# Spatio-angular transfer functions for fluorescence microscopes

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#### Abstract

We investigate how the orientation and position of fluorescent dipole emitters affects microscopic imaging using electromagnetic optics theory. Starting with the thoroughly studied spatio-angular point spread function, we introduce the spatio-angular coherent spread function, coherent transfer function, and optical transfer function as electromagnetic extensions of well-known functions in scalar optics theory. We use these concepts to show that fluorescence microscopes have a spatio-angular band limit.

#### 1 Introduction

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.,  $\hat{\mathbf{s}}$ ; and bold capital roman type for matrices, e.g.,  $\mathbf{R}$ . We use the real spherical harmonic functions

$$y_l^m(\vartheta,\varphi) = \begin{cases} \sqrt{2}K_l^m \cos(m\varphi)P_l^m(\cos\vartheta), & m > 0\\ K_l^0 P_l^0(\cos\vartheta), & m = 0\\ \sqrt{2}K_l^m \sin(-m\varphi)P_l^{-m}(\cos\vartheta), & m < 0 \end{cases}$$
(1)

where

$$K_l^m = \sqrt{\frac{(2l+1)}{4\pi} \frac{(l-|m|)!}{(l+|m|)!}},\tag{2}$$

and  $P_l^m(x)$  are the associated Legendre polynomials. The l=0 and l=1 spherical harmonics are given by

$$y_0^0(\vartheta,\varphi) = \sqrt{\frac{1}{4\pi}},$$

$$y_1^{-1}(\vartheta,\varphi) = \sqrt{\frac{3}{4\pi}}\sin\varphi\sin\vartheta, \qquad y_1^0(\vartheta,\varphi) = \sqrt{\frac{3}{4\pi}}\cos\vartheta, \qquad y_1^1(\vartheta,\varphi) = \sqrt{\frac{3}{4\pi}}\cos\varphi\sin\vartheta.$$
(3)

## 2 Spatio-angular point spread functions

Figure 1 shows a schematic of the fluorescence microscope that we are considering with a summary of our notation. We start by following Backer and Moerner [1] to find the electric field at position  $\mathbf{r}_b$  in the back focal plane due to a single dipole emitter at position  $\mathbf{r}_o$  oriented along  $\hat{\mathbf{s}}_o$  as

$$\tilde{\mathbf{e}}_{b}(\mathbf{r}_{b}; \mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) \propto e^{-i(kn_{o}/f_{o})\mathbf{r}_{b}\cdot\mathbf{r}_{o}} \sqrt{\frac{1}{\rho_{b}}} \begin{bmatrix} \sin^{2}\phi_{b} + \rho_{b}\cos^{2}\phi_{b} & \sin\phi_{b}\cos\phi_{b}(\rho_{b} - 1) & -\frac{r_{b}}{f_{o}}\cos\phi_{b} \\ \sin\phi_{b}\cos\phi_{b}(\rho_{b} - 1) & \cos^{2}\phi_{b} + \rho_{b}\sin^{2}\phi_{b} & -\frac{r_{b}}{f_{o}}\sin\phi_{b} \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \cos\varphi_{o}\sin\vartheta_{o} \\ \sin\varphi_{o}\sin\vartheta_{o} \\ \cos\vartheta_{o} \end{bmatrix} \Pi \left(\frac{r_{b}}{r_{b}^{\max}}\right),$$

$$(4)$$

where we define  $\rho_b \equiv \sqrt{1 - \left(\frac{r_b}{f_o}\right)^2}$ , 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 as dipole emitter is moved in object space, the square root term conserves power before and after the objective lens, the matrix models the dipole emission process and electric field rotation caused by the objective lens, the vector is the

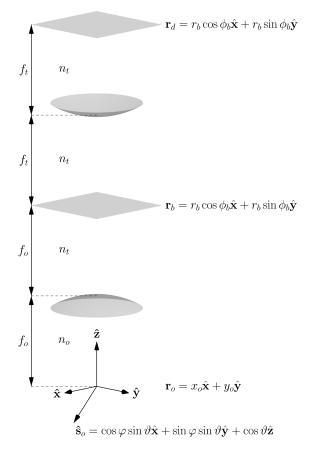


Figure 1: Simplified schematic of a single-view fluorescence microscope. The object is placed near the focal point of an aplanatic objective lens with focal length  $f_o$  in a medium with refractive index  $n_o$ . The object is parameterized by the 2D position vector  $\mathbf{r}_o$  (o for object) and an orientation unit vector  $\hat{\mathbf{s}}_o$ . The light emitted by the fluorescent object is collected and collimated by the objective lens so that the electric fields are purely transverse in the back focal plane. Points in the back focal plane are parameterized by a 2D position vector  $\mathbf{r}_b$  (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  $\mathbf{r}_d$  (d for detector). The back focal plane and detector are in a medium with refractive index  $n_t$ . Note that this schematic is not to scale—we consider the case where  $f_o \ll f_t$ .

dipole orientation unit vector, and  $\Pi\left(\frac{r_b}{r_b^{\max}}\right)$  accounts for the numerical aperture of the lens with  $r_b^{\max} = \frac{f_0}{n_0} \text{NA}$ . We use a tilde to mark single dipole response functions—we will consider the response due to a field of dipoles in the next section.

Although we will not use the result immediately, we can write the electric field in the back focal plane under the paraxial approximation. We expand Eq. 4 in a Taylor series about  $r_b = 0$  and drop all second-order and higher terms to find

$$\tilde{\mathbf{e}}_{b}^{(p)}(\mathbf{r}_{b}; \mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) \propto e^{-i(kn_{o}/f_{o})\mathbf{r}_{b}\cdot\mathbf{r}_{o}} \begin{bmatrix} 1 & 0 & -\frac{r_{b}}{f_{o}}\cos\phi_{b} \\ 0 & 1 & -\frac{r_{b}}{f_{o}}\sin\phi_{b} \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \cos\varphi_{o}\sin\vartheta_{o} \\ \sin\varphi_{o}\sin\vartheta_{o} \\ \cos\vartheta_{o} \end{bmatrix} \Pi \left(\frac{r_{b}}{r_{b}^{\max}}\right). \tag{5}$$

We will continue to use a superscript (p) to mark terms that have used the paraxial approximation.

If the tube lens is weakly focusing  $(f_o \ll f_t)$  then we can find the electric field in the detector plane by

taking the Fourier transform of the electric field in the back focal plane

$$\tilde{\mathbf{e}}_d(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) \propto \int_{\mathbb{R}^2} d\mathbf{r}_b \, \tilde{\mathbf{e}}_b(\mathbf{r}_b; \mathbf{r}_o, \hat{\mathbf{s}}_o) \mathrm{e}^{-i(kn_t/f_t)\mathbf{r}_b \cdot \mathbf{r}_d}.$$
 (6)

By isolating the phase term in Eq. 4 with  $\tilde{\mathbf{e}}_b(\mathbf{r}_b;\mathbf{r}_o,\hat{\mathbf{s}}_o) \equiv e^{-i(kn_o/f_o)\mathbf{r}_b\cdot\mathbf{r}_o}\underline{\tilde{\mathbf{e}}}_b(\mathbf{r}_b;\hat{\mathbf{s}}_o)$ , and plugging into Eq. 6 we find that

$$\tilde{\mathbf{e}}_d(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) \propto \int_{\mathbb{R}^2} d\mathbf{r}_b \, \tilde{\underline{\mathbf{e}}}_b(\mathbf{r}_b; \hat{\mathbf{s}}_o) e^{-i(kn_t/f_t) \, \mathbf{r}_b \cdot [\mathbf{r}_d - M\mathbf{r}_o]}. \tag{7}$$

where  $M = -\frac{n_o}{n_t} \frac{f_t}{f_o}$  is the transverse magnification. By writing the electric field in the detector plane in terms of  $\mathbf{r}_d - M\mathbf{r}_o$ , we have established that the electric field in the detector plane is transverse shift-invariant—a transverse shift of the object creates a magnified transverse shift of the image. We define coordinates on the detector centered on the image of the object  $\mathbf{r}'_d \equiv \mathbf{r}_d - M\mathbf{r}_o = r'_d \cos \phi'_d \hat{\mathbf{x}} + r'_d \sin \phi'_d \hat{\mathbf{y}}$ , then we follow Novotny [2] by writing the integrals in polar coordinates, evaluating the azimuthal integrals, and writing the result concisely in terms of three radial integrals

$$\tilde{\mathbf{e}}_{d}(\mathbf{r}'_{d},\hat{\mathbf{s}}_{o}) \propto \begin{bmatrix} a(r'_{d}) + c(r'_{d})\cos(2\phi'_{d}) & c(r'_{d})\sin(2\phi'_{d}) & 2ib(r'_{d})\cos\phi'_{d} \\ c(r'_{d})\sin(2\phi'_{d}) & a(r'_{d}) - c(r'_{d})\cos(2\phi'_{d}) & 2ib(r'_{d})\sin\phi'_{d} \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \cos\varphi_{o}\sin\vartheta_{o} \\ \sin\varphi_{o}\sin\vartheta_{o} \\ \cos\vartheta_{o} \end{bmatrix}, \tag{8}$$

where  $a(r'_d), b(r'_d)$ , and  $c(r'_d)$  are

$$a(r_d') = \int_0^{\theta_{\text{max}}} d\theta \, \sqrt{\cos \theta} \sin \theta (1 + \cos \theta) J_0(k_b r_d' \sin \theta f_o / f_t), \tag{9}$$

$$b(r_d') = \int_0^{\theta_{\text{max}}} d\theta \, \sqrt{\cos \theta} \sin^2 \theta J_1(k_b r_d' \sin \theta f_o / f_t), \tag{10}$$

$$c(r_d') = \int_0^{\theta_{\text{max}}} d\theta \,\sqrt{\cos\theta} \sin\theta (1 - \cos\theta) J_2(k_b r_d' \sin\theta f_o/f_t). \tag{11}$$

We can identify Eq. 8 as the vector-valued *coherent spread function* (CSF) of the microscope. The more familiar scalar-valued CSF—sometimes called the amplitude transfer function—is only applicable to cases where electromagnetic optics plays no role in the microscope—not true for samples that contain dipole emitters.

