Fluorescence microscopy in biology

Principle of fluorescence microscopy

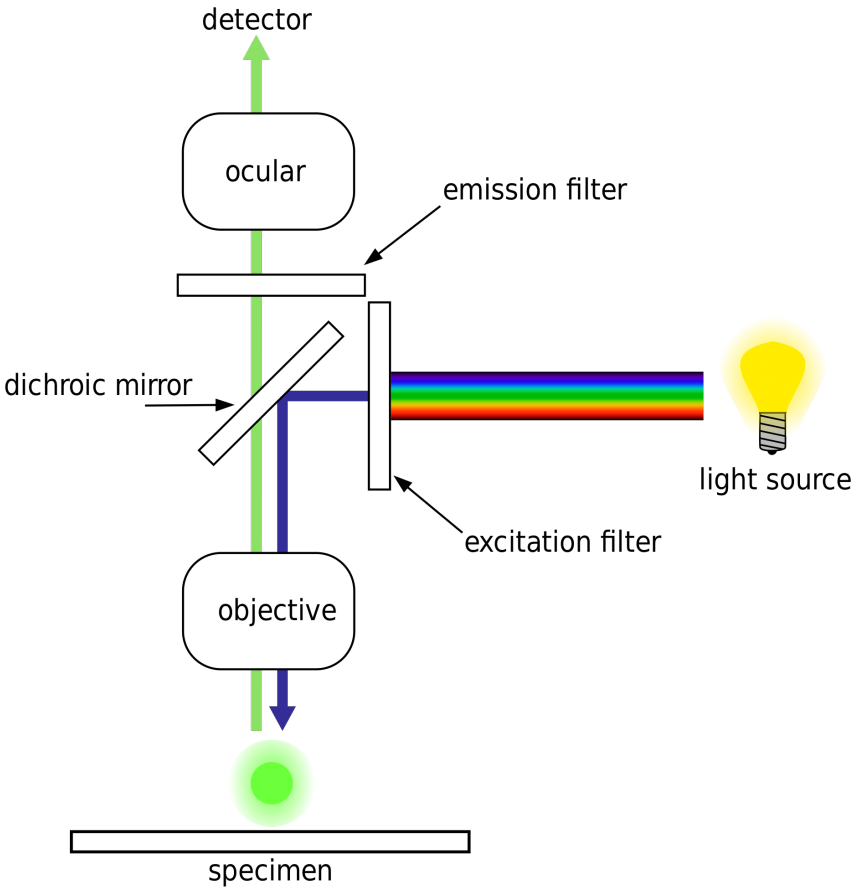
