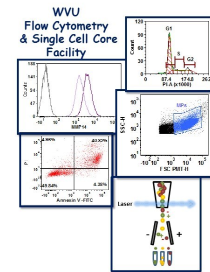
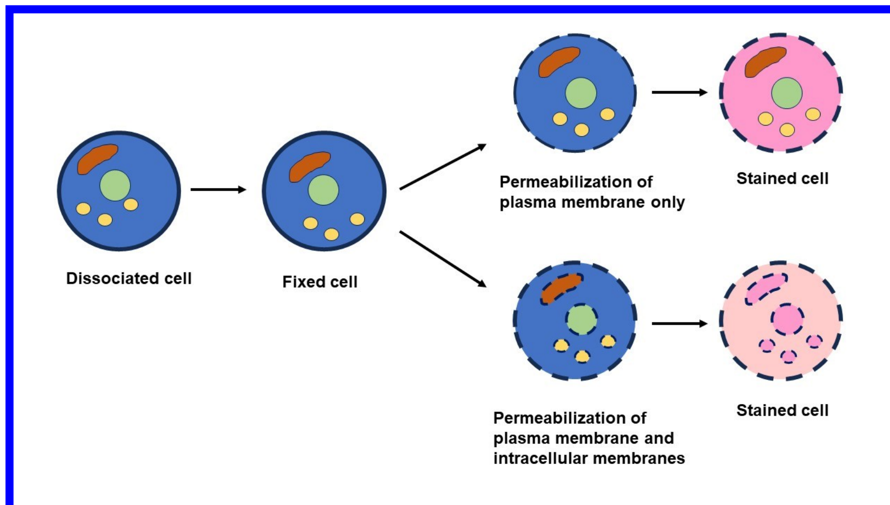


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 11, issue 3 January 2024

The Basics of Intracellular Flow Cytometry



Being able to identify cell types based on expression of surface markers is one of the key uses for flow cytometry. While this is widely available and commonly used, another great use for flow cytometry is identifying proteins in side the cell. Being able to look inside a cell and measure intracellular markers allows for researchers to gain insight into cell signaling and functional responses. Intracellular staining enables researchers to reveal more information about how cells function in relation to diseases, for example what critical processes are dysregulated.

Intracellular proteins have vital roles in almost every biological process making them potential therapeutic targets for various conditions from autoimmune diseases to different types of cancers. For example, the transcription factor hypoxia-inducible factor 1α (HIF-1α), which is expressed at low levels under normal conditions. However, when put under hypoxic conditions, it is up-regulated to drive the transcription of multiple genes that allow for metabolic adaptation to oxygen deprivation. This makes HIF-1α an important target for cancer

Inside this Issue

1-3	The Basics of Intracellular Flow
4	10x Genomics Chromium Controller
5	10x Genomics Transcriptomics
6-7	Flow Cytometers in the Facility
8	Other Instrumentation in the Facility
9	Fee Schedule
10	Upcoming Holidays
10	New Users Guide
10	Information on Acknowledging the Core
10	iLAB Scheduling

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

drugs as it allows for cell survival under hypoxic tumor conditions.

Besides using intracellular staining to examine transcription factors, it can be used for an almost limitless number of applications. Some of these applications include measuring cytokine production, the expression level of transcription factors, or the phosphorylation-driven activation of signaling pathways. Thus, intracellular staining is an essential tool for most researchers because it allows for the correlation between cellular phenotype, which is based on surface marker expression, and cellular function. Additionally, intracellular flow cytometry is quickly becoming a vital tool for epigenetics, which is the study of how histone and DNA modifications affect gene expression without alteration of the genetic code.

Fixation and Permeabilization

When surface staining cells for flow cytometry, the process is relatively straightforward, with three main steps: blocking, staining, and washing and an optional fourth fixation step. However, detecting intracellular markers requires several additional steps. The two most important ones are that the cells **must** be fixed, to prevent any changes from occurring during subsequent handling, and permeabilized, to allow antibody reagents access to their targets.

There are two primary choices for fixation: formaldehyde and methanol. Formaldehyde is most common, as it preserves cellular morphology and reduces the lose of soluble proteins. However, methanol cannot be used in conjunction with certain live-dead stains, like 7-AAD, PI, and DAPI. Instead, fixable live-dead stains should be used. Methanol can also be used as a fixative. An additional effect of using methanol is that will not only fix the cells but also permeabilizing cells, which can save time. A disadvantage of using methanol as a fixative is that it can be disruptive to cellular epitopes, which can inhibit some antibodies from binding to their epitopes. For this reason, it can not be used with some antibodies. Thus, it is important for researchers to double check the datasheets for the antibodies being used and, if time and money allows, perform an optimization/practice experiment.

With the fixation agents in mind, it is important to discuss and consider the choice of permeabilization agent because it can likewise impact antibody specificity and functionality. There are two detergents that work well for intracellular staining: Triton X-100 and saponin. Triton X-100 permeabilizes the plasma membrane and intracellular membranes, like those around the nucleus and mitochondria, allowing access for antibodies. Saponin is more limited, as it only permeabilizes the plasma membrane and is reversible which allows it to be used as antibody diluents if the antibodies are used downstream of permeabilization.

Tips for Successful Experiments

As discussed above, choosing the correct fixative and permeabilization method is crucial for successful intracellular staining. In addition, there are several other things to consider to ensure a successful, accurate, and reproducible intracellular flow cytometry experiment.

