CELL-BASED Assay News
The High-Content Weekly for Drug Discovery and Functional Genomics
4/7/2006 | Volume: 3 | number 14

in this issue

Features

Placing UHT Screening System at Cincinnati Labs Enables Evotec to Plant Commercial Roots in US

The deal, which is also a much-needed windfall for the company, marks the start of an ongoing collaboration with UC and Cincinnati Children's Hospital that may eventually enable the partners to develop a state-funded drug-screening facility for multiple Ohio-based research institutes.

BD Unveils First New High-Content Analysis Tools Since '04 Atto Bioscience Acquisition

The releases also follow a recent trend among HCS vendors to offer a range of low-end and high-end platforms to address distinct emerging markets.

News Scan

Molecular Devices, Arcturus, Sanger Institute, Caliper, Zygogen, DanioLabs, Fox Chase Cancer Center, Dharmacon, Sigma-Aldrich, LI-COR

Phenotype

Columbia's Louis Cleveland on Computer Science Techniques for Automated Microscopy

Patent Watch

UNM, Senomyx, Cadus, UC, and Caliper Among Recent Recipients of US Patents

Products

Invitrogen, Molecular Devices, Beckman Coulter

Placing UHT Screening System at Cincinnati Labs Enables Evotec to Plant Commercial Roots in US

Evotec Technologies this week said that it has sold an ultra-high-throughput screening system worth $2.8 million to the Computational Medicine Center, a collaborative institute between the University of Cincinnati and the Cincinnati Children's Hospital.

For Evotec Tech, which is the tools and technologies division of German drug-discovery firm Evotec OAI, the placement marks the start of an ongoing collaboration with UC and Cincinnati Children's that would allow it to establish its first commercial base of operations in the US. In addition, the deal will enable the three partners to develop a state-funded drug-screening facility for multiple Ohio-based research institutes.

Together, these developments could help Evotec Tech improve its penetration of the US drug-screening market, both through the planned use of the Cincinnati facility as a demonstration site, and because of the strong industrial play of the academic institutions involved.

The deal is also a welcome financial windfall for Evotec Tech, which saw a significant drop in sales last year, primarily due to the introduction of new instrument platforms and the integration into its own drug-discovery platforms of new ultra-high-throughput screening technology acquired last year from Carl Zeiss (see *CBA News, 5/16/2005*).

According to Carsten Claussen, Evotec Tech's CEO, the Computational Medicine Center purchased a plate::explorer ultra-high-throughput screening system consisting of five modules, including a plate::vision multimode CCD-based plate reader and an Opera confocal high-content screening device.

"It's not just a university playground, we think."

The plate::vision and plate::explorer are two of the technologies Evotec Tech acquired from Zeiss in May 2005, and Evotec has said that one of its goals was to integrate these platforms with its own Opera technology. Claussen said that although Evotec Tech has successfully integrated Operas into the ultra-HTS platforms installed at former Zeiss customer locations, the Computational Medicine Center placement is the first totally integrated unit Evotec Tech has sold.

According to Rich Kiley, general manager of the Computational Medicine Center, Evotec's screening system and operations base will be located at the Genome Research Institute, a 300,000-square-foot facility located about 10 miles north of UC and Cincinnati Children's adjacent city campuses.

The GRI is a state-funded collaborative research effort between UC and Cincinnati Children's and houses researchers from both institutes, as well as other collaborators, Kiley said.

Ruben Papoian, director of drug discovery at GRI and research professor at UC, will oversee the screening facility. He told *CBA News* that the facility will not be completely operational until September, but that GRI has solicited research projects that are ready to be scaled up into a high-throughput format.

"We canvassed workshops mostly within UC — and we'll do it with Children's, too — where we invited researchers to present about where they see their research going toward a therapeutic end," Papoian said. "Most of them come with targets, or some molecules that have shown some drug-like activity; some need to do some late-stage validation; and some projects we chose because they are ready for high-throughput screening."

Although the Evotec platform is capable of performing both high-throughput biochemical and high-content

cell-based assays, the majority of projects presented at these workshops so far are high-content cell-based assays that need to be optimized for screening, Papoian said.

Big Deal for Evotec

The news is significant for Evotec Tech on several fronts. First, the sale, worth an estimated $2.8 million, accounts for more than 13 percent of what the unit collected in revenue for all of last year; a welcome boost considering that last week it reported a 12-percent drop in year-over-year revenues (see *CBA News, 3/31/2006*).

