Singular value decomposition of multiframe polarized fluorescence microscopes

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1 Introduction

In these notes we will develop the continuous models for several multiframe polarized fluorescence microscopes. For each design we will calculate the spatio-angular point spread function, optical transfer function, and singular system consisting of the singular values, object-space singular functions, and data-space singular functions.

All of the microscopes we will consider are imaging fields of oriented fluorophores. To a good approximation any field of oriented fluorophores can be represented by a member of the set $\mathbb{U} = \mathbb{L}_2(\mathbb{R}^3 \times \mathbb{S}^2)$—square-integrable functions that assign a scalar value to each position and orientation.

We will be considering multiframe microscopes that capture multiple images of the same object (we assume that the object is static over the imaging time). The data for the ith frame can be represented by a member of $\mathbb{V}_i = \mathbb{L}_2(\mathbb{R}^N)$—square-integrable functions that assign a scalar value to each point in a 2-dimensional Euclidean space. If the microscope collects N frames, then all of the data can be represented by a member of the larger set $\mathbb{V} = \mathbb{L}_2(\mathbb{R}^{2N})$—square-integrable functions that assign a scalar value to each point in a $2N$-dimensional Euclidean space. We can say that the complete data space $\mathbb{V}$ is built by taking the *direct sum* of the data space for each frame $\mathbb{V}_i$

$$\mathbb{V} = \bigoplus_{i=1}^{N} \mathbb{V}_i. \tag{1}$$

Notice that we are assuming that data space is continuous—we are ignoring the effects of finite pixels and a finite field-of-view.

We can model any linear relationship between object space and data space using an integral transform

$$g(\mathfrak{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o \, h(\mathfrak{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \tag{2}$$

where $g(\mathfrak{r}_d) \in \mathbb{U}$ is the data, $f(\mathbf{r}_o, \hat{\mathbf{s}}_o) \in \mathbb{V}$ is the object, and $h(\mathfrak{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) \in \mathbb{U} \times \mathbb{V}$ is the point response function of the imaging system. Notice that $\mathfrak{r}_d$ is a $2N$-dimensional coordinate where the first two dimensions are the coordinates of the first image, the next two dimensions are the coordinates of the second image, and so on. Usually it will be more convenient for us to rewrite Eq. 2 as the sum of N terms

$$g(\mathfrak{r}_d) = \sum_{i=1}^{N} \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o \, h_i(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \tag{3}$$

where $\mathbf{r}_d$ is a 2-dimensional coordinate for each frame. We can also rewrite the forward model as N separate equations

$$g_i(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o \, h_i(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \qquad i = 1, 2, \ldots, N, \tag{4}$$

but this is potentially less convenient than Eqs. 2 and 3 because it hides the fact there is a single integral transform that relates object space to data space.

In these notes we'll only be considering shift-invariant microscopes, so we can simplify the model to

$$g(\mathfrak{r}_d) = \sum_{i=1}^{N} \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o \, h_i(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o). \tag{5}$$

We can rewrite the complete forward model in Eq. 5) more compactly using operator notation

$$g(\mathfrak{r}_d) = [\mathcal{H}\mathbf{f}](\mathfrak{r}_d). \tag{6}$$

In these notes we will ignore magnification—in the previous notes we showed that we can make a change of variables that puts a system with magnification in the form of a system without magnification. In other words, we can drop the primes that indicated magnified quantities in previous note sets. We will also restrict ourselves to the paraxial approximation and drop the (p) superscripts.

2 Polarized epi-illumination with unpolarized epi-detection

We will start by restricting our analysis of epi-illumination microscopes to in-focus objects. This means that our object space is $\mathbb{L}_2(\mathbb{R}^2 \times \mathbb{S}^2)$.

2.1 Point response function

In the previous notes we showed that the excitation point response function for polarized epi-illumination is given by

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

where

$$\tilde{A} \equiv \cos^2(\alpha/2)\cos(\alpha), \tag{8a}$$

$$\tilde{B} \equiv \frac{1}{12}(\cos^2\alpha + 4\cos\alpha + 7), \tag{8b}$$

and $\alpha \equiv \arcsin(\text{NA}/n_o)$.

