FLUORESCENT PROBES LOCALIZATION ANALYSIS AT NANOMETER SCALE.

PTANDON1

READING ASSIGNMENT 2

SUMMARY

The paper discusses the key factors involved in determination of localization precision aims to solve the problems of localization and photon counting precision by using the information from the experimental parameters like the pixel size and background noise. The authors take one step ahead and propose robust computational algorithm for localizing subresolution particles with an equivalent efficiency to that of a least squares fit.

INTRODUCTION

The main idea that drives the work done in the paper is that even though an observed object's size is limited by the microscope's resolution, if the number of photons is sufficient, it is feasible to determine the center of the object with precision. Two kinds of noise play an important role in the sub pixel resolution of particles. These are namely: *photon shot noise* and the *background noise.* Pertaining to these noises, two cases exist for each noise: Shot noise limited case (When noise in each pixel is dominated by the photons emitted but the particle being localized) and the background noise limited case (when the noise is from other sources i.e. CCD, dark current).

For the experiment carried out, 2 fitting algorithms were used namely: Gaussian mask (a simplified version of the nonlinear least squares that ignores shot noise) and the full least squares Gaussian fit. The dependence of the noise on the number of photons along with the derivation of localization precision related to the noise is discussed in the following section.

DETAILED SUMMARY

According to theory, the best estimate of a particle's position suggested by equation of standard error of the mean of shot noise and taking into account pixelating noise is given as:

$< (\Delta x)^2 > = \frac{s^2}{N}$. Adding on the background noise, the expected value of uncertainty in position is: $< (\Delta x)^2 > = \frac{s^2+a^2/12}{N} + \frac{4\sqrt{\pi}s^3b^2}{aN^2}$, where the first term is contributed by the photon noise added with the pixelating noise $a^2/12$. The second term comes into play due to the background noise which is devoid of the pixelating noise because during its calculation from the generic equation of the mean squared error, using integration, the pixel size is considered very small.

This clearly demonstrates the relation between the noise scaling with the number of photons; i.e. Photon noise scales as $\frac{1}{\sqrt{N}}$ and the background noise scales as $\frac{1}{N}$. The transition point where the photon shot noise and the background noise become equal determines which noise dominates based on the value of N. Thus an expression for N_t $(Transition)$ is obtained such that $N_t = \frac{4\sqrt{\pi}s^3b^2}{a\left(s^2+\frac{a^2}{12}\right)}$. An important conclusion that can be drawn here is that if the number of photons is less than this number the noise will be dominated by the background noise otherwise the photon shot noise. Having the expression for N_t, the error for the estimated total number of photons is obtained by differentiating with respect to N, given by $< (\Delta N)^2 > = N + \frac{2\sqrt{\pi}sb^2}{a}$.

In 2 dimensions, the equations transform to:

$$< (\Delta x)^2 > = \frac{s^2+a^2/12}{N} + \frac{8\pi s^4 b^2}{a^2 N^2}, N_t = \frac{8\pi s^4 b^2}{a^2\left(s^2+\frac{a^2}{12}\right)} \text{ and } < (\Delta N)^2 > = N + \frac{4\pi s^2 b^2}{a^2}$$

The experimental comparison results depict that the uncertainty obtained using the Gaussian mask algorithm is 30% greater than the theoretical predictions. This can be attributed to the following reasons: 10% of the error due to analytic interpolation between the limits, very small pixel assumption (no pixelating noise assumed in theory), and it ignores the photon noise.

Regarding the full least squares gaussian fits, theory and the algorithm agree at high N but at low N both show excess errors over the theory (likely due to difficulty in locating the spot). At medium N, theory underestimates the error for which the cause is attributed to analytic interpolation. To analyze more in detail the performance of the algorithms, the number of variables was increased. The 2 cases where the theory and algorithms did not agree were: when number of photons is low and high background noise (i.e. Low S/N ratio), and when the particle is spread over too few pixels (Pixelation noise becomes more apparent).

Although Gaussian mask is a simplification of the non-linear least squares, the simplification actually may lead to loss in localization precision at high N. In general, the theory predicted the outcomes to a large extent, which could thus be useful in guiding a broad range of future experimentation.

The paper suggests that if background noise is cellular auto-fluorescence or from other random sources, confocal laser scanning microscopes can be used to offer advantage in this and also in the fact that they use the wavelength not closer to the excitation wavelength. Other avenues include Quantitative sub-resolution imaging which is gaining popularity of late.

DISCUSSIONS

The paper goes into great depth about how the dependencies exist between the noise and the number of photons. The algorithm derived is very insightful pertaining to the factors that govern sub pixel resolution of particles. Not only the algorithm and the math, the paper also addresses possible avenues to solve the issues associated. Thus it provides a well-structured layout for future experimentation design and how to handle the issues at hand.

REFERENCES

[1] Precise nanometer localization analysis for individual fluorescent probes. Russell E Thompson, Daniel R Larson, Watt W Webb Biophys J. 2002 May; 82(5): 2775–2783.