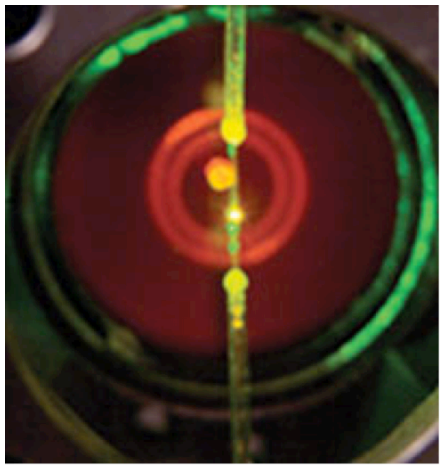
Measuring Cell-to-Cell variability with Flow Cytometry and Fluorescence-Activated Cell Sorting



Q-Bio Summer Lecture Series
August 3, 2010
Babetta L. Marrone
Director, NIH-National Flow Cytometry Resource
Los Alamos National Laboratory

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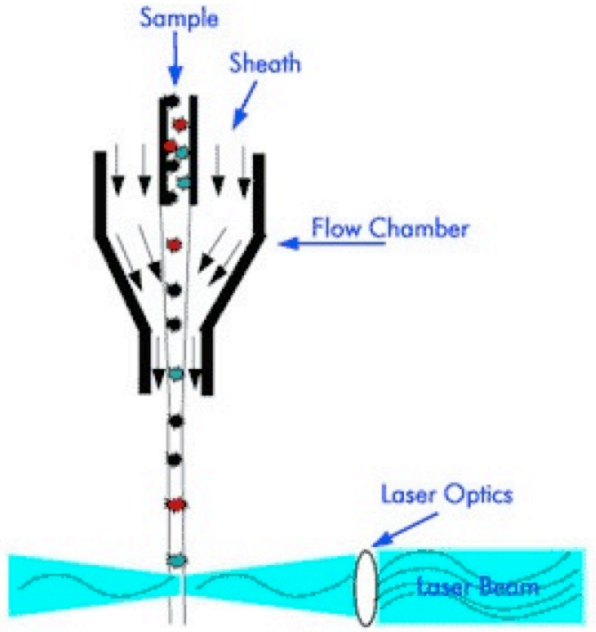
Definition

- Flow cytometry (abbreviated: FCM) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus
  - It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second
  - Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both research and clinical practice
  - A common variation is to physically sort particles based on their properties, so as to purify populations of interest.
    - *From Wikipedia, the free encyclopedia*

How it Works: Fluidics

- Flow cytometry uses the principles of light scattering , light excitation, and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5um to 40um+ diameter.
- Cells are focused before intercepting an optimally focused light source. Lasers are most often used as a light source in flow cytometry.
- Flow cytometers use the principle of hydrodynamic focusing for presenting cells to a laser. The sample is injected into the center of a sheath flow. The combined flow is reduced in diameter, forcing the cells into the center of the stream. This allows cells to pass through the laser beam one cell at a time.



http://biology.berkeley.edu/crl/flow_cytometry_basic.html

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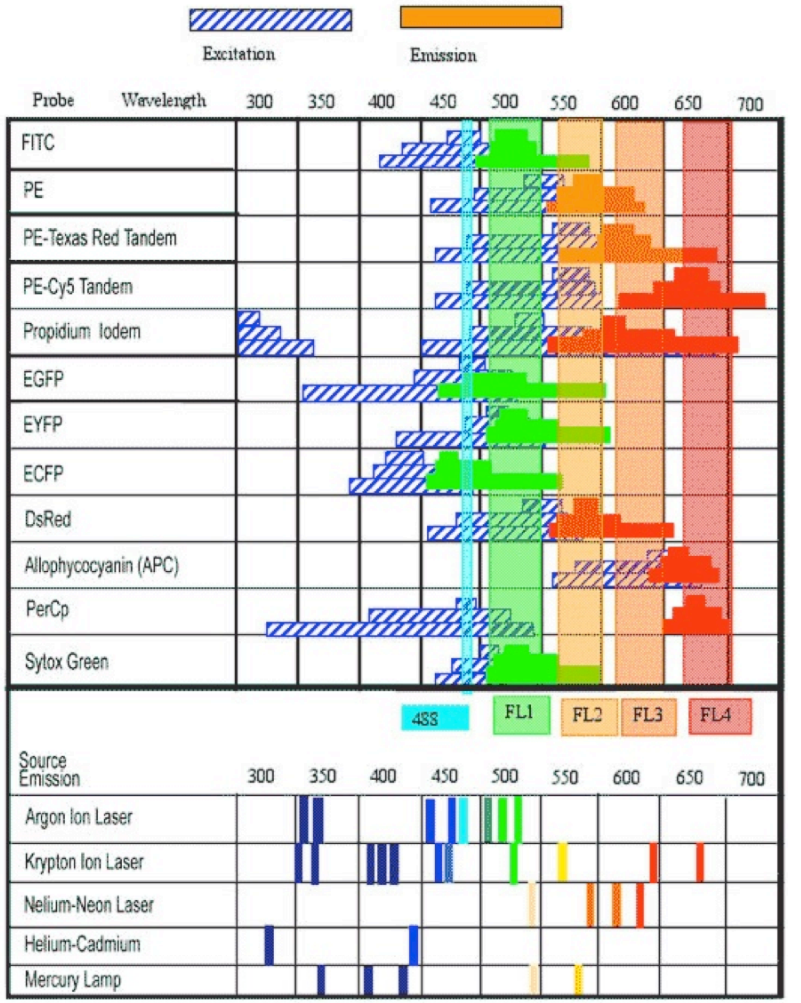
Fluorescence Detection

