

Harmonic Analysis of Fluorophore Orientation Measurement Systems

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Abstract

TODO

1 Introduction

TODO

2 Theory

We use $\hat{\mathbf{r}}$ and (θ, ϕ) interchangeably to represent points on the unit sphere \mathbb{S}^2 using the parameterization

$$\hat{\mathbf{r}} = (\sin \theta \cos \phi, \sin \theta \sin \phi, \cos \theta). \quad (1)$$

Whenever possible we use the notation and vocabulary of Barrett and Myers [1].

2.1 Laplace series

Consider a square-integrable function $f : \mathbb{S}^2 \rightarrow \mathbb{R}$ that maps points on the unit sphere to the real numbers. We can express f as a weighted sum of spherical harmonics called the Laplace series [2, 3]

$$f(\theta, \phi) = \sum_{l=0}^{\infty} \sum_{m=-l}^l F_l^m y_l^m(\theta, \phi) \quad (2)$$

where

$$y_l^m(\theta, \phi) = \begin{cases} \sqrt{2} K_l^m \cos(m\phi) P_l^m(\cos \theta), & m > 0 \\ K_l^0 P_l^0(\cos \theta), & m = 0 \\ \sqrt{2} K_l^m \sin(-m\phi) P_l^{-m}(\cos \theta), & m < 0 \end{cases} \quad (3)$$

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

and $P_l^m(x)$ are the associated Legendre polynomials. We have chosen to use the real spherical harmonics because we are representing real functions only. For convenience we will rewrite equation 2 using a single index $i = l(l+1) + m$ which gives

$$f(\theta, \phi) = \sum_{i=0}^{\infty} F_i y_i(\theta, \phi). \quad (5)$$

We will also rewrite the functions f and y_i as vectors using a single index to represent points on the sphere which gives

$$\mathbf{f} = \sum_{i=0}^{\infty} F_i \mathbf{y}_i. \quad (6)$$

where $\mathbf{f} \in \mathbb{R}^{\infty \times 1}$ and $\mathbf{y}_i \in \mathbb{R}^{\infty \times 1}$

Finally, we express equation 6 in its final form using the notation

$$\mathbf{f} = \mathbf{Y}^T \mathbf{F} \quad (7)$$

where T denotes a transpose, $\mathbf{F} \in \mathbb{R}^{\infty \times 1}$ is an ordered vector of the Laplace series coefficients, and $\mathbf{Y} \in \mathbb{R}^{\infty \times \infty}$ is a matrix with each column consisting of a spherical harmonic \mathbf{y}_i . Note that equation 7 is just a reindexed version of equation 2.

A key property of the spherical harmonics is that they form an orthonormal basis on the sphere—that is

$$\mathbf{Y}^T \mathbf{Y} = \mathbf{Y} \mathbf{Y}^T = \mathbf{I} \quad (8)$$

where \mathbf{I} is the identity matrix. We can exploit this orthogonality to compute the Laplace coefficients \mathbf{F} for a given function \mathbf{f} . If we left-multiply both sides of equation 7 by \mathbf{Y} and use equation 8 to simplify we get

$$\mathbf{F} = \mathbf{Y} \mathbf{f}. \quad (9)$$

We call \mathbf{Y} the *Laplace expansion operator* because it acts on a function \mathbf{f} and generates its Laplace coefficients \mathbf{F} . Note that the Laplace series of a function on \mathbb{S}^2 is analogous to the Fourier series of a function on \mathbb{S}^1 . When we expand a function into a Laplace or Fourier series, we are expressing the function in a convenient orthonormal basis.

2.2 Harmonic analysis of orientation measurements

Consider a single fluorophore with a fixed dipole axis oriented along a direction $\hat{\mathbf{r}}$. To measure the orientation of the dipole axis we set up an illumination system to excite the fluorophore—a light source and a combination of lenses and polarizers—and a detection system to detect the light that the fluorophore emits as it relaxes—a detector and a combination of lenses and polarizers. We are deliberately considering very general orientation measurement systems at this stage.

We can model the complete measurement system using a function $h(\hat{\mathbf{r}}) : \mathbb{S}^2 \rightarrow \mathbb{R}_+$ that maps the dipole orientation to measured intensity values. We call $h(\hat{\mathbf{r}})$ the *point response function* of the measurement system. To find the point response function we can model the measurement system mathematically, or we can build the measurement system and take measurements as we manipulate the orientation of a single fluorophore.

