WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 11, issue 4 April 2024

Fluorescent Minus Ones (FMO) Basics

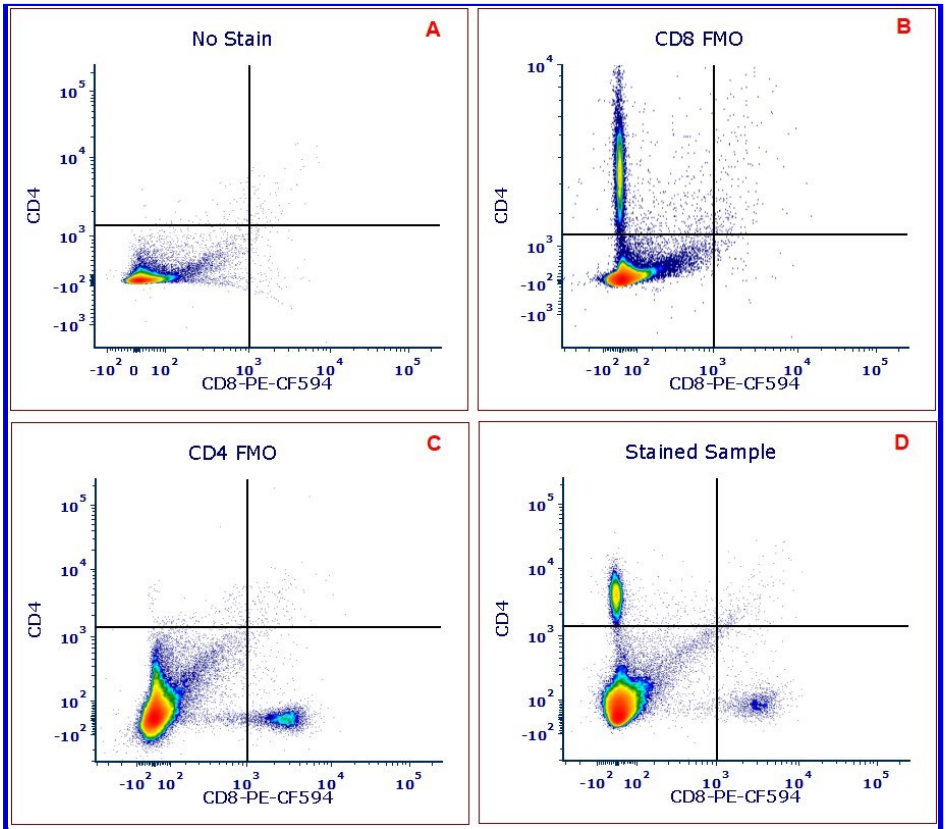

Figure1. FMO controls can aid in proper gating. (A) No stain control, (B) CD8 FMO control, (C) CD4 FMO control and (D)A sample stained with both antibodies.

In flow cytometry, gating is one of the most subjected and important processes performed when analyzing data. It is also the topic that staff in flow cytometry cores spend the most time discussing with users. Gating flow cytometry data can be difficult for many reasons including that the population of interest is very small or staining was done using a large multiple color staining panel with complex compensation. Improper gating can lead to misidentifying populations or not identifying them at all. For

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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:
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these reasons, improper gating can lead to a slew of issues downstream and even affecting future projects.

Typically, no stain and single stain controls are used for proper gate placement. Both are important as the no stain control shows exactly what the cells' background fluorescence looks likes at each detector. The no stain control is used to identify the negative population. Single stain controls are used to identify the positive populations. Sounds simple, however, sometimes even having the correct no stain and single stain controls can still lead to uncertainty. This is when having fluorescence minus one controls, better known as FMOs, come in handy.

A FMO control is a tube that contains every fluorescent antibody in your panel except for one. For example, a PE FMO would contain every antibody except for the one labeled with PE. When a FMO control is analyzed there will be no positive population on any graph or histogram containing PE while all the other expected fluorescent populations will be present. When you compare the PE FMO with the single stain control or a sample labeled with all the antibodies in your panel it will be easy to determine where the gate should be placed for PE. FMOs are an excellent resource for better identifying gating boundaries, especially in panels with multiple colors and complex compensation, as they create a clearer differentiation

