Assignments Matlab - single molecule localization

Goal

In this assignment we will learn how to apply Maximum Likelihood Estimation for finding the position of single molecule emitters.

Background

The image of a single molecule emitter is the PSF of the imaging system centered at the emitter location and distorted by noise. Suppose the image of the molecule is captured on a square Region Of Interest (ROI) of $N_p \times N_p$ pixels (typically N_p between 7 and 17 will do). The measured pixel values are n_k, where the index k takes values between 1 and N_p^2. We will try to model these images with a simplified Gaussian PSF:

$$\mu_k = \frac{N}{2\pi\sigma^2} \exp\left[-\frac{(x_k - x_0)^2 + (y_k - y_0)^2}{2\sigma^2} \right]$$

where (x_k, y_k) are the center coordinates of the pixels, and where the unknown parameters are the emitter coordinates (x_0, y_0), the width of the spot σ, and the photon count N. Maximum Likelihood Estimation (MLE) gives the values for these unknowns as (see Lecture slides):

$$N = \sum_k n_k, \quad x_0 = \frac{1}{N}\sum_k n_k x_k, \quad y_0 = \frac{1}{N}\sum_k n_k y_k,$$
$$\sigma^2 = \frac{1}{2N}\sum_k n_k \left[(x_k - x_0)^2 + (y_k - y_0)^2 \right]$$

The uncertainty of the position estimation for the coordinates appears to be:

$$\Delta x_0 = \Delta y_0 = \frac{\sigma}{\sqrt{N}}$$

We will apply these formulas on simulated images to test the prediction for the localization uncertainty. The key assumption of the MLE fitting procedure is that the only noise source is shot noise, i.e. the distribution of photons over the image is taken to follow Poisson statistics.

Task 1

We first turn to different ways to model noisy images using the command “imnoise”. You can generate a test image by “image = sum(im2double(imread('peppers.png')),3);”. Suppose that the total number of photons captured by the detector and converted to electrons is N. Write a code to simulate images on the detector corrupted by Poisson-noise for $N = 5\times10^4$, $N = 5\times10^5$, $N = 5\times10^6$, and $N = 5\times10^7$. Simulated noise is added by the line “noisy_image = 1e12*imnoise(1e-12*nonoise_image,`poisson’)”. What do you expect that these images will look like? Does this expectation hold true?

Task 2

Now look at “Examplescript_6p2.m” on blackboard. Simulated images of single emitters are stored in the file “emitterPSFs.mat” and read in. Copy this file from blackboard to your directory as well before running “Examplescript_6p2.m”. The images are simulated using the vectorial theory taking effects of high NA and polarization into account. So, the actual PSF is different from the Gaussian PSF that is assumed in our MLE fitting model. How many images are there? And how many pixels are there in each image? Look at the Matlab workspace after you run the code to get the answer. A first step is to make images corrupted with Poisson-noise corresponding to a range of photon count values between 10^2 and 10^4, according to the recipe developed in Task 6.1. The next step is to find values for the unknowns (x_0, y_0, σ, N) for all of the simulated noisy images using the solutions following from the MLE-algorithm. Calculate the standard deviation of the localization error x_0-x_{true} (and likewise for the y-coordinate) over all images for each value of the photon count using the command “std”. The “true” values of the emitter coordinates are also read in from the “emitterPSFs.mat” file. How well does the result fit with the prediction for the localization uncertainty?

Task 3

Modify your code by adding a constant background B to all images before applying the “imnoise” command to introduce Poisson-noise. What happens as a function of B? Try values for B ranging from 0 to 30 photons per pixel.