JANUARY / FEBRUARY 2022 # 103

the Analytical Scientist®

A Peek Behind the Veil

Meet the analytical scientists unravelling myths, mysteries, and masterpieces

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"Unfortunate, but Not a Complete Disaster"

Here's to all event organizers – including those at Pittcon – who've had to make "the tough call"

You need to be a combined epidemiologist, political forecaster, and sociologist – or an outright clairvoyant – to make the right call these days. I have great sympathy for organizers big and small who have felt compelled to cancel physical events for a second or even third time. The latest conference casualty? Pittcon. Its organizers reluctantly announced the move away from a physical event in late January (1), citing health and safety risks spawned by an "ever-increasing communicability of emerging COVID-19 strains." And it's hard to argue with that reasoning.

Assuming Pittcon goes virtual, I'm sure the team will do an excellent job, as they did in 2021 – and I'll certainly enjoy attending. At the same time, I think we all now know, deep down, that nothing beats a physical meetup.

I recently spoke with James Harynuk about the challenges of virtual events – ironically, before he and his colleagues at GCxGC also made the tough call. "Attending a meeting from the comfort of your basement, you find yourself being distracted by other demands – from work, kids who may be trying to attend school remotely, or just trying to conduct a physical life and a virtual life simultaneously in two vastly incompatible time zones," he said.

Time zones are challenging enough when you're flying between them – but when you're stuck in one place, it's (almost) arguably worse. "Being that I live in Hawaii, virtual meetings have really taken a toll on my ability to network and be connected with research colleagues worldwide," Katelynn Perrault from Chaminade University of Honolulu told me. "Often, meetings take place in the middle of the night for my time zone, which makes balancing virtual conferences with other job responsibilities hard – there are some days that I literally do work around the clock."

Perrault also mentioned the feeling of inspiration she gets after traveling for physical conferences, which virtual events haven't – unsurprisingly – been able to replicate. I suppose we're caught between two quite different dictionary definitions of event: "something that happens" versus "a noteworthy happening."

Though we may be over virtual events, we're evidently not over the pandemic. And in some cases, digital will remain a necessary substitute for real life. Faced with that (virtual) reality, one of my new year's resolutions is to block out dedicated time in my calendar and then force myself – but mainly others – to stick to it, while hoping for a chance to meet some of you in person one day – preferably in 2022.

In Harynuk's words – reflecting on the prospect of going virtual: "It would be unfortunate, but not a complete disaster."

James Strachan
Editor

References

1. *Pittcon (2022). Available at: pittcon.org*

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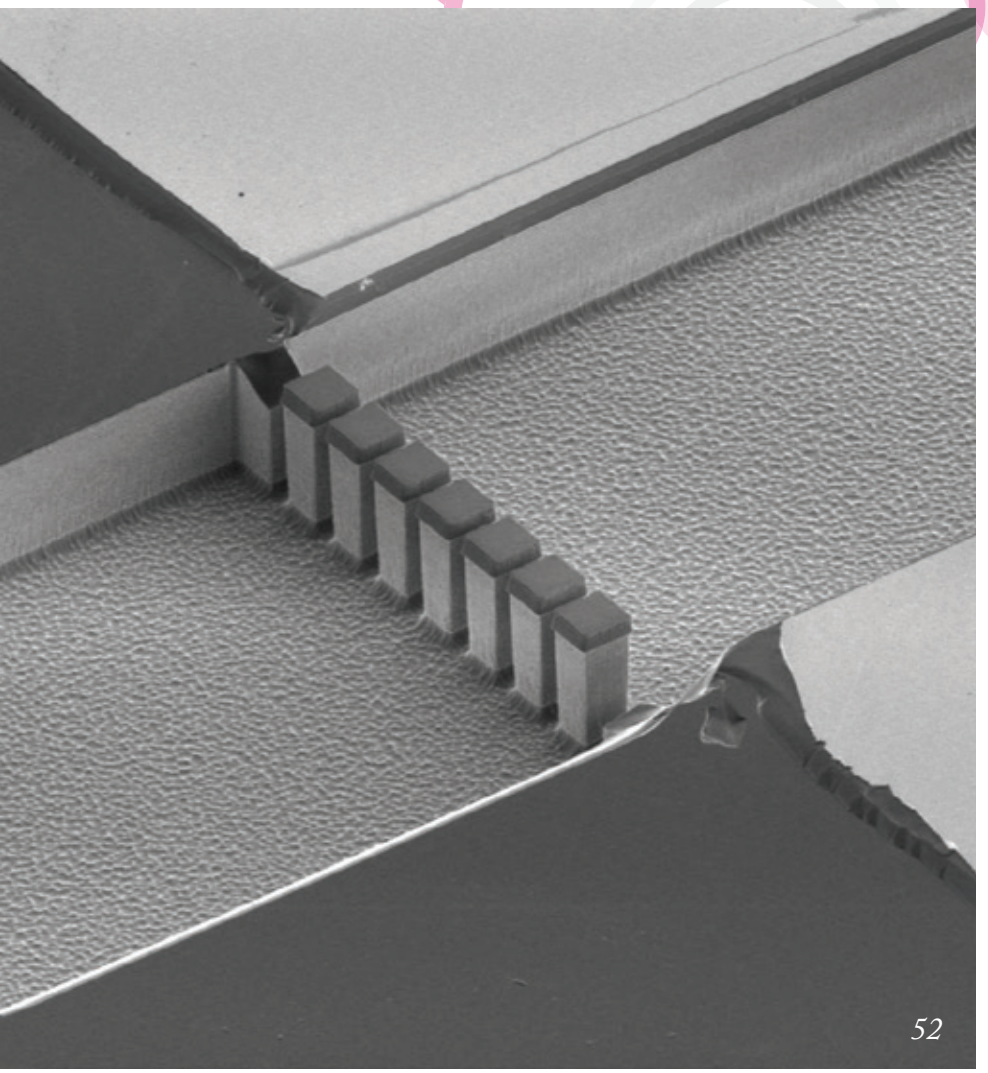

On The Cover

Composite image of Girl with a Pearl Earring from images made during the Girl in the Spotlight project. Credit: Sylvain Fleur and the Girl in the Spotlight team

Core Topics

Sitting Down With

the Analytical Scientist

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Upfront

Research
Innovation
Trends

Unveiling Life's Rich Pattern

Metabolite patterns uncovered by NMR spectroscopy may shed light on patients with non-specific cancer symptoms

Early cancer diagnosis is crucial for ensuring the best possible patient outcome (1). Currently, the referral options for patients with nonspecific symptoms – such as fatigue or weight loss – aren't well defined, which can make selecting the appropriate specialist difficult. This can lead to a loss of time and resources, which in turn may delay diagnosis until tumors are larger or have metastasized (2).

