

Three-dimensional fluorophore orientation imaging with polarized multiview microscopy

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Abstract: We show that polarized fluorescence microscopes make band-limited measurements in the angular frequency domain. We use this result to propose and demonstrate efficient algorithms for reconstructing three-dimensional fluorophore orientations from polarized multiview microscope data.

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1. Introduction

Polarized fluorescence microscopes can measure the orientation of fluorophores in live specimens [1]. Unfortunately, existing techniques are limited to imaging two-dimensional orientations in thin samples, and attempts to generalize these approaches to three-dimensional orientations have faced computational difficulties. We propose the use of the spherical harmonics to simplify the analysis of polarized fluorescence microscopes. In the same way that the Fourier transform simplifies the analysis of spatial imaging systems, the spherical Fourier transform simplifies the analysis of angular imaging systems.

2. Theory

Consider a small volume that contains many non-interacting fluorophores. We define the orientation distribution function $f(\hat{\mathbf{r}})$ as the number of fluorophores per steradian oriented in a direction $\hat{\mathbf{r}}$. The goal of polarized fluorescence microscopy is to estimate $f(\hat{\mathbf{r}})$ in many volumes throughout a three-dimensional sample.

The forward model for an N -measurement polarized fluorescence microscope is given by

$$g_i = \int_{\mathbb{S}^2} d\hat{\mathbf{r}} h_i(\hat{\mathbf{r}}) f(\hat{\mathbf{r}}) \quad \text{for } i = 1, \dots, N \quad (1)$$

where $h_i(\hat{\mathbf{r}})$ is the point response function of the i th microscope configuration, the integral is over the sphere \mathbb{S}^2 , and g_i is the i th intensity measurement. By discretizing $f(\hat{\mathbf{r}})$ and expanding $h(\hat{\mathbf{r}})$ in terms of the spherical harmonics, we can rewrite equation 1 as

$$\mathbf{g} = \Psi \mathbf{B}^+ \mathbf{f} \quad (2)$$

where $\mathbf{f} = [f(\hat{\mathbf{r}}_1), \dots, f(\hat{\mathbf{r}}_R)]^T$ is a vector of R samples of $f(\hat{\mathbf{r}})$; \mathbf{B} is a matrix of the spherical harmonics evaluated at the sample points, $\mathbf{B}_{ij} = Y_j(\hat{\mathbf{r}}_i)$ where Y_j is the j th spherical harmonic; $^+$ is the Moore-Penrose pseudoinverse; Ψ is a matrix of the spherical harmonic coefficients of the point response functions, $\Psi_{ij} = \int_{\mathbb{S}^2} d\hat{\mathbf{r}} h_i(\hat{\mathbf{r}}) Y_j(\hat{\mathbf{r}})$; and $\mathbf{g} = [g_1, \dots, g_N]^T$ is a vector of the N intensity measurements.

The point response functions for a wide class of polarized fluorescence microscopes have been calculated previously [2]. For many polarized fluorescence microscopes the point response function can be expanded in terms of a small number of spherical harmonics. If the point response functions can be expanded in terms of M spherical harmonics then Ψ is an $N \times M$ matrix and \mathbf{B}^+ only needs to be calculated in the first M columns. This simplification reduces Equation 1 from a set of integrals to an efficient series of matrix multiplications.

3. Methods and Results

We labeled the actin filaments of a fixed-cell sample with Alexa Fluor 488 Phalloidin and imaged the sample with an asymmetric 1.1/0.71 NA dual-view inverted selective plane illumination microscope [3] with variable polarizers added to both illumination paths. The dipole moment of Alexa Fluor 488 Phalloidin is known to align parallel to the long axis of actin filaments, so this sample makes a suitable test specimen for our reconstruction algorithm. We imaged the specimen volume eight times—two views with four illumination polarization orientations each.

To reconstruct the orientation in each voxel we solved the following problem

$$\mathbf{f}^* = \underset{\mathbf{f} \in \{\mathbf{e}_i\} i=0,\dots,R}{\operatorname{argmin}} \|\mathbf{g} - \Psi \mathbf{B}^+ \mathbf{f}\|_2^2 \quad (3)$$

where \mathbf{e}_i is a vector with a one in the i th entry and zeros elsewhere. By constraining \mathbf{f} we are assuming that all of the fluorophores in each voxel are oriented in the same direction—a reasonable assumption for this sample.

