ANAT 3231 Cell Biology
Lab12 - Stem Cell Analysis

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Introduction to Flow Cytometry

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What Is Flow Cytometry?

A Flow Cytometer is an instrument designed to measure the scatter and fluorescence signals of single particles (e.g. cells) focused in a liquid stream.

It allows multi-parameter analysis of individual cells.

A Flow Sorter further allows the Separation of cells based on measured properties

FACS = Fluorescence-Activated Cell Sorting
A Flow Cytometer is a combined system of

**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 electronic signals and digitize them for computer analysis
The introduction of a large volume into a small volume in such a way that it becomes “focused” along an axis is called **Hydrodynamic Focusing**.
(2) Optics: Laser

- monochromatic
- in phase (coherent light)
- very bright (high intensity)
- unidirectional

Laser

- Blue laser (Argon) 488 nm
- Red diode laser 635 nm
- Violet diode laser 405 nm
(2) Optics: Detector

- Scatter and Fluorescence Signals are detected in unique **channels** (FSC, SSC, FL1, FL2, FL3,...)

- The detectors are called Photomultiplier (PMT)

- The specificity of detection is controlled by optical filters and mirrors
(3) Electronics

- Interfaces with the computer for data transfer
- Converts optical signals to proportional electronic signals (voltage pulses)
- Analyzes voltage pulse height, area or width
Serotec: Introduction to flow cytometry by Misha Rahman
Measurable parameters of cells

Scatter Signals: Intrinsic Structural Parameters (no probe)
Cell size: small angle light scattering = forward scatter (FSC)
Cytoplasmic granularity: large angle light scattering = side scatter (SSC)

Fluorescent Signals: Extrinsic Structural & Functional Parameters (fluorescent probe)
DNA/RNA content  e.g. propidium iodide (PI) / acridine orange (AO)
Surface proteins  e.g. labelled with antibodies
Intracellular proteins  e.g. labelled with antibodies
Enzyme activity  e.g. fluorogenic substrates (*aldefluor*)
DNA synthesis  e.g. BrdU staining (*proliferation*)
Forward Scatter indicates cell size

- light scattered in the forward direction (along the same axis as laser light)
- intensity of forward scatter is proportional to the size of cells
Side Scatter indicates cell granularity

- light scattered to the side is detected in the side or 90° scatter channel (SSC)
- the intensity of side scatter is proportional to the shape and optical homogeneity (granularity) of cells
What is a Fluorophore?

*a molecule which will absorb energy of a specific wavelength and re-emit energy at a different (but equally specific) wavelength*
**Immunofluorescent Labelling**

**cell surface proteins:**
receptors, adhesion molecules, ligands, ...

**intracellular proteins:**
- cytokines, enzymes, structural proteins, cell signalling molecules, nuclear antigens, ...
- fixation, permeabilization
Absorption & Emission Spectra of Fluorescein (FITC)

Wavelength (nm)

λ_ex = 495nm

λ_em = 520nm

Relative fluorescence

FITC
Excitation and Emission of FITC and Phycoerythrin (PE)
Multiple labeling
How to Isolate Stem Cells? FACS vs MACS®

Magnetic-Activated Cell Sorting

1. Cells Labeled
2. Magnetic Separation
3. Super paramagnetic particles
4. Elution of Positive cells

Flow through
How to Isolate Stem Cells? FACS vs MACS®

Fluorescence-Activated Cell Sorting
Results from Last Week’s Lab

Negative Population

Positive Population

2.39%

23.9%