Noting the need for a new approach to diagnosis, James Larkin from the University of Oxford turned his attention to biofluid metabolomics. Using nuclear magnetic resonance (NMR) spectroscopy and computational analysis, he and his colleagues identified patterns of metabolites present in patients' blood samples. "We discovered some patterns are more common in cancer patients and others are more frequent in patients with non-cancerous disease," Larkin explains. "We can use these changing patterns to determine whether or not a patient has cancer."

Existing approaches to cancer detection work by either physically searching for a tumor or detecting specific markers in the blood or in circulating tumor DNA. "What's unique about our approach is that we're looking only at the pattern of metabolites and seeing how the pattern itself is changing in cancer," says Larkin.

The researchers were able to identify patients with solid tumors with high sensitivity and specificity and were also able to distinguish between patients with metastatic disease and those without. In two individual cases, the metabolomics model also identified early-stage cancers before conventional imaging was able to discern them. The implications of these findings are promising for patients with nonspecific symptoms, potentially enabling cheaper, faster, and more efficient diagnosis.

"Our immediate goal is to develop this technology to the point where it can be used daily by doctors seeing patients," says Larkin. "Right now, there are a lot of cancer patients receiving late diagnoses, which leads to poorer outcomes and more deaths than necessary. Our approach will enable doctors to answer a key question early on: 'Does this patient have cancer?' If the answer is yes, the patient can be referred confidently to existing clinics, cutting down waiting time and improving prognosis."

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1. *R Tucker, Hospital Healthcare Europe (2022). Available at: https://bit.ly/3KLQdsX.*
2. *JR Larkin et al., [Online ahead of print], Clin Cancer Res (2022). DOI: 10.1158/1078-0432.CCR-21-2855.*

SPEAKING OF...

Gender Equality

To celebrate the International Day of Women and Girls in Science 2022 (Feb 11), we've curated a selection of quotes on gender equality from our archive

"Overall, it's about helping people understand that bringing people together from diverse groups actually benefits everyone and creates a space to uncover novel scientific ideas. There are so many solvable diseases that are specific to a single population and not addressed until that group or population are engaged in the research question."

Michelle Reid, *A Network for Progress*

"Diverse teams deliver better results – and because it's our ethical obligation to encourage inclusivity in science. Unfortunately, many people's personal experiences, and much of the available data, suggest that science still suffers from bias and discrimination against underrepresented groups."

Emma Wilson, *Kickstarting Publishing Parity*

BUSINESS IN BRIEF

Bruker acquires Prolab, the FDA turns to Waters for medical products testing tools, and SomaLogic teams up with Illumina to advance proteomics… What's new in business?

- SomaLogic and Illumina have announced a co-development agreement aimed at "democratizing and accelerating our understanding of the human proteome" – in the words of proteomics SomaLogic Chief Executive Officer Roy Smythe. SomaLogic's SomaScan Proteomics Assay will be applied to Illumina's high throughput next-generation sequencing (NGS) platforms. "Proteins play a central role in cellular function and health, and NGS can support a greater understanding of this role by unlocking biological insights at scale," said Illumina Chief Technology Officer Alex Aravanis.
- NanoMosaic, a biotech company developing nanoneedle technology for multi-omics applications, has signed a lease for a standalone building of 30,880 square feet in Waltham, MA, USA, to house its Multi-Omics World Headquarters. "This expansion

allows for large scale, vertically integrated manufacturing of the Tessie instrument, consumables and reagents," said Joe Wilkinson COO of NanoMosaic.
- Sticking with 'omics, Bruker has acquired Prolab Instruments – a Swiss technology company specializing in micro and nano-UHPLC pump technology. Prolab's Zirconium, a nano-LC to cap-LC split-less pump, optimized for proteomics results, is already part of Bruker's nanoElute system, and the companies are planning more tech mashups in the proteomics and metabolomics fields.
- The US Food and Drug Administration's (FDA) Office of Regulatory Affairs has purchased Waters' Empower Chromatography Data Software and NuGenesis Laboratory Management Software to support its medical products testing operations across its five field science laboratories. Waters will also install the hardware, assist with instrument qualification and software validation, and train site analysts.

Introducing "SynTOF"

How a flow cytometry and time-of-flight mass spectrometry mashup helps explore risk factors of neurodegenerative diseases

A recently developed technique, dubbed synaptometry by time-of-flight (SynTOF), combines flow cytometry with time-of-flight mass spectrometry, allowing scientists to examine tens of millions of individual human single-synapse events using 38 antibody probes. Specifically, the technique allowed Stanford University researchers to compare and contrast human brains without pathologic change or with pure Alzheimer's disease (AD) or Lewy body disease (LBD).

By applying machine-learning approaches to the dataset, the technique not only confirmed pre-established differences, such as reduced caudate dopamine transporter in LBD and increased hippocampal pathologic tau in AD, but also offered new insights; for example, increased hippocampal CD47 and lowered DJI proteins in AD, and higher ApoE proteins in AD with dementia.

Given that next-best alternatives for such analyses – namely, array tomography and conventional flow cytometry – hit a ceiling of one million single-synapse events, SynTOF represents "an unparalleled opportunity for multiplex analysis," according to the researchers.

"How can we expect to significantly improve representation of women and minorities in academia when the major role models are white men who regularly work at nights and during weekends?"

