UTHealth™

The University of Texas
Health Science Center at Houston

Medical School

Introduction to Flow Cytometry

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7/28/2015

Flow Cytometry

- Flow cytometry is the measurement of single cells as they pass single file through a beam of light in a fluid stream
- Cells are “flowing” through the instrument (flow cell)
- More control over which cells are being examined (cell sorting)
- Generate data for only the cells you are interested in

Flow Cytometric Immunophenotyping

- Characterization of a cell or group of cells by the presence or absence of certain antigens on their *surface* or in their *cytoplasm*
  - Pre-described immunophenotypes can be used to aid in the diagnosis of hematopoietic neoplasms
  - Pattern recognition + Right context
- Majority of the time, this is what we are talking about when we say “flow cytometry”

Figure 2

MARGINAL ZONE CD19, CD20, CD22, CD79, sIg, FMC7, bcl-2, CD23+ (CD5, CD10, CD43, bcl-6 neg)

NODAL MALT

CLL CD19, CD20, CD43,CD23, sIg+/-, CD5 (CD79 CD10, CD22, FMC7 neg)

HCL CD19, CD20, CD22, sIg++, CD11c, CD25, CD103, CD123 (CD5, CD10, CD23 neg)

T LYMPHOMAS AND ANGIOIMMUNOBLASTIC CD5+/CD10+/CD3+/CD4+

SEZARY CD3, CD2, CD5, CD4, CD7+/-(CD8 neg)

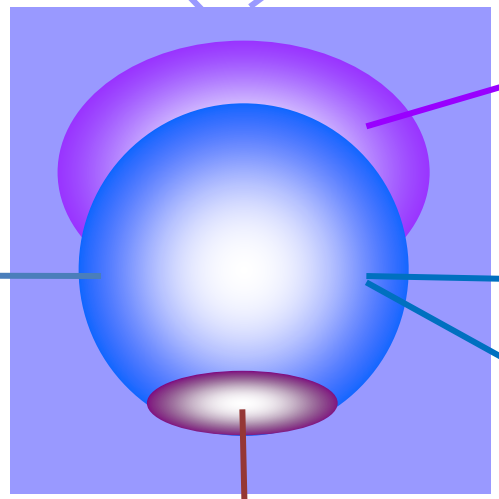
LARGE CELLS ANAPLASTIC CD2, CD4, CD3+, CD30

PERIPHERAL CD2, CD3, CD5, CD4

ANGIOCENTRIC CD2, CD5, CD4 ou CD8, CD56

INTESTINAL CD3, CD7, CD103

ATLL CD3, CD4, CD25, CD45RO, CD7neg



MANTLE ZONE CD19, CD20, CD22, CD79, FMC7, sIg, bcl-1, CD5, CD43,

(CD10, CD23 neg)

BURKITT CD19, CD20, CD22, CD79, sIg, CD10, DR, bcl-6

(CD5, CD23, Tdt neg)

FOLLICULAR CD19, CD20, CD22, sIg+, CD79, CD10, bcl-2

(CD5, CD23, CD43 neg)

WALDENSTROM CD19, CD20+, CD22, CD79 sIg+, cIg, FMC7 (CD5, CD10, CD23 neg)

DIFFUSE LARGE B CELL LYMPHOMAS, sIg+, cIg+, CD19, CD20, CD79, CD22++, CD79, CD10+ (CD5 rare cases, bcl-2, bcl-6, MUM-1 can be useful for further classification)

INTESTINAL OR LUNG MALT

Follicular, mantle zone, marginal zone

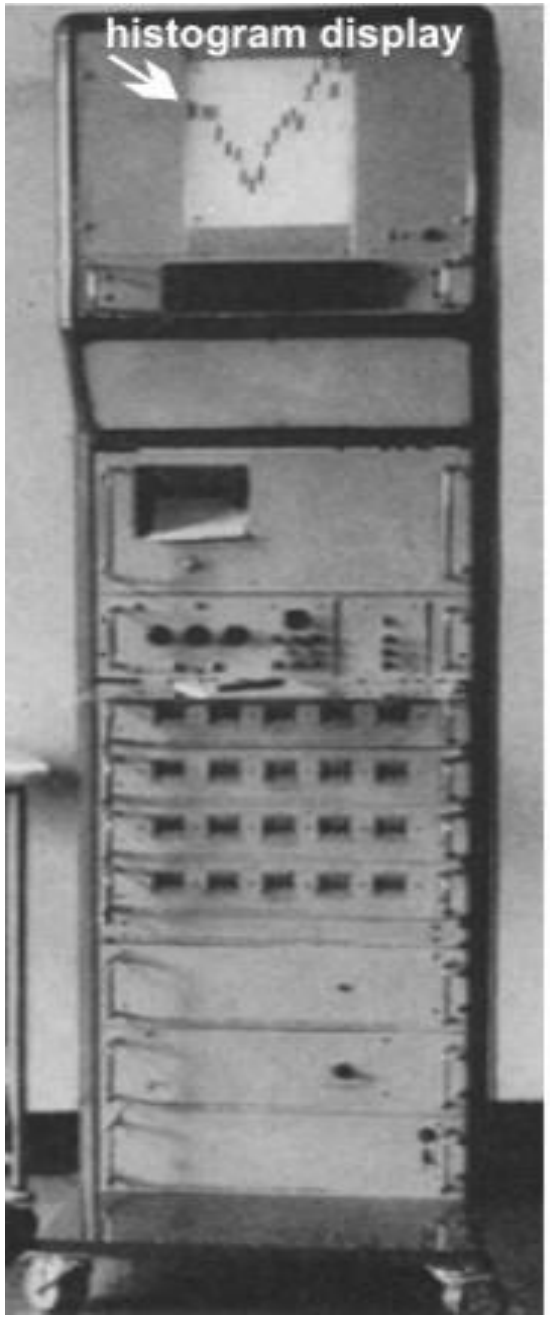
SLVL

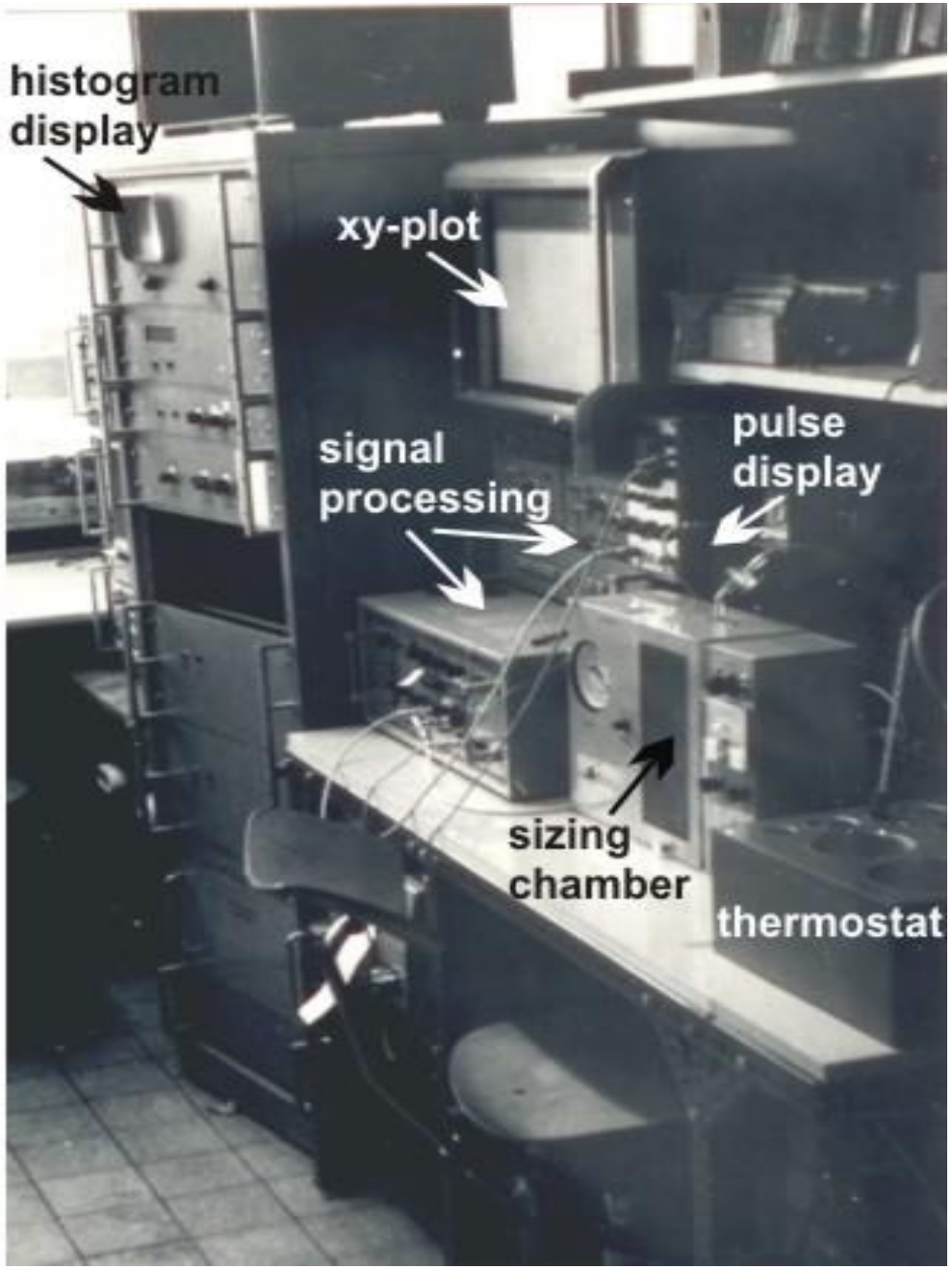
circulating marginal zone cells

MYELOMA cIg, CD56, CD45+, CD38, CD138

(CD19, CD20, CD22 neg)


BD FACSCanto II


histogram display


histogram display
xy-plot
signal processing
pulse display
sizing chamber
thermostat

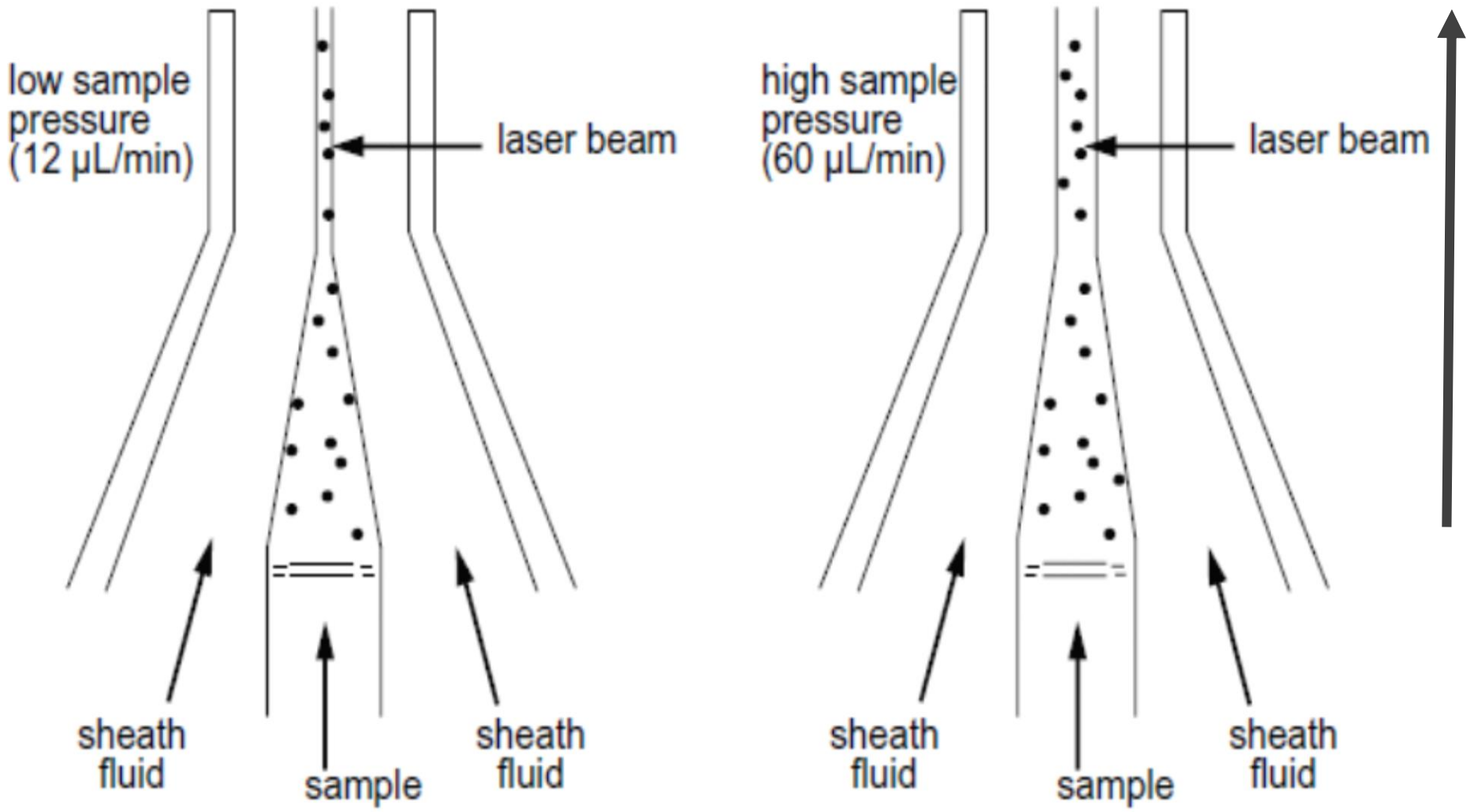

Built-in 19" TFT Screen
Welcome to
CUBE
CyFlow®
Cube 8


WASTE
SHEATH
PARTEC

Why?

