Singular value decomposition of single view structured illumination microscopes

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1 Introduction

In these notes we will find the kernel, transfer function, and singular value decomposition of epi-illumination and epi-detection single view structured illumination microscopes. We will model the relationship between the spatial density of fluorophores in a two-dimensional sample—a member of $\mathbb{L}_2(\mathbb{R}^2)$—and the complete data space—a member of $\mathbb{L}_2(\mathbb{R}^2 \times \mathbb{R}^2 \times \mathbb{S}^1)$. We will show that structured illumination microscopes sample this five-dimensional space—pixels sample a pair of spatial dimensions $\mathbb{R}^2$, illumination pattern orientations and spatial frequencies sample another pair of dimensions $\mathbb{R}^2$, and the phase of the illumination pattern samples a circular dimension $\mathbb{S}^1$.

2 Kernel

Structured illumination microscopes interfere two coherent illumination beams to create a sinusoid squared excitation pattern in the sample. The excitation kernel takes the form

$$h_{\text{exc}}(\mathbf{r}_o, \mathbf{k}, \phi) = \cos^2(\mathbf{k} \cdot \mathbf{r}_o - \phi) = \frac{1}{4}\mathrm{e}^{i2(\mathbf{k}\cdot\mathbf{r}_o - \phi)} + \frac{1}{2} + \frac{1}{4}\mathrm{e}^{-i2(\mathbf{k}\cdot\mathbf{r}_o - \phi)}, \tag{1}$$

where $\mathbf{r}_o$ is the two-dimensional position in the sample, $\mathbf{k}$ is the wave vector of the illumination pattern, and ϕ is the phase of the illumination pattern. The detection process of the microscope is shift invariant, so we can model the detection process with a detection kernel $h_{\text{det}}(\mathbf{r}_o)$. In these notes we will use the paraxial detection model

$$h_{\text{det}}(\mathbf{r}_o) = \left[\frac{J_1(2\pi\nu_o|\mathbf{r}_o|)}{\pi\nu_o|\mathbf{r}_o|}\right]^2, \tag{2}$$

where $J_1(\cdot)$ is a first-order Bessel function of the first kind, and $\nu_o = \text{NA}/\lambda$ is the coherent cutoff frequency. We can easily plug in a more sophisticated detection model if we need to.

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

$$g(\mathbf{r}_d, \mathbf{k}, \phi) = [\mathcal{H}f](\mathbf{r}_d, \mathbf{k}, \phi) = \int_{\mathbb{R}^2} d\mathbf{r}_o \, h_{\text{exc}}(\mathbf{r}_o, \mathbf{k}, \phi) h_{\text{det}}(\mathbf{r}_d - \mathbf{r}_o) f(\mathbf{r}_o), \tag{3}$$

where $g(\mathbf{r}_d, \mathbf{k}, \phi)$ is the five-dimensional data set and $f(\mathbf{r}_o)$ is the two-dimensional density of fluorophores.

The adjoint of the forward operator is given by

$$f(\mathbf{r}_o) = [\mathcal{H}^\dagger g](\mathbf{r}_o) = \int_{\mathbb{S}^1} d\phi \int_{\mathbb{R}^2} d\mathbf{k} \int_{\mathbb{R}^2} d\mathbf{r}_d \, h_{\text{exc}}(\mathbf{r}_o, \mathbf{k}, \phi) h_{\text{det}}(\mathbf{r}_d - \mathbf{r}_o) g_v(\mathbf{r}_d, \mathbf{k}, \phi). \tag{4}$$

3 Transfer function

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

$$G_n(\boldsymbol{\nu}, \mathbf{k}) = H_n(\boldsymbol{\nu}, \mathbf{k}) F(\boldsymbol{\nu}), \tag{5}$$

$$F(\boldsymbol{\nu}) = \int_{\mathbb{R}^2} d\mathbf{k} \sum_{n=0}^{\infty} H_n(\boldsymbol{\nu}, \mathbf{k}) G_n(\boldsymbol{\nu}, \mathbf{k}), \tag{6}$$

where

$$G_n(\boldsymbol{\nu}, \mathbf{k}) = \int_{\mathbb{S}^1} d\phi \, \mathrm{e}^{i2\pi\phi n} \int_{\mathbb{R}^2} d\mathbf{r}_d \, \mathrm{e}^{i2\pi \mathbf{r}_d \cdot \boldsymbol{\nu}} g(\mathbf{r}_d, \mathbf{k}, \phi), \tag{7}$$

$$H_n(\boldsymbol{\nu}, \mathbf{k}) = \int_{\mathbb{S}^1} d\phi \, \mathrm{e}^{i2\pi\phi n} \int_{\mathbb{R}^2} d\mathbf{r}_d \, \mathrm{e}^{i2\pi \mathbf{r}_o \cdot \boldsymbol{\nu}} h_{\mathrm{exc}}(\mathbf{r}_o, \mathbf{k}, \phi) h_{\mathrm{det}}(\mathbf{r}_o), \tag{8}$$

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

Notice that we have taken the Fourier transform of object space variable $\mathbf{r}_o$ and taken the Fourier series of the data space variable ϕ, but we have left the data space variable $\mathbf{k}$ alone because it is "natively" in the frequency domain.

