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Comparison of estimation algorithms in single-molecule localization

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ABSTRACT

Different techniques have been advocated for estimating single molecule locations from microscopy images. The question arises as to which technique produces the most accurate results. Various factors, e.g. the stochastic nature of the photon emission/detection process, extraneous additive noise, pixelation, etc., result in the estimated single molecule location deviating from its true location. Here, we review the results presented by [Abraham et. al, Optics Express, 2009, 23352-23373], where the performance of the maximum likelihood and nonlinear least squares estimators for estimating single molecule locations are compared. Our results show that on average both estimators recover the true single molecule location in all scenarios. Comparing the standard deviations of the estimates, we find that in the absence of noise and modeling inaccuracies, the maximum likelihood estimator is more accurate than the non-linear least squares estimator, and attains the best achievable accuracy for the sets of experimental and imaging conditions tested. In the presence of noise and modeling inaccuracies, the maximum likelihood estimator produces results with consistent accuracy across various model mismatches and misspecifications. At high noise levels, neither estimator has an accuracy advantage over the other. We also present new results regarding the performance of the maximum likelihood estimator with respect to the objective function used to fit data containing both additive Gaussian and Poisson noise. Comparisons were also carried out between two localization accuracy measures derived previously. User-friendly software packages were developed for single molecule location estimation (EstimationTool) and localization accuracy calculations (FandPLimitTool).

Keywords: localization, single molecule, tracking, Cramer-Rao lower bound

1. INTRODUCTION

In recent years, detailed information about intracellular components and various cellular mechanisms that were otherwise obscured by conventional fluorescence imaging techniques has been uncovered by single molecule microscopy techniques.[1–3] A key step in the analysis of single molecule data is determining the locations of single molecules. The accuracy of the analysis is, therefore, dependent on the accuracy with which the locations of single molecules can be determined. Over the last decade, we have also seen the development of super-resolution imaging techniques that rely on exciting and localizing small sparsely distributed subsets of fluorophores.[4–6] In these techniques, the super-resolution image is reconstructed from the estimated locations of the fluorophores. The quality of the super-resolution image, therefore, is also dependent on the accuracy with which fluorophores can be localized.

Determining the location of a single molecule is not straightforward because of the stochastic nature of the photon emission and detection processes, noise from background autofluorescence and camera readout, pixelation, etc. Various techniques for localizing single molecules from microscopy images are available. The question arises as to which technique produces the most accurate results. In a previous comparative study of some of the available techniques,[7] it was concluded that fitting Gaussian profiles using the least squares algorithm produces the most accurate results. However, neither the maximum likelihood estimator, an extensively studied estimator with a well established performance in statistical and signal processing applications,[8] nor the fitting of Airy profiles, which in many cases models single molecule data better than Gaussian profiles, was investigated in this comparison.

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Here we summarize the results by Abraham et. al.[9] of a systematic comparison of the nonlinear least squares and maximum likelihood estimators for various single molecule location estimation scenarios using both Gaussian and Airy profiles to model the data. In addition, we present new results regarding the performance of the maximum likelihood estimator when estimating single molecule locations from data that contains both additive Poisson and Gaussian noise components. In the study by Abraham et. al.,[9] the objective function used with the maximum likelihood estimator correctly modeled the noise component as a mixture of both Poisson and Gaussian noise sources. However, this objective function is computationally intensive. Here we look at the performance of the maximum likelihood estimator using a simpler objective function that assumes the data has only Poisson characteristics, when performing single molecule location estimations on data that contains both additive Gaussian and Poisson noise components.

Since the various techniques for estimating the location of a single molecule produce results with varying accuracies, the question arises as to what the best possible accuracy is with which a single molecule can be localized for a given set of imaging and experimental conditions irrespective of the estimation technique being used. A simple formula, derived on the basis of certain approximations, has previously been published by Thompson et. al.[10] to estimate the localization error and has been used extensively in various studies.[5,6,11,12] We have previously presented a rigorous framework to calculate the localization accuracy for various estimation scenarios using the theory of the Fisher information matrix and the Cramer-Rao lower bound.[13,14] Using this framework, the limit of the localization accuracy, which is the best possible accuracy for a given set of imaging and experimental conditions using an unbiased estimator, can be calculated. Here, we also summarize the results of a comparison between these two approaches for determining the localization error for a given set of experimental and imaging conditions.

