MICRO-561

Fundamentals of Biomicroscopy

Syllabus (tentative)

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Lecture 2	Ray Optics-2 & Matrix Optics-1
Lecture 3	Matrix Optics-2
Lecture 4	Matrix Optics-3 & Microscopy Design-1
Lecture 5	Microscopy Design-2
Lecture 6	Microscopy Design-3 & Resolution -1
Lecture 7	Resolution-2
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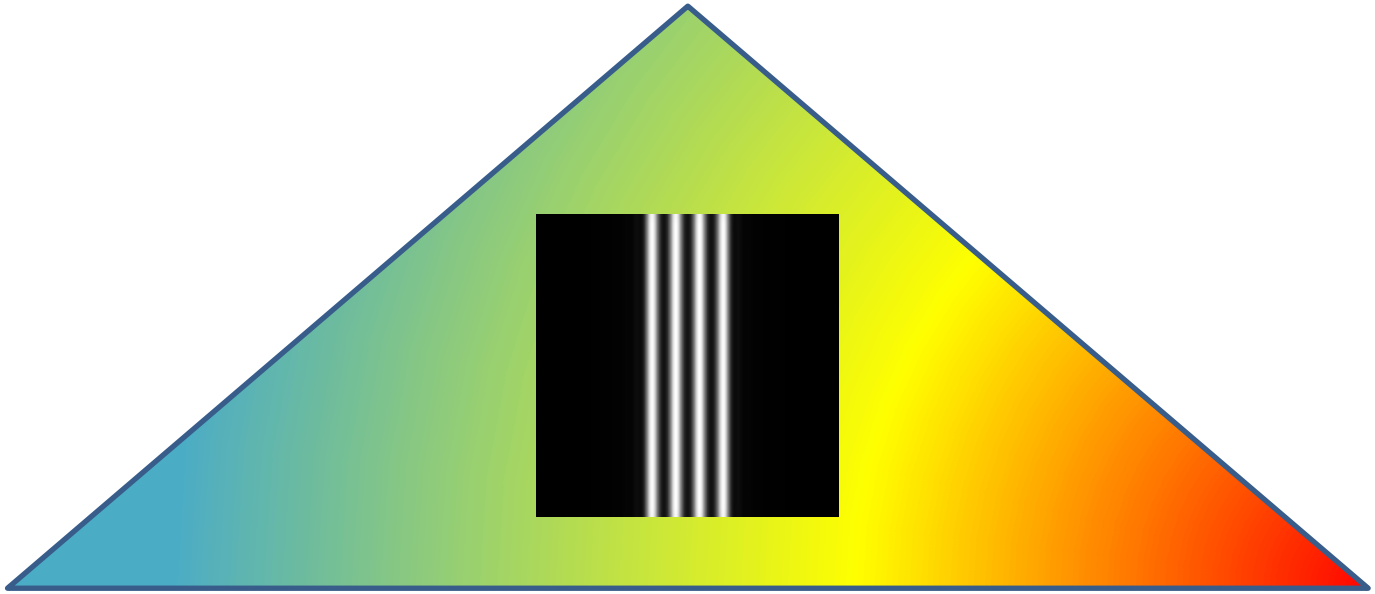
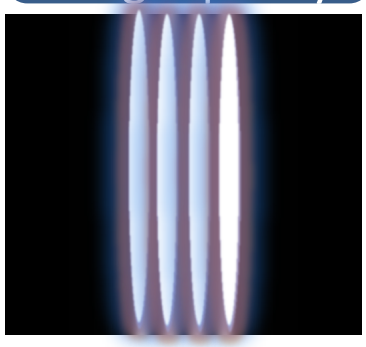
Lecture 9	Contrast & Fluorescence -1
Lecture 10	Fluorescence-2
Lecture 11	Fluorescence-3
Lecture 12	Sources, Filters
Lecture 13	Detectors
Lecture 14	Bio-application Examples

Important aspects in microscopy

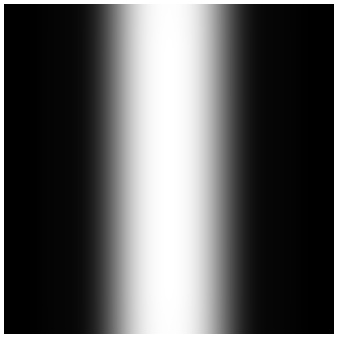
Magnification



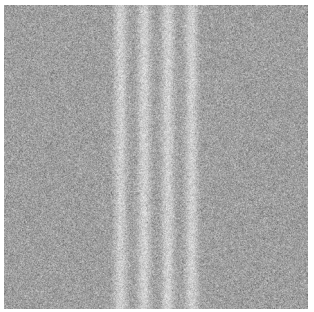
Aberrations – image quality



Resolution



Contrast



Fluorescence microscopy

- **In order to understand basic fluorescence microscopy, we will discuss:**
  - **Principles of fluorescence emission & excitation**
  - Properties of fluorescent dyes
  - Different types of fluorescence markers
  - The important optical components
    - Filters and filter sets
    - Excitation Sources
    - Detectors
    - Also, their proper positioning in the optical train of the microscope

Summary: Important Fluorescence Terms

Excitation (absorption) spectrum → its peak gives the excitation (absorption) wavelength

Emission spectrum → its peak gives emission (fluorescence) wavelength

Stokes shift: The difference in wavelength between the excitation & emission peak wavelengths

Extinction coefficient: A measure of how much light will be absorbed by a given dye/probe concentration and specimen thickness.

Quantum efficiency (yield): Ratio of light absorbed to fluorescence emitted → 0 - 1 (0 - 100%)

Brightness: extinction coefficient * quantum yield

Fluorescence life-time: decay time of a photo-excited fluorophore from excited state to the ground state

Additional Important Fluorescence Terms

Blinking:

- Occurs during continuous excitation of a fluorescent molecule where the emission transitions between “on” and “off” states, like twinkling stars in night.
- The exact underlying mechanism is not well understood.

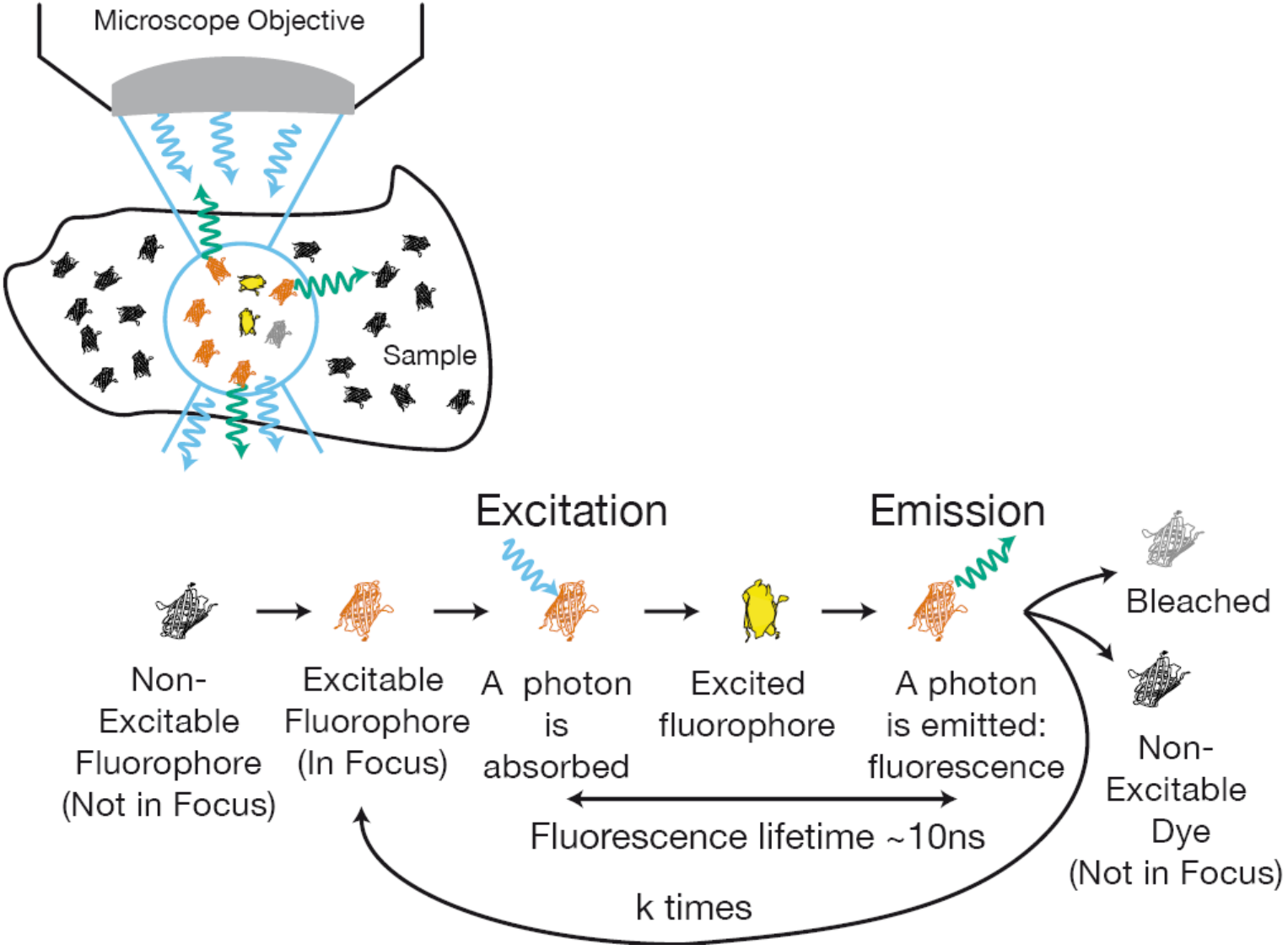
Quenching:

- Arises from variety of competing processes that induces non-radiative relaxation (i.e. without photon emission) of excited state electrons to the ground state.
- These non-radiative transition pathways compete with the fluorescence relaxation, thus resulting in reduced or even complete elimination of the emission.
- A wide variety of basic elements and compounds can behave as quenching agents (i.e. O2, halogens, amines, some polymers, many organic molecules..)

Photo-bleaching (also termed as *fading*):

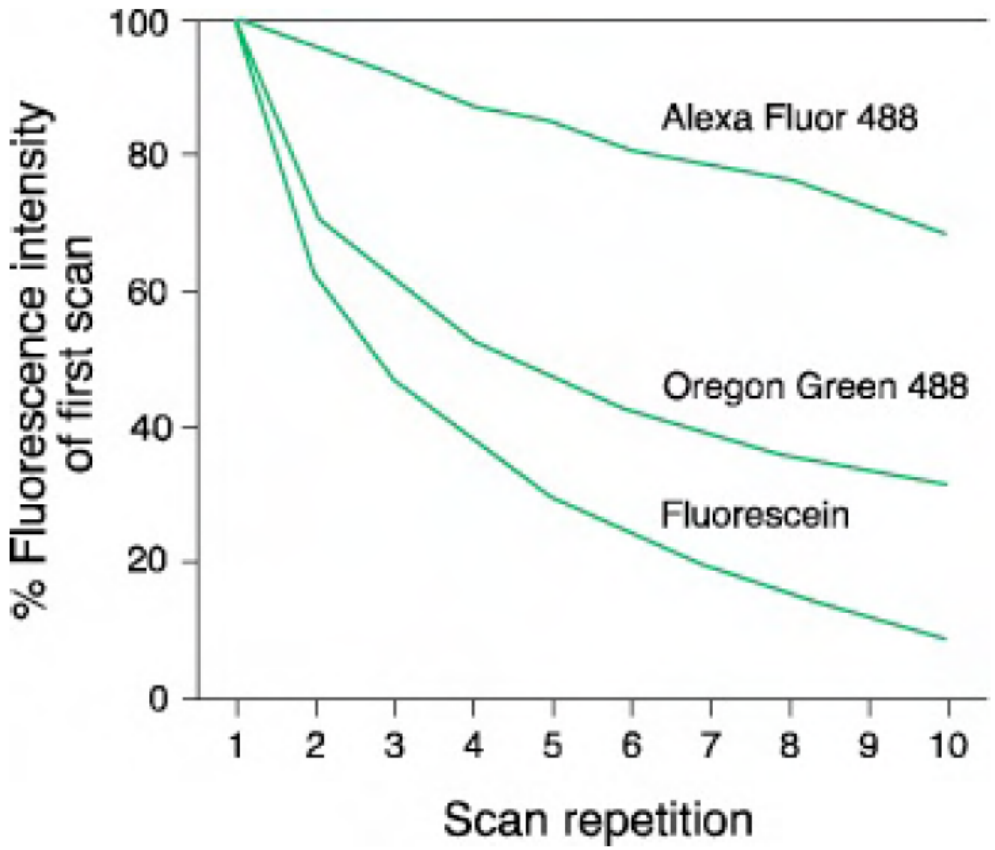
- In contrast to blinking & quenching, photo-bleaching occurs when a fluorophore **permanently** loses its ability to fluoresce due to photon-induced chemical damage and covalent modification.
- The average number of excitation & emission cycles that occur for a particular fluorophore before photo-bleaching is dependent upon the molecular structure & the local environment.
- Some fluorophores can bleach quickly after emitting only a few photons, while others can be more robust and undergo thousands or even millions of cycles before bleaching

