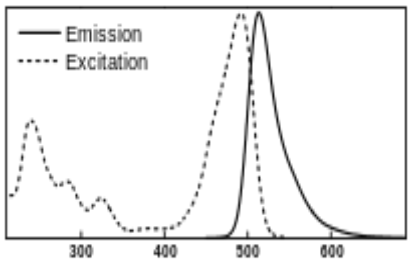
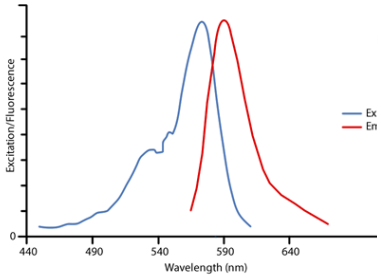
Microscopy Lab

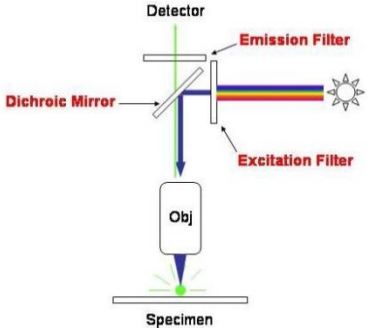
1. Does zooming improve the resolution of the image? What does it change?

   Zooming does not increase the resolution of the image. Zooming gives a magnified image.

2. A sample is co-stained with fluorescein and Texas red. Choose the right excitation source, dichroic beamsplitters and emission filters for detecting the fluorescence signal from each of the two dyes. Explain your selection.







   Available light sources: 488nm, 520nm, 543nm, 633nm, 690nm, 800nm

   Available dichroic beamplitters-cut-off wavelength: 450nm, 490nm, 500nm, 520nm, 590nm, 620nm, 650nm

   Emission filters: 480 +/- 40 nm, 490 +/- 10nm, 520 +/- 40nm, 550 +/- 20nm, 580 +/- 40nm, 600 +/-20nm, 630 +/- 10nm

A. 488nm, 490nm dichroic, 520 +/- 40nm bandpass filter.
B. 543nm, 590nm dichroic, 600 +/- 20nm bandpass filter

   The excitation wavelength needs to match the absorption spectrum of the dye. The dichroic mirror needs to have a cut-off frequency larger than the laser wavelength but smaller than the emission of the dye. The bandpass filter should allow no excitation wavelength to pass through while covering the emission spectrum as much as possible.

3. Recall the images taken with different pinhole sizes and explain how the pinhole affects the image quality. How do the pinhole sizes affect the image quality of a two-photon excited fluorescence microscope? Why?

   Reducing the pinhole size improves resolution but also reduces the amount of signal reaching the detector. There is an optimum pinhole size corresponding to maximum resolution that can be attained while allowing sufficient signal to reach the detector. There is usually no pinhole involved in two-photon excited microscopy (give extra credit if there is any reference to a

potential improvement in resolution when a confocal pinhole is used in two-photon microscopy).

4. Compare the two photon excited fluorescence (TPEF) microscopy to confocal fluorescence microscopy in terms of optical sectioning, laser sources used, resolution, penetration depth and photodamage. What is the main benefit of these technologies related to their application? What is the main difference between these microscopy techniques related to their application?

   Optical sectioning: confocal fluorescence microscopy uses a pinhole to attain optical sectioning, while in TPEF microscopy this is provided through the TPEF effect in the process of laser-tissue interaction.

   Laser sources: Confocal fluorescence microscopy uses a CW laser source while the TPEF microscopy uses a femtosecond pulse laser source.

   Resolution: Confocal fluorescence microscopy provides higher resolution due to the shorter wavelength used compared to TPEF microscopy

   Penetration depth: TPEF microscopy provides enhanced penetration depth in scattering samples due to longer wavelength used compared to confocal fluorescence microscopy.

   Photodamage: Confocal fluorescence microscopy is more prone to photodamage compared to the TPEF microscopy due to the shorter wavelength used (often in the UV range).

   Main benefit related to application: Both microscopy techniques provide optical sectioning, thus they are suitable for imaging thick tissue samples.

   Main difference related to application: Stained samples are usually used in confocal fluorescence microscopy, while endogenous fluorophores in unstained samples can be excited in TPEF microscopy due to reduced photodamage.