Spatio-angular transfer functions for polarized fluorescence microscopes

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1 Introduction

These notes are a continuation of the 2018-02-23 notes on the spatio-angular transfer functions for unpolarized fluorescence microscopes. We will use the same notation and extend those results to microscopes with polarized illumination and detection.

2 Polarized illumination

We can model polarized excitation using a spatio-angular excitation point response function $h_{\text{exc}}(\mathbf{r}_o, \hat{\mathbf{s}}_o)$. In these notes we will only consider spatially uniform excitation patterns $h_{\text{exc}}(\hat{\mathbf{s}}_o)$, but we would need the full function to model structured illumination patterns.

We calculated several spatio-angular excitation point response functions in our Optics Express paper [1] (in the paper we called this function the excitation efficiency for a single dipole, but this is equivalent to the excitation point spread function). If we place a spatially incoherent and spatially uniform light source (or its image) in the aperture plane with the optical axis along the $\hat{\mathbf{z}}$ axis, then the excitation point response function is given by

$$h_{\text{exc}}^{\hat{\mathbf{z}}}(\Theta, \Phi; \phi_{\text{exc}}) = \bar{D}\{\bar{A} + \bar{B}\sin^2\Theta + \bar{C}\sin^2\Theta\cos[2(\Phi - \phi_{\text{exc}})]\}, \tag{1}$$

where $\hat{\mathbf{s}}_o = \cos\Phi\sin\Theta\hat{\mathbf{x}} + \sin\Phi\sin\Theta\hat{\mathbf{y}} + \cos\Theta\hat{\mathbf{z}}$, the illumination polarizer orientation is given by $\hat{\mathbf{p}} = \cos\phi_{\text{exc}}\hat{\mathbf{x}} + \sin\phi_{\text{exc}}\hat{\mathbf{y}}$, and

$$\bar{A} = \frac{1}{4} - \frac{3}{8}\cos\alpha + \frac{1}{8}\cos^3\alpha, \tag{2a}$$

$$\bar{B} = \frac{3}{16}\cos\alpha - \frac{3}{16}\cos^3\alpha, \tag{2b}$$

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

$$\bar{D} = \frac{4}{3(1-\cos\alpha)}, \tag{2d}$$

where $\alpha = \arcsin(\text{NA}/n_o)$. I'm using bars on the constants to avoid notation overlap with the previous note set. We can rewrite this expression in terms of spherical harmonics as

$$\begin{aligned} h_{\text{exc}}^{\hat{\mathbf{z}}}(\hat{\mathbf{s}}_o; \hat{\mathbf{p}}) \propto{}& [2A + (4/3)B]y_0^0(\hat{\mathbf{s}}_o) - \frac{4\sqrt{5}}{15}By_2^0(\hat{\mathbf{s}}_o) + \\ & \frac{4C}{\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]y_2^2(\hat{\mathbf{s}}_o) - (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})y_2^{-2}(\hat{\mathbf{s}}_o)\right\}. \end{aligned} \tag{3}$$

The light-sheet excitation point response function is given by Eq. 3 in the limit of small NA which gives

$$h_{\text{exc}}^{\hat{\mathbf{z}},\text{ls}}(\hat{\mathbf{s}}_o; \hat{\mathbf{p}}) \equiv \lim_{\alpha\to 0} h_{\text{exc}}^{\hat{\mathbf{z}}}(\hat{\mathbf{s}}_o) \propto y_0^0(\hat{\mathbf{s}}_o) + \frac{7}{4\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]y_2^2(\hat{\mathbf{s}}_o) - (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})y_2^{-2}(\hat{\mathbf{s}}_o)\right\}. \tag{4}$$

To find the excitation point response function for illumination along the $\hat{\mathbf{x}}$ axis we need to change every $\hat{\mathbf{x}}$ and $\hat{\mathbf{y}}$ to $\hat{\mathbf{z}}$ and $-\hat{\mathbf{y}}$, respectively. Rotating the spherical harmonics is not trivial, but we show the relevant transformation matrix in Appendix A. The excitation point response function for illumination along the $\hat{\mathbf{x}}$ axis is given by

$$h_{\text{exc}}^{\hat{\mathbf{x}},\text{ls}}(\hat{\mathbf{s}}_o;\hat{\mathbf{p}}) \propto y_0^0(\hat{\mathbf{s}}_o) + \frac{7}{4\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{z}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]\left[\frac{\sqrt{3}}{2}y_2^0(\hat{\mathbf{s}}_o) + \frac{1}{2}y_2^2(\hat{\mathbf{s}}_o)\right] + (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})y_2^{-1}(\hat{\mathbf{s}}_o)\right\}. \tag{5}$$

