



Introduction to Flow Cytometry

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Outline

- Basic Concept of Flow Cytometry
- Introduction to Instrument Subsystems
- Application Examples



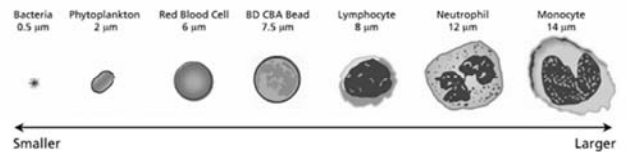
What is Flow Cytometry?

- Flow = Fluid
- Cyto = Cell
- Metry = Measurement
- A variety of measurements are made on cells, cell organelles, and other objects **suspended in a liquid** and flowing at rates of **several thousands per second** through a flow chamber.



Particle Size

- Detection range: 0.5~50um

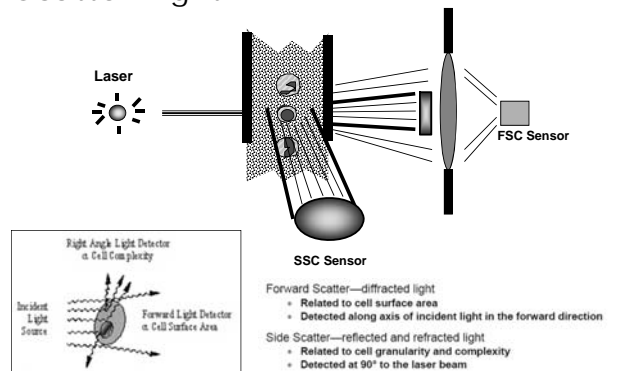


What Can a Flow Cytometer Tell Us About a Cell?

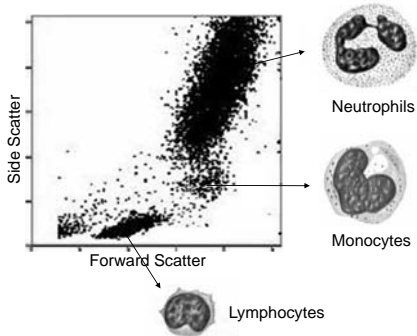
- Its relative size (Forward Scatter—FSC)
- Its relative granularity or internal complexity (Side Scatter—SSC)
- Its relative fluorescence intensity



Scatter Light

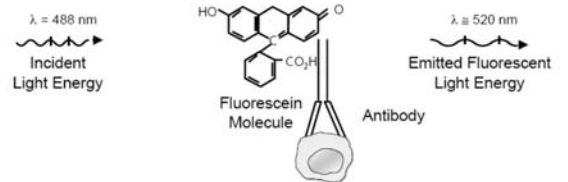


Lysed Whole Blood



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Fluorescence Light

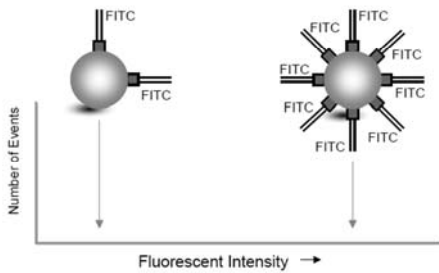


- The fluorochrome absorbs energy from the laser.
- The fluorochrome releases the absorbed energy by:
 - vibration and heat dissipation.
 - emission of photons of a longer wavelength.

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Fluorescence

Emitted fluorescence intensity proportional to binding sites



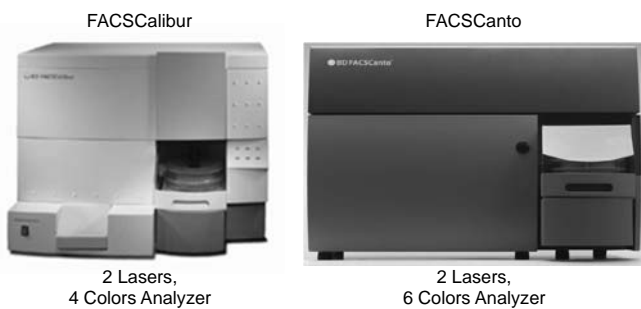
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Applications

- Phenotype Analysis (Cell Surface Antigens/Markers)
- Intracellular Analysis
 - Eg. Cytokines, Signal Transduction molecules...etc.
- DNA Analysis
 - Eg. Viability, Cell cycle, Apoptosis...etc.
- Cell Function Analysis
 - Eg. Free radicals, Ca^{2+} , Reporter genes...etc.
- CBA (Cytometric Bead Array)

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BD Flow Cytometers – Cell Analyzer



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BD Flow Cytometers – Cell Sorter



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Subsystems

Fluidics

To introduce and focus the cells for interrogation.