To build our intuition about the CSF, we will rewrite the matrix multiplication in Eq. 8 in terms of the spherical harmonics. Notice that the l = 1 spherical harmonics are the Cartesian components of the unit dipole axis (see Eq. 3), so the CSF becomes

$$\tilde{\mathbf{e}}_{d}(\mathbf{r}'_{d},\hat{\mathbf{s}}_{o}) \propto \begin{bmatrix}
[a(r'_{d}) + c(r'_{d})\cos(2\phi'_{d})]y_{1}^{1}(\hat{\mathbf{s}}_{o}) + c(r'_{d})\sin(2\phi'_{d})y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + 2ib(r'_{d})\cos\phi'_{d}y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\
c(r'_{d})\sin(2\phi'_{d})y_{1}^{1}(\hat{\mathbf{s}}_{o}) + [a(r'_{d}) - c(r'_{d})\cos(2\phi'_{d})]y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + 2ib(r'_{d})\sin\phi'_{d}y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\
0
\end{bmatrix}.$$
(12)

By applying the paraxial approximation ( $\sin \theta \approx \theta$  and  $\cos \theta \approx 1$ ), the integrals  $a(r'_d), b(r'_d)$  and  $c(r'_d)$  can be evaluated in terms of Bessel functions. We can evaluate  $a(r'_d)$  and  $b(r'_d)$  with the help of  $\int_0^z dx \ x J_0(ax) = z J_1(az)/a$  and  $\int_0^z dx \ x^2 J_1(ax) = z^2 J_2(az)/a$ , respectively, and  $c(r'_d) = 0$  because  $J_2(x)$  is zero to first order. In this case, the CSF simplifies to

$$\tilde{\mathbf{e}}_{d}^{(p)}(\mathbf{r}_{d}',\hat{\mathbf{s}}_{o}) \propto \begin{bmatrix} a^{(p)}(r_{d}')y_{1}^{1}(\hat{\mathbf{s}}_{o}) + 2ib^{(p)}(r_{d}')\cos\phi_{d}'y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ a^{(p)}(r_{d}')y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + 2ib^{(p)}(r_{d}')\sin\phi_{d}'y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ 0 \end{bmatrix}, \tag{13}$$

where the integrals evaluate to

$$a^{(p)}(r'_d) = \frac{J_1(2\pi\nu_o r'_d)}{\pi\nu_o r'_d}, \qquad b^{(p)}(r'_d) = \frac{\text{NA}}{n_o} \left[ \frac{J_2(2\pi\nu_o r'_d)}{\pi\nu_o r'_d} \right], \tag{14}$$

and we have substituted

$$\nu_o \equiv \frac{\text{NA}}{M\lambda}, \quad \text{NA} \equiv n_o \sin \theta_{\text{max}}.$$
 (15)

Under the paraxial approximation the electric fields on the detector created by a single dipole are composed of two parts, a parallel part and a radial part. The coefficients of the  $y_1^0$  spherical harmonic in Eq. 13 create the radial part of the field—the z component of the dipole generates a radial field on the detector with respect to the image point. The coefficients of the  $y_1^1$  and  $y_1^{-1}$  spherical harmonics create a parallel field in the detector plane parallel to the x and y components of the dipole. As the NA increases and the paraxial approximation no longer applies, we begin to see coupling between the x and y components of the dipole with the y and x transverse fields, respectively.

We can find the intensity in the detector plane due to a single dipole by taking the squared modulus of the CSF

$$h(\mathbf{r}_d', \hat{\mathbf{s}}_o) = \left| \tilde{\mathbf{e}}_d(\mathbf{r}_d', \hat{\mathbf{s}}_o) \right|^2. \tag{16}$$

For convenience we keep the CSF written in terms of the spherical harmonics and use a table of spherical harmonic products (see Appendix A) to calculate the intensity in the detector plane as

$$h(\mathbf{r}'_{d}, \hat{\mathbf{s}}_{o}) \propto \left(a^{2}(r'_{d}) + 2b^{2}(r'_{d}) + c^{2}(r'_{d})\right) y_{0}^{0}(\hat{\mathbf{s}}_{o}) - \frac{2\sqrt{15}}{5} a(r'_{d})c(r'_{d})\sin(2\phi'_{d})y_{2}^{-2}(\hat{\mathbf{s}}_{o}) + \frac{1}{\sqrt{5}} \left(-a^{2}(r'_{d}) + 4b^{2}(r'_{d}) - c^{2}(r'_{d})\right) y_{2}^{0}(\hat{\mathbf{s}}_{o}) + \frac{2\sqrt{15}}{5} a(r'_{d})c(r'_{d})\cos(2\phi'_{d})y_{2}^{2}(\hat{\mathbf{s}}_{o}).$$

$$(17)$$

We can identify Eq. 17 as the spatio-angular point spread function (PSF) of the microscope. Writing the spatio-angular PSF in terms of spherical harmonic functions has two advantages. First, it allows us to express the spatio-angular PSF very concisely. Instead of considering the point spread function for every possible dipole orientation, we only need to consider four spatio-angular PSFs—one for each spherical harmonic. Second, the spherical harmonic functions form an orthonormal basis for functions on the sphere—a convenient fact that we will use later.

It is useful to compare Eq. 17 to Backer and Moerner's approach [1]. They expand the spatio-angular PSF in terms of six second moments of the fluorophore distribution  $\{s_x^2, s_y^2, s_z^2, s_x s_y, s_x s_z, s_y s_z\}$ . This approach is very useful—only six precomputed spatio-angular PSFs are required to represent an arbitrary spatio-angular PSF. Instead of expanding in terms of six second moments, we expand onto just four spherical harmonics which, unlike the second moments, are orthonormal functions. In the next section we will use the orthonormality of the spherical harmonics to derive spatio-angular transfer functions for fluorescence microscopes.

The spatio-angular PSF under the paraxial approximation is given by

$$h^{(p)}(\mathbf{r}'_d, \hat{\mathbf{s}}_o) \propto \left(a^{(p)^2}(r'_d) + 2b^{(p)^2}(r'_d)\right) y_0^0(\hat{\mathbf{s}}_o) + \frac{1}{\sqrt{5}} \left(-a^{(p)^2}(r'_d) + 4b^{(p)^2}(r'_d)\right) y_2^0(\hat{\mathbf{s}}_o). \tag{18}$$

We rewrite the PSF in the following form to make plotting and interpretation easier

$$h^{(p)}(\mathbf{r}_d,\hat{\mathbf{s}}_o) = h_0^{0(p)}(\mathbf{r}_d)y_0^0(\hat{\mathbf{s}}_o) + h_0^{2(p)}(\mathbf{r}_d)y_0^2(\hat{\mathbf{s}}_o), \tag{19}$$

$$h_0^{0(p)}(\mathbf{r}_d) \equiv a^{(p)^2}(r_d) + 2b^{(p)^2}(r_d), \tag{20}$$

$$h_0^{2(p)}(\mathbf{r}_d) \equiv \frac{1}{\sqrt{5}} \left[ -a^{(p)^2} (r_d) + 4b^{(p)^2} (r_d) \right]. \tag{21}$$

First, consider the coefficient on the  $y_0^0$  spherical harmonic in Eq. 18. This coefficient is the point spread function for an angularly uniform distribution of fluorophores. The first term of the coefficient  $A^{(p)^2}(r'_d)$  is the familiar Airy disk that arises from the contribution of dipoles oriented in the transverse plane, while the second term  $B^{(p)^2}(r'_d)$  is a smaller factor that arises from dipoles oriented outside of the transverse plane. This leads to an interesting conclusion—a uniform distribution of dipoles has a point spread function that is slightly wider than an Airy disk even in the paraxial approximation. The Airy disk is usually derived using paraxial scalar optics while here we have used paraxial electromagnetic optics. Therefore, we can consider the second term to be an electromagnetic correction to the Airy disk. We will quantify this difference in the next section.

