SINGLE-FLUOROPHORE ORIENTATION DETERMINATION WITH MULTIVIEW POLARIZED ILLUMINATION MICROSCOPY

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ABSTRACT

Polarized-light microscopy can be used to measure the orientation of single molecules in living cells [1]. By exciting and imaging a fluorescent specimen with several polarization orientations in sequence, researchers can calculate the orientation of single molecules and use the results to draw biological conclusions.

Unfortunately, most existing polarized-light microscopy techniques can only measure the orientation of the projection of the dipole moment into the transverse plane. Even methods that are sensitive to the three-dimensional orientation of molecules [2] suffer from degeneracy and highly anisotropic orientation uncertainty. An ideal microscope could measure the orientation of molecules uniquely with a small and uniform uncertainty for all orientations.

In this work we evaluate the ability of multiview polarized illumination microscopes to determine the three-dimensional orientation of fixed single-molecule fluorescence transition dipoles [3]. We find that multiview microscopes have fewer degeneracies and more uniform orientation uncertainty compared to single-view microscopes. We discuss optimal design choices, reconstruction techniques, and preliminary experimental results.

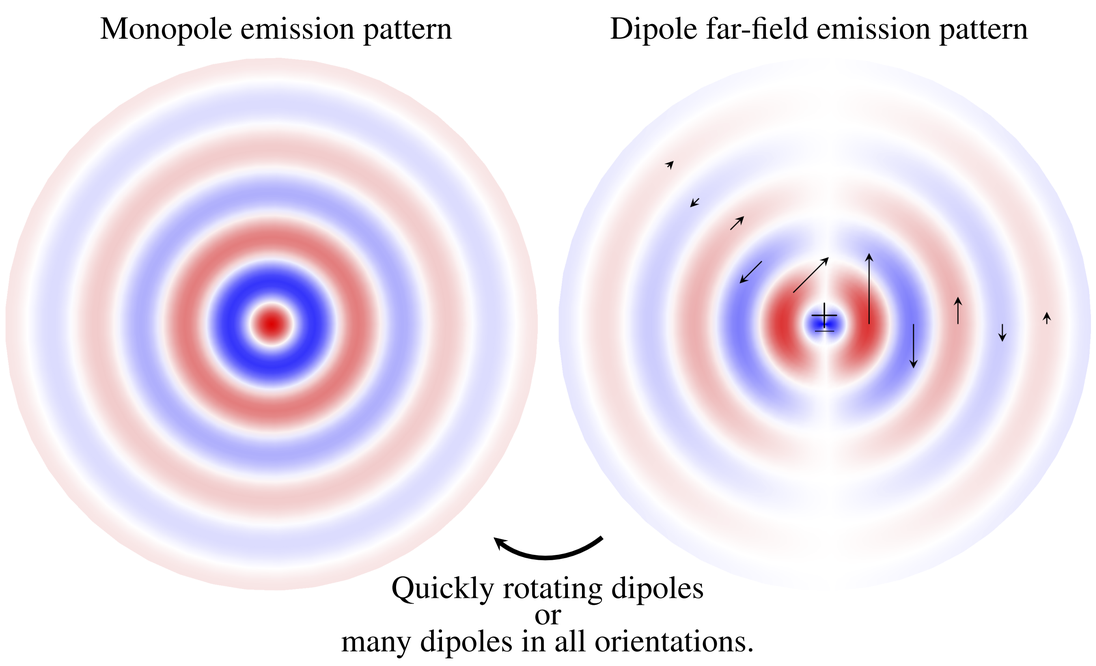

Fig. 1: Monopoles emit unpolarized light isotropically while dipoles emit polarized light anisotropically. A quickly rotating dipole or many dipoles oriented in all directions approximate a monopole emitter. Polarized light microscopes exploit the emission and excitation patterns of dipoles to find their orientation. If a fluorophore is rigidly attached to a structure of interest, the fluorophore's orientation can report valuable information to biologists.

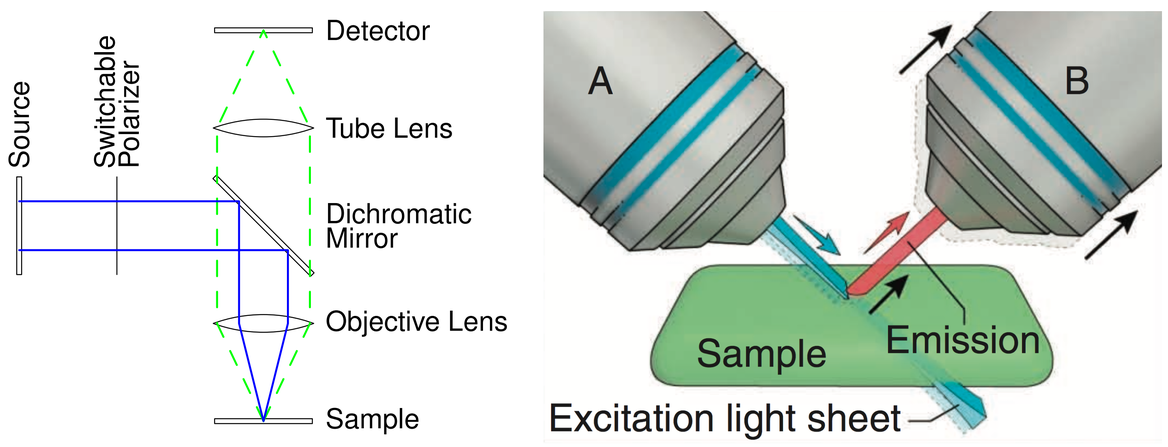

Fig. 2: Left: Single-view and **Right:** dual-view microscopes. Single-view microscopes have poor axial resolution and illuminate out-of-focus regions. Dual-view microscopes can use light-sheet illumination to achieve isotropic resolution and reduce phototoxicity.

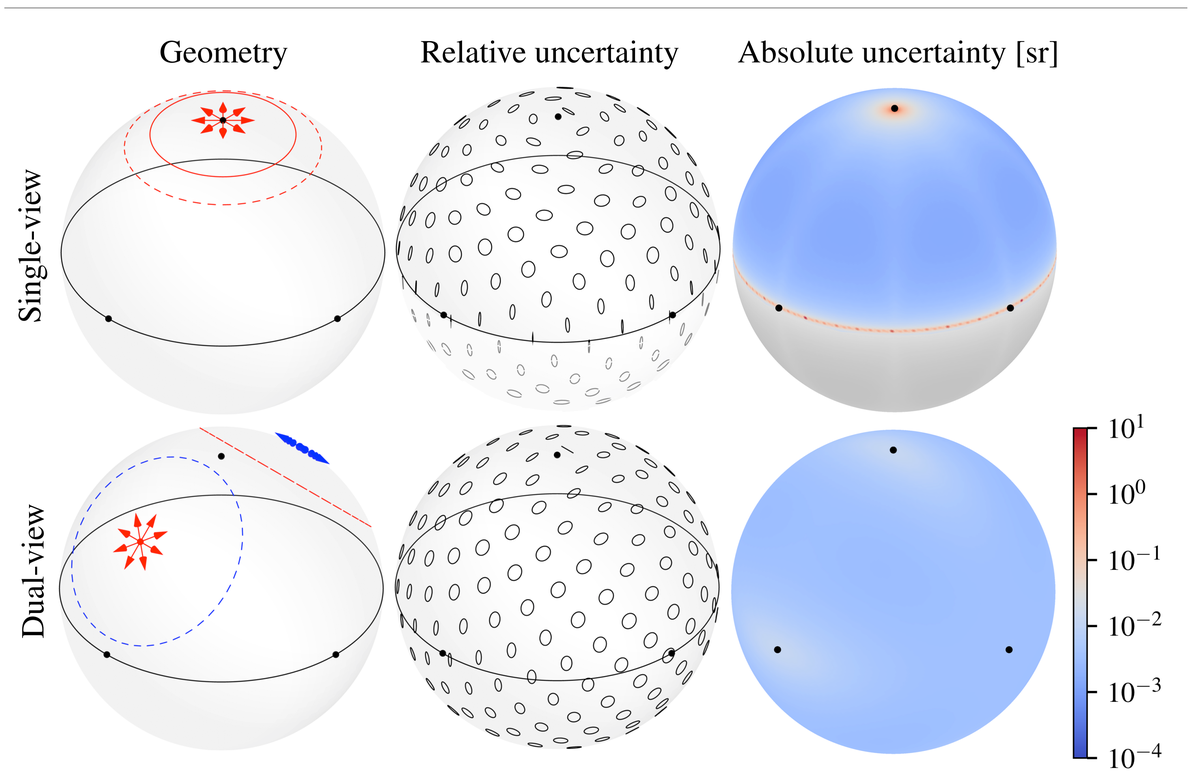

Fig. 3: Cramér-Rao lower bound analysis of **Row 1:** single- and **Row 2:** dual-view microscopes. Dual-view microscopes have fewer degeneracies and more uniform orientation uncertainty compared to single-view microscopes.

