



## Crafting Smaller, Faster Technologies

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The decreasing size and increasing speed of analytic and other tools are helping researchers work more efficiently and productively.

**Patrick McGee**, Senior Editor

Advances in chip production, materials science, and biology have had a great impact across a number of industries, including the pharmaceutical and biotechnology sectors. These advances, coupled with demands that companies be lean and mean, resulted in technologies that continue to shrink in size while performing their designated tasks more and more quickly. Over the last several years, analytical technologies such as spectroscopy, chromatography, imaging, and others evolved in this manner, allowing research labs to squeeze more data out of smaller and smaller samples in the hope that diminishing pipelines can be replenished. These modified versions of older technologies coupled with the development of some new ones will have an effect this year and over the next several years.

### Internalizing microfluidics

"Microfluidics will continue to have an impact. I think that's a technology platform that has really gained credibility and has started to show an impact in the last couple of years," says Ralph Lambalot, PhD, associate director for screening technologies at Pfizer's Research Technology Center, Cambridge, Mass. "There's a lot more that can be done, and we certainly have a lot of interest in partnering with people to further develop microfluidics platforms, not only for biology but also within the area of chemistry." Microfluidics, he says, is having an impact primarily in the area of biological screening for both enzymatic and cell-based assays. Fluidigm Corp., South San Francisco, Calif., a developer and manufacturer of integrated fluidic circuits (IFCs), is also bringing microfluidic technology to structural biology.

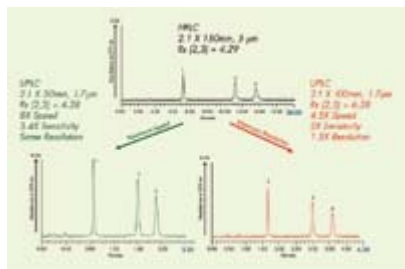
Lambalot's group collaborated extensively with a variety of vendors to produce advanced tools. They worked closely with BioTrove Inc., Woburn, Mass., to develop an instrument for high-throughput mass spectrometry (HTMS). The instrument has been used over the last three years as part of BioTrove's RapidFire Lead Discovery service to screen more than 5 million compounds. In addition to Pfizer, other companies that recently employed the service include Schering-Plough Corp., Kenilworth, N.J., and Amgen, Thousand Oaks, Calif.

The HTMS instrument features a belt that delivers samples to an attached triple-quad MS device. The belt goes through an internal incubator where a proprietary dispenser loads microliter-sized droplets of buffered enzyme and substrate onto the tape. A syringe-based applicator then dispenses test compounds from 96- or 384-well plates into those droplets. The HTMS system allowed them to cut sample preparation time from 30 seconds to as little as three seconds.

"The impact of HTMS exceeded our expectations, in that as we were developing that platform in collaboration with BioTrove, we really thought it was going to have a niche application, that there would be some small percentage of our portfolio that would be able to capitalize or profit from HTMS," Lambalot says. "But we've been surprised. It was one of these situations of 'build it and they will come.' Once word spread that we had this platform, scientists within the therapeutic areas began pulling out targets that they had wanted to work on but couldn't pursue because of the lack of a robust assay platform."

### Faster screening throughput

The technology was developed at the Massachusetts Institute of Technology, Cambridge, Mass., by Ian Hunter, PhD, Colin Brenan, PhD, and others. "The basic idea is that we were able to miniaturize the fluidics such that we could combine high-speed, low-volume chromatography with fast



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**The Acquity Ultra Performance LC was designed to reduce run times by up to 10 times, so it allows researchers to maximize speed and resolution without compromising chromatographic integrity. (Source: Waters Corp.)**

injection into a mass spectrometer to use a mass spectrometer as the screening tool as opposed to optical-based assays," says Brenan, who is now BioTrove's chief technology officer and senior vice president of research and engineering development.

The system's biggest advantage is that the information obtained comes directly from the interaction between the molecules and the reaction because no labeling has to be done. Researchers are directly weighing the molecules and, in the case of enzymatic assays, looking at the turnover of the enzyme directly, Brenan says. In addition, assay development is done more quickly than with a labeled approach, so researchers don't have to worry about the chemistry associated with putting an optical or radioactive label on a substrate.

Brenan says some of the advances BioTrove made reflect those in the industry in general when it comes to MS, chromatography, and other technologies: "The general trend is towards miniaturization and towards speed." He believes MS is underused relative to its potential. For example, once ions are injected, the actual measurement happens very quickly, so quite often, MS instruments are sitting idle. Most of the time is spent in chromatography doing the separation and injection of the sample. He says BioTrove and other companies are working to develop the tools to handle smaller volumes for injection and chromatographic separation.

