New Microscope Images Ions' Ins and Outs

With the scanning ion-conductance microscope, researchers will be able not only to map the topography of a living cell but also to observe the flow of ions out of the cell membrane

MUCH OF THE FUNCTIONING OF A CELL depends upon controlling the passage of ions through the cell's membrane, or outer wall. By opening and closing the pores in this membrane, the cell regulates concentrations of such ions as calcium, potassium, or sodium. This allows the cell to maintain its internal environment and trigger various functions, such as the contraction of a muscle cell or the firing of a neuron. In muscle contraction, for example, opening certain pores allows various ions to flow between the inside and outside of the cell, which in turn leads to cell contraction.

Now a newly developed type of microscope should give biologists a much closer look at the workings of these pores than previously possible. As reported on page 641, the scanning ion-conductance microscope (SICM) will be able both to map the surface of a living cell without damaging it and to monitor ions as they move in and out of the pores.

The SICM is the newest in a series of microscopes that are spin-offs of the scanning tunneling microscope (STM), which won the 1986 Nobel Prize in Physics for its creators, Gerd Binnig and Heinrich Rohrer. The STM, invented in 1982, examines a sample not by exposing it to visible light or other type of radiation but rather by scanning a tiny probe across the sample's surface much as a blind man taps a cane to detect the bumps and cracks in a sidewalk. Other microscopes, such as the atomic force microscope, the scanning capacitance microscope, and now the SICM, also use a sharp probe to scan the contours of a sample but each device uses a slightly different type of probe.

In the case of the SICM, the probe is a glass micropipette with a tip whose inner diameter measures from 0.05 to 0.1 micrometer. Paul Hansma of the University of California at Santa Barbara, the inventor of the SICM, likened it to a miniature eye dropper that is moved over a sample. The trick is to keep the tip of the probe a fixed distance from the surface of the sample as it scans across. This is done as follows:

The sample is placed in a saline solution, and the pipette is filled with the same saline solution and brought up next to the sample. An electrode is placed in the pipette, a second electrode is put in the sample's container, and a voltage is applied across the two electrodes. This creates a current between the electrodes, which is carried by the sodium and chlorine ions supplied by the salt molecules.

Once the tip of the pipette gets close enough to the surface of the sample—within about the same length as the pipette's inner diameter—the tip's opening starts to become blocked, making it more difficult for ions to travel between the two electrodes. This in turn causes the current between the electrodes to drop. In this way, the current provides a precise indication of how close the tip is to the sample.

The rest is easy. The tip is scanned across the surface while a delicate mechanism monitors the current and moves the tip up and down to keep the current constant. By recording the ups and downs of the tip as the probe moves over the surface, the SICM constructs a topographic map of the sample.

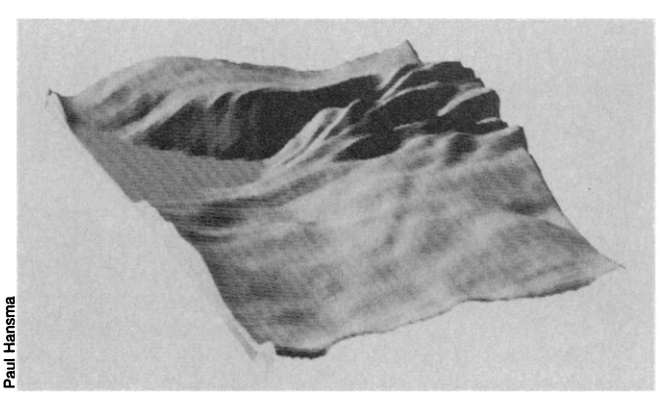
The only difference between this and the STM, Hansma said, is how the probes sense the distance from the surface. The STM uses a tunneling current between the probe and the sample to monitor this distance. A tunneling current is a quantum mechanical current that does not need a conducting medium, so the probe and sample are often placed in a vacuum. Hansma said his lab has one machine with interchangeable heads that serves as an SICM, an STM, and an atomic force microscope.

One major advantage of the new microscope over the STM, Hansma said, is that it will image nonconductors. Because the STM depends on a current between the sample and the probe, the sample must be an electrical conductor, which most organic materials are not. The SICM, on the other hand, measures a current carried by the solution in which the sample is placed. The SICM is also gentle—it does not touch a sample's surface, so it does not damage the sample.

The greatest potential of the SICM, Hansma said, is in monitoring the movement of ions through pores in living cells. With the SICM, he said, "you would be able to see what is coming out of each individual ion channel, plus you would be able to map the topography of the cell around the pore." Hansma said he has tested this function by imaging the pores in a membrane filter, but he has not looked at pores in biological samples.

Although the SICM needs some improvements before it can provide the resolution needed for many of biological applications, Hansma said this should not be too hard. His model used pipettes with diameters no smaller than 0.05 micrometer—"We borrowed a pipette puller from a local microbiologist," he said—but pipettes with inner diameters smaller than 0.01 micrometer have been made by others. The biggest problem with smaller pipettes is that they are fragile and tend to break during a scan, he said. By decreasing the inner diameter of the pipette and improving the location measurements of the tip, Hansma said the SICM should eventually be able to form images with details as small as 0.01 micrometer, or 10 nanometers.

One possible improvement of the SICM would be to use a micropipette with several barrels, each of them sensitive to a different ion, Hansma said. In this way, the microscope could observe the movement out of a cell's wall of a number of different types of ions simultaneously. ■ ROBERT POOL



Paul Hansma

An image of a leaf *formed by the scanning ion-conductance microscope. The image was reproducible, which showed that the imaging process did not damage the surface.*