STANDARD OPERATING PROCEDURE (SOP)

Attune NxT – Flow Cytometer Operation

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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

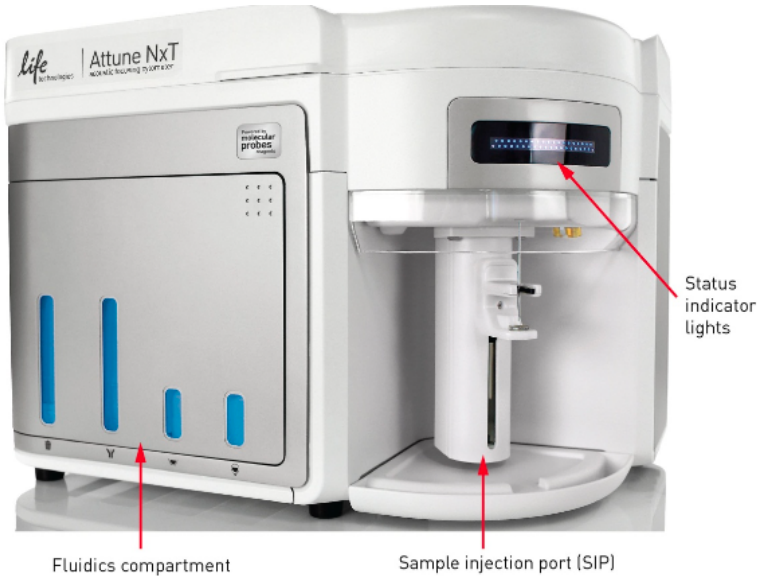
026/300.85.79

- 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

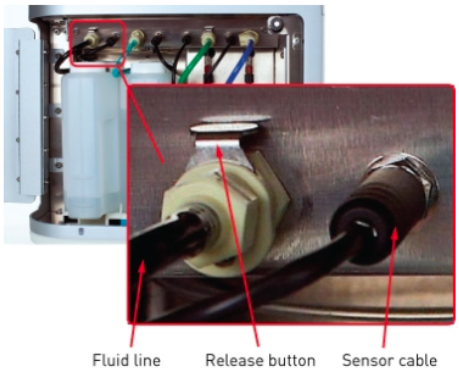
I. General information for the Attune NxT

- The Attune™ NxT Acoustic Focusing Cytometer is a conventional flow cytometer equipped with 4 lasers and 14 detectors. It has a range of flow rates from 12.5 µL/min to 1000 µL/min. Starting from 200 µL/min, some loss of sensitivity may occur, especially for small particles.
- In general, the maximum recommended sample concentration for analysis is 1×10^6 cells/mL.
- The maximum recommended sample concentration for 500 µL/minute and 1,000 µL/minute flow rates is 5×10^5 cells/ml.
- The instrument is running with Focusing fluid as sheath fluid.
- The instrument is equipped with an automatic loader. The loader can be used with 96-wells and 96- deep wells plates formats.



II. Instrument Start-Up

- First user of the day: Start-Up
  - Check the level of all solutions and refill/empty if needed (press the metal release buttons to free the tubing and remove the sensor cable)



    - There are 4 fluidic containers on the Attune NXT equipment (from left to right): Waste, Focusing fluid, Wash solution and Shutdown solution.
    - On the Autosampler there are also two big containers: waste and focusing fluid.
  - In this order: Power on the CytKick™ Autosampler first, the Attune™ NxT Cytometer next, and finally the computer.

  - Log into windows:
    - Session= Administrator, Password = AttuneNxT
  - Double click on the AttuneNxT software's icon located on the desktop.
  - Log In with your Account:
    - Username = Labo_XXX (PI family name in capital letters), Password = XXX
  - Wait for the software to connect with the cytometer.
  - Go under "instrument" tab, then click on "Startup". Follow the instruction and let it work for about 10min.
  - When the machine is ready, the lights are green!
- Following users:
  - Check the level of all solutions and refill/empty if needed.
  - Log In with your Account:
    - Username = Labo_XXX (PI family name in capital letters), Password = XXX

III. Acquisition workflow

- Create an experiment
  - Select File - New Experiment on the Ribbon bar
  - Select if you use tubes or plate (you can mix tube and plate in a plate exp.)
  - Adapt the number of groups and tubes needed
  - Click OK
- Select the instrument settings tabs
  - Select only the needed "parameters"
  - Select A+H+W for SSC and FSC, keep only A for the other parameters
  - Add names to each parameter (color "label" + antibody "target")
- Workspace:
  - Create graphs as needed (minimum is FSC/SSC and each color on one axis), better use the Hyperlog scale.
  - Optimize your instrument settings (voltages), "instrument settings" tab, under "parameters" choices.
  - In the Collection Panel tab, enter the Acquisition Volume, and then set the Flow Rate (25ul/min). Don't go higher than 6000 events/sec.
- Set up your settings:
  - Load your sample (run, not record)
  - Adjust the voltage for FSC & SSC to observe your cells on scale.
  - Lower the voltage for fluorescence channels **only** if off scale due to high fluorescence intensity.
  - Recover unused sample if not enough volume left in tube for subsequent acquisition: click on Recover Volume and then next to use the same tube or supply a fresh empty tube if desired.