"What Evotec found here is a combination of academic research institutions … as well as business partners with a deep commitment towards commercial development"

Total 2005 receipts for Evotec Tech declined to €17 million ($20.8 million) from €19.3 million in 2004, but Evotec's parent last week said that it expects the Tech unit to report increased revenues this year — despite maintaining that it is no longer core to the company.

In addition, Evotec Tech may be able to parlay the UC and Cincinnati Children's deal into additional sales in the US. The first and most important step in this direction is for the Hamburg, Germany-based company to establish a US base at the CMC.

According to Claussen, Evotec Tech currently has about 10 workers in the US — mostly "salespeople and some instrument and applications people." The majority of these employees work either out of home offices, a small lab space in Woburn, Mass., or, in some cases, at customer sites.

"Now, as part of this deal, we will send people from Hamburg to Cincinnati to start operations there, and we also will hire some people to work there," Claussen said. These workers will be on the parent company's payroll, but will be supervised by GRI's Papoian.

"Then we will have instrument demonstrations and also some stock there in Cincinnati, in the middle of the US," Claussen added. "So it will be a showcase for potential customers."

Lastly, Evotec may be able to drum up platform sales specifically through its new connections at the University of Cincinnati and Cincinnati Children's Hospital. One of the reasons Evotec Tech wanted to work with these institutions is their strong top-to-bottom play in academic drug discovery, as well as their strong ties to the pharmaceutical industry.

"What's really important is their focus on translational medicine, from the Genome Research Institute all the way to the patients at the Children's Hospital," Claussen said. "They are really good at target discovery, with animal models, and in patient studies, and underneath that, they will do screening at our drug-discovery center. So they should really be a complete unit with all these modules to hopefully discover more and better drugs. It's not just a university playground, we think."

Furthermore, UC, Children's, CMC, and GRI together have a strong interest in commercial development.

"What Evotec found here is a combination of academic research institutions in Children's and UC, as well as business partners with a deep commitment towards commercial development," CMC's Kiley told *CBA News*. "The founding principle of the Genome Research Institute was really around translational medicine, and how you can accelerate discoveries taking place now into actual therapeutics."

Along with that philosophy comes a great deal of pharmaceutical industry experience within the institutes. For example, Jane Henney, senior vice president and provost for health affairs at UC's Academic Health Center, was commissioner of food and drugs at the US Food and Drug Administration from 1998 to 2001,

and is currently an AstraZeneca director.

George Thomas, interim director of the GRI and genome science department at UC, is on the scientific advisory board of Novartis Oncology; and GRI and UC's Papoian has more than 20 years of pharmaceutical experience with companies such as Novartis and Serono.

In addition, GRI has an ongoing collaboration with Cincinnati-based Procter & Gamble Pharmaceuticals, and the center is geographically very close to current Evotec customer Eli Lilly, which is based in Indianapolis.

Finally, once the screening center at the GRI is up and running, there is a large possibility that it will become a core-type screening facility for other academic entities in the state and region, because the Ohio Third Frontier, a state-run economic development group, funds the CMC, which in turn negotiated the deal with Evotec.

"The Third Frontier program is investing $1.5 billion in accelerating the growth of technology companies in Ohio, and the majority of that is focused on life sciences areas," CMC's Kiley said. "The GRI itself received $10 million when it started up; the Computational Medicine Center, which I manage, received $28 million from this program; and we plan to seek additional funding with the idea that having high-throughput screening capabilities and compound libraries is an extraordinarily expensive asset."

Kiley cited Evotec's collaboration model in Europe, in which it does a great deal of preliminary research with academic institutions, and then "funnels" the final targets and assays to its central screening facility in Germany.

"We think the same model will emerge here, and we think it will be a real benefit to other institutions here like Ohio State, Case Western Reserve, and the Cleveland Clinic, to really have access nearby to that kind of expertise, which is unusual," he added.

In fact, according to Papoian, many of the initial projects for the screening facility were chosen in part because of their translational promise into new drugs.

"The first projects we chose would fall into the area of CNS diseases — some migraine-type targets, and some for opiate tolerance; as well as some metabolic disease stuff," Papoian said.

"We picked those because they fell into different phases, so there wouldn't be a bottleneck," he added. "But we also picked those because they were therapeutic areas where we already had interest from commercial partners in pharma — that they would probably be interested in those projects once we brought them a little further along."

— *Ben Butkus* (bbutkus@genomeweb.com)

BD Unveils First New High-Content Analysis Tools Since '04 Atto Bioscience Acquisition

BD Biosciences this week launched a number of new products for high-content analysis, including two distinct lower-end versions of its Pathway HT automated imaging system, and a set of antibody reagents that have been validated specifically for use with high-content imaging systems.

