THE BIG AND THE BOLD

Some say it looks like a rocket ready for take-off. Others say a pagoda. Either description seems more fitting for the tiered and towering 13-metre structure than the diminutive word 'microscope'.

The electron microscope at Osaka University in Japan is the most powerful of its kind. The technology behind it is nearly 80 years old, and the device itself has been in operation since 1995. But scientists there say that they are only just starting to show what this monstrous machine can do for biology.

Transmission electron microscopes shoot out a beam of electrons, which pass through some parts of a sample and are scattered by others. The emerging beam carries data that can be used to map out the structures it has transited.

Because electron beams have a much shorter wavelength than visible light, they can easily resolve structures just a few nanometres across, far beyond the resolution of a conventional light microscope and better for viewing the details of cells' internal and external topography.

In the 1940s, scientists used some of the first electron microscopes to produce ground-breaking images of cells' mitochondria and other organelles. The detail was breathtaking, but researchers had to slice the tissue so that it was thin enough for the weakly energized electrons to get through: they were imaging dead, two-dimensional slices cut from living, three-dimensional structures. "Electron microscopy gave a framework to lay out physiological biology," says Mark Ellisman, a neuroscientist and microscopy specialist at the University of California, San Diego. "But it was kind of like taking road kill and analysing it."

Ultrahigh-voltage electron microscopes (UHVEMs), which give the electron beam enough energy to pass through and analyse thicker samples, started with two machines that could reach 3,000 kilovolts, built in Toulouse and Osaka in 1970. The new Osaka scope was constructed next door to that older one between 1991 and 1995, with a ¥2.4-billion (US$25-million) budget. It has an electron gun with a 5.7-metre accelerator tube and a maximum 3,500 kiloelectronvolt electron beam, and it can force electrons through biological samples thicker than 5 micrometres. This goes far beyond the 80-nanometre and 500-nanometre upper limits of the 100-kilovolt and 300-kilovolt machines typically found in laboratories.

The Osaka UHVEM is particularly valuable for imaging the diffuse but delicate structures of neurons, which requires an image that spans several micrometres at a resolution of tens of nanometres. Naoko Kajimura, an electron microscopist at Osaka University, has captured three-dimensional images of neuronal cells in the retina to show that those lacking a protein called pikachurin cannot form synapses (S. Sato *et al. Nature Neurosci.* **11,** 923–931; 2008). Had the 2-micrometre section been imaged by conventional electron microscopy, it would have needed to have been cut into some 30 slices, making it difficult to ensure that a part was not being missed.

A decade ago, few life scientists used the Osaka UHVEM, says Hirotaro Mori, director of the Research Center for Ultra-High Voltage Electron Microscopy, which houses the machine. Materials scientists dominated them, especially those who were exploring the properties of thinly sliced semiconductors and integrated devices. But times have changed. Digital imaging has replaced film, slashing the time needed for data collection and analysis. An automated system makes it possible to better control the mechanics and flux of electrons and hence limit damage to tissues and cells. Computer software can correct much of the image distortion. And scientists have now developed electron-tomography methods — in which the sample is tilted under the microscope's electron beam — which generate a series of images from different angles that can be compiled into an accurate three-dimensional structure. Now Mori says that biologists account for half of the machine's basic-research time, which has to be booked up to a month in advance.

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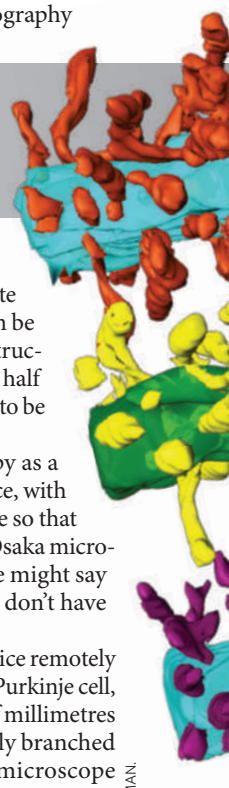
— Mark Ellisman

Microscopists say that electron microscopy as a whole is undergoing something of a renaissance, with attempts to update and automate the technique so that it can image three-dimensional samples. The Osaka microscope is a brute-force solution. "Many people might say bigger is not better," says Ellisman, "but they don't have access to it."

Ellisman and his group, who operate the device remotely from San Diego, have been working to image a Purkinje cell, an expansive neuron that measures a couple of millimetres from the cell body to the tips of the exquisitely branched dendritic arbor. The group first took light microscope images of the cell, marking its location in the cerebral cortex so that it could be indexed in a cell-based brain atlas. The team then sent thick sections to Osaka to get nanometre resolution of the spines on the dendrites. Ninety per cent of the mammalian brain's synapses are made on these mushroom-like projections but, Ellisman says, "until now scientists have thought of them with tinker-toy-level abstraction". The UHVEM image, which shows the number of spines as well as the size and shape of the spines' heads and necks, will be crucial for simulations on how the brain works, he says.

Mori laments that such science experiments account for only half of the machine's use. To balance demand he gives the other 50% to industrial tasks, such as verification of newly designed integrated semiconductor devices, nanoscale characterization of new lubricants, or the measurement of hair damage after a cosmetic treatment. "I would like to see more basic science," says Mori. ■

David Cyranoski



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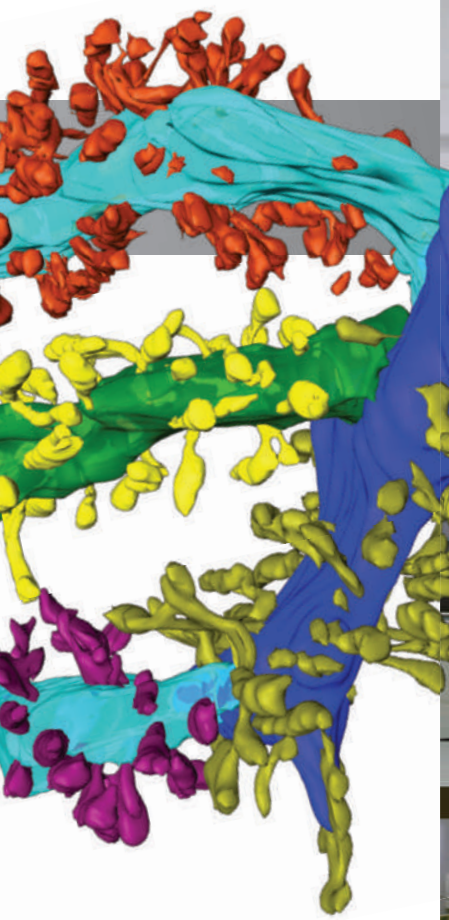
See page 629 and online at http://tinyurl.com/microspecial.



The fine-branching dendritic arbor of a Purkinje neuron (top) has been reconstructed from images taken by the Osaka electron microscope (right).