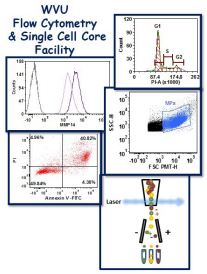
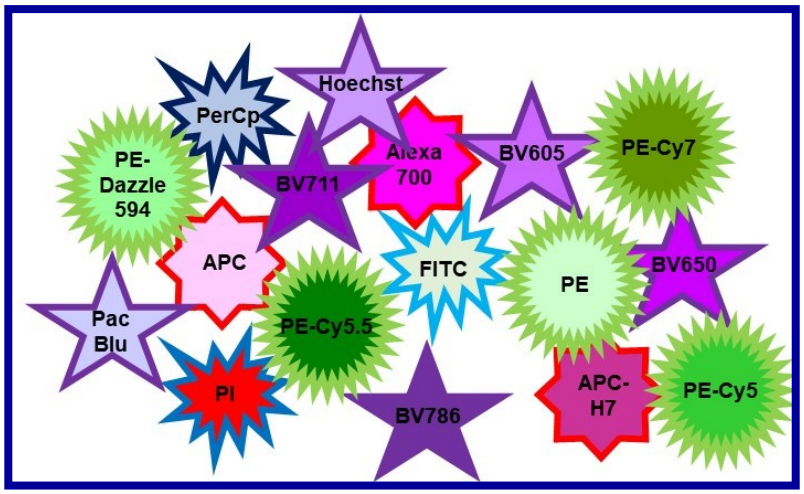
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 7, issue 3 January 2021

Best Practices for Multi-Color Flow Cytometry



Welcome to 2021! Hopefully it will be a better and less eventful year than 2020. To start the new year off on the right foot, lets review the best practices for performing multi-color flow cytometry. In this article, guidelines for sample prep, detector voltage, fluorochrome selection, antibody titration and controls will be presented.

Sample Prep

It can not be emphasized enough that good sample prep is essential for obtaining good flow data. Most Flow Cytometry Core staff will tell you that that if a flow cytometry experiment fails it is almost always because of issues with the sample not the instrument. Here are a few things to keep in mind when preparing samples for flow cytometry:

1. Cells must be in a single cell suspension
2. Samples should not have any visible clumps, they will clog the instrument
3. If trypsin is being used to get adherent cells into suspension remember, trypsin can damage surface proteins so that antibodies will not bind
4. Never scrape adherent cells to detach them from the plastic. Scrapping tends to do a lot of damage to the cell.

Inside this Issue

Facility Location:
Main Lab: 2160 HSCN
Annex Lab: 2184 HSCN

Phone:
304-293-6273

email:
flowcore@hsc.wvu.edu

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

After hours access is available for experienced users with prior approval from Dr. Kathy Brundage

Contact Dr. Brundage at:
kbrundage@hsc.wvu.edu

5. Before fixing cells, make sure they are in a single cell suspension. Once fixed, cells cannot be separated from each other without seriously damaging them.

Detector Voltage

The voltage of each detector can be adjusted and should be prior to recording any data. What you may not know is that periodically, the core staff runs what is known as a voltage walk or voltration using CS&T beads. Usually the voltration is done after the instrument has been serviced and the lasers re-aligned. The voltration allows you to determine the minimal voltage required to get a clear separation of dim fluorescent signals from background instrument noise. Starting in Jan 2021, the optimal linear range of each detector will be posted on the instrument so that you will know the minimal voltage required to get a good separation of dim populations from background.

Fluorochrome Selection

When choosing which fluorochromes to use in a staining panel, the goal is to minimize the amount of compensation without affecting the data quality. The more fluorochromes in the panel, the more likely the spillover will reduce the ability to distinguish the specific signal of one fluorochrome in the presence of the others. Below are a list of things to keep in mind when designing a staining panel as well as a tool for assisting you in the process.

1. For targets that are expressed at low levels use bright fluorochromes i.e. PE, BV421 or APC
2. Since intracellular proteins tend to be less abundant than most surface proteins use bright fluorochromes
3. Think about the population(s) you are interested in and the gating strategy you will employ. For proteins that will be co-expressed use fluorochromes that have distinct emission spectrums and when possible excited by different lasers.

To assist you in generating a staining panel, FluoroFinder is a great tool. Below are the steps to follow when using FluoroFinder:

1. Go to the website — https://fluorofinder.com
2. Log in or register if you are not already registered. Registration is free
3. If not already selected, Enter your institution (West Virginia University)
4. The second box should auto fill with “WVU Flow Cytometry Core”
5. Select the instrument then click “Continue” button
6. “Select Markers” page open. On this page list all the markers/proteins to be detected in the panel, the target species and antigen density (if known). Click “Continue” when done.
7. “Select Products” page opens. On this page will be a table that shows the available fluorochrome conjugated antibody combinations available for each marker entered on the previous page
8. For each marker, select the fluorochrome you want the antibody to be conjugated to
    a. A box opens with a list of antibodies specific for that Marker conjugated to the selected fluorochrome and includes catalog numbers, clone names and prices.
    b. Select an antibody.
    c. Once a fluorochrome is selected, the detector for that fluorochrome is grayed out and cannot be used with any other markers.
    d. 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. 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 markers 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

e. Click “Continue” button when done

9. In the next window, the completed panel will be shown. At this point, the panel can be named (saving the panel), printed, exported to excel or as a pdf. It is also possible to go back and modify the panel or start building a new one.

