Project Assignment 3

Team 1
Siddharth Gurdasani
Easwaran Ramamurthy
Aniket Rao
Patrick Ropp

April 6, 2015

B.2 Part I Particle Feature Detection

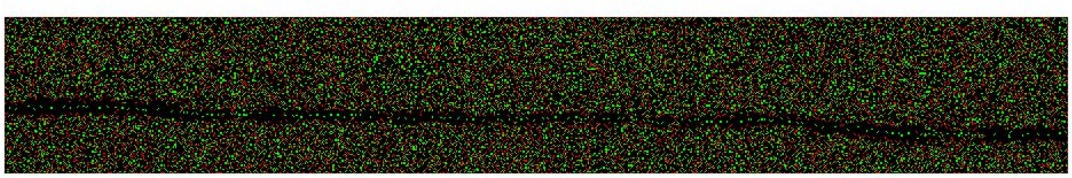
B.2.1 : We manually cropped a rectangular region from one image using MATLAB command "**getrect**" from the image background area. Then we calculated the mean and standard deviation of the selected region from all the images in the sequence using MATLAB commands "**mean**" and "**std**" respectively. Following are the parameters that we get:

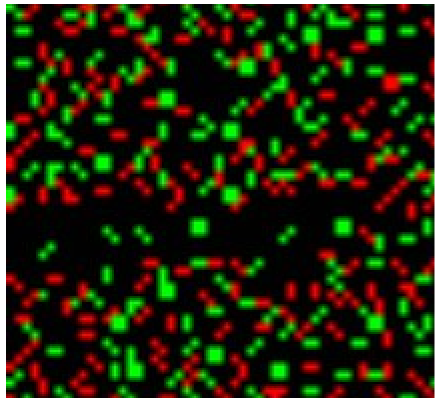
Mean = 301.9245
Standard Deviation = 23.8726

B.2.2: We filtered all the frames in the image sequence using a Gaussian kernel with the standard deviation equal to one third of the Rayleigh radius (as mentioned) and with the given lambda and numerical aperture values as given. Further we used the kernel size as ***6*sigma*** to create the Gaussian kernel using "**fspecial**" MATLAB command and convolved the image with the kernel using MATLAB command "conv2".

For the detection of local maxima and minima, we looped through each image in the sequence pixel by pixel and took the local maximum pixel and local minimum pixel in a 3x3 as well 5x5 region in the image.

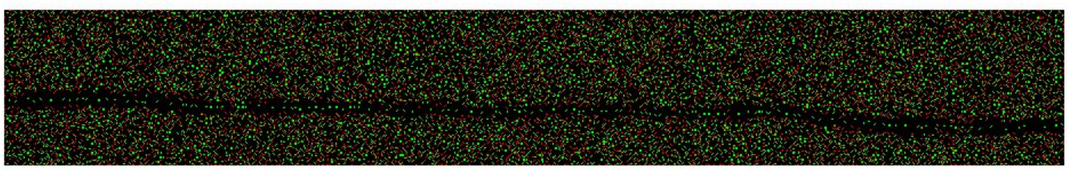
Following is the image for the local minima and maxima using a 3x3 mask:

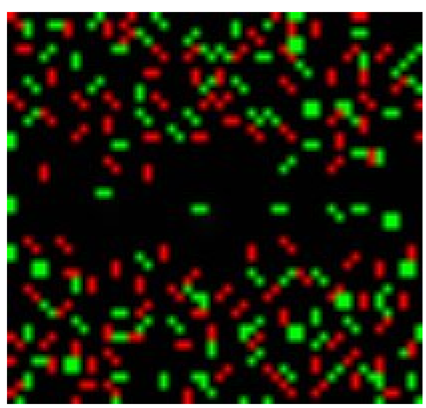




4x zoomed subsection

Following is the image for the local minima and maxima using a 5x5 mask :



