General

Registration with the Flow Cytometry Facility, as well as attending the technical introduction by the facility staff is a prerequisite to use the Cytek Aurora.

Loading more than 3 ml of sample or cleaning fluid leads to carry-over and hardware damage. Please stick to the indicated volumes in the SOP for each step.

Questions / reporting low-priority issues:

Feedback form

Emergencies:

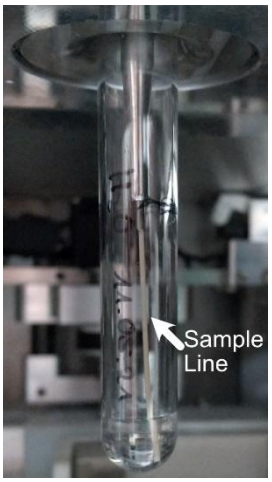
079 252 27 65
079 831 55 96

I. First user of the day: Startup, Daily QC

1. **Start the instrument PC and the support PC**. Log in to the support PC with your core domain user account (e.g. John Doe: username = j.doe).
2. **Check that a water tube is still loaded on the SIP to trigger the connection.**
3. Start the instrument by pressing the button on the left side of the instrument.
4. Start the SpectroFlo Software and **log-in** with user: **Aurora**, password: **borealis**.
5. Open the "Acquisition" module.
6. Make sure that the "Cytometer" indicator in the software (bottom right) has changed from yellow to green and the SIT has retracted into the instrument before removing the water tube:

   Sheath Waste Cytometer

   Red not connected, yellow busy with sample line positioning, green ready to remove tube.



7. **Warm up:**
   - **Let the optics warm up for 30 min.** (Proceed with step 7 while waiting)
     The hardware warmup time only starts after the instrument is connected to SpectroFlo.
     After starting the software, do not close the software again!
   - **Warm up time is used to clean and prepare the instrument for the QC run.**
     During the warmup time, signal intensities and CV values will still change.
     The state in which the QC is run affects settings for all users for the rest of the day.
   - **Never skip the warmup and cleaning before QC!**
8. **Cleaning:**
   - Incubate the instrument with Contrad:
     - Run "Clean Flow Cell" ("Cytometer" in menu on the left) with 2.5 ml of 10% Contrad twice (instead of Bleach + ddH_2O).
     - Wait at least 20 min! Do not perform fluidics operations during that time.
     - Re-run a "Clean Flow Cell" with 2.5 ml ddH_2O twice.
   - Record a water run: Acquisition tab → My Experiments → Open experiment "Water runs" → Sample Group "First user water run" → create a new tube → name it with today's date and your initials (Format 2026-11-01-XY) → Refill water tube with ~1 ml ddH_2O and record for 3min at high flow rate.
   - Event rate may start high and should decrease to ~10 events/s at the end of the recording. If event rate stays high, rerun with a fresh tube of water.

9. **Daily QC (run >30min after software was started):**
   - Run SpectroFlo QC beads (stored in the fridge next to the machine)
     Check if diluted bead tube from previous run is available, otherwise prepare fresh beads.
     To prepare fresh beads: Shake bottle very well (do not vortex bottle) → add 1 drop of beads to 0.3 ml FACSFlow (do not use ddH_2O!) → vortex bead tube. Return remaining beads to fridge after QC.
   - Run QC: Go to "QC & Setup tab" (top right) → "Daily QC"
     Attach the QC bead tube to the SIP, check bead lot number and start the "Daily QC".

If the QC fails:

- Did you prepare the beads in FACSFlow? Don't use water!
- Did you run the QC before the 30 min warmup time was finished?
- Did you perform the required cleaning steps and water run? Did the water run look clean (i.e., less than 10 events / sec at the end of the run)?
- Low bead concentration (less than 100 evts/sec)? Did you shake bead bottle well enough? Old beads? Prepare fresh beads and re-run QC.
- If none of the above matters, restart the instrument by switching off/on the main power button and rerun QC.
- Please report failed QC via the feedback form. Contact us for further help.

II. In between users: Login and New/Template Experiment

1. Log into the instrument PC with your core domain user account. You will be automatically connected to the instrument PC and SpectroFlo should be running. If SpectroFlo was closed by previous user, restart it and run water (in the Water Run Experiment) for 10 min to get the lasers, fluidics and optics running. (please send report via the feedback form if this happened).
2. Go to "QC & Setup tab" (top right) → "Daily QC" and check that the daily QC had been run and passed for the day.
3. Select the "Acquisition" tab.
4. Create a "New" experiment" and go through the tabs to fill in the relevant info.
   - "Fluorescent Tags": Add all the dyes in your panel
   - "Groups": Create a reference group with all the single-color controls either prepared on cells/beads, and create sample group/s.
   - "Markers": It may help you to add the markers for the respective dyes here.
   - "Acquisition": Set the number of events to record and additional parameters for the acquisition.
   - "Keywords": Can be skipped usually.
5. You can alternatively impot a previously created template experiment:
   - To import template/previous experiments, go to "My experiments" → Import "the zipped experiment file". Large experiment files should be copied to the local drive ("D:\Temp Data Export") before importing to avoid long waiting time.
   - Confirm that the "User Setting" (i.e., the FSC/SSC detector gains) was automatically applied to your experiment.

