Accuracy Evaluation and Modification Method for Estimating Point Light Spot Positions with Gaussian Fitting Algorithm

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Abstract. This paper presents an accuracy evaluation method for point light spot position estimation with a Gaussian fitting algorithm combined with a centroid algorithm. Using centroid algorithm in the region around the Gaussian fitting distribution position estimation gets a position estimation value, and it is found that the deviation between this value and the Gaussian fitting algorithm position estimation approximately indicates the deviation between the Gaussian fitting algorithm position estimation and the real one. And at the basis of this finding, a modification method is proposed for position estimation with the Gaussian fitting algorithm. Experimental results show that the accuracy evaluation errors are less than 0.62 nm for 100- and 500-nm diameter fluorescent nanospheres, and the proposed modification method can enhance the accuracy by 3.1 nm.

Keywords: Point Light Spot, Gaussian Fitting Algorithm, Centroid Algorithm.

1. Introduction
Passive microrheological, particle tracking analysis and super-resolution fluorescence microscopy all require the point light spots of nanoparticles or molecules to be absolutely estimated or located in their video images [1-5]. The more accurate the point light spot position estimation, the better the performance of these techniques [6-8]. It was observed that a Gaussian function fitting algorithm can achieve the best position estimation for point light spots [9, 10]. This algorithm estimates the mean squared error (MSE) between the actual intensity distribution of a point light spot and the intensities of the fitting Gaussian function. The lesser the MSE, the better the performance of the Gaussian function fitting algorithm [11-13]. MSE estimation is the only method for evaluating the Gaussian function fitting algorithm, which does not reveal the accuracy of point light spot position estimation with this algorithm. Moreover, noise influence on the estimation accuracy cannot be averted in spite of using a denoising algorithm. In addition, the noise influence is random: the estimation accuracy of a point light spot varies because of variation in the noise level at each pixel position. Therefore, it is best to evaluate the estimation accuracy for each Gaussian function fitting of a point light spot so as to modify each estimation accuracy.

2. Accuracy Evaluation and Modification Method
The intensity distributions of point light spots in the microscopic images of nanoparticles or molecules can be fitted using a Gaussian surface function. The Gaussian surface is given as follows [9, 10]:

\[ f(x, y) = \frac{1}{2\pi \sigma^2} e^{-\frac{x^2 + y^2}{2\sigma^2}} \]
\[ I(x_i, y_j) = A \cdot \exp \left[ -\frac{(x_i - x_0)^2 + (y_j - y_0)^2}{B} \right] \tag{1} \]

where \( x_i \) and \( y_j \) are the coordinates of a pixel on the transverse and longitudinal axes in the microscope image, respectively. \( I(x_i, y_j) \) is the intensity corresponding to each pixel of the point light spot image. \( x_0 \) and \( y_0 \) are the central coordinates. \( A \) and \( B \) are constants. Fitting equation (1) to the intensity distribution of point light spots in a microscope image yields \( x_0, y_0, A, \) and \( B \). The center coordinates \( x_0 \) and \( y_0 \) are the required point light spot position estimations.

Apart from using the Gaussian function fitting algorithm to obtain the point light spot position estimation, another method involves using a centroid algorithm. Equation (2) gives the formula for centroid calculation:

\[
\begin{align*}
C_x &= \frac{\sum_{i=1}^{n} \sum_{j=1}^{m} x_i I(x_i, y_j)}{\sum_{i=1}^{n} \sum_{j=1}^{m} I(x_i, y_j)} \\
C_y &= \frac{\sum_{i=1}^{n} \sum_{j=1}^{m} y_j I(x_i, y_j)}{\sum_{i=1}^{n} \sum_{j=1}^{m} I(x_i, y_j)}
\end{align*}
\tag{2}
\]

In equation (2), \( C_x \) and \( C_y \) are the position estimations obtained using the centroid algorithm. It is firstly to simulate the intensity distribution for nanoparticles or molecules imaged with a charge-coupled device (CCD). An actual point light spot image contains several types of noises, such as, shot noise, readout noise, amplifier noise, dark current noise, etc. All types of noises can be classified into two categories: additive noise and multiplicative noise

\[
\begin{align*}
I_{\text{add, noise}}(x_i, y_j) &= I(x_i, y_j) + \text{noise}_{\text{additive}}(x_i, y_j) \\
I_{\text{mult, noise}}(x_i, y_j) &= I(x_i, y_j)(1 + \text{noise}_{\text{multiplicative}}(x_i, y_j))
\end{align*}
\tag{3}
\]

In equation (3), \( \text{noise}_{\text{additive}}(x_i, y_j) \) and \( \text{noise}_{\text{multiplicative}}(x_i, y_j) \) represent the additive and multiplicative noise values at \( (x_i, y_j) \) pixel, respectively. Below, two types of point light spot distribution of intensities containing noise, \( I_{\text{add, mult}}(x_i, y_j) \) and \( I_{\text{mult, add}}(x_i, y_j) \), are used for simulation analysis.

