Adaptive Optics for Biological Imaging using Direct Wavefront Sensing

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University of California Santa Cruz

Photonics Media Webinar
Adaptive Optics for Microscopy
October 23rd, 2013

Adaptive Optics at UC Santa Cruz

Center for Adaptive Optics A University of California Science and Technology Center

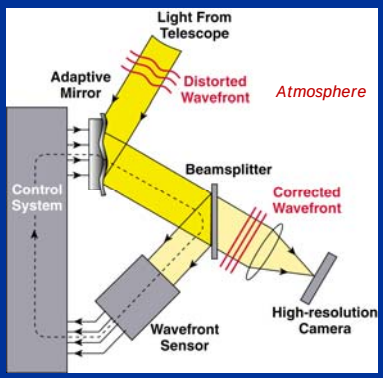
Laboratory for Adaptive Optics, UCO Lick Observatory

W.M. Keck Center for Adaptive Optical Microscopy

Direct Wavefront Sensing & Correction

- **Wavefront Aberrations**
  - **Astronomy (Komolgorov Spectrum)**
  - **Vision Science (Near Sighted, Far Sighted, Astigmatism)**
  - **Biology**
- **Reference Beacons**
  - **Astronomy: Laser Guide-Stars**
  - **Biology: Fluorescent Protein Guide-Stars**
- **Shack-Hartman Wavefront Sensor (SHWS)**
- **Wavefront Correction**
  - **Wide-Field Microscopy**
  - **Confocal Microscopy**
  - **Two-Photon Microscopy**

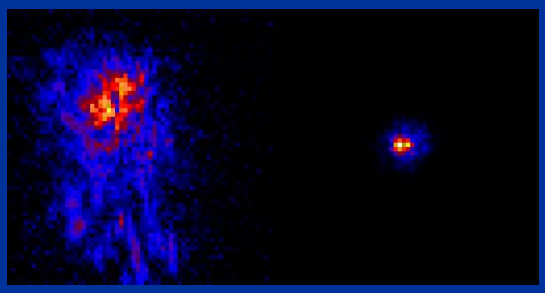
Adaptive Optics in Astronomy



Twinkle, Twinkle Little Star!

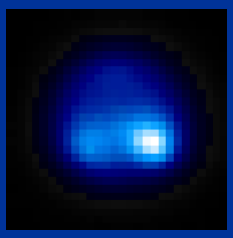
Without AO

With AO

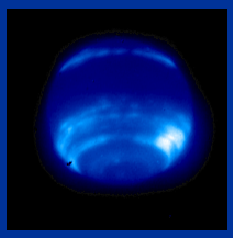


Adaptive Optics in Astronomical Imaging

Neptune



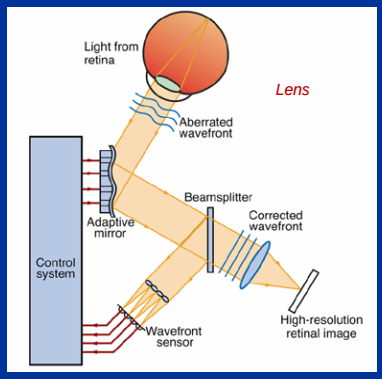
AO Off



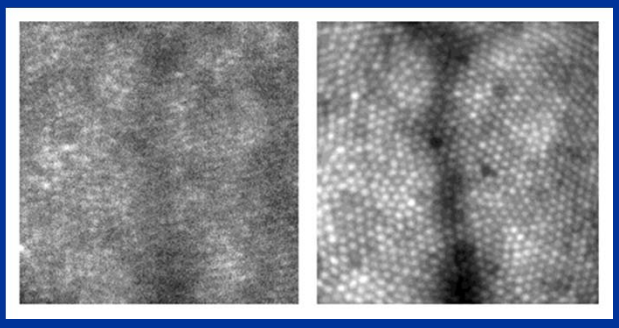
AO On

B. Macintosh et al. (1999)

Adaptive Optics in Vision Science



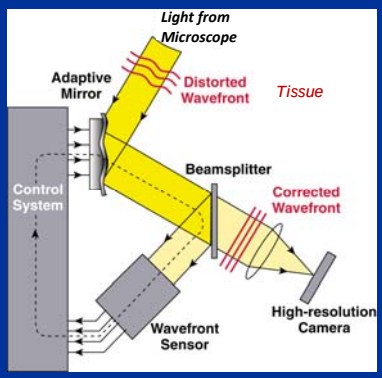
Adaptive Optics in Retinal Imaging



AO Off AO On

Roorda & Williams, 1999

Adaptive Optics in Biology



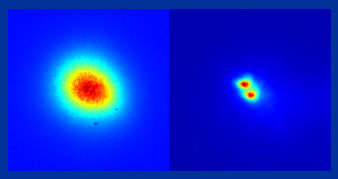
Wide-Field AO Correction of Green Fluorescent Sample Beads

Drosophila Embryo

AO Off AO On

Oscar Azucena, UCSC

AO in Astronomy and Biology

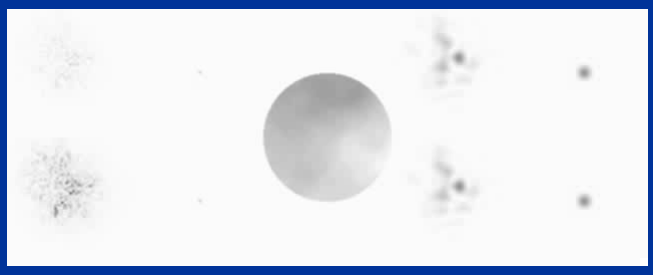


AO reveals a binary star

AO reveals distinct structures

Wavefront Aberrations in Astronomy

1 micron images Phase error 5 micron images
Uncorrected Corrected Uncorrected Corrected