This is the first new product launch from BD Biosciences' high-content cellular-imaging business since it acquired Atto Bioscience, which originally developed the Pathway, nearly two years ago.

The launches also follow a recent trend among HCS vendors such as Cellomics and Molecular Devices to offer a range of low-end and high-end platforms to address distinct emerging markets.

"From a market perspective, this demonstrates BD's commitment to the HCS market," Phil Vanek, director of marketing for bioimaging systems at BD Biosciences, told *CBA News* this week.

The products were unveiled simultaneously at the American Association for Cancer Research meeting in Washington, DC, and the Federation of American Societies for Experimental Biology meeting in San Francisco, BD Biosciences said.

The new instruments are the BD Pathway 435 and 415, lower-end versions of the Pathway HT, which BD Biosciences has also recently renamed the Pathway 855. BD Biosciences put the new numerical labeling systems in place to better designate the different tiers of current and future instruments.

"From a market perspective, this demonstrates BD's commitment to the HCS market."

"The goal is to indicate that any of our high-end, kinetic live-cell imagers will be in the 800 series, while the 400 series will be more of an endpoint instrument — not really entry-level, but a comparable instrument to what you would find from the other endpoint instruments on the market," said Vanek.

Specifically, the Pathway 435 is a confocal bench-top imager that essentially employs the same optics as the Pathway 855. The major difference is that the 435 is a compact standalone imager meant for the bench top, while the 855 is decked out fully for large-scale screening campaigns, complete with liquid handling, environmental control, and advanced image-analysis software.

BD describes the Pathway 415 as a cost-effective alternative to the Pathway 435. It does not feature confocal imaging capabilities, but can be upgraded to confocal, Vanek said.

Both systems also provide a streamlined software interface, Vanek said, called application wizards, "so people can get in and onto the instrument and start working right away."

BD did not disclose how much the instruments would cost.

Emerging Markets

BD launched the new instruments primarily in response to market demand, according to Vanek.

"We still see that 75 or 80 percent of the market is interested in looking at endpoint applications," Vanek said. "I think the reason for that is the data is not as intense, the equipment is not as expensive, and the requisite understanding of the biology — it's not information overload. So there is a lot of value to having more simplified, more streamlined access into automated imaging."

This follows a recent trend in which HCS vendors have found a need to market both a higher-end system, usually for kinetic live-cell imaging, and usually incorporating confocal or confocal-like optics; and a lower-end system typically used for fixed cell endpoint assays. These often, but not always, use CCD camera-based imaging.

Molecular Devices most recently launched a line of low- to high-end imaging products, while Fisher's Cellomics unit also expanded its options with agreements with Applied Precision and Evotec Technologies.

"We still see that 75 or 80 percent of the market is interested in looking at endpoint applications."

This range of instruments is meant to satisfy distinct market segments that have recently developed, although it is still difficult to label those segments. It seems natural to identify them as pharmaceutical and biotech on one side, and academic and government labs on the other. However, if there is a line here, it continues to be blurred, as one need only look at examples such as the NIH's recent establishment of the Molecular Libraries Screening Centers Network, and Evotec's multimillion-dollar partnership with the University of Cincinnati and Cincinnati Children's Hospital, announced just this week [*see accompanying story, this issue*].

Last June, Dietrich Ruehlmann, product manager for imaging and analysis at BD Biosciences, told *CBA News* during a roundtable discussion that BD "[doesn't] differentiate any more based on where the customer is; we differentiate based on what the customer is trying to do." (See *CBA News, 6/27/2005*).

Vanek this week reiterated his colleague's statement, and said that BD Biosciences wouldn't necessarily market the Pathway 855 primarily to pharma and biotech customers.

"I don't think we really differentiate as much by where they are," Vanek said. "The [Pathway 855] is geared towards people who are doing a bit more sophisticated bioimaging — those needing kinetic capability, confocal, and environmental control all wrapped up in one instrument."

He added that the 855 still offers a certain level of flexibility in that "you're not nailed down or pushed into pre-set algorithms or assays, and you have a lot of flexibility to sort of go off the beaten path and explore new biology," something that certainly applies in academic research.

BD Biosciences also introduced a set of a "few hundred" antibody reagents that have been validated for use with the Pathway imaging systems.

"BD has a long history in supporting cell analysis research … and [has] always had a large collection of mostly monoclonal, as well as some polyclonal antibodies that were available for Western blotting, or in some cases immunohistochemistry; but mostly for flow cytometry," Vanek said. "The challenge was that those reagents had never been adequately verified for fluorescence imaging applications, and the market was demanding verified reagents."