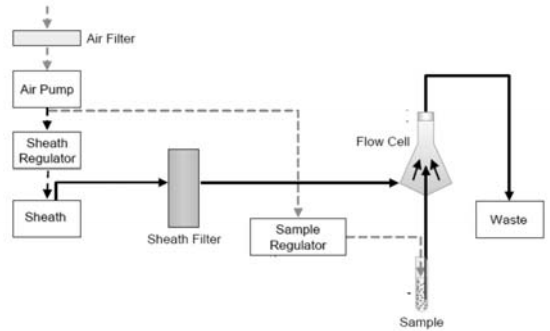
Optics

To generate and collect the light signals.

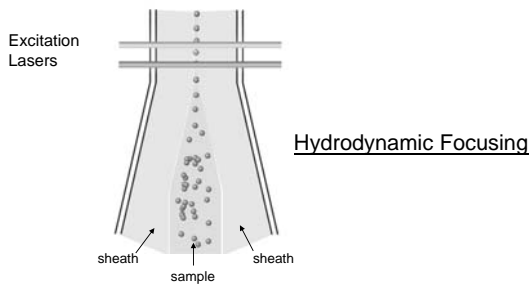
Electronics

To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer.

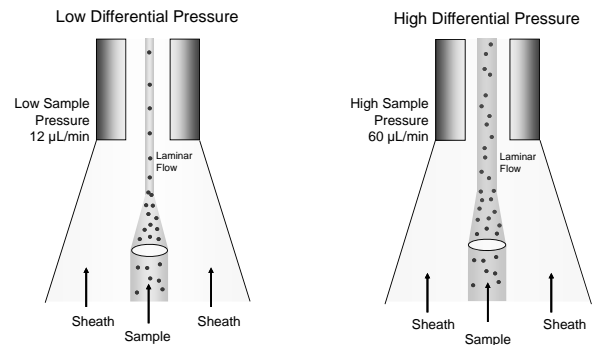
Fluidics — Pressurized System



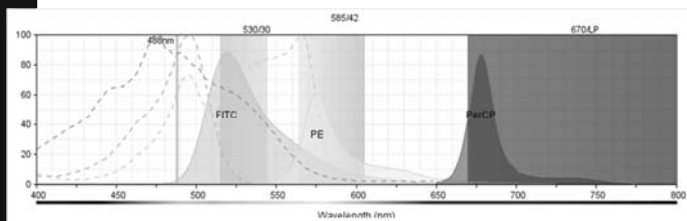
Flow Cell: Sample Flow



Sample Differential



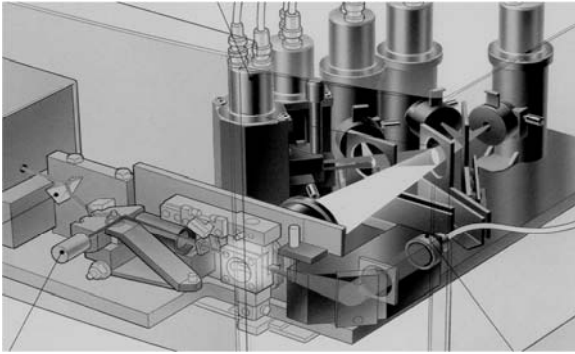
Fluorochrome Spectra



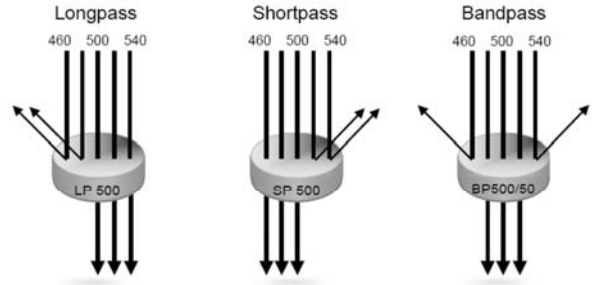
Optics

- Excitation optics
 - Lasers: 488nm(Blue) + 633nm(Red)
 - Lenses to shape and focus the laser beam
- Collection optics
 - A collection lens to collect light emitted from the article-laser beam interaction
 - A system of **optical mirrors and filters** to route specified wavelengths of emitted light to designated **optical detectors**

