

# Bioluminescence, fluorescence and fluorescent proteins

# **Incandescence**

is light generated by **high temperatures.**



# **Luminescence**

Emission of light **without heat**

# Diversi tipi di **LUMINESCENZA**

- **TIPO**

- **CHEMILUMINESCENZA**

- BIOLUMINESCENZA

- FOTOLUMINESCENZA  
(fluorescenza, fosforescenza)

- ELETTROLUMINESCENZA

- RADIOLUMINESCENZA

- **CAUSA**

- **Reazione chimica**

- Reazione enzimatica

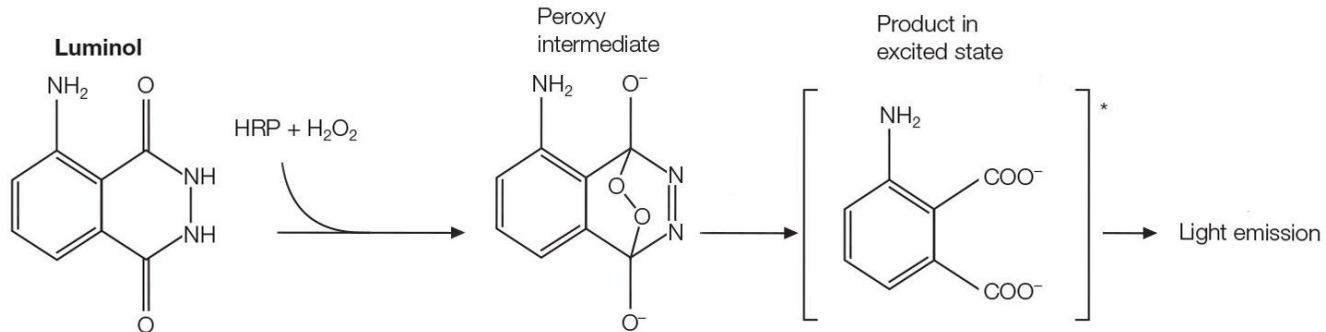
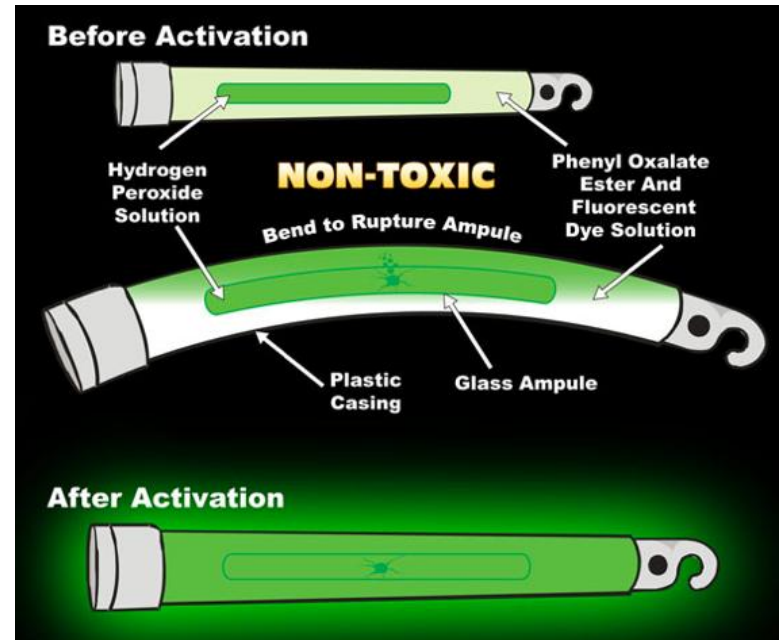
- Assorbimento luce UV-vis

- Corrente elettrica in gas ionizzato

- Materiale radioattivo incorporato nel  
“fosforo”

# Diversi tipi di LUMINESCENZA

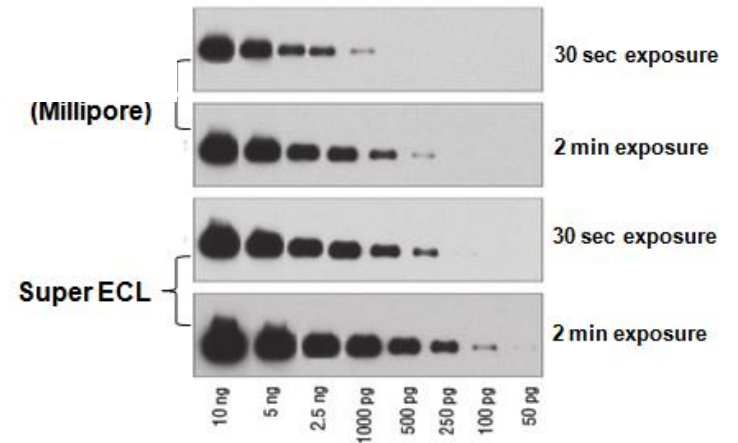
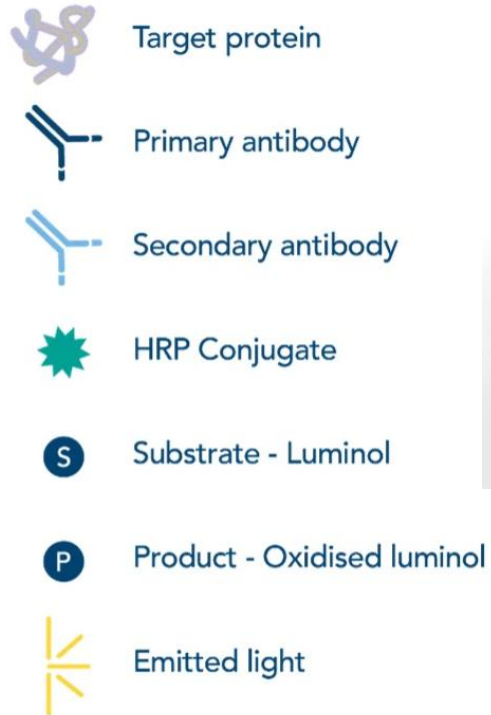
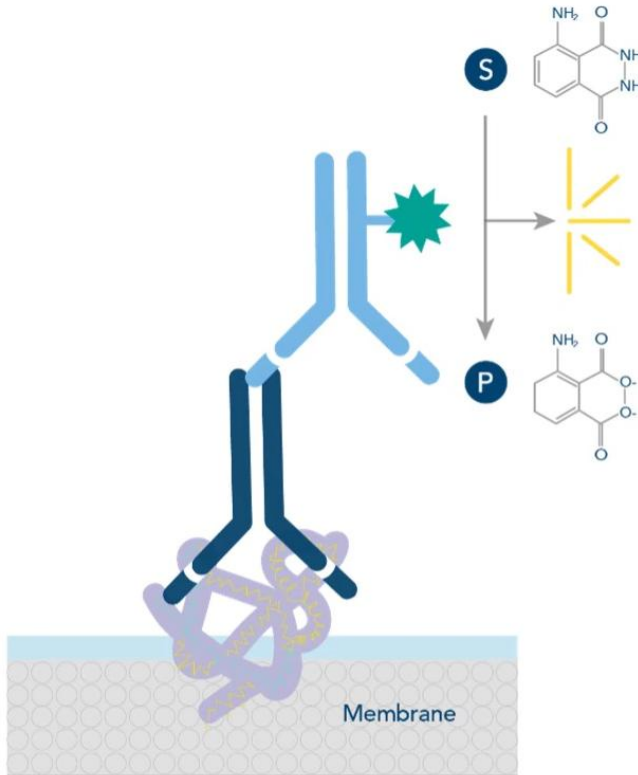
- CHEMILUMINESCENZA
- Reazione chimica



# Diversi tipi di LUMINESCENZA

## • CHEMILUMINESCENZA

## • Reazione chimica



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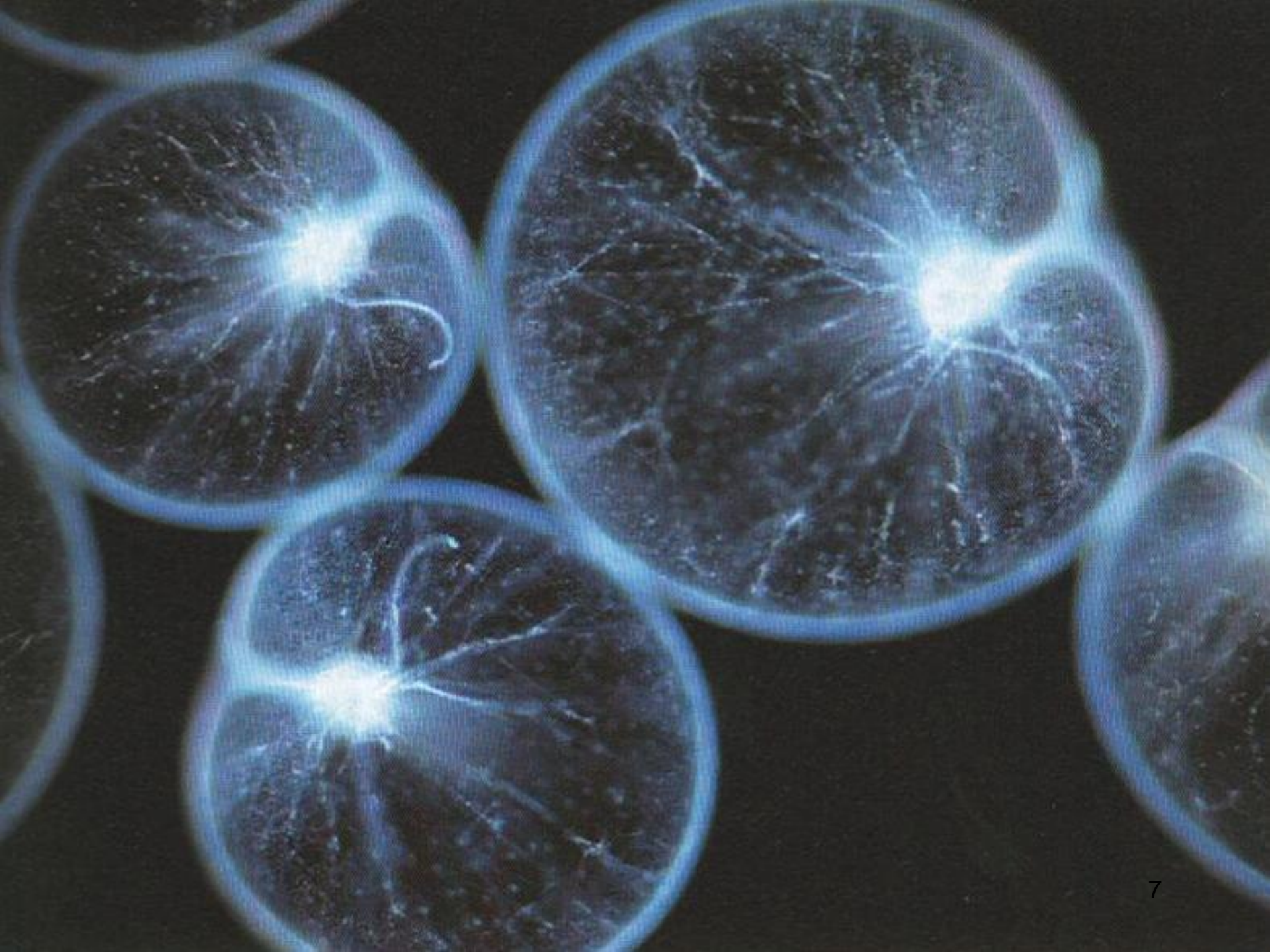
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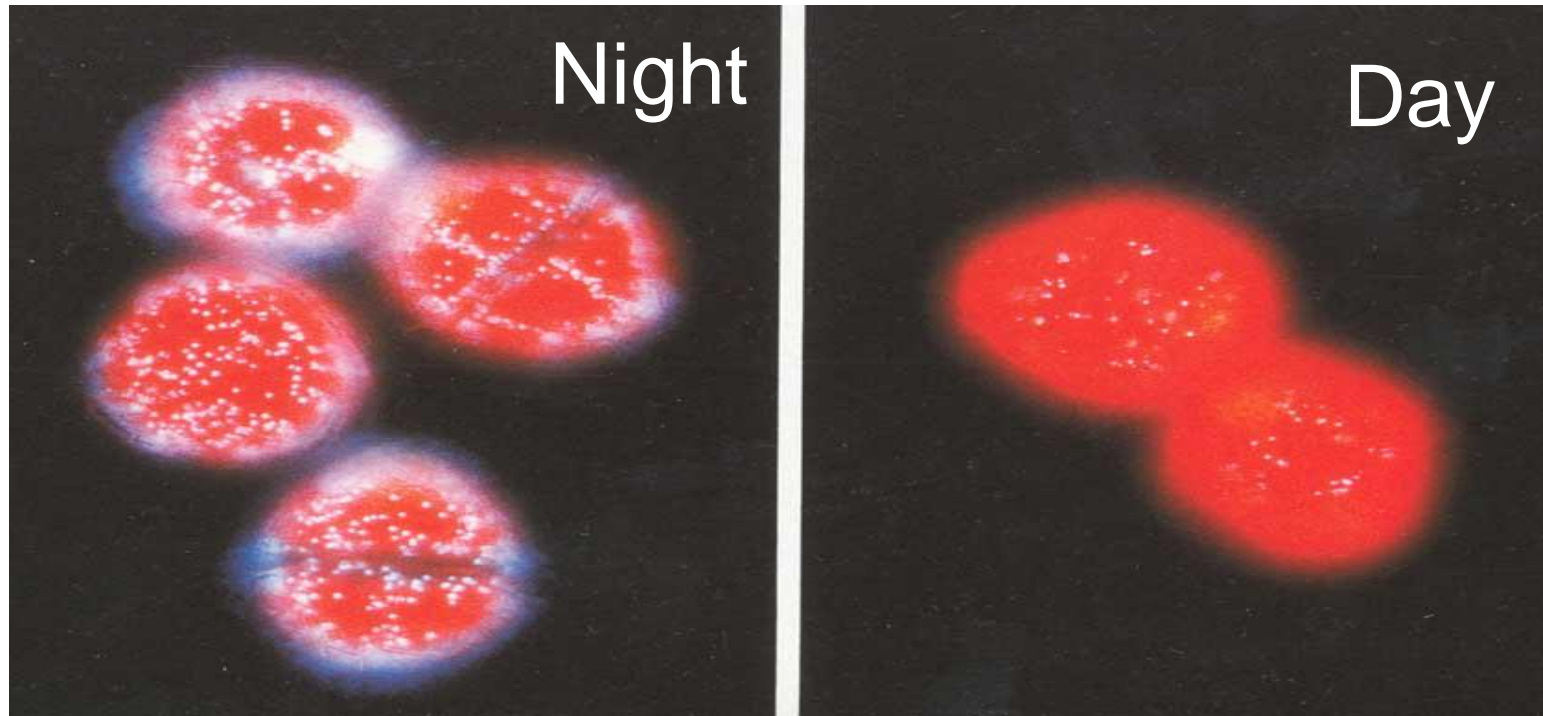
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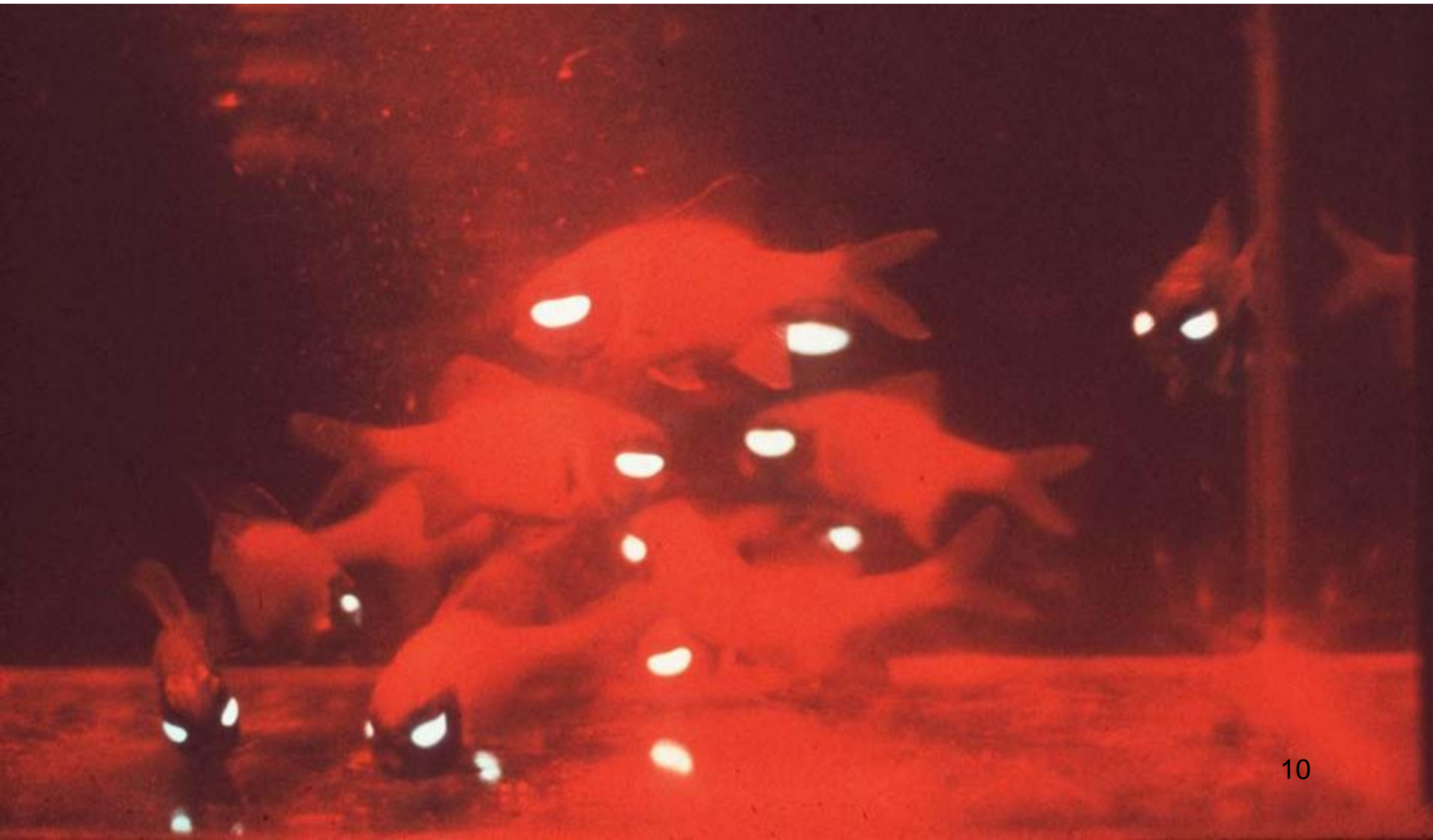
# Gonyaulax: luciferin fluorescence in scintillons