In addition to BioTrove, Pfizer worked closely with other vendors, including Caliper Life Sciences, Hopkinton, Mass., and Fluidigm. Lambalot says Pfizer's relationship with Caliper goes back about five years when they acquired one of Caliper's first-generation high-throughput screening instruments, the 250S. He says the instrument proved very useful both for enzyme-based assays in their off-chip mode, as well as for cell-based assays working in the on-chip mode.

"We got a very comprehensive validation data set, so much so that when we were designated as the kinase center of emphasis within Pfizer just last year, it was pretty easy for us to turn to Caliper and say 'We've already validated this technology. We're ready to apply it further now working with kinases.'" Lambalot says the primary advantage of working with Caliper's platform is that it is a fluorescence-based technology that features a direct continuous-assay method, something that allows researchers to quantitate both the consumption of substrate and the creation of product in real time.

Pfizer has worked closely with Fluidigm as well, and their platform is used by Lambalot's facility and many of Pfizer's structural biology groups. Fluidigm developed IFCs, a platform that allowed them to launch the Topaz system for protein crystallization three years ago. Topaz reduces the time required to generate crystals that produce the high-quality data required to solve protein structures, and many labs have adopted the systems to build large-scale operations. Fluidigm has been collaborating with GlaxoSmithKline researchers in Harlow, UK, to develop a high-throughput protein crystallization platform based on Topaz.

### Separation, analysis shrink

The evolution toward smaller and faster technologies is also evident in liquid chromatography/mass spectrometry (LC/MS) systems and other analytical tools, says Art Coddington, PhD, a recently retired research fellow from Merck Research Laboratories (MRL), West Point, Pa. "Everything, I think, boils down to speed," Coddington says. One system that had an impact in this respect is the Acquity Ultra Performance LC, a system launched by Waters Corp., Milford, Mass., in 2004. The Acquity was designed from the ground up by Waters to reduce run times by up to 10 times by using low-dispersion, high-speed detectors and 1.7 µm small-particle chemistries for columns.

"Your band broadening goes down and efficiency goes up. It goes hand in hand, so you have more plates per unit length of the column. You have more efficiency if you shorten the column, you get lower back pressure, you get all sorts of good things," Coddington says. He adds that the MRL got their first Acquity system in the summer of 2004, and Coddington believes they were the first open-access medicinal chemistry arena in the nation to have one. They have since added another system.

"The chemists come up and say they now try things that they've never tried before or hardly ever tried before because of time constraints and because of the cost of the compound to run a reaction on a certain scale and

have it fail." The system is much more sensitive than previous ones, Coddington says, so sensitive, in fact, that chemists begin to see things in reactions that they have never seen before. "Then I have to sit down and explain to them as an analytical chemist that everything hinges on limit of detection and limit of response and all those sorts of things, and explain to them that because the Acquity now concentrates all of the analytes that you inject onto the column into a smaller volume, the peaks are narrower and taller."

Before the development of Acquity, particle sizes continually decreased in size, so much so that their efficiency challenged the in-strumentation, says John Morawski, MSc, director of worldwide business development for Acquity and the program manager who oversaw its development. Conventional LC systems often had to be customized by researchers to get what they needed, whether it was extending the flow for preparative analysis, modifying the flow cell for more sensitivity, or modifying the system volumes for more efficiency. "Each one of those modifications promised some kind of improvement in performance, but they also offered compromises for what you had to live with. So, as you went to a smaller flow cell, for example, to maintain chromatographic fidelity of your separation, you would at the same time rob yourself of sensitivity, so you'd get resolution at the expense of sensitivity."

### Imaging drug action

Advances in imaging will continue to have an impact in drug discovery and development this year and over the next several years, says Gerald Fox, PhD, senior group leader for neuroscience research at Abbott Laboratories, Abbott Park, Ill. He says pharmaceutical and biotechnology companies have become increasingly interested in using imaging tools such as magnetic resonance imaging (MRI), functional MRI (fMRI), positron emission tomography (PET), and optical imaging to translate preclinical data into successful clinical studies. While companies have invested heavily in developing various modalities, the bulk of the attention seems to be going into different methods for doing MRI and PET, particularly in conjunction with molecular probes, Fox says.



