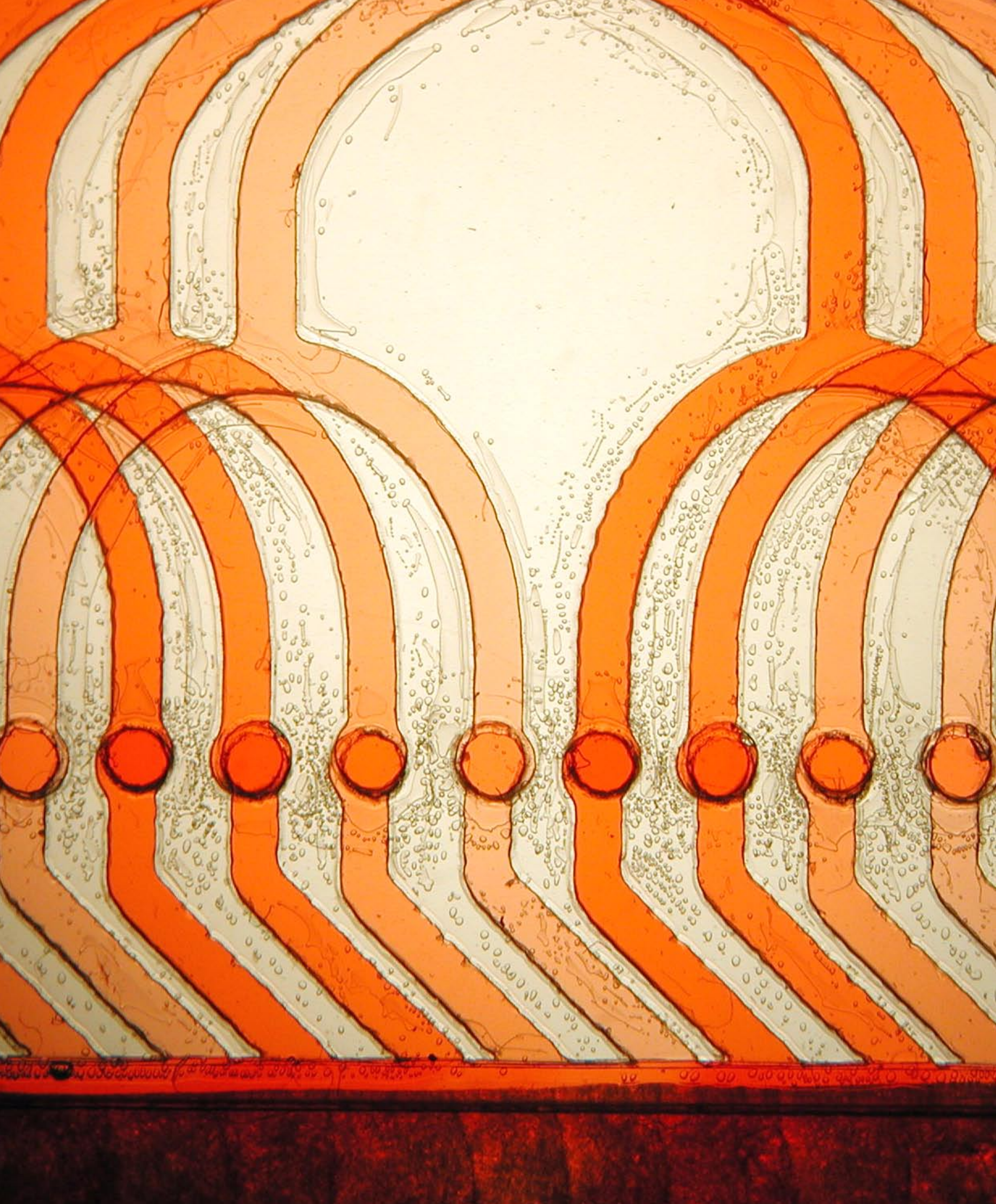


It may look like a painting by Morris Louis, but this magnified photograph shows the many-channeled plumbing of a "lab-on-a -chip" created by University of Washington bioengineer Albert Folch to study cells' reactions to chemicals. Fluids—in this case, dye and water—are pumped through pipes just 500 microns wide, en route to chambers where they will merge to form 16 combinations.

IN THE MICRO LAB:
Plumbing the width of a human hair // channels only microns deep // testing a single drop of saliva //
With speed, efficiency and portability, and that's only the beginning.

