Singular value decomposition of a dual orthogonal-view fluorescence microscope

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1 Introduction

In these notes we will find the kernel, transfer function, and singular value decomposition of a dual orthogonal view fluorescence microscope (diSPIM). We will model the relationship between the spatial density of fluorophores in a three-dimensional sample—a member of $\mathbb{L}_2(\mathbb{R}^3)$—and the two three-dimensional volumes—two members of $\mathbb{L}_2(\mathbb{R}^3)$. We could consider the diSPIM as a microscope that makes two samples of a larger space $\mathbb{L}_2(\mathbb{R}^3 \times \mathbb{S}^2)$, but I don't think this will give us any extra insight at this point.

2 Kernel

The diSPIM illuminates the sample with a uniform-width Gaussian beam (we ignore light-sheet broadening). In path A the light sheet is in the xy plane for viewing by an objective with an optical axis along the z axis. In path B the roles are reversed and the light sheet is in the yz plane for viewing by an objective with an optical axis along the x axis.

We can model the relationship between the object and the data using an integral transform

$$g_{\mathrm{v}}(\mathfrak{r}_d) = [\mathcal{H}f]_{\mathrm{v}}(\mathfrak{r}_d) = \int_{\mathbb{R}^3} d\mathfrak{r}_o \, h_{\mathrm{v}}(\mathfrak{r}_d - \mathfrak{r}_o) f(\mathfrak{r}_o), \qquad \mathrm{v} = \{\mathrm{A}, \mathrm{B}\}, \tag{1}$$

where $g_{\mathrm{v}}(\mathfrak{r}_d)$ is the three-dimensional data collected from the vth view, $f(\mathfrak{r}_o)$ is the three-dimensional density of fluorophores, and $h_{\mathrm{v}}(\mathfrak{r}_d - \mathfrak{r}_o)$ is the kernel (or point-spread function) for the vth view. A typical model for the kernels of the diSPIM is

$$h_{\mathrm{A}}(\mathfrak{r}_o) = \mathfrak{g}(r_x, \sigma_{\mathrm{tr}})\mathfrak{g}(r_y, \sigma_{\mathrm{tr}})\mathfrak{g}(r_z, \sigma_{\mathrm{ax}}), \tag{2}$$

$$h_{\mathrm{B}}(\mathfrak{r}_o) = \mathfrak{g}(r_x, \sigma_{\mathrm{ax}})\mathfrak{g}(r_y, \sigma_{\mathrm{tr}})\mathfrak{g}(r_z, \sigma_{\mathrm{tr}}), \tag{3}$$

where $\mathfrak{g}(x, \sigma) \equiv \exp(x^2/2\sigma^2)/\sqrt{2\pi\sigma^2}$, $\mathfrak{r}_o = r_x\hat{\mathbf{x}} + r_y\hat{\mathbf{y}} + r_z\hat{\mathbf{z}}$ is the three-dimensional position vector in the object, σ_{tr} is the spatial standard deviation of the transverse point spread function (set by the detection NA), and σ_{ax} is the spatial standard deviation of the axial point spread function (width/thickness of the light sheet).

The adjoint of forward operator is given by

$$f(\mathfrak{r}_o) = [\mathcal{H}^\dagger g](\mathfrak{r}_o) = \sum_{\mathrm{v}=\{\mathrm{A},\mathrm{B}\}} \int_{\mathbb{R}^3} d\mathfrak{r}_d \, h_{\mathrm{v}}(\mathfrak{r}_d - \mathfrak{r}_o) g_v(\mathfrak{r}_d). \tag{4}$$

3 Transfer function

We can rewrite the forward and adjoint operators in the frequency domain as

$$G_{\mathrm{v}}(\boldsymbol{\nu}) = H_{\mathrm{v}}(\boldsymbol{\nu})F(\boldsymbol{\nu}), \tag{5}$$

$$F(\boldsymbol{\nu}) = \sum_{\mathrm{v}=\{\mathrm{A},\mathrm{B}\}} H_{\mathrm{v}}(\boldsymbol{\nu})G_{\mathrm{v}}(\boldsymbol{\nu}), \tag{6}$$

where

$$G_{\mathrm{v}}(\boldsymbol{\nu}) = \int_{\mathbb{R}^3} d\mathfrak{r}_d \, g_{\mathrm{v}}(\mathfrak{r}_d) e^{i2\pi \mathfrak{r}_d \cdot \boldsymbol{\nu}}, \tag{7}$$

$$H_{\mathrm{v}}(\boldsymbol{\nu}) = \int_{\mathbb{R}^3} d\mathfrak{r}_o \, h_{\mathrm{v}}(\mathfrak{r}_o) e^{i2\pi \mathfrak{r}_o \cdot \boldsymbol{\nu}}, \tag{8}$$

$$F(\boldsymbol{\nu}) = \int_{\mathbb{R}^3} d\mathfrak{r}_o \, f(\mathfrak{r}_o) e^{i2\pi \mathfrak{r}_o \cdot \boldsymbol{\nu}}. \tag{9}$$

