WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 10, issue 3 January 2023

NovaFluor Dyes from Invitrogen

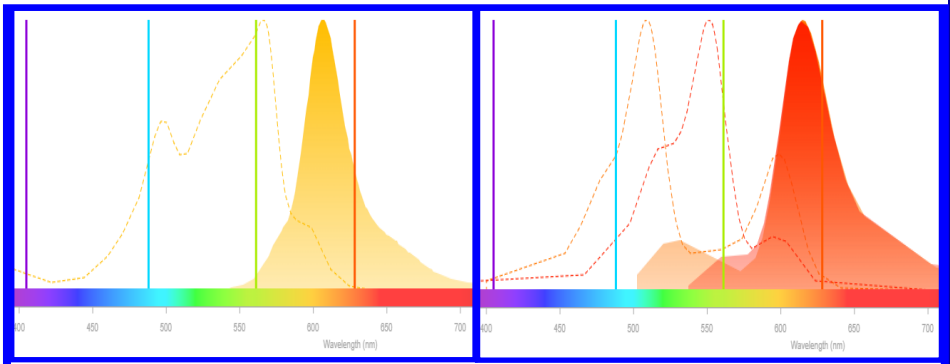

Figure 1. The excitation and emission ranges of PE-eFluor 610 (left) and NovaFluor Blue 610-30 S and NovaFluor Yellow 610 (right). PE-eFluor 610 is excited by both the blue (488 nm) and yellow/green (561 nm) lasers while the NovaFluor dyes are excited by only one laser each. This allows for an additional marker to be added to the panel. Images generated using the Ther-

Have you ever had a panel which you wanted to add additional markers and found the compensation difficult? Do you have tandem dyes in your panel which are preventing you from expanding your panel? Invitrogen's NovaFluor dyes may be your answer. The NovaFluor dyes are designed to have a narrow excitation range for minimal cross-laser excitation meaning less compensation. These dyes are easy to add to pre-existing panels, are compatible with traditional and spectral flow cytometry, and allow for more markers to be detected. Additionally, these dyes are compatible for use in conventional flow cytometers, like the LSR Fortessa and the FACS Aria III, as well as spectral flow cytometers, like the Cytek Aurora.

How it Works

NovaFluor dyes utilize Phiton technology, which is a macrostructure labeled with small molecule fluorophores. This

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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

creates unique fluorescent signatures that aid in avoiding cross-excitation between laser lines. The excitation/emission profiles of these dyes are also designed to avoid spectral spillover into other channels. With this, high-dimensional panel design can be simplified and previously unusable channels can be incorporated. Thus providing higher resolution data for easier analysis.

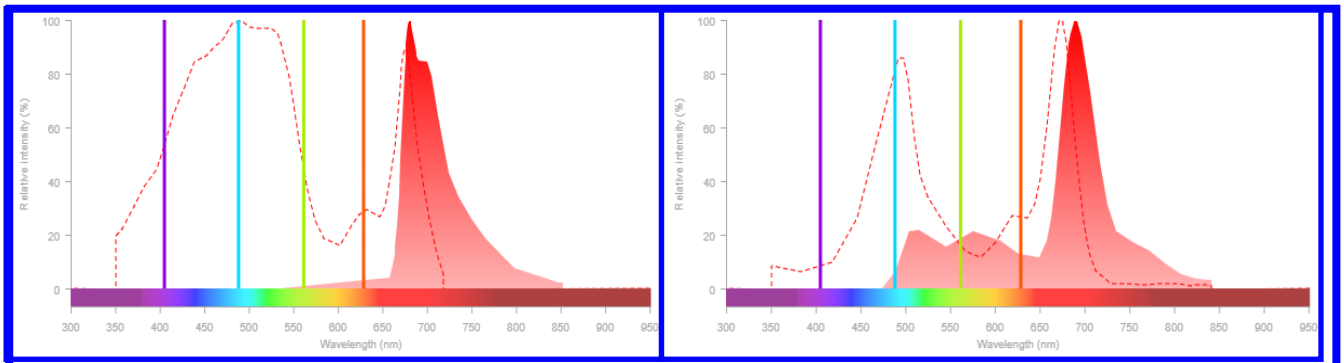


Figure 2. The excitation and emission ranges of PerCP-Cy5.5 (left) and NovaFluor Blue 690 (right). The narrower excitation range of the NovaFluor dye allows for easier compensation and less spillover than when PerCP-Cy5.5 is being used. Images generated using the ThermoFisher Fluorescence SpectraViewer.

