Creative Bioarray

Flow Cytometry

Flow =the motion characteristics of fluids

Cytometry=is a general name for a group of biological methods used to measure various parameters of cells.

Counting of RBCs through capillary tube

1930s

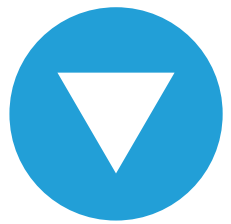
1950s

Development of coulter principle

Development of FACS and other advances

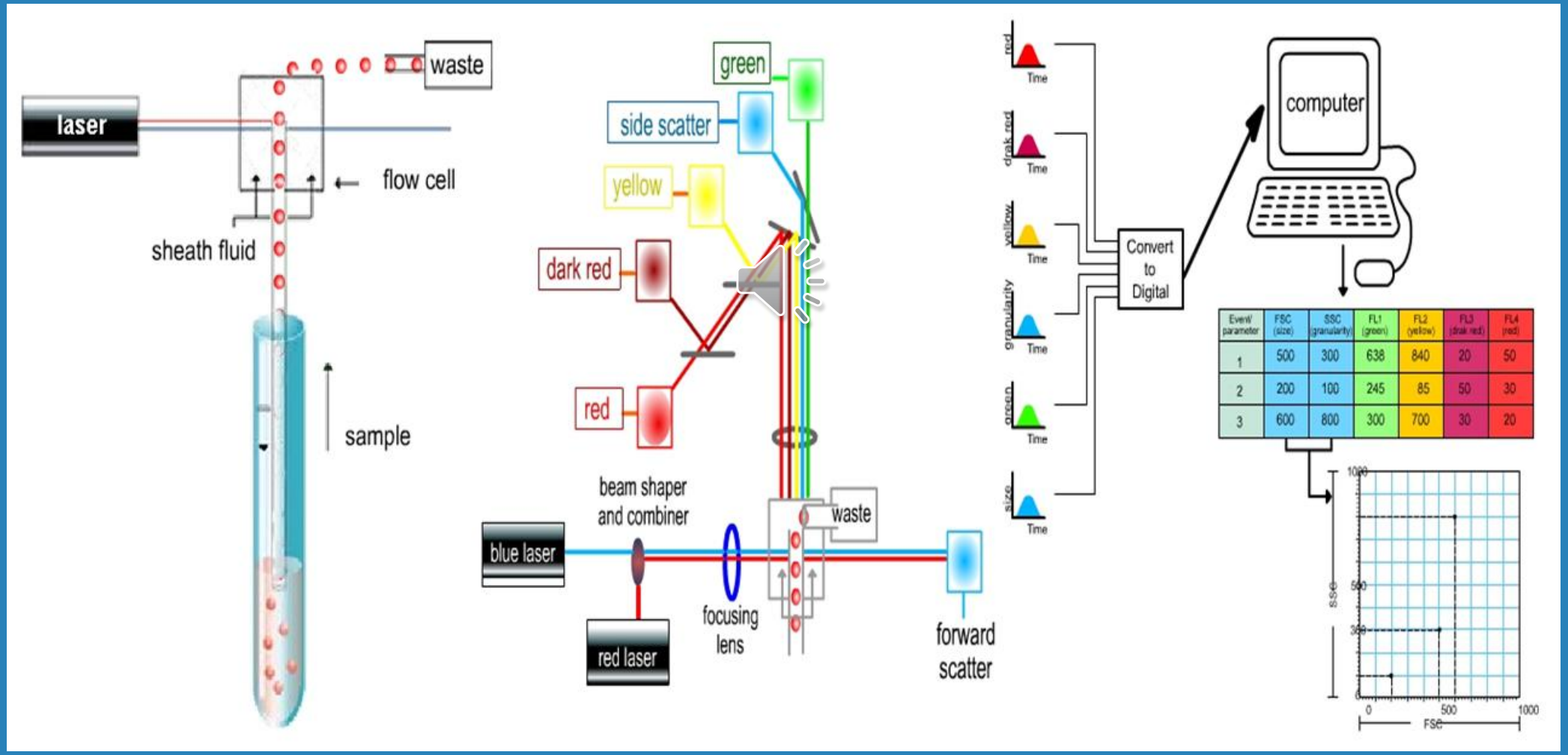
1970s

