PH450 Report 2021-22

Evaluating Spot Finding Methods

Submitted in partial fulfilment for the degree of MPhys

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(Dated: 22nd March 2022)

Abstract

Acknowledgements

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I. Introduction

A. Sub-Pixel Localisation

Super-resolution microscopy is the process of taking the diffraction limit of a microscope, 250nm in the x and y direction, and improving it at a minimum by a factor of 2 although more modern methods improve it by up to a factor of 10. In the past this has been achieved by ensemble techniques like SIM (Structured Illumination Microscopy) and STED (Stimulated Emission Depletion) **insert explanation of ensemble techniques**. An improvement on these methods is single-molecule microscopy, in which molecules are individually fluoresced and imaged instead of ensembles which helps distinguish more detail and produce better results than the diffraction limit. There are two ways that this general method have been implemented; photo-activated localization microscopy(PALM) and stochastic optical reconstruction microscopy(STORM), both rely on fluorophores which are fluorescent chemicals that re-emit light after being excited. This helps as the fluorescence emits the light stochastically so only a subset of molecules "light-up" at once, this is important as if they are separated by at least 200nm then they can be located to nanometre precision. Since the molecules are now separated spatially this process needs to just needs to be repeated until all molecules have been "switched-on", this gives a stack of images with blurry spots which can be located and recombined into a final image with spot-precision on the order of < 20nm.[1] The resolution of a super-resolution image usually doesn't refer to the spot-precision of the located molecule, rather it refers to the structural resolution, this can be calculated along with the density of fluorophores as the Nyquist-Shannon sampling theorem states a minimum number of fluorophores are required to resolve the structure. For example if the resolution is 20nm in one dimension then fluorophores have to be separated at least 10nm apart at a density of $10^4 \mu m^{-2}$.[2][3]

1. Pixels and Resolution

In the Modern Dictionary of Electronics by Graf a pixel is described as a, spatial resolution element. The smallest distinguishable and resolvable area in an image. [4] This was mainly in reference to an analog image but the principle applies to a raster image, which is a NxM matrix of values that display colour and intensity. In the specific case of the spot localisation that the Methods section (III) describe the images are NxN and 16-bit black and white.

Resolution is the capability of resolving two points or lines. Since super-resolution by definition changes the resolution of the input images, the resolution of the output image needs to be quantified. One of the ways to do this is by using the Nyquist-Shannon sampling theorem, this states that **fix reference in bib file**->[5][6]

B. Motivation

The main motivation behind my project is to improve the compute time that it takes to render images through sub-pixel localisation whilst keeping an acceptable level of accuracy. That is to say this project should be aiming to produce a method of spot-finding that either less complex, less computations per localisation, fewer steps or a mixture of all.

This doesn't just have applications in localisation microscopy but also can be used in other technologies, for example a paper published by Castorena and Creusere [7] has the problem of needing a fast super-resolution technique to get higher spatial resolution from their LIDAR (Light Detection and Ranging) system. This system is constrained by the laser spot size and precision of the scanning mechanical unit as the single laser source with sampling rates in the GHz range which achieves high accuracy for depth and range resolution but poor spatial resolution. A consideration

was to decrease the diameter of the laser thus increasing the spatial sampling density, although this was deemed too computationally expensive.

With the almost exponentially increasing launching of small form factor satellites such as the CubeSat, arises the challenge of efficiently utilising the satellites computing resources. This means any segment of code being ran on the satellite needs to run as quickly as possible whilst keeping a certain standard of accuracy, especially for the processes that the satellite depends on to operate like attitude control, power management and calculations for orbital maneuvers. The main method used for orienting(attitude control) CubSat like satellites is by using a Star Tracker, this works by using a camera mounted facing stars that are known to the satellite via a star catalogue and moves based on how aligned or unaligned a reference image is with the actual image seen.[8] The method in which the image is processed so it can be compared to the reference is called spot finding, this entails taking the image and finding each bright spot or star accurately. The motivation for this project is to develop a spot finding method for star tracking and compare it to the state of the art algorithms measuring accuracy, precision and speed.

This technique is also used for super-resolution microscopy, which changes the optical limits of microscopy from 250nm to about 10nm, this is achieved by temporally or spatially spacing the light coming from the specimen being imaged.

still need to add more technologies that use spot localisation

C.   Literature Review (don't call it literature review)

II.   Literature Review

The field of spot finding or star finding is a fairly recent field with papers coming out in the mid 80's from NASA. However the methods haven't changed that much since the main algorithm still used is centroiding since it's a very good compromise between quickness and accuracy being that it can give an answer in the 1-100 microsecond range [9]. Much of the innovation has come from optimising the algorithm or optimising the data going into the algorithm.

