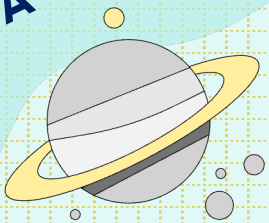
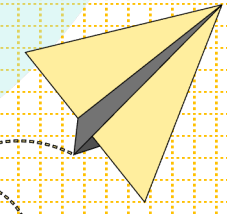
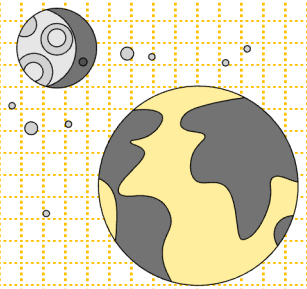
COMPUTATIONAL IMAGING

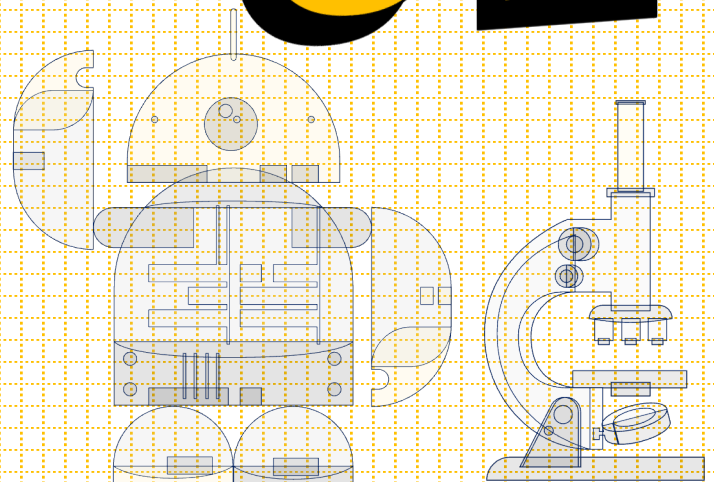






ACTIVITY

02 GAUSSIAN maximization

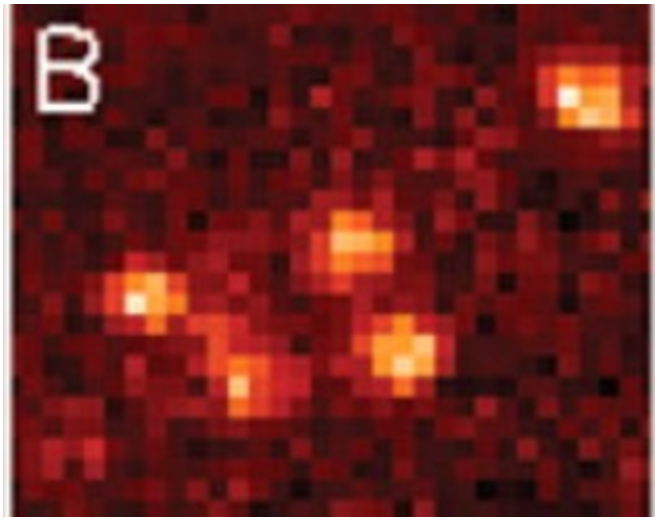


SUBMITTED BY:
RENE L.PRINCIPE JR.

SUBMITTED TO:
DR. MARICOR N. SORIANO

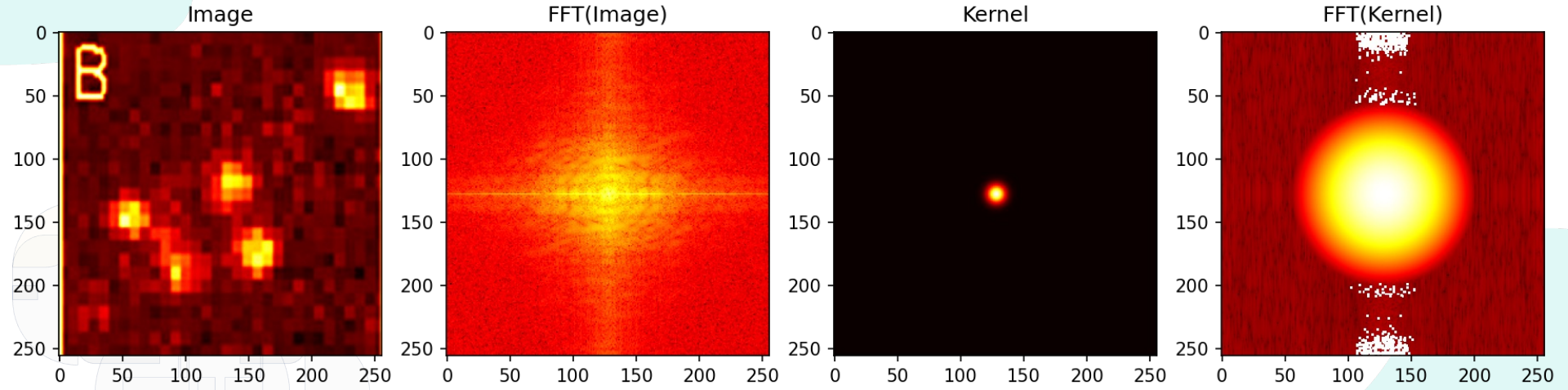
Background

Photoactivated Localization Microscopy (PALM) is an imaging technique that aims to improve understanding of biological dynamics and structures on a smallest level possible, that is beyond the diffraction limit. Fluorescent samples are photoactivated to facilitate its localization by fitting a gaussian. Repeating the localization process multiple times, the collection of the location information constitutes the super-resolving the image.



