Fast and precise algorithm based on maximum radial symmetry for single molecule localization

Hongqiang Ma,[1,2] Fan Long,[1,2] Shaoqun Zeng,[1,2] and Zhen-Li Huang[1,2,*]

[1]*Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics-Huazhong University of Science and Technology, Wuhan 430074, China*

[2]*Key Laboratory of Biomedical Photonics of Ministry of Education, Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China*

**Corresponding author: leo@mail.hust.edu.cn*

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We present an algorithm to estimate the location of single fluorescent molecule with both high speed and high precision. This algorithm is based on finding the subpixel position with maximum radial symmetry in a pixelated single molecule fluorescence image. Compared with conventional algorithms, this algorithm does not rely on point-spread-function or noise model. Through numerical simulation and experimental analysis, we found that this algorithm exhibits localization precision very close to the maximum likelihood estimator (MLE), while executes ~1000 times faster than the MLE and ~6 times faster than the fluoroBancroft algorithm.

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Localization microscopy is increasingly recognized as a valuable tool for direct visualization of molecular events in living cells. This technique relies mainly on repeated localization of active (fluorescent) molecules from hundreds or even thousands of images to build a final super-resolution image [1]. Therefore, it is of particular importance to develop efficient algorithms for molecule localization with sufficient speed and precision. In the past several years, a number of algorithms have been reported for such purpose [2–5]. In particular, a combination use of maximum likelihood estimator (MLE) and graphics processing unit (GPU) parallel computation is capable of real-time processing of experimental images even from fast electron multiplying charge coupled device (EMCCD) cameras [6,7].

With the development of bright fluorescence probes, it is now possible to explore the potentials of combining localization microscopy with scientific complementary metal oxide semiconductor (sCMOS) camera [8] for significantly enhancing the versatility and power of localization microscopy in live cell imaging for which studying dynamics of collective cell migration is highly desired [9]. For this promising combination, which takes advantage of the extremely high data readout rate of sCMOS cameras (up to 560 MHz), the estimated computation duty for real-time molecule localization can be up to 50 times heavier than that from fast EMCCD cameras (~10 MHz). Unfortunately, this demand cannot be met from existing localization algorithms with both satisfactory speed and precision.

We notice that the point-spread-function (PSF) of a properly aligned microscope exhibits naturally radial symmetry [10]; therefore, for a two-dimensional (2D) fluorescence image originated from a single molecule with negligible or evenly distributed fluorescence background, the gradient distribution of the image also would exhibit radial symmetry. In this letter, we utilize the radial symmetry nature of a single molecule image, and derive an algebraic algorithm, termed maximum radial symmetry estimator (MrSE). Through simulation and experimental analysis, we verify that this new algorithm is capable of fast and precise single molecule localization.

Let us start from a pixelated image (A), which can be originated from any PSF profiles with radial symmetry distribution. We employ a rectangular coordinate system whose origin is located at the image center, and the x and y axes run parallel to the right and up directions, respectively. We assume that there is only one fluorescent emitter inside A, and that the molecule is located at pixel (x_0, y_0). In localization microscopy, the intensity of a pixel is contributed mainly by the fluorescence signal emitted by the active molecule and background fluorescence, because a low-light detector with negligible camera noise typically is used to capture the image.

First, we use two optimized gradient operators to convolve with the original pixelated image A, which gives the distribution of image gradient (G):

$$G_x = \begin{bmatrix} -1 & -1 & 0 & 1 & 1 \\ -1 & -1 & 0 & 1 & 1 \\ -1 & -1 & 0 & 1 & 1 \end{bmatrix} * A,$$

$$G_y = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 0 & 0 & 0 \\ -1 & -1 & -1 \\ -1 & -1 & -1 \end{bmatrix} * A. \tag{1}$$

The two operators in Eq. (1) were optimized by presenting better localization precision, and work well for PSF smaller than 10×10 pixels. We calculate the gradient direction of pixel (m, n) with the following equation:

$$\theta(m, n) = \arctan(G_y(m, n)/G_x(m, n)), \tag{2}$$

where $G_x(m, n)$ and $G_y(m, n)$ are image gradients in the x and y directions of pixel (m, n), respectively.

