WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 9, issue 1 January 2022

New in the Core
10x Genomics Chromium Controller

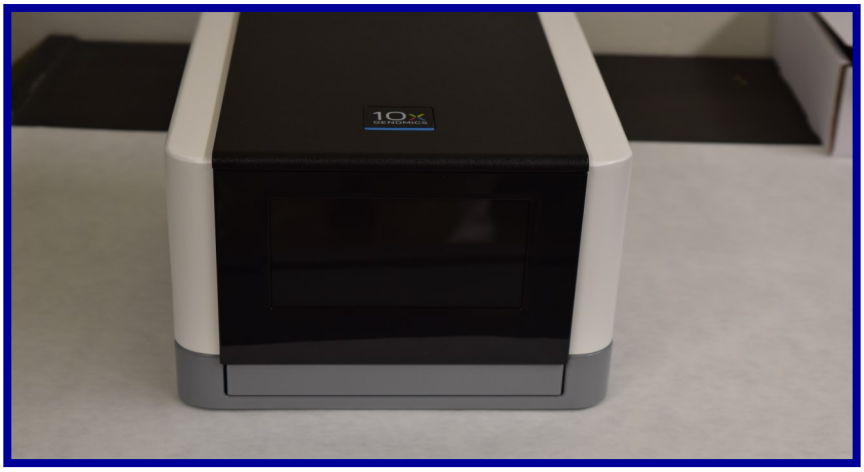

Figure 1. The 10x Genomics Chromium Controller instrument.

The 10x Genomics system uses the its Chromium Controller and Next GEMs (Gel Bead-In Emulsions) Technology, to analyze large numbers of cells at the single cell level. It does this by using unique identifiers to barcode 500 to 10,000 cells in a single reaction. Sample can be either single cells or nuclei from cell lines, fresh tissue or frozen tissue.

The 10x Genomics Chromium Controller (Figure 1) uses a Next Gem Chip and microfluidics to perform single cell partitioning of thousands of cells into individual oil droplets containing a single cell and a gel bead. Each Next GEM Chip can hold up to 8 samples and each sample can barcode 500 – 10,000 individual cells/sample.

The Gel Beads are the powerhouse of this process (Figure 2). Each are coated with a unique oligonucleotide barcode sequence and functionalized sequences to capture the molecule of interest in samples. While the oligonucleotide barcoding does vary between assays, most consist of three sections:

1. A TruSeq Read 1 sequence that is used for preparation and sequencing and is the same across all gel beads

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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

2. A 16-base pair 10x Barcode sequence that serves as the unique molecular address for the bead
3. A 10-12 bp Unique Molecular Identifier (UMI) sequence which used during data processing and is unique to the gel bead.

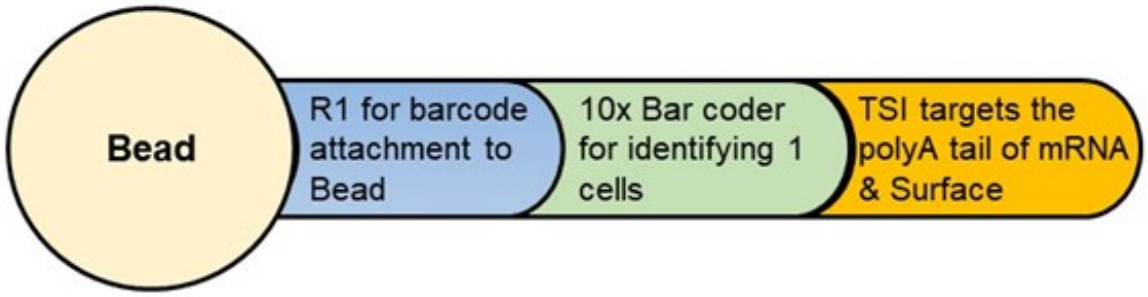


Figure 2. Schematic representation of a gel bead.

The gel beads are necessary for creating the GEMs, which are nanoliter scale "Gel Bead-in-emulsion" droplets that encapsulates the micro-reaction that occurs within the Chromium Controller. Each individual GEM acts as its own isolated reaction.

