STANDARD OPERATING PROCEDURE (SOP)

BC Cytoflex S - Operation

Document No:	CAF-SOP-004-V4	Revision Date:	08.06.2026

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

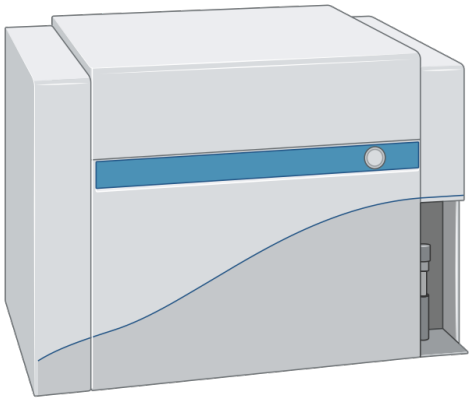
026/300.85.79

- 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

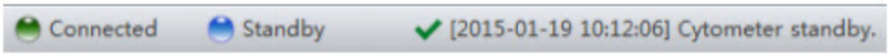
I. General information for the Cytoflex

- The CytoFLEX S is a conventional flow cytometer equipped with 4 lasers and 13 detectors.
- In general, the maximum recommended sample concentration for analysis is 1×10^6 cells/ml.
- The instrument is running with BC Cytoflex sheath Fluid cubitainer (water) as sheath fluid.
- Samples can be injected using an automatic sample loader as well as manually. The automatic loader can be used with 96-wells (V-shape, U-shape) and 96- deep wells plates formats.
- Expected flow rate during acquisition are:
  - Low: 10 ul/min
  - Medium: 30 ul/min
  - High: 60 ul/min
- Minimum sample volumes are:
  - Tube mode = 100ul (10ul dead volume)
  - Plate mode = 50ul (10ul dead volume)
- Data acquisition rate is up to 30'000 events/sec, but you might see a spread of the data starting at 10'000 event/sec in certain cases.



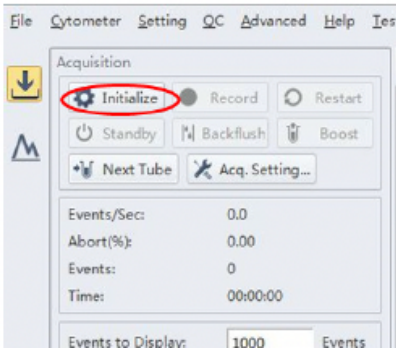
II. Instrument start-up

- **First user of the day: Start-Up**
  - Check the level of Sheath fluid (blue cable front) and replace with a new box if needed (lift to check). The box should be at the same level (height) than the instrument!
  - Empty the waste (yellow cable back) in the red containers.
  - Turn on the power switch on the back cover of the instrument, located just above the power cable.
  - Turn on the computer and login using your private UNIFR credentials.
  - Log into the CytExpert Software with **username = labo_XXX (PI family name), password= XXX (PI family name)**
  - Ensure that the Connected icon on the Status Bar near the bottom-left side of the display is green.



Note: On the lower right corner confirm the Sheath and Waste levels:

- ***Green lights** mean you can proceed with your experiment*
- ***Red lights** and an audible alarm mean the fluid levels are not sufficient. Change it!*



  - Verify that the Sample is on tube mode: Semi-automatic Sampler status icon located in the bottom right. If not, proceed to the Plate-Tube switch section.
  - Select **Initialize** in the Data Acquisition Control screen or select Initialize in the Cytometer menu to initialize the instrument (**out of stand-by mode, will put the tube loader in front position**).
  - Select **Cytometer** in the top menu, click **System Startup Program** and follow request from the software:
    - *Load a 2ml water tube.*
    - *Run it for 10min, then close.*

- **Following users:**
  - Check the level of all solutions and refill/empty if needed.
  - Turn on the computer and login using **your private UNIFR credentials**.
  - Log into the CytExpert Software with **username = labo_XXX (PI family name), password= XXX (PI family name)**

III. Acquisition workflow

Note: The instrument may be in Standby mode (if unused for 10min), initialize it before use!