2. METHOD

Our approach to compare the performance of the maximum likelihood and nonlinear least squares estimators was to simulate multiple images of a single molecule for each estimation scenario, estimate the location of the single molecule from each image using both estimators, and compare the standard deviation of the estimates from each algorithm for each scenario. For each estimation scenario, we also calculate the practical localization accuracy measure (PLAM), which is the square root of the lower bound on the variance of 2D location estimates of a single molecule when detection is carried out with a pixelated detector of finite area and when the signal is potentially corrupted by extraneous noise sources. The equations involved in estimating the locations of single molecules, the Airy and Gaussian profiles, and the calculation of the limit of the localization accuracy, along with the details of the implementations of these calculations can be found elsewhere.[9,13,14] Since it is also important to ensure that both estimators recover the true parameter, i.e. the location of the single molecule, for each estimation scenario we examined the mean of the estimates to ensure that they match the true location coordinate parameters and to ensure that there is no obvious bias in the estimates.

3. RESULTS

3.1 Ideal practical scenario

We first investigated the performance of the two estimators in the ideal practical case where the data has no extraneous additive noise and the model used to the fit the data matches the model by which the data is generated. Our results show that in this scenario the standard deviations of the estimates from the maximum likelihood estimator were smaller than those from the nonlinear least squares estimator for the various photon counts we tested. This was true irrespective of whether the data generation and fitting were done using Airy or Gaussian profiles. Further, the standard deviations of the estimates from the maximum likelihood estimator matched the PLAM in this scenario, indicating that for this case, the maximum likelihood estimator is optimal.

3.2 Single molecules located away from the center of the pixel

Practically, it is highly improbable that a single molecule should be located at the center of the pixel. We have previously shown that the localization accuracy is dependent on the location of the point source relative to the pixel.[13] Therefore, to avoid inconsistencies in the study arising from varying the location of the point source

along with other parameters, we fixed the position of the point source to the center of pixel in all our calculations. As it will be very rare in practice for the single molecule to be located at the center, we also investigated the performance of the two algorithms when the point source is located away from the center. We found that while the maximum likelihood algorithm produced estimates with consistent standard deviations irrespective of the location of the point source, the performance of the nonlinear least squares estimator was dependent on the location. The further away from the center of a pixel, and closer to the edge of the pixel, the point source was located, the larger were the standard deviations of the estimates from the nonlinear least squares estimator. The standard deviations of the estimates from the maximum likelihood estimator, on the other hand, matched the PLAM irrespective of the location of the point source.

3.3 Effect of noise on the performance of the estimators

In a practical situation, the data is invariably corrupted by background auto-fluorescence, modeled as additive Poisson noise, or noise from the readout process, modeled as additive Gaussian noise. To study the effects of noise on the performance of the two algorithms, images of a single molecule with various levels of additive Gaussian and Poisson noise were simulated and the location of the single molecule was estimated from each image using both estimators. Comparing the standard deviations of the estimates to the PLAM, we see that as the noise component in the data increases, the standard deviations of estimates from both algorithms converge to the PLAM. However, at low noise levels, the maximum likelihood estimator has a clear accuracy advantage when compared to the nonlinear least squares estimator.

The objective function for the maximum likelihood estimator can be chosen based on the nature of the underlying data. In our calculations we have used two specific objective functions, one which assumes the underlying data is only Poisson in nature, and the second, which is computationally more intensive than the first and consequently takes much longer to execute, which accounts for the presence of additive Gaussian noise in addition to Poisson noise. Since the execution time of the maximum likelihood estimator with the objective function that assumes only Poisson noise is comparable to the nonlinear least squares estimator, we wanted to study the behavior of the maximum likelihood estimator on data containing both additive Poisson and Gaussian noise components based on the two objective functions. We estimated the location of a single molecule from images containing additive Poisson and Gaussian noise using the maximum likelihood estimator, first with the objective function that assumes the data is only Poisson in nature, and then with the computationally intensive objective function that accounts for both types of noise in the data. The results from this calculation are displayed in Fig. 1.