III.   Methods

A.   Centroiding

The most common way of spot finding for star tracking is to use centroiding algorithms, this is when a subsection of pixels are considered to be a star using a rough calculation. The area of interest is then filtered in such a way that reduces noise and aberrations, finally apply the algorithm in this case it's the center of gravity method (1)(or the moment method)[9][10].

$$(x_b, y_b) = \left(\frac{\sum_{ij} I_{ij} x_{ij}}{\sum_{ij} I_{ij}}, \frac{\sum_{ij} I_{ij} y_{ij}}{\sum_{ij} I_{ij}} \right) \tag{1}$$

As can be seen in equation 1 the centroiding method is fairly trivial, the part that determines the computational operations needed is the i and j terms. These terms are the 'window' of pixels that have been chosen by another rough estimator to get a generalised position, the window is a square around the estimated position so the computation scales like n^2, where n is the window size.[10]

B. Fitting methods

1. Point Spread Function

Point Spread Functions(PSF) is the way an object blurs due to the imaging of a point source of light, it's the reason the diffraction limit of a microscope is 250nm (in the x,y direction) and >450-700nm (in the z direction).[1] The PSF is also the smallest resolvable detail that can be seen with light as other objects that emit light that are smaller than one another all appear to be the same size. Provided that laboratory equipment is set up correctly, i.e. the lens is corrected for aberrations and constants are known such as aperture and angels between lens and samples, methods like the Richards-Wolf model and Gibson-Lanni model will calculate the centre of a spot near perfectly. The problem with these methods is that they are complicated and slow, and also offer an answer that is unnecessarily accurate. [11][12]

2. Gaussian

Where the exact prediction of section (III B 1) fails Gaussian fitting tries to succeed by presuming that, if all equipment is set up correctly, the centre of a PSF of a point source is always going to be in the centre. Thus for 2-D spot finding that can't afford the computational time of the previous method uses the more simple equation:

$$I(x,y) = I_0 \cdot \exp\left(-a \cdot k^2((x-x_0)^2 + (y-y_0)^2)\right) + b$$

Where k is $\frac{2\pi}{\lambda}$, a is the width of the PSF, I_0 is the peak intensity and b is the average background per pixel. [12]

3. Triangular method

The triangle method being used takes inspiration from the Gaussian method, in which, a Gaussian curve is produced and the area is calculated by integrating the function. After this the position of the spot is estimated by a hyper-parameter optimisation method which minimises the residual area left over from the fitting process. This triangle method looks to reduce the computational load by removing the integration step, this can be done as the area of a triangle is just $\frac{1}{2} base \cdot height$. On top of this, the method also sums pixel intensities across axis, firstly it gives a better signal to noise ratio but also it means the triangle only needs to be rendered in 2 dimensions instead of 3, further reducing the computational time.
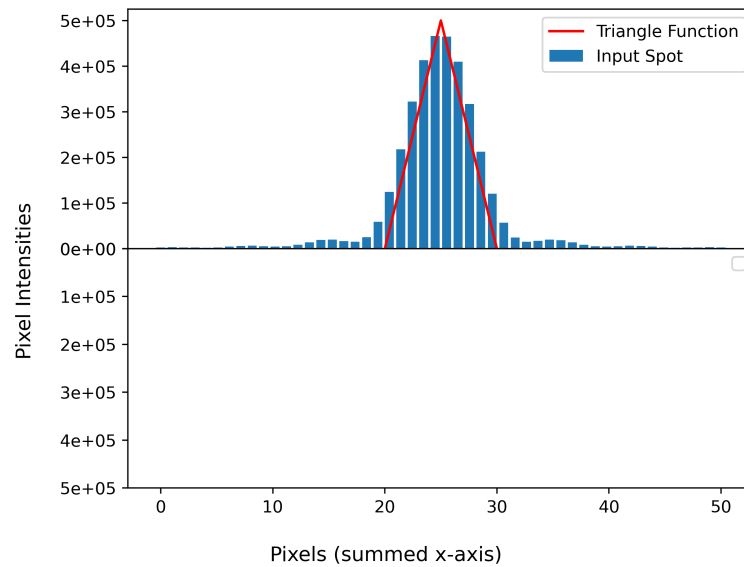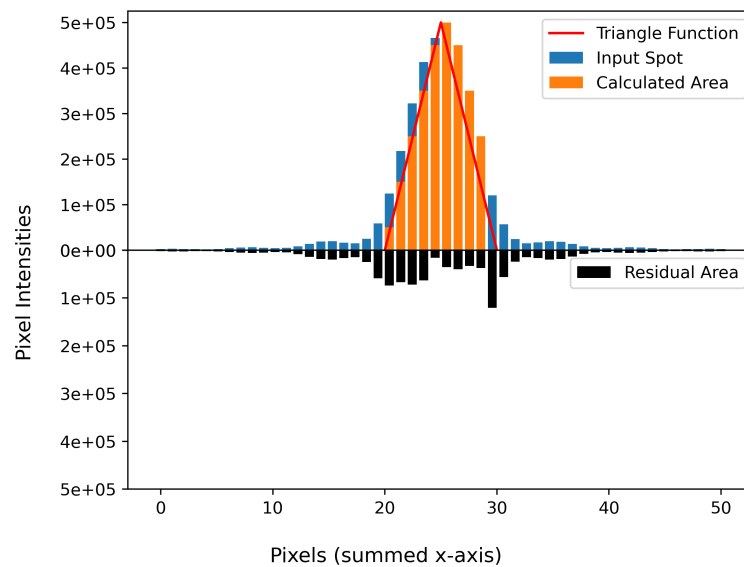
Figure 1. Graph of ideal spot data

