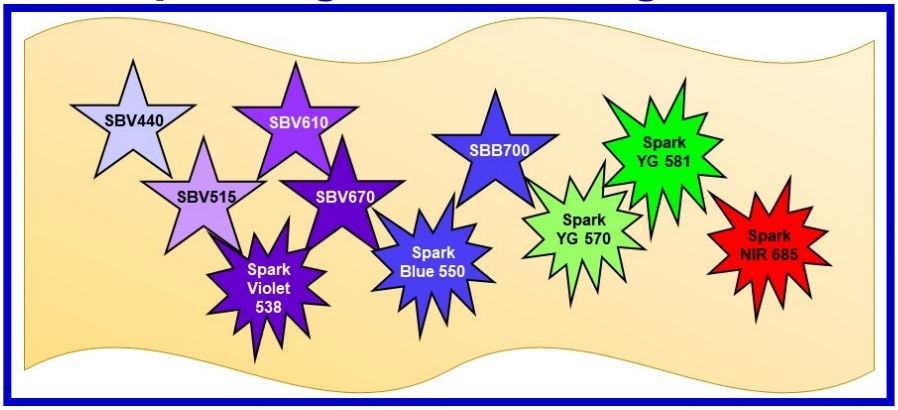
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 7, issue 4 April 2021

StarBright and Spark Dyes: Expanding Your Staining Panel



Over the last few years, the fluorochromes in the flow cytometrist toolbox has expanded at a rapid rate. Flow Cytometrist are no longer limited to the traditional fluorochromes FITC, PE, APC, Alexa Fluor 488 etc. Today, there are a plethora of options many with improved stability, brightness and less spillover. In past newsletters, we have discussed the Brilliant Violet family of dyes from BD and the SuperBright family of dyes from eBioscience. In this issue of the newsletter, we are focusing on two of the newest additions to the flow cytometry tool box, Bio-Rad's StarBright and Biolegend's Spark dyes.

StarBright Dyes from Bio-Rad

The StarBright family of dyes are fluorescent nanoparticles that were developed specifically for flow cytometry. The main features of the StarBright dyes are:

- Narrow excitation and emission spectra with less spillover into other detectors
- Bright dyes are good for detection of rare populations and low density antigens
- No special buffer is needed when multiple family members are used unlike the Brilliant Violet and SuperBright families of dyes
- Very photostable meaning less bleaching do to exposure to the lasers.

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

There are currently 5 members of the family, 1 excited by 488nm blue laser and 4 excited by 405 violet laser with 4 more expected to be released later in 2021 (SBV475, SBV570, SBV710 and SBV790). See the table below for details on the current available options.

Dye	Excitation	Emission	Bandpass Filter	Best Detector on the Fortessa
SBV440	405 nm Violet Laser	440 nm	450/50	PacBlu
SB515	405 nm Violet Laser	515 nm	525/50	BV510
SB610	405 nm Violet Laser	610 nm	610/10	BV605
SB670	405 nm Violet Laser	670 nm	670/30	BV650
SBB700	488 nm Blue Laser	700 nm	695/40	Percp-Cy5.5

Spark Dyes from Biolegend

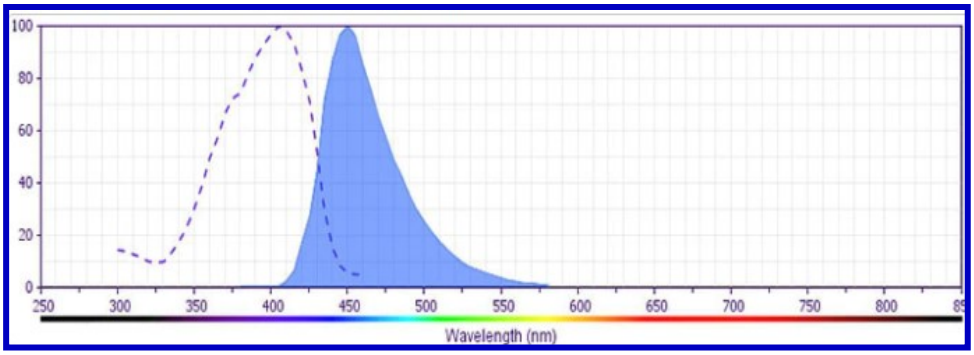
The Spark family of dyes are small, synthetic fluorochromes developed to fill spectral spaces between existing fluorochromes. The main features of the Spark dyes are:

