Fast maximum likelihood algorithm for localization of fluorescent molecules

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A common task in microscopy is to fit an image of a fluorescent probe to a point spread function (PSF), in order to estimate the position of the probe. The PSF is often approximated as a Gaussian for mathematical simplicity. We show that the separable property of the Gaussian PSF enables a reduction of computational time from $O(L^2)$ to $O(L)$, where $L$ is the width (in pixels) of the image. When tested on realistic simulated data, our algorithm is able to localize the probes with precision close to the Cramer-Rao Lower Bound. © 2011 Optical Society of America

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Estimating the position of a fluorescent molecule from a microscope image is a key task in single-molecule biological experiments [1] and superresolution microscopy [2]. Although diffraction causes the width of the image to be on the order of the wavelength of light or larger, the coordinates of the image’s center (corresponding to the coordinates of the molecule) can be estimated with high precision, limited only by the number of photons detected [3]. In the ideal case, when pixellation effects are negligible and the only noise is shot noise, if the same molecule is imaged repeatedly (each acquisition lasting the same time and the molecule emitting photons at a steady rate) the maximum precision (i.e. smallest possible standard deviation of the repeated position estimates) is $\delta x \geq \lambda / (2\pi NA \sqrt{N})$, where $\lambda$ is the wavelength of the light emitted by the molecule, NA is the numerical aperture of the imaging system, and $N$ is the average number of photons emitted by the molecule during image acquisition [3].

The theorem of Cramér and Rao states that this maximum precision is achievable if the position is estimated by fitting the hardware’s point spread function (PSF) to the image via Maximum Likelihood Estimation (MLE) [3], with the molecular coordinates $(x_0, y_0)$ as fitting parameters. Using MLE to determine a molecular position requires that one have a model for both the PSF (e.g. 2D Gaussian) and the noise in light detection (e.g. shot noise). Based on the parameters of the model, one can calculate the likelihood of obtaining the given data, and vary the parameters to maximize the likelihood of the data. MLE has been implemented on graphics processing units to estimate molecular positions at high speed [4]. Other popular approaches, which do not require detailed noise models, include Least Squares fits [5], a Gaussian Mask algorithm based on Least Squares [6], and iterated centroid methods to mitigate the effects of background [7]. However, in all of these methods the computational time is proportional to the number of pixels in the image. The proportionality between time and number of pixels depends on the complexity of the model being fit to and the convergence criteria, but the number of function evaluations in an iteration of MLE or Least Squares will be proportional to the number of pixels.

Although the PSF is (often) closer to an Airy function $(2J_1(\lambda r)/kr)^2$, where $k = 2\pi/\lambda$, fitting to a Gaussian PSF is a common practice [4, 6, 8] that has been shown to give accurate and precise estimates with performance close to the theoretical limit [8]. Here we will perform faster MLE fits by using the separable property of the Gaussian function: $e^{-\alpha k^2((x-x_0)^2+(y-y_0)^2)} = e^{-\alpha k^2(x-x_0)^2}e^{-\alpha k^2(y-y_0)^2}$, where $\alpha$ is a numerical factor that we will assume to be 0.28 [5]. Although this separable property of the Gaussian PSF has been used in deconvolution [9] and estimates of the Cramer-Rao lower bound [10], we are not aware of any published studies of the performance of maximum likelihood position estimators using our approach.

We can model the signal $S$ on a pixel centered at $(x, y)$ as a Poisson random variable, and fit to it an expression $\mu(x, y)$ equal to the sum of a PSF and a background:

$$\mu(x, y) = b + I_0 \iint_{\text{pixel area}} e^{-\alpha k^2((x'-x_0)^2+(y'-y_0)^2)} \, dx' \, dy'$$

where $b$ is the average background count per pixel and $I_0$ is proportional to the integration time and the photon emission rate of the molecule. We assume that the background is a combination of out-of-focus and scattered fluorescence, and is hence a Poisson random variable with mean $b$. Fits are done on a square region of interest (ROI) of $L \times L$ pixels. If the PSF is negligible outside of the ROI, and if the “dead space” between pixels is a negligible fraction of the detector’s surface area, we can approximate the sum of all of the photon counts in the column centered at $x$ by integrating $e^{-\alpha k^2(y-y_0)^2}$ over $(-\infty, \infty)$. The integral over the pixel width is a difference of error functions, so the summed signal $S_x$ in a
column centered at \( x \) has mean \( \mu_s(x) \) given by:

\[
\mu_s(x) = bL + \frac{\pi I_0 L}{2\alpha k^2} (\text{Erf}(x + a/2 - x_0) - \text{Erf}(x - a/2 - x_0))
\]

