STORM Offers Super-Resolution in 3D!

Stephen W. Carmichael[1]
Mayo Clinic
carmichael.stephen@mayo.edu

For the first few centuries of microscopy, spatial resolution was limited by the diffraction barrier. Recently, this barrier has been broken using several different methods. Optical methods that provide better resolution than the diffraction barrier are referred to as super-resolution. Although these techniques have significantly improved resolution in two dimensions (*x* and *y*) or in the axial dimension (z), it has not been possible to achieve substantial improvement in all three dimensions simultaneously. A study by Bo Huang, Wenqin Wang, Mark Bates, and Xiaowei Zhuang demonstrated a breakthrough by achieving a spatial resolution that is 10 times better than the diffraction limit in all three dimensions without using sample or optical-beam scanning.[2]

Their method is a variation in stochastic optical reconstruction microscopy (STORM, in this case called 3D STORM) in the astigmatism imaging mode. This involves placing a weak cylindrical lens in the optical pathway so that the focal planes in the *x* and *y* axes are slightly different. When a fluorophore is at the average focal plane (approximately halfway between the two different focal planes) the image appears to be round. When the fluorophore is above the average focal plane its image is more focused in the y direction, appearing to be ellipsoidal; conversely when it was below the average focal plane it appeared ellipsoidal in a perpendicular direction. Using a Gaussian function, the *x* and *y* dimensions could be determined, as well as determining the position of the fluorophore in the *z* axis unambiguously.

After establishing the validity of their method by imag[illegible] beads, they used quantum dots for calibration. Then Huang *et al.* applied 3D STORM to cultured epithelial cells. Cells were immunostained with primary antibodies to β-tubulin and then secondary antibodies that were double-labeled (with Cy3 and Alexa 647) were introduced. With a proximal Cy3, the Alexa 647 fluorophore could be differentially switched off and on with a red laser (657 nm wavelength) and green laser (532 nm), respectively. During each activation cycle only a small fraction of well-separated Alexa 647 fluorophores were activated, allowing their positions to be accurately determined. Over the course of many activation cycles, the positions of all fluorophores were determined to give a super-resolution image. Multiple layers of microtubule filaments could be observed at high resolution in three dimensions. Experiments to quantify the resolution indicated that their 3D STORM method provided 22 nm resolution in the *x* dimension, 28 nm in *y*, and 55 nm in *z*.

Finally, to demonstrate that 3D STORM can resolve nanoscopic organelles *in situ*, they imaged clathrin-coated pits using a direct immunofluorescence scheme with an antibody labeled with Cy3 and Alexa 647. The pits could be clearly visualized and their dimensions were consistent with those seen with electron microscopy. It is quite possible that the resolution in these experiments on cells was limited by the size of the antibodies and that 3D STORM can provide even better resolution when smaller labels are used. It is exciting to speculate that 3D STORM can be used in real time, providing super-resolution in 4 dimensions in living cells!

1 The author gratefully acknowledges Dr. Xiaowei Zhuang for reviewing this article.

2 Huang, B., W. Wang, M. Bates, X. Zhuang, Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy, *Science* **319**:810-813, 2008.

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Brainbow transgenic mouse brain, expressing many distinct combinations of red, yellow and cyan fluorescent proteins in nervous tissue. In Brainbow mice, neurons randomly choose combinations of the three fluorescent proteins so that they each glow a particular color. The image is a montage of images acquired from the hippocampus, captured using a laser scanning confocal microscope with a 60x 1.45 N.A. oil immersion objective. Image captured by Jean Livet, Harvard University, Cambridge, MA, USA. Honorable Mention, 2007 Olympus BioScapes Digital Imaging Competition.

