STANDARD OPERATING PROCEDURE (SOP)

Cytek AURORA - Operation

Document No:	CAF-SOP-005-V6	Revision Date:	02.06.2026

Policy, purpose and responsibilities

- The goal of this SOP is to ensure a proper operation of the specified instrument.
- Following theoretical and practical introduction courses are mandatory prerequisite to use the instrument.
- The users must accept and sign the usage policies from the CAF before any instrument usage.
- Please immediately report any technical problems or questions to:

cellanalyticsfacility@unifr.ch

or

Sarah.cattin@unifr.ch

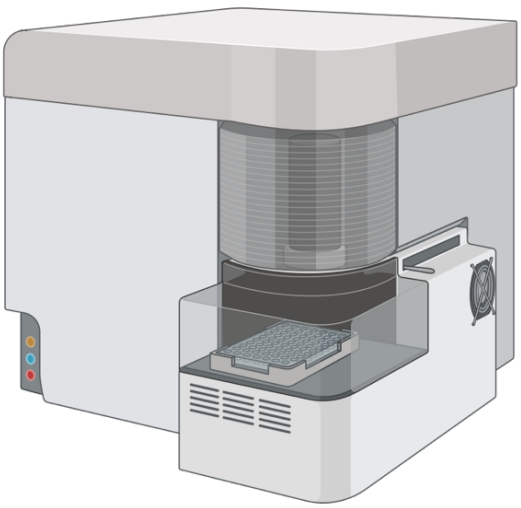
026/300.85.79

- Instrument is deeply cleaned and performance are checked by the CAF each Monday. QC information and follow-up in time are available in the bigdata server, in the CAF folder, open to all users to consult.

Instrument procedure

I. General information for the Cytek Aurora

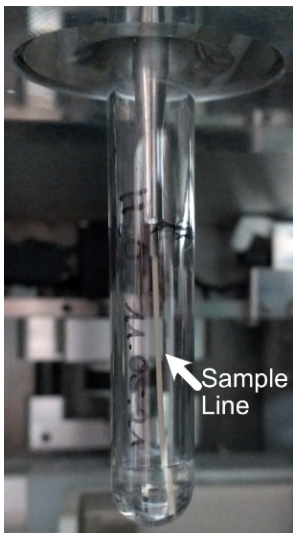
- The Cytek Aurora is a full spectrum flow cytometer equipped with 5 lasers and 64 detection channels. Samples can be injected using an automatic sample loader as well as manually.
- The instrument is running with FACSFlow (PBS) as sheath fluid.
- The loader can be used with a 40-tube rack (for 5ml standard tubes), 96-wells plates and 96- deep wells plates formats.
- Expected flow rate during acquisition are:
  - *Low: 15 ul/min*
  - *Medium: 30 ul/min*
  - *High: 60 ul/min (100 in plate mode)*
- In general, the maximum recommended sample concentration for analysis is 1×10^6 cells/mL.
- Data acquisition rate is up to 35'000 events/sec, but you might see a spread of the data starting at 10'000 event/sec in certain cases.



II. Instrument start-up

- First user of the day: Start-Up
  - Check the level of the sheath fluid and replace with a new box if needed (lift to check).
  - Empty the waste in the red containers
  - Check that a water tube is still loaded on the SIP.
  - Turn on the computer, log into windows. **Password = Welcome#1**
  - Start the instrument by pressing the button on the left side of the instrument.
  - Start the SpectroFlo Software and login with **username = labo_XXX (PI family name), password= XXX (PI family name)**
  - After starting the software, do not close the software again. The hardware connection and warmup time only starts after logging into the SpectroFlo software!
  - Open the "Acquisition" module.
  - 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.

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



  - If loader needed, switch it ON using the side button (dedicated section page 7)
  - On the instrument is connected and the sample line did retract from the water tube, run water during optical warm-up time:
    - On Acquisition tab → Default
    - Insert a 3ml tube with water and run on "high speed" for 20min

- First user of the day: Instrument QC
  - The QC must be run every day of usage of the instrument!
  - Prepare SpectroFlo QC beads (in the fridge next to the sorter):
    - Check if diluted bead tube from previous run is available, otherwise prepare fresh beads.
    - Fresh beads: Vortex bottle and add 1 drop of beads to 0.3 ml FACSFlow (do not use ddH2O!) → vortex bead tube. Volume is sufficient for about 3 QC runs. Return remaining beads to fridge after QC with tight close cap and date on the tube.
  - 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".

