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(C) Sub FGF2 – Sub-optimal FGF2 (to identify inhibitors/potentiators) 500pg/ml FGF2
(D) Max GFs – Maximal EGF + FGF2 (to identify inhibitors/potentiators) 20ng/ml EGF + 20ng/mL FGF2

Results for 20 inhibitors are presented as mean data (of n=4 wells per condition) with error bars indicating standard error of the mean (SEM). The presence of inhibitor K-252a, *Nocardiopsis* sp. (Cat. No. 420297) alone in the culture media resulted in a 10-fold mNS cell viability.

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- CXCL2 MCP1
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NEW Technology Feature!

Read the article in this issue on page 853.

Ebb and Flow: CYTOMETRY for the next generation

Flow cytometry technologies have been rapidly advancing, becoming more portable as devices decrease in size and now offering enhanced cell sorting capabilities with an ever increasing number of fluorescent colors and lasers available for cell detection. The recent combination of cell tagging technology with metals and microscopy has opened the way for potential new uses of flow cytometry devices, which are now being applied in most fields of biology, from botany and stem cells topathology and cell signaling.

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EBB AND FLOW:
Cytometry For The Next Generation

Think there’s nothing new in the world of flow cytometry? Think again. As industry powerhouses like BD Biosciences and Beckman Coulter continually redefine the technology’s bleeding edge, upstarts have emerged with innovative technologies of their own. And not only upstarts. No less a corporate brand than Sony entered the fray this past February with the purchase of flow cytometry firm iCyt. As Richard Konz, director of the University of Massachusetts Medical School Flow Cytometry Core Facility, explains, the technology has shaken off its immunology roots to become a multidisciplinary tool. “Literally every department is using flow for something, and that for me is very exciting,” Konz says. “It’s basically never a dull moment.”

By Jeffrey M. Perkel

“But it isn’t penguins that take Ducklow so far from home. Ducklow is a marine ecologist who studies what he calls “microbial oceanography” in the waters off the West Antarctic peninsula. Basically, he and his team study the microbial food web, the bacteria, protozoa, and phytoplankton that help sustain and define the biology of the oceans. Their job: to collect samples, take a microbial census, and assess some basic biology—how many are alive, how many are motile, and how many are “grazers” that ingest other organisms, for instance.

In past years, his team has collected samples, preserved them, and shipped them back to Massachusetts for analysis, but that approach, he says, was unacceptable; preservation, shipping, and storage conspire to damage the samples and introduce artifacts. But for six weeks in January and February 2010, during the southern summer, he and his team employed a different technology.

As detailed in an abstract presented at the June 2010 meeting of the American Society for Limnology and Oceanography in Santa Fe, Ducklow and his team took a two-laser, four-color Accuri C6 flow cytometer to Antarctica for “Shipboard, near-real-time enumeration of living phytoplankton and bacteria along the West Antarctic Peninsula.”

Though others have taken flow cytometers to sea before—marine biology is something of a niche application for the devices—Ducklow’s was “the first group to have a flow cytometer that far south,” he says. The ocean simply isn’t the best environment for flow cytometry; most flow cytometers are large, temperamental, power-hungry beasts. The compact, low-power, easy-to-operate C6, Ducklow says, “has revolutionized our research.” (How compact? Weighing in at just 13.6 kg the C6, says Richard Konz, is “the size of four shoeboxes put together.”)

Flow cytometry is undergoing something of a revolution too. Once the province of immunologists bent on enumerating blood cell populations by their surface markers, the technology now is being applied across all facets of biology, from botany and stem cells to pathology and cell signaling. “It’s a phenomenal tool,” says Steve McClellan, Senior Biological Scientist in the Flow Cytometry Core Laboratories at the University of Florida. “There’s hardly anything you cannot use flow for.”

GUMMING UP THE WORKS

In flow cytometry, a flowing stream of cells is focused into single-cell, usually by injection into a fast flowing river of sheath fluid—a process called hydrodynamic focusing. (Not everyone uses that approach. EMD Millipore’s EasyCyte cytometers employ silica microcapillaries instead of sheath fluid (Millipore was acquired earlier in 2010 by Merck KGaA). And Life Technologies’ Attune system focuses cells using sound energy; by focusing the cells before they reach the sheath fluid, says Flow Cytometry Systems Business director Mike Olszowy, the system can push cells into the system faster without losing focus, speeding analyses up to 16-fold over conventional approaches.) The cells then pass one or more lasers, which interrogate the cells by exciting attached fluorophore-labeled antibodies directed against surface (or occasionally, intracellular) markers. The population is analyzed as a group of individuals, making it possible to determine, for instance, how many CD4+ T-cells also express CD8. (Cell sorters operate via the same principle, but add the ability to collect specific cell populations of interest; BD Biosciences’ new Influx can sort up to six distinct populations.)

