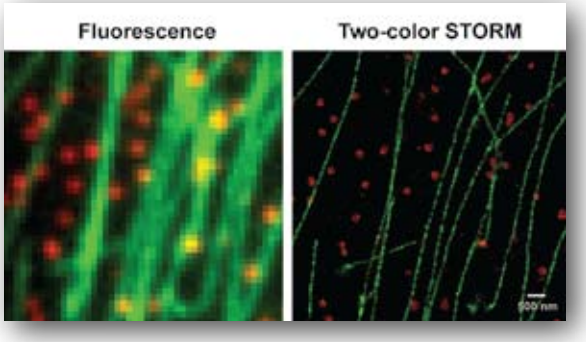
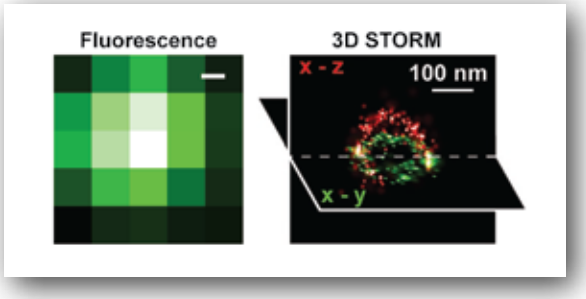
Resolving the blind spot

Seeing the unseen with super-resolution fluorescence microscopy

On the cellular scale, life gets interesting below 200–300 nm. That's the length scale of most intracellular structures and the level at which the cell carries out most of its work. Unfortunately, it's a blind spot for conventional light microscopes. Even when using fluorescent-tagged molecules, light microscopes cannot resolve two objects closer than half the wavelength of the light because of the phenomenon called diffraction. Their images look blurry and overlap no matter how high the magnification. This resolution limit is like the fat man wearing a tall hat in the movie seat in front of you. He's blocking the best part of the picture. Now comes a new "super-resolution" fluorescence microscopy technique that may at least get the fat man to remove his hat.



Fluorescent imaging can only capture a fuzzy image of a filamentous cytoskeleton structure (green) and a round membrane structure (red). STORM clearly resolves these two structures from each other even when they are densely packed.



The 3D STORM image reveals the hemispherical cage shape of clathrin-coated pits, which can be seen from the cross sections in the two perpendicular directions. For comparison, only a featureless spot can be seen in the conventional fluorescence image.

Developed by Bo Huang, Xiaowei Zhuang, and colleagues at Harvard University, stochastic optical reconstruction microscopy (STORM) is one of several higher-resolution fluorescence microscopy techniques invented recently that fundamentally surpass the diffraction limit. STORM can record light emitted from one molecule in the sample. Using probe molecules that can be "photoswitched" between a visible and an invisible state, STORM determines the position of every molecule of interest and then compiles their positions to define a structure.

With STORM, the Harvard researchers laterally resolved cellular features as small as 20–30 nm, an order of magnitude smaller than what conventional fluorescence microscopy can achieve. Huang and colleagues have now adapted STORM to study three-dimensional (3D) structures. They can now visualize a whole cell with an axial resolution of 50–60 nm. Multicolor imaging has been realized using photoswitchable fluorophores made of combined pairs of various activator dyes and reporter dyes. Combined multicolor and 3D STORM images revealed detailed interactions between cell organelles and the cytoskeleton. In brain tissue, the technique revealed fine details of the synaptic structure of the olfactory system.

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Seeing the Unseen in a Cell With Super-Resolution Fluorescence Microscopy

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