Overview of 10x Genomics Technology

So, you may be asking yourself at this point, how does all of this work? It all starts with preparing the sample, either whole cells or nuclei depending on the assay you want to run and the sample source. The samples are loaded onto a Next GEM chip along with the gel beads (containing the oligos with the unique identifiers, barcode sequence and TruSeq Read 1 sequence), and partitioning oil. The chip is loaded into the Chromium Controller instrument which mixes the barcoded Gel Beads with the sample, enzymes, and partitioning oil to form thousands of GEMs (Figure 3). Next, a reverse transcription reaction is performed during which the gel bead dissolves releasing the barcoded oligos, the nuclei/cells are lysed, and the RNA from the lysed cells are labeled with the barcoded oligos. At this point all the RNA fragments from the same nuclei/cell share a common 10x Barcode. The barcoded cDNA of hundreds to thousands of cells are pooled into a single sample for library prep

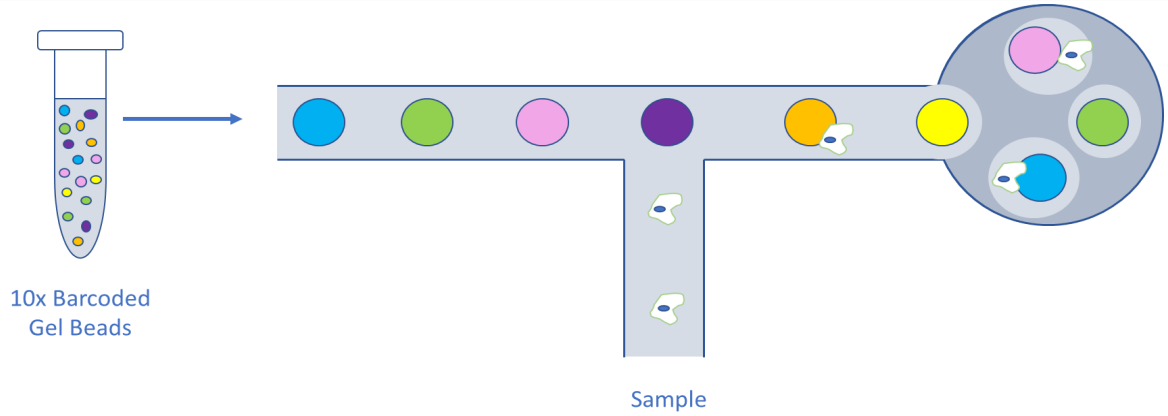


Figure 3. Inside the Chromium Instrument, one barcoded Gel Bead will be bound to a single cell or single nuclei.

(Figure 4). During the library prep, sequence specific adaptors are added to the end of the cDNA fragments via PCR. At this point, the samples are ready for sequencing.

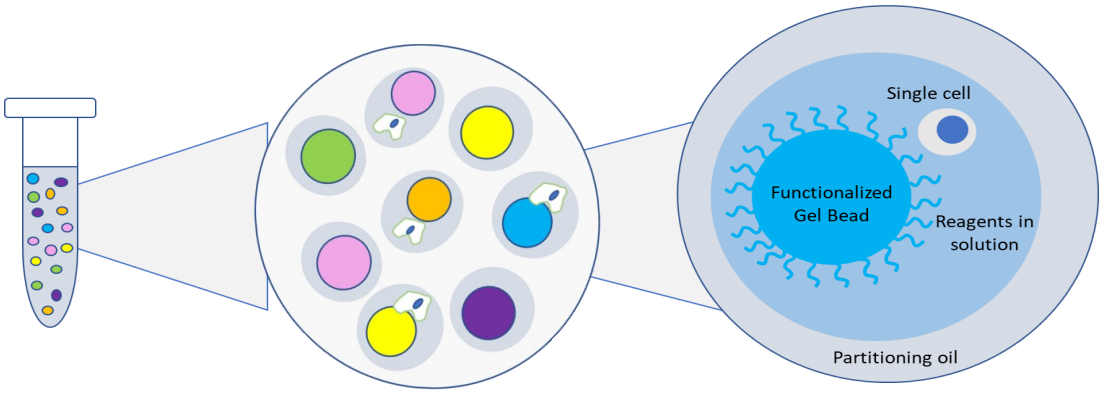


Figure 4. A closer look at the Gel Bead-in-Emulsion (GEMs), in which the sample, the gel bead, and reagents are combined into one drop contained in partitioning oil. The reaction that will occur within this drop in which the gel bead will be dissolved and the targeted molecules of interest from the single cell will be captured and barcoded.

