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Effect of Time Discretization on the Limit of the Accuracy of Parameter Estimation for Moving Single Molecules Imaged by Fluorescence Microscopy

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***Abstract*—In this paper, we consider the problem of the accuracy of estimating the location and other attributes of a moving single molecule whose trajectory is acquired in a sequence of time intervals by a pixelated detector. We present expressions of the Fisher information matrices from which the benchmark for the accuracy of the parameter estimates is obtained. In the absence of extraneous noise, it is shown that time discretization of the image acquisition process results in a limit of the accuracy of the parameter estimates that is better than or at least as good as that acquired without time discretization. This analytical result is also illustrated by simulations. However, in the presence of extraneous noise, simulations show that finer time discretization may not always lead to better limit of the accuracy than that acquired without time discretization of the image acquisition process.**

I. INTRODUCTION

In single-molecule fluorescence microscopy, one of the ways to understand cell dynamics is to optically track the fluorescent-labelled molecules as in single-particle tracking experiments. From the acquired data, we can estimate the locations and other attributes of the molecules of interest. [1]–[5]. However, it has been known that the localization error due to noise that is inherent to single-particle tracking experiments may give rise to misinterpretation of the acquired data [4]. The molecular motion of the molecules also contributes to the localization error [5]. Hence, it is important to have a benchmark from which the accuracy of the parameter estimates can be measured against.

The problem of the accuracy of estimating the parameters of a moving molecule has been addressed in our recent work [6]. There, we have obtained the benchmark, which provides the limit of the accuracy of the parameter estimates, from the Fisher information matrix. Specifically, the limit of the accuracy of the parameter estimates is obtained by taking the square root of the diagonal elements of the inverse of the Fisher information matrix for the underlying random process that characterizes the acquired data. The results obtained in [6] are based on the acquisition of the image of the moving molecule in a single time interval spanning the total acquisition time and thus is inapplicable to single-particle tracking experiments where the images are acquired in a sequence of time intervals.

In this paper we investigate how time discretization of the image acquisition process affects the limit of the accuracy of the parameter estimates. We show that through time discretization of the image acquisition process, we can obtain a limit of the accuracy that is better than or at least as good as that acquired without time discretization. Specifically, we consider the case where the object moves in the two-dimensional (2D) object space and its images are acquired in a sequence of time intervals by a pixelated detector of finite size. We study both the noise-free and noisy (extraneous noise corrupted) cases. The results obtained in this paper provide insights which would enable the experimentalists to optimize their experimental setups for tracking single molecules in order to achieve the best possible accuracy.

The organization of this paper is as follows. In Section II, we provide expressions of the Fisher information matrix for two stochastic models from which the limit of the accuracy of the parameter estimates is obtained: One for the noise-free case and another for the noisy cases. For the noise-free case, by working out an analytical expression for the difference in the two Fisher information matrices for a single time interval spanning the total acquisition time and for a sequence of time intervals respectively, it is shown that time discretization of the image acquisition process provides a limit of the accuracy that is better than or at least as good as that acquired without time discretization. In Section III, simulations are performed to illustrate the analytical results obtained in Section II and to understand how extraneous noise affects the limit of the accuracy. Conclusions are presented in Section IV.

II. EFFECT OF TIME DISCRETIZATION ON THE LIMIT OF THE ACCURACY

In single-molecule fluorescence microscopy, the detection of photons is inherently a random phenomenon and thus the recorded image of the molecules is stochastic in nature. Following [8], the acquired data is modelled as a space-time random process [9] which we will refer to as the image detection process $\mathcal{G}$. The temporal part describes the time points of the photons detected by the detector and is modelled as a temporal Poisson process with intensity function Λ_θ, where θ denotes the parameters that describe the trajectory of the object. The spatial part describes the spatial coordinates of the arrival location of the detected photons and is modelled as a family of mutually independent random variables with probability densities given by $\{f_{\theta,\tau}\}_{\tau \geq t_0}$, where τ denotes the time point of a detected photon. Throughout this paper, we let $t_0 \in \mathbb{R}$ and $\theta \in \Theta$, where Θ denotes the parameter space that is an open subset of $\mathbb{R}^n$ with n being the dimension of θ. The spatial and temporal parts of $\mathcal{G}$ are assumed to be mutually independent of each other and the probability density function $f_{\theta,\tau}$ satisfies certain regularity conditions that are necessary for the calculation of the Fisher information matrix.

