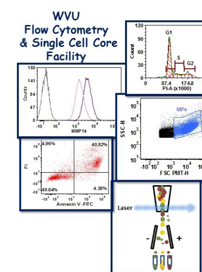

WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 2, issue 4 April 2016

New Flow Cytometry Tool

One of the most important parts of any flow cytometry experiment is choosing the appropriate combination of fluorochromes. While choosing fluorochromes is relatively easy when the experiment requires just a few fluorescent molecules, the process becomes more difficult once you get beyond four fluorochromes. The reason for the increase in difficulty is that it is not uncommon for the emission spectrum of one fluorochrome to overlap with another's. Several companies have versions of "Spectra Viewers", which show you the excitation and emission spectra of different fluorochromes based on the laser and filters sets you select. Unfortunately, these websites, only provide a limited amount of general information.

Recently a new tool called **FluoroFinder (https://fluorofinder.com)** was developed for multi-color flow cytometry panel selection. It is a free tool designed to help you in your fluorochrome-antibody decision making process. It not only tells you which fluorochromes are available for the particular laser and filter set combinations on your institution's flow cytometers, but it will also tell you the catalog number and company that has the antibody-fluorochrome combination for a particular marker/protein. It can also let you know how much spillover the fluorochrome will have into other detectors. The website is designed to reduce the amount of time spent making an antibody panel. With all this being said remember that no system is fool proof so once you have made a panel it is a good idea to double check it prior to purchasing any reagents.

How do you use FluoroFinder?

FluoroFinder is really easy to use. The WVU Flow Cytometry & Single Cell Core Facility's flow cytometers laser and filter configurations are already in the system. Right now they are offering a free T-shirt to everyone that registers. Below is an outline of the steps you need to do in order to generate a panel.

1. Go to the website—https://fluorofinder.com
2. Log in or register if not already a subscriber
3. Click on the green "Build/Enter Panel Now" button

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Facility Location:
2160 HSCN
Phone:304-293-6273
e-mail: flowcore@hsc.wvu.edu

Hours of operation:
9:30 am to 5:00 pm, M-F

After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

4. “Choose a Cytometer” page opens up
   A. Enter your institution—if you type “We” a drop down menu opens. Select West Virginia University
   B. The second box on the page will auto fill with “WVU Flow Cytometry Core”
   C. For the third box select the Cytometer you will be using
   D. Click “Continue”
5. “Cytometer” page opens. This page shows the lasers, filters and suggested fluorochromes that work with each laser and filter set. Click “Continue”
6. “Select Markers” page open. On this page you **must** list all the markers/proteins to be detected in the panel, the target species and antigen density. Additional boxes that you may want to fill-in include the host that the antibody comes from, Isotype and antibody clone name. Click “Continue” when done.
7. “Select Products” page opens. Here you will find a table that shows the available fluorochrome conjugated antibody combinations available for each marker you entered on the previous page
   A. For each marker, select the fluorochrome you want the antibody to be conjugated to
   B. A box opens with a list of antibodies specific for that Marker conjugated to the selected fluorochrome. This box will also tell you the catalog number, the clone name and price.
   C. Select the antibody you want.
   D. Once a fluorochrome is selected, the detector for that fluorochrome is grayed out and cannot be used with any other markers.
   E. Hovering over the selected fluorochrome results in a pop up box that shows you how much spectral overlap the fluorochrome has with other choices.
      1. It is best to select fluorochromes that have the least spectral overlap with each other
      2. This box will also indicate the percent of the fluorochrome’s emission spectra that will be detected at the detector
      3. For low level expressing marker choose a fluorochrome in which the percent detected is as high as possible and has minimal spectral overlap with the other fluorochromes you have chosen
   F. Click “Continue” button when done
8. In the window, your completed panel will be shown. You can name it (saving the panel), print the panel, export the panel to excel or as a pdf, go back and change the panel or build another

Check out the online panel design tool now at www.fluorofinder.com or click here to learn more about this resource. To schedule a free 15 minute, online training demonstration for your lab at your convenience, contact FluoroFinder at demorequest@fluorofinder.com.

