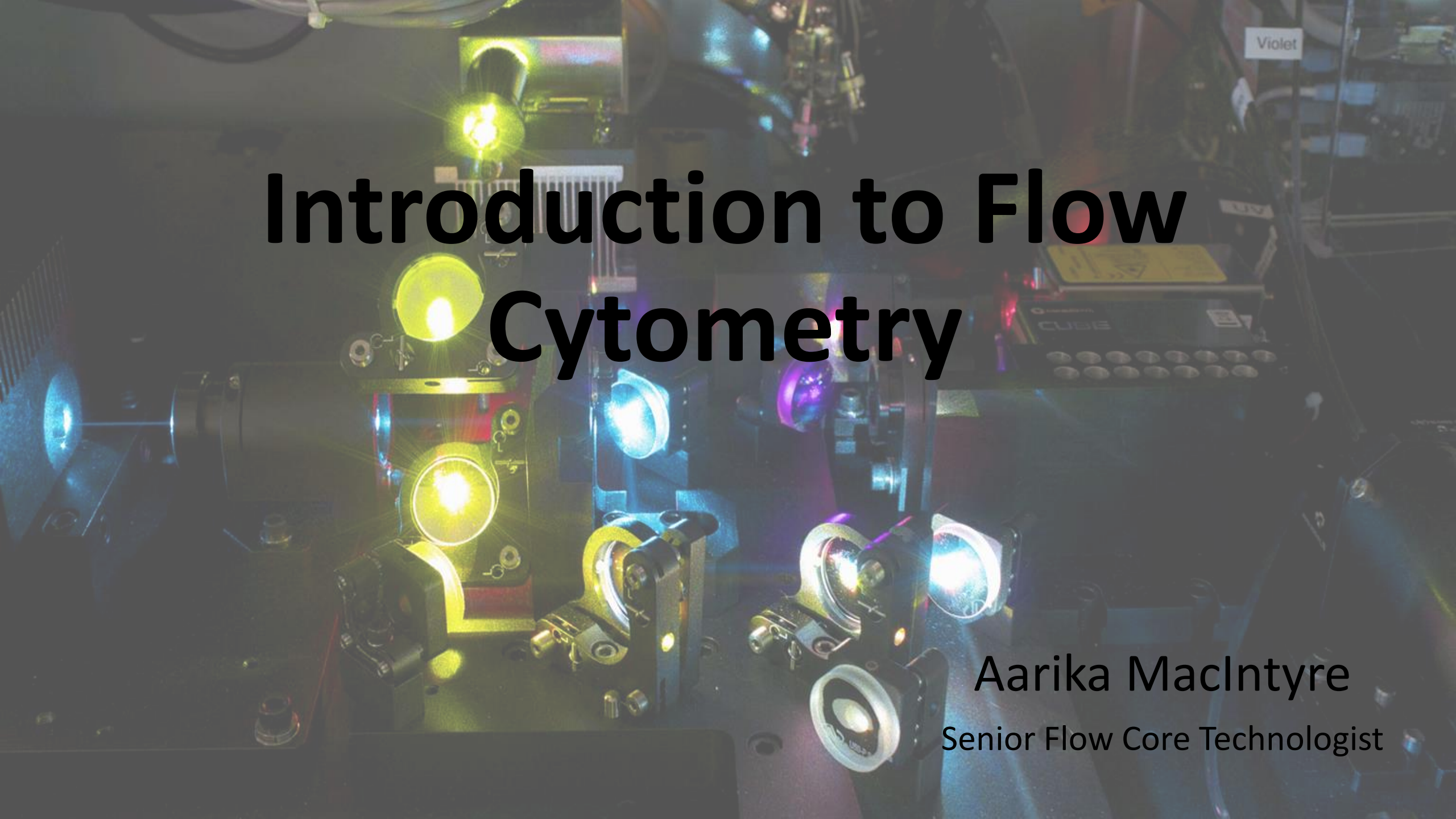


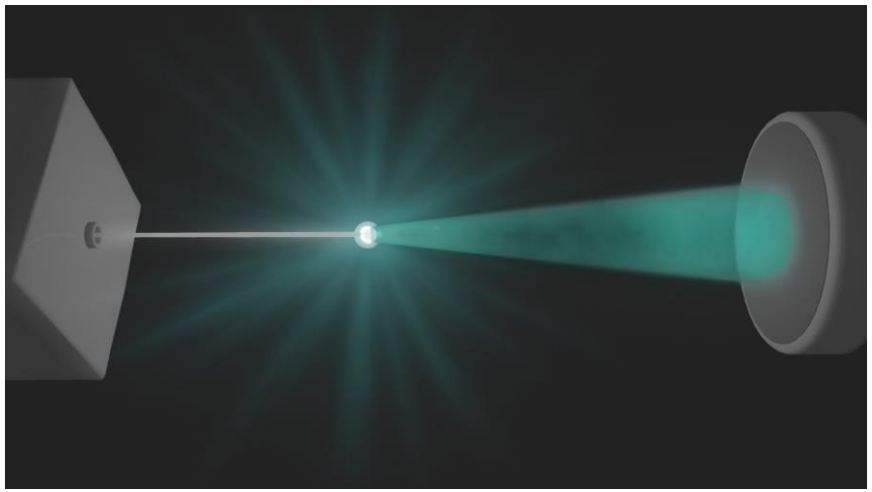
Introduction to Flow Cytometry

Aarika MacIntyre

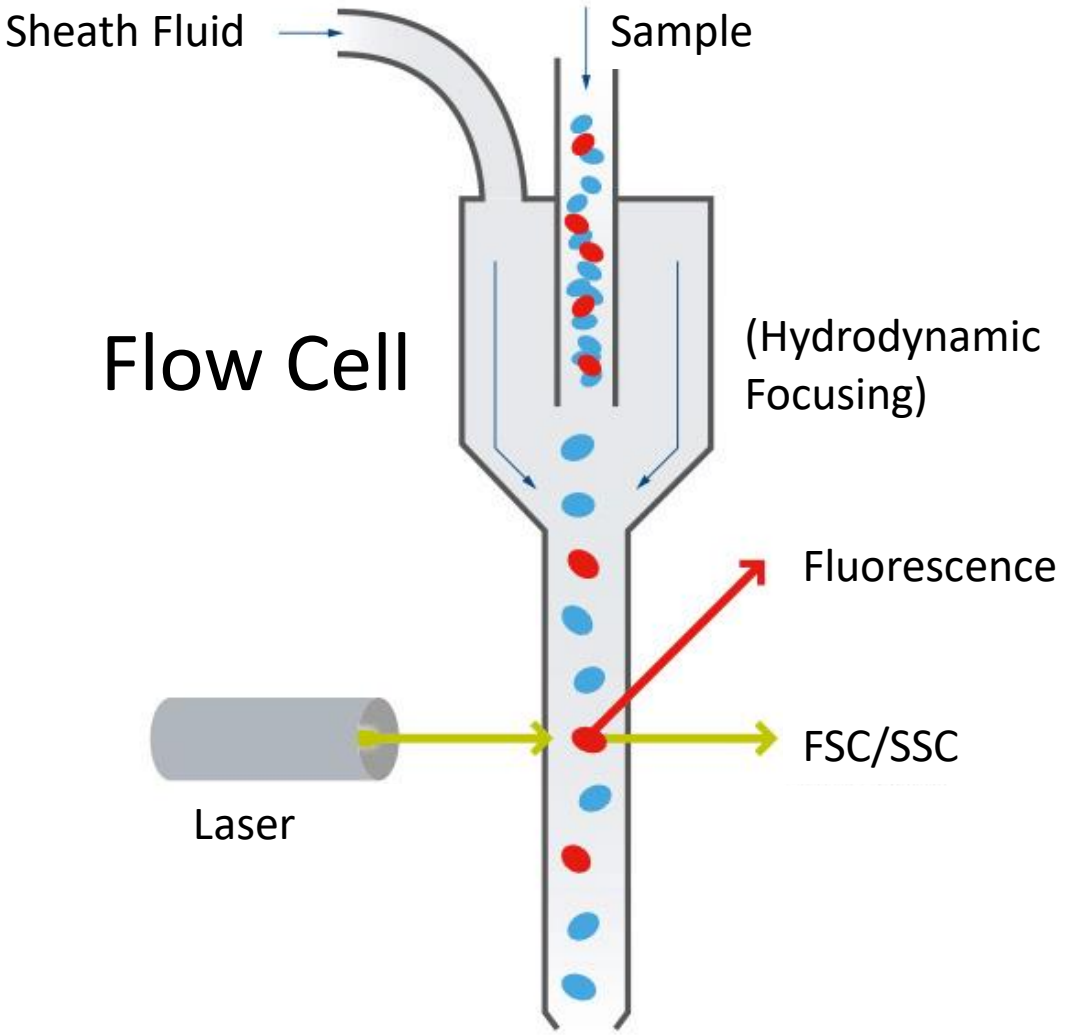
Senior Flow Core Technologist

What is Flow Cytometry?

