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Innovations

Fluidigm

Biochips Get Indoor Plumbing

Today's biotechnology abounds with buzzwords—from genomics and proteomics to biochips and microarrays—and for good reason. These terms were coined to describe the new concepts and tools that are revolutionizing the way biological research is being done. Industrial biology is becoming both smaller and larger at the same time, and the analogies with the revolution in computing power and computing tools in the last century continue to resonate. Miniaturization is bringing the economies of scale into the design of biochips, a market that is already approaching a billion dollars annually. The race is on to develop integrated devices that can perform complex biological assays in a massively parallel format. Not only are these tools boosting throughput, they are vastly reducing the cost of very expensive and often very limited biological samples.

In the last few years, a new technology, and hence a new term, have made their appearance: microfluidics. As biochips become closer in size to the cells and molecules on which they work, so too must the channels, pumps, and valves that manipulate the solutions of reagents and substrates. A chip intended to handle thousands of microquantities of biological samples must be able to store, aliquot, and mix these reagents and then provide some means of measuring the activity of interest. However, as the engineers of macro systems of a century ago found, one quickly runs into the problem referred to as the "tyranny of numbers" —the practical limit to the complexity of assembled systems. A South San Francisco company called Fluidigm appears to have solved this problem—on the micro scale at least—by building upon a powerful new technology to design and create complex, fully integrated, microfluidic devices.

Their solution is to fabricate the pumps and valves directly into the microfluidic circuitry of the chip—by a process dubbed "multi-layer soft lithography." The material they use is a soft silicone elastomer—a pliable, gas permeable, rubbery material that can be spun out on a mold and cured. The mold is a flat surface with raised channels created by photolithography. Two such layers fused face-to-face onto a glass slide can create a three-dimensional, channel-containing chip. The bottom, sample layer contains channels through which flow both reagents and substrates, under the influence of pressure changes in the channels of the upper control layer. A detailed demonstration of Fluidigm's soft lithography process can be found on their website (www.fluidigm.com). Sophisticated design of various control and fluid layers can produce chips that can simultaneously perform multiple, complex reactions on literally thousands of samples—all controlled by manipulating the air pressure within the control channels. The components of the chip are spatially addressable, enabling the results to be read by standard chip-readers. Moreover, the elastomer material itself is gas permeable, allowing cells to thrive for weeks within its confines.

"We have attacked this problem by developing a microprocessor that crystallizes proteins in situ with two orders of magnitude less sample than current techniques and with integrated fluid handling," says Worthington, explaining that "the device makes use of a process called free interface diffusion, and several biologically important proteins resistant to standard techniques have been crystallized with the technology."

The technology originated with the work of Fluidigm founder Stephen R. Quake. Quake studied physics at Stanford, along with good friend and Fluidigm CEO and cofounder Gajus Worthington. Quake was an academic scientist at heart, whereas Worthington had an interest in entrepreneurship. While Quake ascended quickly to a faculty position at Caltech in Southern California, Worthington sought out mentors to teach him the art of building a business. With the aim of "learning by example," he joined a high tech company called Actel. By 1998, Worthington's self-apprenticeship felt complete, and he decided that it was time for him to make a move. "A key attribute essential for entrepreneurship is the sense of when the time is ripe, and the decisiveness to act without hesitation," says Worthington, "and in 1998, I knew that it was time for me to make a move." As it happens, Worthington did not know what it was that he would be doing, but serendipitously, it was just then that he heard from his old pal Quake. "Steve emailed me a preprint of a paper on his microfluidics work, and it was clear to me that the commercial potential of the technology was mammoth."

When asked whether he had any trepidation about going into business with an old friend, Worthington provides an emphatic no. "I had very clear expectations going in that this would require a lot of sacrifice and pain, and the only way to succeed was to work with someone with whom I had only the greatest personal and professional regard." Both Worthington and Quake wanted to develop an idea with longevity and wanted to make real, tangible products. Such an idea was

almost anathema at the time—the internet was white hot, and back-of-the-envelop ideas abounded. "In 1999, most VCs interested in biomedical applications were thinking e-health—they had almost abandoned traditional biotechnology." In this atmosphere, venture funding was out, so the pair went after "angels," private individuals looking for investment opportunities. Angels tend to provide support to businesses that are in a very early stage of development, relying on their gut instincts about the power of an idea or a technology, rather than the exacting demands of the process of due diligence that often come with venture capital.

