Optimizing angular diSPIM measurements

February 5, 2019

1 Introduction

Our goal in these notes is to find optimal angular sampling schemes for the diSPIM with light-sheet tilting. We review the continuous angular forward model, find the sampling schemes available to us, choose an objective function to optimize, then search through the sampling schemes.

2 Continuous forward model

We briefly review the continuous angular forward model—see the previous notes for a more detailed discussion. We can write the continuous-to-continuous forward model in the form

$$\mathbf{g}_c = \mathcal{H}_{cc}\mathbf{f}_c, \tag{1}$$

where $\mathbf{f}_c \in \mathbb{L}_2(\mathbb{S}^2)$ is the angular dipole density, $\mathbf{g}_c \in \mathbb{L}_2(\mathbb{S}^2 \times \mathbb{S}^1)$ is the continuous irradiance data taken by varying the detection optical axis (the $\mathbb{S}^2$ dimension) and the illumination polarizer (the $\mathbb{S}^1$ dimension), and $\mathcal{H}_{cc}$ is a linear continuous-to-continuous Hilbert-space operator between these spaces.

If we choose a delta-function basis for both object and data space then we can rewrite Eq. (1) as

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

where $\hat{\mathbf{s}}_o \in \mathbb{S}^2$ is the object angular coordinate, $\hat{\mathbf{s}}_d \in \mathbb{S}^2$ is the detection optical axis coordinate, $\hat{\mathbf{p}} \in \mathbb{S}^1$ is the polarizer coordinate, and $h(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}; \hat{\mathbf{s}}_o)$ is the kernel of the integral transform. We can separate the kernel into an excitation and detection part since these processes are incoherent

$$h(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}; \hat{\mathbf{s}}_o) = h^{\text{exc}}(\hat{\mathbf{p}}; \hat{\mathbf{s}}_o) h^{\text{det}}(\hat{\mathbf{s}}_d; \hat{\mathbf{s}}_o). \tag{3}$$

The excitation kernel is straightforward—the squared dot product of the polarizer orientation $\hat{\mathbf{p}}$ with the object angular coordinate $\hat{\mathbf{s}}_o$

$$h^{\text{exc}}(\hat{\mathbf{p}} \cdot \hat{\mathbf{s}}_o) \propto (\hat{\mathbf{p}} \cdot \hat{\mathbf{s}}_o)^2. \tag{4}$$

The detection kernel requires more care since we are collecting light over the NA of the objective. In the previous notes we started with the power along a single ray then integrated over the aperture to find that the detection kernel is

$$h^{\text{det}}(\hat{\mathbf{s}}_d \cdot \hat{\mathbf{s}}_o) \propto (1 - \sqrt{\cos\alpha}) P_0(\hat{\mathbf{s}}_d \cdot \hat{\mathbf{s}}_o) + \frac{1}{5}\left[\frac{1}{4}\sqrt{\cos\alpha}(7 - 3\cos(2\alpha)) - 1\right] P_2(\hat{\mathbf{s}}_d \cdot \hat{\mathbf{s}}_o), \tag{5}$$

where $\text{NA} = n_o \sin\alpha$, and $P_\ell(x)$ are the Legendre polynomials.

We can rewrite the mapping in Eq. (1) in an object-space spherical harmonic basis as

$$g(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}) = \sum_{\ell m} H_\ell^m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}) F_\ell^m, \tag{6}$$

where F_ℓ^m is the angular dipole spectrum given by

$$F_\ell^m = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o\, f(\hat{\mathbf{s}}_o) Y_\ell^{m*}(\hat{\mathbf{s}}_o), \tag{7}$$

and $H_\ell^m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}})$ is the kernel in this basis given by

$$H_\ell^m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}) = \sum_{\ell' m'} \sum_{\ell'' m''} (-1)^m G_{\ell,\ell',\ell''}^{-m,m',m''} H_{\ell'}^{m',\text{exc}}(\hat{\mathbf{p}}) H_{\ell''}^{m'',\text{det}}(\hat{\mathbf{s}}_d), \tag{8}$$

