By Thomas Ricci

"Small" Buzz at Pittcon Leads to Some Very Large Scientific Achievements

There's been a very small buzz going on at Pittcon since the late 1990's and it seems to be growing smaller by the day. The buzz is being propagated by several academic leaders whose pioneering research in nanotechnology, lab-on-a-chip, microfluidics and miniaturized instruments have led to commercial products that today are solving some very large scientific problems.

Just a few years ago, Professors Chad Mirkin, Richard Mathies and Graham Cooks presented their early research in these innovative technologies at Pittcon and shared their vision on how these discoveries could be applied in the life science, diagnostic, semiconductor and other areas. At Pittcon 2009, conferees will see how these visions have been realized by start-up scientific companies who have developed commercial products that incorporate these new inventions to solve real-world problems.

This article will provide a brief profile of four of these emerging companies.

Nanotechnology

Nanotechnology has been heralded by many as "the next industrial revolution" and has the potential to provide solutions in just about every industry imaginable, from healthcare to the environment. Many of these early ideas and more recent commercial successes have been well represented at Pittcon since the year 2000. Dr. Chad Mirkin (Figure 1), the George B. Rathmann Professor of Chemistry at Northwestern University, started nanotechnology research in the mid-nineties and frequently presented his group's advances in the area at Pittcon. In 1999, he co-authored a paper which introduced their discovery of Dip-Pen Nanolithography (DPN™) (1) and in 2000, he presented a paper at Pittcon on

Figure 1 *Dr. Chad Mirkin, Northwestern University*

Emerging Nanotechnologies for Chemical Analysis in which he described a nanoscale technique for DNA sequencing and published several papers (2,3).

In 1995, Dr. Mirkin was named director of the International Institute for Nanotechnology which currently includes more than 40 faculty members and 400 students from around the world. Since inception of the institute, 15 companies have been commercialized based on technologies licensed from the Institute and more are sure to come. Dr. Mirkin personally co-founded two of those companies - Nanosphere, Inc. and NanoInk, Inc.

Nanosphere, Inc.

Dr. Mirkin describes Nanosphere, Inc. (Northbrook, IL) as the "biggest commercial success yet in nan-

otechnology." Dr. Mirkin and Northwestern Professor of Chemistry, Dr. Robert Letsinger co-founded Nanosphere in 2000 based upon discoveries made in their Northwestern University chemistry lab. These discoveries made possible the consistent manufacturing and functionalization of gold nanoparticles with oligonucleotides (DNA or RNA), or antibodies that can be used in diagnostic applications to detect nucleic acid or protein targets, respectively.

Since its founding, Nanosphere has made continuous enhancements to the original technology advances by coupling the gold nanoparticle chemistry and capabilities with multiplex array analysis, microfluidics, human engineering and software development to produce a full-solution, diagnostics workstation, called the Verigene® System. The system leverages the company's proprietary nanotechnology to move genetic and protein testing closer to the point of care, thereby overcoming the time-and cost-intensive limitations of conventional polymerase chain reaction tests. Nanosphere received FDA approval for use of the Verigene system in November 2007 for its first test to determine an individual's ability to metabolize the anti-coagulant medication warfarin, information critical to determining safe and appropriate dosing.

"The Pittcon community is not just interested in science, but how the science is transformed into useful technology."

Dr. Chad Mirkin

"In addition to saving costs and speeding diagnostics, the Verigene System greatly simplifies molecular diagnostic testing and makes it accessible to virtually any clinical laboratory," said Bill Moffitt, CEO of Nanosphere, Inc. "The localized testing also can greatly enhance physician decision making and brings complex molecular testing directly into the mainstream clinical process."

The company closed its Initial Public Offering in November 2007 and is now a fully-integrated healthcare company with established cGMP manufacturing operations. They currently have a number of new Verigene tests in the pipeline for genetic diagnostics, pharmacogenetics, and identification of infectious diseases. Mr. Moffitt sees a variety of potential future applications in ultra-sensitive protein diagnostics, research applications, biosecurity, food and animal testing and industrial sensors.

NanoInk, Inc.

Dr. Mirkin founded NanoInk (Skokie, IL) in 2001 to exploit the commercial opportunities presented by a new technique discovered in his lab called Dip-Pen Nanolithography (DPN™). The DPN (patent-pending) process enables the building of nano-scale structures and patterns by literally drawing molecules onto a substrate. Structures less than 20 nm may be built using virtually any material.

Dr. Mirkin described the DPN™ process as "one of the most versatile nanolithographies currently available, allowing one to construct nanostructures not possible via any other technique. It's going to open up a wealth of scientific capabilities and is beginning to enable major technological advances in the life science, pharmaceutical and semiconductor sectors." (4)

NanoInk obtained an exclusive license for DPN technology from Northwestern University in 2001 and secured its first round of venture-backed funding in April of 2002. The company launched its first DPN-based product shortly thereafter. Today, the company's NSCRYPTOR™ technology is used as an enabler for the rapid creation of materials and nanoscale structures in biological research, materials science, device research and nanomanufacturing.