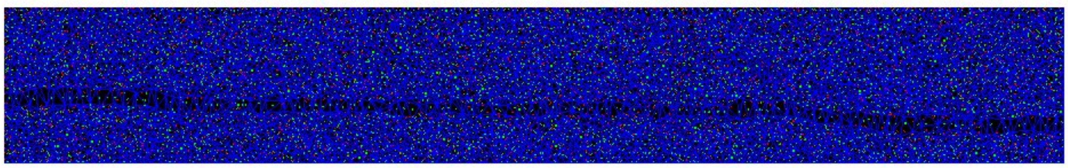


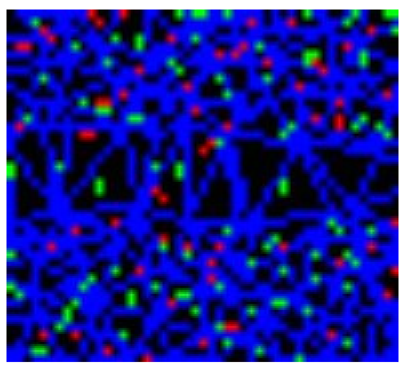
4x zoomed subsection

From the above images, we can observe that choosing a bigger mask in this case 5x5 provides us with fewer local maxima points and minima points than using a 3x3 mask. Most of the maxima/minima points are sort of combined in the 5x5 points and the reason being our consideration of maxima/minima pixels in a bigger area of the image.

B.2.3: We used the in-built MATLAB command "**delaunayTriangulation**" for the association of local maxima and minima points.

Following is the image for the delaunay triangulation, where the vertices (Red) represent the local minima points/particles and in the centre of each triangle there is a local maxima point/particle shown in Green.





4x zoomed subsection

B.2.4: We used the following null and alternative hypotheses from the reference paper [1] where ΔI is the difference between the intensity at local maxima and the mean of background noise obtained from B.2.1.

The null hypothesis states that there is no significant difference between the background and the local maxima intensities, while the alternative hypothesis states that the local maxima intensities are greater and more prominent than the background intensities.

$$H_0 : |\Delta I| = 0$$

$$H_A : |\Delta I| > 0 \qquad \text{where } \Delta I = I_L - I_{BG}$$

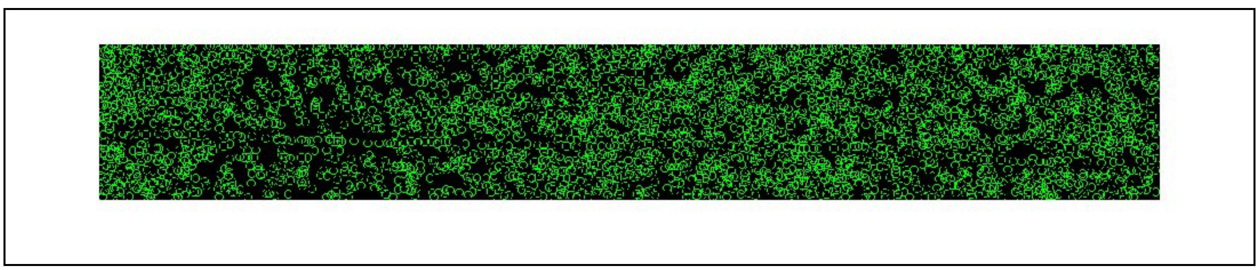
Also, we know that $\sigma(I_L)$ is zero as given, so $\sigma^2(I_L)$ also becomes zero and we are dividing the $\sigma^2(I_{BG})$ by 3 because we are considering 3 nearest neighbors of a pixel for the calculation of the standard deviation $\sigma^2_{\Delta I}$.

and the test statistic $T = \frac{|\Delta I|}{\sigma_{\Delta I}} \propto t_\alpha(0, 1)$
where $\sigma^2_{\Delta I} = \sigma^2(I_L) + \frac{1}{3}\sigma^2(I_{BG})$

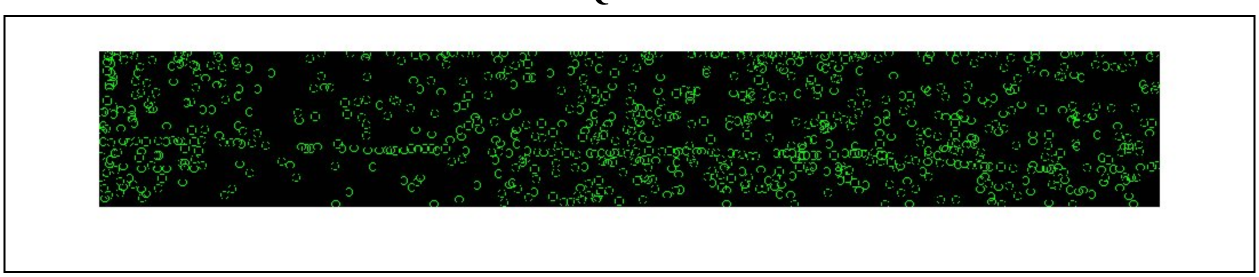
We reject the null hypothesis if the value of test statistic i.e. $T \geq Q_\alpha$ where Q_α is the confidence quantile provided by the user otherwise we don't reject the null hypothesis.

