

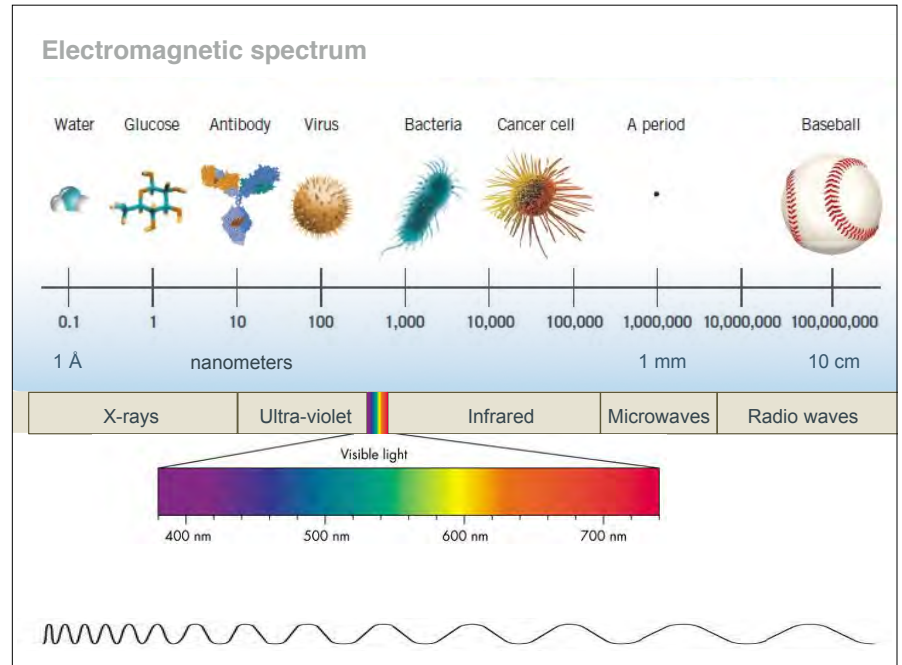
10. Using Light to Learn about Biological Function

Fluorescence fundamentals

1. Spectroscopy & Photophysics
2. Fluorescence
3. Radioactive labels

Niphakis, Cravatt *Annu. Rev. Biochem.* **2014**, 341–377

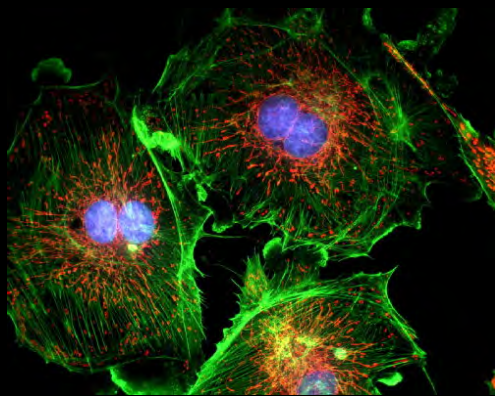
1



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Using light to learn about function

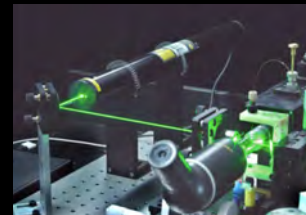
Fluorescence spectroscopy



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1. Spectroscopy & photophysics

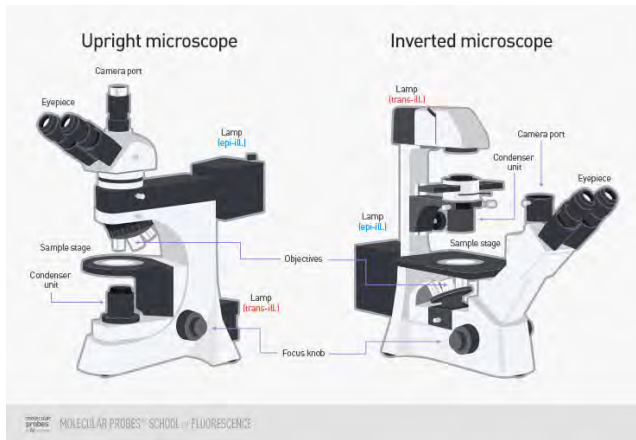
Advent of laser tech



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1. Spectroscopy & photophysics

