SUMMER INSTITUTE CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
						July 02
						Early Arrival Airport Arrivals and Check-in
July 03	04	05	06	07	08	09
Early Arrival Airport Arrivals and Check-in	Airport Arrivals and Check-in 6:00pm: 4th of July Celebrations	9:30am-12pm: Campus tour, Panther ID & ISSS Check-in 12-2pm Lunch 2:00-6:00pm, Shuttle to local grocery store	9:30am-11:30am ISSS, OII, & Housing Orientation & Presentation **2:30-4:30pm:-Welcome Reception and Buddy Meet & Greet Event**	**Classes begin!** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 INTRO - TRAINING**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA PREPARATION**	Free Day
10	11	12	13	14	15	16
12:00-4:00pm: The World Coca-Cola and Georgia Aquarium	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS I**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS II**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm:BIOL4905 PROTEOMICS III** 6:00-10:00pm: Atlantic Station Shopping & Movie (Sign-up)	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS IV ?**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 RNA PREPARATION**	6:00-9:00pm: Dinner in America (Sign-up)
17	18	19	20	21	22	23
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 qPCR & AUTOMATION**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA SEQUENCING**	**MINI BREAK**	9-11:20am: Morning course **CDC TRIP** **1:30-4:30pm: BIOL4905 MICROSCOPY / AFM**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30 - 4:30pm: BIOL4905 NEXT GEN SEQ.** 5:30-7:30pm: Meet & Greet BBQ event @ The Commons	9:00am - 6:00pm: Outlet Mall
24	25	26	27	28	29	30
Free Day	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 MICROARRAY I**	9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 NANOSTRING**	9-11:20am: Morning course 11:20am-2:00pm: Lunch **Notebook Day** **1:30-4:30pm: BIOL4905 NANOSTRING**	**Last day of classes** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 FLOW CYTOMETRY**	**FINALS**	Free Day
31	August 01	02	03	04		
Free Day	Activity Day at the Recreation Center (Sign-up)	Free Day	9:30-11:00am: Georgia Capitol Tour (Sign-up) 2:00-4:00pm: Closing Reception	Departures (check-out at 12:00pm)		

Note: Students may arrive prior to the program date with an extra charge of $35 per night. Earliest day to check-in to University Commons is July 2.

Legend:

Orange: Courses **Blue: Lunch Break.** **Red: Sign-up events**

GSU Biology Core Facility

Supporting Life Sciences at GSU

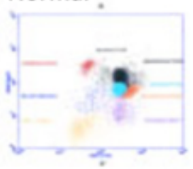
http://biotech.gsu.edu/core_facility/index.html

Georgia State University

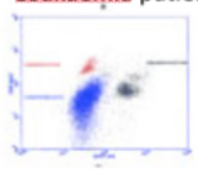
Flow Cytometry

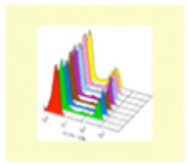
Profiling Cells

Normal



Leukaemia patient

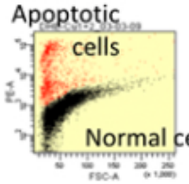




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

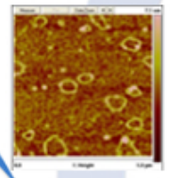


Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis

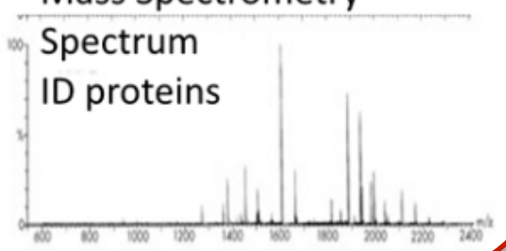


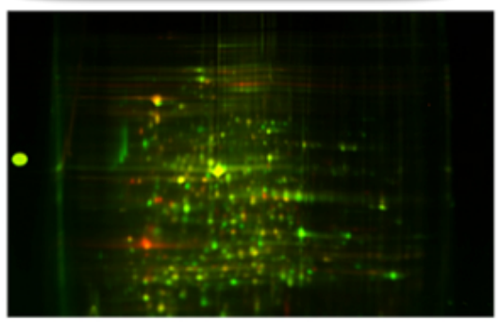
Cellular Functions

Mass Spectrometry

Spectrum

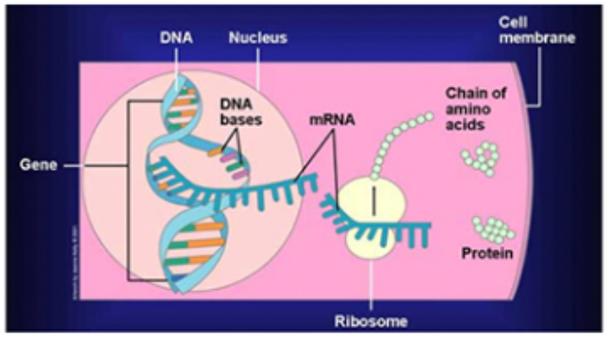
ID proteins





DNA

Replication



Protein

Expression

Proteomics

Profiling Proteins

2D Protein gel

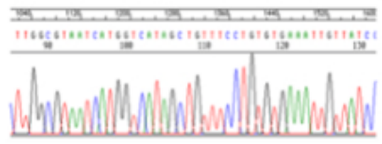
Protein separation using

Electric charge and molecular weight

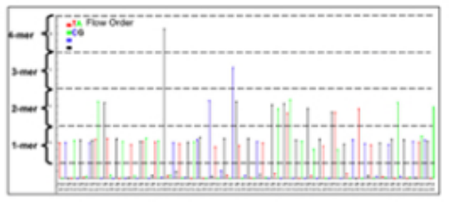
DNA Sequence Analysis:

Profiling DNA

Sanger Sequencing – >800 base pairs/run



High Throughput Genomic Sequencing – 100,000 base pairs/run

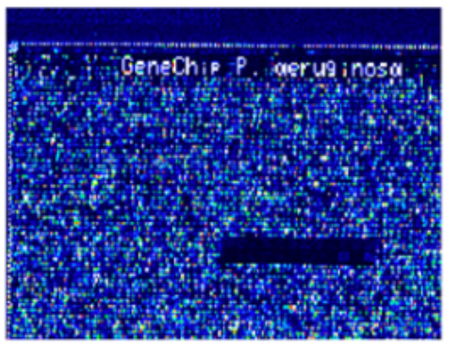


RNA

Expression

Microarray:Analysis

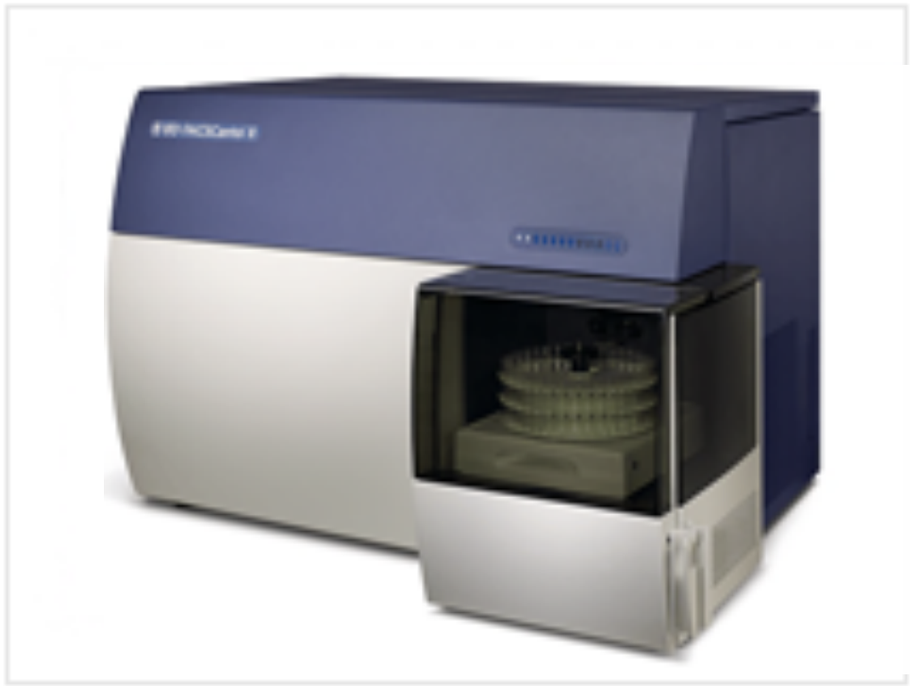
Profiling mRNA



Colour of pin-point dots demonstrates the presence / absence of gene sequences

Cellometer Auto 2000

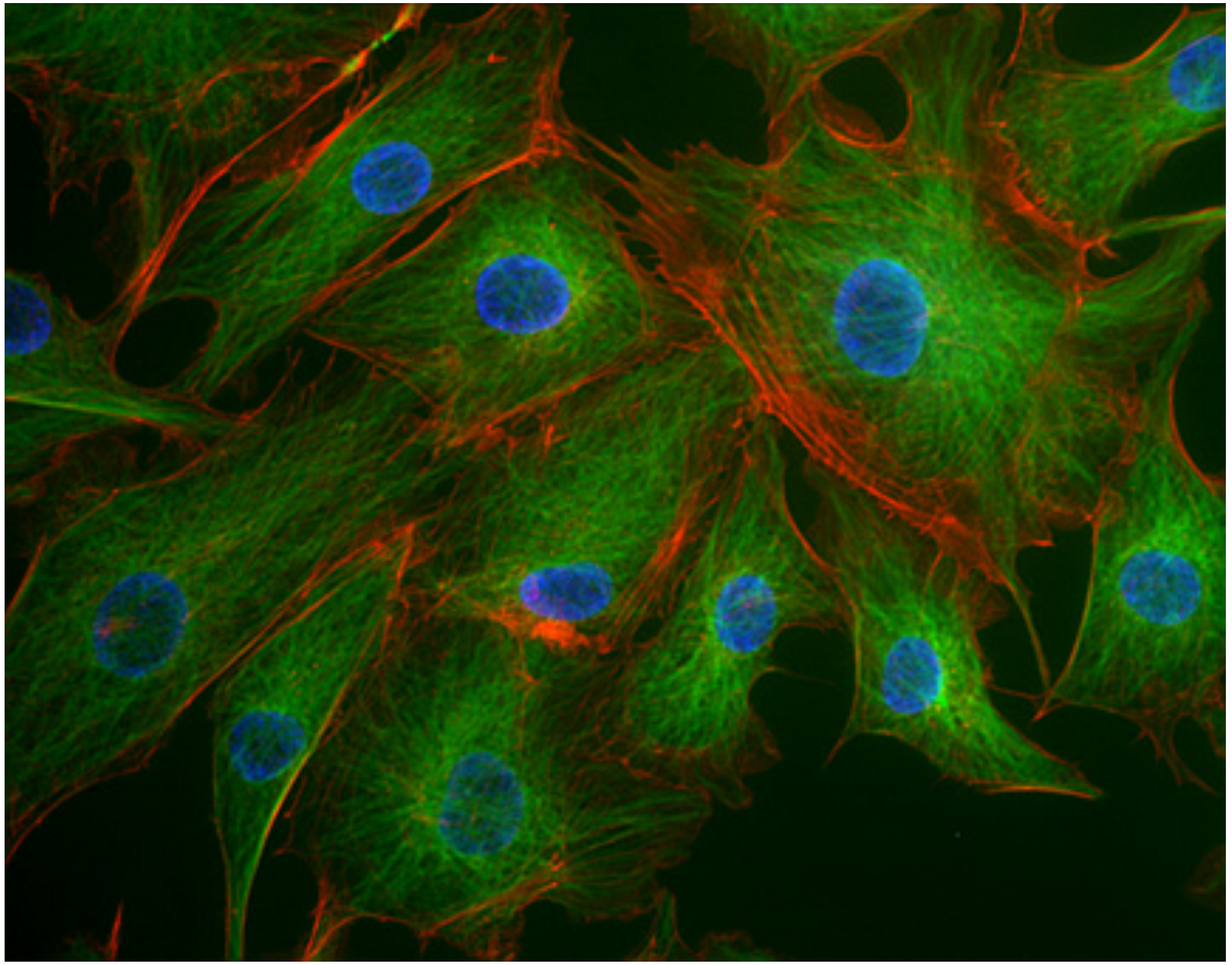
Dual Fluorescence and Bright Field Imaging: allow for rapid analysis of cell viability in heterogenous cell populations



BD FACSCanto™ II

Cell analyzer with proven reliability and high-quality results supporting up to 10 parameters.

Using Fluorescence to Interrogate Cells



Fundamentals of Cytometry

StarCellBio

presents

FLOW CYTOMETRY

Fundamentals of Cytometry

https://youtu.be/EQXPJ7eeesQ

Fundamentals of Cytometry

What is it?

A brief history

What cytometry is / does

How does it work?

Fluidics

Optics

Electronics

What can we do with it?

A quick overview
of some of its applications

BD Accuri™ C6 Plus

New generation Accuri makes flow cytometry even more within reach.

BD FACSCanto™ II

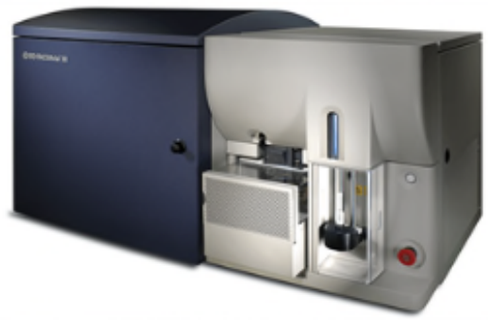
Cell analyzer with proven reliability and high-quality results supporting up to 10 parameters.

BD LSRFortessa™

FACSFortessa is configurable and upgradeable with up to 4 lasers to detect up to 18 colors simultaneously.

Research cell analyzers

Flow cytometers that identify, count, and characterize cells to support cell analysis. needs.

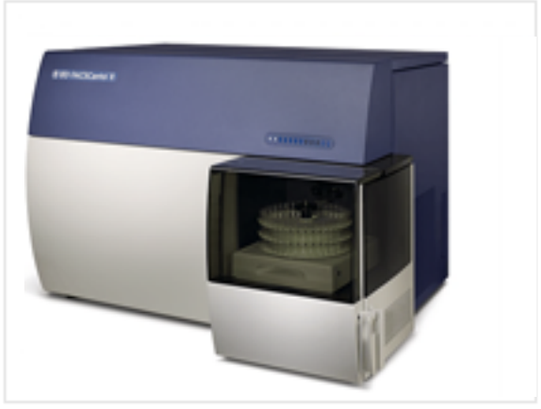


BD FACSAria™ III

A cell sorter with patented technologies that deliver ease-of-use and superior multicolor performance.

BD Accuri™ C6 Plus

New generation Accuri makes flow cytometry even more within reach.



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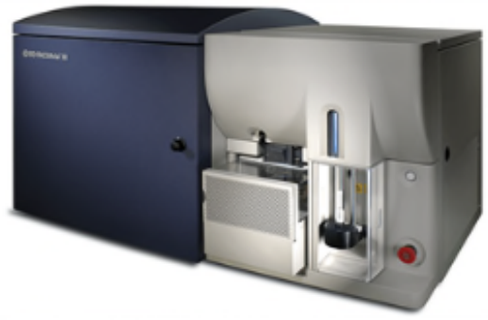

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Flow cytometers that identify, count, and characterize cells to support cell analysis. needs.

BD FACSAria™ III

A cell sorter with patented technologies that deliver ease-of-use and superior multicolor performance.

4 lasers, Blue lasers (405, 488 nm), Red laser (639 nm) and a Green laser (561 nm).

Mounts up to 18 detectors, and measure a maximum of ~22-24 colors simultaneously.

BD Accuri™ C6 Plus

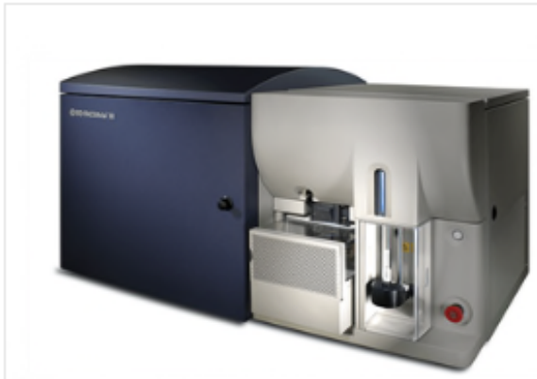
New generation Accuri makes flow cytometry even more within reach.

BD FACSCanto™ II

Cell analyzer with proven reliability and high-quality results supporting up to 10 parameters.

BD LSRFortessa™

FACSFortessa is configurable and upgradeable with up to 4 lasers to detect up to 18 colors simultaneously.



BD FACSAria™ III

A cell sorter with patented technologies that deliver ease-of-use and superior multicolor performance.

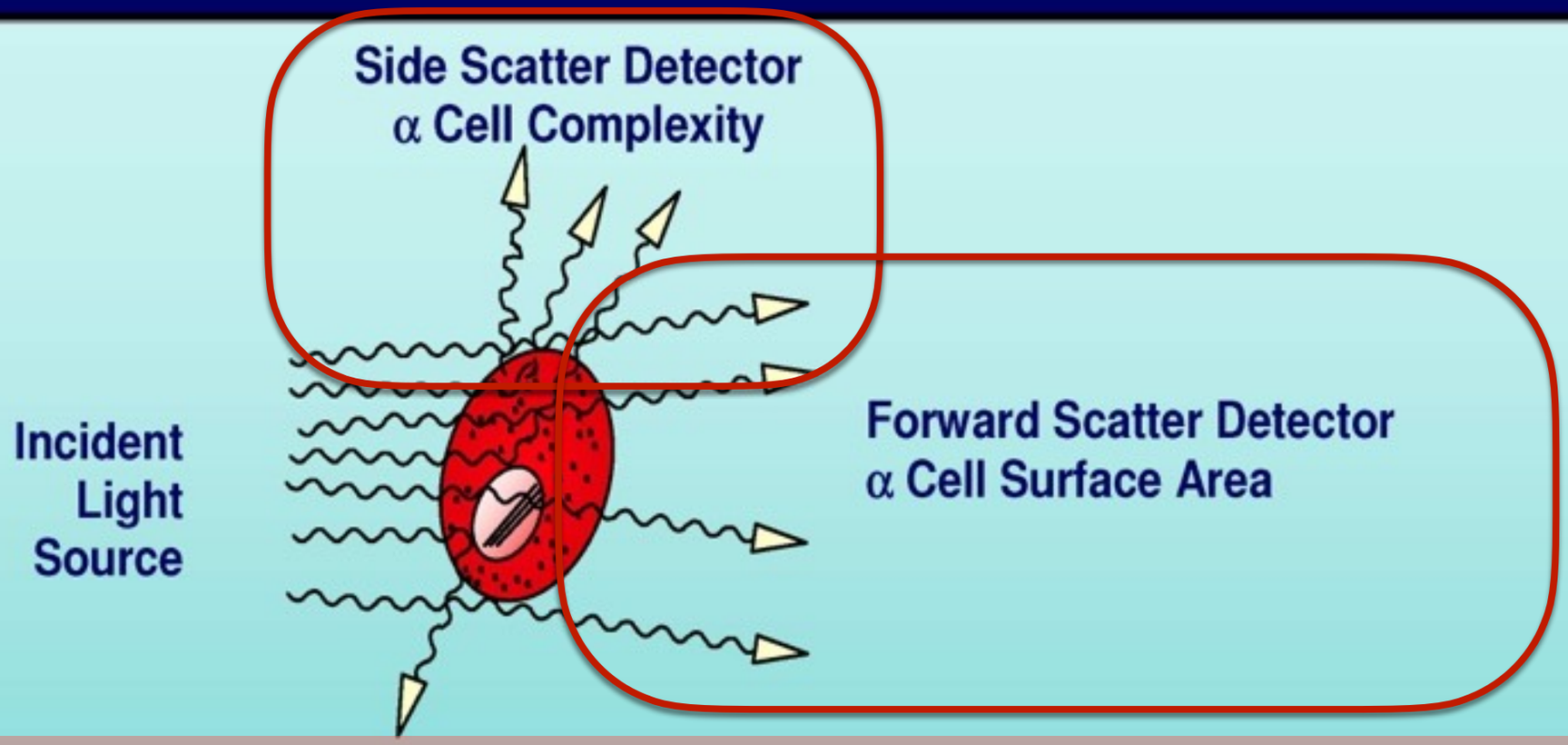
Research cell analyzers

Flow cytometers that identify, count, and characterize cells to support cell analysis. needs.

4 lasers, Blue lasers (488 nm), Red laser (639 nm) and Green laser (561 nm) and a near UV (375-nm).

Mounts up to 20 detectors, and measure a maximum of ~13 dye colors simultaneously.

Properties of FSC and SSC

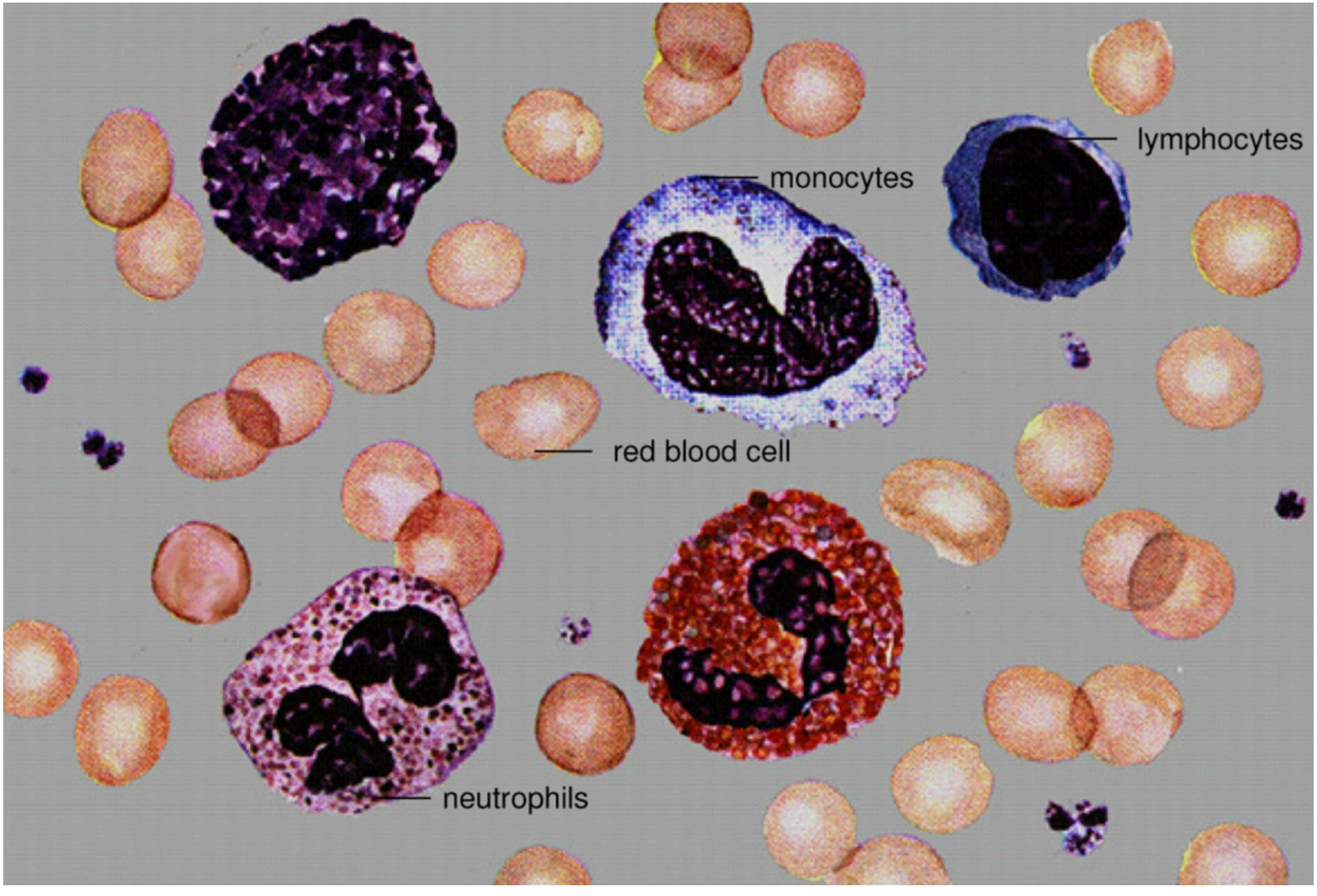


Forward Scatter (FSC)—refracted and diffracted light

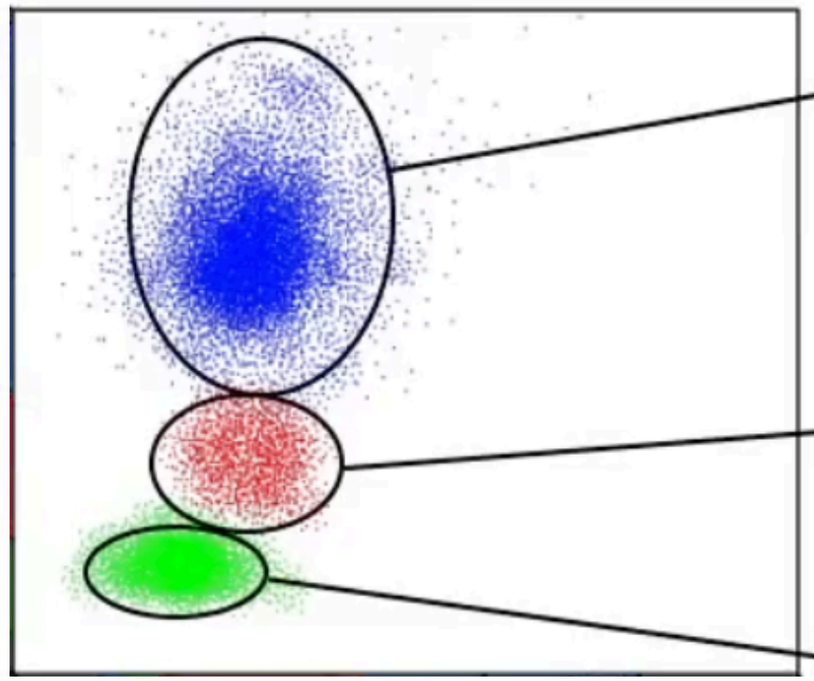
- Related to cell volume
- Measured along axis of incident light in the forward direction

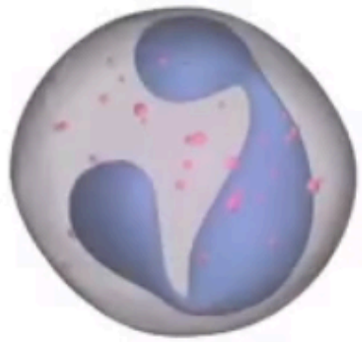
Side Scatter (SSC)—reflected and refracted light

- Related to cell granularity and complexity
- Selected at 90° to the laser beam


lymphocytes
monocytes
red blood cell
neutrophils

Neutrophils

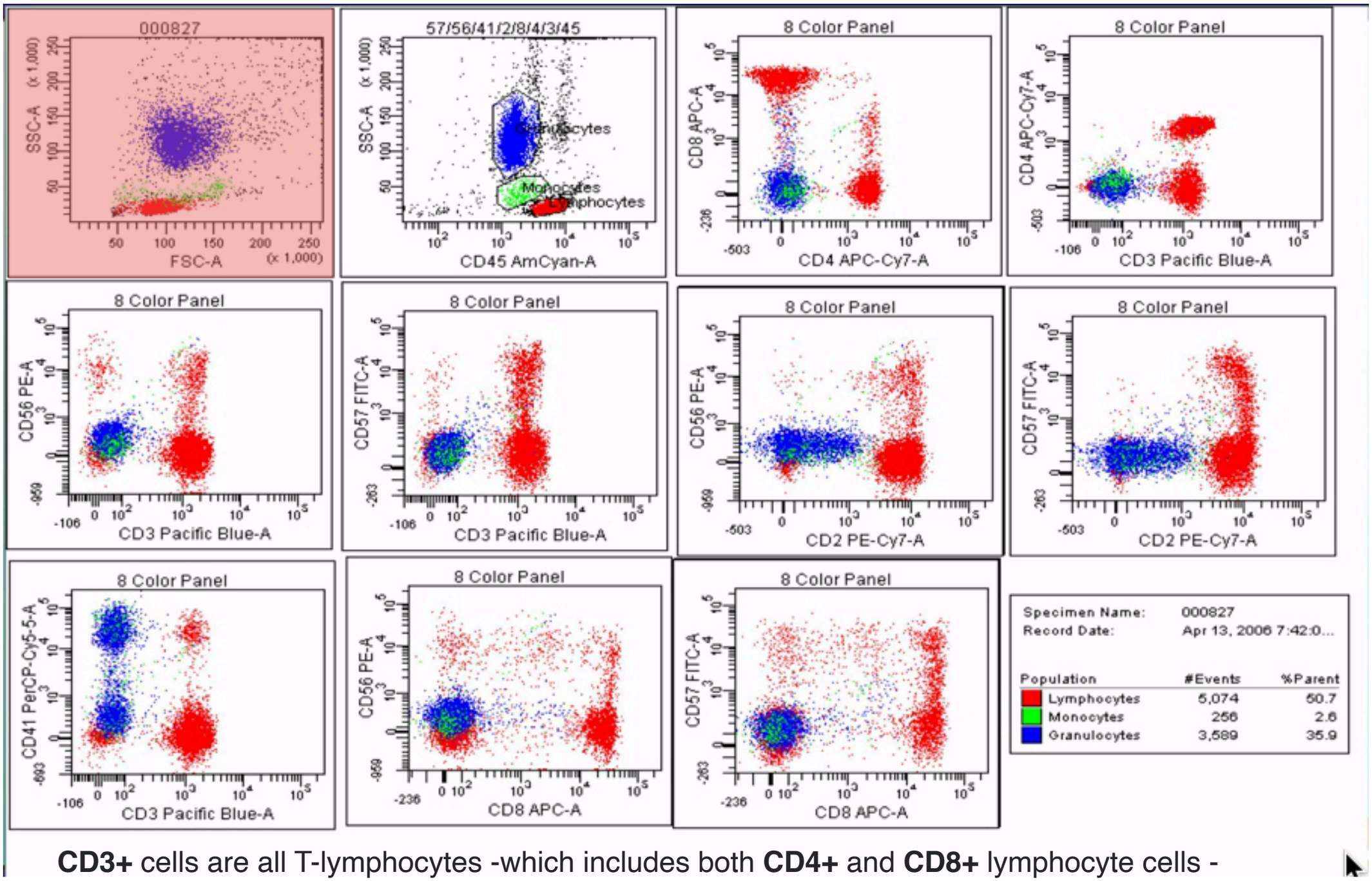




Monocytes

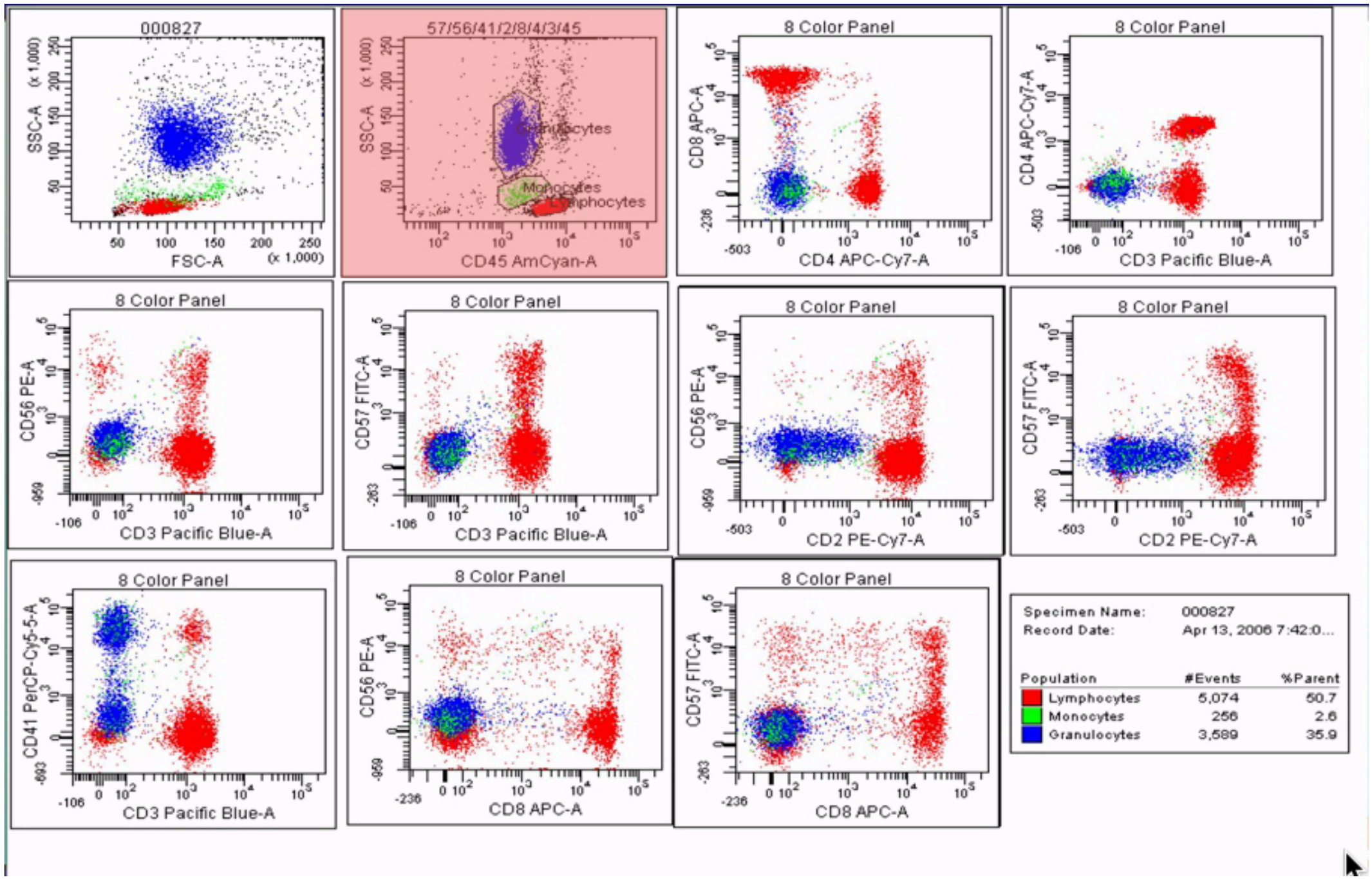
Lymphocytes

8 color T & NK Cell Worksheet



CD3+ cells are all T-lymphocytes -which includes both **CD4+** and **CD8+** lymphocyte cells - along with anomalous **CD56** (High NK cells and large ”granular lympocytes…”), **CD57**-expressing “senescent” T-cells etc.