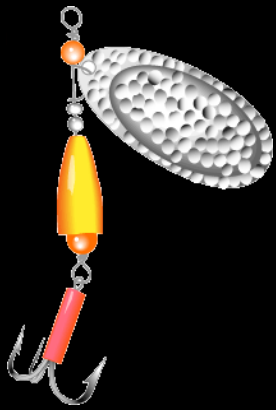
# Flashlight fish, Gulf of Aqaba



# Flashlight fish in aquarium



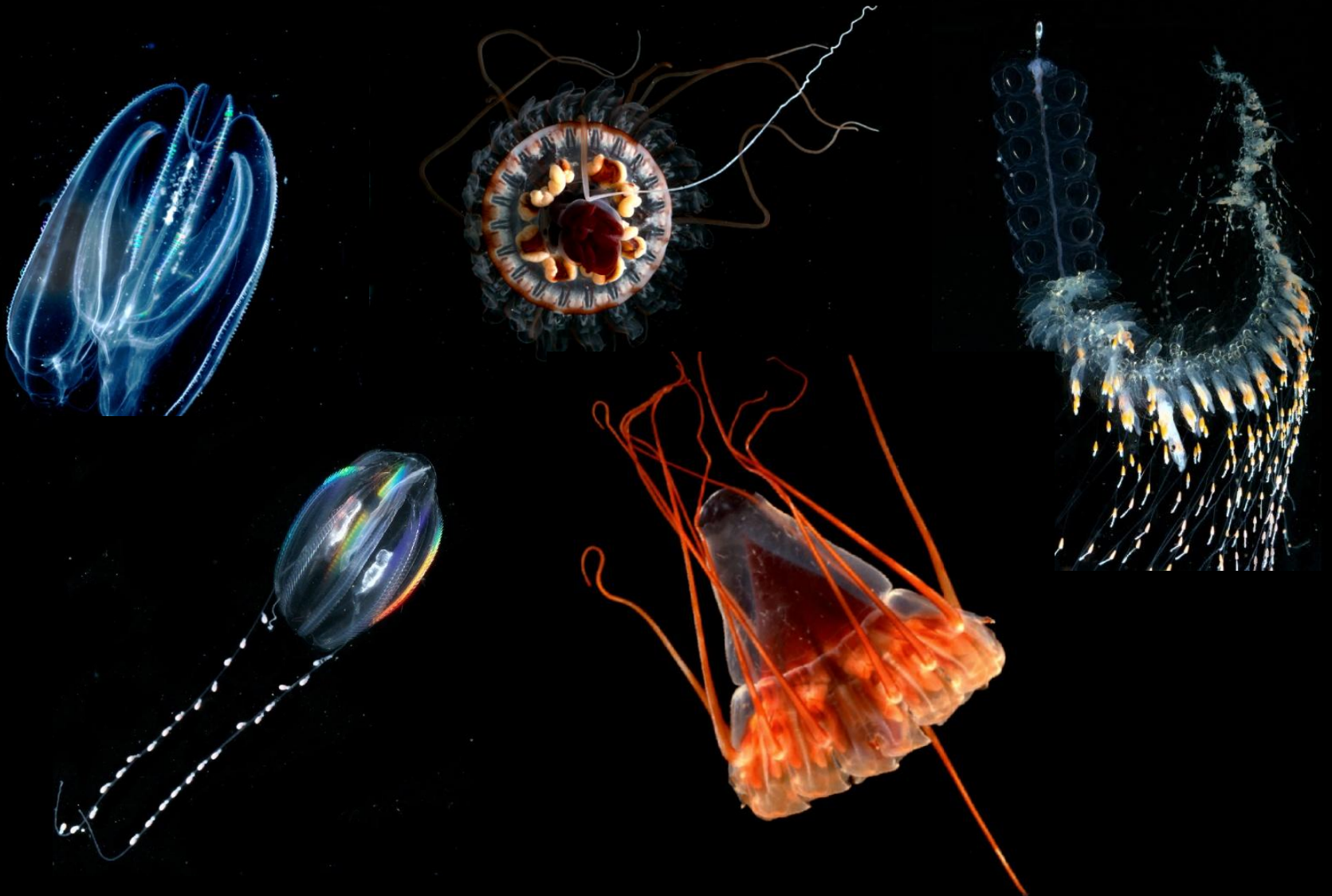


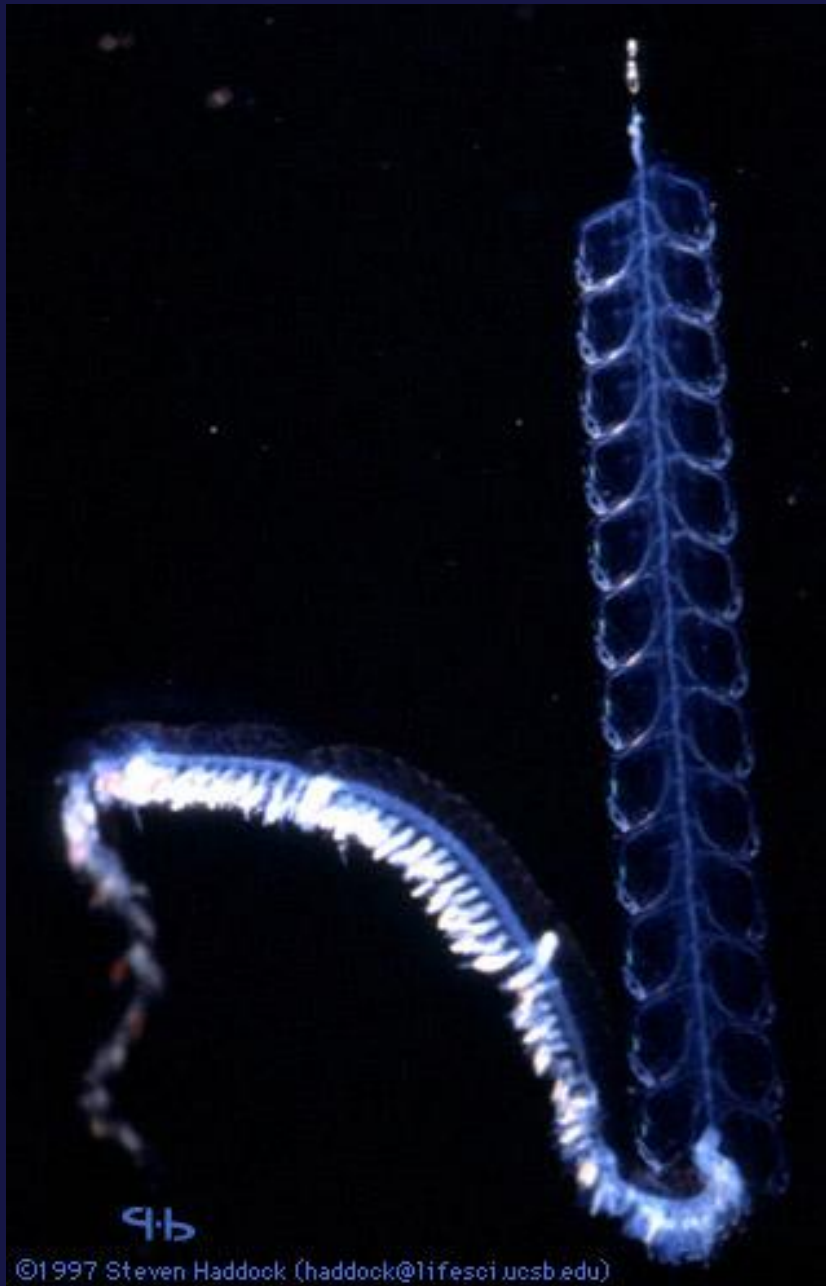


# Biofluorescence in Shark

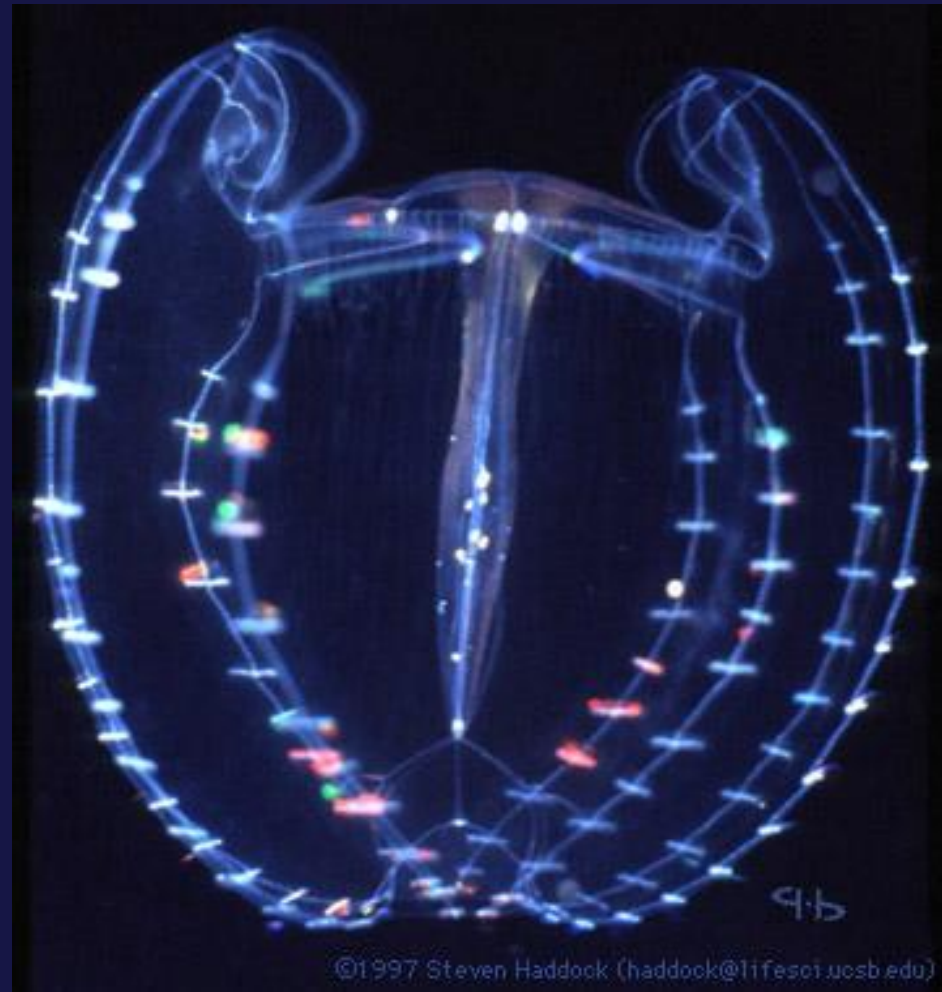


# Fluorescent gelatinous Zooplankton





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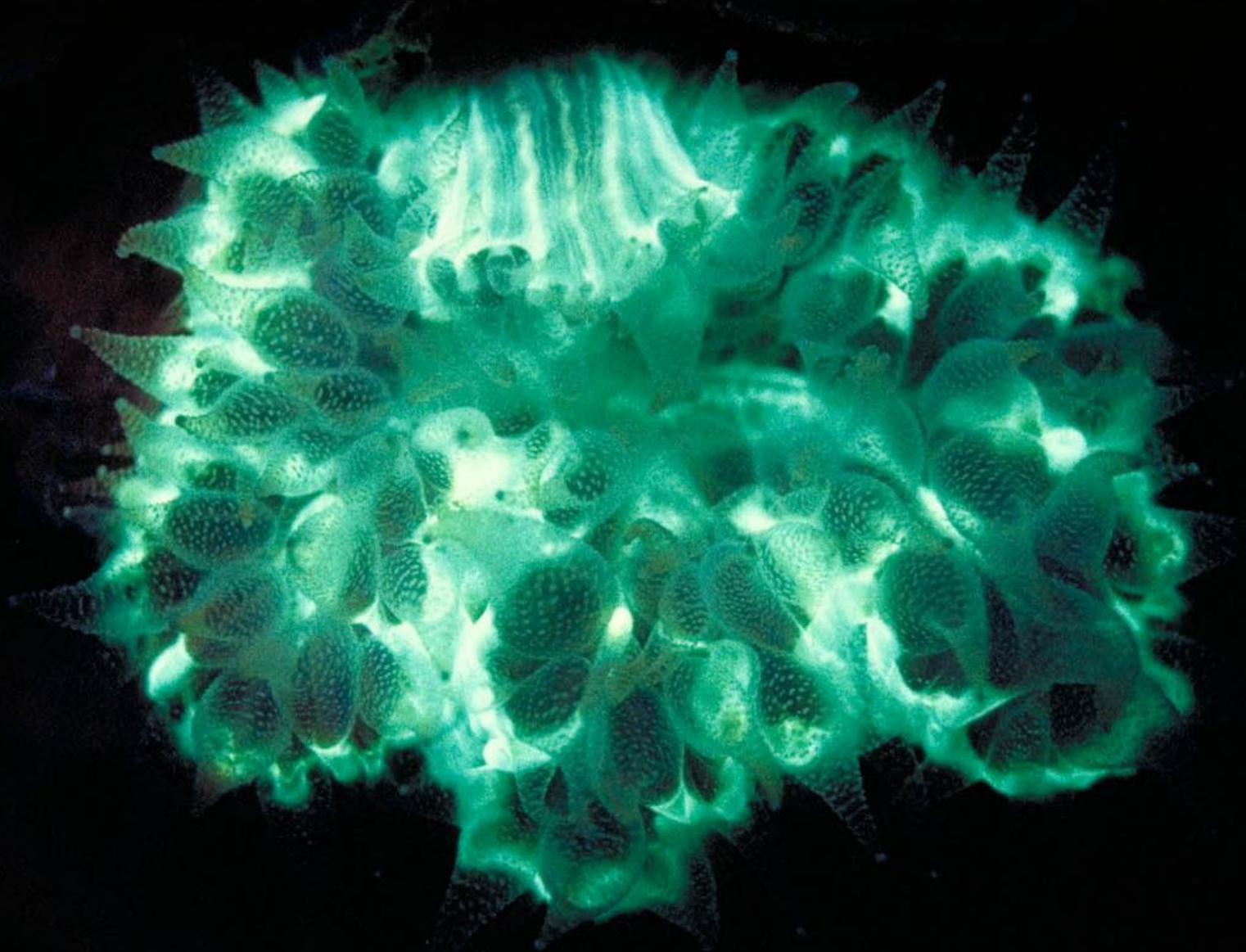


©1997 Steven Haddock (haddock@lifesci.ucsb.edu)

