connect the Sheath line coming down from the instrument to the Sheath Port on the HTS.
12. Put the instrument in **PLATE MODE**, The switch is located on the right side of the instrument.
13. Once Sheath line is connected, set instrument to "**RUN**", with a speed of "**LOW**"
14. Re-Initialize the HTS System
    a. In the toolbar of the FACSDiva Software, Press **HTS>Re-Initialize**
15. Prime HTS Twice
    a. In the toolbar of the FACSDiva Software, Press **HTS>Prime**, Repeat.

16. Create a new PLATE by clicking **Experiment>NEW PLATE.** This will open a menu where you can select your type of plate.
    a. EXAMPLE: If well A1 is control cells, create a SETUP CONTROL. This will be the only time you can adjust for voltages. Set up the experiment with atleast one FSC/SSC graph and histograms of all the fluorophores you have.
        i. Ensure they are all on scale with your cells control.
    b. EXAMPLE: If Wells A2-A12 are samples, highlight wells A2-A12 and then press the blue circle with a syringe inside it. This will create SPECIMIN WELLS. Each Well is representative of a tube.

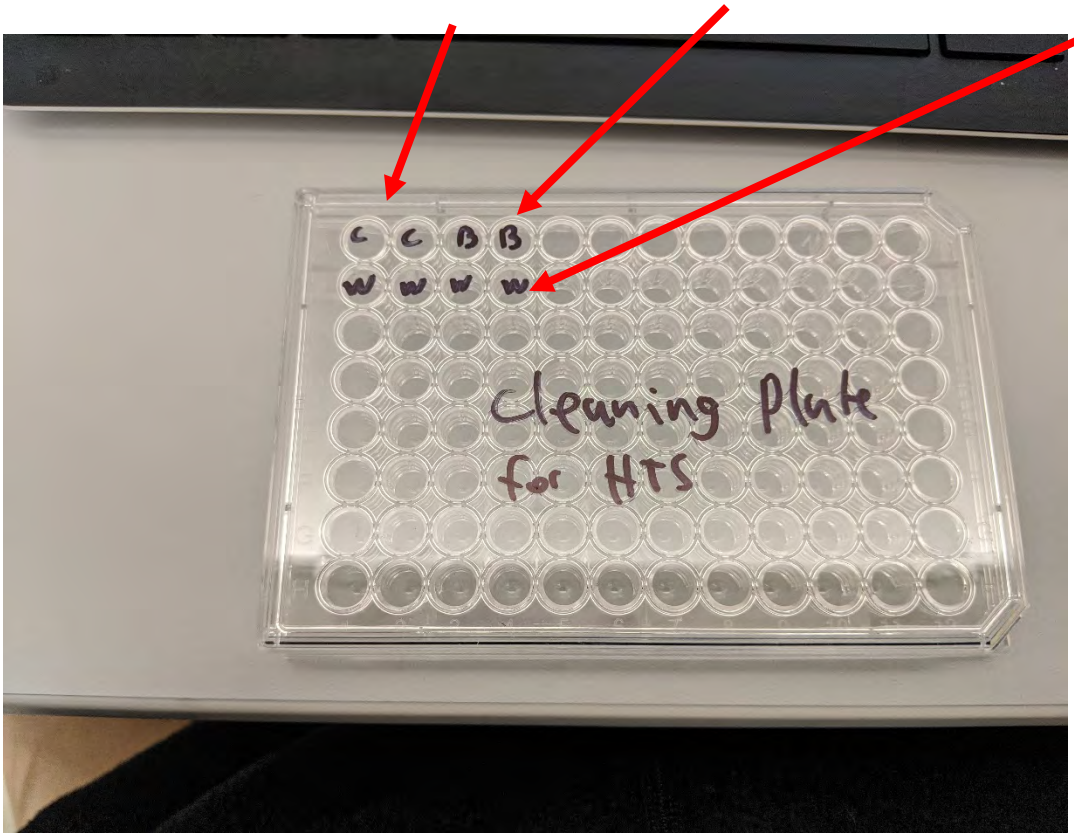


17. Click your "**SETUP CONTROL**" (PINK) and Press ***"RUN WELL"*** ***DO NOT PRESS RUN PLATE YET***