- Narrow excitation and emission spectra with less spillover into other detectors
- High photostability with less bleaching due to exposure to lasers
- Not sensitive to standard fixatives including organic solvents used for phosphor flow thus maintaining their brightness after fixation

There are currently 5 members of the Spark Dye family. See the table below for details:

Dye	Excitation	Emission	Bandpass Filter	Best Detector on the	Antigen Expression
Spark Violet 538	405 nm Violet Laser	538 nm	525/50	BV510	High
Spark Blue 550	488 nm Blue Laser	550 nm	515/30	FITC	High
Spark YG 570	561 nm Yellow-Green Laser	570 nm	585/15	PE	Moderate
Spark YG 581	561 nm Yellow-Green Laser	581 nm	585/15	PE	Moderate
Spark NIR 685	628 nm Red Laser	685 nm	670/30	APC	Moderate

Meet the Fluorochrome: Blue Horizon V450

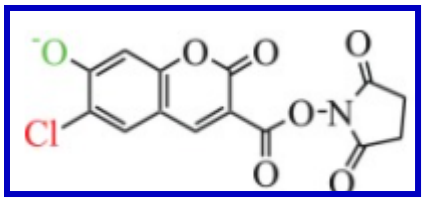


Excitation
404 nm (Violet laser)

Emission max
488nm (Same detector as PacBlu and BV421)

Type of Fluorochrome: Coumarin dye (aromatic lactone compound, water soluble, small molecular weight)

Characteristics: Brighter than PacBlu and Alexa Fluor 405



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

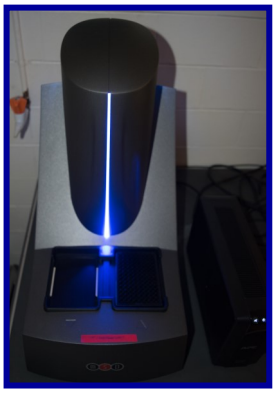
Format: 96 or 384 chambers per chip

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

May 6 –10, 2021	Kathy Out of Lab	Facility open for Experienced Users only No Sorting
May 31, 2021	Memorial Day Holiday	Facility Closed
July 5, 2021	Independence 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

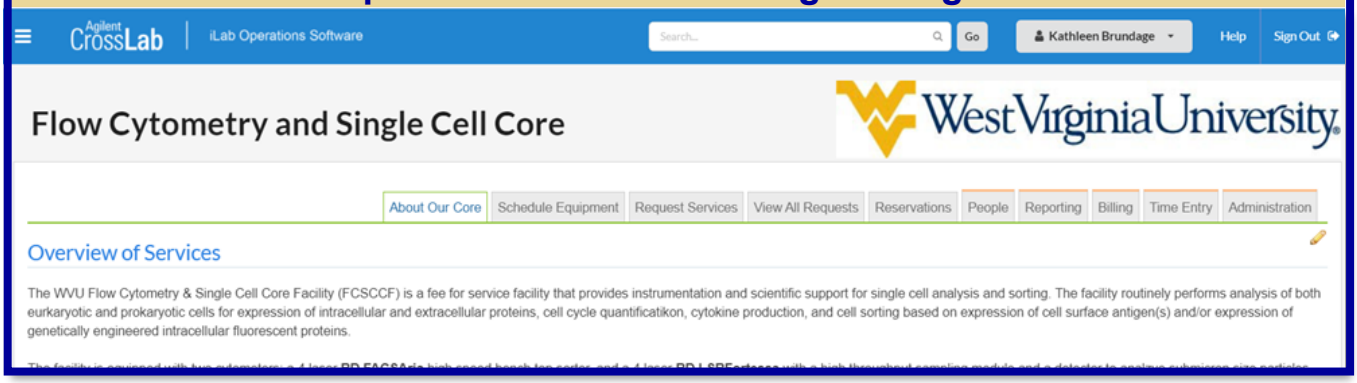
New User Guide

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