SOP.UCF.046 – CYTEK AURORA TROUBLESHOOTING

1. The cytometer does not connect

To avoid connection problems, make sure you turn on the equipment by the recommended order (1st computer and Windows, 2nd cytometer, 3rd SpectroFlo).

If cytometer doesn't connect, close SpectroFlo and open again.

2. Acquisition error on the SpectroFlo software

a. Failed to connect with DAQ boards, due to computer losing connection with the instrument. Restart the computer.

3. Low number / no events displayed

a. Clumpy sample. Filter your sample right before you acquire, and make sure you agitate/vortex the tube before placing it in the SIT.

b. Sample line is touching the bottom of the tube. Run Calibrate SIT. Choose a value that ensures the sample line is not touching the bottom of the tube. The sample line will go down until it touches the bottom of the tube/well, then it will go up until the value you chose (make sure you are paying close attention on this step, since the sample line only stays on this position for about one second), and then it will retract all the way up. Keep testing and adjusting the value if needed.

c. Instrument is dirty or clogged. Run SIT Flush 2 times, followed by a Clean Flow Cell with Clean, and then a Clean Flow Cell with water. If the problem persists, repeat the process and contact the staff.

d. Laser delay not correct. Ensure the laser delay values match those from the latest Daily QC run. If they don't, re-run the QC.

e. Incorrect Threshold applied. Ensure the Threshold is set to the appropriate parameter (usually FSC) and value.

4. Distorted data in scatter parameters

a. Air bubble in flow cell. Run a SIT Flush.

b. Air in sheath filter. Run a Purge Filter and check if the sheath tank is empty. If it is, fill it and Run another Purge Filter.

c. Dirty flow cell. Run a Clean Flow Cell.

d. Low sample viability. Check viability of the cells.

e. Hypertonic buffers. Check the pH of the buffers and fixative.

f. Incorrect instrument settings.

5. Quality control issues

a. Cannot see the beads. There may be a bubble in the SIT: perform SIT Flush twice.

b. Beads show double peak. Re-make beads: ensure tube is clean, PBS is clean, and correct bead lot is added to the tube.

c. High rCVs

- Air bubble in fluidics – run a SIT Flush and a Purge Filter.
- Sample flow rate set too high – set the sample flow rate to Low or Medium.
- Dirty Flow Cell – run Clean Flow Cell; if it persists, run Clean Flow Cell using 30% Contrad 70, followed by DI water.
- Air in the sheath filter – Run a Purge Filter and check if the sheath tank is empty. If it is, fill it and Run another Purge Filter.
- Beads not diluted in the same fluid as the sheath (PBS).

d. QC does not complete

- Wrong QC bead sample – ensure you are running SpectroFlo QC beads.
- Beads not properly mixed.
- Beads too diluted – concentrate beads or prepare a fresh vial.
- Air bubble in the sample line – run a SIT flush.

e. QC fail

Examine the plots for each failing parameter. If today's result is an outlier, perform a fluidic startup by closing and re-opening SpectroFlo, and re-run the QC.

If it still fails, contact a UCF Staff member and, together, follow the next protocols and re-run QC after each of them until problem is solved:

- Air bubble in fluidics or in the sheath filter – Run a Purge Filter and check if the sheath tank is empty. If it is, fill it and Run another Purge Filter.
- Dirty flow cell – run Clean Flow Cell; if it persists, run Clean Flow Cell using 30% Contrad 70, followed by DI water.
- Beads not diluted in the same fluid as the sheath (PBS).
- (ONLY if recommended by a UCF staff member) Complete Fluidics Shutdown with 4 tubes of 30% Contrad, then power off

for 1-2 hours. Start unit up with water on SIT, perform 2 SIT flushes, run water for 5 min, run QC