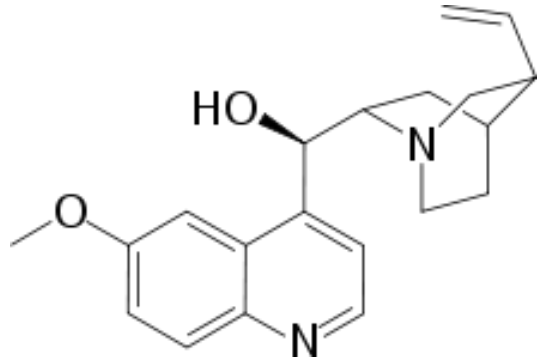
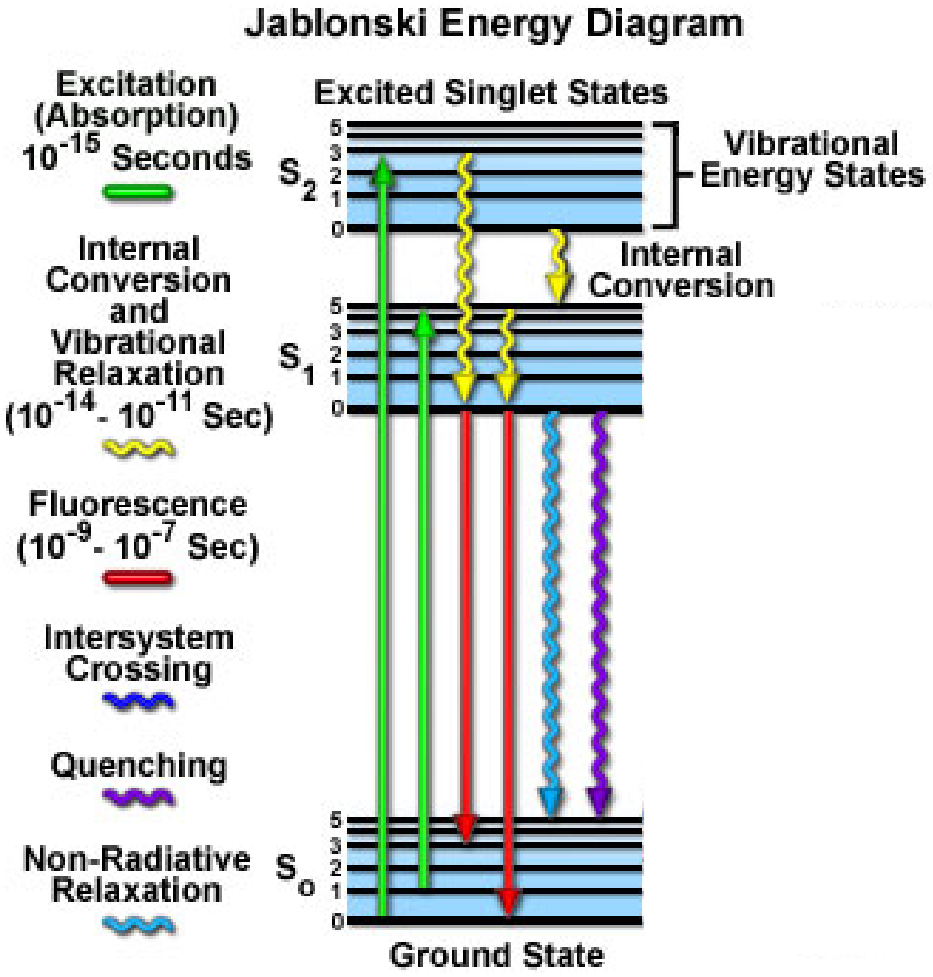
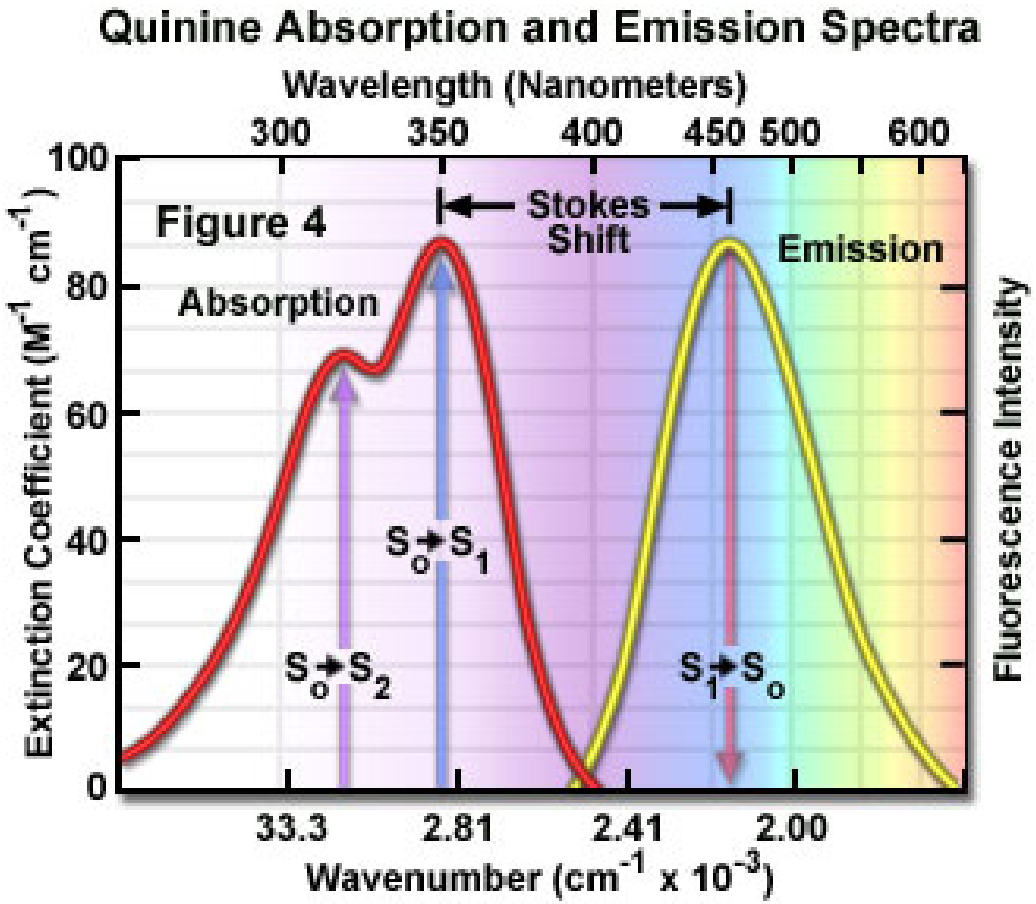
More on fluorescence

- Last class
  - Fluorescence
  - Absorption emission
  - Jablonski diagrams
- This class
  - More on fluorescence
  - Common fluorophores

Jablonski diagrams to spectra

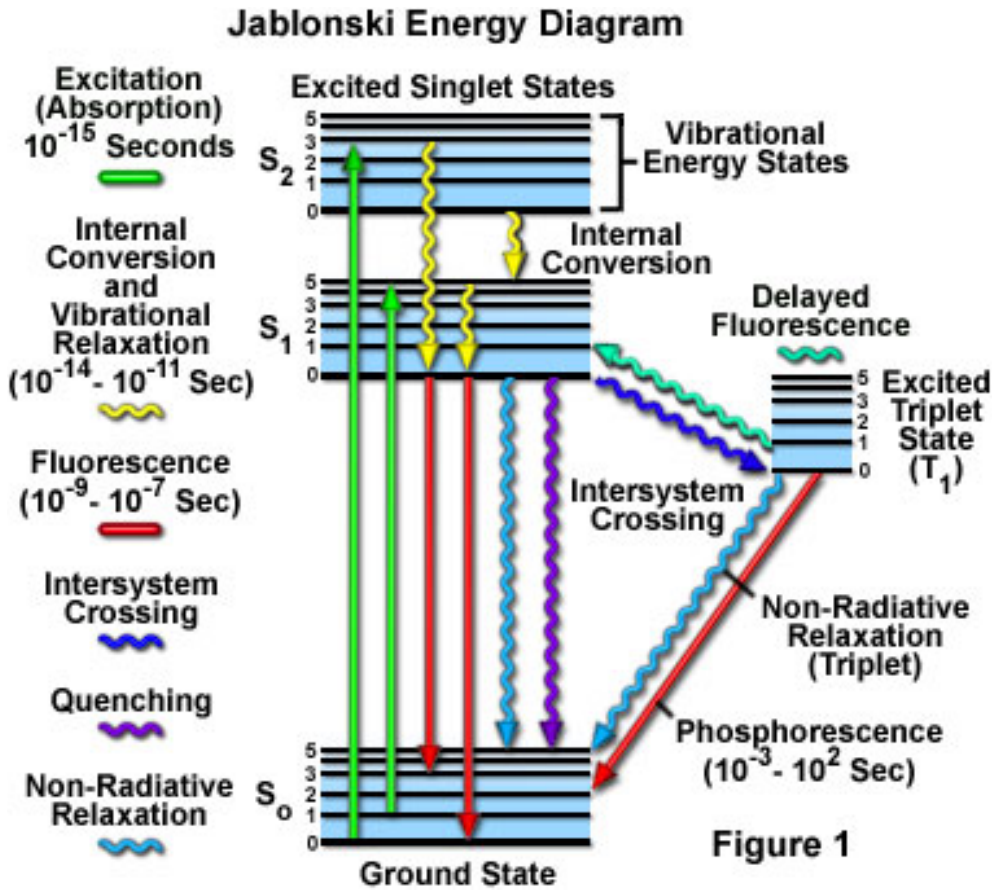






Properties of fluorophores

- Excitation max
- Emission max
- Spectrum breadth
- Molar extinction coefficient
- Quantum yield
- Photostability
- Photons per molecule
- Solubility