(2)

where \( a \) is the pixel width. Eq. (2) depends only on the \( x \) coordinate of the column; we can thus estimate \( x_0 \) and \( y_0 \) by fitting Eq. (2) to \( L \) column sums and \( L \) row sums rather than \( L^2 \) pixels. Consequently, we evaluate functions \( 2L \) times per iteration rather than \( L^2 \) times.

In order to estimate \( x_0 \) via MLE, we must vary a vector of parameters \( \theta = (x_0, b, I_0) \) to maximize the logarithm of the likelihood \( \mathcal{L}(S_s|\theta) \), i.e. the conditional probability of obtaining the data \( S_s \) (given as a vector of the signals from each pixel) given the parameters \( \theta \). (\( \alpha \) may also be varied, if it is unknown, but MLE is not sensitive to small errors in the assumed value of \( \alpha \) [8].) If we assume Poisson noise in photon detection, the log-likelihood is given by [3, 4]:

\[
\log \mathcal{L}(S_s|\theta) = \sum_x S_s(x) \log \mu_s(x; \theta) - \mu_s(x; \theta) - \log S_s(x)!
\]

(3)

where \( x \) is the pixel coordinate and the expected photon count is written \( \mu_s(x; \theta) \) to indicate that it depends on the fit parameters as well as the coordinate of the column. We can efficiently find a maximum by varying elements of \( \theta \) via the Newton-Raphson method [11]. (For details, see the supplemental materials of [4]).

For initial parameter estimates, we will estimate \( b \) by setting the smallest column sum \( S_{s,\text{min}} \) equal to \( bL \), and estimate \( x_0 \) with a background-corrected center of mass:

\[
x_{\text{cm}} = \frac{\sum_x x (S_s(x) - bL)}{\sum_x (S_s(x) - bL)}
\]

(4)

To estimate \( I_0 \), we will set the largest column sum \( S_{s,\text{max}} \) equal to the right side of Eq. (2), using our estimates of \( bL \) and \( x_0 \) and the \( x \) coordinate of the appropriate column. The Newton-Raphson method is then used to iterate from this initial point in parameter space to the maximum of \( \mathcal{L} \). It is important to note that our initial estimate of \( x_0 \) should, in most cases, be very close to the maximum-likelihood estimate: In many cases of practical interest, the photon counts are high enough that the shot noise is approximately Gaussian, we are approximating the PSF as Gaussian, and we have a reasonable first-pass background correction. For Gaussian noise (as opposed to Poisson), a Gaussian PSF, and small pixel size, the center of mass estimator is the MLE for the molecular position [3]. Also, when the initial estimate is close to the maximum, Newton-Raphson is known to require only a few iterations to converge [11]. In most of our work, we found that going beyond \( 2 - 3 \) iterations did not significantly reduce the variance of the position estimates, but \( 6 \) iterations were used for caution.

We tested the performance of this algorithm by estimating \( x_0 \) from simulated images generated with known molecular positions. The performance of MLE in fluorescent probe localization has been extensively studied as a function of photon count and background noise [8]; here we focus on cases where our assumptions (negligible gaps between pixels, PSF magnitude negligible outside ROI) are of marginal validity. We assume shot noise in light detection and additive background fluorescence, and use an Airy PSF integrated (numerically) over the pixel area to generate photon counts. The wavelength of light is 550 nm, the center-to-center pixel spacing is \( d = 110 \) nm, and the number of pixels \( L \) along the width of the ROI is varied, as well as the pixel width \( a \leq d \). We generated the images so that 1000 photons are detected (in the absence of background) when the molecule is located at the center of the ROI in the best case for each figure; shrinking the ROI or displacing the molecule by \((x_0, y_0)\) from the center of the ROI causes a smaller number of photons to be detected because some photons now fall outside the ROI. (However, we do not reduce the number of photons or the background if the pixel area is decreased, assuming that integration time increases commensurately, with proportional effects on signal and background.) We compared the background-free case with a background of 4 photons/pixel (256 or 400 background photons total over the image, i.e. 25% or 40% of the actual signal). For each value of \( x_0 \) in the plots shown, we generated 1600 images to calculate the mean and standard deviation of the \( x_0 \) estimates, thereby determining the bias (if any) and precision of the estimates.