Cycle of a fluorophore



From Michael Liebling, UCSB

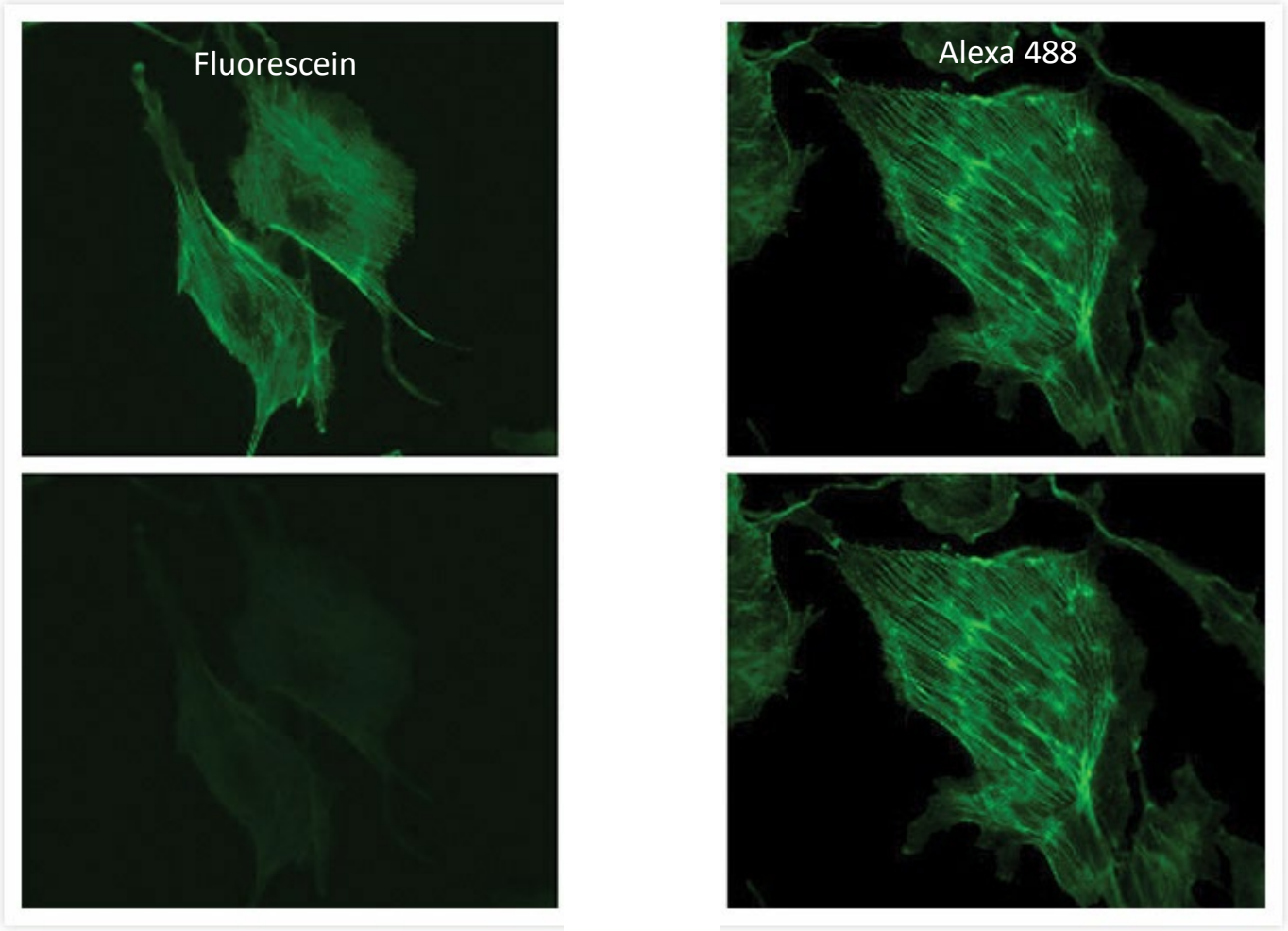
Photobleaching



Photobleaching resistance of the green-fluorescent Alexa Fluor 488, Oregon Green 488 and fluorescein dyes, as determined by laser-scanning cytometry. EL4 cells were labeled with biotin-conjugated anti-CD44 antibody and detected by Alexa Fluor 488 (S11223), Oregon Green 488 (S6368) or fluorescein (S869) streptavidin (Section 7.6). The cells were then fixed in 1% paraformaldehyde, washed and wet-mounted. After mounting, cells were scanned 10 times on a laser-scanning cytometer; laser power levels were 25 mW for the 488 nm spectral line of the argon-ion laser. Scan durations were approximately five minutes apiece, and each repetition was started immediately after completion of the previous scan. Data are expressed as percentages derived from the mean fluorescence intensity (MFI) of each scan divided by the MFI of the first scan. Data contributed by Bill Telford, Experimental Transplantation and Immunology Branch, National Cancer Institute.

A good dye is more photo-stable, thus it photo-bleaches less.

Photobleaching



after
30 seconds

- Bovine pulmonary artery endothelial cells (BPAEC) were labeled with fluorescein phalloidin (left panels, Cat. no. F432), or Alexa Fluor® 488 phalloidin (right panels, Cat. no. A12379), which labels filamentous actin, and mounted in PBS.
- The cells were placed under constant illumination on the microscope with an FITC filter set using a 60× objective.
- Images were acquired at one-second intervals for 30 seconds.
- Under these illumination conditions, fluoresce in photobleached to about 20% of its initial value in 30 seconds; the fluorescence of Alexa Fluor® 488 phalloidin stayed at the initial value under the same illumination conditions.

Fluorophore Properties

Example: Properties of fluorescent protein variants

Table 1 Properties of novel fluorescent protein variants

Fluorescent protein	Excitation maximum (nm)	Emission maximum (nm)	Extinction coefficient per chain[a] ($M^{-1}cm^{-1}$)	Fluorescence quantum yield	Brightness of fully mature protein (% of DsRed)	pKa	$t_{0.5}$ for maturation at 37 °C	$t_{0.5}$ for bleach[b], s
DsRed	558	583	75,000	0.79	100	4.7	~10 h	ND
T1	555	584	38,000	0.51	33	4.8	<1 h	ND
Dimer2	552	579	69,000	0.69	80	4.9	~2 h	ND
mRFP1	584	607	50,000	0.25	21	4.5	<1 h	6.2
mHoneydew	487/504	537/562	17,000	0.12	3	<4.0	ND	5.9
mBanana	540	553	6,000	0.70	7	6.7	1 h	1.4
mOrange	548	562	71,000	0.69	83	6.5	2.5 h	6.4
dTomato	554	581	69,000	0.69	80	4.7	1 h	64
tdTomato	554	581	138,000	0.69	160	4.7	1 h	70
mTangerine	568	585	38,000	0.30	19	5.7	ND	5.1
mStrawberry	574	596	90,000	0.29	44	<4.5	50 min	11
mCherry	587	610	72,000	0.22	27	<4.5	15 min	68

[a]Extinction coefficients were measured by the alkali denaturation method[8,30] and are believed to be more accurate than the previously reported values for DsRed, T1, dimer2 and mRFP1[7]. [b]Time (s) to bleach to 50% emission intensity, at an illumination level that causes each molecule to emit 1,000 photons/s initially, that is, before any bleaching has occurred. See Methods for more details. For comparison, the value for EGFP is 115 s, assuming an extinction coefficient of 56,000 $M^{-1}cm^{-1}$ and quantum efficiency of 0.60 (ref. 30). ND, not determined.

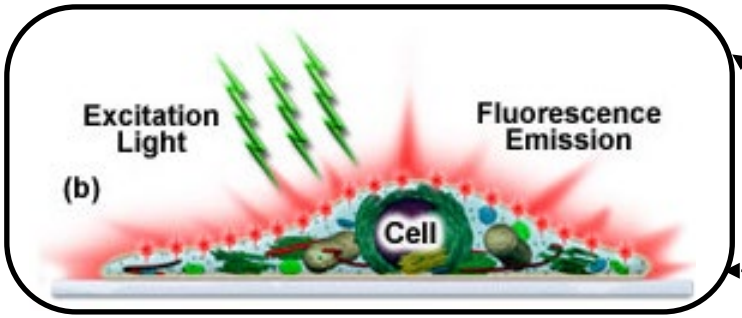
- Brightness: εQ
- Photobleaching characterization

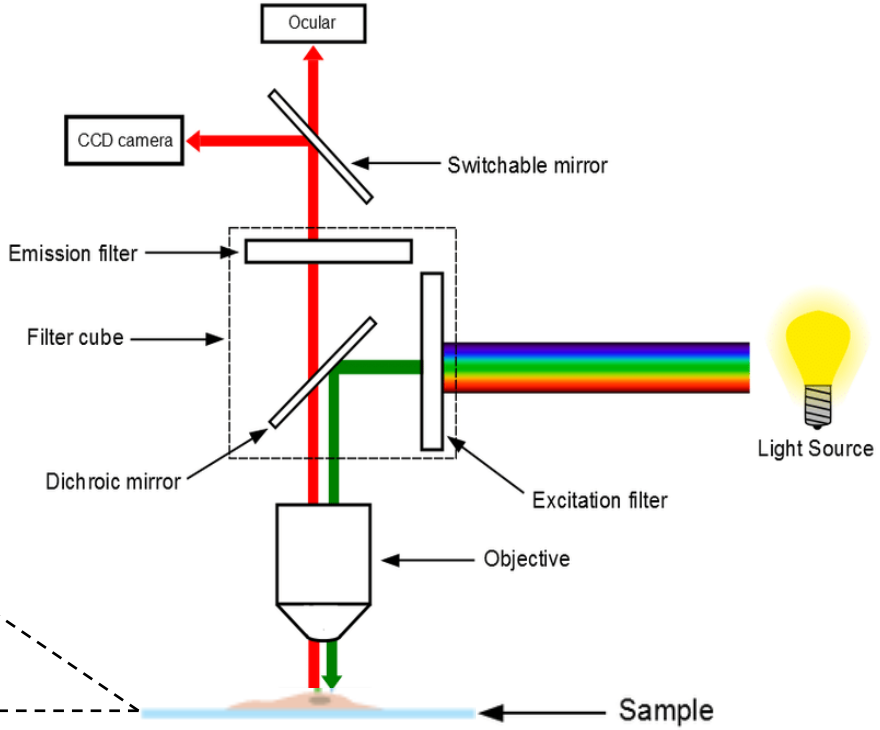
DsRed from
Red Discosoma corals

Outline

- To understand fluorescence microscopy we need to be familiar with:
  - Basic principles of fluorescence
  - Properties of fluorescent dyes
  - **Different kinds of fluorescence markers**
  - The important optical components
    - Filters and filter sets
    - Excitation Sources
    - Detectors
    - Also, their proper positioning in the optical train of the microscope

Fluorescence labelled cell:

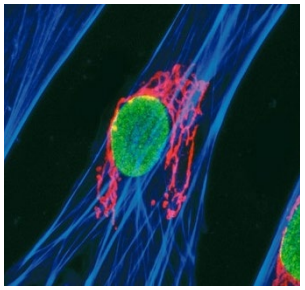




There are different types of fluorescence probes

Organic fluorophores:

1. Synthetic dyes

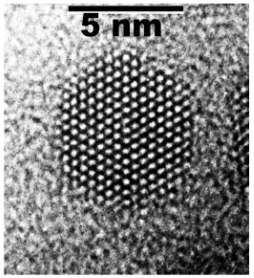


2. Fluorescent proteins

Inorganic fluorophores:

1. Lanthanides

2. Quantum dots



Probes, markers, labels, tags, dyes ..

Synthetic Dyes: fluorescent markers

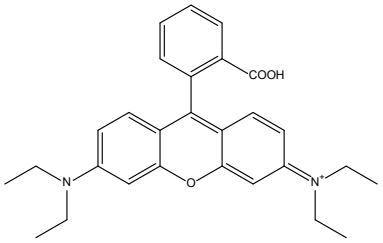
- These dyes are typically small molecules with molecular weight < 1000 Da [Dalton]

- Small molecule dyes penetrate easily through cell membranes.

- Small molecule dyes minimally disturb the molecule that they are attached to.
  - **Example:** They are widely used in DNA staining where small DNA molecule folding or hybridization can be disturbed by larger marker molecules.

Examples of commonly used dyes in biomicroscopy

Rhodamine dyes



rhodamine B

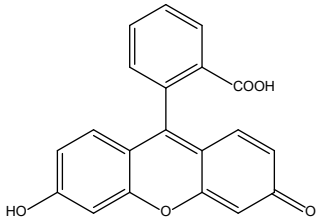
λ_{abs}=542nm

λ_{em}=579nm

Q = 0,50

ε = 106’000 $M^{-1}cm^{-1}$

Fluoresceines



fluoresceine

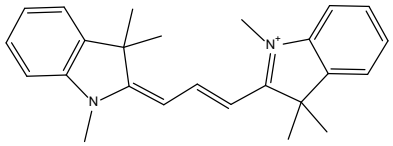
λ_{abs}=489nm

λ_{em}=534nm

Q = 0,73

ε= 92’300 $M^{-1}cm^{-1}$

Cyanines



Cy3

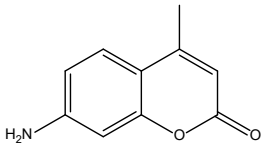
λ_{abs}=546nm

λ_{em}=571nm

Q = 0,15

ε= 271’000$M^{-1}cm^{-1}$

Coumarins



coumarin 440

λ_{abs}=354nm

λ_{em}=434nm

Q = 0,73

ε= 23’500$M^{-1}cm^{-1}$

Synthetic dyes can come almost in every color!

