Sharper Image

No consumer toy, here is a researcher's dream—a light microscope that goes where none has gone before, down to the level of individual protein molecules.

MORE THAN A CENTURY AGO, GERMAN PHYSICIST ERNST ABBE DISCOVERED A FUNDA*mental limitation on how sharply a conventional light microscope can focus on extremely small objects. Because of the way light moves in the realm of the infinitesimal, no matter how powerful the microscope, it cannot distinguish particles closer than about 200 nanometers (one-quarter the diameter of a typical bacterium) as separate objects. ¶ The problem is that the wave nature of light becomes more apparent in* the microscopic world. Particles inside cells distort light waves in much the same way that raindrops falling into a pond form concentric ripples. With so many structures packing cells, high-magnification microscope images become torrents of overlapping light patterns.

As a graduate student in the 1980s, Eric Betzig wasn't satisfied living within Abbe's constraints, so he developed an imaging technique called near-field microscopy to circumvent them. Honing the method at Bell Labs, Betzig achieved unprecedented resolutions of 30–50 nanometers.

"A new photo-activatable fluorescent label was the missing element that sparked the PALM concept."

HARALD HESS

But even at that level, images weren't sharp enough to meet what Betzig calls the First Holy Grail of optical microscopy: imaging individual protein molecules within cells. He and his Bell Labs colleague Harald Hess realized that, by discerning individual proteins, cell biologists could keep more intimate tabs on what those proteins were doing and how they interacted.

Last year, the two inventors—both unemployed at the time—developed an elegant procedure out of an idea they had begun exploring together in the 1990s. Instead of having a muddle of fluorescently labeled proteins glowing at once, sloshing light waves everywhere, they found a way to turn on just a few molecules at a time. That way, the concentric waves surrounding each molecule wouldn't overwhelm the image.

It's easy to pinpoint where a pebble falls in a pond by looking at the center of the ripples. Likewise, Betzig and Hess reasoned, by making just a few molecules in the cell visible at a time, they could pinpoint those molecules and computationally erase the ripples from the image. Then they could do another exposure on a different set of molecules in the same field. With automated optics and electronics, they could repeat the operation, snapping thousands of exposures, until they captured virtually every labeled molecule. The composite image that emerged by overlaying these pictures would reveal the locations of all the individual molecules.

The key to making the method work is a new generation of photoactivatable fluorescent protein labels developed by Jennifer Lippincott-Schwartz and George Patterson at the National Institutes of Health (NIH), with whom Hess and Betzig collaborated. Before they can fluoresce, the probes must be activated by exposure to violet light. With genetic engineering techniques, any protein can be tagged with the photoactivatable labels. By shining just a small amount of violet light on cells, the researchers can activate and image a different random sample of labeled protein molecules in every snapshot.

The whole discovery was inspired by a visit to researchers at Florida State University. "A new photoactivatable fluorescent label that we learned about from Michael Davidson at Florida State was the missing element," says Hess. "It dovetailed with work that we did years ago at Bell Labs and sparked the PALM concept."

After two months in a makeshift lab constructing the photoactivated localization microscope (PALM), the researchers shifted focus to Lippincott-Schwartz's lab in Bethesda, Maryland, where the team soon saw the images they had hoped for. The pictures show cellular structures at a resolution comparable to that of electron microscopy, but with the added benefit of being specific to only the desired target protein localized to 2- to 25-nanometer precision. "It knocks the socks off of standard fluorescence microscopy from a resolution perspective," Betzig says.

The team revealed the technique at an April meeting at NIH, and their paper,

Mark Harmel

with its spectacular images, appeared August 10, 2006, in the advance online edition of *Science*, and in the print version on September 15.

Securing the First Holy Grail wasn't the only cause for celebration for Hess and Betzig, who are no longer unemployed. Last October, Betzig joined HHMI's Janelia Farm Research Campus (JFRC) as a group leader. Hess was appointed shortly thereafter as director of Janelia's applied physics and instrumentation group. Revolutionary as PALM is, Betzig says, "We have a long way to go to apply it routinely to biology." For example, they want to make PALM compatible with a larger palette of photoactivatable dyes so researchers can label multiple proteins at once. "Knowing [the intracellular location of] one protein is nice," Betzig says, "but what you really want to know is how protein A is interacting with protein B."

