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Moving diagnostics from the bench to the bedside

Researchers have been able to miniaturize elements of potential diagnostics, but what will it take to put them together into a workable device?

Jim Kling

The past decade has brought impressive advances in surface and materials science and engineering, as well as in the development of new microelectronic components. These tools hold the promise of miniaturizing diagnostic devices, which could dramatically reduce costs and increase throughput and sensitivity of a wide range of diagnostic tests. They also raise the tantalizing possibility of so-called point-of-care diagnostics, in which an entire test is self-contained in a hand-held device, delivering on-the-spot medical advice.

But the promises of miniaturization have so far gone largely unfulfilled. A few point-of-care products that focus on small molecules have been commercialized, such as Redwood City, California-based Cygnus's GlucoWatch and Abbott Park, Illinois-based Abbott's i-STAT 1 system, which measures troponin I. (This device can tell quickly if an emergency room patient with chest pain is suffering a heart attack.) But more complex miniaturization has remained elusive. The culprit is not the technology. Most agree that microfluidics, nanotechnology, microarrays and other such devices have made steady progress and are sufficiently advanced to act as individual components of an integrated device. The problem has been standardizing components and getting them to work together.

"Look at the way a chip is used. It's hooked up to a computer, and valves, and lasers, and microscopes. It's buried in this mass of much more expensive support infrastructure. We need to figure out what is necessary and what can get downsized. I'm optimistic that all kinds of [miniaturized diagnostics] will be commercialized, but it's not something you can say has found its place," says Harvard University's George Whitesides, whose research encompasses microfluidics, fluidic optics and nanotechnology, among other disciplines.

Technical barriers

Microarrays are the furthest along of the miniaturization technologies and have made inroads as diagnostics with Basel-based Roche's AmpliChip, which identifies variations in two cytochrome P450 genes, enabling physicians to determine a patient's drug metabolism profile and select appropriate antidepressants, antipsychotics, beta-blockers and some chemotherapy drugs. Microarrays are potential components in a wide variety of miniaturized diagnostics, but they suffer from some limitations, including a lack of robustness and reproducibility.

Microfluidics has slowly matured over the past twenty years, leading some to hope for a 'lab-on-a-chip' device capable of purifying, isolating and characterizing samples in one neat package. The devices promise greatly reduced sample and reagent volume. Classical devices use glass surfaces, which can accept a large range of fluid types, and the chemistry of capture reagents is well understood. But it is difficult to control fluid flow, making it impossible to design complicated networks. Stephen Quake, of Stanford University, uses polydimethylsiloxane (PDMS) plastic, developed by Whitesides, to manufacture microfluidic systems capable of moving nanoliter volumes of fluid with high flexibility. Quake has constructed PDMS networks with hundreds of valves and pumps. "It's pretty trivial to make these devices," says Adrian Ozinsky, of the Institute of Systems Biology in Seattle, who collaborates with Quake. "The downside is they don't handle organic samples... for proteomics experiments you need to use acetonitrile, for example. There are efforts to discover variants of these plastics with different properties."

Quake has used PDMS in a number of applications, including one that gets around one of the limitations of microarrays—their reliance on diffusion, which leads to an 8- to 24-hour hybridization time and decreased efficiency. Quake's team designed a PDMS microfluidic device with built-in pumps that produce chaotic mixing of components (mixing streams of steady pressure-driven flows in microchannels). The device improved sensitivity by an order of magnitude when operated over the same length of time as a conventional microarray. The device also sped up hybridization time by a factor of three or more. Other key challenges in microfluidics development include cell manipulation, lysis, separation, sample concentration and detection.

As with microfluidics, micro-electro-mechanical systems (MEMS) encompass the fabrication of microscopic electronic devices using techniques akin to those used in making silicon computer chips. Devices have been built with reservoirs, pumps, cantilevers, rotors, channels, valves, sensors and other structures from biocompatible materials. MEMS have already hit the market in the form of Yoqneam, Israel-based Given Imaging's PillCam, a pill containing a battery, a light-emitting diode and a complementary metal-oxide semiconductor (CMOS) video camera that records images as it passes through a patient's gastrointestinal tract. Also enclosed is a transmitter that broadcasts the images to an external receiver.

Microfluidics and MEMS provide plumbing and platforms. The next challenge is to improve detection. According to Scott Manalis

of MIT, technical challenges include achieving seamless integration with other microfluidic components, improving sensitivity, avoiding the use of labels and optics and developing schemes that are low in cost and mass producible.

Some researchers are working on a new generation of sensors that detect binding events through changes in electrical or mechanical properties of a material. For example, Charles Lieber and colleagues at Harvard University developed a device in which nanowires are linked to surface receptors and incorporated into arrays (*Nat. Biotechnol.* **23**, 1294–1301, 2005). The conductance of the nanowire changes perceptibly when a biomolecule binds to the surface receptor. The system picks up protein signals at femtomolar concentrations with high selectivity. The arrays have detected prostate-specific antigen (PSA) in serum to concentrations as low as 0.9 picograms per milliliter.

