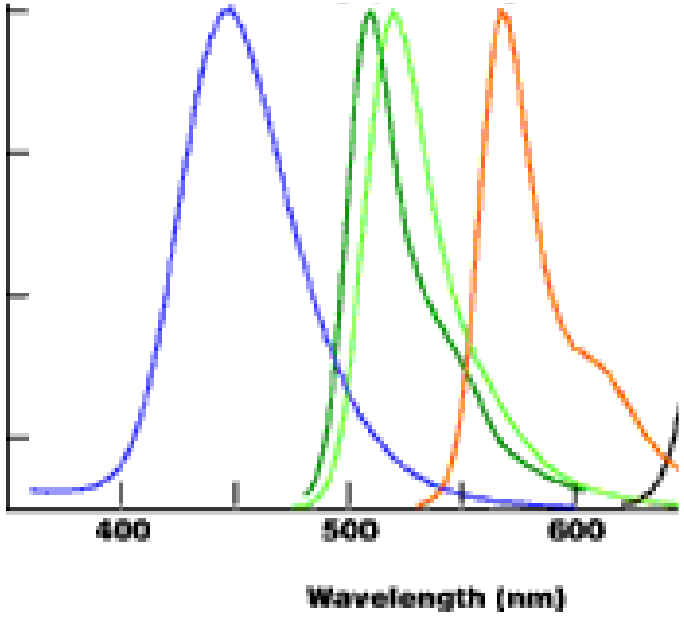
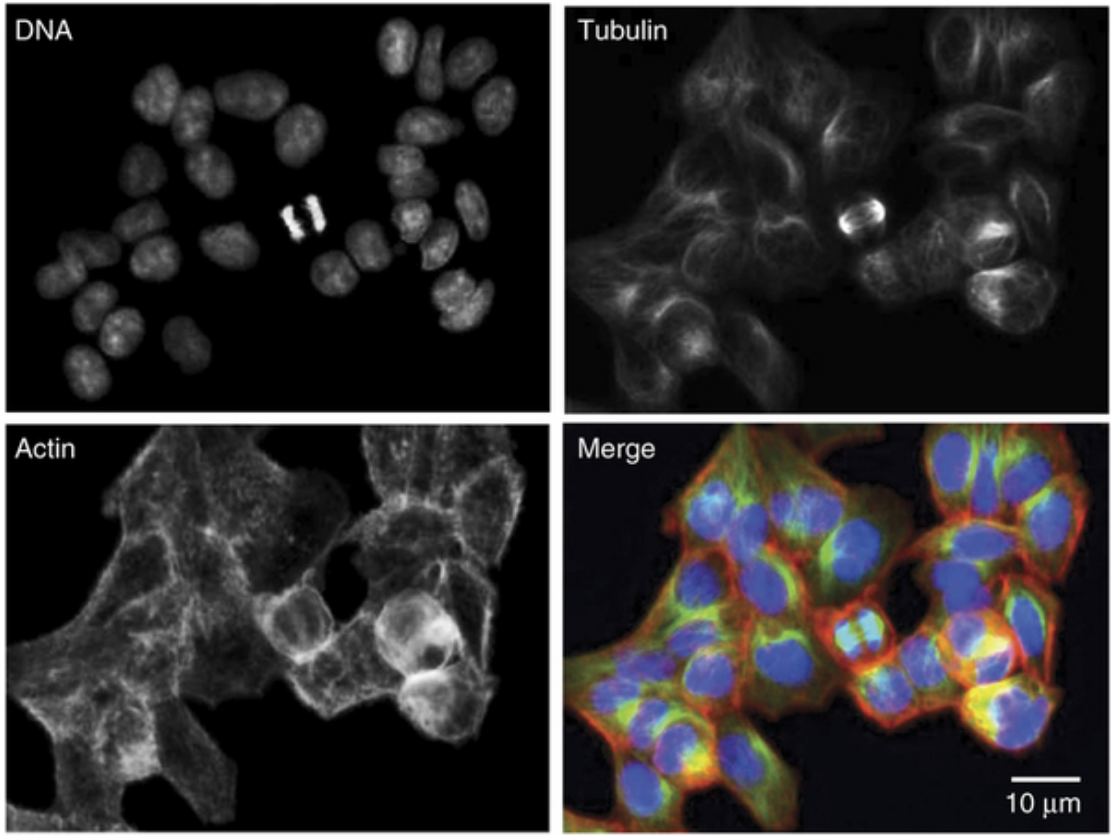
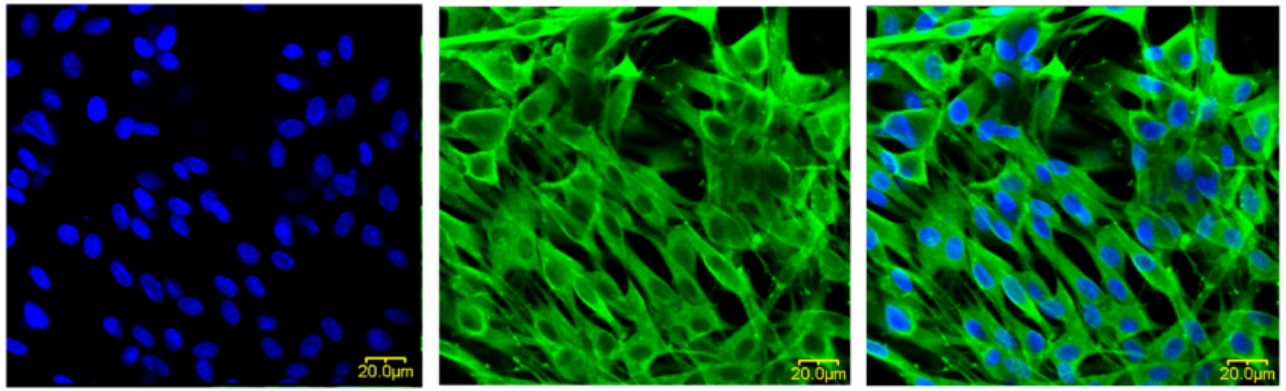
Fluorescent proteins, more applications

- Last class
  - Organic dyes
  - DAPI/FITC/TRITC spectra
- This class
  - GFP based probes
  - Exotic genetically encoded fluorophores

Organic dyes - DAPI-FITC-TRITC

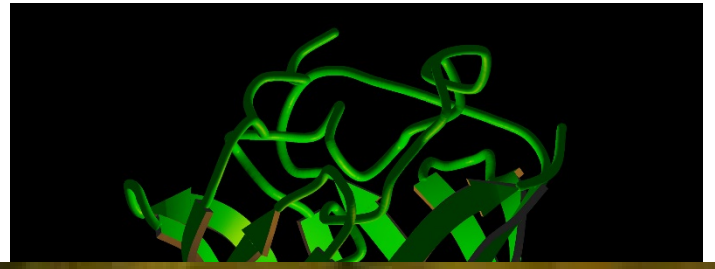


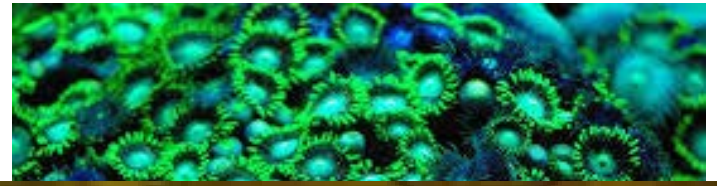




FPs

- Never as good as the dyes
- Lower brightness and photostability
- However, we don't have to make them, so they're very cheap

