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December 10-14, 2013 • San Antonio, TX

**Sixth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved**
December 6-9, 2013 • Atlanta, GA

**AACR-IASLC Conference on Molecular Origins of Lung Cancer**
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Meeting Global Challenges: Discovery and Innovation
Scientific discovery and innovation are helping to drive solutions to current and future global challenges. Economic progress in every community worldwide has meanwhile become increasingly interdependent with advances in science and technology. Challenges related to ensuring sufficient food for a growing population, quality healthcare, renewable fuels, and a sustainable and enriching environment demand innovation and international dialogue. Addressing these challenges depends upon discoveries emerging from the convergence of physical, life, engineering, and social sciences in innovative ways that are most useful to society.

In a weakened global economy, many countries have begun to limit their investments in the future. Yet, investments in innovations – including funding for education as well as basic and applied research – represent our best prospect for a sustainable environment and increased economic growth. Economists estimate, after all, that innovation in science and technology are the source of more than half of the economic growth in many countries. By increasing innovation in sustainable products and processes, world economies can continue to enhance human welfare across society.

Innovation springs from the translation, production, and distribution of discovery and invention to society. In the contemporary world, this is not a linear process, but rather, a matrix of interactions. Societies, with support from public and private sectors and institutions, struggle to integrate the necessary disciplines and interests into this matrix. Within the scientific and engineering community, we need to better integrate different disciplines and voices into a consensus supporting innovation. Developed and developing countries that accomplish this will become the economies of the future.

At the same time, it is imperative that we work in ways that are transparent and open to a diversity of contributors and ideas. Assessing risk versus benefit in adopting an innovation is complex and depends upon an open dialogue. Only then will we realize the promise of furthering scientific discovery and innovation to meet pressing global challenges and improve quality of life.

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An Expanding Color Palette for Biotechnology

In This Issue
Think of any multiplexed biological technique. RT-PCR. DNA sequencing. Flow cytometry. Microscopy. All work on different principles. Yet all have one common factor: fluorescence. The ever-expanding variety of fluorescent molecules, nanoparticles, and proteins has expanded the palette, so to speak, of biological experimentation. The pages of research journals may be filled mostly with reds and greens, but today’s researchers have literally dozens of hues available from which to choose. Such variety has expanded the spectrum of applications and instrumentation available to the research community. The future of fluorescence multiplexing has never seemed so, ahem, bright.

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Upcoming Features
The Microbiome—May 10
Proteomics: MALDI Imaging—May 31
Data Management: Cloud-Based—June 14

Opening Industry-Academic Partnerships

In This Issue
Research and development today is about networking, sharing, and partnering. Collaborations between industry and academia are promoted by open innovation programs, which have become a near-universal model for R&D. Pharmaceutical and biotechnology companies offer university researchers access to resources and funding. Academic scientists bring in-depth expertise and basic research data to the table. Open innovation has exploded into megapartnerships of academia, industry, government agencies, and private organizations. These consortia have the potential to solve major medical and public health issues, if they can set terms and goals that reward all parties.

See the full story on page 225.

Upcoming Features
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An Expanding Color Palette for Biotechnology

Think of any multiplexed biological technique. RT-PCR, DNA sequencing. Flow cytometry. Microscopy. All work on different principles. Yet all have one common factor: fluorescence. The ever-expanding variety of fluorescent molecules, nanoparticles, and proteins has expanded the palette, so to speak, of biological experimentation. The pages of research journals may be filled mostly with reds and greens, but today’s researchers have literally dozens of hues available from which to choose. Such variety has expanded the spectrum of applications and instrumentation available to the research community. The future of fluorescence multiplexing has never seemed so, ahem, bright. By Jeffrey M. Perkel

Mario Roederer, senior investigator in the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID), knows something of the promise and limitations of fluorescent dyes. Roederer has been studying T cell responses to infection and vaccines for nearly 20 years, first as a postdoc in Len Herzenberg’s lab at Stanford University and later as a group leader at NIAID. In the early-to-mid-1990s, Roederer says, he and his colleagues in Herzenberg’s lab were using flow cytometry to try to understand the impact of HIV-1 infection on the immune system.

