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Microfluidics: in search of a killer application

Companies and academic researchers are developing more and more microfluidic devices. But what the technology stakeholders really want is an application that will trigger widespread adoption of microfluidics by biologists. Nathan Blow reports.

The field of microfluidics is now at a point where the search is on for a ‘killer application’—or a ‘killer app’ as computer people would say. Over the past ten years microfluidics, the art of precisely controlling microliter volumes of fluid, has evolved from ‘cool chips’ built by a small community of academics with degrees in biotechnology and physics into a technology with serious commercial potential, exploited by companies providing instruments for DNA, RNA and protein applications. “We are now very good at most biological applications. PCR, protein crystallization, *in vitro* transcription and translation, and bioreactors for microbial and eukaryotic tissue culture work pretty well for most groups around the world,” says Stephen Quake of Stanford University. And even for those assays for which microfluidic solutions are not available, adventurous researchers have avenues to fabricate their own ‘lab on a chip’.

Still, all this good news raises a troubling question: why are more researchers not embracing microfluidic solutions? George Whitesides of Harvard University believes the answer is in the applications: “We need to find applications where it is the result that is the value, not the device.”

The killer app may be lurking undiscovered among the current crop of microfluidic applications or may yet have to be described. But if such an application is found, it could lead to a new direction in biology, where lab-on-a-chip solutions finally become universal, and all biologists go small-scale.

Potential killers in our midst

One of the first commercial companies to offer microfluidic devices to biologists was Caliper Life Sciences, which now offers two microfluidics chip platforms—planar and sipper chips. Planar chips are manual solutions that require the user to pipette



The LabChip90 is a microfluidics platform based on sipper chip technology. (Courtesy of Caliper Life Sciences.)

reagents into reservoirs on the chip. Caliper has licensed the planar chip technology to Agilent, for use in the 2100 Bioanalyzer, and to BioRad, for use in with the Experion automated electrophoresis system. Mark Roskey, vice president of reagents and applied biology at Caliper Life Sciences says, “the main driving applications of the planar chip today are the separation of DNA, RNA and proteins.” But Agilent has also used planar chips to develop cell-based assays.

Sipper chips, in contrast, use integrated capillaries allowing the chip to be loaded directly from a microtiter plate or other outside source. This makes sipper chips a high-throughput solution as ‘sipping’ through the capillaries can be repeated many times. Caliper’s LabChip90, based on sipper chip technology, can be used to separate and quantitate DNA and protein samples. “The electrophoresis is fully automated in the Lab

Chip, and the software automatically quantitates the size of the product and creates a digital map of the separation,” explains Roskey.

According to Roskey, much of today’s microfluidics market is driven by large biotechnology and pharmaceutical companies, and the key to larger adoption of microfluidics solutions is to make the devices simpler. “It should be one box that researchers put sample in and get an answer out,” says Roskey. Putting this theory into practice, Caliper is developing a new platform for drug discovery that is capable of profiling the reactions of multiple kinases against a compound of interest in a single experiment. Called DeskTop Profiler, it will come with the assay, chips and instruments packaged together in a turn-key version.

Whereas Caliper has focused much energy in the past on providing microfluidic chips for separation and analysis of DNA and RNA fragments, other companies are starting to tackle the staple of molecular biology laboratories throughout the world: PCR. Just when researchers thought that PCR could not become anymore high-throughput, several microfluidics companies are promoting alternative solutions to do more, quicker.

The Open Array technology of BioTrove relies on ‘passive nanofluidics’ to allow users to perform thousands of nanoliter-volume PCRs. The basis of the Open Array technology is a steel plate with 3,072 individual holes coated with a proprietary hydrophilic protein, and the remainder of the plate is made hydrophobic. “Therefore, when any liquid is brought in proximity to the holes, the liquid will be sucked into the holes and held by surface tension,” explains Kevin Munnely, senior director of genome products at BioTrove. The steel plates are supplied with dried-down primers and probes to customer’s specifications, and PCR mixes can

BOX 1 MICROFLUIDICS EYES THE FUTURE

From novel cell-based assay development to integrated microfluidic solutions, such as the Microchip BioTechnologies single chip for DNA sequencing, companies are looking to the future in the microfluidics market. SpinX Technologies is one such company offering a twist on the creation of valves for microfluidic devices.

