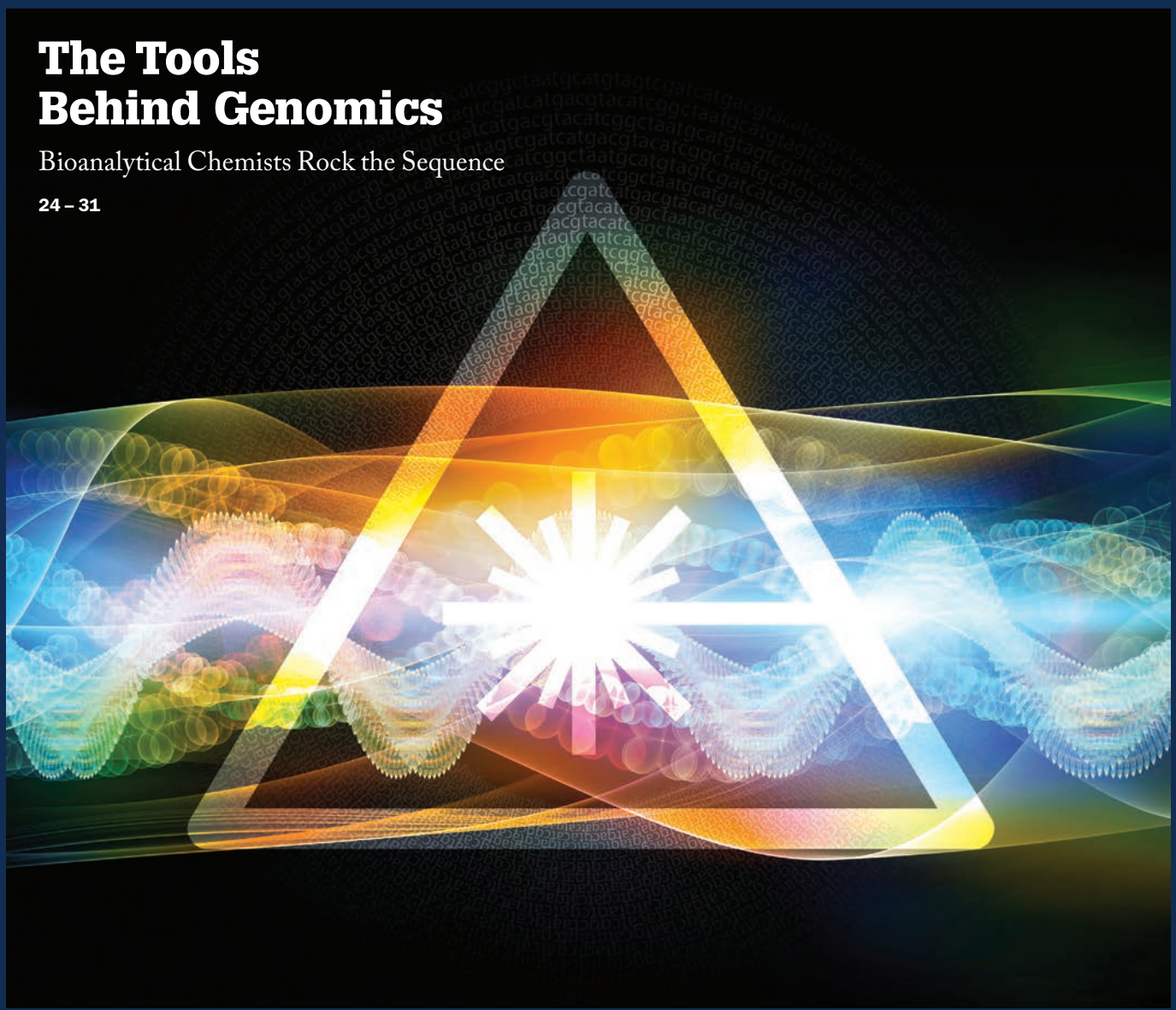
JANUARY 2013 # 01

the Analytical Scientist

The Tools Behind Genomics

Bioanalytical Chemists Rock the Sequence



Online this Month

Talking Point

Each week we host a discussion on a key area of the analytical sciences. Have your say and find out what others are thinking by signing up for our email alert at link.theanalyticalscientist.com/alerts

This month's Talking Points:

- When will carbon nanotubes switch from lab curiosity to industry stalwart?
- By Trust or By Fear: Do you tailor your management approach to individual staff members?
- Innocents Abroad: give your tips on helping foreign PhD students/new staff to settle in unfamiliar surroundings.

Video

Fasha Mahjoor offers candid thoughts on his career, his company and the field.
Go to theanalyticalscientist.com/issues/0113/602

Website

Scan the code with your smartphone to access The Analytical Scientist website.
Or go to www.theanalyticalscientist.com

Spotlight on Entrepreneurs

Online-only articles take you inside the companies showcased in this month's feature.
Go to theanalyticalscientist.com/issues/0113/404

App

Content at 30,000 feet.
The Analytical Scientist iPad app is now available to download at the Apple App Store. Offering the ability to download and read content offline, it's easy to take The Analytical Scientist wherever you go.
To download the app and for updates on the upcoming Android version, visit link.theanalyticalscientist.com/app

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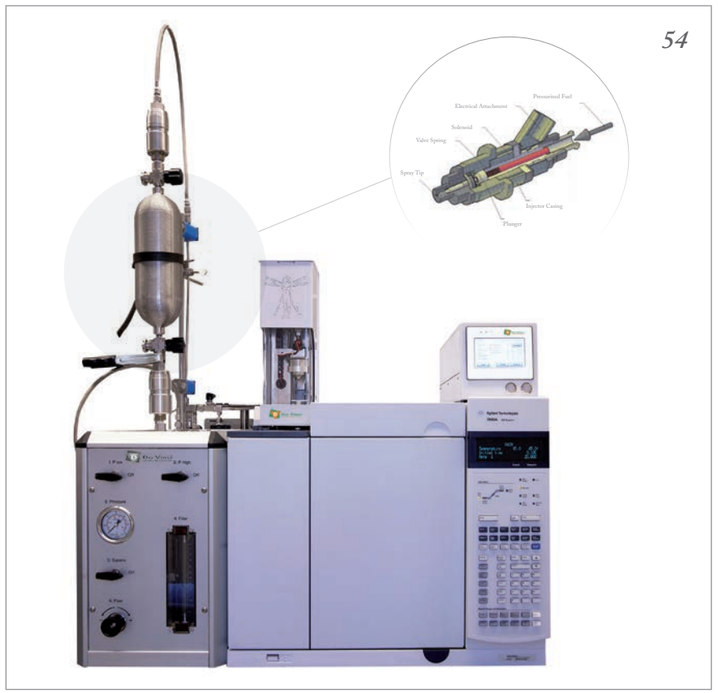
Contents



54

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32

the Analytical Scientist

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Recording, Scrutinizing, Celebrating

If you are a professional in the analytical sciences, read on: this is your magazine.

Welcome to the first issue of The Analytical Scientist. Our aim is to provide you with useful, credible and stylish content, and a forum for interaction, in print and online.

Analytical science demands such a forum. It is the engine that drives some of the most significant areas of basic science and it plays a key role in ensuring safe water, food security, effective medicines and diagnostics, efficient and sustainable energy, and the necessary tools for forensics and national security. Analytical science is central to our lives, as scientists and as citizens, and it deserves to be recorded, scrutinized and celebrated.

Four characteristics set The Analytical Scientist apart:

First, it will tell stories. We will cover analytical science in part by delving into the hopes, fears, motivations and aspirations of the key figures in the field. Only through exploring why analytical scientists do what they do can we gain a full understanding of the subject.

Second, it will adopt a solutions-based approach. This reflects the fact that analytical science is no longer just a series of individual techniques, but a group of integrated and complementary methods.

Third, it will address professional development in addition to science, technology and business topics. We will offer advice aimed at senior researchers and managers in the analytical sciences, focusing on such issues as how to manage your career, how to promote innovation within your team and how to run an efficient lab.

Fourth, it will endeavor to engage the entire community. The content that we publish and post is a starting point, not an end point. We want your feedback, your suggestions and your submissions. From this, a network of ideas, debate and personal interactions will emerge that connects and enhances research, and helps industry to tune new products, services and applications.

You can enjoy the The Analytical Scientist magazine in print and in digital versions optimized for computer, tablet and smartphone. Online, at www.theanalyticalscientist.com, we will be developing a rich array of additional content, much of it interactive, including debates and discussions, podcasts and videos, an application notes library and webinars.

We at The Analytical Scientist are tremendously excited by this undertaking. Our success will be measured in how well we serve you, the analytical sciences community, so please don't hesitate to let us know what you like, what you don't and what you want to see us cover. It's your publication.

Richard Gallagher
Editorial Director

Samuel Kounaves

Shortly before joining the chemistry faculty at Tufts University in 1988, Samuel Kounaves received a Ph.D from the Université de Genève, Switzerland. "Though I spent my childhood watching Star Trek and wanting to explore other planets, my first taste of the unknown came in the first year of my MS degree at Cal State San Diego in 1976, using sensors in submarines to measure trace metals at the bottom of the Pacific Ocean." Kounaves did not realise then that it was the start of his career in "extreme" analytical science. "In the summer of 2008, we were analysing the soil on the surface of Mars – finally I was doing what I had only imagined 30 years earlier." See page18.

Norm Dovichi & Amanda Hummon

Norm Dovichi's postdoctoral fellowship at Los Alamos Scientific Lab introduced the concept of single molecule detection, leading to the development of a capillary array DNA sequencer that became the workhorse tool used in the human genome project. Amanda Hummon similarly blurred the boundary between analytical chemistry and biology. Her PhD thesis research focused on mass spectrometry and bioinformatic strategies to predict and identify neuropeptides, leading her to play a seminal role in annotation of the honeybee genome. Currently colleagues at the University of Notre Dame in Indiana, USA, Norm and Amanda here join forces to describe current and future technologies in genome sequencing.
See page 24.

Willlem van Raalte

Following several management positions in the laboratory instrument manufacturing sector, Willem van Raalte established Da Vinci Europe Laboratory Solutions B.V. in 2000 to provide laboratory support in the Benelux region. True to his entrepreneurial spirit, van Raalte quickly realized his company could answer additional market demands. "As our customers needed more than just support and instruments, Da Vinci moved towards providing analytical solutions." Twelve years later, and Da Vinci has expanded its offerings to a worldwide customer base through a robust network of partners. See page54.

Caroline West

Caroline West, now an associate professor in analytical chemistry, ICOA, University of Orléans, France, only heard derisive comments when she first start working with supercritical fluid chromatography: "Why the hell are you wasting your time with this non-existent technique?" Now, the tide has turned. West discovered SFC when she was only a "baby" analytical chemist, so she can no longer live without it. "Unravelling its surprising phenomena and projecting the quasi-unlimited possibilities of this separation method is keeping my neurons 150% busy. I've no time to fall into the boredom of using long and fastidious methods with unsatisfactory resolution." See page 20.

Upfront

Reporting on research, personalities, policies and partnerships that are shaping analytical science.

We welcome information on interesting collaborations, C-level appointments or research that has really caught your eye, in a good or bad way. Email rich.whitworth@texerepublishing.com.

Carbon Youtubes

Nanotubes can be tailor-made for personal medicine, the ripening of fruits and a myriad of other uses. But when will they make it to market?

Last summer, fifteen-year-old Jack Andraka hit the headlines with a test for pancreatic cancer that was faster, cheaper and more sensitive than current diagnostics. To create the test, he applied antibody-bound carbon nanotubes to filter paper: when the target antigen bound to the antibodies, small changes in conductance of the nanotubes was recorded.

The test wasn't exactly new. At least one research paper (Nanotechnology (19 (46), 2008) used a similar "nanoscale oncometer" for breast cancer. But it did highlight the exciting possibilities of new materials and edged us closer to cost-effective personalized healthcare.

The big question, for medicine and for other applications, is: when will this 'youtube' technology be commercialized?

That question also came to mind this past autumn, when another carbon nanotube marvel hit the mainstream news: a sensor-drawing pencil. MIT researcher Katherine Mirica created a functioning gas sensor by literally drawing a carbon nanotube line between printed gold electrodes using a pencil tip shaped from carbon nanotube powder. The target gases are detected by monitoring changes in electrical conductivity.

"The potential of our findings lies in the simplicity, versatility, and the solvent-free nature of this method […] It requires no specialized facilities or highly trained technicians. And nanotube-based pencils have the potential to be very stable formulations of sensing materials," says Timothy Swager, leader of the MIT Chemistry group conducting the research. "Making sensors by drawing carbon nanotubes on the surface of paper could be very cost effective. We believe that the characteristics of our technology will accelerate the development of carbon nanotube-based sensors, and will lower the barrier to their commercial entry."

As with most truly innovative technologies, commercial success is not straightforward. "One step for further development is adapting this method to selective gas sensors for specific applications," Swager continues. "Another is the thorough characterization of sensors for stability and the ability to operate at a variety of environmental conditions."

The group already has extensive experience with covalent and non-covalent chemical functionalization of the nanotubes. For example, they recently reported on the selective and sensitive detection of a fruit-ripening hormone (ethylene gas) using carbon nanotubes functionalized with a copper-containing compound. "We are now working towards applying these types of strategies for fabricating nanotube-based sensors by drawing," says Swager.

If development goes to plan, Swager believes the nanotube pencil-based diagnostics could hit the market within the year. *RW*

Carbon nanotube pancreatic cancer test on YouTube: goo.gl/6K25K
Carbon nanotube sensor drawing pencil on YouTube: goo.gl/DWpgd

Talking Point: What are the potential applications and challenges of carbon nanotube-based sensors? Follow the discussion and share your opinions on these technologies online at: theanalyticalscientist.com/issues/0113/209

In Sync

New and notable partnerships in the world of analytics

Mass Spec's March on Clinical Diagnostics

Who: Thermo Fisher Scientific and Immundiagnostik.
What: Immundiagnostik's antibody technology will be used to optimize sample preparation by isolating low-level analytes ahead of Thermo Fisher Scientific's LC-triple quadrupole mass spectrometry systems.
Why: To create new commercial assays of complex mixtures for the diagnosis of cardiovascular diseases and bone metabolism disorders.
How: Thermo will contribute several bits of kit (high-throughput mass spectrometric immunoassay technology, TSQ Vantage triple quadrupole MS, Orbitrap Elite high-resolution accurate mass MS) while Immundiagnostik will worry about the details: identification of diagnostic parameters, validation of LC-MS/MS products and making headway on commercial development.

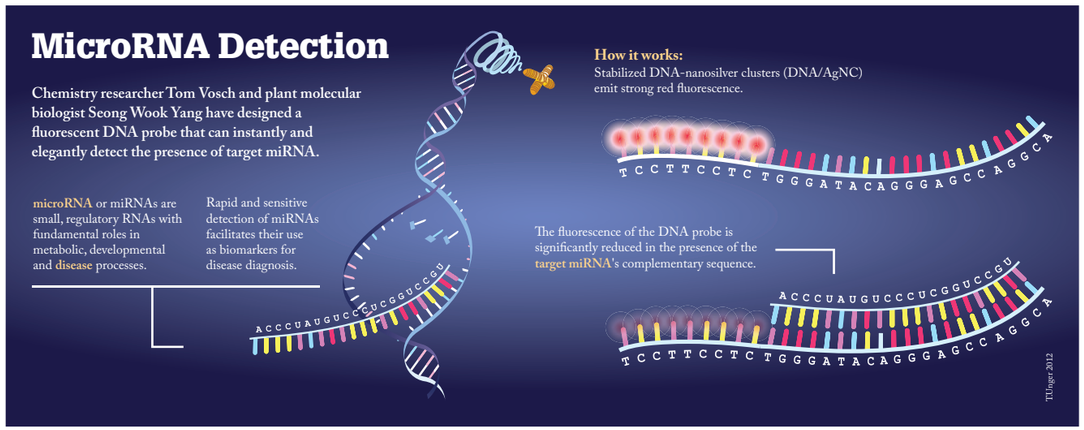
Texas Two-Step, Part One: The IRT

Who: University of Texas at Arlington and Shimadzu Scientific Instruments.
What: A $25.2 million collaboration, the Institute for Research Technologies (IRT) builds on the existing Shimadzu Center for Advanced Analytical Chemistry to introduce a Center for Imaging and the new Center for Environmental, Forensic and Material Analysis.

What draws the Japanese giant to Texas? According to Shimadzu president Shuzo Maruyama, "Shimadzu sees in UT Arlington an exciting energy, great vision and the potential to transform scientific research and education".
Selling point: The deal brings in analytical equipment to IRT that is not found elsewhere in North America, some of which will be made available to nearby UT Dallas and UT Southwestern Medical Center.

Texas Two-Step, Part Two: Quantifying Crude

Who: Agilent Technologies and University of Houston's Department of Earth and Atmospheric Sciences.
What: Agilent is providing $1 million worth of state-of-the-art analytical systems to researchers to further study the geology and composition of crude oil.
How: Microwave plasma-atomic emission spectroscopy (MP-AES), inductively coupled plasma-optical emission spectroscopy (ICP-OES), and several advanced GC-MS instruments will all be used to study geological specimens and to separate and quantify the thousands of compounds found in crude oil.
Plus: The three-year research project will also look into currently controversial shale gas extraction – better known as fracking – to evaluate potential but also to provide insight into any detrimental environmental impact. *RW*



Applied Zoo-ology

How one company's scientific know-how is helping a black rhino breeding program

Analysing black rhino faeces might not be every scientists' idea of fun, but for Sue Walker, wildlife endocrinologist at Chester Zoo, UK, there is valid method behind apparent madness.

There are less than 600 East African black rhinos left in Kenya, which make up 70 per cent of the global population. Therefore, maintining a genetically healthy and viable captive population is incredibly important; however, managing and breeding black rhinos in captivity is not straightforward.

"When working with wildlife, we try to do everything non-invasively. We don't even want to touch the animals, so we use faeces, urine – anything we can easily get our hands on," says Walker. But stepping away from traditional blood sample analysis causes problems, especially when it comes to studying the hormones that can help improve breeding program success.

"If you analyse blood from the animal, the hormones are equivalent to human progesterone and oestrogen. In other words, native hormones that are quite easy to identify with enzyme immunoassay (EIA) tests," Walker explains. "But in our complex samples, those hormones have been broken down, and we need to confirm that the metabolites measured with EIA are reflective of what's going on in the animal's endocrine system."

Linking unknown metabolites to their parent hormones with EIA alone is difficult, because of antibody cross-reactivity. The complex relationship between hormones related to reproductive health (progesterone, oestrogen and testosterone) and those known to indicate the impact of potential stress (glucocorticoids) further clouds the issue. Confirmation of the results is essential.

Improved analysis = better breeding

"Anytime something comes up that's out of the ordinary, we jump at the chance to help," says Tim Liddicoat, a Thermo Fisher Scientific Support manager and key member of the collaboration.

