Basics of a Flow Cytometer

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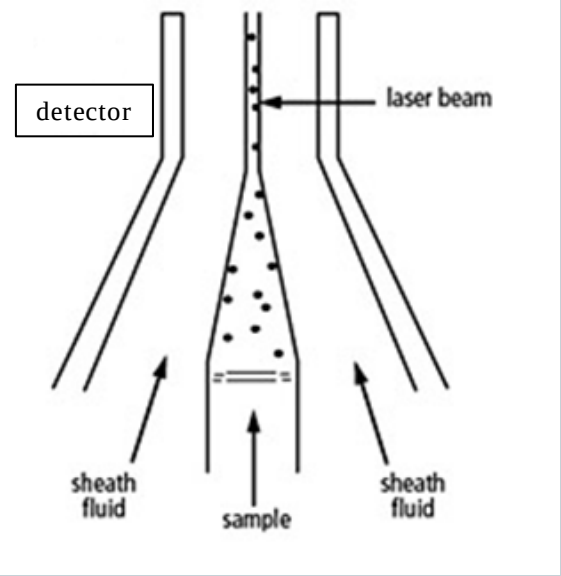
http://www.usask.ca/pharmacy-nutrition/research/groups/FlowCytometryUserGroup/Home.php

What is Flow Cytometry

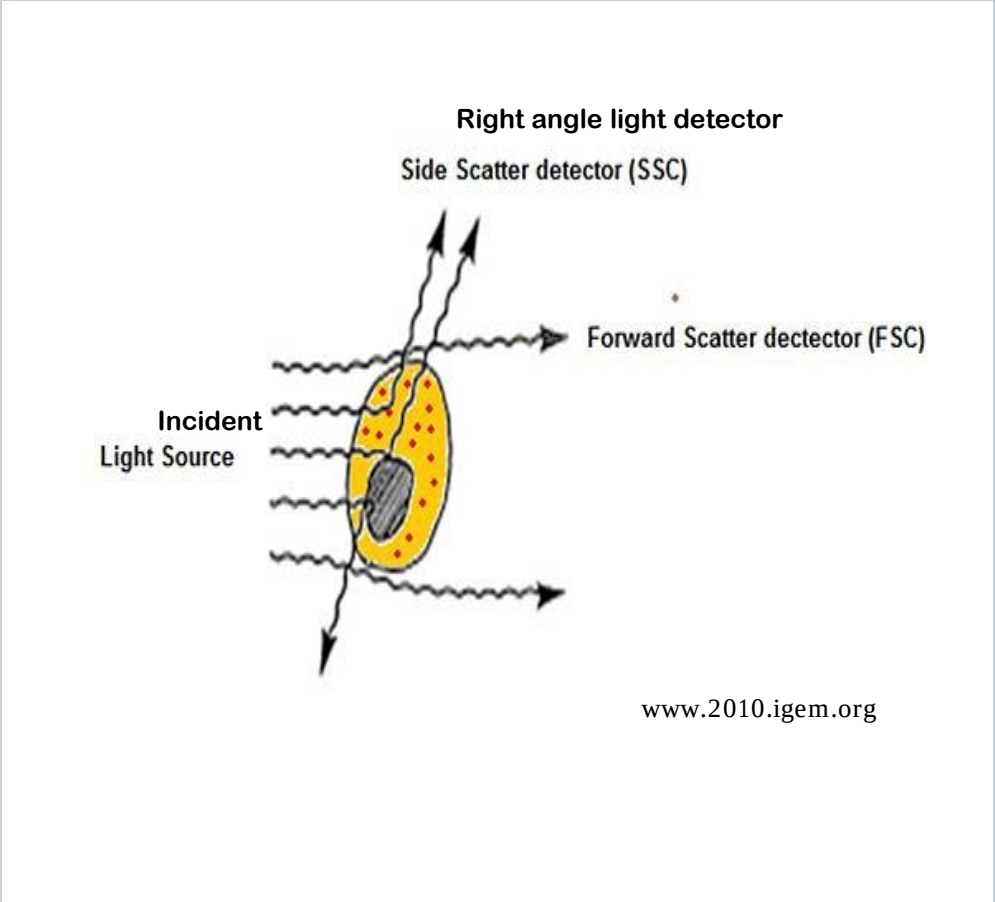
- flow cytometry is the measurement of cells/particles in a flow system, which delivers the cells/particles (0.2 to 150 µm) singly past a point of measurement.

- Points to consider
  - Flow
  - Light
  - Detection

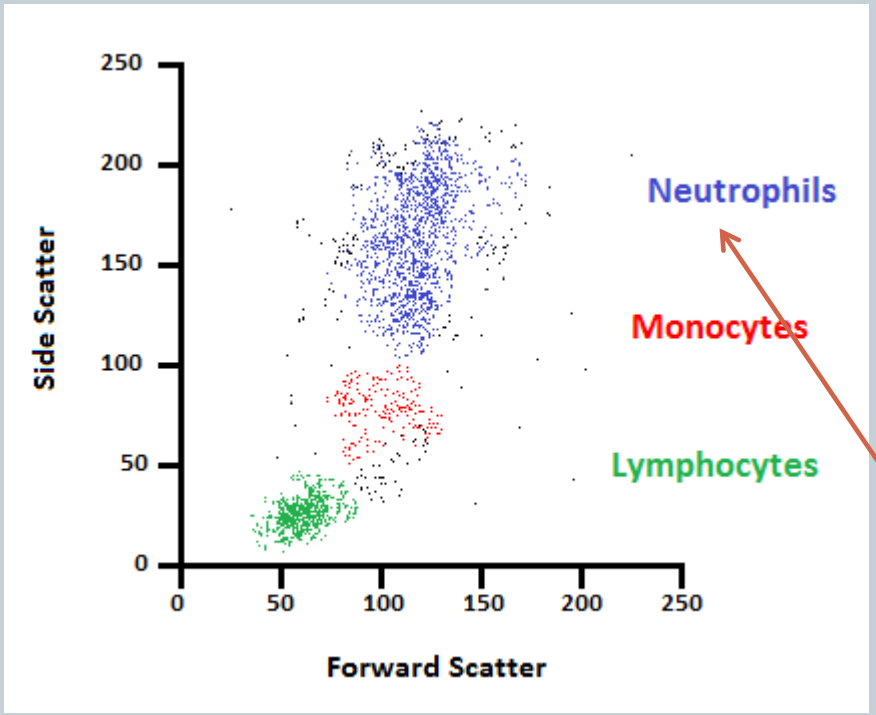


What can a Flow Cytometer Tell Us About a Cell/Particle?

- Relative size by **Forward Scatter**
- Relative granularity or internal complexity by **Side Scatter**
- Research question by **Relative fluorescence intensity** (ie marker)