Next, consider the coefficient on the  $y_2^0$  spherical harmonic in Eq. 18. This coefficient is the spatial PSF for a distribution of fluorophores proportional to  $3\cos^2\vartheta_o - 1$ . Counterintuitively, this fluorophore distribution cannot exist because it would require a negative number of fluorophores along some orientations; but if this distribution could exist, then this coefficient would be its spatial PSF. Considering negative distributions of fluorophores in our calculations should not be cause for concern. The spherical harmonics span the space of functions on the sphere, so any positive fluorophore distribution can be represented by the spherical harmonics and we never need to consider negative fluorophores.

Finally, consider all of the spherical harmonics that have a zero coefficient. These spherical harmonics span the angular null space of our measurement system—fluorophore distributions that lie in the null space do not affect the measured intensities. Under the paraxial approximation all of the non-zero coefficients are rotationally symmetric (m=0) spherical harmonics, which means that the transverse orientation of the dipoles does not affect the PSF. In the high NA case this is no longer true—two m=2 spherical harmonics have non-zero coefficients and the transverse orientation of dipoles does affect the PSF.

### 3 Spatio-angular transfer functions

Consider a thin object that consists of fluorescent dipoles in arbitrary positions and orientations. We can represent the entire object using a function  $\mu(\mathbf{r}_o, \hat{\mathbf{s}}_o)$  that returns a complex-valued vector for each position  $\mathbf{r}_o$  and direction  $\hat{\mathbf{s}}_o$ . The magnitude of the complex-valued vector is the magnitude of the dipole moment and the elements of the vector give the orientation and phase of the radiating dipole moment. Because  $\mu(\mathbf{r}_o, \hat{\mathbf{s}}_o)$  includes the relative phases of different points and orientations in the object,  $\mu(\mathbf{r}_o, \hat{\mathbf{s}}_o)$  can represent coupled dipoles that are emitting partially or completely coherently.

We can find the electric field on the detector created by this object by multiplying  $\mu(\mathbf{r}_o, \hat{\mathbf{s}}_o)$  with the CSF then integrating over all positions and orientations in the object

$$\mathbf{e}_{d}(\mathbf{r}_{d}) = \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{P}^{2}} d\mathbf{r}_{o} \ \tilde{\mathbf{e}}_{d}(\mathbf{r}_{d} - M\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) \boldsymbol{\mu}(\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}). \tag{22}$$

Note that these integrals represent a vector sum—the coherence of the electric fields radiated by the object can cause cancellations of the fields created on the detector.

We can simplify this expression by expanding the CSF in terms of spatio-angular harmonics

$$\tilde{\mathbf{e}}_d(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} \, \mathbf{E}_l^m(\boldsymbol{\nu}) y_l^m(\hat{\mathbf{s}}_o) \, \mathrm{e}^{i2\pi(\mathbf{r}_d - M\mathbf{r}_o) \cdot \boldsymbol{\nu}}, \tag{23}$$

where  $\mathbf{E}_l^m(\boldsymbol{\nu})$  is the spatio-angular spectrum of the CSF given by

$$\mathbf{E}_{l}^{m}(\boldsymbol{\nu}) \equiv \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{o} \,\tilde{\mathbf{e}}_{d}(\mathbf{r}_{d} - M\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) y_{l}^{m}(\hat{\mathbf{s}}_{o}) \,\mathrm{e}^{-i2\pi(\mathbf{r}_{d} - M\mathbf{r}_{o}) \cdot \boldsymbol{\nu}}. \tag{24}$$

We can change variables to make this expression easier to evaluate

$$\mathbf{E}_{l}^{m}(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{o} \,\tilde{\mathbf{e}}_{d}(\mathbf{r}_{d}^{\prime}, \hat{\mathbf{s}}_{o}) y_{l}^{m}(\hat{\mathbf{s}}_{o}) \,\mathrm{e}^{-i2\pi\mathbf{r}_{d}^{\prime}\cdot\boldsymbol{\nu}}. \tag{25}$$

By plugging Eq. 23 into Eq. 22 we find that

$$\mathbf{e}_{d}(\mathbf{r}_{d}) = \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{o} \left[ \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^{2}} d\boldsymbol{\nu} \, \mathbf{E}_{l}^{m}(\boldsymbol{\nu}) y_{l}^{m}(\hat{\mathbf{s}}_{o}) \, \mathrm{e}^{i2\pi(\mathbf{r}_{d} - M\mathbf{r}_{o}) \cdot \boldsymbol{\nu}} \right] \boldsymbol{\mu}(\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}). \tag{26}$$

We can rearrange this equation into the following form

$$\mathbf{e}_{d}(\mathbf{r}_{d}) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^{2}} d\boldsymbol{\nu} \, \mathbf{E}_{l}^{m}(\boldsymbol{\nu}) \left[ \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{o} \, \boldsymbol{\mu}(\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) y_{l}^{m}(\hat{\mathbf{s}}_{o}) \, e^{-i2\pi M \mathbf{r}_{o} \cdot \boldsymbol{\nu}} \right] e^{i2\pi \mathbf{r}_{d} \cdot \boldsymbol{\nu}}.$$
(27)

We recognize the term in square brackets as the spatio-angular spectrum of the object, so we define

$$\mathbf{M}_{l}^{m}(\boldsymbol{\nu}) \equiv \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{o} \,\boldsymbol{\mu}(\mathbf{r}_{o}, \hat{\mathbf{s}}_{o}) y_{l}^{m}(\hat{\mathbf{s}}_{o}) \,\mathrm{e}^{-i2\pi M \mathbf{r}_{o} \cdot \boldsymbol{\nu}}. \tag{28}$$

and write the electric field on the detector as

$$\mathbf{e}_{d}(\mathbf{r}_{d}) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^{2}} d\boldsymbol{\nu} \, \mathbf{E}_{l}^{m}(\boldsymbol{\nu}) \mathbf{M}_{l}^{m}(\boldsymbol{\nu}) e^{i2\pi \mathbf{r}_{d} \cdot \boldsymbol{\nu}}. \tag{29}$$

Eq. 38 shows that the electric field on the detector can be found by resolving the object into its spatio-angular components  $\mathbf{M}_{l}^{m}(\boldsymbol{\nu})$ , weighting each component by  $\mathbf{E}_{l}^{m}(\boldsymbol{\nu})$ , then summing over all spatio-angular components. Therefore, we identify  $\mathbf{E}_{l}^{m}(\boldsymbol{\nu})$  as the spatio-angular coherent transfer function (CTF).

In Appendix B we calculate the paraxial CTF for a single-view fluorescence microscope as

$$\mathbf{E}_{l}^{m(p)}(\boldsymbol{\nu}) = \begin{bmatrix} \delta(l-1, m-1) + \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} \nu \cos \phi_{\nu} \delta(l-1, m) \\ \delta(l-1, m+1) + \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} \nu \sin \phi_{\nu} \delta(l-1, m) \\ 0 \end{bmatrix} \Pi\left(\frac{\nu}{\nu_{o}}\right). \tag{30}$$

The CTF shows that single-view coherent microscopes have a spatial band limit at  $\nu = \nu_o$  and an l=1 angular pass band. It is useful to compare the paraxial CTF in Eq. 30 to the paraxial electric field in the back focal plane in Eq. 5. The CTF is just a rescaled version of the electric field in the back focal plane, and this leads to an extremely valuable interpretation. The CTF of a single-view fluorescence microscope is constrained to the three members of the l=1 band. The value of the CTF for each m is a rescaled version of the electric field due to a single dipole oriented along one of the Cartesian axes.

At the risk of being too explicit, the m=1 CTF is a rescaled version of the electric field due to a dipole oriented along the x axis, the m=-1 CTF is a rescaled version of the electric field due to a dipole oriented along the y axis, and the m=0 CTF is a rescaled version of the electric field due to a dipole oriented along the z axis. Therefore, we can reason about the CTF by thinking about the electric field that single x-, y-, and z-oriented dipoles create in the back focal plane.

With this interpretation in mind, the form of the CTF is not surprising. Under the paraxial approximation x- and y-oriented dipoles uniformly fill the back focal plane with an electric field parallel to the dipole axis. A z-oriented dipole creates a radial electric field with linearly increasing amplitude towards the edges of the back focal plane.

The spatial band limit can be increased by increasing the NA of the microscope, so what parameter sets the angular band limit? The angular band limit in the current microscope is set by the dipole emission process—only l=1 terms show up in the CTF for dipole emitters. Adding polarizing filters to the microscope will not extend the band limit of the microscope because filters only block electric fields. However, polarized illumination can be used to extend the band limit. The excitation process is completely incoherent with the emission process,

so the spatio-angular transfer functions of the two processes will multiply. We can use the multiplication rules in Appendix A to find the band limit of a new microscope that uses selective excitation.

