Update On 3D Orientation Reconstruction

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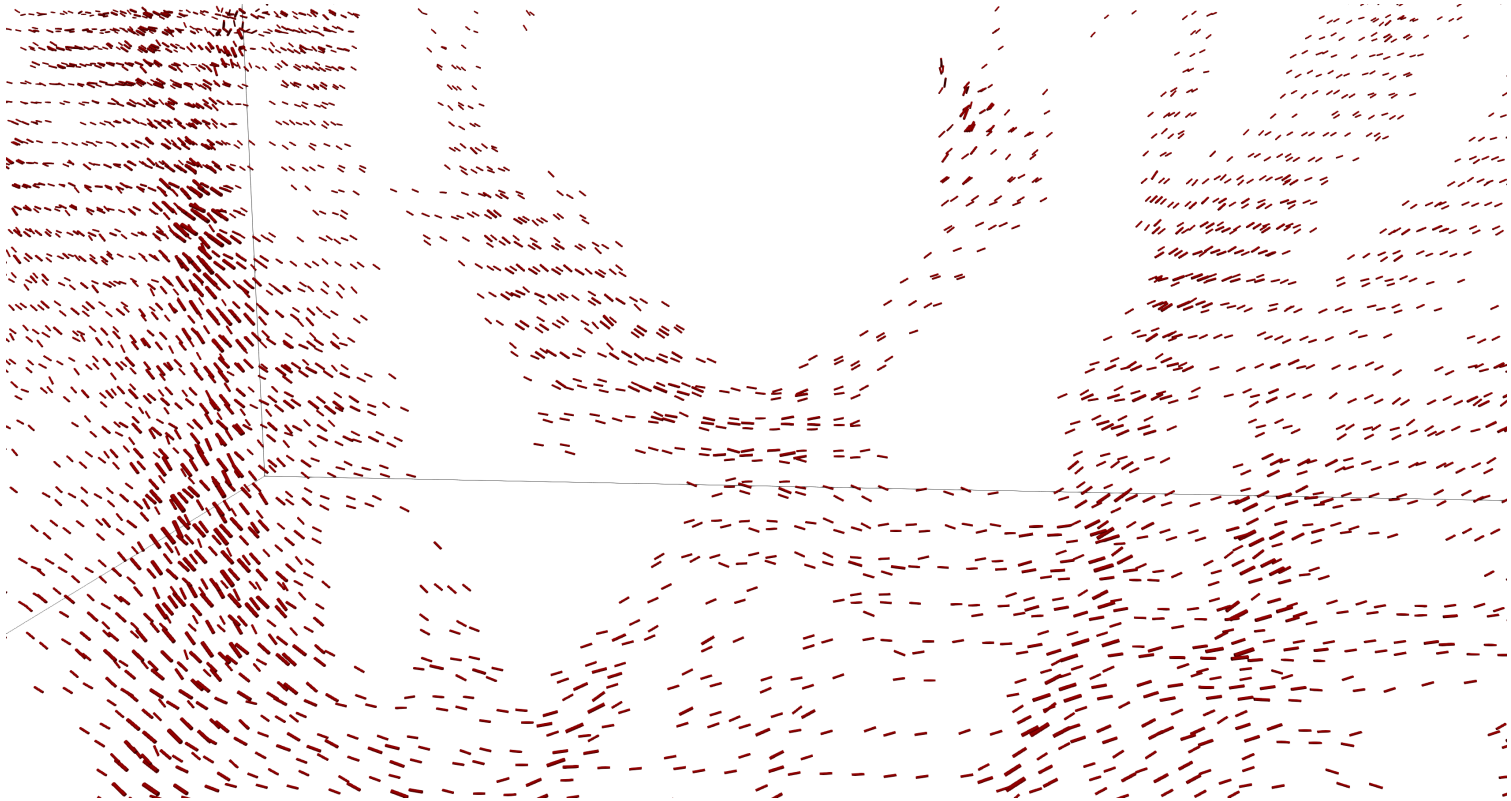
Polarized diSPIM data—Summer 2017

Since last time:

- Much faster reconstructions. Reconstructed 350K voxels in 1 min on my laptop. Bottlenecks are file I/O (not much I can do here) and visualization (chokes a bit on my computer, but live renders on diSPIM computer and RCC).
- Previously I was just reconstructing the orientation in each voxel. Now I'm reconstructing the orientation and the "number" of fluorophores in each voxel. In each voxel I assign an oriented glyph scaled by the number of fluorophores in that voxel.