If we plug the kernels in Eqs. 1 and 2 into Eq. 8 and evaluate the integrals we find the transfer function is

$$H_n(\boldsymbol{\nu}, \mathbf{k}) = \frac{1}{4} H_{\mathrm{det}}(\boldsymbol{\nu} - 2\mathbf{k}) \delta_{n,2} + \frac{1}{2} H_{\mathrm{det}}(\boldsymbol{\nu}) \delta_{n,0} + \frac{1}{4} H_{\mathrm{det}}(\boldsymbol{\nu} + 2\mathbf{k}) \delta_{n,-2}, \tag{10}$$

where $\delta_{n,m}$ is a Kronecker delta,

$$H_{\mathrm{det}}(\boldsymbol{\nu}) = \frac{2}{\pi} \left[\arccos\left(\frac{|\boldsymbol{\nu}|}{2\nu_o} \right) - \frac{|\boldsymbol{\nu}|}{2\nu_o} \sqrt{1 - \left(\frac{|\boldsymbol{\nu}|}{2\nu_o} \right)^2} \right] \Pi\left(\frac{|\boldsymbol{\nu}|}{2\nu_o} \right), \tag{11}$$

is the detection transfer function for a fluorescence microscope without structured illumination, and $\Pi(\cdot)$ is a rect function.

4 Singular value decomposition

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

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

where $u_{\boldsymbol{\rho}}(\mathbf{r}_o)$ 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

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

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

$$\int_{\mathbb{R}^2} d\mathbf{k} \sum_{n=0}^{\infty} H_n(\boldsymbol{\rho}, \mathbf{k}) H_n(\boldsymbol{\rho}, \mathbf{k}) U(\boldsymbol{\rho}) = \mu_{\boldsymbol{\rho}} U(\boldsymbol{\rho}). \tag{14}$$

Therefore, the eigenvalues are given by

$$\mu_{\boldsymbol{\rho}} = \int_{\mathbb{R}^2} d\mathbf{k} \sum_{n=0}^{\infty} H_n^2(\boldsymbol{\rho}, \mathbf{k}), \tag{15}$$

and the eigenvectors are constants

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

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

$$\sigma_{\boldsymbol{\rho}} = \sqrt{\int_{\mathbb{R}^2} d\mathbf{k} \sum_{n=0}^{\infty} H_n^2(\boldsymbol{\rho}, \mathbf{k})}, \tag{17}$$

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

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

Finally, the data space singular functions are given by

$$v_{\boldsymbol{\rho}}(\mathbf{r}_d, \mathbf{k}, \phi) = \frac{1}{\sigma_{\boldsymbol{\rho}}} \mathcal{H}_{\mathrm{v}} u_{\boldsymbol{\rho}}(\mathbf{r}_o) = \frac{\sum_{n=-\infty}^{\infty} e^{i2\pi n\phi} H_n(\boldsymbol{\rho}, \mathbf{k})}{\sqrt{\int_{\mathbb{R}^2} d\mathbf{k} \sum_{n=0}^{\infty} H_n^2(\boldsymbol{\rho}, \mathbf{k})}} e^{i2\pi \mathbf{r}_d \cdot \boldsymbol{\rho}}. \tag{19}$$

Looking closer at the singular value spectrum lets us see the limits of structured illumination microscopy. If we can illuminate the sample with all possible $\mathbf{k}$ vectors, then the singular spectrum would be a constant for all $\boldsymbol{\rho}$. This is not practically possible because real microscopes are limited by the illumination NA of the microscope. In this case, the integral is over the illumination NA of the microscope, and we can clearly see that the singular value spectrum will have twice the bandwidth of a widefield microscope.

Note that we can find a closed form for the singular value spectrum, but I haven't done this. I think that the main insight from these notes is that the singular value spectrum is the square root of the sum of the squared transfer functions for each individual illumination pattern. This result applies to a (more realistic) discrete sampling of $\mathbf{k}$ and ϕ space as well.

Also note that the Fourier series expansion of the phase dimension plays a role in the transfer function and singular value decomposition. I suspect that the Fourier series expansion should play a role in an efficient reconstruction as well (does it already?).