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 technical problems:

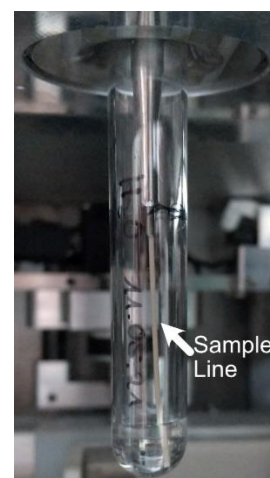
Feedback form

Emergencies:

079 252 27 65 or 079 83155 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. For instance, 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. **Warm up:**
   - **Let the optics warm up for 30 min.** (Proceed with step 7 while waiting)
     The hardware warmup time only starts after login into the SpectroFlo software!
     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 instrument QC is run affects settings for all users for the rest of the day → Never skip the warmup and cleaning before QC!
   - 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. **Cleaning:**
   - Incubate the instrument with Contrad:
     - Run a "Clean Flow Cell" ("Cytometer" in menu on the left) with 2 x 2.5 ml 30% Contrad (ignore the prompt to load Bleach + ddH$_2$O instead).
     - Wait at least 20 min! Do not perform fluidics operations during that time.
     - Run a "Clean Flow Cell" with 2 x 2.5 ml ddH$_2$O.
   - On 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 2020-12-04-XY) → Refill water tube with ~1 ml ddH$_2$O and record for 3min at high flow rate.
   - Event rate usually starts high and should decrease to ~10 events/s at the end of the recording. If event rate stays high, prepare a fresh tube with water and rerun.
8. **Daily QC (run >30min after software was started):**
   - Run SpectroFlo QC beads (in the fridge in the hallway): Check if diluted bead tube from previous run is available, otherwise prepare fresh beads.
     Preparing fresh beads: Shake bottle very well (do not vortex bottle) and add 1 drop of beads to 0.3ml FACSFlow (do not use ddH$_2$O!) → vortex bead tube. Return remaining beads to fridge after QC.

- Run QC: Go to "QC & Setup tab" (top right) → "Cytometer QC" (left) → "Daily QC"
  Attach the QC bead tube to the SIP, select the correct bead lot 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. For instance, John Doe: username = j.doe. After logging in, you will be automatically connected to the instrument PC and SpectroFlo should be running. If SpectroFlo was closed by previous user, restart it but wait for >15min before you start recordings (please send report via the feedback form if this happened).
2. Go to "QC & Setup tab" (top right) → "Cytometer QC" (left) → "Daily QC" and check that the daily QC was run by the first user and passed.
3. Select the "Acquisition" tab.
4. Create a "New" experiment" or use an imported "Template". If you want 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. 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 this affects 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, 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. Verify that **bubble/clog detection** is enabled (Preferences → Cytometer → Fluidics).
5. Press "**Start**" after activating the correct tube in SpectroFlo (green arrow).
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.
7. Adjust the **gains for FSC & SSC** to observe your cells on scale. FSC: 50 is a good starting point.

8. Optimize the **gains for the fluorescence channels** if necessary. Default settings work for most antibody-staining.
9. Record your single stains.
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).

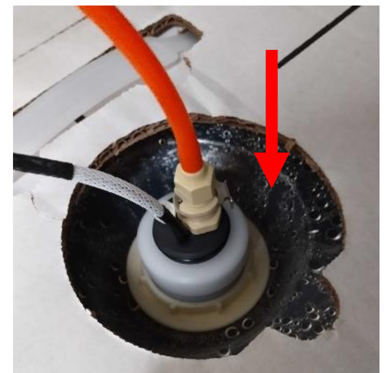
IV. Fluid Supply and Waste Handling

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.