Table of some characteristics of fluorophores **used in fluorescence microscopy and also in flow cytometry:**

Probe	Ex (nm)	Em (nm)	MW	Notes
Reactive and conjugated probes				
Hydroxycoumarin	325	386	331	Succinimidyl ester
Aminocoumarin	350	445	330	Succinimidyl ester
Methoxycoumarin	360	410	317	Succinimidyl ester
Cascade Blue	(375);401	423	596	Hydrazide
Pacific Blue	403	455	406	Maleimide
Pacific Orange	403	551		
Lucifer yellow	425	528		
NBD	466	539	294	NBD-X
R-Phycoerythrin (PE)	480;565	578	240 k	
PE-Cy5 conjugates	480;565;650	670		*aka* Cychrome, R670, Tri-Color, Quantum Red

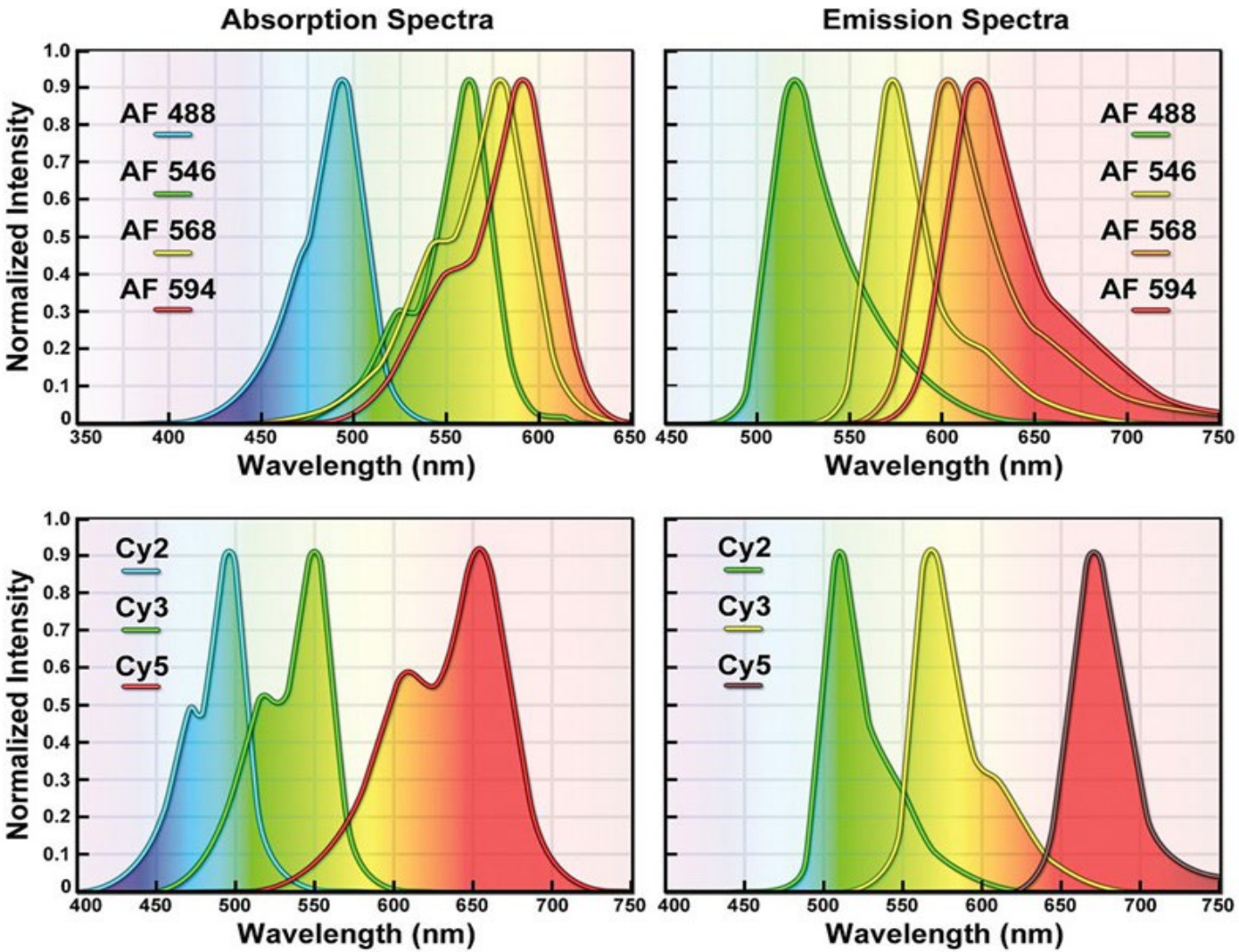
Ex: Peak excitation wavelength (nm)
Em: Peak emission wavelength (nm)
MW: molecular weight

From Salk Institute CCMI (Flow Cytometry)

Synthetic dyes can come almost in every color!

Probe	Ex (nm)	Em (nm)	MW	Notes
Reactive and conjugated probes				
PE-Cy7 conjugates	480;565;743	767		
Red 613	480;565	613		PE-Texas Red
PerCP	490	675		Peridinin chlorphyll protein
TruRed	490,675	695		PerCP-Cy5.5 conjugate
FluorX	494	520	587	(GE Healthcare)
Fluorescein	495	519	389	FITC; pH sensitive
BODIPY-FL	503	512		
TRITC	547	572	444	TRITC
X-Rhodamine	570	576	548	XRITC
Lissamine Rhodamine B	570	590		
Texas Red	589	615	625	Sulfonyl chloride
Allophycocyanin (APC)	650	660	104 k	
APC-Cy7 conjugates	650;755	767		PharRed

Two popular commercial synthetic dyes: Alexa and Cyanine series



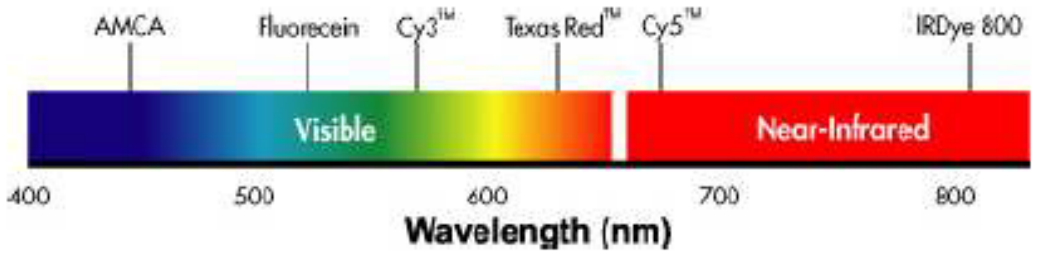
Invented by Molecular Probes, now a part of Thermo Fisher Scientific, and sold under the Invitrogen brand name.

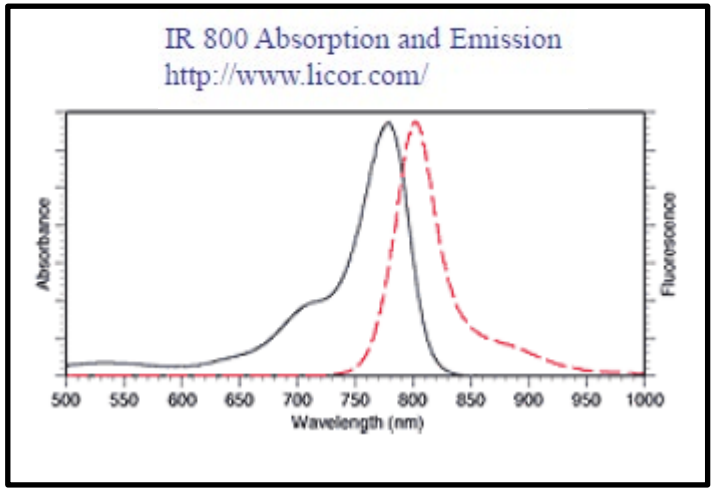
Cyanines were first synthesized over a century ago. They were originally used, and still are in photography.

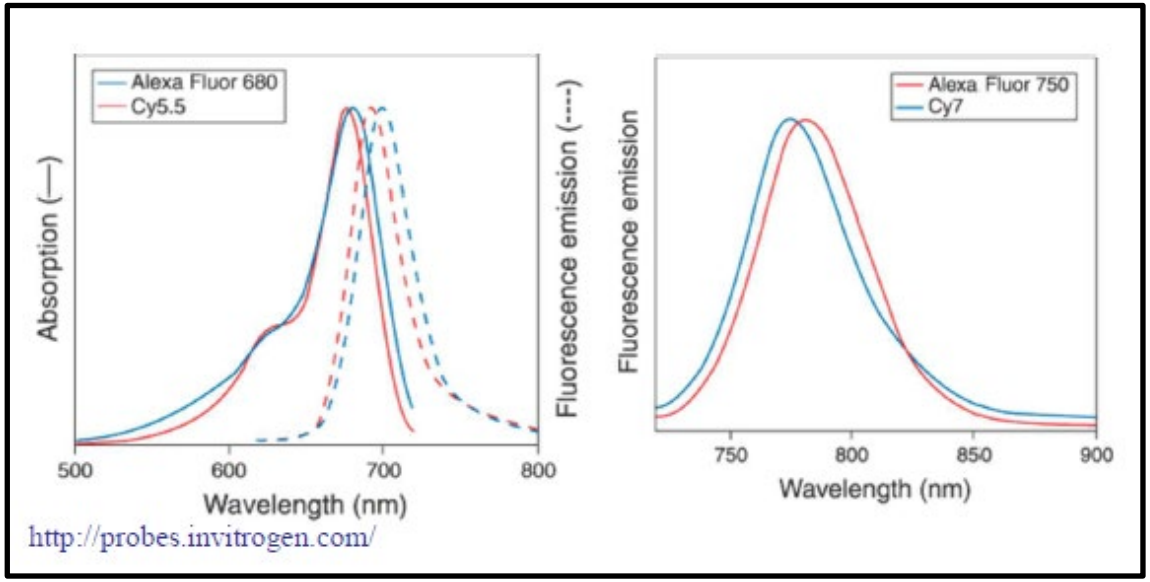
Alan S. Waggoner et al. of Carnegie-Mellon University filed a key patent for its biotech use. The IP was licensed to GE Healthcare.

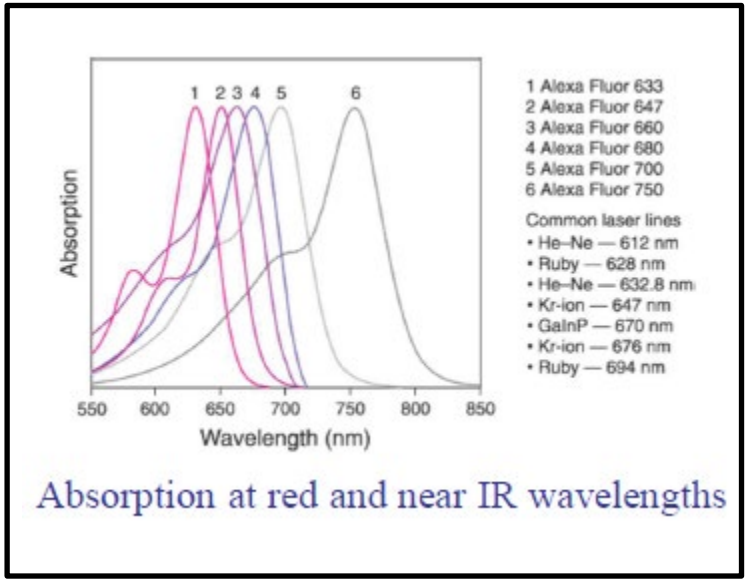
Near-Infrared (Near-IR) Dyes

- High extinction coefficients (typically 160'000 250'000 cm-1M-1)
- Typically a small stokes shift
- Examples: Alexa 680, Alexa750, Cy5.5, Cy7, IR800 ...







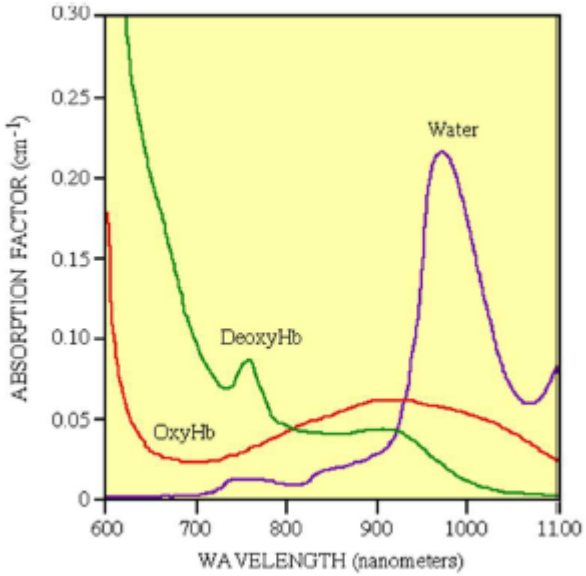


Near-Infrared (Near-IR) Dyes

Near IR dyes are good for live tissue imaging

- **Near-IR window**:
  - Low absorption of water and hemoglobin
  - Low auto-fluorescence from background allows good SNR in fluorescence

- **Good tissue penetration**



Synthetic Dyes: fluorescent markers

- These dyes are typically small molecules with molecular weight < 1000 Da [Dalton]

- Small molecule dyes penetrate easily through cell membranes.

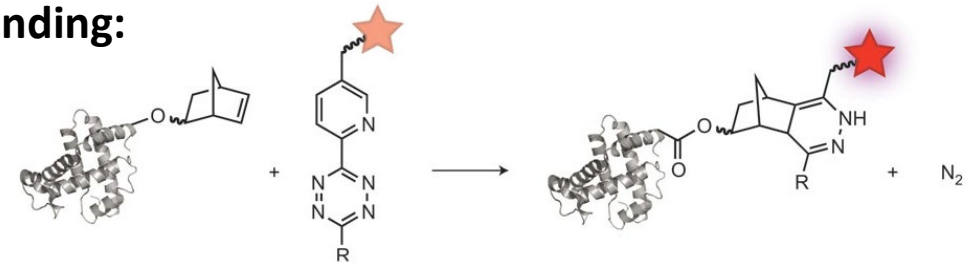
- Small molecule dyes minimally disturb the molecule they attach to.
  - **Example:** They are widely used in DNA staining where small DNA molecule folding or hybridization can be disturbed by larger marker molecules.

