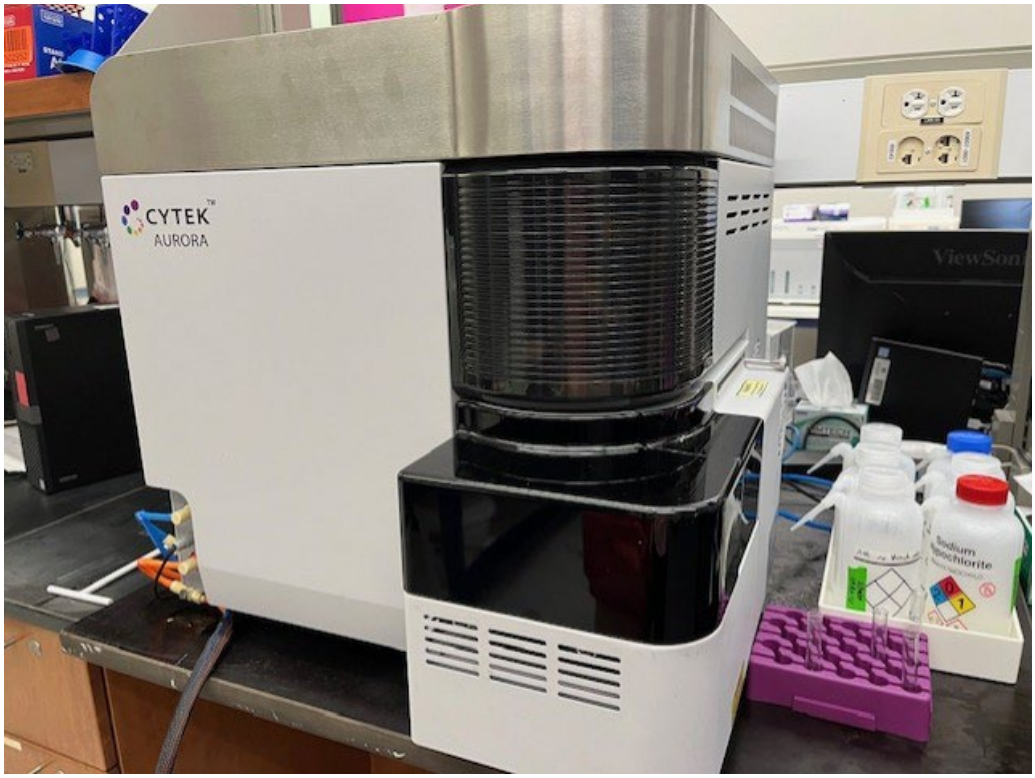
GCC Flow and Mass Cytometry Shared Resource: Facility Contact Details

Analyzer Room:	CN 4158C	1-5468
Data Analysis Room:	CN 4158D	
Sort Room:	CN 4146C	1-8473
Dr. Rebekah Tritz (Research Manager):	CN 4158E	1-5468
David Hansen (Senior Research Assoc.):	CN 4121A	1-8880



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Important Points:

- If an issue arises which you are unsure how to resolve, please do NOT try and fix it yourself – Please contact a GCC Flow and Mass Cytometry SRL staff member (leave a note and send an email if the problem arises outside of business hours)
- All users MUST be trained by the GCC Flow and Mass Cytometry SRL staff to use the instrument and be familiar with the SRL policies. Disregarding SOPs or abuse of the machines will result in suspension of SRL access. For training, please contact the GCC Flow and Mass Cytometry SRL staff.
- Correct shutdown and cleaning procedures MUST be followed. Failure to do so can result in instrument malfunction and affect data quality of the next user.
- AttuneNxt software can be accessed on a PC in 4158C. Please contact SRL staff to setup your lab's account in the software. If you need any assistance, please contact SRL staff.

Sample Preparation:

