Analysis Questions:

- 1. Considering the Jablonski diagram...
 - a. Draw in and label energy flow for photon absorption, vibrational relaxation, fluorescent emission, and non-radiative decay.
 - b. What is the wavelength of emitted light?
 - c. What is the wavelength of light to excite the second electromic state?
 - d. If the fluorescent lifetime is 1 ns, what is the rate of photon emission?
 - e. If the non-radiative decay has a lifetime of 3 ns, what is the quantum yield?
 - f. Why is the triplet state so much longer lived as compared to fluorescent emission rate?



- 2. Consider the following image and structured element.
 - a. Draw in the 1/0 of a dilated image
 - b. Draw in the 1/0 of an eroded image
 - c. What will happen to in image of cells if you perform an opening with a structured element larger than every cell?

Image													Strel		
0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1 1
0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1 1
1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	
1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	
0	1	1	1	1	0	0	0	0	0	1	1	1	1	1	

3. Fluorophores

- a. What are 4 important properties of organic dye fluorophores to consider?
- b. What are 3 additional properties of fluorescent proteins to consider?
- c. For the following spectra, what are 2 potential fluorophores for each?
- d. What is the energy lost (in eV) to vibrational relaxation for each?
- e. What are two environmental factors that will tune the properties of each?



4. Filters:

- a. What are the wavelengths passed by a 490/50 filter?
- b. If the filter blocks other wavelengths with an OD 3, what percentage of photons of get through?
- c. Why are excitation filters typically less expensive than emission filters?
- d. Is a dichroic mirror needed for trans-illumination fluorescence?

Matlab Questions:

- 1. Open up blobs.png.
 - a. Isolate all the horizontal lines > 8 pixels. Plot a histogram of lengths of the horizontal lines.
 - b. Isolate all objects with circles > 6 pixel radius. Plot a histogram of the area of the objects
- 2. Open printedtext.png. Correct for the background illumination and noise. See if you can write the entire text.