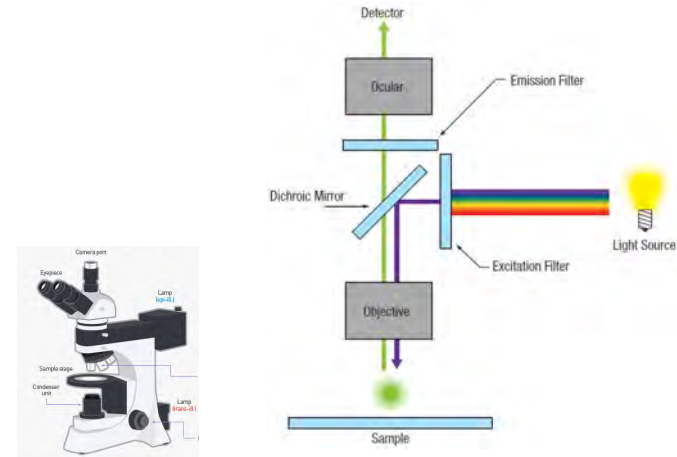
Fluorescence microscopy



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1. Spectroscopy & photophysics

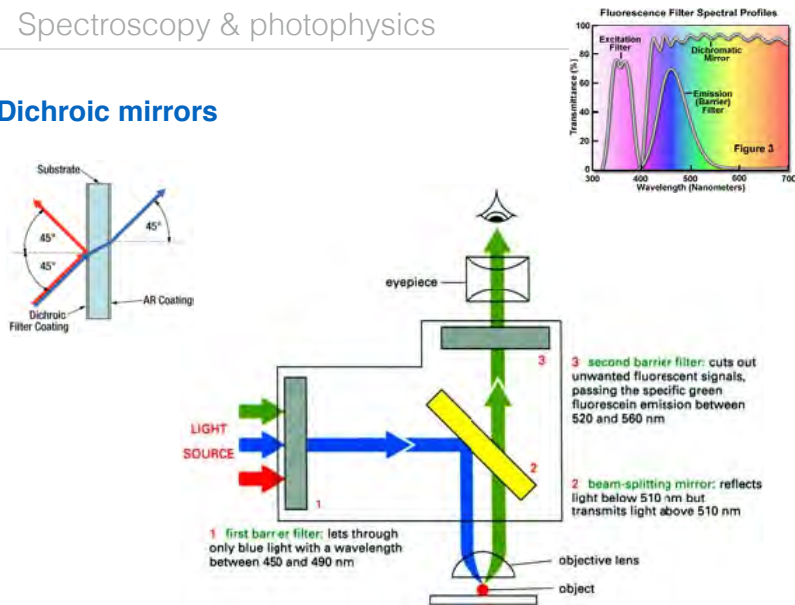
Fluorescence microscopy



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1. Spectroscopy & photophysics

Dichroic mirrors



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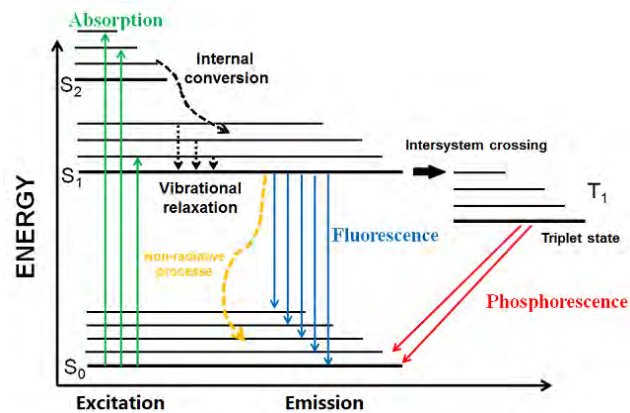
Fluorescence fundamentals

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Niphakis, Cravatt *Annu. Rev. Biochem.* 2014, 341-377