8 color T & NK Cell Worksheet



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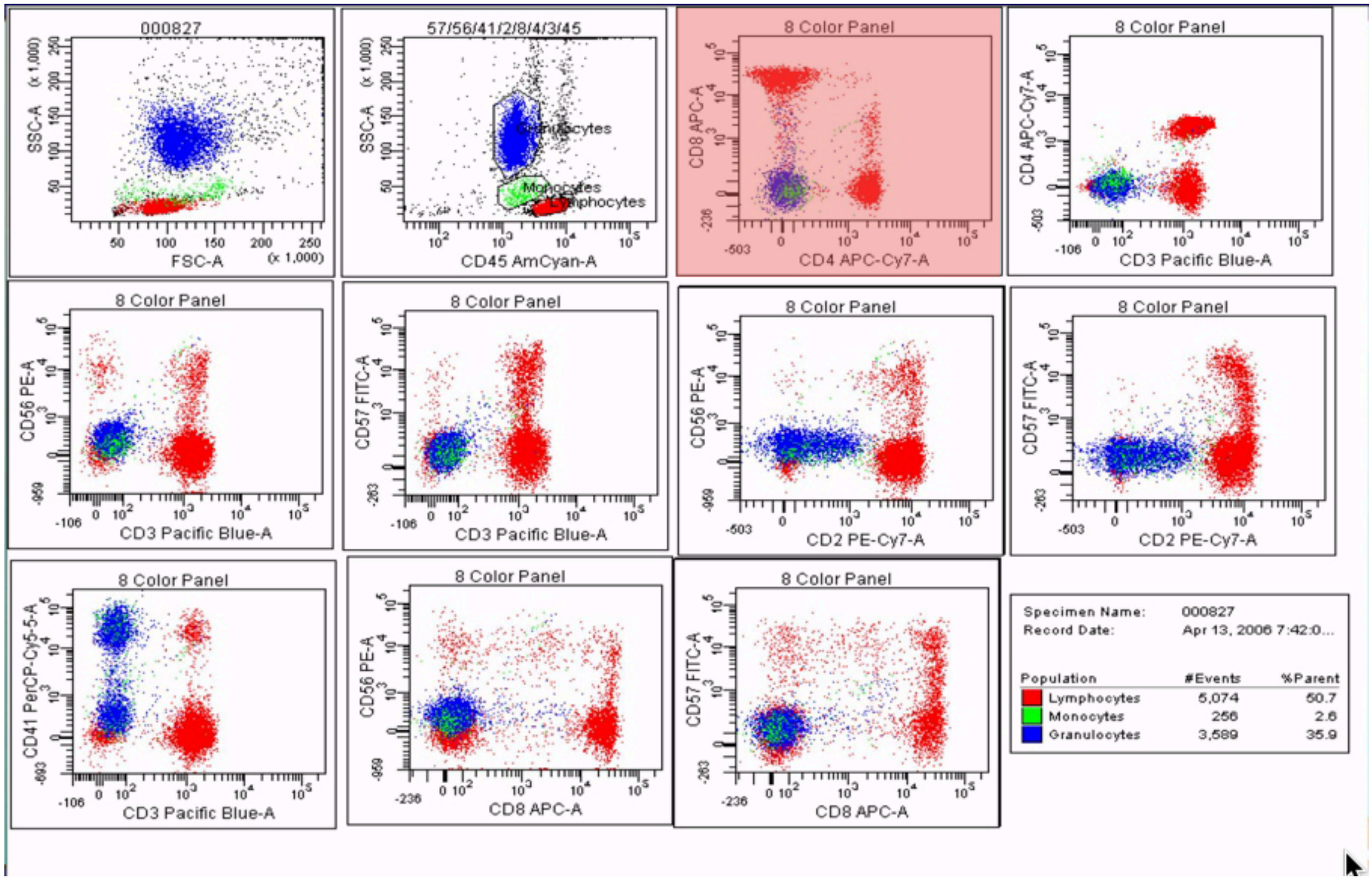
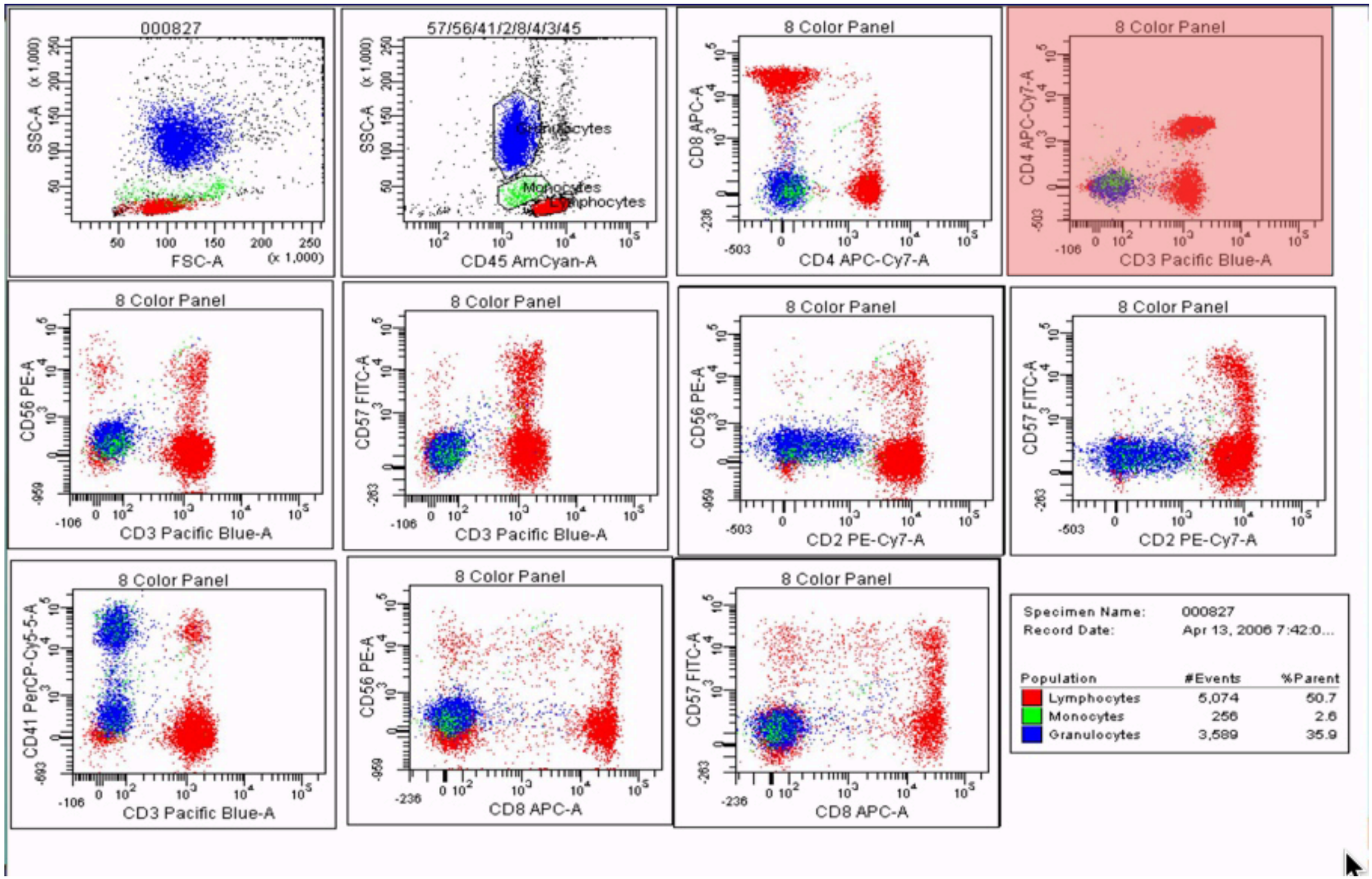


8 color T & NK Cell Worksheet



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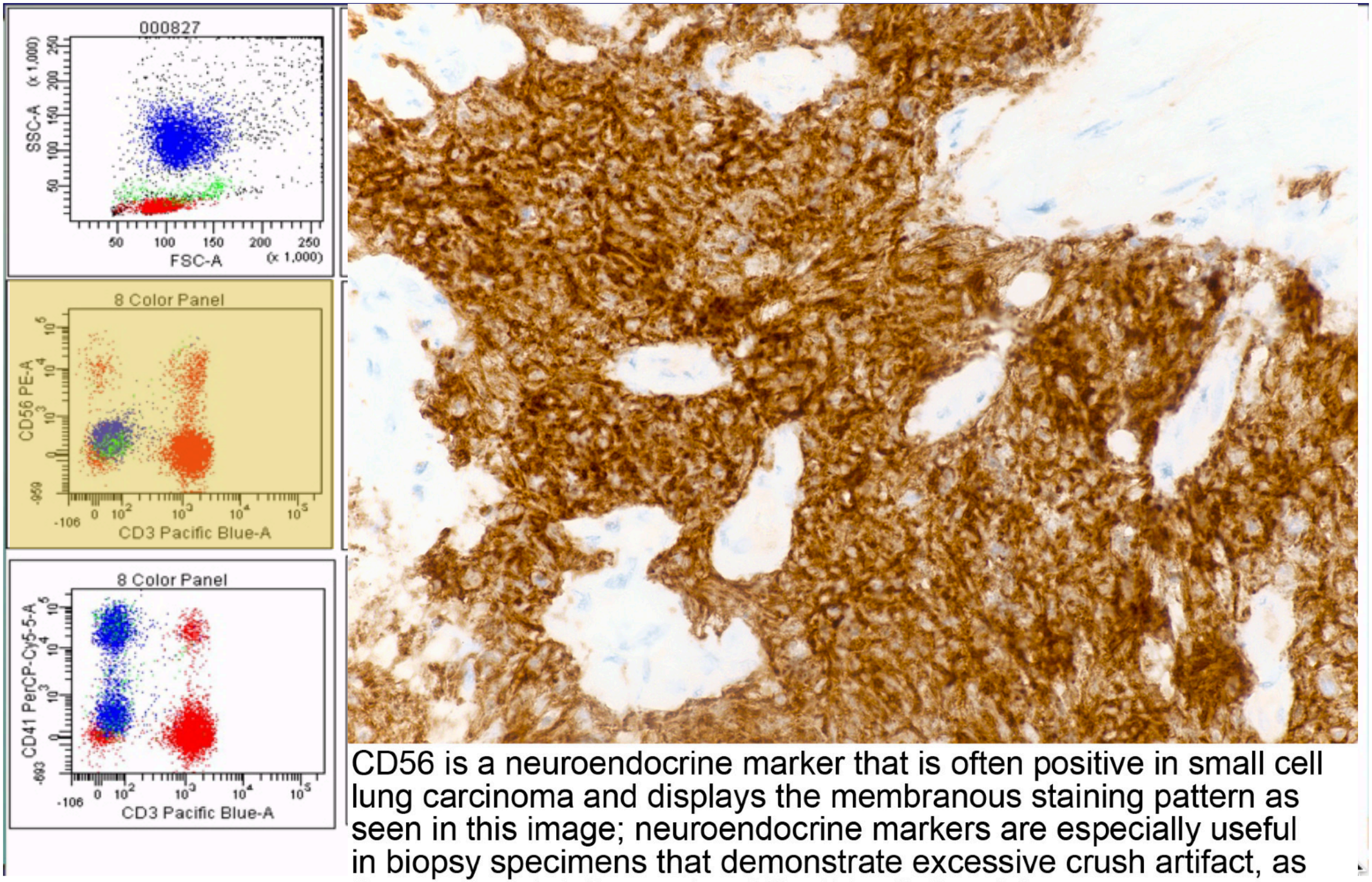
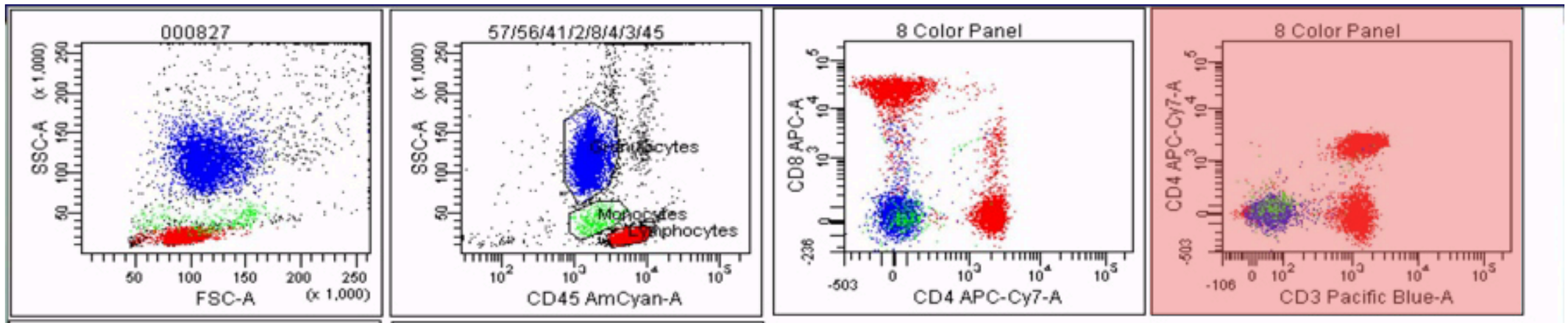


CD56 is a neuroendocrine marker that is often positive in small cell lung carcinoma and displays the membranous staining pattern as seen in this image; neuroendocrine markers are especially useful in biopsy specimens that demonstrate excessive crush artifact, as the nuclear details can be difficult to distinguish

8 color T & NK Cell Worksheet



Basic Flow Cytometry System

Fluidics

Carries particles to the laser intercept

Optics

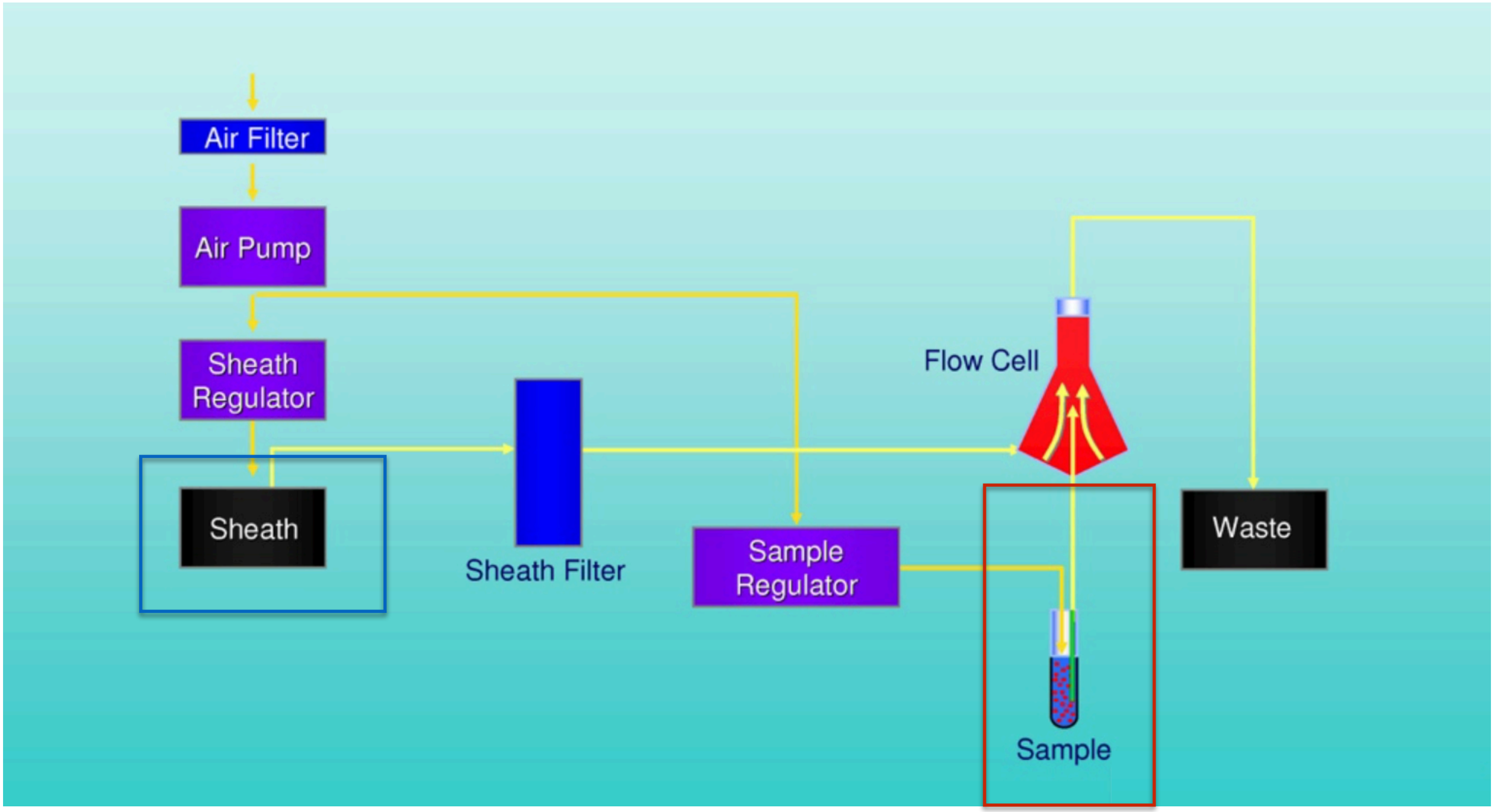
Light source for creating FSC & SSC as well as fluorescence

Optics for detecting fluorescence

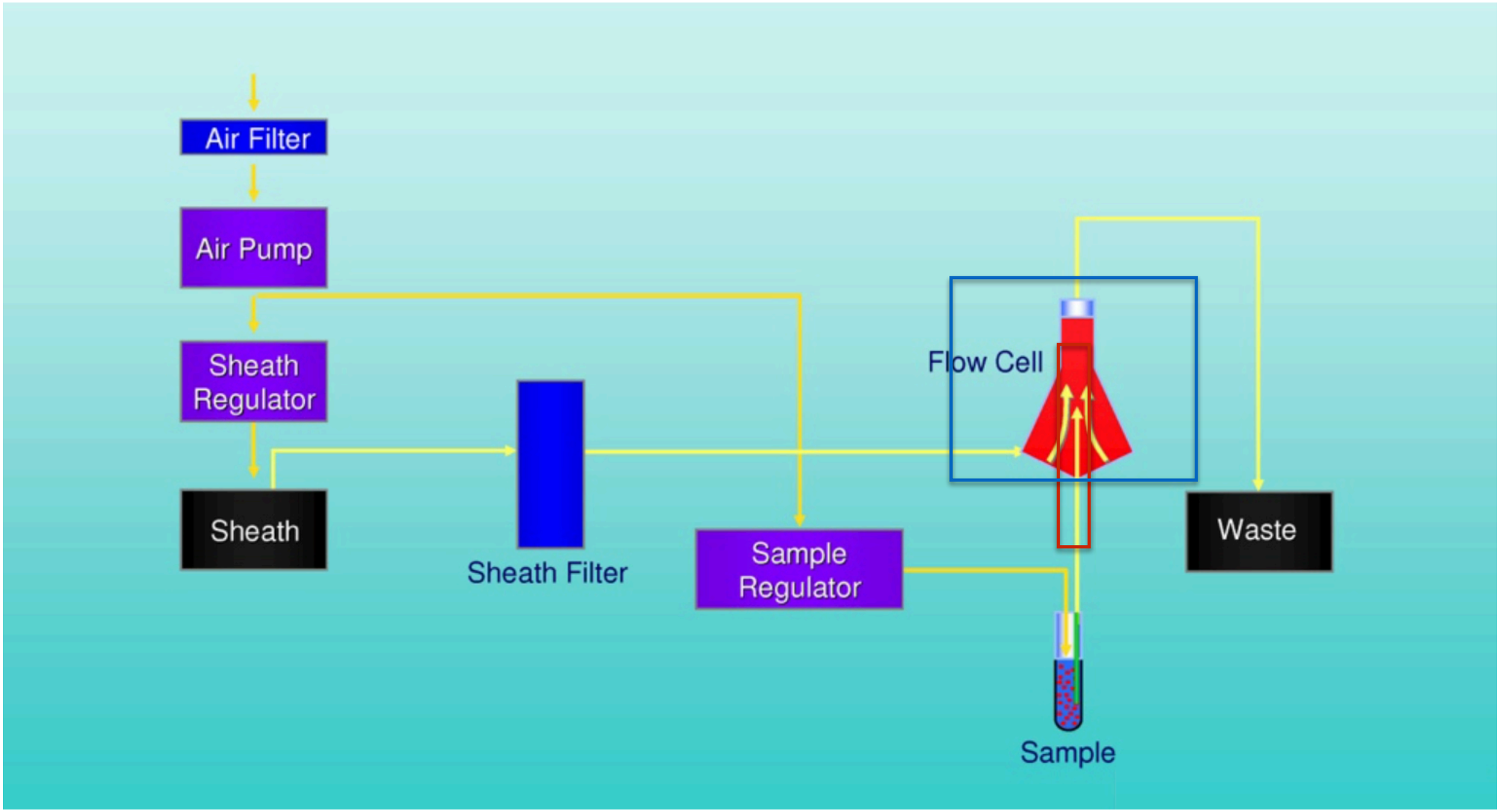
Electronics

Creating digital signals from the optics and displaying these signals on a computer

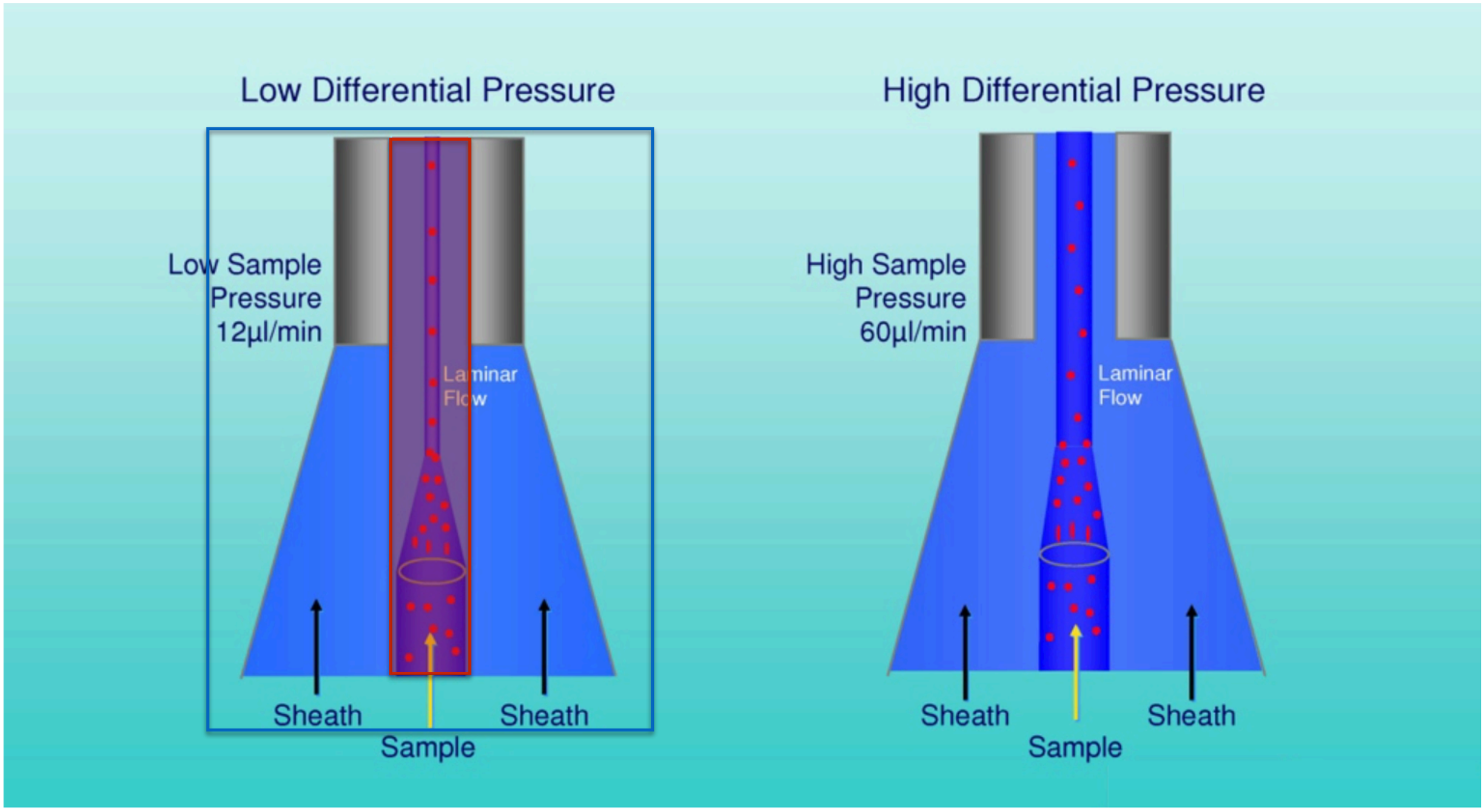
Basic Fluidics



Basic Fluidics



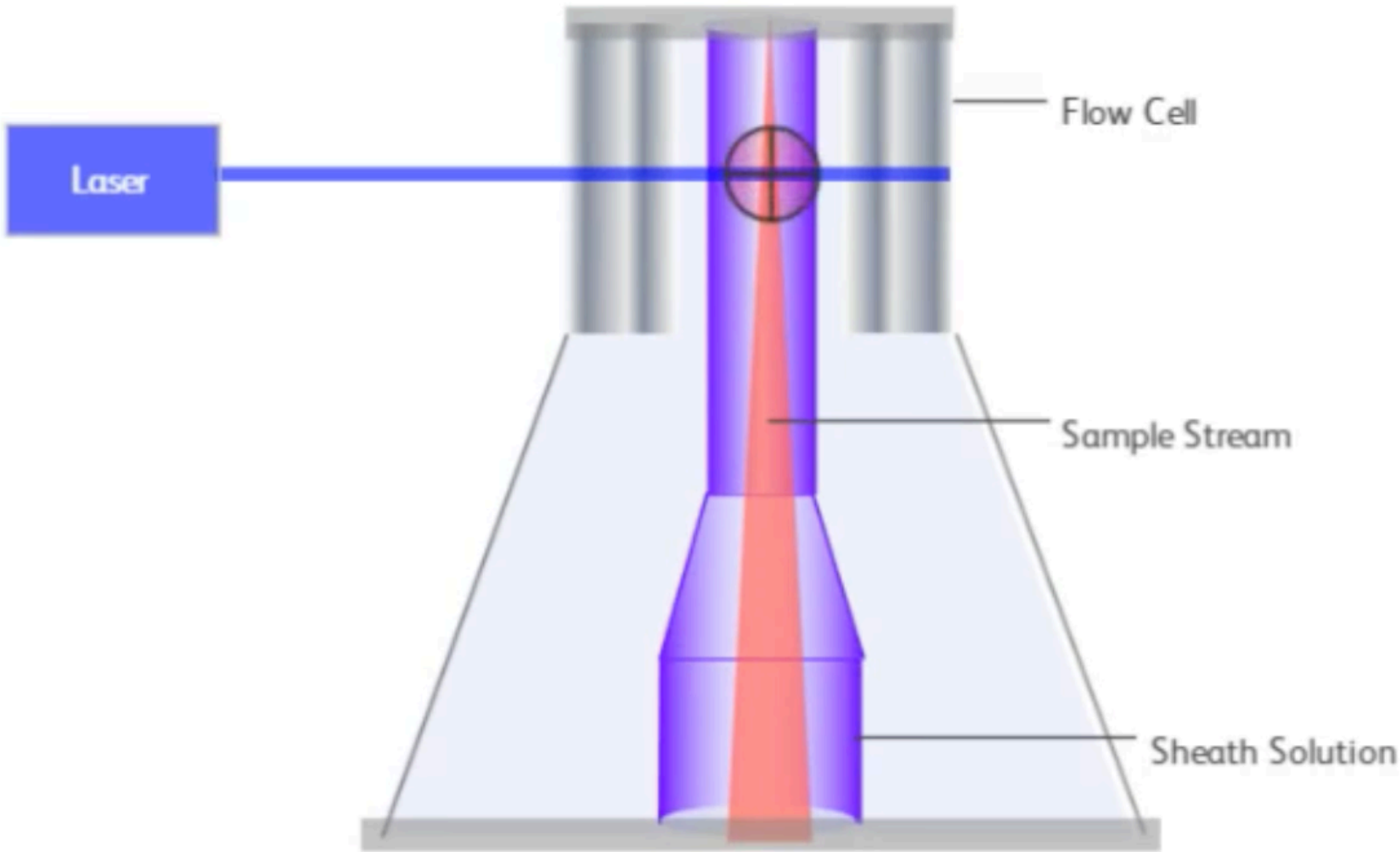
Sample Flow



Laminar flow

In fluid dynamics, laminar flow is characterized by fluid particles following smooth paths in layers, with each layer moving smoothly past the adjacent layers with little or no mixing. At low velocities, the fluid tends to flow without lateral mixing, and adjacent layers slide past one another like playing cards.

Interrogation Point



Basic Optical Systems

Excitation optics

Lasers

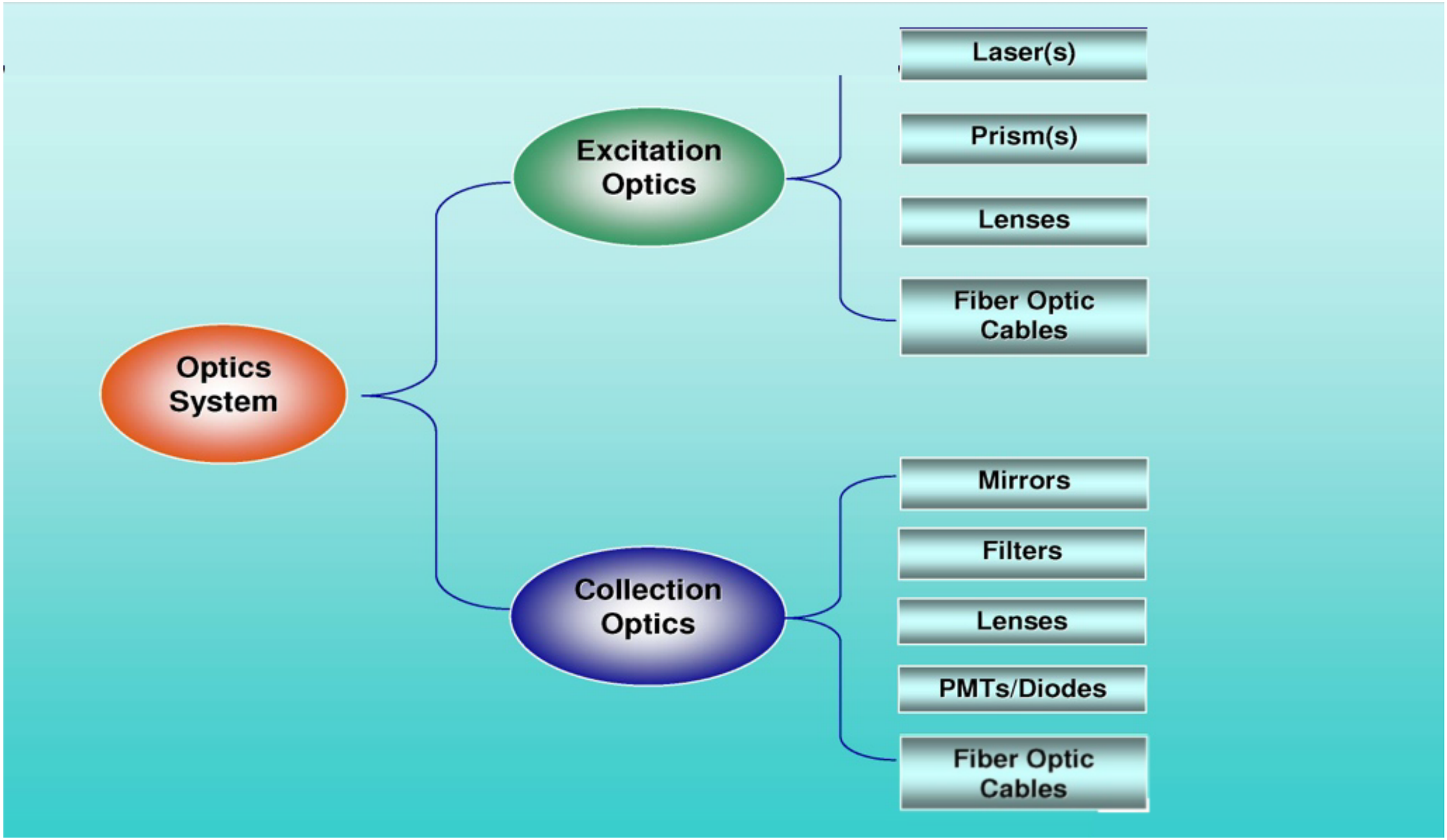
Lenses to shape and focus the laser beam

Collection optics

A collection lens to capture light emitted from the particle-laser beam interaction

A system of optical mirrors and filters to direct specified wavelengths of the captured light toward designated photon sensitive detectors

Basic Optical Systems



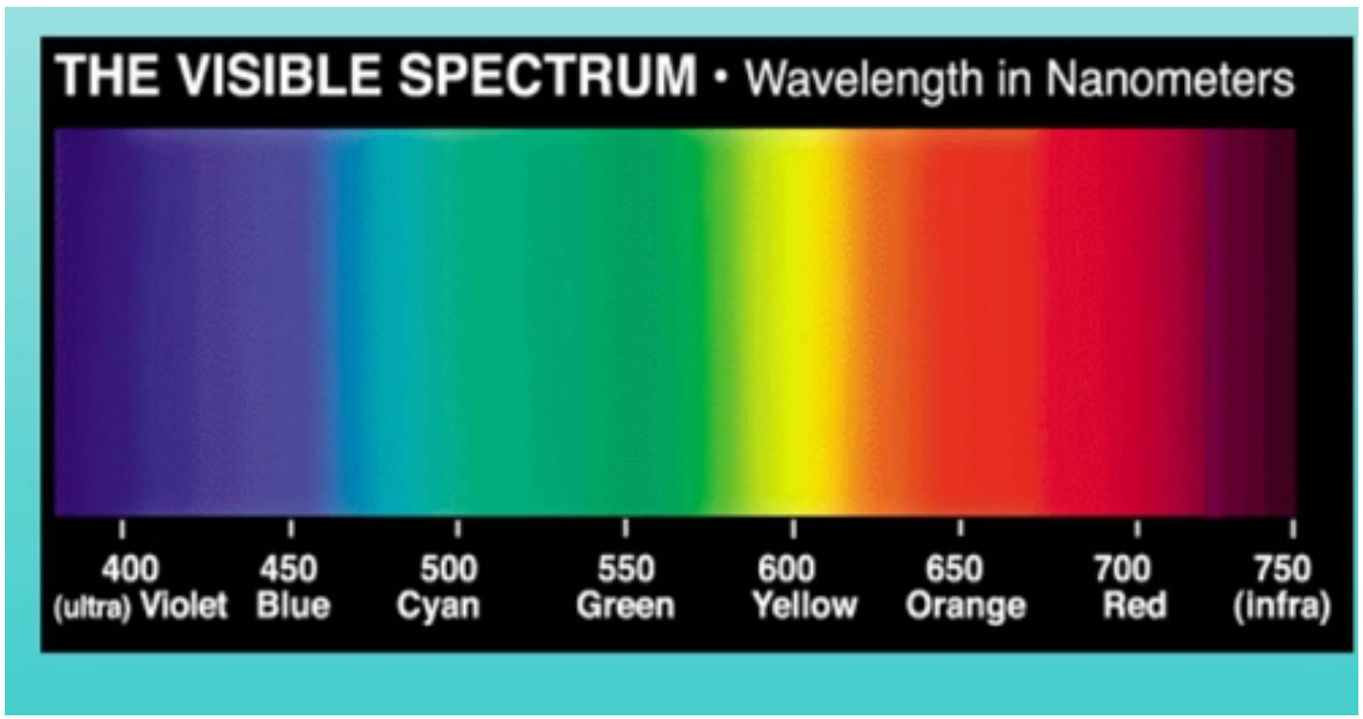
Lasers

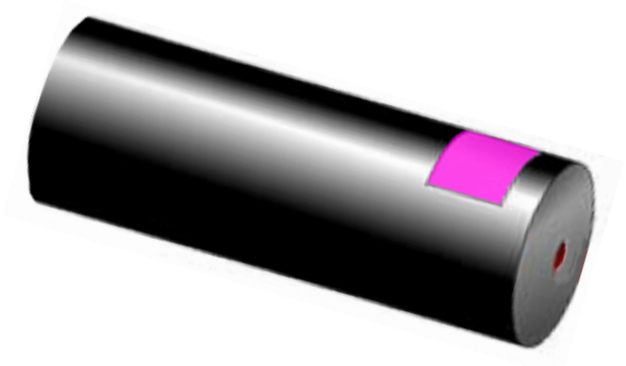
Light Amplification by Stimulated Emission of Radiation

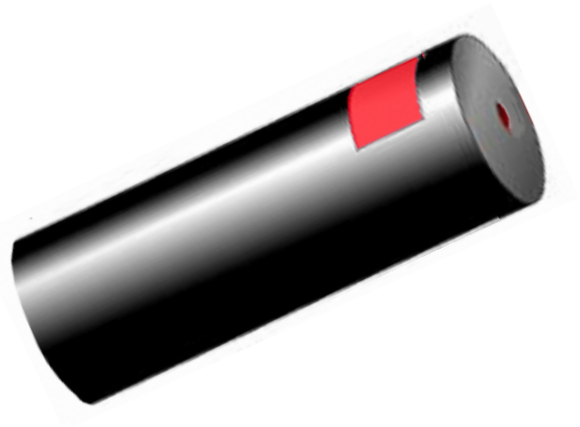
Light output from a a laser

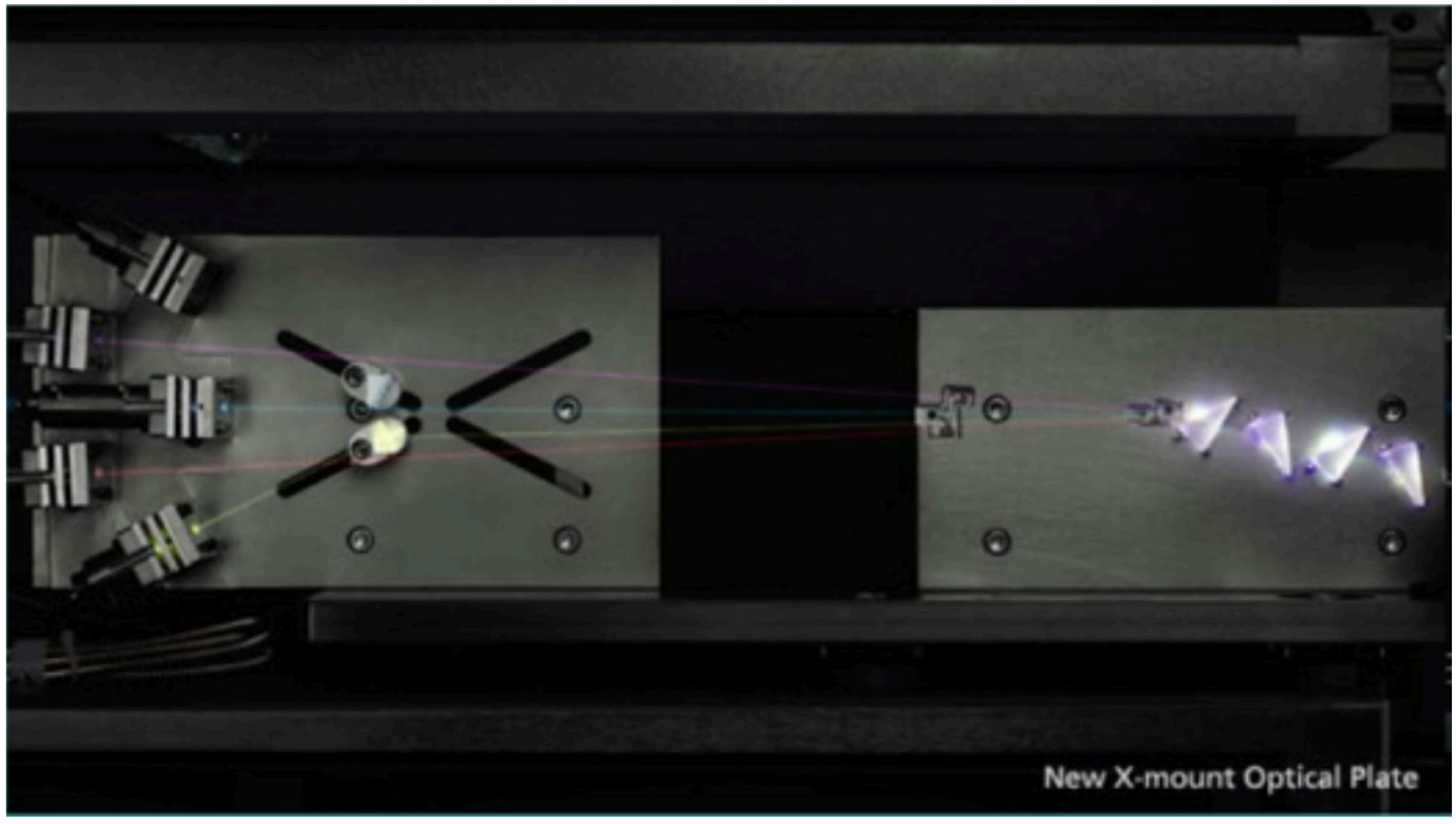
Monochromatic

Unidirectional







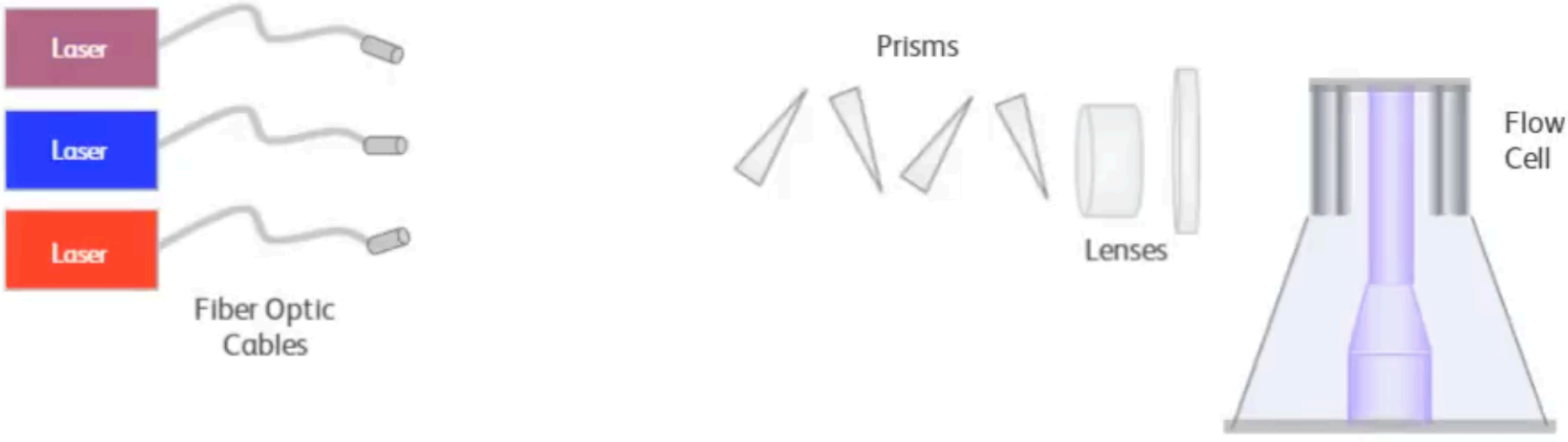


Basics of Cytometry

Excitation optics

Lasers

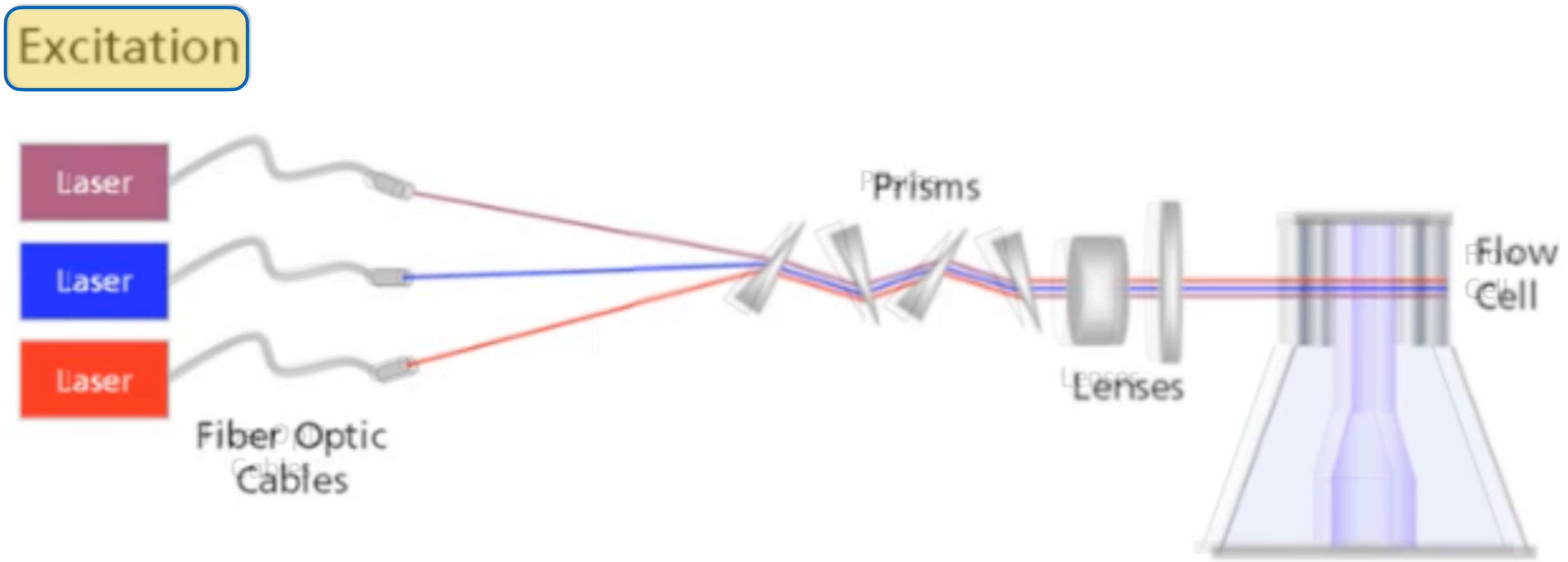
Lenses to shape and focus the laser beam