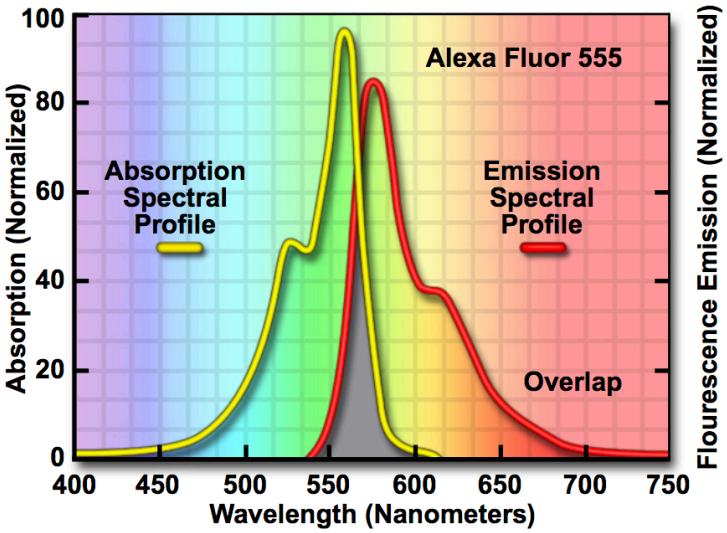
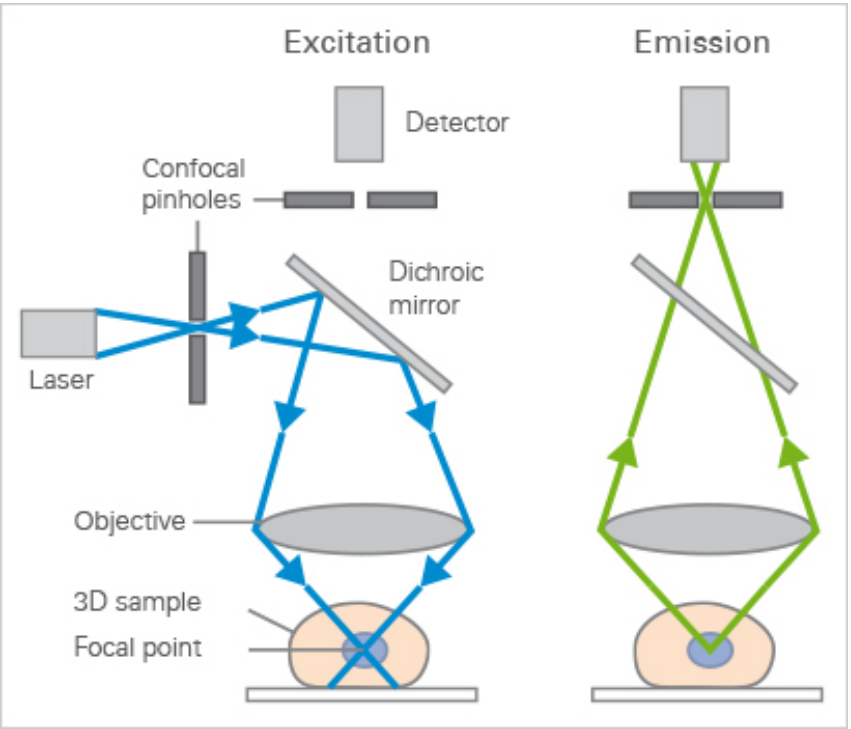