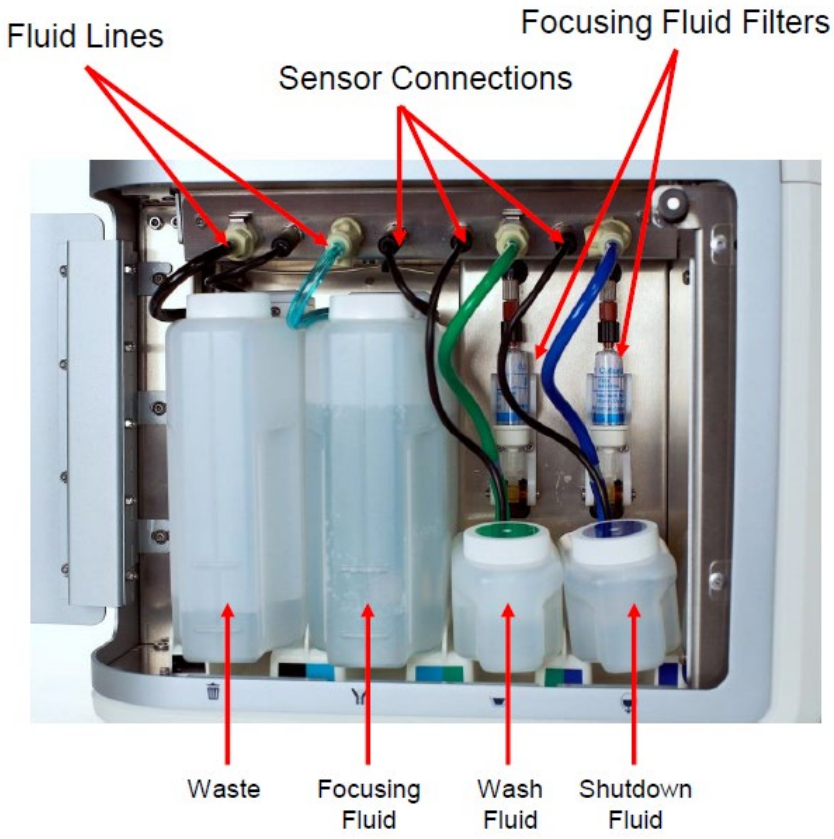
1. Instrument –
    a. AttuneNxt: equipped with 405nm, 488nm, 561nm and 640nm lasers, allowing detection of signals in 14 fluorescent channels.
2. Instrument specifications are available at https://www.augusta.edu/cancer/research/shared-resources/flow/equipment.php.
3. The SRL facility recommends using FluoroFinder (https://fluorofinder.com/) for custom panel design specific to GCC Flow Cytometry SRL machines. If you require assistance developing a panel or navigating panel building sites please contact SRL staff.
4. **ALL** samples that are not from whole blood MUST be filtered through a (minimum) 70uM mesh filter before acquisition – NO EXCEPTIONS. Specimens must be inactivated of all pathogens before they are brought into the facility. Commonly, 1-2% paraformaldehyde is used.
5. All samples must be resuspended at an appropriate concentration to not block the SIP (sample injection probe). Users who continue to block the SIP through neglectful sample preparation may have their usage revoked.
6. Samples may be brought in 12x75mm tubes or standard sized 96 wells plate.

Required solutions:

1. Attune Focusing Fluid – buffered, azide-free carrier (sheath fluid)
2. Attune wash/ shutdown solutions – used for start-up, sanitizing and shutdown functions
3. FACs clean/ MiliQ water – used for cleaning and sanitizing
4. Debubble solution – used for "Debubble" function in software

Instrument Start-up:

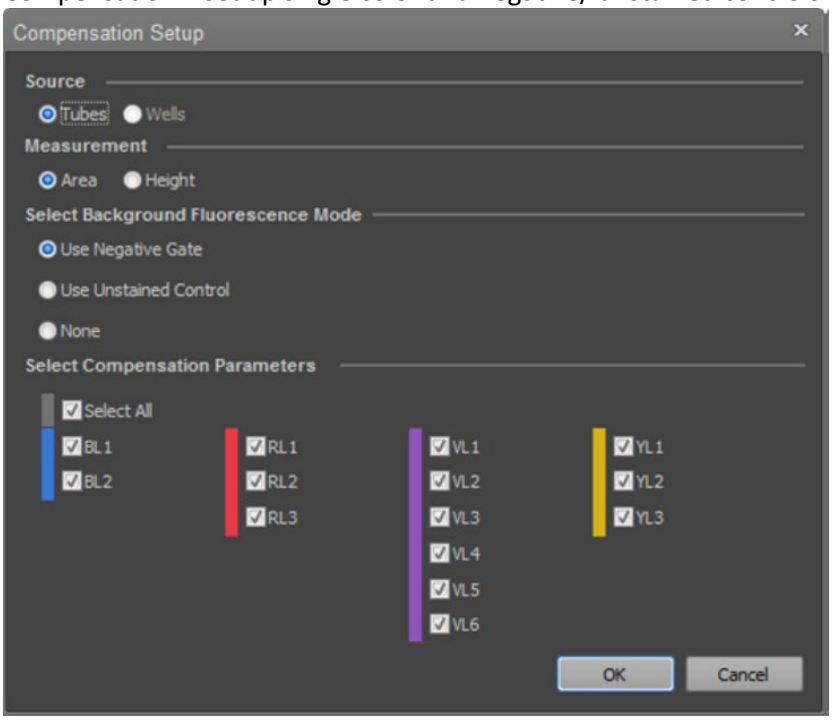
1. AttuneNxt machine stays powered on, but in a standby/ sleep mode. If you are the first one to run on the machine start with #2. If running after someone else, skip to #4.
2. Check fluid levels:
    a. Waste container – when full, empty into sink and replace 100mL bleach
    b. Focusing fluid (sheath) – replacements on shelf above sharps container
    c. Wash (green)/ shutdown (blue) fluid – replacements on shelf next to focusing fluid