Examples of application of forward and side scattering



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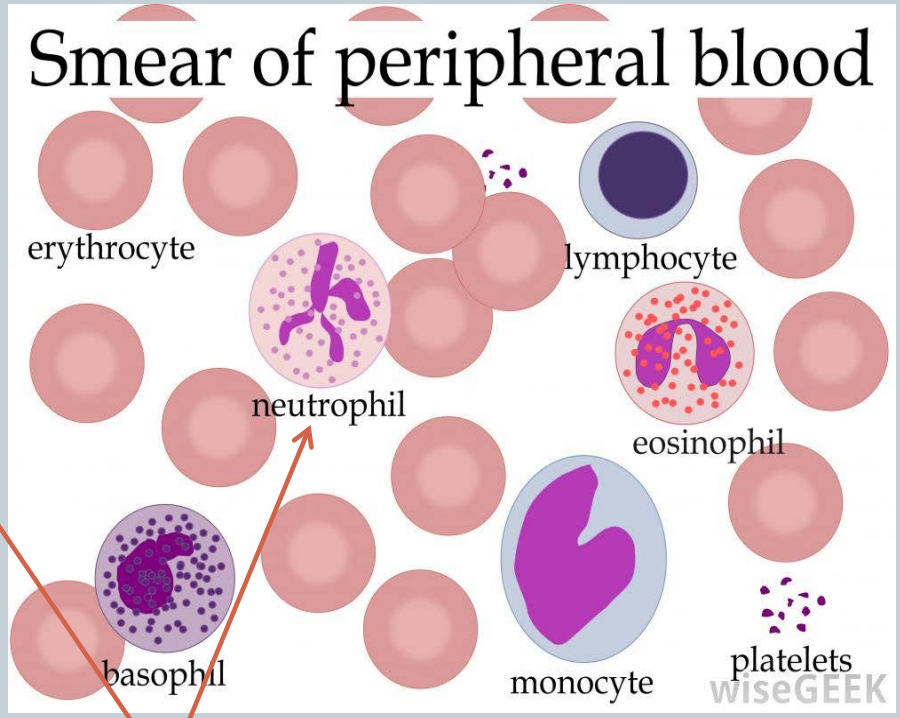
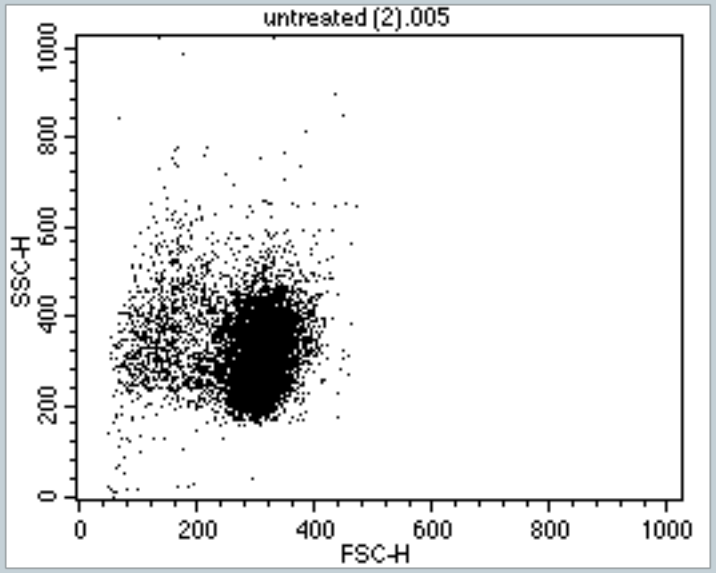
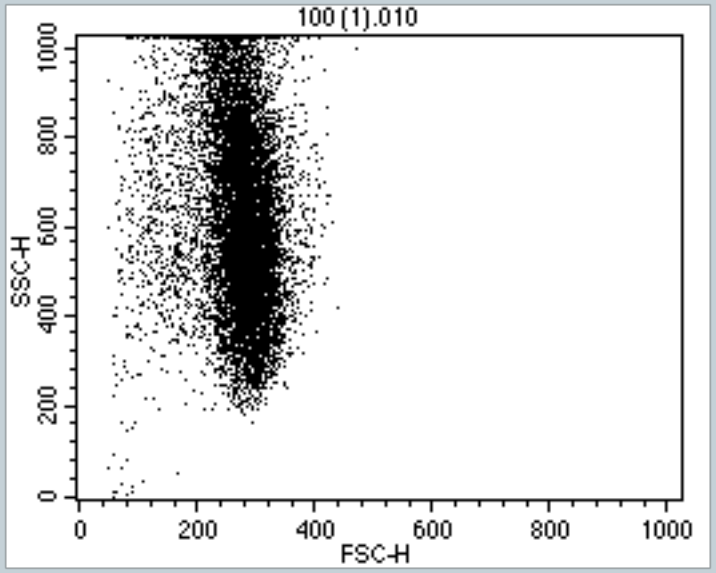


high internal complexity and large size

Examples of application of forward and side scattering

- Nanodiamond and titanium oxide particles in cellular uptake studies using HeLa cells increases the side scatter with little effect on forward scattering

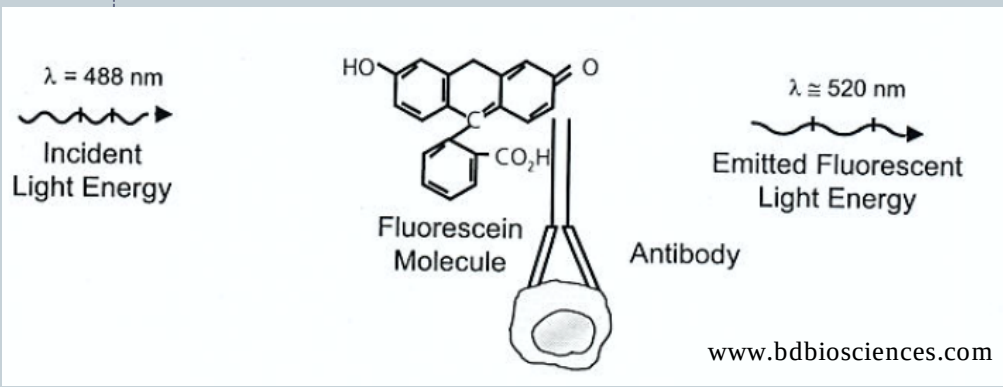


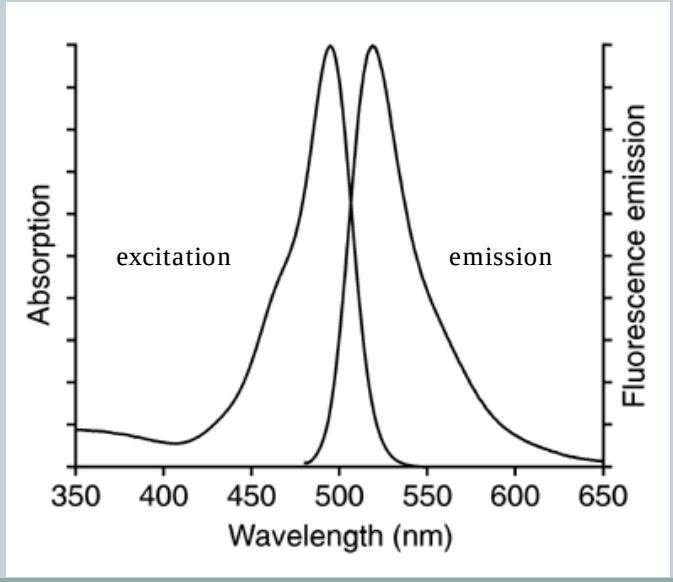


S. Alwani and Dr. Badea research

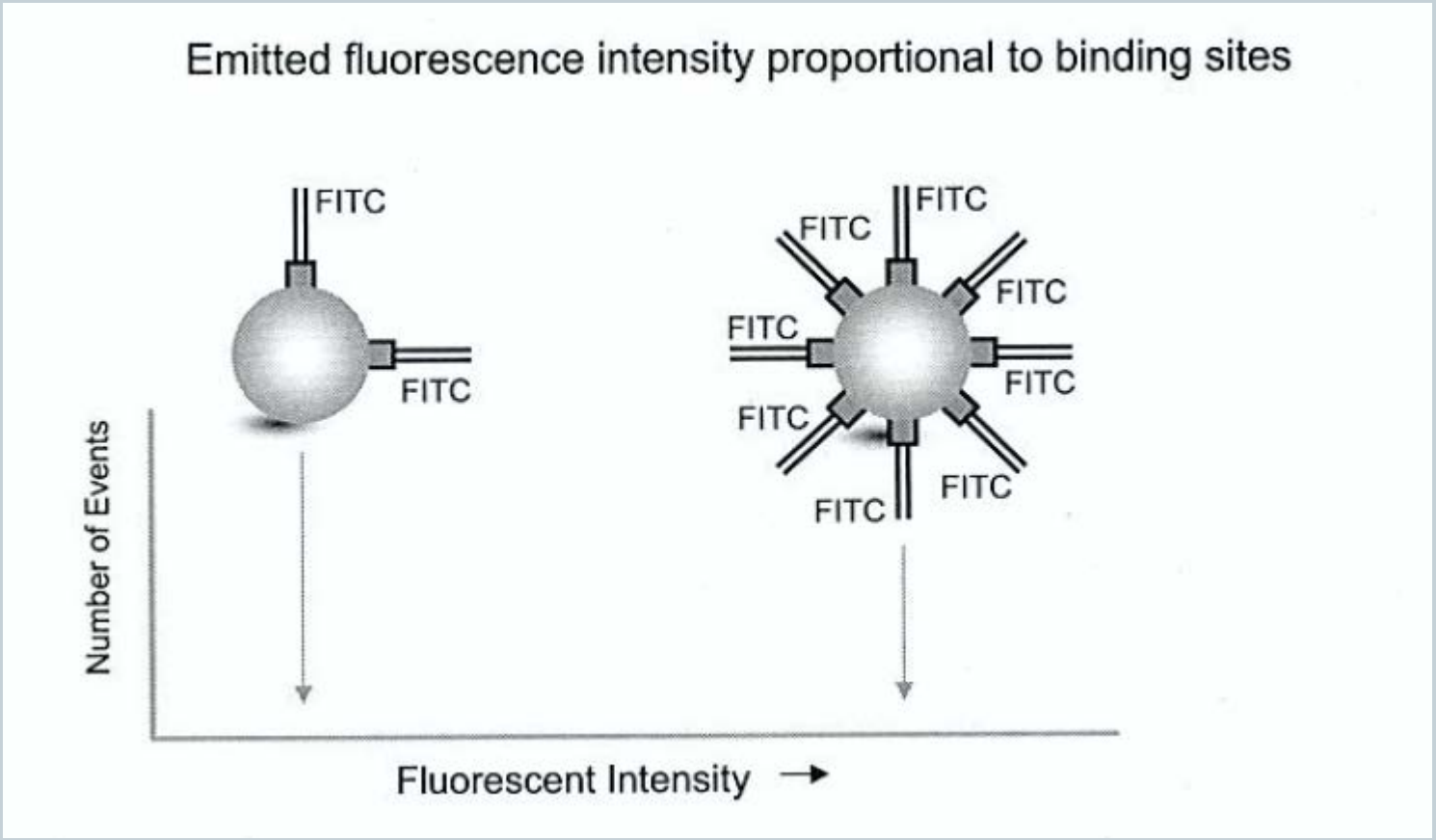
What is Fluorescent Light?