$$H_{\ell'}^{m',\text{exc}}(\hat{\mathbf{p}}) \propto Y_{\ell'}^{m'*}(\hat{\mathbf{p}}) \left[\delta_{0\ell'} + \frac{2}{5}\delta_{2,\ell'}\right], \tag{9}$$

$$H_{\ell''}^{m'',\text{det}}(\hat{\mathbf{s}}_d) \propto Y_{\ell''}^{m''*}(\hat{\mathbf{s}}_d) \left[(1 - \sqrt{\cos\alpha})\delta_{0,\ell''} + \left(\frac{1}{4}\sqrt{\cos\alpha}(7 - 3\cos(2\alpha)) - 1\right)\delta_{2,\ell''}\right]. \tag{10}$$

with the Gaunt coefficients given by

$$G_{\ell,\ell',\ell''}^{m,m',m''} = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \, Y_\ell^m(\hat{\mathbf{s}}_o) Y_{\ell'}^{m'}(\hat{\mathbf{s}}_o) Y_{\ell''}^{m''}(\hat{\mathbf{s}}_o). \tag{11}$$

It's useful to compare Eq. (2) to Eq. (6)—in a delta function basis we need to compute an angular integral, while in the object-space spherical harmonics basis the kernel has a finite number of terms so we can compute it exactly.

It's also useful to compare Eq. (3) to Eq. (8)—in a delta function basis the kernel is the product of the excitation and detection kernels, while in the object-space spherical harmonic basis the kernel is a generalized convolution of the excitation and detection kernels.

3 Sampling schemes

We can write the sampling operation as a continuous-to-discrete Hilbert space operator $\mathcal{D}_w$

$$\mathbf{g}_d = \mathcal{D}_w \mathbf{g}_c = \mathcal{D}_w \mathcal{H}_{cc} \mathbf{f}_c. \tag{12}$$

We can write the sampling operation in a delta function basis as

$$g_m = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_d \int_{\mathbb{S}^1} d\hat{\mathbf{p}} \, w_m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}) g(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}). \tag{13}$$

where g_m is the mth discrete measurement and $w_m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}})$ is the mth sampling aperture. For now we will restrict ourselves to delta-function sampling apertures

$$w_m(\hat{\mathbf{s}}_d, \hat{\mathbf{p}}) = \delta(\hat{\mathbf{s}}_{d,m} - \hat{\mathbf{s}}_d)\delta(\hat{\mathbf{p}}_m - \hat{\mathbf{p}}), \tag{14}$$

where $\hat{\mathbf{s}}_{d,m}$ and $\hat{\mathbf{p}}_m$ are the mth sampling points. This restriction means that we will leave the polarizer setting and tilt angle fixed during each volume acquisition. We could consider scanning the polarizer setting and tilt angle in the future for faster acquisitions, but I think these sampling schemes will always decrease "angular SNR".

So far we've formulated everything quite generally and haven't made any reference to the diSPIM geometry. Now we find the constraints that the diSPIM geometry places on the values of $\hat{\mathbf{s}}_{d,m}$ and $\hat{\mathbf{p}}_m$. If we define the $\hat{\mathbf{x}}$ and $\hat{\mathbf{z}}$ axes as the optical axes of the two objective then we have the following sampling constraint

$$\hat{\mathbf{s}}_{d,m} \in \{\hat{\mathbf{x}}, \hat{\mathbf{z}}\}. \tag{15}$$

In words, this constraint says that we can only detect along one of two discrete and orthogonal axes.

Next, we constrain the polarizer sampling points $\hat{\mathbf{p}}_m$. We introduce a variable $\hat{\mathbf{s}}_{e,m}$ to denote the excitation optical axis for the mth measurement. This variable does not affect the forward model (we've formulated in terms of $\hat{\mathbf{p}}$ only on the excitation side), but it will allow us to conveniently constrain $\hat{\mathbf{p}}$. First, we constrain the excitation optical axis to be perpendicular to the detection optical axis

$$\hat{\mathbf{s}}_{e,m} \cdot \hat{\mathbf{s}}_{d,m} = 0. \tag{16}$$

This is a fairly loose constraint, but it ensures that the light sheet is in focus across the field of view.