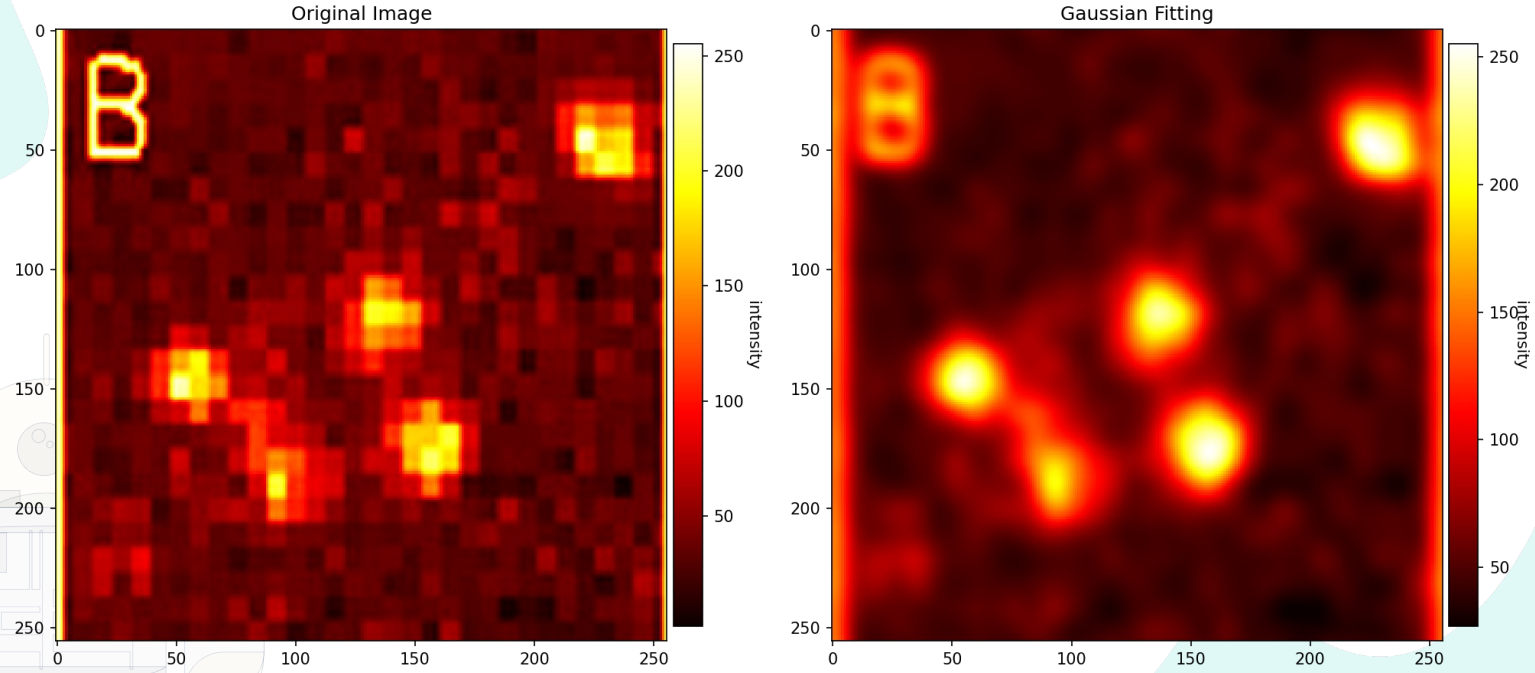
Overview

Typically, gaussian fitting algorithms does the job to localize the peaks, however, I wanted to the test if a good old Fast Fourier Transform (FFT) correlation technique can do the job. Essentially, it is a template matching technique that finds the similarities between a template kernel (i.e. a gaussian function) and the test image (activated cells), hence the correlation. Shown below are the test image, the gaussian kernel, and their corresponding FFTs.



Overview

FFT correlation exploits the complex conjugates of the frequency domain between the two images, returning a correlation map that emphasizes the regions in the test image that closely reassembles the template kernel. From these smoothened image, localization can be done by setting a threshold or through centroid detections.



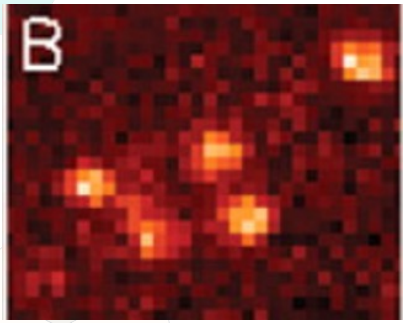
FFT Correlation

Mathematically, the correlation p(x,y) between the test image f(x,y) and a template g(x,y) is given by:

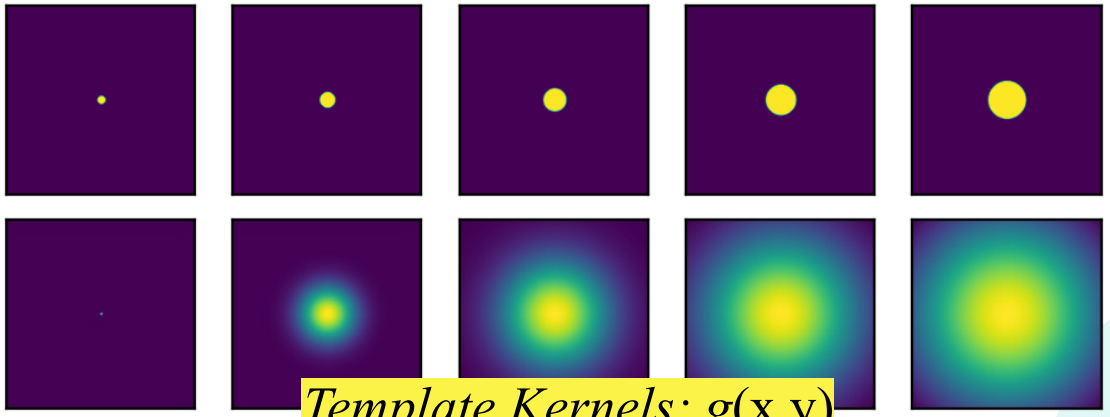
$$p = f \odot g = \int\int f(x', y')\, g(x + x', y + y')\, d\,x'\, dy'$$

In the Fourier space, the correlation P(u,v) is related to the fourier transforms of f(x,y) and g(x,y) given by

$$P = F^{*}\, G.$$



Test Image: f(x,y)



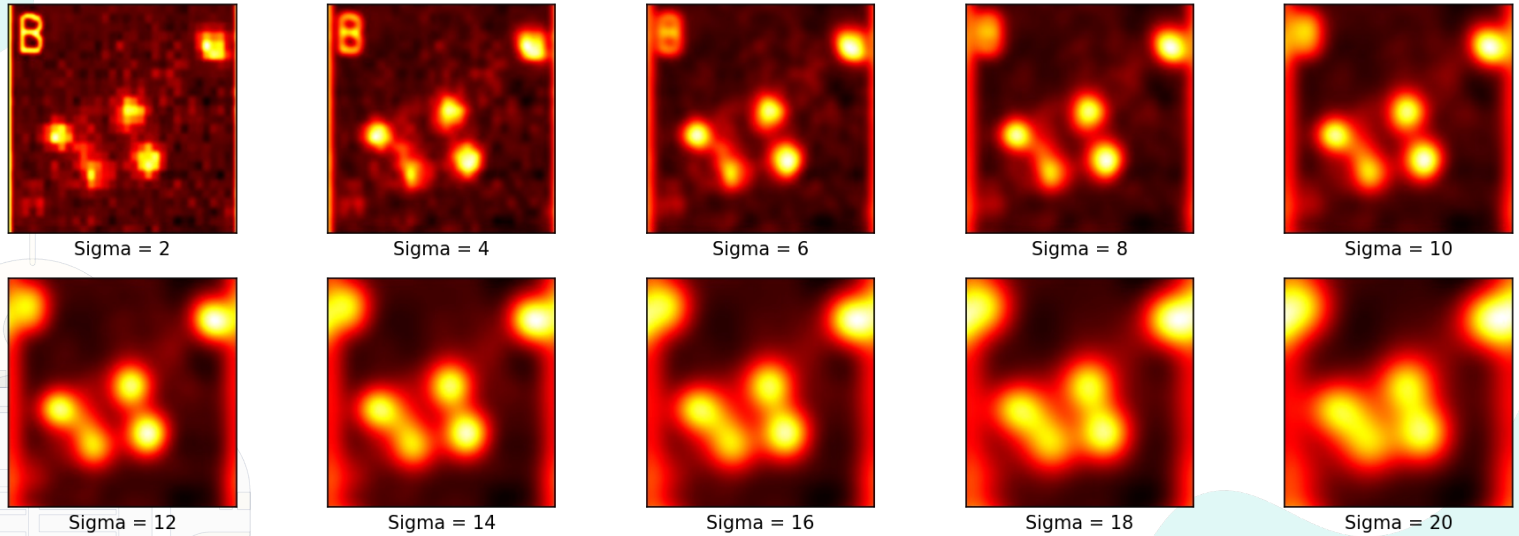
Template Kernels: g(x,y)

The correlation map is then recovered by taking the inverse FT of P.

Results

Shown in the following pages are the correlation maps returned by using templates such as (1) gaussian kernels of increasing variances and (2) defocus kernels of increasing extent sizes. Unlike gaussian maximization which fits perfect gaussians to the test image's localized peaks, FFT correlation technique preserve the cell's irregularity while emphasizing the peaks and smoothening the entire image.