To this end, BD Biosciences took a portfolio of several hundred of its antibody reagents and ran screening experiments with them using a Pathway 855 "to actually make sure that under two fixation and permeabilization methods, and in three different cell lines, we get adequate labeling," Vanek said.

"Based on those criteria, if an antibody makes it through our applications challenge, then we will put it into the special category of imaging certified," he added, which is what the new products are. Vanek said that BD also continues to develop new antibodies that may have application in imaging.

— *Ben Butkus* (bbutkus@genomeweb.com)

News Scan

Molecular Devices Buys Arcturus' Laser Capture Microdissection Business for $10M

Molecular Devices has acquired the laser capture microdissection business of Arcturus Biosicence for $10 million in cash, the company said this week.

Under the terms of the agreement, Molecular Devices acquired Arcturus' LCM-related assets and hired 42 Arcturus employees. LCM operations will immediately move to Molecular Devices' headquarters in Sunnyvale, Calif. The company expects that the acquisition will increase its revenues by $8 million to $10 million this year.

"We believe that offering LCM systems with our existing genomics and cellular imaging products is a natural fit for our life sciences research customers as LCM is typically an upstream step for genomic or imaging analysis," said Molecular Devices CEO Joseph Keegan in a company statement. "We also believe that we can improve the distribution of the LCM platform by leveraging our worldwide distribution infrastructure."

Arcturus has more than 1,000 LCM systems installed worldwide. The company's LCM platform includes reagents for sample preparation, instrumentation and consumables for visualization and excision, and additional reagents for post-capture processing.

Sanger Institute Buys a Caliper LabChip Unit for Protein Expression Project QC

The Wellcome Trust Sanger Institute has purchased a Caliper LabChip 90 Automated Electrophoresis System for quality control of DNA and proteins in Sanger's Atlas of Protein Expression project, Caliper said this week.

Specifically, the Sanger Institute will use the LabChip 90 system to generate quantitative sizing, concentration, and purity data for PCR products, expressed proteins, and antibodies used in immunohistochemistry experiments, Caliper said.

Financial details were not disclosed.

The goal of the Atlas of Protein Expression project is to systematically study protein expression in cells and tissues to create a database of protein-expression profiles that will be accessible to researchers worldwide.

Zygogen Licenses Transgenic Zebrafish Tech to DanioLabs for Drug Discovery

Zygogen has issued a non-exclusive license to DanioLabs for Zygogen's Z-Tag transgenic zebrafish technology, the companies said this week.

Under the terms of the agreement, DanioLabs will use the technology for internal drug screening purposes. The agreement calls for Zygogen to receive license and royalty payments from DanioLabs.

Additional financial details were not disclosed.

The Z-Tag technology combines the zebrafish's transparent organogenesis with physiologically relevant expression of modified genes and markers to allow the development of quantitative assays for automated compound screening.

Zygogen uses Z-Tag to provide in vivo preclinical drug-discovery services, including target validation, compound screening, and toxicity testing, and is the exclusive licensee of the technology.

Fox Chase Cancer Center Joins Dharmacon's Global RNAi Alliance

Dharmacon, a unit of Fisher Biosciences, said this week that Philadelphia's Fox Chase Cancer Center has joined its Genome-Wide RNAi Global Initiative.

Fox Chase becomes the 11th member of the alliance, and the first new member since the initiative was formed in October.

The alliance aims to use Dharmacon's complete siRNA library to conduct functional genomics screens of genes throughout the human genome.

Sigma-Aldrich Acquires Chinese Distributor, Expands in Shanghai

Sigma-Aldrich has increased its Shanghai operations and acquired Beijing Superior Chemicals and Instruments, the company said this week

The acquisition of Beijing Superior — Sigma's main distributor in China with locations in Shanghai, Beijing, and Guangzhou — will increase the number of Sigma employees in China almost 10-fold, to 87. Since 75 percent of Beijing Superior's $22 million in annual revenues result from Sigma products, the acquisition will not immediately impact Sigma's sales in China.

Sigma said it has also established a Wholly Foreign Owned Enterprise, called Sigma-Aldrich (Shanghai) Trading Company; expanded its administrative offices in Shanghai; and opened a leased transit warehouse in Shanghai.

LI-COR to Donate Odyssey Infrared Imaging System

LI-COR Biosciences will donate a complete Odyssey infrared imaging system to a public or private research institution based on a random drawing, the company said this week.

The system will include the company's new Odyssey MousePod small animal-imaging accessory, Odyssey application and analysis software, and installation. Registration for the drawing is open from April 1 to May 30 to entrants in the United States and Canada only, LI-COR said.