Studies in Miniature

BY LOGAN WARD

A single drop of saliva contains almost every substance present in human blood—including 1,166 proteins, and unknown numbers of hormones, carbohydrates, electrolytes and mineral ions—but at concentrations 10 to 100 times lower. Last spring, researchers from three California institutions catalogued the complete set of saliva proteins, in part to identify many that are disease indicators, or biomarkers. Above-normal C-reactive protein, for example, indicates acute inflammation that has been linked to rheumatoid arthritis, lupus and even heart disease, whereas HIV antibodies (also proteins) can signal the presence of the condition. So—in theory, at least—a simple spit test might tell as much about a person's health as a full-scale blood profile, but without the painful stick or the need for a phlebotomist to draw blood.

The problem is that current spit tests in most instances aren't practical. The analytical equipment is too expensive, the specialized labor too costly and the wait time for results too prolonged. But now that may change, as the field of microfluidics, also known as lab-on-a-chip technology, comes into its own. Sandia National Laboratories in Livermore, Calif., has designed a palm-size device that can measure how far certain biomarkers in saliva travel through a gel the length of a fingernail—and by doing so, can spot periodontal disease even before the onset of symptoms and can potentially detect illegal drugs. What's more, the results are available in a matter of minutes, rather than the hours or days required for a typical laboratory test performed on saliva or blood. In addition, Micronics, a company located in Redmond, Wash., has created a microfluidics device the size of a credit card that can diagnose malaria and dengue fever from a fingerprick drop of blood within minutes. Micronics expects to add more diseases to its capabilities soon.

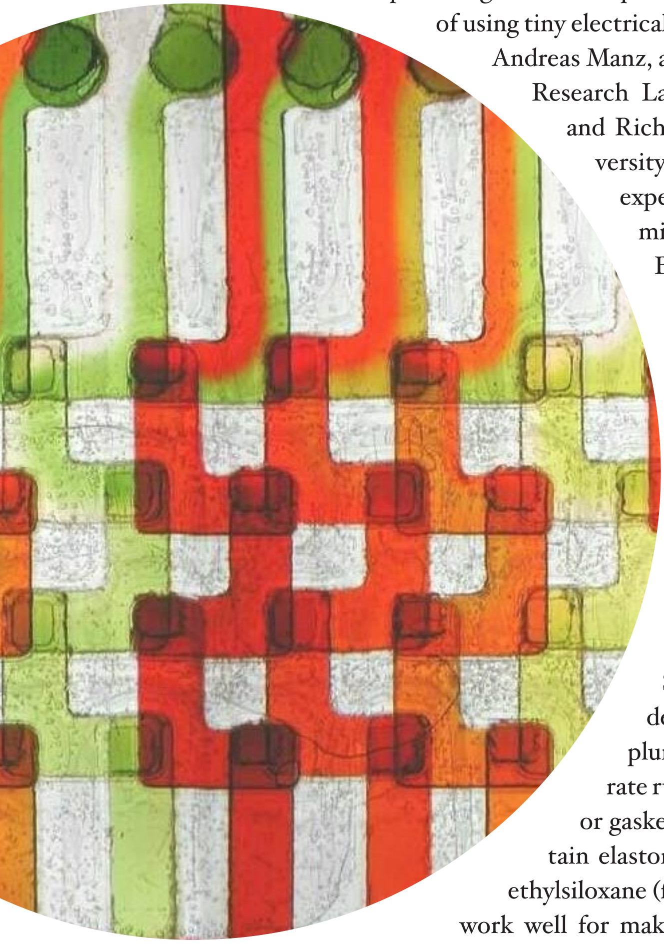
When chemical analyses and biological assays are done on such a tiny scale, they yield quicker results that are also less expensive because the equipment can be mass produced, or is even disposable and requires just microliters of samples and testing compounds. Even more remarkable, thanks to the peculiar laws of fluid dynamics, liquids in very small spaces operate more smoothly and predictably, enabling researchers to scrutinize such microscopic developments as the growth of nerve cells in muscle tissue and to grow perfect protein crystals—a delicate process, essential in the search for effective drug compounds, that had been relegated to the zero-gravity environment of NASA space missions. Microfluidics, in short, is opening avenues that, on our unwieldy human scale, were never possible, let alone affordable.

