SPATIO-ANGULAR RESTORATION OF FLUORESCENCE MICROSCOPY DATA

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ABSTRACT

Polarized light microscopy is routinely used to estimate the orientation of fluorophores in biological samples. By imaging a specimen under several illumination polarizations and reconstructing the orientation with pixel-wise arithmetic, researchers can watch the orientation of fluorophores change in real time. Unfortunately, these reconstruction techniques are incompatible with spatial deconvolution, so the contrast in the reconstructed image is strongly dependent on spatial frequency.

Meanwhile, the rapidly-growing field of single-molecule fluorescence microscopy performs spatio-angular restorations by fitting measured intensities to a precomputed library of patterns. This approach can be used to estimate the orientation and position of single molecules, but the reconstructions are computationally expensive, and building intuition about the angular resolution of these methods is challenging.

We propose to solve these issues by decomposing the spatio-angular point spread function into spatio-angular harmonics—complex exponentials and spherical harmonics. We show that existing fluorescence microscopes have a spatio-angular band limit which allows us to perform spatio-angular restorations efficiently. We discuss restoration techniques for polarized light imaging, multiview imaging, single-molecule imaging, and combinations thereof. Finally, we discuss possibilities for spatio-angular super-resolution microscopy.

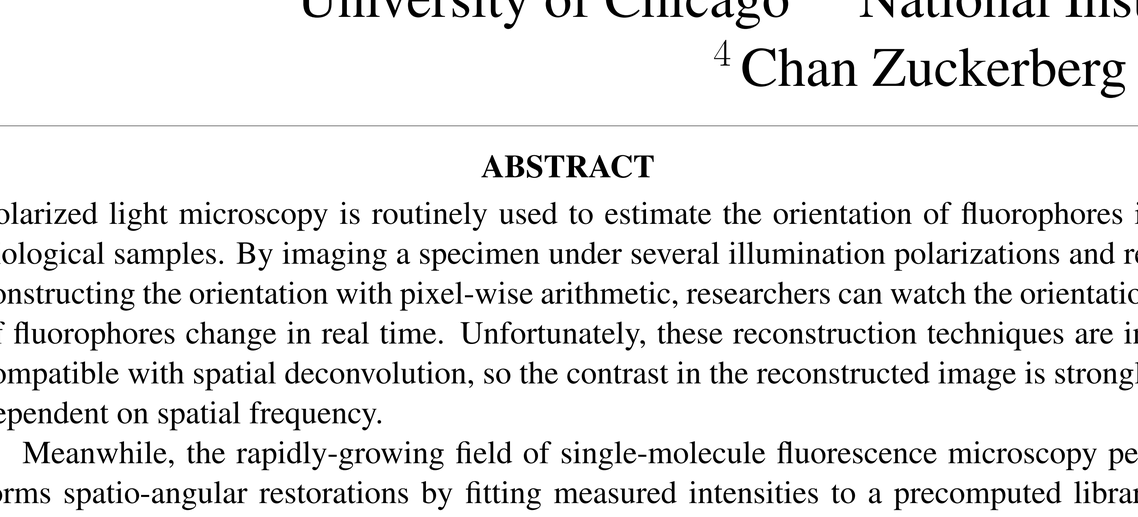

Fig. 1: Left: Single-view polarized illumination microscope. **Right:** We collect four images of the sample under four illumination polarization orientations. Polarized illumination selectively excites fluorophores with dipole axes parallel to the polarization orientation.

