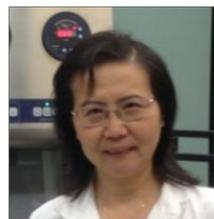


Flow Cytometry Shared Resource

Director: Rebecca Martin, PhD



Julie Farnsworth, BA
Resource Manager and Flow Operator



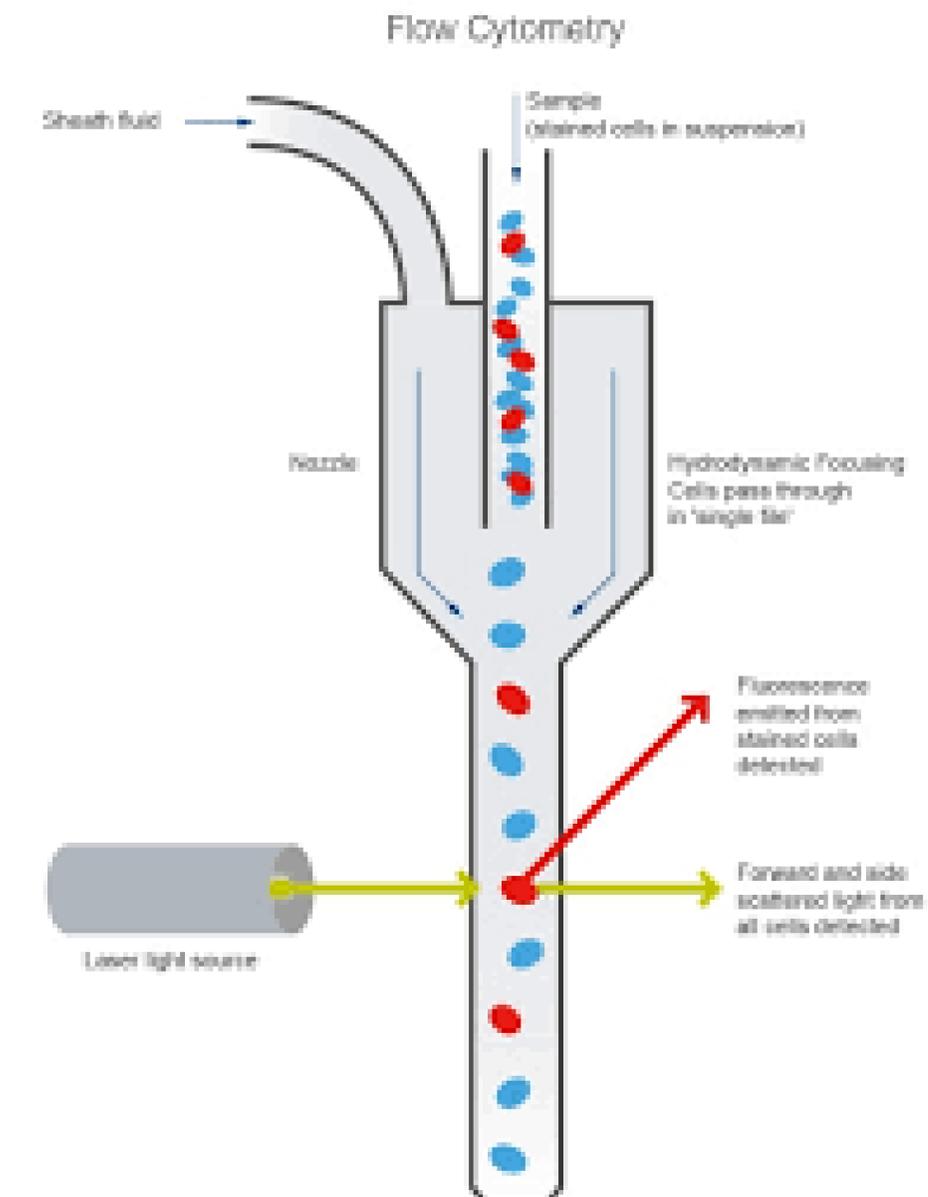
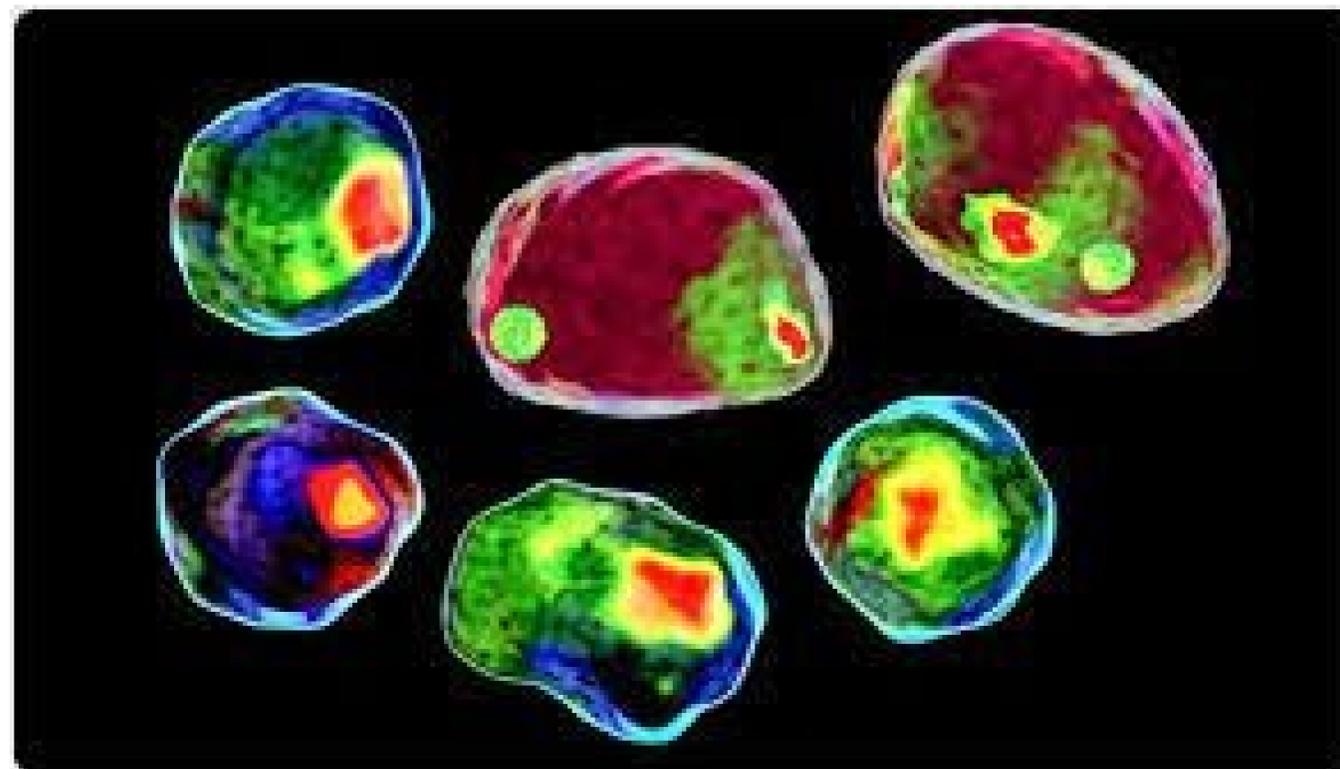
XinYan Pei, MD
Flow Cytometry Specialist



