



PH450 Report 2021-22

Evaluating Spot Finding Methods

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Abstract

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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 by a factor of 2. 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 whatever is being imaged overcomes the diffraction limit by being fluorescent. 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 you just need to repeat this process temporally 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.[2] 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 $10^4 \mu m^{-2}$.[3][4]

B. reasoning behind my project

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.

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 work 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.[5] 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. There are two ways of doing this 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 but they also emit the light stochastically so with a fast enough camera the light spots can be independently seen. This also requires a method of finding and recombining each spot in each frame to obtain a final sharper image than before.[6]

II. State of the field/lit 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 [7]. Much of the innovation has come from optimising the algorithm or optimising the data going into the algorithm, in a 2011 paper by Zhai et al[8] Nyquist sampling was used to reconstruct an accurate point spread function(PSF) which helped achieve micro-pixel accuracy.

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)[7][9].

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

A powerful tool for image processing is ImageJ, this is an open source tool that can be used for anything from noise reduction, fourier transforms and finding maxima to analysis such as histograms to visualisations. Even better because it's open source you can either find plugins or create one. [10]

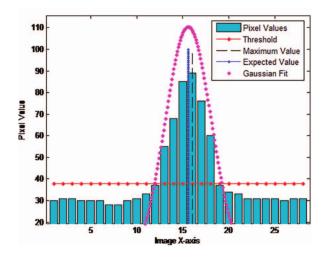


Figure 1. Intensity of real star data (Gienah) showing visually how data is pruned. [1]

B. Various fitting methods

- 1. Gaussian
- 2. Triangular method

IV. Theory (probably don't need)

V. Results

A. Centroiding

B. Triangle Fitting

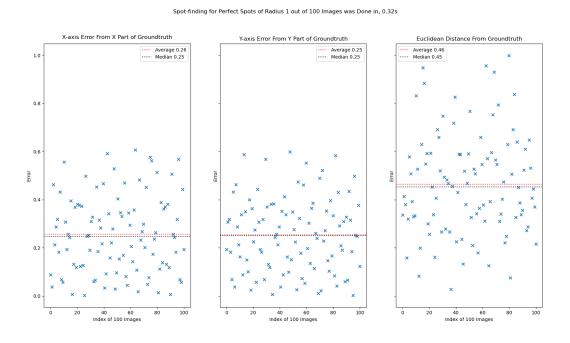


Figure 2.

Spot-finding for Perfect Spots of Radius 1.41 out of 100 Images was Done in, 0.32s

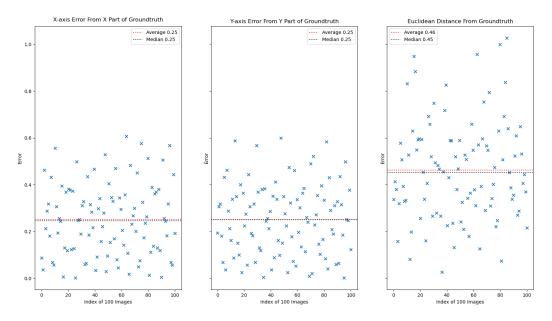


Figure 3.

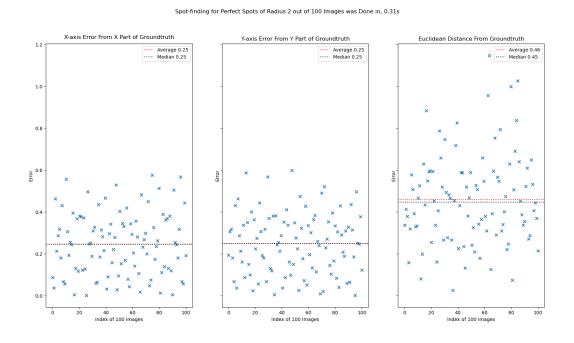


Figure 4.

Spot-finding for Perfect Spots of Radius 2.83 out of 100 Images was Done in, 0.31s

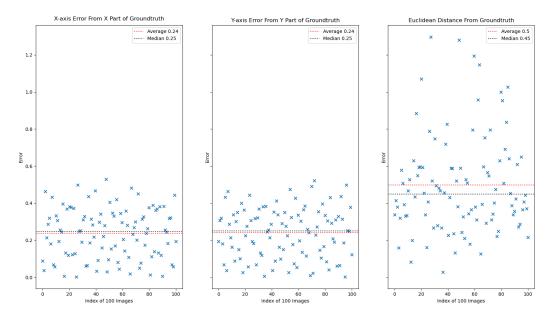


Figure 5.

