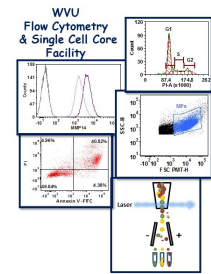


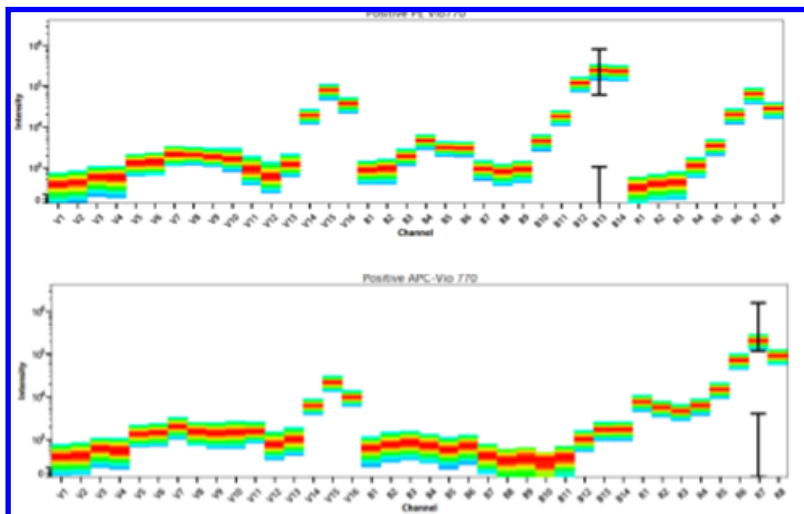
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 11, issue 2

October 2023

Vio and Vio Bright Dyes from Miltenyi Biotec



When expanding a panel, one of the hardest tasks is to find fluorochrome that are both stable and bright as well as compatible with the fluorochromes you already have. Miltenyi Biotec has developed a set of dyes known collectively as the Vio and VioBright Dyes.

These dyes are a family of fluorochromes which have high fluorescent intensities and low spillover, allowing them to be ideal for flow panels with a large number of colors. Additionally, the Vio and VioBright dyes are great for using with some of the most popular, traditional fluorochromes like APC, FITC, PerCP, and PE.

Currently there are eleven total dyes available. These dyes can be excited by the four most common lasers used in flow cytometry, blue, violet, yellow-green, and red. Thus, ensuring easy integration into existing panels. All of the dyes are compatible with traditional paraformaldehyde fixation. In addition, some are compatible with other less common fixative i.e. acetone and methanol.

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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

For the violet laser, there are three dyes available: VioBright V423, VioBlue, and VioGreen. Of these three, VioBright V423 is the brightest, being five times brighter than VioBlue. It also has minimal spillover and excellent resolution of rare cells and dim antigens which makes it a great choice for larger multicolor panels.

Laser	Dye	Brightness (Scale 1 (dim) - 5 (bright))	Compatible Fixa- tive	Excitation max	Emission max
Violet (405 nm)	Vio Bright V423	5	PFA, methanol	399 nm	420 nm
	VioBlue	3	PFA, ethanol, methanol, ace- tone	400 nm	452 nm
	VioGreen	1	PFA, ethanol, methanol, ace- tone	388 nm	520 nm

For the blue laser, there are three dyes available: Vio B515, Vio Bright FITC, and Vio Bright B515. The Vio Bright dyes here stand out for being innovative as the technology allows for an increased number of fluorochrome molecules per antibody compared to traditional conjugation. This allows for dyes that emit a signal detected in the standard FITC channel with brightness levels similar to PE making Vio Bright FITC and Vio Bright 515 excellent alternatives for the FITC channel. Their high brightness makes them a great choice for detecting low-expressed markers, rare cells, and dim or uncharacterized markers. Vio Bright B515 also stands out for its high photostability regardless of fixative agent used. Unlike PE and APC, Vio Bright B515 has great stability in paraformaldehyde- and methanol-based fixatives with minimal impacts on brightness.

