

BD FACS Symphony Start-up, Shut down, and QC SOP

Equipment: BD FACS Symphony (6 lasers: B/R/V/YG/UV)
S. No: R660964C3001
Location: TRB Fourth Floor A4-143
Description: It comes with 355nm UV, 405nm violet, 488nm Blue, 561nm yellow-green, and 640nm red lasers enabling the simultaneous measurement of up to 29 different characteristics of a single cell.
Location: TRB Fourth Floor A4-143A

Materials:

- CST Beads, BD Biosciences Catalog Number# **LOT number may vary**
- Sheath fluid
- Working FACS Cleaning Detergent, CAT#340345
- Distilled water

Consumables:

- BD 5 ml FACS tube, polystyrene

1.1 Before Start-up:

1. Check the waste tank located under the instrument. Make sure the waste tank is empty. To dump the waste tank, please follow the following instructions:
 - If the waste tank is full, it is necessary to dump the fluid. **IMPORTANT:** before draining, disinfect the waste fluid.
 - Change the waste tank and connect an empty waste tank.
 - Add bleach tablet; 1 tablet per 7 Liter of waste fluid.
 - Label the tank and add the date of bleaching.
 - Leave the bleached tank overnight and drain the waste into the sink.
2. Check the level of sheath fluid located under the instrument. If needed, refill the tank with sheath fluid which is available beside the equipment (in 10L White boxes). Turn on the instrument, the green button on the right side of the device.

FIGURE 1: SYMPHONY INSTRUMENT

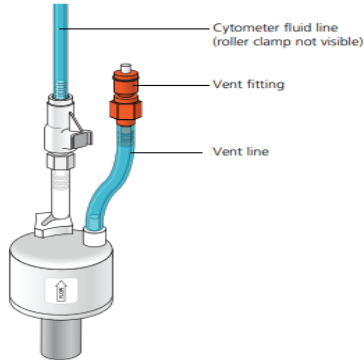


3. Press once the RESTART button on the fluid rack located under the instrument.
4. Make sure there is a tube with water in the sample injection port.
5. Press the PRIME button on the front control panel (as shown in the figure) and check if air bubbles are coming out of the nozzle.

Removing trapped air bubbles:

To remove air bubbles:

1. Check the sheath filter for trapped air bubbles.

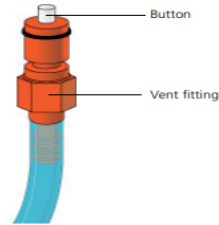


2. If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.



Caution! When removing air bubbles, do not vigorously shake, bend, or rattle the sheath filter or you might damage it.

3. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.



4. Tilt the filter and verify that no trapped air remains in the filter.
5. Repeat steps 3 and 4 until no air is observed in the filter.
6. Check the sheath line for air bubbles.
7. Open the roller clamp at the fluidics interconnect (if necessary) to bleed off any air in the line. Collect any excess fluid in a waste container.
8. Close the roller clamp.

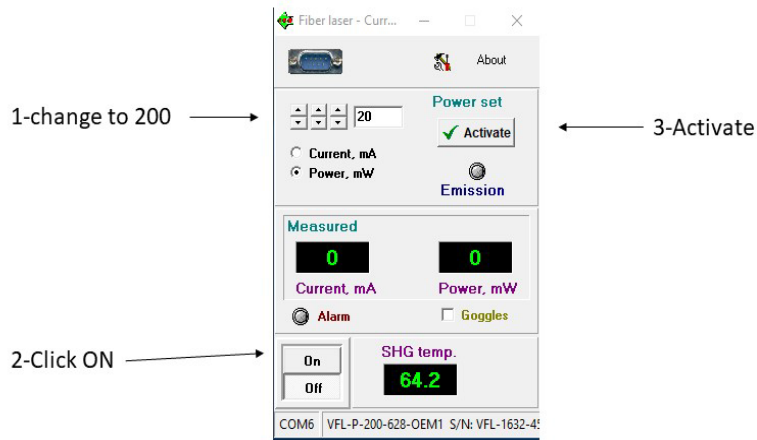
PRIME



1.2 Cytometer startup and tracking:

1. Log in to the BCCHR using your username and password
2. CST beads vial designated for BD FACS Symphony is kept in the fridge (#00375) in Room A4-143A. Please communicate with Lisa Xu to know which lot is in use.
3. Once you are informed about the lot# of CST beads, you need to make a working dilution. Take a FACS tube, add 1 ml of DW, add three drops of CST beads, then vortex for a few seconds. Label the tube with the lot #, the date, and the instrument. Keep the diluted beads in the fridge.
4. Start cleaning the tubing by adding 3-4 ml of FACS cleaning solution into a FACS tube, insert it into the nozzle, set the fluid speed on high, and press the RUN button on the instrument's front control panel. 1 min with arm aside and 5 min with arm in the position.
5. Start washing with DW: add 3-4 ml of DW in a FACS tube, insert it into the nozzle, set the fluid speed on HIGH, and press RUN button, 1 min with arm aside and 5 min with arm in the position.

