

## 02740 Project #2: Particle Detection

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### B.1 Image data

The image sequence was downloaded

### B. 2. Part I: Particle feature detection

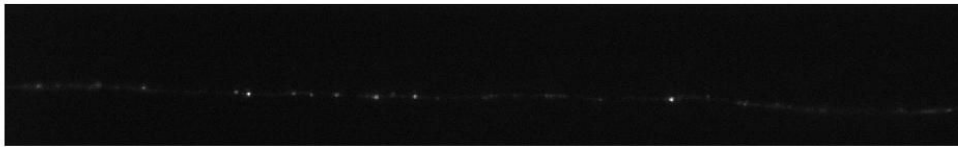
#### B.2.1 Calibration of dark noise

The mean and standard deviation of the background was found to be  $\sim 307$  and  $\sim 24$ , respectively.

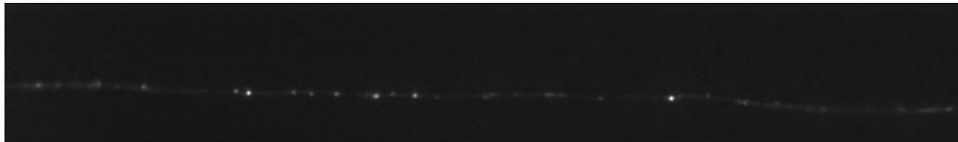
#### B.2.2 Detection of local maxima and local minima

The goal in this section is to filter the image into points (coordinates) of local maxima and local minima with a mask.

#### *Original image*



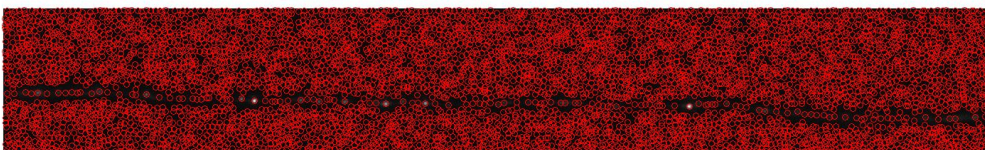
#### Convolved with gaussian mask



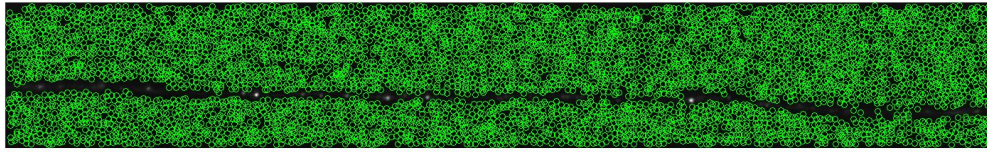
Using the equation  $3\sigma = 0.61\lambda/\text{NA}$ . Since one pixel is 65nm wide, then  $\sigma$  is about 1.18 pixels. 1.18 was used to calculate the Gaussian Mask which was a 3X3 matrix. We then compared the performance of using a 3X3 mask vs. a 5X5 mask for finding local maximums.

It is observed that there are more local maxima and local minima found in 3x3 than in 5x5. This makes sense due to smaller masks result in more positions getting picked.

#### Local maxima

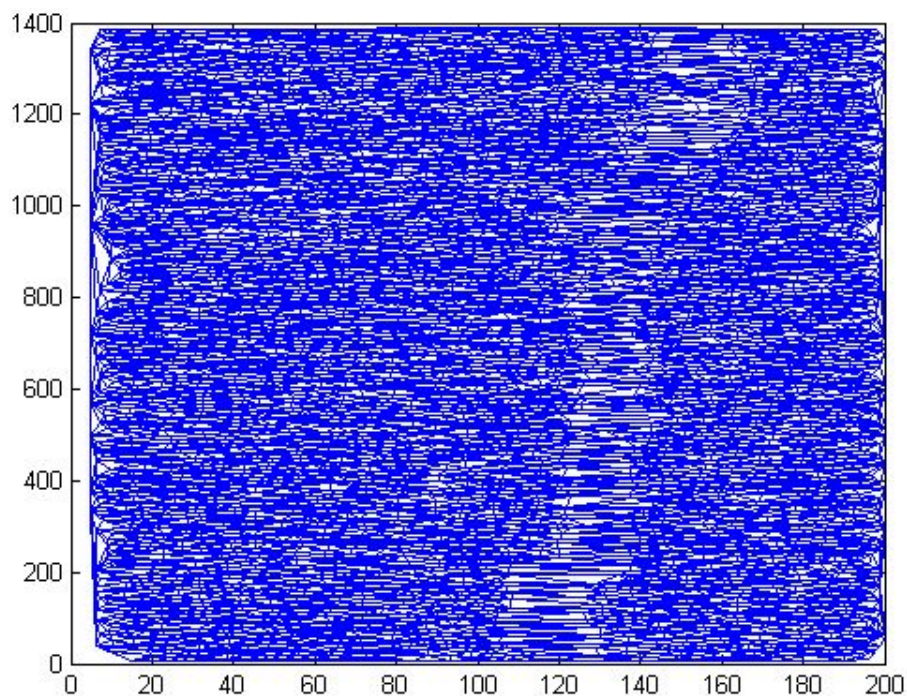


## Local minima



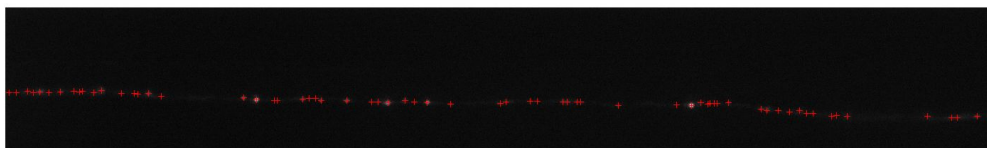
### B.2.3 Establishing the local association of maxima and minima

We use Delaunay triangulation to establish corresponding between local maxima and local minima. By checking the position of local minima (labeled in the previous process), we get the vectors for its x coordinate and y coordinate, then using *delaunayTriangulation()* function. Since the image itself is very large and consequently the number of local minima is quite big before t test, we would see that the triangulation appears very compact in the space.