- The fluorochrome absorbs energy from the laser
- The fluorochrome releases the absorbed energy by emission of photons of a longer wavelength





Fluorescence

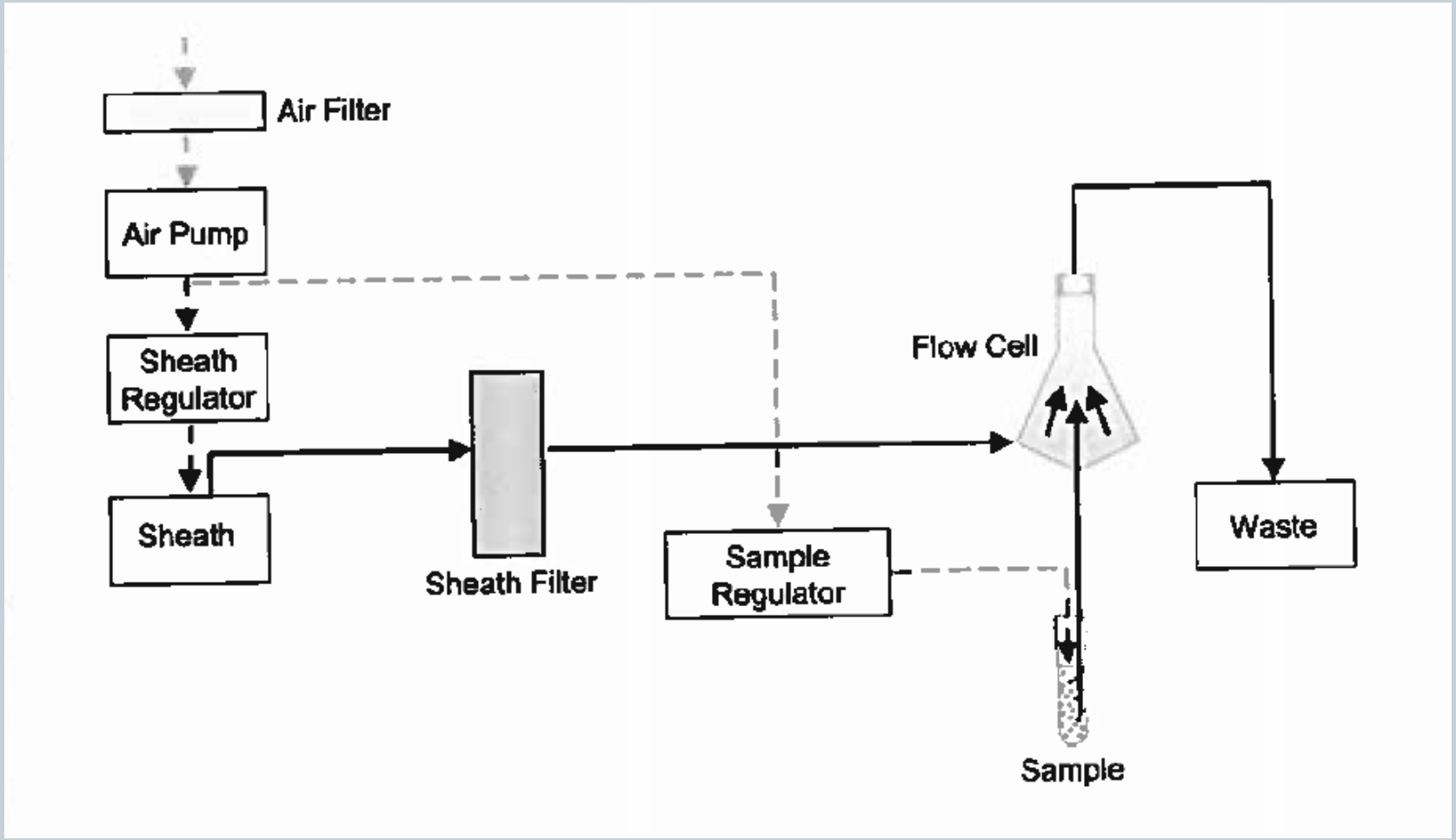


www.bdbiosciences.com

FACSCalibur

- Fluidics
  - Introduces and focuses the cells for interrogation
- Optics
  - Generates and collects the light signals
- Electronics
  - Converts the optical signal to digital signal, processes the signal and communicate with the computer

Fluidics


Air Filter
Air Pump
Sheath Regulator
Sheath
Sheath Filter
Sample Regulator
Flow Cell
Waste
Sample

Hydrodynamic focusing

- Slower moving sample stream is injected into a faster moving sheath stream
- Surface tension and laminar flow causes the sample to be “wicked off” into a narrower faster moving stream within the sheath stream (stream within a stream)
- Alignment of cells within this stream are controlled by velocity of the two streams

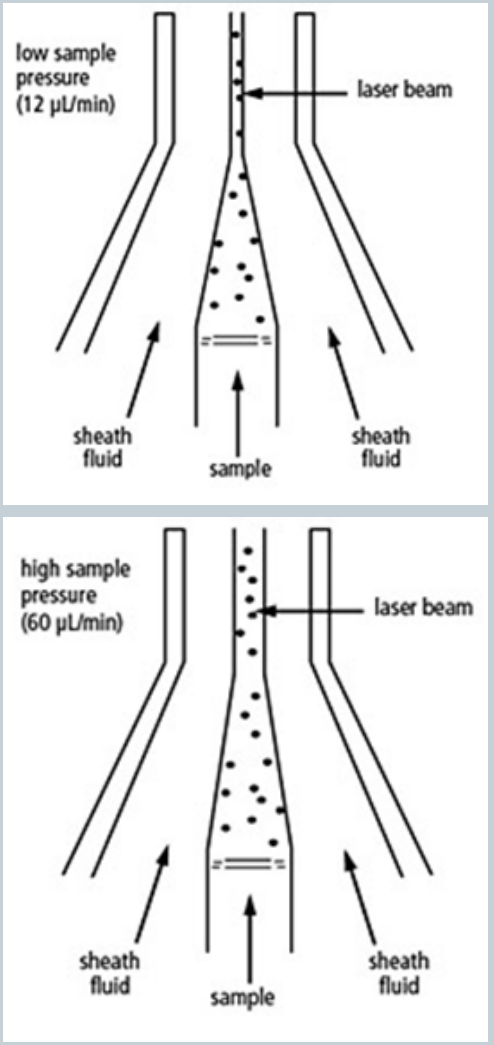
Flow cell

Higher resolution

(Quantitative)

Lower resolution

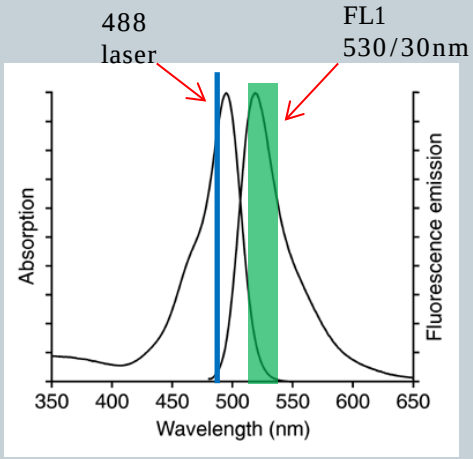
(Qualitative)



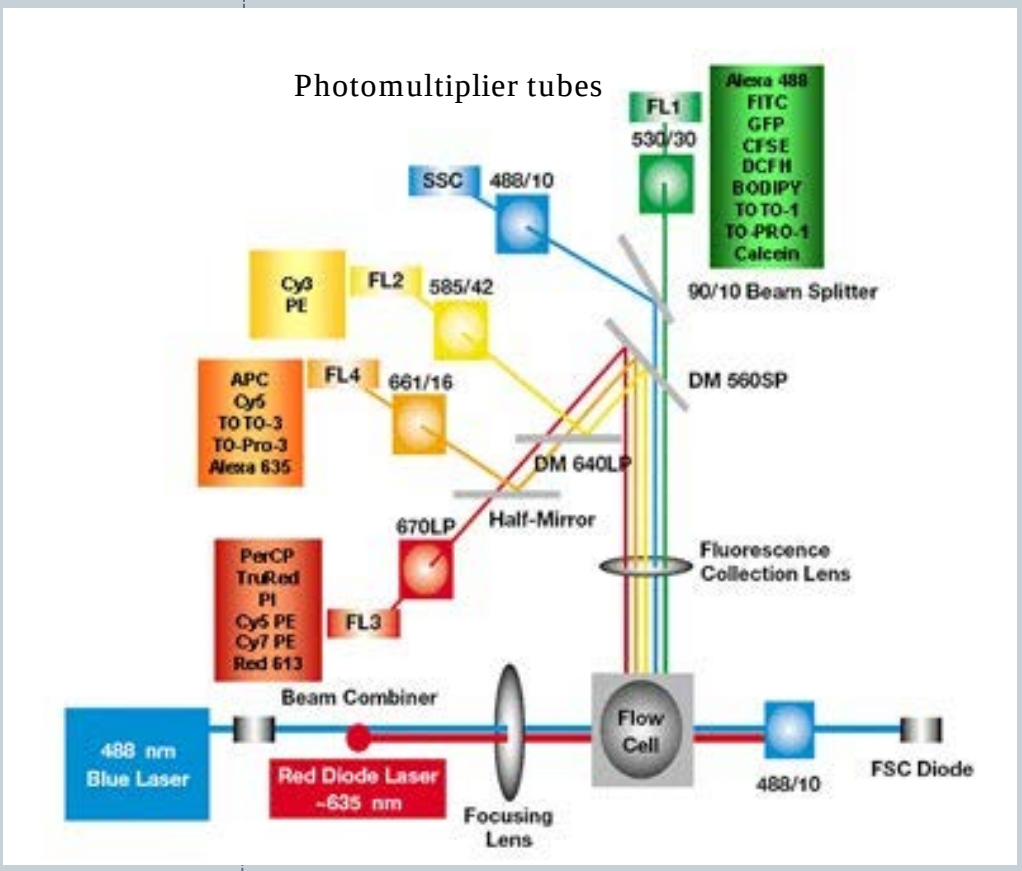
Optics

Excitation/Emission

- 2 lasers (488 and 635nm)
- 4 filters BP and LP (FL1 to 4)



