General note

Please report all issues that you could **solve or not solve** using our feedback form. If you cannot eliminate the problem independently try to call one of the emergency numbers.

Questions / reporting technical problems:

Feedback form

Emergencies:

044 635 02 03
044 635 53 36

I. Only three lasers shown in BD Coherent Connect software

1) Computer was running before Symphony was switched on. Shut down Symphony and computers.
2) Start Symphony first, then the computers.

II. No events when on Acquire or Record.

1) Make sure the instrument is on “Run” and not in “Standby”
2) Double check FSC voltage and thresholds. Run FACSClean and set FSC to 400 V. At an FSC threshold of 5000 you should see events.
3) Load a different sample tube or a tube with beads. If you observe events now, check the previous tube for cracks or liquid on the inner tube rim, which can prevent tube pressurization.
4) Take tube off the instrument and ensure that the black o-ring above the sample needle is clean and dry. If the o-ring is wet or slippery with e.g. cleaning fluids due to overfilling of cleaning tubes, the tube cannot be presurized. Wipe o-ring with a wet tissue und dry it afterwards.

5) Take tube off the instrument and ensure that the grey conical plastic cap above the sample needle is tightened correctly. If it is loose, tighten it (finger tight is sufficient!).
   Note: Do not remove tubes from the instrument with a twist, this loosens the cap over time
6) Check the wet cart. Do you see any red light(s) on?
   - Yes: Exchange FACSFlow or/and waste tank(s) according to the “Wet Cart SOP”. If this does not help continue with **“Symphony run dry section”**.
   - No: Lift up the 20l sheath supply to double check the sheath level. If it is empty replace and continue with **“Symphony run dry section”**.

7) Unload tube and check if you see drops appearing at the sample needle tip (from backflush).
   - Yes: If you can see drops the sample needle is not clogged.
     Confirm by performing a "Prime" and check for the appearance of air bubbles.
     Note: Air bubbles are expected and normal during "Prime".
   - No: Confirm the instrument is on "Run". Take a water tube and dip the tip of the sample needle into the water (do not load the tube!) and press "Prime". Do you see a stream of air bubbles?
     - Yes: The sample line is free → Proceed to point 8).
     - Yes but very little: You probably have a partial clog of the sample needle.
       - Load a FACSClean tube, mark the liquid level with a marker and run on high flow rate for >10 min.
         Use Rinse if your sample contains lipids i.e. brain or fatty tissue.
       - Check for improved air bubble stream, then continue your experiment.
     - No: Sample needle is probably clogged.
       - Prime instrument 3x.
       - Run FACSClean on high flow rate. If you are getting events now continue washing with FACSClean for >10 min.
         Use Rinse if your sample contains lipids i.e. brain or fatty tissue.
       - Check for improved air bubbles, then continue your experiment.

   If no bubbles can be observed after standard cleaning procedure try these:

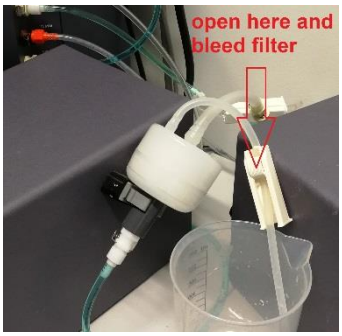
   - Try to unblock the sample needle by applying negative pressure.
     - Open the sample port arm. Connect a syringe (stored in Fortessa behind right door above control panel) via the silicon tube to the sample needle and pull the syringe plunger.
     - Liquid appearing in the syringe indicates that you have loosened the clog.
     - Perform a prime and install a tube with FACSClean.
       If you are getting events continue washing with FACSClean for > 10 min on high flow rate → 3 min FACSRinse → 1min H_2O → continue experiment. Otherwise repeat procedure.
   - Run FACSClean for extended time on high flow rate > 30 min
   - Carefully load a tube with hot water (>60°C, DI H_2O) run 5 min at high flow rate. Prime 1x and check if you see events after loading a FACSClean tube.
   - Run Contrad diluted 1:5 for extended time on high flow rate.

   If these interventions did not solve the issue you have either misdiagnosed another problem as a clog or you need somebody from the Cytometry Facility to unclog the machine for you!