Polarized diSPIM data—Summer 2017



Fly-around video here: `https://www.dropbox.com/s/szvssvu9fzxsajj/orientation-anim.avi?dl=0`

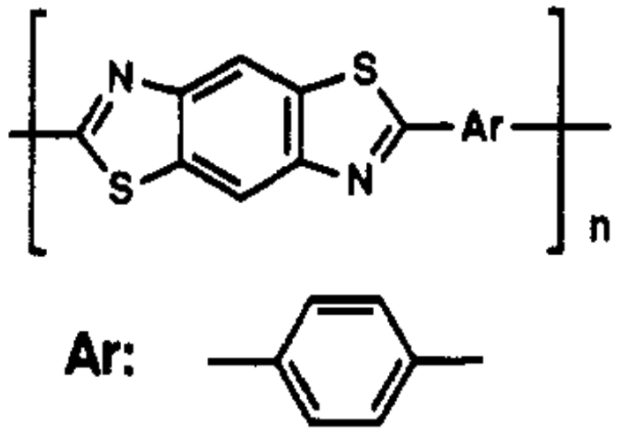
Polarized diSPIM data—Summer 2017

Notes:

- The video shows the entire volume $68 \times 108 \times 46$ μm^3.
- I reconstructed $10\times$ as many voxels as the ones shown in the video then thresholded the smallest ones away. In Paraview I can change the threshold with a slider and choose slice planes quickly.
- I'd estimate that a majority of voxel orientations align parallel with the actin fibers within 10 degrees. The fiber with 25-30 degree error that we saw before is one of the worst cases. The orientations also clearly follow the edges of the cells, although the individual fibers are not resolvable in these regions. I'm still skeptical of our data ordering, but the results are encouraging.