www.uni-leipzig.de

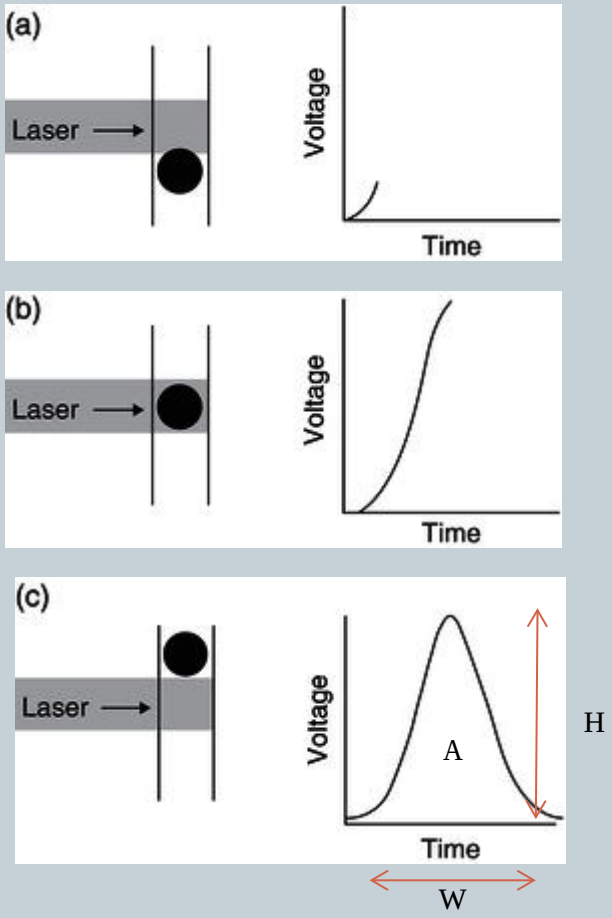


www.cnbc.pt

Pre-Amplifier

Levels:
E-1
E00
E01
E02
E03

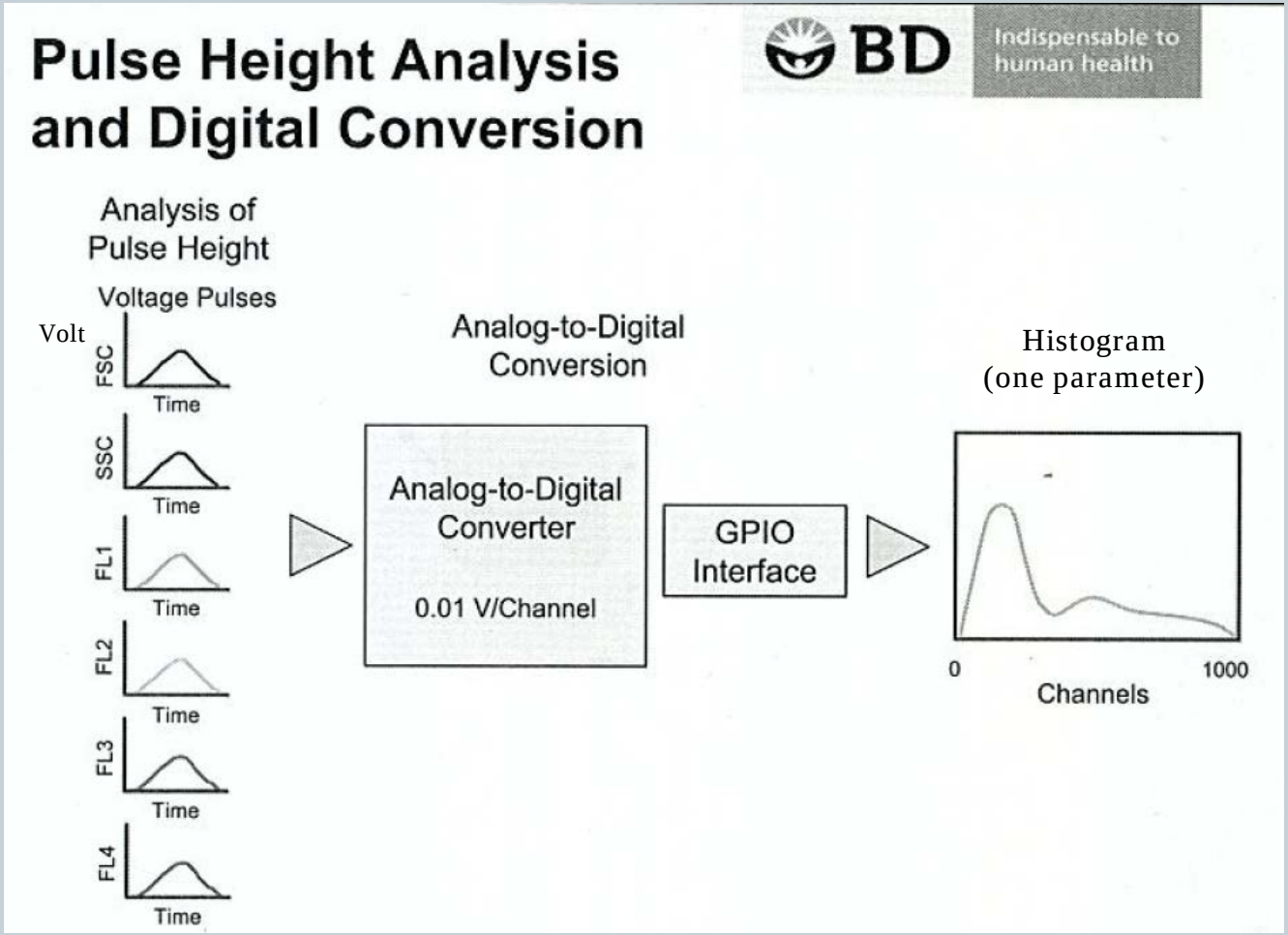
Electronics



Practical Flow Cytometry Haematology Diagnostics

- Pulse height (H), area (A) and width (W)
- Converts analog signals to proportional digital signals

Electronics



Plotting Data

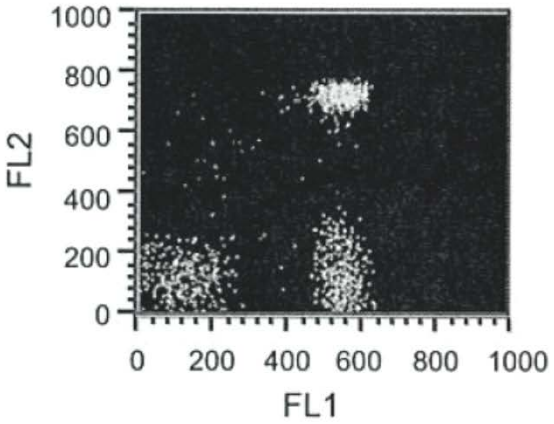
BD Indispensable to human health

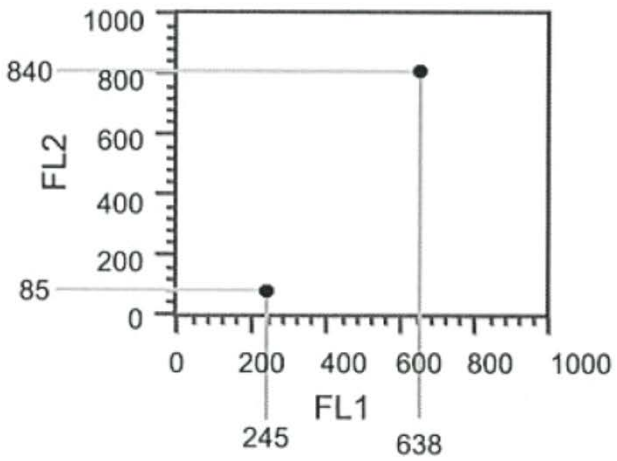
Dot Plot

List-Mode Data

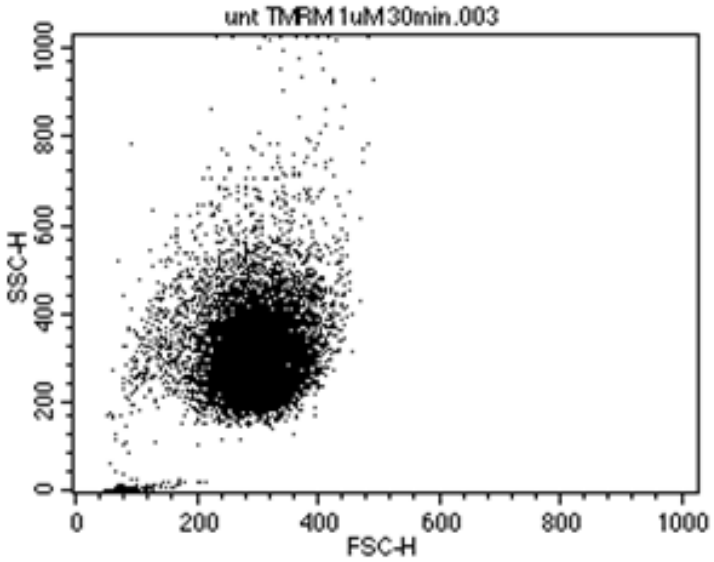
	FSC	SSC	FL1	FL2
Event 1	30	60	638	840
Event 2	100	160	245	85
Event 3	300	650	160	720

