Biomicroscopy I - Exercise Sheet 12-13

December 9, 2025

1 Fluorescence Microscopy

A. How does the spectrum of a quartz tungsten-halogen lamp change with increasing applied voltage? If one needs to dim down its intensity by a factor of 10 without affecting the illumination spectrum, what would you recommend to do?

B. Name a few commonly used types of optical filters in fluroescence microscopy (related to the wavelength response).

C. Calculate the transmission efficiency of neutral density filters with optical density (OD) values of 0.3, 0.6, 0.9, 1.3 and 2.3.

D. Sketch the spectra of a short-pass, long-pass and band-pass optical filters.

E. Sketch the spectra of short-pass and long-pass based dichoic mirrors.

2 Quantum dots

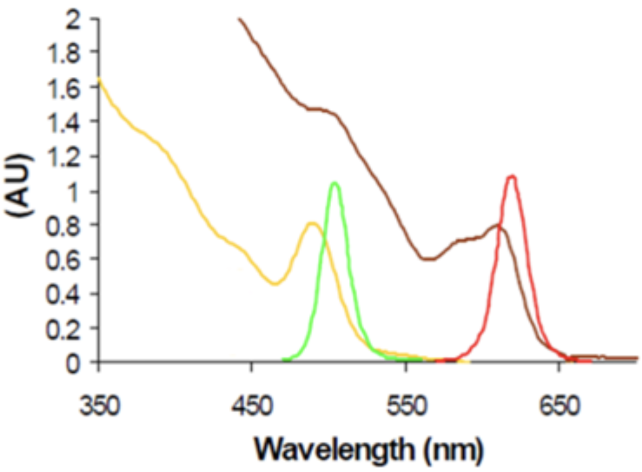

Figure 1: Absorbance and emission spectra of two different sized CdSe-based quantum dots

Figure 1 shows the absorbance and emission spectra of two different sized CdSe based quantum dots, QD_1 and QD_2, to be used in fluorescence microscopy. Here, yellow and green curves are the spectra of QD_1, while brown and red curves are the spectra of QD_2.

A. Out of the four curves, which ones correspond to the absorption spectra?

B. Out of the four curves, which ones correspond to the emission spectra? What are the emission peaks and bandwidths?

C. Which quantum dot has a larger size: QD_1 or QD_2? Explain your reasoning in a few short sentences.

D. What is the maximum suitable wavelength one can use to excite QD_1?

E. What is the maximum suitable wavelength one can use to excite QD_2?

F. What is the maximum suitable wavelength one can use to excite both QD_1 and QD_2 simultaneously?

3 Jablonski diagram

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

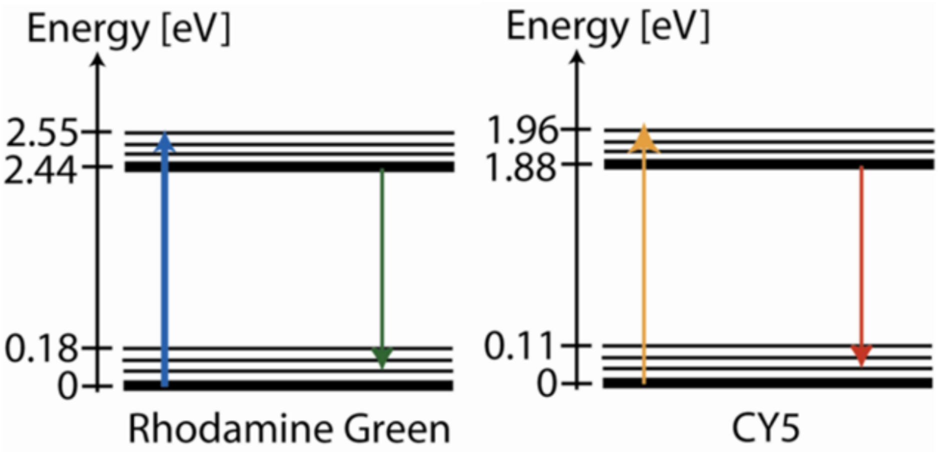

Figure 2: Jablonski diagram of Rhodamine and Cyanine (CY5). Note that energy axes have different scales.

Draw the spectral characteristics of a suitable excitation and emission filter set that you can suggest for separating the corresponding spectra of each fluorophore in Figure 3: Rhodamine in left and Cy5 in right.