Following are the images that we got using the confidence quantiles. The number of peaks located for each are Q2 = 2885, Q4 = 795, Q7 = 101 and Q10 = 51.

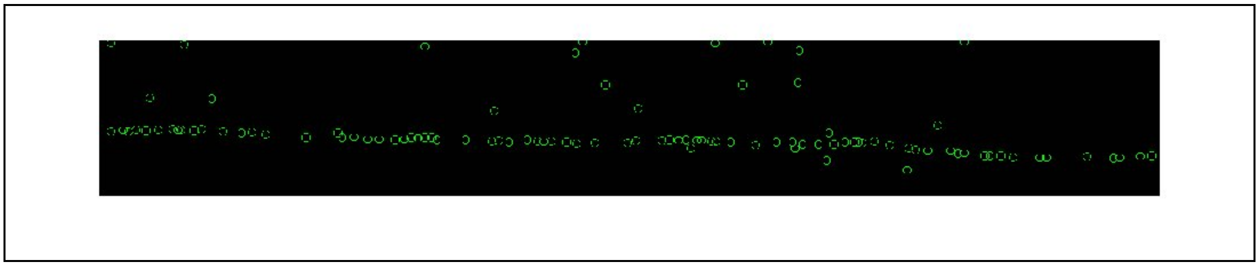
Q = 2



Q = 4



Q = 7



Q = 10

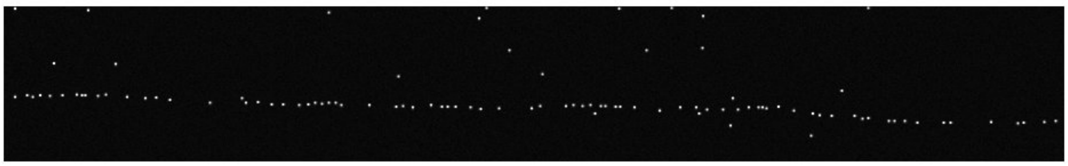
Confidence Quantile (Q)	Number of local maxes
10	51
7	101
4	795
2	2885

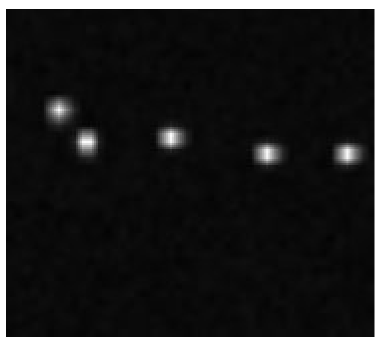
From the above images and table, we can observe that the confidence quantile Q = 7 gives a better result in terms of specificity and sensitivity for detecting the significant particles. We can see that in the image for Q = 10, some of the particles are missed out and so the number of true negatives is high in this case.

The results for each frame of the image sequence are saved as mat files in the folder "**saved_mat_files**" with each frame named as the "**001_a5_002_t214_matFile.mat**" i.e. the original file name with a suffix **"_matFile.mat"**.

B.3 Part II Subpixel Resolution Particle Detection

B.3.1: After performing a statistical filter on our maxima, we then took the resulting binary matrix and convoluted it with the gaussian kernel as outlined in section B.2.2. This was multiplied by a normalization factor equal to the maximum value of the original image times the inverse of the maximum value of the convoluted and filtered image. This gives us the synthetic peaks. We then created a matrix of values drawn from a normal distribution based on the background values identified in B.2.1.





4x zoomed subsection

B.3.2: After interpolating the filtered image using interp2 to a much finer detail (i.e. pixel sizes that are 1/5th (13 nm) of the original pixel size (65 nm)), we looked at each local maximum that was found in the image. A gaussian was fit to the 5x5 grid surrounding the local maximum using the 'lsqcurvefit' function and the resulting coordinates were converted back to the original pixel coordinate. Such a method allows for detection at subpixel level and is used in particle tracking experiments. The code is submitted in file **'gaussianFitting.m'**.

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.