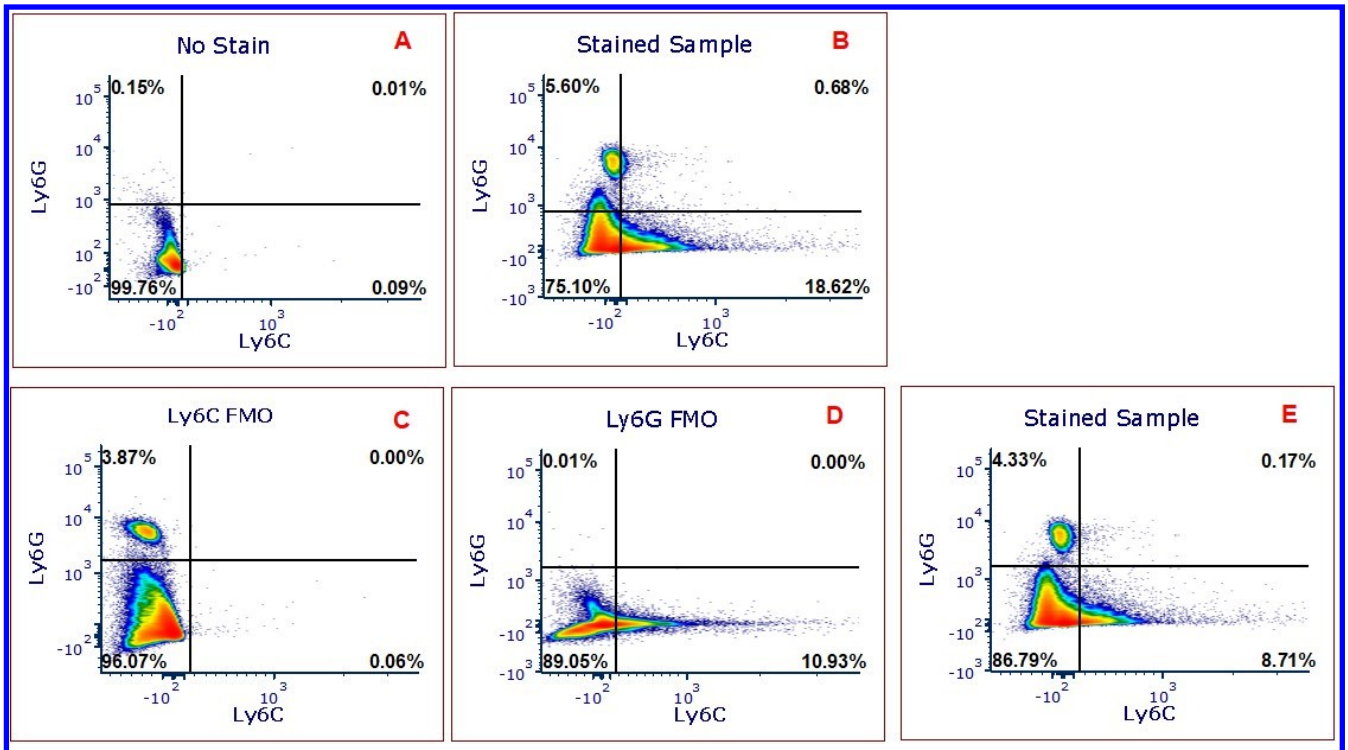


Figure 2. Density plots showing the value of FMO controls. (A) No stain control sample. (B) Sample stained with both Ly6G and Ly6C using the no stain control to set the quadrants. (C) Ly6C FMO control (D) Ly6G FMO control and (E) Sample stained with both Ly6G and Ly6C using the FMO control to set the quadrants. The percent of cells in each quadrant are shown in each plot

between the positive and negative populations of a fluorochrome.

In Figure 2, the no stain control is shown for Ly6C vs Ly6G expression in a plot (Figure 2A). Using only the no stain control to determine where the negative populations are for both Ly6C and Ly6G leads to the gating shown in Figure 2B. For these two plots the quadrants were placed based on the no stain control sample (shown in Figure 2A). The reasoning for placing the quadrants where we did is that we wanted less than 1% of the events in the upper left (Ly6G(+) only), upper right (Ly6G(+)Ly6C(+)), lower right (Ly6C(+) only) quadrants on the no stain control plot (Figure 2A). The lower left quadrant (double negative population) should be the one with the majority of events.

Now look at Figure 2C, 2D and 2E. Figure 2C displays the FMO control for Ly6C and Figure 2D displays the FMO control from Ly6G. As you can see in these plots Ly6G has two very distinct populations, Ly6C does not. As such, while it is easier to properly place the gate for Ly6G, it is harder to do so with Ly6G. This is why having the FMO control for it is so helpful. While the percentage of Ly6G positives does not drastically change between the two, the percentage of Ly6C positives does. Figure 2B, which is gated using only the no stain control sample, shows 12.73% of events are Ly6C positive Figure 2E, which is gated using the FMO controls, shows 9.48% of events are Ly6C positive. That's a difference of 3.25% of events and has the potential to have a major impact on proper data interpretation.