$$QY = \frac{\#Fl\ photons}{\#Abs\ photons}$$

$$QY = \frac{k_f}{k_f + k_{NR}}$$

Spectra of fluorophores

- Absorption – the ability to absorb photons at a given wavelength
- Excitation – ability to excite fluorescence at a given wavelength
- Emission – ability to emit photons at a given wavelength

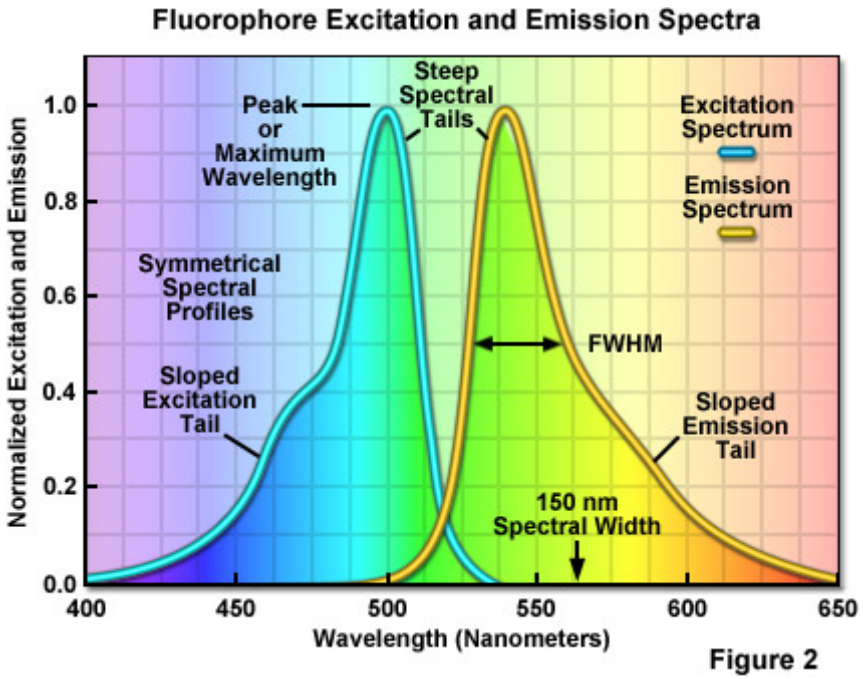
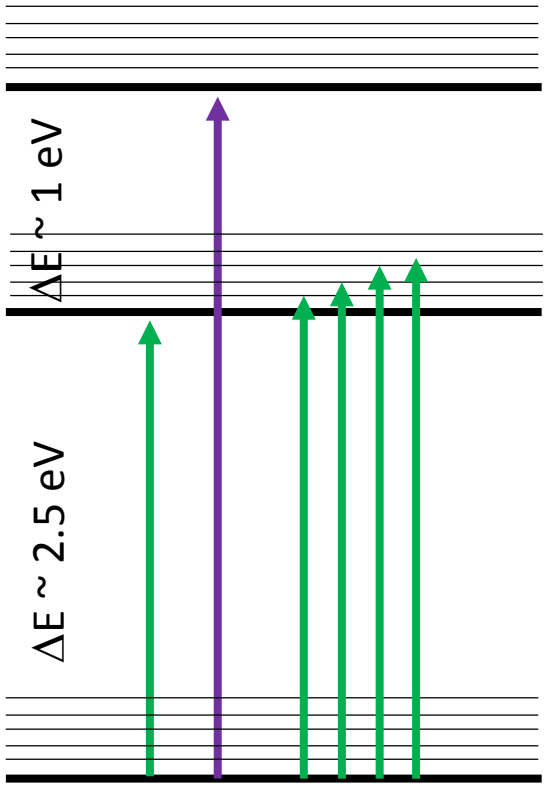
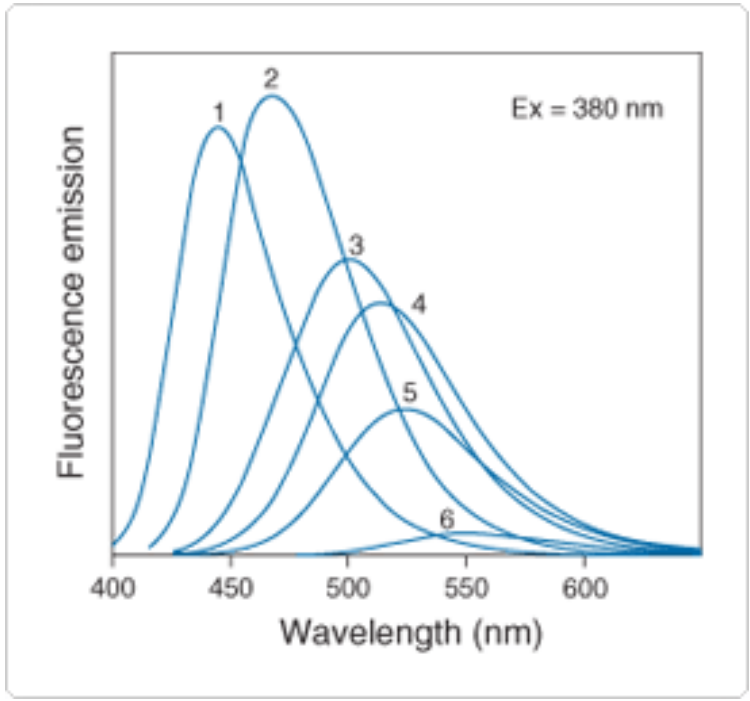


Figure 2



Fluorescence emission is determined by local environment

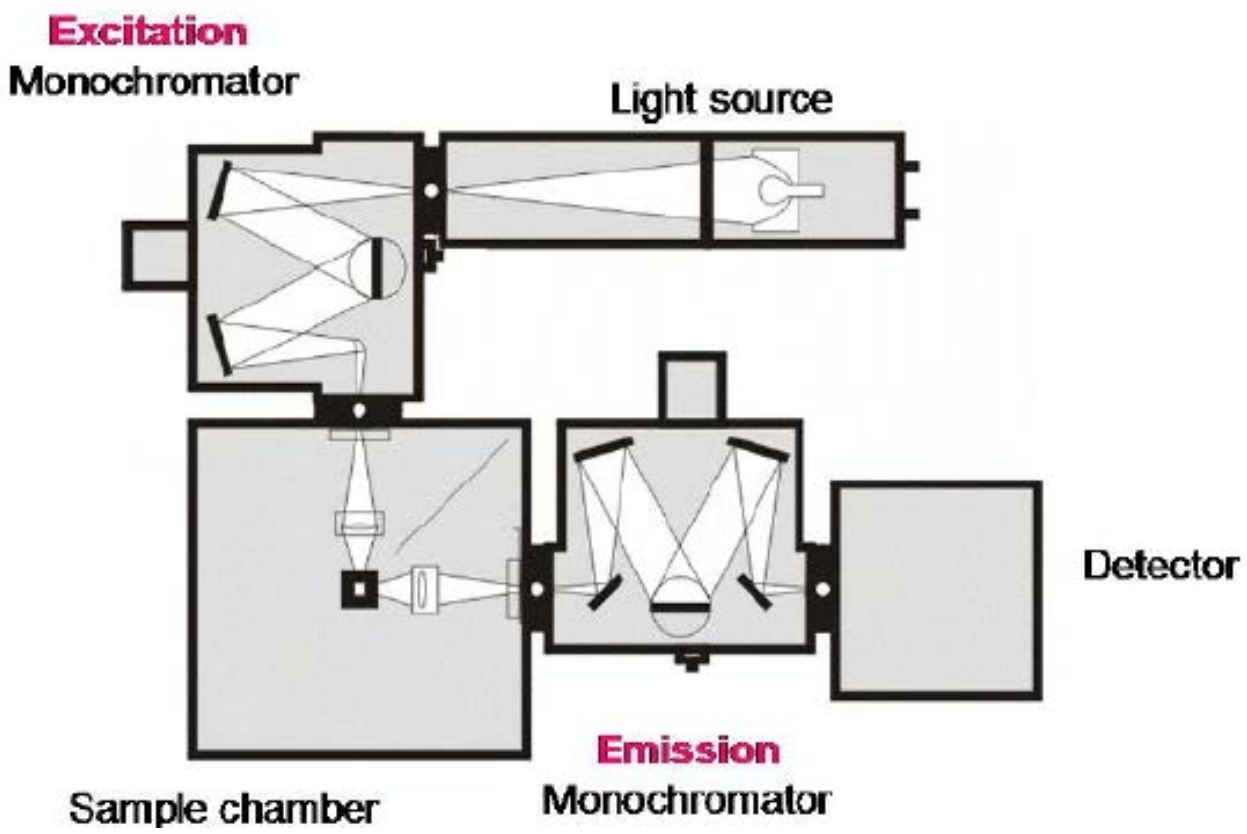
- Temperature
- pH
- Oxygen content
- Solvent
- Concentration of fluorophore



