

MICROFLUIDICS: BRINGING NEW THINGS TO LIFE SCIENCE

Most everything we know about fluids is based on macroscale observation. But things are different at the microscale, where “channels” can be built with two walls instead of four, turbulence doesn’t exist, and fluids can flow side by side without mixing. For years, microfluidics has been the subject of hyperbole, with some speculating that laboratory process would one day be miniaturized onto a chip. Current commercial offerings leverage microfluidics’ most obvious advantages: small sample volumes, rapid results, and lower costs. But as researchers have discovered, microfluidics also has a second benefit: enabling experiments that cannot otherwise be accomplished. **By Jeffrey M. Perkel**

As a new assistant professor, Adela Ben-Yakar found a way to use low-energy laser pulses to sever a single neuron in the millimeter-sized nematode, *Caenorhabditis elegans*. The technique is called femtosecond laser axotomy, and according to Ben-Yakar, it has been well received.

“We developed nanoaxotomy in 2004,” says Ben-Yakar, of the **University of Texas in Austin**, “and at a *C. elegans* meeting in June there was a whole session on using it.”

Unfortunately, the technique is far too unwieldy for large-scale genetics. Between picking up the animal, positioning it on the microscope, anesthetizing it, and the surgery itself, each worm takes about 10 minutes, says Ben-Yakar, who has a Ph.D. in mechanical engineering and a postdoc in applied physics.

She could have thrown more students at the project, like so much laboratory cannon fodder, but that would have occupied them for years, she suspected. “I thought maybe I could make a microfluidic device and do the work with far fewer people in much less time.”

What she created was a so-called “lab-on-a-chip” device capable of capturing a worm, holding it steady during surgery, and shunting the animal to any of three recovery chambers. The process takes under a minute per animal, she says.

It is an enabling technology for Ben-Yakar, who says she now can screen for new genes and chemicals that might influence neuroregeneration in vivo. It also is a development that, in a sense, encapsulates the promise of microfluidics, a field that is about more than miniaturization, lower reagent volumes, and cost.

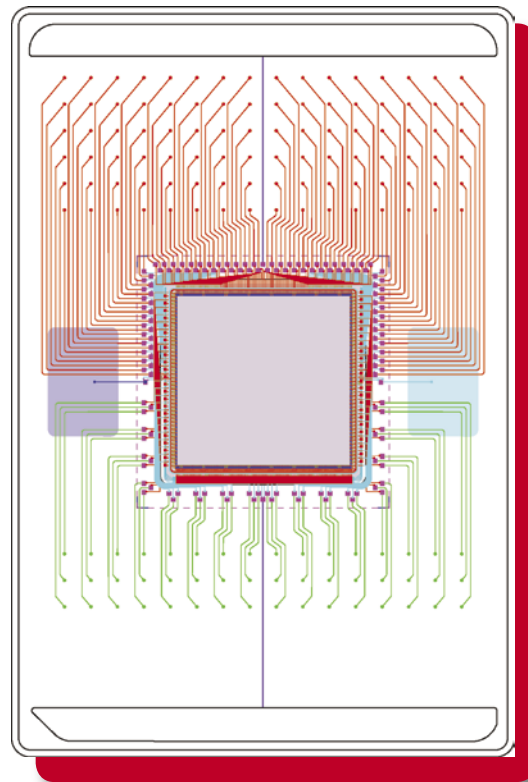
“The desire to use less sample is important and it is what got people excited,” says Nate Coper, marketing director at **Caliper Life Sciences**. “But really what’s different is you can now do things you previously couldn’t do.”

Thinking Small

“The trick with microfluidics is, you have to think small,” says Glenn Walker, assistant professor of biomedical engineering at the **University of North Carolina (UNC) and North Carolina State University**. “You can’t just take something that works big, shrink it down, and expect it to work.”

That’s because fluid dynamics don’t scale, he says. Fluids that flow like water at the macroscopic level behave more like honey at the microscale. Inertial forces decline while shear forces become important. Turbulence becomes a nonissue, while interactions between the fluid and the channel wall become critical. Even the seemingly simplest of processes can become problematic. For instance, since magnetic forces also don’t scale well, magnetic-based separations generally aren’t as effective on a chip.

On the other hand, the physics of microfluidics is also enabling, Walker says. Two parallel fluid streams in a microchannel, if they flow fast enough, will [continued >](#)



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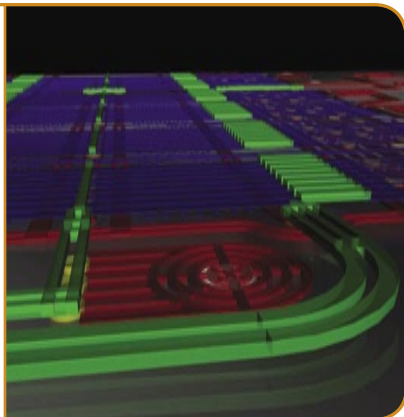
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Lab on a Chip/Microfluidics

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not mix, for example. “There will be a very sharp interface [between them],” Walker says. Thus, it is possible to bathe just one side of a cell with a chemical, to see how it reacts.

