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## New Microscopy Technique Transforms Millions of Points of Light into Detailed 3-D Images

Recent advances in fluorescence microscopy have enabled scientists to see inside cells with far better clarity than traditional optical imaging techniques, which are limited by the wavelength of light. Now, Howard Hughes Medical Institute (HHMI) researchers have added a whole new dimension to that newfound clarity.

In the February 8, 2008, issue of the journal *Science*, HHMI investigator Xiaowei Zhuang and colleagues at Harvard University announce an advance that allows researchers to examine the complete three-dimensional morphology of many of the tiniest structures of cells. The new technique is an expansion of a method Zhuang's team first developed in 2006 to enable super-resolution imaging - 10 times better than what is attainable with conventional light microscopy - in two dimensions. Bo Huang, a postdoctoral researcher in Zhuang's lab, is the first author of the paper.

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In 2006, Zhuang and colleagues developed stochastic optical reconstruction microscopy (STORM), a super-resolution technique that uses fluorescing probes that can be turned on and off to let researchers zero in on some of the finest details of cellular structures. Other methods of super-resolution imaging include photoactivated localization microscopy (PALM), developed in 2006 by Eric Betzig and Harald Hess, scientists at HHMI's Janelia Farm Research Campus and by Samuel Hess at the University of Maine; stimulated emission depletion (STED) by Stefan Hell at Max Planck Institute; and saturated structured illumination microscopy (SSIM) by Mats Gustafsson at the University of California, San Francisco.

To create an image with STORM, researchers label the molecules they want to study with fluorescent probes, and then use a burst of light to activate the

fluorescence in a small percentage of labeled molecules. The microscope captures an image of the fluorescing probes. The technique is designed to activate a sufficiently low percentage of the probes to allow the image of each fluorescing molecule to be seen separately. This allows the molecules to be localized individually. The process is repeated many times, capturing a different subset of molecules with each iteration. A final compilation of the images shows each molecule in its precise location in the cell with nanometer accuracy.

With a two-dimensional resolution of 20-30 nanometers - about 2,000 times smaller than the diameter of an average cell - STORM allows researchers to look inside cells and discern between molecules that are so close together that, using conventional light microscopy, they would appear as one. But as good as STORM is, Zhuang explained, some important information was still missing. We keep running into people asking: 'What if these two things that you say are close are not that close in the depth dimension?' she said.

So the team set out to demonstrate that STORM can achieve super-resolution in all three dimensions. Zhuang explained that when the images of individual molecules are collected to generate a two-dimensional STORM image, those images also contain information that reveals the molecules' positions in the third dimension. The center position of the image gives you x and y, and the shape of the image tells you where in the z dimension it is, she said. At a specific depth, a fluorescing molecule will appear round through the microscope. Zhuang and her colleagues inserted a cylindrical lens into the imaging path of the microscope, so that above and below that point, the circle distorts, becoming more and more elliptical in shape as the distance increases.

Zhuang says the instrumentation needed for 3D STORM is readily available. Chances are, any imaging center with a fluorescence microscope setup can use it for this method with just little bit of modification. The data analysis needed to generate an image, however, is more complex. A complete STORM image is actually the compilation of millions of points, representing the positions of the molecules of interest, each of which must be precisely localized in three dimensions. Zhuang and her colleagues are continually refining the complicated algorithms they developed for this data analysis, but hope to eventually develop user-friendly software that will make 3D STORM readily accessible to other researchers.

The addition of the third dimension of super-resolution is one of a series of improvements to the original STORM technique, Zhuang said. In 2007, her group introduced new fluorescent labels that are compatible with STORM imaging, enabling researchers to track different types of proteins at the same time using multi-color imaging. Next, she says, she and her colleagues will work to speed up the imaging process. Currently, collecting the data to create a STORM image can take tens of seconds to minutes, which is too long to catch most of the cell's dynamic processes in action, but Zhuang is optimistic that they can improve this substantially.