Chemistry 201

Fluorescence

NC State University

Fluorescence

Fluorescence is the process of emission of light from an excited state created by absorption. Fluorescence is very sensitive, and can even be measured from single molecules. A key parameter is the fluorescence quantum yield, which gives the efficiency of emission following excitation.

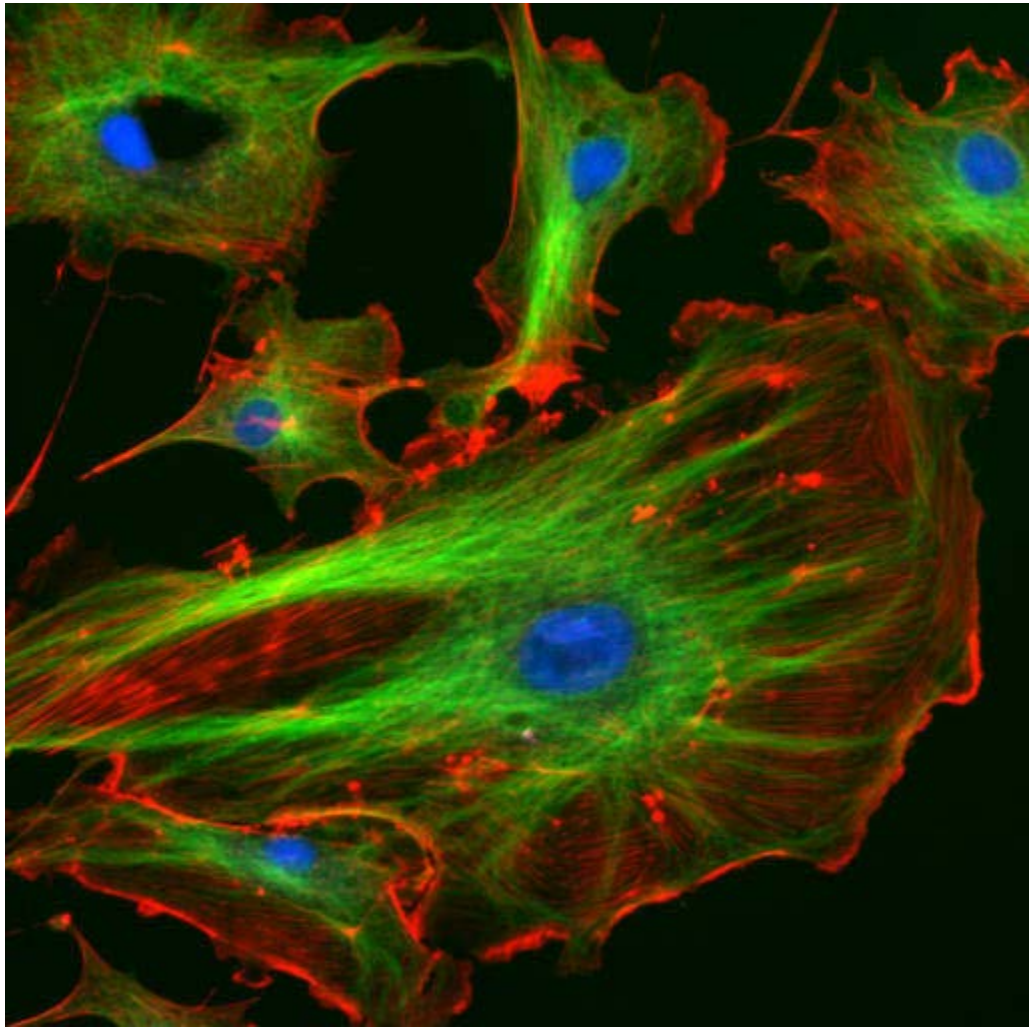
$$\Phi = \frac{k_f}{k_f + k_{nr}}$$

Fluorescence quantum yield

Think of fluorescence quantum yield as a kind of efficiency. It tells you how much light is emitted per light absorbed. It is a fraction. Highly fluorescent dyes are in demand since they are “bright” and they can be used to detection binding, intermolecular interactions, molecular motions etc. The quantum yield is formally defined in terms of the microscopic rate constants for fluorescence and non-radiative relaxation.