The idea behind SpinX Technologies virtual laser valve (VLV) started with a simple observation says CEO and founder Bart Van de Vyver. "When we looked at the microfluidics field, we saw that one thing that was missing was the ability to change protocols or even perform different protocols on the same device," says Van de Vyver. This led to the development of the VLV system, in which the valves in the chips are not fabricated—they are created for each assay using a laser. Van de Vyver notes that the creation of valves according to the user's exact specification for each assay adds flexibility to microfluidic assays.

VLV technology takes advantage of a single laser to puncture a hole between a fluid-containing chamber in the horizontal plane and a channel in the vertical plane, allowing fluid movement or mixing. Users have the ability to create any number of valves they desire using the system. Although it is not a new assay, Van de Vyver is quick to point out that they "really want to be the underlying glue that allows existing technologies to perform assays in nanoliter volumes."

The applications for this technology are far-reaching as the creation of valves on the fly is useful for any application in which fluid mixing is crucial. Dennis Church, head of customer applications at SpinX Technologies, indicates that applications from preclinical drug discovery to bacterial detection were being explored now by SpinX.

pair differences between the capture probe and target DNA can be discriminated. The real value of the platform, says Hodko, is the open nature of the technology that integrates fluidics and electric field-assisted transport on the array. The array is delivered to the user as a 'blank slate' on which the researcher can 'electronically print' whatever probes they desire. "We have around 200 users that used the platform to develop their own assays," says Hodko.

Protein analysis is also on the minds of several microfluidics companies. Fluidigm's Topaz system is a microfluidic protein crystallization system that automates the process of nanoscale free interface diffusion. The chips allow proteins to move from areas of high reagent concentrations to lower reagent concentrations by taking advantage of large numbers of valves. The technology has proven very effective at obtaining crystals for X-ray crystallography studies¹.

Other companies providing microfluidic solutions for protein applications include BioTrove with the RapidFire system, an integrated mass spectrometry solution that uses a microfluidic device to clean and purify samples before analysis, and Biacore, which produces several systems that rely on fundamental flow cell-based fluidics principles to study protein interactions.

Nothing available? Make the solution!

Many researchers have specific assays that they want miniaturized and put on a chip, for which there is no off-the-shelf system available. With some help and investment of time, it is possible for these brave souls to fabricate a microfluidic device for their assay. "Every biologist that we have worked with, who has taken the time to learn the techniques, finds

thus be placed directly onto the plate to set up all reactions in a couple of minutes.

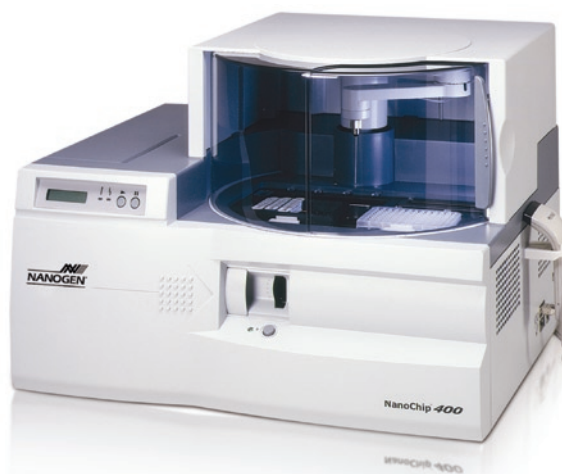
Fluidigm, a company cofounded by Stephen Quake, is developing microfluidic strategies based on the concept of integrated fluidic circuits. The company's technology is largely based on Quake's work on microfluidic large-scale integration, using large numbers of valves and channels to perform multiple assays on a single chip. Fluidigm offers the BioMark 48.48 dynamic array, a microfluidic solution to perform quantitative real-time PCR. Using integrated fluidic circuits, the BioMark 48.48 array is capable of partitioning and combining reagents and samples into 2,304 reactions. Fluidigm's devices have demonstrated that valves are a precious commodity for integrated systems. Other companies are looking into alternative ways of making valves (Box 1).

