WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 6, issue 2 October 2019

Getting to Know the Brilliant Violet (BV) Fluorochromes

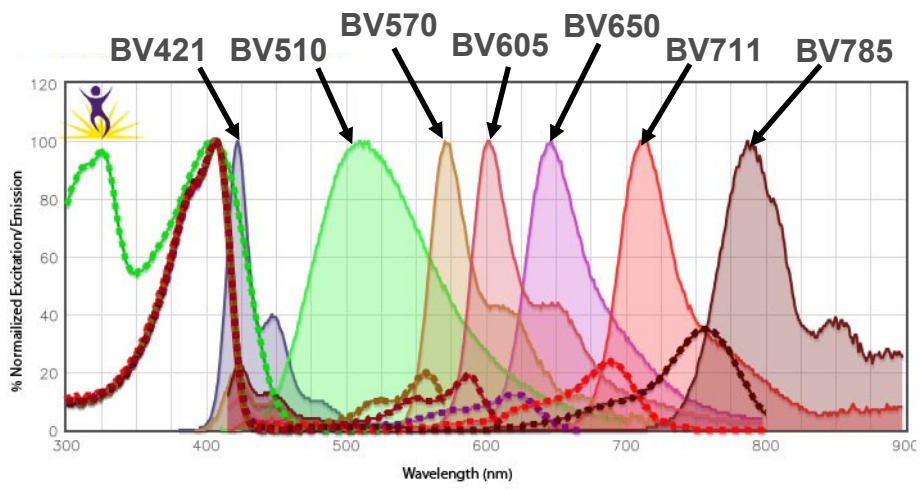

Figure 1. Excitation and emission spectra of BV Fluorochromes

As many of you know the BD LSRFortessa has recently under gone an upgrade resulting in the addition of 4 photo-multiplier tubes (PMTs) to the violet laser detection array. In means in practical terms is:

1. You can use 6 different violet excited fluorochromes in a single experiment
2. A maximum 16 total fluorochromes can be detected at once on the LSRFortessa
3. All of the available Brilliant Violet (BV) fluorochromes can be detected

BV Fluorochromes

BV fluorochromes are polymer dyes excited by lasers that emit strongly in the violet light range (390nm - 419nm). The first BV conjugated antibody was developed by Sirigen and Biolegend and released for commercial use in 2011-2012. Today there are 7 BV fluorochromes, some are made up of just base polymers like BV421 and BV510 while others are tandem dyes (BV570, BV605, BV650, BV711 and BV786) (Figure 1). Several of the BV fluorochromes are

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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

even brighter than PE, one of the brightest fluorochromes available (Table 1).

Table 1. Characteristics of BV Fluorochromes

BV Name	Emission (nm)	Brightness Compared to PE	Panel Design Considerations
BV421	421	3x	Best for antigens with low expression
BV510	510	0.6x	Best for antigens with moderate expression
BV570	570	Similar to PE	Best for antigens with low to moderate expression
BV605	605	Similar to PE	Best for antigens with low to moderate expression Significant spillover into PE and PE-CF594 detectors
BV650	650	2x	Best for antigens with low to moderate expression. Moderate spillover into APC, Alexa700, and BV711 detectors
BV711	711	1.75x	Best for antigens with low expression Moderate spillover into PerCP-Cy5.5, Alexa700, and BV786 detectors
BV786	786	1.5x	Best for antigens with low expression

BV Staining Buffers

One potential issue when using more than one BV fluorochromes in a staining panel is that staining artifacts can occur where a population that should be negative for a marker would have low to moderate levels of non-specific background staining. These artifacts are a result of some of the antibodies interacting with each other. The amount of interaction has been determined to be a function of: 1) the antibody conjugates in the panel, 2) the antibody concentration, and 3) the number of antibody conjugates in the panel.

The solution to minimize these artifacts is to use a commercially available staining buffers when 2 or more BV fluorochrome conjugated antibodies are being used in the same staining panel. BD Biosciences has a buffer called "*Brilliant Stain Buffer Plus* " (catalog # 566385). eBioscience also has a buffer called "*Super Bright Complete Staining Buffer*" (catalog #SB-4401-42). Both buffers work and are only used as the dilution buffer for the antibodies when you are making a master mix or cocktail of antibodies.

If you are interested in learning more about BV fluorochromes or would like to use them in an upcoming staining panel stop by the lab or send me an email (kbrundage@hsc.wvu.edu). I will be happy to assist you with incorporating these reagents into your experimental designs.

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

LSR Fortessa

Operator: User of 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 16 fluorochromes

Applications:

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

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

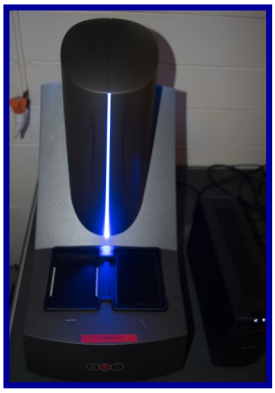
Format: 96 or 384 chambers per chip

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

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
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

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/account/login

Upcoming Holidays & Events

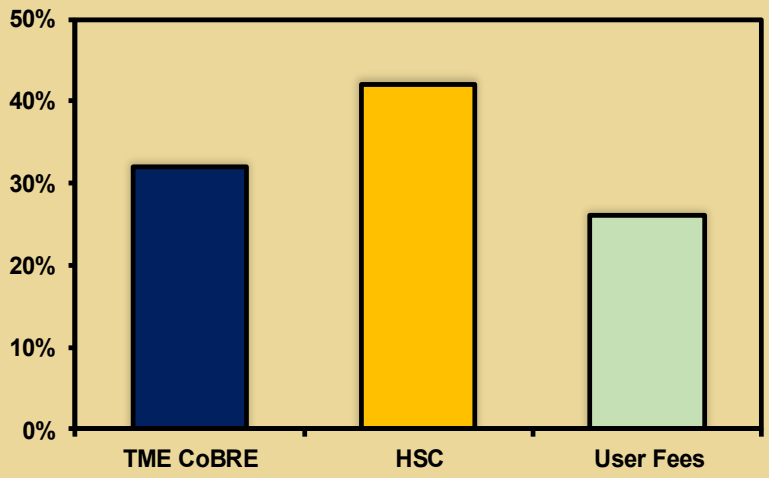
October 21—November 1, 2019	LumaCyte Radiance	Instrument demo
November 27 –29, 2019	Thanksgiving Break	Facility Closed
December 24 –26	Winter Holiday Break	Facility Closed
December 31, 2019 - January 1, 2020	New Year's	Facility Closed

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.

Revenues sources for WVU Flow Cytometry & Single Cell Core Facility (FY2019)



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