Figure 2. Graph of data from figure 1 with the calculated area and residual

As can be seen in figure 2 the area of the simulated spot are subtracted from the area under the triangle and a residual is left over, this residual parameter is the variable to be minimised for in the optimisation routine.
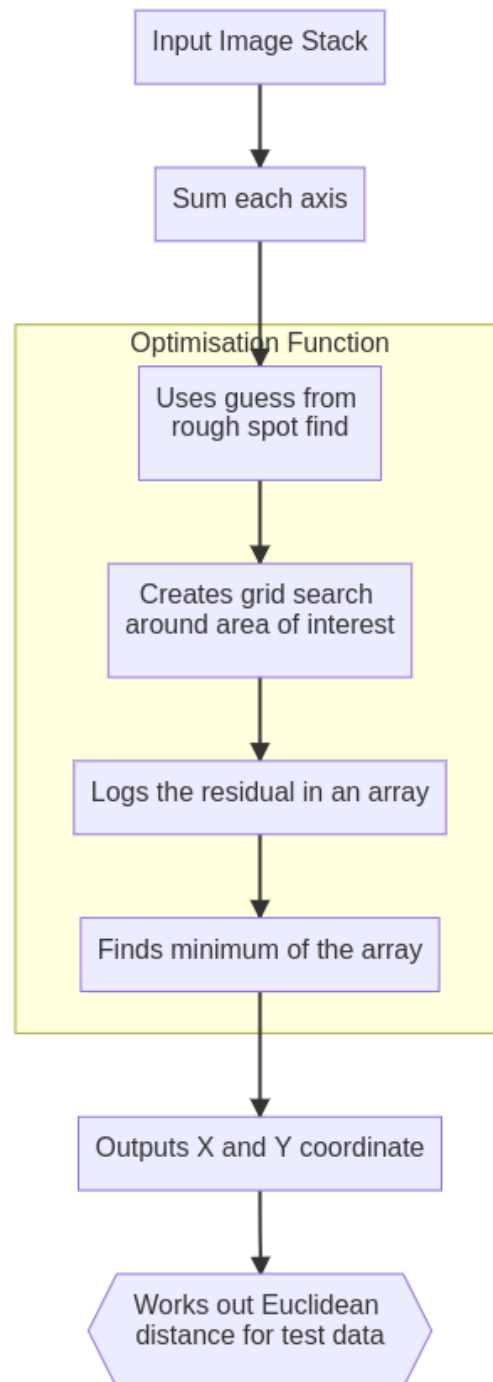
Figure 3. Flowchart describing how the code for the triangular fitting method works

