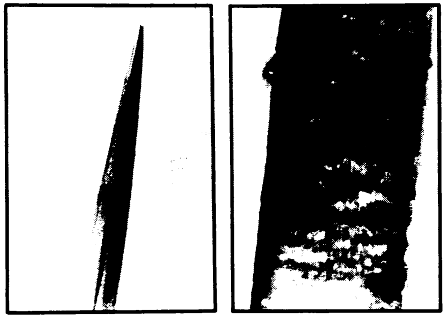
Tiniest tools probe a cell's molecules

Here's a team for the Guinness records book: a heater one-twenty-fifth the diameter of a human hair, and a companion thermometer only half as big around as the heater. According to biophysicist Frederick Sachs, their inventor at the State University of New York at Buffalo, the thermometer is theoretically capable not only of discerning temperature changes as small as 10^{-5}°C, but also of doing it in a record-breaking 100 microseconds.



Tip of thermometer (left) is about one-fiftieth the diameter of a human hair (right).

"Among the things we're going to measure are the temperature changes in nerve cells when they're stimulated," Sachs says. He suspects they may reveal "cycles of temperature as heat flows in and out of the cell." But these tools could have a number of more practical applications as well, he says. For example, they could be used to measure blood flow in capillaries or the rate of air flowing through a single alveoli (the tiniest air sac) in the lung.

Sachs wasn't out to set smallness records with his microtools (though he did). They were merely the means to an end—a probing of the ion channels in cell membranes. "Ion channels are little protein pores that control the flow of current in a cell," Sachs explains. When they're open, current flows through them; when they close, the current stops.

Sachs wanted to measure the kinetics of these channels — how fast they would open or close. "In real time, we're talking about measuring the shape of one molecule," he says. And channel movements are temperature sensitive, he points out, because these channels normally open or close in response to being kicked about at random by neighboring molecules.

The researchers also wanted to measure the temperature dependence of the movement of ions through an open channel: At higher temperatures they move faster. "The idea was to catch the channel while it was open and then to 'freeze' it by turning off the heater," Sachs says. In its immediately cooler state, a channel would tend to stay open — at least for a while — giving the researchers time to study it. But it had to be cooled fast, "and the rate of cooling depends very strongly on the size of the heater," he explains, because it determines how small the initially heated area is.

Developed by Sachs, Tony Auerbach, James Neil and Rich McGarrigle, the heater is made out of an open-tipped cylindrical glass pipette filled with a low-melting-point alloy of indium and bismuth. The metal core comes right to the edge of the tip, which is about 2 microns in diameter. (There are about 25,000 microns to the inch.) A layer of gold just 50 molecules thick covers the entire device, providing an electrically conducting contact between the outside and the inner metal. When the heater is hooked up to a power source, current driven down the inside returns through the tip and up the outside. As the system drives current in, it measures the resistance at the tip. "That serves to indicate the temperature," Sachs says, "because the resistance of the metal goes up as the temperature goes up."

Sachs invented the thermometer to monitor those minute temperature changes in the ion channels they were stimulating with the heater. It had to be small to respond quickly enough to catch tiny oscillations in temperature. So Sachs made it *very* small, roughly 1 micron in diameter at the tip and a mighty 2 millimeters at the back end. (There are 1,000 microns in a millimeter.)

Like the heater, it started as a cylindrical glass micropipette. But the thermometer's pipette is closed at the bottom and is divided by a partition running down its entire length. The partition separates two wells of an ionic fluid — saltwater. In a standard outdoor thermometer, temperature is measured by the distance a fluid expands up a closed glass cylinder. Sachs's thermometer records temperature as the speed at which ions in solution move. As temperature increases, their speed increases. And their speed is inferred from electrical resistance.

For now, the heater can be varied in 0.1°C increments to 100°C. If it gets any hotter it risks melting out the metal core. However, by substituting quartz for the glass pipette and another metal for the core, Sachs believes it should be capable of going to 1,000 or 1,500°C. The thermometer now can record temperatures over a range from about −60°C to perhaps 300°, but with a different ionic fluid, and again a substitution of quartz for its glass housing. Sachs suspects it might handle the temperature profiling of flame fronts in combustion chambers, such as those used to model auto engines. Details about both devices are due to appear in the fall Methods in Enzymology.

Like a new telescope or microscope, these high-resolution microtools offer a new way of probing uncharted domains. And until you've mapped those domains, he says, you don't know what you're missing. *—J. Raloff*

Forcing an unexpected attraction

That oil and water don't mix is hardly surprising. Yet, unexpected results are coming out of recent direct measurements of the force that drives hydrocarbons in water to aggregate into droplets or distinct layers. These experiments show that the attractive force between two wide, flat hydrocarbon surfaces immersed in water is 10 to 100 times stronger than predicted by classical theory.

"We have established that there is a force that is extremely long-range and much larger than one would expect," says D. Fennell Evans of the University of Minnesota in Minneapolis.

In the Sept. 13 Science, Evans, Richard M. Pashley of the Australian National University in Canberra and their colleagues report that this force appears to depend on the dimensions and geometry of the surfaces. It also reflects the ordering of water molecules near the hydrocarbon layers.

The results suggest that biological systems can take advantage of a wide range of attractive forces that operate between hydrophobic (water-repelling) parts of molecules, the researchers say. These forces may drive the folding of proteins and the formation of biological structures such as micelles, vesicles and lipid bilayers.

For their experiments, equivalent to observing the coming together of two oil drops in water, the researchers use a special apparatus consisting of two silvered glass plates covered by thin, smooth sheets of mica. The polar ends of long, double-chained alkylammonium acetate molecules adsorb onto the mica sheets, exposing the hydrophobic ends of the molecules to form large, planar, electrically neutral, water-repelling surfaces. A spring mechanism allows the measurement of the force between the surfaces when the two plates, immersed in water, are parted in steps as small as 1 angstrom.

"This apparatus allows you to measure directly the kinds of forces that govern a lot of what goes on in biological self-assembly," says Evans. Nevertheless, the apparatus is very expensive and delicate. "It's extraordinarily difficult to use," he says.

The researchers are now studying more complicated surfaces and the effect of solvents other than water. Says Evans, "We're looking at the kinds of interactions that one sees in membranes." *— I. Peterson*