Consider a pixelated detector $\mathcal{C}_p$ which is a collection $\{C_1, \ldots, C_{N_p}\}$ of open, disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels that constitute the pixelated detector. The acquired data comprises not only detected photons from the object of interest and the background component but also the measurement noise from the readout process. The detected photons from the object of interest and the background component are Poisson distributed while the measurement noise is Gaussian distributed. We refer to the detected photons from the background component as the Poisson noise and the measurement noise as the Gaussian noise. Since the acquired data is modelled as a space-time random process, following [10], we let $\mathcal{G}^{(1)}(\Lambda_\theta^{(1)}, \{f_{\theta,\tau}^{(1)}\}_{\tau \geq t_0}, \mathcal{C}_p)$ and $\mathcal{G}^{(2)}(\Lambda^{(2)}, \{f_{\tau}^{(2)}\}_{\tau \geq t_0}, \mathcal{C}_p)$ denote the image detection processes that model the detected photons from the object of interest and background component, respectively.

The images of the object of interest are assumed to be acquired in N time intervals $[t_{i-1}, t_i]$, $i = 1, 2, \ldots, N$, over the total acquisition time $[t_0, t_N]$. Assuming that $n_{k,i}$ denotes the total number of detected photons from the object of interest in the pixel C_k for the time interval $[t_{i-1}, t_i]$, then the total number of detected photons from the object of interest for the total acquisition time $[t_0, t_N]$ is given by $\sum_{i=1}^{N} \sum_{k=1}^{N_p} n_{k,i}$. It can be shown that $n_{k,i}$ is independently Poisson distributed with mean

$$\mu_{\theta,k,i} = \int_{t_{i-1}}^{t_i} \int_{C_k} \Lambda_\theta^{(1)}(\tau) f_{\theta,\tau}^{(1)}(r) dr d\tau,$$
$$k = 1, 2, \ldots, N_p, \ i = 1, 2, \ldots, N, \ \theta \in \Theta. \quad (1)$$

Similarly, the number of detected photons from the background component in the pixel C_k for the time interval $[t_{i-1}, t_i]$ is independently Poisson distributed with mean

$$\beta_{k,i} = \int_{t_{i-1}}^{t_i} \int_{C_k} \Lambda^{(2)}(\tau) f_{\tau}^{(2)}(r) dr d\tau,$$
$$k = 1, 2, \ldots, N_p, \ i = 1, 2, \ldots, N. \quad (2)$$

Since the images acquired during each time interval are independent of one another, the Fisher information matrix of $\mathcal{G}$ for the total acquisition time $[t_0, t_N]$ can be expressed as the sum of the Fisher information matrices for each time interval [7], i.e., $\mathbf{I}(\theta) = \sum_{i=1}^{N} \mathbf{I}_i(\theta)$. In the case where there is no extraneous noise, we simply let $\beta_{k,i} = 0$ and the standard expression for the Fisher information matrix of a Poisson distribution [9] becomes

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \sum_{i=1}^{N} \frac{1}{\mu_{\theta,k,i}} \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta}\right)^T \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta}\right), \ \theta \in \Theta. \quad (3)$$

However, when extraneous noise is present, we use (2) for the Poisson noise and assume a mean $\eta_{k,i}$ and variance $\sigma_{k,i}^2$, $k = 1, 2, \ldots, N_p$, $i = 1, 2, \ldots, N$, for the Gaussian noise in each pixel during each time interval $[t_{i-1}, t_i]$. Thus, the Fisher information matrix corresponding to the total acquisition time $[t_0, t_N]$ in [8] is re-formulated as