- As the cells or particles intercept the light source they scatter light and fluorochromes are excited to a higher energy state. This energy is released as a photon of light with specific spectral properties unique to different fluorochromes.

- The Table has a listing of commonly used fluorescent dyes and their excitation and emission spectra. This table also includes the most common laser light sources with their multiple lines of emission.



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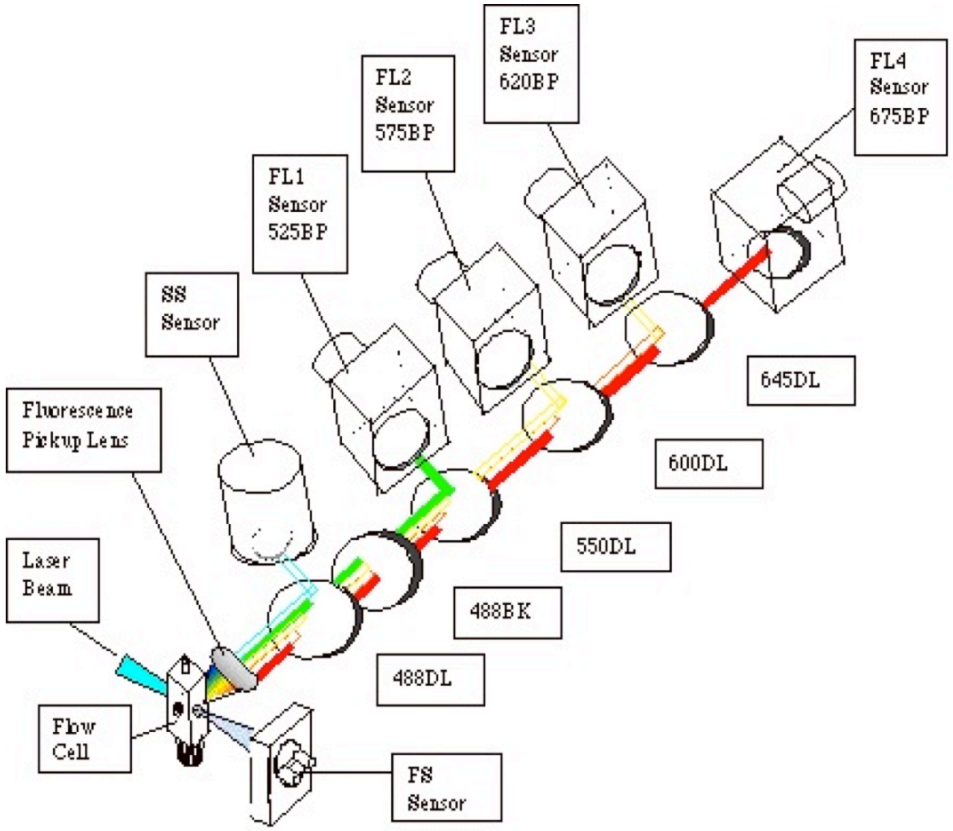
Optics

- Scattered and emitted light from cells and particles are converted to electrical pulses by optical detectors.
- Light is sent to different detectors by using optical filters. For example, a 525 nm band pass filter placed in the light path prior to the detector will only allow "green" light into the detector.
- The most common type of detector used in flow cytometry is the photomultiplier tube (PMT).
- This figure shows a basic layout of the optical components in a flow cytometer.