Question:
How can we introduce synthetic fluorescence labels on the sample?

There are multiple methods for introducing fluorescence labels

Three commonly used approaches to couple a fluorescence label to a **specific** biomolecule:

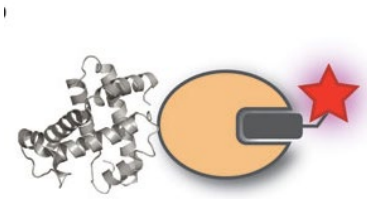
1) Crosslinking with covalent bonding:



Some of the most used functional targets and reactive groups in crosslinking:

- Primary amines (–NH2)
- Carboxyls (–COOH)
- Sulfhydryls (–SH)
- Carbonyls (–CHO)

2) Dye molecule is linked to a specific antigen that binds to a target biomolecule



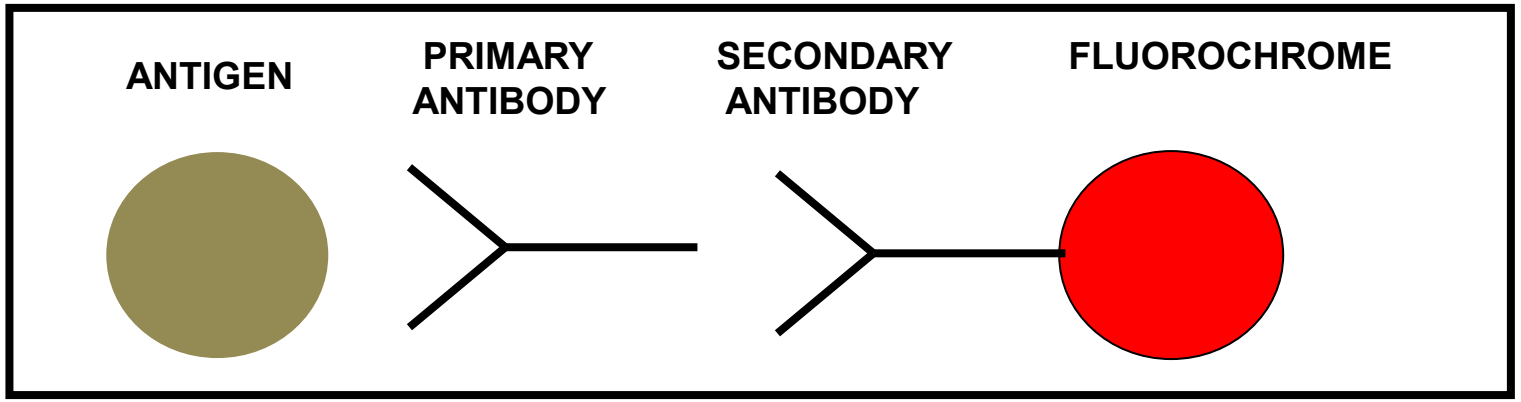
There are multiple methods for introducing fluorescence labels

3) Immuno-fluorescent staining.
This method uses antibodies to locate and identify the patterns of protein expression in cells:

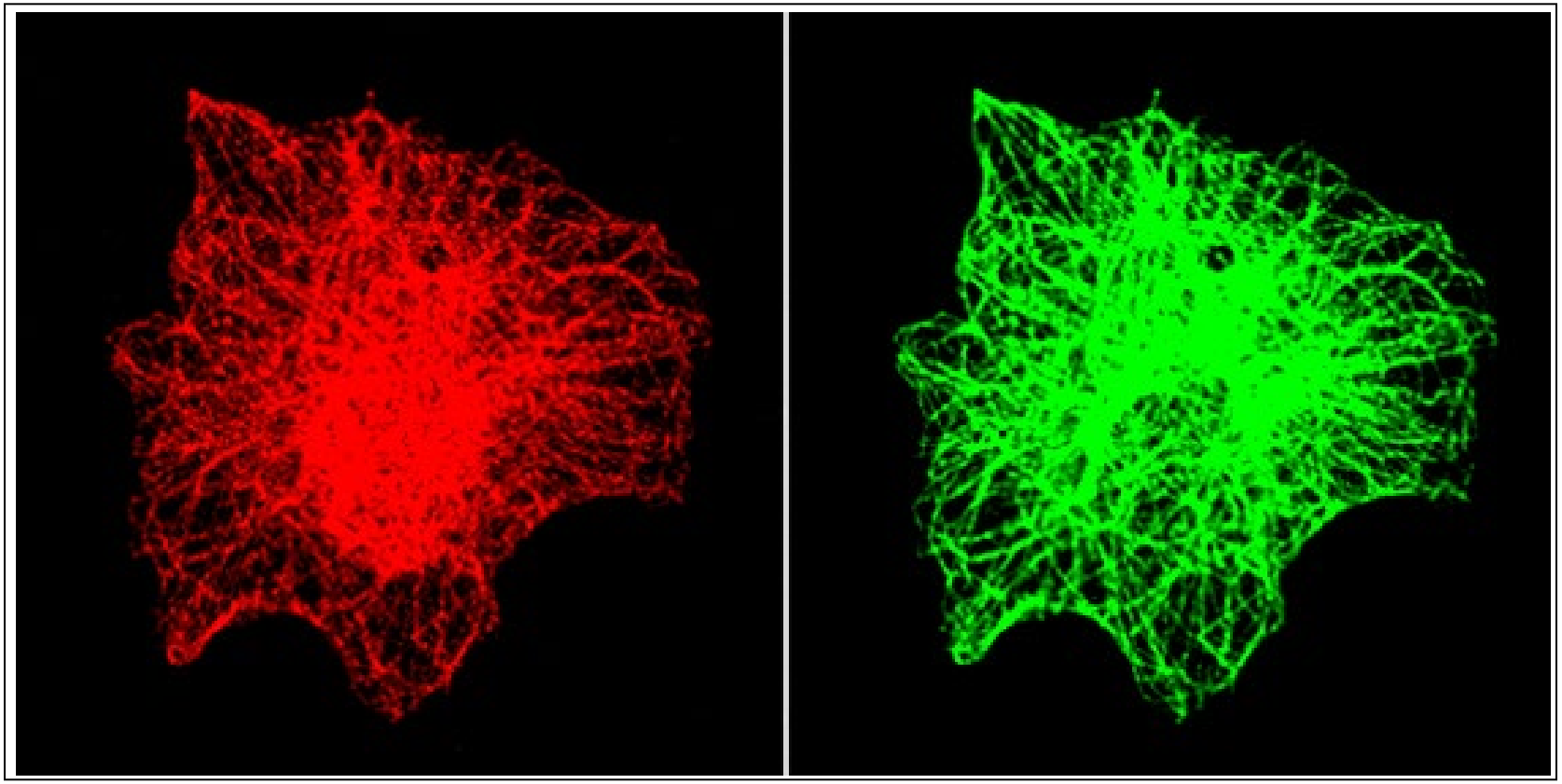
i) Primary antibody binds to antigen.

ii) Antibody-antigen complex bounds to a secondary antibody that is conjugated to a fluorochrome.

iii) Upon absorption of proper light, the fluorophore emits light at its own characteristic wavelength, thus allowing the detection of the antigen-antibody complexes.



Example for immuno-fluorescent staining



PRIMARY ANTIBODY
sheep anti-p53 polyconal

SECONDARY ANTIBODY
Texas Red conjugated anti-sheep

PRIMARY ANTIBODY
mouse anti-α tubulin monoclonal

SECONDARY ANTIBODY
FITC conjugated anti-mouse

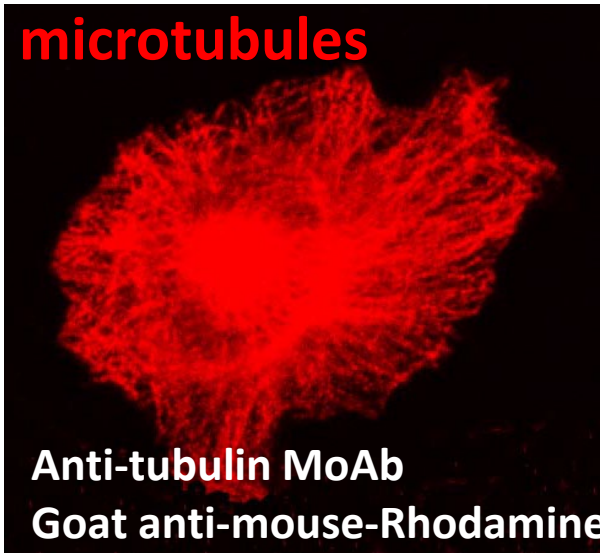
Giannakakou et al., Nature Cell Biology,2000

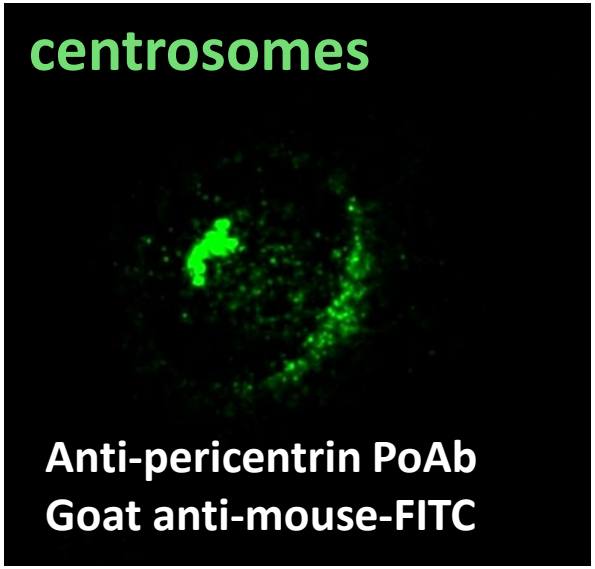
Fluorescence labels can offer target (biomolecular) specificity: direct staining of specific cell structures

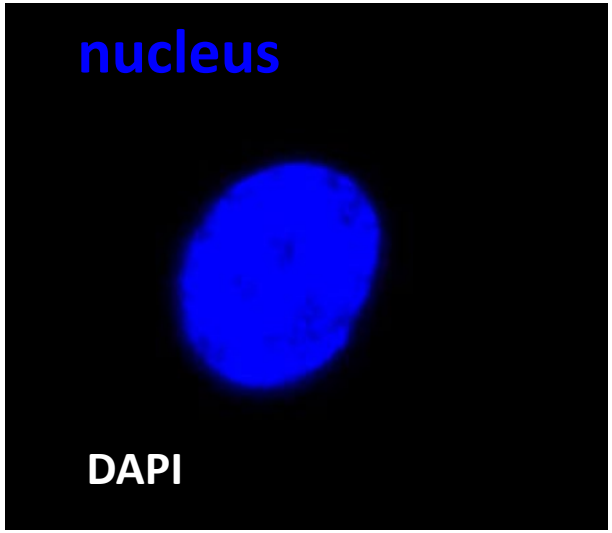
Target	Fluorescent Probe	Mechanism
Mitochondria	MitoTracker	mitochondrial membrane potential
Lysosomes	LysoTracker	hydrolytic activity of enzymes
ER and Golgi	Lectin conjugates	lipid composition
Stress fibers	Phalloidin-conjugaes	bind F-actin
Nuclei	DAPI	binds to minor groove of ds-DNA

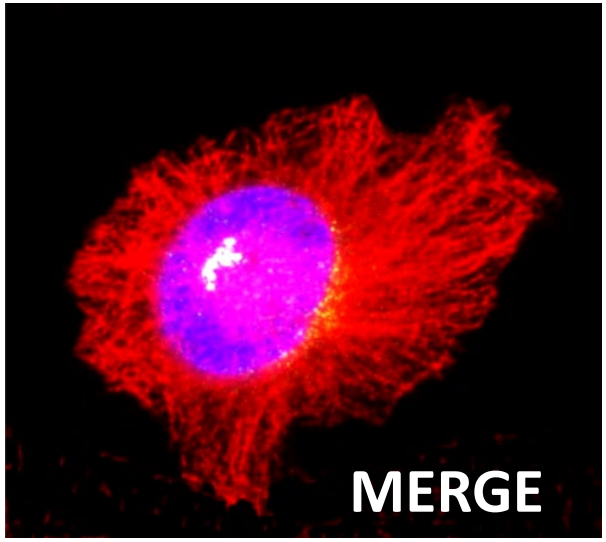
Multi-Color Imaging & Specificity

Differently colored dyes can be addressed to the different parts of the cell:

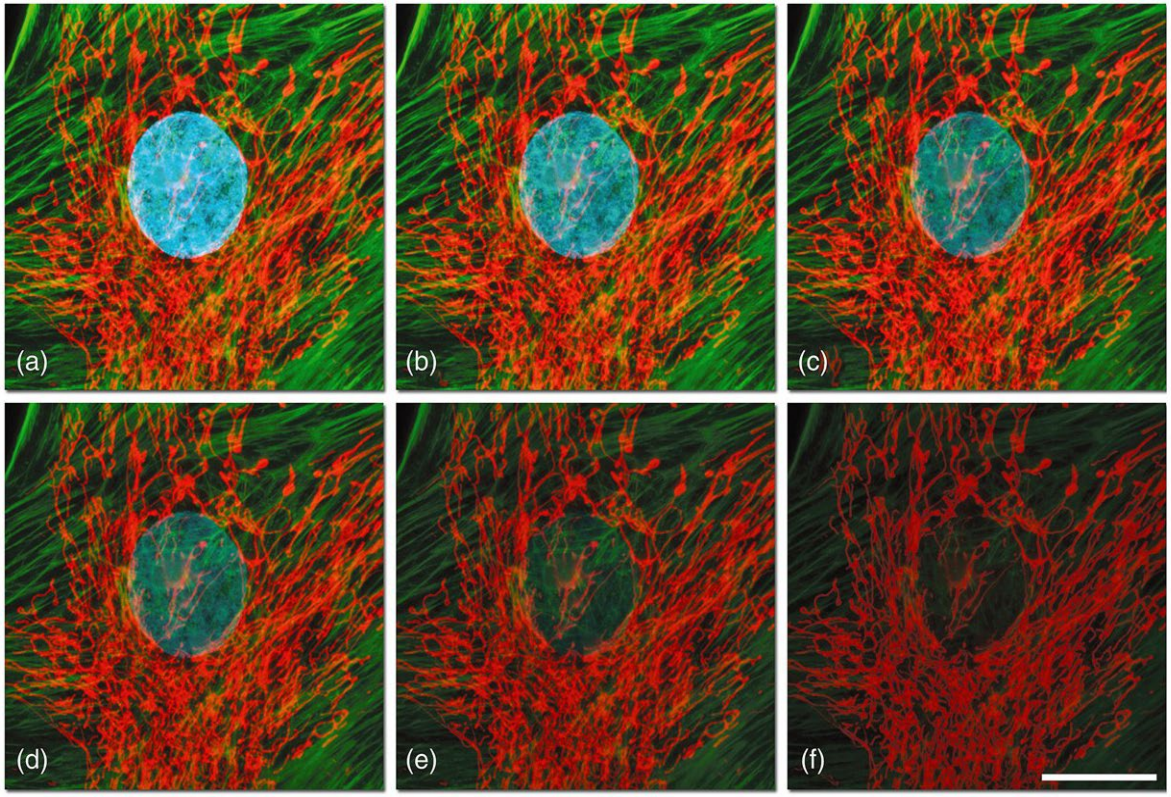








Concern in multi-color fluorescence: photobleaching



- Photobleaching observed in a series of bioimages captured at different time points for a stained culture of Indian Muntjac deerskin fibroblast cells.
  - The nuclei were stained with DAPI (blue fluorescence)
  - The mitochondria were stained with MitoTracker Red (red fluorescence)
  - The actin cytoskeleton were stained with Alexa Fluor 488 (green fluorescence).
- Time points were taken in 2-minute intervals by exciting the three fluorophores simultaneously while also recording the combined emission signals.
- Note that all three fluorophores have a relatively high intensity in panel a, but the DAPI (blue) intensity starts to drop rapidly at 2 minutes and is almost completely gone at 8 minutes.
  The mitochondrial and actin stains are more resistant to photobleaching, but the intensity of both drops over the course of the timed sequence (10 minutes).

(a) t = 0. (b) t = 2 m. (c) t = 4 m. (d) t = 6 m. (e) t = 8 m. (f) t = 10 m. Bar = 20 μ m.

Environmental and external effects can change fluorescence properties

Effect of coupling:

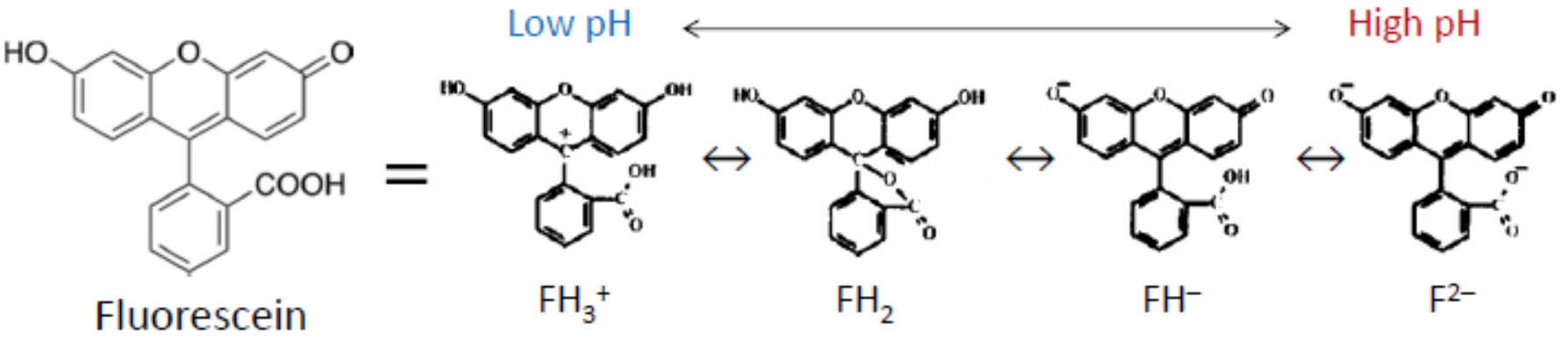
- Emission spectra may shift by coupling a dye to a protein or bacteria.

- Quantum efficiency may change by coupling a dye to a protein.
  - **For example:** coupling Fluorescein to a protein may reduce the dye quantum yield Q by 60% and ε by 10 %.

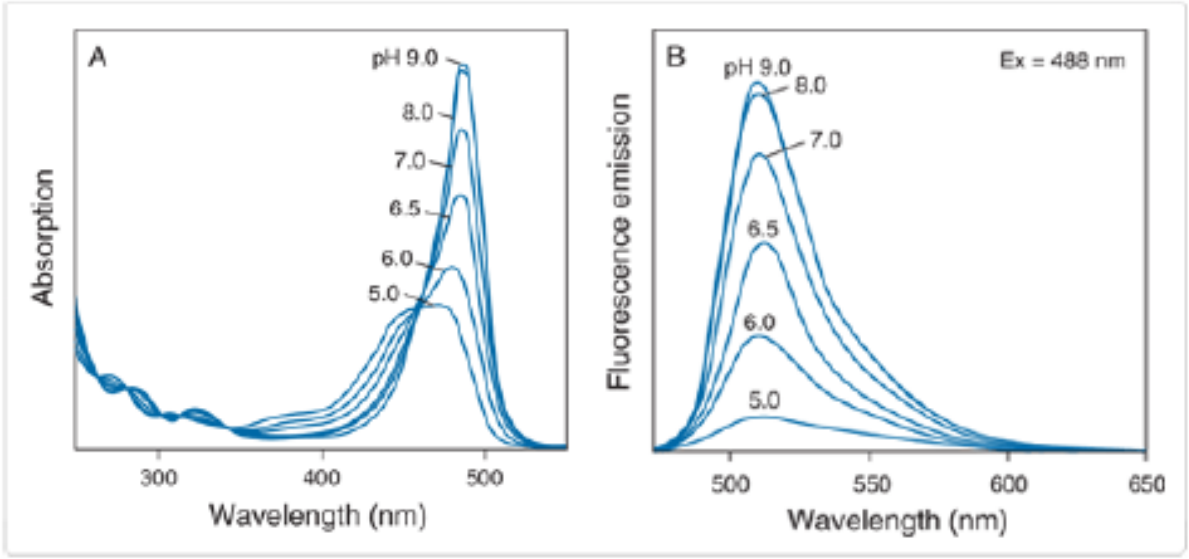
Other environmental and external factors:

- Solvent pH, solvent type, solvent polarity, and other quenching species can change fluorescence properties.

Example: The influence of pH (environment) on dye brightness



Fluorescein



Invitrogen

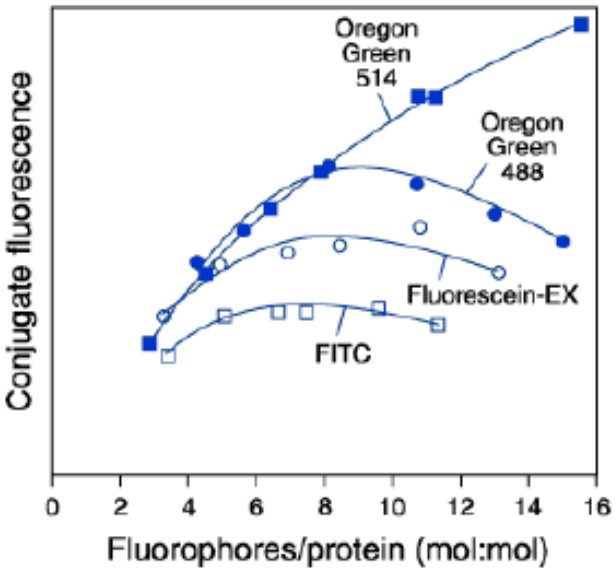
Examples: The influence of dye concentration & excitation light intensity

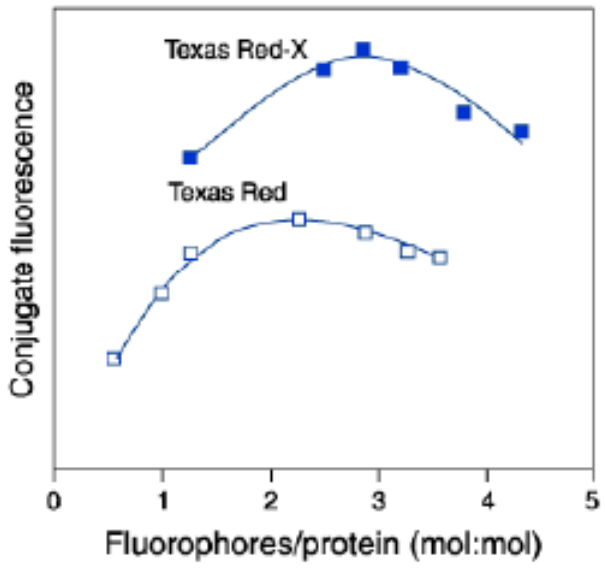
- **At high concentration of dye:**

Quantum efficiency (ϕ_F) thus emission intensity may decrease due to:

- Increased dye-dye interaction.
- Self-quenching: self-absorption will happen at overlap regions between excitation and emission spectra.

Increasing dye concentration: increasing # of dyes per protein





- **Under high intensity illumination:**

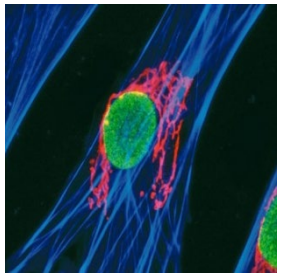
Irreversible destruction or photo-bleaching of the excited dye limits emitted intensity.

Outline

- **There are different types of fluorescence probes.**
- **Let's cover next fluorescence proteins**

Organic fluorophores:

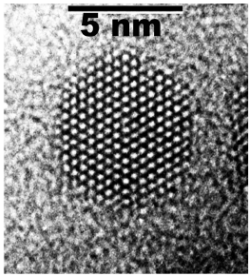
1. Synthetic dyes



2. Fluorescent proteins

Inorganic fluorophores:

1. Quantum dots
2. Lanthanides

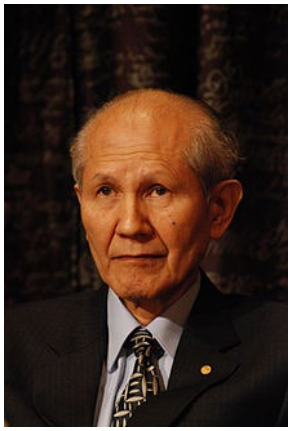


Fluorescence Proteins

- Fluorescent proteins are discovered first in the form of "**Green Fluorescent Protein**, GFP" in the early 1960s.
- Dr. Shimomura worked in the Department of Biology at Princeton to study bioluminescent jellyfish Aequorea Victoria that he collected at the Friday Harbor Laboratories of the University of Washington. In 1962, he isolated GFP from Aequorea Victoria.

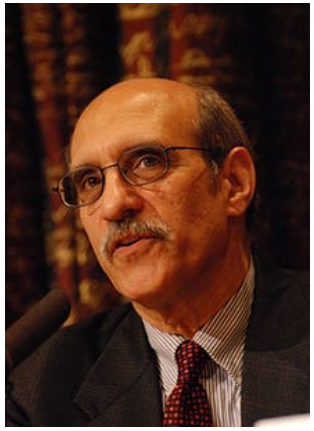
- Fluorescent proteins opened up **a new era in cell biology**:
  - They enabled to apply molecular cloning methods, fusing the fluorophore moiety to a wide variety of protein and enzyme targets, in order to **monitor cellular processes in living systems using optical microscopy.**

2008 Nobel Prize for chemistry is awarded for the discovery & development of GFP



Osamu Shimomura
Organic chemist & marine biologist

Roger Tsien
biochemist

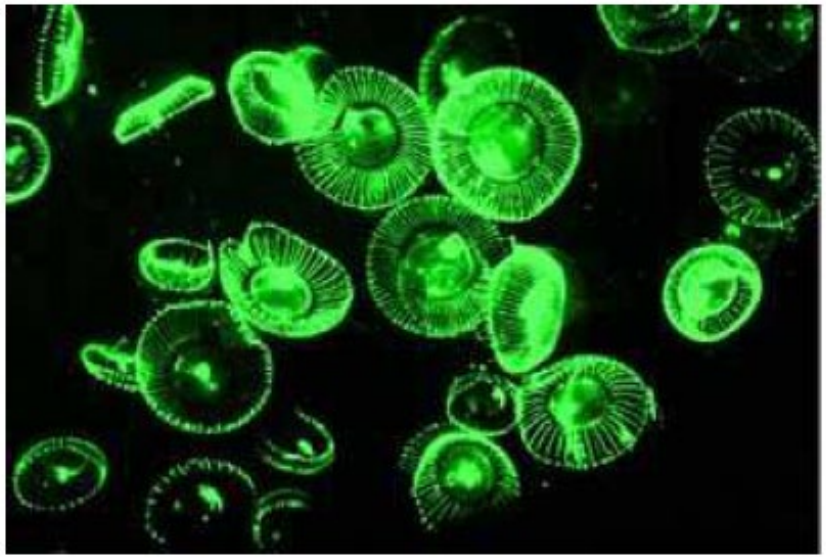


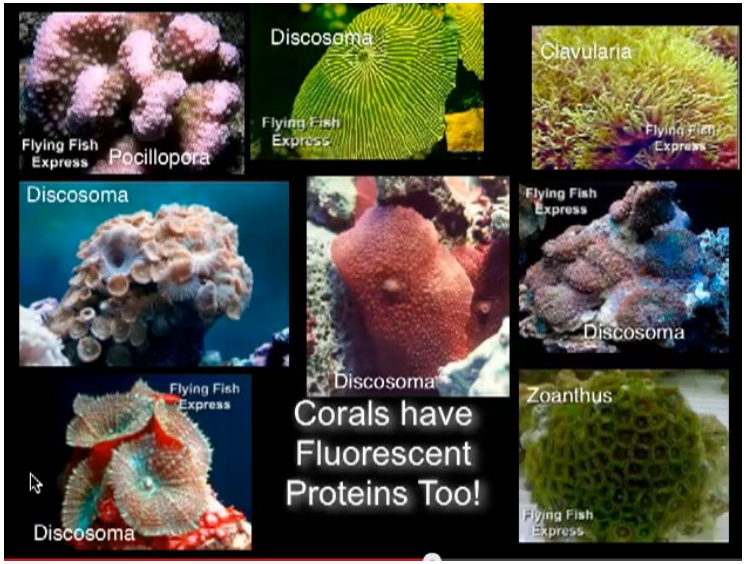
Martin Chalfie
neurobiologist

Green Fluorescent Protein - GFP

- GFP is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range.

