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Telescopes, stars, and cells: adaptive optics microscopy

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A technology derived from astronomy holds promise for imaging live deep tissue at the resolution of single cells.

In the late 1980s, well before the digital revolution reached microscopy, a weekly job in our cell biology lab was to immerse black-and-white 35mm film in a tank of pressurized hydrogen gas. This was a trick borrowed from astronomers. The hydrogen-sensitized film enabled the long exposures required for imaging faint objects in deep space. Similarly, we used the sensitized film to pick up faint fluorescent signals from deep within cells. Now, some two decades later, cell and developmental biologists are again turning to an imaging technique pioneered by astronomers, known as adaptive optics (AO).

Adaptive optics has transformed imaging from ground-based telescopes. The technology continuously corrects wavefront distortions produced from starlight passing through the earth's turbulent atmosphere. A key component of the imaging system is a deformable mirror operating at rates of 30Hz to over 1kHz that restores refracted wavefronts to plane waves through an array of actuators underlying the mirror. These systems rely on a Shack-Hartman sensor to precisely measure the wavefront distortions and either a natural or laser-induced guide star to provide a known point source of light. Over the past five years, a group of us (engineers, astronomers, and biologists) at the University of California, Santa Cruz, have made significant progress applying AO technology to microscopy.[1,2]

The past two decades have seen dramatic changes in light microscopy. Previously, to obtain high-quality fluorescent images, samples were fixed in formaldehyde or other crosslinking reagents. With the advent of confocal microscopy and a new generation of probes, namely, green fluorescent protein, obtaining live high-resolution time-lapse fluorescent images from living tissues has become routine. This has been a tremendous advance for biologists studying the complexities of living systems at single-molecule resolution.

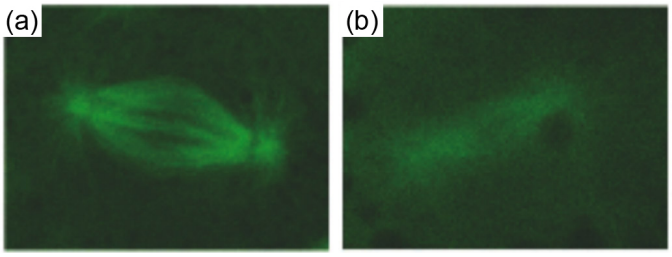

Figure 1. *Image aberration in a Drosophila melanogaster embryo. (a) Mitotic spindle at the surface of an embryo versus (b) distorted mitotic spindle ∼30μm deep inside.*

The problem now facing biologists is that, as one probes deeper into tissues, the image quality rapidly deteriorates. Figure 1 shows images of a mitotic spindle located at the surface and 30μm below the surface of a *Drosophila melanogaster* (fruit fly) embryo. Even with this modest increase in depth, image quality degrades in short order. While other approaches, such as magnetic resonance imaging, can image deeply through multiple tissues, currently no technology is capable of capturing live deep tissue at the resolution of a single cell.

A major factor contributing to degradation with depth is that the wavefront of the fluorescent emission signal is distorted as it traverses the turbulent cytoplasm of living tissues. This challenge to biological imaging is similar to that faced by astronomers viewing objects in space from ground-based telescopes. Incoming light from space becomes severely distorted once it enters the earth's turbulent atmosphere. To correct for these distortions, AO was applied to ground-based telescopes. Due to the similarity in imaging challenges, our interdisciplinary group has begun using AO principles and technology to improve deep-tissue fluorescent biological imaging. One problem that immediately arose in considering this approach was how much of the degradation was due to refraction versus scatter. AO can correct the former but not the latter. We addressed this issue by injecting fluorescently labeled dextran beads in the large