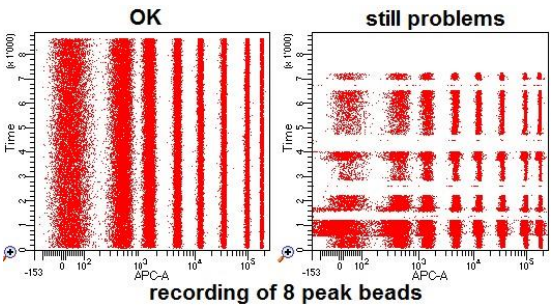
8) Set FSC voltage to > 550 V. Do you see events now?
   - Yes: Reduce FSC voltage to 400. Load a tube with 8 peak beads (check the fridge for "Rainbow Calibration Particles, 8 peak", if you have to prepare a fresh tube shake bottle vigorously, don't vortex, add 1 drop to ca 300 µl H_2O or PBS, then vortex).
     Do you get signals for scatter and fluorescent parameters?
     - Yes: Switch back to your sample and retry recording.
     - No: Restart Diva.

III. Symphony run dry

1) Does the wet cart show an alarm?
   - Yes: Is the alarm “waste full” or “FACSFlow empty”?
     a. Waste full: empty waste, if waste is actually empty remove sensor from waste tank, clean sensor with water and EtOH, wipe dry. Reinstall sensor, PRESS “RESTART” ON WETCART!
     b. FACSFlow empty: Check sheath supply and if empty refill with a fresh 20l box of FACS Flow, PRESS “RESTART” ON WETCART!
   - No: Check sheath supply. If the box is empty remove sheath sensor and clean sensor and lid from salt precipitates & liquid. If the 3 ends of the sensor touch each other carefully bend them apart. Install a new box of FACS flow, PRESS “RESTART” ON WETCART!
2) After pressing “Restart” button on wet cart all red alarm signs should turn off.
3) Press “Prime” button on the wet cart and hold until “Pump Fill” lamp turns off & pumping sounds stops (can take ~2-5 min if intermediate tank was completely empty).



4) Air bubbles in sheath line or sheath filter?
   Bleed the filter of the intermediate sheath tank by rolling open the rolling clamp until no air bubbles are visible, tip and tilt filter accordingly to pool and remove air bubbles.
5) Prime the instrument 3 x without a tube attached to the sample loading port.
6) If you see a continuous slow run of drops from the sample needle (backflush) the system is ready to use.
7) To be on the safe side run a control sample and plot one channel against time in a dot plot. A channel where you expect a strong fluorescence signal from most cells is most suitable otherwise use the FSC. If you observe a steady signal over some minutes the instrument runs correctly free of air. An unstable run points to remaining air bubbles in the fluidics. In this case, repeat bleeding the filter and perform additional priming of the instrument.



IV. There are events but signal is missing in one or more channels

1) If the Acquisition Dashboard shows events, but you see none displayed in the plots: Make sure you are in the Global Worksheet, or in a Normal Worksheet connected to your current tube. To toggle between Global and Normal Worksheets, press the leftmost icon in the Worksheet window.
2) Check the fridge for “Rainbow Calibration Particles, 8 peaks. If you have to prepare a fresh tube shake bottle vigorously, don’t vortex, add 1 drop to ca 300 µl H_2O or PBS, then vortex.
3) Create a new experiment, do not delete any channels from the “Cytometer Settings”, set FSC voltage to 400.
4) Generate at least one 2D plot for each laser with channels for the respective laser. Make sure you include the channel you want to test for.
5) Run beads and record some events. 8 peak beads show fluorescence in all channels and allow to distinguish up to 8 separate populations depending on the channel resolution.

Result 1: You miss signals in all channels that are excited by the same laser

- Reason a) The laser is not running. Check the laser status and output power in the BD Coherent Connect software.
- Reason b) The fluidics if unstable and the laser delay is off. Go to Cytometer window → laser tab → Increase window extension to 20, press enter. If you see signal now, you probably have fluidics instabilities. Proceed to II. “Symphony run dry”

Result 2: Only one or a few channels do not show 8 peak bead signals.

- Make sure that the voltage for these channels is at least 300 V.
- Otherwise it could be either a problem with the PMT of the respective channel or with an electronics board.
- Switch off the cytometer via the green button, wait about one minute and restart the instrument. If the problem persists you will need to contact the facility.

Result 3: 8 peak bead signals are observed in the channel that showed no signal for the sample.
→ Change back to your sample. If there is still no signal detectable the problem is probably the staining.

V. Symphony does not connect with Diva

1) Try to reconnect via “Cytometer” → “Connect” in Diva (wait 1 min).
   If this does not help:
2) Switch off the cytometer via the green button and turn off the computers.
3) Wait about one minute and then restart the Symphony and then the computers.
4) If necessary repeat a second time.