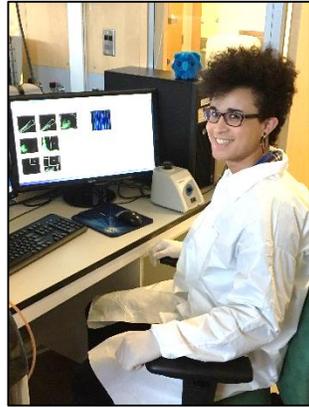




Welcome Aboard!

In October, we brought on a new research technician, Ariel Arus-Altuz. Ariel obtained her bachelor's degree in biochemistry from NC State, and most recently worked with Proctor & Gamble as a quality control analyst. In her position with the DHVI, Ariel will split her time between working in the facility to maintain and QC our analyzers, and working in the lab on DHVI research efforts. Say hi the next time you see her!



The Analysis Suite is Moving

There is much excitement at the DHVI surrounding the new GMP suite coming to the second floor of MSRB2. To accommodate this new facility, the Flow Facility's analysis suite (containing the L01, L02, and F01) will be relocated. The analyzers will be moved to RP105, which is located in RP1 next to GSRB2. Our sorters (A01, A02, and N01) will not be relocated.

We understand that this move will inconvenience some of our users, especially those who work in MSRB2, but we are working to offset the increased distance with enhanced capability. For example, in our current location in MSRB2, we do not have the safety measures to analyze live human or non-human primate cells. In the new RP1 location, we will house the L02 in a room with appropriate safety measures to run BSL2+ samples. This new capacity will allow, for example, analyses of calcium flux in human cells or cell lines.

The facility will relocate in early 2018, although the exact date remains to be determined. Look for emails from us with details on the move!

Flow Cytometry Tips and Tricks

Compensation Basics

By Patti McDermott

Fluorescence compensation corrects for overlapping emission spectra of fluorochromes. Proper compensation ensures that signals from neighboring channels are appropriately subtracted so that there is no spillover between channels. Without compensation, for example, the positive signal obtained with PE CD4 labeling would also appear in the PE-Texas Red channel, leading to a false positive result.

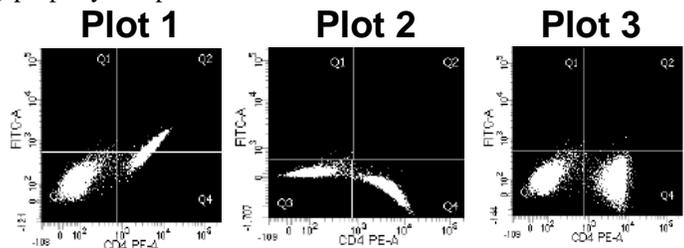
To calculate compensation, samples stained with individual fluorochrome-labeled reagents must be run on the cytometer. If you run a 6-color antibody panel, you will need 6 compensation samples.

When is compensation correctly set? When the median fluorescence intensity (MFI) of the negative population is equal to the MFI of the positive population *in the spillover channel*.

For example, if the PE and FITC channels in the plots below are correctly compensated, then the PE positive and PE negative populations will have the same MFI *in the FITC channel*. If the FITC MFI of the PE positive population is higher than the FITC MFI of the PE negative population, then compensation needs to be increased. If the FITC MFI of the PE positive population is lower than that of the PE negative population, then compensation must be reduced.

For practice, use the data obtained from a PE compensation tube below to determine which plot is:

- a) over compensated
- b) under compensated
- c) properly compensated

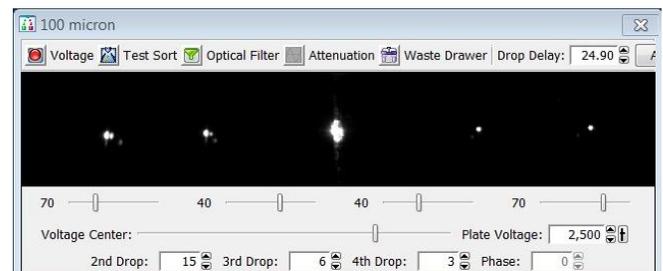


(See answers at the bottom of the next page)

Sort Tip: Select the Best Nozzle Size

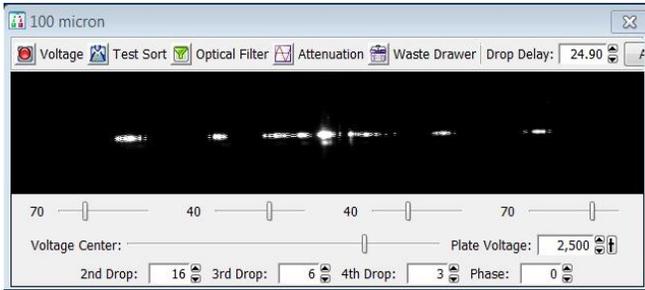
By Steve Slater

Fanning is good for keeping cool on a hot summer's day, but while sorting cells, it can mean the difference between a good and a bad sort. To understand fanning, take a look at this Diva window from a typical 4-way sort:



In the picture above, the five white points represent vertical streams of droplets illuminated by a horizontal laser. The four smaller white dots are the streams of droplets containing sorted

cells sent to collection tubes. The bright center stream contains droplets of cells going to the waste. In the photo below, do you notice anything different?