In 2006, Harald Ritchie, Director, Business Development at Thermo Fisher Scientific, was touring the zoo's vet facility and asked Walker if she would ever consider using HPLC. "I pretty much jumped up and down and said 'I would love to,' but we didn't have the money or the support," says Walker. HPLC can fractionate the sample and allow different EIA tests to be run in parallel, which eliminates cross-reactivity and provides confirmation, despite an unknown target.

For the female black rhino, long and short reproductive cycles have been identified. Analysis using HPLC and EIA have confirmed that long cycles are linked to increases in adrenal function as reflected by higher concentrations of glucocorticoids. Research is now underway to understand why these long cycles occur. Despite this unknown, the HPLC and EIA analysis is currently being used to determine the most appropriate time to introduce males to the females without aggression. The accompanying photo illustrates the tangible benefits of getting this timing right.

Recently, the work has gone beyond HPLC and into mass spectrometry (MS). The flexibility of MS allows qualitative and quantitative analysis of extremely complex samples. "Initial results by quadrupole MS have been very promising. If we consider using an instrument capable of accurate mass scanning it will not be necessary to know which metabolites you are looking for because you can reexamine the data at a later date and identify compounds you did not initially target," says Liddicoat. Walker's dream, and the subject of continued collaboration between the Zoo and Thermo Fisher Scientific, is being able to measure multiple hormones from a single sample.

Sample stability

Another notable development from the collaboration is the eyebrow-raising use of consumables. "Our sample preparation is novel," Liddicoat explains. "We store rhino faecal extracts on solid phase extraction (SPE) cartridges. It's a really inventive thing to do".

Walker's team have shown in tests that faecal extract can be stored for about six months on SPE cartridges, and that has particular benefits in the field. For example, a PhD student from the University of Nottingham, UK who is tracking wild elephants in Malaysia is using the cartridges to collect and store faecal extract on the trail. Back in the lab, the sample can be pulled from the cartridges for further analysis.

The cartridge loading procedure is fairly standard but with some important differences: it is performed in the field with no electricity or expensive equipment and the methanol concentration is higher than a typical reverse-phase extraction because of the need to extract compounds of interest from the sample.

The complete process allows for easy storage and shipping. "I am aware that this type of protocol has been carried out previously on on-line SPE cartridges for storage of pharmaceutical library samples, but not routinely and not with biological samples. But it does make sense as chemists have known about the stability of solid-supported compounds for a while," says Liddicoat.

An additional benefit? "It's great when someone asks me what I do for a living and I'm able to describe the project at the zoo without sending them to sleep in 20 seconds flat," he says. "Having something good to talk about at the pub? You can't buy that." *RW*

For more information about Chester Zoo's ongoing projects, visit goo.gl/1n6GT

Off the Menu

A look at recent reports of chemical contaminants in the food supply

Leaden Eggs

A fresh egg rapidly sinks and lies flat on its side in cold water. And if it's laced with heavy metal? Unfortunately, most food analysis requires significantly more sophisticated instrumentation than a water-filled bowl. New York State Department of Health Research scientist Henry Spliethoff recently discovered lead in urban-farmed eggs.

"I collaborated with chemists at the NYS Department of Agriculture & Markets Food Laboratory. We homogenized the edible portion of whole eggs, digested with nitric acid and hydrogen peroxide and analyzed using inductively coupled plasma mass spectrometry [Agilent 7500 CX ICP-MS]," says Spliethoff.

The work was a component of Healthy Soils, Healthy Communities, a drive to identify foodstuffs contaminated by urban soils. The full report will be published later this year, but the New York Times reported that, of 58 eggs tested, 28 contained lead in concentrations of 10–73 ppb, with one hitting over 100 ppb. Safe limits for lead are generally assessed on a case by case basis, but the US Environmental Protection Agency's guidance on tap water states that if more than 10% of samples exceed 15 parts per billion, action must be taken. *goo.gl/eEDaK*

Arsenic-Laced Rice

Arsenic has also hit the headlines, having been shown to be present in many different rice products. Chickens again (or rather chicken farmers) may be to blame. Consumer Reports, a nonprofit and independent US organisation, recently raised the alarm about levels of both inorganic and organic arsenic found in rice products. Total arsenic content was measured using inductively-coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), with arsenic species (As(III), As(V), DMA and MMA) elucidated through the addition of ion chromatography.

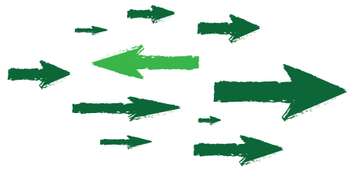
Meanwhile, the US Food and Drug Administration is in the middle of its own analysis, with FDA Deputy Commissioner for Foods Michael Taylor stating: "It is critical to not get ahead of the science […] The FDA's ongoing data collection and other assessments will give us a solid scientific basis for determining what action levels and/or other steps are needed to reduce exposure to arsenic in rice and rice products." *goo.gl/zRgNm goo.gl/g2xin*

Bubble Tea Bust-Up

After the shock of lead and arsenic in your egg-fried rice, you may need to sit down with a nice cup of tea.

Initial claims of carcinogenic agents in 'bubble tea' by Manfred Möller from the Institute of Hygiene and Environmental Medicine at the University Hospital Aachen have, perhaps unsurprisingly, been disputed by Possmei Corporation, a Taiwanese manufacturer of the tapioca pearls used in the beverage. Chinese media reports that retests on bubble teas by German authorities were also clear of carcinogens. *goo.gl/1Ilsm*

RW



Our Open Source Future: Hardware…

Is free, collaborative development about to have a major impact on analytical instrumentation?

Open source. It's a term we are all familiar with, certainly in the context of software. Anyone who has dabbled with the Linux operating system will have already recognized the benefits, challenges and pitfalls of stepping away from the mainstream and into a free, collaborative approach. Fewer will know that the world's top ten supercomputers are based on Linux (Top500.org), which may shake the myth that open source equates to under-performance.

But surely, when it comes to analytical instrumentation, options are limited? Not so, says Joshua Pearce, an associate professor at Michigan Technological University, who is pioneering 'FOSH' or free, open source hardware.

"Most analytical scientists are familiar with the sometimes absurd prices we pay for scientific equipment," says Pearce.

"In the old days you had no choice – you had to buy proprietary tools to participate in state-of-the-art research or develop everything from scratch."

What broke the camel's back for Pearce was a dishonest vendor. A salesman had promised Pearce that he would have the ability to make alterations to a highly specialized tool as he developed new materials. After a few months, a minor modification was needed but the vendor demanded thousands of dollars.

So Pearce set out to solve the problem without recourse to the manufacturer. The answer lay in combining three-dimensional printing with open-source software-driven microcontrollers. "For the same several thousand dollars we took some open-source designs and re-developed the tool we needed ourselves – and we have the source code so we can make changes in the future for free."

There is currently no central hub for all FOSH scientific equipment but there are dozens (or even hundreds) of groups sharing projects on university and company websites or those specific to a particular subfield. Thingiverse.com features designs from all walks of life, which makes a quick browse most enjoyable. Pearce's "things" are shared there for all to see.

"Now we have joined a virtuous cycle. As we share our designs of research equipment with others, they make them better and we all benefit," says Pearce. "The genie is out of the bottle. Open-source development changes everything – there is no stopping it." *RW*

…and Software

An Open Source platform promises wizardry in the analysis of proteomics mega datasets

Proteomics – the overarching study of protein populations – has been transformed by modern mass spectrometry's ability to provide accurate and high-resolution analysis of intact proteins and the peptides derived from them.

But something else that advanced mass spectrometry does very well is to produce vast amounts of data. And large datasets are difficult and time consuming to analyze, especially when working in a cross-platform environment with proprietary, closed formats. Consequently, research teams are spending more and more of their time developing custom data analysis software.

Now, perhaps, there is a magic wand to deal with the problem. ProteoWizard is a free central resource where proteomics software developers can share high-quality source code and benefit from the work done by others; a proteomics toolkit, if you will.

Initiated in 2007 by researchers at the Mallick Lab at the Spielberg Family Center for Applied Proteomics, Stanford University, ProteoWizard is reaching critical mass with active contributions from the MacCoss group at University of Washington and the Tabb group at Vanderbilt University.

Parag Mallick, professor at Stanford, web designer and president of the ProteoWizard Software Foundation, believes open source offers the ultimate in collaboration. "By openly distributing ProteoWizard we are able to collaborate with labs all over the world to create an ecosystem that spurs innovation in proteomics and helps democratize the field," he says.

Three key principles underpin ProteoWizard's potential for widespread success. The first is robustness; ProteoWizard software is of commercial quality or better. This is ensured by strict adherence to current software design principles, giving users confidence in the stability of products and support for them.

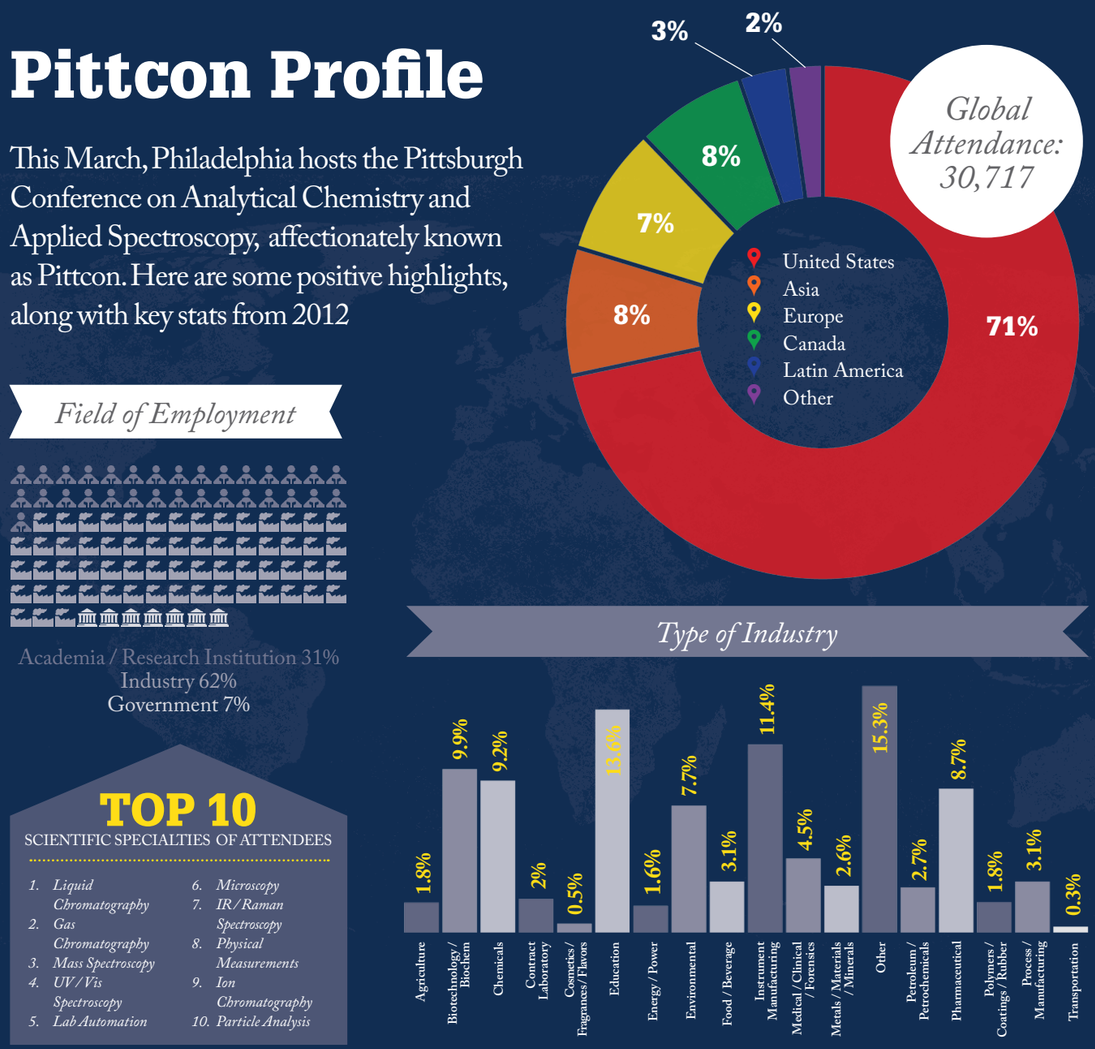
Second, ProteoWizard is cross-platform in nature, compatible with Windows, Linux and Macintosh systems.

Third, and most importantly, its core is fully open source, and its development is open to, indeed dependent upon, widespread collaboration. Mallick welcomes newcomers with open arms: "We've been fortunate to have dozens of researchers contribute to ProteoWizard and are actively encouraging new developers to join the effort". *RW*

For more information about contributing to ProteoWizard or to download the toolkits available, visit proteowizard.sourceforge.net

Pittcon Profile

This March, Philadelphia hosts the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, affectionately known as Pittcon. Here are some positive highlights, along with key stats from 2012



Field of Employment

Academia / Research Institution 31%
Industry 62%
Government 7%

TOP 10
SCIENTIFIC SPECIALTIES OF ATTENDEES

1. *Liquid Chromatography*
2. *Gas Chromatography*
3. *Mass Spectroscopy*
4. *UV/Vis Spectroscopy*
5. *Lab Automation*
6. *Microscopy*
7. *IR/Raman Spectroscopy*
8. *Physical Measurements*
9. *Ion Chromatography*
10. *Particle Analysis*

Type of Industry

Editor's Highlights for Pittcon 2013, March 17th–22nd, Philadelphia

- Short courses in management
- Networking Events

Sunday 17th
- Career Placement for Managers and Scientists.
- Chemical Imaging: Current Applications and Future
- Changing Requirements for Metals Testing in Pharmaceuticals.

Monday 18th
- Bioanalytical Separation by Asymmetric Flow Field-Flow Fractionation (AsFIFFF).

Tuesday 19th
- Coaching as a Powerful Leadership Tool
- Robust LC Separations at Extremes of pH and Temperature.

Wednesday 20th
- Green Analytical Chemistry
- How Can Your Business Do More to Support the Scientific Community?

Thursday 21st
- Improving Teamwork and Task-Focus in the Analytical Laboratory

For full list of courses and events, visit pittcon.org

In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can up to 600 words in length, written in the first person.

Contact the editors at edit@theanalyticalscientist.com

The Epic Saga

Quantitative analysis and the essence of doing good science

By Samuel Kounaves, Professor of Chemistry at Tufts University.

For decades, the majority of chemists have taken undergraduate courses oft-entitled "Analytical Chemistry". Historically, such courses covered topics that many chemists label as "classical": error analysis, gravimetry, titration, acid/base buffers, redox reactions and some instrumentation. The philosophy? Chemists needed to separate, identify and quantify chemical substances.

Having taught Analytical Chemistry since the late 80s, it became clear to me that it was not subject matter pertinent only to skilled chemists. And during the past few years, students from biology, chemical engineering, geology, environmental science and even physics started to take the course. The motivation behind this increased interest is the realization that, in addition to using many of the same instruments, the same fundamental skills for making reliable and accurate measurements are required, whether you are measuring dopamine in rat brain cells, CO^2 in the atmosphere, hydrocarbon fractionation in petroleum – or the soluble salts in martian soil…

In fact, my own research focuses on fundamental questions in planetary science by using modern in situ or on-site analytical systems that are custom designed to make accurate and relevant measurements of biogeochemistry in extreme environments – Mars. As a co-investigator and wet chemistry laboratory lead scientist for the Phoenix Mars mission, my group and I were able to perform the first wet chemical analysis of martian soil. The analyses revealed an alkaline soil containing a variety of soluble minerals, including, somewhat unexpectedly, perchlorate. This surprising discovery prompted us to analyze soils in Antarctica, and we found that the soils of the entire continent also contained perchlorate, and that its extent is probably as global on Earth as it is on Mars. This finding has global implications; perchlorate is currently the target of environmental regulations in many countries. The question must now be: what exactly constitutes a "pollutant", when it appears to be produced by nature?

The same type of sensor array developed for Mars is now being used to analyze the chemistry of deep-sea thermal vents in the Atlantic. Our longer-term goal is to use similar analytical devices to investigate the surface and sub-glacial oceans on other planetary bodies such as Jupiter's moon, Europa.

So, you see, analytical science is truly ubiquitous. It is practiced in forensics, agriculture, pharmaceutics, commerce, manufacturing, space exploration – every industry imaginable. It affects everything from the food we eat to the cars we drive to the very environment in which we live.

To address these sweeping changes in application, my analytical chemistry course slowly morphed to the point where, not only did its name change to "Quantitative Analysis", but one-third of it is now devoted to understanding scientific method and doing "good science" – covering more rigorously error analysis, precision, accuracy, statistics, sampling, presentation of data and beyond. The rest of the course is devoted to using several types of modern instruments, along with hands-on laboratory experiences where classroom topics are put into practice.

For me, "quantitative analysis" is the epic saga and essence of science; the art and core of doing good science is observation and measurement. From the very first observations made by humans thousands of years ago, our knowledge of the world and universe has grown by the accumulation of millions of persistent and repeatable measurements.

Humans are, from birth, analytical and scientific beings, and left to their own devices will take measurements with all five senses and act to control their environment. The synergy of hypothesizing, observing and measuring [science], transforming these into useful tools and applications [engineering], and using these new tools [technology] to make new observations and measurements, continues to help form culture and civilization. It is the underlying force that drives scientific progress.

Now more than ever, there is a need to make reliable analytical measurements, so I find it disquieting that many universities have been dropping courses in analytical science over the past few decades; all too often, the material is integrated into lower level courses. But considering the overcrowded first-year curriculum, I doubt that enough emphasis and time is spent on ensuring that students learn how to measure analytically or obtain enough hands-on experience. This lack of analytical science increasingly appears in top journals, where error analysis is often omitted or inaccurately completed.

For me, the launch of The Analytical Scientist is a welcome addition, mainly in the hopes that it will contribute to a new focus on "good science". The art of making accurate and reliable measurements, whether in chemistry, biology, physics, geology – or any other domain, underpins improved understanding of ourselves and our universe.

We are all, in many ways, analytical scientists. The challenge is to make sure we all learn and use these analytical abilities critically, rationally and responsibly.

How To Personalize Medicine

Two new studies demonstrate the power of advanced metabolomics; progress in instrumentation will propel it into the clinic

By Ian Jardine, VP and CTO of Life Sciences Mass Spectrometry at Thermo Fisher Scientific.

The measurement of individual organic molecules to determine inborn errors of metabolism, diagnose disease and monitor therapy and recovery is not new; indeed, it has a long and illustrious history. Can continuing advances in metabolomics answer growing calls for understanding 'health' more deeply and drive personalized medicine development? I believe so.

Molecules that are routinely evaluated in the clinic include testosterone and other hormones; vitamin D3; peptides; classes of compounds related to metabolic function, such as amino acids, organic acids, sugars and acylcarnitines; and many

other organic molecules, such as lipids. Yet, there are hundreds, and more likely thousands, of molecules that are routinely ignored, either because a clear association has never been made to genetic variation, disease or well-being, or because they have been too difficult to measure in an affordable multiplexed fashion.