Next we find the intensity on the detector plane  $g(\mathbf{r}_d)$  by taking the modulus squared of the electric field. Using Eq. 22 we find that

$$g(\mathbf{r}_d) = |\mathbf{e}_d(\mathbf{r}_d)|^2 = \left| \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o \ \tilde{\mathbf{e}}_d(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) \boldsymbol{\mu}(\mathbf{r}_o, \hat{\mathbf{s}}_o) \right|^2.$$
(31)

If the dipoles emit incoherently—a fair assumption for fluorescent emitters that are not within homo-FRET distance—then we can take the squared modulus of the object and CSF independently, which gives

$$g(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o |\tilde{\mathbf{e}}_d(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o)|^2 |\boldsymbol{\mu}(\mathbf{r}_o, \hat{\mathbf{s}}_o)|^2.$$
(32)

We recognize that  $|\tilde{\mathbf{e}}_d(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o)|^2$  is the PSF of the microscope  $h(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o)$ , and we introduce a new function  $f(\mathbf{r}_o, \hat{\mathbf{s}}_o) \equiv |\mu(\mathbf{r}_o, \hat{\mathbf{s}}_o)|^2$  to represent the object

$$g(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o \ h(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o). \tag{33}$$

We can interpret  $f(\mathbf{r}_o, \hat{\mathbf{s}}_o)$  as the spatio-angular density of dipoles—it is a function that is proportional to the number of fluorophores at position  $\mathbf{r}_o$  oriented in direction  $\hat{\mathbf{s}}_o$  per unit volume per unit solid angle. Notice that h and f are scalar functions—we removed all phase information when we took the modulus squared.

We can follow our previous work with the electric field and simplify Eq. 33 by expanding the PSF in terms of spatio-angular harmonics

$$h(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} \, H_l^m(\boldsymbol{\nu}) y_l^m(\hat{\mathbf{s}}_o) \, e^{i2\pi(\mathbf{r}_d - M\mathbf{r}_o) \cdot \boldsymbol{\nu}}, \tag{34}$$

where  $H_l^m(\boldsymbol{\nu})$  is the spatio-angular spectrum of the PSF given by

$$H_l^m(\boldsymbol{\nu}) \equiv \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o h(\mathbf{r}_d - M\mathbf{r}_o, \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o) e^{-i2\pi(\mathbf{r}_d - M\mathbf{r}_o) \cdot \boldsymbol{\nu}}.$$
 (35)

We can change variables to make this expression easier to evaluate

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_d' h(\mathbf{r}_d', \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o) e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}.$$
 (36)

By plugging Eq. 34 into Eq. 33 we find that

$$g(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o \left[ \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} H_l^m(\boldsymbol{\nu}) y_l^m(\hat{\mathbf{s}}_o) e^{i2\pi(\mathbf{r}_d - M\mathbf{r}_o) \cdot \boldsymbol{\nu}} \right] f(\mathbf{r}_o, \hat{\mathbf{s}}_o).$$
(37)

We can rearrange this equation into the following form

$$g(\mathbf{r}_d) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} \, H_l^m(\boldsymbol{\nu}) \left[ \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o \, f(\mathbf{r}_o, \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o) \, e^{-i2\pi M \mathbf{r}_o \cdot \boldsymbol{\nu}} \right] e^{i2\pi \mathbf{r}_d \cdot \boldsymbol{\nu}}.$$
(38)

We recognize the term in square brackets as the spatio-angular spectrum of the object, so we define

$$F_l^m(\boldsymbol{\nu}) \equiv \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o f(\mathbf{r}_o, \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o) e^{-i2\pi M \mathbf{r}_o \cdot \boldsymbol{\nu}}.$$
 (39)

and write the intensity on the detector as

$$g(\mathbf{r}_d) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} H_l^m(\boldsymbol{\nu}) F_l^m(\boldsymbol{\nu}) e^{i2\pi \mathbf{r}_d \cdot \boldsymbol{\nu}}.$$
 (40)

Eq. 47 shows that the intensity on the detector can be found by resolving the object into its spatio-angular component  $F_l^m(\nu_o)$ , weighting each component by  $H_l^m(\nu_o)$ , then summing over all spatio-angular components. Therefore, we identify  $H_l^m(\nu_o)$  as the spatio-angular optical transfer function (OTF). Notice that the CSF and CTF are vector-valued functions, while the PSF and OTF are scalar-valued functions.

In Appendix C we calculate the paraxial OTF for a single-view fluorescence microscope as

$$H_l^{m(p)}(\nu) = H_0^{0(p)}(\nu)\delta(l,m) + H_2^{0(p)}(\nu)\delta(l-2,m), \tag{41}$$

$$H_0^{0(p)}(\nu) \equiv \frac{A^{(p)}(\nu) + 2B^{(p)}(\nu)}{1 + NA^2/(2n_o^2)},\tag{42}$$

$$H_2^{0(p)}(\nu) \equiv \frac{-A^{(p)}(\nu) + 4B^{(p)}}{\sqrt{5} \left[1 + NA^2/(2n_o^2)\right]}.$$
(43)

where

$$A^{(p)}(\nu) = \frac{2}{\pi} \left[ \arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right), \tag{44}$$

$$B^{(p)}(\nu) = \frac{\text{NA}^2}{2\pi n_o^2} \left[ \left( 1 - \frac{\nu^2}{\nu_o^2} \right) \arccos\left(\frac{\nu}{2\nu_o}\right) + \left( 1 + \frac{\nu^2}{2\nu_o^2} \right) \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right). \tag{45}$$

Notice that the paraxial OTF is rotationally symmetric, so we can write in terms of  $\nu$  instead of the complete spatial frequency vector  $\nu$ . This will no longer be true when we leave the paraxial approximation or add polarizing filters to the system. Also, notice that  $A^{(p)}(\nu)$  is the spatial OTF for an incoherent imaging system under the paraxial and scalar approximations [3].

If our sample consists of isotropic distributions of fluorophores only, then we can ignore the  $H_2^{0(p)}(\nu)$  term in the OTF. As the NA approaches zero,  $B^{(p)}(\nu)$  disappears and we recover the spatial OTF from the spatioangular OTF as expected. For non-zero NA the difference between the OTF with and without the scalar approximation is  $2B^{(p)}(\nu)$ . We can interpret this difference as an electromagnetic correction to the scalar OTF. The correction is proportional to  $\frac{NA^2}{n_s^2}$ , so it is a small term for all but high NA objectives.

Next, consider the  $H_2^{0(p)}(\nu)$  term for NA approaching zero. In this limit  $H_2^{0(p)}(\nu)$  approaches the isotropic scalar paraxial OTF multiplied by a factor of  $-1/\sqrt{5} \approx -0.45$ . We can interpret the  $H_2^{0(p)}(\nu)$  term as the spatial transfer function if our object was made entirely of fluorophores distributed like the  $y_2^0$  spherical harmonic given by  $y_2^0 = \sqrt{5/16\pi}(3\cos^2\vartheta - 1)$ . The  $y_2^0$  spherical harmonic consists of positive fluorophores near the z axis and negative fluorophores near the transverse plane. All of these fluorophores contribute to the transfer function, but the negative fluorophores in the transverse plane contribute the most (fluorophores in the transverse plane emit the most radiation along the optical axis) so the total transfer function is negative.

We can use the spatio-angular OTF to derive the spatial OTF for any distribution of fluorophores. As an example, let's derive the spatial OTF for a z-oriented fluorophore. The object is  $f(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \delta(\mathbf{r}_o)\delta(\hat{\mathbf{s}}_o - \hat{\mathbf{z}})$ . We can calculate the angular spectrum of the object as  $F_l^m(\boldsymbol{\nu}) = \int_{\mathbb{R}^2} d\mathbf{r}_o \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \delta(\hat{\mathbf{s}}_o - \hat{\mathbf{z}}) y_l^m(\hat{\mathbf{s}}_o) \mathrm{e}^{-i2\pi M \mathbf{r}_o \cdot \boldsymbol{\nu}} = y_l^m(\hat{\mathbf{z}})$ . Finally, we can plug in the angular spectrum into equation 47

$$g(\mathbf{r}_d) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \int_{\mathbb{R}^2} d\boldsymbol{\nu} H_l^m(\boldsymbol{\nu}) y_l^m(\hat{\mathbf{z}}) e^{i2\pi \mathbf{r}_d \cdot \boldsymbol{\nu}}, \tag{46}$$

then plug in the spatio-angular OTF from Eq. 41 and evaluate the sum to find

$$g(\mathbf{r}_d) \propto \int_{\mathbb{R}^2} d\nu \left[ H_0^0(\nu) y_0^0(\hat{\mathbf{z}}) + H_2^0(\nu) y_2^0(\hat{\mathbf{z}}) \right] e^{i2\pi \mathbf{r}_d \cdot \nu}. \tag{47}$$

So the spatial OTF for a z oriented dipole is given by

$$H_{\hat{\mathbf{z}}}(\nu) \propto \sqrt{\frac{1}{4\pi}} H_0^0(\nu) + \sqrt{\frac{5}{4\pi}} H_2^0(\nu).$$
 (48)

The main result is that the spatial OTF for any dipole distribution is a linear combination of the OTFs for the spherical harmonics. I am partially hesitant to refer to  $H_{\hat{\mathbf{z}}}(\nu)$  as an OTF because it is object dependent, but the vocabulary is fine if it is understood that the sample only contains z-oriented dipoles. For general distributions, the complete spatio-angular OTF should be used instead of deriving the transfer function for every individual fluorophore.

Fig. 2 shows the spatio-angular OTF for a microscope with NA = 0.8 and n = 1.33. The most important feature of this figure is that  $H_0^{0(p)}(\nu)$  is a low-pass filter compared to  $H_0^{2(p)}(\nu)$  with a maximum difference of  $\sim 7\%$  (with larger difference for higher NA). If the difference between  $H_0^{0(p)}(\nu)$  and  $H_0^{2(p)}(\nu)$  is not completely obscured by noise, we will be able to exploit the difference to estimate the out-of-plane angle of dipoles with a single image (putting aside the ill-posedness of the problem).