Backed by Quake's technology and Caltech's endorsement, they managed to interest a leading entrepreneur in the southern California area, Bruce Burrows, who brought $3 million to the table. The cash allowed them to flesh out areas for product application and to create the tools to integrate the valves and pumps on the chip. They were also able to recruit renowned biotechnology IP attorney Bill Smith as their general counsel. By 2000, biotechnology was back in vogue with the VC community, allowing the duo to raise $11.5 million on the strength of the technology, their strong IP position, and the applications they envisaged in industrial biology. The group continued to develop the technology, demonstrating a 100-fold increase in the number of valves integrated into chips. They established a pilot production facility, filled out their executive team, and secured their first partnership with big pharma giant GlaxoSmithKline.

Fluidigm's commanding IP position afforded the company a technology platform that was difficult for competitors to replicate. What they now needed, according to Worthington, was the "killer application." Various devices have been developed, at least in prototype, including a "genetic microprocessor" able to perform 20,000 PCR reactions in parallel for use in genotyping, a microfluidic cell sorter, and a miniature immunoassay array. More recently, Quake's laboratory has developed a microfluidic memory storage device whose behavior is reminiscent of random access memory (see Science Online, doi:10.1126/science.1076996). The device is able to address and recover the contents of one among thousands of picoliter chambers within the chip for further investigation, "opening up the possibility of many new applications, both scientific and commercial," according to Quake.

But the "killer ap" of choice is the Topaz product for protein crystallization, currently available as a prototype product and expected to be widely launched sometime next year. Drug developers have a keen interest in determining the three-dimensional structure of proteins involved in human pathogenesis. Although great advances in technology have been made recently, the process remains costly and cumbersome—and still very much an esoteric art. "We have attacked this problem by developing a microprocessor that crystallizes proteins in situ with two orders of magnitude less sample than current techniques and with integrated fluid handling," says Worthington, explaining that "the device makes use of a process called free interface diffusion, and several biologically important proteins resistant to standard techniques have been crystallized with the technology." Free interface diffusion is widely regarded as superior to conventional crystallization techniques because the process is effectively equivalent to testing a multitude of conventional crystallization conditions simultaneously. "It is like mapping out the contour of a mountain with roads instead of one discrete point at a time," Worthington explains. "We are able to miniaturize the process because of our on-chip valve technology." A key advantage of the device is the ability to test a wide array of crystallization conditions with miniscule amounts of precious protein sample. Production of these protein samples is a critical bottleneck in the crystallization process. The product has already shipped out to several undisclosed test sites in big pharma, the biotech industry, and in academia.

In this age of dwindling bank accounts for many biotech companies, with scant hope of a top-up for many, it is important to note that at the end of 2001, Fluidigm did succeed in raising an additional $37 million in Series C financing from a group of venture capital investors led by Lehman brothers, providing needed capital to begin bringing its products to market. It has also signed an agreement to provide GlaxoSmithKline with microfluidic systems. Of course, Fluidigm is not the only act in town, as Aclara Biosciences and Caliper technologies have developed more traditional microfluidic devices based on molded plastic and glass, respectively. Both groups use electric current or pressure differentials to move fluid through their devices, which are already on the market and make use of commercially available robots and readers. Surface Logix (www.surfacelogix.com) in Boston also uses soft lithography to create a complementary set of chips for biological assays, but they have not integrated microfluidics into their design at this point.

The proof is in the pudding, as they say, and Fluidigm's future depends largely on the outcome of the tests now underway of its inaugural Topaz product. Worthington and Quake are optimistic, as the results so far are better than expected. They are also confident that this is only the beginning for the technology and the company. "Our pace of integration has been dramatically faster than even Moore's law," says Worthington. "Since invention of the first valve, Fluidigm and the Quake lab at Caltech have increased integration by a factor of 10 each year." Only 2 years have passed, however, but the results do compare favorably to Moore's law, which holds that microprocessor complexity doubles every 18 months. Today, the group is able to fabricate devices with up to 25,000 working valves, and they foresee reaching 100,000 before not too long. "That level of integration will allow us to market devices that eliminate the robots in use in many labs today," says Worthington. "We plan to end the 'tyranny of pipetting,'" jokes Quake.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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