A New Wave of Microfluidic Devices

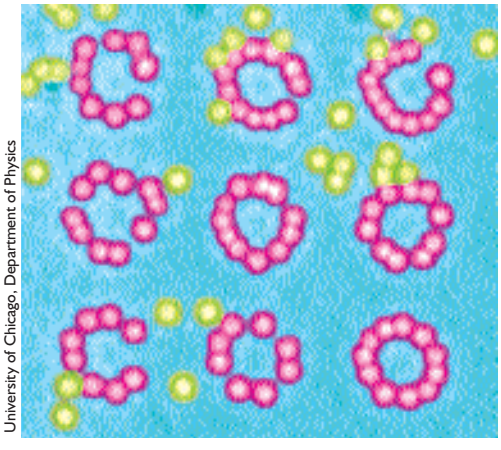
FEATURE

by Jennifer Ouellette

Over the past few years, microfluidics devices have enjoyed success in certain niche applications, notably ink-jet printers and lab-on-a-chip assays. However, recent advances and innovations could make the technology ubiquitous. Potential applications include pharmaceuticals, biotechnology, the life sciences, defense, public health, and agriculture, each of which has its own needs. Hence, the next generation of microfluidics devices now entering the marketplace emphasizes flexibility and usefulness in a variety of contexts—a trait that many in the industry deem crucial to the devices' commercial success.

Flexibility and a variety of uses are key

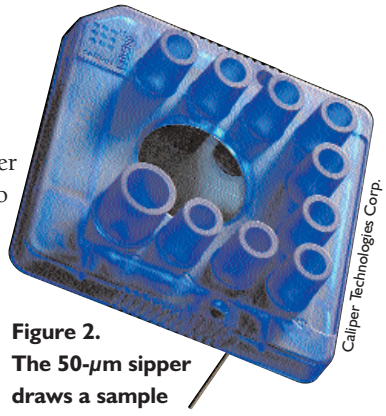
Microfluidics refers to a set of technologies that control the flow of minute amounts of liquids or gases—typically measured in nano- and picoliters—in a miniaturized system. "Unlike microelectronics, in which the current emphasis is on reducing the size of transistors, microfluidics is focusing on making more complex systems of channels with more sophisticated fluid-handling capabilities," says George Whitesides, Mallinckrodt Professor of Chemistry and Chemical Biology at Harvard University (Figures 4, 5, and 7). Although micro- and macrofluidics systems require similar components—including pumps, valves, mixers, filters, and separators—the small size of microchannels causes their flow to behave differently (see "Micro versus macro," page 17). Hence, microscale components require new fabrication methods.



University of Chicago, Department of Physics

Figure 1. Polystyrene spheres, each 1 μm in diameter, are trapped in a 3 × 3 array of optical vortices created by nine helical rings of light stemming from a single Gaussian laser beam using a computer-generated hologram. The particles rotate at hundreds of rpm by orbital angular momentum transferred from the helical beams and entrain rapid flows from the surrounding fluid, acting as a micrometer-scale mixer.

Microfluidics devices, first developed in the early 1990s, were initially fabricated in silicon and glass using photolithography and etching techniques adapted from the microelectronics industry, which are precise but expensive and inflexible. The trend recently has moved toward the application of soft lithography—fabrication methods based on printing and molding organic materials (see *The Industrial Physicist*, August/September 2002, pp. 16–19)—to build microdevices. These techniques enable the construction of three-dimensional networks of channels and components, and they provide a high level of control over the molecular structure of channel surfaces. Most importantly, says Whitesides, "they bring the technology needed to fabricate complex microfluidics devices out of clean rooms and into the laboratories of the biologists and chemists who use them."



Caliper Technologies Corp.

Figure 2. The 50-μm sipper draws a sample into a quartz microfluidics chip on the underside of this plastic caddy, which has wells for reagents on top. DNA fragments are automatically stained with a fluorescent dye, electrophoretically separated, and laser-detected.

Microfluidics' appeal lies in the fact that the microchips require only a small amount of sample and reagent for each process—only a few tens or hundreds of nanoliters compared with the 100 ml required by existing plate assays. Microscale reactions also occur much faster because of the unique physics of small fluid volumes, and microfluidics technologies are easily automated to do routine assay and sample preparation on standardized chips with little human intervention. Such chips hold the promise of combining multiple functions on a single chip, including purification, labeling, reaction, separation, and detection. Microfluidics would guide the sample automatically from one station to another on the chip.