- For the same reason we look at a sample under a microscope:
- In a heterogeneous collection of cells:
- Determine the presence/absence of cell(s) of interest
- Determine the characteristics of the cell(s) present:
- “Parameters”
- Size, granularity, immunophenotype, proliferation rate, etc.
- MPC: multi-parameter flow cytometry

FLOW CELL

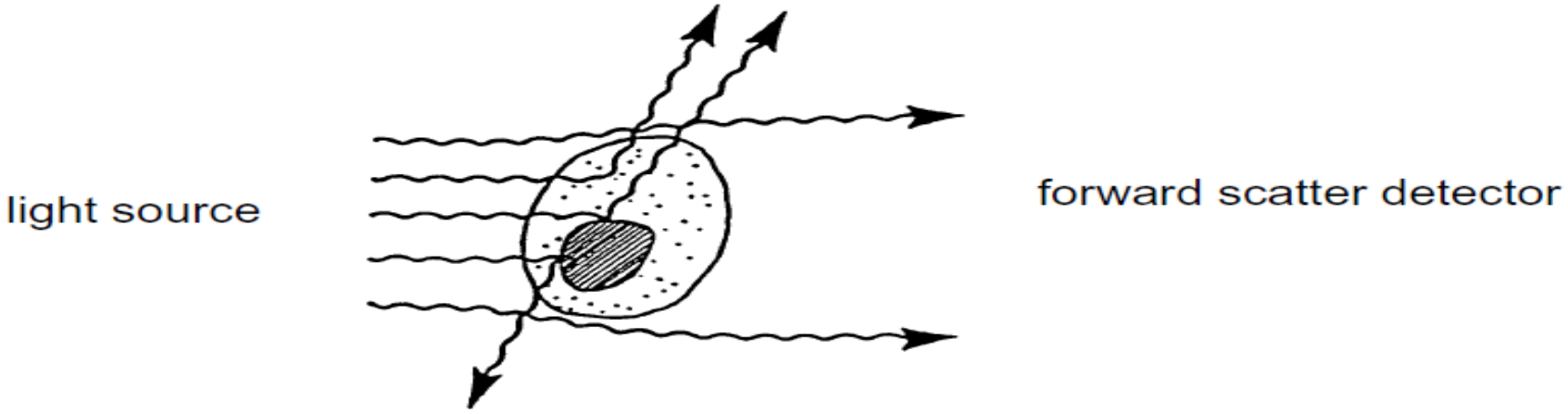


COAXIAL FLOW

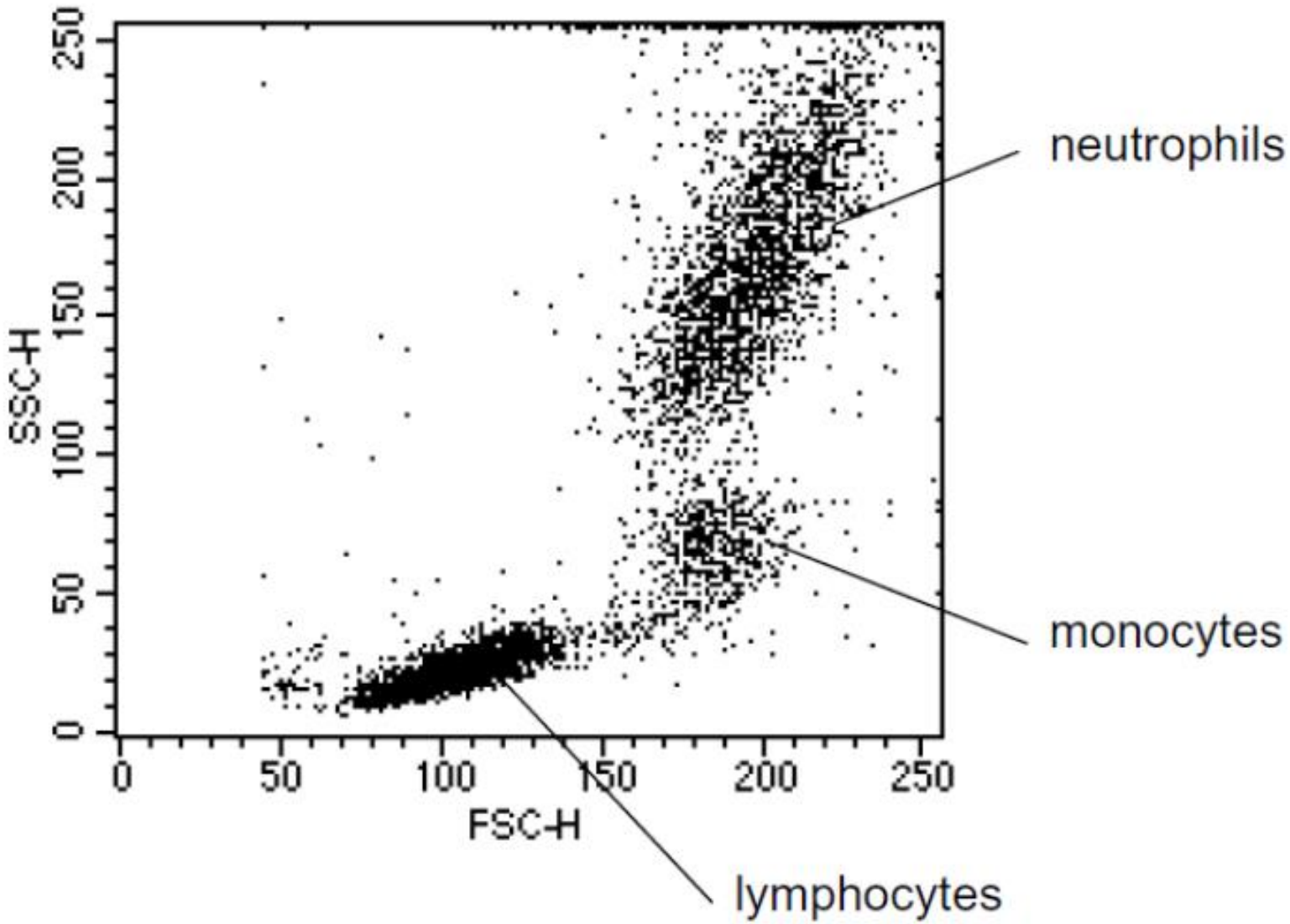
Light Scatter

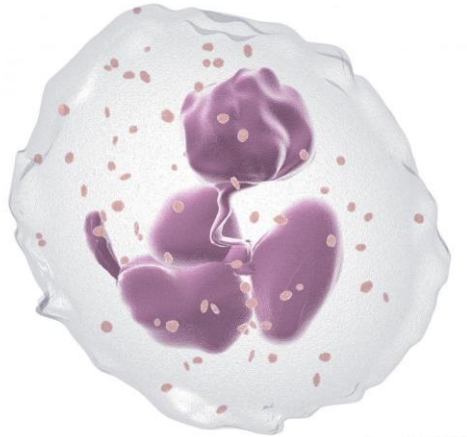
- Light passing in a “straight line”
- Deflection of light from its straight path is light scatter
  - Requires some kind of interaction with matter
  - Wavelength (energy) of the light
  - Characteristics of the matter
- If we control everything else (wavelength, etc.) we can use light scatter to determine characteristics of matter
  - Cells in our case