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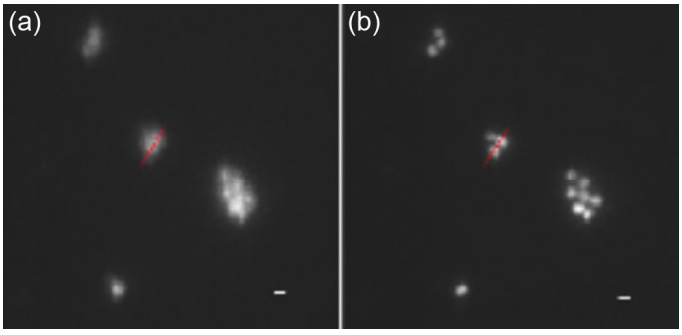

Figure 2. *Fluoresecent dextran beads underneath a Drosophila embryo, either without (a) or with (b) AO correction. (Photo courtesy of the Optical Society of America.[1])*

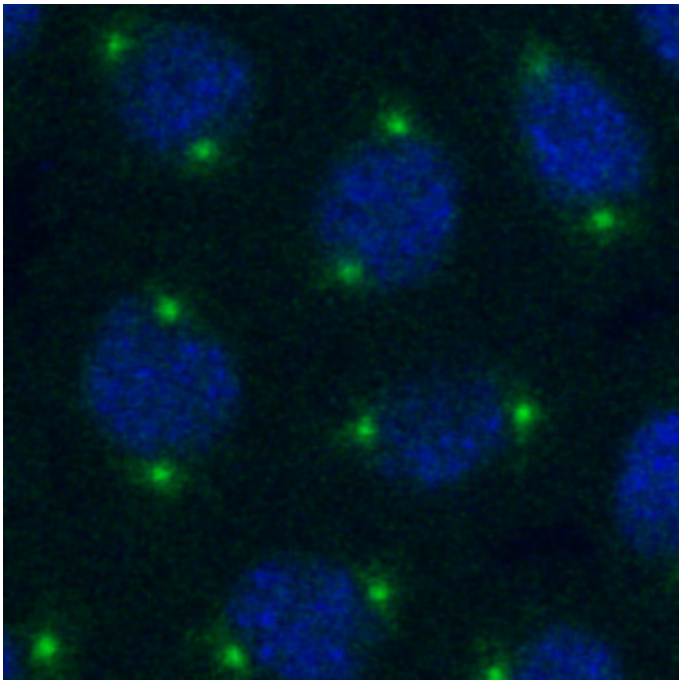

Figure 3. *Drosophila embryo immediately following fertilization, showing points of localization of the protein kinase Polo (green) suitable for a potential 'guide star.' DNA is shown in blue.*

Drosophila embryos. Our measurements indicated that at depths of 150–200μm, a little over 50% of the image degradation is due to optical aberrations that are correctable through AO. How this value will vary with cell and tissue type is unknown, but these studies clearly demonstrate the potential of this approach.

Developing an AO microscope for biological imaging also requires identifying the biological equivalent of a guide star. Astronomers use either a natural star close to the object being observed or a strong laser to excite the sodium layer in the upper atmosphere to create an artificial guide star. We have had excellent success using 1μm fluorescently labeled dextran beads injected into *Drosophila* embryos. Imaging these beads at a depth of 100μm allows a 10-fold improvement in the ratio of the observed to theoretical maximum peak intensity (Strehl ratio) and results in a corrected field of view of almost 40μm in diameter centered around the guide star.[2] As shown in Figure 2, these beads have been used to measure and correct wavefront aberrations induced by the yolky cytoplasm of a *Drosophila* embryo (left and right panels with and without AO, respectively).[1]

The field of AO is in its infancy, and much work remains. For example, while fluorescent dextran beads serve as suitable biological guide stars, they must be injected, which makes their distribution and position difficult to control. Consequently, there is a need for a more universal guide star. Obvious candidates are green-fluorescent fusion proteins. Because these are genetically encoded, they can be easily spliced into virtually any protein and their position and distribution in the tissue precisely defined. Figure 3 provides an example of a highly conserved protein kinase, Polo, which concentrates at the centrosomes and would likely serve as an excellent biological guide star (Polo in green, nuclei in blue). For AO microscopy to become broadly applicable, we must also characterize wavefront distortions in a wide variety of cell and tissue types, which is where we will focus our next steps.

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