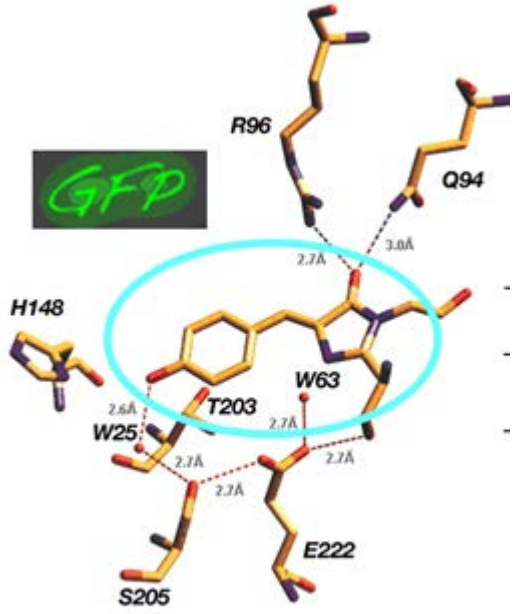




Metrics

1. Excitation/emission
2. Quantum yield
3. Brightness
4. Photostability
5. Toxicity
6. Maturation time
7. Monomeric?

GFP chromophore

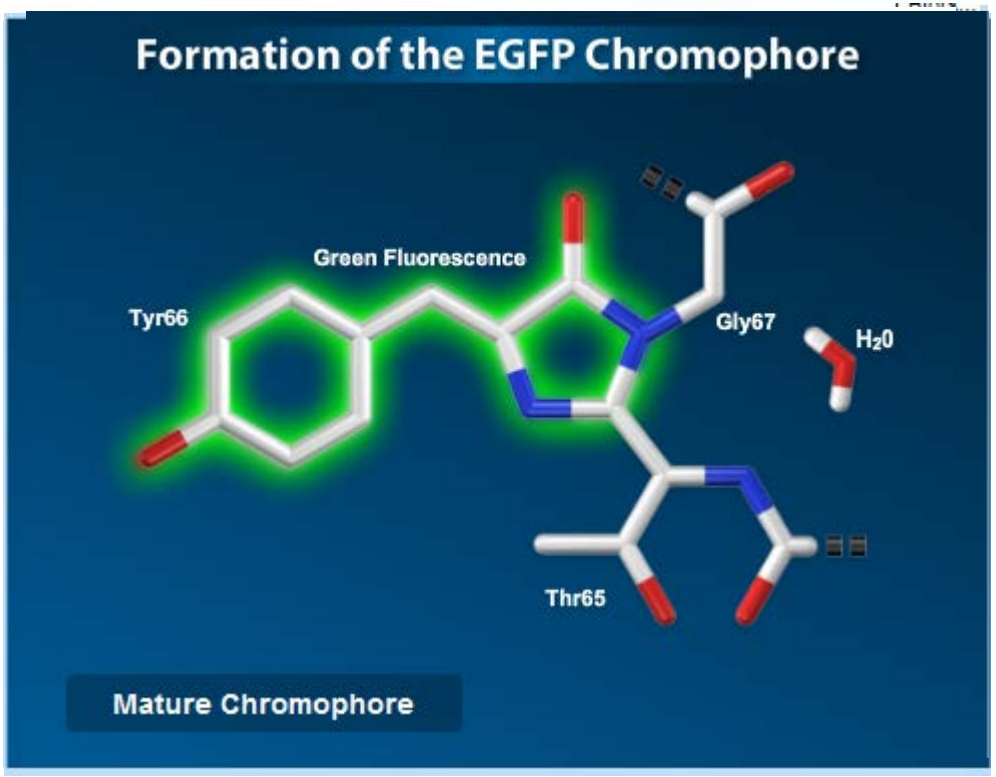


Chromophore also has to have extended electron backbone, but made entirely of amino acids.

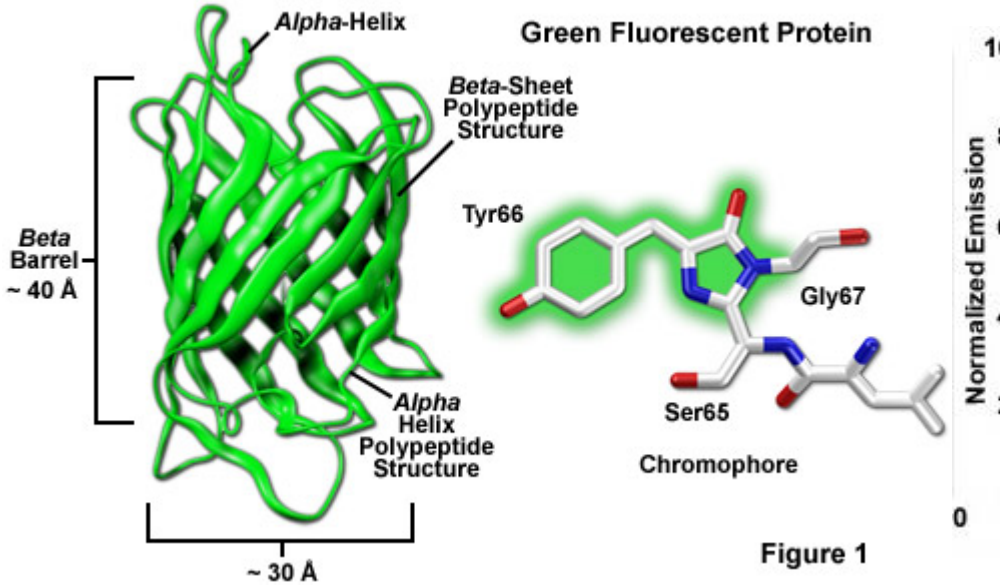
Complicated dance to make it happen

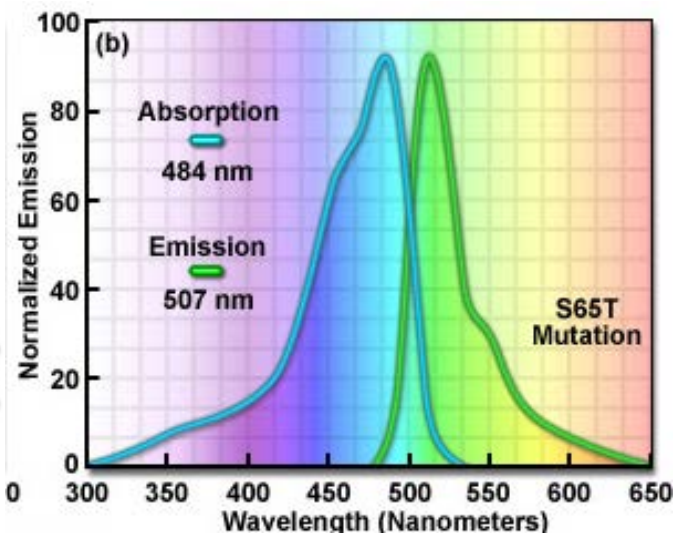
Maturation takes time to completely form

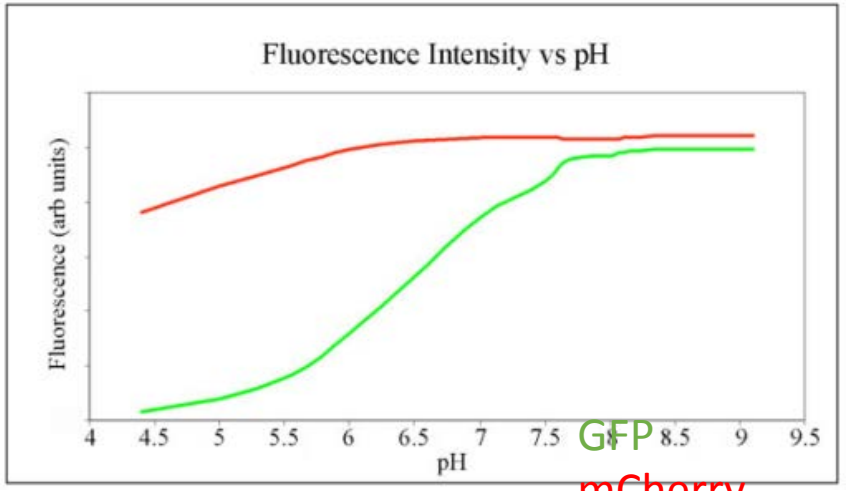
Another thing to keep in mind when evaluating fluorophores



GFP is the archetype







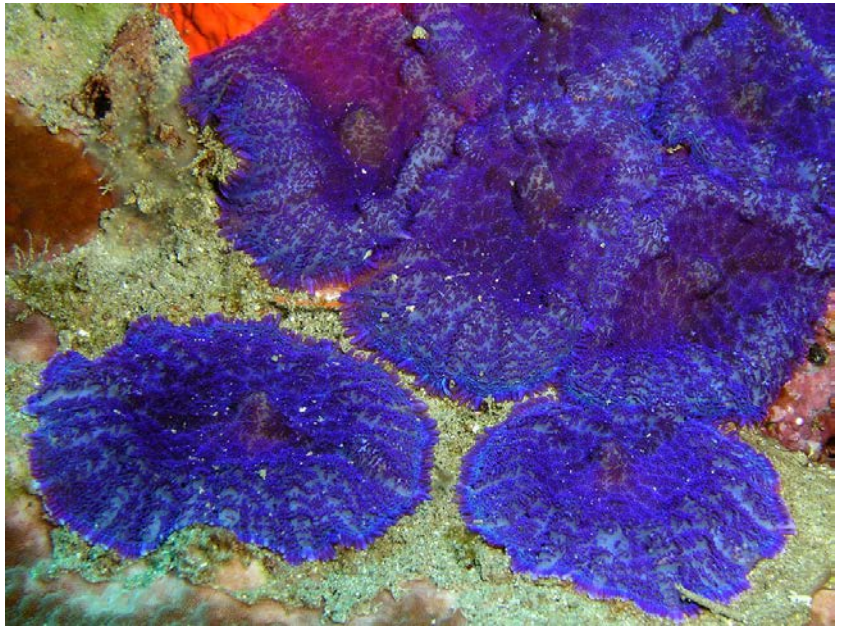
Laser line = 488 nm

GFP

Cy dye (5X)

~230 AA, or ~700 bp
Relatively small protein
MW = 27 kDa
FITC MW = 350 Da, 100 times smaller

