Biomicroscopy I - Solutions Exercise Sheet 11

November 26, 2024

1 Beer-Lambert law and fluorescent dyes

In this and further tasks utilising Beer-Lambert law you can use powers instead of intensities, since incoming and outcoming light usually come through the same cross-section.

A. (a) $I_{out} = 1W \cdot e^{-7 \cdot 10^4 \cdot 10^{-1} \cdot 10^{-4}} = 0.5\text{W}$.
Note: always check that the factor of the exponent is dimensionless: $\left[\frac{1}{M \cdot cm} \cdot M \cdot cm = 1\right]$

$I_{absorbed} = I_{in} - I_{out} = 0.5\text{W}$.

(b) Part of the absorbed light will be converted to emission:

$$Q = \frac{I_{emitted}}{I_{absorbed}} = \frac{0.39}{0.5} = 0.78$$

B. $I_{out} = 0.37\text{W}$

$I_{absorbed} = 0.63W$

$Q = \frac{0.1}{0.63} = 0.158$

C. The brightness B is $B \sim Q \cdot \varepsilon$

$B_{A.} = 54600$; $B_{B.} = 15800$

Dye $A.$ is brighter, as it emits more light for the same incident intensity.

2 Fluorescence microscopy

A. Photobleaching occurs when a fluorophore permanently loses the ability to fluoresce due to photon-induced chemical damage and covalent modification. The average number of excitation and emission cycles that occur for a particular fluorophore before photobleaching is dependent upon the molecular structure and the local environment. Some bleach quickly after emitting only a few photons, while others are more robust and undergo thousands or even millions of cycles before bleaching.

B. Some commonly used synthetic dyes are:

- Alexa Fluor: AF488, AF546, AF568, AF594
- Cyanine dyes: Cy2, Cy3, Cy5, SYBR green
- DAPI

C. GFP, Yellow fluorescent protein

D. For a fluorescence microscope, we need:

- A light source
- A set of filters, often comprising a filter cube
- An objective
- A florescently labelled sample
- A detector

E. A filter cube typically contains three filters: an excitation filter, a dichoic mirror and an emission filter.

F. Ion arc lamps, metal halide lamps, LEDs, lasers.

G. Quartz tungsten-halogen lamp has a black-body radiation spectrum (continuous frequency broad spectrum, see Figure 1). A mercury lamp also has a continuous spectrum, but has multiple emission peaks (Fig. 2). Finally, LED sources will emit over narrow bandwidths, typically of 20-50nm (Fig. 3).

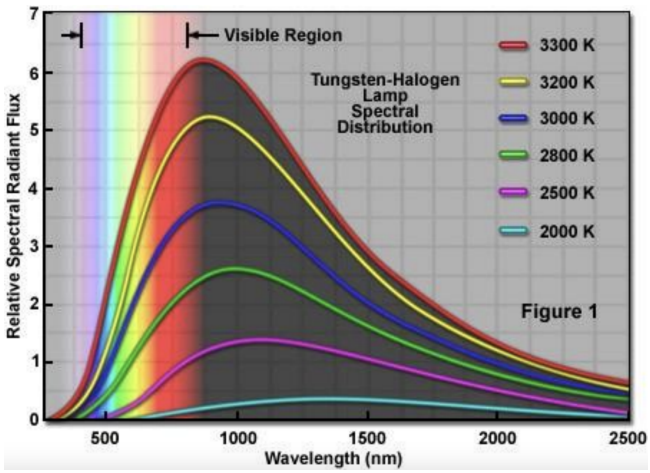

Figure 1: Spectrum of a Quartz tungsten-halogen lamp

3 Quantum dots

A.

$$E = \frac{(6.626 \cdot 10^{-34}\text{J} \cdot \text{s}) \cdot (3 \cdot 10^{8}\text{m/s})}{(700 \cdot 10^{-9}\text{m})} = 2.84 \cdot 10^{-19}\text{J} \approx 1.77\text{eV}$$

B. Given that the frequency $f \propto \frac{1}{\lambda}$ and the energy $E = hf$, Solution 1 has a larger energy and larger frequency.

$$\frac{E_1}{E_2} = \frac{h \cdot c \cdot \lambda_2}{\lambda_1 \cdot h \cdot c} = \frac{\lambda_2}{\lambda_1} = \frac{560 \cdot 10^{-9}}{475 \cdot 10^{-9}} \approx 1.2\text{x larger}$$