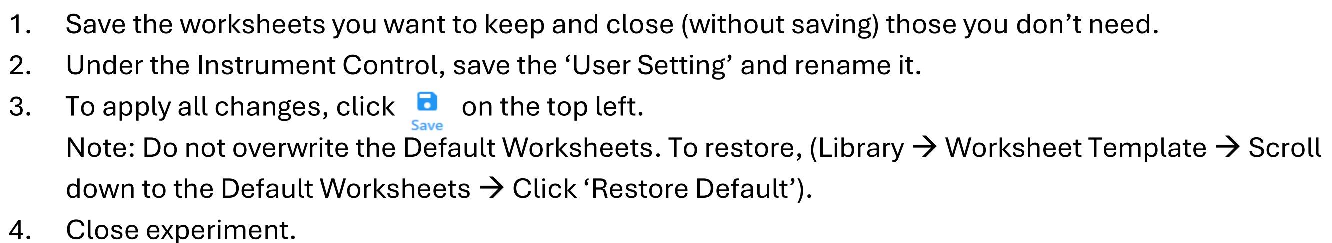
Full waste container:



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. Bring the full waste to the cell culture lab (44G03H) and add 200 ml Na-hypochlorite (Bleach/Javel). Please label the container with "Bleach added + date".
4. Leave the waste to incubate overnight.

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, (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 your data folder on files.core.uzh.ch.
4. All files will be automatically deleted once a month.

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 template experiment as described above.

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. On 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 2020-12-04-XY) → Refill ddH_2O tube and run 3 min ddH_2O at high flow rate and record this water run.
4. If you observe events from your sample, repeat the Clean Flow Cell and rerun the water recording.

VIII. Handover / Shutdown

1. Check the instrument booking calendar if somebody is booked after you.
2. **Between different users of the day:**
   a) Clean the instrument (see Cleaning).
   b) Make sure that a water tube remains attached to the SIP.
   c) Do NOT close the SpectroFlo software. Do NOT log out of the instrument PC. Only disconnect the session by minimizing the blue bar on top of the screen.
   d) Log out of the support computer. Please note that your log out time may be used for billing.
   e) 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 "Fluidics Shutdown" (Cytometer tab). Shut down the instrument when prompted to by the on-screen message.
   c) Make sure that a 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) First, switch off the instrument PC, then 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 run the fluidic shutdown and shutoff the instrument.

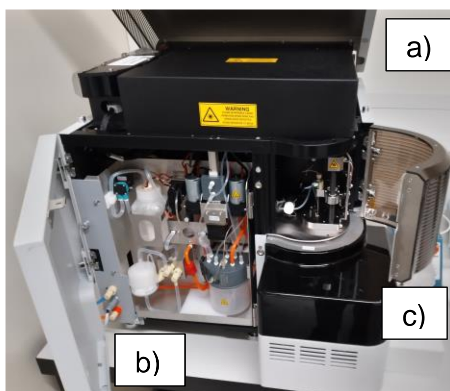
Note: Violation of the SOP rules will result in penalty points (see SOP "Penalty points")!

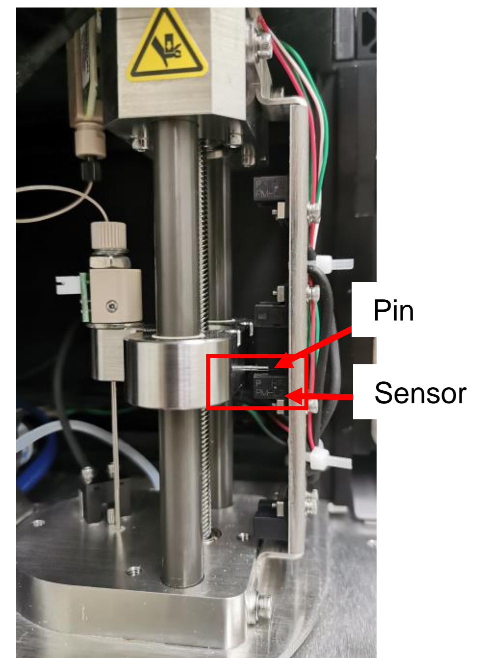
X. Troubleshooting

- 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.
- 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).
- Cells are not taken up well by the machine (relevant for larger cells)
  →Optimize the SIT position for your tubes (Cytometer → Calibrate SIT).
  Perform a Calibrate SIT with a higher Lift Distance. Default value is 0.75 for tubes.