1) Compare time-correlated single photon counting (TCSPC) and time-gating methods of fluorescence lifetime imaging microscopy (FLIM) methods. Note differences in detectors, data analysis, photon efficiency, and imaging speed.

2) Briefly describe the key difference(s) between time-domain FLIM and frequency-domain FLIM.

3) Above are 3 images: (left) phasor plot; (right, top) fluorescence intensity image; (right, bottom) fluorescence lifetime image. Please answer the following questions.

   a) What is plotted in the phasor plot?
   b) Is the lifetime decay of the fluorescence in these cells single-exponential or multi-exponential? Or, is there not enough information presented to answer. Briefly, explain your answer.
   c) The intensity image seems to be brighter in the cell nucleus, especially for the cell on the right in the images. However, the lifetime image of the same cell seems to be fairly uniform. Why might that be?

4) When using FLIM to measure FRET, would one measure changes in the donor lifetime or the acceptor lifetime? Why?

5) Consider two proteins that can interact with one another expressed in a cell. One wishes to measure the change in the fraction of proteins that interact in a particular subcellular location by measuring changes in FRET efficiency. Explain briefly how this is done using both intensity-based measurements and lifetime-based measurements. Is FLIM more accurate for measuring FRET efficiency? Why or why not?