### B.2.4 Statistical selection of local maxima

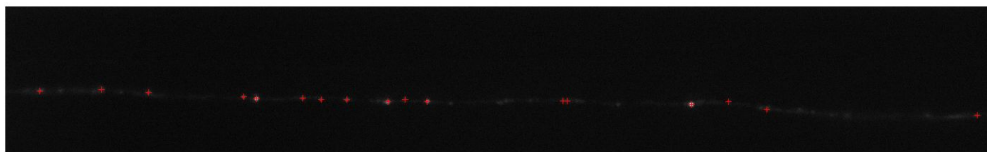
We perform the t-test for all the local maximum using the formular mentioned in reference 1.



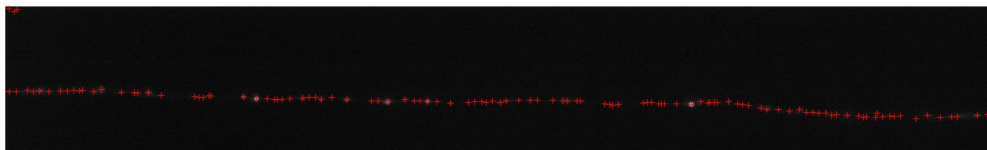
We calculated the local background of a specific local maximum using the mean of nearest triangle's local minimums.

```
if (localmax(i,j) == 1)
    mean=trianglemean(j,i,DT,x,y,image);
    T = image(i,j) - mean;
    tSet = [tSet T];
    if(T < Q*sigma)
        localmax(i,j) = 0;
    end
```

$Q = 10.0$



$Q = 2.5$



## B.3 Part II: Sub-pixel resolution particle detection

### B.3.1 synthetic image generation

The goal of this section is to visualize ground truth of the original image using previously found local maxima. See result after running the code.

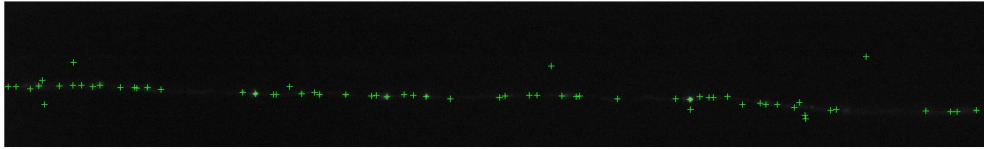
### B.3.2 sub-pixel resolution detection using pixel oversampling

We perform the oversampling on each local maxima remained after the t-test. First we get the vectors and matrix needed as the input of *interp2* function, taking the nearest 8 neighbors of the target local maxima as the first three arguments of *interp2* function. Then we divide the pixel into 5X5 parts and set the index of each part. Further, we use these 25 new image intensity values and the corresponding indexes to perform the Gaussian fitting (using *lsqcurvefit* for least-square fitting) for the local maxima and we can get the sub-pixel top point coordinates of the Gaussian.

The result contain the absolute position of the center ( $x_0, y_0$ ) in the 25 sub-pixels.

From the result we can observe that several points has a obvious displacement comparing to its original positions. The possible reasons for this may be that the intensity of these points(after minis the background) may be not so remarkable and it is more likely to cause its deviation of position. Another factor may be that we use the nearest 8 neighbors for the interpolation and it

may lead to bias. However, it is clear that most of those points with remarkable intensity are on its original position.



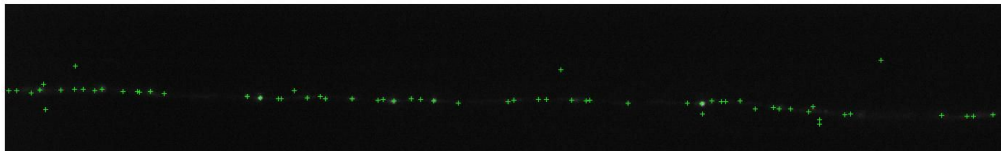
### **B.3.3 Benchmarking sub-pixel resolution particle detection**

Run the code testpart3.m to check it.

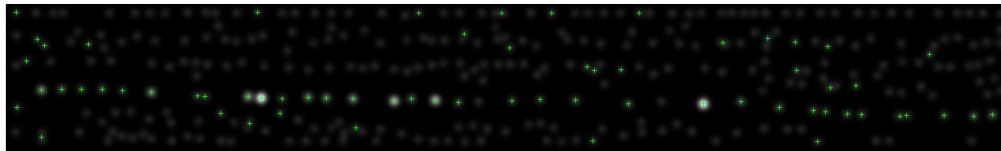
The “accuracy” of the original image from ground truth is (-0.752455%, 14.386521%)

The “precision” of the original image from ground truth is (1.808743%, -63.422931%)

#### ***Result from 3.2***



#### ***Result from 3.3***



### **References**

- [1] A. Ponti, P. Vallotton, W. C. Salmon, C. M. Waterman-Storer, and G. Danuser, Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy, *Biophysical Journal*, 84:3336-3352, 2003.
- [2] M. K. Cheezum, W. F. Walker, and W. H Guilford, Quantitative comparison of algorithms for tracking single fluorescent particles, *Biophysical Journal*, 81:2378-2388, 2001.