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Out of the shadows

The accolades have been rolling in for the use of magnetic resonance in biological imaging. Richard Ernst received a Nobel for contributions to the development of high-resolution nuclear magnetic resonance spectroscopy, and last year Kurt Wüthrich got another for application of the phenomenon in determining the three-dimensional structure of biological macromolecules in solution. Just last month, Paul Lauterbur and Sir Peter Mansfield also received their summons to a fancy dinner in Stockholm for demonstrating the use of gradients in a magnetic field for creating two-dimensional images of internal structures and its refinement through mathematical analysis—findings that provided the foundation for magnetic resonance imaging, a diagnostic tool now in use in over 20,000 clinics and laboratories worldwide.

But what of light microscopy, for so long the ubiquitous and routine imaging tool of the amateur and professional biological investigator? This issue of *Nature Biotechnology* presents a series of articles describing recent developments in the optical imaging field. And great things are clearly afoot.

Since its initial description by Robert Hooke, the light microscope has been augmented with all manner of gadgets and innovations: phase contrast, differential interference contrast, laser confocal scanning systems, video, solid-state cameras, lasers and image analysis software to name a few. And yet, two seemingly insurmountable constraints on the technology have remained.

The first of these is Abbe's resolution limit (or the diffraction limit)—the smallest distance that can be resolved between two lines by optical instruments. The best that most confocal microscopes with single or even multiphoton excitation can achieve is a (spatial) resolution of 180 nm in the focal plane (x,y) and only 500–800 nm along the optic (depth) axis (z). For biologists, unfortunately, most macromolecular complexes and signaling domains have dimensions of ~5–500 nm and the largest virus (pox virus) has a diameter of 250 nm. Thus, we have lacked the means to image, in real time and in live samples, biologically relevant molecules and entities at a resolution less than 200 nm.

The good news is that several pioneering super-resolution technologies, including I^5 microscopy, 4Pi microscopy and stimulated emission depletion microscopy (see p. 1347), are now taking the resolution of light microscopes beyond this limit. Lensless technology, such as scanning near-field optical microscopy (see p. 1378)—a technique that crosses the boundary between atomic force microscopy and optical microscopy and provides information about surfaces at spatial (x,y) resolutions down to 50 nm and to 10 nm in the axial (z) plane—is also breaking new ground. Unfortunately, these techniques are also rather rough on their labeling agents, causing photo-bleaching (essentially light-mediated destruction of the label), which could potentially compromise attempts to improve resolution.

The other major problem for optical imaging is that biological tissues are very good at absorbing and scattering light. This limits analysis of cellular events to just a few hundred micrometers below the surface. In this respect, microscopes that use near infrared, longer wavelength light, multiphoton absorption or optical coherence tomography (p. 1361) are now achieving greater tissue penetration (up to 2–3 mm) than traditionally thought possible, with the additional benefits of reduced photodamage of tissues and longer probe lifetimes. And while some are working to broaden the palette of reporters available (*e.g.*, through mutagenesis of fluorescent proteins to extend excitation peaks and emission maxima to longer wavelengths), others are focusing on technologies that dispense with reporters altogether, attempting instead to visualize cellular structures through the measurement of intrinsic fluorescence.

Looking ahead, the current renaissance in optical imaging technologies bodes well for biology and medicine. Until now, most light microscopy has focused on probes that report transcriptional activity. As it becomes increasingly clear that a large proportion of the signaling pathways and regulatory mechanisms in the cell act not at the level of transcription but rather at the level of protein-protein interactions and within specific cellular compartments, optical techniques for monitoring a protein's local physico-chemical environment and the proteins in its immediate vicinity will become increasingly important.

Microarrays and other global assays of gene expression activity that have dominated biotech in recent years will be increasingly complemented by imaging technologies for visualizing a much greater spectrum of cellular processes, including mRNA turnover, protein phosphorylation and glycosylation states, translation initiation and progress, and DNA structural and chemical modification. As the technology is both extended from molecular imaging to the visualization of cell, tissues, anatomy and physiology, and combined with other types of imaging (*e.g.*, positron emission tomography, computed tomography and ultrasound), its promise for improving the speed and accuracy of disease diagnosis is quite real and definitely not the stuff of biotech entrepreneurial dreams.

The latest $9.5 billion endorsement of this promise came in October when the world's largest company by market value, General Electric, bought Amersham. Wondering what is one of Amersham's core businesses? Contrast agents for enhancing the imaging of organs and tissue.