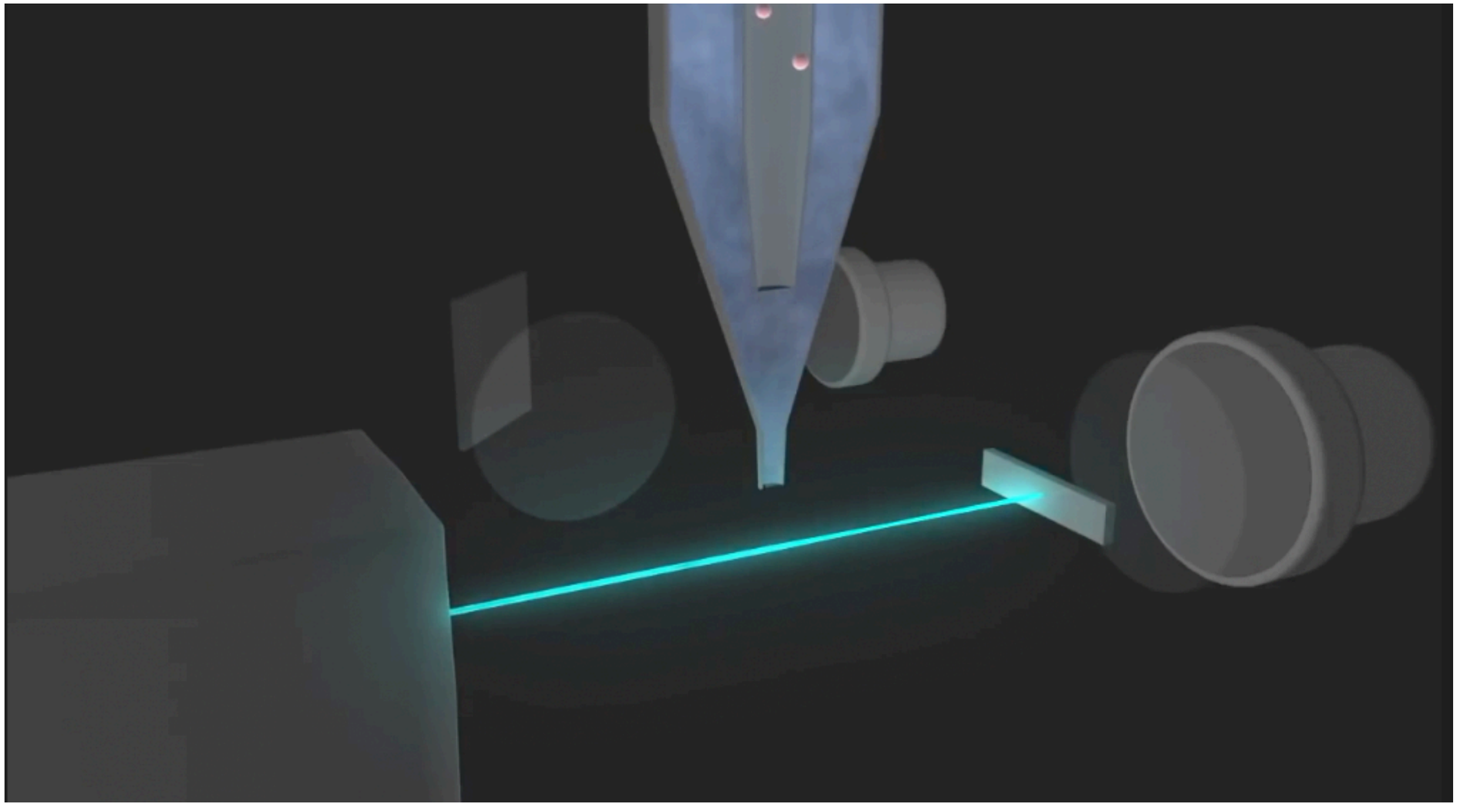
Excitation optics

Lasers

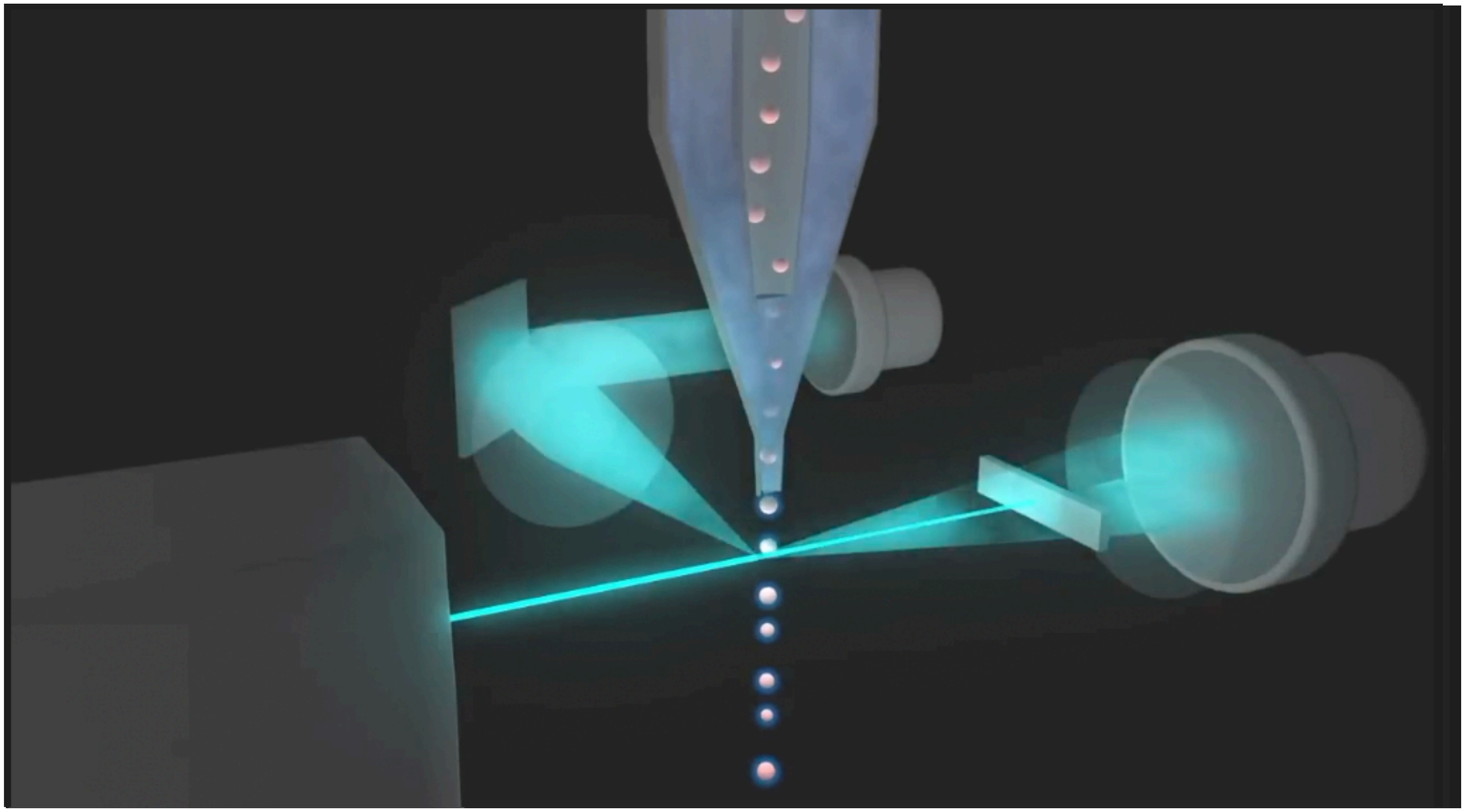
Lenses to shape and focus the laser beam



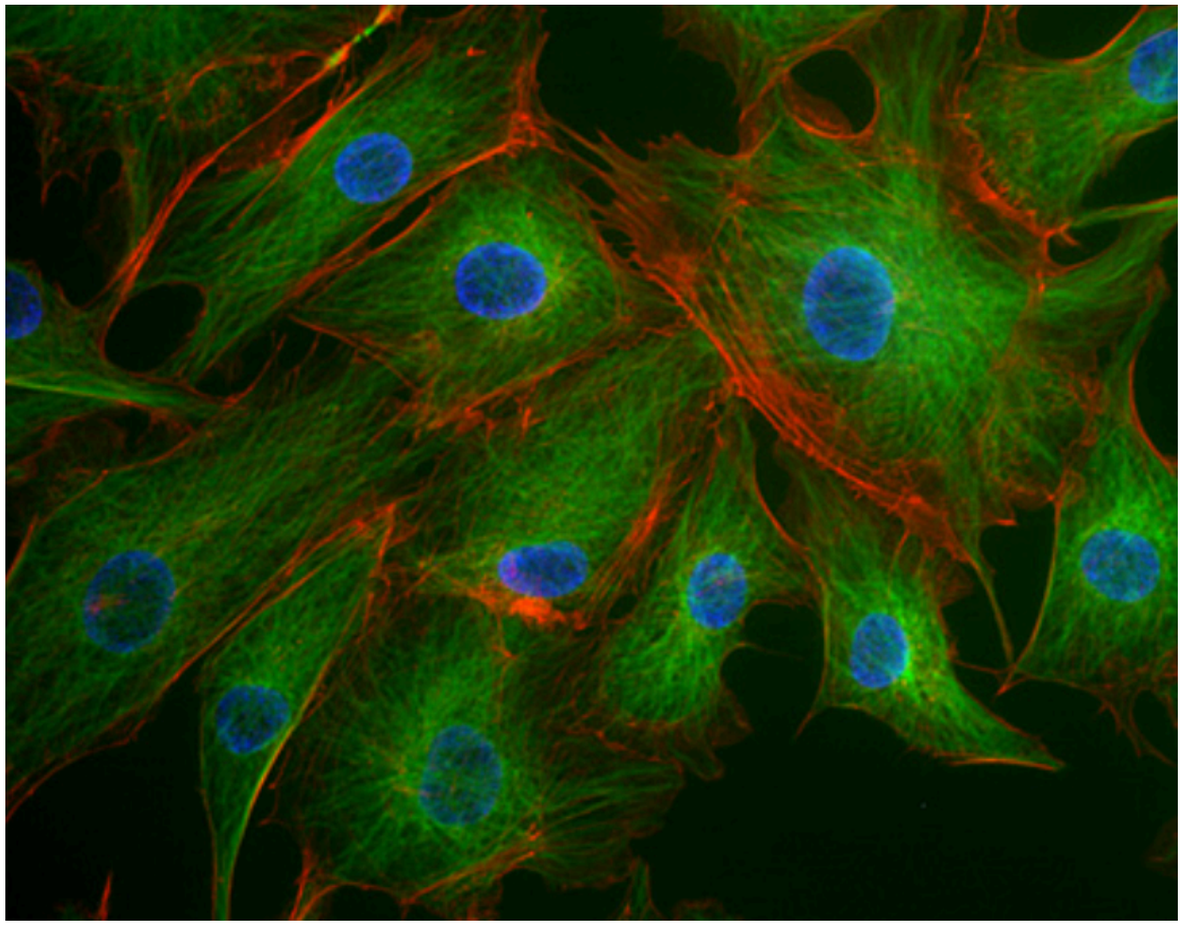
Excitation Optics

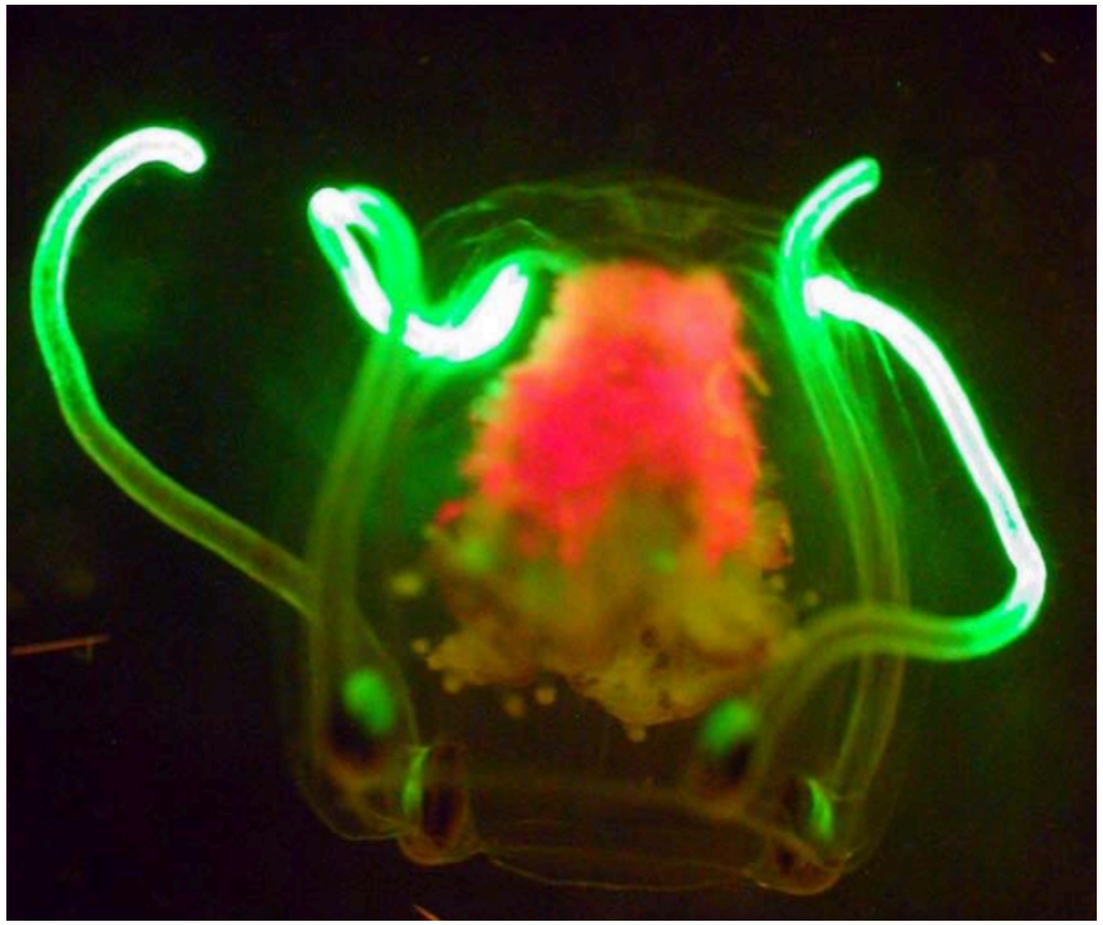


Collection Optics



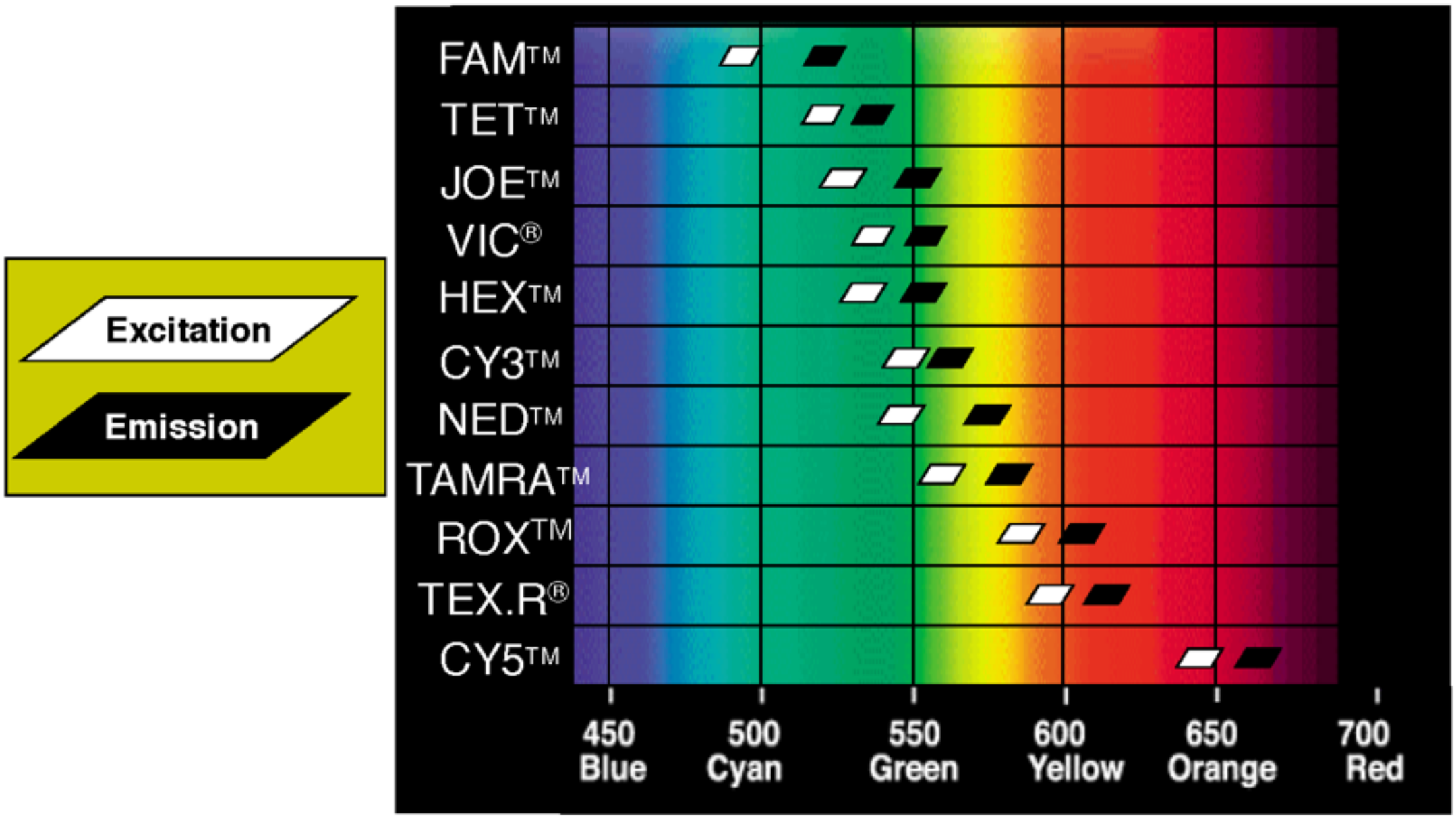
What is Fluorescent light?





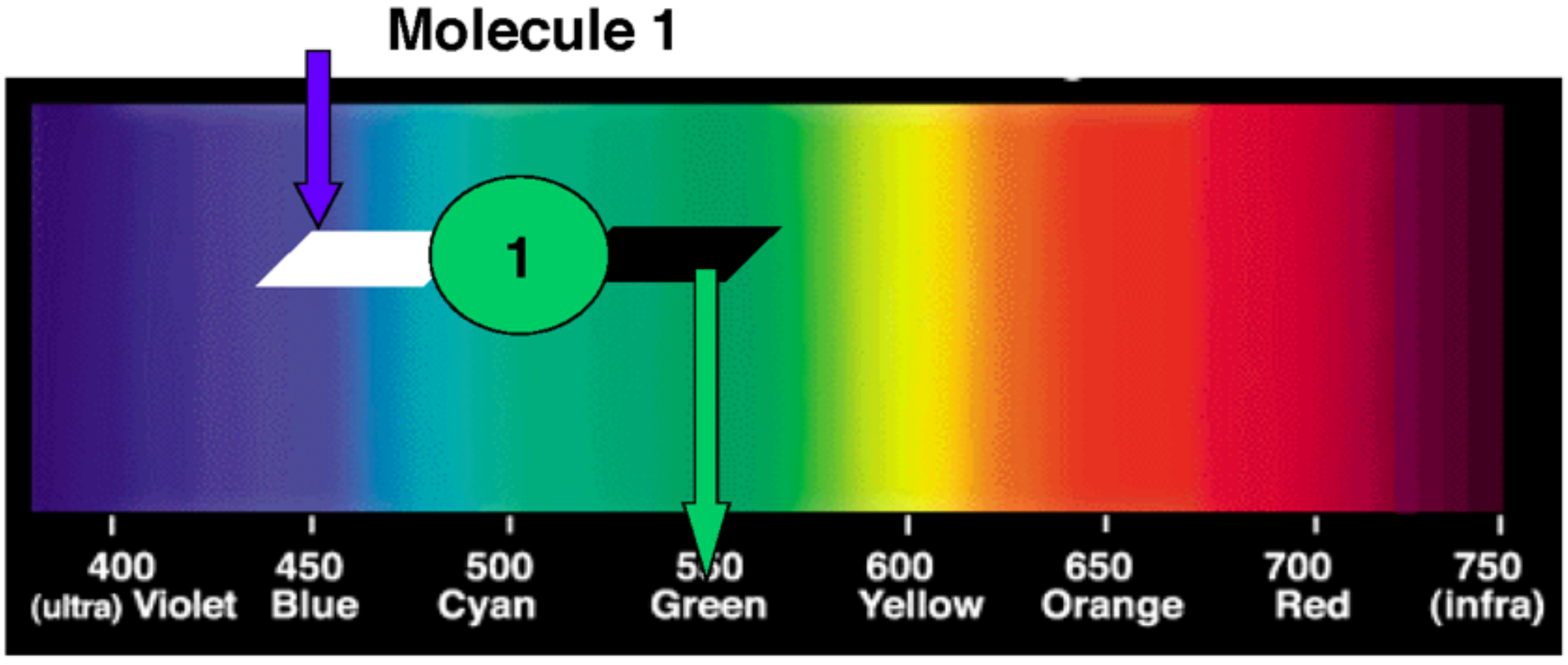
The visible or invisible **radiation emitted by certain substances** as a result of incident radiation of **a shorter wavelength**

Fluorescence molecules absorb light and emit light at a longer wavelength (ie lower energy)



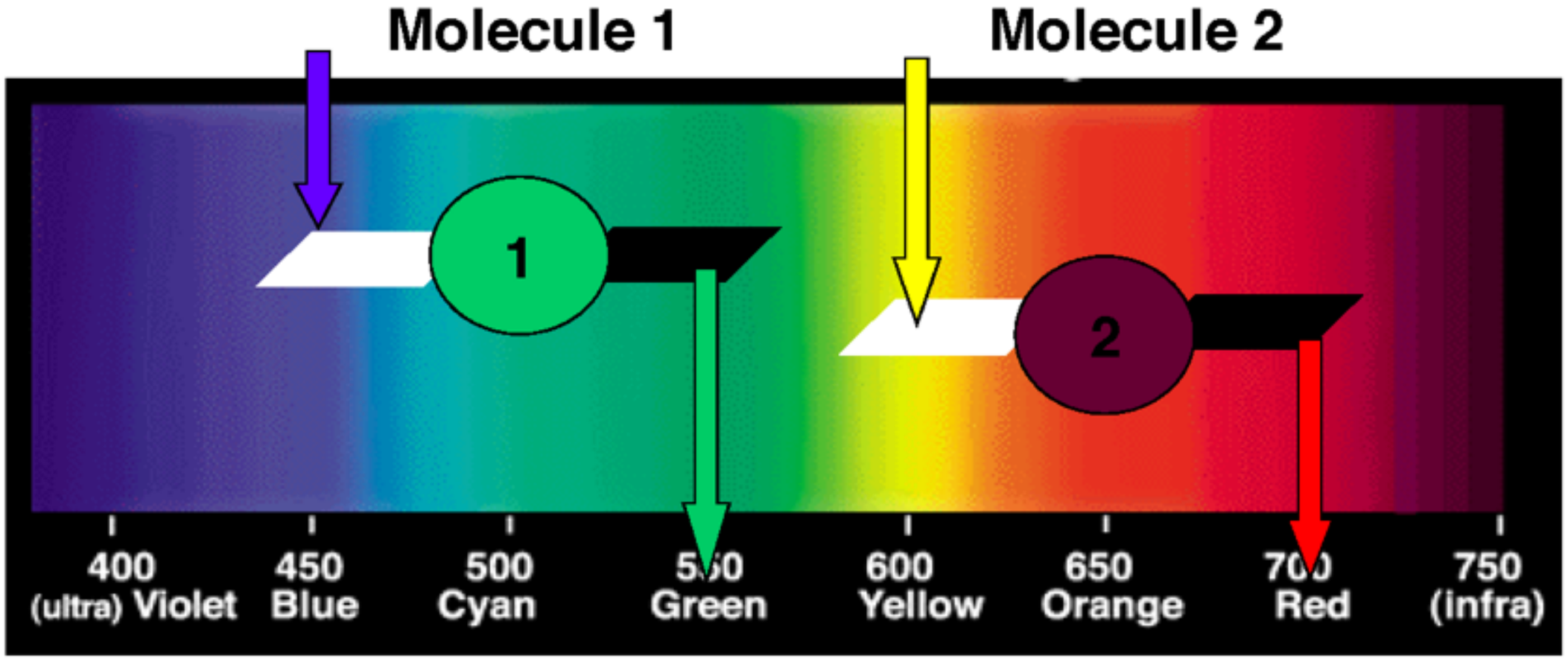
Note: these are approximations only

Behavior of 1 fluorescent molecule



Molecule 1 absorbs blue and emits green

Behavior of 2 fluorescent molecules

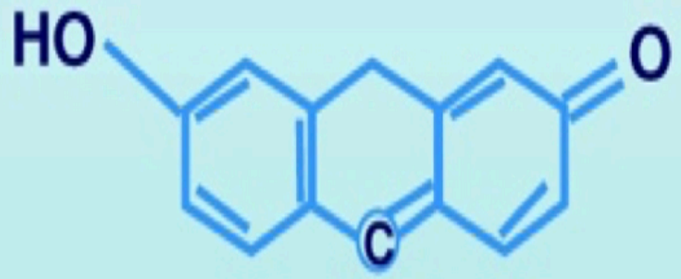


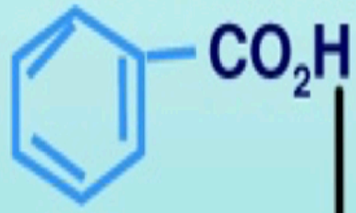
Molecule 1 absorbs blue and emits green

Molecule 2 absorbs yellow and emits red

λ = 488 nm

Incident
Light Energy





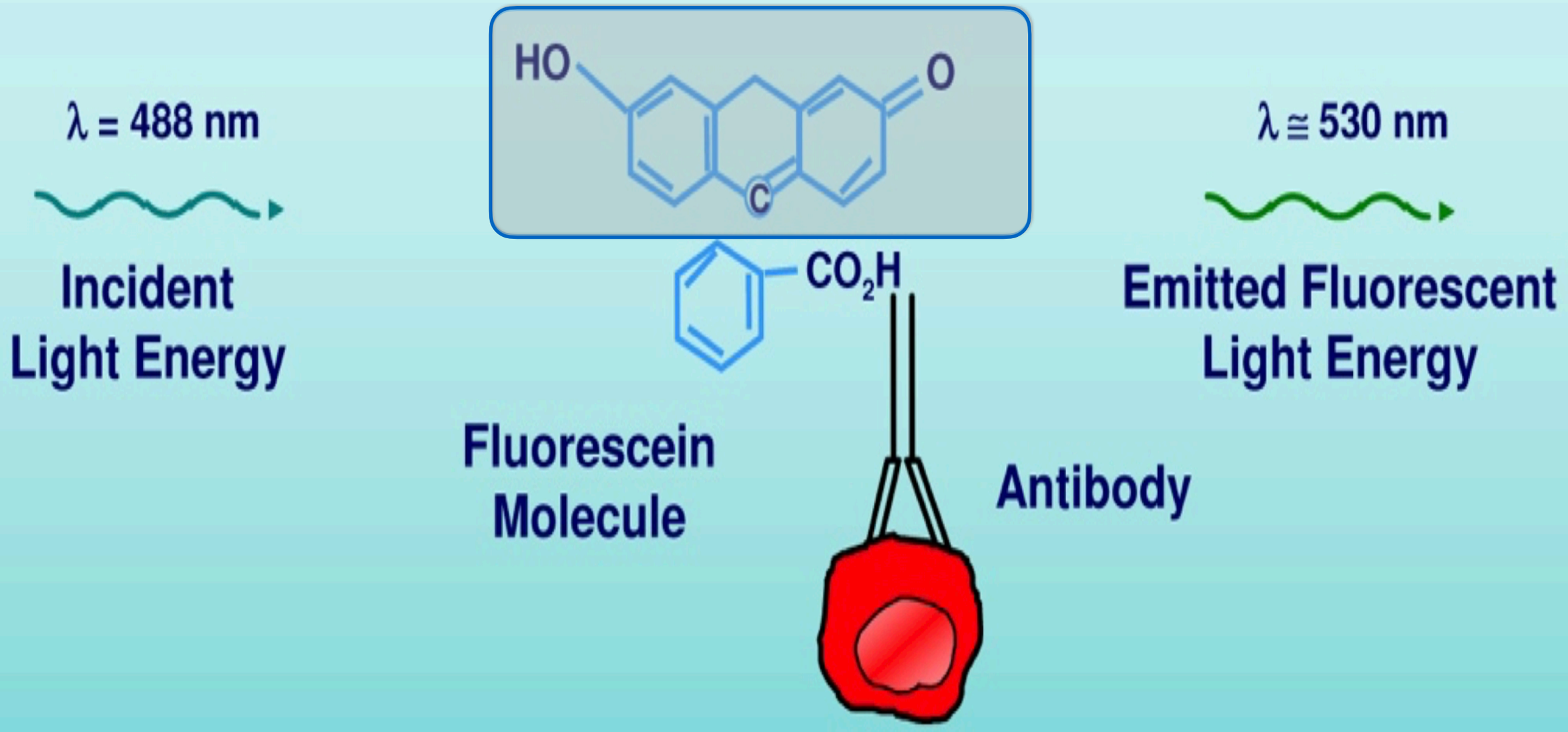
Fluorescein
Molecule

Antibody

$\lambda \cong$ 530 nm

Emitted Fluorescent
Light Energy

- **The fluorochrome absorbs energy from the laser**
- **The fluorochrome releases the absorbed energy by:**
  - Vibration and heat dissipation
  - Emission of photons of a longer wavelength



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  - Vibration and heat dissipation
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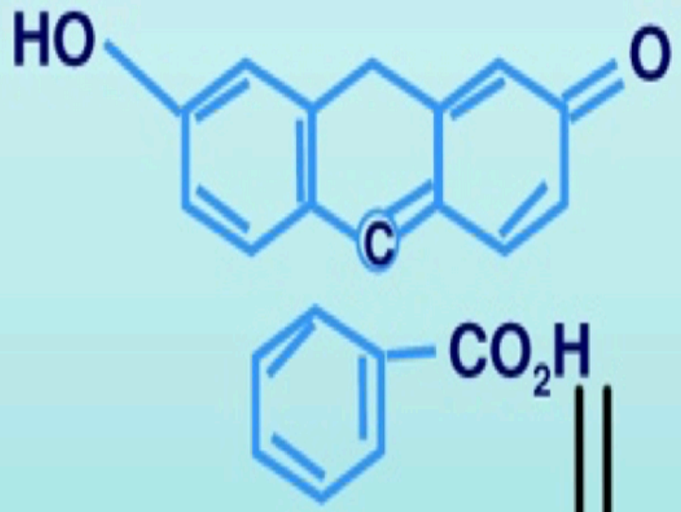
λ = 488 nm

Incident
Light Energy



Fluorescein
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Emitted Fluorescent
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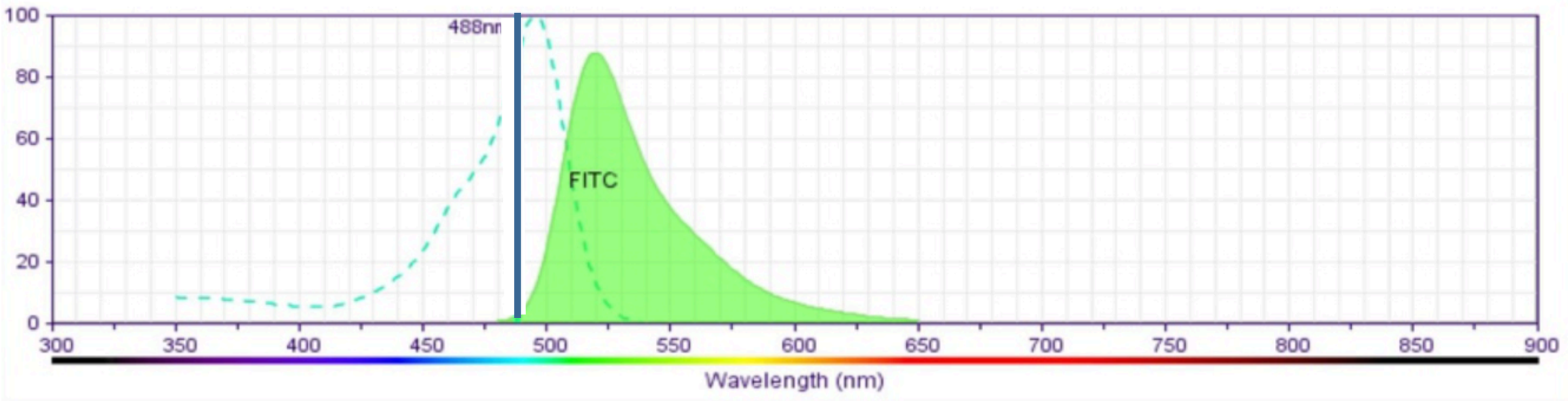
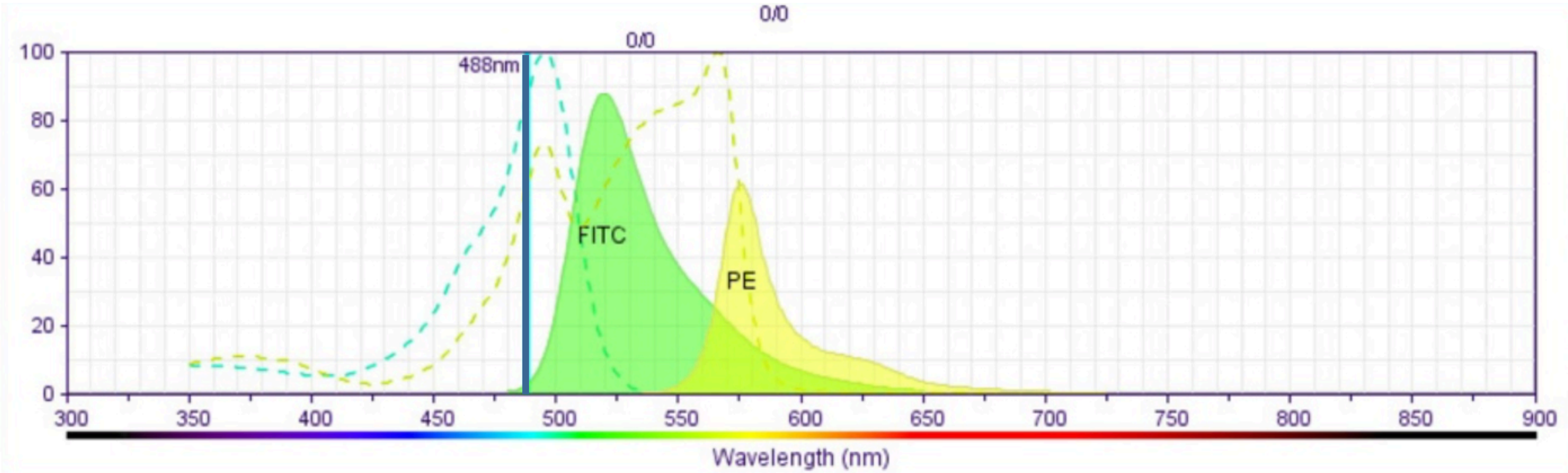
FITC Spectra



Fluorescein (FITC) can be excited using a 488 nm BLUE Laser with a “peak” **emission** at ~525 nm

