JULY 2017 # 54

the Analytical Scientist

Upfront
Virtual reality shakes up the chemistry classroom
10

In My View
Are younger scientists more productive?
18

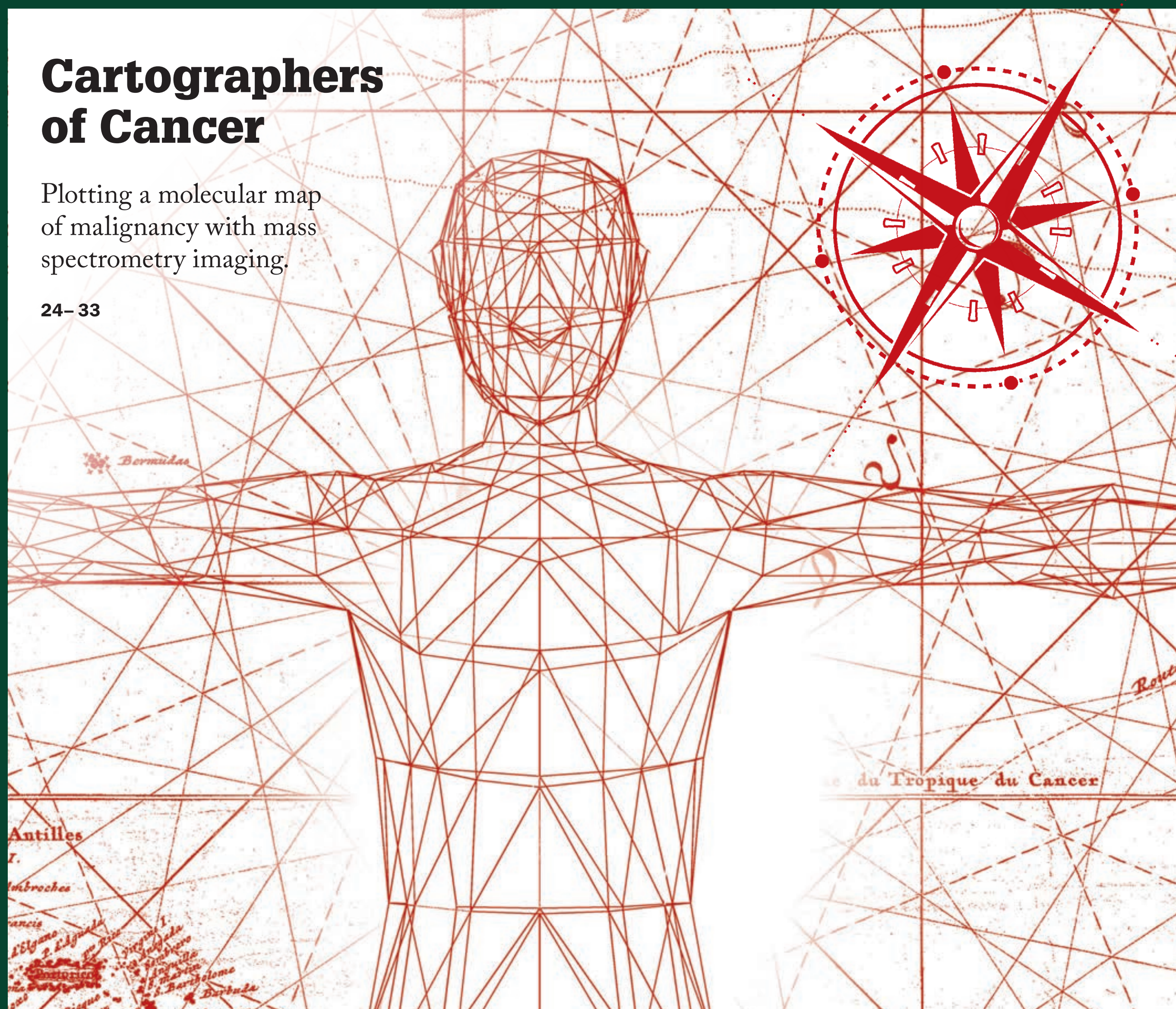
Feature
Unearthing the future of forensic science
34 – 41

Sitting Down With
Curator of good science, Ian Wilson
50 – 51

Cartographers of Cancer

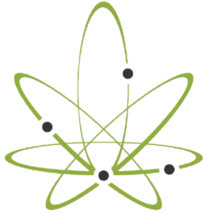
Plotting a molecular map of malignancy with mass spectrometry imaging.

24– 33



www.theanalyticalscientist.com

Use Promo Code TEXERE for 25% Off Registration!!!



CANNABIS
SCIENCE
CONFERENCE

Hosted by

August 28-30, 2017

Portland, Oregon

OREGON Oregon Convention Center

The World's Largest Cannabis Science Conference returns to downtown Portland for an incredible gathering of analytical scientists, medical professionals, cannabis industry experts and novices interested in learning more about cannabis science!

Contact Josh Crossney at josh@jcanna.com for more info.

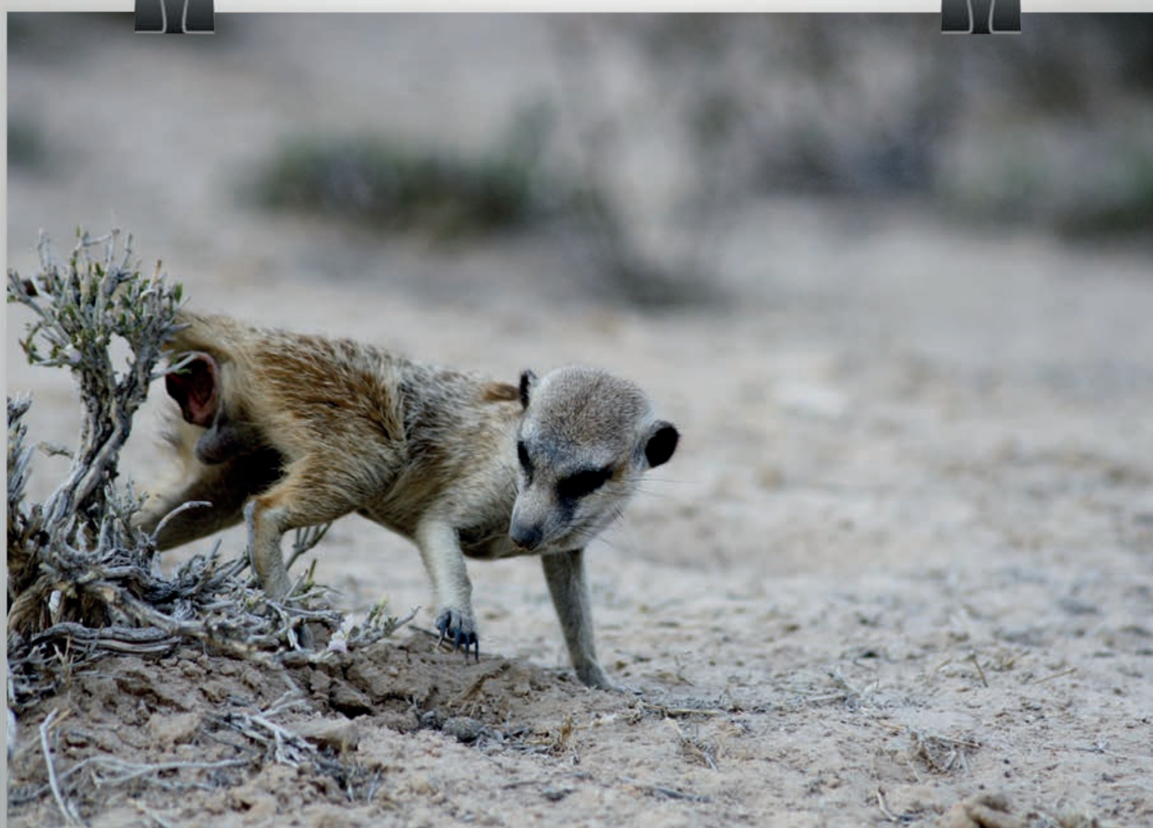
CannabisScienceConference.com

jCannaInc

jCanna_Inc

@jCanna_Inc

Image of the Month



Love at First Glands

Think you've had a rough week? At least you haven't had to swab the anal glands of a meerkat, like one group of researchers (1). To identify the origins of the animals' distinctive odor, the team used GC-MS to analyze the smelly "paste" that acts as their calling card to friends, rivals and potential mates. They identified volatile compounds in the glandular secretions and compared them with samples from the anal pouches of the meerkats, determining that each animal's delicately balanced "bouquet" is a result of shared bacteria – rather than shared genes – a finding that's likely to be important for social interaction.

Reference 1. S Leclaire et al., "Social odours covary with bacterial community in the anal secretions of wild meerkats", Scientific Reports, 7 (2017). PMID: PMC5468246

Credit: Lydia Greene, Duke University

Would you like your photo featured in Image of the Month? Send it to charlotte.barker@texerepublishing.com

Contents

03 **Image of the Month**

09 **Editorial**
Future Separations: Redux, by Rich Whitworth

Upfront

10 Daydreaming in the Classroom

11 From Chemical to Clinical Analysis

11 Nanofluidic PAT

12 Whither on the Vine?

12 Long Time Coming...

13 Reid All About It

14 Small Samples; Big Promises

On The Cover

On a journey to build the most detailed ever molecular map of cancer.

the Analytical Scientist

ISSUE 54 - JULY 2017

Editor - Charlotte Barker
charlotte.barker@texerepublishing.com
Deputy Editor - Joanna Cummings
joanna.cummings@texerepublishing.com
Scientific Director - Frank van Geel
frank.vangeel@texerepublishing.com
Content Director - Rich Whitworth
rich.whitworth@texerepublishing.com
Editorial Director - Fedra Pavlou
fedra.pavlou@texerepublishing.com
Publishing Director - Lee Noyes
lee.noyes@texerepublishing.com
Business Development Manager - Sam Blacklock
sam.blacklock@texerepublishing.com
Head of Design - Marc Bird
marc.bird@texerepublishing.com
Junior Designer - Hannah Ennis
hannah.ennis@texerepublishing.com
Digital Team Lead - David Roberts
david.roberts@texerepublishing.com
Digital Producer Web/Email - Peter Bartley
peter.bartley@texerepublishing.com
Digital Producer Web/App - Abygail Bradley
abygail.bradley@texerepublishing.com
Audience Insight Manager - Tracey Nicholls
tracey.nicholls@texerepublishing.com
Audience Project Associate - Nina Duffissey
nina.duffissey@texerepublishing.com
Traffic and Audience Associate - Lindsey Vickers
lindsey.vickers@texerepublishing.com
Traffic Manager - Jody Fryett
jody.fryett@texerepublishing.com
Social Media / Analytics Associate - Ben Holah
ben.holah@texerepublishing.com
Events Manager - Alice Daniels-Wright
alice.danielswright@texerepublishing.com
Marketing Manager - Katy Pearson
katy.pearson@texerepublishing.com
Financial Controller - Phil Dale
phil.dale@texerepublishing.com
Accounts Assistant - Kerri Benson
kerri.benson@texerepublishing.com
Chief Executive Officer - Andy Davies
andy.davies@texerepublishing.com
Chief Operating Officer - Tracey Peers
tracey.peers@texerepublishing.com

Editorial Advisory Board
Monika Dittmann, *Agilent Technologies, Germany*
Norman Dovichi, *University of Notre Dame, USA*
Gary Hieftje, *Indiana University, USA*
Emily Hilder, *University of South Australia, Australia*
Ron Heeren, *Maastricht University, The Netherlands*
Tuulia Hyötyläinen, *University of Örero, Finland*
Hans-Gerd Janssen, *Unilever Research and Development, The Netherlands*
Robert Kennedy, *University of Michigan, USA*
Samuel Kounaves, *Tufts University, USA*
Marcus Macht, *SCIEX, Germany*
Luigi Mondello, *University of Messina, Italy*
Peter Schoenmakers, *University of Amsterdam, The Netherlands*
Robert Shellie, *Trajan Scientific and Medical, Australia*
Ben Smith, *University of Florida, USA*
Frantisec Svec, *University of California at Berkeley, USA*
Ian Wilson, *Imperial College London, UK*
Frank Bright, *University at Buffalo, USA*
Chris Harrison, *San Diego State University, USA*

Change of address
nina.duffissey@texerepublishing.com
Nina Duffissey, The Analytical Scientist, Texere Publishing Ltd, Haig House, Haig Road, Knutsford, Cheshire, WA16 8DX, UK

General enquiries
www.texerepublishing.com | info@texerepublishing.com
+44 (0) 1565 745 200 | sales@texerepublishing.com

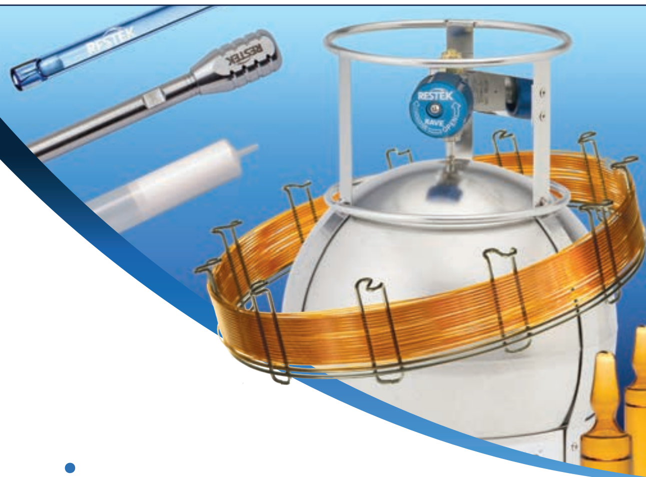
Distribution:
The Analytical Scientist (ISSN 2051-4077), and The Analytical Scientist North America (ISSN 2514-7544), is published monthly by Texere Publishing Ltd and is distributed in the US by UKP Worldwide, 3390 Rand Road, South Plainfield, NJ 07080
Periodicals postage paid at South Plainfield, NJ
POSTMASTER: Send US address changes to (Title), (Publisher) C/O 3390 Rand Road, South Plainfield NJ 07080.
Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com)
Annual subscription for non-qualified recipients £110

Reprints & Permissions – tracey.nicholls@texerepublishing.com
The opinions presented within this publication are those of the authors and do not reflect the opinions of The Analytical Scientist or its publishers, Texere Publishing. Authors are required to disclose any relevant financial arrangements, which are presented at the end of each article, where relevant.
© 2017 Texere Publishing Limited. All rights reserved. Reproduction in whole or in parts is prohibited.

texere publishing ltd

Available on the App Store

Restek® Enviro



YOUR GUIDE FOR

Environmental Sampling and Analysis

Backed by a long history of supporting environmental labs, Restek's high-performance chromatography supplies and world-class technical service equip you to achieve:

- **Faster throughput and lower cost per sample.**
- **Accurate, reliable, and legally defensible data.**
- **Hassle-free compliance with method requirements.**

Visit us at **www.restek.com/enviro**

Restek® products come with an unbeatable guarantee.

That is Restek® Pure Satisfaction.

RESTEK®

Pure Chromatography

Scale 1:2,3

Their handheld
Raman Spectrometer

Their handheld
Raman Spectrometer

Their handheld
Raman Spectrometer

Their handheld
Raman Spectrometer

Mira M-3
Metrohm
Raman
Metrohm
Raman

Mira M-3 handheld Raman Spectrometer

True single-handed operation

Barely larger than a smartphone, the Mira M-3 is one of the most convenient to use handheld Raman spectrometers in the market. The Mira M-3 is ...

- **Super compact** – 13 (h) x 8.5 (w) x 4 (d) cm
- **Super fast** – verify identity of materials in seconds
- **Fully compliant** with FDA 21 CFR Part 11

www.metrohm.com/mira

42

50

In My View

16 **Catherine Cockcroft** explores an emerging dilemma in food testing.

17 **Carmen Nitsche** says we must find a better way to share data on hazardous chemicals.

18 Is age related to scientific productivity? asks **Victoria Samanidou**.

20 Don't succumb to mass spectrometry mania, says **Ian Wilson**.

Report

22 Keeping Afloat in Modern Water Analysis

Features

24 **Cartographers of Cancer**
Using mass spectrometry imaging, a multidisciplinary team will create a detailed "Google Earth view of cancer" – charting a course towards new options for prevention, diagnosis and treatment.

34 **The New Face(s) of Forensic Science**
Meet the scientists working tirelessly to make sure the courts have the full facts.

42 **Upping the (Analytical) Ante**
Separation science still has a few aces up its sleeve, according to three speakers from this year's "Riva in Texas" symposium.

Departments

46 **Solutions:** Tandem Triumph, by Alun Cole

Sitting Down With

50 **Ian Wilson**, Chair in Drug Metabolism and Molecular Toxicology, Faculty of Medicine, Department of Surgery & Cancer, Imperial College London, UK.

HUMANITY IN SCIENCE AWARD

The Nomination date has now closed for the 2017 Humanity in Science Award

What is the Winning Project for 2017?

Recognizing Altruism and Innovation

The Humanity in Science Award recognizes and rewards a scientific project that has the potential to make the world a better place.

More information:
www.humanityinscienceaward.com

humanityinscienceaward.com info@theanalyticalscientist.com @humanityaward humanityinscienceaward humanityinscience

Future Separations: Redux

In Issue 03 of The Analytical Scientist, Emily Hilder and Rob Shellie boldly predicted that the next wave of separation technology would be "smaller, faster and smarter." How far have we come?

In fairness, it's only been four years since Emily and Rob's predictions – but, here and there, I'm starting to experience a little déjà vu. At the highly successful HPLC 2017 meeting in Prague, for example, I thoroughly enjoyed my conversation with the PharmaFluidics team, which is promising a "silicon revolution in chromatography" with μPAC technology. The new nano-LC "column" is essentially a microchip device that is manufactured using lithographic micromachining techniques – much like those used in the electronics industry – and is the commercialized result of the work from Gert Desmet's group at the Free University of Brussels.

I was shown a whole silicon wafer on which the array of perfectly ordered microcolumns existed and it looked distinctly... futuristic. In particular, I was intrigued by how smart electronics and separation science suddenly looked far more compatible. (And I secretly wondered if Alphabet Inc. or any other technology giants had already made surprise visits to Belgium). How disruptive will the technology be? Hard to say – but it appears to have all the hallmarks of a game changer. The first iteration of the technology certainly got people talking. And it's only early days; the PharmaFluidics team appears to have big plans, the right ideas, and a few tricks up its sleeves... Expect an article exploring the technology and expectations very soon.

Funnily enough, on chatting with Emily Hilder (now aptly the Director of the Future Industries Institute at the University of South Australia) during the coffee break, she reached for her smart device, 'Googled' the PharmaFluidics homepage, and tapped "About" for another dose of déjà vu:

"In a visionary contribution to [The Analytical Scientist] on the future of chromatography, Hilder and Shellie postulated in 2013 that the next wave of separation technology will be smaller, faster and smarter. At that time, the PharmaFluidics team was working hard to prepare for the introduction of a revolutionary technology for liquid chromatography. One year later, seed investment funds were secured [...]"

But that's just one flavor of the future. Imagine my delight when I read the thought-provoking contributions to "Upping the (Analytical) Ante" (see page 40), which mentions several further tantalizing tastes: microfabricated GC-HPMS systems, the integration of separations and sensors in disposable microfluidic microtiter plates, credit card-sized GC, and the disruptive potential of SLIM for ion mobility spectrometry.