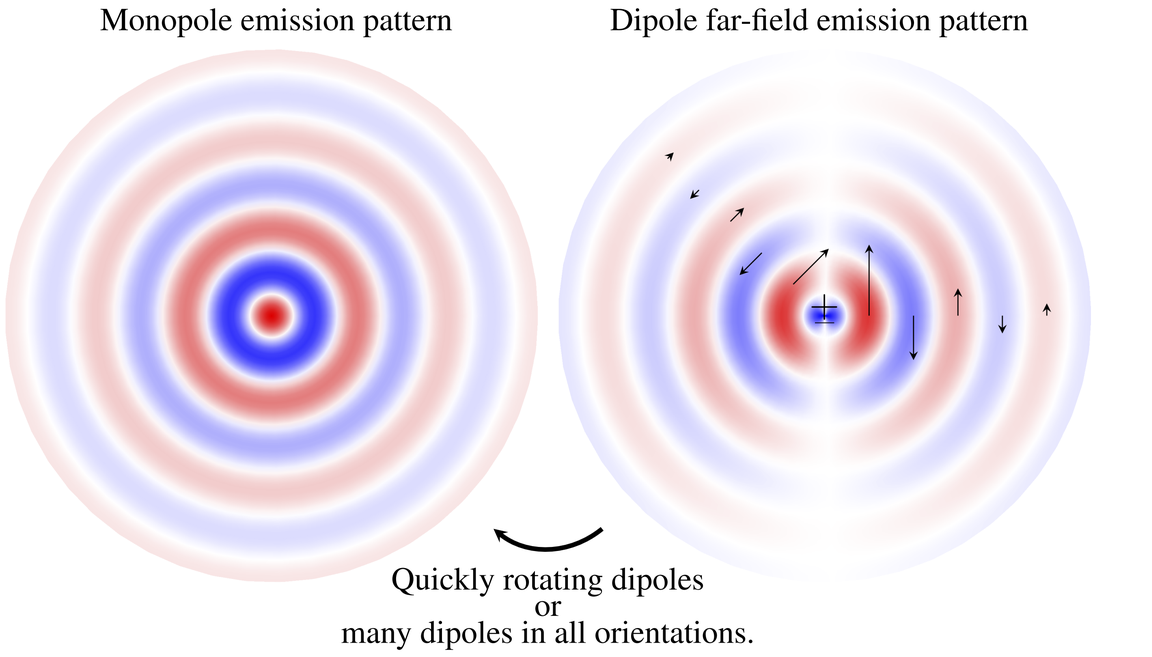

Fig. 2: Monopoles emit unpolarized light isotropically while dipoles emit polarized light anisotropically. A quickly rotating dipole or many dipoles oriented in all directions approximate a monopole emitter. If a fluorophore is rigidly attached to a structure of interest, the fluorophore's orientation can report valuable information to biologists.

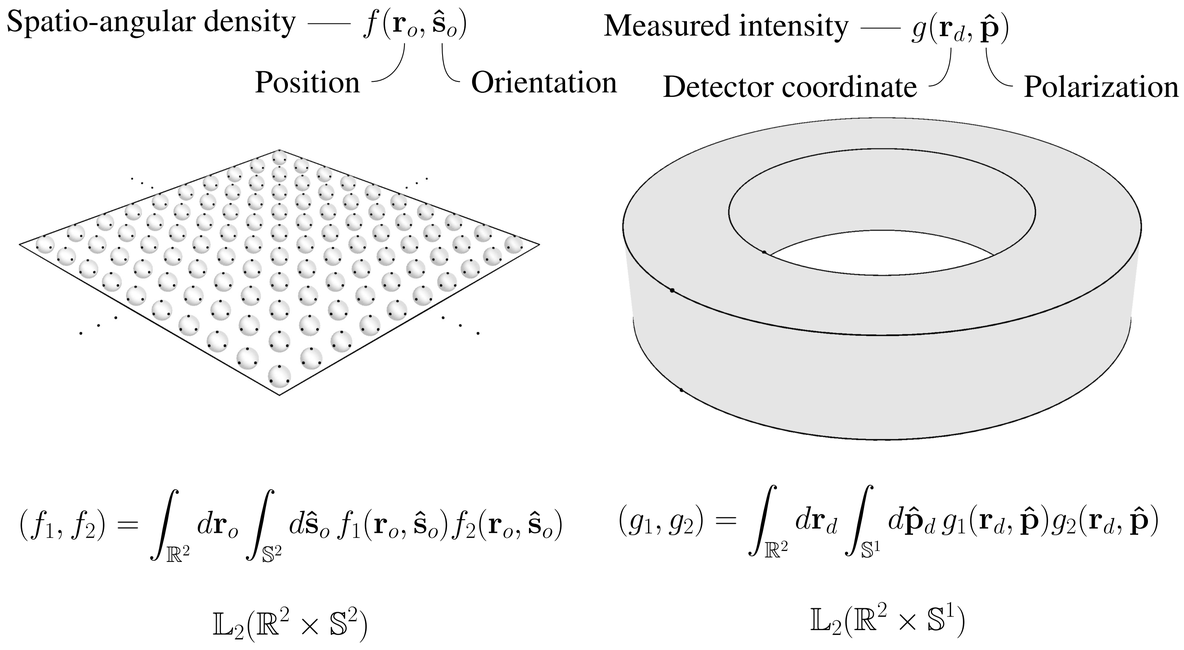

Fig. 3: Left: We are trying to reconstruct the spatio-angular density of fluorophores throughout a sample—members of the Hilbert space $\mathbb{L}_2(\mathbb{R}^2 \times \mathbb{S}^2)$. **Right:** We collect an image of intensity values for each polarization orientation on a circle—members of the Hilbert space $\mathbb{L}_2(\mathbb{R}^2 \times \mathbb{S}^1)$.