Isabelle Kohler, *Mind the (Gender) Gap*

"I've gone into meetings where a young woman comes up with an idea that no one is interested in, but then, all of a sudden, some guy says the same thing and now it's his idea. The fact that discrimination is society-wide makes it very hard to tackle in a specific industry."

Ellen Miseo, *Towards Meritocracy*

"About the time I was trying to decide whether or not to do a PhD, a supervisor flatly told me, 'You have to decide whether you want to do a PhD or get married and have kids.' Either/or."

Ingeborg Petterson, *Beyond the 'Old Boys Club'*

"I believe that the majority of young chemists do not see the gender gap. We have come through our undergraduate degree as a mix of men and women, all treated equally, and it has resulted in us expecting to be treated equally in future positions."

Lisa Miller, *On Balance*

In My View

Experts from across the world share a single strongly held opinion or key idea.

How Can We Grow Diversity in STEM?

We need to get more diverse people into STEM, help them stay there, and ensure their hard work is fairly rewarded at the top of their professions

By Lisa Jones, Associate Professor of Pharmaceutical Sciences, University of Maryland, Baltimore, USA

Increasing diversity in STEM subjects is a complicated challenge, but that just means we have to come at it from several angles. We've found that targeted intervention at middle school age is key because children aged 11–12 are old enough to retain what you're teaching them, but young enough for programs to make a real difference.

My activities in this regard include involvement in the UMB CURE Scholars program, through which we work with three West Baltimore middle schools in a predominantly African-American neighborhood. The idea is to introduce children to STEM in a more meaningful way than normally occurs in the classroom. We focus on middle school students, because studies show that elementary school is too early for retention in STEM subjects, and high school is too late. The best time to reinforce the STEM fields is when kids are thinking about their future careers, and that's middle school. With that aim, the CURE program provides year-round activities with students and support science teaching at the schools. My laboratory hosts children for summer research projects; we have about 22 students in the lab each year.

We make the project relevant for them; for example, by focusing on diseases they would see in their community, such as asthma and diabetes. A typical day for these students would start with a short lecture on the disease – we make it short and fun to keep them engaged – followed by some experimental work. In the case of asthma, the children might build a healthy lung, observe how the air flows through it, and then mimic restriction of the airway. Whatever we do, we always emphasize innovation; we want them to be comfortable with trying something new, not scared of it. Finally, we train them in soft skills, including communication and community awareness. This latter part is important; we want them to be able to go home and discuss diseases that are prevalent in their community, and how those conditions can be prevented or treated. So far, about 65 percent of our Core Scholars have ended up wanting to major in STEM subjects, so our program has been very successful.

But once you've got diverse people into the system, the next big challenges is helping them stay there. Historically, STEM subjects have suffered from the "leaky pipeline" problem – diverse people come in, but then leave. We aim to fix that leak!

I'm also involved in the NIH-funded initiative for maximizing student development (IMSD), which aims to

"Whatever we do, we always emphasize innovation; we want them to be comfortable with trying something new."

improve diversity in STEM subjects at PhD level. Within this initiative, I am co-director – along with Mike Summers and Rachel Brewster – of the Meyerhof graduate fellows program (one of the largest IMSD programs). Our objective is not only to recruit diverse students into PhD programmes but also to support them through graduate school. Support might include mental health workshops that help them deal with racial microaggressions or grant-writing workshops. At the same time, we train the broader faculty to support diverse students – one example being bias training. It's a serious endeavor, but it's been a lot of fun. And in 2018 we celebrated the 100th Meyerhof PhD student, so we can certainly say we've improved diversity in STEM students!

But these kinds of programs are not enough to fix all the problems we face. In particular, academics of color still have issues at the professor level – getting tenure, for example. Part of the reason is an unbalanced and unrecognized workload; if you are the only person of color in a department, students of colour automatically gravitate towards you for support – and the time you spend mentoring them comes out of time you'd otherwise spend researching and writing. And universities typically only judge your research output – in particular, how many publications and grants you got. Similarly, if you are the only woman or the only person of color in the department, you'll end up being asked to join every research committee so they can demonstrate diversity. And that means you can add lots of unrecognized committee work to your unrecognized mentorship…

In my view, universities should either protect faculty of color from all committee duties – or recognize and reward those duties as an integral part of their tenure package. One or both of those things must happen. You can bet that every person of color in the faculty is excelling just as much as their white counterparts, but they are being held back because of those extra duties.

If we want more people of color in STEM, we need to make sure we inspire children (at the middle school age in particular), then ensure those who do make it to graduate school are supported, and finally eliminate the workload imbalance faced by academics at the tops of their professions. This latter point will cascade down – providing more role models in leadership positions to encourage early career academics and even children that their hard work will be fairly rewarded.

(NA)DES: Delivering on a Green Promise?

The COVID-19 pandemic has been the focus of scientists and politicians for the past 18 months, but not enough is being done to combat another widespread issue with serious health implications: food fraud

By Ernest Teye, Senior Lecturer, School of Agriculture, University of Cape Coast, Ghana

It is not scaremongering to suggest that the problem of food fraud has reached pandemic proportions. For those unfamiliar with the topic, food fraud can be defined simply as the intentional adulteration of food (or food ingredients) for economic profit (1). According to PWC's Food Fraud Vulnerability Assessment (2), it is a threat that costs the global food industry between $30 - $40 billion per year. Not only does food fraud have a significant economic impact, but, more concerningly, it has serious health implications for those who ingest adulterated products.

There's no doubt food fraud is a global issue, but my personal focus is tackling the issue in West Africa and bringing it to the forefront of policymakers' agendas. I'm on a mission to foster international collaboration between food academics, chemists, engineers, and scientists to tackle the problem head on.

Why West Africa specifically? Firstly, Ghana is my home country and where I have established my career and expertise – I lecture at the University of Cape Coast's School of Agriculture in Ghana, and I lead the Africa Centre for Food Fraud and Safety. Secondly, the combination of various factors makes the problem of food fraud in West Africa particularly prevalent. Africa imports $35 billion in food annually (3), so communities can be flooded with fraudulent foodstuffs from abroad. The complexity of our global supply chain, inconsistent regulations, lack of robust testing frameworks, and a lack of support from policymakers all serve to amplify the problem. Consequently, the need for innovative and accessible solutions to test food authenticity is vital.