We also showed that the point response function for unpolarized epi-detection is given by

$$h_{\text{det}}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = [a^2(r_o) + 2b^2(r_o)] y_0^0(\hat{\mathbf{s}}_o) + \frac{1}{\sqrt{5}}\left[-a^2(r_o) + 4b^2(r_o)\right] y_2^0(\hat{\mathbf{s}}_o), \tag{9}$$

where

$$a(r_o) = \frac{J_1(2\pi\nu_o r_o)}{\pi\nu_o r_o}, \qquad b(r_o) = \frac{\text{NA}}{n_o}\left[\frac{J_2(2\pi\nu_o r_o)}{\pi\nu_o r_o}\right], \tag{10}$$

and

$$\nu_o \equiv \frac{\text{NA}}{\lambda}, \qquad \text{NA} = n_o \sin\alpha. \tag{11}$$

The excitation and detection processes are incoherent, so to find the complete point response function we can multiply the excitation and detection point response functions which gives

$$h(\mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}) = h_{\text{exc}}^{\hat{\mathbf{z}}}(\hat{\mathbf{s}}_o; \hat{\mathbf{p}}) h_{\text{det}}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \frac{1}{\tilde{N}} \sum_{l=0}^{\infty} \sum_{m=-l}^{l} h_l^m(\mathbf{r}_o; \hat{\mathbf{p}}) y_l^m(\hat{\mathbf{s}}_o), \tag{12}$$

where $\tilde{N} = \frac{\tilde{A}}{10} + \frac{1}{2}$ is a normalization constant and the terms in the series are given by

$$h_0^0(r_o) = \left[\frac{\tilde{A}}{10} + \frac{1}{2}\right] a^2(r_o) + \left[-\frac{2\tilde{A}}{5} + 1\right] b^2(r_o), \tag{13}$$

$$h_2^{-2}(r_o; \hat{\mathbf{p}}) = \left[\frac{9\sqrt{15}}{70} a^2(r_o) + \frac{3\sqrt{15}}{35} b^2(r_o)\right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \tag{14}$$

$$h_2^0(r_o) = \left[-\frac{\sqrt{5}\tilde{A}}{14} + \frac{\sqrt{5}}{10}\right] a^2(r_o) + \left[-\frac{11\sqrt{5}\tilde{A}}{35} + \frac{2}{\sqrt{5}}\right] b^2(r_o), \tag{15}$$

$$h_2^2(r_o; \hat{\mathbf{p}}) = -2\left[\frac{9\sqrt{15}}{70} a^2(r_o) + \frac{3\sqrt{15}}{35} b^2(r_o)\right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \tag{16}$$

$$h_4^{-2}(r_o; \hat{\mathbf{p}}) = \left[-\frac{3\sqrt{5}}{70} a^2(r_o) + \frac{6\sqrt{5}}{35} b^2(r_o)\right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \tag{17}$$

$$h_4^0(r_o) = \frac{3\tilde{A}}{35}[a^2(r_o) - 4b^2(r_o)], \tag{18}$$

$$h_4^2(r_o; \hat{\mathbf{p}}) = -2\left[-\frac{3\sqrt{5}}{70} a^2(r_o) + \frac{6\sqrt{5}}{35} b^2(r_o)\right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \tag{19}$$

and all other h_l^m terms in the series are zero. Finally, the complete forward model for this class of microscope is given by

$$g(\mathbf{r}_d) = \sum_{i=1}^{N} \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o\, h(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}_i) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \qquad i = 1, 2, \dots, N. \tag{20}$$