Microfluidics was born when the worlds of chemistry and silicon chips collided, says Harvard University professor and chemist George Whitesides, "developing as a cooperation between people who like to make small things and people who need small things." In the 1980s, as analytical chemists and molecular biologists delved ever deeper into the molecular structure of compounds, they yearned for a

COURTESY FOLCH LABS

In bioengineer Albert Folch's chip, each pair of fluids meets at a circle chamber (the green junction at the top of the photograph, left), but thanks to the special properties of liquids in microscopic spaces, there is almost no mixing until the fluids pass through the "serpentine mixer"—three right-angle turns shown in the plaidlike areas of both photographs that don't conform to the rest of the chip's flat plane, encouraging an even blend.

technology that would do for their lab experiments in genetic sequencing and protein analysis what computers had done for their data calculations. Because their work revolved around analyzing substances, that meant creating a way for fluids to move on a microscale.



The first microfluidics chips borrowed fabrication techniques straight from computer chip makers. But instead of using tiny electrical circuits, pioneers such as Andreas Manz, at the Ciba-Geigy Central Research Lab in Basel, Switzerland, and Richard Mathies, at the University of California, Berkeley, experimented with etching microchannels into silicon. By the 1990s, Whitesides and others, including a young Oxford University physics Ph.D. named Stephen Quake, were experimenting with other materials, including rubber.

"Microfluidics is essentially miniaturized plumbing," says Quake, who today co-chairs Stanford's bioengineering department. And most plumbing systems incorporate rubber in the tubing, valves or gaskets. Quake found that certain elastomers, especially polydimethylsiloxane (found in plumber's caulk), work well for making miniature valves and are cheap enough to allow for trial and error.

All that experimentation is paying off as microfluidics moves toward commercial applications. And it's about time, says Quake, who considers even today's most automated laboratories too big, expensive and inefficient. The problem, he says, relates to the "tyranny of numbers"—the practical limit to the complexity of systems such as early computers, which consisted of thousands of vacuum tubes, used hundreds of kilowatts of power and weighed as much as 30 tons.

An analogous situation now exists in labs. After a nurse draws blood from a patient filling one of those Vacutainer tubes with the colored tops— the sample begins an odyssey into a world of pipette-wielding technicians running hundreds of thousands of dollars' worth of appliance-size machines. Consider, for instance, the search for circulating tumor cells (CTCs), which indicate that cancer may be spreading into a new area of the body. The first step involves separating platelets and red and white blood cells out of blood plasma in a high-speed centrifuge. Next, a technician decants, or draws off, the plasma. Finally, the plasma is run through a flow cytometer, a cell-sorting machine that detects microscopic particles suspended in fluid by aiming a laser beam and highly sensitive photodetectors at a stream of the fluid and waiting for the CTCs to emit light. Because this rigorous testing often damages or destroys the fragile CTCs it has an accuracy rate of less than 50%.

When Mehmet Toner, a member of the surgery department and director of the BioMicroElectroMechanical Systems Resource Center at the Massachusetts General Hospital, learned that clinicians were trying to detect the presence of CTCs using outdated, off-the-shelf cell-sorting equipment, he wasn't surprised by such inconclusive results. And it's a crucial task, considering that metastases cause 90% of human cancer deaths. To find a better way, Toner partnered with Ronald Tompkins, a surgeon at the MGH, and Daniel Haber, director of the hospital's cancer center, to create a team of clinicians, engineers and scientists. The result is a device the size of a business card that has at its core a silicon chip etched with 80,000 tiny pegs, each coated with an antibody to a particular protein found on a certain type of solid tumor. When a standard three- to eight-milliliter blood sample is pumped across the chip, blood cells bounce past the microscopic array of pegs, but any tumor cells stick to the antibody "glue" on the posts.

When Toner was designing his CTC chip, he decided to bypass centrifuging and other damaging procedures and send whole blood samples straight from test tube to test. But because CTCs are so rare, he needed to be able to detect a single tumor cell among, say, a billion blood cells. Microfluidics gave him that extreme sensitivity. According to results published last December in the journal *Nature*, the chip found CTCs in all but one of 116 samples from 68 patients with metastatic cancers—a 99% sensitivity rating. And it found the cells by the thousands—several orders of magnitude higher than previous tests performed with flow cytometry and other

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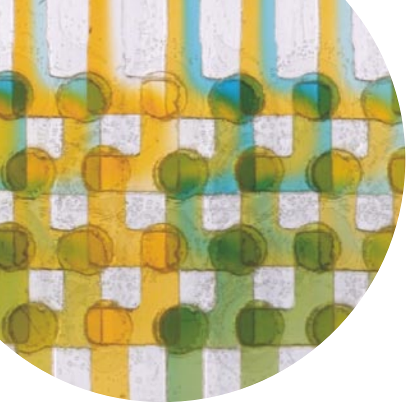


The chip found circulating tumor cells in all but one of 116 samples from 68 patients with metastatic cancers—a 99% sensitivity rating.

standard methods. What's more, in samples taken from 20 healthy participants, the chip found no CTCs, scoring a 100% accuracy rate. In an additional study, the chip isolated CTCs in seven of seven patients with early-stage prostate cancer.

