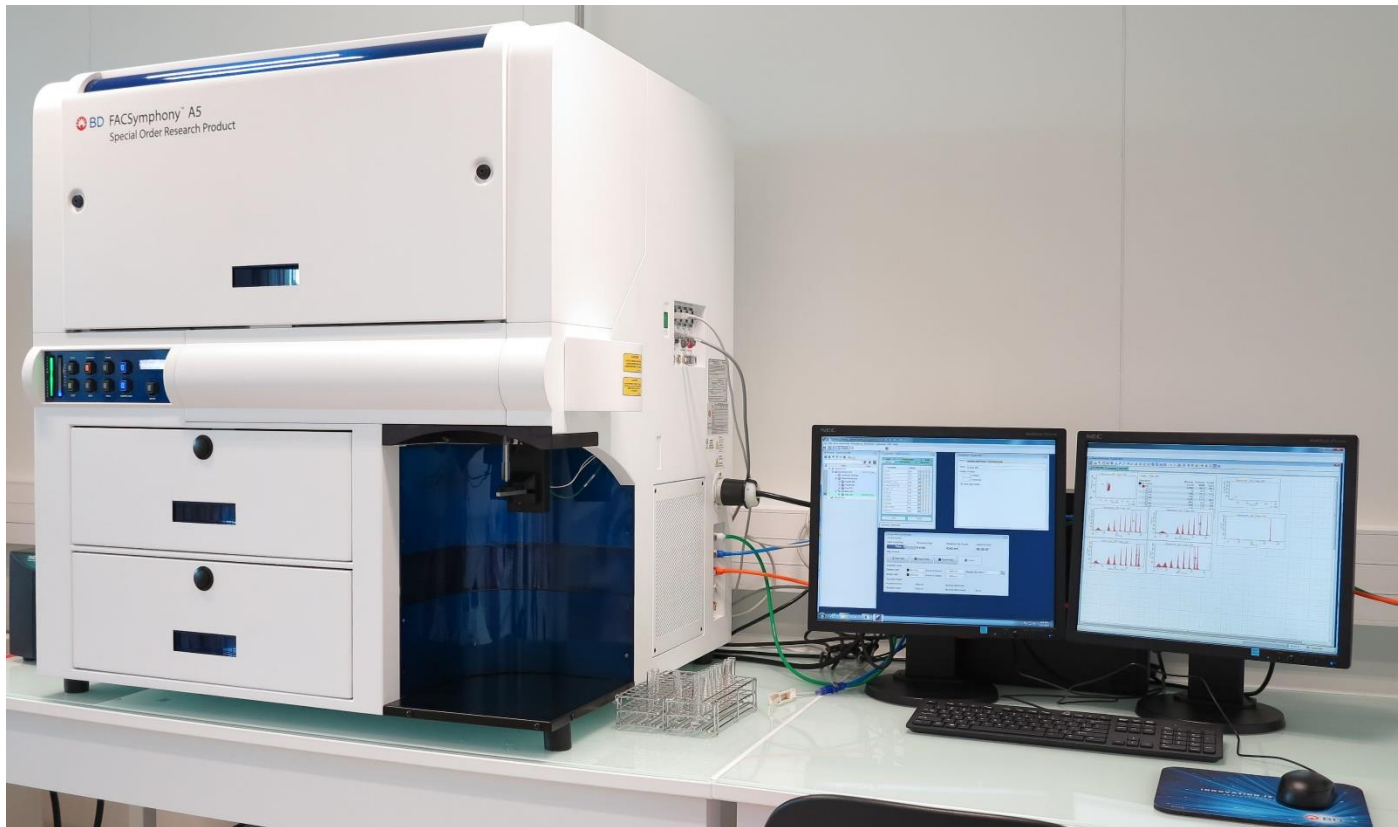# Flow Cytometry

Dr. Esmaeil Sadroddiny

Medical Biotechnology

Tehran University of Medical Sciences

"Flow cytometry": What stands for?

- Measurement (metry) of cells (cyto) in a fluid (flow)

Flow cytometry applications

- Cell size
- Cell shape
- DNA,RNA and protein content
- Internal and external receptors
- Cell membrane structure
- Apoptosis
- Calcium flux

Advantages

- It is very **FAST**: you can measure tens of thousands of particles per second.
- There is a **LOT of information** to be gained. Many physical and fluorescent parameters are measured simultaneously allowing for the resolution of distinct populations and cellular functions in complex and heterogeneous mixes.
- Data is **qualitative, quantitative** and unbiased.
- **Pure, sterile, live cells** of interest can be retrieved for downstream applications such as culture, functional assays, transplant, imaging and "omics" including DNA and RNA sequencing, proteomics, metabolomics, etc…
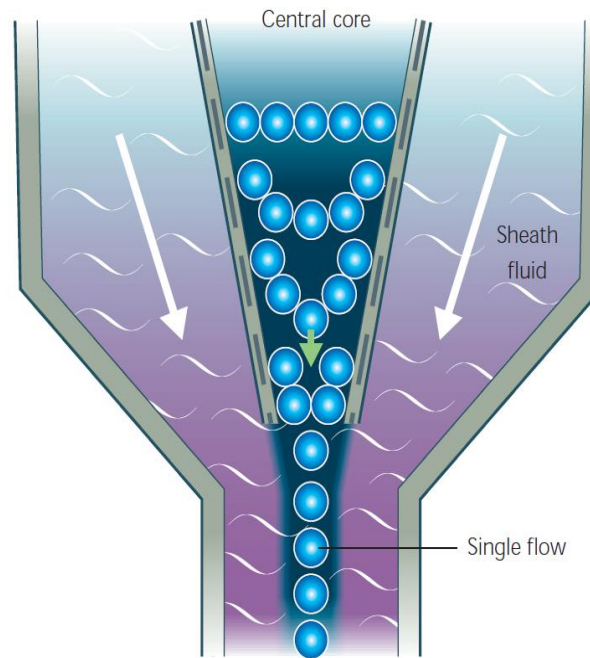
Main parts

- Fluidic system
- Optical system
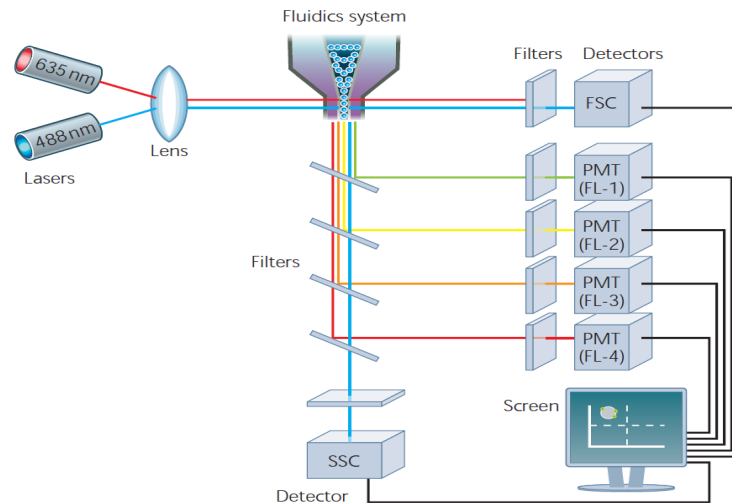- Electronic parts

Typical Flow cytometer set up
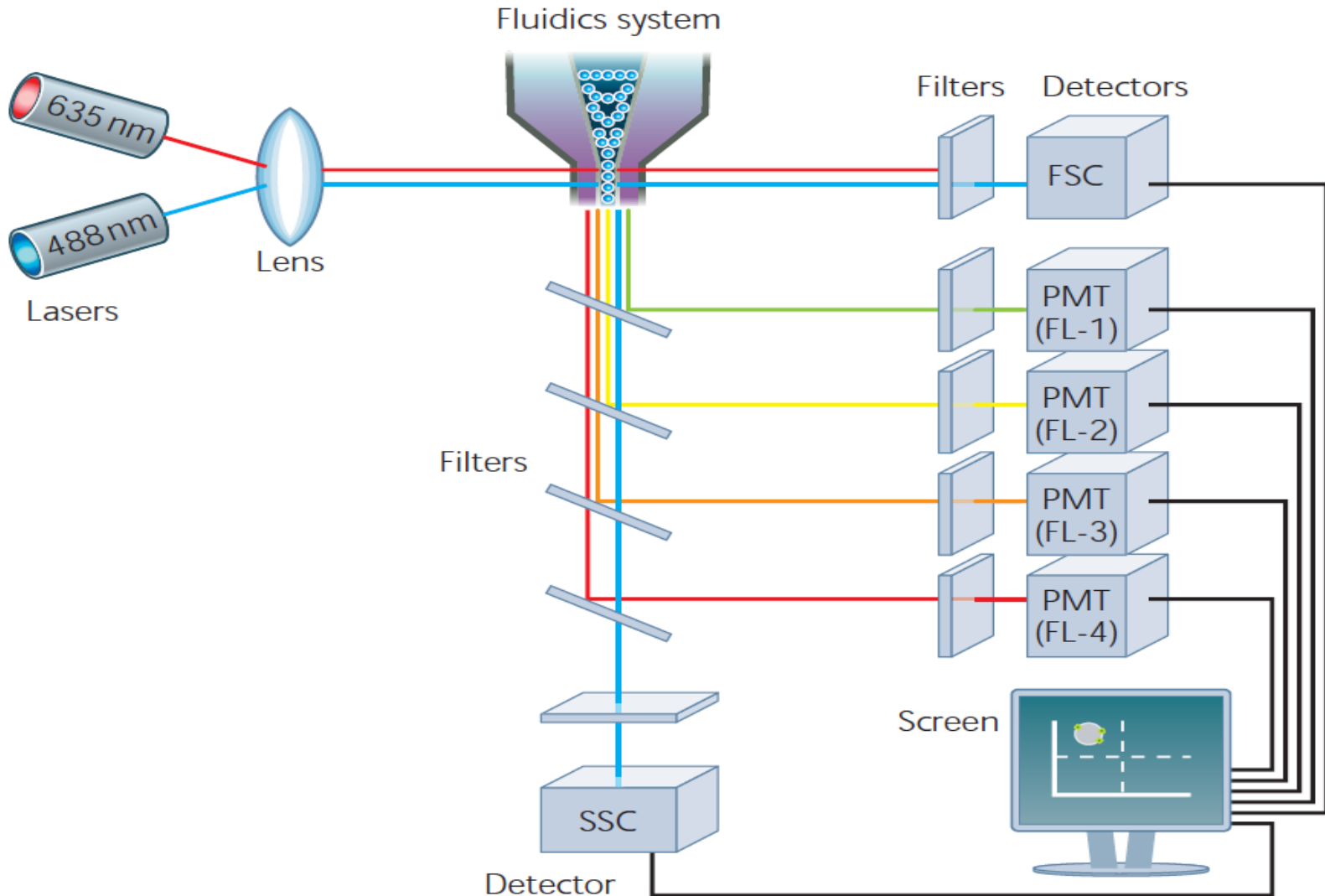
Fluidic system