What	*Flow rate*	*Plate*	*Tube*
Dead volume (lost)	up to 200 µL/min	30 µL	50 µL
	500–1000 µL/min	50 µL	75 µL
Minimum volume		20 µL	40 µL

- Compensation
  - **In tube:** Double-click in your compensation node in your experiment (or in Experiment Explorer)
  - **In plate:** "Setup comp".
  - Select the needed parameters (in plate, attribute a well number) + where the negative must be taken (if US control)
  - Load each tube as requested and record them
  - Put the neg and pos gates on each peak
  - When the last compensation control is recorded, Matrix automatically calculates.
  - Click OK to apply the compensation values to the dataset.

- Start your acquisition:
    - Enter the collection criteria in the Collection Panel. Set limits to collection by the number of events for specified gates, total sample volume analyzed, or by elapsed time.
    - Clicking «Apply to Experiment» on the main button applies the current Run Protocol to all Samples in the current Experiment.
    - Select the Sample of interest (double click) from the Experiment Explorer panel.
    - Make sure you have filtered and vortexed your samples well. When analyzing large cells, filter sample with 0.45µm filter directly before analysis.
    - Install the tube containing the sample and lift-up the tube loader to the active position.
    - Click «Run». Click «record» to start data collection.

IV. Data exportation

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

- Right click on the experiment/ file that you want to export and select "export"
- Export both "Experiment" and "FCS files"
- Click «Browse» to specify the destination folder in which the experiment data is to be exported.
- Click "Export"
- Export your data as FCS files (3.0 or 3.1; never as 2.0!) to the "data" folder on the user folder. Important: Open the destination folder and check that the size of each FCS file is > 0 kb. If files have 0 kb this may indicate a corrupted data export, and you will need to repeat the export procedure!
- The Attune NxT is not connected to internet. You will then need a USB key to harvest your data.

- **Before closing the software, you must remove all experiments from the database! Only keep templates.**

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 each user.
  - On the Instrument ribbon, Select the "Sanitize Attune SIP" if tubes were used, select "Auto Sampler SIP (standard)" if plates were used
  - Follow instruction (load a tube of bleach + a plate for cleaning with water in A2)
  - Click on Next and let the program clean (10min)
  - Lower the tube lifter
  - Under the file ribbon, select "log-out"
  - Leave instrument and computer running for the next user.
  - Clean the work area - don't leave used tubes, gloves, etc. behind.

- If the instrument seems dirty:
  - If you are running problematic samples, you might have to clean in between your experiments to avoid clogging:
    - If it looks dirty: click the "rinse" function in the instrument tab.
    - If it looks clogged: On the Instrument ribbon, click "Unclog" and follow instructions

- Last user of the day:
  - Perform the Sanitize SIP with Bleach first (see "in between users" previous section)
  - Go under "instrument" tab, then click on "Shutdown" – Quick (25min)
  - Check the level of the liquids and follow the instructions (several tube changes)
  - Don't close session or software during shutdown, otherwise it will stop!!
  - After the shutdown, the instrument is in stand-by (blue lights).
  - Quit the software, switch off the computer, the instrument and the autosampler.
  - Clean the work area – don't leave used tubes, gloves, etc. behind.

VI. Optical configuration of the instrument

Laser	Channel Name	Filter	Main colors examples
Violet (405nm)	VL1	440/50	Spark Violet 423, V500, **V450**, PacBlue, AF405, **BV421**, VioBlue, *DAPI*, BFP, *LD/Zombie violet*
	VL2	512/25	*LD/Zombie Aqua*, PacGreen, **BV510**, AF430, PacOrange
	VL3	603/48	BV605, BV570, *LD/Zombie Yellow*
	VL4	710/50	BV750, **BV711**, SB702
Blue (488nm)	BL1	526/52	**AF488**, AF514, GFP, **BB515**, FITC, YFP, CFSE, Spark blue 515, *LD/Zombie Green*
	BL2	590/40	AF546, RB613, Pe-Vio615, *LD Red*
	BL3	694/40	BB700, PerCP, *7AAD*, *DRAQ7*, **RB670**
Yellow-green (561nm)	YL1	586/15	AF532, AF555, dTomato, dsRED, **Pe**, **Spark YG570**, Spark YG581, **RY586**
	YL2	620/15	Pe-Dazzle594, mCherry, RFP, Pe-Texas Red, *PI*, **RY610**
	YL3	695/40	Pe-AF700, Pe-Cy5, Pe-Cy5.5, *7AAD*, RY655, **RY703**
	YL4	780/60	**RY775**, **Pe-Cy7**, *DRAQ7*
Red (640nm)	RL1	670/14	**AF647**, **APC**, APC-Cy5, APC-Cy5.5, AF680, RR688
	RL2	720/30	**R718**, AF680, AF700, APC-Cy5.5, Spark NIR 685, *Zombie NIR*
	RL3	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	30.09.2025	Initial Release	Sarah Cattin
V2	10.11.2025	Protocol update	Sarah Cattin
V3	24.11.2025	Detailed procedure update	Sarah Cattin
V4	02.06.2026	Structural changes	Loïc Chentout / Sarah Cattin