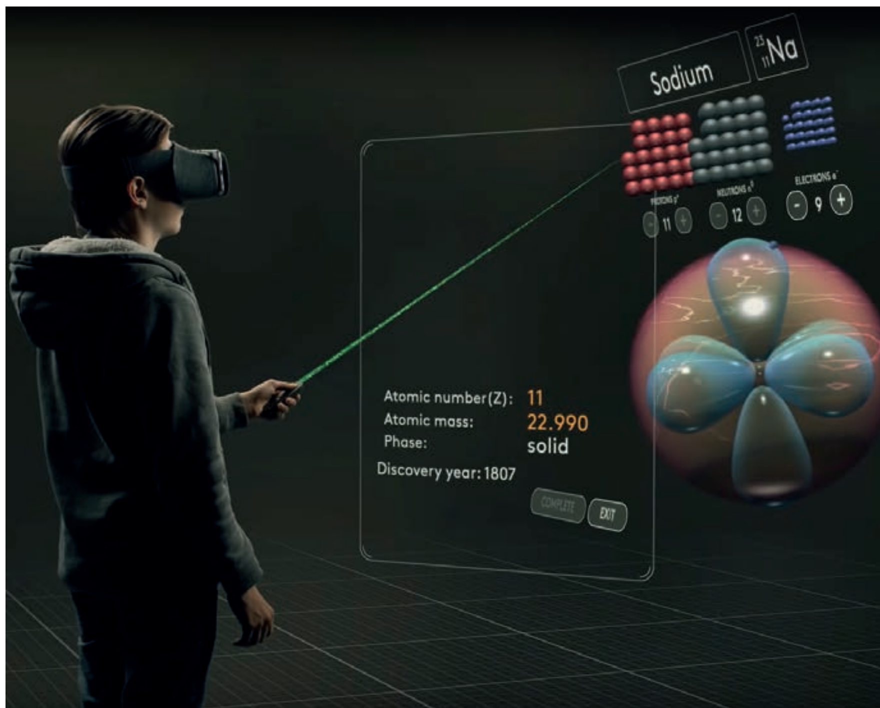
The future looks fascinating. But the present isn't a bad place to be either...

Rich Whitworth
Content Director

Upfront

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

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker@texerepublishing.com



Daydreaming in the Classroom

Virtual reality lessons are helping kids get their heads around complex chemistry. Could the analytical community learn a thing or two?

MEL Chemistry VR – a new series of virtual reality science lessons launched for Google Daydream in June 2017 – aims to help high school students learn by "immersing" themselves in atom-level chemistry. "Chemistry is filled with abstract concepts that are difficult for young minds to grasp," explained company founder Vassily Philippov in a recent press release. "VR is perfect for placing kids inside a chemical reaction, to see how these molecules interact with each other," and is likely to be somewhat safer than Ian Wilson's childhood dabblings – see page 50...

But don't fret. Philippov has no desire to replace wet chemistry with a VR version: "Real hands-on experiments are more engaging for kids. You see science. You touch science. You smell science. Every time I do experiments with kids, I see their eyes light up. We don't want to take that away from them."

MEL Science – the company behind MEL Chemistry VR (https://melscience.com/vr/) – believes VR is a much more efficient way of helping young people gain a deeper understanding of complex subjects, cutting down on explanation time and encouraging curiosity. Philippov elaborated: "Instead of memorizing how nitric acid reacts in five different conditions, they will understand how it interacts. They will understand what is happening with the molecules, ions and atoms in this reaction. They will see for themselves why it interacts differently in different conditions."

So, the big question: who's volunteering to plead with Philippov to create MEL Analytical Chemistry VR?

From Chemical to Clinical Analysis

What's new in business?

In our regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. This month saw yet more innovative solutions being showcased during the busy summer conference season, and Eurofins made some significant acquisitions across Europe and Canada.

Products

- Fortis Technologies launches the SpeedCore C18-PFP LC column
- Chinese IVD approval is gained for SCIEX' Triple Quad 4500MD system
- Bruker announces novel NMR phenomics research capabilities at Metabolomics 2017
- Agilent unveils new solutions at HPLC 2017 in Prague
- Thermo Fisher Scientific introduces the Cascadion SM clinical analyzer and automated chemistry analyzers for veterinary diagnostics
- Waters UPLC and MS systems are approved for IVD use in Brazil
- SepSolve launches the Insight Flow Modulator for GC×GC

Investment & acquisitions

- Analytik Jena sells AJ Blomesystem to GUS Group
- Eurofins acquires Genoma Laboratory Group in Italy, an environmental testing lab in Slovenia, and Canadian CRDMO Alphora Research Inc.
- Shimadzu acquires French analytical standards firm AlsaChim
- PerkinElmer to acquire Euroimmun for $1.3b in cash

Left to right: Yasunori Yamamoto, President Shimadzu Europa, Toufik Fellague, Managing Director AlsaChim; Jean-Francois Hoeffler, President AlsaChim; Juergen Kwass, Managing Director, Shimadzu Europa

Collaborations

- DiaSorin and Tecan partner on MDx platform development
- CiToxLab and KaLy-Cell partner on metabolism tests

For links to original press releases and more business news, visit the online version of this article at: tas.txp.to/0717/BUSINESS

Nanofluidic PAT

Is continuous, real-time analysis of biologics during manufacturing on its way?

Applying quality control to living organisms is tricky at best – but also crucial: the quality of biopharmaceuticals has a clear impact on both safety and efficacy. And so quality assurance is typically conducted at the end of the (lengthy and costly) biomanufacturing process – but is that logical? "If the manufacturing system produces low-quality or abnormal biologics, it is hard to see whether the product quality and system operation are normal or not during the manufacturing process through conventional analytics systems," says Sunghee Ko, Postdoctoral Associate of Jongyoon Han's laboratory at the Massachusetts Institute of Technology. "Because of this, current quality measurements (for example, release analytics) can lead to money loss and a disruption of biologic supplies when manufacturing has problems."

The logical solution? Monitoring biologics during the manufacturing process. Han's lab has taken on the challenge and created a nanofluidic device that they plan to directly link to a bioreactor to monitor purity and bioactivity with high sensitivity, resolution, and speed. "This is one of the preferable monitoring methods to realize process analytical technology (PAT) defined by FDA, and allows us to respond rapidly if there is a change in bioreactor conditions that affects the quality," says Ko.

The device is based on a series of nanoscale filters – or, to be more precise, patterned nanochannel arrays of varying depths and protein electrical potentials – that separate molecules by size (from 14–200 kDa). The team's paper (1) demonstrated multiparameter quality monitoring of three 20μl biologic samples within 50 minutes, but also shared a prototype on-line sample-preparation system that could make at-line monitoring – and therefore real-time quality assurance of biologics – a reality. *WA*

Reference

1. *SH Ko et al., "Nanofluidic device for continuous multiparameter quality assurance of biologics", Nat Nanotechnol, [Epub ahead of print] (2017). PMID: 28530715.*

Whither on the Vine?

PTR-MS analysis of VOCs in Pinot Noir can pin down its origin

How well do you know your Pinot Noir? A new method could provide a rapid "fingerprint" of the volatile organic compounds (VOCs) in this silky smooth, complex little number…

When it comes to wine analysis, gas chromatography-mass spectrometry (GC-MS) is a regular at the table, offering accurate analysis and differentiation of VOCs. The word on the 'vine is, there are faster alternatives – but the presence of ethanol can reduce sensitivity.

A team of New Zealand researchers from the Department of Food Science, University of Otago, NZ, hit the vineyards in an attempt to achieve rapid differentiation of wines from different sites, while maintaining sensitivity (1). The team had already sampled several wines for analysis with GC-MS at two different stages during the winemaking process (2) – immediately before being barreled, and after being aged in barrels for six months. In the current study, the VOC profile of each sample was differentiated by proton-transfer reaction mass spectrometry (PTR-MS; Ionicon Analytik) combined with manual headspace dilution – to minimize the effects of the ethanol.

The results? In the published paper, the authors conclude that PTR-MS analysis of wine, while less able to identify specific compounds than GC-MS, "may be a useful technique for rapid VOC fingerprinting to discriminate samples from different geographical origins." They add that "the similarities and differences expressed in the wines' VOC profiles may help winemakers to reveal the potential of individual vineyard sites to produce wines of certain character." In other words: using PTR-MS may well make life easier (and analysis quicker) for winemakers and those fighting wine fraud…which can only be good news for all the oenophiles out there. *JC*

References

1. *C Schueuermann et al., "PTR-MS volatile profiling of Pinot Noir wines for the investigation of differences based on vineyard site", J Mass Spectrom, [Epub ahead of print], (2017) PMID: 28598532.*
2. *C Schueuermann et al., "GC-MS metabolite profiling of extreme Southern pinot noir wines: Effects of vintage, barrel maturation, and fermentation dominate over vineyard site and clone selection", J Agric Food Chem, 64, 2342–2351 (2016).*

Long Time Coming…

The world's longest-running synchrotron light experiment reaches day 1,000.

The aptly named Long Duration Experimental (LDE) facility at the UK's Diamond Light Source allows researchers to conduct repeated experiments over time using synchrotron light.

The first researcher to make use of the LDE (1,000 days ago) was Claire Corkhill from the University of Sheffield, who is using X-ray powder diffraction to investigate the hydration of cements used to encapsulate nuclear waste. The samples are set up on a robotic bench and automatically passed into the beam every week, to see what minerals are formed as the cement reacts with water. This long-term data will be a valuable contribution to our understanding of materials critical to the safe disposal of nuclear waste, and could help design more robust cement mixes for the future. *CB*

Reid All About It

The International Reid Bioanalytical Forum's collegiate atmosphere and carefully curated sessions have earned it a dedicated following of discerning bioanalysts. We caught up with Forum Chair Tim Sangster (Charles River Laboratories) to get the lowdown on the event.

What is the origin of Reid Forum?

The Forum has been running every two years since 1975. It was conceived by the late Eric Reid, who directed the Wolfson Bioanalytical Unit at the University of Surrey, as a forum for bioanalytical scientists to discuss the issues of the day in an open, collaborative environment. I first attended in 1997 and learnt a huge amount from discussions with wonderful scientists like Howard Hill, Ian Wilson, Derek Stevenson and Eric Reid himself. This year, I am following in their footsteps by chairing the meeting – it's a great honor.

What's special about the event?

There is a great sense of community that you don't typically get at other, larger meetings. We encourage early career scientists and students to come along and mix with some of the biggest names in the field. The small size and active social program mean that by the end of the three days, you can easily come away knowing every attendee by name. We firmly believe that collaboration is the key to moving the field forward, and creating social networks has been a key principle of Reid from the beginning. We are moving to a new location this year, and we've pulled out all the stops for our social events – from a fun pub quiz to a meal in one of Cambridge's oldest dining halls.

Reid Forum is also unusual amongst academic or industry conferences in that we actively encourage people to not just celebrate their successes, but also share their failures – something that is made possible by the supportive environment fostered by the event.

What are you looking forward to in this year's program?

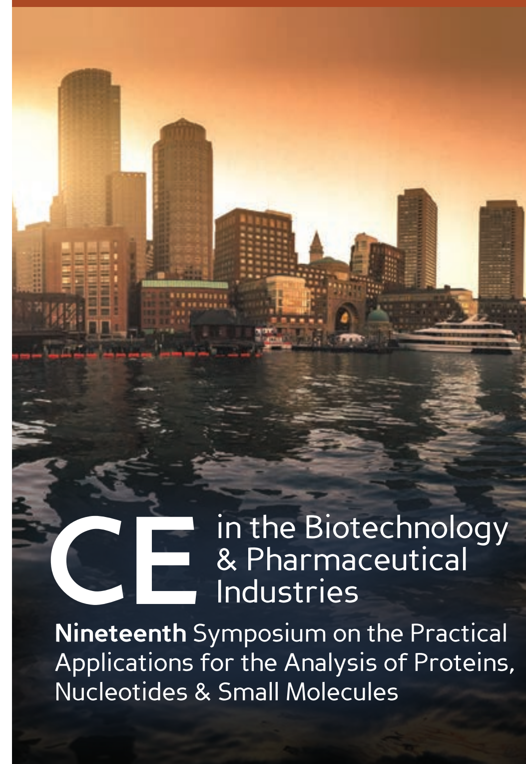
Tony Edge will host a session in which five vendors will present their vision for the future of bioanalysis, and answer questions about where they see the field going. It's always interesting to hear from regulators, and this year Stephen Vinter from the MHRA will be covering some of the hot topics in bioanalysis from the regulatory angle.

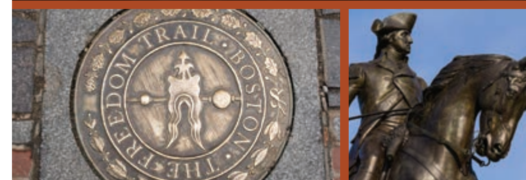
There is a pre-conference training course on large-molecule analysis by chromatography on September 4, which we expect to be very popular with industry scientists.

We have an entire session dedicated to immunochemistry and immunology. As bioanalysts, many of us are being stretched to look at molecules outside our own area of expertise, and this session will offer lessons learned from those who have made the transition from small to large molecule analysis.

The 2017 International Reid Bioanalytical Forum will be held September 4–7 at the Cambridge Belfry, Cambourne, UK. For more details or to register see http://www.chromsoc.com/ChromsocEvents.aspx

September 17-20, 2017
Boston Park Plaza Hotel, Boston, MA





Abstract Submission Deadline:
Oral Deadline: June 23, 2017
Poster Deadline: August 18, 2017

Symposium Co-chairs:
Steffen Kiessig, *F. Hoffman-La Roche Ltd.*
David Michels, *Genentech, a Member of the Roche Group*

CASSS™
SHARING SCIENCE **SOLUTIONS**

For program updates, hotel information and sponsor information, please scan the QR code or visit www.casss.org.

Small Samples; Big Promises

Ultrasensitive mutation analysis could boost liquid biopsy

It's hard to look at a laboratory medicine journal without seeing the words "liquid biopsy" these days. Small wonder the technique is such a hit – it's simple, noninvasive, and makes use of emerging molecular techniques to tell us more than ever about the diseases patients face. But with all of these advantages, liquid biopsy does face one challenge – sensitivity.

"The main issue with analyzing circulating cell-free DNA is that its concentration is low, and DNA of tumor origin is present at very low frequencies – sometimes only individual molecules," says Anders Ståhlberg, docent in molecular medicine at the University of Gothenburg's Sahlgrenska Cancer Center. "Standard techniques are not sensitive enough to find these rare molecules," he continues, "but with new approaches such as our SiMSen-Seq technique, this is now possible."

SiMSen-Seq allows the detection of circulating tumor DNA (ctDNA) in the blood with up to 1,000-fold more sensitivity than the methods currently in use. Ståhlberg and his colleagues accomplish this feat by adding a molecular barcoding step. "In molecular DNA barcoding, a unique

GAME-CHANGING COATINGS™

For your entire analytical flow path

sequence is added to each individual DNA molecule that enables us to track all sequencing reads back to the original DNA molecule. By aligning reads with the same barcode, it is then possible to differentiate between true mutations and those resulting from polymerase errors."

SiMSEn-Seq is not the only liquid biopsy method to use barcoding, but Ståhlberg says that each method carries its own limitations. "Our contribution is that we managed to develop a cost-effective method that is simple to use, flexible to adjust, and can be used with minimal DNA input."

What are the researchers doing with the technique now? Ståhlberg outlines a number of clinical investigations applying ultrasensitive mutation detection to liquid biopsy, including patients with childhood sarcomas, melanomas and breast cancers. He and his team are also applying their approach to areas beyond cancer, including chronic obstructive pulmonary disease and immunological responses. Nonetheless, he warns against jumping into liquid biopsy too fast. "The potential of circulating cell-free DNA is very high, but validation studies are important to prove its clinical value. You may find mutations without a disease – so we need to learn how and when to perform this type of analysis."

Ståhlberg next plans to learn exactly which liquid biopsy applications gain the greatest clinical value from ultrasensitive mutation analysis. He and his colleagues have recently received funding from several collaborating organizations to start a translational genomics platform (3) working with liquid biopsies and ultrasensitive mutation analysis. And he's optimistic about the future of liquid biopsy: "By analyzing patient-specific mutations in blood plasma, we anticipate improvements in diagnosis, treatment selection, prognosis, treatment monitoring and relapse detection." *MS*

References

1. *A Ståhlberg et al., "Simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing", Nat Protoc, 12, 664–682 (2017). PMID: 28253235.*
2. *A Ståhlberg et al., "Simple, multiplexed, PCR-based barcoding of DNA enables sensitive mutation detection in liquid biopsies using sequencing", Nucleic Acids Res, 44, e105 (2016). PMID: 27060140.*
3. *"Translational Genomics Platform" (2017). Available at: http://bit.ly/2rMqmwi. Accessed June 20, 2017.*

Discover how SilcoTek® inert coatings help you maximize system reliability by eliminating the negative effects of a metal flow path.

www.SilcoTek.com/analytical-scientist

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

To Test or Not to Test

There is a dilemma facing the food industry: technological advances allow us to detect an ever-growing number of potentially harmful emerging organisms. But when is the right time to start testing?

By Catherine Cockcroft, Head of Microbiological Services, Eurofins Food Testing UK Limited, UK.

New health threats concerning the food industry feature regularly in the media. But differentiating true emerging organisms of concern from the background noise is difficult – and addressing those threats within food businesses even more of a challenge. Those in the industry must recognize the hazards relevant to their products, assess the risks, and then manage them through prerequisite programs (the foundation of good hygiene practices) and the use of food safety hazard analysis and critical control point (HACCP) principles.

Microbiological testing can provide verification that HACCP and Good Manufacturing Practices are working. However, with some emerging organisms of concern, it may be challenging to carry out verification testing, and even more difficult to interpret the results.

Foodborne viruses, including norovirus, hepatitis A and hepatitis E, cannot grow or multiply on foodstuffs, but some products, such as bivalve mollusks, leafy vegetables and berries contaminated with water containing infected human waste, can act as vectors for their transmission to humans (via the fecal–oral route). Resulting illness can vary from self-limiting gastrointestinal symptoms to more serious liver inflammations. And though the true burden of illness attributable to contaminated food is not known, it is estimated that norovirus is the most common cause of foodborne illness in the European region, with close to 15 million cases each year, causing more than 400 deaths (1).

Foodborne virus testing in foods is challenging, particularly when it comes to the recovery of low levels of strongly adherent viral particles, which may be protected in microscopic crevices or within the digestive gland of bivalve mollusks. Even the best methods available may only recover one percent of the viral particles present.

Complex molecular techniques detect the presence of viral particles, and results are expressed in numbers of viral genome copies. Detection in itself does not necessarily mean that people consuming the food are at risk of foodborne illness, of course. The infective dose from foods is not

> *"Given the information gaps that currently exist, should food businesses already be testing for foodborne viruses to verify the effectiveness of the controls they have in place?"*

known, though may be as low as ten viral particles. Furthermore, the presence of viral RNA does not necessarily mean that the particle is capable of infectivity.

Another consideration is the cost of performing the analysis. Molecular techniques, unlike conventional cultural microbiology methods, are expensive and complex to perform, increasing the cost per test from a few pounds (GBP) to perhaps a few hundred pounds.

Clearly, testing does not assure food safety, and producers/manufacturers already have procedures in place that minimize the risk of contamination of foods by foodborne viruses.

Given the information gaps that currently exist, should food businesses already be testing for foodborne viruses to verify the effectiveness of the controls they have in place? If viral particles are detected on foodstuffs, what remedial action should food businesses take? Is there a risk that product will be removed from sale when it doesn't present a true risk to the consumer? Or is the risk greater to the consumer if food businesses choose not to perform any verification testing?