$$\Phi = \frac{k_f}{k_f + k_{nr}}$$

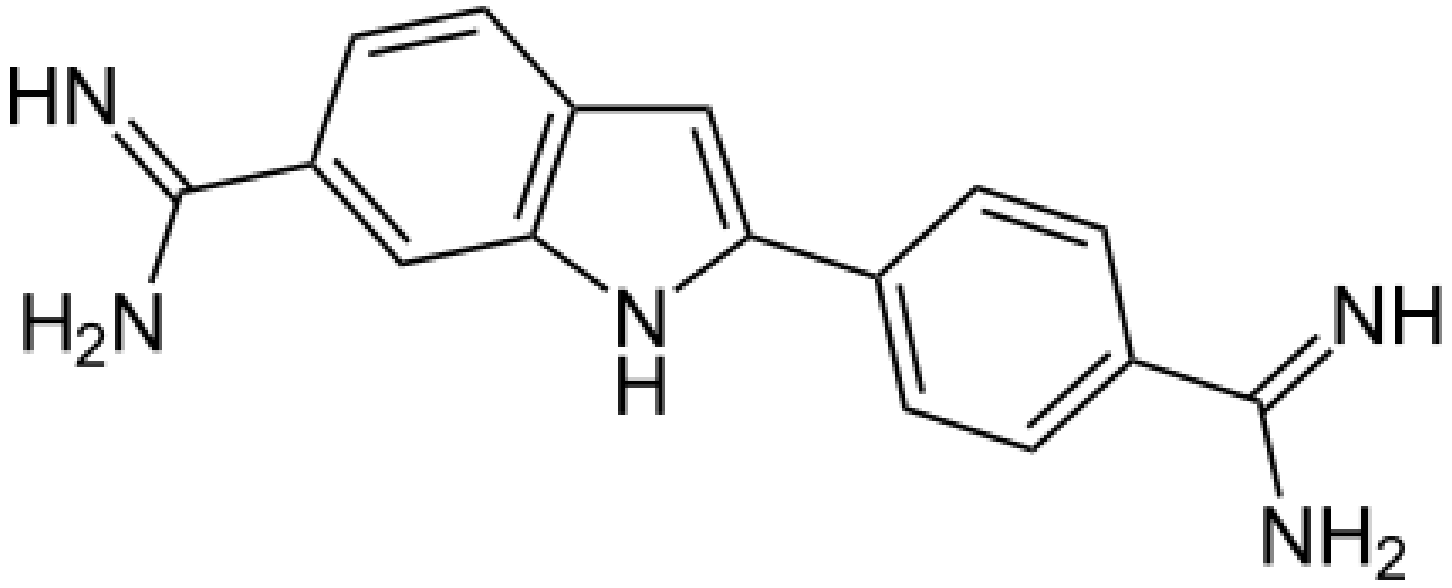
Fluorescently labeled cell



Specific fluorescent labels for cells

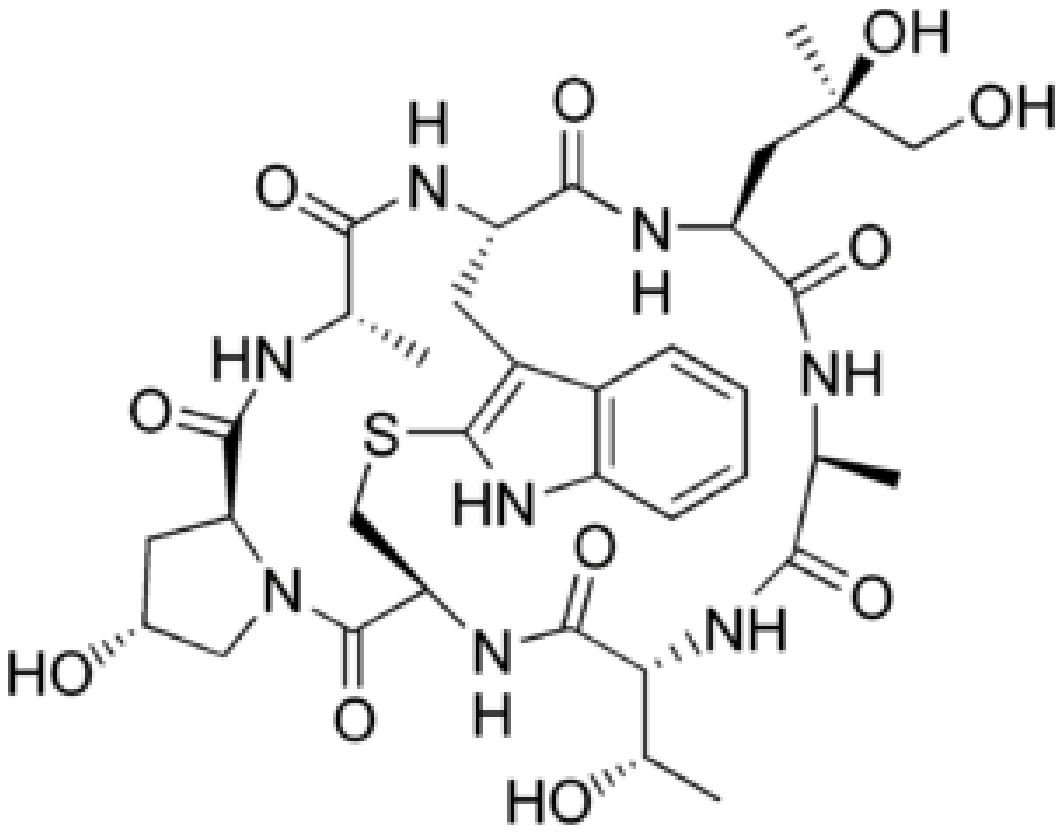
There are a number of different colored fluorescent molecules used for staining cells in a confocal fluorescent microscope. These include blue DAPI dye for nuclear staining. Some dyes are proprietary so we cannot provide their structure. For example, the red dyes used for mitochondrial staining are anti-bodies with a red label. Actin staining is accomplished using a Fluorescently-labeled mushroom toxin known as phalloidin. This combination of dyes permits visual-ization of the state of a cell and differentiation of organelles and the nucleus.