If we use the kernels in Eqs. 2 and 3 then the transfer functions are

$$H_{\mathrm{A}}(\boldsymbol{\nu}) = \mathfrak{g}(\nu_x, 1/\sigma_{\mathrm{tr}}) \mathfrak{g}(\nu_y, 1/\sigma_{\mathrm{tr}}) \mathfrak{g}(\nu_z, 1/\sigma_{\mathrm{ax}}), \tag{10}$$

$$H_{\mathrm{B}}(\boldsymbol{\nu}) = \mathfrak{g}(\nu_x, 1/\sigma_{\mathrm{ax}}) \mathfrak{g}(\nu_y, 1/\sigma_{\mathrm{tr}}) \mathfrak{g}(\nu_z, 1/\sigma_{\mathrm{tr}}), \tag{11}$$

where $\boldsymbol{\nu} = \nu_x \hat{\mathbf{x}} + \nu_y \hat{\mathbf{y}} + \nu_z \hat{\mathbf{z}}$ is a three-dimensional spatial frequency vector.

4 Singular value decomposition

To find the singular value decomposition of the imaging operator we need to solve the eigenvalue problem

$$[\mathcal{H}^\dagger \mathcal{H}] u_{\boldsymbol{\rho}}(\mathfrak{r}_o) = \mu_{\boldsymbol{\rho}} u_{\boldsymbol{\rho}}(\mathfrak{r}_o), \tag{12}$$

where $u_{\boldsymbol{\rho}}(\mathfrak{r}_o)$ are the object space singular functions of the system. This eigenvalue problem is easily solved in the frequency domain, so we write the object-space singular functions as

$$u_{\boldsymbol{\rho}}(\mathfrak{r}_o) = U(\boldsymbol{\rho}) e^{i2\pi \mathfrak{r}_o \cdot \boldsymbol{\rho}}. \tag{13}$$

Plugging Eqs. 5, 6, and 13 into Eq. 12 gives a new eigenvalue equation

$$\sum_{\mathrm{v}=\{\mathrm{A,B}\}} H_{\mathrm{v}}(\boldsymbol{\rho}) H_{\mathrm{v}}(\boldsymbol{\rho}) U(\boldsymbol{\rho}) = \mu_{\boldsymbol{\rho}} U(\boldsymbol{\rho}). \tag{14}$$

Therefore, the eigenvalues are given by

$$\mu_{\boldsymbol{\rho}} = \sum_{\mathrm{v}=\{\mathrm{A,B}\}} H_{\mathrm{v}}^2(\boldsymbol{\rho}), \tag{15}$$

and the eigenvectors are constants

$$U(\boldsymbol{\rho}) = 1. \tag{16}$$

The singular values are given by the square root of the eigenvalues

$$\sigma_{\boldsymbol{\rho}} = \sqrt{\sum_{\mathrm{v}=\{\mathrm{A,B}\}} H_{\mathrm{v}}^2(\boldsymbol{\rho})} = \sqrt{H_{\mathrm{A}}^2(\boldsymbol{\rho}) + H_{\mathrm{B}}^2(\boldsymbol{\rho})}, \tag{17}$$

and the object-space singular functions are the usual Fourier components of the object

$$u_{\boldsymbol{\rho}}(\mathfrak{r}_o) = e^{i2\pi \mathfrak{r}_o \cdot \boldsymbol{\rho}}. \tag{18}$$

Finally, the data space singular functions are given by

$$[v_{\boldsymbol{\rho}}]_{\mathrm{v}}(\mathfrak{r}_d) = \frac{1}{\sigma_{\boldsymbol{\rho}}} \mathcal{H}_{\mathrm{v}} u_{\boldsymbol{\rho}}(\mathfrak{r}_o) = \frac{H_{\mathrm{v}}(\boldsymbol{\rho})}{\sqrt{H_{\mathrm{A}}^2(\boldsymbol{\rho}) + H_{\mathrm{B}}^2(\boldsymbol{\rho})}} e^{i2\pi \mathfrak{r}_d \cdot \boldsymbol{\rho}}. \tag{19}$$

5 Operator decompositions

We can use the singular vectors and singular values to decompose the forward and adjoint operators of the microscope. Table 1.2 of Barrett is particularly helpful.