At this early stage in the understanding of these micro-organisms, caution is advised before rushing into full-scale routine testing. Producers and manufacturers should anticipate how they will react to detection of these organisms, and be ready to enact those processes should the need arise. In the meantime, research continues to better understand these viruses, and methods for their testing are being refined and improved upon. In three to five years, we may even be in a position to include these organisms in routine verification testing of at-risk foodstuffs.

Reference

1. *http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/*

When Experiments Go Wrong

Laboratory safety is a priority for all. We need to get better at sharing data on hazardous chemical reactions.

By Carmen Nitsche, Business Development Consultant, The Pistoia Alliance, USA.

In February 2017, a PhD student at the University of Bristol in the UK was conducting a routine experiment. An unanticipated reaction created triacetone triperoxide – a highly explosive substance – and the emergency services were called to carry out a controlled explosion. Fortunately, no one was hurt, but the incident highlights how easy it is to unintentionally create a hazardous chemical or unwanted reaction, particularly in a research institution.

A chemical reaction doesn't have to create an explosion to be hazardous. Depending on the scale of the reaction, reagents can violently interact to shatter glassware, spew forth toxic gases or burst into flame. There are numerous books, databases and other resources available that outline reagent safety information, but what would be more beneficial is a searchable, freely available database on unintended reaction incidents and near-misses. Such practical information does exist of course – but it's often locked in internal silos, where it is difficult to find and share even within a company, much less across organizations (nobody likes to admit when an experiment has gone horribly wrong...).

As the life sciences industry relies on experimentation to develop new products, there is no way to eliminate risks entirely. However, the same negative incidents should never happen twice. Researchers need access to previously reported dangers. To this end, The Pistoia Alliance has recently developed the Chemical Safety Library Service. The service allows the research community to submit, store and share hazardous chemical reaction information.

"Depending on the scale of the reaction, reagents can violently interact to shatter glassware, spew forth toxic gases or burst into flame."

The library has been seeded by members of The Pistoia Alliance, with a number of incidents from their archives. Members can add and share their chemistry reaction-related incidents and learnings – and the content is free to download and integrate for use with internal informatics systems, such as electronic lab notebooks or inventory systems. These systems can also be configured to alert scientists if there is a potential known safety risk before they

carry out an experiment.

Since the majority of safety information falls in the precompetitive arena, sharing this kind of experience should be straightforward. Moreover, in cases that do involve proprietary components, the Chemical Safety Library offers a function to convey these important safety learnings without revealing company intellectual property.

The Pistoia Alliance is a global not-for-profit organization that intends to help lower the barriers to innovation in life sciences R&D – and one of our key focuses is collaboration. Our library service could help increase laboratory safety, but we need the life sciences community to embrace this effort.

Following the launch of the Chemical Safety Library Service in March 2017, requests for access have been overwhelming. The positive response shows just how much the industry is looking for such a resource. But looking is not enough! Ultimately, the more data the Chemical Safety Library contains, the more useful it becomes to the entire industry. We need companies to move beyond their reticence to share and to add data on hazardous chemical reactions. The process only takes a few minutes. Safety is everyone's concern and now every researcher can embrace the responsibility and do something constructive about it.

For more information, visit www.pistoiaalliance.org/projects/chemical-safety-library/

They Shoot Horses, Don't They?

Age-based stereotypes exist, even in scientific communities. But is age related to research productivity – and, if so, to what extent?

By Victoria F. Samanidou, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece.

The relationship between age and productivity is not a simple one to quantify. Older workers are assumed to be less effective and industrious than their younger colleagues when it comes to more physical tasks (1,2). But what about science in particular?

In scientific communities, opinions on the net effect of age on productivity are varied. Several factors influence the productivity rate of researchers or academics; experience, health status, position, rank, and many more. It also begs the questions: what exactly is "productivity" and how do we measure it? In academic communities, it is often measured by the number of publications, along with the number of self-excluded citations and the h-index; the former relating to quantity and the latter to the quality and impact of the work. Do older scientists publish less or more? It is difficult to make an estimation – the determinants of individual productivity are extremely complex and I doubt whether typical metrics are in any way useful. However, I can say that authorship is not always directly related to actual productivity.

Perhaps rather than trying to guess the productivity of individuals, it is more useful to reflect on the "typical" path in a scientist's career. In short, it can take a long time to get to the top. On the path to recognition, I have witnessed three typical turning points in the career of academics; the first occurs at around the age of 35-40 years, where researchers are expected to step up their productivity to reach a higher position. A second inflection point comes at the age of 50-55, when the rate of productivity can reach a plateau or decrease slightly (3).

> *"Perhaps rather than trying to guess the productivity of individuals, it is more useful to reflect on the 'typical' path in a scientist's career."*

The third turning point, I believe, comes when researchers are approaching retirement age. As researchers move up the stratified hierarchy of science, recognition reaches a peak, leading to collaboration with more productive groups, greater success in gaining access to funding and more likely publication in scientific journals with a higher impact – all boosting perceived productivity. However, there is another trend in this age bracket; older professors publish far

fewer first-authored papers and instead move to the end of the list of co-authors, as they are more likely to be the leaders of their own groups.

No one can deny that with time, physical power decreases. In addition, technological developments and innovations are not always easily integrated by older scientists. On the other hand, a significant number of older scientists stay active in research, keep their productivity at a high level until their retirement and continue to inspire the young, still playing an effective role in the production of high impact papers. Indeed, if one is able to inspire 10 or more team members to be more efficient (while striving for high quality), the overall effect is an increase in productivity for the group, perhaps far outweighing the potential of a single individual.

So are older scientists more productive than their younger peers? I would argue that the most important aspect, whatever the age of the scientist, is the degree of satisfaction that they gain from collaboration with others – and, even more important, their passion for furthering research. And I don't believe either of those aspects have anything to do with how old you are. There are more than a few examples of scientists – young and old – who have simply lost interest; they require a change in attitude or should consider an alternative profession...

"All scientific research relies on collaboration – and so researchers of all ages need to play a significant role in its dynamic."

All scientific research relies on collaboration – and so researchers of all ages need to play a significant role in its dynamic. With understanding on both sides, it's a multi-way process; when we are surrounded by young people – eager students in academia or dynamic young scientists in research institutes or industry – it can be easier for us to maintain a "youthful" outlook; in turn, younger colleagues can benefit from the great experience, knowledge and tenacity of their superiors. To my mind, when it comes to age, it's less of a generation "gap" and more of a spectrum.

References

1. *Times Higher Education, "Are older academics past their productive peak?" (2016). Available at: http://bit.ly/1QZK9bt. Accessed July 7, 2017.*
2. *Taylor and Francis Online, "Explaining the increase in publication productivity among academic staff: a generational perspective", Available at: http://bit.ly/2tQ0EYv. Accessed July 7, 2017.*
3. *Y Gingras et al., "The Effects of Aging on Researchers' Publication and Citation Patterns", PLoS ONE, 3, [online only], (2008).*

Behind every great (U)HPLC system…

is a great column…

and you can expect:

Reproducibility
• day to day • column to column • lab to lab

Robustness
• pH
• temperature
• 100% aqueous eluents

Scalability
• UHPLC ↔ HPLC ↔ PREP
• easy method transfer

Selectivity
• RP, NP, HILIC
• Chiral, SFC
• IEX, SEC

Discover more at: www.ymc.de

Managing MS Mania

Mass spectrometry has certainly changed the face of analytical science, but it's not a panacea.

By Ian Wilson, Chair in Drug Metabolism and Molecular Toxicology, Faculty of Medicine, Department of Surgery & Cancer, Imperial College London, UK.

It's a great time to be analytical scientist. Technology in this field is developing rapidly, with ever-increasing capabilities. However, the increasing reliance on mass spectrometry (MS) that I see, especially amongst young scientists, makes me uneasy. Is MS becoming so dominant that people forget that there are other ways of analyzing things?

Already, some people in my own field of metabonomics are reluctant to move outside the confines of mass spectrometry. There is an attitude that if it can't be done by LC-MS, then it can't be done at all. Of course, that is simply untrue. There are many answers beyond LC-MS, but you have to be willing to try a different (and possibly less sexy) approach.

I am by no means suggesting that we go back to the past (though I do have a museum of old analytical equipment, if you're interested – page 50). But consider that, in the space of 30 years, mass spectrometry has progressed from a specialist instrument requiring intensive training and lengthy analysis, to something that any competent analytical chemist can use. When I did my first mass spectrometric analyses, it took a whole day to analyze a single spectrum (printed on photosensitive paper – we counted the mass units by hand!) The power and ease of today's mass spectrometers is wonderful by comparison.

So yes, it would be ridiculous to turn our backs on the wonderful power and ease of use of modern mass spectrometry. But… we must also be aware of its limitations, and keep an open mind to alternatives. If our starting point is always to assume that we will analyze the sample by LC-MS, we can forget to ask the most important question – what are we trying to learn from our analysis?

MS is remarkably sensitive (though this is structure dependent). But if you find that you have to dilute a urine sample 10,000 times to get the analytes you want to measure into the linear range of the instrument, it's time to ask yourself if your approach is the best one. If all you want to do is quantify a particular molecule, why not use something like LC-UV? Since the 1970s, and assuming a suitable chromophore, we've been analyzing samples with LC-UV at 1 ng/mL with great selectivity, precision and accuracy. Plus, for the cost of one LC-MS system, you could buy ten LC-UV systems.

"No doubt, over time, pragmatism will prevail and techniques currently out of favor will find their place again."

I work with colleagues at Imperial College who use both ^{1}H NMR spectroscopy and LC-MS for both small and large-scale metabonomic analyses. At first sight the use of NMR spectroscopy and LC-MS for the same analysis seems a bit strange, as it is a common perception that the former is rather insensitive - so how could it compete with MS? However, in my experience, the combination of the two is brilliant. They are quite orthogonal in the metabolites they access, and the information provided is complementary. In addition ^{1}H NMR spectroscopy is inherently quantitative, wonderfully reproducible, contains a lot of structural information and is not subject to ion suppression! Add in LC-MS and you have a very powerful combination for the analysis of complex mixtures such as biofluids.

No doubt, over time, pragmatism will prevail and techniques currently out of favor will find their place again. And perhaps in a few years, a new technique may even come along to steal mass spectrometry's crown and shake us all up again. It's one of the things I love about analytical chemistry. All I ask is that while we welcome the latest and greatest, let's not lose our perspective and forget the old favorites.

FRITSCH

FRITSCH. ONE STEP AHEAD.

www.fritsch-us.com

SAMPLE PREPARATION

Milling Sieving Dividing

- Various types of grinding principles
- Simple operation and cleaning
- Exact grinding results down to the nano range
- Consistent reproducibility
- Dry and wet grinding

PARTICLE SIZING

From 0.01 µm up to 20 mm

- High measurement precision, consistent reproducibility
- Static light scattering
- Dynamic Image Analysis
- Short measuring times
- Fast and automatic particle analysis

FRITSCH Milling and Sizing, Inc.
57 Grant Drive · Suite G · Pittsboro, NC 27312 · Phone 919-229-0599 · info@fritsch-us.com

Keeping Afloat in Modern Water Analysis

Ashley Sage, Senior Manager, Applied Markets Development (EMEAI) at SCIEX, considers the trends, challenges – and solutions – that are driving the future of water analysis.

We recently surveyed the readers of The Analytical Scientist to uncover some of the challenges that keep water analysts up at night – and some of their expectations and hopes for the future. Here, I offer my thoughts on the findings.

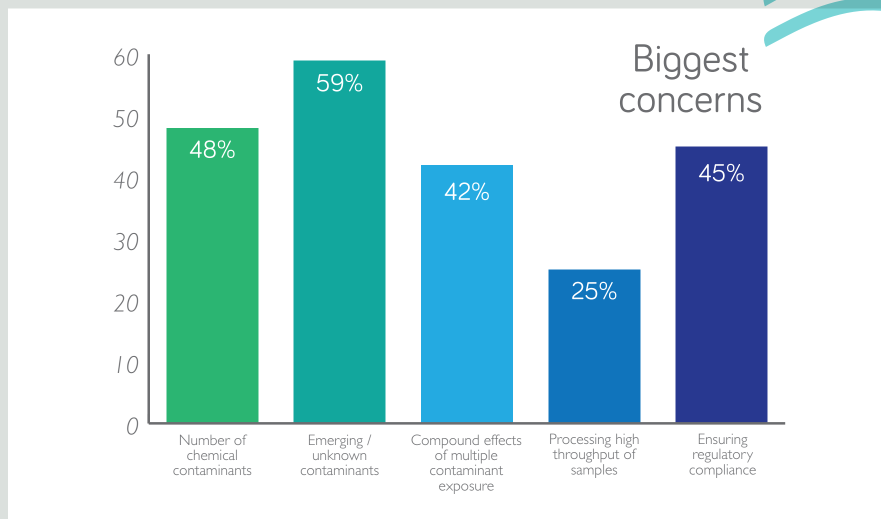


Figure 1. What are the biggest challenges facing water analysis? (Percentage of survey respondents, multiple selections allowed).

Biggest concerns

First of all – proving that water analysis isn't as straightforward as it first seems – it's interesting to note that only four percent of respondents felt that they faced no challenges in water analysis (and I'm not sure what their respective line managers would say about that!). On the other hand, 40 percent of respondents faced three or more challenges and 56 percent of respondents faced at least one or two challenges.

When I've spoken with customers in water analysis, it's clear that keeping pace with regulations lies at the heart of many of the main challenges – and almost half of our respondents note the regulatory burden directly. But, as with many other analytical fields, detecting more compounds at lower concentrations – and the emergence of new contaminants – are major concerns in water analysis. In short, the number of samples is increasing (and likely to continue increasing) – and, more worryingly, the analytical challenge is not always met by the required level of instrument sophistication or the necessary skill level within the laboratories that need to do the work.

Water analysis laboratories are not alone in needing the right tools to do the job – and instrument manufacturers have to step up and take the lead by offering not only the right solutions but also the right level of support.

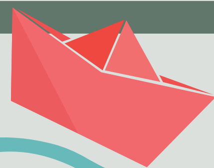
I believe that today's instruments and methods can certainly meet regulatory requirements, so the barrier is actually the adoption of potentially transformative technology in these routine laboratories. But resistance can only last so long in the face of increasing regulatory scrutiny... Simpler instrumentation and fast, foolproof methods (for emerging and unknown contaminants, for example) enable an easier transition, as can the level of support on offer. Education and training is part of that support network, and so SCIEXUniversity was set up to offer free training programs and a large database of online self-paced eLearning courses.

Daily workflow challenges

It's interesting to see sample preparation and budget constraints stand out from the crowd when it comes to major workflow challenges. Sample preparation is still a critical throughput-limiting step in many routine laboratories, and one possible solution is direct analysis. However, direct analysis (after sample dilution to remove matrix interferences) demands higher performance instruments, which unfortunately conflicts with the other major challenge – budget constraints; after all, higher performance instruments cost more to develop and manufacture. That said, many customers are less constrained when it comes to capital expenditure – so purchasing an instrument is relatively easy; overall operational costs are more of a concern. Clearly, we are trying to develop instruments that are smaller, faster and cheaper (through more streamlined manufacture), but I've found that the key attribute for most lab managers is whether instruments are 'fit for purpose' – and that's something we are very focused on across all of our platforms.

What is crucial for success?

Almost half of respondents consider "quick and efficient confirmatory analysis" as being crucial to the success of their laboratory – and that echoes the conversations I've had with customers. When it comes to municipal water companies, in particular, the laboratories are run almost like factories, so speed and efficiency are essential – as is reliable and robust instrumentation. And because of the sheer number of samples, the ability to perform multiplexed



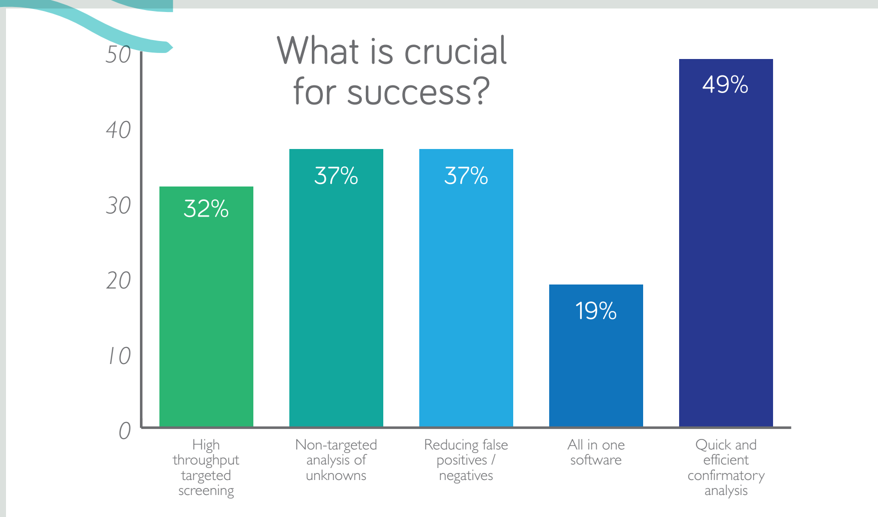


Figure 2. Which of the following are crucial to your laboratory's success? (Percentage of survey respondents, multiple selections allowed).

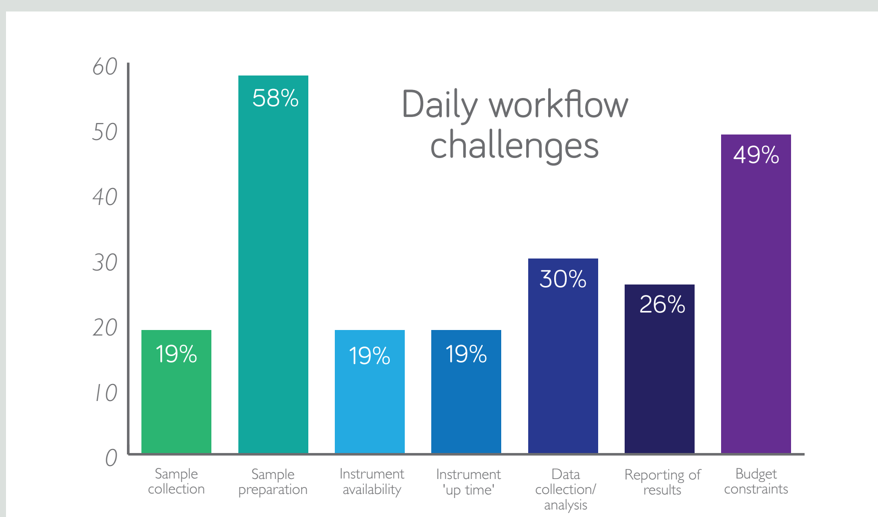


Figure 3. What are the main challenges in your daily workflows? (Percentage of survey respondents, multiple selections allowed)

analysis and different assays (for example, trizenes and acid herbicides) on the same instrumental set up is key.

Given that water analysis is driven by safety but under resource pressures, it's no surprise that reducing false negatives and false positives is also high on the agenda.

Non-targeted analysis is a trend across several application areas. In water analysis, there is a regulated list of compounds that must be adhered to – but that doesn't account for new and emerging contaminants. What's in the water that we don't know about? Water companies increasingly have to prove that they have the analytical capability to perform non-targeted analysis and, once again, though the technology (accurate mass QTOF instrumentation alongside SWATH acquisition, for example) and methods are available, data interpretation can be particularly challenging – especially at high-throughput. And the reality is that there are very few trained mass spectrometrists in these highly routine laboratories. In short, routine labs can only benefit from more intuitive data interpretation solutions.