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \sum_{i=1}^{N} \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta}\right)^T \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta}\right)$$
$$\times \left(\int_R \frac{\left(\sum_{l=1}^{\infty} \frac{[\upsilon_{\theta,k,i}]^{l-1} e^{-\upsilon_{\theta,k,i}}}{(l-1)!} \frac{1}{\sqrt{2\pi}\sigma_{k,i}} e^{-\frac{1}{2}\left(\frac{z-l-\eta_{k,i}}{\sigma_{k,i}}\right)^2} \right)^2}{p_{\theta,k(z),i}} dz - 1 \right) \quad (4)$$

where $\upsilon_{\theta,k,i} = \mu_{\theta,k,i} + \beta_{k,i}$ and the Poisson-Gaussian mixture probability density function $p_{\theta,k(z),i}$ is given by

$$p_{\theta,k(z),i} = \frac{1}{\sqrt{2\pi}\sigma_{k,i}} \sum_{l=0}^{\infty} \frac{[\upsilon_{\theta,k,i}]^l e^{-\upsilon_{\theta,k,i}}}{l!} e^{-\frac{1}{2}\left(\frac{z-l-\eta_{k,i}}{\sigma_{k,i}}\right)^2},$$
$$z \in \mathbb{R}, \ k = 1, 2, \ldots, N_p, \ i = 1, 2, \ldots, N. \quad (5)$$

Numerical methods are used to compute the inverse of the Fisher information matrix expressed in (3) and (4), from which the respective limit of the accuracy of the parameter estimates is obtained. With this limit of the accuracy, the experimentalists can evaluate the extent to which the experimental settings such as the detector array size, the pixel size, extraneous noise, have on the experiments. In the event where the accuracy of the parameter estimates obtained from experiments surpasses the limit of the accuracy, the validity of the results becomes questionable since according to the Cramér-Rao inequality, the (co)variance (matrix) of any unbiased estimator of an unknown vector parameter is bounded from below by the inverse of the Fisher information matrix [7].

To illustrate the effect of time discretization on the limit of the accuracy of the parameter estimates, we consider the case where an object moves in a 2D plane in the absence of extraneous noise. Using (3) and assuming that the photon detection rate of the object of interest is a constant, i.e., $\Lambda^{(1)}(\tau) = \Lambda_0 \in \mathbb{R}^+$, we formulate the Fisher information matrix $\mathbf{I}_1(\theta)$ for the case where the image is acquired in a single time interval spanning the total acquisition time $[t_0, t_N]$ and another $\mathbf{I}_N(\theta)$ where N $(N > 1)$ time intervals are used. Denote $\delta\mathbf{I}_{N-1}(\theta)$ as the difference of these two Fisher information matrices, i.e., $\delta\mathbf{I}_{N-1}(\theta) := \mathbf{I}_N(\theta) - \mathbf{I}_1(\theta)$.

For $k = 1, 2, \ldots, N_p$, $u = 0, 1, \ldots, N-1$, $v = 1, 2, \ldots, N$, let $a_{k,u,v} := \int_{t_u}^{t_v} \int_{C_k} f_{\theta,\tau}^{(1)}(r) dr d\tau \in \mathbb{R}^+$ and $A_{k,u,v} := \int_{t_u}^{t_v} \int_{C_k} \frac{\partial f_{\theta,\tau}^{(1)}(r)}{\partial \theta} dr d\tau \in \mathbb{R}^{1\times n}$.

For $k = 1, 2, \ldots, N_p$, $m, i = 1, 2, \ldots, N-1$, let $W_{k,m,i} := a_{k,m,m+1} \cdot A_{k,i-1,i} - a_{k,i-1,i} \cdot A_{k,m,m+1} \in \mathbb{R}^{1\times n}$.