The forward operator decomposes into

$$\mathcal{H}_{\mathrm{v}} f(\mathfrak{r}_o) = \sigma_{\boldsymbol{\rho}} \mathbf{v}\mathbf{u}^{\dagger} f(\mathfrak{r}_o) = \mathcal{F}^{-1}\left\{H_{\mathrm{v}}(\boldsymbol{\rho})\mathcal{F}\left\{f(\mathfrak{r}_o)\right\}\right\}. \tag{20}$$

This result is essentially the Fourier-convolution theorem—it means that we can apply the forward operator by taking the Fourier transform of the object, multiplying the result by the Fourier transform of the kernel, then taking the inverse Fourier transform.

The adjoint operator decomposes into

$$\mathcal{H}^{\dagger} g_{\mathrm{v}}(\mathfrak{r}_d) = \sigma_{\boldsymbol{\rho}} \mathbf{u}\mathbf{v}^{\dagger} g_{\mathrm{v}}(\mathfrak{r}_d) = \mathcal{F}^{-1}\left\{\sum_{\mathrm{v}=\{\mathrm{A,B}\}} H_{\mathrm{v}}(\boldsymbol{\rho})\mathcal{F}\left\{g_{\mathrm{v}}(\mathfrak{r}_o)\right\}\right\}. \tag{21}$$

Once again this result is not too surprising, and I suspect that this is how the existing MLEM/RL reconstructions have implemented the adjoint operator.

A potentially useful result is to decompose the Moore-Penrose pseudoinverse operator

$$\mathcal{H}^{+} g_{\mathrm{v}}(\mathfrak{r}_d) = \frac{1}{\sigma_{\boldsymbol{\rho}}} \mathbf{u}\mathbf{v}^{\dagger} g_{\mathrm{v}}(\mathfrak{r}_d) = \mathcal{F}^{-1}\left\{\sum_{\mathrm{v}=\{\mathrm{A,B}\}} \frac{H_{\mathrm{v}}(\boldsymbol{\rho})}{H_{\mathrm{A}}^2(\boldsymbol{\rho}) + H_{\mathrm{B}}^2(\boldsymbol{\rho})}\mathcal{F}\left\{g_{\mathrm{v}}(\mathfrak{r}_o)\right\}\right\}. \tag{22}$$

This gives us a (new?) one-step reconstruction algorithm. In words:

1. Calculate or measure the kernel and transfer function of the microscope for both views and precalculate two filters: $H_{\mathrm{A}}(\boldsymbol{\rho})/[H_{\mathrm{A}}^2(\boldsymbol{\rho}) + H_{\mathrm{B}}^2(\boldsymbol{\rho})]$ and $H_{\mathrm{B}}(\boldsymbol{\rho})/[H_{\mathrm{A}}^2(\boldsymbol{\rho}) + H_{\mathrm{B}}^2(\boldsymbol{\rho})]$.
2. Take the 3D Fourier transform of the data from each view, multiply the result by the corresponding precalculated filter from step 1, then add the results.
3. Take the inverse Fourier transform.

This reconstruction is fast—it only requires three 3D Fourier transforms and a single product—so it might be useful for live reconstructions during imaging.

The reconstruction has two limitations compared to MLEM/RL. First, the Moore-Penrose pseudoinverse solution is a minimum-norm least-squares solution so it assumes Gaussian noise and does not account for Poisson noise at low counts. Second, the reconstruction is not constrained to positive values so noisy data may cause a (usually slightly) negative value in the reconstructed object. These limitations might not be a problem if an online reconstruction can help guide the experiment while its happening and a complete MLEM/RL reconstruction can be performed offline.

6 Discussion

The singular values of the dual view microscope confirm a result that matches our intuition—the singular spectrum for a dual view microscope is the root sum of squares of the transfer functions for each view. If we can describe the imaging system as linear-shift invariant, I expect that this result will hold for other multiview designs—the three-view diSPIM, possibly the mirror diSPIM(?), and possibly unpolarized light-field designs.

An analogous calculation for the light-field microscope might yield a reconstruction algorithm that is much faster than Broxton's spatial-domain reconstruction. TBD.

We've considered the diSPIM as a CC-CD imaging system and shown that the CD part of the microscope is described by a rank-1 operator. Compare this with polarized light microscopes where the angular part of the microscope is described by a rank-3 (single-view) or rank-6 (dual-view) operator.

Finally, notice that the Moore-Penrose pseudoinverse operator in Eq. 22 reduces to a simple inverse filter if we only use the data from one view.