The Incredible Shrinking Laboratory

The analytical laboratory of the future may scrutinize DNA or test a ground-water sample for pollutants within reaction chambers and channels etched on a silicon chip

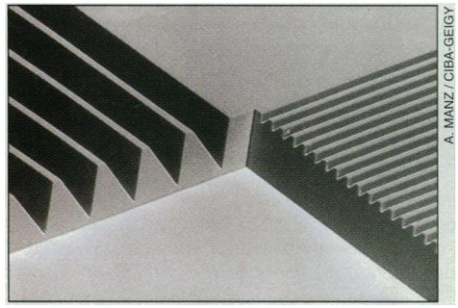
Microchips have miraculous power to miniaturize. First silicon chips compressed computers from rooms full of vacuum tubes and relays into devices the size of a hardback book. Now microchips of another sort are poised to perform the same feat of downsizing on analytical laboratories. Sorting out a sample's chemical constituents, sequencing DNA, or searching for mutations in a gene now requires benches laden with equipment. But if several researchers have their way, these tasks may soon require nothing more than a battery pack, some cunningly crafted microchips, and a digital readout.

Already, some of these researchers have succeeded in shrinking the tubes and beakers of ordinary analytical equipment into channels and chambers etched on the surface of postage-stamp–sized silicon and glass chips. These devices still require large-scale supporting equipment, but instrument-makers are hard at work on full-fledged "labs on a chip" that could have an impact in disciplines ranging from medicine to environmental chemistry, says John Sninsky, director of research at Roche Molecular Systems in Alameda, California. Such microlabs will "allow testing to occur outside of sophisticated research and clinical laboratories," he says. This could allow doctors to test for genetic diseases such as hereditary breast cancer in their offices and ecologists to check river water for pollutants on site.

The work could bring other boons. As Sninsky points out, "Miniaturization allows reactions to occur much more rapidly than in conventional instruments," because microinstruments require minuscule volumes of reactants. This easily sated appetite for expensive reagents should also save money. But researchers still need to overcome some macrohurdles. Like computer engineers, who have had to miniaturize not only the computer's processor, but also memory and data-storage devices, they will have to scale down and integrate every component in an analytical process.

And that's no small task, because each step currently requires its own bulky machinery, such as centrifuges for preparing samples and lasers for detecting the results. But researchers are taking heart from their progress so far. "All the pieces are there," says Jeff Ives, a bioanalytical chemist at Molecular Tool, a Baltimore-based DNA analysis company. "We just need to put them together."

One piece is taking shape in the laboratories of Ciba-Geigy in Basel, Switzerland, where researchers are laboring to shrink a workhorse technique of analytical chemistry known as high-pressure liquid chromatography (HPLC), which separates and identifies components in a diverse mixture of chemicals. The technique works by combining the sample with an inert carrier solution, then forcing the mixture through a stack of tiny particles in a narrow tube. The sample's components emerge from the tube at different times, allowing them to be identified.

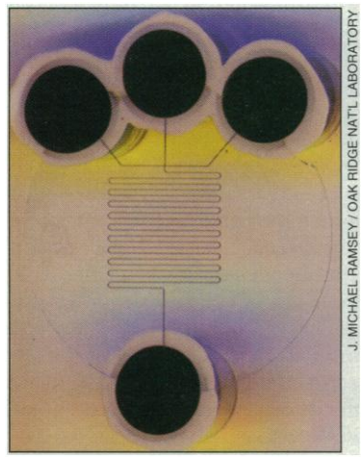


A. MANZ / CIBA-GEIGY

Good plumbing. Submillimeter channels in a chip carry fluids for free-flow electrophoresis.

Ordinarily HPLC machines are the size of a large color television set. But, using standard techniques for etching silicon, the Ciba-Geigy researchers, led by Andreas Manz, miniaturized key steps of the separation process. They carved a tiny channel roughly 300 microns wide and 2 centimeters long in a silicon wafer, then packed it with minute beads of a common HPLC filter. Finally, they cut a series of small channels at either end to feed liquid into the Lilliputian separation column and steer the separated molecules that emerge from it into a chamber where they are detected.