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One example of a molecular probe used in PET imaging is Pittsburgh Compound B (PIB), which is a simple molecule that binds to the abnormal amyloid plaques in the brain characteristic of Alzheimer's disease. When imaged with a PET scan, PIB, which was developed at the University of Pittsburgh, showed researchers pathological changes in the brain that could be the earliest signs of the disease, perhaps as many as 10 years before patients experience serious memory loss.

**Researchers at Abbott Laboratories are using functional MRI on awake rats to map changes in brain activity following administration of a drug that acts on the central nervous system.**  
(Source: Gerald Fox, PhD)

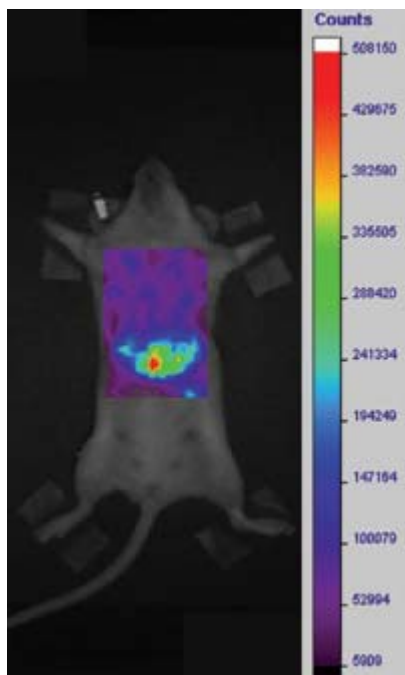
"In the future one can envision the use of this technology to follow the progression of the disease. There's a lot of interest right now in intervention strategies for Alzheimer's, and one could follow the effect of the therapeutic intervention over time in the same patients. The disadvantage of PET, of course, is that you have to use a radiotracer, and it's more invasive than MRI in that regard. Also, your spatial resolution is not great."

Because of increased sensitivity, MRI could be used in the future to visualize amyloid plaques at the microscopic level using probes similar to PIB. Instead of being radioactive, they would be scanned with a paramagnetic tag that would be detectable in the magnet, allowing researchers to see individual plaques. In addition to increased sensitivity, the convergence of chemistry and molecular probes is having an impact, as is the increased understanding of the pathophysiology of diseases such as Alzheimer's. "Computing power and software analytic tools have also improved considerably and that really helps with data analysis."

### In vivo imaging

In their facility at Abbott Laboratories, Fox and colleagues are exploring the use of fMRI, an *in vivo* imaging technique for detecting cerebral hemodynamic changes in response to alterations in neural activity. It provides high spatial and temporal resolution and has been applied to human and animal psychopharmacology studies, an approach that has been called pharmacological MRI (phMRI). Fox says they encountered a number of challenges from the outset because fMRI is a relatively new technology used a great deal clinically, but is underused preclinically.

They developed a technique to perform imaging in animal models, and one of the first challenges they faced was that the rats they were using would have to be anesthetized to avoid motion artifacts. But the



**Researchers at the University of Pennsylvania are developing near-infrared emissive polymersomes that are ideal for *in vivo* optical imaging directly through deep tissue via non-invasive fluorescence-based imaging.**

(Source: Peter Ghoroghchian, PhD)

anesthetics produced direct and indirect confounding effects on pharmacological action and cerebral hemodynamics. To get around this obstacle, they developed a technique for imaging awake animals that involved training the animals over a period of time to remain still in a special restrainer with built-in coils to detect brain signals. Using this technique, they found their sensitivity increased and their data was highly reproducible.

And the effects they detected were subtle, Fox says. While such experiments invariably use high doses of drugs such as cocaine or amphetamines that have a dramatic effect on the brain, their experiment sought to detect the effect of low doses of ABT-594, an agonist for nicotinic acetylcholine receptors. "This is a very selective compound for a particular class of receptors, and we showed we could block those effects with an antagonist, which is important to show our specificity. We're one of the first, if not the first, to be able to show this kind of an effect in an awake rat and to be able to then relate the brain activity patterns that we saw to all of the behavioral and other data that we have generated on this compound already."

Peter Ghoroghchian, an MD/PhD candidate in the department of biomedical engineering at the University of Pennsylvania, Philadelphia, is developing near infrared (NIR)-emissive polymersomes. These are robust polymer vesicles that uniquely incorporate, and uniformly distribute, numerous large, hydrophobic, NIR-fluorophores (NIRFs) exclusively in their lamellar membranes. "NIR-emissive polymersomes define a family of organic-based, soft matter quantum dot analogues that are ideally suited for *in vivo* optical imaging," Ghoroghchian says.

