Adaptive Optics for Biological Imaging using Direct Wavefront Sensing

This presentation will be on the use of adaptive optics (AO) with direct wavefront sensing for biological imaging. Adaptive optics have been used in ground based astronomy to correct image aberrations caused by refraction as light passes through Earth's turbulent atmosphere. As shown on the left in Figure One, light from the telescope has a distorted wavefront, as indicated by the wavy lines. A wavefront sensor measures these distortions and applies the opposite shape on an adaptive mirror using a feedback control system. After reflection from the adaptive mirror a corrected wavefront is generated and is recorded by a high-resolution camera. An image of the planet Neptune before and after AO correction is shown on the right in Figures 1(a) and 1(b). After correction the cloud structure on Neptune can be resolved in 1(b).

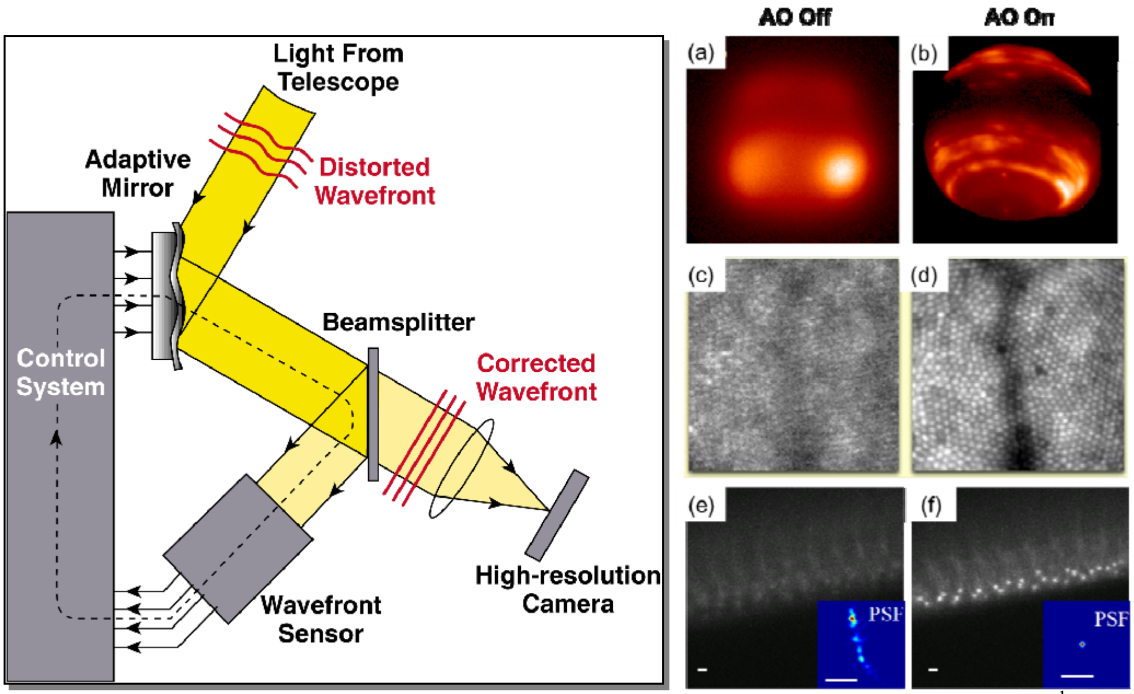


Figure One. Adaptive optics to correct for wavefront aberrations in astronomy (a)&(b)[1], vision science (c)&(d)[2], and biological imaging (e)&(f)[3]. Scale bars are 2 μm.

The same approach has been used in vision science to correct for the aberrations caused by the eye when imaging the retina. An image of the retina without AO is shown in Figure 1(c), and with AO correction in 1(d). The individual rods and cones in the retina can be resolved with AO correction, allowing for studies of retinal diseases such as age related macular degeneration and retinitis pigmentosa. We have extended the direct wavefront sensing approach used so successfully in astronomy and vision science to biological imaging, as shown in Figures 1(e) and 1(f). Here the process of mitosis is studied in a *Drosophila* embryo with GFP-polo labeled centrosomes at a depth of 83 μm below the coverslip. Without AO correction the centrosomes are not visible, as shown in 1(e). With AO correction the centrosomes can be resolved as small white dots along the edge of the embryo, as shown in 1(f), enabling live *in-vivo* studies, at depth, at the diffraction limit of the optical system.