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



DAPI is a nuclear stain that interacts with DNA and has blue fluorescence. This representation of the molecule shows all of the carbon atoms as points. Hydrogen atoms bonded to carbon atoms are implied since carbon is always tetravalent.

Phalloidin for actin staining

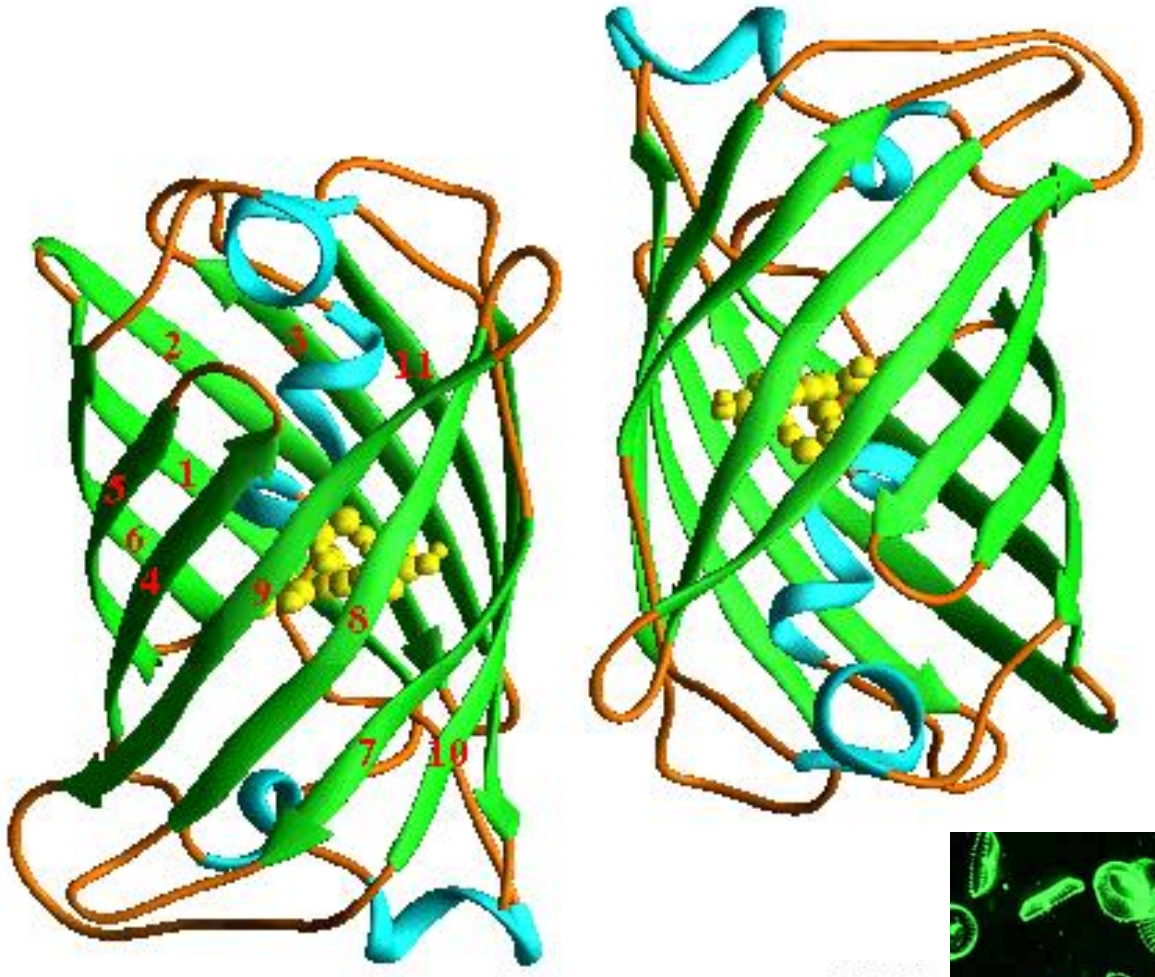


Phalloidin is a toxin that binds to F-actin and can easily be conjugated to fluorescent molecules. This representation of the molecule shows all of the carbon atoms as points. Hydrogen atoms bonded to carbon atoms are implied since carbon is always tetravalent.

Genetically encoded fluorophores

Green fluorescent protein has revolutionized cell biology. The fluorophore forms spontaneously once the GFP protein is expressed in the ribosome. Thus, one can attach the GFP gene to another gene and GFP will act as a fluorescent label when the protein is