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Super-Resolution Microscopy Captures Molecules in Motion

A new twist on a sophisticated light microscopy technique is enabling researchers to capture short videos of fast-moving cellular processes while delivering super high resolution images of whole cells.

The new microscopy technique, developed by scientists at the Howard Hughes Medical Institute's Janelia Farm Research Campus, captures up to 11 images per second. It is reported in the May 2009 issue of the journal *Nature Methods*.

"This technique gives you twice the resolution of the normal microscope, looking at a whole cell at 10 frames per second, and that's just plain cool."

- Mats G.L. Gustafsson

Optical microscopes—the kind that use light and glass lenses—have been around since the late 1500s. Although ideal for looking at live samples, these scopes all run up against the same wall: they cannot distinguish objects that are closer to each other than about 200-nanometers—one-500th of the width of a human hair. That may sound small, but the complicated molecular machinery inside cells is even smaller.

The basic problem is something called the “diffraction limit.” Light is a wave and the microscope runs into trouble when the distance between objects of interest is less than half the wavelength of the light. Until the 1990s, the diffraction limit was considered a law of nature. Once scientists realized they could in effect break the law, the field of super-resolution light microscopy took off.

One HHMI researcher leading the charge is Mats Gustafsson, who is now a group leader at Janelia Farm. In 2000, while at the University of California, San Francisco (UCSF), Gustafsson introduced structured-illumination microscopy or SIM. The technology takes advantage of moiré patterns, which are produced by overlaying one pattern with another. Two rows of chain-link fencing seen from a distance can produce a moiré pattern, as can overlapping layers of gauzy curtains.

In structured-illumination microscopy, the sample under the lens is observed while it is illuminated by a pattern of light (more like a bar code than the light from a lamp). Several different light patterns are applied, and the resulting moiré patterns are captured each time by a digital camera. Computer software then extracts the information in the moiré images and translates it into a three-dimensional, high-resolution reconstruction. The basic idea has been around since the 1960s, but wasn't fully realized until recently.

At UCSF, Gustafsson used SIM to visualize the molecular scaffolding that holds the shape of cells, with two-fold better resolution than a conventional microscope. Since then, his group has improved its resolution and also introduced three-dimensional SIM, making it possible to see parts of cells that go undetected using most light microscopes.

Until now, SIM was too slow to image living cells, whose inner parts are constantly in motion. "I've given talks on structured illumination microscopy as a means of generating high resolution images of fixed cells for several years," says Gustafsson. "The first question after my talks is always, 'Can you do this live?' We wanted to answer that question with 'Yes.'"

Now he can, thanks in part to the addition of a liquid crystal spatial light modulator, a half-inch-sized mirror-like device that generates patterns of light using thousands of pixels that the researchers can control individually. Spatial light modulators are similar to liquid crystal displays in televisions and laptops, except that Gustafsson used a version with a much faster (sub-

millisecond) response time. "That is precisely what we needed," Gustafsson says. With this part in place, a microscope can generate new patterns of light about 1,000 times faster than the original SIM equipment.

After adding the spatial light modulator to their microscope, they used it to visualize microtubules—long, thin filaments that provide structure and support to cells—in living fruit fly cells. They could see individual microtubules moving as the cell's skeleton reorganized itself to prepare for cell division. At 100-nanometer resolution, SIM revealed much more detailed images than could be obtained by traditional methods for live cell imaging.

To see if they could capture a faster moving target, Gustafsson's team tried live SIM on kinesins, proteins that carry cellular supplies along microtubule "tracks" at a blistering pace of about a micron per second. Microtubules and kinesins also help get cells ready to divide.

By setting the light intensity and exposure frequency just right, the researchers could see the kinesin zipping over the microtubule. They recorded the event by capturing images from the microscope at 11 frames per second for several hundred frames. "I was excited to see that we could image moving kinesin, which is one of the most rapidly moving processes in a living cell," Gustafsson says. "If we can image kinesin, we should be able to image most other cell processes."

In recent years, other groups have tweaked super-resolution methods, each of which has unique advantages, Gustafsson says. For example, researchers who developed Stimulated Emission Depletion (STED) microscopy have made it possible to take videos of live cellular processes with 60 –nanometer resolution. Unlike SIM, however, STED is limited to a small field of view. "We think this technique fits a niche where you want to simultaneously have high frame rates and large imaging areas," he says.

The technique can be adjusted to fit individual researchers' needs. Gustafsson has started collaborations with researchers who plan to use SIM to see how cells migrate toward or away from chemical targets. And although the research reported in *Nature Methods* used 2D-SIM, 3D-SIM is also on the horizon. Gustafsson says this will require making adjustments to the hardware and taking more images per frame.

Gustafsson acknowledges that the technical details of his new technique may be a bit obscure, but the point, he says, is not: "Look, this technique gives you twice the resolution of the normal microscope, looking at a whole cell at 10 frames per second, and that's just plain cool."