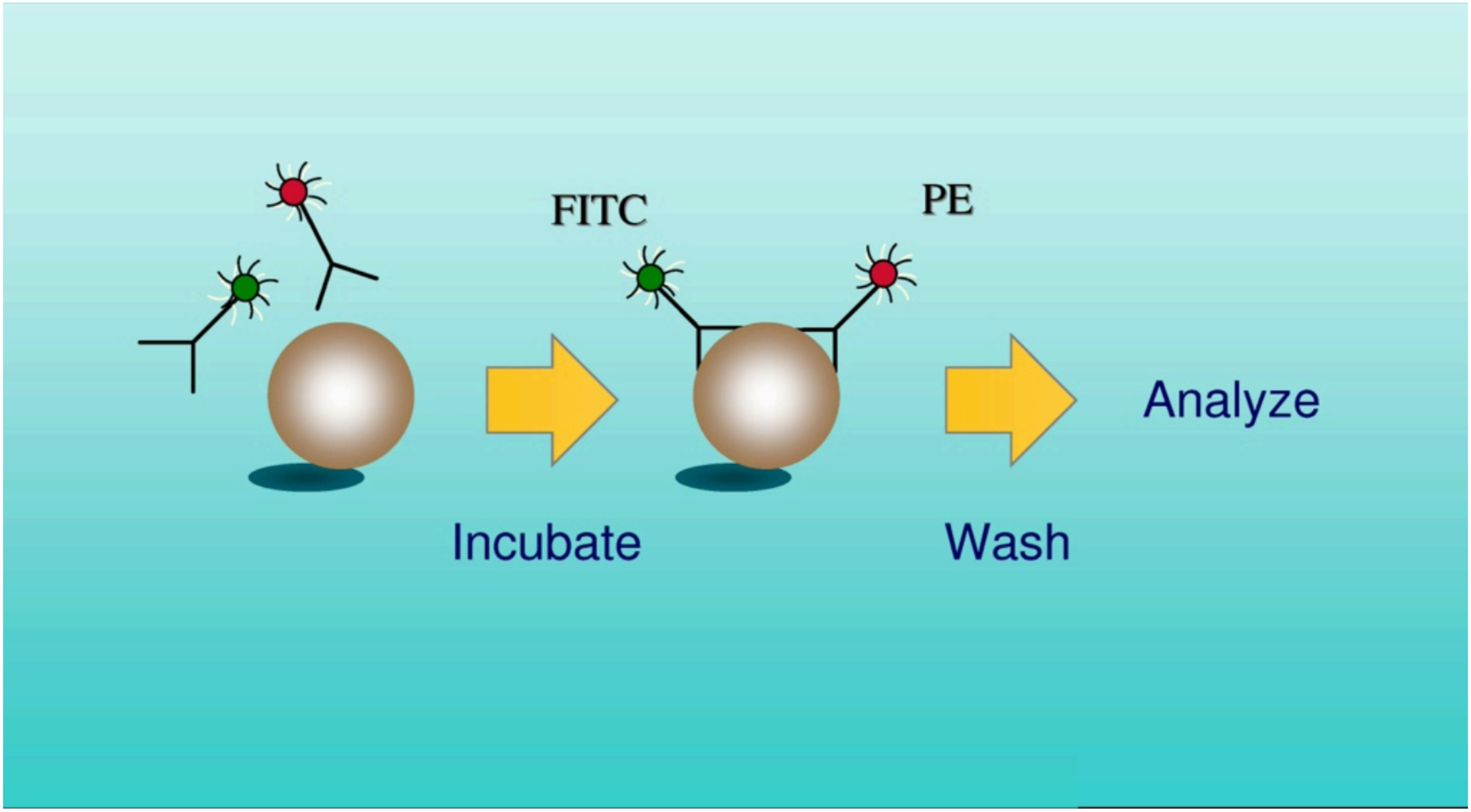
2 Colour Spectra

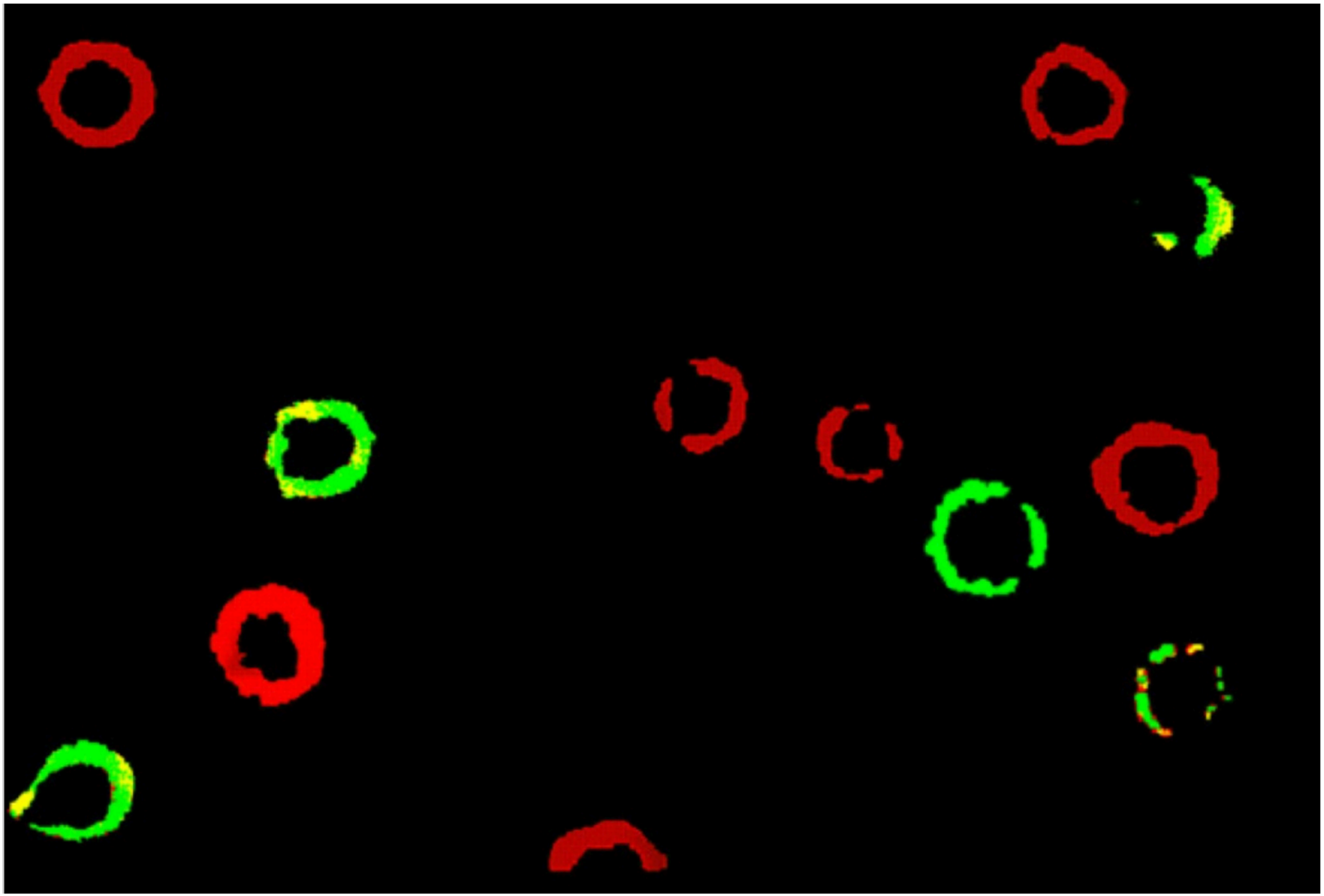


Fluorescein (FITC) can be excited using a 488 nm BLUE Laser with a “peak” **emission** at ~525 nm

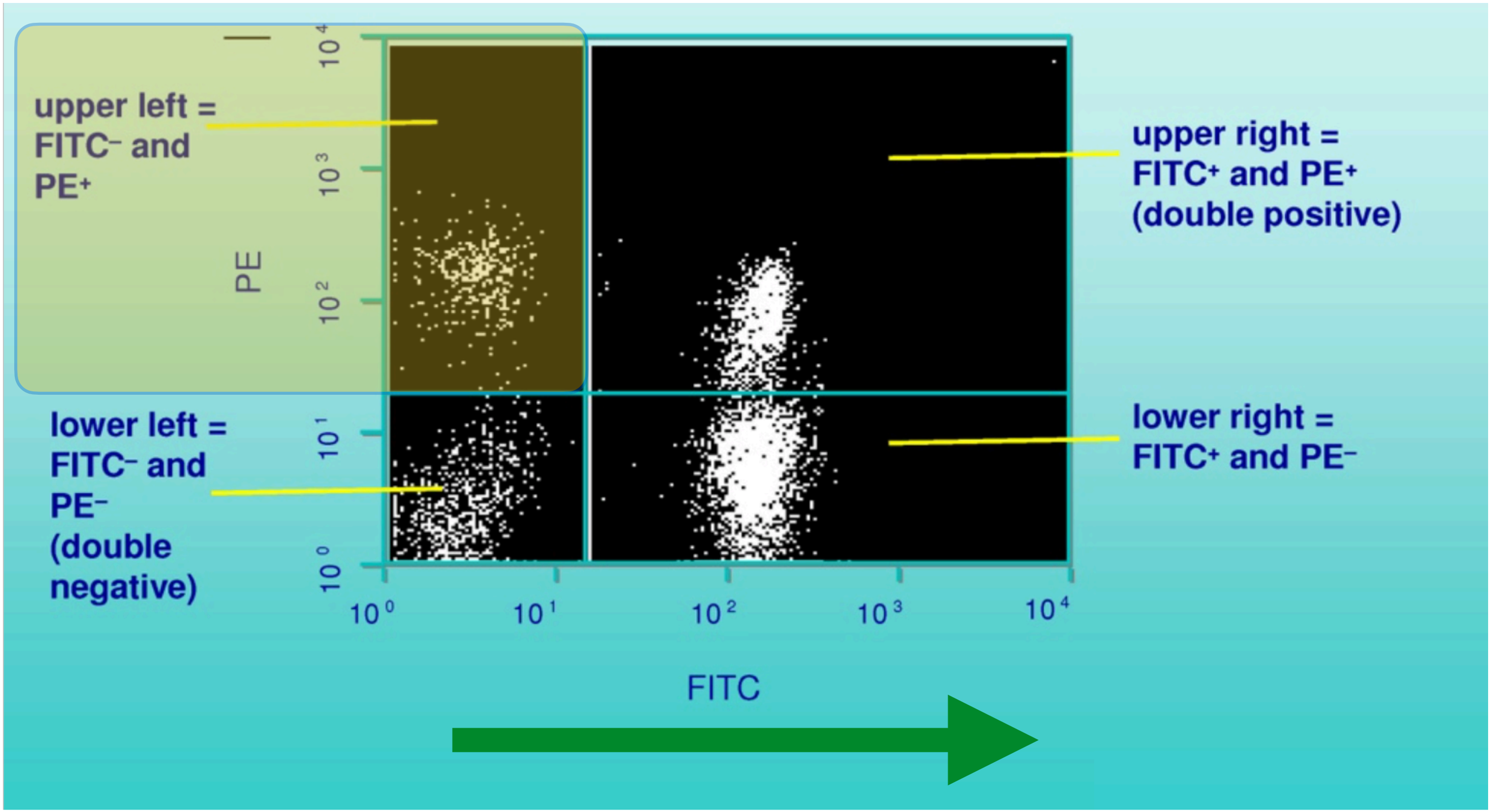
Phycoerythrin (PE) can also be excited using a 488 nm BLUE Laser with a “peak” **emission** at ~578 nm

2 Colour Direct Staining

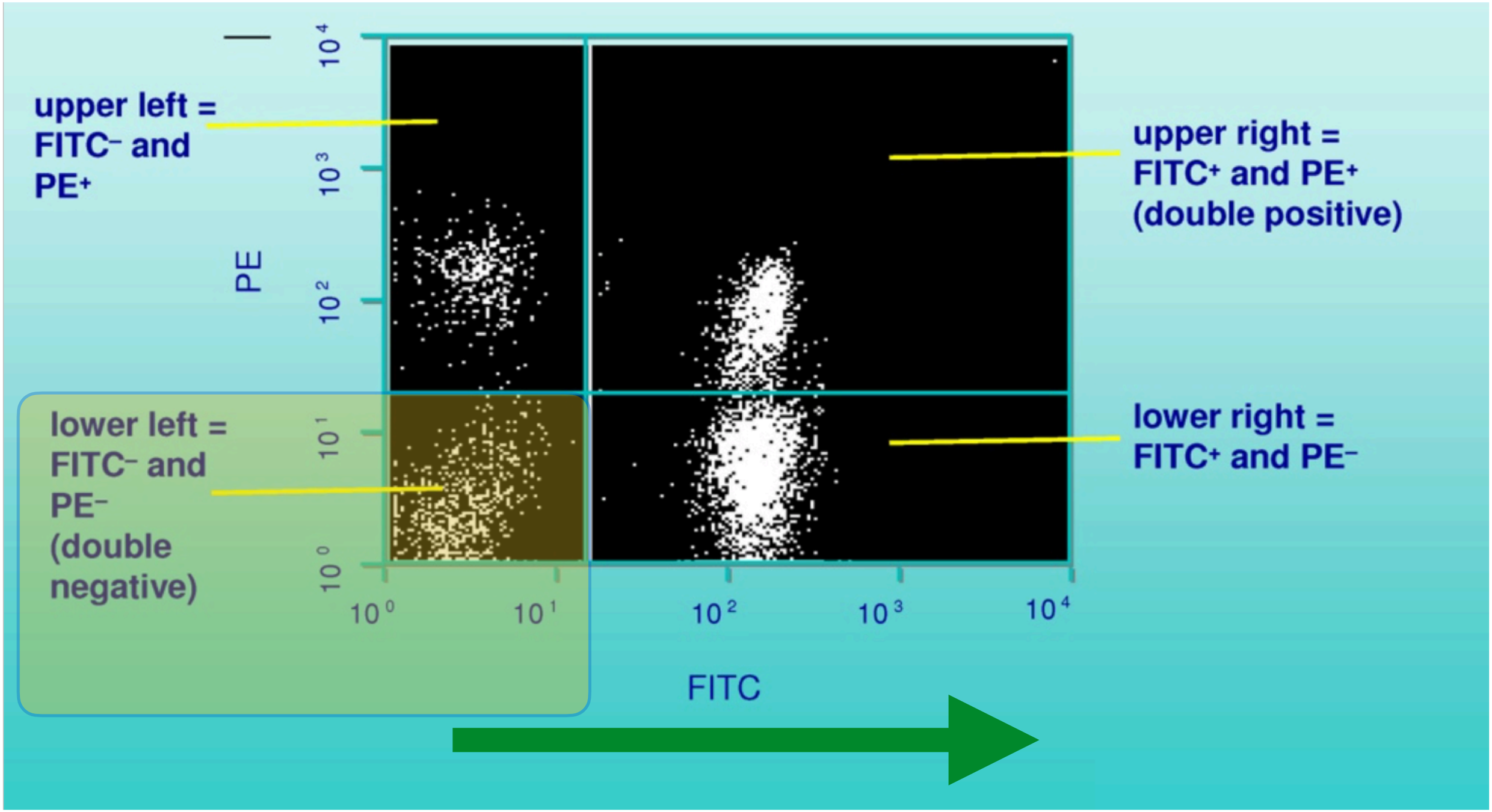




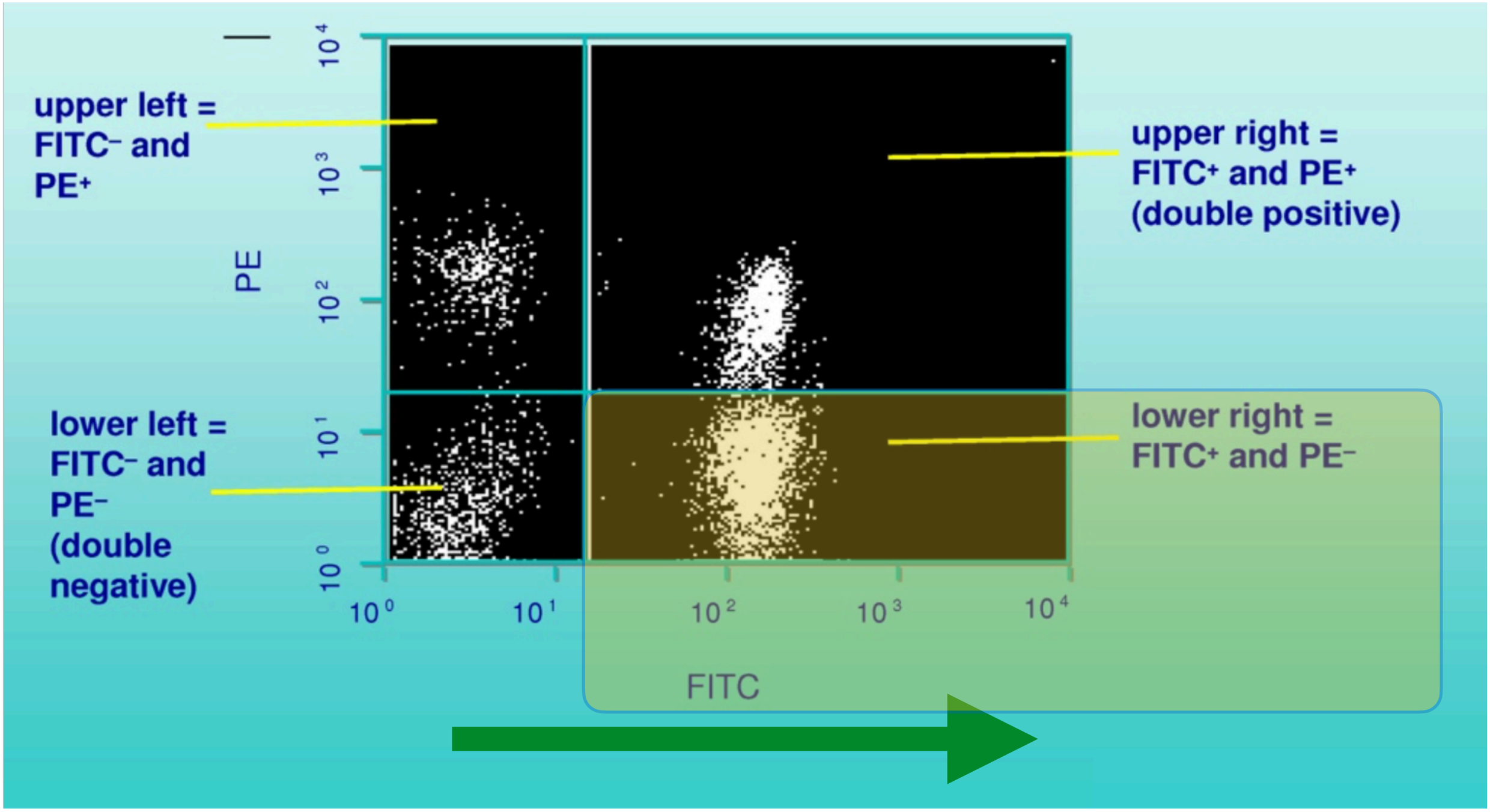
2 Colour Cell Analysis



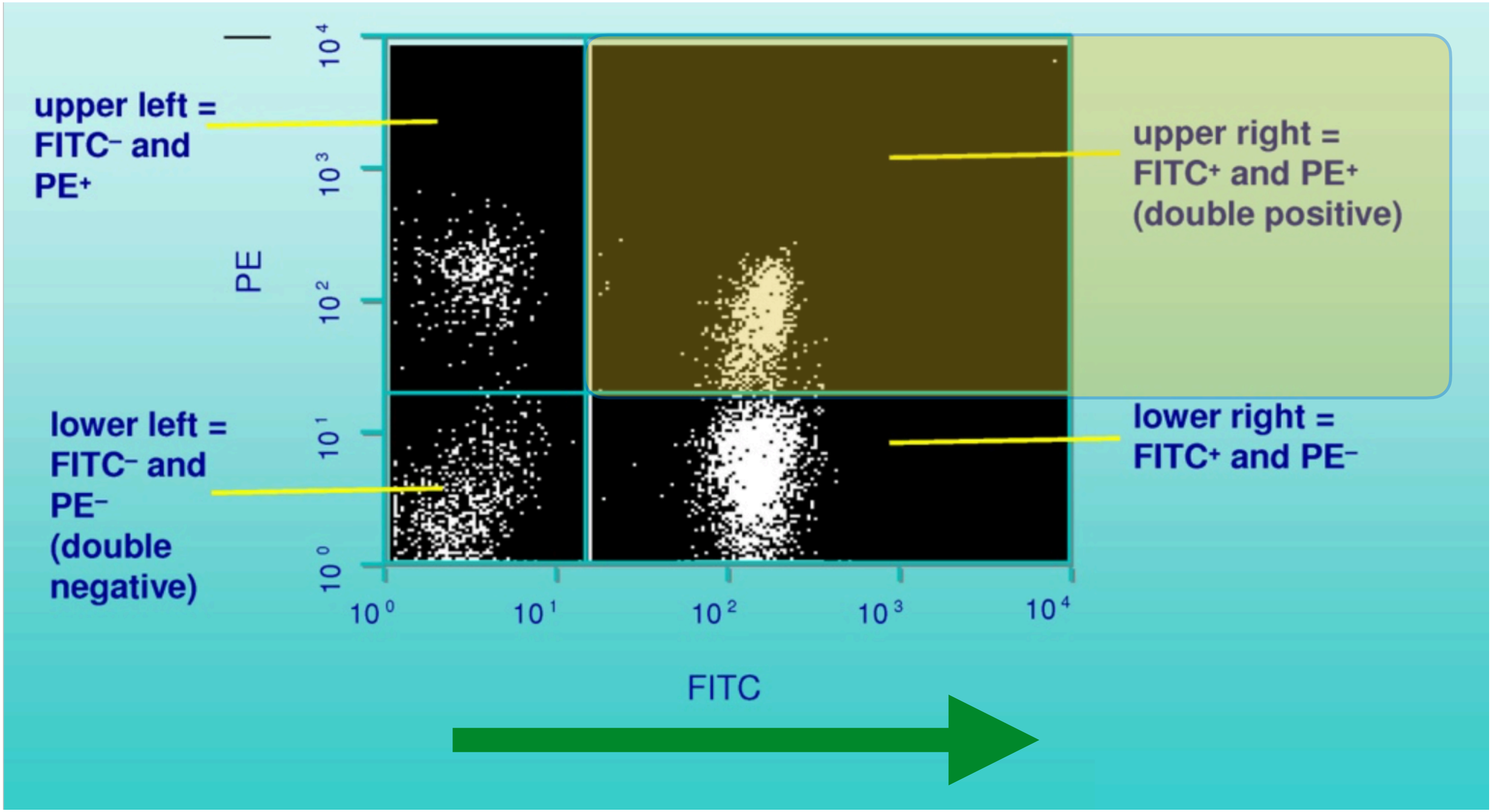
2 Colour Cell Analysis



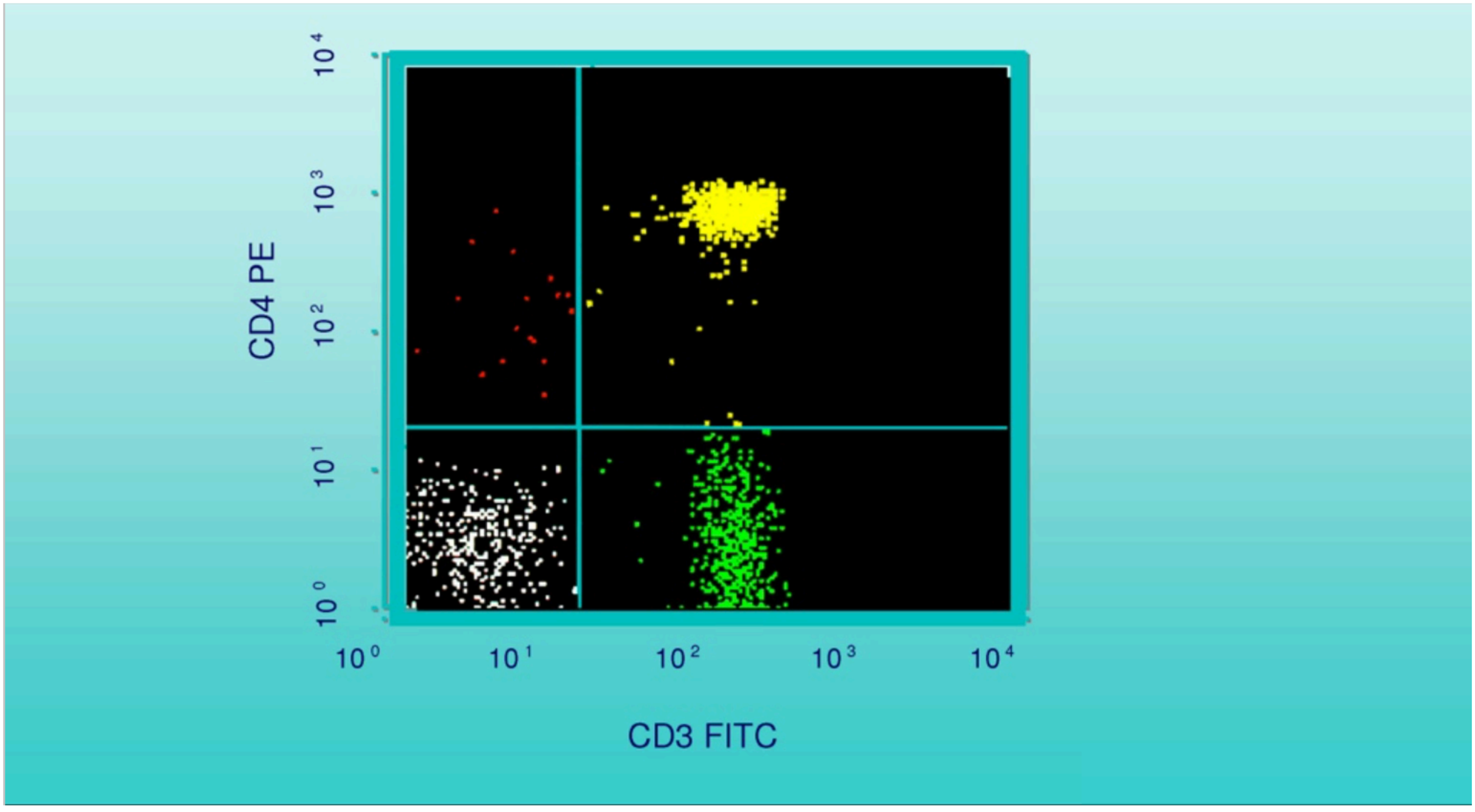
2 Colour Cell Analysis



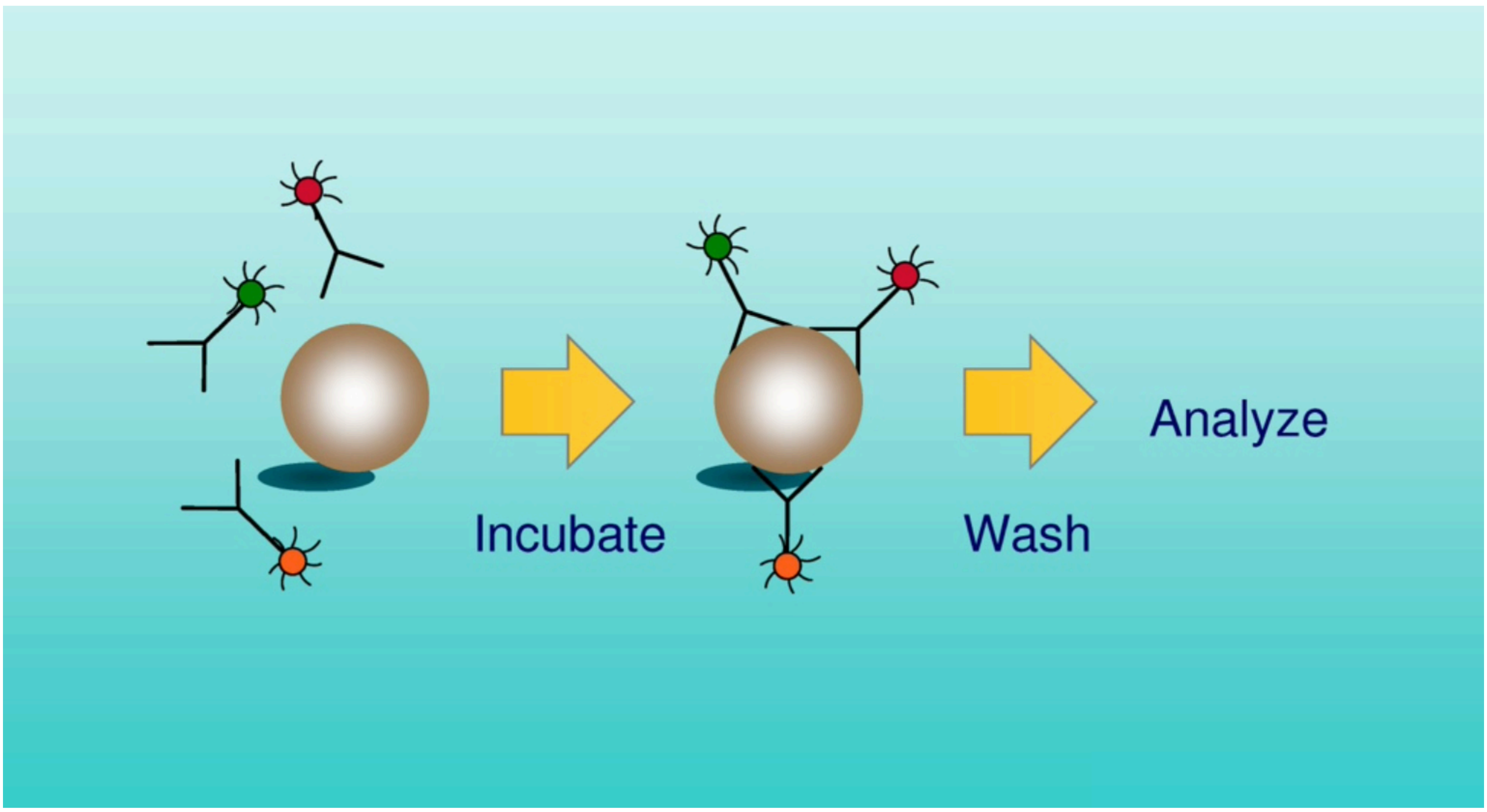
2 Colour Cell Analysis



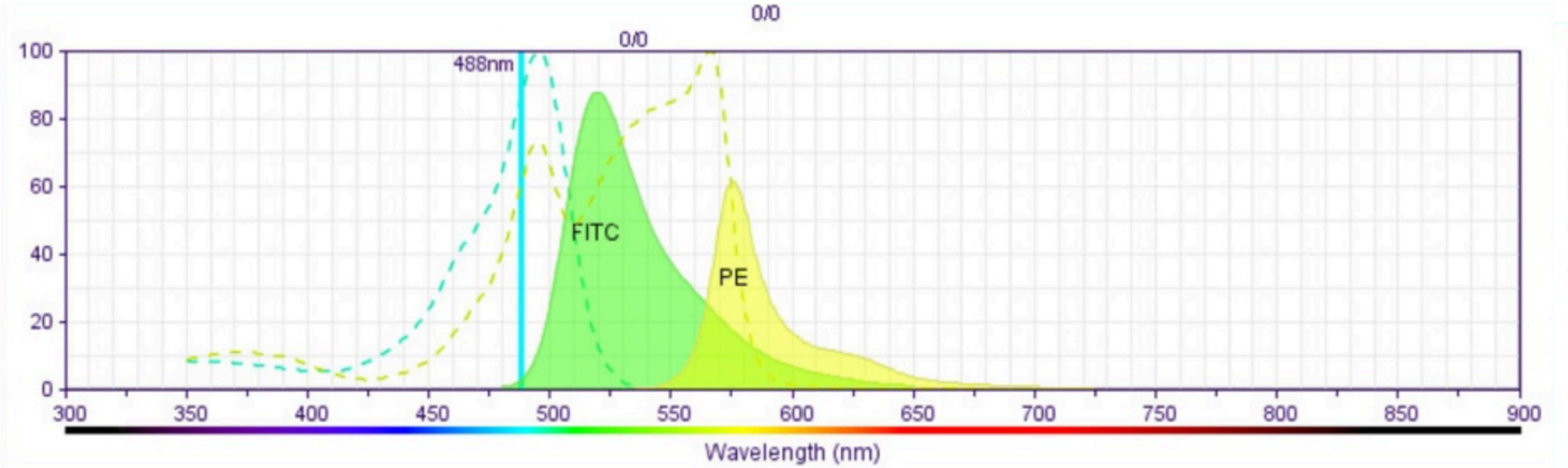
2 Colour Cell Analysis



Examples…


Incubate
Wash
Analyze

3 Colour Spectra

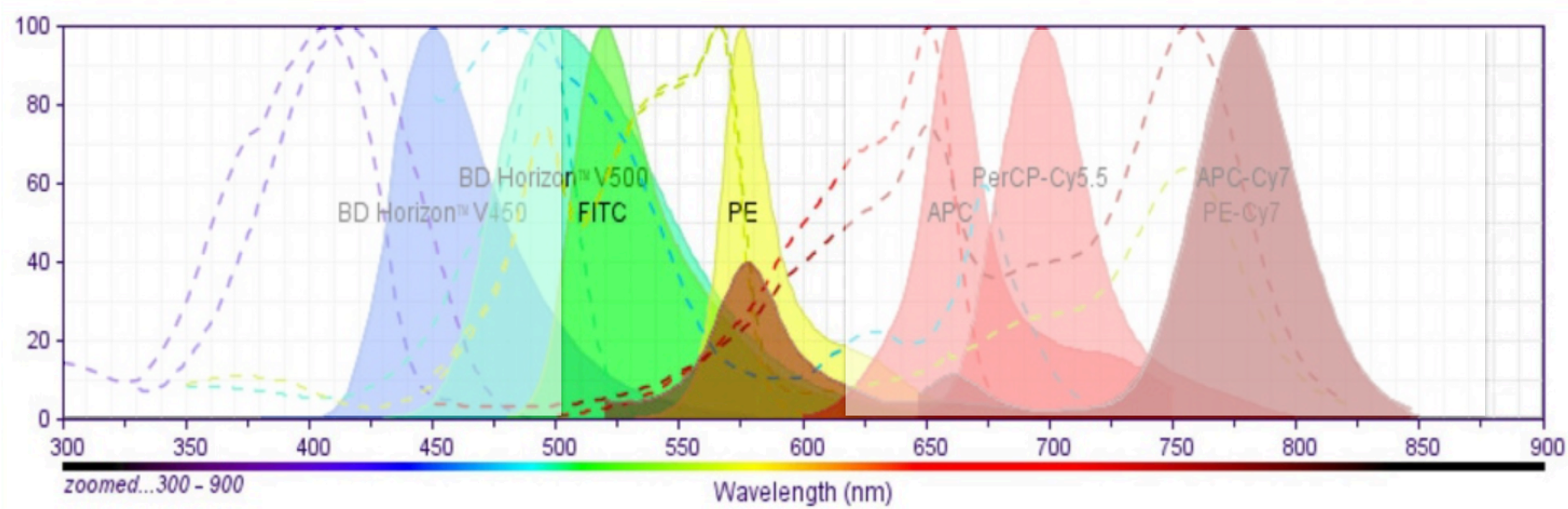


Fluorescein (FITC) can be excited using a 488 nm BLUE Laser with a “peak” **emission** at ~525 nm

Phycoerythrin (PE) can also be excited using a 488 nm BLUE Laser with a “peak” **emission** at ~578 nm