Metabolomics – the nonbiased identification and quantification of all metabolites in a biological system – is on the verge of transforming measurement in the clinic. Today, metabolomics of human biology is a rapidly growing field precisely because it is crucial for the development of personalized medicine. Two particularly striking recent papers (1, 2) epitomize the importance of whole metabolome analysis.

Some background

Nuclear magnetic resonance (NMR) was applied to the problem of multiplexed metabolite measurement from the mid-1980s, and particularly by Jeremy Nicholson of Imperial College London. Initial successes included insight into the diagnosis and treatment of diabetes mellitus. Though quantitative, the sensitivity and lack of large scale multiplex analysis has generally found this technology lacking, and the prohibitive system size and associated cost per analysis indicates that NMR will not enter routine clinical chemistry labs any time soon.

"Recent rapid advances have enabled striking increases in the number of metabolites that can be routinely measured in small biological samples."

On the other hand, recent rapid advances in another technology have enabled striking increases in the number of metabolites that can be routinely measured in small biological samples. That technology is quantitative ultra-fast, highly sensitive, high resolution/high mass accuracy liquid chromatography with tandem mass spectrometry. Hundreds of metabolites are now accessible, and a few thousand may soon be routinely measurable using LC-HRAM-MS/MS.

Interestingly, these LC-MS/MS systems were predominantly developed for proteomic analysis, mainly for quantitative sequence analysis of peptides resulting from protein mixture digestion. Entire human cellular proteomes of >10,000 proteins, including post-translational modifications such as phosphorylation, can be measured using today's instrumentation and software.

The protein analysis problem, which once seemed intractable, is now seen as relatively routine because all analytes are peptides, the sequence of which can be determined from gene coding. The metabolome is arguably a much more difficult analytical problem; organic metabolite structures are extremely diverse, are not predictable from genetic information, and every sample contains a multitude of non-human metabolites, for example, from bacteria and other organic material, or from food, drugs, and a multitude of other organic contaminants. So, how does LC-HRAM-MS/MS stack up to this challenge?

Recent advances

The first paper (1) describes genome-wide association studies (GWAS) that have identified a number of genetic loci associated with blood metabolite concentrations. These findings provide new functional insights for many disease-related associations, including those for cardiovascular and kidney disorders, type 2 diabetes, cancer, gout, venous thromboembolism and Crohn's disease. The study advances our knowledge of the genetic basis of metabolic individuality in humans and generates many new hypotheses for biomedical and pharmaceutical research. This study only employed LC ion-trap MS/MS analysis, without the application of HRAM. Upcoming publications, where the application of HRAM has been added to the MS/MS methodology, will expand these associations dramatically.

The second paper (2) presents an integrative personal omics profile (iPOP) analysis that combines extremely high coverage of genomic, transcriptomic, proteomic, metabolomic, and autoantibody profiles from a single individual over a 14 month period. The iPOP analysis revealed various medical risks, including type 2 diabetes. It also uncovered extensive, dynamic changes in diverse molecular components and biological pathways across healthy and diseased conditions. This study, which does employ LC-HRAM MS/MS, albeit not the latest technology, demonstrates that longitudinal iPOP can be used to interpret healthy and diseased states by connecting genomic information with additional dynamic omics activity, such as metabolomics.

To continue with this dramatic progress, the desires of metabolomics researchers are straightforward: they want to identify every metabolite in their samples, they want to do it quickly, and they want to do it quantitatively. To the instrument community, this translates into demand for more resolution, more reproducibility and the ability to identify unknowns, even at very low concentrations. Researchers can expect rapid advances in separations and mass spectrometry technology to continue, addressing major challenges like the tremendous structural diversity of these small molecules, the need for robust analysis across large numbers of samples and the need to identify unknowns. Moving this new advanced metabolomics capability into the clinic will be inevitable.

References

1. *K. Suhre et al., "Human metabolic individuality in biomedical and pharmaceutical research," Nature, 477, 54–60 (2011).*
2. *R. Chen et al., "Personal omics profiling reveals dynamic molecular and medical phenotypes," Cell, 148, 1293–1307 (2012).*

Greening SFC

Supercritical fluid chromatography (SFC) is often proclaimed to be a green technology. How well does the reality match up to the claims?

By Caroline West, Institut de Chimie Organique et Analytique (ICOA), University of Orléans, France.

Green chemistry is defined as chemical processes that reduce or eliminate negative environmental impacts. SFC is perceived to be "green", as compared to classical high-performance liquid chromatography (HPLC), based on two features that appear to match the definition. First, CO_2 is nontoxic and safe to use. Second, SFC reduces solvent consumption and waste. The favorable impression of SFC is further supported by the economical aspect of reduced solvent consumption and waste disposal, and the comparatively low cost of CO_2 – environmental and economic arguments are readily confused. These factors, alongside the technological improvements now available on the market, have prompted a re-birth of SFC.

How compelling is the supposed greenness of the method? Having practiced SFC for ten years, I am willing to question the point.

Typically, when a new SFC method is developed to replace an older HPLC method, performance is the sole criterion. Performance is measured via resolution or via quantitative information, such as sensitivity and linearity. If high-throughput is required, the short cycle time afforded by SFC's high flow rates and short equilibration is a plus point, while for preparative separations, the system's productivity is the selling point. Environmental aspects are hardly ever considered. SFC does have inherent features that make it environmentally friendly. However, in everyday practice these are rarely considered, and much effort is needed to make SFC the green technology that it is proclaimed to be.

Greening Guidelines

Since CO_2 is essentially a by-product of the fermentation industry, SFC systems are often presented as "good recyclers" of CO_2: SFC chromatography, the argument goes, uses CO_2 produced by others. While this is true, it does not change the fact that this very same

CO_2 still ends up being released into the atmosphere. Should it be recycled? To put things into perspective, one analytical SFC system operating for one working day only releases about the same amount of CO_2 that an eco-friendly car produces in 20 km. This we can overlook. However, the amount of CO_2 released by preparative-scale SFC systems is much larger and certainly worth recycling or capturing, something that is still far from being systematic practice.

Beyond carbon dioxide, the use of eco-friendly solvents and additives, such as ethanol and water, should be encouraged. In achiral SFC, the effects of changing the co-solvent are minor, so ethanol could replace methanol and acetonitrile for a large proportion of analytical applications. And water has been proven to be a very interesting additive (or even co-solvent, in ternary mixtures with carbon dioxide and ethanol) to enhance solubility and peak shapes of polar compounds.

Method development strategies should be revised to include only orthogonal chromatographic systems, ensuring that the final method will be developed in the shortest time possible. Most users are still relying on essentially redundant systems that make screening processes unnecessarily long. Besides, I have often found that several solutions exist for one separation problem, and one of them has significantly advantages over the others, in terms of solvent consumption and analysis time.

Green chemistry applies innovative scientific solutions to real-world environmental situations. For SFC, innovative solutions are still required to:

- Limit the energy required for the production and recycling of CO_2.
- Limit the energy required to cool down CO_2 for pumping.
- Improve sample transfer between the back-pressure regulator and the fraction collector without the need for a large make-up flow rate.
- Allow for multi-detection of several samples analyzed in a parallel or simultaneous fashion.
- Ensure good mass spectrometric sensitivity without the need for additional liquids.

SFC does have the potential to be a very green separation and purification method. It is in the hands of manufacturers and users to make it such.

Accelerating Food Analysis

Rapid, high-resolution, information-rich analytical methods to determine mineral oil contamination of foodstuffs, including baby foods are needed. Here's how it could be done

By Luigi Mondello and Peter Tranchida. Luigi Mondello is Professor of Analytical Chemistry, and Peter Tranchida is Professor of Food Chemistry, at the Dipartimento Farmaco-chimico of the University of Messina, Italy.

Contamination of foodstuffs with petroleum-derived mineral oils is, unfortunately, a common occurrence. Mineral oil saturated hydrocarbon (MOSH; linear and branched alkanes, naphthenes) and aromatic hydrocarbon (MOAH; mainly alkylated) contamination have both been described in numerous investigations. In rats, bioaccumulation of MOSH can lead to the formation of microgranulomas in the liver [1]. A limit of 0.6 mg/kg, for MOSH (from n-alkane C10 to C25) contamination in foods, has been recently proposed by the German Federal Ministry of Food, Agriculture and Consumer protection (BMELV).

Over two decades ago, Grob and co-workers pinpointed lubricating oils, release agents and mineral batching oil used for the production of jute as sources of contamination [2, 3]. Later, it was discovered that mineral oil can be transferred to dried baby foods from the ink printed on cardboard containers [4] and recent research has further highlighted MOSH and MOAH in foods packaged in cardboard [5]. MOSH have even been found in a high proportion of human milk samples [6] and continue to enter babies bodies throughout weaning, in the form of homogenized baby foods [7].

Most studies have used rather complicated, time- and solvent-consuming multidimensional liquid-gas chromatography (LC-GC) methods, with flame ionization detection (FID). It is well known that such applications generate humps, defined as unresolved complex mixtures (UCM). No identification information is usually reported on the composition of these UCMs, which is regrettable since they may be important to define the presence of interferences, toxicity and the source of contamination.

With interest in, and awareness of, the toxicological presence of mineral oil in food increasing, we believe that faster, more environmentally-friendly and higher-resolution methods are required, including detection using mass spectrometry.

"We believe that faster, more environmentally-friendly and higher-resolution methods are required, including detection using mass spectrometry."

Previously, we developed a fast LC-GC-FID method in a study on vegetable oils. This had a run-to-run time of 14 min [8], used a programmed temperature vaporizor as the GC sample introduction system and consumed limited quantities of organic solvents (hexane/CH_2C_{12}). Disadvantages? We wouldn't describe the approach as environmentally friendly. In addition, the method suffered from the "blindness" of the FID; however, even an MS system would be challenged by UCMs.

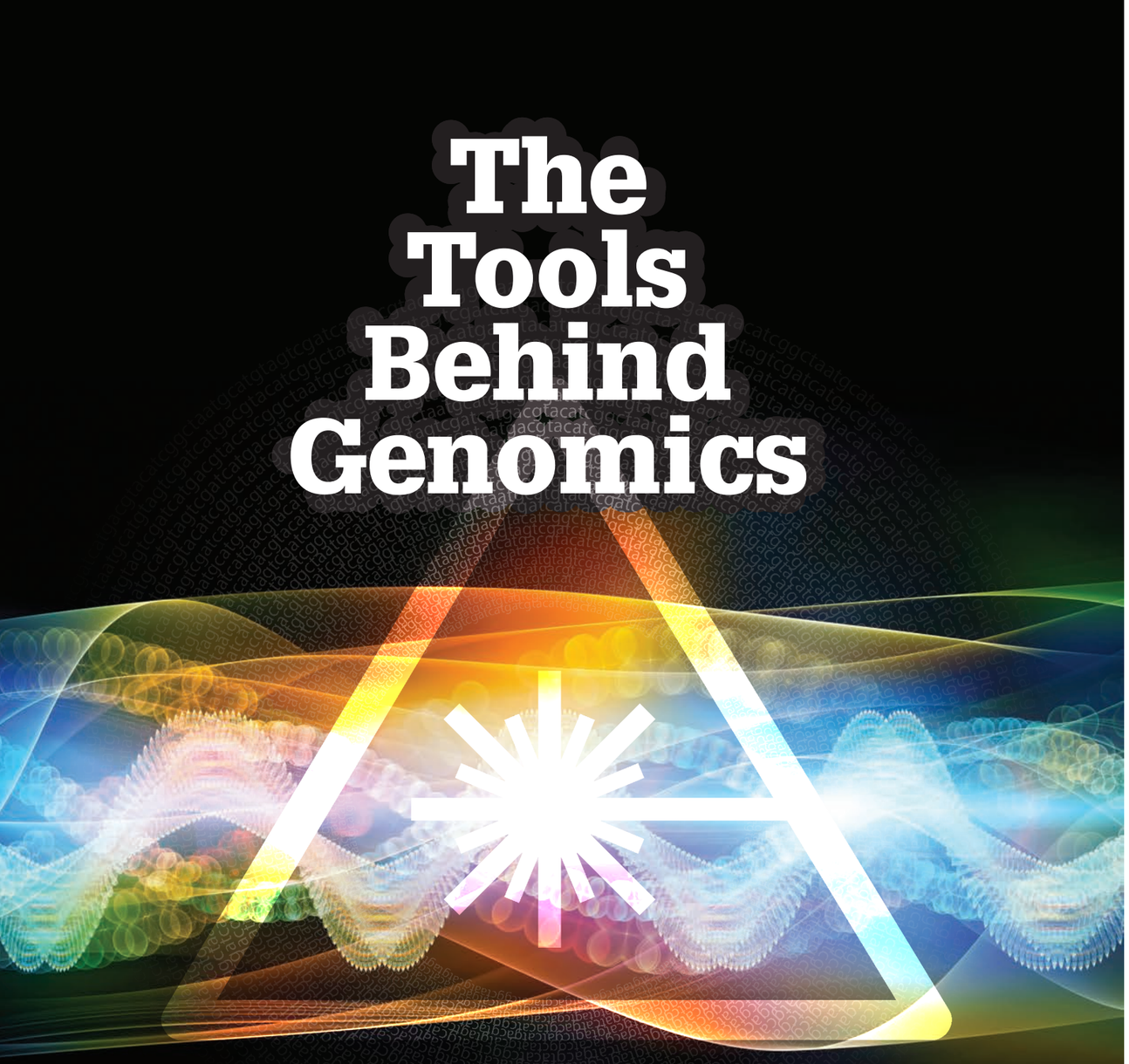
For baby-food, we initially employed this optimized fast LC-GC-FID method. Once MOSH contamination was encountered and quantified (levels up to 2 mg/kg were found, considering the BMELV proposed hydrocarbon range), we generated more detailed information using an off-line LC-comprehensive GC (GC×GC)-MS approach. Cryogenic GC×GC, a high-resolution two-dimensional technique, was performed by using an apolar-polar column set. The MOSH components of the UCM found in baby food were nicely separated on the two-dimensional chromatogram: linear and branched alkanes eluted along the same line (separated in the first dimension), while the naphthenes were resolved in the second dimension. At that point, interpretation of the mass spectral data became much easier.

Of course, an off-line method cannot be defined as rapid but there is no real technological obstacle in the construction of an on-line LC-GC×GC-MS instrument. Ideally, such instrumentation would include an FID unit, with the effluent directed to both detectors. This would provide rapid (fast GC×GC methods can be developed), high-resolution, and information-rich analysis of food contamination by mineral oil. What remains a problem is how to reduce or eliminate the consumption of toxic organic solvents.

References

1. *Scientific Opinion on Mineral Oil Hydrocarbons in Food, EFSA Journal, 10, 6 (2012).*
2. *K. Grob et al., Food contamination by hydrocarbons from lubricating oils and release agents: Determination by coupled LC-GC, Food Addit. Contam., 8, 437 (1991).*
3. *K. Grob et al., "Determination of food contamination by mineral oil from jute bags using coupled LC-GC", J. AOAC Int., (1991), 74, 506.*
4. *C. Droz and K. Grob, "Determination of food contamination by mineral oil material from cardboard using on-line coupled LC-GC-FID", Z. Lebensm. Unters. Forsch., (1997), 205, 239.*
5. *S. Moret et al., "Rapid and sensitive solid phase extraction–large volume injection–gas chromatography for the analysis of mineral oil saturated and aromatic hydrocarbons in cardboard and dried foods", J. Chromatogr. A, 1243, 1 (2012).*
6. *N. Concin et al., Mineral oil paraffins in human body fat and milk, Food Chem. Toxicol., 46, 544 (2008).*
7. *L. Mondello et al., "Determination of saturated-hydrocarbon contamination in baby foods by using on-line liquid–gas chromatography and off-line liquid chromatography–comprehensive gas chromatography combined with mass spectrometry", J. Chromatography. A, 1259, 221 (2012).*
8. *P. Q. Tranchida et al., "A rapid multidimensional liquid–gas chromatography method for the analysis of mineral oil saturated hydrocarbons in vegetable oils", J. Chromatogr. A, 1218, 7476 (2011).*

The Tools Behind Genomics



What underpinned three decades of explosive growth in genomics? Instrumentation and sample handling technologies developed by bioanalytical chemists and engineers, say two leading experts. Here, they summarize recent technological progress and anticipate the developments that will have the greatest impact in the near future.

By Norman J. Dovichi and Amanda B. Hummon

Progress in genomics, powered by advances in analytical chemistry, is transforming the study of life from a qualitative to a quantitative science. The continuous introduction of new approaches has enabled speed and sequencing capacity to rocket while costs have plummeted (see "Three Decades of Growth" [page 27]). It cost several billion dollars to sequence the first human genome. Today, twelve years later, entire genomes are being sequenced for $10,000 US dollars. Undoubtedly, the pace will accelerate still further as analytical chemists and engineers develop faster and cheaper sequencing technologies. This revolution will likely require generations of medical scientists to interpret, but ultimately will deliver major advances in health care.

Here, we look back at the origins of DNA sequencing, describe the range of approaches that are in use today and sketch the technologies that will drive the next phase of genome sequencing.

A Brief History

The field of genomics began with the discovery of DNA structure by Watson and Crick in 1953 (1). That structural model has served as a template for our understanding of the function of the molecule, although details remained elusive for two decades until the development of two powerful methods for the determination of nucleotide sequences in DNA. Initially, the chemical degradation method of Maxam and Gilbert was the tool of choice (2). However, that reaction used rather hazardous chemicals, and was supplanted in the late 70's by Sanger's dideoxy chain termination method, once the necessary reagents became commercially available (3).

"The continuous introduction of new approaches has enabled speed and sequencing capacity to rocket while costs have plummeted."

Sanger's method employs biochemical synthesis; a primer (which provides a starting point for DNA synthesis) is annealed to one strand of DNA in the presence of DNA polymerase, deoxynucleotide triphosphates, and a small amount of a single dideoxynucleotide. The DNA polymerase adds deoxynucleotides to the primer, synthesizing the complementary strand of DNA. Occasionally, a dideoxynucleotide is incorporated, which produces a strand that cannot be further extended; this addition is called a chain-termination reaction. Four separate reactions can be performed with different dideoxynucleotides. The reaction products are then separated in different lanes in a polyacrylamide gel. Sanger employed radioactive nucleotides and detected the fragments by autoradiography. Autoradiography-based sequencing throughput is ultimately limited by the visual interpretation of sequencing gels, which is a tedious and error-prone step in the procedure.

The next giant leap in sequencing technology came a decade later with the development of fluorescence-based automated sequencers. Leroy Hood's group at Cal Tech (4) and James Prober's group at DuPont (5) extended Sanger's method by replacing the radioactive label with fluorescent tags, and replacing autoradiography with laser-induced fluorescence detection.