Recent examples of food fraud reported in local and regional media include palm oil laced with banned food colorant Sudan IV, and meat and fish treated with an embalming agent (formalin) to keep it unnaturally fresh. Despite growing reports, awareness of such fraud within the community and its negative effects on human health remains limited. And that leaves the door wide open for dishonest trade, ultimately limiting the right of African consumers to clean, healthy food – instead, exposing them to the risk of illness or even death. Since the COVID-19 pandemic, there has been a further spike in food fraud due to panic buying, price inflation, and food shortages. Climate change is also compounding the problem: over 100 million people in Africa are at risk (4). In short, the need to eradicate food fraud grows greater with time.

To provide solutions to the issue of food fraud, I have been fortunate enough to collaborate with researchers from the Institute of Global Food Security, Queen's University Belfast (IGFS-QUB) on Agilent-funded research led by Professor Chris Elliott.

We developed a two-tier approach for screening food, specifically rice in this project. Handheld near-infrared spectrometers can scan large numbers of samples in the field, with suspected fraudulent foodstuffs being sent for confirmatory analysis in the laboratory using LC-MS, GC-MS, and ICP-MS. My goal is to use these tried and tested methodologies across West Africa to mitigate food fraud. We're already looking at the use of portable sensor devices for rapid onsite and non-destructive screening of palm oil, with 15.7 percent of palm oil imported worldwide being delivered to Africa (5).

The opportunity to share our expertise across borders has helped us develop practical, effective long-term solutions to combat food fraud in Africa and across the globe. Together we have hosted workshops that showcase our findings, and in turn revealed how uninformed stakeholders in Africa were about the problem of food fraud. And that led us to establish the Africa Centre for Food Fraud and Safety.

The collaboration between the Africa Centre for Food Fraud and Safety and IGFS-QUB has created an important platform. We have been able to generate awareness and attract talent to combat food fraud in West Africa and beyond. African communities should not be a dumping ground for harmful and fraudulent foodstuffs. Our mission is simple but essential: To make Africa's food safe.

Good Job Security and Increased Pay in the Chemical Sciences, Pay Report Shows

Every two years, the Royal Society of Chemistry (RSC) asks its members to share their experiences of working in the chemical sciences. In 2021, 4,298 members shared feedback to build a picture of the employment landscape, the challenges and skills needed within the sector.

By Sarah Salter RCDP, Career and Professional Development Adviser, Royal Society of Chemistry

The Royal Society of Chemistry's latest employment research is part of our continuing drive to uncover disparity and be the voice of the chemical sciences. The 44th edition of this authoritative report reflects responses from our members across the world to provide benchmarking data for individuals, employers, and recruiters – helping to inform career pathways or inspire the next step for career development.

Beyond that, it's more than a salary a survey

The report provides evidence that can help drive positive change within the chemical sciences and beyond. The research helps us to understand the challenges, barriers and specific needs of our community, to help develop and deliver support initiatives to continue advancing the chemical sciences.

The full report provides significant detail, but the highlights include:

- Pay has increased for survey respondents compared with 2019
- Job security has increased by five percent (up to 70 percent) compared with 2019
- Leadership, networking and influencing are still the top skills survey respondents want to develop
- Home working opportunities have increased compared with 2019

Inclusion and diversity in the chemical sciences

Ensuring a fair and equal career progression across the chemical sciences is crucial, and the report informs an evidence-based approach to tackling inclusion and diversity. It explores pay gaps, differing experiences for women, people from minoritized ethnic groups, disabled people, LGBT+ people, carers, and those who have taken career breaks.

It highlights that the chemical sciences still need to address equality issues as women, disabled people, those with caring responsibilities, and LGBT+ people were less likely to agree there are equal opportunities for all where they work, or to feel that their working environment is diverse and inclusive.

For instance, only 21 percent of disabled respondents held a position of high responsibility compared with 37 percent of non-disabled respondents. As a result of the COVID-19 pandemic, disabled people experienced more difficulties in developing their skills. The research within this report contributes to making grants and funding available to support these needs and reduce the barriers to skills development. Find out more by visiting rsc.li/funding.

Influencing change

The report findings support the strategy and services offered by our benevolent fund, the Chemists' Community Fund, our wider membership and our professional development activities. The report plays a key role in our response to current political topics, including advising the UK government on immigration changes, teacher retention, the UK Government's comprehensive spending review, and other policy priorities. This is part of our wider strategic goal to advocate and be the voice of the chemical sciences community.

The report has evolved from its roots to serve a larger and more diverse community today. However, the fundamental reasons for collecting and publishing it have not changed since its origins over 100 years ago. The pay and reward survey gives our members the opportunity to showcase the chemical sciences and the many and varied possible career pathways. This is incredibly useful to undergraduate students and those considering a career in chemistry. The data also helps their peers make evidence-based decisions about their careers.

Key Stats

- Highest paying sectors are the industrial or commercial company (£51,200), public sector (£51,000) and consulting (£47,000).
- The top three motivations for work: Being passionate about what I do; Doing work that has a positive impact on society; Having autonomy and work-life balance

Download your copy today

If you're a member, you can access the authoritative report at the following link: www.chemistryworld.com/members. If you're not a member, you can find out more about joining or purchasing the 2021 Pay and Reward Report by visiting rsc.li/whatchemistsearn If you have any queries about the report, or if you're a member who would like to speak with the Career Management team please contact us (careers@rsc.org).