SCIEX has certainly invested (and will continue to invest) a great deal of resources in software development to enable seamless data interpretation in more routine applications – but we also understand the growing interest in open source software. In fact, we've collaborated with enviMass, which is an open source code structure that can import our high-resolution MS data and perform trend detection, isotope grouping and homologue detection – to help with data deconvolution. I believe the mass spectrometry community still has a long way to go when it comes to software – and it's likely that continued investment alongside collaborative efforts offer us all the fastest route forward.

Sage and SCIEX

I've been in the analytical science world for over 20 years. I did my PhD in analytical chemistry (with a focus on environmental pollutants) followed by a postdoctoral fellowship at the University of Leeds, before working my way through a number of roles at several big analytical instrument manufacturers. I joined the senior management team at SCIEX in 2013, and today try to keep a keen ear to the ground to listen to what our customers want and need in several key markets, including environmental analysis. I believe SCIEX is well known in the pharmaceutical, clinical and food application areas, but people may be less familiar with the solutions and new technology that we've developed to support the environmental space.

Bermudas

du Tropique du Cancer

Route

Antilles

Barbuda

Antegua

Descada

Marigalante

Dominica

Martenique

St. Lucia

St. Vincent

Barbades

Bequia

Grandillos

Granada

Tabago

Isles

Caribes

I. Trinidad

R. Oronoque

FERME.

GUIANA

CARTOGRAPHERS OF CANCER

Meet the researchers using mass spectrometry imaging to plot a molecular map of malignancy.

From Ptolemy to Google Maps, humans have been driven to record the landscape around them. By committing our world to paper, we can understand it, order it, and maybe even control it. A blank on a map is intriguing, but unnerving; medieval mapmakers, faced with unknown territories, filled them with ferocious monsters and deadly storms. These mythical beasts were vanquished as intrepid explorers charted the wilderness, filling in the gaps in our knowledge.

Can a new kind of cartography help us face down another terror? Cancer is much better understood than it was 50, or even five years ago, but for the millions of people diagnosed with cancer every year – and the doctors who treat them – there are still many troubling uncertainties. Have we caught it in time? Will it spread? What is the best course of treatment?

An ambitious five-year project led by the UK's National Physical Laboratory (NPL) will record the most detailed map yet of the molecular landscape of a tumor. By combining new and existing mass spectrometry imaging techniques, the multidisciplinary team will create a "Google Earth view of cancer" – from whole-tumor down to subcellular level – with the hope of charting a course towards new options for prevention, diagnosis and treatment.

A Google Earth View of Cancer

Lead investigator Josephine Bunch shares details of an ambitious project to image all the molecules associated with cancer, in an interview with Charlotte Barker.

In 2015, I heard a program on BBC Radio 4 about the Cancer Research UK (CRUK) Grand Challenge – a series of five-year, £20 million awards for multidisciplinary teams willing to take on some of the toughest challenges in cancer research. One of the problems they described was mapping tumors at a molecular and cellular level – creating a Google Earth view of tumors. Traditionally, mapping of tumors was performed using standard histology and histopathology tools, such as microscopy of stained tissues. The problem is that to stain for a molecule, you have to know what it is. To create a comprehensive map, we have to be able to find all molecules, including the unexpected. As an analytical scientist, I saw that what CRUK was describing was fundamentally a measurement challenge, and felt immediately that the way forward would be through mass spectrometry imaging (MSI).

Admittedly, I may have been somewhat biased. From the moment I first encountered mass spectrometry, I was hooked. I love the sheer variety of instruments available; the many ways we can create and transmit ions gives us a huge number of different combinations. Then there's the breadth of applications – mass spectrometry measurements are being recorded everywhere from oceans to operating theaters to missions on Mars. At the time, I had been Co-Director of the National Physical Laboratory (NPL)'s National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI) for three years, leading our efforts in ambient MSI and matrix assisted laser desorption/ionization (MALDI).

Friends and colleagues encouraged me to contact CRUK to see if they would consider a MSI-based project. CRUK confirmed that there were no preconceived ideas about how the challenge should be solved, and I decided to go for it.

Google Translate

First, I put together a consortium of researchers, some of whom I already knew or had heard of, and others who were recommended to me. The team consists of experts in the relevant cancers, world-leaders in developing genetic models, inventors and innovators of techniques, and specialists in various aspects of tumor biology and metabolism.

We will take samples from breast, pancreatic and colorectal cancer, from patient biopsies and mouse models, and we will use them to build chemical images – molecular maps – at a range of different scales, from single cells up to whole tumors. The reason we often use CRUK's clever analogy of "Google Earth for tumors" is because it is so important to be able to explain our work in accessible terms; partly to communicate the value of our research to the public, but also so that our team of analytical chemists, physicists, biologists and medics are able to articulate shared goals.

We all want to achieve the same thing but we don't always speak the same language. For example, if you ask a biologist about the biggest challenge in mapping a tumor, they are likely to mention the difficulties of obtaining samples for molecular or metabolic studies, and interpreting the information. A mass spectrometrist may have a completely different answer, focusing on the huge sample numbers involved or the problem of building instruments with the resolution required. The Google Earth analogy has guided us in designing our pipeline and bringing the right investigators and techniques on board. I believe that the better you can break the project down into accessible descriptions across disciplines, the better the science.

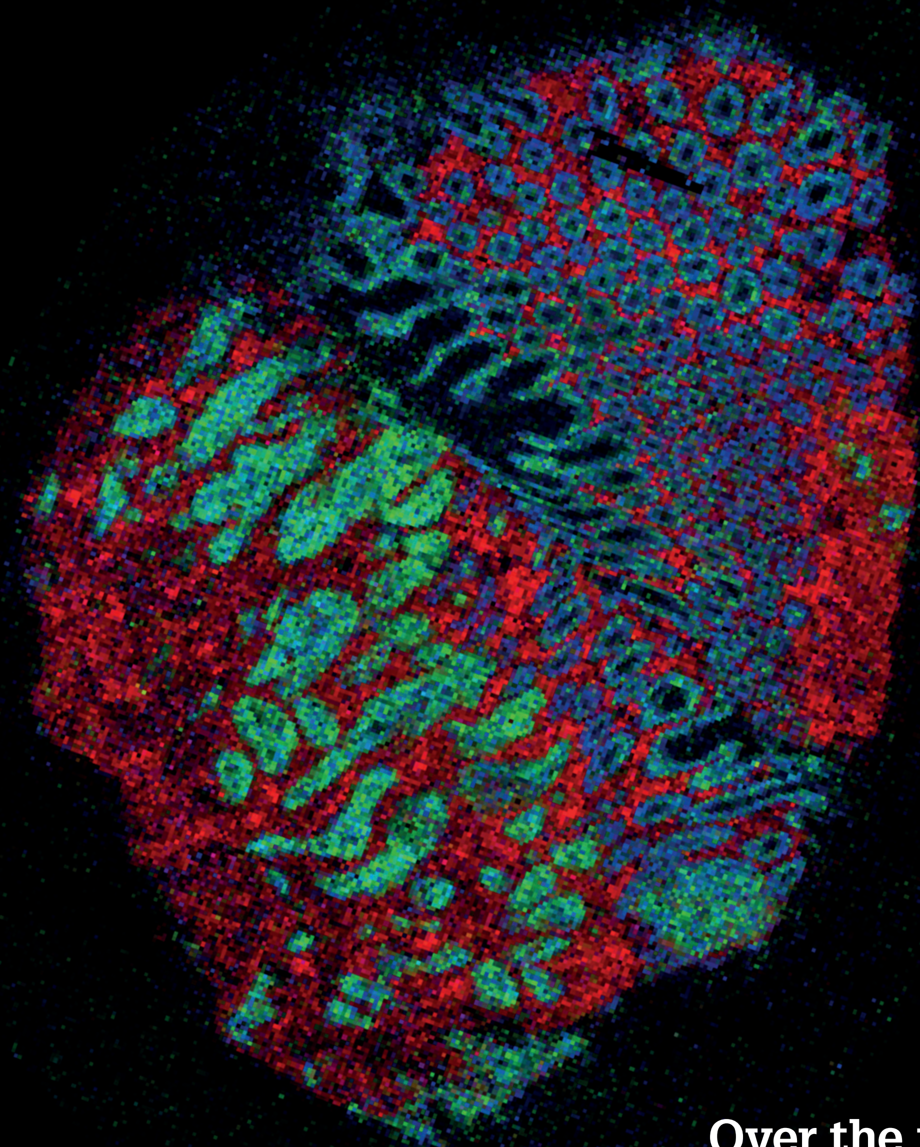
A grand measurement challenge

Getting the call to say we'd been successful in our bid was incredibly exciting. NPL is leading the consortium and will be analyzing samples with MALDI, desorption electrospray ionization (DESI), secondary ion MS (SIMS), Nano-SIMS and OrbiSIMS. We will also be coordinating imaging performed at other institutions, managing the data generated and disseminating the resulting protocols and instrumentation.

We launched the project officially in May 2017, and even before that we were getting our instruments ready and gathering preliminary data. Our results so far have shown the extraordinary amount of data possible when we combine different MSI techniques. However, there is also an abundance of challenges, from ensuring that we have sensitivity at the highest resolutions for key metabolites to maintaining the quality of each and every measurement. But mining the enormous data sets we collect is perhaps the biggest challenge of all.

Our priority for now is to build a framework to ensure quality measurements across the huge number of samples we plan to analyze. From sample collection to data analysis, there are so many factors that could introduce variation, especially when working with multiple techniques. NPL and the Grand Challenge consortium are extremely passionate about generating reproducible data and repeatable measurements. We don't want to produce beautiful pictures that cannot be reproduced or don't accurately represent the underlying cancer biology.

We have designed pilot studies assessing the performance of the plethora of different MSI instruments we're using – and we've made some measurements at several different sites to understand how much it affects the results. This work represents an important foundation in being able to quote the performance of the different techniques in combination. We have also been assessing the various

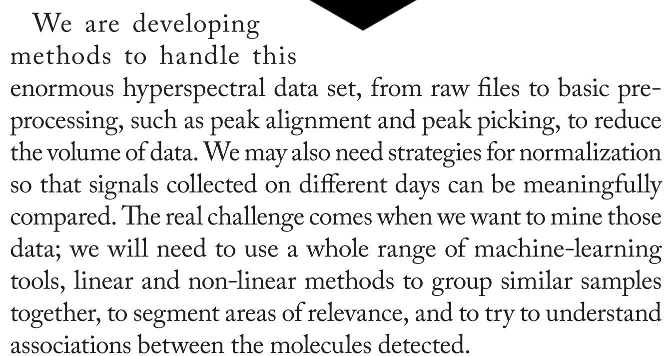


A bowel cancer sample imaged using MSI.

Credit: Zoltan Takats, Renata Filipe-Soares (Imperial College London); Nicole Strittmatter, Gregory Hamm, Richard Goodwin (Astra Zeneca); Rory Steven, Adam Taylor, Alan Race, Spencer Thomas, Rasmus Havelund, Josephine Bunch (NPL).

“**Over the five years,** we hope to share results **so exciting** that other labs are inspired **to use our techniques.**”

The team plans to zoom in using different instruments to inspect important areas of the tumor. *Credit: Zoltan Takats, Renata Filipe-Soares (Imperial College London); Nicole Strittmatter, Gregory Hamm, Richard Goodwin (Astra Zeneca); Rory Steven, Adam Taylor, Alan Race, Spencer Thomas, Rasmus Havelund, Josephine Bunch (NPL).*



parts of our pipeline to ensure absolute consistency. It is perhaps not the most glamorous work, but it's vital that samples from different sites are being collected, stored, transported and analyzed in the same way, and that we have robust pipelines in place for handling our data all the way from raw files to the curated data that will be available to the public.

Right now, I'm most excited about making measurements that no-one has ever made before whilst working with an extraordinary consortium of researchers – all of whom are there to ensure that we are making measurements that matter. I'm also looking forward to the second phase of the project: interpreting our data, sharing it and broadening our network. Over the five years, we hope to share results so exciting that other labs are inspired to use our techniques. And we will be ready to help them acquire quality data as quickly as possible.

Next-level MSI

The past few years have seen fantastic work from around the world using MSI as a powerful method for mapping multiple molecules in the same tissue. It might still be an emerging technique, but it already has a fantastic pedigree of excellent results. The Grand Challenge project will build on that success by using several MSI techniques in combination to widen the range of molecules examined, and so gain an in-depth understanding of tumor metabolism – linking genes, proteins, peptides, lipids and metabolites.

We will be making use of significant recent advances in technology. An example is the 3D OrbiSIMS instrument, which combines SIMS with an Orbitrap mass analyzer. As Ian Gilmore describes in "The Super-Resolution Revolution," the hybrid instrument allows very high resolution. Going back to the Google Earth analogy, OrbiSIMS is like peering through the window of a house in Street View to see where the sofa is...

At that level of detail you can't get through enormous numbers of samples – just as you wouldn't want to record the position of every sofa in the world. Instead, we will use other techniques to identify cells and regions within the tumor that we want to pay special attention to. MALDI and DESI give a street and city view – they can get down to pixel sizes small enough for a street-level look but can also rapidly create a basic "city map" of a tumor section.

Other techniques will help us localize our search to specific "cities". In addition to MSI methods, such as SIMS, MALDI and DESI, we are also using techniques for in vivo analysis and imaging of metabolites – such as the iKnife (REIMS) and MRI.

A common aspect of all the techniques is that they will produce a series of mass spectra acquired at discrete locations across the tissue.

We are developing methods to handle this enormous hyperspectral data set, from raw files to basic pre-processing, such as peak alignment and peak picking, to reduce the volume of data. We may also need strategies for normalization so that signals collected on different days can be meaningfully compared. The real challenge comes when we want to mine those data; we will need to use a whole range of machine-learning tools, linear and non-linear methods to group similar samples together, to segment areas of relevance, and to try to understand associations between the molecules detected.

Measuring success

Our ultimate goal is to gain new insights into tumor progression that might help diagnose and treat cancer. If we can help biologists understand exactly how tumors grow and spread, that knowledge can be translated to make sure that patients are diagnosed earlier, and can be given the right treatments at the right time. Of course, it will take time to translate our findings into the clinic. Concentrating on the next five years, I will be satisfied if:

- The tumor biologists on the team have gained fresh understanding.
- We have significantly improved the performance of the techniques we're using.
- Our measurements are being adopted as standard in research labs.
- Our data have helped to produce new in vitro models that are more representative of real human tumors.

Like the cartographers of cities, countries and continents, we want to fill in the blanks on our map of cancer, and unlock the secrets of tumor metabolism.

The Grand Challenge Team

A diverse group of analytical chemists, physicists, biologists and medics have come together to make the vision of a Google Earth for tumors a reality.

Josephine Bunch
Bunch will lead the Grand Challenge consortium. She is Co-Director of the National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI) at NPL.

Ian Gilmore
Gilmore is a Senior NPL Fellow and Head of Science at NPL. He is the founder of the National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI) at NPL, where he conceived of the 3D OrbiSIMS instrument and led the project to build it. The Gilmore group will lead the high-resolution 2D and 3D SIMS imaging for the Grand Challenge.

John Marshall
A professor of tumor biology at Barts Cancer Institute, Queen Mary University in London, Marshall is an expert in tumor invasion and the role of adhesion molecules. In the Grand Challenge, the Marshall group will deliver imaging CyTOF (mass cytometry) analysis.

Owen Sansom
Sansom is interim director of the Cancer Research UK Beatson Institute. He has been instrumental in determining the molecular hallmarks and cell of origin of epithelial cancers (colorectal and pancreatic). The Sansom laboratory will provide the Grand Challenge team with in vivo models.

Richard Goodwin
A Principal Scientist at AstraZeneca, Goodwin leads a MSI group studying the distribution of drugs. Within the Grand Challenge team, his role is to help perform inter-site experiments, translate the findings into an industry setting and disseminate for maximum impact on the development of new oncology medicines.

Mariia Yuneva
Yuneva leads a group at the Francis Crick Institute dedicated to oncogenes and tumor metabolism. In the Grand Challenge, her group will provide in vivo and ex vivo models of mouse and human primary breast cancers and their metastases.

George Poulogiannis
Poulogiannis joined the Institute of Cancer Research (ICR) in 2014 and now leads the Signaling and Cancer Metabolism team. His contribution to the Grand Challenge will be to study the therapy sensitivity pattern of metabolically-distinct tumor phenotypes using genetic and pharmacological approaches.

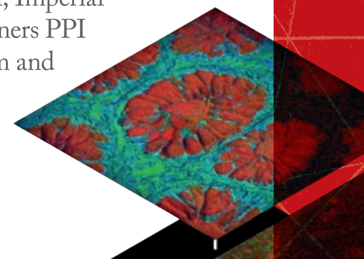
Zoltan Takats
The inventor of multiple analytical methods for direct analysis of biomolecular systems, including the iKnife, Takats is Professor of Analytical Chemistry at Imperial College London. In the Grand Challenge, the Takats group will help deliver multi-modal MSI, and lead REIMS and iKnife studies.

Kevin Brindle
Brindle is Professor of Biomedical Magnetic Resonance at the University of Cambridge and a senior group leader in the CRUK Cambridge Institute. In the Grand Challenge, the Brindle group will be responsible for hyperpolarized 13C imaging in the clinic, collection of tumor material in surgery and production of patient-derived orthotopic tumor xenografts.

Simon Barry
Barry is a Senior Principal Scientist in the IMED Oncology group at AstraZeneca. His research focuses on the cross talk between the tumor and its micro-environment. AstraZeneca's Oncology group will support the Grand Challenge with specialist technical and scientific contributions.

Kelly Gleason
For the last 12 years, Gleason has led a team of research nurses in the field of oncology clinical research. She has also supported the Imperial Patient and Public Involvement (PPI) Group for the Imperial CRUK Centre for the past five years.

Harry C. Hall
After a colorectal cancer diagnosis in 2002, Hall became a founder member and chair of W London Cancer Network Partnership Group. He now sits on the NIHR Imperial BRC PPI Panel, Imperial College & Partners PPI Research Forum and the CRUK Imperial Centre PPI Group.



The Super-Resolution Revolution

A unique feature of the Grand Challenge is the inclusion of a brand new technique – 3D OrbiSIMS. In an interview with Charlotte Barker, Ian Gilmore explains why he decided to combine SIMS with an Orbitrap, and how his dream of super-resolution metabolic imaging is being realized.

I grew up wanting to be a surgeon, a laudable ambition with one fatal flaw – I am extremely squeamish. Luckily, I loved physics just as much as biology. Completing my PhD here at the UK's National Physical Laboratory (NPL) taught me just how important measurement science is – and sparked my fascination with mass spectrometry imaging (MSI). Over the next 20 years, I developed a world class capability in secondary ion mass spectrometry (SIMS). Most of our work was in devices, organic semiconductors, and advanced manufacturing, but I could see huge potential for these techniques in biology.

Five years ago, I established the National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI), with the goal of bringing physical metrology to the life sciences. Now, a large amount of my research is with the pharma industry, trying to better understand how drugs interact with cells, and so reduce drug attrition. The Centre started with just five people, and it's now grown to one of the biggest MSI centers worldwide, with 22 staff and 20 PhD students.

In vino veritas

These days, my ultimate research goal is to achieve "super-resolution metabolic imaging". Super-resolution microscopy has been absolutely transformational for the life sciences; it lets us peer into the machinery of life – the proteins that make up our cells. With MSI, I believe we can do the same with metabolites and drugs.