When examining the standard deviations of the estimates from the maximum likelihood estimator using the objective function that assumes the data is strictly Poisson in nature, we see that for low Poisson or high Gaussian noise levels the standard deviations of the estimates are considerably higher than those from the nonlinear least squares estimator and the maximum likelihood estimator with the objective function that correctly models the noise. On the other hand, for high Poisson noise levels or low Gaussian noise levels, the standard deviations of the estimates from the maximum likelihood estimator using the objective function that assumes the data is strictly Poisson in nature matches the standard deviations of the estimates from the maximum likelihood estimator that correctly models the noise component. This can be explained by the inability of the objective function that assumes the data to be strictly Poisson in nature to account for negative data values.

While the digital count corresponding to each pixel is never negative, the photon count corresponding to a pixel can be negative after camera gain conversion and background or camera offset subtraction, depending on the specified background or offset. Unlike the objective function that accounts for both Gaussian and Poisson noise components, the objective function that assumes the data to have only Poisson characteristics runs into problems when fitting data with negative values. During an estimation, the implementation of this objective function incorrectly weights pixels with negative values, which appear more towards the edge of the single molecule profile, thus causing the peak of the single molecule profile to be estimated with lower accuracy, resulting in larger standard deviations. This is confirmed by truncating negative pixel values to zero in the data before fitting. In this case, as the results show, the standard deviations are smaller than in the case where the data containing negative pixel values as the incorrect weighting is eliminated leading to a more accurate estimate of the peak of the single molecule profile. This suggests that if the objective function that assumes the data is

strictly Poisson in nature is to be used, care must be taken in background subtraction to eliminate problems with negative values and data truncation.

3.4 Effect of width misspecification

To reduce the time taken to complete estimates and to avoid problems that can arise from simultaneously estimating the width parameter in addition to other parameters, the width parameter value is specified. However, the specified width parameter value can deviate from the true value due to various factors. For example, the sample may be away from the true plane of focus by a few hundred nanometers because visual depth discrimination is poor close to the plane of focus. In our experience, this can cause the width parameter of the PSF to deviate by up to 50%, which we have also verified through simulations. Deviations in the width parameter can also arise from failing to consider deviations in the optical properties of the optical elements from their design specifications.[15] Therefore, it is important to understand what the effect of misspecifying the width parameter is on the accuracy of estimates from the two estimators. Our results show that for both Airy and Gaussian profiles, the maximum likelihood estimator produces results with consistent accuray. However, the accuracy of the results of the nonlinear least squares estimator is dependent on the way the width parameter has been misspecified. If the specified width parameter causes the profile being fit to be wider than the profile of the single molecule, the standard deviations of the estimates from the nonlinear least squares algorithm are comparable to those from the maximum likelihood estimator. However, if the specified width parameter causes the profile being fit to be narrower than the profile of the single molecule, the standard deviations of the estimates from the nonlinear least squares estimator are significantly larger than the standard deviations of the estimates from the maximum likelihood estimator.

3.5 Effect of model mismatches

The Airy and Gaussian profiles are commonly used to model the image of a single molecule. In many situations where the Airy profile is a more accurate model, Gaussian profiles have been used to the fit the data to estimate single molecule locations.[16] Our results show that in such situations, the nonlinear least squares estimator provides more accurate results than the maximum likelihood estimator, showing the greater sensitivity of the maximum likelihood estimator to the model. However, it must be pointed out that the standard deviations from the nonlinear least squares algorithm in this case do not attain the PLAM, suggesting that to achieve the best accuracy it is important to use the maximum likelihood algorithm with the correct model. If in addition to mismatching the model, the width parameter is misspecified, the maximum likelihood estimator again produces results with consistent accuracy while the results from the nonlinear least squares estimator are dependent on the nature of the misspecification.

3.6 Comparison of two analytical approaches to predicting localization accuracies

As mentioned earlier, Thompson et. al. have previously provided a formula to estimate the localization error[10] which has been used extensively. We have previously presented a more rigorous approach to calculating the localization error based on the theory of the Fisher information matrix. The results of the calculations reviewed here indicate that the theoretically optimum standard deviations as derived by the Fisher information matrix based approach can in fact be attained by the maximum likelihood estimator. We therefore wanted to compare the localization accuracies predicted by the two approaches.