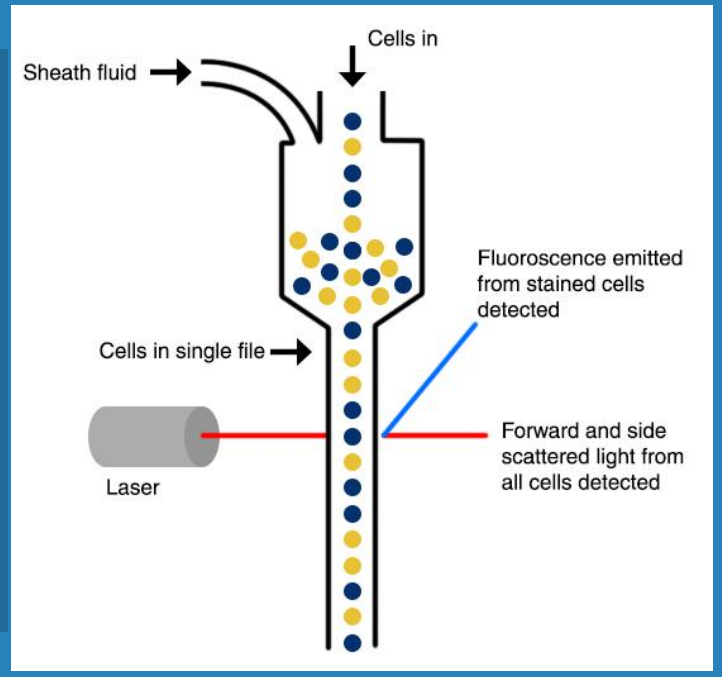
How Flow Cytometry Work



Components of Flow Cytometry

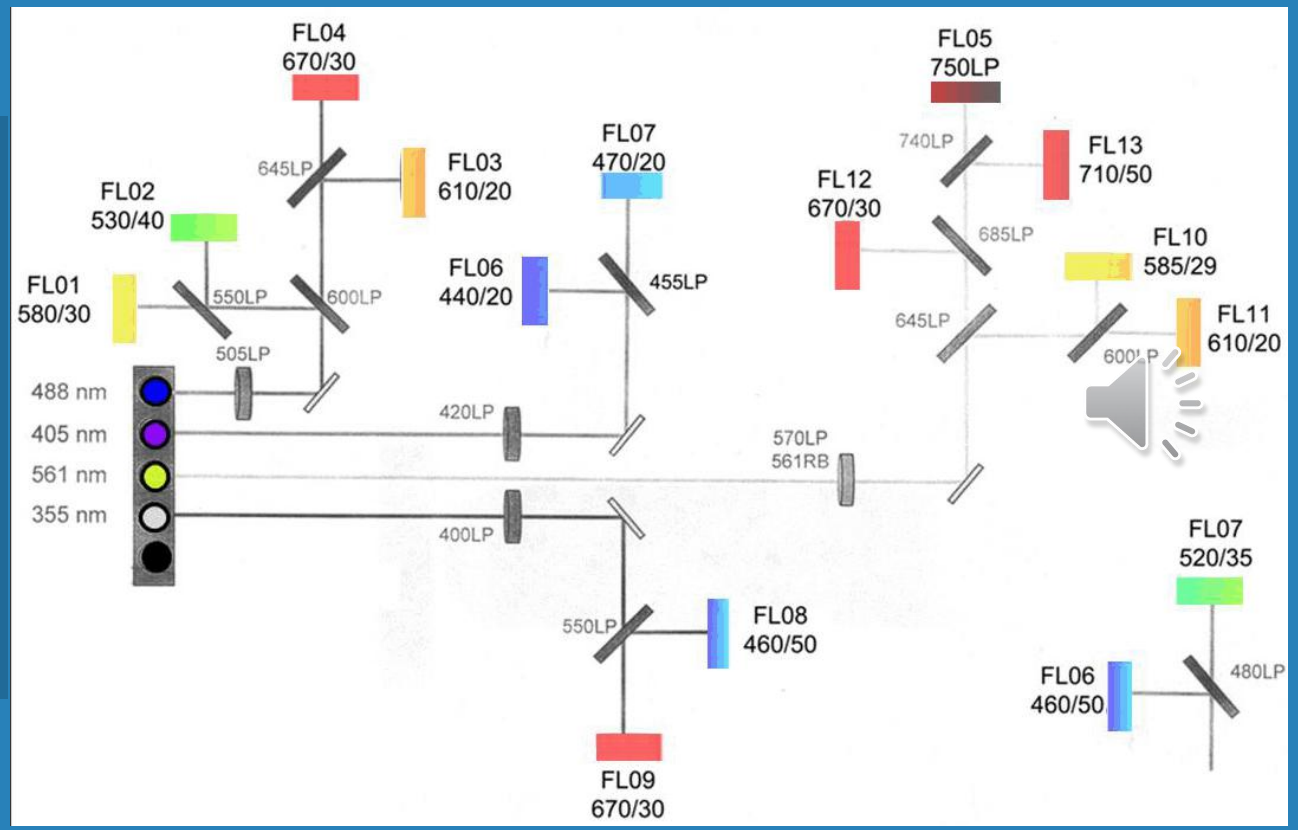
Fluidics Optics Electronics





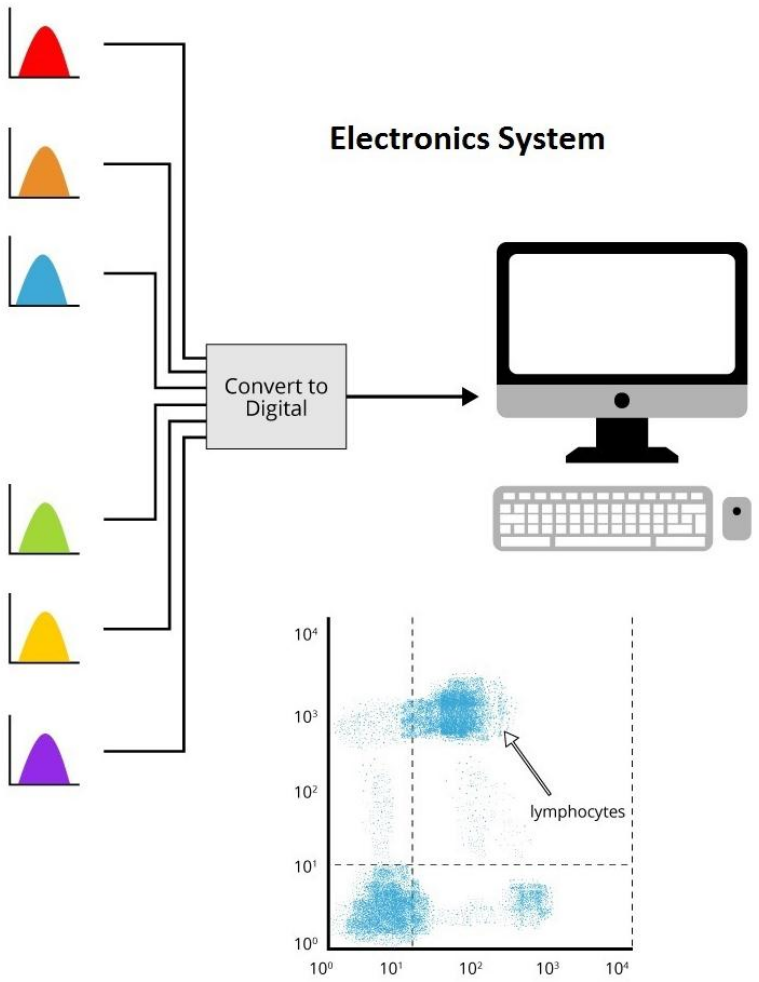
The fluidics system transports particles in a stream to the laser beam for interrogation.

Objective is to have one cell pass through the laser intercept at a time.



The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.

Fluorescent and SSC signals are collected at right angles to the excitation laser are progressively picked off to facilitate multiple fluorochrome use.



The electronics system converts the detected light signals into electronic signals that can be processed by the computer.

The fluorescence intensity measured is proportional to the number of fluorescent molecules bound to the cell.