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Regular Mail to:
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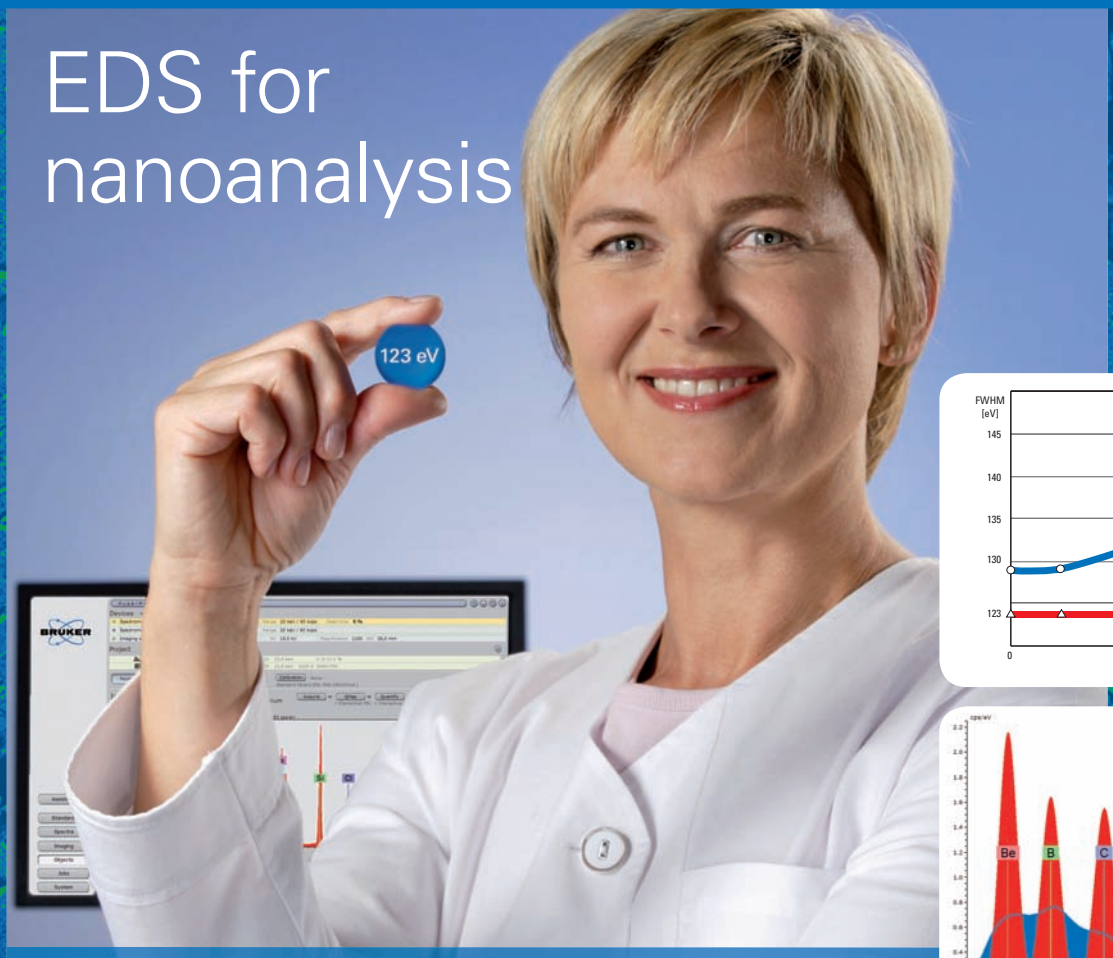

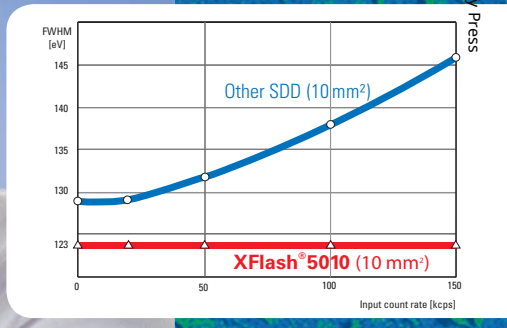

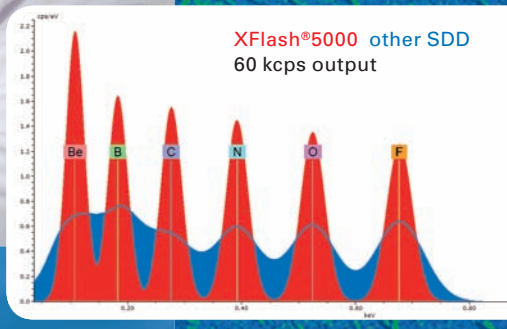

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