

Aurora Reference Guide

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Updated 12/14/2023

Fluidics:

- i. ALWAYS check at start
- ii. Supply:
 - a. Use autoclaved water in glass bottles
 - b. Use quick disconnect to pop off tubing, fill from glass bottles, reconnect tubing
- iii. Waste:
 - a. Use quick disconnect to pop off tubing, leave sensor connected, rotate bottle (not lid) and empty in sink.
 - b. Add bleach to bleach fill line, it's under the sink and labelled Unified Flow Core
 - c. Empty the waste when it reaches the Empty fill line, around $\frac{3}{4}$ full
 - d. Replace cap by rotating bottle (not lid) - reconnect tubing
- iv. Software will tell you when sheath is empty or waste is full – you should never see this warning if you are following steps i, ii, and iii.

Auto Sampler Loader (ASL):

- i. Calibrate: Acquisition > Left control panel bottom icon for ASL Calibration > Select Plate type > Select A1 position > Click arrows to center position of probe over A1
- ii. Plates (Pink, Yellow, and Black only)
- iii. 40 tube holder (Pink and Yellow only)
- iv. Sample shake/agitation
 - a. Maximum volume 175ul in 96-well plates

Loading a tube

- i. Sensor knows when tube is not on, orange light will come on (behind curved black door)
- ii. Make sure light does come on when you take off a tube, if it doesn't, the sensor might be stuck, inform a Flow Core staff member

Software:

- i. QC
 - a. Flow Core staff will QC and setup each weekday morning.
 - b. Friday's QC settings will work for Saturday and Sunday, if you want to learn how to QC the machines on weekends talk to a Flow Core staff member.
- ii. Acquisition
 - a. Default > quickly check fully stained sample to make sure not off the charts bright
 - b. New > new experiment
 - c. Template > may also save experiments as a template
 - d. Import > Flow Core doesn't save experiments, we save FCS files only, to re-use experiment must re-import, Flow Core deletes experiments twice a month, to save experiment, export to your own storage

iii. Data

- a. Please export your experiments to your own storage device, the experiment folder will contain your FCS files and any files needed to re-import the experiment
- b. Only FCS files are kept, experiment files are deleted every 2 weeks
- c. FCS files can be found on external back-up drive after 2 weeks

New Experiment:

- i. Create Wizard > easy to follow; next, save, or cancel
- ii. Name > Experiment Name w/ Date
- iii. Fluorochromes arranged by exciting laser, if you are unsure you may use search function. Double Click to add to Exp, or click Add.
- iv. Viability and fluorescent proteins have their own sections
- v. Groups > references are single color stains
- vi. Create Reference Group > choose cells for negative control (always use cells for negative control), may choose beads/cells individually for each reference, but we highly recommend using cells whenever possible.
- vii. Set up Exp, click Group tab, add tubes for samples
- viii. Markers > drag or type in markers for each color at the experiment or group level
- ix. Keywords > skip
- x. Acquisition > change samples to unmixed worksheet = compensated; can change stopping gate values or gate selected. Don't change the storage gate, anything outside of the storage gate will not be recorded, leave it on all events. (since we haven't created gates yet there are none to choose, you can come back and Edit later)
- xi. Save & Open

Acquiring:

- i. Acquisition Control: no data saved until recorded
- ii. Instrument Control: rarely change individual gain (voltage), only if off scale, but if needed, don't change individual gain, lower laser by percentage. If you need to change gain for a channel, ask a Flow Core staff member.
- iii. Threshold: exclude debris from RBC derived samples, be careful because any events excluded by threshold are not saved.
- iv. Controls: Unstained – change FSC + SSC, can do so between samples with no effect on **unmixing** (compensation). Click start, probe descends to take up sample.
- v. Speed: low 15-20ul/s < medium 30-40ul/s < high 60-80ul/s. If speed drops too low there's a clog, event rate will drop fast as well
- vi. Look at Unstained, change FCS and SSC until population is in center of plot. Doesn't matter where negative is on histograms as long as there's space for positive peak. Record Unstained.
- vii. Look at fully stained next before recording controls. Don't record yet, just confirm everything's on scale.
- viii. Always wait a few second after hitting acquire to start recording, fluidics flux
- ix. Finish recording all controls.
- x. Remember to add negative beads, when needed, to controls. Controls must have a negative population.