The FCSR is centrally located on the 4th floor of the Molecular Medicine Research Building. In total, it occupies approximately 1,500 square feet of space within a short walking distance of Massey Cancer Center, Sanger Hall, The School of Pharmacy, and the Bus line that connects Monroe Park Campus to The School of Engineering.

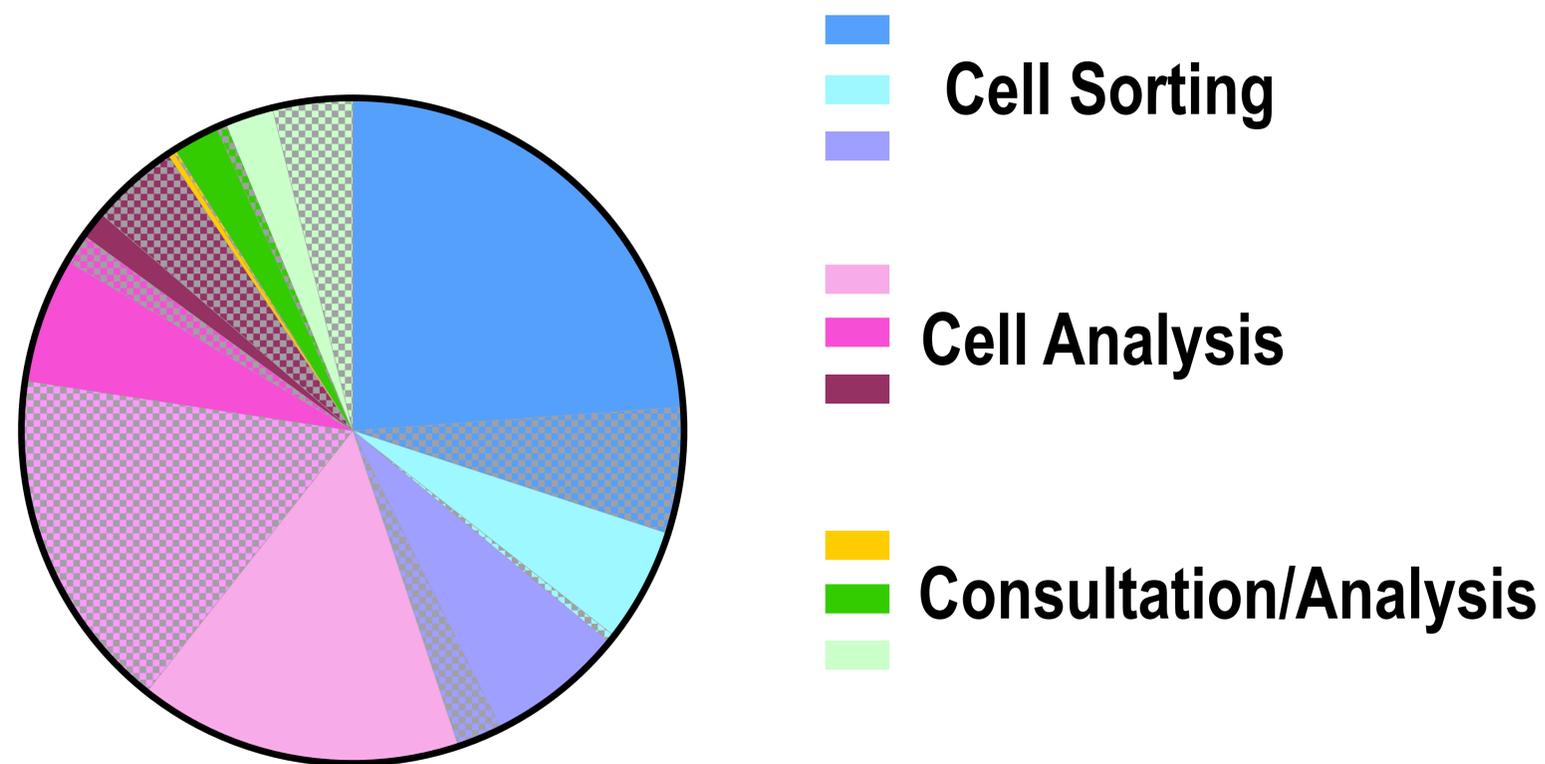
What is Flow Cytometry?

Single cell analytics- The ability to resolve a single cell in a cell suspension



Who uses the Flow Core?

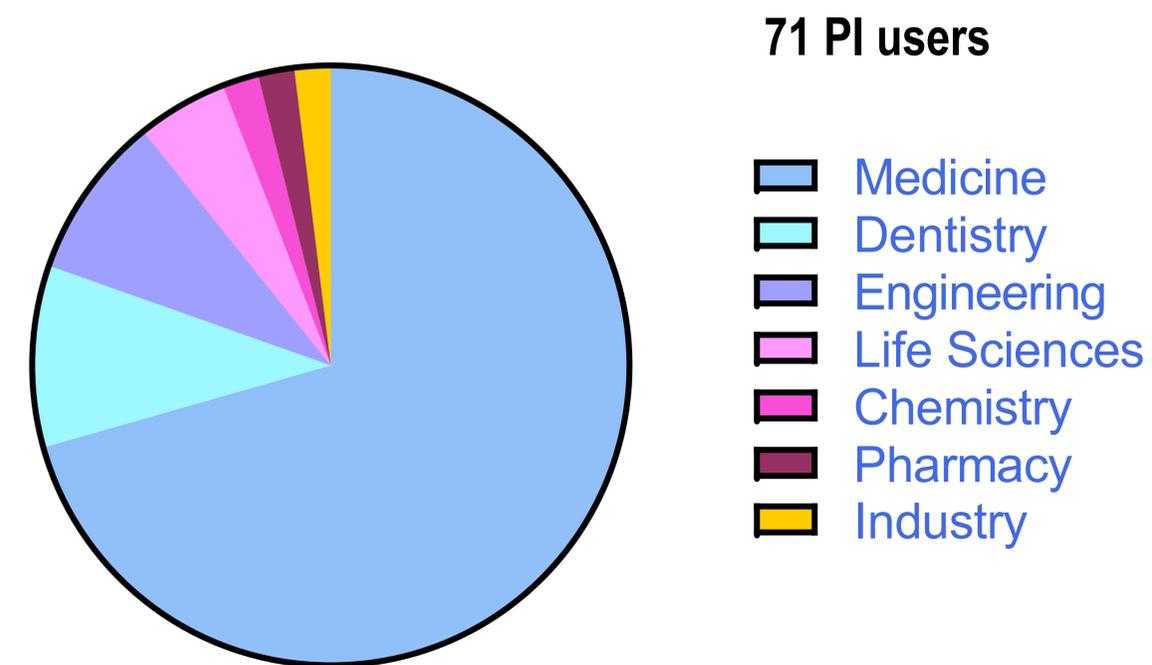
What do we do?