In Hood's technology, a primer is labeled with one of four fluorescent tags. Each dye-labeled primer is used in a chain-termination reaction with a single dideoxynucleotide. For example, a primer labeled with a red dye might be used with the dideoxyadenosine chain terminating reaction, a green dye used with the dideoxycytidine reaction, a yellow dye with the dideoxyguanosine reaction, and a blue dye with the dideoxythymidine reaction. The reactions products are pooled and separated in a single lane of a polyacrylamide gel. A four-color spectrograph records the fluorescence intensity as fragments migrate past the fixed detector. The identity of the terminating nucleotide is determined based on the spectral characteristics of the dye. Prober developed a more sophisticated version of this technology where the chain terminating reaction is performed simultaneously with the four labeled dideoxynucleotides, simplifying the overall reaction.

Hood's and Prober's technologies replaced the laborious and error-prone steps of visual interpretation of an autoradiogram generated from a sequencing gel. And it was this automated reading of DNA sequence that catalyzed the human genome project. In 1986, a group of visionary biochemists realized that this new analytical technology could be used for the very large and ambitious goal of determining the sequence of the entire human genome. Initial efforts were funded first by the Department of Energy and then the National Institutes of Health in the U.S. Gradually, it became an international effort, drawing on scientific and financial contributions from many countries.

Despite the success of fluorescence-based sequencing, the systems were not fully automated. Preparation of the sequencing gels remained extremely laborious, particularly when envisioning such an ambitious program, so a modest

Table 1: Comparison and Relative Cost of Next Generation Approaches

Sequencer	*454 GS FLX*	*HiSeq 2000*	*SOLiDv4*	*Sanger 3730xl*
Sequencing Mechanism	Pyrosequencing	Sequencing by synthesis	Ligation and two-base coding	Dideoxy chain termination
Read Length	700 bp	50SE, 50PE, 101PE	50+35 bp or 50+50 bp	400~900 bp
Accuracy	99.9%*	98%, (100PE)	99.94% *raw data	99.999%
Reads	1 M	3 G	1200~1400 M	-
Output Data / Run	0.7 gb	600 gb	120 gb	1.9~84 kb
Time / Run	24 Hours	3~10 Days	7 Days for SE, 14 Days for PE	20 Mins~3 Hours
Advantage	Read length, fast	High Throughput	Accuracy	High quality, long read length
Disadvantage	Error rate with polybase more than 6, high cost, low throughput	Short read assembly	Short read assembly	High cost low throughput
Price	Instrument $500,000, $7000 per run	Instrument $690,000, $6000 / (30x) human genome	Instrument $495,000, $15,000 / 100 gb	Instrument $95,000, about $4 per 800 bp reaction
CPU	2* Intel Xeon X5675	2* Intel Xeon X5560	8* processor 2.0 GHz	Pentium IV 3.0 GHz
Memory	48 GB	48 GB	16 GB	1 GB
Hard Disk	1.1 TB	3 TB	10 TB	280 GB
Automation in Library Preparation	Yes	Yes	Yes	No
Other Required Device	REM e system	cBot system	EZ beads system	No
Cost / Million Bases	$10	$0.07	$0.13	$2400

KEY: SE: single-end sequencing, PE: paired-end sequencing, bp: base pairs, gb: gigabase, tb: terabase.
Data taken from Liu et al. "Comparison of Next-Generation Sequencing Systems" J. Biomedicine & Biotechnol. 2012, Article ID 251364

portion of the genome project funded improvements in the analytical technology. A number of approaches were investigated, including mass spectrometry, scanning probe microscopy, and microfabricated technologies. Unfortunately, those approaches lacked sufficient sensitivity, speed, robustness, or resolution and were abandoned.

Ultimately, technology based on capillary electrophoresis was developed by academic and industrial researchers, and successfully commercialized by Applied Biosystems and Amersham. The flexibility and robustness of fused silica capillaries facilitated the automation of the electrophoretic separation, in addition to the reading of the sequence using laser-induced fluorescence. The Applied Biosystems instrument, the model 3700 DNA sequencer, introduced in 1998, was the most successful and generated the lion's share of the sequence data for the first human genomes. That instrument was based on technology developed by Hitachi in Japan (6) and the University of Alberta in Canada (7).

In an interesting business decision, Applied Biosystems created a new company, Celera, whose goal was to use whole-genome shotgun sequencing, wherein a huge number of relatively short fragments were sequenced using ABI's model 3700 instrument. The overlap of these shotgun sequences was used to assemble the data into a coherent sequence. This technology had been pioneered by J. Craig Venter in earlier efforts to sequence smaller genomes. In contrast, the publically funded effort employed a series of mapping and cloning steps using a range of vectors in a more ordered approach. The commercial and publically funded projects proceeded in parallel, and they ended in a dead heat, describing their results in a set of publications that appeared in Science and Nature in February 2001. Over a billion dollars was invested in the sequencing effort to determine the three billion bases of sequence in the human genome, which was completed well ahead of schedule and well under budget.

The use of extensive mapping and cloning of a large genome has not been replicated. Instead, all modern sequencing efforts employ some form of whole-genome shotgun sequencing.

Next Generation DNA Sequencing

Capillary electrophoresis remains a useful tool for routine sequencing of small fragments. However, a suite of new technologies has been developed, providing spectacular improvements in analytical performance. Four such technologies are compared in Tables 1.

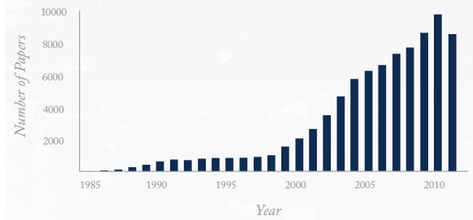
Pyrosequencing

A group at the Royal Institute of Technology in Stockholm developed an alternative to Sanger sequencing (8). The fragment to be sequenced is immobilized on a solid support along with a primer. DNA polymerase and a single deoxynucleotide triphosphate are then added. If the deoxynucleotide is incorporated into the strand, a diphosphate group (pyrophosphate) is produced. This molecule is converted to ATP by sulfurylase. The ATP acts as a substrate for luciferase, generating a flash of light upon incorporation of the nucleotide. If the nucleotide is not incorporated, no light is observed. The DNA sequence is then determined by treating the sample with different nucleotide triphosphates in series, and recording which nucleotide generates luminescence. The sequence of several hundred nucleotides can be determined in a single run.

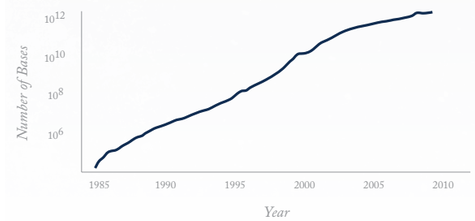
Pyrosequencing forms the basis of one of the earliest next-generation sequencing methods, which was developed by 454 Life Sciences, a subsidiary of Roche (9). It is a massively parallel form of pyrosequencing where millions of templates are sequenced simultaneously. Genomic DNA is fragmented and bound to beads under sufficiently dilute conditions to ensure that no more than one fragment is attached to a bead (see Figure 1). Beads are compartmentalized in an oil emulsion and the polymerase chain reaction (PCR) is used to amplify the fragments, creating a coat of 10^7 copies of the original sequence on the bead. Beads containing amplified fragments are enriched and captured into wells on a slide. Appropriate enzymes required for pyrosequencing are added to the wells and each of the four deoxynucleotides is pumped in succession over the slide and luminescence recorded. Although each step is relatively slow, sequencing

3 decades of growth

Only consumer electronics and computation have undergone the rapid technological growth seen in genomics and proteomics. That growth is illustrated by the number of publications indexed by Medline (upper panel) and the growth in the size of Genbank, the public repository for genomic sequence (lower panel). Bases deposited doubles every 18 months, an extreme example of Moore's law, a computing term from the1970's which held that processing power for computers would double every two years. And the Genbank data are the tip of the iceberg; much more sequencing data reside in private databases. The journal Nature reported in July 2012 that 10,000 human genomes have been sequenced, which corresponds to 3×10^{13} bases of finished sequence. When considering the redundancy with which genomic sequence is obtained, it is likely that well over 10^{15} bases of genomic sequence have been generated, the vast majority in the past 12 months.



Papers indexed in Medline under the topic of Genomics



Size of Genbank, the public repository for genomic sequence.

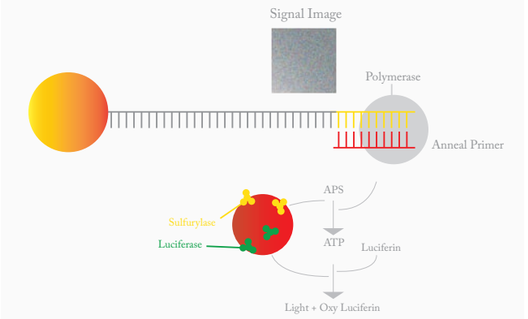


Figure 1: One Fragment = One Bead = One Read. In the 454 sequencing platform, a single-stranded template DNA library is generated via emulsion-based clonal amplification on sepharose beads. The amplified strands are then read by pyrosequencing.

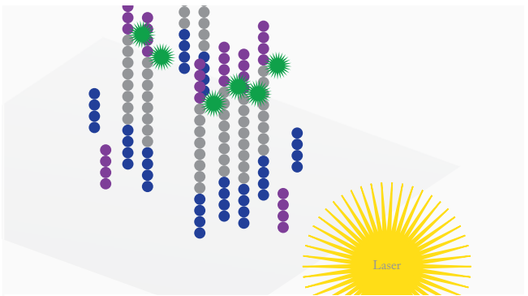


Figure 2: In Illumina sequencing, single strands of DNA are amplified into clonal clusters. Those clusters are sequenced by the addition and detection of reversible terminator labled nucleotides. As each of the reversible dye terminators has a distinct fluorescent tag, all four nucleotides can be interrogated at a single time.

data is generated from tens of millions of samples in parallel producing a gigabase of sequence per run, which is six orders of magnitude higher throughput than that produced by capillary electrophoresis instruments.

Reversible Terminator Sequencing

Illumina offers another next-generation sequencing method (10). Sequencing templates are immobilized on a surface to generate a "lawn of sequencing primers" (see Figure 2). A clever surface-based version of PCR is used to amplify each of the sequencing fragments and to immobilize those fragments in a micrometer region on the surface, generating clonal clusters. Sequencing is performed by hybridizing a primer to the immobilized fragments, with the addition of DNA polymerase and a modified deoxynucleotide triphosphate. This modified nucleotide contains a fluorescent tag that allows detection of the nucleotide once it has been incorporated onto the growing complementary strand. It also has a blocking group that prevents addition of another nucleotide. Excess labeled nucleotides are washed from the system, and a laser-based fluorescence detector images the surface. Those templates that incorporated the modified nucleotide are detected based on their fluorescence. The label and the blocking group are then removed chemically, and the process is repeated with another nucleotide, building up the sequence of each of the immobilized templates. This technology produces huge amounts of data, ~5 x 10^{10} bases per run, but each template only generates ~100 bases of sequence, which can challenge the assembly of the individual sequences into a complete genomic sequence; the instrument is ideal for resequencing known genomes rather than de novo sequencing of novel organisms. Because of this massive data output, Illumina currently controls around two-thirds of the sequencing market share.

Ligation-Based Approaches

SOLiD (Sequencing by Oligonucleotide Ligation and Detection, see Figure 3) also generates short sequences from large numbers of templates (11). Like the 454 technology, templates are amplified and immobilized on a solid particle based on emulsion PCR. Tens to hundreds of millions of beads are deposited on a glass slide and the sequence read by addition of a fluorescently labeled oligonucleotide. A ligase is used to covalently attach the oligonucleotide to the primer. Only those oligonucleotides whose sequence matches the complementary portion of the template are attached. The slide is imaged by laser-induced fluorescence, and clever chemistry is used to remove the fluorescence dye. The procedure is repeated, gradually extending the complementary strand. This procedure interrogates two nucleotide positions at a time, examining the 4th and 5th nucleotide in the template. Once the procedure is completed, the synthesized strand is removed, and a new primer is added which has an additional base, allowing the determination of bases that were missed in the first pass. A set of five primers is used to determine the sequence across ~25 to ~50 nucleotides. The instrument is capable of generating over 2 x 10^{10} bases of sequence per day, and of sequencing a genome in a few days.

Proton Detection

Ion Torrent (see Figure 4) employs similar chemistry to pyrosequencing and relies on the scalable manufacturing pipeline developed by the semiconductor industry (12). Unlike the other detection schemes, however, Ion Torrent utilizes non-photonic detection. The addition of a deoxynucleotide triphosphate to a growing oligonucleotide results in the formation of a proton in addition to the pyrophosphate group. This proton causes a drop in the pH of the solution, which can be detected with a high sensitivity pH meter. Ion Torrent deposits pH sensors at the bottom of a huge number of wells on a microfabricated device. Sequencing proceeds by amplifying large numbers of DNA fragments, depositing them into the wells, and then flowing DNA polymerase and a deoxytriphosphate over the device. Those wells that contain a fragment that incorporated the nucleotide will experience a pH decrease, which is sensed by the electronics. Even though only 20-40 percent of the wells are used in an Ion Torrent chip, the massively parallel nature of the design still results in high data output. The manufacturer claims that over 4 x 10^{11} bases of sequence are generated in a two-hour operation, with over 99.5 percent accuracy.

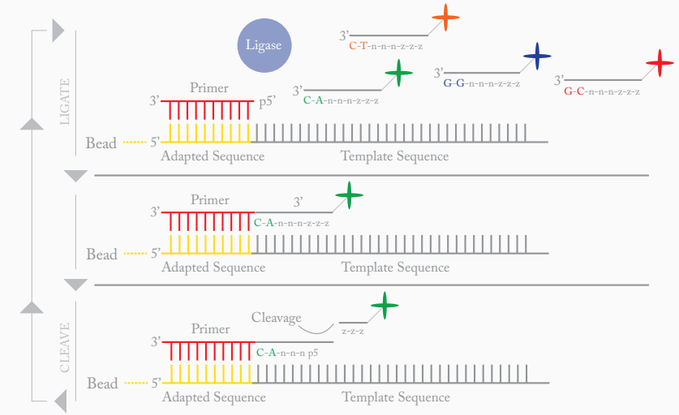


Figure 3: SOLiD sequencing. In sequencing by ligation, adapter sequences are added to the 5' end of the DNA strand of interest. Primers are hybridized to the adapter sequence and then fluorescently labeled di-base probes compete for ligation next to the sequencing primer. The specificity of the di-base probe is achieved by interrogating every first and second base in each ligation reaction. The DNA strand is fully sequenced and then all primers are removed. The process is repeated with a second primer shifted by one nucleotide. In this way, every position is interrogated twice.

Figure 4: Ion Torrent. A bead with clonal, amplified sequences is deposited in a pore. Sequencing primers and DNA polymerase are added. As nucleotides are added to the sequence, protons are released and detected. Based on the quantized signal, the number of protons, and hence, the number of nucleotides added, can be determined.

Is Single Molecule Detection The Future?

There has been significant interest in extending whole genome sequencing to the general population as a guide to individualized medicine. The National Human Genome Research Institute (NHGRI) has invested heavily in translating the genomic sequence into guidance for the treatment of patients at their bedside. However, for individual genomic sequencing to be a reality, costs must drop by one to two orders of magnitude. To this end, NHGRI has active funding solicitations with the goal of obtaining whole genome sequences for $1000.

In 2008, Helicos became the first company to offer what it termed "True Single Molecule Sequencing" with its HeliScope technology (13). A short DNA strand (25 bp) is prepared and a PolyA primer added to the 3' end. Up to a

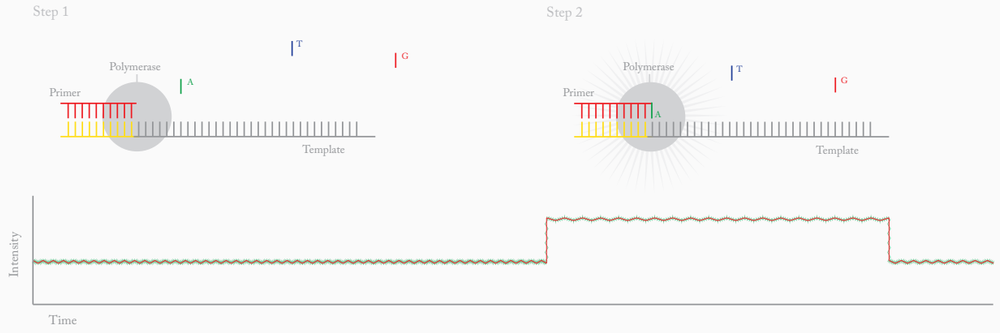


Figure 5: SMRT Sequencing. A DNA polymerase is immobilized with a template single strand of DNA. Primers and fluorescently labeled nucleotides are added. As nucleotides are incorporated with the nascent DNA strand, they are excited by the laser and emit a distinct microsecond emission. Once the nucleotide is added, the dye is cleaved and the signal diminishes.

billion DNA strands are hybridized to a DNA flow cell with Oligo dT capture sites. Sequencing reactions are detected from individual molecules with the addition of fluorescent-labeled nucleotides. In its marketing, Helicos placed great emphasis on freedom from amplification, citing errors in the PCR process as a major flaw with other sequencing approaches. Unfortunately, Helicos was unable to generate sufficient cash flow to service their debt, and they filed for Chapter 11 bankruptcy on November 15, 2012.

Pacific Biosciences has also developed a single molecule detection approach, termed SMRT for Single Molecule Real Time sequencing (14). SMRT and PacBio, as it is commonly called, initially generated a lot of buzz in the genomics world. The SMRT system (see Figure 5) offers unlimited read length, no need for PCR amplification, and fast detection. The methodology is based on using DNA polymerase as a real-time sequencing engine. Multiple DNA polymerases are immobilized in Zero Mode Waveguides and then monitored via fluorescence for the addition of labeled nucleotides. The approach is very promising, but has not been readily adopted by the genomics community because of the expensive instrumentation, coupled with a higher relative error rate compared to other sequencing approaches.

Nanopore: newest (and brightest?)

In February, 2012, Oxford Nanopore announced the development of their sequencing platform, based on passing single DNA strands through protein nanopores embedded in a polymer membrane (see Figure 6). As the nucleotides pass through the protein channel, a detectable change in the membrane current occurs (15). Each of the four nucleotides produces a distinct signal, allowing discrimination and ultimately, a DNA sequence. If a hairpin structure is present, both the sense and antisense strands can be read for each DNA molecule. The approach can also be performed in an "exonuclease-sequencing" fashion, where an exonuclease is positioned over the nanopore and the individual nucleotides are cleaved and translocated through the pore one at a time.

Nanopore is currently generating interest for several reasons. The nanopore technology does not appear to have read length limitations. During the initial press conference, Oxford Nanopore announced that they had sequenced a 48 kb virus genome in a single pass. Another appealing aspect is cost: Users do not need to purchase expensive instruments, buying only a disposable sequencer, which is about the size of a USB memory stick, for an estimated $900. At the time of writing, the Nanopore system was not available for sale nor has a peer-reviewed article demonstrating the capabilities of the technology been published – two events that are eagerly anticipated by the genomics community.

What next?

Nearly three decades of technology advances have now reduced the cost and speed of genomic sequence to the point where whole genome studies are routine. Further advances are on the horizon, but we are approaching the point of diminishing returns; each individual genome is generally static, so there is little need to resequence. The obvious exception is in tracking cancer progression, where loss of tumor suppressor genes carries a grim prognosis. Genomic analysis is likely to reach maturity within a decade, when the cost of sequencing a human's genome drops to ~$100.

We have not addressed epi-genomics, which deals with post-replication modification of specific nucleotides in the genome. Most common is the incorporation of a methyl group in cytosine residues, which has implications in gene expression. In comparison to genomics, epi-genomics is in its infancy. There is a real need for improved technologies to produce low cost, high-speed analysis of the epi-genome, which will be invaluable in understanding developmental biology and disease progression.

We have also not addressed transcription analysis. The use of reverse transcription and either hybridization arrays or whole-genome sequencing have become powerful tools in transcriptomics. Real-time PCR provides quantitation across many orders of magnitude. Technology for transcriptomics is likely to reach maturity within a decade of the maturation of genomics.

Conclusion

Freeman Dyson said "New directions in science are launched by new tools much more often than by new concepts" (16). The astonishing advances in genomics over the past two decades are a striking demonstration of this statement. Within living memory, the determination of a few bases of DNA sequence would require a graduate student's entire career. Today, whole genome sequences are generated within a few days at core facilities located on many university campuses.

This advance in technology results from a set of wise choices first by the Department of Energy, then by the National Institutes of Health, and now by the private sector. First, these funders identified a problem and articulated it clearly. Second, they provided significant funding levels for a wide range of technologies. Third, they culled unsuccessful technologies. Fourth, they nurtured those technologies that showed progress toward the solution of the problem. This model has proven to be remarkably robust in the development of analytical instrumentation, and has provided an astonishing return on investment to the public and private sectors. ••

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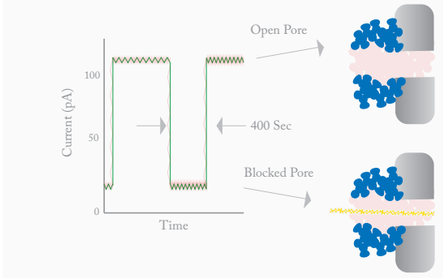


Figure 6: Nanopore strand-sequencing using ionic current blockage. DNA/RNA are negatively charged and are electrophoretically driven through the nanopore channel.

References

1. *J. D. Watson and, "Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid," Nature, 171: 737–8 (1953).*
2. *A. M. Maxam and W. Gilbert, "A new method for sequencing," Proc. Natl. Acad. Sci. USA, 74: 560–4 (1977).*
3. *F. Sanger, S. Nicklen and A.R. Coulson, "DNA sequencing with chain-terminating inhibitors," Proc. Natl. Acad. Sci. USA, 74: 5463–7 (1977).*
4. *L. M. Smith, et al., "Fluorescence detection in automated DNA sequence analysis," Nature, 321: 674–9 (1986).*
5. *J. M. Prober, et al., "A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides," Science, 238: 336–41 (1987).*
6. *H. Kambara and S Takahashi, "Multiple-sheathflow capillary array DNA analyser," Nature, 361: 565–6 (1993).*
7. *D. Y. Chen, et al., "Low-cost, high-sensitivity laser-induced fluorescence detection for DNA sequencing by capillary gel electrophoresis, J. Chromatogr., 559: 237–46 (1991).*
8. *M. Ronaghi, M. Uhlén and P. Nyrén, "A sequencing method based on real-time pyrophosphate," Science, 281: 363–365 (1998).*
9. *D. A. Wheeler et al., "The complete genome of an individual by massively parallel DNA sequencing," Nature, 452: 872–876 (2008).*
10. *E. R. Mardis, "Next-generation DNA sequencing methods," Annu. Rev Genomics Hum. Genet., 9: 387–402 (2008).*
11. *J. Shendure, et al. "Accurate multiplex polony sequencing of an evolved bacterial genome," Science, 309: 1728–32 (2005).*
12. *J. M. Rothberg, et al. An integrated semiconductor device enabling non-optical genome sequencing. Nature, 475: 348–52 (2011).*
13. *T. D. Harris, et al.," Single-molecule DNA sequencing of a viral genome," Science, 320: 106–9 (2008).*
14. *J. Eid et al., "Real-time DNA sequencing from single polymerase molecules," Science, 323: 133–8 (2009).*
15. *S. Howorka, S. Cheley and H. Bayley, "Sequence-specific detection of individual DNA strands using engineered nanopores," Nat. Biotechnol., 19: 636–9 (2001).*
16. *F. J. Dyson, "Imagined Worlds," Harvard University Press, Cambridge MA, 1998.*

Futuromics: The Proteome *Don't miss Dovichi and Hummon's overview of analytical aspects of the proteome in our April issue*

The Analytical Entrepreneurs

Here's how five successful analytical science businesses were developed from scratch. Within a framework of essential good practice, each company developed with a uniqueness that mirrors its founder(s). The lesson? To be an entrepreneur, you can blaze your own trail.

By Richard Gallagher

Who among us doesn't occasionally dream of going it alone? Of taking our hard-won expertise, our relationships with the movers and shakers, and our sure-fire idea for a product or service, and setting up our own company?

The five trailblazers (and their four companies) featured here did just that. Below, and in the accompanying online articles and podcast, they tell their stories: how – and why – they became entrepreneurs, how their experiences differ from working in industry or academia, and how they continue to be challenged, exhilarated and deeply satisfied by their work.

Origins

What catalyzes that move from daydreaming about a new venture to actually starting one?

Eric Yeatman, a researcher at Imperial College in London who set up Microsaic with two colleagues over a decade ago, was driven by the desire to get his ideas out into the world. "In a practical field like engineering, that's a strong motivating factor," he says. Top technical universities want to demonstrate an economic impact, so Imperial was encouraging, Yeatman explains, and signed over intellectual property rights in return for an equity stake. The die was cast.

Terry Berger cites "Stubbornness, and a belief in the technology and myself," as the drivers behind Berger Instruments. In the 1980's, Berger was assigned by his employers, Hewlett Packard (now Agilent), to look at the market for Supercritical Fluid Chromatography (SFC). He came to the conclusion that its sweet spot was as a competitor to reversed phase HPLC and not capillary SFC, which was the prevailing view. A decade later, HP launched an instrument that tried to fulfill both functions, with mixed success. Despite being "huge and expensive," the product had "revolutionary performance that was slowly changing the way people saw the technology," Berger says. "So I bought out the SFC business when HP restructured."

"The idea to start my own company was ignited while I was head of sales in mainland Europe for International and American Laboratory magazines," says Marco Koenen. "I was in touch with a lot of entrepreneurs in the field of chromatography and had the opportunity to 'look into many kitchens,' as we say in The Netherlands, finding out about manufacturing, distributing and selling laboratory equipment." His attempt to get the e-commerce platform, Labworld, off the ground in 2002 failed in the wake of 9/11 and the dot-com bubble burst, but Koenen was undaunted. That same year, he started JSB along with Fedde Keegstra, with Koenen handling the commercial arm of the business and Keegstra taking care of the technical side.

Divisions of labor were also used by husband-and-wife team Alun Cole and Liz Woolfenden, who launched Markes International in 1997 after spotting an opening in

the market. “We realised that a full-functionality thermal desorption system for single sample tubes would be ideal for academia and smaller laboratories – markets that were not catered for by the product ranges available at that time,” says Woolfenden. While working very closely together, Liz took primary responsibility for interactions with customers as the company got up and running, with Alun handling business and financial aspects – assignments that have endured.

Finances

Approaches to funding are as varied as the businesses themselves.

JSB worked with a local bank in Eindhoven to secure finance. “There are a lot of high tech companies in the region so the bank has technical specialists,” Koenen explains. Last year Eindhoven was selected the ‘smartest’ region in the world. “Our account manager even worked with chromatography in a previous life,” he says. "Given that JSB is a value-added reseller of Agilent Technologies with a focus on hyphenated gas chromatography or HPLC solutions, that’s quite a plus."

Berger’s story has a rather more pioneering feel. “We got by on a wing and a prayer,” he says. Mark Shuman, Berger’s business partner, had a golfing companion who owned an old converted church that housed eight small businesses. He became a third partner and Berger Instruments launched in the church basement with deferred rent and an answered phone. “Neither Mark or I took any salary for the first year, we lived on personal savings,” Berger recalls. Worse still, at the company’s darkest moment the two borrowed against their personal independent retirement accounts. “There was no penalty if you put the money back within 60 days,” he explains. “Mark borrowed against his, then 59 days later I borrowed against mine, to repay his, with no guarantee I would get it back to repay the loan. We did this at the end of one year and then repeated it early the next year. In this way, Mark and I provided the cash flow for the company for eight months, which got us to the next level.” Scary stuff!

Others have enjoyed a smoother ride. Markes International has been privately funded throughout, and consistently profitable since the initial nine-month start-up phase. “Cash flow is by far the most important factor throughout the life of any company,” says Alun Cole. “It allows you to remain in charge, and gives you the flexibility to develop the business in a way that makes the best sense in the long-term.”

Eric Yeatman alone has experience in raising capital and going public. “When we first raised funds in 2006, the environment was fantastic,” he says. “We had a quick valuation and raised a decent amount of money, which kept us going for several years.” By the time Microsaic started thinking about further fundraising it was 2009, the situation had changed completely and the risk appetite of investors had pretty much disappeared. “It was very, very difficult and the market has been bumpy ever since,” Yeatman says. Despite this, the company launched a successful Initial Public Offering, (IPO, the first sale of stock by a private company to the public) in the spring of 2011. “We caught a bit of a window and did our homework, so we managed to get a few investors enthusiastic about backing it for floatation,” he explains.

"Absolutely nothing stands still for more than five minutes. It’s like the painting of the Forth Bridge – as soon as you reach one end, you have to start again at the other."

Growth

Once the business is animated, a period of growth and development can ensue – or not – depending on the decisions taken (see ‘A 15-Point Plan for Entrepreneurship’ [page 39]). What crucial actions did our entrepreneurs take?

“Anticipating success,” says Alun Cole. “When we set up, even when we were just four or five people, we had the framework of a large organization.” Structuring the business by discipline – research, production, sales, finance, etc., – right from the off, avoided the pitfall of focusing on a ‘pet’ aspect of the business, he says.

“Knowing how the market is developing, and then creating focus within your organization to address those developments,” is what Marco Koenen sees as a key factor in JSB’s success. He warns against small companies developing

Alun Cole and Liz Woolfenden, Markes International Limited

Age, Location: 56 and 52 years; Llantrisant, Wales
Tweet your company: Providers of instrumentation for monitoring and analyzing trace-level chemicals in air and gas streams across diverse application areas.
History: Founded in 1997 with two employees and two contract engineers. Currently, 80 employees.
Major customers: Our global customer base includes major industry, government agencies, academia and the service laboratory sector.
Origin of name: We have four children, and Markes comes from the initial letters of all our names: Martyn, Alun, Rebecca, Kate, Elizabeth and Simon.
Most satisfying moment: Building a market-leading company while making a real contribution to the science of thermal desorption and TOF MS.

Marco Koenen, JSB Group

Age, Location: 45 years; Eindhoven, The Netherlands
Tweet your company: A high end system integrator of Agilent Technologies, providing innovative and creative solutions focused on gas chromatography and HPLC.
History: Started in 2002 by myself and Fedde Keegstra. Now has staff of 20.
Major customers: The petrochemical, environmental, pharmaceutical and research institutes in the Benelux countries, Germany, UK and Scandinavia.
Origin of name: JSB stands for Just Simply Better. It's what we try to be for our customers.
Most satisfying moment: Becoming the preferred supplier of a UN-related organization. We configured complex mobile GC-MS systems and installed them all over the world.

a 'supermarket mentality,' and encourages start-ups to have the confidence to step away from business opportunities that do not fit their focus. "Leave them to partners, or even to competitors," he advises. By targeting its products and services, JSB became a stakeholder in finding solutions for customers. "This has created more sales and faster growth despite turning down some opportunities."

Clearly, deciding what products to develop and what markets to aim at are crucial in the growth phase. Koenen adds a third factor to the mix: with whom you choose to partner. "It is essential to do a thorough analysis to ensure you select a reliable partner that will match your own goals." JSB aimed high, working with Agilent.

One thing that every new company needs is a detailed, comprehensive business plan. "But you cannot project longer than about three years ahead," Liz Woolfenden notes. Indeed, she recommends a rolling three-year plan that is constantly updated. "Absolutely nothing stands still for more than five minutes. It's like the painting of the Forth Bridge – as soon as you reach one end, you have to start again at the other. It's not that your plan was wrong last time around, but the market and your company are constantly evolving, so the plan needs to evolve too."

"It is essential to do a thorough analysis to ensure you select a partner that will be reliable and a good match to your own goals"

Where several of our companies exploded out of the blocks with a definite product in mind, Microsaic took time to weigh up the different options in its portfolio. "In the first few years we were quite broad and explored technologies in optical telecoms and electronic switching," Eric Yeatman explains. "We concluded that mass spectrometry held the most promise, then did our first round of fund raising in 2006 and started to build up the product development function." Microsaic were bold enough to take a second critical decision, switching from gas to liquid analysis, to focus on the pharma market. "That was a big leap and quite a risky one because we had a lot of technology development to go from one to the other."

Another requirement in the early stages is to get the word out about the new company. Koenen is in no doubt about the most effective method. "Mouth-to-mouth advertising is best," he says. "Having your customers talk about the high quality of service that you are delivering is priceless."

To gain such powerful endorsement means, of course, that you must fulfill customers' expectations. This is the one item mentioned by all of our entrepreneurs as a key factor in growing a successful company. Flexibility, speed of service and quality of support are essential, says Koenen, while Terry Berger notes that "You must truly understand, at the deepest level, what the customer needs." He also emphasizes outstanding service. "There is nothing worse for your business than having a customer who can't work because your instrument is broken."

Strong advertising campaigns and useful newsletters are also part of the mix, as is a presence at tradeshows. Koenen believes that it is important to stand out: during a recent trade show, for example, JSB had a Formula One racing car on its booth. "The applications we had on display were all linked to the sport, such as fuel and paint analysis, and pyrolysis-GC of tires analysis," he explains. "These are real life customer applications and they illustrated what we can deliver. Plus, it was a lot of fun and we gave visitors the chance to win a day of racing."

People

Building a company means expanding staff numbers while keeping the spirit of the startup intact. What advice do our entrepreneurs have on identifying and attracting the right people?

"I like to keep an eye open for talent," says Yeatman. "I'm constantly encountering people in business and personal life, and being involved in academia offers a third group. When I spot someone who fits the bill, I try to get them interested in the company."

Academic connections can make it relatively easy to get technical staff with the right skills and background, but Berger, whose career is in industry, also believes in hiring people that he knows well. "I think it is pretty easy to identify real contributors and 'true believers' in the technology," he says.

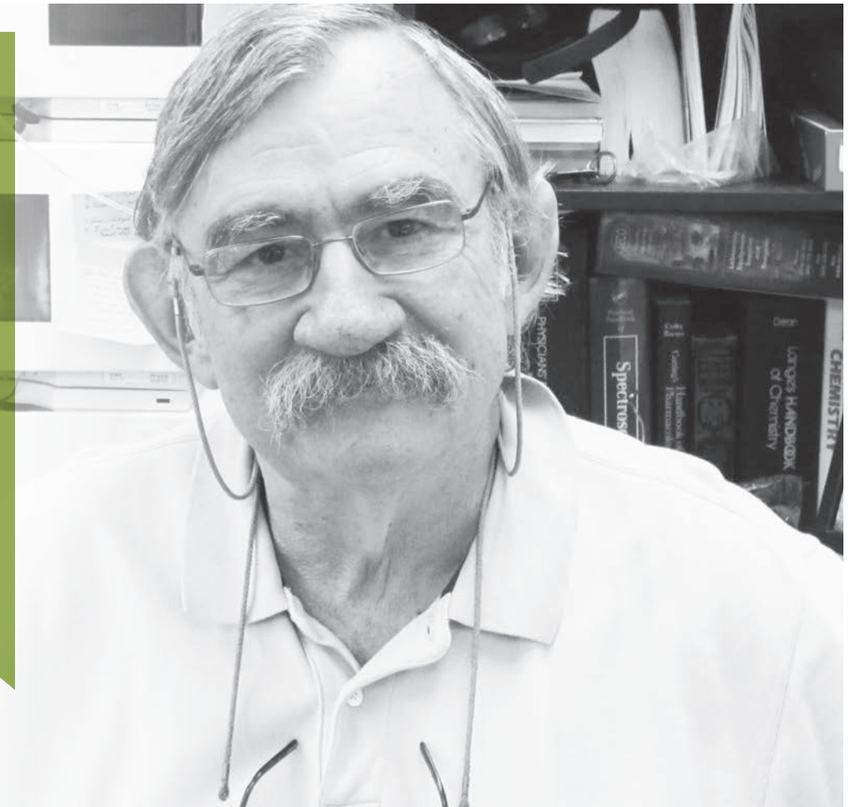
It's also important to attract new blood – people who add skills and perspectives that complement your own, says

Eric Yeatman, Microsaic Systems

Age, Location: 49 years; Woking, England
Tweet your company: Developing and manufacturing miniature mass spectrometers to put analytical power into the hands of many more users than have it today.
History: Started 11 years ago by three colleagues in electrical engineering at Imperial College with a focus on silicon micro-engineering. Focus on mass spec since 2006, now has 30 employees.
Major customers: Currently concentrating on the pharmaceutical and related sectors.
Origin of name: Microsaic is a contraction of 'Microsystems at Imperial College.'
Most satisfying moment: Delivering our first system to a customer.

Terry Berger, Berger Instruments

Age, Location: 66 years; Company was based in Newark, DE, USA
Tweet your company: A start-up promoting Supercritical Fluid Chromatography (SFC), a new technique in separation science.
History: Started in 1995, sold at the end of 2000. Forty-three employees plus several distributors at time of sale.
Major customers: Pharma discovery and drug development.
Origin of name: My partners decided that my scientific reputation was a differentiator and suggested the name.
Most satisfying moment: People at a trade-show saying the product we sold them is the best thing ever.



A 15-Point Plan for Entrepreneurship

1. If your only goal is to get rich quick, don't bother. If you seek job satisfaction, learning opportunities and excitement, read on.
2. Look for a niche market – quality is the key, not quantity.
3. Know your market. Figure out what you can do differently to meet customer needs and differentiate yourself from the competition.
4. Before you start, demonstrate your dedication to yourself by mastering the technology side of your business.
5. Be very sure that you can handle the pressure and appreciate what you are asking your family to go through – they are taking the risks with you.
6. Control your cash flow. This allows you to remain in charge, and gives you the flexibility to develop the business in the way that you know makes best sense long-term.
7. Invest in communication and self-management skills. Exude confidence and, if needed, display a little irrational optimism.
8. Try to keep product positioning, structures and processes simple, and focus on your people.
9. Do not immediately adjust your strategy because a sudden opportunity seems to promise a faster way to success: consistency, continuity and focus are important. Be patient, it's going to take longer than you thought.
10. Be passionate about doing the best possible job in all areas of your business, whether you're already familiar with it or not.
11. Continuously update your knowledge and know-how, and seek (and accept) advice from more knowledgeable people, including colleagues and stakeholders.
12. Don't be afraid of failure and always have a plan B. And a plan C.
13. Only take on as much as you can cope with: have the courage to say "no".
14. When using consultants, ask for recommendations and use only those with a proven track records and relevant experience.
15. Truly understand your customers and give them the best possible product and service quality. Get their feedback, and do something with it.