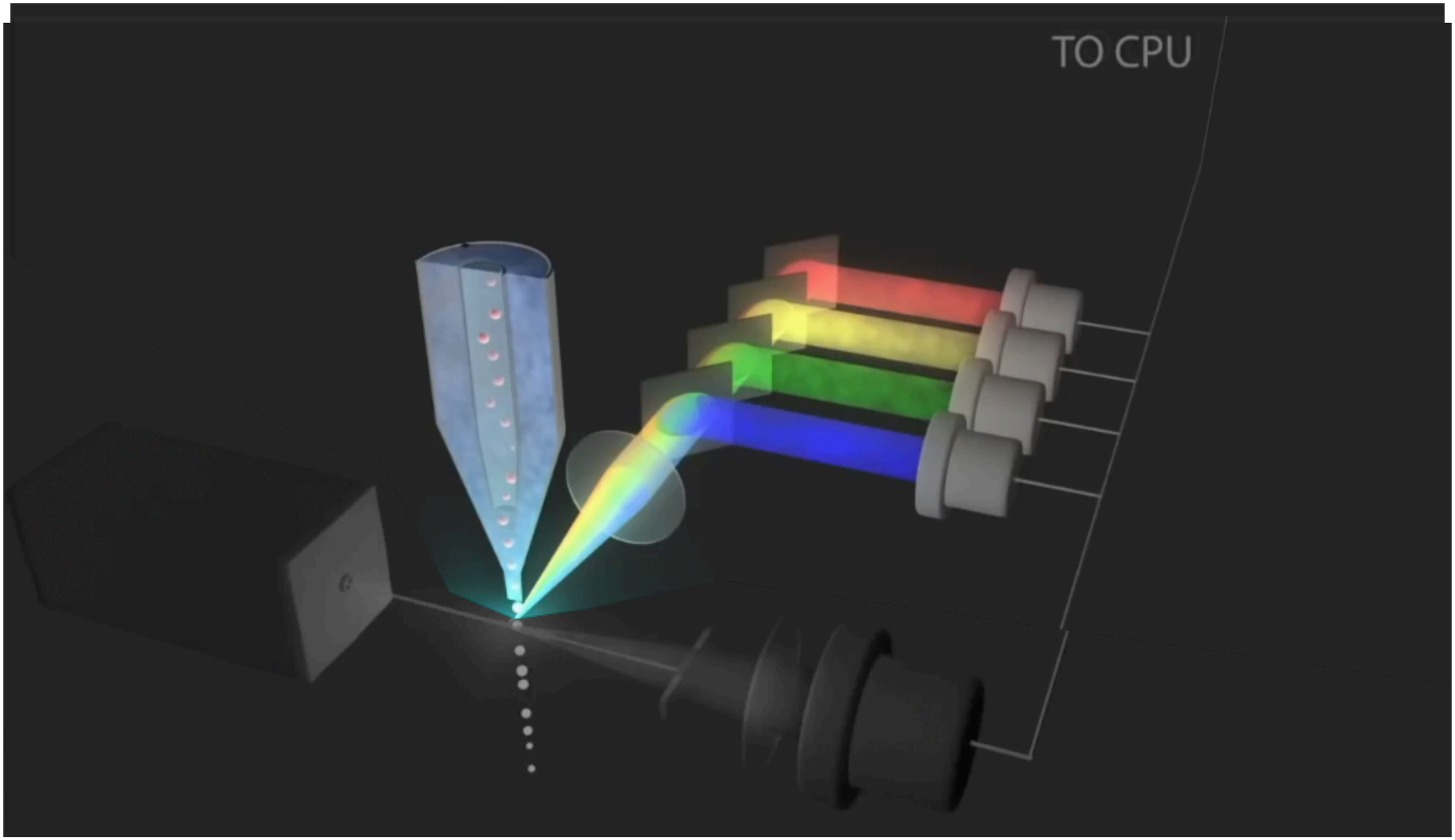
PerCP has a “peak” **emission** ~695 nm

Multiple Colour Spectra

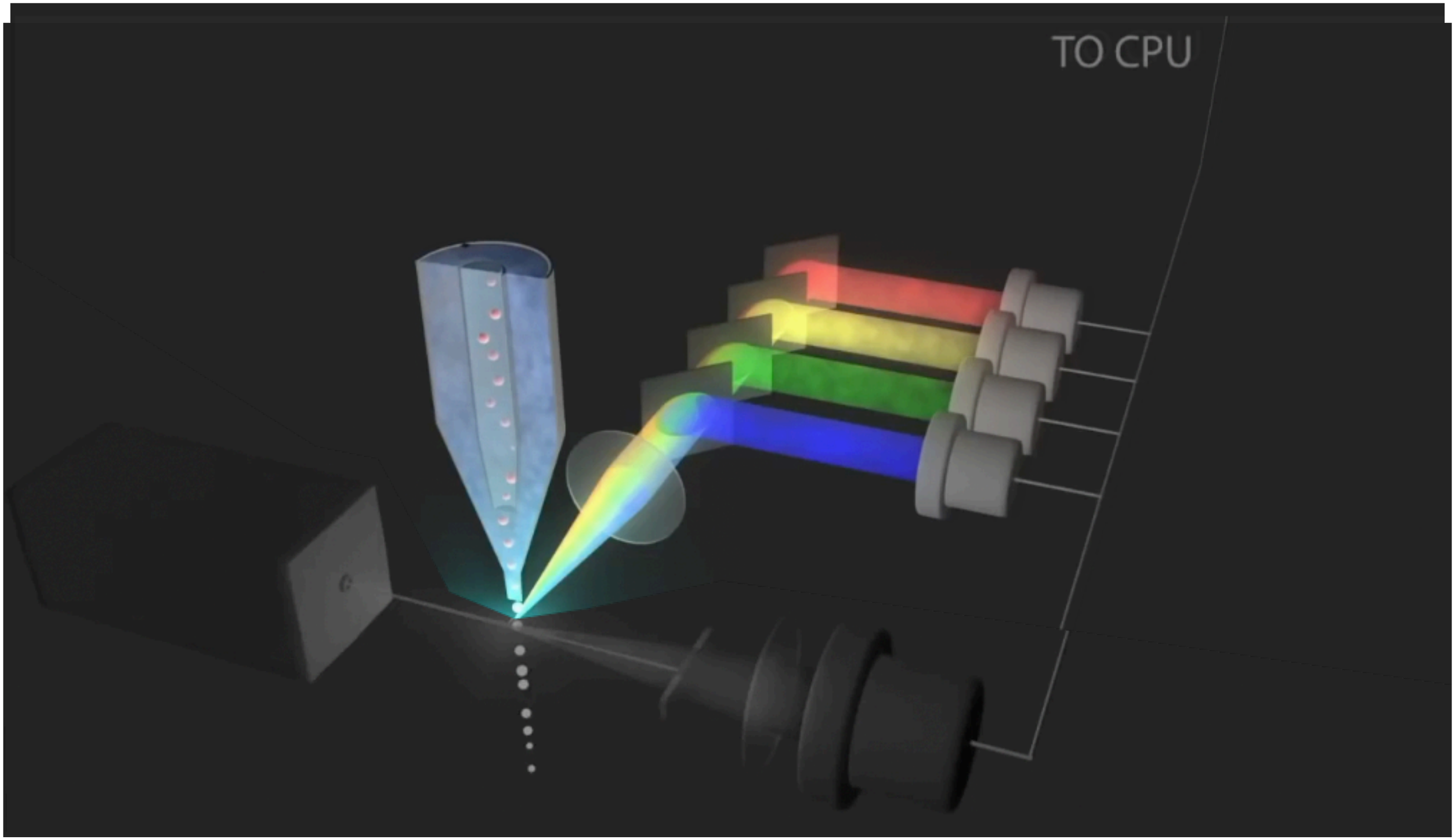


Several fluorescent colours with their **excitation**
and **emission** peaks (dotted lines) derived from different lasers

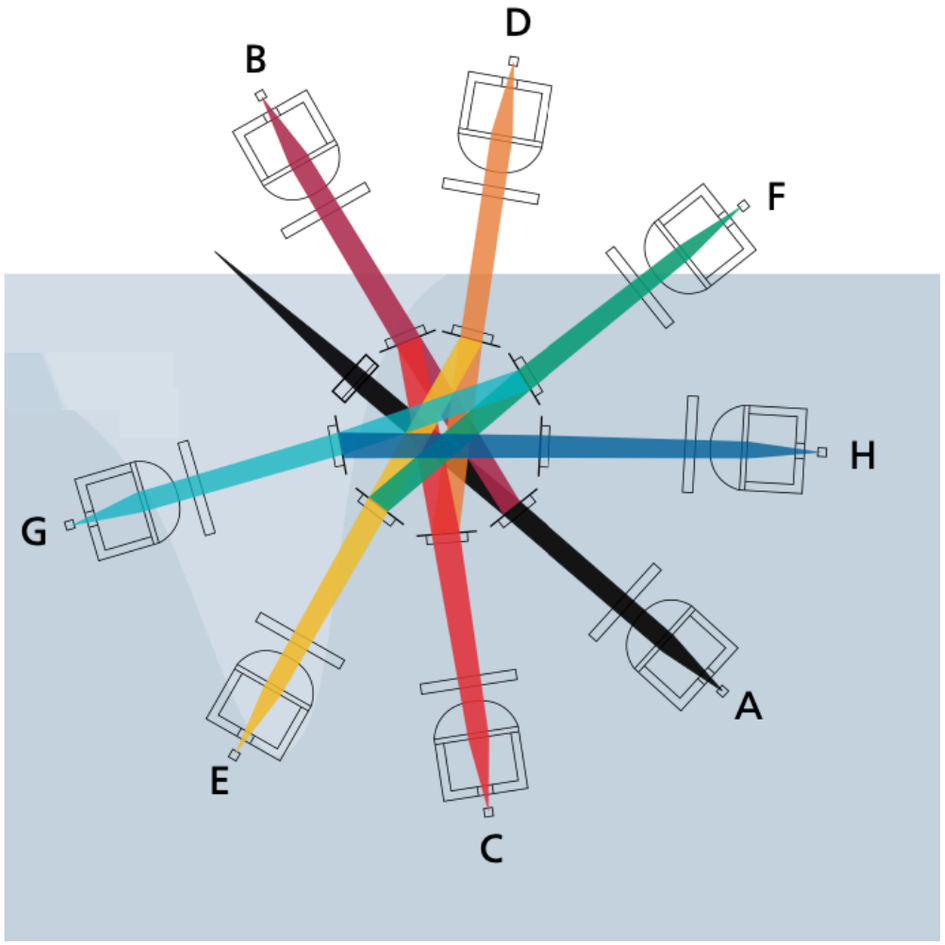
Collection Optics



Collection Optics



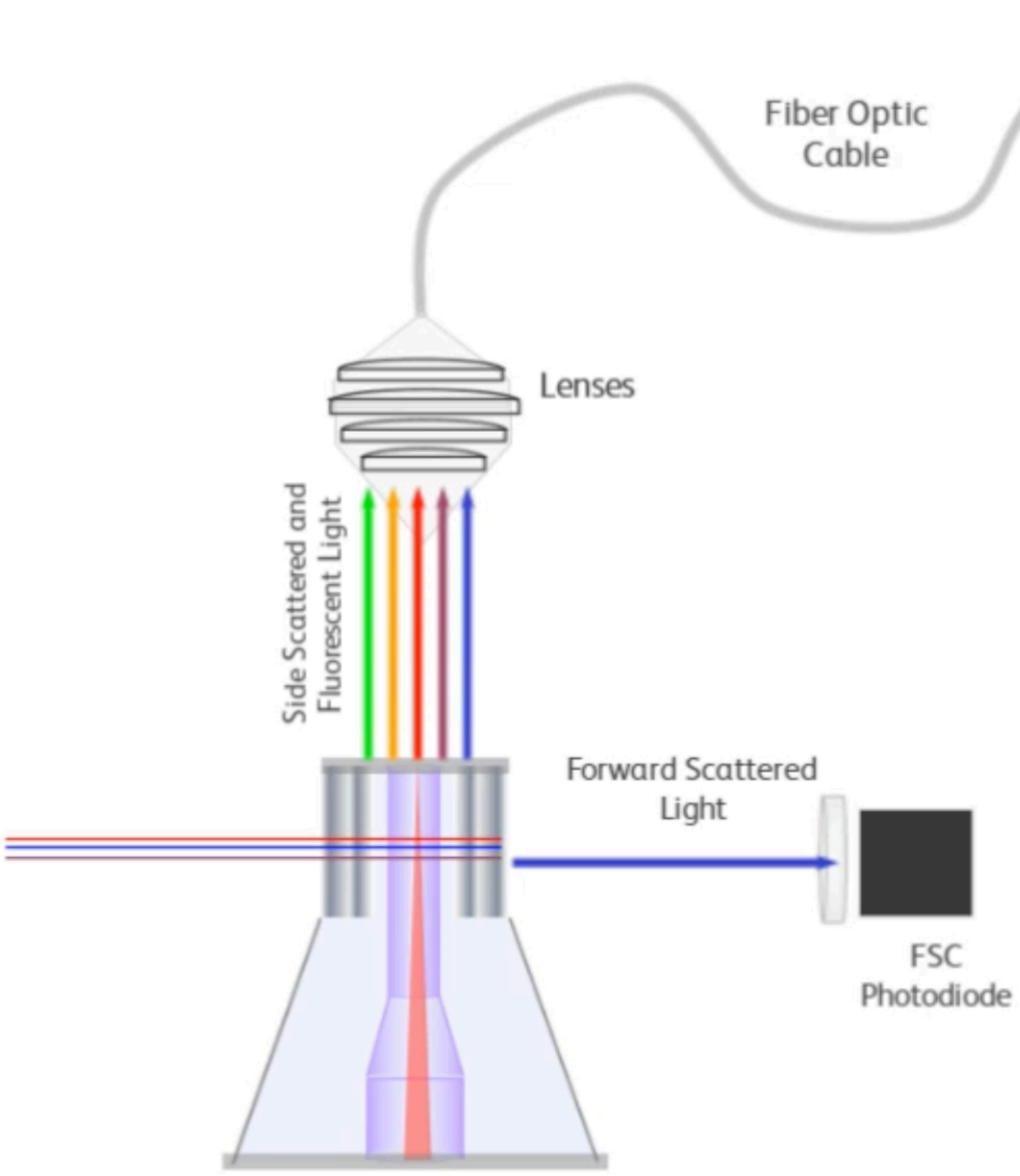
Collection Optics

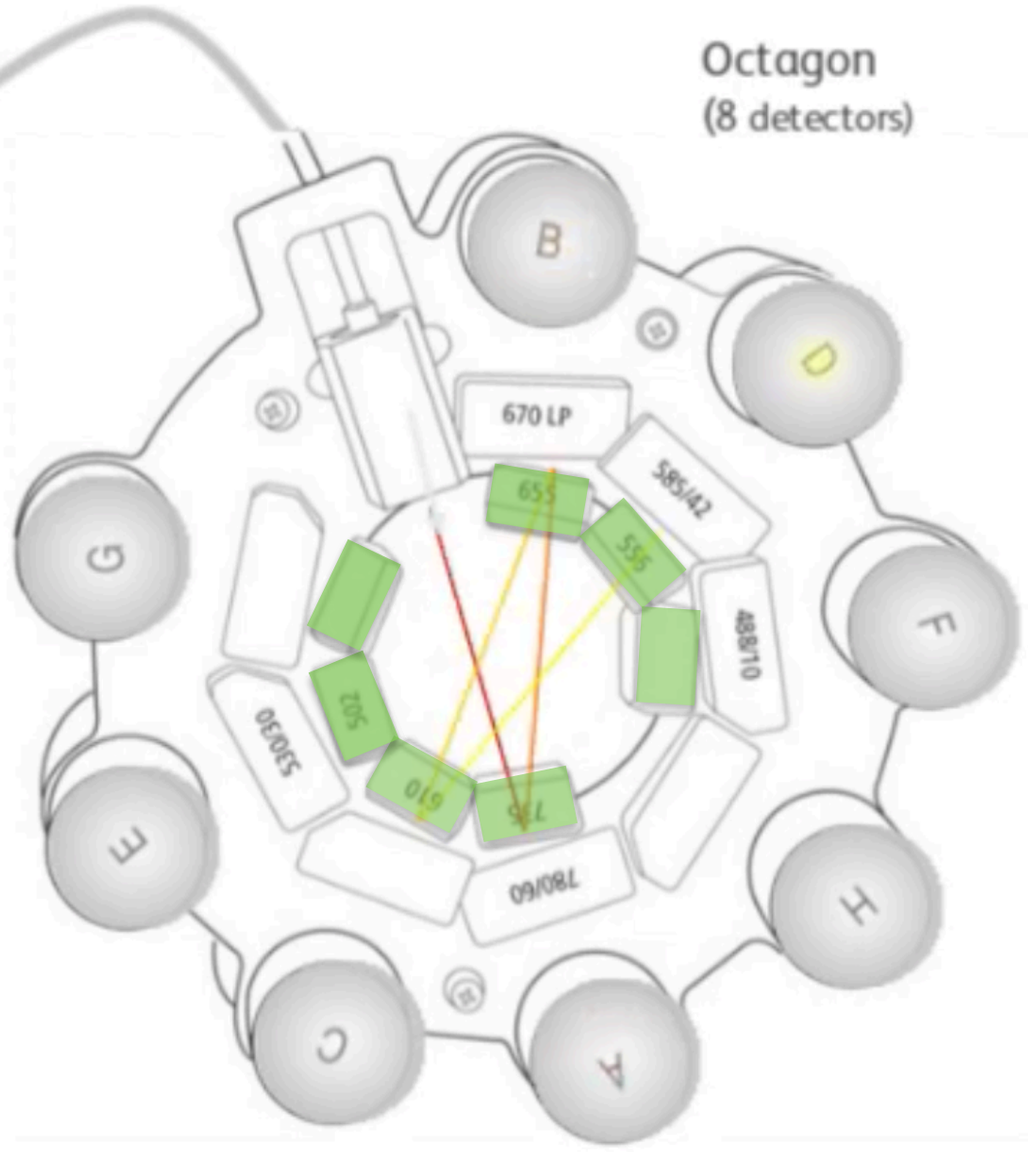


BD LSRFortessa™

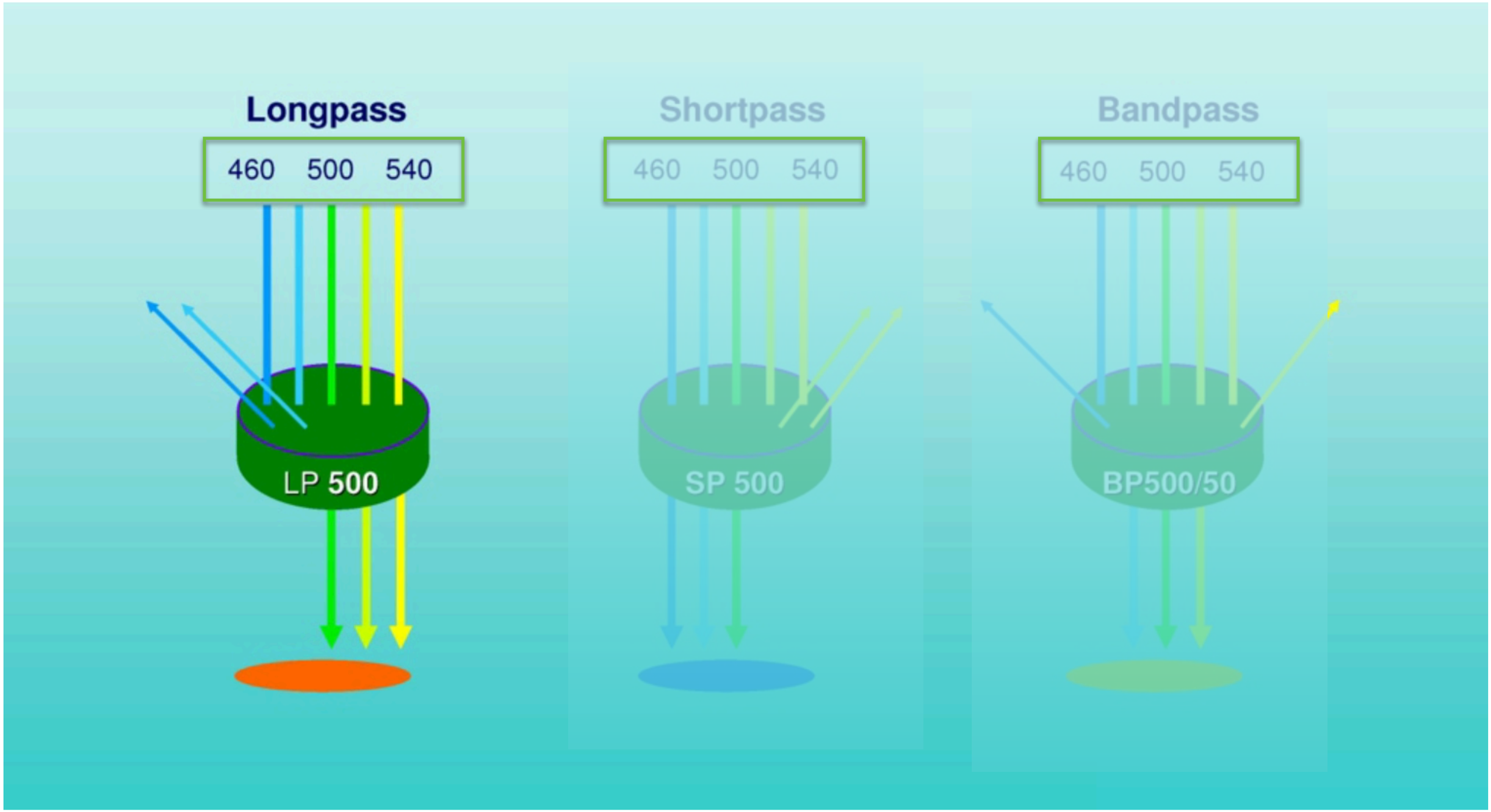
FACSFortessa is configurable and upgradeable with up to 4 lasers to detect up to 18 colors simultaneously.

Collection Optics… Octagonal Arrangement

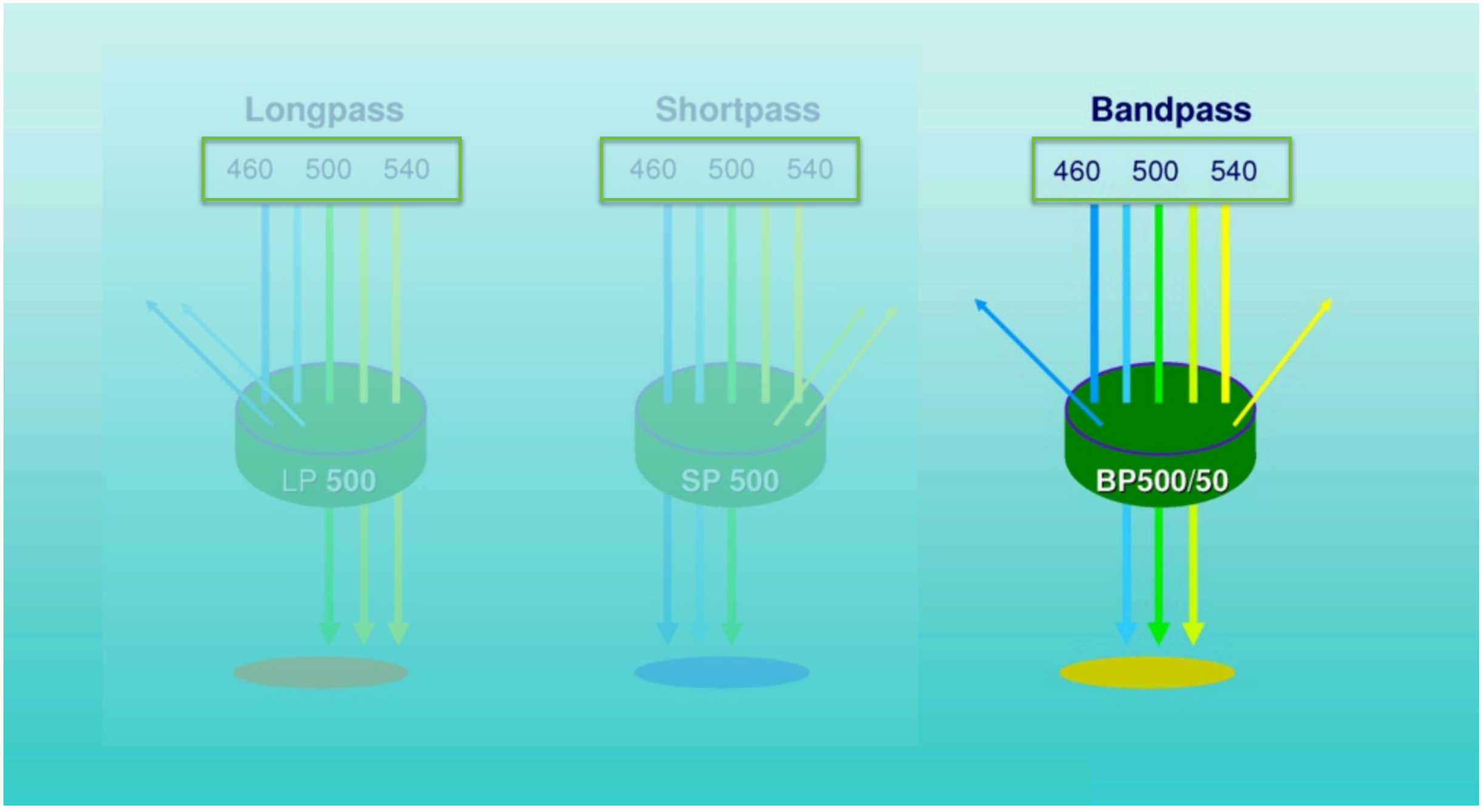




Optical Filters

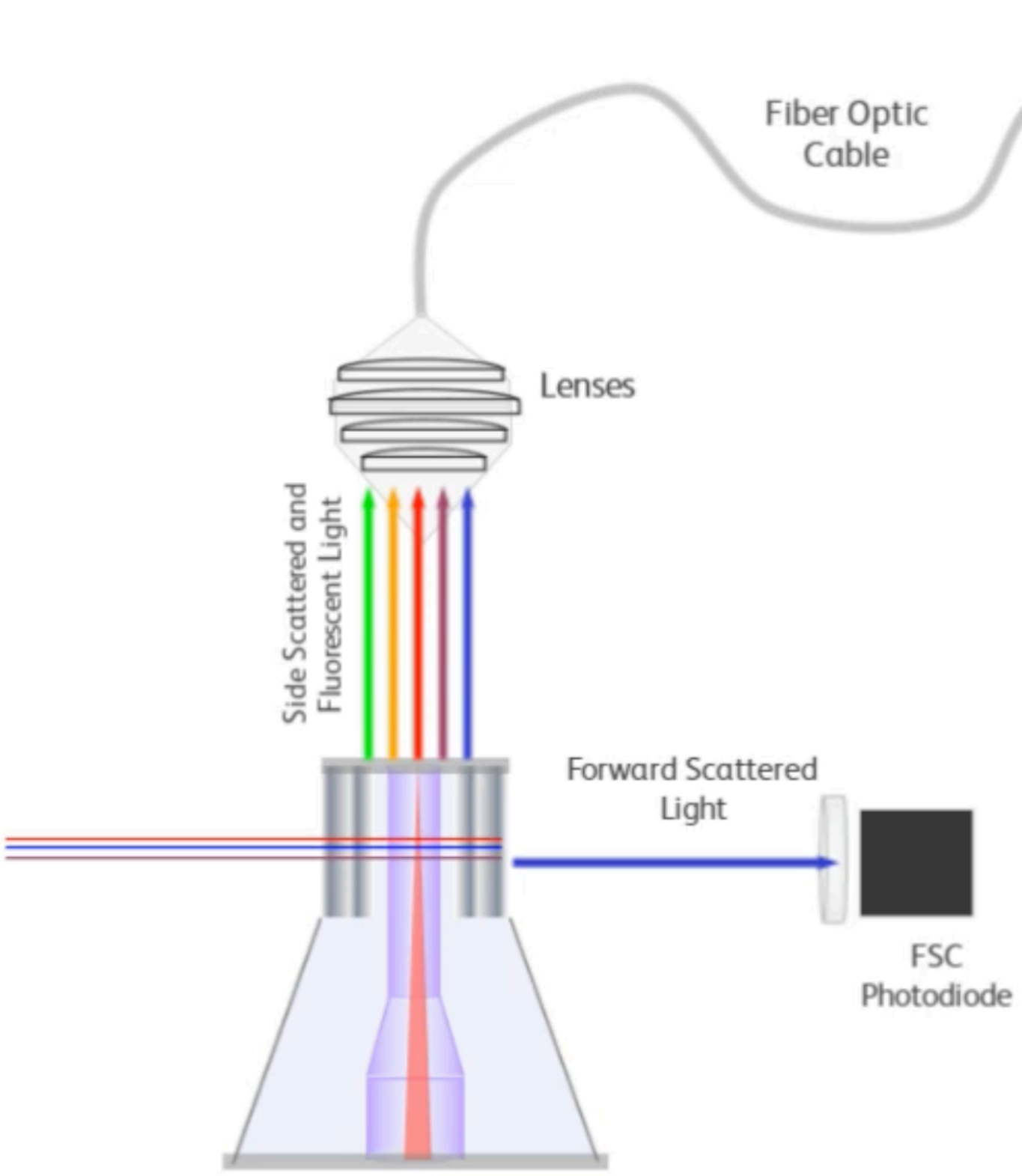


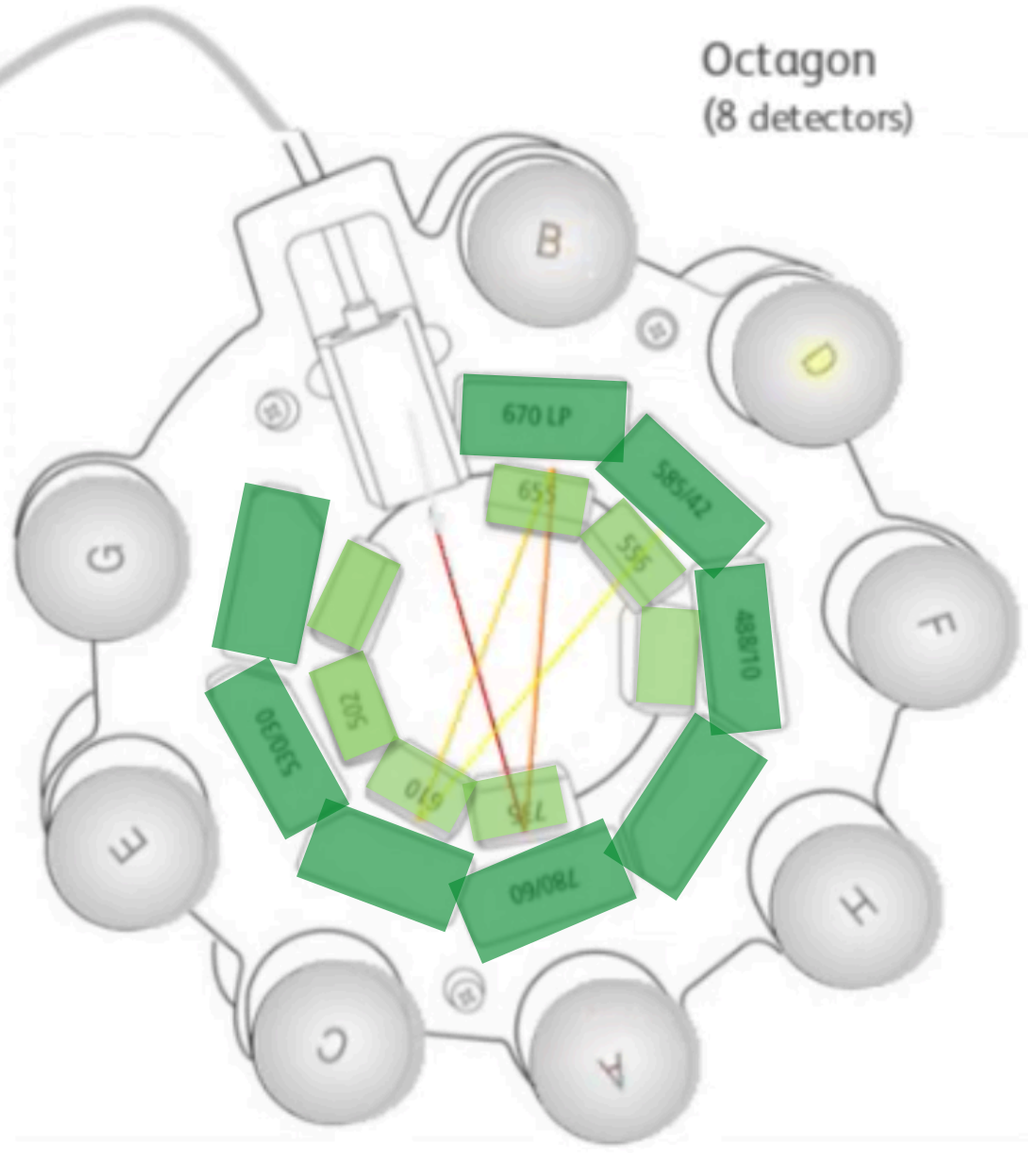
Optical Filters



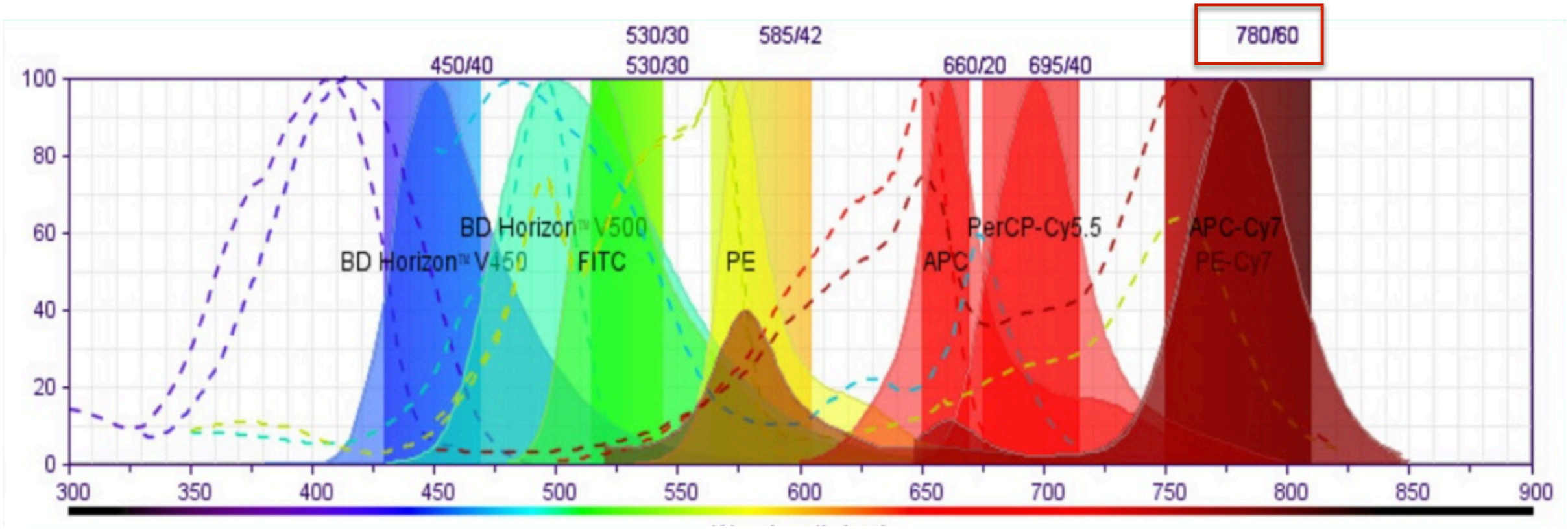
Note: most of the filters that are used here are
“Bandpass” wavelength nm / range

Collection Optics… Octagonal Arrangement





Optical Filters



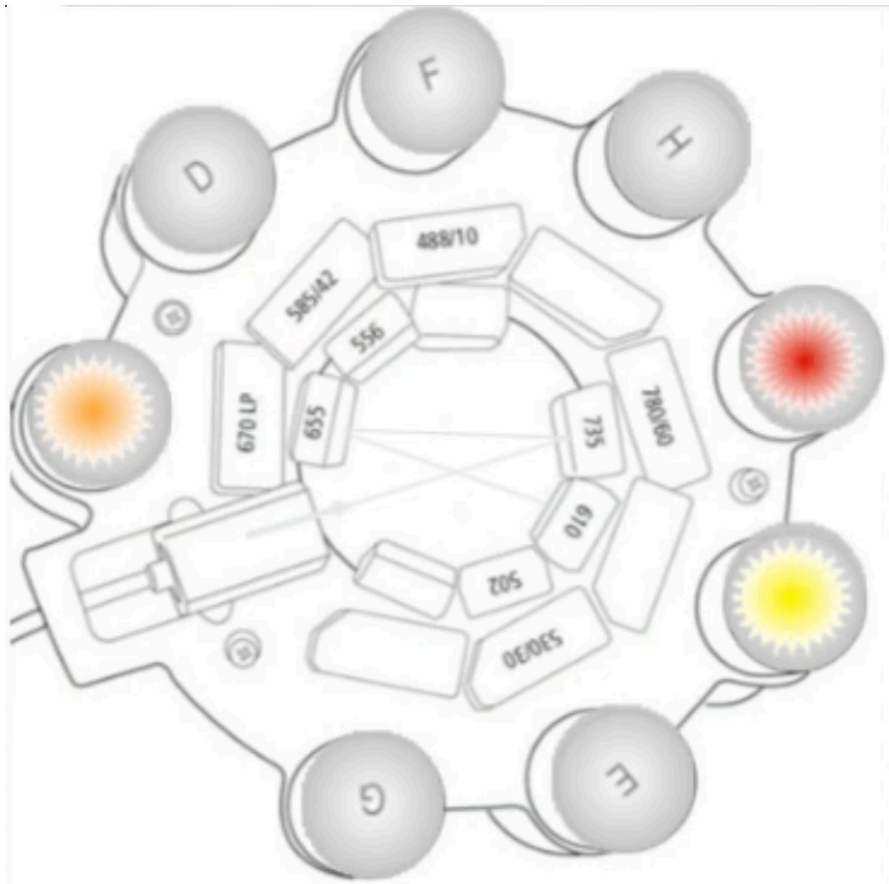
Note: most of the filters that are used here are
“Bandpass” wavelength nm / range
eg. 780 / 60

Fluorescent Protein Properties

Class	Protein	Excitation (nm)		Emission (nm)		Fluorescence Quantum Yield			Extinction Coefficient ($M^{-1}cm^{-1}$)			Brightness (x 10^{-3} $M^{-1}cm^{-1}$)		pK_a			Reference
		Literature	Our Data	Literature	Our Data	Literature	Our Data	s.d.	Literature	Our Data	s.d.	Literature	Our Data	Literature	Our Data	s.d.	
Blue	EBFP2	383	386	448	448	0.56	0.53	0.01	32,000	39,000	725	17.92	20.67	5.3	4.4	0.07	43
	mTagBFP2	399	400	454	454	0.64	0.48	0.01	50,600	76,000	4,000	32.38	36.48	2.7	2.4	0.02	44
Cyan	mTurquoise	434	434	474	474	0.84	0.84	0.02	34,000	31,000	400	28.56	26.04	4.5	3.5	0.02	45
	mTurquoise2	434	434	474	473	0.93	0.92	0.03	30,000	31,000	300	27.90	28.52	3.1	3.6	0.01	46
	mCerulean	434	434	475	475	0.49	0.51	0.02	33,000	28,000	1,100	16.17	14.28	4.5	3.9	0.12	47
	mCerulean3	433	433	475	475	0.80	0.80	0.01	40,000	29,000	730	32.00	23.20	3.2	3.4	0.01	48
	mTFP1	462	467	492	492	0.85	0.85	0.02	64,000	53,000	1,000	54.40	45.05	4.3	4.3	0.12	49
UV-Excitable Green	mT-Sapphire	399	396	511	509	0.60	0.59	0.00	44,000	34,000	1,100	26.40	20.06	4.9	4.8	0.05	50
Green	EGFP	488	488	507	508	0.60	0.67	0.02	56,000	56,000	1,300	33.60	37.52	6.0	6.1	0.25	12
	mEGFP	NA	489	NA	508	NA	0.74	0.01	NA	62,000	1,550	0.001	45.88	6.0	5.8	0.14	17
	Emerald	484	483	509	509	0.68	0.75	0.01	57,500	62,000	1,150	39.10	46.50	6.0	4.6	0.02	51
	mEmerald	NA	483	NA	510	NA	0.79	0.01	NA	62,000	1,500	0.001	48.98	6.0	4.7	0.16	17
	sfGFP	485	487	507	509	0.65	0.72	0.01	83,300	53,000	1,750	54.15	38.16	5.5	5.8	0.09	14
Yellow-Green	mPapaya	NA	528	NA	540	NA	0.74	0.02	NA	62,000	1,600	0.00	45.88	NA	6.6	0.02	6
	YPet	517	517	530	527	0.77	0.76	0.01	104,000	132,000	1,950	80.08	100.32	5.6	5.5	0.01	52
	Citrine	516	515	529	526	0.76	0.70	0.01	77,000	117,000	2,000	58.52	81.90	5.7	5.4	0.08	15
	mCitrine	NA	515	NA	528	NA	0.74	0.01	NA	120,000	2,600	0.001	88.80	5.7	5.6	0.13	17
	Venus	515	515	527	526	0.63	0.65	0.01	110,000	126,000	2,000	69.30	81.90	6.0	5.6	0.05	36
	mVenus	515	515	527	528	0.64	0.67	0.01	105,000	127,000	3,750	67.20	85.09	6.0	5.4	0.08	36
	Topaz	514	515	527	527	0.57	0.71	0.02	94,500	113,000	4,000	53.87	80.23	NA	6.3	0.12	51
	mTopaz	NA	515	NA	527	NA	0.68	0.02	NA	108,000	1,900	0.001	73.44	NA	5.9	0.16	17
	Clover	505	505	515	517	0.76	0.88	0.02	111,000	105,000	2,500	84.36	92.40	6.2	5.9	0.08	53
	mClover	NA	505	NA	516	NA	0.84	0.01	NA	105,000	1,800	0.001	88.20	NA	5.9	0.06	53
	mNeonGreen	506	504	517	517	0.80	0.80	0.01	116,000	113,000	1,900	92.80	90.40	5.7	5.4	0.01	54
Orange	mOrange	548	548	562	563	0.69	0.64	0.02	71,000	112,000	7,750	48.99	71.68	6.5	6.3	0.10	6
	mOrange2	549	550	565	564	0.60	0.56	0.02	58,000	73,000	800	34.80	40.88	6.5	6.5	0.14	18
	mKO	548	547	559	560	0.60	0.77	0.02	51,600	134,000	4,700	30.96	103.18	5.0	4.9	0.15	55
	mKO2	551	551	565	565	0.57	0.71	0.02	63,800	105,000	3,100	36.37	74.55	5.5	5.5	0.13	56
Orange-Red	tdTomato	554	555	581	581	0.69	0.55	0.02	138,000	92,000	7,400	95.22	50.60	4.7	4.5	0.05	6
	TagRFP	555	556	584	581	0.48	0.33	0.02	100,000	130,000	4,100	48.00	42.90	3.1	3.0	0.15	57
	TagRFP-T	555	557	584	583	0.41	0.32	0.01	81,000	106,000	6,000	33.21	33.92	4.6	4.3	0.12	18
	DsRed2	563	561	582	583	0.55	0.53	0.02	43,800	77,000	690	24.09	40.81	NA	4.2	0.12	4, 58
Red	mRuby	558	558	605	587	0.35	0.38	0.01	112,000	109,000	1,800	39.20	41.42	4.4	4.4	0.05	59
	mRuby2	559	559	600	590	0.38	0.37	0.01	113,000	107,000	2,800	42.94	39.59	5.3	4.4	0.05	53
	mApple	568	569	592	591	0.49	0.46	0.02	75,000	75,000	1,000	36.75	34.50	6.5	6.5	0.09	18
	mRFP1	584	586	607	609	0.25	0.35	0.01	50,000	55,000	1,500	12.50	19.25	4.5	3.8	0.20	60
	mCherry	587	586	610	610	0.22	0.30	0.01	72,000	85,000	2,000	15.84	25.50	< 4.5	3.8	0.11	6
	FusionRed	580	577	608	604	0.19	0.30	0.01	83,000	85,000	1,800	15.77	25.50	4.6	4.2	0.01	61
Far-Red	mKate2	588	587	633	623	0.40	0.42	0.02	62,500	57,500	600	25.00	24.15	5.4	5.5	0.05	62
	mNeptune	600	599	650	640	0.20	0.23	0.01	57,500	55,000	1,300	11.50	12.65	5.4	5.3	0.04	63
	mCardinal	604	603	659	651	0.19	0.18	0.00	87,000	79,000	1,550	16.53	14.22	NA	5.3	0.12	64
	mPlum	590	588	649	645	0.10	0.13	0.01	41,000	80,000	1,100	4.10	10.40	< 4.5	4.6	0.05	65

Supplementary Table 1. Spectral Properties of Fluorescent Proteins. Data were acquired as described in the Methods. Quantum yield standards used were: 1-aminoanthrcene in cyclohexane (QY = 0.61); fluorescein in 0.1 M NaOH (QY = 0.85); rhodamine B in EtOH (QY = 0.65); cresyl violet in EtOH (QY = 0.54). Also included are the original references for the creation of each variant.