Going Above and Beyond with Low-Flow UHPLC

How the Thermo Scientific™ Vanquish™ Neo UHPLC system embodies a new era of proteomics

Presenting highlights from the webinar "Beyond the boundaries of LC-MS proteomics with next-generation all-in-one nano-, capillary- and micro-flow LC," which is now available to view on demand at: https://bit.ly/3Gf0xX1

After listening to feedback from researchers, day-to-day instrument users, laboratory managers, and engineers, Thermo Fisher Scientific identified a number of crucial attributes for the next generation of low-flow LC systems:

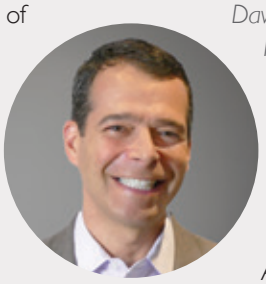
i) performance: getting deeper into quantitative proteomics, bioanalysis or biopharma while maintaining high sensitivity and separation power,

ii) robustness: delivering high-quality results 24/7,

iii) ease of use: ensuring results are easy to obtain for users with varying levels of LC-MS expertise.

And the Vanquish Neo UHPLC system has it all. Indeed, this nano-, capillary- and micro-flow system for high-performance LC-MS workflows enables long-term, trouble-free analysis and seamlessly integrates with the Thermo Scientific MS portfolio. The new system is designed for a range of applications, from single-cell analysis to routine quantification of peptides and posttranslational modifications. Below, David Perlman, Bernhard Kuster, and Karl Mechtler share their experience of using the Vanquish Neo UHPLC system in the wild.

A platform for ultrasensitive and single-cell proteomics



David Perlman is the Senior Principal Scientist and Director of Ultrasensitive Proteomics, Merck Exploratory Sciences Center, Cambridge, MA, USA

Tell us a bit about your work with the Vanquish Neo…

As you can tell from the title of my talk, my team is extremely interested in single-cell proteomics. We are very excited by the potential for this new field to have an enormous impact on our understanding of the cellular functional heterogeneity that defines human health and disease. Every technology that helps us further towards this goal is more than welcome.

We ran a technical assessment of the Vanquish Neo and it demonstrated extraordinary reliability, performance flexibility, and ease-of-use through our entire testing period. It has enabled the comparative assessment of high-performance column systems and their flow rate optimization, including testing the cost and benefits of ultra-low flow rates. Its consistent performance has really facilitated our MS run parameters to maximize not only identification but also peptides quantification. In terms of our single-cell application, we created a family of single-cell proteomics quality control standards for multiplex SCoPE-MS type experiments using commercially available peptide digest products from two separate human cell lines.

Since the webinar, you've had more time with the Vanquish Neo. How has that been?

We continue to be impressed with the ongoing robustness of the Vanquish Neo platform. It has been in continuous use in our lab 24/7 for 9 months now and counting. We use it in challenging high-pressure nano-flow applications with multiple different column systems that we swap in and out, as well as multitude of different gradients. So far, we have had flawless operation with not a single day of downtime or service required. That track record is impressive, particularly in the historic context of nano-flow systems. The Vanquish Neo autosampler itself is masterfully engineered. I've had a great deal of experience with other systems in the past; maintaining continuous high-performance operation month after month was frequently a challenge, and upkeep was

laborious. The Vanquish Neo has reset my own expectations for productivity on our platform and helped identify where our weakest links may lie.

Have you heard any feedback from other users?

Because we were among the first to test the Vanquish Neo – and because it has only just recently entered the market – we have only really been able to share our own experiences. I know that my positive feedback has been welcome news – everyone likes to hear of solutions to problems that they are also experiencing. And I might add our early feedback is backed up by our own purchasing choices: once the Vanquish Neo became commercially available, we ordered two more systems for my own group, and are planning to purchase one more unit this quarter. Nothing speaks louder than that – and it's simply a reflection of the degree of our own personal excitement for this product.

Deep dive proteome profiling

Karl Mechtler is Head of the Protein Chemistry Facility, Research Institute of Molecular Pathology, Vienna Biocore Facilities, Austria

What experiments have you run with the Vanquish Neo system?

We've used the system for deep-dive proteome profiling with nanoLC-MS and high-performance columns, comparing the Thermo Scientific™ PepMap™ Neo column, PharmaFluidics µPAC™ column, and IonOpticks Aurora column. As an example, with the PepMap column, we saw accelerated sample loading, which reduced the overhead time by around two times in comparison with constant flow loading. We also saw increased acquisition speed, highly reproducible run-to-run results, and a max identification rate already being achieved at 1 µg loading.

What features of the Vanquish Neo system particularly benefit your work?

The new pump can now transport liquids at a range of one nanoliter per minute to 100 microliters a minute. There is also constant pressure sampling and calibration up to 1500 bar. The autosampler has a precision from 10 nanoliters – an important feature for single-cell proteomics. Other important features are auto-detection for injections and sample pressurization to reduce the shock during the injection. There are also different programs available to minimize carryover.

Overall, the Vanquish Neo UHPLC system provides versatility to operate different high-performance column types by precisely controlling pressure and flow parameters. The optimization of LC-MS acquisition conditions enables routine identification of more than 7000 protein groups in single-shot DDA analysis on different column types. Robust UHPLC operation, negligible carryover, stable performance of columns and emitter extend the boundaries of deep routine proteome profiling.

Analyzing thousands of proteomes with micro-flow LC-MS/MS

Bernhard Kuster, Professor and Chair of Proteomics, Technical University of Munich (TUM), Munich, Germany

Tell us about your lab's research…

Our lab covers research in three areas: mapping proteomes, understanding how drugs work, and building proteomics tools. This last area is where the Vanquish Neo system comes in. Generally, nano-flow LC is great in terms of sensitivity for proteomics applications, but the chromatography is challenging and it's also limited in throughput. We wanted to see if we could reconcile this somehow, by looking at how to improve micro-LC separations for use in high throughput and high-quality proteomics. We've managed to achieve that with the Vanquish Neo for protein profiling of body fluids.

Have you had any questions about your time with the system at conferences?

We managed to get across for ASMS – and we had some interesting conversations. The proteomics community typically uses nano-LC separations, so once you deviate from that and try to show what's possible with micro-LC, people immediately raise questions about sensitivity. And though there is a small loss in sensitivity, it is entirely acceptable for most applications because you can simply use more sample. Besides that, people seem to be most excited about the robustness of the system – the columns last for ages. There's also been a lot of positive feedback around the versatility of these systems and how simple they are to use.

What are the standout benefits from your perspective?

