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Influence of Prior Knowledge on the Accuracy Limit of Parameter Estimation in Single-Molecule Fluorescence Microscopy

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***Abstract*—In estimation theory, it is known that prior knowledge of parameters can improve the Cramér-Rao lower bound (CRLB). In this paper, we study the influence of prior knowledge on the CRLB of the estimates of the parameters that describe the trajectory of a moving object (single molecule). Since the CRLB is obtained from the inverse of the Fisher information matrix, we present a general expression of the Fisher information matrix in terms of the image function, the object trajectory and the prior knowledge matrix. Applying this expression to an object moving linearly in a two-dimensional (2D) plane with two distinct cases of prior knowledge, explicit CRLB expressions are derived. From these expressions, we show that the improvement in the CRLB of the parameter estimates is dependent on which parameters are known.**

I. Introduction

In single-molecule fluorescence microscopy, one of the ways to understand cell dynamics is to optically track the fluorescent-labelled molecules as they move over time and to analyze the results of the acquired data [1]. However, misinterpretation of results may arise due to the localization error of the molecules of interest. This localization error is due to the inherent noise of the detection process [2] and the motion of the molecules [3]. Hence, it is essential that the accuracy limit of the location and other attributes of the molecules of interest is known in order to ascertain the validity of the results obtained.

The issue of the limit of the accuracy of the parameter estimates for parameters that describe the trajectory of a moving object has been addressed in our recent work [4]. We obtained the accuracy limit of the parameter estimates from the Cramér-Rao lower bound (CRLB) [5]–[7] for the case where no prior knowledge of the parameters is assumed [4]. Since in estimation theory that the use of prior knowledge may lead to a more accurate estimate [5], it is of interest to know how the accuracy limit presented in [4] may improve with prior knowledge of some of the parameters. For simplicity, in this paper we will consider the special case of prior knowledge in which some of the parameters are known, that is, we will have fewer parameters to be estimated. In such a case, it will simplify the computation of the CRLB due to the reduction of the size of the Fisher information matrix, and also improve in the accuracy limit of the parameter estimates.

The organization of this paper is as follows. In Section II, we generalize the expression of the Fisher information matrix in [4] by incorporating the prior knowledge matrix. We then give explicit expressions of the accuracy limit of the parameter estimates for two different cases of prior knowledge when an object is moving along a straight line. Conclusions are presented in Section III.

II. Influence of Prior Knowledge on the Fundamental Limit of Accuracy

In a basic microscope setup, we consider an object of interest moving in the object space, imaged by a lens system and its image captured by a detector in the detector space. The detector detects photons emitted by the fluorescent-labelled object during a fixed acquisition time. Since this detection process of the emitted photons is inherently a random phenomenon, the recorded image of the object 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. Although not explicitly denoted, the probability densities $\{f_{\theta,\tau}\}_{\tau \geq t_0}$ can also be dependent on the focus level $z_\theta(\tau)$ and orientation $o_\theta(\tau)$, $\tau \geq t_0$, of the object of interest. Throughout the 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

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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 (see [8] for details).

To account for prior knowledge of some of the parameters, we adopt the approach in [10] where a prior knowledge matrix K is used. It is expressed as $K = \partial\theta/\partial\tilde{\theta}$, $\tilde{\theta} \subseteq \theta \in \Theta$, where $\theta = (\theta_1, \theta_2, \ldots, \theta_L)^T$ corresponds to all the parameters that describe the trajectory of the object of interest and $\tilde{\theta} = (\theta_1, \theta_2, \ldots, \theta_M)^T$ corresponds to those to be estimated. By incorporating the prior knowledge matrix to the expression of the Fisher information matrix in [4], we obtain a general expression in the following theorem. It should be noted that the photon detection rate is assumed to be independent of θ and the emitted photons are detected by a non-pixelated detector of infinite size, i.e. $\mathcal{C} = \mathbb{R}^2$, in the absence of extraneous noise.