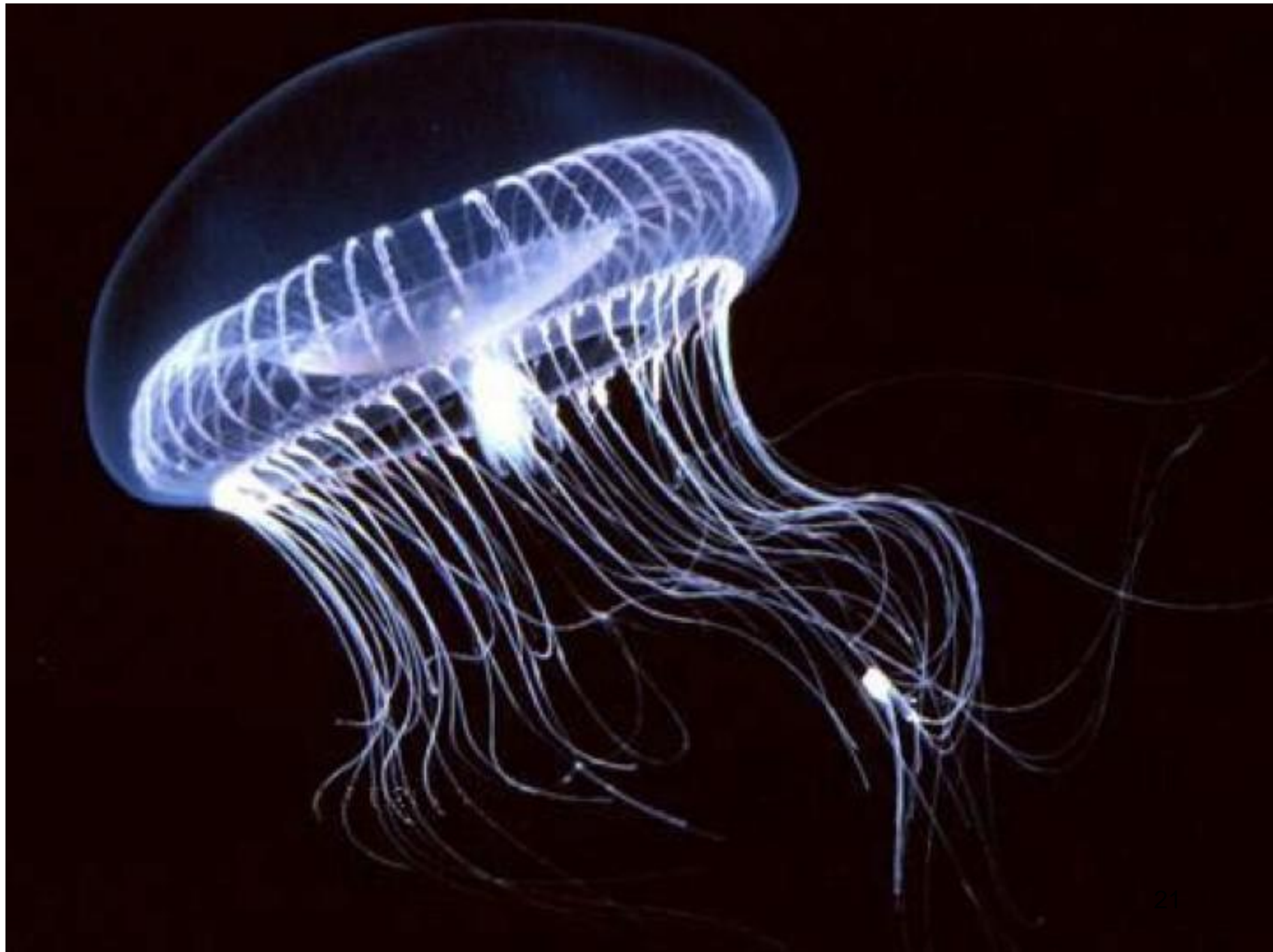




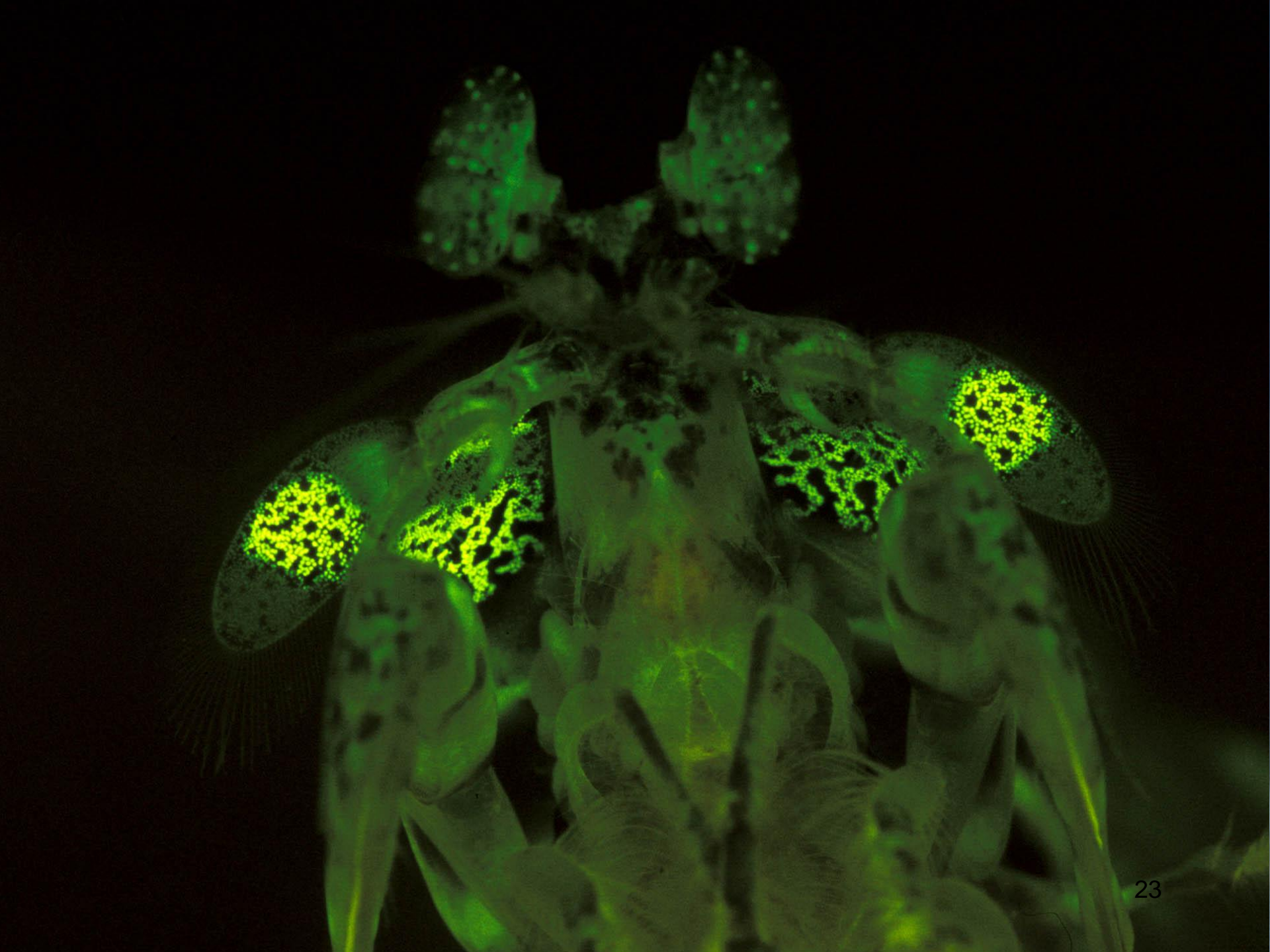












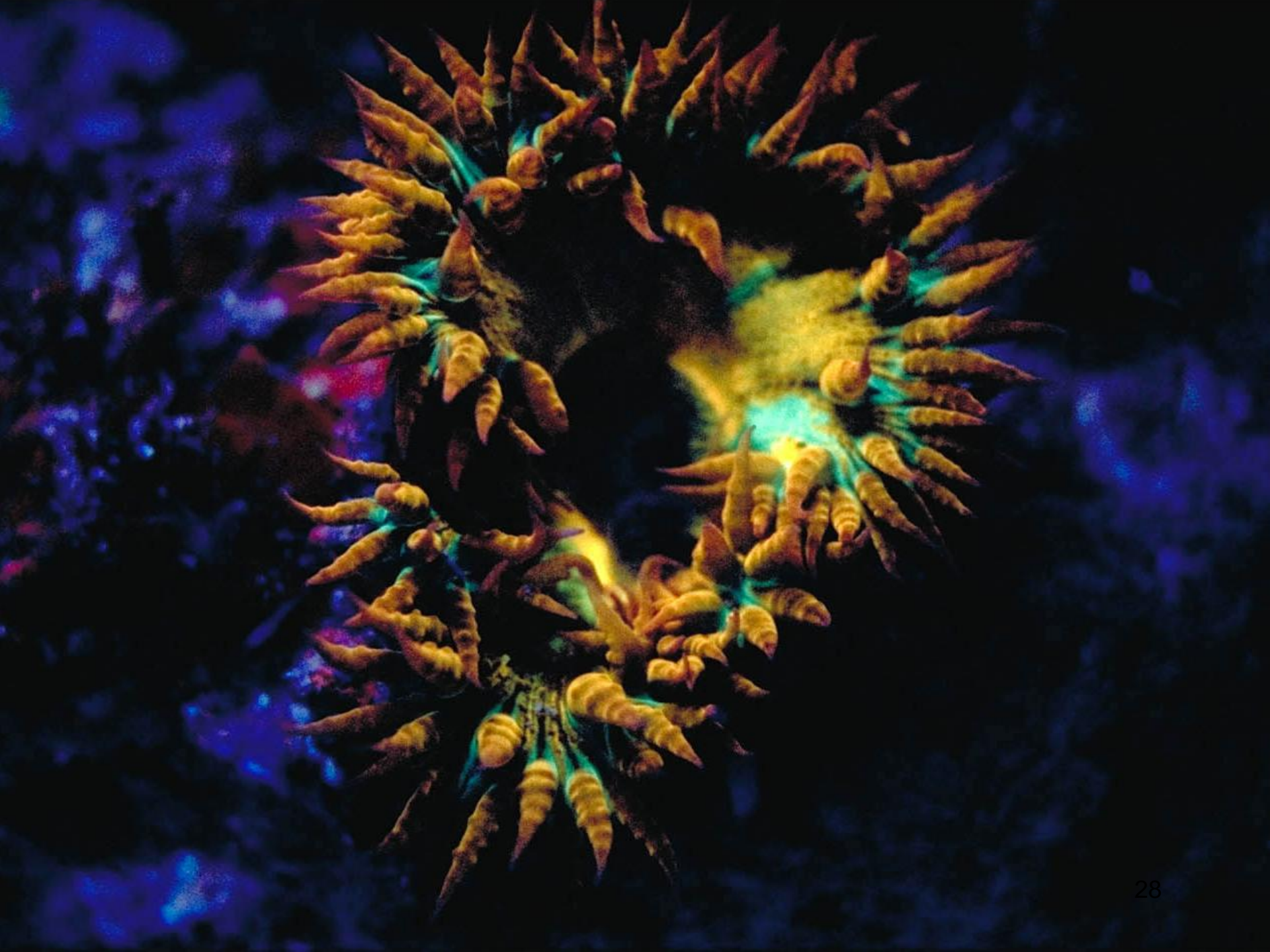
# Shrimp scaring a fish



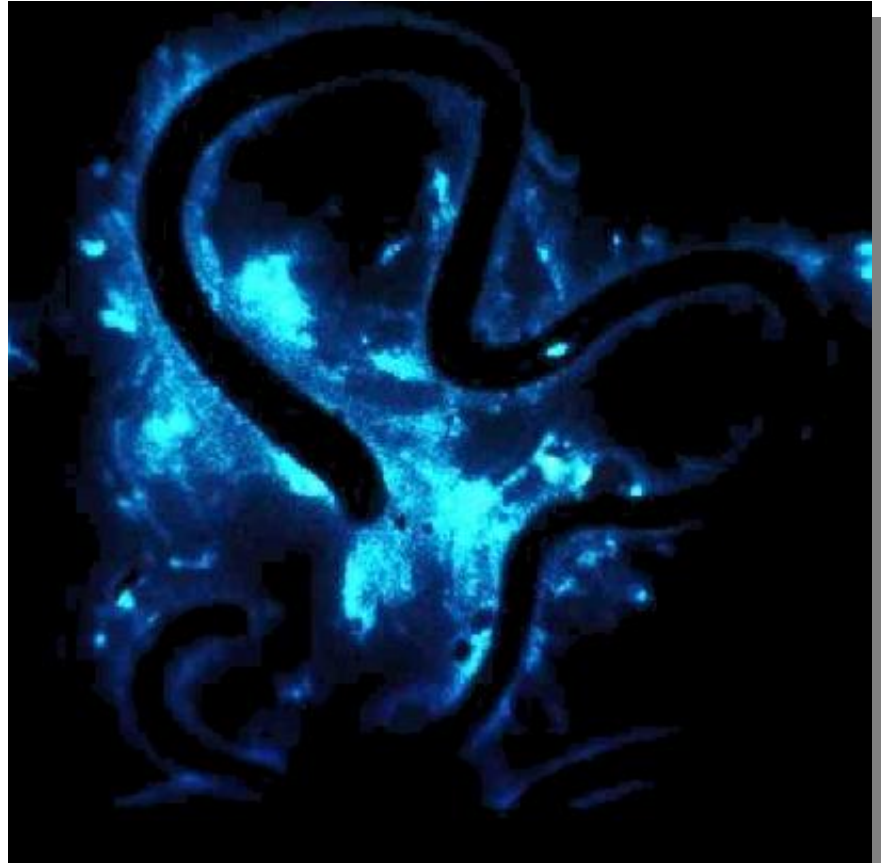
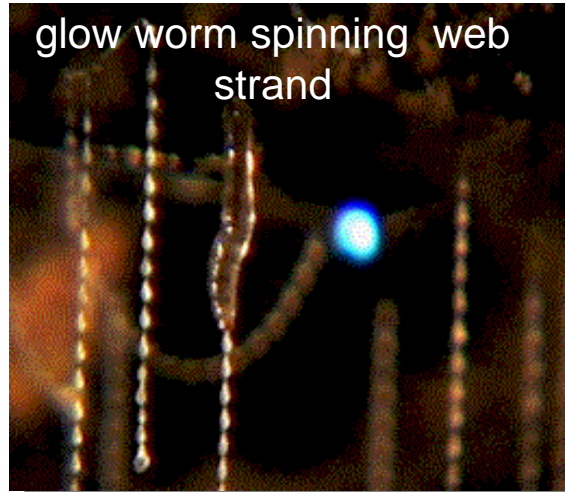




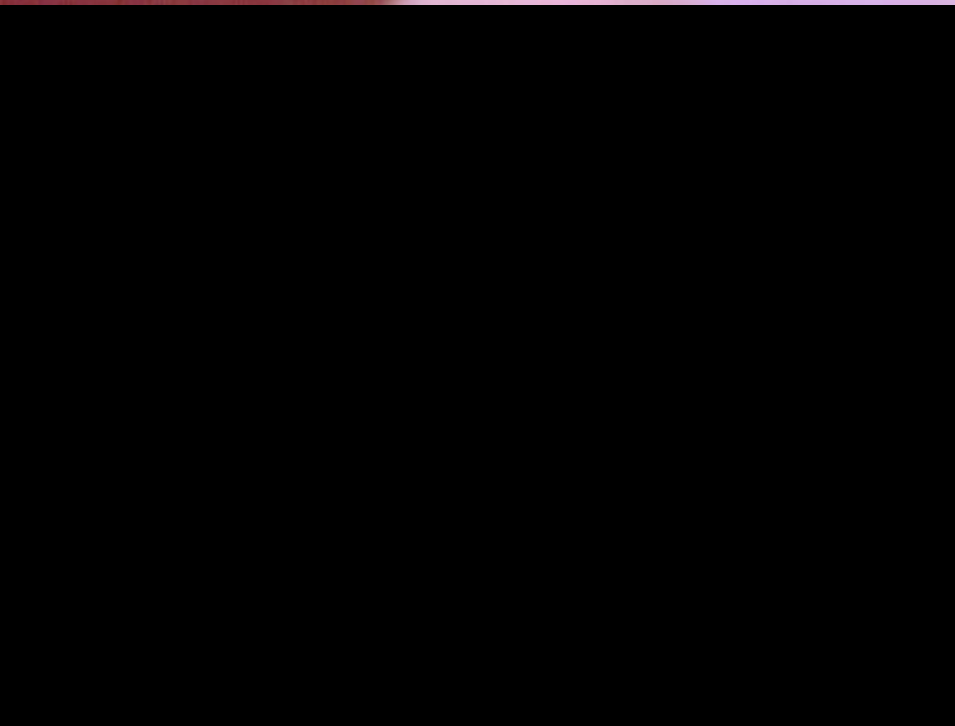
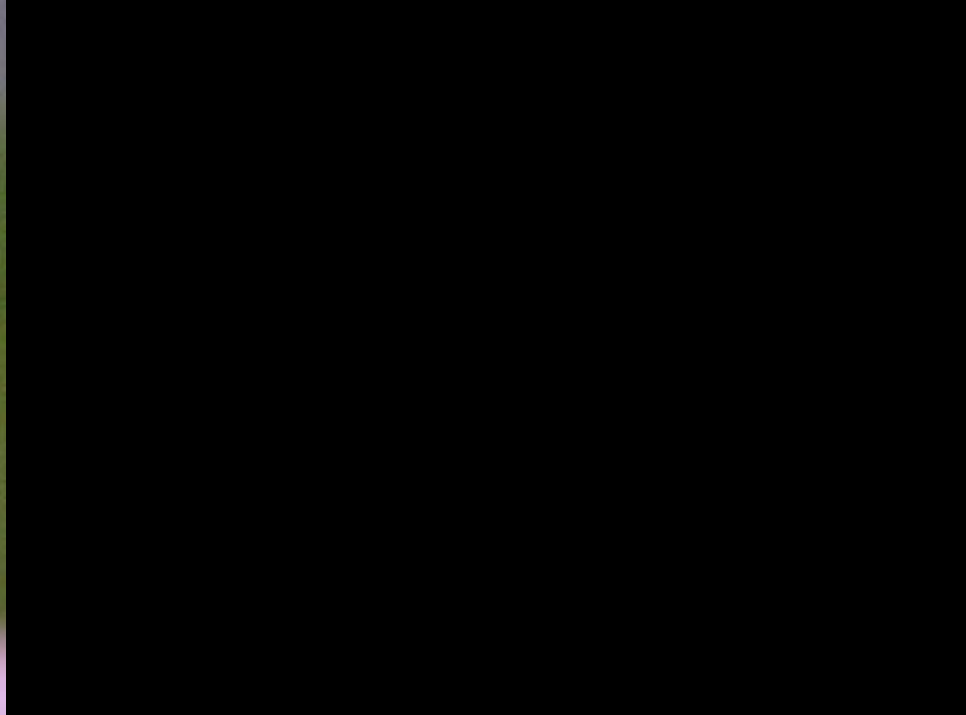




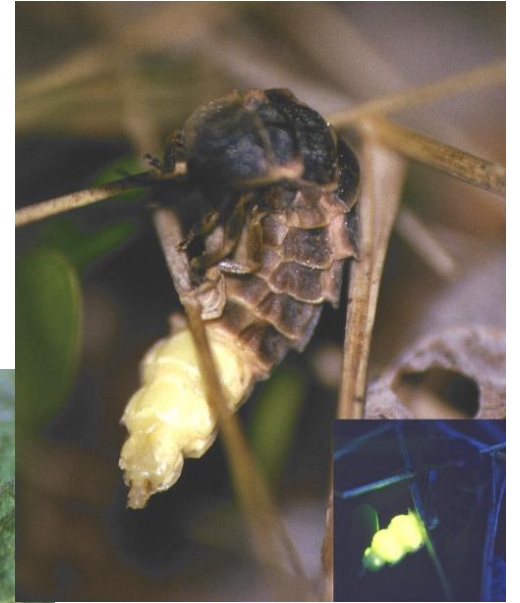
# Bioluminescent worms



Some earthworms exude a luminescent sticky, continuously glowing slime



# Firefly superfamily

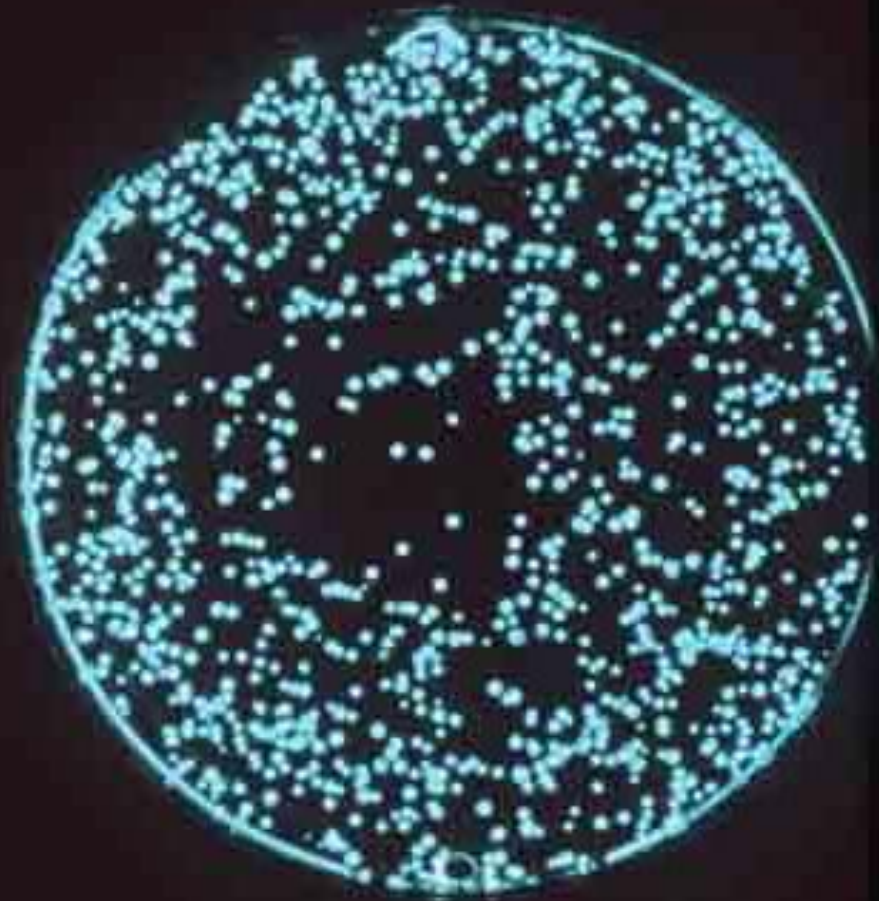


# History

- **500 BC** Anaximenes of Miletus, noticed that when he was rowing at night his oar blade gave off a strange light as it broke the water.
- Aristotle (**384-322 BC**) described 180 marine species and was the first to recognise "cold light" (luminescence).
- **Christopher Columbus** (1492). referred to mysterious lights in the water before reaching San Salvador
- In **1634 English** explorers mistook the light from firefly beetles for the lights of Spanish campfires and decided to avoid landing on Cuba, perhaps altering the history of the new world.

# Bioluminescent species

- 17 phylae and over 700 genera contain bioluminescent species
- bacteria, fish, squid, fungi, jellyfish, protozoa, sponges, worms, centipedes and millipedes, crustaceans, snails, click beetles and fireflies
- Not mammals, reptiles, birds, amphibians, spiders or scorpions



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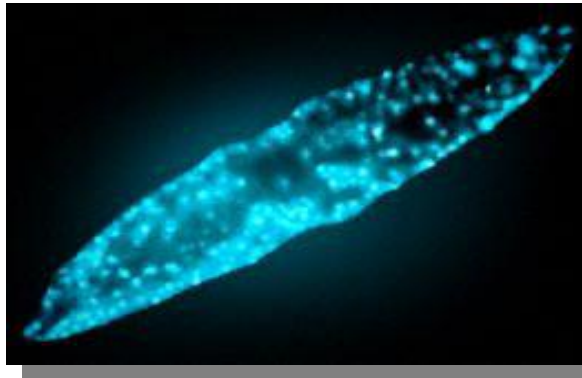
Luminescence

Bioluminescence

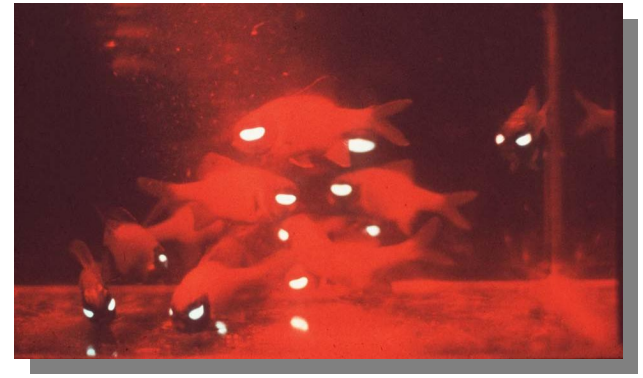
**Makes its own light**



Fireflies!



Dinoflagellates



Flashlight fish

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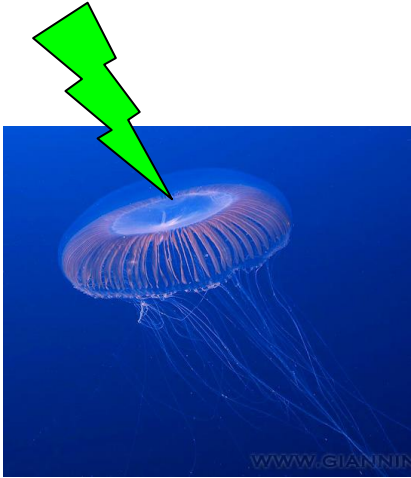
Luminescence

**Fluorescence**

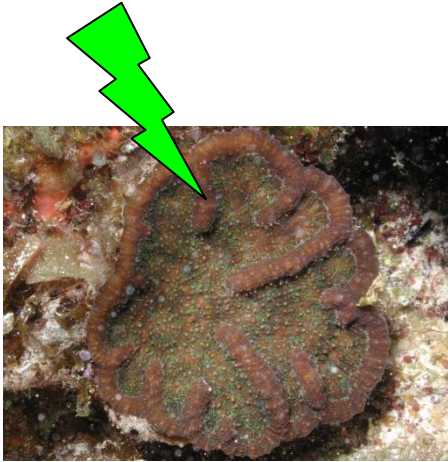
**Glow**s when you **shine light** on it



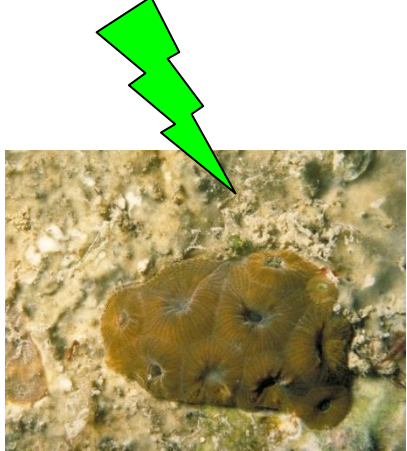
ON



Jellyfish



Corals



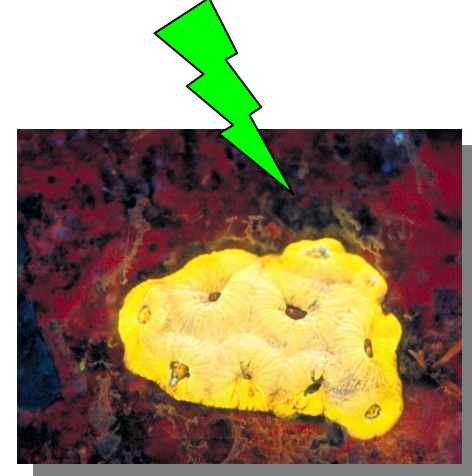
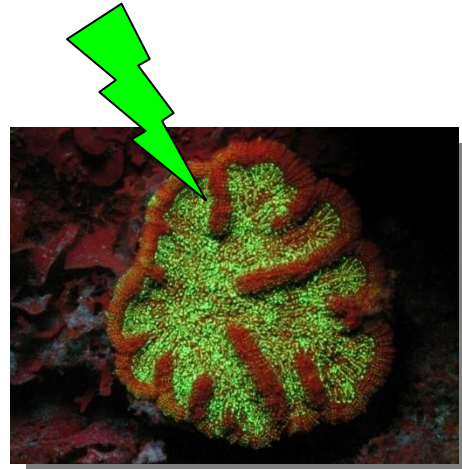
Luminescence

**Fluorescence**

**Glow stops when you turn off the light**



Jellyfish



Corals



Luminescence

**Glow**s when you  
**shine light** on it

Fluorescence

**Glow stops** when you turn off **the light**



Jellyfish

Corals

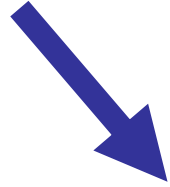


# Fluorescence and phosphorescence

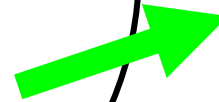
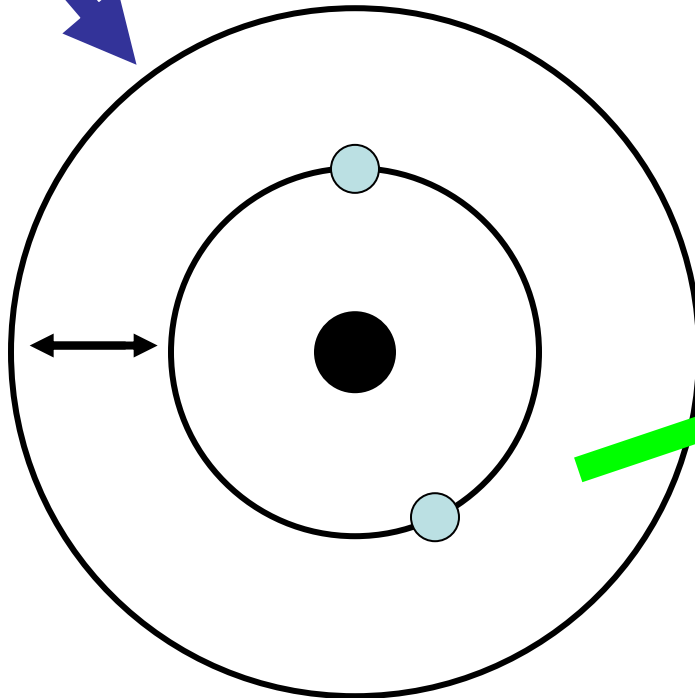
- In **phosphorescence** the light is re-emitted **over a longer time period**
- In **fluorescence** the light is **immediately re-emitted**
- **Fluorescence** is
  - the absorption of electromagnetic radiation (light) **at one wavelength**,
  - its re-emission at another, **longer wavelength**