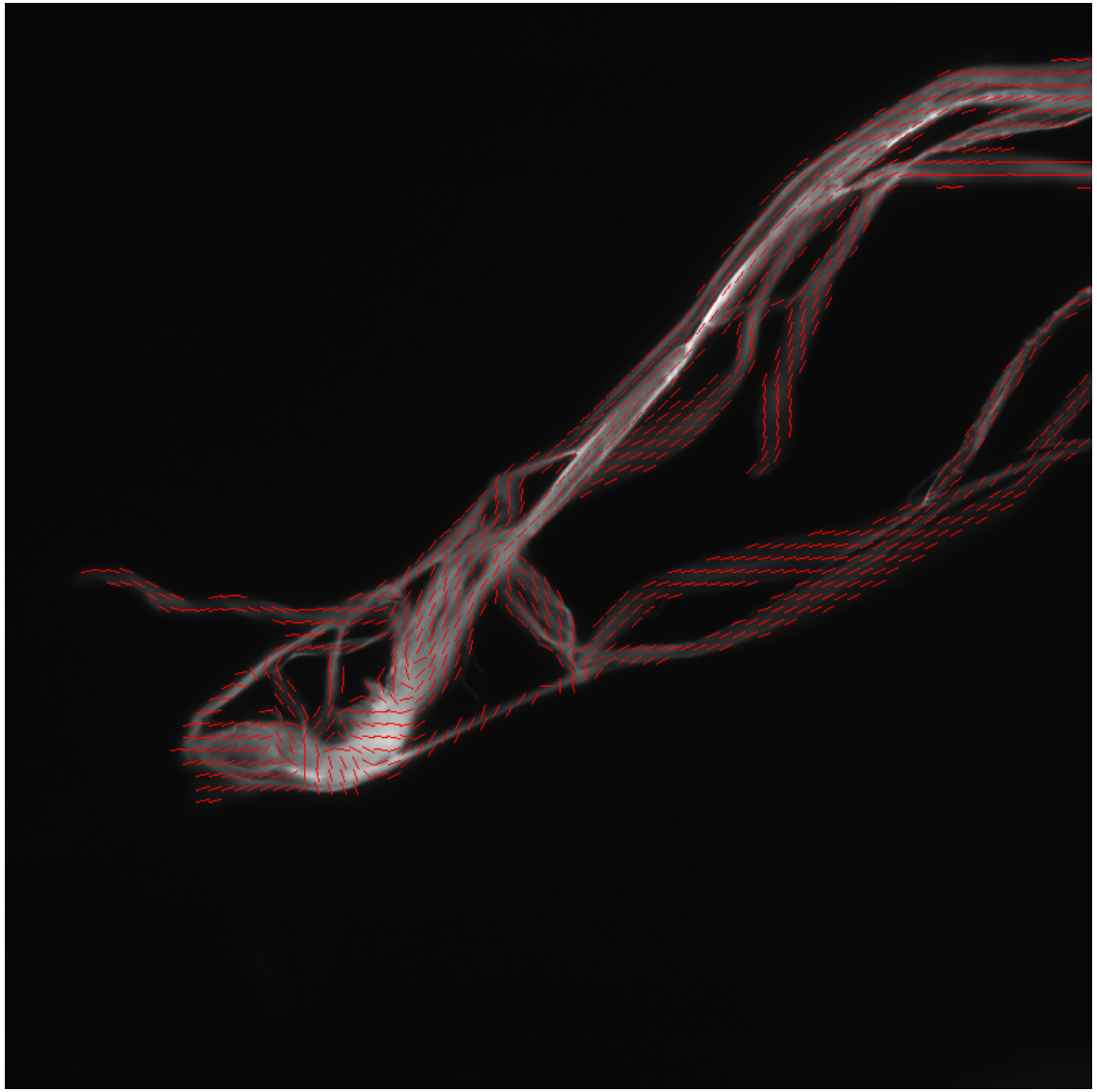
PBT Film via Fred Lanni

poly(1,4-phenylene-2,6-benzo-bis-thiazole)



- **Goal**: evaluate this sample as a test specimen for 3D reconstructions
- Broad excitation and emission spectra
- Stable and insoluble
- No published data on fluorescence anisotropy—only Fred's verbal reports

PBT Film, Single-view FluoPolscope
20×/0.5 NA, 325×325 μm^2
February 6, 2018



PBT Film, Single-view FluoPolscope
20×/0.5 NA, 325×325 μm^2
February 6, 2018

Notes:

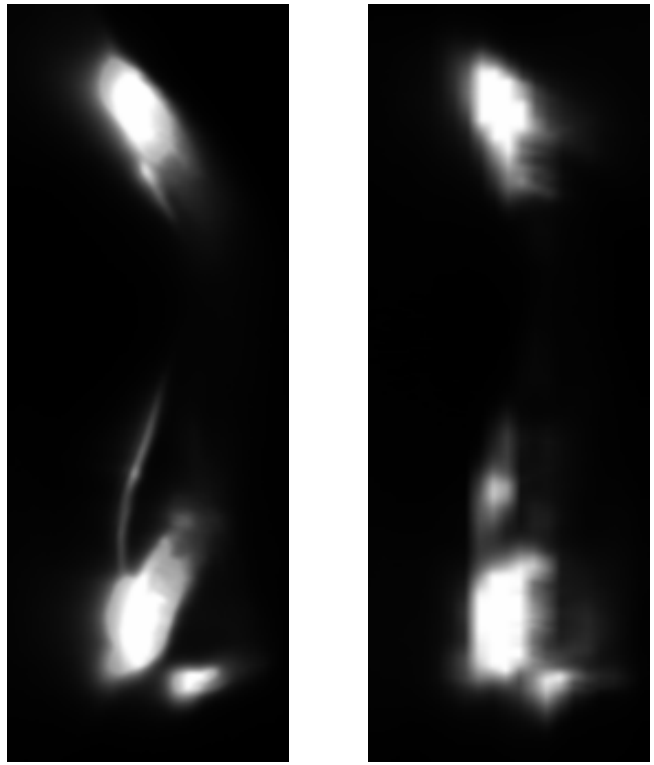
- I sandwiched the PBT film between a slide and cover slip so the fibers are approximately flat.
- I calculated the orientation lines using the existing FluoPolscope algorithms and overlaid the lines on the average intensity image.
- Good alignment along the fibers as expected (except for one pesky fiber in the bottom center?).

Uncontrolled polarization dual-view data 0.8/0.8 NA, February 3, 2018

Notes:

- We took two datasets with the PBT film on the symmetric diSPIM without polarizers. The illumination light was partially polarized on one arm, and almost completely polarized on the other arm—I don't expect any quantitative results given that we didn't use polarizers.
- During our first collection I accidentally saturated the detector for most parts of sample.
- During the second collection I turned down the laser power to avoid saturation.
- The two views from the first (saturated) dataset registered easily.
- The two views from the second (unsaturated) dataset didn't register. I can't visually match up any of the features in the two datasets either. This could be because of orientation dependence, shadowing, something else?

Saturated data—possible registration issues?



- In the saturated registered dataset I noticed that the deformed dataset (right) is significantly degraded compared to the undeformed dataset (left). I'd expect some degradation on the order of a few pixels, but this seems much larger. Do these results match your experience, Min?

Conclusions

- The PBT film is a convenient sample that displays fluorescence anisotropy.
- I think it will be a useful sample for testing the diSPIM with polarizers. We will be able to fray the sample into all orientations, and we won't have to worry about labeled cells being too flat or GUVs being too fragile.

Next Up

Next week:

- Thesis proposal document

After that:

- Formulate joint spatio-angular restoration problem
- Answer lingering questions about relating these reconstructions to existing work. How is the "anisotropy" related to these reconstructions? Can we understand Rudolf's existing reconstructions using spherical harmonics?