

Biomicroscopy I - Exercise Sheet 11

November 26, 2024

1 Beer-Lambert law and fluorescent dyes

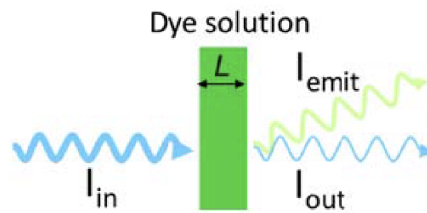


Figure 1: Absorption, transmission and emission of light by a dye solution.

The Beer-Lambert law relates the absorption of light to the properties of the material through which the light is traveling. Figure 1 shows the experimental set-up in which light is passing through a sample containing a fluorescence dye solution. The intensities of incident (I_{in}), transmitted (I_{out}) and emitted (I_{fluor}) light can be measured experimentally. The Beer-Lambert law states:

$$I_{out} = I_{in}e^{-\varepsilon cL}.$$

where I_{in} and I_{out} are the intensities of the incident and transmitted light, respectively. L is the thickness of the dye sample, c is the concentration of the dye solution and ε is the molar absorption coefficient of the dye.

- A. Assume that the sample solution is containing $1\mu\text{m}$ thick fluorescein dye at a molecular concentration of 0.1M . The molar absorption coefficient of fluorescein is $\varepsilon \sim 70,000\text{M}^{-1}\text{cm}^{-1}$. The incoming power and power of emitted emission are measured as 1W and 0.39W respectively.
- (a) Calculate the power of the absorbed light by the dye.
 - (b) What is the quantum efficiency of this dye?
- B. Repeat A. if the fluorescein sample is replaced with another dye at the same concentration with $\varepsilon \sim 100,000\text{Mcm}^{-1}$. For the same incident intensity of 1W , fluorescence light is measured as 0.1W .
- C. Which of the two dyes is brighter?

2 Fluorescence Microscopy

- A. What is photobleaching and what is its effect in fluorescence microscopy? Explain in a few short sentences.
- B. Name a few commonly used synthetic proteins in fluorescence microscopy.
- C. Name a few commonly used fluorescence proteins in fluorescence microscopy.
- D. List the most important optical and opto-electronic components in fluorescence microscopy.
- E. A filter cube is at the heart of a fluorescence microscope. How many filters does it typically contain? What are these filters?
- F. Name at least four commonly used illumination sources for fluorescence microscopy.
- G. What are the major differences in the spectra of a quartz tungsten-halogen lamp, mercury arc lamp and LED? Approximately sketch the spectra of these three sources.

3 Quantum Dots

- A. A quantum dot solution is emitting a red color with a wavelength of 700 nm. What is the energy associated with this wavelength of light?
- B. Assume you have two quantum dot solutions. Solution 1 emits a blue color of approximate wavelength 475 nm. Solution 2 emits green color light of approximate wavelength 560 nm. Which solution emits photons with larger energy and larger frequency? How much larger is its energy?

4 Jablonski diagram

The Jablonski diagrams of two fluorophores are represented in Fig. 2.

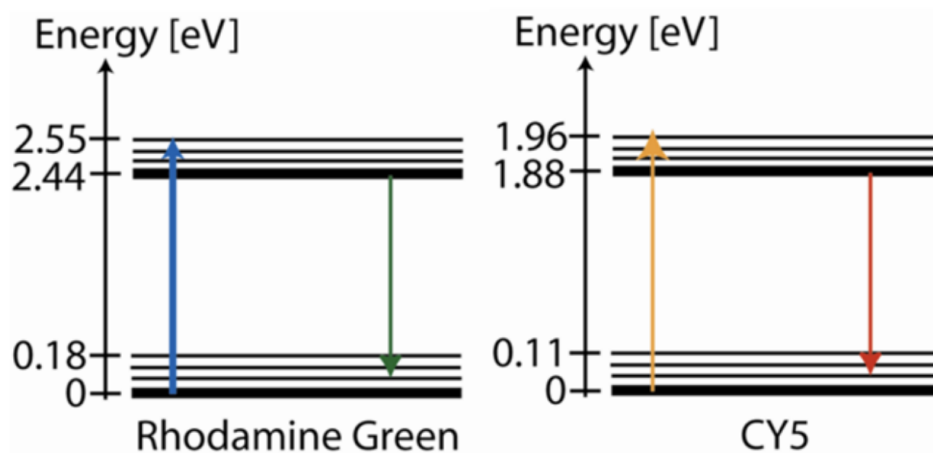


Figure 2: Jablonski diagram of Rhodamine and Cyanine (CY5). Note that energy axes have different scales.

- A. Which laser wavelength would you use to excite those fluorophores?
- B. What are the emission wavelengths for each dye and what will be the emission spectral widths?