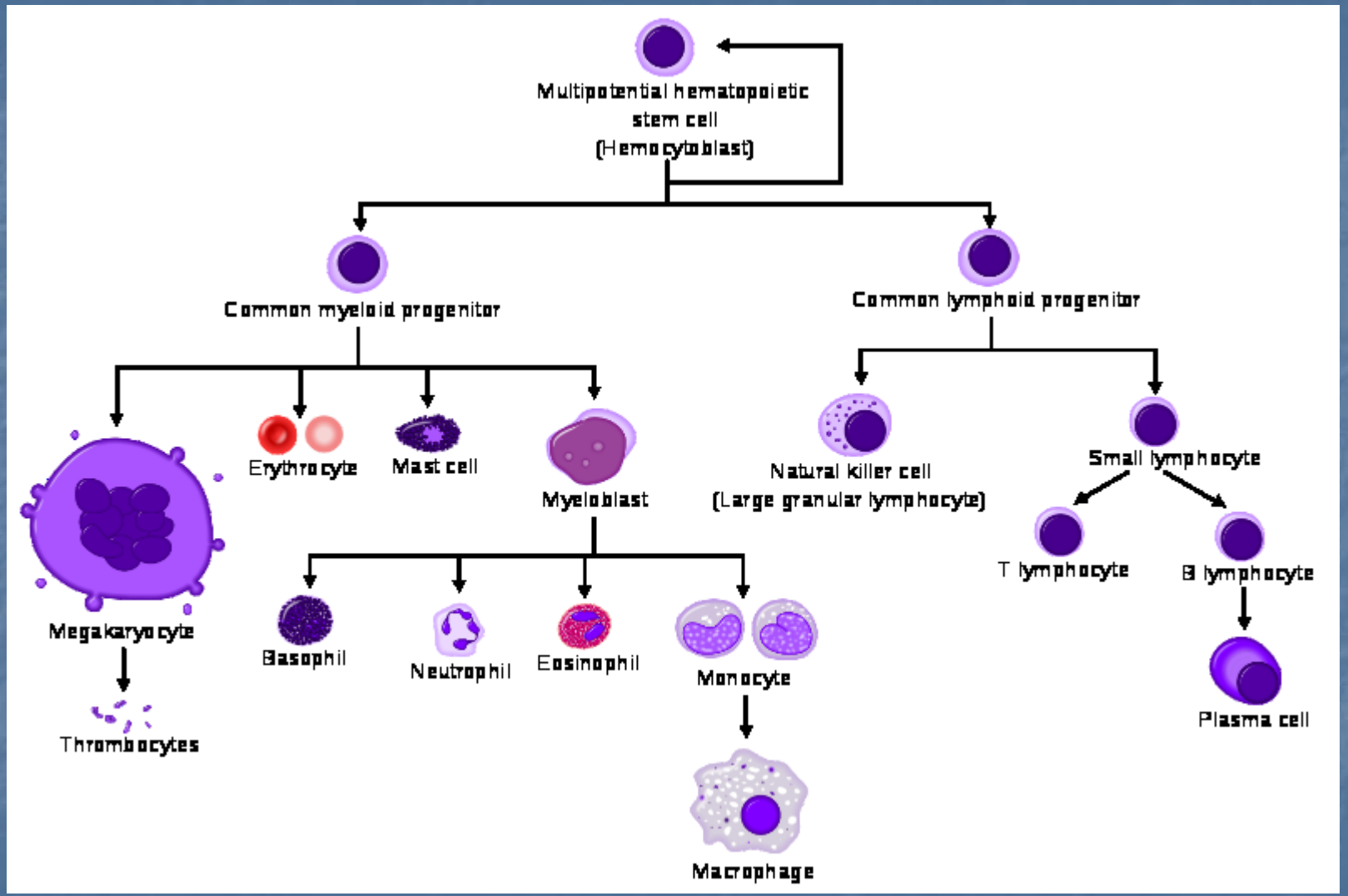
Flow Cytometry
FACS
(Fluorescence-Activated Cell Sorter)

Flow Cytometry
Leonard Herzenberg

- technique for analyzing populations of cells
- cells are measured individually but in large numbers
- cells are incubated with fluorescently labeled monoclonal antibodies directed against different antigens (cell surface, intracellular, nuclear)
- CD numbers (clusters of differentiation)
  - CD1-CD364 (Nov 2014)
  - Lineage specific markers
  - Used in phenotyping (leukaemias)


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

CD-2	nezralé T-lymfocyty
CD-3	všechny T-lymfocyty (součást **TcR**), kromě NK buněk
CD-4	helperské T-ly
CD-7	T-ly nacházející se v thymu
CD-8	cytotoxické T-ly
CD-14	monocyty a makrofágy
CD-15	neutrofily, eosinofilní granulocyty
CD-16	NK buňky, neutrofily
CD-19	B-lymfocyty
CD-34	lymfoidní a myeloidní progenitorové buňky
CD-38	plazmatické buňky
CD-40	B-ly (izotypový přesmyk za přítomnosti CD-40 ligandu)
CD-45	panleukocytární antigen
CD-56	NK buňky
CD-58	endothelie, buňky prezentující antigen T-lymfocytům (adhese s CD-2)
CD-64	Fc γ receptor (makrofágy, neutrofily…)
CD-68	dendritické buňky
CD-80 a 86	buňky prezentující antigen T-lymfocytům
CD-95	FAS receptor
CD-203	bazofilní granulocyty

Common phenotypes and AML

FAB Subtype	Common Phenotype
M0	DR, CD13, CD33, CD34, $CD7^{-/+}$, $TdT^{-/+}$
M1	Similar to MO except $CD15^{-/+}$
M2	DR, CD13, CD33, more CD15 and less CD34 than M1
M3	DR(−), CD13, CD15, CD33, $CD34^{-/+}$, CD2 occasionally
M4, M5	DR, CD15, $CD14^{+/-}$, CD33 > CD13, $CD34^{-/+}$, CD4 weak
M6	DR, $CD13^{-/+}$, $CD33^{+/-}$, CD34, CD45 weak
M7	$DR^{-/+}$, $CD33^{+/-}$, CD34, CD41, CD61

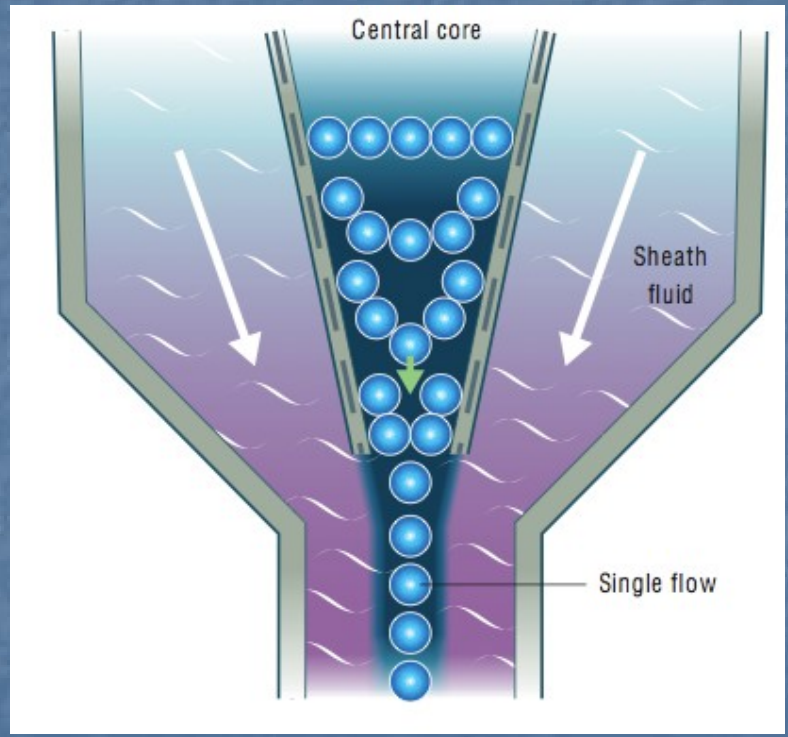
Blood October 15, 1997 vol. 90 no. 8 2863-2892