NIH R01GM114274, NIH R01EB017293

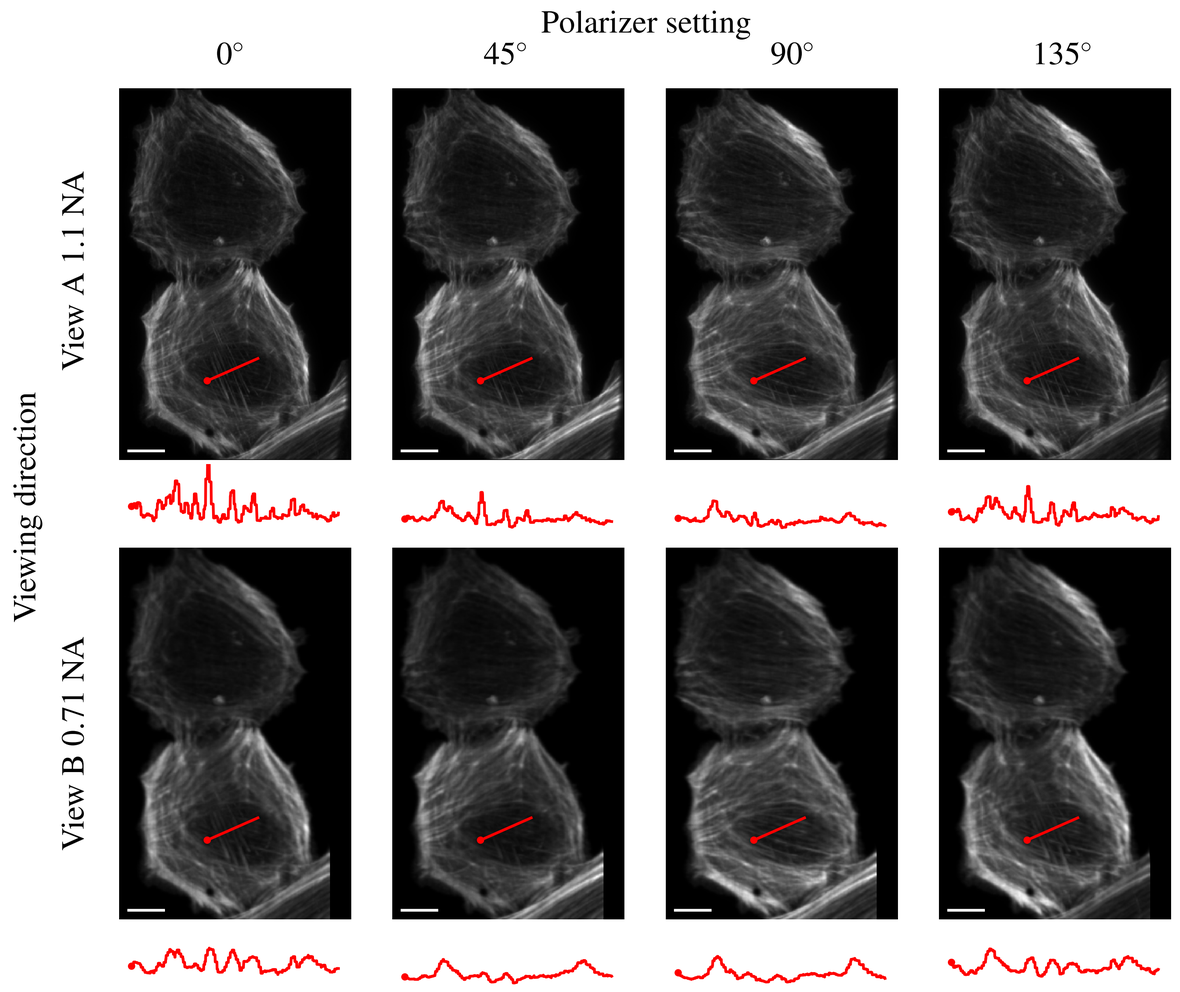

Fig. 4: We imaged fixed U2OS cells stained with Alexa Fluor 488 Phalloidin using an asymmetric 1.1/0.71 NA dual-view light-sheet microscope with polarized illumination. **Above:** Maximum-intensity projections through the eight collected volumes. We collected each volume with a different view (rows) and illumination polarization (columns). **Red:** Profiles through each volume show that changing the view and polarization modulates the measured intensity. Scale bars = 10 μm.

$$\underbrace{g_i(\mathbf{r}_d)}_{\text{Detected intensities}} = \underbrace{\int_{\mathbb{R}^3} d\mathbf{r}_o \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o}_{\text{Integral over position and orientation}} \underbrace{h_i(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o)}_{\text{Spatio-angular point spread function}} \underbrace{f(\mathbf{r}_o, \hat{\mathbf{s}}_o)}_{\text{Spatio-angular density}}$$

Eq. 1: Spatio-angular forward model assuming independent dipole emitters.

$$\mathbf{f}^* = \underbrace{\operatorname*{argmin}_{\mathbf{f} \in \{\mathbf{e}_i\}\ i=0,\dots,R}}_{\text{Constrained to a single orientation}} \underbrace{||\,\mathbf{g} - \Psi\,\mathbf{B}^{+}\mathbf{f}\,||_2^2}_{\substack{\text{Angular density estimate} \\ \text{Measured intensities} \\ \text{Transfer function} \\ \text{Discrete spherical Fourier transform} \\ \text{Variable}}}$$

Eq. 2: Angular reconstruction assuming that the spatial and angular problems are separable.

