HW7
DUE: 11/17/2017 – 11:30 AM
MCDB 4312/5312

**Analysis Questions:**

1. Considering the differences between PALM and STORM:
    a. What sets the ultimate resolution limit for reconstruction microscopy?
    b. Which technique gives higher resolution, and why?
    c. Which technique would you choose for live cell imaging?

2. Considering a SIM experiment:
    a. What is the physical principle that enables high frequency capture?
    b. What is the ideal improvement in resolution?
    c. Why are 9 images required to reconstruct the SIM image?
    d. What happens if a single image (of the 9) is corrupted by motion?

3. Considering a STED experiment
    a. What sets the ultimate resolution limit in STED?
    b. Assuming you're illuminating Atto647N, what is the widefield diffraction limit at an NA of 1?
    c. What about if you illuminate with a STED beam at 100x the saturation intensity?
    d. " " at 1000x the saturation intensity?

4. Read the minflux paper:
    a. What enables tracking ribosomes at such a high precision for such long periods of time?
    b. Why do they suggest ~6 nm resolution is the best that anyone can do with fluorescence?

**Matlab Questions:**

5. Single molecule localization
    a. Load in the .tif file from the movie. In each frame there is 1 single molecule that is moving. During each frame, the molecule can move ONLY in the x direction, ONLY in the y direction, or stay still.
    b. For each frame use the Gaussian fit to identify sub-pixel localization (ie, get an x-y value for the center of the SM).
    c. For each frame, calculate the distance the particle moved in the x and y direction.
    d. Make a histogram for the x-steps, and the y-steps.