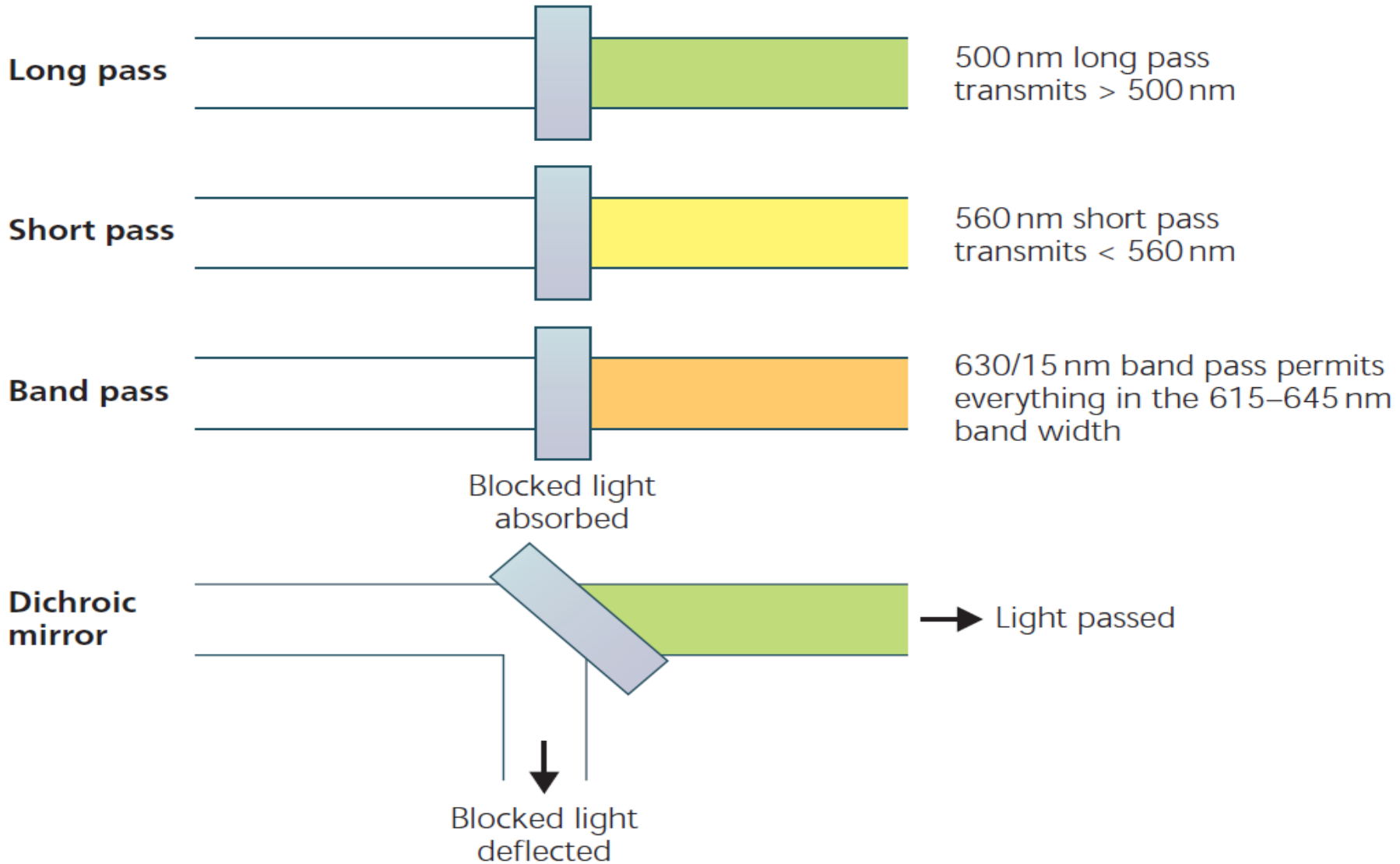- What function for "hydrodynamic focusing"?

Optics and detection system

- **Light source**: Laser or arc lamp

- **Lens and filters:**

- **Detectors**: either silicon photodiodes or photomultiplier tubes (PMTs)

Types of filters

**Long pass** — 500 nm long pass transmits > 500 nm

Short pass — 560 nm short pass transmits < 560 nm

Band pass — 630/15 nm band pass permits everything in the 615–645 nm band width

Blocked light absorbed

Dichroic mirror — Light passed

Blocked light deflected

Signal Processing

- Log and linear scaling
- **Parameter**: The measurement from each detector, forward scatter (FSC), side scatter (SSC) or fluorescence (FL).
- **Event**: The data acquired in each parameter, refer to the number of cells displaying the physical feature or marker of interest

Forward Scatter Channel(FSC) and Side Scattering Channel (SSC)

Cell images from WebMD
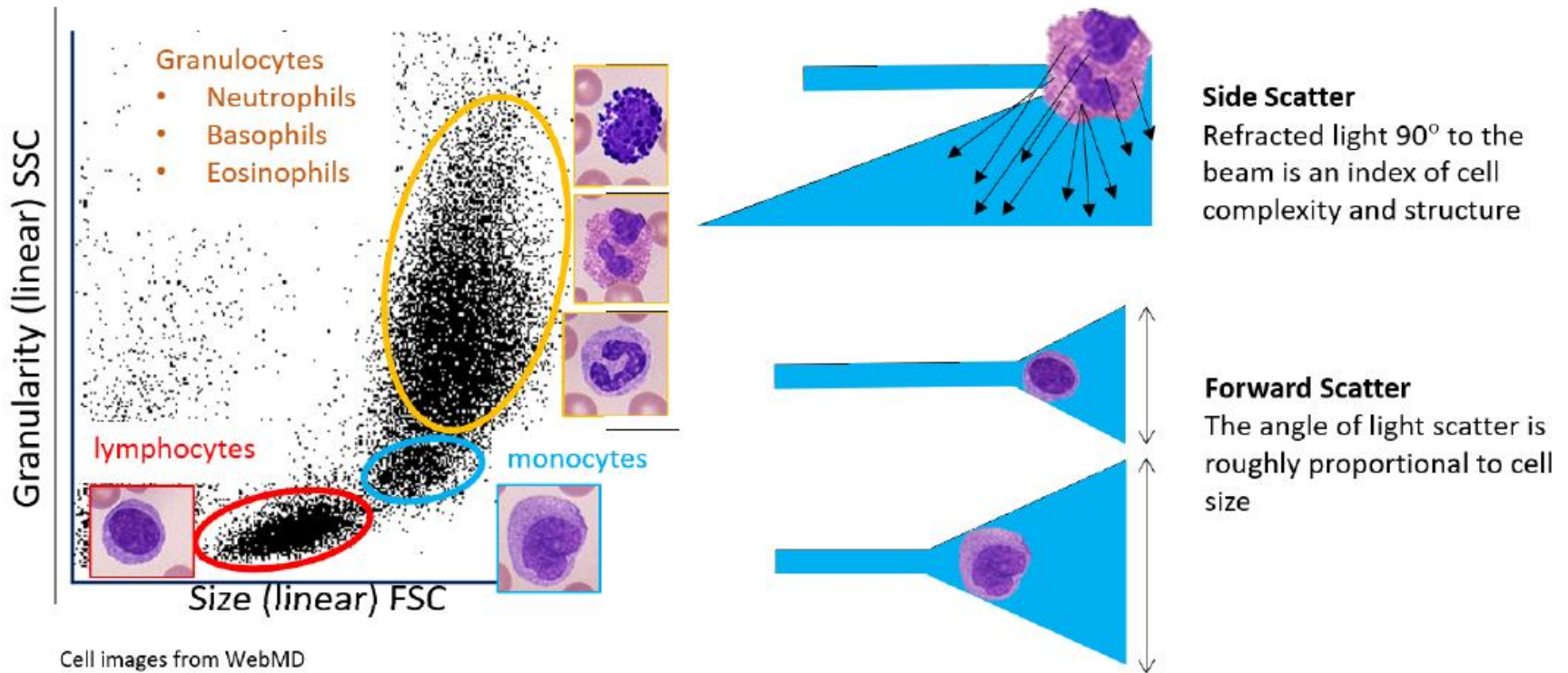
Cell sorting

- FACS^{TM}
- Break-off point
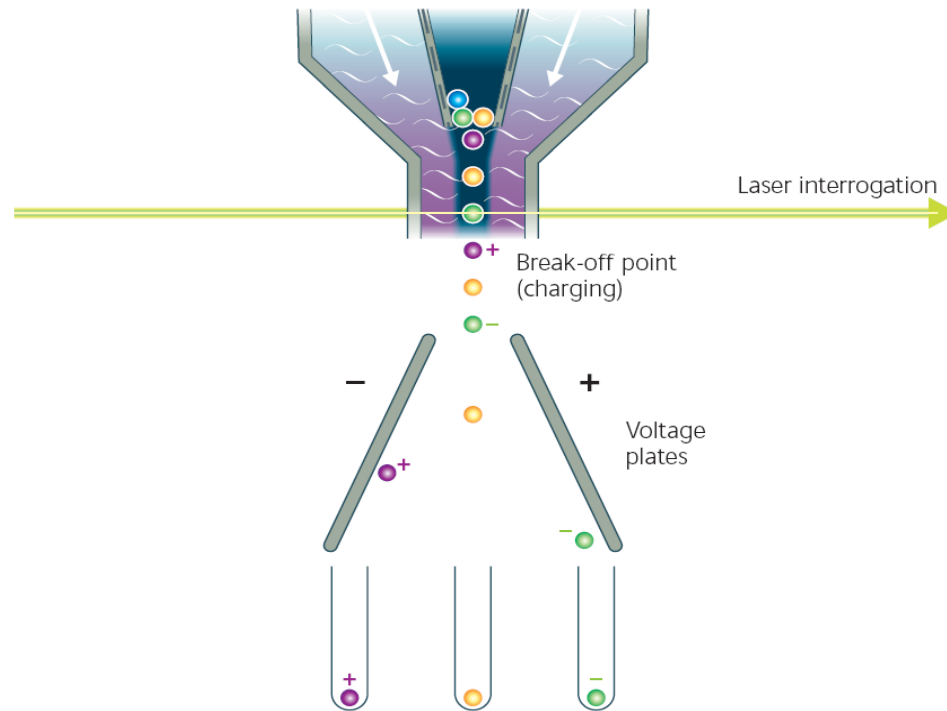
Principles of fluorescence

- Fluorochromes: dyes, which accept light energy at a given wavelength and re-emit it at a longer wavelength.

