A NOVEL RESOLUTION MEASURE FOR OPTICAL MICROSCOPES: STOCHASTIC ANALYSIS OF THE PERFORMANCE LIMITS

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ABSTRACT

In the recent past, the increased use of optical microscopes in quantitative studies, such as single molecule microscopy, has generated significant interest in quantifying its performance limit. Here, by adopting an information-theoretic stochastic framework, we present expressions to calculate performance limits that quantify the capabilities of optical microscopes. We revisit the resolution problem from the stochastic framework and derive a new resolution measure. Our result, unlike Rayleigh's resolution criterion, predicts that the resolution of an optical microscope is not limited, but that the resolvability depends on the detected photon count. Analytical expressions are also given that take into account the effect of deteriorating experimental factors such as pixelation and noise sources. We also consider the location estimation problem, which is of relevance for particle tracking applications.

1. INTRODUCTION

The optical microscope has been an invaluable tool for biological research. The recent past has witnessed an increased use of optical microscopy as a cellular imaging modality. Optical microscopes perform tasks ranging from long term (hours time scale) three dimensional imaging of live cells to fast imaging (milliseconds time scale) of molecular interactions within a cellular environment even at the single molecule level ([1, 2]). In several biological applications the data acquired through an optical microscope requires extensive quantitative analysis ([2]). In order to carry out such studies, it is important for an experimenter to know the capabilities of the instrument. This not only provides insight into determining the feasibility of a particular experiment, but it also helps in designing an optimal experimental setup.

In this paper we present results to calculate performance limits that quantify the capabilities of an optical microscope. Due to the random nature of the acquired data, we adopt a stochastic framework and use the tools of statistical estimation theory ([3]) to determine the performance limit. We consider a data model in which the photon emission (detection) process is modeled as a random process (shot noise process). We take into account the pixelation of the detector and additive noise sources, such as Poisson and Gaussian noise sources, that are typically present in the acquired data ([4]). We note that our results are applicable to several microscopic techniques such as fluorescence microscopy, bright-field microscopy, etc.

We investigate the resolution problem, which is an important problem in optical microscopy. The classical resolution criterion of Rayleigh, although widely used, is well known to be based on heuristic notions ([5]) that are incompatible with current imaging approaches. By using the above stochastic framework, we derive a new resolution measure that overcomes the limitations of Rayleigh's criterion. According to our new resolution measure, the resolution of an optical microscope is not limited and it can be improved by increasing the expected number of detected photons. We note that our new resolution measure is in contrast to other results ([6, 7]) that are not applicable to photon-limited applications, since they are based on deterministic data models that only consider the additive Gaussian noise component. We also investigate the location estimation problem, which is of current interest in biological applications such as particle tracking.

2. GENERAL RESULTS

Our approach to calculating the performance limit is based on the statistical theory concerning the Fisher information matrix ([3, 8]). According to the Cramer-Rao inequality ([8]), the variance of any unbiased estimator $\hat{\theta}$ of an unknown parameter θ is always greater than or equal to the inverse Fisher information matrix, i.e., $\mathrm{Var}(\hat{\theta}) \geq \mathbf{I}^{-1}(\theta)$. Since the performance of an unbiased estimator is typically specified in terms of its standard-deviation, the above inequality implies that the square-root of the (corresponding leading diagonal element in the) inverse Fisher information matrix provides a bound to

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the accuracy with which the unknown parameter θ can be determined. Therefore, the performance limit to determining a particular object attribute is defined as the square root of the inverse Fisher information matrix calculated for that attribute.

For a general parameter estimation problem in optical microscopy, the expression of the Fisher information matrix corresponding to the acquisition time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^{t} \int_{\mathcal{C}} \frac{1}{\Lambda_\theta(\tau) f_{\theta,\tau}(r)} \left(\frac{\partial [\Lambda_\theta(\tau) f_{\theta,\tau}(r)]}{\partial \theta} \right)^T \times \frac{\partial [\Lambda_\theta(\tau) f_{\theta,\tau}(r)]}{\partial \theta} dr d\tau, \quad \theta \in \Theta. \tag{1}$$

The above result (see [9] for details) is a generalization of the Fisher information matrix given in [10, chap 4] to the time varying case. Here Λ_θ denotes the intensity function of the Poisson process, which models the time points of the detected photons, $f_{\theta,\tau}$ denotes the density function of the independent random variables that model the spatial coordinates of the detected photons and $\mathcal{C}$ denotes the detector. It is assumed that the spatial and the temporal components are mutually independent of each other. In deriving eq. 1 no specific assumptions have been made regarding the functional form of $f_{\theta,\tau}$ or Λ_θ. Therefore, the above expression of $\mathbf{I}(\theta)$ is applicable to a wide variety of imaging conditions, such as (in)coherent/polarized illumination and detection, etc. We note that an implication of the time dependence of the density function $f_{\theta,\tau}$ is that the above equation is applicable to moving objects.

