

Research Flow Cytometry Core

Quarterly Newsletter



Information Provided by your CCHMC RFCC

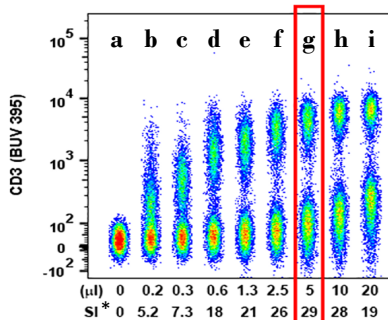
Let's Talk About It

Reagent Titration

You may have heard that you should always titrate antibodies in a multicolor flow experiment, but why is it so important and, practically, how to do it?

For example, consider a BUV395 conjugated anti-CD3 antibody. The goal of titration is to find a staining concentration where as many CD3 molecules as possible are bound to an antibody molecule — but *nonspecific* binding of the antibody is prevented or minimized.

Look up the vendor's recommended staining concentration and start with a concentration 2 fold higher. Do a 2:1 serial dilution of at least 4-5 steps. Ideally you should do enough steps to see where the nonspecific background staining starts to increase and where the specific positive staining starts to decrease.



a-e. Ab concentration too low.

f-g. Optimal staining concentration.

h-i. Ab concentration too high, nonspecific staining.

Nemoto, S; et al. Cytometry Part A. 89A: 427-429, 2016
*SI=stain index: (pos median - neg median)/(2*neg SD)

You will often find the optimal concentration is far lower than the vendor recommends, which means your expensive reagents last longer!

Check it Out!

Software, software, software!

Interested in cell cycle analysis? The ModFit LT software on our PC2 Workstation provides a more efficient analysis for you to use! Need to perform high parameter analysis? Compare FlowJo to FCSEXPRESS on our PC2 or PC3 Workstations.

Access the Sony Software for analysis on our PC3 workstation! Interested in getting trained to sort autonomously? Email [Celine](#) for a consultation!

The RFCC workstations provide you FREE access to: FACSDiva, FlowJo, ModFit LT, FCS express, SpectroFlo, Sony Cell Sorter Software, and the ImageStream Machine Learning Module. Make sure you use [Stratocore](#) to book the [workstation](#) that suits your needs!

Spectral Accessibility Peaks!

Thanks to all who filled out our annual instrument survey. Previous feedback has allowed us to acquire another Cytek Aurora, Maleficent! You can find both spectral analyzers in R5509 (2 lab bench bays over). Trained Aurora users have already been granted rights to Maleficent. The PC3 workstation with SpectroFlo will move to that area of the lab shortly.

Helpful Hints

How Can You Help Yourself?

Online courses are right at your fingertips! Need a refresher on basic flow cytometry? See Course 1. RFCC Policies and Procedures can be found in Course 2. Using BD FACSDiva? Check out Course 3 for an overview of the software, and Course 4 to review how to run an experiment. CCHMC badge holders can find our courses on the [ELM](#) by signing in with your CCHMC credentials. External users can check out any of these videos on our website, [HERE](#).

Troubleshooting

Scan this QR Code for instructions on how to create and save a template!



Also, take note of the troubleshooting guide located at each BD instrument!



Help Us Help You!

The Spring Survey is on its way! Keep an eye out for this survey to release in June. Your feedback provides us insight into what services we are providing well, and how we can serve you better!

Dates to Note

- 04/12 - 04/14: ImageStream Workshop with Rob Thacker, PhD, from Luminex.
- 04/27: ORVCA Meeting 1-2 PM ~ Presentation of the LeviCell by Levitasbio & VeloCyt by Bennubio.
- 04/27 - 04/28: Laminar Wash System demo with Ohimai Unoje, PhD, from Curiox.
- 05/25: ORVCA Meeting 1-2 PM ~ Presentation of the CytoFLEX SRT sorter by Beckman Coulter.
- 06/03 - 06/07: Cyto 2022 Annual Conference.
- 09/16 - 09/18: GLIIFCA XXXI Annual Scientific Meeting in Indianapolis, IN. FACSria cell sorters will only be available to autonomous users these days. Autonomous users please contact us to book.
- 09/29: Annual ORVCA Meeting with John Irish as the Keynote Speaker.