WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 8, issue 1 July 2021

New in the Core
Cytek Aurora Full Spectrum Flow Cytometer

Figure 1. The 3 laser Cytek Aurora installed in the WVU FCSCCF

As may already be aware, there have been a number of new additions to the WVU FCSCCF in the last few months. For this issue of the newsletter, you will be introduced to the newest flow cytometer in the facility, the 3 laser full spectrum Cytek Aurora (Figure 1). This instrument was purchased with a NIH S10 Equipment Grant in May 2021.

Full Spectrum Flow Cytometers

You may be asking yourself, what does full spectrum flow cytometer mean and how does the Aurora differ from the other flow cytometers in the facility?

Let's start by discussing the optical design and detectors in the Aurora and compare them to the LSRFortessa that we are all familiar with. Both instruments have spatially separated lasers (Aurora: 405 mm, 488nm and 640 nm) (LSRFortessa: 405 nm, 488 nm, 561 nm and 628 nm) with dedicated detector arrays. The detectors in the LSRFortessa are photo-multiplier tubes (PMTs) while the detectors in the Aurora are avalanche photodiodes (APDs). APDs are very small, about the size of a

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

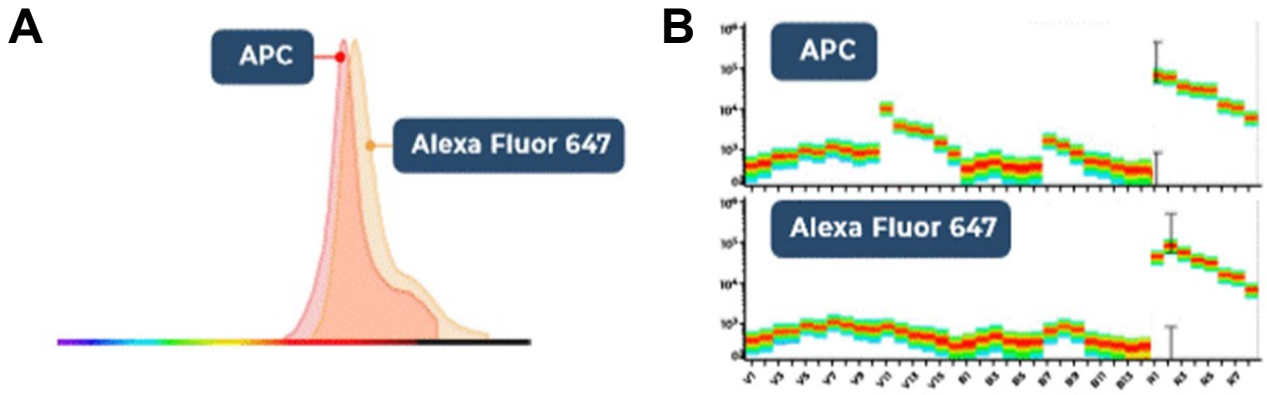


Figure 2. The emission spectrum of the fluorescent dyes APC and Alexa Fluor647 on (A) a traditional flow cytometer using PMTs and (B) on the full spectrum Cytek Aurora.

pencil eraser, compared to PMTs and have higher sensitivity and linearity at higher wavelengths them PMTs. In the LSRFortessa, there are 8 detectors for the 405 nm, 488 nm and 561 nm lasers and 3 detectors for the 628 nm laser. In the Aurora, the detector array for each laser is set up to cover the entire light spectrum starting 15 nm above the laser wavelength and ending at 829 nm with the spacing between detectors between 15 –34 nm. The result of this configuration is that there are 16 detectors for the 405 nm laser, 14 for the 488 nm laser and 8 for the 640 nm laser for a total of 38 detectors. FSC is detected off the blue (488 nm) laser and there are two SSC detectors one off the blue (488 nm) laser and the other off the violet (405 nm) laser for better sensitivity in detecting submicron particles. So what does this all mean? It means that instead of getting a single fluorescent signature for the dye, you get the whole spectrum or what I like to think of as a "fingerprint" of the dye. Because you get a whole emission spectrum of the dye, you can use dyes like APC and Alexa647 in the same sample and distinguish them on the Aurora (Figure 2). This can not be done on the LSRFortessa since on the LSRFortessa both dyes would be detected by the same detector. Figure 3 has two examples of how well the full spectrum Aurora can easily distinguish between APC staining from Alexa Fluor 647 in the same sample.

