IMAGING

Simultaneous multicolor localization microscopy

Point spread function engineering streamlines multicolor super-resolution imaging and single-particle tracking.

Single-molecule localization microscopy techniques, such as PALM and STORM, and single-particle tracking techniques allow for precise analysis of biological molecules and structures. Over the past several years, improvements in these methods have led to advances in imaging in three dimensions and in multicolor imaging of multiple structures in the same cell.

A major challenge for localization microscopy techniques is achieving axial resolution that is as accurate and precise as the lateral resolution. One elegant solution to this problem has come in the form of point spread function (PSF) engineering. In this approach a phase-shaping element such as a phase mask is used to modify the microscope's PSF. This changes the shape of the image of an emitting fluorophore in a predictable manner that is dependent on the precise axial position.

W.E. Moerner, postdoctoral fellow Yoav Shechtman and colleagues at Stanford University extended PSF engineering to tackle the challenge of multicolor localization microscopy. Although multicolor localization microscopy and 3D particle tracking have been accomplished, multicolor imaging typically requires imaging of each color sequentially, using multiple cameras, or imaging different fields of view in different channels. Although useful, each of these options can have its own drawbacks in terms of speed and ease of use.

In this work, the researchers designed phase masks that produced distinct patterns based on the emission wavelength of the fluorophore. Some benefits of this approach are that registration of the different channels is straightforward because all the light goes through the same optical elements, and that the different channels can be imaged simultaneously to decrease the overall imaging time and increase temporal resolution.

The team demonstrated the power of their approach by designing phase masks that allowed them to simultaneously track red and green microspheres in 3D as they diffused randomly in solution. Using another phase mask, they demonstrated two-color STORM imaging in fixed mammalian cells.

Although the localization microscopy implementation is currently limited to 2D multicolor imaging, the researchers note that improvements could extend the method to 3D imaging, and to imaging of up to five colors simultaneously.

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RESEARCH PAPERS
Shechtman, Y. *et al.* Multicolour localization microscopy by point-spread-function engineering. *Nat. Photonics* **10**, 590–594 (2016).