Total=\$109,321

90% increase in business since 2016

Who is the FCSR user?



71 PI users

User Breakdown
2019-2020

Goals

- Provide access to state-of-the-art flow cytometry instrumentation, cost-effective flow cytometry services, and skilled technical assistance
- Provide consultation on assay development, technical information, troubleshooting, data interpretation, data analysis, support for scientific publications and grant applications
- Train faculty, staff, and trainees of all levels on the use of the instruments within the FCSR and provide education on flow cytometry theory, data analysis methods, and software

Major Equipment & Services

Cell Sorting

BD FACSAria Fusion SORP High-Speed Cell Sorter (Q1 2018)

- 355 nm, 405 nm, 488 nm, 561 nm, 640 nm
- Up to 22 parameters analyzed simultaneously
- 4-way sorting
- 96-well plate sorting
- Single cell sorting



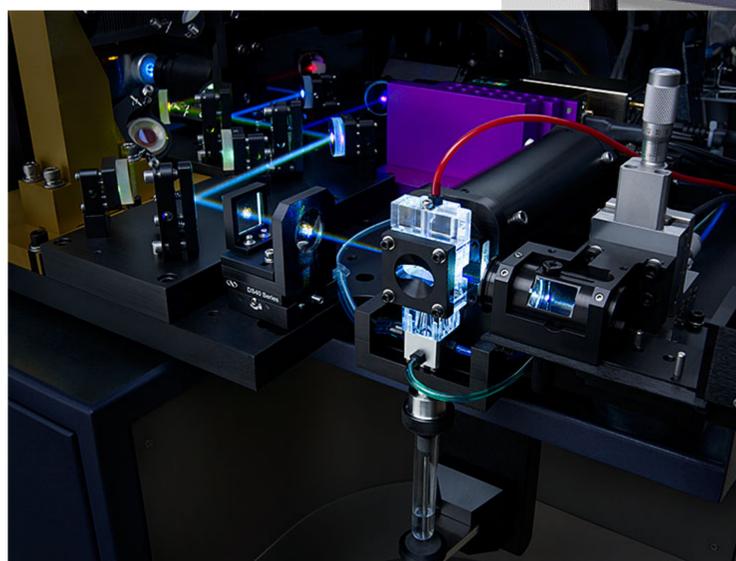
What can we Sort?

Nuclei from neurons, tumor cells, coral cells, immune cell populations, transfected cells, delicate cells, and human clinical samples.

We can do it at 10,000 events per second!

Major Equipment & Services

Cell Analysis



Cell Analysis

5 lasers- up to 22 parameters , 18 fluorescent markers

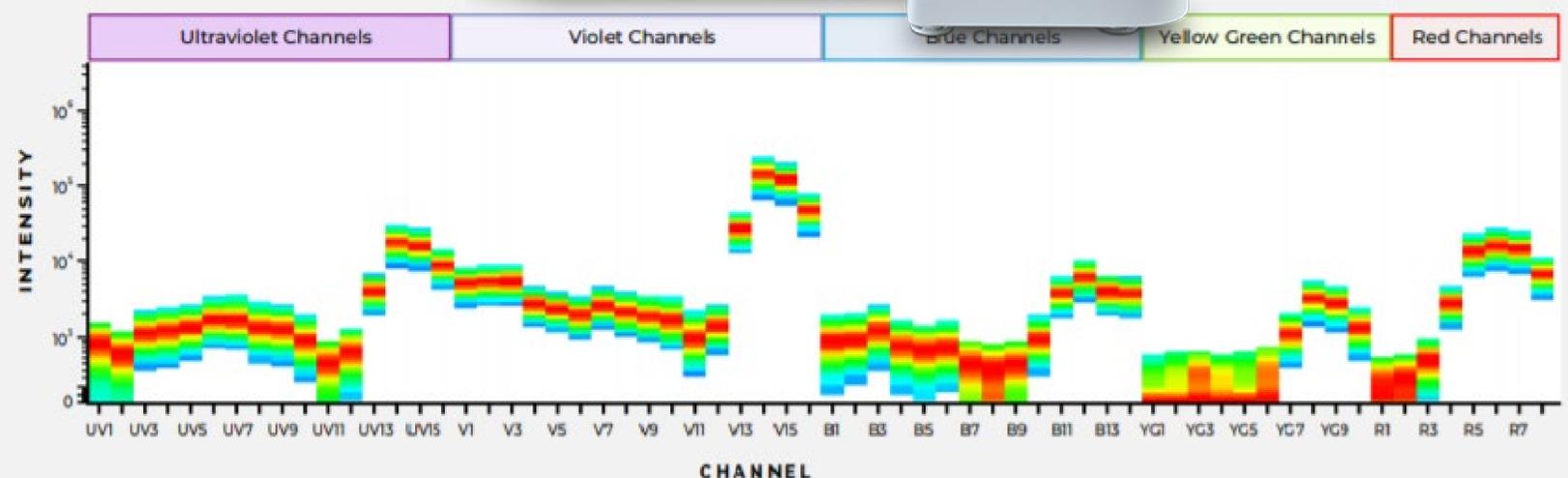
- This instrument is capable of multiparameter traditional flow cytometry
- Can visualize all types of cells, including bacteria flow