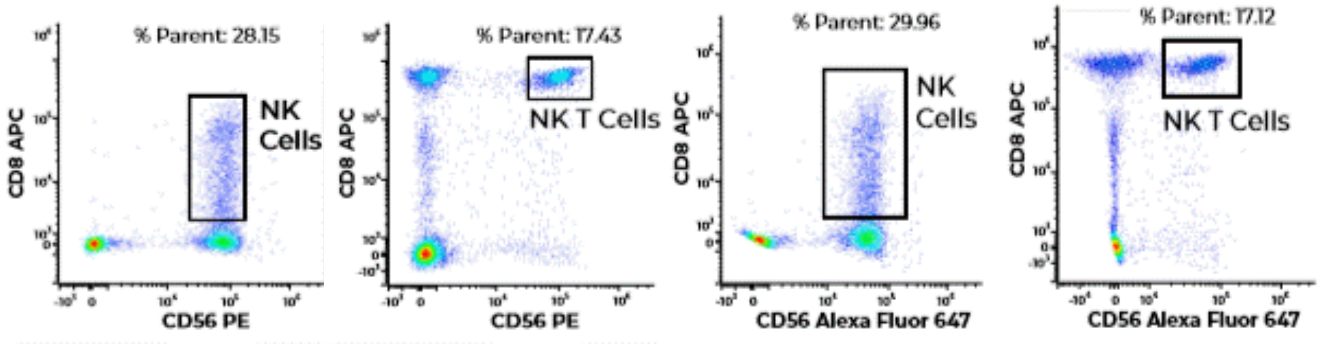


Figure 3. Human peripheral blood mononuclear cells stained with antibodies to CD8 and CD56 to identify NK and NKT cells. The top plots show cells stained with two dissimilar fluorochromes, CD8-APC and CD56-PE clearly identifying the NK and NK T cells. The bottom two plots show cells stained with CD8-APC and CD56-Alexa Fluor 647. (Figure from Cytek Biosciences, Inc. 2020 poster on website)

Unmixing vs Compensation

When running a multi-color experiment on a traditional flow cytometer like the LSRFortessa, compensation is done to remove the emission spillover of each color in the other colors' detectors. With the Aurora, there is no compensation, which should make many of you very happy. Instead a process called unmixing is performed. The good news is the unmixing is done by the software and can be done after running the single stain controls but before any multi-color samples are run or after all the single stain and multi-color samples have been run. The unmixing process uses an ordinary least square calculation to calculate the contribution of each known fluorochrome spectra to the total collected emission signal. Both the raw data and the unmixed data are saved. It is the unmixed fcs files that are used for data analysis. An added feature of full spectrum flow is that autofluorescence can extracted as well making data analysis cleaner.

Keys to a Good Experiment using the Aurora

Like any flow cytometry experiment, samples run on the Aurora require some preliminary work before attempting a "real" experiment. Outlined below are some of the key steps:

1. **Designing a staining panel**

   Careful panel design is extremely important. This can not be emphasized enough. Beware that not every fluorochrome has a distinct enough fluorescent signature to allow them to be used together. For example, PE-Cy7 and PE-Vio770 when analyzed on a 3 laser Aurora have almost identical fluorescent signatures. To help with panel design, the configuration of the Aurora has been added to the West Virginia University instrument choices in Fluorofinder. In addition, the Cytek website (www.cytekbio.com) has a spectral viewer that will give you a similarity index of any combination of fluorochromes. In addition, the WVU FCSCCF staff are more than happy to assist in designing your staining panels.