The approach by Thompson et. al.[10] assumes that the image of a single molecule is adequately modeled by a Gaussian profile. In this case, the localization error calculated using the Fisher information matrix approach matches the localization error calculated using the formula provided by Thompson et. al. If, however, the Airy profile is a better model of the data, then the results from the Fisher information matrix approach differ from the results calculated using equivalent parameters with the formula provided by Thompson et. al. A simple comparison, in this case, of the results from the two approaches in the absence of deteriorating influences such as pixelation and noise shows that there is approximately a 30% difference in the localization accuracy predicted by the two approaches. When the effects of pixelation are also considered, the Fisher information matrix based approach predicts lower standard deviations than the approach by Thompson et. al. for small pixel sizes and conversely for larger pixel sizes.

4. CONCLUSIONS

In all scenarios we examined, both the maximum likelihood and nonlinear least squares estimators are, on average, able to recover the true location of the single molecule without any discernible bias. From the various scenarios, we see that the comparative accuracy of the two estimators depends on the specific estimation scenario. Comparing the standard deviations of the estimates from both algorithms, we see that in many important situations, the standard deviations from the maximum likelihood algorithm are smaller than those from the nonlinear least squares algorithm, indicating that the maximum likelihood estimator is more accurate. In fact, in these scenarios, the standard deviations of the estimates from the maximum likelihood estimator attain the PLAM, indicating that they are optimal for those estimation scenarios. When the data contains a large noise component, our results show that neither algorithm has an accuracy advantage over the other. However, at low signal levels we see that the maximum likelihood estimator again produces more accurate results than the nonlinear least squares estimator. This observation is consistent with results reported in the deconvolution literature with regard to the performance of these two algorithms in the realm of quantum limited data.[17–19] While the accuracy advantage of the maximum likelihood estimator does not always hold when performing estimations with model mismatches or misspecifications, it should be noted that this estimator still produces results with consistent accuracies across the various model mismatches and misspecifications we examined. It should also be noted that in the presence of such distortions, neither algorithm should be expected to behave properly. If a certain type of modeling uncertainty or mismatch is expected and highly accurate results are required, an analysis similar to this one may need to be conducted to understand the effects of such distortions on the accuracy of the results from the various estimation techniques.

5. SOFTWARE

The calculations involved in estimating the location of a single molecule are relatively complex. Calculating the limit of the localization accuracy also involves many non-trivial computational steps. The lack of appropriate software to address the computational complexity has sometimes prevented the use of some of these methods. The EstimationTool and FandPLimitTool software packages were developed in MATLAB to facilitate single molecule localization calculations and limit of the localization accuracy calculations respectively. The EstimationTool supports both the Airy and Gaussian profiles and both the nonlinear least squares and maximum likelihood estimators using both objective functions discussed here. The FandPLimitTool similarly supports both the Airy and Gaussian profiles for various estimation scenarios.

