Bounding parameter uncertainty in single molecule localization

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We will adopt the Gaussian PSF approximation (image function):

$$q(x,y) = \frac{1}{2\pi\sigma^2}\exp\left(-\frac{(x-x_0)^2+(y-y_0)^2}{2\sigma^2}\right)$$

and define the number of photoelectrons at a pixel k as a sum of three random variables

$$H_{\theta,k} = S_{\theta,k} + B_{\theta,k} + W_{\theta,k}$$

where $S_{\theta,k}$ and $B_{\theta,k}$ are Poisson processes for signal and background while $W_{\theta,k}$ represents Gaussian noise of a CMOS array. Unless otherwise specified we will assume that $W_k \sim \mathcal{N}(m_k, \sigma^2_{w,k})$. The mean of the variable $H_{\theta,k}$ is found by measuring m_k and evaluating the following three equations

$$\langle H_{\theta,k}\rangle = \mu_{\theta,k} + \beta_k + m_k$$

$$\mu_{\theta,k} = \int_{t_0}^{t}\Lambda(\tau)\int_{C_k} q(x,y)dxdyd\tau$$

$$\beta_{\theta,k} = \int_{t_0}^{t}\Lambda(\tau)\int_{C_k} b(x,y)dxdyd\tau$$

where $b(x,y)$ is a spatially dependent background function. $\Lambda(\tau)$ is the emission rate as a function of time which could, for example, be exponential decay for photobleaching. We now need to show the complete form of $P(H_k)$ which requires that we first know $P(S_k)$. Let's say that we have a fluorophore that emits photons according to a Poisson process with rate η which therefore has mean $\mu = \eta\Delta t$ where Δt is the exposure time. If that photon were to arrive at a particular CMOS pixel k, it can be detected with a probability equal to the quantum efficiency $0 \leq \gamma \leq 1$ of the detector. Given that the photon indeed arrives at pixel k, S_k is then a product of two random variables

$$M_k \sim \text{Poisson}(\eta) \qquad N_k \sim \text{Bern}(\gamma, 1-\gamma)$$

According to the first distribution, the probability M_k photons will be detected in the exposure time Δt is Poisson, which has the form

$$\text{Poisson}(n; \eta) = \frac{\exp\left(-\eta \Delta t\right) (\eta \Delta t)^n}{n!}$$

There are two variables: whether or not a photon was emitted and whether or not a photon can be detected. The number of detected photons is $S_k = M_k N_k$.

$$P(S_k, M_k, N_k) = P(S_k | M_k = m_k, N_k = n_k) P(M_k | N_k = n_k) P(N_k = n_k)$$

Note that $m_k, s_k \in \Omega$ where $\Omega = \{z \in \mathbb{Z} : z \geq 0\}$ We can marginalize this distribution over M_k and N_k

$$P(S_k) = \sum_{m_k} \sum_{n_k} P(S_k | M_k = m_k, N_k = n_k) P(M_k | N_k n_k) P(N_k = n_k)$$

Expanding this expression out over the support of n_k gives

$$\begin{aligned} P(S_k) = \sum_{m_k} & P(S_k | M_k = m_k, N_k = 0) P(M_k | N_k = 0) P(N_k = 0) \\ & + P(S_k | M_k = m_k, N_k = 1) P(M_k | N_k = 1) P(N_k = 1) \end{aligned}$$

At this point it is quite clear that we have

$$\begin{aligned} P(S_k) &= \sum_{m_k} \gamma \delta(s_k - m_k) P(M_k = m_k) + (1 - \gamma) \delta(s_k) P(M_k = m_k) \\ &= \gamma P(M_k) + \delta(s_k)(1 - \gamma) \end{aligned}$$

This result tells us that we reduce probability mass by a factor of γ and inflate the value at zero by adding $1 - \gamma$. Next, we can can use this to find the distribution over the corrupted signal $P(H_k)$