How to Incorporate NovaFluor Dyes

The best way to utilize NovaFluor dyes in a panel is to replace dyes that are excited by multiple lasers. One example of this is PE-eFluor 610, which is excited by both the blue and yellow lasers. By replacing it with NovaFluor Blue 610-30 S and NovaFluor Yellow 610, spillover is minimized and additional marker can be added (Figure 1). However, it is important to note that NovaFluor dyes cannot be used with nucleic acid binding dyes such as 7-AAD, PI, and DAPI. Live/Dead Fixable dyes are recommended instead if you want to determine cell viability.

The brightness of fluorophores is very important as is how much spillover occurs . NovaFluor dyes have very little spillover and very clean signals. They have very low spread which makes them good replacements for other dyes. For example, NovaFluor Blue 690 can be used instead of PerCP-Cy5.5 (both excited by the blue laser). Both of these fluorochromes have the same excitation/emission but NovaFluor Blue 690 has less spread which allows for easier compensation (Figure 2).

Using CellBlox Blocking Buffer with NovaFluor Dyes

Invitrogen offers the CellBlox Blocking Buffering which is formulated to block nonspecific binding of the NovaFluor labeled antibodies to cells. This buffer reduces non-specific background labeling of cells. Invitrogen recommends using this buffer anytime NovaFluor dyes are being used.

CellBlox buffer does require some changes to staining protocols. Add 5 uL of the buffer directly to cell suspensions containing 10^3 to 10^8 cells along with antibodies for a final staining volume of 100 uL. Alternatively, CellBlox buffer may also be added to an antibody cocktail

prior to labelling cells by adding 5 uL of the buffer for every stained samples to be labeled with a final staining volume of 100 uL. CellBlox buffer is compatible with other blocking reagents like Fc Block, Brilliant Stain Buffer, Super Bright Complete Staining Buffer, and blocking proteins. The buffer is not required when labeling antibody-capture beads .

New Service from Cytek: the Cytek Cloud

For those of you using the Cytek Aurora, there is a new service called “The Cytek Cloud”. It is from Cytek and it is supposed to aid in experiment design and running samples. There are two components to the Cytek Cloud: the Panel Builder and the Experiment Builder.

1. **The Panel Builder** allows users to visualize your fluorochrome selection which can then be easily compared and optimized. Overall , this will allow for easy and quick panel design which then can be moved to the Experiment Builder.
2. **The Experiment Builder** allows users to create their experiment layout prior to running samples by labeling the fluorochromes, selecting and labeling sample wells/tubes, and adjusting acquisition and loader settings. When you are ready to begin running samples, rather than having to create the experiment while on the instrument, you can upload your experiment from the Cytek Cloud and get to running immediately.

To sign up for a free account, go to: cloud.cytekbio.com.
The WVU FCSCCF is in the process of developing an SOP for users of the Cytek Cloud