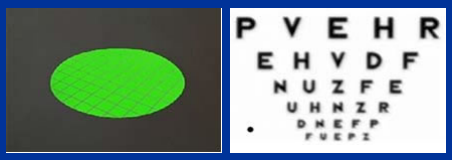


Top row – instantaneous snapshots Bottom row – 5 second integrations

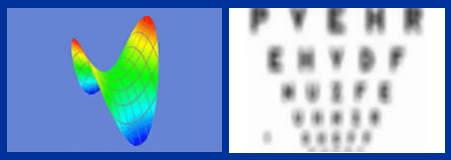
Simulations with an 8.4 m diameter telescope under good seeing conditions

Michael Lloyd-Hart

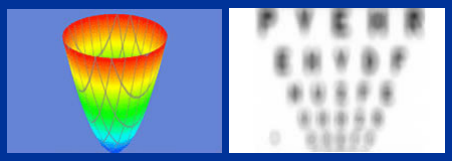
Wavefront Aberrations in Vision Science



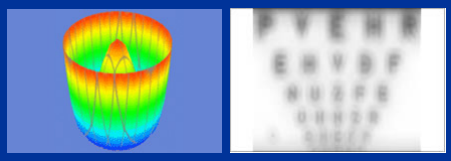
The emmetropic (“normal”) eye



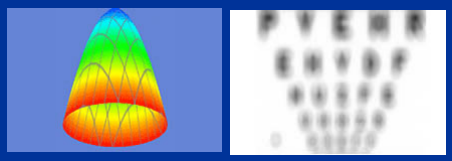
The astigmatic (“football-shaped”) eye



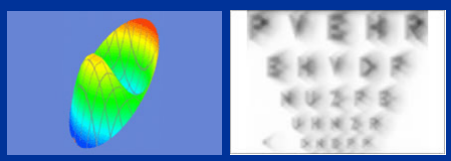
The myopic (“nearsighted”) eye



An eye with spherical aberration



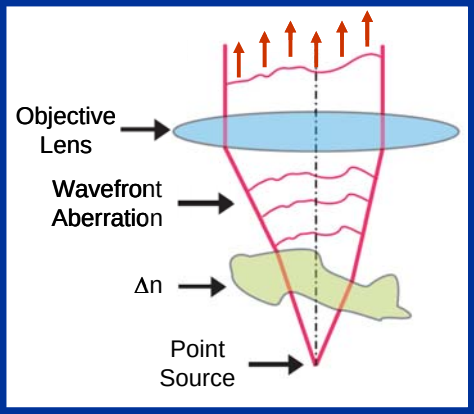
The hyperopic (“farsighted”) eye



An eye with high coma (i.e., keratoconus)

Wavefront Aberrations in Microscopy

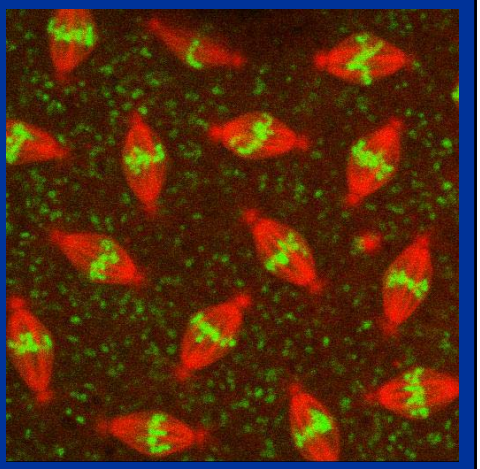
Wavefront Aberrations in Microscopy



Wavefront aberrations due to a change in a sample's refractive index Δn

M. Schwertner, M.J. Booth & T. Wilson, *Specimen-induced distortions in light microscopy*, Journal of Microscopy, Vol. **228**, Pt 1, pp. 97–102 (2007)

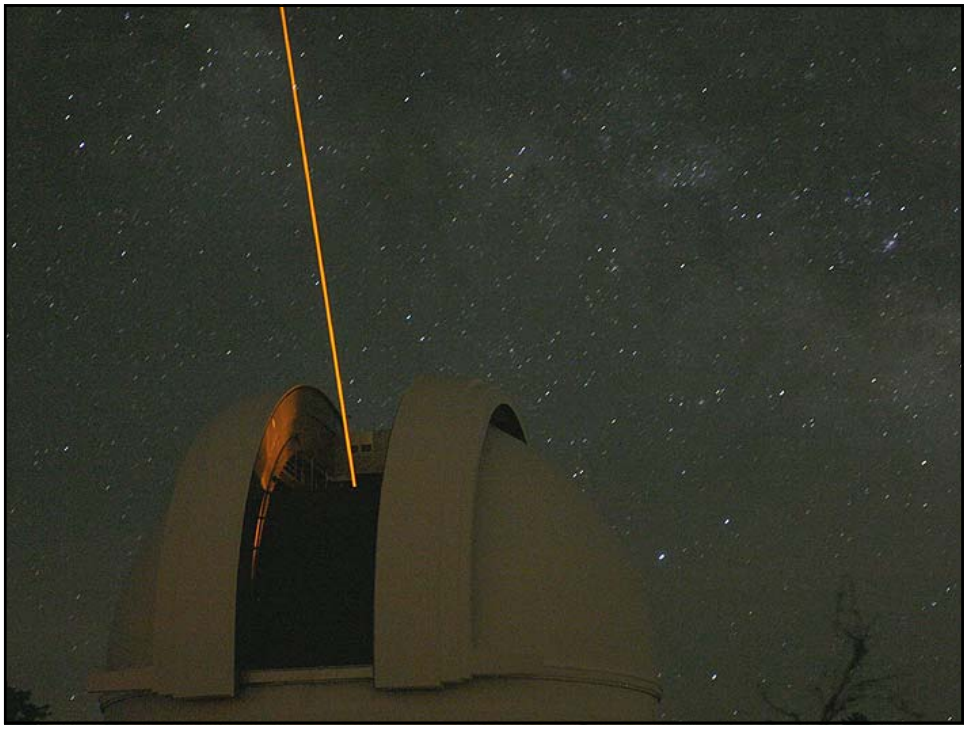
The Need for Speed!