In the picture above, the streams are doing something that is known in cytometry as “fanning.” Fanning is not desirable, and can decrease cell purity and viability.

So, what causes fanning? For any cell sorter on the market today, the maximum cell/particle diameter that can safely fit through a nozzle will be 15-20% of the nozzle’s diameter (with 20% being the maximum particle size—do not exceed). If we use a 70 µm nozzle, then the safe maximum particle diameter is (70 µm x 15%) 10.5 µm. The 20% maximum would be 14 µm in this example. Once a particle reaches 10.5 µm in diameter, the outer side streams will start to fan. Once a particle reaches 14 µm in diameter, all streams will fan, and your sort may not produce results as expected. Once the 20% upper threshold is exceeded, the nozzle will most likely clog.

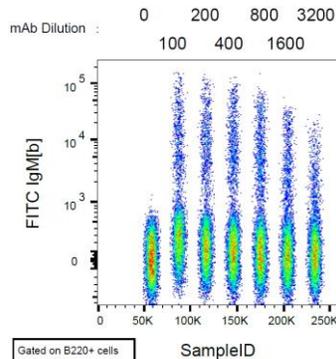
In planning your next sort, keep in mind the diameter of your cells, as well as the physics of the cells. Some cells expand under pressure, while other cell types may unfurl, thus increasing the diameter of the cell while under pressure. Some cells may clump together to form aggregates and, while not sorting these, they can still cause fanning. Proper planning with these simple facts in mind will help ensure the best sort possible.

FlowJo® Tip: Showing Titration Data

By Derek Cain

Looking for an easy way to show your titration data? Use FlowJo’s concatenation feature to plot data from multiple FCS files in a single dot plot (see below). To make a plot like this in FlowJo10, follow these steps:

1. Organize your samples into a new Group.
2. Create appropriate gates.
3. Select the gate in the first FCS file in the group.
4. Click Edit→Select Equivalent Nodes.
5. Click File → Export/Concatenate→ Export/Concatenate Populations.
6. In the “Populations” window that opens, select “Concatenate”. Choose the save destination – this will be the location of a new FCS file.
7. Click “Concatenate.”
8. When prompted with “Load Files into:”, select “Existing Workspace”, and then click Close.
9. Back in the “Populations” window, click cancel.



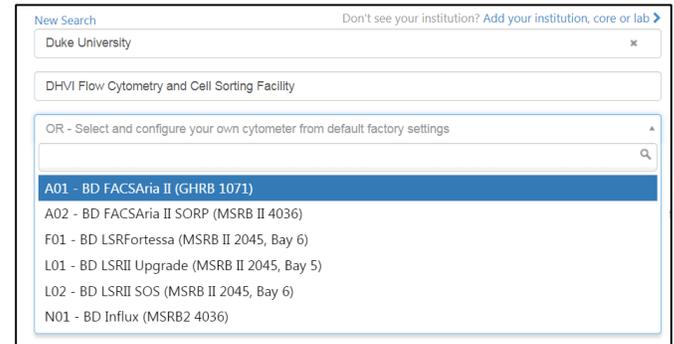
10. A new FCS file with the prefix “concat” will appear in the group. Open this file, and put the parameter of interest on the y-axis and “SampleID” on the x-axis.

Antibody Panel Design with FluoroFinder®

By Derek Cain

Fluorofinder® is a free, online application to aid researchers in the design of antibody panels for flow cytometry. The DHVI Flow Facility has partnered with Fluorofinder® to offer two services to facilitate cytometry experiments:

1. Build an antibody panel based on the specific configuration of our instruments. When building a new panel, click on the “Cytometer” tab and then enter the following information (for the bottom field, select the cytometer you plan to use):



When building your panel, you will now be able to select fluorochromes that match the lasers and filter sets of our specific cytometers.

2. Group Panel Collaboration Tool: We are compiling antibody panels designed by our users. To join the DHVI Flow Facility group, please email the director (derek.cain@duke.edu) with a membership request for our Fluorofinder group.

Once you have joined the group, you can view our repository of antibody panels. Click Experiment Center -> View Group Experiments-> Cytometers. If you have a panel you would like to share with fellow flow folks, please save your panel to the group!

Visit <https://fluorofinder.com> to check it out, or contact the facility director (derek.cain@duke.edu) for more details.

Fun Stuff

We’ve listed quotes from our favorite holiday movies/shows below. Send us an email at dhviflo@dm.duke.edu with the correct movie/show match (Subject Line: Holiday Fun) – correct entrees will be entered into a random drawing for a free hour of cytometry!

1. **Patti:** “Here we only got one rule. Never ever let it cool! Keep it cookin’ in the pot, Soon, ya got hot choc-o-lat!”
2. **Steve:** “It’s Christmas Eve. It’s the one night of the year when we all act a little nicer, we smile a little easier, we cheer a little more. For a couple of hours out of the whole year we are the people that we always hoped we would be.”
3. **Ariel:** “I’m sorry I ruined your lives and crammed eleven cookies into the VCR.”
4. **Derek:** “Randy lay there like a slug. It was his only defense.”

Answers to Compensation Basics: Plot 1, b; Plot 2, a; Plot 3, c