# Fluorescence

Ligth



Energy gap

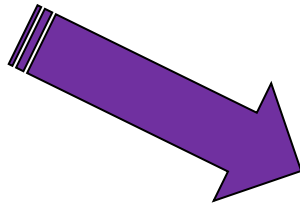


Ligth

# Electromagnetic Spectrum

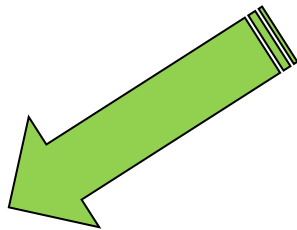
Absorb

at **high energy**

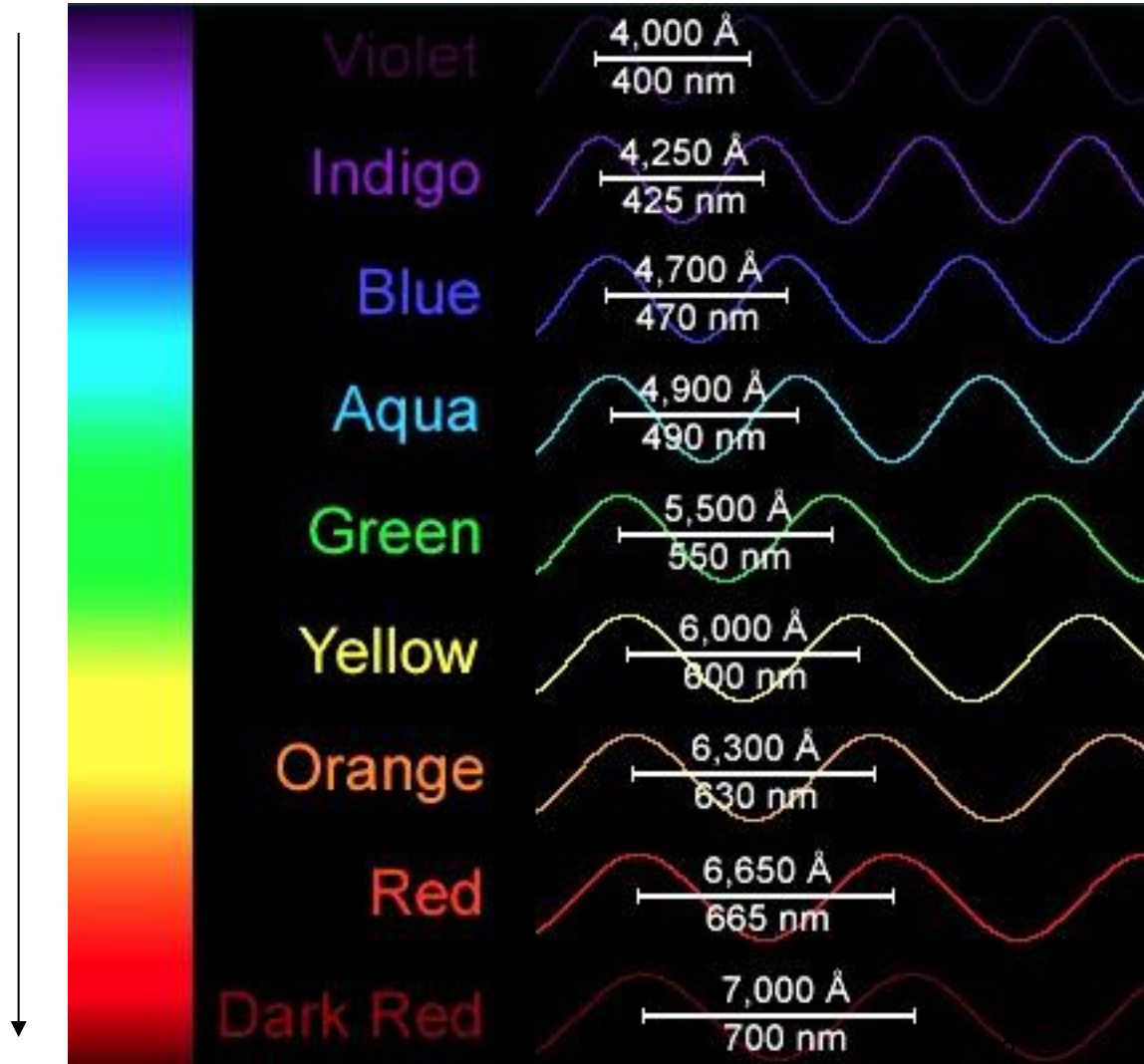


Emit

at **low energy**

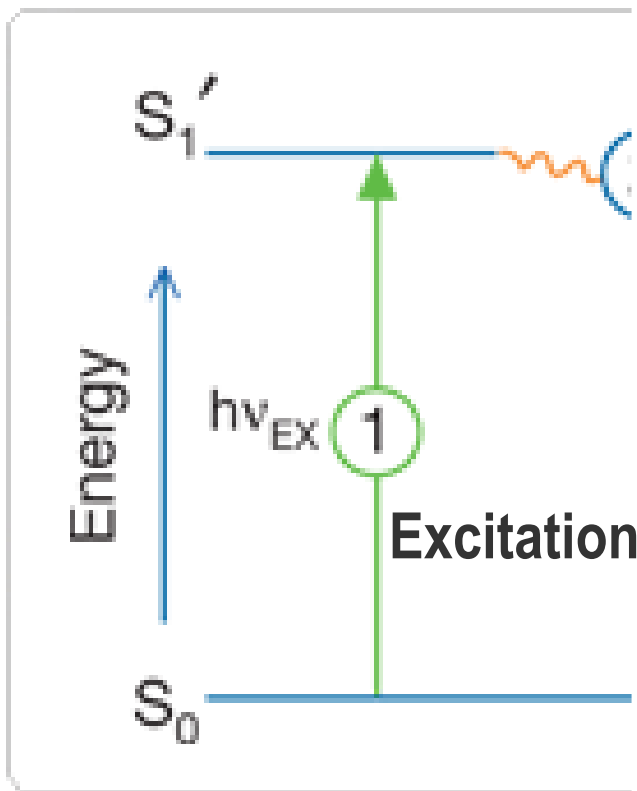


Decreasing energy



# FLUORESCENZA

- **Emissione di radiazioni** di una molecola che torna, da uno stato elettronico **eccitato** grazie all'assorbimento di luce, a quello **fondamentale**
- L'emissione è spostata **verso il rosso** rispetto alla eccitazione
- Parametri misurabili:
  - **Spettri di eccitazione ed emissione**
  - Intensità di fluorescenza
  - Tempo di vita
  - Polarizzazione



### Stage 1: Excitation

A photon of energy  $h\nu_{EX}$  is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state ( $S_1'$ ).

### Stage 2: Excited-State Lifetime

The excited state exists for a finite time (typically 1–10 nanoseconds)

### Stage 3: Fluorescence Emission

A photon of energy  $h\nu_{EM}$  is emitted, returning the fluorophore to its ground state  $S_0$

# Resa quantica

$\Phi_f$  = quantum yield

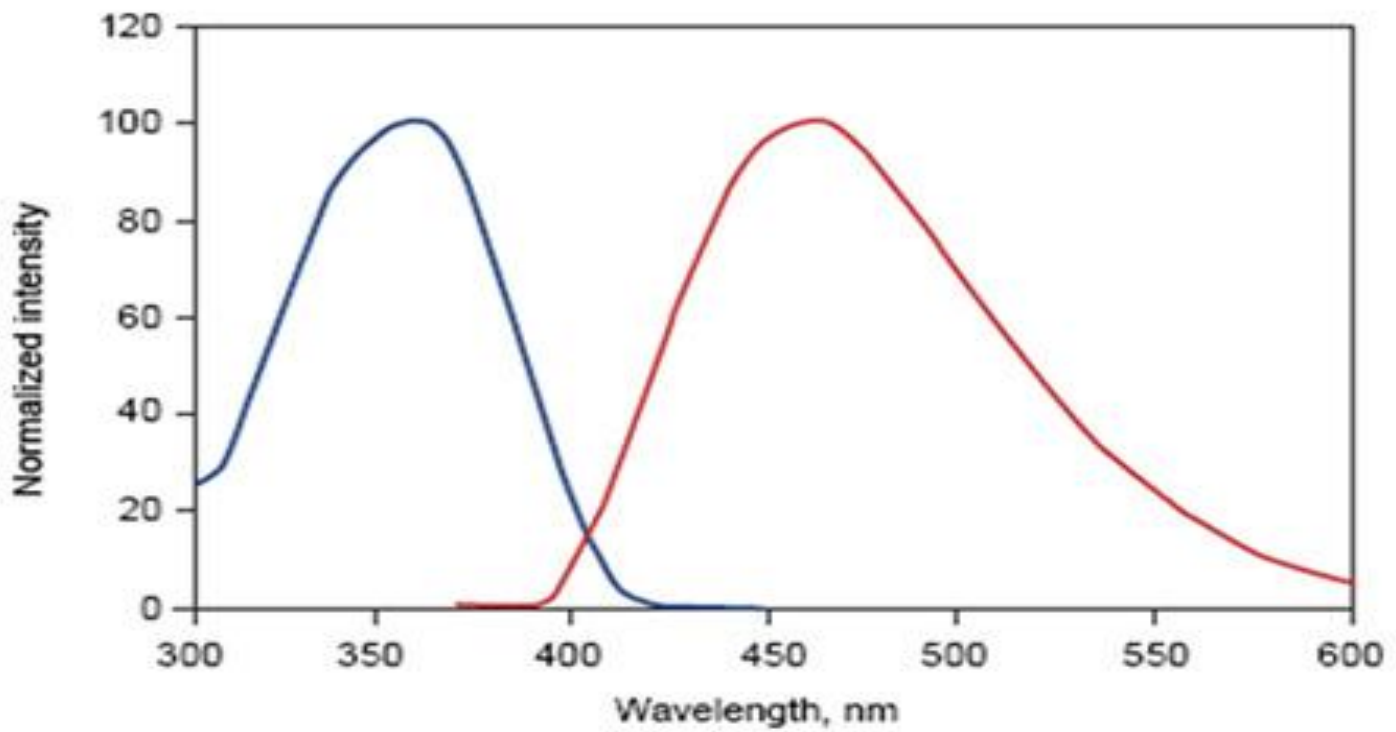
La resa quantica (rendimento quantico, efficienza quantica) della molecola.

$$\Phi_f = \frac{\text{Numero di fotoni emessi}}{\text{Numero di fotoni assorbiti}}$$

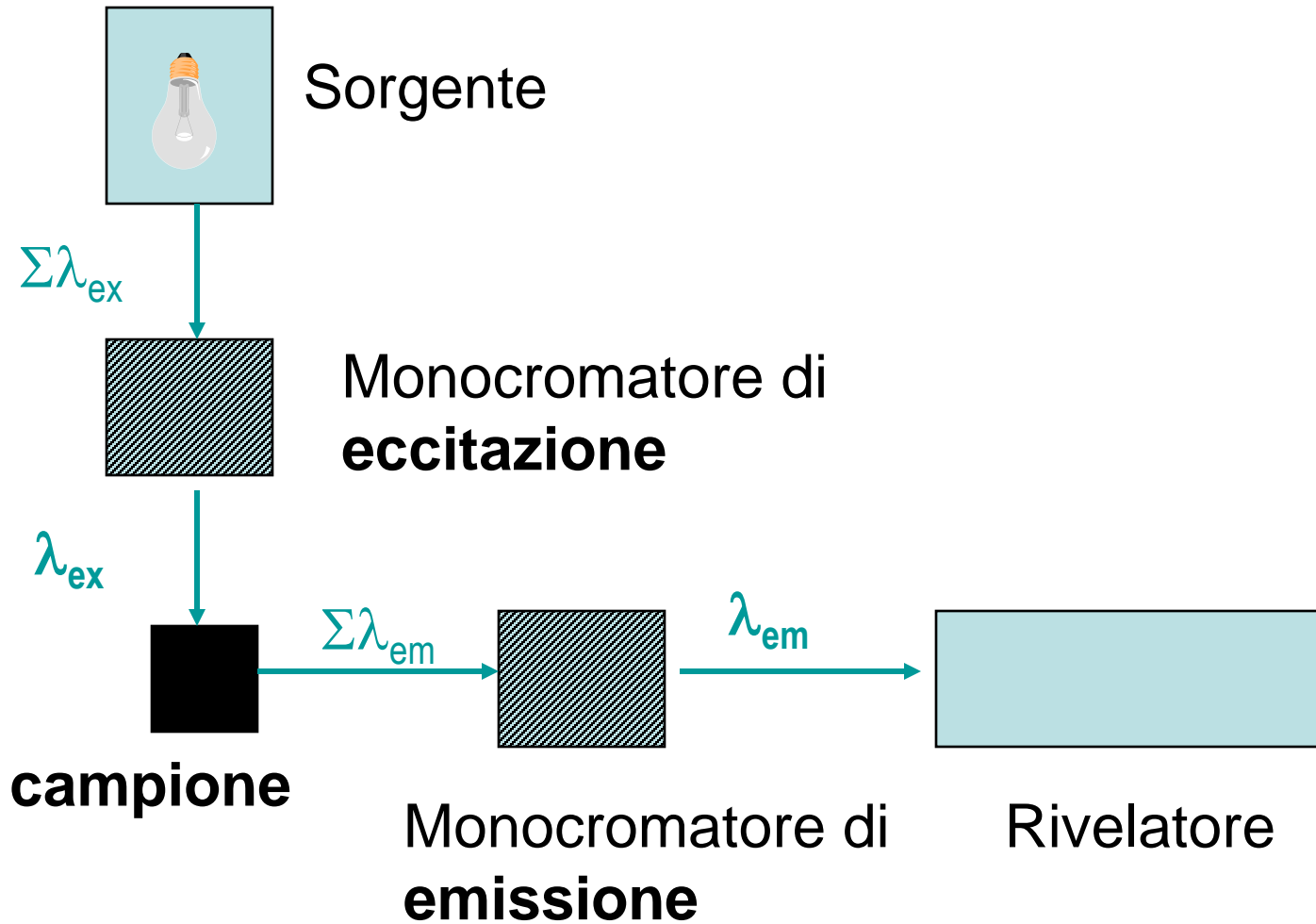
$$0 \leq \Phi_f \leq 1$$

- Per  $\Phi_f = 0$  la molecola non è fluorescente (in quelle condizioni).

La **resa quantica** rappresenta la frazione di molecole de-eccitate per fluorescenza



# Diagramma di uno spettrofluorimetro



# Spettri in soluzione

- Spettro di emissione

- Si illumina il campione con luce a lunghezza d'onda costante e si fa una scansione del monocromatore di emissione.

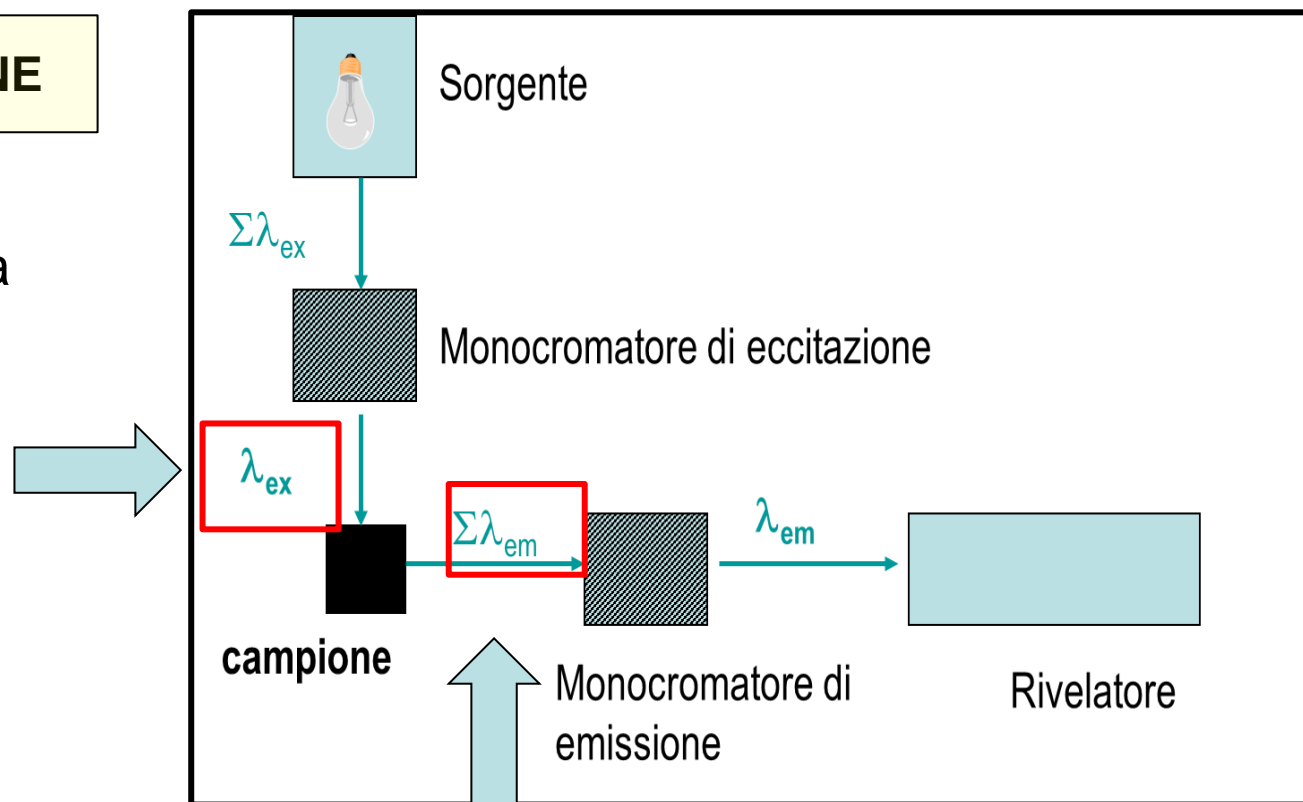
- Spettro di eccitazione

- Si fa una scansione del monocromatore di eccitazione tenendo fissa la lunghezza d'onda del monocromatore di emissione.

## Spettri in Soluzione

### SPETTRO DI EMISSIONE

- Si illumina il campione con luce a **lunghezza d'onda costante**



- si fa una **scansione** del monocromatore di emissione

# Spettri in soluzione

- Spettro di emissione

- Si illumina il campione con luce a lunghezza d'onda costante e si fa una scansione del monocromatore di emissione.

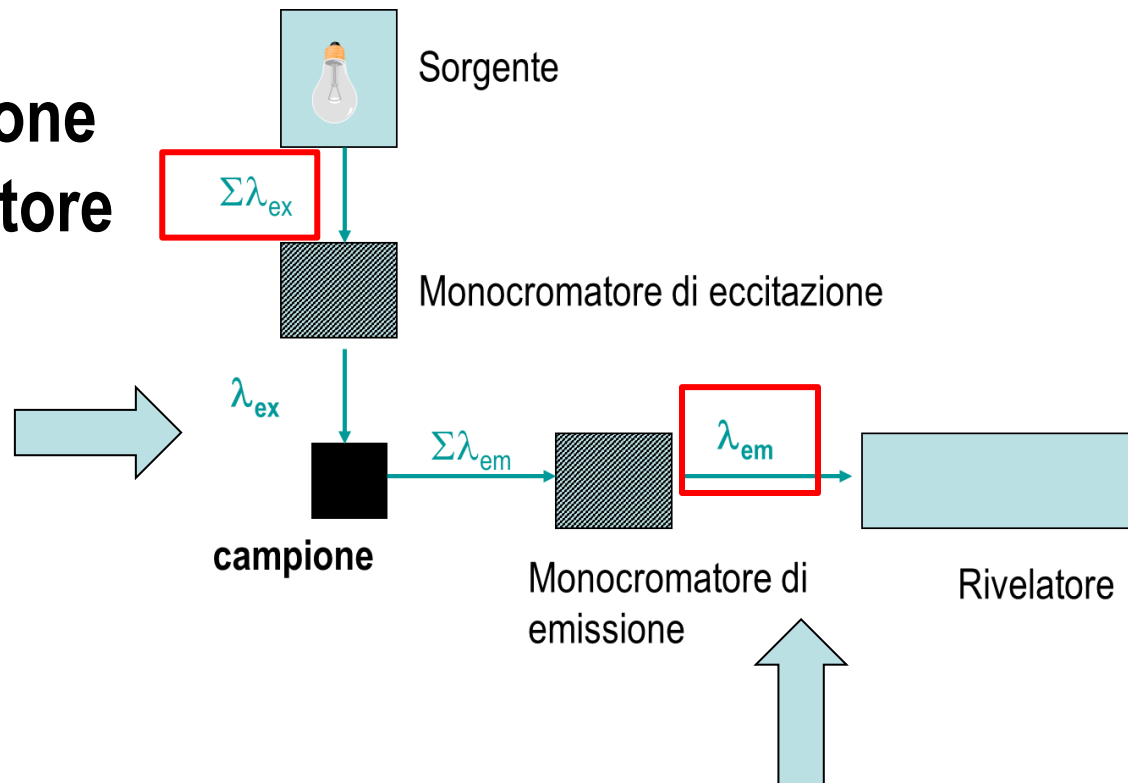
- Spettro di eccitazione

- Si fa una scansione del monocromatore di eccitazione tenendo fissa la lunghezza d'onda del monocromatore di emissione.

# Spettri in Soluzione

## SPETTRO DI ECCITAZIONE

- si fa una scansione del monocromatore di eccitazione



- Si misura a lunghezza d'onda costante nel monocromatore di emissione

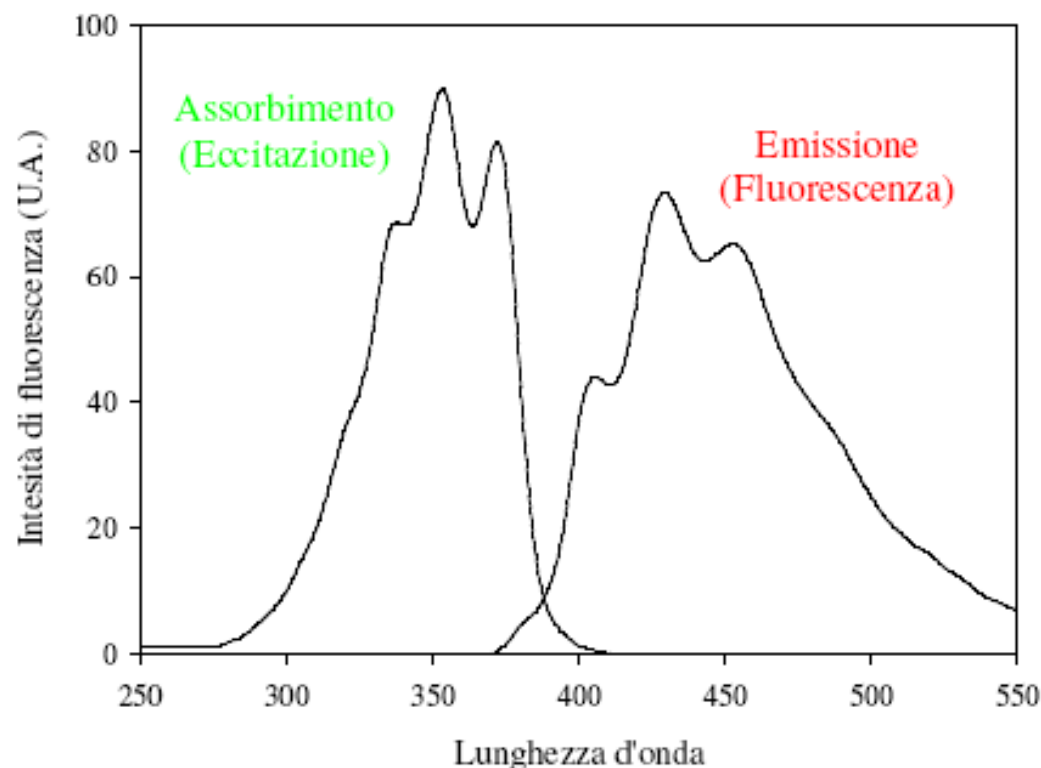
# Spettri eccitazione e di emissione

- **Spettro di emissione:**

- $\lambda_{\text{ex}} = 340 \text{ nm}$ , scansione dell'emissione da 370 nm a 550 nm

