Improving microscopy by following the astronomers' guide star

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A corrective strategy used by astronomers to sharpen images of celestial bodies can now help scientists see with more depth and clarity into the living brain of a mouse. Eric Betzig, a group leader at the Howard Hughes Medical Institute's Janelia Farm Research Campus, will present his team's latest work using adaptive optics for biology at the annual meeting of the American Association for the Advancement of Science in Washington, D.C. during a press conference on Thursday, Feb., 17, and a panel discussion on Friday, Feb. 18.

A key problem in microscopy is that when the light shines on a biological sample, such as a slice of brain tissue, light waves hit the cells and bounce off in different directions. The larger the piece of tissue, the more interesting and diverse its collection of parts, which makes the light waves bend and scatter in unpredictable ways.

For the past decade, researchers have been trying, with limited success, to sharpen blurred images of biological specimens using a method astronomers call adaptive optics. Recently, however, Betzig and postdoctoral researcher Na Ji, have made large strides toward improving resolution deep into tissue by combining a new approach to adaptive optics with an imaging technology called two photon fluorescence microscopy. Their results, published in 2009 in the journal Nature Methods, describe the first applications of adaptive optics to improve images of brain slices taken from mice.

Astronomers apply adaptive optics by shining a laser high in the atmosphere in the same direction as the star or other object they want to observe. The light returning from this so-called guide star gets distorted as it travels through the turbulent atmosphere back to the telescope. By using a tool called a wavefront sensor, astronomers can measure this distortion directly, and then use these measurements to deform a telescope mirror to cancel out the atmospheric aberrations. The correction gives a much clearer view of the target. Today, adaptive optics instrumentation accompanies all of the world's major telescopes.

Unlike in astronomy, microscopists can't place a wavefront sensor within a live animal to directly measure the distortions of light deep within tissue. To get around this problem, Betzig reasoned that the perfect focus is nothing more than a bunch of rays converging from many different directions to the same identical point. The heterogeneity of tissue means that ray is deflected differently so they no longer meet at a single point. Betzig figured that if they could study the rays individually, they could correct their deflections and steer them back to a single focus.

For their 2009 study, Betzig and Ji buried fluorescent beads underneath thick slices of mouse brain. The beads act as guide stars to help measure the deflections of the rays. To do that, the pair use a one-inch display called a spatial light modulator. The display allows them to turn on one ray at a time and then take an image of the bead. They can then determine how much the ray is deflected from the amount the bead's image is shifted relative to the desired focal point. The display is then used like a small, tiltable mirror to steer the ray back to the focal point. The process is then repeated with each of the other rays. This strategy improves the fluorescent signal and recovers optimal resolution through a chunk of tissue up to 400 micrometers thick. "Another advantage is that it's very efficient in terms of how little light is needed," Betzig says. "Light is not completely noninvasive, so as microscopists we have to be very careful not to damage our specimens."

Ji, who will soon begin her own lab as a fellow at Janelia Farm, has recently taken the technique to the next level: live brain imaging in mice. To do this, she uses genetic engineering to label the brains of mouse fetuses with a fluorescent marker of

neurons while at the same time injecting fluorescent bead guide stars. After the mice mature, Ji builds a window into the brain by removing a piece of skull and replacing it with a clear glass cover. She then uses the adaptive optical microscope she and Betzig built to peer through the glass and image the individual neurons underneath.

One of the concerns the researchers faced when imaging a live animal was how long a correction would persist. In astronomy, stars twinkle so fast that up to a thousand corrections are needed per second. Fortunately, Ji and Betzig learned that, for an anesthetized mouse, a single correction would remain valid for nearly an hour. Another question was whether the correction made at a single guide star bead could be applied to surrounding areas. Although the answer varies from sample to sample, Ji's work has shown that a single correction will often apply to a space of over 100 micrometers in each direction, a volume that can fit dozens of neurons. "It would be prohibitively slow if you had to correct at every point throughout an entire volume," Betzig says. To address this same problem in the field of astronomy, scientists employ a series of deformable mirrors that each look at guide stars in different directions. Betzig hopes to use a similar strategy to widen the correction even further in his microscope.

Since arriving at Janelia Farm in 2005, Betzig and his colleagues have pioneered new super high-resolution imaging techniques and shared them with biologists. One of them, photoactivated localization microscopy or PALM, maps individual protein molecules to produce images with 10-20 times the resolution of a traditional light microscope. PALM and other types of imaging, such as confocal microscopy and wide-field imaging, can be greatly improved with adaptive optics, he says. This is only the beginning. "What we do is primitive compared to the sophistication of what they do in the astronomy community," Betzig says. "I still feel we have a lot to learn from astronomers."

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