We can examine

- Cell surface markers-receptors, ligands
- Cytoplasmic markers-cytokine release
- Nuclear markers-transcription factors
- Secreted Cytokines using multiplex assays
- Phospho flow, if that western blot isn't working for you and you can't get a pure population, Phospho flow normalized it per cell in a mixed population.
- Calcium signaling
- Cellular Metabolism- Fatty Acid uptake or Glucose uptake
- Proliferation, Apoptosis, Cell Cycle and more!

Major Equipment & Services

Cell Analysis Cytek Aurora



Cell Analysis

5 lasers- up to 44 parameters , 40 fluorescent markers (we have performed assays of 36 parameters here at VCU!)

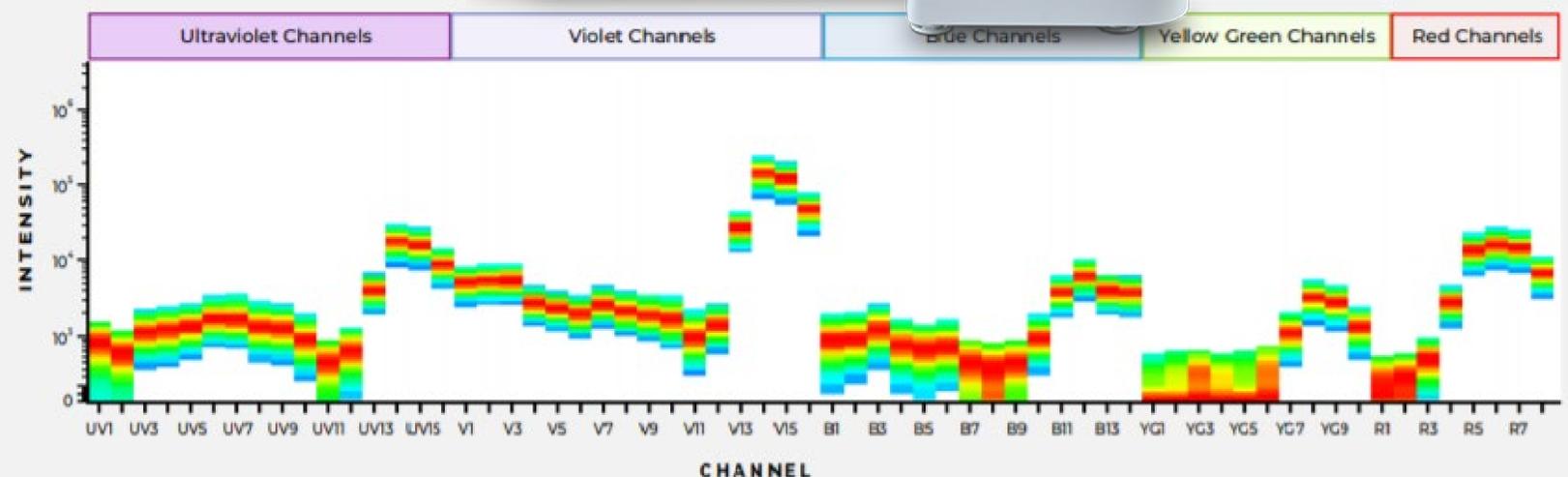
- This instrument is cutting edge spectral flow cytometry and becoming the new standard

We can examine

- Cell surface markers-receptors, ligands
- Cytoplasmic markers-cytokine release
- Nuclear markers-transcription factors
- Secreted Cytokines using multiplex assays
- Phospho flow, if that western blot isn't working for you and you can't get a pure population, Phospho flow normalized it per cell in a mixed population.
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Major Equipment & Services

Cell Analysis Cytek Aurora



Cell Analysis

5 lasers- up to 44 parameters , 40 fluorescent markers (we have performed assays of 36 parameters here at VCU!)

