Spatio-angular Kernels and Transfer Functions for Fluorescence Microscopes

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1 Introduction

In these notes we will consider a single-view fluorescence microscope **with no polarizing filters** that uses TIRF excitation to excite fluorophores in all orientations equally. This excitation scheme allows us to focus exclusively on the detection process. We will find the spatio-angular kernel of this microscope and analyze it in the frequency domain using the spatio-angular Fourier transform. Along the way we will show that uniform excitation fluorescence microscopes have spatial and angular band-limits.

We use plain roman type for scalars, e.g., x, y, z; bold lowercase roman type for two-dimensional vectors, e.g., $\mathbf{r}$; hats for unit vectors, e.g., $\hat{\mathbf{s}}$; bold lowercase gothic type for three-dimensional vectors, e.g., $\mathfrak{r}$; and bold capital roman type for matrices, e.g., $\mathbf{R}$.

2 General Forward Model

Consider a three-dimensional field of oriented fluorescent dipoles. We can represent the entire object using a function $f(\mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})$ that returns the number of dipoles at position $\mathfrak{r}_\mathbf{o}$ per unit volume oriented in direction $\hat{\mathbf{s}}_\mathbf{o}$ per unit solid angle.

The intensities measured using a general linear measurement system can by modeled using

$$g_i(\mathbf{r_d}) = \int_{\mathbb{R}^3} d\mathfrak{r}_\mathbf{o} \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_\mathbf{o}\ h_i(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) f(\mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) \tag{1}$$

where $g_i(\mathbf{r_d})$ is the camera frame collected under the ith measurement configuration (i indexes polarizer settings or views), $\mathbf{r_d}$ is the two-dimensional detector coordinate, and $h_i(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})$ is the spatio-angular kernel of the ith measurement configuration. Our goal is to reconstruct the object $f(\mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})$ from intensity measurements $g_i(\mathbf{r_d})$.

3 Spatio-angular Kernel

In this section we will write out the kernel $h(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})$ for a non-polarized single-view fluorescence microscope with angularly uniform TIRF illumination. This section mostly restates the results of XXXBacker and Moerner 2014XXX, so I recommend reading that paper for a more complete understanding.

First, we find the electric field $\mathbf{e}$ at position $\mathbf{r_b} = r_b \cos\phi_b \hat{\mathbf{x}} + r_b \sin\phi_b \hat{\mathbf{y}}$ in the back focal plane created by a single dipole at position $\mathfrak{r}_\mathbf{o} = x_o\hat{\mathbf{x}} + y_o\hat{\mathbf{y}} + z_o\hat{\mathbf{z}}$ and oriented in direction $\hat{\mathbf{s}}_\mathbf{o}$

$$\mathbf{e_b}(\mathbf{r_b}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) \propto \mathrm{e}^{in_o k(x_o x_b + y_o y_b + z_o \rho_b)} \sqrt{\frac{n_o}{n_b \rho_b}} \begin{bmatrix} \sin^2\phi_b + \rho_b \cos^2\phi_b & \sin\phi_b \cos\phi_b(\rho_b - 1) & -r_b \cos\phi_b \\ \sin\phi_b \cos\phi_b(\rho_b - 1) & \cos^2\phi_b + \rho_b \sin^2\phi_b & -r_b \sin\phi_b \\ 0 & 0 & 0 \end{bmatrix} \hat{\mathbf{s}}_\mathbf{o} \Pi\left(\frac{r_b}{r_b^{\max}}\right) \tag{2}$$

where we have defined $\rho_b \equiv \sqrt{1 - r_b^2}$ and $\Pi(x)$ is a boxcar function that returns 1 when $|x| < 1$ and 0 otherwise. We can understand this expression term by term: the exponential term accounts for the phase objective, the square root term conserves power before and after the objective lens, the matrix is a position-dependent rotation matrix that models the electric field rotation caused by the objective lens, $\hat{\mathbf{s}}_\mathbf{o}$ is the dipole orientation unit vector, and $\Pi\left(\frac{r_b}{r_b^{\max}}\right)$ accounts for the numerical aperture of the lens with $r_b^{\max} = \frac{f_0}{n_0}\mathrm{NA}$.

The next step is to find the electric field in the detector plane. We can use the paraxial approximation here because the focal length of the tube lens is long. In this case the electric field in the detector plane is the Fourier transform of the electric field in the back focal plane

$$\mathbf{e_d}(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) = \int_{\mathbb{R}^2} d\mathbf{r_b}\ \mathbf{e_b}(\mathbf{r_b}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) \mathrm{e}^{i(kn_b/f_t)\mathbf{r_b}\cdot\mathbf{r_d}}. \tag{3}$$

Finally, we can find the intensity measured in the detector plane (the kernel) by taking the modulus squared of the electric field

$$h(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o}) = |\mathbf{e_d}(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})|^2. \tag{4}$$

Unfortunately, the Fourier transform in Eq. 3 cannot be written in closed form, but equation 4 can be rearranged so that it can be evaluated using six precomputed 2D Fourier transforms per z_o position.

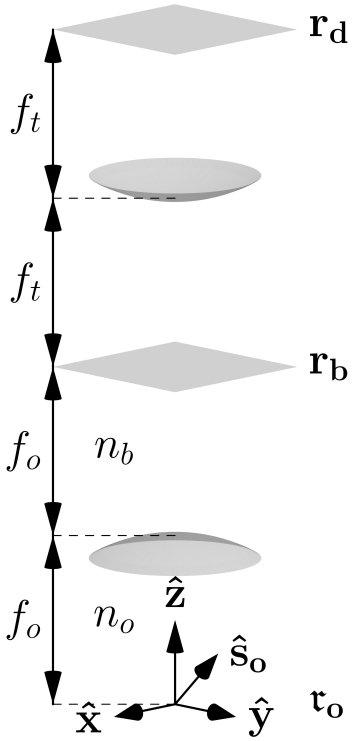

Figure 1: Simplified schematic of a single-view fluorescence microscope. The object is placed near the focal point of an objective lens with focal length f_o in a medium with refractive index n_o. The object is parameterized by the 3D position vector $\mathfrak{r}_\mathbf{o}$ ($\mathbf{o}$ for object) and a orientation unit vector $\hat{\mathbf{s}}_\mathbf{o}$. The light emitted by the fluorescent object is collected and collimated by the objective lens so that the electric fields are purely transverse in back focal plane. Points in the back focal plane are parameterized by a 2D position vector $\mathbf{r_b}$ ($\mathbf{b}$ for back focal plane). Finally, the tube lens with focal length f_t refocuses the light onto a detector. Points on the detector are parameterized by a 2D position vector $\mathbf{r_d}$ ($\mathbf{d}$ for detector). The back focal plane and detector are in a medium with refractive index n_b. Note that this schematic is not to scale—in typical microscopes $f_o \ll f_t$ to provide magnification.

TODO: Show transverse shift invariance.

TODO: Program up an efficient implementation of $h(\mathbf{r_d}; \mathfrak{r}_\mathbf{o}, \hat{\mathbf{s}}_\mathbf{o})$.

TODO: Plot select kernels.

4 Spatio-angular Transfer Function

TODO: Show angular band limit by taking angular Fourier transform only.

TODO: Show spatial band limit by taking the spatial Fourier transform of the first angular term.

TODO: Evaluate and plot spatio-angular transfer function numerically. This will be expensive, but it only has to be done once for each microscope.

5 Reconstruction

6 Conclusions