Effects of pixelation and noise

The Fisher information matrix $\mathbf{I}(\theta)$ given in eq. 1 considers a detector that provides the time points and the spatial coordinates of every detected photon. We next consider a pixelated detector $\{C_1, \ldots, C_{N_p}\}$ in which the acquired data consists of the number of photons detected at each pixel C_k ($C_k \subseteq \mathbb{R}^2$), where N_p denotes the total number of pixels. It can be shown that the photon count from the object of interest at the k^{th} pixel is independently Poisson distributed with mean μ_θ, which is given by $\mu_\theta(k,t) := \int_{t_0}^{t} \int_{C_k} \Lambda_\theta(\tau) f_{\theta,\tau}(r) dr d\tau$, where $k = 1, \ldots, N_p$, $\theta \in \Theta$, $[t_0, t]$ denotes the acquisition time interval and Λ_θ and $f_{\theta,\tau}$ denote the intensity function and the density function, respectively. We consider two independent additive noise sources, namely Poisson and Gaussian noise sources. Poisson noise (mean $\beta(k,t)$) is used to model the effects of autofluorescence and scattering at the k^{th} pixel, and Gaussian noise (mean η_k, variance $\sigma^2_{w,k}$) is used to model the measurement noise at the k^{th} pixel due to the readout process.

In the absence of the additive noise sources (Poisson and Gaussian noise), the expression of the Fisher information matrix for a pixelated detector follows from a well known result for Poisson random variables (see [9, 10]). In the presence of additive noise sources the expression for the Fisher information matrix for a pixelated detector is given by ([9])

$$\mathbf{I}(\theta) := \sum_{k=1}^{N_p} \left(\frac{\partial \mu_\theta(k,t)}{\partial \theta} \right)^T \frac{\partial \mu_\theta(k,t)}{\partial \theta} \times \left(\int_{\mathbb{R}} \frac{\left(\sum_{l=1}^{\infty} \frac{[\nu_\theta(k,t)]^{l-1} e^{-\nu_\theta(k,t)}}{(l-1)!} \cdot \frac{1}{\sqrt{2\pi}\sigma_{w,k}} e^{-\frac{1}{2}\left(\frac{z-l-\eta_k}{\sigma_{w,k}}\right)^2} \right)^2}{p_{\theta,k}(z)} dz - 1 \right), \tag{2}$$

where $\theta \in \Theta$, $\nu_\theta(k,t) := \mu_\theta(k,t) + \beta(k,t)$, $k = 1, \ldots, N_p$, $\theta \in \Theta$, μ_θ and β are as given above, and

$$p_{\theta,k}(z) := \frac{1}{\sqrt{2\pi}\sigma_{w,k}} \sum_{l=0}^{\infty} \frac{[\nu_\theta(k,t)]^l e^{-\nu_\theta(k,t)}}{l!} e^{-\frac{1}{2}\left(\frac{z-l-\eta_k}{\sigma_{w,k}}\right)^2},$$

where $\theta \in \Theta$ and $z \in \mathbb{R}$. Analogous to eq. 1, the above expression of the Fisher information matrix is also applicable to a wide variety of imaging conditions.

3. RESOLUTION PROBLEM

The resolution problem is concerned with the task of determining the distance of separation d between two point sources from the data acquired with an optical microscope setup. Here we consider imaging conditions analogous to those assumed in Rayleigh's criterion, i.e., the point sources are in focus and emit incoherent, unpolarized light.

The intensity function is given by $\Lambda_\theta(\tau) = \Lambda_1(\tau) + \Lambda_2(\tau)$, $\tau \geq t_0$, where Λ_i denotes the photon detection rate of the point source, $i = 1, 2$, and the density function $f_{\theta,\tau}$ is given by

$$f_{\theta,\tau}(r) := \frac{1}{2M^2} q\left(\frac{x}{M} - \frac{d}{2}, \frac{y}{M} \right) + \frac{1}{2M^2} q\left(\frac{x}{M} + \frac{d}{2}, \frac{y}{M} \right), \tag{3}$$

where $(x, y) \in \mathbb{R}^2$, $\theta \in \Theta$, $\tau \geq t_0$, M denotes the total lateral magnification of the microscope setup, and q denotes the image function of the point source. The image function is defined as the image of an object that is imaged at unit magnification when the object is located at the origin of the coordinate axes in the specimen space. By definition, the image function satisfies the integral identity $\int_{\mathbb{R}^2} q(x,y) dx dy = 1$. In the above equation we consider a simple arrangement in which the point sources are separated by a distance d and are located equidistant from the origin on the x axis.

