

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:

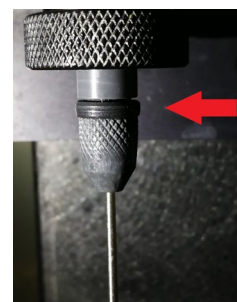
☎ 044 635 02 06

☎ 044 635 02 17

☎ 044 635 53 36

I. Fortessa does not show events!

- 1) Make sure the instrument is on “Run” and not in “Standby”
- 2) Double check FSC voltage. Run FACSClean at FSC 450, 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 this prevents pressurization of the tube. 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 “**Fortessa run dry section**”.
 - No: Lift up the 20l sheath supply to double check the sheath level. If it is empty replace and continue with “**Fortessa 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: 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 H₂O → 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 H₂O) 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 unplug the machine for you!

8) Set FSC voltage to 800. 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 H₂O or PBS, then vortex).

Do you get signals for scatter and fluorescent parameters?

- Yes: Switch back to your sample and retry recording.
- No: Close Diva and shut down the instrument via the main green power switch. Restart the computer and log in to Diva **before** restarting the instrument. If this does not help, the instrument requires technical inspection by Cytometry Facility staff.

II. Fortessa run dry!

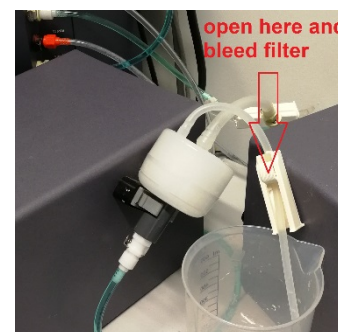
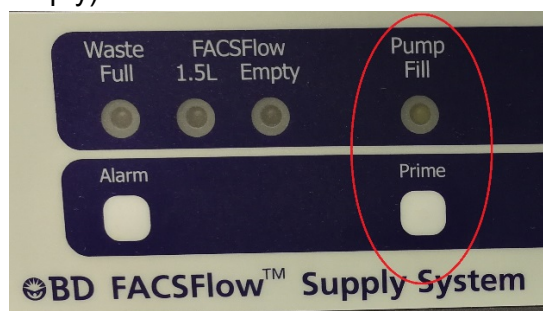
1) Does the wet cart show an alarm?

- 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!**

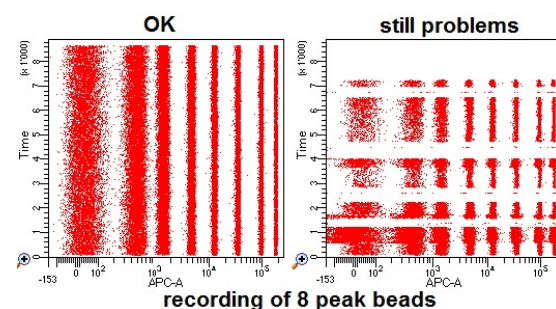
2) After pressing “Restart” button on wet cart all red alarm signs should turn off.



- 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.



III. There are events but I miss positive signals in one or more channels

- 1) 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 μ l H₂O or PBS, then vortex.
- 2) Create a new experiment, do not delete any channels from the “Cytometer Settings”, set FSC voltage to 400.
- 3) Generate plots that display channels which use the same excitation laser (e.g. PE+PE-Cy5 or FITC+PerCP). Make sure you include the channel you want to test for.
- 4) 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.

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

- Reason a) The fluidics if unstable and the laser delay is off. Go to Cytometer window → laser tab → Increase window extension to 20, press enter. Do you see signals now?
 - Yes? → You probably have fluidics instabilities!
Proceed to II. “Fortessa run dry!”
 - No? Reset window extension to original value and proceed to Reason b)
- Reason b) The laser is not running. → Switch off the cytometer via the green button (Minimum downtime for lasers 2 min before switching cytometer back on!).

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

- This can be either a problem with the PMT of the respective channel or an electronic board.
- Switch off the cytometer via the green button (Minimum downtime for lasers 2 min before switching cytometer back on!). If the problem persists you will need to contact the facility.

Result 3: Now I can see 8 peak bead signals in the channel that I had trouble with before

→ Change back to your sample. If there is still no signal detectable the problem is your staining.

IV. Fortessa does not connect with computer!

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

Minimum downtime for lasers 2 min before switching cytometer back on!

3) In the meantime close Diva and restart the computer.

4) Log into the computer with your core domain login, start the Diva software and log into Diva with your Diva login.

5) Now switch the cytometer back on via the green button and wait for software and cytometer to connect. If necessary repeat a second time.