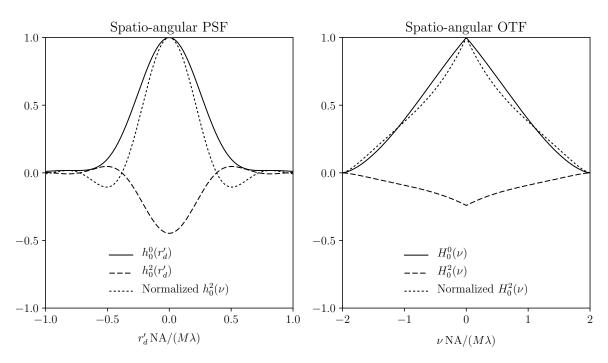


Figure 2: **Left:** Paraxial spatio-angular point spread function for a single-view fluorescence microscope with NA=0.8 and  $n_o$ =1.33. The solid line is the PSF for  $y_0^0$  distributions and the dashed line is the PSF for  $y_2^0$  distributions. The dotted line is the  $y_2^0$  PSF normalized for comparison with the  $y_0^0$  PSF. The  $y_2^0$  distributions includes "negative" fluorophores, so it gives rise to a negative PSF. **Right:** Numerical paraxial spatio-angular optical transfer function for the same microscope. The  $y_0^0$  term has a comparatively low-pass response while the  $y_2^0$  term has a comparatively high-pass response.

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### A Products of real spherical harmonics

The six products of l = 1 spherical harmonics are

$$y_1^{-1}y_1^{-1}\sqrt{\pi} = \frac{1}{2}y_0^0 - \frac{\sqrt{5}}{10}y_2^0 - \frac{\sqrt{15}}{10}y_2^2, \tag{49}$$

$$y_1^{-1}y_1^0\sqrt{\pi} = \frac{\sqrt{15}}{10}y_2^{-1},\tag{50}$$

$$y_1^{-1}y_1^1\sqrt{\pi} = -\frac{\sqrt{15}}{10}y_2^{-2},\tag{51}$$

$$y_1^0 y_1^0 \sqrt{\pi} = \frac{1}{2} y_0^0 + \frac{\sqrt{5}}{5} y_2^0, \tag{52}$$

$$y_1^0 y_1^1 \sqrt{\pi} = \frac{\sqrt{15}}{10} y_2^1, \tag{53}$$

$$y_1^1 y_1^1 \sqrt{\pi} = \frac{1}{2} y_0^0 - \frac{\sqrt{5}}{10} y_2^0 + \frac{\sqrt{15}}{10} y_2^2.$$
 (54)

The fifteen products of l=2 spherical harmonics are

$$y_2^{-2}y_2^{-2}\sqrt{\pi} = \frac{1}{2}y_0^0 - \frac{\sqrt{5}}{7}y_2^0 + \frac{1}{14}y_4^0 - \frac{\sqrt{35}}{14}y_4^4$$
 (55)

$$y_2^{-2}y_2^{-1}\sqrt{\pi} = -\frac{\sqrt{15}}{14}y_2^1 + \frac{\sqrt{10}}{28}y_4^1 + \frac{\sqrt{70}}{28}y_4^3$$
 (56)

$$y_2^{-2}y_2^0\sqrt{\pi} = -\frac{\sqrt{5}}{7}y_2^{-2} + \frac{\sqrt{15}}{14}y_4^{-2}$$
 (57)

$$y_2^{-2}y_2^1\sqrt{\pi} = -\frac{\sqrt{15}}{14}y_2^{-1} - \frac{\sqrt{70}}{28}y_4^{-3} + \frac{\sqrt{10}}{28}y_4^{-1}$$
(58)

$$y_2^{-2}y_2^2\sqrt{\pi} = \frac{\sqrt{35}}{14}y_4^{-4} \tag{59}$$

$$y_2^{-1}y_2^{-1}\sqrt{\pi} = \frac{1}{2}y_0^0 + \frac{\sqrt{5}}{14}y_2^0 - \frac{\sqrt{15}}{14}y_2^2 - \frac{2}{7}y_4^0 - \frac{\sqrt{5}}{7}y_4^2$$
 (60)

$$y_2^{-1}y_2^0\sqrt{\pi} = \frac{\sqrt{5}}{14}y_2^{-1} + \frac{\sqrt{30}}{14}y_4^{-1}$$
(61)

$$y_2^{-1}y_2^1\sqrt{\pi} = -\frac{\sqrt{15}}{14}y_2^{-2} - \frac{\sqrt{5}}{7}y_4^{-2}$$
(62)

$$y_2^{-1}y_2^2\sqrt{\pi} = -\frac{\sqrt{15}}{14}y_2^{-1} + \frac{\sqrt{70}}{28}y_4^{-3} + \frac{\sqrt{10}}{28}y_4^{-1}$$
(63)

$$y_2^0 y_2^0 \sqrt{\pi} = \frac{1}{2} y_0^0 + \frac{\sqrt{5}}{7} y_2^0 + \frac{3}{7} y_4^0 \tag{64}$$

$$y_2^0 y_2^1 \sqrt{\pi} = \frac{\sqrt{5}}{14} y_2^1 + \frac{\sqrt{30}}{14} y_4^1 \tag{65}$$

$$y_2^0 y_2^2 \sqrt{\pi} = -\frac{\sqrt{5}}{7} y_2^2 + \frac{\sqrt{15}}{14} y_4^2 \tag{66}$$

$$y_2^1 y_2^1 \sqrt{\pi} = \frac{1}{2} y_0^0 + \frac{\sqrt{5}}{14} y_2^0 + \frac{\sqrt{15}}{14} y_2^2 - \frac{2}{7} y_4^0 + \frac{\sqrt{5}}{7} y_4^2$$
 (67)

$$y_2^1 y_2^2 \sqrt{\pi} = \frac{\sqrt{15}}{14} y_2^1 - \frac{\sqrt{10}}{28} y_4^1 + \frac{\sqrt{70}}{28} y_4^3$$
 (68)

$$y_2^2 y_2^2 \sqrt{\pi} = \frac{1}{2} y_0^0 - \frac{\sqrt{5}}{7} y_2^0 + \frac{1}{14} y_4^0 + \frac{\sqrt{35}}{14} y_4^4$$
 (69)

### B Paraxial coherent transfer function

In this appendix we calculate the paraxial CTF for a single-view fluorescence microscope. We start by plugging Eq. 13 (the paraxial CSF) into Eq. 25

$$\mathbf{E}_{l}^{m(p)}(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^{2}} d\hat{\mathbf{s}}_{o} \int_{\mathbb{R}^{2}} d\mathbf{r}_{d}' \begin{bmatrix} a^{(p)}(r_{d}')y_{1}^{1}(\hat{\mathbf{s}}_{o}) + 2ib^{(p)}(r_{d}')\cos\phi_{d}'y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ a^{(p)}(r_{d}')y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + 2ib^{(p)}(r_{d}')\sin\phi_{d}'y_{1}^{0}(\hat{\mathbf{s}}_{o}) \end{bmatrix} y_{l}^{m}(\hat{\mathbf{s}}_{o}) e^{-i2\pi\mathbf{r}_{d}'\cdot\boldsymbol{\nu}}.$$
(70)

We split the integral into two parts by writing

$$\mathbf{E}_l^{m(p)}(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, \mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o), \tag{71}$$

$$\mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) \equiv \int_{\mathbb{R}^2} d\mathbf{r}_d' \begin{bmatrix} a^{(p)}(r_d') y_1^1(\hat{\mathbf{s}}_o) + 2ib^{(p)}(r_d') \cos \phi_d' y_1^0(\hat{\mathbf{s}}_o) \\ a^{(p)}(r_d') y_1^{-1}(\hat{\mathbf{s}}_o) + 2ib^{(p)}(r_d') \sin \phi_d' y_1^0(\hat{\mathbf{s}}_o) \end{bmatrix} e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}.$$
 (72)

Substituting  $a^{(p)}(r'_d)$  and  $b^{(p)}(r'_d)$  using Eq. 14 gives

$$\mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_{o}) = \int_{\mathbb{R}^{2}} d\mathbf{r}_{d}' \begin{bmatrix} \frac{J_{1}(2\pi\nu_{o}r_{d}')}{\pi\nu_{o}r_{d}'} y_{1}^{m}(\hat{\mathbf{s}}_{o}) + 2i\frac{\mathrm{NA}}{n_{o}} \left[ \frac{J_{2}(2\pi\nu_{o}r_{d}')}{\pi\nu_{o}r_{d}'} \right] \cos\phi_{d}' y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ \frac{J_{1}(2\pi\nu_{o}r_{d}')}{\pi\nu_{o}r_{d}'} y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + 2i\frac{\mathrm{NA}}{n_{o}} \left[ \frac{J_{2}(2\pi\alpha r_{d}')}{\pi\nu_{o}r_{d}'} \right] \sin\phi_{d}' y_{1}^{0}(\hat{\mathbf{s}}_{o}) \end{bmatrix} e^{-i2\pi\mathbf{r}_{d}'\cdot\boldsymbol{\nu}}.$$
(73)

