MICROSCOPY

Better resolution for structured-illumination microscopy

The use of a photoswitchable fluorescent protein allows nonlinear structured-illumination microscopy of cellular structures at 50 nanometer resolution.

In the quest for improved resolution in fluorescence light microscopy, the technique of structured-illumination microscopy (SIM) independently pioneered by Mats Gustafsson, Rainer Heintzmann and others has filled a unique niche. Whereas developers of techniques based on localizing individual fluorophores under wide-field illumination or decreasing the size of the scanning spot in scanned illumination strove to achieve ever better resolution of structures in cells, SIM—owing to the physics it is based on—has been limited to providing about a twofold improvement in resolution. Improvements in SIM have instead focused on increasing both two-dimensional and three-dimensional imaging speed of whole cells.

Preliminary work with SIM using polystyrene beads showed that by exploiting the nonlinear response of saturated fluorophores through high-intensity illumination it was possible to improve resolution about fourfold. Unfortunately, fluorophores bleach so quickly under those conditions that imaging of biological structures in cells is unfeasible. Researchers concluded that some other nonlinear process would need to be exploited, and they proposed that photoswitchable fluorophores would be an appropriate alternative.

Now, the late Mats Gustafsson with Hesper Rego and colleagues demonstrate photoswitch-based nonlinear SIM imaging of biological structures in whole mammalian cells. They chose the photoswitchable fluorescent protein Dronpa because of its reported high numbers of photoswitching cycles. Although they could not obtain the expected performance from Dronpa, it worked well enough for imaging fixed cells with a resolution of about 50 nanometers.

Rego and colleagues demonstrated the technique by imaging two nuclear pore proteins fused to Dronpa and saw different localization patterns. They also created a Dronpa-LifeAct fusion and used it to image the two-dimensional network of actin fibers in an entire mammalian cell.

There is still much room for improvement. The use of spatial light modulators for faster switching of the structured illumination patterns and the application of better-performing photoswitchable proteins should make the technique suitable for live-cell super-resolution imaging and such work is undoubtedly under way.

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RESEARCH PAPERS

Rego, E.H. *et al.* Nonlinear structured-illumination microscopy with a photoswitchable protein reveals cellular structures at 50-nm resolution. *Proc. Natl. Acad. Sci. USA* advance online publication (12 December 2011).