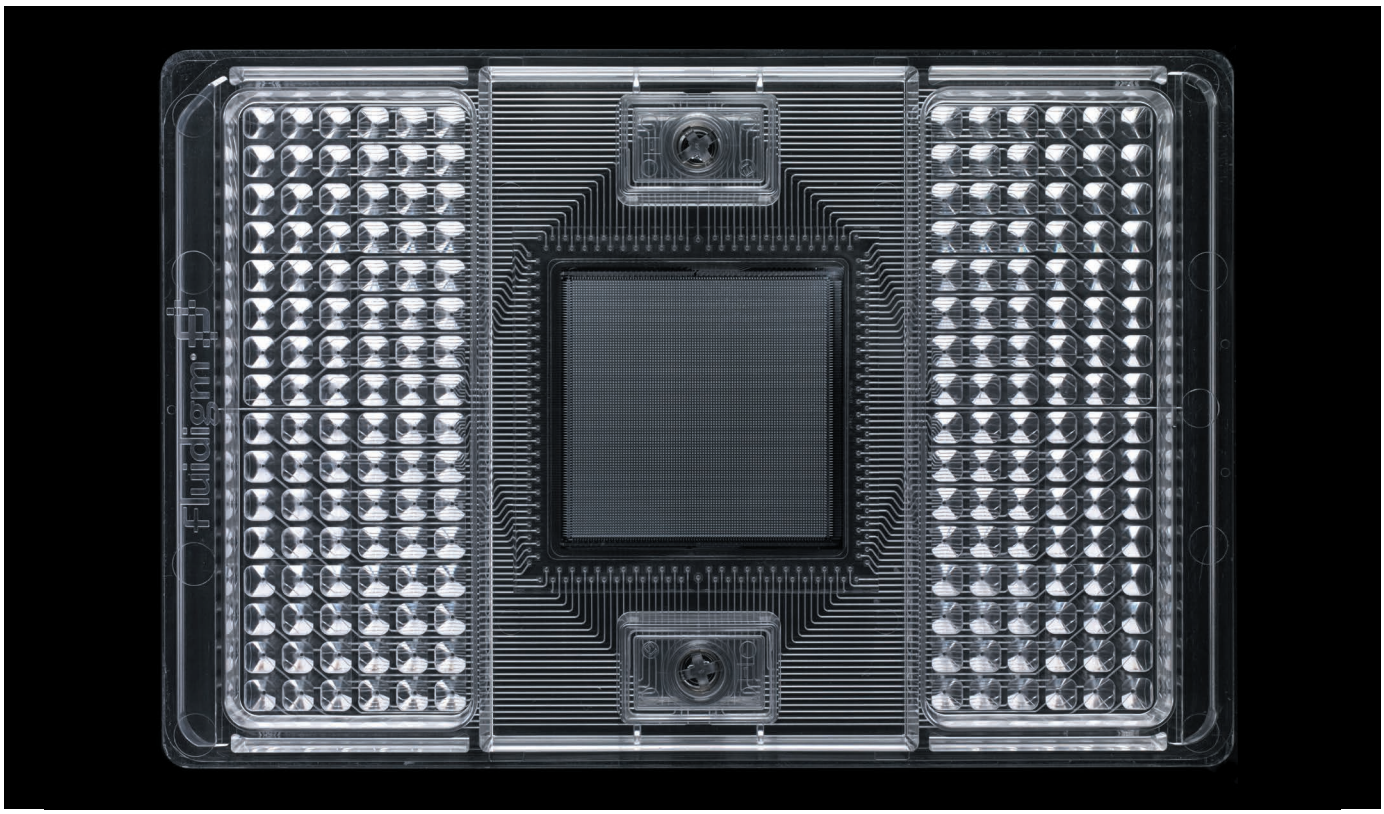
TECHNOLOGY FEATURE

THE MAKING OF A MEDICAL MICROCHIP

Microfluidic devices filled with intricate channels that exploit fluid behaviour promise to make it easier to diagnose genetic disease.

FLUIDIGM



This chip, developed by Fluidigm, can be used in a microfluidics system to analyse genomic information from samples as small as a single cell.

BY AMBER DANCE

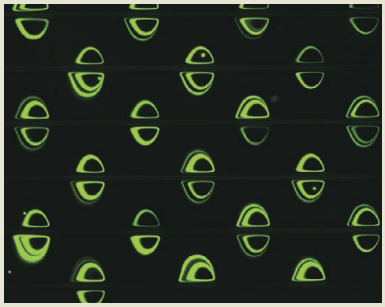
The bioengineers in Dino Di Carlo's lab at the University of California, Los Angeles, spend a lot of time wrapped in head-to-toe suits and looking a bit jaundiced. The engineers work in a clean room, where a steady flow of filtered air removes particulates. Blue or purple light would harden the photosensitive material with which they work, so they limit lighting in the room to butter-yellow.

