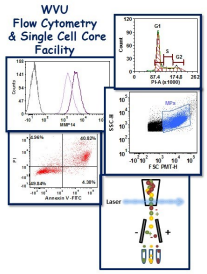
WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



Newsletter Volume 10, issue 1 **October 2022**

New Core Services: Visium & MultiMACs Cell24

The Flow Cytometry and Single Cell Core Facility is excited to announce two new services for users. The first is the 10x Genomics Visium Spatial Gene Expression, which aids in expanding understanding of gene expression in tissue samples. The second is the Miltenyi Biotec MultiMACS Cell24 Separator Plus, which allows for parallel separation of up to 24 samples at once. Both services are available now through the core. More information about theses services can be found below.

10x Genomics Visium Spatial Gene Expression

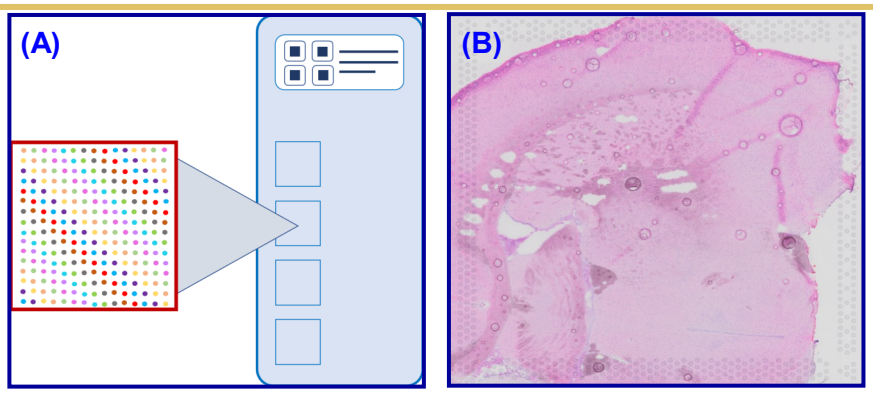


Figure 1. A Visium Spatial Gene Expression slide. (A) Schematic representation of the slide and its four capture areas. Each capture area is 6.5 x 6.5 mm with a total of 5,000 spots. (B) Is an example of a slice of mouse brain in one of the capture areas.

The newest service the Core is providing comes from 10x Genomics' Visium Spatial Gene Expression kit. The Visium technology uses special oligo printed slides for mapping gene expression on a tissue slice. This technology can be used on fresh frozen or FFPE tissues allowing for the whole transcriptome of the sample to be analyzed. The slides have been demonstrated to be compatible with a wide variety of organs and species.

The key to the technology is the the Visium Spatial Gene Expression slide. This slide contains demarcated regions for four individual sections. Each region measures 6.5 x 6.5 mm and contain 5,000 "spots". Each "spot" has a cluster of oligos with a unique

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

Contact Dr. Brundage at:
kbrundage@hsc.wvu.edu

identify so that the sequenced mRNA can be mapped back to its original location in the tissue section. Each “spot” is 55 µm in size giving a resolution of 1– 10 cells/spot depending on the tissue.

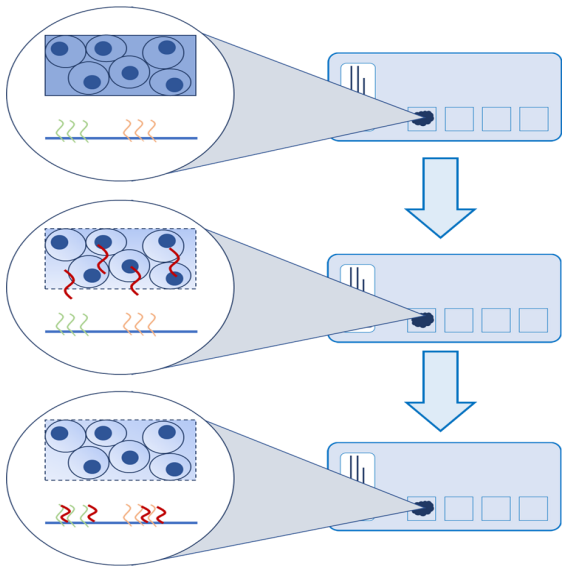


Figure 2. The process of staining and imaging, permeabilization, and cDNA synthesis. The stained tissue is shown on the top slide above the barcoded oligonucleotides. The slide will also be imaged before progressing to the next step. The tissue is then permeabilized, allowing for the mRNA to be released. The final slide shows the captured mRNA which will then be used for cDNA synthesis and sequencing.