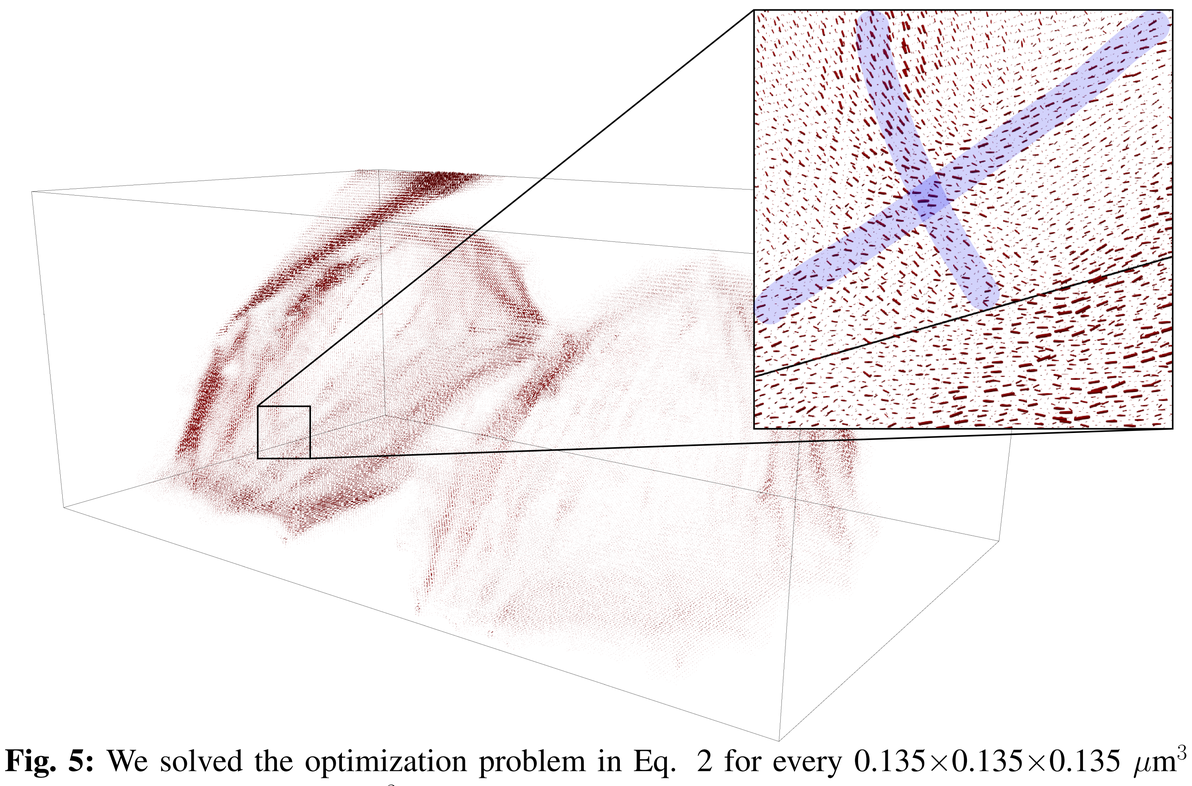

Fig. 5: We solved the optimization problem in Eq. 2 for every $0.135\times0.135\times0.135$ μm^3 voxel in the $68\times108\times46$ μm^3 volume of data shown in Fig. 4. We visualize the result by assigning a scaled and oriented cylinder to approximate the number and orientation of fluorophores in each voxel. **Inset:** Two manually highlighted actin fibers. Our reconstructed orientations are approximately aligned with the long axes of the actin fibers as expected.

[1] S. Weiss, "Fluorescence spectroscopy of single biomolecules," *Science*, vol. 283, no. 5408, pp. 1676–1683, 1999.

[2] J. T. Fourkas, "Rapid determination of the three-dimensional orientation of single molecules," *Opt. Lett.*, vol. 26, no. 4, pp. 211–213, Feb. 2001.

[3] T. Chandler, S. Mehta, H. Shroff, R. Oldenbourg, and P. J. La Rivière, "Single-fluorophore orientation determination with multiview polarized illumination: Modeling and microscope design," *Opt. Express*, vol. 25, no. 25, pp. 31 309–31 325, Dec. 2017.

IEEE International Symposium on Biomedical Imaging (ISBI), Washington, DC, April 4–7, 2018