FACSCalibur Optics

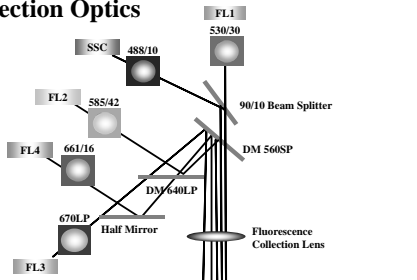


Optical Filters



FACSCalibur Optics

Collection Optics



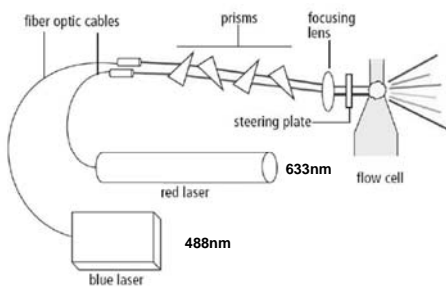
Excitation Optics



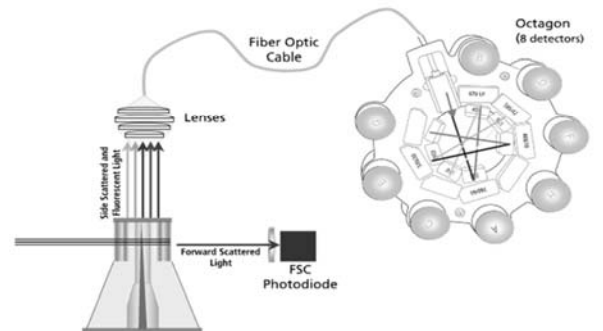
FACSCalibur Configuration

| Detector | Filter | Color | Fluorochrome |
|----------|----------|---------------|--------------------|
| FL1 | 530/30nm | Green | FITC |
| FL2 | 585/42nm | Yellow/Orange | PE |
| FL3 | 670nm LP | Dark Red | PerCP, PerCP-Cy5.5 |
| FL4 | 661/16nm | Red | APC |

FACSCanto Excitation Optics



FACSCanto Collection Optics





Detector Blocks—Octagon and Trigon

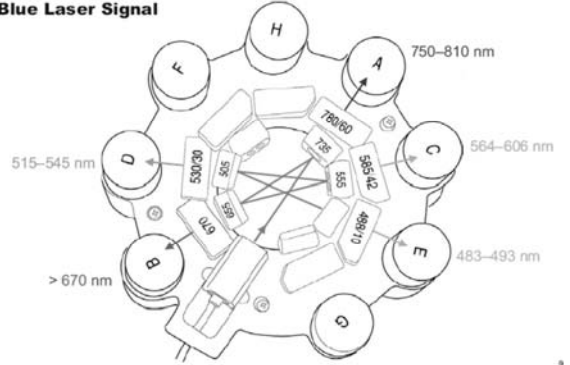


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Collection Optics—Octagon

Blue Laser Signal

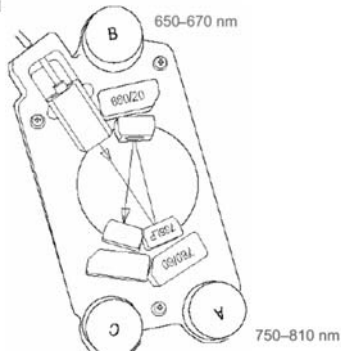


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Collection Optics—Trigon

Red Laser Signal



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Optics-- Configuration

| Wavelength (nm) | PMT Position (nm) | Intended Dyes | Other Dyes |
|-----------------|-------------------|--------------------|----------------------|
| 488 (blue) | A | PE-Cy7 | PI, PE-Cy5.5, DSRRed |
| | B | PerCP, PerCPCy5.5 | PI |
| | C | PE | PI |
| | D | FITC | GFP |
| | E | Side Scatter (SSC) | |
| 633 (red) | A | APC-Cy7 | |
| | B | APC | Alexa Fluor® 633 |

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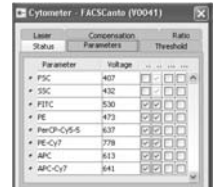
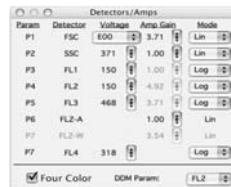
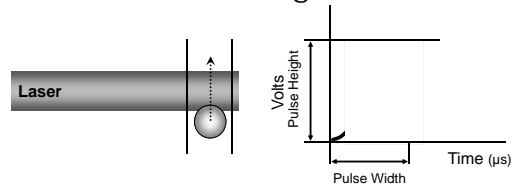
Electronics

- PMTs and preamps convert photons to voltage pulses.
- Analog-to-digital converters translate analog signals to proportional digital signals.
- Compute area and height for each pulse.
- Perform compensation and calculate ratios and width.
- An embedded computer interfaces with the computer workstation for data transfer.