Coral based FPs



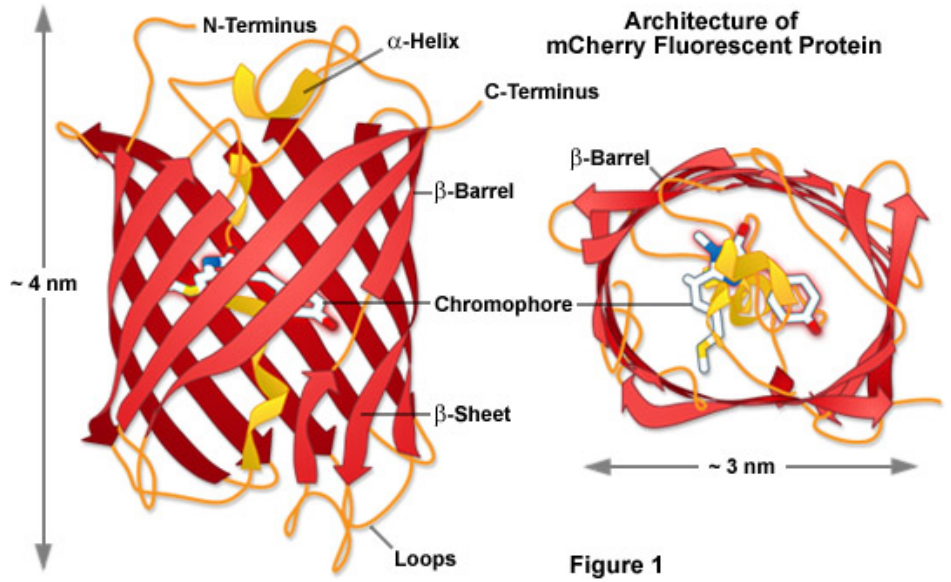
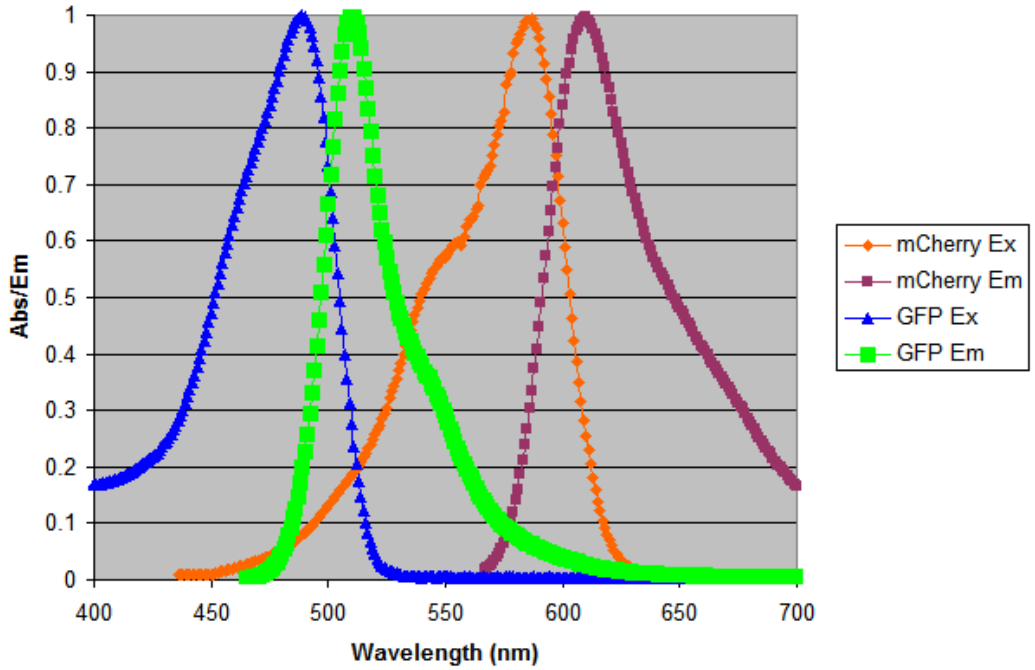
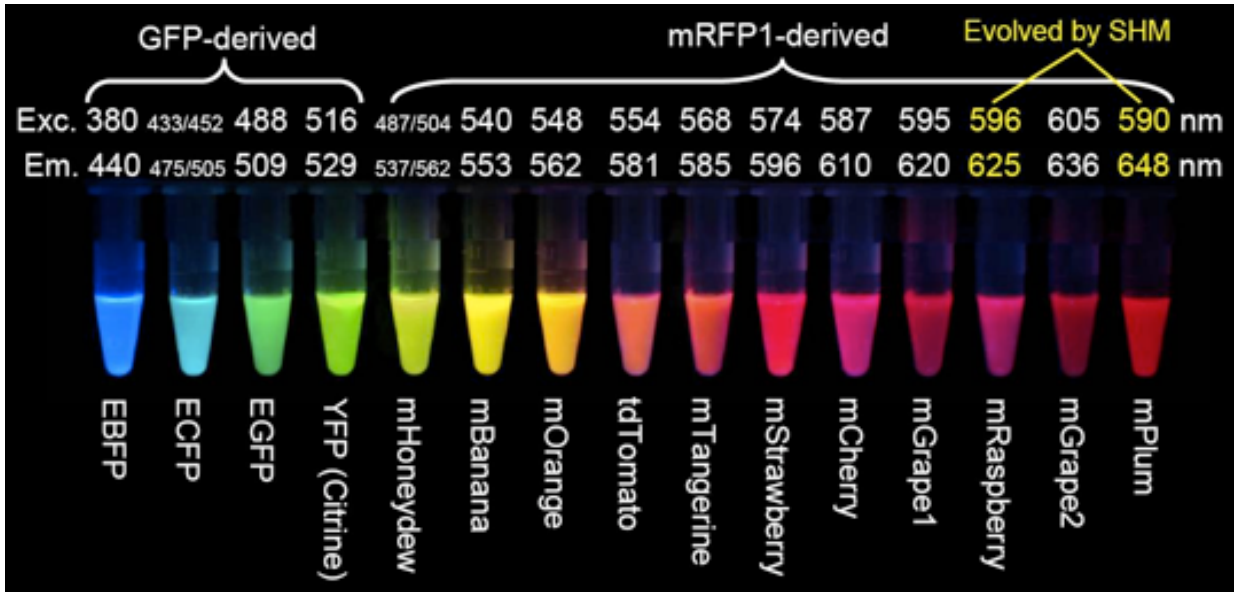