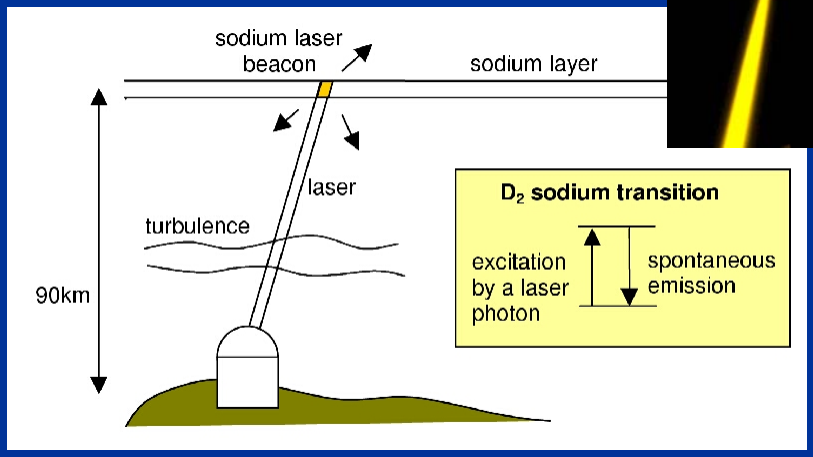
Live Analysis Is Essential for Studying Dynamic Cellular Events