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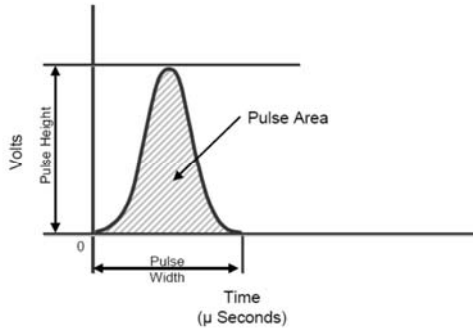


Creation of a Voltage Pulse

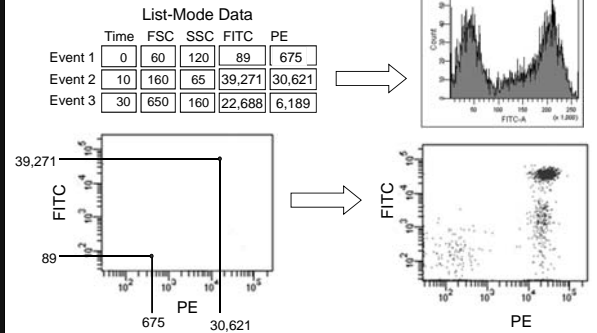


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Quantification of a Voltage Pulse

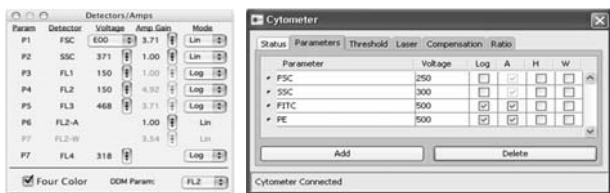


Data Storage

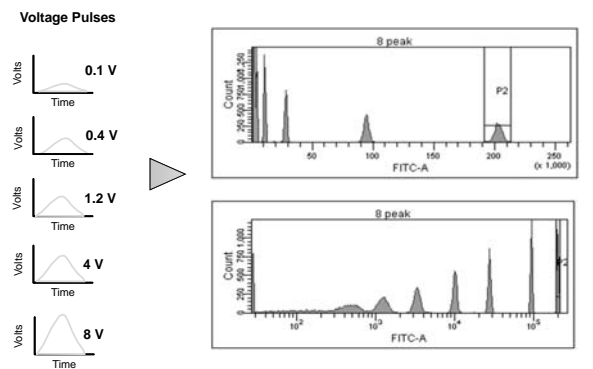


Data Display

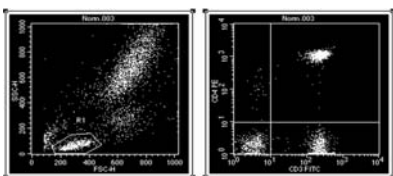
- Linear Scaling
- Log Scaling



Linear v.s Log

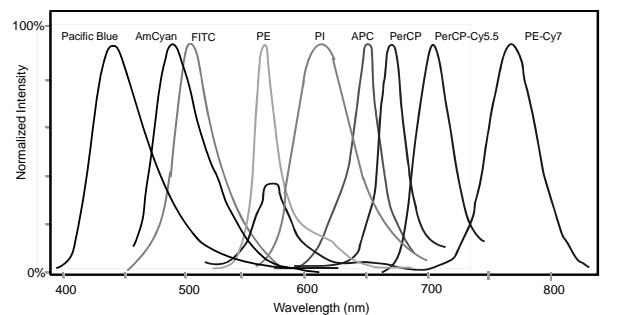


Linear v. Log Amplification



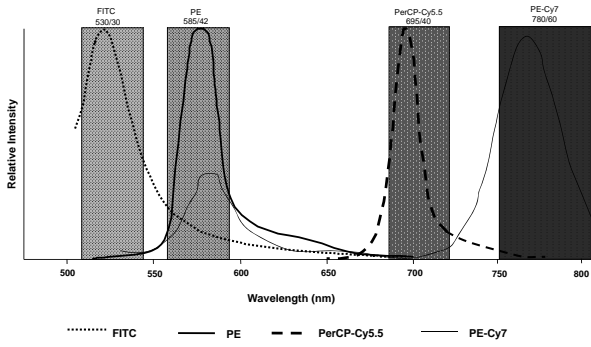
- **Linear** amplification is usually used for light scatter parameters and DNA analysis.
- **Log** amplification is used for fluorescence signals with a large dynamic range.