- Why is it better and what can it do for the user?
- It uses new technology that visualizes each dye across the spectrum, allowing a fingerprint to be used to detect the dye.
- Auto fluorescent cells or auto fluorescent drugs can be visualized as if it were a marker
- It can resolve unique dyes
- It has special lasers that can resolve very tiny particles from the background. Exosome flow and Viral flow is possible on the machine

The FCSR, Moving Research Forward at VCU-Cytek Aurora

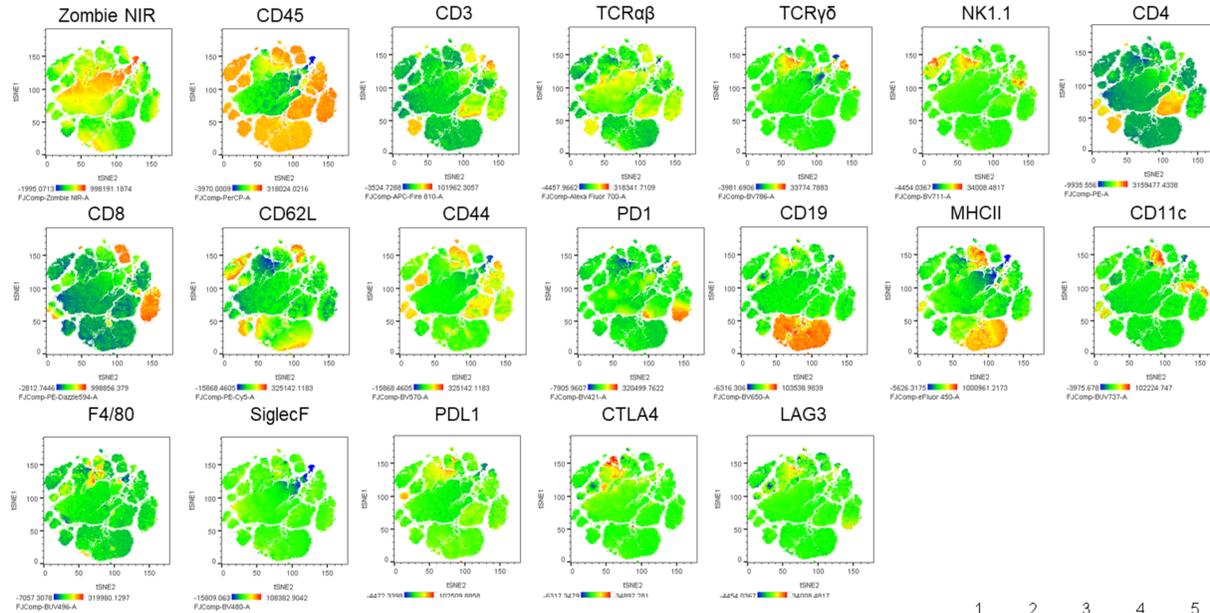
High Dimensional Spectral Flow

- Unsupervised Flow Analysis
- Full immuno-profiling in mouse and human cancers
 - Plate-based drug screens
- Large plate-based assays run as fully automated drug screens. No need to mind instrumentation.
- Easily visualized to determine viability or other surface marker changes with whole-plate heat maps.
- 96-wells run in about an hour. Visual analysis in about 10 minutes.