Sullivan Lab, UCSC

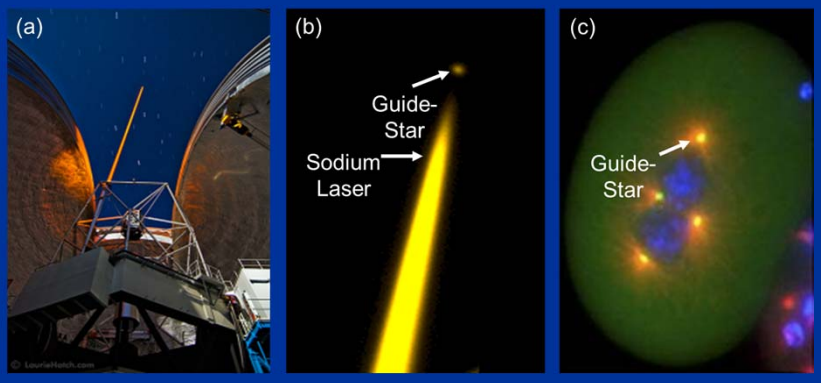
Reference Beacons



Laser Guide Star in Astronomy



Fluorescent Protein Guide-Stars

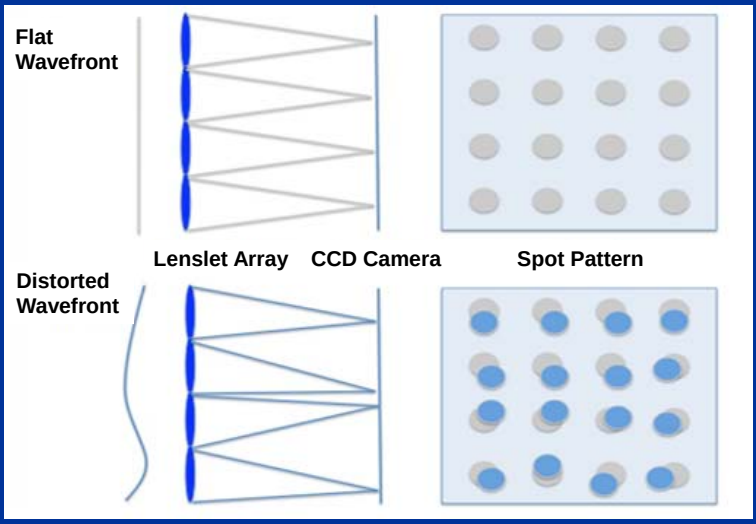


GFP-labeled centrosomes for biological guide-stars in adaptive optic microscopy

Prof. Roy, McGill University

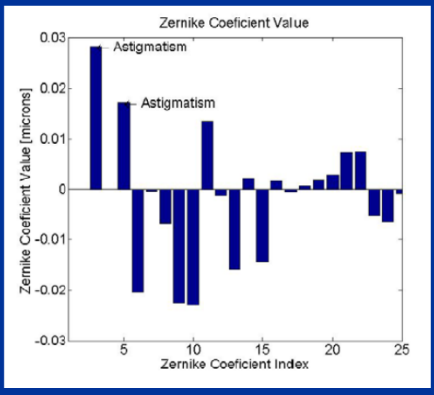
Shack-Hartmann Wavefront Sensor

Shack-Hartmann Wavefront Sensor



http://www.adaptica.com

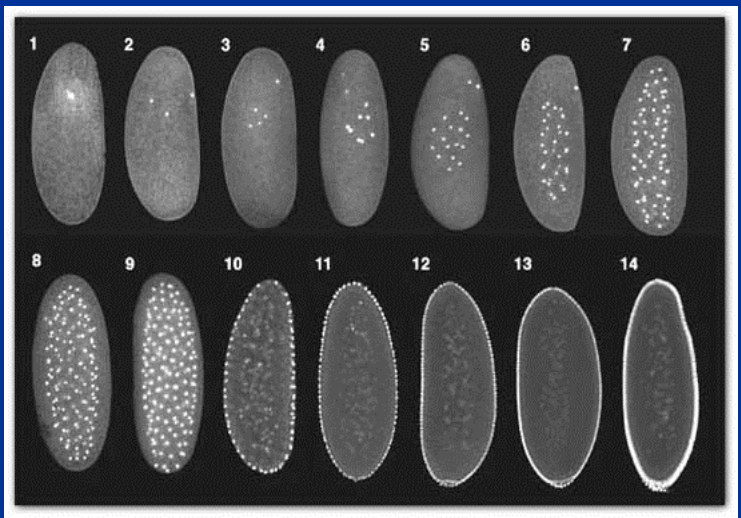
Wavefront Aberrations Measured in Drosophila Embryo



Oscar Azucena, UCSC

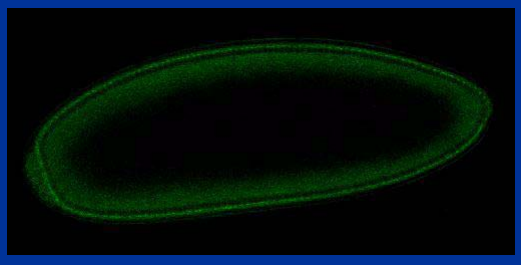
AO Wide-Field Microscope

Mitosis Cycles



Prof. William Sullivan, UCSC MCD Biology

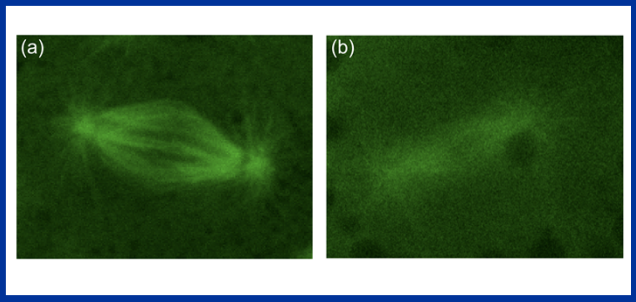
The need for depth!



Loss of signal with depth in a *Drosophila* embryo

Prof. William Sullivan, UCSC MCD Biology

Confocal Images of GFP Labeled Tubulin in Drosophila Embryos

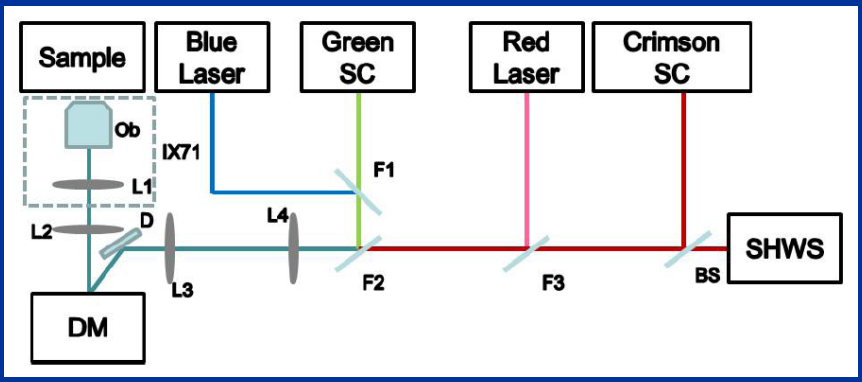


Surface

30 μm below the surface

Prof. William Sullivan, UCSC MCD Biology

AO Wide-Field Microscope

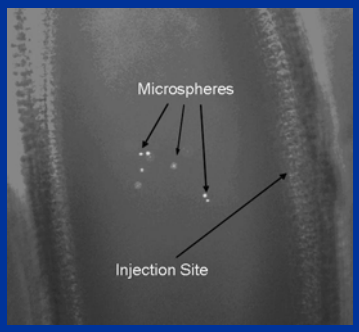


Oscar Azucena, UCSC

Injection of Fluorescent Bead Reference Beacons in Drosophila Embryo

Prof. William Sullivan, UCSC MCD Biology

Injection of Fluorescent Bead Reference Beacons in Drosophila Embryo

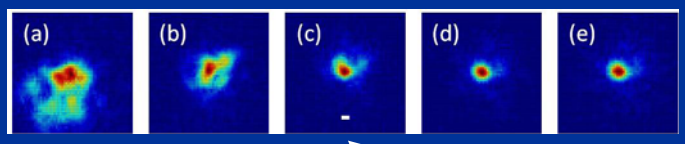


1 μm crimson beads

Oscar Azucena, UCSC

Wide-Field AO Correction of Crimson Reference Beacon

Correction of 1 μm microsphere 100 μm beneath surface embryo



Uncorrected image of a bead

40% correction

The length of the bar is equal to the diffraction limit of the 40X (0.75 NA) objective lens, 0.45 μm.