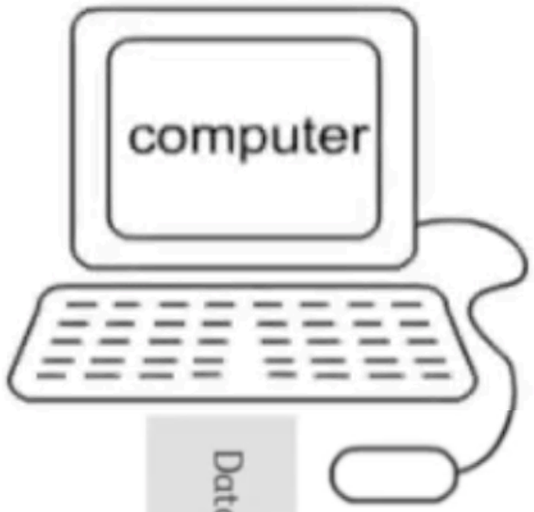
Electronics



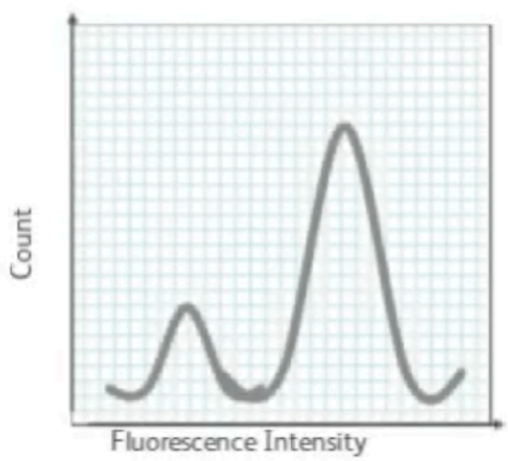
Converts light to electronic signals

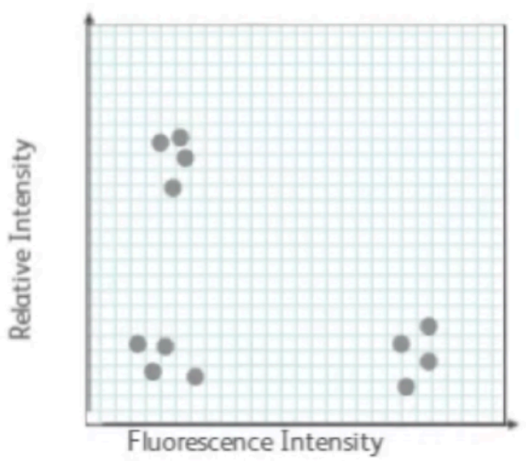
Data		
Event	X	Y
1	33260	120
2	32395	132
3	328	117
4	261853	260226
5	34428	160

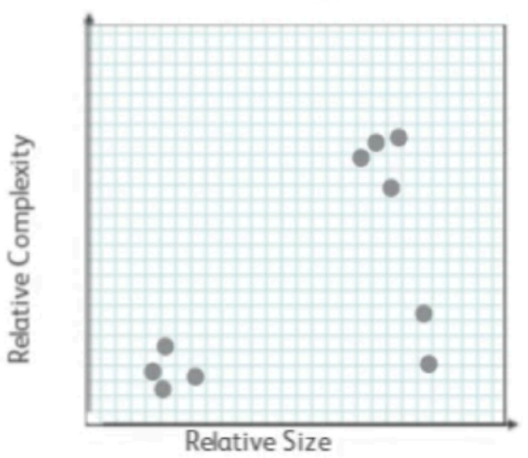
Digitizes data



Data Analysis





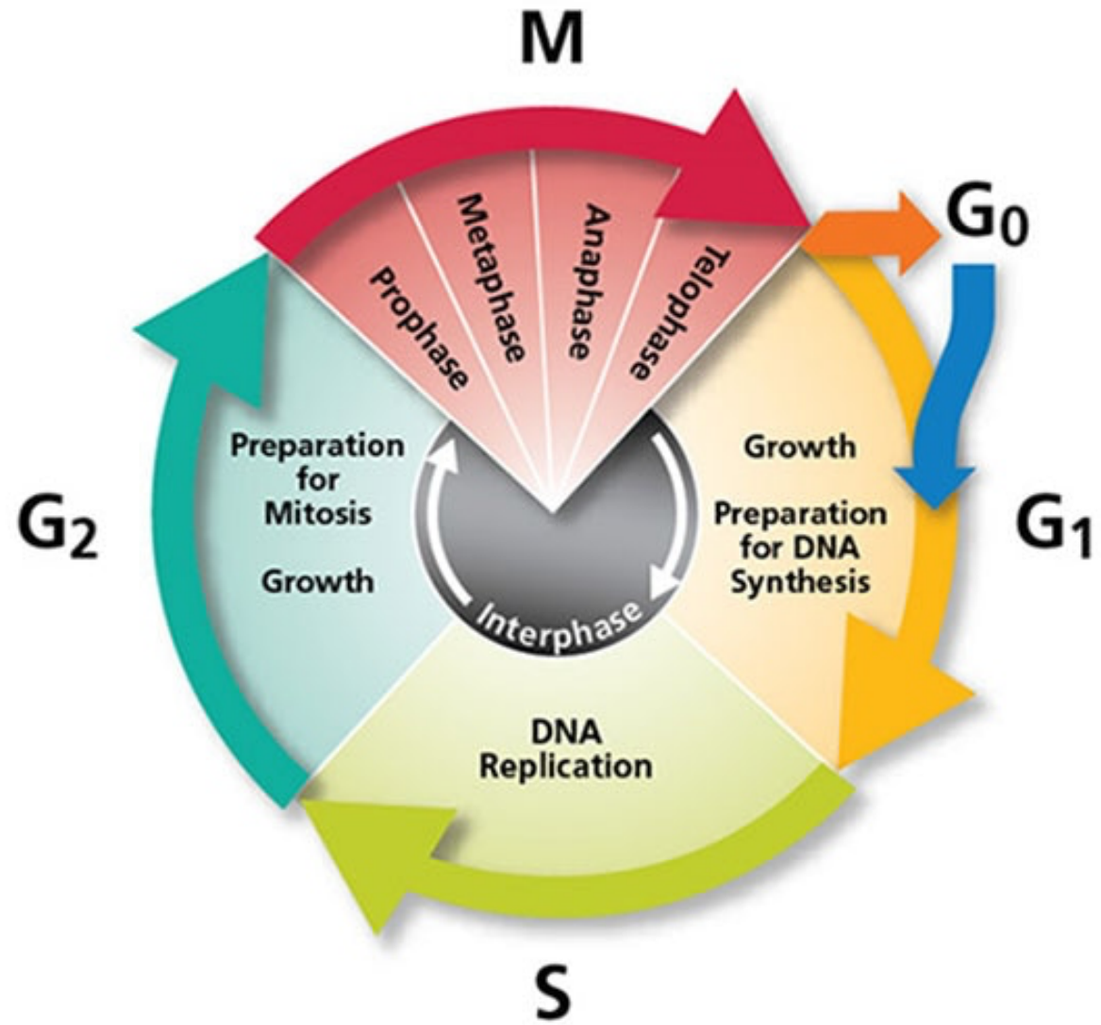


Applications

Cell Cycle Analysis via Flow Cytometry

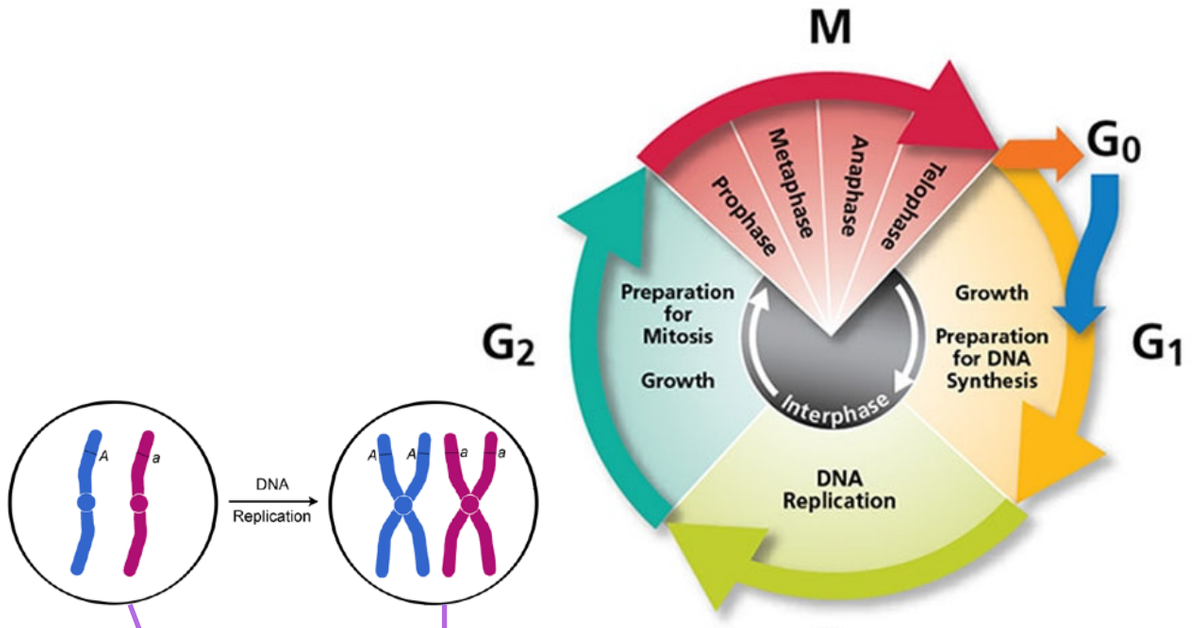
Cell Cycle Compartments

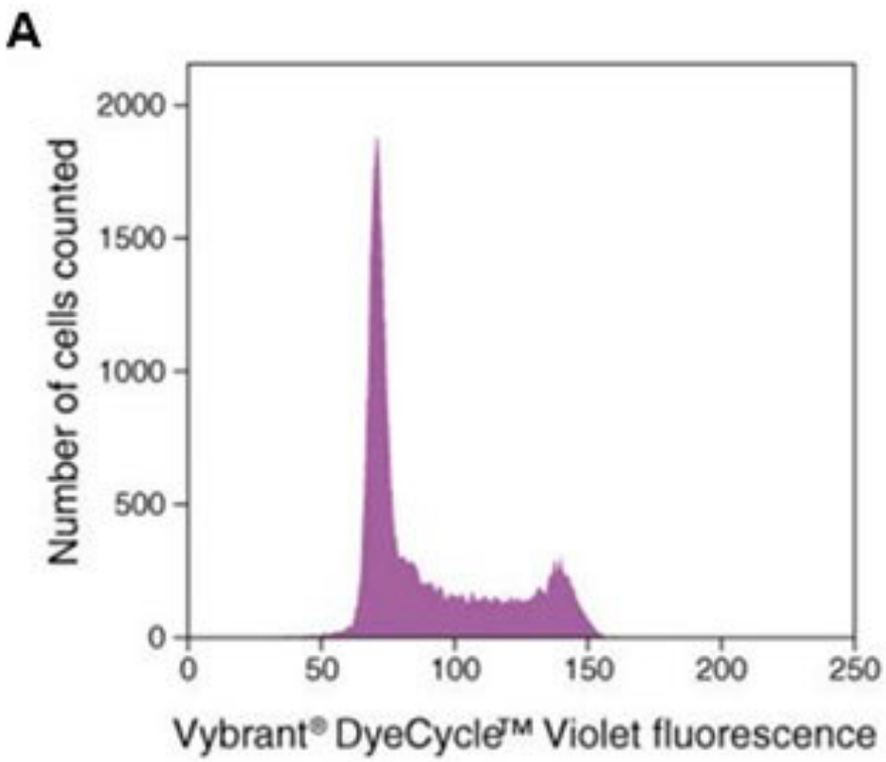
such analysis requires some version of membrane permealization, using alcohol (?) or detergents etc.

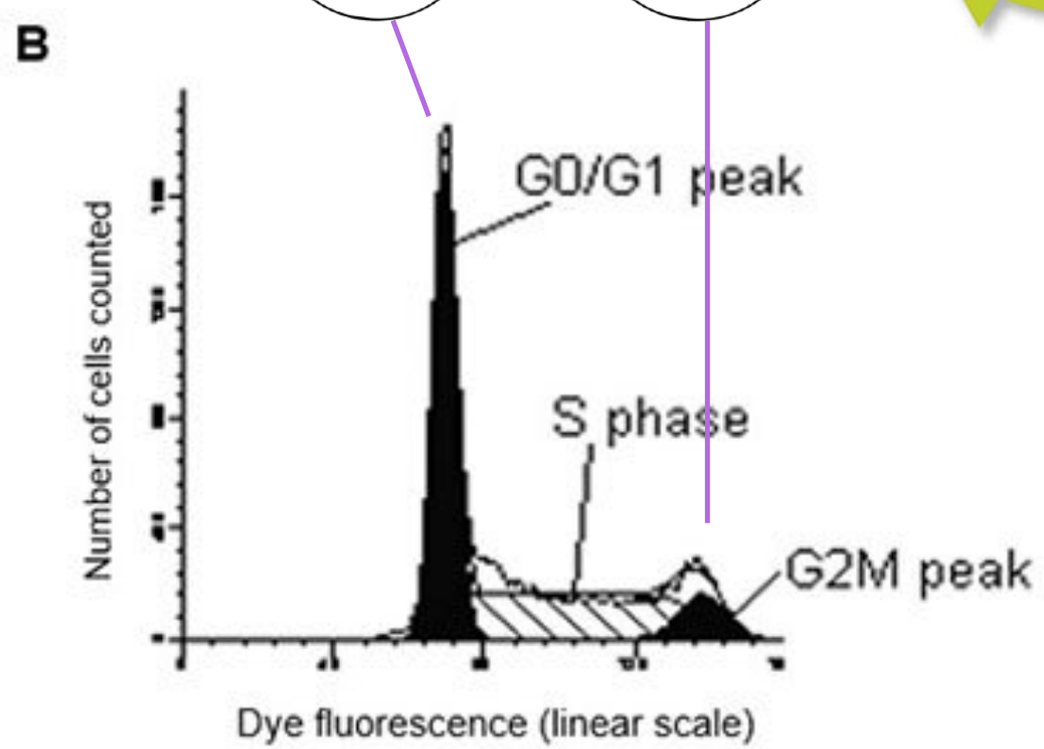


Applications

Cell Cycle Analysis via Flow Cytometry

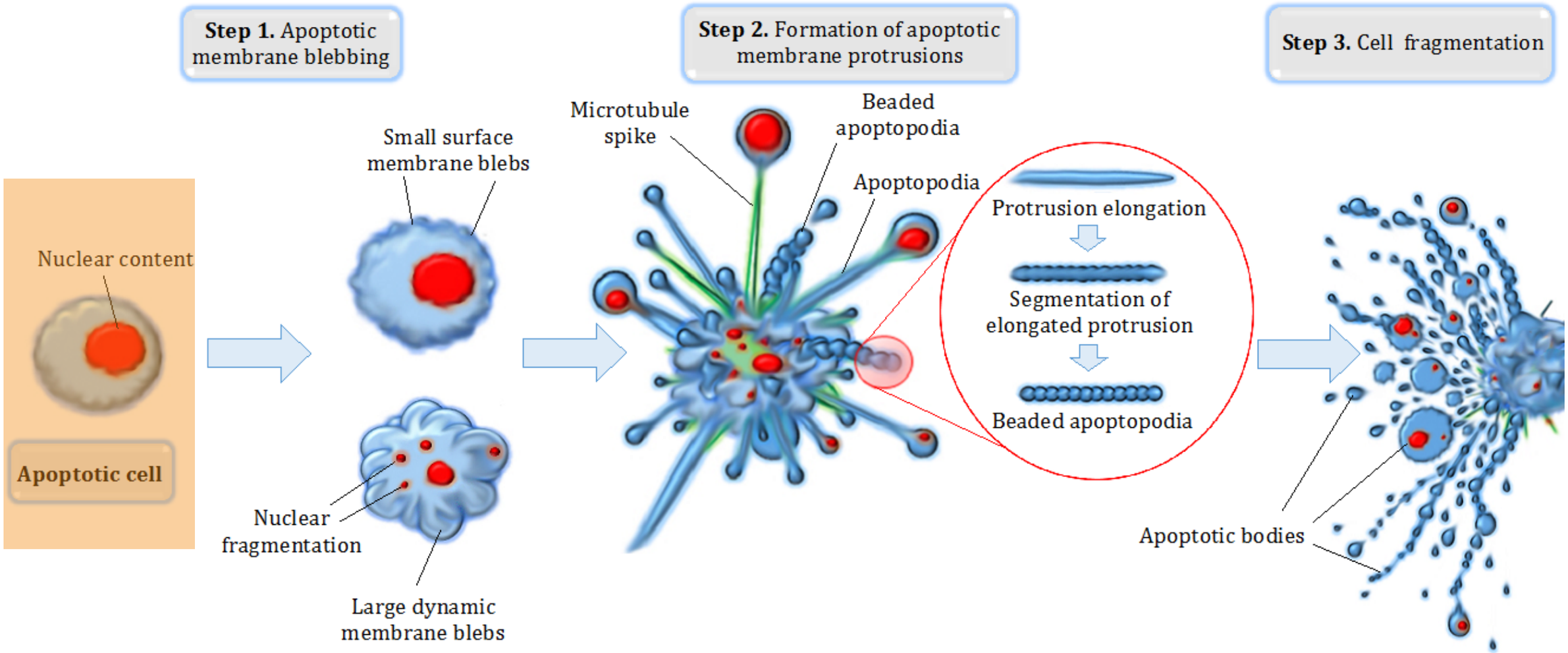






Applications

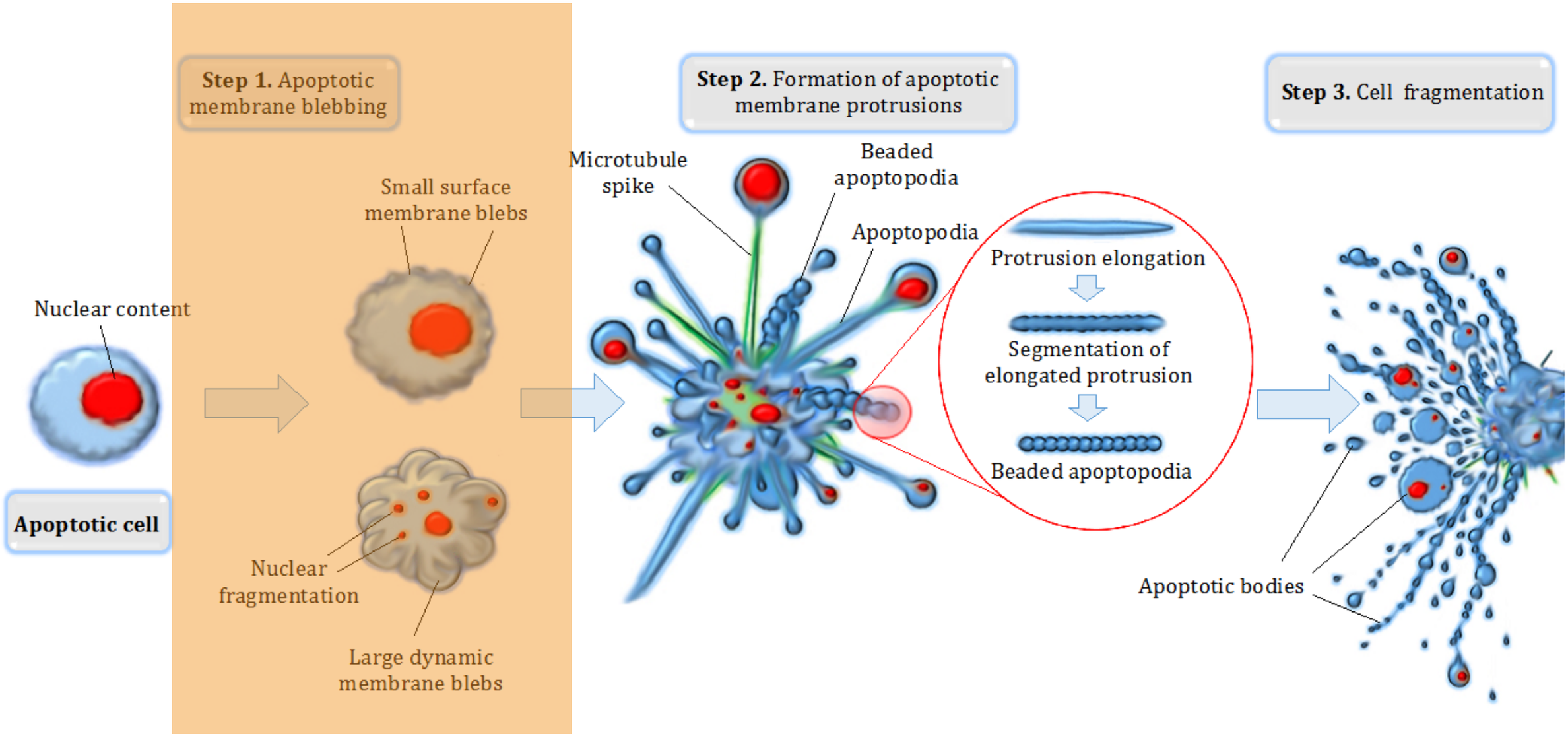
Apoptosis via Flow Cytometry



Different steps in apoptotic cell disassembly.

Applications

Apoptosis via Flow Cytometry

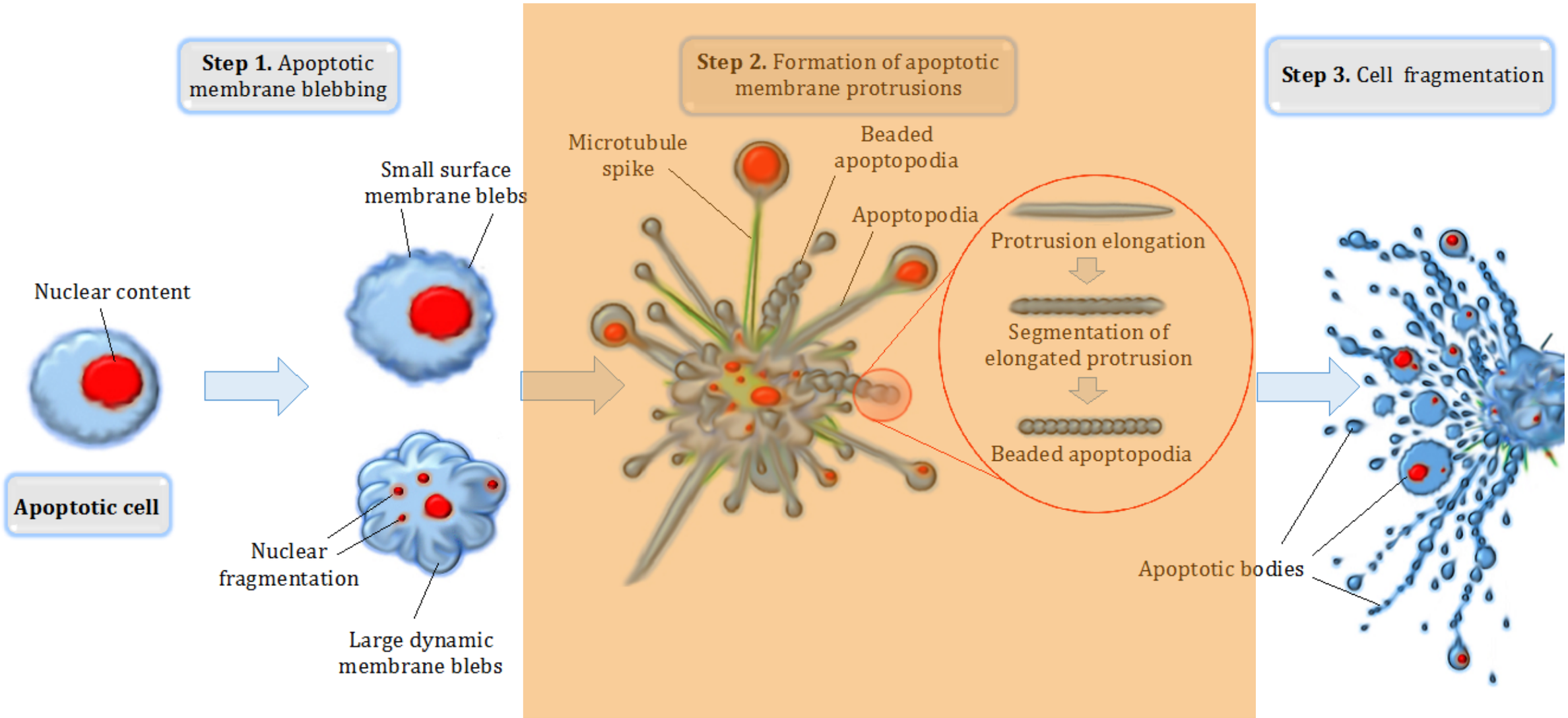


Different steps in apoptotic cell disassembly.

Applications

Apoptosis via Flow Cytometry

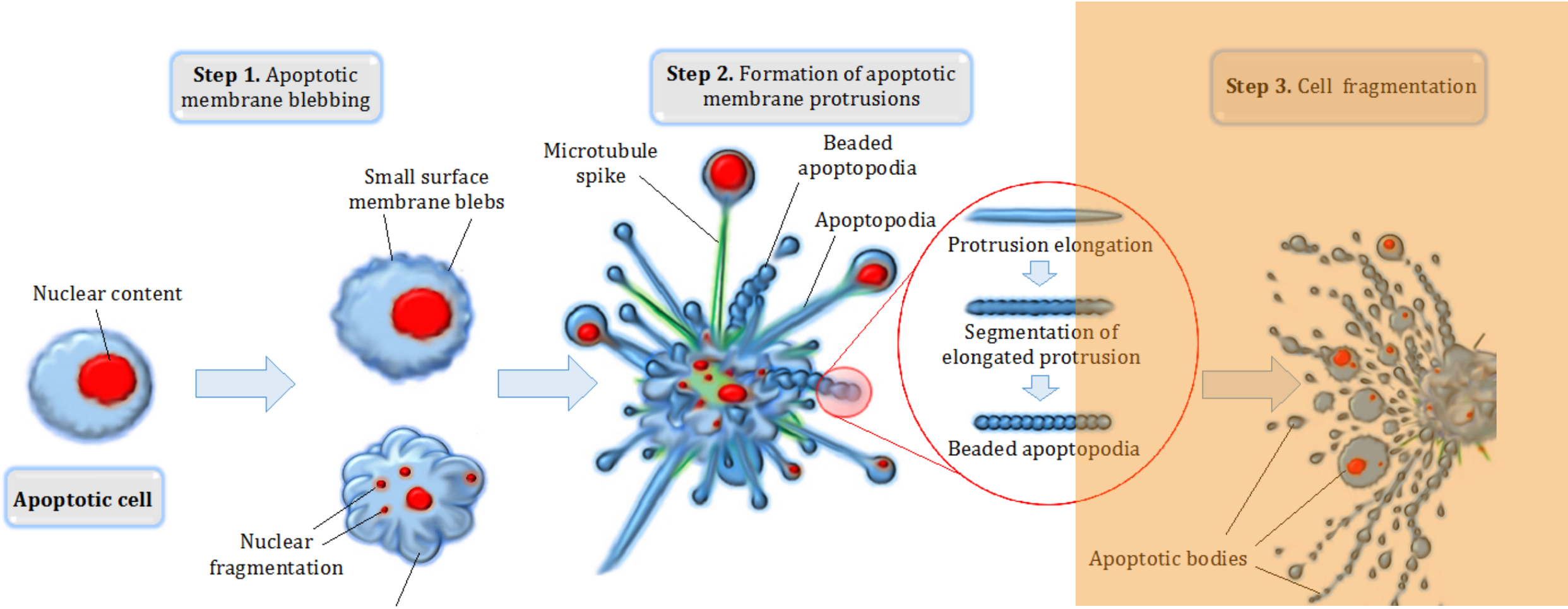
Different steps in apoptotic cell disassembly.



Applications

Apoptosis via Flow Cytometry

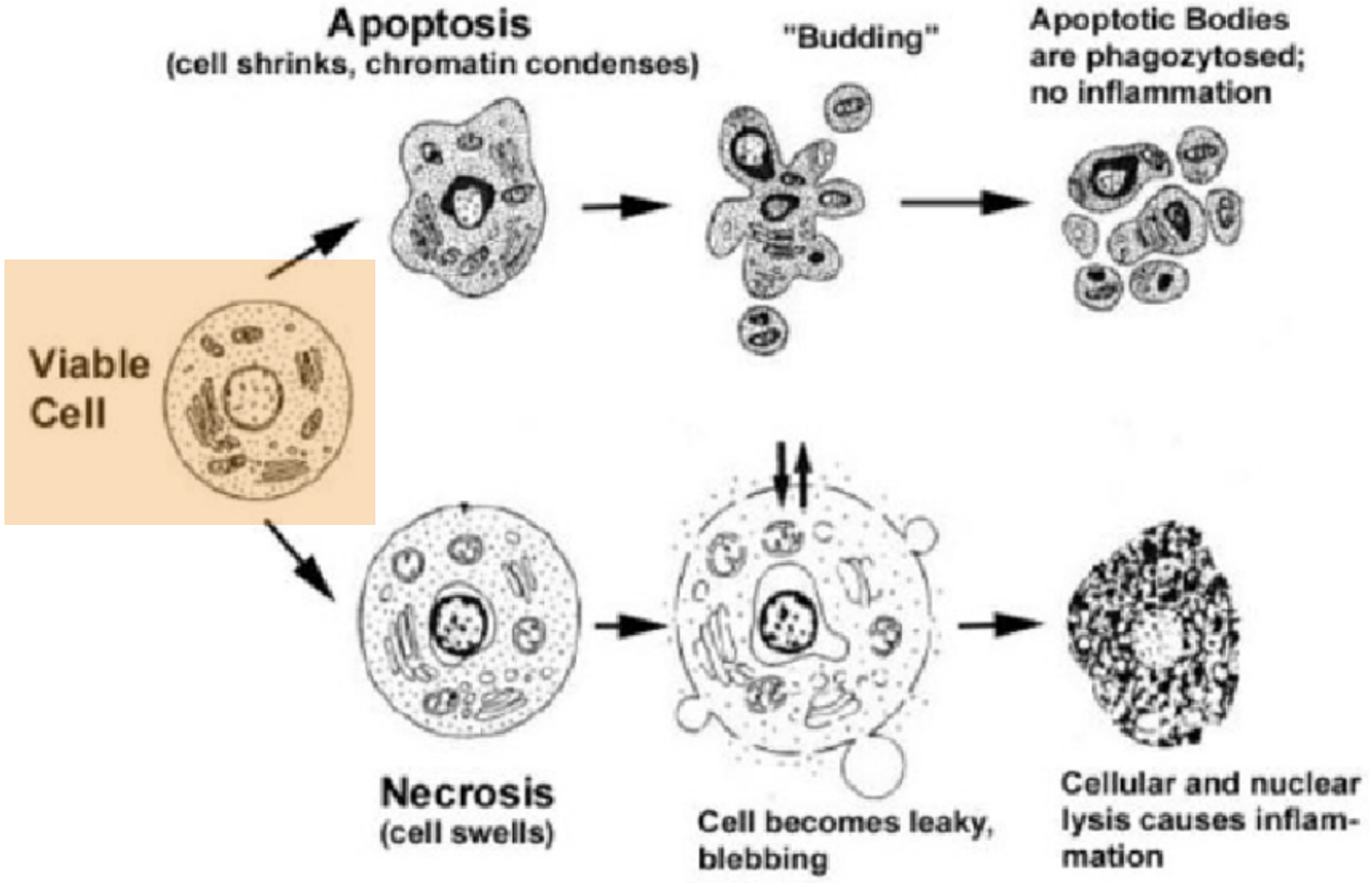
Different steps in apoptotic cell disassembly.



12 caspases

Caspases (cysteine-aspartic proteases, cysteine aspartases or cysteine-dependent aspartate-directed proteases) are a family of protease enzymes playing essential roles in programmed cell death. ... These are signalling molecules that allow recruitment of immune cells to an infected cell or tissue.

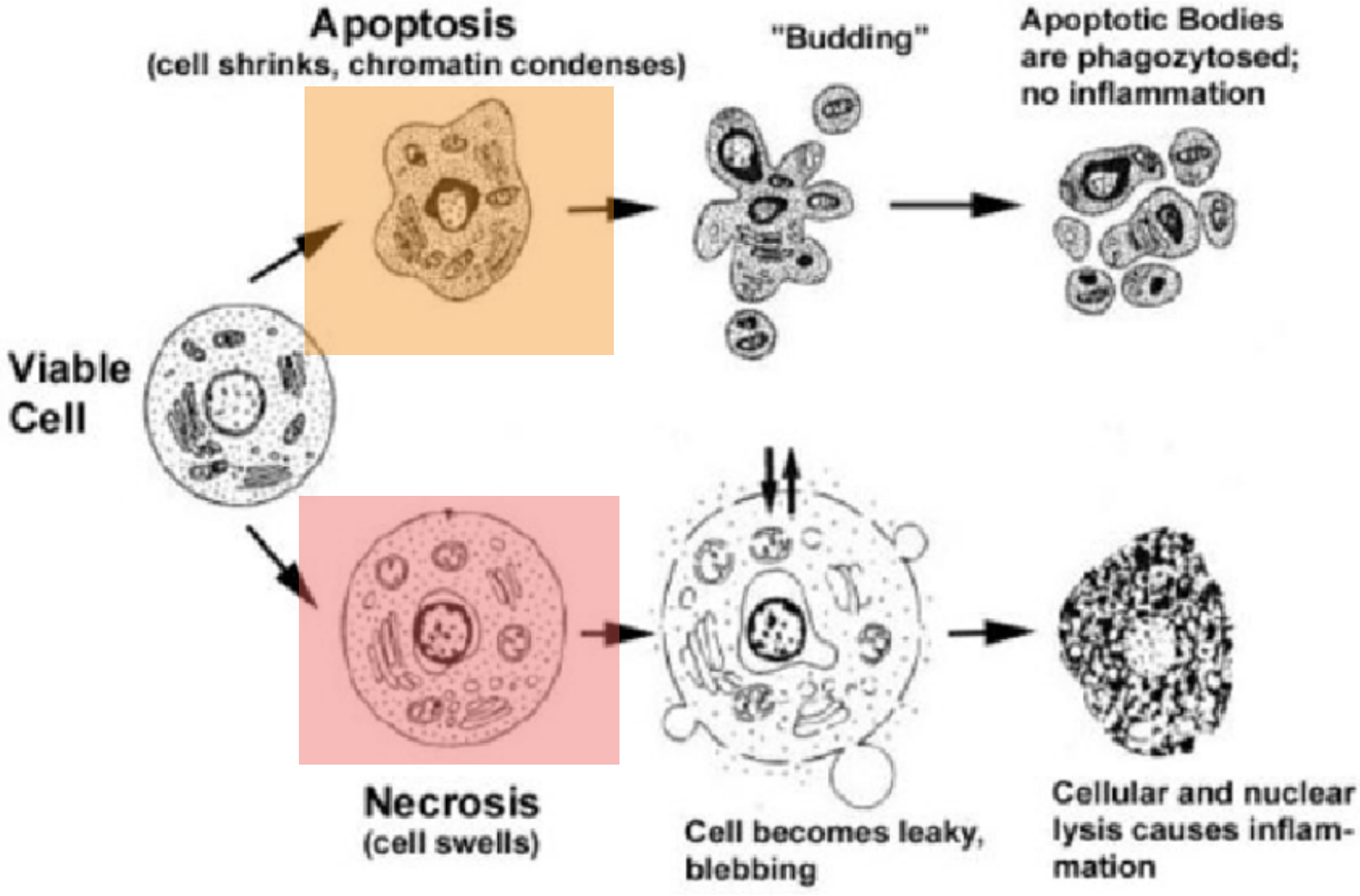
Apoptosis vs. Necrosis



Hallmarks of the apoptotic and necrotic cell death process **Apoptosis** includes **cellular shrinking**, chromatin condensation and margination at the nuclear periphery with the eventual formation of membrane-bound apoptotic bodies that contain organelles, cytosol and nuclear fragments and are phagocytosed without triggering inflammatory processes.