Theorem 1. *Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that*

(A1) *there exists an image function $q_{z_\theta(\tau),o_\theta(\tau)}$ such that for the lateral magnification $M > 0$, the photon distribution profile of a moving object is given by $f_{\theta,\tau}(x,y) = 1/M^2 q_{z_\theta(\tau),o_\theta(\tau)}\left(x/M - x_\theta(\tau), y/M - y_\theta(\tau)\right)$, $(x,y) \in \mathbb{R}^2$, $\tau \geq t_0$,*

(A2) *the time dependent trajectory of the object $(x_\theta(\tau), y_\theta(\tau))$ are uniformly bounded for $t_0 \leq \tau \leq t$,*

(A3) *$\partial q_{z_\theta(\tau),o_\theta(\tau)}\left(x/M - x_\theta(\tau), y/M - y_\theta(\tau)\right)/\partial p(\tau)$ exists for $(x,y) \in \mathbb{R}^2$, $z_\theta(\tau)$, $o_\theta(\tau) \in \mathbb{R}$, $\tau \geq t_0$ where $p(\tau) := \begin{bmatrix} x & y & z_\theta(\tau) & o_\theta(\tau) \end{bmatrix}^T$,*

(A4) *$V_\theta(\tau) := \begin{bmatrix} -\frac{\partial x_\theta(\tau)}{\partial\theta} & -\frac{\partial y_\theta(\tau)}{\partial\theta} & \frac{\partial z_\theta(\tau)}{\partial\theta} & \frac{\partial o_\theta(\tau)}{\partial\theta} \end{bmatrix}^T$, $\tau \geq t_0$.*

Then for $\tilde{\theta} \in \Theta$, the Fisher information matrix $\boldsymbol{I}(\tilde{\theta})$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$\boldsymbol{I}(\tilde{\theta}) = \int_{t_0}^{t} \Lambda(\tau) K^T V_\theta^T(\tau) \left(\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{U_\theta(\tau)^T U_\theta(\tau)}{q_{z_\theta(\tau),o_\theta(\tau)}(x,y)} dxdy \right) V_\theta(\tau) K d\tau \tag{1}$$

where $U_\theta(\tau) = \frac{\partial q_{z_\theta(\tau),o_\theta(\tau)}(x,y)}{\partial p(\tau)}$, $K = \frac{\partial\theta}{\partial\tilde{\theta}}$, $\tilde{\theta} \subseteq \theta \in \Theta$.

The general expression in Theorem 1 allows us to calculate the Fisher information matrix of the underlying random process that characterizes the acquired data when some or all of the parameters that describe the trajectory of the object are unknown. When $\tilde{\theta} = \theta$, i.e., all the parameters are unknown, the prior knowledge matrix becomes an identity matrix of size that is equivalent to the total number of parameters that describe the trajectory. This general expression pertains to the case where an object moves in the three-dimensional (3D) space. From its inverse, we can therefore obtain the lower bound to the best possible accuracy for the parameter estimates.

This lower bound is referred to as the fundamental limit of the accuracy for the particular parameter vector, or in short, the fundamental limit. The term fundamental is used to describe the fact that the model which underlies the expressions for calculating the lower bound does not take into account any deteriorating effects of the acquisition system such as pixelation of the detector and the various noise sources that typically occur in experimental settings. The fundamental limit has practical value as it provides us with a quantity of what is theoretically possible in the absence of deteriorating factors and thus serves as a benchmark for practical cases.

In the case where the object of interest moves in a 2D focus plane or in a relatively flat structure, we consider the image function $q_{z_\theta(\tau),o_\theta(\tau)}$ to be independent of the focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$, and assume that it is radially symmetric such that $q(x,y) = \tilde{q}(r^2)$ for a function $\tilde{q} : \mathbb{R}^2 \to \mathbb{R}$. Thus, the general expression in Theorem 1 simplifies to an expression comprising the product of two separable integrals as shown in the following proposition.