Figure 3 - *Fluorophore Absorption and Emission Profiles*



Confocal fluorescence microscopy

Confocal laser scanning microscopy



Spinning disk confocal microscopy

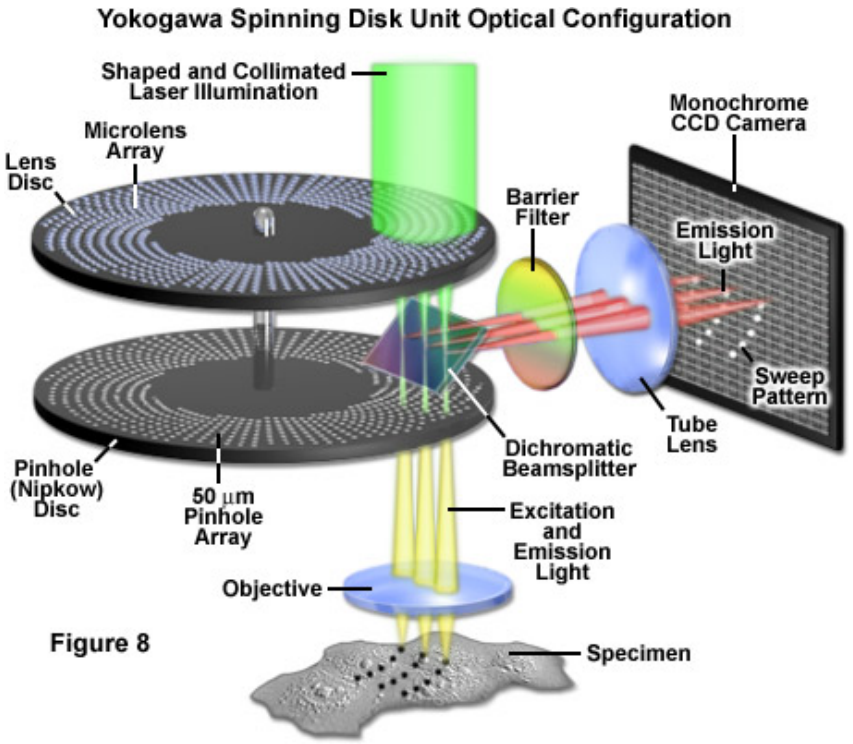
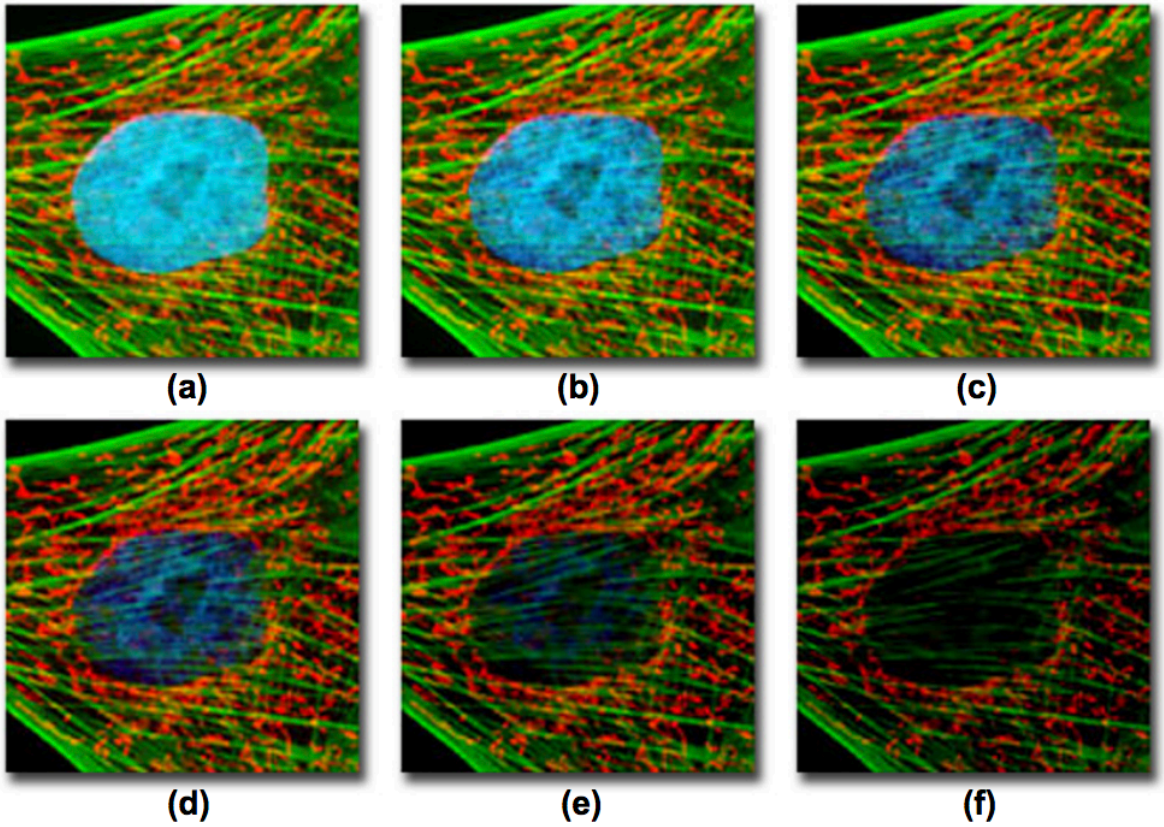