Spectral Overlap- Compensation Theory





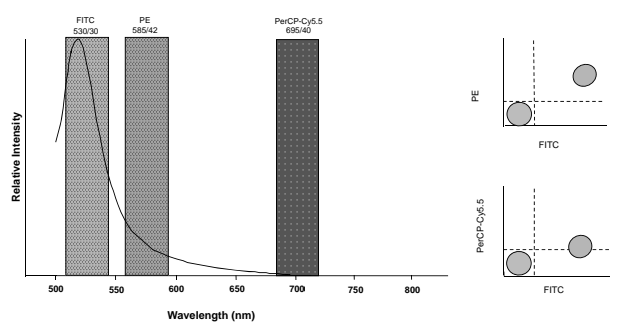
Spillover



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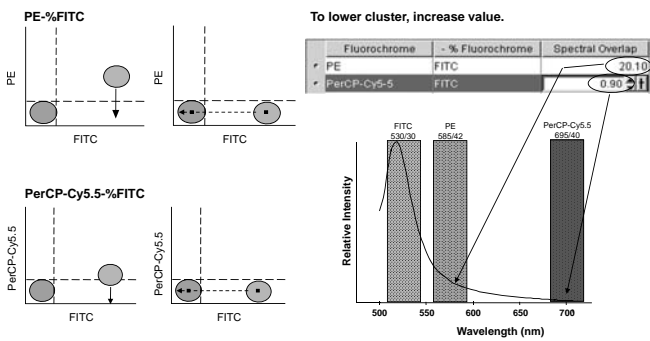
FITC Spillover



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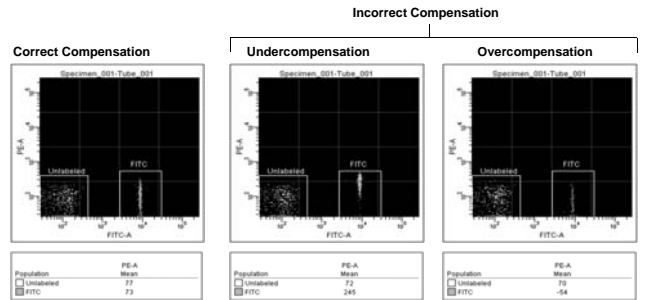
FITC Compensation



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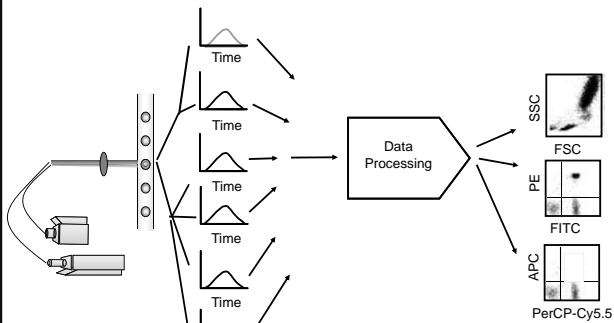
Compensation Examples



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Review



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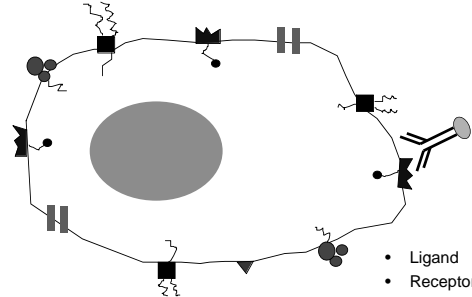
Application Examples

Applications

- Phenotype Analysis (Cell Surface Antigens/Markers)
- Intracellular Analysis
 - Eg. Cytokines, Signal Transduction molecules...etc.
- DNA Analysis
 - Eg. Viability, Cell cycle, Apoptosis...etc.
- Cell Function Analysis
 - Eg. Free radicals, Ca²⁺, Reporter genes...etc.
- CBA (Cytometric Bead Array)

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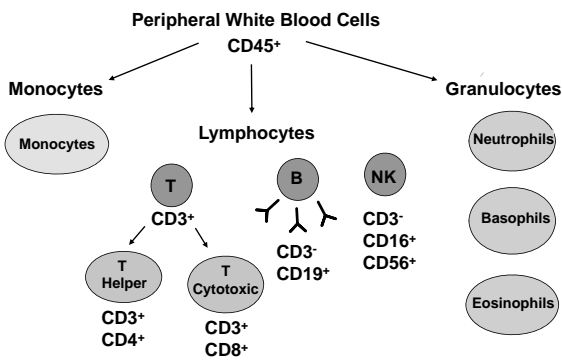
Phenotype Analysis