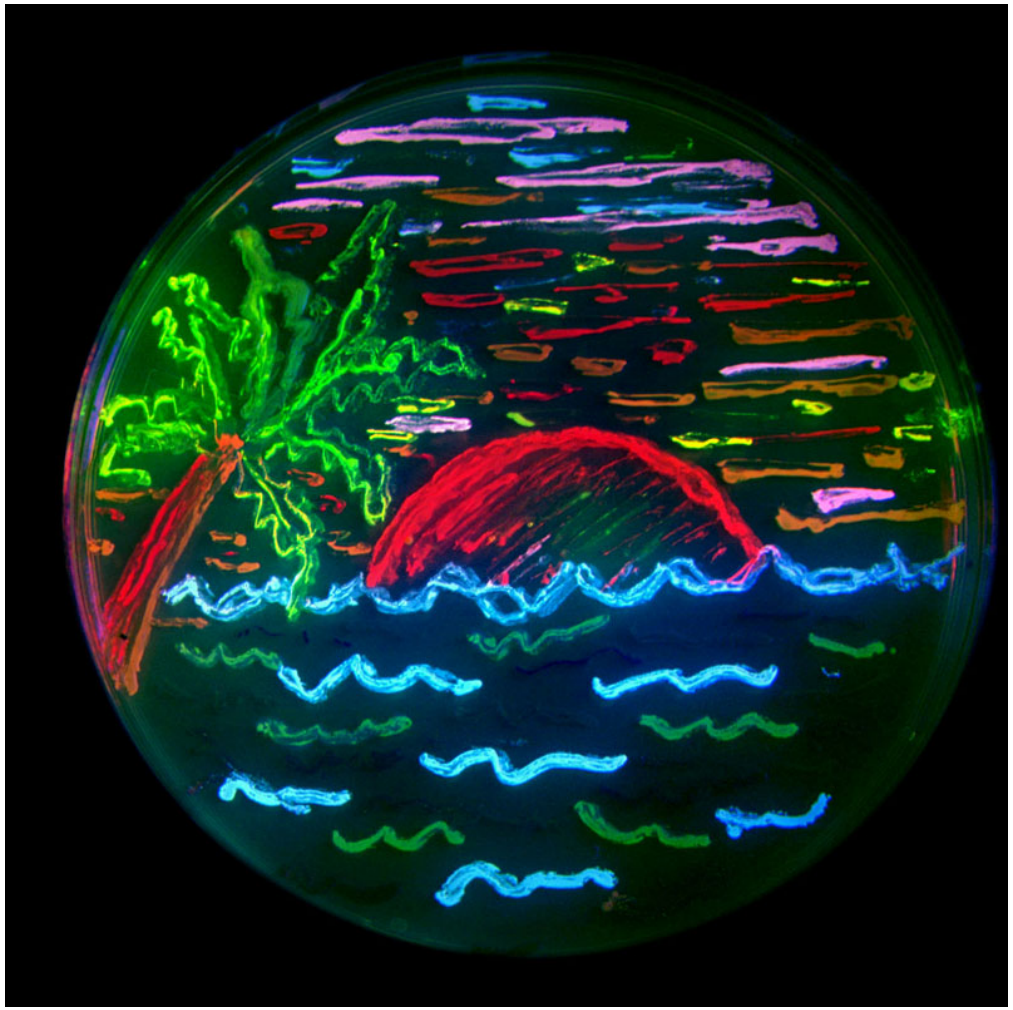
Figure 1



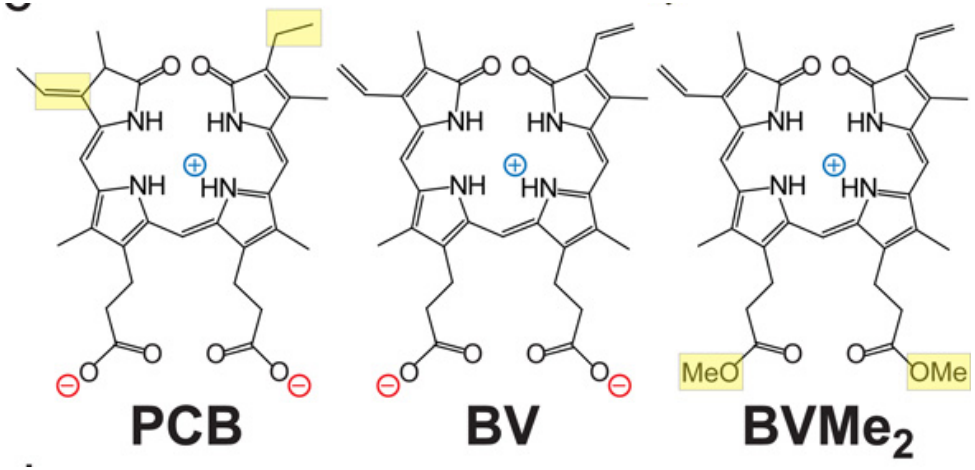
Host of fluorescent proteins out there

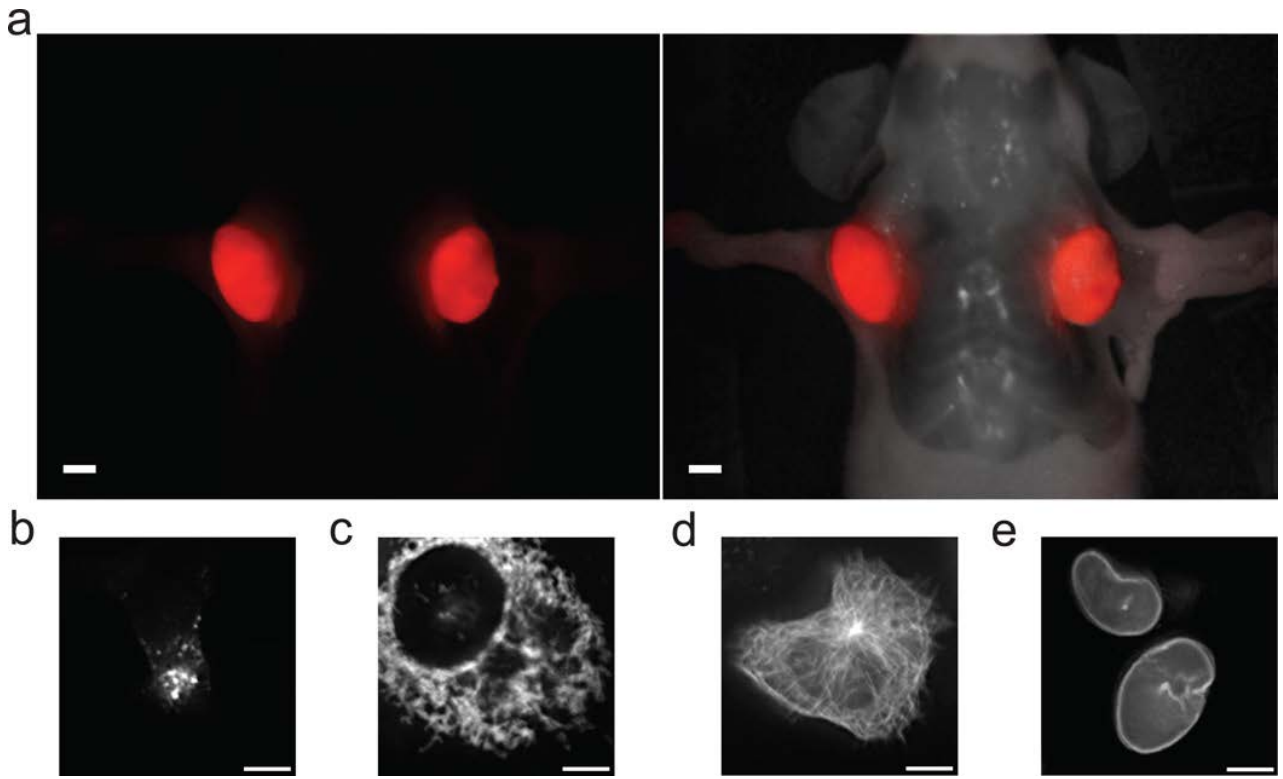


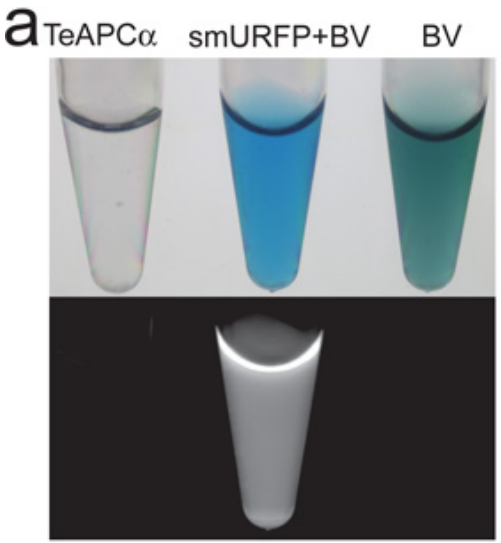
All the proteins have been evolved from 2 main scaffolds.