The chromatographic resolution is the clear winner for us as we're analyzing very complex mixtures – so peak capacity is key. In the micro-LC system we tested, the Vanquish Neo delivered sharp peaks – and the higher signal on the electrospray offsets quite a bit of the anticipated loss compared with nano-flow. I've already mentioned excitement around the robustness – and that's also important for us. Even three years ago, if someone had said we would be able to analyze 10,000 samples, I wouldn't have believed them. But now, such projects are running in our lab. Amazing progress!

Abbie Vandivere (Mauritshuis) and Annelies van Loon (Mauritshuis/Rijksmuseum) set up the macroscopic X-ray fluorescence (MA-XRF) scanner to scan *the Girl*.

Credit: Martijn Beekman

MYTHS, MYSTERIES, *and* MASTERPIECES

Meet the analytical scientists working to enhance our understanding of the past, preserve our cultural heritage, and uncover the secrets behind artistic masterworks. Find out: how Abbie Vandivere uncovered the secrets of the *Girl with a Pearl Earring*, why Richard Hark sought to put the Vinland Map debate to bed, and what it took for Luciana Carvalho and James McCullagh to unravel the mystery of the Sicán mask.

a *NEW LOOK* at the *OLD MASTERS*

Abbie Vandivere was granted the opportunity to examine Vermeer's masterpiece, Girl with a Pearl Earring, with an international group of scientists. Which techniques did they employ to uncover the secrets of the Girl?

Can you tell us about yourself and the current project?

I'm a paintings conservator at the Mauritshuis in The Hague, the Netherlands. My expertise includes restoration and conservation of artworks but also, crucially, the application of advanced analytical techniques. After all, whenever we want to restore an artist's work, we must first understand the materials and techniques the artist used. In fact, research and analysis are an increasingly important part of our work at the Mauritshuis.

Recently, Edwin Buijsen, the Head of Collections, asked me to examine one of our most iconic paintings – Girl with a Pearl Earring, which was painted by Johannes Vermeer around 1665. I couldn't believe that I was being offered such an amazing opportunity! But it also gave me a lot to think about; in addition to the usual challenges, the Mauritshuis wanted us to conduct the examination in front of museum visitors because of the painting's high profile. We had three key questions to answer: What kind of expertise do we need for the examination? How can we access that expertise? How might we safely carry out the investigation in a public way?

The press ready to capture the first moment of the examination with MA-XRF within an enclosure in the Golden Room of the Mauritshuis.
Credit: Ivo Hoekstra, Mauritshuis

And how did you approach such an important piece of artwork?

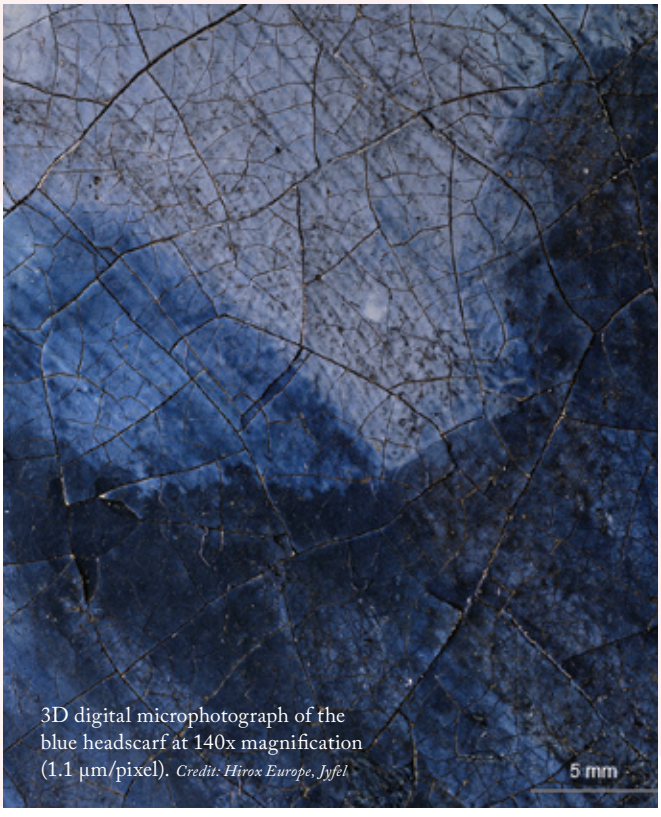
Analytical science is constantly advancing, as you well know, so we were keen to apply a suite of technologies that were simply unavailable during the last restoration – back in 1994. Indeed, one significant development over the last 25 years has been the advent of non-invasive technologies; no longer must we rely on methods that demand sample removal. For instance, we employed macro-X-ray fluorescence scanning (MA-XRF) to scan the painting's surface and establish which elements are located in different areas. But, at the same time, we could perform more sophisticated analyses on the samples that had to be removed from the painting in 1994; for example, we were able to apply advanced elemental analysis for the first time. These techniques included scanning electron microscopy (SEM) with energy dispersive x-ray analysis (EDX), which allows us not only to identify different paint layers, but also to conduct elemental analysis of each pigment type in each layer. Another technique, focused ion beam scanning transmission electron microscopy (FIB-STEM), allows us to determine the exact elemental make-up of the tiniest particles in a thin slice of paint. We also sent some 1994 cross-sections for synchrotron analysis at a particle accelerator, which allowed us to distinguish between different types of pigment at the molecular scale. Overall, we benefited from improved versions of the technologies available in 1994 – multispectral infrared analysis, reflectance imaging spectroscopy, 3D scanning and various forms of photography. Furthermore, improvements in computing and microscopy technologies have taken us to the next level; for example, we now have a 10 billion pixel image of *the Girl*, which allows us to zoom in remarkably – all the way down to the level of individual pigment particles! A great deal has changed since 1994 – even the way we share results (via the Internet) was not possible back then…

All the individual technologies are exciting in their own right, but I believe the really novel aspect of our current analysis of *the Girl* was the holistic approach that was enabled by using so many different analytical techniques. By combining elemental identification with precise determination of element locations throughout the painting, we literally and figuratively got the full picture! Of course, this holistic approach was only made possible through collaboration with many different

Emilien Leonhardt (Hirox Europe) examining the painting using the Hirox 3D digital microscope.
Credit: Vincent Sabatier: Hirox Europe, Jyfel

A 3D digital microphotograph shows the pearl at 140x magnification (1.1 µm/pixel).
Credit: Hirox Europe, Jyfel

Head researcher Abbie Vandivere examines Girl with a Pearl Earring.
Credit: Ivo Hoekstra: Mauritshuis



3D digital microphotograph of the blue headscarf at 140x magnification (1.1 µm/pixel). *Credit: Hirox Europe, Jyfel*

specialist institutions. Fortunately, conservation science is a very collaborative field, and *the Girl* is a very famous painting – so people were eager to help!

