

AUGUST 16, 2007

## Researchers Zoom in on Life in Technicolor

A colorful new palette of fluorescent tags promises to give researchers better view of life at the molecular level. The tags, which are compatible with recent advances in super-resolution microscopy, allow researchers to label different molecules with distinct tags and simultaneously track them with nanometer resolution within cells.

Howard Hughes Medical Institute investigator Xiaowei Zhuang and her colleagues at Harvard University created an assortment of fluorescent probes that offer researchers greater flexibility when using fluorescence microscopy techniques that pinpoint individual molecules inside cells. Zhuang and coworkers created one of these techniques, known as stochastic optical reconstruction microscopy (STORM), last year. The new multicolor STORM research was published in the August 16, 2007, edition of *Science Express*, an advanced online publication of the journal *Science*.

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— Xiaowei Zhuang

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Fluorescent microscopy is a crucial tool in molecular biology. Relatively noninvasive, optical imaging is rapid enough to allow researchers to spy on cellular processes as they occur. Fluorescent probes can label specific proteins, or nucleic acids such as DNA and RNA.

Until fairly recently, the resolution of fluorescent microscopy was limited by the wavelength of light. Recent advances have broken this light barrier and allowed scientists to see structures clearly that are separated by less than the traditional limit of 200 nanometers. For example, STORM uses fluorescing probes that can be activated and deactivated to let researchers zero in on many of cells' tiniest structures. The microscope collects a large number of images, each with a different subset of probes activated, then compile the images to generate a clear picture of how fluorescently tagged molecules are distributed within a cell.

Last year, Eric Betzig and Harald Hess, scientists at HHMI's Janelia Farm Research Campus, developed a technique called photoactivated localization microscopy (PALM), which also uses photoactivatable probes to create images with nanometer resolution.

Until now, however, these techniques have lacked one of the most powerful features of fluorescence microscopy: the ability to track and distinguish multiple types of proteins simultaneously. Few suitable probes were available, meaning researchers could not use more than two colors at a time. But multiple colors would allow researchers to color-code several proteins or genes in a cell and watch how they interact. That way, they could truly see life in action.

Now, Zhuang and her colleagues Mark Bates, Bo Huang, and Graham Dempsey have developed probes in nine new colors. Using these, they have shown that it's possible to image biological structures using multiple colors at once, with a resolution of about 20 - 30 nanometers—about the diameter of a tiny poliovirus.

This is the first example of super-resolution imaging in three colors, said Zhuang, senior author of the paper and developer of STORM. We also developed photoswitchable probes in many colors.

Photoswitching—turning probes off and on with a pulse of light—is what makes super-resolution techniques like STORM possible. For a densely labeled sample, if all of the probes fluoresced simultaneously, the glow emitted by nearby probes would overlap, and resolution would be lost. To avoid this problem, STORM employs fluorescing probes that can be turned off by a specific wavelength of light. Shine the light on the sample, and the field goes dark. Then, a different wavelength of light is used to activate only a small subset of the probes. These probes can then be imaged, their locations determined, and switched off. Another subset is then activated. Ultimately, the repetitions result in a super-resolution, highly accurate image.

The specific probes that Zhuang and coworkers developed are each made of two dyes. One dye, called the photoswitchable reporter, emits light. The other dye is called the activator because it helps to activate the photoswitchable reporter. By combining each of three light emitters with each of three activators, Zhuang's lab created nine colors.

Fluorescent probes typically emit a relatively broad spectrum of light, she said. If you have a visible light (wavelength) range of 400 nanometers to 700 nanometers, you cannot distinguish that many probes. But each of our probes has two parts that can be independently tuned—the color of light that activates the probe, determined by the activator, and the color of light emitted, determined by the reporter. That means researchers can combine colors just as artists do, creating blue, green and red, but also magenta, gold and brown, among others.

Zhuang and her colleagues used the new probes to simultaneously image microtubules—long, cylindrical structures that help give a cell its

structure—and clathrin-coated pits on the surface of the cell, used to bring molecules from a cell's external environment inside the membrane. The researchers attached one activator-reporter pair to the microtubules, and a different activator-reporter pair to clathrin. When they imaged the labeled cells with STORM, the green filamentous structures of the microtubules and red spheres of clathrin-coated pits were revealed with remarkable clarity. In contrast, when the cells were imaged with traditional fluorescence microscopy, the densely packed filaments were unresolved in many regions of the cell and the pits showed little discernable shape.

From there, the team went on to show that three colors could be imaged at the same time. Using three of their new probes, they labeled three different types of DNA molecules and spread them on a microscope slide at a high density. Traditional microscopy generated only an indistinct glow from the tagged DNA, but using STORM, the group clearly saw clusters of red, blue and green—each representing an individual molecule of DNA.

Although the research was not done in live cells, Zhuang is confident that the technology will one day be used in live-cell imaging, allowing researchers to create color-coded images of cells and to catch their actions with molecular-scale resolution.

The method we use is ready for proteins on the surface of live cells, Zhuang said. We are working on modifications to allow the dyes to enter a cell and be targeted to a specific protein sequence.

Another hurdle to imaging live cells in this way is the speed of the process. A STORM image is really a series of frames constructed from multiple on/off cycles. One can be created in several seconds to several minutes, but this is still too slow for many cellular processes. However, with some simple tweaks, Zhuang is confident that she could create a super-resolution STORM image in one second.

To get faster than that will take some development, she said, but I think it's possible.