3. Run instrument start-up (warms lasers, initializes pumps, primes fluidics, informs user of system status).
    a. Lower the tube lifter and remove 96-well plate – when lowering or raising the tube lifter use two fingers, do not force
4. Run SIP sanitize function with FACs clean, then with Attune wash solution

Create an Experiment:

1. Decide whether experiment will be tube-only or plate. A plate experiment includes 1 plate and tube groups/samples.
2. Compensation – set up single color and negative/ unstained controls



3. Software wizard will walk you through setting up compensation, groups and samples.
    a. Collection criteria – events, volume or time
    b. Flow rate –
        i. Low flow rates (12.5uL – 25uL): mostly hydrodynamic focusing
        ii. High flow rates (100uL – 500uL): both hydrodynamic and acoustic focusing

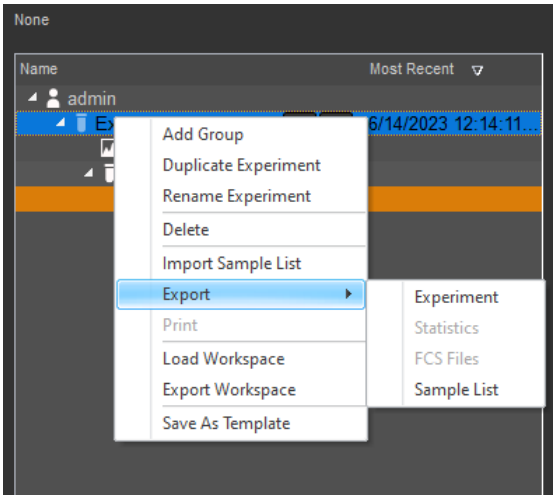
Sample flow rate	*New to the Attune?* Recommended sample concentration (~3,500 ev/sec)	*Accurate counts?* Maximum concentration (~8000 ev/sec)	*Fastest?* Maximum sample concentration (~35,000 ev/sec)	Cell/particle size
1000 µL/ minute	2.1 x 10^5 cells/mL	0.48 x 10^6 cells/mL	2.1 x 10^6 cells/mL	- Particles > 4 µm - Predominantly acoustic focusing
500 µL/ minute	4.2 x 10^5 cells/mL	0.96 x 10^6 cells/mL	4.2 x 10^6 cells/mL	- Particles > 2 µm - Predominantly acoustic focusing
200 µL/ minute	6.7 x 10^5 cells/mL	1.5 x 10^6 cells/mL	6.7 x 10^6 cells/mL	
100 µL/ minute	1.3 x 10^6 cells/mL	3 x 10^6 cells/mL	1.3 x 10^7 cells/mL	
25 µL/ minute	5.4 x 10^6 cells/mL	1.2 x 10^7 cells/mL	5.4 x 10^7 cells/mL	- Small particles < 2 µm - Best resolution from background for dimly positives assays - Smallest sample core - Predominantly hydrodynamic focusing
12.5 µL/ minute	1.0 x 10^7 cells/mL	2.4 x 10^7 cells/mL	1.0 x 10^8 cells/mL	

4. Install sample on tube loader and raise loader to run sample
5. Optimize voltages for FSC, SSC and fluorescent channels before recording any data (Run mode)
6. Once settings have been optimized, record data to collect FCS file (Record mode)

Post-experiment/ Shutdown procedure:

a. Unclog x1
b. Sanitize Attune SIP 1x with FACs clean
c. Sanitize Attune SIP 1x with Attune was solution
d. For dirty/ sticky samples – run “Deep Clean” (quick = 30min)
e. Last experiment of the day – run “Shutdown” (standard = 60min)

Exporting Data: data, workspace and settings can be exported



Data Analysis: Data can be analyzed either on the Attune or the offline computer with the AttuneNxt software. The exported FCS files can be analyzed with FlowJo. If concentrated help is needed on analysis, please contact SRL staff.