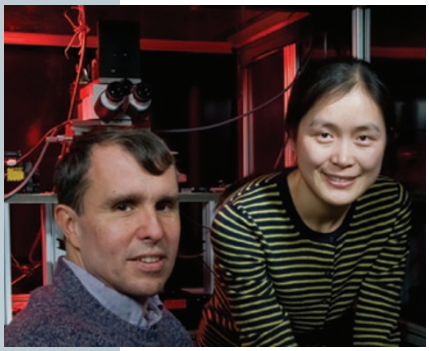
THE AUTHOR FILE

Eric Betzig and Na Ji

Adapting optics: techniques for seeing stars scale to cells.

As a boy who dreamed of being an astronaut, Eric Betzig hung a poster of the Crab Nebula in his bedroom. That image is a fuzzy blur compared to the sharpness and detail that can be captured by today's ground-based telescopes. Much of the clarity comes from techniques, collectively termed adaptive optics, which actively correct distortions that occur as light travels through the atmosphere.



Just as kilometers of air distort light travelling through it and into a telescope, tissues also distort light in microscopy. Variations within tissue, including the structures researchers most want to see, bend light rays so that they do not all converge sharply at a focal point. Betzig and his postdoc Na Ji thought adaptive optics approaches similar to those that bring the heavens into focus might also apply for cells in thick tissue slices.

Betzig is perhaps best-known for developing methods that let microscopes overcome a barrier known as the diffraction limit, which normally leaves cellular structures blurry if they are smaller than 250 nanometers (half the length of a small mitochondrion). But conversations with neuroscientist colleagues at Janelia Farm in Ashburn, Virginia, USA led him to consider bigger-scale problems. Rather than looking closely at a few cultured cells, these researchers want to peer deep into mouse brains. But when dealing with thick tissue, ordinary microscopy techniques often do not work, let alone Betzig's special 'super-resolution' techniques. "In biological tissue or in other organisms, you don't even get diffraction-limited resolution because of the optical properties of the sample," explains Ji.

Ji began with experiments to determine just how bad the optics inside the brain are. This involved *in utero* surgery to get tiny fluorescent beads into mouse brains. Buried inside cortical tissue, beads only half a micrometer in diameter can appear as 20-micrometer-long smears.

In addition to showing just how distorted the images could be, the bead experiments also suggested an intuitive solution. Structures such as mitochondria, blood vessels and nuclei all diffract to different degrees, creating overlapping distortions that are difficult to detangle. Taking individual swaths of light rays and swinging them back to a common focus one by one could fix the distortion.

But identifying an intuitive fix and implementing it can be very different things. Betzig and Ji were not sure how many corrections would need to be applied or how often or how much computational power these corrections would require. In astronomy, for example, adaptive optics techniques to adjust for a flickering atmosphere require very quick corrections, on the order of a thousand per second. In tissues, aberrations seem stable over hours. But spatial corrections can be much more challenging.

Betzig and Ji used a commercially available spatial light modulator with over two million pixels, each of which could be independently adjusted to tilt light beams. In fact, light could be tilted so much that it fell completely outside the microscope objective. This feature allowed the researchers to scan the sample with only one small group of rays at a time, identify the displacement of the image and determine exactly what tilt could be applied to bring those rays to the focus. To control the beams and correct the images, Ji and Betzig collaborated with software engineer Daniel Milkie of Coleman Technologies.

"We can see deeper into the brain with better detail." —Na Ji

The results are the elimination of ghost images and sharper focus in calibration samples of fixed brain slices (page 141), and Ji is already collaborating with neuroscientists at Janelia Farm. "We're just now getting *in vivo* data," she says. "We can see deeper into the brain with better detail."

It is too early to try to figure out how such tools will be distributed, Betzig says, adding: "Where we are now is a really good milestone for proving the validity of the approach, but there's still a lot of hard work to be done." Compared to the astronomy community, microscopy is still in the infancy of what can be done with adaptive optics. That will change, Betzig predicts. "In ten years time or thereabouts, [adaptive optics] will be ubiquitous in microscopy for studying anything that's beyond the thickness of a single cell."

Monya Baker

Ji, N., Milkie, D.E. & Betzig, E. Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues. *Nat. Methods* **7,** 141–147 (2010).