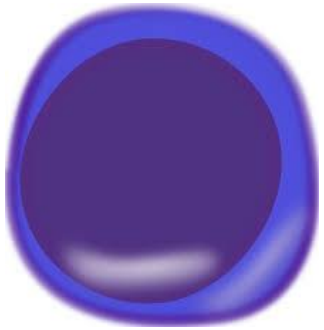
CYTOPLASMIC GRANULARITY

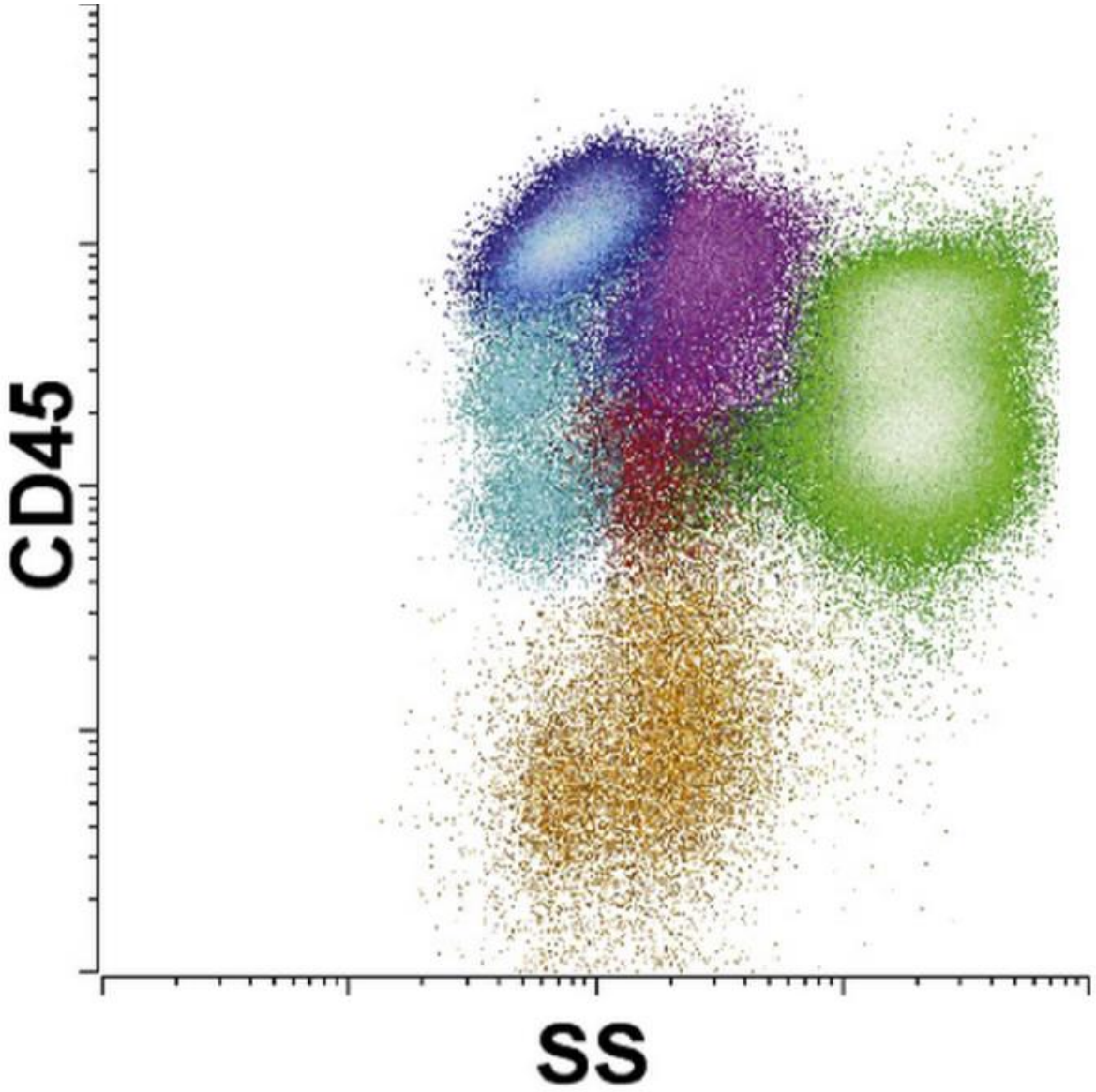


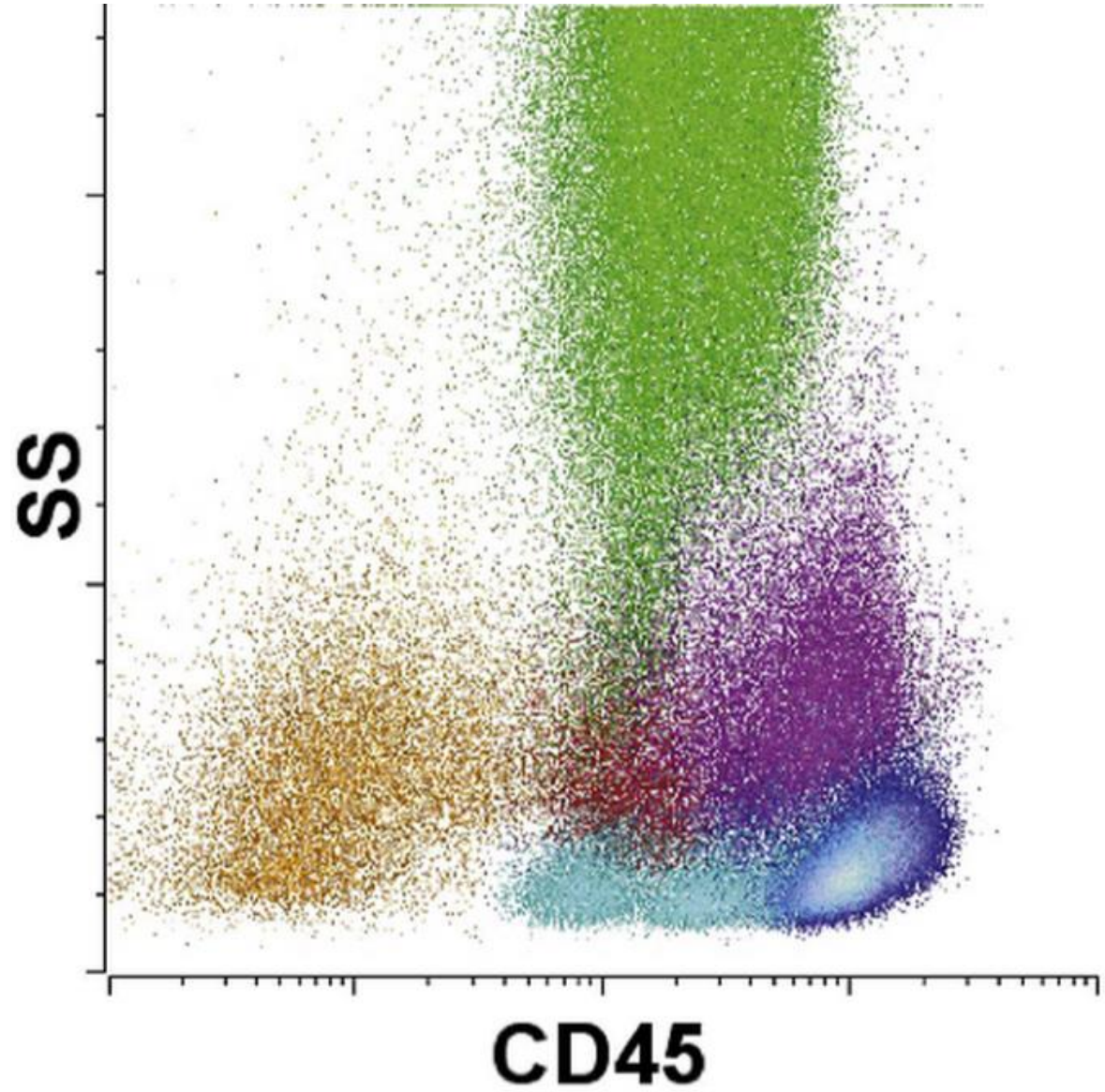
CELL SIZE


SSC-H
FSC-H
0
50
100
150
200
250
neutrophils
monocytes
lymphocytes


wiseGEEK




CD45
SS


SS
CD45

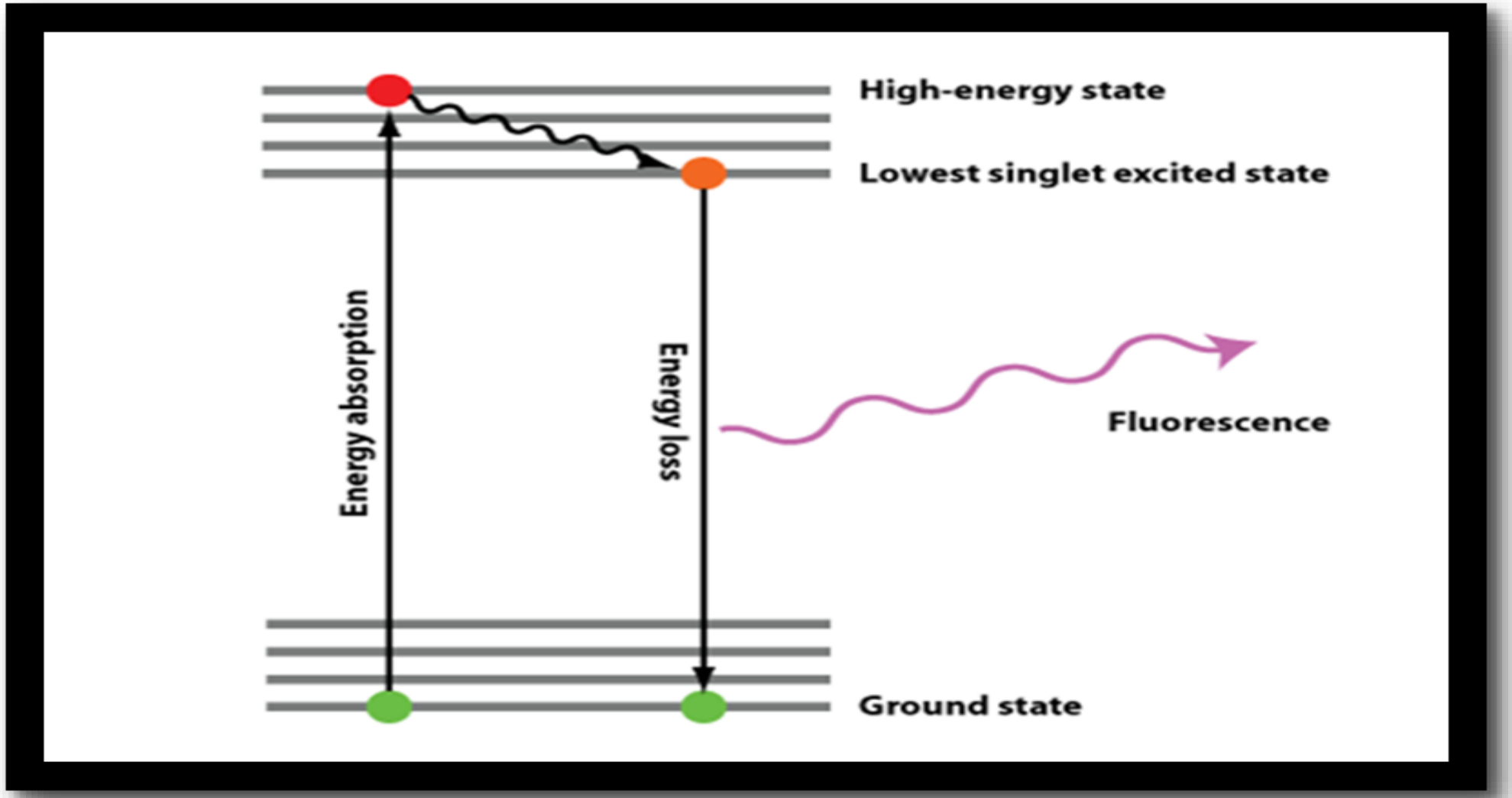
Fluorescence

Absorption of light at a certain wavelength(s)

Photon of light hits electrons in matter->Excitation of electrons to higher energy state->Quick decay to ground state->Excess light is released as a new photon

Emission of light a (usually) a longer (lower energy) wavelength(s)

$$E = hc/\lambda$$


High-energy state
Lowest singlet excited state
Energy absorption
Energy loss
Fluorescence
Ground state