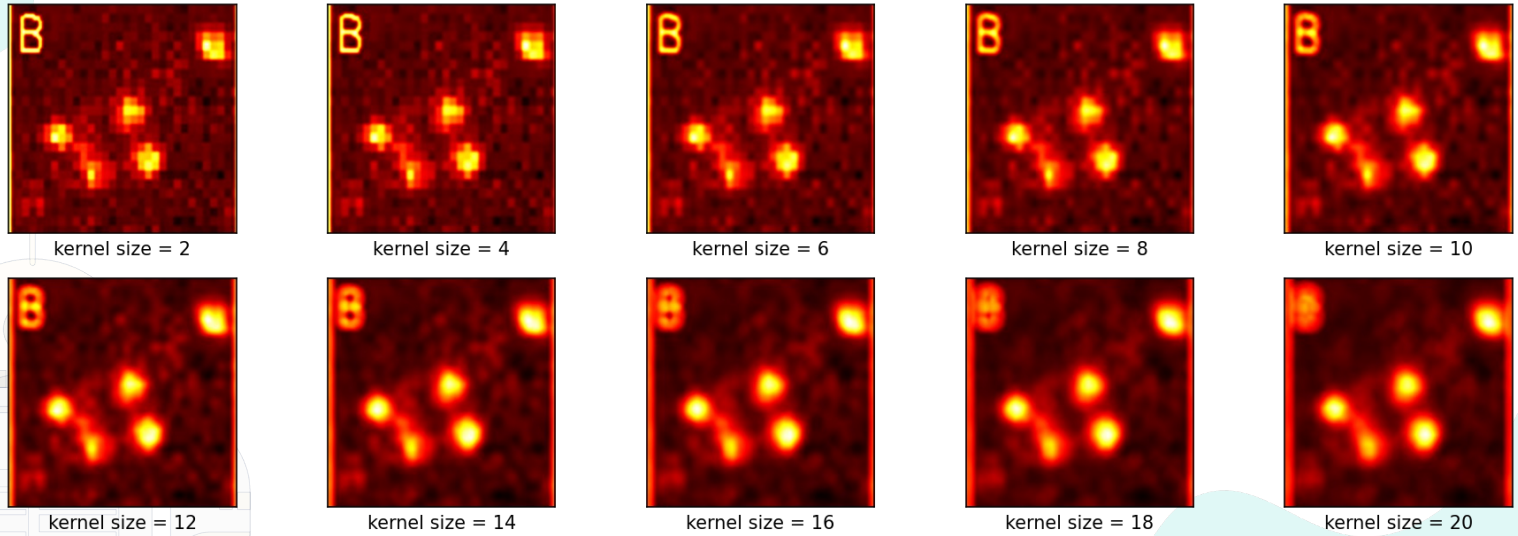
Gaussian Kernels: $g(x,y)$



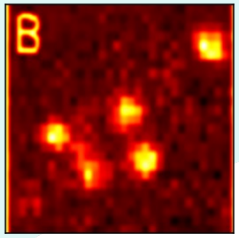
Results

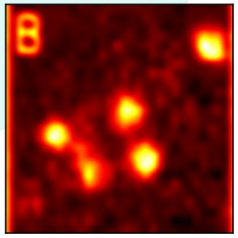
FFT correlation map using gaussian template yielded sufficient results however, at large variances (way beyond the dimensions of the cells), a gaussian blur becomes prominent. Meanwhile, using a defocus kernel circumvents this problem as it emphasized the peaks and super-resolved the test image even at very large kernel sizes.

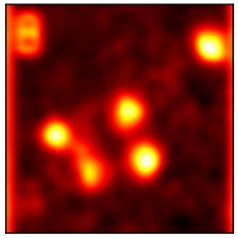
Defocus Kernels: g(x,y)