1) toluene, 2) chloroform, 3) acetonitrile, 4) ethanol, 5) methanol and 6) water

Brightness = ε * QY/1000

Measuring fluorescence



Start with known concentration of fluorophore

Run a quick excitation and emission scan

Compare to known fluorophore

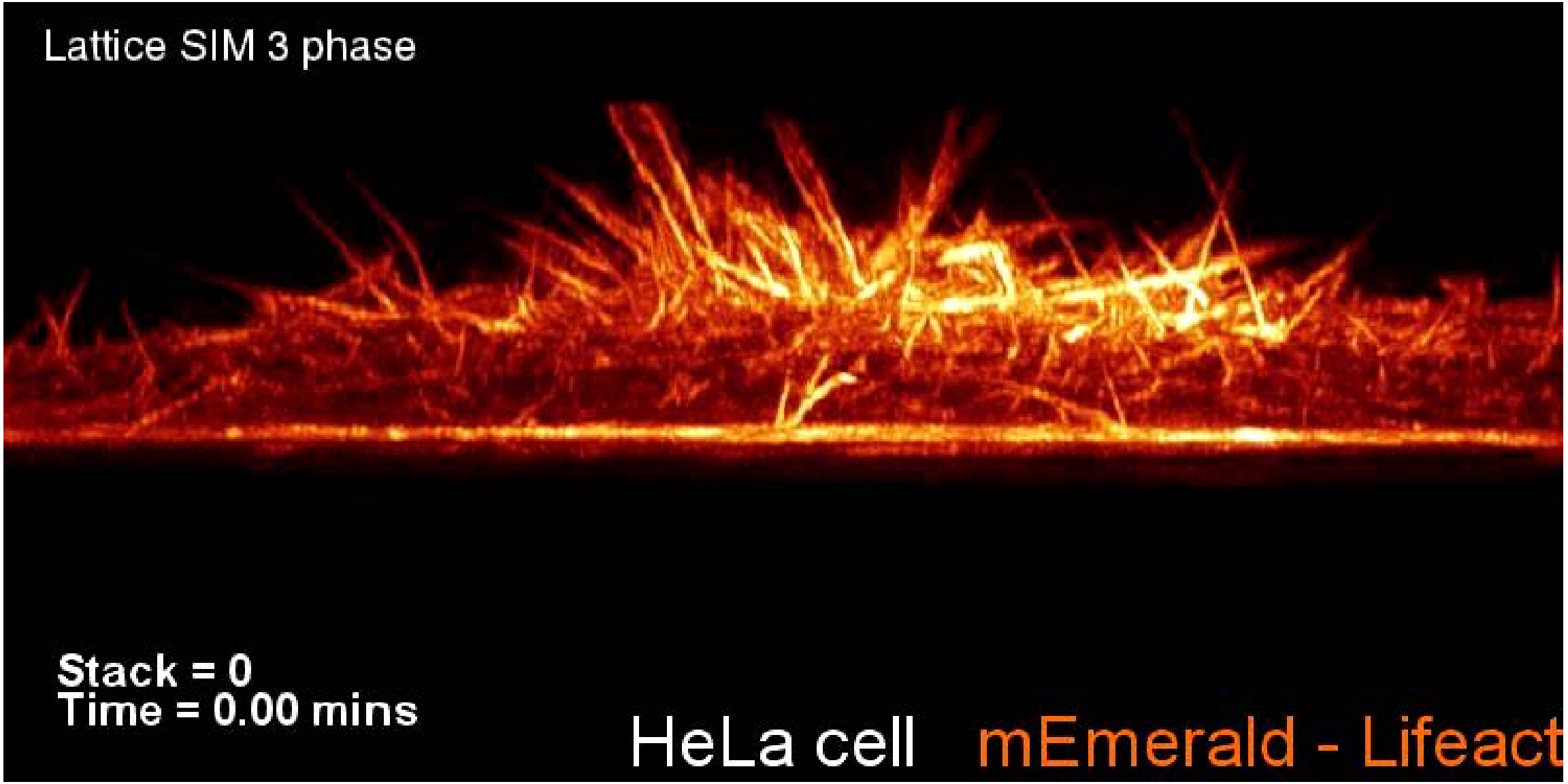
$$\frac{QY_1 abs_1}{em_1} = \frac{QY_2 abs_2}{em_2}$$

Abs = absorbance at specific wavelength
Em = integrated emission
QY = quantum yield

Fluorescence imaging

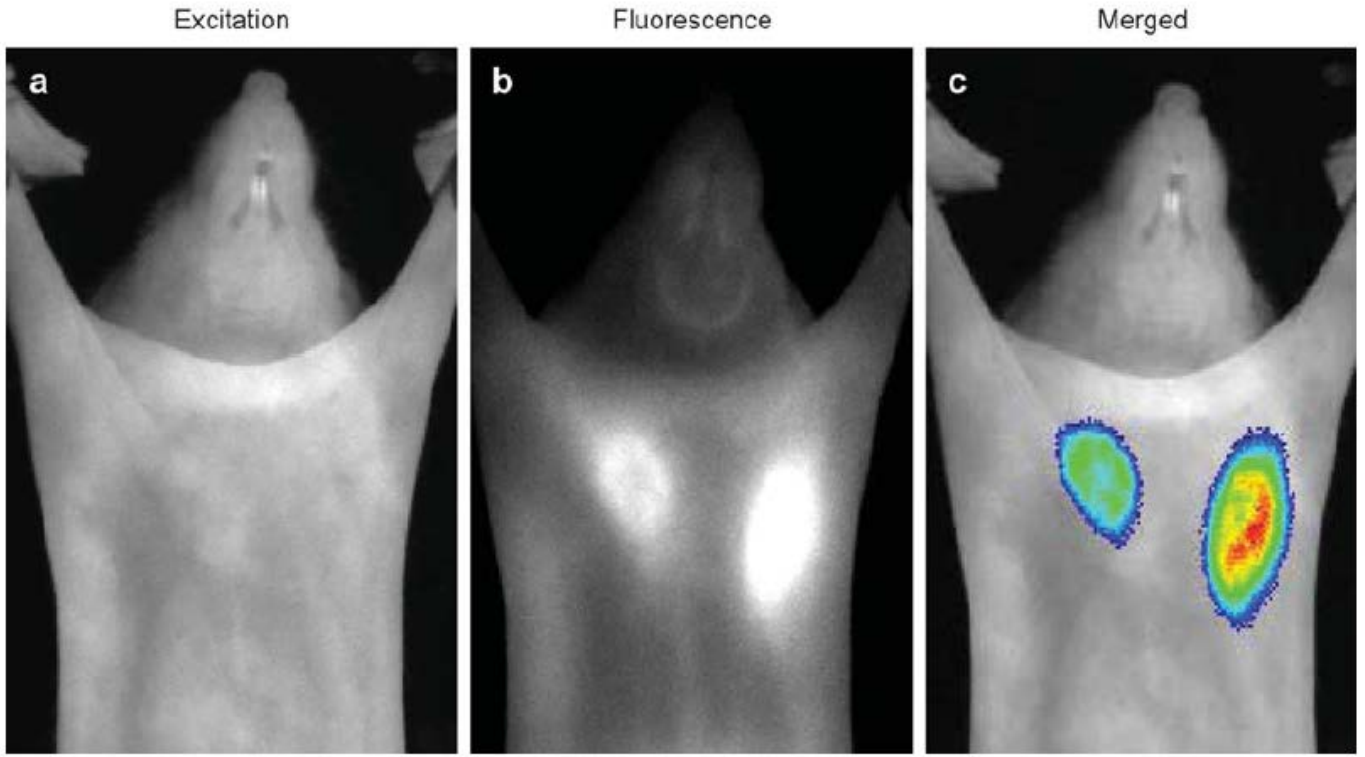
- Why do we use it so much?
- Color separation gives it power
- We can use filters to look for ONLY
  the emitted light
- Low background -> High contrast
- Works in live cells
- Small sizes
- We are good at dealing with the visible spectrum