Proposition 2. *Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that there exists a radially symmetric image function q, i.e., $q(x,y) = \tilde{q}(r^2)$, for a function $\tilde{q} : \mathbb{R} \to \mathbb{R}$, that does not depend on $z_\theta(\tau)$ and $o_\theta(\tau)$ such that for $M > 0$, the photon distribution profile $f_{\theta,\tau}$ of a moving object is given by $f_{\theta,\tau}(x,y) = 1/M^2 q\left(x/M - x_\theta(\tau), y/M - y_\theta(\tau)\right)$, $(x,y) \in \mathbb{R}^2$, $\tau \geq t_0$. Then for $\tilde{\theta} \in \Theta$, the Fisher information matrix $\boldsymbol{I}(\tilde{\theta})$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by*

$$\boldsymbol{I}(\tilde{\theta}) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial(r^2)} \right)^2 dr \times \int_{t_0}^{t} \Lambda(\tau) K^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix} K d\tau, \tag{2}$$

where $K = \partial\theta/\partial\tilde{\theta}$, $\tilde{\theta} \subseteq \theta \in \Theta$.

The significance of the expression in Proposition 2 is that it greatly simplifies the calculation of the Fisher information matrix when some or all of the parameters that describe the trajectory are unknown. From the Fisher information matrix, we can then obtain the fundamental limit. For some specific trajectories, e.g., linear and circular trajectories, explicit analytical expressions of the fundamental limit can be derived from the expression in Proposition 2. We illustrate this with an example where an object with radially symmetric image function moves linearly in a 2D plane and prior knowledge of some of the parameters that describe its trajectory are available. These expressions are then used to study the influence of the prior knowledge of the parameters on the fundamental limit.

For a short exposure interval, the trajectory of a moving object can be assumed to be linear. By letting $\gamma^2 = 4\pi \int_0^\infty r^3/\tilde{q}(r^2) \left(\partial\tilde{q}(r^2)/\partial r^2\right)^2 dr$, the expression of the Fisher information matrix in Proposition 2 can be further simplified and rewritten as

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \int_{t_0}^{t} \Lambda(\tau) K^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix} K d\tau, \tag{3}$$

where $K = \partial\theta/\partial\tilde{\theta}$, $\tilde{\theta} \subseteq \theta \in \Theta$. Since the parametric expressions for the linear trajectory of the object of interest for the time interval $[t_0, t]$ are given by $x_\theta(\tau) = x_0 + v(\tau - t_0)\cos\phi$, $y_\theta(\tau) = y_0 + v(\tau - t_0)\sin\phi$, $t_0 \le \tau \le t$, where (x_0, y_0) is the starting location of the object, ϕ is the direction of movement, i.e., the angle between the linear trajectory and the $x-$axis and v is the speed of the object, then for $\theta = (x_0, y_0, \phi, v) \in \Theta$,

$$\begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} = \begin{bmatrix} 1 & 0 & -v(\tau - t_0)\sin\phi & (\tau - t_0)\cos\phi \\ 0 & 1 & v(\tau - t_0)\cos\phi & (\tau - t_0)\sin\phi \end{bmatrix}, \quad \tau \ge t_0. \quad (4)$$

By substituting the prior knowledge matrix K of the specific case and expression (4) into (3), we obtain the Fisher information matrix for the unknown parameter vector $\tilde{\theta}$. By inverting the Fisher information matrix and taking the square root of its diagonal, we obtain the fundamental limit of the parameter estimates.

For the case where prior knowledge of v is available, the corresponding prior knowledge matrix for the unknown parameter vector $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ is given by $K = \begin{bmatrix} I_3 & 0 \end{bmatrix}^T$, where I_3 is a 3×3 identity matrix and 0 is a null vector. Using the fact that the matrix multiplication is an associative operation, we can first reduce the matrix size before subjecting the expression to further algebraic manipulations such that

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \begin{bmatrix} a_1(t) & 0 & -va_2(t)\sin\phi \\ 0 & a_1(t) & va_2(t)\cos\phi \\ -va_2(t)\sin\phi & va_2(t)\cos\phi & v^2 a_3(t) \end{bmatrix}, \quad (5)$$

where $a_1(t) = \int_0^{t-t_0} \Lambda(\tau + t_0) d\tau$, $a_2(t) = \int_0^{t-t_0} \Lambda(\tau + t_0)\tau d\tau$ and $a_3(t) = \int_0^{t-t_0} \Lambda(\tau + t_0)\tau^2 d\tau$. For $a_i(t) \not\equiv 0$, $i = 1, 2, 3$, an inverse exists for (5). Thus if the photon detection rate is a function of time, i.e., $\Lambda(\tau)$, $\tau \le t_0$, the fundamental limit of $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ for the time interval $[t_0, t]$ is as follows.

