THE AUTHOR FILE

Erik Jorgensen

Fluorescent proteins can be located in electron micrographs.

Just five years ago, two things Erik Jorgensen at the University of Utah wanted most as a scientist seemed condemned by physics to exist in separate spheres. He was trying to discover where exactly certain proteins were in synapses, but techniques for imaging these structures could not be used to identify the proteins.

Electron microscopy can reveal morphological detail as small as one nanometer, but specific proteins cannot be discerned. Genetic engineering can add fluorescent tags to proteins, but standard fluorescence microscopy can localize them only to within about 250 nanometers, several times the width of the synaptic cleft.

Wayne Davis

Jorgensen toyed with the idea of combining electron and fluorescence microscopy, but dismissed it as impossible. Properties of light itself prevent high resolution. According to a principle known as the limit of diffraction, optical lenses cannot typically reveal details much smaller than half the wavelength of light passing through them.

Jorgensen learned of Stefan Hell's work while visiting a colleague in Göttingen, Germany. "He leaned across the table and said, 'there's a guy here who can break the diffraction limit'," Jorgensen recalls. "It was almost like he didn't want to speak too loud." Jorgensen's impression was of "highfalutin physics" that would never be applicable to cell biology. Later, he heard Hell speak about his work on super-resolution microscopy. "It was like a lightening bolt," he says. "I'd been thinking about this problem that was insurmountable, and here was the solution." If fluorescent proteins could be preserved, electron micrographs could be matched with images from light microscopy on a nanometer scale, the scale that matters for proteins.

First Jorgensen had to be sure he could keep fluorescent proteins viable. Conditions that make the best samples for electron microscopy are hostile for proteins. Acid treatments quench fluorescence. Fixatives that cross-link cell components cleave proteins, and embedding cells in plastic dehydrates them and denatures their proteins.

Jorgensen credits first author Shigeki Watanabe with finding the right preparation protocols. "He'd go through a sample 'prep' and see at what point the fluorescence was killed. He just picked the problems off one by one until he solved it." The process, says Jorgensen, was not unlike climbing a mountain made up of a series of cliffs.

They also had a few big breaks. A search of the literature identified a plastic from the early days of electron microscopy that had been abandoned because of its tendency to absorb water. The plastic makes samples more difficult to cut but, as researchers in Jorgensen's laboratory discovered, preserves fluorescent proteins.

Choosing scanning electron microscopy (SEM) was also crucial. SEM, says Jorgensen, makes stunning pictures of ant antennae and fly eyes, but most cell biologists stick to transmission electron microscopy, which is what he initially planned to use. But after a chat with a SEM enthusiast, Jorgensen realized that, unlike transmission electron microscopy, SEM could work with samples mounted flat on a slide, a convenient preparation for fluorescence microscopy.

The next step was to see whether the same samples that worked for electron microscopy could also be used for super-resolution microscopy. Hell immediately agreed to try imaging with his technique, stimulated emission depletion (STED), and Jorgensen determined that researchers in his own lab would pursue another super-resolution technique, called photoactivated localization microscopy (PALM). The latter required careful planning. Zeiss is still commercializing PALM, and at the time of the research, the company had only five microscopes available for beta testing. Jorgensen recalls convincing the Zeiss representative that his lab should get the machine destined for North America. "I bought him a good German beer and showed him our preliminary results," he says.

Meanwhile, tension was building with Jorgensen's collaborators in Germany. No matter how scientists in Jorgensen's lab packed the samples, when they arrived in Germany they were no longer fluorescent. "I could detect the irritation in the emails," Jorgensen recalls. Then, by accident, Watanabe let one of his slides dry out and wondered what would happen if he added a bit of water. To everyone's surprise, fluorescence returned, and the samples in Germany could also be revived. "That was the most magical moment," says Jorgensen, "when you saw the color come back into this black, dead sample."

The main obstacle now, says Jorgensen, is the scarcity of suitable microscopes, but he expects that problem to be solved soon. "It's going to be a couple of years and then we can all start tearing the cell apart."

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