Substituting for $f_{\theta,\tau}$ and Λ_θ in eq. 1, the general expression for the Fisher information matrix corresponding to the resolution problem is given by

$$\mathbf{I}(d) := \frac{1}{4} \int_{t_0}^{t} \int_{\mathbb{R}^2} \frac{1}{\Lambda_1(\tau) q(x + \frac{d}{2}, y) + \Lambda_2(\tau) q(x - \frac{d}{2}, y)} \times \left(\Lambda_1(\tau) \frac{\partial q(x + \frac{d}{2}, y)}{\partial x} - \Lambda_2(\tau) \frac{\partial q(x - \frac{d}{2}, y)}{\partial x} \right)^2 dx dy. \tag{4}$$

According to optical diffraction theory the image of an in-focus point source that emits incoherent, unpolarized light is described by the Airy profile ([11]), which, in terms of

an image function is given by $q(x,y) = J_1^2(\alpha\sqrt{x^2+y^2})/(\pi(x^2+y^2))$, $(x,y) \in \mathbb{R}^2$, where J_1 denotes the first order Bessel function of the first kind, $\alpha = 2\pi n_a/\lambda$, n_a denotes the numerical aperture of the objective lens and λ denotes the wavelength of the detected photons. Substituting for q in eq. 4 and assuming that the photon detection rate of the point sources is a constant i.e., $\Lambda_i(\tau) = \Lambda_0$, $\tau \geq t_0$, $i = 1, 2$, the fundamental resolution measure (fundamental performance limit to determining the distance d) is given by

$$\delta_d := \frac{1}{\sqrt{\mathbf{I}(d)}} = \frac{1}{\sqrt{4\pi \cdot \Lambda_0 \cdot (t-t_0) \cdot \Gamma_0(d)}} \cdot \frac{\lambda}{n_a}, \quad (5)$$

where $\Gamma_0(d)$ is given by

$$\Gamma_0(d) := \int_{\mathbb{R}^2} \frac{1}{\frac{J_1^2(\alpha r_{01})}{r_{01}^2} + \frac{J_1^2(\alpha r_{02})}{r_{02}^2}} \left((x+\frac{d}{2}) \frac{J_1(\alpha r_{01}) J_2(\alpha r_{01})}{r_{01}^3} \right.$$

$$\left. -(x-\frac{d}{2}) \frac{J_1(\alpha r_{02}) J_2(\alpha r_{02})}{r_{02}^3} \right)^2 dxdy, \quad (6)$$

with J_n denoting the n^{th} order Bessel function of the first kind, $r_{01} := \sqrt{(x+d/2)^2+y^2}$ and $r_{02} := \sqrt{(x-d/2)^2+y^2}$. The above result pertains to the best case scenario, since the stochastic framework used to obtain $\mathbf{I}(d)$ considers a microscope setup in which the detector provides the time points and the spatial coordinates of every detected photon without adding any extraneous noise sources. For any imaging condition, this is an idealization of current imaging detectors in which the presence of finite-size pixels and measurement noise deteriorate the acquired data. By definition, δ_d is a bound to the smallest possible standard deviation of any unbiased estimator of the distance, and therefore, eq. 5 gives a result that is fundamental to the imaging conditions that are analogous to those of Rayleigh's criterion. We note that the expression for δ_d shows how the resolution measure is affected by deterministic properties such as the numerical aperture of the objective lens n_a, the wavelength of the detected photons λ and the acquisition time $t - t_0$, and stochastic parameters such as the photon detection rate Λ_0.

Fig. 1 shows the behavior of the fundamental resolution measure δ_d as a function of the distance of separation d for a pair of point sources with $\lambda = 600$ nm and that are imaged with a 1.45 NA objective lens. According to Rayleigh's criterion, the two point sources cannot be resolved if their distance of separation is below 261 nm ($\approx 0.61\lambda/n_a$). From the figure we see that for distances well below Rayleigh's criterion ($50 - 250$ nm), the numerical value of δ_d is relatively small thereby predicting high accuracy in estimating d. However, for very small distances of separation ($d < 50$ nm), the numerical value of δ_d becomes large thereby predicting relatively poor accuracy in estimating d. For example, the fundamental resolution measure predicts an accuracy not better than ± 7.88 nm to resolve a distance of 10 nm between the two point sources when the expected photon count per point source is 2500. On the other hand, the fundamental resolution measure predicts an accuracy not better than ± 2.5 nm to resolve a distance of 200 nm for the same expected photon count per point source.

