SINGLE FLUORESCENT PARTICLE TRACKING ALGORITHMS: A COMPARATIVE STUDY

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READING ASSIGNMENT 2

SUMMARY

Single particle tracking. Many applications. Nanometer resolution algorithms for videos but never tested. Here 4 common tracking algorithms tested. Namely: Cross correlation, Sum-absolute difference (SAD), Centroid, and Direct Gaussian fit. Images of varying sizes (from point source to 5um) are generated, noise added and the algorithms made to perform while comparing each one of them for accuracy and precision. Results indicate that for large size particles, cross correlation is the most accurate. The most accurate and precise algorithms overall is Direct Gaussian for point sources. And both of them fail as S/N approaches 4.

INTRODUCTION

Single particle tracking refers to motion tracking of fluorescent particles at um level. In general, all methods for tracking follow 2 basic stems: Segmentation (using multiple particles followed by discrimination), and the Tracking algorithm. The main idea behind the current day tracking algorithms is as follows: Perform cross correlation of subsequent images, followed by calculation of center of mass of the relevant object, then fit Gaussian curves to intensity profile.

There are 2 kinds of errors associated with tracking algorithms: Determinate (Algorithmic inaccuracies. Biases towards the incorrect results) and Indeterminate (Fluctuations in individual measurements due to noise). These define the accuracy and prediction of the algorithm. The general method for finding the efficacy of algorithm is usually by tracking the position of a stationary object which is improved by estimating the position of the object and matching it with what is observed.

DETAILED SUMMARY

The protocol followed in the paper first generates images of fluorescent particles of varying sizes, followed by convolving them with appropriate PSF. They are then resampled with known sub particle displacement into lower resolution array representing a video camera, shot noise is added to give SNR between 1.3 and infinity and a comparison is done by performing 1000 iterations of image pairs with each of the four algorithms discussed below. The relationship of this study to fluorophore imaging is based on the fact that the signal to noise ratio is usually 3-4 in those studies.

The tracking algorithms can be divided into 2 categories: Those that estimate the absolute position of the particle in each image independently (Centroid, Direct Gaussian) and those that estimate the change in position of a particle by comparing an image to one subsequent. *(cross-correlation and SAD algorithms).*

The Centroid method is based on comparing the center of mass across successive images. It assumes that the object intensity is much greater than the background intensity. Thus there is a need to remove as much background noise as possible (generally done by using a threshold) in this method. The Gaussian method uses simplex algorithm and a least squares estimator to fit.

The equation for the Gaussian fit is given as follows: $G(x, y) = A.\exp\left[-\frac{(x-x_0)+(y-y_0)}{B}\right]$ where x_0 is the position of the curve center and A and B are arbitrary constants. Like in centroid algorithm, a difference is computed with the x0 and y0 fitted values to get the change in position.

The Correlation method is based on finding a correlation between values in the Kernel K (of the successive images) and the image I. It tends to match the brightest portion of the images, the expected error for which can be minimized by normalization using root mean square values. The sum absolute difference (SAD) algorithm is based on minimizing the sum of absolute differences between the overlapping pixels and using this information to determine the translation of Image relative to the Kernel K. There is another category of methods called the Interpolation methods which focus mainly on sub pixel resolution. It uses three main functions: Paraboloid, Cosinusoid, Gaussian

For all the algorithms, the bias was found to be approximately zero when the pixels were in multiples of 0.5. The COR was found to be less biased by an order than the other algorithms whereas the direct Gaussian fit was least biased. The Centroid algorithm biased towards the midpoint of adjacent pixels. Regarding precision as a function of actual distance moved, direct Gaussian fit standard deviation was independent of the actual distance moved followed by COR which had the second least s.d.

Comparing the algorithms in terms of Bias and the signal to noise ratio, the bias in case of centroid is approximately equal to the actual distance moved, COR and SAD choose random locations in the matrix Gaussian fit was found free to return values outside the actual boundaries. Comparing the algorithms in terms of precision and S/N, for centroid, the s.d. increases with increase in S/N whereas it decreases for the rest of the algorithms with best performance by Gaussian fit (s.d. < 1 at S/N = 4).

The main reason why Gaussian fit works with bias less than the COR is because, they are functionally very similar to each other. Both involve matching to an underlying pixel intensity matrix. The only difference is that the Gaussian kernels are noise free. Thus it can operate at a lower S/N.

DISCUSSION

The paper goes into great depth about the comparison between the currently existing algorithms for particle tracking algorithms. Not only they discuss the results that they obtained but they compare the results from similar studies and explain why the results differ clearly. They discuss about the limiting S/N ratio that how much is enough for the algorithms to function. Thus the paper has lot of applications in the fields of sub pixel resolution and single molecule imaging.

REFERENCES

[1] Quantitative comparison of algorithms for tracking single fluorescent particles. M K Cheezum, W F Walker, W H Guilford Biophys J. 2001 October; 81(4): 2378–2388.