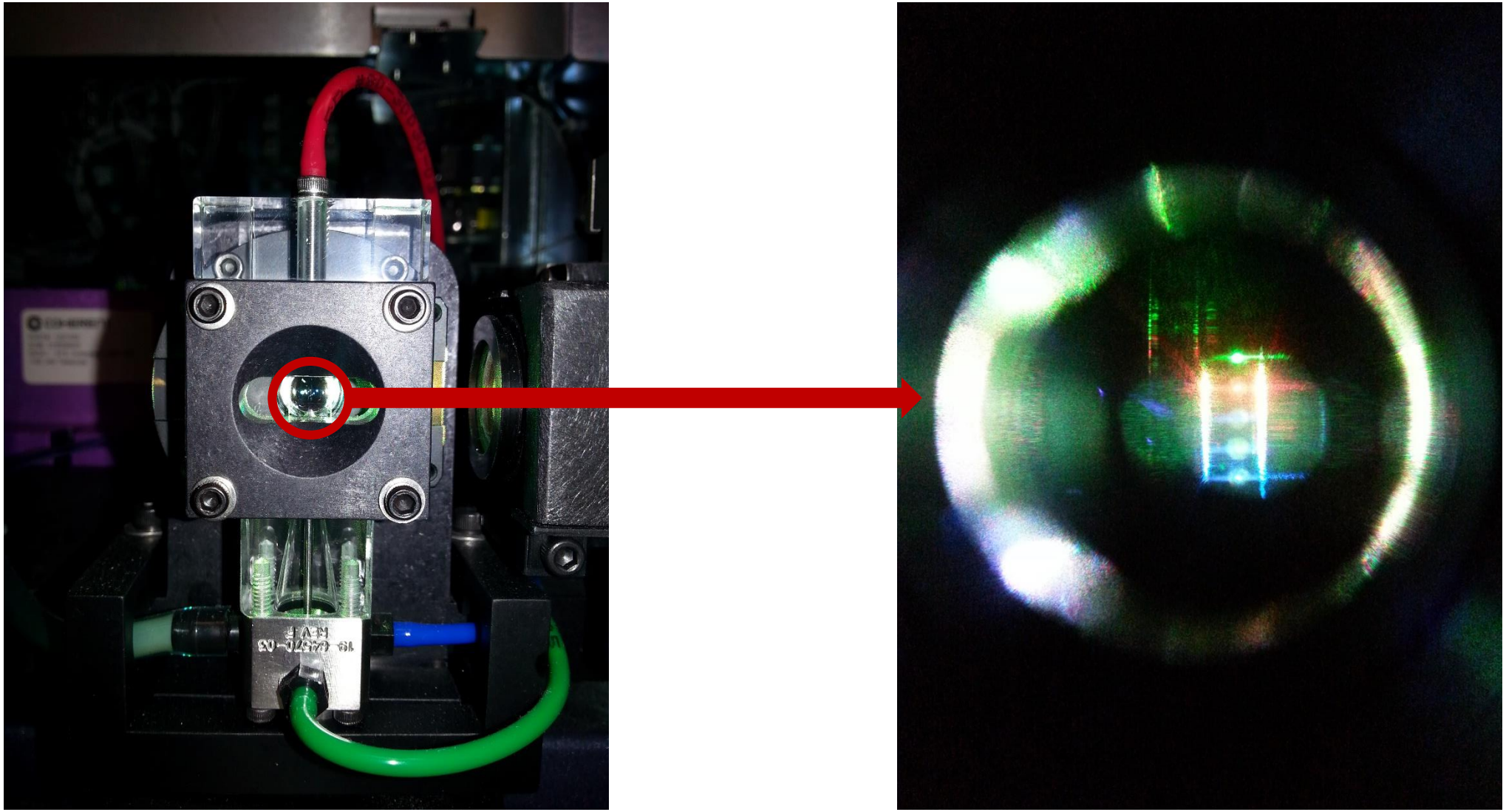
- Single-cell analysis.
- Uses monoclonal antibodies to tag markers on/inside the cells.
- Fluorescent molecules (flurochromes) are bound to the antibodies.
- Flurochromes are excited by lasers at specific wavelengths.
- Fluorochromes emit light at a higher wavelength, which is read by the cytometer.



