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Facility Manager: Geoffrey Lyon

# Yale Flow Cytometry Core

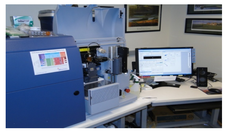
## Purpose

To provide comprehensive cytoflourometric analysis and sorting to the YCC investigators. The facility not only provides, maintains, and operates the instruments, but also trains users, develops techniques, provides protocols, and manages access and the financial aspects of the Shared Resource

## Key Services

- Multiparameter flow cytometric analysis (user operated)
- Multiparameter fluorescence activated high speed cell sorting (operator-assisted)
- Training in the use of FACS analyzers and the FACSAria sorters.
- Consultation on experimental design and interpretation.
- Data analysis stations with state-of-the-art analysis software

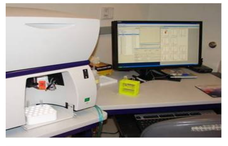
## Major Equipment and Locations



**BD FACS Arias**
•Locations - 300 George St. 2320H, Amistad 416, and TAC 617 and 633
•Temperature controlled sorting
•Tube, Index-sorting, and Slide or Plate-sorting
•70, 85, 100, 130-micron nozzles

**FACS Aria Sorters in Bio-safety Cabinets**
•Locations - LEPH 901B and Amistad 416
•Sorting Unscreened Human Material
•LEPH: BSL-2 and BSL-3 Pathogen Sorting

**MoFlo**
•Location – TAC S617
•3-laser, 8-compensated color sorting
•Tube, slide, or plate sorting
•Sorting Concentrations up to 6x10^7



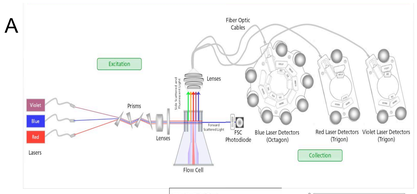
**LSRII**
•Locations – 300 George St. 2320H, Amistad 416, and TAC 533 and 613
•3, 4, and 5 laser systems
•12 to 18-PMTs for fluorescence detection

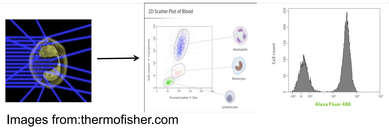
**Stratedigm**
•Locations – TAC S533 and S613
•3 and 4 lasers systems
•8 or 13 PMTs for fluorescence detection
•Plate loader acquisition capability

**Amnis**
•Location – TAC S613
•Rapid acquisition of flow cytometric data and high resolution images of individual cells
•20x, 40x, and 60x objectives

## Fluorophore Detection

A




Images from:thermofisher.com

B

| Lasers | Fluorochromes |
|---|---|
| 355nm UV Lasers | BUV 395, BUV 737, DAPI, AF350, |
| 405nm Violet Lasers | BV421, BV711, BV605, AF405, AF430, AF605, QDot605, CFP, AmCyan, Pacific Orange, Cascade Blue, Pacific Blue, Sky Blue, BFP, CFP, Hoechst |
| 488nm Blue Lasers | PerCP, EMA, PercP-Cy5.5, FITC, GFP, PKH67, CFSE, AF488, YFP* |
| 532nm Green Laser | PE-Cy7, PE-AF750, PE-Cy5.5, PE-Cy5, PE-AF647, AF568*, AF594*, PE-Texas Red, PI, PI, PE, DsRed, RFP, Pyronin-Y, PKH26 |
| 640nm Red Laser | AF647, AF750*, AF680, AF700, APC, APC-Cy7 |

C

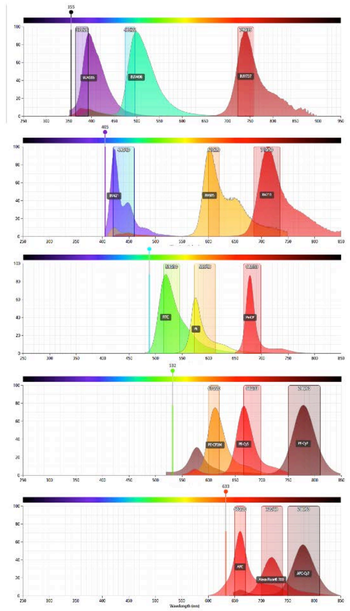
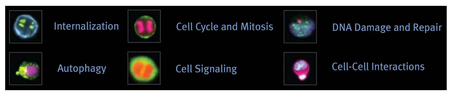


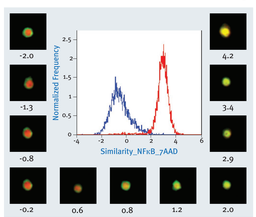

Fig 1. (A) Optical path of a standard flow cytomer, allowing for detection of distinct populations based off FSC, SSC and fluorescence, (B) List of common fluorochromes and associated laser best suited for their excitation, (C) Emission spectrum and detection channels of fluorochoromes excited sequentially by lasers (bottom to top) 355nm, 488nm laser, 407nm laser, 532nm laser, and 633nm laser.