2. **Single stain controls aka Reference Controls (Aurora)**

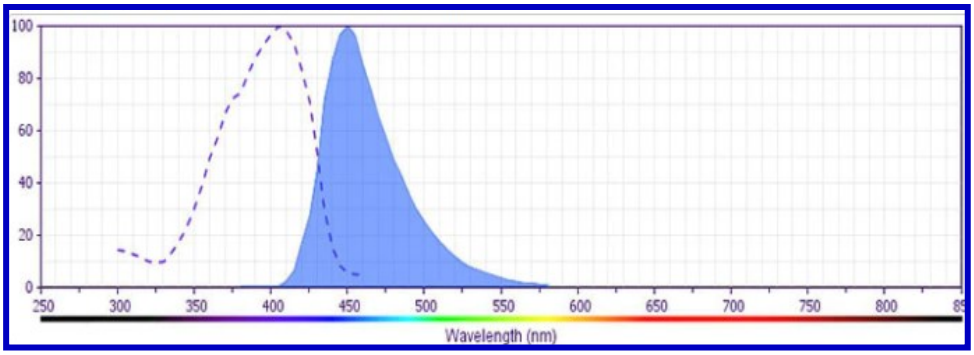
   It is really important that you have a good positive signal for each single stain control. On the Aurora, it is perfectly acceptable to use comp beads, cells, or a mix of beads and cells for the single stain control samples. The key is that the single stain controls have to be stained with the identical antibody used on your samples. Also, if you use beads for some and cells for other single stain controls you will need to include a no stain for both beads and cells.

3. **Determine antibody titers**

   Regardless of instrument, it is always recommended that you titer the antibodies in your staining panel in order to determine the optimum antibody concentration needed in your experiments. The more fluorochromes you use in an experiment, the more important it is not to use excessive amounts of antibody. In many cases, the amount of antibody needed is less than what the manufacturer recommends, so tittering has the added benefit of saving money.

To assist users of the Aurora, we are currently working on SOPs for designing a staining panel, tittering antibodies, operation of the instrument in plate mode to name a few. If you would like to learn more and/or have a look at the instrument stop by the lab. We would be happy to show it to you. To learn more about how the Aurora would work for your experiments, please contact us at kbrundage@hsc.wvu.edu or 304-293-6273.

Meet the Fluorochrome: Blue Horizon V450

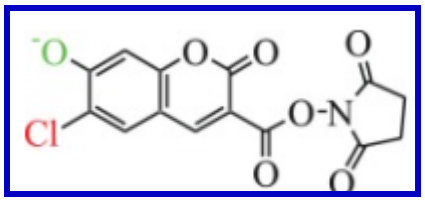


Excitation
404 nm (Violet laser)

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

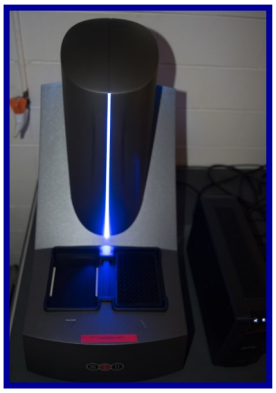
Format: 96 or 384 chambers per chip

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

September 6, 2021	Labor Day	Facility Closed
September 20 –24, 2021	Kathy Out of Lab	Facility Open—Instrumentation available
November 24 –26, 2021	Thanksgiving	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

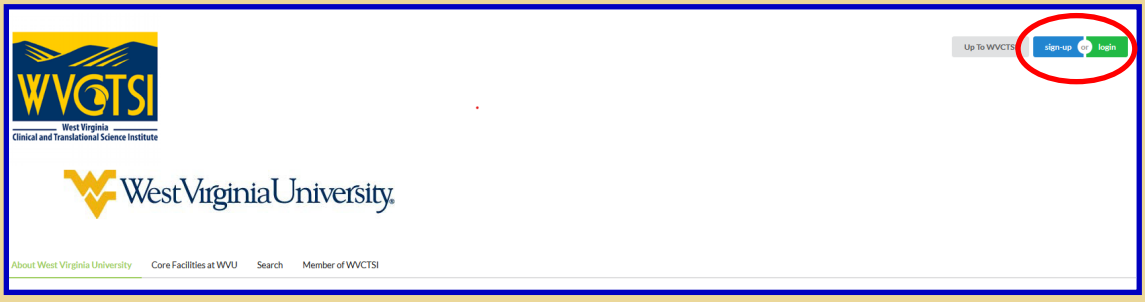
New User Guide

Hands-on training for LSRFortessa, 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
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
	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