Closed Form Expressions for the Excitation and Detection Efficiency of Single Dipoles Under Polarized Wide-Field Illumination

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1 Introduction

In these notes I will develop the relationships between the excitation/detection efficiencies and the dipole orientation, polarizer orientation, and microscope geometry for wide-field illumination microscopes. First, I will write out the relationships for the epi-illumination and epi-detection case, then I will generalize the relationships for oblique or orthogonal geometries.

2 Detection Efficiency

In the 2017-04-25 notes I followed Fourkas and showed that the detection efficiency (the fraction of emitted power that we collect) for a polarized wide-field microscope is

$$\eta_{\text{det},\phi_{\text{det}}} = \frac{\int_{\Omega} d\hat{\mathbf{r}} \, \left|\hat{\mathbf{P}}_{\text{det}} \cdot \tilde{\mathbf{R}}(\hat{\mathbf{r}})(\hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}})\right|^2}{\int_{\mathbb{S}^2} d\hat{\mathbf{r}} \, |\hat{\mathbf{r}} \times \hat{\boldsymbol{\mu}}_{\text{em}} \times \hat{\mathbf{r}}|^2} \tag{1}$$

where

$$\hat{\mathbf{r}} = \sin\theta\cos\phi\hat{\mathbf{i}} + \sin\theta\sin\phi\hat{\mathbf{j}} + \cos\theta\hat{\mathbf{k}} \quad \text{is the dummy integration vector,} \tag{2}$$

$$\hat{\boldsymbol{\mu}}_{\text{em}} = \sin\Theta\cos\Phi\hat{\mathbf{i}} + \sin\Theta\sin\Phi\hat{\mathbf{j}} + \cos\Theta\hat{\mathbf{k}} \quad \text{is the emission dipole moment,} \tag{3}$$

$$\tilde{\mathbf{R}}(\hat{\mathbf{r}}) = \begin{bmatrix} \cos\theta\cos^2\phi + \sin^2\phi & (\cos\theta - 1)\sin\phi\cos\phi & -\sin\theta\cos\phi \\ (\cos\theta - 1)\sin\phi\cos\phi & \cos\theta\sin^2\phi + \cos^2\phi & -\sin\theta\sin\phi \\ \sin\theta\cos\phi & \sin\theta\sin\phi & \cos\theta \end{bmatrix} \tag{4}$$

$$\text{is the rotation matrix that models the objective lens,} \tag{5}$$

$$\hat{\mathbf{P}}_{\text{det}} = \cos\phi_{\text{det}}\hat{\mathbf{i}} + \sin\phi_{\text{det}}\hat{\mathbf{j}} \quad \text{is the pass axis of the polarizer,} \tag{6}$$

$$\Omega = \{(\phi,\theta) | \phi \in (0, 2\pi] \text{ and } \theta \in (0, \alpha]\} \quad \text{is the cap of the sphere collected by the objective.} \tag{7}$$

Plugging equations 2–7 into equation 1 and simplifying gives Fourkas' result with an extra factor of $\frac{3}{2}$

$$\eta_{\text{det},0}(\Theta,\Phi,\alpha) = A + B\sin^2\Theta + C\sin^2\Theta\cos 2\Phi \tag{8a}$$

$$\eta_{\text{det},45}(\Theta,\Phi,\alpha) = A + B\sin^2\Theta + C\sin^2\Theta\sin 2\Phi \tag{8b}$$

$$\eta_{\text{det},90}(\Theta,\Phi,\alpha) = A + B\sin^2\Theta - C\sin^2\Theta\cos 2\Phi \tag{8c}$$

$$\eta_{\text{det},135}(\Theta,\Phi,\alpha) = A + B\sin^2\Theta - C\sin^2\Theta\sin 2\Phi \tag{8d}$$

where

$$A = \frac{1}{4} - \frac{3}{8}\cos\alpha + \frac{1}{8}\cos^3\alpha \tag{9a}$$

$$B = \frac{3}{16}\cos\alpha - \frac{3}{16}\cos^3\alpha \tag{9b}$$

$$C = \frac{7}{32} - \frac{3}{32}\cos\alpha - \frac{3}{32}\cos^2\alpha - \frac{1}{32}\cos^3\alpha. \tag{9c}$$

If we remove the polarizer from the detection arm the detection efficiency is

$$\eta_{\text{det},\times}(\Theta,\alpha) = \eta_{\text{det},0} + \eta_{\text{det},90} = 2A + 2B\sin^2\Theta \tag{10}$$

where $\times$ denotes "no polarizer".