The MousePod accessory fits over the standard Odyssey scanning surface for in vivo imaging of up to three mice, according to the company. MousePod features temperature regulation and connection to any gas anesthesia system.

LI-COR's IRDye reagents for in vivo imaging include infrared dyes for labeling probes, an IRDye 800 CW labeling kit, and the new IRDye 800CW EGF Optical Probe.

Researchers may submit an entry via the company's website, at LI-COR exhibitions during April and May, or by mail, the company said. A winner will be selected by random drawing on June 1, with the winner announced by June 15 on LI-COR's website.

Columbia's Louis Cleveland on Computer Science Techniques for Automated Microscopy

Louis Cleveland
Research scientist, department of medicine
Columbia University

At A Glance

Name: Louis Cleveland

Position: Research scientist, department of medicine, Columbia University; director, Laboratory for Molecular Mechanisms in Human Diseases, St. Luke's-Roosevelt Hospital Center

Background: Various staff, then faculty positions in departments of microbiology and medicine, Columbia University, 1980-present.

Education: PhD, chemistry, Rutgers University, 1974; Postdoc, Immunology, Columbia University, 1975-1979.

Throughout his career, Louis Cleveland has been interested in immunology and the molecular underpinnings of diseases ranging from chronic lymphocytic leukemia to schizophrenia. More recently, Cleveland and colleagues at Columbia University have taken an interest in using computer science techniques to automate biological imaging as a means to facilitate their molecular biology research. The researchers published a paper on their work in the April 2006 issue of Computers in Biology and Medicine [2006 Apr; 36(4): 339-62], on which Cleveland was corresponding author. *CBA News* caught up with him this week to discuss his work.

This paper was just published in April, but your group submitted it in September 2004. How long has this project been going on?

It's been in the works for quite a while. In 1997, I had funding from the National Cancer Institute to develop an immunotherapeutic vaccine for chronic lymphocytic leukemia. During the course of that project, I made a manual device for capturing cells and monitoring gene expression. That gave me a tangible sense of what the possibilities would be if we were to build an automated system. Since that time, it has been my goal to build a robotic system that would facilitate the monitoring of gene expression at the single-cell level in viable cells. It was difficult to get funding for this highly interdisciplinary project. However, a proposal submitted to the National Cancer Institute in collaboration with Larry Yao was eventually successful.

From the beginning, it has been my intention to build a system that is as automated as possible. Ideally, one would like to replace the human operator with computer vision and other types of algorithms. A critical first step towards this goal is the development of an algorithm that can recognize cells in microscope images of cultures. In our grant proposal, we initially planned to use traditional image-analysis algorithms that would recognize fluorescent-stained nuclei. We also planned to have a human operator review the algorithm's results because we weren't confident that they'd be all that accurate. Only after human review would the image-derived information be used to control the hardware.

Soon after our grant application was funded, my collaborator, Larry Yao, and I were fortunate to recruit a graduate student, Xi Long, who had a strong background in computer vision. As discussions evolved, I decided to do something much more ambitious than we originally planned in our grant proposal. Essentially, I asked Xi to explore the use of statistical learning machines for cell recognition and localization. These have

two major advantages. First, the end user simply trains the machine with pre-classified samples. Therefore, there is no need for an end user to struggle with the esoterica of traditional image analysis. Second, there is a high degree of flexibility. To work with a different cell type, one simply retrains the machine. This is in contrast with traditional image analysis approaches, where a detailed optimization must be done for each cell type.

I also asked Xi to try to avoid the use of fluorescent staining. This was important, because we're interested in studying gene expression with fluorescent probes. With green fluorescent fusion proteins, for example, one can monitor gene expression in real time with a fluorescence microscope. In many experiments, one needs to monitor multiple biologically relevant parameters with fluorescent probes. Unfortunately, in most microscopes, there are typically six colors that can be monitored simultaneously (or eight sequentially). It is therefore highly desirable to use the limited number of fluorescence channels to study something like gene expression rather than to use them just for cell identification. Initially, Xi Long used an artificial neural network with a novel preprocessing algorithm. The results exceeded any expectations I had at the beginning. Accuracy was in the low 90-percent range. Even cells in clumps could be recognized. With regard to accuracy, this algorithm is quite adequate for many practical applications. However, artificial neural networks are not ideal for biologists as end users, since they can be difficult and time-consuming to optimize. To get around this problem, Xi Long explored the use of support vector machines, which have the advantage of being much easier to optimize than artificial neural networks.