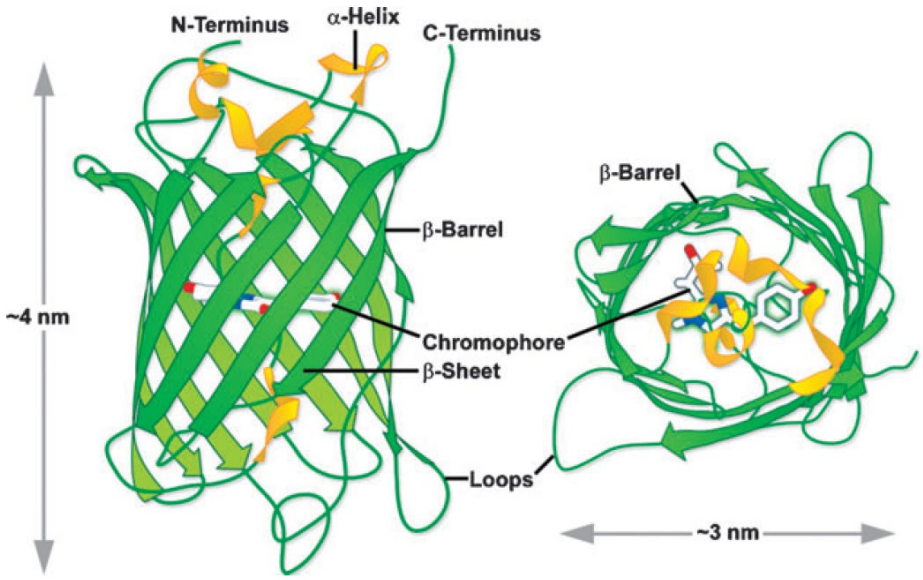
- Although the protein is first isolated from the **jellyfish** Aequorea Victoria, many other marine organisms, like coral reefs have similar fluorescent proteins.

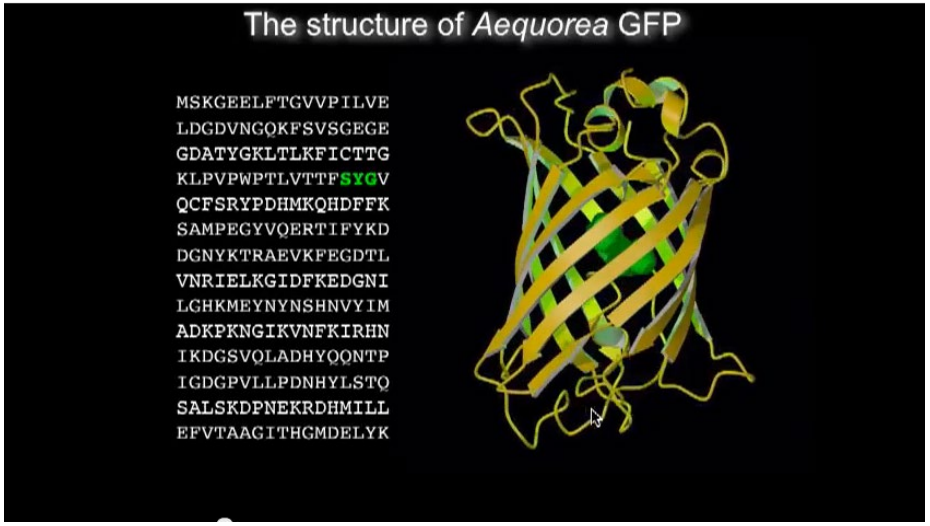




Green Fluorescent Protein - GFP

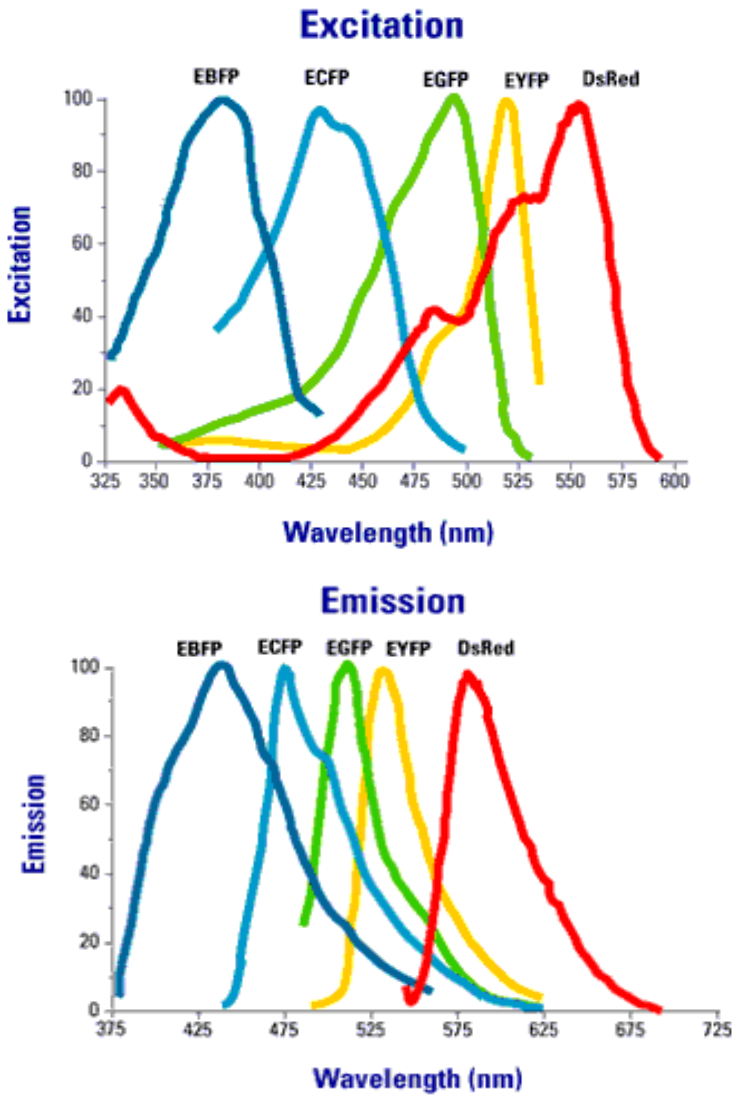
- The fluorophore of GFP sits in the center of the β-barrel
- The chromophore is formed (from Ser-65, Tyr-66, Gly-67) upon folding of the polypeptide chain, without the need for enzymatic synthesis.

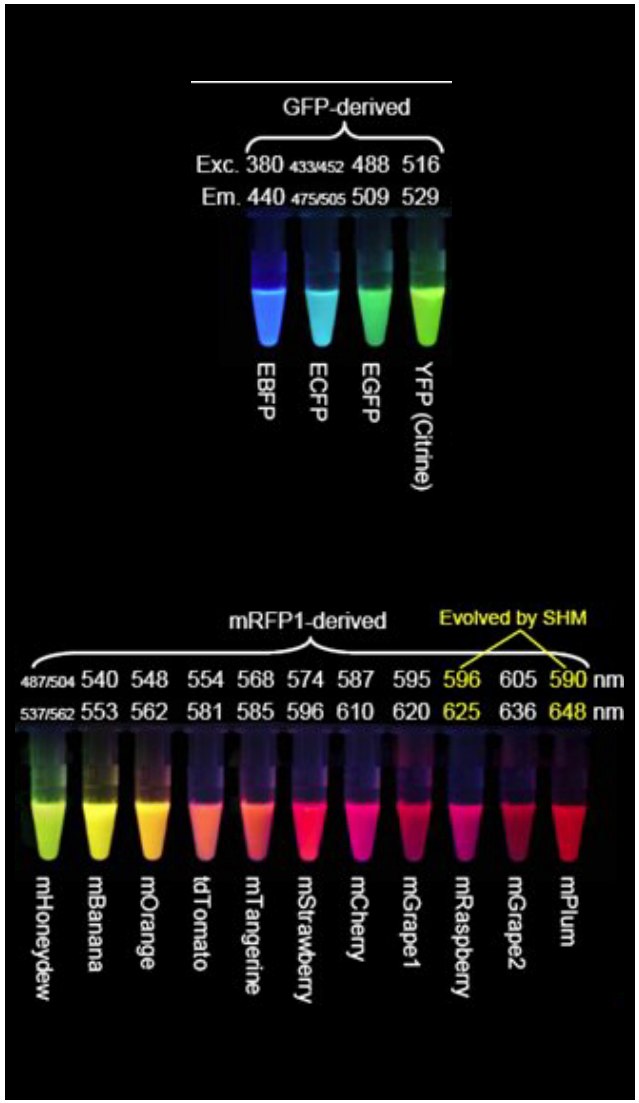




The fluorescent protein palette: tools for cellular imaging.
Richard N. Day and Michael W. Davidson
Chem. Soc. Rev., 2009, 38, 2887–2921.

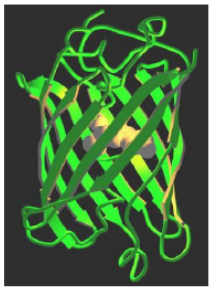
Fluorescent proteins can have different colors

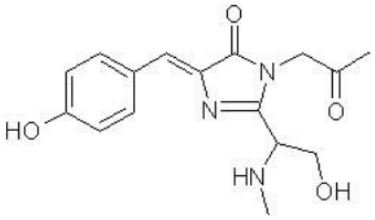




Advantageous of Fluorescent Proteins-1

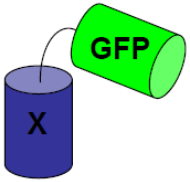
- If the DNA vectors are provided (purchasable), they can be used to construct fluorescent proteins that can be observed in cells **after transfection with the engineered vectors**.
- This technique avoids the problem of purifying, tagging, and introducing labelled proteins into cells or having to produce specific antibodies.
- In cell and molecular biology, the GFP gene is frequently used as a reporter of expression.
- **Thus, fluorescent proteins are suitable for studying protein dynamics in living cells.**



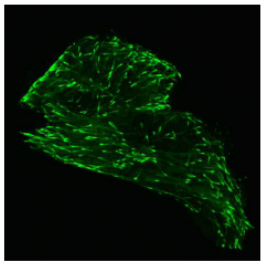


GFP chromophore
from Ser-Tyr-Gly

Link GFP sequence to gene of your favourite protein



GFP folds
and becomes
fluorescent



GFP lights up your
favourite protein in cell

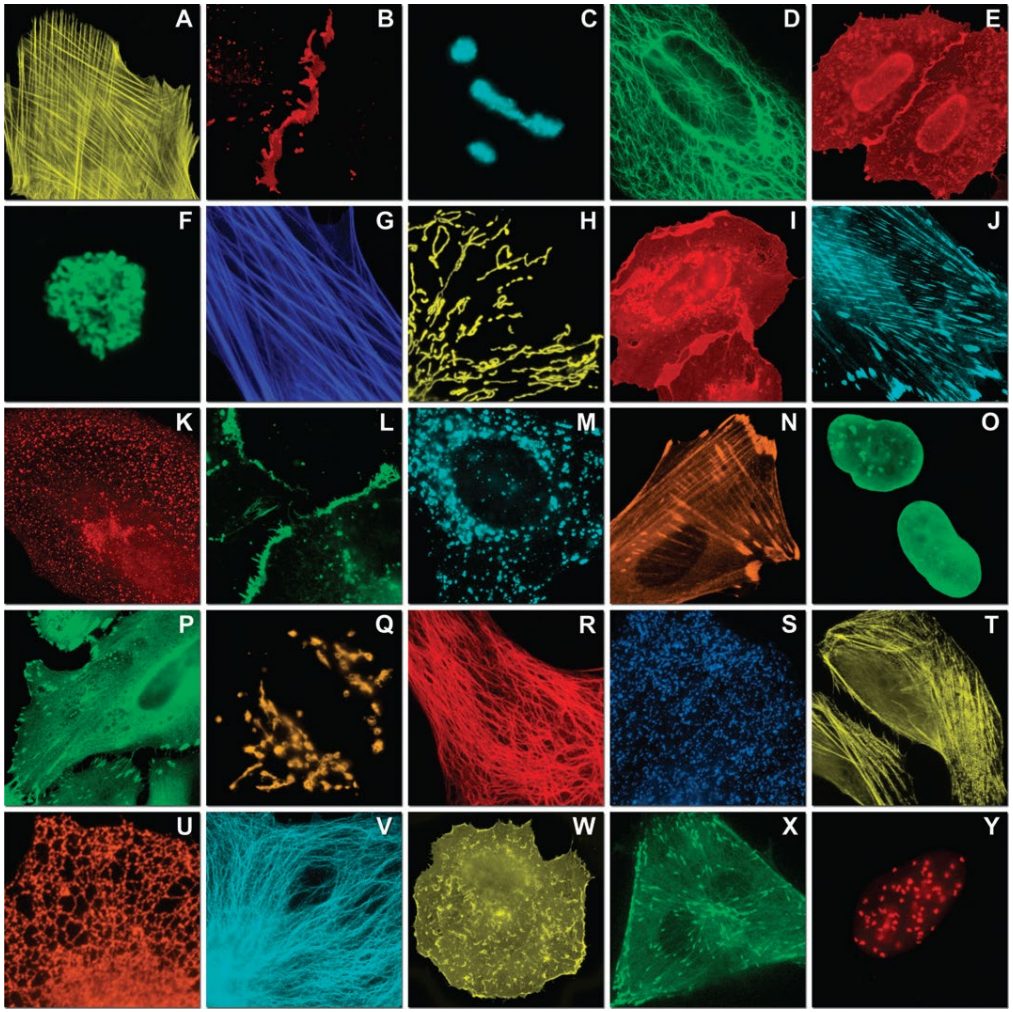
Advantageous of Fluorescent Proteins -2

GFP gene can also be inserted into living organisms and maintained in their genome through breeding…

Example: Applications of fluorescent proteins

The fluorescent protein palette: tools for cellular imaging.

