MICROSCOPY

A curveball for microscopists

A curved beam of light provides microscopists with a new tool to overcome imaging challenges.

Even a baseball pitcher with a strong fastball will find situations when a slow pitch with a curved trajectory will perform better. Two independent studies now demonstrate how a curved beam of light known as an Airy beam can provide advantages for microscopy at small and large scales.

At the small scale, early examples of super-resolution imaging by localization-based methods such as stochastic optical reconstruction microscopy (STORM) were limited to two dimensions (*xy*). But unless the structure being imaged is near the surface of a sample, three-dimensional information deep in the sample is highly desirable. Various optical designs have been described that allow the *z* position of each fluorophore to be estimated alongside the *xy* position. These methods mostly rely on using *z* position–dependent changes in the shape of the imaged spots to estimate their *z* positions but are often limited to short distances from the coverslip or have relatively low localization precision in *z*. Additionally, some methods are easier to implement than others.

When Shu Jia joined Xiaowei Zhuang's lab at Harvard University, he came with considerable experience using Airy beams in other applications and realized that an Airy beam could also be used to estimate the *z* positions of molecules in STORM imaging. A curved beam of light is ostensibly a poor choice for imaging anything because the curved trajectory of the light emitted from a fluorophore generates entanglement of its lateral and axial position information. Jia solved this problem by generating two Airy beams that curved away from one another such that the true *xy* position of the imaged molecule was halfway between the two images of it. Moreover, the distance between the images encoded the *z* distance of the molecule from the focal plane. By using beams that curved a proper amount, they obtained nearly identical localization precision in *x*, *y* and *z*.

Importantly, unlike the Gaussian beam typically used in microscopy, the Airy beam has a long propagation distance. The localization precision of ~10 nm at the focal plane decreased only to ~15 nm at 3 μm away, with 2,000 photons detected from each fluorophore. The authors showed that STORM imaging using the Airy beams not only provided excellent isotropic resolution of immunolabeled microtubules and mitochondria in mammalian cells, but also imaged structures sufficiently far away from the focal plane that Gaussian-based imaging failed to detect them.

Because the two beams are symmetrical above and below the focal plane, it is not possible to focus inside a sample and image both above and below the focus simultaneously. Either the entire sample or the illuminated region of the sample (using, for example, light-sheet illumination) has to be placed on one side of the focal plane. But the 3-μm imaging depth obtained from just one side of the focus is still impressive, and the method should be reasonably easy to implement because it relies on a readily available and easily programmed spatial light modulator.

In addition to having a long propagation distance, Airy beams also exhibit self-healing properties in scattering media. Scattering is rarely a concern for super-resolution imaging, but it is often a serious problem for large-scale imaging with light-sheet fluorescence microscopy.

In this issue (p. 541), Kishan Dholakia and colleagues at the University of St. Andrews in Scotland report using an Airy beam for light-sheet microscopy in scattering media to achieve high contrast and high resolution with low photobleaching and up to a tenfold increase in the field of view compared to that of a traditional Gaussian beam or the self-healing Bessel beam. The performance comparison relied on using a single imaging system with matched illumination and detection objectives that did not account for the many different ways that light-sheet microscopy can be implemented, but there is no question that the method can provide substantial advantages in many situations.

The curved Airy beam provides microscopists with a valuable new tool across a wide range of imaging applications, and we expect to see more researchers incorporating it into their repertoire just as pitchers often incorporate a curveball into theirs.

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RESEARCH PAPERS
Jia, S. *et al.* Isotropic three-dimensional super-resolution imaging with a self-bending point spread function. *Nat. Photonics* **8**, 302–306 (2014).