   Note: To speed up the experimental setup, you may use the virtual machines to prepare your experiment template beforehand.

III. Acquisition

Note: The sample line may be a little bent. If you are afraid, it may affect your acquisition, please reach out to the facility staff for help.

1. Make sure you have **filtered and vortexed your samples** well.
   Filter your samples using a filter appropriate for your cell size to remove larger aggregates (typically 35 – 70 µm mesh size).
2. Attach your **sample tube** to the SIP. Never load tubes with more than 3 ml fill level!
3. If you are running low sample volumes (<100 µl), you should turn off the **Fluidic Boost** (Preferences → Cytometer → Fluidics → Tick box 'Skip Fluidics Boost when…').
   Note: This only works for Medium and High flow rates; Fluidics Boost is always activated by default on Low flow rate. Fluidic Boost facilitates faster stable acquisition, but you'd lose 35 µl of your sample.
4. Check that the green arrow is activated on the correct tube.
5. Press "**Start**" to start acquiring samples.
6. Change **sample flow rate** if needed. High flow rate results in lower resolution.
   Low: 10 µl / min Medium: 30 µl / min High: 60 µl / min
   To change the flow rate during recording, you need to pause the recording.
   If you run into an *"Acquisition Error: bubble or clog is detected"* → stop the run → remove the tube for the automated SIT flush → re-run the tube.
7. Adjust the **gains for FSC & SSC** to observe your cells on scale. FSC: 70 is a good starting point.
8. Default **gain settings for the fluorescence channels** work for most antibody-staining. If you have a bright intrinsic marker (e.g., GFP, tdTomato) or highly autofluorescent samples, you may need to lower the fluorescence detector gains.
9. Record your single-color controls.
10. Click on "**Unmix**", perform the necessary gating and select "Live Unmixing" at the end.
11. Create a new **unmixed worksheet** to make your plots.
12. Record your samples and view them on the unmixed worksheet.
    Note: To automatically open the unmixed worksheet, save the worksheet, and apply it to the Acquisition Table (Edit → Acquisition → Worksheet → Select your saved unmixed worksheet for your samples).

IV. Fluid Supply and Waste Handling

If you get a notification on the software to exchange the sheath or waste:

Empty FACSFlow container:

1. Unscrew the lid from the empty FACSFlow container (blue tubing).
2. Replace the empty container with a fresh one.
3. Insert the tubing into the new container and screw the cap on.
4. Double check that you did not accidentally disconnect the connectors on the lid.
5. Leave the empty container next to the instrument as a future waste tank.

Full waste container:

1. Unscrew the lid from the full waste container (orange tubing).
2. Attach the waste lines to an empty container. **Push the cap in** to avoid overfilling the tank!
3. Put the full waste by the sink and add 200 ml Na-hypochlorite (Bleach/Javel). Please label the container with "Bleach added + date".

V. Data Saving and Export

Data Saving:

1. Save the worksheets you want to keep and close (without saving) those you don't need.
2. Under the Instrument Control, save the 'User Setting' and rename it.
3. To apply all changes, click Save on the top left.
   Note: Do not overwrite the Default Worksheets. To restore go to Library → Worksheet Template → Scroll down to the Default Worksheets → Click 'Restore Default'.
4. Close experiment.

Data Export:

1. Go to "My Experiments", select your experiment and export.
2. Export the experiment to the D: drive in the local D:\Temp Data Export folder.
3. Copy the exported experiment "ZIP" file to the server: files.core.uzh.ch (Z:) → data → your personal folder
4. All files will be deleted during the monthly maintenance (dates can be found on the instrument booking calendar).

QC Export:

If you plan to reuse your reference controls for future experiments, you will need to export the QC data as described in the QC export/import SOP.

Note: For safety reasons, the use of USB sticks is blocked, data export is only possible via our server.

VI. Template Creation and Export

1. Go to "My Experiments", right click and "Duplicate without Data"
   (Optional: you may keep the Reference Group Data to reuse single-stained controls).
2. Export empty experiment as described above in the "Data Export" section.

VII. Cleaning after your experiment

Note: Do not exceed tube fill levels of 3 ml!

1. **Every user must clean the instrument after their acquisition**. If you are running problematic samples, you might have to clean in between samples to avoid clogging.
2. Run a "Clean Flow Cell" (Cytometer tab) with 2.5 ml FACSClean and 2.5 ml ddH_2O.
3. Record water run: Acquisition tab → My Experiments → Open experiment "Water runs" → Sample Group "All users water run" → create a new tube →name it with today's date and your initials (Format 2026-11-01-XY) → Refill ddH_2O tube and record for 3min at high flow rate.
4. If you observe events from your previous samples, repeat the Clean Flow Cell and rerun the water recording.