Figure 4 *- Photobleaching Rates in Multiply Stained Specimens*

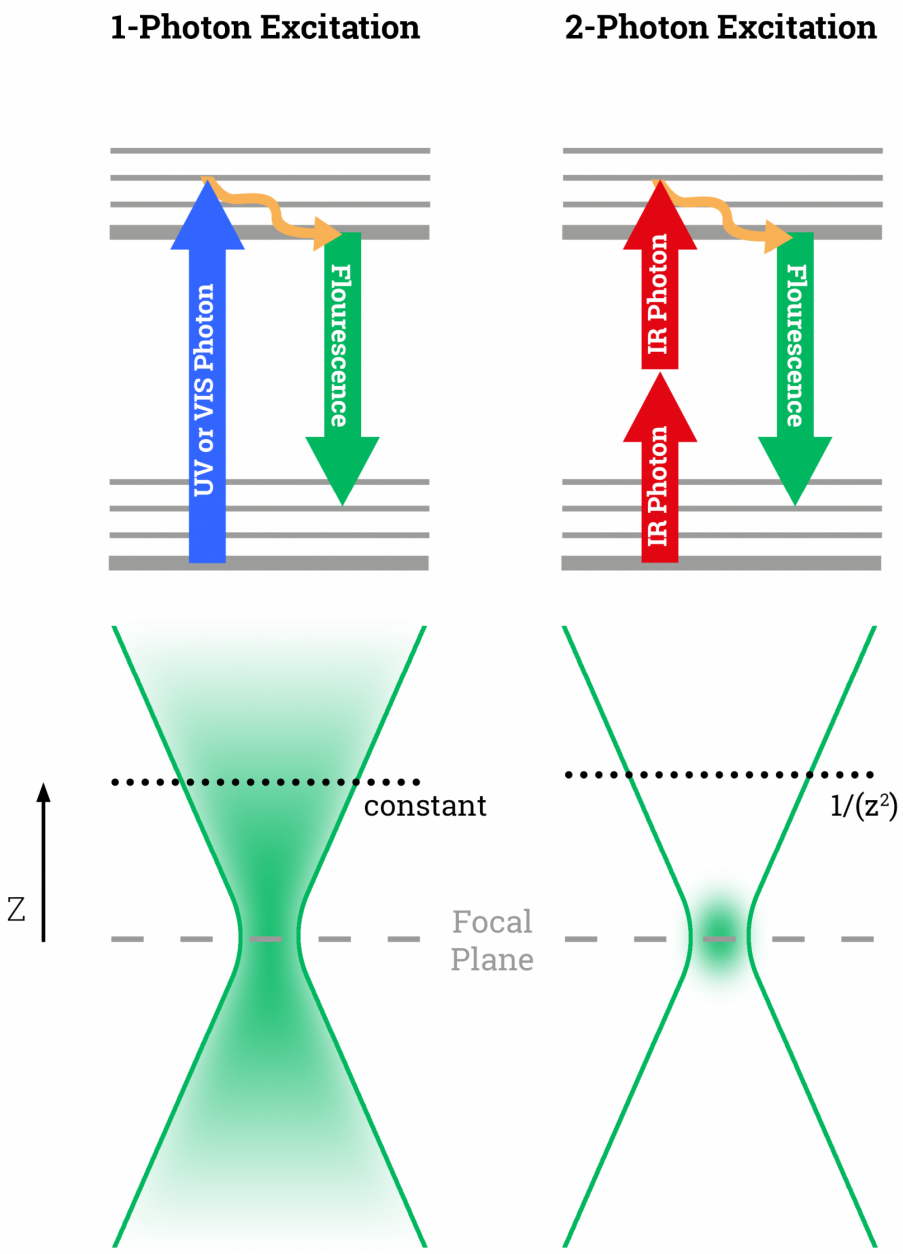


2-photon fluorescence

Two IR photons within a few fs can excite electrons in the fluorophore

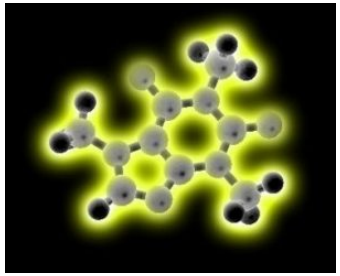
Advantages

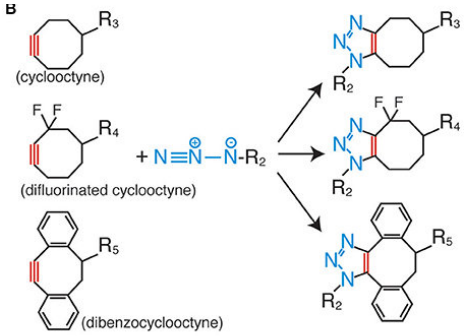
- Non-linear process => excitation probability ~ intensity^2 =>
  - localized imaging in plane without confocal pinholes
  - less bleaching and phototoxicity
- IR light scatters less than short wavelength photon => imaging deeper in tissue