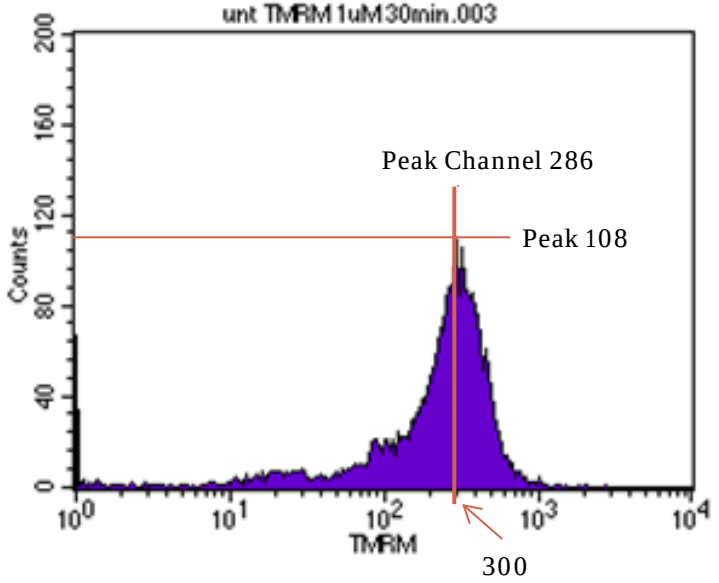
Channel values





Plot Stats (Cell Quest Pro)





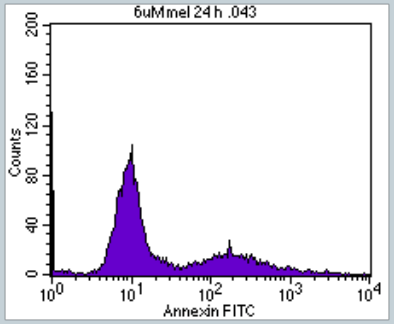
Sample ID: unt TMRM 1uM 30min
Gate: No Gate
Total Events: 10000
Acquisition Date: 27-Aug-14
Gated Events: 10000
X Parameter: TMRM (Log)

Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak	Peak Ch
10000	100.00	100.00	270.55	212.47	54.49	266.55	108	286

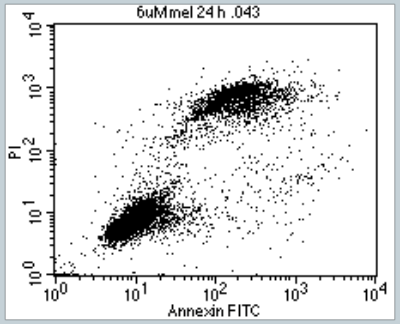
$$Mean = \frac{x_i}{n}$$

$$Geo\ Mean = 10^{\frac{\sum log(x_i)}{n}}$$

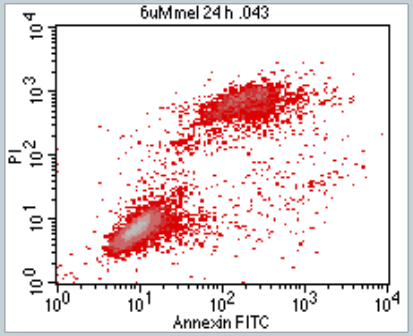
Types of Plots



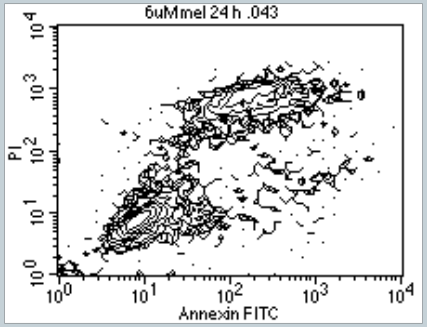
Histogram



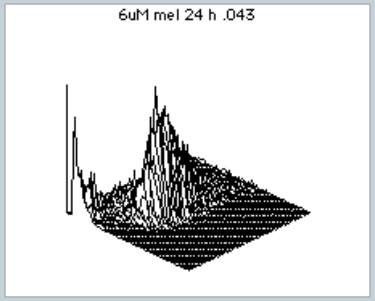
Dot plot



Density plot



Contour plot



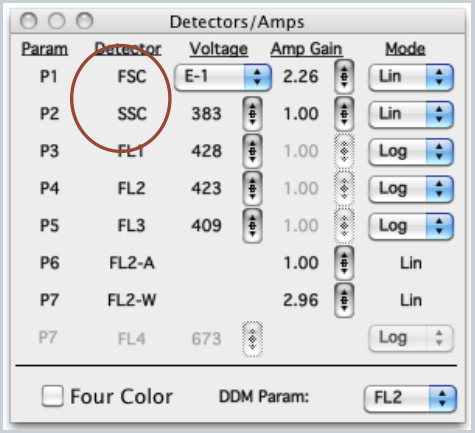
3D plot

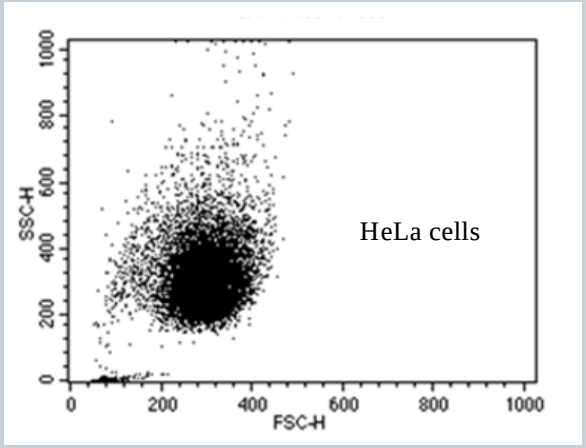
Flow Cytometer Setup

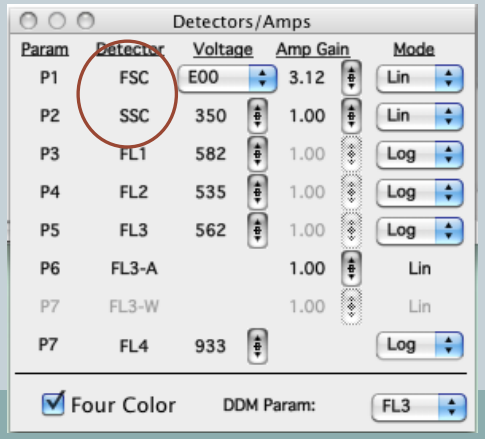
- Set the forward and side scatter detectors for your untreated, unstained cell population of interest
- Set fluorescence detectors sensitivities
- Using more than one fluorescence marker? Do you need to correct spectral overlap with compensation?
- Collect data from your samples

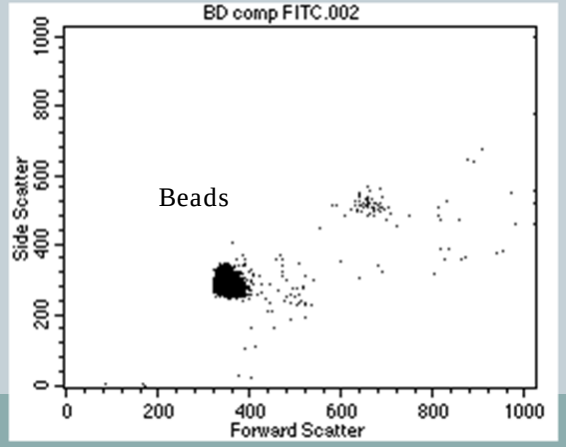
Setting FSC and SSC

- Set forward and side scatter detectors to untreated cells.