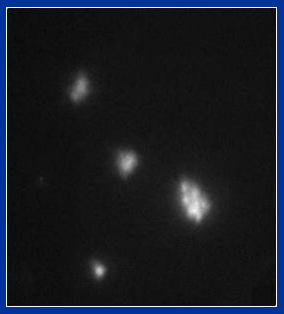
10X improvement in relative Strehl ratio

The Strehl ratio is defined as the ratio of the peak image intensity from a point source compared to the maximum attainable intensity using an ideal optical system limited only by diffraction over the system's aperture.

Oscar Azucena, UCSC

Wide-Field AO Correction of 1 μm Green Sample Beads

Drosophila Embryo



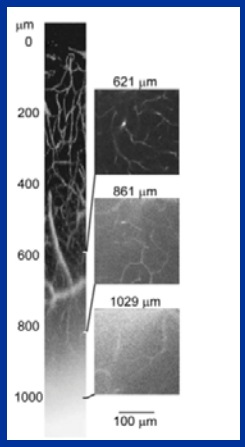
AO Off

AO On

Oscar Azucena, UCSC

AO Confocal Microscope

Deep tissue image degradation



Current Commercial Microscopy

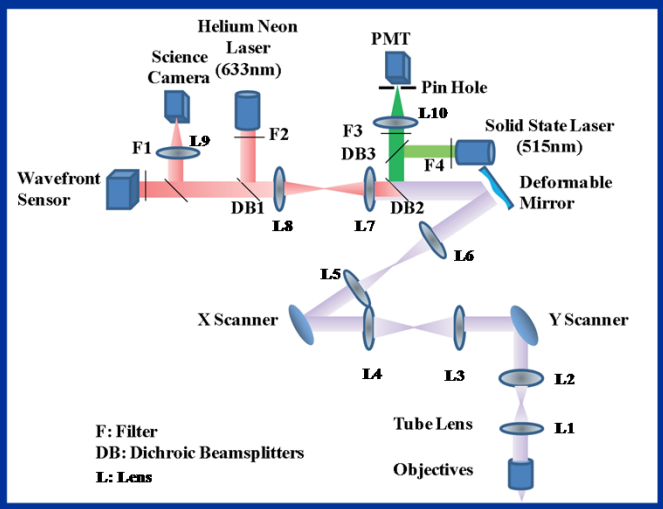
Cross-Sectional Image

~1mm

Deep tissue image degradation

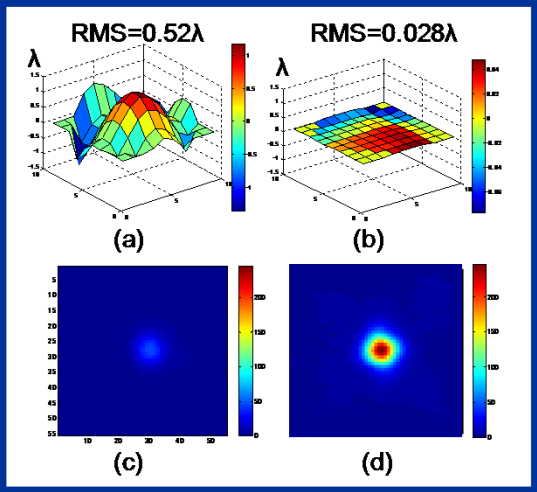
Prof.Yi Zuo, UCSC MCD Biology

AO Confocal Microscope



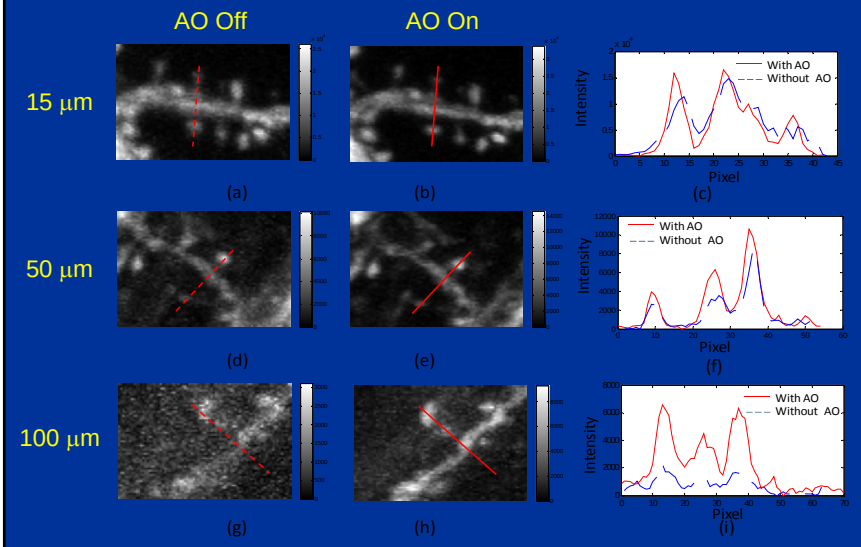
Xiaodong Tao, UCSC

Wavefront measurements from a 1 μm fluorescent microsphere through 100 μm thick brain tissue



