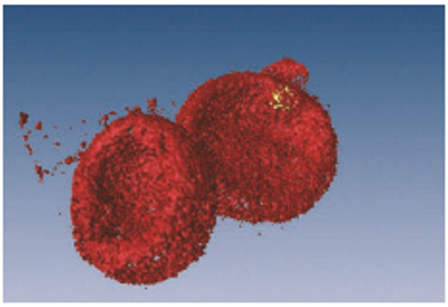
Something to see

Light microscopy is undergoing a renaissance, with a huge range of tools and techniques for gathering biological data with unprecedented speed and resolution. Michael Eisenstein takes a closer look.

Confocal microscopy is now a well-established technique for the three-dimensional imaging of cellular structures. But despite its success, the technique has its limitations when imaging live cells. The scanning process can greatly reduce imaging speed, for example, and the powerful lasers involved can damage the cells. Some manufacturers have addressed these problems by designing alternative systems such as 'restoration' microscopy (see 'Achieving clarity', below), whereas others have strived to improve confocal technology. Leica Microsystems of Wetzlar, Germany, for instance, still uses standard point-rastering in its high-end TCS SP5 confocal instrument, but it incorporates a resonant scanner for real-time 'true confocal' imaging with little cell damage.

An alternative approach uses a rapidly rotating disk, called a Nipkow spinning disk, which has numerous apertures to illuminate hundreds of spots simultaneously. This allows faster imaging with reduced photobleaching, although it does suffer from some loss in resolution. Perkin-Elmer Life and Analytical Sciences of Boston, Massachusetts, was among the first companies to develop instruments using this technology.



LEICA MICROSYSTEMS

High-resolution image of healthy (left) and malarial red blood cells taken with Leica's TCS-4Pi.

Its UltraVIEW ERS microscope uses parts from leading Nipkow-disk manufacturer Yokogawa in Tokyo, Japan. The system is designed for live-cell confocal imaging and can be fitted with a sensitive electron-multiplying CCD (EMCCD) camera for detection. "This offers frame-rates in excess of 100 frames per second, if you're looking at relatively small areas in your sample," says product leader Paul Orange. Olympus of Tokyo also makes a spinning-disk system, called the DSU, which features disks with slits instead of holes. Five different disks are available that vary in terms of slit number and spacing. "You can match the slit spacing to the numerical aperture of your objective," says product manager Nicolas George. The DSU can also incorporate high-resolution EMCCD cameras for imaging live specimens at up to 150 frames per second.

The LiveScan SFC from Nikon Instruments in Melville, New York, uses arrays of pinholes or slits for multipoint imaging. These remain stationary while mirrors sweep the beam spots over the sample — a process known as swept-field confocal imaging, developed by Prairie Technologies of Madison, Wisconsin. Nikon is also introducing controlled light emission microscopy, which uses feedback from the detection process to modulate laser intensity to prevent oversaturation or unnecessary illumination of signal-free regions. "You sacrifice a little bit of temporal speed," says Stan Schwartz, vice-president of Nikon's microscopy division, "but the casual user can make correct and beautiful images, and you significantly reduce photobleaching and phototoxicity."

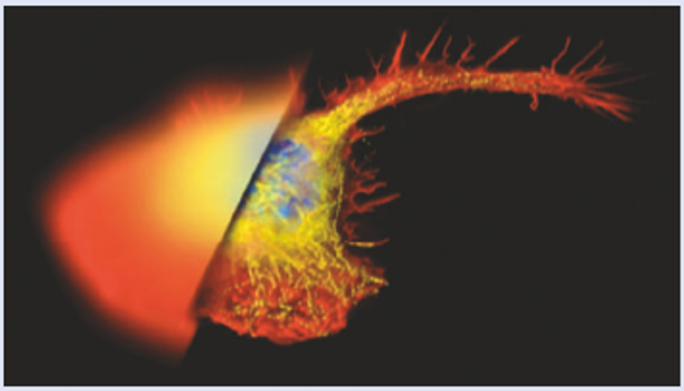
The LSM 5 *LIVE* from Carl Zeiss MicroImaging in Jena, Germany, uses a line-scanning approach that can image an area 512×512

ACHIEVING CLARITY

A key strength of confocal imaging is the elimination of emitted light from outside the focal point, such as that from tissue autofluorescence. But there are situations when non-confocal imaging is a better option. Deconvolution or restoration microscopy, in which computational algorithms remove artefactual blurring from actual image fluorescence while restoring out-of-focus fluorescence to its proper location, is one solution.

Applied Precision of Issaquah, Washington, was the first company to offer a complete real-time restoration-microscopy instrument, the DeltaVision RT. This combines a motorized stage with deconvolution software to collect and process two-dimensional image sections for the real-time assembly of 'restored' three-dimensional image projections.

Although restoration microscopy is sometimes presented as an economical alternative to confocal, many confocal users find advantages in computational image correction, including compensation for potential resolution loss with spinning-disk instruments. Confocal manufacturers have responded by incorporating deconvolution into their software packages. For example, Nikon Instruments in Melville, New York, offers a '2D-RT decon' module that removes blurring from two-dimensional sections to clean up three-dimensional confocal images in real time, and Olympus of Tokyo uses deconvolution tools developed by Intelligent Imaging Innovations in Denver, Colorado.



J. EVANS

Huygens software from Scientific Volume Imaging restored the detail to this macrophage imaged by wide-field microscopy.

Many users also opt for dedicated deconvolution software, such as the Huygens suite from Scientific Volume Imaging of Hilversum, the Netherlands. "Our software offers as much knowledge as we dare to put in about microscope image formation and noise characteristics," explains founder Hans van der Voort, "and with that we can recover as much as possible about the original object."

Huygens is regularly updated to tackle the data being generated by new imaging techniques — an onerous task for an expanding field. Above all, the challenge is keeping the final image clean and true-to-life. "What everybody hates is a restored image that is an artefact," says van der Voort. "You can make an image like a photograph, which is nice to see. But at second glance, if you really want to analyse your data, what you want is reliability." M.E.