Biophysics 210: Biological Light Microscopy Kurt Thorn Syllabus

Discussion section meets Tuesdays from 1-2:30pm in MH2100 Labs meet Thursday or Friday from 2-5pm (location varies)

Week 7: Optical Sectioning: Deconvolution and Light Sheet

Goals: Understand the principles of deconvolution and light sheet microscopy. Compare and contrast these approaches to optical sectioning to confocal and two-photon microscopy. Learn several different methods for constructing light sheet microscopes and understand the strengths and weaknesses of each.

Discussion Section: May 12th

Labs: May 14th and 15th

Lectures (watch before discussion section):

- Deconvolution Microscopy
- Light Sheet Sectioning
- <u>Dual-View Inverted Selective Plane Illumination (diSPIM)</u>

Reading required for discussion section:

- Phillipp J. Keller and Misha B. Ahrens. Visualizing Whole-Brain Activity and Development at the Single-Cell Level Using Light-Sheet Microscopy. Neuron (2015) 85(3):462-483
- Yicong Wu, Peter Wawrzusin, Justin Senseney, Robert S Fischer, Ryan Christensen, Anthony Santella, Andrew G York, Peter W Winter, Clare M Waterman, Zhirong Bao, Daniel A Colón-Ramos, Matthew McAuliffe and Hari Shroff. Spatially isotropic four-dimensional imaging with dual-view plane illumination microscopy. Nature Biotechnology (2013) 31(11):1032-1038

Additional Reading (optional):

- Wallace, W., Schaefer, L. H. and Swedlow, J. R. A Workingperson's Guide to Deconvolution in Light Microscopy. BioTechniques 31: 1036-1097 (2001).
- Molecular Expressions: Deconvolution Microscopy

- Emmanuel G Reynaud, Jan Peychl, Jan Huisken and Pavel Tomancak. Guide to light-sheet microscopy for adventurous biologists. Nature Methods (2015) 12:30-34
- Jan Huisken, Jim Swoger, Filippo Del Bene, Joachim Wittbrodt, Ernst H.K. Stelzer.
 Optical sectioning deep inside live embryos by selective plane illumination
 microscopy. Science (2004) 305:1007-1009
- Jan Huisken and Didier Y. R. Stainier. Even fluorescence excitation by multidirectional selective plane illumination microscopy (mSPIM). Optics Letters (2007) 32(17):2608-2610
- Keller, Philipp J., et al. "Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy." Science 322.5904 (2008): 1065-1069.

Discussion Section Topic: Instead of our usual problem set format, this weeks discussion section will be devoted to a discussion of the various approaches to 3D imaging that we have learned about over the last two weeks, their pros and cons, and how they can be used together.

Lab: We will demonstrate light sheet imaging and deconvolution microscopy. (Nikon Imaging Center)