Compared with earlier attempts to detect CTCs, "this is clinically actionable information," says Toner, who, following some design tweaks and ongoing clinical trials, hopes to have a $20 version of the device in doctors' hands within two to three years. "Being able to find these cancer cells changes the whole paradigm of oncology," he says.

In both basic research and clinical applications, other paradigms are also shifting. University of Washington bioengineer Albert Folch uses microfluidics devices as miniature cell-culture tools to replace the petri dish. For example, in a project to study synapses, the connection points between nerve ends, a team from Folch's lab used a device to observe the role the protein agrin plays in the development of neurotransmitter receptors in muscle tissue. Instead of bathing cultured muscle tissue with agrin in a dish, the researchers mimicked the influence of a neuron during the birth of a muscular synapse by subjecting only parts of the muscle cells to a stream of agrin beneath a channel etched on a chip. The experiment proved that local exposure to agrin leads to clustered neurotransmitter receptors only at the site of agrin stimulation, yielding a key piece of information about how nerves and muscles communicate.

"Essentially, humans are microfluidic," Folch says. "Each of our cells is exposed to a different molecular environment, and to try to recreate that—to build artificial cell culture systems that are more faithful to the actual conditions—requires very precise fluid handling techniques on a very small scale." But in a petri dish, cells are laid out uniformly on a flat surface and then bathed with a homogenous fluid, thereby losing many levels of complexity that exist in the microenvironments of our bodies.

Diagnosis in the Fast Lane //

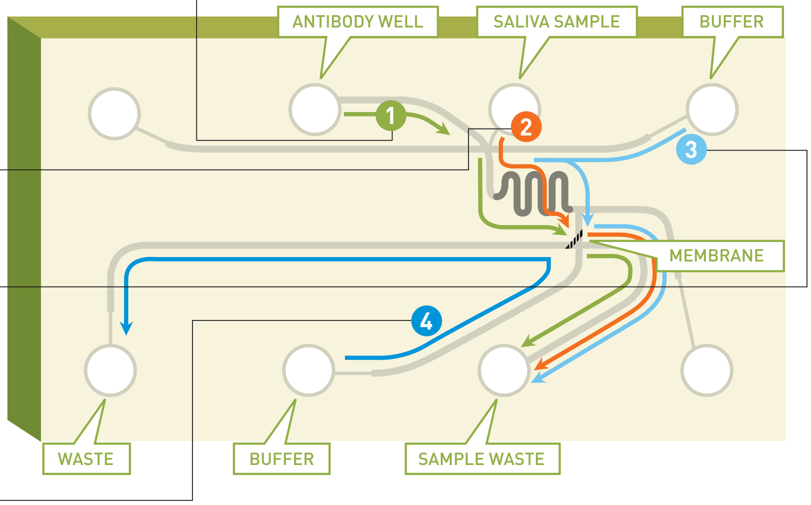
With two microscope slides, one computer chip and nine volts of electricity, a new saliva analyzer can return results in eight minutes, as opposed to several hours. Here's how it works.

Minutes 0 to 2: A negative electrode is attached to the antibody well (full of fluorescent antibodies to protein biomarkers present in the disease being tested for), and a positive one to the sample waste well. When a current is applied, the antibodies are drawn by the positive charge into a membrane, where they lodge, too large to pass through.

Minutes 2 to 4: Voltage drives the saliva to the membrane, where any proteins get stuck alongside the antibodies. If any of the proteins are biomarkers, they will bind to the antibodies.

Minutes 4 to 5: Voltage is applied to drive buffer fluid to the sample waste well, flushing out any remaining saliva.

Minutes 5 to 8: Voltage drives the antibodies and proteins up a long channel, which contains sieving gel. Each molecule, when subjected to a charge, travels a certain distance through the gel based on its mass. Because these distances are quantifiable, the device can recognize which proteins are bound to antibodies. The device then measures the ratio of bound proteins (signifying the presence of the disease) to unbound (healthy) proteins.