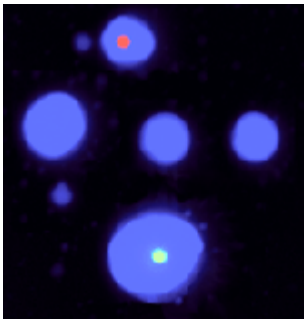
Microfluidics, MEMS, nanowires and similar technological approaches are advancing, but they face some technical challenges. One is seemingly pedestrian but nonetheless troublesome: messy biological samples. Stool and tissue samples and even serum must be pretreated before any analysis. "It's something that you would think would be trivial, but it's not. Suppose you're looking for something in feces or in blood—what do you do with that so that it ends up being 100 microliters of fluid? That interface between the microfluidic system and the macroscopic world is one that people are still working on," says Whitesides.

Biological molecules also present a challenge to materials science. "Some of these devices, with valves and motors on them, are becoming quite sophisticated, but biological molecules, being quite hydrophobic, are really sticky. If you don't pay close attention to that in the materials science of your miniaturization device, you could end up with your biological molecule sticking to the device before it gets to the reading chamber," says John Sninsky of Celera Diagnostics of Alameda, California.

Other approaches to miniaturization don't fit into neat categories. Bert Vogelstein, from Johns Hopkins Kimmel Cancer Center, is working on an approach designed to combat the problem that most of the molecules found in a serum sample come from normal cells. "We're faced with the challenge of detecting one mutant among the ten thousand wild-type or normal molecules," he says.

With his colleague Kenneth Kinzler, also at Hopkins Kimmel Cancer Center, Vogelstein developed a method to miniaturize PCR reactions to count each DNA fragment individually. The technique, called BEAMing (for beads, emulsion, amplification and magnetics), amplifies all of the fragments in circulation, thus eliminating false negatives (Fig. 1). Each fragment is amplified in its own aqueous compartment within an emulsion. Each compartment contains a magnetic bead attached to a primer, polymerase and all the reagents required for PCR. Single-base extension then labels wild-type fragments with one dye, and the mutant form with another. A flow cytometer separates the labeled beads, counting the number of mutants in the sample. "The product we're looking at (on each bead) is either completely wild type or completely mutant, which means you have a very high signal-to-noise ratio," says Vogelstein.

Figure 1. Beads in a bubble.



BEAMing technology captures individual DNA molecules in bubbles containing fluorescently labeled beads. Of the seven aqueous compartments visible in this picture, two contain beads. (Reprinted with permission from Dressman *et al., Proc. Natl. Acad. Sci. USA* **100**, 8817–8822 (2003).) Copyright 2003, National Academy of Sciences, USA.

Full Figure and legend (158K)

His team applied the method to search for a mutant form of adenomatous polyposis coli (APC) associated with colorectal cancer. Everyone with advanced cancer who was tested showed significantly elevated levels of the mutant, and more than 60% of individuals with early-stage disease had elevated levels.

Vogelstein's method boasts near-perfect specificity and demonstrates how miniaturization of diagnostics could get around a key limitation of current diagnostics: false positives. In a typical cancer screen, the false positives are likely to far outnumber the actual positives, resulting in a lot of unnecessary anxiety and invasive procedures. In fact, financially and emotionally expensive red herrings are a key barrier to screening for diseases of low incidence. "In a screen it's okay not to be 100% sensitive, as long as your specificity is good. For example, if we could determine presymptomatic cancers in two-thirds of people, with no false positives, we could potentially reduce deaths from cancer by two-thirds, with no new therapy," says Vogelstein. The technology has been licensed to Exact Sciences of Marlborough, Massachusetts (Table 1).

Table 1. Selected companies in the miniaturization industry

Full Table

Nontechnical barriers

Despite the various challenges still facing miniaturization technologies, many agree that much of the basic work has been done. There will continue to be refinements, of course, but "the proofs of concept have been done. We can do sequencing, measure RNA. The microfluidics can handle picoliter volumes. We can do multiplexing with (high) sensitivity. We can do the core things that one wants to do in bulk samples," says Ozinsky.

The problem, he says, is that the various devices haven't been combined into cohesive, user-friendly units. "The limitations are in having it in a format that you can hand to a technician and say 'press this button,' and it will work." Celera's Sninsky agrees, especially for point-of-care applications. "These tests are in some cases very sophisticated, and as you push testing closer and closer to the bedside, you are pushing it to individuals who are less strong technologically. Those assays have to become even more robust."

Such engineering should be done by industry, says Ozinsky. "But companies have been cautious because they're waiting to see what the market is. There's a sort of break in the flow."

For their part, large diagnostic companies are also concerned about high development costs associated with miniaturization, according to Gerd Grenner of Roche Diagnostics in Indianapolis. "If you go into a new microtechnology platform, [developing it and] bringing it to the market involves a lot of money." It is much simpler and cheaper to add on to existing platforms. "What you have to do for the first ten tests on a menu is much more than the next ten, and the next. And in the end, the new miniaturized platform has to perform better than the old platform. There has to be a major difference," Grenner says.