## Amnis Imagestream

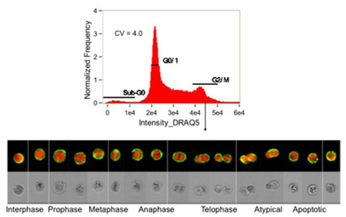
**Amnis Imagestream Imaging Cytometer**
- 20x, 40x, 60x objectives
- Violet, Blue, Green, and Red lasers
- Staff support for data acquisition and analysis
- Up to 5000 cells/second with real-time compensation
- Intuitive image analysis that incorporates traditional flow cytometry gating




A. Nuclear translocation during cell signaling




B. DNA content and cell cycle analysis




C. Analysis of DNA fragmentation

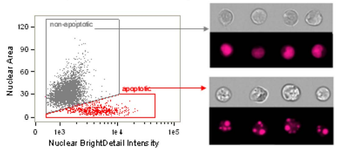
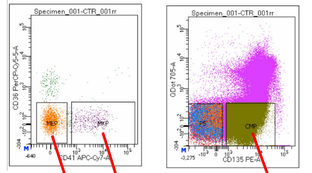



Fig 2. (A) Nuclear translocation of NFkB in response to LPS. Treated (red) and control cells (blue) were stained for NFkB and the DNA binding dye 7AAD. Extent of fluorescence overlap of the two channels is expressed as a similarity score. (B) Cell cycle analysis. In addition to DNA content, the size and aspect ratio of each cell can also be quantified. Images of individual cells within a gate can be further examined. (C) Analysis of DNA fragmentation. Cell features determined by image analysis can also be plotted against other analytic features. In this example, the nuclear area is plotted against the fluorescent intensities of brighter punctate spots within that nucleus.

## Specialty Sorting

The FCSR cells sorters have a variety of special
Features not realized by many users:

- Temperature Controlled collection (cooling or heated collection)
- Plate, Eppendorf, and slide deposition
- Single-cell plate sorting
- Amplifying sort collection
- Multiple nozzle options to accommodate a cells from 0.5 to 65-microns using sheath pressures from 5 to 70+ PSI

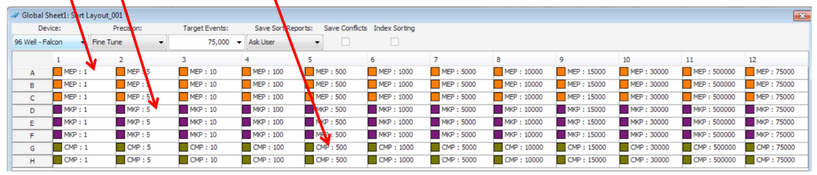




Fig 3. (Above) – Various sort collection devices for the BD FACS Aria; Eppendorf, 5-ml, and 15-ml collection holders; 96-well and slide collection holders. (Below) 96-well plate sort layout. This sort is for a graduated collection of larger numbers of gated cells. The plate was segmented by three populations (MEP, MKP, and CMP cells) and collected by increasing numbers of cells per well from a single cell to 75,000 per well.

## Index Sorting

Index sorting provides sort information on event-by-event basis. The index sort mode creates an FCS file containing data on the X and Y coordinates of the cells that can be traced back to the flow characteristics of the cell or combination of cells sorted, providing a complete flow phenotype for every sorted cell. Index sorting can be an important tool in stem cell sorting, clonal selection, single-cell sequencing, and drug testing.

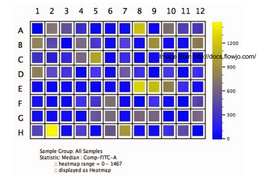




Fig 4. (Above) – An index sort layout of single cells deposited in individual wells. Fluorescent intensity information is retained, allowing for back-gating later. (Left) – A Flowjo derived heat map that displays values based on a yellow-blue gradient.

## Remote Data Access

- A data mirror system allows users to access their exported data remotely. A server synchronizes with the .fcs export folder of each of the LSRII computers daily. Copies of the export folders are created each morning between 4-6 am. The data is then accessible for users to retrieve remotely from the server or from our stand alone computers workstations outside of TAC S613.

- It is also possible to export data within Diva software at a hallway workstation. Sample acquisition can take longer than expected, so when time is short at the cytomer, data export can now be completed away from the cytometer. This also obviates the need to reserve more time on the equipment later simply to export your data.