IV. Results and analysis

A. Centroiding

B. Triangle Fitting

In figure 4 it can be seen that while using simulated data the mean accuracy of the localisation is 0.4 of a pixel whilst being executed in 0.34s.
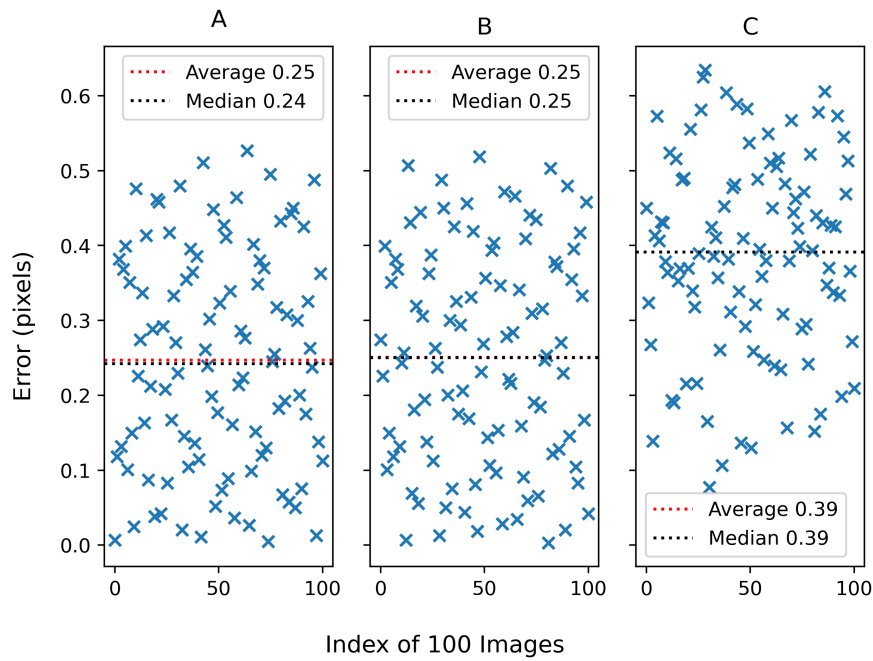
Figure 4. Simulated spots with R1.00, A: Error in the X direction, B: Error in the Y direction, C: Absolute error from the ground-truth

Part C of figure 4 has been replicated for all simulated spots given in figure 5 to compare how the change in spot size changes accuracy.
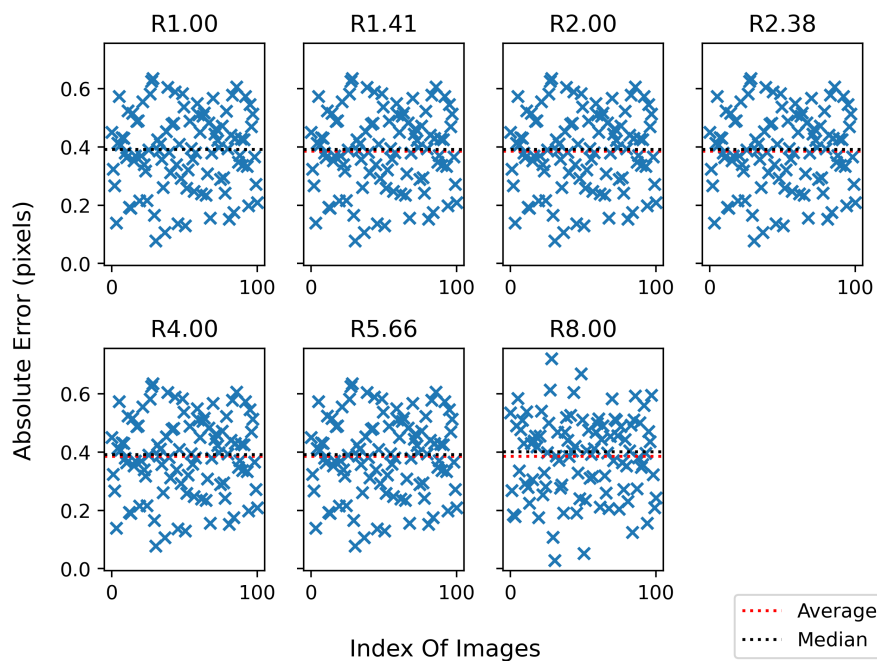