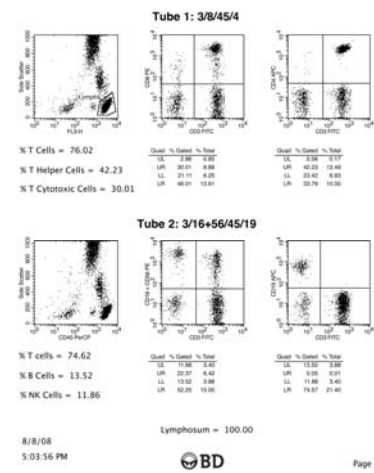
- Ligand
- Receptor
- Adhesion molecule
- ...etc

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Lymphocyte Immunophenotyping



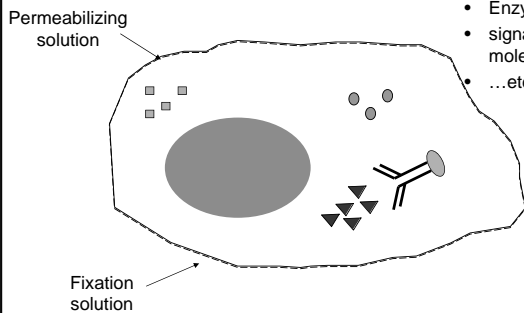
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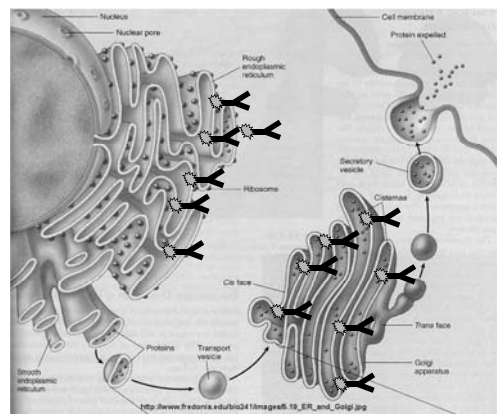
Intracellular Analysis

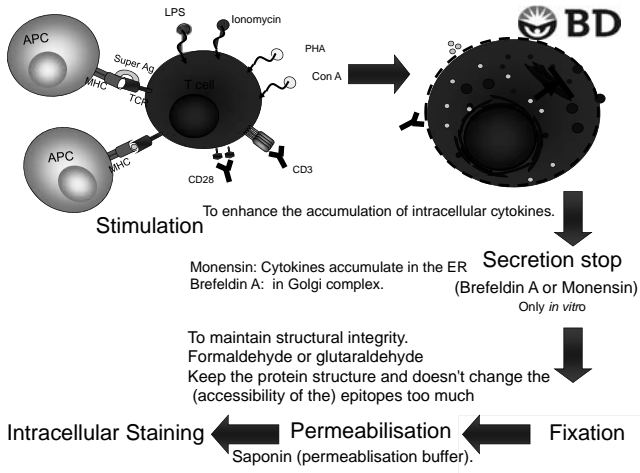


- Cytokine
- Enzyme
- signal transduction molecule
- ...etc.

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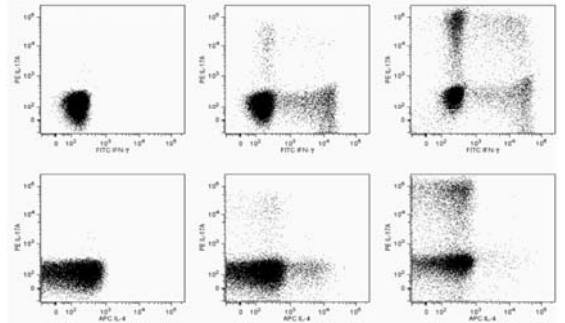
Cytokine Detection

Picture From www.fredonia.edu



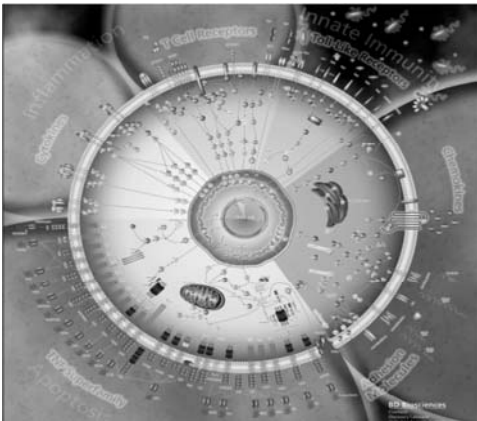
Combination of Cell Surface and Cytoplasmic Staining