In a forthcoming article in the journal *Analytical Methods and Instrumentation*, Manz and his colleagues describe an early test run in which they used the device to separate two different fluorescent dyes. "It's a very interesting demonstration" that the complex plumbing on the chip works as intended, says Jed Harrison, a microdevice-maker at the University of Alberta in Edmonton. "But it's not a perfect HPLC yet." And Manz concedes that, before the device is ready for field use, the group needs to shrink other components, such as the pump that drives fluids through it.



J. MICHAEL RAMSEY / OAK RIDGE NATL LABORATORY

All in one. Capillary electrophoresis chip can mix reagents, separate sample components in a sinuous channel, and detect them.

Another hard-working technique that is shrinking under the miniaturizers' efforts is the polymerase chain reaction, or PCR, a method for copying a minute sample of DNA millions or billions of times that has revolutionized genetics research. The process begins when technicians mix the sample with free nucleotides, a DNA-copying enzyme, and short nucleotide sequences called primers, which bind to DNA and delineate a section to be copied. The mixture is heated and cooled repeatedly. Heating unzips the paired strands of DNA into two separate nucleotide chains; cooling allows the primers to bind to the appropriate spots on the separate chains. The enzyme then attaches free nucleotides end to end, starting at the primers, to copy the stretch of DNA. As the cycle is repeated the number of copies grows exponentially.

In current laboratories, this procedure happens in a machine the size of a microwave oven, costing many thousands of dollars. In the last 2 years, however, groups led by M. Allen Northrup at Lawrence Livermore National Laboratory and Peter Wilding at the University of Pennsylvania have shrunk the process onto chip-sized devices, opening the way to cheaper and more portable PCR machines that might find their way into doctors' offices. The chips, incised with channels and chambers to hold the reactants, are heated electrically. And because they are so small, they cool down much faster than conventional PCR machines, speeding the thermal cycling and shortening the procedure's running time from an hour in fast full-sized machines to as little as 15 minutes.

So far the mini-PCR chips have succeeded primarily in copying single DNA segments. But at a

meeting on genetic screening held in San Francisco last month, Northrup reported that his group had used a battery-powered, hand-held device to simultaneously amplify eight different gene segments containing the most common mutation sites for cystic fibrosis. This multiple copying, Northrup explains, requires very precise temperature control to insure that all of the sequences are copied at the same rate. His group achieved the needed control, he says, by building a tiny heating element directly into the reaction chamber.

When the copying was complete, the researchers could check each fragment for mutations that can cause cystic fibrosis by applying the amplified sequences to simple paper test strips. "For the first time we have a battery-powered, hand-held system that can be used to check for common genetic disorders," says Northrup. But he acknowledges that PCR-on-a-chip isn't ready for the doctor's office. One reason is that extracting DNA from blood or other samples still requires complex sample preparation procedures that have not yet been scaled down.

Getting it all together

Microscale HPLC and PCR are only two results of this rush toward miniaturization. Separation techniques known as free-flow electrophoresis and capillary electrophoresis have undergone the same downsizing. And researchers at biotech start-ups such as Affymetrix in Santa Clara, California, and Hyseq Inc. in Sunnyvale, California, are making chip-based devices that carry an array of immobilized nucleotide sequences, designed to identify disease-causing sequences in DNA (*Science*, 3 June 1994, p. 1400).

Because individual analytical components like these can be faster and cheaper than their current counterparts, instrument-makers are already linking them to their full-scale laboratory instruments, says Harrison. But analysis-on-a-chip won't realize its full potential, researchers agree, until all the functions needed to carry out a particular reaction—including batteries to drive the system, reaction chambers and filters to prepare the sample, and detectors to read the results—can be miniaturized as well. Says Harrison: "We're only beginning to put this whole series of elements together."

This integration may have come farthest for capillary electrophoresis (CE), which separates snippets of DNA or amino acids. Unlike HPLC, which sorts molecules in liquids based on their interactions with solid particles in the separation column, CE does so by using an electric field to draw them through a narrow channel. The molecules' different sizes and charges cause them to migrate through the channel at different rates, so they emerge separately at the far end. Over the last few years several groups have succeeded in putting the separation step onto a chip threaded with a tiny channel. And they've even begun adding sample preparation and detection steps to their chips.