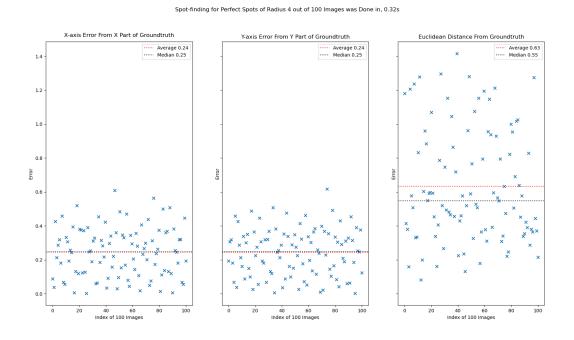


Figure 6.

Spot-finding for Perfect Spots of Radius 5 66 out of 100 Images was Done in 0.32s

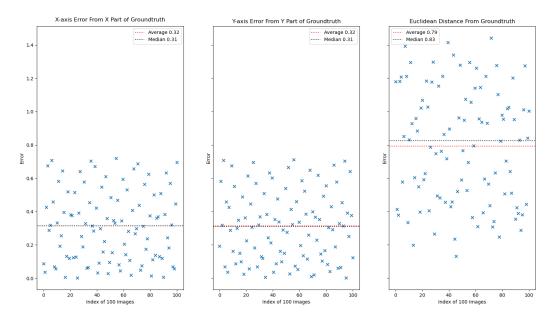


Figure 7.

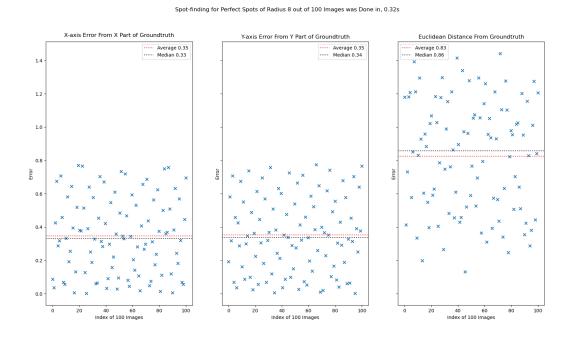


Figure 8.

Spot-finding for Perfect Spots of Radius 2 (with noise) out of 100 Images was Done in, 0.31s

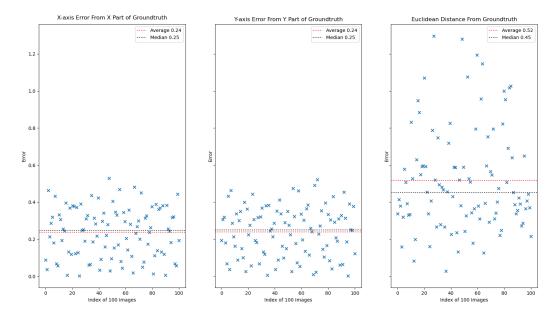


Figure 9.

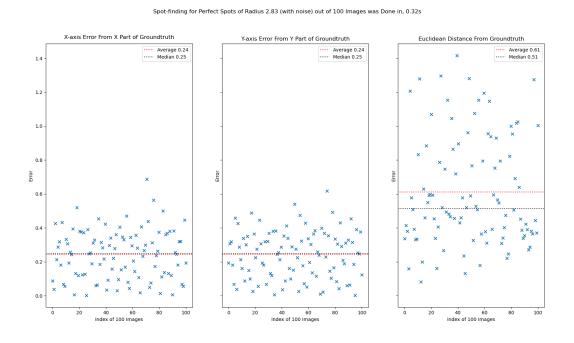


Figure 10.

Spot-finding for Perfect Spots of Radius 5.66 (with noise) out of 100 Images was Done in, 0.31s

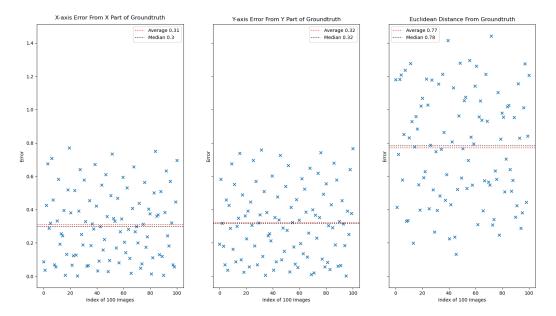


Figure 11.

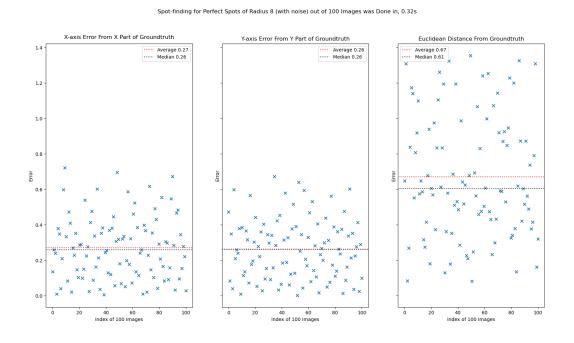


Figure 12.

VI. Analysis (maybe don't need)

VII. 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

VIII. Conclusion

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