

Original Issue:

In the Raw Data provided by the grayscale images that were taken, there was a significant amount of noise that was present making the data very cluttered.

*Dynamic Offset implemented

Gaussian Fitting:

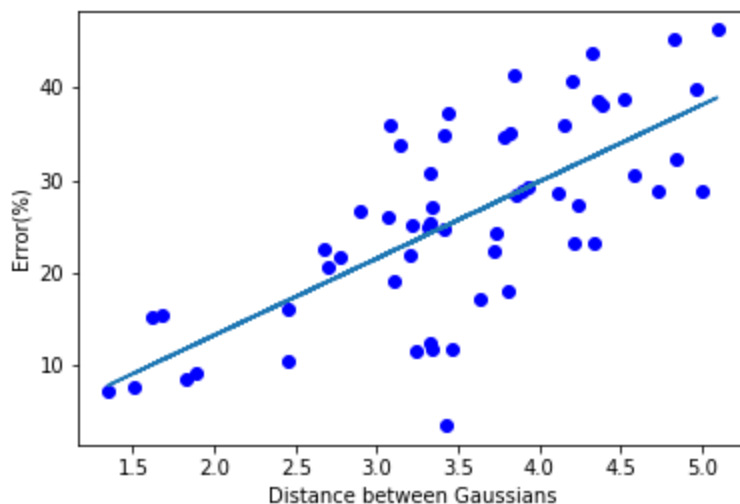
1 Gaussian:

When dealing with the two spots that were being replicated, I originally tried to fit it with a single 2 dimensional Gaussian which fit the data with an average error of around 18%.

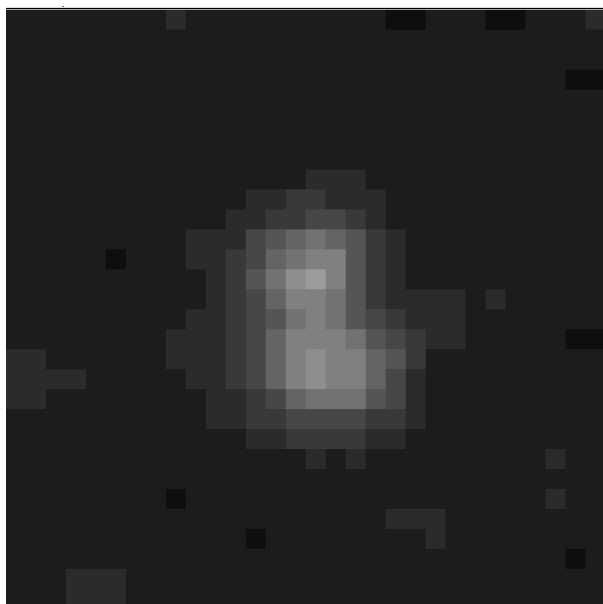
2 Gaussians:

When using two gaussians to fit the raw data, it was much more useful as a good estimate of the spot was provided when using a modified fitting technique from the original one gaussian. In this way, the exact center of the gaussian, the height and width were easily found using the scipy optimize least_squares (not to be confused with leastsq which does not enable the use of bounding for parameters which can result in negative values).

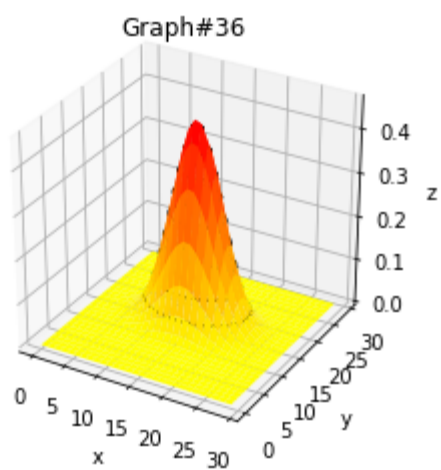
On real data, the error seen by using the sum of the peaks of the two gaussian distribution over the one gaussian distribution in relation to the distance between the centers of the gaussians can be seen below:



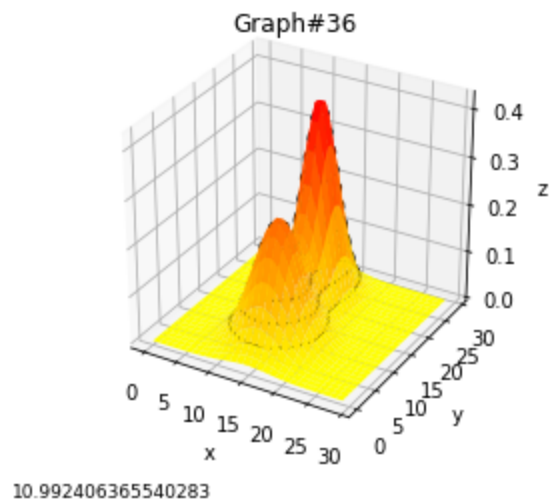
Ex.(Image #36 of Dataset 1)