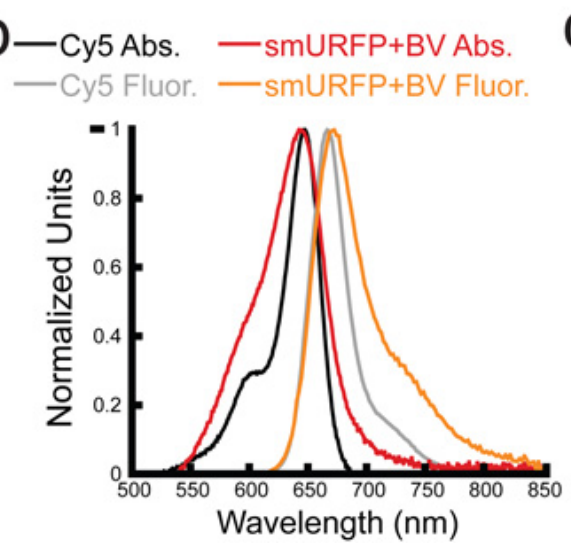


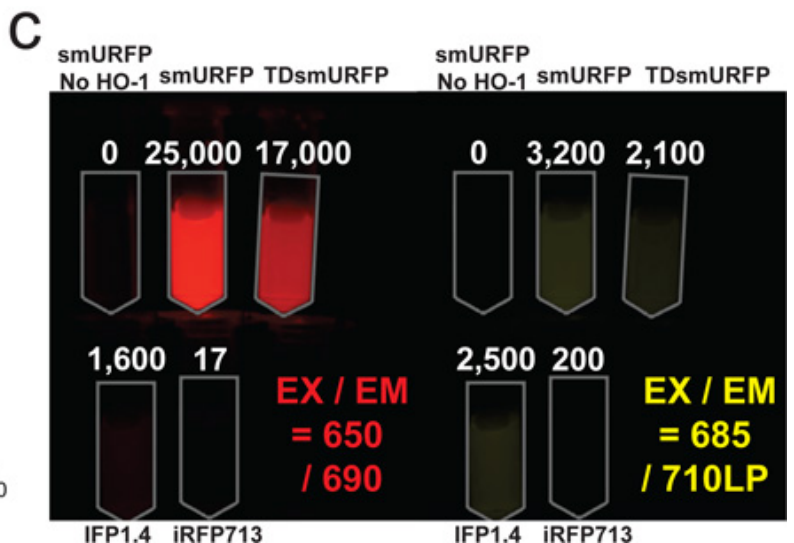
Far red proteins







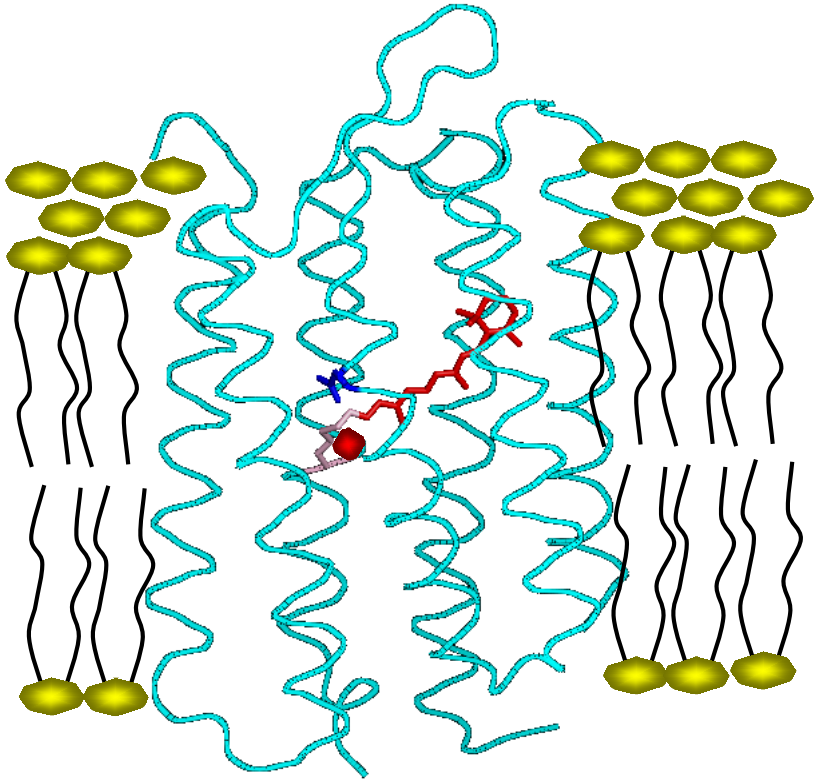


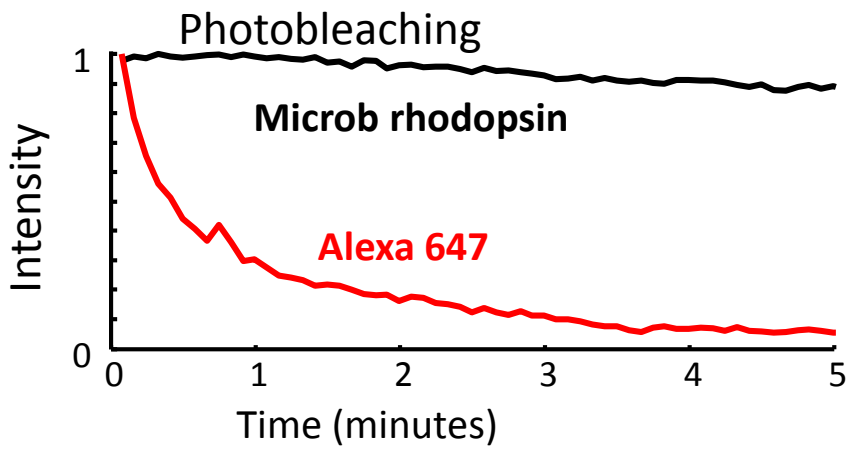


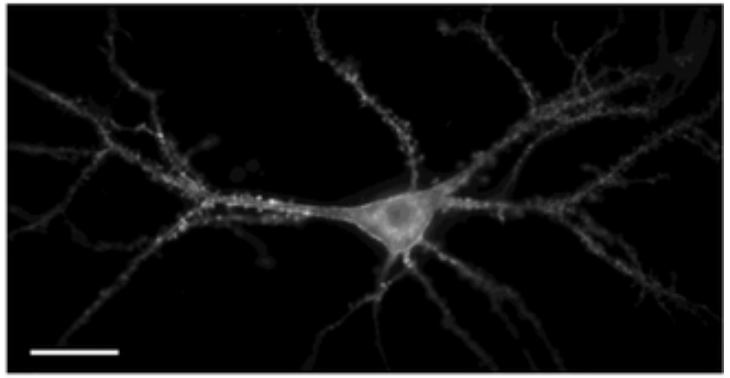
Microbial rhodopsins

- Only fluorescent membrane protein
- Dim relative to GFPs and dyes
- Excitation and emission in the red

$\lambda_{excitation}$	590-640 nm
$\lambda_{emission}$	~710 nm
Quantum Yield	10^{-3} - 10^{-2}

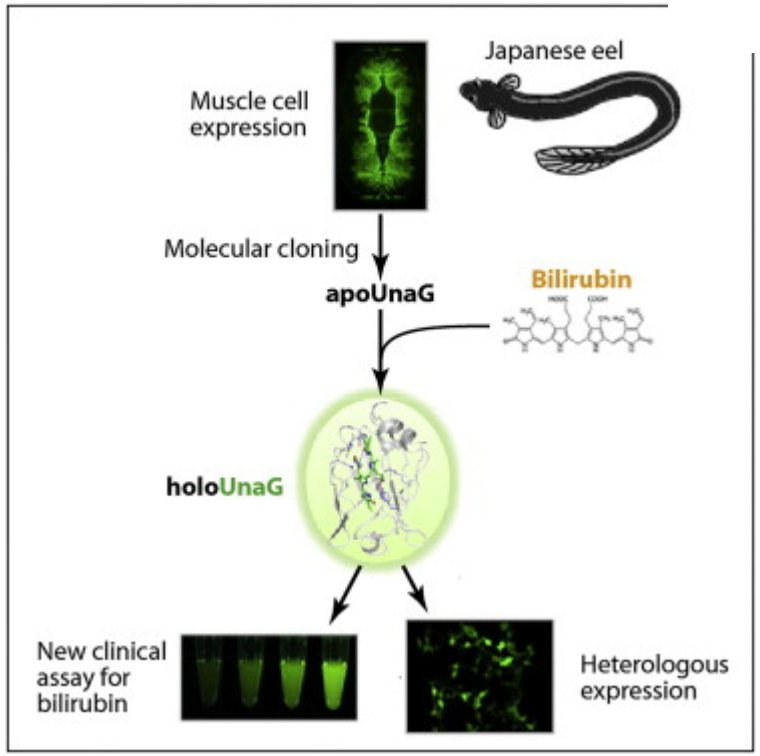


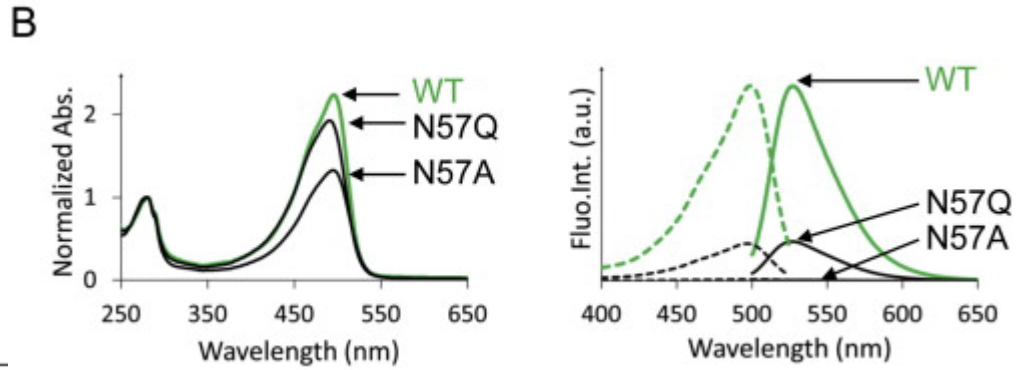




Bilirubin based fluorophores - UnaG

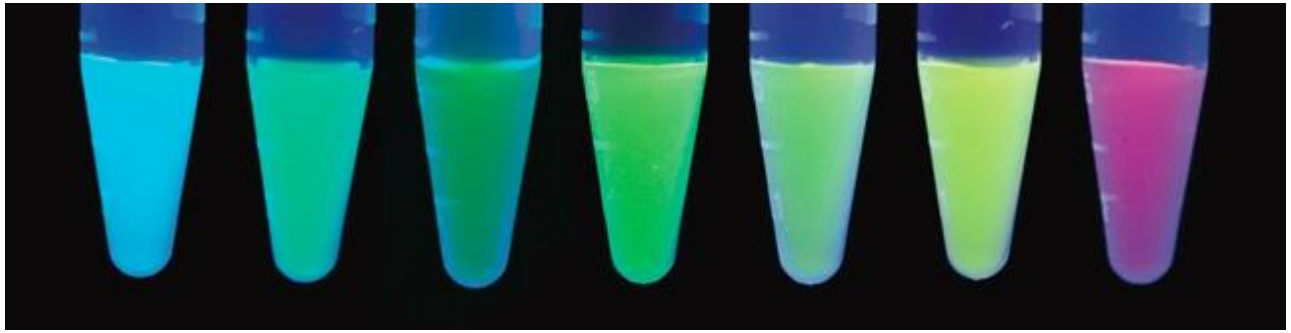
- New fluorescent protein identified in eel muscle
- Uses bilirubin as a chromophore

