Microscopy Lab Questions

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

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 beamsplitters-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
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?

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?