VIII. Handover / Shutdown

1. Check the instrument booking calendar to see if somebody is booked after you.
2. **Between different users of the day:**
   a) Clean the instrument (see Cleaning).
   b) Do NOT close the SpectroFlo software. Do NOT log out of the instrument PC. Minimize the blue bar on top of the screen.
   c) Log out of the support computer using the shortcut on the desktop. Please note that your log out time may be used for billing.
   d) Leave instrument and computers on for the next user & clean the work area.
3. **Last user of the day:**
   a) Clean the instrument (see Cleaning).
   b) Perform a "Daily Clean" (Cytometer tab). Shut down the instrument when prompted to by the on-screen message.
   c) Make sure that the water tube remains attached to the SIP, the SIT is lowered to the tube bottom, and the SIT tip should be submerged in water.
   d) Using the shortcut on the desktop, switch off the instrument PC, followed by the support PC. Please note that your log out time may be used for billing.
   e) Clean the work area.

IX. Booking cancellation as first/last user

- If you cancel or do not use your booking as the **first user** of the day, inform the user after you that they can factor in the additional time needed for the startup.
- If you cancel or do not use your booking as the **last user** of the day, inform the user before you, to perform the fluidic shutdown and shut off the instrument.

Note: Violation of the SOP rules will result in penalty points (see SOP "Penalty points")

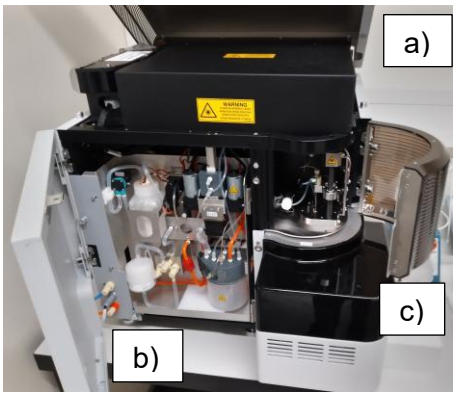
X. Troubleshooting

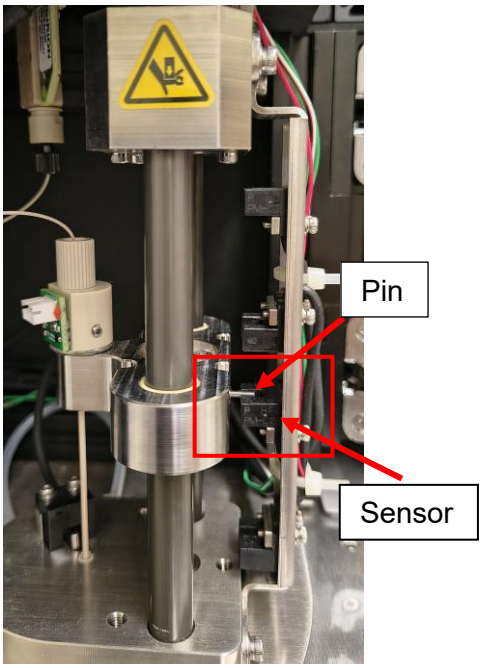
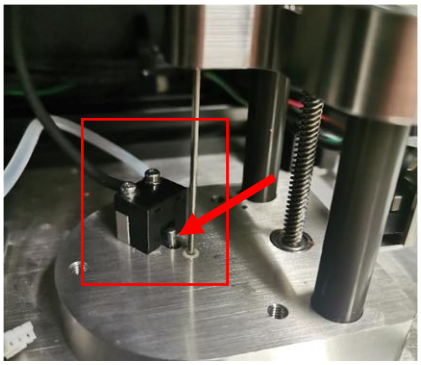
- Software does not connect with the instrument.
  → Check that a tube is attached at the SIP.
- No or little liquid is taken up during the Clean Flow Cell or Fluidics Shutdown procedures.
  → Run SIT Flush and repeat the cleaning procedure.
- You finished the liquid in your sample, but the machine continues recording on high event rate.
  → Verify that **bubble/clog detection** is enabled (Preferences → Cytometer → Fluidics).
- The measured sample flow rate is significantly lower than the defaults (10/30/60 µl) or you are getting frequent "Clog detected" warnings.
  → **increase** the "**SIT Lift Distance**" from the default 0.75 to 1 (menu on the left "Cytometer" → Calibrate SIT →SIT Lift Distance).
- The machine repeatedly reports a bubble = empty tube, but there is always still sample left in the tube.
  → **lower** the "**SIT Lift Distance**" (menu on the left "Cytometer" → Calibrate SIT →SIT Lift Distance).
  Check that the sample line does not bend during acquisition (indication for touching the bottom!).

- The sample line is not lowered anymore when trying to acquire samples and the instrument makes a **loud grinding noise**. → **Switch off the instrument as fast as possible**!

After instrument is switched off you need to manually reset the sample line position.

1. Open the instrument
   a) lift the cover
   b) open the left door
   c) open the rounded black door.
2. Hold the shiny metal sledge and move the sample line down (yellow arrow). The pin protruding on the right should be just above the lower sensor at half height (red box).
3. Attach the water tube to the SIP.
4. Turn on the instrument, wait for connection and continue recordings.





- Software reports that a tube is attached even though there is none.
  → Manually push down the tube sensor flag as detailed below.
  1. Open the instrument (see steps a-c above).
  2. Push the sensor flag down (red arrow).