Biomicroscopy I - Exercise Sheet 10

November 18, 2025

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 $c \approx 3 \times 10^8$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 1μm.

B. Calculate the energy of red light.

C. Calculate the energy of light if its wavelength is 2.48μm.

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**?

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

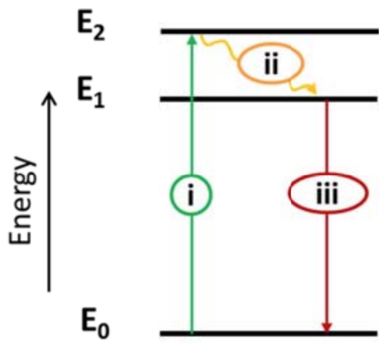

Figure 1: The fluorescence three-level process.

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 D., 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 **r**adiatively transit back to the ground state by emitting a photon at a rate of k_r or **n**on-**r**adiatively transit by transferring its energy to collisions at a rate of k_{nr}.

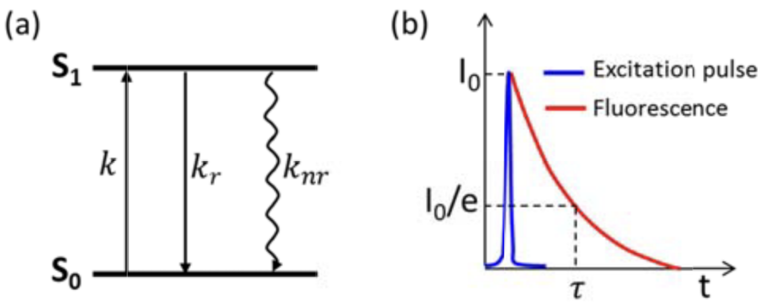

Figure 2: Two-level system for a fluorescent molecule.

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?