$$C = \left[\frac{Ib - Is}{Ib}\right] * 100\%$$

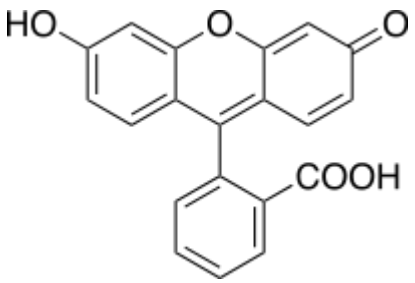

Lattice SIM 3 phase
Stack = 0
Time = 0.00 mins
HeLa cell mEmerald - Lifeact

Applications of fluorescence in biology

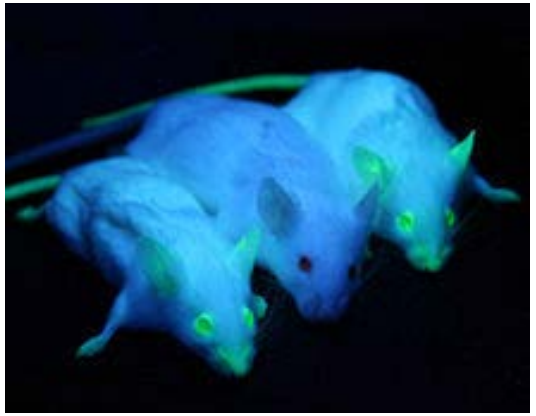
- Intracellular distribution
- Intracellular dynamics
- Protein interactions
- Intracellular sensing
- Organelle marking/status
- Enzyme reactions
- Cell physiology
- Neuronal tracing
- Cell fate measurements
- Cell tracking
- Cell cycle



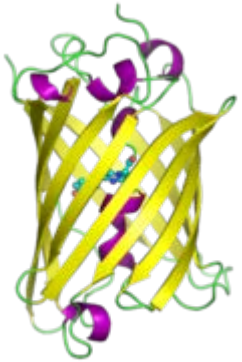
Types of fluorophores



Dyes



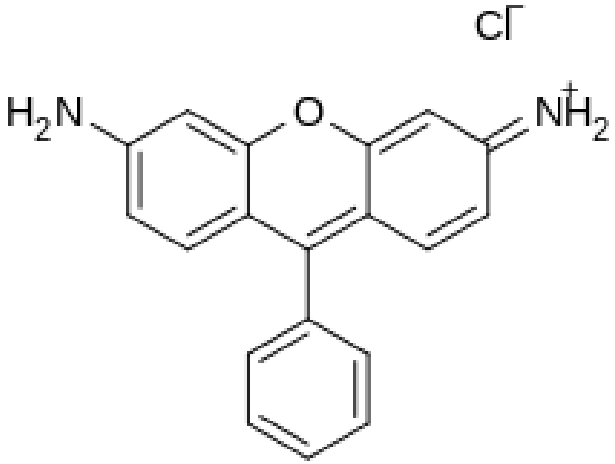
Proteins



Quantum dots

Dyes

- Must have extended polar electron chain
- Extended electron density



Cheap, common

	Excitation	Emission
DAPI	358	461
FITC	495	519
TRITC	558	576
Texas red	589	615
Cy3	550	570
Cy5	650	670
Cy7	743	767

Metrics

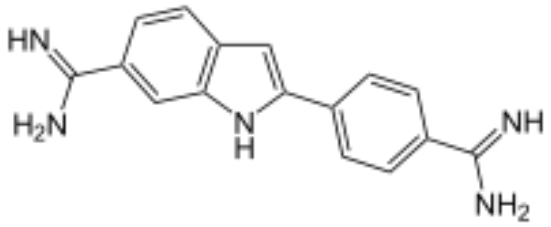
1. Excitation/emission
2. Quantum yield
3. Brightness
4. Photostability
5. Toxicity
6. Solubility
7. Permeability

Alexa Fluor series

	Color	Absorb	Emit	MM	ε	Quantum Yield [6]
Alexa Fluor 350	blue	346	442	410	19,000	-
— 405	violet	401	421	1028	35,000	-
— 430	green	434	541	702	15,000	-
— 488	cyan-green	495	519	643	73,000	0.92
— 500	green	502	525	700	71,000	-
— 514	green	517	542	714	80,000	-
— 532	green	532	554	721	81,000	0.61
— 546	yellow	556	573	1079	112,000	0.79
— 555	yellow-green	555	565	~1250	155,000	0.1
— 568	orange	578	603	792	88,000	0.69
— 594	orange-red	590	617	820	92,000	0.66
— 610	red	612	628	1172	144,000	-
— 633	Far-red	632	647	~1200	159,000	-
— 635	Far-red	633	647	-	140,000	-
— 647	Far-red	650	665	1155.06[7]	270,000	0.33
— 660	Near-IR	663	690	~1100	132,000	0.37
— 680	Near-IR	679	702	~1150	183,000	0.36
— 700	Near-IR	702	723	~1400	205,000	0.25
— 750	Near-IR	749	775	~1300	290,000	0.12
— 790	Near-IR	782	805	-	260,000	-

DAPI – nuclear stain

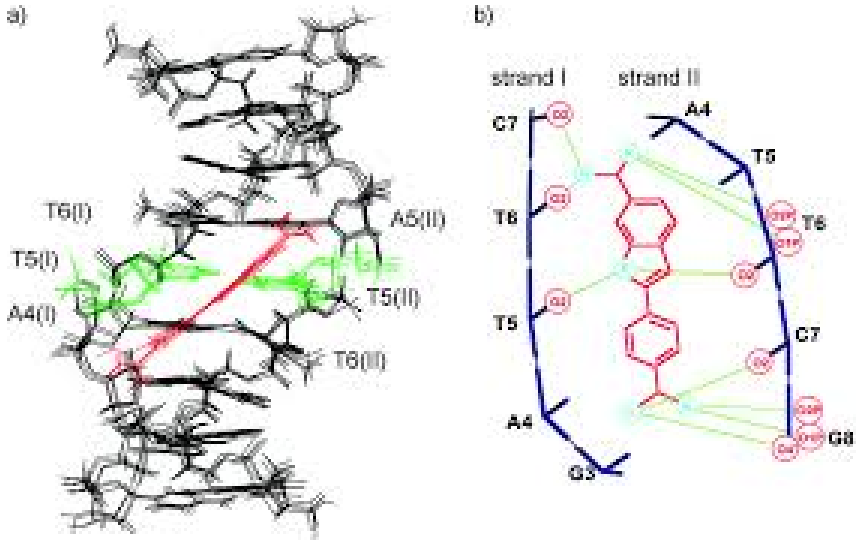
(**4',6-diamidino-2-phenylindole**)

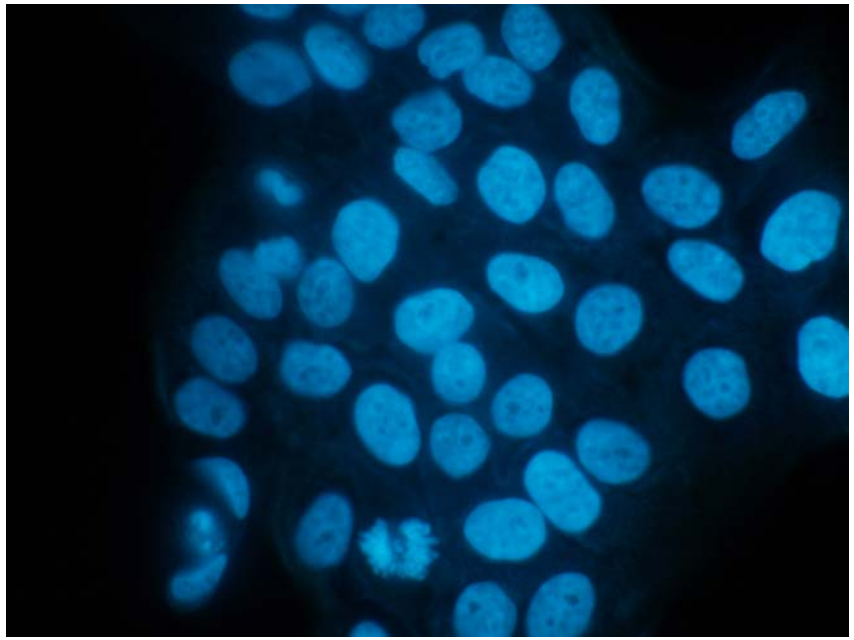


Binds in AT rich regions of DNA
Increases fluorescence upon nucleic acid binding

Excitation/Emission – UV/blue

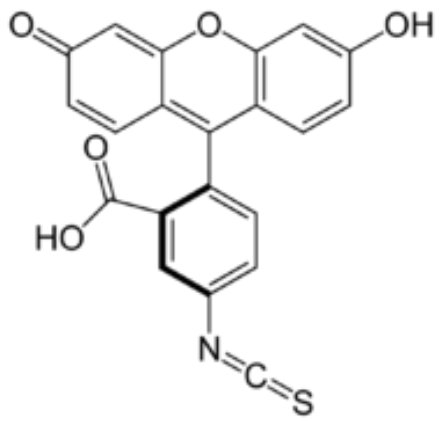
Very common to label nuclei in fixed cells. Can be used in live cells, but toxic