Richard N. Day and Michael W. Davidson, *Chem. Soc. Rev.*, 2009, 38, 2887–2921.



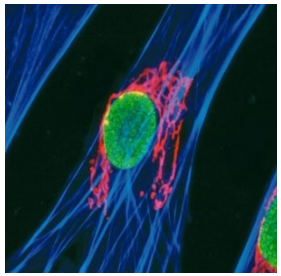
(A) mOrange2-b-actin-C-7.
(B) mApple-Cx43-N-7.
(C) mTFP1-fibrillarin-C-7.
(D) mWasabi-cytokeratin-N-17.
(E) mRuby-annexin (A4)-C-12.
(F) mEGFP-H2B-N-6.
(G) EBFP2-b-actin-C-7.
(H) mTagRFP-T-mitochondria-N-7.
(I) mCherry-C-Src-N-7.
(J) mCerulean-paxillin-N-22.
(K) mKate-clathrin (light chain)-C-15.
(L) mCitrine-VE-cadherin-N-10.
(M) TagCFPlysosomes-C-20.
(N) TagRFP-zyxin-N-7.
(O) superfolderGFP-lamin B1-C-10.
(P) EGFP-a-v-integrin-N-9.
(Q) tdTomato-Golgi-N-7.
(R) mStrawberry-vimentin-N-7.
(S) TagBFP-Rab-11a-C-7.
(T) mKO2-LC-myosin-N-7.
(U) DsRed2-endoplasmic reticulum-N-5.
(V) ECFP-atubulin-C-6.
(W) tdTurboRFP-farnesyl-C-5.
(X) mEmerald-EB3-N-7.
(Y) mPlum-CENP-B-N-22.

Outline

- **There are different types of fluorescence probes.**
- **Let's focus next on inorganic fluorophores.**

Organic fluorophores:

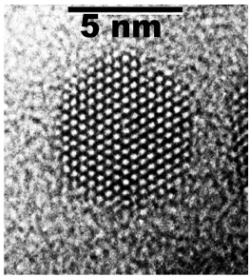
1. Synthetic dyes



2. Fluorescent proteins

Inorganic fluorophores:

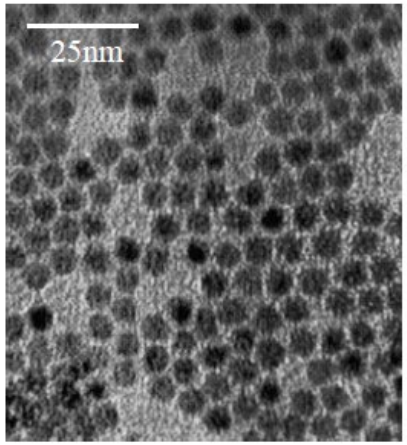
1. Quantum dots
2. Lanthanides

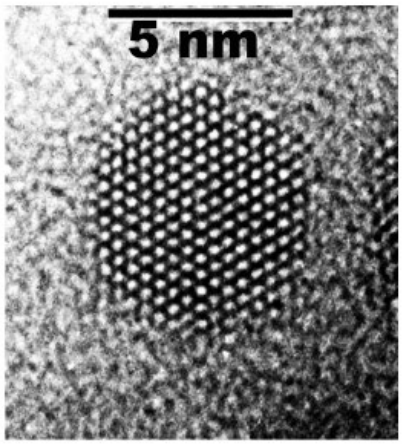


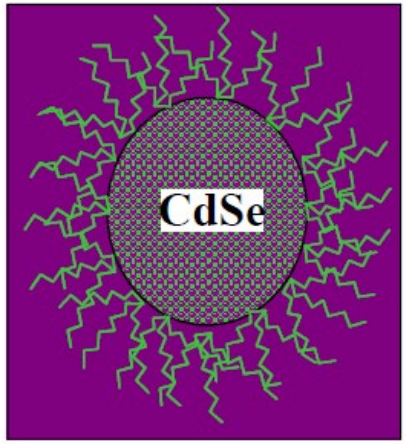
Quantum Dots (QD)

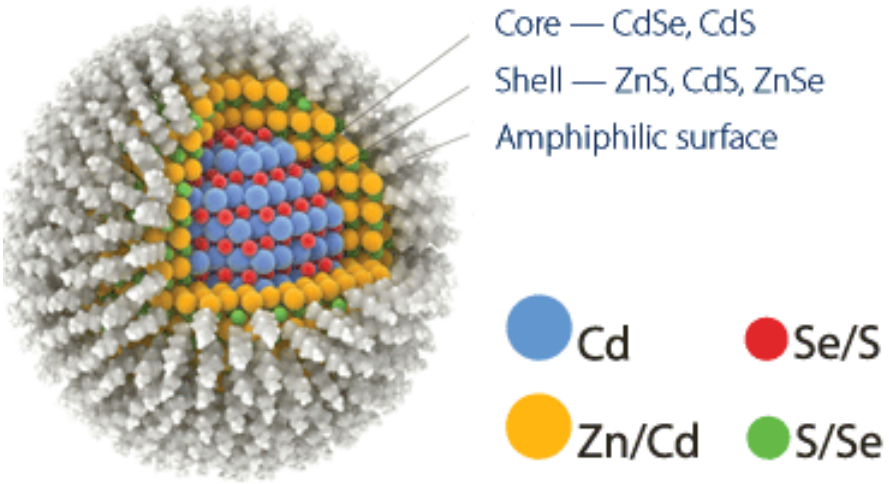
QD are man-made molecular-sized semiconductor nanocrystals that fluorescence

TEM images







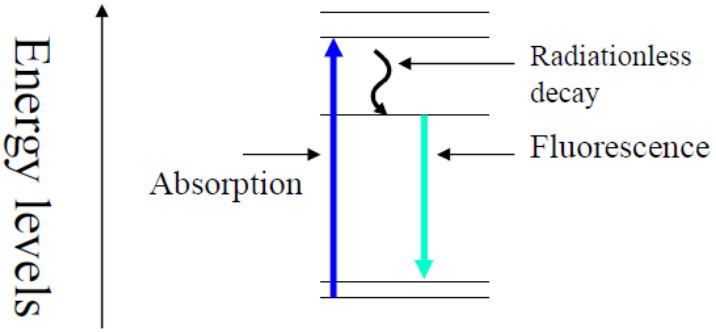


Size: Typically tuned from ~2-10 nm
Structure: highly crystalline

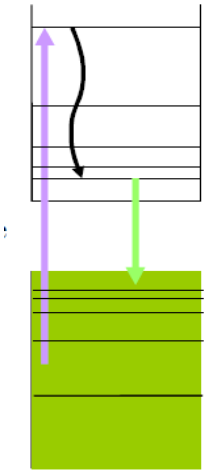
Properties:

- *Higher extinction coefficient than organic fluorophores*
- *High photostability and resistance against photobleaching.*

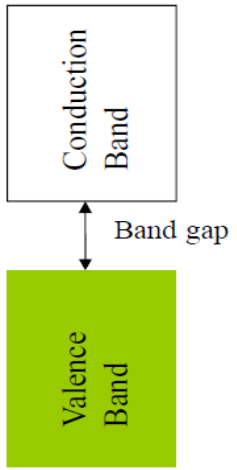
Quantum Dots: Fluorescent semiconductor nanoparticles



a) Atoms & Molecules:
Discrete energy levels

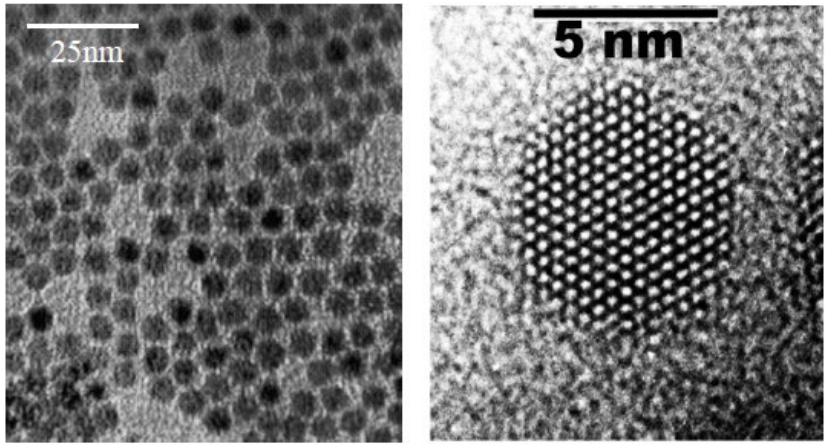


b) Q-dots:
Discrete energy levels (like molecules)



c) Semiconductors:
No energy discretization (i.e. quantization)
Energy levels are so close that they form "bands"

Semiconductor nanoparticles with diameters lower than the exciton Bohr radius, often called **"artificial atoms"**.



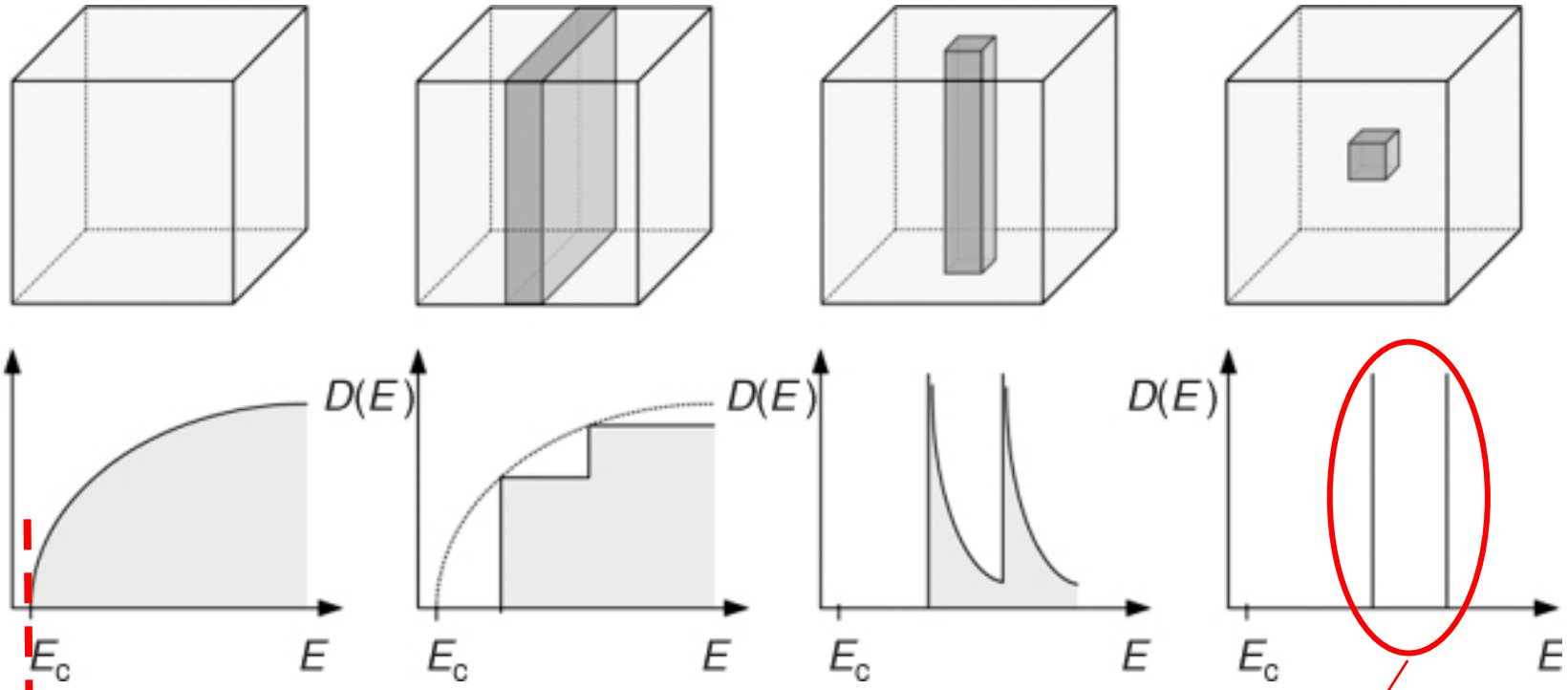
Quantum Confinement

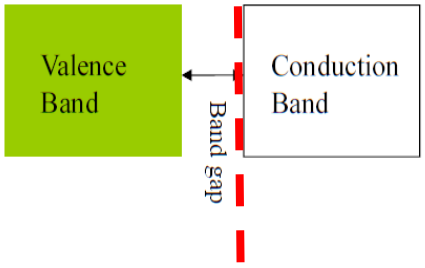
No Confinement
Bulk material (i.e. semiconductors)