Harvard University professor and chemist George Whitesides, a pioneer in microfluidics, took the lessons of computerization to the lab, miniaturizing unwieldy testing processes.

So to replicate actual conditions, Folch explains, requires precise fluid-handling techniques on a very small scale.

Transforming laboratory environments is also essential to the world of drug discovery, in which the best way to understand a protein related to a particular disease is to take it out of solution and trap it in a solid crystal—an ordered array of molecules. To form crystals, researchers mix samples of a particular protein with other chemical compounds called reagents. They may try hundreds of reagents before finding one that produces an ideal crystal. Then, using X-ray diffraction to examine the crystal, they map out the protein's atomic structure to home in on places at which a drug might bind to it and perform a certain function—blocking the creation of an enzyme that leads to the replication of a virus, for instance, which is what the class of HIV drugs known as protease inhibitors do. The larger and better-formed the crystal, the easier it is to search for an appropriate target site.

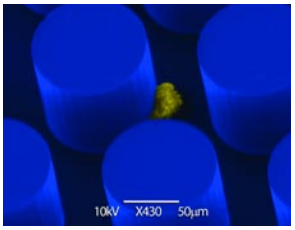
> "Essentially, humans are microfluidic. Every cell is exposed to a different molecular environment."

The most useful crystals grow in utter stillness, absent turbulence or vibration of any sort, including the movement of molecules resulting from convection (caused by temperature differences) and the subtle force of sedimentation caused by gravity. Because sedimentation distorts crystals into flawed shapes, researchers have experimented with cultivating crystals in space, supplying protein samples to NASA for its shuttle missions.

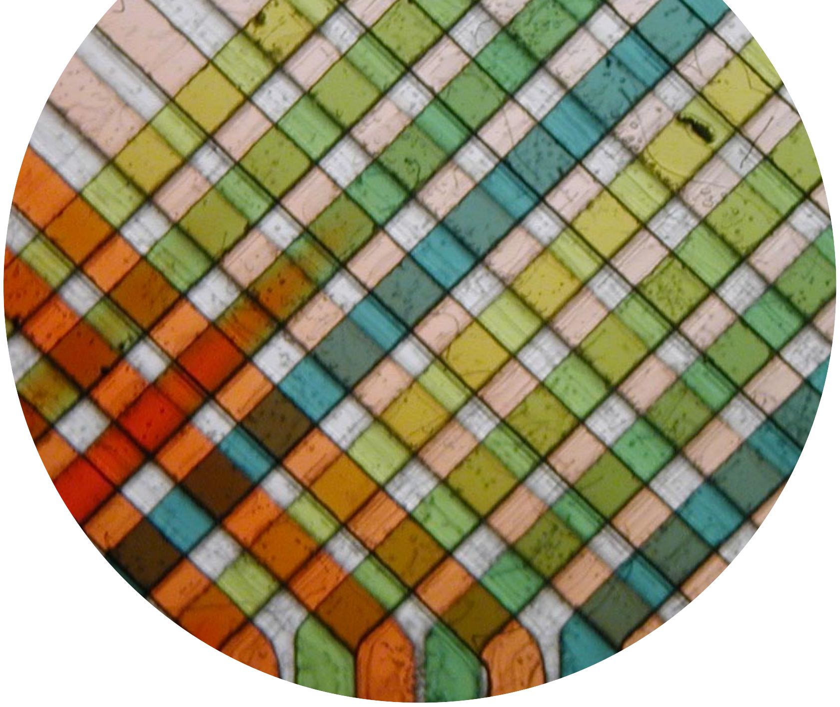
Now, biochemists are employing microfluidics to grow better crystals in earthbound laboratories. Using a protein-crystallization chip made by Fluidigm (a company co-founded by Quake), Hyock Kwon, at the University of Texas Southwestern Medical Center, recently completed a study examining the protein PCSK9, which prevents the removal of LDL (bad cholesterol) from the blood—research that could lead to an entirely new approach to reducing cholesterol.

"Under a microscope," says Kwon, "you can see all these channels and chambers that can be opened and closed for mixing different solutions." In an experiment requiring the utmost precision, this device automatically mixes minute amounts of protein samples and reagents, measuring compounds by the nanoliter—a quantity one-thousandth as large as the microliters of traditional testing. Without the device, researchers would need to perform the task either by hand or using pipette-wielding robotic machines.