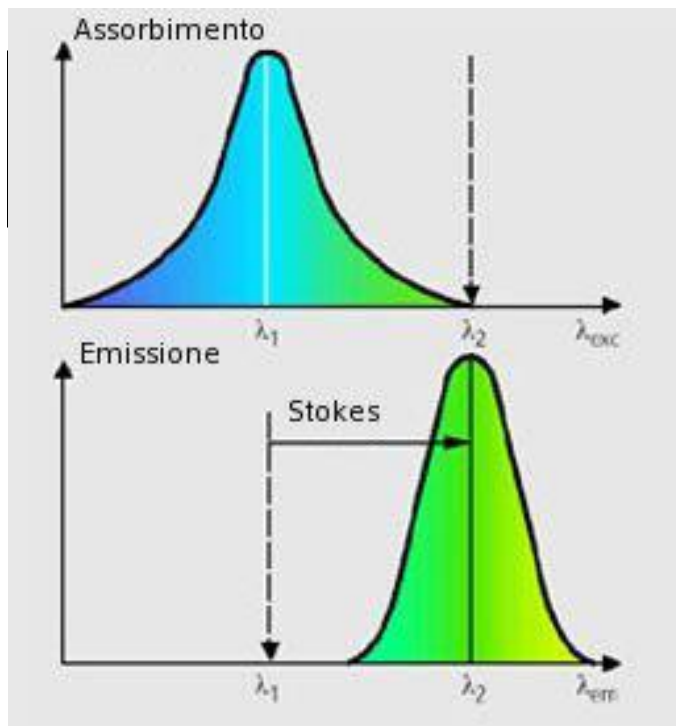
- **Spettro di eccitazione:**

- $\lambda_{\text{em}} = 430 \text{ nm}$ , scansione dell'eccitazione da 250 nm a 420 nm

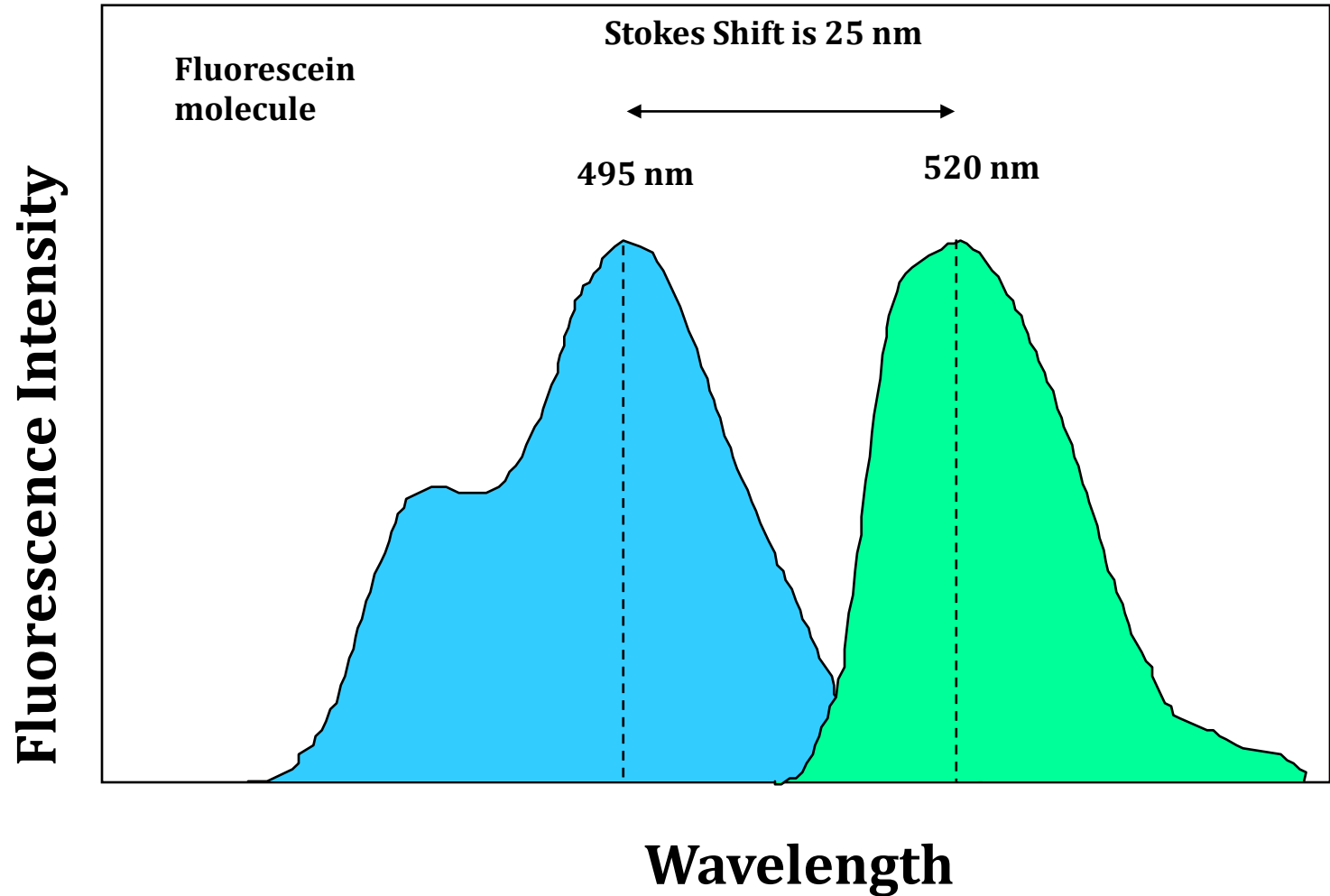


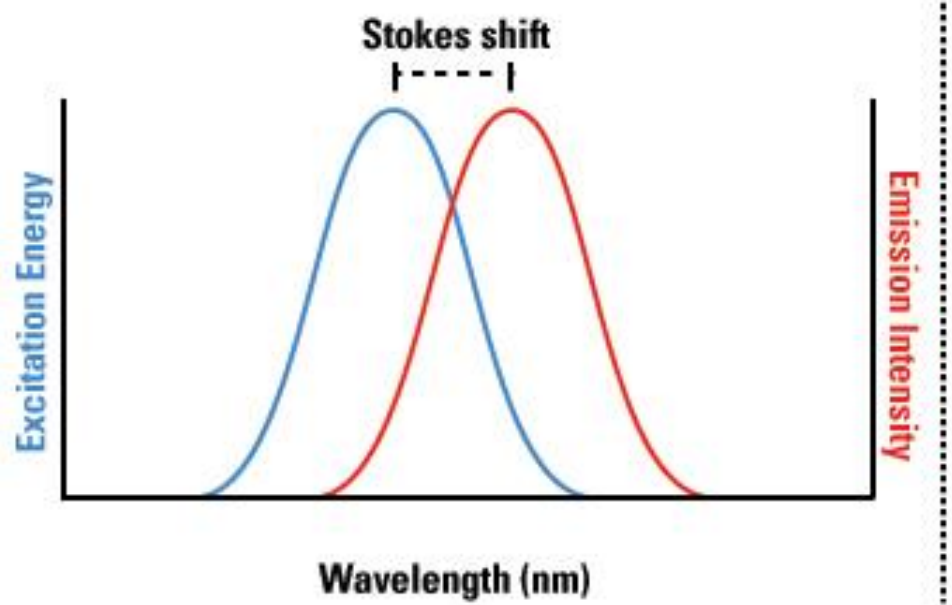
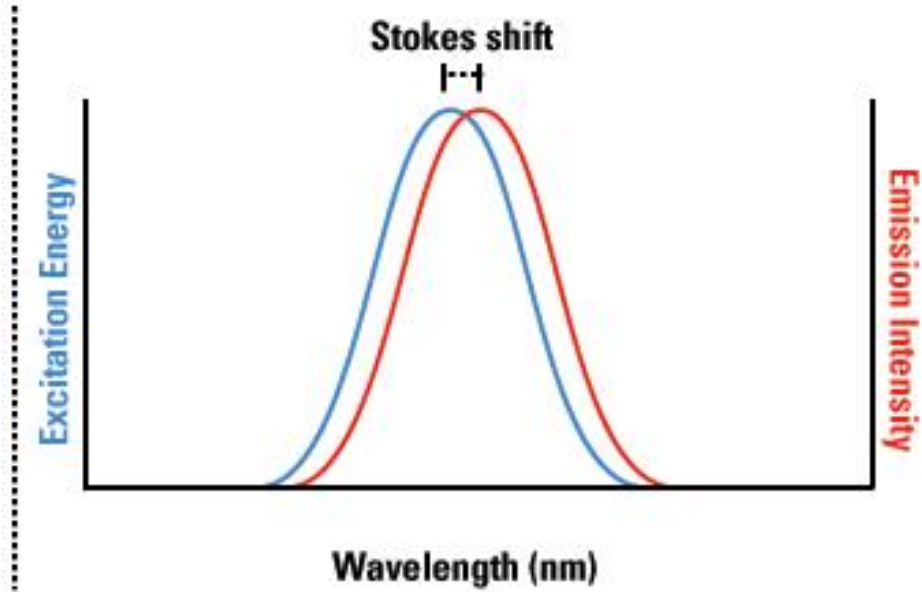
# Spettri di assorbimento ed emissione

- I fenomeni di disattivazione non radiativa ( $k_f$ ) dissipano l'energia accumulata dallo stato eccitato.
- L'emissione avverrà quindi ad energia più bassa (maggiore lunghezza d'onda).
- La differenza in energia è detta "Stokes' shift".

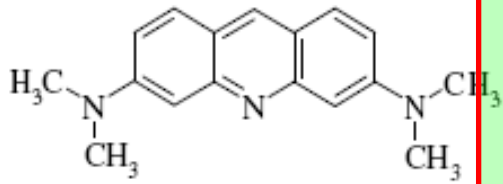


# Stokes Shift

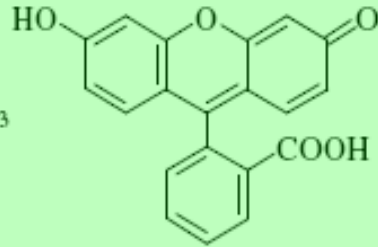




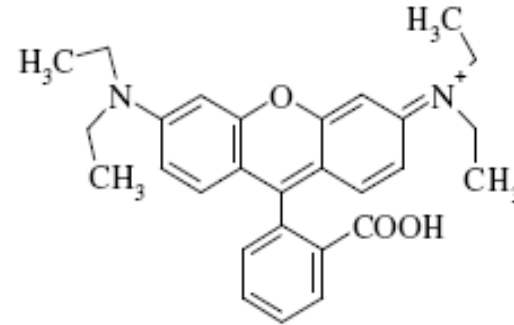
# Fluorofori estrinseci



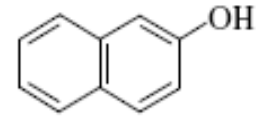
*Arancio di acridina*



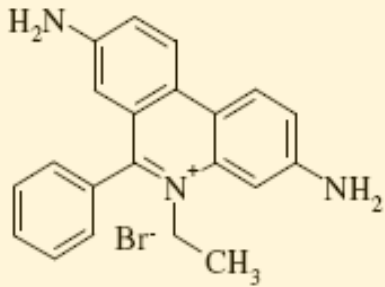
*Fluoresceina*



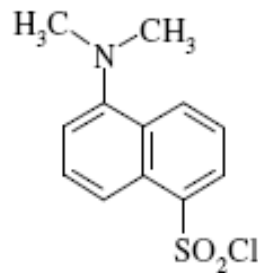
*Rodamina B*



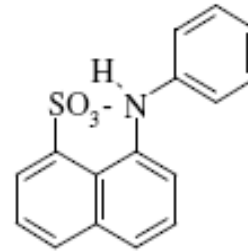
*Naftolo*



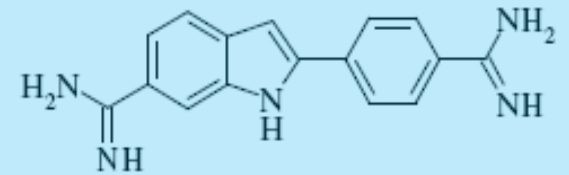
*Bromuro di etidio*



*Dansile*

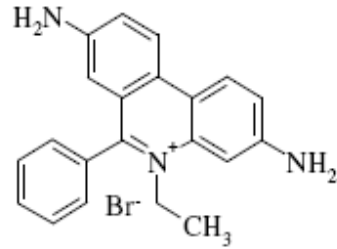
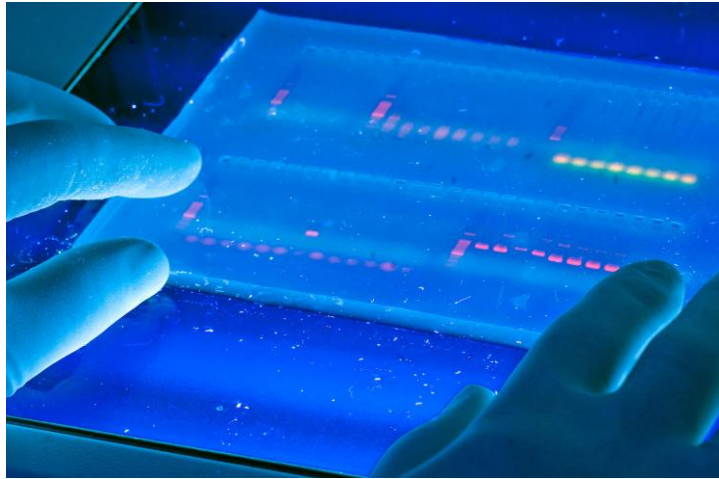


*ANS*

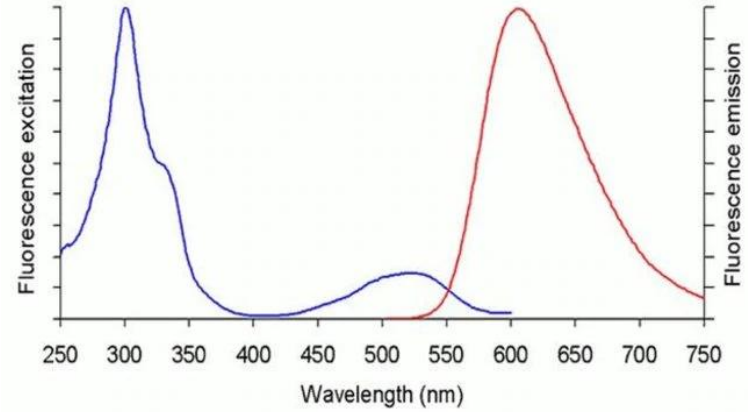


*DAPI*

4',6' diamidino-2-fenilindolo

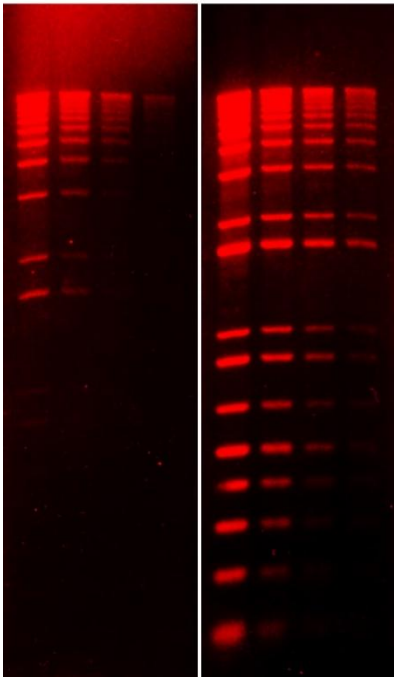


*Bromuro di etidio*

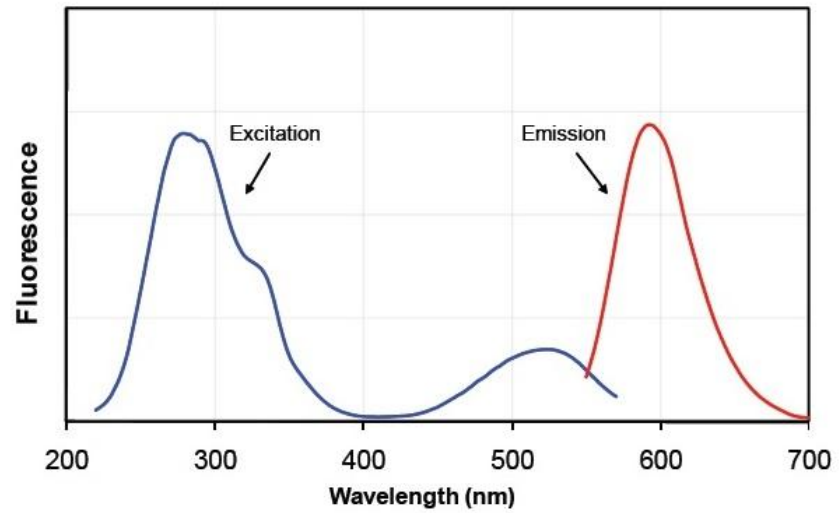


EtBr

GelRed®

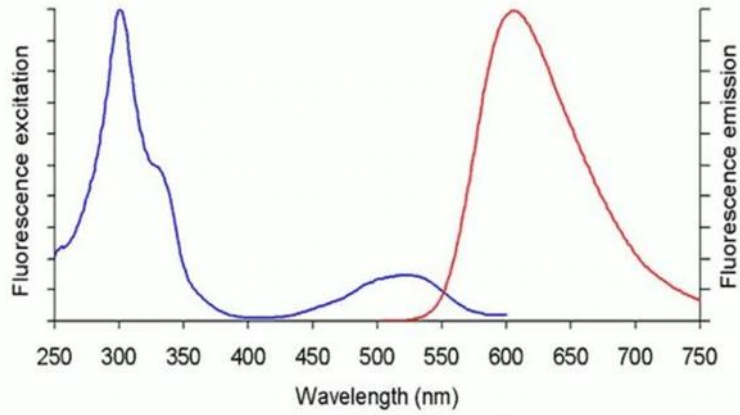


### Spectral Properties

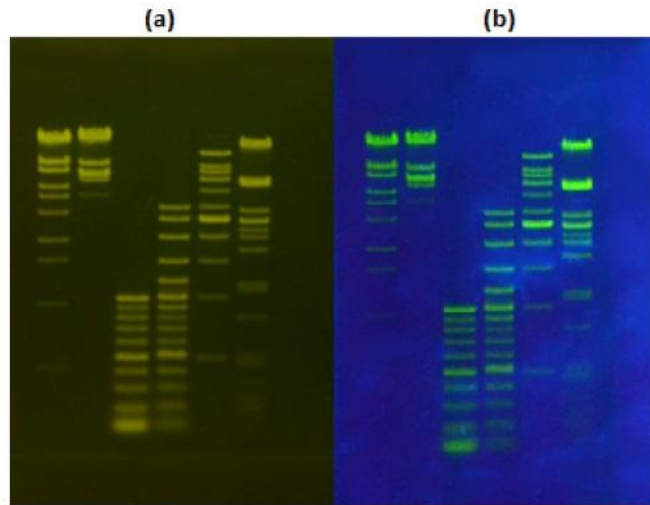
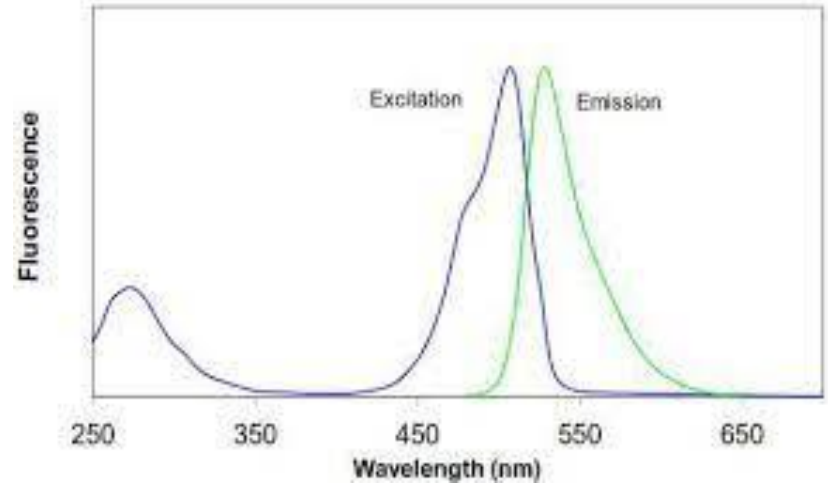




# EtBr



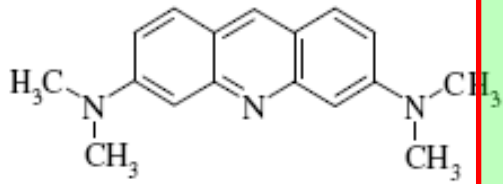
# Gel green



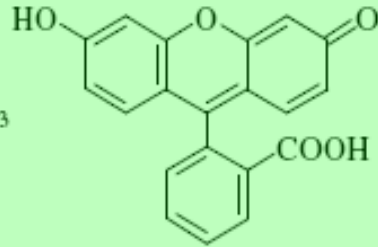
## Post-staining for Agarose Gel

Figure 1: Various ladders and makers run at 1.5% TBE agarose gel. The agarose gel is post-stained with ViSafe Green Gel Stain. The gel is visualized using transilluminator with (a) blue light; (b) UV light.