3 Excitation Efficiency

Here we calculate the excitation efficiency (the fraction of incident power that excites the fluorophore). We calculate the power that excites the dipole by taking the 3D Jones vector that is incident on the condenser $\hat{\mathbf{P}}_{\text{exc}}$, pass it through the condenser using a rotation matrix $\tilde{\mathbf{R}}$, take the dot product with the absorption dipole moment $\hat{\boldsymbol{\mu}}_{\text{abs}}$, take the modulus squared to find the intensity, then integrate over the cone of illumination. Finally, we find the excitation efficiency by dividing by the total power incident on the fluorophore. The final expression is

$$\eta_{\text{exc},\phi_{\text{exc}}} = \frac{\int_\Omega d\hat{\mathbf{r}} |\hat{\boldsymbol{\mu}}_{\text{abs}} \cdot \tilde{\mathbf{R}}(\hat{\mathbf{r}})\hat{\mathbf{P}}_{\text{exc}}|^2}{\int_\Omega d\hat{\mathbf{r}}} \tag{11}$$

where

$$\hat{\mathbf{r}} = \sin\theta\cos\phi\hat{\mathbf{i}} + \sin\theta\sin\phi\hat{\mathbf{j}} + \cos\theta\hat{\mathbf{k}} \quad \text{is the dummy integration vector,} \tag{12}$$

$$\hat{\boldsymbol{\mu}}_{\text{abs}} = \sin\Theta\cos\Phi\hat{\mathbf{i}} + \sin\Theta\sin\Phi\hat{\mathbf{j}} + \cos\Theta\hat{\mathbf{k}} \quad \text{is the absorption dipole moment,} \tag{13}$$

$$\tilde{\mathbf{R}}(\hat{\mathbf{r}}) = \begin{bmatrix} \cos\theta\cos^2\phi + \sin^2\phi & (\cos\theta - 1)\sin\phi\cos\phi & -\sin\theta\cos\phi \\ (\cos\theta - 1)\sin\phi\cos\phi & \cos\theta\sin^2\phi + \cos^2\phi & -\sin\theta\sin\phi \\ \sin\theta\cos\phi & \sin\theta\sin\phi & \cos\theta \end{bmatrix} \tag{14}$$

$$\text{is the rotation matrix that models the condenser lens,} \tag{15}$$

$$\hat{\mathbf{P}}_{\text{exc}} = \cos\phi_{\text{exc}}\hat{\mathbf{i}} + \sin\phi_{\text{det}}\hat{\mathbf{j}} \quad \text{is the pass axis of the excitation polarizer,} \tag{16}$$

$$\Omega = \{(\phi, \theta) | \phi \in (0, 2\pi] \text{ and } \theta \in (0, \alpha]\} \quad \text{is the cap of the sphere illuminated by the condenser.} \tag{17}$$

Plugging equations 12–17 into equation 11 and simplifying gives

$$\eta_{\text{exc},0}(\Theta, \Phi, \alpha) = D(A + B\sin^2\Theta + C\sin^2\Theta\cos 2\Phi) \tag{18a}$$

$$\eta_{\text{exc},45}(\Theta, \Phi, \alpha) = D(A + B\sin^2\Theta + C\sin^2\Theta\sin 2\Phi) \tag{18b}$$

$$\eta_{\text{exc},90}(\Theta, \Phi, \alpha) = D(A + B\sin^2\Theta - C\sin^2\Theta\cos 2\Phi) \tag{18c}$$

$$\eta_{\text{exc},135}(\Theta, \Phi, \alpha) = D(A + B\sin^2\Theta - C\sin^2\Theta\sin 2\Phi) \tag{18d}$$

where

$$D = \frac{4}{3(1 - \cos\alpha)} \tag{19}$$

The excitation efficiency is the same as the detection efficiency with an extra factor D.