- Create an experiment:
  - Click on File>New Experiment.
  - Save the experiment in your group data folder with a date and a name (D drive please).
  - Put the instrument in the injection mode that is needed: tube or plate mode.
- Set up your settings:
  - Right click on the tube, select set channels, and tick the needed parameters + give them names (antibody + dye used), then OK.
  - Create the different dot plot that you need (minimum is FSC/SSC and each color on one axis),
  - Load an unstained sample tube, put the speed in medium, and click on RUN in the Acquisition panel (left)
  - Click on **Acquisition Settings**, then click on QC gain
  - Click on **Run** and adjust the gain (voltages) for FSC & SSC to observe your cells on scale.
  - Gate around your cells using a polygonal gate. You can add other gates depending on your needs.
  - Click on the Hierarchy icon  to visualize your gating and rename them if needed.
  - Check that all channels are looking negative (should be in the first half of the graph).
  - Stop the acquisition and load a fully stained sample in the same tube.
  - Click on **Run** in the Acquisition panel (left), **overwrite the data**.
  - Check that all channels are looking positives where expected, and that nothing is out of scale.
  - If this is not true, you only adjust the gain of the parameter that is out of scale. To do so, you can either open the **Acq. Settings Tab** and manually change the value or use the Gain Hand. 
  - Adjust the threshold settings if needed (using  in the plot control area).
  - Continue or save the experiment settings if you want to perform new compensation: Click on Export to File… in the Acq. Settings tab and save it in your Experiment folder.
- Compensation:

Note: The compensation is another type of experiment, independent. Save your experiment before starting.

  - Select **Compensation Matrix** in the Setting menu to open the Compensation Matrix window to compensate for the fluorescence spillover.
  - If a new automated compensation is needed, proceed as follows:
    - Prepare all necessary unstained and single staining (beads or cells).

- Select **New Compensation** in the **File** menu and save it in your compensation folder.
- Select the corresponding tubes (unstained also) in the pop-up Compensation Setup window, then select OK.
- CytExpert automatically creates a list of matching empty tubes in the Tube panel as well as all the plots needed to gate the positive and negative populations.
- Under **acquisition settings**, import the gain from file that you just exported from your experiment settings.
- Load the corresponding unstained or single staining, and click on Run, then record.
- Repeat these steps for all tubes.
- Select the positive and negative population in each tube (on the first plot always only).
- After collecting data for all necessary compensation samples, generate the compensation matrix by selecting **Compensation Calculation** in the Setting menu.
- Save your compensation as a file.

  - In the File menu, Close the compensation experiment and open your experiment.
  - In the Compensation Matrix tab (on top of the tubes), you can import a compensation file you've created.
  - When you import the compensation, a pop-up window will ask you how you want to import the compensation, click on the first "matrix and convert based on the current gain".

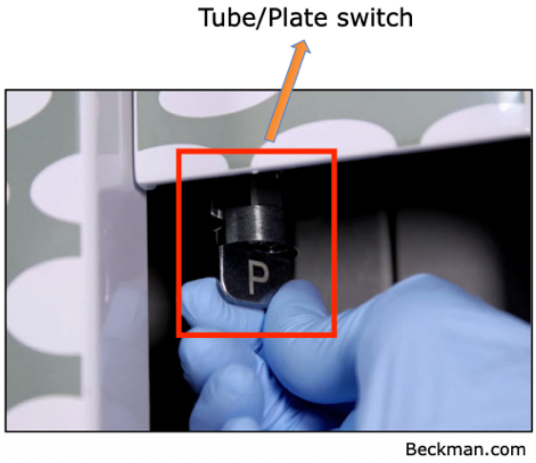
- Start your acquisition:
  - In the first tube: Select at least two stopping rules for your experiment (It is important to set a limit in case the event limit cannot be reached by the machine otherwise it will wait indefinitely).
  - Create all your tube and rename them (right click).
  - Right click on the first tube created:
    - "Apply acquisition settings to all tubes"
    - "Apply to all tubes" naming of the channels
  - Vortex the sample and place it on the holder.
  - Select Run to load the sample, wait 2 sec
  - Select Record to save the data

Note: You can also push the load button on the front of the instrument to automatically start the run and record the data.