It's a big challenge. Unlike microscopy, MSI is typically label-free – labels can interfere with drug dynamics, and would be impossible for metabolites, which are constantly changing. There are other problems, too; SIMS allows us to focus down into the super-resolution space (under 250 nm). However, the mass spectra we get are of poor quality, so we cannot always identify the molecules accurately.

One evening in May, 2011 I was enjoying a glass of Pinot Noir, while preparing a presentation for a forthcoming conference. My presentation discussed why the spectra we get from SIMS are so complex and hard to interpret. Largely, it's because the MS instruments we use prioritize speed over accuracy. Speed is crucial to cover the millions of pixels needed for 3D imaging, but to do any serious work in life sciences, we need accuracy too. Unfortunately, the constraints of physics mean that it's not possible to combine speed and accuracy into a single analysis – all MS is a compromise. To illustrate my point, I plotted a graph with speed on one axis, and accuracy on the other. I placed different mass spectrometer designs on this chart, from the super-fast time of flight (TOF) analyzers often used in SIMS, to the much slower but more accurate Fourier-transform mass spectrometers, such as the Orbitrap. As I looked at my chart, I had an idea – why not combine two mass spectrometers, one from each end of the spectrum, and so get the best qualities of both?

At NPL, we take a lot of time and trouble to understand the measurement principles we're working with; it allows us to see the big picture and spot gaps – and opportunities. It was clear to me that a hybrid instrument was the only way to get the qualities we needed, so that's what we set out to create. The result was OrbiSIMS, which combines TOF and Orbitrap mass spectrometers.

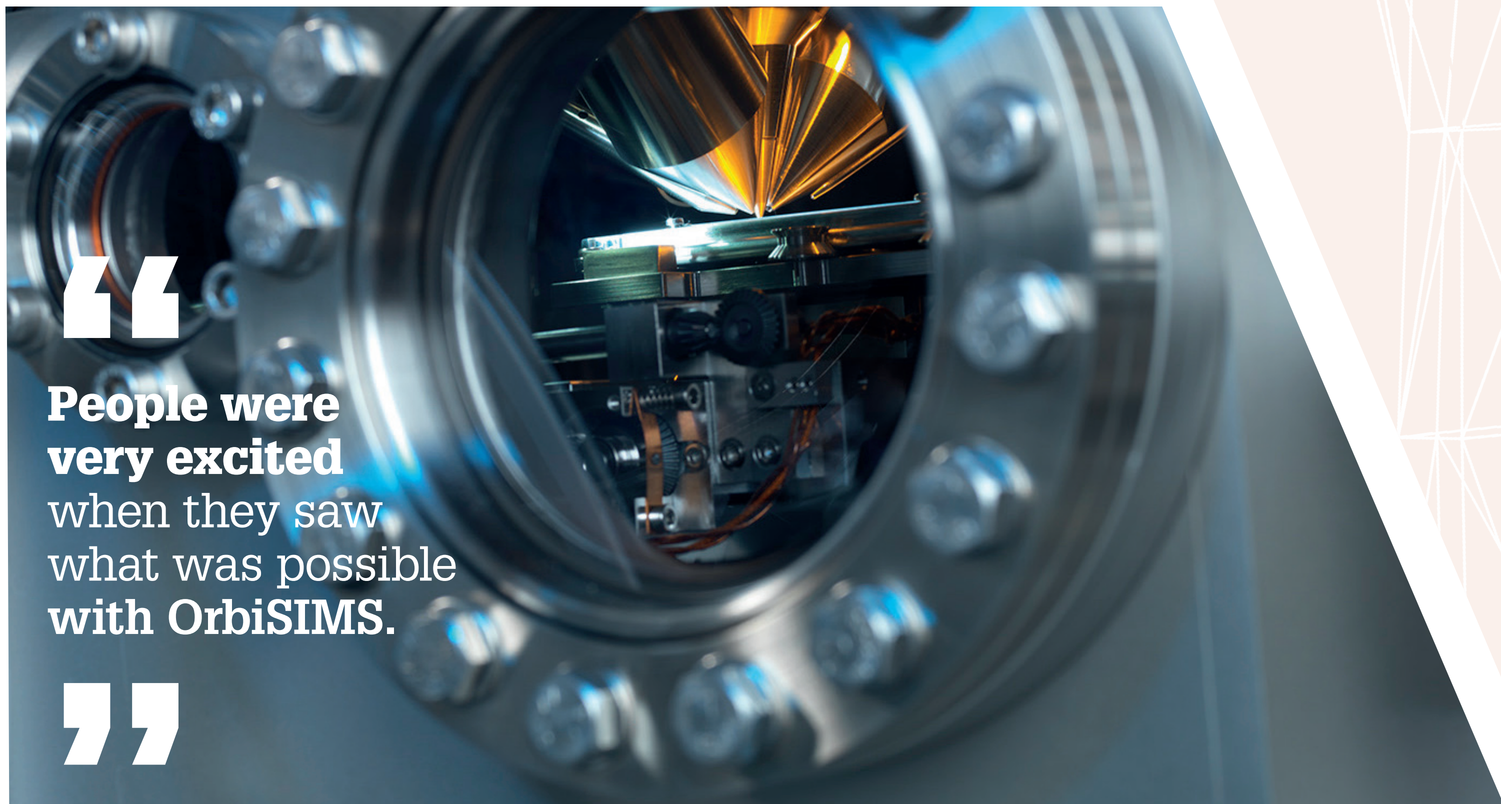
Orbitrap is well known in the life sciences for its high mass accuracy and mass resolving power, allowing us to find the smallest saplings within a forest of peaks. However, it is too slow for the 3D imaging we want to do; TOF-MS provides the speed. The combination of the two confers some important advantages, some of which only became apparent during development. It's a little like a hybrid car – the fusion of a petrol engine and electric power gives a combination of qualities that would otherwise be unachievable, like rapid acceleration and fuel efficiency.

Better together

For readers who are not familiar with SIMS, it involves scanning a focused ion beam over the surface to be analyzed. Each time it hits a pixel on the surface it causes "sputtering", liberating molecules from the top surface of the material – in a cell, the outer cell membrane. The molecules enter the TOF mass spectrometer, giving us a mass spectrum for that one pixel. We repeat the process until we have generated a 2D image. We can then pull up the image for any of the mass peaks in the mass spectrum – for example, we might want to look at a particular lipid, drug molecule or metabolite. To generate a 3D image, we use another ion beam to carefully remove a thin layer of the surface, like a microtome slice. In a normal SIMS instrument, the material removed is discarded, but in OrbiSIMS it is analyzed by the Orbitrap, increasing our sensitivity and specificity. We then repeat the whole process for the newly revealed surface, before removing another layer.

To put it another way – imagine you are digging in your garden. You take a digital photo of the plot you are about to dig, then get your spade and dig out a layer of soil, before taking another picture. If you keep taking photos as each layer is dug out, you eventually build up a basic 3D image of the plot. That's SIMS. With OrbiSIMS, we not only take photos of the layers of soil as they are revealed (using TOF), but also

Top: Josephine Bunch with the Orbitrap and MALDI ion source. Bottom: 3D OrbiSIMS.



analyze the soil that is removed (using Orbitrap technology).

To make the instrument, we brought together two of the leading mass spectrometry companies – Orbitrap-maker Thermo Fisher Scientific and imaging TOF-SIMS specialists IONTOF. GlaxoSmithKline is an essential partner in the project – helping to ensure a successful outcome that will have impact in the pharmaceutical industry to reduce drug attrition and improve our understanding of drug up-take at a single-cell level.

Sharing OrbiSIMS

The first results were presented in a plenary talk at the SIMS XX conference, Seattle, USA in September 2015. People were very excited when they saw what was possible with OrbiSIMS, and we are now working with a number of groups who plan to install an OrbiSIMS in their own labs.

We have taken a great deal of care to make the workflow as simple as possible for people with a biology or life sciences background. Alexander Makarov made a huge contribution to the life sciences when he invented the Orbitrap, and it has become a de facto standard for proteomics and metabolomics studies. People are very comfortable with the technology and its capabilities, so the skills are already there to move into imaging with OrbiSIMS. If you can use an Orbitrap, you are halfway to being able to operate OrbiSIMS. To learn the imaging side of things takes longer, but people with some research experience can usually get up and running fairly quickly.

Another nice feature of this instrument is what we call "cryo-SIMS". If we want to examine cells down to the organelle scale, we need to be able to preserve the ultrastructure of the cells. Researchers in transmission and scanning electron microscopy have done so much beautiful work on preparing samples for a vacuum-based instrument – we have copied their achievements with pride. Our instrument is compatible with Leica sample preparation systems, so anyone with previous experience of TEM and SEM can easily prepare samples for OrbiSIMS.

New insight

We took delivery of the first OrbiSIMS in November 2016. It has already started to give us some unique insights, and we have many more projects planned. The applications for OrbiSIMS are many and varied, but a big focus for us is the development of new drugs. There are three key questions we must answer about any potential drug:

1. Does the drug reach its target?
2. Does it bind with the target?
3. Does it have a pharmacological effect?

Our work with the pharmaceutical industry is all about providing answers that help unsuitable candidate drugs "fail fast" – before the company spends US$1.8 billion bringing it to a medicine. Imaging studies can shed light on fundamental biological processes, and reveal how and why a candidate might fail. For example, we published a paper recently showing the first direct evidence of drug-induced phospholipidosis (excess accumulation of phospholipids in tissues). It is a known side effect of a number of drugs, but the mechanisms (and clinical significance) are not well understood. The drug we studied, amiodarone, was already known to accumulate in the lysosomes, but we were the first to image the subsequent upregulation of lipids to form excess multi-layer lamellas – the clinical sign of phospholipidosis.

Another good example is tracking drug distribution within individual cells. OrbiSIMS can provide not only high-resolution images showing where the drug is, but also data that confirm the identity of the drug and show upregulation of metabolites. With this level of detail at an individual cell level, you can see variation within cell populations. Why do some cells take on more drug than others? Do they all have the same metabolic mechanisms? How do the cells communicate? OrbiSIMS can help us get the answers to these and many more questions. It's great to feel that we have introduced a new capability to life sciences.

Room at the bottom

We're very excited to be part of the Cancer Research UK Grand Challenge project headed by Josephine Bunch. Most of the Grand Challenge partners focus on the multicellular or tissue scale, so we have an opportunity to add something unique by using 2D and 3D SIMS and OrbiSIMS for metabolic imaging at the single cell, cell-cell interaction and subcellular levels. In the words of physicist Richard Feynman, "There is plenty of room at the bottom."

Preserving the structure of cells, and their constantly changing metabolites, is a big challenge, which is why cryo-SIMS is so important. We must instantly freeze cells and keep them at -80 °C until we can analyze them, otherwise metabolites will change. Our Grand Challenge project colleague at Cambridge University, Kevin Brindle, will use liquid nitrogen cooling to freeze biopsy samples immediately and ship them to us, so we can take a snapshot of the metabolites in time. Connecting the in vivo measurements Kevin makes using hyperpolarized MRI with our subcellular analyses will give us a cryogenic snapshot of tumor metabolism.

High-resolution imaging takes time – even with OrbiSIMS. We can't analyze whole tumors or organs in the timeframe available, so we will be guided by what Josephine and others find in their wider-resolution imaging.

It's a very exciting project and the whole team are hugely enthusiastic. For me, it comes back to my childhood desire to become a clinician; it's great to have come full circle and be doing something that could ultimately improve or extend people's lives.

What's next?

We're now in the second phase of the OrbiSIMS project; the instrument is already proving its value, but to achieve super-resolution metabolic imaging we need to significantly increase sensitivity. At the moment, the vast majority of molecules released by sputtering from an ion beam are neutral, so we don't see them in the mass spectra. At the moment only around 1 in 10^5 molecules are ionized, which limits sensitivity. If we could go up to 1 in 10^3, we could increase spatial resolution by a factor of 10 – jumping from 1 μm to 100 nm resolution and putting us well within the super-resolution bracket (under 250 nm).

We are attempting to reach that goal in two ways. First, we recently filed a patent for a novel in situ deposition matrix. In MALDI, a matrix is used to enhance the ion yield, but the matrix contains a solvent that de-localizes the molecules. That's not such a big issue in MALDI since resolution is typically no better than 10 μm, but it's no good for subcellular imaging. So we invented a method that allows us to deposit matrix molecules onto the surface (in situ, while taking our 3D image). It gives us up to a tenfold increase in signal, and could get us to about 300 nm resolution with suitable ion beams.

Second, we will be developing post-ionization methods to give us that final boost in resolution. We already have a portable TOF-MS (Kore Technology, UK) that we call the "baby OrbiSIMS," which will be traveling around a number of different laser facilities, so we can quantitatively measure the fundamental processes of laser post-ionization, and decide which laser system to integrate into the instrument.

While generating all this amazing data, we must make sure we have the tools to manage it. One of my colleagues is developing machine-learning methods, combined with standard informatics tools, to help us identify more of the peaks in our spectra. In the past, we have often had to guess the identity of peaks from SIMS, but with the additional data coming from the Orbitrap, we can use the wonderful techniques developed by the informatics community to give us a definite ID.

What we can do with OrbiSIMS already is amazing and we're going to keep pushing to break through the 250 nm barrier. It's a 10-year goal but if we can achieve that, I think we will be able to bring those same levels of transformation that we saw with the advent of super resolution microscopy.

THE NEW FACE(S) OF FORENSIC SCIENCE

Forensic science is glamourized onscreen,but often misrepresented. Meet the real stars of the field: the people delving into DNA profiles, trialing new technologies – and communicating an altruistic passion for the power of forensic science.

The Science and Nothing But the Science

With Craig O'Connor

In recent years, forensics has come under increasing scrutiny – and rightly so; the goal is to get evidence into a court of law, which can ultimately affect whether someone goes to jail. It goes without saying that we want to make sure we are putting the best science out there. Over the years, the field has all too often been overly influenced by the law (it might be easier to get it into court if we don't do X or do Y instead). But as changes in technology give us the ability to do more with less, we have an opportunity to put the science firmly back into forensic science – by which I mean, making data-driven decisions without any undue secondary influences.

Meeting the challenge

I work as a criminalist at the Office of the Chief Medical Examiner in New York City. We cover all five boroughs of NYC – eight million people. There's a lot of crime, and therefore a great need for forensic scientists in the crime lab. We process upwards of 12,000 cases a year (most crime labs process far fewer than that). We see new pieces of evidence daily, from samples of firearms to other weapons, even half-eaten food. Anything you can think of, we've probably had to deal with.

We are also one of the very few laboratories in the country fortunate enough to have a research and validation group within our lab. Here, our main goal is to validate new techniques to see if they're fit for use, and then apply them to casework. We also look at 'up-and-coming' research and techniques, to see if they could work in a forensic setting.

My day-to-day is pretty varied. I could be examining crime scene evidence, looking for blood, semen, saliva and skin cells, taking samples, doing preliminary or screening tests for bodily fluids, and conducting DNA analysis.

DNA and PCR

Back at college, basic DNA extraction and quantitation was one of the simplest analyses we did. Over the last 15 or so years, however, many things have changed. In the 1990s, the main challenge was to get enough DNA from a sample to be able to compare it to an individual, so the focus was on body fluids (blood, semen, or saliva), and most techniques used nanograms or micrograms of DNA – in our world, that's a lot of DNA. As the years went by, the ability to extract DNA improved, and we began working with lower and lower amounts of DNA. Fast-forward to 2010, and many labs started assessing what we call "touched items" – looking at skin cells rather than bodily fluid deposits. You get much fewer cells and, therefore, a lot less DNA – in the picogram range. But the challenge is not only being able to detect small amounts of DNA; it must be analyzed and interpreted. We can detect DNA on a shirt or the handle of a knife, but there's no test that's going to tell us how it got there. One can only postulate. And we also can't tell how long DNA has been on an item.

Science meets law

Forensics covers a wide range of different techniques from fingerprint analysis to shoeprint analysis to bitemarks and DNA analysis. There's a misconception that forensic science is poorly regulated. But at least when it comes to DNA, we are highly regulated, through both accreditation and national standards. New techniques have to go through rigorous testing, to check if they are fit for use; we have to go through validation, following quality assurance standards put out by the FBI; we have to get approval from our state commissions as well as intra-agency commissions – all before we start using it for casework.

As an example: massive parallel sequencing, which has been used in the biomedical field for over a decade, is only now making its way into forensics, because of the hurdles we need to clear to get it admitted into court. And naturally, we have to show that whatever technique we use gives the correct answer each and every time.

It's also part of our job to testify, though not all cases make it to court. I've testified over 60 times, and although it does get easier, each case is different – as is every piece of evidence, each result and, of course, each attorney you're dealing with. Our justice system is very adversarial by nature. But I enjoy it, and it's the part of the job that most analysts like; it gets you out of the lab, first of all, and second, you get to see the criminal justice system in action. In the end, the meeting of science and the law is just another challenge that comes with the territory.

Between scene and screen

Within the medical examiner's office, we handle many technologies – forensic biology is just one portion. We have a toxicology department, medicolegal investigators that are "on scene" every time there's a death, a molecular genetics department that deals with new and emerging technologies for looking at sudden death syndrome, and one very few labs working on body fluid identification. Within forensic biology, the newest technology is advanced statistical analysis – what's called probabilistic genotyping.

It's a varied and exciting role, without a doubt. But on a day-to-day or case-to-case basis, we must only focus on the science. Do our positive or negative controls pass? Is the test fit for purpose and likely to give the right result? In a broader sense, knowing that a result can somehow lead to justice is really rewarding. We're working for the people of New York City – the victims, the suspects, and the criminal justice system as a whole.

Craig O'Connor is Criminalist IV at the Office of the Chief Medical Examiner, New York, USA.

One Piece of the Puzzle

With Kacey Cliburn

On August 1, 2002, I started my career in forensic science with a Forensic Chemist position at the Oklahoma Office of the Chief Medical Examiner. During my time there, I completed my Masters in Forensic Science before going on to work for the Oklahoma State Bureau of Investigation. I am now a Research Toxicologist at the Federal Aviation Administration (FAA).

I work in the Bioaeronautical Sciences Research Lab as part of the FAA's Civil Aerospace Medical Institute (CAMI) in Oklahoma City. It is the only forensic toxicology lab for the FAA that performs analysis for aviation accidents in the US. We provide toxicology results for accidents to both FAA investigators and National Transportation Safety Board (NTSB) investigators. These results are part of the investigation and data collection that could affect regulations that make air travel safer. The NTSB is charged with determining the probable cause of transportation accidents; thus, the toxicology reports are helpful in identifying substances that may have played a role in the accident. The FAA's mission is to "provide the safest, most efficient aerospace system in the world", and by assisting with the development of regulations and policy, the Office of Aviation Safety helps ensure that's the case.

As part of CAMI, the lab also supports the mission of the FAA by conducting research on, for example, the incidence of drugs found in post-mortem specimens, and by developing new and innovative ways to perform toxicological analyses. Research programs at CAMI are designed to stay up-to-date with human safety risk issues and to promote collaborative scientific discovery within aerospace medical research.

We employ a range of analytical extraction methods: liquid-liquid extraction, solid phase extraction, immunoassay, and headspace analysis. And we typically couple these extractions with gas chromatography-mass spectrometry (GC-MS)

and liquid chromatography-mass spectrometry (LC-MS). Our aim is to detect a wide variety of substances, including controlled substances (methamphetamine, cocaine, and tetrahydrocannabinol), prescription medications, over-the-counter medications, and ethanol, that were in someone's body at the time of a plane crash.