Aragonit Crystal

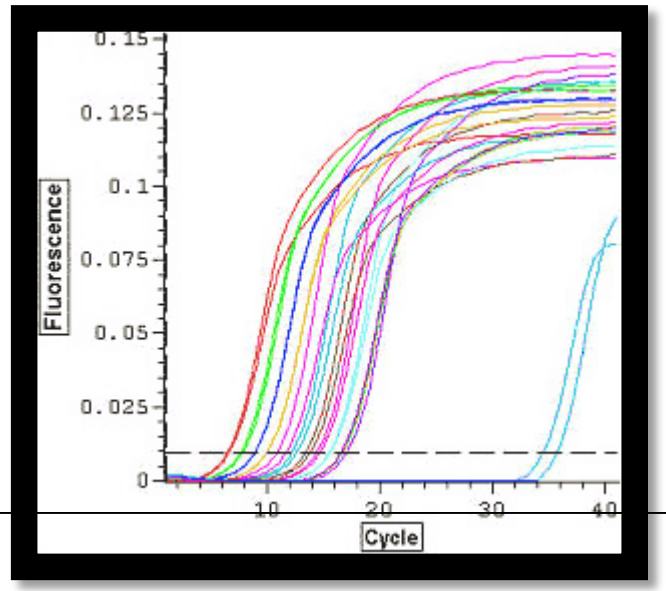
Higher Energy Wavelength Absorption

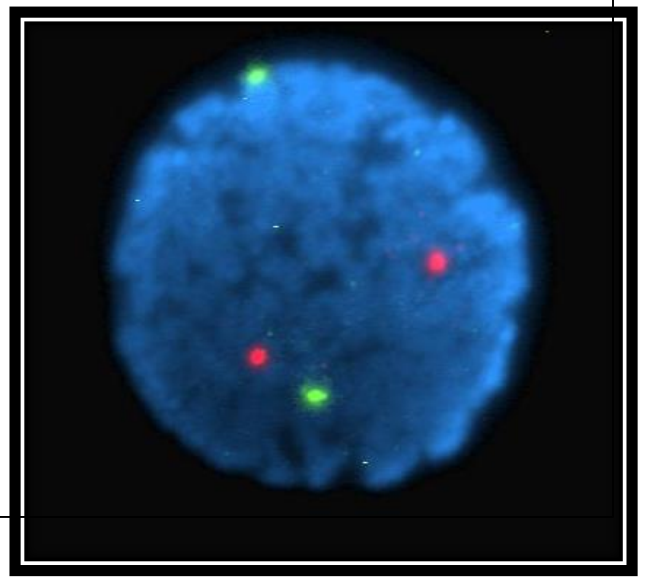
Lower Energy Wavelength Emission

Fluorochromes

Fluorescent chemical compounds

Used alone or as a substrate for enzymatic reaction, probe, antibody

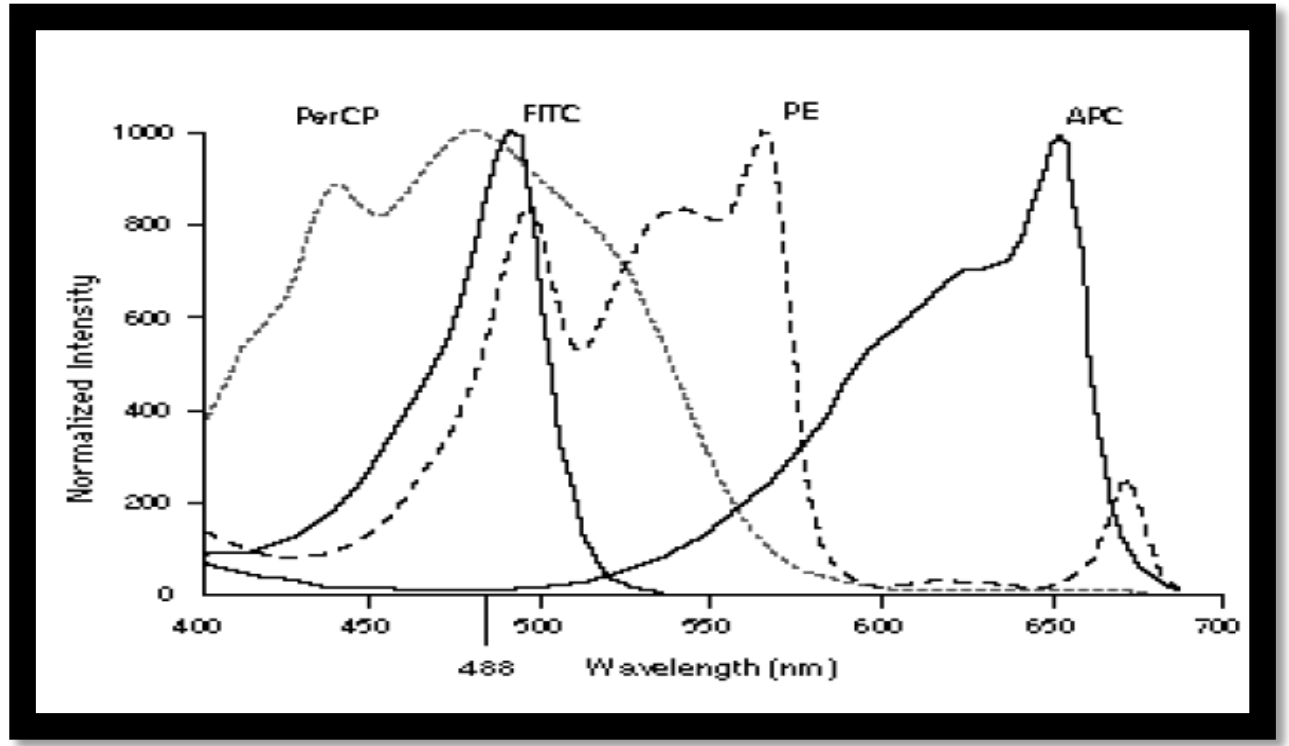


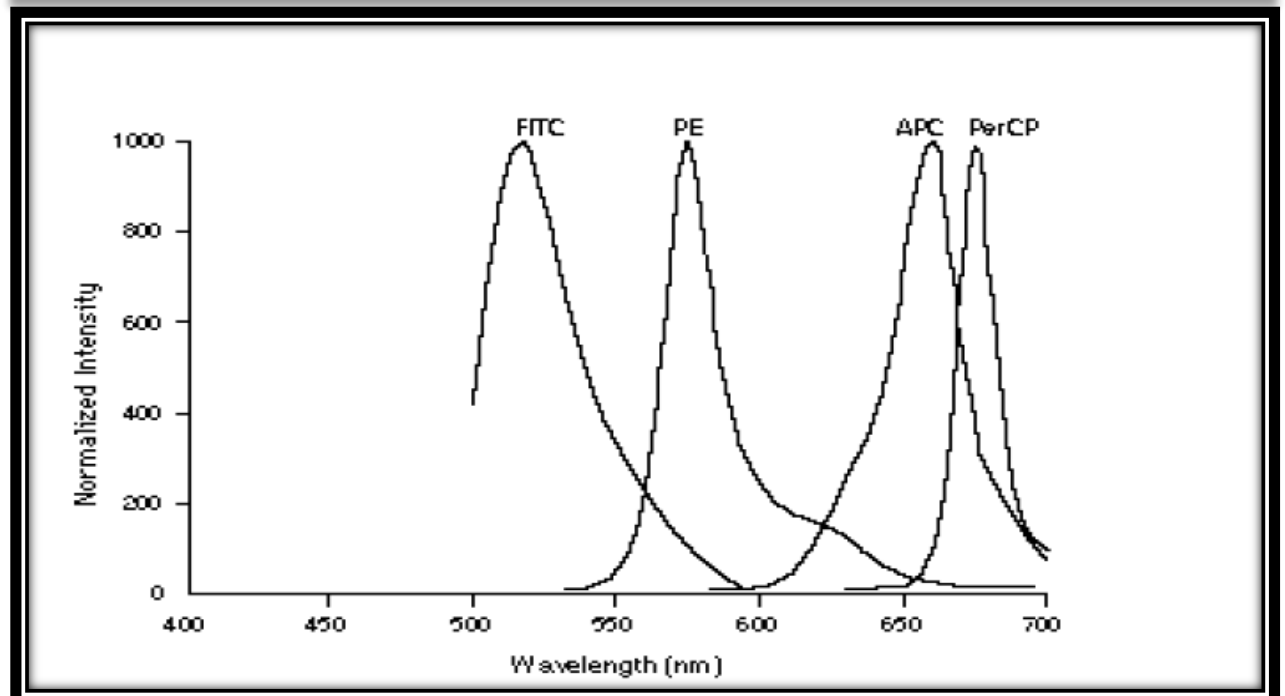


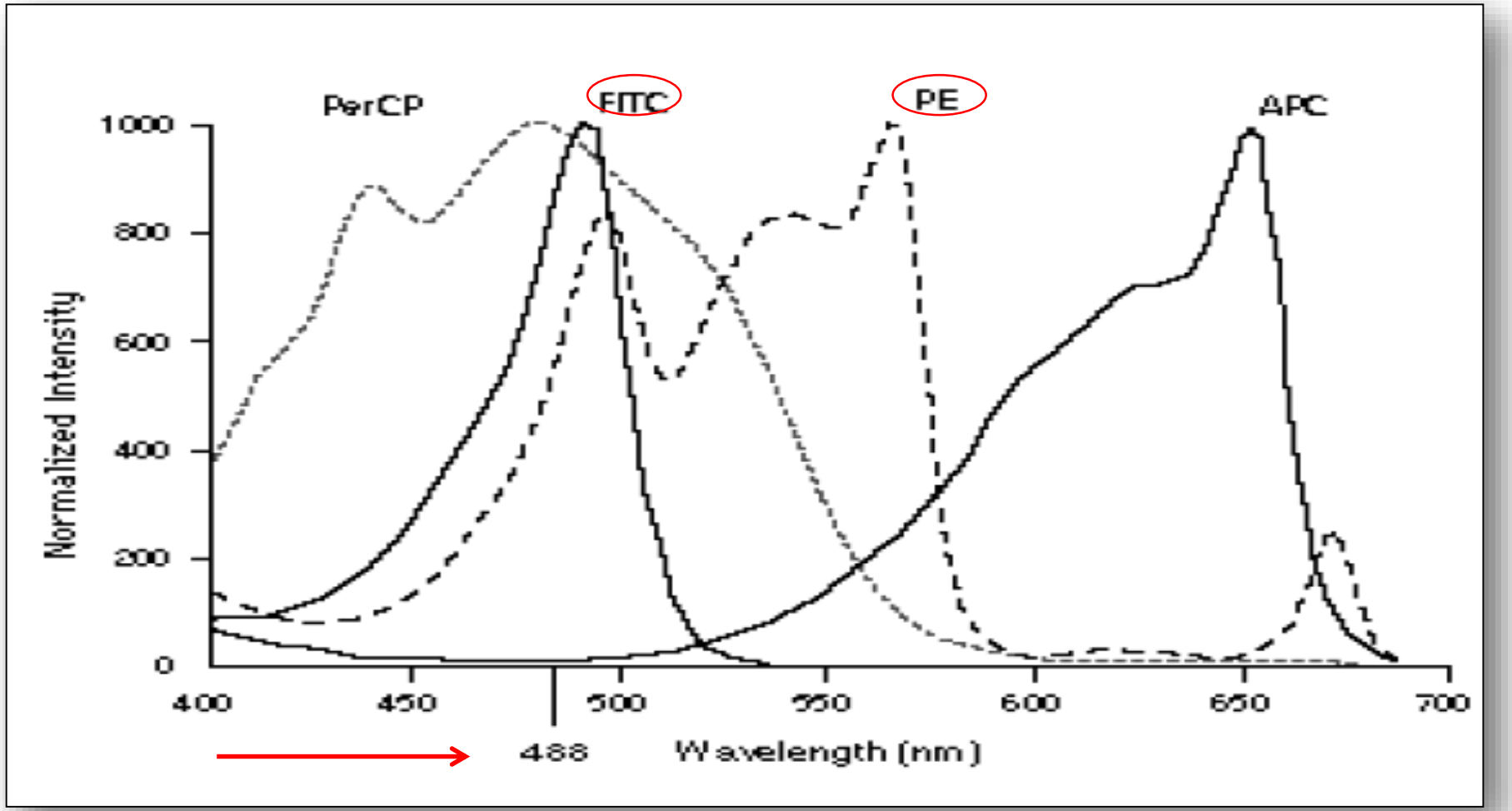
Fluorochromes

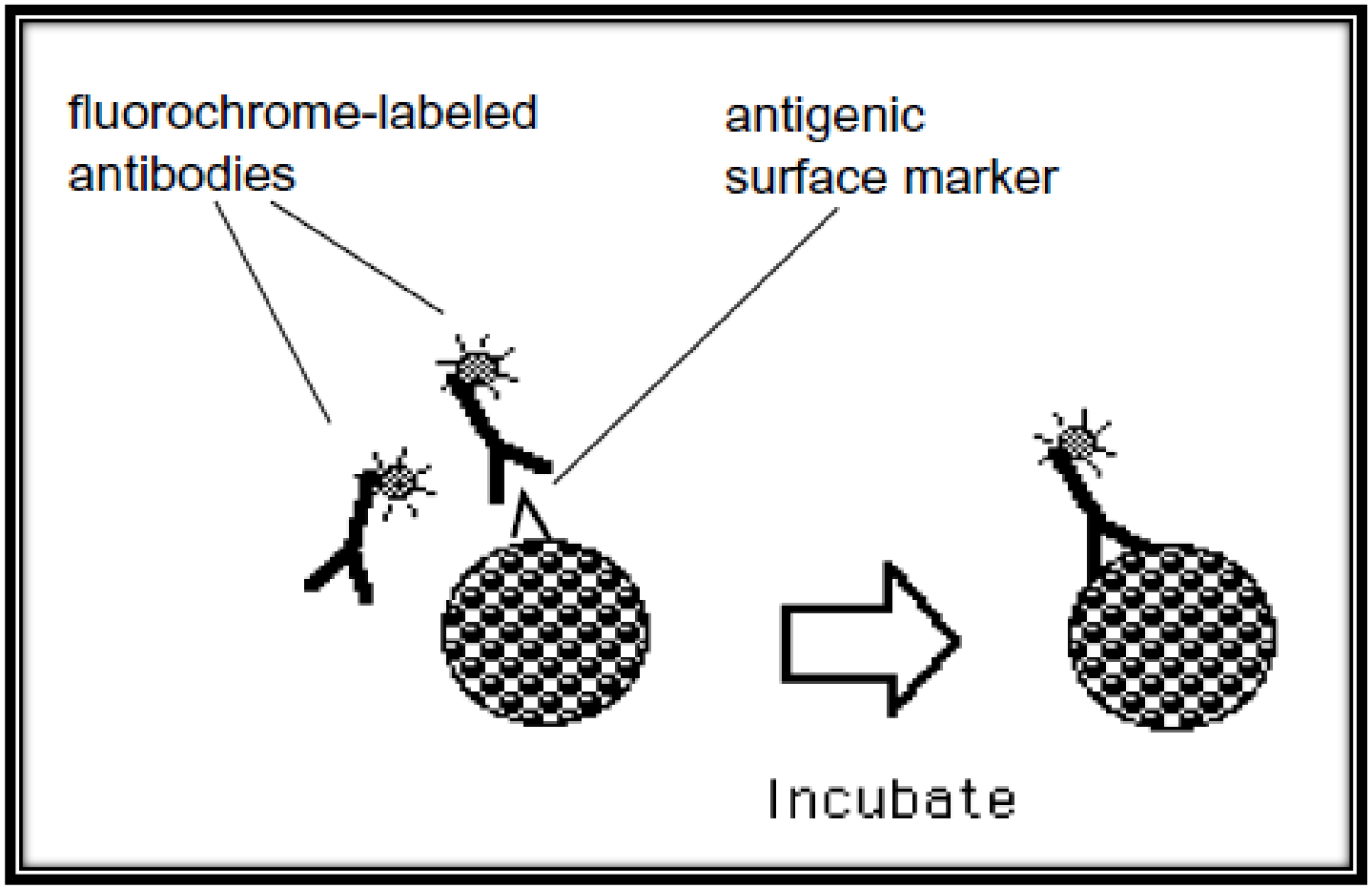
- Wavelength range in which a substance absorbs light is its absorption spectra
- Wavelength range in which that substance emits light is its emission spectra
- Difference between peak absorption wavelength and peak emission wavelength is called “Stoke’s Shift”

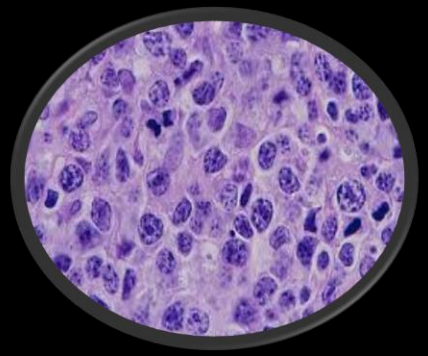
FITC = fluorescein isothiocyanate
PE = phycoerythrin
PerCP = peridinin chlorophyll protein
APC = allophycocyanin






