Flow Cytometry Core Facility (FCCF)

Science Down to the Single Cell

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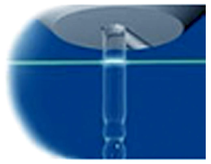


Introduction

The Flow Cytometry Core Facility (FCCF), located in Pinn Hall (Room 2011 and 2013), provides all investigators at the University of Virginia access to high quality, cost effective flow cytometry services. By providing these services, as well as the scientific expertise necessary to effectively use this technology, the facility serves to enhance the scope and quality of scientific research performed at the University.

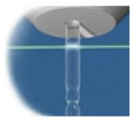
The FCCF works closely with investigators to identify their research needs, develop an appropriate flow cytometric approach, assist in the implementation of the experimental design, and guide in the data analysis and presentation of the data. The staff of the FCCF consider themselves **"partners in your single cell analysis needs"**. We provide a wide variety of tools to support everything from the simplest to the most complex of single cell studies.

Services

- **Consultative Services**
  - Experimental Design
  - Data Analysis
  - Panel Design
  - Data Presentation
  - Letters of Support

- **Educational Services**
  - Full week course quarterly
  - One-on-One training
  - Seminars
  - Workshops

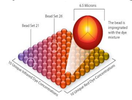
- **Cell Sorting**
  - Magnetic antibody cell sorting
  - 4-6 way population sorting
  - Single cell sorting
  - BSL1-2+ sorting
  - Sorting for RNA



- **Conventional analytical benchtops**
  - Assisted acquisition
  - Trained unassisted acquisition
  - 96 well plate acquisition
  - 1-17 colors; 2-4 lasers

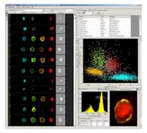
Services (cont.)

- **Luminex (Multiplex bead assays)**
  - Cytokine, Chemokine, Apoptosis, Cell signaling multiplex assays
  - MagPix training
  - Assay performance



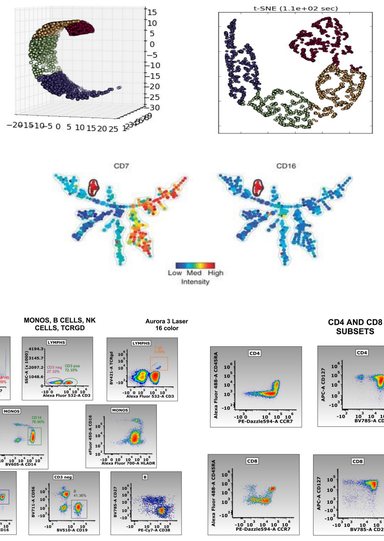
- **Mass Cytometry (CyTOF)**
  - Panel design
  - Metal conjugated antibody bank
  - Metal tag Conjugation services
  - Data acquisition

- **Imaging Flow Cytometry (ImagestreamX MKII)**
  - Panel/experimental design
  - Assisted/unassisted acquisition
  - Data analysis
  - Training



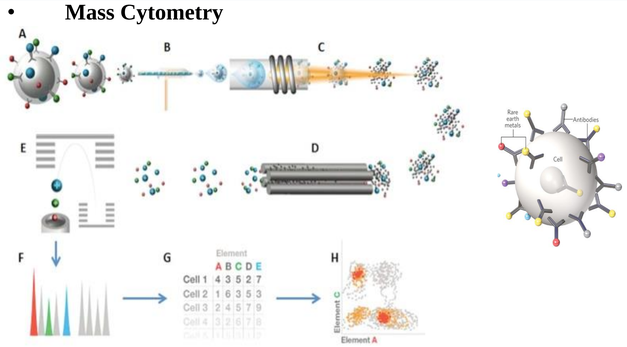
- **Spectral Flow Cytometer (Aurora)**
  - Panel/experimental design
  - Assisted/unassisted acquisition
  - Training
  - >20 colors

- **Data Analysis**
  - FCSExpress software site license
  - Assisted data analysis
  - Computational and Bioinformatics service (Astrolabe Diagnostics)
  - Software training/educational seminars



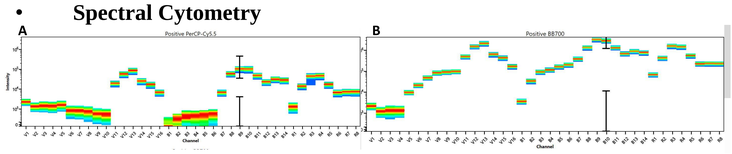
Technology Highlights: High Dimensional Cytometry

- **Mass Cytometry**



A liquid sample containing cells labeled with antibodies tagged with heavy metal isotope probes **(A)** are introduced into a nebulizer creating an aerosol **(B)** which is directed towards a plasma torch **(C)** where the cells are vaporized. Low mass ions are removed **(D)**, and the ion cloud enters the TOF chamber where probes are separated based on their mass to charge ratio as they accelerate towards the detector **(E)**. The time-resolved detector measures a mass spectrum that represents the identity and quantity of each isotopic probe on a per-cell basis **(F)**. Data is generated in FCS format **(G)** and can be analyzed using third party software **(H)**.