Nanogen offers a microfluidics-based array platform for the old technology of DNA hybridization. Their take on this technology allows users to selectively capture probes on a chip and then probe target DNA. To this end, Nanogen's NanoChip 400 consists of an array of 400 individual platinum electrodes, each electrode 50 μm in diameter and covered with a layer of silicon dioxide, exposing only the platinum. On top of this array of electrodes is a hydrogel permeation layer containing streptavidin. Biotinylated DNA probes are added to the array and electrodes

are energized; because of the charge of DNA, the probes move toward the energized electrodes and are captured and held in place via the biotin-streptavidin interaction.

Each electrode can be individually energized, allowing users to specify the location of a specific 'capture' DNA probe. "You can call this process electronic printing," says Dalibor Hodko, director of advanced technologies at Nanogen.

After this 'electronic printing' of capture probes, target DNA samples are added to the chip and binding of the capture probe to the target DNA can be determined. Hodko says that using this technology single-base-

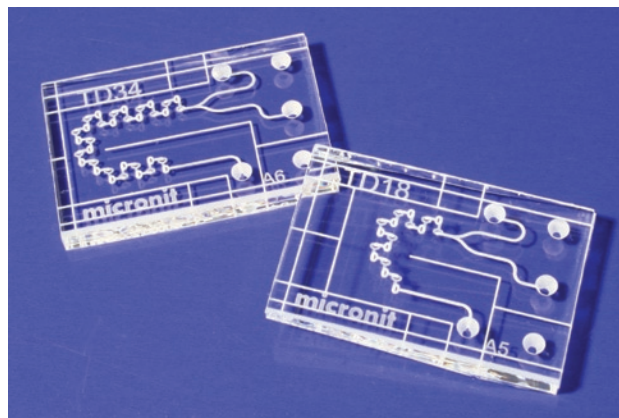


The NanoChip 400 uses charged electrodes to perform electronic microarrays. (Courtesy of Nanogen.)

them very useful,” says Whitesides, who continues to work on original solutions to microfluidics challenges (**Box 2**).

Micronit Microfluidics is a commercial supplier and fabricator of microfluidic devices for researchers worldwide. The company was founded by Micha Mulder and Ronny van't Oever in 2000 with the goal of supplying microfluidic chips to both commercial and academic researchers. Since day one, Mulder and van't Oever have opted for glass as the primary microfabrication surface. Mulder says it was the most logical choice because it is chemically inert compared to plastic, it has an indefinite shelf life, and chemists have used it for hundreds of years—so it is known how glass behaves with different solvents.

In addition to producing glass chips to a client's specific design details, Micronit also offers options to get customers started in the world of microfluidics right away. Several off-the-shelf options are available, including twelve different capillary electrophoresis-based chips, six different microreactors, four different micromixers and four different microplates. All these chips can be connected



An example of a passive mixer in which fluids are mixed by chaotic advection. (Courtesy of Micronit Microfluidics.)

to the outside world using Micronit's Lab-on-a-Chip kit. "You can use standard capillaries and teflon or silica tubing, which are used everywhere, and you get nice leak-free connections," says Mulder.

Micronit often works with companies on designing prototype microfluidic devices for many different applications. From this

experience, Mulder is hopeful that the 'killer app' will soon be coming: "Many of the companies that we have worked with are at the end of their prototyping phase, and will be moving into the market in one or two years with their products," he says.

Jessica Melin directs the Stanford Microfluidics Foundry, a resource open to

the scientific community that fabricates polydimethylsiloxane (PDMS) microfluidics chips based on the principle of multilayer soft lithography. Soft lithography, developed by Xia & Whitesides², replicates structures using elastomeric materials, and multilayer soft lithography refers to adding elastomer layers on top of each other. The benefit of these additional layers is the ability to generate 'plumbing' tools on the chip, such as valves³.