Flow cytometry is the workhorse of immunology labs, enabling researchers to interrogate cells one by one to identify those that do or do not express specific cell surface markers, such as CD3, CD4, and CD8. Using a special kind of flow cytometer called a cell sorter, researchers can even isolate and expand cell populations of interest. The problem was, when Roederer began his postdoc, there simply were not enough usable fluorescent colors available to sift through the dizzying complexity of the human immune system.

“We realized one of our limitations was that we weren’t able to interrogate the immune system sufficiently,” he says.

At the time, flow cytometry was limited by a lack of useful, separable fluorescent labels to four color channels, far too few to delve into immune cell subtypes. By the time Roederer left the lab, however, the number of available channels had risen to 12, the result of improvements in hardware, software, and dye chemistries, and by the early 2000s it was up to 18 thanks to a new class nanoparticulate quantum dots from Quantum Dot (now part of Life Technologies).

Using those 18-color panels, Roederer’s group has been able not only to identify cells of interest, but probe their behavior as well. “You need six or eight colors just to identify the cells,” he explains, “and then you need other colors to see what they are doing.” In one recent paper, Roederer’s lab characterized stem cell-like memory T cells as a subpopulation of CD95+, IL-2R[β][γ], CXCR3+, LFA-1+ cells within the larger CD45RO+, CCR7+, CD45RA+, CD62L+, CD27, CD28+, IL-7R[α]− T cell “compartment.”

Today, Roederer’s 18-color panels represent essentially the state-of-the-art in fluorescent flow cytometry (and, for that matter, for fluorescent experiments in general). But the state-of-the-art isn’t static: New fluorescent dyes promise to boost his multiplexing capability to 25- or even 30-plex within the next few years, meaning he and other immunologists will be able to tease apart immune cell responses with ever more sophistication. Yet researchers using other fluorescence techniques are not being left out in the cold: new hardware and reagents are enabling higher levels of multiplexing and greater sensitivity for a range of biological applications.

Brilliant New Dyes

To squeeze more colors from his already strained BD Biosciences LSRII, Roederer is going to need some new fluorophores. Enter Brilliant Violet dyes from Sirigen (acquired by BD Biosciences in August 2012).

Unlike quantum dots, which are nanoparticles, and small molecule organic dyes like Life Technologies’ Alexa Flours, Sirigen’s fluorophores are actually light-harvesting polymers. They rely on the same electrically conductive polymer chemistry that was recognized with the 2000 Nobel Prize in Chemistry, and are used either directly or as “tandems,” in which the polymer non-radiatively passes its energy to another dye that shifts its emission further into the red. In this case, the company has over

Upcoming Features

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the past year and a half developed and released a series of seven violet laser (407 nm)-excitable fluorophores, called Brilliant Violet dyes, which emit at wavelengths from 421 nm through 785 nm.

According to Robert Balderas, vice president of biological sciences at BD Biosciences, the Sirigen dyes possess many of the qualities one would want in a good fluorophore. They are photostable and resist quenching, and most importantly, are exceptionally bright, up to 10-times brighter than phycoerythrin (PE), which is one of the brightest dyes available. And that’s important because in flow cytometry especially, the trick is coupling dye brightness with target abundance. To label an abundant protein, researchers can use a relatively dim dye, as the sheer number of fluorophores on any given cell will overcome the weak signal. For rare proteins, though, bright dyes are best, and until recently only a few were available. Now, says, Roederer, there is at least one more, Brilliant Violet 421; other dyes in the series are almost equally as strong.

“The Sirigen dyes are really remarkable,” says Roederer, who had thought with QDots that he had maxed out fluorescence multiplexing with 18 channels. Now he is slowly rotating the Sirigen dyes into his cytometry panels in lieu of QDots, producing panels that are no more complex than before, but that are far brighter and thus more sensitive. “So while our ability to multiplex hasn’t increased with the new dyes, our sensitivity and ability to make precise measurements has increased in a way that I haven’t seen in 20 years,” he says.

Development of new colors, though—for instance, polymers that excite in the ultraviolet or far in the red ends of the spectrum—will increase Roederer’s multiplexing ability even more. “I am optimistic in the next five years we could do 25 or even 30 color measurements, which is really fantastic.”

According to Balderas, Sirigen is already hard at work making that expansion happen. And that could significantly alter the flow cytometry market, he says, by allowing the development of multiple colors off individual lasers, while at the same time pushing the envelope on higher-end instrumentation. “If we could build multiple dyes off multiple lasers, imagine what we could do with an instrument that has five lasers.”