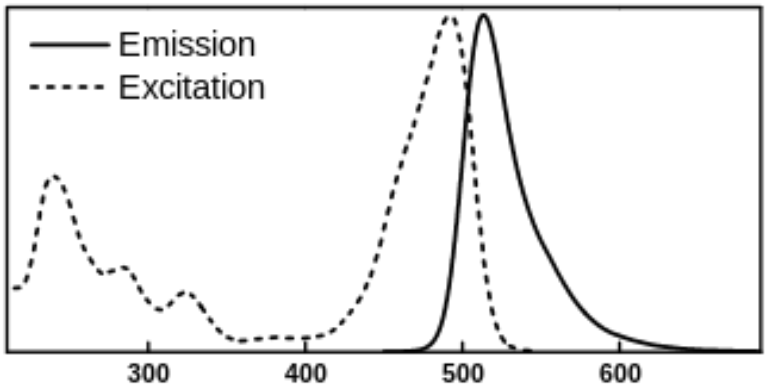




Laser line = 405 nm

FITC - fluorescein





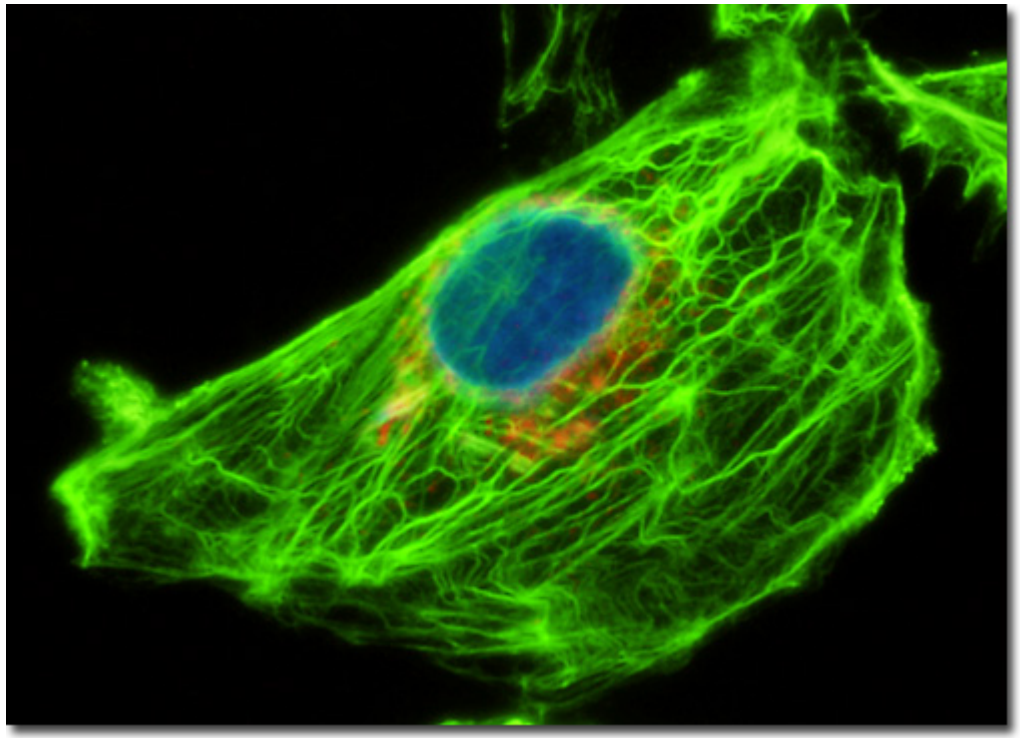
Laser line = 488 nm

Excitation/Emission – 495/515 nm

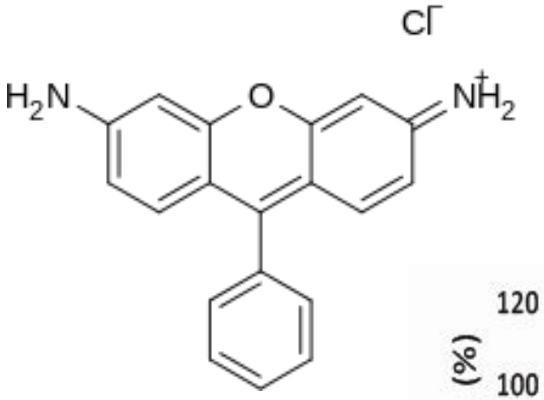
Very common fluorescent dye – super cheap. Similar spectrum to GFP.

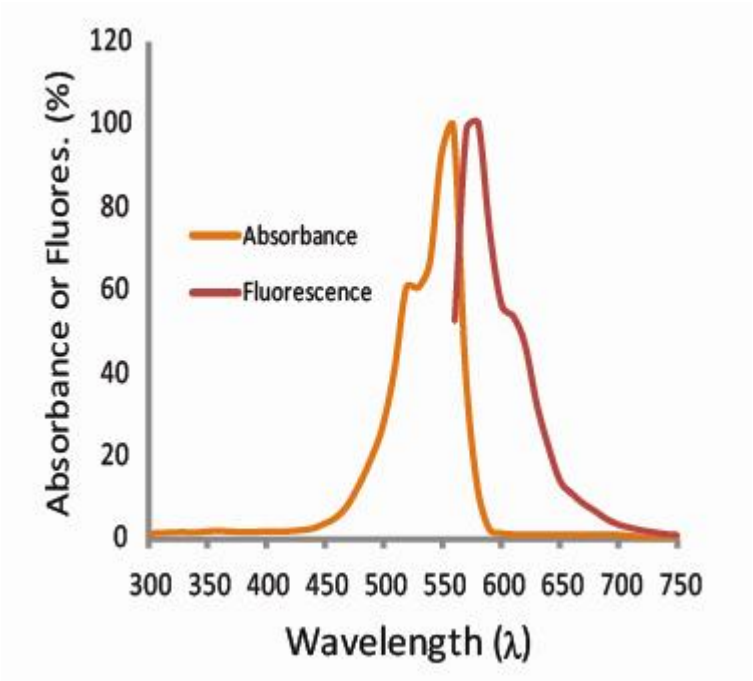
Highly sensitive to pH, not very photostable