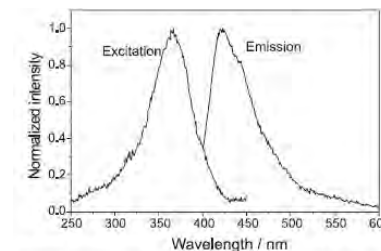
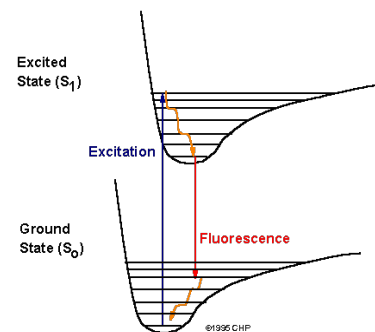
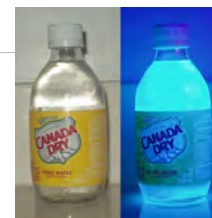
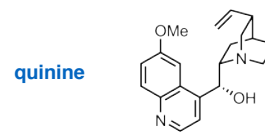
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2. Fluorescence



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2. Fluorescence

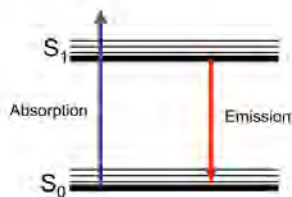


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2. Fluorescence

Stokes Shift

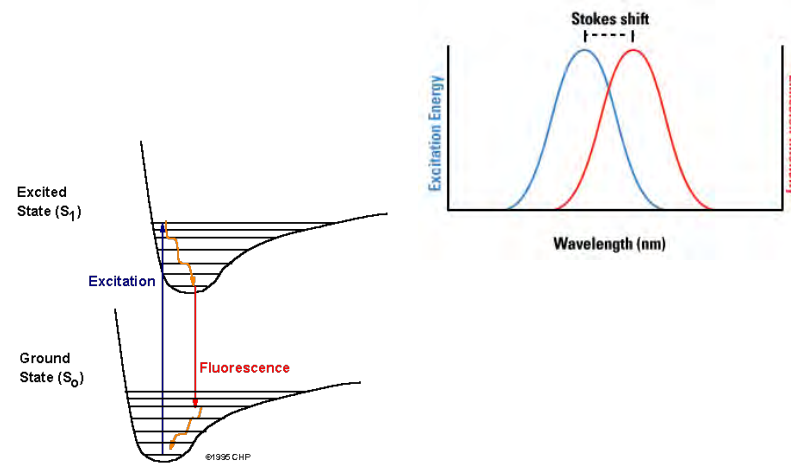
- Difference between excitation energy and emission energy
 - Molecule is excited by high energy (low λ) photon
 - Emitted photon must be lower energy (higher λ) than absorbed one
- **ALLOWS EMITTED PHOTONS TO BE DISTINGUISHED FROM EXCITATION PHOTONS**
 - Basis for obtaining low signal **BACKGROUND** for detecting fluorophors attached to proteins, DNA, cells, etc