We can rewrite this in terms of three two-dimensional Fourier transforms

$$\mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) \propto \begin{bmatrix} t_1(\boldsymbol{\nu}) y_1^1(\hat{\mathbf{s}}_o) + t_2(\boldsymbol{\nu}) y_1^0(\hat{\mathbf{s}}_o) \\ t_1(\boldsymbol{\nu}) y_1^{-1}(\hat{\mathbf{s}}_o) + t_3(\boldsymbol{\nu}) y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix}$$
(74)

where

$$t_1(\boldsymbol{\nu}) \equiv \frac{1}{\pi \nu_o} \int_{\mathbb{R}^2} d\mathbf{r}_d' \frac{J_1(2\pi \nu_o r_d')}{r_d'} e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}, \tag{75}$$

$$t_2(\boldsymbol{\nu}) \equiv \frac{2i}{\pi \nu_o} \frac{\text{NA}}{n_o} \int_{\mathbb{R}^2} d\mathbf{r}_d' \frac{J_2(2\pi \nu_o r_d')}{r_d'} \cos \phi_d' e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}, \tag{76}$$

$$t_3(\boldsymbol{\nu}) \equiv \frac{2i}{\pi \nu_o} \frac{\text{NA}}{n_o} \int_{\mathbb{R}^2} d\mathbf{r}_d' \frac{J_2(2\pi \nu_o r_d')}{r_d'} \sin \phi_d' \, e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}. \tag{77}$$

All three of the functions to be transformed are separable in polar coordinates, so we can rewrite the Fourier transform as a sum of weighted Hankel transforms [3]. In general, if a function  $g(r,\theta)$  is separable in polar coordinates then we can rewrite it as  $g(r,\theta) = g_R(r)g_{\Theta}(\theta)$  and its two-dimensional Fourier transform is given by

$$\mathcal{F}\{g(r,\theta)\} = \sum_{k=-\infty}^{\infty} c_k(-i)^k e^{ik\phi} \mathcal{H}_k\{g_R(r)\}$$
(78)

where

$$c_k = \frac{1}{2\pi} \int_0^{2\pi} d\theta \, g_{\Theta}(\theta) e^{-ik\theta}$$
 (79)

and  $\mathcal{H}_k\{\}$  is the Hankel transform of order k given by

$$\mathcal{H}_k\{g_R(r)\} = 2\pi \int_0^\infty dr \, r g_R(r) J_k(2\pi r \nu). \tag{80}$$

First, we evaluate  $t_1(\boldsymbol{\nu})$ . The Fourier transform will be in polar coordinates, so we define polar coordinates in frequency space as  $\boldsymbol{\nu} \equiv \nu \cos \phi_{\nu} \hat{\mathbf{x}} + \nu \sin \phi_{\nu} \hat{\mathbf{y}} \equiv \nu_{x} \hat{\mathbf{x}} + \nu_{y} \hat{\mathbf{y}}$ . The angular part of  $J_z(\nu_o r'_d)/r'_d$  is a constant, so  $c_k = \delta(k)$  which means we only need to evaluate the zero-order Hankel transform

$$t_1(\nu) = \frac{1}{\pi \nu_o} \mathcal{H}_0 \left\{ \frac{J_1(2\pi \nu_o r_d')}{r_d'} \right\}.$$
 (81)

From tabulated Hankel transforms we find that

$$\mathcal{H}_{\mu} \left\{ \frac{J_{\mu+1}(2\pi\nu_{o}r_{d}^{\prime})}{r_{d}^{\prime}} \right\} = \frac{1}{2\pi}\nu_{o}^{-\mu-1}\nu^{\mu} \Pi\left(\frac{\nu}{a}\right)$$

$$\tag{82}$$

when  $\nu_o > 0$  and  $\text{Re}(\mu) > -\frac{3}{2}$  [4]. Applying this result we find that

$$t_1(\boldsymbol{\nu}) = \frac{1}{2\pi^2 \nu_o^2} \Pi\left(\frac{\nu}{\nu_o}\right). \tag{83}$$

To evaluate  $t_2(\boldsymbol{\nu})$  and  $t_3(\boldsymbol{\nu})$  we need to be careful with the angular part. For  $t_2(\boldsymbol{\nu})$  the angular part of the function is  $\cos \phi'_d = \frac{1}{2} \left( e^{i\phi'_d} + e^{-i\phi'_d} \right)$ . Therefore,  $c_k = \frac{1}{2}\delta(k-1) + \frac{1}{2}\delta(k+1)$  and we have to evaluate two Hankel transforms given by

$$t_2(\boldsymbol{\nu}) = \frac{2i}{\pi\nu_o} \frac{\text{NA}}{n_o} \left[ ie^{-i\phi_{\nu}} \mathcal{H}_{-1} \left\{ \frac{J_2(2\pi\nu_o r_d')}{\pi\nu_o r_d'} \right\} - ie^{i\phi_{\nu}} \mathcal{H}_1 \left\{ \frac{J_2(2\pi\nu_o r_d')}{\pi a r_d'} \right\} \right]. \tag{84}$$

To put the Hankel transforms in the form of Eq. 82 we apply  $\mathcal{H}_{\mu} = (-1)^{\mu}\mathcal{H}_{-\mu}$  to get

$$t_2(\boldsymbol{\nu}) = \frac{2i}{\pi\nu_o} \frac{\text{NA}}{n_o} \left[ -ie^{-i\phi_{\nu}} \mathcal{H}_1 \left\{ \frac{J_2(2\pi\nu_o r_d')}{\pi\nu_o r_d'} \right\} - ie^{i\phi_{\nu}} \mathcal{H}_1 \left\{ \frac{J_2(2\pi\nu_o r_d')}{\pi\nu_o r_d'} \right\} \right]. \tag{85}$$

Applying Eq. 82 and simplifying gives

$$t_2(\boldsymbol{\nu}) = \frac{1}{\pi^2 \nu_o^3} \frac{\text{NA}}{n_o} \nu \left( e^{-i\phi_{\nu}} + e^{i\phi_{\nu}} \right) \Pi \left( \frac{\nu}{\nu_o} \right)$$
 (86)

Finally,

$$t_2(\boldsymbol{\nu}) = \frac{1}{\pi^2 \nu_o^3} \frac{\text{NA}}{n_o} \nu \cos \phi_{\nu} \Pi\left(\frac{\nu}{a}\right)$$
 (87)

Similarly,

$$t_3(\mathbf{\nu}) = \frac{1}{\pi^2 \nu_o^3} \frac{\text{NA}}{n_o} \nu \sin \phi_\nu \Pi\left(\frac{\nu}{a}\right) \tag{88}$$

Plugging Eqs. 83, 87, and 88 into Eq. 74 and normalizing gives

$$\mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) = \begin{bmatrix} y_1^1(\hat{\mathbf{s}}_o) + \frac{2}{\nu_o} \frac{\mathrm{NA}}{n_o} \nu \cos \phi_{\nu} y_1^0(\hat{\mathbf{s}}_o) \\ y_1^{-1}(\hat{\mathbf{s}}_o) + \frac{2}{\nu_o} \frac{\mathrm{NA}}{n_o} \nu \sin \phi_{\nu} y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix} \Pi\left(\frac{\nu}{\nu_o}\right). \tag{89}$$

Finally, we find the paraxial CTF by evaluating the angular integral in Eq. 72

$$\mathbf{E}_{l}^{m(p)}(\boldsymbol{\nu}) = \begin{bmatrix} \delta(l-1, m-1) + \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} \nu \cos \phi_{\nu} \delta(l-1, m) \\ \delta(l-1, m+1) + \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} \nu \sin \phi_{\nu} \delta(l-1, m) \\ 0 \end{bmatrix} \Pi\left(\frac{\nu}{\nu_{o}}\right). \tag{90}$$

# C Paraxial optical transfer function

In this appendix we calculate the paraxial OTF for a single-view fluorescence microscope. We start with Eq. 91

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_d' h(\mathbf{r}_d', \hat{\mathbf{s}}_o) y_l^m(\hat{\mathbf{s}}_o) e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}.$$
 (91)

We could plug in the paraxial PSF and evaluate the integrals, but this will lead us to Fourier transforms that cannot be found in tables. Instead we use a trick from scalar Fourier optics and write the OTF in terms of the detected electric field [3]

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_d' \left| \mathbf{e}_d(\mathbf{r}_d', \hat{\mathbf{s}}_o) \right|^2 y_l^m(\hat{\mathbf{s}}_o) e^{-i2\pi \mathbf{r}_d' \cdot \boldsymbol{\nu}}.$$
 (92)

Next, we manipulate this equation to

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) \int_{\mathbb{R}^2} d\mathbf{r}_d' \, \mathbf{e}_d^{\dagger}(\mathbf{r}_d', \hat{\mathbf{s}}_o) \mathbf{e}_d(\mathbf{r}_d', \hat{\mathbf{s}}_o) \, e^{-i2\pi\mathbf{r}_d' \cdot \boldsymbol{\nu}}, \tag{93}$$