PerCP
FITC
PE
APC
1000
800
600
400
200
0
Normalized Intensity
400
450
500
550
600
650
700
488
Wavelength (nm)


fluorochrome-labeled
antibodies
antigenic
surface marker
Incubate

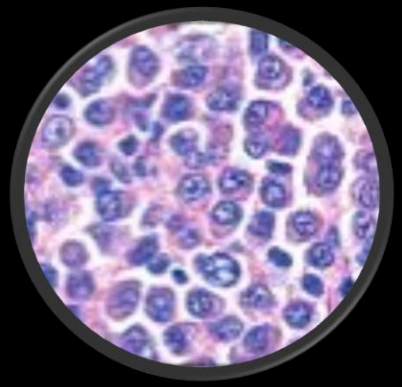


Kappa

Lambda

sKappa FITC
sLambda PE

cKappa FITC
cLambda PE

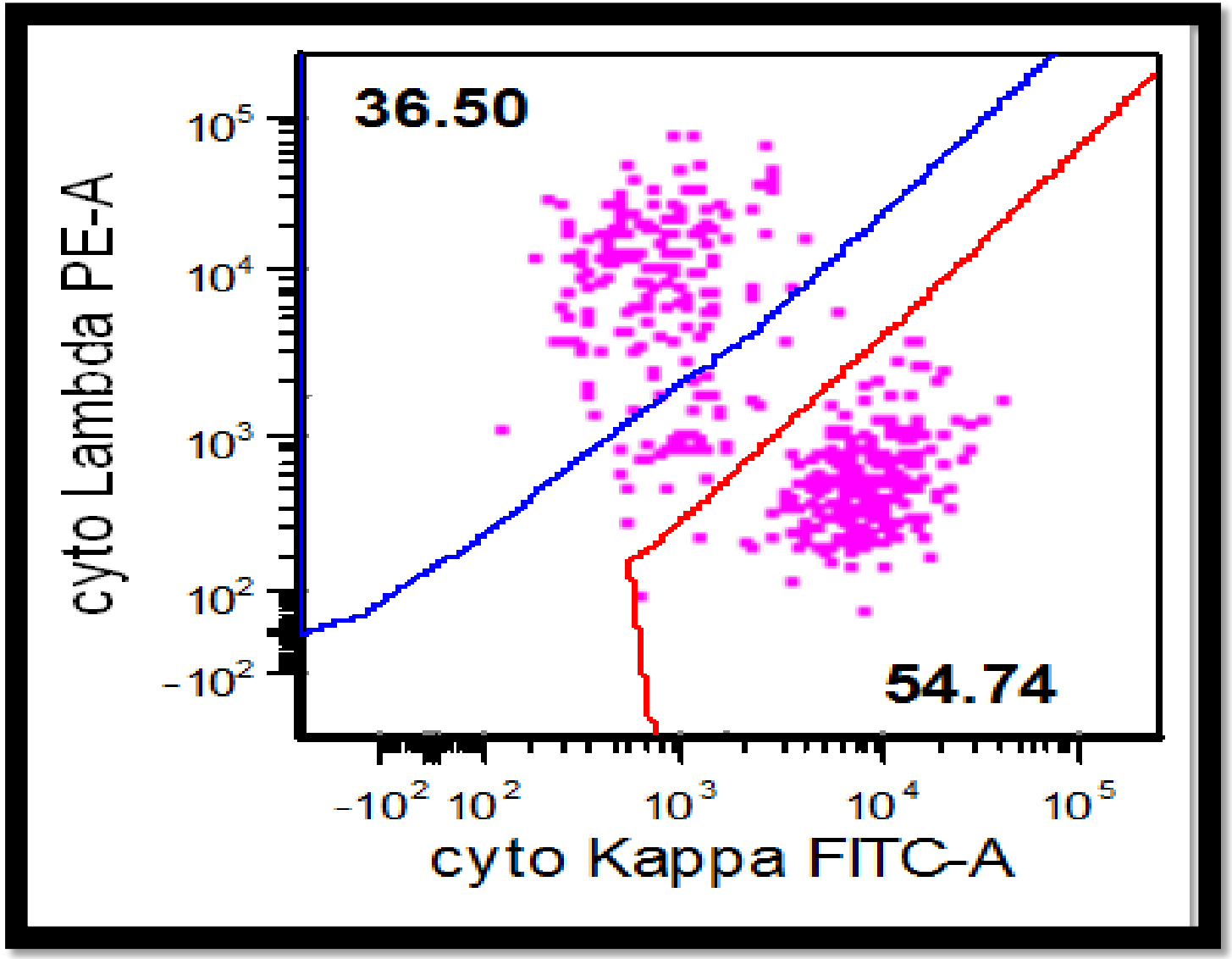


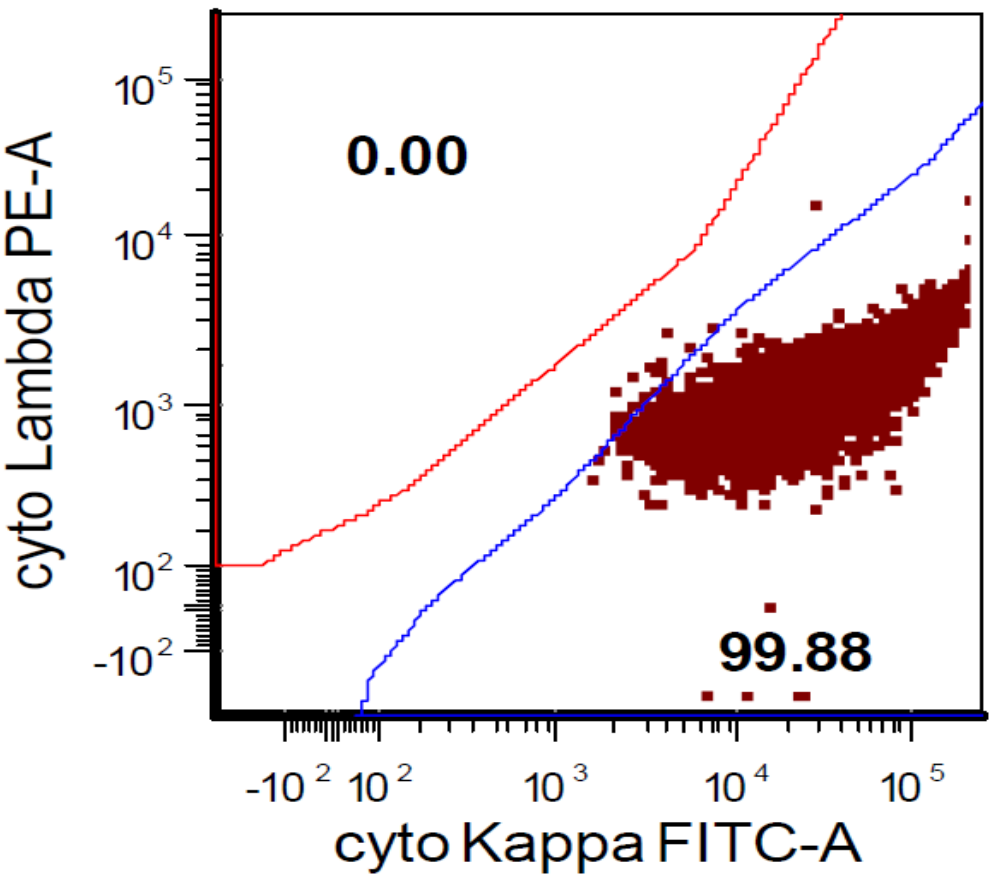
Permeabilization

Fixation

Kappa

Lambda


36.50
54.74
cyto Lambda PE-A
cyto Kappa FITC-A
10^5
10^4
10^3
10^2
-10^2
-10^2 10^2 10^3 10^4 10^5

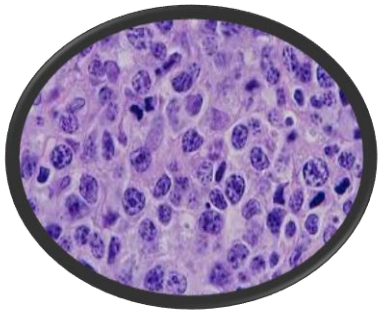

cyto Lambda PE-A
10^5
10^4
10^3
10^2
-10^2
0.00
99.88
-10^2 10^2 10^3 10^4 10^5
cyto Kappa FITC-A

Fluorochrome	Fluorescence Emission Color	Em-Max (nm)
Brilliant Violet™ 421	Blue	421
BD Horizon™ V450	Blue	448
Pacific Blue™	Blue	452
BD Horizon™ V500	Green	500
Alexa Fluor® 488	Green	519
FITC	Green	519
PE	Yellow	578
BD Horizon PE-CF594	Orange	612
APC	Red	660
Alexa Fluor® 647	Red	668
PE-Cy™5	Red	667
PerCP	Red	678
PerCP-Cy™5.5	Far Red	695
Alexa Fluor® 700	Far Red	719
PE-Cy™7	Infrared	785
APC-Cy7	Infrared	785
BD APC-H7	Infrared	785

10^4 to 10^6 per tube

CD8-CD19-CD4-CD34-CD3-CD14-CD56-CD45

KAPPA-LAMBDA-CD22-CD19-CD10-CD20 -CD5 BV 421-CD45



CD43-CD200-CD5-CD19-CD10-CD20-CD38 BV 421-CD45

CD11c-CD23-CD22-CD19-CD79b- -CD5 BV 421-CD45

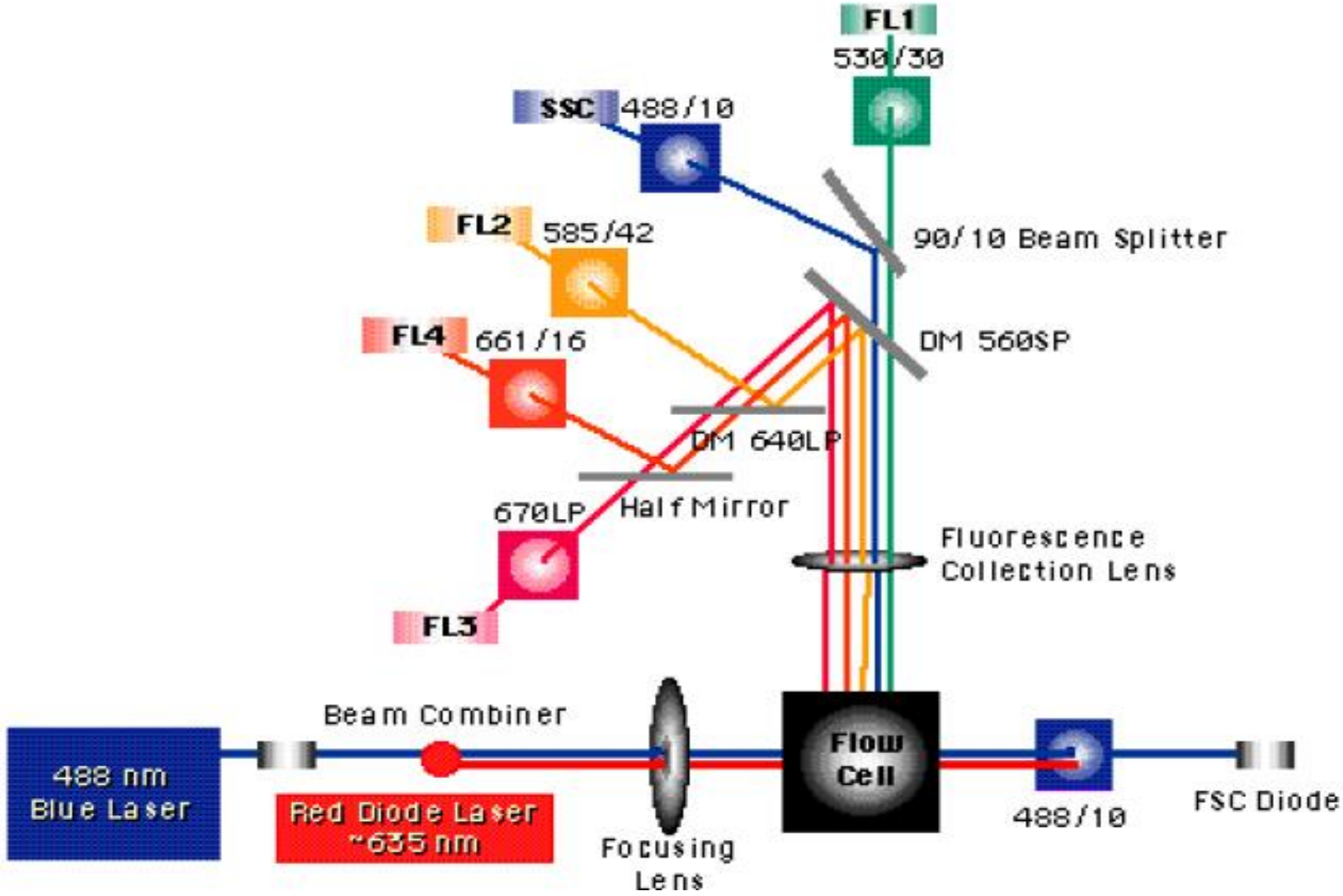
Laser(s)