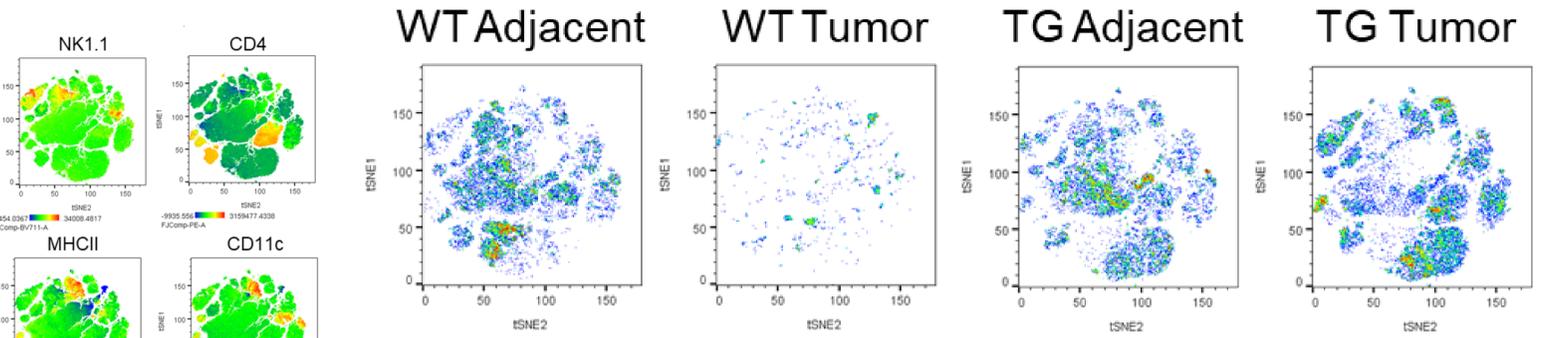
Data Analysis and Consultation

Traditional data analysis and figure preparation

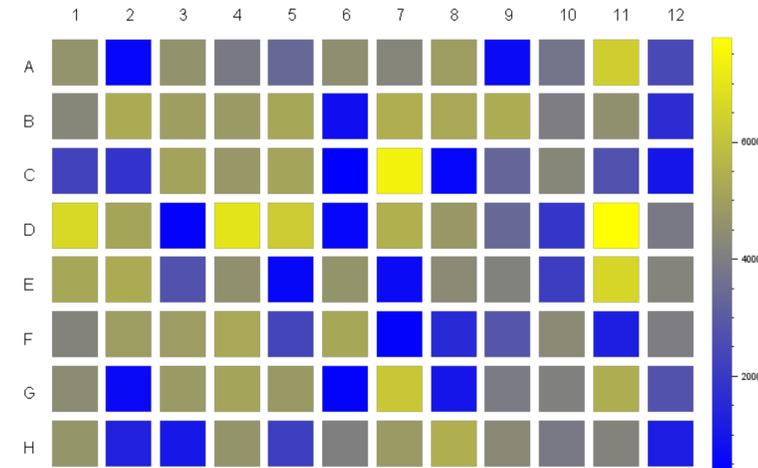
Unbiased data analysis, TSNE and UMAP preparation



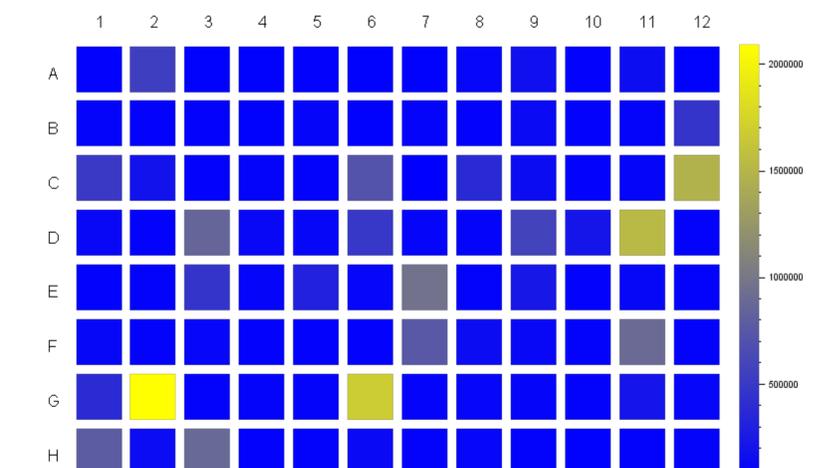
**Plate-based drug screens
(Viability and Surface Expression of a marker of interest on the same 96-well plate side-by-side)**



Unsupervised Flow Analysis of Immune Populations in the Tumor and Adjacent Tissue (25 Parameter Analysis)



Sample Group: All Samples
Statistic: Lymphocytes/Single Cells/Median : Comp-PE-A
:: heatmap range = 38800 - 7.81E5
:: displayed as Heatmap



Sample Group: All Samples
Statistic: Lymphocytes/Single Cells/Median : Comp-Zombie Aqua-A
:: heatmap range = 53477 - 2.10E6
:: displayed as Heatmap