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) \int_{\mathbb{R}^2} d\mathbf{r}_d' \, \int_{\mathbb{R}^2} d\mathbf{r}_d'' \, \mathbf{e}_d^{\dagger}(\mathbf{r}_d', \hat{\mathbf{s}}_o) \mathbf{e}_d(\mathbf{r}_d'', \hat{\mathbf{s}}_o) \delta(\mathbf{r}_d'' - \mathbf{r}_d') \, e^{-i2\pi \mathbf{r}_d'' \cdot \boldsymbol{\nu}}, \tag{94}$$

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) \int_{\mathbb{R}^2} d\boldsymbol{\tau} \, \int_{\mathbb{R}^2} d\mathbf{r}_d' \, \int_{\mathbb{R}^2} d\mathbf{r}_d'' \, \mathbf{e}_d^{\dagger}(\mathbf{r}_d', \hat{\mathbf{s}}_o) \mathbf{e}_d(\mathbf{r}_d'', \hat{\mathbf{s}}_o) \mathbf{e}^{-i2\pi\boldsymbol{\tau}(\mathbf{r}_d''-\mathbf{r}_d')} \mathbf{e}^{-i2\pi\boldsymbol{r}_d''\cdot\boldsymbol{\nu}}, \tag{95}$$

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) \int_{\mathbb{R}^2} d\boldsymbol{\tau} \left[ \int_{\mathbb{R}^2} d\mathbf{r}_d' \, \mathbf{e}_d^{\dagger}(\mathbf{r}_d', \hat{\mathbf{s}}_o) e^{i2\pi\mathbf{r}_d' \cdot \boldsymbol{\tau}} \right] \left[ \int_{\mathbb{R}^2} d\mathbf{r}_d'' \, \mathbf{e}_d(\mathbf{r}_d'', \hat{\mathbf{s}}_o) e^{-i2\pi\mathbf{r}_d'' \cdot (\boldsymbol{\nu} + \boldsymbol{\tau})} \right], \tag{96}$$

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) \int_{\mathbb{R}^2} d\boldsymbol{\tau} \, \mathbf{E}^{\dagger}(\boldsymbol{\tau}; \hat{\mathbf{s}}_o) \mathbf{E}(\boldsymbol{\tau} + \boldsymbol{\nu}; \hat{\mathbf{s}}_o). \tag{97}$$

We have followed the proof for the Wiener-Kinchin theorem [5, 6] to explicitly show how the angular integral affects the usual scalar calculation. Eq. 97 shows that we can calculate the OTF by taking the vector autocorrelation of the spatial CTF then projecting the result onto the spherical harmonics.

We will rewrite Eq. 97 in two parts

$$H_l^m(\boldsymbol{\nu}) \propto \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, y_l^m(\hat{\mathbf{s}}_o) H(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) \tag{98}$$

$$H(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) \equiv \int_{\mathbb{R}^2} d\boldsymbol{\tau} \, \mathbf{E}^{\dagger}(\boldsymbol{\tau} - \boldsymbol{\nu}/2; \hat{\mathbf{s}}_o) \mathbf{E}(\boldsymbol{\tau} + \boldsymbol{\nu}/2; \hat{\mathbf{s}}_o). \tag{99}$$

Notice the change of variable to simplify the autocorrelation. In the previous section we calculated the CTF in the paraxial case as

$$\mathbf{E}^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_o) = \begin{bmatrix} y_1^1(\hat{\mathbf{s}}_o) + \frac{2}{\nu_o} \frac{NA}{n_o} \nu \cos \phi_{\nu} y_1^0(\hat{\mathbf{s}}_o) \\ y_1^{-1}(\hat{\mathbf{s}}_o) + \frac{2}{\nu_o} \frac{NA}{n_o} \nu \sin \phi_{\nu} y_1^0(\hat{\mathbf{s}}_o) \\ 0 \end{bmatrix} \Pi\left(\frac{\nu}{\nu_o}\right).$$
(100)

By introducing  $\tau \equiv \tau_x \hat{\mathbf{x}} + \tau_y \hat{\mathbf{y}} \equiv \tau \cos \phi_\tau \hat{\mathbf{x}} + \tau \sin \phi_\tau \hat{\mathbf{y}}$  we can plug Eq. 100 into Eq. 99 and find that

$$H^{(p)}(\boldsymbol{\nu};\hat{\mathbf{s}}_{o}) \propto \int_{\mathbb{R}^{2}} d\boldsymbol{\tau} \begin{bmatrix} y_{1}^{1}(\hat{\mathbf{s}}_{o}) + \frac{2}{\nu_{o}} \frac{NA}{n_{o}} (\tau_{x} - \frac{1}{2}\nu_{x}) y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + \frac{2}{\nu_{o}} \frac{NA}{n_{o}} (\tau_{y} - \frac{1}{2}\nu_{y}) y_{1}^{0}(\hat{\mathbf{s}}_{o}) \end{bmatrix}^{\dagger} \begin{bmatrix} y_{1}^{1}(\hat{\mathbf{s}}_{o}) + \frac{2}{\nu_{o}} \frac{NA}{n_{o}} (\tau_{x} + \frac{1}{2}\nu_{x}) y_{1}^{0}(\hat{\mathbf{s}}_{o}) \\ y_{1}^{-1}(\hat{\mathbf{s}}_{o}) + \frac{2}{\nu_{o}} \frac{NA}{n_{o}} (\tau_{y} + \frac{1}{2}\nu_{y}) y_{1}^{0}(\hat{\mathbf{s}}_{o}) \end{bmatrix} \\ \Pi\left(\frac{\tau - \nu/2}{\nu_{o}}\right) \Pi\left(\frac{\tau + \nu/2}{\nu_{o}}\right). \tag{101}$$

Expanding the inner product gives

$$H^{(p)}(\boldsymbol{\nu};\hat{\mathbf{s}}_{o}) \propto \int_{\mathbb{R}^{2}} d\boldsymbol{\tau} \left\{ y_{1}^{1}(\hat{\mathbf{s}}_{o}) \right\}^{2} + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) \right\}^{2} (\tau_{x} - \frac{1}{2}\nu_{x})(\tau_{x} + \frac{1}{2}\nu_{x}) + \left\{ y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\}^{2} + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) \right\}^{2} (\tau_{y} - \frac{1}{2}\nu_{y})(\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{x} - \frac{1}{2}\nu_{x}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{x} + \frac{1}{2}\nu_{x}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\} (\tau_{y} + \frac{1}{2}\nu_{y}) + \left\{ \frac{2}{\nu_{o}} \frac$$

All of the terms with a single  $(\tau, \pm \frac{1}{2}\nu)$  term are correlations of an odd function with an even function, so they are zero. Therefore

$$H^{(p)}(\boldsymbol{\nu}; \hat{\mathbf{s}}_{o}) \propto \int_{\mathbb{R}^{2}} d\boldsymbol{\tau} \left\{ y_{1}^{1}(\hat{\mathbf{s}}_{o}) \right\}^{2} + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) \right\}^{2} (\tau_{x} - \frac{1}{2}\nu_{x})(\tau_{x} + \frac{1}{2}\nu_{x}) + \left\{ y_{1}^{-1}(\hat{\mathbf{s}}_{o}) \right\}^{2} + \left\{ \frac{2}{\nu_{o}} \frac{\mathrm{NA}}{n_{o}} y_{1}^{0}(\hat{\mathbf{s}}_{o}) \right\}^{2} (\tau_{y} - \frac{1}{2}\nu_{y})(\tau_{y} + \frac{1}{2}\nu_{y}) + \Pi\left(\frac{\tau - \nu/2}{\nu_{o}}\right) \Pi\left(\frac{\tau + \nu/2}{\nu_{o}}\right).$$

$$(103)$$

After expanding the spherical harmonics we can rewrite this equation in terms of two autocorrelations

$$H^{(p)}(\nu; \hat{\mathbf{s}}_o) \propto \left[ A^{(p)}(\nu) + 2B^{(p)}(\nu) \right] y_0^0(\hat{\mathbf{s}}_o) + \frac{1}{\sqrt{5}} \left[ -A^{(p)}(\nu) + 4B^{(p)}(\nu) \right] y_2^0(\hat{\mathbf{s}}_o)$$
(104)

where

$$A^{(p)}(\nu) \equiv \frac{1}{\pi \nu_o^2} \int_{\mathbb{R}^2} d\tau \, \Pi\left(\frac{\tau - \nu/2}{\nu_o}\right) \Pi\left(\frac{\tau + \nu/2}{\nu_o}\right), \tag{105}$$

$$B^{(p)}(\nu) \equiv \frac{\text{NA}^2}{\pi \nu_o^4 n_o^2} \int_{\mathbb{R}^2} d\tau \, \left[ (\tau_x - \nu_x/2)(\tau_x + \nu_x/2) + (\tau_y - \nu_y/2)(\tau_y + \nu_y/2) \right]$$

$$\Pi\left(\frac{\tau - \nu/2}{\nu_o}\right) \Pi\left(\frac{\tau + \nu/2}{\nu_o}\right). \tag{106}$$

I've used the constant  $\frac{1}{\pi\nu_o^2}$  in front of both autocorrelations so that the results are normalized, but these constants are not essential. Also, notice that both autocorrelations are rotationally symmetric, so we can write them as a function of  $\nu$  instead of  $\nu$ . We can evaluate these integrals with the help of the geometric construction shown in Figure 3 [3].