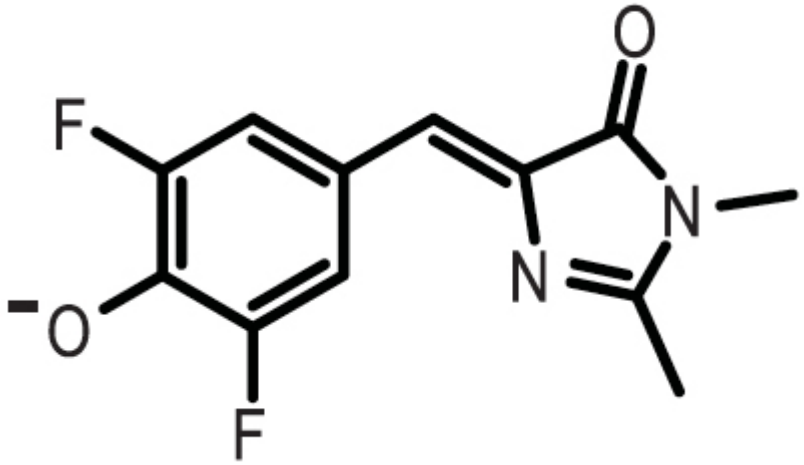


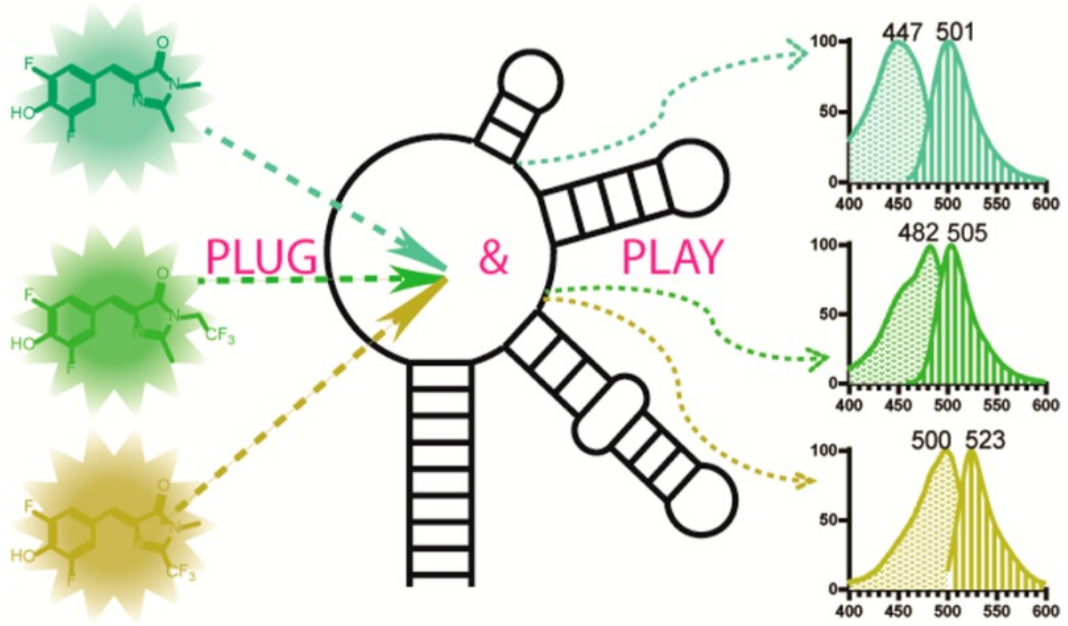


RNA aptamer fluorescence - spinach

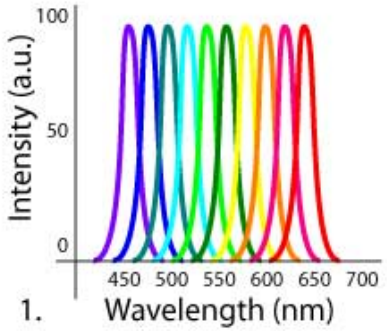
- Spinach holds 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI)

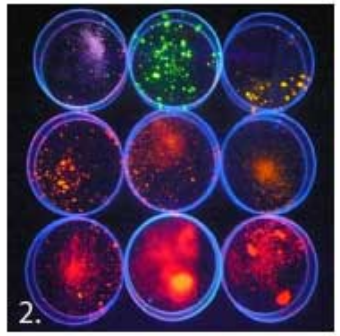






Quantum dots





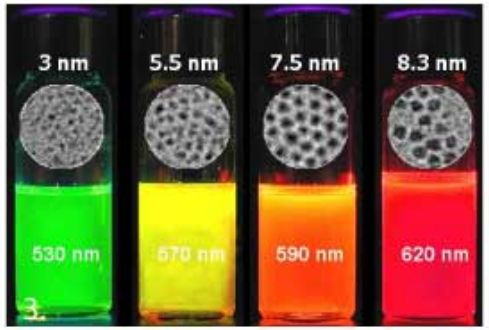
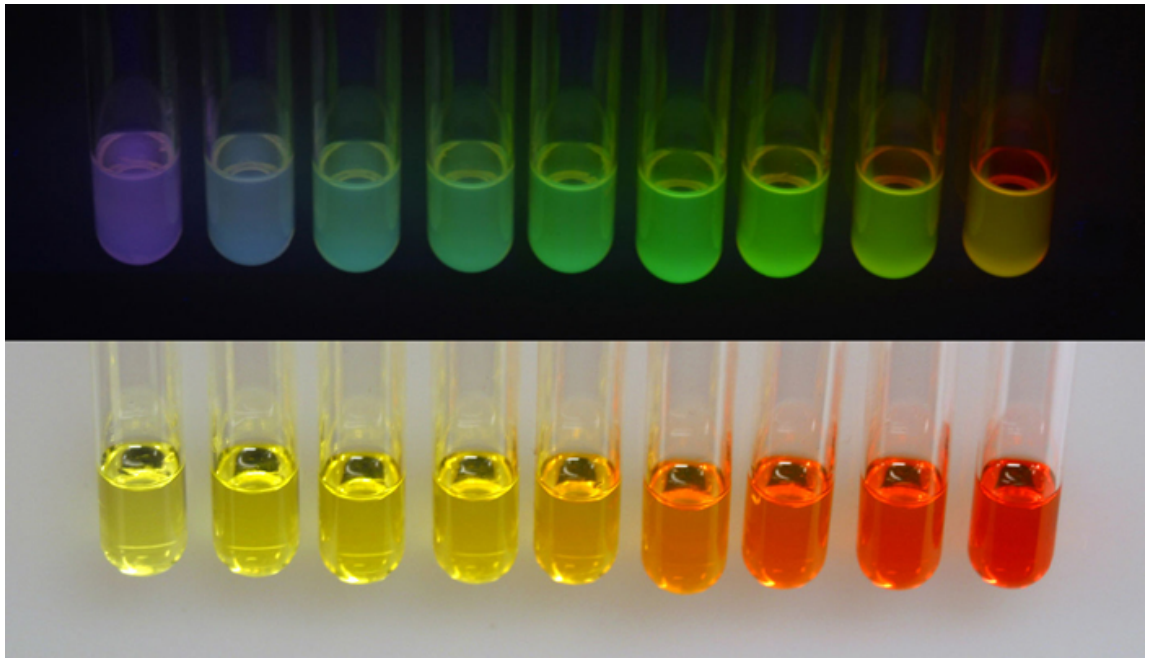
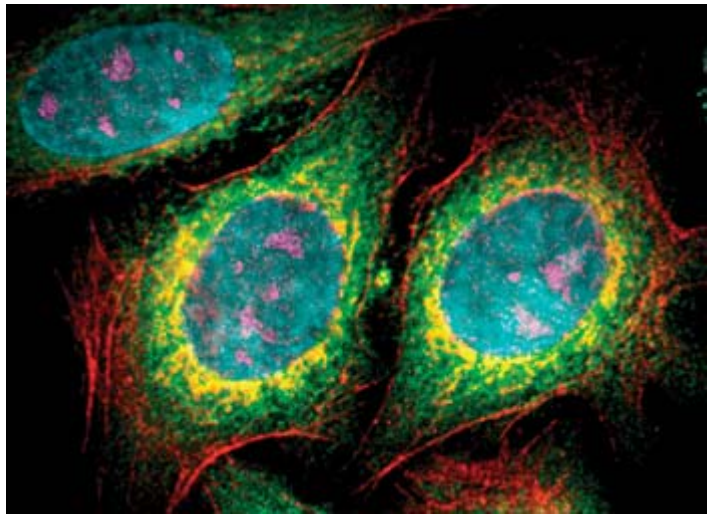


Figure1. Photoluminescence spectra of the quantum dots at wavelength of emission.
Figure2. Ocean's quantum dots in powder form.
Figure3. TEM of Ocean's quantum dots and their corresponding colors at the wavelength of emission.

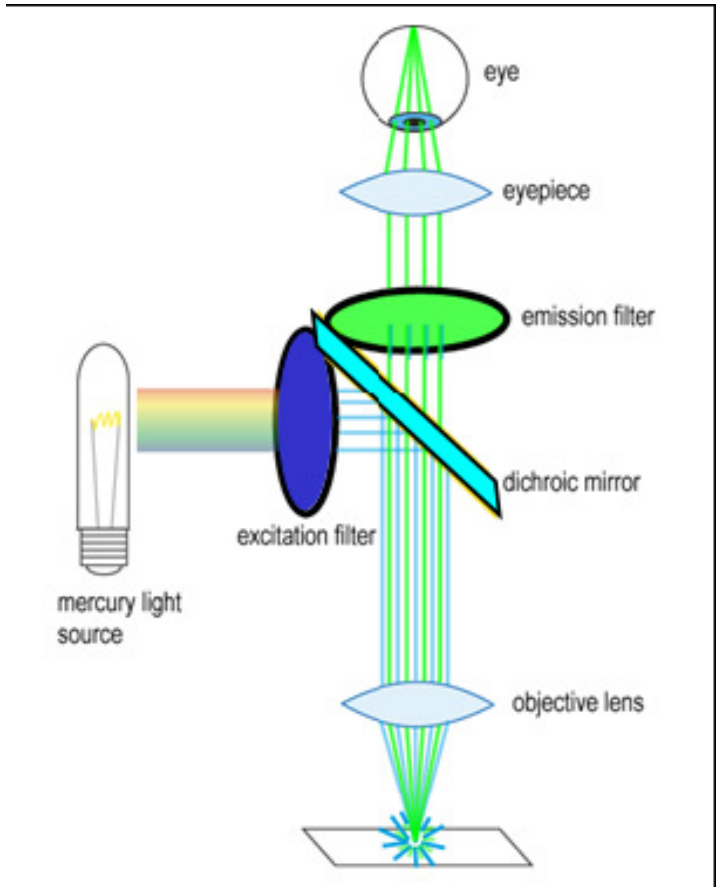
- Un-photobleachable
- Each one has a single excitation band at ~400 nm
- Emission color dependent solely on size





Filters – how do we isolate fluorescent photons?