**Bead-Based Megaplexing**

Another application for fluorescence multiplexing is analyte quantitation. And no such tool is quite so popular as Luminex’s xMAP beads.

xMAP beads enable a kind of highly multiplexed, solution-based ELISA. Where an ELISA measures single analytes individually in each well of a microtiter plate, xMAP technology uses fluorescently coded beads to, in essence, pool multiple wells into one and distinguish them optically.

Key to these process are xMAP beads, micrometer-sized polystyrene spheres loaded with varying amounts of two or three fluorescent dyes. The precise amounts of each dye serves as a kind of fluorescent fingerprint for the beads, and Luminex currently supports as many as 500 such fingerprints.

In an xMAP assay, each fingerprint is associated with a specific biomarker. For instance, a panel for assaying immune cell responses in blood sera might have one fingerprint associated with an antibody to IL-2, a second for IL-4, and so on. (A separate set of Luminex beads, called xTAG beads, enable nucleic acid-based assays.) Beads corresponding to the different analytes to be tested are mixed in a single well of a microtiter plate and incubated with the biological sample. The antibodies capture their specific targets. Then, after washing, a mixture of fluorescently tagged secondary antibodies is added, after which the reaction is read.

In the traditional Luminex workflow, the beads are read on a dedicated instrument, essentially a flow cytometer, that uses lasers to interrogate each bead one by one, first to read its fingerprint—that is, identify the analyte being measured—and then the abundance of antigen it bound. In 2010, however, the company released a new instrument, the MAGPIX, for labs looking for a smaller, less-expensive, and less-multiplexed alternative.

The Luminex MAGPIX is a fluorescence imager that uses paramagnetic microspheres (hence the name) and holds them in place on a solid substrate with a magnet to create a fixed, albeit temporary, solid-phase bead array. (The magnetic beads also simplify sample processing and improve bead recovery, says Jehangir Mistry, head of multiplex and immunoassays at EMD Millipore, which markets MILLPLEX-branded xMAP assays.) The instrument then excites that array with LEDs and captures the resulting fluorescence on a CCD camera.

Unlike the Luminex 200 (which can distinguish up to 100 different bead types) and the FLEXMAP 3D (which can recognize 500), the MAGPIX is limited to 50 different bead fingerprints, says Matthew Grow, director of marketing at Luminex. But that’s more than enough for most assays, he notes. In fact, the MAGPIX (along with the Luminex 200) is approved for use in both Canada and Europe with the company’s 15-pathogen xTAG Gastrointestinal Pathogen Panel. EMD Millipore’s largest panel, says Mistry, is a 42-plex human cytokine and chemokine panel. (Most Luminex assays are actually created and sold by its partners, including EMD Millipore, Bio-Rad Laboratories, R&D Systems, and more than a dozen others. The company recently launched an iPhone application, xMAP Kit Finder, to help researchers identify kits targeting specific biomarkers.)

**Bioassays On A View-Master**

Quanterix Corporation, a Boston-based firm founded in 2007, has taken an approach not all that different from the MAGPIX to effect highly sensitive multiplexed analyte detection.

Quanterix was founded by Tufts University professor David Walt, who also co-founded Illumina, and that corporate heritage is evident in Quanterix’s single-molecule array (Simoa) technology. continued>
Before it became known as a sequencing firm, Illumina developed a bead-based microarray called the BeadArray, which blends the bead-based approach of Luminex with planar microarray detection. Similarly, Simoa assays are carried out on small “Simoa Discs,” which look like high-tech reboots of the ViewMaster reels so popular in the 1960s and 1970s. Each disc holds 24 arrays of 216,000 holes on its periphery, with each array corresponding to one well of a microtiter plate. The holes are just large enough to hold the 3 μm beads Quanterix uses for its antigen-capture assays.

As with xMAP, each bead is fluorescently fingerprinted with one of four dyes, and each has a unique capture antibody on its surface. After incubation with the sample and washing, those beads are incubated with secondary antibodies and then labeled with an enzyme. They are then flowed onto the Simoa Disc, where the beads are captured in wells and then sealed. That step, explains David Duffy, the company’s vice president for research, makes the Quanterix system exceptionally sensitive, as the enzyme converts a dark substrate to a fluorescent one in a femtoliter-sized volume, rather than allowing the fluorophores to diffuse into a larger space.

