

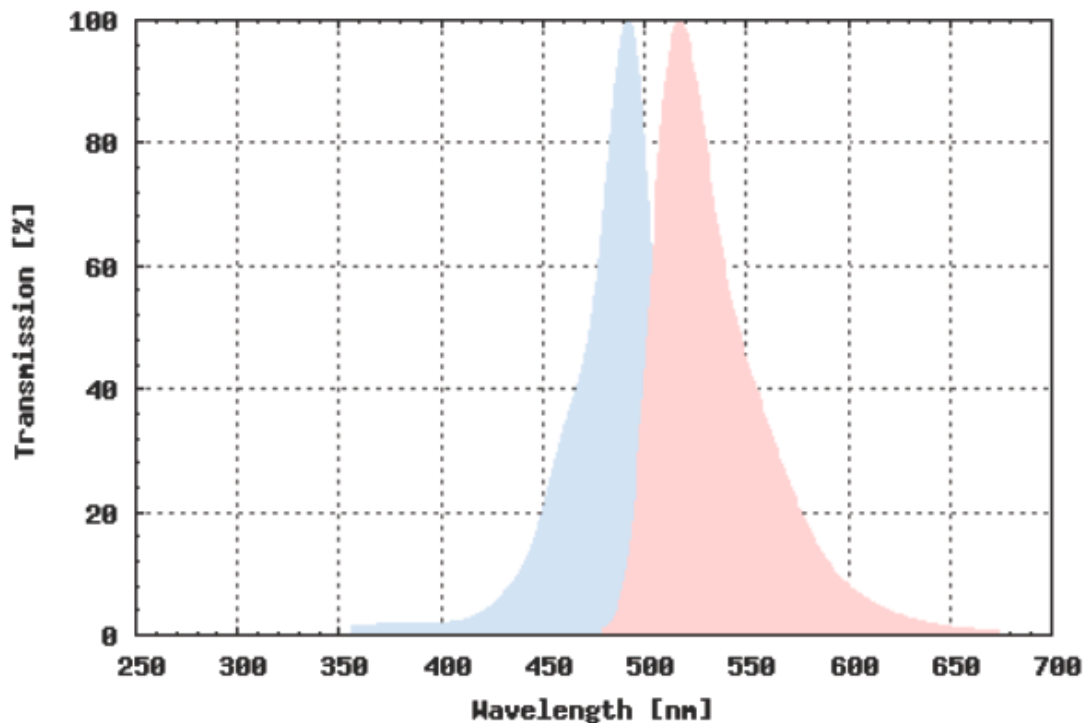
Exam 4, Part B – Bioimaging (Open Book)

Name: _____

Points: _____ (Maximum 6)

You may use a calculator. You may use lecture notes, books, etc. Work alone. Recommended time for this part of the exam: 50 minutes. Total time for both parts of the exam: 90 minutes or 10 after the first person turns in the second part of the exam, whatever comes first.

1. (2 points) You are interested in selecting filters and a dichroic mirror for an epifluorescent microscope that will be used to detect the presence of 5-carboxyfluorescein (5-FAM) in HeLa cells. The excitation and emission spectra are given below. Draw a diagram of an epifluorescent microscope. Draw hypothetical transmission profiles of the filters that you wish to purchase. Make this drawing similar to those provided by a commercial manufacturer (e.g. Omega Optical). How will you determine the fraction of excitation and emission photons will be lost at each filter and dichroic?



2. (2 points) (a) What are the smallest dimensions of an object containing 5-FAM that can be visualized using a microscope equipped with a 60X, NA=1.4, oil-immersion objective? Assume that the index of refraction for the oil used on the sample is 1.5. (b) In a confocal approach, what would be the z-resolution for this system? (c) If a CaCo₃ cells is 8- μ m thick, how many confocal sections must be taken in order to fully cover the entire cell thickness? (d) In selecting a CCD camera to visualize subcellular structures close to the x-y resolution of the microscope, what is the maximum pixel size that can be used (Hint: remember Niquist).

3. (2 points) The specifications for two CCD cameras, C9100-01 and C9100-11 are given below. (a) How many 5-FAM photons per pixel are needed to obtain a $S/N > 5$ for each camera? Assume that readout noise at minimum gain is the limiting factor. Take into account the quantum efficiency of the camera. (b) Given the full well capacity of this camera, how many electrons correspond to each ADU in each camera? How many photons correspond to this ADU in each camera? (c) What would be the quantum noise associated with the detection of 20,000 photons? (d) Would this camera be adequate to image objects containing 5-FAM that have dimensions close to the resolution limit?