What novel insights did your holistic approach reveal?

In particular, by using ultra-high performance liquid chromatography (UHPLC), we established that Vermeer had used cochineal in the red of *the Girl's* lips – and not just any cochineal; we showed that it was cochineal of Mexican or North American origin. In other words, he was relying on a product that must have been imported by sea. This single finding told us as much about international trade and which materials were available in Delft in the 17th century as it did about Vermeer's choices. I think it's amazing that we can learn so much about global-scale activity from one tiny sample.

One of our big research questions concerned the origin of Vermeer's materials, so we were especially pleased with the cochineal finding. On a similar note, we have also been investigating lead white pigments in detail for some time. By analyzing lead white samples from artworks and historic mines all over Europe, we contributed to a database of the various types of lead white pigments and where they came from. Subsequently, we performed lead isotope analysis of the white pigment in *the Girl*, and compared the results with the database entries. And I can tell you that Vermeer's lead white pigment originated in England's Peak District – a good example of how specific we can get. This particular exercise was only made possible in this case by a micro-invasive sampling tool that was recently developed specifically for artwork analysis. The technique allowed us to remove 5 to 20 µg of pigment from a paint cross-section. Incidentally, another change we've seen in the last 25 years is the development of technologies specifically for the artwork analysis field!

Were you surprised by what you found?

Vermeer's palette wasn't exactly unexpected, as it was similar to those of his contemporaries: lead white from England, cochineal from Mexico or North America, and other locally sourced pigments. He also used indigo, but we are not yet sure of its origin. There are perhaps 800 varieties of indigo from all over the world and we don't yet have a database that

Left: Visible light photograph
Credit: René Gerritsen Art & Research Photography
Right: Underlayers beneath the surface revealed using MS-IRR.
Credit: John Delaney and Kate Dooley, National Gallery of Art, Washington.

clearly defines each variety and its respective origin. We can learn more from further UHPLC-based pigment analysis – we already know that Vermeer's pigment is not likely to have been derived from the Asian plant Indigo tinctoria – the most common form of indigo. Like the cochineal, it may be from the Americas, but we don't yet know for sure. This indigo example illustrates the importance of pigment databases. Collaboration is key to this endeavor; fortunately, there's a lot of interest in conservation science now, and museums are very willing to have their artworks analyzed in this way. Our databases are growing very rapidly – and that will allow us to provide more accurate and detailed analyses.

One excellent example of an unexpected finding relates to how we revealed elements of the painting that are no longer visible. For instance, the painting's background currently appears grayish-black – but we now know that it was made up of a black charcoal underlayer beneath a glaze of blue and yellow pigments, which would originally have made an intense green. And the really new information generated by our current project is more subtle still – it turns out that the original background wasn't flat and formless, but represented the folds of a curtain! That information came from analysis using a combination of several technologies: some measured the thickness of the layers, some showed the distribution of the elements, and all together they showed us something that we had no idea existed.

How will your work guide subsequent restoration decisions?

Our analytical work not only enables us to imagine how the painting looked when Vermeer painted, it also gives us a record of how it is today. By preserving a record of this painting at this moment in time, we have a reference we can use to chart any changes that might happen in the future. But we have to accept that the painting has had a rough history – it was not really appreciated until the early 20th century. For example,

Composite image of *Girl with a Pearl Earring* from images made during the Girl in the Spotlight project. *Credit: Sylvain Fleur and the Girl in the Spotlight team*

"And the really new information generated by our current project is more subtle still – it turns out that the original background wasn't flat and formless, but represented the folds of a curtain!"

there are cracks in the paint surface which we just have to accept; they're not going to get better, and they may get worse. All paintings change, whether we like it or not. But we do our best to make inevitable changes occur as slowly as possible – the painting is protected by glass, and environmental factors, such as temperature and humidity, are carefully controlled. Furthermore, we are attempting to predict changes that might happen over time, and perhaps better mitigate them.

Could your approach help establish the authorship of an artwork?

The technologies we use to identify an artist's materials and techniques are neutral – they don't establish authorship in themselves. But they can identify commonalities or differences between paintings, and show whether a painting of disputed provenance was produced using, say, the pigments available to the artist in question. The more analysis we do, the more confident we can be about establishing authenticity – but the aim of this project was technical examination, not establishment of authenticity.

How do you expect things to change over the next 5–10 years?

We will increasingly rely on the combination of data gathered using many different technologies. This, together with the application of data science and visualization tools for comparative analysis, will give us unprecedented amounts of information. And I think that's how we'll have the greatest impact – not just by developing new technologies, but by getting more information from the data we can already generate. That's how we approached *the Girl* – and the project has gone much further than many other studies. I'd like to think it will become a benchmark for future research on other paintings. But I appreciate that not every painting in every museum will be able to benefit from the range of technologies available to *the Girl* – the iconic nature of the piece opened many doors!

In fact, our work on *the Girl* is not finished – we're still crunching data. And we are still working on a deeper exploration of how *the Girl* would have looked when she was first painted – for a project called "The Girl in 1665." I'm working with scientists, academics and computer graphics specialists to reconstruct her original appearance – based on the scientific data we have gathered (it's not just a case of tweaking some settings in Photoshop!). In brief, using digital technology, we are generating a range of possibilities of how she might have appeared in the original painting. And looking further ahead, we are investigating the possibility of using 3D-printing approaches to generate reconstructions of artworks like *the Girl*. It is a very exciting time!