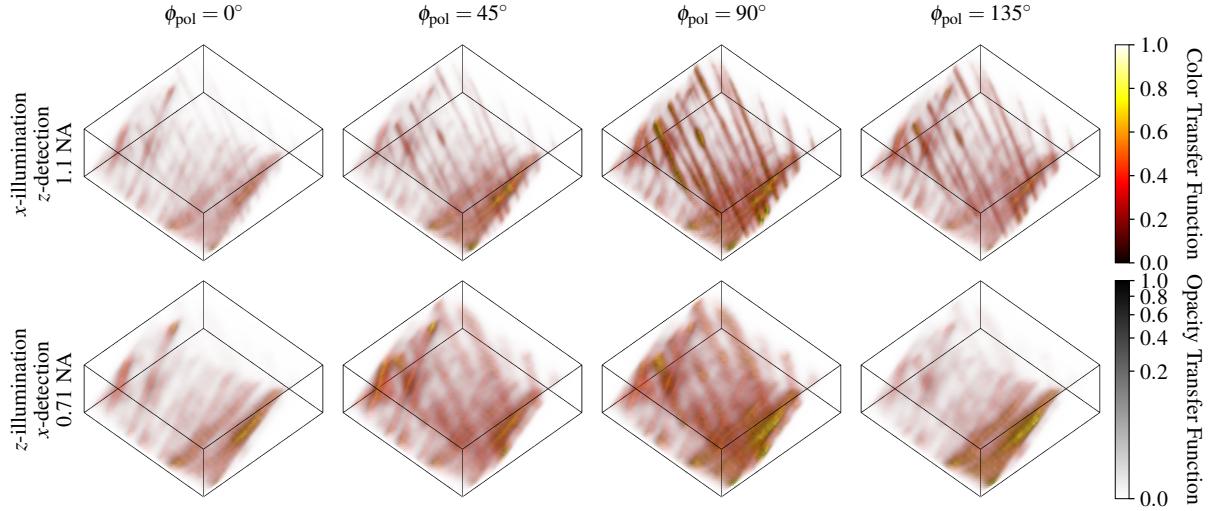


Fig. 1. Intensity data from a $13.5 \times 13.5 \times 5.4 \mu\text{m}^3$ volume from two views (rows) and four illumination polarization orientations (columns).

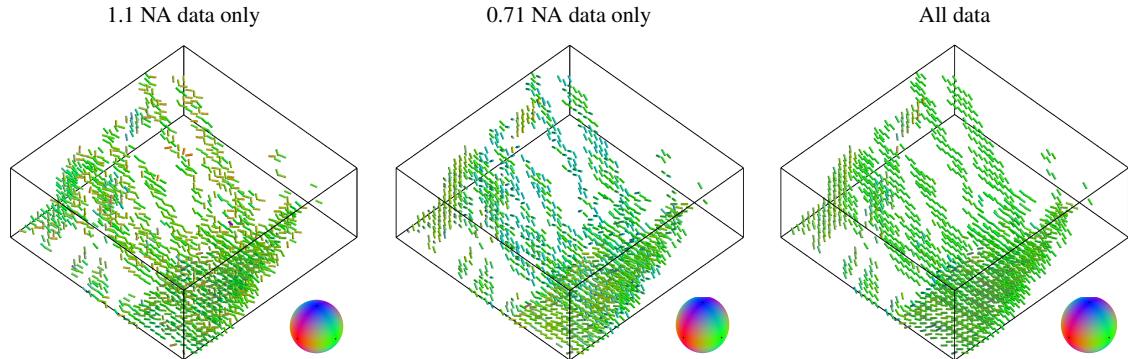


Fig. 2. Orientation reconstructions using (left) only data from the 1.1 NA view, (center) only data from the 0.71 NA view, and (right) data from both views. Data from both views is required to correctly reconstruct the three-dimensional orientation of fluorophores.

References

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