- Excitation and emission

- Light: frequency and wavelength

Higher energy   Lower energy

400 nm   500 nm   600 nm   700 nm

Ultraviolet   Infrared

Visible spectrum
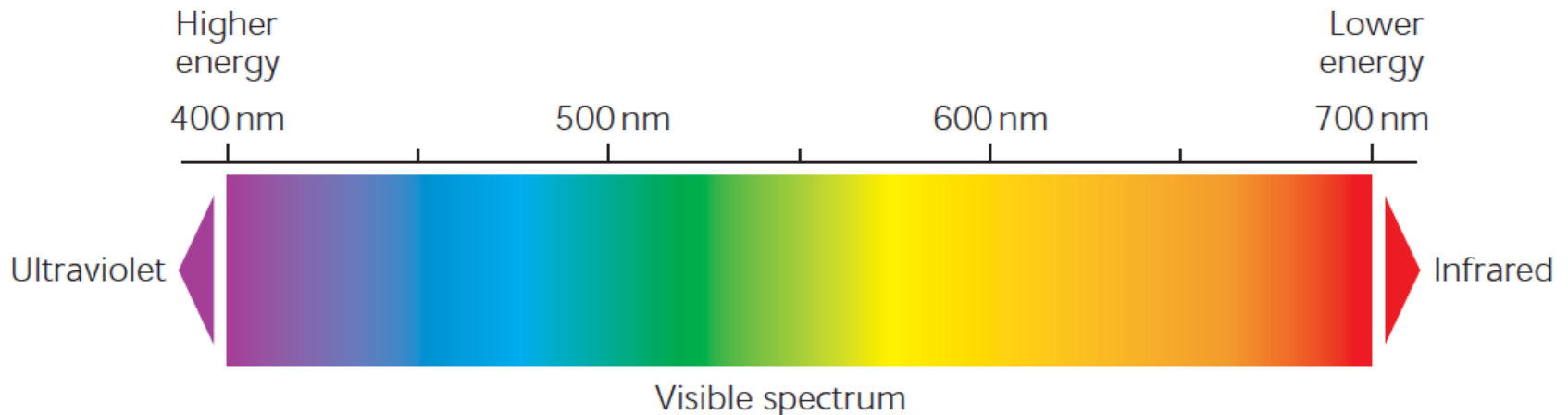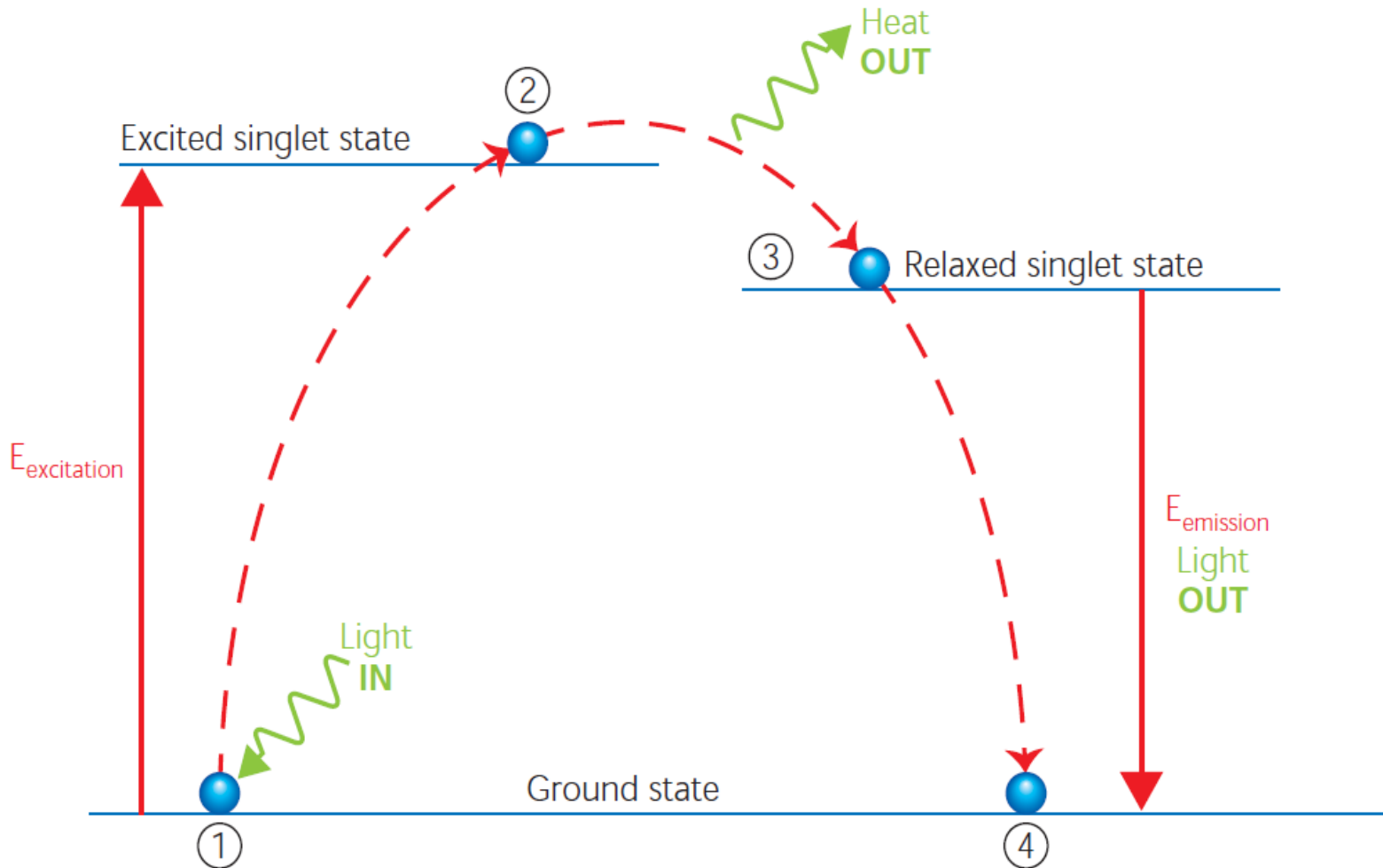
Stokes shift

Stokes shift
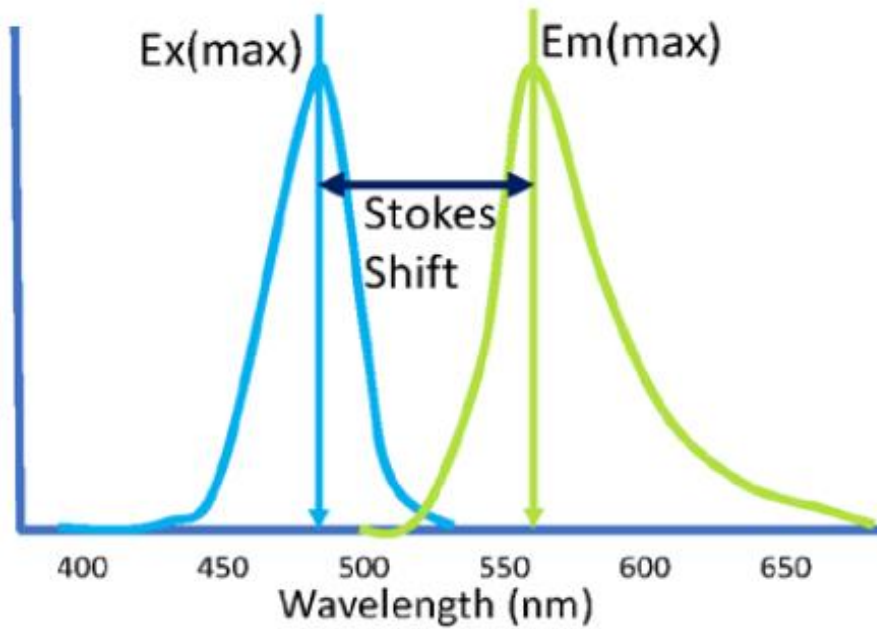
Importance of Stokes shift

- Single dye vs Tandem dye: FRET (fluorescence resonance energy transfer): a clever way to achieve higher Stokes Shifts and, therefore, increase the number of colors that can be analyzed from a single laser wavelength.
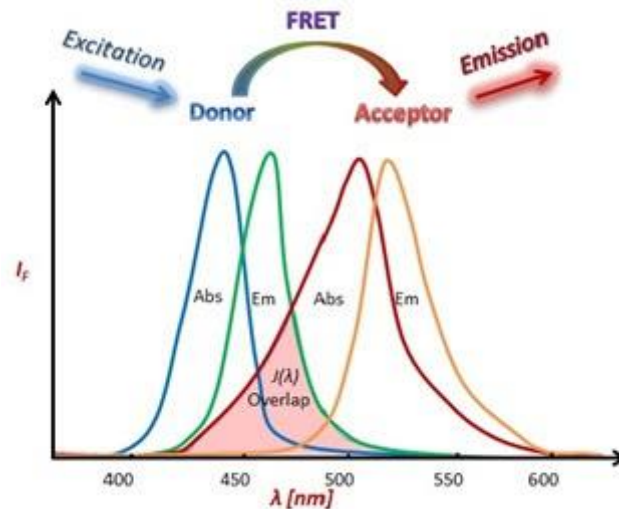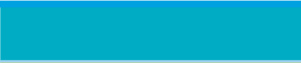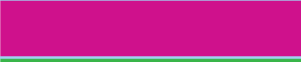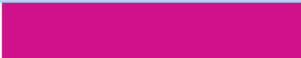
Maximal absorbance, maximal emission
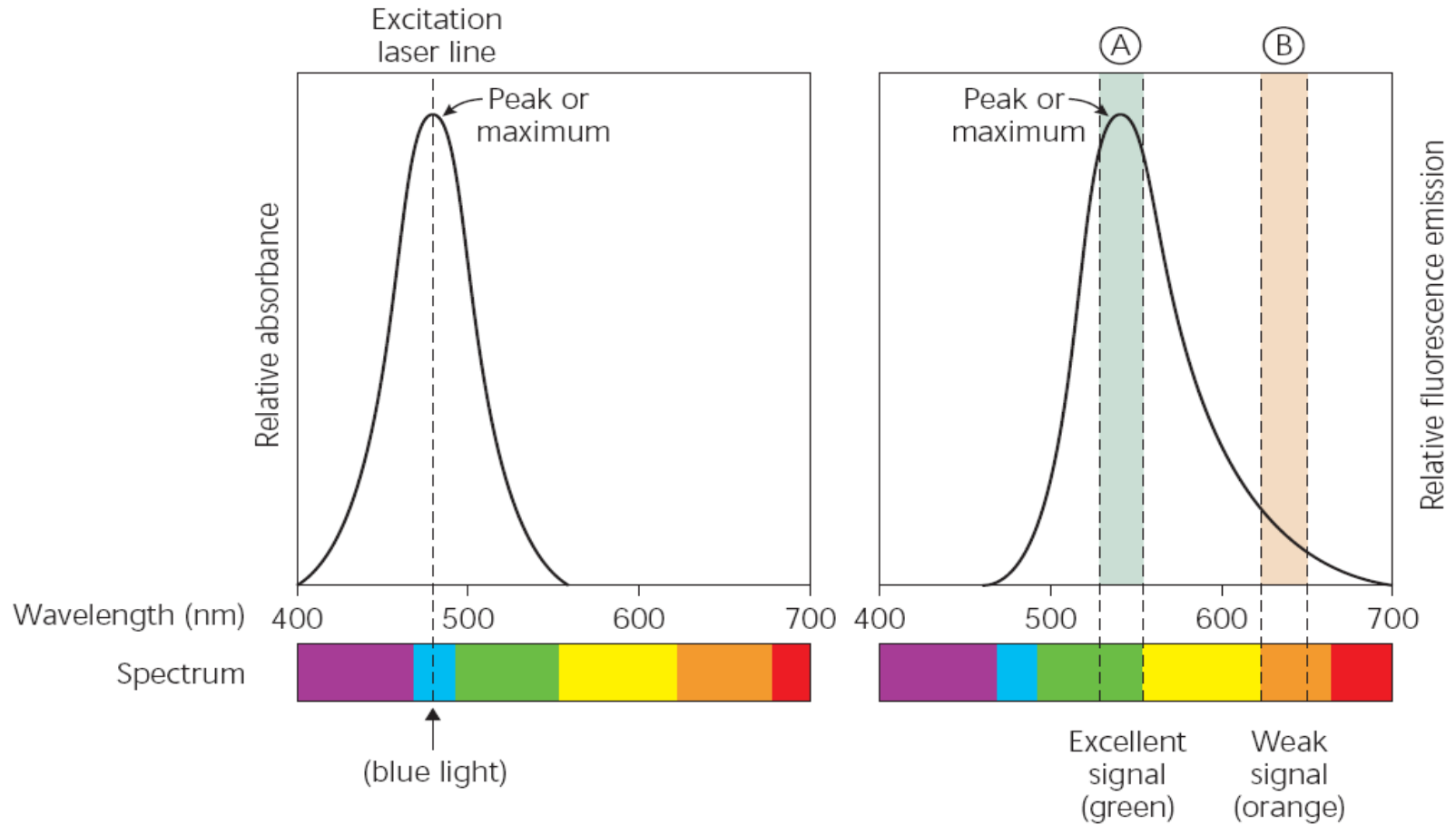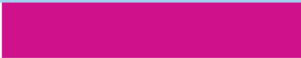
Single dyes