Next, we obtain a radial line that passes through the midpoint of pixel (m, n) in a direction parallel to its gradient direction $\theta(m, n)$:

$$y = K(m, n)x + C(m, n), \tag{3}$$

where $K(m,n)$ denotes $\tan(\theta(m,n))$, and $C(m,n)$ is determined by $(n - K(m,n) \times m)$.

In a noise-free, nonpixelated image, the gradient at any position will point exactly to the emission center, and the radial lines will all pass through the exact position of the molecule. Unfortunately, for a noisy and pixelated image, the radial lines may deviate from the exact position, indicating that it is not practical to obtain the exact molecule position directly by finding the crossing point of these lines. In this case, according to a well-established least squares method, we estimate the molecule location by finding a subpixel point that has the minimum sum of squares of the deviations (D_{sum}) to those radial lines (see Fig. 1), using the following equation:

$$D_{\text{sum}}(x,y) = \sum_m \sum_n \left(\frac{|K(m,n)x - y + C(m,n)|}{\sqrt{1 + K^2(m,n)}} \right)^2. \quad (4)$$

Mathematically, D_{sum} achieves minimum at the position where the following criterion is met:

$$\begin{cases} \frac{\partial D_{\text{sum}}(x,y)}{\partial x} = 0 \\ \frac{\partial D_{\text{sum}}(x,y)}{\partial y} = 0 \end{cases}. \quad (5)$$

Therefore, the estimated molecule position could be easily calculated from the linear equations in Eq. (5).

Finally, beginning from raw images, the complete procedures of using MrSE for single molecule localization are summarized as follows.

Step 1:

Subregion extraction as described in Quan *et al.* [6] and Hedde *et al.* [11].

Step 2:

Determine radial lines using Eqs. (1)–(3).

Step 3:

Find molecule location using Eqs. (4) and (5).

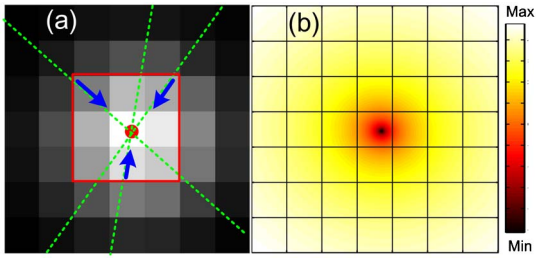

Fig. 1. (Color online) Principle of MrSE. (a) Single molecule fluorescence image, where the dot indicates the exact molecule position, the arrows show the gradient directions of the pixels beneath them, and the lines indicate the corresponding radial lines; (b) distribution of D_{sum} from an arbitrary subpixel point to the radial lines of all pixels inside the square in (a). Note that the point with minimum value (the darkest point) demonstrates the estimated position of the molecule.

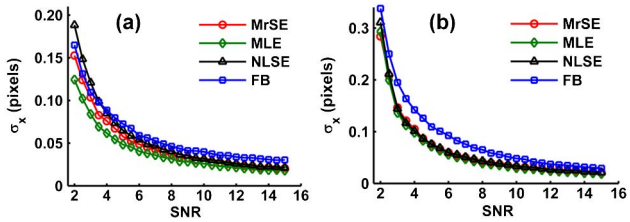

Fig. 2. (Color online) Localization precision comparison with (a) simulated images with no background and (b) a background fluorescence (I_b) of 10 photons per pixel [6,7]. Note that the comparison is quantified as the standard deviation of localization error and is specified in x direction (σ_x) only.

The localization precision and speed of MrSE were compared numerically and experimentally with three well-known methods, including the MLE [2], nonlinear least squares estimator (NLSE) [2], and fluoroBancroft (FB) algorithm [3]. Quan *et al.* [6] and Smith *et al.* [7] were used to select the necessary parameters for MLE and NLSE, and Shen and Andersson [3] was used for FB. All analyses were performed using MATLAB (Math Works) on the same desktop computer (2.33 GHz Core2 Quad).

In numerical simulation, images with 13×13 pixels were generated according to Ober *et al.* [2], for which (1) the x and y coordinates of the molecule are randomly distributed between −1 pixel and 1 pixel, respectively, with a minimum interval of 0.01 pixel; (2) the PSF was modeled with a Gaussian function; and (3) the width of Gaussian kernel (σ_{PSF}) is set to be 1 pixel, corresponding to a PSF of 6×6 pixels. We define the signal-to-noise ratio (SNR) of an image as the ratio of I_0 to the square root of $(I_0 + I_b)$ where I_0 is the peak signal intensity after background subtraction and I_b is the intensity of fluorescence background [4,6]. For each SNR level, 3000 images were generated and analyzed. Subregions of 7×7 pixels were extracted for all algorithms, whereas MrSE and FB analyzed a subset of 3×3 pixels in the center. The criteria for box size selection are to obtain best localization precision [3,6].

With simulated images, we compared the localization precision of all the four algorithms. Surprisingly, we found that our MrSE achieves almost the same localization precision as MLE when the background fluorescence is 10 photons per pixel [see Fig. 2(b)], and only slightly lower precision than MLE when there is no background fluorescence [see Fig. 2(a)]. MrSE is consistently superior to the other algebraic FB algorithm irrespective of the background fluorescence level and SNR range under studied. We believe that the excellent localization precision of MrSE comes from a combination use of the gradient operator and least squares method, which largely reduces the effect of pixilation and noise on the localization precision of algebraic algorithms.

Next, we evaluated the localization speed of these algorithms and found that MrSE runs several times faster than FB, and more than 1000 times faster than the fitting-based NLSE and MLE (see Table 1). Previously, in molecule localization, it was observed that the GPU-based MLE algorithm runs about ~50 times faster than the standard MLE [6,7], which is mainly determined by the number of stream processors of GPU. Therefore, current comparison results clearly indicate the superior

Table 1. Average Time Consumption per Molecule Localization[a]

Method	Simulation		Experiment	
	Time (ms)[b]	Gain[c]	Time (ms)[d]	Gain[c]
MrSE	0.0065	1	0.0078	1
FB	0.042	6.5	0.053	6.8
NLSE	8.2	1260	8.0	1020
MLE	8.8	1350	8.5	1090

[a]Note that the execution times for FB, NLSE, and MLE are comparable to literature [3].
[b]Averaged from 3000 simulated molecules.
[c]The speed gain of MrSE over other algorithms.
[d]Averaged from 5000 experimental molecules that were extracted from the first 200 TIRF images studied in Fig. 3. The subregion boxes were the same as those in[b].

localization speed of MrSE, especially after taking the difficulty in GPU hardware development into consideration.

It would be of great beneficial to evaluate the performance of MrSE in analyzing real experimental data. Therefore, we captured a series of TIRF images where the actin bundles in fixed HeLa cells were labeled with fluorescence protein d2EosFP [12]. Experimental details were reported previously [8]. Briefly, fluorescence from d2EosFP were collected by an objective (UAPON 100XOTIRF, Olympus), filtered with a dichroic mirror (Di01-R488/561, Semrock) and a long-pass filter (BLP01-561R-25, Semrock), and detected by an EMCCD (iXon 897, Andor). The pixel size at sample plane was 160 nm and thus an Airy disk covers 3×3 pixels.

We employed a well-accepted approach, which is based on analyzing the molecule counting distribution profile of a tiny structure [6], to characterize the localization precision performance of the algorithms. We found that MrSE is capable of processing experimental images with resolution (101 nm) close to that from NLSE and MLE (~87 nm), and better than that from FB [see Fig. 3(c)]. Simulation study [see Fig. 3(e)] verified that such resolution differences are due to the fact that algebraic algorithm is more sensitive to insufficient sampling than fitting-based algorithm, because a lower sampling density (3×3 pixels) increases the effect of pixilation on localization precision [2]. On the other hand, we observed that MrSE analyzes experimental images with a significant faster speed (more than 1000 times) than the fitting-based NLSE and MLE algorithms, which is consistent with our previous finding with simulation data (see Table 1).