In many applications, the 2D Gaussian profile is often used instead of the Airy profile due to the relative ease of simulating Gaussian profiles. The Gaussian image function is given by $q(x,y) = (1/(2\pi\sigma^2)) \exp(-(x^2+y^2)/(2\sigma^2))$, $(x,y) \in \mathbb{R}^2$, where σ denotes the width of the Gaussian profile. Setting $\Lambda_i(\tau) = \Lambda_0$, $\tau \geq t_0$, $i = 1, 2$, and substituting for q and Λ_i in eq. 4, the fundamental resolution measure corresponding to the Gaussian profile is given by

$$\frac{1}{\sqrt{\mathbf{I}(d)}} = \frac{\sigma}{\sqrt{\Lambda_0(t-t_0)}} \frac{1}{\sqrt{\Psi_0(d)}},$$

where

$$\Psi_0(d) = \frac{1}{8\pi\sigma^4} \int_{\mathbb{R}^2} \frac{1}{e^{-\frac{(x+\frac{d}{2})^2+y^2}{2\sigma^2}} + e^{-\frac{(x-\frac{d}{2})^2+y^2}{2\sigma^2}}} \times$$

$$\left((x+\frac{d}{2}) e^{-\frac{(x+\frac{d}{2})^2+y^2}{2\sigma^2}} - (x-\frac{d}{2}) e^{-\frac{(x-\frac{d}{2})^2+y^2}{2\sigma^2}} \right)^2 dxdy.$$

We next consider the case of a pixelated detector with square pixels of length W. To calculate the Fisher information for the pixelated detector we require the expression for $\mu_\theta(k)$ - the mean photon count of the point sources at the k^{th} pixel, and the expression for $\partial\mu_\theta(k)/\partial\theta$. For the Airy profile, the expression for $\mu_\theta(k)$ and $\partial\mu_\theta(k)/\ \partial\theta$ have to be evaluated through numerical integration. For the Gaussian profile, the expression for $\mu_\theta(k)$ and $\partial\mu_\theta(k)/\partial\theta$ can be expressed in terms of the error function and are given by

$$\mu_\theta(k) = \mu_\theta(i,j) := \frac{\Lambda_0(t-t_0)}{4} \left(\left[\text{erf}(\alpha_i^-) - \text{erf}(\alpha_{i-1}^-) \right] + \right.$$

$$\left. \left[\text{erf}(\alpha_i^+) - \text{erf}(\alpha_{i-1}^+) \right] \right) \left(\text{erf}(\delta_j) - \text{erf}(\delta_{j-1}) \right), \quad \theta \in \Theta,$$

$$\frac{\partial\mu_\theta(i,j)}{\partial d} := \frac{\Lambda_0(t-t_0)}{4\sqrt{2\pi}\sigma} \left(\left[e^{-(\alpha_{i-1}^-)^2} - e^{-(\alpha_i^-)^2} \right] \right.$$

$$\left. + \left[e^{-(\alpha_i^+)^2} - e^{-(\alpha_{i-1}^+)^2} \right] \right) \left(\text{erf}(\delta_j) - \text{erf}(\delta_{j-1}) \right),$$

where erf denotes the error function, $\alpha_j^\pm = (iW \pm Md/2)/(\sqrt{2}M\sigma)$, $i = 1, \ldots, N_x$ and $\delta_j = (jW)/(\sqrt{2}M\sigma)$, $j = 1, \ldots, N_y$. In the above expression, (i,j) denotes the row and the column number of the k^{th} pixel, N_x (N_y) denotes the number of pixels in the pixel array along the x (y) direction, $k = 1, \ldots, N_p$ and $N_p = N_x + N_y$. The above expressions are obtained by making use of the identity $(\partial/\partial x)\text{erf}(f(x)) = (2/\sqrt{\pi}) e^{-f^2(x)} \frac{\partial f(x)}{\partial x}$, $x \in \mathbb{R}$.