\[
\begin{align*}
I_{\text{add, mult}}(x_i, y_j) &= I(x_i, y_j) + \text{noise}_{\text{additive}}(x_i, y_j)(1 + \text{noise}_{\text{multiplicative}}(x_i, y_j)) \\
I_{\text{mult, add}}(x_i, y_j) &= I(x_i, y_j)(1 + \text{noise}_{\text{multiplicative}}(x_i, y_j)) + \text{noise}_{\text{additive}}(x_i, y_j)
\end{align*}
\tag{4}
\]

In equations (4) and (5), the noise at \( (x_i, y_j) \) pixel of both \( \text{noise}_{\text{additive}}(x_i, y_j) \) and \( \text{noise}_{\text{multiplicative}}(x_i, y_j) \) conform to normal distribution. The Gaussian function in equation (1) for fitting the point light spot distribution of intensities provides the MSE. If there is no noise when the central point coordinate \( (x_0, y_0) \) and surface shape (depending on \( A \) and \( B \)) of the obtained Gaussian fitting function are close to the real ones, the MSE becomes increasingly smaller. However, in the presence of noise, the actual central point coordinate and surface shape deviate from the actual values; this results in poor performance of the optimization method used in the Gaussian fitting algorithm (such as, the gradient descent algorithm, Levenberg–Marquardt algorithm, or Gauss–Newton iteration method). The optimization method makes both the central point coordinate and surface shape holistically converge to optimal possibilities for obtaining the minimum MSE; our aim is to obtain the accurate central point coordinate (position estimation) of point light spot distribution intensities and not the accurate surface shape. Therefore, the MSE is not a criterion for evaluating the accuracy of position estimation. The centroid algorithm (equation (2)) is susceptible to image noise and the shape of the point light spot. Here, we used the position estimated with the Gaussian fitting algorithm as the center point for the centroid algorithm, which is given as
In equation (6), \((x_{\text{Gauss}}, y_{\text{Gauss}})\) is the position estimated with the Gaussian fitting algorithm, and \(\gamma\) is the radius (the unit is pixel) of the operating area of the centroid algorithm in the point light spot image. Assuming that \(\gamma\) can be equal to 2, 3, 4……R, where R is the maximum radius ranging from \((x_{\text{Gauss}}, y_{\text{Gauss}})\) to the edge of the point light spot, the averages of \(C_{x_{\gamma}}(x_i, y_j)\) and \(C_{y_{\gamma}}(x_i, y_j)\) are represented as \(C_{x_{\gamma}}\_\text{average}\) and \(C_{y_{\gamma}}\_\text{average}\), respectively.

\[
\begin{align*}
C_{x_{\gamma}} &= \sum_{i=1}^{n} \sum_{j=1}^{m} (x_i, I(x_i, y_j)) / \sum_{i=1}^{n} \sum_{j=1}^{m} I(x_i, y_j) \\
C_{y_{\gamma}} &= \sum_{i=1}^{n} \sum_{j=1}^{m} (y_j, I(x_i, y_j)) / \sum_{i=1}^{n} \sum_{j=1}^{m} I(x_i, y_j)
\end{align*}
\text{subject to } \sqrt{(x_i - x_{\text{Gauss}})^2 + (y_j - y_{\text{Gauss}})^2} \leq \gamma
\tag{6}
\]

Figure 1. Distance between \((C_{x_{\gamma}}\_\text{average}, C_{y_{\gamma}}\_\text{average})\) and \((x_{\text{Gauss}}, y_{\text{Gauss}})\) and that between \((x_{\text{Gauss}}, y_{\text{Gauss}})\) and the real positive estimation with varied simulation coefficients. (a) sequence of the first-type simulation. (b) sequence of the second-type simulation.

Assuming a sequence of varied coefficients (-2:0.2:2), in the first-type simulation \(I_{\text{add\_mult}}(x, y)\) and second-type simulation \(I_{\text{mult\_add}}(x, y)\), the mean value sequence and standard deviation sequence of \(\text{noise\_additive}(x, y)\) are \(10+\text{varied coefficient}\times5\) and \(0.2+\text{varied coefficient}\times0.1\), respectively, and those for \(\text{noise\_multiplicative}(x, y)\) are \(0.2+\text{varied coefficient}\times0.1\) and \(0.2+\text{varied coefficient}\times0.1\), respectively. As observed in figure 1, the distance between \((C_{x_{\gamma}}\_\text{average}, C_{y_{\gamma}}\_\text{average})\) and \((x_{\text{Gauss}}, y_{\text{Gauss}})\) is slightly greater than or equal to the deviation caused by the influence of noise on the Gaussian fitting algorithm. It approximately indicates the position estimation accuracy of the Gaussian fitting algorithm. Specially, when the range of varied coefficients is from -2 to 0, the error of the distance between \((C_{x_{\gamma}}\_\text{average}, C_{y_{\gamma}}\_\text{average})\) and \((x_{\text{Gauss}}, y_{\text{Gauss}})\) is less than 0.05 pixels. The obtained central point of the Gaussian fitting distribution of point light spots is made to traverse each position in the indicated accuracy deviation region with tiny mesh steps; determination of the central point with the minimum MSE in all these traversed Gaussian fitting distributions yields the modified point light spot position estimation. In the proposed modification, the tiny mesh step is given by deviation indication/\(n\), where \(n\) is a positive integer; it was set as 20.
Figure 2. Position estimation modifications for the two types of simulations $I_{\text{add mult}}(x_i,y_j)$ and $I_{\text{mult add}}(x_i,y_j)$. (a) first-type simulation. (b) second-type simulation.