Figure 5. Spot localisation (like graph C in figure 4) of different radii, R, on perfect data (executed in 2.4s)
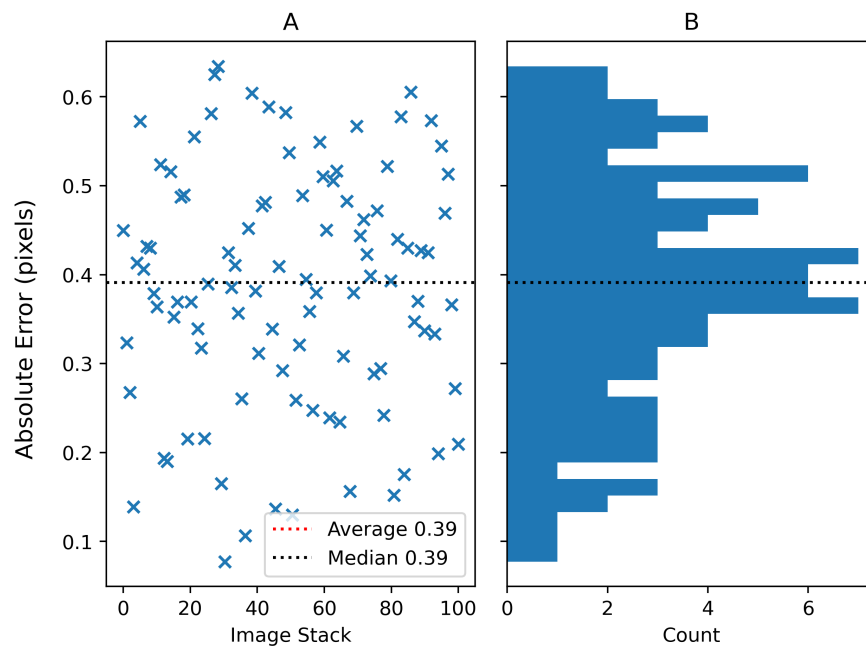
Figure 6. A: Absolute error from the ground-truth for R1.00, B: A histogram of errors from graph A
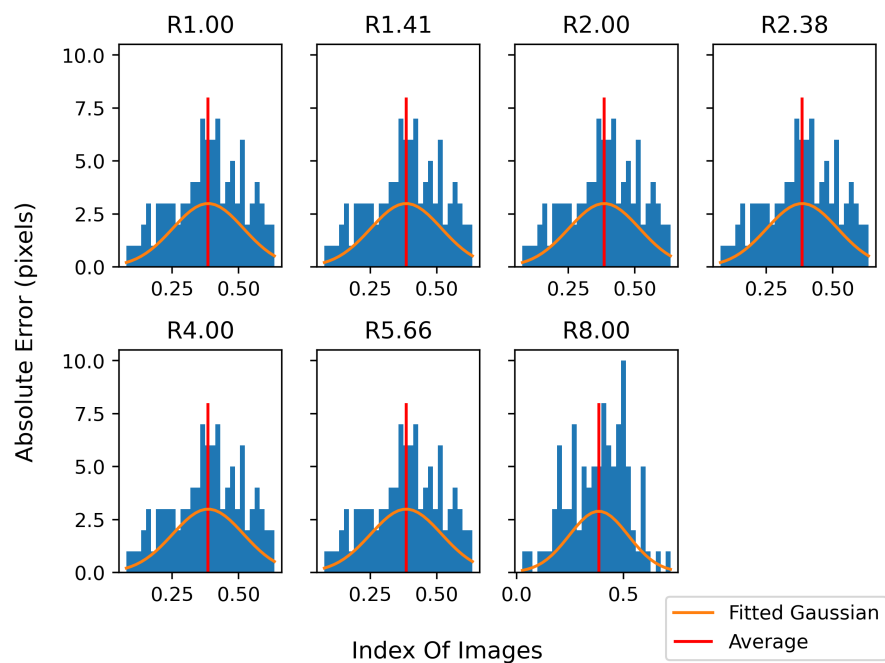

Figure 7. Histograms of the absolute error from each radii

Radii	Average	Variance
1.00	0.3850	0.0177
1.41	0.3850	0.0177
2.00	0.3850	0.0177
2.83	0.3850	0.0177
4.00	0.3850	0.0177
5.66	0.3850	0.0177
8.00	0.3847	0.0189

Table I. Values of figure 7

Figures 6 & 7 and Table I are a demonstration of how the absolute error of the spots are in a general Gaussian or normal distribution.

V.   Discussion

A.   Results in context of my aims

B.   Results in comparison with other studies/industry standard

C.   Explanations for unexpected results

D.   Discuss improvements

VI.   Conclusion

References

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