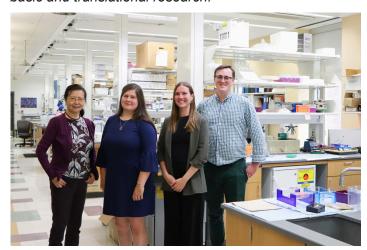




Flow Cytometry Shared Resource

The FCSR provides access to state-of-the-art instrumentation in a cost-effective manner and to deliver skilled technical assistance, training and support to Massey researchers in basic and translational research.



Welcome to the latest update from the VCU Flow Cytometry Shared Resource (FCSR)! We're excited to share some groundbreaking advancements and expanded capabilities designed to enhance the services we provide to the VCU research teams we currently serve, as well as expand our portfolio to working on future projects and with more researchers!

FCSR team

This newsletter highlights our new and emerging services, including the integration of spectral cytometers for high-parameter staining and cell sorting. This new technology features a specialized spectral flow cytometer dedicated to nano flow cytometry, enabling in-depth analysis of viruses, bacteria, small particles and extracellular vesicles (EVs).

Building on the success of our "Mouse Immunophenotyping Panel," we're also thrilled to introduce a new, comprehensive 23-parameter "Human Immunophenotyping Panel," complete with expert training on data acquisition and analysis to empower your studies.

Read on to learn more about what's new and exciting with the FCSR. We're eager to collaborate with you!

Sincerely, Rebecca K. Martin, Ph.D. Flow Cytometry Shared Resource Director

About the Core

The FCSR provides access to state-of-the-art instrumentation in a cost-effective manner and delivers skilled technical assistance, training and support to researchers in basic and clinical research.



A wide range of services are available, from routine cell sorting, imaging flow cytometry and fluorescence analysis to custom design of complex staining panels that address the specific needs of individual investigators. The resource manager closely follows the analyses performed by the core facility, ensuring quality control and advising facility users on approaches to experimental design and data analysis.

This shared resource maintains a stable base of instrumentation, expertise and trained personnel to provide services and training for multiple users.

Services

Flow cytometry analysis

- Apoptosis assays
- DNA and cell cycle analysis
- Cell viability/cytotoxicity assays
- Cells ranging from bacterial cells to muscle cells can be analyzed
- Particles, exosomes and extracellular vesicles can be analyzed down to 80nm
- Isolated mitochondrial assessment with enumeration and membrane potential
- Internal free Ca2+ measurement
- Examination of cell surface antigens, internal antigens and nuclear antigens
- Multiplex cytokine/chemokine assay analysis
- Phospho-flow cytometry
- Multi-parameter analysis that extends up to 22-parameters, with 5-lasers
- Multi-parameter spectral analysis that extends beyond 43-parameters, with 5lasers
- 96-well plate loader automation
- Image cytometry analysis that extends up to 6-parameters, with 4-lasers
- Image cytometry for internalization, cellular compartment co-localization or nuclear localization

High speed cell sorting

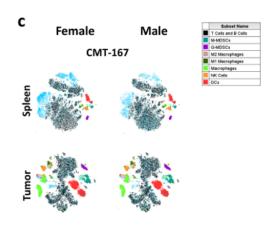
- Sorting of viable cell under sterile BSL2 conditions
- Single-cell sorting into 96-well plates
- Sorting of cells extends up to 20parameters, 4-ways, with 5 lasers with traditional cell sorting
- Sorting of cells extends up to 44 plus parameters, 6 ways, with 5 lasers with spectral cell sorting

Data analysis and consultation

- FlowJo software (most up to date version) available for annual license purchase or on-site usage for data analysis
- FCSExpress v5 available for remote and on-site usage for data analysis
- Data Analysis and figure preparation services
- Troubleshooting and experimental design consultation
- One-on-one training in data analysis or instrument operation
- Resources available to all users on the Flow Cytometry Shared Resource Server under the "Current Protocols" folder include:
 - Data analysis help, links to videos for instrument operation, FCSR basics course video, an extensive experimental protocol library and troubleshooting guides

FCSR Updates and News

Multiplexing Cytokines and Chemokines with Flow Cytometry: The Cytek Aurora system offers a streamlined approach to biological assays through its integrated 96-well plate loaders. This automation significantly boosts the efficiency of high-throughput screening. A key application of this technology is its compatibility with Biolegend's LegendPlex assay, enabling the simultaneous detection of up to 14 distinct analytes, such as cytokines or chemokines, from a single sample. This capability provides a comprehensive analysis of multiple biomarkers, offering valuable insights in various research fields.



