

A New Clarity

APPLYING A NEW MICROSCOPY TECHNIQUE TO DETECT INDIVIDUAL MOLECULES IN THREE DIMENSIONS.

The intricate molecular insides of cells are coming into focus, thanks to HHMI investigator Xiaowei Zhuang at Harvard University. Zhuang has developed a three-dimensional version of her high-resolution stochastic optical reconstruction microscopy (STORM) technique, allowing scientists to find the location of cellular molecules with better resolution than conventional light microscopy.

To get a glimpse of cells' inner workings, scientists typically tag molecules with proteins or dyes that give off fluorescence. But images of this fluorescence have a resolution that's limited to a few hundred nanometers by the diffraction of the light in all directions.

With this conventional method, "if you have a very interesting structure but it's smaller than the resolution, it just looks like a

featureless dot," says Zhuang. Multiple fluorescent molecules blur together. So she and her colleagues came up with a trick.

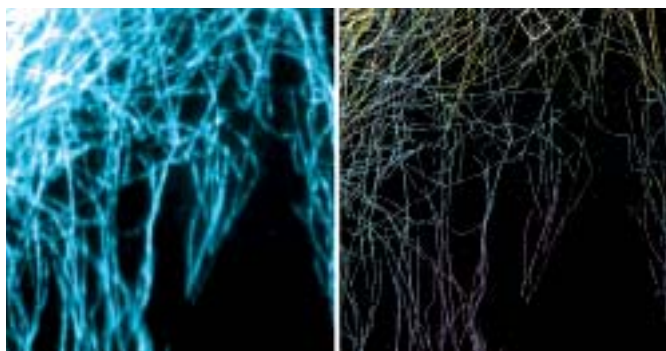
Zhuang's STORM technique, first described in 2006, had been used only for two-dimensional imaging until now. STORM involves tagging molecules with a fluorescent label, or fluorophore, that can be switched on and off.

Her team avoids the problem of overlapping fluorescence by using low amounts of light to switch on only a small percentage of fluorophores at once. Through the microscope, researchers can pinpoint a molecule's location by calculating where the center of each dot is.

Now, Zhuang has also developed a way to determine where the molecule is in the third dimension—by analyzing the size and shape, or blurriness, of each dot. Repeating the process many times, randomly turning on fluorophores during each iteration, can reveal the precise location of all the tagged molecules in a cell.

"These cellular images are now 10 times sharper in all three dimensions," says Zhuang, who described the technique in the February 8, 2008, issue of *Science* and has used it to look at the proteins that help viruses enter cells—a process involving minuscule complexes of molecules that had never before been resolved by light microscopy.

"We can solve many problems that were previously beyond our reach, but there are still things that we can't reveal," she says. "And the closer the resolution gets to true molecular scale, the more questions arise." ■ —SARAH C.P. WILLIAMS



Microtubules visualized with a new microscopy technique (right) appear sharper than when viewed using conventional microscopy (left).

IN BRIEF

process is hindered by mutations in spastin, the microtubules in neurons—where spastin may be especially active—can't rearrange themselves.

DECODING THE MOLECULAR SIGNATURE OF PROSTATE CANCER

Prostate cancers display a molecular signpost that alerts the body to their presence, HHMI researchers have discovered. Understanding how this flag signals the immune system to respond to tumor cells may help researchers develop new ways to fight cancer.

Scientists led by HHMI investigator James P. Allison of Memorial Sloan-Kettering Cancer Center chopped up tumors and exposed the tissue to a mixture of immune T cells, all carrying different random receptors. T cells carrying one receptor in particular replicated in response to the tissue, indicating that this receptor recognized a marker on the tumor cell surface.

To the researchers' surprise, however, the T cells carrying the tumor receptor were also activated by other types of chopped-up tissue, suggesting that the

marker was not specific to cancer cells. Yet in living mice, T cells carrying the receptor of interest attacked just prostate tumors.

To clear up this discrepancy, the scientists searched for the molecule that the T cells recognize. They pinpointed histone H4, a protein found ubiquitously inside the nuclei of cells—explaining why all ground-up tissues activate the T cells. In prostate tumor cells, they determined, histone H4 leaves the nucleus and is displayed on the surface of the cells.

The team next hopes to learn exactly how histone H4 goes from being inside cell nuclei to being a flag for the immune system, as well as whether the protein has value in predicting prostate cancer.

DNA EXPANSION LEADS TO NEUROLOGICAL DISORDER

The inherited neurodegenerative disease spinocerebellar ataxia type 1 (SCA1) can be explained by a molecular tug-of-war, researchers have discovered. It was known that an expansion of a repetitive region of DNA in the gene ataxin 1 (ATXN1) led to SCA1, characterized by neural degeneration

that begins at around age 30. Now, HHMI investigator Huda Zoghbi of Baylor College of Medicine has shown why this expansion is so toxic to cells.

Zoghbi and her colleagues wanted to explore a recent observation that a separate, single mutation in ATXN1 can cancel out the disease-causing effects of the expansion. So they screened proteins to find ones that interacted preferentially with one form of ATXN1 or the other.

The researchers found two proteins—RBM17 and CIC. The disease-causing version of ATXN1 prefers to bind to RBM17, leaving CIC with less ATXN1 to bind to. RBM17 and CIC are constantly struggling to spend time with ATXN1, says Zoghbi. The inherited expansion shifts this balance in RBM17's favor, and that is what causes disease, the researchers conclude in a report published online in *Nature* on March 12, 2008.

Next, Zoghbi and her collaborators hope to deduce the functions of both RBM17 and CIC, to determine why ATXN1's increased interactions with RBM17 cause neural degeneration.