All calculations in Figure 1 were done in Matlab, except that standard deviations in a “best case” were compared with the “Practical Localization Accuracy Measure” (PLAM), which was calculated with the program FandPLimitTool [8]. In our ideal case, FandPLimitTool assumed that fitting was done on a 2D image rather than column sums, that there is no gap between pixels, that \( y_0 = 0 \) (so that the ROI is correctly centered), and that the ROI is \( 12 \times 12 \) (i.e. 2.4x across, large enough to capture the entire central maximum of the PSF). In the other calculations, we considered worse cases: a smaller ROI (\( 8 \times 8 \) or \( 10 \times 10 \), corresponding to widths of \( 1.6 \lambda \) or \( 2\lambda \)), an off-center ROI (\( y_0 = d \) or \( 2d \)), and substantial gaps between pixel edges (either 10% or 20% of the center-to-center spacing \( d \)).

In both graphs, the PLAM for the ideal case is approximately constant as a function of \( x_0 \), because small displacements do not significantly reduce the number of photons falling in the ROI. The precision (standard deviation of estimates) is close to the PLAM for molecules near the center of the ROI, even if the molecule is displaced from the center of the ROI by two and a half pixels (indicating a poorly-drawn ROI). In all cases shown here the bias in the \( x_0 \) estimate is smaller than the standard deviation. In a few cases the variance of our estimates is about 2% lower than the PLAM; we attribute this to the fact that when estimating the variance of a distribution, the estimate of the standard deviation will have a fractional uncertainty of order \( 1/\sqrt{n} = 2.5\% \) for a sample of
visual confirmation that our estimator is unbiased and is still 550 nm. The performance in Fig. 2 provides background photons per pixel is 4. (The wavelength is 600, the pixel size is 150 nm, and the number of molecules. To increase the spread of the estimates indicates the position estimates from repeated imaging dots indicate actual molecular positions, and blue dots show the results of repeated application of this algorithm (available at molecule-localization-plugin/) in Figure 2 we see that in the absence of background the standard deviation starts at a lower value (as expected) and becomes worse (especially for a large ROI), while in the case with background the standard deviation starts at a higher value but rises more slowly. We attribute this to model mismatch: We are making the common approximation of fitting a Gaussian to data generated with an Airy PSF. This approximation is least accurate in the tails of the PSF, and a very far tail of the PSF will be found on the left side of the image for large and a large ROI. Fitting this poorly-matched model to our data will affect the log-likelihood score. This suggests that while our approximation of integrating over all might be less valid for a small ROI, the breakdown of that approximation for a small ROI is mitigated by the avoidance of model mismatch effects.

Finally, we have created an ImageJ plugin that implements our localization algorithm (available at http://code.google.com/p/molecule-localization-plugin/) in Figure 2 we show the results of repeated application of this algorithm to an image of 3 fluorescent molecules. The red dots indicate actual molecular positions, and blue dots indicate the position estimates from repeated imaging of the molecules. To increase the spread of the estimates for display purposes, the photon count per molecule is 600, the pixel size is 150 nm, and the number of background photons per pixel is 4. (The wavelength is still 550 nm.) The performance in Fig. 2 provides visual confirmation that our estimator is unbiased and performs well irrespective of the molecule’s position relative to the pixel edges.

In conclusion, we have shown that Gaussian fitting via maximum likelihood estimate can be sped up from $O(L^2)$ to $O(L)$ by taking advantage of the separable property of the Gaussian PSF. The algorithm yields performance comparable to standard MLE approaches even when the key assumptions behind the algorithm (no gaps between pixels, and a PSF completely contained within the ROI) are violated.

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References
Bibliography with article titles (for review purposes)

References