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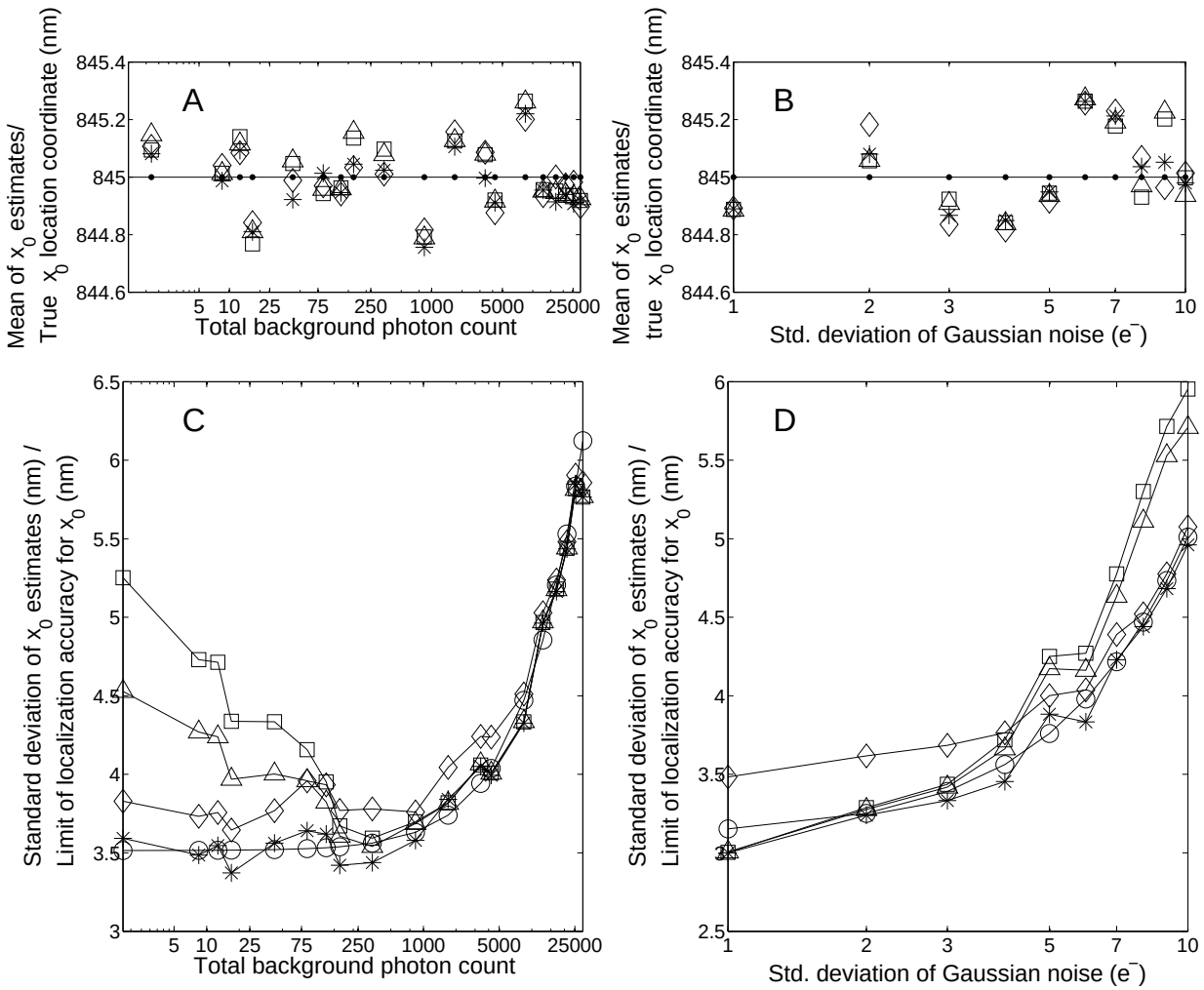

Figure 1. Comparison of the maximum likelihood and nonlinear least squares estimators in the presence of additive Poisson and Gaussian noise. Panels A(C) and B(D) show the mean(standard deviation) of the estimates from the nonlinear least squares estimator ($\Diamond$), the maximum likelihood estimator using the objective function that assumes the data has only additive Poisson noise, both when negative values in the data are truncated to zero ($\triangle$) and when they are not ($\Box$), and the maximum likelihood estimator using the objective function that accounts for both additive Poisson and Gaussian noise in the data ($*$). ($\bullet$) indicates the true x_0 coordinate value, and ($\circ$) indicates the PLAM or the limit of the localization accuracy of x_0. In panels A and C, 1000 images of a stationary single molecule were generated using the Airy pixelated profile and readout noise with standard deviation of $4e^-$ for each value of the total background photon count. In panels B and D, 1000 images of a stationary single molecule were generated using the Airy pixelated profile and fixed background of 2 photons/pixel/s for each value of the standard deviation of Gaussian noise. The mean of the Gaussian noise component was set to zero in all cases. Location coordinates (x_0, y_0) were estimated from each image using the nonlinear least squares estimator, the maximum likelihood estimator using the objective function that assumes the data has only additive Poisson noise, and the maximum likelihood estimator using the objective function that accounts for both additive Poisson and Gaussian noise in the data. Values for the width, photon detection rate, and background parameters, were fixed to the values used to generate the images. The following numerical values were used when simulating the single molecule images. Pixel size: 13 $\mu m \times 13$ μm, pixel array size: 13×13, expected number of photons from the single molecule at the detector plane: 1000 photons, magnification $M = 100$, wavelength $\lambda = 520$ nm, numerical aperture $n_a = 1.3$. The single molecule image was centered within the pixel array.