Finally, we'll confirm that the excitation efficiency reduces to Malus' law when $\Theta = \pi/2$ and as $\alpha \to 0$.

$$\begin{aligned}
&= \lim_{\alpha\to 0} \eta_{\text{exc},0}(\pi/2, \Phi, \alpha) \\
&= \lim_{\alpha\to 0} D(A + B + C\cos 2\Phi) \\
&= \lim_{\alpha\to 0} \left(\frac{4}{3(1-\cos\alpha)}\right)\left(\frac{1}{4} - \frac{3}{16}\cos\alpha - \frac{1}{16}\cos^3\alpha + \left(\frac{7}{32} - \frac{3}{32}\cos\alpha - \frac{3}{32}\cos^2\alpha - \frac{1}{32}\cos^3\alpha\right)\cos 2\Phi\right) \\
&= \lim_{\alpha\to 0} \frac{\frac{1}{4} - \frac{1}{4}\cos\alpha - \frac{1}{12}\cos^3\alpha + \left(\frac{7}{24} - \frac{1}{8}\cos\alpha - \frac{1}{8}\cos^2\alpha - \frac{1}{24}\cos^3\alpha\right)\cos 2\Phi}{1 - \cos\alpha}
\end{aligned}$$

Applying L'Hospital's rule gives

$$\begin{aligned}
&= \lim_{\alpha\to 0} \frac{\frac{1}{4}\sin\alpha + \frac{1}{4}\sin\alpha\cos^2\alpha + \left(\frac{1}{8}\sin\alpha + \frac{1}{4}\sin\alpha\cos\alpha + \frac{1}{8}\sin\alpha\cos^2\alpha\right)\cos 2\Phi}{\sin\alpha} \\
&= \frac{1}{2} + \frac{1}{2}\cos 2\Phi = \boxed{\cos^2\Phi}.
\end{aligned}$$

4 Epi-illumination and Epi-detection Forward Model

If we excite with polarized illumination from above and detect without a polarizer from the same direction the intensity we expect to collect is

$$I_{\phi_{\text{exc}}} = I_{\text{tot}}\eta_{\text{exc},\phi_{\text{exc}}}\eta_{\text{det},\times} \tag{20}$$

where I_{tot} is the intensity collected from the fluorophore if we had an experimental setup that had an excitation and detection efficiency of one.

If we collect frames with four polarization settings we get the following expressions

$$I_0(\Theta,\Phi,\alpha) = I_{\text{tot}}D(A + B\sin^2\Theta + C\sin^2\Theta\cos 2\Phi)(2A + 2B\sin^2\Theta) \tag{21a}$$
$$I_{45}(\Theta,\Phi,\alpha) = I_{\text{tot}}D(A + B\sin^2\Theta + C\sin^2\Theta\sin 2\Phi)(2A + 2B\sin^2\Theta) \tag{21b}$$
$$I_{90}(\Theta,\Phi,\alpha) = I_{\text{tot}}D(A + B\sin^2\Theta - C\sin^2\Theta\cos 2\Phi)(2A + 2B\sin^2\Theta) \tag{21c}$$
$$I_{135}(\Theta,\Phi,\alpha) = I_{\text{tot}}D(A + B\sin^2\Theta - C\sin^2\Theta\sin 2\Phi)(2A + 2B\sin^2\Theta). \tag{21d}$$

5 Oblique or Orthogonal Arms

The expressions above assume that the optical axis is aligned with the $\hat{\mathbf{z}}$ axis. If the optical axis is along a different axis, we could modify the expressions by (1) changing the limits of integration in equations 1 and 11 and recalculating or (2) performing a change of coordinates in equation 18. Approach (2) is much easier and uses the change of spherical coordinates derived in the 2017-06-09 notes. The main results are reproduced here

$$\Theta' = \arccos\left(\sin\psi\cos\Phi\sin\Theta + \cos\psi\cos\Theta\right) \tag{22a}$$
$$\Phi' = \arccos\left(\frac{\cos\psi\cos\Phi\sin\Phi - \sin\psi\cos\Theta}{\sqrt{1-(\sin\psi\cos\Phi\sin\Theta + \cos\psi\cos\Theta)^2}}\right) \tag{22b}$$

$$\Theta = \arccos\left(-\sin\psi\cos\Phi'\sin\Theta' + \cos\psi\cos\Theta'\right) \tag{23a}$$
$$\Phi = \arccos\left(\frac{\cos\psi\cos\Phi'\sin\Phi' + \sin\psi\cos\Theta'}{\sqrt{1-(\sin\psi\cos\Phi'\sin\Theta' + \cos\psi\cos\Theta')^2}}\right) \tag{23b}$$

The forward model for an oblique detection or excitation arm can be found by plugging equation 23 into equation 21.