17 January, 2022 Issue #3

Research Flow Cytometry Core

Quarterly Newsletter

Information Provided by your CCHMC RFCC

How Do I...?

Optimizing & Confirming Sorting Success

There are numerous steps you can include to optimize your sort! Prior to sorting, it is important to know the cell expression of your markers. If you're not sure, review the vendor data regarding your specific antibodies. Well expressed, abundant markers should be paired with dim fluors; poorly expressed should be paired with bright fluors. Check your panel on our analyzers to ensure acceptable resolution prior to sorting.

Additionally, it is crucial to have controls on-hand; even if you have a well-established template. Single color controls with each experiment can be critical for compensation calculations. Incorporating Fluorescence Minus One (FMO) samples can be extremely useful as gating controls for data analysis, as well as controls to validate the expression of rare or low-expressing markers. We DO NOT suggest the use of FMOs as a replacement for single color controls.

Utilization of a viability dye to exclude dead cells is a great way to better identify healthy-cells within your target population. Another beneficial exclusion method is to exclude aggregates by utilizing both forward and side scatter doublet discrimination plots in your gating strategy. In addition to proper controls and exclusion methods, running a post sort for analysis allows you to verify the purity of your sort for publications.

Core Concepts to Consider

Sorting Recommendations

It is crucial that the collection tube you have selected is appropriate for the number of cells you are sorting. An increase in nozzle size impacts the number of cells you can collect, due to an increase in droplet size. For example, the 70um nozzle can sort ~1.25 x 10^6 cells per Eppendorf tube; the 100um nozzle can sort that amount into a 5mL FACS tube. In general, if you expect a few thousand cells, it is best to use Eppendorf tubes instead of 15mL or 5mL tubes.

General Recommendations

Be certain to carefully read the technical data sheet for your viability dye! Not all viability dyes label dead cells in the same manner (Ex. DNA dyes vs amine dyes).

Sorting Updates

Mary Mullen and Anastasia Brenn have joined our RFCC! We thank you for your patience as they integrate into our team.

Receive updates about what is going on in our core by registering to the research Flow Core email list! If you want to be updated on our ORVCA monthly meeting, sign up for the ORVCA email list!

Did You Know?

If you are a part of the preparation of cell samples and/or bringing the cells to our core, you need to have a sorting consultation with Celine. Make sure you have a Stratocore account with a budget number, and contact Celine to schedule a consultation!

Our annual instrument survey is open. Please participate to help us determine your instrument and data analysis needs! Survey ends **JANUARY 21ST**.

We can sort 60-well Terasaki plates on the Sony instruments now! Email Abbey for training to plate sort on the SH800 & MA900.

Check out the ORVCA meeting February 23rd where Rob Thacker, PhD. with Luminex, is presenting data analyzed with our new machine learning module, currently on the IDEAS analysis workstation. If you're willing to provide either old or new data that Rob could analyze and present, please contact Sarah! If you're interested in participating in an upcoming ImageStream workshop email Sarah! Let her know if you'd want to acquire a new experiment with Rob, or focus on data analysis. Thanks in advance for your participation!

Dates to Note

01/17: Martin Luther King Jr. Day, No Staff on-site.

01/26: ORVCA Zoom Meeting @ 1PM ~ Option available to remove debris/dead cells from your single-cell preps (Curiox Biosystems, Akadeum, StemCell, Miltenyi, and BioLegend).

02/08: FlowJo workshop 10AM - 1PM in S 1.203, 26 available seats. REGISTER HERE.

02/09: FlowJo workshop 10AM - 12PM in S 1.203, 26 available seats. REGISTER HERE.

02/23: ORVCA Zoom Meeting @ 1PM ~ ImageStream update and machine learning module.

04/12 - 04/14 OR 04/20 - 04/22: ImageStream Workshop (official date to be released).

Our Cell Sorters | Schedule a sort on Stratocore | Check out our Team | Contact us with Questions