4 Filter sets for fluorescence microscopy

Assume a fluorescence microscope with a sample containing fluorescein molecules as seen in the lectures and having excitation and emission spectra shown in Figure 3 (a). The suggested filter set to use can be seen in Figure 3 (b).

A. According to Figure 3b, write write down the types of filters used for excitation, emission and dichroic mirror. Indicate their spectral characteristics (*e.g.* cut-off/cut-on/center wavelengths, bandwidth).

B. Is this the only possible filter set configuration we could use?

C. Do you think the fluorescence microscope will function if we use a SP500 filter as for excitation? Note that SP stands for short-pass.

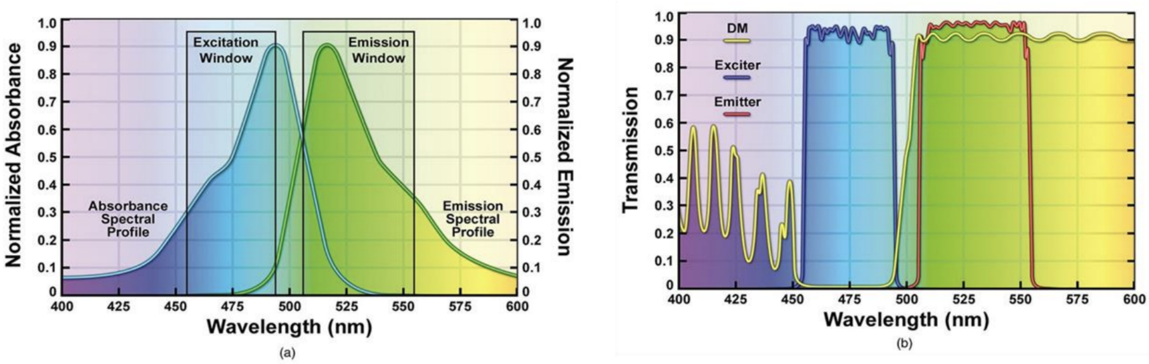

Figure 3: **(a)** Absorption and emission spectral profile of fluorescein. **(b)** Spectra of the suggested filters

D. Do you think the fluorescence microscope will function if we skip using the suggested emission filter?

E. Do you think the fluorescence microscope will function if we use a LP500 filter as for emission? Note that LP stands for long-pass.

F. If you have a spare blue LED source available with 475nm center wavelength and 35nm bandwidth, how could you use it in your microscope? Should you still use a BP475nm if you choose LED as excitation source? Note that BP stands for band-pass.

