Photon Counting Histogram: One-Photon Excitation

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Calculation of the observation volume profile

In a lens system, the 3D image of a point object is called the point spread function, \( \text{PSF}(r) \). Dimensionless optical units are defined as

\[
\begin{align*}
  u & = z \cdot \frac{2\pi}{\lambda_{\text{ex}}} n \sin^2 \alpha \\
  v & = r \cdot \frac{2\pi}{\lambda_{\text{ex}}} n \sin \alpha
\end{align*}
\]

(S1)

for excitation and

\[
\begin{align*}
  u' & = r \cdot M^2 \frac{2\pi}{\lambda_{\text{ex}}} \sin^2 \alpha \\
  v' & = r \cdot M \frac{2\pi}{\lambda_{\text{em}}} \sin \alpha
\end{align*}
\]

(S2)

for emission, where \( z \) is the coordinate along the optical axis, \( r = \sqrt{x^2 + y^2} \), \( \lambda_{\text{ex}} \) and \( \lambda_{\text{em}} \) are the excitation and emission wavelengths, \( n \) is the index of refraction of the sample medium, \( \alpha = \sin^{-1}(\text{NA} / n) \) is the half cone angle of the objective with \( \text{NA} \) being the numerical aperture, and \( M \) is the magnification of the objective. For the excitation point spread function, we use the complex integration
representation for the diffraction proposed by Richards and Wolf:

\[ I_0(u,v) = \int_0^\alpha A(\theta) \cdot \sin \theta (1 + \cos \theta) \cdot J_0 \left( \frac{v \sin \theta}{\sin \alpha} \right) \cdot \exp \left( \frac{iu \cos \theta}{\sin^2 \alpha} \right) \, d\theta \]

\[ I_1(u,v) = \int_0^\alpha A(\theta) \cdot \sin^2 \theta \cdot J_1 \left( \frac{v \sin \theta}{\sin \alpha} \right) \cdot \exp \left( \frac{iu \cos \theta}{\sin^2 \alpha} \right) \, d\theta \]

\[ I_2(u,v) = \int_0^\alpha A(\theta) \cdot \sin \theta (1 - \cos \theta) \cdot J_2 \left( \frac{v \sin \theta}{\sin \alpha} \right) \cdot \exp \left( \frac{iu \cos \theta}{\sin^2 \alpha} \right) \, d\theta \]  \hspace{1cm} (S3)

where \( J_n(x) \) is the \( n \)th order Bessel function, and

\[ \text{PSF}_{\text{ex}}(u,v) = |I_0|^2 + 2|I_1|^2 + |I_2|^2 \]  \hspace{1cm} (S4)

for unpolarized or circularly polarized light. The formula for linearly polarized excitation is more complex and involves much more computation time; thus, we only calculate the unpolarized or circularly polarized case. The apodization function \( A(\theta) \) describes the amplitude distribution for the electric field on the plane after the objective:

\[ A(\theta) = \cos^2 \theta \cdot \exp \left( -\beta^2 \frac{\sin^2 \theta}{\sin^2 \alpha} \right) \]  \hspace{1cm} (S5)

where the under filling factor \( \beta \) is the ratio of the radius of the objective back aperture to the \( e^2 \) radius of the excitation laser.\(^{[2]}\)

For the emission point spread function, we do the same integration as in Eq. (S3) over the tube lens of the microscope, with the integration upper limit \( \alpha' = \sin^{-1}(\sin \alpha/M) \). This apodization function in this case is given by the isotropic fluorescence emission:\(^{[3]}\)

\[ A(\theta) = \cos^2 \theta \left( 1 - M^2 \sin^2 \theta \right)^{\frac{1}{2}} \]  \hspace{1cm} (S6)
We then calculate the observation volume profile by multiplying the excitation PSF and the integration of the emission PSF over the circular pinhole:

\[ W_c(u,v) = \text{PSF}_{\text{exc}}(u,v) \cdot \int_{\text{Pinhole}} \text{PSF}_{\text{em}}(\gamma \cdot u, |\mathbf{r}_D - \gamma \cdot v|) d\mathbf{r}_D \] (S7)

where \( \gamma = \frac{\lambda_{\text{ex}}}{n \lambda_{\text{em}}} \) reflects the fact that the magnification is independent of wavelength.\(^3\)

In numerical computation, the Bessel functions are evaluated using rational approximations,\(^4\) all the integrations are calculated using the Romberg method\(^4\) with an accuracy of \(10^{-5}\). Both the point spread functions and the observation volume profiles are calculated and stored in grids of 0.1 optical units with \( u \) up to 200 optical units and \( v \) up to 100 optical units. Bilinear interpolation is employed in extracting a value from the saved functions.

References: