

HW6

DUE: 10/27/2017 – 11:30 AM

MCDB 4312/5312

Analysis Questions:

1. Consider a single molecule
 - a. What sets the limits on the position uncertainty when fitting to the PSF?
 - b. Look up how many photons a GFP can emit before photobleaching?
 - c. Roughly how many could you detect on your camera?
 - d. What is the ideal uncertainty for a GFP?
 - e. If you are using a 60x, NA 1.45 objective on a camera with 6.5 μm pixels with no background, what is the uncertainty limit?

2. Assuming 620 nm light imaged with an objective with NA 1.2:
 - a. What is the widefield lateral diffraction limit?
 - b. What is the scanning confocal lateral diffraction limit?
 - c. What is the axial scanning confocal diffraction limit?
 - d. What is one disadvantage of confocal microscopy as compared to widefield?

3. Assuming a scanning confocal experiment:
 - a. If the dwell time per pixel is 2 μs , how long would it take to build up a 512 x 512 image?
 - b. If you detect 100 photons per pixel, what is the minimum number of GFP molecules in that spot (hint: use the lifetime and QY to calculate a maximum rate).

4. TIRF
 - a. What is the minimum numerical aperture to set up TIRF in air?
 - b. “ “ in water?
 - c. What is the steepest angle you can send in light with a 1.49 NA objective?
 - d. What is the penetration depth for 630 nm light going into cells at that angle?
 - e. At a distance of 500 nm, what is the relative intensity to right at the coverslip?

5. Geometric operators:
 - a. What is the difference between a mean filter and median filter?
 - b. In an edge filter, what are the two components necessary to call edges?
 - c. Briefly describe the process used by a LoG filter, and why step 1 is needed.

Matlab Questions:

6. Functions

- a. Write a function to align frames in a movie. The input should be a 3D matrix, and the function should align frames 2:end with the first frame. The output should be an 3D matrix with the aligned images. Send me your alignment.m function with your email.
- b. Run your alignment function on drift_movie.tif.
- c. Take a mean across time of both the unaligned movie and the aligned movie, and display them.