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: Emergencies:

⌨ **Feedback form**   ✆ **044 635 02 03**
          ✆ **044 635 02 06**

I. Startup, Daily QC and Waste Disposal

Special duties for the first user of the day:

1. Check if there is a waste tank in decontamination on the sink from the day before. If yes, empty it into the sink.
2. **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. After logging in, you will be automatically connected to the instrument PC.
3. **Check that a water tube is still loaded on the SIP.**
4. Start the instrument by pressing the button on the left side of the instrument.
5. Start the SpectroFlo Software and **login** with user: **Aurora**, password: **borealis**.
6. After starting the software, do not close the software again.
7. Open the "Acquisition" module.
8. **Warm up, cleaning and QC run:**
   - **Let the optics warm up for 30 min.**
     The hardware warmup time only starts after logging into the SpectroFlo software!
   - During the warmup time, signal intensities and CV values will still change.
     Warm up time is used to clean and prepare the instrument for the QC run.
     Important: 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:

Sample Line

     ✅ **Sheath**   ✅ **Waste**   ✅ **Cytometer**

     Red not connected, yellow busy with sample line positioning, green ready to remove tube.
   - Incubate the instrument with Contrad:
     - Run a "Clean Flow Cell" ("Cytometer" in menu on the left) with 2 x 2.5 ml 30% Contrad (instead of Bleach + ddH$_2$O).
     - 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 Acquistion 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 ddH$_2$O tube to contain about 0.5 ml and run 3 min ddH$_2$O at high flow rate and record this water run.
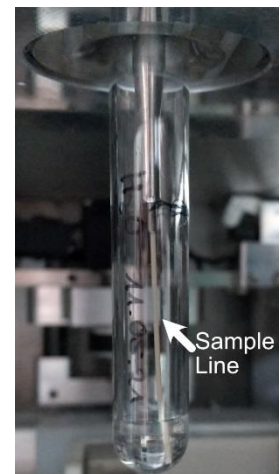
- Prepare SpectroFlo QC beads (in the fridge in the hallway): Check if diluted bead tube from previous run is available, otherwise prepare fresh beads.
  Fresh beads: Shake bottle very well by hand (do not vortex bottle) and add 1 drop of beads to 0.3 ml FACSFlow (do not use ddH$_2$O!) → vortex bead tube. Volume is sufficient for about 3 QC runs. Return remaining beads to fridge after QC.
- Note: While you wait for the complete 30 min warm up time to finish you can now begin to set-up your experiment before running the daily QC. You can use the virtual machines to prepare your experiments beforehand and import them when you come to the instrument.
- Select the correct Bead Lot for the current beads.
- 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? Add another drop of beads to the tube.
- Too high CV value warnings? Run a "Clean Flow Cell" and try running the QC again.

II. Login, Software Start and Import

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.
2. Log in to the SpectroFlo Software with user: **Aurora**, password: **borealis**. Never close the software unless you must restart it due to technical problems.
3. 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.
4. If you want to import previous settings etc., go to the "**Library**" module and select
   - o "User Settings" to import instrument gain settings UST files.
   - o "Worksheet Templates" to import plot worksheets WTML files.
   - o "Experiment Templates" to import experiment layout zip files.
   - o "Loader Settings" to import settings for the plate loader files.
5. Select the "Acquisition" tab.
6. Create a "New" experiment" or use an imported "Template".
7. Open "Instrument Control" and select your imported "User Settings" if applicable.
8. Switch to "Worksheets" (on the left) and create new worksheets as necessary or "Open Worksheet Template".

III. Acquisition

Note: The sample line is not perfectly straight and can appear to be slightly curved. This is normal. But notify us if you see any kinks in the sample line.

1. Make sure you have **filtered and vortexed your samples** well.
   Filter your samples using a filter mesh size appropriate for your cell size to remove larger aggregates (generally ca 35 – 70 µm).
2. Attach your **sample tube** to the SIP. Never load tubes with more than 3 ml fill level!

