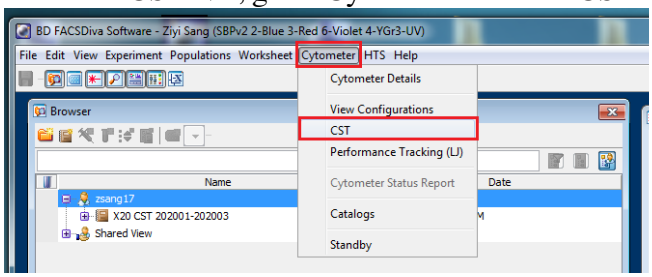
CST Quick Guide rev20200401

1. Turn on the cytometer and log into your FACSDiVa account as usual.

2. All supplies are in white mini-fridge under FACSCalibur workstation.
   2.1. Bead dilutions are stable ≥1 week if kept refrigerated and protected from light.
   2.2. Use existing tube of CST beads if volume is ≥300ul and age is ≤7 days.

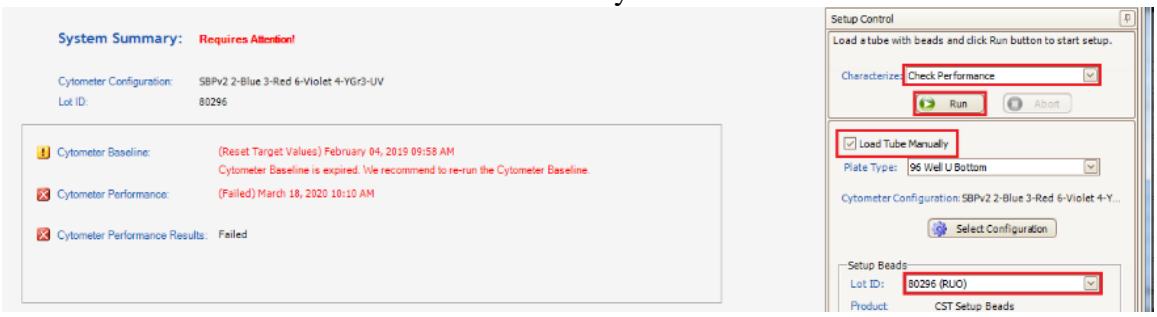
3. To make new tube of CST beads (all supplies in white mini fridge):
   3.1. Use new 5ml polystyrene FACS tube with cap
   3.2. Label tube with "CST", lot#, date: e.g. "CST 80296 3/30/20"
   3.3. Vortex blue CST dropper bottle for 2-3s
   3.4. Squeeze 1 drop CST bead stock into labeled tube
   3.5. Add 600 ul DI H2O and vortex 2-3s
   3.6. Immediately return blue CST dropper bottle to

4. In FACSDiVa, go to Cytometer Menu > CST.



5. A CST window will pop up, and it will take up to 1 min to connect. The bottom right corner will switch from "Connecting" to "Connected".

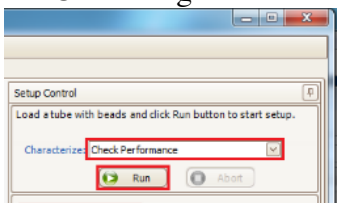
6. In the new CST app window
   6.1. Set Characterize to "Check Performance"
   6.2. Lot ID matches blue dropper bottle "80296(RUO
   6.3. Check the box for "Load Tube Manually".



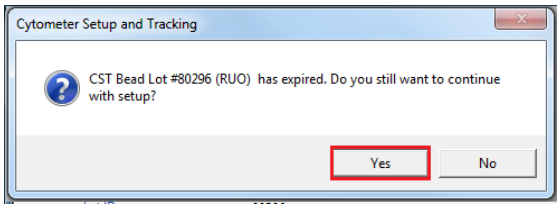
7. Set fluidics to RUN, LOW, and rotate fine adjustment 5 full turns from either end.

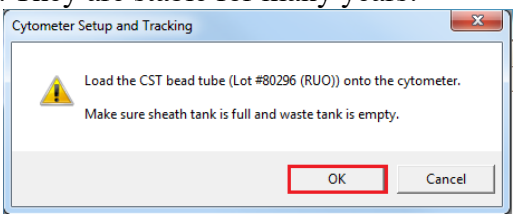
8. Vortex bead tube and load on cytometer

9. Click the green “Run” button.



9.1. Select “yes” if bead lot has expired. They are stable for many years.

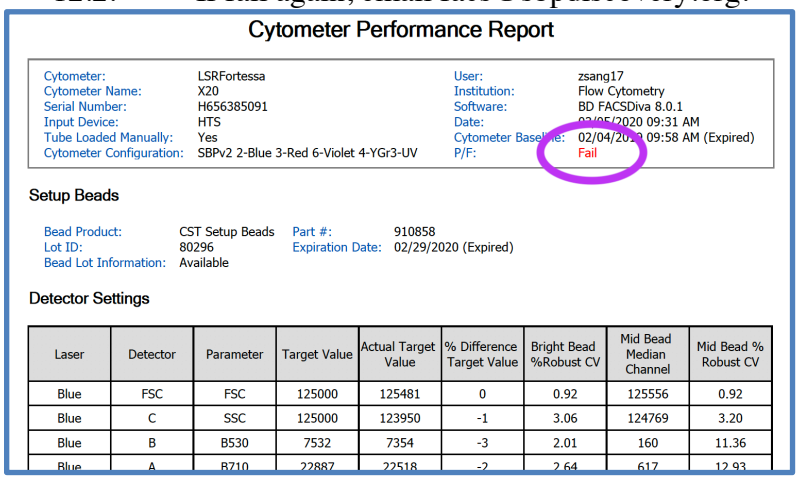




10. CST will detect beads and calculate results (can take up to 15min.) If liquid in tube gets too low before the CST completes, click the red “Abort” button, make a new tube of beads as instructed in step 4, and repeat steps 8-9.

11. When CST finishes, “Unload the CST bead tube” will pop up.
    11.1. Select “OK”.
    11.2. Unload the tube and put it back to the fridge for the next user.

12. A generated PASS/FAIL report will show stats for each channel.
    12.1. If fail, PRIME then rerun CST (using beads made today).
    12.2. If fail again, email facs@sbpdiscovery.org.



Cytometer Performance Report

Cytometer:	LSRFortessa	User:	zsang17
Cytometer Name:	X20	Institution:	Flow Cytometry
Serial Number:	H656385091	Software:	BD FACSDiva 8.0.1
Input Device:	HTS	Date:	03/05/2020 09:31 AM
Tube Loaded Manually:	Yes	Cytometer Baseline:	02/04/2020 09:58 AM (Expired)
Cytometer Configuration:	SBPv2 2-Blue 3-Red 6-Violet 4-YGr3-UV	P/F:	Fail

Setup Beads

Bead Product:	CST Setup Beads	Part #:	910858
Lot ID:	80296	Expiration Date:	02/29/2020 (Expired)
Bead Lot Information:	Available		

Detector Settings

Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead %Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	125000	125481	0	0.92	125556	0.92
Blue	C	SSC	125000	123950	-1	3.06	124769	3.20
Blue	B	B530	7532	7354	-3	2.01	160	11.36
Blue	A	B710	22887	22518	-2	2.64	617	12.93

13. Close the window

14. 3min bleach +3 min DI water clean, then proceed with your experiment.