The basic experiment is simple enough to be learned in a day. But the devil is in the details, as they say, and there’s a reason experts like McClellan and Konz have jobs. It takes skill to align and maintain lasers, calibrate the system, prepare the samples, and analyze the data. Added colors only compound complexity, cont. on p. 854»

UPCOMING FEATURES
Apoptosis—December 3
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Both in experimental design and downstream data analysis. There are, for instance, literally hundreds of different 2D scatter plots one can generate from 16-color data, says Alan Saluk, director of the flow cytometry core at the Scripps Research Institute in La Jolla.

Instrument operation also takes finesse. They can, and often do, “gum up,” says Saluk. “Anyone can run a flow cytometer once it’s set up and working perfectly. The moment it does not work is when the expertise comes in,” he says.

All of which explains why, despite the excitement low-cost, turnkey systems like the C6 generate—certainly, a $44,000 cytometer can help democratize the field—many worry about the implications of putting these systems in the hands of people who are not properly trained to use them. Thirty-two-year flow cytometry veteran J. Paul Robinson, director of the Purdue University Cytometry Laboratories, is one such individual.

“I have no problem with the smaller instruments,” Robinson explains. “What I have a problem with is the implication that you don’t need to understand anything about the technology.” At best, he says, researchers may end up disappointed with their results; at worst, by improperly manipulating their data, they could commit “unintentional [scientific] fraud.”

His advice: Seek out an expert. “If you don’t understand a field, the smartest thing to do is work with someone who does and have them make sure that the designs of your experimental protocols are correct, that the data analysis is correct, and that what you report accurately reflects the biology,” he says. “If you don’t do that, then you open yourself up to error.”

**COLORFUL CONUNDRUM**

One such expert is Stanford University School of Medicine professor Garry Nolan. Nolan, who has been doing flow cytometry for 30 years, was a graduate student with industry pioneer Len Herzenberg. He recalls using a three-color instrument that is “literally in the Smithsonian Institution.”

Today’s flow cytometers have considerably more options than Nolan and Robinson had three decades ago—anything from simple one- or two-laser turnkey systems like the C6 and Stratedigm SE100, to high-end hot rods like the BD LSRRF Fortessa, an analyzer that can be configured with up to four lasers and 16 color channels. Beckman Coulter’s MoFlo XDP can sort based on up to 7 lasers and 27 colors at 200,000 cells per second, says Nigel Llewellyn-Smith, the company’s director for Strategic Marketing for flow cytometry reagents; the soon-to-be-released Astrios cell sorter will feature up to 49 colors. “We call it the mother of all sorters,” he says.

Most analyzers, though, are more modest affairs, a reflection of the fact that few researchers use more than between four and 10 colors at once; McClellan estimates that 95 percent of his facility’s business involves four or fewer colors, and 50 percent involves just one—researchers checking transfection efficiency of GFP, for instance. That said, six-to-10-color cyometry may be the new four, says Llewellyn-Smith, who estimates that 80 percent of researchers are working in that range, and that most of those “have graduated away from two, three, or four colors.” (Beckman Coulter’s new Gallois is a 10-color analyzer; Life Technologies’ Attune can handle six.)

In contrast, maybe just one or two percent of cytometrists use 23 colors, Llewellyn-Smith says, and it isn’t even physically possible today to do much more. That limitation is imposed by fluorescent dyes’ overlapping spectral signatures. It takes serious technical skill and time to assemble an antibody panel and “get everything copacetic,” says McClellan, even one as “small” as 12 colors. “You can’t just pick 12 different antibodies with 12 different fluorophores,” he explains. “Sometimes you get weird interactions.”

The typical approach is trial and error: be cautious in matching dyes to antibodies, use the right controls, and apply downstream compensation algorithms to subtract spectral bleedthrough. Compensation and autofluorescence, says Nolan, “are the two 800-pound gorillas in any flow facility.” In high-color experiments, he adds, “You can easily spend more time on [picking] fluorophores and compensation than on data analysis.”

Recently, though, Nolan has found a way to tackle 35-parameter experiments routinely that require no such spectral compensation, and 50-plex studies are in the works.

**CyTOF TO THE RESCUE**

Nolan’s group studies intracellular signaling pathways in immune cells and immunologic diseases. To get the global view of signaling events they want, they need to track 50 or so surface markers, plus another 80 intracellular ones, at the same time and under a variety of “perturbation” conditions. Clearly, a non-fluorescent approach is called for.

Enter CyTOF.