$$\underbrace{g(\mathbf{r}_d, \hat{\mathbf{p}})}_{\text{Detected intensities}} = \underbrace{\int_{\mathbb{R}^2} d\mathbf{r}_o \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o}_{\text{Integral over position and orientation}} \underbrace{h(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{p}}, \hat{\mathbf{s}}_o)}_{\text{Spatio-angular kernel}} \underbrace{f(\mathbf{r}_o, \hat{\mathbf{s}}_o)}_{\text{Spatio-angular density}}$$

Eq. 1: The forward model is a linear integral transform.

$$\underbrace{\begin{bmatrix} | \\ g \\ | \end{bmatrix}}_{\text{Data}} = \underbrace{\begin{bmatrix} | & | & & | \\ u_1 & u_2 & \dots & u_R \\ | & | & & | \end{bmatrix}}_{\substack{\text{Data space} \\ \text{singular functions}}} \underbrace{\begin{bmatrix} \sigma_1 & & & \\ & \sigma_2 & & \\ & & \ddots & \\ & & & \sigma_R \end{bmatrix}}_{\text{Singular values}} \underbrace{\begin{bmatrix} — & v_1^T & — \\ — & v_2^T & — \\ & \vdots & \\ — & v_R^T & — \end{bmatrix}}_{\substack{\text{Object space} \\ \text{singular functions}}} \underbrace{\begin{bmatrix} | \\ f \\ | \end{bmatrix}}_{\text{Object}}$$

Eq. 2: Singular value decomposition of the forward model. The object space singular functions $\{v_i\}$ span the measurement space of the imaging system.

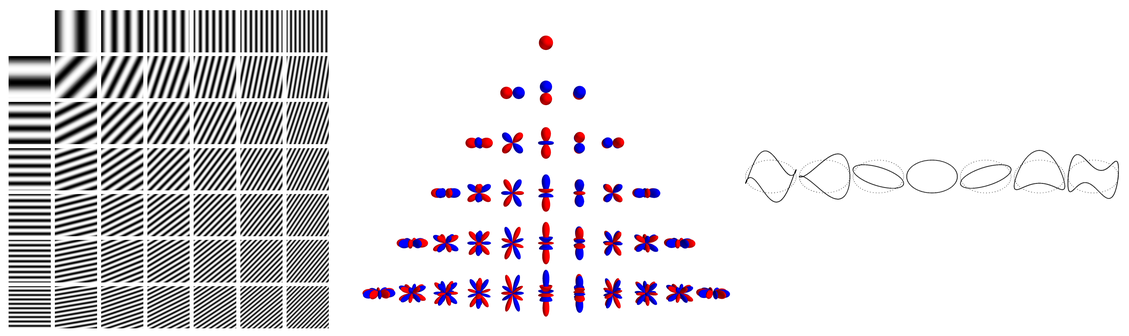

Fig. 4: Decomposing into spatial, spherical, and circular harmonics allows us to calculate the singular value decomposition of the forward model analytically.

