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University of Oregon; Fall 2024

Physics 410/510 Image Analysis: Homework 4

Partial – there will be more! (And the numbering may change!)

Due date: Wednesday October 23 by 11:59 pm, submitted through Canvas. You'll see in "Assignments" a place to submit a **PDF**.

Reading: All PDFs are on Canvas in \Readings

- [optional] This describes how to make a simulated image of a point source, similar to what we discussed in class: The first few sections of Materials and Methods, page 2 through "noise model" on page 3, of: M. K. Cheezum, W. F. Walker, W. H. Guilford, "Quantitative comparison of algorithms for tracking single fluorescent particles," *Biophys J* **81**, 2378-2388 (2001). https://www.cell.com/biophysj/fulltext/S0006-3495(01)75884-5 PDF: cheezum guilford tracking algorithms 2001.pdf
- [Very Optional, for those who want more a detailed treatment of the optics of the point spread function.] A few pages on point-spread-functions: Chapter 3, through "circular lenses." M. Gu, *Advanced Optical Imaging Theory*, Springer, 1999. A PDF is posted: "Gu Chapter 3 through 3p2p1 PointSpreadFunction.pdf". **Note** that λ here is not the free-space wavelength, but the wavelength in the medium: λ_{free}/n.

Upcoming, in case you want to get an early start:

- Sections 1-3 of Parthasarathy and Small, "Superresolution Localization Methods," *Annu. Rev. Phys. Chem.* **65:**107-125 (2013). (*http://www.annualreviews.org/doi/abs/10.1146/annurev-physchem-040513-103735*)
- R. J. Ober, S. Ram, E. S. Ward, "Localization Accuracy in Single-Molecule Microscopy," *Biophys. J.* **86**, 1185–1200 (2004). *https://www.cell.com/biophysj/fulltext/S0006-3495(04)74193-4*
- [Optional] Regarding super-resolution and the challenge of finding objects, you might like this video, *http://www.ibiology.org/ibioseminars/cell-biology/xiaowei-zhuang-part-1.html*, which gets at the methods described in the first section or two of the Parthasarathy and Small paper.

1 Quantized aggregates. (8 pts.) Download "emitters_33px_100ph.png," a simulated image of 100 point-sources arranged on a grid. Each emitter is located at a multiple of (33, 33) pixels from the top left. Imagine that these are images of single molecules, a protein linked to green fluorescent protein for example. You want to know: do these proteins exist as monomers (one unit), dimers (two units), trimers (three units), ..., or a combination of these? In other words, is the brightness of the dots quantized, and if so, how many quanta are there per dot? Is this the same for all of them? Your task is to figure this out from the image.

(a) Clearly explain each step of your assessment in addition to stating the answer. (Pasting code without explanations will get zero points.) Except for the placement on a grid, this is a very "real-world" problem – in fact, it was inspired by a graduate student talk I attended!

(b) Show a histogram of the intensities of the molecules. (Why? Think about this, or it may become obvious after making it.)

(c) Of the 100 molecules, how many do you think are monomers, dimers, trimers, ...?

Notes:

(*1*) You're only interested in brightness for now, so this problem doesn't require any of the localization concepts we're about to learn – in fact, I could have assigned it last week.

(*2*) There are, of course, background intensities of various sorts that you'll have to figure out how to deal with. Take the spacings, multiples of (33, 33) px as a given, though.

(*3*) The same sort of image but without any background or noise except photon noise, and with brighter molecules, is at "emitters_33px_1000ph_noNoise.png.". ***Suggestion:*** Try your analysis on this first! You should find 69 monomers, 20 dimers, 11 trimers, and nothing higher.

(*4*) I am deliberately not spelling out how to solve this – just like in real life, you'll have to figure it out! You have all the tools in hand...

(*5*) If you're confused, work backwards: Suppose you had an image with 50 monomers and 50 dimers. What would it look like?

