

Biomicroscopy I - Exercise Sheet 10

November 19, 2024

1 Energy and wavelength of a photon

According to quantum optics, light is constituted of *quanta* (particles) called “photons”. The energy E of these quanta is related to the wavelength of light λ as follows:

$$E = h\nu = \frac{hc}{\lambda} = \hbar\omega$$

where $c \approx 3 \times 10^8 \text{m/s}$ denotes the speed of light, $h \approx 6.63 \times 10^{-34} \text{J} \cdot \text{s}$ is the fundamental Planck’s constant and $\hbar = \frac{h}{2\pi}$ (pronounced as *h-bar*) is the reduced Planck’s constant.

By plugging the values of h and c , the relationship between the wavelength λ (in μm) and energy (in eV^1) is given as:

$$E = \frac{1.24(\text{eV} \cdot \mu\text{m})}{\lambda(\mu\text{m})}$$

Using these relationships and Table 1, complete the following tasks:

Violet	Blue	Green	Yellow	Orange	Red
~ 400	~ 475	~ 510	~ 570	~ 590	~ 650

Table 1: Wavelength values of the visible spectrum (in nm)

- A. Calculate the energy of light if its wavelength is $1\mu\text{m}$.
- B. Calculate the energy of red light.
- C. Calculate the energy of light if its wavelength is $2.48\mu\text{m}$.
- D. Calculate the wavelength of light (in nm) if its energy is 2 eV.
- E. Roughly determine the color of light if its energy is 3.1 eV.

2 Fluorescence as a three-stage process

Fluorescence can be described with the ‘three-level’ process show in Figure 1.

- A. Which process corresponds to excitation: **i**, **ii** or **iii**?

¹1 eV is the work of the electric field with the voltage $U = 1\text{V}$ spent for the displacement of elementary charge $\Rightarrow 1\text{eV} = 1.6 \cdot 10^{-19}\text{J}$.

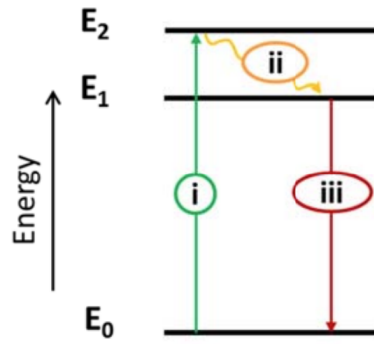


Figure 1: The fluorescence three-level process.

- B. Which process corresponds to fluorescence signal generation: **i**, **ii** or **iii**?
- C. Which process corresponds to energy loss in the system: **i**, **ii** or **iii**?
- D. Assume that the energy values of this simple molecule system are $E_0 = 0\text{eV}$, $E_1 = 2.17\text{eV}$ and $E_2 = 2.61\text{eV}$. If you shine the molecule with orange light ($\lambda = 590\text{nm}$), can the molecule be excited?
- E. Under the same energy values as in C., would the molecule be excited if you shine it with violet light ($\lambda = 400\text{nm}$) instead?
- F. Assume that the molecule is excited to the excited state E_2 and relaxed immediately to E_1 before radiative decay. What will the energy, wavelength and color of the emitted light?
- G. What is the amount of energy lost in the excitation-emission process for this molecule?

3 Fluorescence lifetime and decay rates

Consider a fluorescent molecule which is simplified with a two-level system shown in Figure 2a. With absorption (*i.e.* excitation) the molecule transits at a rate of k from the ground truth state S_0 to the excited state S_1 . It can either radiatively transit back to the ground state by emitting a photon at a rate of k_r or non-radiatively transit by transferring its energy to collisions at a rate of k_{nr} .

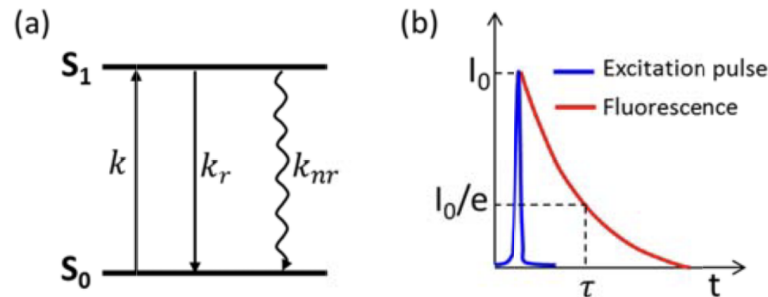


Figure 2: Two-level system for a fluorescent molecule.

Let us assume that $k_{nr} = 3 \cdot 10^8\text{s}^{-1}$ and $k_r = 7 \cdot 10^8\text{s}^{-1}$. Answer the following questions:

- A. What is the radiative lifetime (τ_r) of this molecule?

- B. What is the non-radiative lifetime (τ_{nr}) of this molecule?
- C. What is the total decay rate ($k = k_r + k_{nr}$) of this molecule?
- D. The fluorescence lifetime, τ (which is $\frac{1}{k}$) can be experimentally determined by exciting the molecule with a very short pulse (close to a δ -pulse) at $t = 0$ and then measuring the decay of fluorescence intensity with a fast detector. The excitation and fluorescence curves are given in Figure 2b. Fluorescence intensity decays as:

$$I = I_0 e^{-\frac{t}{\tau}}$$

What is the fluorescence lifetime (or *total lifetime*) of this molecule?

- E. What is the quantum efficiency of this molecule?