6. Open the electric current control panel.
7. A window pops out, change the attributes as shown below:



1.3 Turning on Lasers and FACS Diva:

1. Click on 
2. A window pops out, turn on all lasers:

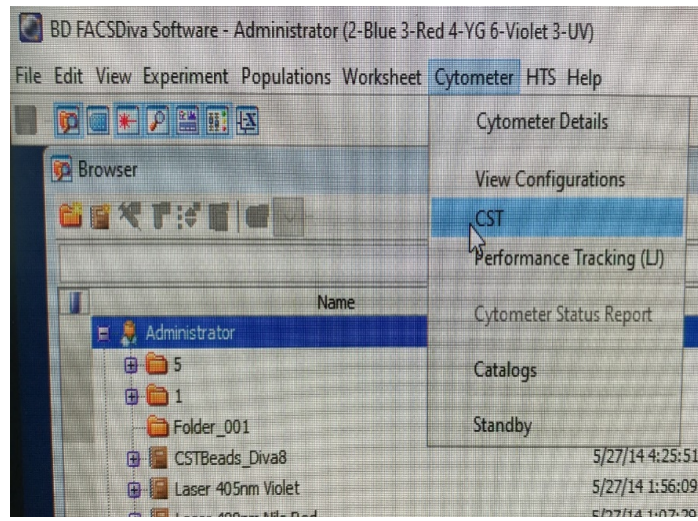


3. If any laser is not activated concurrently, then manually move the cursor up (white arrow)

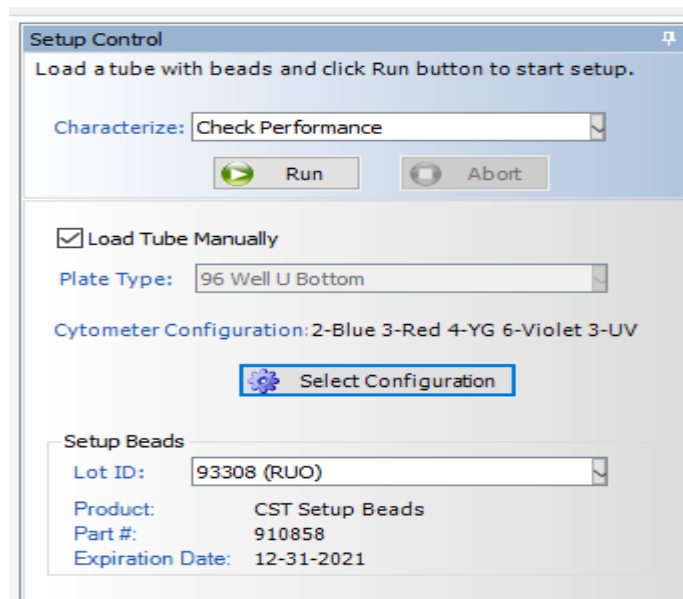
- Click on FACS Diva software, then log in to your laboratory username and password. The research assistant responsible for QC may use the Core Facility username and password.



- On the default FACS Diva open page, select cytometer > CST



6. Wait until the RUN sign on the FACS Diva software turns green.



7. Then press the LOW button on the front control panel of the instrument and turn backward five times the fine tubing nob. Now, insert the CST beads tube into the nozzle, wait for 30 seconds, and click the green RUN on the FACS Diva.
8. The software automatically calculates the CV of the signals. It may take about five min. If everything goes well, a report shows up saying that QC passed. If it does not pass with warnings, it is necessary to start the troubleshooting process (Section 1.4).
9. Take out the diluted CST beads tube, put it back in the fridge, and run cleaning as described earlier (i.e. Start washing with DW: add 3-4 ml of DW in a FACS tube, insert it

into the nozzle, set the fluid speed on HIGH, and press RUN button, 1 min with arm aside and 5 min with arm in the position.)

10. Press the LOW BUTTON, then press the STAND BY button on the control panel.

Suppose there is no booking on the instrument, exit CST. Diva will go back to the default page, and a window comes up. Click on USE CST SETTINGS.

11. Click on FILE on the left corner, choose to log out.

12. Log off from the computer and turn off the instrument; otherwise, leave it on for the following user.

1.4 Troubleshooting

ISSUE	ACTION
PASSED WITH WARNING	Ignore the warning IF the CV in the report is less than 7-8% on a few channels. However, it depends on your specific requirements for the experiment
IT DID NOT PASS; THE NUMBER OF BEADS IS TOO LOW	Make new diluted CST beads tube & rerun CST QC
DID NOT PASS	Run cleaning again and repeat CST QC
DID NOT PASS	Run the deep clean process and redo CST QC
DID NOT PASS	COMMUNICATE WITH LISA XU
Warnings on <2 channels %rCV <10	<ul style="list-style-type: none"> • Proceed with using a cytometer • Observe: is this a consistent trend (same parameter? Similar %rCV to previous checks?)
Warnings on >2 channels %rCV >10, <15	<ul style="list-style-type: none"> • Clean machine as described in point 1 above and check visually before proceeding • If the warning does not clear up, assess whether the affected parameter will affect the resolution
High CV on >2 channels %rCV >15	<ul style="list-style-type: none"> • Call BD Field Service hotline at 1(866)542-6663

- Note and report to BD trends in peaks and CV – are they always moving in the same direction? Jumping around?