$$\begin{aligned} \delta_{x_0} &= \frac{1}{\gamma}\sqrt{\frac{a_3(t) - \frac{a_2^2(t)}{a_1(t)}\cos^2\phi}{a_1(t)a_3(t) - a_2^2(t)}}, \delta_{y_0} = \frac{1}{\gamma}\sqrt{\frac{a_3(t) - \frac{a_2^2(t)}{a_1(t)}\sin^2\phi}{a_1(t)a_3(t) - a_2^2(t)}}, \\ \delta_\phi &= \frac{1}{\gamma v}\sqrt{\frac{a_1(t)}{a_1(t)a_3(t) - a_2^2(t)}}. \end{aligned} \quad (6)$$

Since the fluorescent-labelled molecule is much smaller than the optical resolution of the imaging system, it can be modelled as a point source. Thus, its image when in focus is a diffraction-limited spot describes by an Airy profile. However, this profile is often approximated with a 2D Gaussian profile in practice because it provides a good approximation in the central region [11]. Hence, in the case of the 2D Gaussian image function $q(x, y) = 1/(2\pi\sigma^2)\exp\left(-(x^2 + y^2)/(2\sigma^2)\right)$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$, $\gamma := 1/\sigma$ [12]. As for the Airy image function $q(x, y) = J_1^2(2\pi n_a\sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 + y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma := 2\pi n_a/\lambda$ [12], where n_a and λ denote the numerical aperture and emission wavelength respectively and J_1 denotes the first order Bessel function of the first kind.

If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \ge t_0$, then

$$\begin{aligned} a_1(t) &= \Lambda_0(t - t_0),\ a_2(t) = \frac{\Lambda_0}{2}(t - t_0)^2, \\ a_3(t) &= \frac{\Lambda_0}{3}(t - t_0)^3, \quad t > t_0. \end{aligned} \quad (7)$$

Substituting the above expressions into (6), the fundamental limit of $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ for the time interval $[t_0, t]$ simplifies to

$$\begin{aligned} \delta_{x_0} &= \frac{1}{\gamma}\sqrt{\frac{4 - 3\cos^2\phi}{N}},\ \delta_{y_0} = \frac{1}{\gamma}\sqrt{\frac{4 - 3\sin^2\phi}{N}}, \\ \delta_\phi &= \frac{2}{\gamma v(t - t_0)}\sqrt{\frac{3}{N}}, \quad t > t_0, \end{aligned} \quad (8)$$

where $N := \Lambda_0(t - t_0)$ denotes the expected number of detected photons for the time interval $[t_0, t]$.

In the case where prior knowledge of ϕ is available, the corresponding prior knowledge matrix of the unknown parameter vector $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ is given by

$$K^T = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}. \quad (9)$$

Substituting (9) and (4) into (3),

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \begin{bmatrix} a_1(t) & 0 & a_2(t)\cos\phi \\ 0 & a_1(t) & a_2(t)\sin\phi \\ a_2(t)\cos\phi & a_2(t)\sin\phi & a_3(t) \end{bmatrix}. \quad (10)$$

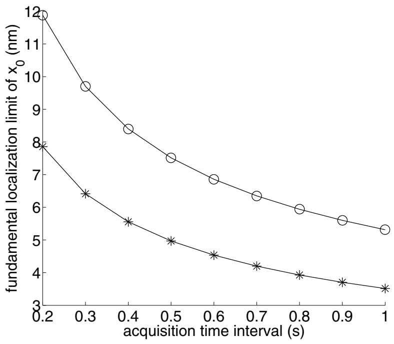
Similar to the preceding case, if the photon detection rate is a function of time, i.e., $\Lambda(\tau)$, $\tau \le t_0$, then the fundamental limit of $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ for the time interval $[t_0, t]$ is given as follows.