No analytical field is without its challenges, and many faced by our lab are similar to other post-mortem forensic toxicology labs. We may receive highly putrefied samples that make extraction techniques difficult or, because of the violent nature of the accident, we may only receive tiny biological specimens on which to perform analyses. However, there are unique aspects to aviation cases; our job is to detect and quantify levels of drugs that are generally in the therapeutic range – whereas a Medical Examiner's Office toxicology lab often detects and quantifies high levels of drugs in potential overdose cases. To accomplish this low detection requirement, our lab must use the most sensitive chemical techniques and instrumentation possible. And, of course, it's imperative that we regularly research new methods to enhance our analytical capabilities. I have already seen a shift in the analytical instruments used; 15 years ago, almost all of our methods were based on GC-MS, but now more methods are being developed for LC-MS.

In the last decade, the forensic toxicology community has had to react to the introduction and surge in usage of synthetic cannabinoids and novel psychoactive substances. Because these substances are new and ever-changing, forensic laboratories have to continually develop and validate methods that can detect them. I would like to see more of these methods developed – and more case reports published – so that forensic toxicologists can understand the pharmacology and toxicology of these substances.

Forensic toxicology is one piece to the forensic puzzle – and may sometimes be the key. At the FAA, forensic toxicology may help investigators in determining if any drug or substance played a role in the cause of the accident; at the Medical Examiner's Office, forensic toxicology may provide the answer as to the cause of death – which might help a grieving family understand what happened to their loved one. That's important to me.

My dad always told me to "find a passion, and not a profession" and after starting my career in forensic toxicology, I understood what he meant. People within the forensics field are diligent, detail-oriented, and good problem solvers. If I ever have a problem or need help with an issue in the lab, I can email people in other parts of the country and someone will offer a suggestion or idea that will help me. The nature of the cases that we handle means that this job is not for everyone, but, from the moment I started, I knew I would be in this field for the rest of my career.

Kacey Cliburn is Research Toxicologist at the Federal Aviation Administration, Oklahoma, USA.

Fly in the Face of Evidence

Analysis of insect eggs on corpses at different stages of development can provide a time window for forensic experts – but it can be difficult to distinguish similar species without using expensive and often time-consuming techniques such as DNA profiling. Now, an organic chemist and forensic entomologist have teamed up to develop a quicker and cheaper method for analyzing the eggs of different blow fly species. Jennifer Rosati, Professor of Forensic Entomology in the Department of Sciences at John Jay College of Criminal Justice, New York, USA, tells us more.

How did your research begin?

Rabi Musah (Associate Professor, University of Albany, New York, USA) and I met at a forensic symposium – she presented her work on using DART-MS for the identification of psychotropic compounds in plant material, while I presented my work on understanding blow fly behavior and its importance in post-mortem interval (PMI) estimations. She approached me to offer her chemical expertise to my study system and suggested that DART could also be useful in forensic entomology. From there we began to forge our relationship and are in the process of incorporating the use of DART-MS in many aspects of forensic entomology.

Could you tell us a little more about your method?

Freshly laid eggs were collected from multiple necrophagous fly species, including representatives from the blow fly family (Calliphoridae), specifically Calliphora vicina, Lucilia sericata, L. coeruleiviridis, and Phormia regina species as well as the Phoridae and Sarcophagidae families. We analyzed the eggs by DART-HRMS, determining that species-specific differences are correlated to the amino acid profiles of the insects. The presence of these free amino acids in the egg samples was also confirmed through the use of MALDI-SpiralTOF-HRMS, as well as thin-layer chromatography.

What impact will this discovery have on forensics?

Current practices in the field of forensic entomology involve many hours devoted to insect rearing and species identification, which can be difficult, particularly during the immature stages of development. In fact, very few identifications are carried out on egg or larval samples. This technique could offer quick and rapid identification for all life stages, as well as verification for adult identifications. Our published findings are really just the tip of the iceberg when it comes to using DART-MS in the field of forensic entomology. This technique could easily be utilized in many other forensic fields, from forensic toxicology to fingerprint analysis. Our next step is validating this technique for other forensically relevant insect species and also looking at its use in entomotoxicology.

Has this technique been used in a real life setting?

I recently took on a case where a large egg mass was collected and preserved from human remains, which is typically unusable evidence. Though I have already reared the larvae that were also collected from the remains, I plan to use this technique to verify my adult identifications and to also determine the species composition of the egg mass. A forensic entomologist is frequently questioned on the stand regarding their ability to carry out species identification correctly. By utilizing this technique, I'll provide independent validation of my species identification – which will remove any subjectivity in my analysis and allow me to reliably incorporate the proper developmental data into my colonization estimate. To be able to employ this research technique immediately into an applied forensic setting is very exciting.

Reference

1. *JE Giffen et al., "Species identification of necrophagous insect eggs based on amino acid profile differences revealed by direct analysis in real time-high resolution mass spectrometry", Anal Chem, (2017). Available at: http://bit.ly/2uMuvhz. Accessed July 11, 2017.*

Battling the Backlog: Part I

With Sarah Lum

I started my career in capillary zone electrophoresis (CZE) instrument development with laser-induced fluorescence detection, for environmental applications. While attending the Microscale Separations and Bioanalysis Conference in Canada in 2016 to present my research, I went to a forensics session and was shocked to hear about the major backlog in sexual assault cases in the US. As it stands, there's tens of thousands of rape kits awaiting analysis in the US – that's tens of thousands of victims whose lives could be on hold while they await justice. The injustice of it deeply affected me. Afterwards, I asked the presenter if anyone had attempted the separation using capillary electrophoresis. He said, "No! Why don't you give it a try?" So I started working on it in my spare time.

Scientifically, this is a separations issue; the main challenge in the analysis of sexual assault kits is the separation of sperm cells (containing male DNA), from the epithelial cells in the sample. The epithelial cells grossly outnumber the sperm cells in most samples, which makes it difficult to get a clean DNA profile of the perpetrator. The current method of differential extraction is very inefficient. The process uses a series of solutions and detergents to wash the sample off the swab, targeting the fragile epithelial cells first and the hardier sperm cells last. However, each of these fractions contain DNA from both perpetrator and victim and produce mixed profiles that are often difficult to interpret. Furthermore, the process requires a lot of analyst interaction with the sample, which increases the risk of sample contamination or loss.

CE is already used in every crime lab for DNA analysis, and is known to produce efficient separations of DNA and small molecules. In my previous work, I pushed the upper limits of CZE by separating mixtures of bacteria for environmental applications. But could CZE be used to separate mixtures containing epithelial cells, which were over 40 times the size of the E. Coli I was previously working with? The scientists I spoke with at the conference were concerned that the epithelial cells would clog the capillary. In response, I spent a few months working on sample preparation – testing different buffers to remove the sample from the swab, and manipulating CZE injection and separation parameters to overcome this challenge. Then, I interfaced the CZE separation with an automated fraction collector developed in my lab. I could then inject mock sexual assault samples into the CZE system, separate intact sperm cells from epithelial cells and lysed cellular debris, and collect purified fractions.

With this technology, we can get very specific separation in under 15 minutes, and I'm continually striving to achieve an even faster separation with equal efficiency. This is a more effective alternative to the current method of differential extraction, which requires samples to incubate for a few hours and often overnight. Furthermore, there is very little human interaction with the sample since there are no wash steps. I've been using a visual analysis method to quantitatively determine my yield and evaluate the separation, but I would like to switch to something more in-depth such as real-time PCR coupled with fluorescence detection in the near future.

The University of Notre Dame's Tech Transfer Office will be looking to commercialize the technology. My job is to improve the instrument and to continue running experiments to show that it not only can work on fresh samples, but it can also handle the backlog. I'm currently doing a time study to ensure system effectiveness with three-month-old mock sexual assault swabs – I'd like to go back up to a year and test different storage conditions (temperature and humidity) since many counties do not have ideal storage facilities. I aim to demonstrate that

speed, simplicity and sensitivity make this method worthwhile for every lab.

The University of Notre Dame does not have a forensics program, so everything I've done has been very reliant on collaboration and communication with other research institutions. The forensics community have been very supportive. We're all passionate about finding ways we can help people – that's what we're in this job for. It's not about fame, making money, or beating your competition – it's about working together to solve society's problems.

I'm very hopeful about the future. There are a lot of people passionate about making progress in forensic science, bringing justice to our communities and lowering crime rates – I want to be part of that.

Sarah Lum is Bioanalytical Chemistry PhD student and Graduate Research Assistant at the University of Notre Dame, Indianapolis, USA.

Battling the Backlog: Part II

With Charlie Clark

I became enamored with acoustic differential extraction (ADE) at graduate school at the University of Virginia. I joined the Landers Research Lab in 2014, and I have since been working on the development of a microfluidic technology (SONIC) that uses acoustic force to separate sperm cells from epithelial cells in sexual assault samples.

Small-scale chemistry

The SONIC system originates from a collaboration that started with Prof Thomas Laurell at Lund Univ and incorporates ADE on a microfluidic device – essentially using sound waves to apply pressure and separate particles. The acoustic trapping principle is the application of a standing sound wave through a microfluidic channel filled with liquid. Those sound waves create low-pressure nodes where they intersect, and high-pressure anti-nodes where the sound waves are out of phase. If you flow particles through that acoustic trapping site, they'll follow the path of least resistance into the low-pressure nodes. And if you tune the frequency of the sound waves properly, you can actually trap and hold particles of a certain size, while everything else flows around it.

Different cell types in the human body vary drastically in terms of size, shape and function. Sperm cells are very well conserved across humans – they're all around 6.0 micrometers in size (at the head) and ~50 micrometers long (head-to-tail), , with roughly the same shape and features. That means we can tune our trapping site very precisely to sperm cells. Once we've flowed our sample through and are holding those sperm cells in place, we have multiple downstream avenues that go to different chambers; we can let all of our sample waste go to one, then switch the flow and release sperm cells into another, thereby purifying those cells that we want to capture.

The conventional method used to separate sperm cells from other cells (primarily epithelial cells) is simple differential extraction. You spin your sample containing multiple cell types at 18,500 x g for 10 or 12 minutes, and the sperm cells will pellet out to the bottom. The analyst removes the supernatant, re-suspends it, and repeats this spin and wash step until they get a purified sperm fraction. It still surprises me that conventional analysis is so manual and thus, how variable this can make the process in handling these types of samples.

In essence, what we're trying to do in the Landers Lab is automate that separation process – taking it out of the hands of the user to make it more uniform. With our methods, you simply load your sample; the metering, fluidic control, trapping, and manipulation are all handled by the instrument – and you are presented with a small vial of purified sperm cells from your sample.

Baby steps

The response to SONIC from the community has generally been positive, although people don't always appreciate the steps that need to be taken in a project like this. When I describe it to other forensic or analytical scientists, they often jump straight to posing convoluted scenarios: "What if you get a sample that has cells from five different people, with four different suspected attackers?" I have to explain that we're not addressing that yet; it takes baby steps to get to that point. What we're doing might not change the types of samples you can look at, but it could open the door to more reproducible male capture – and, in this field in particular, that's crucial.

One of our biggest challenges – and this was unexpected – has been getting reliable information from the rest of the forensic community. We don't have access to real casework. It was really hard, for example, to find out the ratio of female to male cells in a typical sample – we were given numbers that ranged from 1:1 to 600:1.

Probing on Palm Beach

An exciting new development for me was going down to work with Palm Beach County Sheriff's Office (PBSO) in Florida, to observe some of their forensic techniques, train them on using the instrument that we developed, and then compare different extraction methods.

They handed me a list of adjudicated samples – tank tops, sheets, condoms, cheek swabs – all kinds of samples and substrates and cell types that I wasn't ready for. It was much more of a challenge than I thought, but a great opportunity to try the instrument with real samples. One gratifying moment was when they presented us with an adjudicated sample – a cutting from a sheet that had been stored since 2009. We pulled it off the shelf, resuspended it, and were able to separate sperm cells from that sample using the instrument. From our sample, we were able to generate a DNA profile that matched the reference profile that they obtained via their own method eight years earlier. Perhaps not the most challenging sample, but a great moment for us nonetheless.

The trip was really eye-opening. It struck me how unique every lab is; there are different national and state guidelines on how you handle samples, and how you handle these types of investigations. PBSO is a very well-funded state lab, so they have the best instrumentation. It seems like other labs who have obtained less funding may not be able to handle as many samples or hire as many analysts – which means that having new technology that expedites analysis is even more important.

Translating forensics

I'd really had no exposure to forensics before working with this group, but what really hooked me was how easy it is to convey the importance of what I'm working on. Everyone I talk to agrees that it's important to help address the backlog of samples in solving these crimes by speeding up the analysis process. Forensics is in some ways more visible than other areas of analytical science.

Does our technique have scope beyond forensics? We believe so. A recently graduated student from our lab has applied this acoustic isolation technique to the separation of cancer cells. Circulating tumor cells appear in very low numbers in the bloodstream; if you can focus on the differences of those cells – be it in size, shape or compressibility – and separate them using our acoustic technique, then you have the potential to tailor the treatment to the type of cancer the patient has. It's the same principle, but a whole other set of parameters and instrumentation being applied to a new field.

Charlie Clark is a PhD candidate at the Landers Group, Department of Chemistry, University of Virginia, USA.

MARKES international

Since 1997 20 YEARS ANNIVERSARY

World leaders in **thermal desorption** for environmental air monitoring

Tailor your TD system to your requirements – canisters, on-line and sorbent tubes

- Maximise efficiency by running VVOC to SVOC on one platform
- Sample splitting and re-collection for easier validation and method compliance
- Reduce your costs – avoiding use of liquid cryogen

Find out more
http://chem.markes.com/TD

A company of the SCHAUENBURG International Group

Upping the (Analytical) Ante

We catch up with three speakers from the 41st International Symposium on Capillary Chromatography and 14th GC×GC Symposium in Fort Worth (fondly known as "Riva in Texas") to find out what got people talking – and discover that analytical science still has more than a few aces up its sleeve.

Consuming Passion

Vincent Remcho shares personal highlights of the event – and his new consumables concept.

What's the latest from your lab?

We're producing novel high-throughput screening consumables that leverage existing laboratory tools. At the symposium, I spoke about our recently developed disposable microfluidic microtiter plates. We can add reagents to certain wells and interconnect them; those reagents can then be dried so that you have a consumable that can be used in any plate reader, whether UV-vis or fluorescence, depending on the assay. It's a way of embedding separations and sensors together into a microfluidic platform that fits into existing plate readers, so that a broader cross-section of end users can access the technology.

The potential impact of the work is high and the University has been quick to protect the IP, so we have only recently been able to share information on it. Primarily, we have focused on two fields of application for the technology. One is medical diagnostics – the sensing of multiple biomarkers/target analytes for disease diagnostics. The second is the detection of heavy metals and other toxins in the environment.

What were the key trends at ISCC?

There was a resurgence of interest in ion separations/analysis, partly as a result of environmental concerns in the USA. In 2015, a reservoir of contaminated water from an old mine was released into the Animas River in the western United States (the Gold King Mine waste water spill). The spill included toxic lead and cadmium, bringing public attention to the importance of metal analysis of water. The renewed interest was reflected in a number of talks on ion separations at ISCC – everything from capillary electrolytic eluent generation for glycan separation to trace analysis of ions by matrix elimination. A particular highlight was the Giorgia Nota Award lecture from Sandy Dasgupta at University of Texas at Arlington on ion chromatography: "Open Tubular Ion Chromatography. Two Decades of Pursuit: Quo Vadis Domine?" He led with a tribute to Giorgia Nota, who sadly died within a year of retiring, and had some wise words on appreciating and enjoying our colleagues while we have them, both professionally and personally.

Multidimensional separations were of course a strong theme, including an impressive session on chemometrics for GC×GC, with standout lectures on comprehensive chemical fingerprinting for wine analysis by Stephen Reichenbach from the University of Nebraska, and exploring the capabilities of post-column chromatography with FID by Andrew Jones at Activated Research Company (ARC). The latter described a relatively new product, the Polyarc system, which uses an inorganic catalyst to reduce organic molecules to methane and so allows almost universal detection of organic molecules with FID, while a consistent response factor between analytes makes calibration far easier (for more on Polyarc, see tas.txp.to/0617/POLYARC).

Unsurprisingly, proteomics and biomarkers continue to be hot topics, with great talks on capillary zone electrophoresis as a tool for ultrasensitive bottom-up proteomics (Norman Dovichi, University of Notre Dame), tracking chronic lung disease progression through volatile biomarkers (Heather Bean, Arizona State University), the detection of Mycobacterium bovus in lung infection, and rapid diagnosis of invasive aspergillosis.

On the GC side, novel sorbents were much discussed. There was still some talk of monoliths, but attention seems to be turning more to ionic liquids, as covered by Len Sidisky of MilliporeSigma.

What challenges face the field?

One of the big challenges for the field right now is one that faces all areas of scientific endeavor: the lack of interest on the part of governments to invest in research. It's a disconcerting trend but it was good to see it being addressed in such a clear and scientific way at ISCC – with genuine concern and honest evaluation. In my opinion, a piece of the solution lies in better informing the public of the value we add as measurement scientists. One of the beautiful aspects of analytical science is that it is such a practical field – questions about the environment and health are of concern to most people – and analytical scientists answer those questions. It puts us in a wonderful position to communicate the value that our research adds and how it positively impacts on the public.

How will things change by Riva 2027?

I certainly expect to see a continuing trend towards miniaturization and low-cost analytical devices. Mike Ramsey (UNC Chapel Hill) opened a session on microanalysis by talking about his work on microfabricated GC-HPMS, while Adam Woolley (BYU) is using microfluidic devices to analyze preterm birth biomarkers, and his group has continued to make really good progress.

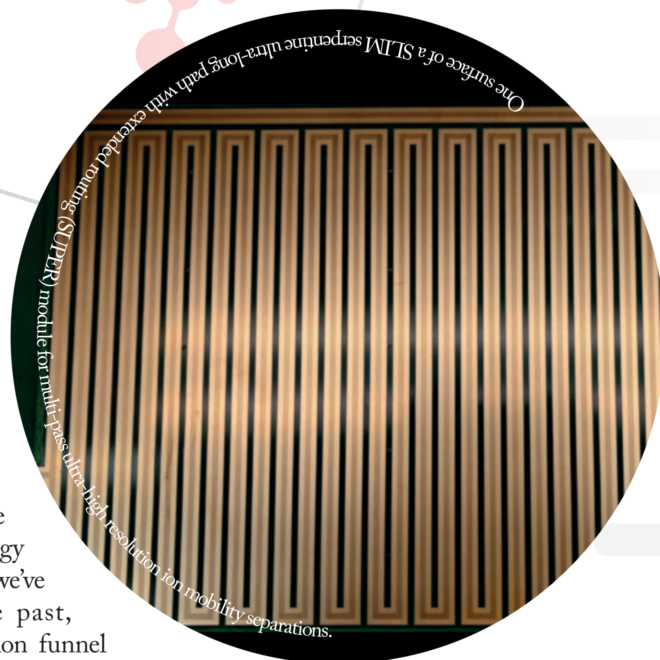
We can expect to see continued integration of chromatography and mass spectrometry. A plenary presentation by Richard Zare (Stanford) on drop-by-drop analysis using mass spectrometry covered not only the work of his own lab, but that of labs around the world.

I also had a great conversation with Kevin Thurbide from the University of Calgary about the revival of an interesting topic – supercritical fluid chromatography (SFC). SFC has faded from attention (though not from importance) in recent years and Kevin spoke about a pH-tunable water stationary phase for SFC and GC, which could be a real advance.

Vincent Remcho is Professor and Patricia Valian Reser Faculty Scholar at Oregon State University Department of Chemistry.

Upward Mobility

Richard D. Smith's new approach to IMS-MS is making waves. We caught up with him after his ISCC plenary.



One surface of a SLIM serpentine ultra-long path with extended routing (SUPER) module for multi-pass ultra-high resolution ion mobility separations.

What's the latest from your lab?

We're exploring better, faster, more effective ways to characterize a wide range of biological systems, including those affecting human health and the environment. I've had a longstanding interest in combining different separation techniques with mass spectrometry, including LC, SCF, capillary electrophoresis and capillary LC. Right now, my group is continuing that interest by combining MS with very high-resolution ion mobility spectrometry (IMS).

IMS has a great deal of potential for analytical science, but lack of resolution has limited its use. My lecture at ISCC focused on a new approach for IMS-MS based upon what we call Structures for Lossless Ion Manipulations (SLIM) – a new form of ion optics. SLIM are constructed from electric fields generated by arrays of electrodes on evenly spaced planar surfaces, to which various RF and DC electric potentials can be applied, and used to enable a broad range of ion manipulations. We exploit the robustness and ruggedness of mature technology developed to support electronics, but instead of moving electrons around a circuit, we're using electric fields to manipulate ions in the gas phase.

The lossless ion transmission provided by SLIM provides the basis for exceptionally high sensitivity and we use this along with the ability to create very long path ion mobility separations – long, serpentine paths that allow us to achieve very high resolution. The combination of high resolution, sensitivity and speed are very attractive for many measurements. We have been able to separate a lot of previously indistinguishable isomers; for example, peptides modified with a phosphate group at different sites. We are also developing an application to look at peptides that contain a D rather than an L amino acid – diastereomers or epimers. These molecules are biologically interesting, but hard to resolve with standard techniques. Another potential application is to separate peptide isomers containing leucine versus iso-leucine amino acid residues, which are almost always indistinguishable by mass spectrometry; when we can separate them, we can characterize them effectively. Essentially, we're addressing blind spots in biological separations. The enhanced resolution with SLIM means we can pull apart things that have almost identical mass spectra and that are difficult or impossible to separate by LC. The separations are extremely fast, typically under a second, and the reproducibility that we get using ion mobility is rock-stable. All we need to do is control temperature and pressure very precisely to achieve very high reproducibility. It's an important development for many practical applications.

I would say it's a significant departure from the way things have been done. But it has its roots in some of the technology development we've done in the past, such as the ion funnel for aiding sensitivity in MS measurements.

What caught your eye at ISCC 2017?

The work Dan Armstrong has been doing on D and L amino acids in various biological systems is fantastic, and at ISCC he reported intriguing work in mouse brains and other tissues. I share his belief that these compounds and other related epimers are highly biologically significant, but the roles are generally poorly understood at present. That's a fun area to watch. (For more on chiral amino acids, see tas.txp.to/0617/CHIRAL).

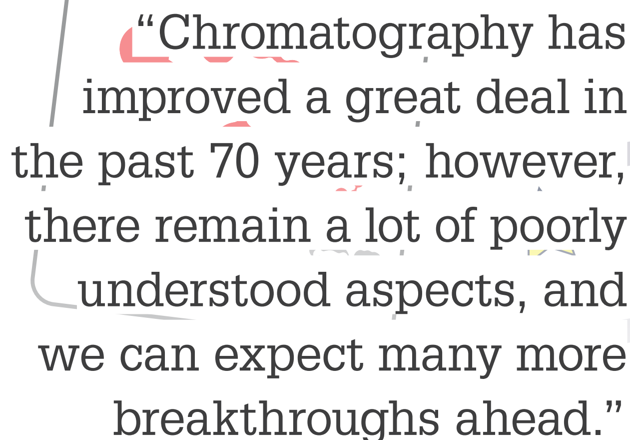
What's next for your work – and the field?

There continue to be fantastic developments in, and a need for improved analysis of, biological samples. People are working with smaller and smaller samples – and are already talking about the single-cell level. Genomics is great but it doesn't tell us much of what is going on in biological systems. Proteomics and metabolomics measurements are still expensive and slow, with many blind spots. Over time, proteomics and metabolomics will take on some of the speed of genomic approaches, and so will have a greater impact.

I truly believe that the work we are doing with SLIM is going to be disruptive in mass spectrometry-based measurements. In some cases, SLIM may displace LC ahead of MS; in others, SLIM could be inserted between the LC and MS steps.

At ISCC, I concentrated on using SLIM for ion mobility separations but we really see it as a much broader platform – we can not only separate but also store ions for extended periods, and carry out all kinds of reactions. The greatest opportunities are in what I like to call a "gas phase ion chemistry workbench," where we can separate, store, react and manipulate ions – providing the basis to do things we could not even imagine in the past.

Richard D. Smith is Battelle Fellow at the Pacific Northwest National Laboratory.



> "Chromatography has improved a great deal in the past 70 years; however, there remain a lot of poorly understood aspects, and we can expect many more breakthroughs ahead."

Multidimensional Character

With 40 years in analytical chemistry, Carlo Bicchi is perfectly placed to reflect on the past, present and future of separation science.

What is the goal of your lab?

Our lab mainly works with volatile fractions of plant matrices of interest to the food, cosmetic and pharmaceuticals fields. This includes "sensomics" – the science of flavor and fragrance, including chemistry and sensory perception. We have two main lines of research: one dedicated to aroma and food, and one to natural products. The main technique we use is GC, and the core goal of our lab is to develop GC×GC, GC-MS and sample preparation methods to advance the study of natural products, and aroma and fragrance.

What were the key trends at this year's symposia?

In GC×GC, the most important advances discussed included a better understanding of how the modulator works, exploring the possibilities of the second dimension, and improving data elaboration.

As I explained in my talk – "Comprehensive 2D-GC in the flavor and fragrance fields: simply an additional tool or a backbone of new strategies?" – new technologies fall into two categories. Some give you better or faster results, but don't fundamentally change your strategy (tools). However, others give you an added value – results that you were unable to obtain with conventional techniques (backbone). In my talk, I argued that GC×GC is a backbone technique, since it allows better separations of biologically active volatiles that occur in very small amounts but may have powerful effects. This is a real step-change.

When it comes to bioanalysis, LC has also seen major advances in combination with mass spectrometry. It's unbelievable how far mass spectrometry has evolved over the past 10 years or so, and how much extra information can now be obtained; for example, when studying complex mixtures.

The importance of sample preparation is often underestimated, even though it has always been – and remains – the bottleneck of modern technologies. The evolution of sample prep needs to be accelerated and here, automation is playing an important part. Automation has progressed rapidly, and there are exciting possibilities ahead in robotic technologies and miniaturization.

How will things change by Riva 2027?

Many people say that LC and GC are now mature techniques. I disagree. Chromatography has improved a great deal in the past 70 years; however, there remain a lot of poorly understood aspects, and we can expect many more breakthroughs ahead.

Miniaturization will also be important. We now have in our hands the technologies, ideas and tools to develop smaller instruments, such as portable GC. A column with 40,000 theoretical plates can be obtained with a 1.5cm^2 chip, and a full GC can be contained within the space of a credit card. When I started in the field, to achieve just 3,000 plates was a tremendous feat, requiring a huge instrument.

What challenges face the field?

I believe in separation before detection – LC or GC before MS. Of course, you can do a lot of analysis using MS alone, but separation is still a fundamental step for complex mixtures. In my opinion, more attention must be given to ensuring that tomorrow's analytical scientists have a full grasp of separation techniques, rather than being over-reliant on MS (read Ian Wilson's article on "Managing MS Mania" on page 20).

Though I believe increasing computing power and more sophisticated data elaboration techniques are important, there is a risk that the computer can end up driving you. A computer will always give you a number, but that number must be translated into a result – and that requires training.

Carlo Bicchi is Full Professor of Pharmaceutical Biology at the Faculty of Pharmacy of University of Turin.

Tandem Triumph

Getting a 2016 Analytical Scientist Innovation Award (TASIA) was a crowning achievement for the team behind Markes International's Tandem Ionisation technology – and also the fruit of many years' hard work. Here's the story behind the solution.

By Alun Cole

The problem

Historically, the use of soft ionization for gas chromatography-mass spectrometry (GC-MS) has been limited by time-consuming hardware changes and optimization, as well as the additional expertise required for interpretation of results. These drawbacks led to its use as a 'last resort' rather than in routine workflows.

We wanted to know: could we gain the benefits of soft ionization without the hassle?

Background

Our lab chemists, like pretty much everyone running GC-MS methods, have for a long time depended upon electron ionization (EI) at 70 eV to generate the vast majority of their mass spectra.

But that doesn't mean that lower ionization energies don't have a place in the analytical chemist's toolbox – in fact, so-called "soft ionization" can be really useful. Lower energies reduce the amount of ion fragmentation, which means you get bigger ion fragments at the detector, and so better information on the identity of the target molecule.

So why don't GC-MS analysts routinely use soft ionization? A key factor is the inconvenience of the most common approach – chemical ionization (CI). CI uses a different ion source configuration from EI, and it needs additional pressurization and reagent gases. As a result, switching between EI and CI is impractical for most people, relegating CI to 'last resort' status.

The story that ultimately led to the release of Tandem Ionisation in 2016 began almost a decade earlier, when, shortly after establishing Markes International, my co-founder Elizabeth Woolfenden and I became aware of the activities at Five Technologies. A start-up company based in Munich, Five Technologies were working on GC detection techniques – in particular, time-of-flight (TOF) mass spectrometry in high-sensitivity sensors.

Although the core of Markes was (and remains) thermal desorption-gas chromatography (TD-MS), the majority of TD applications use MS as a detection technique; so when, in 2001, Five Technologies developed a design for a TOF mass spectrometer with a new ion source (Figure 1) that offered improved sensitivity while maintaining mass resolution, we were naturally excited. As a result, we started a partnership with them in 2004, whereby we funded research on the application of their TOF technology to GC, and in return we acquired the rights to develop, manufacture and sell the resulting products.

As part of this venture we established a company, ALMSCO, through which development activities were funded. ALMSCO is led by two talented scientists – Pierre Schanen and Gerhard Horner – who are essentially independent researchers, and so less likely to fall into a common trap: "we'll do it this way because we've always done it this way."

Figure 1. The ion source of the BenchTOF instrument, incorporating technology that ultimately allowed Tandem Ionisation.

> *"Once the concept had been proven on paper, it was remarkably easy to turn into reality – about eight months from the basic idea to a working demonstration!"*

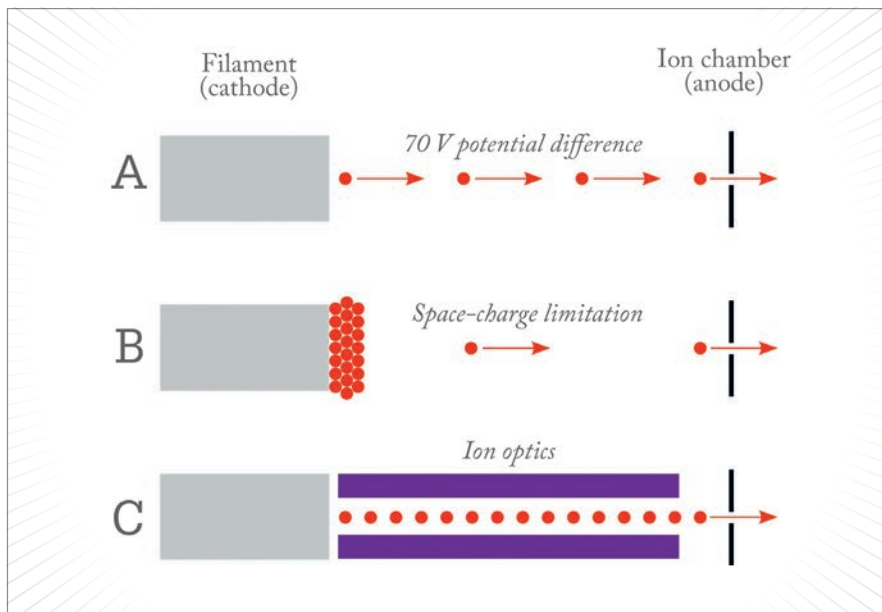


Figure 2. The operation of an electron ionization ion source using (A) conventional (70 eV) ionization energies, (B) low ionization energies, (C) our Select-eV ion-source design.

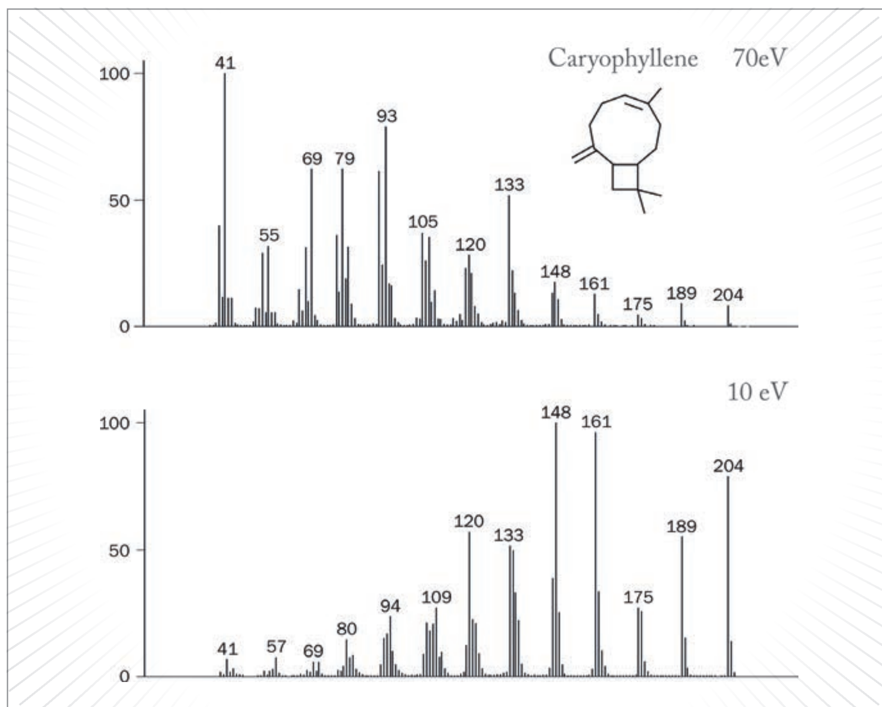


Figure 3. Comparison of spectra obtained using Select-eV for caryophyllene, showing greater responses for the higher-m/z ions at low ionization energy. An additional benefit of Select-eV is provided by the different fragmentation patterns at progressively lower energies, which can provide additional information to distinguish between very similar molecules, such as terpenoids or hydrocarbon isomers.

As a result, they've been free to use their initiative to come up with new solutions from first principles. It was this atmosphere of innovation, together with expertise from other Markes staff members, including Nick Bukowski, that ultimately led to the launch of our TOF mass spectrometer in 2008. Known as the BenchTOF because of its compact dimensions, this instrument has proved highly popular amongst our target audiences, first in academia, then increasingly in the petrochemical, food and fragrance industries. Our customers like its high sensitivity and its ability to generate mass spectra that closely match those in quadrupole-acquired spectral libraries.

The solution

Where does soft ionization come into the story?

Throughout the development of BenchTOF, we were aware that the instrument's design would allow us to do interesting things with the ion optics at a later date. So, once the product was launched, we set about investigating options that might deliver soft electron ionization.

At the time, the very idea would have sounded a strange to many analysts; after all, soft electron ionization had been tried before – and deemed unworkable. The major problem is that the smaller potential difference doesn't effectively pull the electrons away from the filament (where they are generated). Fewer electrons reaching the ion source means that fewer ions are formed, leading to a collapse in sensitivity.

The Road to Tandem Ionisation

2001	BenchTOF designed by Five Technologies
2004	Markes acquires BenchTOF technology
January 2008	BenchTOF launched
2008	Development of Select-eV begins
January 2014	Launch of Select-eV
December 2014	Select-eV wins TASIA
May 2016	Launch of Tandem Ionisation
December 2016	Tandem Ionisation wins TASIA

Pierre and Gerhard realized that they could draw on the BenchTOF's unique design – incorporating a gated electron beam – to overcome the problem. The concept: to use a high potential difference to accelerate electrons away from the filament, but then reduce their energy before they arrived in the ion chamber (Figure 2). The result? A steady flow of lower-energy electrons, but with sensitivity back to acceptable levels.

Once the concept had been proven on paper, it was remarkably easy to turn into reality – about eight months from the basic idea to a working demonstration!

But then came the all-important period of instrument refinement, developing manufacturing processes, and beta-testing with collaborators in academia. Launched as Select-eV (also a TASIA winner in 2014), the technique allows the user to carry out runs using an ionization energy of their choice – either 70 eV "hard" ionization for regular library-matching or "soft" ionization between 10 and 20 eV.

The mass spectra generated using Select-eV show significantly reduced fragmentation, depending on the molecule's structure and the exact ionization energy (Figure 3). As well as providing stronger signals from the higher-mass fragments that enable similar compounds to be discriminated, there's reduced interference from GC background and ionized carrier gases, resulting in much cleaner spectra. In turn, higher signal-to-noise ratios can be obtained, compensating for the inherently lower ionization efficiency (given the lower-energy electrons), and bringing sensitivity back up to the level needed for the most demanding applications. Achieving all this without hardware changes is a major benefit in busy laboratories.

Many analysts immediately saw the benefit of soft ionization with Select-eV, and were keen to obtain an instrument to see what it could do for their own samples. But almost immediately they were asking us: "Is there any way we can do soft and hard ionization in a single run?" We already knew that analysts would be familiar with the concept of "switching" between ionization modes, given the well-established positive/negative ion capability of mass spectrometers. However, we didn't want to run before we could walk, so we focused on the core soft ionization capability before we started trying anything more sophisticated...

Two years of development later and we had modified the way the ion optics worked so that the electron energies rapidly switched between soft and hard ionization, meaning that ions from even the narrowest GC peaks were generated at both energies. The most challenging aspect was actually the electronics, which had to be carefully refined to allow switching of multiple voltages at up to 100 times a second, which is necessary for fast GC separations, such as those used in GC×GC.

The outcome of all this work, launched at the Analytica tradeshow in May 2016, was Tandem Ionisation. Incorporated into the BenchTOF instrument, it was able to generate two datasets in a single GC run (Figure 4) – much to the delight of our customers. Software has also been a key aspect of the project, and we made sure that the raw data could be split into two separate data files in real-time, meaning that hard and soft ionization data can be interrogated as soon as it's generated, saving valuable time in method development.