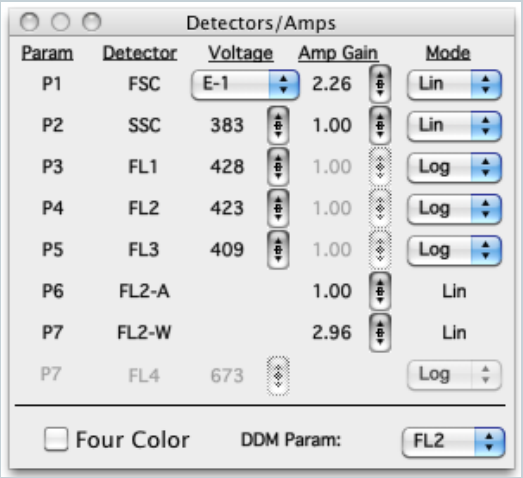


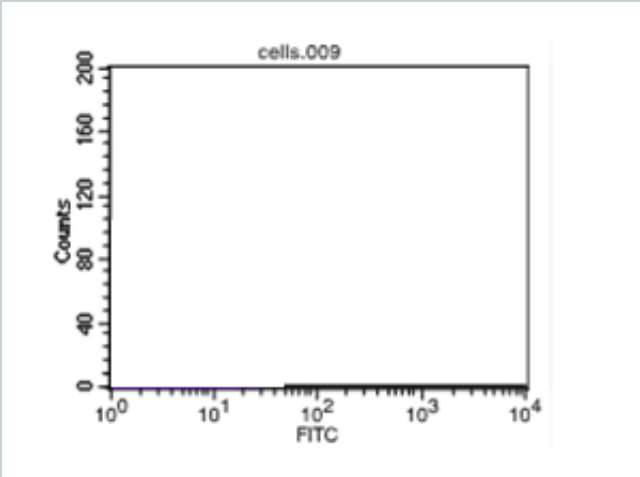


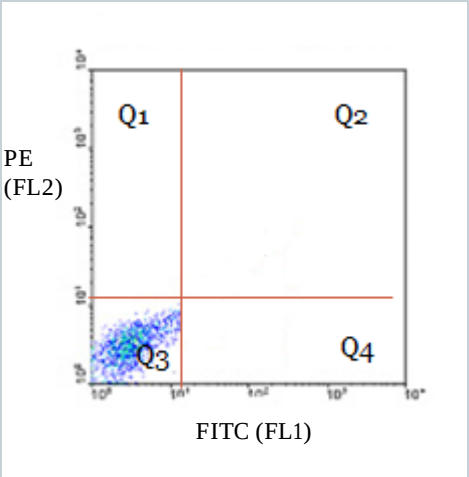


Setting Fluorescence Detectors

- Set FL1 and FL2 detectors so that the auto fluorescence from unstained cells are set within the first log decade (10^0 to 10^1)

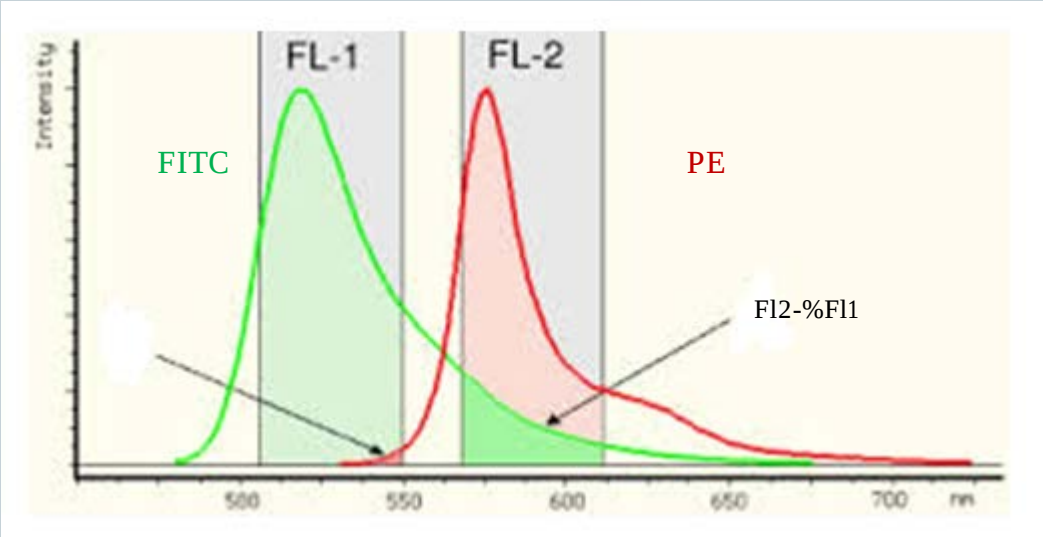






Compensation

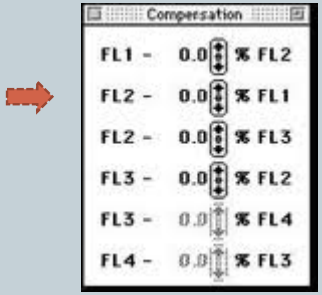
- When analysing more than one color be careful of spectral overlap
- Digital FACS have software for this.

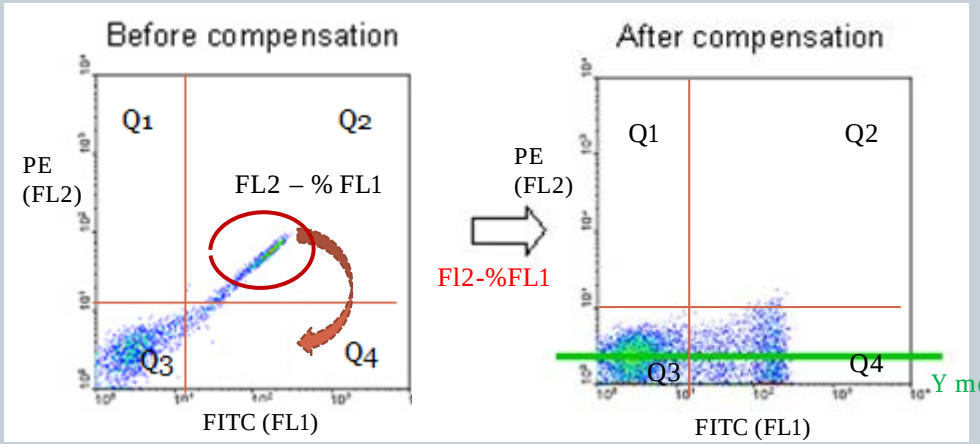


Flowcyt.salk.edu

Compensation

Cells + FITC

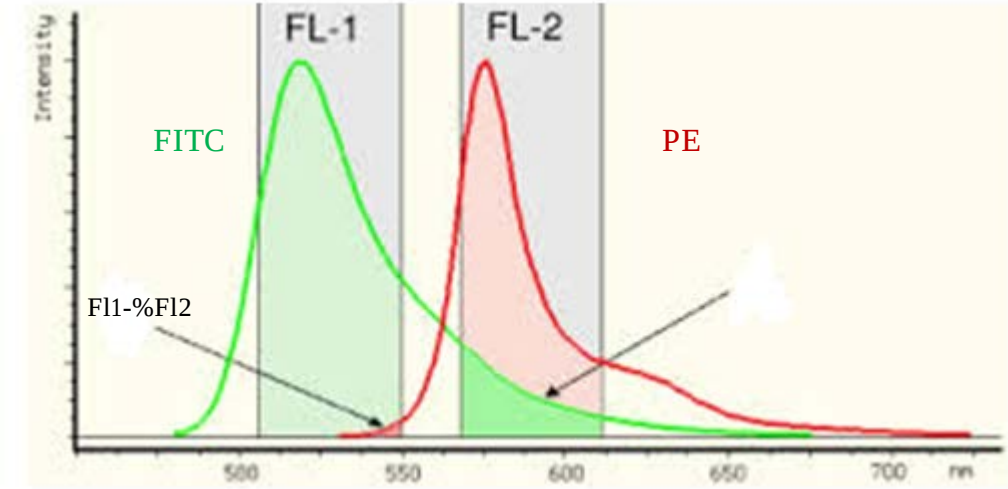




Quadrant Statistics

Quad	Events	X Mean	Y mean
Q1	0	***	***
Q2	0	***	***
Q3	7198	3.87	3.27
Q4	2786	336.3	3.32

Compensation



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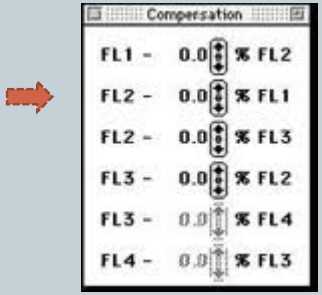
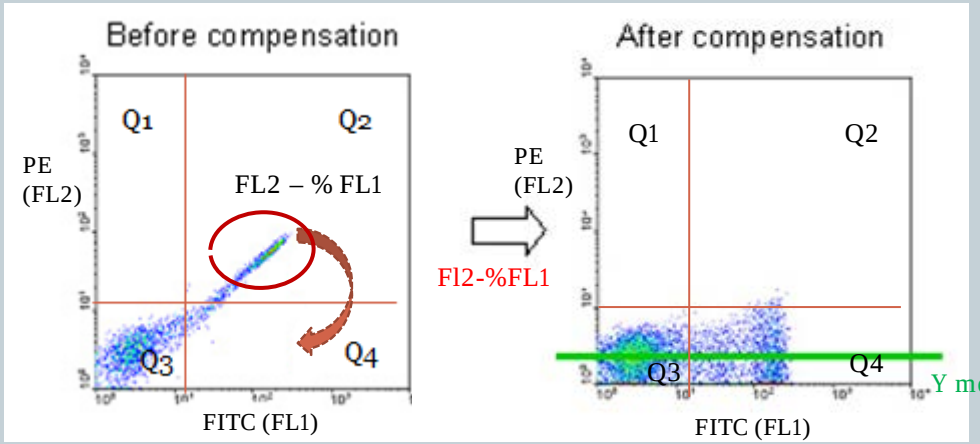
Compensation