They and others in the field are building tools for preparing and analysing blood and other fluid samples to diagnose genetic anomalies, such as the mutations carried by cancer cells. Few such tools require a clean room, but these ones depend on the ability of fluids to travel through channels so small that even one speck of dust could block them — a field of technology development called microfluidics. In theory, these assays, encapsulated in chips the size of a microscope slide, could allow for rapid and automatic diagnosis: sample in, answer out; so easy that a novice could use it. In practice, the devices rarely work this way, and usually, some pre-processing of the sample is required.

Researchers such as Di Carlo are working to address those shortcomings, making the chips easier to manufacture and experimenting with materials and designs. They are tackling challenges such as predicting the behaviour of fluids in small places, and determining how to make the chips both effective and affordable. Solving these problems requires an interdisciplinary approach, notes Amy Shen, a chemical engineer at the Okinawa Institute of Science and Technology Graduate University in Japan. The payoff could run from cost and time savings in the lab to medical devices that speed diagnosis of genetic and infectious diseases.

Microfluidic circuits enable scientists to work with samples that are precious or in limited supply, and to squeeze more results out of expensive reagents. Working with minute volumes makes it possible to conduct many analyses in parallel — and often rapidly. Because only machines can manipulate such tiny volumes, microfluidics is conducive to automation, which reduces human error. Ideally, even minimally trained technicians would be able to perform testing.

That goal remains elusive. Developers so ▶



DINO DI CARLO/AIP PUBLISHING

Orbits of beads in laminar microvortices formed in a microfluidic Vortex chip.

Tips for chips

Building a microfluidics chip generally starts with software such as AutoCAD, Adobe Illustrator or SolidWorks. "We can draw out the path of the pipes," says Dino Di Carlo, a bioengineer at the University of California, Los Angeles.

Fluid flow in a microchannel is predictable, he says. But crunching the numbers requires supercomputer time. Most researchers prefer to keep building iterations of a chip until they get their desired flow, although software can still help. The microfluidics company Fluigent near Paris offers software tools to help researchers to adapt their chip design, says its scientific founder, Jean-Louis Viovy at France's basic-research agency, the CNRS, in Paris.

Di Carlo's group built its own simulation tool called uFlow. Recognizing that microfluidics chips often include repeating elements, such as S-curves or columns that partition the liquid, they used a supercomputer once for each element, to work out how it changes flow. uFlow uses those data to treat the endpoint of each element as the starting point for the next, which saves processing power while it simulates complex channel shapes.

Once researchers have their desired chip design, they have several options to obtain it. They can engineer it themselves, or order custom chips. Shannon Stott and her colleagues at Massachusetts General Hospital in Charlestown contracted with a division of the Japanese electronics giant Sony, now owned by Stratec, to make blood-sorting chips using the same machinery that the company uses to create Blu-ray video discs.

There are also standard microfluidics chips available to perform common functions. Providers abound: popular options include Agilent Technologies in Santa Clara, California; Dolomite of Royston, UK; Fluidigm of South San Francisco, California; and Fluigent. A.D.

far have focused on miniaturizing processes used to analyse DNA or RNA in blood and other bodily fluids, such as by creating miniaturized versions of polymerase chain reaction (PCR) machines to copy and quantify rare gene sequences, or hybridizing to link nucleic acids with fluorescent probes. As a result, the microchip method often requires biological materials to have already undergone some processing, for example, to remove components that would interfere with the reactions. The major bottleneck, says Jean-Louis Viovy, research director at France's basic-research agency, the CNRS, in Paris and scientific founder of the nearby microfluidics company Fluigent, is "trying to expand the toolbox of microfluidics to be able to go from the real sample to the results, all in microfluidics".

CHIPPING AWAY AT DISEASE

Di Carlo's lab developed a method[1] for a specific kind of sample preparation: isolating circulating tumour cells (CTCs) — bloodborne hallmarks of cancer that can reveal a tumour's origin and the mutations that make it tick. To produce the chips, the lab uses a common technique called photolithography to make microchips out of PDMS, a transparent rubber. In the clean room, engineers spread a liquid mixture onto a circular plate of silicon — the material used for computer microchips. Then, mimicking the semiconductor industry, they cover the polymer with a printed black 'photomask' that contains clear portions in the shape of their desired channels. They then expose it to ultraviolet light to harden the liquid in only the exposed sections, creating an inverse cast of the chip.

Di Carlo's engineers then move to their normal lab. To create a chip, they pour liquid PDMS over the cast and bake it at 65 °C to harden it. Finally, they fuse a glass slide to the bottom of the PDMS, creating a chip prototype that has the look and feel of clear, extra-firm jelly. The whole procedure takes about a day.