1 gaussian fit



2 gaussian fit

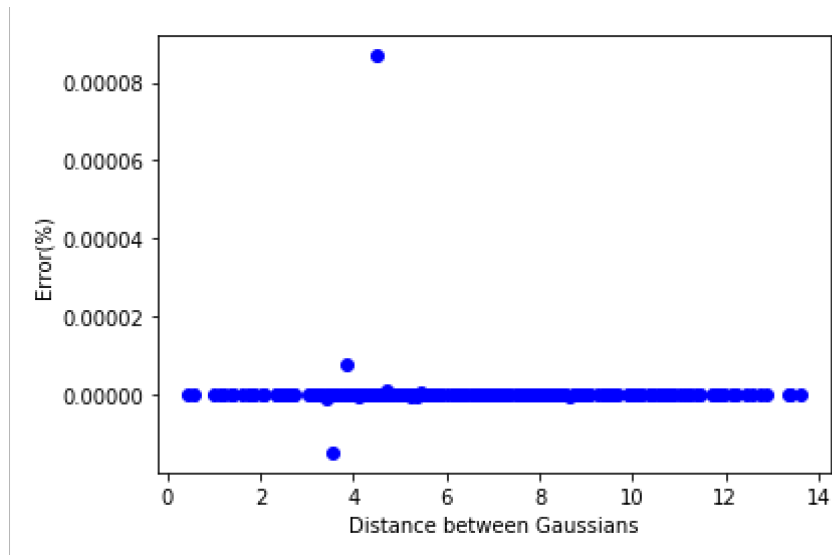


*Note the number indicates the distance between the centers of the two gaussians in pixels

Plots to make (simulated data):

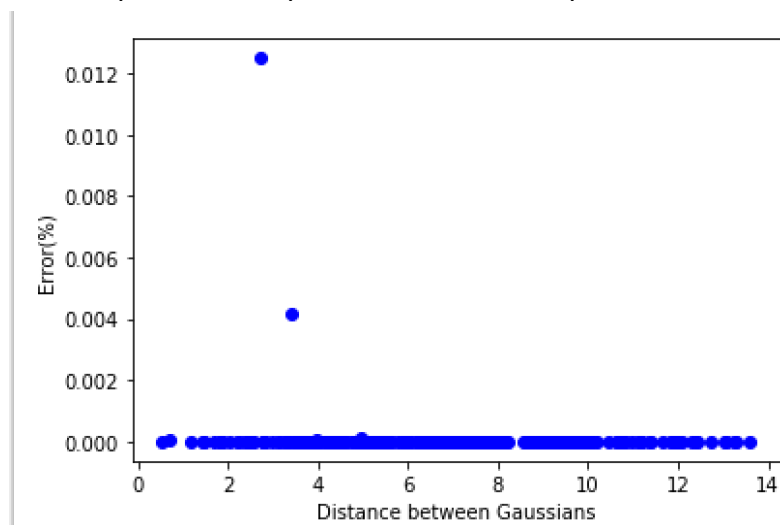
1. Amplitude prediction

- a. True total amplitude vs. predicted (fit) amplitude



Above is simulated data with no deviation

- b. True vs. predicted amplitude for individual spots.



2. Position prediction

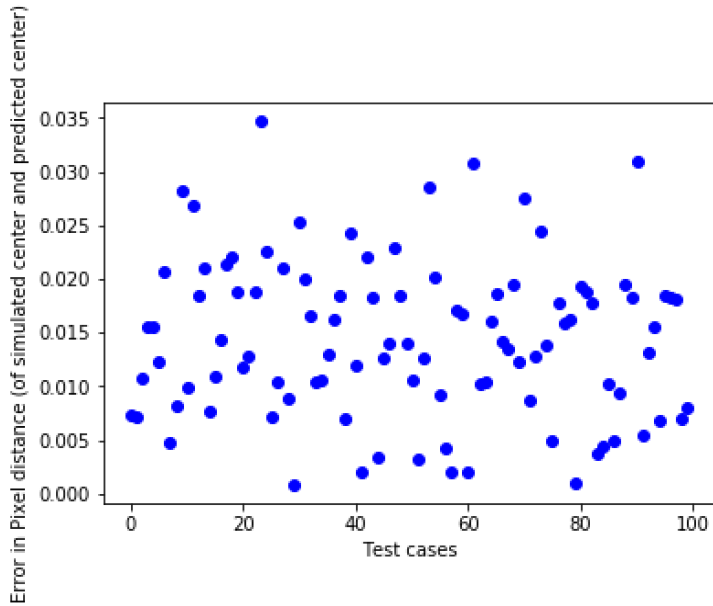
- a. Total difference between predicted and actual spot positions vs. spot separation