“That in particular is a really good example of a new, enabling tool that lets you do something you cannot do with pipettes and flasks,” Walker says. “The ability to treat only part of a cell with a chemical is really powerful.”

Yet there is something of a disconnect between microfluidics experts like Walker and the intended end-users of their engineering creativity. Despite a few notable commercial successes, such as Agilent Technologies’ Bioanalyzer 2100, the majority of microfluidic advances remain bound up in the literature, beyond the reach of the life science community.

“The technology is in its early adolescence,” says George Whitesides, professor of chemistry and chemical biology at **Harvard University**. “To me one of the big issues is—whether users are in analytical work or in biopharmaceuticals—that, quite correctly, they don’t want to be in the business of making the device. Their expertise is not in microfabrication,” Whitesides says. “So as with electronics, what happens is there is a critical mass of applications where it becomes worth it for manufacturers to get into the area, to make products that are commercially available.”

“It’s like a computer or a car: Most people cannot build one or fix one, but they can learn how to use them,” says Stephen Quake, professor of bioengineering at **Stanford University** and the **Howard Hughes Medical Institute** and co-founder of microfluidics firm **Fluidigm**. “When a technology reaches that point, you can say it has arrived. And that’s where we are right now for some applications.”

Material Support

At least some of that success stems from advances in materials. While some build chips out of glass or hard plastic, many others (including Fluidigm) prefer poly(dimethylsiloxane) (PDMS), a rubbery material also used to make soft contact lenses. According to Whitesides, who heads one of two groups that first worked with the polymer, PDMS has become a popular choice for microfluidic device fabrication for its ease of use.

Glass and hard plastics are easy to break and hard to etch and bond, he says. In contrast, “PDMS is just very easy to work with. There’s a very simple procedure to go from design to master to mold, and you can seal the layers together almost effortlessly,” he says. A rubbery elastomer, PDMS won’t break if dropped. It also breathes, so gases can exchange with the environment beyond the chip, while the material’s springiness enables pneumatic control.

Pneumatic control circuits, which Quake developed while at the California Institute of Technology and licensed to Fluidigm, comprise two layers. Fluid flows through channels in the bottom layer. In the top layer, separated from the bottom by a thin layer of PDMS, empty control channels run perpendicular to the fluid channels. Pressurization of the control channels with air closes the fluid channels below, just as stepping on a hose will staunch the flow of water.

Ben-Yakar’s nanoaxotomy chip uses this process to trap individual worms in a microfluidic “surgical suite” as if with a finger. For Fluidigm, pneumatic valves also enable system integration on an unprecedented level. The company’s 96.96 Dynamic Array, for instance, uses more than 25,000 valves and about a million features to run 9,216 parallel 6.7-nl polymerase chain reactions in just three hours.

That’s just miniaturization. But the company’s newest chip is truly enabling, says Gajus Worthington, Fluidigm’s co-founder, CEO, and president. “The [BioMark 12.765] Digital Array allows you to do things you could never do before. It literally does digital PCR.”

“Suppose you have a 5- μ l sample with a single molecule you want to test,” he explains. “We break it into 1,000 different compartments, and we effectively increase the concentration of what you are looking for 1,000 times. That makes it 1,000 times easier to find.”

That means it might be possible to, for instance, screen for evidence of fetal aneuploidy in maternal blood, a test that currently cannot otherwise be run, says Worthington.

Making the Impractical Practical

Swedish microfluidics firm **Cellectricon** targets a decidedly smaller niche with its Dynaflow system.

A microfluidic perfusion system intended for drug developers who use patch clamping for optimization of potential ion channel compounds, Dynaflow “is an add-on that enables the customer to rapidly control fluids around the patch-clamped cells,” says Matthias Karlsson, chief technical officer and scientific co-founder. The cell is patch-clamped as normal and placed in the chip; then the system simply switches the fluid channel to which the cell happens to be exposed.

According to Karlsson, existing technology does not allow for controlled, rapid changes in the fluid environment around a clamped cell, and that can be a problem, because signaling events occur in milliseconds.

“If you cannot monitor with millisecond resolution, you will not see the reaction of the drug with the channel,” he says, adding, “Dynaflow makes these experiments possible in an industrial setting.”

According to Karlsson, “The benefit of having this on a microfluidic system is that the only mixing is by diffusion, not by turbulence, so the transition from one compound to another is very sharp, like a step function.”