Lenses & Mirrors

Filters

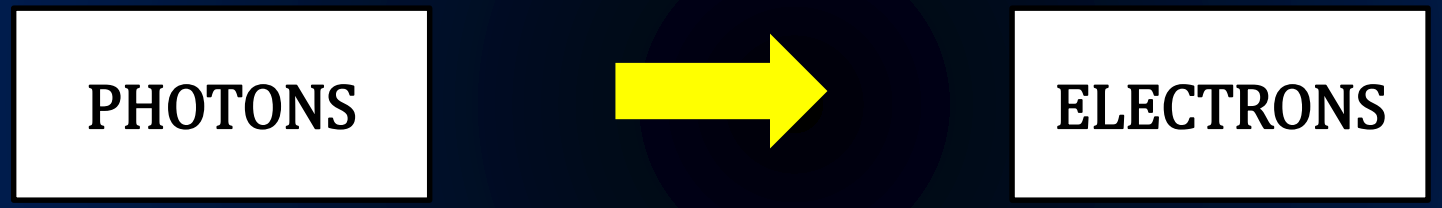
Detectors

Diodes & PMT



Electronic System

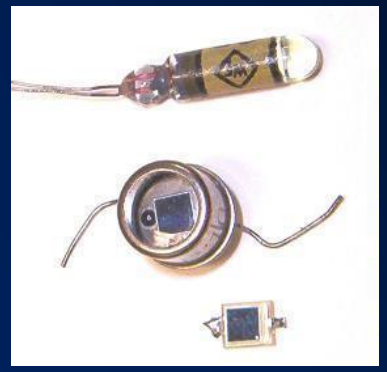
- Converts light signals into numerical data for analysis



- Cell or “event” hits laser
- Generates a pulse

Electronic System

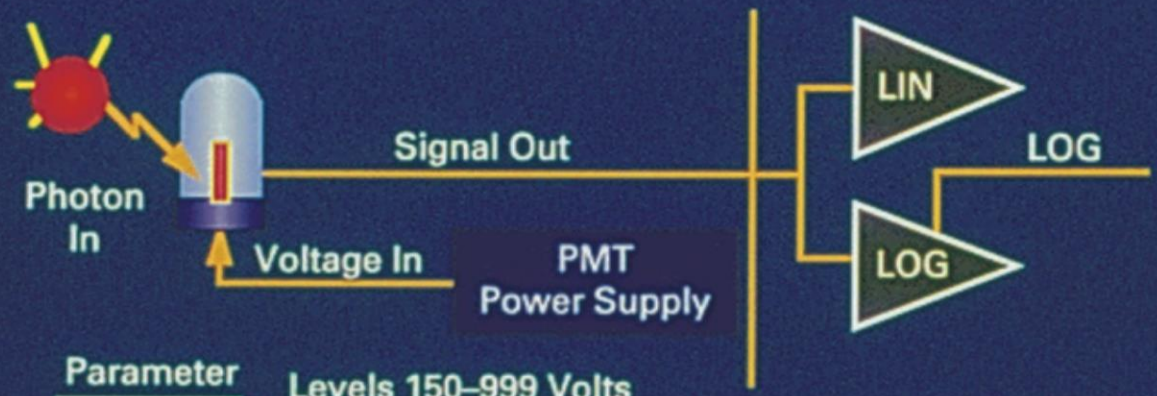
- Photodiodes (FSC and SSC)
- Photomultipliers (Fluorescence)
- Convert light signal (photons) into electrical signals (pulses)
- Thresholds
- Voltage (amplification)



Electronic System

- Each event gets a numeric value (pulse height, width, area) and assigned a channel number
- Raw data stored as “list mode data”
- Each channel number is assigned point on a dot plot
- Linear or logarithmic scale

Conversion of Optical Signals to Proportional Electronic Signals

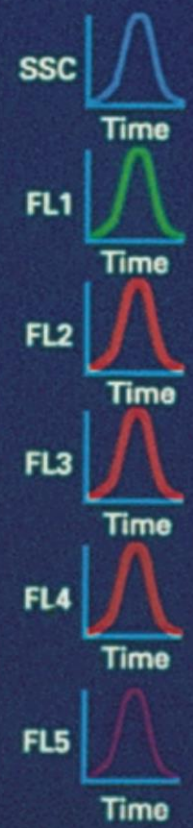


Parameter

Levels 150–999 Volts

SSC
FL1
FL2
FL3
FL4
FL5

Voltage Pulses

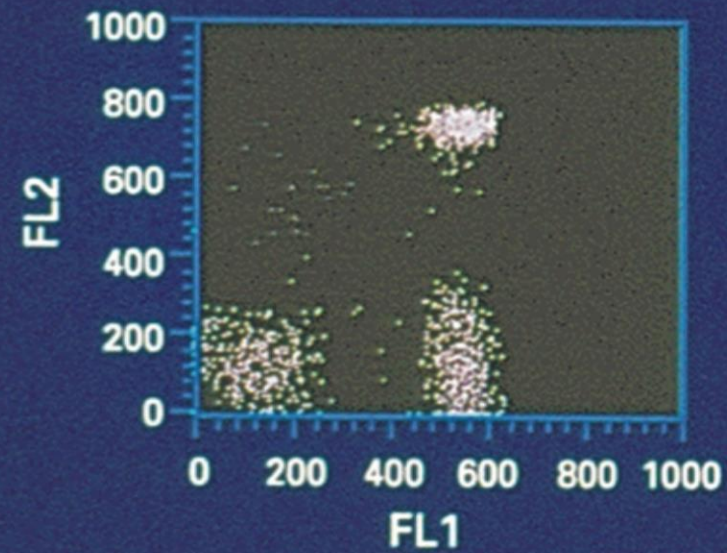


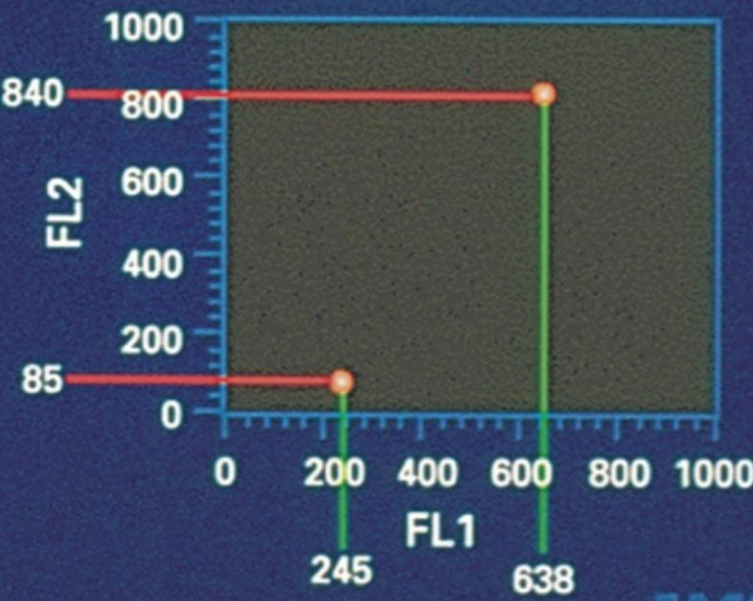
FACS Academy

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





FACS Academy

Fluidics and optics system allows the simultaneous generation of 8 signals from each event
“10 dimensions and beyond”

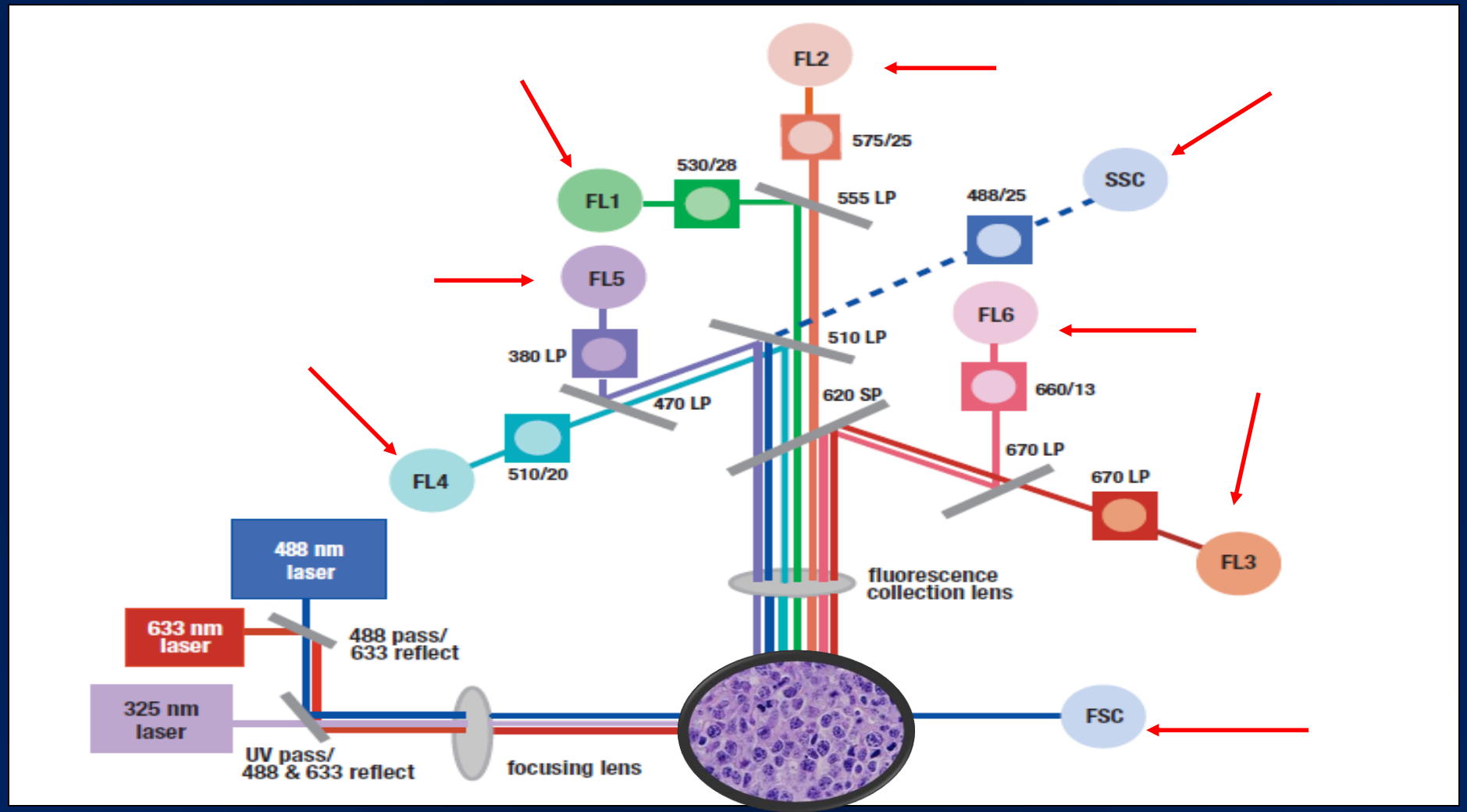
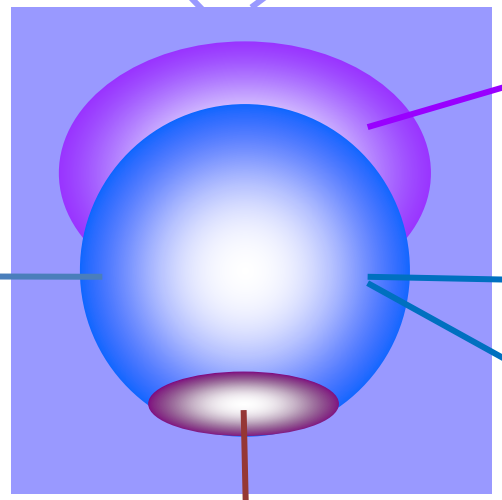


Figure 2



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NODAL MALT

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HCL CD19, CD20, CD22, sIg++, CD11c, CD25, CD103, CD123 (CD5, CD10, CD23 neg)

T LYMPHOMAS AND ANGIOIMMUNOBLASTIC CD5+/CD10+/CD3+/CD4+

SEZARY CD3, CD2, CD5, CD4, CD7+/-(CD8 neg)

LARGE CELLS ANAPLASTIC CD2, CD4, CD3±, CD30

PERIPHERAL CD2, CD3, CD5, CD4

ANGIOCENTRIC CD2, CD5, CD4 ou CD8, CD56

INTESTINAL CD3, CD7, CD103

ATLL CD3, CD4, CD25, CD45RO, CD7neg

MANTLE ZONE CD19, CD20, CD22, CD79, FMC7, sIg, bcl-1, CD5, CD43,

(CD10, CD23 neg)

BURKITT CD19, CD20, CD22, CD79, sIg, CD10, DR, bcl-6

(CD5, CD23, Tdt neg)

FOLLICULAR CD19, CD20, CD22, sIg+, CD79, CD10, bcl-2

(CD5, CD23, CD43 neg)

WALDENSTROM CD19, CD20±, CD22, CD79 sIg±, cIg, FMC7 (CD5, CD10, CD23 neg)

DIFFUSE LARGE B CELL LYMPHOMAS, sIg±, cIg+, CD19, CD20, CD79, CD22++, CD79, CD10± (CD5 rare cases, bcl-2, bcl-6, MUM-1 can be useful for further classification)