Melin has seen a variety of attitudes in people coming to the foundry. "Some people come to us and have a specific design in mind, others have questions about how they could realize a type of design," says Melin, who sees the foundry as a good way to spread the knowledge of microfabrication and microfluidics in general.

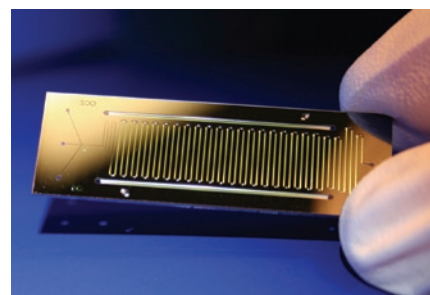
The Stanford Microfluidics foundry has been busy since opening the doors in 2005. That first year the foundry produced over 2,500 chips for researchers and another 500 functional molds. Although most of the business was from the Stanford University community, Melin says 30% of the chips were requested for academic researchers outside Stanford.

Is the killer app in disguise?

Some companies have moved away from DNA- and protein-based microfluidic applications to tackle cell-based microfluidics. Cellix, founded in 2004, is based on collaboration between the physics and clinical medicine departments at Trinity College in Dublin, Ireland. "Our technology is based on disposable microfluidic biochips, which very closely mimic the *in vivo* conditions of blood capillaries," says Dmitry Kashanin chief technology officer and cofounder of Cellix.

The Cellix microfluidic platform consists of a disposable microfluidic biochip, with eight channels, each 100 μm deep by 400 μm wide, a pumping device to provide flow and cell-analysis software. By coating the sides of the channels with different ligands, attachment of cells can be directly monitored. Kashanin says that the platform is applicable to many diseases that cause an inflammatory response. "All these processes are similar; it is only the cells and the ligands that are different."

Kashanin says that the Cellix platform is presently used by several pharmaceutical companies to characterize lead compounds



A microfluidics chip constructed using both glass and silicon. (Image courtesy of Micronit Microfluidics.)

targeted against autoimmune diseases. Cellix is not the only company that is now moving into the world of cell-based microfluidic assays; both Caliper Life Sciences and BioTrove have also moved in that direction. Some researchers actually think the biggest payout for microfluidics will be in the area of cell-based applications. "If you are going to work with primary human cells, I do not see how you are going to get a lot of them, and it requires microfluidic solutions," says Whitesides.

Roskey says that Caliper is focusing on cell-based assays using microfluidics, including culture of primary human cells and generation of response curves for single cells exposed to treatment. BioTrove and its collaborators have explored the potential of cell assays using their OpenArray technology as well. "In a nutshell, you can think of the Open Array as microplate, but giving you a high content. You can grow cells in the holes and perform staining on the cells in each individual hole in a high-content fashion," says Munnally.

The 'killer app' for microfluidics has yet to be found. Maybe it will surface soon as more and more microfluidic solutions are described not only in the scientific literature but also in the catalogs of various companies. Everyone in the field has hope. As Whitesides puts it, "everywhere you look, you see clever solutions to interesting problems, capabilities that were not there before, but getting it to the point where it is routinely useful as opposed to collections of interesting academic papers is the trick."

1. Hu, S.H. *et al. Proc. Natl. Acad. Sci. USA* **104**, 8773–8778 (2007).
2. Unger, M.A. *et al. Science* **288**, 113–116 (2000).
3. Xia, Y. & Whitesides, G.M. *Angew. Chem. Int. Ed. Engl.* **37**, 551–575 (1998).

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BOX 2 SIR, THERE IS A BUBBLE IN MY SYSTEM!

Bubbles in a closed system are not always the most desirable things. In the microfluidic world, however, bubbles may just be what the scientist ordered. "Basically these isolated little compartments turn out to be broadly useful for a number of applications in microfluidics," says Whitesides, a microfluidics pioneer who is exploring the use of bubbles for different microfluidic applications.