How does the assay work?

Four individual intact fresh frozen tissue sections are cut from the specimen and placed in the four spotted areas. Slides containing tissues can be stored up to 1 month at –80°C. On the day of the assay, the tissues are fixed in cold methanol and H&E stained. The stained sections are imaged on the Olympus VS120 Slide Scanner (located in the Imaging Facility) using a 10x objective. If desired, you can do fluorescent staining of the tissue instead of H&E. After imaging the tissue is permeabilized which releases the cell’s mRNA. The released mRNA then binds to the barcoded oligonucleotides coated on the sections of the slides. The captured mRNA is used to synthesize barcoded cDNA which can be sent directly for standard DNA sequencing. The 10x Barcoded library retains information about spatial location allowing for visualization of spatial gene expression within the individual tissue sections.

There are several steps that need to be taken prior to running your first slide. These steps are essential for obtaining good quality results in the end. Outlined below are the pre-requisite steps that will ensure you get publishable data:

1. Freeze the tissue in OCT
2. Determine quality of the RNA from the frozen tissue by cutting 10 sections and placing them in a microfuge tube. Isolate RNA using a Qiagen RNease Mini Kit. Determine the RIN number using a BioAnalyzer (needs to be ≥ 7)
3. Determine the permeabilization time for your tissue using the Visium Tissue Optimization kit

The WVU FCSCCF along with the WVU Genomics Core have set up a pipeline to make the whole process as painless as possible. If you provide slides with the tissue sections on them the Flowcore staff will do the optimization to determine the optimal permeabilization time for your tissue. They will also run the real slides from fixation through the barcoding step. After which, Ryan Percifield (Genomics Core) will generate the libraries. If you need help in determining the quality of the RNA, Ryan will also provide assistance.

For those interested in learning more, the Core is hosting **a seminar on November 2nd at 2 PM in HSC North room 2094 with 10x Genomics**. Please contact Dr. Brundage for more information.

MultiMACS Cell24 Separator Plus

Figure 3. The Miltenyi Biotec Multi-MACS Cell 24 Separator Plus. This instrument is stored in the Core's annex lab. This image displays how the instrument can be used with 12 columns, the touchscreen menu, the elution station, and the waste collection block.

The newest piece of equipment available for users is the Miltenyi Biotec MultiMACS Cell24 Separator Plus. Similar to the autoMACS Pro Separator, the MultiMACS saves users time by allowing for the separation of up to 24 samples at once.

The MutliMACS Cell24 Separator Plus is a semi-automated cell separator that allows for magnetic separation. MACS MicroBeads and MACS Columns allow for parallel multi-sample magnetic cell isolation. The instrument is versatile as it can be upscaled and downscaled depending on the number and volume of samples being run at one time. Designed for isolation of almost any cell type from any cell source, the instrument provides reliable and standardized results and is compatible with other downstream applications like molecular analysis, cell-culture assays, and flow cytometry.

The primary benefits of this instrument are the versatility and speed when running samples and the ability to run up to 24 samples at once. There are four column types available for usage and all are dependent on the type of cells being isolated.

The main feature is the Multi-24 Column Block, which allows for the positive selection and depletion of up to $1x10^8$ magnetically labeled cells from up to 24 samples at once. The instrument comes with a Single-Column Adapter for use with up to 12 LS, LD, and Whole Blood Columns for usage with fewer or different types of samples. LS Columns are optimized for positive selection and depletion of up to $1x10^8$ strongly magnetic labeled cells. LD Columns are optimized for depletion of up to $1x10^8$ strongly magnetic labeled cells. Whole Blood Columns are optimized for cell isolation directly of up to 10 mL of whole blood, buffy coat, or bone marrow. Regardless of the number of samples being run, all are parallelly processed which saves time for users.