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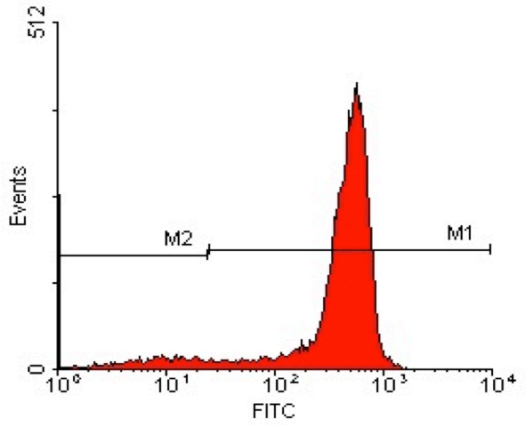
Data Processing

- The electrical pulses originating from light detected by the PMTs are then processed by a series of linear and log amplifiers.
- Logarithmic amplification is most often used to measure fluorescence in cells. This type of amplification expands the scale for weak signals and compresses the scale for “strong” or specific fluorescence signals.
- After the different signals or pulses are amplified they are processed by an Analog to Digital Converter (ADC) which in turn allows for events to be plotted on a graphical scale (One Parameter, Two parameter Histograms).
- Flow cytometry data outputs are stored in the form of computer files using the FCS 2.0 or 3.0 standard.



One-parameter histograms

- A one-parameter histogram is a graph of cell count on the y-axis and the measurement parameter on x-axis. All one-parameter histograms have 1,024 channels. These channels correspond to the original voltage generated by a specific "light" event detected by the PMT detector. In other words, the ADC assigns a channel number based on the pulse height for individual events. Therefore, brighter specific fluorescence events will yield a higher pulse height and thus a higher channel number when displayed as a histogram.

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2-parameter Data Analysis

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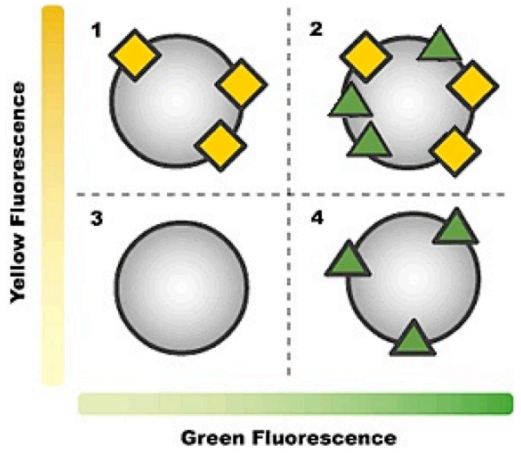
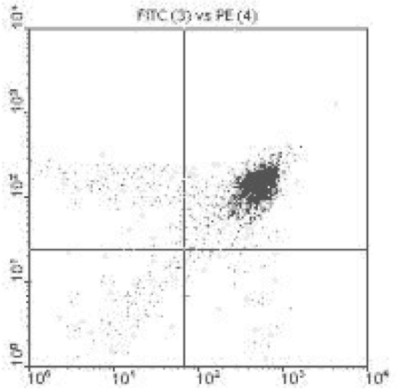
Two-Parameter Histograms

- A graph representing two measurement parameters, on the x- and y-axes, and cell count height on a density gradient. This is similar to a topographical map. You can select 64 or 256 channels on each axis of two-parameter histograms. Particle counts are shown by dot density or by contour plot.