Common in immunofluorescence



TRITC - tetramethylrhodamine





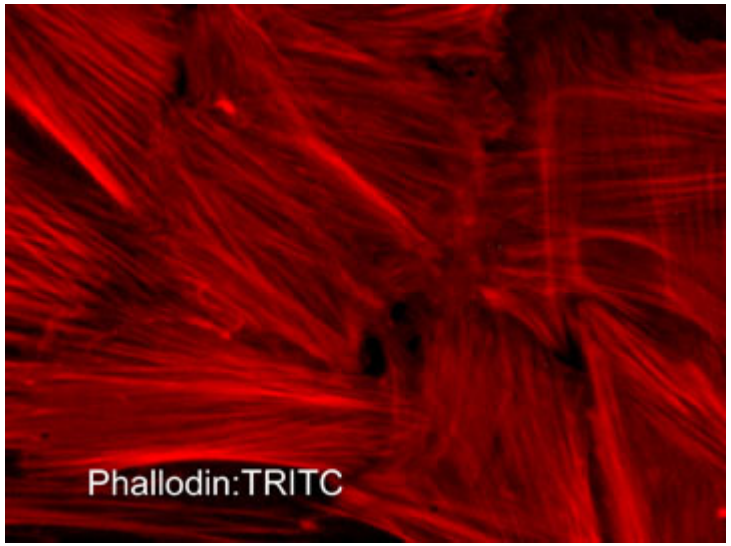
Laser line = 532 nm, 561 nm

Excitation/Emission – 550/579 nm

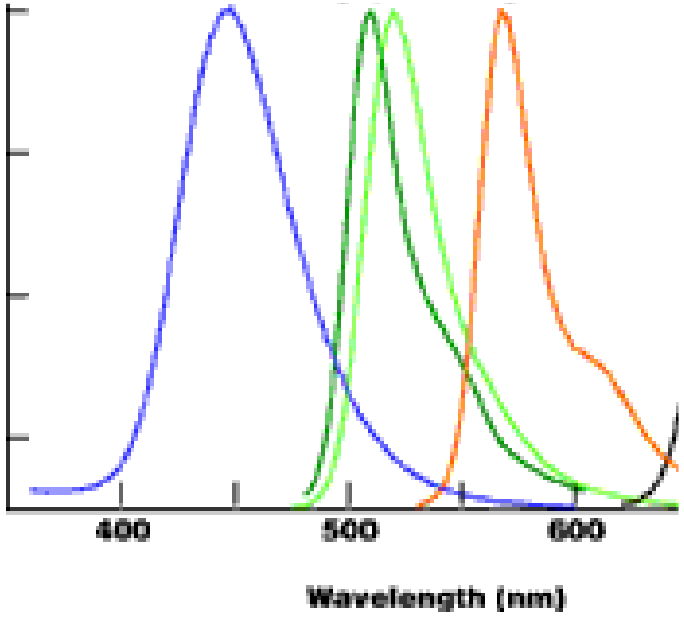
Very common fluorescent dye – super cheap. Similar spectrum to mCherry.