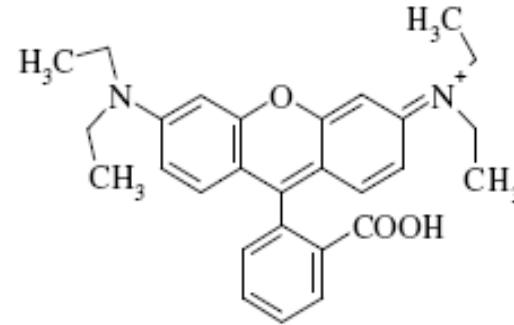
# Fluorofori estrinseci



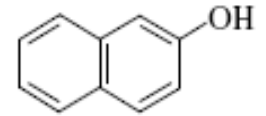
*Arancio di acridina*



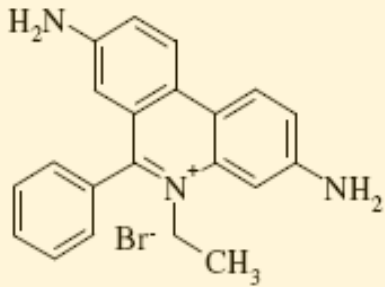
*Fluoresceina*



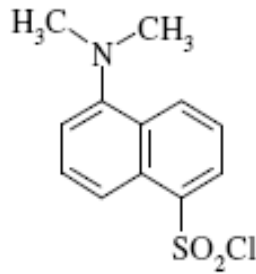
*Rodamina B*



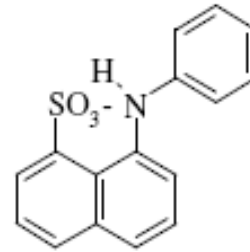
*Naftolo*



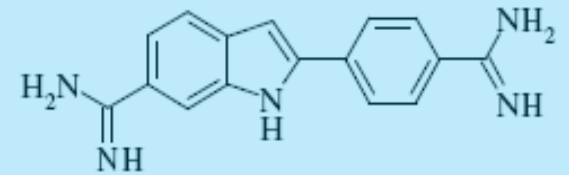
*Bromuro di etidio*



*Dansile*



*ANS*



*DAPI*

4',6' diamidino-2-fenilindolo

**Absorb light at one wavelength and emit light at a specific and longer wavelength**

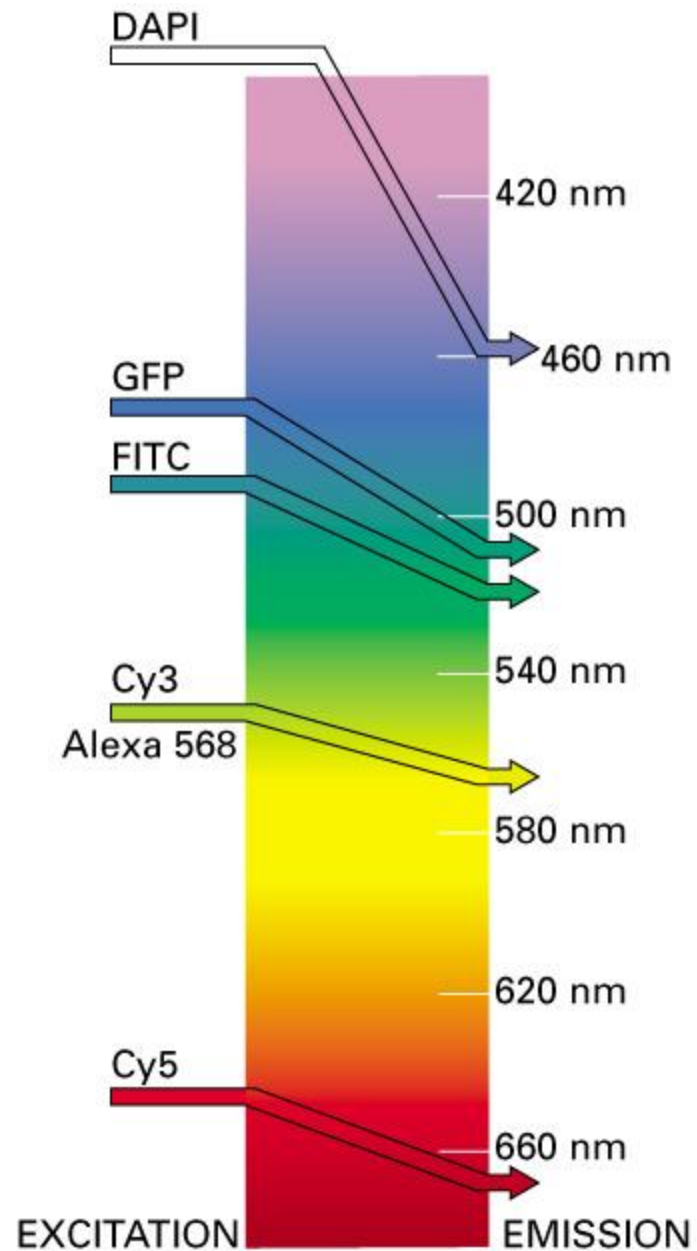
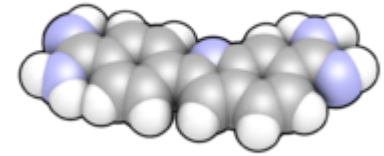
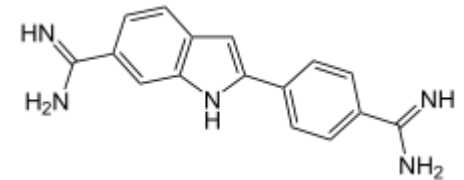


Figure 9–13. Molecular Biology of the Cell, 4th Edition.

## DAPI (4',6-diamidino-2-phenylindole)



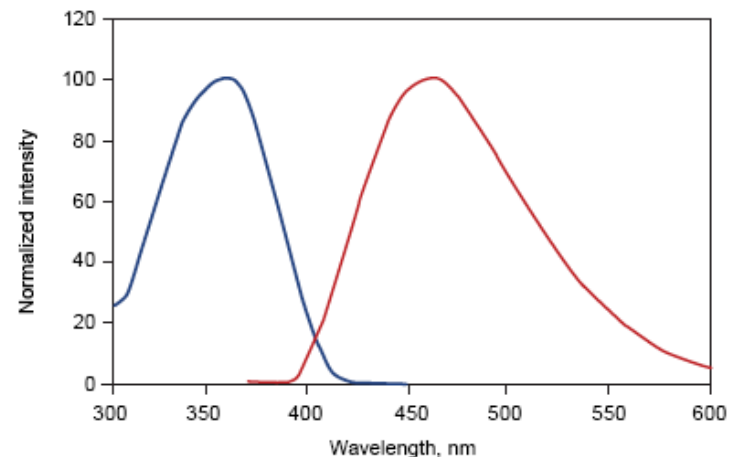
is a **fluorescent stain** that binds strongly to **A-T rich regions in DNA**.  
It is used extensively in **fluorescence microscopy**.



As DAPI can pass through an intact cell membrane, it can be used to stain both **live and fixed cells**, though it passes through the membrane less efficiently in live cells and therefore the effectiveness of the stain is lower.

**DAPI** has

- absorption maximum at a wavelength of **358 nm (ultraviolet)**
- emission maximum is at **461 nm (blue)**.



# DAPI Protocol for Fluorescence Imaging

## Cell Analysis Protocols

Search all cell analysis protocols

## Protocols by Platform

Microscopy Protocols

Flow Cytometry Protocols

High Content Screening Protocols

Microplate Reader Protocols

## Protocols by Application

Cell Proliferation Protocols

Cell Viability Protocols

Cell Structure Protocols



## Nuclear counterstain for fluorescence microscopy

A popular nuclear and chromosome counterstain, DAPI (4',6-diamidino-2-phenylindole) emits blue fluorescence upon binding to AT regions of DNA.

### This protocol can be used for:

- Nucleic acid (nuclear) staining in fluorescence microscopy

### This protocol should not be used for:

- Flow cytometry

### You will need the following for this protocol:

- Cells growing in culture
- [DAPI, hydrochloride \(Cat. No. D1306\)](#)
- Phosphate-buffered saline (PBS) or other suitable buffer
- Fluorescence microscope



Contact Us

First, fix and permeabilize cultured cells with a protocol appropriate for your sample.



1. Wash the cells 1–3 times in PBS as needed.



2. Add sufficient 300 nM DAPI stain solution to cover the cells.



3. Incubate for 1–5 minutes, protected from light.



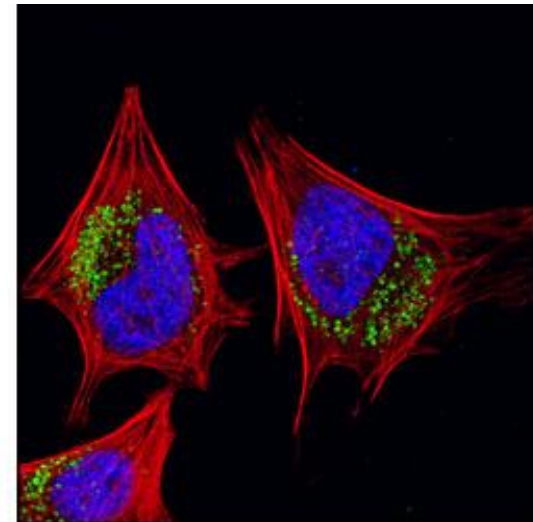
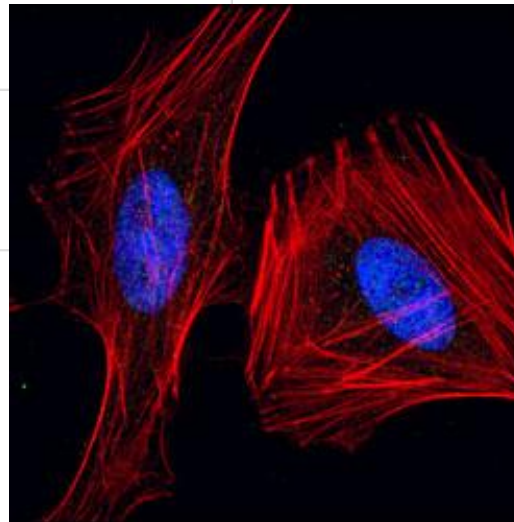
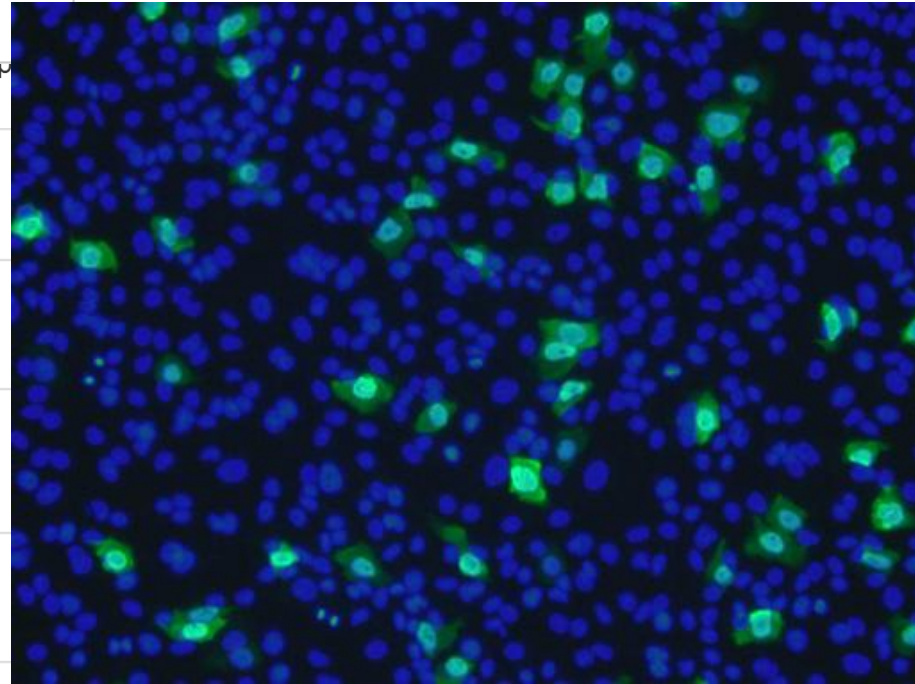
4. Remove the stain solution.



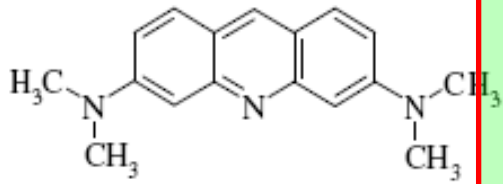
5. Wash the cells 2–3 times in PBS.



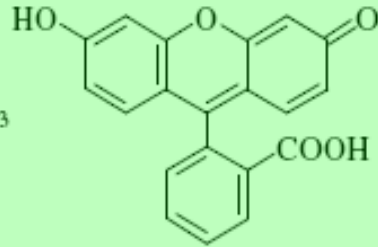
6. Image the cells.



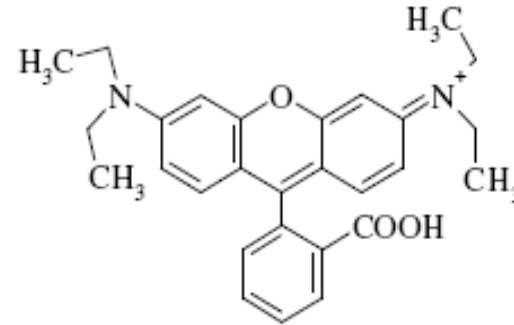
# Fluorofori estrinseci



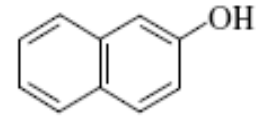
*Arancio di acridina*



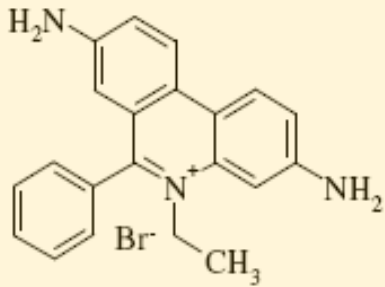
*Fluoresceina*



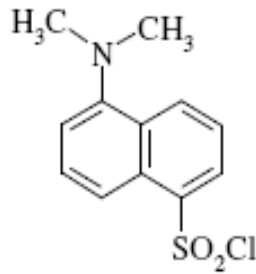
*Rodamina B*



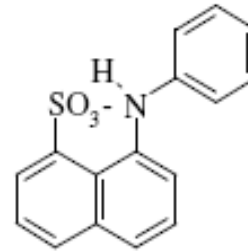
*Naftolo*



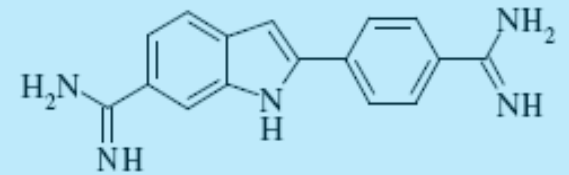
*Bromuro di etidio*



*Dansile*



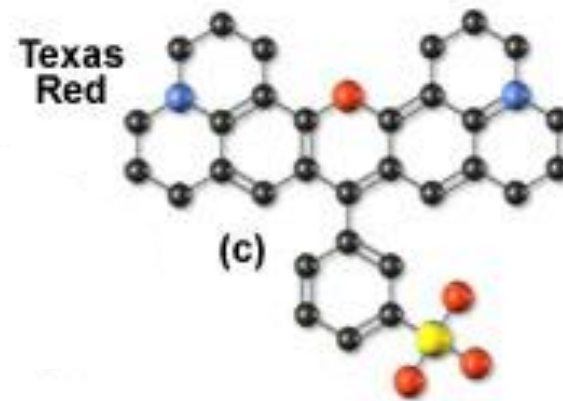
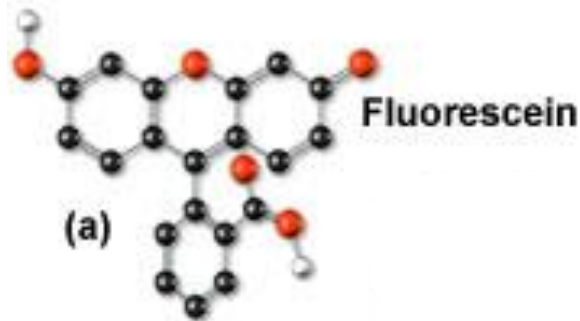
*ANS*



*DAPI*

4',6' diamidino-2-fenilindolo

# Common Fluorophores in Widefield and Confocal Microscopy



**Figure 1**

● Phosphorous     ● Nitrogen     ● Hydrogen  
● Oxygen            ● Carbon

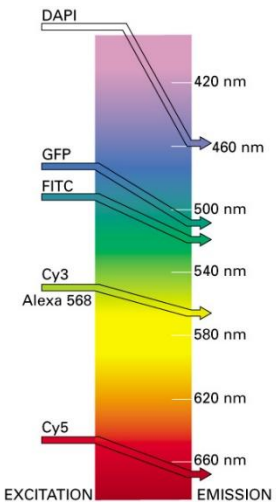
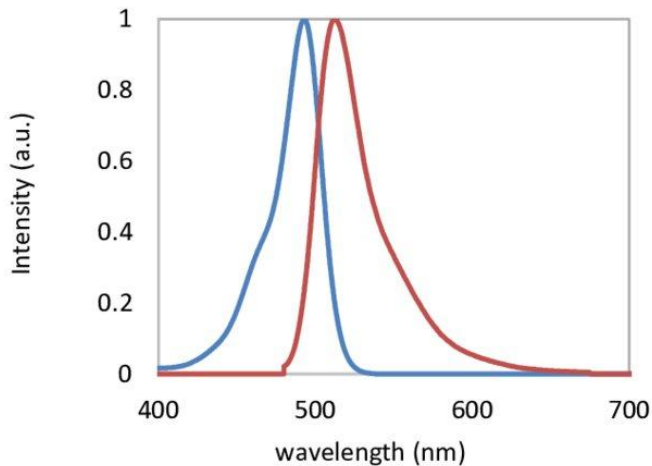


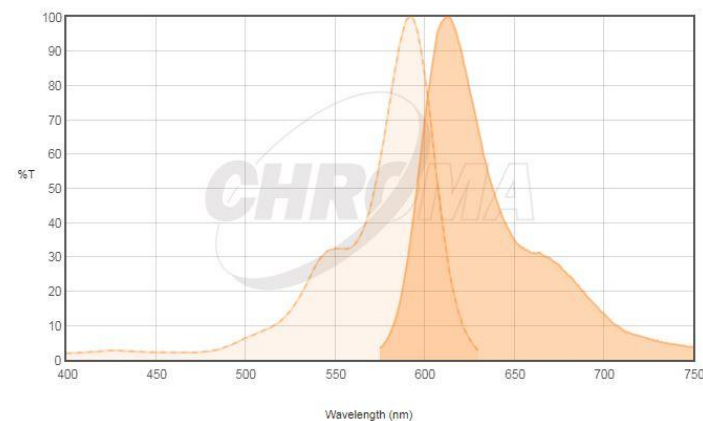
Figure 9-13. Molecular Biology of the Cell, 4th Edition.