Applications

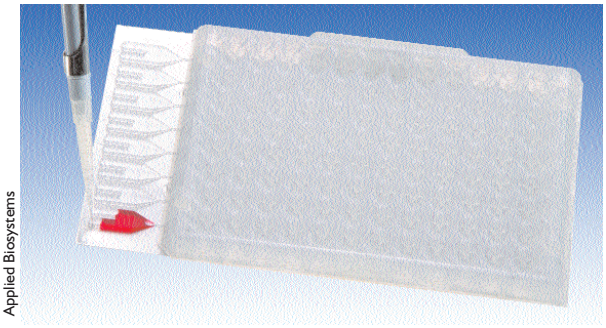
The most mature application of microfluidics technology is ink-jet printing, which uses orifices less than 100 μm in diameter to generate drops of ink. Today, ink-jet printing is moving out of the office and into biotechnology to deliver reagents to microscopic reactors and deposit DNA into arrays on the surface of biochips.

Biochips have been in the marketplace in various formats for several years. Improvements in microfluidics technology currently in development could have a revolutionary impact on the next generation of assays, particularly as nanotechnology moves into wider application. "Biotechnology is increasingly about large numbers of experiments, such as analyses of DNA or drugs, screening

of patients, and combinatorial synthesis, all of which are processes that require handling fluids," says Whitesides. "As the number of experiments has grown, the devices used to carry them out have shrunk, and the strategy of 'smaller is better' has begun to transform the world of fluidics as it transformed the world of electronics."

Most microfluidics devices today use electrokinetic and pressure methods to move small amounts of fluid around a microchip. However, although such techniques are useful for certain niche markets, they lack the flexibility required for universal application—a key selling point to scientists seeking to reduce capital investment. Fluidigm Corp. (San Francisco, CA) believes that multilayer soft lithography (MSL) provides a solution. Developed in the late 1990s by Caltech biophysicist Stephen Quake, MSL enables the company to fabricate three-dimensional structures from multiple layers of soft elastomer by imprinting each layer and then binding them together to form the pumps, valves, and channels integral to the chip. This enables a single chip to serve many functions, including sample preparation, manipulation of live cells, perfusion of reagents, and analyte detection. That kind of flexibility gives any microfluidics technology a competitive edge in emerging markets.

Flexibility is also the key to three core product lines of Caliper Technologies Corp. (Mountain View, CA). Andrea Chow, a chemical engineer with the company, likens the products to modern video games. "The box is the same, except that with video games you put in a different game cartridge, whereas in this case you put in a different LabChip and run different software," she says. "People



Applied Biosystems

Figure 3. Quantitative gene expression results using a polymerase chain reaction can be obtained with this microfluidic card by pipetting in reaction mixes, centrifuging, sealing, removing the reservoir, and thermal cycling.

can't use the software to develop something new, but the applications we offer can replace several instruments."

The Bioanalyzer 2100, a collaboration between Caliper and Agilent Technologies (Palo Alto, CA), targets individual researchers working with small samples of DNA, RNA, proteins, and cells. Caliper's AMS-90 line is geared toward higher-throughput applications, such as the quality control of DNA sorting, during which hundreds of samples are run at a time. The Caliper 250 is configured for drug-discovery applications, in which large numbers of compounds must be screened to identify potential candidates with specific desired properties. The latter two product lines are semiautomated and use robotics to perform hundreds to tens of thousands of experiments a day (Figure 2).

Innovations

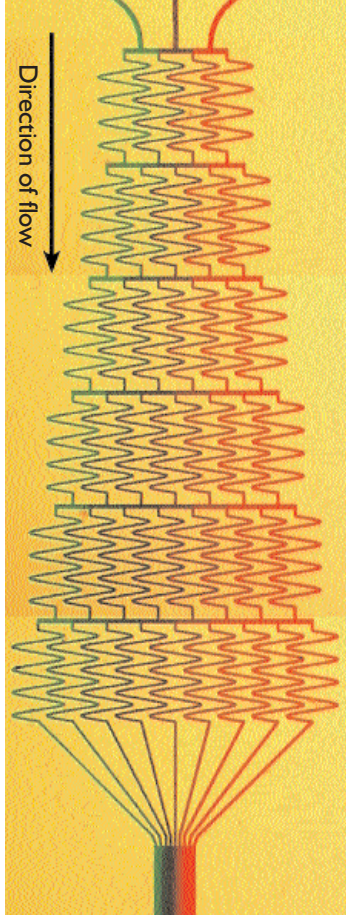
Although microfluidics devices are entering the marketplace, no industry standard exists, even for the simplest components. "The field remains open for exploration, facilitated by the move toward simple fabrication methods, such as rapid prototyping based on molding of elastomers, that reduce costs and delays," says Whitesides. The potential of microfluidics provides much of the driving force behind many new innovations and the research boom in academia and industry. Most major research institutions have groups working in the area, and the technology is being commercialized by several companies for applications such as rapid DNA sequencing, chemical-analytic systems, and cell manipulation.

