Imaging Cellular Structure and Dynamics from Molecules to Organisms

MONDAY 9:00 AM **ROOM: America's Ballroom (2nd Level)**

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Eric Betzig obtained a B.S. in Physics from Caltech and a Ph.D. in Applied Physics at Cornell. In 1988, he became a PI at AT&T Bell Labs where he extended his thesis work on near-field optical microscopy, the first method to break the diffraction barrier. By 1993, he held a world record for data storage density and recorded the first super-resolution fluorescence images of cells as well as the first single molecule images at ambient temperature. Frustrated with technical limitations and declining standards as more jumped into the field, he quit science and by 1996 was working for his father's machine tool company. The commercial failure of the technologies he developed there left him unemployed in 2003 and looking for new directions. This search eventually culminated in his co-invention of the super-resolution technique photo-activated localization microscopy (PALM) with his best friend, Bell Labs colleague Harald Hess. For this work, Betzig was co-recipient of the 2014 Nobel Prize in Chemistry along with Stefan Hell and William E. Moerner. Since 2005, he has been a Group Leader at the Janelia Research Campus, developing new optical imaging technologies for biology.

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The hallmark of life is that it is animate. Every living thing is a complex pocket of reduced entropy through which matter and energy flow continuously. Thus, although structural imaging is informative, a more complete understanding of the molecular basis of cellular physiology requires high-resolution imaging of the dynamics of the cell in its native state across all four dimensions of spacetime simultaneously.

Unfortunately, several factors conspire to render such unperturbed, physiological 4D imaging difficult. First, as powerful as genetically encoded fluorescent proteins have become, until recently they have rarely been used at endogenous expression levels, and therefore can upset the homeostatic balance of the cell. New genome editing technologies, specifically CRISPR / CAS9, address this problem. Second, conventional live cell imaging tools such as spinning disk confocal microscopy are too slow to study fast cellular processes across cellular volumes, create out-of-focus photo-induced damage and fluorescence photobleaching, and subject the cell at the point of measurement (i.e., the excitation focus) to peak intensities orders of magnitude beyond that under which life evolved. In the past few years, we have used "non-diffracting" beams, specifically Bessel beams and 2D optical lattices, to create ultra-thin light sheets capable of imaging of sub-cellular dynamics in 3D across whole cells and small embryos with near-isotropic resolution at up to 1000 image planes/sec over hundreds of time points ([1], Fig. 1). We have worked with over fifty different groups to apply these tools in areas including: mitotic spindle alignment during asymmetric stem cell division [2]; actomyosin contractions driving the initial gastrulation of *C. elegans* embryos [3]; binding kinetics of single transcription factor molecules to DNA in live stem cells [4]; dynamic, heterogeneous remodeling of P granule proteins in *C. elegans* embryos [5]; asymmetric formation of clathrin-coated pits on the dorsal /ventral surfaces at the leading edge of motile cells [6]; rapid 3D redistribution of actin in T cells during the formation for the immunological synapse [7]; and spatiotemporal quantification of microtubule growth tracks throughout the cellular volume at all mitotic stages [8].

Finally, much of the contribution of optical microscopy to cell biology has come from observing individual cells cultured onto glass substrates, and yet it is certain that they did not evolve there. True physiological imaging likely requires studying cells in their parent organisms, where all the external environmental cues that drive gene expression, and hence their structural and functional phenotypes, are present. However, such imaging is compromised by the highly inhomogeneous refractive index of most biological tissues, which distorts light rays and thereby degrades both resolution and signal. We have adopted methods of adaptive optics (AO), initially developed in astronomy, to recover diffraction-limited performance deep within living systems ([9], Fig. 2, left and bottom), and have recently combined AO on both the excitation and detection arms of our lattice light sheet microscope to image sub-cellular dynamics noninvasively within multicellular systems such as developing zebrafish embryos (unpublished, Fig. 2, upper right).

References:

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[2] S.J. Habib, *et al.,* Science **339**, (2013), p. 1445.
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[4] J. Chen, *et al.,* Cell **156**, (2014), p. 1274.
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[6] C. Kural, *et al.,* Mol. Biol. Cell **26**, (2015), p. 2044.
[7] A.T. Ritter, et al., Immunity **42**, (2015), p. 864.
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[9] K. Wang, *et al., Nat. Meth*. **11**, (2014), p. 625.

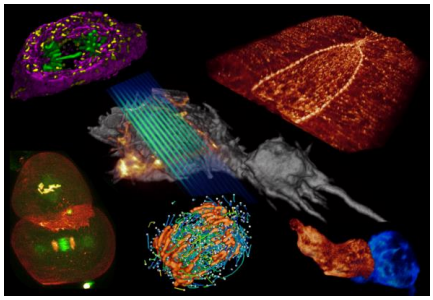

Figure 1. In lattice light sheet microscopy, an ultrathin illumination plane (blue-green, center) excites fluorescence (orange) in successive planes as it sweeps through a specimen (gray) to generate a 3D image. Applications in mitosis, embryonic development, and immunology are shown in several surrounding examples [1].

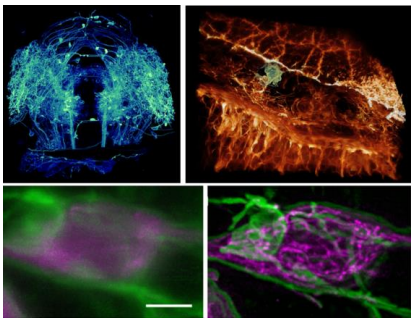

Figure 2. Top Left: Adaptive optical (AO) two-photon image of a sparse set of neurons across 240 x 240 x 270 µm in the developing zebrafish brain [9]. Bottom: Two color confocal images of plasma membranes (green) and mitochondria (magenta) in a neuron 150 µm deep, before (left) and after (right) AO correction [9]. Top Right: AO lattice light sheet microscopy of different cell types in the developing zebrafish ear, showing skin cells (top layer), the fluid-filled perilymphatic space (middle) containing a neutrophil (light blue), and hindbrain neurons (bottom layer).