## Fluorescein



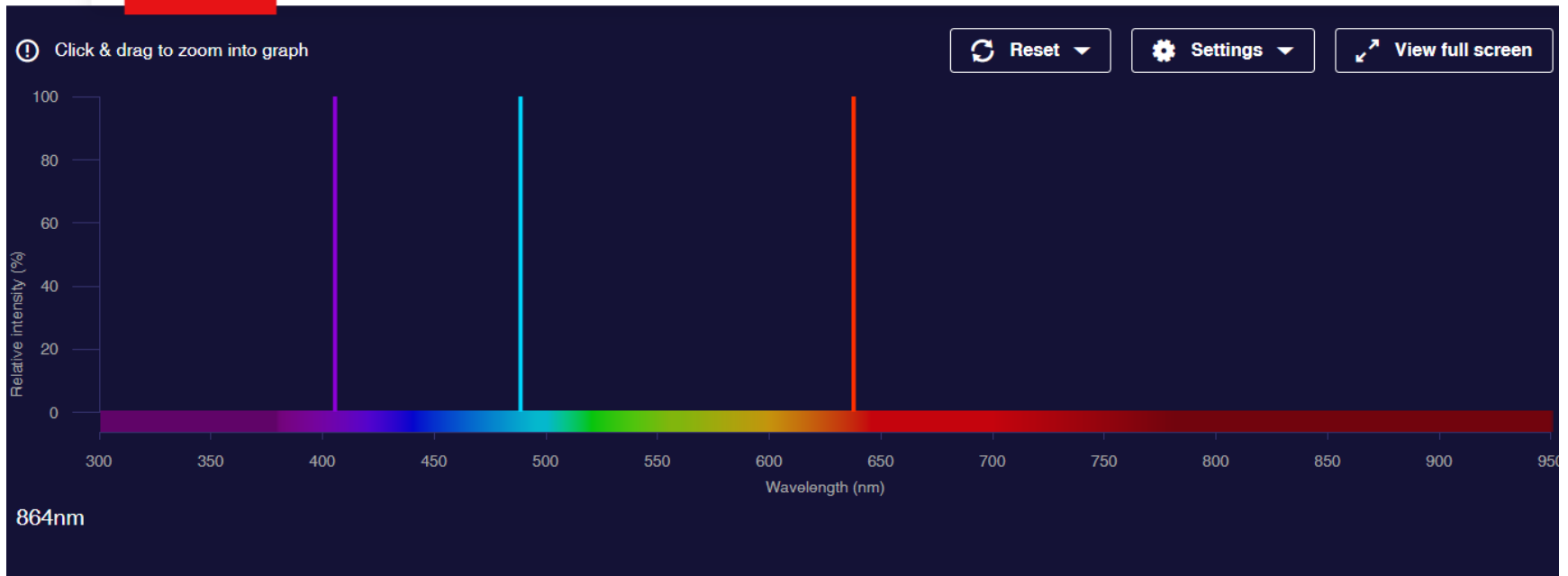
Excitation (nm)	Emission (nm)
498	517

## Texas red



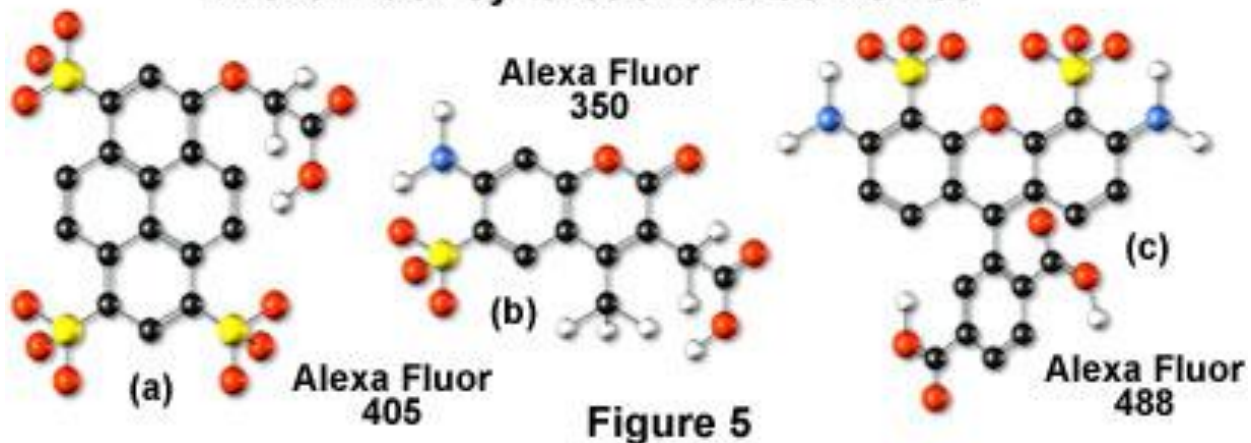
Excitation (nm)	Emission (nm)
595	620

To activate a fluorophore a LASER is usually used

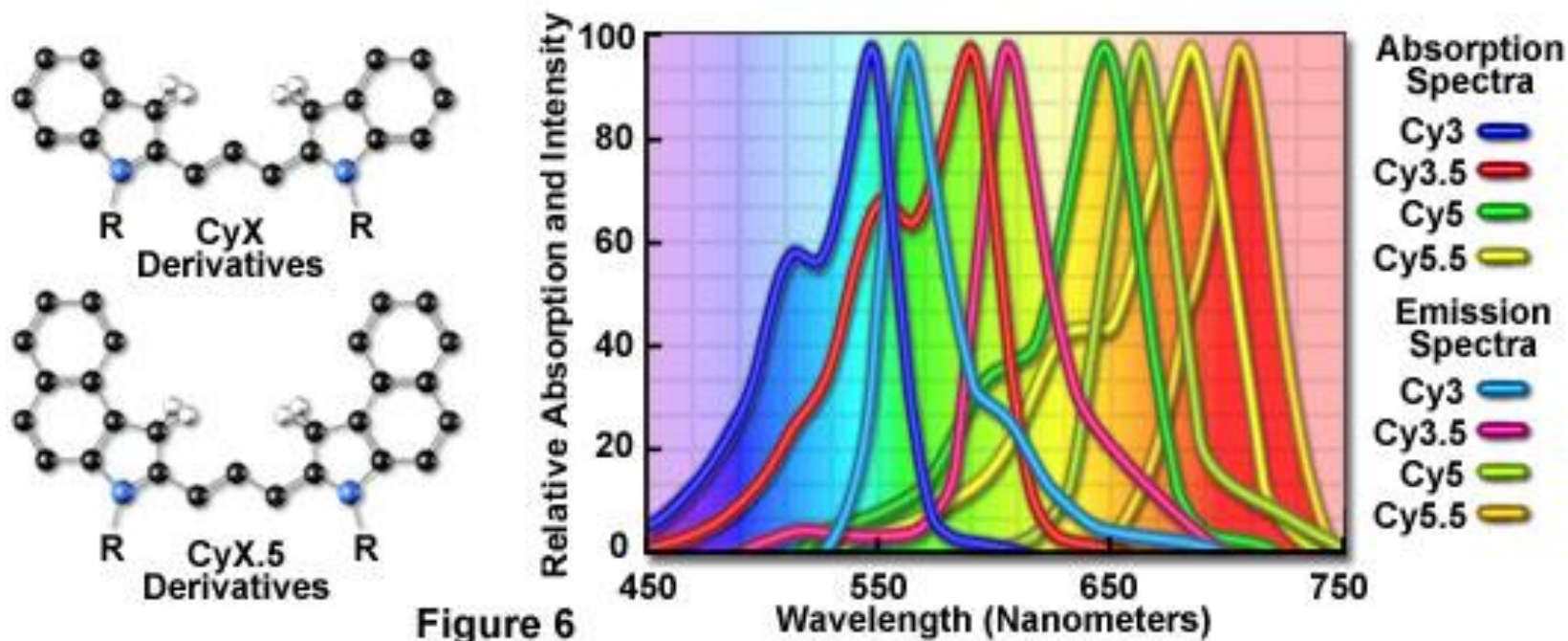


Light source <span>3</span>		Excitation filter <input type="checkbox"/>	Emission filter			
Show <input checked="" type="checkbox"/>	Lasers	Wavelength	Normalize	Custom name		
<input checked="" type="checkbox"/>	Violet	405	<input checked="" type="radio"/>		×	
<input checked="" type="checkbox"/>	Blue	488	<input type="radio"/>		×	
<input checked="" type="checkbox"/>	Red	637	<input type="radio"/>		×	

## Alexa Fluor Synthetic Fluorochromes

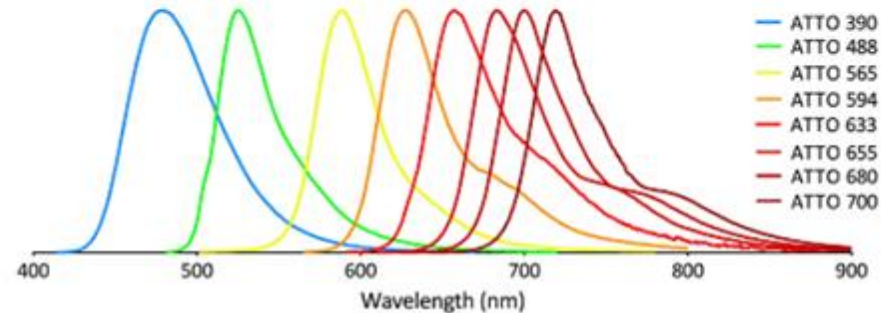


## Structure and Spectral Profiles of Cyanine Fluorochromes



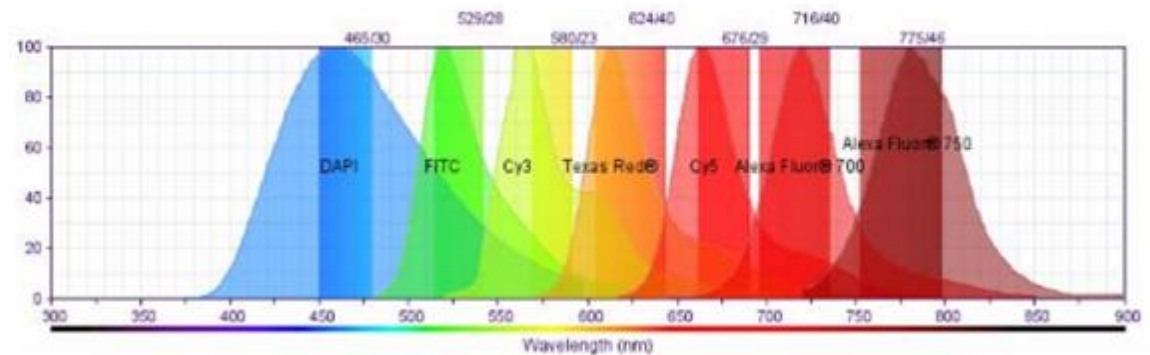
# Modern variants

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

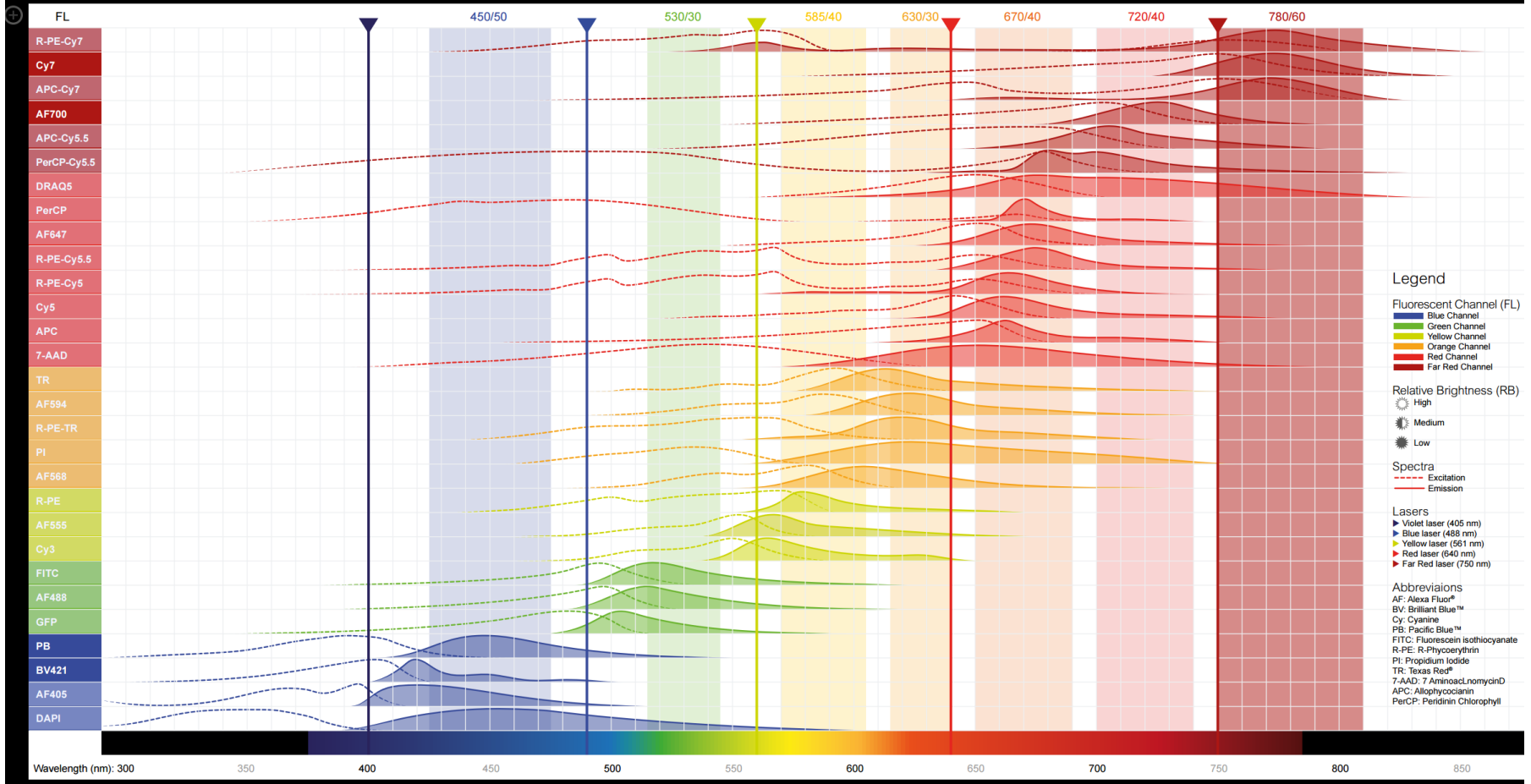


More money =

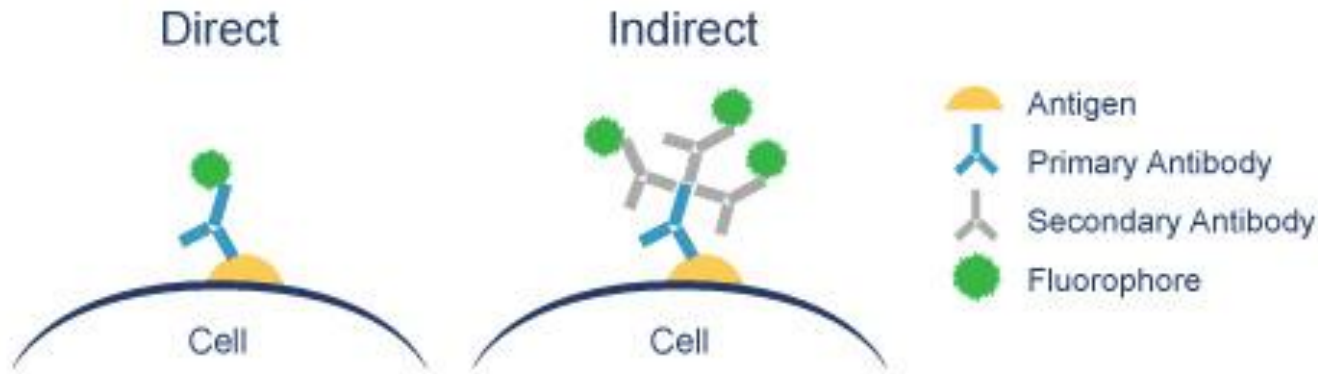
1. Higher brightness
2. Photostability
3. Water solubility
4. Broad range of colors



# Fluorochrome chart

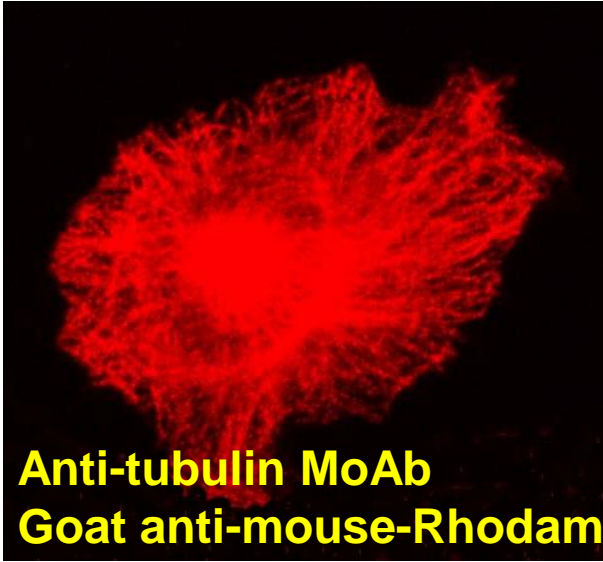


# Fluorescence Microscopy

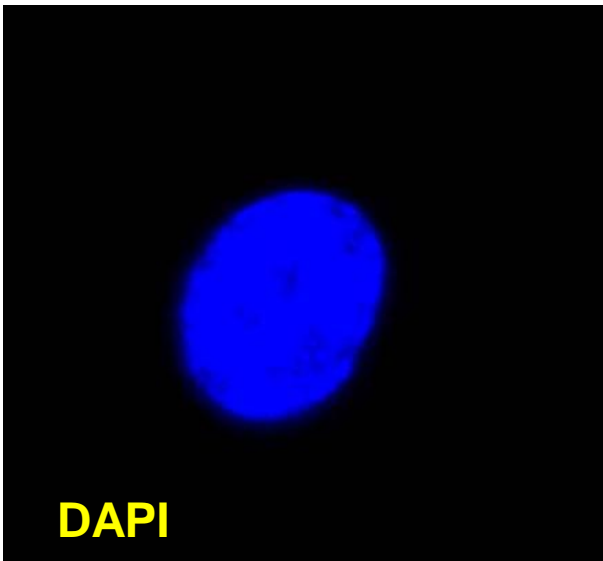
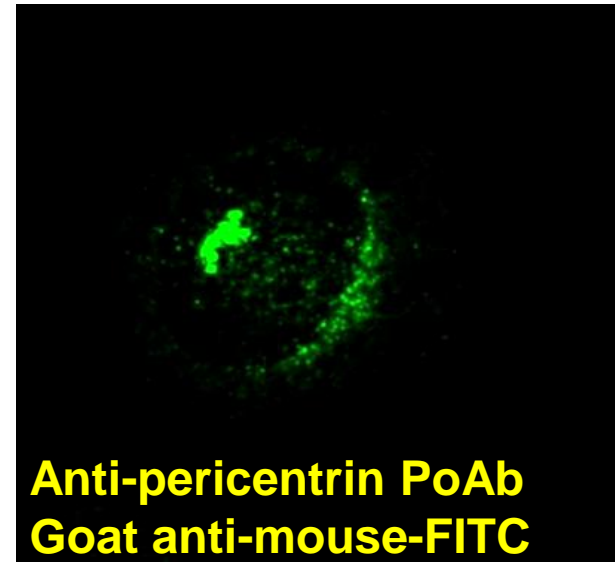


- Major Function: Localization of specific cellular molecules – example proteins
- **Major Advantages:**
  - Sensitivity: “glow” against dark background
  - Specificity: immunofluorescence
  - Cells may be fixed or living
- **Fluorescent dyes or proteins (Fluorochromes)**
  - fluorochromes may be **indirectly or directly** associated with the cellular molecule
  - **Multiple fluorochromes** may be used simultaneously

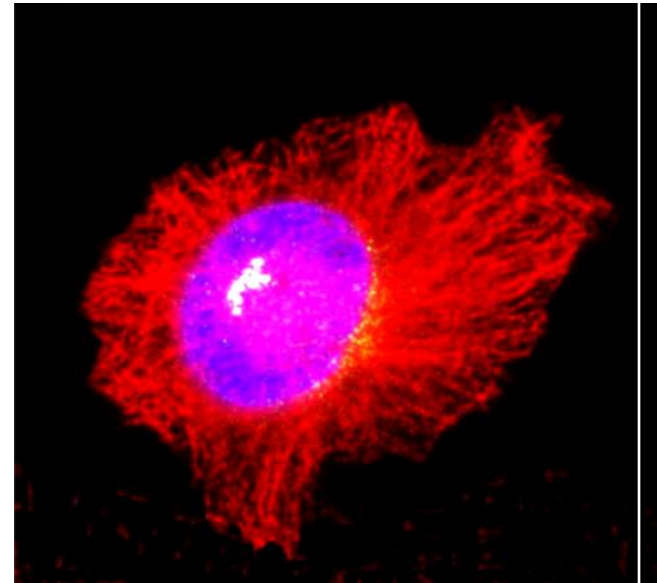
## microtubules



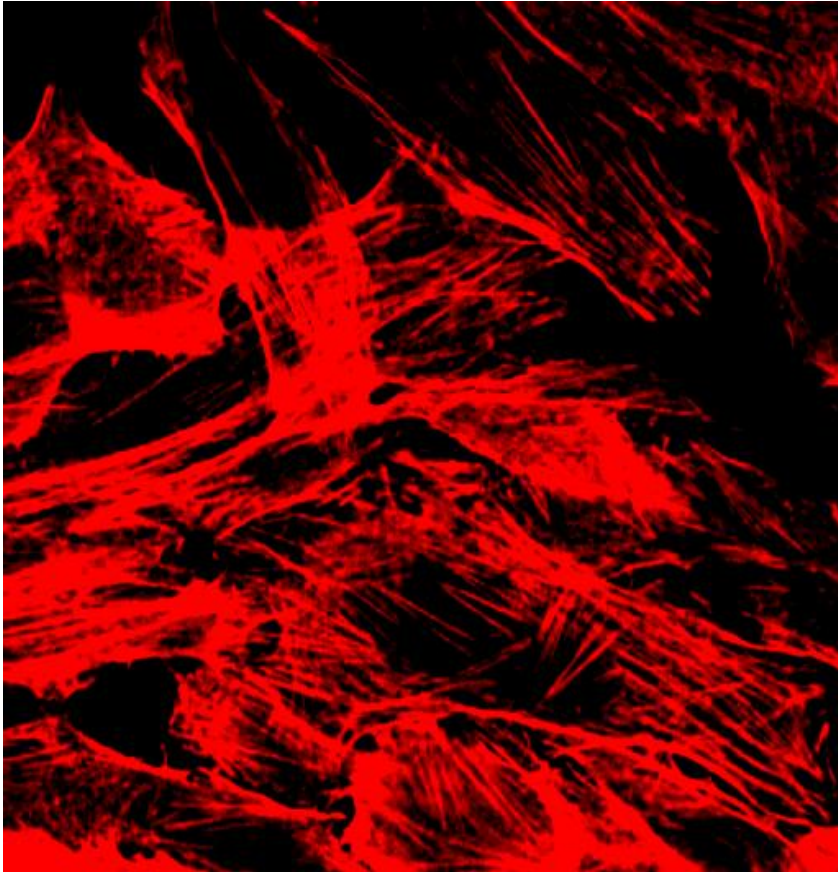
## centrosomes



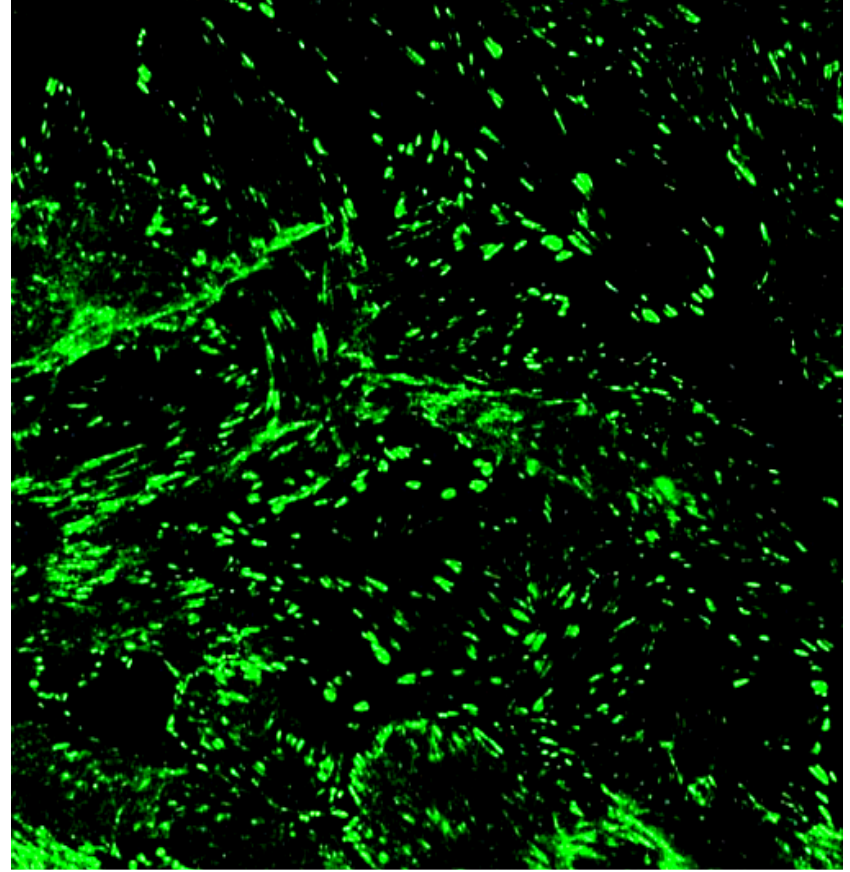
## nucleus



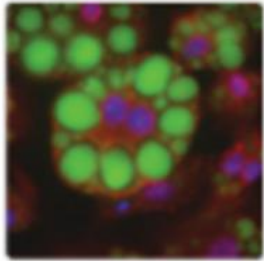
## Stress Fibers



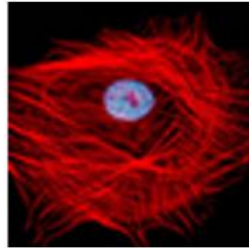
## Focal Adhesions



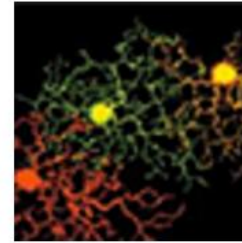
# Find organelle-specific antibodies and organelle stains