Dye	Laser excitation line (nm)	Maximal absorbance (nm)	Maximal emission (nm)	Fluorescence color
Alexa Fluor® 405	405, 407	401	421	
Alexa Fluor® 430	405, 407	433	541	
Alexa Fluor® 488	488	495	519	
Alexa Fluor® 633	633, 635, 647	632	647	
Alexa Fluor® 647	633, 635, 647	650	665	
Alexa Fluor® 660	633, 635, 647	663	690	
Alexa Fluor® 680	633, 635, 647	679	702	
Alexa Fluor® 700	633, 635, 647	702	723	Infrared
APC	633, 635, 647	650	661	
FITC	488	490	525	
Pacific Blue™	405, 407	410	455	
PerCP	488	490	675	
Phycoerythrin	488	490, 565	578	

FRET (fluorescence resonance energy transfer)

Tandem dyes

Dye	Laser excitation line (nm)	Maximal absorbance (nm)	Maximal emission (nm)	Fluorescence color
APC-Alexa Fluor® 750	633, 635, 647	650	779	Infrared
APC-Cy5.5	633, 635, 647	650	695	
APC-Cy7	633, 635, 647	650	785	Infrared
PerCP-Cy5.5	488	496, 546	695	
PE-Alexa Fluor® 610	488	496, 546	627	
PE-Alexa Fluor® 647	488	496, 546	667	
PE-Alexa Fluor® 680	488	496, 546	702	
PE-Alexa Fluor® 700	488	496, 546	723	Infrared
PE-Alexa Fluor® 750	488	496, 546	779	Infrared
PE-Cy5.5	488	496, 546	695	
PE-Cy5	488	496, 546	667	
PE-Cy7	488	496, 546	785	Infrared
PE-Texas Red®	488	496, 546	615	

Fluorescence compensation
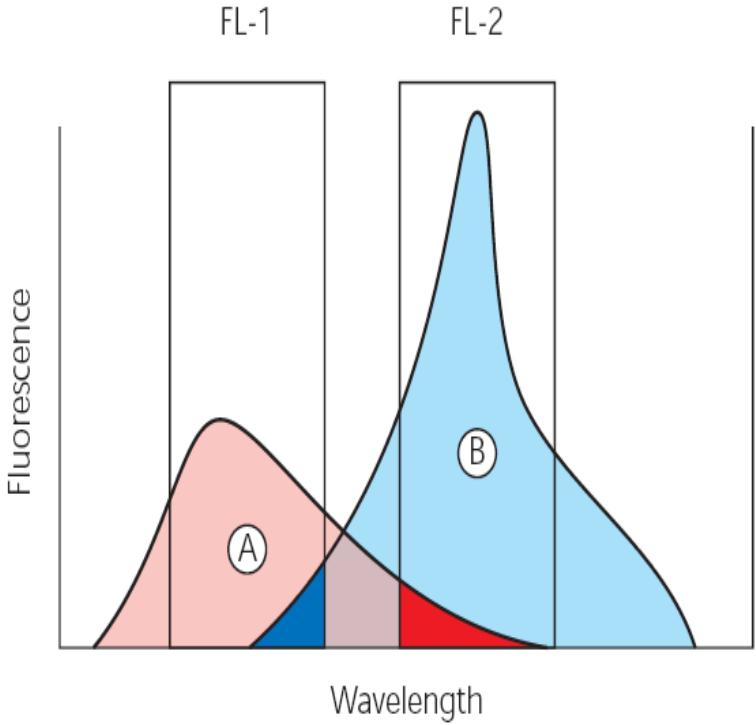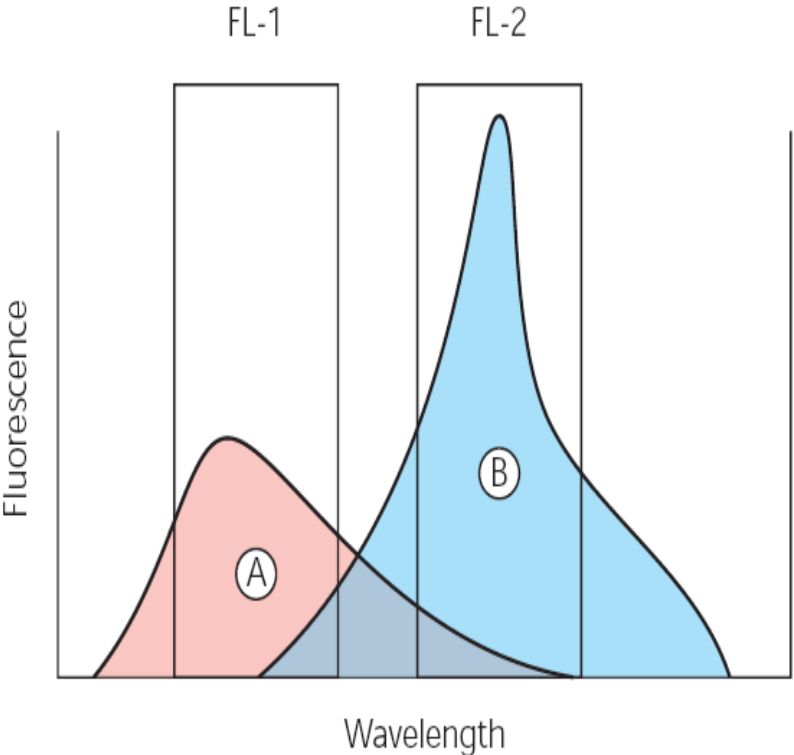
Data analysis