Filters in fluorescence



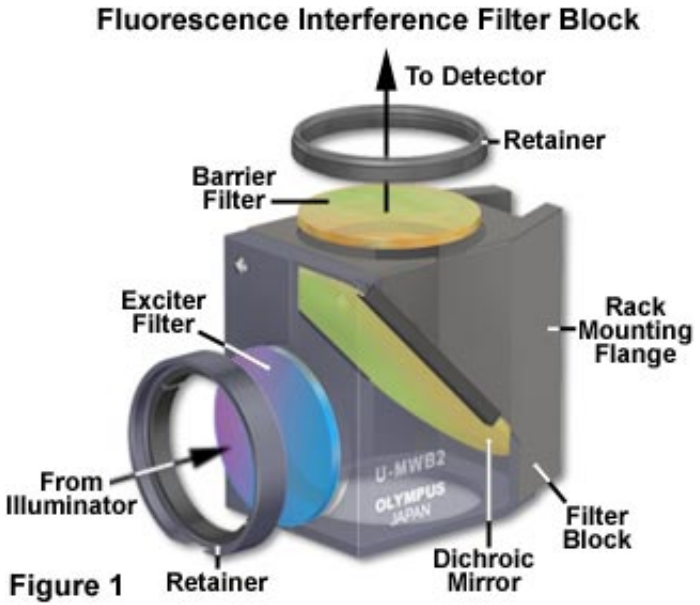
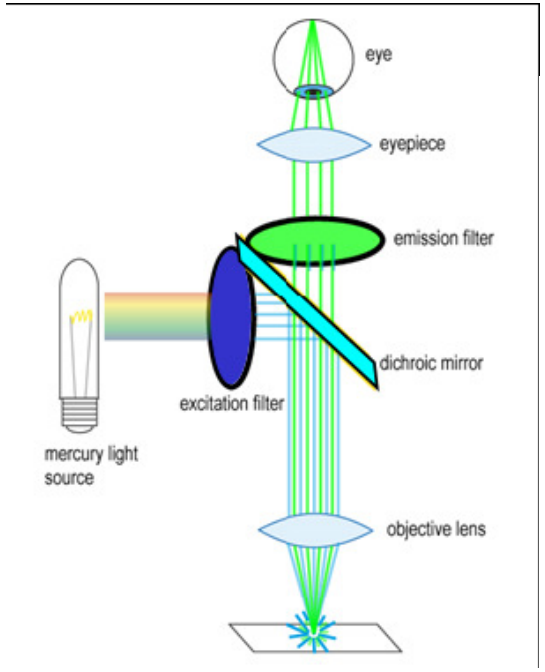


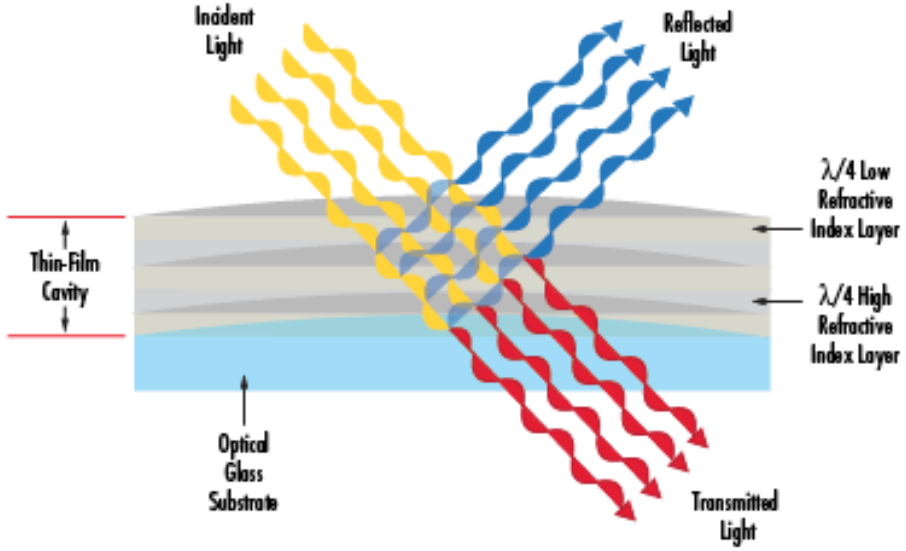
Figure 1

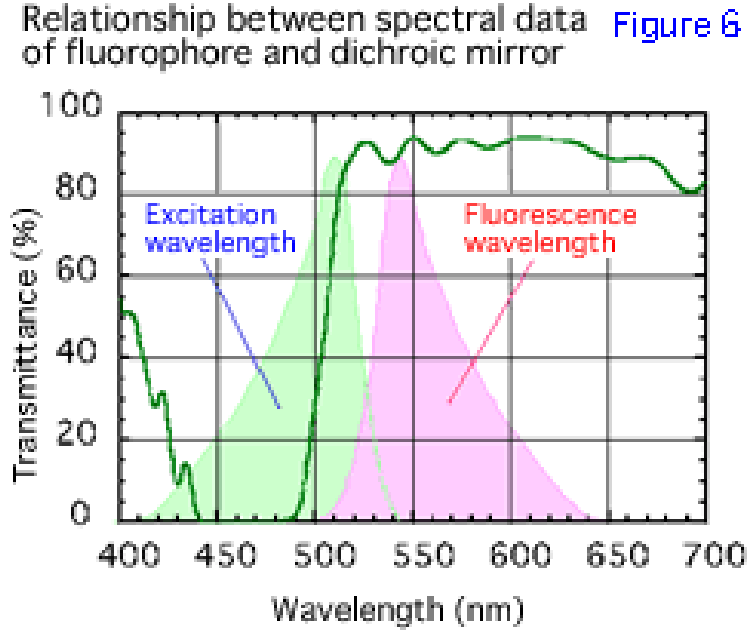
3 Pieces:

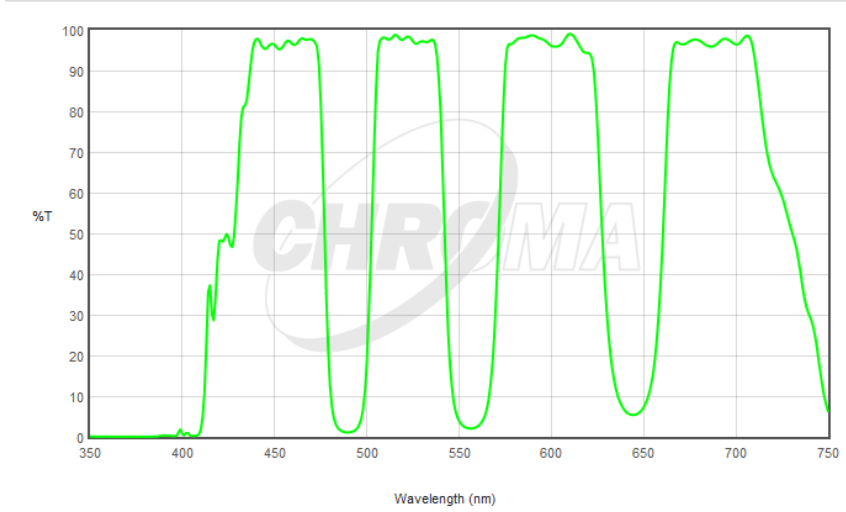
1. Excitation filter – selects incoming light
2. Dichroic mirror – reflects excitation light, transmits fluorescence emission
3. Emission filter – selects fluorescent light