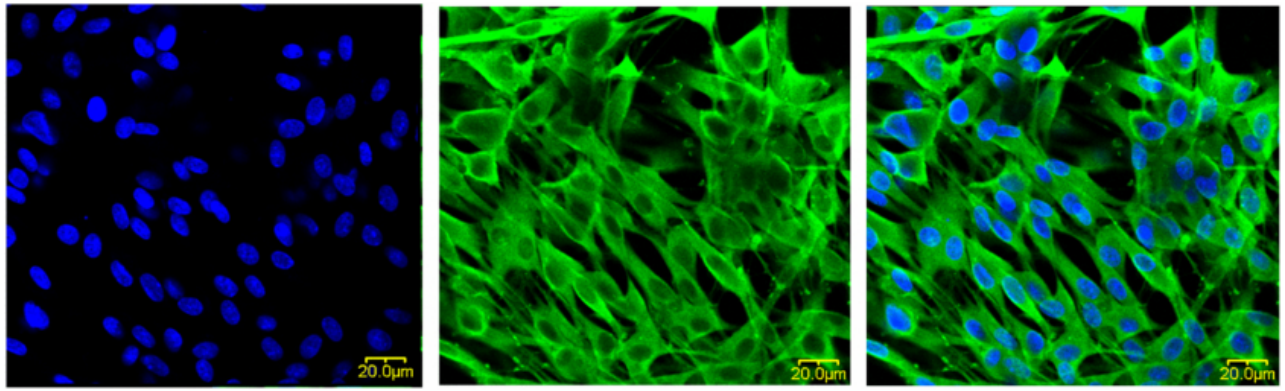
Not very photostable

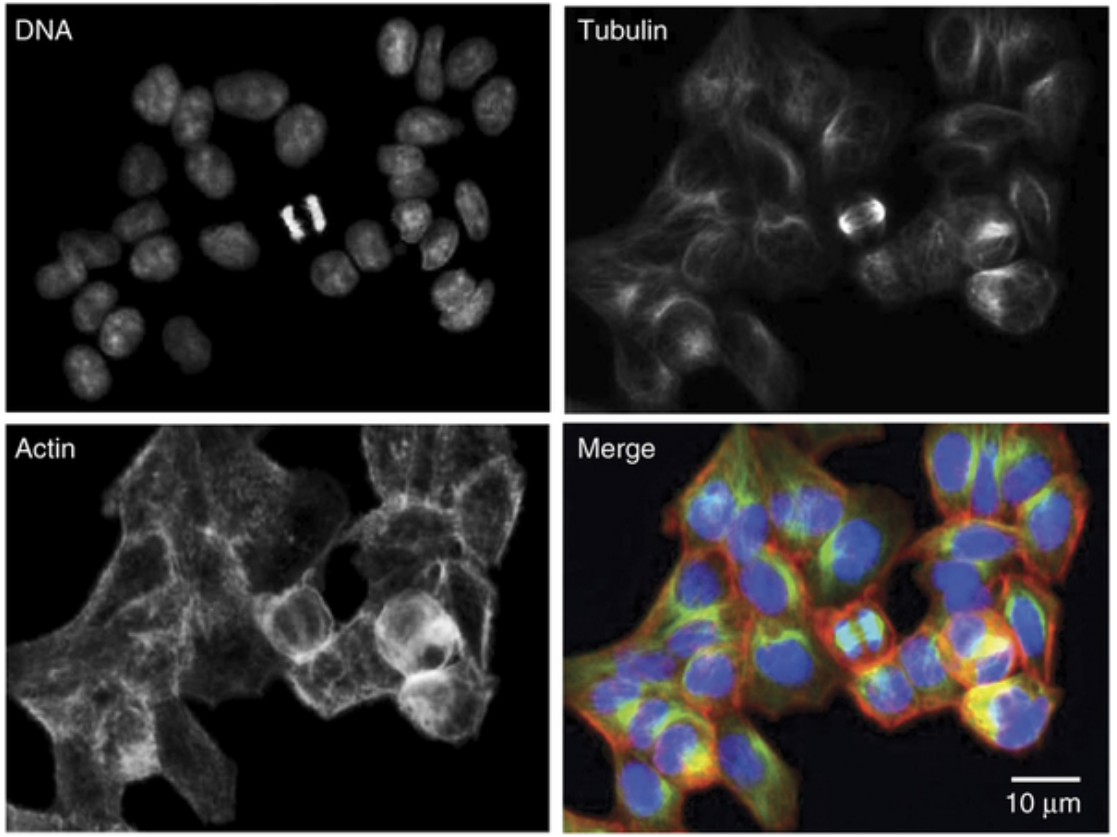
Common in immunofluorescence



DAPI-FITC-TRITC are a common set

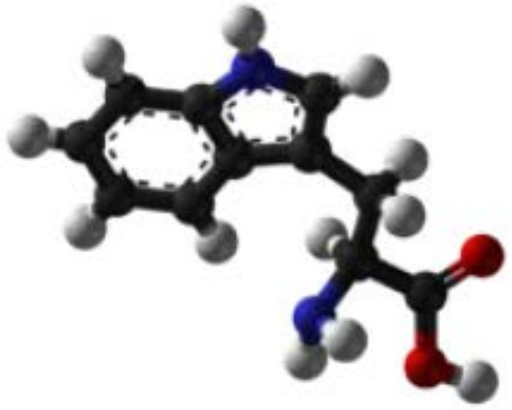


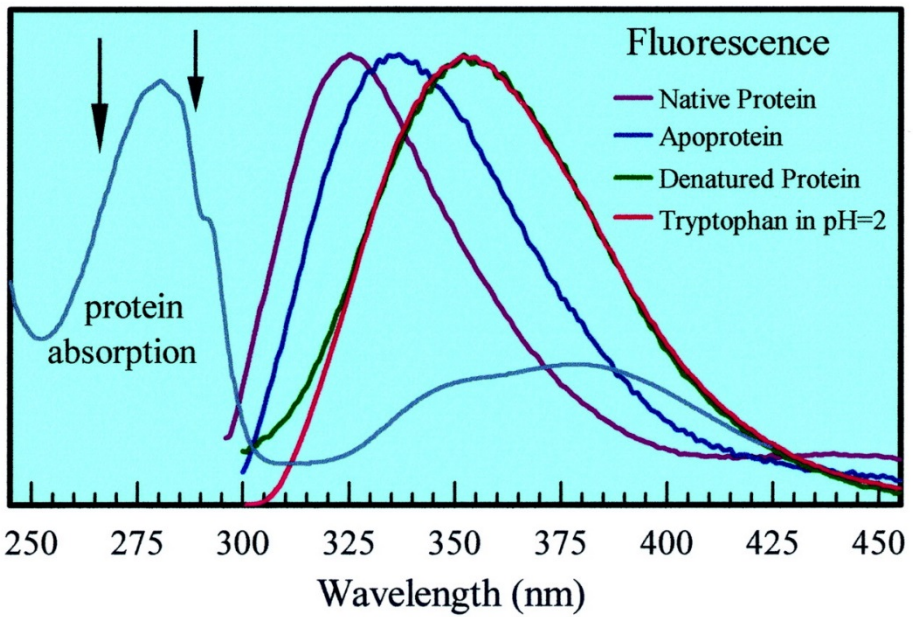


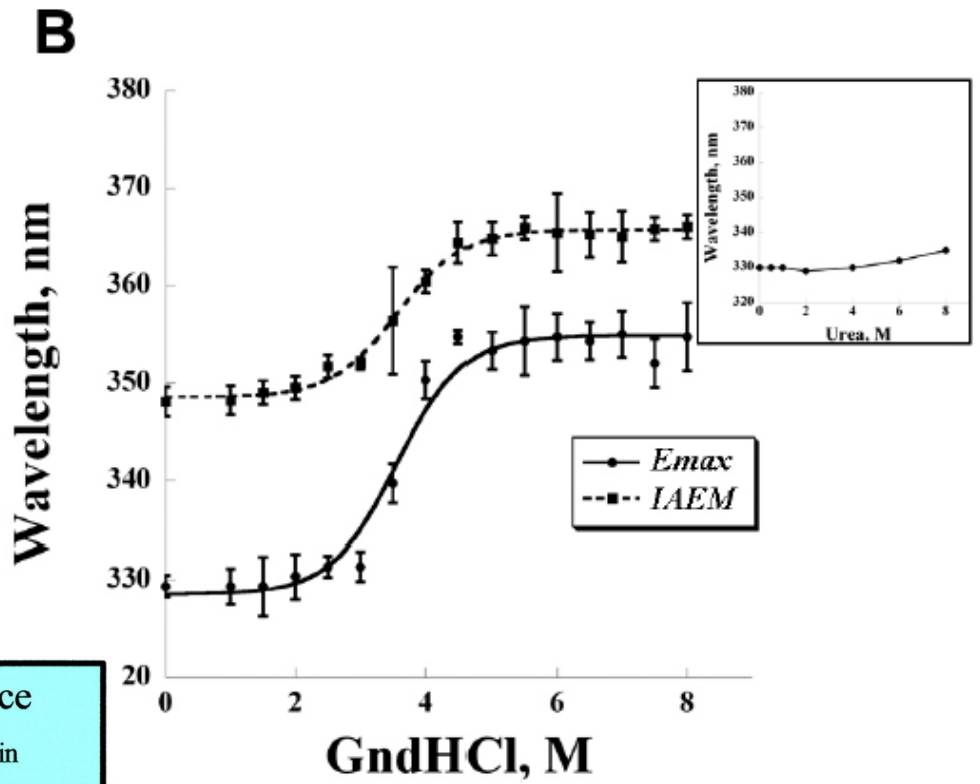


Tryptophan fluorescence

- Aromatic amino acid
- Emits at 330 nm in hydrophobic environment
- Emits at 365 nm in polar environments
- Can be used to track protein folding

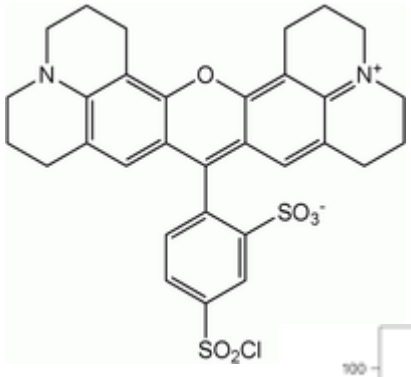


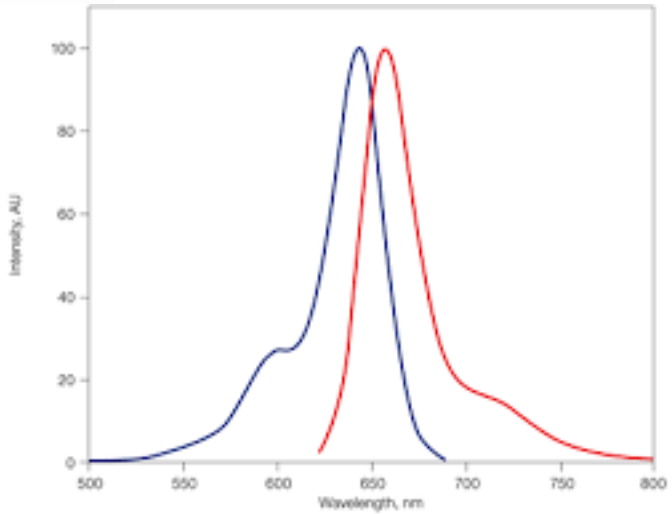




Texas Red

Sulforhodamine 101





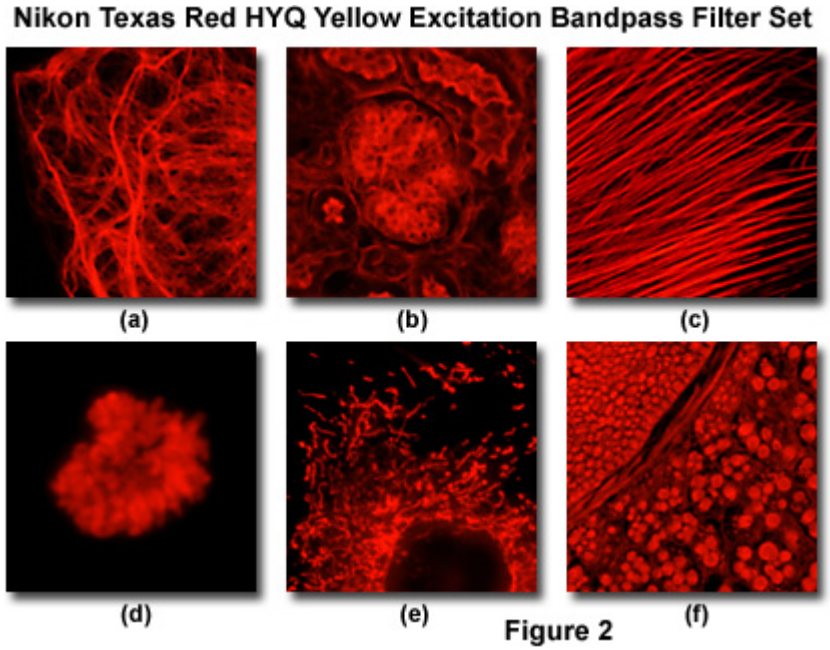
Laser line = 594 nm

Excitation/Emission – 590\610 nm

Common fluorescent dye
Somewhat photostable

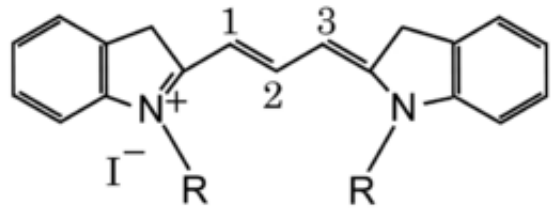
Common in immunofluorescence

Used to extend range of visible light,
but can't be used with TRITC

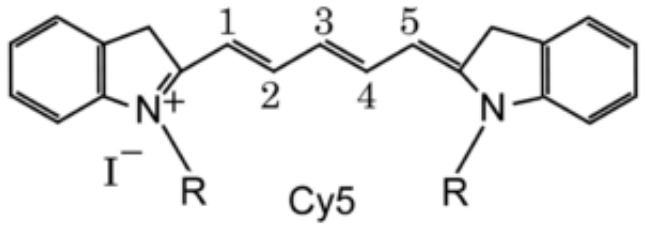


Cyanine variants

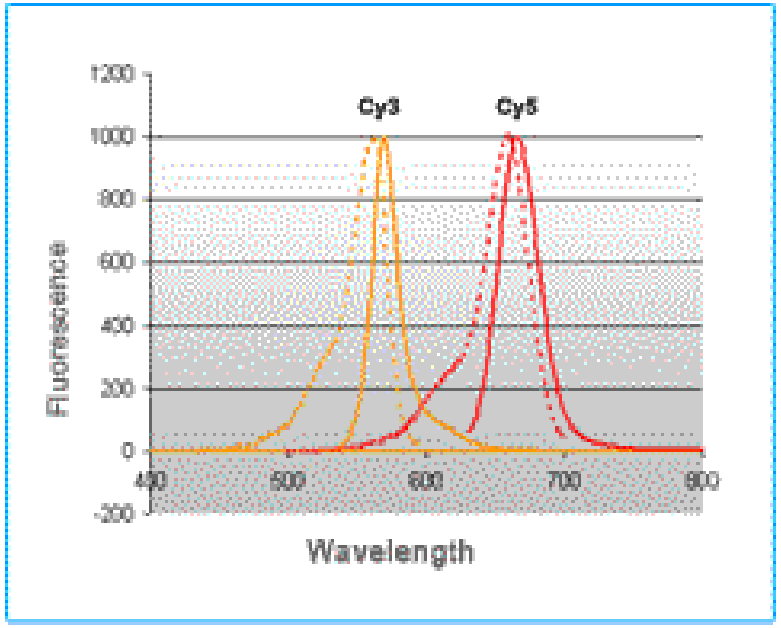
Probe	Ex (nm)	Em (nm)	MW	Quantum yield
Cy2	489	506	714	QY 0.12
Cy3	(512);550	570;(615)	767	QY 0.15 [5]
Cy3B	558	572;(620)	658	QY 0.67
Cy3.5	581	594;(640)	1102	QY 0.15
Cy5	(625);650	670	792	QY 0.27[5]
Cy5.5	675	694	1128	QY 0.28[6]
Cy7	743	767	818	QY 0.28