Laser	Dye	Brightness (Scale 1 (dim) - 5 (bright))	Compatible Fixa- tive	Excitation max	Emission max
Blue (488 nm)	Vio B515	4	PFA, ethanol, methanol, acetone	488 nm	514 nm
	Vio Bright FITC	4	PFA, ethanol, methanol, acetone	496 nm	522 nm
	Vio Bright B515	5	PFA, ethanol, methanol, acetone	488 nm	514 nm

There are three dyes available for the yellow-green laser: PerCP-Vio 700, PE-Vio 615, and PE-Vio 770. All three are tandem conjugate dyes and their high brightness allows for easy detection of dim, rare, and uncharacterized markers. These dyes work by having the PE or PerCP act as donor dyes while the Vio acts as an acceptor dye, allowing for efficient donor

-to-acceptor energy transfer and low spillover into the donor dye detection channel.

Of all of the dyes in the Vio Dye family, PE-Vio 770 provides the greatest fluorescent intensity with great separation of positive and negative populations of stained cells. When compared to other spectrally similar tandem conjugates, such as PE-Cy7, PE-Vio 770 exhibits significantly higher fluorescent intensities, making it a good alternative.

Laser	Dye	Brightness (Scale 1 (dim) - 5 (bright))	Compatible Fixative	Excitation max	Emission max
Yellow-green (561 nm)	PerCP-Vio 700	3	PFA	482 nm	704 nm
	PE-Vio 615	5	PFA	565 nm	620 nm
	PE-Vio 770	4	PFA	565 nm	775 nm

The red laser has the largest selection, with five dyes available: APC-Vio 770, Vio R667, Vio Bright R667, Vio R720, and Vio Bright R720. Of these five, Vio Bright R667 is the brightest, allowing for improved separation of positive and negative cell populations which makes it ideal for detecting dim populations. This dye is also a better alternative to the APC conjugate as it has a two-fold increase in brightness over APC. Vio Bright R720 is also a better alternative for Alex Fluor 700 as it is brighter and has significantly less background noise. APC-Vio 770 is a tandem conjugate dye and provides strong fluorescent staining for accurate identification and analysis of cellular populations.

Laser	Dye	Brightness (Scale 1 (dim) - 5 (bright))	Compatible Fixative	Excitation max	Emission max
Red (635 nm)	APC-Vio 770	3	PFA	652 nm	775 nm
	Vio R667	5	PFA, ethanol, methanol, acetone	645 nm	688 nm
	Vio Bright R667	5+	PFA, ethanol, methanol, acetone	645 nm	668 nm
	Vio R720	3	PFA, ethanol, methanol	695 nm	720 nm
	Vio Bright R720	4	PFA, ethanol, methanol	695 nm	720 nm

So next time, you are designing a new panel or expanding an existing panel check out these dyes on the Miltenyi Biotec's website: <https://www.miltenyibiotec.com/vio>

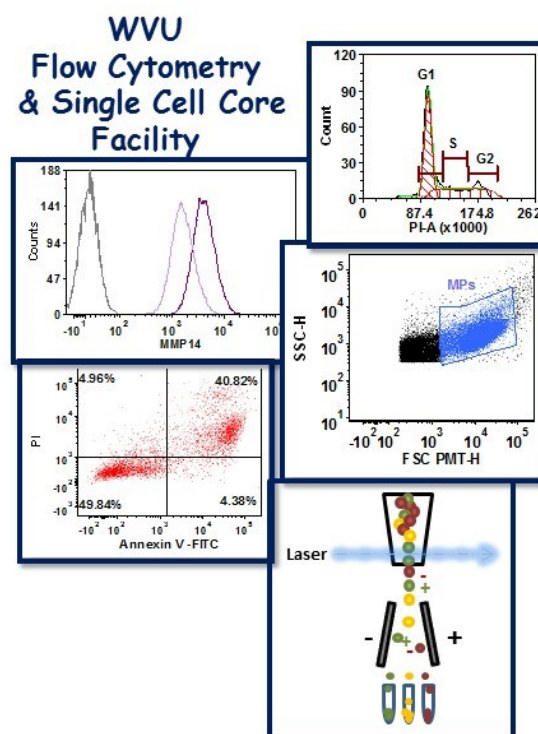
Letter to Users on the Upcoming Increase to User Fees

WVU Flow Cytometry & Single Cell Core Facility Users,

I would like to inform you of pending changes to the fee structure for the WVU Flow Cytometry & Single Cell Core Facility. As you are well aware, the cost of doing research has been rising exponentially since covid. One of the biggest cost to the facility is service contracts. In the last year alone, the service contracts on all the instruments have gone up by 10% or more and the cost of supplies to maintain the instruments have substantially increased as well. As a result, the operating expenses for the facility have increased quite a bit. Currently, the Health Sciences Center provides 46% of the operating budget, TME CoBRE grant provides 21%, and user chargebacks makes up the rest (33%). In the past, we have been able to absorb the smaller yearly increases in service contracts and supplies without increasing user fees. In fact, user fees have stayed the same since 2017. Unfortunately, the facility does not have the funds to absorb the current increases in the facility's expenses. After careful consideration, we have decided to increase the user fees **effective January 1, 2024**. This increase in fees will allow us to continue to provide state of the art instrumentation and service to your lab. On the following pages containing the prices for service, the current prices are in black text and the new prices are in red text.

