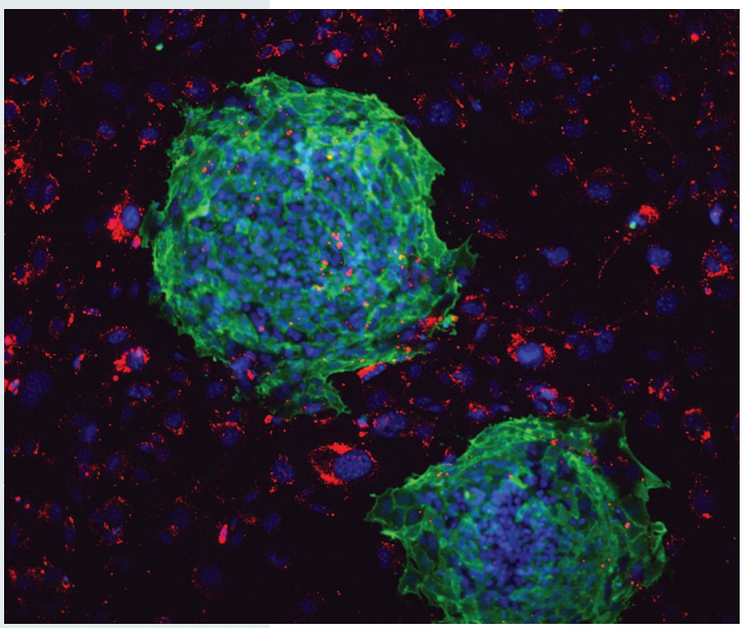
New Techniques Facilitate New Methods

by Mike May

Technology has always facilitated scientific discovery, but perhaps never as significantly as it does today. High-throughput genotyping pushed ahead the human genome project. Microarrays sparked functional genomics. And microfluidics ushered in labs on chips. To stay competitive, today's scientists must evolve with technology and apply the latest methods.

At the University of California at Berkeley, Tom Alber studies the structural and biochemical mechanisms of virulence and signaling in tuberculosis. He also develops new methods for X-ray crystallography. Alber says, "We're constantly adopting and inventing new methods.



Invitrogen is using fluorescent nanoparticles to discriminate between human embryonic stem cell (hES) colonies and feeder cells. Mouse feeder cells were labeled with Q-Tracker 655 nanoparticles (red-labeled cells), and then used to support growth of BGO1V hES cells. The hES colonies were stained with a stem-cell specific antibody, SSEA4, and appear green in this photomicrograph. The nuclei from all cells were stained with the nuclear-specific stain, DAPI, and appear blue.

In the last year, we began using Gateway cloning, multiwell fluorescence assays, small-angle X-ray scattering measurements, automated monitoring of crystallizations, and automated crystal mounting for synchrotron X-ray data collection." While that may seem like a lot of new techniques to adopt in one year, it is often necessary to do so to stay in the game of modern science.

The battle to push science ahead through technology goes on in academic, government, and industrial laboratories. Academic scientists try out new tools, and invent more of their own. Government labs also take on new products and create new techniques. Industry uses new tools to make even better ones. It's a never-ending cycle: develop a new technique, discover new science, and develop even more advanced approaches.

Fighting terrorism

Jonas Winchell works at the Bioterrorism Rapid Response and Advanced Technology (BRRAT) Lab of the Centers for Disease Control and Prevention (Atlanta, GA). He says, "Our laboratory has three primary functions: provide a 24/7 response to bioterrorism events for the purposes of rapid identification of suspect agents so that appropriate actions can be taken; perform research and development for diagnostic assay development and implementation; and disseminate and provide support for developed and validated diagnostic procedures and technology to a network of public-health laboratories, known collectively as the Laboratory Response Network." Doing that work, says Winchell, demands constant exploration for advanced technologies. Recently, the BRRAT lab added a Bio-Plex assay (Bio-Rad Laboratories Hercules, CA) that allows for the simultaneous detection of many threat agents using just one experimental test that does not take much time. Winchell says, "The technology greatly enhances our ability to test multiple samples for multiple agents at once."

For the past eight years, the BRRAT lab has exploited various applications of real-time PCR. "This technology allows us to specifically amplify small amounts of target nucleic acid in real-time," says Winchell. "We have been able to take this approach one step further by manipulating the assay to provide greater dis-

criminatory power in order to learn more about the nature of certain bioterrorist threat agents." That approach is representative of a common form of technological evolution: take an existing technique and improve it.

In looking for new techniques, says Winchell, "We are dedicated to examining technologies that provide faster results, give greater sensitivity, are easy to operate, are less expensive, and ultimately provide superior results over the currently used technologies."

Industrial advances

Industrial scientists also use advanced techniques to develop even better ones. Jonathan D. Chesnut of Invitrogen (Carlsbad, CA) works on stem cells—especially developing new tools to identify and analyze embryonic and adult stem-cell populations. He says, "One of our goals is to create a comprehensive, standardized set of tests that can be used to gauge the differences between stem cell cultures from cell line to cell line, from day to day, and from lab to lab." For example, Chesnut and his colleagues are applying quantum-dot labeling technology to easily discriminate between human embryonic stem cells and feeder cells in mixed populations. Many approaches to keeping stem cells in culture use a so-called feeder layer, which often consists of mouse fibroblasts. These animal cells can get mixed in with the stem cells, and scientists need ways that enable them to tell one from the other.