Grenner says that Roche is following the miniaturization companies closely even as it works on its own programs in microarrays, nucleic acid testing and point-of-care testing. He sees a certain disconnect between the diagnostics industry and those developing miniaturization technologies. "They are people who come from the plastics injection molding or semiconductor industry, but they have a limited understanding of diagnostics. We've looked at them... we haven't found companies so far that are attractive, that have a complete solution. They may have something that serves a niche, but we can only look for solutions for big markets."

He agrees with Ozinsky that the key is integration. "If you want to make a product that's useful, you've got to combine those technologies. If your assay has five steps, and each step has a different technology, if you combine each of those things and put it together, it gets complex. Sometimes when you go microscopic you can combine two steps into one because now they're in close proximity. That's where the art begins," says Grenner. "Simplifying, combining double steps into one. Only then do you gain. If you just shrink everything, you may actually introduce more complexity."

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> Gerd Grenner, of Roche Diagnostics
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Large companies often shy away from the major investment it takes to develop a fully integrated diagnostic, whereas academic groups have their own challenges, according to Manalis. "Part of the problem is, as with most advances that come out of research groups, that the manufacturing expertise often leaves the group when the student graduates. Then the next challenge is to make it compatible with something else. You want to keep that knowledge and then integrate it. That's really hard for universities to do," says Manalis.

Manalis hopes to solve both problems by working with a foundry to standardize the MEMS systems that he uses in a novel method to measure mass by observing the shifts in a microfluidic channel's resonance frequency that occur when molecules accumulate on the channel's inside walls. The group works with Santa Barbara, California-based Innovative MicroTechnology, a company that specializes in MEMS microfabrication. "Now it's standardized. If you give them design rules and a layout, they'll send you a part. This preserves the manufacturing knowledge and allows us to establish a platform." Quake has set up a similar arrangement with his microfluidics work using the PDMS plastic. "It's another really good example of crossing that barrier. It enables interdisciplinary research collaborations because you have a platform that you can build upon.... I think it will help drive (the field) forward," says Manalis.

Market considerations

Miniaturization also faces challenges within the diagnostics market itself. The current diagnostics system could be a serious barrier to point-of-care devices, at least in the doctor's office. Currently, a patient typically gives up a blood sample that gets sent off to a lab, with results available in a day or two. "That has proven to be a very cost-effective model. At the end of the day it's easier for doctors to send it all out. That's what point-of-care diagnostic testing is up against," says Michael G. McCully, director and senior analyst at Recombinant Capital of Walnut Creek, California.

Of course, point-of-care devices would be perfectly suited to other applications, such as public health settings in the developing

world, ambulances or even at-home testing. But these are niche markets that might be hard pressed to reward the development costs.

Others believe that miniaturization itself will create new niches, sometimes profitable ones. "As far as tests that you can wait 72–96 hours for the results, the clinical laboratories have a pretty good lock on those. But we'll see what happens in ten years. What about a blood test that I could do for a guy who falls down in a parking lot? How do you know it's not a [heart attack]? I still think there are undiscovered markets," says Northwestern University's Thomas Meade, who has developed miniaturized DNA and protein diagnostics. In January, FDA approved the eSensor DNA detection system for the detection of cystic fibrosis, which he developed and commercialized through Clinical MicroSensors (since sold to Osmetech of Roswell, Georgia). Sample DNA is labeled with ferrocene, an organometallic compound, and then hybridized to capture probes attached to electrodes on a circuit board. Applied voltage then elicits a current from the ferrocene. The applied voltage that is required to get a current varies with mutant and wild-type DNA fragments.

Miniaturization pioneers dream of a hand-held device to provide instant diagnoses of any disease imaginable, and they may well get there. But in the meantime, it is likely to require baby steps dictated by technical and economic realities. And miniaturization isn't all or nothing, of course. Many of the individual technologies can advance diagnostics, even if they don't integrate all of the processes. "Suppose the integrated system is five years out, but in year two we have a batch-fabricated detector that we can make millions of, cheaply. If you have a robust detector that can match the performance of optics, all on a chip with just wires coming out, that can be attractive," says Manalis.

Others believe that miniaturization will solve problems in unexpected ways and possibly even change the economics of diagnostics, which are traditionally a low-margin business. Quake cites the example of a novel chip that synthesizes positron emission tomography (PET) contrast agents, developed by Fluidigm of S. San Francisco, California, and based on his microfluidics systems. These short–half-life glucose analogs can distinguish between tumors based on their metabolism, but they rely on radioisotopes and must be synthesized and used swiftly. Current practice is to isolate the radioisotopes in a cyclotron and quickly synthesize the glucose analog. Fluidigm has developed a microfluidics-based chip that can synthesize the analog much more quickly than traditional synthetic methods. Swapping out chips could lead to a much larger array of reagents, potentially redefining the distribution network for these agents and demanding high margins. Munich-based Siemens has licensed the technology and plans to commercialize it. "It flies in the face of the traditional low-margin view of diagnostics," says Quake.

Seattle

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