expressed. GFP can be used to understand how genes are “turned on” during the development of organisms. It can be used to monitor cellular responses and as a genetically encoded stain like the artificial stains discussed previously.

Green fluorescent protein



Originally derived from a jellyfish

Family of fluorescent proteins

Genetic modification of the GFP gene leads to red, blue, cyano, yellow, and many other fluorescent proteins. The world of fluorescent proteins is an enormous boon to cell biology since these molecules can be programmed into a cell. Multiple colors permit tracking of several different simultanoues events. It also permits energy transfer (also known as fluorescent resonant energy transfer or FRET), which is a distance dependent process that permits one to see whether two fluorescent molecules are close to each other in space.

Applications of GFP

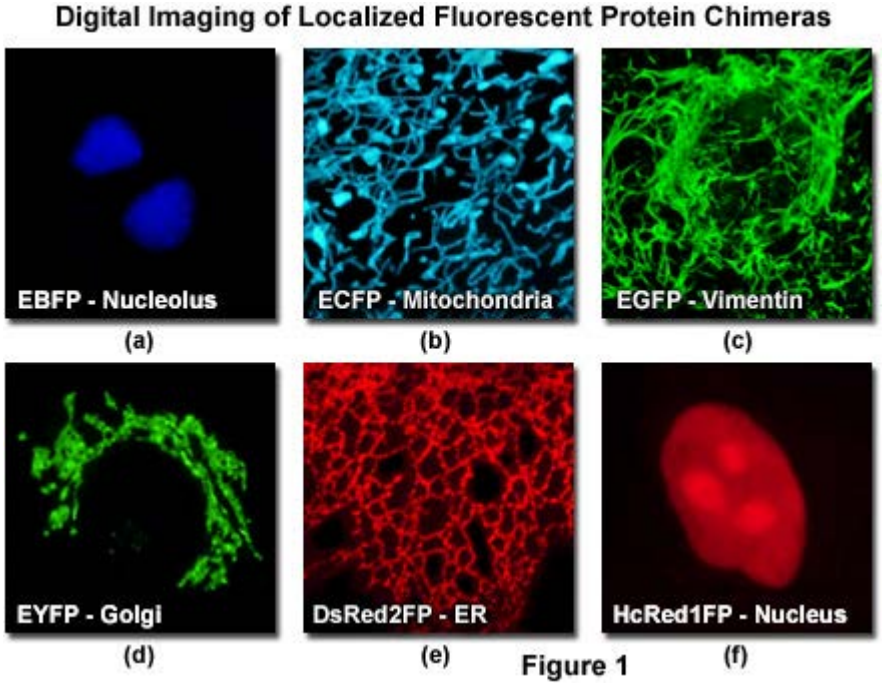


Figure 1