The necrotic cell swells, becomes leaky and finally is disrupted and releases its contents into the surrounding tissue resulting in inflammation. Modified from (Van Cruchten et al. 2002)

Apoptosis vs. Necrosis



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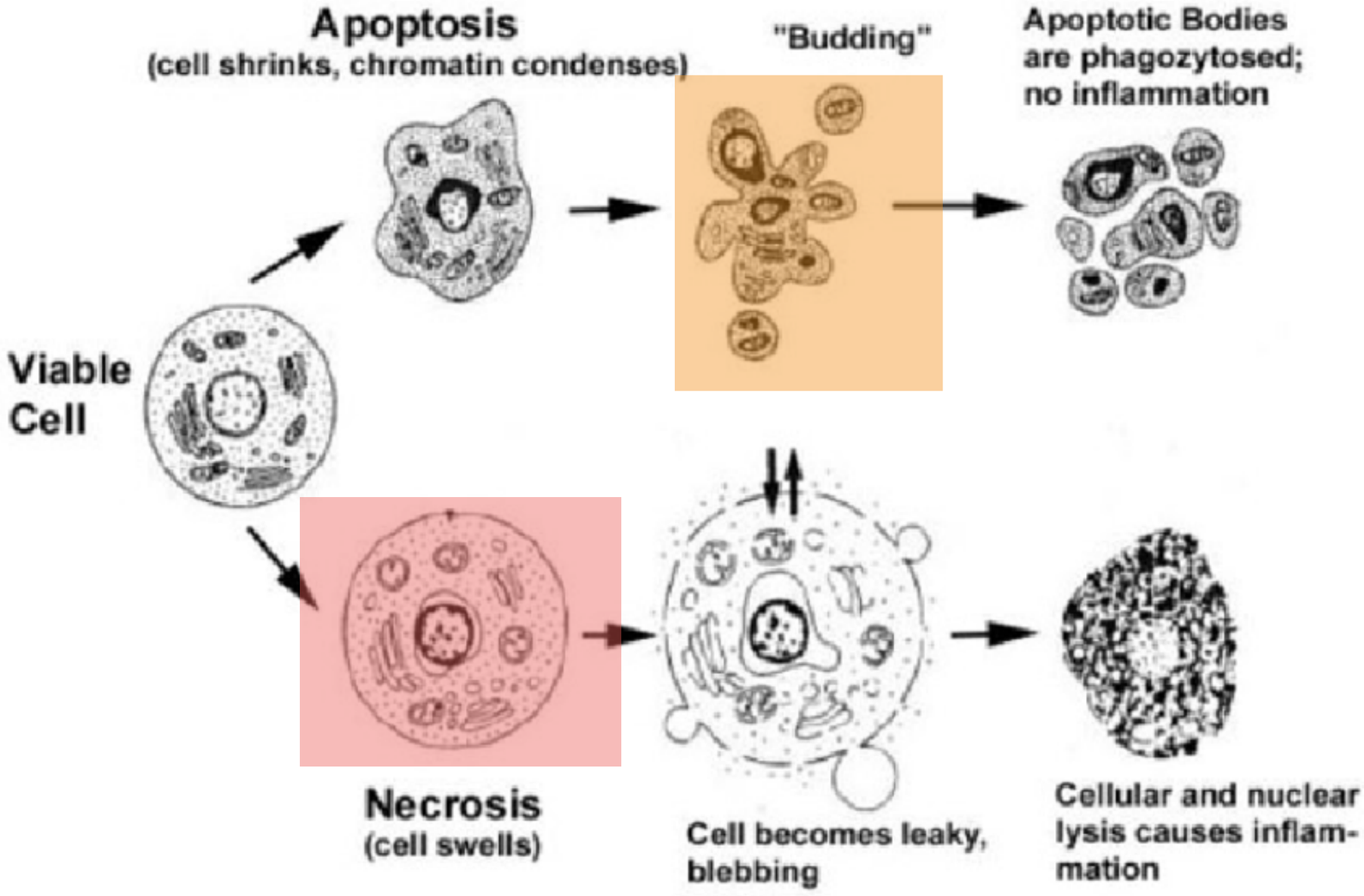

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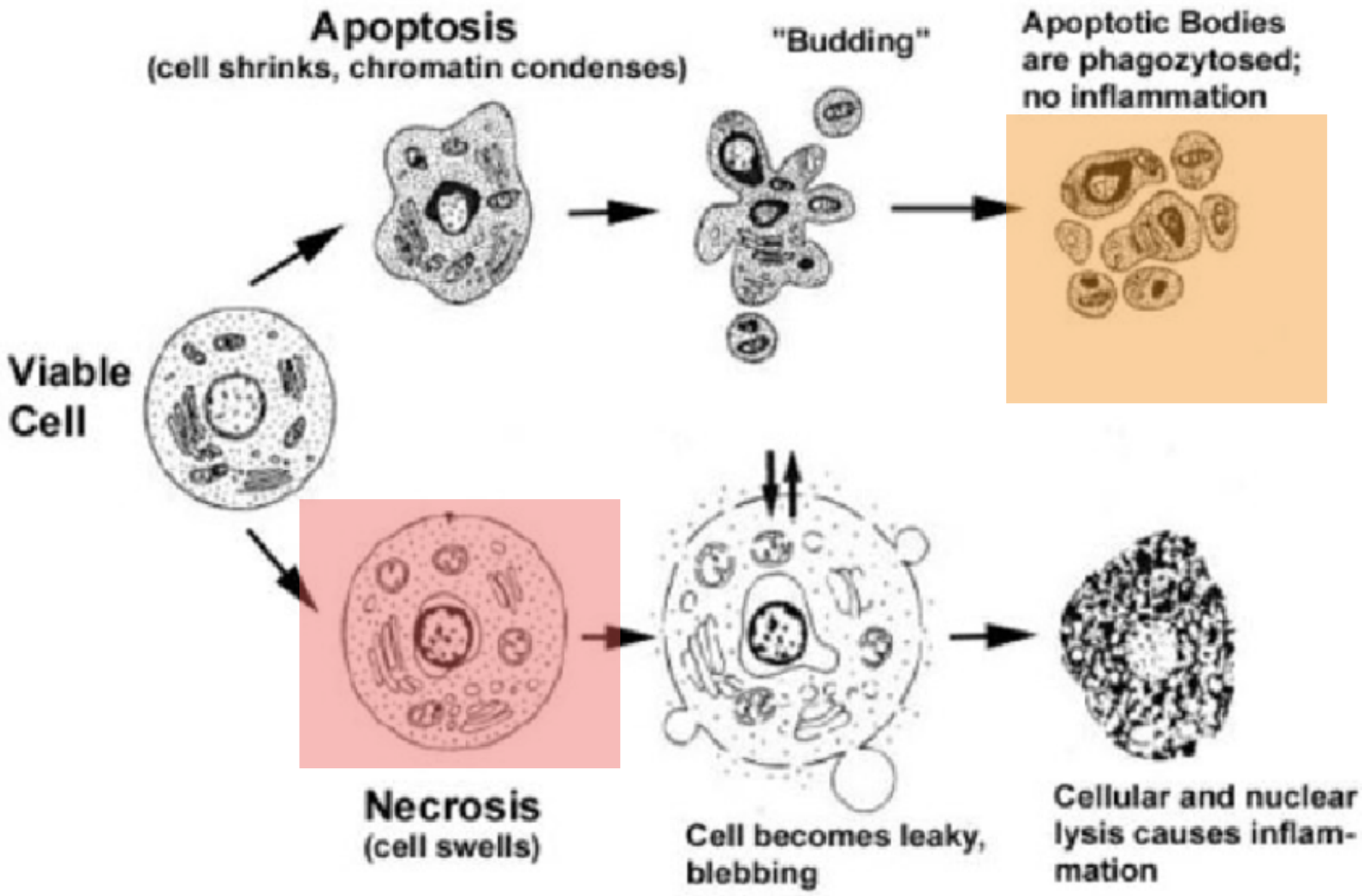

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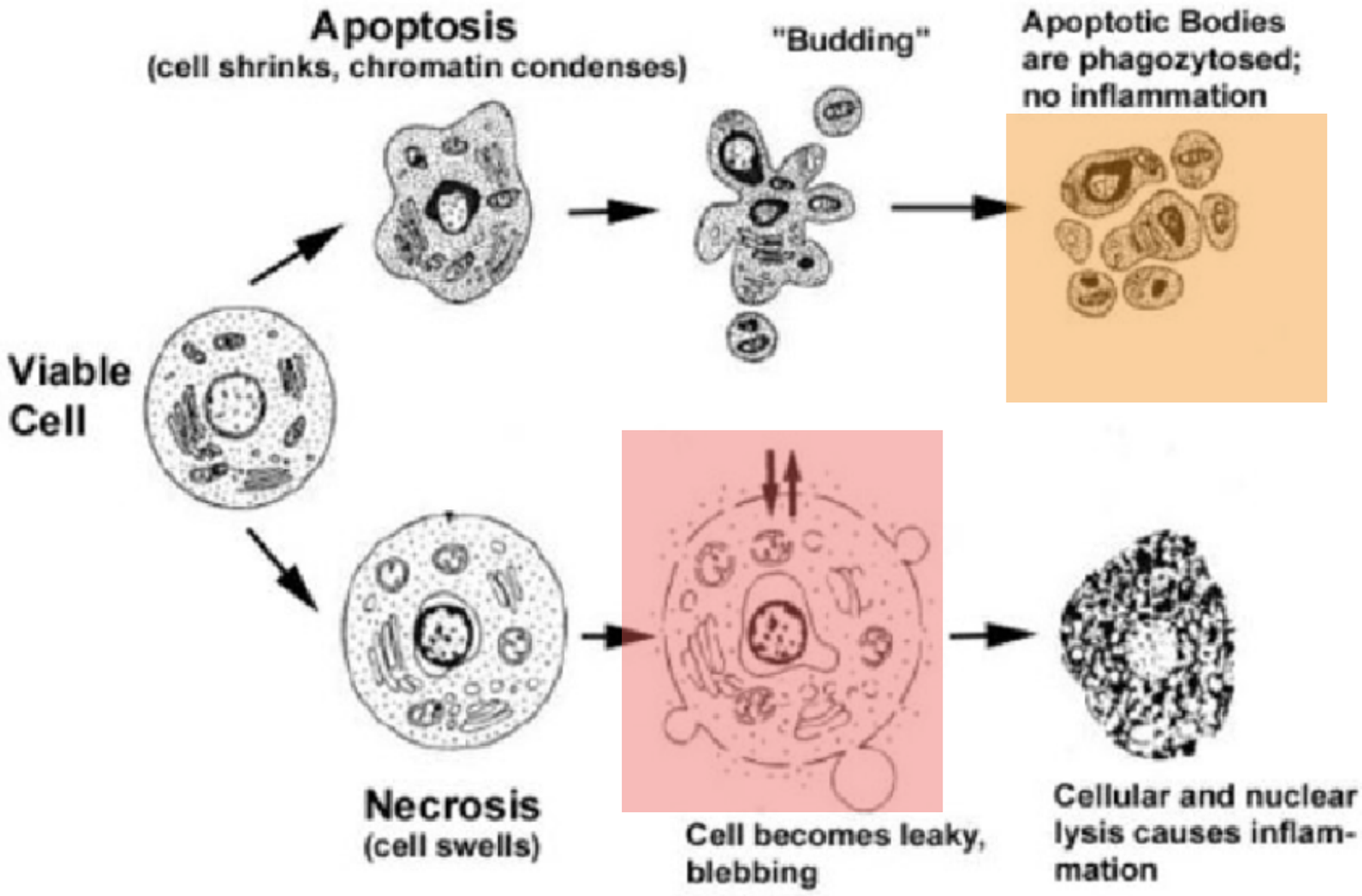

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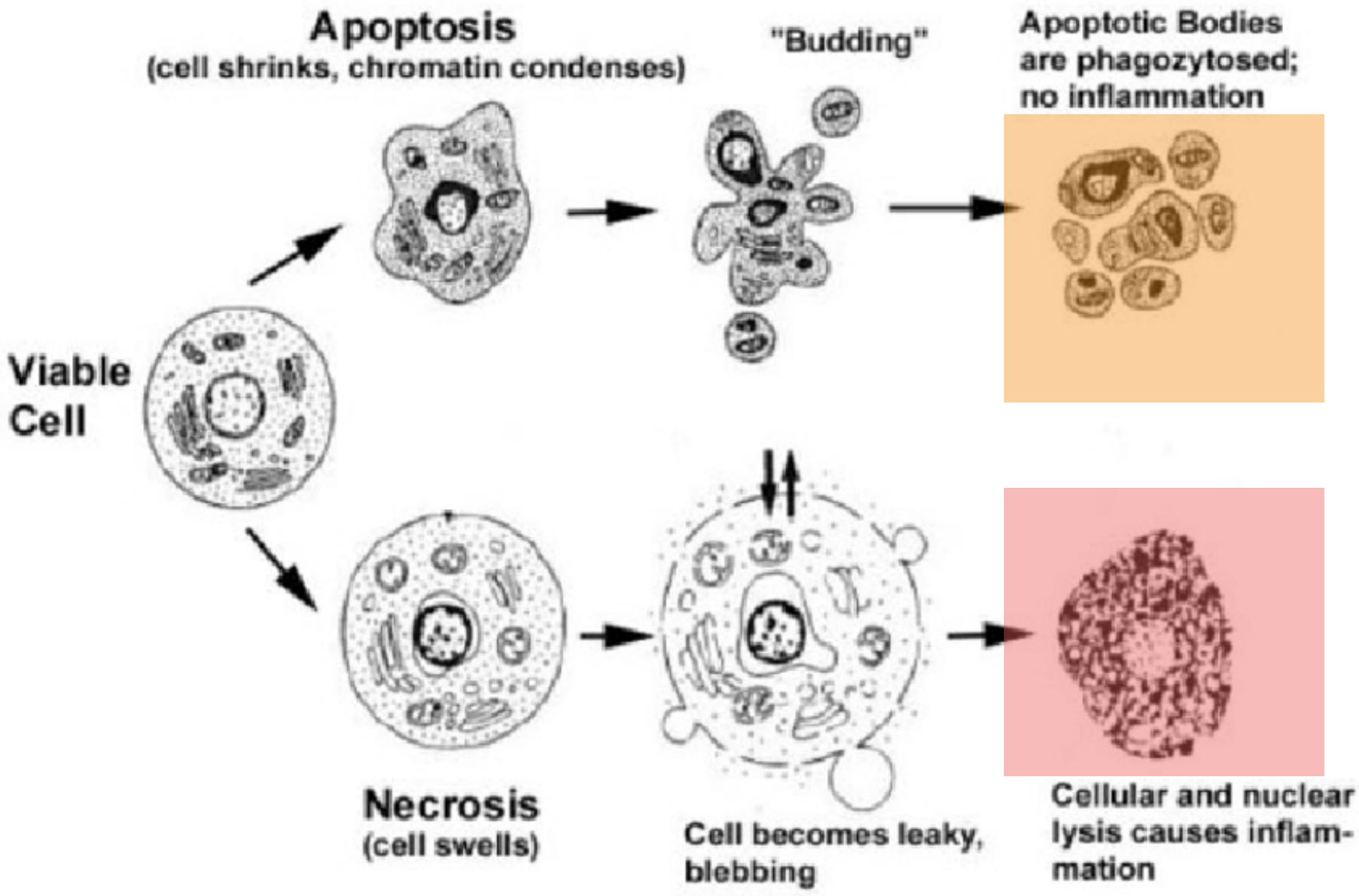

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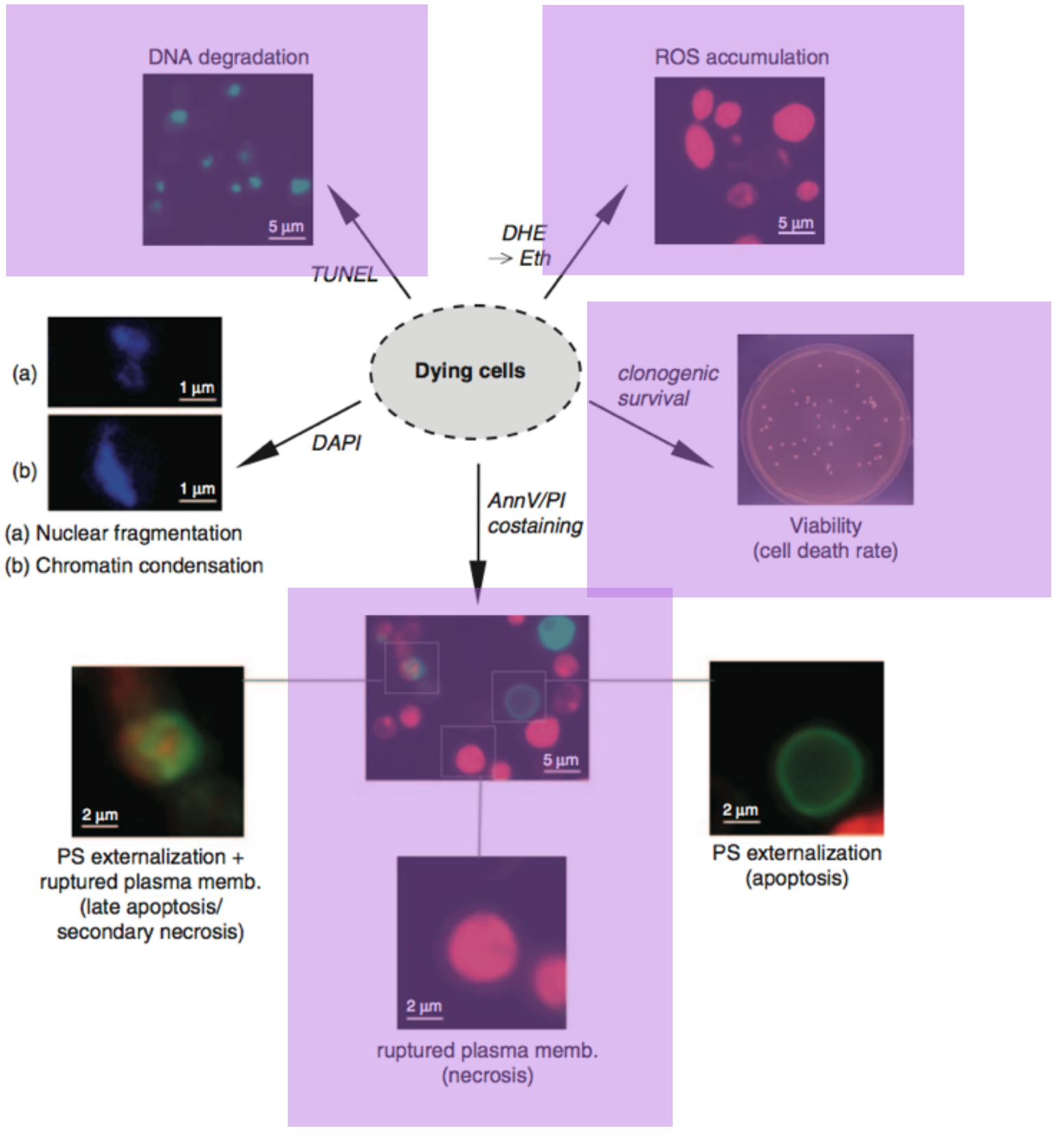
The necrotic cell swells, becomes leaky and finally is disrupted and releases its contents into the surrounding tissue resulting in inflammation. Modified from (Van Cruchten et al. 2002)

Apoptosis vs. Necrosis

- **Apoptosis**
- Process of programmed cell death
- Programmed cell death involves a series of biochemical events leading to characteristic changes in cell morphology and death
- Changes to the cell membrane include loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation
- Apoptosis, confers advantages during an organism's life cycle.
  - the differentiation of fingers and toes in a developing human embryo occurs because cells between the fingers apoptosis; the result is that the digits are separate.
  - Between 50 and 70 billion cells die each day due to apoptosis in the average human adult.

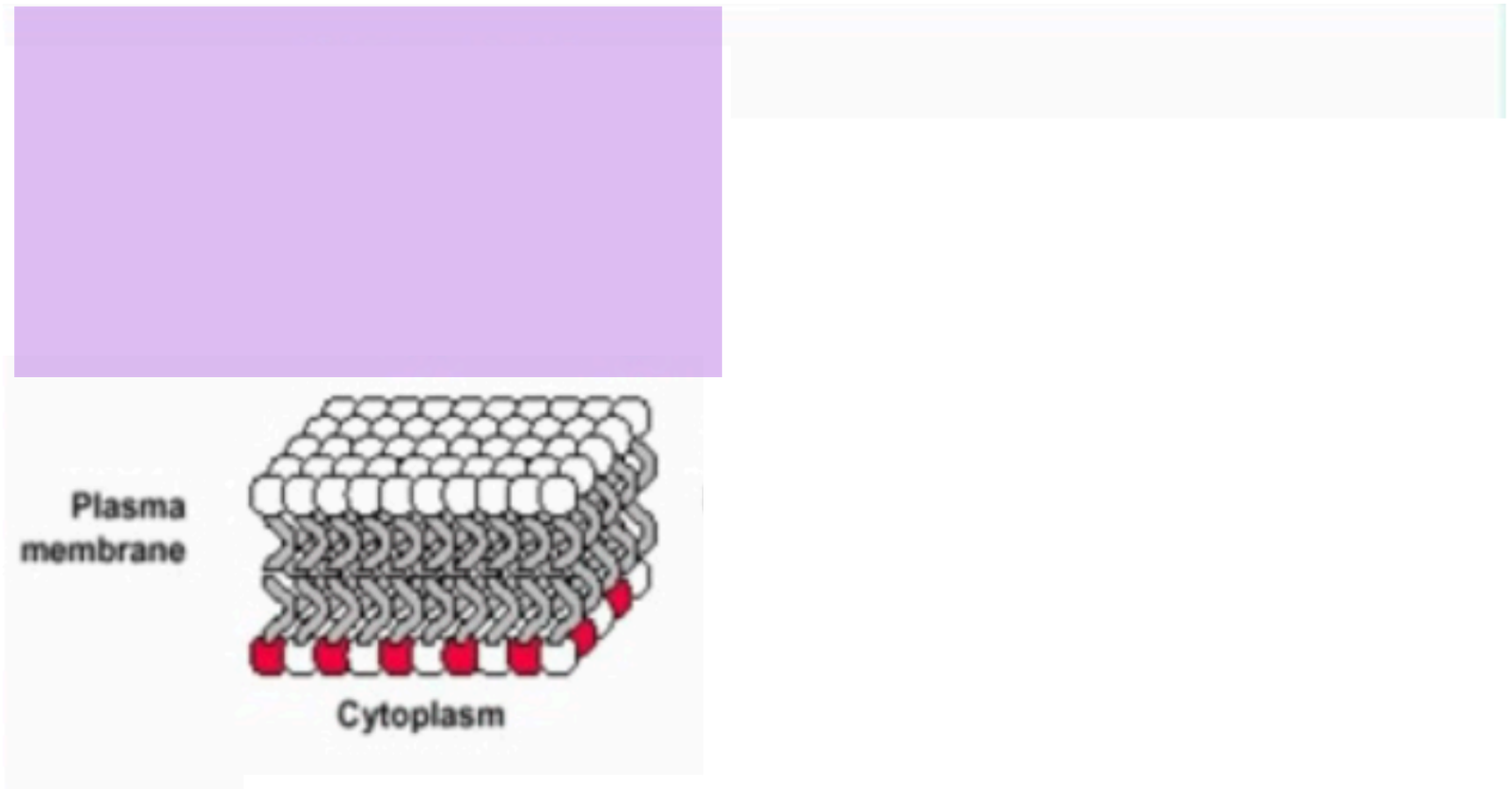
- **Necrosis**
- Traumatic cell death that results from acute cellular injury,
- Processes of disposal of cellular debris do not damage the organism
- Various applications can be used to differentiate between Apoptosis and Necrosis


DNA degradation
5 µm
ROS accumulation
5 µm
TUNEL
DHE
→ Eth
Dying cells
(a)
1 µm
(b)
1 µm
DAPI
(a) Nuclear fragmentation
(b) Chromatin condensation
AnnV/PI
costaining
clonogenic
survival
Viability
(cell death rate)
5 µm
2 µm
PS externalization +
ruptured plasma memb.
(late apoptosis/
secondary necrosis)
2 µm
ruptured plasma memb.
(necrosis)
2 µm
PS externalization
(apoptosis)

Applications

Apoptosis via Flow Cytometry

Annexin V is a surface marker, which detects early membrane changes associated with apoptosis; namely the “externalization of the the inner leaflet of the lipid membrane



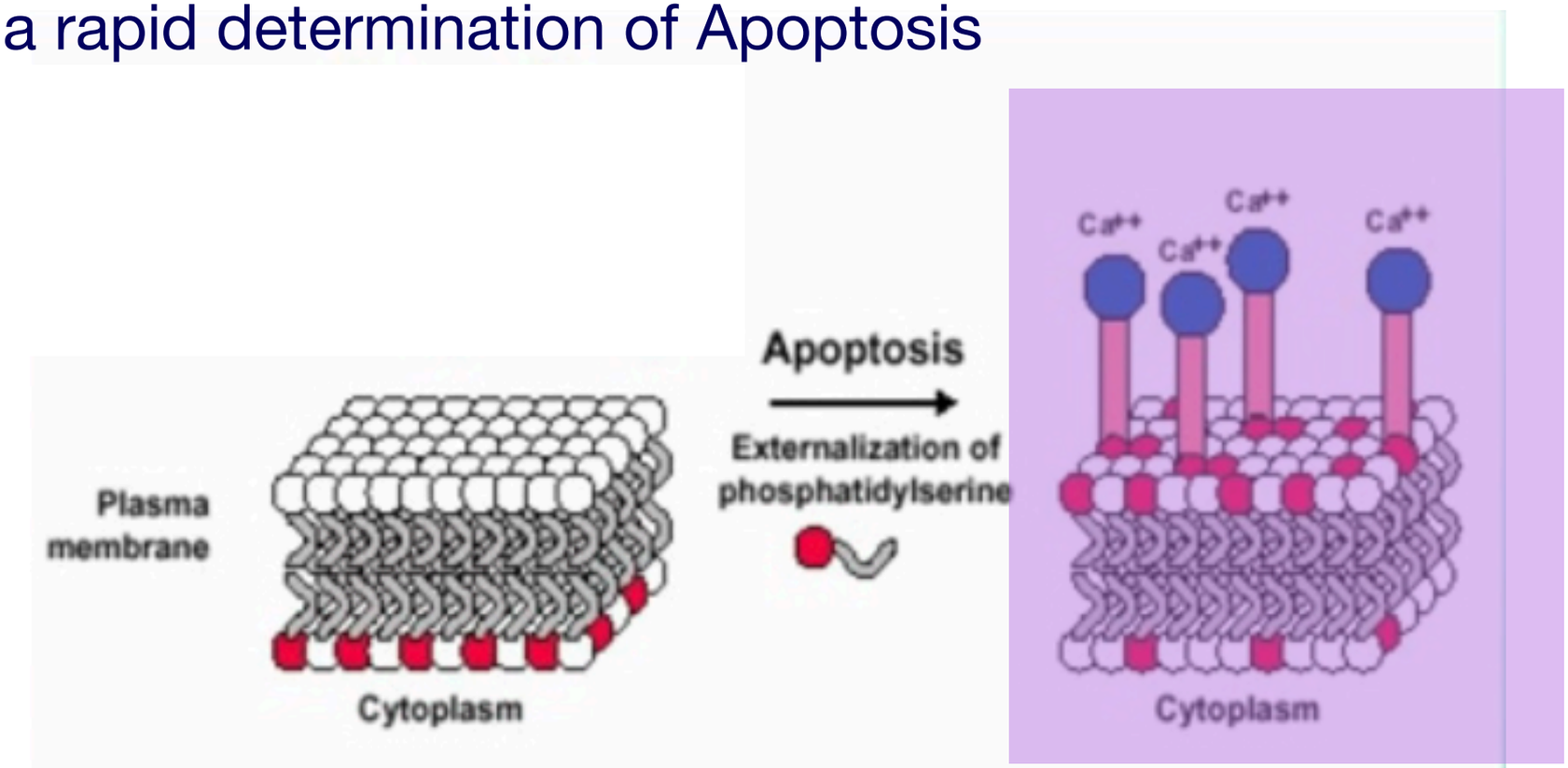
Schematic Representation of the **Annexin V** assay

Applications

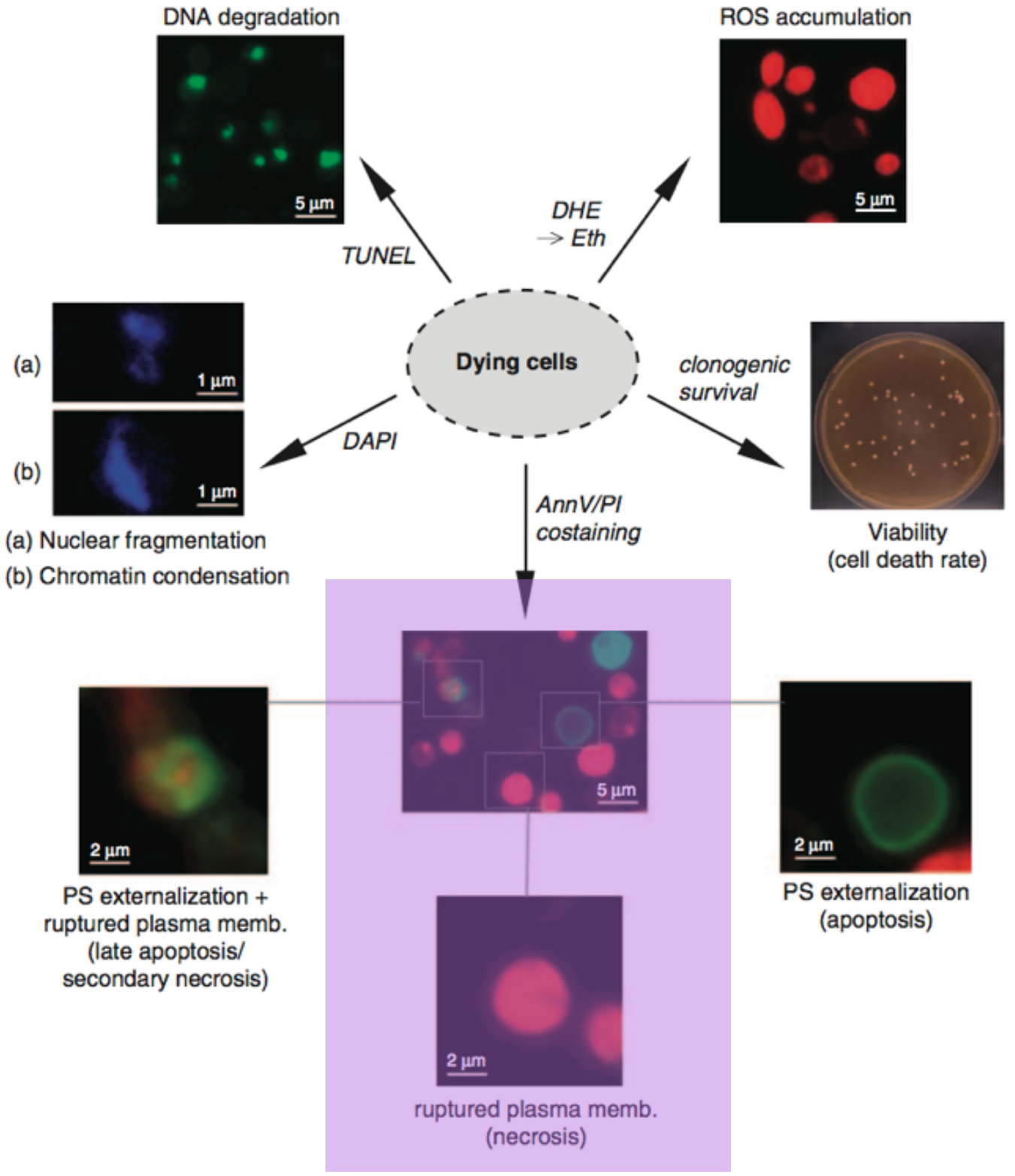
Apoptosis via Flow Cytometry

Annexin V is a surface marker, which detects early membrane changes associated with apoptosis; namely the “externalization of the the inner leaflet of the lipid membrane.

As, such it is a rapid determination of Apoptosis



Schematic Representation of the **Annexin V** assay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(late apoptosis/
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2 µm
PS externalization
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2 µm
ruptured plasma memb.
(necrosis)

Applications

Apoptosis via Flow Cytometry

12 caspases

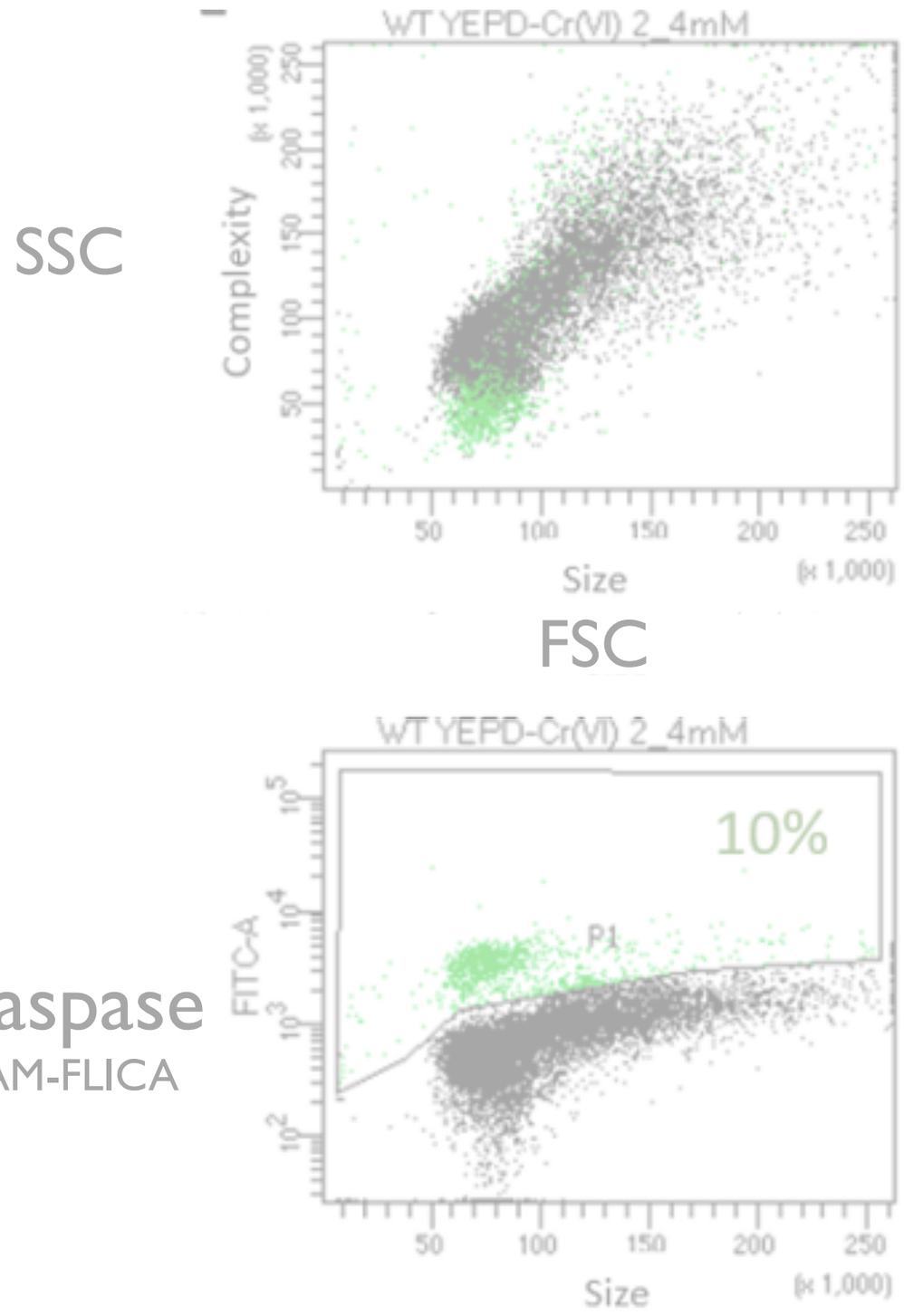
There are 12 **caspases** in humans alone, which have been classically grouped on the basis of sequence homology, domain architecture, and cell biology as inflammatory (**caspase**-1, **caspase**-4, **caspase**-5, and **caspase**-11), apoptotic initiators (**caspase**-2, **caspase**-8, **caspase**-9, and **caspase**-10), or executioners (**caspase**-3, ... Apr 5, 2016

1 caspase

There is ONLY 1 metacaspase in *S. cerevisiae*, “budding yeast”

Applications

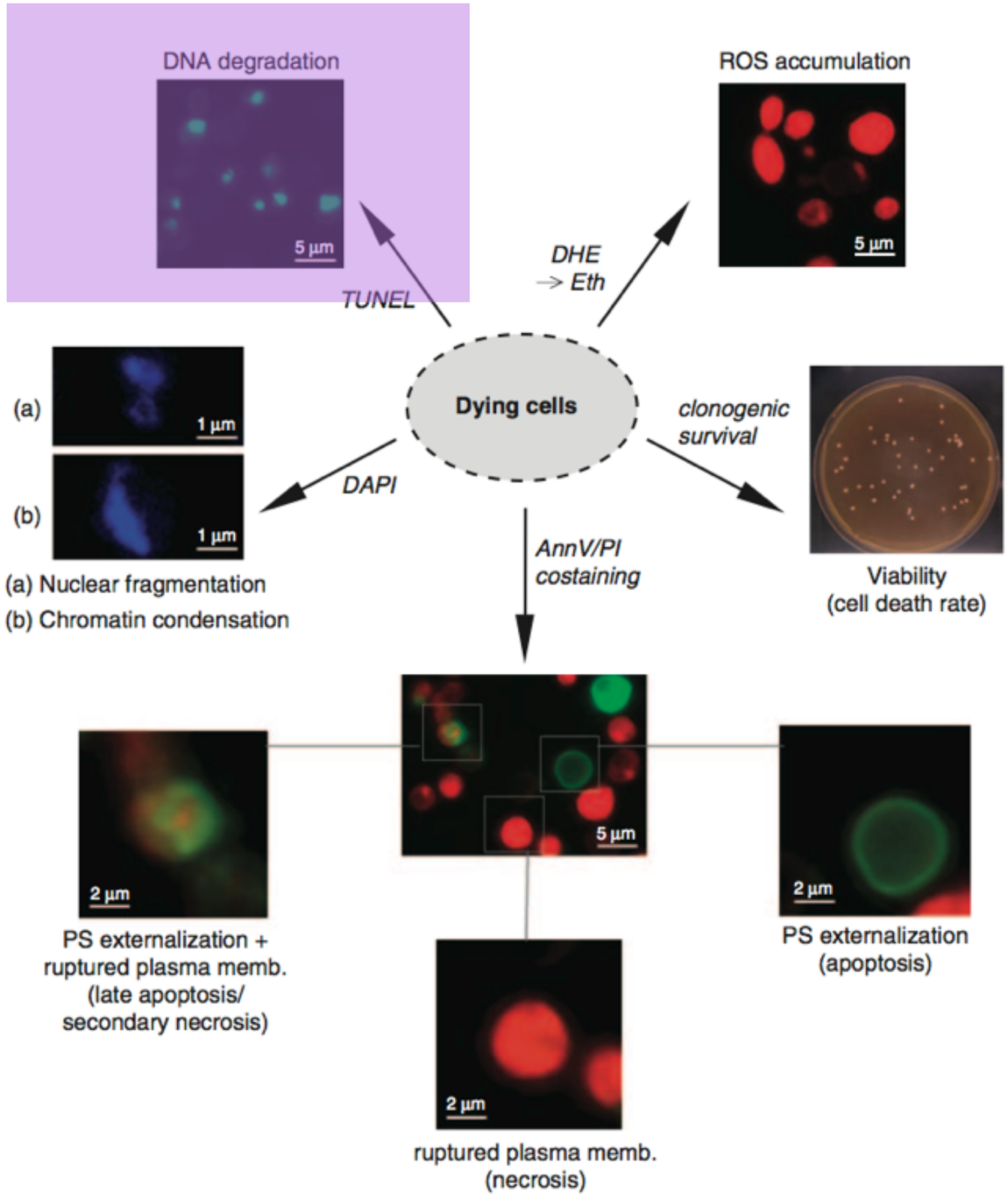
Apoptosis via Flow Cytometry



Yeast cells treated with Heavy Metal & stained with

FAM-FLICA (caspase)