Flow Cytometer Instrumentation

- four general components
  - Fluidics
  - Optics
  - Detectors
  - Electronics
- Understanding how a flow cytometer operates is critical to the design of your experiments

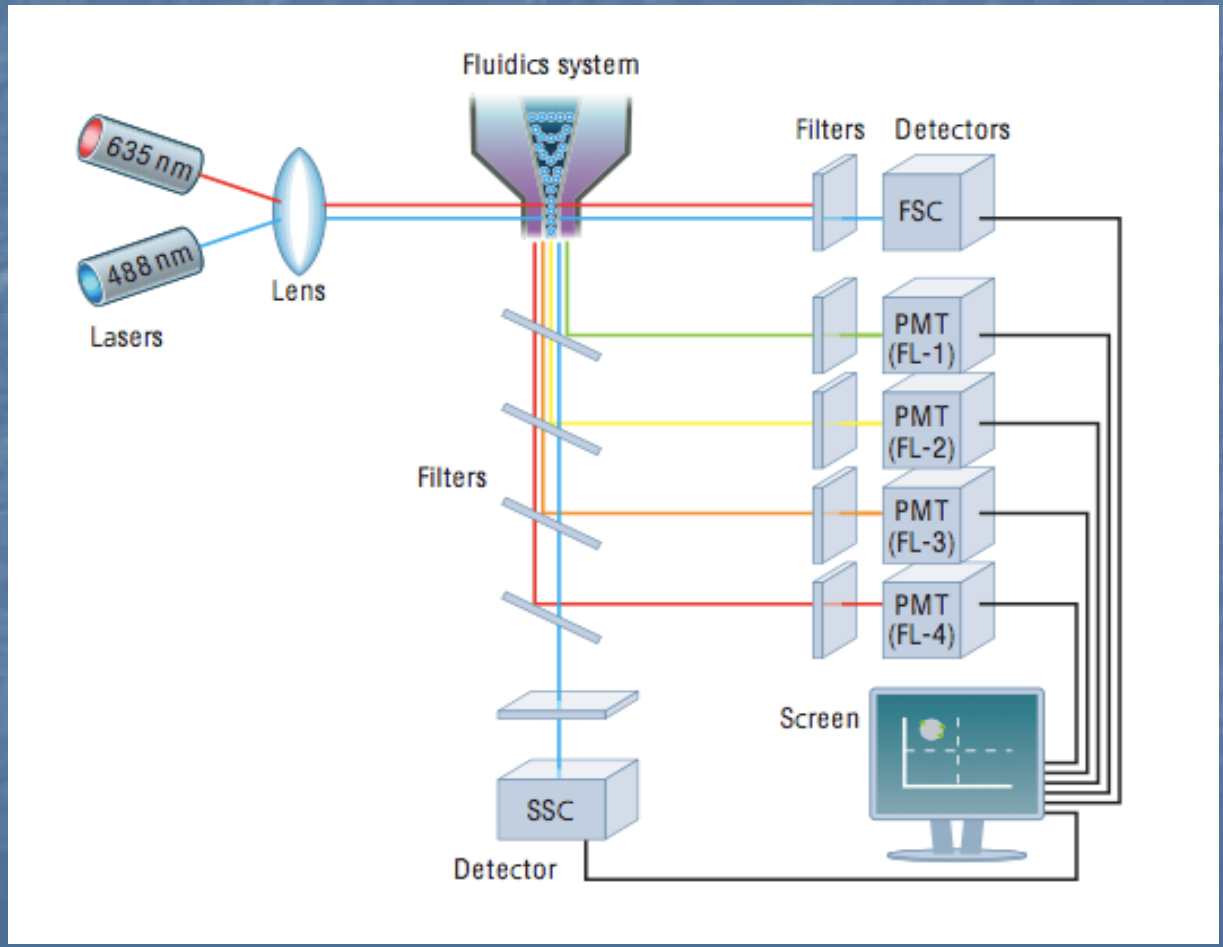
Flow Cytometry Fluidics

- The cell sample is injected into a stream of sheath fluid
- Labeled cells are accelerated and individually pass through a laser beam
- its resulting fluorescence and angle deflection detected by a photocell