In study with support vector machines, I asked Xi to reach for an even more ambitious goal, namely the discrimination of unstained viable and nonviable cells in images obtained with brightfield microscopy. To get a practical level of accuracy, it was necessary to develop a new strategy for training support vector machines, which we refer to as CISS, or compensatory iterative sample selection. With this strategy, the results again greatly exceeded expectations. Accuracy was in the low 90-percent range. At this point, we believe that we have an algorithm that is both robust and practical for end users.

Prior to this, were there other methods to detect and count viable cells besides fluorescence or manually counting?

The time-honored method for determining cell viability with a microscope is to use a stain such as nigrosine or trypan blue. As someone with decades of experience observing cells with an ordinary tissue-culture brightfield microscope, I can generally determine if an unstained cell is dead or alive. However, even with extensive experience, a human observer is not sufficiently reliable for rigorous measurements. For example, when I add nigrosine to a culture, I usually find surprises — some cells that looked dead before adding the stain may exclude dye, indicating their viability. Similarly, there may be some cells that take up dye even though they looked viable before adding the stain. Accordingly, published results on cell viability are virtually always dependent on use of a stain.

What do the terms 'support vector machine' and 'CISS' mean?

Starting in the late 1970's, Vapnik began development of the so-called support vector machine, or SVM. SVMs are statistical learning machines that use supervised learning, meaning they are trained with a training set that is prepared by an independent means. SVMs classify data samples that are represented as vectors. A vector is a mathematical quantity that is simply an ordered set of numbers. A familiar example can be found in high school algebra where vectors are represented as the xy-coordinates in a Cartesian plane. With support vector machines, the vector spaces are abstract, and they can have large, even infinite, numbers of dimensions.

In the case of an SVM — let's say you're trying to solve a binary classification problem, where you have two classes: what you want, and everything else. There is a decision function that separates these two classes in a way that maximizes the margin. The graph of this function in the abstract vector space is a decision surface. The vectors that are closest to the decision surface are called support vectors. SVM technology has evolved

over quite some time. It is now very sophisticated and is being used in diverse applications, ranging from face recognition to fraud detection. It's a very general technology.

So how have you applied these to cell detection and identification?

In our case, we take images of cultured cells, and generate vectors with what we call pixel patch decomposition. You take a typical image — say 640 by 480 pixels — and move a small rectangle across the image, covering all possible positions. The size of the rectangle is chosen so that it fits around the largest cell that we're dealing with — say 25 by 25 pixels. For each position of the rectangle, we get a vector consisting of the 625 pixel values in that rectangle. We're interested in pixel patches that have a centered cell. If we know that the cell is centered, then we know where the cell is in the image, since we know the position of the center of the rectangle. With this strategy, the classifier is taught to recognize pixel patches that contain a centered cell.

The pixel patch decomposition technique generates a large number of vectors, about 290,000 in the above example. On the other hand, the number of pixel patches with centered cells might be 100 or so. Essentially, we have a needle-in-a-haystack problem because a large fraction of the pixel patches you get are not what you're looking for. The first step in solving this problem is to reduce the dimensionality of the vectors. It turns out that you don't need 625 dimensions to capture the complexity of the objects being classified. To reduce dimensionality, we use a standard technique — principal component analysis — which reduces the dimensionality to a much lower number, typically 10. The support vector machine is then trained to classify the set of vectors having reduced dimensionality. Fortunately, this is a tractable problem for existing PC hardware.

To prepare a training set, the vectors are divided into two classes: viable cells and everything else — needles and the rest of the haystack. One problem that has kept this technology from being used is that the training set is much larger than you can possibly handle with existing computer hardware. Consequently, you end up randomly choosing a portion of the training set. The problem is that the chosen set may not be a representative sample. This is especially a problem for the large "haystack" set.

To solve the above problem, we developed the CISS technique. In this technique, you iteratively correct the set chosen for training. If a sample not in the initial training set is misclassified in a test experiment, you use it to replace correctly classified samples in the initial training set and repeat the training procedure. With this procedure we have gotten convergence to quite high accuracy. The CISS procedure is an important contribution from our perspective, since it facilitates the use of SVMs with the highly unbalanced training sets generated with pixel patch decomposition. I think it will be useful for anybody using an SVM with a needle-in-a-haystack problem.

In the paper, you said this method outperformed several commonly used methods. Can you talk about this?

What we said is a little bit misleading, because it implies there are other techniques out there that people are using to do the same thing. We should have said more clearly that, within our own studies, we've compared an SVM plus CISS to an SVM without CISS, and to an artificial neural network. We found CISS to be essential for success when using an SVM. To our knowledge, statistical learning machines have not previously been used to distinguish between unstained viable and nonviable cells.