Koenen. "I look for accomplished people who display drive and dynamism, and who enjoy having a lot of freedom." Berger agrees. "Hire the best people you can find, give them the tools they need, then get out of their way."

Given the size of teams at young companies, every new appointment is vital. Even so, Yeatman points out that hiring decisions must focus on long-term contributions, rather than on short-term tactical considerations. "Hire the person who is going to chip in over years, even if he or she may not make the greatest difference the first day they walk in the door," he says. "Surveys of business leaders show that the staff members with the biggest impact are the ones with the right attitude and generic traits, rather than those who were specifically qualified for a particular role."

Once you've found the right people, the challenge becomes involving them as much as possible in the vision and work of the company. Leadership style is important. "You must establish the vision of the company and make sure everyone is on board with it," says Berger. "This means leading by example. It also means trusting your people to do the right thing, but fix mistakes – theirs and yours – as soon as you can.

Alun Cole and Liz Woolfenden's prescription for successful team building includes:

- Holding regular team-building meetings and developing internal communications channels, such as company newsletters, to engage staff and keep everyone in the loop.
- Encouraging a culture where mistakes are acceptable, provided that they are acknowledged and that lessons are learnt from them.
- Expecting the best from people and being tough on sloppiness.
- Instilling a culture that demands the best possible service for customers – one that customers will remember and keep coming back for.
- Not tolerating any form of office politics.

All five view setting up their own company as a life-enhancing experience. Woolfenden and Cole describe it as "incredibly rewarding," and Koenen says, "This job gives me enjoyment and fulfillment on a daily basis". And when asked whether or not they would go through it all again, for Yeatman, "it's in my blood now," while Berger responds that he already has – several times.

SPME Goes Mainstream

Solid-phase microextraction combines fast and simple analyte extraction with detection in the parts per trillion range. Here, pioneers of the technology predict that it is set to break out of the bioanalytical lab and into the clinic.

By Barbara Bojko and Janusz Pawliszyn

The development of solid-phase microextraction (SPME) applications began almost 25 years ago (see solid-phase microextraction 101, opposite, for an explanation of the technique) and has slowly expanded in reach.

In the early days, the primary application was in gas chromatography (GC) platforms to analyze volatile compounds. This established SPME as one of the standard methods for air analysis, and these environmental credentials were enhanced by water analysis, which today includes both spot and passive water sampling and in vivo fish sampling [3]. One example of the utility of SPME is that, previously, monitoring environmental exposure and calculating bioaccumulation required that fish be sacrificed for sample collection, tissue homogenization and extraction with organic solvents. Using SPME, we simply place a small fiber in the muscle for a few minutes to extract analytes. The fiber is then removed for analysis and the fish is released. This low-invasive, non-lethal approach generates the same data as standard procedures.

Another established application for SPME is in the food industry. The analysis of volatile compounds can profile trace contaminants, monitor ingredients and identify the sample's origin. GC platforms dominate these analyses, and the attraction of combining these with SPME, and in particular headspace SPME, lies in the simplicity of the method and the availability of automated SPME autosamplers, which save time and increase precision. SPME is increasingly being coupled with liquid chromatography (LC) analysis as the range of commercially available coatings compatible with LC solvents expands (see 'Where SPME Wins [page 42] and Table 1 [page 43]).

More recently, attention has turned to biomedical applications, such as the monitoring of drug concentration or screening of metabolites. The development of high-resolution mass spectrometers combined with the microextraction capabilities of SPME makes SPME-MS a powerful analytical tool.

Solid-Phase Microextraction 101

Solid-phase extraction (SPE) separates compounds in a mobile phase by their affinity for a particular solid in the stationary phase. In solid-phase microextraction (SPME), the polymeric sorbent (the extraction phase) can be solid or rubbery and is attached to various solid support structures (see Figure 1). Fibers, the most commonly used form of solid support, are typically used to extract volatile compounds for GC applications and non-volatiles for LC. Other SPME preparations include thin films, coated membranes, coated vessels and suspended particles [1]. Most recently, SPME has been used as coated mash to improve sample clean-up; this improves sensitivity signals for a target analyte when coupled directly to a Direct Analysis in Real Time (DART)–MS/MS platform [2].

SPME relies on the diffusion of analytes from the sample matrix to the extraction phase with the goal of reaching equilibrium between phases (see Figure 2). In extractions from complex biological matrices, that is, biofluids and tissues, the biological system itself acts as a 'buffer' for the extraction phase, preventing saturation of the coating and competition between hydrophilic and hydrophobic analytes. In simple terms, the free-fraction concentration of polar compounds is high in aqueous biological systems, whereas non-polar species tend to be bound in complexes with macromolecules like proteins. Another important factor that contributes to balanced coverage of analytes is the affinity between the extraction phase and the compound. SPME coatings are mainly hydrophobic and therefore have high affinity for non-polar compounds and low affinity for more abundant hydrophilic species.

Clinical growth

Our group now has a significant focus on preclinical and clinical applications of SPME, spanning therapeutic drug monitoring to pharmacokinetics, and global screening of the metabolome to capturing the characteristic changes induced by stimuli, such as disease state or response to therapy.

Thanks to a collaboration with physicians at Toronto General Hospital (TGH), Canada, we have begun to validate SPME in clinical practice. Working with the Department of Anesthesia, we are studying two groups of patients: one is undergoing various surgical procedures, the other includes patients at different stages of renal dysfunction. This is the first time that the technique has been used in relatively large-scale studies in a clinical setting; we have analyzed hundreds of samples, showcasing SPME's automated, high throughput capabilities. With samples being taken from authentic cases where our colleagues at TGH need our support to solve clinical problems, SPME was truly tested because it was not possible to cherry pick the matrix, drug or concentration to be studied. Sometimes the solutions were relatively straightforward; on other occasions we encountered previously unknown difficulties.

Where SPME Wins

- Improved sample clean-up versus protein precipitation (PPT) and ultrafiltration (UF) methods
- Open-bed format eliminates cartridge-clogging issues seen with solid-phase extraction (SPE)
- One-step extraction of hydrophilic and hydrophobic compounds versus time-consuming two-step liquid-liquid extraction (LLE)
- Easy automation for GC and LC couplings
- On-site and in vivo applications do not require pumps and tubing, in contrast to microdialysis (MD) and UF
- Non-selective and highly selective extraction, depending on sorbent
- Flexible extraction phase geometry, for example, fiber and thin film (blade) for direct immersion in vivo studies.
- Low invasiveness – no sample collection required for in vivo analysis
- Direct extraction from complex solid matrices allows monitoring of spatial distribution

Recently, we have been working on the pharmacokinetic profile of tranexamic acid, an anticoagulant administered to patients undergoing heart surgery alongside a cardiopulmonary bypass. The drug is often the only anticoagulant used in such cases, and its dosing regime is based on mathematical predictions. We revealed that the concentration of the drug varies from patient-to-patient, with a higher than expected average value. This suggests that some corrections to dosing may be required [4]. In parallel, we monitored changes in the metabolome profile of these patients. This helped us understand the metabolite response to the surgery and pharmacotherapy, and also uncovered some notable individual response, which is of interest in terms of personalized medicine.

A further collaboration, with a group of thoracic surgeons at TGH, focuses on in vivo profiling of the lung metabolome during organ transplantation. The aim is to identify new biomarkers. This surgical team is a world leader in novel methods of lung preservation and transplantation and we hope that the planned development of SPME-based rapid diagnostic tools will further improve their results and those of other surgical teams worldwide.

Drug testing

One of our most topical challenges is a project sponsored by the World Anti-Doping Agency (WADA). We are one year into the development of high-throughput methods for the simultaneous determination of over 120 doping substances of varying chemical and physical properties and the results are very promising.

There are a number of methods to screen those substances already, but most procedures focus on a certain group of compounds with similar properties. Our objective is to achieve the requisite coverage, and satisfactory recovery, in a single analysis. To do so, we use a high-throughput automated system to perform 96 analyses simultaneously. WADA criteria state that compounds must be detected at sub-ppb or -ppt levels. To meet these, we use a thin film geometry SPME device, called a blade. To date, urine analysis has been optimized and we have started working on plasma and blood. The protocol requires minimum sample handling compared with currently-used approaches.

In light of recent news reports, including Lance Armstrong's alleged offenses, it is safe to say that there is space for a new tool to monitor doping substances in sport. We hope that SPME can be a part of this.

Biomedical analysis

In biomedical analysis, SPME is still being predominantly used in an academic setting. Although the number of published papers is growing, the technique appears to be too new to have become established in routine analysis.

That said, SPME can be considered to be an alternative for some current assays, particularly those that require time-consuming sample preparation, such as drugs or toxins. Another niche application is where automation is difficult and analysis throughput is low. SPME offers simple automation for both volatile and non-volatile compounds – particularly in the latter case where autosamplers for leading GC platforms offer integrated SPME systems for high throughput analysis.

The main roadblock for introduction of SPME into hospitals is that mass spectrometry instrumentation, to which it is commonly coupled, is not standard. However, the powerful combination of SPME and MS is already drawing attention in therapeutic monitoring of drugs or biomarkers, which points to a major desire of the clinician: receiving information in real time. One trend in medical diagnostics is bedside analysis in place of lab analysis, which is cost-effective and user-friendly in addition to time-saving. Chip-based, microfluidic devices have been designed to be used on site by medical personnel, but these have limitations, such as

Table 1: SPME versus solid-phase extraction (SPE)/protein precipitation (PPT)

Feature	*SPME*	*SPE / PPT*
Nature of extraction	"Soft", non-exhaustive	Exhaustive
Selectivity	Based on differences in *k* and/or *h*, *d*	Non-selective (PPT) or selectivity based on chemistry of sorbent (SPE)
Sensitivity	Good; quantitative transfer to GC	Very good
Environment	Solvent-free technique with GC; reduced solvent with LC	Greater solvent consumption than SPME
Flexibility in configuration	Flexible	Limited
Automation	Available	Available (mainly vacuum assisted)
Convenience in handling on site and in-vivo	Very convenient	Difficult to handle on-site; in vivo application not feasible
Integration of sampling and sample preparation steps	Yes	No
Free concentration determinations	Yes	No
Allows binding, speciation studies and reaction monitoring	Yes	No; requires combination with different method (i.e., ultrafiltration)
Calibration	Unconventional: diffusion-based coefficient or Henry constant; conventional based on sample volume	Conventional

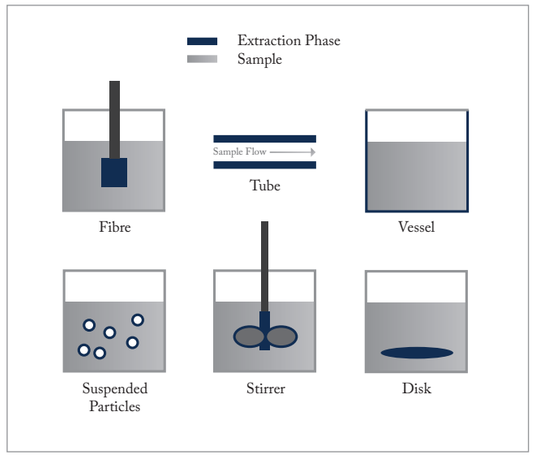


Figure 1. Various formats of SPME devices.

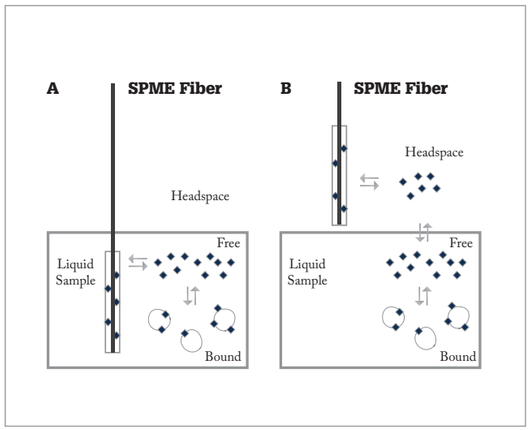


Figure 2. SPME using direct immersion (A) and headspace (B) for the determination of free concentrations.

Potential Pitfalls

Competition can occur in headspace mode during the extraction of volatile compounds. This sampling issue is well known and a number of publications have already addressed the issue by showing how to optimize experimental conditions, particularly how to select the appropriate fiber and extraction time to achieve reproducible and satisfactory results.

Some matrices, such as blood and food, are challenging to work with because occlusion of the fiber by matrix remains can occur. While we can overcome this issue, new projects may well bring new surprises. For example, in vivo studies in which sampling from solid tissues requires relatively long extraction times of 20–30min, it can be challenging to find a true compromise between the length of the experiment and sensitivity.

high detection limits, carry-over and cross-selectivity. They are useful for qualitative or semi-quantitative prescreening, but do not replace powerful analytical platforms. The field of clinical diagnostics is, therefore, crying out for innovative solutions—the more tools in the toolbox, the better the chances of effective diagnosis. Coupling SPME directly with mass spectrometers to obtain fast, reproducible and accurate results is one such tool [2, 5-9].

Direct MS analysis is nothing new in analytical chemistry, but the use of complex matrices without prior sample preparation causes ionization issues and, consequently, unreliable quantitation. By integrating sampling, sample preparation and solvent-free extraction in a single step, SPME is as an excellent candidate for rapid on-site analysis. A recent illustration couples Extracted Blood Spot analysis (EBS) with DART to determine drugs in whole blood [2]. The principles of EBS are similar to Dried Blood Spot analysis (DBS); a drop of blood is deposited on solid support that allows transport to an analytical laboratory. The main difference between the two approaches is the support material: for DBS, cellulose is the most common choice, while for EBS, as with SPME, a polymeric coating is used. This means that, for EBS, small molecules are extracted and introduced to the ionization source, while all interferences are removed in a quick wash step following extraction. Using this approach, we can normalize the matrix, meaning there is no significant difference between blood, plasma, urine, tissue or water. In contrast, using DBS, contaminants from the biological matrix and the support go through to the MS, affecting the quality of the data.

In vivo SPME is an alternative to EBS: first, a fast pre-equilibrium extraction (<2 min for fluids) is performed followed by a rinse step, then the fiber is placed in front of the mass spectrometer.

In vivo studies

The technique most commonly used today to sample small molecules in living systems is microdialysis (MD). There are three main differences between MD and SPME:

1. Metabolite coverage. MD is usually used to monitor polar species and ions. SPME coatings have better affinity for hydrophobic and semi-hydrophobic compounds.
2. Temporal resolution. MD allows constant monitoring of the dialysate content, while SPME uses sampling at regular intervals or an average concentration.
3. Spatial resolution. MD can only monitor different regions by using distinct probes, each connected to separate system of pumps and tubing. In SPME, segmented fibers [10] can be used to obtain information about compound concentration in different regions of an organ, for example, striatum and cortex of the brain, in a single experiment.

Given these differences, MD and SPME are highly complementary approaches to the study of living systems.

Outlook

The uses of SPME–MS for in vivo tissue monitoring and as a rapid diagnostic tool are in the early stages of development, but the preliminary data are very promising. We believe that these are likely to be major SPME applications.

Of course, the more sophisticated the application, the greater the technological demand, so the development of new extraction phases and sampling devices must become a real focus. In terms of extraction phases, we need coatings characterized by better analyte coverage for untargeted metabolomics; molecularly imprinted polymer (MIP) sorbents for selective extractions of target analytes (drugs and biomarkers); and advances in aptamer-based phases to broaden the range of SPME applications.

From a sampling point of view, real-life situations are teaching us how SPME can be made more convenient for the user. As a result, new device designs specific to certain applications, such as tissue samplers and connections to medical devices will be developed.

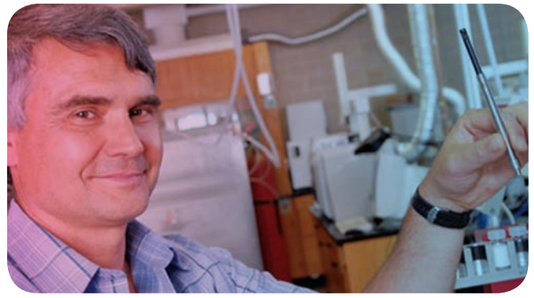
Perhaps the biggest challenge is to convince not only the scientists working in hospitals and bioanalytical labs, but also those who perform clinical analysis on a daily basis about the potential of SPME. Current techniques are well established and the applied protocols are an integral part of routine analysis. Even if a new technique offers a more convenient approach or the ability to 'tune' results, a reliance on "tried and tested" methods is difficult to break. SPME must wait in line to be slowly accepted as a routine method unless one particular application becomes the gold standard. Perhaps pharmacokinetics or monitoring of unstable biomarkers?

Janusz Pawliszyn is leader and Barbara Bojko is researcher at the Pawliszyn Group, Department of Chemistry, University of Waterloo, Canada.

References

1. *J. Pawliszyn, Handbook of Solid Phase Microextraction, Chemical Industry Press, Beijing, 2009.*
2. *F.S. Mirnaghi and J. Pawliszyn. Reusable solid-phase microextraction coating for direct immersion whole-blood analysis and extracted blood spot sampling coupled with liquid chromatography-tandem mass spectrometry and direct analysis in real time-tandem mass spectrometry. Analytical Chemistry 84(19), 8301-9 (2012).*
3. *O.P. Togunde et al. Optimization of solid phase microextraction for non-lethal in vivo determination of selected pharmaceuticals in fish muscle using liquid chromatography-mass spectrometry. Journal of Chromatography A. 1261, 99-106 (2012).*
4. *B. Bojko et al , Ther. Drug Monit. 34, 31–37 (2012).*
5. *Y. Wang, S. Nacson, J. Pawliszyn. The coupling of solid-phase microextraction/surface enhanced laser desorption/ionization to ion mobility spectrometry for drug analysis. Analitica Chimica Acta. 582(1), 50–54 (2007).*
6. *Y. Wang et al. Solid-phase microextraction combined with surface-enhanced laser desorption/ionization introduction for ion mobility spectrometry and mass spectrometry using polypyrrole coatings. Rapid Communications in Mass Spectrometry. 18(2), 157-162 (2004).*
7. *H. Tong et al. Solid phase microextraction with matrix assisted laser desorption/ionization introduction to mass spectrometry and ion mobility spectrometry. Analyst. 127(9), 1207-1210 (2002).*
8. *Y. Wang et al.. High-performance SPME/AP MALDI system for high-throughput sampling and determination of peptides. Analytical Chemistry. 77(24), 8095-8101 (2005).*
9. *S. Perera et al. Coupling solid-phase microextraction and laser desorption ionization for rapid identification of biological material. Rapid Communications in Mass Spectrometry. 26(7), 853-862 (2012).*
10. *X . Zhang et al. Tissue-specific in vivo bioconcentration of pharmaceuticals in rainbow trout (oncorhynchus mykiss) using space-resolved solid-phase microextraction. Environmental Science & Technology 44(9), 3417-22 (2010).*

Janusz Pawliszyn: The Sweet Smell of Success



As inventor of SPME, I once received an excited call from the Caribbean. "You have made me a millionaire," said the gentleman on the other end of a static-laden line. An olfactory chemist, he thought that he had exhausted all options for finding an unstable fragrance ingredient that had disappeared from the slurry during extraction of the collected plant material. However, application of SPME recovered the lost ingredient by sampling directly from a living flower in its natural habitat and facilitated efficient transfer of the component to the analytical instrument for characterization. How that single fragrance resulted in such material wealth remains unknown to me. Perhaps he should tell his story in these pages.

