New type of optical microscopy attains near-molecular resolution

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A new type of microscopy invented by Xiaowei Zhuang and colleagues at Harvard University and the Howard Hughes Medical Institute delivers spatial resolution more than 10 times better than that of conventional optical microscopes, putting scientists tantalizingly close to the first crisp, ultra-resolution, real-time imaging of living biomolecules and cells.

The new technique can currently resolve objects as small as 20 nanometers, or 20 billionths of a meter, a resolution Zhuang says could be pushed, with further improvements, to molecular scale.

Zhuang, along with Michael J. Rust and Mark Bates at Harvard, describes stochastic optical reconstruction microscopy, or STORM, in the Aug. 9 online edition of the journal *Nature Methods*.

"We believe this technique has the potential to transform molecular and cellular imaging in biology," says Zhuang, an HHMI investigator and professor of chemistry and chemical biology in Harvard's Faculty of Arts and Sciences. "High-resolution bio-imaging is now accomplished using electron microscopy, which requires a 'dead' sample. The scientific community has long hoped for a technique offering STORM's attributes: touchless, gentle, real-time imaging of live biological samples at the molecular scale."

At the heart of STORM's success are fluorophores, glowing molecules that can be driven between a fluorescent and a dark state hundreds of

times with repeated exposure to light with different colors. Zhuang, Bates, and colleague Tim Blosser discovered such a fluorophore and described it in Physical Review Letters in 2005.

"Scientists have long known that individual fluorophores can be localized to nanometer accuracy," Zhuang said. "However, light from fluorophores that are close to each other cannot easily be separated, making it very difficult to resolve these objects. After we discovered this wonderful switchable fluorophore, we realized that it offers one solution to this problem."

Their solution, described this week in *Nature Methods*, is to only activate a small fraction of the fluorophores at a time, imaging them and determining their location to nanometer resolution.

Rust and Bates attached fluorophores to antibodies, which can be engineered to attach in turn to many types of biomolecules. Exposure of a fluorophore-bound biological sample to successive flashes of light of varying wavelengths activates different subsets of fluorophores, revealing their locations. After many such still images are taken, they are merged into a single image -- a sea of glowing fluorophores clearly resolvable, for instance, along a strand of DNA or protein filament.

"The whole STORM imaging process currently takes several minutes to create a crisp imaging of a biological sample," Zhuang said, "but we are fairly confident that we can ramp up the speed to virtually real-time. Our next step is a molecular resolution, multi-color, real-time STORM for live object imaging."

Source: Harvard University