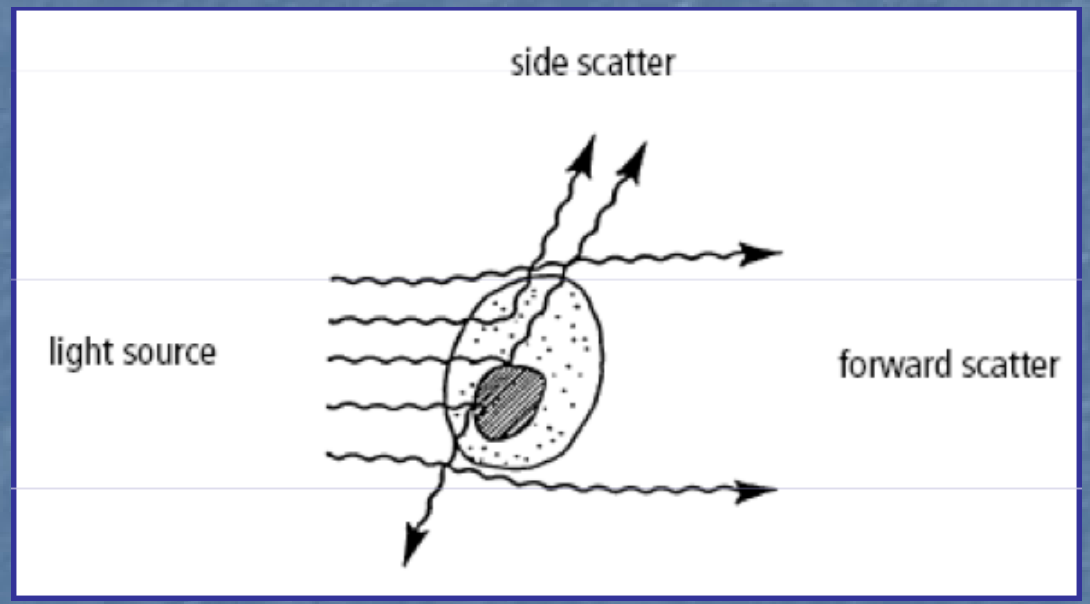
Flow Cytometer Optics

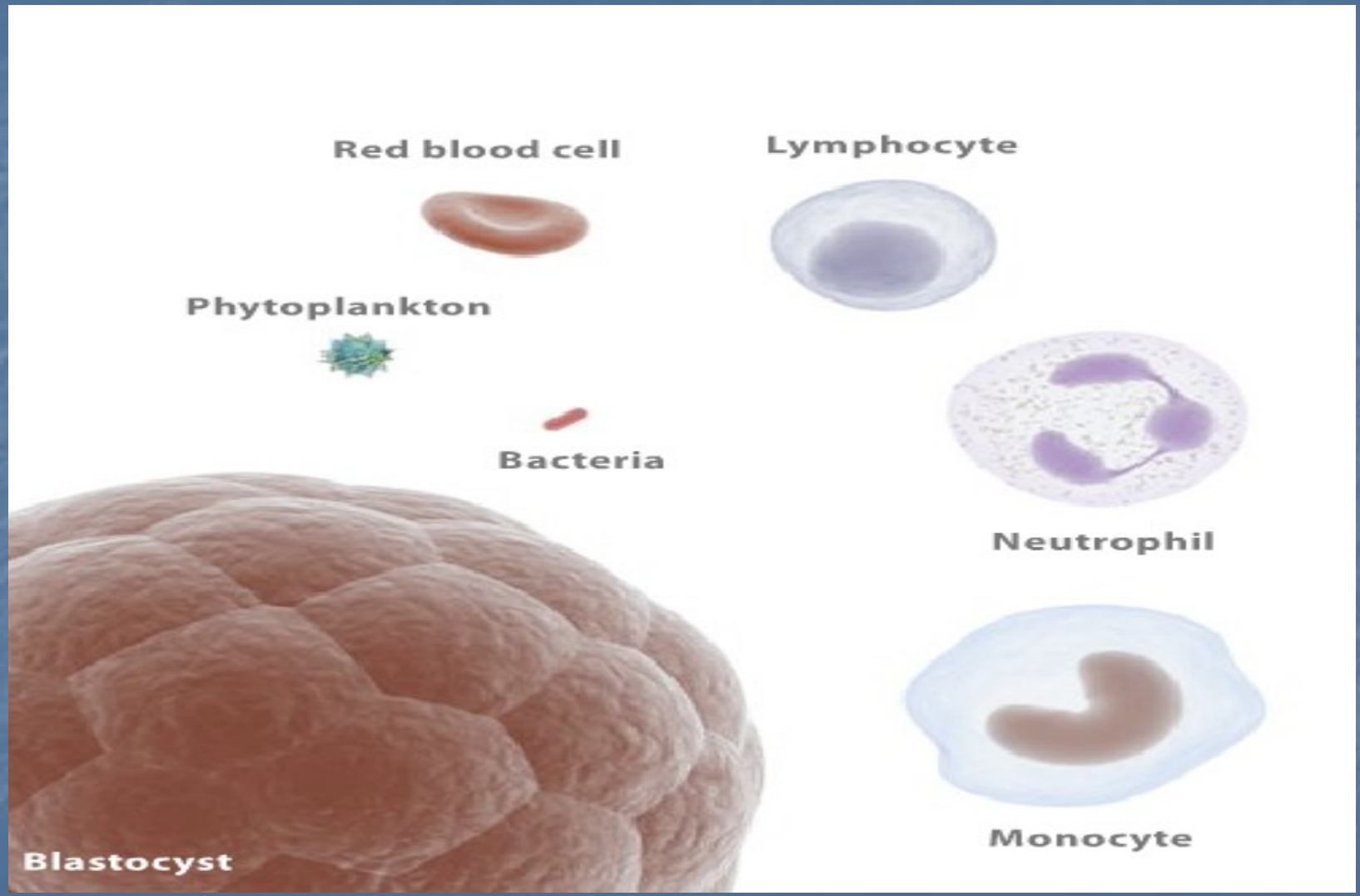
- Light emitted from the interaction between the cell and the laser beam is collected by a lens
- The light moves through a system of optical mirrors and filters
- Specified wavelengths are then routed to optical detectors



What can you measure with a Flow Cytometer?

- 1. Size and complexity of cells
  - Forward-scattered light (FSC)
    - is proportional to the surface area or size of a cell
  - Side-scattered light (SSC)
    - is proportional to the granularity or internal complexity of a cell
- 2. Cell surface molecules
- 3. Nuclear antigens
- 4. Protein expression/localization
- 5. Copy number variation (Flow-FISH)
- 6. Cell pigments



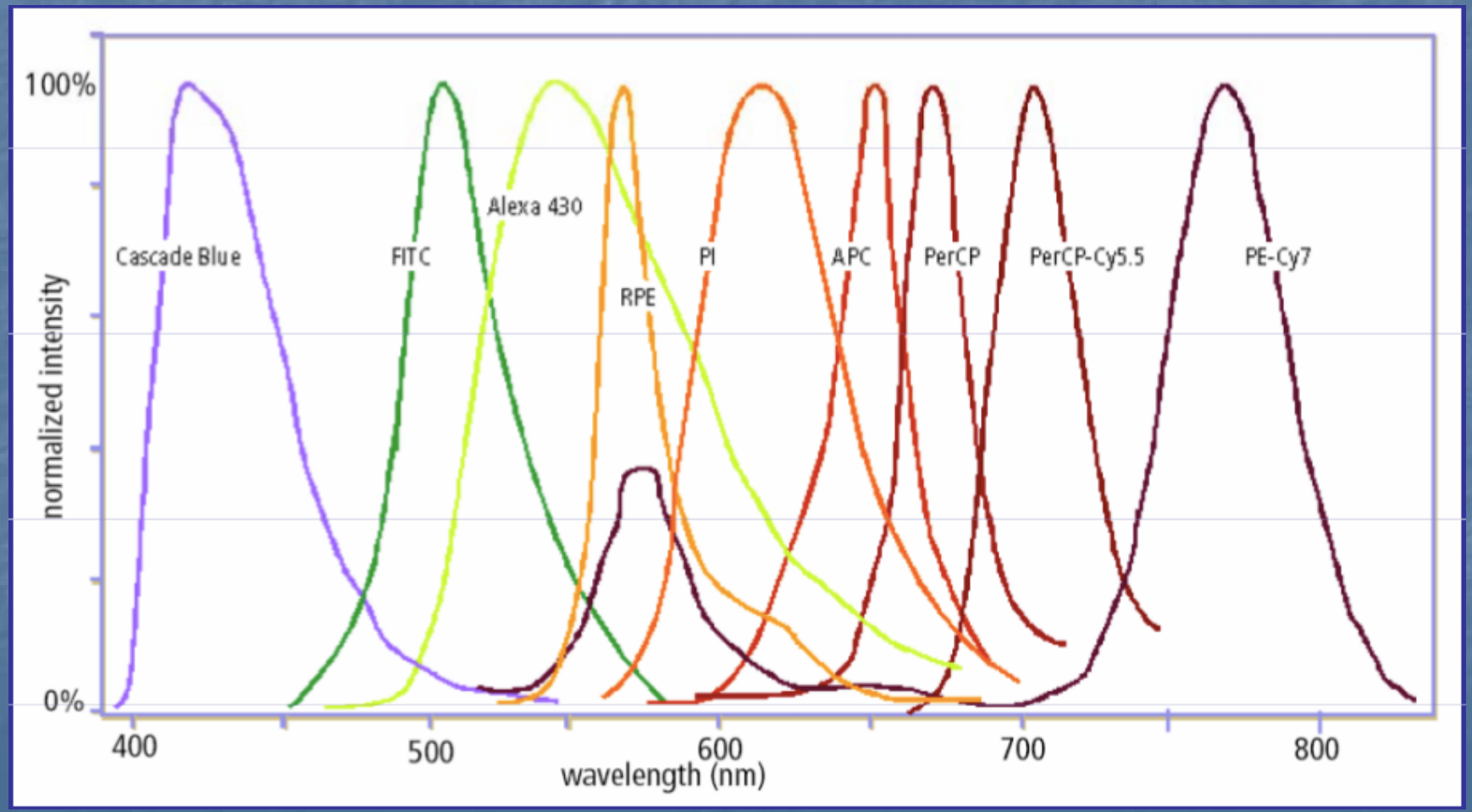

Red blood cell
Lymphocyte
Phytoplankton
Bacteria
Neutrophil
Monocyte
Blastocyst

Fluorochrome Emission

- The laser beam excites the fluorochrome at a specific wavelength and the fluorochrome emits light at a separate wavelength (emission)
- Property of any fluorescent dye; 2 things:
  - Excitation spectra
  - Emission spectra

  - If your laser functions at 488nm, find dyes that have excitation spectra at that λ