- oregonstate.edu/flow_07





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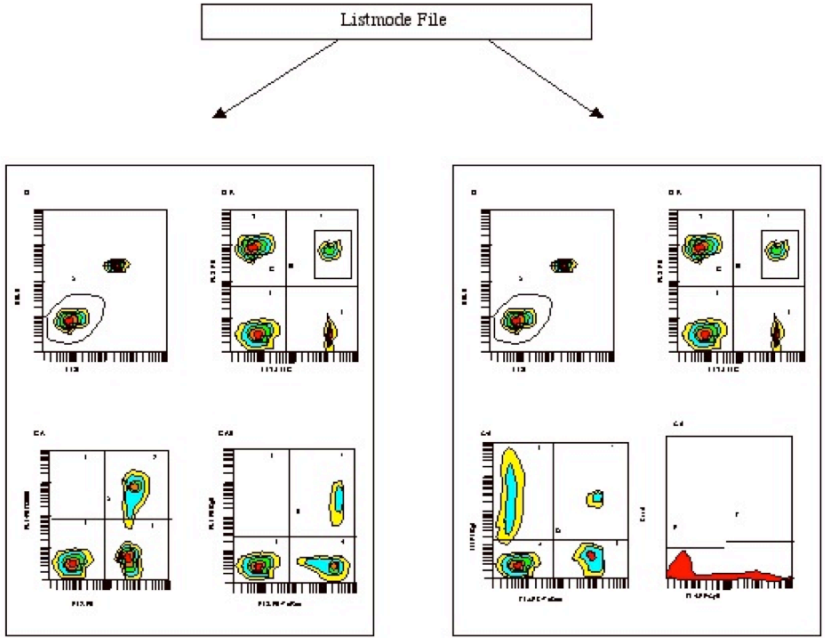
Listmode Files

Data corresponding to one sample can be stored as a histogram file and/or as a listmode file.



- Listmode files consist of a complete listing of all events corresponding to all the parameters collected, as specified by your acquisition Protocol. Raw listmode data files can be opened or replayed using any program designed for analysis of flow cytometry data.

- Once your data has been collected and written into a listmode file you can replay the file either using the specific Protocol used for collection or any other program specifically designed for analysis of flow cytometry data.

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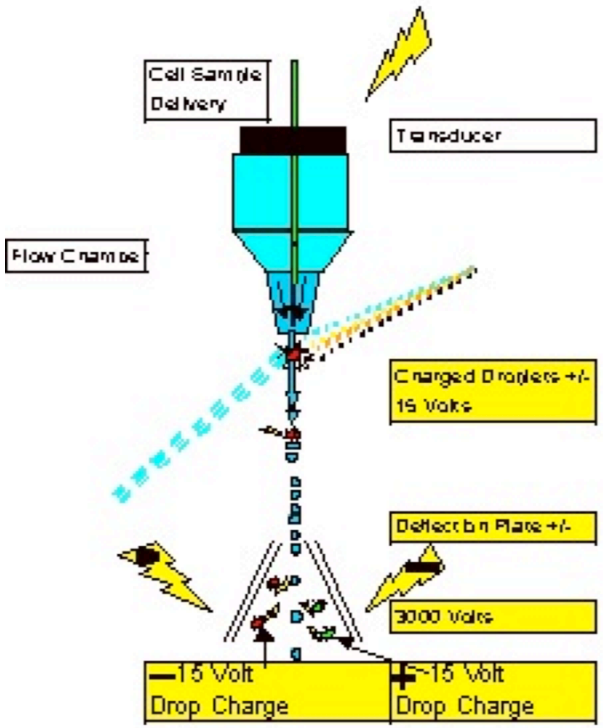
Cell Sorting



- Flow cytometry analysis of a single cell suspension yields multiparameter data that allows researchers to identify and characterize various subpopulations of cells. The process of separating cells using flow cytometry multiparameter data, is referred to as sorting.
- Sorting is a specialized process that requires sophisticated electronic components not incorporated into most bench-top instruments
- Sorters include the following components:

  -A tunable transducer which permits the breaking of the fluid sheath into individual droplets. These individual droplets will encapsulate single cells.

  - Electric charge delays for charging individual droplets.

  -Deflection plates for deflecting individually charged droplets into collection tubes.

  -Software settings for defining sorting criteria, these include regions defining populations to be sorted.

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History

- 1953, Wallace Coulter, first impedence-based flow cytometer (Coulter principle)
- 1965 Mack Fulwyler (Los Alamos National Laboratory)
  - Science paper
  - Flow sorter based on analysis by Coulter principle

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Advantages

- Analyzes single cells or particles
  - 0.5 micron (bacteria) to 100 micron
  - Cell to cell variability
  - Heterogeneity of sample
- Rapid-1000 to 20,000 cells/sec
- Quantitative
- Multiparameteric
- Allow physical separation of cells (sorting) based on selected characteristics

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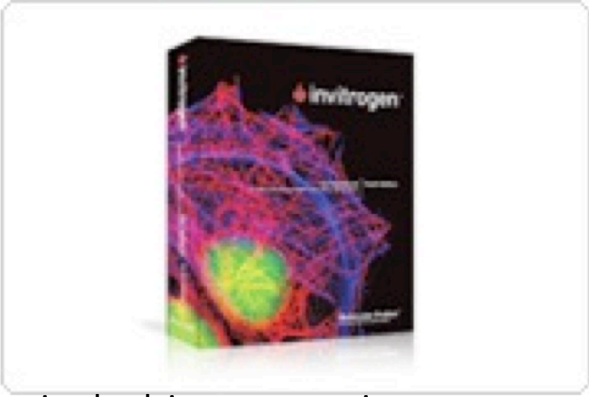
What it Measures

This list is very long and constantly expanding.