Scientists at Sandia National Laboratories (Livermore, CA) are developing ChemLab, a portable, handheld chemical-analysis system for homeland security, defense, and environmental and medical applications. Currently a prototype, ChemLab can detect chemical-warfare agents and proteins, as well as biotoxins such as ricin, staphylococcal enterotoxin B, and botulinum toxin. It can also identify viruses and bacteria using protein fingerprinting. Sandia expects to commercialize the system within the next two years.

Physicist David Grier of the University of Chicago has developed a new technique known as holographic optical tweezers. In this approach, a laser beam is sent into a hologram and divided into myriad subbeams, which can independently suspend and manipulate many tiny particles for transportation, mixing, or reacting. Using this technique, ensembles of microspheres can be moved into patterns and set to spinning by the holographically sculpted light fields (Figure 1).

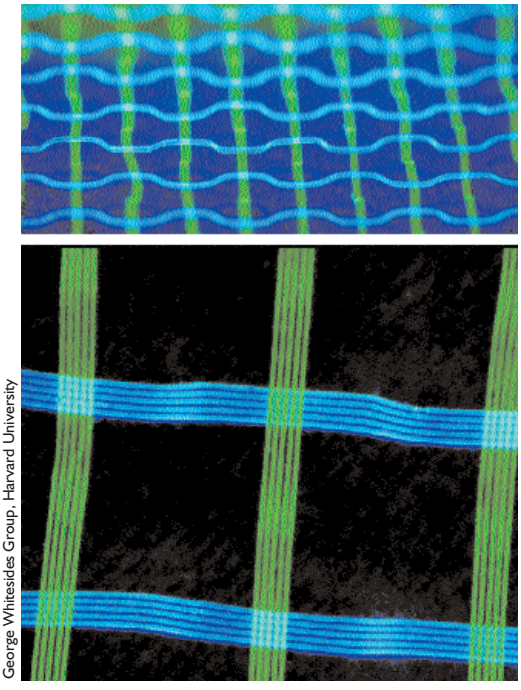
When applied to fluid samples of biomolecules, this holographic multiplexing produces what Grier has dubbed "optical fractionation," an optical equivalent of gel electrophoresis, the workhorse of the biotechnology industry. It enables electric fields to differentially drive and separate macromolecules. However, Grier's approach is more flexible than gel electrophoresis because it does not require a viscous gel. By merely tweaking the computer-generated hologram or the laser wavelength, the user can sort objects ranging from the 10-nm (important for viruses) up to the 100-μm scale.

Other advantages of optical fractionation include con-



George Whitesides Group, Harvard University

Figure 4. Photograph of a microfluidic device used for studying concentration gradients in a network of branching serpentines, in which three dyes are injected at the top before combining in a single channel at the bottom.



George Whitesides Group, Harvard University

Figure 5. A microfluidic network with a basket-weave geometry created in a 500-μm-thick poly(dimethylsiloxane) membrane on a 2-mm grid, in which the channels are filled with an aqueous solution of fluorescein (green) or Cascade Blue and illuminated with ultraviolet light.

tinuous rather than batch-mode operation, and optimal performance simply requires varying the laser power, laser wavelength, and/or the geometry of the trap arrays. Thus, the same apparatus can sort samples on the basis of size, surface charge, dielectric constant, magnetic permeability, and shape. The technique "offers exponential sensitivity for fractionation by size," says Grier. "This is unprecedented. Other techniques offer linear, quadratic, or slightly better selectivity, whereas optical fractionation is qualitatively more selective."

Cell sorting

Grier's innovation forms the basis for the core technology of Arryx, Inc. (Chicago, IL), which holds exclusive license to all techniques based on holographic optical tweezers. Arryx's BioRyx 2000 system, introduced in March 2002, enables researchers to manipulate hundreds of microscopic objects independently and simultaneously in three dimensions. Its new MatRyx, now in development, is a high-throughput cell sorter initially slated for use in the cattle industry to sort X and Y sperm for breeding.