3. **Calibrate the SIT** position for your tubes (Cytometer → Calibrate SIT). Only needed if using other tubes than standard 12 x 75-mm FACS tubes like the small inlay tubes. The tube height must not differ more than a couple of millimeters from standard FACS tubes! Standard value for most applications is 0.75.
4. If you have low sample volumes, you can turn of the **Fluidic Boost** (Preferences → Cytometer → Fluidics) that delivers your sample faster to the flow cell but can consume up to 35 ul of sample. Skip Fluidic Boost only works for Medium and High flow rates, not for Low.
5. Verify that **bubble/clog detection** is enabled (Preferences → Cytometer → Fluidics)
6. Press "**Start**" after activating the correct tube in SpectroFlo (green arrow).
7. Adjust **sample flow rate** if needed. Lower flow rates result in higher resolution.
   Low: 10 µl / min   Medium: 30 µl / min   High: 60 µl / min
   To adjust the flow rate during recording, you have to pause the recording.
8. Adjust the **gains for FSC & SSC** to observe your cells on scale.
9. Optimize the **gains for the fluorescence channels** if necessary.
10. After recording your single stains, click on "**Unmix**", perform the necessary gating and select "Live Unmixing" at the end.
11. Create a new **unmixed worksheet** for your samples, if needed, to see the unmixed signals.
12. Record your samples.

IV. Fluid Supply and Waste Handling

- **Empty FACSFlow container:**
  1. Unscrew the lid from the empty FACSFlow container (blue lines). Take the tubing out of the container.
  2. Replace the empty container with a fresh one. If no new FACSFlow containers are left under the table, get one from the Fortessa room (WAD-K-126).
  3. Insert the tubing into the new container and screw the cap on**.**
  4. Double check that you did not accidentally disconnect the quick 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 lines). Take the sensor out of the container.
  2. Attach the waste lines to an empty container. **Push the cap in** to avoid overfilling the tank!
  3. Put the full waste to the sink next to the cell sorter and add 200 ml Na-hypochlorite (Bleach/Javel). Remember to label the container with "Bleach added + date".
  4. Leave the waste to incubate overnight.

V. Template Creation and Export

We recommend saving all templates and settings to your home directory on files.core.uzh.ch.

- To save gain settings:
  o Select "Save As" in the instrument controls.
  o Go to "Library → User Settings" and export the settings.
- To save worksheet templates:
  Note: worksheets are also saved in experiment templates.
  o Go to the "Worksheet" tab and select "Save As" to save the worksheets you want to export.

- o Go to "Library → Worksheet Templates" and export the worksheet.
- To save experiment layouts:
  Note: experiment templates do not contain the gain settings!
  - o Select "Save As" for your experiment.
  - o Go to "Library → Experiment Templates" and export the template.

VI. Data Export and DB Cleanup

Note: Delete all your data from the database before you log off!

1. Under "Worksheets", save those worksheets you want to be included in your export.
2. Under "Acquisition", close your experiment ⊗ (if still open). Only save those Worksheets and User Settings that you want to keep. **Do not overwrite the Default Worksheets!**
3. Go to "Acquisition" and select "My Experiments".
   a) For smaller datasets (< GB):
      i. Select your experiment from the list and click "Export". Export the experiment directly to your data folder on files.core.uzh.ch.
   b) For larger datasets (> GB, can take very long to export otherwise):
      i. Export the experiment to the D: drive in the local D:\Temp Data Export folder.
      ii. Copy the experiment "ZIP" file to your data folder on files.core.uzh.ch.
      iii. All files in the local "Temp Data Export" folder will be automatically deleted once a month.
4. Right-click on your experiment(s) and select "Delete".
5. Go to "Library" and export your saved "User Settings" (if applicable).
6. Go to "Library" and delete all templates that you created under "User Settings", "Worksheet Templates" and "Experiment Templates".
7. Before closing SpectroFlo, make sure to remove all your experiments, templates and settings from the database! Do not delete the "Water runs" experiment.
8. If you run experiments over multiple days using the same reference controls you will need to export the QC data as described in the QC export/import SOP on our website.

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

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. Calibrate the SIT for normal FACS tubes (Cytometer → Calibrate SIT). Only needed if you worked with plates or non-standard tubes before.
3. Run a "Clean Flow Cell" (Cytometer tab) with 2.5 ml FACSClean and 2.5 ml ddH$_2$O. Wipe off any spills from the SIP before switching to ddH$_2$O.
4. On Acquistion 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$_2$O tube and run 3 min ddH$_2$O at high flow rate and record this water run.
5. Attach a tube with clean ddH$_2$O and acquire it at high flow rate. No fixed number of maximum event count yet. For now, just make sure that no events from your sample show up.

