systems are starting to be appreciated and people are looking at what can be done with large amounts of data from these kinds of instruments — at multiparametric studies of cells and what kinds of coordinations you can derive between different families of cells," says Andrew Olson, product line manager for high-throughput cellular imaging at Axon.

Electron microscopes

Cell biologists are also turning to electron microscopy to understand supramolecular structures at the atomic level. Transmission electron microscopy (TEM) has long been used to visualize cell ultrastructure in plastic-embedded sections of biological material, but it is now increasingly being used for three-dimensional imaging of whole cells, cell organelles and protein complexes.

To understand how protein complexes execute biological functions, researchers need to know the structures of these large, fragile constructions, and have deployed techniques such as nuclear magnetic resonance (NMR) spectroscopy, which offers only limited resolution, and X-ray crystallography, which requires large numbers of molecules in a crystalline array. TEM offers a more flexible solution.

"Over the past five years, the proof of concept has been given that TEM can be very instrumental in determining the three-dimensional structure of these huge protein complexes," says Werner Hax, life-sciences business development manager at electron-

Tecnai G2 Polara from FEI.

microscope manufacturer FEI of Hillsboro, Oregon. "TEM can tell you exactly where in the cell these proteins and protein complexes are located, so you can bring everything into perspective."

The Tecnai G2 Polara from FEI is a TEM optimized for structural biology tomography and single-particle analysis. "Imaging of frozen hydrated biological samples within a TEM has to be done with the greatest care, as the electron beam can damage the object under investigation," says Hax. "The Tecnai G2 Polara allows observation of proteins at temperatures below 10 K and provides the other conditions to minimize beam damage."

The Tecnai instruments are fully digitally controlled, with accessories such as cameras and X-ray detectors embedded, allowing them to be integrated into automated processes. "A lot of data have to be acquired and a lot of computational technology has to be applied to reconstruct the 3D representation of each protein in the cell," Hax says. "To do that within an acceptable period of time, particularly if you want to get this applied in industry in drug discovery, automation is an absolute must. Without that, TEM will never have the dominant role it can play."

Less specialized electron microscopes are finding wider use as an everyday tool for biological research. Delong Instruments of Brno, Czech Republic, has produced what it claims is the world's smallest TEM. The LVEM5 is intended primarily for biological and medical research, and provides high-contrast images of thin sections and particles such as viruses, enzymes, ribosomes, proteins and DNA, without the need for staining with heavy metals.

"This is practicable only by a considerable decrease of the energy of the imaging beam electrons," says Delong's Michal Drsticka. "When the energy is decreased roughly ten times, the contrast is ten times higher. The microscope therefore can be used both in university research and biological objects diagnostics."

The LVEM5 is around a tenth of the size of a conventional TEM, and could join the optical microscope as an everyday benchtop instrument. The bioscience researcher has never had such a choice of ways of seeing. ■

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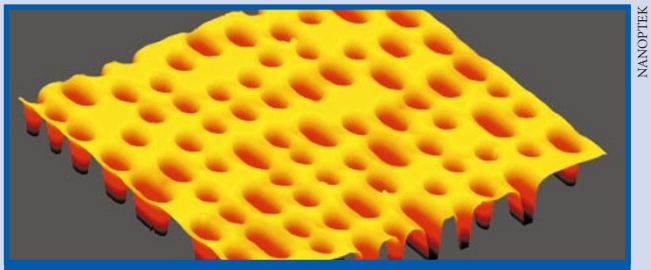
SCANNING THE SURFACE

Photon tunnelling microscopy (PTM) is a unique surface-scanning technology that can produce real-time three-dimensional images at unparalleled resolution of biological samples, including live cells. The basic technology can be used with common optical microscopes, and can achieve better vertical resolution than a scanning electron microscope without the need for a scanning tip.

The system addresses a fundamental problem of optical imaging — the smaller an object is relative to the wavelength of incident light, the larger the angle of the diffracted light. For very small objects, the light is diffracted at such a large angle that it cannot leave the object, and is bound in an 'evanescent field', the intensity of which decays exponentially with distance. To see such a small object, you have to take the lens to within a few hundred nanometres of the object's surface without damaging either the instrument or the sample. And unless you operate in a 'clean room', you will have to deal with dust that is many times larger than this distance.

The technology that enables PTM was developed by John Guerra in his former role as senior principal engineer at Polaroid. In June 2002, Guerra launched his own company, Nanoptek, based in Concord, Massachusetts, to develop and commercialize the technology. The key to Nanoptek's approach is a square of soft, flexible polymer film, 75 mm across and 15–20 μm thick, called a transducer, which is applied over the sample. This is made optically continuous to the near-field lens with a fluid coupling. "This allows you to move around this 75-mm area very quickly without damaging the microscope or the sample," Guerra explains. The film also covers any dust particles on the sample.



NANOPTEK

DVD surface imaged by photon tunnelling microscopy.

Over the 400-nm vertical range of the evanescent field, the coupling goes from total to zero, creating a grey-scale image in the microscope. A 12-bit CCD camera would give a vertical resolution of 0.1 nm — better than that achievable with scanning electron microscopy (SEM), and equal to that of atomic force microscopy (AFM), with the added advantage of real-time imaging.

"You can get 200-μm fields of view at video rates. You can watch chemotaxis in progress or cells moving," says Guerra. At about 100 nm, lateral resolution is not as good as with SEM or AFM, but is better than that of confocal or phase-shifted interference microscopy. Nanoptek is also planning to combine PTM with phase-shifting to increase the lateral resolution to 0.1 nm. "That will be the next exciting development," Guerra says. "You will be able to image viruses and very, very small biological materials." **T.C.**