2 A high-resolution PSF. (4 pts.) Write a function that calculates the point spread function, outputting it as an N x N array as a function of input parameters: N, the wavelength of light, the numerical aperture, and the pixel scale (i.e. the distance in the focal plane that each pixel corresponds to.) You'll use this function in various exercises in this and future problem sets, so make it a good one! Note that for a circular lens or aperture, the point spread function

$$PSF = 4\left(\frac{J_1(v)}{v}\right)^2$$

where:

- J_1 is a Bessel function of the first kind of order 1 (see note below)
- $v = (2\pi/\lambda)\,\mathrm{NA}\,r$
- λ = free-space wavelength of light
- NA = the numerical aperture
- r = position in the focal plane (in the same units as λ, of course)

Submit three images (i.e. three PSF arrays), each spanning approximately 1 μm x 1 μm: **(i)** Use λ = 0.5 μm (green light), NA = 0.9, N = 101; **(ii)** the same as (i), but λ = 0.4 μm (blue light); **(iii)** the same as (ii), but NA = 0.5.

Also submit the code for your function.

Notes:

- J_1 in Python can be calculated with the scipy package: `scipy.special.j1` . The whole `scipy` package is too large to import, so use `import scipy.special as scipy_special` and `scipy_special.j1(`v`)` . In MATLAB, $J_1(v)$ is `besselj(1,`v`)`.
- For $v \rightarrow 0$, $J_1(v) / v \rightarrow 0.5$, so PSF($v$ == 0) = 1.

Suggestion: First make an N x N array of distances from the center (r), then make an array of v values, and then calculate the PSF array.

Please see the "Additional Notes" document!

3 A worse worm image. (4 pts.) Download from Canvas "fetter_Celegans_cellfig10.jpg", shown below, a transmission electron micrograph of a slice of a *C. elegans* embryo, from Rick Fetter and Cori Bargmann (source: http://www.wormbook.org/chapters/www_intromethodscellbiology/intromethodscellbiology.html). Note the scale bar. Create an image that shows what the slice would look like if imaged with visible light (e.g. wavelength 0.53 μm) rather than electrons. Use your PSF function from Problem #2, and NA = 0.7. Don't simulate noise or pixelation, just consider the consequences of diffraction (i.e. the wave-nature of light). Submit the simulated optical image.

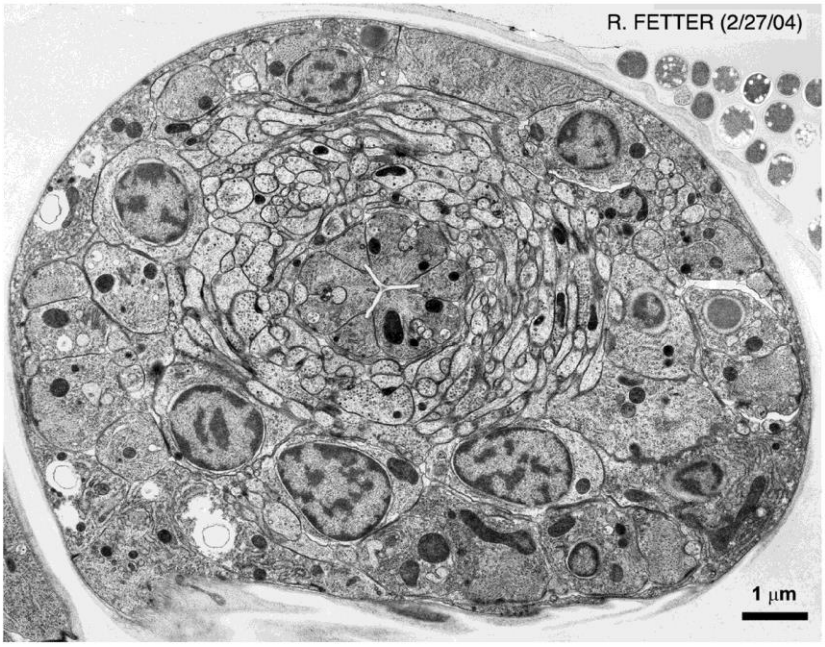

***Figure**. A TEM image of a slice of a C. elegans embryo. Source: Rick Fetter and Cori Bargmann.*