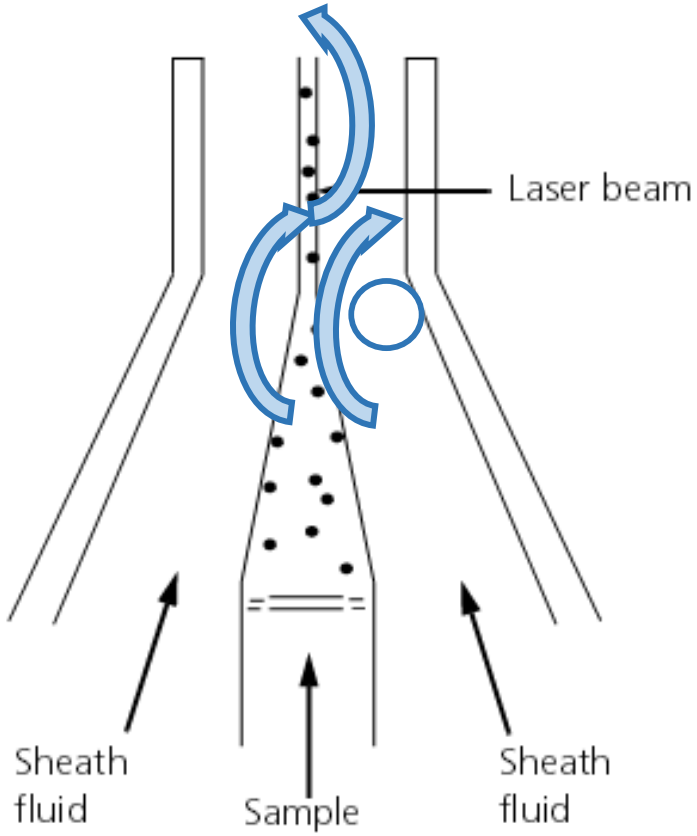
Basics of Flow – Fluidics

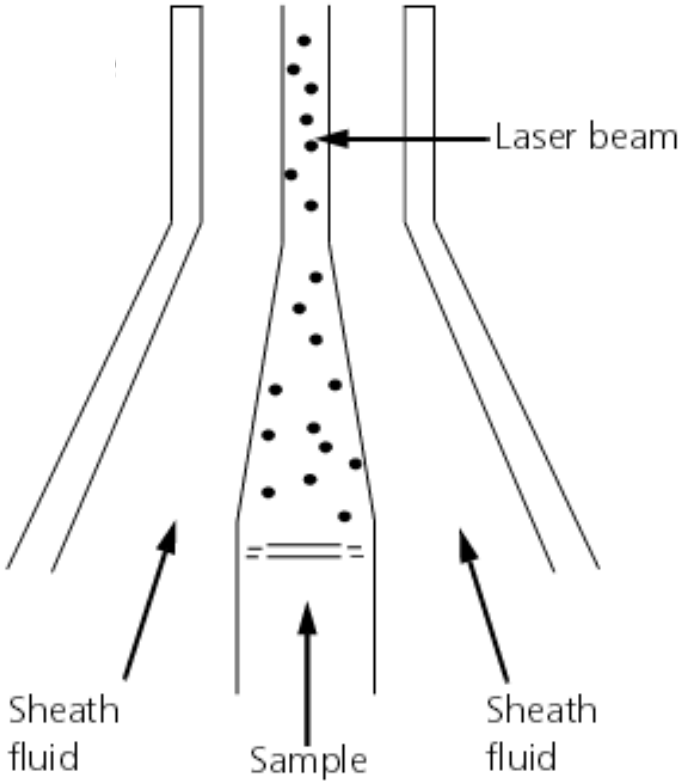


Basics of Flow - Fluidics

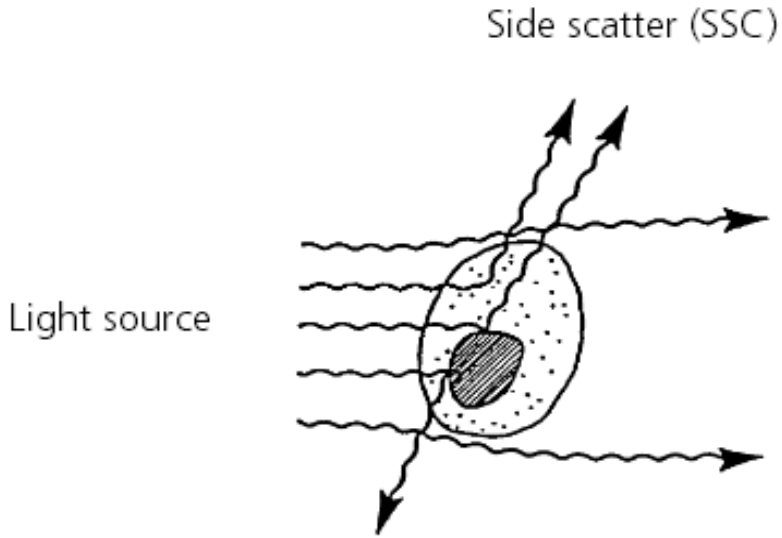


Basics of Flow - Fluidics

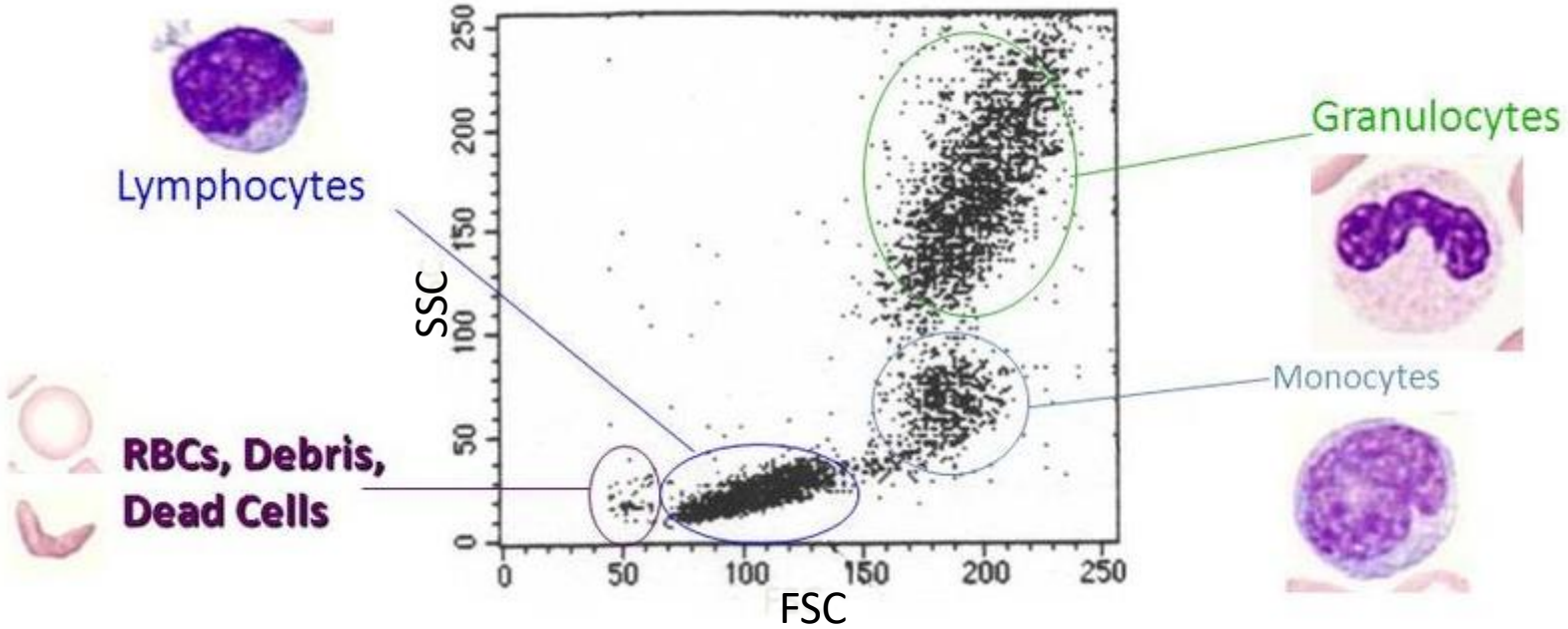




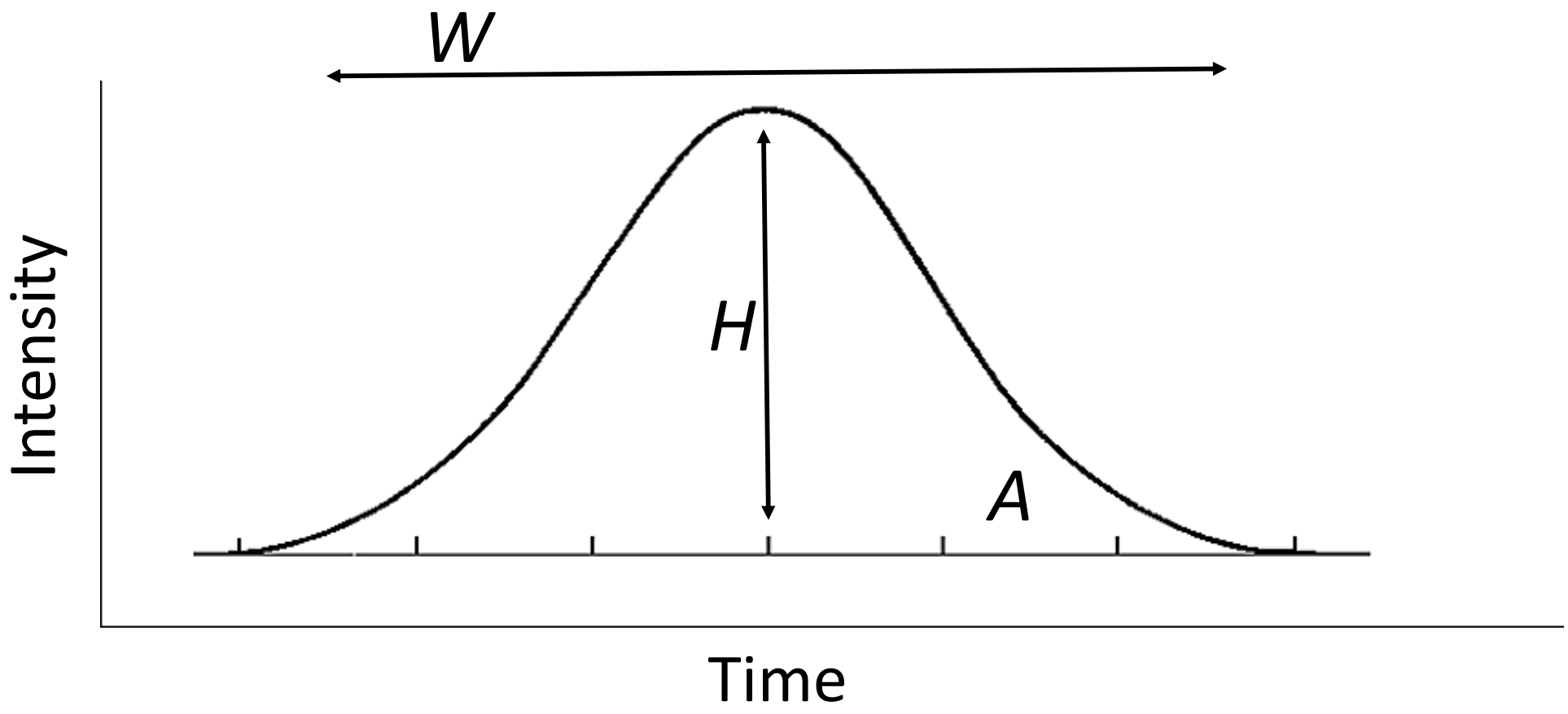
Basics of Flow



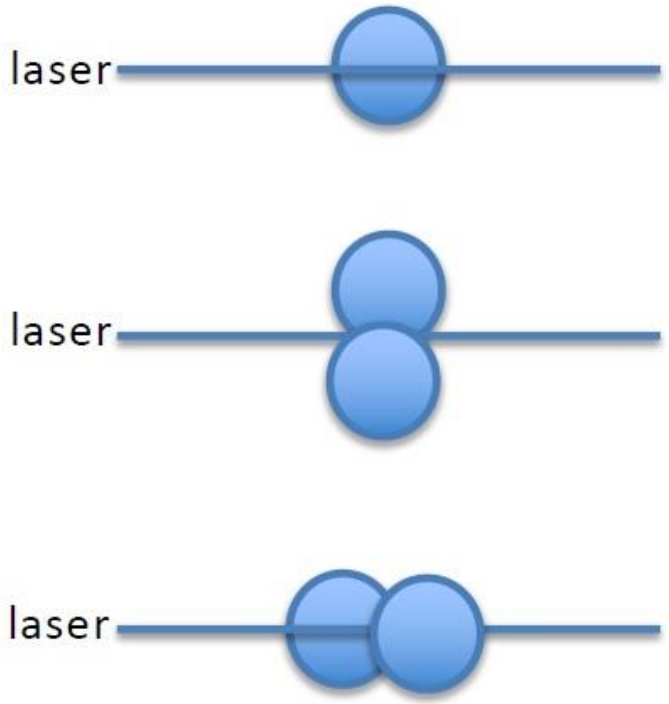
Mammal whole blood/bone marrow

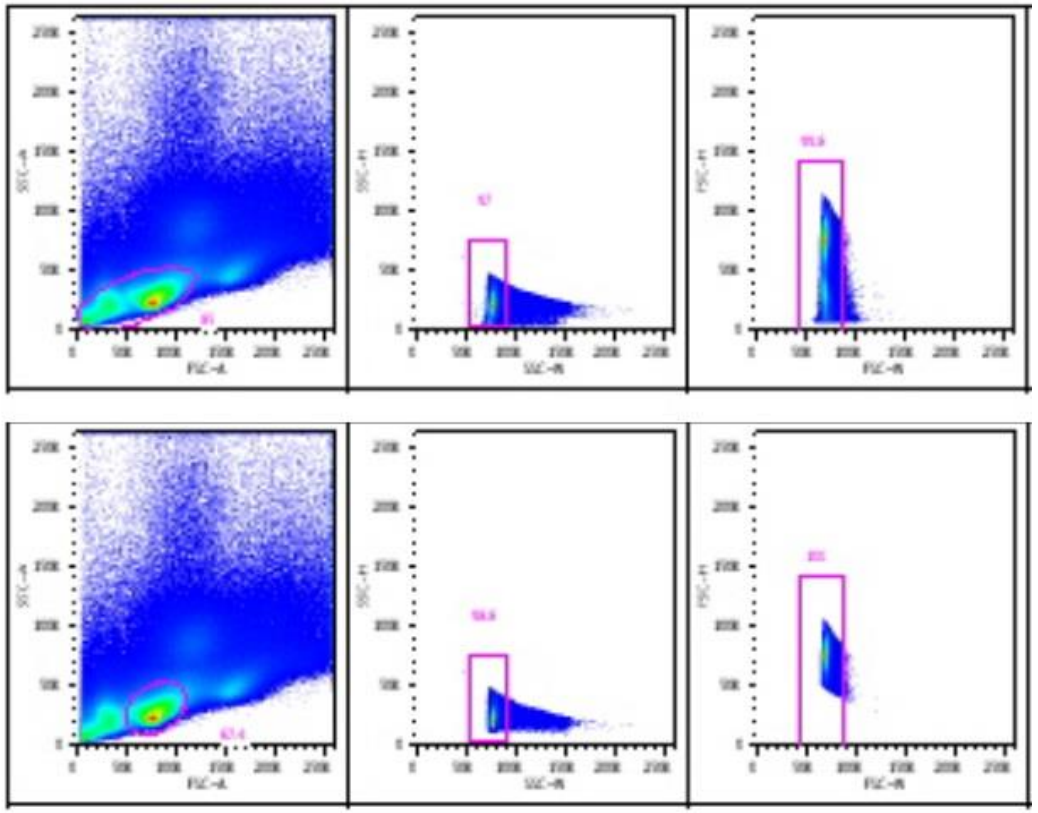


Basics of Flow



Basics of Flow

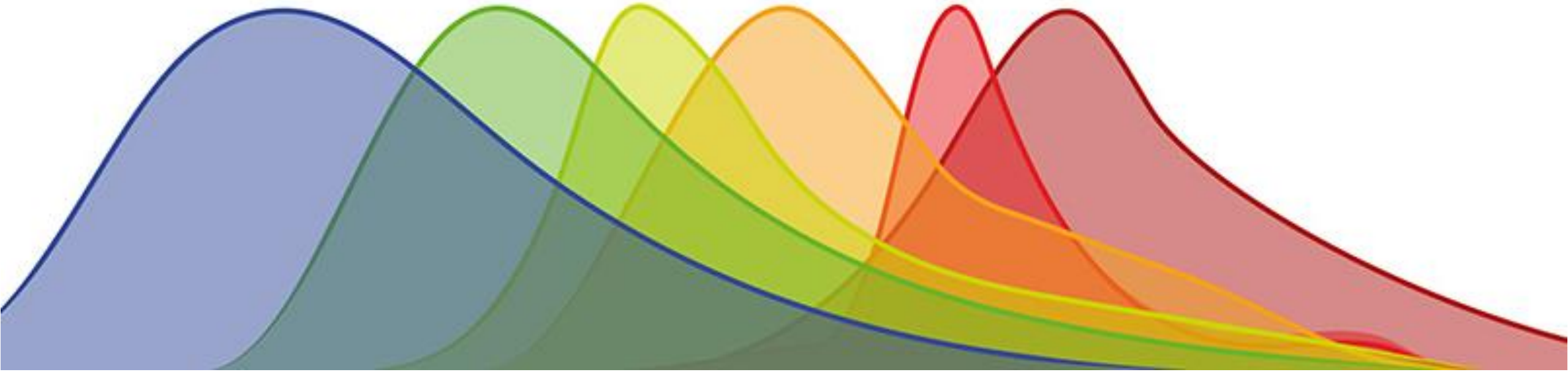


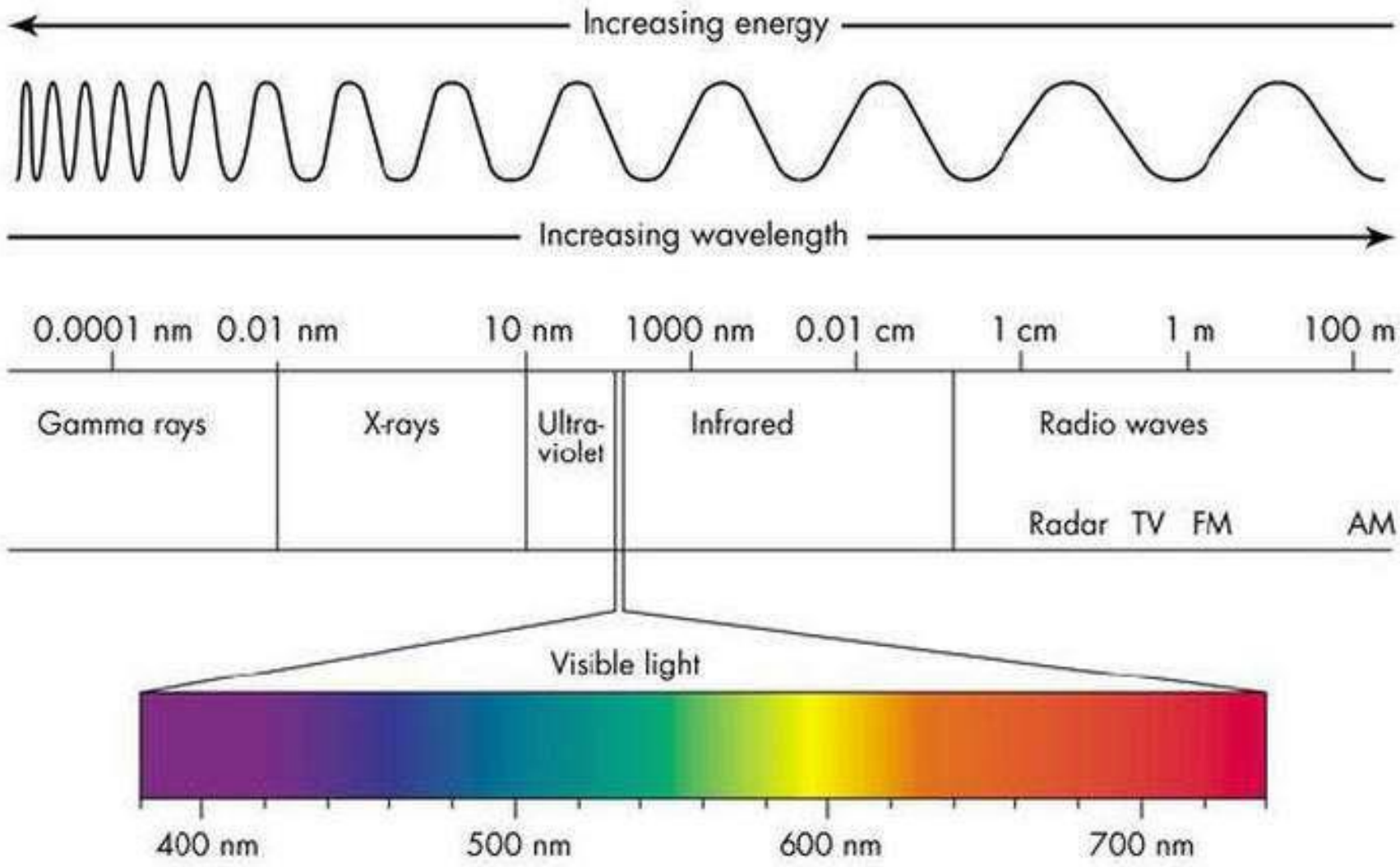


SSC-H vs SSC-W

FSC-H vs FSC-W