“You go from needing millions of enzymes to a single enzyme generating about 3,000 fluores, and you can detect a single protein molecule,” Duffy says.

Duffy claims the Quanterix system (called the Simoa HD-1 Analyzer) can detect as little as 0.02 pg/mL of signaling molecules like TNF-alpha, which is one-to-two orders of magnitude better than comparable Luminex assays. The system, which is set to launch in July 2013, can theoretically multiplex up to 12 different analytes. But in practice, he says, the company will focus on panels of five or so targets to speed assay development and minimize antibody cross-reactivity. Initial panels will target applications such as oncology, neurology, and inflammatory and infectious diseases.

Quantifying RNA In Vivo

Another new development in fluorescent multiplexing comes from Chad Mirkin’s lab at Northwestern University. Called Nanoflares by Mirkin and commercialized by AuraSense and EMD Millipore (which calls them SmartFlare RNA Detection Probes), the technology enables researchers to quantify RNA as in an RT-PCR assay, but within living cells.

Nanoflares are 13 nm gold particles coated with 20-base oligonucleotides complementary to the RNA of interest—a structure Mirkin calls a “spherical nucleic acid.” A second, shorter oligonucleotide is bound to the targeting oligo and end-labeled with a fluorophore. Initially that fluor is dark, as gold is an extremely efficient fluorescence quencher. When these constructs encounter a complementary RNA, however, a short “flare” is released, producing a fluorescent signal.

According to Mirkin, the beauty of spherical nucleic acids (which his team first developed some 16 years ago) is that they are naturally and rapidly taken up by cells via scavenger receptors, so no transfection agents are required. When coupled with the Nanoflare design, this property allows researchers to quantify RNAs in living cells, apparently without toxicity.

“One of the real wins in nanoscience has been the discovery that if you take what I think is arguably the world’s most important molecule ever synthesized, DNA, and you arrange it in a highly oriented spherical form, you get properties that are radically different, and one of those is the ability to enter cells,” he says.

Mirkin’s team published a duplex version of the Nanoflare approach in early 2012, using one color channel to quantify an RNA of interest and the second to measure a housekeeping control. Up to three or four color multiplexing is possible, he says.

According to David Giljohann, chief operating officer and principal scientist at AuraSense, Nanoflares can be used as an alternative (albeit a less sensitive and quantitative one) to RT-PCR, for instance, to monitor transgene expression or siRNA efficacy. Just add the flares to a dish of cells, wait 12 to 16 hours, and measure the results using flow cytometry. EMD Millipore already has over 100 SmartFlare detection probes available, Mistry says, and more are coming.

For cell biologists testing the efficacy of short regulatory RNAs or measuring transgene efficacy, such tools could prove to be a godsend, especially as positive cells can be isolated and put back into culture using cell sorting. That could even benefit immunologists like Roederer, who could, for instance, use the approach to compare immune responses in RNAi–treated and untreated cells.

It truly is a bright time for fluorescence multiplexing.

Jeffrey M. Perkel is a freelance science writer based in Pocatello, Idaho.

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A new range of high-quality cyanine dye products support the demand for fluorescent labeling in biological imaging and molecular diagnostics. In addition to a new range of 3’-CPG solid supports modified with Cyanine 540 and Cyanine 650, Link Technologies now also manufactures and supplies the commonly used phosphoramidites, allowing researchers to source an extended range of cyanine reagents. Until now, 3’-cyanine modification of an oligonucleotide could only be done postsynthetically, normally to an amino-modified oligo, or by the use of a cyanine phosphoramidite onto a universal or modified support that will not interfere with the intended use of the oligo. With the innovative 3’-modified 1,000Å CPG supports, cyanine dyes can be added directly to oligos of interest without additional modification steps. Used as fluorescent markers in oligonucleotide synthesis, dyes are central to numerous detection techniques, such as real-time polymerase chain reaction, fluorescence in situ hybridization, SERRS-based DNA detection assays, and FRET studies.

Link Technologies

For info: +44-(0)-1698-849911 | www.linktech.co.uk
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Cytokine and growth factor signaling doesn’t stop at the cell surface. R&D Systems offers a full line of signal transduction reagents to complement our industry-leading cytokines and growth factors. In the upcoming months we will be adding rich signal transduction content to our website. Follow us on social media to receive updates.