Th1/Th2/Th17 Phenotyping Kit



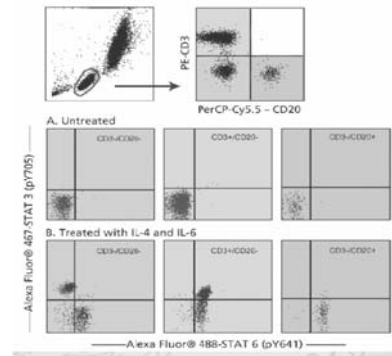
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Signal Transduction



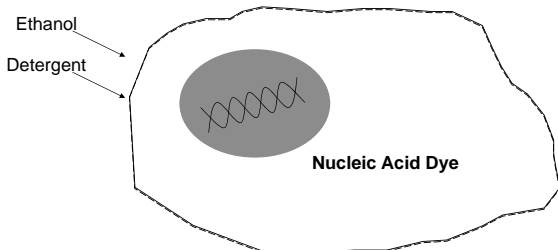
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Intracellular Staining in Activated Lysed Whole Blood



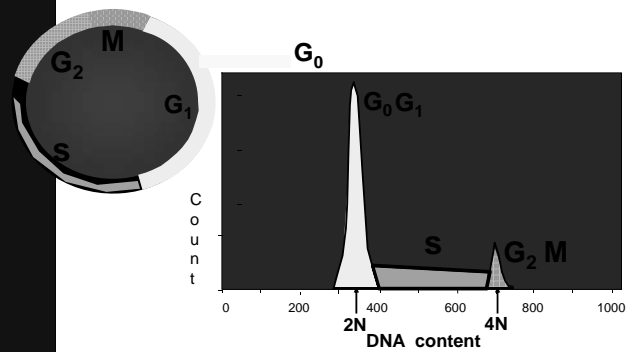
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DNA Analysis



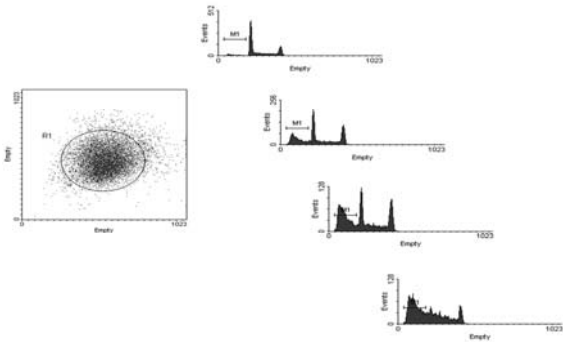
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Cell Cycle Analysis



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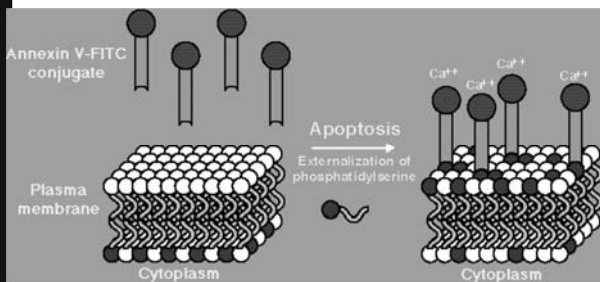
Apoptosis (Sub G1)



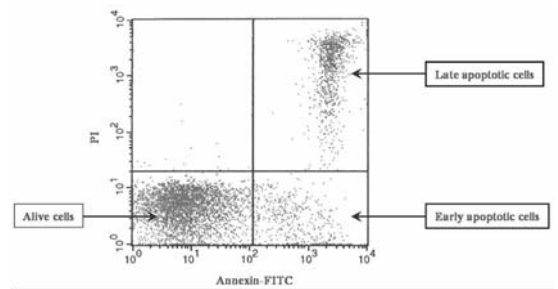
Cell Function Analysis

- Membrane Potential (DiOC6, JC-1)
- Oxidative Metabolism (Free Radicals)
- Intracellular PH Value (Snarf-1)
- Ca⁺⁺ Influx (Fluo-4/Fura Red, Indo-1)
- Phagocytosis
- Cell Proliferation (PI, BrdU, Intracellular Cyclins)
- Apoptosis (Annexin V, active Caspase-3)