Featured Barcode Technology

Before discussing the different assays, a brief discussion of the featured barcode technology that can be employed as part of some of the assays is necessary. Feature Barcodes are oligonucleotide barcodes sequences that can be used to label CRISPR guides or label antibodies for simultaneous measurement of gene expression and any additional cellular features within the same single cell. These can be used only with the Chromium Single Cell Gene Expression assay and the Chromium Single Cell Immune Profiling assay. More information about the use of Feature Barcodes will be given in those assays' respective sections.

Assays

Currently, there are four assays available. Nuclei can be used for all four assays while whole cells can only be used in two of the assays.

Single Cell Gene Expression Assay

This assay can be used with whole cell or nuclei samples and allows for analysis of gene expression, surface protein expression, and CRISPR perturbations within single cells. The functionalized sequences on the Gel Beads in this assay, which are Poly(dT) and two types of Capture Sequences, enable the capture of 3' mRNA. Feature Barcode can be used with this assay and will provide either labeled antibodies for cell surface protein analysis or labeled CRISPR guides to link edits with cell phenotypes.

Single Cell Immune Profiling assay

This assay can be used with both whole cell and nuclei samples. It allows for the analysis of full-length pairs of B-cell or T-cell receptors, surface protein expression, antigen specificity, and 5' gene expression, all at a the single cell level. The Switch oligo functionalized sequences on the Gel Beads in this assay enabling the capture of 5' mRNA. Feature Barcodes can be used with this assay and will

provide either labeled antibodies for cell surface protein analysis or labeled peptide-MHC multimers to measure antigen specificity.

Single Cell ATAC Assay

This assay can only be used with nuclei samples. It allows for the analysis of chromatin and will provide either labeled antibodies for cell surface protein analysis or labeled CRISPR guides to link edits with cell phenotypes.

Single Cell Multiome ATAC + Gene Expression Assay

As with the Single Cell ATAC Assay, this assay can only be used with nuclei samples. It allows for simultaneous profiling of gene expression and opening chromatin from the same single cell. Similar to the previously mentioned assay, this assay also allows for characterization of cell types and a better understanding of gene regulation. The Poly(dT)VN and Spacer functionalized sequences on the Gel Beads in this assay enables the dual capture of accessible chromatin fragments and 3' mRNA.

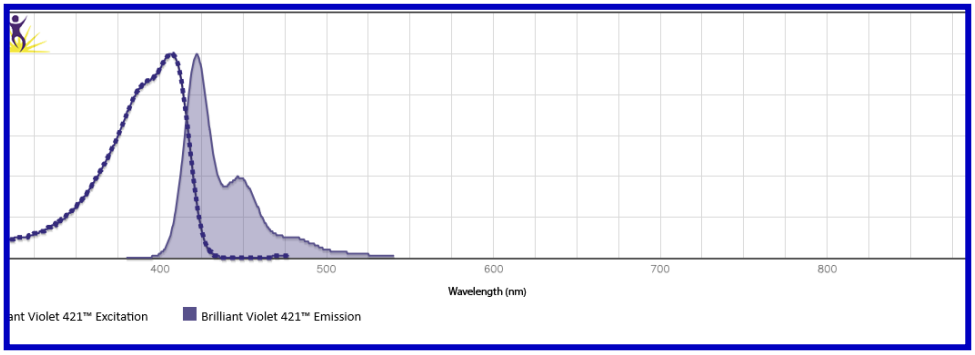
Using 10x Genomics in Your Research

If you think you might like to perform one of the assays to further your research, here are some suggested steps:

Steps	
1	Meet with Dr. Kathy Brundage to discuss the potential experiment
2	Have a zoom meeting with the 10x Genomics Sales Rep (Jessie Duller) and Science and Technical Advisor (Bradley Toms) along with Dr. Brundage to learn more about the technology and which assay(s) may be best for your experiment
3	Optimize sample prep and determine if flow sorting will be needed
4	Schedule the experiment with Dr. Brundage
5	Bring samples on designated day/time to Dr. Brundage for loading on the chip, generating GEMs, and barcoding
6	Ryan Percifield will finish the processing of the samples and generate the library
7	Send samples out for sequencing either to Marshall University or another facility

If you would like to learn more about 10x Genomics technology please contact the core staff. They can point you to some very useful webinars and literature related to the technology and how other researchers are using it in their work.

Meet the Fluorochrome: Brilliant Violet 421



Excitation

405 nm (Violet laser)

Emission max

421 nm (Same detector as Alexa Fluor 405 and Cascade Blue)

Type of Fluorochrome: Non-tandem polymer dye (the core is used as donor molecule, compatible with other dyes on the 405-lviolet laser, can be used on the BD LSRFortessa, BD Aria III, and Cytek Aurora)

Characteristics: One of the brightest fluorochromes, twice as bright as PE, and works best with the dimmest markers of the panel

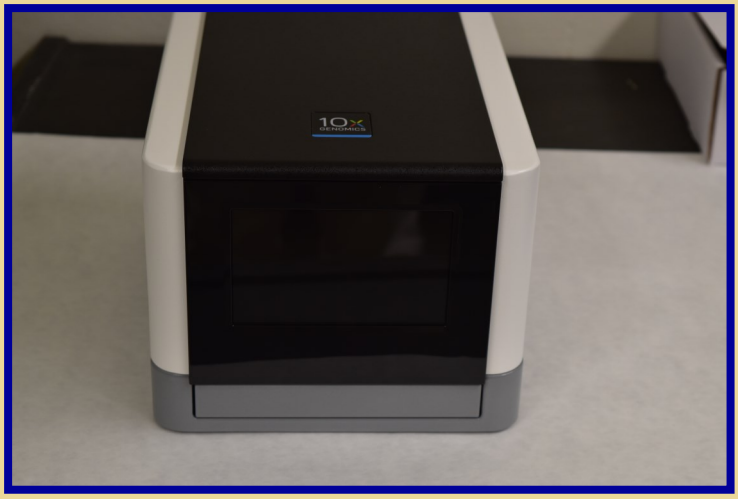
Also New in the Core

Hello! My name is Raven Forshee and I am the new lab assistant for the Flow Cytometry Core. If you have not met me yet, here is a little bit of info about me:

- **Graduated in May 2021 from West Virginia University with a Bachelor's of Science in Biology with a minor in Religious Studies.**
- **Previously conducted research with Dr. Rita Rio in the biology department.**
- **Began working in the Flow Core in August 2021.**
- **Hobbies include crochet and enjoying horror movies and novels.**

On the dunes outside Huacachina, Peru during a May 2019 trip.

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	$275
Chip*	$260	$260
Reagents	$50/sample	$80/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	$80/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

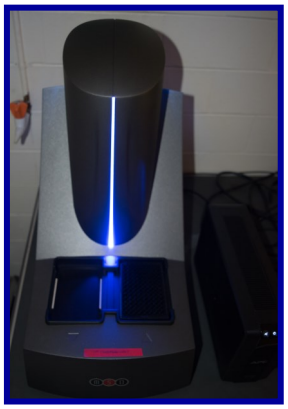
Format: 96 or 384 chambers per chip

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

January 17, 2022	Martin Luther King, Jr. Day	Facility Closed
February 4-13, 2022	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
March 28-31, 2022	Kathy out of lab	Facility Open and All Services Available Except 10x Genomics
April 15, 2022	Spring Holiday	Facility Closed
May 10, 2022	Primary Election Day	Facility Closed
May 30, 2022	Memorial 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