- **Spectral Cytometry**



Evaluating fluorescence across the entire spectrum, from 400-850nm, allows the use of fluorochromes that normally would not be usable together with a conventional flow cytometer. In the example above, PerCP-CY5.5 **(A)** and BB700 **(B)** in a conventional cytometer would be detected in the same channel and therefore could not be used in the same panel. With spectral cytometry, the fluorescence fingerprint across the entire spectrum of these two fluors are different enough to be unmixed. The power of spectral of unmixing opens many new possibilities of fluorescent probe combinations and panel expansions to >20 colors with just three lasers! Similarly, closely related fluorescent proteins, such as YFP and GFP, are now usable together **(C)**

C

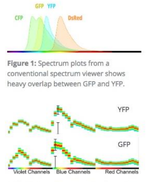


Figure 1: Spectrum plots from a conventional spectrum viewer shows heavy overlap between GFP and YFP.

Figure 2: Spectrum plots from Aurora show distinct signatures across three lasers.

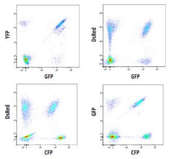
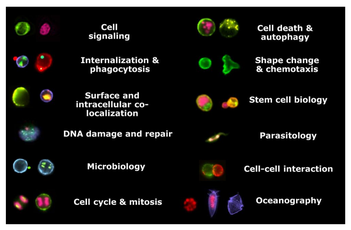
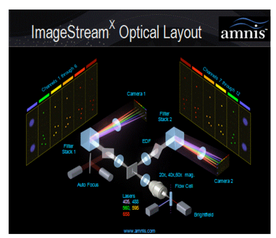


Figure 3: Sp2/0 cells were transfected with GFP, YFP, CFP and/or DsRed (alone or in combination) and run on the Aurora (plots are gated on FSC vs SSC). Each population is clearly identified.

- **Imaging Flow Cytometry**





(Left) Examples of applications enabled by this powerful technology. **(Right) How it works: ImageStream^X Layout:** The fluidics work in a similar way to all flow cytometers with a sheath stream to focus the cells before they pass through the lasers. The fluorescence and brightfield images are separated on to the detector camera. In order to create a higher image contrast the cells are tracked pixel by pixel down the detector surface and reconstructed by the software (Inspire).

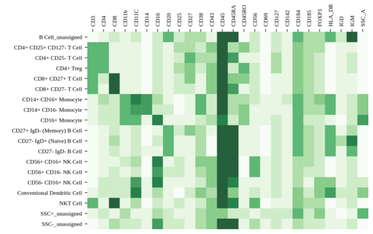
Specialty Services

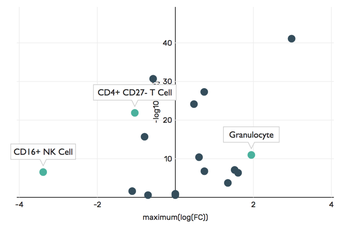
- **Antibody Conjugation Services**
  - Fluorochrome conjugation
  - Metal isotope conjugation (CyTOF)

- **Mass Cytometry Antibody Bank**
  - Metal tagged antibodies
    - mouse and human
  - Metal conjugation kits
  - DNA intercalators
  - Viability reagents
  - Crowd sourced or direct purchase

- **Full Service CyTOF**
  - Panel design
  - Sample labeling
  - Data acquisition
  - Data analysis

- **Computational Analysis**
  - Flow and mass cytometry (CyTOF)





Expertise

- **Laboratory Director:** Joanne Lannigan, M.S., DLM, QCym (ASCP); >35 years experience
- **Laboratory Manager:** Michael Solga, M.S., SCym (ASCP); >20 years experience
- **Senior Laboratory Specialist:** Claude Chew, B.S., SCym (ASCP); >10 years experience
- **Laboratory Specialist:** Alex Wendling, B.S., SCym (ASCP); >8 years experience
- **Administrative Coordinator:** Lesa Campbell, A.A.S.; >9 years experience

For more information visit our website: https://med.virginia.edu/flow-cytometry-facility/ Follow us on Facebook: @uvaflow or email us: fccf-staff2@virginia.edu Call us: 434-243-2711