- Gating: e.g. according to physical characteristics
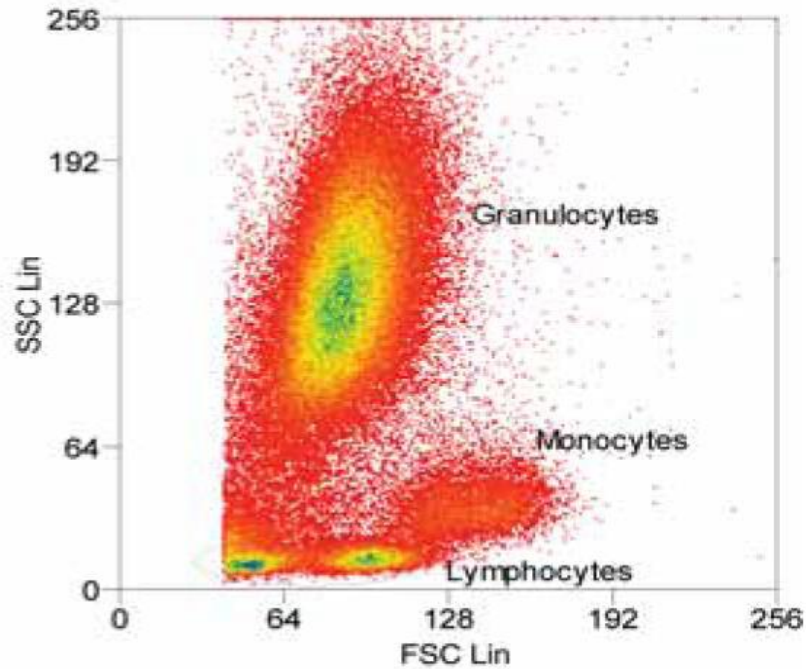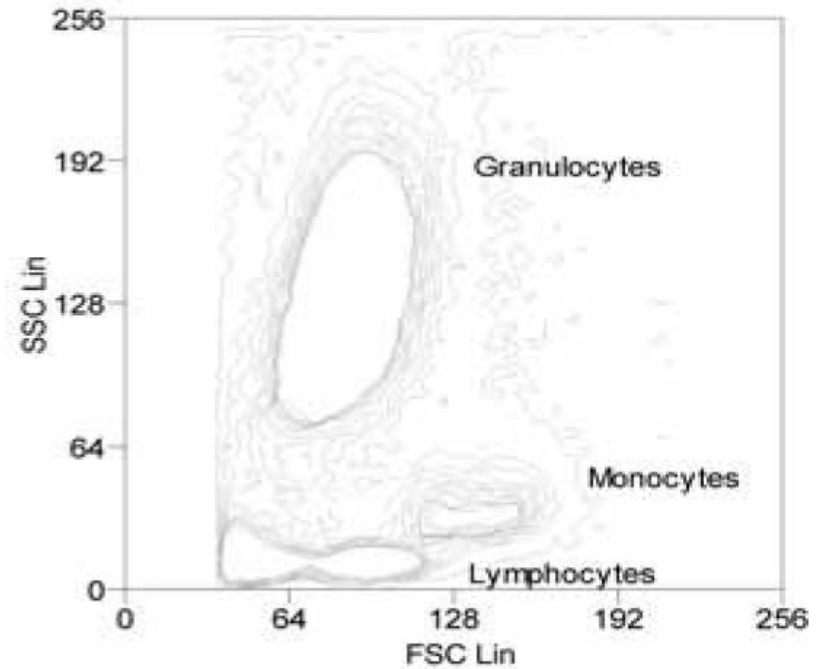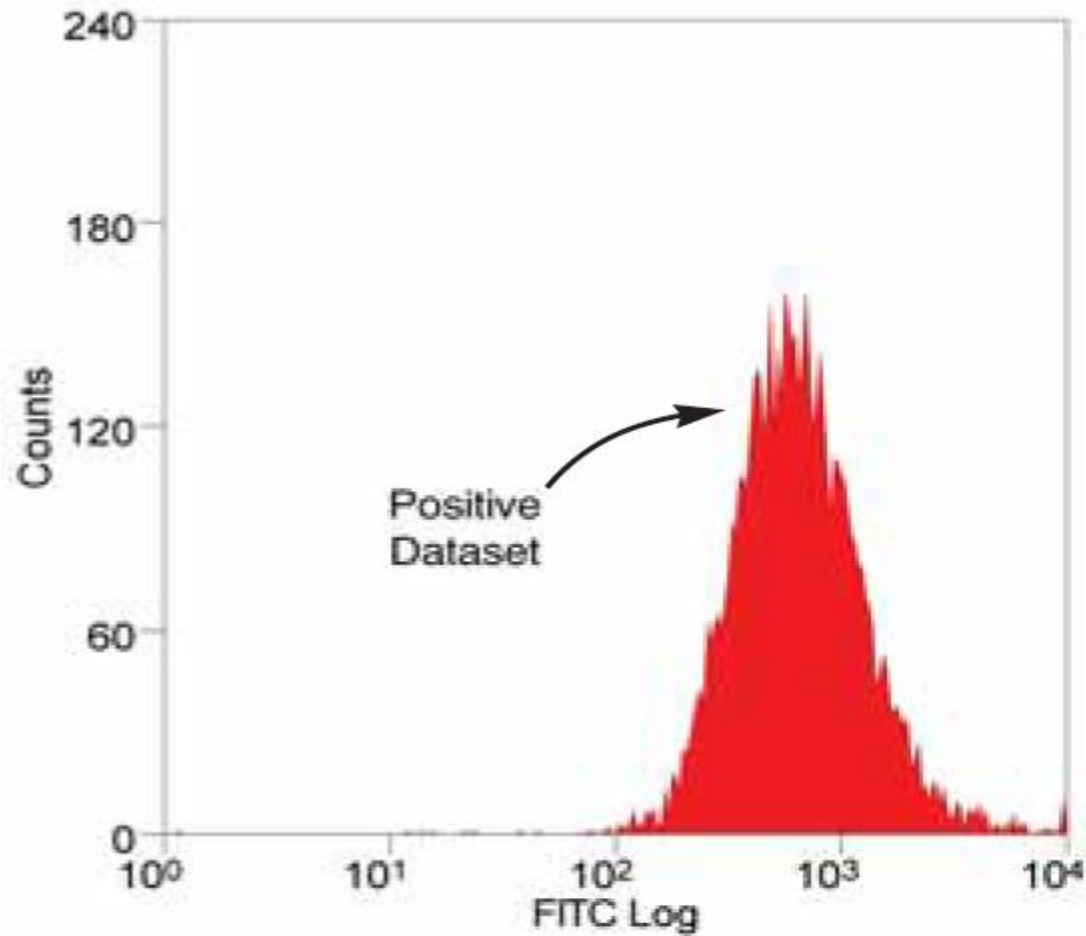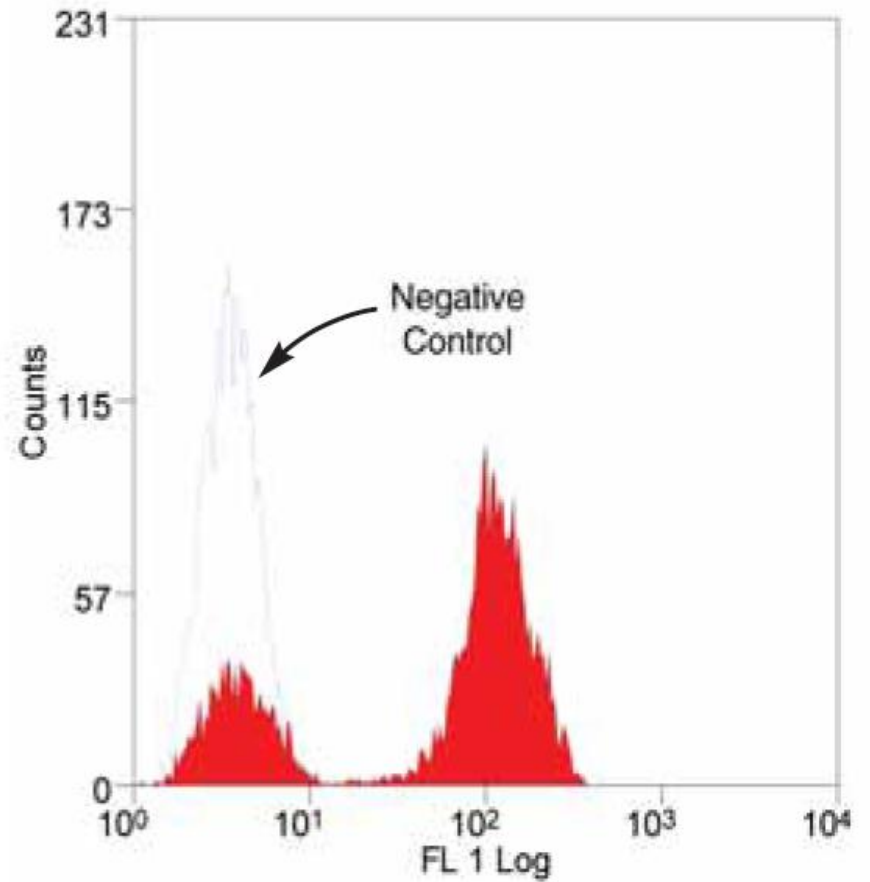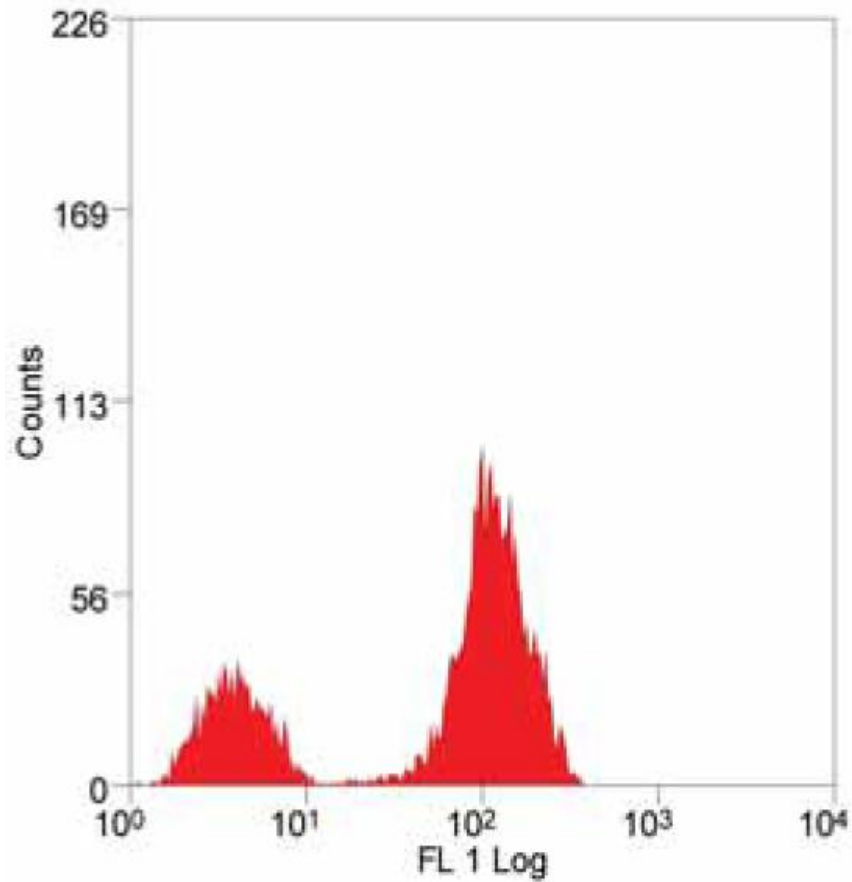
Analysis of CD markers
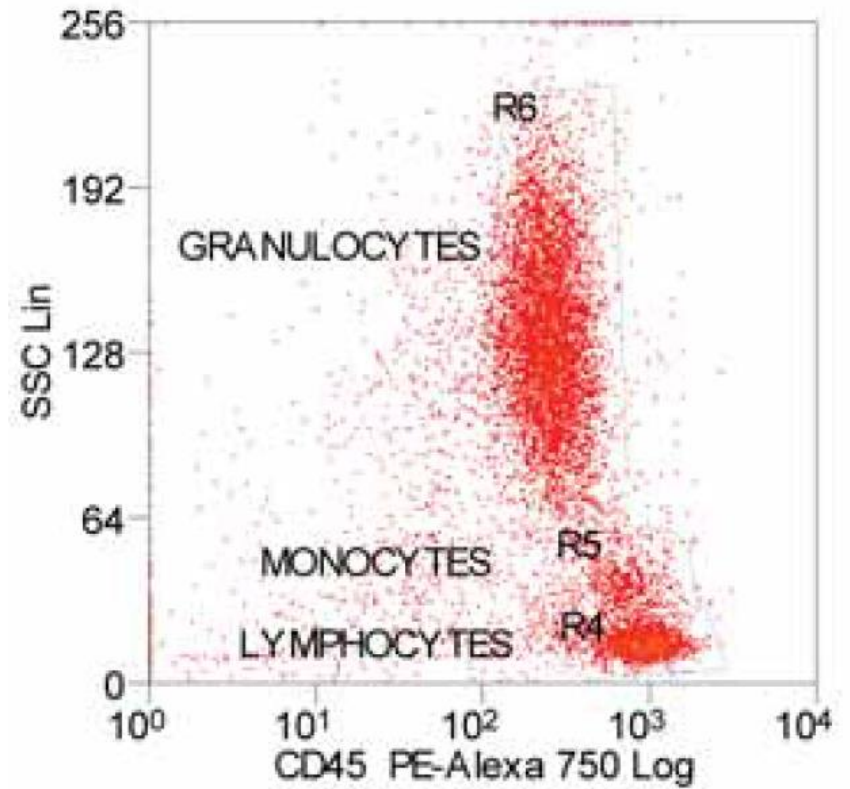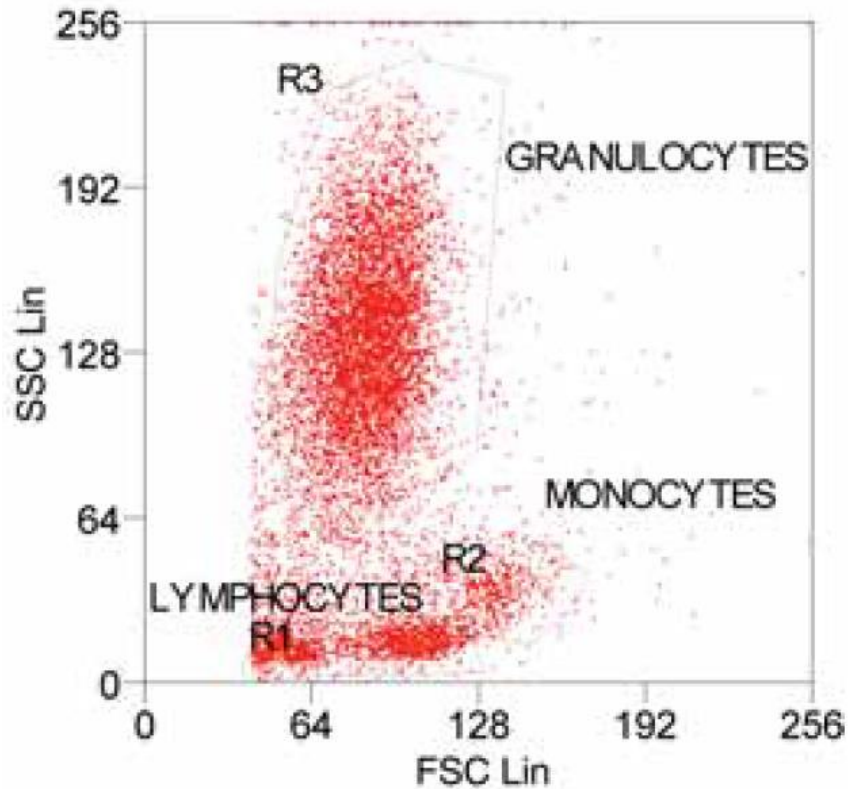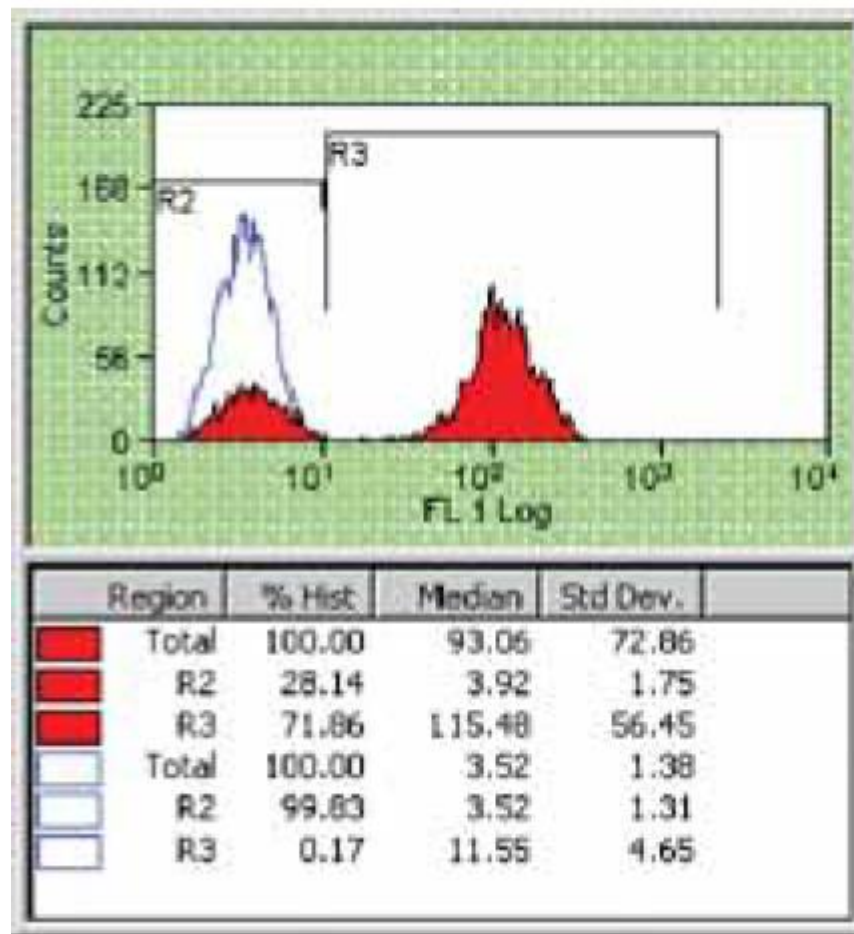
Discriminating doublets
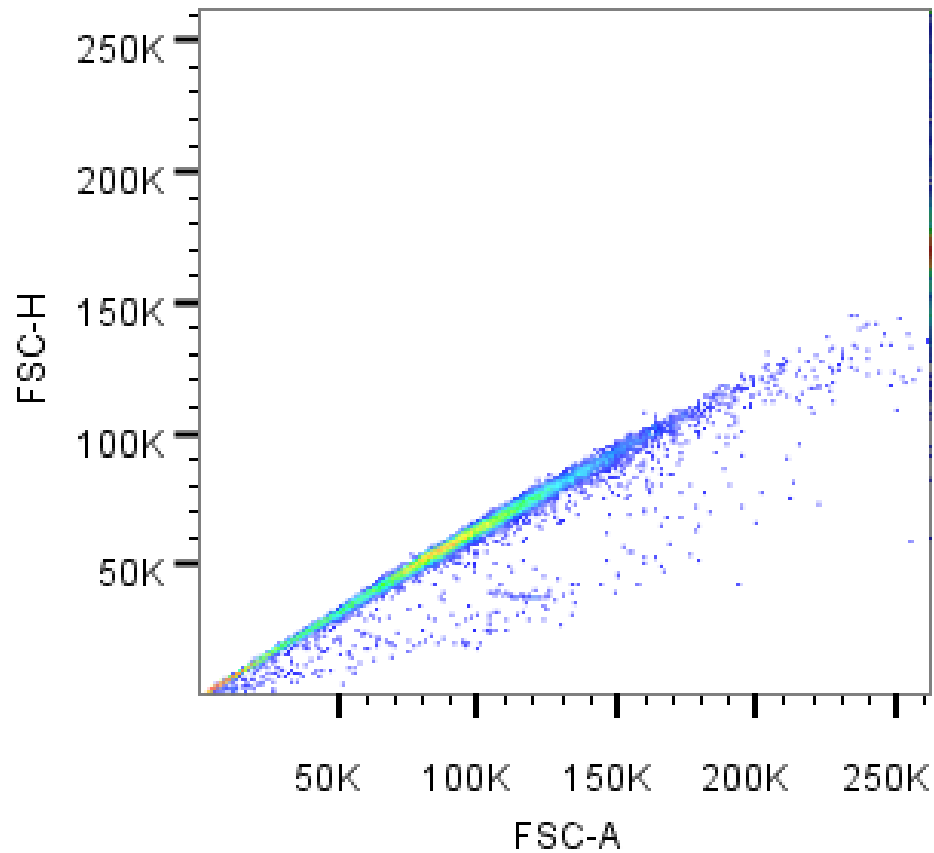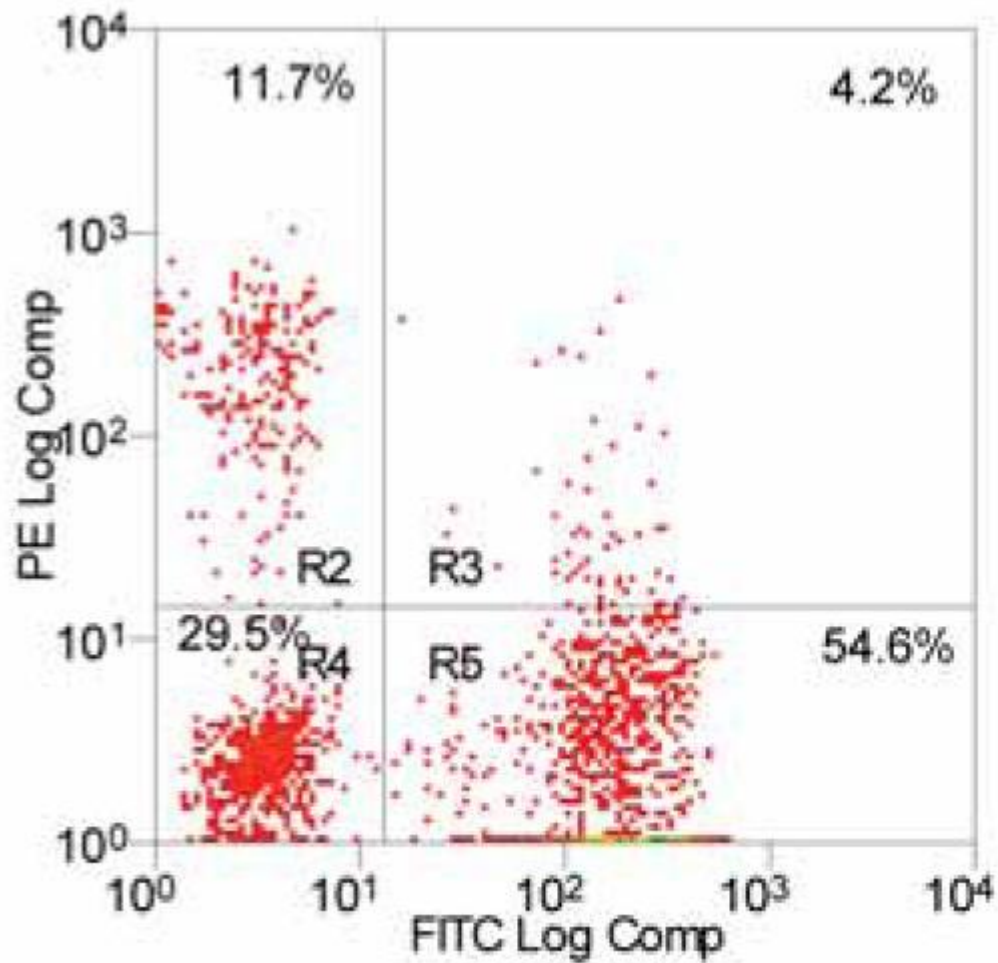
Single parameter histogram