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2. Fluorescence

Stoke shift

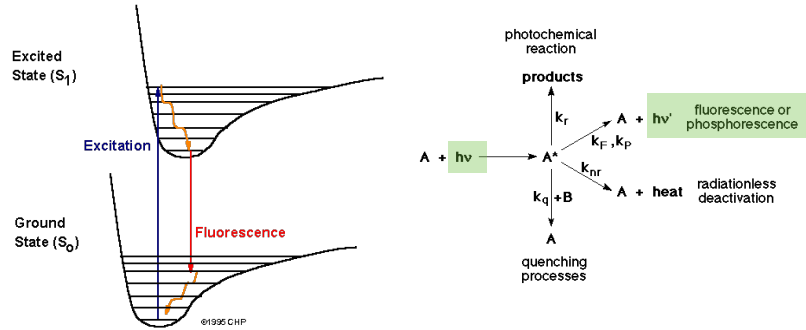


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2. Fluorescence

Quantum yield

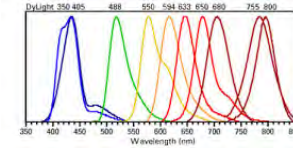
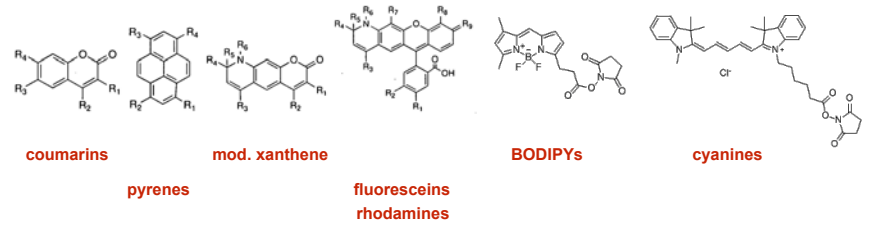
$$\Phi = \frac{\# \text{ photons emitted}}{\# \text{ photons absorbed}}$$



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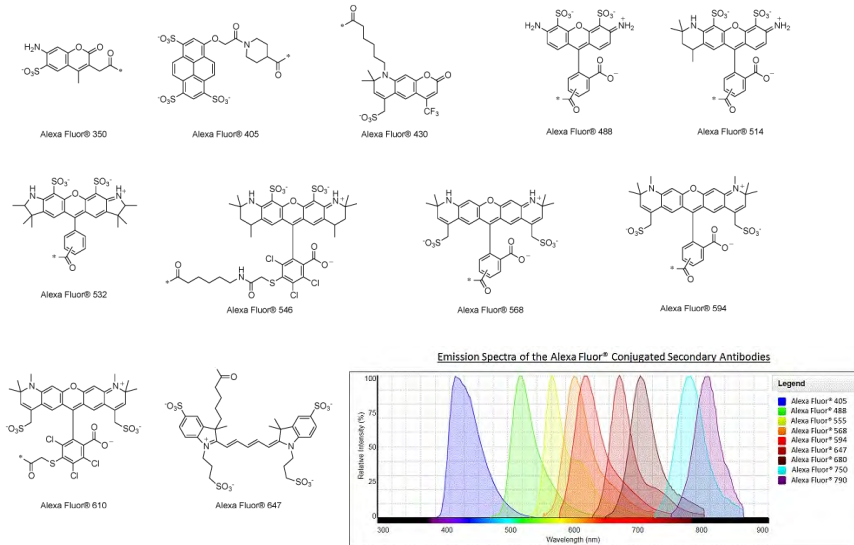
2. Fluorescence

Common fluorescent dyes



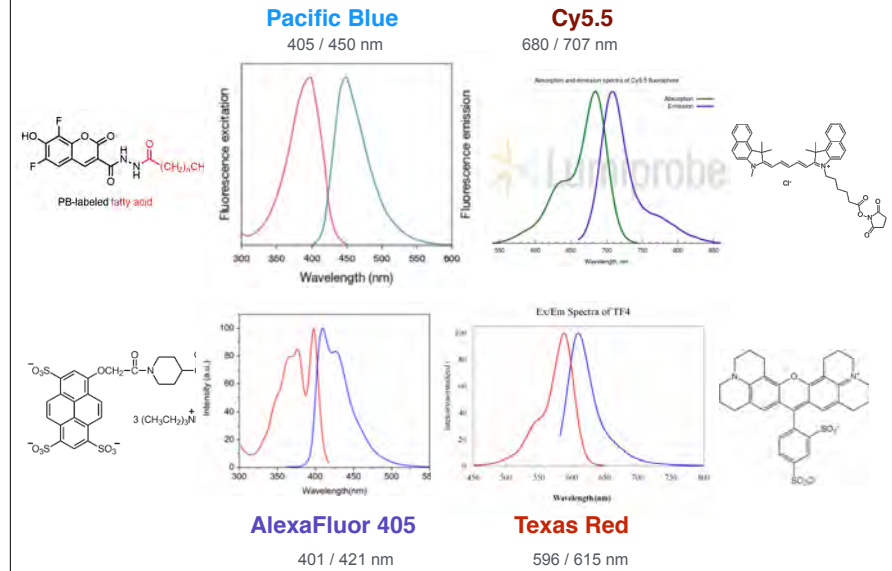
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The Alexa palette