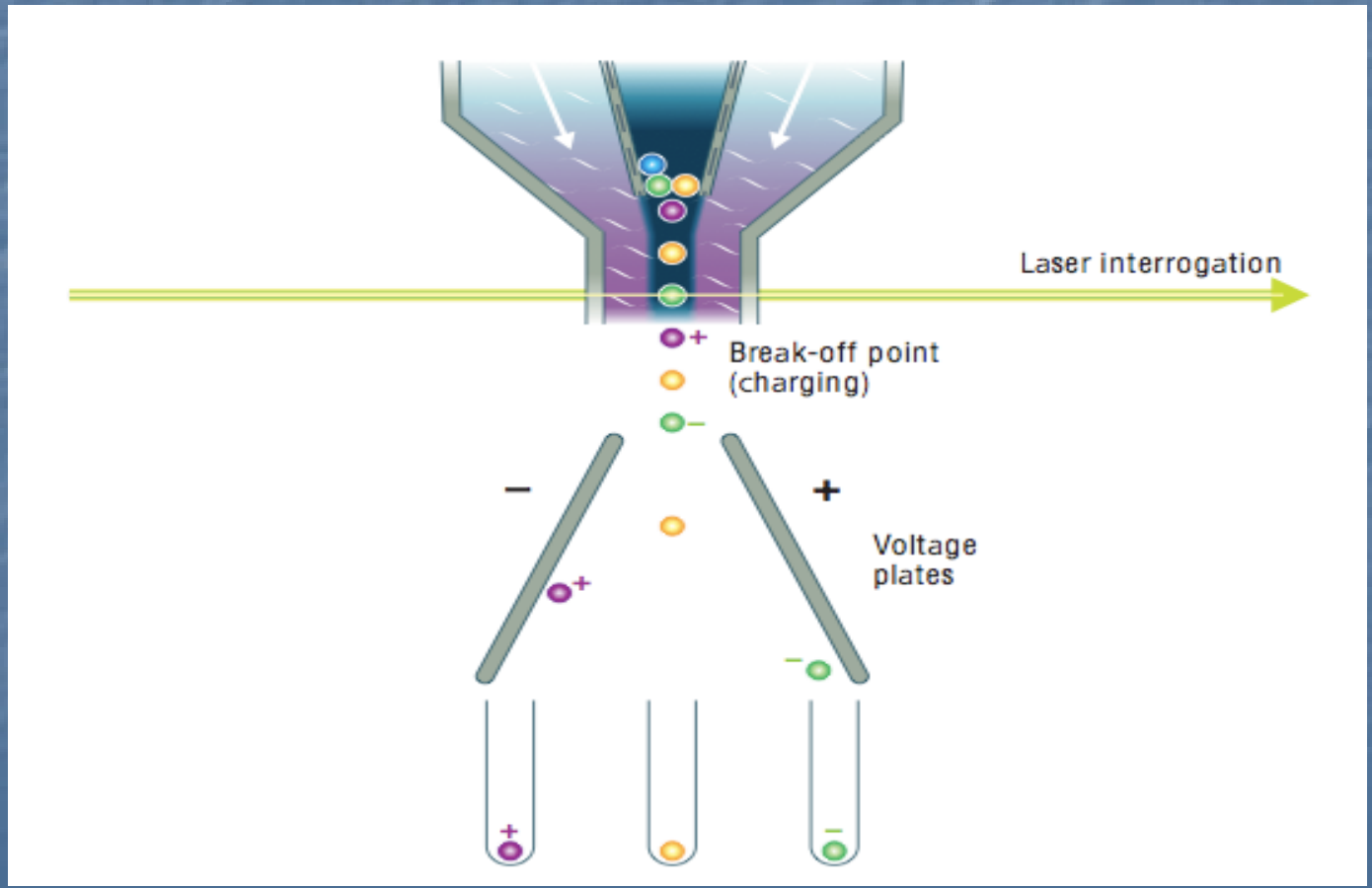
Flourochromes Have Overlapping Emission Wavelengths



Technical Components

- Detection Systems
  - Photomultiplier Tubes (PMTs)
    - Historically 1-2
    - Current Instruments 3-9
- Illumination Systems
  - Lasers (Blue 488 nm, Green 532 nm, Red 640 nm, violet 405 nm)
    - BD FACS Calibur
      - Argon ion (488)
      - HeNe (633)
    - BD FACS Canto II
      - Solid state (488)
      - HeNe (633)
      - Violet (405)
    - FACS Aria sorter

Electrostatic Flow Sorting



How do we detect the signal?

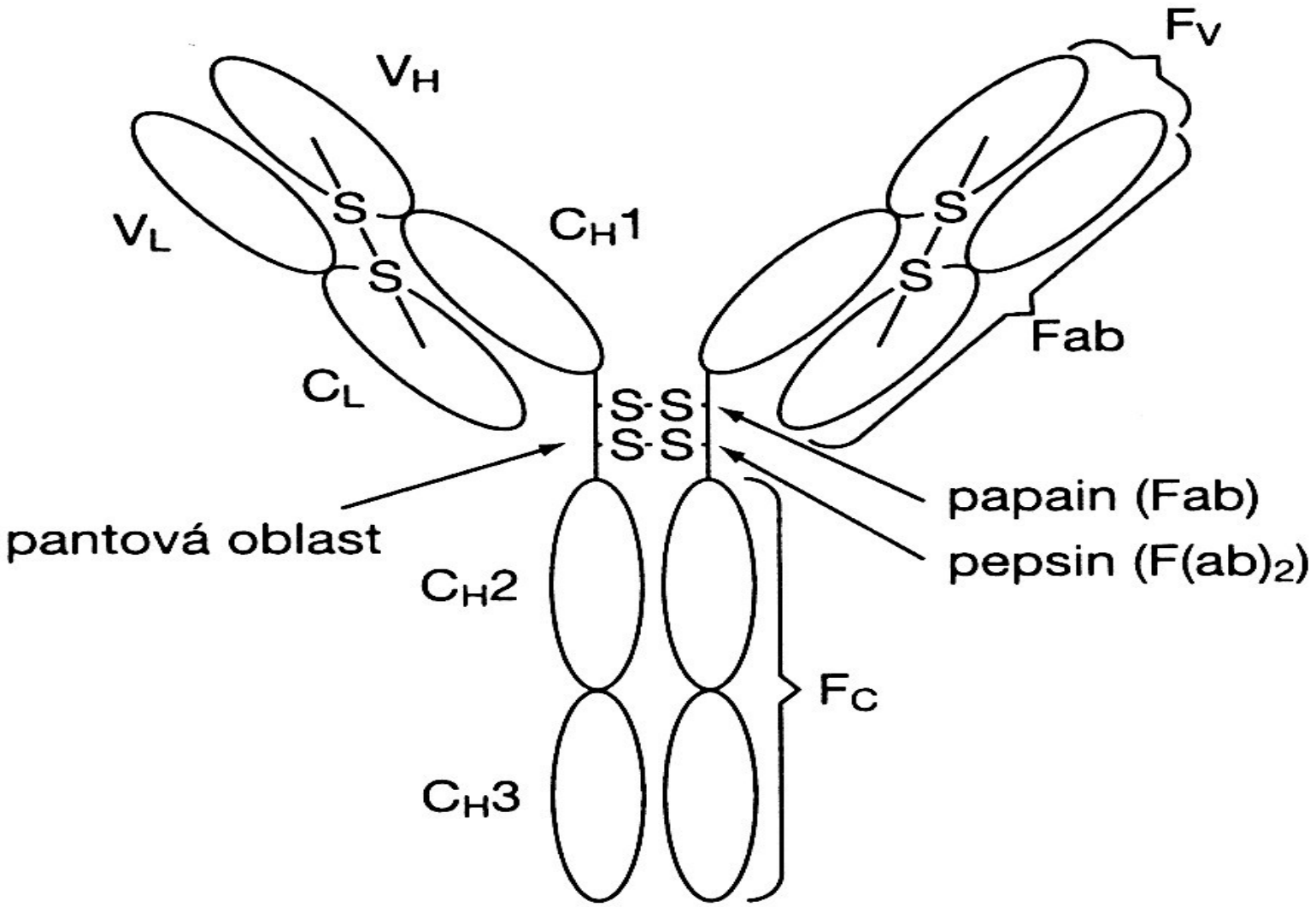
- Photomultiplier tubes (PMT) and lenses
- FL-1 lets light through at 500-560 nm
- FL-2 lets light in at 560-611 nm
- FL-3 610-660 nm
- FL-4 >660 nm
- Picking dyes too close in λ results in no detection