- QC can be run under 40tubes rack mode or single tube manually!

- If the QC fails:
  - *Did you prepare the beads in FACSFlow? Don't use water!*
  - *Low bead concentration (less than 100 evts/sec)? Did you shake bead bottle well enough? Add another drop of beads to the tube.*
  - *You used diluted beads ready? Retry preparing the beads freshly*
  - *Too high CV value warnings in the last part of each laser? The cytometer is dirty Run a "Clean Flow Cell" and try running the QC again.*

- Following users:
  - Check the level of all solutions and refill/empty if needed.
  - Log In with your Account:
    - Username = labo_XXX (PI family name), password = XXX (PI family name)
  - All QC information and follow-up in time are available in the bigdata server, in the CAF folder, open to all users to consult.

III. Acquisition workflow

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.

- Open/create an experiment:
  - Select the "Acquisition" tab
  - If your template is ready or if you want to open a previous experiment, go under "My Experiments", then "import" or "Open" the corresponding experiment.
  - Only zipped experiment (by SpectroFlo) can be imported!
  - Otherwise, click on "New experiment"
  - Give a name, starting by your initials and date (ex: SC_20260310)
  - Add the fluorescent tags of your experiment (should appear on the right "selection")
  - If your dye is not in the list, you can add it in the library before (please ask Sarah Cattin)
  - Create your groups:
    - Reference group is mandatory; it's for single staining! You should choose beads or cells. If you have both, click on "add" and select the correct colour in beads. Insert the corresponding label (antibody) for all.
    - You can then create other groups for your samples and rename them (one per autofluorescence or cells type)
    - Right-click on the group and add "unstained control" to insert the unstained tube of the group. IMPORTANT, otherwise no AF extraction will be done!

- Insert all your markers/labels from antibody for all your tubes.
- Select all acquisition options (can be changed later).

- Open "Instrument Control" and select your imported "User Settings" (if applicable). Very important to compare experiments over time!!
- *If needed:* Switch to "Worksheets" (on the left) and create new worksheets as necessary or "Open Worksheet Template" if already prepared.
- Set up your settings:
  - Make sure you have **filtered and vortexed** your samples well.
  - Attach your sample tube to the SIP. Never load tubes with more than 3ml.
  - Press "Start" after activating the correct tube in SpectroFlo (green arrow).
  - Adjust sample flow rate if needed. Lower flow rates result in higher resolution.
  - Adjust the gains for FSC & SSC to observe your cells on scale (no recording, set SSC and FSC around 30 to start).
  - Check you unstained sample for auto fluorescent signal, then all the colour that you will use (or the full stained control directly)
  - *If needed (signal out of scale):* Optimize the gains for the fluorescence channels, using only % of the entire laser (still no recording.
  - *If needed:* right-click on a tube to import it from your storage folder (must be unzipped)
  - Standardization: **Once your settings are set and will be used for several experiment to compare, "save as" the CytekAssaySettings in "instrument control" and give them your cells name!! To reuse for each repetition of the experiment to compare.**
- Start your acquisition:
  - Start to record your single stains (all references controls tubes).
  - Start to record your samples. For this, you have two possibilities:
    - **Raw recording** (only raw data generation):
      - Set your gates and the cell number you would like to measure (back in "edit" your experiment)
      - Simply record everything by clicking on "record" after loading the correct tube. Unmixing will be done later.
    - **Live Unmixed recording** (raw + unmixed data generation):
      - After recording your single stains, click on "Unmix", perform the necessary gating and select "Live Unmixing" at the end.
      - Create a new unmixed worksheet for your samples, if needed, to see the unmixed signals.
      - Set your gates and the cell number you would like to measure (back in "edit" your experiment)
      - Simply record everything by clicking on "record" after loading the correct tube.

IV. Data exportation

Note: Data will be deleted each Monday, be certain to have exported yours!!