There is one drawback when it comes to FMO controls and that is the cost. The larger the panel, the more FMO controls needed and thus the more antibody is used per experiment. When you have panels of 8 or more this can quickly add up. To try to keep the cost down, the WVU Flow Cytometry & Single Cell Core Facility staff recommends making a complete set of FMO controls the first time you use the antibody panel. Look at the data and identify the antibodies whose positive populations are easy to identify (usually there is a large distinct population compared to the negative population) and those that are more difficult to see. Next time you use the panel, you only have to have FMO controls for those antibodies whose positive populations are difficult to gate i.e. is very small or not distinct population from the negative population. Doing a limited number of FMO controls will cut down on the amount of antibody needed and result in fewer tubes to run on the cytometer.

So the next time you are creating a large multicolor panel or the populations of interest is not very distinct or you are struggling with compensation, consider incorporating FMO controls. It will make you more confident in your gating by providing a much clearer identification of positive populations. If you would like to learn more about FMO controls or have questions, don't hesitate to reach out to the WVU Flow Cytometry & Single Cell Core Facility for help.

Fluorochrome Word Search

N	D	P	A	C	I	F	I	C	B	L	U	E	M
C	E	O	C	A	E	P	I	R	O	I	E	A	I
A	F	A	A	N	P	F	D	R	C	E	T	U	T
A	G	Q	R	T	T	C	N	T	T	P	Y	Q	O
S	D	I	D	I	T	A	I	F	F	C	R	A	T
B	R	B	P	S	R	F	E	G	C	T	R	E	R
E	P	E	T	E	X	A	S	R	E	D	E	I	A
E	E	D	R	E	P	E	O	C	O	R	H	B	C
P	R	E	M	D	C	E	F	R	T	I	C	M	K
R	C	R	A	Z	T	T	P	H	A	U	M	O	E
I	P	E	A	R	L	R	C	Z	M	C	P	Z	R
B	D	L	C	E	C	R	E	T	O	T	L	C	R
M	T	I	E	R	D	T	K	D	T	U	A	E	E
T	R	N	T	R	I	T	C	F	D	I	I	Y	D

WORD BANK

PE TEXAS RED
GFP
PERCP
MCHERRY
DTOMATO
PACIFIC BLUE
NILE RED
ZOMBIE AQUA
FITC
MITOTRACKER RED
DID
TRITC
NEAR IR
APC

Solution to word search will be published in July 2024 newsletter.

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

WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Instrument Fee	$190/run	$315/run
Chip*	$300/chip	$460/chip
Reagents**	$55/sample	$95/sample
cDNA Tracer Tape	$11.50/sample	$20/sample
Sample Prep (nuclei isolation)	$248/cell line	$380/cell line
	$300/frozen tissue	$460/frozen tissue
Labor***	$55/h	$92/h

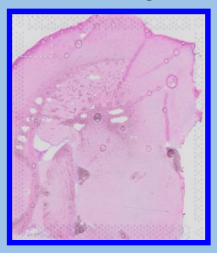
*Holds up to 8 samples
**Does not include cost of the 10x Genomics reaction kit.
***The GEM generation & Barcoding takes about 2 – 2.5 hours. The generation of the library can take 3.5 – 10 hours depending on the assay you are running.

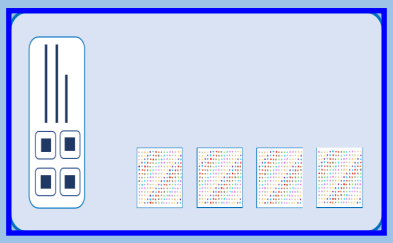
10x Genomics Visium Transcriptomics

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

WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Visium Optimization Slide (Manual)	$385/slide	$640/slide
Reagents for spatial gene expression (does not include cost of slide or kit)	$66/sample	$110/sample
Labor for spatial gene expression	$55/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

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

Luminex MAGPIX

Operator: User or Facility Staff

Optics:

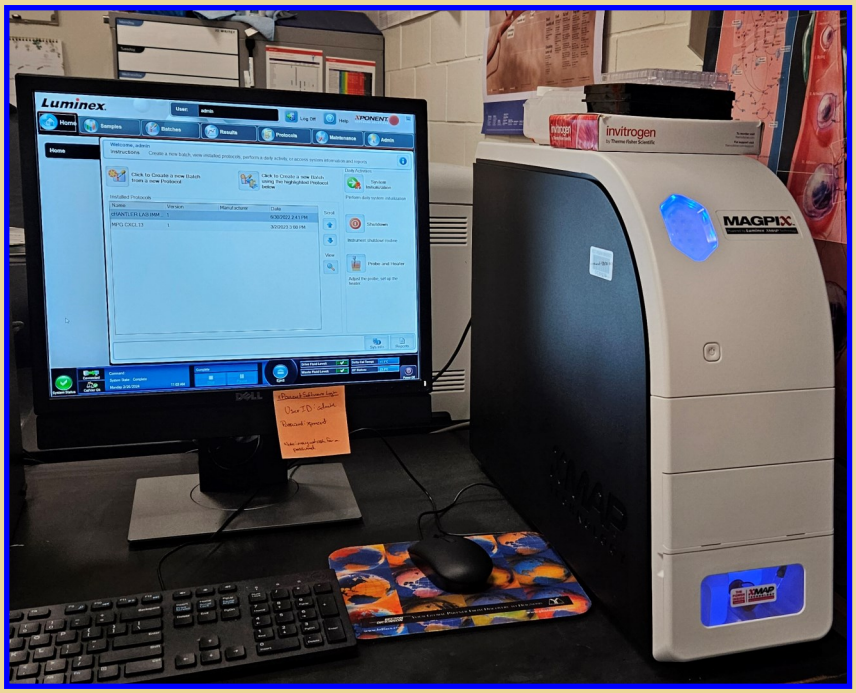
- Light Emitting Diodes
- CCD Camera

Detection Parameters:

- Reads up to 50 unique magnetic bead regions

Applications:

- Qualitative and quantitative analysis of proteins and nucleic acids



Other Instrumentation Available in the Facility

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:

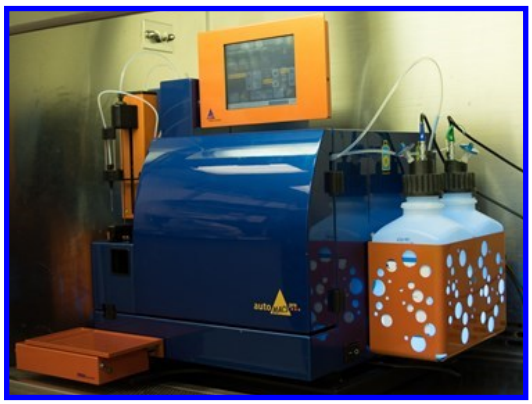
- Tissue disassociation into single cell suspension for culture or flow cytometry assays
- Tissue homogenization for molecular biology applications

AutoMACS Pro Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell sorting

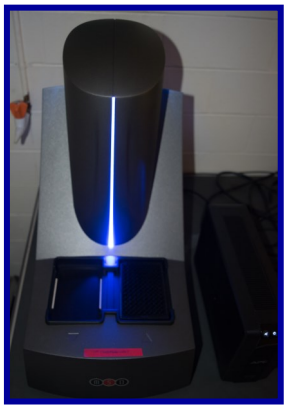


MSD Multi-Array Platform

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 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

WVU FCSCCF Usage Fees

FLOW CYTOMETERS		
WVU FCSCCF Usage Fees	**FOR WVU USERS**	**FOR NON-WVU USERS**
Flow Cytometers for analysis – User operated	$40/h	$60/h
Flow Cytometers for analysis – Facility operated	$58/h	$90/h
Sorting	$85/h	$133/h
Sort setup fee	$22	$34

OTHER EQUIPMENT		
WVU FCSCCF Usage Fees	**FOR WVU USERS**	**FOR NON-WVU USERS**
AutoMACs Pro	$5/sample	$8/sample
gentleMACs	$11.75/sample	$20/sample
Luminex MAGPIX — User Operated	$45/h	$70/h
Luminex MAGPIX — Facility Operated	$65/h	$90/h
MSD QuickPlex SQ120	$12/h	$20/h
MultiMACS 24 Separator Plus	$3.50/separation	$5/separation
NanoSight NS300 – User Operated	$47/h	$75/h
NanoSight NS300 – Facility Operated	$67/h	$102/h
Zetasizer Nano Z – User Operated	$27/sample	$43/sample
Zetasizer Nano Z – Facility Operated	$27/sample + $55/h	$43/sample + $92/h

Upcoming Holidays & Events

May 2 to 14, 2024	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
May 14, 2024	Primary Election Day	University Holiday - Lab Closed
May 27, 2024	Memorial Day	University Holiday - Lab Closed
July 4, 2024	Independence Day	University Holiday - Lab 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

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 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