Next, we constrain the excitation optical axis to within the available tilt angles of the instrument. If we use $\mathcal{R}$ to denote an "axis swapping operator" ($\mathcal{R}(\hat{\mathbf{z}}) = \hat{\mathbf{x}}$ and $\mathcal{R}(\hat{\mathbf{x}}) = \hat{\mathbf{z}}$), then the tilting constraint can be written as

$$\hat{\mathbf{s}}_{e,m} \cdot \mathcal{R}(\hat{\mathbf{s}}_{d,m}) \leq \Delta, \tag{17}$$

where Δ is the maximum tilt angle. For the current instrument $\Delta = 15^\circ = \pi/12$.

Finally, we constrain the excitation polarization $\hat{\mathbf{p}}_m$ to be perpendicular to the excitation optical axis

$$\hat{\mathbf{s}}_{e,m} \cdot \hat{\mathbf{p}}_m = 0. \tag{18}$$

For convenience we will denote individual sampling points with $\mathfrak{s}_m = (\hat{\mathbf{p}}_m, \hat{\mathbf{s}}_{d,m})$, and we will denote the set of all sampling points $\mathfrak{s}_m$ that satisfy Eqs. (15)–(18) by $\mathcal{S}$. With this notation we can denote a complete sampling scheme with M samples by $\mathfrak{s} \in \mathcal{S}^M$ (M copies of the set $\mathcal{S}^M$).

4 Objective functions

Our goal is to choose an M-sample sampling scheme $\mathfrak{s} \in \mathcal{S}^M$ that is optimal in some sense. We propose that we optimize the Schatten 2-norm of the continuous-discrete imaging operator

$$\mathfrak{s}^* = \underset{\mathfrak{s} \in \mathcal{S}^M}{\operatorname{argmax}} ||\mathcal{D}_{\mathfrak{s}} \mathcal{H}_{cc}||_2. \tag{19}$$

The Schatten p-norm is defined as the $\mathbb{L}_p$ norm of the singular values of the operator, so the Schatten 2-norm is a reasonable measure of the amount of information that the imaging system can pass. It favors sampling schemes that have many large singular values, so it will prefer sampling schemes that measure many orthogonal parameters of the object that are insensitive to noise.

It's useful to interpret the Schatten 2-norm by analyzing a spatial imaging system. The "effective OTF" that appears in many fluorescence microscopy papers (including Hari and Yicong's 2018 SIM review) is a specific example of a continuous singular value spectrum. We can calculate the Schatten 2-norm by taking the effective OTF, squaring its value at each spatial frequency, integrating over the spatial frequencies, then taking the square root. The Schatten 2-norm will be largest for imaging systems that have many singular values (a high spatial frequency bandwidth) that are large (we prefer effective OTFs that are large and constant over the bandwidth).

Eq. (19) will optimize the number and size of the singular values without considering how interpretable the corresponding parameters are. One of our motivations for adding light-sheet tilting was to measure rotationally invariant parameters of the object which will be much more interpretable than rotationally variant parameters. To achieve this objective we need to measure at least the $\ell = 0$ and $\ell = 2$ rotationally invariant subspaces—a 6-dimensional subspace of $\mathbb{L}_2(\mathbb{S}^2)$. This will require at least 6 measurements. To ensure that a sampling scheme has measured this rotationally invariant subspace, we require that

$$\operatorname{rank}(\mathcal{D}_{\mathfrak{s}} \mathcal{H}_{cc} \mathcal{P}_{\ell_c=2}) \geq 6 \tag{20}$$

where $\mathcal{P}_{\ell_c=2}$ is a projection operator onto the $\ell = 0$ and $\ell = 2$ subspace. For convenience we will denote the set of sampling schemes that satisfy the constraint in Eq. (20) by $\mathcal{Z} = \{\mathfrak{s} \,|\, \operatorname{rank}(\mathcal{D}_{\mathfrak{s}} \mathcal{H}_{cc} \mathcal{P}_{\ell_c=2}) \geq 6\}$.

