SENSORS AND PROBES

More dyes enter the realm of nanoscopy

Widely used dyes for conventional microscopy of subcellular structures can also be used for super-resolution imaging.

Most super-resolution imaging has involved protein-based labeling of intracellular proteins, via either fluorescent-protein fusions or dye-labeled antibodies. But the efforts of Richard Haugland and his company Molecular Probes—now part of Life Technologies Corporation—provided microscopists with a variety of fluorescent dyes that display excellent selective labeling of cellular structures. Although these dyes weren't intended to be photoswitched, live-cell switching-based super-resolution imaging is possible when the dyes are imaged under suitable conditions. Earlier this year, researchers demonstrated such imaging of nuclear and mitochondrial DNA using the double-stranded DNA detection dye PicoGreen (Benke and Manley, 2012).

Other fluorescent dyes bind to specific cellular membrane structures such as the endoplasmic reticulum, mitochondria, lysosomes or plasma membrane.

Now Zhuang and colleagues demonstrate stochastic optical reconstruction microscopy (STORM) imaging of several of the most commonly used cellular-membrane dyes. As expected, the photoswitching performance they obtained was not as good as that of the best-performing STORM dyes. However, the high density and good mobility of the membrane dyes did allow good-quality STORM imaging. This was aided by the fact that individual dyes could be localized multiple times as they moved within the membrane.

Using DiI, they imaged moving filopodia and dendritic spines in neurons at 409-nanometer resolution with a 15-second imaging rate and 70-nanometer resolution if imaging speed was increased to 5 seconds for each reconstructed image in a movie series. When imaging mitochondria with MitoTracker Red, they were able to increase the imaging speed to 2 seconds per image at 40-nanometer resolution by localizing overlapping molecules using a multi-emitter fitting algorithm. Similar performance was obtained when imaging endoplasmic reticulum or lysosomes with ER-Tracker Red and LysoTracker Red, respectively. Finally, using ER-Tracker Red and MitoTracker Red, they achieved dual-color imaging of these organelles.

Bringing these dyes into the realm of sub-diffraction imaging adds powerful tools to the expanding community of nanoscopists.

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RESEARCH PAPERS

Benke, A & Manley, S. Live-cell dSTORM of cellular DNA based on direct DNA labeling. *Chembiochem* **13**, 298–301 (2012).

Shim, S.H. *et al.* Super-resolution fluorescence imaging of organelles in live cells with photoswitchable membrane probes. *Proc. Natl. Acad. Sci. USA.* **109**, 13978–13983 (2012).