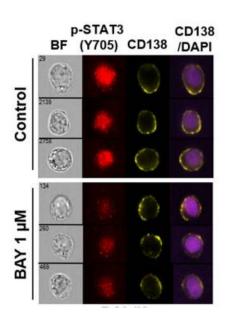
Cytek Imagestream Flow Cytometer: The ImageStreamX Mark II is a sophisticated instrument that, while less commonly known, functions similarly to a traditional fluorescent microscope. However, its key advantage lies in its ability to quantify fluorescence with high precision. This quantitative capability makes it exceptionally well-suited for experiments investigating dynamic cellular processes such as protein phosphorylation, receptor internalization, and molecular co-localization.

For example, research from Steven Grant's, M.D., lab has utilized this instrument to demonstrate the phosphorylation of the STAT3 pathway, as published in a peer-reviewed journal. This illustrates the ImageStreamX Mark II's power in providing quantifiable insights into complex biological mechanisms.



Data Analysis: FCSR provides comprehensive training in both fundamental and advanced data analysis techniques using FlowJo software. Services extend to performing in-depth data analysis for researchers, which includes the preparation of publication-ready figures and the generation of complex visualizations such as t-distributed stochastic neighbor embedding (tSNE) and Uniform Manifold Approximation and Projection (UMAP) plots.

For instance, an exemplary tSNE analysis was conducted by Lauren May, a former Ph.D. student of Joseph Landry, Ph.D., and was subsequently published in the journal Cancer Research.



Upcoming Events

Saturday, July 19 from 9:30 a.m. - 2 p.m.: Closing the Gap: Coping with Cancer Summit Tuesday, Aug. 5 from 10 - 11 a.m.: Flow Cytometry Shared Resource Walk-in Clinic Tuesday, Sept. 2 from 10 - 11 a.m.: Flow Cytometry Shared Resource Walk-in Clinic

Have questions about data analysis? The monthly Flow Data Office Hours walk-in clinic to discuss general tips and best practices for flow cytometry data analysis including:

- Software Assistance: Learn the ins and outs of analysis software like FlowJo, SpectraFlow and/or IDEAS
- Figure Presentation: Assistance in designing figures for publications
- Basic and High-Dimensional Analysis: Help with anything from basic gating strategies, MFI and cell cycle analysis to complex data analysis like U-MAP and tSNE plots

The walk-in clinic is available for all Massey and non-Massey VCU affiliates.

Questions? Contact Alex Wendling at wendlinga@vcu.edu.

FCSR Researcher Highlight

The Landry Lab investigates the crucial functions of novel chromatin regulators in both healthy development and cancer progression. This research employs a combination of genetic, molecular and biochemical approaches, primarily utilizing mouse models and tissue culture systems.

Through utilizing the Flow Cytometry Shared Resource, Dr. Landry was able to secure significant funding to continue studying the interface between epigenetics, cancer therapies and tumor immunology.

"The flow core team has been very helpful in the development of cytometry and sorting experiments for all of my funded projects. Topnotch facilities, training and analysis support. Everyone from Rebecca to XinYan, Madison, and Alex are very helpful."

Publications of this research:

- pubmed.ncbi.nlm.nih.gov/39312191/
- pubmed.ncbi.nlm.nih.gov/33811160/
- www.biorxiv.org/content/10.
 1101/2022.07.15.500220v1



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Flow Cytometry Shared Resource

Molecular Medicine Research Building (MMRB): Room 4-001 and 4-005 Card Access will be granted after instrument training.