Location estimation problem

We next consider the problem of estimating the location of

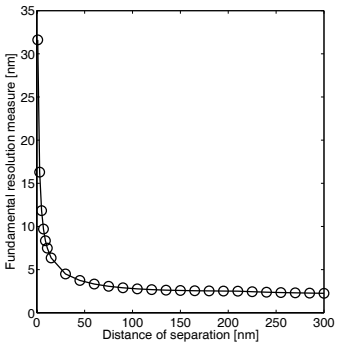

Fig. 1 Behavior of the fundamental resolution measure (eq. 5) as a function of the distance of separation. Here, the expected number of detected photons per point source is set to be $\Lambda_0(t-t_0) = 2500$ photons, the wavelength of the detected photons is set to be $\lambda = 600$ nm and the numerical aperture of the objective lens is set to be $n_a = 1.45$.

an object. Here, we use the Gaussian profile to describe the image of the object (see [12] for results pertaining to the Airy profile). The unknown parameter is set to be $\theta = (x_0, y_0, \sigma, \Lambda_0)$, where (x_0, y_0) denotes the location of the object, σ denotes the width of the Gaussian profile and Λ_0 denotes the photon detection rate of the object. The density function $f_{\theta,\tau}$ is given by

$$f_{\theta,\tau}(r) := \frac{1}{2\pi(M\sigma)^2} e^{-\frac{(x-Mx_0)^2+(y-My_0)^2}{2(M\sigma)^2}}, \quad (x,y) \in \mathbb{R}^2,$$

where $\theta \in \Theta$, $\tau \geq t_0$ and M denotes the total lateral magnification of the microscope setup. The intensity function Λ_θ is given by $\Lambda_\theta(\tau) := \Lambda_0$, $\tau \geq t_0$. Substituting for Λ_θ and $f_{\theta,\tau}$ in the Fisher information matrix given in eq. 1 and inverting it, we get

$$\mathbf{I}^{-1}(\theta) := \begin{bmatrix} \frac{\sigma^2}{\Lambda_0(t-t_0)} & 0 & 0 & 0 \\ 0 & \frac{\sigma^2}{\Lambda_0(t-t_0)} & 0 & 0 \\ 0 & 0 & \frac{\sigma^2}{4\Lambda_0(t-t_0)} & 0 \\ 0 & 0 & 0 & \frac{\Lambda_0}{t-t_0} \end{bmatrix}, \tag{7}$$

where $\theta \in \Theta$.

We next consider a pixelated detector with square pixels of length W. To calculate the Fisher information matrix, we require the expression for $\mu_\theta(k)$ and $\partial\mu_\theta(k)/\,\partial\theta$, which, for the Gaussian profile are given by

$$\mu_\theta(k) = \mu_\theta(i,j) = \frac{\Lambda_0 t}{4}\Big(\text{erf}(\epsilon_i) - \text{erf}(\epsilon_{i-1})\Big)\Big(\text{erf}(\kappa_j) - \text{erf}(\kappa_{j-1})\Big),$$

$$\frac{\partial\mu_\theta(i,j)}{\partial x_0} = \frac{\Lambda_0 t}{\sqrt{8\pi}\sigma}\Big(e^{-\epsilon_{i-1}^2} - e^{-\epsilon_i^2}\Big)\Big(\text{erf}(\kappa_j) - \text{erf}(\kappa_{j-1})\Big),$$

$$\frac{\partial\mu_\theta(i,j)}{\partial y_0} = \frac{\Lambda_0 t}{\sqrt{8\pi}\sigma}\Big(\text{erf}(\epsilon_i) - \text{erf}(\epsilon_{i-1})\Big)\Big(e^{-\kappa_{j-1}^2} - e^{-\kappa_j^2}\Big),$$

$$\frac{\partial\mu_\theta(i,j)}{\partial\sigma} = \frac{\Lambda_0 t}{2\sqrt{\pi}\sigma}\Big[\Big(\epsilon_{i-1}e^{-\epsilon_{i-1}^2} - \epsilon_i e^{-\epsilon_i^2}\Big)\Big(\text{erf}(\kappa_j) - \text{erf}(\kappa_{j-1})\Big)$$

$$+\Big(\text{erf}(\epsilon_i) - \text{erf}(\epsilon_{i-1})\Big)\Big(\kappa_{j-1}e^{-\kappa_{j-1}^2} - \kappa_j e^{-\kappa_j^2}\Big)\Big],$$

$$\frac{\partial\mu_\theta(i,j)}{\partial\Lambda_0} = \frac{1}{\Lambda_0}\mu_\theta(i,j),$$

where erf denotes the error function, $\theta \in \Theta$, $\epsilon_i := \frac{iW - Mx_0}{\sqrt{2}M\sigma}$, $\kappa_j := \frac{jW - My_0}{\sqrt{2}M\sigma}$ for $i = 1, \ldots, N_x$ and $j = 1, \ldots, N_y$.

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