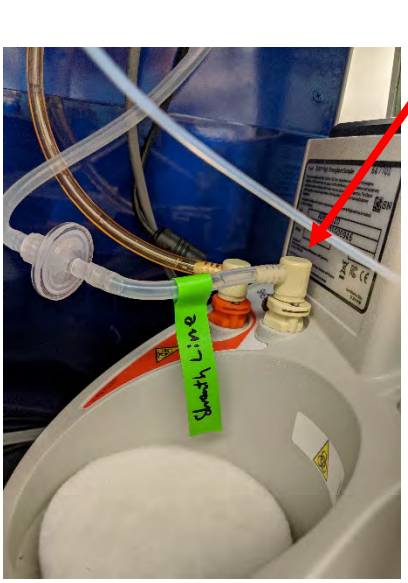


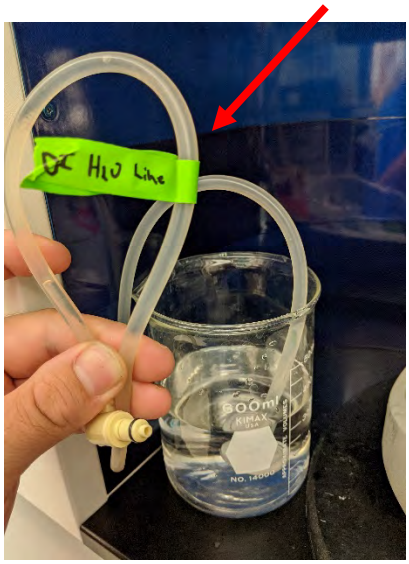
18. Adjust your voltages quickly while acquiring sample.
19. Once voltages are appropriately set, Create **"SPECIMIN WELLS"** (Blue) where you have samples you would like to record/run.
20. Set your run settings as desired
    a. The Maximum volume acquired per sample for High Throughput mode is 10ul/sample.
    b. The Maximum volume acquired per sample for Standard is 200ul/sample.
21. Once your Run settings are set select the first SPECIMIN WELL (BLUE CIRCLE) and press "***RUN PLATE***"

22. When plate is done running and you are ready to shut down, Remove plate and insert the cleaning plate.
    a. Fill Wells A1-A2 with contrad, A3-A4 with Bleach, and Fill Wells B1-B4 with DI H20.

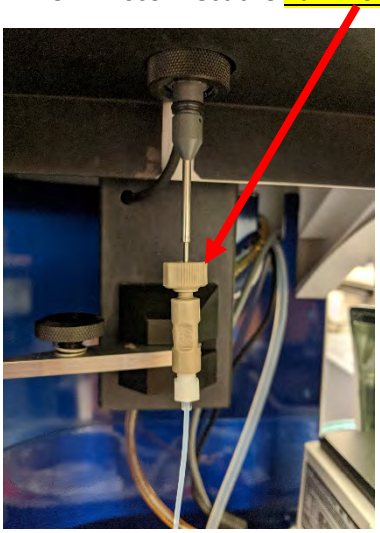


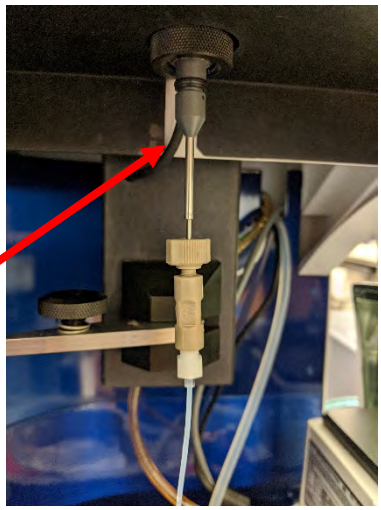
23. Press **HTS>Clean**
    a. This will create a new plate template for the cleaning of the HTS.
24. Load the Cleaning Plate onto the HTS.
25. Press "OK" once the Plate has been loaded and replace the plastic cover to the HTS.
26. Wait for System to complete the cleaning protocol.
27. When finished, Detach the sheath line that is attached to the HTS and Reattach H20 Line.



