Very, Very, Very Small Scale

Analytical chemistry techniques are being developed to make measurements on the scale of individual molecules

By JANET RALOFF

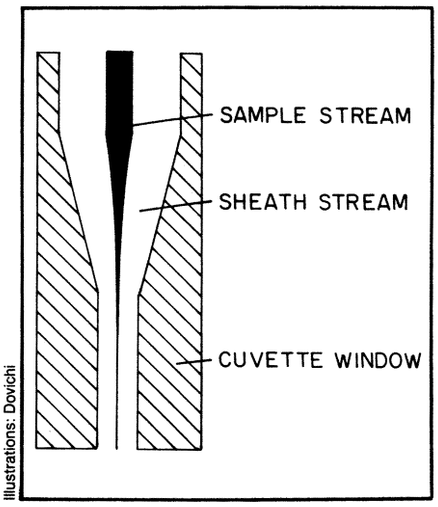
Norman J. Dovichi likes to think small. Very small. It's necessary in his business, which is the development of devices to study and quantify attograms—quantities on the scale of a millionth of a millionth of a millionth of a gram.

"To get a feel for what we're measuring," says the University of Alberta chemist in Edmonton, "imagine something that has the same volume as a grain of salt dissolved in your average olympic swimming pool." What you end up with is a "per drop" concentration of about 10^{-18} grams—or an attogram. And those drops are small. It takes a trillion to fill a teaspoon.

While the detection limits of his instruments may qualify for the *Guinness Book of World Records*, Dovichi hasn't pursued them merely for the challenge. Nature sometimes packages samples of materials that researchers want to study in very small quantities. His aim, with a series of laser-based devices, is to allow detailed quantitative inspection of those minute packages.

Dovichi's efforts began several years ago with the adaptation of the sheath-flow cuvette (a device first developed around World War II) to measure laser-induced fluorescence. While commercial sheath-flow cuvette systems are normally used to count individual cells (such as cancer cells) flowing through the device, Dovichi is modifying his instrument to quantify much smaller units of biological material — such as peptides, amino acids and proteins isolated from complex mixtures of materials by chromatographic or electrophoretic techniques.

In order to be "seen," these materials must be stained with a fluorescent coloring agent. Dovichi's device has been tailored to recognize fluorosine isothiocyanate, a yellow dye frequently used to label proteins and peptides for immunological analyses. Two streams of liquid are pumped through his cuvette system: a square stream of pure solvent — something that could be as simple as water — from 1 to 250 microns across, and down the center of that solvent a stream of the stained sample material traveling through the cuvette at a millionth of a liter per minute.



Illustrations: Dovichi

Sheath-flow cuvette: Sample is injected into the center of a flowing sheath stream. As cuvette's internal cross-section narrows, so does sample's radius.

An argon-ion laser focused into the sample stream induces the stained material to fluoresce. A microscope focused on the sample stream, at right angles to the laser beam, collects the fluorescent light and directs it to a photomultiplier tube for quantification.

