how it works

A LOW-COST, DISPOSABLE, SILICON-BASED PLATFORM OUTPERFORMS CONVENTIONAL SYSTEMS FOR DOING MOLECULAR DIAGNOSTICS.

DNA lab on chip rests on MEMS foundation

By Michele Palmieri, STMicroelectronics

A CHEMICALLY CHISELED silicon biochip is at the heart of a tiny portable DNA laboratory that promises to reshape the landscape of instruments for analyzing microbiological samples. The biochip, now in a prototype stage, is the primary element of a disposable cartridge that more quickly and less expensively analyzes DNA than do conventional instruments by integrating target nucleic-acid amplification and detection on one chip (**Figure 1**). Commercial development will improve the treatment and lower the cost of common infections by reducing hospital stays and the misuse of antibiotics.

Moreover, the chip's layout and manufacturing process, which relies on microfluidic-MEMS (microelectromechanical-systems) technology, allows users to customize the device to accommodate a range of analyses, including multiple concurrent ones, and positive and negative controls. Consequently, researchers at STMicroelectronics, which developed the chip, say applications abound in areas beyond medical diagnostics to include drug development; testing crops and livestock for genetic diseases; detecting bacteria, toxins, or allergens in food; testing water supplies for dangerous microorganisms; and oncology and disease prevention.

The first-generation lab-on-chip platform comprises a disposable cartridge and a benchtop-sized control instrument and reader that manages the interface between the operator and the biochip. Compared with working with bulky conventional instruments and protocols, which is time-consuming, the miniature system uses fewer reagents and less power, and it delivers the results of a DNA analysis in minutes rather than hours or—in the case of growing bacterial cultures—days.

The ability to more quickly obtain results means that doctors can respond more quickly to infectious diseases with lifesaving treatments. The biochip also provides more accurate results than do conventional means, in turn providing doctors with the information they need to prescribe narrow, targeted antibiotics, thus slowing the spread of drug-resistant bacteria. The second-generation lab-on-chip platform envisions a higher level of integration in the disposable cartridge and an even smaller instrument that will mean portability and the ability

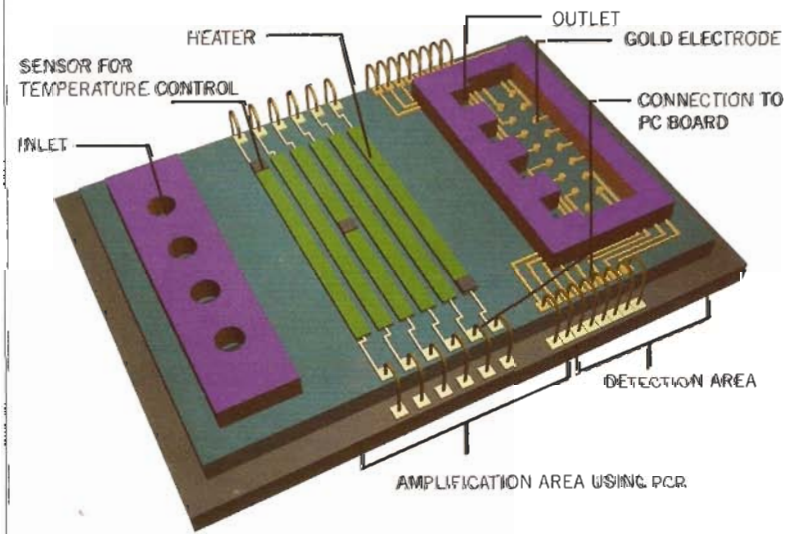

Figure 1 A lab on chip includes a section for detection and another for amplifying DNA samples by precisely controlled heating. The silicon devices draw on MEMS expertise from ink-jet-printer technology and well-understood IC-fabrication techniques.

to directly deliver and test results at the point of care, rather than sending samples to a laboratory.

The high degree of integration of the miniature lab reduces the level of manual intervention. A graphical user interface monitors the analysis in progress and guides the operator through all the necessary steps: simply loading a DNA sample for analysis and inserting the cartridge into the instrument.

All chemical reactions occur inside the biochip's proprietary buried channels or on its surface. And because the cartridge that carries the chip is self-contained and disposable, the system strongly reduces the cross-contamination risks of conventional multistep protocols.

AMPLIFIED DNA

Of key importance is the biochip's ability to perform both DNA amplification, through a PCR (polymerase-chain-reaction) process and detection of one substrate (see **sidebar** "The ABCs of DNA amplification"). In this process, a researcher mixes a DNA sample with a polymerase enzyme and DNA primers and passes it through a bank of 12 microscopic channels, each measuring 150×200 microns, within the silicon. Electrical heating elements in the silicon—essentially resistors—heat the channels, cycling the mixture through three precise predetermined temperatures that amplify the DNA sample.

The system then fluidically moves the amplified DNA into the biochip's detection area, which contains DNA fragments attached to the surface probe. There, matching DNA fragments in the sample, target DNA attach themselves to the fragments on the electrodes, whereas DNA fragments without matching patterns fall away. The system achieves accuracy by temperature control. It detects the presence of the DNA fragments by illuminating them with a laser and observing which electrodes fluoresce.

The development of this unique and innovative biochip builds on the experience researchers gained from a variety of fluidic applications, such as optical-bubble switches and ink-jet-printer heads. In the case of MEMS-technology ink-jet-printer heads, microscopic channels etched in the silicon connect hundreds of ink-filled chambers to a chip's surface. Each chamber contains a resistive heating element that quickly vaporizes part of the ink, propelling tiny droplets toward the paper.