Whitesides started examining bubbles as part of his research into complexity, studying objects with components that interact with one another nonlinearly. According to him, bubbles are the simplest components that you can find in nature. "We have now done a lot of work on the very basics of looking at bubbles, and it turns out that as you look at them, you begin to see a number of applications," says Whitesides. One of the applications was using bubbles as a substitute to mixers in microfluidic devices.

Traditional microfluidic mixers come in two varieties: passive and active. Passive mixers use the geometry of the system to move fluids in or out of plane to create chaotic advection of the fluids. Active mixers can be controlled externally and varied by the user. Both have difficulties associated with their fabrication: passive mixers usually require at least two layers of fabrication, whereas active mixers require additional, complicated microfabrication to be incorporated into the device. Whitesides says that bubbles can be easier, because no additional fabrication is necessary when mixing with bubbles. "The virtue of bubbles is that, if you can get a bubble in a channel, you can get mixing quite rapidly and quite efficiently."

Bubbles are not perfect, though. Several issues related to the use of bubbles as mixing devices have to be resolved. "There is a particular bit of the puzzle that needs to be added, which will not be hard to do but it has not been done yet—that is, bubble on demand," says Whitesides. Bubbles on demand are necessary to provide that single mixing bubble into the necessary channel to allow efficient mixing. The machine to perform this function has not been invented yet, but Whitesides thinks it is only a matter of time.

SUPPLIERS GUIDE: COMPANIES OFFERING MICROFLUIDICS SOLUTIONS

Company	Web Address
Abbott Laboratories	http://www.abbott.com
Advanced Liquid Logic	http://www.liquid-logic.com/
Agilent Technologies	http://www.agilent.com
Applied Biosystems	http://www.appliedbiosystems.com/
Aviva Biosciences	http://www.avivabio.com/
Biacore	http://www.biacore.com
Bioident	http://www.bioident.com/
Bioprocessors	http://www.bioprocessors.com/
Bio-Rad	http://www.bio-rad.com
Biotrove	http://www.biotrove.com
Caliper Life Sciences	http://www.caliperlifesciences.com/
Cellix	http://www.cellixltd.com
Cepheid	http://www.cepheid.com
Ciphergen	http://www.ciphergen.com
Cole-Parmer	http://www.coleparmer.com
Dionex	http://www.dionex.com
Eksigent Technologies	http://www.eksigent.com
Erie Scientific Company	http://www.eriemicrofluidics.com/
Evotec Technologies	http://www.evotec-technologies.com/
Fluidigm	http://www.fluidigm.com
Gyros	http://www.gyros.com
Handy Lab Inc.	http://www.handylab.com
Helicos Biosciences Corporation	http://www.helicosbio.com
Hewlett-Packard	http://www.hp.com
Ibidi Integrated BioDiagnostics	http://www.ibidi.de/
Invitrogen	http://www.invitrogen.com
Iq Micro Inc.	http://www.iq-micro.com/
Liquidia Technologies	http://www.liquidia.com/
Micalyne Inc.	http://www.micalyne.com/
Microchip Biotechnologies Inc	http://www.microchipbiotech.com
Microfluidics	http://www.microfluidicscorp.com/
Micronics	http://www.micronics.net
Micronit Microfluidics BV	http://www.micronit.com/
Monogram Biosciences	http://www.monogrambio.com/
Nanogen	http://www.nanogen.com
Nanostream	http://www.nanostream.com
Nanoterra	http://www.nanoterra.com/
Network Biosystems	http://www.networkbiosystems.com
Orchid Biosciences	http://www.orchid.com
Pyrosequencing AB	http://www.pyrosequencing.com
Roche 454	http://www.roche.com
Spin X Technologies	http://www.spinx-technologies.com/
Surface Logix	http://www.surfacelogix.com
Tecan	http://www.tecan.com
Tronics Microsystems	http://www.tronics-mst.com/