With an installed base of more than 60 instruments worldwide, NanoInk CEO Jim Hussey sees a bright future for NSCRYPTOR technology. "Our early adopters were from leading university and government research and we're now seeing a lot of interest in DPN technology from industry as well," he said

The company's Nanoencryption technology is used to place nano-scale codes on virtually any material, including pharmaceutical capsules and tablets, to protect brands with covert, conclusive authentication

to fight counterfitting and other illegal diversion of products. Dr. Hussey noted that counterfitting issues are growing in the pharmaceutical and biotech areas and the company received FDA approval for their nanoencryption system in May of 2008.

NanoInk plans to introduce two new systems at Pittcon 2009: the DPN-5000 which incorporates new advancements in NSCRYPTOR technology which will enhance application speed and the NLP-2000 which is being launched specifically for creating nanostructures for biological applications. "Biochemists are typically dealing with extremely sensitive biomolecules and the NLP-2000 is an extremely easy to use instrument which can deliver nanoscale sized deposits of biomolecules over 40 mm x 40 mm areas," Mr. Hussey noted.

Dr. Mirkin has been selected by the Society for Analytical Chemists of Pittsburgh to receive the 2009 Pittsburgh Analytical Chemistry Award which will be presented at Pittcon 2009, where he will present new innovations in nanopatterning technology and nanoparticle-based molecular diagnostics. "The Pittcon community is not just interested in science, but how the science is transformed into useful technology. It is not unusual to hear interesting scientific research in the technical program that will ultimately appear as a major technological capability or instrument on the exposition floor later," he said.

Microfluidics

Research in microfluidics appeared at Pittcon in the early 1990's and new advances in the technology have been presented every year. The technology deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, micron length and nanoliter volume scale. Microfluidic structures include micropneumatic systems, i.e. liquid pumps, valves, mixers and routers for the manipulation and preparation of fluidic samples and microfluidic structures for the on-chip analysis of nano- and pico-liter volumes. Recent advances in microfluidics technology are revolutionizing molecular biology procedures for enzymatic analysis (e.g., glucose and lactate assays), DNA analysis (e.g., polymerase chain reaction and high-throughput sequencing), and proteomics. The basic idea of microfluidic biochips is to integrate assay operations such as detection, as well as sample pretreatment and sample preparation on one chip.

Recently, the technology has advanced to the commercial stage and instrumentation has been introduced that provide significant benefits to the research community.

Figure 2
Professor Richard Mathies, University of California, Berkeley

Microchip Biotechnologies, Inc.

Microchip Biotechnologies Inc. (Dublin, CA) is an early-stage privately-held company located in Dublin, California that is developing advanced nanofluidic sample preparation and analytical instrumentation for Life Sciences, Applied Sciences, and Diagnostics markets. Their solutions are based on MBI's proprietary "NanoBioProcessor™ platform and associated "Microscale-on-Chip-Valves" (MOV™) that were developed by Professor Richard Mathies (Figure 2) and his team at the University of California, Berkeley.

Professor Mathies, the G.N. Lewis Professor of Chemistry and Dean of the College of Chemistry, and his group have been involved in developing lab-on-a-chip technology that will enable portable and completely integrated chemical processing systems, from sample preparation through analysis. "Lab-on-a-chip technology research has been ongoing since early 1990's, but until recently, had not really fulfilled its promise," said Professor Mathies. "The greatest challenge to this point was to create a new set of robust tools to integrate sample preparation of nanoliter quantities of fluids with the analytical portion of the instrument reliably and economically."

In 2003, Professor Mathies group achieved a major breakthrough and their subsequent publication documented the feasibility of a microfabricated device for high throughput DNA sequencing that coupled isolation, template amplification, Sanger extension, purification and electrophoretic analysis?all in a single microfluidic circuit. (5) The breakthrough was enabled by the development of pneumatically-operated membrane valves and pumps that could be controlled and operated at the nanoliter scale. (6)

Professor Mathies today believes that the dramatic breakthrough that occurred just 5 years ago has caused a paradigm shift by demonstrating the feasibility that chemical processing typically performed in the lab with large systems, robotic stations and other traditional equipment can now be performed with volumes an order of magnitude smaller?and now, all on a chip.

"Pittcon is the nexus of formal science and instrumentation and provides a close connection between what's being talked about and what's real."

Dr. Graham Cooks

Later in 2003, Professor Mathies collaborated with Drs. Stevan Jovanovich and Dennis Harris who shared a common vision that microfluidics was an enabling technology with enormous potential. They saw immediate opportunity in the sample preparation area where they could eliminate the biggest bottleneck in preparing and moving a sample from the source to the instrument cheaply and economically. They believed that integrated microfluidic processing dramatically reduces analysis time and reagent consumption, and eliminates costly and unreliable macroscale robotics and laboratory apparatus.

The three co-founded Microchip Biotechnologies, Inc. (MBI) later that year after obtaining an exclusive license agreement with the University of California for "Fluid Control Structures in Microfluidic Devices" and related patent applications. Their initial focus is on use of nanofluidic technologies to simplify sample preparation, address the issue of volume scale and ultimately develop complete "sample-to-answer" solutions with a much smaller footprint than is available today.