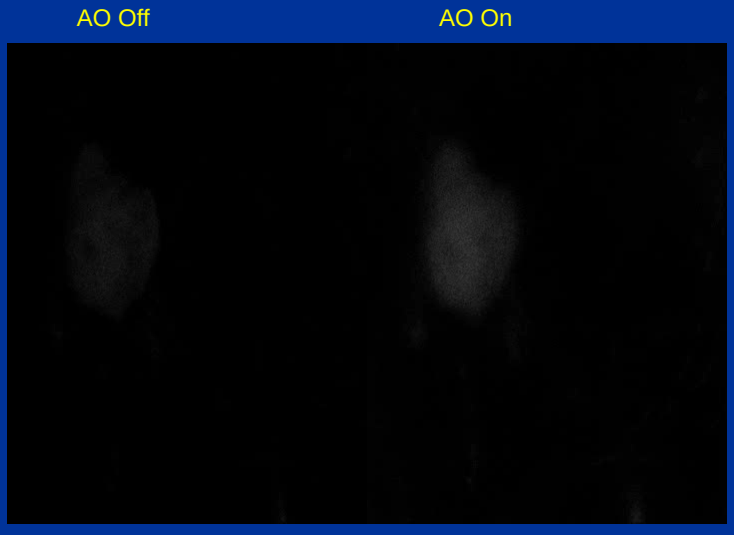
Xiaodong Tao, UCSC

Confocal Images of Mouse Brain Tissue

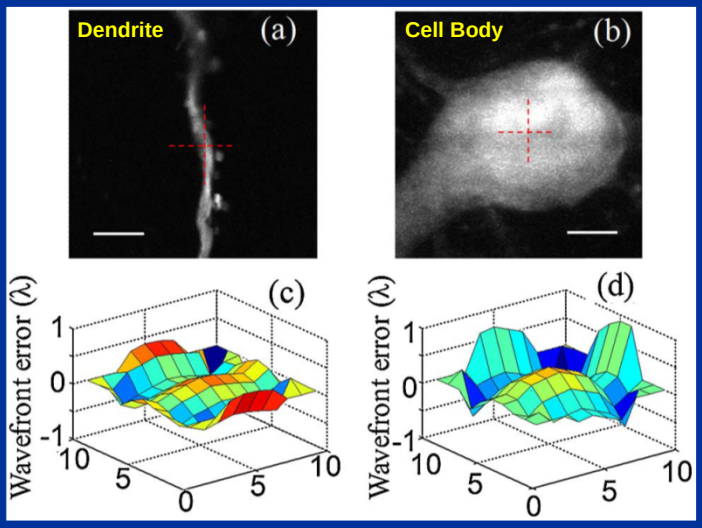


Xiaodong Tao, UCSC

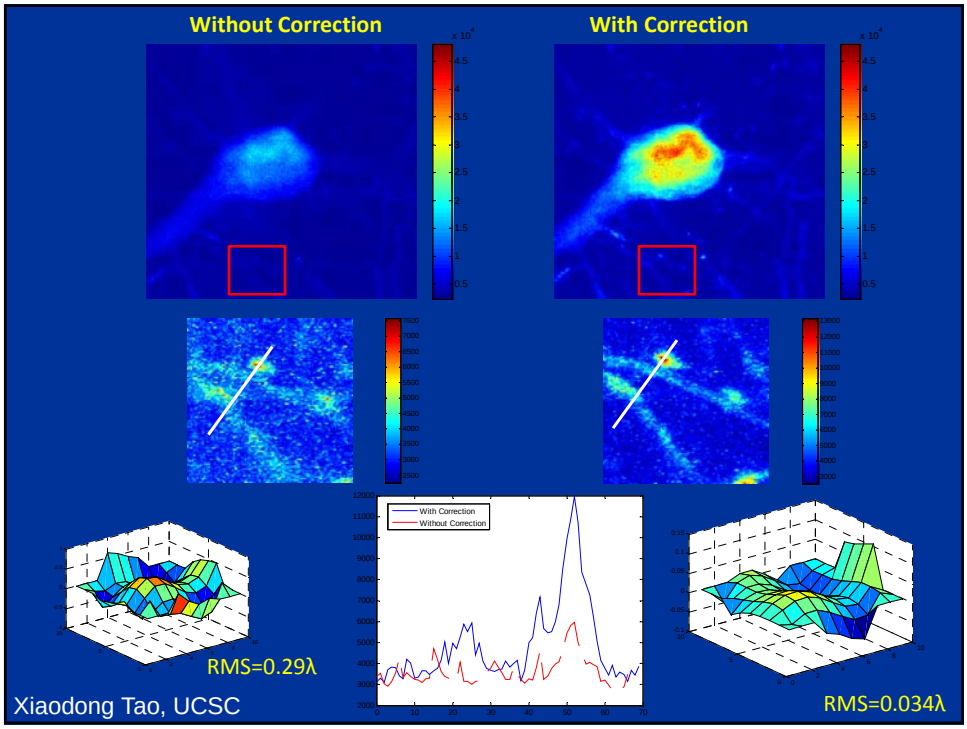
Cell Body



Fluorescent Protein Guide-Stars (YFP)