In April, Micronics Inc., (Redmond, WA), patented a credit-card-sized microfluidics device to perform immunoassays, which it achieves by exploiting molecular binding reactions and differential diffusion rates in microchannels (Figure 6). The microassay is applicable to a wide range of analytes, including therapeutic drugs, molecular biological markers, and environmental contaminants. And University of Michigan researchers, led by Gary Smith of the department of obstetrics and gynecology, have developed a prototype microfluidics device that rapidly and automatically sorts sperm and isolates the most viable swimmers for injection into an egg. Their ultimate goal is to create a self-contained, at-home test for men to screen for infertility or to judge the success of vasectomy or vasectomy-reversal procedures. The device sorts sperm on the basis of their speed.

Surface Logix (Cambridge, MA) targets its microfluidics systems to advanced cell-based assays for drug research. It is developing multiplexed cell-based assays that provide precise control over cell location, culture condition, and reagent delivery. Benefits include the ability to isolate, manipulate, and monitor individual cells; real-time analysis of physiological changes; and compatibility with standard assay formats and detection devices.

With Fluidigm up and running, Quake has turned to developing another microfluidics device. This one allows the careful metering of reagents to facilitate protein crystallization under a variety of conditions, including pH, viscosity, surface tension, or various solvents. The device can produce 144 parallel reactions and requires only 10-nl protein samples. It transforms proteins into crystals in hours rather than days, and X-ray bombardment can then determine their molecular structure. Another Caltech team, headed by Carl Hansen, has devised a complex microfluidics test that contains thousands of micromechanical valves and hundreds of chambers. Hansen's device is also highly integrated. A single chip has 1,000 250-pl chambers with valves for controlling flow and mixing, which makes it useful for large-batch protein-crystal growth and other biomolecular studies.

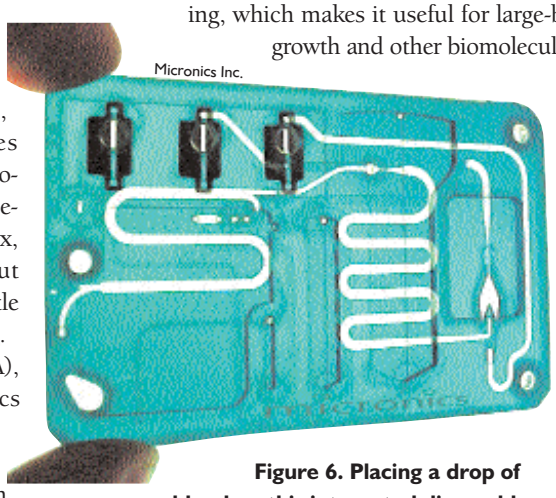


Figure 6. Placing a drop of blood on this integrated disposable circuit of various microfluidic elements allows a white blood cell count that might need to be monitored in the event of chemical or biological warfare.

At the University of Leipzig in Germany, researchers have invented an optical stretcher in which cells are sorted and studied by squeezing them. The fluid-borne cells are exposed to laser beams, which stretch them to probe their elasticity, and the device can detect cancer, according to team leader Jochen Guck. The process works because cancer cells are softer than healthy ones, and the optical stretcher can differentiate between the two at significantly faster rates than current elastisizing methods—several hundred per hour compared with 10 cells per day.

Another area where microfluidics could prove valuable is structural genomics (Figure 3). This market sector will grow at a compound annual rate of 32% to an estimated $1.4 billion over the next five years, according to a 2002

MICRO VERSUS MACRO

Microfluidics hardware requires different methods of construction and design. Conventional devices cannot simply be scaled down because the basic physics changes at the microscale. When the dimensions of a device or system become small enough, particles suspended in a fluid become comparable in size to the device itself, which dramatically alters system behavior.

Although the fluid properties remain the same at the microscale, surface tension, viscosity, and electrical charges can become dominant forces on a fluid because the surface-to-volume ratio is much greater than for macroscale systems. Also, no one fully understands how heat transfer and mass transfer function at the microscale, and what effect they might have on the device.

"At large scales, the inertia of the fluid is important, whereas at smaller scales, it is not important at all," explains Brian Kirby, a staff scientist at Sandia National Laboratories. "At the macroscale, the notion of applying a voltage to a fluid and expecting it to have an impact is ridiculous; we use mechanical pumps at the macroscale that exploit inertia. When you get down to length scales on the order of 10 μm, applying voltages to fluids encased in a channel can be used to manipulate them."

study by Front Line Strategic Consulting, Inc. (San Mateo, CA). Chow reports that Caliper is currently developing a prototype microfluidics system for genomics analysis, with the initial focus on single-nucleotide polymorphism analysis, which can detect the substitution of one amino acid for another in a protein. "By adding the correct reagent to a channel containing a genomic DNA sample, then selectively amplifying a certain gene segment, we can study the characteristics of that particular segment of interest." Other lab-on-a-chip companies exploring this area include Nanogen (San Diego, CA) and Orchid Biosciences (Princeton, NJ).

