DHVI Flow Facility Newsletter

Spring 2017 Duke Human Vaccine Institute Flow Cytometry Facility

Welcome Aboard!

In January, we brought on a new flow engineer, Steve Slater. Steve previously worked for BD, and he brings over eleven years of experience with cytometry instrumentation, electronics, optics, and software. On top of it all, he's a heck of a nice guy, and we are excited to have him on board. On a typical day, you will most likely find Steve running a sort on the A01, tweaking a laser alignment on a LSRII, or blowing minds with an 18-color, 6-way sort on the Influx. Say hi the next time you see him.

Core Research Transition

Last December, we migrated all scheduling and billing services to Core Research. We greatly appreciate the patience of our users during this transition, and we are continually working with Core Research developers to improve the functionality and ease of this platform. With any change, however, there are bound to be growing pains. Here are some scenarios we encounter frequently:

User Scenario 1: "I could have sworn that I made a reservation yesterday, but now it's not on the calendar. Either I'm crazy, or Core Research is!"

Likely Issue: After saving the reservation, you may have forgotten to push "Submit For Approval." Reservations that have been saved but not submitted are automatically deleted after 10 minutes. These ineffectual, unapproved reservations appear as "Carolina blue" on the calendar.

User Scenario 2: "My PI wants me to use a new fund code for an upcoming flow session, but when I enter that fund code in the new reservation in Core Research, I don't see my name on the list of users."

Likely Issue: You have not been linked to that fund code within the Core Research system. Unfortunately, flow staff cannot make these links. The following personnel can link users to fund codes: the PI of the fund code, a PI-delegate, or a financial administrator. Talk to these folks to get linked.

User Scenario 3: "I really love getting emails from Core Research, but the huge batch that shows up in my inbox at the beginning of every month is a little ridiculous…"

Issue: For better or worse, Core Research generates an email to the user at every step of the reservation life cycle. Think of each email as a milestone for that experiment. When the final email arrives that your reservation has been billed, enjoy the cathartic release of sending it to your Deleted Items folder.

Sorting on the BD Influx

Ever wished you could sort more than 4 cell populations at a time? Do you think 18-color panels are sooo 2016? Does your favorite cell type require more than 8 levels of gating? If these questions keep you up at night, then contact the DHVI Flow Facility about setting up an experiment for the BD Influx. This cell sorter features 5 lasers, 23 fluorescent parameters, and 6-way sorting. Its modular design offers flexibility to meet your research needs. Please email the facility director (derek.cain@duke.edu) for more information.

DHVI Flow Facility Website – New and Improved!

https://shared-resources.dhvi.duke.edu/dhvi-core-facilities/dhvi-flow-cytometry

In the fall of 2016, we unveiled our new website. From the home page, you can:

- Request training to be an independent user of our analyzers (see "Get Started")
- View our instrument lasers and optical configurations (see "Instruments & Services")
- Request a FlowJo license (see "Instruments & Services")
- Download protocol sheets, instrument guides, policies and procedures, and SOPs (see "Download Library")
- Find links to flow cytometry-related resources and educational tools (See "Useful Info")
- View and download experimental protocols (See "Useful Info"). Got a protocol to share with the rest of the Duke flow cytometry community? Send it to us (dhviflo@dm.duke.edu)!

Antibody Panels on Fluorofinder

We are working with Fluorofinder to build a repository of antibody panels, developed by DHVI Flow Facility users, that have been validated on our instruments. This repository will be available through the Fluorofinder "Groups" application. Users will be able to share their panels - with pertinent information on antibody clones, fluorochrome conjugates, optimal titers, and ordering information - with other members of the Duke flow cytometry community. We are currently building the repository – if you have an antibody panel to share with your fellow flow-ers, please contact the facility director (derek.cain@duke.edu). Likewise, if you are interested in viewing panels in the repository, please send the director an email request for membership to the group. Keep an eye out for more information in the near future.

Tell us about your Publications!

We would love to hear about your publications that use flow data acquired in our facility. In fact, we will feature your published article on our website. Contact the facility director for details (derek.cain@duke.edu). And don't forget about us in your Acknowledgments section!

FlowJo Training Workshop

On April 26, we will host John Quinn from FlowJo for our annual training. This FREE workshop will be a great opportunity for new and experienced users to learn the various tools in FlowJo to analyze and display cytometry data. Feel free to attend the workshops most useful for you. The workshops will be held in MSRBI Room 001.

Workshop Schedule

9:00–10:30 am: FlowJo for Beginners. This workshop will deal with FlowJo basics. The focus will be FlowJo version 10, but much of the material will apply to version 9.

10:45-11:30 am: FlowJo (version 9) for Advanced Users

11:30-12:15 pm: FlowJo (version 10) for Advanced users

2:30-3:30 pm: SeqGeq. This is a new platform in FlowJo to integrate analysis of single-cell sorts and RNA-seq data.

Updated Protocol Sheets

At the beginning of 2017, we updated our instrument protocol sheets. Please download the new sheets from the website (link below) and use them in your upcoming experiments!

https://shared-resources.dhvi.duke.edu/dhvi-core-facilities/dhvi-flow-cytometry/downloads

Facility FAQ's

One of the most common questions we receive - especially from new users - is, "How much time should I book for my first sort?" To answer this question, we've compiled this guide:

1. For every ml of sample (@10^7 cells/ml) to sort: 1 hour
2. For every fluorochrome in your panel, add 5 minutes
3. If sorting at low pressure (45 or 20 psi), add 30 minutes
4. If sorting into 96-well plates, add 30 minutes (disregard if sorting at low pressure)

Example: Bob plans to sort cells from 2 tubes, each containing 10^7 cells in 1 ml of buffer. Bob's panel has 8 colors, he wants to sort at 20 psi, and he will collect his sorted cells in 5 ml tubes (not plates). The first time he comes to sort, he should book:

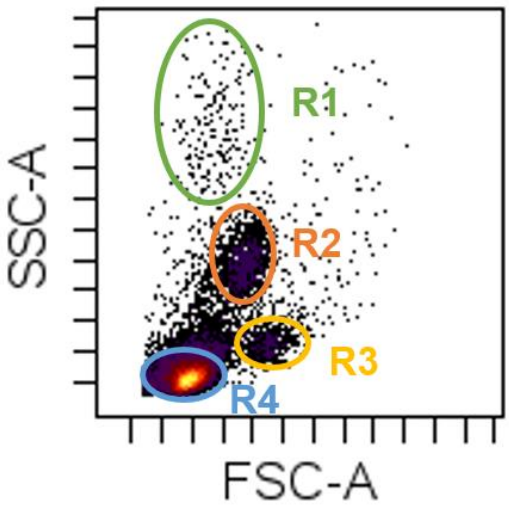
1. 2 ml of cells @ 1 ml/hr = 2 hours
2. 8 colors @ 5 minutes/fluorochome = +40 minutes
3. Sort at 20 psi = +30 minutes

Total: 2 hrs + 40 min + 30 min = 3 hours and 10 minutes

This guide is a conservative estimate. As you (and we) become familiar with your samples, you will be able to make better estimates for the amount of time needed for your sorting needs.

Test your Cytometry Skills

1. Mouse blood was harvested and red blood cells were lysed. From the FSC and SSC profile below, identify the cell types in each gate.



Answers: R1=eosinophils, R2=neutrophils, R3=monocytes, R4=lymphocytes