Live-cell biology with three-dimensional fluorescence orientation microscopy

Fluorescence microscopy is an extremely valuable tool in biology—by introducing a fluorescent probe into a live organism and measuring the position of the probe, researchers can watch cellular processes as they occur. An extraordinary amount of effort has been expended towards improving the spatial resolution of fluorescence microscopes, and recent breakthroughs have allowed microscopes to achieve resolutions beyond the diffraction limit, which has enabled new biological insights [1].

In addition to the position of fluorophores, the *orientation* of fluorophores can report on biological processes [2]. Single fluorophores absorb and emit light like *dipoles*—they absorb and emit polarized light anisotropically—so their orientation can be measured using polarized light microscopes. If these fluorophores are rigidly attached to molecules of interest, then the fluorophores can provide valuable information on the orientation of those molecules. Unfortunately, current techniques can only measure the transverse orientation of fluorophores in relatively thin specimens. These constraints severely limit the number of biological questions that can be answered using available orientation measurement techniques.

We propose the use of polarized multiview microscopes to measure the three-dimensional orientation and position of fluorophores in thick living specimens. We are currently developing two polarized multiview microscopes with complementary strengths—a dual-view light sheet microscope and a light-field microscope. We will discuss these designs, propose a spatio-angular reconstruction algorithm, and look closer at several possible applications. We believe that this class of techniques will enable new insights in structural and functional biology. Ultimately, our goal is to develop tools that will enable biological discovery for an improved understanding of human health.

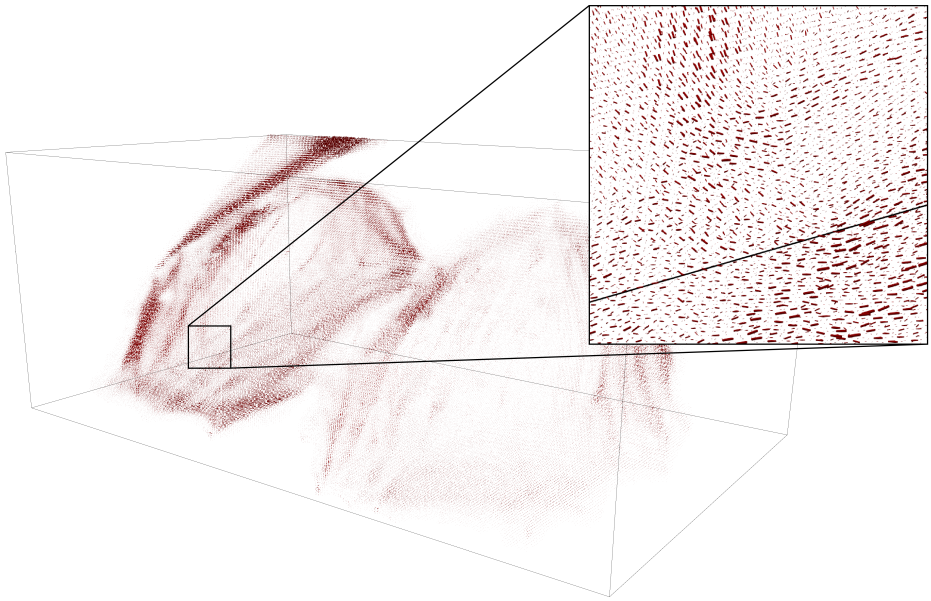

Figure 1: Orientation reconstruction of a $68\times108\times46$ μm^3 volume of fixed cells stained with Alexa Fluor 488 Phalloidin and imaged with an asymmetric 1.1/0.71 NA dual-view light-sheet microscope. We assign a scaled and oriented glyph to each voxel to indicate the quantity and orientation of fluorophores in that voxel. The inset shows two crossed actin fibers, and our reconstructed orientations are aligned with the long axes of these fibers as expected.

[1] L. Mockl, D. C. Lamb, and C. Brauchle, "Super-resolved fluorescence microscopy: Nobel Prize in Chemistry 2014 for Eric Betzig, Stefan Hell, and William E. Moerner," *Ange. Chem. Int. Ed.*, vol. 53, no. 51, pp. 13 972–13 977, 2014.

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