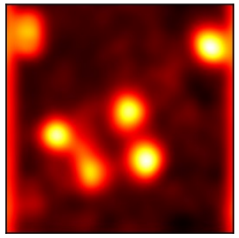


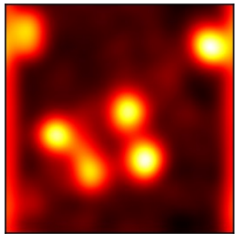
Gaussian Kernel

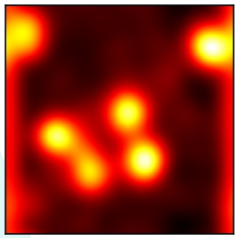

Sigma = 2

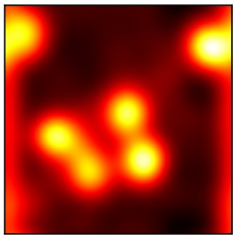

Sigma = 4

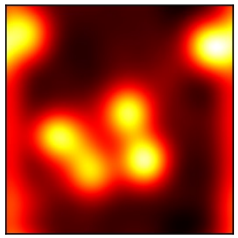

Sigma = 6

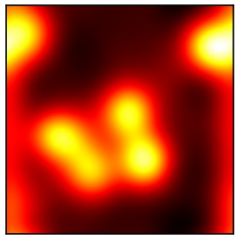

Sigma = 8

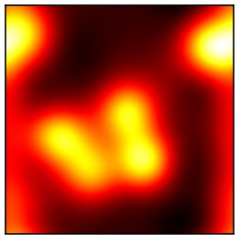

Sigma = 10

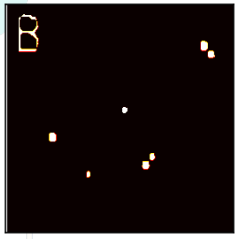

Sigma = 12


Sigma = 14

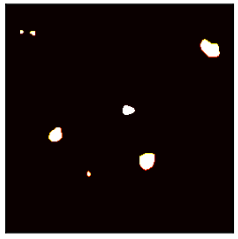

Sigma = 16

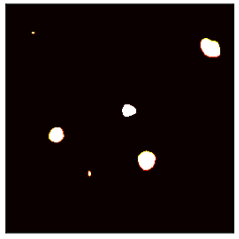

Sigma = 18

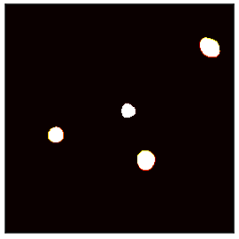

Sigma = 20

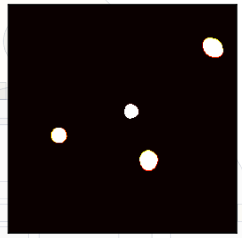

Sigma = 1

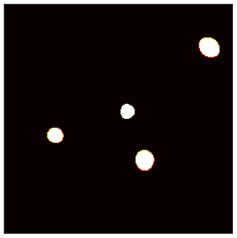

Sigma = 2

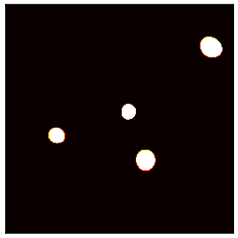

Sigma = 3

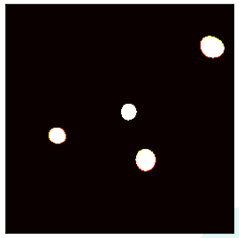

Sigma = 4

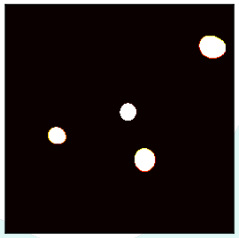

Sigma = 5


Sigma = 6

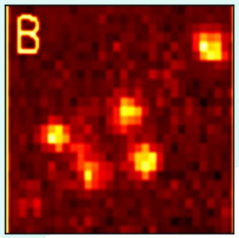

Sigma = 7

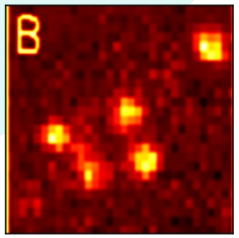

Sigma = 8

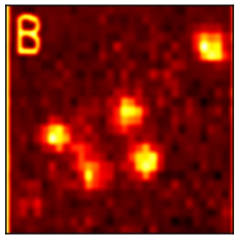

Sigma = 9

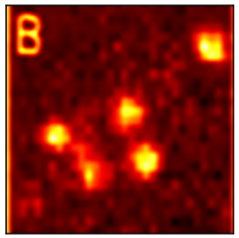

Sigma = 10

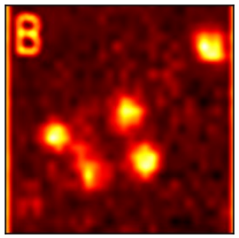
Defocus Kernel

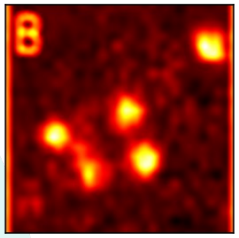

kernel size = 2

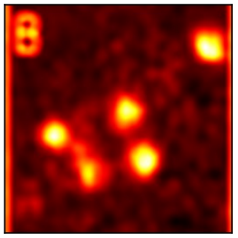

kernel size = 4

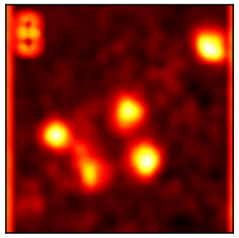

kernel size = 6