Finally, I would like to say thanking for supporting the WVU Flow Cytometry & Single Cell Core Facility over the years. Your use of the facility has allowed us to expand into spectral flow cytometry, bulk single cell RNAseq, and spatial transcriptomics. As always, Raven and I strive to provide your lab with high-end instrumentation and expertise in order for you to generate high quality data that will move your research forward.

Kathleen Brundage, Ph.D.
Director, WVU Flow Cytometry & Single Cell Core Facility



10x Genomics Chromium Controller

Operator: Facility Staff

Applications:

- ◆ Single cell barcoding for 500 to 10,000 cells
- ◆ Whole cell or nuclei samples
- ◆ Gene Expression Analysis
- ◆ Immune Profiling
- ◆ ATAC
- ◆ Multiome ATAC + Gene Expression
- ◆ Spatial Gene Expression



10x Genomics Chromium Controller Fees

****NOTE: Prices will be increasing on January 1st, 2024. Increased prices shown in red.****

WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Instrument Fee	\$175/run → \$190/run	\$290/run → \$315/run
Chip*	\$260/chip → \$300/chip	\$430/chip → \$460/chip
Reagents**	\$50/sample → \$55/sample	\$85/sample → \$95/sample
cDNA Tracer Tape	\$9/sample → \$11.50/sample	\$14/sample → \$20/sample
Sample Prep (nuclei isolation)	\$225/cell line → \$248/cell line	\$350/cell line → \$380/cell line
	\$270/frozen tissue → \$300/frozen tissue	\$420/frozen tissue → \$460/frozen tissue
Labor***	\$50/h → \$55/h	\$83/h → \$92/h

*Holds up to 8 samples

**Does not include cost of the reaction kit.

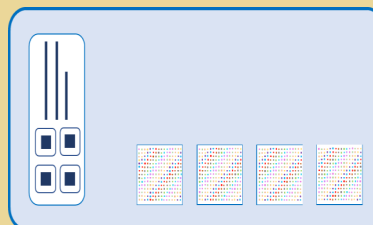
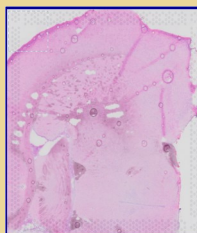
***The GEM generation & Barcoding takes about 2 – 2.5 hours. The generation of the library can take 3.5 – 10 hours depending on the assay you are running.

10x Genomics Visium Transcriptomics

Operator: Facility Staff

Applications:

- Gene expression mapping on tissue
- Fresh-frozen or FFPE tissues
- Analysis of whole transcriptome within select tissue section



10x Genomics Visium Transcriptomics Fees

****NOTE: Prices will be increasing on January 1st, 2024. Increased prices shown in red.****

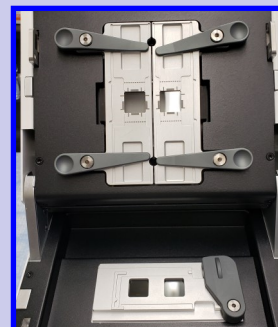
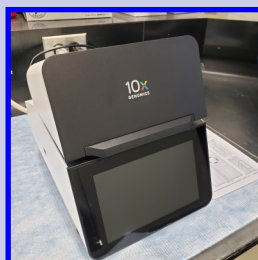
WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Visium Optimization Slide (Manual)	\$350/slide → \$385/slide	\$580/slide → \$640/slide
Reagents for spatial gene expression (does not include cost of slide or kit)	\$60/sample → \$66/sample	\$100/sample → \$110/sample
Labor for spatial gene expression	\$50/h → \$55/h	\$78/h → \$92/h

Spatial Transcriptomics with 10x Genomics CytAssist

Operator: Facility Staff

Applications:

- Simplifies Visium workflow
- Fresh-frozen or FFPE tissues
- Analysis of whole transcriptome of whole tissue section