Antibody Titration

Titrating antibodies is highly recommended before running a full experiment. Tittering antibodies has several benefits including

1. Minimizes non-specific binding
2. Increases signal detection
3. Minimizes compensation/spillover
4. In many instances, you may be able to use less antibody then recommended by the manufacturer, saving on the amount of antibody you need to use in an experiment as well as saving money.

When titrating antibodies always start with the manufacturer's recommended concentration and then do 3 two fold dilutions down from there. If the manufacturer does not provide a recommended concentration, then start at 1μl and dilute down.

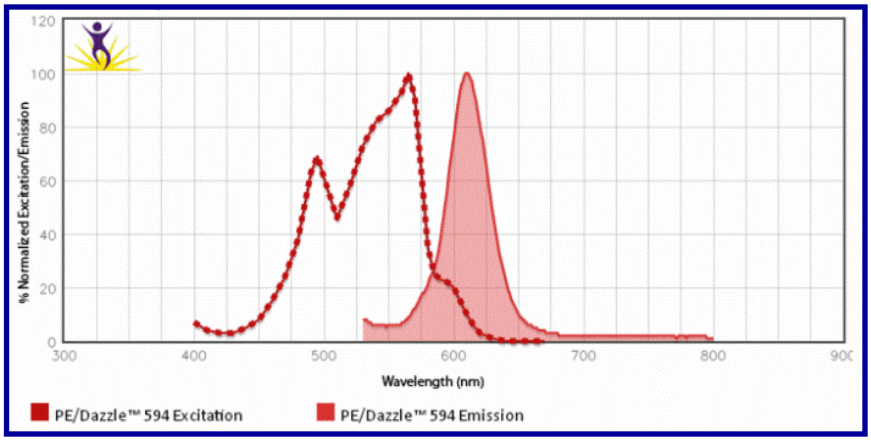
Controls

It is the opinion of the WVU FCSCCF staff that there really is no such thing as too many controls. In flow cytometry, several different types of controls are recommended including:

1. No stain controls.
    a. These are cells that have gone through the whole staining protocol but at the step where fluorescently labeled antibody is added, an equivalent amount of buffer is added instead.
    b. Are used to identify the autofluorescence of the cells
    c. Indicate where the cells that do not bind antibody are located
2. Single stain controls
    a. The cells in these tubes are only stained with a single antibody or dye
    b. There should be one for each fluorochrome, fluorescent protein or dye in the panel
    c. Are used for compensation and setting gates
3. Fluorescence minus one (FMO) controls
    a. The cells are stained with all the antibodies except one
    b. There should be one for each fluorochrome in the panel
    c. Are used for gating and checking for antibody interactions that may make identifying sub-populations difficult
4. Isotype controls
    a. The cells are stained with an antibody conjugated to the same fluorochrome as one in the staining panel but the antibody should not bind to the cells.
    b. It is used to check for non-specific binding due to protocol issues

The WVU FCSCCF staff hopes the recommendations above will be useful to you. They should provide you with ways to improve you flow cytometry data as well as give you more confidence in your experimental results. As always if you want to discuss anything written in the newsletter or other things related to flow cytometry please stop by the WVU FCSCCF.

Meet the Fluorochrome: PE-Dazzle 594



Excitation

561 nm (Yellow/Green laser)

488 nm (Blue laser)

Emission max

610 nm (Y/G laser detector same as PE-Texas Red; Blue laser detector same as PI)

Type of Fluorochrome: Tandem Dye

Characteristics: Very bright similar to PE-Texas Red and PE-eFluor 610
Extremely photostable
Little spillover into other detectors

Flow Cytometers in the Facility

FACSAria III Cell Sorter

Operator: Facility Staff

Lasers:

- 488 nm solid state
- 561 nm solid state
- 633 nm solid state
- 407 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 13 fluorochromes

Applications:

- Cell Sorting (Aseptic)
- Single Cell Sorting
- Cell Phenotyping
- Cell Viability
- Cell Cycle Analysis

Flow Cytometers in the Facility (continued)

LSR Fortessa

Operator: User or Facility Staff

Lasers:
- 405 nm solid state
- 488 nm solid state
- 561 nm solid state
- 628 nm solid state

Detection Parameters:
- Forward Scatter
- Side Scatter
- Simultaneous detection of 17 fluorochromes

Applications:
- Cell phenotyping
- Cell Viability
- Cell Cycle analysis
- Apoptosis Assays