Cy3



Cy5



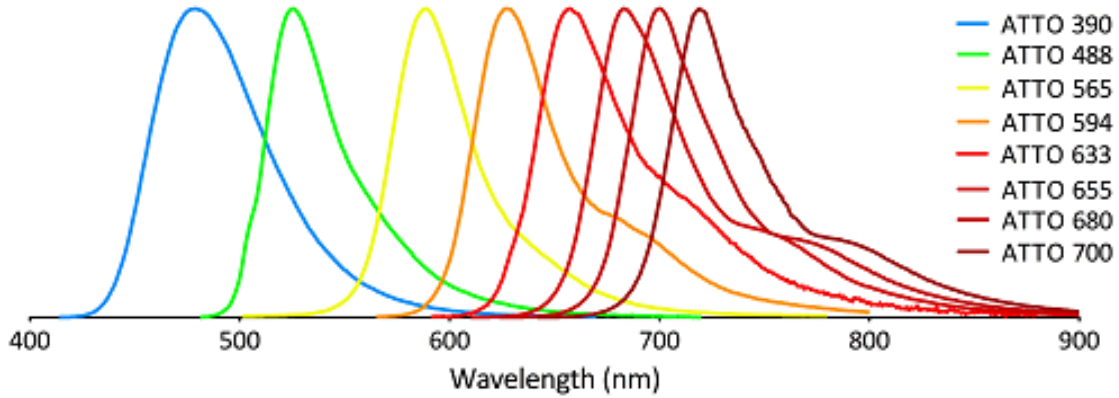
Cy3 is a newer TRITC equivalent

Cy3 and Cy5 are by far the most common

Cy5 can add a 4th color:
DAPI, FITC, TRITC (or Cy3), Cy5

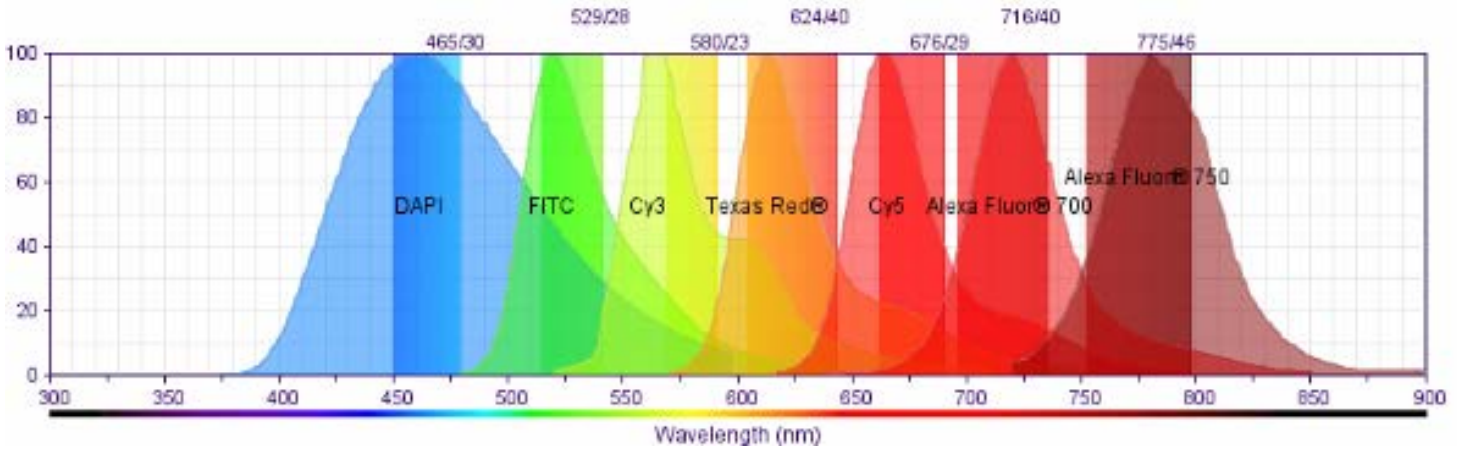
Modern variants

- Cyanine dyes
- Alexa fluor series
- Atto series
- Dylight series



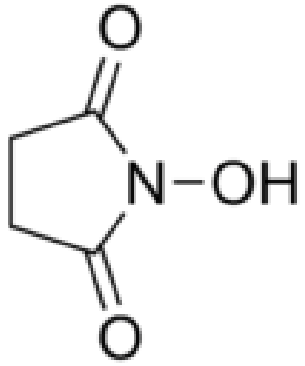
More money =

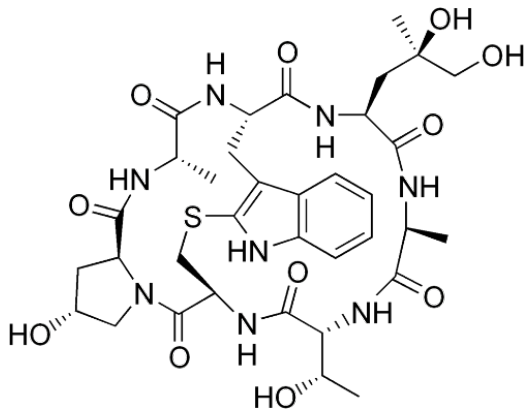
1. Higher brightness
2. Photostability
3. Water solubility
4. Broad range of colors

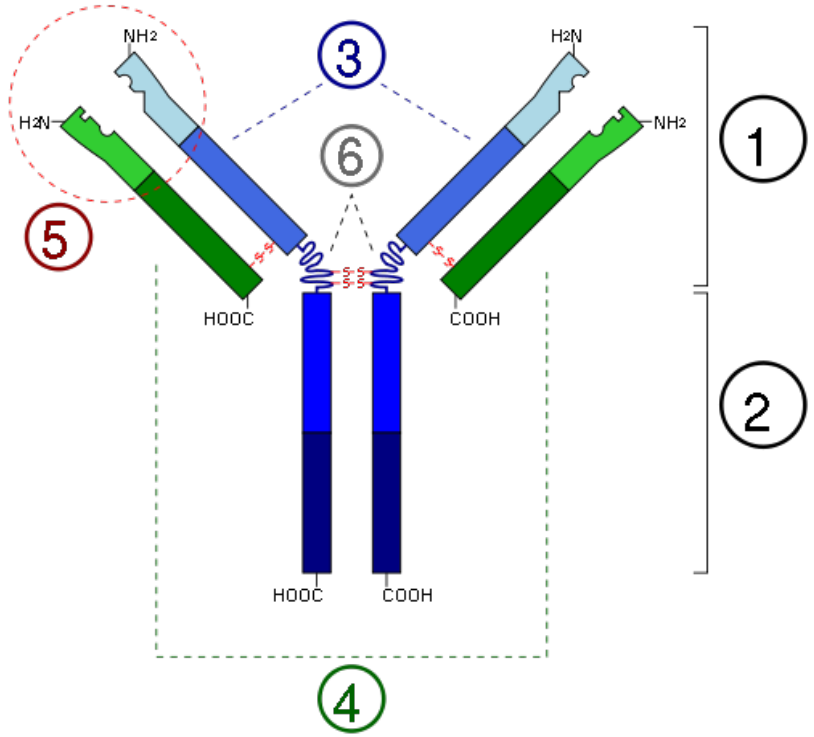


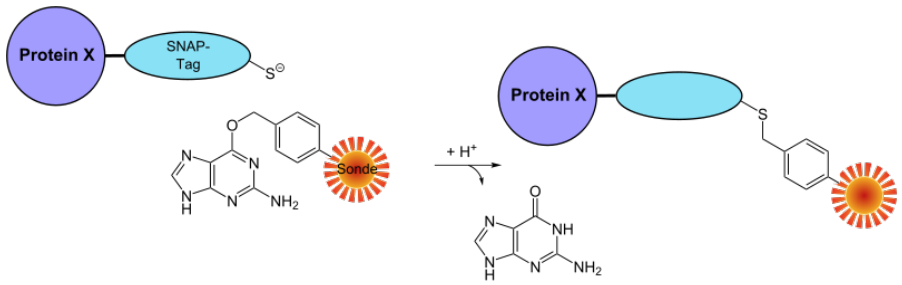
Targeting dyes

- NHS ester or maleimide – react to proteins
- Antibody conjugates
- Toxin conjugates (phalloidin)
- SNAP CLIP HALO tags









And on to Matlab…