Industrial scientists also focus on developing new techniques that scientists can use. For example, Lee Lomas of Ciphergen (Fremont, CA) says, "Our group oversees the development of new biochip chemistries that are used in surface-enhanced laser-desorption/ionization mass spectrometry. We also develop new methods that more effectively separate complex protein mixtures to allow the detection of low-abundance proteins." To reach these goals, Lomas and his colleagues keep developing new technologies. Lomas says, "We recently described a new protein-equalization technology that uses peptide combinatorial libraries." Lomas says that this technology concentrates lower-abundance proteins from a biological sample in the presence of a background of higher-abundance proteins. Moreover, this approach does not remove the high-abundance proteins. Lomas says, "It retains representation of all protein members." He adds, "Our current focus is to detect, characterize, and develop quantitative assays for protein analytes that are correlated with disease." Using reproducible methods that detect lower-abundance proteins improves the odds of discovering proteins that are highly correlated with disease.

PerkinElmer's ATR FT-IR Image accessory provides attenuated total reflectance on its Spotlight 300 FT-IR Imaging system.

Essentially all companies must constantly bring out new techniques to stay viable. For instance, Richard M. Eglen of PerkinElmer Life and Analytical Sciences (Wellesley, MA) and his colleagues recently developed two new AlphaScreen platforms: Phosphosensor and *SureFire*. "Phosphosensor," Eglen says, "allows antibody-free screening assays to be conducted on a critical class of drug targets, kinases." The SureFire assay, which was developed by TGR BioSciences (Thebarton, Australia) works in the G protein-coupled receptor area, which makes up another large class of druggable targets. This assay detects the phosphorylation of cellular ERK (extracellular signal-regulated kinase), which Eglen describes as "a signaling molecule that can also be used to detect cellular activation by some kinases."

PerkinElmer also develops new devices, like the ATR FT-IR Image accessory. PerkinElmer's Sharon Williams says, "This enables attenuated total reflectance—or ATR—images to be collected on our Spotlight 300 FT-IR Imaging system." Williams also points out that they've improved the repeatability of ATR results.

Instead of just making new technologies, though, industrial scientists also use them. At Wyeth, Madison, NJ, Sunil Nagpal works on nuclear-receptor drug discovery research. The activity of these receptors could be modified with small molecules. So he looks for molecules that suppress the transcription of these

"This lets us put compounds in various categories. Instead of taking individual compounds, we can test groups in *in vitro* and *in vivo* assays."

"Phosphosensor allows antibody-free screening assays to be conducted on a critical class of drug targets, kinases."

receptors to treat diseases. To advance this research, Nagpal and his colleagues keep exploring new approaches. They recently brought in multiplex-cofactor interaction assays to look at a spectrum of factors simultaneously. Nagpal says, "This lets us put compounds in various categories. Instead of taking individual compounds, we can test groups in *in vitro* and *in vivo* assays." They are also adding new biochemical techniques, including AlphaScreen (amplified luminescent proximity homogeneous assay) and FRET (fluorescence resonance energy transfer). "We are also bringing in gene-card technology from Applied Biosystems (Foster City, CA)," says Nagpal. "This way, we get a PCR expression profile of multiple genes." This will help the company move from developing simple agonists and antagonists to tissue- and gene-selective compounds.

Keep on inventing

Sometimes, instead of bringing in off-the-shelf technologies, scientists return to the old-fashioned approach: They invent them. Like most scientists, Jeffrey P. Townsend of Yale University (New Haven, CT) does a fair amount of inventing. He explores population genetics, functional genomics, and evolutionary biology. He is currently working on the population genetics and functional genomics of the model fungi *Saccharomyces cerevisiae* (wine yeast) and *Neurospora crassa* (bread mold). To enhance his work, he creates new bioinformatic and statistical techniques to analyze DNA microarrays.

Other scientists also require improved computational techniques. Kathleen Matthews of Rice University (Houston, TX) studies interactions between proteins and DNA that regulate gene expression. In the last year, says Matthews, "The key change has been to couple theoretical/computational models with our experimental work. This was most important in our exploration of the folding mechanism for a monomer derivative of a lactose repressor." In this study , Matthews and her colleagues—Cecilia Clementi, Pernilla Wittung-Stafshede, Corey Wilson, and Payel Das—compared their theoretical model with experimental data on the folding and unfolding of the repressor, which they obtained with a Pi-Star spectrometer from Applied Photophysics (Leatherhead, UK). In work on molecular dynamics, Matthews and her colleagues also employed the Amber simulation package, which was developed by scientists in academics and industry, together with a simplified protein model. Such computational tools play a huge role in today's data-rich times.

The reasons to keep technology moving probably go without saying, but Tom Alber says it quite simply and clearly: "It is to increase the speed of research and open new windows on nature." It is a battle that never ends. When asked if he has plans to add other methods or techniques in the coming year, he says, "You bet—higher-throughput cloning and protein-purification methods."

As the technological advances continue—across academics, government, and industry—scientists will grab every improved approach that they can. Today's discoveries often hinge on new angles on methods and improved and novel instruments.

MANUFACTURER'S INDEX