As shown by the insets to Figures 1(e) and 1(f), the Point Spread Function (PSF) can be severely distorted when imaging *in vivo* through intervening tissue. This can be a problem for imaging through tissue at the single molecule level using super-resolution imaging techniques such as Photo-Activation Localization Microscopy (PALM) and Stochastic Optical Reconstruction

Microscopy (STORM) where a Gaussian must be fit to the PSF. It is not clear how to fit a Gaussian to the PSF shown in Figure 1(e), but it is straightforward for the corrected PSF shown in Figure 1(f). Similar to the binary stars that are resolved using adaptive optics in astronomy, as shown in Figure 2(a) and 2(b), adaptive optics in biology allows structures at the diffraction limit to be resolved in deep tissue imaging, as shown in Figures 2(c) and 2(d).

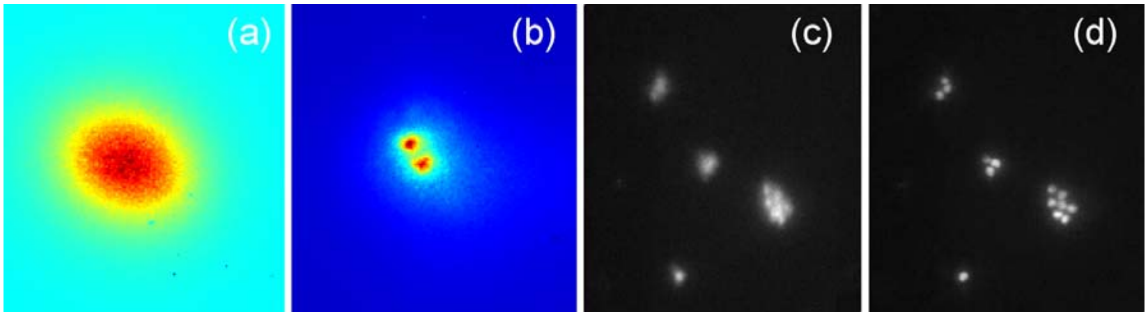
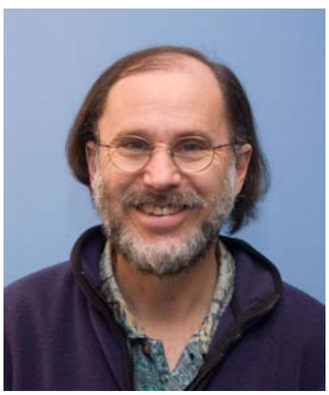


Figure Two. *Adaptive optics enables diffraction limited imaging in astronomy and biology.* (a) Binary star imaged without AO. (b) With AO.[4] (c) 1 micron fluorescent beads imaged through 20 microns of *Drosophila* embryo tissue without AO. (d) With AO[5].

We will discuss our results using adaptive optics in biological imaging to correct for refractive image aberrations and how we are extending this approach to compensate for scattering in tissue.



Joel Kubby is a Professor and Department Chair of Electrical Engineering in the Baskin School of Engineering at the University of California at Santa Cruz. His research is in the development and use of adaptive optics for biological imaging. Prior to joining the University of California at Santa Cruz in 2005, he was an Area Manager with the Xerox Wilson Center for Research and Technology and a Member of Technical Staff in the Xerox Webster Research Center in Rochester New York (1987-2005). Prior to Xerox he was at the Bell Telephone Laboratories in Murray Hill New Jersey working in the area of Scanning Tunneling Microscopy (STM).

---

[1] Macintosh, B.; Max, C. E.; Roe, H.; Gibbard, S.; Gavel, D.; Acton, S.; Lai, O.; Wizinowich, P.; de Pater, I.; Ghez, A.; Baines, K., *Adaptive Optics Imaging of Neptune with the W.M. Keck Telescope*, American Astronomical Society, DPS meeting #31, #65.06 (1999).
[2] Roorda, A. and Williams, D.R., *The arrangement of the three cone classes in the living human eye*, Nature **397**, pp. 520-522 (1999).
[3] Tao, Xiaodong; Crest, Justin; Kotadia, Shaila; Azucena, Oscar; Chen, Diana C; Sullivan, William; Kubby, Joel, Optics Express **20**, *Live imaging using adaptive optics with fluorescent protein guide-stars*, pp.15969-15982 (2012).
[4] http://ao.jpl.nasa.gov/Palao/AdditionalResults.html
[5] O. Azucena, J. Crest, S. Kotadia, W. Sullivan, X. Tao, M. Reinig, D. Gavel, S. Olivier, and J. Kubby. *Adaptive Optics Wide-Field Microscopy Using Direct Wavefront Sensing*, Optics Letters **36**, pp.825-827 (2011).