Fluorochromes



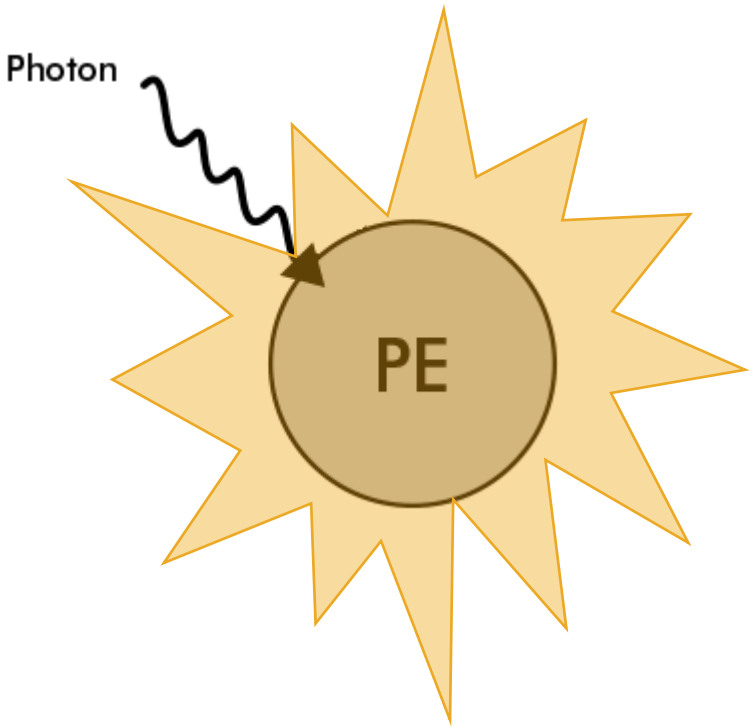

Increasing energy
Increasing wavelength
0.0001 nm
0.01 nm
10 nm
1000 nm
0.01 cm
1 cm
1 m
100 m
Gamma rays
X-rays
Ultra-violet
Infrared
Radio waves
Radar
TV
FM
AM
Visible light
400 nm
500 nm
600 nm
700 nm

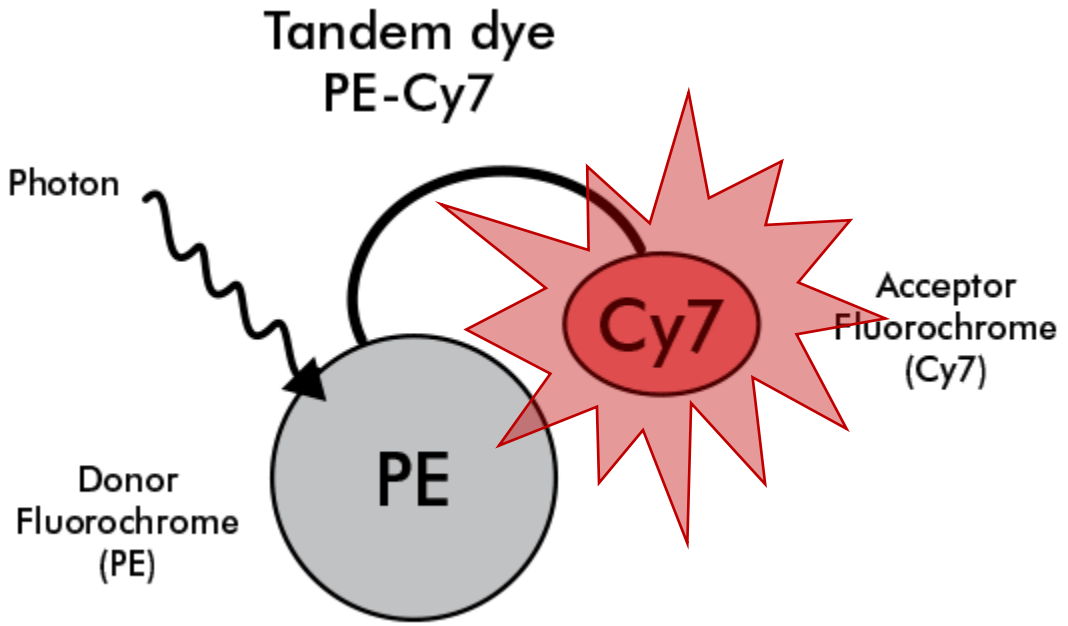
Fluorochromes

- Many “colors” to choose from.
- Each fluorochrome has two properties: excitation and emission.
- Excitation: wavelength at which the fluorochrome absorbs the most energy.
- Emission: wavelength at which the fluorochrome produces the most energy.