Because the manufacturing techniques of the lab on chip are similar to those that silicon ICs use, device designers can draw on four decades' worth of reliable, mass-manufacturing know-how and give new life to depreciated larger-geometry-IC-production facilities. Also, engineers can focus on the design-rule-based approach that most microelectronic products use instead of simultaneously designing both the device and its manufacturing process. This approach leads to the development of cost-effective devices in line with the highly competitive medical-diagnostic market.

THE HEAT IS ON

The chip's many advantages over current plastic and glass substrates stem from the fact that the thermal properties of silicon make it extremely well-suited for the biochip application, which combines fluidic, mechanical, electrical, and optical elements. For example, silicon has a high thermal conductivity, allowing the chip to maintain a uniform temperature over a wide area, and low thermal capacity, affording fast thermal cycling during the PCR process. These features make the reaction time shorter than that of conventional macroscopic thermocyclers and improve on the performance of microscale systems having substrates with a lower thermal conductivity. For the same reason, the silicon substrate precludes the need to transfer the sample between separate areas of the chip, each at a different temperature. Instead, the fluid remains stationary, and the chip heats and cools it in place, greatly simplifying the chip's microfluid design.

Silicon also allows electronic controls on the same chip. In this case, the heaters and temperature-sensing ele-

ments are integrated into the chip's surface, giving accurate, real-time readings and closed-loop feedback. As a result, the chip continuously monitors and adjusts the parameters of the reaction. In addition, the proximity of the reaction channel to the sensing elements—just a few microns—guarantees that the readings closely follow the actual temperatures seen by the samples.

Once the system amplifies the DNA, the chip performs detection on a dedicated portion of the biochip's surface. Here, engineers can customize both the number of probes and their placement to meet the needs of an application. Process latitudes can accommodate the needs of even the most demanding molecular diagnostic application.

Moreover, whereas the current prototype of the reader and detection system depends on optical detection, the product road map envisions electronic detection, further improving the process. Although optical-DNA detection is common, it needs costly and bulky devices, which are ill-suited for portable instruments.

Researchers have proved electronic detection in laboratory settings, although manufacturers have not yet deployed it in medical applications. The technique would decrease the need for bulky equipment by integrating that function, making better use of the silicon and resulting in easier to use, lower cost, and more portable instruments.□

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THE ABCs OF DNA AMPLIFICATION

The PCR (polymerase-chain-reaction) process is behind a useful technique for "amplifying" minute DNA samples for analysis. This process combines a DNA sample with DNA building blocks, or "primers," and a polymerase enzyme. The mixture repeatedly cycles through 94, 60, and 72°C temperatures, causing the original DNA double helix to separate and replicate using the primers and enzyme. Each cycle through the three temperatures doubles the amount of DNA. After 20 cycles, therefore, one molecule of DNA yields 1 million copies; 30 cycles yields 1 billion.

In the first step of the process, denaturation, the mixture heats to 94°C, separating the two strands of the DNA's double helix (**Figure A**). At 60°C, annealing occurs, in which the single-strand primers, comprising short chains of DNA building blocks—adenine, cytosine, guanine, and thymine—bind to their complementary single-stranded bases on the denatured DNA. The last step, extension, occurs at 72°C. In this step, the thermos-acquaticus polymerase enzyme synthesizes the DNA, extending the single-stranded template that the primers started. This process results in two helixes where one originally was.

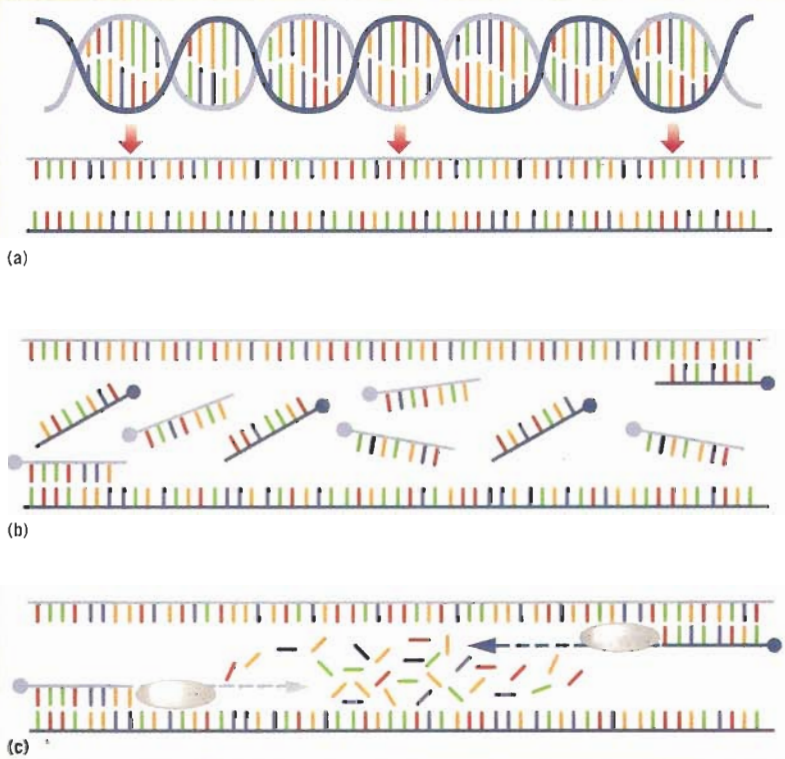

Figure A In step one of the PCR process, denaturation occurs. By heating the DNA to an optimal temperature of 94°C, the double strand melts and opens to single-stranded DNA (a). In the second step, annealing occurs at an optimal temperature of 60°C. The single-stranded primers bind to their complementary single-stranded bases on the denatured DNA (b). In step three, extension occurs at an ideal temperature of 72°C. The polymerase attaches and starts to copy the template. The result is two helixes in place of the first (c).