3. Plots for visual inspection of fit

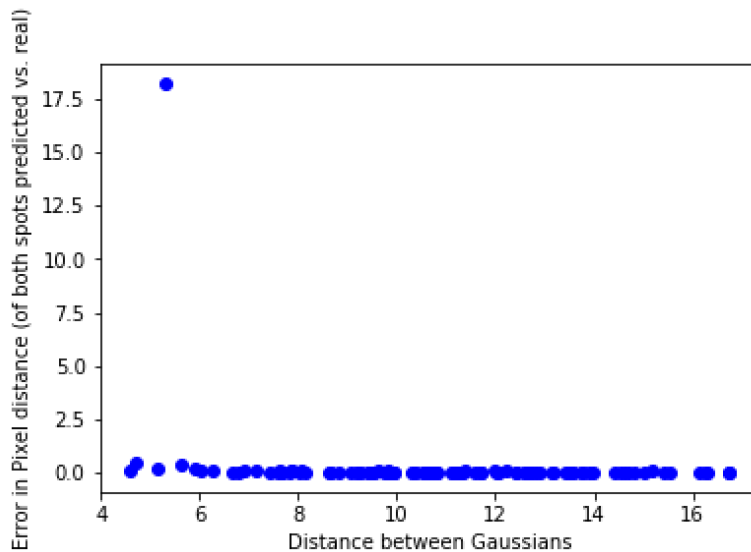
- a. Heatmap or grayscale of “true” data with predicted spot centers and “isoclines”
b. Difference heatmaps

Pixel Distances:

The Error when looking at the center of a single spot with a 5% deviation(noise added to each individual pixel) of simulated data(100 datapoints) X axis is just random trial cases:



The Error when predicting centers of two spots (sum of the differences of the error) with a 5% deviation of simulated data vs. the Distances between the centers of the two Gaussians(100 datapoints) - X Axis:



*Note: Random jumps are sometimes seen in this case for some unknown reason? Usually happens about 1 time every 100 cases and no evident pattern is really seen as to which cases cause that bad of an error

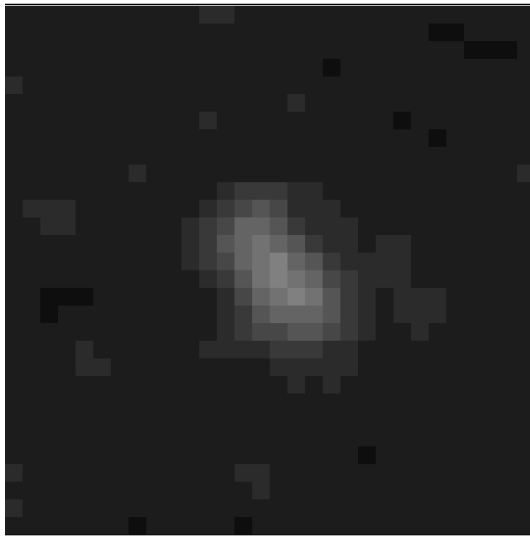
Real Data Predictive spots:

Using the real data from the dataset, I overlaid the predicted centers of the Gaussians over the original image:

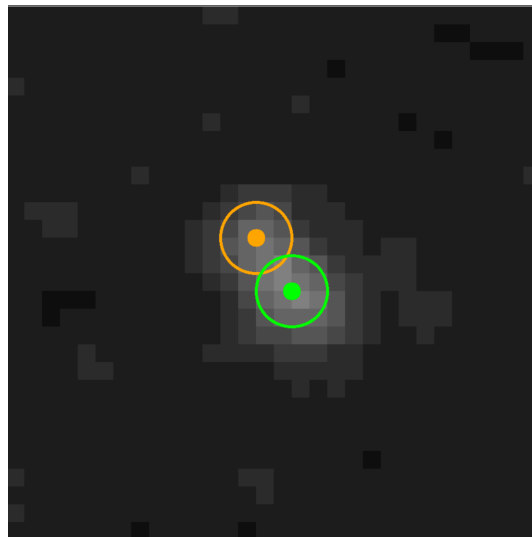
(The spot is located with an accuracy in the decimals so it is hard to display on a 30 by 30 pixel map so the image is resized to a much larger scale. The dot is the center of the gaussian and the ellipse signifies the gaussian boundaries)

Ex. Dataset 1 Image 24=

Orig Image:



Predicted Gaussian Centers:



*Library of snips is stored in folder in the file -> also updated for dynamic centers based on the amplitude

Spot Tracking

3D Gaussian Fitting

Tracking Spots Over Time

Spot Movement Statistics