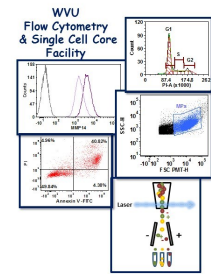


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 7, issue 1

July 2020

Super Bright Polymer Dyes

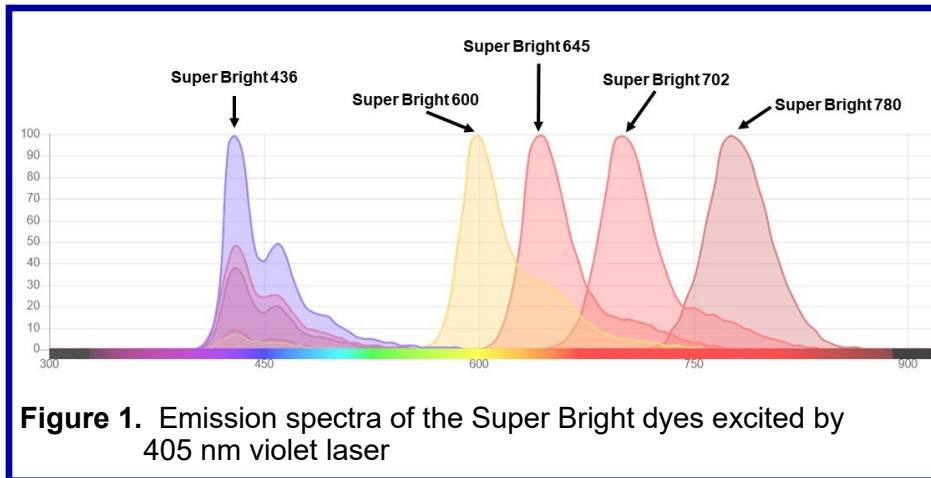


Figure 1. Emission spectra of the Super Bright dyes excited by 405 nm violet laser

In the Oct 2019 issue of the newsletter, we discussed the Brilliant Violet (BV) family of dyes which are excited by a violet laser (405 nm) and emit over a broad range. In this issue we discuss another family of dyes excited by the violet laser, the Super Bright dyes.

Super Bright Dyes

Super Bright dyes are a family of tandem polymer dyes excited by a violet laser. They were originally developed by eBioscience as photo stable, bright alternatives to the available violet laser excited fluorochromes, i.e. PacBlu, V450. The development of the Super Bright fluorochromes expanded the number of fluorescent molecules that could be detected off the underutilized violet laser. Currently, there are 5 family members that emit over a broad spectrum (Figure 1). The Super Bright fluorochromes are named with their peak emission wavelength making it easy to determine which detector should be used to detect the emission signal. On the next page is a table showing some of the properties of the Super Bright dyes.

Like the BV dyes, when two or more Super Bright dyes are used in a staining cocktail a special commercial staining buffer is recommended. The manufacturer recommends the Invitrogen eBioscience Super Bright Staining Buffer. This buffer minimizes nonspecific interactions that may occur between polymer based dye conjugates. If you are using one or more BV dyes in your panel in combination with one or more Super Bright

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Facility Location:
2160 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

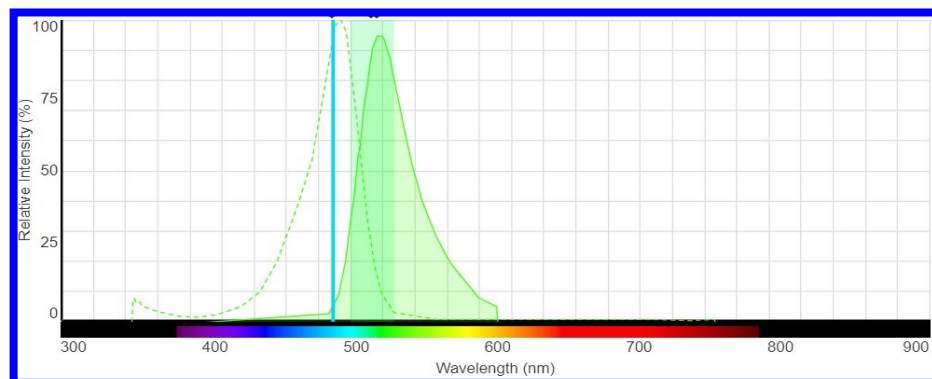
Contact Dr. Brundage at:
kbrundage@hsc.wvu.edu