- xi. SSC is detected by the violet laser, SSC-B is detected by the Blue laser
- xii. Machine looks at all signals across entire spectrum

Unmixing:

- i. Unmix Wizard
 - a. Select control, software asks if you'll use today's or from library, may import FCS file from previous Exp in a pinch, but very inadvisable. Just make a reference library.
- ii. Gates
 - a. Ctrl key moves all gates
 - b. Software will choose correct channel usually, but if it doesn't, just drag and drop onto correct channel
 - c. If peak is wide, just take brightest point, minimum 200 events
 - d. Refer to guide printout for correct channel, even if it's bright in other channel, just choose the correct channel. Remember the software is looking at the spectral signature.
- iii. Live Unmixing: **always choose live unmixing!!!**
 - a. Software should save experiment in the background, but click save just in case, little X to close, yes to save
 - b. If you are happy with the gains used during the experiment, remember to save your user settings with the date or name of experiment. You may choose to reuse those user settings/gains at a later date.
 - c. Go to My Experiment, where experiments are stored
 - d. Highlight your experiment > export to your drive or OneDrive
 - e. If large experiment size, export to Desktop first, then to your USB (much faster)
 - f. Exports to Zip Folder
 - Raw Data (to reimport experiment)
 - Unmixed (FlowJo)
 - FCS 3.1 files
 - Experiment file, can import into SpectroFlo
 - Assay settings
 - Raw + Unmixed worksheets

Troubleshooting:

- i. Cytometer Tab > Left side
 - a. SIT flushes between samples, please do an extra SIT flush between sticky, red, or dirty samples
 - b. SIT Calibration: .75mm from bottom of a 5ml tube, if you add a bullet tube change distance to 1.25, click calibrate, save by clicking ok > calibration you set is saved for the rest of your session. It won't save if you just click cancel.
 - c. Purge filter: don't use
 - d. Clean Flow Cell: if SIT flush doesn't unclog, follow software directions. If bleach doesn't work use 10% Contrad instead and run again. If still clogged – contact Flow Core.
 - e. Long Clean: **don't run!** Flow Core runs routinely to keep machine performing well.

- f. Fluidics Shutdown: follow software directions, you must stay until the end to press the round power button on the left side of the Machine. Use 10% Contrad even though the software says to use 30%

Last User:

- i. Check Schedulebook.com before leaving the machine, every time, the person after you may cancel and leave you as last user of the day.
- ii. If you are last user of the day you must run Fluidics Shutdown and stay to turn the machine off via the round power button on the left side of the machine. (This counts as cleaning the machine, when Flow Core staff monitor to make sure all users are cleaning we check if you are last user of the day if we don't see recordings in your CLEAN experiment.)
- iii. Don't shutdown the computer, Core staff takes care of computer updates, etc.
- iv. Check waste, if it's full empty it.
- v. Check sheath, if it's below half full, fill it.

Not Last User:

- i. Import CLEAN Experiment from desktop
 - a. Record 3 minutes running BLEACH on high
 - b. Record 3 minutes running WATER on high
- ii. Sign Out when done
 - a. Charged the greater time: reserved or used > please shorten your reservation if you finish early so that others may use that run time.
- iii. Check waste, if it's full empty it.
- iv. Check sheath, if it's below half full, fill it.

Plates/Tube Rack:

- i. Switch on loader, rocker switch on the outside of the loader
- ii. In experiment: choose format, it defaults to single tube > add rack/plate
- iii. Plates: once you've installed your plate in the loader, and selected plate in the software, grasp and pull the gate shifter forward, it acts as a guide for the SIT to ensure it dips into the correct well.
- iv. Remember to turn the loader off when you are finished.