Can this method be applied to automated fluorescence microscopy, as well, like what is used in high-content screening?

Absolutely. It's precisely what we intend it for. My laboratory is focused on human diseases, and I'm interested in things like schizophrenia and chronic lymphocytic leukemia. We are developing this technology to facilitate molecular studies on cells from people who have these illnesses. Back in 1997, we found that

manual devices were absolutely hopeless. The development of an automatic machine has been a major goal since that time. What I envision is not just a robotic microscopy system with some automatic features. Rather, the key word is 'autonomous.' Once you press the start button, the system should run on its own for quite some time before a human operator is needed. Given the extraordinary discriminative power of SVMs, I think we're on the verge of a new era of autonomous systems that will involve fluorescence microscopy and other microscope techniques. A human operator looking into the microscope or even at a computer screen will not be needed in these systems.

To develop an autonomous system based on microscopy, several problems need to be solved. One of the hardest problems is cell identification without the use of stains. Once the needles in the haystack — or cells — are found, we want to use pattern recognition algorithms to recognize sub-cellular features of biological relevance, especially gene expression in real time. If you're doing complex time-lapse experiments, it's possible the machine would benefit from being able to make decisions, so we see decision-making algorithms as being an integral part of autonomous machines. It's not as if this device is extremely futuristic — I see it as a companion development to the autonomous vehicles that recently won the DARPA-sponsored competition. The basic building blocks needed for autonomous systems are now available.

Is there an interest in commercializing this?

Yes, all of what we do is ultimately protected through patent applications, and the Columbia University Science and Technology Ventures office is handling the commercialization. Although we now have NCI funding, the level of funding is only appropriate for proof-of-concept work. At a certain point, bringing this technology to practical devices that can be put in the hands of researchers is going to require industrial partners. Up until recently, it's been premature to pursue that, but we're at a point now where we're poised to move in that direction. We certainly see our device as being valuable in multiple applications: drug discovery, clinical diagnostics, manufacturing of protein therapeutics, and as a generic tool for cell biology research.

Patent Watch

The Science and Technology Corporation at the University of New Mexico has been awarded **US Patent No. 7,018,846**, "Display of receptors and analysis of binding interactions and drug libraries."

Inventors listed on the patent are **Larry Sklar, Eric Prossnitz, Janeen Vilven,** and **Donna Neldon**.

According to its abstract, the patent protects a display and method of preparing 7-transmembrane and other receptors for real-time kinetic analysis of binding interactions. The invention includes display on beads and in micelles for multi-well and flow cytometric analysis. The invention is useful for ligand discovery and drug action discovery, and G-protein response in particular, the abstract states.

Senomyx has been awarded **US Patent No. 7,022,488**, "Functional coupling of T1Rs and T2Rs by Gi proteins, and cell-based assays for the identification of T1R and T2R modulators."

Inventors listed on the patent are **Guy Servant, Mark Ozeck, Paul Brust,** and **Hong Xu**.

According to its abstract, the patent protects cell-based assay methods for identifying compounds that modulate the activity of specific T1R or T2R taste receptors or which modulate the effect of other T1R or T2R modulators on T1R or T2R activity. These assay methods preferably detect the effect of a putative T1R or T2R modulator compound on MAPK activation, cAMP accumulation, or adenylyl cyclase activity or another signaling pathway regulated by Gi proteins. The level of MAPK activation, cAMP accumulation, or adenylyl cyclase is preferably determined by immunoassay methods that use ligands (monoclonal or polyclonal antibodies) that specifically bind an activated (phosphorylated) MAPK, cAMP, or adenylyl cyclase, the abstract states.

Cadus Technologies has been awarded **US Patent No. 7,022,513**, "Cell-based signal generation."

Inventors listed on the patent are **Jun Xu** and **Joshua Trueheart**.

According to its abstract, the patent protects a rapid, reproducible, robust assay system for screening and identifying pharmaceutically effective compounds that specifically interact with and modulate the activity of a cellular protein, e.g., a receptor or ion channel. The assay enables rapid screening of large numbers of compounds to identify those which act as an agonist or antagonist to the bioactivity of the cellular protein. In particular, the assay makes use of a cell that harbors a protein that is responsive to a cellular signal transduction pathway. The protein is operatively linked to a polypeptide which causes a detectable signal to be generated upon stimulation of the pathway, e.g., when a compound interacts with and modulates the activity of a cellular receptor or ion channel of the cell. Thus, the cell provides a signal generation means comprising a novel fusion protein, the expression of which is independent of stimulation/activation of the signal transduction pathway, but the activity of which is responsive to the signal transduction pathway, the abstract states.