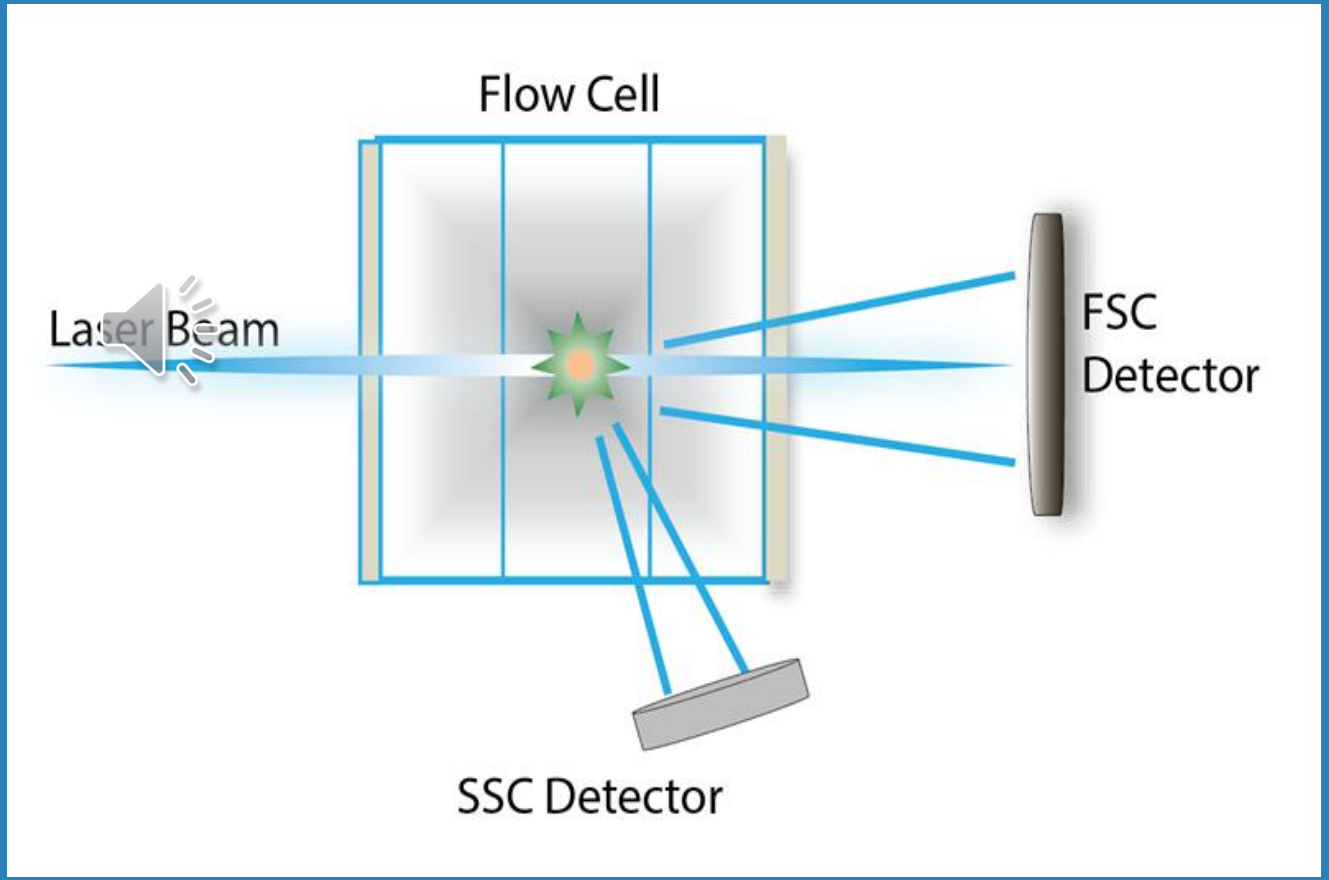
For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisions to charge and deflect particles.

Scatter Signals and Fluorescent Signals

- Scatter
  - — Forward Scatter (FSC)
  - — Side Scatter (SSC)
- Fluorescence
  - — FITC, PE, APC, GFP, DAPI

Incident light scattered at small angles (0.5-2.0°) is called Forward Scatter (FSC)

Incident light scattered at an angle of 90° is called Side Scatter (SSC)