To find $P(H_k)$, we first evaluate the joint density $P(S_k, H_k)$

$$\begin{aligned} P(S_k, H_k) &= P(H_k | S_k = s) P(S_k = s) \\ &= \frac{1}{Z} \exp\left(-\frac{(H_k - g_k s - \mu_k)^2}{\sigma_k^2}\right) \frac{\exp\left(-\Lambda_k\right) \Lambda_k^s}{s!} \end{aligned}$$

Marginalizing over S_k gives the desired distribution over H_n

$$P(H_k) = \frac{1}{Z} \sum_{s=0}^{\infty} \frac{\exp\left(-\Lambda_k\right) \Lambda_k^s}{s!} \exp\left(-\frac{(H_k - g_k s - \mu_k)^2}{\sigma_k^2}\right)$$

Consider the general prescripton of maxmimum likelihood parameter estimation:

$$\mathcal{E}_{\mathrm{MLE}} : \theta^* = \underset{\theta}{\operatorname{argmax}}\ \ell(\mathcal{D}|\theta)$$

where $\ell = \log \mathcal{L}$ is the log-likelihood function
Question: can we derive a theoretical lower bound on our uncertainty in θ^* for an arbitrary estimator $\mathcal{E}$?
Start by defining the *score* of ℓ with respect to θ as

$$\mathcal{S} = \underset{x\sim p}{\mathbb{E}}\left[\frac{\partial}{\partial\theta}\ell(x|\theta)\right]$$

Since x is a continuous random variable, we have to consider the average score

The Fisher Information $I(\theta)$ is defined as the variance of the score

$$I(\theta) = \underset{x\sim p}{\mathbb{E}}\left[\frac{\partial}{\partial\theta}\left(\ell(x|\theta)\right)\right]^2 = \underset{x\sim p}{\mathbb{E}}\left[\frac{\partial^2}{\partial\theta^2}\left(\ell(x|\theta)\right)\right]$$

for $x \in \mathcal{D}$. The variance takes this from because it can be shown that $\mathcal{S} = 0$
Intuitively, if the likelihood is insensitive changes in θ, then $\mathcal{D}$ does not provide very much information about θ

When there are many parameters, the Fisher Information (second moment of the score) is a covariance matrix

$$I_{ij}(\theta) = \underset{x\sim p}{\mathbb{E}}\left[\frac{\partial}{\partial\theta_i}\left(\ell(x|\theta)\right)\frac{\partial}{\partial\theta_j}\left(\ell(x|\theta)\right)\right]$$

We have shown that the model for the number of photoelectrons at a pixel is

$$P(H_k) = \frac{1}{Z}\sum_{s=0}^{\infty}\frac{\exp\left(-\Lambda_k\right)\Lambda_k^s}{s!}\exp\left(-\frac{(H_k - g_k s - \nu_k)^2}{\sigma_k^2}\right)$$

Notice that ν_k is dependent on $\int q(x,y)dxdy$ and therefore the PSF parameters $\theta = (\mu_x, \mu_y, \sigma)$. These can be plugged into the following Fisher information matrix

$$
\begin{aligned}
I_{ij}(\theta) &= \mathop{\mathbb{E}}_{H\sim P}\left[\frac{\partial}{\partial\theta_i}\left(\log\prod_k P(H_k)\right)\frac{\partial}{\partial\theta_j}\left(\log\prod_k P(H_k)\right)\right] \\
&= \mathop{\mathbb{E}}_{H\sim P}\sum_k\left[\frac{\partial}{\partial\theta_i}\log P(H_k)\frac{\partial}{\partial\theta_j}\log P(H_k)\right]
\end{aligned}
$$

We need to then calculate the derivatives with respect to the parameters θ_i.

Computing the mean value at a pixel involves computing an integral $\int_{C_k} q(x,y)dxdy$. This integral is difficult to compute, but a 2D image is really just a 2D histogram. So if we can find the PSF $q(x,y)$ from the objective lens, we can use Monte Carlo integration (sample from the normalized PSF) and multiply by the emission rate ν to compute the mean of the Poisson process at a pixel k