To find the excitation transfer functions we need to calculate

$$H_{l,\text{exc}}^m = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o\, h_{\text{exc}}(\hat{\mathbf{s}}_o)y_l^m(\hat{\mathbf{s}}_o). \tag{6}$$

This calculation is straightforward now that we've expressed the excitation point response functions in terms of spherical harmonics. The excitation transfer functions are

$$H_{l,\text{exc}}^{m,\hat{\mathbf{z}}}(\hat{\mathbf{p}}) \propto [2A + (4/3)B]\delta(l,m) - \frac{4\sqrt{5}}{15}B\delta(l-2,m)(\hat{\mathbf{s}}_o) + \frac{4C}{\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]\delta(l-2,m-2) - (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})\delta(l-2,m+2)\right\}, \tag{7}$$

$$H_{l,\text{exc}}^{m,\hat{\mathbf{z}},\text{ls}}(\hat{\mathbf{p}}) \propto \delta(l,m) + \frac{7}{4\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]\delta(l-2,m-2) - (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})\delta(l-2,m+2)\right\}, \tag{8}$$

$$H_{l,\text{exc}}^{m,\hat{\mathbf{x}},\text{ls}}(\hat{\mathbf{p}}) \propto \delta(l,m) + \frac{7}{4\sqrt{15}}\left\{[(\hat{\mathbf{p}}\cdot\hat{\mathbf{z}})^2 - (\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})^2]\left[\frac{\sqrt{3}}{2}\delta(l-2,m) + \frac{1}{2}\delta(l-2,m-2)\right] + (\hat{\mathbf{p}}\cdot\hat{\mathbf{x}})(\hat{\mathbf{p}}\cdot\hat{\mathbf{y}})\delta(l-2,m+1)\right\}. \tag{9}$$

3 Polarized detection

In the previous note set we found the spatio-angular detection point spread function for unpolarized fluorescence microscopes. In this section we will find the spatio-angular detection point spread function when there is a linear polarizer in the detection path.

The electric field in the back-focal plane of a fluorescence microscope under the paraxial approximation is given by

$$\tilde{\mathbf{e}}_b^{(p)}(\mathbf{r}_b;\mathbf{r}_o,\hat{\mathbf{s}}_o) \propto \begin{bmatrix} y_1^1(\hat{\mathbf{s}}_o) - \frac{2r_b}{f_o}\cos\phi_b y_1^0(\hat{\mathbf{s}}_o) \\ y_1^{-1}(\hat{\mathbf{s}}_o) - \frac{2r_b}{f_o}\sin\phi_b y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix}. \tag{10}$$

If we place a polarizer oriented along $\hat{\mathbf{p}}_d$ in the back focal plane then the electric field becomes

$$\tilde{\mathbf{e}}_b^{(p)}(\mathbf{r}_b;\mathbf{r}_o,\hat{\mathbf{s}}_o) \propto \hat{\mathbf{p}}_d \cdot \begin{bmatrix} y_1^1(\hat{\mathbf{s}}_o) - \frac{2r_b}{f_o}\cos\phi_b y_1^0(\hat{\mathbf{s}}_o) \\ y_1^{-1}(\hat{\mathbf{s}}_o) - \frac{2r_b}{f_o}\sin\phi_b y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix}. \tag{11}$$

To find the electric field in the detector plane we need to take the two-dimensional Fourier transform of each component. The dot product with the polarizer is linear, so we can pull the Fourier transform inside and use the same result from the previous notes

$$\tilde{\mathbf{e}}_d^{(p)}(\mathbf{r}'_d,\hat{\mathbf{s}}_o;\hat{\mathbf{p}}_d) \propto \hat{\mathbf{p}}_d \cdot \begin{bmatrix} a^{(p)}(r'_d)y_1^1(\hat{\mathbf{s}}_o) + 2ib^{(p)}(r'_d)\cos\phi'_d y_1^0(\hat{\mathbf{s}}_o) \\ a^{(p)}(r'_d)y_1^{-1}(\hat{\mathbf{s}}_o) + 2ib^{(p)}(r'_d)\sin\phi'_d y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix}. \tag{12}$$

Finally, we can find the spatio-angular point spread function by taking the squared modulus of the electric field on the detector.

