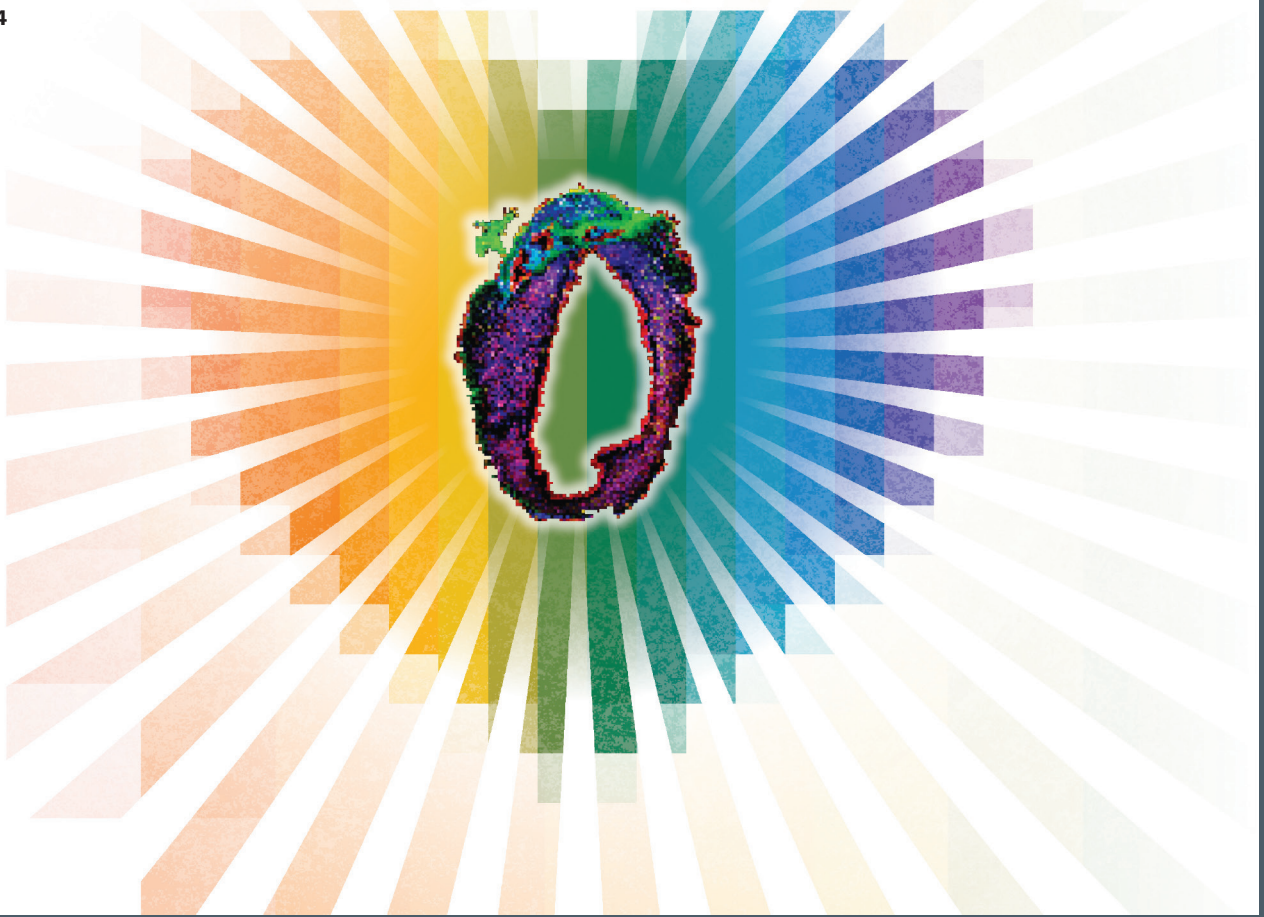
JANUARY 2014 # 12

the Analytical Scientist

MS Imaging Targets the Clinic

Ron Heeren and Axel Walch critically appraise a flourishing field



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Online this Month

Care to Comment?

Publication of articles is often the starting point for an informed debate. Join other readers who are already sharing their viewpoints – positive and negative:

Quality by Disagreement (tas.txp.to/0114/QbD)

"Disagree. It is rather interesting to see that the author has biased opinion about Pharmacopeias. USP always welcomes comments from NDA holders, ANDA holders or DMF holders. When any of these companies submit a request for revision, USP reviews those requests by focusing on the advantage of the proposed new procedure, working with the Expert Committee to forge a path forward. There are lots of examples where USP has replaced an outdated procedure, be it HPLC, GC, or any other techniques, with procedures using modern technology."
– Ravi Ravichandran, USA.

"QbD in analytical development is best used with high-throughput UPLC. Considering the dominance of HPLC in the industry, it would take a while for QbD to be fully fledged in a lab."
- Jerry Jin, USA.

The Laws of Physics (tas.txp.to/0114/Physics)

"A truly delightful and insightful essay! Regarding physics and physicists: first, we must not forget the great chemist Ernest Rutherford who once said, 'All of science is either physics or stamp collecting!' Think about that in the context of the time he made the statement: chemistry and the periodic table; biology and the family trees... Now, all scientists are doing physics in one manner or another. Only a few keep the title, however, to avoid the appellation 'Smarty Pants!'

Regarding publications (one of this writer's favorite topics): the plethora of multiple author papers is disgraceful, yet continues to pollute the literature because it ensures large numbers of 'authors' able to generate even larger numbers of citations. The growth of scientific journals in excess of 3 percent per year ensures that there will always be a place for every paper irrespective of its significance. Indeed, over 40 percent of all such published papers are never cited except by their own authors! That seems to indicate that more than 40 percent of all those scientific works should never have seen the light of day. What a waste!" – *Philip Wyatt, R&D Director/Manager, USA.*

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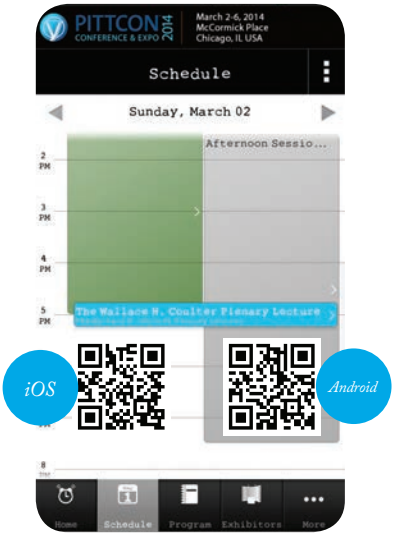

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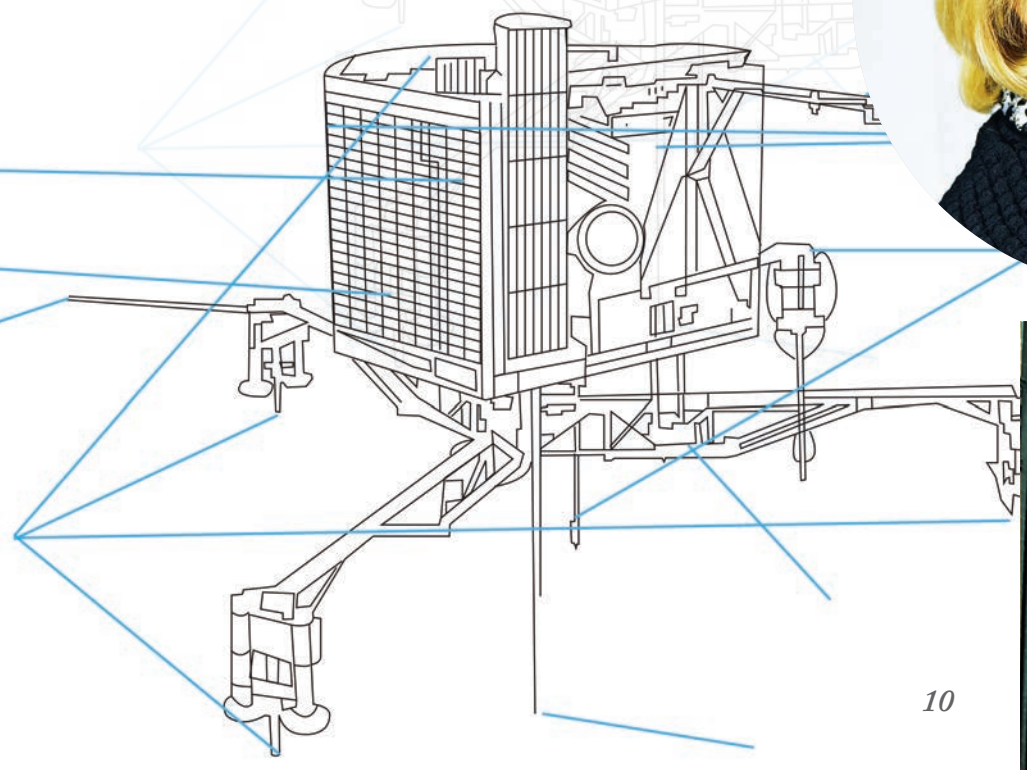

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On The Cover

"Pixelated Heart" represents MS imaging in the clinic. Overlaid with an actual MS image of a rat heart section (courtesy of AMOLF).

Features

Departments

Sitting Down With

the **Analytical Scientist**

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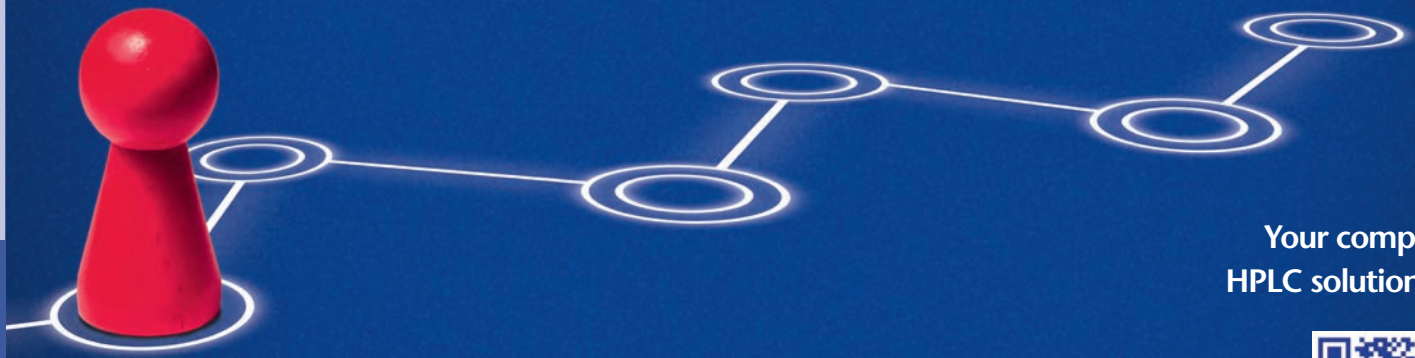

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Time for Reflection

Are your interests mirrored by our content? With one year behind us, we want to know what you like and what you don't like about The Analytical Scientist

In her years as an au pair in the UK (before we met), my wife once received a precious gift from the lady of the house – a sign of her appreciation. It was a small but beautiful porcelain box, with the phrase "In here you see, what pleases me" inscribed on the lid. Intriguing words. When my wife opened the box she discovered that the bottom was made of mirrored glass, and so what she saw was a reflection of her own face. What a lovely and subtle way of telling someone that you are fond of them. My wife treasures that box to this day.

As editors of The Analytical Scientist, our goal is to offer you something approaching a "precious gift" each and every month. We aim to present a selection of articles that mirror your interests and make you pause for reflection. If we succeed in this goal, then we can legitimately consider ourselves to be a useful, and integral part of the analytical sciences community – which is not always the case for trade publications.

Over the course of 2013, we spoke with many readers of The Analytical Scientist at meetings. This generated a considerable amount of feedback, which helped us enormously in shaping the development of the publication. Our sense from these interactions is that the publication is on the right track.

Now, with a year's worth of issues under our belt, it would be extremely valuable to take a deeper dive into readers' reactions to the publication. And so we kindly request that you make your thoughts and wishes known in our online reader's survey, at **tas.txp.to/0114/survey**.

The 2014 Reader Survey
tas.txp.to/0114/survey

The survey comprises fewer than 20 questions, with space at the end for you to add suggestions for specific articles (which you may or may not offer to write). It shouldn't take more than five minutes to complete.

In return, we will publish a snapshot of the results in an upcoming issue and, to thank you for participating, we are offering a prize draw with a couple of intriguing prizes – click on the survey link to find out more.

Please help us by completing the survey – and ensure that every time you open The Analytical Scientist you'll find content that interests, engages and pleases you.

Frank van Geel
Scientific Director

Contributors:

Joeri Vercammen

"I have never felt like a true scientist," says Joeri Vercammen, "It just takes so long to get things done. I consider myself more as a facilitator. I bring the right people together to get things done. Science, technology or marketing? They're all mutually important to me." No wonder Joeri enjoys working at Interscience, a Benelux-based distributor of GC and GC-MS instrumentation. "I advise our teams on how to differentiate themselves by applying smart chromatography". With his team at the "The Uninspired Scientist", he develops training programs to make scientists and their institutions think more like entrepreneurs. He shares his wisdom with you on page 26.

Kim H. Esbensen

Kim Esbensen is a research professor in Geoscience Data Analysis and Sampling at GEUS (National Geological Surveys of Denmark and Greenland), chemometrics professor with the ACABS research group, Aalborg University, Denmark, and external professor of process analytical technologies (PAT) at the Telemark Institute of Technology, Norway. Somehow, Kim also finds time to be a member of seven international societies and chairman of the DS-Forum 205 taskforce, responsible for writing the world's first horizontal (matrix-independent) sampling standard. "I like to get involved…" he says, "And I have devoted all my research to the theme of representative sampling of heterogeneous systems and PAT." To see how strongly Kim feels about the subject, see page 21.

Alexander Bünz

"I do not believe in coincidences. Rather, I very much enjoy watching how life assembles itself like a beautiful puzzle." It is with this philosophy that Alexander Bünz's career developed. He became devoted to chromatography during high school, received his PhD on thermodynamic properties of supercritical fluids, and discovered new media for the electrophoretic separation of DNA fragments as a postdoc at UC Berkeley. Alexander then got bitten by the business bug and worked in several positions in the chemical industry before receiving his MBA at the TSM Business School in the Netherlands. In 2007, he became managing director of Knauer, Germany. Ever since then, he's been breaking all the rules (page 20).

Robert Trengove

After graduating with a PhD in physical chemistry, Robert Trengove took up a postdoc in chemical engineering and chemical technology at Imperial College, London. "In 2003, I began research with colleagues on fungal metabolomics and published the first Australian fungal metabolomics paper in 2006," Rob says. "This collaboration resulted in the establishment of a Metabolomics Australia Node". Rob is now working on diverse metabolomics projects, and developing the nexus between exposure and health using metabolomics. Rob runs through the tools of the trade on page 36.

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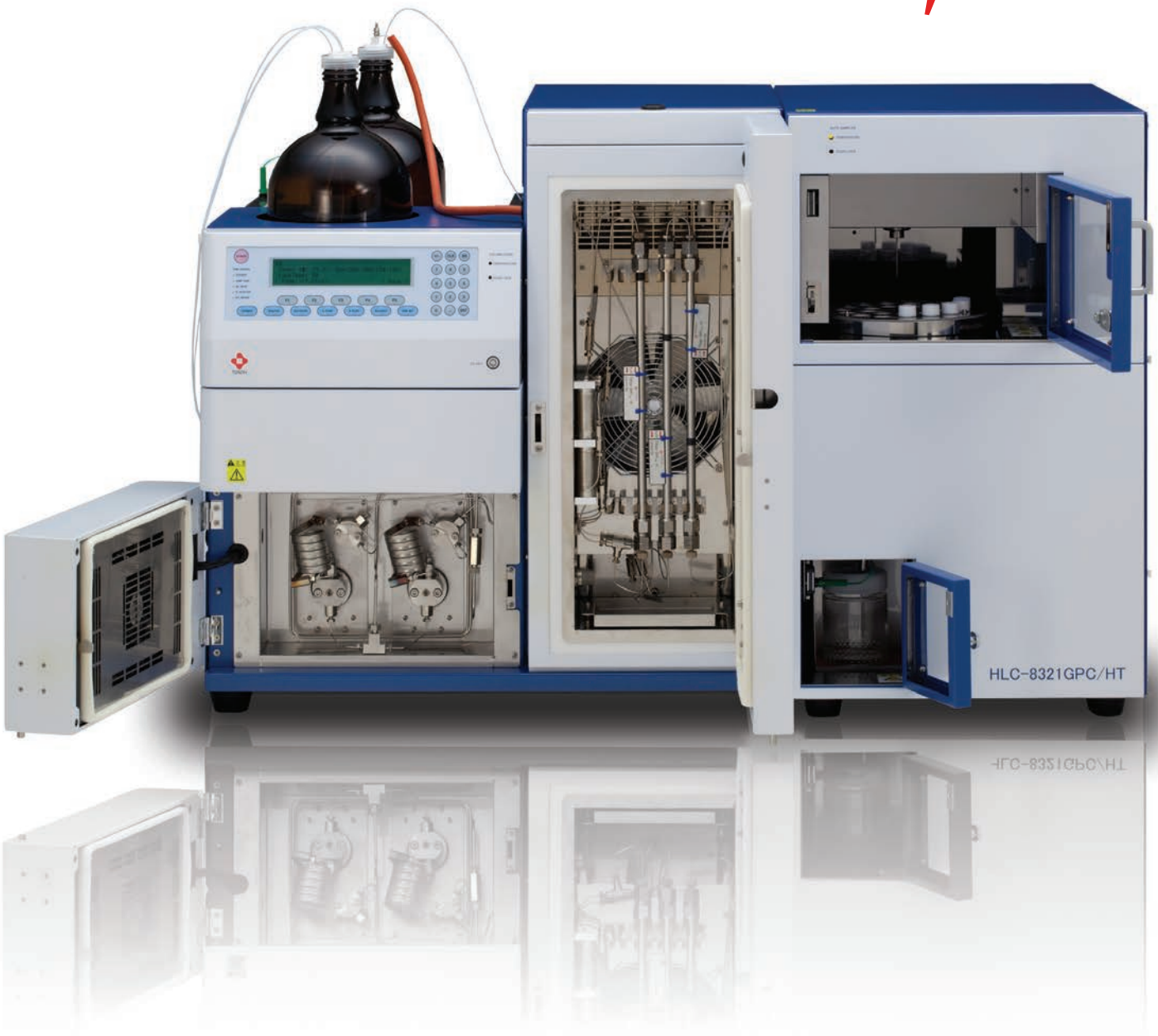

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Upfront

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

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The Comet Chaser

Main engines: check. Communications: check. Solar power: check. GC-MS system on board: check.

In 2004, the European Space Agency's Rosetta Mission launched from French Guiana. Objective: to catch Comet 67P/Churyumov-Gerasimenko (discovered in 1969) and accompany it to the inner solar system. After ten years of nail biting, comet chaser Rosetta, now some 500 million miles away, has awoken from hibernation and phoned home. Meanwhile, Ian Wright (interviewed below), professor of planetary sciences at The Open University, UK, and principal investigator for the Ptolemy instrument, is patiently awaiting the first ever comet soft landing and his moment of glory. Ptolemy, part of the Philae Lander's science package, is the size of a small shoebox, weighs under 5 kg, and contains the "miniaturized" gas chromatography-mass spectrometry system developed by Wright some years ago…

How do you feel about this once in a lifetime mission?

"I'm not sure what the emotion is, but I am eternally grateful to my university and the UK funding agencies for giving me the chance to experience it. Of course, there is a certain amount of excitement, but I'm also hoping to experience the relief and gratification that comes with success. And although Rosetta is currently very much in focus, it is necessary to be thinking about the next part of one's own "lifetime"; along the way I've done many things besides Rosetta, but it feels strange to be actively contemplating the next big project before this one has finished."

What were the main challenges in developing Ptolemy?

"Bearing in mind the ten years that will have elapsed between launch and eventual arrival at the comet, I recall that the instrument was being put together just as the millennium bug was about to end civilization as we knew it. But I had personally started on the project more than ten years before that. Back then, the average UK household had a car running on leaded petrol, only five TV channels (satellite TV was a rarity), possibly a "computer" of some sort with a dial-up modem (operating, on a good day, at 0.01 Mbits/s) and, if business executives were in residence, a cell phone (something of brick-like beauty and appearance). It's fair to say that some of the challenges for Ptolemy were the challenges of a previous era (or century) in addition to the overall timescale of the mission itself."

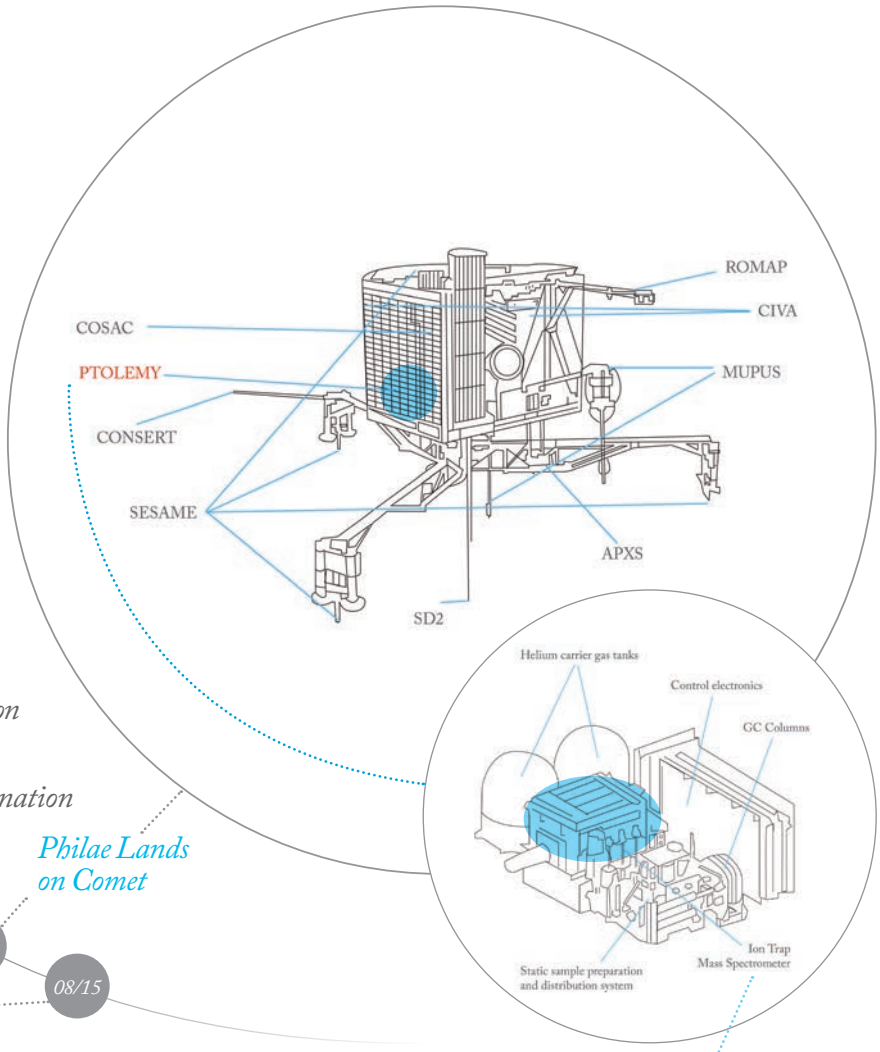
What mission outcome would give you the greatest satisfaction?

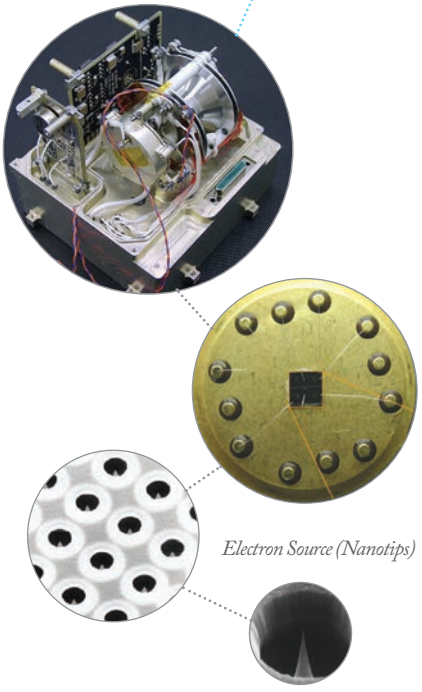
"In many ways there have already been rewarding outcomes; some of the people who had involvements with the mission now have interesting jobs and careers of their own. Furthermore, some of the expertise developed on the project has been, and continues to be, deployed in ventures that are either academically or commercially relevant. For those still actively working on Rosetta, the greatest satisfaction will be the return of some valid electromagnetic signals that ultimately have the capacity to convey some new scientific meaning and knowledge."

In addition to Ptolemy (aka *MODULUS*: methods of determining and understanding light elements from

Mission Milestones

- 03/04 — *Launch*
- 07/05 — *Deep Impact Observation*
- 09/08 — *Asteroid Steins Flyby*
- 07/10 — *Asteroid Lutetia Flyby*
- 07/11 — *Enter Hibernation*
- 01/14 — *Exit Hibernation*
- *Philae Lands on Comet*
- *Rendezvous with Comet C-G* — 05/14
- 11/14
- 08/15 — *Rosetta & Comet enter Perihelion*





unequivocal stable isotope compositions), the Philae surface science package (total mass 21 kg) also includes an alpha-proton-X-ray spectrometer to determine elemental composition, another GC-MS system (the cometary sampling and composition experiment or COSAC) for chemical analysis of core material, and a host of other acronymic wonders:

SESAME: surface electrical, seismic, and acoustic monitoring experiments will acoustically investigate surface material and measure the environment's dielectric properties.

CONSERT: comet nucleus sounding experiment by radiowave transmission will measure electrical characteristics of the nucleus bulk material and internal structure.

MUPUS: multi-purpose sensors for surface and subsurface science will study the comet's physical properties.

ROMAP: Rosetta lander magnetic field investigation and plasma monitor will investigate the comet's magnetic field as well as its interaction with the solar wind.

CIVA: Comet nucleus infrared and visible analyzer will characterize samples an record IR spectra.

Philae also boasts a couple of other in-situ imaging systems and the all-important sample drill and distributor (SD2), which feeds samples to Ptolemy, CIVA and COSAC. Let's hope it all works after ten years in deep space.

For more information on Rosetta Mission: tas.txp.to/0114/Rosetta and Ptolemy: tas.txp.to/0114/Ptolemy.

The Alternative Impact Factor

Could online activity assessments of academic papers be a viable alternative to impact factor or h-index?

Altmetric (www.altmetric.com) is a UK-based start up with a mission to "track and analyze the online activity around scholarly literature". Its list of the Top 100 academic research papers were published at the end of 2013.

How the data were collected and the process used to collate the Top 100 can be found on the company's blog (1), but a quick scan of the list reveals a number of articles that (in some cases intentionally) attract a certain kind of attention – not necessarily the right kind. Is online response predominantly driven by toilet humor? Perhaps. Evidently, the system is in its infancy, with room for improvement.

Despite potential drawbacks, it was both pleasing and surprising to find two entries that represent the analytical sciences – testimony to the wide reaching impact of our field on society.

#36: Towards practical, high-capacity, low-maintenance information storage in synthesized DNA (2)

What? Computer files totalling 739 kilobytes of hard-disk storage was converted into DNA code, which was then synthesized.
Result? The synthesized DNA was sequenced and the original computer files reconstructed with 100% accuracy.
Featured in The Analytical Scientist: www.theanalyticalscientist.com/issues/0413/digital-versatile-dna/

#3: Direct Imaging of Covalent Bond Structure in Single-Molecule Chemical Reactions (3)

What? Noncontact atomic force microscopy used to investigate reaction-induced changes in the internal bond structure of oligo-(phenylene-1,2-ethynylenes).
Result? Images revealed complex surface reaction mechanisms behind thermally-induced cyclization cascades of enediynes.

How important is online response to your work? Please let us know by commenting on this article – online!

References

1. *www.altmetric.com/blog/the-2013-top-100-list*
2. *N. Goldman et al., Nature (2013) doi:10.1038/nature11875 (Altmetric rank: 36).*
3. *Dimas G. de Oteyza et al., Science, 340 (6139), 1434-1437 (2013) (Altmetric rank: 3).*

Protein Proxies

Can correlations between micronutrient levels and proteomic profiles help development of more rapid tests for malnutrition?

Take plasma samples from 500 Nepalese children, analyze them for nutrient levels using conventional biochemical methods and for proteins using quantitative mass spectrometry. Compare the results. You may just have the answer.

Keith West, Bob Cole and colleagues at the Bloomberg School of Public Health and School of Medicine, Johns Hopkins University, Baltimore, USA, did precisely that as part of a Gates Foundation funded-project that aims to investigate the potential for a more rapid, universal platform for screening micronutrient levels (1). Cole provided insight into the analytical aspects of the research.

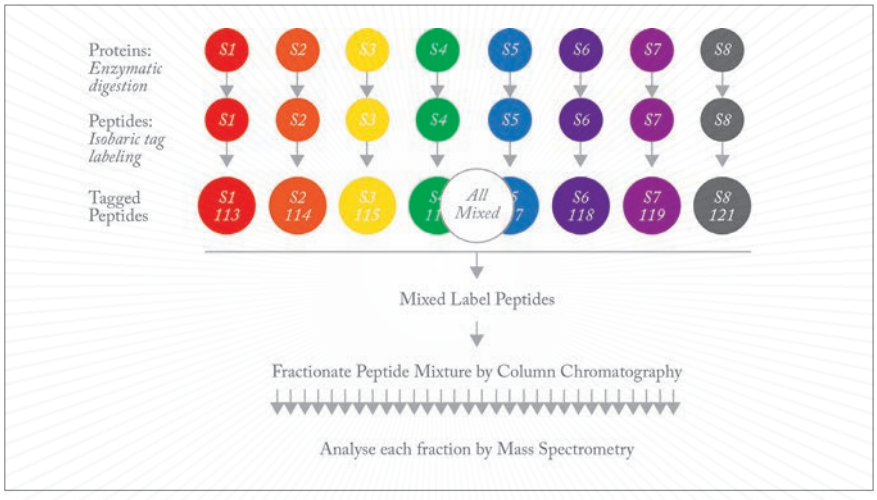


Figure 1. Workflow for analyzing eight samples in one experiment using iTRAQ reagents in an isobaric mass tag assay.

"We figured that proteins are most likely up- and down-regulated in relation to levels of micronutrients in the body, and that their correlation could be

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found in blood," says Cole. With that thought in mind, the group set out to find correlations between nutrient levels and individual proteomic profiles and, "Amazingly enough, we actually did."

The first protein and nutrient that showed a strong correlation in the system was the well-known pairing of vitamin A and retinol-binding protein (RBP). "It was very satisfying to see the correlation pop right out of the whole analysis in this way; we weren't directly looking to measure RBP4," says Cole, "we were actually measuring everything. Seeing RBP4 levels change relative to the amount of vitamin A proved we'd got something and validated the assay".

"The biggest challenge for me was the sheer number of samples that Keith wanted to analyze in order to gain sufficient statistical power." To lighten the load, Cole used iTRAQ reagents in an isobaric mass tag approach (see Figures 1 and 2). "Multiplexing the analysis allowed us to look at more than one sample per assay. That saves a lot of time. It also provides direct comparison of those samples with each other". In each run, eight samples were individually digested and labeled with a different iTRAQ tag, then combined and fractionated using HPLC. "Standardization of each step was very important; when we analyzed each of those fractions on our MS platform (Thermo Orbitrap Velos), we needed the results to be as reproducible as possible."

The team identified 4705 proteins, with 982 found in at least 50 of the children. "Employing a linear mixed effects model, we observed a number of correlations in addition to vitamin A and RBP4 (r = 0.88), namely, 25-hydroxyvitamin D and vitamin D-binding protein (r = 0.58); α-tocopherol and apolipoprotein C-III (r = 0.64); copper and ceruloplasmin (r = 0.65); and selenium and selenoprotein P isoform 1 (r = 0.79)".

Clearly, traveling around the rural areas of developing nations with a huge and complex system is not the end goal. "The objective was always to identify sets of proteins directly associated with nutrients in plasma to allow us to design a much simpler platform that measures those biomarkers," says Cole, "To that end, we are in talks with experts at the forefront of simplified microassays for proteins."

Beyond the potential to benefit populations in the developing world, there are whispers of extending the application to personalized medicine. Watch this space.

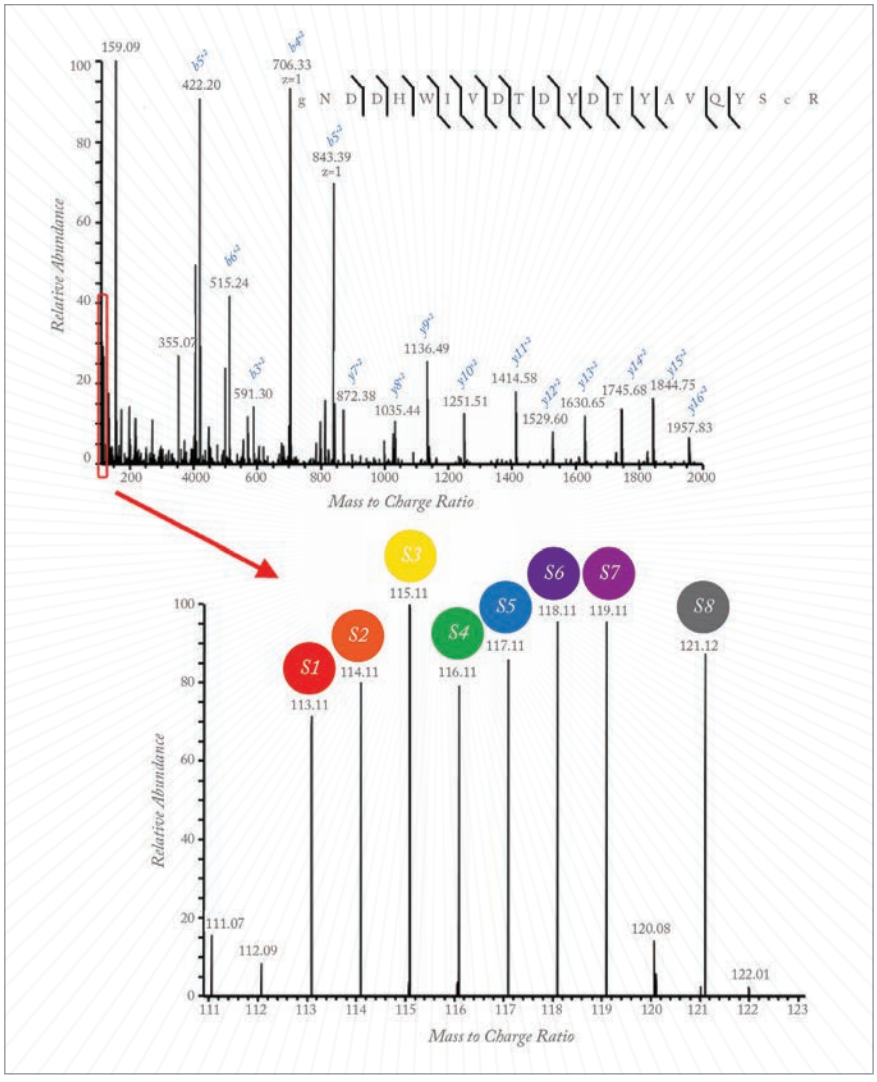

Figure 2. Relative quantification of a retinol binding protein 4 peptide, a protein proxy for vitamin A (retinol). Relative intensity of the reporter ions (113-121) shows the relative amount of this peptide in eight different samples. g=iTRAQ tagged glycine; c=alkylated cytiene.

Reference

1. *R. N. Cole et al., "The Plasma Proteome Identifies Expected and Novel Proteins Correlated with Micronutrient Status in Undernourished Nepalese Children", J. Nutr. 143, 1540-1548 (2013) DOI:10.3945/jn.113.175018*

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What's for Dinner, Mummy?

GC-MS shows that high status Egyptians used exotic balms to mummify food for a slap-up meal in the after life

Mummies are an obvious and tempting target for organic residue analysis. And, over the years, Richard Evershed, professor of biogeochemistry at the University of Bristol, UK, has published several papers that add to the known chemistry of mummification and thus the trends of the process. But his most recent research focuses on something a little more unusual: meat mummies (1).

Research interest in mummies follows a natural hierarchy. Humans, unsurprisingly, come first. Animal mummies – pets for the dead - were produced in the millions, and so have also been extensively studied. Food mummies, on the other hand, have patiently awaited their chance in the research spotlight. Some foods, such as fruits, seeds and bread, are relatively common in tombs because they survive simply through desiccation rather than complex mummification. "Meat offers a different challenge. We wanted to see if the mummification processes for humans and animals were replicated. Unfortunately, meat mummies tend only to be found in high status tombs, which means they're rare and somewhat precious," says Evershed.

Only 40 boxes of mummified food were found in Tutankhamun's tomb – perhaps the most popular of all mummies (now even more famous given recent findings on the religious reasons behind his erectly embalmed appendage). And while Evershed couldn't get his hands on King Tut's tasty treats, he was able to secure samples from the tombs of Yuya and Tjuiu (1386–1349 BC), the parents of Queen Tiye (wife of Pharaoh Amenhotep III, if we're name dropping). Evershed analyzed the samples with his usual armory of techniques and used isotope analysis to identify the origin of fat residues.

"We are dealing with very precious samples; we keep manipulation to a minimum and work at the microscale," says Evershed. The extracts were trimethylsilylated and injected through a high-temperature GC system with a thin-film (non-polar) column. "This setup allows us to take a look at the full range of compounds, from fatty acids up to high molecular weight waxes."

Evershed's biggest surprise was a package identified by X-ray in Cairo to contain part of a rack of beef ribs. "We were lucky to receive some of the bandaging, which had a lot of organic balm applied. When extracted and analyzed by GC-MS, the major compounds were found to be fatty acids," says Evershed. "Wax esters – possibly derived from bees wax – were also present. But the real surprise was the distinctive triterpenoids, which point to a source in the pistachio tree. This is a resin that was certainly known in the Mediterranean in those times, and there's evidence of it being traded around, but it's very rarely been found in mummies."

In fact, Evershed's balm pre-dates previously recorded use of the pistachia balm by about 700 years. The limited use of the balm, perhaps only in higher status tombs, indicates its exotic nature; indeed, very few high status mummies have been analyzed in this way – most of them are still kept under wraps (pun intended) in Egypt.

The recent research adds to a growing body of data that helps gain a deeper understanding about the evolution of the embalming process. It also forms part of a "bigger picture"; Evershed's group has been active in the field of archaeological organic residue analysis since the 1980s. "That was when people started to develop more of a realization that, if you applied organic chemistry using modern analytical methods, such as GC-MS, you could identify molecules that, in turn, allowed insight into some of the more intractable materials found at archaeological sites."

"The use of analytical chemistry adds many more pieces to the archaeological puzzle – pieces that other archaeological methods, like staring down a microscope, simply cannot provide," concludes Evershed.

Reference

K. A. Clarka, S. Ikram, and R. P. Evershed, "Organic chemistry of balms used in the preparation of pharaonic meat mummies", Proc. Natl. Acad. Sci., 110 (51), 20352-20353 (2013). DOI: 10.1073/pnas.1315160110.

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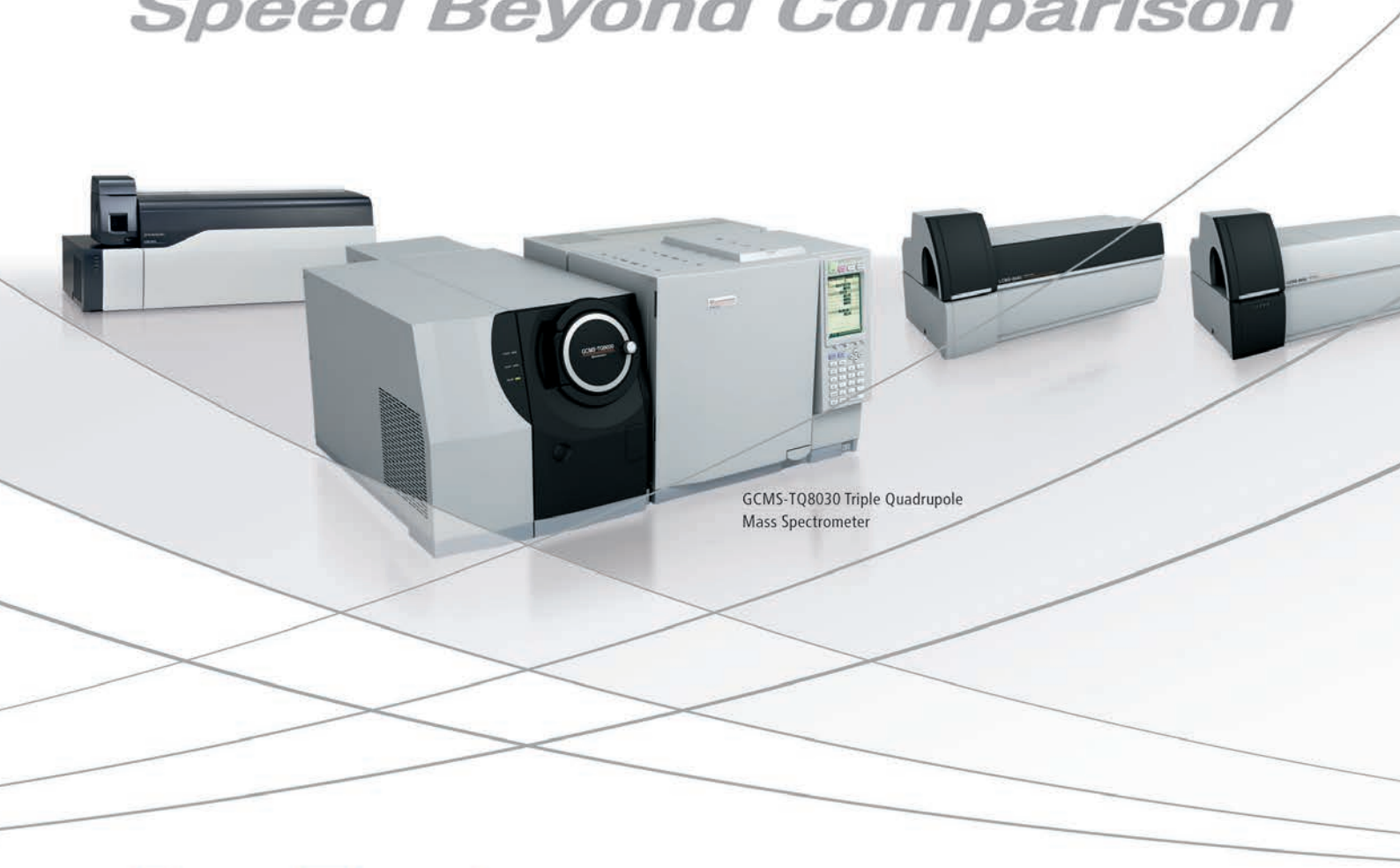

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Pittcon Prelude

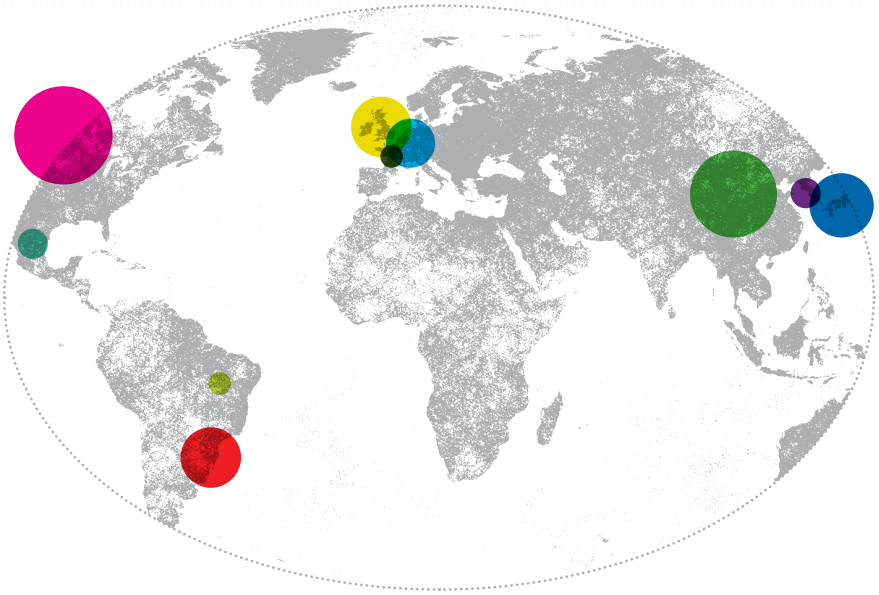
The Windy City is set to welcome "the world's largest annual premier conference and exposition on laboratory science", but are you prepared? Here, we look at historical attendance trends and offer our top picks.

Much like The Analytical Scientist, "Pittcon's target audience is not just 'analytical chemists,' but all laboratory scientists – anyone who identifies, quantifies, analyzes or tests the chemical or biological properties of compounds or molecules, or who manages these laboratory scientists." With that mission firmly in mind, Pittcon offers an almost dizzying array of technical programs and short courses covering the entire field of analytical science, in addition to packing in an exhibition and poster session that will test your fitness levels.

How best to navigate it? Certainly the app (see page 3) is very handy – you can start planning for the big show right now using the personalized agenda builder.

We hope some of the information presented below will help you decide what might be hot in 2014.

If you do find yourself with any free time at the show, don't forget to stop by The Analytical Scientist at 4151 to say hello to the team and pick up a copy of the latest issue.
Pittcon 2014 will be held March 2–6 at McCormick Place, Chicago, USA.
www.pittcon.org

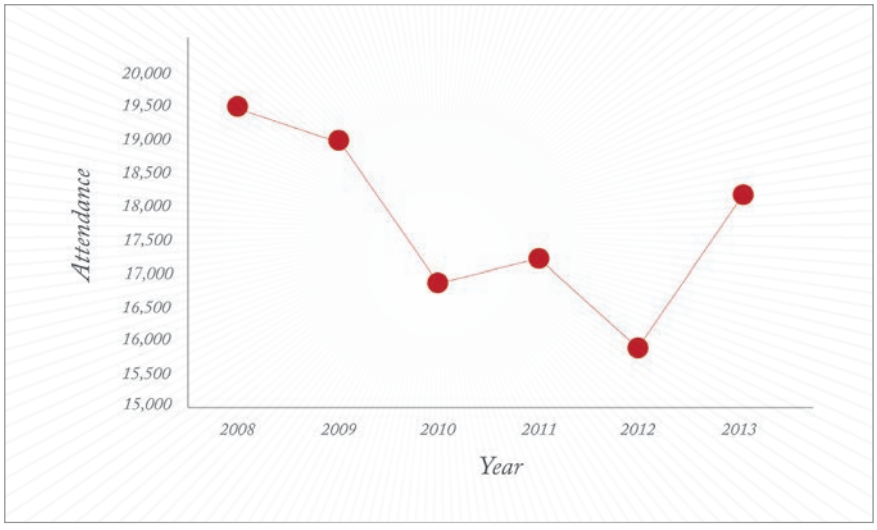


Global Networking

Canada	*2.6%*	*UK*	*1.6%*	*Brazil*	*0.6%*
China	*2.3%*	*Germany*	*1.3%*	*France*	*0.6%*
Japan	*1.9%*	*South Korea*	*0.8%*		
Uruguay	*1.7%*	*Mexico*	*0.8%*		

Attendance: Riding the Great Recession (2008-2012)

2013 proved to be the bounce-back year for Pittcon, with attendance numbers climbing towards pre-recession numbers



Top Ten Most Popular Short Courses of 2013

- Essentials of Modern HPLC/UHPLC 2: Operation, Troubleshooting and Method Development
- Introduction to ICP Mass Spectrometry
- Analytical Organic Mass Spectrometry
- Essentials of Modern HPLC/ UHPLC 1: Fundamentals and Applications
- Analytical Method Validation, Verification and Transfer: Using a QbD Approach to Method Lifecycle
- Qualification and Validation of Laboratory Instruments and Equipment for Regulatory and QS Compliance
- Application of ICP-AES Spectrometry
- Root Cause Analysis
- Elemental Impurities in Pharmaceuticals
- Industrial Problem Solving Using Thermal Analysis Techniques

Editorial Top Five

Symposia

- The Science and Impact of Transformative Technologies on Forensic Science (March 2)
- Capillary Liquid Chromatography - A Powerful Tool in Analytical Chemistry (March 3)
- Design and Application of Smart Materials for Chemical Sensing and Analysis (March 4)
- Quantitative Glycomic and Glycoproteomic Strategies (March 5)
- Fiber-Based Analytical Platforms (March 6)

Oral Sessions

- Methods for Metabolomics, Lipidomics, and Proteomics (March 2)
- Nanotechnology: Sensors and Electrochemistry (March 3)
- Analysis of Bioagents and Explosives (March 4)
- Chemometrics (March 5)
- Capillary Electrophoresis: Small Molecules and Neurotransmitters (March 6)

Organized Contributed Sessions

- Orthogonal and Risk-Based Sensing Systems for Homeland Security Applications (March 2)
- Spectroscopy for Everyone – Smaller, Cheaper, in the Field (March 3)
- High Throughput Analysis for Food Safety and Cosmetics (March 4)
- Novel Application of Terahertz and Millimeter Waves in Spectroscopy and Imaging (March 5)
- SAS: Women in Spectroscopy (March 6)

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 be up to 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

Breaking The Rules

Bending standard corporate strategy sounds like risky business, but can be rewarding in more ways than one.

By Alexander Bünz, Managing Director, Knauer, Berlin, Germany

I love to rethink behavior patterns and business processes, especially if they have become well accepted by the majority of market participants. Why? Because I truly believe that in a rapidly changing world, the rules of business also need to change. Therefore, I fully advocate 'breaking the rules' with my staff and have proved that it can be of significant value to both our customers and our company. Here, I describe two examples of rule breaks that we have successfully employed and used intensively.

When we analyzed the sales process in our market, we recognized that selling was still very much focused on offering customers a dizzying array of options, comprising hardware, software and services from the portfolio of the supplier. Apparently, most sales people genuinely believe that, regardless of the needs of the customer, they will have a product or service that matches perfectly. The product catalogue is very often the sales person's main tool and is used to impress the customer and win their business.

However, if instead we let customers precisely describe their situation, the challenges they are facing, and the financial consequences they are shouldering, a different scenario often emerges. How do we build a deeper relationship with customers? It's simple: by asking open questions that allow people to think and collect together their ideas. It is fascinating how much can be learnt by asking the right questions. As a result, we have pretty much abandoned the use of product catalogues in our sales process and, because of that, our sales force don't always assume that we have the right or best product or service to fulfill the customers needs – a fact that is a quiet truth for many, if not all, service providers.

Taking the above philosophy a step further could be considered dangerous, but is actually quite logical. With such in-depth knowledge being shared with our sales people, we feel comfortable about offering all possible solutions and, by doing so, enable customers to better manage the challenges of the future. It is typically a surprise to our customers that, included in our proposed list of possible solutions, there may very well be a recommendation to connect with one of our direct competitors. Why do we include this option and risk losing business? We do it because – shelving profit for a moment (difficult I know) – the deeper relationship forged with our customer warrants the sharing of best knowledge. Likewise, most people would not feel comfortable about recommending a product that didn't match a friend's needs or budget – trust is at stake. And with trust comes the potential bonus of long-term business rather than one-time sales.

The second rule break is about challenging something that is ingrained into us all from our earliest days at school in order to change the very

"I truly believe that in a rapidly changing world, the rules of business also need to change."

culture of our company. To prosper in the HPLC market, we rely heavily on innovation. This means that it is important for everybody involved in this creative process to explore new fields – and, therefore, to take risks and fail. And while many companies claim to accept mistakes as part of their culture, we once again take things a slightly scary step further. We actually demand that everybody takes ownership of at least one major mistake each year, which is cheerfully acknowledged in our annual appraisal meetings. Why do we give this idea so much weight and go as far as asking the managing directors to openly discuss the mistakes they have made? It is because we strongly believe that, alongside the willingness to fail and thus make mistakes, we generate the potential to develop and successfully market truly new products and solutions.

For us at least, breaking the rules is working. It differentiates us from competitors and has increased our credibility as a partner. On those grounds, I'm always on the look out for new rules to break – or at least bend.

The Critical Role of Sampling

Far too little respect for, and competency in, representative sampling is making a mockery of analytical error.

By Kim H. Esbensen, Research Professor, Geological Survey of Denmark, and Professor, Greenland and Aalborg University, Denmark

When confronted by a large material lot, say 200 tonnes, with a sampling task (scoop, shovel, or whatever in hand) it is natural for the uninitiated to ask: "how big a sample do I need in order for it to be representative?" For lots of this size, the question can be very daunting. Surprisingly, in the light of the theory of sampling (TOS), this is the wrong question, at the wrong time, in the wrong place! How wrong can you be?

The wrong question. Representativeness is not related to sample size, but to the sampling process. Ascertaining whether a particular sample is representative or not can never be resolved by characterization of the sample itself, only by characterization of the sampling process.

The wrong time. The question should have been considered long before the actual sampling process.

The wrong place. Unless you have already started learning the bare minimum of proper sampling principles, you are most likely considering taking just one sample – a procedure termed 'grab sampling' in TOS. However, the most important tenet of TOS is that grab sampling is always wrong. Only composite sampling works, where a sufficient set of individual increments covering the entire volume of the lot is essential. Numerically determining "sufficient" is part of the definition of representativeness, and is, in fact, the answer to the question, "how big a sample". For many, this inconvenient truth is at odds with practicality and economic feasibility. However, if a sample cannot be documented as being representative, what is the purpose of subjecting it to analysis?

Lot materials can vary enormously, be this in their overall size, shape and material composition, their constituent units (particles, grains, molecules, number of physical phases), the unit's own compositional differences (constitutional heterogeneity) and their spatial distribution within the lot at all scales from the sampling increment to the full lot size (distributional heterogeneity). A full definition of heterogeneity characteristics is not necessarily a straightforward issue. For illustration, I'll pick a few examples from the world of science, technology and industry, where representative sampling is a must: food and feed (sampling for quality, compositional compliance, or health control); coal (sampling for determination of calorific value, moisture, or 'percentage fines'); genetically modified organisms (sampling for regulatory compliance); commodity or product quality control (sampling for control of specification). All such sampling targets (along with many other examples) are different in almost all of the above aspects. In fact, they are so vastly different that it follows that representative sampling should be material dependent, meaning that a bewildering number of different

approaches are needed: if not one for each material then one for each material class.

For a very long time, representative sampling has been too complex for comfort, with the majority hoping the issue would go away by itself. For more than 60 years, TOS has been predominantly ignored or even actively criticized, with a few salient exceptions; for example, in the mining and cement industries, where faulty product and process decisions cannot be accepted because of the enormous tonnages of material at stake. Here, TOS has been an integral part of the decision-making process for years.

What is the relationship between sampling and the validity of an analytical result? Well, let me counter with another question: what is the purpose of analyzing a 'sample' whose provenance is not known? Any analytical result is, strictly speaking, only valid for the (often) miniscule analytical aliquot volume; however, decisions based upon the result pertain to the entire original lot. Typical aliquot/lot mass ratios involved in practical sampling range from 10^3 to 10^{12}, but even the smallest ratio is far from trivial. What if all this mass-reduction – sub-sampling – is not representative? In a word: disaster! Everything hinges on the sampling process and whether it is representative or not. Evidence shows that sampling error effects dominate over analytical error effects, often dwarfing them in the total uncertainty budget (1-4).

So, amidst all the complexity, is everything lost? Most emphatically, no! The last ten years have seen very encouraging changes, with even more momentum gained in the last three years or so. What happened? The surprise is that TOS outlines why and how sampling issues are not overly complex, but instead can be understood with only a little, albeit focused, effort. It turns out that lots are only different with respect to one salient characteristic: heterogeneity. And TOS helps counteract heterogeneity in the sampling process. The principles behind representative sampling are scale-invariant, so, once mastered, can solve all sampling problems, at all scales, for all types of lot and material. In fact, TOS states that there is no such thing as an impossible lot or material to sample, there are only sampling situations that are wrongly viewed as "too complex", "too laborious", or "too expensive" relative to existing grab-sampling purposes. While dominant in today's general sampling perception, these arguments are all totally wrong.

September 2013 saw the publication of the world's first standard dedicated to sampling, "DS 3077 - Representative Sampling – Horizontal Sampling" (3), in which all principles behind representative sampling are now for the first time collated, described in sufficient detail, and given context. DS 3077 forms a very convenient starting point for everyone interested in getting to the bottom of the (otherwise totally unsolvable) sampling problem.

References

1. *K. H. Esbensen and L. P. Julius, "Representative sampling, data quality, validation - a necessary trinity in chemometrics", in Comprehensive Chemometrics (Wiley Major Reference Works. Vol. 4, Oxford) p. 1-20. (2009)*
2. *Esbensen, K.H. & P. Paasch-Mortensen (2010). Theory of Sampling – the Missing Link in Process Analytical Technologies (PAT). DOI: 10.1002/9780470689592.ch3*
3. *http://webshop.ds.dk/product/M278012/ds-30772013.aspx*
4. *http://www.impublications.com/tos-forum*

Where is the MSI Software Messiah?

Common data file formats are readily available for mass spectrometry imaging (MSI) data. Now, the community must agree on an extensible cross-platform software solution that everyone can use.

Guillaume Robichaud, David Muddiman, Kenneth Garrard, and Jeremy Barry, North Carolina State University, Raleigh, USA.

Once upon a time in the history of proteomics and liquid chromatography-mass spectrometry (LC-MS), instrument manufacturers encoded data in vendor-specific formats and offered proprietary software tools for data analysis, with limited or zero data-exporting capabilities. For a long time, there was no common data file format, making it very difficult to share, compare, and analyze MS data obtained from different platforms. And so, to perform custom data analysis, scientists would often have to reinvent the wheel, spending precious time writing programs for common tasks, such as peak picking, deconvolution, peak area calculation, and data visualization.

Initial efforts from the Seattle Proteome Center (mxXML format) and later from HUPO-Proteomic Standard Initiative (mzML format) were key contributions to the field because they provided standard, vendor-neutral formats for MS data. Most vendors joined the parade and provided support or even tools to convert proprietary data into vendor-neutral formats. A second major contribution to the field was the Proteowizard project, which provided the community with a robust, validated modular set of free, open-source tools and libraries to perform analysis of MS and proteomics data. Scientists could finally focus their time and energy on developing novel algorithms and tools that significantly advance the field.

So, what is happening within the field of MSI? More than 15 years after its introduction, there is still no widely accepted tool for viewing and performing basic data processing (peak picking, feature recognition, data extraction, normalization). Lessons learned from the history of software development in proteomics and LC-MS suggests that a common, shareable data file format is one prerequisite for such tool to exist. The imzML format has now been around for several years and has been accepted by the MSI community as the common data file format for MSI. Free, robust data file converters from vendor formats to imzML are also widely available.

"There is still a lot of work to do before MSiReader becomes the definitive tool."

But what about MSI software? After a quick census, we found 20 different MSI tools, eight of which are commercial products. The disconcerting part is that most of the free software was released in the last two years or is currently "in development". It is safe to assume that most of the time spent coding these 12 interfaces was not spent developing novel data-processing algorithms, but instead building the user interface and implementing the same basic but necessary MSI tools. On the other hand, brilliant research is focusing on the development of new algorithms for the analysis of MSI data, such as peak picking, automatic feature extraction, data normalization, spatial segmentation, clustering, resampling, but until there is a common, open source MSI platform, they cannot be easily implemented.

Earlier this year, our group introduced MSiReader, an open-source interface for viewing and analyzing MSI data. The fully customizable interface can load most common MS/MSI data file formats, process high mass resolving power data, and comes with a plethora of great data visualization and analysis tools. Currently used by hundreds of scientists in over 50 (academic and industry) laboratories around the world, it already incorporates several important features, such as data export tools, peak extraction, batch processing, spectra viewing, normalization, image co-registration, baseline correction. Do we think MSiReader is the ultimate solution? Well, from the positive response we've received from the community so far, we firmly believe that it is at least a very honest attempt. However, there is still a lot of work to do before MSiReader becomes the definitive tool. In fact, we can already think of a small list of improvements: implementation of a comprehensive statistical analysis toolset, optional data resampling, 3D MSI, and co-registration with more data formats.

So, let's all work together and make it happen! We see all feedback and user input as essential to the future development and success of MSiReader.

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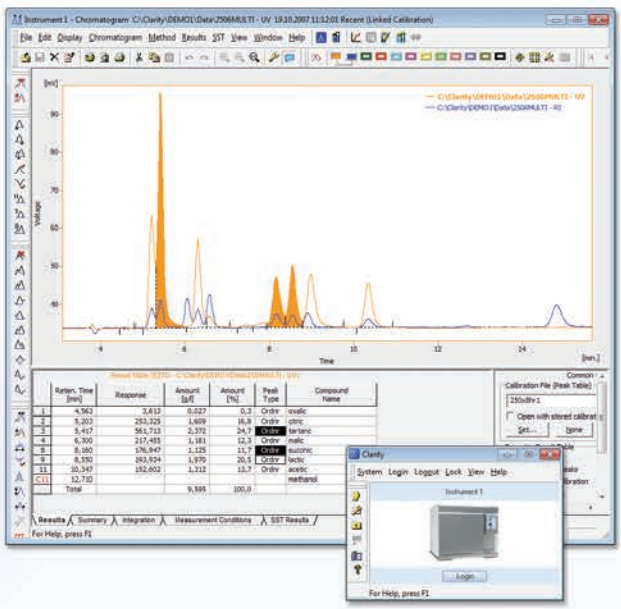

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The Entrepreneurial Scientist

In the face of increasing competition, standing out from the crowd requires more than good science. We must embrace self-marketing.

By Joeri Vercammen, managing expert at IS-X, Louvain-la-Neuve, Belgium.

I still remember the first time I searched the Internet. It was 1996 – a time when dinosaurs, such as Netscape, AltaVista, and AOL, ruled the virtual world. A couple of years later, during my PhD, I personally contributed (very slightly) to the implosion of the music industry (apologies to Lars Ulrich). Indeed, our faculty had the dubious honor of crashing the university's main servers for the first time, simply by downloading MP3s from Napster…

Just as Napster revolutionized the music industry, quite remarkable things are occurring in all areas. Today, platforms rather than websites are beginning to dominate the virtual world. SlideShare, for example, is a community for sharing presentations online (which I like a lot), hosting more than ten million presentations, documents and infographics. And with over 60 million unique visitors and 130 million monthly page views, it ranks in the world's top 150 most popular websites. Due to this popularity, each uploaded presentation possesses the intrinsic capability to rank highly in Google searches. Triggered (though skeptical) by this potential, I uploaded a presentation on gas chromatography carrier gases last year. If you type 'van Deemter' in Google you will most likely find it on the first page. The result? My presentation, "The van Deemter Indoctrination", has been viewed more than 2600 times – something that would not have happened if I had only published it on my own blog. And for a small annual fee, you can become a "pro" user with access to analytics that track presentation performance and trace viewers.

Compare this approach to the current state of scientific publishing and the contrast cannot be more vivid. Articles are generally published six months after submission, authors still have to pay and sign away copyright. And article-level analytics information? No such thing. Clearly, twenty-first century science would greatly benefit from a serious shift in gear.

We are all well aware that competition is getting fiercer by the year. As an example, in the year 2000, only six articles with co-authors from Chinese institutions were published in Nature journals. By 2012, Chinese scientists accounted for 8.5 percent of articles. That's a staggering increase of 5000 percent! At the same time, the global financial crisis has put severe pressure on scientific funding. Europe has been hit particularly hard; the Horizon 2020 research program suffered a budget cut of over 12 percent before its acceptance by the European Council. The general public is not always guaranteed to be supportive of scientific endeavors either; did you know that only 20 percent of Americans believe that human beings evolved from less advanced life forms without the guidance of a God? (In fact, 37 percent believe that God created human beings in their current form in the last ten thousand years. Source: YouGov.)

And yet, I remain hopeful. New approaches, such as crowdsourcing and open access journals, remain marginal, but are generating momentum that even classic scientific publishers cannot deny. A couple of weeks ago, I received an email from Elsevier proudly announcing a new service called 'AudioSlides'. I immediately checked the website: "AudioSlides are short, webcast-style presentations that are shown next to the online article on ScienceDirect". It's basically a low-tech version of SlideShare that gives authors the opportunity to present their research in their own words, helping readers to quickly understand what a paper is about, to appreciate its relevance.

The consequences for science practitioners are clear. Standing out and getting noticed is the key message. In order to do so, it's crucial to stop thinking like a typical scientist, get out of the lab and start acting like an entrepreneur. Not for you? Just take a look at these five qualities of a successful entrepreneur (2):

1. Unwavering passion
2. Open mind towards learning new things
3. Desire to be an expert
4. Forward-looking approach
5. Constant flow of ideas

Sounds familiar, doesn't it? So start marketing yourself; design yourself a logo, write a blog, and make beautiful presentations. Think outside the box and consider crowdsourcing for your next project. You'll be an innovator, no doubt, but isn't that what we all ought to be? The status quo is no longer an option.

References

1. *http://today.yougov.com/news/2013/07/22/belief-in-evolution-up-since-2004/*
2. *http://www.entrepreneur.com/article/227776*

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Authors intending to submit papers for the symposium will be required to adhere to the following deadlines:

- A 300 word abstract must be received no later than **February 1, 2014**. For abstract submission see the website.
- Notification of acceptance will be mailed to the authors by **March 1, 2014**.

REGISTRATION FEE

Advanced registration, prior to April 1, 2014	
Registration 38th ISCC	450,00 €
Registration 11th GCxGC	200,00 €
Combined Registration (38th ISCC/11th GCxGC)	600,00 €
* Student Registration 38th ISCC	225,00 €
* Student registration 11th GCxGC	100,00 €
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Registration fees include entrance to all technical sessions and the exhibition, a copy of the final program, a book of abstracts and participation in social events.

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MS Imaging Targets the Clinic

The field of mass spectrometry imaging is enjoying constant – and exciting – progress. Here, Ron Heeren and Axel Walch highlight current research applications and anticipate the impact that the technology will have on clinical practice.

Give us a snapshot of mass spectrometry (MS) imaging

Axel Walch: From a clinical perspective, MS imaging – unlike most imaging methods – removes the need for target-specific labeling whilst measuring the required broad spectrum of endogenous and exogenous analytes in tissues within the histological context. The highly multiplexed analyses offered by MS imaging enables not only diagnostic (targeted) assays but also the potential for biomedical discovery applications. Multi-class analysis capability (from proteins and peptides to drugs, lipids and beyond) is a further strength of MS imaging. The practical simplicity of MS imaging and its ability to gain reliable information, even from the smallest tissue samples, means that it has the potential to complement traditional histopathologic evaluation for assisting in diagnosis, risk assessment, or response prediction to therapy.

Ron Heeren: Right now, there is a strong push for higher information content imaging to unravel the molecular complexity of biological surfaces. This includes a drive for higher resolution techniques – both higher spatial resolution and higher molecular resolution. The latter is being pushed by high-resolution mass spectrometry (MS) techniques (Fourier transform (FT)-MS, TOF systems and so on), as well as the incorporation of ion mobility separation to enrich the wealth of molecular information obtained from complex organic surfaces.

Another important development is the move towards rapid quantitative MS imaging strategies that use selective reaction monitoring (SRM) approaches and dedicated internal standards. In particular, this is a requirement for pharmaceutical applications, such as pharmacokinetic and pharmacodynamic (PK/PD) studies.

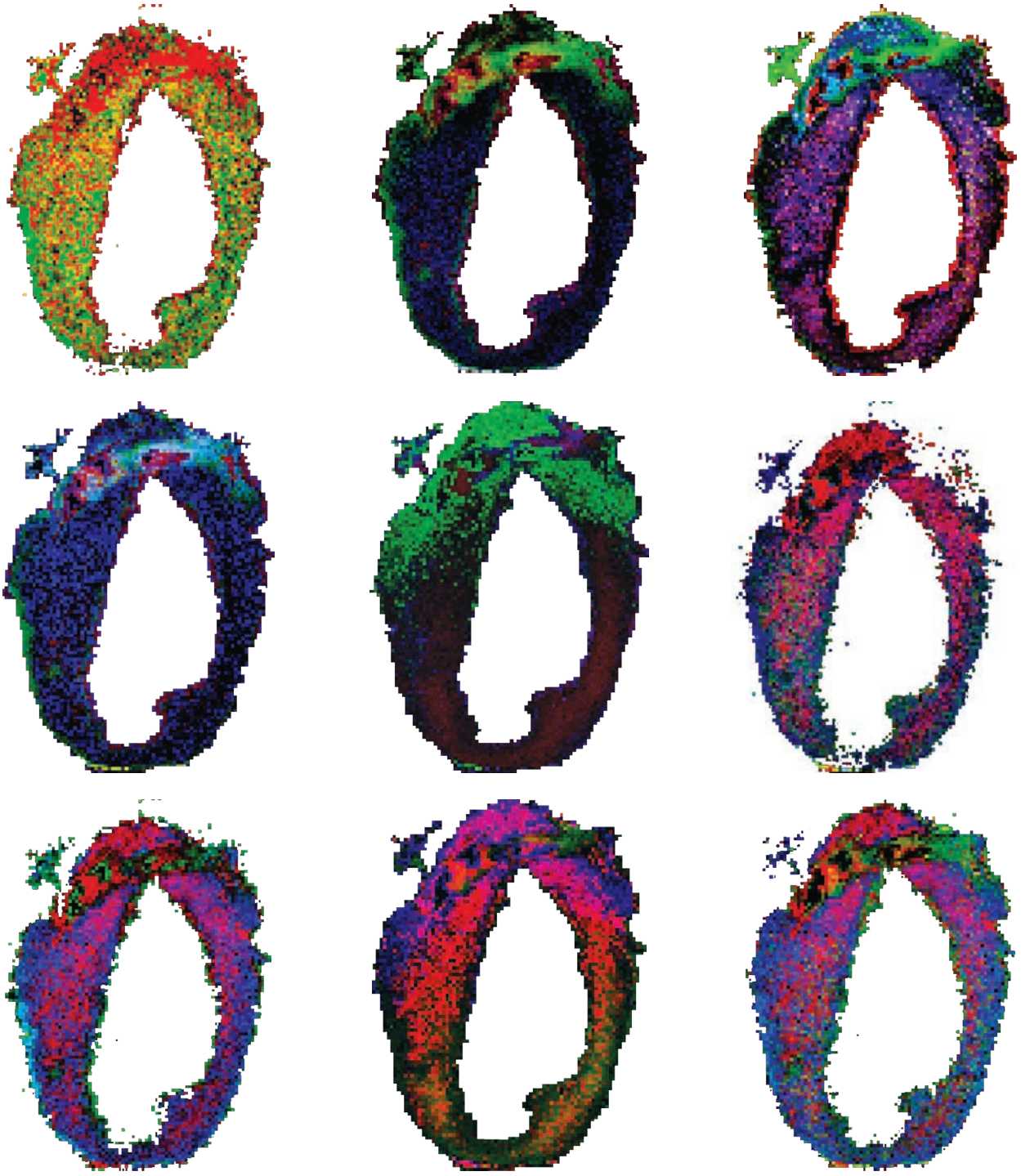
Ambient imaging technologies are opening up a whole new area of MS imaging. Essentially, samples that are not vacuum-compatible can now be imaged at the molecular level. In some cases, such as with laser ablation electrospray ionization (LAESI), multiply-charged ions can be produced, and that offers various analytical advantages. This step change now allows true top-down imaging to be developed.

What trends do you see in the application of MS imaging?

RH: MS imaging is developing into an analytical tool for the fundamental discovery of signaling pathways of disease, leading to validated molecular disease profiles in the area of clinical molecular imaging. These disease profiles can be used for staging a disease, that is, for diagnostic and prognostic purposes. Even more importantly, the breadth of molecular information that MS imaging offers is a key input parameter for personalized medicine. In reality, it is rare to find a single molecule or biomarker that can be used to determine the course of a disease. Rather, it is an understanding of the interplay between proteins, lipids and other small molecules that offers the true solution, and that is exactly what MS imaging offers. It is this that will define its role in the future.

The heterogeneity of tumors can be fully disclosed using MS imaging. Lipids, primary metabolites and peptide/protein images reveal the tumor margins and provide insights into molecular signaling pathways. This makes MS a key tool for various aspects of oncology, such as identfying surgical margins, developing personalized therapies and tumor staging.

Other areas of medicine will also benefit from the fundamental understanding of the spatial molecular composition. One example is cardiovascular research: atherosclerotic plaques and the interaction of stents with arterial walls can be much more fully explored. Another is neurodegenerative diseases: altered molecular signals that lead to tissue loss or the formation of protein plaques in the brain can be dissected. The power to examine all molecules simultaneously will provide true mechanistic understanding of disease.



Mass spectometry images of rat heart tissue sections.

In the more distant future, I predict that there will be discovery-based approaches, drug distribution and effect monitoring… possibly even MS imaging within the GP's office.

AW: To answer that, let me offer a brief description of the current status of pathology in personalized medicine. The task of the pathologist is to assist physicians in the correct diagnosis of diseases at the earliest possible stage; this allows the optimal treatment strategy to be developed for each patient. Surgical pathology (the traditional tissue diagnosis of biopsies or surgical resection specimens) is simply a tool based on histology. "Molecular morphology" aims to combine novel molecular and genetic information with the well-established histomorphologic features that continue to define cancer and many other types of diseases mentioned by Ron. Certain molecular and genomic technologies for tissue analysis are already useful in diagnosis of disease, taking us towards treatment tailored to individual patients. Personalized medicine – the future of patient health care – demands personalized pathology. This would integrate the flood of new molecular data with traditional surgical pathology, digital histopathology, and clinical data in electronic medical records. MS imaging of tissues has a central role in combining traditional tissue diagnosis by histopathology with highly multiplex molecular analysis. MS imaging data sets are enormously complex, comprising thousands of unique ion images. These images can be registered to high-resolution digitized histology images, correlating molecular information and adding a new dimension of understanding to the histopathology and thus disease.

The future for personalized pathology is that molecular signatures of proteins, peptides, lipids, and molecules of cell metabolism obtained by MS imaging directly from patient tissues, will improve diagnosis and therapy response prediction for improved patient stratification. In PK/PD, the application of MS imaging to determine the tissue distribution of drugs and their metabolites will have a dramatic impact on both drug discovery and development.

What have been the biggest MS imaging breakthroughs of the last decade?

RH: The birth of atmospheric desorption and ionization techniques and their combination with mass spectrometry have opened up a new field of imaging and analysis. This has allowed analysis to be performed in situ, for instance during surgery and has also enabled researchers to study non-vacuum-compatible samples. Another huge advance was the recent development of top-down imaging MS in which large proteins are localized and, importantly, identified in the same high resolution MS experiment. The recent application of active pixel detectors coming from high-energy physics has increase throughput and sensitivity. In the secondary ion mass spectrometry (SIMS) community, new particle beams (C60, Argon and H2O clusters) have enabled detailed depth profiling and 3D imaging studies with larger intact molecular species. All very exciting breakthroughs.

AW: From a preclinical and clinical research point of view, there has been a plethora of methodological publications about MS imaging, but only a limited number of studies clearly demonstrated an impact on MS imaging in biomedical and clinical research applications – my main area of interest. The following impressive highlights show the potential for biomedical discovery:

- Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues (1)
- Mammalian heart renewal by pre-existing cardiomyocytes (2)
- Ambient mass spectrometry for intraoperative molecular diagnosis of brain tumors (3)
- Imaging mass spectrometry reveals modified forms of histone H4 as new biomarkers of microvascular invasion in heptocellular carcinomas (4)
- Chemo-informatic strategy for imaging mass spectrometry-based hyperspectral profiling of lipid signatures in colorectal cancer (5).

"Personalized medicine – the future of patient health care – demands personalized pathology"

What are the greatest challenges facing MS imaging?

AW: MS imaging technology has huge potential, but do we want to see improved spatial resolution, the detection of higher molecular weight compounds, greater sensitivity, better quantitation, and increased molecular coverage? Of course we do! And, as with other analytical techniques that have made the leap, increased system stability, reproducibility, and user-friendliness will be essential, if we are considering MS imaging for routine use in a clinical setting. This is where vendors can help; to ensure a successful transition, fully integrated systems are an absolute must. Some are moving in the right direction, but a complete solutions platform – from sample prep to MS analysis to bioinformatics – is not yet available. I would love to know what's in the pipeline…

RH: While the technology is evolving rapidly and resolution and speed are improving annually, challenges still exist. Research is striving for ever more detailed insights but we are falling orders of magnitude shy of the challenges in bioinformatics and clinical validation of results. Standardization and validation are two issues that must be addressed for clinical maturation. More and more data is being generated using protocols that are fit-for-purpose rather than being standardized, making it difficult to generate large-scale, validated e-biobanks with assured quality. There is a clear need for standardization of protocols and tissue standards to evaluate the quality of the data being entered into the databases. The Human Tissue Atlas is a perfect example of how immunohistochemistry (IHC) images are consolidated around different tissues and diseases. Something similar for MS tissue imaging would be an incredible clinical resource. The projection – or fusion – of MS images onto existing tissue atlases is another challenge that needs to be (and is being!) tackled by the MS imaging community. One example is the Allen Brain Atlas (www.brain-map.org), currently used by researchers involved in brain tissue imaging with MS. European network initiatives, such as the Cooperation in Science and Technology (COST) action on MS imaging, have the potential to address these challenges.

Clearly, you both envisage a strong role for MS imaging in a clinical setting…

RH: The vast amount of molecular information generated by multimodal tissue imaging experiments is ideal for generating biobanks for different diseases. MS delivers a lot of immediate information on many different levels which can be used to make, for instance, well-informed clinical decisions during surgery or selecting personalized therapies. However, as stated earlier, this is only possible if the clinician can assess the information in context or immediately compare it to molecular disease profiles obtained from existing knowledge stored in an appropriate biobank or database. Once this infrastructure is established, the clinical diagnostic and prognostic applications of MS imaging will bloom. Biobanks will quickly be generated by analyzing large amounts of tissues and profiling approach (a concise set of quick local analyses) will be used to personalize therapy or adapt surgical procedures.

AW: MS imaging does have huge clinical potential, especially in surgical pathology but also in other areas. I agree with Ron's remarks on the need for development, standardization, and validation. Take the example of sample preparation, something we all hope to spend less time and effort on. While certain solutions have been developed, for example, pre-coated slides, spotting robots and spray coaters, much less has been done to standardize usage across laboratories or platforms. In order for diagnostic assays to become routine:

- Validated methodologies (for a statistically significant sample set) must be developed
- Methods for analyte identification must be improved
- Assay specificity must be confirmed (identification of the analyte in situ provides final confirmation that the assay sufficiently specific).

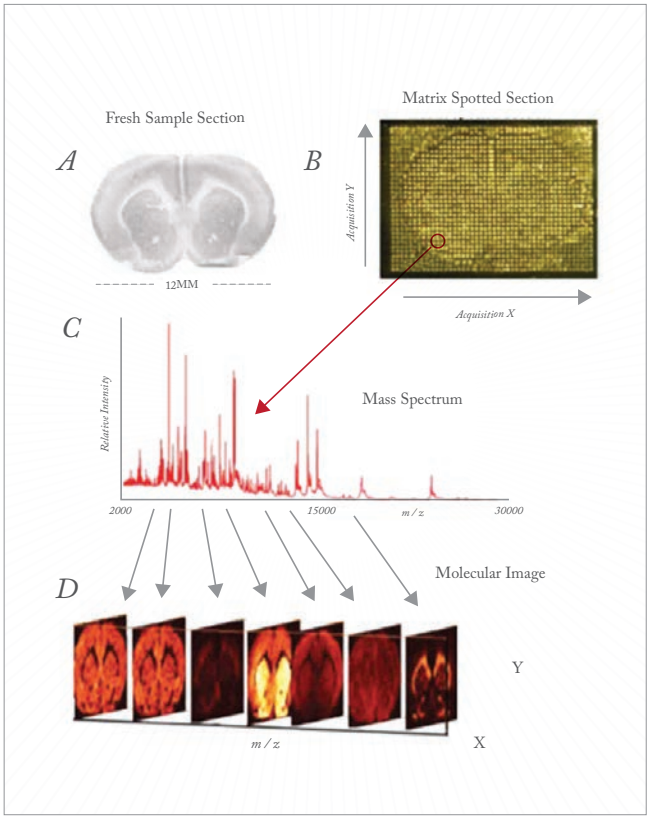
Can you comment on quantitative aspects of MS?

AW: Improvements are essential. While qualitative assessment can distinguish disease states based on the presence or absence of certain molecules, increasing numbers of applications require the ability to discriminate between subtle changes in analyte abundance. In these cases, we need absolute quantitation to make a valid clinical decision. This will require improvements to sample preparation, such as the addition of standards to the tissue, alongside increased instrumental range and more robust data analysis to advance MALDI imaging quantitation.

For early drug discovery, quantitative MS imaging could be deemed to be adequate. However, making accurate quantitative judgments (on a pixel-by-pixel basis) is some way off and must be addressed.

From a data analysis point of view, a number of software packages are attempting to address the time-consuming nature of both analysis and calibration. This will have a positive impact on the potential of quantitative analysis. Validation will be key to unlocking the true applicability of quantitative MS imaging – robustness is of paramount importance.

RH: The quantitative aspects of many MS-based surface analytical techniques are understudied. We still do not have sufficient insight into why certain molecules ionize well in one tissue environment but not at all in another. This mechanism is referred to as tissue suppression or, more colloquially, "the matrix effect." That's a term well known by those using other MS analyses, employed here to indicate that we have few clues as to what determines which molecules we can and cannot image with MS based microscopes. The difficulty can, in part, be ameliorated by the use of clever internal standards or multiple reaction monitoring (MRM)-based approaches. Forensic applications are beginning to benefit from this.



Imaging mass spectrometry. From Seeley & Caprioli, PNAS, 105, 18126 (2008)

However, there are still simply too many unknowns to make MS imaging quantitative and other targeted approaches currently offer a better solution when the compound of interest is known. In this light, MS imaging is a discovery tool for looking for the "unknown players" or the specific combination of molecules that make something happen in a living system.

What is the most tantalizing analytical application?

RH: From the many possible responses, I will offer three. One, in conservation science, MS imaging can be applied to the study of paint cross-sections. Two, investigations of bacterial colonies under environmentally-challenged conditions can be employed to investigate bioremediation of polluted environments. These studies can also offer insight into efficiently producing bio-fuels.

And three, study of the interfaces between new biomaterials and living systems will enable better biocompatible materials and drug delivery systems to be designed.

AW: The most tantalizing analytical applications of MS imaging are those that otherwise could not be performed directly with intact tissue in the natural histological context. Examples include the multiplex analysis of drugs, drug combinations and their associated metabolites; and imaging of cell metabolism molecules in situ, such as energy metabolism or the citric acid cycle. The simultaneous or sequential analysis of these different analytes on the same tissue section is possible, further increasing the uses of the technology. Such analyses at the level of individual cell populations within tissues could be not performed before the advent of MS imaging.

What specific choices need to be made to push progress?

RH: We stand at a transition point. The technology has matured enough to be applied in the clinic, but small-scale efforts will lead to disappointments; a large-scale infrastructure is needed to take full advantage of the benefits. Investment is essential, as is the concerted effort of all involved. Large-scale molecular imaging centers, with participation of national science and national health councils will result in more efficient translational research. The imaging centers in Harvard and Vanderbilt universities are key examples that I hope Europe will follow, developing large-scale multimodal imaging approaches complemented by solid bioinformatics and e-biobanking infrastructure. All of this requires integration at the national and international level (EU/Horizon 2020 and WHO).

Lastly, talk us through the next decade

AW: We've discussed challenges and limitations here. Despite these, MS imaging has seen exponential growth in both technological development and potential applications. I can only imagine it continuing to do so, joining a host of other established imaging techniques to form an ever more powerful toolbox combining broad coverage and exquisite specificity for the molecular analysis of tissues. MS imaging has already demonstrated its usefulness for biological discovery, and applications that display real clinical value are starting to surface more frequently.

Given continued progress in the challenging areas mentioned above, MS imaging will increase its foothold in biomedical research and, as we move towards personalized medicine, begin to adopt its greatest role in personalized pathology.

RH: New high throughput detectors, such as the IonPix detector from Omics2Image, and new high resolution imaging modes will be introduced. Parallel detection combined with imaging of increasingly large molecules or complexes will become possible and will take off with these new detectors, directly impacting the speed of analysis. In addition to new technologies, novel and efficient bioinformatics approaches are needed and, therefore, inevitable. All large-scale analytical data collected will be embedded in e-biobanks and made available to the larger medical community. This in turn will enable the introduction of small-scale MS-based analytical devices into health care systems. From here it is not hard to imagine a future where GPs have more informative tools at hand to quickly screen patients or determine drug levels and adjust therapy. Ultimately, this should reduce the cost of health care and improve quality of life for patients and their immediate environment.

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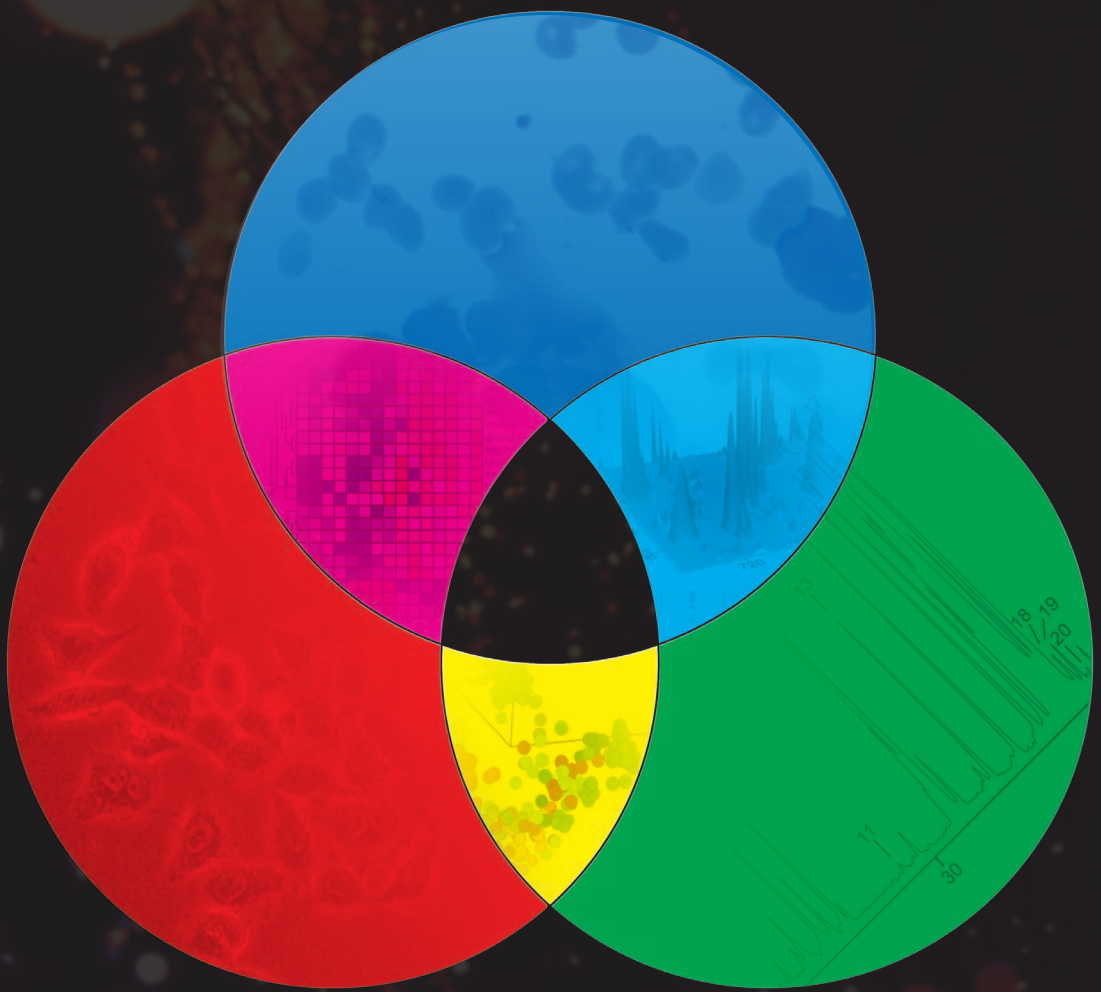

Tools of the Metabolomics Trade

How we go about the systematic study of the unique chemical fingerprints that specific cellular processes leave behind – and what we can learn from them.

By Robert Trengove

Jim Watson, co-discoverer of the structure of DNA and one of the driving forces behind the Human Genome Project, recently said (1), "If I was starting a PhD today, I would do it in metabolomics." What is exciting to Watson – and to all of us in metabolomics – is that the field is starting to move beyond simply identifying differences between control and treated/disease samples to mapping out pathways and the ways in which they change in response to the treatment or disease.

Let's track the development of the field. The current schema for metabolome investigations began around 2000, although the concept of targeted metabolomics has earlier origins (2). Initiation of the field is generally credited to Robinson and Pauling in 1971 (3) but the term was only coined in 1998 by Oliver et al. (4). Fourier transform infrared spectroscopy (FTIR) was used in that paper, while the first published use of single quadrupole GC-MS was in the field of plant metabolomics in 2000 (5); this work led to the development of GC-MS-based mass spectral libraries, and the Golm metabolome database (6, 7). Whilst this early research in plants was termed metabolomics, Nicholson and colleagues (8) led a parallel approach in whole organisms, which they termed metabonomics. Today these approaches have completely converged. Shortly after these initiatives began, the Human Metabolome Project emerged, led by David Wishart at the University of Alberta. It resulted in the development of the Human Metabolome Database (HMDB), a freely accessible web resource (9), which has recently been upgraded to version 3.0 (10) and now contains more than 40,000 annotated metabolite entries.

Since 2007, Wishart and coworkers have quantitatively measured several metabolomes (11-14), including that of cerebral spinal fluid, human serum, human urine and bovine ruminal fluid. In determining these biofluid metabolomes, they first mined the literature to determine the number of compounds that could be expected to be present. The experimental data subsequently used multiple instrumental platforms to maximize metabolome coverage, with quantitative data being established for the majority of metabolites. All of the experimental biofluid metabolome studies detected and identified fewer compounds than literature mining, but they also revealed compounds that had not previously been reported, as well as reporting concentration ranges for the metabolites. The most recent study, on the human urine metabolome, included the measurement of inorganic ions using ICP-MS and utilized BIOCRATES kits for targeted analysis.

The need for multiple platform analysis to maximize metabolome coverage is well known, as illustrated in Figure 1 (from Ref. 13). Equivalent conclusions have been drawn from non-human metabolome studies (15,16). The strengths and weaknesses of each instrumental platform for different biofluid types have been highlighted through these multiplatform studies.

Instrument Platforms

Ever since the life science community began to drive developments in separation science and mass spectrometry, the mantra has been "better separations, faster". Routine use of mass analyzers for metabolite "separation" following partial or minimal chromatographic separation is now common practice. This desire to undertake minimal sample preparation results in routine analysis of samples that would have previously been considered "too dirty" for mass spectrometry analysis; however, the need for metabolite limits of detection comparable to those achieved after extensive sample preparation remains, which places substantial demands on the chromatographic instrument, the mass spectrometer source and the initial ion optics. As a result, instrument manufacturers have invested significantly in research and development of source design to make them more robust, under the constraint of maximizing sensitivity. The techniques that are routinely used, together with emerging techniques, are shown in Figure 2.

The ideal metabolomics consortium would have access to all of these technologies, and a clear approach that plays to the strengths of each configuration for the sample types under study. Here, rather than address each chromatography and MS configuration, we focus on the strengths and shortcomings of each mass analyzer.

Single Quadrupole Instruments

The period from 2000 onwards has seen substantial developments in instrument and software performance. For GC-MS, single quadrupole systems can now operate at more than 15 scans per second, matching fast oven ramping to improve sample throughput, compared with 2-3 scans per second in 2000. In addition to faster scan rates, the instruments now have substantially improved limits of detection, even in full-scan mode. However, one must be cognizant of the type of samples being analyzed and the impact of these on the ability to achieve higher throughput analysis. For instance, plants have many different sugars, with very similar mass spectra, which dictates that they must be chromatographically resolved, reducing sample throughput compared with, for example, a plasma sample.

Time of Flight Instrument

Perhaps the most consistent developments in metabolomic analysis have come from Q-TOF mass analyzer instruments, even though the focus has been on HPLC- or UPLC-based systems. Mass accuracy, mass resolution, scan speed and dynamic range of mass analyzers have all improved significantly over the last decade, to the extent that this can be considered the age of the TOF. TOF and hybrid TOF instruments have been widely used. Their key benefit is broad metabolome coverage; however, only some of the most recent instruments are capable of polarity switching and data-dependent MS/MS, and MS/MS without loss of mass resolution and mass accuracy is limited to the very newest instruments. Accurate isotopic patterns are now achievable with most instruments, which, together with 1 ppm mass accuracy, offers the ability to determine unambiguous stoichiometry up to 500 amu. This killer combination in the latest generation instruments, under 1 ppm mass accuracy and over 50,000 mass resolution, delivers MS/MS without loss of mass accuracy or mass resolution and accurate isotope patterns; it represents substantial increased capability for untargeted metabolomics.

However, post-processing can be extensive with the latest generation instruments. Whilst cumbersome, it is possible to manually curate GC-MS single quadrupole untargeted data (where, typically, more than 400 compounds can be resolved) but this is simply not viable for QTOF data, where it is routine to resolve several thousand features. Software tools are needed to undertake the first pass and subsequent curation steps, and to identify "problem" metabolites in each sample, as well as facilitating visualization of the metabolite peak. Previously, third party software was used, but it is now being incorporated into vendor software. In addition, the software needs to facilitate the removal of features that are background to the system and artifacts from sample preparation methodologies.

Until very recently, dedicated GC-TOF instruments have largely been nominal mass instruments with fast scanning capability. Dedicated "high" resolution GC-QTOF MS instruments have only recently become available, and they currently do not have the capabilities of the latest generation LC-QTOF MS systems. The GC-QTOF instruments are limited in mass resolution to around 12,500 and in mass accuracy to 5 ppm. In the low mass range where they operate, there is a clear need for capability improvement. for capability improvement.

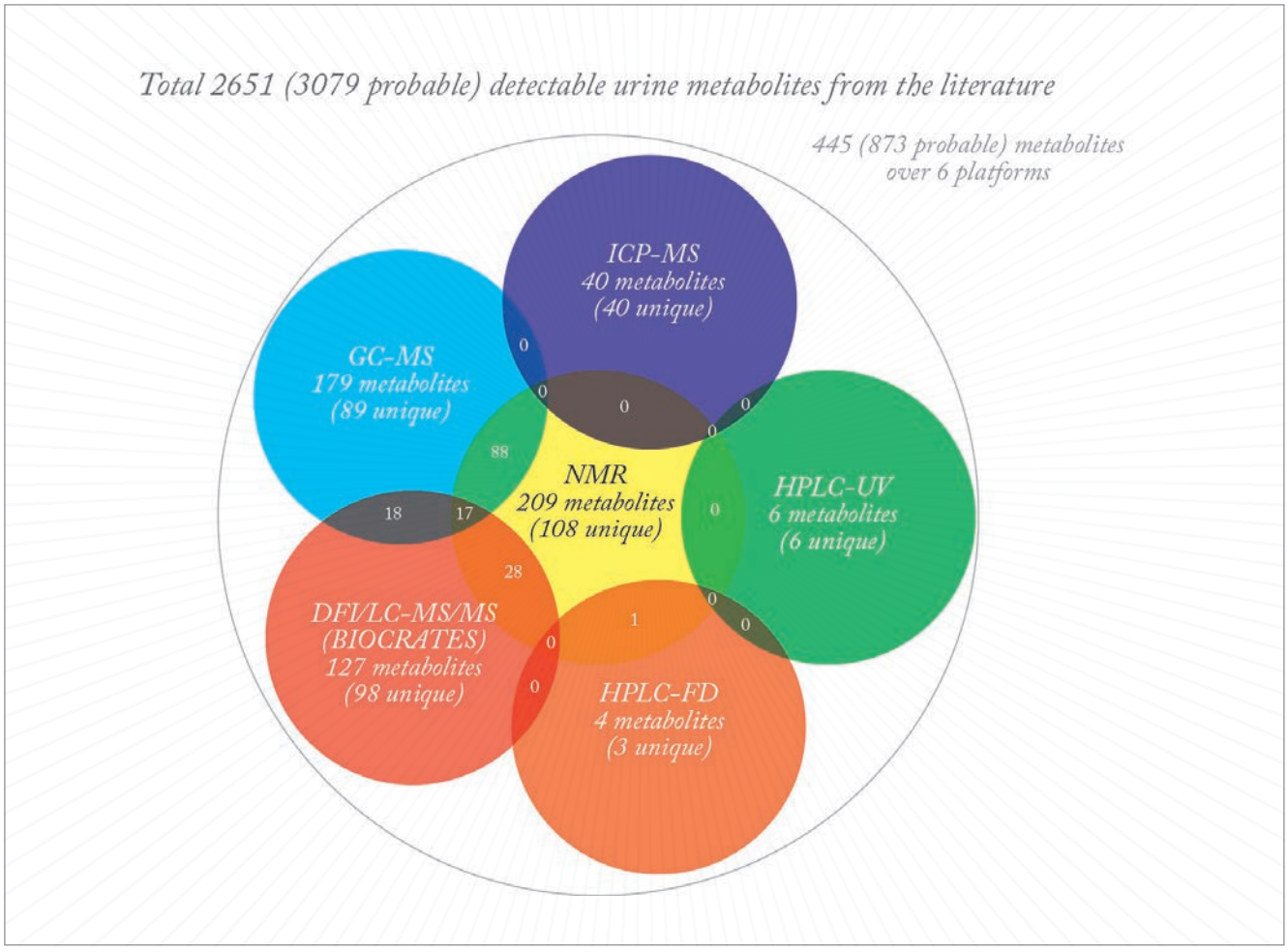

Figure 1. Venn diagram of urine metabolite overlap of metabolomics tools (adapted from Ref. 13).

GCxGC TOF MS

We discuss GCxGC separately as it is a technique that promises a great deal but has not been fully utilized by the metabolomics community because of data analysis complexity and the difficulty in building automated pipelines. Typically, switching from a GC-MS single quadrupole system to a GCxGC TOF MS system results in detection of 2-3 times more metabolites. GCxGC instruments are significantly more expensive to purchase than GC-MS single quadrupole systems, while requirements for liquid nitrogen cryo-cooling significantly add to operating costs, which has been a factor in the slower uptake. The cost issue has been partly addressed by consumable-free configurations, but these have some limitations with metabolites of very high volatility. Furthermore, the data format used for these instruments was not readily imported into third party software packages until recently. Most likely, the full capability for metabolomics will be realized as the next generation of GCxGC systems with sub part per million (ppm) accurate mass become available in 2014.

Figure 2. The Metabolomics Toolbox. Techniques in green provide bulk metabolomics information and those in grey provide spatial metabolite information.

Triple Quadrupole Instruments

Triple quadrupole (QQQ) instruments have traditionally been considered only for analysis based on selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) as the sensitivity for full-scan was inferior to single quadrupole instruments. However, latest generation instruments have excellent full-scan sensitivity, only surpassed by the latest generation single quadrupole instruments. When MRMs are established, QQQ-based instruments are capable of metabolite MRM analysis of far more than 500 compounds in a single run. This offers the potential for development of large targeted analysis workflows, referred to as a 'targeted untargeted' approach. Through the development of metabolite MRM atlas platforms in parallel with the peptide SRM atlas (www.srmatlas.org), it will be possible to select pathways to be analyzed by choosing the appropriate metabolites. This will be a significant step in the development of quantitative metabolomics, and help establish metabolomics as the new clinical chemistry.

Fourier Transform (FT)MS

FT instruments are now considered to be the gold standard for mass spectrometry, as sector instruments are gradually

disappearing. FT instruments command a significant price premium but have always been considered to provide ultimate performance.

FT-ion cyclotron resonance (ICR)-MS instruments have undergone significant developments in the last two years, with the switch from the solution to the absorption mode of operation. This instantly doubled the instrument's resolution and, coupled with the development of the ParaCell, provides resolution up to 10,000,000. With a number of source options (electrospray ionization (ESI), nano spray ESI, atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization (APPI), GC/API and matrix-assisted laser desorption/ionization (MALDI)), and MS imaging, FT-ICR-MS has become a very flexible metabolomics solution when used in a 7 Tesla magnet configuration.

The current generation of Orbitrap analyzers – the Orbitrap Fusion Tribrid – combines quadrupole, ion trap, and Orbitrap technology, and has a mass resolution of 450,000, with under 1 ppm mass accuracy, up to15 MS/MS scans per second, and with the options of ESI, nano spray ESI, APCI, and APPI sources.

Capillary electrophoresis-MS

CE-MS has recently re-emerged as sheathless MS interfaces become commercially available. With sheathless interfaces, the dilution effect of the sheath liquid is removed and the resulting MS signals are approximately two orders of magnitude higher. This has significantly improved metabolome coverage and allows users to take advantage of the anion-neutral-cation coverage within a single metabolomics analysis. CE has always been considered a very high-resolution chromatographic technique and requires the scanning speed of a QTOF mass analyzer to cope with the chromatographic resolving power.

NMR

Nuclear magnetic resonance (NMR) is suited to urine analysis because it offers the opportunity to simply balance pH and analyze – no further sample preparation is required. Deconvolution is used to determine the metabolites that are present, and their levels. The strengths of NMR are its ability to identify compounds with great confidence and the fact that it is a quantitative technique. However, it has an inferior detection limit compared with mass spectrometry. This inability of NMR to detect and differentiate the changes in low-level metabolites that mass spectrometry techniques routinely monitor is a considerable drawback for the metabolomics community. Despite this fact, the recent human urine NMR metabolome data from the Wishart group (13) demonstrates the ability of NMR to monitor and quantify 200-plus metabolites in urine samples. And recent developments in NMR magnet and probe technology have significantly improved the temporal stability of chemical shift, with claims of stability lasting several years.

Folded Flight Path

Folded Flight Path (FFP) mass analyzer instruments have recently been introduced to the mass spectrometry market. They offer high mass resolution (of up to 50,000), mass accuracy of less than 1 ppm, accurate isotope patterns and fast scanning rates (up to 200 scans per second). Due to be released with GCxGC capability later this year, FFP instruments will be valuable complementary additions to the metabolomics toolbox. Instruments using this technology are currently undergoing evaluation in the hands of the metabolomics community.

Bottlenecks and Opportunities

The Data Bottleneck

Data analysis and informatics processes, critical sections in the metabolomic workflow, must now be considered to be the bottleneck in metabolomics (taking over from sample preparation, the traditional analytical chemistry bottleneck). Data processing, informatics and bioinformatics each contribute to the problem and these days successful metabolomic studies devote at least half of the total project time to these issues.

One of the key workflow processes is the curation of data prior to any statistical treatment, which specifically involves deconvolution, integration, and internal standard correction and normalization. Normal variation in metabolites can result in levels for individual samples low enough that the software deconvolution and integration algorithms do not detect certain metabolites in some samples. There are two camps in the data analysis debate with

respect to the handling of missing values for metabolites in data sets when dealing with untargeted metabolomics studies. One camp advocates for, and the other against, the replacement of missing values with weighted averages of values from the other samples for that metabolite using an automated pipeline. This approach is very much at odds with the traditional targeted analytical chemistry approach and will continue to be a point of disagreement between biologists, who require an automated pipeline, and traditional analytical chemists.

MS Imaging

All early studies were "bulk metabolomics", in which a tissue sample was extracted and the composition of the extracted metabolites was representative of the bulk compositional information. In contrast, Richard Caprioli and coworkers have pioneered the development of MS imaging with an initial focus on proteins and peptides. This has been expanded to include lipids and metabolites, and there is an enormous development effort going into this approach, which will provide specific tissue location data.

Quantitative Metabolomics

Untargeted metabolomics studies are primarily semi-quantitative. The cost of quantitative untargeted metabolomic studies can be prohibitive, as most techniques will detect ≥ 400 compounds. Stable isotope dilution analysis requires labeled standards for each metabolite and even at a cost of just $1 per metabolite, costs mount up quickly. A more affordable approach utilizes a labeled metabolite for each class of metabolite. The standard addition approach requires that every sample has up to five analysis runs, adding a significant time and cost burden to studies.

Clinical Chemistry

Clinical chemistry analysis compares the levels of metabolites in patient samples with the normal population range, and typically each test looks at just 2-3 metabolites, which are used as biomarkers associated with a disease. However, metabolomics has the capability to measure biomarkers associated with not just a single disease but those from many diseases simultaneously. Furthermore, broad metabolomic coverage offers the opportunity to study disease progression in greater detail, making the prospect of personalized medicine a reality.

Current Challenges

In order of priority, some of the major challenges that face the metabolomics community, and, therefore, offer potential opportunities are:

- Compound Identification: the confident assignment of metabolites is critical to advancing metabolomics.
- Standards: for many metabolites standards are difficult to obtain.
- Pathway Mapping: the real promise of metabolomics is in establishing which biochemical pathways are modified in response to a disease in humans or animal models, or to biotic and abiotic stress in plants. This provides the ability to monitor disease progression and the response to treatment.
- Large Sample Sets: studies with cohorts of several thousand will become the norm; there is a clear need to establish the ability to analyze these large sample sets without instrument bias.
- Bioinformatics: as outlined earlier.

In addition, metabolomics as a field needs to make advances in the following areas:

- Metabolite MRM libraries
- Organ- and organism-specific mapped pathways, using an agreed format
- Integrated targeted and untargeted methodologies
- Upward integration of metabolomics with lipidomics and proteomics
- Data visualization tools which provide the ability to walk through pathways and between pathways with links to lipid and protein switches or shunts.

Finally, I note that a great deal of scientific literature appears to be invisible to the research community unless it is electronically accessible – certain research projects are therefore in danger of missing critical information or repeating experiments that have already been undertaken. We should not re-invent the wheel.

Robert Trengove is Director of the Separation Science and Metabolomics Laboratory, Murdoch University, Perth, Australia.

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Philanthropy and Analytical Science

What does your employer or favorite vendor contribute to the good of society? How important is that to you? And do you have the opportunity to volunteer your time and expertise to help others?

By Richard Gallagher

Look deep within ourselves and we all discover a love of humanity: a desire to care for, nourish, develop and enhance the lives of less fortunate fellow human beings. For people like analytical scientists, much of whose time and attention is taken up by work, the philanthropic programs of employers and key suppliers can form an important element of how we fulfill this philanthropic urge.

Corporate philanthropy can be defined as the donation of profits and resources to nonprofit organizations. There are two main approaches: cash donations, which often take the form of companies matching personal gifts by staff members; and the provision of facilities and/or volunteer time. The latter may take advantage of the competencies of the company – for example, an organization that employs a lot of scientists could become involved in science education – this called "strategic philanthropy." Alternatively, the contribution may be purely humanitarian – responding to a natural disaster, for example.

Many of the leading companies in analytical science have considerable financial clout and other resources that allow them to engage in strategic philanthropy, which addresses problems at their core. Here, we describe the philanthropic programs of a selection of them: Agilent Technologies, Thermo Fisher Scientific, and Waters Corporation; thanks to Cynthia Johnson, Taryn Corbino and Jeff Tarmy, respectively. The sidebar "Setting a Good Example," (page 46) profiles the charitable and philanthropic work of Phenomenex, as described by CEO Fasha Mahjoor.

What is your philanthropic strategy?
Agilent: It is focused on scientific education, but covers other areas, such as health and the environment. One-third of the employees lend their talent and time to charities, while being paid up to four hours per month.
Thermo: Helping build stronger communities through hands-on service programs and charitable contributions that align with our mission and engage our employees by being involved and making a difference.
Waters: Our charitable giving is primarily focused on supporting organizations dedicated to science, education and health care. We are also actively involved in supporting local organizations dedicated to strengthening our communities in the arts, health and human services, recreation, education and the environment.

Why do companies give?
The charter of our philanthropic program is to promote science education by supporting initiatives that inspire students to pursue studies in science, technology, engineering, and math.
To improve the quality of life in the communities in which we work and live. This is mostly done through financial support to selected non-profit organizations operating within those communities.
It is part of our culture and the way we do business around the world to help make our communities better. Employees want and expect to be part of giving back.

Is it just about "doing good" or is there self-interest on the company's part too?
It is both. There is definitely a self-interest, driven by our employees' interest and our desire to harness our own expertise to make communities where we live and work better, healthier places to live.
We consider it to be an investment in the future of science – we rely on individuals who are passionate about science both as our future employees and customers. It's a logical fit.

Who decides on strategy?
Our Charitable Giving strategy is set by Waters Corporation' Executive Committee led by our CEO.
CEO and top executives, led by VP of Corporate Relations set strategy. Individual communities have employee panels that decide on the local activities/support. Our programs are active in about 40 countries.
The strategy is defined by our CEO with the support and guidance of our SVP of Corporate Development and Strategy, our VP of Corporate Communications, and our SVP of Human Resources.

Do you offer company products and services as part of your philanthropic mission?
We receive requests for in-kind donations from many different areas of the business. Typically, these donations are facilitated through our Community Action Councils, which oversee local giving and involvement. In addition to providing financial and in-kind donations, these employee-led councils provide our employees with hands-on volunteer opportunities in their local communities. These can range from support of a community science day to donation of instruments for an academic laboratory.
Yes, for universities. We donate to universities based on invited proposals, and separately, we match employees' personal donations of cash and equipment up to $20k list price of equipment per employee, per institution per year.

Do you partner in giving with employees?
Yes. Our employees are encouraged to contribute individually through

our Matching Gift Program, which matches employee contributions to qualified non-profit organizations.

How do you measure the impact/value of your projects?

Major projects have regular milestone meetings in collaboration with recipients. For smaller, local grants, we rely on employee volunteers to provide feedback and support.

We are continually monitoring the impact we have on our local communities. From improved test scores to college preparedness, we ensure our volunteer work and financial contributions are supporting students, teachers and educational institutions with the greatest need.

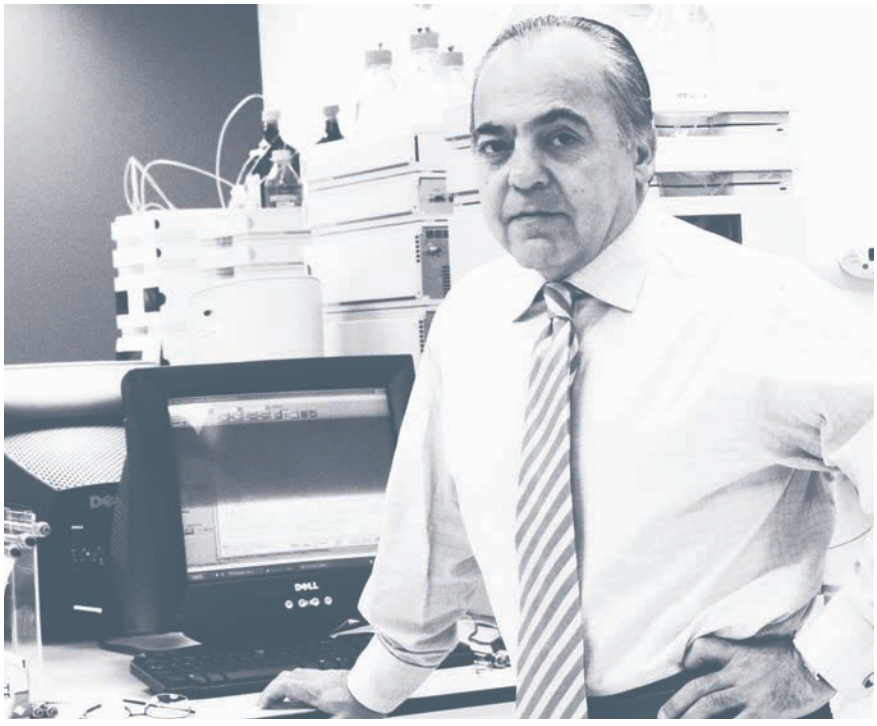
Describe one project that was particularly successful.

In China, after the Sichuan earthquake several years ago, Agilent adopted and re-built a school that was destroyed. Our employees have traveled to the school every holiday and provided equipment, and have led the students in innovative programs.

We participated in the Massachusetts Math & Science Initiative (MMSI), a $30 million initiative organized jointly by Mass Insight Education and the Commonwealth of Massachusetts to help close the achievement gap for underserved students. Our $1 Million contribution is having a measureable impact on STEM education: MMSI has grown from enrolling less than 5,000 students in 2008 to nearly 11,000 in 2012.

In 2006, Waters Corporation received the Circle of Humanitarians Award from the American Red Cross for our corporate contribution and employee

Setting a Good Example



Fasha Mahjoor is a relentless humanitarian. In the past year, he has been awarded the Ellis Island Medal of Honor and elected to the Board of Directors for the American Red Cross, Los Angeles Region; when this magazine last encountered him, he had just abseiled down Europe's tallest building to raise funds for a favorite cause. So it comes as no great surprise that Mahjoor views philanthropy and charity as "part of the DNA" of Phenomenex, the company that he set up in 1982.

"There's never been a (philanthropy) strategy as such. It's simple with us: about 25 years ago, I put pen to paper to write a mission statement that has never changed. It concludes with, 'it is our responsibility and foremost mission to promote the growth, prosperity and well-being of … our customers, our employees and humanity'," he explains. A sense of duty to the underprivileged is "part and parcel of who we are," he says.

He pauses to give the example of his company's work with an organization called Feed My Starving Children. "While we were working with them, our managing director for Italy was on a visit here. He became so inspired that he brought a sister organization, Stop Hunger Now, to Italy. Over the past two years, we have together prepared 360,000 meals for children in Haiti, the Philippines, North Korea and so on. On one occasion, 600-700 people worked around the clock to do this, with many staff members accompanied by family

engagement efforts in support of a Hurricane Katrina disaster relief fund.

What has been the response of employees?

The feedback has been positive. Our employees share a sense of pride that the company they work for is giving back in a sustainable manner.

Employees are highly supportive and many tell us it is one of the reasons they want to work at Agilent.

The companies approached for this article clearly have well thought-out and executed philanthropic strategies. What do you think? Are we/they doing enough? Do you have a personal philanthropy experience and, if so, did your passion and talent translate into positive results? Do you have suggestions for increasing the impact of analytical scientists on education and other areas of philanthropy? Is there someone you know who sets a great example to the community? Let us know! Join the conversation at: tas.txp.to/0114/giving

and friends. It was a wonderful occasion."

Phenomenex's corporate giving strategy is characterized by two things: first, the support for charitable causes, rather than the more strategic philanthropic projects that larger companies can get involved in, leveraging their greater size; and second, the extent of involvement of the staff. Mahjoor says that for some projects up to 80 percent of employees have volunteered to participate. And he believes that the company's excellent staff retention is partly down to a shared belief in what it stands for.

So, is Mahjoor driving the program? "Not at all," he says, "We have philanthropic teams that meet every other week at all our subsidiaries, and they decide on what to support. This is sometimes agreed collectively, across companies, but it is often local or national." When there are natural disasters, such as Typhoon Haiyan in the Philippines last November, Phenomenex teams worldwide collaborate to make the biggest impact possible.

Local projects can sometimes generate attention in all 15 countries that Phenomenex has a presence in. One of these is in India. "We started our company there two-and-a-half years ago," Mahjoor explains, "And soon started to help out at orphanages. These are heartbreaking institutions." The current focus is on one particular orphanage that has 40 children below the age of 12. "Our employees are very involved, on a weekly basis; teaching, feeding, and going on field trips. The children have monthly visits to the company office, which the employees love; everyone tries to be there for the visits. We finance their education in private schools. We even painted and decorated the buildings and provided mattresses and blankets."

What advice does Mahjoor offer other companies thinking of developing a more hands-on approach to giving? "Do it. Don't just send a check – get everyone physically involved." Is there an upside for the company as well as for recipients? "The return to the company is intangible, but to my mind it is enormous, for the joy and motivation it brings to our employees," he insists. "It brings us, as well as the projects that we support, nothing but good."

Extraction of Antiepileptic Drugs from Serum and Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

Antiepileptic drugs (AED) are prescribed to suppress seizures in epilepsy patients. The ability to therapeutically monitor these drugs in patients is necessary for maintaining optimal medical care and managing any adverse effects of the drug. In this application note ISOLUTE SLE+ is demonstrated as an effective way to extract AEDs from serum and urine. The method was developed using a 96–well plate format to facilitate a high throughput workflow model

Sample Preparation for Neutral Antiepileptic Drugs in Serum and Urine:
Format: ISOLUTE® SLE+ 400 µL 400 Supported Liquid Extraction plate, part number 820-0400-PO1
Sample Pre-treatment: Pipette serum/urine (blank, calibrator or patient (100 µL)) into a container. Add ammonium acetate (5mM, pH 2.9, 250 µL). Add up to 50 µL of internal standard. Mix.
Sample Processing: Load up to 400 µL of pre-treated serum/urine sample onto the ISOLUTE SLE+ 96-well plate. Apply a pulse of positive pressure or vacuum and allow samples to sit for 5 minutes.

Sample Preparation for Neutral Antiepileptic and Zwitterionic Drugs in Serum and Urine:
Format: ISOLUTE® SLE+ 400 µL 400 Supported Liquid Extraction plate, part number 820-0400-PO1
Sample Pre-treatment: Pipette serum/urine (blank, calibrator and patient (100 µL)) into a container. Add 50% aqueous formic acid (100 µL). Add up to 100 µL of internal standard.
Sample Processing: Load up to 300 µL of pre-treated serum sample onto the ISOLUTE SLE+ 96-well plate. Apply a pulse of positive pressure or vacuum and allow samples to sit for 5 minutes.
Analyte Elution All: Elute analytes with methyl tert-butyl ether containing 1% (v/v) trifluoroacetic acid (conc) solution (2 x 700 µL). Allow sample to flow through by gravity and collect eluent. Apply positive pressure or vacuum as needed to facilitate a constant flow of approximately 1 mL/min (10–12 drops).
Post Extraction All: Evaporate to dryness (45 °C for 15 mins) and reconstitute sample in mobile phase.

HPLC Conditions
Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)
Column: Phenomenex Gemini C18, 150 mm x 4.6 mm (5 µm)
Mobile Phase: Solvent A: 5mM Ammonium Formate with 0.01% (v/v) Formic Acid
Solvent B: Methanol: Acetonitrile (50:50m v/v)

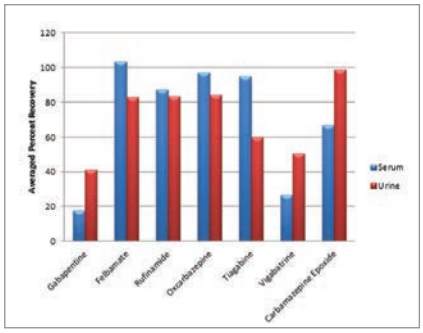


Figure 1.Plot of average recoveries for neutral and zwitterionic antiepileptic drugs in Serum and urine at 20 ng/mL, pretreated with 5 mM ammonium acetate.

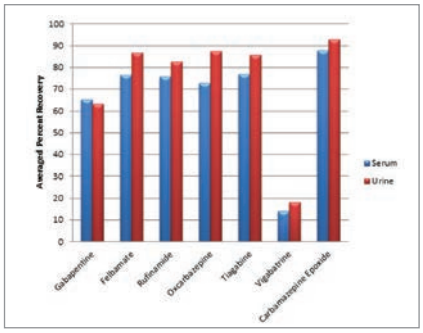


Figure 2.Plot of average recoveries for neutral and zwitterionic antiepileptic drugs at 20 ng/mL in serum and urine, pre-treated with 50% aqueous formic acid.

Mass Spectrometry Conditions
Instrument: Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.
Ion Source Temp: 700 °C

Results and discussion
The first pre-treatment strategy calls for 5 mM ammonium acetate to be added to the fortified matrix at a 1:2.5 (v/v) ratio. Recoveries for the AEDs using this pre-treatment were good for all of the neutral AEDs in urine and serum and lower for the zwitterionic AEDs, particularly in the serum matrix (Figure 1) .The lower recovery for the gabapentin and vigabatrin was attributed to their lower log P values (<1).
The second pre-treatment strategy calls for the addition of a 50% aqueous formic acid buffer to the fortified matrix at a 1:1 (v/v) ratio. This particular strategy increases the recovery for the gabapentine significantly (Figure 2). The vigabatrin does not benefit from formic acid pre-treatment. The neutral AED recoveries were good using this strategy.

Biotage

For full HPLC and MS conditions, download application note AN805 from biotage.com

Characterization of Diesel Fuel Using a Modular Raman System

Identify. Authenticate. Verify.

By Yvette Mattley, Ph.D.

Biodiesel is a non-petroleum-based diesel fuel made from vegetable oil or animal fats. Biodiesel can be used in most diesel engines with little to no engine modification required, and can be blended with petroleum diesel fuel to provide a cleaner burning, lower emission diesel fuel.

As biodiesel production increases, Raman spectroscopy will become a useful analytical tool during the refining process to assess incoming raw materials, monitor the production process and confirm the quality of the final product.

Measurement Conditions

Today's Raman measurement options range from handheld and benchtop systems such as the Ocean Optics IDRaman series, to modular, "build your own" Raman systems including spectrometers, lasers, probes and sample holders.

The Raman measurements described here were made with a modular setup comprising the Maya2000 Pro-NIR spectrometer, a 785 nm Raman laser, a Raman-coupled probe for 785 nm Raman and a sample holder. Acquisition parameters were a 500 millisecond integration time with no scans averaged and no boxcar smoothing. Samples of corn oil (sometimes used as a diesel alternative) and petroleum-based diesel were placed in small glass vials for analysis.

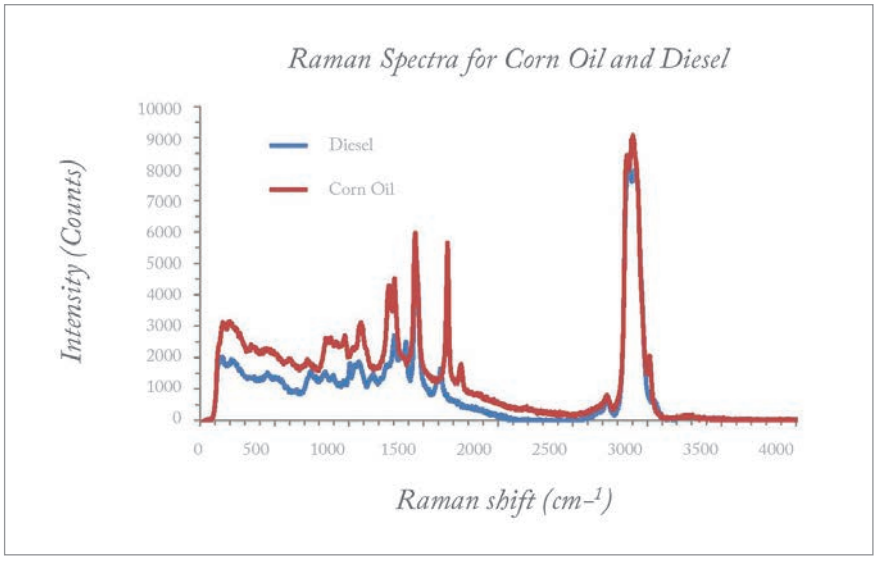


Figure 1: Raman is an excellent method for distinguishing corn oil-based biodiesel fuels from petroleum-based diesel fuels.

Results

The Raman spectra for corn oil and diesel are shown in Figure 1. While these spectra share some features due to the hydrocarbon content of the samples, there are several spectral differences observed in the fingerprint region from 500–2000 cm^{-1}. Even though both samples are suitable for use as fuel in diesel engines, they have distinct Raman spectra that distinguish the corn oil-based biodiesel fuel from the petroleum-based diesel fuel. Note that in addition to identifying the fuel type based on its Raman fingerprint, one could obtain more quantitative information from the spectra by applying the appropriate chemometrics models.

One notable difference for the spectrum of corn oil is the presence of stearate (a form of fatty acid found in animal and vegetable fats and oils) in the region from 1600-1800 cm^{-1}. While stearate artifacts and other spectral differences allow for easy discrimination of these fuels, samples with even more closely aligned spectral peaks can be distinguished. Indeed, by using a narrower slit in the spectrometer optical bench, you will achieve a higher Raman shift resolution over a narrower spectral range.

Conclusion

With their unique hydrocarbon composition, fuels are well suited for identification and characterization using Raman analysis. The wealth of spectral features in the Raman spectra for fuels can be used in a range of applications including determination of critical fuel parameters, fuel classification and detection of counterfeit fuels.

With all the choices available, the modular approach to Raman measurements provides a nearly endless choice of setups with the ability to change or add components to meet your evolving measurement needs.

Ocean Optics

Mother of Ambition

Sitting Down With… Marja-Liisa Riekkola,
Professor of Analytical Chemistry, University of Helsinki, Finland

Why analytical chemistry?

When I was young, I didn't believe I would be a scientist because I didn't like the idea of being locked alone in my "tower", only focused on a research project. But then I realized that scientists can create their own world. At university, I wasn't sure what to specialize in, but my Bachelor's research project (developing a new method for determination of metals using gas chromatography) was so interesting that I continued it all the way through to my PhD. I used many instrumental techniques during that time and became hooked. There is a clear and strong link between analytical chemistry and the real world; basic research is clearly tied to applications. And while many other fields use analytical chemistry, we must always remember that it is a discipline in its own right – not simply a tool.

You became a full professor just four years after completing your PhD. How?

In Finland, positions for professorships are completely open. I was one of ten competitive applicants – and the only woman. The other nine were already associate professors. The application process uses outside evaluators – in our case, four very well respected and well-known international professors – whose role is to independently assess the quality of publications. They decided that, even though I was young, I was of the right caliber. But the faculty makes the final decision based on those assessments and, to tell the truth, I was the second choice; the first, an electrochemist, accepted a professorship at his current university, and so the position was offered to me. Even still, I feel the faculty was quite modern in its thinking back then.

The issue of equality in science was raised in one of our editorials…

Yes, I noticed that there were only eight women in your Power List. How do we increase the representation of women in science? That's a good question – especially for me as the mother of two clever daughters. But, actually, I've never really faced the kind of opposition that others appear to – I've always received full support. Though I must admit that back in the early days, when people came to my office, they assumed I was the secretary and asked for Professor Riekkola – it annoyed me then, but now I just laugh.

Enforced support could be one answer. When faced with the choice of two good but equal candidates, should we always choose the female? In our physics department, they have elected to follow that kind of politics. Unfortunately, I think women have a tendency to be too self-critical – if we believe in ourselves and our abilities more, it is easier to strive for higher level positions. I think role models are also very important.

Would you consider yourself a role model for women in science?

Well, I was the first female full professor in Finland – and when I started I was also pregnant. The majority of the science faculty was male and they didn't really know how to handle a pregnant professor. But I proved that gender didn't matter – it is the results that count. I was also involved in an EU project – "Advanced Training for Women in Scientific Research" – where I mentored a younger female scientist in career development amongst other things. Actually, the interesting discussions we had were also helpful for me.

Your research is divided into two areas...

Yes – environmental and bioanalytical. For the former, our laboratory belongs to the Academy of Finland's Centre of Excellence in Atmospheric Science, where we are interested in the chemical composition of nanoparticles and the development of sampling devices and portable instruments. On the bio side, we've been working on lipoproteins and extracellular matrix components for a long time. More recently, we've started to look at the use of new biomaterials in innovative separation devices.

You're also an editor for Journal of Chromatography A. What research has caught your eye?

There are lots of new things going on right now – miniaturization and biochip systems, for example. Mike Ramsey's work is fascinating. I'm also very interested in those who are making the most of the exciting work being done by excellent scientists in the materials field. Computational and molecular dynamics modeling and their integration into analytical techniques is another hot area. But we must remember that we always need strong theoretical people to maintain fundamental research so that we can understand why we get certain results.

Being an editor forces me to keep a finger on the pulse of research around the world – I can see how research is taking giant steps forward in China, for instance. As I check the manuscripts that come in, I can witness the advancements that are being made by groups all over the world – it's just like being at the cinema, watching a movie of progress!

How has analytical chemistry changed over the years?

In many fields, researchers have understood not only the value of chemistry, but that analytical chemistry and its associated techniques are an absolute requirement. Each year, I notice the increase of students from different discplines attending my chromatography lectures. Biology, ecology, physics – they have all started to understand how important analytical chemistry is. I think we are much more appreciated nowadays.

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