Guava easyCyte HT

Operator: User or Facility Staff

Lasers:
- 488 nm solid state

Detection Parameters:
- Forward Scatter
- Side Scatter
- Simultaneous detection of 3 fluorochromes

Applications:
- Cell Counts
- Apoptosis Assay
- Cell Cycle Analysis

Other Instrumentation Available in the Facility

AutoMACS Pro Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell 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 downstream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

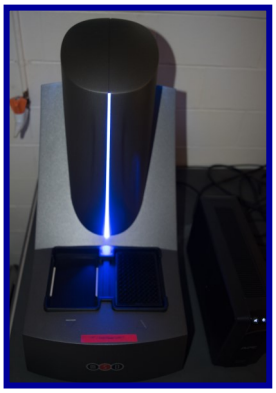
Format: 96 or 384 chambers per chip

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



Nanosight NS 300

Operator: User or Staff

Application:

- Determines the size and concentration of particles 10 nm to 1 microns in size
- Equipped with 4 lasers (405 nm, 488 nm, 532 and 642) to detect fluorescently labeled particles

Zetasizer Nano Z

Operator: User or Staff

Application:

Measures the zeta potential of particles in a solution using laser Doppler micro-electrophoresis

Upcoming Holidays & Events

January 1, 2021	New Year's Day	Facility Closed
January 18, 2021	Martin Luther King's day	Facility Closed
April 2, 2021	Spring Holiday	Facility Closed

Note to Users

Please remember to acknowledge the support of the HSC Research Office and NIH grants that support the WVU Flow Cytometry & Single Cell Core in all your publications. The grant numbers are listed below:

TME CoBRE grant: P20GM121322

WVCTS grant: GM104942 important if you used the Miltenyi AutoMACS pro (installed 6/29/18)

WV InBRE grant: GM103434

Fortessa S10 grant: OD016165

NanoSight NS 300 use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

ZetaSizer NanoZ use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

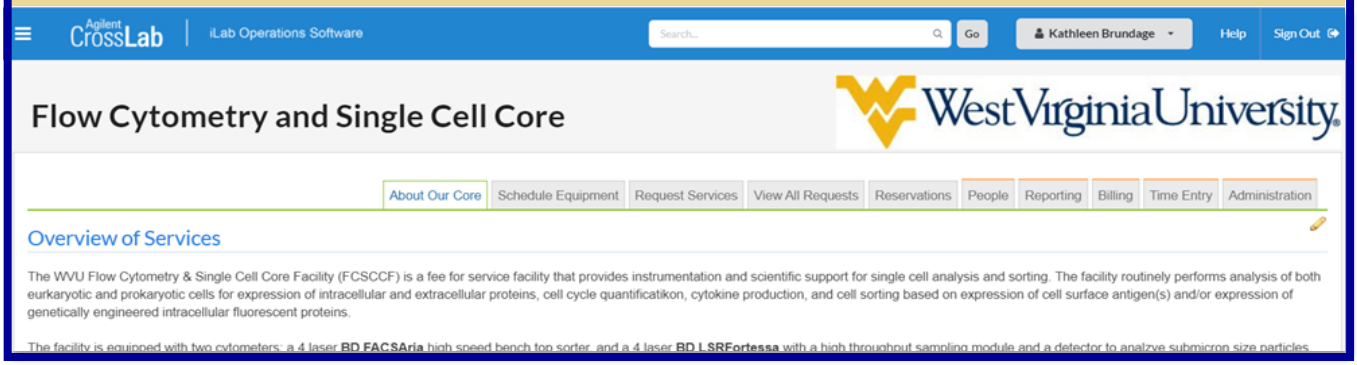
New User Guide

Hands-on training for LSRFortessa, C1 Single Cell Auto Prep System, NanoSight NS300 and Zetasizer Nano Z is **mandatory** for all new users and must be scheduled by consultation with the facility director.

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. Sorting on the FACSAria is by facility staff only.

The facility uses iLAB scheduling/billing software from Agilent to manage the use of the facility's instrumentation. If you would like to use the instruments housed in the facility please use the URL shown below to register as a WVU User and to login to reserve an instrument.

https://wvu.corefacilities.org/landing/984



Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS Pro	Facility Staff or User	$4.50 / separation	$6.85 / separation
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$320/plate
	User	No Cost	$115/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$80/h
	Analysis: User	$34.65/h	$53/h
	Sorting	$77.70/h	$120/h
	Sorting Setup	$19.43/sort	$30/sort
gentleMACS	Facility Staff or User	$10.50/sample	$16/sample
Guava easyCyte	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
LSRFortessa	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
MSD QuickPlex SQ120	Facility Staff or User	$10.50/h	$16/h
NanoSight NS300	Facility Staff	$61.00/h	$93/h
	User	$42.50/h	$65/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$39/sample + $80/h
	User	$25/sample	$39/sample + $16/h