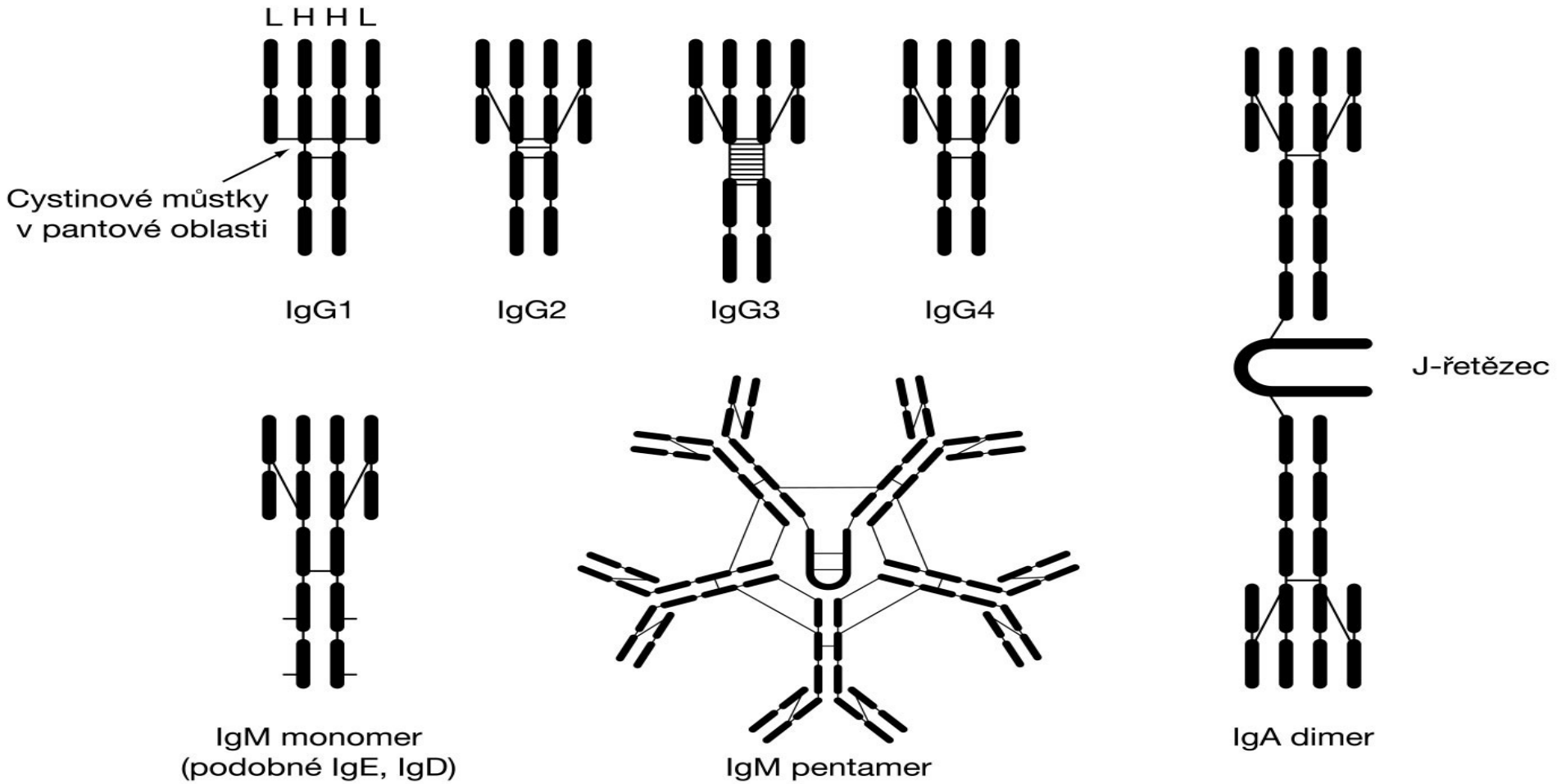
Flow Cytometer Electronics

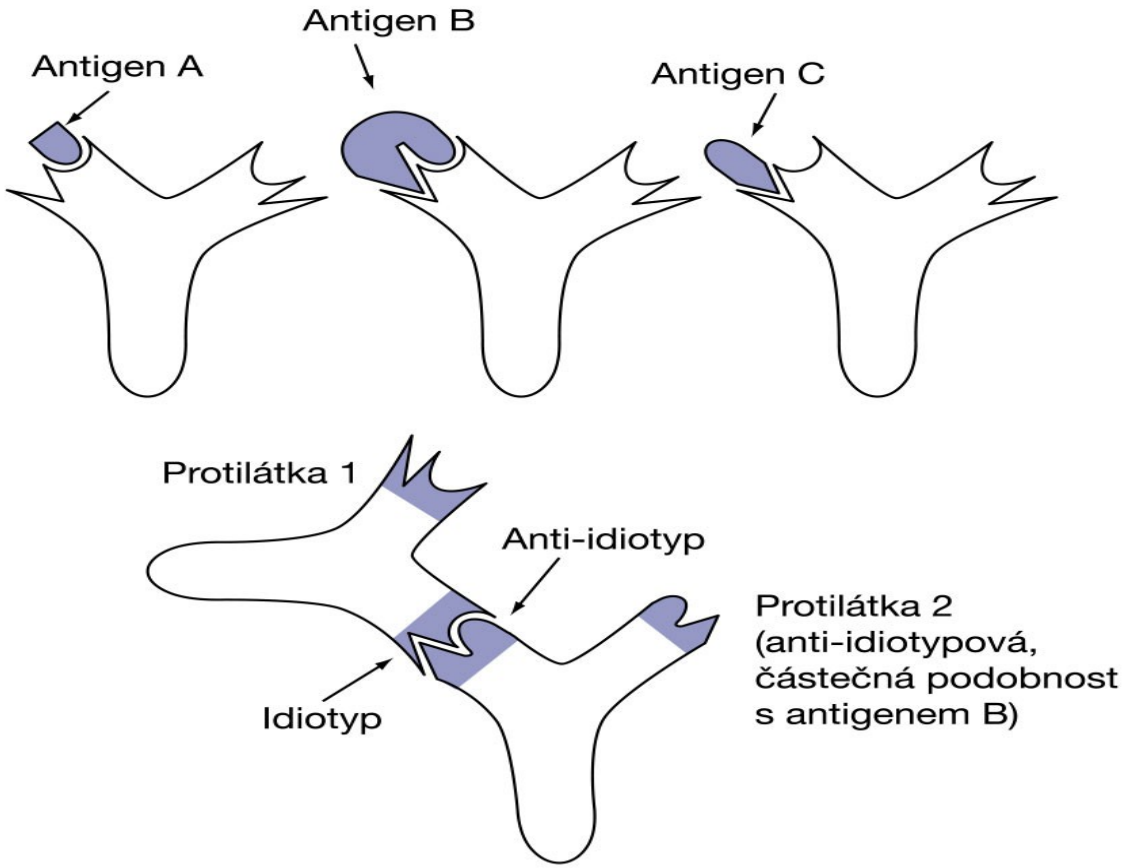
- The voltage pulse height, width, and area are determined by the particle's size, speed, and fluorescence intensity
- parameters are acquired and analyzed in real-time by a computer

Antibodies (immunoglobulins)