The technique has certainly provided me with new experiences and research opportunities. I appreciate being recognized as an inventor and to receive innovation prizes, and it is gratifying to consult and help solve practical problems. But what is the most rewarding is when I see faster and better quality data being produced in the lab and on-site. The story of the fragrance is a good example and in the future I expect impacts in clinical and environmental applications. Indeed, that is my mission.

The promise of future SPME developments is to move away from distinct steps in sampling, sample prep, separation and detection. SPME and related technologies aid in the integration of these individual steps, constituting a paradigm shift in the field by facilitating rapid, environmentally friendly and low cost analysis.

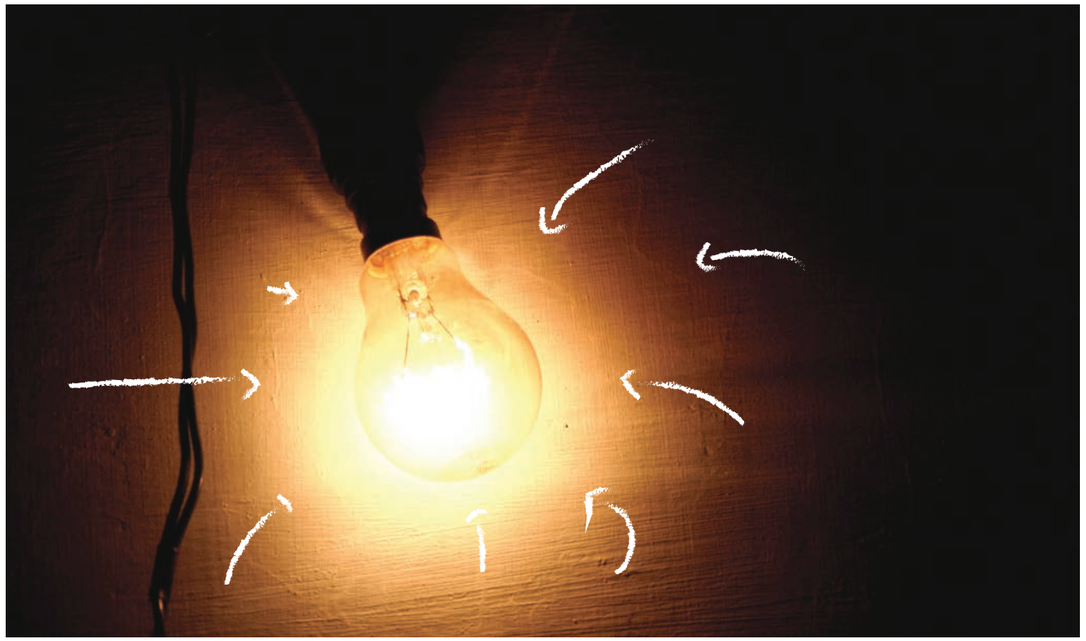
Business

Economic drivers
Emerging trends
Business strategies

Switch on to Open Innovation

Today, knowledge is widely distributed. Companies must integrate expertise, inventions and intellectual property from multiple sources to advance their business models. It's called open innovation and it offers a golden opportunity for small, talented and nimble organizations.

By Razi Imam



No company can afford to rely solely on internal sources for ideas and breakthroughs, according to Henry Chesbrough, author of the best selling book, 'Open Innovation'. In today's R&D environment, the logic of an earlier era, where innovation was closed off from outside ideas, technologies, and assets, no longer holds. In its place, the new paradigm of open innovation opens up corporations to collaboration and partnering along the entire value chain.

The Path to Open Innovation

Between 1945 and 1985, according to David Mowery of the University of California, Berkeley, research and development was mainly performed in centralized corporate research facilities. But from the mid-1980s onwards, investment in R&D departments became anemic. The focus moved from developing new products and processes to supporting business units that were profitable. And these units needed evolutionary breakthroughs rather than game-changing revolutionary discoveries. Many companies shuttered pure research initiatives and either refocused their R&D departments on immediate issues, such as customer problems, or eliminated them completely.

The dramatic reduction in investment led to massive layoffs and scientists being moved to other parts of the businesses. However, the volume of work for researchers did not abate greatly, creating a backlog of projects. Programs that had high visibility within the management team got preferential treatment while projects that were strategic, and may have provided many years of profitable income, often got shelved.

This created the conditions for a new breed of entrepreneurship to flourish. Independent analytical labs, complete with latest equipment, sprouted up to support the R&D efforts of large corporations. These analytical labs began, and have continued, to focus on providing testing services, performing as a support arm to the corporate R&D function. Concentrating on routine analysis and overflow projects proved to be a successful strategy. The sector has experienced strong, continual growth and analytical lab companies, such as RJ Lee Group, have flourished.

While the corporate strategy of substantially reducing R&D investment helped corporations to deal with immediate issues of profitability and maintaining earning per share ratios, it has not been without longer-term problems. Products are increasingly commoditized, meaning that they compete primarily on price, as their lifespans lengthen. In this global market, competitors in Asia, Europe and North and South America are looking to win on price. More concerning still is the capacity of companies worldwide to develop and market new products that may make existing instruments obsolete.

Corporations are actively seeking to adopt novel approaches to innovation to address this competitive landscape. One of the most promising approaches is open innovation, which is beginning to see widespread adoption in the analytical sciences sector as well as areas that rely on rapid innovation, such as consumer electronics and packaged goods.

Making It Work

Clearly, we are not going back to the days of centralized R&D. The corporations that develop analytical instruments realize that if they are to remain competitive they have to go outside the boundaries of their companies to find technologies that will help them to develop new products.

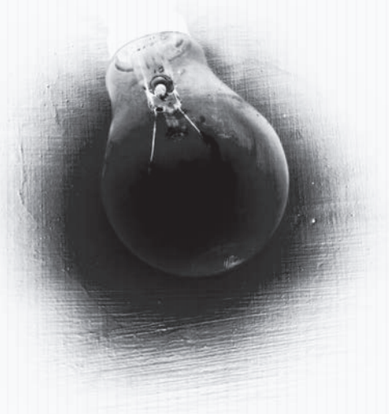
This provides a perfect opportunity for analytical labs to reposition themselves from simply being a testing facility to becoming an innovation partner. The opportunity to move up the value chain and support this powerful innovation process is real and current. Open innovation has already generated considerable momentum in other fields but there have been barriers to implementation in fields such as material sciences, life sciences and chemistry. Here, discoveries and technologies take a long time to come to fruition, huge reams of data have to be analyzed, scalability for mass production needs to be worked out, and EPA, FDA, and other regulatory bodies have to be satisfied.

Far from being barriers, these issues represent real opportunities for analytical labs to develop powerful solutions and services that assist corporate R&D to adopt open innovation. Moving up the value chain

A Guide to Open Innovation

12 Characteristics of Open Innovation

1. Ideas can come from anywhere, and anyone.
2. Ideas need time to incubate; they are not instantaneous.
3. Spaces and environments are needed for ideas to collide.
4. Follow hunches – and hunches about hunches. Connect. Exchange ideas.
5. Encourage dissent. Play 'the devil's advocate'. Form eclectic teams. Allow things to get a little bit crazy.
6. Develop an ecosystem. Reach out to experts, partners, customers.
7. Experiment. Iterate. Improve. Innovate.
8. Lead from the top – get management buy-in.
9. Nurture and manage staff and projects.
10. Accept failure, and learn from it.
11. Institute a reward system that provides internal and external recognition.
12. Think differently: heed the black swan. (1).



A Guide to Open Innovation

How to Implement Open Innovation

- Understand your internal innovation culture
- Understand the type of people you will need on your team
- Understand where ideas come from
- Develop a technology search platform
- Develop an innovation process

How to Build an Open Innovation Culture

- Establish a clear mandate
- Identify a strong strategic purpose
- Develop an innovation language
- Layout the resources and authority to be given to the innovation team
- Clarify how potential conflicts are to be handled
- Build your team

Personality Attributes of the Open Innovation Team Members

- Holistic point of view
- Talent for networking
- Strong communication skills
- Full of optimism
- Strong passion and drive
- Curiosity
- Belief in change
- Sense of urgency
- Ability to deal with uncertainty

means helping companies find, develop and launch new products.

Here are three examples of sought-after capabilities that would strengthen a company's position as an open innovation partner:

Development of Comprehensive Technology Research/Assessment Platforms

There is one trillion dollars' worth of unused intellectual property in our universities, federal labs, and corporations (2). Analytical labs that develop a software research platform to access these resources and validate the findings of the researchers will be sought-after partners, as this service is key to open innovation for corporate R&D departments. It will require an expansion of internal software development capabilities, possibly requiring many man-years of development. The key challenge is that there are over 5000 sources of information, each with a unique structure and database convention.

Validating Technology Readiness

Professors, scientists, and researchers at universities and federal labs have a strong tendency to be overly optimistic about the maturity of their discovery: they often have a fervent belief that their discovery is ready for prime time when it is still a preliminary finding from an experiment at the bench. Here, analytical labs can play a translational role, taking ideas that have significant promise and addressing the challenges of scalability and regulatory compliance. The number and range of discoveries that fall into this gray area is enormous. Plugging the development gap offers an excellent opportunity to small, nimble, specialist companies.

Development of Crowdsourced Analytical Platforms

One of the key challenges of analytical testing is handling the huge volumes of data that are being generated; these need to be managed, parsed, indexed, categorized, cleaned and validated. Today's software programs, often archaic legacy solutions, fail to do the job well. An opportunity exists to access crowd intelligence to increase speed and accuracy. For example, a protein researcher at the University of Washington, struggling to predict the native structures of proteins, designed Foldit (www.fold.it). In this game, users play with three-dimensional structures and, following certain rules, fold them into protein structures. Today, more than 250,000 users are playing the game and it has transformed the field.

Another program, which is called Eterna (http://eterna.cmu.edu) and was developed by Carnegie Mellon University and Stanford University, has over 30,000 players contributing RNA sequencing designs. So far, more than 306 RNA designs have been synthesized for in vitro testing.

Analytical labs can be similarly transformative in their thinking. There are many other capabilities that they could develop that would add to the repertoire of open innovation. The adoption of open innovation by corporate R&D is changing the game. Analytical labs that move first have the chance to be the industry stars of tomorrow.

Razi Imam is an award–winning innovator, entrepreneur and author, and founder of 113 Industries (113industries.com).

References

1. *Nassim Nicholas Taleb, The Black Swan: The Impact of the Highly Improbable, Penguin, 2007.*
2. *Kevin G. Rivette and David Kline, Discovering New Value in Intellectual Property, Harvard Business Review, 2000.*

Profession
Leadership
Talent Development
Career Planning

By Trust or By Fear?

Whether you're a team leader in a small European toxicology lab or a director at a giant American measurement solutions provider, understanding and managing the motivation of your staff is the key to success.

By Hans Versnel

When did you last seriously examine your management style and the organizational structure of your company? With the workplace environment rapidly evolving, placing increasing emphasis on problem-solving and creativity, is the productivity of your staff being adversely affected by a traditional management approach?

At the deepest level, management is a straight choice between trust and fear. Organizational culture and management style fracture into those who trust employees and allow them the discretion to act flexibly and responsibly, and those who exercise control for fear of the poor outcomes that the quality of employee judgments and attitudes might bring. It is my contention that neither approach alone is sufficient. To get the most from your staff requires a nuanced combination of the two, implemented by department, job function and sometimes even tailored to the individual employee.

A lesson in philosophy

After a little philosophical digging, it becomes clear that the two schools of management rehash an 18th century dispute between so-called consequentialists and non-consequentialists on the question "what is good ethical behavior?" Early English liberals like Jeremy Bentham believed that 'good' must be judged from the effects of behavior. In contrast, the Prussian, Immanuel Kant, argued that it is impossible to foresee the full effect of our actions and therefore we must look at the intentions behind those actions. Can you, he asked, really judge a person who caused an avalanche simply because a single stone slipped from under his shoe?

The implications of these competing philosophies are much greater than you might expect, especially when it comes to management and organization. The concept of full responsibility drives some bosses, notably in English-speaking nations, to exert extreme degrees of control because they perceive the liabilities to be so huge. It is this sense of responsibility that gives these same bosses the feeling that they are entitled to a large share of profits in times of success. However, in complex organizations, as exemplified by the science and technology sectors, there are down sides to the fear-driven responsibility model. It can easily become impossible to fully control employees who are specialists or must cope with ever-changing circumstances. In my work as an organization analyst, I see a lot of employee frustration caused by rules, regulations, checklists and reports that are perceived as irrelevant or simply do not make sense.

Compliance or responsibility?

It is natural that managers want their employees to be accountable for their actions, but compliance systems that make people feel that they are being treated like morons can be expensive for their employers. "Am I not allowed to think for myself?" is the typical reaction. "OK, then, you can solve the problems!"

Broadly speaking, and in my experience, management practice follows the ancient philosophical split: the fear–compliance model tends to be favored by English-speaking nations, while a trust and responsibility approach is preferred in mainland Europe. Of course, implementation is not black and white. For example, in Europe, the finance industry utilizes increased compliance while in the UK and US, modern high-tech businesses benefit from fully engaging the skills of their specialist employees. Take Apple, Google, or eBay, for instance. Steve Jobs focused on ensuring that his staff knew what they were doing, and why – traditionally the Northern European model.

In this system, managers spend considerable amounts of time discussing goals, roles and strategy, to ensure that their employees are engaged in their work. They want staff to be able to cope with all kinds of situations, embedded within a shared set of beliefs. The unwritten rule is to always explain goals and responsibilities, and to only talk about 'how' when asked. It's an approach that was developed in Germany, contrary to the popular view of the country elsewhere. Indeed, while it may not be a popular subject, you can draw parallels to the relatively small force that took over much of Europe in the early 1940s; the German army was flexible and agile because it gave discretionary power to low-ranking but well-educated officers.

Going back to the example of the European finance industry, the more machine-like the organization, the better the compliance model fits. If inputs, processes and outputs can be standardized, then such a model can be superb. But, in general, this is not the way that the world is developing. Work that can be standardized can often be mechanized or computerized; the tasks left to humans require insight, flexibility, and the ability to perform in changing circumstances. That's the essence of work in the 21st century and it seems to match increased employee trust and responsibility perfectly. This may explain why, per capita, northern Europeans make about the same amount of money as their American counterparts, but Americans have to work 40 percent longer.

Does this mean that there is no place for command and control management? Well, not quite.

What drives you makes you stronger

As developers of systems that measure motivational patterns, or drives, my colleague Machiel Koppenol and I have collected data for more than 100,000 people worldwide. Our instruments are used to gain insight that can help improve leadership and team performance. With RealDrives (see 'What Drives Us?') we identified factors that help determine the balance between people, work and management styles. We distinguish between six drives, using the categorization

What Drives Us?

Each pattern indicates a person's "Real Drive": what they find important.

A people-person, to whom ideas and solidity are important.

Six values, six colors
Everyone has a distinct RealDrive pattern formed by colored values.

The value that wants to care and contribute to a sense of safety and security. Key words: helpfulness, unity, caring.

The value that strives for a private domain and provides speed but also fervour. Key words: fast, alert, intrepid.

The value that strives for order and certainty. Key words: clarity, discipline, reliability.

The value that strives for the human touch and puts mutual harmony first. Key words: equality, openness, sharing.

The value that strives for results and progress. Key words: ambition, goal-orientedness, flexibility.

The value that wants to analyse freely and act based on insight. Key words: knowledge, freedom, self-management, vision.

developed by Clare W. Graves in the 1970s, namely the drive:

- to preserve traditions
- for power
- for certainty
- to connect to others
- for progress
- to understand

It is possible to distinguish many different patterns, but there are just two truly important dimensions when it comes to getting a match between people, work and leadership. The first is between concrete or abstract tasks: some people want to be given concrete tasks and concrete guidance, while others feel more comfortable with abstract roles and responsibilities. The second dimension, which is 'how' versus 'what', is even more important. Again, there is a sharp divide: some people want to be given goals and targets, and to be free in how they achieve them; these same people get frustrated if they are forced to comply with exhaustive rules and procedures. But the other half of your staff may want clarity on how they are expected to work; for them, agreements and clear procedures are perfectly acceptable.

If you look more closely at your organization, you may discover that departments can be very different when it comes to these two dimensions. Your accountants, for example, are likely to be different to your sales people – if they were not, you could well have a problem! The question is, then, why do you treat them as if they are the same? Even within the accounts or sales departments there may be a diversity of personality types, and you would do well to recognize and manage these appropriately.

Respect diversity; promote positive behavior

Failure to recognize the driving force behind your employees results in a loss of productivity. In one study, we measured the drive patterns of 1,100 sales people to find out which profiles worked best in different sales contexts: some did simple commodity sales, some sold complex solutions, and some sold within a relationship-oriented market. We compared the drive patterns of individuals in different sales categories with their results. For each group, successful patterns were observed but we also identified a number of underperforming patterns. These individuals had the 'right' drive pattern but they sold 40% less than their colleagues. When we drilled down to figure out why, we discovered that almost every underperformer had a boss of the wrong type in terms of the 'how or what' dimension.

Every drive has a positive and a negative behavioral style (see 'Drives and Behaviors'). If a person is managed well, positive behavior results. If managed badly, creativity turns into cynicism, responsibility becomes "I just do what is in my job description", and so on. This is not a minor consideration. For a large company, the cost of negative behavior throughout the entire organization can be immense. And for a small company, it can be a matter of life and death.

So, how can you as a manager promote positive behavior? First, don't assume that extra force will help. In reality it is much more likely to make things worse. Second, start to trust people more and encourage a greater degree of ownership. And last, but certainly not least, find out what drives each of your staff – which means understanding what they really find important.

Hans Versnel is a system maker and co-owner of Hatchfund. For more information about RealDrives, visit www.realdrives.com

Drives and Behaviors

Negative Behavior	*Drive*	*Positive Behavior*
gets cynical, criticizes	knowledge, insight	develops ideas, helps thinking
complains, gossips, whines	harmony, equality	involves others, communicates
self protective, cautious	result, success	shows ambition, takes on challenges
sticks to job description	structure, certainty	creates structure and order
becomes defensive, aggressive	domain, respect	sets the pace, shows courage
gets anxious, fearful	security, unity	creates safety and family atmosphere

Solutions

Real analytical problems
Collaborative expertise
Novel applications

It's All About the Residue

Da Vinci Laboratory Solutions and a global oil and gas corporation address a tough analytical challenge associated with liquefied petroleum gas (LPG).