* volume and morphological complexity of cells
* cell pigments such as chlorophyll or phycoerythrin
* total DNA content (cell cycle analysis, cell kinetics, proliferation, etc.)
* total RNA content
* DNA copy number variation (by Flow-FISH)
* chromosome analysis and sorting (library construction, chromosome paint)
* protein expression and localization
* Protein modifications, phospho-proteins
* transgenic products in vivo, particularly the Green fluorescent protein or related fluorescent * cell surface antigens (Cluster of differentiation (CD) markers)
* intracellular antigens (various cytokines, secondary mediators, etc.)
* nuclear antigens



- enzymatic activity
  * pH, intracellular ionized calcium, magnesium, membrane potential
  * membrane fluidity
  * apoptosis (quantification, measurement of DNA degradation, mitochondrial membrane potential, permeability changes, caspase activity)
  * cell viability
  * monitoring electropermeabilization of cells
  * oxidative burst
  * characterising multidrug resistance (MDR) in cancer cells
  * glutathione
  * various combinations (DNA/surface antigens, etc.)
  * cell adherence (for instance pathogen-host cell adherence)

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From: Wikipedia

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Applications

- Immunophenotyping
  - CD markers (100+)
  - HIV/AIDS treatment/ progression
- Ploidy Analysis
  - Cell cycle
  - Proliferation
- Sorting
  - Purification-stem cells
  - Protein engineering
- Cell counting
  - Blood analysis
  - Environmental studies, water analysis
  - Chromosome analysis
- Functional assays
  - Viability
  - Reporter genes/expression

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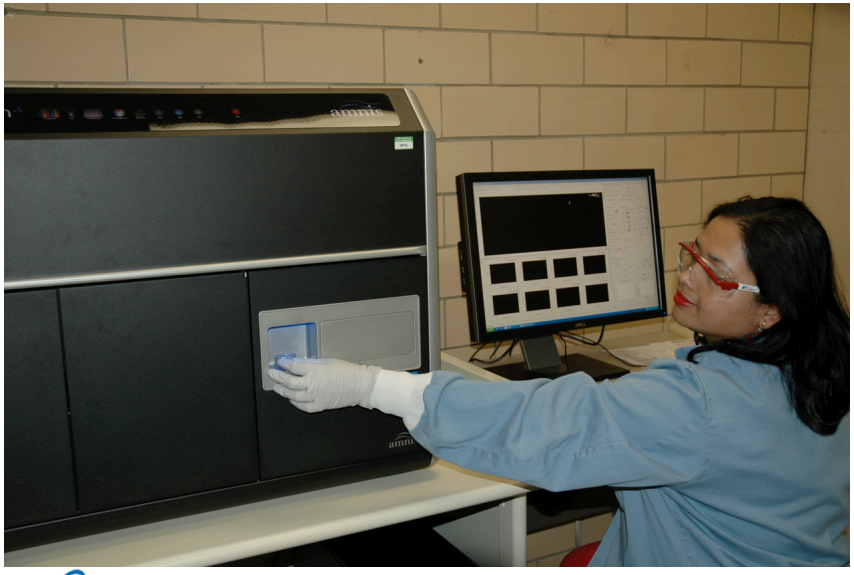
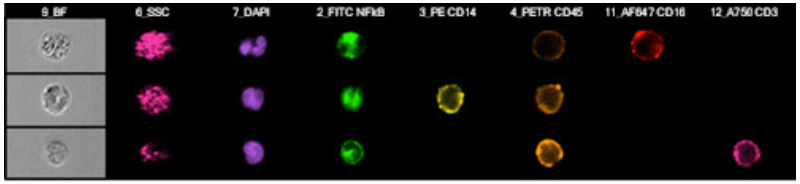
New Advances

amnis





Human whole blood cells stained with FITC anti-NFkB, PE-anti CD14,PE-TexasRed anti-CD45, AF647 anti-CD-16, APCA-AF750 anti-CD3 and DAPI

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