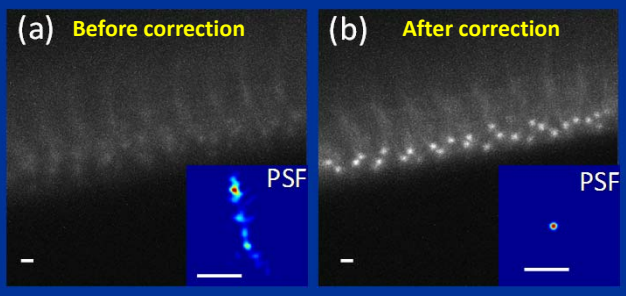
Xiaodong Tao, UCSC



Xiaodong Tao, UCSC

Drosophila Embryo

Depth: 83 μm

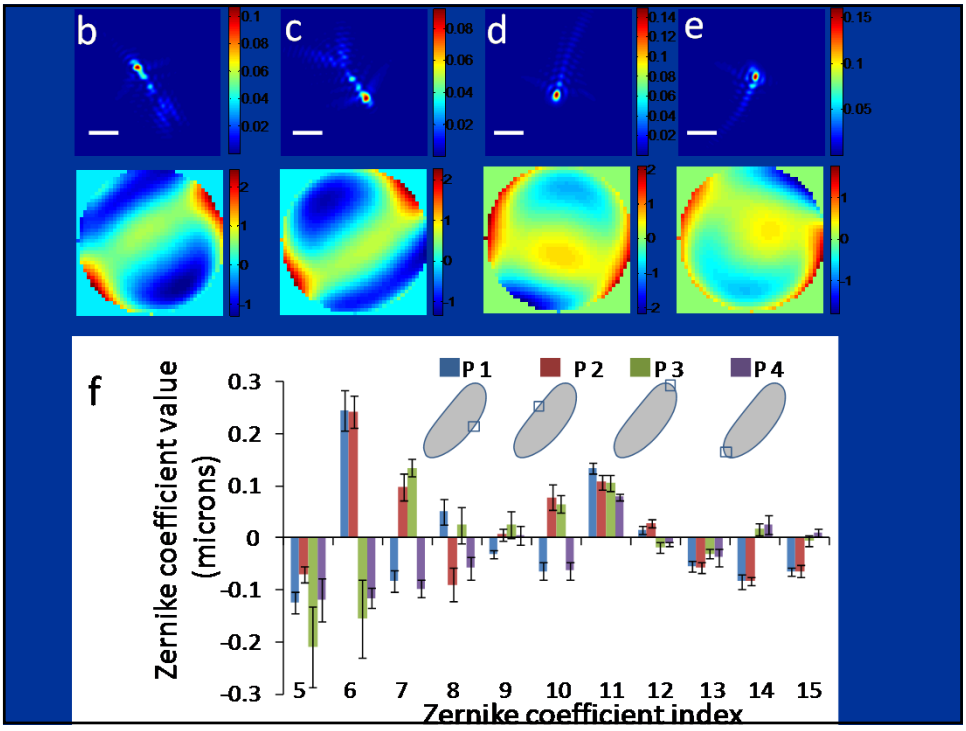


Cannot see the centrosomes

Can see the centrosomes

The images and PSF without (a) and with (b) correction for a cycle 14 fruit fly embryo with GFP-polo at the depth of 83 μm. Scale bars, 2 μm