Dichroic mirrors – enabling epi-fluorescence





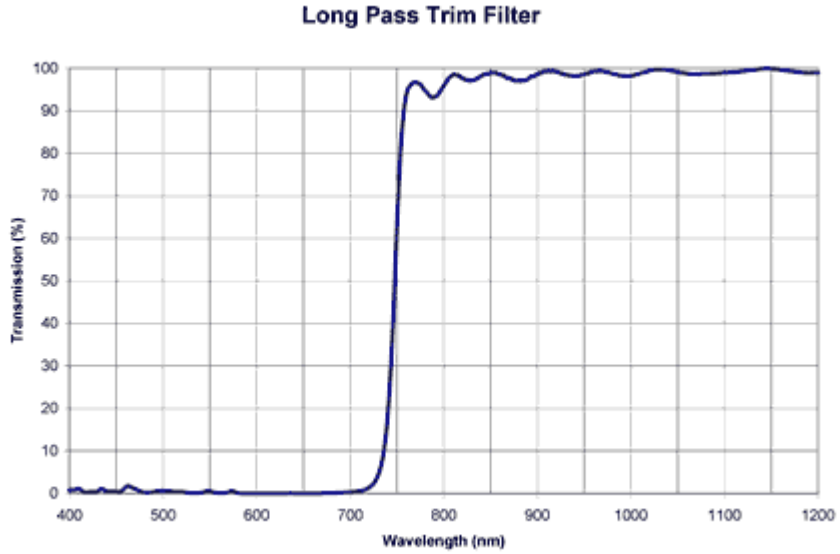


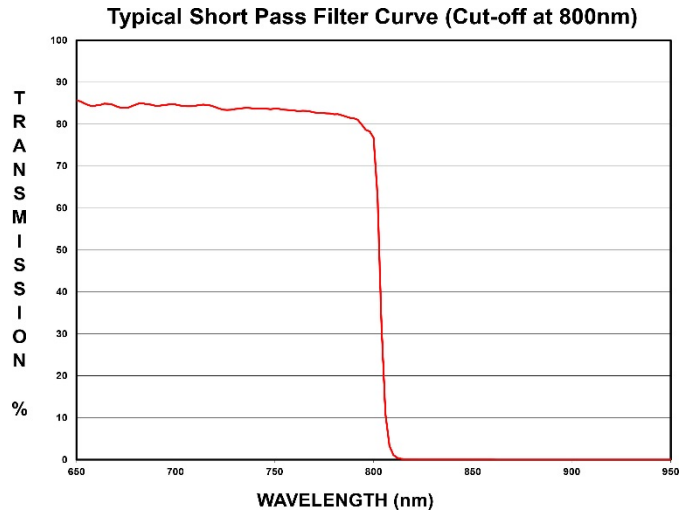


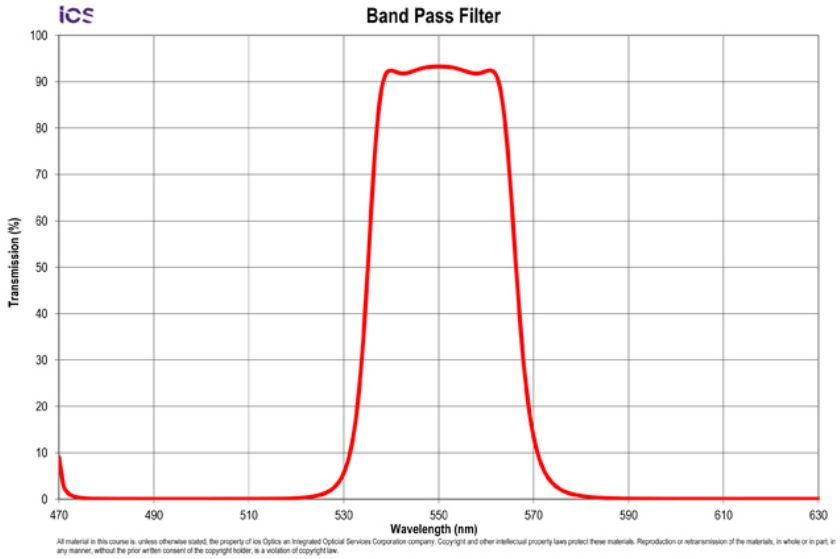
Allows separation based on color
Stokes shift has changed fluorescence

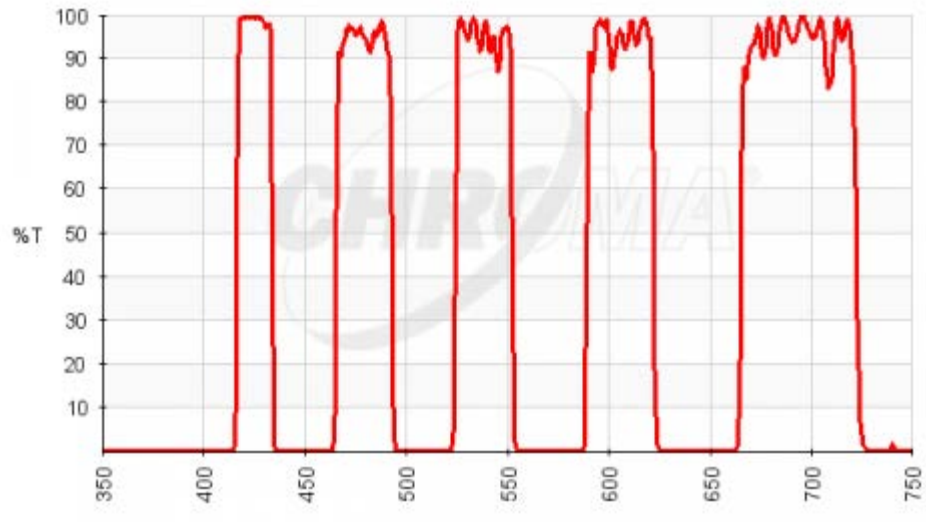
Filters

- Long-pass – 530 LP
- Short-pass – 495 SP
- Band-pass – 530/40
- Multi-band – 530/40 + 610/50



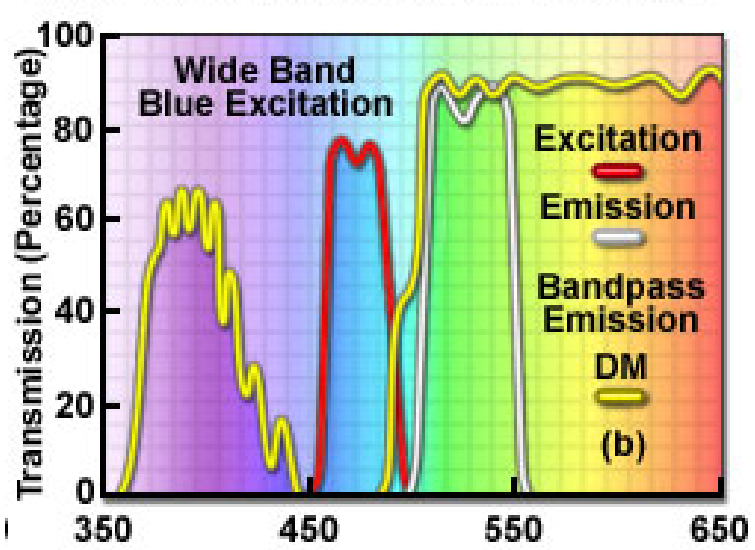






Excitation filters

- Used to select the excitation light
- Typically have small band passes or short pass
- Transmission doesn't have to be especially high
- Ensure that white light source hits at 90˚



Filter lingo:

500/50 – The central transmission is at 500 nm, and the whole band is 50 nm. Transmits from 475 – 525 nm.

Emission filters

- Used to select the emission light
- Typically have larger band passes to collect as many photons as possible
- Transmission should be exceptionally high
- Tilted at a few degrees to prevent back reflections onto the sample
- Can be long pass or bandpass

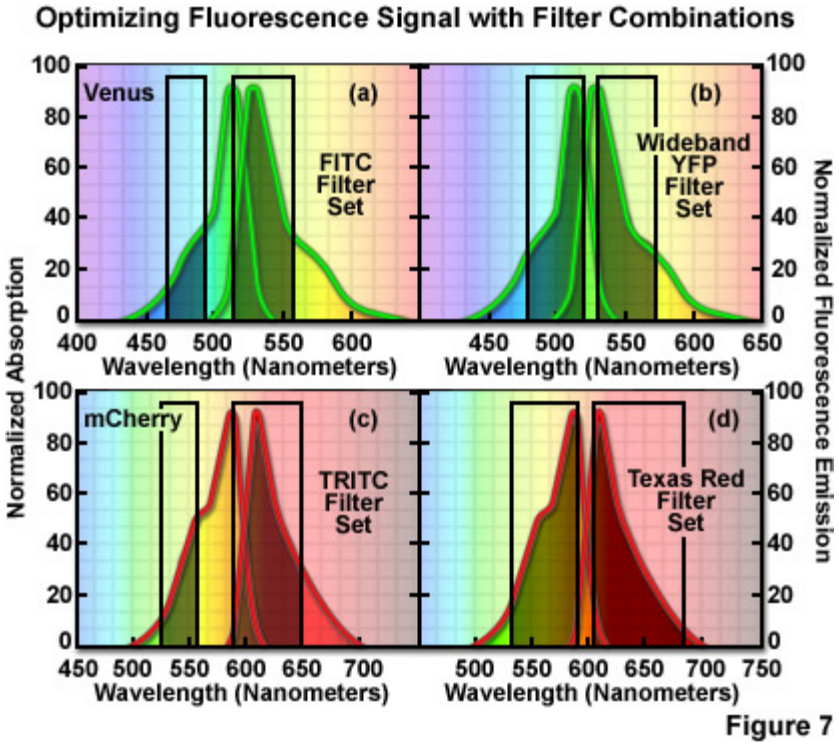


Figure 7

And on to Matlab…