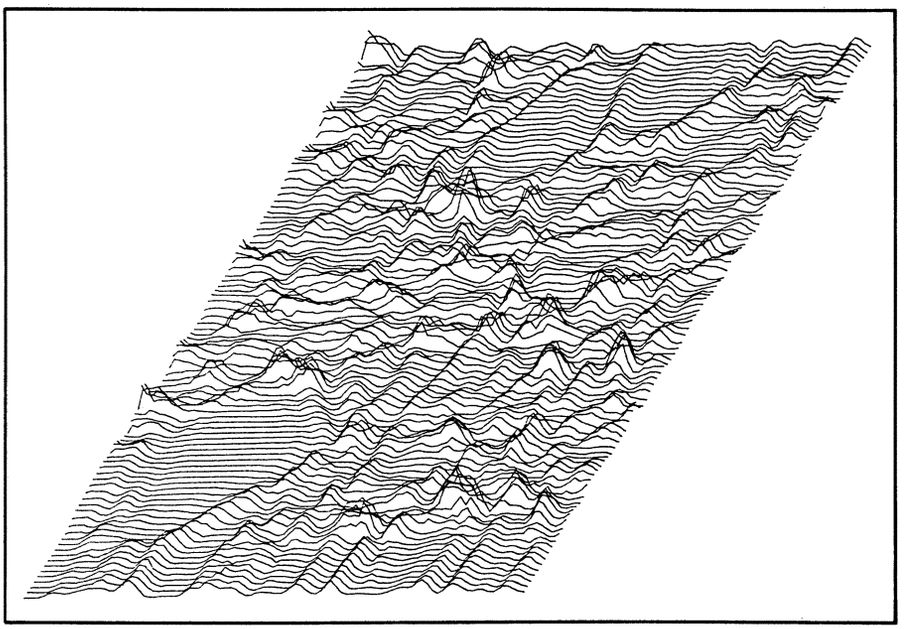
Since the amount of fluorescing sample running through the microscope's field of view in any second (the response time of the system's electronics) is only about 10^{-11} liters, or 0.1 millionth of a millionth of a liter, Dovichi says he can come close to detecting the signal emitted by a single molecule. In fact, he says, he already has the equipment necessary to make the counting of single molecules possible. All that remains is for him to reduce the background "noise" in his system, speed up its electronics and add a few other refinements. Such changes could be completed within a few years, he says.

When they are, this device will offer "the ultimate quantitative analysis," he believes, since "one can do no better than to count every molecule in one's sample."

A related light-scattering detector to measure the size of extremely small particles combines the sheath-flow cuvette with something called laser-doppler velocimetry—a two-laser technique for measuring the velocity of flowing liquids. As before, two streams flow through a cuvette system: one of pure solvent, another of solvent to which the particles that will be measured have been added. Two laser beams of equal intensity are focused on the flowing stream of particles, intersecting the sample stream at roughly 40° angles to each other. By diluting the suspension of particles in solvent, Dovichi can ensure that these particles pass through this study region in single file.

Where the laser beams cross, a set of optical fringe patterns occurs. Particles passing through this fringe region generate a burst of scattered light, the frequency of which is related linearly to the particle's speed. Electronics measure how the intensity of light scattering varies with time, providing a reading of their size.

Dovichi describes this system as the "least expensive, simplest and quickest" way to measure particles down to 45 nanometers (thousandths of a millionth of a meter) in radius. Moreover, he adds, "this is the most precise technique for discriminating between a mix of different-sized particles." Dovichi anticipates his new system could ultimately find use in such tasks as measuring the size of respirable pollutants (submicrometer particles have been implicated in causing black-lung, brown-lung and asbestos-related disease), studying viruses and subcellular biological components, and analyzing the purity of solvents used in the semiconductor industry.



Micrograph generated by photothermal microscope of 100 × 100-micron cross-section of a basswood plant stem. This image represents the rate of heat flow (thermal diffusivity) through the material. Similar images can be made of minute color differences in stained biological materials. Dovichi expects that such micrographs depicting cross-sectional slices of human tissue may one day help pathologists identify cancerous lesions in seconds – avoiding the 12- to 24-hour sample-preparation time it takes today.

His newest development is a device he calls the photothermal microscope. Able to discriminate between very minute variations in the the color density of materials, it can not only be used to study highly transparent materials – those containing a millionth of the color contrast needed for conventional imaging systems – but it can also, uniquely, measure the thermal properties of the sample and resolve these data for selected point-like, three-dimensional regions (areas containing only about 120 colored molecules) within a large, bulk sample. Moreover, he says, it does not damage the sample in the process. And by recording data for a series of adjacent points as the probe is moved through the sample, a researcher can assemble an image of a cross-sectional slice through the uncut, bulk material.