Major Equipment & Services

Cell Analysis



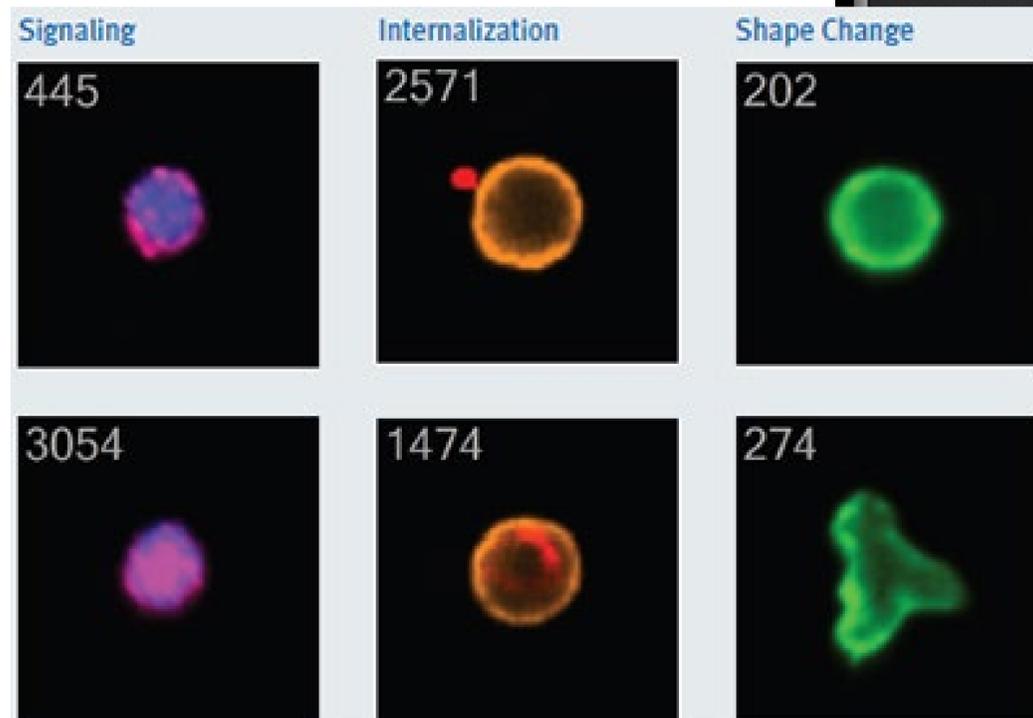
Cell Analysis

Amnis ImageStream X Mk II Imaging Cytometer

- 405 nm, 488 nm, 561 nm, 642 nm- 4 lasers and brightfield
- This instrument is a microscope and a flow cytometer
- It gives you quality pictures (60x) of each cell and quantitative data too!

We can examine

- Cell internalization, cellular compartment co-localization, or nuclear localization, and shape changes.



Goals

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- Train faculty, staff, and trainees of all levels on the use of the instruments within the FCSR and provide education on flow cytometry theory, data analysis methods, and software

Major Equipment & Services

FCSR Instrumentation

BD FACSAria Fusion SORP High-Speed Cell Sorter (Q1 2018)

- 355 nm, 405 nm, 488 nm, 561 nm, 640 nm
- Up to 22 parameters analyzed simultaneously

BD Aria II sorter High-Speed Cell Sorter

- 405 nm, 488 nm, and 640 nm
- Up to 13 parameters analyzed simultaneously

BD LSRFortessa-X20 cell Analyzer (Q1 2017)

- 355 nm, 405 nm, 488 nm, 561 nm, 640 nm
- Up to 22 parameters analyzed simultaneously

BD FACSCanto II Analyzer

- 488 nm, 633 nm
- Up to 11 parameters analyzed simultaneously

Amnis ImageStream X Mk II Imaging Cytometer

- 405 nm, 488 nm, 561 nm, 642 nm

*New Cytex Aurora Spectral cell Analyzer (Q1 2021)

- 355nm, 405nm, 488nm, 561nm, 640nm
- Up to and beyond 64 parameters analyzed simultaneously

Reichert SR7500DC SPR

- 2-channels, monitors label-free ligand-receptor interaction



FCSR Services

Cell Analysis

- Multiparameter, simultaneous detection of up to 22 parameters, 18 fluorescent markers used to examine cell surface, internal, and nuclear antigens with traditional flow cytometry
- Spectral flow cytometry of 64 parameters, and over 38 fluorescent markers performed currently in-house to examine cell surface, internal, and nuclear antigens with normal and plate-based acquisition.
- DNA and cell cycle analysis
- Internal free Ca²⁺ measurement
- Cell viability/cytotoxicity
- Mitochondrial staining
- Multiple cytokine/chemokine analysis in serum/cell supernatants using multiplex assays
- Image flow cytometry for internalization, cellular compartment co-localization, or nuclear localization

Data Analysis and Consultation

- Traditional data analysis and figure preparation
- Unbiased data analysis, TSNE and UMAP preparation
- Troubleshooting and experimental design consultation
- One-on-one training in data analysis or instrument operation