Negative isotype control
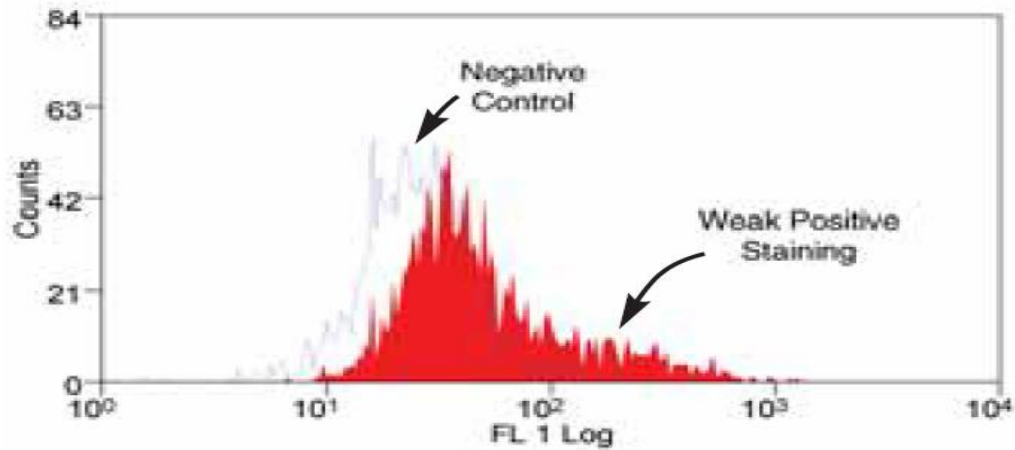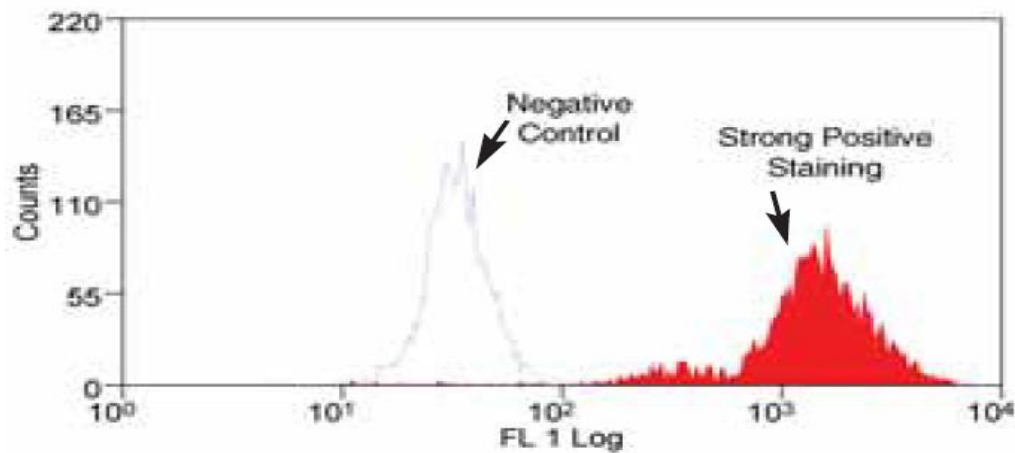
Statistical analysis