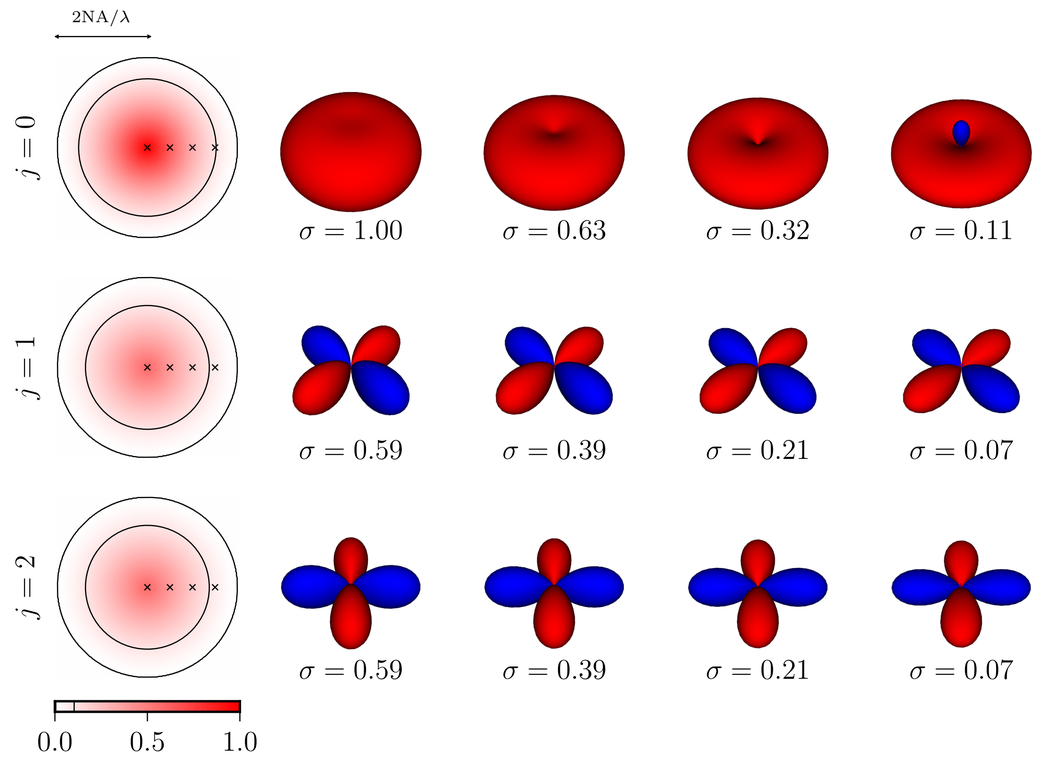

Fig. 5: Column 1: Singular value spectrum of a single-view polarized illumination microscope with NA = 0.8. The position in each plot corresponds to a spatial frequency, and the outer contour is the spatial band limit of the microscope. **Columns 2–5:** Angular part of the object space singular vectors at the points marked with crosses in column 1. **Rows:** All three angular branches of the singular spectrum.

Quantum mechanics	**Image science**
$\mathcal{H}$ = Hamiltonian	$\mathcal{H}$ = Forward operator
$\mathcal{H}\psi_k = W_k\psi_k$	$\mathcal{H}^\dagger\mathcal{H}u_k = \mu_k u_k$
$\mathcal{T}_i\mathcal{H} - \mathcal{H}\mathcal{T}_i = 0$	$\mathcal{T}_i\mathcal{H}^\dagger\mathcal{H} - \mathcal{H}^\dagger\mathcal{H}\mathcal{T}_i = 0$
$\mathcal{T}_i$ form the symmetry group of the Hamiltonian.	$\mathcal{T}_i$ form the symmetry group of the imaging system.

Identical singular values lead us to the symmetry group of the imaging system.
See *Foundations of Image Science*, Section 6.7, Barrett & Myers.

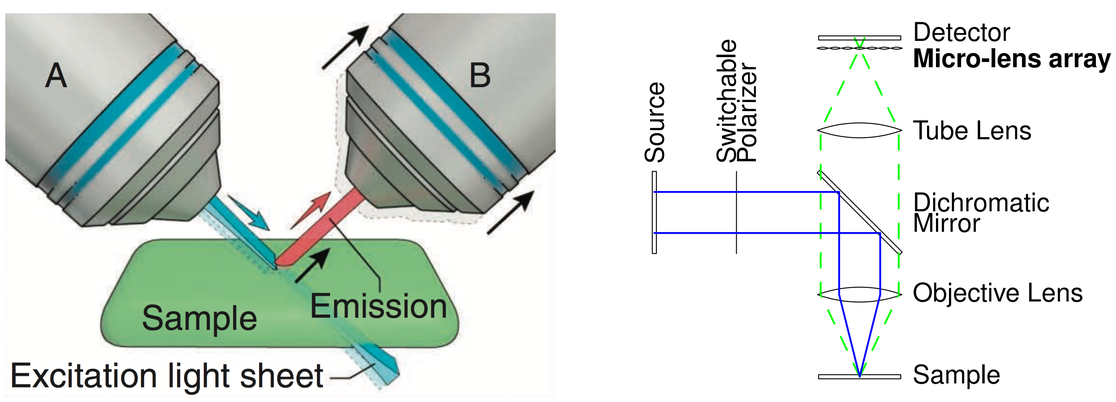

Fig. 6: Future directions. **Left:** Dual-view microscopy. **Right:** Light-field microscopy.

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