Flow Cytometry Core: What's New in Cytometry??

Joanne Lannigan, M.S[1]., Michael Solga, M.S[2]., Claude Chew, B.S[2]., Brian Capaldo, Ph.D.[2], Lesa Campbell, A.A.S.[2], Timothy Bender, Ph.D.[1]

University of Virginia, School of Medicine, [1]Department of Microbiology, Immunology & Cancer Biology, [2]Office of Research Core Administration

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.

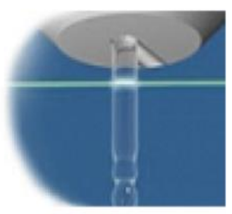
The FCCF works closely with investigators to identify their research needs, develop an appropriate flow cytometric approach, assist in the implementation of the experimental design, and guide in the data analysis and presentation of the data. The staff of the FCCF consider themselves **"partners in your 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

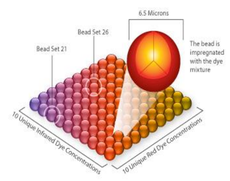
- **Cell Sorting**
  - Magnetic antibody cell sorting
  - 4-6 way population sorting
  - Single cell sorting
  - BSL1-2+ sorting
  - Sorting for RNA



- **Analytical benchtops**
  - Assisted acquisition
  - Trained unassisted acquisition
  - 96 well plate acquisition

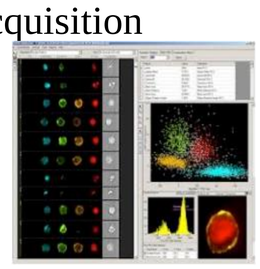
Services (cont.)

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

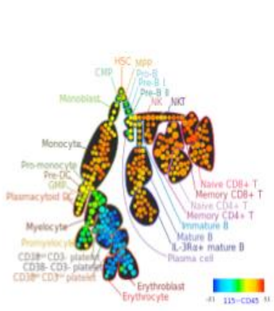


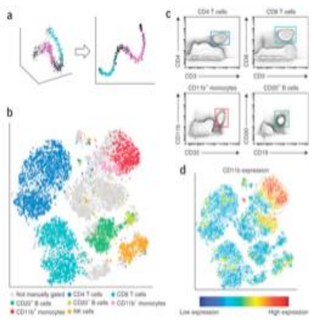
- **Mass Cytometry (CyTOF)**
  - Panel design
  - Metal conjugated antibody bank
  - Metal tag Conjugation services
  - Data acquisition

- **Imaging Flow Cytometry**
  - Panel/experimental design
  - Assisted/unassisted acquisition
  - Data analysis
  - Training



- **Data Analysis**
  - FlowJo, FCSExpress software site license
  - Assisted data analysis
  - Computational and Bioinformatics service
  - Software training

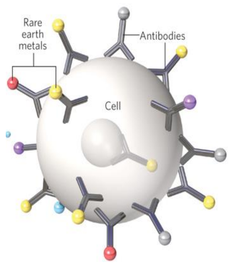




Specialty Services

- **Antibody Conjugation Services**
  - Fluorochrome conjugation
  - Metal isotope conjugation (mass cytometry)

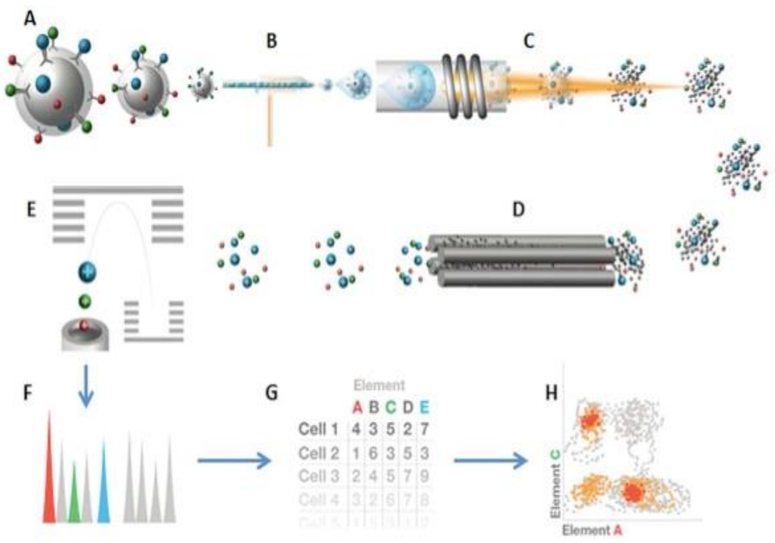
- **Mass Cytometry Antibody Bank**
  - Metal tagged antibodies
    - mouse and human
  - Metal conjugation kits
  - DNA intercalators
  - Viability reagents
  - Crowd sourced or direct purchase



- **Computational Analysis**
  - Flow and mass cytometry (CyTOF)
  - Proteomics/Genomics

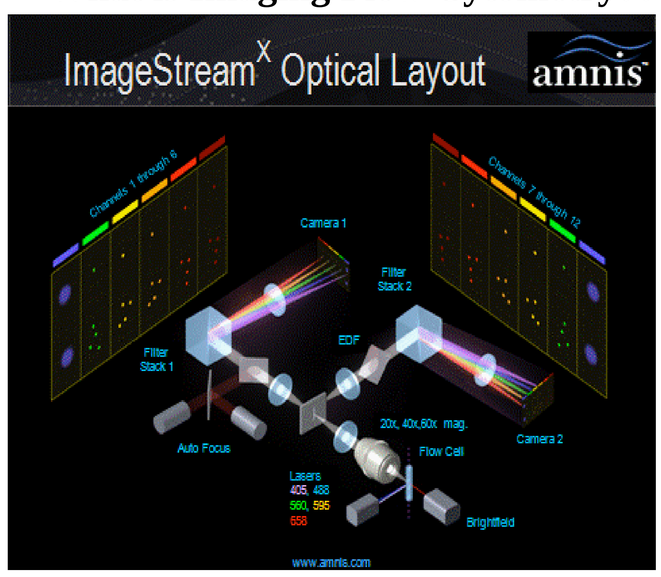
Technology Highlights

- **What is 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)**.

- **What is Imaging Flow Cytometry??**



ImageStream^X Layout: The fluidics work in a similar way to all flow cytometer's with a sheath stream to focus the cells before they pass through the lasers. The fluroscence 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).

Expertise

- **Laboratory Director:** Joanne Lannigan, M.S., ASCP (DLM), (Qcym); 35 years experience
- **Laboratory Manager:** Michael Solga, M.S., CCy; 20 years experience
- **Laboratory Specialist:** Claude Chew, B.S., CCy; 10 years experience
- **Computational Analyst:** Brian Capaldo, Ph.D.; 5 years experience
- **Administrative Coordinator:** Lesa Campbell, A.A.S.; 9 years experience