Dual color histogram

Intracellular markers
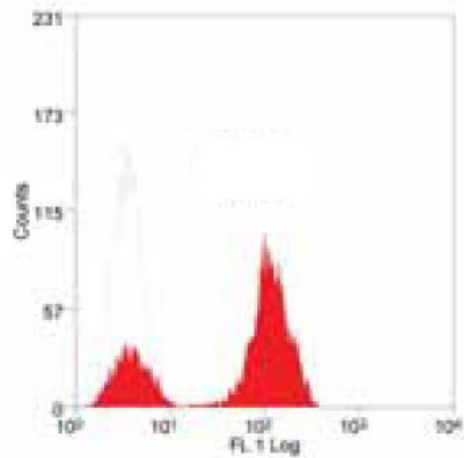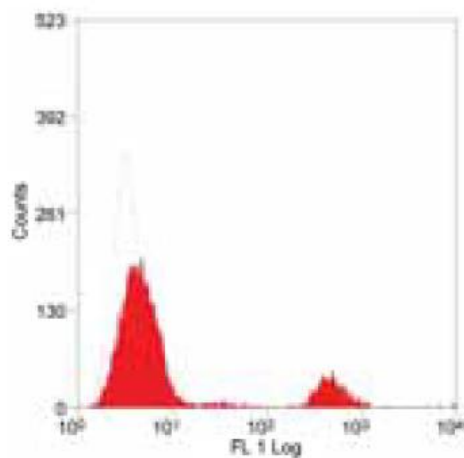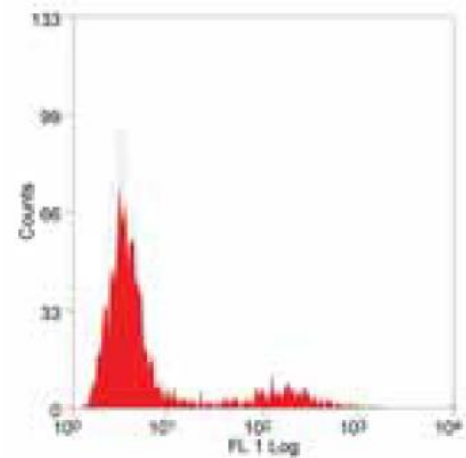
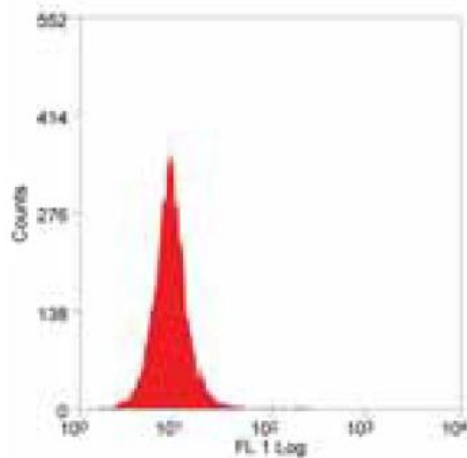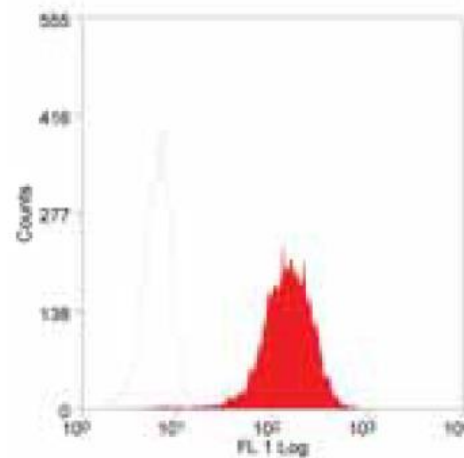# Cell population shift
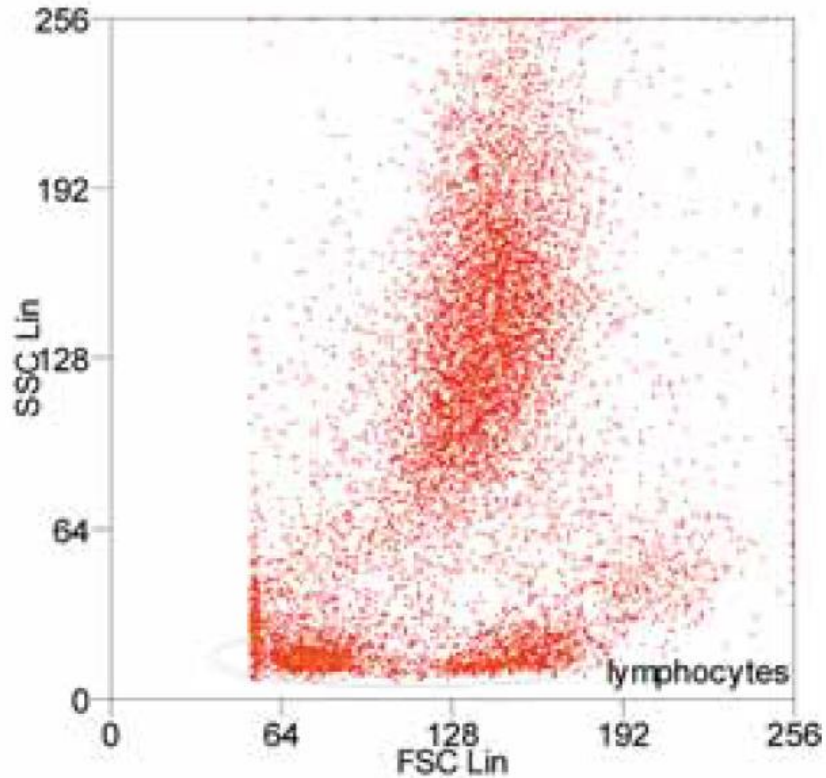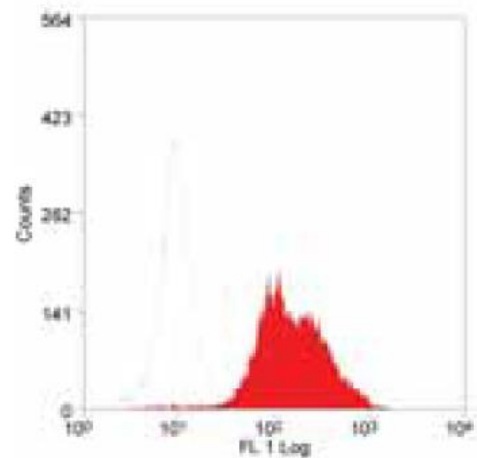