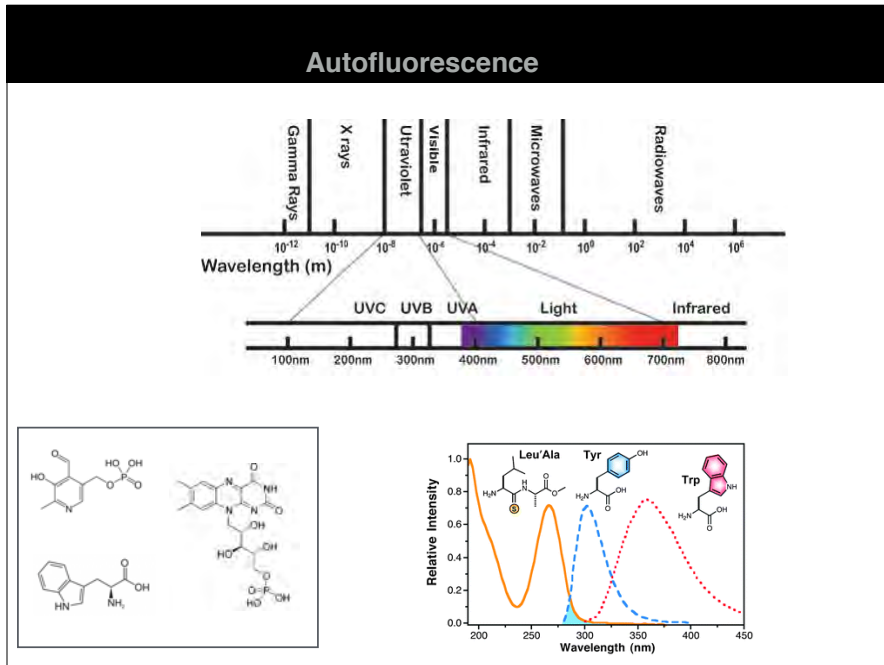


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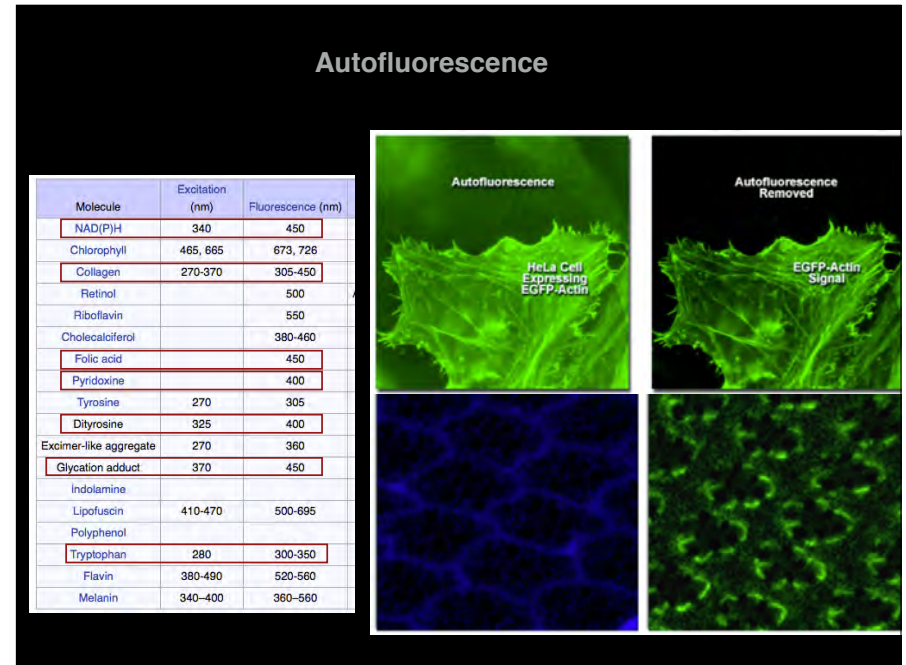
2. Fluorescence



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3. Radioisotope labels

Positron Emission Tomography (PET)

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3. Radioisotope labels

Stoke shift