- To export your experiment:
  - Close your experiment. **Do not overwrite the Default Worksheet.**
  - Go under "my experiments"
  - Select the corresponding experiment and click on "export"
  - Select the folder for the exportation. It should be in the bigdata server of the CAF (shortcut on the desktop of the computer), in your research group folder.
  - Let it export (can take some time). The result is a zip file containing your data + worksheets + instrument settings.

- To export directly your FCS file:
  - In your experiment, select the corresponding tubes
  - Right-click and select "export FCS files"
  - Select the folder for the exportation. It should be in the bigdata server of the CAF (shortcut on the desktop of the computer), in your research group folder.
  - Only the FCS files will be exported (no settings), but this is the only way to get a naming with group and tube name per file.

- To save gain/voltages settings:

Note: settings are also saved in your experiment.
  - Select "Save As" in the instrument controls.
  - Go to "Library → User Settings" and export the settings with your initials and cell type.

- To save worksheet templates:

Note: worksheets are also saved in your experiment.
  - Go to the "Worksheet" tab and select "Save As" to save the worksheets you want to save (don't overwrite Default Worksheet).
  - Go to "Library → Worksheet Templates" and export the worksheet.

- **After exportation, before closing SpectroFlo, Right-click on your experiment(s) and select "Delete".**

V. Instrument cleaning and shutdown

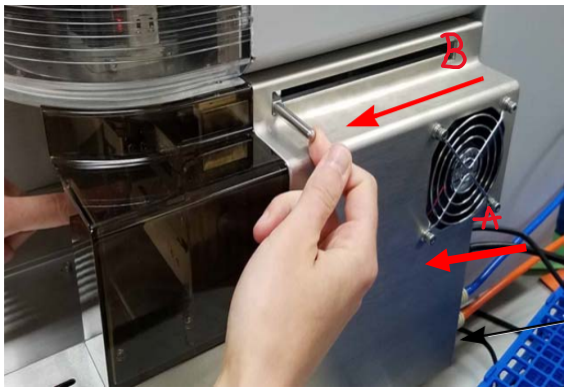
- Check on Open IRIS if somebody is booked after you: http://iris.science-it.ch
- In between users:
  - The instrument must be cleaned between every user, and if clogging happened.
  - Go under the "Cytometer" tab
  - Run a "Clean Flow Cell" with 2.5 ml FACSClean and 2.5 ml water.
  - Attach a tube with clean ddH2O and acquire it at high flow rate for 1min.
  - Clean water should show a threshold rate of 0-50 events/sec. If not, repeat the cleaning procedure.
  - Logout the SpectroFlo Software (arrow on the top corner right).
  - Leave instrument and computer running for the next user.
  - Clean the work area - don't leave used tubes, gloves … behind.

- Last user of the day:
  - The shutdown MUST be performed in tube mode (no rack)
  - Go under the "Cytometer" tab
  - Perform a "Fluidic shutdown" following instruction (4 tubes to pass, 1min each)
  - Shut down the instrument when prompted to by the on-screen message.
  - Make sure that a water tube remains attached to the SIP, the sample line is lowered to the tube bottom, and the tip is submerged in water.
  - Close SpectroFlo software and shut down the computer.
  - Clean the work area - don't leave used tubes, gloves … behind.

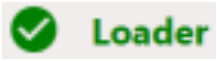
Using the Sample Loader: Specific information

I. Compatible plates and tubes

- The loader works with:
  - *Standard 96 well (U-, V- or flat-bottom) plates.*
  - *Nunc and Brand 96 deep well plates (1, 1.3, 2 and 2.2 ml)*
  - *Rack for 5ml standard FACS tubes (40 positions)*
- You can work in loader mode or in single tube mode
- No cooling or heating of the loader
- Cells are resuspended before acquisition via shaking of the stage (meaning, don't completely fill your wells)



II. Setting up the loader

- Switch on (A) the plate loader via the switch at the back.
- Wait for the Loader status display in the software to turn green: 

- Make sure that there is no tube on the SIP and that the sample line is pulled into the instrument.
- **When using plates:** → Pull the lever (B) to the front.
- **When using the tube rack:** → Leave the lever (B) in the back position.
- Go to "Preferences" → "Cytometer" and choose what should be done in case of bubble or clog detection (by default: enable).
- Put your plate or rack on the tray. A1 position is at the front left.
- If plate tray is not at the front, click the "Eject" button in the "Cytometer "tab or the Acquisition Control.
- **Load the plate/rack (move it to the back of the loader) by clicking "Load"** in the Acquisition Control or the Cytometer tab. IMPORTANT, don't start first, load first!!
- The loader is now ready to use.