5 Commonly used optical microscopes in biology

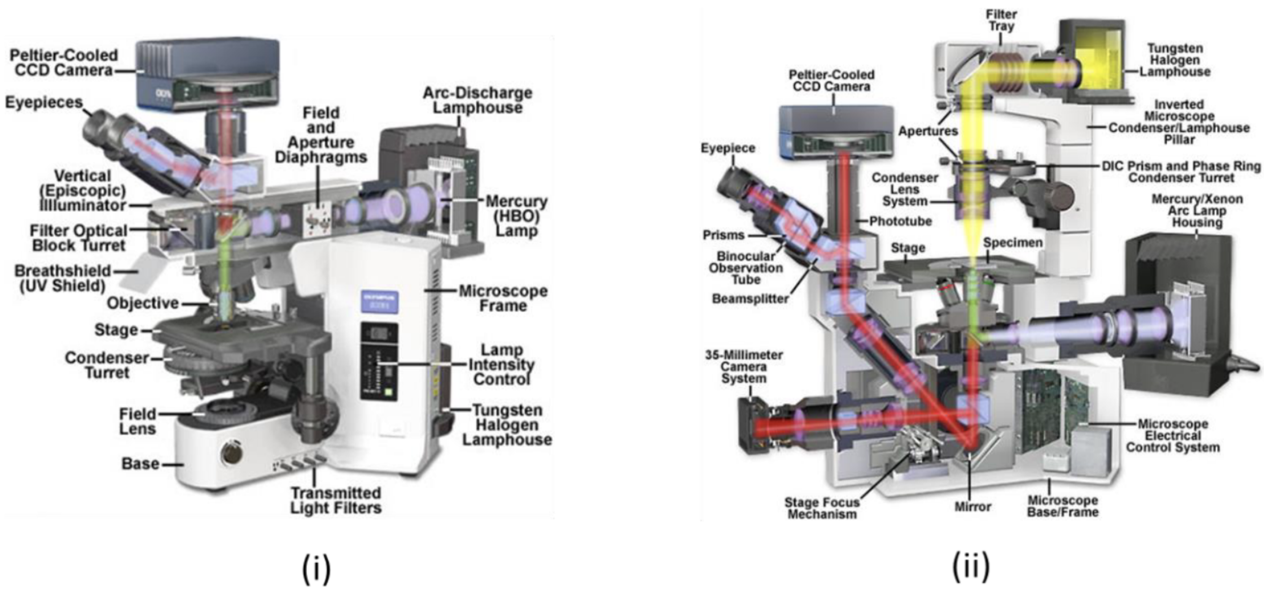

Figure 4: Cross-sectional scheme of two commonly used microscopes

A. Determine the upright and inverted microscope out of these two.

B. For each microscope type, trace the optical pathway for bright-field microscopy in transmission configuration: start from illumination, next to the sample, and then to detector/eye. What is the bright-field microscopy illumination source? Pay attention to other optical components that you encounter along the pathway (filters, apertures, detectors etc).

C. For each microscope type, trace the optical pathway (start from illumination, next to sample, and then to detector/eye) for fluorescence microscopy. Is it in reflection or transmission configuration? What is the fluorescence microscopy illumination source? Pay attention to other optical components that you encounter along the pathway (filters, apertures etc).

6 Selecting filters and illumination for fluorescence microscopy

Fluorescent proteins and Alexa Fluor dyes are frequently used in molecular and cellular biology. The efficiency of exciting a fluorophore is dependent on the selection of the right fluorescence filter sets and light source. We offer in the Annex specifications for two different types of lasers and three LEDs and introduce a table with different filters from Thorlabs (an optics company) in Figure 6. The emission and excitation spectra of two types of fluorescent proteins and Alexa Fluor dye are shown in the Figure 5.