10x Genomics Spatial Transcriptomics with CytAssist Fees

WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
CytAssist Instrument Usage	\$100/slide	\$155/slide
Reagents for spatial transcriptomics (does not include cost of slide or kit)	\$66/sample	\$110/sample
Labor for spatial transcriptomics	\$55/h	\$92/h

Flow Cytometers in the Facility

FACSria 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



Cytek Aurora Full Spectrum Flow Cytometer

Operator: User or Facility Staff

Three lasers:

- 405 nm Solid State violet
- 488 nm Solid State blue
- 640 nm Solid State red

Twenty-seven parameter analysis:

- Forward Scatter on blue laser
- Side Scatter on blue laser and violet laser
- 24 different fluorochromes

Applications:



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

MultiMACS Cell24 Separator Plus

Operator: User
Application:
High throughput manual separations
1-24 samples
Positive and negative cell separation

gentleMACS Octo Dissociator with Heaters

Operator: User
Application:
Tissue disassociation into single cell suspension for culture or flow cytometry assays
Tissue homogenization for molecular biology applications

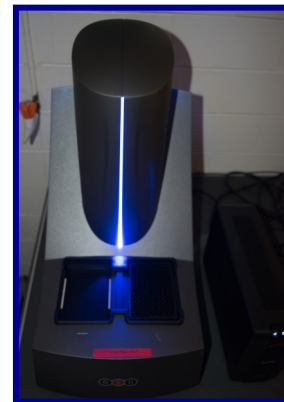
AutoMACS Pro Magnetic Bead separator

Operator: User
Application:
Single extracellular marker cell sorting
Depletion/negative cell sorting



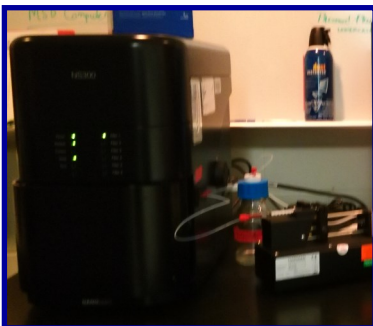
MSD Multi-Array Platform

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 μ l 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



WVU FCSCCF Usage Fees

****NOTE: Prices will be increasing on January 1st, 2024.
Increased prices shown in red.****

FLOW CYTOMETERS		
WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Flow Cytometers for analysis – User operated	\$34.65/h → \$40/h	\$53/h → \$60/h
Flow Cytometers for analysis – Facility operated	\$52.50/h → \$58/h	\$80/h → \$90/h
Sorting	\$77.70/h → \$85/h	\$116.55/h → \$133/h
Sort setup fee	\$19.43 → \$22	\$29.15 → \$34
OTHER EQUIPMENT		
WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
AutoMACs Pro	\$4.50/sample → \$5/sample	\$6.85/sample → \$8/sample
gentleMACs	\$10.50/sample → \$11.75/ sample	\$15.75/sample → \$20/ sample
MSD QuickPlex SQ120	\$10.50/h → \$12/h	\$15.75/h → \$20/h
MultiMACS 24 Separator Plus	\$3/separation → \$3.50/ separation	\$4.65/separation → \$5/ separation
NanoSight NS300 – User Operated	\$42.50/h → \$47/h	\$63.75/h → \$75/h
NanoSight NS300 – Facility Operated	\$61.00/h → \$67/h	\$91.50/h → \$102/h
Zetasizer Nano Z – User Operated	\$25/sample → \$27/sample	\$38/sample → \$43/sample
Zetasizer Nano Z – Facility Operated	\$25/sample + \$52.50/h → \$27/sample + \$55/h	\$38/sample + \$78.75/h → \$43/sample + \$92/h

Upcoming Holidays & Events

November 3, 2023	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
November 22-24, 2023	Thanksgiving Break	Facility Closed
December 1, 2023	Kathy out of lab	Facility Open and All Services Available Except for 10x Genomics
December 22-26, 2023	Christmas Break	Facility Closed
January 1, 2024	New Year's Day	Facility Closed
January 15, 2023	Martin Luther King, Jr., Day	Facility Closed
Jan 31 to Feb 10, 2024	Kathy out of lab	Facility Open and All Services

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

WV InBRE grant: GM103434

WVCTS grant: GM104942

Aurora S10 grant: OD028605

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, Cytek Aurora, 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.

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>