		Effective Filter Range (nm)		
Excitation Source	Detector Position	Low	High	Common Fluorochromes
488 nm Blue	A	667.5	702.5	Cy5.5-PerCP
	B	500	550	FITC, CFSE, AF488, GFP
	C	483	493	Side Scatter
633nm Red	A	750	810	Cy7-APC, AF750-APC
	B	707.5	752.5	Cy5.5-APC, AF700, AF680
	C	650	670	APC, AF647
405 nm Violet	A	750	810	QDot800, BV786
	B	670	740	QDot705, BV711
	C	655	670	QDot655, BV650
	D	595	625	QDot605, BV605
	E	570	595	QDot585
	F	557	570	QDot565
	G	505	550	Pac Orange, AF430, QDOT 54, BV480
	H	425	475	Pac Blue, AF405, BV421
532 nm Green	A	750	810	Cy7-PE, AF750-PE
	B	690	735	Cy5.5-PE
	C	650	670	7AAD, Cy5-PE
	D	600	620	AF610-PE, TxRed-PE
	E	562.5	587.5	PE, AF 532

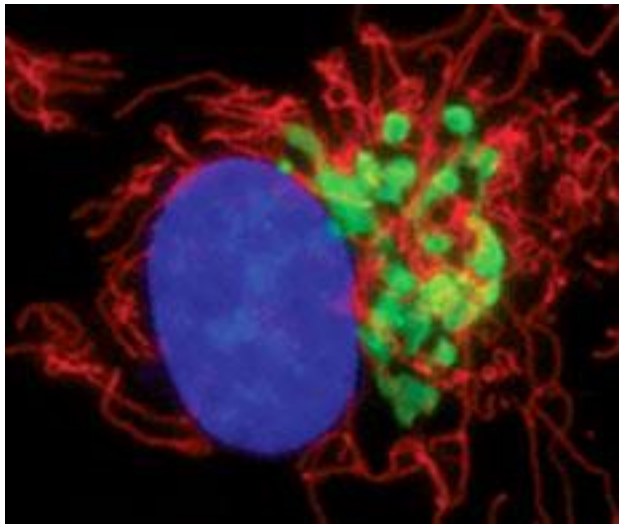
Fluorochromes

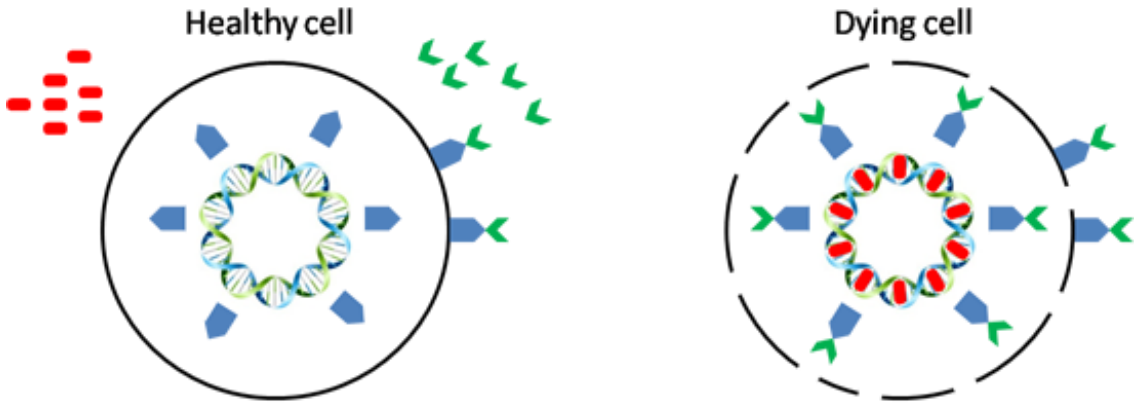


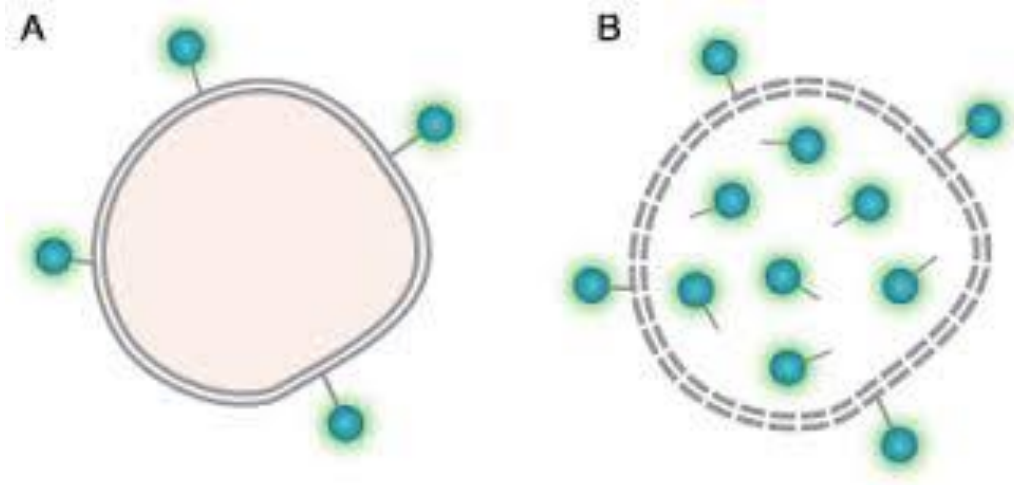


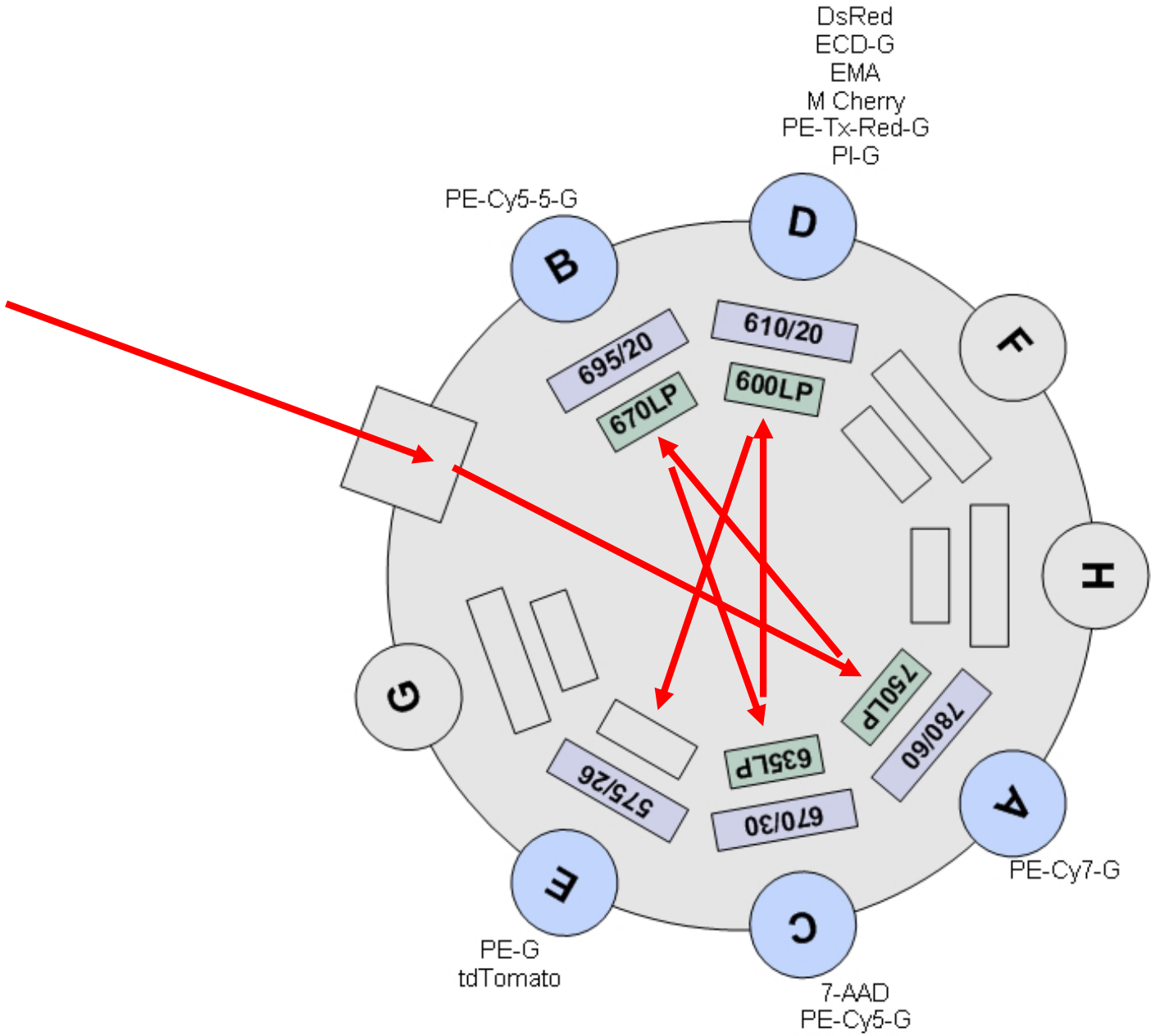
Fluorochromes

- Fluorescent proteins
  - GFP, YPF, CFP, RFP
- Viability Dyes
  - DNA Dyes – DAPI, PI, 7AAD, etc.
  - Fixable Viability Dyes