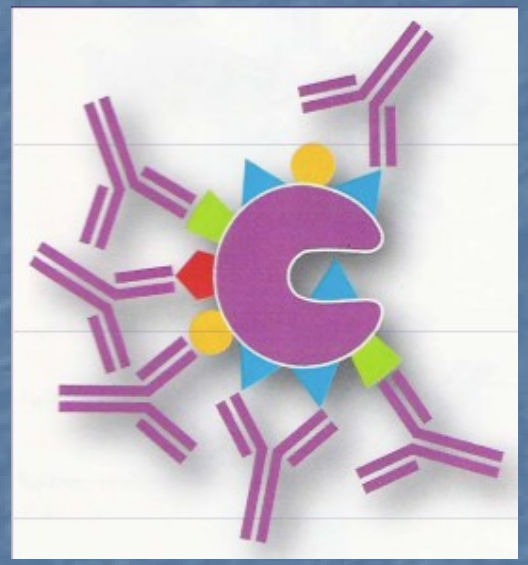
- Clone
- Isotype
- Mouse, rat, rabbit, goat
- Reactivity
- Specificity
- Fluorochrome
- Catalogue number and cost: $$$ £££ €€

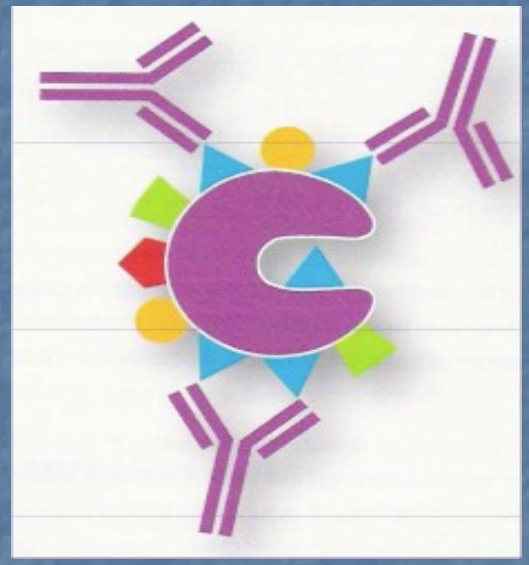

V_H
V_L
C_H1
C_L
F_V
Fab
S
S
S
S
S-S
S-S
pantová oblast
papain (Fab)
pepsin $(F(ab)_2)$
C_H2
C_H3
F_C


L H H L
Cystinové můstky
v pantové oblasti
IgG1
IgG2
IgG3
IgG4
J-řetězec
IgM monomer
(podobné IgE, IgD)
IgM pentamer
IgA dimer


Antigen A
Antigen B
Antigen C
Protilátka 1
Anti-idiotyp
Idiotyp
Protilátka 2
(anti-idiotypová,
částečná podobnost
s antigenem B)

Polyclonal vs Monoclonal antibodies





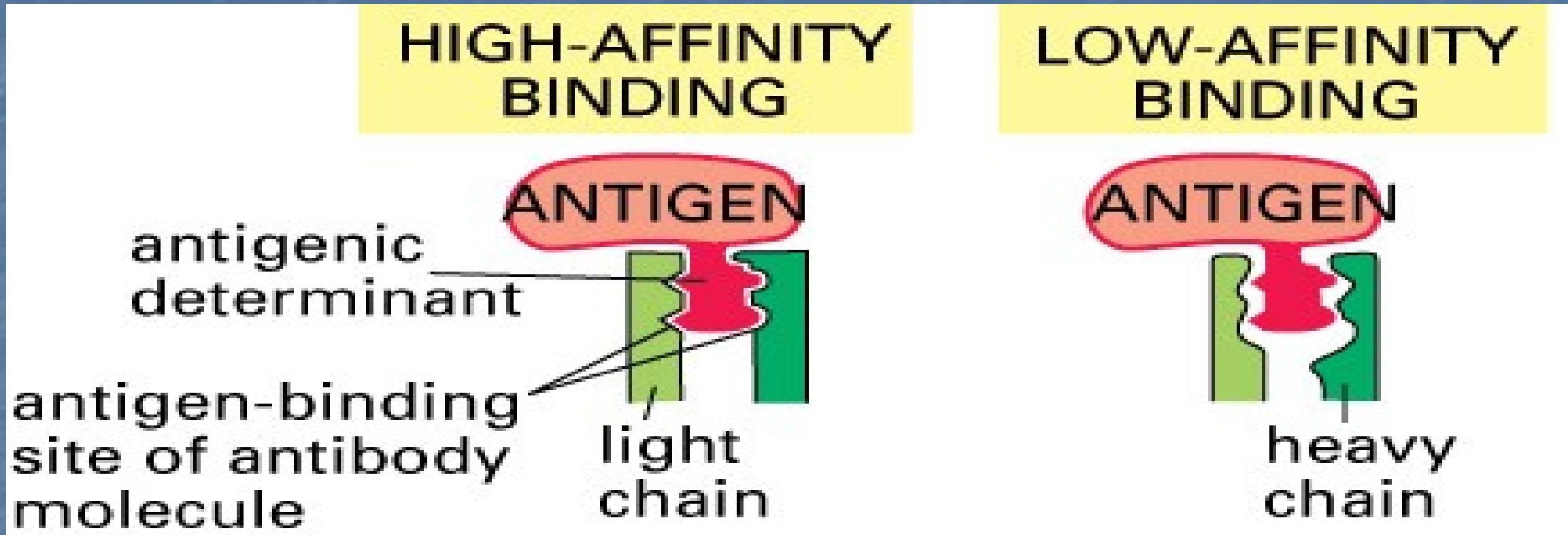


Figure 24–28. Molecular Biology of the Cell, 4th Edition.