Beyond the solution

Tandem Ionisation represents a significant breakthrough – a technology allowing low-eV ionization and regular

> *"Our ultimate goal is to make soft-ionization mass spectrometry an everyday tool rather than a last resort for the GC-MS analyst."*

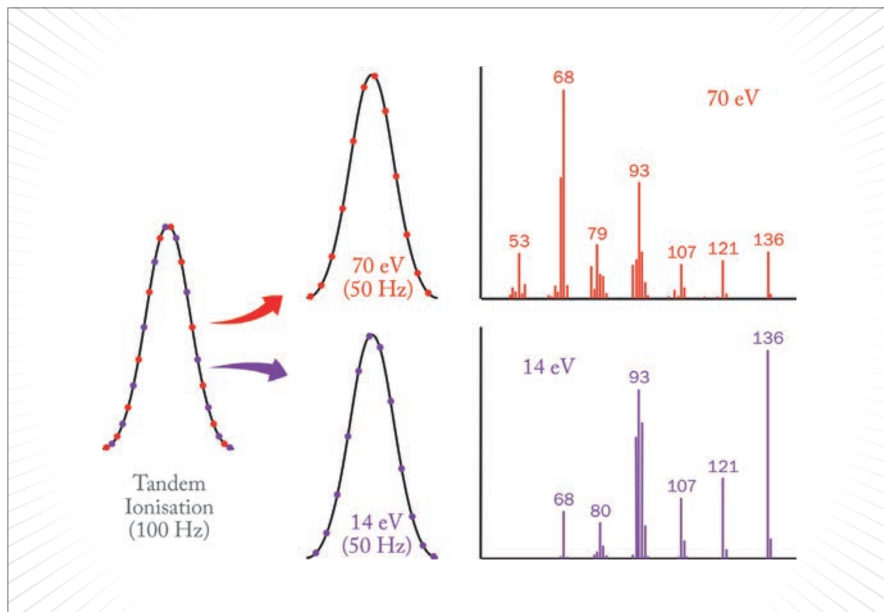


Figure 4. Illustration of the Tandem Ionisation process, whereby multiplexing of the ionization energy enables a single acquisition to generate both hard and soft ionization data files.

70 eV ionization with no inherent loss in sensitivity, no complicated hardware changes, and no need to run the sample twice.

The benefits of soft ionization on BenchTOF instruments have already been demonstrated in the scientific literature; in a paper on GC×GC fingerprinting of hydrocarbons in motor oil (1), Salim Alam and colleagues at the University of Birmingham concluded that “the combination of retention times in two dimensions and mass spectra at low and high ionization energies confers unparalleled power to identify specific isomers within the chromatograms.”

And more recently, a team led by Jef Focant at the University of Liège, Belgium, used soft ionization to help identify challenging compounds in blood headspace (2). The team concluded, “The combination of low and high ionization energies [...] improved the identification of challenging compounds for blood VOC profiling.” (Read about the latest research from the Focant group at tas.txp.to/0417/SMELL).

Work on Tandem Ionisation continues today – specifically on software tools to make it easier to use in high-throughput laboratories. You could say our ultimate goal is to make soft-ionization mass spectrometry an everyday tool rather than a last resort for the GC-MS analyst. Thanks to scientific insight and a willingness to embrace new ideas, I think we are well on the way to making that a reality.

Alun Cole is Founding Director of Markes International, Llantrisant, UK.

References

1. *MS Alam et al., “Using variable ionization energy time-of-flight mass spectrometry with comprehensive GC×GC to identify isomeric species”, Anal Chem, 88, 4211–4220 (2016).*
2. *LM Dubois et al., “Thermal desorption comprehensive two-dimensional gas chromatography coupled to variable-energy electron ionization time-of-flight mass spectrometry for monitoring subtle changes in volatile organic compound profiles of human blood”, J Chromat A, 1501, 117–127 (2017).*

September 19-22, 2017
Boston Park Plaza Hotel, Boston, MA

14th Symposium on the Practical Applications of Mass Spectrometry in the Biotechnology Industry

ABSTRACT SUBMISSION DEADLINE:
May 26, 2017 oral presentation
August 18, 2017 poster presentation

SYMPOSIUM CO-CHAIRS:
Michael Boyne
BioTechLogic, Inc.
Eef Dirksen
Synthon Biopharmaceuticals B.V.

CASSS™
SHARING SCIENCE **SOLUTIONS**

For program updates, hotel information and sponsor information, please scan the QR code or visit www.casss.org.

Curator of Good Science

Sitting Down With... **Ian Wilson**, Chair in Drug Metabolism and Molecular Toxicology, Faculty of Medicine, Department of Surgery & Cancer, Imperial College London, UK.

Did you always want to be a scientist?
Like most little boys of my generation, I started off wanting to be a fighter pilot, but in my teens some excellent teachers got me hooked on science. My parents encouraged my passion – first buying me chemistry sets, and later a garden shed that I turned into a rudimentary laboratory. I spent many happy hours there experimenting with different chemicals, including such wholesome substances as bromine and chlorine.

What turned you on to analytical science?
I got my first taste of analytical chemistry while studying mitochondrial biogenesis in yeast, which involved using preparative LC columns to isolate the DNA. Later, I decided there was no future in molecular biology (prophecy was never my strong point) and went to work with Keele University's David Morgan on insect molting hormones as potential pesticides. We extracted the hormones with solvents before conducting GC with electron capture detection – the sensitive detector of its era. As it turned out, the hormones were useless as a pesticide, at least against locusts, but the experience turned me into an analytical chemist. There were few jobs available to analytical chemists-cum-entomologists so, after a short post doc at University College Hospital London, I moved into the pharmaceutical industry for the next 30-plus years.

What did you enjoy about working in pharma?
In short: working with other highly motivated scientists in a multidisciplinary team on a meaningful goal. It was nice to have access to all the latest analytical equipment, too. These days, I worry about the future of pharma. Many major pharma companies have now contracted, merged or disappeared – in my view, that's not success, it's circling the wagons. It's getting harder and harder to discover new drugs, get them into the market, and recoup the costs.

But, from the point of view of analysis, pharma has done a lot to drive the development of analytical science; for example, the rise of LC-MS began in applications arising from the needs of bioanalysts for very sensitive detection of drugs in biofluids.

What's your current focus?
I'm still doing a lot of work on drug metabolism and toxicology, but also collaborate with colleagues at the MRC-NIHR National Phenome Centre on metabonomic (also known as metabolomic) studies. Originally set up to take advantage of the analytical equipment left over from anti-doping testing for the 2012 Olympic Games, the Phenome Centre was designed for large-scale metabolic phenotyping (metabonomics/metabolomics) of samples obtained in epidemiological studies, for example, using NMR spectroscopy and LC-MS.

What are some of the most interesting developments in analytical science right now?
The mantra of "smaller, better, faster" is still very much what drives us. Some people say that we've gone as far as we can go with LC, but they are talking nonsense. After all, nothing much seemed to be happening in LC towards the end of the 20th century; then UHPLC came along, and I saw the whole field change overnight. Is UHPLC the be-all and end-all of LC? I doubt it – I think there are many innovations to come. The ambition is still there to do more – just look at the Million Peaks Project led by Peter Schoenmakers at the University of Amsterdam (see tas.txp.to/0416/Million).

What's your proudest achievement?
Probably the most influential work I have been part of was the development of a tool for quality control (QC) in metabonomics: at its simplest, these QCs are prepared as you aliquot your racks of samples, when you put a little of each sample into a "gestalt" or pool sample. Then you analyze that sample at regular intervals throughout the run. If your analytical method is perfect, the gestalt sample would appear in your principal components analysis as a single central spot. Of course, no method is perfect, so you actually end up with a cloud of spots. Broadly speaking, the tighter the clustering of the QCs, the better. It's been great to see this approach being widely adopted.

"Is UHPLC the be-all and end-all of LC? I doubt it – I think there are many innovations to come."

However, as you get older you realize that your best achievement is the people that you work with and mentor/develop, or the PhD students that you train. I have always held that the secret to success is hiring people smarter than you...

Do you still have a science shed?
These days I have a cellar, which I have filled with "historic" chromatographic instruments. As time went on I saw the whole history of chromatography being thrown in the trash, and I felt it was important to preserve some of it. The smaller instruments like LC pumps and gas chromatographs are readily portable, so I started rescuing them and taking them home. Soon people starting donating interesting instruments, and I now have a collection of around 60 chromatographs. As the number grew, my wife was indulgent enough to let me convert our cellar into a museum of sorts, and I've recently started working with a colleague, who is an excellent photographer, to document the collection.

What is SONAR?

We're talking mass spectrometry rather than submarines, but how does it work, what are the advantages and who can benefit? We catch up with Jim Langridge (Director, Scientific Operations) and John Chipperfield (Senior Systems Evaluation Scientist) to find out.

SONAR in Short

With John Chipperfield, Senior Systems Evaluation Scientist at Waters.

SONAR is an elegant solution to a very specific problem; if you've got high complexity in your sample and you need more reliable information, then this is the mode for you. SONAR is a data independent acquisition mode that generates MS/MS data – at UPLC speeds – for everything in the sample indiscriminately, using a resolving quadrupole that scans rapidly over a given mass range. Essentially, SONAR enables users to quantify and identify components from a single injection. It's actually a pretty straightforward implementation of a simple idea – it just so happens that it's very powerful.

For users to be able to stay ahead of the game, they need to be able to achieve more with less time – and that means gaining as much information from a single analysis as possible. But there's an important point to make here: speed is nothing without confidence and reliability. Analysts need to be able to trust their data – they need to know that they have correctly identified a given compound – and that's a crucial issue that we aimed to address with the SONAR acquisition mode.

SONAR is an incredibly powerful qualitative technique that can generate a tremendous amount of information for a given sample – and it does this faster and much more thoroughly than you could before. But, to reiterate, you can also pull quantitative information straight out of that dataset.

Importantly, it's just as easy to process SONAR data as any other data that we produce – you can just plug it into Progenesis or UNIFI. And having listened to our customers, we've also made it compatible with third-party software – Skyline from the MacCoss lab, for example.

If we compare SONAR with our major competitor in the area of data independent acquisition, there's an important distinction to make: because we can scan so fast using a resolving quadrupole, we gain selectivity and we're able to assign fragments to precursors very accurately. But we're also able to do it quickly enough to get the optimum number of points across a peak to enable quantification. I don't think our competitors can match up on that particular aspect.

Navigating the Future of Mass Spectrometry

With Jim Langridge, Director of Scientific Operations at Waters

What's your background?

I started out in biochemistry and analytical chemistry. While I was working on traditional biochemistry techniques – ELISA and fluorescence assays, and so on – we began to explore the potential of mass spectrometry to replace some of these methods. The technology was going through something of a revolution at the time and seemed to be accelerating faster than traditional biochemistry. So that was my route into mass spectrometry.

I've been with Waters for 23 years, taking on a variety of different roles during that time; I worked on the original Q-TOF that we developed in 1997, and also on the early incarnations of the ion mobility technology that we've pioneered in the SYNAPT platform. I've also spent a lot of time looking at protein and peptide analysis using chromatography and mass spectrometry, and more recently metabolite and lipid analysis, as these are becoming increasingly important. I'm now Director of Scientific Operations at Waters in Wilmslow, but also hold an honorary professorship at Imperial College

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

London in the Department of Surgery and Cancer. The latter role came about through my involvement in projects on desorption electrospray ionization (DESI) and rapid evaporative ionization mass spectrometry (REIMS) technology, developed by Professor Zoltan Takats, who works with Professor Jeremy Nicolson at Imperial.

You've seen more than a couple of decades of mass spectrometry development – how have you seen the field change during that time?

If you go back 25 or 30 years, most people were interested in investigating single analytes that held specific biochemical significance. I think the biggest change that we've seen is the drive towards profiling a large number of analytes – almost taking a snapshot of a particular biological process by investigating the proteins and underlying metabolites. Today's researchers want to use this information to derive biological context – mapping pathways and interactions to understanding how that relates to a disease state, for example. Such analysis has only been made possible by advances in electronics and computers. As power and speed have increased, we're now able to scan faster, with better sensitivity, than I could have imagined – and that's opened up huge future opportunities in biology and health.

So we need to look at the bigger picture?

Exactly. And you can see how research is already starting to change. In the future, people will be more interested in flux-based studies, looking at how molecules and systems change over time. That's part of the reason we developed our SONAR technology – to give researchers a tool that not only offers very powerful qualitative information (something that allows you to get specific information for metabolite identification), but also quantitative information, which is essential for flux studies and understanding how analyte concentrations are changing between different sample sets.

At Waters, we innovate by taking a slightly different pathway – and in doing so, we are able to bring new capabilities – such as SONAR – to the market. That's probably why I've stayed with the company for over 20 years... SONAR is the latest in a very long line of exciting developments that, for me, started with the SYNAPT platform, and includes REIMS and DESI. It's a great time to be in science and it's a great time to be at Waters.

What do you consider to be the key challenges facing modern research?

Perhaps the most relevant word is "complexity" – especially in terms of sheer numbers of analytes in a given sample. Dynamic range also comes into play; very complex samples tend to include analytes over a very wide dynamic range, which can put stress on typical approaches, and cause potentially important analytes to be missed. Even today, we can't see all analytes, but each iteration of technological improvement peels away another layer of complexity, allowing us to see many more components than before. Speed and sample throughput is also an issue – with high complexity comes the need for more rigorous results, which demands analysis of an ever-higher number of samples. Gaining robust data across large numbers of samples is essential if we are ever to understand the underlying biology. Without such an approach, we risk making all sorts of assumptions that aren't valid.

I'm pleased to say that this field of research seems to be moving away from studies with limited samples. Savvy researchers realize that you need to analyze a large number of samples to find something significant. It turns out biology is a lot more complicated than we once thought! Determining the (changing) concentrations of many analytes in large numbers of complex samples is hugely challenging. But as technology advances, researchers are able to do much better and more comprehensive studies.

What makes SONAR special?

Data independent analysis has been around for over ten years – driven by the need for unbiased approaches that don't rely on making decisions on which ions to sample or fragment, but instead record information on all ions. SONAR has two advantages: first, the scanning nature of the quadrupole gives us increased specificity of what we're selecting; because we have a resolving quad, we know what we are passing through at any given point in time, which gives us selectivity – useful when you're trying to associate fragment ions to precursor ions in qualitative work. The second interesting aspect – and what's different about SONAR – is that we don't need the quadrupole to be at a very high resolution; instead we can actually de-resolve it, transmitting a wider window to effectively gain in sensitivity. Those two aspects give us a very flexible acquisition mode, which gains its true power through informatics processing.

How does SONAR address the throughput issue?

The fact that modern electronics now allow us to scan quadrupoles at 10,000 amu per second means that we can move the quad extremely quickly, which, in practical terms, allows us to collect more than ten spectra per second across a chromatographic peak. Consider modern separation science, where the peaks are getting narrower

More Information

SONAR: An expert's perspective

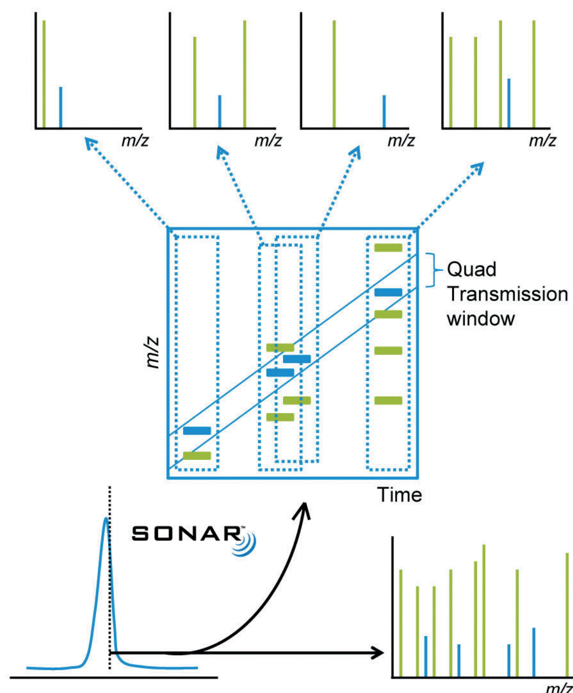
either because people are trying to gain better resolution or increase throughput. The number of points we get across that peak is important both from a qualitative and quantitative perspective.

You mentioned the power of informatics processing – could you go into more detail?

One of the big changes we've made in terms of company philosophy is our decision to open up our software environment so that people can access the data and use them in a variety of different software packages. We had a big push at ASMS 2017 on that very subject.

We've worked – particularly in the research environment – with a wide variety of scientists who have different requirements. They wish to look at data in different ways and use different programs and open source tools. The community has definitely driven our decision, but we also recognized that if someone uses an open source tool to get the information and the results they need, our facilitating that process can only be good for us and our customers. We realize that we cannot provide every single aspect of functionality demanded by such a broad spectrum of different applications and requests.

Skyline came out of MacCoss Lab at the University of Washington, and when I first saw it, I remember thinking, "Wow! What a neat approach. This guy has some great ideas." At an early stage, when Skyline was struggling to gain traction, we were very supportive and worked hard to get our software to produce data in the right format.



Interestingly, our effort also encouraged other vendors...

SONAR is a simple concept – why has it not been done before?

It is simple, but was only made possible by the work we'd done to access data independent acquisition through ion mobility on the SYNAPT platform, which demanded the development of a novel acquisition system that could acquire up to 2,000 spectra per second. For the XEVO and SONAR, we basically used the same acquisition system but, instead of using ion mobility, we used a scanning quad in front of the time-of-flight, which allows us to store data in 200 bins per quad scan. In other words, the idea is simple, but the execution was only made possible by a very talented development team!

Who's most excited by the potential of SONAR?

Definitely the lipidomics community, where we feel there is a big opportunity in terms of an unmet need, namely increased selectivity. Lipids are very close in mass-to-charge, and there are many structural isomers. SONAR allows you to do one acquisition, but still pull those apart quite nicely. Proteomics is another big area.

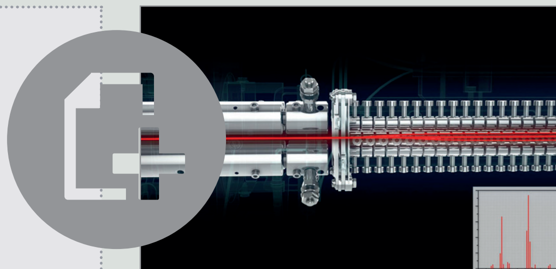
We're also starting to see it move into other areas as we further develop the technique; the mass spectrometry imaging community, for example. We actually had a presentation at ASMS 2017 based on the use of SONAR with DESI imaging.

What about outside of health science?

Environmental analysis and foodomics is a natural progression. Like most technologies, when we first develop them we apply them to the most challenging application area – in this case, proteomics, metabolomics, and lipidomics. But challenges are often mirrored in other fields. Another area that we're starting to explore is biopharmaceutical characterization. Here, reliability and reproducibility of results is absolutely critical, which plays to the key strength of SONAR.

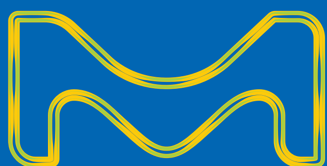
More Information

SONAR Whitepaper:



Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Millipore®
Filtration, Separation & Preparation



thinking of you.

We're committed to filtration tools – and the scientists who use them.

Merck's filtration and analysis tools are the pacesetters in membrane technology with well-established Amicon®, Millex® and Stericup® product lines. So, finding ways to improve wasn't easy—but the answer came from thinking of how we could make filtration easier and reduce environmental impact. The result? Redesigns that feel good to use – inside and out.

How did we do it?

- Easy-to-open, informative and sustainable cut disc membrane packaging
- Faster and more ergonomic Millex® filtration with the Samplicity® G2 vacuum-driven system
- Improved vacuum filtration with our new alignment sleeve and ergonomic clamp

Need the right filter at your fingertips?
Request our new Millex® dispenser to keep your filters sorted and handy.
Have yours hand-delivered: merckmillipore.com/onemillex

Dependable filtration tools. Redesigned, with you in mind.
www.merckmillipore.com

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Merck and the vibrant M are trademarks and Millex, Samplicity, Amicon, Stericup and Millipore are registered trademarks of Merck. 2017 - 01482 04/17 Copyright © 2017 Merck KGaA. All Rights Reserved.

Merck