Single fluorophores with fixed dipole axes only appear in a very small set of samples—samples with few fluorophores fixed to immobile structures or samples with photoactivatable fluorophores. Most samples of interest contain many rotating fluorophores, and we would like to measure the quantity and orientation distribution of these fluorophores. Let $f(\hat{\mathbf{r}}) : \mathbb{S}^2 \rightarrow \mathbb{R}_+$ be a function that maps a direction (a point on the sphere) to half the number of fluorophores pointing along that direction. We call f the *orientation distribution function*. For a single fluorophore oriented along $\hat{\mathbf{r}}_0$ the orientation distribution function is

$$f_{\text{single}}(\hat{\mathbf{r}}) = \frac{1}{2} [\delta(\hat{\mathbf{r}} - \hat{\mathbf{r}}_0) + \delta(\hat{\mathbf{r}} + \hat{\mathbf{r}}_0)] \quad (10)$$

because dipoles are antipodally symmetric. When we add fluorophores to the measurement volume we add the individual orientation distribution functions

$$f_N(\hat{\mathbf{r}}) = \sum_{i=0}^N \frac{1}{2} [\delta(\hat{\mathbf{r}} - \hat{\mathbf{r}}_i) + \delta(\hat{\mathbf{r}} + \hat{\mathbf{r}}_i)] \quad (11)$$

As the number of fluorophores increases the orientation distribution function approaches a continuous function.

The total intensity measured by the system is given by multiplying the point response function with the orientation distribution function and integrating over the sphere

$$g = \int_{\mathbb{S}^2} d\hat{\mathbf{r}} h(\hat{\mathbf{r}}) f(\hat{\mathbf{r}}). \quad (12)$$

Equation 12 assumes that the measurement system is *linear*—if $\int_{\mathbb{S}^2} d\hat{\mathbf{r}} h(\hat{\mathbf{r}}) f_1(\hat{\mathbf{r}}) = g_1$ and $\int_{\mathbb{S}^2} d\hat{\mathbf{r}} h(\hat{\mathbf{r}}) f_2(\hat{\mathbf{r}}) = g_2$ then $\int_{\mathbb{S}^2} d\hat{\mathbf{r}} h(\hat{\mathbf{r}}) [af_1(\hat{\mathbf{r}}) + bf_2(\hat{\mathbf{r}})] = ag_1 + bg_2$. This requirement is satisfied in most experimental cases as long as the fluorophores and detectors are not saturated. Note that this requirement does not mean that h and f have to be linear functions.

We simplify our notation by reindexing h and f and rewriting equation 12 as

$$g = \mathbf{h}^T \mathbf{f}. \quad (13)$$

If we expand \mathbf{h} and \mathbf{f} into their respective Laplace series using equation 7 then

$$g = (\mathbf{Y}^T \mathbf{H})^T (\mathbf{Y}^T \mathbf{F}) \quad (14)$$

where $\mathbf{H} \in \mathbb{R}^{\infty \times 1}$ and $\mathbf{F} \in \mathbb{R}^{\infty \times 1}$ are vectors made of the Laplace coefficients of \mathbf{h} and \mathbf{f} , respectively. Exploiting the orthogonality of the spherical harmonics (equation 8) gives

$$g = \mathbf{H}^T \mathbf{F} \quad (15)$$

Equation 15 gives us a fundamental insight into fluorophore orientation measurement systems—the measured intensity is a sum of the Laplace coefficients of the orientation distribution function weighted by the Laplace coefficients of the point response function.

Most orientation measurement systems make multiple intensity measurements with different point response functions by using multiple detectors or by changing the geometry of the measurement system. We can represent an orientation measurement system that takes N measurements using the compact notation

$$\mathbf{g} = \Psi \mathbf{F} \quad (16)$$

where $\mathbf{g} \in \mathbb{R}_+^N$ is a vector of the intensity measurements, and $\Psi \in \mathbb{R}^{N \times \infty}$ is the *system matrix* where the n th row of the system matrix consists of the Laplace coefficients of the n th point response function. We can also rewrite equation 16 as

$$\mathbf{g} = \mathcal{H} \mathbf{f} \quad (17)$$

where $\mathcal{H} \equiv \Psi \mathbf{Y}^T \in \mathbb{R}^{N \times \infty}$ is the continuous-to-discrete forward operator of the orientation measurement system.

In general, equation 16 is a product of an $N \times \infty$ system matrix with a $\infty \times 1$ vector. Luckily, all orientation measurements systems are *band-limited*—the system matrix only consists of M non-zero columns which simplifies equation 16 to a product of an $N \times M$ system matrix with an $M \times 1$ vector.