Annexin V Assay

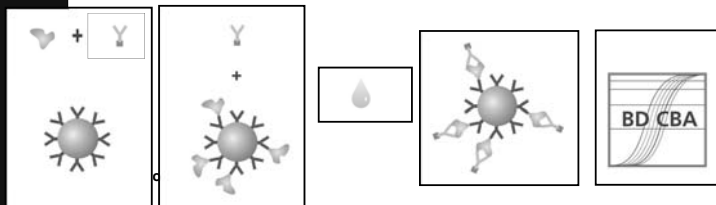


Annexin V/PI Double Staining

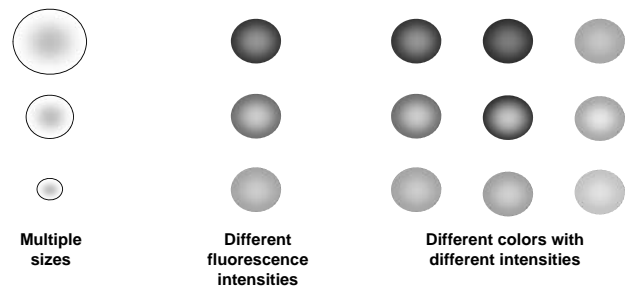


Bordón et al. *Radiation Oncology* 2009 4:58

Cytometric Beads Array (CBA)



Beads Provide a Flexible Platform

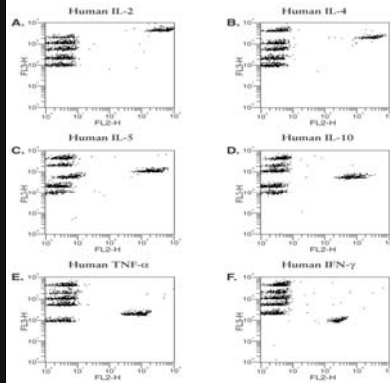




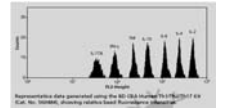
Advantages of Bead-Based Immunoassays

- Analyze multiple analytes simultaneously
- Reduced sample volume requirements
- Reduced hands-on time by parallel analysis of samples
- Wide dynamic range of fluorescence detection (requires fewer sample dilutions)

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- Proteins Measured
- A. Interleukin (IL)-2
 - B. IL-4
 - C. IL-5
 - D. IL-10
 - E. Tumor Necrosis Factor- α
 - F. Interferon- γ

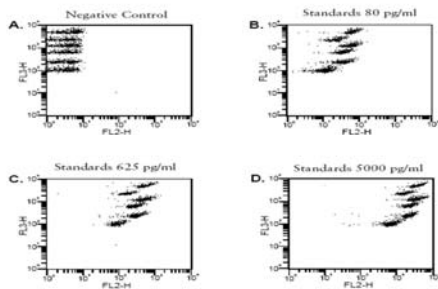


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Cytometry Beads Array (CBA)

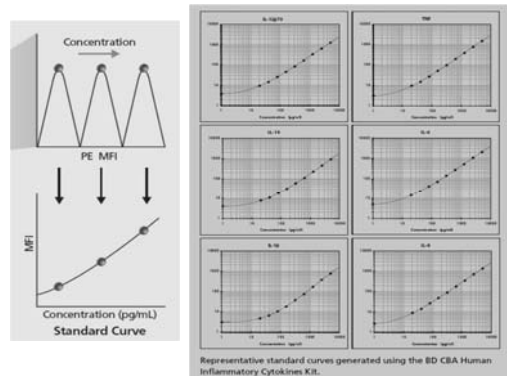
Typical Data



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Standard Curves



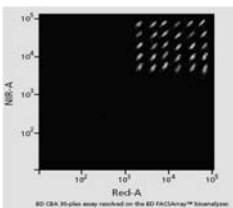
Representative standard curves generated using the BD CBA Human Inflammatory Cytokines Kit.

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CBA Flex Sets

- Open configuration (Up to 30 plex)
- Clustering based on Red and NIR fluorescence intensity
- Need to be used at dual-laser (488nm blue v.s 633nm red) instrument

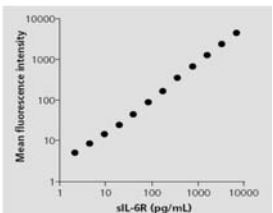
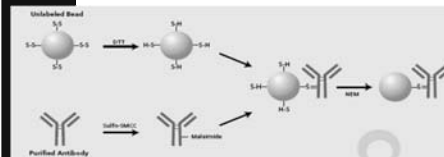


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CBA Functional Beads

Can be conjugated with any Ab or Ag



Standard curve for a soluble IL-6 receptor assay generated using BD CBA Functional Bead E4 following the conjugation procedure in the BD CBA Functional Bead Conjugation Buffer Set manual. Data courtesy of Joseph Cannon and Gloria Sloan, Medical College of Georgia.

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