Cells + FITC





Quadrant Statistics			
Quad	Events	X Mean	Y mean
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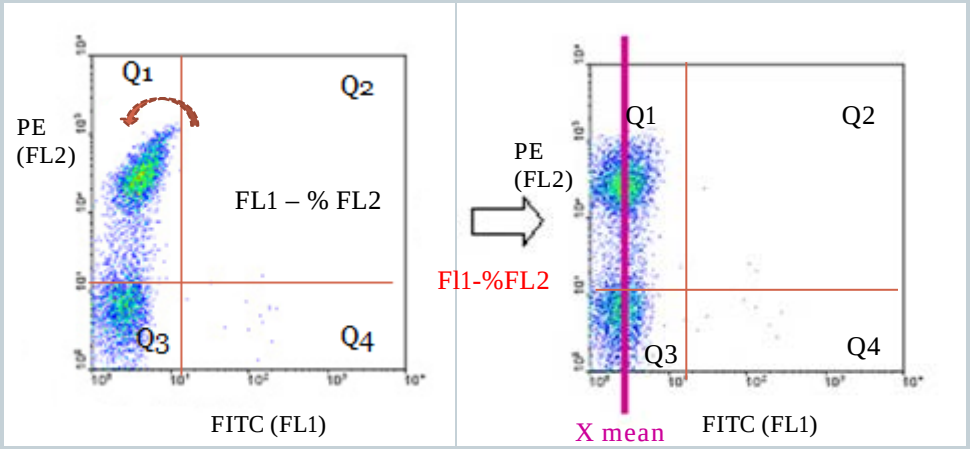
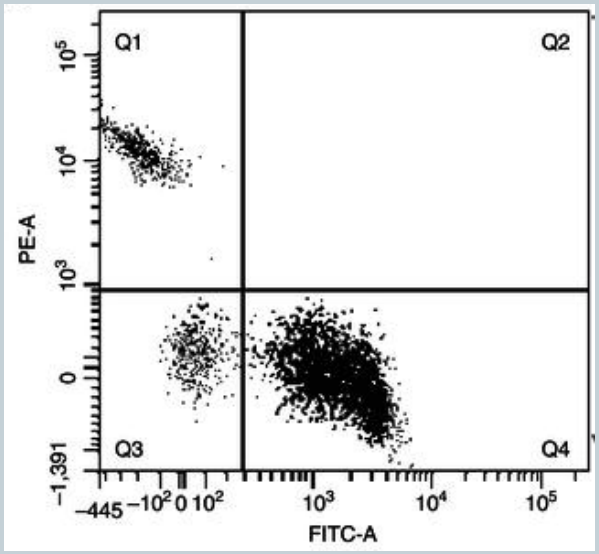
Q3	7198	3.87	3.27
Q4	2786	336.3	3.32

Cells + PE



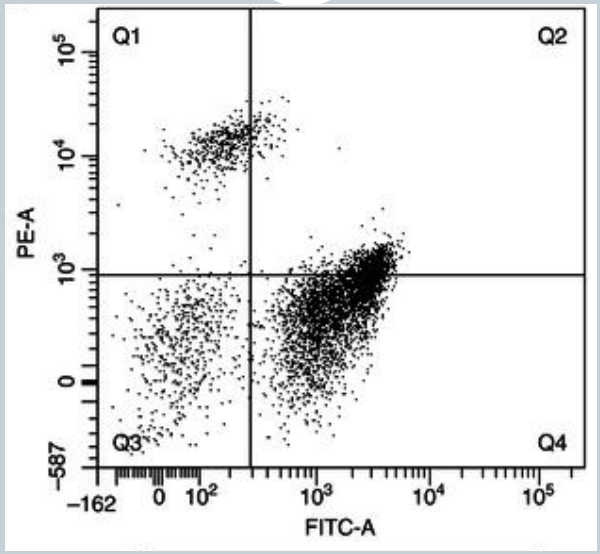
Quadrant Statistics			
Quad	Events	X Mean	Y mean
Q1	318	3.51	600.71
Q2	0	***	***
Q3	1035	3.86	4.03
Q4	0	***	***

Quiz



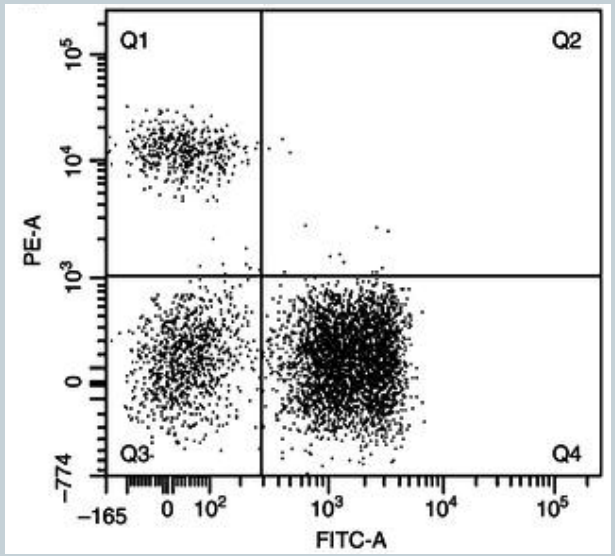
Population	FITC-A Mean	PE-A Mean
Q1	−202	12,685
Q2	1,553	1,539
Q3	59	217
Q4	1,835	−109

Over compensated



Population	FITC-A Mean	PE-A Mean
Q1	164	12,399
Q2	2,728	2,598
Q3	63	238
Q4	1,610	483

Under compensated



Population	FITC-A Mean	PE-A Mean
Q1	49	12,786
Q2	1,398	5,330
Q3	44	173
Q4	1,784	176

Compensated

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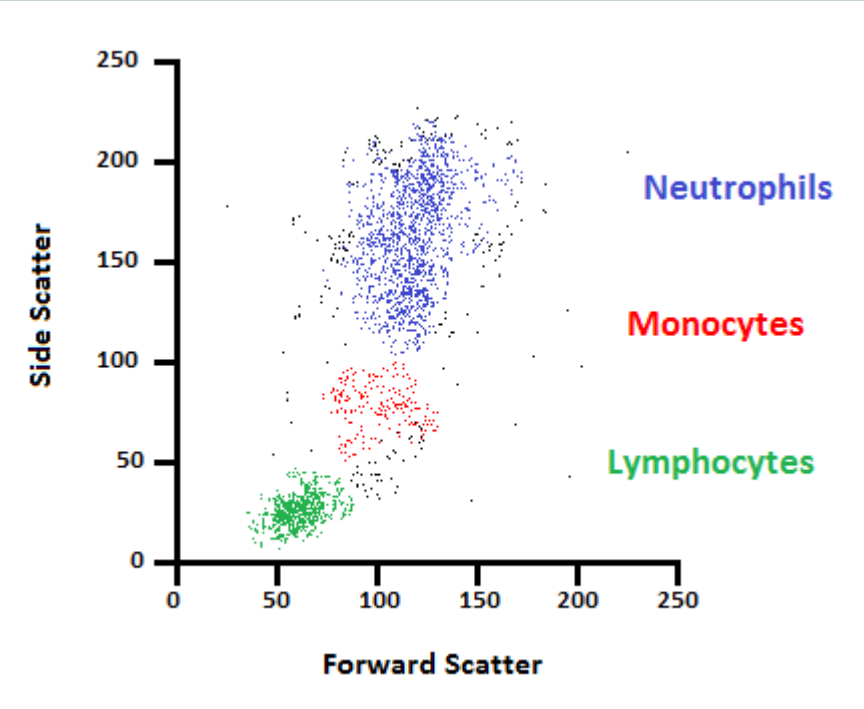
Gating

- Isolate populations of interest
- Gating an area will make your analysis more specific
- Can remove dead cells and debris
- Cannot discriminate between cells with the same scattering properties