Developed by Ontario-based DVSciences, the CyTOF, says president and CEO Scott Tanner, is a “mass cytometer,” an ultrasensitive ICP-MS (inductively coupled plasma mass spectrometer) that interrogates cells not by their fluorescence, but by their atomic composition.

Relaying as it does on a 7000-Kelvin argon plasma stream that effectively cooks input samples into their most fundamental components, ICP-MS is almost never applied to fragile biological specimens, Tanner explains. Instead, it is typically used to measure, say, the level of arsenic in water or beryllium in nuclear fuel rods.

Yet the technology could be used for flow cytometry, Tanner saw, because it is relatively trivial for mass spectrometers to distinguish elements differing by just one mass unit. The key: swap fluorophores for metals.

DVS scientists developed a way to couple antibodies to metal chelators that can bind lanthanides like gadolinium and neodymium. Because the 13 lanthanides come in 37 isotopic flavors, says Tanner, “if you have a chemistry to bind one lanthanide to an antibody, you automatically have 37 different probes.” The company has developed and will shortly launch a second method to tag antibodies with noble metals such as iridium and palladium, increasing the multiplexing possibilities to 67.

The technology is “a true quantum step,” says Nolan, who, as an enthusiastic evangelist purchased the first commercial CyTOF and
plans to purchase several more. (Nolan has recently been added as an advisor to the company, and shortly will be joining its board of directors, says Tanner.)

“Some [researchers] can occasionally, by standing on their head, on a full moon, get 17 [colors] or so. But panel development takes months to get right,” Nolan says. “So imagine suddenly doubling that number, and you can design your panel the day before and be ready to go. You can imagine the excitement.”

His team is now applying the CyTOF to acquire “an ’omics level of understanding” of how individual cells can position themselves to respond so quickly to so many disparate stimuli. “With this device we’ve doubled the number of parameters compared to what had been accomplished in 30 years with traditional flow cytometry, and there are tricks we’ve thought up that will get us way beyond 100 [parameters],” he says.

Nolan’s enthusiasm notwithstanding, the CyTOF does have limitations, writes Howard Shapiro, author of Practical Flow Cytometry, in an e-mail. “Chief among them is its relative inefficiency in data collection.” According to Tanner, only about a third of introduced cells make it to the analyzer; the rest coat the walls of the chamber like the inside of a dirty oven. “This is a substantial liability when one attempts to deal with rare cell populations,” Shapiro notes. The system also can neither size cells nor measure their granularity, parameters measured in fluorescent instruments by forward and side scatter, respectively.

On the other hand, says Tanner, the CyTOF yields cleaner data than fluorescent instruments, because it requires no compensation in highly multiplexed experiments. “Compensation fuzzes the data,” Tanner says. “Intuitively, if you don’t do that you get better separation at low signal levels.” Those experiments also are far less expensive, Nolan adds, because they require less optimization.

That said, one task the CyTOF cannot do is sort—not surprising, given that the cells are charred to a crisp during the analysis. But Nolan apparently has come up with a workaround to that problem. “Talk to me in six months,” he says. “We’ve figured out how to do it absent a Star Trek reintegrator.”

LOCATION, LOCATION, LOCATION

Paul Smith, Professor of Cancer Biology at Cardiff University, and current president of the International Society for the Advancement of Cytometry, doesn’t have a CyTOF in his lab, but he does have access to an Accuri, a MoFlo, and a whole raft of BD hardware.

Despite the different brand names and capabilities, those systems all operate via the same principle and output the same data: fluorescent intensity and light scatter. Sometimes, though, a researcher wants a more nuanced perspective.

For that, Smith’s collaborators at Swansea University turn to the Amnis ImageStream, which, as its name suggests, adds a visual dimension to flow cytometry. “We use that because it’s one of the few instruments that allows us to actually verify that what we think is really happening, is happening,” he says.

The problem with most cytometers, explains Amnis CEO David Basiji, is that their output is just numbers; researchers cannot see what they are analyzing. Like the real estate mantra, location, location, location, sometimes a protein’s physical address is more important than whether or not it’s expressed. “In a standard flow cytometer all you get is intensity, not an image,” Basiji says.

From that perspective, the ImageStream offers the best of two worlds. Like the cross between a flow cytometer and a fluorescent microscope, the ImageStream rapidly images and interrogates cells as they file past a camera. The original ImageStream 100 collected up to six images per cell at 100 cells per second (five fluorescent plus brightfield and/or darkfield images); the newer ImageStreamX (launched in 2009) processes 1,000 cells per second and, with up to five lasers and two cameras, yields up to 12 images per cell.

“What the ImageStream can do, and other flow cytometers cannot, is provide low-resolution morphologic detail in addition to the whole cell fluorescence and scatter measurements conventionally associated with cytometry,” Shapiro says. “Its adherents are therefore investigators who need to localize fluorescently labeled targets within cells.”