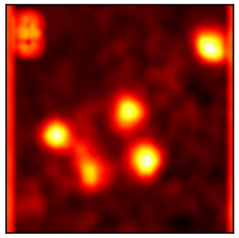

kernel size = 8

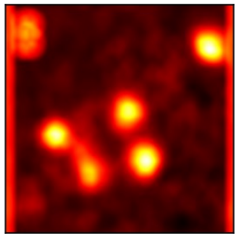

kernel size = 10

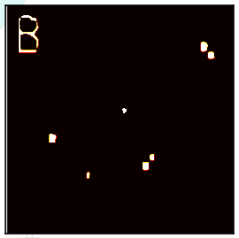

kernel size = 12

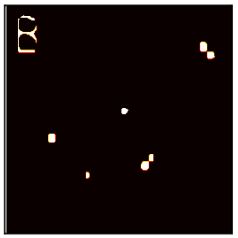

kernel size = 14


kernel size = 16


kernel size = 18


kernel size = 20


Kernel Size = 2

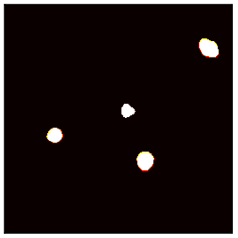

Kernel Size = 4

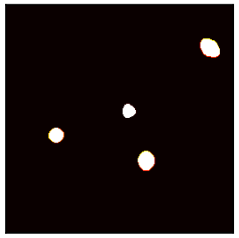

Kernel Size = 6

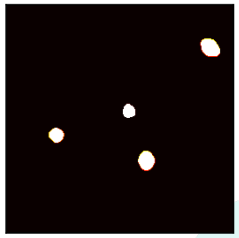

Kernel Size = 8

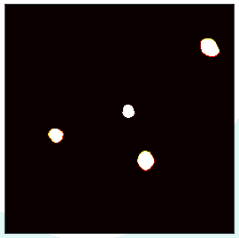

Kernel Size = 10


Kernel Size = 12

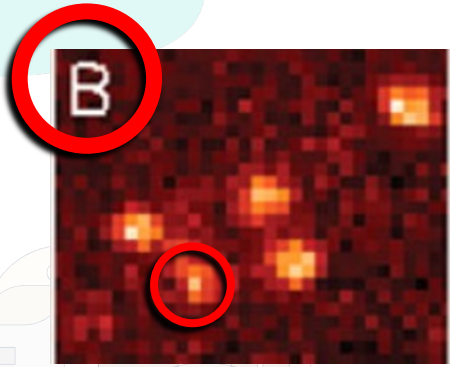

Kernel Size = 14


Kernel Size = 16

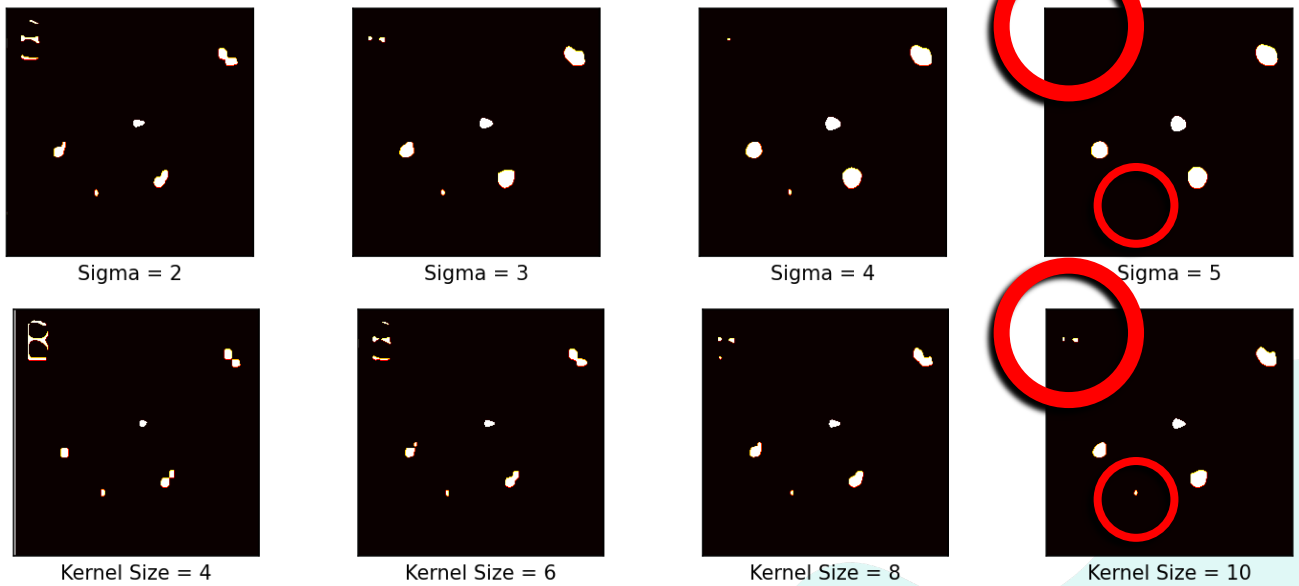

Kernel Size = 18


Kernel Size = 20

Interestingly, thresholding the results for gaussian template eliminated the artifact correlation detection of the letter “B” on the upper left corner of the image right away on increasing variance. The thresholding results for the defocus kernel was able to eliminate the artifact at k = 14. Unfortunately, on both instances, the fifth faint blob on the lower left vanished in the correlation map as soon as the artifact letter was not mapped either. Hence, FFT correlation is effective on highly regular / relatively circular blobs.



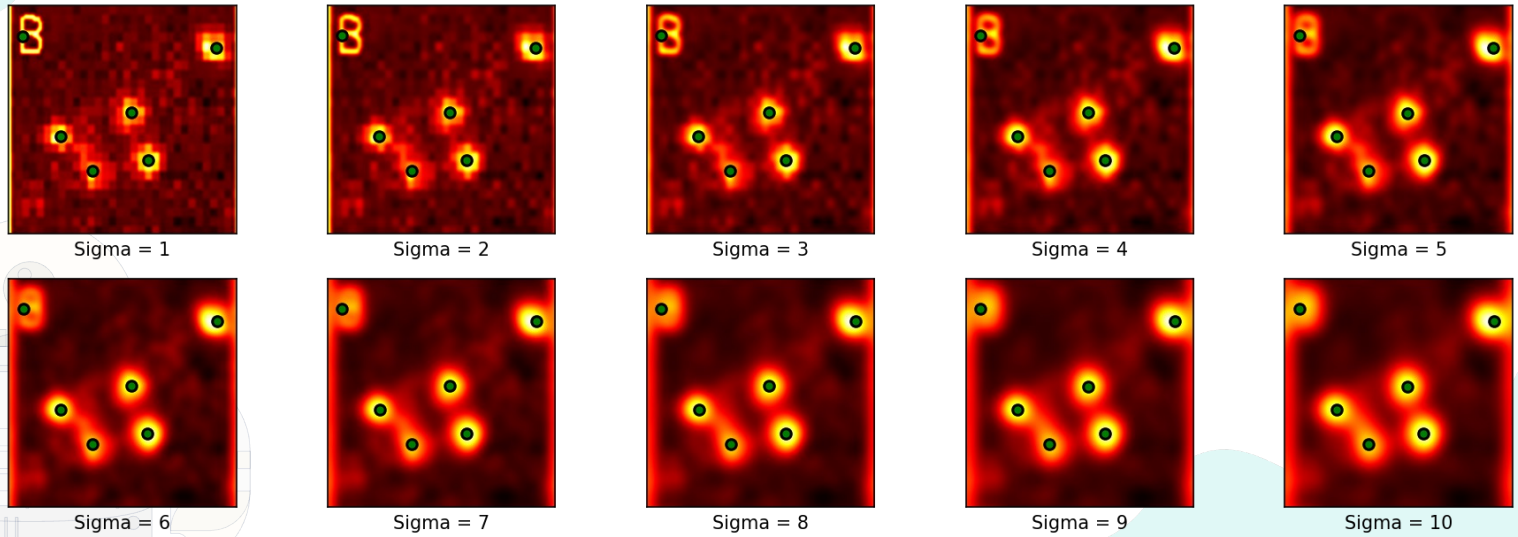
Test Image: f(x,y)