Gating Example



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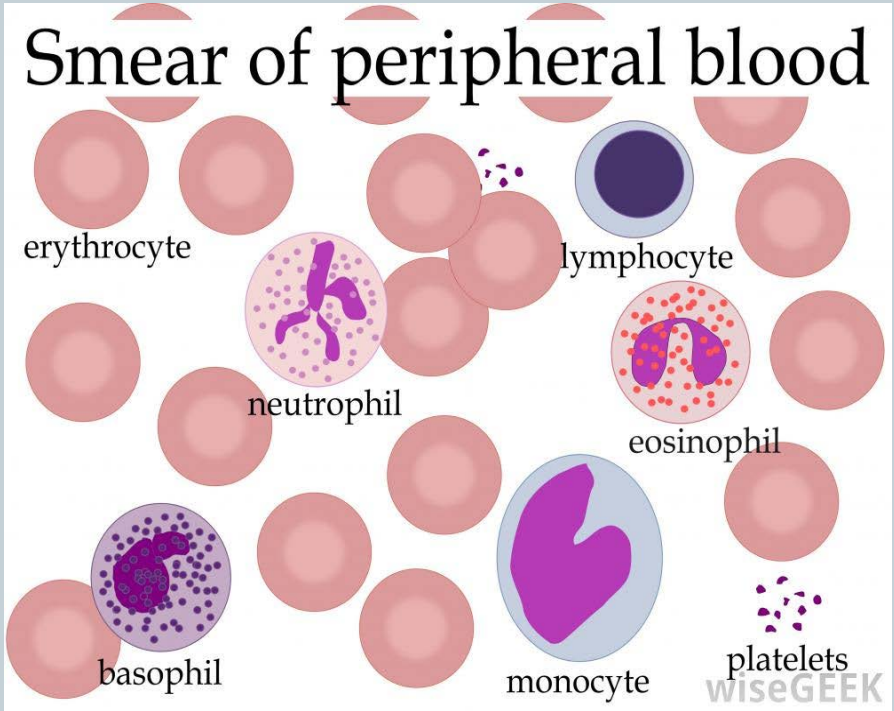
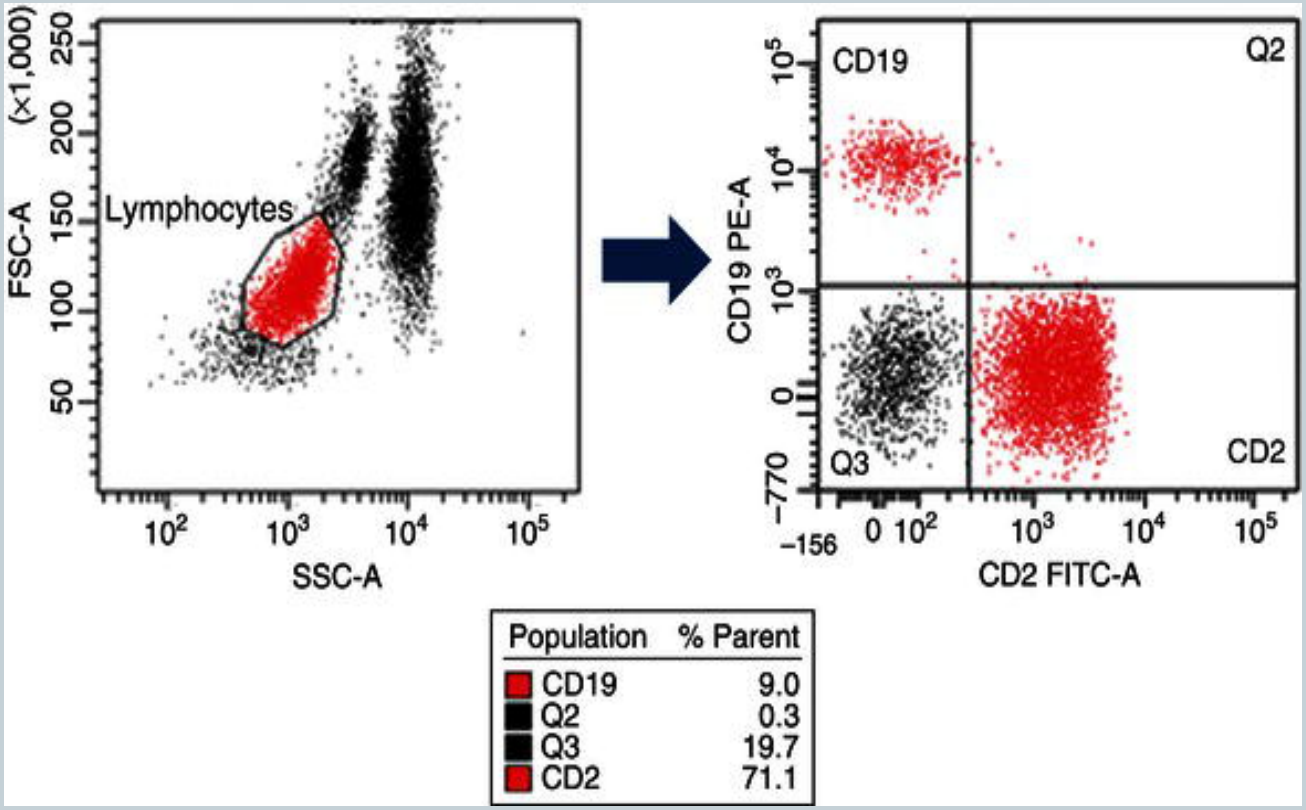


Isolating populations of interest

Gating Example



Cannot discriminate between cells with the same scattering properties

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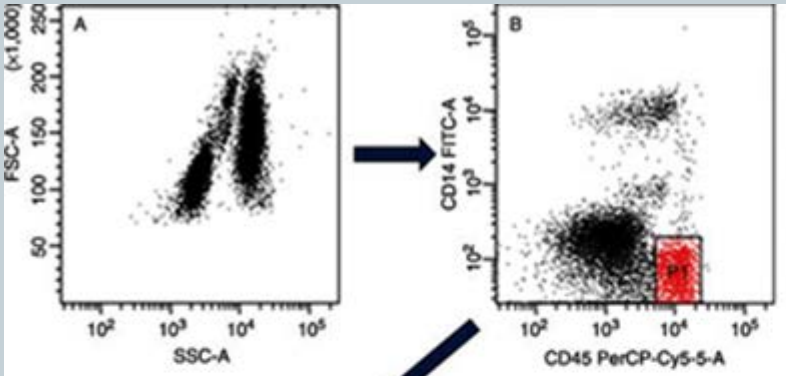
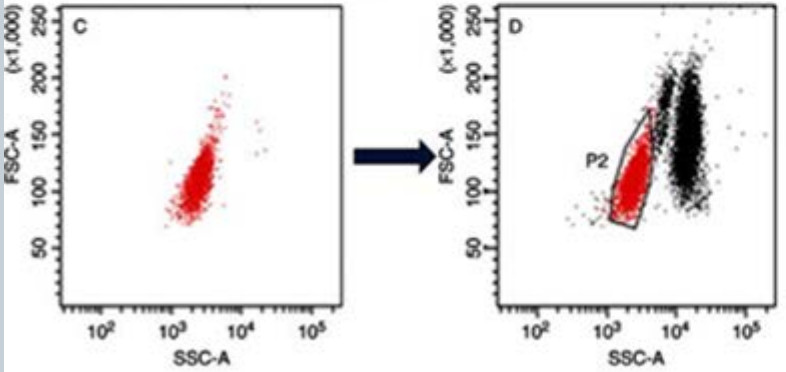
Back Gating Example



Back gating of P1



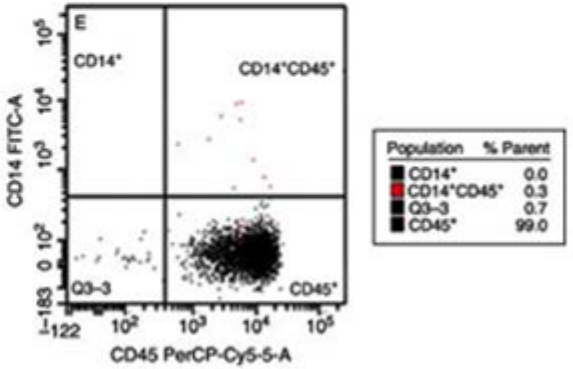
Which population is Lymphocytes?

Leucocytes CD45+

Monocytes CD14+

Lymphocytes CD14-, CD45+Bright

Fluorescence of P2



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Applications

- Immunophenotyping / Intracellular antigens measurement
- DNA/RNA: cell cycle, aneuploidy, endoreduplication, kinetics
- DNA base ratios
- Chromatin structure
- Apoptosis (DNA degradation, mitochondrial membrane potential, permeability changes, caspase activity)
- Membrane potential
- Membrane fluidity
- Membrane fusion/runover
- Intracellular calcium (ions) flux
- Intracellular pH
- Sulfhydryl groups/glutathione
- Cell viability
- Cell tracking and proliferation
- Intracellular reactive oxygen species (Oxidative burst)
- Cell proliferation
- Cell enumeration
- Cell volume and morphological complexity
- Cell pigments (f.ex. chlorophyll or phycoerythrin)
- Drug delivery
- Multidrug resistance (MDR)
- Phagocytosis
- Pathogen-host cell adherence
- Differentiation
- Identification of “stem cells”
- Reticulocyte, platelet etc analysis
- Microparticles analysis
- Assessing infection/transfection levels
- Monitoring of the electropermeabilization of cells
- Cytotoxicity assay
- Enzymatic activity
- Cell activation
- Protein-protein interactions (FRET, split-GFP)
- Protein modifications, phospho-proteins
- Activation of signalling pathways
- Cytokine Secretion
- Sorting (f.ex sperm sorting for sex preselection)
- Karyotyping
- Telomere length

Build Your Own Flow Cytometer