SPATIO-ANGULAR FLUORESCENCE IMAGING WITH A POLARIZED-ILLUMINATION LIGHT-SHEET DUAL-VIEW MICROSCOPE

Talon Chandler[1], Min Guo[2], Abhishek Kumar[2], Shalin Mehta[1,3,4], Hari Shroff[2,3], Rudolf Oldenbourg[3], Patrick J. La Rivière[1,3]

[1]University of Chicago, Chicago, Illinois 60637, USA

[2]National Institutes of Health, Bethesda, Maryland 20892, USA

[3]Marine Biological Laboratory, Woods Hole, MA 02543, USA

[4]Chan Zuckerberg Biohub, San Francisco, CA 94404, USA

Email: talonchandler@talonchandler.com

KEY WORDS: Fluorescence microscopy, polarized-light microscopy, light-sheet microscopy, molecular orientation, dipole imaging, diffusion.

If a fluorophore is rigidly attached to a molecule of interest, then the orientation of the fluorophore can report valuable information about the orientation and diffusive behaviour of the molecule. To measure and interpret this information we have developed a new set of techniques for imaging and reconstructing the positions and orientations of fluorophores throughout a three-dimensional sample.

Our acquisitions consist of (1) illuminating a fluorescent sample with a polarized light sheet then (2) measuring the emitted fluorescence via an orthogonal detection arm. Next, we (3) scan the sample to image a volume and (4) scan the illumination polarization to selectively excite fluorophores in different orientations. Finally, we (5) repeat the procedure with the roles of the illumination and detection arms reversed. The instrument can complete a scan with $500\times500\times100$ spatial measurements, 3 polarization measurements, and 2 views in less than 5 seconds.

Next, we jointly estimate the positions and orientations of fluorophores using a newly developed spatio-angular reconstruction scheme. Figure 1 shows the output of a reconstruction—we plot the quantity of fluorophores at each point and in each orientation.

We have successfully imaged living cells labeled with cytoskeletal and membrane dyes, and we are beginning to tackle new biological problems with our techniques. We will discuss our imaging protocol, reconstruction scheme, theoretical advances, and upcoming challenges.

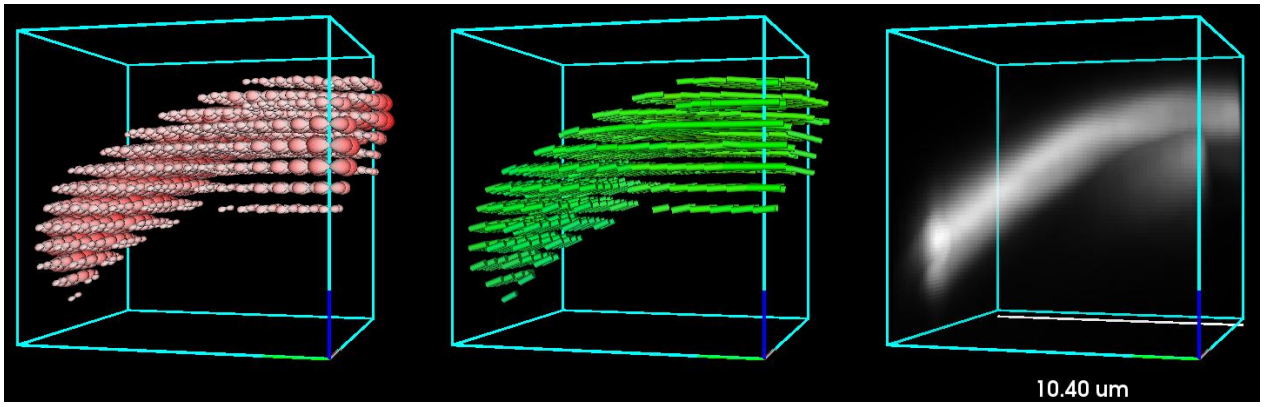

Figure 1: Tobacco plant xylem cells with cellulose labeled by Fast Scarlet. **Left:** Reconstructed orientation distribution function (ODF). The radius of the ODF indicates the quantity of fluorophores at each position and orientation. **Center:** Oriented cylinders indicate the most probable direction for fluorophores in each voxel. **Right:** Maximum intensity projection of the fluorophore density. The reconstructed dipole moments lie parallel to the long axis of the cellulose fibers.