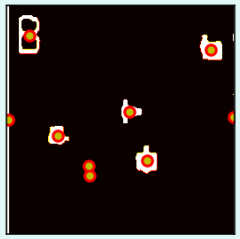


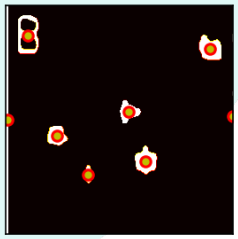
Template Kernels: g(x,y)

Centroid Detection

In the maximization technique as implemented by Reinier Ramos, individual gaussian fittings can sometimes merge two distinct blobs, while multivariate fittings can fit the gaussians well however, this requires a priori knowledge on how many peaks are there to be expected. With centroid detection applied on FFT correlations, the five peaks (excluding the “B” artifact which should not be present on experimental images) are well detected, the test image is retained but just super-resolved.




Sigma = 1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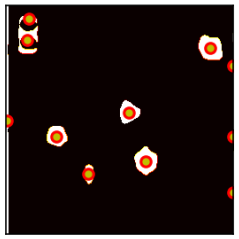
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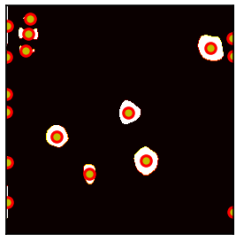
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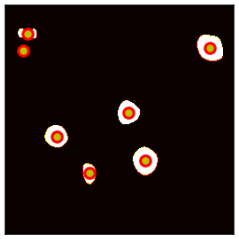
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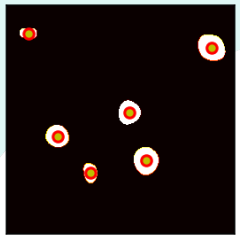
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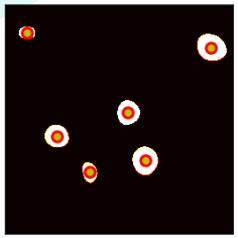
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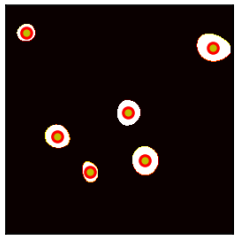
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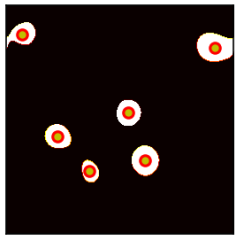
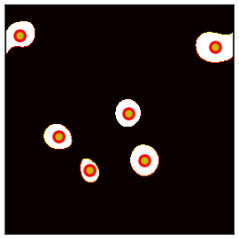
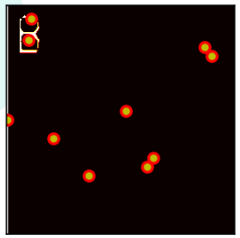

Sigma = 9


Sigma = 10

gray image threshold = 150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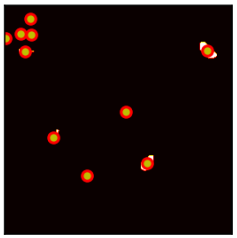
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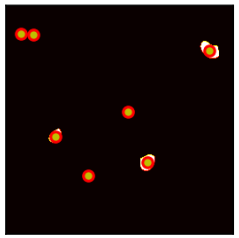
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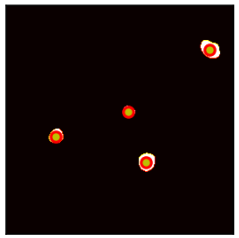
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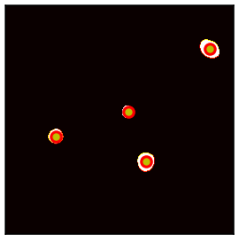
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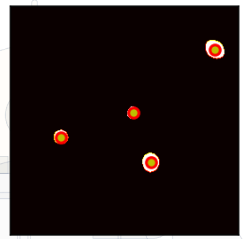
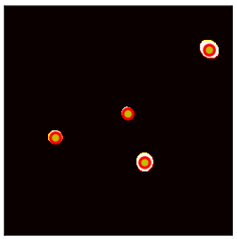