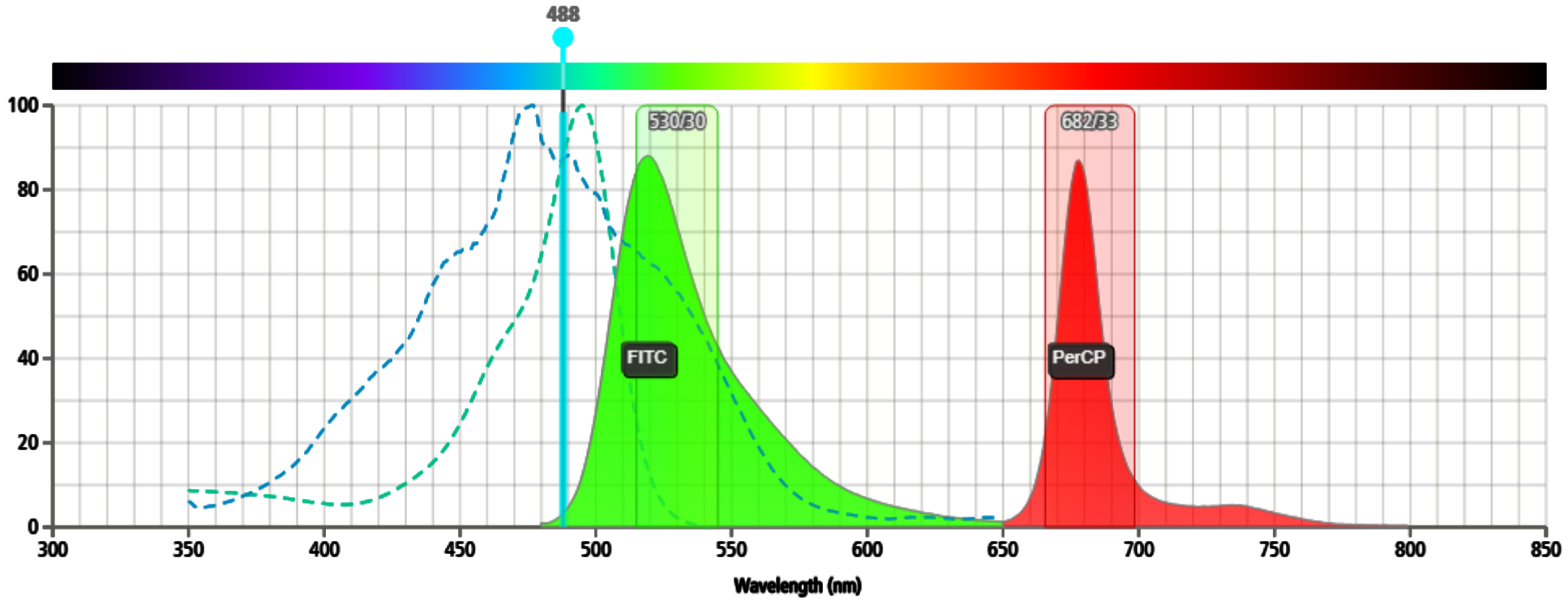




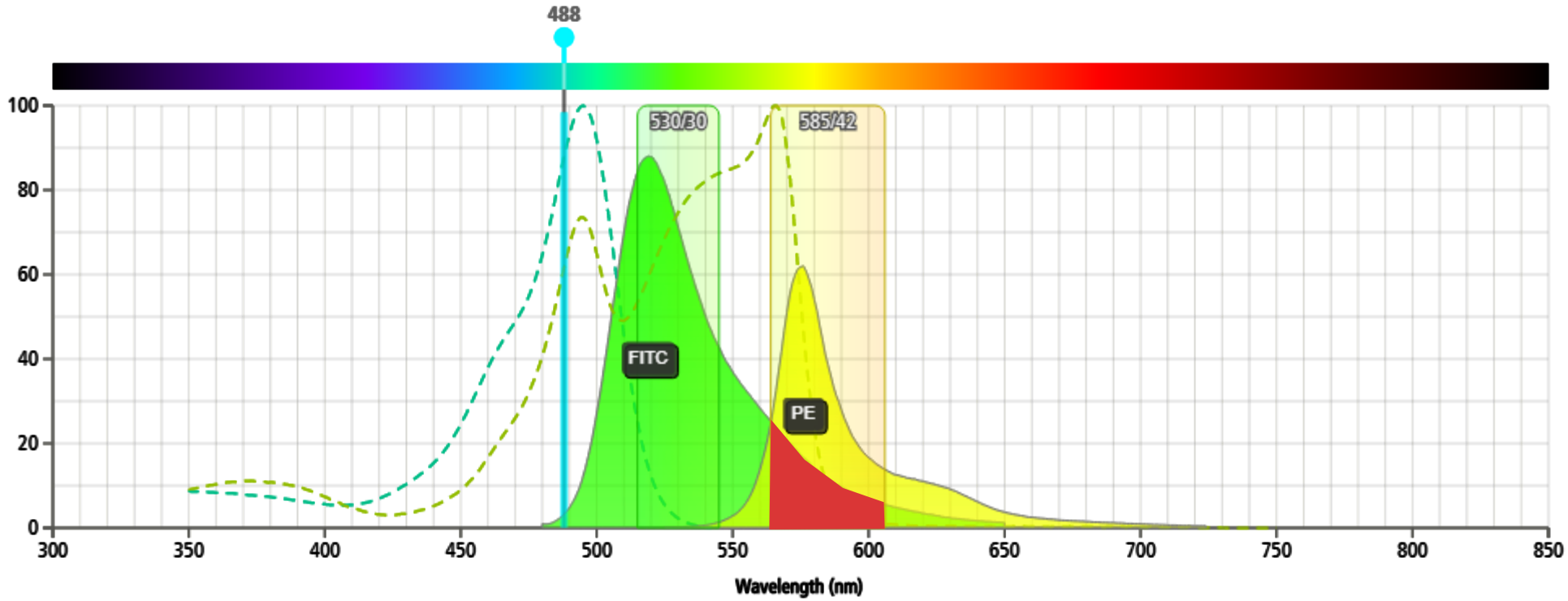



DsRed
ECD-G
EMA
M Cherry
PE-Tx-Red-G
PI-G
PE-Cy5-5-G
D
B
F
H
A
C
E
G
610/20
600LP
695/20
670LP
750LP
780/60
635LP
670/30
575/26
PE-Cy7-G
PE-G
tdTomato
7-AAD
PE-Cy5-G

