GCC Flow and Mass Cytometry Shared Resource: Facility Contact Details

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| Analyzer Room: | CN 4158C | 1-5468 |
| Data Analysis Room: | CN 4158D | |
| Sort Room: | CN 4146C | 1-8473 |
| Rebekah Tritz (Research Manager): | CN 4158E | 1-5468 |
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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 adhered followed. Failure to do so can result in instrument malfunction and affect data quality of the next user.
- NovoExpress software can be accessed on all PCs in 4158D. Please contact SRL staff to setup your lab's account in the software. If you need any assistance, please contact SRL staff.

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**Sample Preparation:**

1. Instruments –
    a. Quanteon 1/2: equipped with 405nm, 488nm, 561nm and 640nm lasers, allowing detection of signals in 25 fluorescent channels.
    b. Penteon: equipped with 349nm, 405nm, 488nm, 561nm and 640nm lasers, allowing detection of signals in 30 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 FluoroFinder 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 plate (6, 12, 24, 48, 96 wells). For any non-standard plate please contact SRL staff to determine if machine can be calibrated for new plate.

**Instrument Start-up:**

1. Locate silver power button on the front left-side of the instrument and press to turn on. This will warm-up the lasers and being priming of the fluidics system. Start-up takes about 6 minutes.
2. While the machine is warming up, proceed with checking the fluids. The fluidics cart is located at the end of the table near each machine. Amount of fluids is monitored by weight sensors in the fluidics cart.

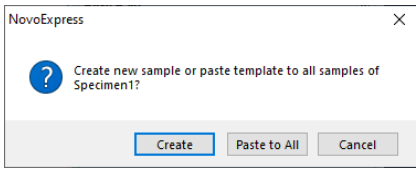


   a. Sheath:
      i. Both Quanteons and the Penteon use sheath fluid made from Mili-Q water and NovoFlow (12L containers are pre-made by SRL staff and located on the table next to the machine)
      ii. When the sheath needs to be refilled the status light (upper left) will turn orange or red, and the corresponding status light on the analyzer will also change.
      iii. When the sheath needs to be added, remove the green cap and pour in more sheath. No need to remove the container off the cart.
   b. Waste:
      i. When the waste is too full the status light will change on the fluidics cart and machines in similar fashion to the sheath. Disconnect the black cord (electronics) first and then the fluidics tube. Empty the waste container in the sink and add 300mL undiluted bleach and reconnect the waste connector to the fluidics cart.
   c. Rinse and Clean solution:
      i. When the Rinse (blue) or Clean (yellow) solution needs to be refilled remove the small container after disconnecting the fluid cable. Place empty container near

sink and replace with a pre-made bottle of solution from the stock on table next to machine.

**NovoExpress Software Navigation (sample set-up, acquisition in a new experiment)**

1. Login using your University ID to the computer and begin your session in iLab (Kiosk).
2. Once the iLab session has been started you will be able to open the NovoExpress software (found on desktop). Login to your lab's NovoExpress account. The software will take 1-2 minutes to connect to the cytometer.
3. The software will automatically open a new experiment, "untitled". Experiments will automatically save to your lab's folder on the hard drive. DO NOT save or acquire directly to Box. This will corrupt your data as it is acquired.
4. Experiment set-up:
    a. Compensation (if needed) – click "Auto Compensation" and select all color channels that you will be using. Make sure to indicate if you have an unstained sample.
    b. Cytometer Setting – after selecting the appropriate detectors during compensation set-up, those detectors will be selected in the list of parameters.
        i. At this point "height" may be selected in addition to "area".
        ii. In this tab you may also select "Stop Conditions" and "Flow Rate".
        iii. Do not make adjustments to the "Threshold" unless previously advised by the SRL staff to do so, as determined by your type of samples.
    c. Plate Manager –
        i. Under the plate drop tab, make sure that the appropriate plate or rack is selected.
        ii. Unless needed, uncheck the "Absolute Count" box. This draws up an extra 30uL of sample.
        iii. In order to conserve sample, make sure to check the "Recover Sample" box
        iv. Samples may be added either through right clicking on the plate layout or through the "Work List" (small blue box, upper left corner). Samples and Specimens may be named either in the Work List or the Experiment Manager.
    d. Experiment Manager – this tab will show the experiment name and the related specimens and samples
        i. Samples:
            1. Empty tube (white): samples with no data
            2. Filled tubes (black): samples where data has been recorded
            3. Cytometer Setting, Compensation, Report and Analysis can be specialized for each Sample. If you want to apply settings from one sample to an entire specimen, drag the sample up to the specimen and "Paste to All"



5. Experiment run:
    a. Cytometer Control – shows "live feed" of the sample being run
        i. Run Single Well: will run the sample currently selected in "Experiment Manager" (red arrow)
        ii. Run Plate: will bring up plate map of samples and allow user to select either all or some of the samples.
        iii. This tab will also allow user to initiate an extra mix or move to the next sample.
    b. "Plot" tab – where the user can create new and manipulate existing plots
        i. For each sample create at least 2 plots: FSC-H vs SSC-H (main population) and SSC-H vs SSC-A (doublet discrimination). This can be done before or during sample acquisition. Gates can be set at any point of acquisition, and the controls/options can be found in the "Plot" tab and "Gate" tabs.
    c. Additional notes –
        i. If additional events are required in a given sample, select the sample and hit "Run Single Well" and select "Append".
        ii. Gain changes can NOT be changed after the sample has finished running. The events must be deleted and the sample re-run.
        iii. Data can be viewed in linear, log and bi-exponential scale.
        iv. If the incorrect plate type was selected and data has been recorded, the plate type can NOT be changed. All events must be deleted, or a new experiment must be started. Therefore it is important to double check the correct plate type has ben chosen.
6. Experiment end:
    a. For regular samples –
        i. Rinse x 2 ("Instrument" tab)
        ii. Clean x1 ("instrument" tab)
    b. For dirty/sticky samples or those with PI –
        i. Run one minute of bleach
        ii. Run one minute of DI water
        iii. Clean ("Instrument" tab)
7. Data removal:
    a. Do NOT acquire sample data directly into Box
    b. Once experiment is finished transfer file to Box then delete off hard drive
    c. Experiments/data, figures and statistical charts will be removed off hard drive EVERY month. This includes anything left on the desktop for every user. Experimental templates (in lab's NovoExpress folder) will be left on hard drive.
    d. THE FLOW CYTOMETRY SHARED RESOURCE LAB IS **NOT** RESPONSIBLE FOR YOUR DATA