Figure 3. Setup of fluorescent nanosphere microscopic system.

Figure 2 shows the position estimation modifications for 30 simulation operations with constant mean values and standard deviations of $\text{noise additive}(x_i,y_j)$ and $\text{noise multiplicative}(x_i,y_j)$ for the $I_{\text{add mult}}(x_i,y_j)$ and $I_{\text{mult add}}(x_i,y_j)$ simulations. Here, the mean value sequence and standard deviation sequence of $\text{noise additive}(x_i,y_j)$ are 10 and 0.2, respectively, and those for $\text{noise multiplicative}(x_i,y_j)$ are 0.2 and 0.2, respectively. Figure 2 shows that the maximum modifications were approximately 0.3 and 0.05 pixels for the $I_{\text{add mult}}(x_i,y_j)$ and $I_{\text{mult add}}(x_i,y_j)$ simulations, respectively. If one pixel is 3 µm, the estimation accuracies are enhanced by 18 nm and 3 nm, respectively, for an amplification factor of 50×. Although most simulation operations were performed without modification, the results of these operations remained nearly unchanged before and after modification; for all increases and decreases less than 0.005 pixels, the results were considered unchanged. This proposed modification method is the best method to ameliorate the position estimation of the Gaussian fitting algorithm in the presence of noise. Even if it does not enhance the position estimation accuracy in some cases, it also does not deteriorate the accuracy.
3. Experiment Result

In figure 3, the magnification of the particle tracking measurement system is 40×. The fluorescent nanosphere sample is placed below the objective and fixed on the two-dimensional precision displacement stage. Here, fluorescent nanosphere samples of two different sizes (with nominal average diameters of 100 nm and 500 nm) are used. Each nanosphere sample was firmly fixed between the coverslip and object slide. In the microscopic system, the pixel size of the CCD is 1.25 μm, and the fiber laser head serves as the excitation source for the fluorescent nanospheres; in addition, the LED source can change the luminous intensity to provide external disturbance light and hence alter the microscopic noise. The horizontal coordinate value of figure 4 is a sequence (1:1:20) in millicandela (mcd) of the luminous intensity of the LED source. With no light from the external LED source and assuming that the obtained position estimation with the Gaussian fitting algorithm is the original position estimation, the ordinate of figure 4 is the deviation of the position estimation of the sequenced luminous intensity condition from the original. In figure 4, the estimated deviation from the estimation accuracy is nearly equal to the estimated deviation obtained using the Gaussian fitting algorithm. One pixel represents 1.25 μm; it also represents a 31.25 nm distance under the 40× magnification condition. The accuracy errors in figure 4 are less than 0.02 pixels (0.62 nm). This accuracy evaluation method can thus indicate the position estimation accuracy of the Gaussian fitting algorithm.

![Graph](image-url)

**Figure 4.** Positive estimation with accuracy evaluation and modification for Gaussian fitting algorithm with two polystyrene latex sphere samples. (a) 100 nm diameter. (b) 500 nm diameter.

The position estimations using the proposed modification algorithm are equal to less than the position estimations with the Gaussian fitting algorithm. Although in some cases, the results of Gaussian fitting nearly remain unchanged before and after the modification, the proposed modification algorithm can enhance the accuracy of the Gaussian fitting algorithm by approximately 0.1 pixels (3.1 nm) when the luminous intensity of the LED source increases, i.e., the microscopic noise becomes strong.
4. Conclusion
In summary, this paper presents the accuracy evaluation of point light spot position estimation with a Gaussian fitting algorithm combined with a centroid algorithm. Using the centroid algorithm in the region around the Gaussian fitting distribution for position estimation yields a position estimation value; it was found that the deviation between this value and the position estimated using the Gaussian fitting algorithm approximately indicated the deviation between the Gaussian fitting algorithm position estimation and the actual one. This finding enables us to evaluate the estimation accuracy for each Gaussian function fitting of a point light spot so as to modify each estimation accuracy. However, the theoretical mechanism of this technique is not clearly understood and requires further investigation. In the future, the proposed method can be incorporated in the optimization method used in the Gaussian fitting algorithm, which provides an accuracy estimation for each convergence step during the optimization process, so as to achieve better optimization result.

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