Compensation

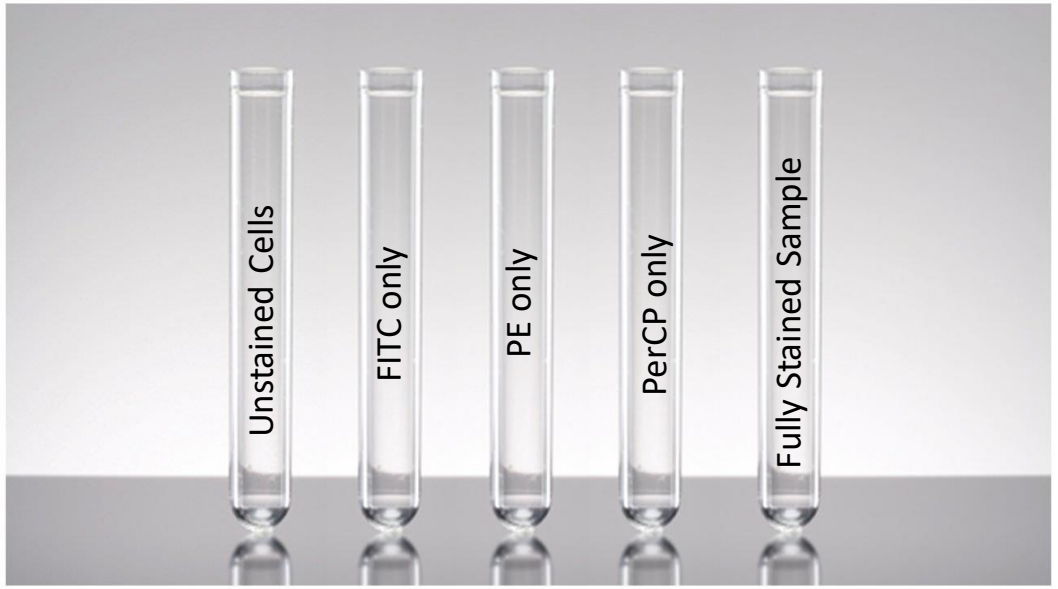


Compensation

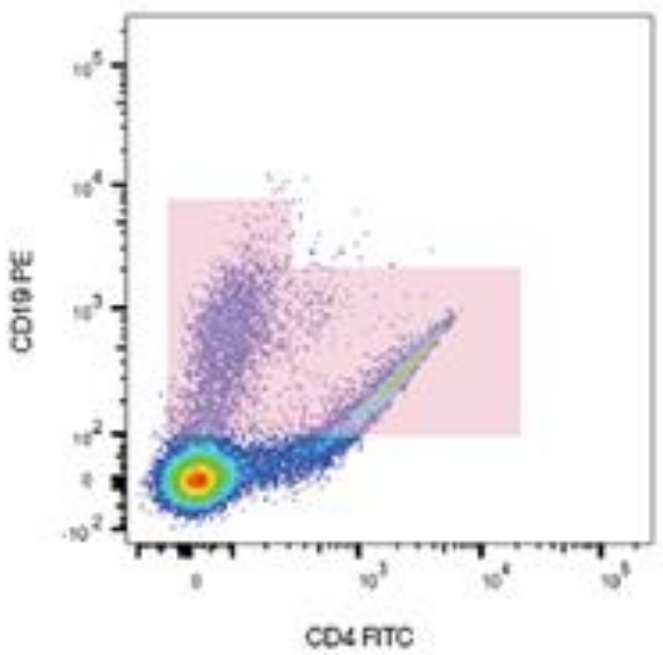


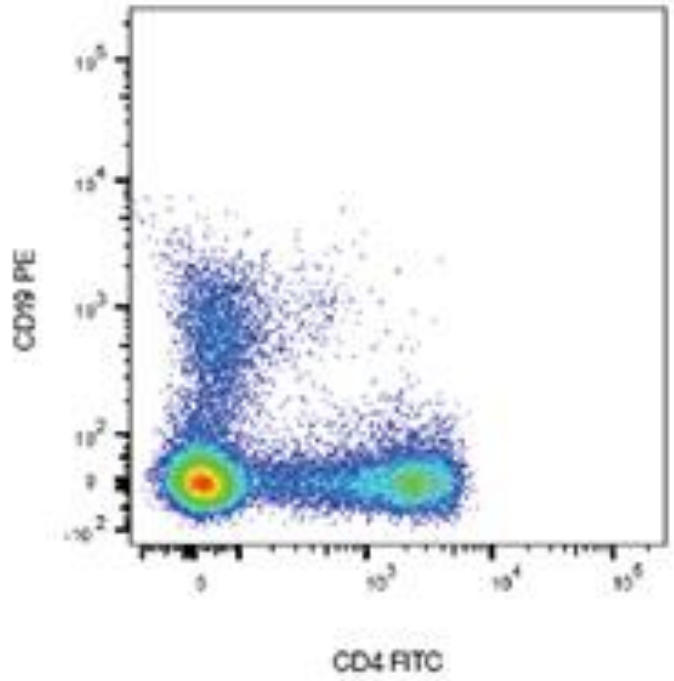
Compensation

- Single-stained controls are required; one for each fluorochrome.
  - Cells
  - Beads
- Unstained control also necessary.
  - Cells are ideal
- Software will calculate the percent overlap for each color.



Compensation



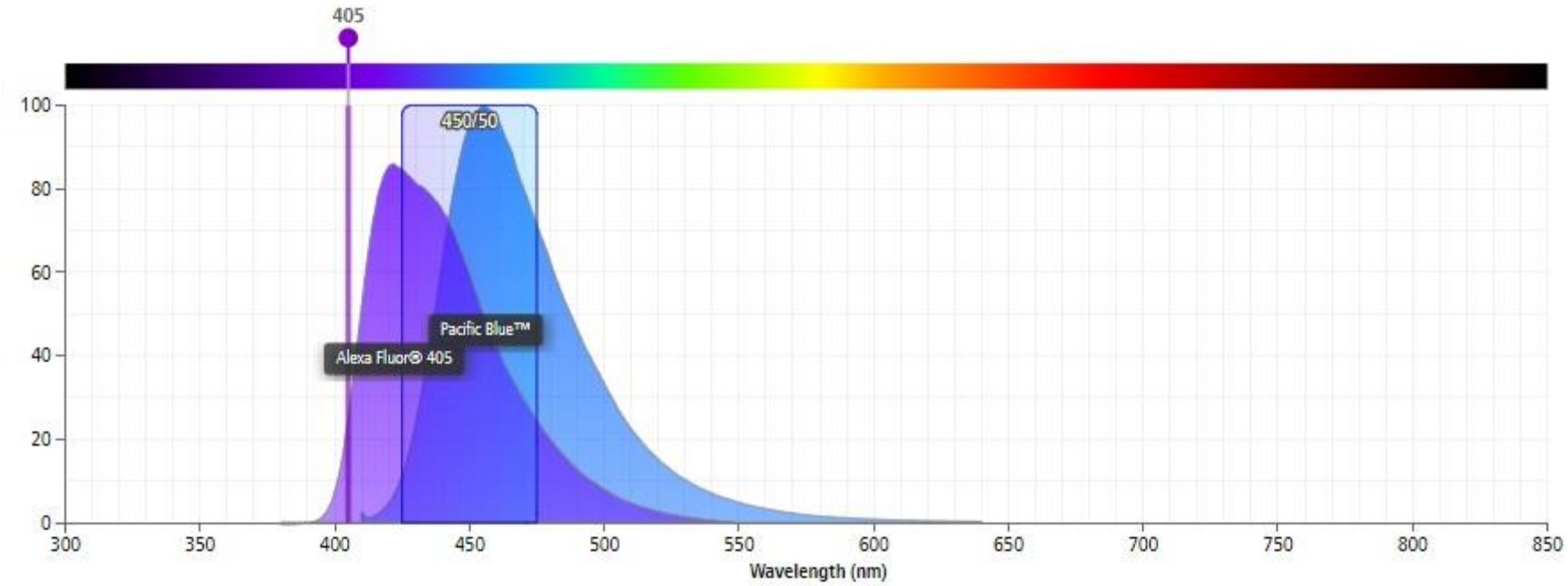


Compensation

- Compensation problems usually caused by poor panel design.
- In general, if two fluorochromes:
  a) are excited by the same laser, and
  b) use the same filter for detection, then

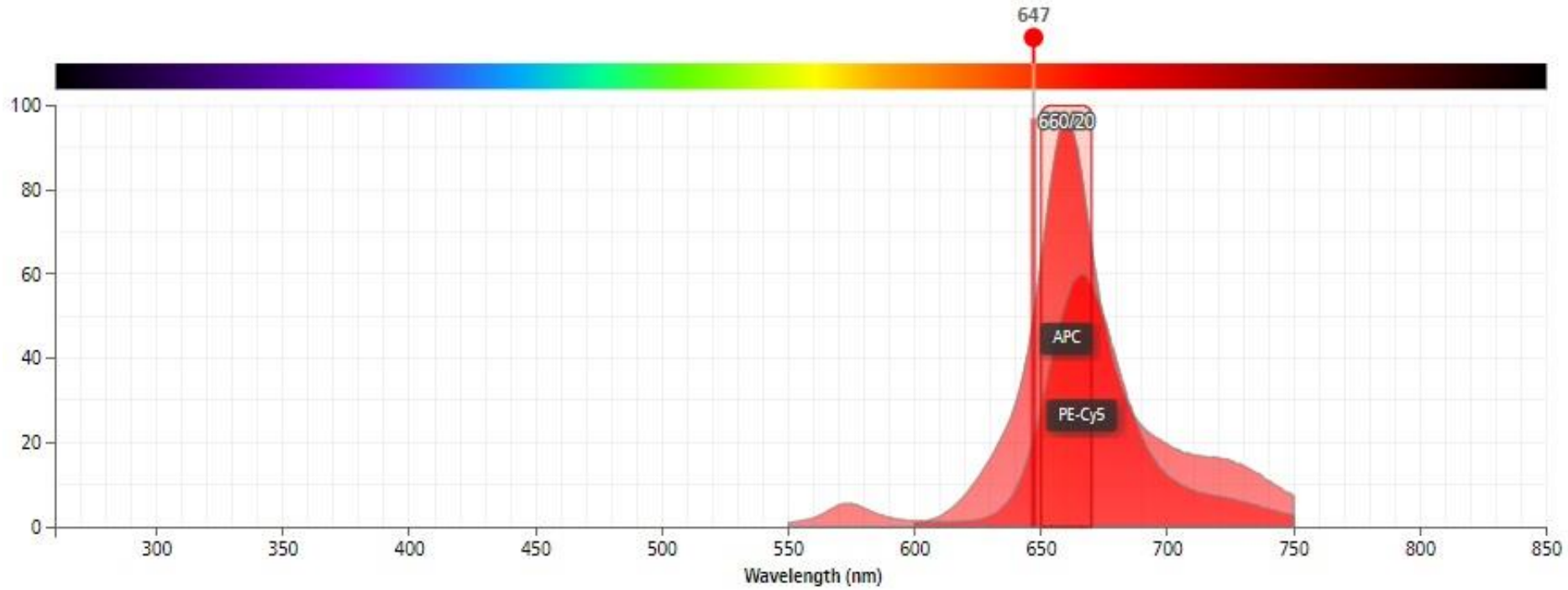
  they cannot be run together on a conventional cytometer.

Compensation



Compensation

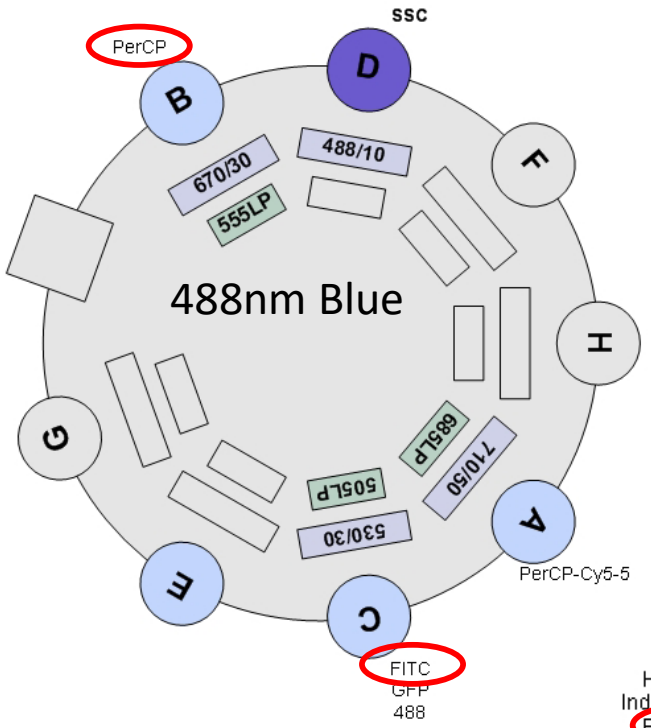
- Sometimes colors that *should* work together don't.

