

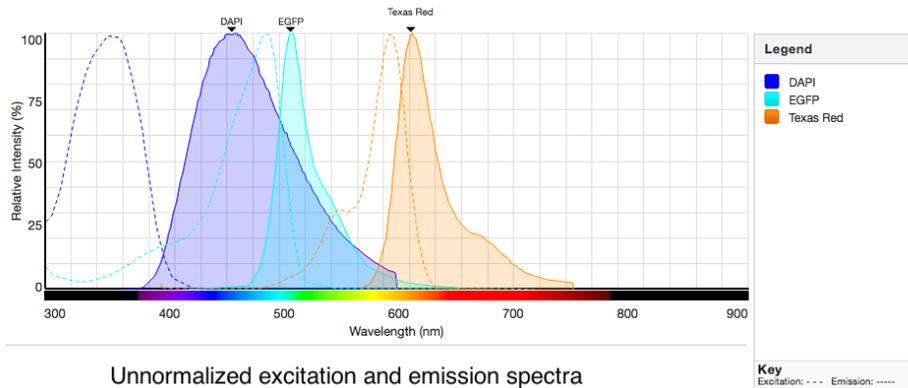
Name _____

Physics 390 In-Class Activity 6

Designing a Multi-color Imaging Experiment

Successful multi-color imaging requires generating a robust signal from each fluorophore and avoiding significant contamination of a signal from one fluorophore by signals from the other fluorophores. In the case of laser-based illumination, this involves choosing appropriate laser lines and emission filters and choosing between options that include simultaneous and sequential imaging.

Consider a sample labeled with the three fluorophores, DAPI, EGFP, and Texas Red. The excitation (dashed lines/unfilled areas) and emission (solid lines/filled areas) spectra for these three fluorophores are shown below. To generate robust signals, you want to choose a laser line that overlaps well with each fluorophore's excitation spectrum. Similarly, you want to choose an emission filter that will pass a significant fraction of each fluorophore's emission with (hopefully) minimal signal contamination from other fluorophores.



Your laser line options are:

405 nm, 458 nm, 488 nm, 514 nm, 561 nm, and 633 nm.

Your emission filters options are:

350/50 nm, 450/50 nm, 490/50 nm, 525/50 nm, 590/50 nm, and 615/50 nm.

Note: The notation 350/50 nm means that the filter passes EM radiation with wavelength ranging from 325 to 375 nm (i.e., 25 nm on either side of 350 nm).

a) Choose the best excitation line for each of the three fluorophores and enter your choice into the table.

Note: DAPI is the hardest to excite with these laser lines, but one choice will work with low efficiency.

Fluorophore	Excitation Line	Emission Bandpass
DAPI		
EGFP		
Texas Red		

b) Choose the best emission filter set for each fluorophore and enter your results into the table.

c) In general, fluorophores in multi-color samples are imaged sequentially. Sequential imaging involves directing only one laser line onto the sample at a time, collecting the resulting fluorophore emission, and then repeating this process for the other fluorophores. For living samples, it can be preferable to image fluorophores “simultaneously,” which involves using multi-laser excitation and then collecting the emission simultaneously using a different detector for each fluorophore. Avoiding significant contamination of a signal from a fluorophore by signals from other fluorophores is much more difficult to achieve with simultaneous imaging, which is one reason this approach is less commonly used.

For each fluorophore combination below, determine if simultaneous and/or sequential imaging is viable with your filters and place a “check mark” in the table when the method is an option.

Fluorophore	Simultaneous	Sequential
DAPI/EGFP		
DAPI/Texas Red		
EGFP/Texas Red		
DAPI/EGFP/Texas Red		