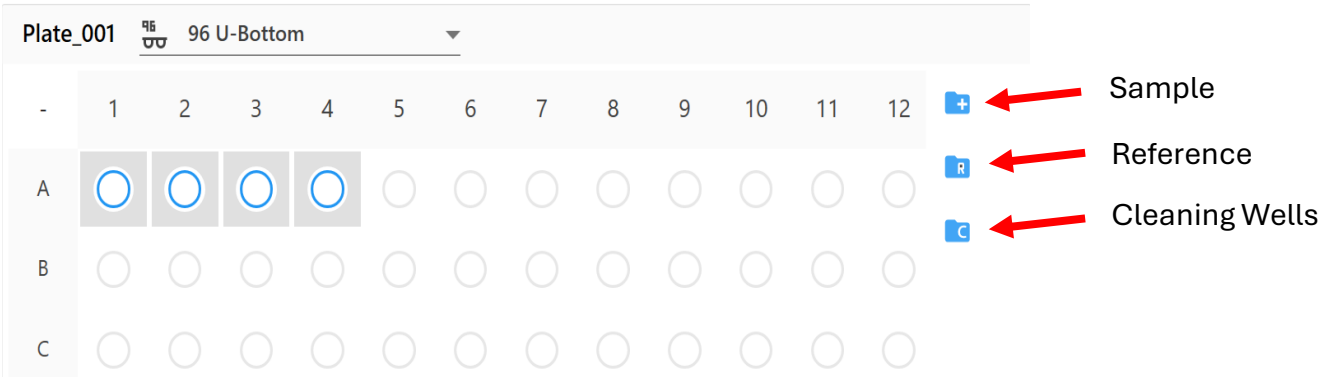
III. Verify SIT calibration

Note: The plates are all calibrated by the facility. Use this part only in case of an issue if no CAF staff is around to do it for you!!

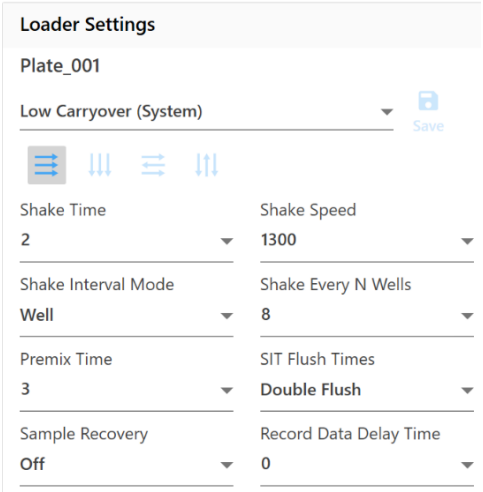
- This step is recommended whenever you use a certain type of plate for the first time.
- Fill a well with water and load the plate.
- If the measured flow rate is too low or you get a "Clog detected" message, go to the "Cytometer" tab and perform a "Calibrate SIT" with a higher Lift Distance.
- Repeat steps until you get the desired sample flow rate.

IV. Set up plate/rack in your experiment

- Create a new experiment or edit an existing one.
- Go under "Edit" → "Groups", add the plates you want to acquire:
- Select the appropriate positions and click the buttons on the right to create groups for normal samples, reference controls or cleaning wells/tubes.
- **Right click on a well to add the unstained control in a specific group (should have a little R). Important, otherwise no AF extraction will be done!**
- Go under "Edit" → "Acquisition", select all settings for each well/tube (gates, events to record…)



- Set the loader settings. Choose from Default, High Throughput or Low Carryover or create your custom settings.