The solutions of Eq. (19) will not necessarily satisfy Eq. (20), and simply adding an extra constraint to Eq. (20) will not ensure that we are optimally measuring the parameters in the subspace that we want to measure. Instead, we propose an alternative optimization problem

$$\mathfrak{s}^*_{\ell_c=2} = \underset{\mathfrak{s}\in\mathcal{S}^M\cap\mathcal{Z}}{\text{argmax}}\,||\mathcal{D}_{\mathfrak{s}}\mathcal{H}_{cc}\mathcal{P}_{\ell_c=2}||_2, \tag{21}$$

which ensures that the objective function is independent of singular values that measure parameters outside of the objective subspace. Note that the set $\mathcal{S}^M\cap\mathcal{Z}$ will definitely be empty for $M \leq 6$, and we are not sure what value of M will yield solutions.

5 Practical implementation details

The two optimization problems above are sufficient to propose optimal sampling schemes, but they lack many of the details required for an implementation. In this section I'll go through some of the practical work of converting the abstract forms into finite-dimensional optimization problems.

First, we need to choose a basis and find matrix representations for the operators $\mathcal{D}_{\mathfrak{s}}$ and $\mathcal{H}_{cc}$. At first glance this seems difficult—$\mathcal{D}_{\mathfrak{s}}$ is a CD operator and $\mathcal{H}_{cc}$ is a CC operator so they do not have finite-dimensional matrix representations individually. Luckily, the combination $\mathcal{D}_{\mathfrak{s}}\mathcal{H}_{cc}$ is a band-limited compact operator, so it has a finite-dimensional representation as a $15 \times M$ matrix that we will call H. The entries of this matrix are given by

$$\mathsf{H}_{jm} = H_j(\hat{\mathbf{s}}_{d,m}, \hat{\mathbf{p}}_m), \tag{22}$$

where j is a single index over the spherical harmonics, and Eq. 8 shows us how to calculate the entries in terms of the excitation and detection kernels.

Next, we need a matrix representation of $\mathcal{P}_{\ell_c=2}$. This is simple in a spherical harmonics basis—we create a 15×15 matrix called P, fill the first six diagonal elements with ones, and place zeroes elsewhere

$$\mathsf{P}_{ij} = \delta_{ij}, \qquad i,j = 0,1,2,3,4,5. \tag{23}$$

Next, we need a way to calculate the Schatten 2-norm efficiently. We could calculate the singular values of each 15×15 matrix, but this would be slow. We can take a shortcut and replace the Schatten 2-norm with the Frobenius norm—the $\mathbb{L}_2$ norm of the matrix entries. Note that this replacement is only valid for finite-dimensional representations of the operators.

Finally, we need an efficient set of scalar parameters to search through the set of sampling schemes $\mathcal{S}^M$. We will parameterize each sample with a discrete view index $v \in \{0, 1\}$ to indicate the detection axis, a tilt angle defined from the nominal excitation axis $\delta \in [-\Delta, \Delta]$, and a polarizer angle defined from the detection axis $\phi \in [0, 2\pi)$.

This parameter space is challenging to search because it is continuous in some parameters and discrete in others. It's not clear if it's possible to take derivatives with respect to the continuous parameters, so as a first pass we will discretize the continuous parameters and use a search heuristic. Discretization has the advantage of providing solutions that will be easier to implement experimentally since we can constrain our solutions to sampling schemes that are easy to align, but of course we can't guarantee that our sampling scheme solutions will be globally optimal.

We will discretize the tilt angle into 5 angles $\delta \in \{-\Delta, -\Delta/2, 0, \Delta/2, \Delta\}$, and we will discretize the polarizer angle into 8 angles $\phi \in \{0, \pi/8, \ldots, 7\pi/8\}$. This means that a brute-force approach will need to search $(2\,\text{views} \times 5\,\text{tilts} \times 8\,\text{polarizer settings})^M$ possible measurement schemes which becomes computationally infeasible for M larger than about 5 or 6.

To avoid a brute-force search we will use a simple hill-climbing algorithm. In words, we (1) start with an initial guess for each of the $3M$ parameters and evaluate the objective function, (2) increment and decrement the first parameter and move in the direction that improves the objective function, (3) repeat (2) for each parameter one by one, (4) repeat (3) and keep cycling parameters until no movement improves the objective function or a fixed number of objective function evaluations is reached. Although the algorithm is extremely coarse and provides no guarantees, it is simple to implement and it can be stopped at any time and will always return its best guess.

6 Results