10-YEAR ANNIVERSARY

The limits of light

Despite the vital role that conventional and confocal light microscopy have had in driving advances in cell biology, their resolution is ultimately limited by the diffraction of light. Light microscopy cannot resolve beyond ~200 nm and, given that most molecular complexes are smaller than this, much of what we want to see has remained beyond our grasp. The development of super-resolution fluorescence microscopy techniques over the past decade allowed this diffraction barrier to be bypassed.

In 2000, Klar *et al*. demonstrated that one way to increase resolution was by deactivating the fluorophores at the periphery of the excitation focal spot of a scanning microscope with a beam of light for stimulated emission depletion (STED) of their excited state. They further showed that this technique worked in bacteria and yeast cells, improving three-dimensional resolution to ~100 nm. They were able, for example, to improve the resolution of labelled vacuolar membranes in live *Saccharomyces cerevisiae* cells, resolving features not recognized by conventional or confocal microscopy.

An alternative solution, photoactivated localization microscopy (PALM), was found in 2006 by Betzig *et al*. PALM and related techniques, such as stochastic optical reconstruction microscopy (STORM), developed by Rust *et al*., work on the principle that sparse subsets of labels can be individually photoactivated and then bleached, and their positional information collected *en masse* can then be assembled into a full image with a more precise position. This approach allowed Betzig *et al*. to image diverse structures in fixed cells, including focal adhesions and mitochondria, with increased resolution.

These techniques and more recent variations have been embraced in diverse fields and are providing unprecedented insights into the organization and function of complex subcellular structures. The hope is that their improved use in live-cell analysis will further increase their potential.

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