MICROSCOPY

Faster, sharper electron microscopy

A technique combining laser and electron pulses is used to achieve nanometer and femtosecond resolution in biological imaging.

Researchers who hope to see fine cell details often must resort to techniques that disrupt the processes or surfaces they most want to visualize. Electron microscopy, for example, frequently requires researchers to coat thin specimens in sheets of gold. Even when such preparation is not essential, microscopy can require exposure times of a few seconds, long enough to blur fast changes. Now, researchers led by Ahmed Zewail at the California Institute of Technology describe an imaging technique that can be used to resolve biological structures at nanometer and femtosecond resolution, without requiring labeling or sophisticated preparation techniques (Flannigan *et al*., 2010). "What is unique," says Zewail, "is that it integrates the spatial resolution of electron microscopy with the ultrafast time resolution of optical imaging."

The method relies on four-dimensional ultrafast microscopy, which exposes the sample to both laser pulses and electron beams. Electrons that travel near the structure to be visualized absorb photons in such a way that this can be detected strongly from background. "When both the photons and the electrons coincide in time and space on the cell, the membrane or nanostructures are selected for imaging," says Zewail. Moreover, structures imaged with this method do not absorb the laser light used for visualization, limiting photothermal damage.

Zewail's group first described the technique, photon-induced, near-field electron microscopy (PINEM), a year ago and used it to image carbon nanotubes and silver nanowires (Barwick *et al*., 2009). Now they use the method to look at protein vesicles of about 500 nanometers in diameter, with increased brightness on the vesicles' 50-nanometer outer shell. Images of whole *Escherichia coli* cells revealed the gap between the outer and inner membranes as well as other structures. The location to be imaged in a cell can be controlled by such factors as the polarization of the lasers and orientation of cell layers probed, which could potentially allow visualization of individual particles such as ribosomes.

Zewail, who says the technique is being commercialized by the electron microscopy company FEI, is currently using the technique to probe antibody structures and believes that the spatial resolution can be improved to as little as a nanometer.

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RESEARCH PAPERS

Barwick, B. *et al*. Photon-induced near-field electron microscopy. *Nature* **462**, 902–906 (2009).

Flannigan, D.J. *et al*. Biological imaging with 4D ultrafast electron microscopy. *Proc. Natl. Acad. Sci. USA* **107**, 9933–9937 (2010).