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) Delete your experiments, settings and templates in SpectroFlo.
   d) Do NOT close the SpectroFlo software. Do NOT log out of the instrument PC. Only disconnect the session by closing the blue bar on top of the screen.
   e) Log out of the support computer. Please note that your log out time may be used for billing.
   f) Leave instrument and computers on for the next user.
   g) 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 is submerged in water.
   d) Delete your experiments, settings and templates in SpectroFlo and close the software.
   e) First, switch off the instrument PC, then the support PC. Please note that your log out time may be used for billing.
   f) 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: Any violation of these rules will result in penalty points (see SOP "Penalty points")!
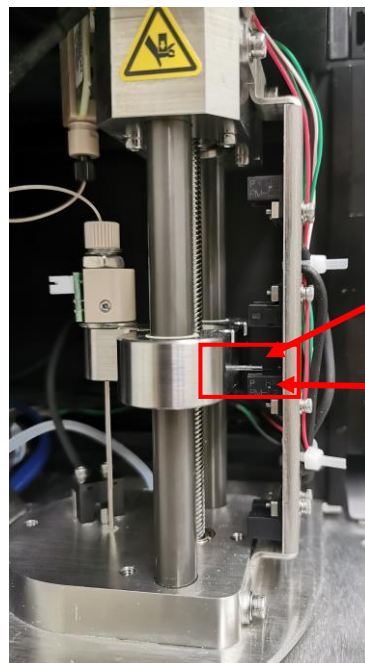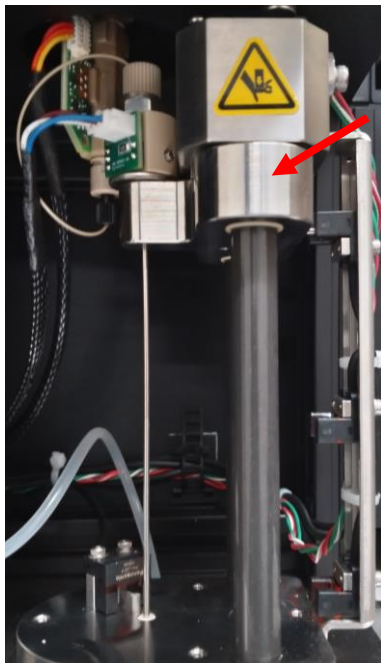
X. Troubleshooting

- 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.
- Software thinks that a tube is attached even though there is none.
  → Manually push down the tube sensor flag as detailed below.
    1. Open the instrument to access the tube sensor: a) lift the cover, b) open the left (white) door, c) open the rounded black door.

2. Push the sensor flag down (red arrow).
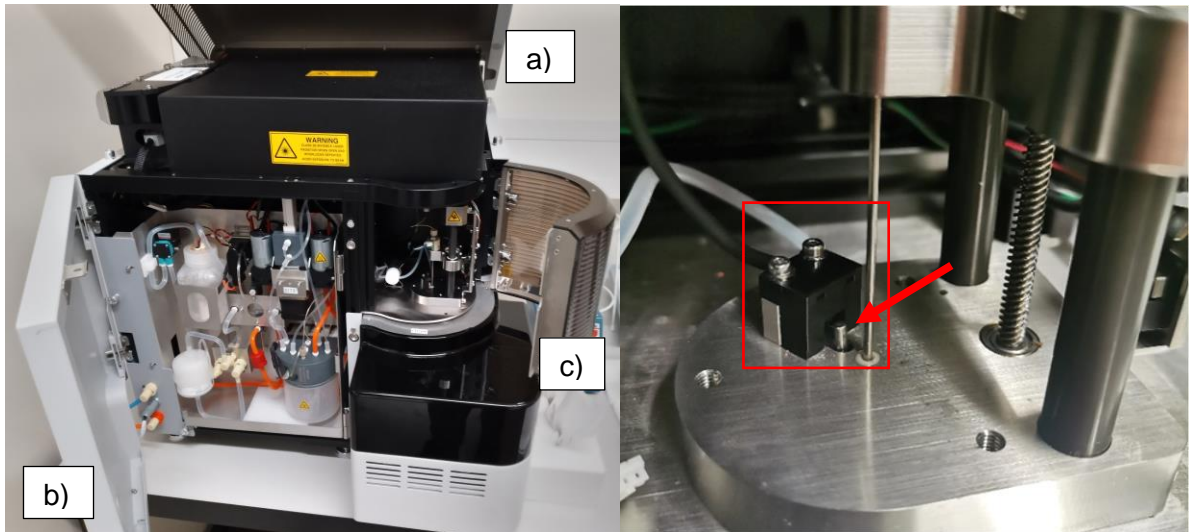
- The sample line is not lowered anymore when trying to acquire samples and the instrument makes a loud grinding noise after running a Calibrate SIT.
  → Manually reset the sample line position.
     1. Switch off the instrument as fast as possible!
     2. Open the instrument (see above).
     3. Hold the shiny metal sledge and move the sample line down (red arrow). The pin protruding on the right should be just above the lower sensor at half height (red box).
     4. Turn on the instrument.