Scatter Signals and Fluorescent Signals

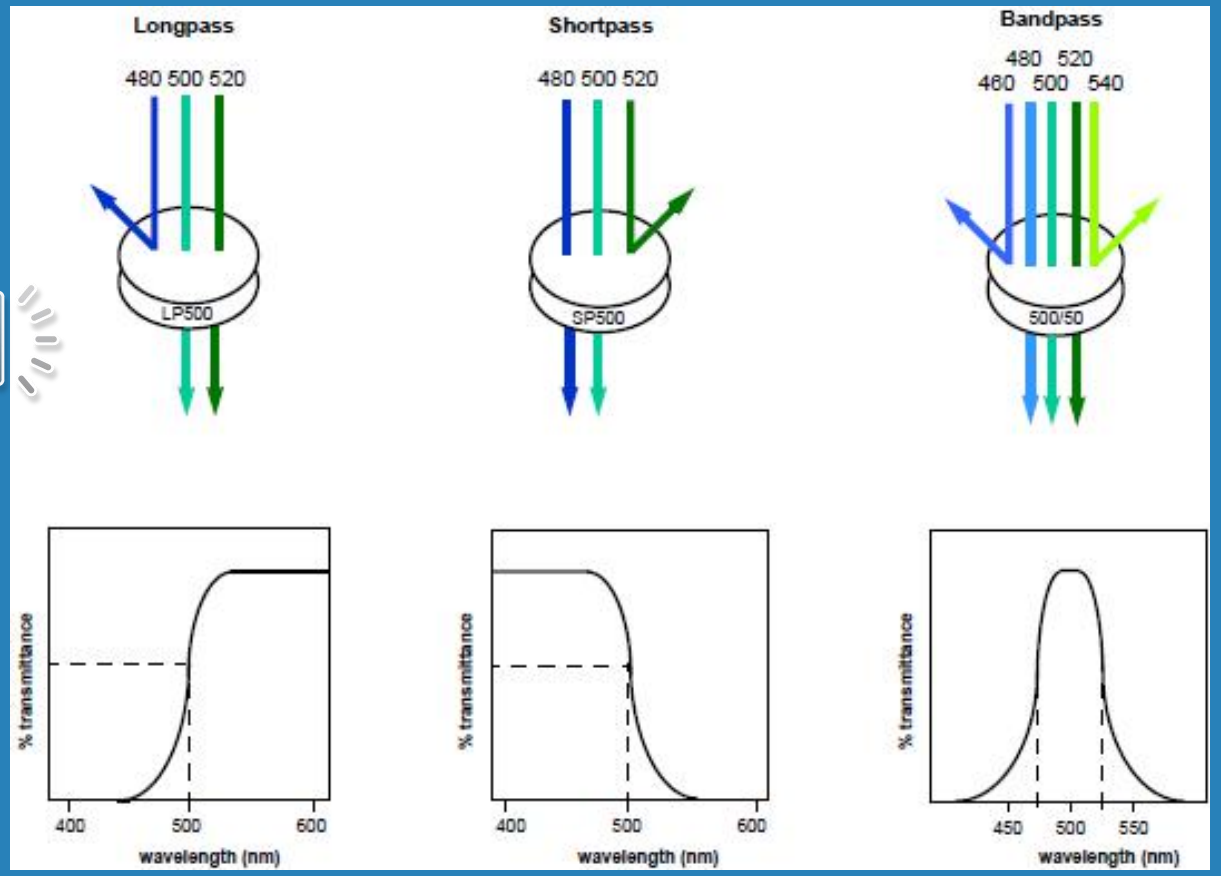
Forward Scatter (FSC)

Rough measure of size, influenced by the wavelength of light, and the angle, lenses and apertures that light is collected at and with.

Different flow cytometers will give slightly different FSC measurements.

Most flow cytometers measure FSC with a photodiode. Bacteria – Photomultiplier tube (PMT)

Dead cells may have lower FSC measurements than live cells. Osmotic swelling can increase cell volume, and decrease light scatter.