Once they settle on a design that works for their purposes, they order plastic versions of the chips, made using the same process used to manufacture plastic toys, says Di Carlo.

Most techniques for fabricating microchips produce 2D designs. But sometimes, a 3D structure is valuable. In one chip design he's working on, Di Carlo uses a magnetic field to pull liquid from a narrow channel into a higher, wider one. As the fluid begins to expand in the larger chamber, surface tension causes it to form a sphere, which buds off as a droplet. "This now is a nanolitre pipette, basically, and that's impossible to do by hand," says Di Carlo. Such partitioning enables the chip to separate fluids such as blood into multiple, discrete reaction chambers so that many tests can be performed simultaneously[2].

To make a 3D chip, scientists have generally had to stack successive layers of a polymer into the photolithography moulds. But 3D printing is changing that, because it requires neither much expertise nor much equipment, say the designers of one entry-level method. Vittorio Saggiomo, a chemist at Wageningen University in the Netherlands, happened upon the idea at home. Saggiomo 3D-prints plastic tools, such as small lamps or pipette holders, as well as fun stuff, such as bird houses. One day, he submerged a 3D-printed *Star Wars* helmet in acetone to smooth out the surface, but left it in too long — and the whole piece dissolved. He realized that he could fashion microchannels in the same way.

Saggiomo and his colleague Aldrik Velders, also a chemist, adapted the process for the lab. They use a 3D printer to create the shape of their desired channel, and suspend that piece of plastic in PDMS. They then soak it in acetone overnight to dissolve the plastic, which leaves behind a ready-to-use microchip[3]. Saggiomo and Velders are playing with this strategy, producing coils or interwoven channels that would otherwise be difficult to make. For example, they designed a chip with a straight channel surrounded by a coiled one. Users could run hot or cold liquid through the coil, says Saggiomo, and thereby change the temperature of a sample in techniques such as PCR.

Even with standard manufacturing procedures, chip designers are getting creative, using channel layouts such as chevrons, angles and squiggles. And although the field is starting to develop standardized designs, Di Carlo says, there's a lot of room for variety in planning the fluid's narrow path.

Chip designers also struggle to predict fluid dynamics at this level. "The underlying physics at this scale, it's completely different from the water in your bathtub," says Walter Minnella, an engineer at the Elvesys Innovation Center, a microfluidics company in Paris. Some forces, such as gravity, become negligible, whereas the high surface-area-to-volume ratio gives surface tension and the interaction between the fluid and channel walls an outsized influence. Aqueous solutions turn viscous, similar to honey, but there's no turbulence, says Di Carlo. As a result, fluid motion becomes predictable — but it still might take a supercomputer a day or two to solve, Di Carlo estimates, which makes repeated simulations impractical. Most scientists opt instead for an empirical approach: build, test, repeat (see 'Tips for chips').

BLOOD BREAKDOWN

Mechanical engineer Shannon Stott at Massachusetts General Hospital in Charlestown and her team built multiple iterations of one chip before settling on its current form. They are pursuing liquid biopsies, a method for detecting and diagnosing disease from genetic clues in the blood. Their goal was to create a system that can purify and analyse CTCs from a minimally invasive blood sample[4]. They call their design the CTC-iChip — 'i' for 'inertial focusing', the technique used to line cells up in single file so that the chip can separate out the CTCs from other blood cells (see 'Anatomy

of a chip'). Among other things, the chip enables the team to count CTCs in patient blood samples and study their genetic composition.

Built out of plastic, the CTC-iChip consolidates three steps into one device. In the first stage, the chip eliminates unwanted blood components. Scientists label white blood cells with magnetic beads and then send the fluid through a chamber containing a series of plastic posts. The smaller bits, such as red blood cells and proteins, whizz through like a moth flying through a dense forest. The larger cells — the white blood cells and the rarer CTCs — are more like lumbering bears. As they bounce off the posts, the large cells are funnelled into stage two — the S-curves, or 'wigglers', as Stott calls them, which line the cells up in single file. In stage three, the device uses a magnet to yank the white blood cells out of line, leaving behind the CTCs.

Di Carlo's lab has developed its own microfluidic methods to sort blood samples[1], using channels dotted with a series of side chambers, like transepts in a church. His former student SJ Claire Hur, a mechanical engineer now at Johns Hopkins University in Baltimore, Maryland, noticed that bigger cells become trapped in vortices created by the widening of a microfluidic channel, much as leaves and rubbish accumulate around bends or rocks in a river[5]. The group designed a system, now manufactured by Vortex Biosciences in Menlo Park, California, that exploits this property to isolate CTCs for further analysis. The researchers are running clinical studies using the Vortex machine to identify markers on CTCs that might indicate how well a tumour will respond to specific immunotherapies.