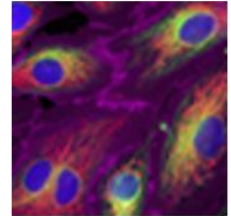
**Adiposomes**



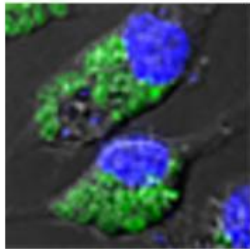
**Cytoskeleton**



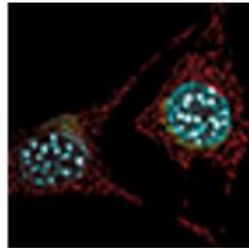
**Cytosol/cytoplasm**



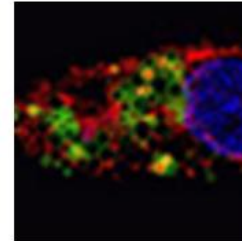
**Endoplasmic reticulum**



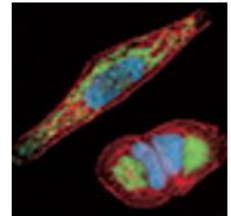
**Endosomes**



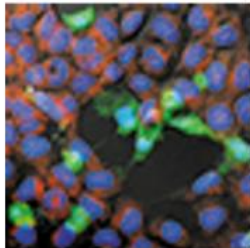
**Golgi complex**



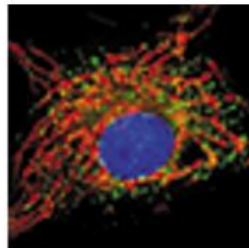
**Lysosomes**



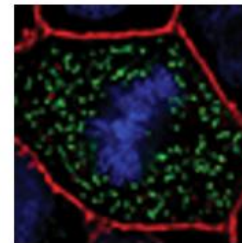
**Mitochondria**



**Nucleus/nucleolus**



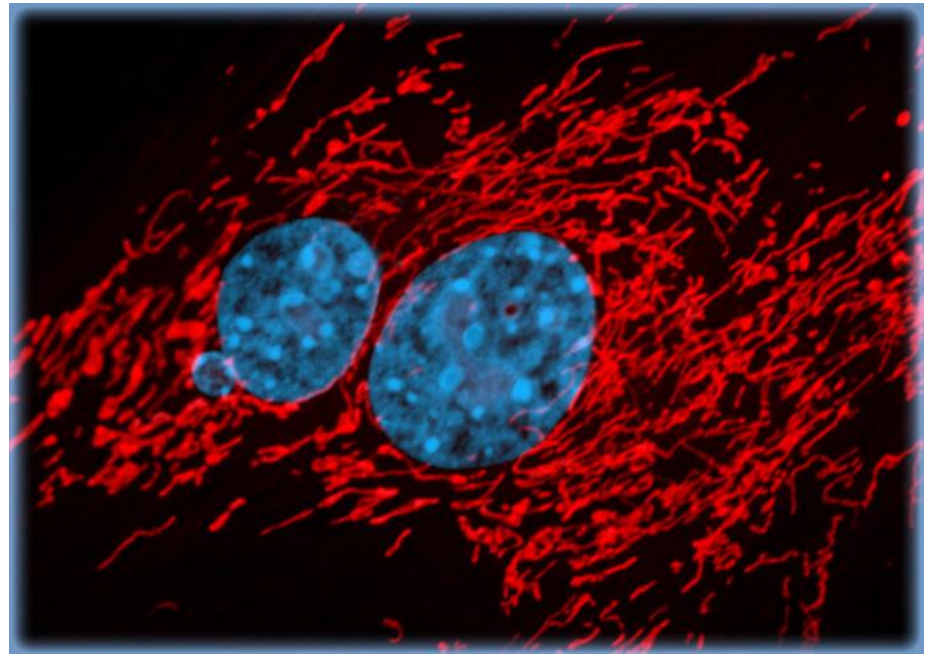
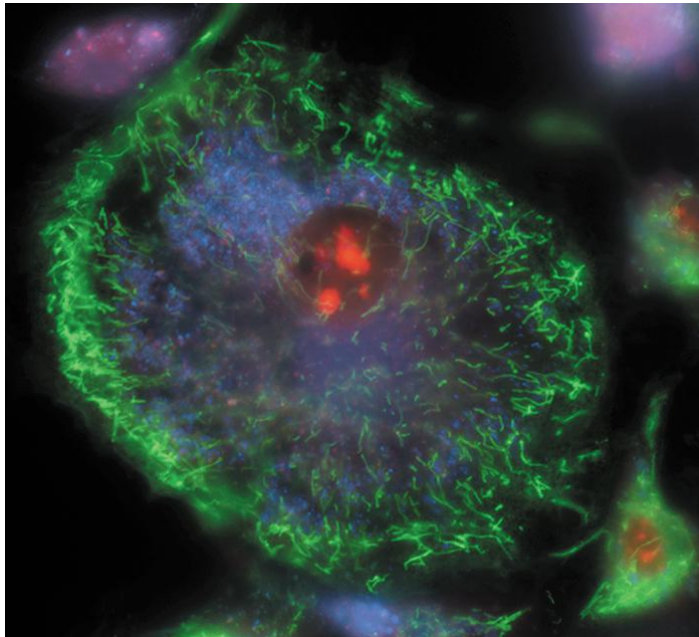
**Peroxisomes**



**Plasma membrane**

## MitoTracker® probes for live cells

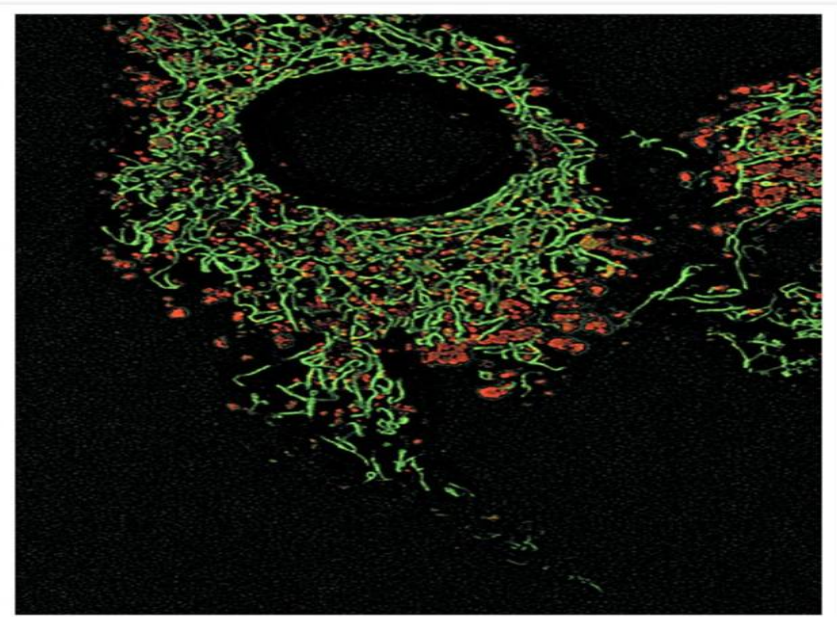
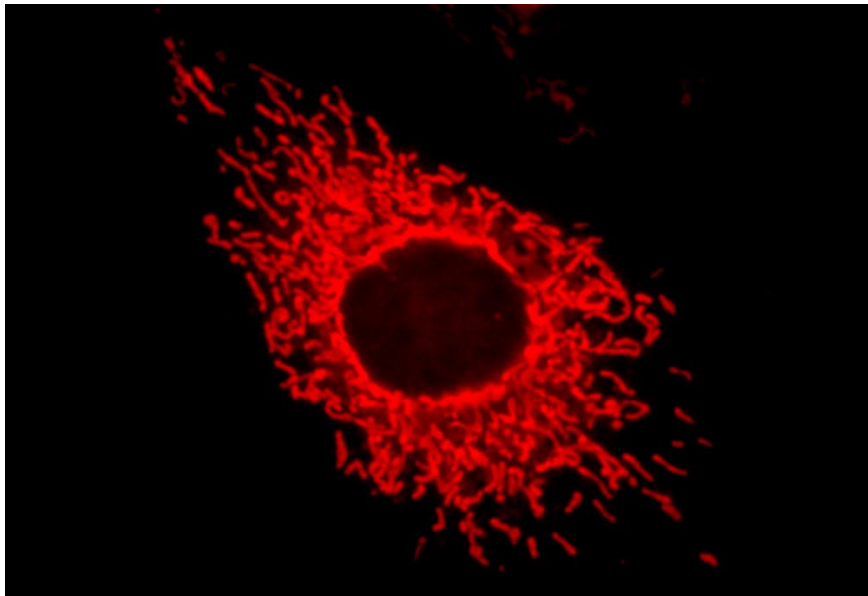
MitoTracker® probes are cationic dyes that are **selectively sequestered by mitochondria in live cells** because of their charge—like other mitochondrial dyes, they depend on the membrane potential for loading



**MitoTracker** dyes are cationic fluorophores that **accumulate electrophoretically into mitochondria in response to the highly negative mitochondrial membrane potential.**

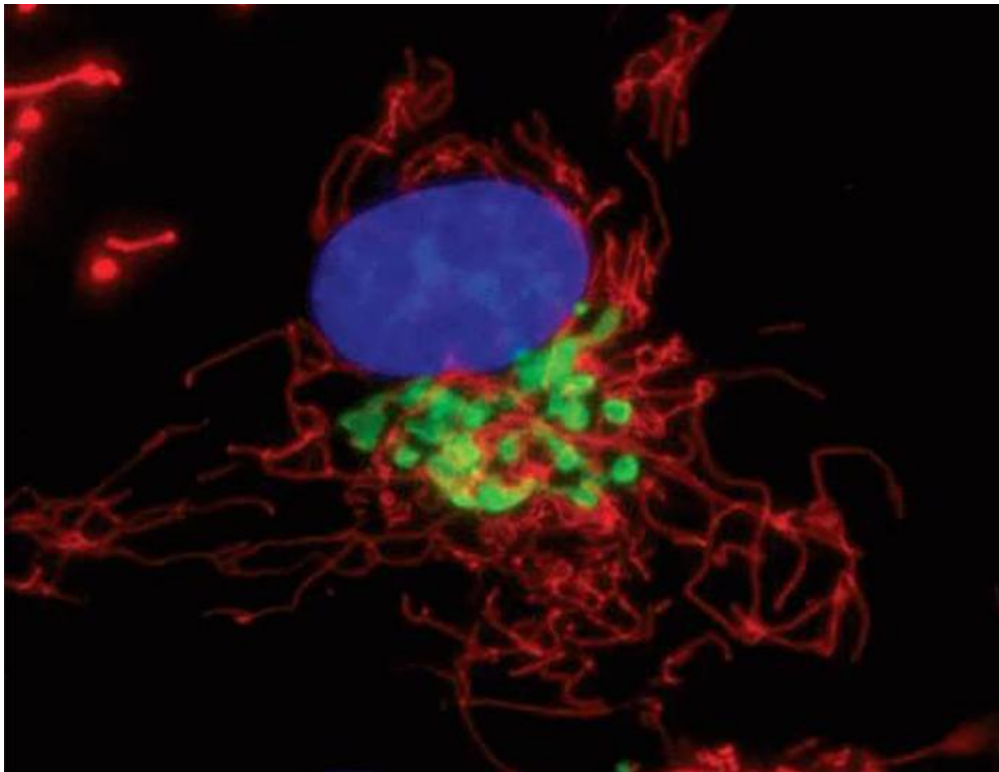
However, unlike rhodamine MitoTracker dyes possess a reactive chloromethyl group that forms **a covalent bond with thiols on proteins and peptides, which traps MitoTracker dyes within mitochondria.**

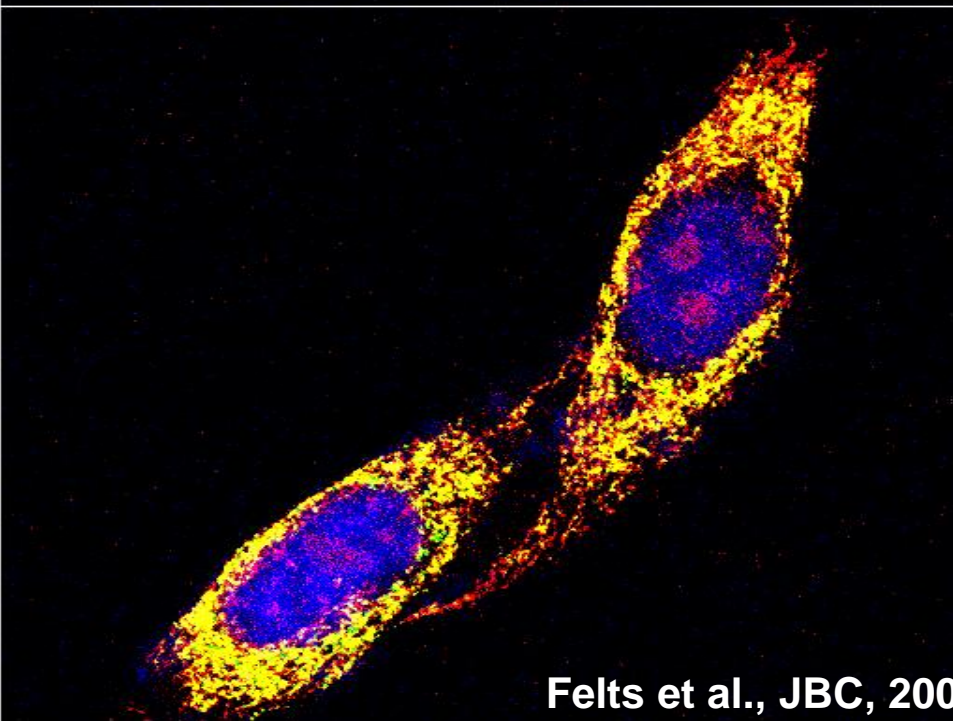
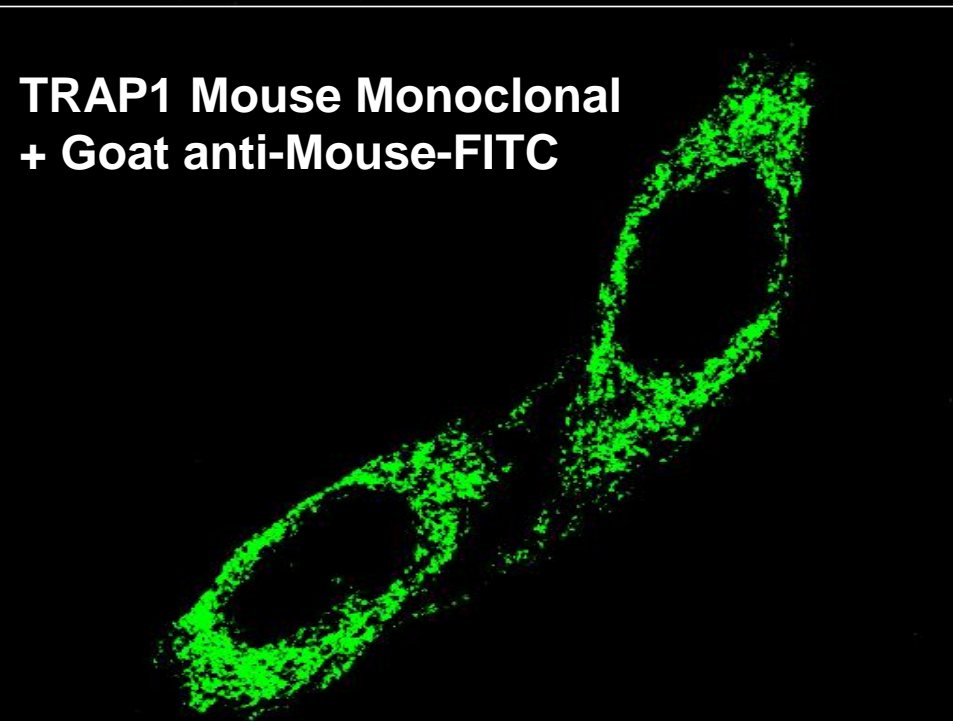
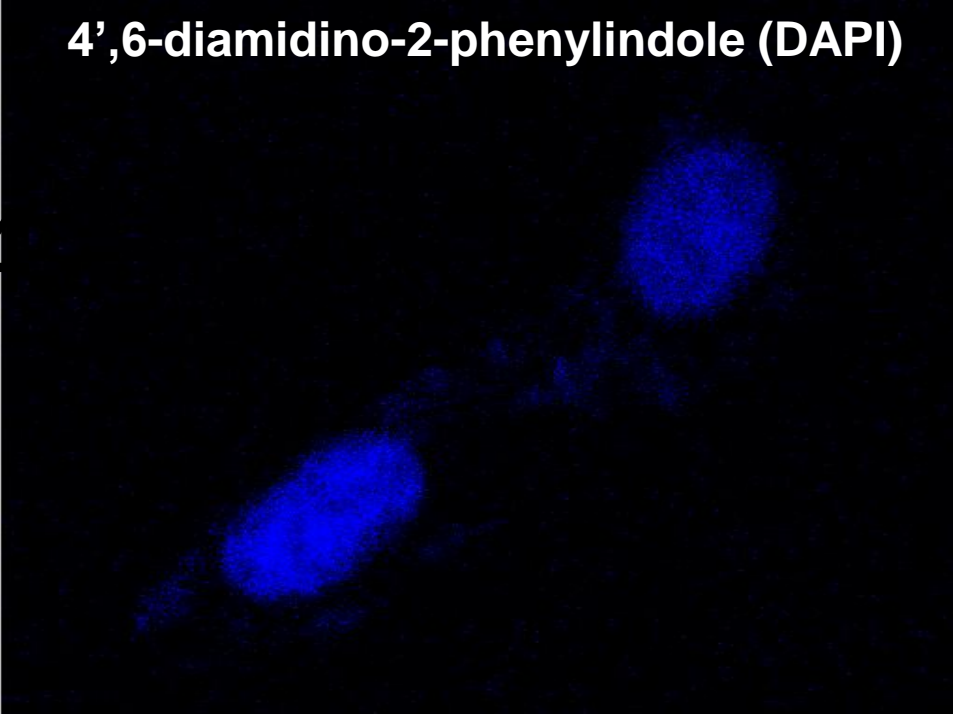
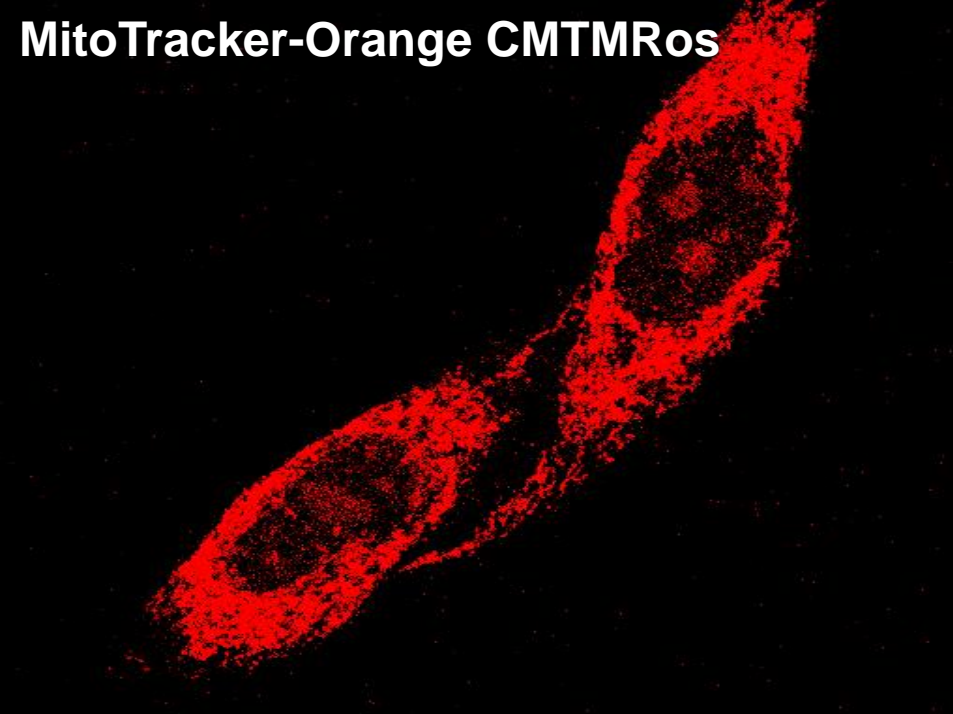
Two of the more popular probes are MitoTracker® Deep Red and MitoTracker® Green.



## CellLight® reagents for Golgi complex

Use CellLight® reagents when you want to label the Golgi complex in live cells to follow the dynamics of intracellular behavior.





# THE HUMAN PROTEIN ATLAS

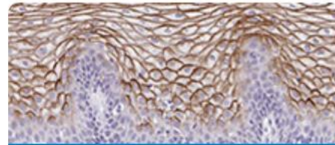


☰ MENU HELP NEWS

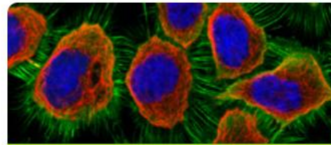
SEARCH

Search Fields »

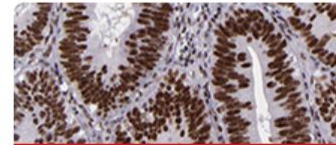
e.g. RBM3, insulin, CD36



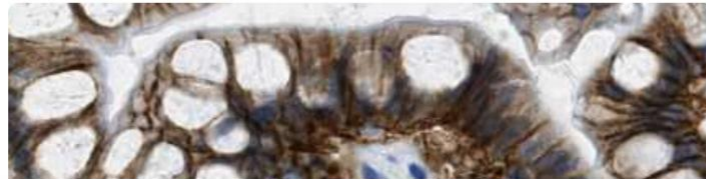
TISSUE ATLAS



CELL ATLAS



PATHOLOGY ATLAS



## Recent news

Fri, 8 Mar 2019  
[Thymus and T cells of the Adaptive Immune System](#)

Mon, 4 Feb 2019  
[The Fertilizing Fallopian Tubes](#)