Scatter Signals and Fluorescent Signals

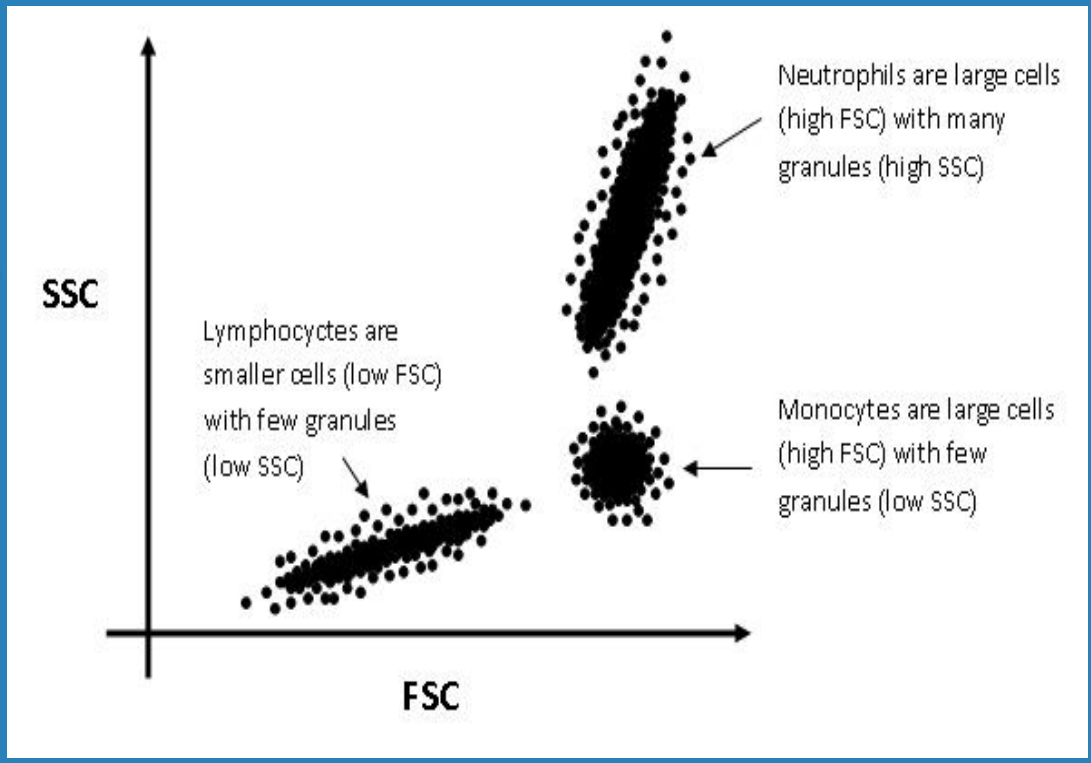
Side Scatter (SSC)

SSC is the measure of light scattered at an angle of 90º (orthogonal).

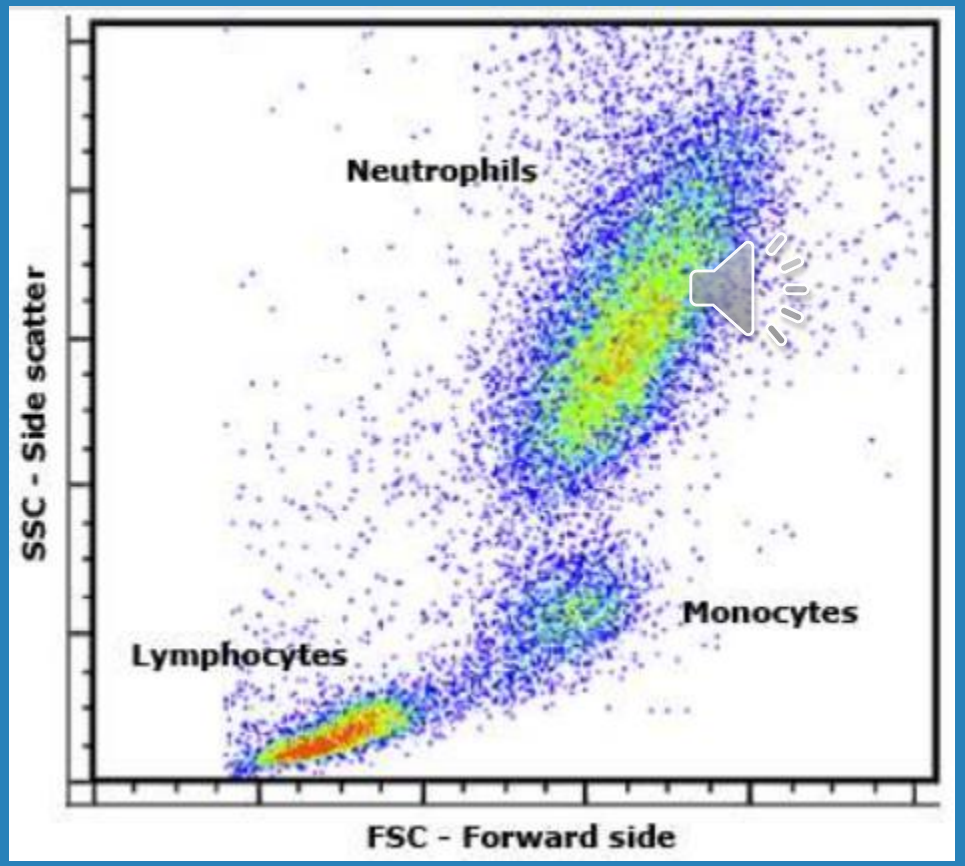
SSC is a measure of the complexity of the cell's internal structures.