The throughput is about 200 compound wells per day, Karlsson says, or “10 to 20 times faster than what a skilled electrophysiologist could do now.” The company’s next-generation system, due late 2009, will bump that up to around 5,000 wells per day.

J. Michael Ramsey, Goldby Distinguished Professor of Chemistry at UNC and co-founder of Caliper Life Sciences, uses glass microfluidic devices to enable yet another highly impractical process: single-cell kinase assays.

Ramsey’s collaborator, Nancy Allbritton, had previously developed a method to load cells with a fluorescent kinase substrate, lyse those

cells one at a time using a laser, and then run capillary electrophoresis on the contents to measure enzymatic activity.

“They could do eight to 10 cells per day,” says Ramsey. Using a microfluidic device built of glass, Ramsey’s team has made the assay realistically useful. “We can do 10 cells per minute,” he says, “and we could probably speed that up.”

He explains, “We have a continuous flow of cells that stochastically arrive at a point where they are lysed, and the lysate is directed into a capillary electrophoresis channel and separated.” It is the same assay Allbritton devised, except “it has essentially been automated.”

Unique Advances

Sometimes microfluidic devices enable richer data from traditional assays. Caliper’s Cospier says kinase assays run on the company’s LabChip systems yield more than a number, for instance; because the reaction components flow through a separation channel, the data are both quantitative and qualitative.

“In a typical single-pot assay, you can get false positives or negatives because things interfere with the reaction and cause you to misinterpret the assay,” he says. By running that same assay on the LabChip platform, “you get the same separation quality as in HPLC, to get very high quality data without artifacts. You cannot do that in a standard microwell.”

Gyros has successfully commercialized a microlaboratory in compact disc format, utilizing flow-through immunoassay principles for protein quantification for the development of protein therapeutics. The Gyrolab system allows assays to be performed at nanoliter scale enabling users to extract more information from minute sample volumes, allowing repetitive sampling from individual animals, during the early development stages of protein therapeutics.

Gyrolab provides a completely different way of interacting analytes with capture reagent. “In our case, this is done under constant flow,” says Mats Inganäs, Gyros’ director of applications and technology assessment. “With ELISA, a sample needs to incubate in a static situation for hours to get the same reaction completed. Our system reduces both assay time—over a hundred assays can be processed in under one hour—and matrix effects.”

New Takes on Old Techniques

David Beebe, professor of biomedical engineering at the **University of Wisconsin, Madison**, uses lab-on-a-chip technology to gain new insights into cell behavior.

Beebe’s lab focuses on cell-cell communication, especially in cancer. One project studies the interaction between epithelial cells and the underlying stroma. “It’s very difficult to do this with standard methods,” he says, as these require either conditioned media or transwell systems, both of which will miss short-lived factors as well as reciprocal signaling events.

Instead, Beebe uses simple microfluidic alternatives to uncover novel phenomena not previously seen in a Petri dish. “For instance, we see a bimodal distribution of proliferative capacity [in primary mouse mammary cells],” he says, which he suspects reflect stem cells. “It is a cell behavior no one has observed in vitro before.”

For Juan Santiago, associate professor of mechanical engineering at Stanford University, microfluidics also enables new approaches to PCR. He and his student, Alexandre Persat, are pursuing a process they call chemical-cycling PCR, which denatures DNA with a denaturant like formamide instead of heat. “Our chip works by cycling chemistry rather than temperature,” Santiago explains. “We

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inject clouds of denaturant while holding the DNA stationary in an electric field.”

Besides obviating heating and cooling elements (and thus, making PCR machines smaller and lighter), this approach is also faster than traditional PCR—Persat has amplified 194-bp targets in just 18 30-second cycles—lower-volume, and possibly more quantitative, Santiago says. In addition, because the amplified DNA is already in a microchannel, it can immediately be reacted or separated.

“As the PCR cycles occur, we are already separating the products,” he says. “No one has ever done that before in real time.” Santiago says the approach could find use in areas like forensics, where sample volume, sensitivity, and contamination are primary concerns.

Santiago says he “strictly” uses off-the-shelf chips, mostly available from Caliper for about \$50, for his work. For those who want a more customized solution, PDMS fabrication is relatively straightforward, says Quake, and can easily be done in a lab, or even a garage, though a clean room is preferable. Stanford’s microfluidics foundry (thebigone.stanford.edu/foundry/) can create microfluidics chips on demand.

Alternatively, you can try Mark Burns’s approach. A professor of chemical engineering and biomedical engineering at the **University of Michigan**, Burns and student Minsoung Rhee developed a toolkit of 15 “microfluidic assembly blocks,” essentially PDMS “LEGO” pieces that users can assemble into any desired structure.

“It’s like making a mosaic,” Burns explains. “You put each tile down, and it makes a picture when you’re done. Only, instead of a picture, it is actually a functioning device.”

An enabling technology for an enabling technology? Only time will tell.

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