In practice the intensity measurements made in equation 16 are corrupted by Poisson noise and background counts, so the complete forward model is given by

$$\mathbf{g} \sim \text{Pois}(\mathcal{H} \mathbf{f} + \mathbf{b}) \quad (18)$$

where $\mathbf{b} \in \mathbb{R}_+^N$ is a vector of background intensity measurements.

2.3 Analysis and design

We can use the formalism developed in the previous section to characterize and optimize an orientation imaging system. The first task for the designer is to find the point response function for each intensity measurement $\{h_1, h_2, \dots, h_M\}$ via modeling or direct measurement. The second task is to find the Laplace series coefficients for the point response functions and use them to assemble the system matrix Ψ . The third task is to calculate the *crosstalk matrix* defined as

$$\mathbf{B} \equiv \Psi^T \Psi. \quad (19)$$

The crosstalk matrix is an infinite positive semidefinite matrix that characterizes the aliasing properties of the measurement system. The off-diagonal entries $\beta_{i,j}$ measure the degree of aliasing between the i th and j th Laplace components—a zero

indicates that the components are orthogonal, and a one indicates that the components are linearly dependent. The diagonal entries $\beta_{i,i}$ indicate how efficiently the i th Laplace component is transferred by the measurement system—a zero indicates that the component cannot be recovered from the data, and a one indicates that the data is efficiently transmitted. The ideal crosstalk matrix is the identity matrix—such a system could transmit all Laplace components without aliasing and could be used to reconstruct all possible fluorophore distributions.

The final task of the designer is to optimize the system based on the information in the crosstalk matrix. The designer may want to add extra measurements so that extra Laplace components are transmitted by the system. The designer may also have access to other parameters—polarizer orientations, numerical aperture, illumination and detection geometry—that may be optimized to extend and diagonalize the crosstalk matrix.

2.4 Reconstruction methods

Our goal is to estimate the orientation distribution function from a set of intensity measurements \mathbf{g} . If nothing is known about the orientation distribution function and the noise on the measurements is small, then we can use the minimum-norm least-squares (MNLS) estimate

$$\hat{\mathbf{F}}_{\text{MNLS}} = \Psi^+(\mathbf{g} - \mathbf{b}) \quad (20)$$

where Ψ^+ is the Moore-Penrose pseudoinverse of Ψ . Once we have an estimate of the Laplace coefficients, we can find the estimated orientation distribution function with

$$\hat{\mathbf{f}}_{\text{MNLS}} = \mathbf{Y}^T \hat{\mathbf{F}}_{\text{MNLS}}. \quad (21)$$

The MNLS algorithm is very efficient, but it amplifies noise when Ψ has small singular values. In low-noise applications this is not an issue, but in many applications amplifying noise is not acceptable.

A more generally applicable algorithm is the Richardson-Lucy (RL) algorithm with updates given by

$$\hat{\mathbf{F}}^{(i+1)} = \text{diag}(\mathbf{H}^T \mathbf{1})^{-1} \text{diag} \left[\Psi^T \text{diag}(\Psi \hat{\mathbf{F}}^{(i)} + \mathbf{b})^{-1} \mathbf{g} \right] \hat{\mathbf{F}}^{(i)}. \quad (22)$$

Once the RL algorithm has converged to an estimate of the Laplace coefficients $\hat{\mathbf{F}}_{\text{RL}}$, we can find the estimated orientation distribution function with

$$\hat{\mathbf{f}}_{\text{RL}} = \mathbf{Y}^T \hat{\mathbf{F}}_{\text{RL}}. \quad (23)$$

2.5 Reconstruction with priors (in progress)

We would also like the ability to incorporate priors into our reconstruction algorithms. For example, we may know that we are measuring a single dipole, or we may know that the orientation distribution function is rotationally symmetric. All such priors can be formulated as a restriction on the set of possible orientation distributions. Our goal is to find a way to map our estimates of the measurable Laplace coefficients to a member of the set of possible orientation measurements.