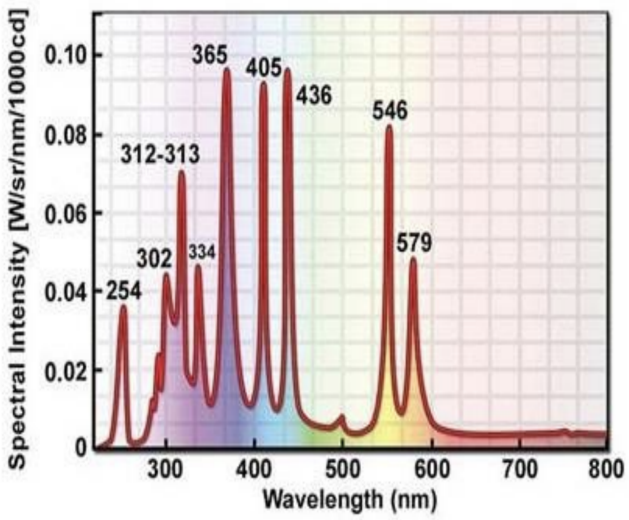

Figure 2: Spectrum of a mercury arc lamp

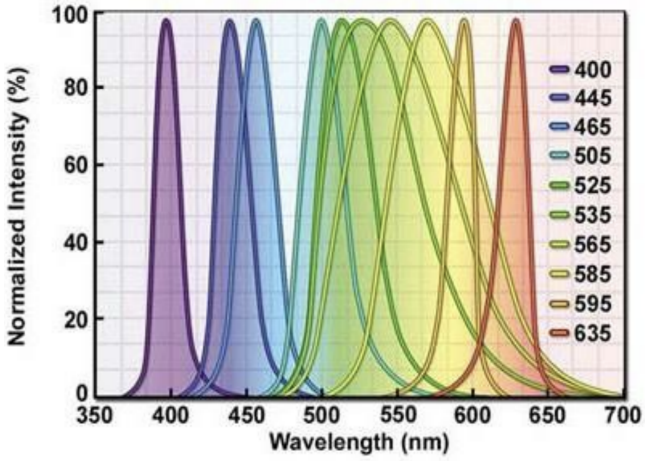

Figure 3: Spectrum of a LED source

4 Jablonski Diagram

A. The excitation for Rhodamine Green starts from the ground state and jumps to the energy state between 2.44 eV and 2.55 eV, which generates a span of the excitation wavelengths. The wavelengths relative to these two energy states are:

$$\lambda_{\text{1RGex}} = \frac{1.24\text{eV} \cdot \mu\text{m}}{E_{\text{1RGex}}} = \frac{1.24}{2.44}\mu\text{m} \approx 508 \text{ nm}$$

$$\lambda_{\text{2RGex}} = \frac{1.24\text{eV} \cdot \mu\text{m}}{E_{\text{2RGex}}} = \frac{1.24}{2.55}\mu\text{m} \approx 486 \text{ nm}$$

Thus the excitation wavelength span lies between 486 nm and 508 nm. We can simply take the average as a peak wavelength which is 497 nm to excite this dye.

Similarly, for CY5:

$$\lambda_{\text{1 CYex}} = \frac{1.24\text{eV} \cdot \mu\text{m}}{E_{\text{1 CYex}}} = \frac{1.24}{1.88}\mu\text{m} \approx 660 \text{ nm}$$

$$\lambda_{2\ \mathrm{CYex}} = \frac{1.24\mathrm{eV}\cdot\mu\mathrm{m}}{E_{2\ \mathrm{CYex}}} = \frac{1.24}{1.96}\mu\mathrm{m} \approx 633\ \mathrm{nm}$$

We can use the average which is 647 nm to excite this dye.

B. The emission for Rhodamine Green starts from the state of 2.4 4eV and jumps back to the energy state between 0.18 eV and ground state, which also generates a span of the emission wavelengths. The wavelengths relative to these two energy states are:

$$\lambda_{1\mathrm{RGem}} = \frac{1.24\mathrm{eV}\cdot\mu\mathrm{m}}{E_{1\mathrm{RGem}}} = \frac{1.24}{2.44-0.18}\mu\mathrm{m} \approx 549\ \mathrm{nm}$$
$$\lambda_{2\mathrm{RGem}} = \frac{1.24\mathrm{eV}\cdot\mu\mathrm{m}}{E_{2\mathrm{RGem}}} = \frac{1.24}{2.44-0}\mu\mathrm{m} \approx 508\ \mathrm{nm}$$

Thus the emission wavelength span lies between 508 nm and 549 nm. We can simply take the average as a peak wavelength for emission spectra which is 529 nm. Similarly, for CY5:

$$\lambda_{1\mathrm{CYem}} = \frac{1.24\mathrm{eV}\cdot\mu\mathrm{m}}{E_{1\mathrm{CYem}}} = \frac{1.24}{1.88-0.11}\mu\mathrm{m} \approx 701\ \mathrm{nm}$$
$$\lambda_{2\mathrm{CYem}} = \frac{1.24\mathrm{eV}\cdot\mu\mathrm{m}}{E_{2\mathrm{CYem}}} = \frac{1.24}{1.88-0}\mu\mathrm{m} \approx 660\ \mathrm{nm}$$

We can use the average which is 681 nm as the emission wavelength.

The spectral widths of Rhodamine Green is $549-508 = 41$ nm;

The spectral widths of CY5 is $701-660 = 41$ nm;