The more 'granular' a cell is the higher its SSC will be.

A neutrophil is much more granular than a lymphocyte.



Scatter Signals and Fluorescent Signals



FSC has some similarities to size

SSC has some similarities to granularity and complexity

Fluorochromes

- Fluorochromes are substances that can be excited by certain light source (such as laser) and emit a fluorescent signal at a single wavelength.
- Fluorescent dyes can directly bind to certain cellular content, such as DNA and RNA, and allow us to perform quantitative analysis on individual cells.
- However, in most cases fluorochromes are conjugated with monoclonal antibodies, which specifically target cellular antigens/markers.

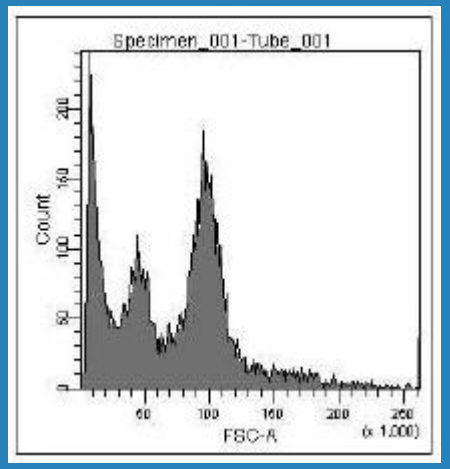
Synthetic, organic dyes	Proteins
FITC	PE
Cy dyes	APC
Horizon dyes	Green fluorescent protein
eFluor dyes	
Pacific blue	
Brilliant violet	

How to Choose Fluorochromes

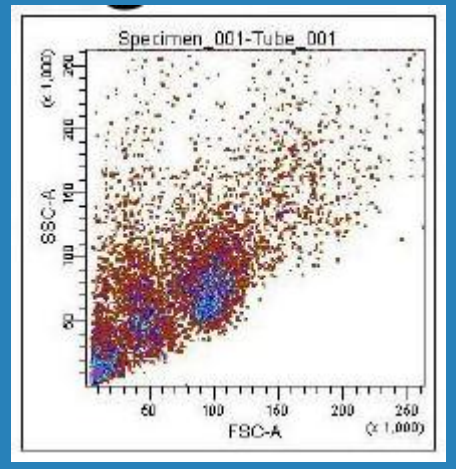
- Antibody availability
- Function — *i.e.* Mcherry VS GFP
- Fluorochrome brightness
- Excitation source
- Emission filters
- Other fluorochromes

Fluorochromes	Excitation (nm)	Emission (nm)
FITC	488	525
PE	488	575
PI	488	630
Cy5	488	675
PerCP	488	675
APC	633	660
APC-Cy	633	767

Visualizing Flow Cytometry Data

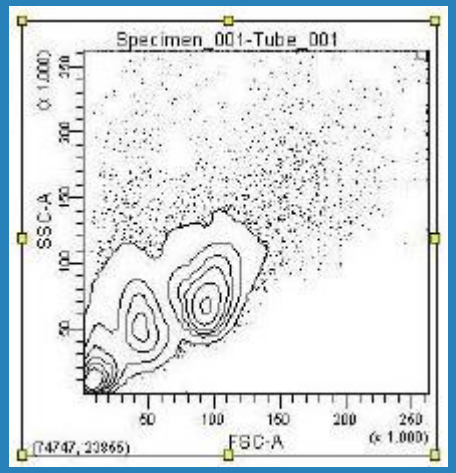


Histogram plot: one parameter only

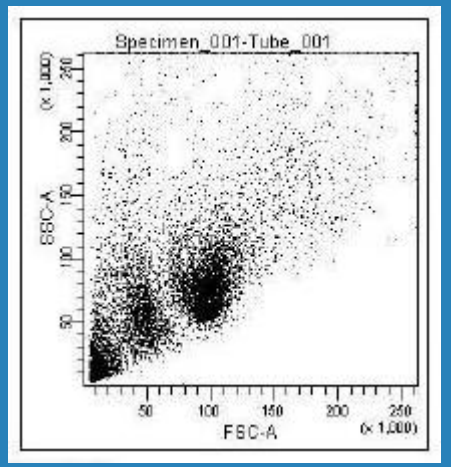


Density plot: viewing the frequency of subpopulations

Contour plot: showing the probability contouring



Dot plot: one parameter vs another



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