The **University of California** has been awarded **US Patent No. 7,022,826**, "Non-oligomerizing fluorescent proteins."

Inventors listed on the patent are **Roger Tsien, David Zacharias,** and **Geoffrey Baird**.

According to its abstract, the patent protects a non-oligomerizing fluorescent protein, which is derived from a fluorescent protein having at least one mutation that reduces or eliminates the ability of the protein to oligomerize. The non-oligomerizing fluorescent protein can be derived from a naturally occurring green fluorescent protein, red fluorescent protein, or other fluorescent protein related thereto. The patent also protects a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. In addition, the patent discloses a polynucleotide encoding a non-oligomerizing fluorescent protein, and a recombinant nucleic acid molecule, which includes a polynucleotide encoding a non-oligomerizing fluorescent protein operatively linked to at least a second polynucleotide. Vectors and host cells containing such polynucleotides also are provided, as are kits containing one or more non-oligomerizing fluorescent proteins or encoding polynucleotides or constructs derived therefrom. The patent also provides methods of making and using the proteins and polynucleotides, the abstract states.

Caliper Life Sciences has been awarded **US Patent No. 7,024,281**, "Software for the controlled sampling of arrayed materials."

Garrett Unno is the lone inventor listed on the patent.

According to its abstract, the patent protects computer-implemented methods for contacting or sampling materials in arrays having x materials sites with microfluidic devices having n capillary elements. Devices, integrated systems, and computer program products for performing these methods are also provided, the abstract states.

Products

Invitrogen this week launched **Omnia Kinase Assays** at the American Association for Cancer Research meeting in Washington, DC.

Omnia Kinase Assays use a fluorescent peptide substrate-based technology for rapid, homogenous, and sensitive detection of enzymatic activity under physiological ATP concentrations, Invitrogen said. By mixing reagents, the company said, researchers are able to read kinetic or real-time fluorescence.

Molecular Devices this week introduced the **SpectraMax M5e** multi-detection microplate reader, an extension of its SpectraMax M5 system.

SpectraMax M5e is a monochromator-based reader developed in collaboration with **Cisbio International,** and is certified to run Cisbio's **HTRF assays**. Users can choose any wavelength between 250 and 850 nanometers, and can select the best-suited parameters for their assays, MDCC said. Like the SpectraMax M5 reader, the M5e has primary applications for fluorescence, time-resolved fluorescence, fluorescence polarization, absorbance, and luminescence assays. MDCC said that endpoint, kinetic, spectrum, multi-wavelength, and well-area scanning reading methods can be used to run assays including cell viability and proliferation, kinase, reporter gene, ELISA, nucleic acid and protein quantification, and enzymatic. The new instrument uses MDCC's SoftMax Pro GxP software.

Beckman Coulter this week launched **IOTest CD85d-(ILT4)-PE conjugated monoclonal antibodies**.

ILT4 is selectively expressed on myelomonocytic cells and binds to classical and non-classical human leucocyte antigen class I molecules, Beckman said. It is believed to play a number of roles in cellular function, including the modulation of APC functions, control of inflammatory response, and maternal tolerance against the fetal semi-allograft, Beckman said.

The new antibody is useful in basic research on leucocyte function, dendritic cells, immunosuppression, tolerogenicity, and maternofetal tolerance, Beckman said.

Cell-Based Assay News

Ben Butkus, Editor
bbutkus@genomeweb.com

Julia Karow, DPhil
Contributing Editor
jkarow@genomeweb.com

Bernadette Toner
Editorial Director
btoner@genomeweb.com

Kirell Lakhman
News Editor
klakhman@genomeweb.com

Elena Coronado
Senior Production Designer
ecoronado@genomeweb.com

SUBSCRIPTIONS

ISSN 1549-9766

Cell-based Assay News is published weekly (50 times annually) by GenomeWeb LLC
Annual subscription rate: $985

Allan Nixon
Director of Subscription Sales
+1.212.651.5623
anixon@genomeweb.com

ADVERTISING

Judy Block
Associate Publisher
+1.212.651.5629
jblock@genomeweb.com

REPRINTS AND WEB RIGHTS

+1.212.651.5618
reprints@genomeweb.com

GENOMEWEB LLC
125 Maiden Lane
Floor 2
New York NY 10038
Phone +1.212.269.4747
Fax +1.212.269.3686
www.GenomeWeb.com

Dennis P Waters PhD
Chairman and Publisher