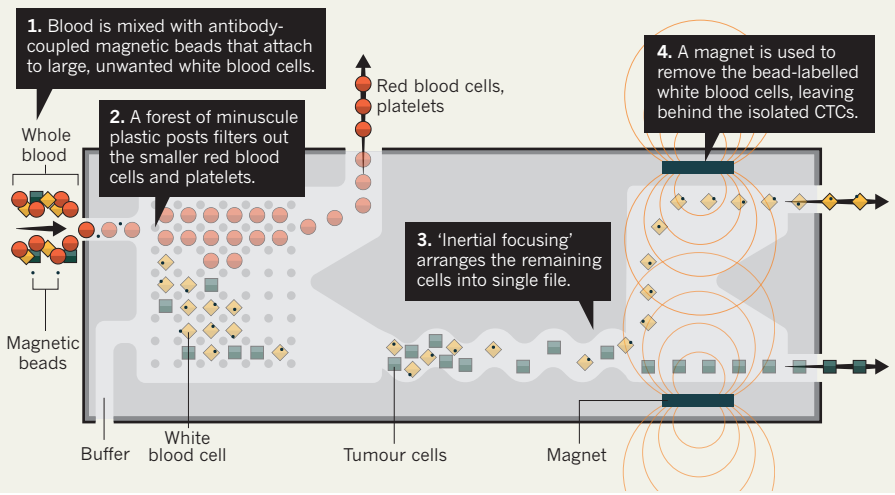
The Vortex microchip itself fits in the palm of Hur's hand, but the system also includes external tubing and pumps to feed the sample through the system, plus fraction collectors to recover the purified CTCs. The whole apparatus is a bit bigger than a microwave, making it less of an all-in-one 'lab-on-a-chip' — as many scientists want —and more of a 'chip-in-a-lab'.

Often, a chip-in-a-lab design is just fine, says Di Carlo. It still saves money over conventional

SOURCE: REF. 4

ANATOMY OF A CHIP

The CTC-iChip uses three successive structural elements to separate circulating tumour cells (CTCs) from other blood components. Here's how it works:



methods and improves results by minimizing experimenter variation. But a true lab-on-a-chip device would make rapid genetic testing possible for clinics or field stations in developing countries, where purchasing and running PCR machines or centrifuges to separate blood samples might be impractical.

LABS ON THE GO

Engineers have come up with a variety of possible solutions. Some, for example, are developing inexpensive devices made out of paper, which can amplify and detect the genes of infectious microbes in blood samples[6]. Hain Lifescience of Nehren, Germany, has designed strip-based tests that can detect specific DNA sequences. Some can identify a person's risk for Alzheimer's disease by searching for variants of a gene called *APOE*. Another can report on genes related to ankylosing spondylitis, a form of arthritis that affects the spine.

Syed Hashsham, an environmental engineer at Michigan State University in East Lansing, is developing a chip-based device for genetic diagnosis in the fields of cancer and infectious disease. "We have to simplify everything," he says. To make production cheaper, and to enable field scientists to make the chips airtight, he switched from silicon-based chips, which were difficult to seal under field conditions, to plastic ones that are cut using lasers and sealable with film.

Another challenge was how to amplify rare genetic material enough for it to be detected in the field. The standard method, PCR, requires repeatedly heating and cooling a sample to precise temperatures. But it's difficult to design a small, portable, inexpensive machine that can switch between those temperatures, says Hashsham. "In the field, thermocycling never works," he says.

He adopted an alternative method of sequence amplification to use in his handheld microfluidic 'Gene-Z' device, which identifies and quantifies known sequences such as the microRNAs that indicate cancer, or the genes of infectious organisms. Called loop-mediated isothermal amplification, the reaction uses a different enzyme from the one in PCR, and requires no temperature cycling. Researchers mix a body-fluid sample, such as spit, with a fluorescent dye that will be incorporated into any DNAs made in the reaction, and then use a syringe to push it into a channel leading to 16 individual chambers. There, the DNA-amplification reagents are preloaded, dried and ready. After the reactions are complete, the device uses light-emitting diodes and sensors to detect the dyes, which indicate a positive reaction[7].

The whole device runs off an iPod Touch and costs no more than US$200 to make, says Hashsham. Each disposable chip, comprising 64 chambers, for a total of four samples, costs less than a dollar. He has validated Gene-Z's results for more than 100 diseases. The challenge now, he says, is persuading funders to manufacture a device that won't be immediately profitable, because he wants to deploy it in regions such as Africa, where quick diagnoses could change the practice of medicine and save lives.

It can be difficult to translate ideas into the commercial space, agrees Shen, who points out that companies might not go for a design if it's too expensive or doesn't work with their existing manufacturing process. That leaves microfluidics a long way from the lab-on-a-chip promise. "There is still a gap, but I think we're slowly bridging that gap," she says. "Eventually, we will get there." ■

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