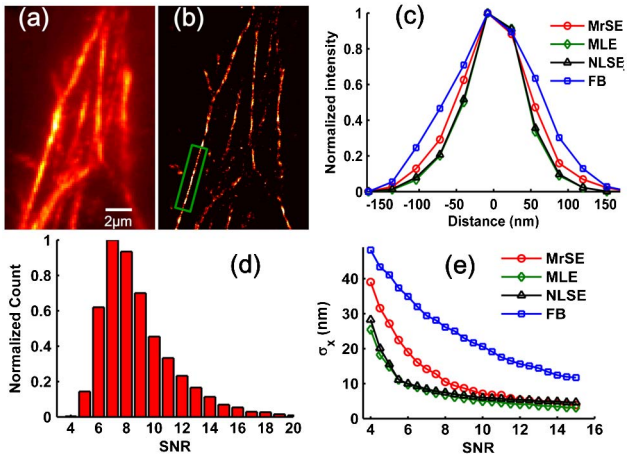

Fig. 3. (Color online) Localization performance of the algorithms for experimental [(a)–(d)] and simulated (e) images: (a) overlay of the first 1000 original TIRF images, (b) super-resolution image reconstructed from MrSE, (c) normalized distributions of fluorescent molecules [in the box of (b)] deviating from their mean contour line, (d) SNR distribution from the experimental images, and (e) estimated localization precision in x direction using simulated images whose parameters were set to be the same as the real experiment.

Finally, we note that (1) although MrSE extracts only the positions of single molecules, other parameters (σ_{PSF} background noise, etc.) can be obtained from a similar strategy used in FB [11] and (2) MrSE works well for images with sparsely distributed fluorescence emission, for which molecule density is less than 1 molecule per μm^2.

In conclusion, we describe an algebraic MrSE algorithm for single molecule localization. MrSE is based on the radial symmetry nature of a single molecule fluorescence image, which can be ensured from optical microscope with proper alignment. We demonstrate that MrSE is capable of processing images with precision close to the standard MLE across a wide range of SNR, while executing more than 1000 times faster. We believe that this algebraic algorithm has great potential in pushing forward toward the goal of online data analysis in localization microscopy with faster detectors.

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References

1. B. Huang, H. Babcock, and X. W. Zhuang, Cell **143**, 1047 (2010).
2. R. J. Ober, S. Ram, and E. S. Ward, Biophys. J. **86**, 1185 (2004).
3. Z. L. Shen and S. B. Andersson, IEEE Trans. Signal Process. **59**, 4041 (2011).
4. B. Yu, D. N. Chen, J. L. Qu, and H. B. Niu, Opt. Lett. **36**, 4317 (2011).
5. R. Starr, S. Stahlheber, and A. Small, Opt. Lett. **37**, 413 (2012).
6. T. W. Quan, P. C. Li, F. Long, S. Q. Zeng, Q. M. Luo, P. N. Hedde, G. U. Nienhaus, and Z. L. Huang, Opt. Express **18**, 11867 (2010).
7. C. S. Smith, N. Joseph, B. Rieger, and K. A. Lidke, Nat. Meth. **7**, 373 (2010).
8. Z. L. Huang, H. Y. Zhu, F. Long, H. Q. Ma, L. S. Qin, Y. F. Liu, J. P. Ding, Z. H. Zhang, Q. M. Luo, and S. Q. Zeng, Opt. Express **19**, 19156 (2011).
9. P. Friedl and D. Gilmour, Nat. Rev. Mol. Cell Biol. **10**, 445 (2009).
10. J. B. Pawley, *Handbook of Biological Confocal Microscopy*, 3rd ed. (Springer, 2006).
11. P. N. Hedde, J. Fuchs, F. Oswald, J. Wiedenmann, and G. U. Nienhaus, Nat. Meth. **6**, 689 (2009).
12. K. Nienhaus, G. U. Nienhaus, J. Wiedenmann, and H. Nar, Proc. Natl. Acad. Sci. USA **102**, 9156 (2005).