Like his light-scattering detector, this device requires two laser beams. Here, however, they cross at right angles within the sample. The energy from one, called the pump laser, is absorbed by the sample, which causes a heating and localized expansion within the probed region. The expansion produces a density change in the material, which in turn induces a refractive-index perturbation relative to the adjacent unheated material. These refractive-index perturbations, capable of bending light rays, actually defocus the second laser beam.

What's happening to this second beam "is exactly analogous to what occurs in a mirage," Dovichi explains. "Essentially, we're forming a controlled mirage within our sample." A decrease in the electrical output signal of a small silicon solar cell, placed in the center of the second laser beam, quantifies that beam's defocusing.

One of this microscope's advantages, Dovichi says, is that the strength of the signal is inversely proportional to the radius of the pump-laser beam. As a result, looking at small areas is easier and yields more precise data than focusing on larger regions. And it can yield nonoptical data, like measurements of how good a thermal conductor the material is, which may help in identifying unknown substances. Finally, the instrument can glean data about points inside a large uncut sample, like a rock, so long as that sample is relatively transparent to the laser's radiation. Currently, the system is limited to studying materials that are not opaque to visible light. But it might eventually be refined to study metals with X-rays, Dovichi says.

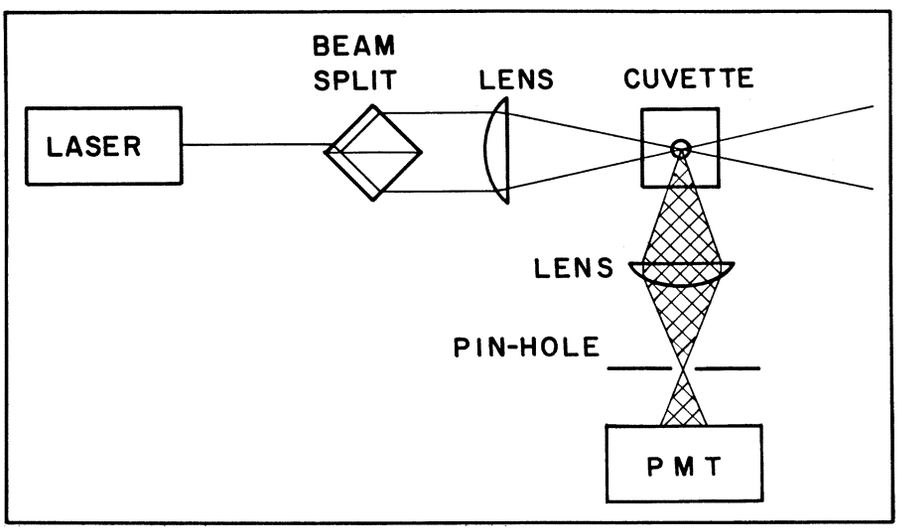


Diagram for light-scatter detector. Laser's beam is split in two, then recombined at the center of the cuvette (shown here from above), creating light and dark fringe patterns. Particles in cuvette sample stream passing through these fringes alter light signal, which is detected by photomultiplier tube (PMT).

He thinks the microscope's most exciting application, however, will be in medicine. Currently, pathologists interested in studying tissues must generate thin-section slices for analysis – a task that can require 12 to 24 hours of preparation. Clearly, one can't easily keep a patient on the operating table awaiting the results of these tissue analyses. But with Dovichi's microscope, one or more cross-sectional slices through the tissue can be optically generated, allowing relatively quick pathological analysis of whether a tissue is, for example, cancerous. "Right now it takes about 30 minutes," Dovichi told SCIENCE NEWS. "In the future, it will take only seconds."

Dovichi is one of just a handful of researchers opening up the atto-scale world to scientific exploration. But he says it doesn't require a stretch of the imagination to see that devices such as these could one day find potent routine applications in fields such as biological analysis, environmental and occupational health, electronics and the semiconductor industry. That's why he argues that it pays to think small. □