To begin using the instrument, order the appropriate beads and columns from Miltenyi Biotec. The Core provides all necessary holders and adaptors, but columns must be purchased from the company as they are not provided.

Live Dead Staining: What It Is and How to Determine the Best Dye to Use

Live/Dead staining should be an essential component of every flow cytometry staining protocol. The reasons it is important to exclude dead cells is:

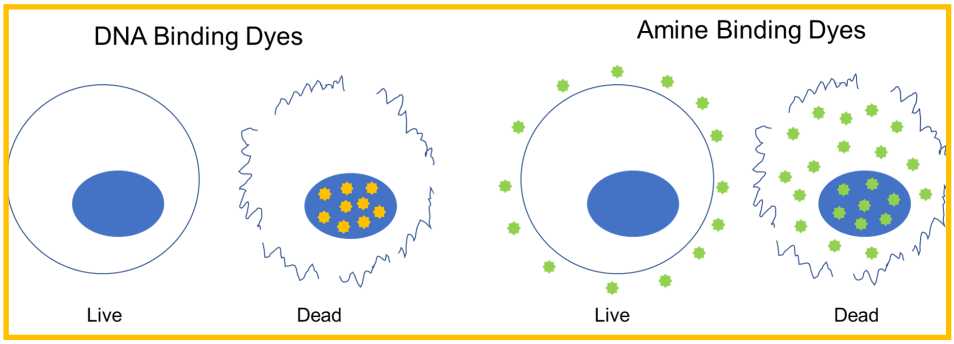
- They have a tendency to bind antibodies in a non-specific manner,
- Having a high degree of autofluorescence
- Tend to clump together

So, including a Live/Dead stain in your panel will make it easier when it comes to data analysis. When sorting, a Live/Dead stain will ensure the cells you isolate are live.

Cells	Recommended dyes	When to add the dye
Live with no fixation	PI, 7-AAD, Zombie, Ghost, or Phantom Dyes	Anytime
Fixed before antibody staining	Zombie, Ghost, or Phantom Dyes	Add prior to fixation
Stain with antibodies then fix	Zombie, Ghost, or Phantom Dyes	Add prior to staining and fixation
Fix and permeabilize for intracellular staining	Zombie, Ghost, or Phantom Dyes	Add prior to fixation
Already fixed	No dye available	N/A

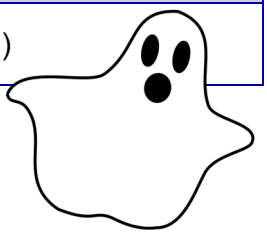
Live-dead staining is possible on both fixed and unfixed samples (see Table above). For samples that will not be fixed, high affinity DNA binding dyes are best for live-dead staining. These DNA-binding dyes enter dead/dying cells through their compromised cell membrane. A live cell with an intact cell membrane will excluded the dye. These dyes include PI (Propidium iodide), 7-AAD, and DAPI.

If samples need to be fixed, there are many other dyes available for aiding in the identification and exclusion of dead cells. Unlike PI, 7-AAD, and DAPI, fixable dyes work by binding to primary amine groups. The dyes will only bind to the cell surface proteins on live cells (see Figure below). Because dead cells have compromised membranes, the dye will be able to bind more protein groups and result in significantly brighter fluorescence than live cells. It is also important to note that fixable dyes can be used on unfixed lives cells.