Let $\{\mathbf{z}_1, \mathbf{z}_2, \dots, \mathbf{z}_p\}$ denote the *prior set*—the (possibly infinite) set of orientation distribution functions that could be present in the sample. We can assemble the set into the rows of a matrix $\mathbf{Z} \in \mathbb{R}^{p \times \infty}$ and apply the Laplace expansion operator to obtain a matrix $\mathbf{Z} \in \mathbb{R}^{p \times \infty}$ with the Laplace coefficients of each prior in each row

$$\mathbf{Z} = \mathbf{Y} \mathbf{z}. \quad (24)$$

When \mathbf{Z} acts on vectors of Laplace coefficients it returns the expansion of those Laplace coefficients in terms of the Laplace expansion of members of the prior set. Therefore, if we have an estimate of the directly measurable Laplace coefficients $\hat{\mathbf{F}}$ (from equation 20 or 22), we can estimate the corresponding Laplace coefficients in the prior set by multiplication with the matrix $\mathbf{Z}^T \mathbf{Z}$

$$\hat{\mathbf{F}}_{\text{prior}} = \mathbf{Z}^T \mathbf{Z} \hat{\mathbf{F}}. \quad (25)$$

Multiplication by $\mathbf{Z}^T\mathbf{Z}$ amounts to projecting the Laplace coefficients onto the nearest member of the prior set. As usual, we can find the estimated orientation distribution function with

$$\hat{\mathbf{f}}_{\text{prior}} = \mathbf{Y}^T \hat{\mathbf{F}}_{\text{prior}}. \quad (26)$$

We need to choose our prior set carefully for a given measurement system. If we choose a prior set that is too large for a measurement system, then multiple members of the prior set will give rise to the same intensity measurements—they will be indistinguishable. We call members of the prior set that give rise to the same measurements *degenerate*.

2.6 Single-molecule prior (in progress)

I strongly suspect that if we can measure all of the spherical harmonics in a single band and we have a single-molecule prior, then we can reconstruct the orientation of a single molecule in any orientation. I’m still developing a proper argument for this.

From [1] section 6.7.7: “ $\text{SO}(3)$...has a set of irreducible representations usually denoted by an index l and the l th irreducible representation has $2l + 1$ dimensions.” These irreducible representations correspond to the rows in Figure XXX.

2.7 Rotational symmetry prior (in progress)

2.8 Cramér-Rao lower bounds (in progress)

In the previous sections we have broken down the reconstruction into three steps

1. Estimate the measurable Laplace coefficients using equation 20 or equation 22.
2. If applicable, use equation 25 to incorporate priors.
3. Use the Laplace coefficients to calculate the orientation distribution function.

If our goal is to estimate the orientation distribution function from intensity measurements, then we would like to know how noise on our measurements affects the estimate of the orientation distribution function.

3 Methods (in progress)

To demonstrate the value of using harmonic analysis for designing orientation measurement systems we will work through several examples of orientation measurement systems.

For each system we will calculate the point response functions, the system matrix, and the crosstalk matrix. Next, we will optimize the orientation measurement system and make measurement recommendations. Finally, we will analyze the ability of the systems to measure the orientation of single fluorophores.

4 Results (in progress)

4.1 Point detectors

Consider an excited dipole being measured by a single point detector. The point response function for this measurement system is given by

$$h_1(\theta, \phi) = \sin^2 \theta \quad (27)$$

where θ is the angle between the dipole axis and the detector. We can expand the point response function in a Laplace series given by

$$h_1(\theta, \phi) = \frac{2\sqrt{\pi}}{3} y_0^0(\theta, \phi) + \frac{4\sqrt{5\pi}}{15} y_2^0(\theta, \phi). \quad (28)$$

In other words, a small single detector measures a linear combination of two spherical harmonics of the orientation distribution function. As expected, we cannot learn much about the orientation distribution function from this measurement unless we have a prior that either (1) restricts the orientation distribution functions to one of the spherical harmonics y_0^0 or y_2^0 or (2) restricts the orientation distribution function to set that directly maps to either y_0^0 or y_2^0 .

If we add two more point detector along orthogonal directions their point response functions and Laplace expansions are

$$h_2(\theta, \phi) = \cos^2 \theta + \sin^2 \theta \sin^2 \phi = \frac{4\sqrt{\pi}}{3} y_0^0(\theta, \phi) - \frac{\sqrt{30\pi}}{15} y_2^{-2}(\theta, \phi) + \frac{2\sqrt{5\pi}}{15} y_2^0(\theta, \phi) - \frac{\sqrt{30\pi}}{15} y_2^2(\theta, \phi) \quad (29)$$

$$h_3(\theta, \phi) = \cos^2 \theta + \sin^2 \theta \sin^2 \phi = \frac{4\sqrt{\pi}}{3} y_0^0(\theta, \phi) + \frac{\sqrt{30\pi}}{15} y_2^{-2}(\theta, \phi) + \frac{2\sqrt{5\pi}}{15} y_2^0(\theta, \phi) + \frac{\sqrt{30\pi}}{15} y_2^2(\theta, \phi) \quad (30)$$

These detectors add two more measurements, but they also introduce two more unknowns.