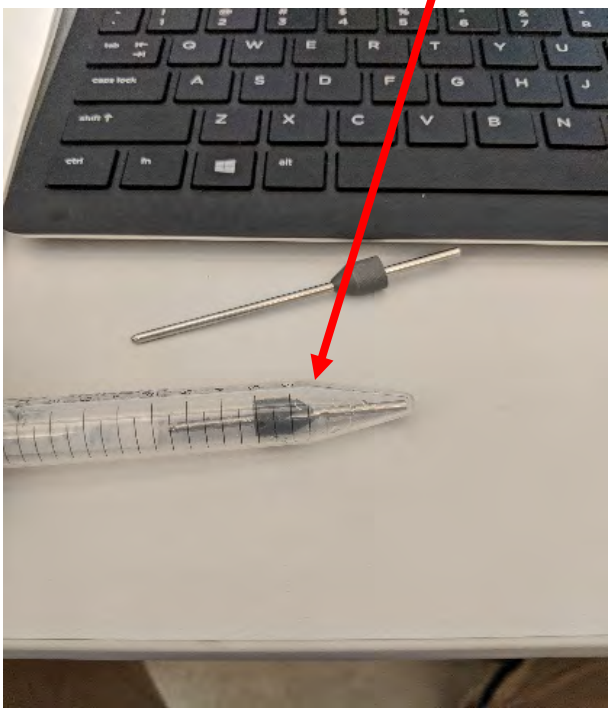


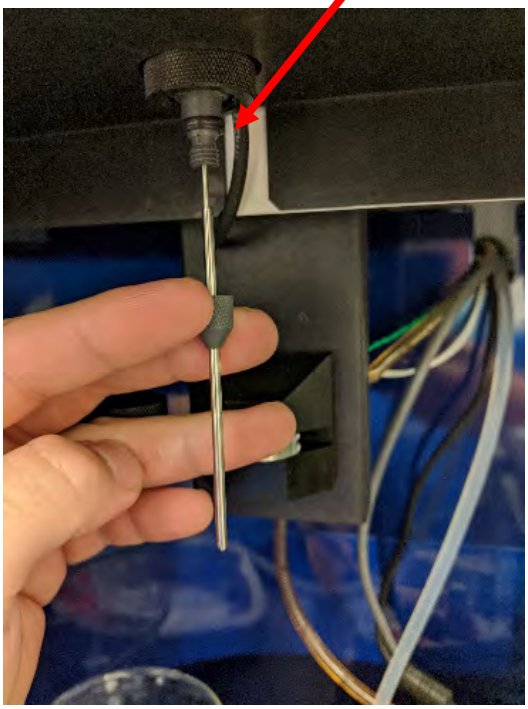
28. Ensure the DI Water cup has at least 300ML of DI H20 in it.
29. Prime HTS Twice
    a. In the toolbar of the FACSDiva Software, Press **HTS>Prime**, Repeat.
30. Now put the instrument in "Standby".
31. Disconnect the Tan Probe From the SIP.





32. Unscrew the Short metal Probe, and Reconnect the Long metal Probe.
    a. Replace the Short Metal Probe inside the 15 ML Conical, return to drawer.





33. Put the instrument back into Tube mode.
34. Put the DI H20 Tube back on the SIP.
35. Ensure the Instrument is in Standby.
36. Exit out of software and Log out of Instrument.