INTESTINAL OR LUNG MALT

Follicular, mantle zone, marginal zone

SLVL

circulating marginal zone cells

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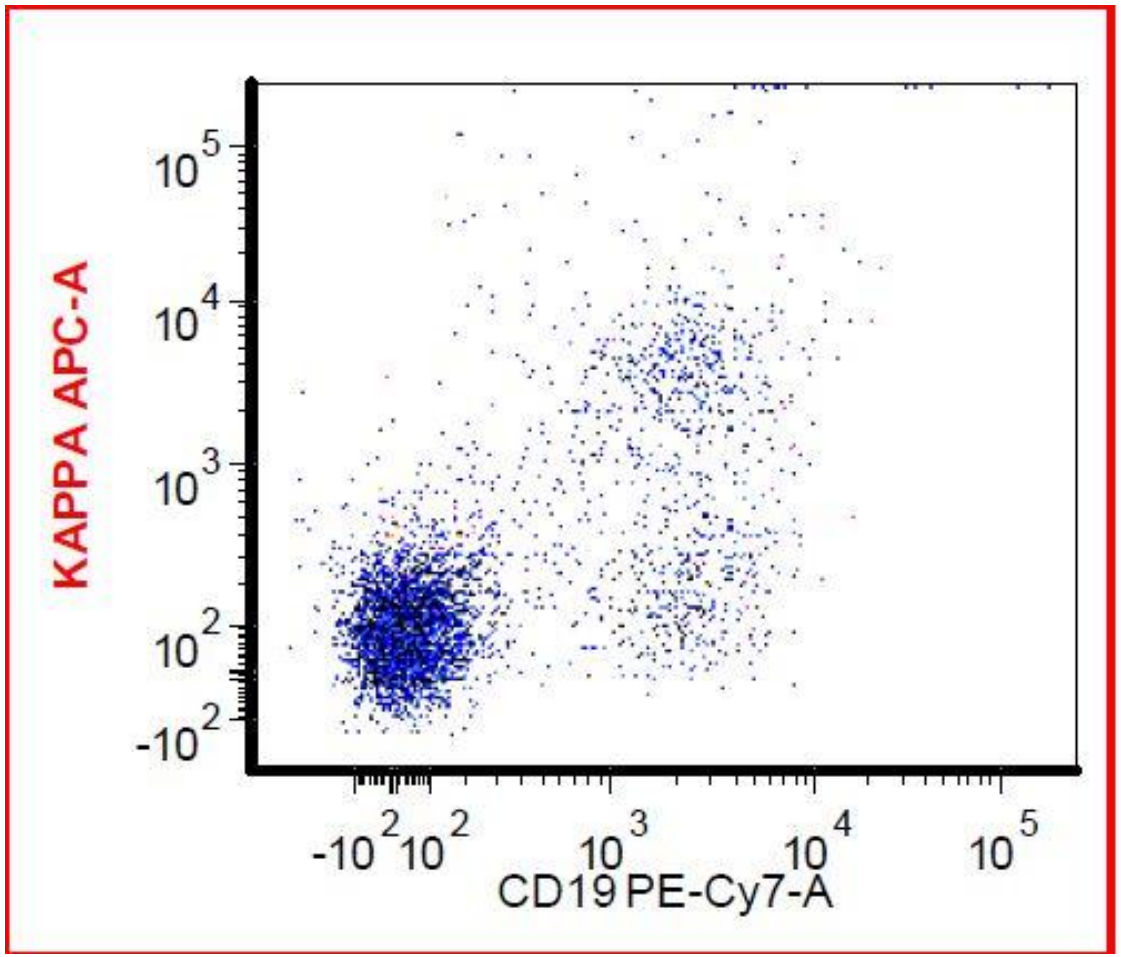
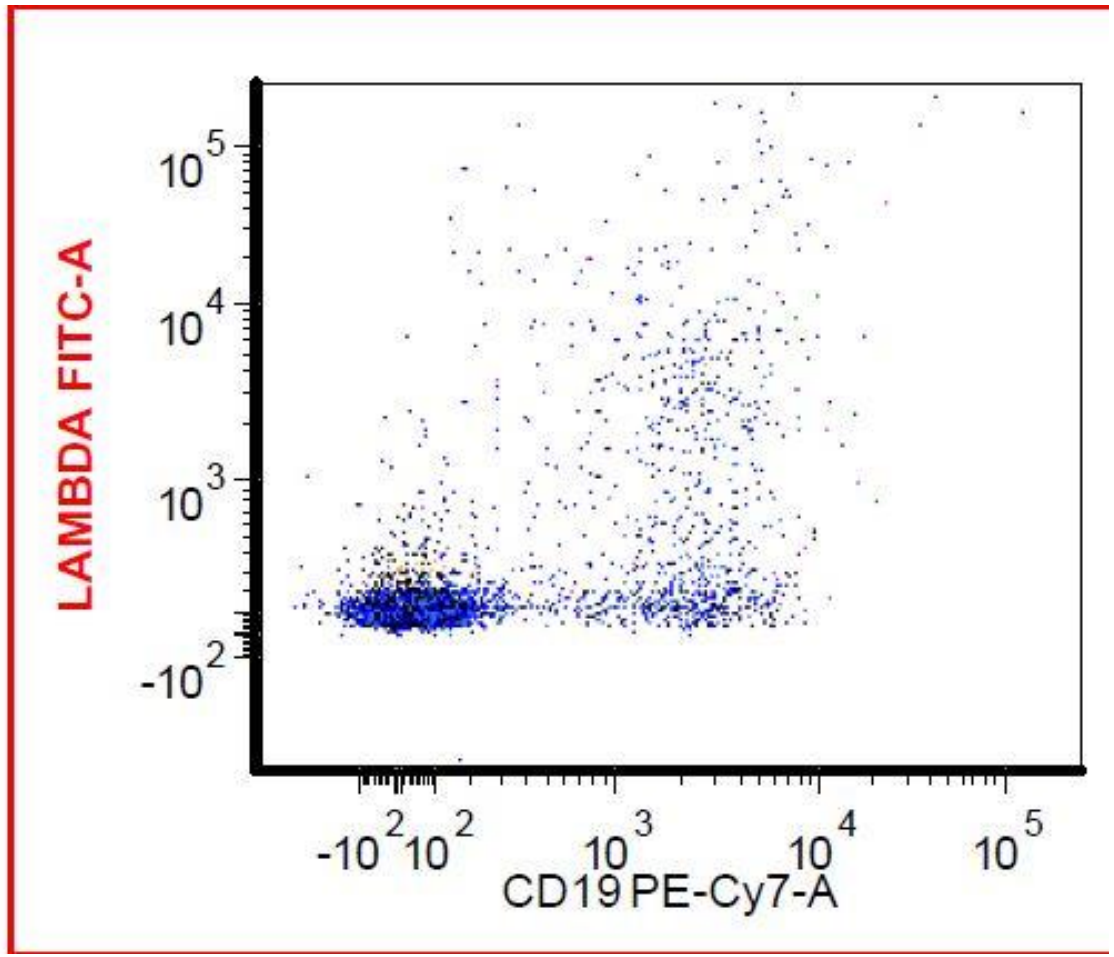
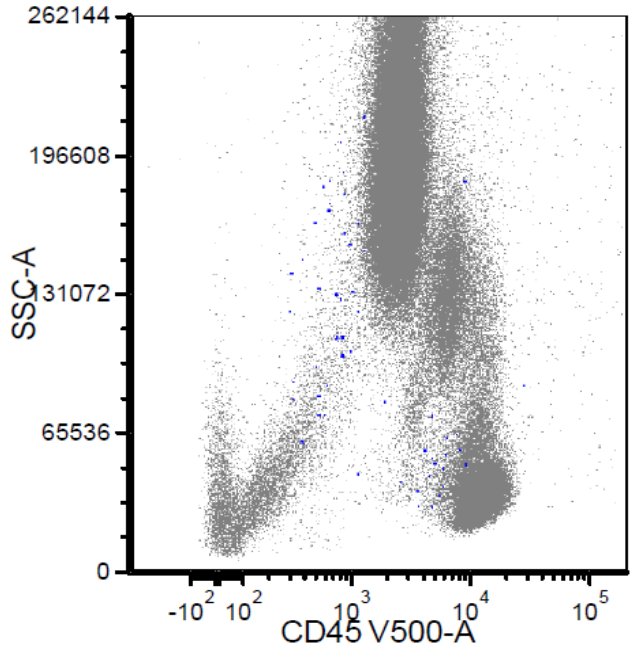
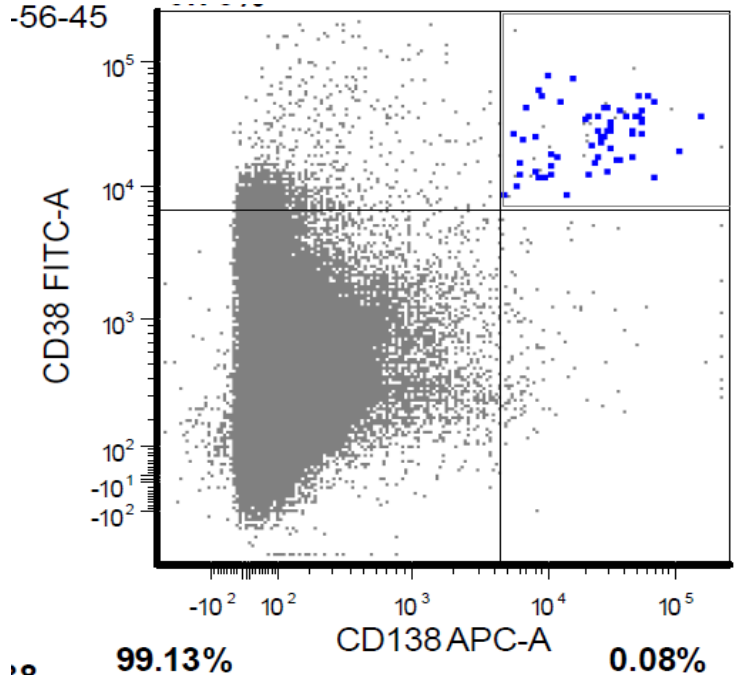
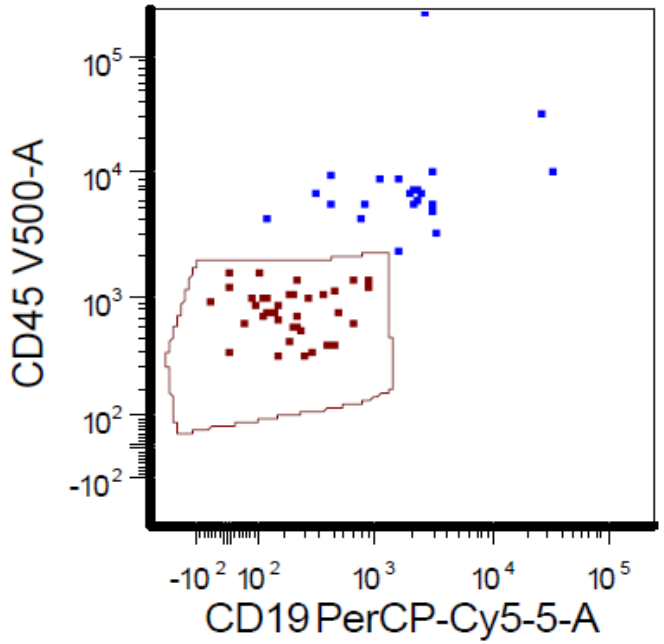
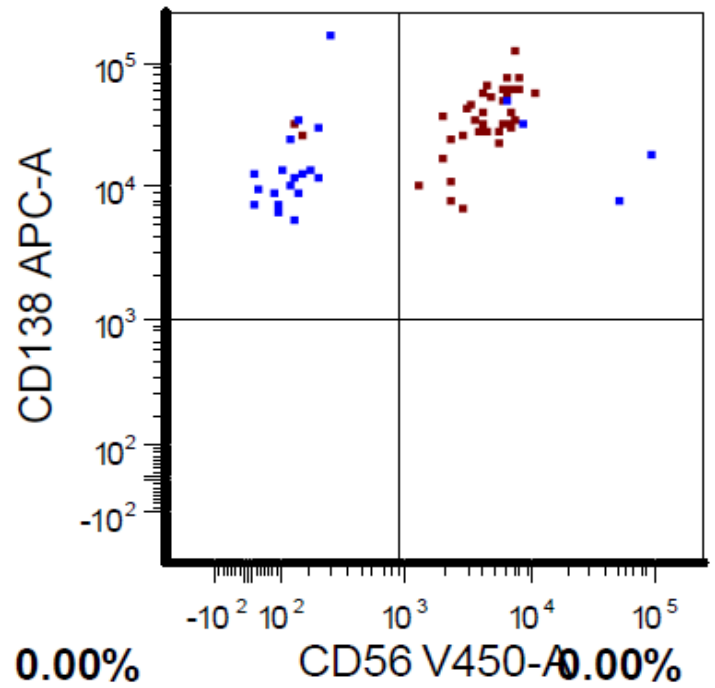