Acquisition order	• row from left to right (A1-A12, B1-B12, etc.) • column from top to bottom (1A-1H, 2A-2H, etc.) • row from left to right, then right to left (A1-A12, B12-B1, C1-C12, etc.) • column from top to bottom, then bottom to top (1A-1H, 2H-2A, etc.)
Shake Time	Select the time (in seconds) to shake the plate/tube rack. You can also disable shake time.
Shake Speed	Select the speed of the orbital shaker (in RPM).
Shake Interval Mode	Select whether you would like to shake every N well or after a specified period. You can also disable shaking.
Shake Every N Wells, or Shake Interval	Select how often (number of wells or time in seconds) to shake the plate/tube rack.
Premix Time	Select the time (in seconds) to shake the plate/tube rack before acquisition of the first tube/well.
SIT Flush Times	Number of SIT flushes performed after each acquisition.
Sample Recovery	Allows any remaining sample that is left in the SIT after acquisition to be deposited back into the well.
Record Data Delay Time	Time in seconds before recording starts (to e.g., exclude the boost phase).

V. Change back to manual tube loading

- Please always change back to manual loading mode at the end of your session to prevent problems for users not familiar with the loader.

- Make sure that the sample line is retracted into the instrument.
- Remove your plate/rack from the loading tray.
- Click on "Load" in the Acquisition Control or in the "Cytometer" tab to move the tray to the back.
- Switch off the plate loader.
- *If plates were used:* Move the lever to the back

VI. Optical configuration of the instrument

Laser	Excitation	Power (mW)	Channels for detection	Detector names
Ultra-Violet	355 nm	20	16	UV1–UV16
Violet	405 nm	100	16	V1–V16
Blue	488 nm	50	14	B1–B14
Yellow-Green	561 nm	50	10	YG1–YG10
Red	640 nm	80	8	R1–R8

Laser	Channel	Center (nm)	Bandwidth (nm)	Start (nm)	End (nm)	Laser	Channel	Center (nm)	Bandwidth (nm)	Start (nm)	End (nm)
Ultra-Violet	***UV1***	373	15	365	380	Blue	***B1***	508	20	498	518
	UV2	388	15	380	395		***B2***	525	17	516	533
	UV3	428	15	420	435		***B3***	542	17	533	550
	UV4	443	15	436	451		***B4***	581	19	571	590
	UV5	458	15	451	466		***B5***	598	20	588	608
	UV6	473	15	466	481		***B6***	615	20	605	625
	UV7	514	28	500	528		***B7***	661	17	653	670
	UV8	542	28	528	556		***B8***	679	18	670	688
	UV9	582	31	566	597		***B9***	697	19	688	707
	UV10	613	31	597	628		***B10***	717	20	707	727
	UV11	664	27	651	678		***B11***	738	21	728	749
	UV12	692	28	678	706		***B12***	760	23	749	772
	UV13	720	29	706	735		***B13***	783	23	772	795
	UV14	750	30	735	765		***B14***	812	34	795	829
	UV15	780	30	765	795	Yellow-Green	***YG1***	577	20	567	587
	UV16	812	34	795	829		***YG2***	598	20	588	608
Violet	***V1***	428	15	420	435		***YG3***	615	20	605	625
	V2	443	15	436	451		***YG4***	661	17	653	670
	V3	458	15	451	466		***YG5***	679	18	670	688
	V4	473	15	466	481		***YG6***	697	19	688	707
	V5	508	20	498	518		***YG7***	720	29	706	735
	V6	525	17	516	533		***YG8***	750	30	735	765
	V7	542	17	533	550		***YG9***	780	30	765	795
	V8	581	19	571	590		***YG10***	812	34	795	829
	V9	598	20	588	608	Red	***R1***	661	17	653	670
	V10	615	20	605	625		***R2***	679	18	670	688
	V11	664	27	651	678		***R3***	697	19	688	707
	V12	692	28	678	706		***R4***	717	20	707	727
	V13	720	29	706	735		***R5***	738	21	728	749
	V14	750	30	735	765		***R6***	760	23	749	772
	V15	780	30	765	795		***R7***	783	23	772	795
	V16	812	34	795	829		***R8***	812	34	795	829

SOP revision (annually at minimum)

Revision	Date	Description of Changes	Reviewed By
V1	01.03.2022	Initial release	Sarah Cattin
V2 – V4	25.11.2025	Correction, small adaptations	Sarah Cattin
V5	10.05.2026	Fuse SOP with Loader in a new standard	Loïc Chentout
V6	02.06.2026	Structural adaptation	Sarah Cattin