At last month's Pittsburgh Conference, an analytical instruments meeting held in New Orleans, separate teams led by Alberta's Harrison, Ciba-Geigy's Manz, and J. Michael Ramsey of Oak Ridge National Laboratory reported some of the most extensive integration to date. Ramsey's group, for example, demonstrated the first lab-on-a-chip that applies CE to the common DNA analysis technique known as restriction fragment analysis. To perform the chip-based version of the technique, Ramsey and his colleagues started by putting DNA and specialized enzymes into different chambers on the chip. They then used the same electric fields that draw molecules through the CE channel to pump liquids carrying the DNA and enzymes into a reaction chamber, where the enzymes cut the DNA into "restriction fragments"—snippets of different lengths. Applying the electric field again caused the chopped DNA fragments to migrate to the separation channel, where they were sorted by size and tagged with fluorescent dyes for detection.

Those reports and others bring fully miniaturized analysis one step closer, but "much work still remains to be done," says Satyam Cherukuri of the David Sarnoff Research Center in Princeton, New Jersey, who is leading efforts there to integrate chip-based devices. One "jugular issue," says Cherukuri, is the size difference between the milliliter-sized samples doctors and chemists are used to working with and the millionfold smaller quantities that suffice for a lab on a chip. Finding a representative fraction of a sample to feed into a microlab could be a problem for a test designed to find, say, a rare viral sequence.

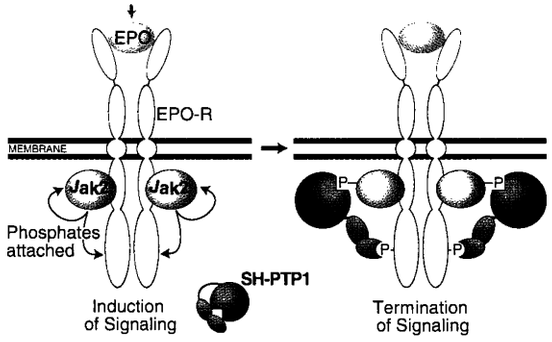
But with the example of computer scientists to inspire them, many researchers believe even that hurdle can be surmounted. "Miniaturization is inevitable in analytical techniques," says Ives. "There's nothing inherent in chemistry or engineering that prevents it." And that green light suggests the mantra of analytical chemistry will soon echo that of computing: Small is beautiful.

–Robert F. Service

CELL BIOLOGY

An "Off Switch" for Red Blood Cells

In biological systems, the brake is as important as the gas pedal. Take the production of red blood cells. In 1989, when the gene for the erythropoietin receptor (EPO-R) was cloned, biologists gained a much better understanding of the signaling mechanism that spurs production of red blood cells in human bone marrow. But the "off switch" that halts this process and thereby maintains the body's precise control over the number of red blood cells in the bloodstream remained unclear.



SOURCE: H. LODISH *ET AL.*, *CELL*

Stop sign. SH-PTP1 turns off the "develop" signal sent by phosphorylated Jak2.

Now scientists in the same Boston laboratory that cloned EPO-R have located the brake: the spot on the mouse version of the receptor where an important down-regulating enzyme docks. And, proving that basic research has serendipitous rewards, in the process they've solved a decades-old medical mystery from Finland that may have played a role in the awarding of three gold medals at the 1964 Winter Olympics.

In a recent issue of *Cell*, a team led by Harvey Lodish at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, including cell biologists Ursula Klingmüller, Ulrike Lorenz, Lewis Cantley, and Benjamin Neel, report that the binding of an enzyme called SH-PTP1 to a specific site on the mouse EPO-R slows the maturation of multipotential "stem cells" in the bone marrow into red blood cells. James Darnell, a molecular biologist at Rockefeller University in New York, says, "It's an important connection. ... The output of the receptor is being regulated somewhat surprisingly."

The surprise is that the switch that turns off hematopoiesis (production of red blood cells) isn't on the EPO-R molecule itself, but on an associated enzyme called Jak2. In both mice and humans, EPO-R is a 550-amino-acid-long protein that spans the stem cell's outer membrane. Lodish and his colleagues believe that when the hormone erythropoietin binds to the receptor's outer domain, it causes Jak2, which binds to the inner portion of the receptor, to attach phosphate groups to itself and to certain amino acids on the receptor. The addition of the phosphates creates docking sites for other proteins that pass the signal "develop into a red blood cell" on to the interior of the stem cell.