Chemistry

Kevin Killeen, project manager of microfluidics and sensors at Agilent, contends that the field has barely tapped the possibilities of microfluidics flow-through processes in chemistry. "Thus far, we have only been scratching the surface by emulating what is currently done in batch mode," he says. "But microfluidics means we can prepare samples for chemical separation and detection in a different way than a lab bench chemist would have (see 2003 Industrial Physics Forum, page 30, "Polymer microfluidics for chemical analysis," Kevin Killeen).

Killeen also foresees a convergence between microfluidics technology and the electronics industry, particularly in the development of optical and electronic parallel detection systems. "Ultimately, we would like to have the detection right on the chip so that it would be part of the chip's architecture," he says. "We need both miniaturization and massively parallel chemical detection capabilities in order to do that."

The advent of the nanotechnology revolution opens even more opportunities for innovation. For example, Caliper's Chow foresees combining microfluidics processing with emerging devices such as nanotechnology-based sensors to create new types of assays with new functions that have no analogy in the macroscopic world. "There are two ways to think about miniaturization," she says. "First, you can take existing technology and simply make it smaller. But it is much more powerful to create something that can only be done in the miniaturized world—this will be a truly enabling technology."

"It might not be the exact equivalent of what happened in the integrated circuit (IC) industry," says Killeen of microfluidics' future. "But I do think it will, in a similar way, do for chemistry what the IC industry did for electronics. Microfluidics is going to revolutionize the way things are done. It is going to make processes more efficient. It's going to miniaturize chemistry and make possible chemical reactions that can't be done in batch mode today because they are either too unstable or the chemicals are too precious. It is hard to say exactly what will happen, but I know it will touch virtually every aspect of our lives." Ω

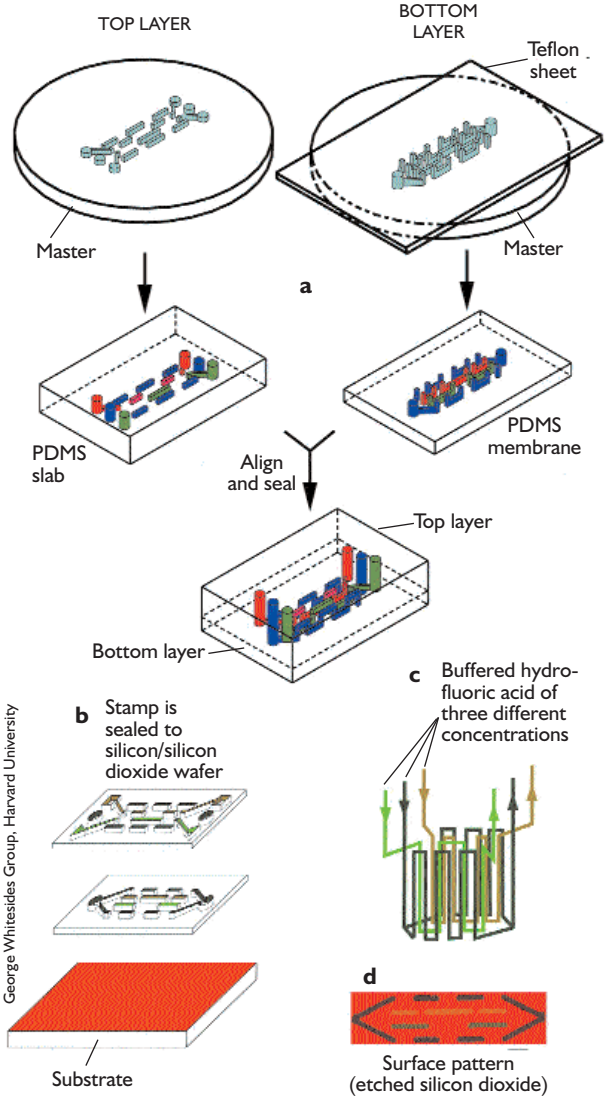


Figure 7. A three-dimensional microfluidic stamp is made from two poly(dimethylsiloxane) (PDMS) layers that are independently molded and cured, aligned so the vertical channels go through, oxidized, and sealed (a). The stamp is gently sealed to a silicon/silicon dioxide wafer (b) and buffered hydrofluoric acid flows through the channels (c), etching away the silicon dioxide, when in contact with the surface, to various depths, as shown by the different colors (d).