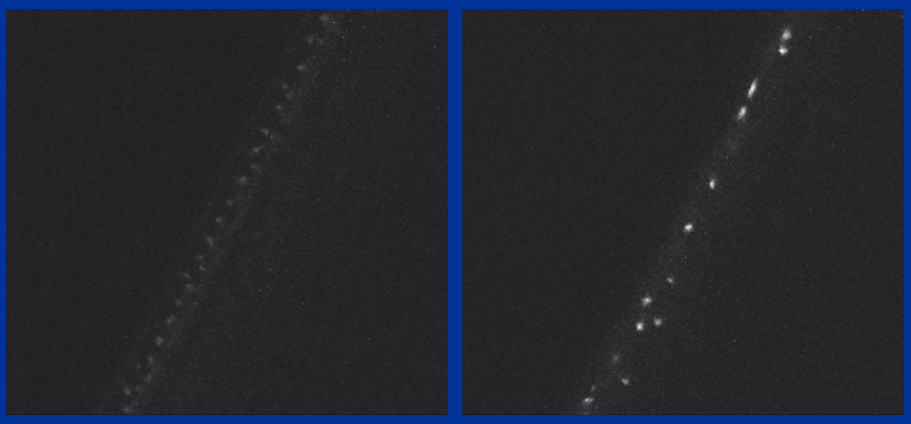
Xiaodong Tao, UCSC



Live Imaging of Centrosomes in Drosophila Embryo

Before correction

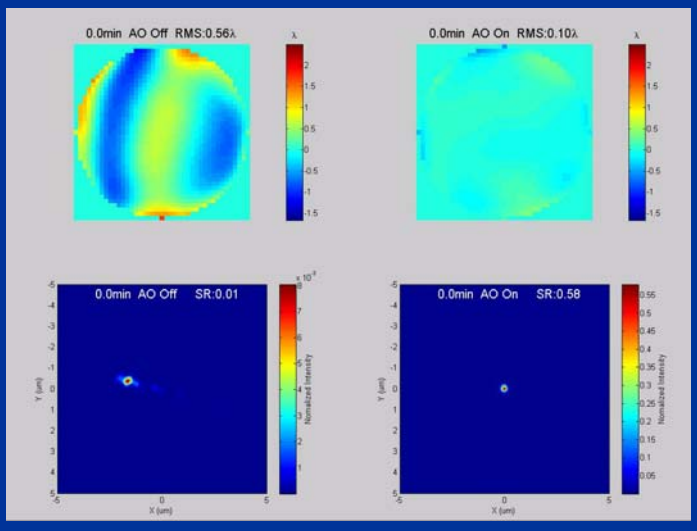
After correction



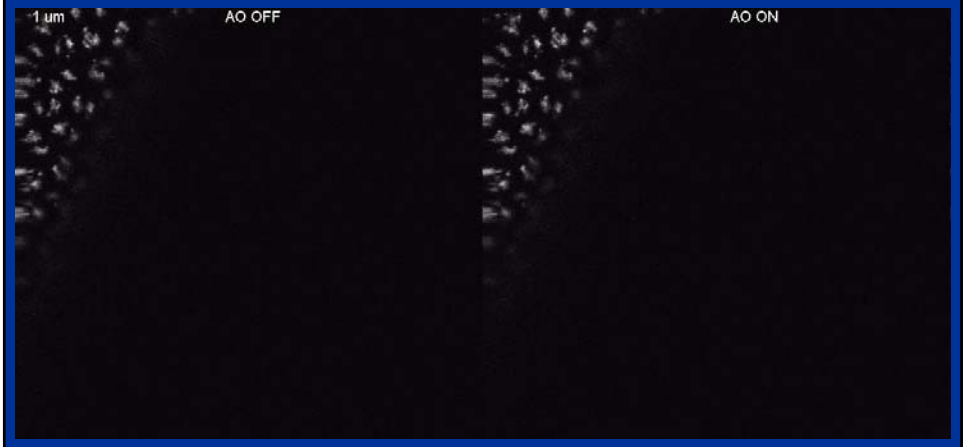
Depth: 80 μm

Xiaodong Tao, UCSC

Live Imaging of Centrosomes in Drosophila Embryo

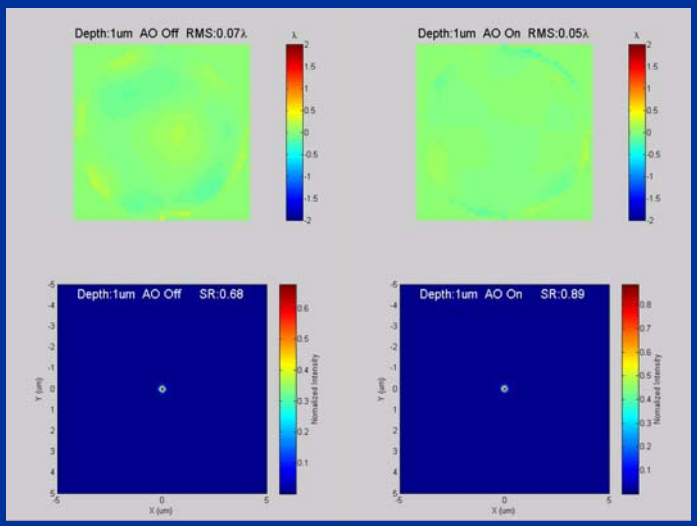


Improvement in Deep Tissue Imaging



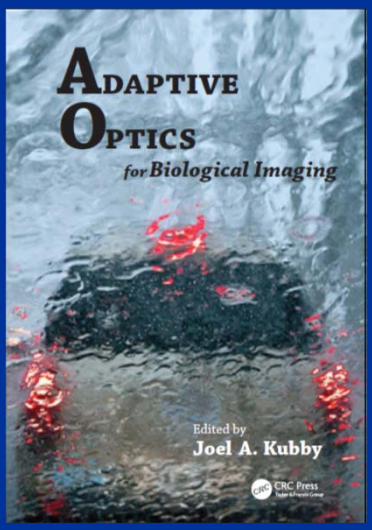
Xiaodong Tao, UCSC

Improvement in Deep Tissue Imaging



Xiaodong Tao, UCSC

Advertisement



Conclusions

- Fluorescent proteins can be used as reference beacons for wavefront measurements in adaptive optics
- Improve relative Strehl ratio in 20 μm thick Drosophila embryo by up to 10x
- Improve relative Strehl ratio in 100 μm thick mouse brain tissue imaged by AO confocal microscope by up to 4.7x
- Currently imaging GFP (*Drosophila*) and YFP (mouse brain tissue) labeled samples
- Extending Measurements to Live Imaging

Collaborators

- Engineering
  - MEMS Group
    - » Oscar Azucena
    - » Xiaodong Tao
    - » Bautista Fernandez
    - » Ziah Dean
  - Laboratory for Adaptive Optics
    - » Don Gavel
    - » Darren Dillon
    - » Marc Reinig
  - Lawrence Livermore National Laboratory
    - » Scot Olivier
    - » Diana Chen
- Biology
  - William Sullivan Group (*Drosophila* embryo)
    - » Jian Cao
    - » Justin Crest
    - » Sheila Kotadia
  - Yi Zuo Group (Mouse brain)
    - » Denise Garcia
    - » Min Fu
- Biophysics
  - John Sedat Group (UCSF)
    - » Peter Kner (now at University of Georgia)

Funding

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- AO Wide-Field Microscope funded by California Institute for Reproductive Medicine (CIRM) Tools and Technologies grant number RT1-01095-1
- AO Confocal Microscope funded by NSF MRI grant number 0852742
- Microscope design funded by UC Lab Fees Program grant number 09-LR-07-118598-KUBJ
- AO Two-Photon Microscope funded by the W.M Keck Foundation.