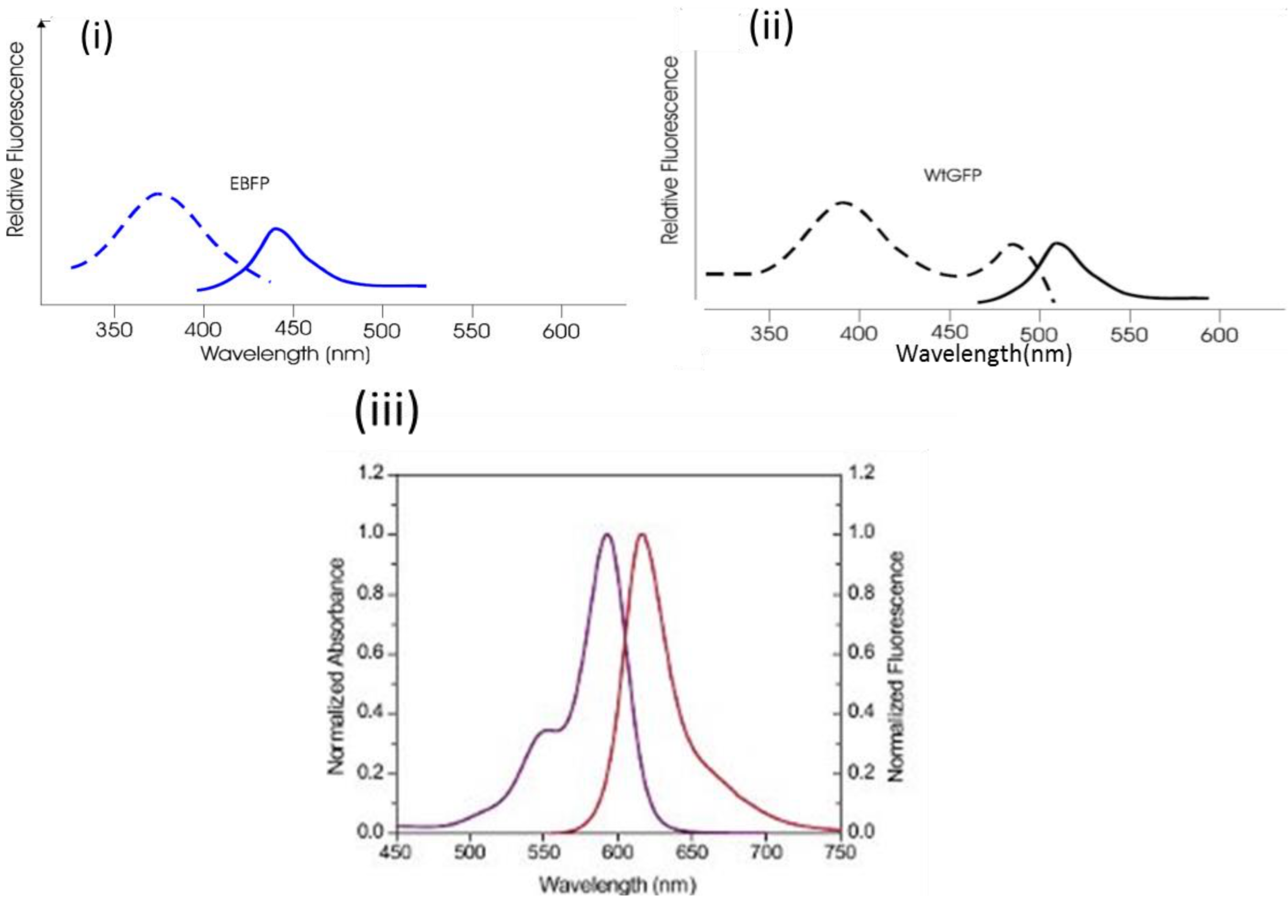

Figure 5: Excitation and emission spectra of (i) EGFP, (ii) WtGFP and (iii) Alexa Fluor 594

A. What is the Stokes shift of the Alexa Fluor 594 dye?

B. The Annex shows a datasheet of several different light sources, *e.g.* supercontinuum fiber laser from Fianium, single wavelength lasers form Dynamic Laser, and LEDs from Thorslabs. Which light source you would choose to excite each of these fluorophores?

Item #	Filter Type	Center Wavelength	FWHM	Reflection Band	Transmission Band	Transmission Data[a]
MF390-18	Excitation	390 nm	18 nm	-	-	
MF460-60	Emission	460 nm	60 nm	-	-	
MD416	Dichroic	-	-	360 - 407 nm	425 - 575 nm	
MF434-17	Excitation	434 nm	17 nm	-	-	
MF479-40	Emission	479 nm	40 nm	-	-	
MD453	Dichroic	-	-	423 - 445 nm	460 - 610 nm	
MF445-45	Excitation	445 nm	45 nm	-	-	
MF510-42	Emission	510 nm	42 nm	-	-	
MD480	Dichroic	-	-	415 - 470 nm	490 - 720 nm	
MF469-35	Excitation	469 nm	35 nm	-	-	
MF525-39	Emission	525 nm	39 nm	-	-	
MD498	Dichroic	-	-	452 - 490 nm	505 - 800 nm	
MF475-35	Excitation	475 nm	35 nm	-	-	
MF530-43	Emission	530 nm	43 nm	-	-	
MD499	Dichroic	-	-	470 - 490 nm	508 - 675 nm	
MF497-16	Excitation	497 nm	16 nm	-	-	
MF535-22	Emission	535 nm	22 nm	-	-	
MD515	Dichroic	-	-	490 - 510 nm	520 - 700 nm	
MF542-20	Excitation	542 nm	20 nm	-	-	
MF620-52[b]	Emission	620 nm	52 nm	-	-	
MD568	Dichroic	-	-	525 - 556 nm	580 - 650 nm	
MF565-24	Excitation	565 nm	24 nm	-	-	
MF620-52[b]	Emission	620 nm	52 nm	-	-	
MD588[c]	Dichroic	-	-	533 - 580 nm	595 - 800 nm	
MF559-34	Excitation	559 nm	34 nm	-	-	
MF630-69	Emission	630 nm	69 nm	-	-	
MD588[c]	Dichroic	-	-	533 - 580 nm	595 - 800 nm	

Figure 6: Spectral information of available filters

C. Assume now that you use excitation sources which have the spectral bands overlapping perfectly with the excitation spectra from fluorophores (different sources for different fluorophores). In the table in Figure 6, you will find different filter choices. Which filter set is the most appropriate to be used to detect the fluorescent signal for each fluorophore?