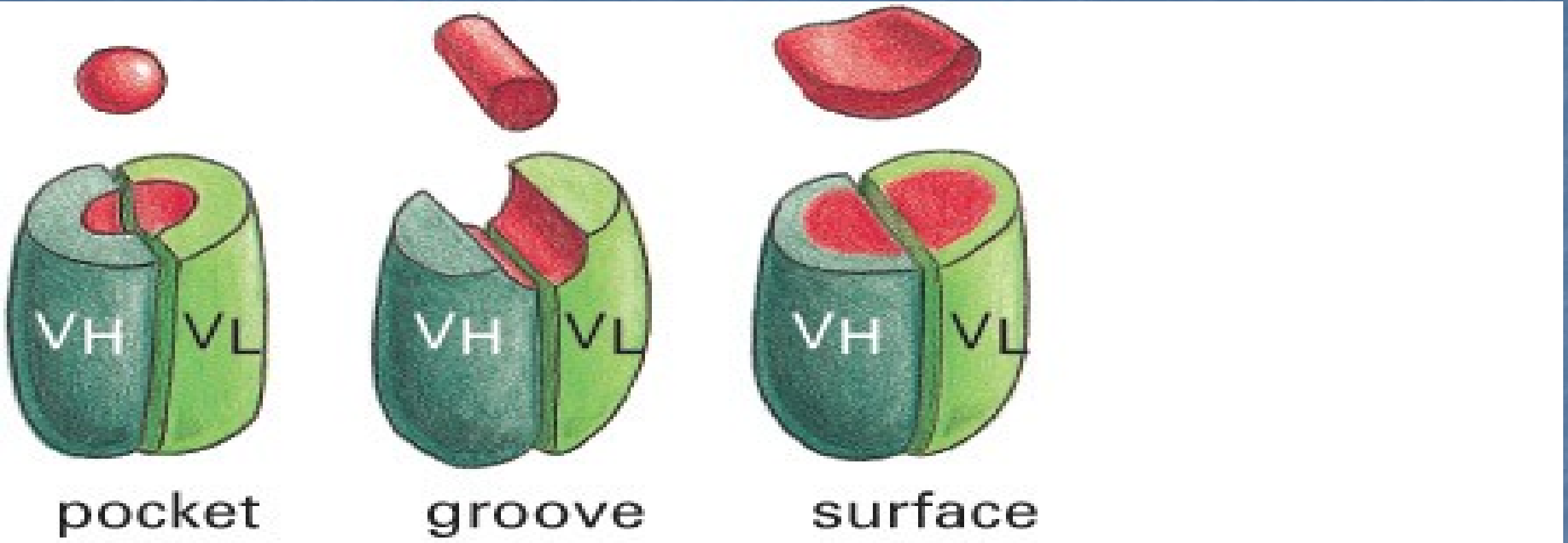
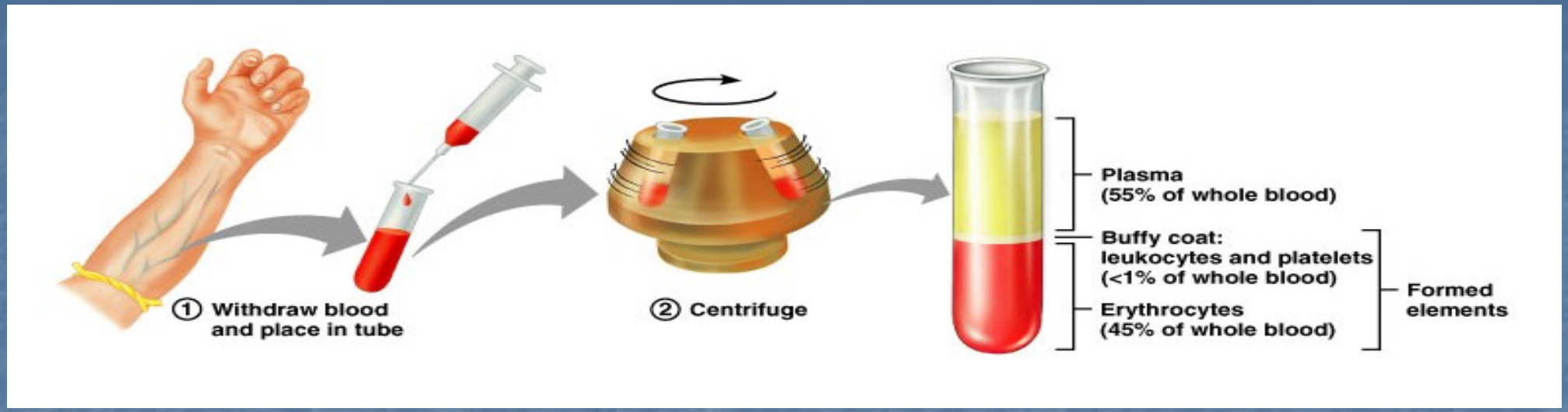


Figure 24–35. Molecular Biology of the Cell, 4th Edition.

