OVERVIEW

• Flow Cytometry
  • Definition
  • Advantages
  • Applications

• Technology
  • Fluidics: Hydrodynamic focusing, Flow Rate
  • Optics: Light sources, Light scatter, Fluorescence, Excitation and emission

• Cell sorting
WHAT IS FLOW CYTOMETRY?

**Flow Cytometry** basic definition: Measurement of physical and chemical properties of cells, flowing in a stream of fluid

- Flow cytometry can be used to analyze **different parameters simultaneously** of large numbers of cells or particles at a single cell level.

- These parameters are based on **cell morphology** (size, internal complexity), **extracellular components** (e.g. membrane proteins) and **intracellular components** (e.g. DNA/RNA content, ...)

- Up to **30 parameters** can be analyzed at the same time! (FSC, SSC and 28 fluorescence parameters)

- Possibility to physically separate your cells of interest by means of **FACS**: Fluorescence Activated Cell Sorting

KEY ADVANTAGES OF FLOW CYTOMETRY

- Population data
  - Measure thousands of cells/particles per second
  - Measure multiple parameters simultaneously.

- Detection of extremely rare populations

- Cell sorting
  - High purity, speed, and yield.

**Flow Cytometry** does not...

- Locate where a certain component is within a cell
- Measure distribution of cellular components
APPLICATIONS OF FLOW CYTOMETRY

- Immunophenotyping
- Cell proliferation
- Apoptosis and cell death
- Cell cycle, DNA synthesis
- Cell activation
- Calcium flux
- Cytokine Secretion
- Activation of signalling pathways
- Levels of intracellular ROS
- Marine and microorganism identification
- Absolute counting
- Transfection/transduction confirmation
- ...

FLOW CYTOMETER IN A NUTSHELL

Cells in suspension flow in single file through...

Fluidics

an illuminated volume where they scatter light and emit fluorescence that is filtered, collected and...

Electronics

converted to digital values that are processed and analyzed in a computer.

VIB

SCIENCE MEETS LIFE
FLUIDICS IN FLOW CYTOMETERS

INTERROGATION POINT
SIMPLIFIED SCHEMATICS

FLUIDICS: FLOW RATE

- Differential pressure between sample and sheath determines flow rate
  - LOW, MED or HIGH speed buttons on BD machines
  - Try to measure below 10000 events/sec on BD machines
- Flow rate is application specific
  - e.g. cell cycle analysis--- slower is better
- Too fast: what happens?
  - Spread of the data will increase due to positions of the cells within the laser interrogation point.
  - The number of coincident events will increase as more cells pass the intercept while the previous cell is being processed

http://expertcytometry.com/fluidics/
FLUIDICS: FLOW RATE

Samples should be run at the lowest flow rate (differential pressure) as possible.

If increasing flow rate...
- has no impact on population distribution: Use high flow rate.
- changes population distribution: Use low flow rate and increase cellular concentration.

OPTICS
EXCITATION - LASERS

MULTIPLE LASER SYSTEMS

Spatially Separated Laser Beams

- Time delay between lasers must be determined for each instrument for a given sheath pressure.
- Changes in Sheath Pressure will impact measurements.
EMISSION - LIGHT SCATTER

Light is reflected towards all directions

Low angle: Forward Scatter (FSC)
High angle: Side Scatter (SSC)

FORWARD SCATTER (FSC)

The magnitude of Forward Scatter is roughly proportional to the size of the cell.
Side Scatter is caused by granulosity and structural complexity inside the cell.

Histogram and Scatter Plot or Dot Plot
FLUORESCENCE

Fluorophore

Energy Levels
FLUORESCENCE

- Low Energy
- High Energy

- Lower Wavelength
- Higher Frequency
- Higher Energy

- Higher Wavelength
- Lower Frequency
- Lower Energy

FLUORESCENT MARKERS IN FLOW

- Each Fluorochrome has its own specific Excitation and Emission Spectra!
- Example: http://www.bdbiosciences.com/eu/applications/s/spectrumguidepage

ADSORPTION AND EMISSION SPECTRA

Each Fluorochrome has its own specific Excitation and Emission Spectra, allowing for the discrimination and quantification of different cell populations. The Excitation Spectra indicate the wavelength at which the fluorochrome absorbs light, while the Emission Spectra show the wavelength range of fluorescence emitted upon excitation. This enables the use of multiple dyes in a single sample, facilitating the analysis of various cellular markers in a flow cytometry experiment.
EXCITATION-EMISSION SPECTRUM

INTERPRETATION

Excitation/Emission spectrum of PE (Phycoerythrin)

Excitation maxima  Emission maxima

Wave length of light (nm)

Phycoerythrin (PE)

Exited
Excitation-max 550 nm

Excitation at different wavelengths decreases intensity of emission.
FLUORESCENCE CHARTS ONLINE

PE-Alexa Fluor 700

Lots of tandem dyes!!!

PE-Cy5  PE-TexasRed  APC-Cy7  BV605  BUV496
PE-Cy7  PE-eFluor610  APC-H7  BV650  BUV536
PE-AF700  PE-CF594  APC-eFluor780  BV711  BUV737
PerCP-Cy5.5  PE-Dazzle594  APC-R700  BV766  BUV805

TANDEM DYES
**REACTIVE AND CONJUGATED PROBES**

- Cascade Blue
- Pacific Blue
- Pacific Orange
- Alexa Dyes
- Lustre yellow
- NBD
- R-Phycoerythrin (PE)
- PE-Cy5 conjugates
- PE-Cy7 conjugates
- PE-Texas Red
- PerCP
- TrueRed
- PerCP-Cy5.5 conjugate
- Fluorescein (FITC)
- BODIPY-FL
- X-Rhodamine
- X-Rhodamine
- Texas Red
- Allophycocyanin (APC)
- APC-Cy7 conjugates
- Etc...

**Immunohistochemistry**

- Direct method
- Indirect method


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**DNA AND CELL FUNCTION PROBES**

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FLUORESCENT PROTEINS

San Diego beach scene drawn with living bacteria expressing 8 different colors of fluorescent proteins

http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE-PLATE-Beach.jpg

Roger Tsien, Nobel Lectures, 2009

FLUORESCENCE ACTIVATED CELL SORTING

Physical separation of a cell or particle of interest from a heterogeneous suspension of cells or particles.

Drop Generation

A cell sorter sorts drops, not cells!

Deflect Charged Drop
Flow cytometry is one of the most widely used techniques in cell biology research.

Source: Georgia Regents University, William King