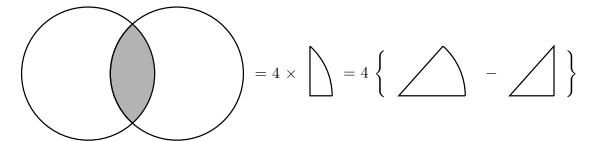


Figure 3: Geometric construction for evaluating  $A^{(p)}(\nu)$  and  $B^{(p)}(\nu)$ . We need to find the boundaries of integration for the overlapping region of two circles with radius  $\nu_o$  and distance  $\nu$  between their centers. The region is given by four times the difference in area between a sector of angle  $\arccos\left(\frac{\nu}{2\nu_o}\right)$  and a triangle with base  $\nu/2$  and hypotenuse  $\nu_o$ .

Starting with  $A^{(p)}(\nu)$  we find that

$$A^{(p)}(\nu) = \frac{4}{\pi \nu_o^2} \left[ \left( \int_0^{\nu_o} d\tau \, \tau \int_0^{\arccos\left(\frac{\nu}{2\nu_o}\right)} d\phi_\tau \right) - \left( \int_0^{\nu/2} d\tau_x \int_0^{\tau_x \frac{2\nu_o}{\nu}} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right) \right] \Pi\left(\frac{\nu}{2\nu_o}\right)$$
(107)

$$A^{(p)}(\nu) = \frac{4}{\pi \nu_o^2} \left[ \left( \int_0^{\nu_o} d\tau \, \arccos\left(\frac{\nu}{2\nu_o}\right) \right) - \left( \int_0^{\nu/2} d\tau_x \tau_x \frac{2\nu_o}{\nu} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right) \right] \Pi\left(\frac{\nu}{2\nu_o}\right)$$
(108)

$$A^{(p)}(\nu) = \frac{2}{\pi} \left[ \arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right). \tag{109}$$

Notice that  $A^{(p)}(\nu)$  is the normalized OTF for fluorescence microscope with isotropic samples under the paraxial and scalar approximations. Next we evaluate  $B^{(p)}(\nu)$  using the same geometric construction. This integral is rotationally symmetric, so we start by rewriting it as

$$B^{(p)}(\nu) = \frac{4NA^{2}}{\pi\nu_{o}^{4}n_{o}^{2}} \left[ \int_{0}^{\nu_{o}} d\tau \, \tau \int_{0}^{\arccos\left(\frac{\nu}{2\nu_{o}}\right)} d\phi_{\tau}(\tau\cos\phi_{\tau} - \nu/2)(\tau\cos\phi_{\tau} + \nu/2) \right]$$

$$- \int_{0}^{\nu/2} d\tau_{x} \int_{0}^{\tau_{x}\frac{2\nu_{o}}{\nu}} \sqrt{1 - \left(\frac{\nu}{2\nu_{o}}\right)^{2}} d\tau_{y}(\tau_{x} - \nu/2)(\tau_{x} + \nu/2) \right],$$

$$B^{(p)}(\nu) = \frac{4NA^{2}}{\pi\nu_{o}^{4}n_{o}^{2}} \left[ \int_{0}^{\nu_{o}} d\tau \, \tau \int_{0}^{\arccos\left(\frac{\nu}{2\nu_{o}}\right)} d\phi_{\tau}(\tau^{2}\cos^{2}\phi_{\tau} - \nu^{2}/4) \right]$$

$$- \int_{0}^{\nu/2} d\tau_{x} \int_{0}^{\tau_{x}\frac{2\nu_{o}}{\nu}} \sqrt{1 - \left(\frac{\nu}{2\nu_{o}}\right)^{2}} d\tau_{y}(\tau_{x}^{2} - \nu^{2}/4) \right].$$

$$(111)$$

For the first inner integral we make use of

$$\int_{0}^{\arccos(z)} d\phi \cos^{2} \phi = \frac{1}{2} z \sqrt{1 - z^{2}} + \frac{1}{2} \arccos(z).$$
 (112)

This results in

$$B^{(p)}(\nu) = \frac{4\text{NA}^2}{\pi \nu_o^4 n_o^2} \left[ \int_0^a d\tau \left( \frac{\tau^3}{2} \left( \frac{\nu}{2\nu_o} \right) \sqrt{1 - \left( \frac{\nu}{2\nu_o} \right)^2} + \frac{\tau^3}{2} \arccos\left( \frac{\nu}{2a} \right) - \frac{\tau \nu^2}{4} \arccos\left( \frac{\nu}{2\nu_o} \right) \right) - \int_0^{\nu/2} d\tau_x \left( \tau_x^3 - \frac{\tau_x \nu^2}{4} \right) \frac{2\nu_o}{\nu} \sqrt{1 - \left( \frac{\nu}{2\nu_o} \right)^2} \right] \Pi\left( \frac{\nu}{2\nu_o} \right), \tag{113}$$

$$B^{(p)}(\nu) = \frac{4\text{NA}^2}{\pi \nu_o^4 n_o^2} \left[ \left( \frac{\nu_o^4}{8} \left( \frac{\nu}{2\nu_o} \right) \sqrt{1 - \left( \frac{\nu}{2\nu_o} \right)^2 + \frac{\nu_o^4}{8} \arccos\left( \frac{\nu}{2\nu_o} \right) - \frac{\nu_o^2 \nu^2}{8} \arccos\left( \frac{\nu}{2\nu_o} \right) \right) - \left( \frac{\nu^4}{64} - \frac{\nu^4}{32} \right) \frac{2\nu_o}{\nu} \sqrt{1 - \left( \frac{\nu}{2\nu_o} \right)^2} \right] \Pi\left( \frac{\nu}{2\nu_o} \right),$$
(114)

$$B^{(p)}(\nu) = \frac{4\text{NA}^2}{\pi \nu_o^4 n_o^2} \left[ \frac{\nu \nu_o^3}{16} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} + \frac{\nu_o^4}{8} \arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu_o^2 \nu^2}{8} \arccos\left(\frac{\nu}{2\nu_o}\right) + \frac{\nu^3 \nu_o}{32} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right), \tag{115}$$

$$B^{(p)}(\nu) = \frac{\text{NA}^2}{2\pi n_o^2} \left[ \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2a}\right)^2} + \arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu^2}{\nu_o^2} \arccos\left(\frac{\nu}{2\nu_o}\right) + \frac{\nu^3}{4\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right), \tag{116}$$

$$B^{(p)}(\nu) = \frac{NA^2}{2\pi n_o^2} \left[ \left( 1 - \frac{\nu^2}{\nu_o^2} \right) \arccos\left(\frac{\nu}{2\nu_o}\right) + \left( 1 + \frac{\nu^2}{2\nu_o^2} \right) \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2a}\right). \tag{117}$$

Now that we've evaluated the autocorrelations we can complete the OTF calculation. By plugging Eq. 104 into Eq. 98, performing the angular integral, and normalizing, we get the complete spatio-angular transfer function

$$H_l^{m(p)}(\nu) = H_0^{0(p)}(\nu)\delta(l,m) + H_2^{0(p)}(\nu)\delta(l-2,m), \tag{118}$$

$$H_0^{0(p)}(\nu) \equiv \frac{A^{(p)}(\nu) + 2B^{(p)}(\nu)}{1 + NA^2/(2n_o^2)},\tag{119}$$

$$H_2^{0(p)}(\nu) \equiv \frac{-A^{(p)}(\nu) + 4B^{(p)}}{\sqrt{5} \left[1 + NA^2/(2n_o^2)\right]}.$$
 (120)

where

$$A^{(p)}(\nu) = \frac{2}{\pi} \left[ \arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right), \tag{121}$$

$$B^{(p)}(\nu) = \frac{\text{NA}^2}{2\pi n_o^2} \left[ \left( 1 - \frac{\nu^2}{\nu_o^2} \right) \arccos\left(\frac{\nu}{2\nu_o}\right) + \left( 1 + \frac{\nu^2}{2\nu_o^2} \right) \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2} \right] \Pi\left(\frac{\nu}{2\nu_o}\right). \tag{122}$$

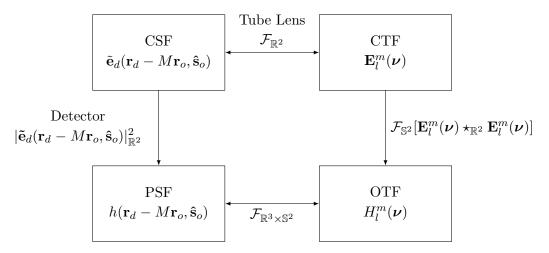


Figure 4: Summary of relationships between the CSF, CTF, PSF, and OTF where  $\mathcal{F}_D$ ,  $|\cdot|_D$ , and  $\star_D$  denote the Fourier transform, norm, and autocorrelation over the set D, respectively. See [3] and [7] for analogous diagrams under scalar optics approximations.