In progress. Calculate the spatio-angular PSF then the spatio-angular OTF.

A Rotation of spherical harmonics

Given a spherical function and its spherical harmonic coefficients c_j (we're using a single index over the spherical harmonics), the spherical harmonic coefficients of the rotated function c_i' can be computed with the linear transformation

$$c_i' = \sum_j M_{ij} c_j, \tag{13}$$

where the elements of the linear transformation can be computed with

$$M_{ij} = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_j(\mathbf{R}\hat{\mathbf{s}}_o) y_i(\hat{\mathbf{s}}_o), \tag{14}$$

where $\mathbf{R}$ is the rotation matrix that maps the original function to the rotated function [2]. If we assemble the elements of the linear transformation into a matrix $\mathbf{M}$, then the matrix is block sparse

$$\mathbf{M} = \left[\begin{array}{c|ccc|cccccc}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots \\ \hline
0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & 0 & 0 & 0 & 0 & 0 & \cdots \\
0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & 0 & 0 & 0 & 0 & 0 & \cdots \\
0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & 0 & 0 & 0 & 0 & 0 & \cdots \\ \hline
0 & 0 & 0 & 0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \cdots \\
0 & 0 & 0 & 0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \cdots \\
0 & 0 & 0 & 0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \cdots \\
0 & 0 & 0 & 0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \cdots \\
0 & 0 & 0 & 0 & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \mathbf{X} & \cdots \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots
\end{array}\right]. \tag{15}$$

This means the spherical harmonic coefficients from each band transform independently from the other bands.

To efficiently find the point spread functions and transfer functions for the diSPIM we will compute the spherical harmonic coefficient transformation matrix for a rotation that maps the $\hat{\mathbf{z}}$ axis to the $\hat{\mathbf{x}}$. The rotation matrix is given by

$$\mathbf{R}_{\hat{\mathbf{z}}\to\hat{\mathbf{x}}} = \begin{bmatrix} 0 & 0 & 1 \\ 0 & 1 & 0 \\ -1 & 0 & 0 \end{bmatrix}, \tag{16}$$

and the spherical harmonic coefficient transformation matrix is given by

$$\mathbf{M}_{\hat{\mathbf{z}}\to\hat{\mathbf{x}}} = \left[\begin{array}{c|ccc|cccccc}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots \\ \hline
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & \cdots \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots \\
0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots \\ \hline
0 & 0 & 0 & 0 & -1/2 & 0 & 0 & \sqrt{3}/2 & 0 & \cdots \\
0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & \cdots \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & \cdots \\
0 & 0 & 0 & 0 & \sqrt{3}/2 & 0 & 0 & 1/2 & 0 & \cdots \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & \cdots \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \ddots
\end{array}\right], \tag{17}$$

when the matrix elements are ordered as $[y_0^0|y_1^1, y_1^{-1}, y_1^0|y_2^0, y_2^1, y_2^{-1}, y_2^2, y_2^{-2}]$. For more complicated transformations (higher order coefficients or arbitrary rotations) we can use recurrence relations to find the spherical harmonic coefficient transformation matrix [3].

References

[1] Talon Chandler, Shalin Mehta, Hari Shroff, Rudolf Oldenbourg, and Patrick J. La Rivière. Single-fluorophore orientation determination with multiview polarized illumination: modeling and microscope design. *Opt. Express*, 25(25):31309–31325, Dec 2017.

[2] Jan Kautz, Peter-Pike Sloan, and John Snyder. Fast, arbitrary BRDF shading for low-frequency lighting using spherical harmonics. In *Proceedings of the 13th Eurographics Workshop on Rendering*, EGRW '02, pages 291–296, Aire-la-Ville, Switzerland, Switzerland, 2002. Eurographics Association.

[3] Joseph Ivanic and Klaus Ruedenberg. Rotation matrices for real spherical harmonics. direct determination by recursion. *The Journal of Physical Chemistry*, 100(15):6342–6347, 1996.