Note: If you forget to click on "record" the already passed data will be saved anyway. Don't overwrite them. Data saved during the run has a blue label, or a green label if recorded.

IV. Using the sample loader: Specific information

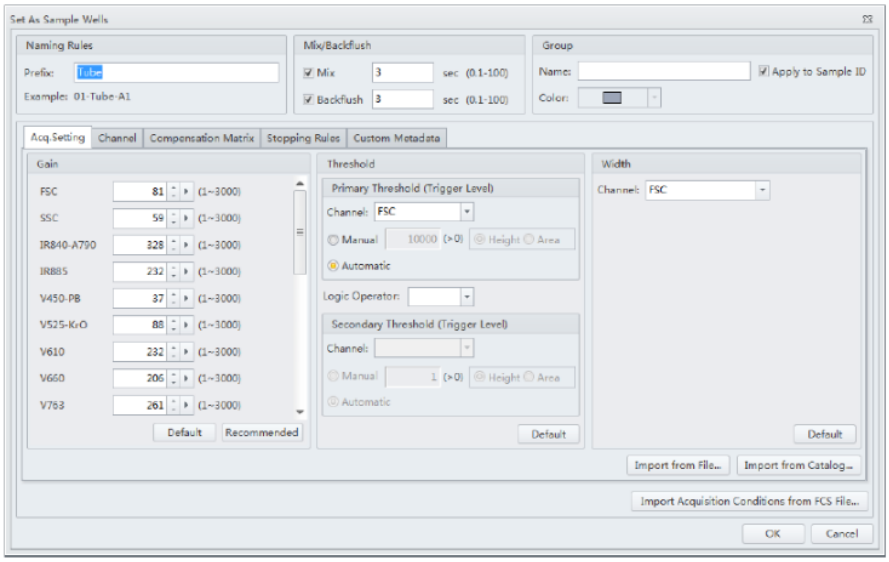
- **Changing tube-plate mode**



  - In the Cytometer menu, select "Sample Injection Mode":
    - *Semi-Automatic for tubes (NOT Manual)*
    - *Plate for plates*
  - A restarting warning prompt will appear, select OK.
  - Turn the silver switch from P (plate) or T (tube) depending on your needs:
  - Switch off the cytometer using the main switch at the back and restart it directly.
  - Initialize the instrument.

- **Set up plate in your experiment**

  - Put the instrument in plate mode (see previous section)
  - In your new experiment, select the plate logo (down)
  - Select the correct plate type and click OK.
  - Select the first well that you want to run and Set as Sample Wells.



  - Go to the **Channel Tab**, select the needed channels and rename them.
  - Create the plots.
  - You can prepare your gain/voltage settings as explained in the acquisition workflow part of this SOP.
  - Once settings exported, you can create the compensation experiments but using plate mode and defining the wells. All samples can be recorded automatically by clicking on Auto Record.
  - After completion, you can calculate the compensation matrix, export it and close your compensation experiment.

- Back in your experiment, you must create your plate template by clicking on the plate logo.
- The Plate window appears. Add a plate with the dropdown menu, be careful to pick up the right plate type, click OK. By default, CytExpert will create an empty plate.
- Once the plate is created, you will have to define the wells to run. Left click and drag your mouse to highlight the desired wells. You can also CTRL left click to select/unselect.
- Set As Samples the desired wells. Each tab will be used to define different parameters for your well:
    - In the Channel tab, select the channel you will use and name the associated labels, uncheck the channel you don't need.
    - In the Compensation Matrix tab, you can import a compensation file you've just created.
    - In the Stopping Rules Tab: Select at least two stopping rules for your experiment. **The dead volume for 96 well plates is 10 ul (V bottom, U bottom) or 20 ul (flat bottom) and the minimum volume is 50 ul per well. Set a rule smaller than your actual volume (your volume -20ul).**
- Once all is validated, click OK.
- You can now select "Auto Record" to start running the plate.
- You can directly insert some cleaning wells inside of your plate for automatic cleaning of the plate sample line (3 cleaning wells + 4 water wells)

V. Data exportation

Note: Data will be deleted each Monday, be certain to have exported yours on the bigdata server!!

- Select Export FCS File from the File menu. The Export FCS File window appears.
    - Select the tubes to export.
    - Check that FCS3.0 is selected.
    - 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).

VI. 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. If you are running problematic samples, you might have to clean in between your samples to avoid clogging.
    - The cleaning must be done always in the mode that you used for acquisition (semi-automatic or plate)
    - Select Cytometer>Daily Clean. Follow the requested cleaning settings:
        - In tube:
            - *Run FlowClean cleaning fluid for 3 minutes (blue solution).*
            - *Run DI water for 5 minutes.*
        - In plate:
            - *Run 3 wells of FlowClean cleaning fluid (blue)*
            - *Run 4 wells of DI water*
    - Clean the bench with 70% EtOH, making sure it is ready for the next user.
    - Empty waste / Refill sheath tank if necessary (put first in standby the instrument).
    - Close the software and log-out of your UNIFR session

- Last user of the day:
    - Follow the "in between users" cleaning procedure.
    - After the daily clean, put back the instrument in semi-automatic injection mode in the software.
    - Shutdown the computer.
    - Switch Off the instrument (at the back)
    - Clean the bench with 70% EtOH, making sure it is ready for the next user.
    - Empty waste / Refill sheath tank if necessary (put first in standby the instrument).

VII. Optical configuration of the instrument

Laser	*Channel Name*	*Filter*	*Main colors examples*
Violet (405nm)	Violet 450/45	450/45	Spark Violet 423, **V450**, PacBlue, AF405, **BV421** VioBlue, *DAPI* , CFP, BFP, *LD/Zombie violet*
	Violet 525/40	525/40	**BV510**, PcOrange, V500, Spark violet 538
	Violet 610/20	610/20	AF430, BV605, BV570, *LD/Zombie Yellow*
	Violet 710/50	710/50	BV750, **BV711**, SB702
Blue (488nm)	Blue 525/40	526/52	**AF488**, AF514, GFP, **BB515**, FITC, YFP, CFSE, Spark blue 515, *LD/Zombie Green*
	Blue 690/50	695/50	BB700, PerCP, *7AAD, DRAQ7* , **RB670**
Yellow-green (561nm)	YG 585/42	585/42	AF568, AF594, dTomato, dsRED, **Pe, Spark YG570,** Spark YG581, **RY586**
	YG 610/20	610/20	Pe-Dazzle594, mCherry, RFP, Pe-Texas Red, *PI* , **RY610**
	YG 690/50	690/50	Pe-Fire700, Pe-Cy5, Pe-Cy5.5, *7AAD* , RY655, **RY703**
	YG 780/60	780/60	**RY775, Pe-Cy7,** *DRAQ7*
Red (640nm)	Red 660/10	660/10	AF635, **AF647**, **APC**, APC-Cy5, APC-Cy5.5, AF680, RR688
	Red 712/25	712/25	**R718**, AF680, AF700, APC-Cy5.5, Spark NIR 685, *Zombie NIR*
	Red 780/60	780/60	AF750, **APC-Cy7, APC-H7**, APC-Fire750, APC-Fire 810, *Zombie NIR*

SOP revision (annually at minimum)

Revision	Date	Description of Changes	Reviewed By
V1 – V3	15.04.2024	Initial release and minor updates	Sarah Cattin
V4	09.06.2026	Structural changes	Loïc Chentout / Sarah Cattin