MICROSCOPY

Advancing imaging

In vivo imaging enables the study of complex, dynamic subcellular processes in three dimensions; however, limitations of existing tools include low resolution, slow imaging speed and high phototoxicity. Betzig and colleagues addressed these issues by developing the use of multiple non-diffracting Bessel beams to create ultrathin light sheets. These light sheets excite fluorescence in successive planes as they scan through a specimen to generate a three-dimensional (3D) image. This approach decreases photobleaching and out-of-focus background, leads to high axial resolution and illuminates the entire field of view.

The authors went on to demonstrate the performance advantage of this technique by imaging various dynamic biological processes in cells and small embryos in three dimensions. Using lattice light sheet microscopy, they imaged the single-molecule binding kinetics of the SOX2 transcription factor in a spheroid of mouse embryonic stem cells, microtubule instability during cell division in HeLa cells and the spatiotemporal relationship between the endoplasmic reticulum (ER), mitochondria and chromosomes during mitosis. In addition, they were able to visualize cell–cell and cell–matrix interactions, exemplified by immunological synapsis formation and neutrophil migration through the extracellular matrix, respectively. Finally, the authors used this approach to image the localization of a chromosomal passenger complex protein, actin distribution and dynamics during embryogenesis in *Drosophila melanogaster* and *Caenorhabditis elegans* embryos. In summary, this new technique enables dynamic processes to be visualized at high speed and high spatiotemporal resolution.

This advance was published shortly following the announcement that the 2014 Nobel Prize in Chemistry was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy" (The Nobel Foundation, 8 Oct 2014). The three laureates overcame the physical limit of the maximum resolution of traditional optical microscopy: Stefan Hell (Max Planck Institute for Biophysical Chemistry, Germany) developed stimulated emission depletion (STED) microscopy, and Eric Betzig (Janelia Research Campus, Howard Hughes Medical Institute, Virginia, USA) and William Moerner (Stanford University, Stanford, California, USA) laid the foundation for single-molecule microscopy. "The new imaging developments suddenly gave us a more than tenfold increase in resolution", said Stefanie Reichelt, Cancer Research UK Cambridge Research Institute, and "we can begin to understand much more clearly what is happening in important biological processes" (The Guardian, 8 Oct 2014).

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ORIGINAL RESEARCH PAPER Chen, Bi-C. *et al.* Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science* http://dx.doi.org/10.1126/science.1257998 (2014)

WEB SITE

Vimeo album Lattice Light Sheet Microscope: http://vimeo.com/album/3098015

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