Notice that we have used a single function $h(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}_i)$ to describe the complete imaging system, and we have shifted the index i to the illumination polarization. A typical choice of frames is given by $N = 4$ and

$$\hat{\mathbf{p}}_1 = \hat{\mathbf{x}}, \qquad \hat{\mathbf{p}}_2 = \frac{1}{\sqrt{2}}(\hat{\mathbf{x}} + \hat{\mathbf{y}}), \qquad \hat{\mathbf{p}}_3 = \hat{\mathbf{y}}, \qquad \hat{\mathbf{p}}_4 = \frac{1}{\sqrt{2}}(\hat{\mathbf{x}} - \hat{\mathbf{y}}). \tag{21}$$

The point response function of the microscope now contains seven terms in the $l = 0$, 2, and 4 bands. Each term is radially symmetric which means that we expect a radially symmetric point spread function for any angular distribution of fluorophores. Notice that the h_l^0 terms do not depend on the polarizer orientation, while the h_l^{-2} and h_l^2 do depend on the polarizer orientation. This is because the fluorophore distributions corresponding to the h_l^0 terms are rotationally symmetric about the optic axis, while the other terms are not rotationally symmetric.

2.2 Optical transfer function

The optical transfer function for this microscope is given by

$$H_l^m(\nu; \hat{\mathbf{p}}) = \frac{1}{\tilde{M}} \sum_{l'=0}^{\infty} \sum_{m'=-l'}^{l'} H_{l'}^{m'}(\nu; \hat{\mathbf{p}}) \delta(l - l', m - m'), \tag{22}$$

where $\tilde{M} = \left[\frac{\tilde{A}}{10} + \frac{1}{2}\right] + \left(\frac{\text{NA}}{n_o}\right)\left[-\frac{2\tilde{A}}{10} + \frac{1}{2}\right]$ is a normalization constant, and

$$H_0^0(\nu) = \left[\frac{\tilde{A}}{10} + \frac{1}{2}\right] A(\nu) + \left[-\frac{2\tilde{A}}{5} + 1\right] B(\nu), \tag{23}$$

$$H_2^{-2}(\nu; \hat{\mathbf{p}}) = \left[\frac{9\sqrt{15}}{70} A(\nu) + \frac{3\sqrt{15}}{35} B(\nu)\right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \tag{24}$$

$$H_2^0(\nu) = \left[-\frac{\sqrt{5}\tilde{A}}{14} + \frac{\sqrt{5}}{10}\right] A(\nu) + \left[-\frac{11\sqrt{5}\tilde{A}}{35} + \frac{2}{\sqrt{5}}\right] B(\nu), \tag{25}$$

$$H_2^2(\nu; \hat{\mathbf{p}}) = -2\left[\frac{9\sqrt{15}}{70} A(\nu) + \frac{3\sqrt{15}}{35} B(\nu)\right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \tag{26}$$

$$H_4^{-2}(\nu; \hat{\mathbf{p}}) = \left[-\frac{3\sqrt{5}}{70} A(\nu) + \frac{6\sqrt{5}}{35} B(\nu)\right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \tag{27}$$

$$H_4^0(\nu) = \frac{3\tilde{A}}{35}[A(\nu) - 4B(\nu)], \tag{28}$$

$$H_4^2(\nu; \hat{\mathbf{p}}) = -2\left[-\frac{3\sqrt{5}}{70} A(\nu) + \frac{6\sqrt{5}}{35} B(\nu)\right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \tag{29}$$

all other H_l^m terms in the series are zero, and

$$A(\nu) = \frac{2}{\pi}\left[\arccos\left(\frac{\nu}{2\nu_o}\right) - \frac{\nu}{2\nu_o}\sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2}\right] \Pi\left(\frac{\nu}{2\nu_o}\right), \tag{30}$$

$$B(\nu) = \frac{1}{\pi}\left(\frac{\text{NA}}{n_o}\right)^2 \left[\arccos\left(\frac{\nu}{2\nu_o}\right) - \left[3 - 2\left(\frac{\nu}{2\nu_o}\right)^2\right] \frac{\nu}{2\nu_o}\sqrt{1 - \left(\frac{\nu}{2\nu_o}\right)^2}\right] \Pi\left(\frac{\nu}{2\nu_o}\right). \tag{31}$$

2.3 Singular system

References