Sigma = 6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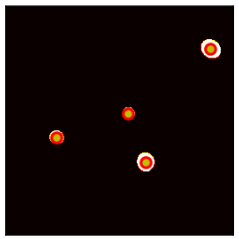
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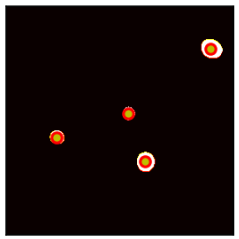
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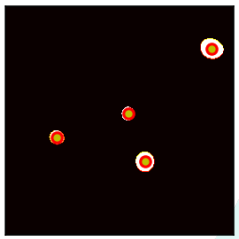
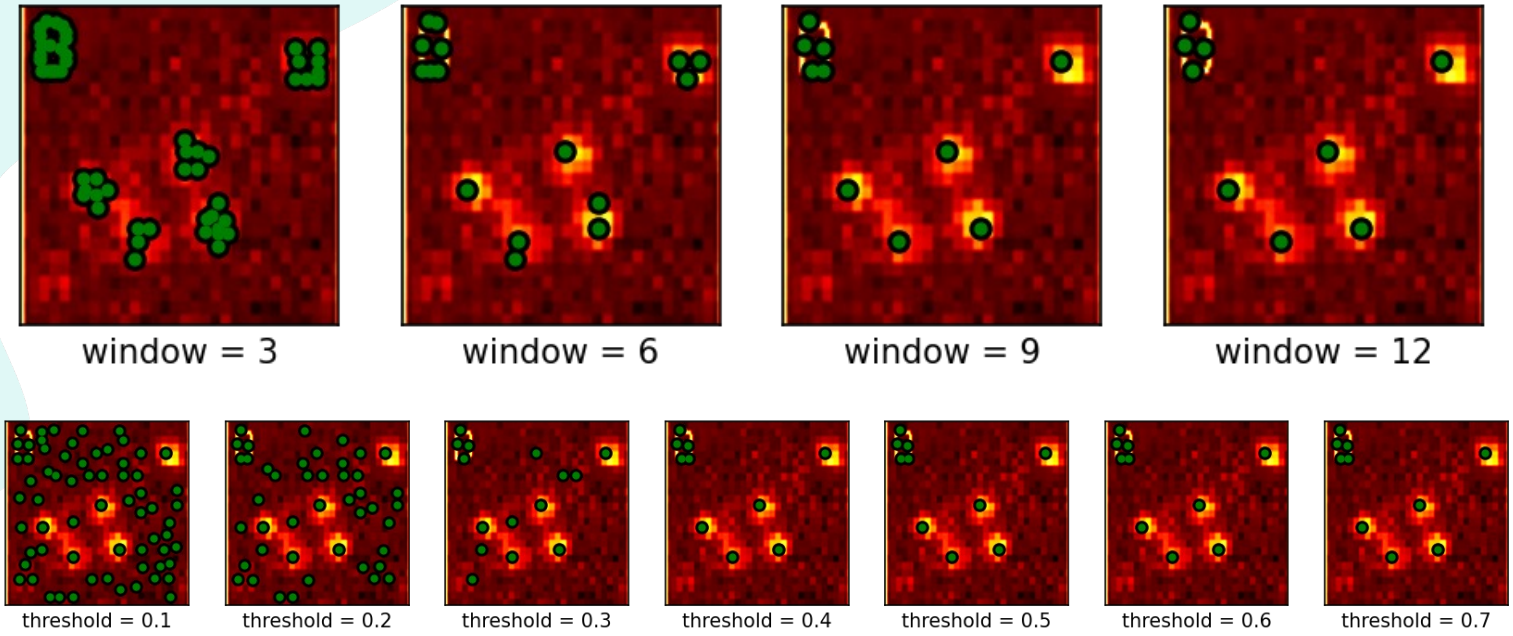

Sigma = 10

gray image threshold = 200

Peak local maximum detection on the raw test image right away struggles to localize the peaks and requires fine-tuning the window and threshold parameters as shown below.



Overall, it was demonstrated how the simple algorithm and concept FFT correlation does the job of super-resolving an image of photoactivated cells, simply by matching gaussian/defocus kernels to identify the positions and similarity.

reflection

This activity and the last made me appreciate the elegant power of Fourier transforms. Convolution and correlation concepts using FFT were used and in the end, I demonstrated how spatial super-resolution can be achieved by exploiting the frequency space alone. I have elaborated the parameter effects in the generated FFT correlation maps, applied thresholding and centroid detection techniques, with the end goal of localizing the peaks and super-resolving the image. I counterchecked these results to Reinier Ramos's implementation of individual and multivariate gaussian expectation maximization.

With that said, I'd give myself a score of **105/100.**

references

- [1] M. Soriano, Physics 305 – PALM Superresolution, (2023).
- [2] Betzig, E., Patterson, G. H., Sougrat, R., Lindwasser, O. W., Olenych, S., Bonifacino, J. S., ... & Hess, H. F. (2006). Imaging intracellular fluorescent proteins at nanometer resolution. science, 313(5793), 1642-1645.

SOURCE CODE

https://github.com/reneprincipejr/Physics-305/tree/main/Activity%202%20-%20Super-resolution%20Gaussian%20Maximization