The expression of the difference matrix $\delta\mathbf{I}_{N-1}(\theta)$ for the total acquisition time $[t_0, t_N]$ is given as follows

$$\delta\mathbf{I}_{N-1}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \sum_{m=1}^{N-1} \sum_{i=1}^{m} \frac{W_{k,m,i}^T \cdot W_{k,m,i}}{a_{k,i-1,i} \cdot a_{k,m,m+1} \cdot a_{k,0,N}}. \tag{6}$$

It can be seen from the above expression that $\delta\mathbf{I}_{N-1}(\theta)$ is the sum of $N_p N(N-1)/2$ positive semidefinite matrices and hence also positive semidefinite. The implication of this is that the diagonal elements of the inverse of $\mathbf{I}_N(\theta)$ are smaller than or at most the same as the corresponding elements of the inverse of $\mathbf{I}_1(\theta)$. Thus, it implies that through time discretization of the image detection process in the absence of extraneous noise, we can obtain a limit of the accuracy that is better than or at least as good as that without time discretization.

III. Simulation results

We next conduct simulations for the noise-free and noisy cases using (3) and (4), respectively, in order to gain insights into how experimental conditions affect the limit of the accuracy of the parameter estimates. For the noise-free case, we study the effect of pixelation without the confounding effect of the extraneous noise. By comparing the results of (3) and (4), we then study the effect of extraneous noise on the limit of the accuracy of the parameter estimates. For the simulations, we assume that the object is moving with a constant speed v from an initial position (x_0, y_0) at a direction of movement ϕ with respect to the $x-$axis. The photon detection rate of the object of interest is assumed to be a known constant, i.e., $\Lambda^{(1)}(\tau) = \Lambda_0^{(1)} \in \mathbb{R}^+$, $\tau \geq t_0$ and its image function is Gaussian, i.e., $q^{(1)}(x, y) = 1/(2\pi\sigma^2) \exp(-((x/M - x_\theta(\tau))^2 + (y/M - y_\theta(\tau))^2)/(2\sigma^2))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$, where $M > 0$ denotes the lateral magnification and $(x_\theta(\tau), y_\theta(\tau))$, $\tau \geq t_0$, denotes the trajectory of the object. When detected photons from the background component are present, we assume its photon detection rate to be a known constant $\Lambda^{(2)}(\tau) = \Lambda_0^{(2)} \in \mathbb{R}^+$, $\tau \geq t_0$ and the detected photons to be uniformly distributed.

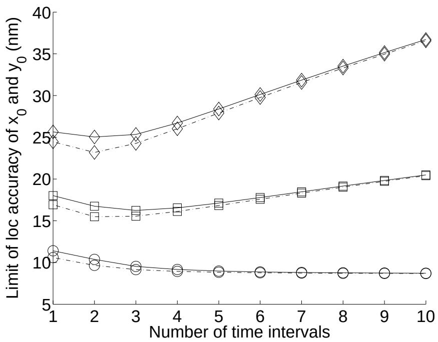
From Fig. 1, it can be seen that in the absence of extraneous noise, the limit of the accuracy of the parameter estimates improves monotonically with increasing number of time intervals used in acquiring the images. In other words, by acquiring the images in N $(N > 1)$ time intervals, the limit of the accuracy of the parameter estimates is better than that acquired in a single interval spanning the same total acquisition time. This result corroborates the implication of (6).