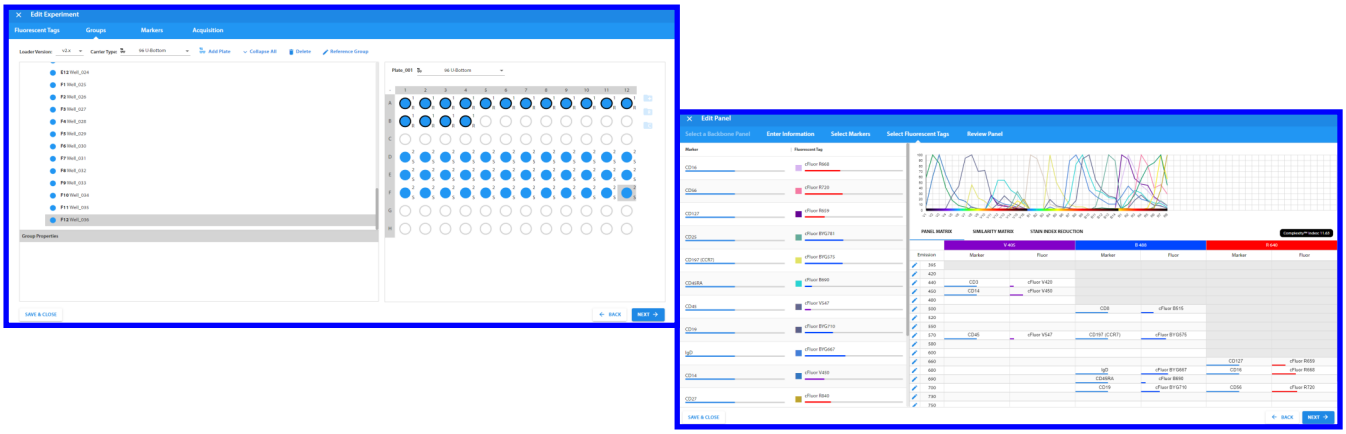


Figure 1. Screenshots of the Cytek Cloud website showing the Experiment Builder (left) and the Panel Builder (right).

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

	For WVU Users	For Non-WVU Users
Instrument Use	$175	$290
Chip*	$260	$430
Reagents	$50/sample	$85/sample
cDNA Tracer-tape	$9/sample	$14/sample
Multiome/ATAC Sample Prep	$225/cell line sample	$350/cell line sample
	$270/frozen tissue sample	$420/frozen tissue sample
Labor**	$50/hour	$83/hour

*Holds up to 8 samples

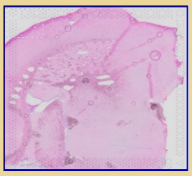
**The GEM generation & Barcoding takes about 2 – 2.5 hours. The generation of the library can take 3.5 – 10 hours depending on the assay you are running.

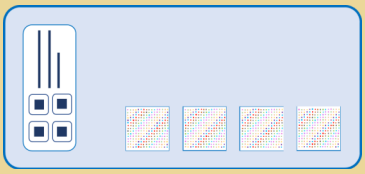
10x Genomics Visium Transcriptomics

Operator: Facility Staff

Applications:

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





10x Genomics Visium Spatial Transcriptomics Fees

	For WVU Users	For Non-WVU Users
Optimization Slide	$350	$540
Reagents for spatial gene expression	$60/sample (4 samples per slide)	$93/sample (4 samples per slide)
Labor for spatial gene expression	$50/hour (process takes approximately 12 hours)	$78/hour (process takes approximately 12 hours)

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

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

AutoMACS Pro Magnetic Bead separator

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

MultiMACS Cell24 Separator Plus

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

gentleMACS Octo Dissociator with Heaters

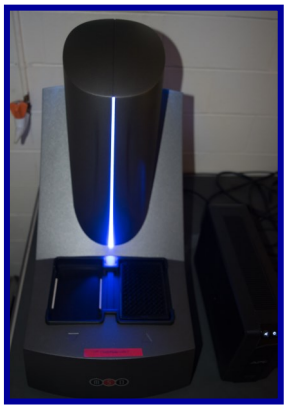
- Operator: User
- Application:
  - Tissue disassociation into single cell suspension for culture or flow cytometry assays
  - Tissue homogenization for molecular biology applications

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
Aurora	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
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
	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
MultiMACS Cell24 Separator Plus	Facility Staff or User	$3/separation	$4.65/ separation
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

Upcoming Holidays & Events

February 3—11, 2023	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
April 7, 2023	Spring Holiday	Facility Closed
April 29-May 13, 2023	Kathy out of lab	Facility Open and All Services Available Except for 10x Genomics
May 29, 2023	Memorial Day	Facility Closed
July 4, 2023	Independence Day	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

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 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