By Willem van Raalte

The Problem

How can the components of oily residues in LPG, and the likely source of these contaminants, be identified using a fast, robust method?

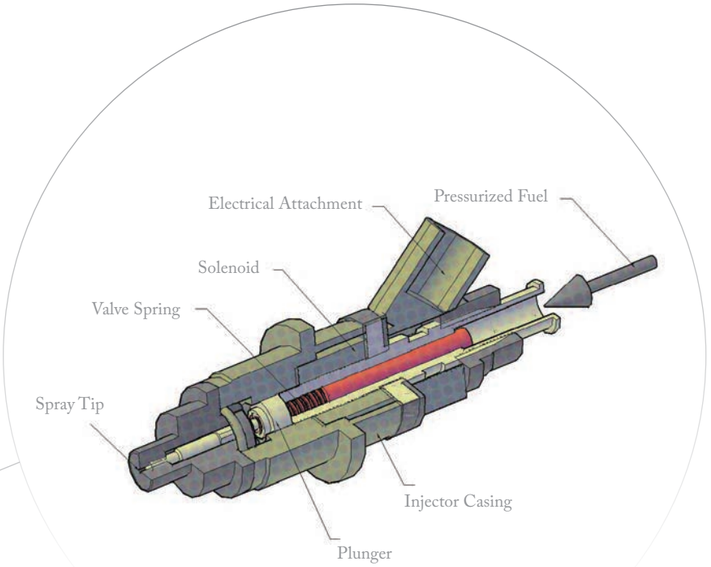
Background

If you work in a petrochemical plant, a refinery, in LPG production, shipping, handing, or at an analytical laboratory charged with LPG analysis, you'll know a thing or two about oily residues—both the associated analytical problems and the negative impact they can have on machinery.

LPG is predominantly a mix of propane (C_3H_8) and butane (C_4H_{10}). Compositional analysis is generally performed using GC-based methods, but propane and butane (C_3/C_4) sample streams may also contain contamination from soluble hydrocarbons from C_{10} up to C_{40} – the typical constituents of oily residue. A number of conventional methods for the analysis of oily residues in LPG have been set out by standards institutions, such as ASTM and CEN (namely, ASTM D2158, EN 15470 and EN 15471). These methods have been in use for many years but they have known limitations. Lab managers must deal with the fact that they are labor intensive, time consuming and hazardous. In addition, the tests are environmentally unfriendly, requiring the evaporation of 100g to 2 kg of LPG. Equally damning, they offer no way of identifying the source of contamination, which makes troubleshooting difficult.

In 2007, Da Vinci Laboratory Solutions (DVLS) was approached by Shell Global Solutions in Amsterdam, the Netherlands, to work in close cooperation on the development of an alternative solution for the analysis of oily residue in LPG using gas chromatography (GC).

In particular, the analytical problem that we were tasked with was to quantitatively detect high boiling components in low-boiling matrices. For some applications, a gas chromatographic solution was available, but sample introduction into the analyzer was a weak point, because uncontrolled amounts of heavy components were retained and/or absorbed by the transfer lines from the sampler to the analyzer.



Schematic overview of the Gasoline Direct Injector used in the DVLS LGI

The Solution

Through our discussions it became clear that the market needed a safe, reliable and robust method to determine LPG residue – qualitatively and quantitatively – in less than half an hour.

This required the development of a high-pressure injection system with the following qualities:

- Large volume injection
- Ability to handle pressure up to 25 Bar
- No absorption
- No discrimination
- No fractionation
- Sample size capabilities from 5-100 µl
- Low or no dead volume
- Low maintenance and user-friendliness

Analyses needed to be sensitive to ppm amounts; exhibit enhanced accuracy; provide information on contamination type and source; and reduce the risk of injury or fire due to sample handling and the physical demands of the test procedure.

The method also had to meet specific practical criteria, including:

- The fast turnaround of container cargo (be it by truck, barge or pipeline).
- The elimination of unnecessary LPG evaporation, to reduce environmental impact.
- The incorporation of a proven sample introduction device for high-pressure on-column injection.

Novel Injector

In close collaboration with Shell, Da Vinci's director of R&D Lenny Kouwenhoven and her team were inspired by the well-proven gasoline direct injector (GDI) used to inject fuel into the automotive engine combustion chamber. The prototype

LPG: A Complex Character

The Good:
Liquefied petroleum gas has become something of a "darling" alternative fuel in the last years. Why? It's more cost effective than its cousins, gasoline and diesel, and much more environmentally friendly. In comparison to gasoline, the flammable mixture of hydrocarbons that make up LPG produce 50 percent less carbon monoxide, 35 percent less nitrogen oxides and have just half of the lower ozone-forming potential. Furthermore, its price is less dependent on crude oil, meaning that LPG is more robust in the marketplace. LPG is wonderfully versatile, and can be used for heating, power generation, refrigeration, cooking and vehicles.

The Bad:
LPG is prone to oily residues that accumulate to form troublesome deposits that corrode or plug fuel filters, pressure regulators, fuel mixers or control solenoids. It can become contaminated by oily residue in just about any part of the production and transportation lifecycle. Transport contamination alone can occur in shared pipelines, valves and trucks used for the distribution of other products.

The Ugly:
Until recently, laboratory analysis of LPG contamination had proven to be labor intensive, time consuming, environmentally unfriendly and even hazardous. In addition, there was no way to obtain an indication about the source of the contamination, a fact that left LPG stakeholders with question marks as to how to fix the problem.

injector used a modified GDI connected to a standard GC injector needle that could be inserted into existing large-volume, on-column GC injection systems.

The DVLS Liquefied Gas Injector (LGI), unveiled in 2010, can inject liquefied gases at room temperature directly on-column at up to 25 bar. Solenoids with millisecond timing activate to transfer the pressurized sample through the needle. A vapor exit flushes the LPG light end fraction and the oily residue remains in the column and is separated in order of boiling point. The result is reported as a concentration in mg/kg (mass ppm). The analysis range covers 10–600 mg/kg with a repeatability of below 5% and a relative standard deviation of between 2.4 and 4.7%. The required analysis time is less than 30 minutes.

In summary, the LGI has many desirable traits:

- Direct on-column injection on to GC up to 25 Bar
- Faster than conventional methods
- Repeatability less than 5%
- Proven GDI technology
- Sample size from 5–100 µl
- Results reported in mg/kg
- Chromatogram indicates the source of the contamination.
- Environmentally friendly (eliminates the evaporation of large volumes of LPG into the atmosphere)
- Directly addresses safety concerns for lab personnel (no open air evaporation of LPG)

New ASTM method

Developing a new GC injector that addressed the needs of the industry was a good first step. But equally important was working with standards organizations to determine whether a new method would help customers in the analysis of LPG residues. This proved to be the case and in December 2011, ASTM released a new method: "Residues in Liquefied Petroleum Gases by Gas Chromatography with Liquid, On-column Injection" (ASTM D7756-11). The benefits of ASTM D7756-11 include:

- no open air evaporation of LPG
- analysis in under 30 mins
- effective waste management
- standard GC analysis
- indication of contamination.

Beyond the solution

When the Shell team first called, we had no idea about the true potential of the solution. Then, customers started requesting support in related application areas. Our development team is currently working on methods of analysis for:

- Di-isopropanolamine (DIPA) in LPG
- 4-Vinylcyclohexene (VCH) in butadiene
- 4-Tert Butylcatechol (TBC) in butadiene
- n-Methyl-2pyrrolidone (NMP) in butadiene

So, despite the title of this article, analysis of oily residues in LPG is only the beginning of the story. Collaborating on a novel solution to a specific problem in one field has opened doors in other interesting application areas. And borrowing an engineering solution from the automotive industry proved to be the inspirational spark.

Willem van Raalte is Managing Director of Da Vinci Laboratory Solutions. www.davinci-ls.com

2007

DVLS approached by Shell Global Solutions in Amsterdam, Netherlands.

2007-2010

DVLS team works with Shell Global Solutions. First DVLS Liquefied Gas Injector prototype built and validated for application. Second prototype built and put into use.

2009

First meeting with ASTM in December.

2010

DVLS Liquefied Gas Injector introduced.

DVLS Liquefied Gas Injector awarded Best New Technology at Gulf Coast Conference in Galvestion, Texas, USA.

2011

The new ASTM method for "Residues in Liquefied Petroleum Gases by Gas Chromatography with Liquid, On-column Injection" released in December.

2012

DVLS' Director of R&D, Lenny Kouwenhoven, received award from ASTM in appreciation of her work.

LC-MS/MS Method for the Determination of Diclofenac in Human Plasma

J. Jones, Thermo Fisher Scientific, Runcorn, Cheshire, UK

A liquid chromatography-tandem mass spectrometry method for the determination of diclofenac in human plasma was developed, giving good chromatographic peak shape and linearity over the required dynamic range of 1 to 1000 ng/mL. Sample preparation was fast and efficient and gave excellent recovery levels using Thermo Scientific™ SOLA™ cartridges and plates. Separation was carried out using a Thermo Fisher Scientific Accucore™ RP-MS column with a cycle time of 1 minute.

Introduction

SOLA products revolutionize solid phase extraction (SPE). This first-in-class SPE product range introduces next generation, innovative technological advancements that give unparalleled performance compared to conventional SPE, phospholipid and protein precipitation products. This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Lower elution volumes
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices, particularly in high-throughput bioanalytical laboratories where reduced failure rates, higher analysis speed and lower solvent requirements are critical. The increased performance gives higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high-coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

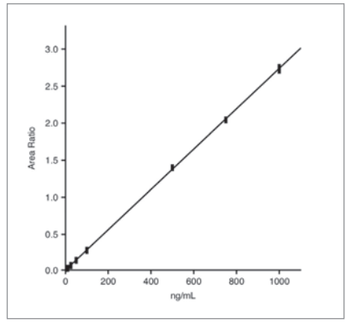


Figure 1: Diclofenac linearity over the dynamic range 1 to 1000 ng/mL

Standard	Concentration (ng/mL)	Average Calculated Concentration (n=6)	Average %Diff	Average Precision (%CV)	% Recovery
Quality Control	15	14.7	-2	4.4	86

Table 1: Average precision data for six replicate QCs for diclofenac

Results

Extracted diclofenac standards from human plasma gave a linear calibration curve over the dynamic range of 1 to 1000 ng/mL with an r^2 coefficient of 0.999 (Figure 1). Six replicates of a 15ng/mL Quality Control (QC) sample were analysed. The precision of the QC was less than 4.4% CV (Table 1). Overspikes were run in triplicate at a concentration of 15 ng/mL and used to calculate the 85.8% percentage recovery level for diclofenac.

Conclusion

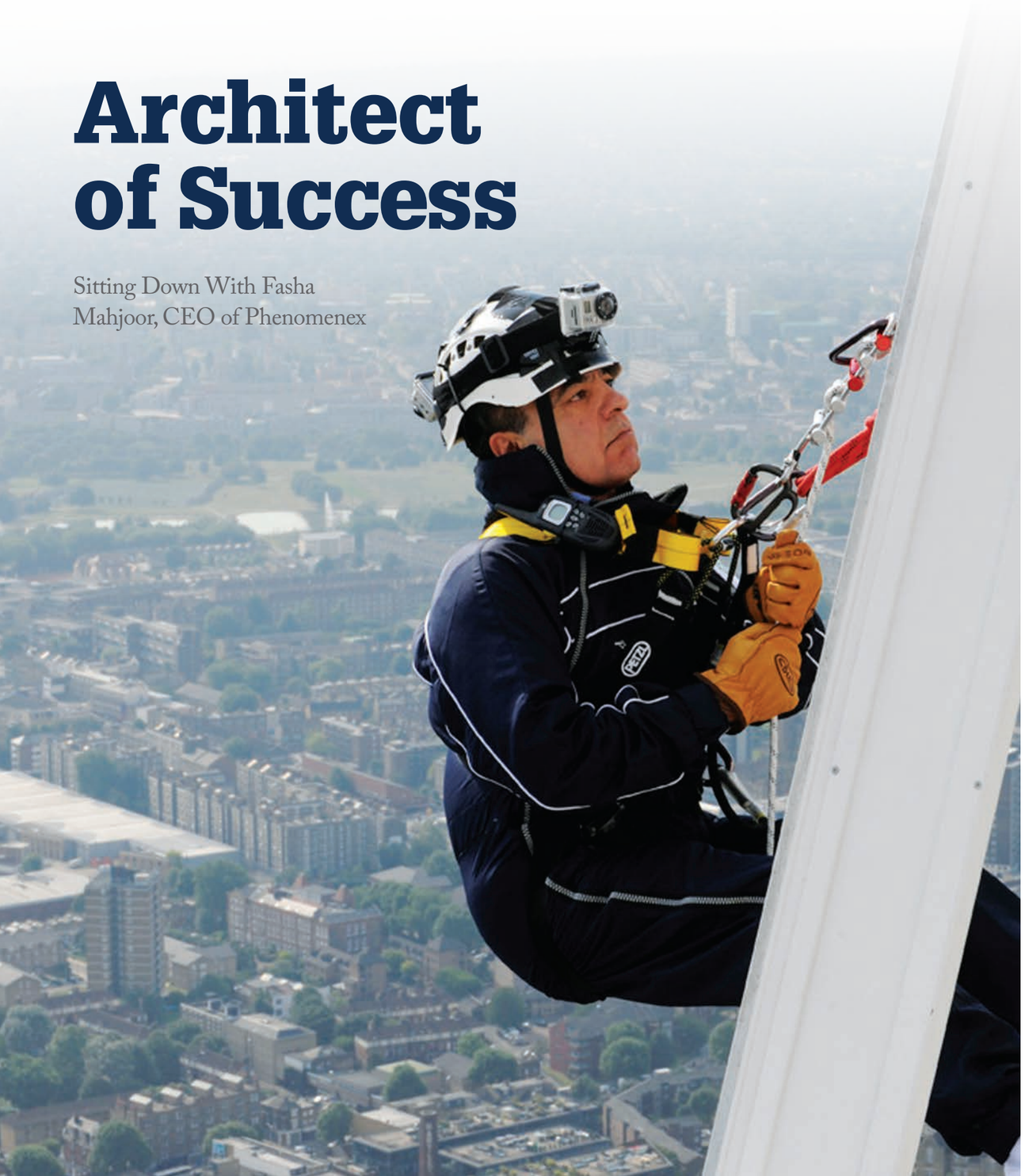
SOLA SPE in combination with Accucore RP-MS columns allow for a simple extraction and quantification of diclofenac from human plasma reaching the desired lower limit of quantitation of 1 ng/mL. Extraction recovery was suitably high and the method showed excellent precision with a percentage coefficient of variation of less than five percent (n=6).

For more information, please see the full application note at: theanalyticalscientist.com/issues/0113/701

Thermo SCIENTIFIC

Architect of Success

Sitting Down With Fasha Mahjoor, CEO of Phenomenex



Describe your company in the length of a tweet.
A global leader in developing novel analytical chemistry solutions that solve the separation and purification challenges of researchers.

How would you describe the culture at Phenomenex?
Progressive, energetic and very friendly. Dynamic. The team spirit is absolutely incredible. It's also perfectionist, we have very high standards.

Is this a reflection of your own personality and values?
I don't want to take too much credit, but I am a perfectionist. The friendship and camaraderie is how I feel I behave normally, so it does reflect a bit of who I am. Being neither a scientist nor thinking of myself as a businessman, I found myself doing things that I enjoyed and made sense to me. Today you read in magazines that the most go-ahead companies have sleeping rooms, gyms, and brightly colored workspaces. Before Google even existed, Phenomenex was doing all sorts of things like that.

"Scientists don't like flowers? Don't like art? Don't need natural light? No, quite the opposite."

How did Phenomenex come about?
I had come to America to finish my Masters in architecture. One of my oldest school friends had started a company involved in chromatography. He wanted to penetrate the US market and asked me to become his distributor – even after I reminded him that I failed every chemistry class at school.

The first office was in Beverly Hills. Actually, it was my architectural office and was 10 feet by 10 feet. I remember calling my first customer at Dow Chemical in Texas and he asked me why we were based there. "Because we do designer columns," I told him. He called me back the next day with a $3,000 order.

That was three decades ago.

You say you came to America. Where are you from?
I was born in Persia. At a young age, I went to England, grew up at a boarding school, went to university there and then came to the USA. Subconsciously, I picked up three very different cultures and each has helped form my character. I consider myself an incredibly lucky man. That background is mirrored in Phenomenex; it is a conglomerate of many different nationalities.

How did the company develop?
It started from humble beginnings – with just me: no science, no technology, no money or finance of any type. Originally, it was all sales and marketing. By the fifth year, I had enough funds to hire scientists to begin the development of our own products. By Year 15 we had undergone complete metamorphosis, transforming into a scientific company.

Today, I am proud to say that we are leaders in certain areas and the main thrust of the company is science. One of our most successful products was introduced close to 15 years ago, the Luna [HPLC column]. Technology-wise, Kinetix [core-shell particles], launched three years ago, and has been a bit of a game-changer. Efficiency, speed and resolution are all improved; solvent-saving is astronomical.

We have a lot of research projects in the pipeline, and not just in the way of HPLC. At any one time we have 50-60 ongoing research products, and probably one in ten will come to fruition.

Do you exercise your architectural roots?
I've been to many companies – competitors, suppliers and customers – and often they are grey and uninspiring. The work environment should be beautiful, or at least pleasant, with lots of natural light. I love art and antiques, and will fly 6,000 miles to get involved in combining old artifacts with a modern space. Scientists don't like flowers? Don't like art? Don't need natural light? No, quite the opposite, they probably enjoy them more than anybody else.

You, and the staff generally, are heavily involved in philanthropic projects…
It is such a privilege to be in a position to give back, to help others. It is part and parcel of who we are. In all our offices, the staff and their families thoroughly enjoy it.

But abseiling down The Shard?!
The Outward Bound Trust, an education charity, wanted to do something a bit different to raise funds, and it was actually Prince Andrew, who is the Chairman, who came up with the idea. It was an extraordinary event. Even standing at the top of the [310 m] tower looking down, the view is so beautiful I didn't have second thoughts and within 10 seconds I was fully preoccupied with the rope in my hand. It took about 12 minutes to complete.

Back on Earth, what do you get from being the CEO of Phenomenex?
Satisfaction in looking back on how far we've come, and more importantly, the privilege of being able to assist other people – colleagues, the community. That gives me an absolute high.

FAST AND FURIOUS

GPC/SEC Solutions and Expert Support

Macromolecular characterization from the experts

GPC/SEC is our passion. We at PSS are fully dedicated to the advancement of macromolecular liquid chromatography by means of developing true solutions and providing competent and personal support.

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