To study the effect of extraneous noise on the limit of the accuracy of the parameter estimates, we consider two different levels of the Gaussian noise while maintaining a constant Poisson noise. At low Gaussian noise, i.e., 2 e^-/pixel, there are several values of N (the number of time intervals for a given acquisition time) that provide better limit of the accuracy of the parameter estimates than that by acquiring the image in a single interval spanning the same acquisition time. However, the range of N corresponding to better limit of the accuracy of the parameter estimates than that by acquiring the image in a single interval spanning the same acquisition time reduces when the Gaussian noise is doubled, i.e., 4 e^-/pixel. The results in the presence of extraneous noise in Fig. 1 is due to the trade-off between the amount of acquired data and the extraneous noise for each time interval. As the number of time intervals used in acquiring the images increases for a fixed total acquisition time, the time interval becomes shorter and thus fewer photons from the object of interest are detected per interval. As for the extraneous noise, specifically the Gaussian noise, it is assumed to be constant per pixel. Thus, the amount of Gaussian noise is dependent on the number of pixels that constitute the pixelated detector and is independent of the time interval. As such, the Gaussian noise eventually overwhelms the detected photons as the time interval reduces and this leads to the deterioration of the limit of the accuracy of the parameter estimates. Comparing the results of the two Gaussian noise levels, it can be seen that not only is the limit of the accuracy of the parameter estimates poorer when there is more Gaussian noise, it also deteriorates at a faster rate. This rate of deterioration is evident from the diverging trends between the results of the two Gaussian noise levels. An interesting observation can also be made in Fig. 1 with regards to the difference between the limit of the accuracy of x_0 and y_0. As the number of time intervals increases, the difference between the limit of the accuracy of x_0 and y_0 diminishes. This phenomenon also occurs when the extraneous noise is absent.

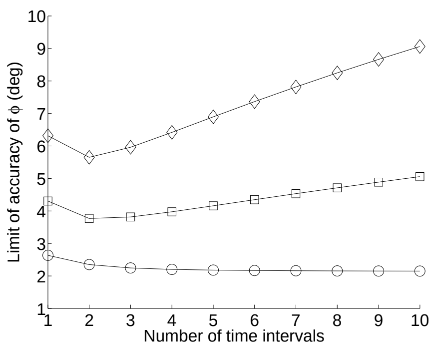
From the results of the simulation, it can be seen that the Gaussian noise has an adverse effect on the limit of the accuracy. Given the fact that the limit of the accuracy becomes poorer with increasing amount of Gaussian noise, deterioration of the limit of the accuracy also occurs at finer time discretization of the image acquisition process.

IV. Conclusions

In this paper, we have presented the expressions of the Fisher information matrix for both the noise-free and noisy cases from which their respective limit of the accuracy of



(a)



(b)

Fig. 1: Limits of the accuracy of the parameter estimates as a function of the number of time intervals used in acquiring the images of a linearly moving object. Panel (a) shows the limit of the accuracy of x_0 (—) and y_0 $(- \cdot -)$. Panel (b) shows the limit of the accuracy of ϕ. The marker (○), (□) and (◇) correspond to the case where there is no extraneous noise, where the Poisson noise $(\Lambda_0^{(2)})$ is 2 photons/pixel/s and the Gaussian noise (σ_k) is 2 e^{-}/pixel are present, and where the Poisson noise $(\Lambda_0^{(2)})$ is 2 photons/pixel/s and the Gaussian noise (σ_k) is 4 e^{-}/pixel are present, respectively. For the object, $\sigma = 84$ nm, its speed $v = 1000$ nm/s, its direction of movement $\phi = 30^o$, its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the detector, and the total acquisition time is 0.4 s. The photon detection rate $\Lambda_0^{(1)} = 1000$ photons/s, magnification $M = 100$, pixel size is 6.8 μm $\times$ 6.8 μm, and the array size is 19×19 pixels.

the parameter estimates can be obtained. We have shown that through time discretization of the image acquisition process in the noise-free case, a limit of the accuracy of the parameter estimates that is better than or at least as good as that acquired without time discretization can be obtained. Simulations have been carried out to investigate the effect of time discretization on the limit of the accuracy in parameter estimation for the noise-free and noisy cases. In the absence of extraneous noise, the limit of the accuracy of the parameter estimates improves monotonically with increasing number of time intervals. However, in the presence of extraneous noise, time discretization may not always improve the limit of the accuracy, especially with the increase of Gaussian noise. Finally, it should be noted that the expressions of the Fisher information matrix and the insights gained from the simulation enable the experimentalists to optimize their experimental setups for tracking single molecules in order to achieve the best possible accuracy.

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