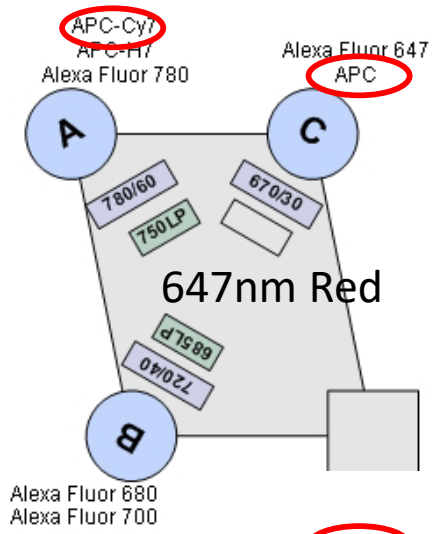


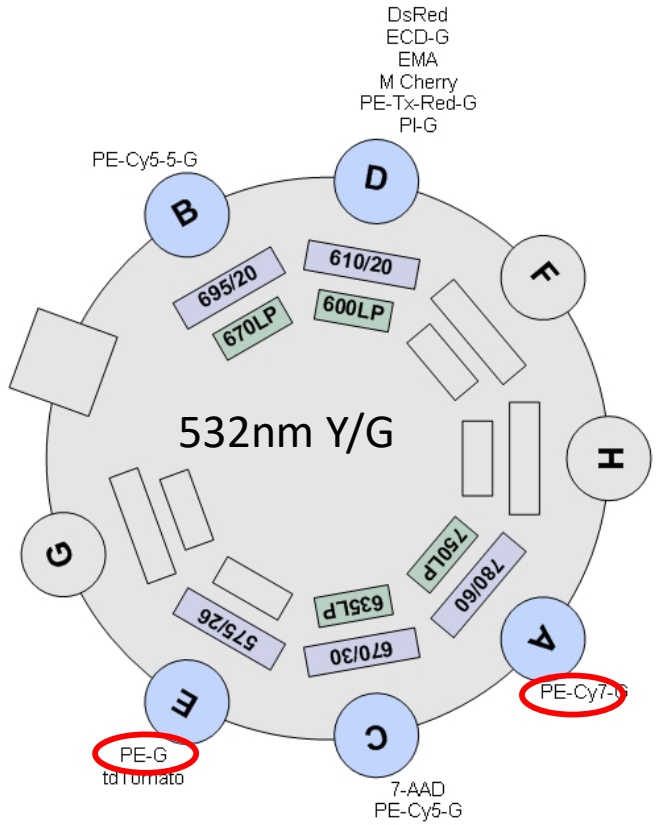
Panel Design

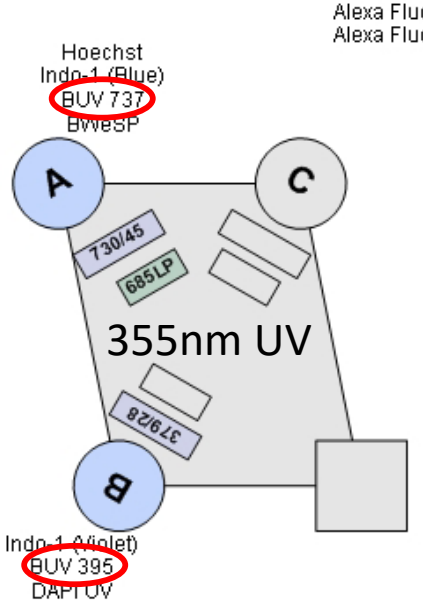
- Spread color choices across available excitation lasers.
- Choose colors with emissions as far apart as possible.
- Match brighter colors with dimmer markers.
  - Some colors fluoresce brighter than others.
  - Some markers are less frequently found on the cell surface.
  - Come to Heidi’s presentation to learn more!

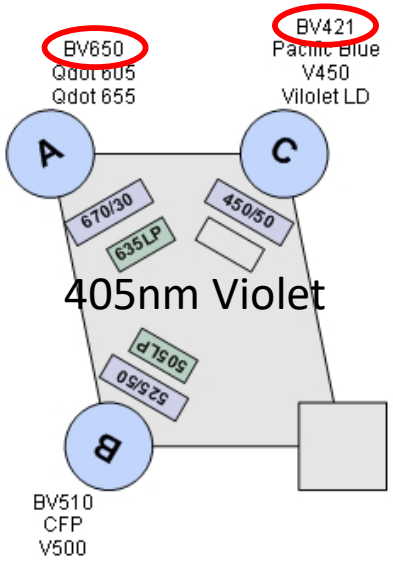
Panel Design

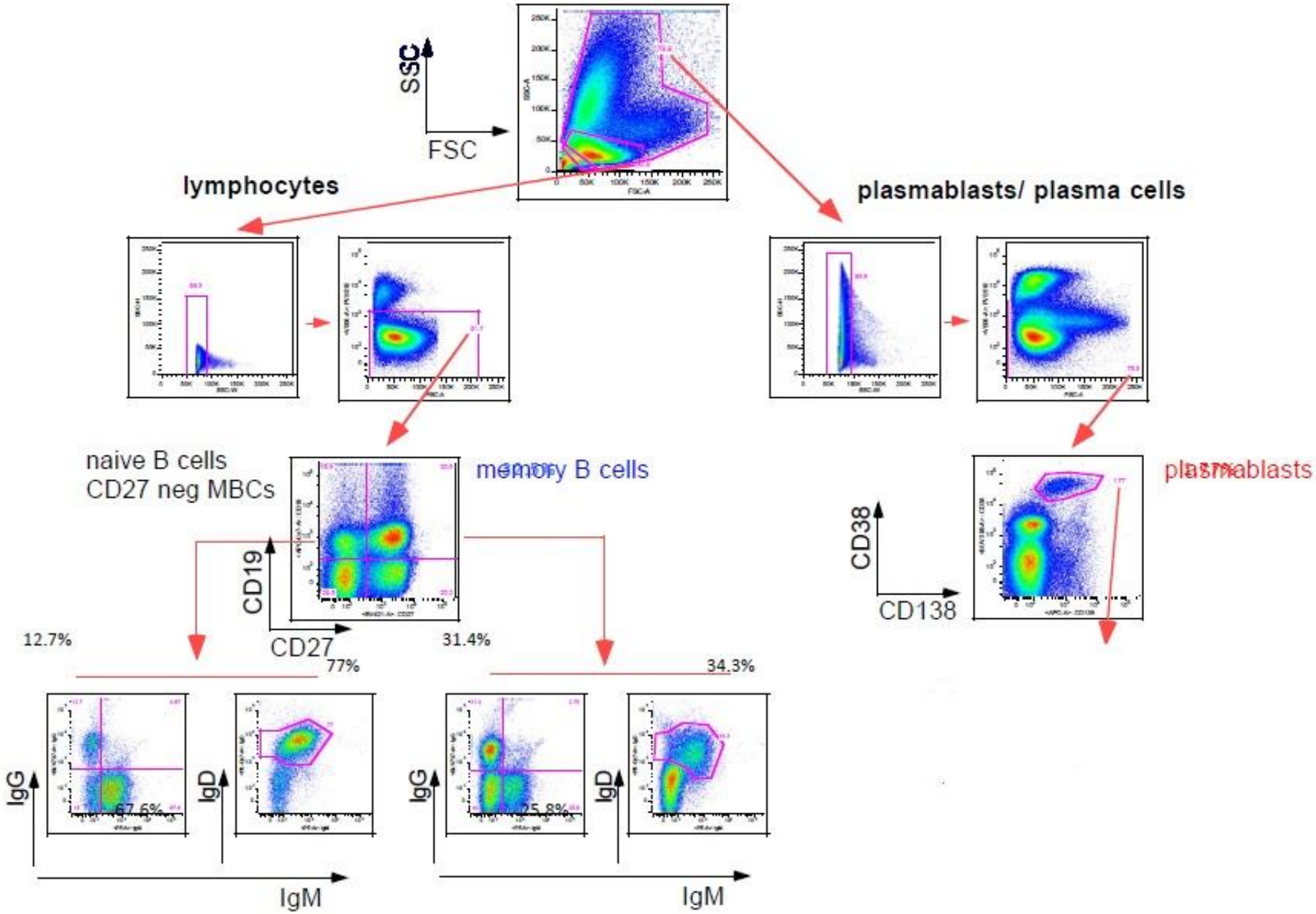











SSC
FSC
lymphocytes
plasmablasts/ plasma cells
naive B cells
CD27 neg MBCs
memory B cells
CD19
CD27
12.7%
77%
31.4%
34.3%
CD38
CD138
IgG
IgD
IgM
67.6%
25.8%

Sample Prep

- Single-cell suspension
- 10^6 cells/mL, minimum 250µL
- 12x75 Polystyrene tube
- Filter at the instrument – bring a pipette!

For help with a staining protocol or tissue prep, see Ailing or other members of the Flow Core.

Thank you!

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