Identifying Your Target

Identifying and researching the exact target is the vital first step for planning out an intracellular flow cytometry experiment. It's important to research where in the cell the target is found, how abundant it is, and whether or not some form of treatment is needed to stimulate its expression. Additionally, the location of the target will help in determining which permeabilization agent should be used, which in turn will determine the antibody to used.

Determine if Sequential Staining Should be Performed

If a combination of extracellular and intracellular markers need to be identified in the same experiment, then sequential staining should be considered. Sequential staining involves blocking, staining for surface markers, fixation, permeabilization, and then staining for intracellular targets. Cell Signaling Technology has a decision tree on their website that can be utilized to determine the steps for the experiment.

Choosing High Quality Antibodies

When choosing antibodies for the experiment, its best to go with those that you have used previously to detect the target of interest in the selected species. If you decide to use an unfamiliar antibody, it is highly recommended to titer the antibody prior to using it in your real experiment.

Carefully Assign Fluorophores

As with extracellular flow cytometry, it is very important to pay close attention to panel design. Extra care needs to be made for intracellular flow cytometry as multiple targets will need to be detected, so spillover must be kept as minimal as possible. Bright fluorophores should be assigned to markers with known small populations and dim fluorophores should be assigned to markers with known large populations. Additionally, the question of which permeabilization agent comes into play as well. For example if methanol is being used, then protein fluorophores like APC and PE must be added after permeabilization is complete, as methanol can denature those fluorophores.

Consider Using Reagents Made Specifically for Intracellular Flow Cytometry

While most antibody reagents used for extracellular staining can be used for intracellular staining, there are reagents made specifically for intracellular flow cytometry. These reagents can be worth the investment, especially for those who know they will be performing a lot of intracellular flow cytometry. One such example is that of fixable viability dyes, like the Ghost Dyes from Tonbo Biosciences, which can be used in place of common non-fixable viability dyes like 7-AAD, PI, and DAPI. For more information on fixable viability dyes, you can consult the FCSCCF October 2022 Newsletter, which can be found on the Newsletter tab of our website at **https://flowcore.hsc.wvu.edu/**

As always, the WVU FCSCCF staff are available to assist you in all aspects of your intracellular flow cytometry experiments.

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

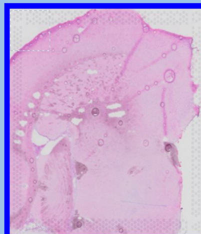
WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Instrument Fee	$190/run	$315/run
Chip*	$300/chip	$460/chip
Reagents**	$55/sample	$95/sample
cDNA Tracer Tape	$11.50/sample	$20/sample
Sample Prep (nuclei isolation)	$248/cell line	$380/cell line
	$300/frozen tissue	$460/frozen tissue
Labor***	$55/h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

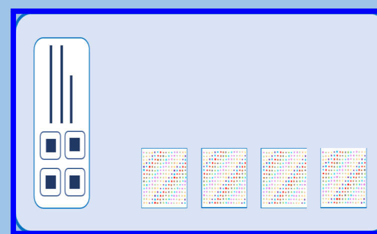
*Holds up to 8 samples
**Does not include cost of the reaction kit.
***The GEM generation & Barcoding takes about 2 – 2.5 hours. The generation of the library can take 3.5 – 10 hours depending on the assay you are running.

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**





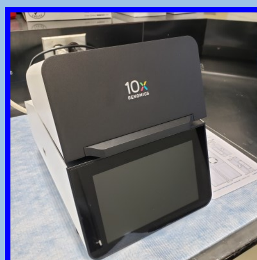
10x Genomics Visium Transcriptomics Fees

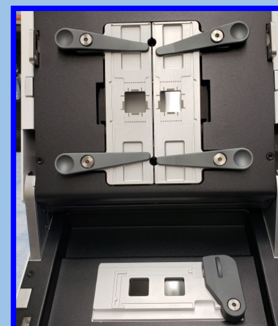
WVU FCSCCF Usage Fees	FOR WVU USERS	FOR NON-WVU USERS
Visium Optimization Slide (Manual)	$385/slide	$640/slide
Reagents for spatial gene expression (does not include cost of slide or kit)	$66/sample	$110/sample
Labor for spatial gene expression	$55/h	$92/h

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

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 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:

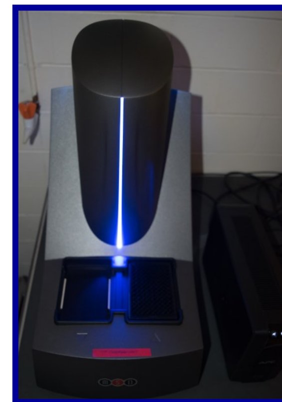
- Single extracellular marker cell sorting
- Depletion/negative cell sorting

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

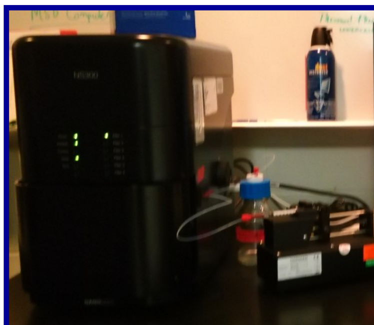


Nanosight NS 300

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
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

January 31 to February 11, 2024	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
March 29, 2024	Spring Holiday	Facility Closed for University
April 22 to 25, 2024	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics

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