The speed and automation of microfluidics is also helping create a generation of point-of-care diagnostic devices that experts say will bring medicine into the twenty-first century—and bring twenty-first century medicine to some of the planet's most remote populations. Already, Sandia's portable analyzer has been used to diagnose periodontal disease long before gums show signs of inflammation. At its core is a microchip, into which one-tenth of a milliliter of saliva is injected by syringe. The saliva enters one of six tiny reservoirs. Tagged with a fluorescent dye, antibodies specific to three or four proteins present in periodontal disease fill another reservoir. At the press of a button, a series of computer-automated



A circulating tumor cell (above, in green) can't resist one of 80,000 antibody-coated posts on a chip developed by Mehmet Toner (right), a biomedical engineer at the Massachusetts General Hospital.



Though they combine visually to create vivid color combinations, the fluid in the two sets of diagonal lines actually run in separate planes, not meeting until they reach circle chambers (just out of the frame).

OPPOSITE PAGE AND THIS PAGE: ASIA KEPKA FOR *PROTO*

tests commences. Instead of physically pumping the fluids, the device uses an electric charge to force the fluids through channels that are about the size of a human hair.

One by one, the saliva sample and each antibody solution are forced together inside a channel in which the fluorescent antibodies attach to any biomarkers that may be present. Another jolt of electricity forces all of the antibodies through a channel filled with a gel that serves as a sieve. Molecules of different masses travel different distances through the gel, making it possible to distinguish particular disease-causing particles. The device measures the level of fluorescence to determine their quantity, returning a detailed diagnosis within 20 minutes. If the quantity of protein biomarkers exceeds a certain level, it suggests that the patient has (or will get) periodontal disease. Sandia is currently working on a similar device that analyzes blood, and future diagnostic tests may include ones for heart disease and cancer.

"The key to this test is portability and speed," says Glenn Kubiak, Sandia's senior manager of biologic and microfluidic sciences. At the standard laboratory scale, the molecules in such a test might travel 10 inches after being given a jolt of electricity, a passage that could take hours to complete, whereas at the micro scale they cover distances of a few millimeters in less than a minute and yield equivalent results.

And because microfluidics is potentially orders of magnitude cheaper, such a device might provide ready access to diagnostic tests that have proved prohibitively expensive. Or, Kubiak says, "it might bring sophisticated medical diagnostics to underserved areas, in which patients can't get to a medical facility for a full battery of tests."

Says oral diagnostics expert Daniel Malamud, a professor at New York University's College of Dentistry: "You can do these tests in the lab, but the problem is that the patient isn't in the lab. What I'm most excited about is being able to export devices to the developing world, where many epidemics start, because it's a lot easier to send chips than to build labs."

That's exactly the approach taken by Micronics. Unlike Sandia, whose target market is medical and dental offices in the United States, the chip manufacturer aims to place its device in the offices of doctors and dentists, Micronics hopes to take its line of disposable infectious-disease diagnosis cards as far as Africa and Asia. "We expect to have devices going to the patient, instead of the patient having to go to the test," says Karen Hedine, the company's president and CEO.

The Micronics system uses the same polymerase chain reaction (PCR) technology now used in laboratories to test for infectious diseases, except that it miniaturizes the process by employing a disposable reagent-embedded card that Hedine estimates will cost as little as $5. The card is inserted into a portable, battery-powered, laptop-size device—estimated cost: less than $5,000—that extracts nucleic acids from the blood sample's DNA or RNA and searches for gene sequences that identify malaria or dengue fever. Tests return results within 15 minutes—"and you don't need a Ph.D. to run them," Hedine says.

Those advantages—being able to test faster, more cheaply and at the patient's bedside, as well as opening up new avenues in drug discovery—could help microfluidics transform health care. "Microfluidics has been promising for a long time, but there were no good applications," Toner says. "Now the field is ready to blossom." ■

→ DOSSIER

1. **"The Origins and the Future of Microfluidics," by George M. Whitesides, *Nature*, July 27, 2006.** A pioneer muses on the field's successes and growing pains.
2. **"Isolation of Rare Circulating Tumour Cells in Cancer Patients by Microchip Technology," by Sunitha Nagrath et al., *Nature*, Dec. 20, 2007.** A detailed account of the first clinical study involving Mehmet Toner's circulating-tumor-cell chip, with findings that MIT cancer researcher and Nobel laureate Phillip Sharp calls "stunning."
3. **"Microfluidic Immunoassays as Rapid Saliva-based Clinical Diagnostics," by Amy E. Herr et al., *Proceedings of the National Academy of Science*, March 27, 2007.** A detailed description of a new point-of-care diagnostic device that microfluidics made possible.