Fluorescent labels in general

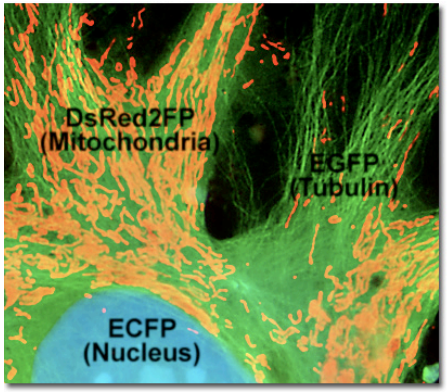
Fluorescent molecules

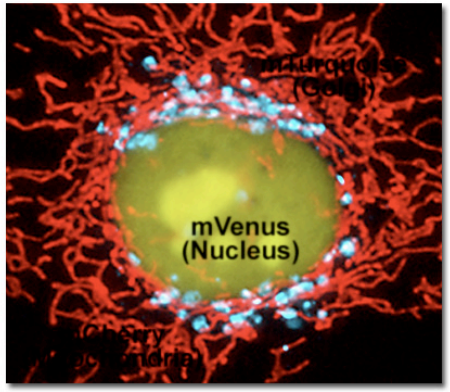




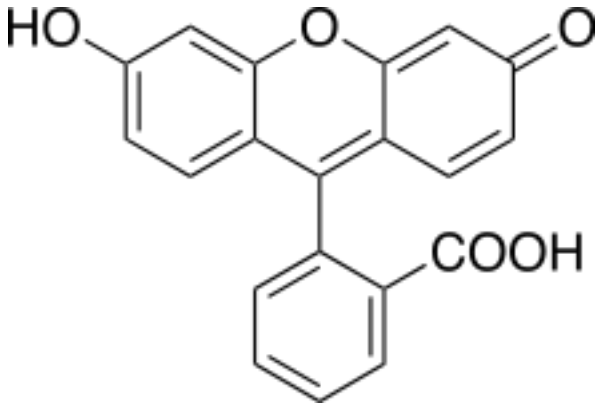
Are attached to biological molecules

The biological molecules are introduced into the cell at their specific sites



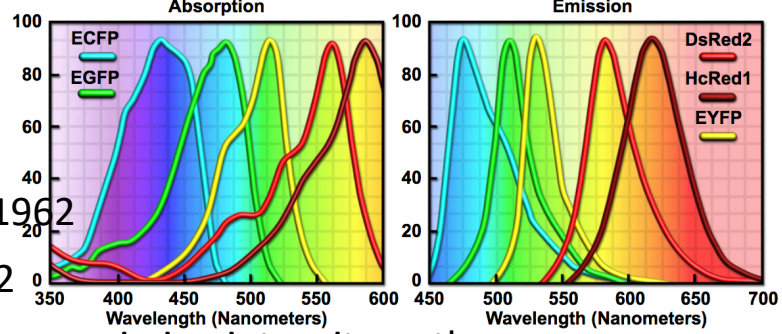


Fluorescein



- Fluorescent is an organic molecule
- biologically active molecules (such as antibodies) may also be attached to fluorescein, allowing biologists to target the fluorophore to specific proteins or structures within cells.
- Fluorescein can also be conjugated to nucleoside triphosphates and incorporated into a probe enzymatically for in situ hybridisation.
  - **nucleoside triphosphates** are the building blocks of both DNA and RNA
  - ***In situ* hybridization (ISH)** is a type of hybridization that uses a labeled complementary DNA, RNA or modified nucleic acids strand (i.e., probe) to localize a specific DNA or RNA sequence
  - In situ hybridization is used to reveal the location of specific nucleic acid sequences on chromosomes or in tissues, a crucial step for understanding the organization, regulation, and function of genes.

Fluorescent Proteins



- green fluorescent protein (GFP) was discovered in jellyfish in 1962
- the gene for green fluorescent protein was first cloned in 1992
- Optical highlighting: modifications of proteins -> change color or emission intensity as the result of external photon stimulation or the passage of time.
- Fluorescent proteins are quite versatile and have been successfully employed in almost every biological discipline from microbiology to systems physiology.
- These ubiquitous probes have been extremely useful as reporters for gene expression studies in cultured cells and tissues, as well as living animals.
- In live cells, fluorescent proteins are most commonly employed to track the localization and dynamics of proteins, organelles, and other cellular compartments.
- A variety of techniques have been developed to construct fluorescent protein fusion products and enhance their expression in mammalian and other systems.
- The primary vehicles for introducing fluorescent protein chimeric gene sequences into cells are genetically engineered bacterial plasmids and viral vectors.
- Fluorescent protein gene fusion products can be introduced into mammalian and other cells using the appropriate vector (usually a plasmid or virus) either transiently or stably.
    - In transient, or temporary, gene transfer experiments (often referred to as **transient transfection**), plasmid or viral DNA introduced into the host organism does not necessarily integrate into the chromosomes, but can be expressed in the cytoplasm for a short period of time.
    - In many cases, the plasmid DNA can be incorporated into the genome in a permanent state to form stably transformed cell lines.

https://www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescent-proteins

In situ hybridization

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Transfection

- **Transfection** is the process of deliberately introducing naked or purified nucleic acids into eukaryotic cells.

Transduction

- **Transduction** is the process by which foreign DNA is introduced into a cell by a virus or viral vector.