27 July, 2021 Issue 1

Research Flow Cytometry Core

Quarterly Newsletter

Information Provided by your CCHMC RFCC

What's new?

New sorters!

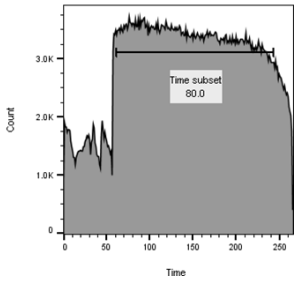
BD FACSAria Fusion – This 5-laser sorter can detect up to 18 fluorescent parameters at once and sort up to 4 populations simultaneously. It has replaced Ariel. Don't worry: any experiment you designed and optimized for Ariel will work on the new instrument, though be aware that if you were using a template from Ariel you will need to create a new one on the Fusion.

ThermoFisher Bigfoot spectral cell sorter – This cutting edge sorter can sort up to 6 populations simultaneously and is capable of spectral unmixing with 48 fluorescent detectors. It can also be configured to use the traditional 1 detector per dye paradigm. The Bigfoot is equipped with a carousel style autosampler so it can be operated remotely. Sort devices include 384 well plates and 10x genomics chips. It is available for staff-assisted sorts now. Please contact Ken to schedule. Training is on the way.

Core concept to consider

Improve your data analysis with time gating

Good flow cytometry data depends on each cell interacting with the laser in the same way. This is why we recommend running samples on the lowest flow rate possible while acquiring data. In addition to scatter and fluorescence parameters, modern flow cytometers also record the time at which each event was recorded. By plotting this time parameter and drawing a gate based on it, you can identify when a fluidics issue might have caused cells to not cross the laser in a consistent way and exclude those events from your analysis.



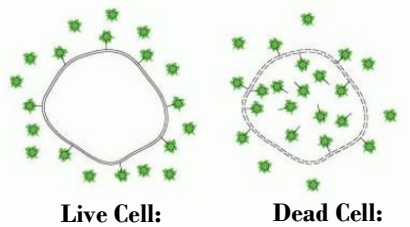
ExCyte advanced flow cytometry course

If you are interested in advancing your flow cytometry skills, the RFCC will be sponsoring a two-day course covering panel design, experimental controls, and data analysis among other topics. Date is TBD but space is limited so please contact Celine if you are interested.

Helpful hints

Improve your live/dead staining

Viability dyes such as fixable live/dead dyes from ThermoFisher and Zombie dyes from Biolegend work differently from nucleic acid stains such as DAPI and 7-AAD. They stain free amino groups found in proteins; live cells with intact membranes will stain only surface proteins while dead cells will stain much brighter because both surface and cytoplasmic proteins are labeled. Practically that means the staining conditions should be different! Fixable live/dead dyes should be added in buffer that *does not* contain BSA or FBS and subsequently washed out before adding any antibody conjugates. Following this procedure will greatly reduce your background staining and improve separation of the dead population.



Live Cell: Stain cannot permeate the membrane = low signal

Dead Cell: Stain permeates the membrane = brighter signal

Dates to Note

8/24: ORVCA Imaging and Cytometry Research Day
9/6: Labor Day no RFCC staff on site