Dr. Jovanovich presented progress on the technology during a Pittcon 2008 Symposium, Next Generation Genome Sequencing Technologies - Automated Microfluidic Sanger Sequencing Sample Preparation. MBI will exhibit their first commercial product, the Apollo 100 STAR™, employing this technology at Pittcon 2009.

"With the Apollo 100 System we are able to automate two labor intensive processes involved in sample preparation for DNA sequencing, which can free up as much as 3 hours of an operator's time," said Dr. Jovanovich. "In addition, for some applications, the system can reduce reagent costs by 5 - 10 fold over conventional sample preparation methods."

MBI is current working on the Apollo 200 System which employs similar microfluidic technology and will address applications for DNA analysis in the forensics area. "The Apollo 200 system will be a suitcase size device that integrates the entire DNA analysis workflow into a single process. The system will have application for Homeland Security, military, and law enforcement areas," he said. MBI hopes to demonstrate a prototype of the next generation system by the end of 2009.

Professor Mathies sees potential for microfluidics and lab-on-a-chip technology to be employed in almost every scientific area. The technology developed in his lab has been selected to be part of the European Space Agency's ExoMars-Pasteur Mission scheduled for launch in 2013 which is designed to carry out a series of in situ experiments on Mars that will look for chemical signs of life on the red planet (7).

"We are developing a fully integrated chemical processing system using the micro valve structures that will be carried in a rover on the surface of Mars. It is the most extreme remote process ever attempted," said Professor Mathies.

Professor Mathies has presented his work at Pittcon since the early nineties and it is likely that you will be able to stay abreast of further developments on the Mars project and other advances in nanofluidics from Professor Mathies group at Pittcon in the future.

Instrument Miniaturization

Instrument miniaturization is one way of addressing the issues of sensitivity, speed, throughput, and cost of analysis in DNA diagnostics, proteomics, and related areas such as forensics and environmental testing. The small footprint of these microfabricated structures leads to instrument designs suitable for high throughput field analyses (8). Increased efforts are being made to develop miniature mass spectrometers, including those which are hand-portable, and to retain the performance characteristics of traditional laboratory instruments as much as possible in the miniature instruments. (9)

One collaboration has resulted in the development of the first commercial field enabled GC-MS instruments.

Griffin Analytical Technologies

Griffin Analytical Technologies (West Layfayette, IL), a business unit within the Detection Division of ICx Technologies, Inc., is an emerging company and commercial developer of field-enabled analytical instrumentation, including MS and GC/MS instruments. The company was founded by two graduate students(10) from the Purdue University Chemistry Department: Garth Patterson who worked for lab director R. Graham Cooks (Figure 3), the Henry B. Hass Distinguished Professor - Analytical Chemistry, and Dennis Barket, who worked with Dr. Paul Shepson in the same lab.

Dr. Cooks group focuses on mass spectrometry, including fundamental phenomena, instrumentation and analytical applications. In the mid-1990's, most commercial development activities were developing larger and more powerful mass spec instruments. Dr. Cooks took the opposite approach and started what he called a mass spec "weight loss program." Their goal was to miniaturize the instruments so they could be used for fast screening applications away from a diagnostic lab and right in the hospital, doctor's office or even in an operating room.

Figure 3 *Dr. Graham Cooks, Purdue University*

The research was being performed as part of Garth Patterson's thesis project and they developed the first miniature Cylindrical Ion Trap (CIT) array that became one of the enablers to miniaturize other mass spec components as well. Shortly after they published their work in 2002 (11) and before Patterson completed his thesis, Patterson and Barket licensed the technology and formed Griffin Technologies. The company's mission is to develop miniature mass spec instruments that perform analyses with the same precision and quality as a conventional laboratory mass spec.

The company first appeared at Pittcon 2003 (12) and the following year introduced its MINOTAUR 300™, a desktop GC-MS and the MINOTAUR 400™, a "field-ready GC-MS." The novel field instruments were nominated for the Pittcon 2004 Editor's Award. Today, Griffin is developing novel field-enabled mass spectrometry products for chemical, explosives trace, and biological sensors in the homeland security, defense, environmental and health markets.

Dr. Cooks, a Pittcon contributor for more than 30 years, received the Pittsburgh Spectroscopy Award from the Spectroscopy Society of Pittsburgh at Pittcon 2000 and will be honored with the Ralph N.

Adams Award at Pittcon 2009. "Pittcon is the nexus of formal science and instrumentation and provides a close connection between what's being talked about and what's real," he said. At Pittcon 2009, Dr. Cooks will present his group's most recent work in desorption electrospray ionization (DESI) which allows mass spectra to be obtained almost anywhere at ambient temperatures, from inert surfaces or directly from living tissues with no sample preparation. He envisions that this research will soon lead to the commercial development of a miniature mass spectrometer that would enable rapid tissue analyses in situ, during surgery.

Prosolia, Inc. (Indianapolis, IN) obtained the licensing rights to the proprietary Desorption Electrospray Ionization (DESI) technique developed in Dr. Cooks lab in 2005 and launched its first products, Omni Spray™ Ion Sources products, in 2006. They will exhibit their products at Pittcon 2009 as well.

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