1D Confinement
"Quantum well"

2D Confinement
"Quantum wire"

3D Confinement
"Quantum dot"

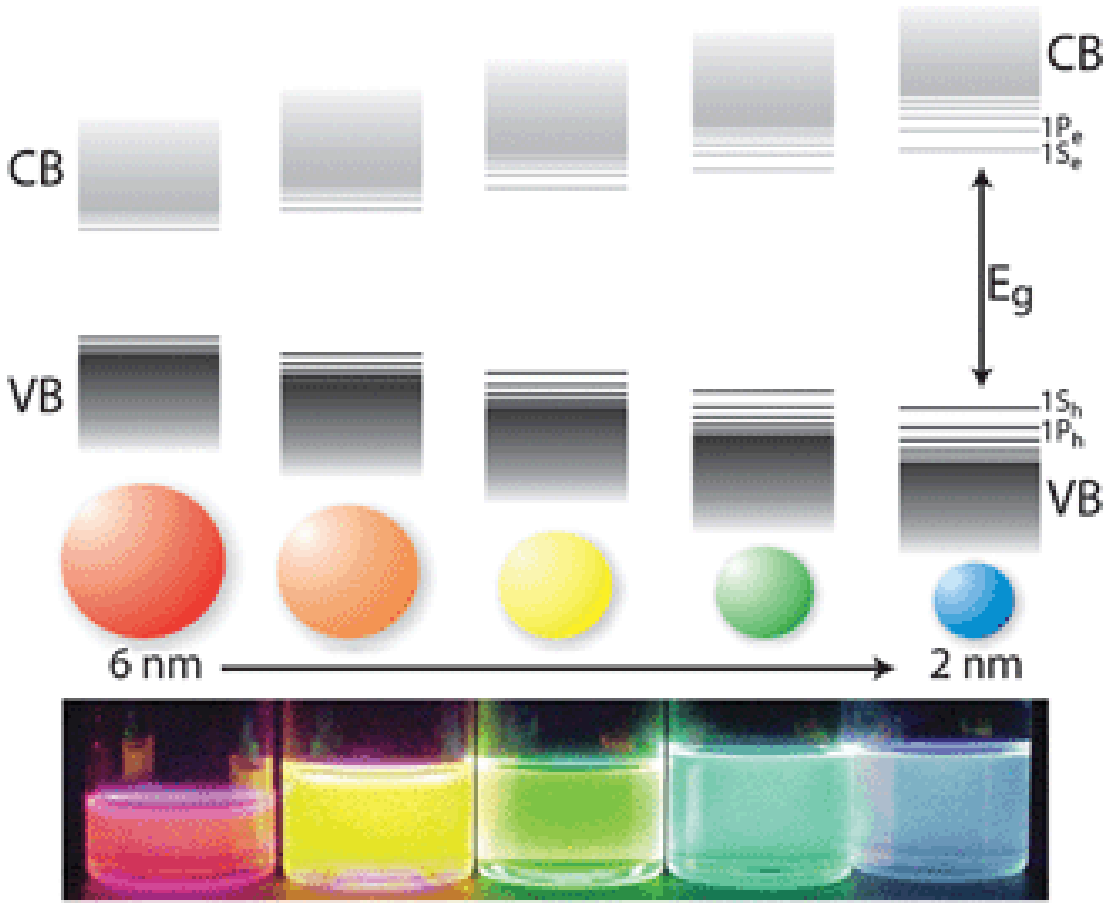




E_c is conduction band edge
D(E) is density of states as a function of frequency

3D confinement results in energy quantization as in "atoms"

Tuning of Quantum Dot Emission



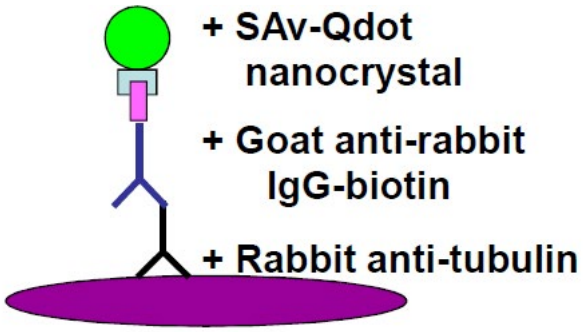
Optical properties of Q-dots:

- The energy levels depend on the particle size.
- With size reduction (which required forcing the electrons to confine better in a small space), the energy gap increases.

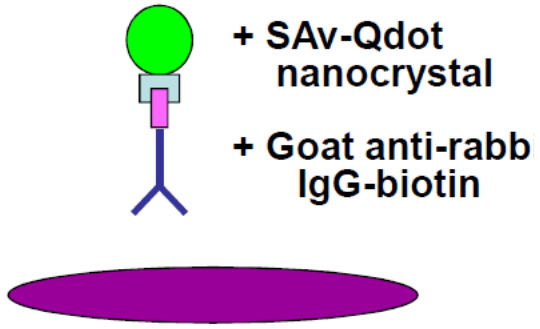
➔ **Smaller sizes result in "blue" tuning.**

Example: specific cellular staining by immuno-fluorescence

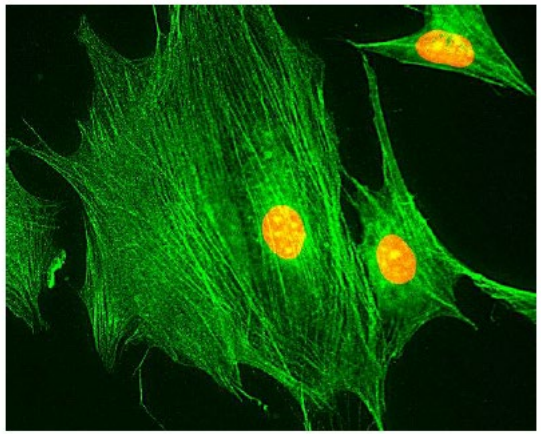
Tubin staining with Quantum Dot (Qdots)

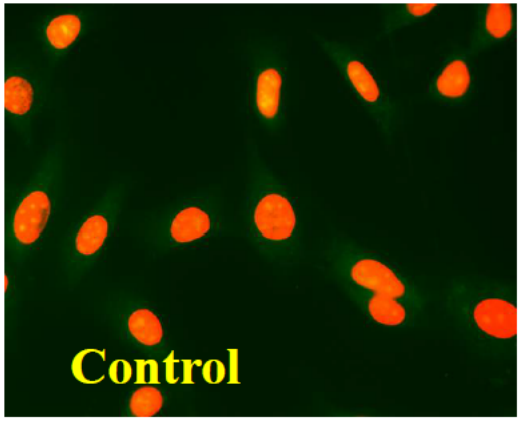


SPECIFIC BINDING



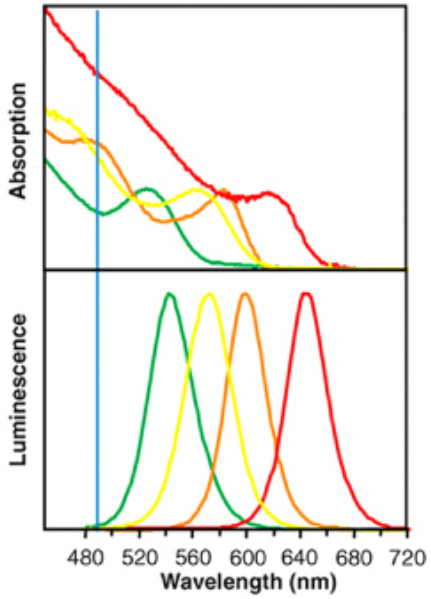
NEGATIVE CONTROL

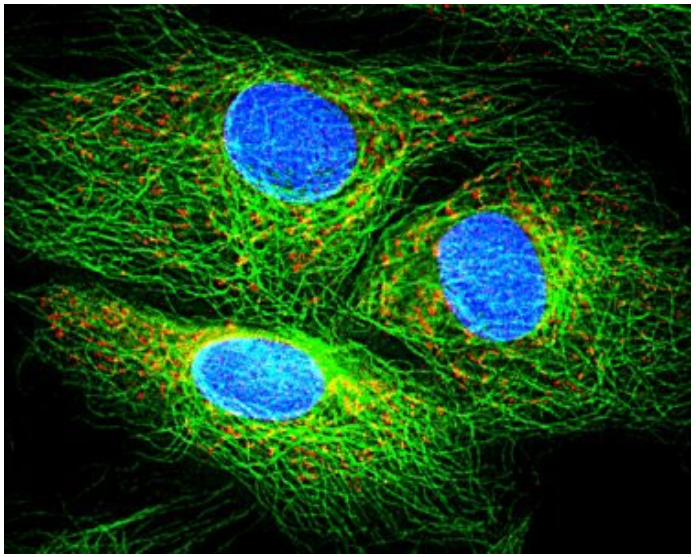




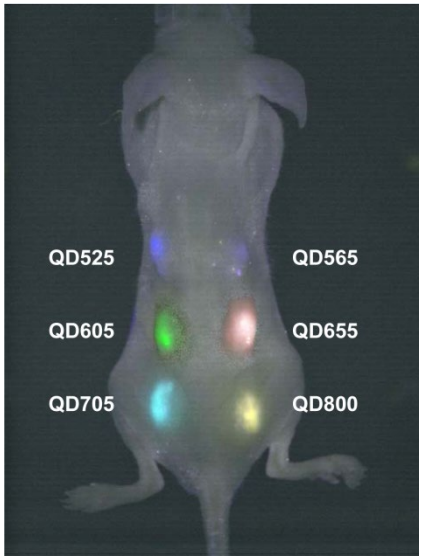
Streptavidin: is a 52.8 kDa protein purified from the bacterium *Streptomyces avidinii*. Streptavidin has an extraordinarily high affinity for biotin (aka vit. B7 or vit. H). With a dissociation constant (K_d) of ~10^{-14} mol/L, biotin-streptavidin binding is one of the strongest non-covalent interactions known in nature.

Bio-imaging with fluorescent quantum dots





X. Wu et al, Nat. Biotech. (2003)



- Excitation in the UV for all particle sizes allows easy color multiplexing.
- Long photostability allows the analysis of particle biodistribution
- **But, toxicity issues** require quantum dot synthesis using other semiconductors without Cd or Se, for instance. Capsulation of core (containing Cd) with shell & protein layers can be alternative.

Lanthanide Chelate Complexes

- The fluorescence of lanthanide complexes is very long-lived with lifetime between a few 100s to a few ms.
- They are typically excited by UV light and emit fluorescence in the visible region.
- Large stokes shift
- Their emission profile is very sharp, and the wavelength is specific to each metal.

  Example: Eu^{3+} complexes can emit at 615 nm & Tb^{3+} complexes can emit at 545 nm

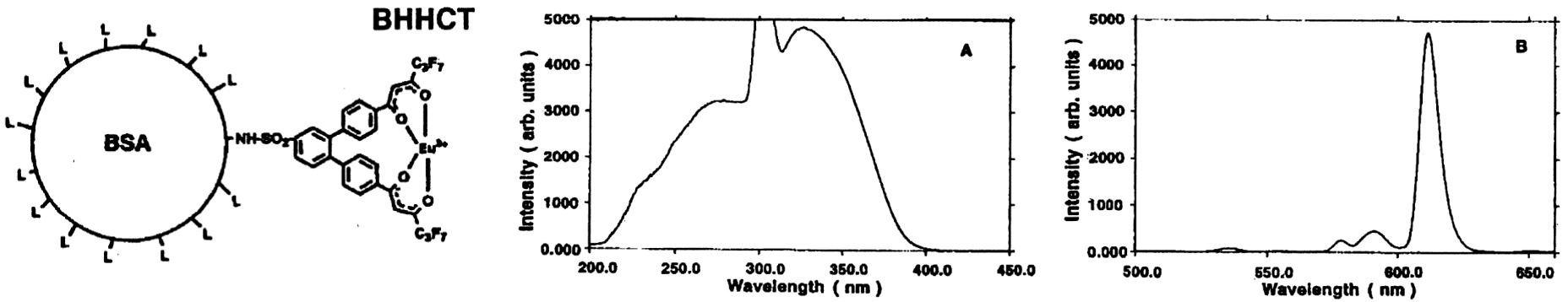
	lifetime	Stokes shift	λex,max	λem,max
	ns	nm	nm	nm
Fluorescein (FITC)	4.5	28	492	520
Rhodamine (RBITC)	2	35	550	585
Eu(β-NTA)3[a)]	500,000	276	339	615

Matsumoto et al. Fluorescent Lanthanide Chelates for Biological Systems. Macromol. Symp. 186, 117-121 (2002)

Cho U. & Chen J.K. Lanthanide-based optical probes of biological systems (2020) Cell Chemical Biology

Lanthanide Chelate Complexes

Excitation (A) and emission (B) spectra of Bis-heptafluoro-hexanedion-chlorosulfo-o-terphenyl (BHHCT) labelled with BSA in the presence of Eu^{3+}.



Example: BHHCT-Eu^{3+} is suitable for immunoassay & DNA hybridization

Matsumoto et al. Fluorescent Lanthanide Chelates for Biological Systems. Macromol. Symp. 186, 117-121 (2002)