"To date, we have focused on the development of NIR-emissive polymersomes capable of binding to various molecular markers of cancer via introduction into the blood stream. One can then image these nanoparticles, in real time, from outside the body and quantitatively determine the spatial localization of viable molecular therapeutic targets at various stages of disease progression." Ghoroghchian says the major applications of this technology would be to enable fluorescence-based optical imaging for drug discovery and development, to offer tools that would transcend various stages from *in vitro* to *in vivo*, and possibly even in human trials.

Vendors are getting in on the act as well. GE Healthcare, Chalfont St. Giles, UK, recently announced a licensing agreement to distribute Hexvix made by PhotoCure ASA, Oslo, Norway. Hexvix (hexaminolevulinat) is an optical molecular imaging agent intended for the diagnosis and monitoring of bladder cancer. Hexvix received approval for the diagnosis of bladder cancer in a large number of European countries, but is not currently approved in the United States. However, a new drug application was submitted last June, and if Hexvix is approved it would be the first optical molecular imaging agent of its kind available in the United States.

### **Nanotech, tissue engineering**

Nanotechnology has created quite a buzz—some would say hype—over the last few years, and its impact is being felt in the pharmaceutical and biotech industries. "Putting aside the hype for a second, nanotechnology offers the opportunity to pursue 21st-century medicine. It's a little way off, but there's a lot of good work going on, primarily at academic institutions," says Zahed Subhan, PhD, CEO of Marillion Pharmaceuticals, Philadelphia. Marillion is a biotechnology company that conjugates tumor-homing molecules to naturally occurring nanoparticles responsible for lipid transport throughout the body.

While aspects of nanotechnology have been around for a number of years, its applications in biotechnology and drug delivery are now coming to the forefront, says Aliasger Salem, PhD, an assistant professor in the division of pharmaceuticals at the University of Iowa, Iowa City. "The advantages that nanotechnology has is that you can manipulate things on a biological scale that you've never been able to do previously."

Prior to coming to Iowa, Salem was a postdoctoral fellow in biomedical engineering in the laboratory of Kam Leong, PhD, at Johns Hopkins University, Baltimore, where they investigated the use of multifunctional

metallic nanorods for gene delivery. "We could put a cell-targeting protein on one side and a plasmid DNA, which would encode a therapeutic gene, on the other side so that they didn't interfere with each other and they were in spatially defined locations. Because of their size, they could be taken up by cells," he says. The plasmid DNA would be released into the cytoplasm and migrate to the nucleus, where it would encode the cell with therapeutic gene. Because the size and composition of the nanorods can be controlled, they could be attractive carriers for delivering DNA into cells with a potential application in genetic immunization.

One focus of Salem's work at the University of Iowa is the use of nano-technology to attack tumors. They take resected tumors, harvest the lysate and proteins, and load them onto biodegradable nanoparticles. They have shown that the nanoparticles are taken up by antigen presenting cells (APCs) such as dendritic cells and macrophages, which then go on to stimulate T cells to act against the cancer from which the antigen was derived. One problem they encountered is that particle delivery prompts a polarized T helper 2-type response, but they have not been getting a very strong T helper 1-type response.

But they found that if they encapsulate immunostimulatory molecules such as CpG ODN, which is like a ligand nucleotide, into the nanoparticles with the tumor-specific proteins, it elicits a strong response from both types of cells. "We get a very, very strong immune response against those cancers and we see three- to fourfold decreases in the size of tumors compared to conventional treatments." Salem says he has a great deal of preliminary data on this approach but is doing further work to ensure the results are repeatable.

In addition to nanotechnology, Salem is very involved in tissue engineering. He is interested in developing methods for growing synthetic functional tissue for *in vitro* toxicology testing for pharmaceutical applications. For example, if hepatocytes are cultured alone, they are not viable for very long, but if they are co-cultured with fibroblasts they are much more functional and therefore representative of actual normal tissue, and they are viable for a longer period. "If you can fabricate synthetic tissues that mimic real tissues, then you avoid much of the animal testing that is necessary at the moment."

Salem says companies have been founded to develop such products, but many are still at the early stages. Some products, however, are already on the market. Testskin II from Organogenesis Inc., Canton, Mass., has been available for several years. It mimics key morphological and biochemical properties of human skin and can be used for *in vitro* research, mainly for toxicology screening.