Still investigating I suspect that we need ≥ 6 detectors along independent axes to create an invertible system that can reconstruct the orientation of single molecules in any orientation. Single detectors measure linear combinations of spherical harmonics in the $l = 0$ (one harmonic) and $l = 2$ (five harmonics) bands for a total of six harmonics.

4.2 Single view microscopes

4.2.1 Epi-illumination and epi-detection

Point response functions for a wide range of microscopes have been calculated in [4]. First, consider a epi-illumination microscope with polarized illumination and epi-detection. The point response function is

$$h_{\text{epi-epi}}(\theta, \phi) = 2D\{A + B \sin^2 \theta + C \sin^2 \theta \cos[2(\phi - \phi_{\text{exc}})]\}(A + B \sin^2 \theta) \quad (31)$$

where A, B, C, D are constants depending on the numerical aperture and ϕ_{exc} is the excitation polarization angle. The Laplace expansion is given by

$$\begin{aligned} h_{\text{epi-pol-epi}}(\theta, \phi) = & \frac{2\sqrt{\pi}D}{15}(15A^2 + 20AB + 5AC + 8B^2 + 4BC)y_0^1(\theta, \phi) \\ & - \frac{\sqrt{30\pi}CD}{105}(7iA \sin(2\phi_{\text{exc}}) - 7A \cos(2\phi_{\text{exc}}) + 6iB \sin(2\phi_{\text{exc}}) - 6B \cos(2\phi_{\text{exc}}))y_2^{-2}(\theta, \phi) \\ & - \frac{2\sqrt{5\pi}D}{105}(28AB + 7AC + 16B^2 + 8BC)y_2^0(\theta, \phi) \\ & + \frac{\sqrt{30\pi}CD}{105}(7iA \sin(2\phi_{\text{exc}}) - 7A \cos(2\phi_{\text{exc}}) + 6iB \sin(2\phi_{\text{exc}}) - 6B \cos(2\phi_{\text{exc}}))y_2^{-2}(\theta, \phi) \\ & - \frac{2\sqrt{10\pi}BCD}{105}e^{-2i\phi_{\text{exc}}}y_4^{-2}(\theta, \phi) \\ & + \frac{8\sqrt{\pi}BD}{105}(2B + C)y_4^0(\theta, \phi) \\ & - \frac{2\sqrt{10\pi}BCD}{105}e^{-2i\phi_{\text{exc}}}y_4^{-2}(\theta, \phi) \end{aligned}$$

We can see that adding illumination polarizers extends our measurements into the $l = 4$ band.

I am surprised to see complex constants show up here. This could indicate an issue with my integration program or integrating around poles?

4.2.2 Orthogonal illumination and detection

The point response function for low-NA polarized illumination (light sheet illumination) with an orthogonal detector is

$$h_{\text{orthopol-epi}} = 2(\sin^2 \theta \cos^2(\phi - \phi_{\text{exc}}))(A + B(1 - \cos^2 \phi \sin^2 \theta)). \quad (32)$$

It's Laplace expansion is

$$\begin{aligned} h_{\text{orthopol-epi}} = & \cdots y_0^0(\theta, \phi) \\ & + \cdots y_2^{-2}(\theta, \phi) + \cdots y_2^0(\theta, \phi) + \cdots y_2^2(\theta, \phi) \\ & + \cdots y_4^{-4}(\theta, \phi) + \cdots y_4^{-2}(\theta, \phi) + \cdots y_4^0(\theta, \phi) + \cdots y_4^2(\theta, \phi) + \cdots y_4^4(\theta, \phi) \end{aligned}$$

4.3 Dual view microscopes

4.3.1 Epi-illumination and epi-detection

4.3.2 Orthogonal illumination and detection

5 Discussion and conclusions (in progress)

- Single detectors measure members of the $l = 0$ and $l = 2$ bands. Changing the axis of the detector changes what members of the $l = 2$ band are measured.
- Polarized illumination with a single detector measures members of the $l = 0$, $l = 2$, and $l = 4$ bands.
- **In progress** Polarized illumination and detection measures members of the $l = 0$, $l = 2$, $l = 4$, and $l = 6$ bands.

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Disclosures

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