$$\begin{aligned} \delta_{x_0} &= \frac{1}{\gamma}\sqrt{\frac{a_3(t) - \frac{a_2^2(t)}{a_1(t)}\sin^2\phi}{a_1(t)a_3(t) - a_2^2(t)}}, \delta_{y_0} = \frac{1}{\gamma}\sqrt{\frac{a_3(t) - \frac{a_2^2(t)}{a_1(t)}\cos^2\phi}{a_1(t)a_3(t) - a_2^2(t)}}, \\ \delta_v &= \frac{1}{\gamma}\sqrt{\frac{a_1(t)}{a_1(t)a_3(t) - a_2^2(t)}}. \end{aligned} \quad (11)$$

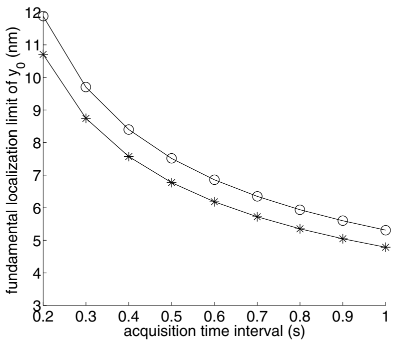
If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \ge t_0$, then the fundamental limit of $\tilde{\theta}$ for the time interval $[t_0, t]$ simplifies to

$$\begin{aligned} \delta_{x_0} &= \frac{1}{\gamma}\sqrt{\frac{4 - 3\sin^2\phi}{N}},\ \delta_{y_0} = \frac{1}{\gamma}\sqrt{\frac{4 - 3\cos^2\phi}{N}}, \\ \delta_v &= \frac{2}{\gamma(t - t_0)}\sqrt{\frac{3}{N}}, \quad t > t_0. \end{aligned} \quad (12)$$

From the analytical expressions in (8) and (12), we can easily see how prior knowledge of the parameters that describe the trajectory affects the fundamental limit by comparing them to the expression of the fundamental limit in [4]. It can be seen that there is no change in the fundamental limit of either ϕ or v when either prior knowledge of v or ϕ is available. However, improvement can be observed for the fundamental localization limits albeit a difference exists between the limits of the two



(a)



(b)

Fig. 1: Fundamental localization limit as a function of the acquisition time interval for an object with linear trajectory. Panel (a) Fundamental limit of x_0. Panel (b) Fundamental limit of y_0. ($\circ$) corresponds to the case where all the parameters that describe the trajectory $\theta = (x_0, y_0, \phi, v)$ are unknown. ($*$) corresponds to the case where prior knowledge of v is available such that $\tilde{\theta} = (x_0, y_0, \phi)$. For the moving object, $\sigma = 84$ nm, magnification $M = 100$, its direction of movement $\phi = 30^o$, its speed $v = 1000\ nm/s$ and the photon detection rate $\Lambda_0 = 1000$ photons/s.

coordinates as shown in Fig. 1. With prior knowledge of either v or ϕ, the fundamental localization limit becomes dependent on the direction of movement ϕ. It is interesting to note that the best fundamental limit of x_0 occurs when prior knowledge of v is available and the object is moving horizontally with respect to the detector. Incidentally, this is also the condition where the worst fundamental limit of y_0 occurs. When the object moves perpendicularly to the horizontal on the 2D plane, the results of the fundamental limit of x_0 and y_0 are inverted. They are only equal when the object moves diagonally across the 2D plane. When prior knowledge of ϕ is available, the outcome of the fundamental limit of x_0 and y_0 is in contrary to the preceding case where prior knowledge of v is available. Hence, from these observations, which coordinate has a better fundamental limit will depend on which of the two parameters is known and the direction of movement of the object.

III. Conclusion

In this paper, we have provided a study of the influence of prior knowledge of the parameters that describe the trajectory on the accuracy limit in parameter estimation. A general expression of the Fisher information matrix for the underlying detection process is formulated in terms of the image function, the trajectory and the prior knowledge matrix.

Using the explicit analytical expression of the fundamental limit for the case of a linear trajectory, we have considered the availability of the prior knowledge of each parameter in turn in order to study their influence on the performance limit. It has been shown that prior knowledge of either the speed or the direction of movement only improves the fundamental localization limit when compared to the case where prior knowledge is unavailable. In addition, the fundamental localization limit becomes dependent on the direction of movement which results in a disparity between the accuracy limit of the coordinates. It should be noted that the results here are essentially independent of the application in single-molecule microscopy and can be applied to the general problem of tracking an object using quantum limited detectors.

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