Capturing 10,000 frames to create a single picture takes hours, so Betzig and his JFRC colleagues are now designing another microscope in pursuit of his Second Holy Grail: high-resolution video images of live cells, captured noninvasively, *in real time*. That goal poses major hurdles. First, there's the light problem. "You can only get so many photons out of a cell before it won't give up any more," Betzig says. And collecting multiple video frames quickly drains the photon supply. Then there's the data problem: high-resolution video microscopy requires monumental computing power and software savvy. But Betzig savors the challenge. On returning from his first visit to Janelia Farm, he told Hess, "I've just been to heaven." ■ – PAUL MUHLRAD

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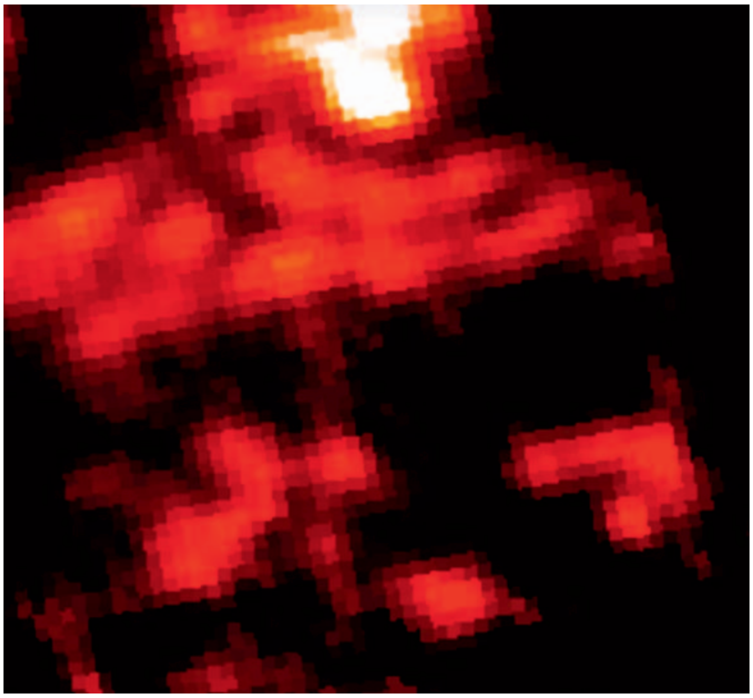
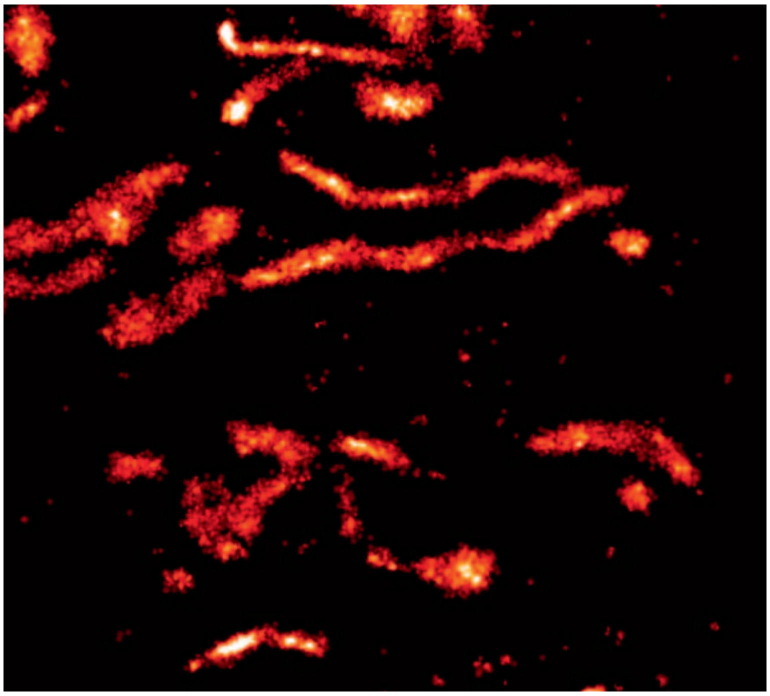
ERIC BETZIG





LEFT: *Viewing a mitochondrion using conventional diffraction-limited microscopy offers a resolution (200 nanometers) barely sufficient to visualize the mitochondrial internal membranes.* **RIGHT:** *Viewing the same mitochondrion by imaging sparsely activated fluorescent molecules one at a time—using PALM—provides much better resolution (20 nanometers), producing a detailed picture of the mitochondrion's internal membranes.*

Images from Science 2006 Sep15; 313(5793):1642-5. Epub 2006 Aug 10. Reprinted with permission from AAAS Photo: Paul Fetters