To log in and reserve a flow cytometer, C1, AutoMACS, MSD or analysis computer, please point your browser to the following URL

https://cores-wvu.mis.vanderbilt.edu/login.cfm

Flow Cytometers in this facility

FACSAria Cell Sorter

Operator: Facility Staff

Lasers: 488 nm Sapphire(SS)
633 nm HeNe
407 nm Violet(SS)

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 11 fluorochromes

Applications:

- Cell Sorting (Aseptic)
- Cell phenotyping
- Cell Viability
- FISH, FRET, SPA

FACSCalibur Analyzer

Operator: User

Lasers: 488 nm Argon
633 nm Red Diode

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 4 fuorochromes

Applications:

- Cell phenotyping
- Cell cycle analysis

LSR Fortessa

Operator: User

Lasers: 405 nm OBIS LX
488 nm Sapphire (SS)
561 nm Sapphire (SS)
628 nm OEM

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes

Applications:

- Cell phenotyping
- Cell Viability
- Cell Cycle analysis
- FISH, FRET, SPA

Other Instrumentation in this facility

AutoMACS Magnetic Bead separator

Operator: User

Application:

- single marker (extracellular) sorting
- depletion sorting

gentleMACS Octo Dissociator with Heaters

Operator: User

Application:

- Dissociation of tissues into single cell suspension for culture or flow cytometry assays
- Homogenizes tissues for downstream molecular biology applications

C1 Single Cell Auto Prep System

Operator: User or Staff

Application:

- Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for down stream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

Format: 96 or 384 chambers per chip

MSD Multi-Array Platform

Operator: User

Applications:

- Detection of cytokines, cell signaling proteins
- Multiplexed assay design (1-10 analytes/plate)

Detection range:

- 1 – 10,000 pg/ml

Sample volumes:

- 25 μl or less

Assay Time:

- 4—6 hours depending on analytes being detected

Update: Sorting co-Pay to continue through June 2016

The HSC Research Office is generously providing the facility with a limited amount of funds to subsidize/co-pay users' sorting costs.

From now through June 2016, the cost of sorting for a WVU researcher is $15/hour.

Fee Schedule (2015-2016)

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	$33/h	$50/h	$74/h	$14/use	$10/use	No Cost	No Cost	$10/sample
Non-WVU User	$49.50/h	$75/h	$111/h	$21/use	$15/use	$75/plate	$225/plate	$15/sample

New User Guide

Hands-on training for FACSCaliber, LSRFortessa and C1 Single Cell Auto Prep System is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting as well as data acquisition on FACSAria is by facility staff only.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own.

Validation of Antibodies

As many of you are aware, NIH is now requiring "Authentication of Key Biological and/or Chemical Resources" as part of all grant applications. One of these key biological resources that needs to be validated are the antibodies we all use in our experiments. Relying on a company's validation is not acceptable. So the big question in the research community is how best to validate an antibody in your lab. An article entitled **"Exercises for Your Abs" published Feb 1, 2016 in The Scientist Magazine** discussed this issue. In the article, David Rimm, professor of pathology at Yale University discusses a few highlights from a paper he published in **Biotechniques (47:197-209, 2010)** that describes a detailed algorithm to verify antibodies. One of his recommendations is a high signal to noise ratio. For Western blots he recommends that the antibody should only detect a nice distinct band with few extra bands. For immunohistochemistry the antibody should only label tissues in which the protein resides. For immunofluorescence the antibody should identify the part of the cell where the protein is known to be located and it should not bind a negative control. Ideally and when possible, a second antibody should be used with similar results. Several antibody companies are also getting involved in validating the antibodies they sell. Proteintech is using RNA interference to test all 12,000 plus in there catalog. Abcam is doing the same and their CEO indicated that several of their antibodies failed the test and they are no longer being sold. Finally, it is important to share your findings.

If you go to our website (http://flowcore.hsc.wvu.edu/) under the Useful Links tab you will find a list of antibody websites where you can post your findings as well as find reviews of antibodies tested by others.

Upcoming Holidays & Events

May 10, 2016	Election Day Holiday	Facility Closed
May 30, 2016	Memorial Day	Facility Closed
June 8—17, 2016	Kathy out of the Lab	Facility open but no sorting performed

Note to users:

Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):

FACSAria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

C1 System users: Experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers U54GM104942 (CTR), P30GM103488 (CoBRE) and P20GM103434 (INBRE).