dyes, you can use either the Invitrogen eBioscience commercial staining buffer or one of the Brilliant Stain commercial buffers. Regardless of which buffer you use, it only has to be used when making a master cocktail of antibodies. A standard staining/wash buffer can be used for all the other steps. One final point about the commercial buffers, they do not interfere with other more traditional fluorochromes.

If you are interested in using the Super Bright fluorochromes and would like to learn a little more about them stop by the lab or send me an email (kbrundage@hsc.wvu.edu). I would be more than happy to assist you with incorporating these fluorochromes into your staining panel.

Table 1. Characteristics of Super Bright Dyes			
Name	Emission (nm)	Brightness Compared to Other Super Bright Fluorochromes	Brightness Compared to Other Fluorochromes
Super Bright 436	436	<ul style="list-style-type: none"> • Similar brightness to Super Bright 780 • Not as bright as Super Bright 600, Super Bright 645 & Super Bright 711 	<ul style="list-style-type: none"> • Similar brightness to BV421 • Brighter than eFluor450 & Pac Blu
Super Bright 600	600	<ul style="list-style-type: none"> • Similar brightness to Super Bright 645 & Super Bright 711 • Brighter than Super Bright 436 & Super Bright 780 	<ul style="list-style-type: none"> • Similar brightness to BV605 • Brighter than Pac Orange
Super Bright 645	645	<ul style="list-style-type: none"> • Similar brightness to Super Bright 645 & Super Bright 711 • Brighter than Super Bright 436 & Super Bright 780 	<ul style="list-style-type: none"> • As bright and sometimes brighter than BV650
Super Bright 711	711	<ul style="list-style-type: none"> • Similar brightness to Super Bright 645 & Super Bright 711 • Brighter than Super Bright 436 & Super Bright 780 	<ul style="list-style-type: none"> • Similar brightness to BV711
Super Bright 780	780	<ul style="list-style-type: none"> • Similar brightness to Super Bright 780 • Not as bright as Super Bright 600, Super Bright 645 & Super Bright 711 	<ul style="list-style-type: none"> • As bright and sometimes brighter than BV785 and BV786

Meet the Fluorochrome: Kiravia 520 from Biolegend



Excitation max

485 nm (Blue laser)

Emission max

518 nm (same detector as the one used for FITC)

Type of Fluorochrome: Organic polymer with a fluorescent dye loaded on a novel organic polymer backbone

Characteristics: Brighter than Alexa 488 and FITC
More photo stable than FITC

Upcoming Holidays & Events

July 3, 2020	Fourth of July Holiday	Facility Closed
July 7, 2020	Kathy out of lab	Facility Open for experienced users only
July 20 –24, 2020	Kathy out of lab	Facility Open for experienced users only
September 7, 2020	Labor Day Holiday	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 FACS Aria is by facility staff only.

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

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 17 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:

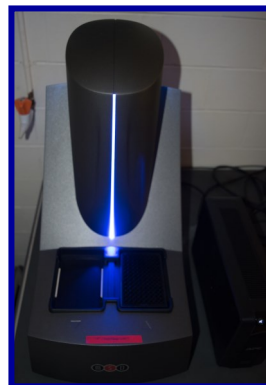
Detection of cytokines, cell signaling proteins

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 /	\$6.85 /
C1 Single Cell Auto Prep System	Facility Staff	\$210/plate	\$320/plate
	User	No Cost	\$115/plate
FACS Aria 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
LSR Fortessa	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 +	\$39/sample +
	User	\$25/sample	\$39/sample +



From Insight to Outcome

Internal WVU user :

Click [here](#) to login or register using your institute login and password.

Not a WVU user?

Login using iLab credentials

If you don't have an account, please [register](#) for an iLab account.

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>