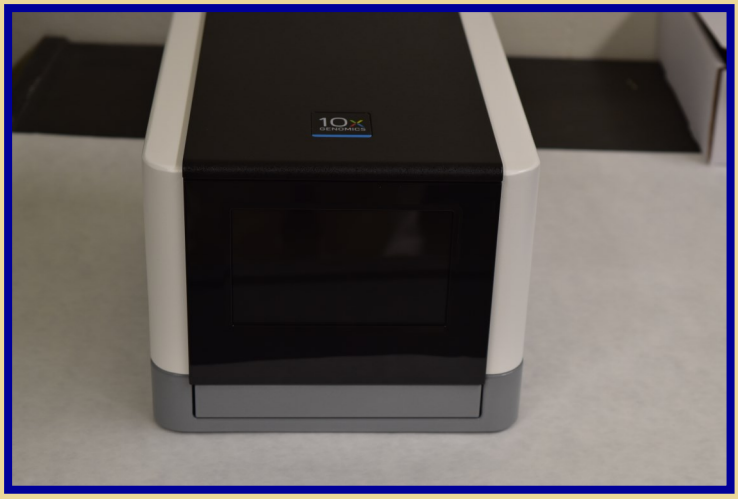


There are multiple fixable dyes available on the market including Zombie (BioLegend), Ghost Dyes (Tonbo Biosciences), and Phantom Dyes (Proteintech). All fixable dyes are available in a variety of excitation/emission ranges to allow for versatility (see Table below). It should be noted that amine dyes must be added prior to fixation of cells. Because fixation can alter the cell's membrane allowing the dye into the cell. To best determine which type of dye to use in your panel, refer to the table below.

Dye Name	Excitation/ Emission	Laser
Zombie Aqua	405/516	Violet (405 nm)
Zombie Violet	405/423	Violet (405 nm)
Zombie Yellow	405/572	Violet (405 nm)
Phantom Dye Violet 540; Ghost Dye Violet 540	405/537	Violet (405 nm)
Phantom Dye Violet 510; Ghost Dye Violet 510	405/510	Violet (405 nm)
Phantom Dye Violet 450; Ghost Dye Violet 450	405/450	Violet (405 nm)
Live/Dead Fixable Violet Stain	416/451	Violet (405 nm)
Live/Dead Fixable Lime Stain	405/506	Violet (405 nm)
Live/Dead Fixable Aqua Stain	367/526	Violet (405 nm)
Live/Dead Fixable Yellow Stain	400/575	Violet (405 nm)
PI	488/617	Blue (488 nm)
7-AAD	488/647	Blue (488 nm)
Zombie Green; Ghost Dye Blue 516; Phantom Dye Blue 516	488/515	Blue (488 nm)
Live/Dead Fixable Green Stain	495/520	Blue (488 nm)
Live/Dead Fixable Olive Stain	480/557	Blue (488 nm)
Zombie Red	561/624	Yellow/Green (561 nm)
Live/Dead Fixable Orange Stain	580/602	Yellow/Green (561 nm)
Live/Dead Fixable Red Stain	595/615	Yellow/Green (561 nm)
Zombie NIR	633/746	Red (628 nm)
Phantom Dye Red 780; Ghost Dye Red 780	633/780	Red (628 nm)
Phantom Dye Red 710; Ghost Dye Red 710	633/710	Red (628 nm)
Live/Dead Fixable Near-IR (780) Stain	633/785	Red (628 nm)



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.**

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

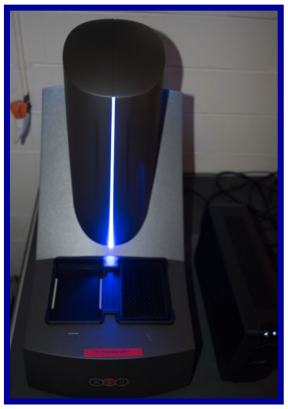
- Operator: User
- Application:
  - Tissue disassociation into single cell suspension for culture or flow cytometry assays
  - Tissue homogenization for molecular biology applications

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

November 2, 2022	10x Genomics FFPE Visium Seminar	2 PM to 3 PM in 2094 HSC-North
November 4, 2022	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
November 8, 2022	General Election Day	Facility Closed
November 23—25, 2022	Thanksgiving Holiday	Facility Closed
December 23—27, 2022	Christmas Holiday	Facility Closed
January 2, 2022	New Year's 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