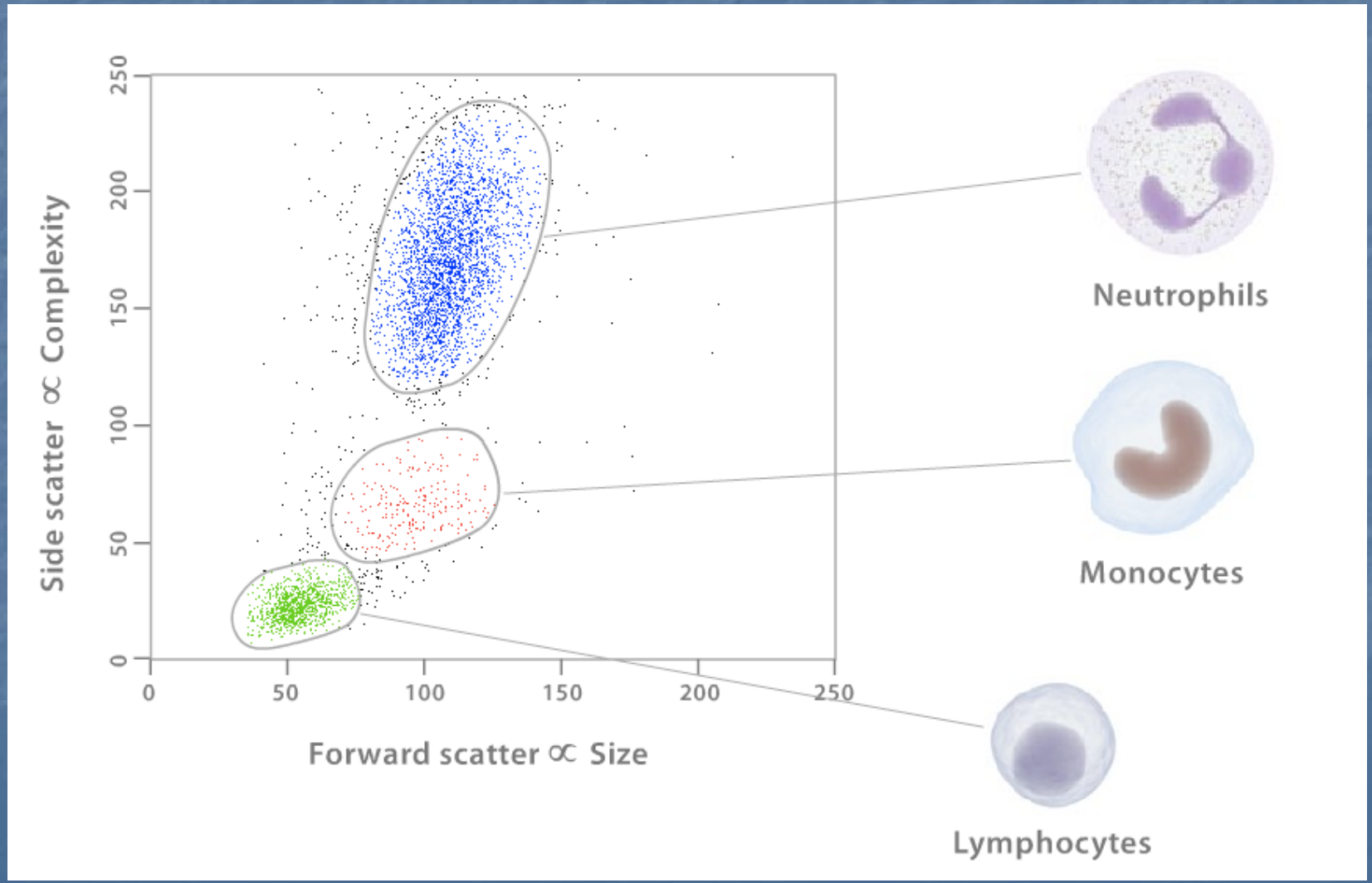


-Cells incubated at 4 C, 37°C or room temperature

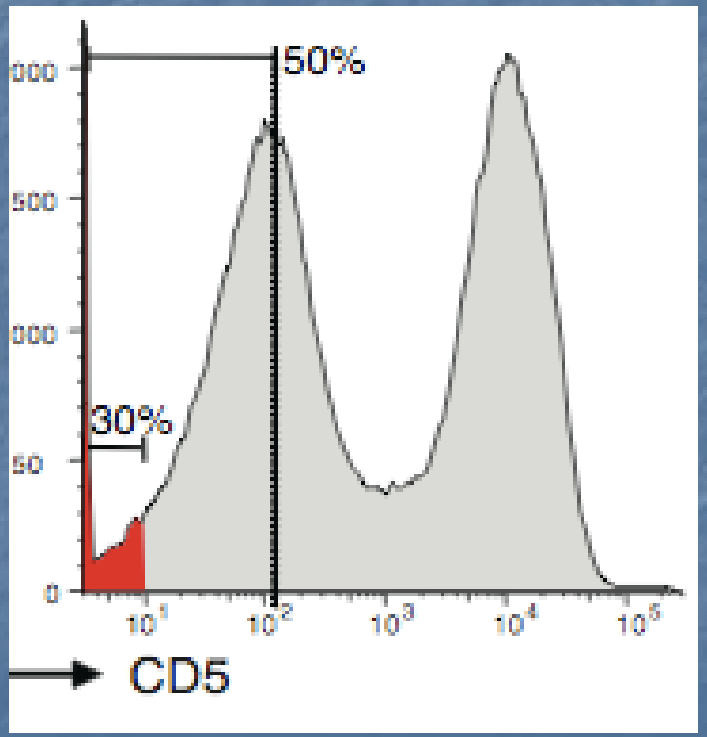
-avoid non-specific binding

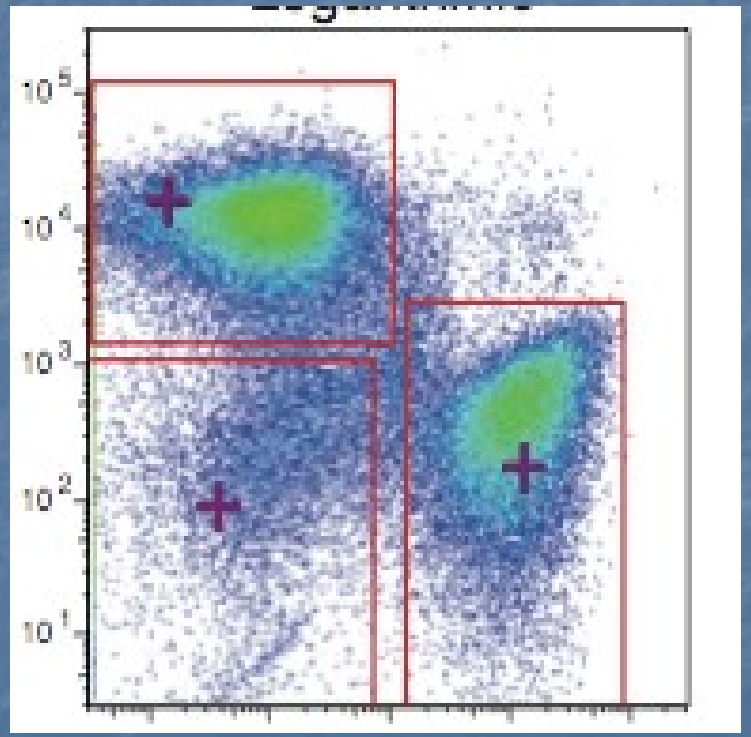
-always have a negative control

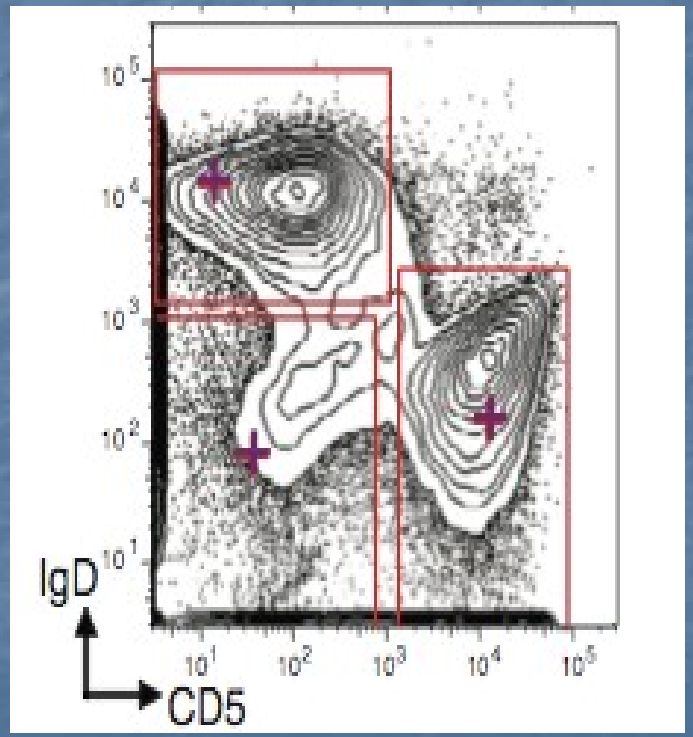
Flow Cytometry (Data)



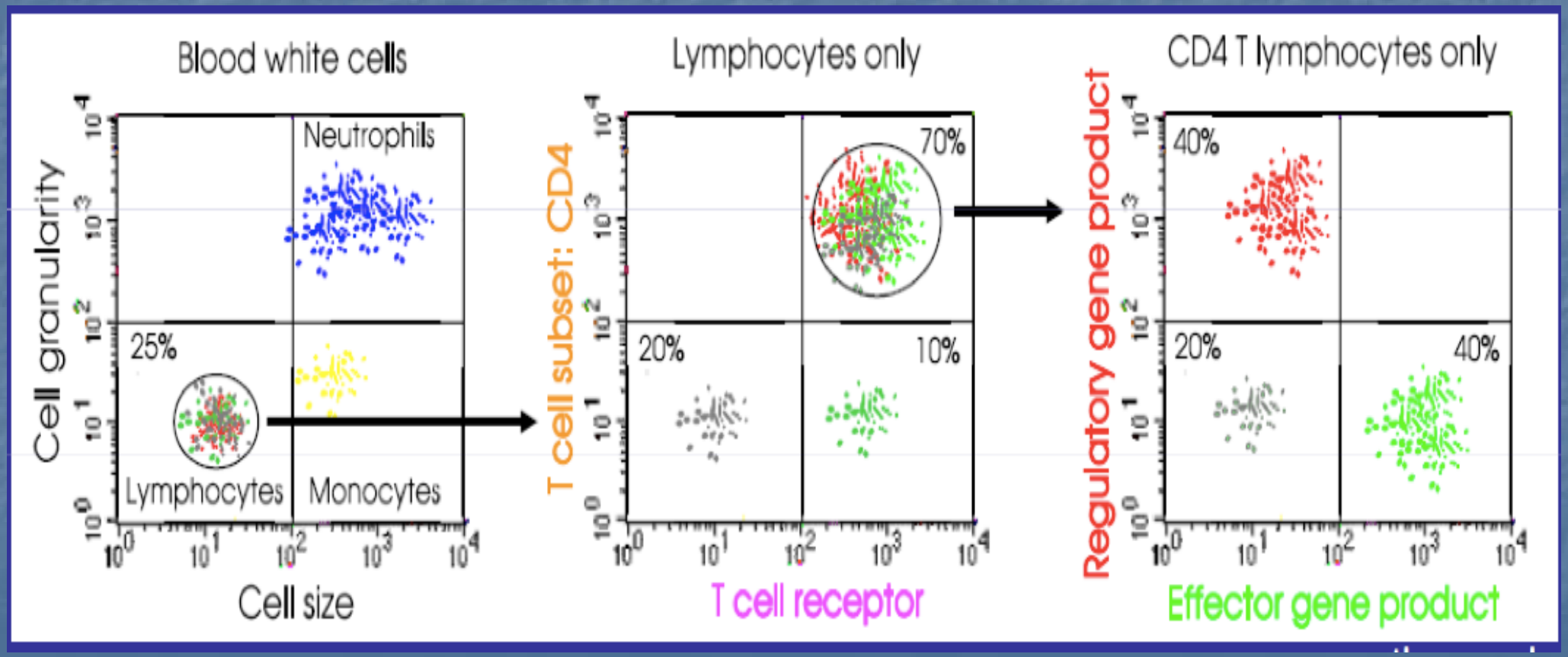
Flow Plots (1D histogram, 2D scatter plots, 2D contour plots)







Gating


Blood white cells
Neutrophils
25%
Lymphocytes
Monocytes
Cell granularity
Cell size
Lymphocytes only
70%
20%
10%
T cell subset: CD4
T cell receptor
CD4 T lymphocytes only
40%
20%
40%
Regulatory gene product
Effector gene product

Application of FC

- Phenotyping
- Cell function; proliferation, apoptosis, cell cycle
- Intracellular staining; IFN
- Nuclear staining
- Clinical
  - detection of malignant cells