Yeast + heavy metal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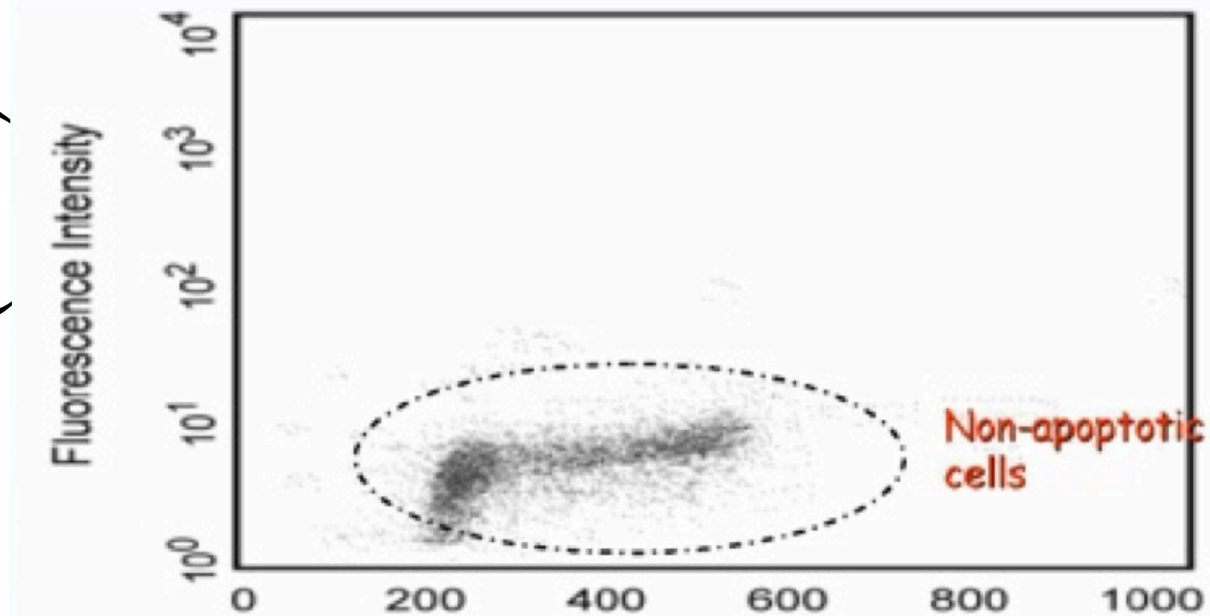
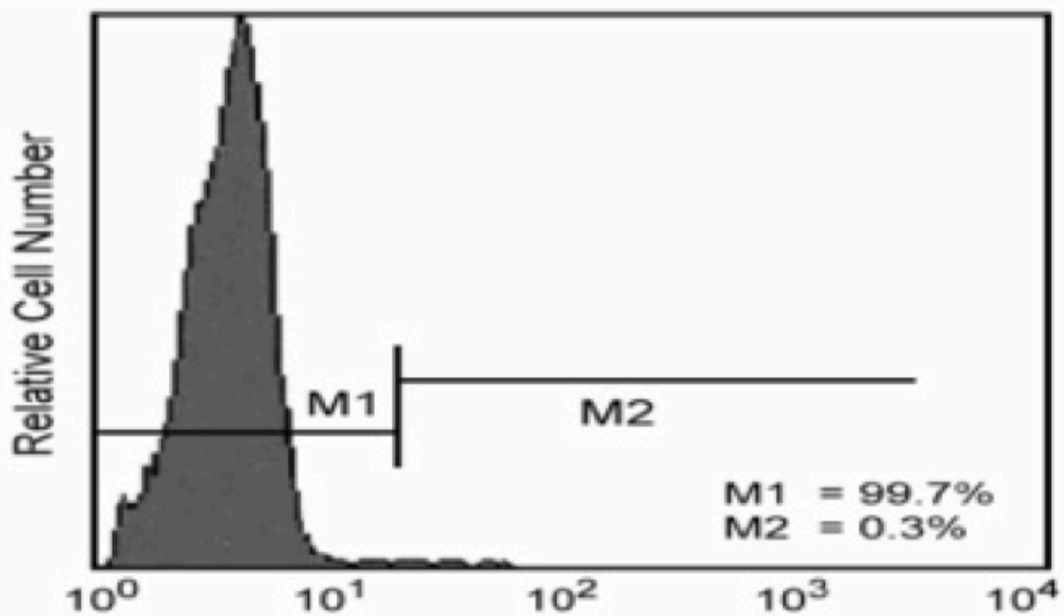
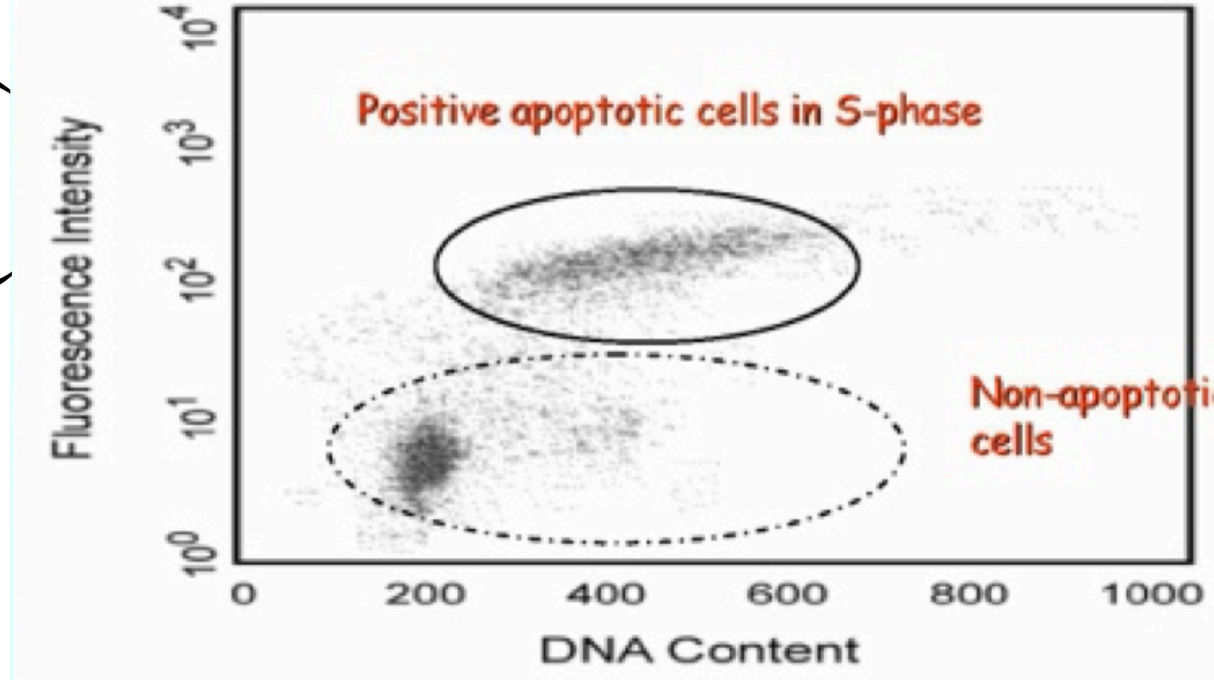
2 µm
PS externalization +
ruptured plasma memb.
(late apoptosis/
secondary necrosis)
5 µm
2 µm
ruptured plasma memb.
(necrosis)
2 µm
PS externalization
(apoptosis)

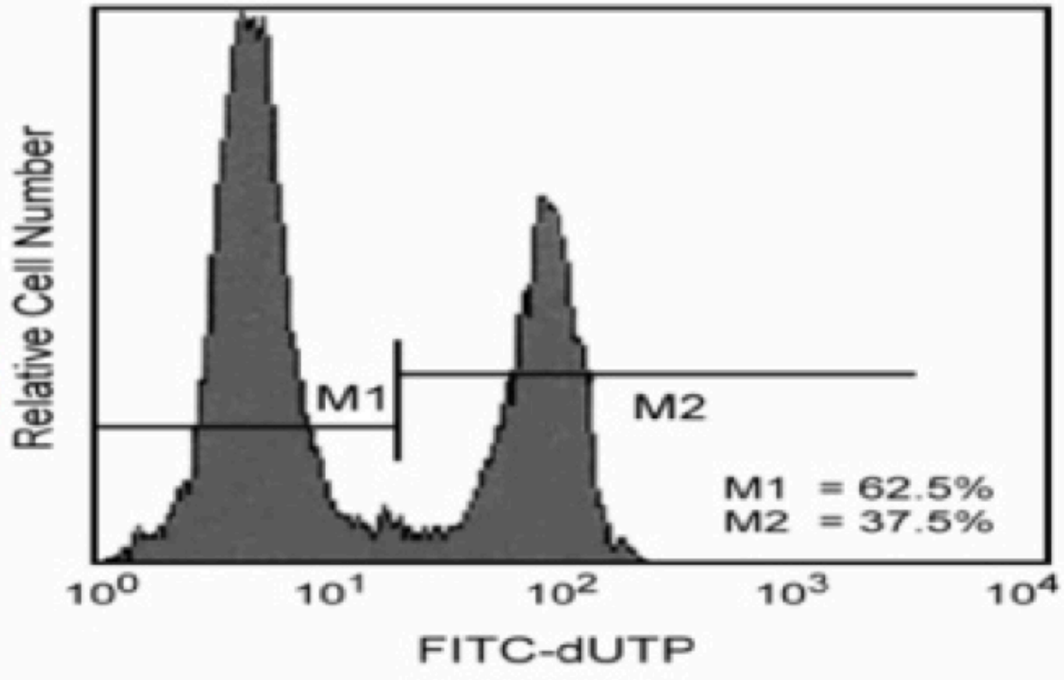
Detection of DNA fragmentation using "TUNEL" Assay

FITC (dUTP)



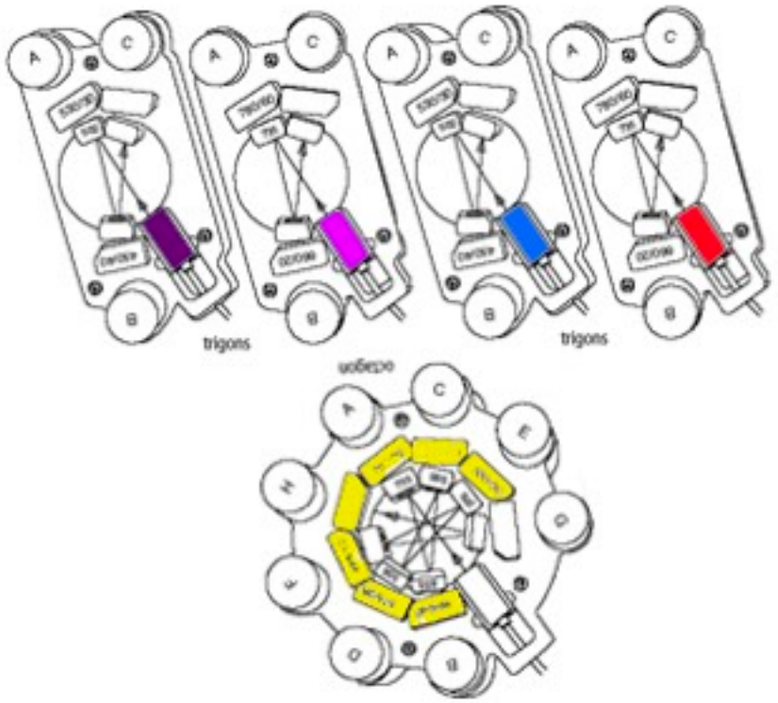


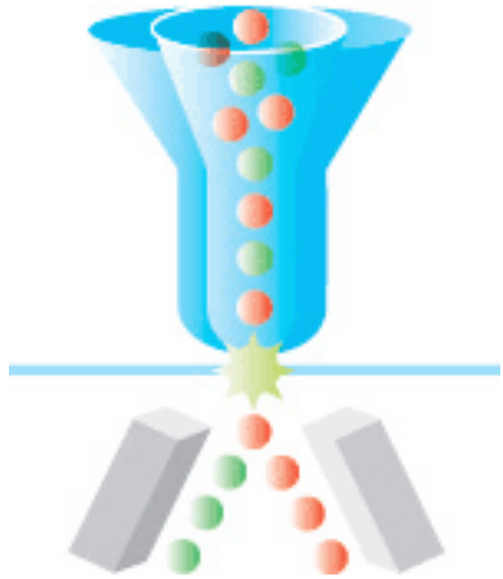


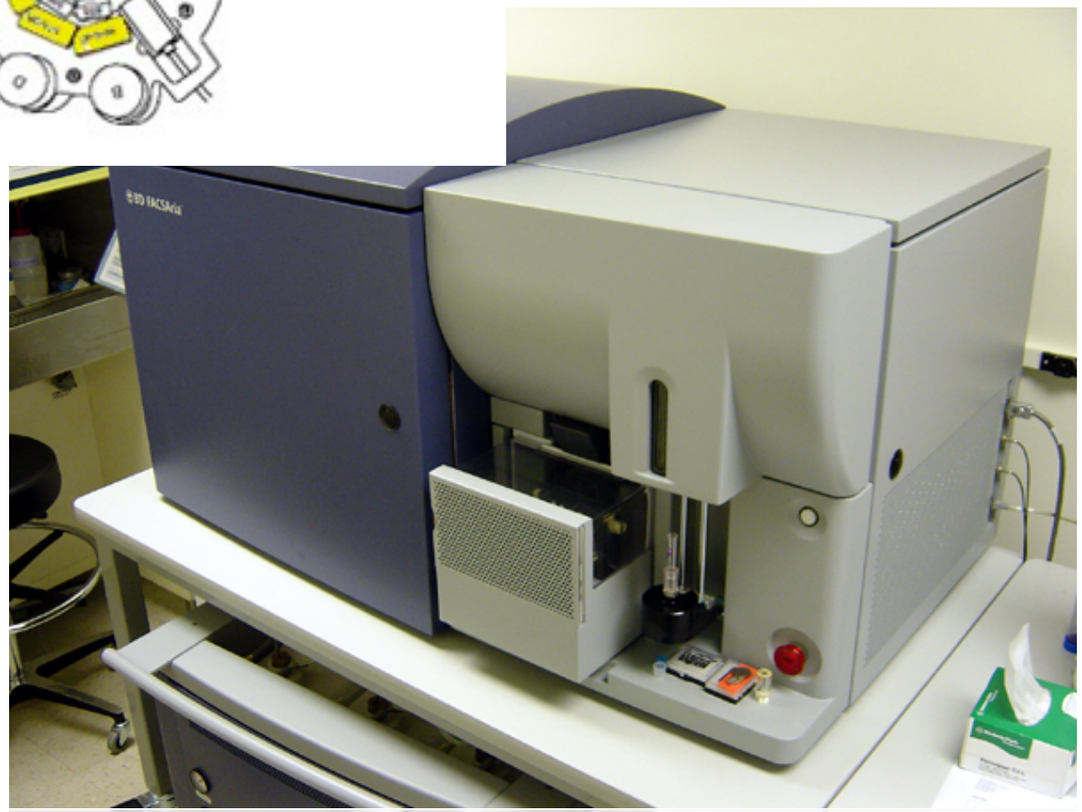


Propidium Iodide

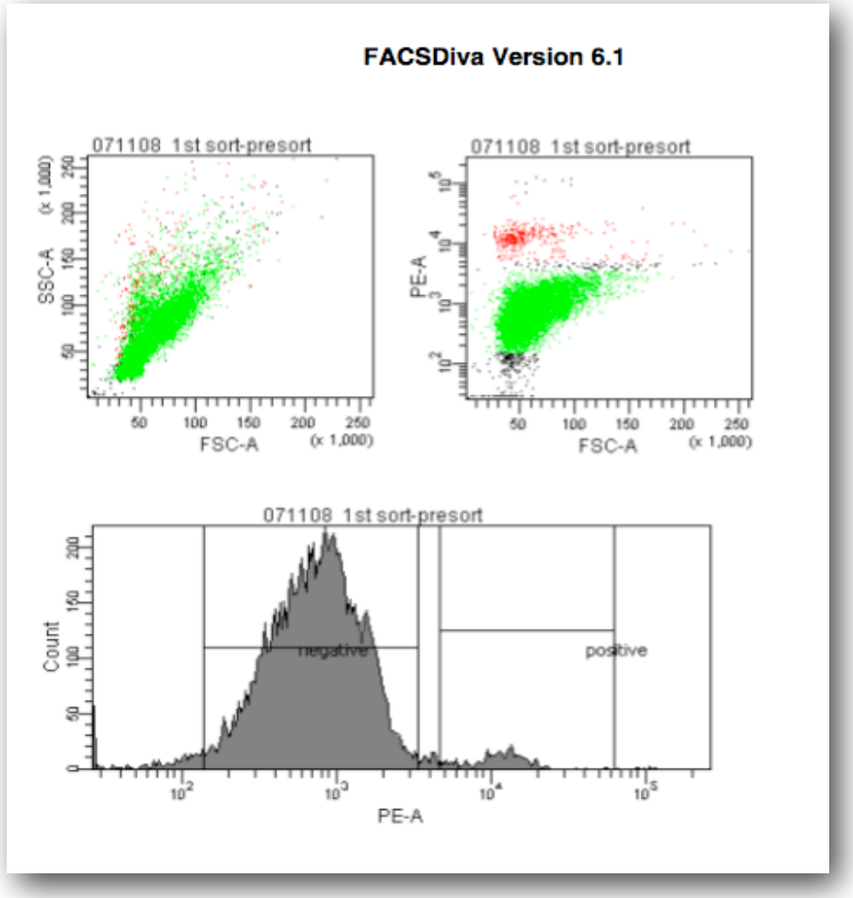
Cytometry - Cell Sorting



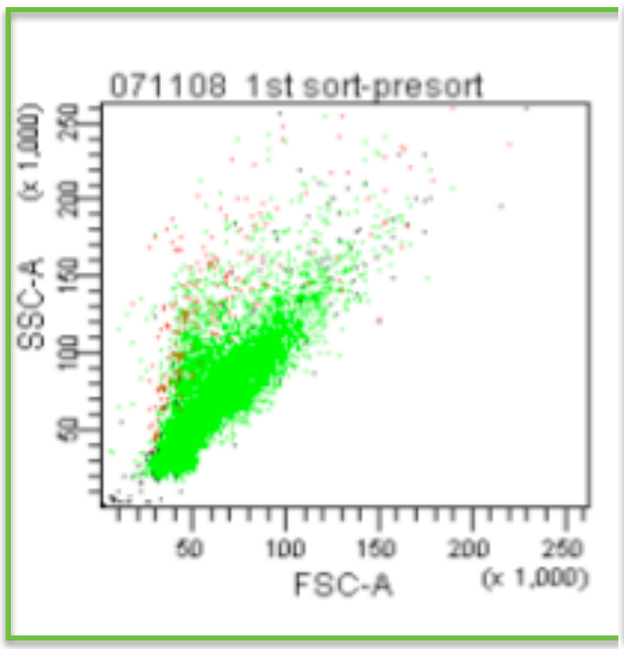


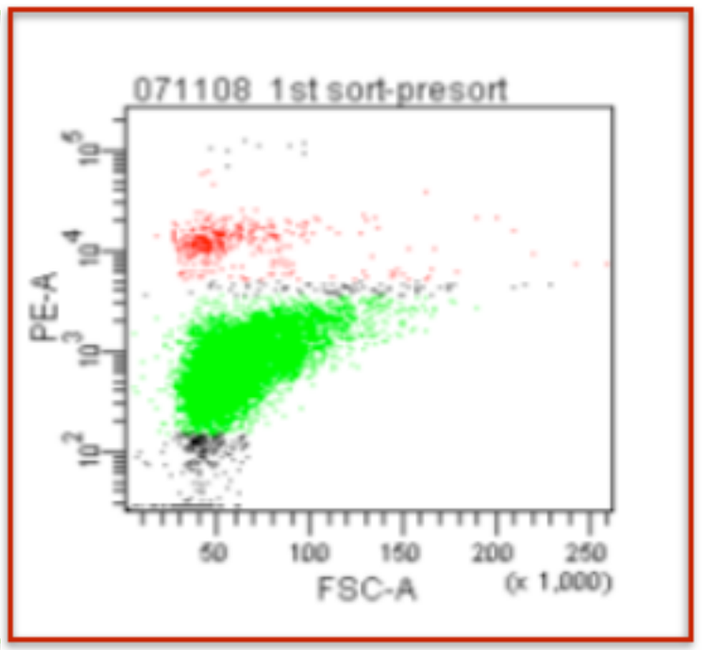


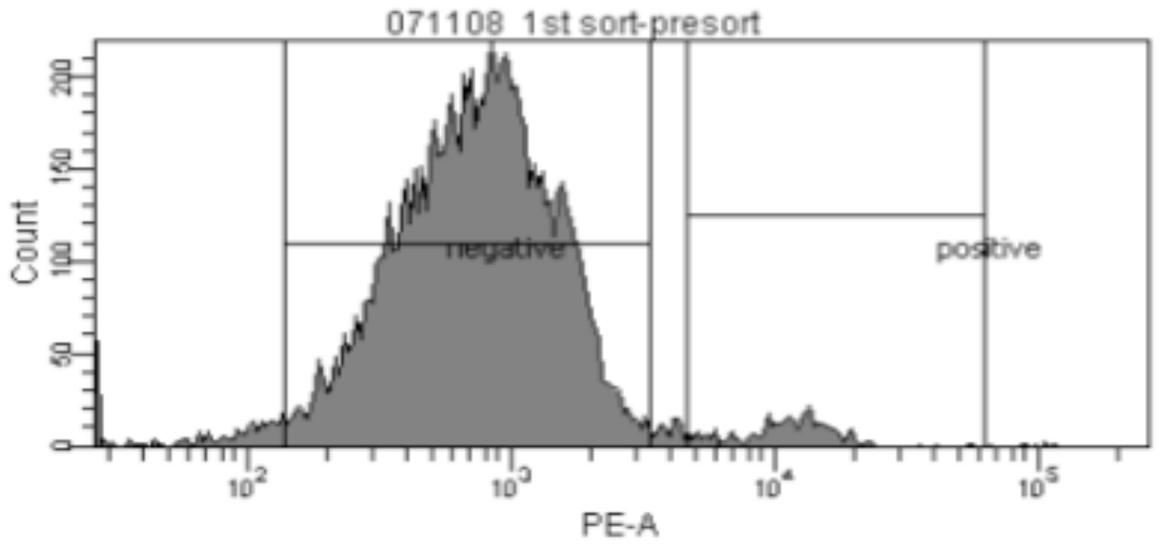
FACS Aria II (Becton Dickinson)

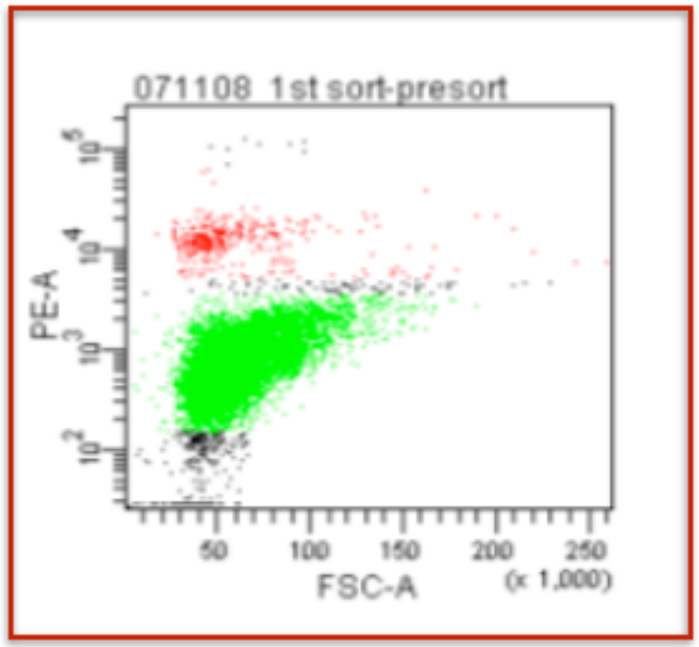


FACSDiva Version 6.1



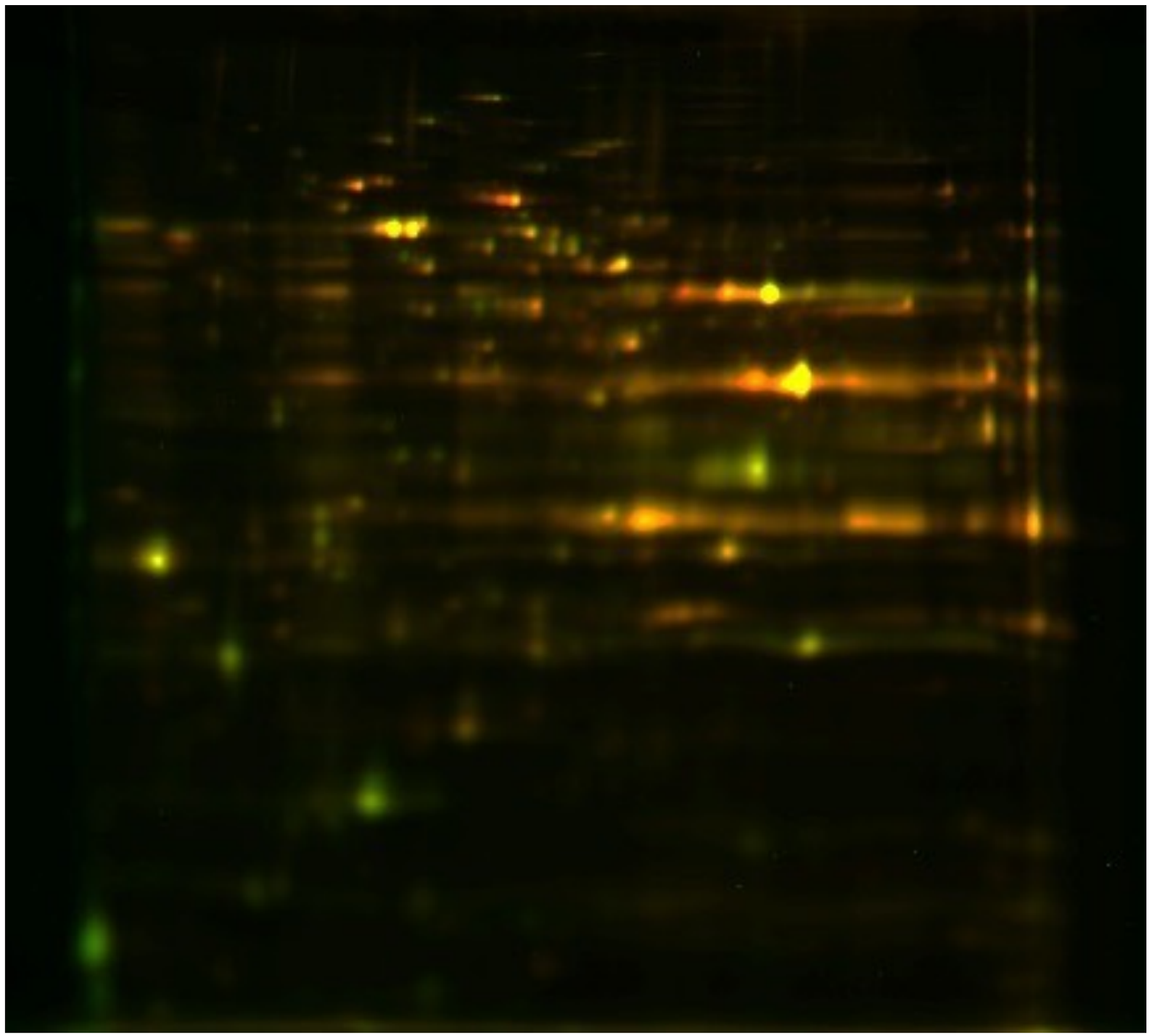






Extract proteins from Cells

2D Gel electrophoresis



2-DE DIGE gel of copper (II)-treated S. cerevisiae wild type proteins of apoptotic and non-apoptotic sub-populations, differentially labeled using CyDye… **Apoptotic cell proteins** were labeled with Cy5 and **non-apoptotic** cell protein were labeled with **Cy3**

GSU Biology Core Facility

Supporting Life Sciences at GSU

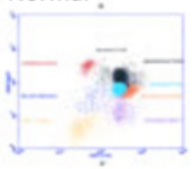
http://biotech.gsu.edu/core_facility/index.html

Georgia State University

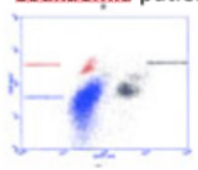
Flow Cytometry

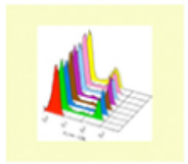
Profiling Cells

Normal



Leukaemia patient

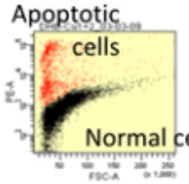




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

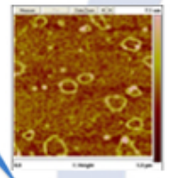


Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis

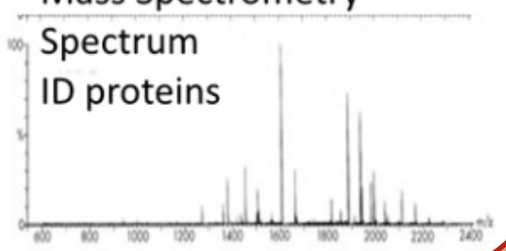


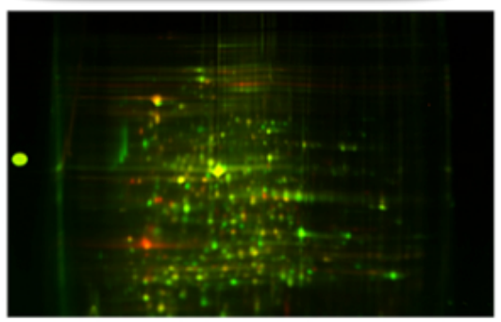
Cellular Functions

Mass Spectrometry

Spectrum

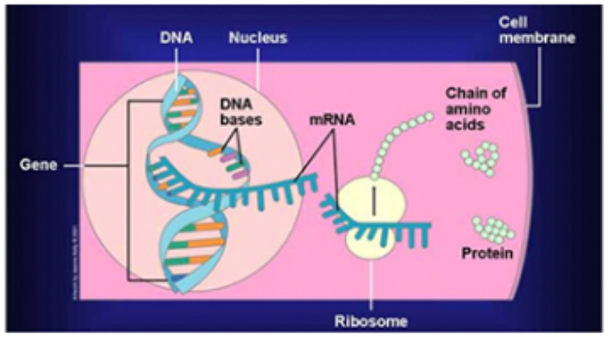
ID proteins





DNA

Replication



Protein

Expression

Proteomics

Profiling Proteins

2D Protein gel

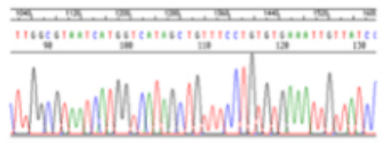
Protein separation using

Electric charge and molecular weight

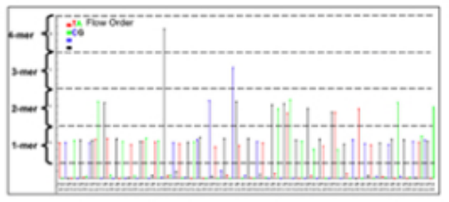
DNA Sequence Analysis:

Profiling DNA

Sanger Sequencing – >800 base pairs/run



High Throughput Genomic Sequencing – 100,000 base pairs/run

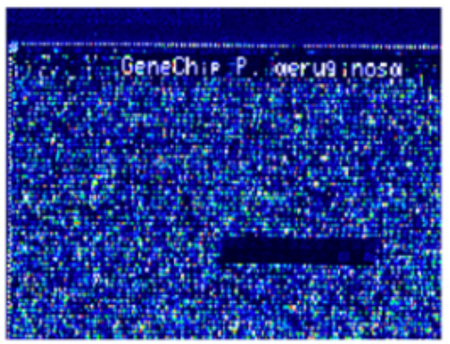


RNA

Expression

Microarray:Analysis

Profiling mRNA



Colour of pin-point dots demonstrates the presence / absence of gene sequences

GSU Biology Core Facility

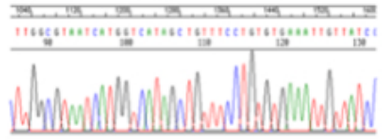
Supporting Life Sciences at GSU

http://biotech.gsu.edu/core_facility/index.html

Georgia State University

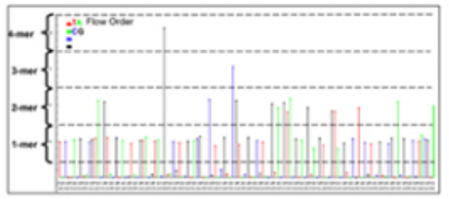
DNA Sequence Analysis:

Profiling DNA



Sanger Sequencing –
>800 base pairs/run

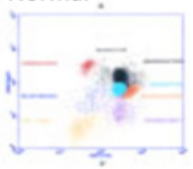
High Throughput Genomic Sequencing –
100,000 base pairs/run



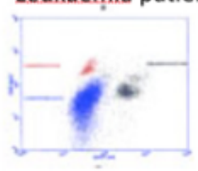
Flow Cytometry

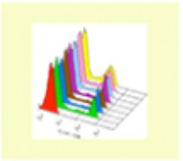
Profiling Cells

Normal



Leukaemia patient

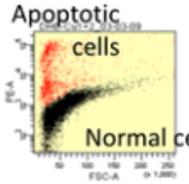




See effects of different drugs on Cell cycle

Apoptotic cells

Normal cells

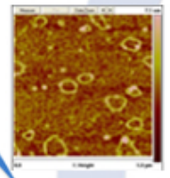


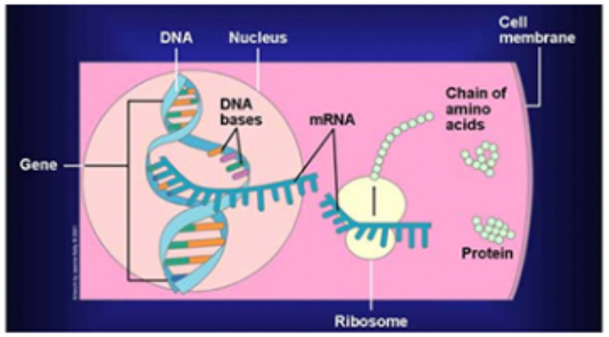
Apoptosis -programmed cell death

Atomic Force Microscopy

Imaging at the Ångström level

Protein structure analysis





DNA

Replication

RNA

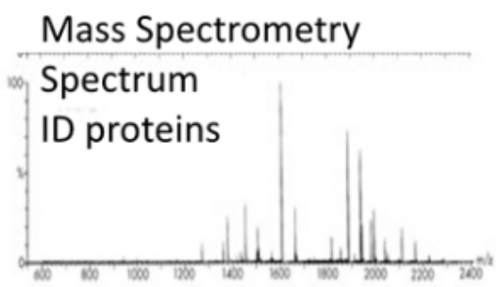
Expression

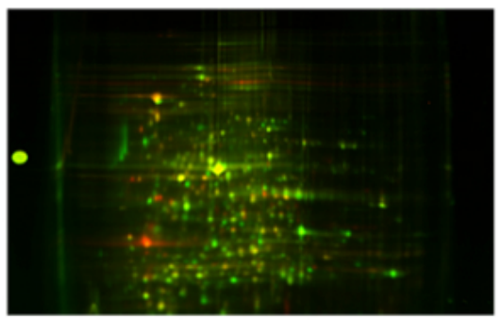
Cellular Functions

Mass Spectrometry

Spectrum

ID proteins





Protein

Expression

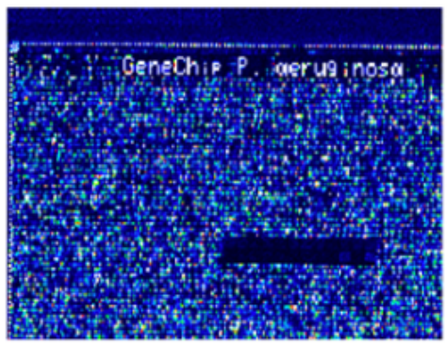
Proteomics

Profiling Proteins

2D Protein gel
Protein separation using
Electric charge and molecular weight

Microarray:Analysis

Profiling mRNA



Colour of pin-point dots demonstrates the presence / absence of gene sequences

Questions:

How long does the RNA “hybridization step” of the RNA to the chip bound DNA take in in Microarray Analysis? [“Microarray_1 video]

How many potential **Genes per Chip** are now available on a **10µM Feature Size** Genechip? [Microarray Lecture pdf]

What is the resolution limit of a light microscopy? [Microscopy Lecture pdf]

What is the resolution limit of an AFM? [Microscopy Lecture pdf]

What is “Abbe’s diffraction limit” ? Why is it important? [Microscopy Lecture pdf]

What is the HIGHEST magnification “objective lens” that we have on our BZX700 microscope? [“How to use the BZX700” video]

Which lasers do we have in the LSR Fortessa? [Cytometry in the ABCore (video)]? Why is this important?