Introduction to Flow Cytometry
-- BD FACSCanto II™

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Outline

- Basic Concept of Flow Cytometry
- FACSCanto II System Introduction
- 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: 0.5~50μm
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

Laser

FSC Sensor

SSC Sensor

Right Angle Light Detector
\( \alpha \) Cell Complexity

Incident Light Source

Forward Light Detector
\( \alpha \) Cell Surface Area

- Forward Scatter—diffracted light
  - Related to cell surface area
  - Detected along axis of incident light in the forward direction

- Side Scatter—reflected and refracted light
  - Related to cell granularity and complexity
  - Detected at 90° to the laser beam
Lysed Whole Blood

- Forward Scatter
- Side Scatter
- Neutrophils
- Monocytes
- Lymphocytes
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.
Fluorescence

Emitted fluorescence intensity proportional to binding sites
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)
• Others
BD FACSCanto II™: 2 Laser, 6 Color
## Configuration

<table>
<thead>
<tr>
<th>Laser</th>
<th>Primary Fluorochrome</th>
<th>PMT</th>
<th>Dichroic Mirror</th>
<th>Bandpass Filter</th>
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<tr>
<td>488 nm (blue)</td>
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<tr>
<td>Side Scatter</td>
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<tr>
<td>FITC</td>
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<td>502LP</td>
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<td>PE</td>
<td>D</td>
<td>556LP</td>
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<tr>
<td>—</td>
<td>C</td>
<td>610LP</td>
<td>blank optical holder</td>
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<tr>
<td>PerCP or PerCP-Cy5.5</td>
<td>B</td>
<td>655LP</td>
<td>670LP</td>
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<tr>
<td>PE-Cy7</td>
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<td>735LP</td>
<td>780/60</td>
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<td>633 nm (red)</td>
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<td>C</td>
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</table>

**Other Fluorochrome**

- GFP
- PI
- PI, PE-Cy5.5, 7-AAD
- Alexa Fluor® 633
Phenotype Analysis

- Ligand
- Receptor
- Adhesion molecule
- ...etc
Lymphocyte Immunophenotyping

Peripheral White Blood Cells

CD45+

Monocytes

Lymphocytes

T

CD3+

CD3+

CD4+

CD3+

CD8+

T Helper

T Cytotoxic

B

NK

CD3-

CD19+

CD3-

CD16+

CD56+

Granulocytes

Neutrophils

Basophils

Eosinophils
Tools for Immunophenotyping

**BD Lyoplate™ Screening Panels** support the rapid, cost-effective immunophenotyping of stems cells and stem cell–derived cells.

<table>
<thead>
<tr>
<th>Description</th>
<th>Format</th>
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<th>Cat. No.</th>
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<tr>
<td>BD Lyoplate Human Cell Surface Marker Screening Panel</td>
<td>Alexa Fluor® 647</td>
<td>5 tests</td>
<td>560747</td>
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<td>Contents: 242 human cell surface markers and 9 isotype controls (5 tests of each lyophilized purified mAb per well)</td>
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<tr>
<td>Alexa Fluor® 647-conjugated goat anti-mouse Ig and goat anti-rat Ig detection reagents</td>
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<td>BD Lyoplate Mouse Cell Surface Marker Screening Panel</td>
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<td>Streptavidin Alexa Fluor® 647 detection reagent</td>
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- Plate-based format is compatible with automation and multichannel pipetting
- The proprietary lyophilized format allows for room temperature storage, long shelf life
- Open wells permit the use of additional markers of choice
- Compatible with BFP, CFP, GFP, YFP, OFP, and RFP expressing cells
Surface Marker Screening

Figure 5. Flow assay optimization. Flow cytometry data from THP-1 cells presented as histograms (blue) overlaid with control antibody (pink). Panel A: CD1a (negative control). Panel B: CD4 (positive control).

Figure 6. Expression of BD Lyoplate panel markers on THP-1 monocytes and macrophages. The data from a flow cytometry screen on THP-1 monocytes and high-content imaging screen on THP-1 macrophages compiled as percent positive cells.
Intracellular Analysis

- Cytokine
- Enzyme
- Signal transduction molecule
- …etc.
Cytokine Detection

http://www.fredonia.edu/bio241/images/5.19_ER_and_Golgi.jpg
Stimulation

Monensin: Cytokines accumulate in the ER
Brefeldin A: in Golgi complex.

To enhance the accumulation of intracellular cytokines.

Secretion stop
(Brefeldin A or Monensin)
Only *in vitro*

To maintain structural integrity.
Formaldehyde or glutaraldehyde
Keep the protein structure and doesn't change the (accessibility of the) epitopes too much

Intracellular Staining ← Permeabilisation ← Fixation
Saponin (permeabilisation buffer).
Combination of Cell Surface and Cytoplasmic Staining
Signal Transduction
Intracellular Staining in Activated Lysed Whole Blood
DNA Analysis

Ethanol

Detergent

Nucleic Acid Dye
Cell Cycle Analysis

- G0
- G1
- S
- G2
- M

DNA content

- 2N
- 4N

Count

BD
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-FITC conjugate
- Plasma membrane
- Cytoplasm
- Apoptosis
- Externalization of phosphatidylserine
- Cytoplasm
Annexin V/PI Double Staining

Bordón et al. Radiation Oncology 2009 4:58
Cell Proliferation

Divisions:

3          2        1          0
Cytometric Beads Array (CBA)
Beads Provide a Flexible Platform

Multiple sizes

Different fluorescence intensities

Different colors with different intensities
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)
Proteins Measured
A. Interleukin (IL)-2
B. IL-4
C. IL-5
D. IL-10
E. Tumor Necrosis Factor-α
F. Interferon-γ
Cytometry Beads Array (CBA)

Typical Data
Standard Curves

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

- Can be conjugated with any Ab

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.*
Fluorescent Cell Barcoding (FCB)

Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling

Peter O Krutzik & Garry P Nolan

Figure 6.31.1. Fluorescent Cell Barcoding protocol. Cell samples are labeled with an amine-reactive fluorescent dye (FCB dye) at different concentrations. After covalent labeling has occurred, cells are washed to remove unbound dye, then combined into one tube and stained with antibodies against intracellular or surface antigens. The combined tube is then acquired on the cytometer. After acquisition, the original cell samples are identified by gating populations that display discrete fluorescent intensities in the FCB channel.
BD Phosflow Violet Fluorescent Cell Barcoding Kit