KAPPA APC-A
10^5
10^4
10^3
10^2
-10^2
-10^2 10^2 10^3 10^4 10^5
CD19 PE-Cy7-A


LAMBDA FITC-A
10^5
10^4
10^3
-10^2
-10^2 10^2 10^3 10^4 10^5
CD19 PE-Cy7-A


262144
196608
131072
65536
0
SSC-A
-10^2 10^2 10^3 10^4 10^5
CD45 V500-A


-56-45
10^5
10^4
10^3
10^2
-10^1
-10^2
CD38 FITC-A
-10^2 10^2 10^3 10^4 10^5
CD138 APC-A
99.13%
0.08%


10^5
10^4
10^3
10^2
-10^2
CD45 V500-A
-10^2 10^2 10^3 10^4 10^5
CD19 PerCP-Cy5-5-A


10^5
10^4
10^3
10^2
-10^2
CD138 APC-A
-10^2 10^2 10^3 10^4 10^5
CD56 V450-A
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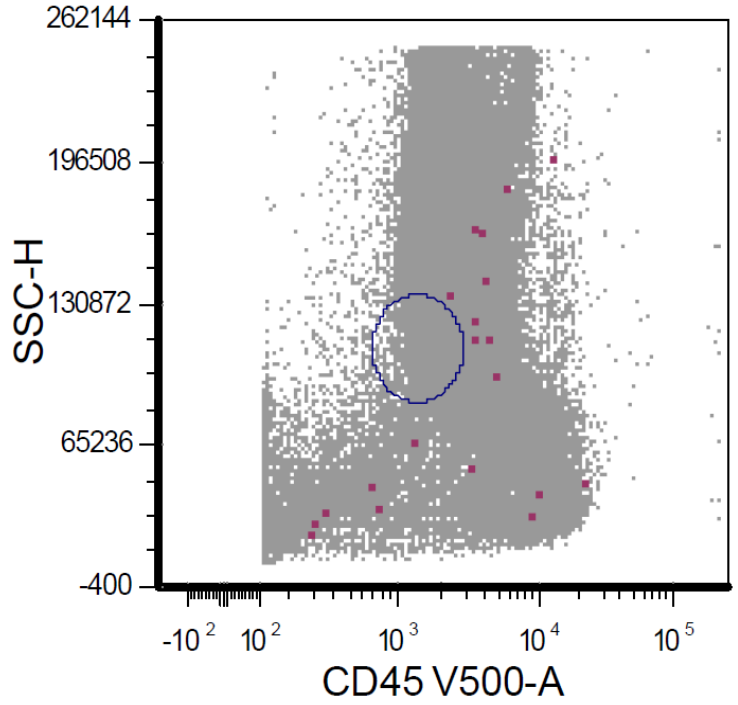
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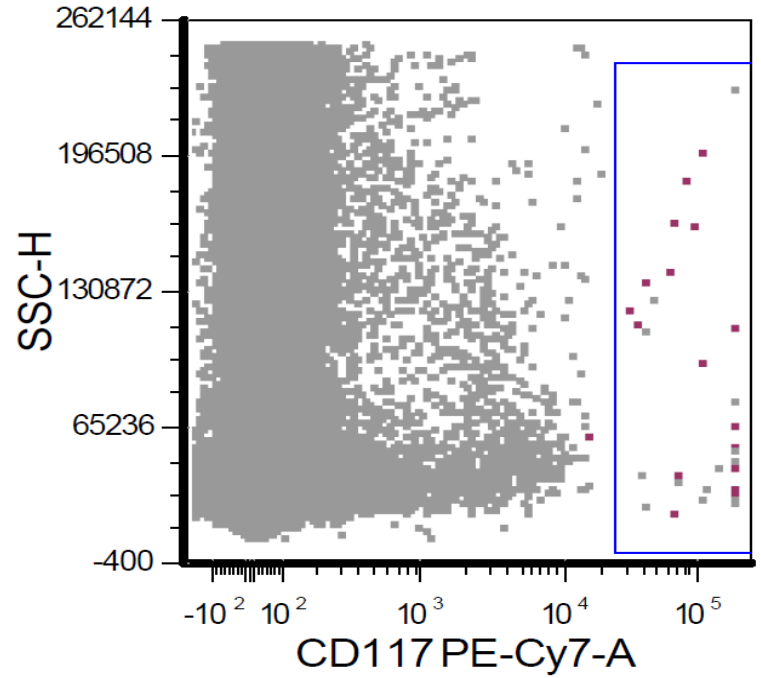
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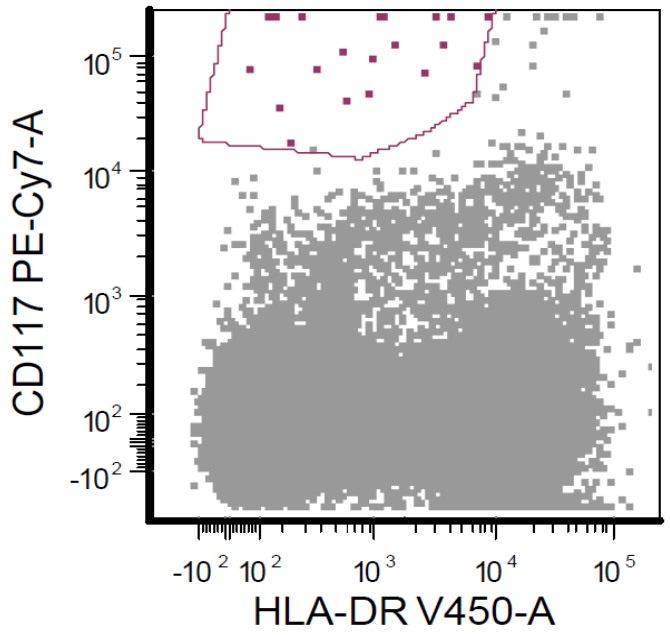
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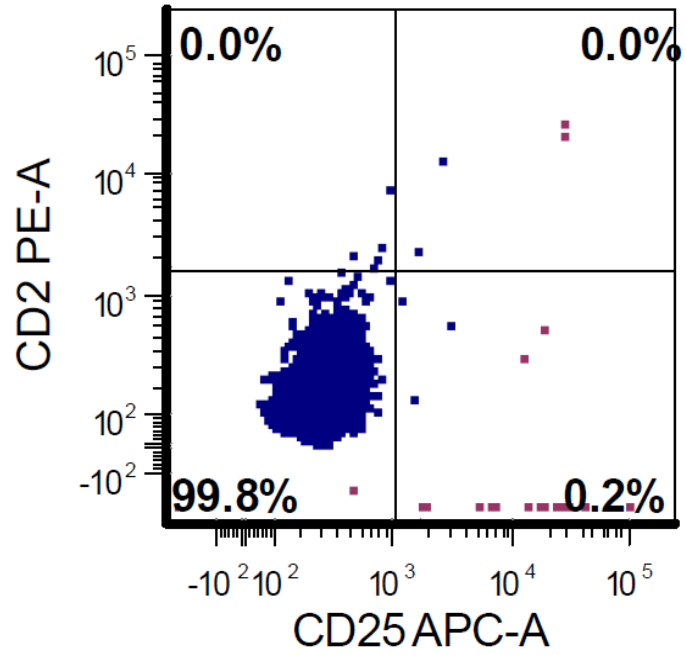
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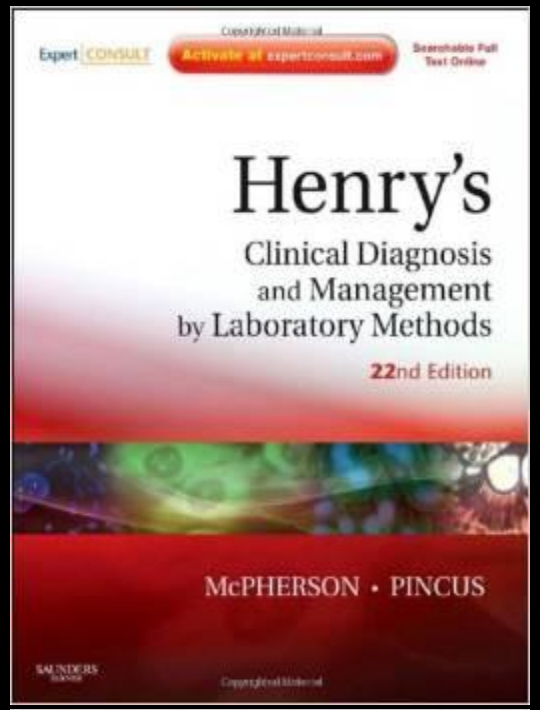
10^4
10^3
10^2
-10^2
CD2 PE-A
-10^2 10^2 10^3 10^4 10^5
CD25 APC-A

Stop Here

- Review hand outs

- Will disuses use of FCIP for diagnosis of acute leukemia and lymphoma in subsequent lectures (if you invite me back)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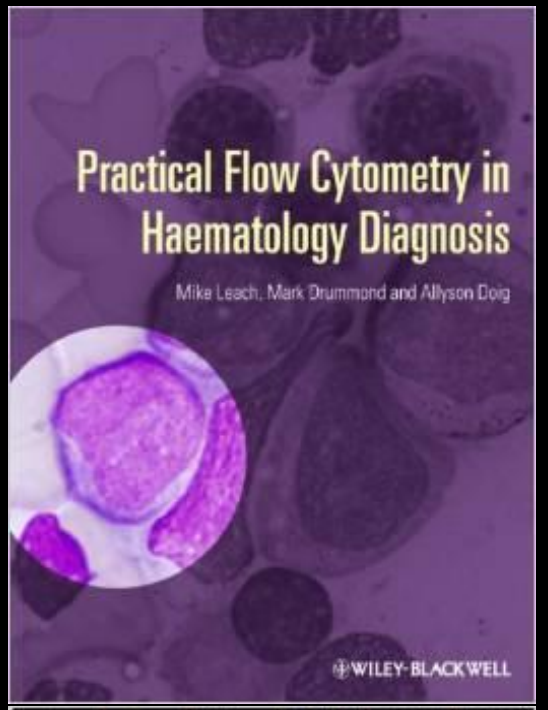
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Mike Leach, Mark Drummond and Allyson Doig
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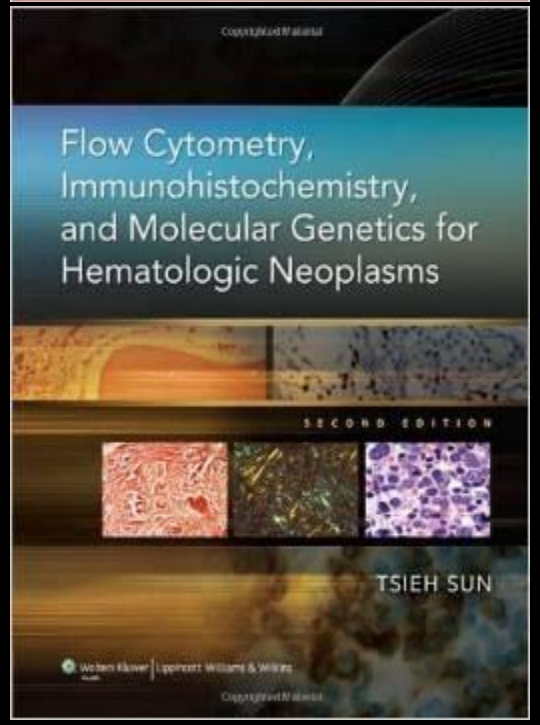
Flow Cytometry, Immunohistochemistry, and Molecular Genetics for Hematologic Neoplasms
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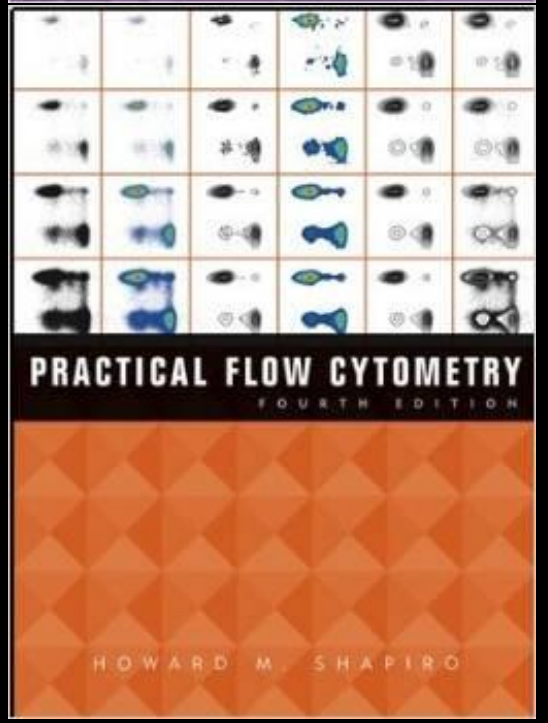
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FOURTH EDITION
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Questions?

- Jesse.M.Jaso@uth.tmc.edu