Users can ask questions such as, in which cellular compartment is a given protein located? Or, has a marker of interest been internalized? Jonathan Katz of the Cincinnati Children’s Research Foundation, and colleagues, recently used an ImageStream to identify “the long-sought [antigen-presenting cell] responsible for breaking peripheral tolerance to cell [antigens] in vivo,” a key event underlying type-1 diabetes. Smith and his collaborators use the ImageStream to track asymmetric cell division in stem cells using quantum dot probes.

Flow cytometry, Smith says, “is a discipline rather than an engineering technology.” Yet it is a discipline driven by engineering. And that engineering is evolving. Many see Sony’s entry into the market—Smith says a harbinger of low-cost, point-of-care cytometers-on-a-chip based on the consumer giant’s Blu-Ray technology. “Change is coming,” says iCyt CEO Gary Durack. “There will be a change in this market, and I think the users of the technology are going to benefit dramatically.”

Never a dull moment, indeed.

Jeffrey M. Perkel is a freelance writer based in Pocatello, Idaho.
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The new Influx Cell Sorting System can be configured with up to seven lasers to support two-way, four-way, and six-way sorting, giving researchers the flexibility to meet specific application or environmental requirements. The system’s modular architecture along with exchangeable detector options, hands-on controls, and sorting options make it adaptable to a wide variety of site and application needs. The Influx System uses parallel electronics to reach a throughput rate of 200,000 events per second, independent of the number of lasers or parameters. Its fluidics design features a special acoustical coupling in the nozzle assembly to reliably create droplets for sorting, while ensuring low shear stress to optimize cell viability, even at high pressures. FACS Accudrop technology simplifies setup and eliminates manual calculations normally required for drop-delay determination. To support aseptic sorting, the system’s disposable fluidics allows researchers to replace a sample line or the complete fluidics path from sheath tank to nozzle tip. In addition, the Influx software provides comprehensive control of the cell sorter from configuration and compensation setup to acquisition, sorting, and analysis. Software wizards and controls can assist researchers to classify cell populations, perform compensation, monitor sorting, and analyze results.

BD Biosciences

ANALYSIS SOFTWARE

The new flow cytometry analysis software, Kaluza 1.1, processes multicolor files of up to 10 million events in real time and offers an analytical speed that is dramatically faster than other commercially available software. With the cutting edge NVIDIA Tesla Supercomputer option, Kaluza 1.1 sets a new standard for flow cytometry data processing speed. The software supports data analysis from a variety of platforms, including MoFlo Series sorters, CyAn ADP and Gallios flow cytometry analyzers, and systems such as the iCys and the iCyte automated imaging cytometers. This version of Kaluza is the first software in the industry to be offered in a variety of translations. Kaluza Flow Cytometry Analysis Software version 1.1 is for research use only and is compatible with Windows XP, Windows Vista, and Windows 7 (32- and 64-bit) operating systems.

Beckman Coulter
For info: 800-526-3821 | www.kaluzasoftware.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information. Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by Science or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.
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Create Knockout Rats and Mice in as few as 5 months

**Learn More at Neuroscience 2010 Symposium**

**Title:** Genetically Modified Rats in Neuroscience Research – Perspectives from the Field

**Chair:** Edward Weinstein, Ph.D.

**Date:** Tuesday, November 16, 2010

**Time:** 6:30 p.m. - 9:00 p.m.

**Place:** San Diego Convention Center, Room 2

**Scientific Poster Presentations**

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<thead>
<tr>
<th>Poster No.</th>
<th>Title</th>
<th>Date and Time</th>
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<tr>
<td>147</td>
<td>Rat models to study genetic disorders of cognition and behavior</td>
<td>Nov 14, 8:00 a.m. - 12:00 p.m.</td>
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<tr>
<td>250</td>
<td>Knockout rat models for Parkinson’s disease</td>
<td>Nov 14, 1:00 - 5:00 p.m.</td>
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<tr>
<td>309</td>
<td>Knockout rat models for the study of Alzheimer’s disease</td>
<td>Nov 14, 1:00 - 5:00 p.m.</td>
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<td>536</td>
<td>Creation of neurological rat models using zinc finger nucleases</td>
<td>Nov 16, 8:00 - 11:00 a.m.</td>
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<td>766</td>
<td>Knockout rat models for schizophrenia and related disorders</td>
<td>Nov 17, 8:00 a.m. - 12:00 p.m.</td>
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<td>780</td>
<td>Knockout rat models for the study of pain and sensorial systems</td>
<td>Nov 17, 8:00 a.m. - 12:00 p.m.</td>
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