Normal person — Leukemia patient
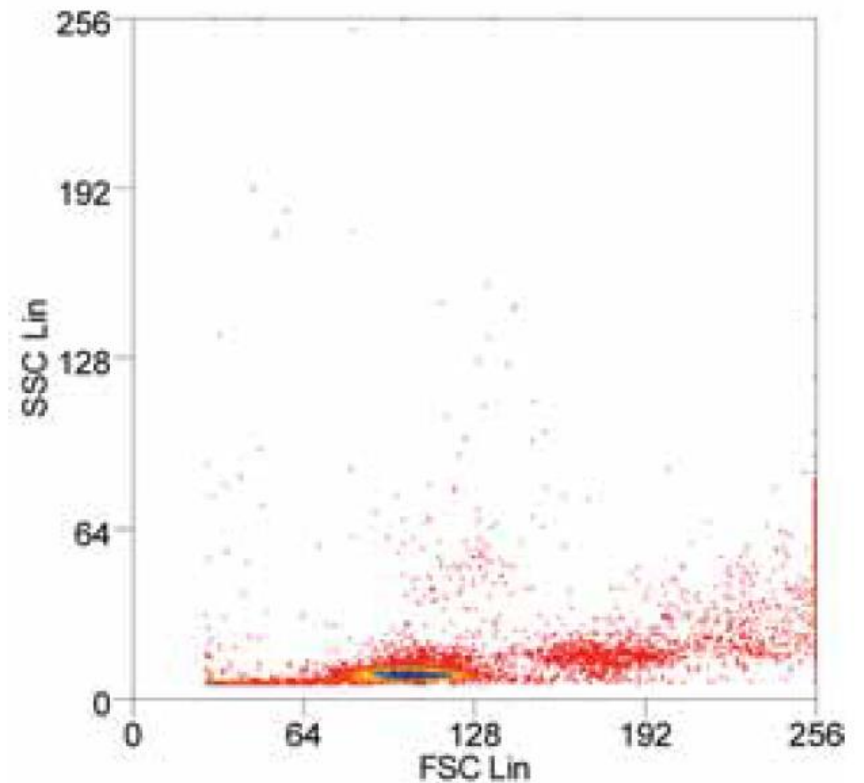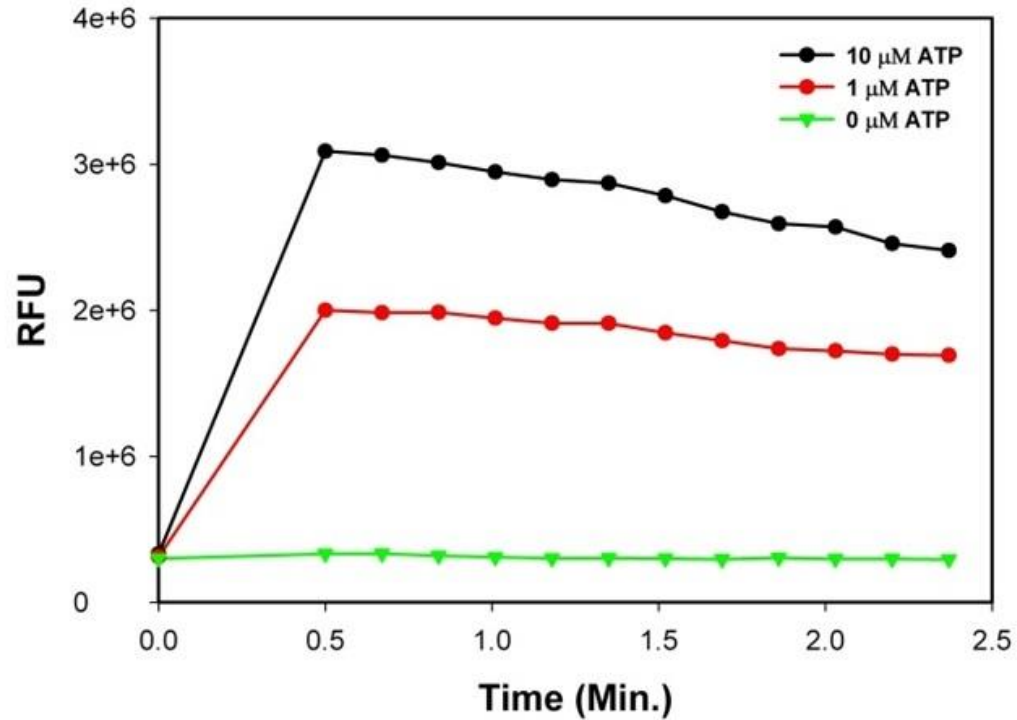
Normal person

CD3

CD20

HLA-DR

Leukemia patient

CD3

CD20

HLA-DR

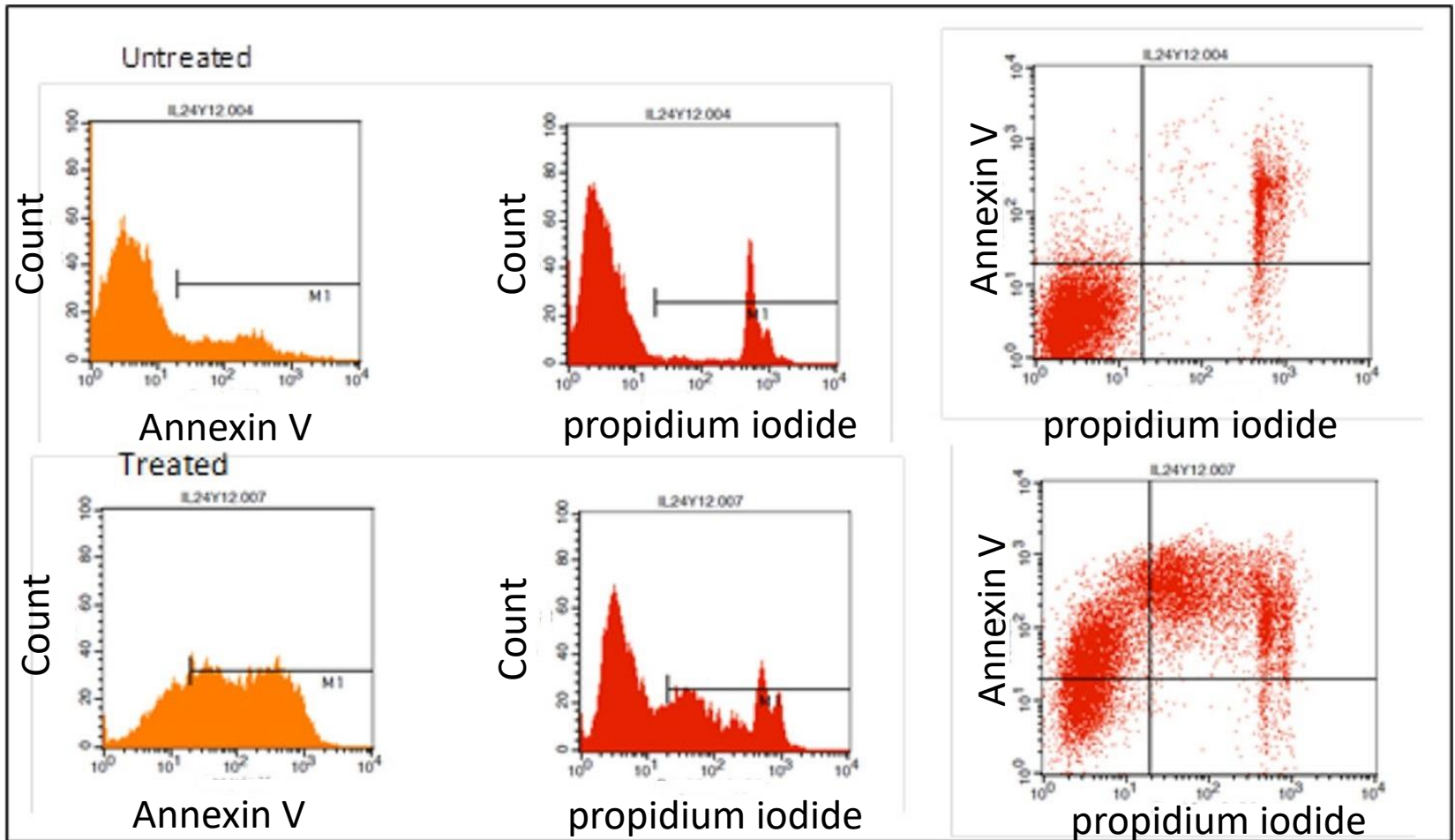# Calcium flux assay
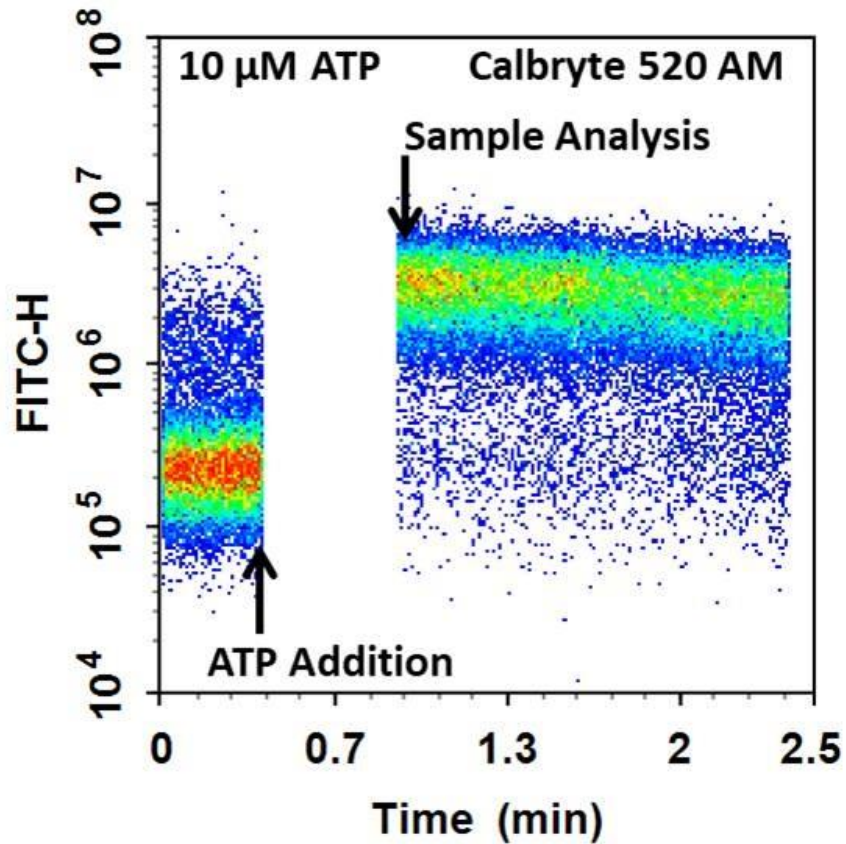
Live and dead cells assay

propidium iodide,
DAPI
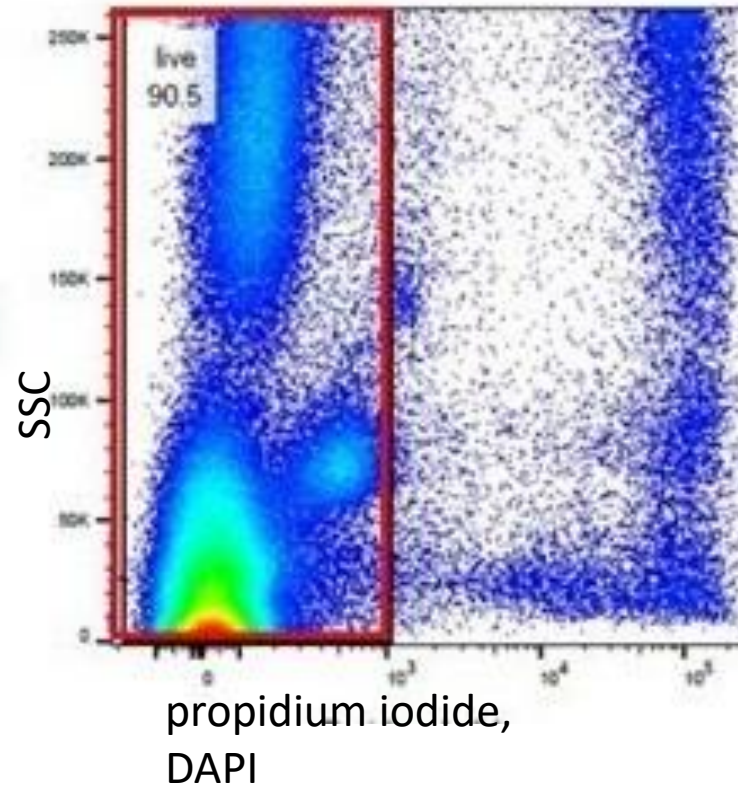
Apoptotic and necrotic cells

Thank You