The Girl in the Spotlight was a Mauritshuis initiative and involved a team of internationally recognised specialists associated with the Netherlands Institute for Conservation+Art+Science+ (NICAS: Rijksmuseum, TU Delft, the Cultural Heritage Agency of the Netherlands (RCE) and the University of Amsterdam), together with the University of Antwerp, Vrije Universiteit Amsterdam, Shell Technology Centre Amsterdam, Hirox Europe, the National Gallery of Art, Washington and many other partners.

A collection of ten articles about the technical examination of the Girl has been published in the open-source journal Heritage Science: https://bit.ly/3G3A6mX

A handheld SciAps LIBS unit in action with a Chinese ceramic object

ANALYTICAL Mythbusting

Richard Hark, LIBS expert and conservation scientist at Yale's Institute for the Preservation of Cultural Heritage, reveals his detective work in the cultural heritage sphere

How did you end up working on the preservation of cultural heritage?

During my PhD at the University of Pennsylvania, I worked on the development of reagents to assist in visualizing latent fingerprints – a project done in conjunction with the US Secret Service. So I was more forensically oriented in my early career. I then spent 25 years as a chemistry professor at small liberal arts colleges, which involved more traditional work in synthetic organic chemistry.

In 1999, I became aware of a workshop offered by the National Science Foundation that connected chemistry and art. And as I was always interested in applications of chemistry – the chemistry of chocolate and things of that nature – I thought it would be interesting. So I took the week-long course and was so inspired that I decided to offer a chemistry of art course the very next fall. And that led me on a path towards getting involved in the analysis of cultural heritage objects. But initially, it was just an opportunity to teach a course for undergraduates.

In 2003, I became aware of laser-induced breakdown spectroscopy (LIBS). I thought that it would have great application in forensic analysis, given its advantages (high sample throughput, ability to see the elemental distribution in heterogeneous materials, no need for sample preparation). And I also thought it could be useful in the analysis of artworks – at least certain types of artworks. Indeed, it had already been used in this context; I read several papers at the time. I got a grant to purchase a LIBS unit (one of the early Ocean Optics instruments) and began doing some research. Around that time, I established a collaboration with scientists at the Army Research Laboratory, who were applying LIBS for hazardous material identification and so on. I then teamed up with a colleague to work on geological applications of LIBS – and that's where you'll find most of my publications.

To bring you to the modern day, I had two sabbaticals in the field of art and cultural heritage analysis. The first was at University College London and the Victoria and Albert Museum, where I used Raman spectroscopy and X-ray fluorescence (XRF) spectroscopy to analyze various artworks. More recently, I attended a week-long technical art analysis workshop at Yale, which led to a sabbatical in the Institute for the Preservation of Cultural Heritage (IPCH) – and then a more permanent position here. The group has since purchased a handheld LIBS instrument, which we've been applying to the analysis of cultural heritage objects.

Can you tell us about your work on the Vinland Map?

The Vinland Map was acquired by Yale in the mid-1960s and purports to be a 15th-century map of the world. It depicts the pre-Columbian "Vinlanda Insula" – a section of North America's coastline southwest of Greenland. It's been a rumbling source of dispute for Yale since the 1960s, with some claiming its authenticity and others – the majority – claiming that it is a fake. But when I joined Yale in 2017, they still hadn't performed their own conclusive analysis. Other groups had analyzed the map; for example, in the 1970s, one analysis found that its ink contains anatase, a form of titanium dioxide first used commercially in the 1920s…

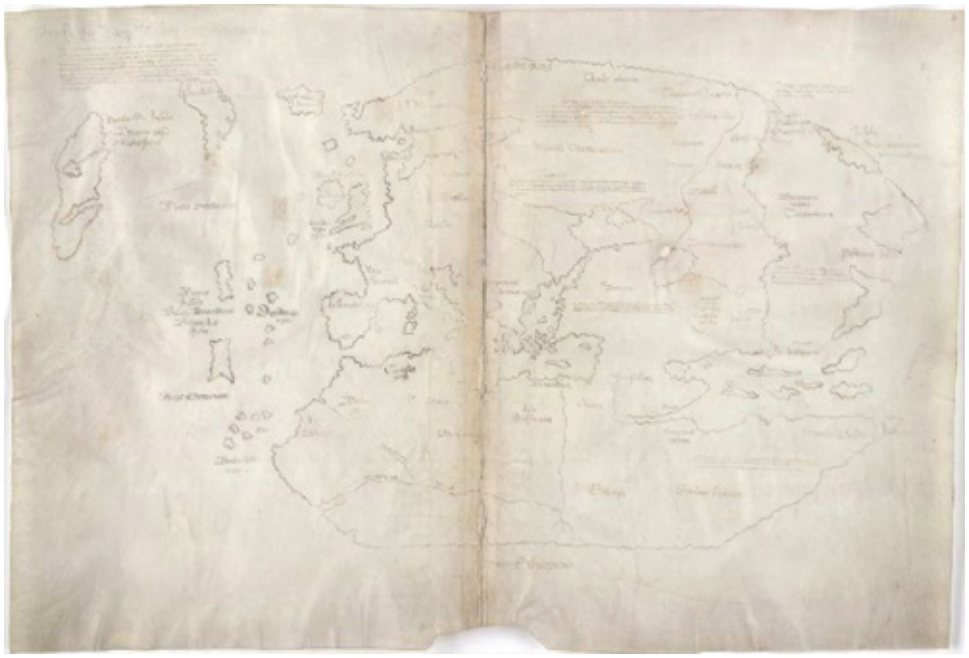
"The goal was to use the new tools the group had acquired to unveil any additional secrets and to perhaps prove, once and for all, that it was a forgery."

I had taught the history of the Vinland Map during my lectures as an example of a potential fake or forgery – and how we can use scientific tools to get to the truth. It was this experience that led me to become associated with the project. The goal was to use the new tools the group had acquired to unveil any additional secrets and to perhaps prove, once and for all, that it was a forgery. Until our project, some people argued that the analyses to date hadn't hit all the right points – even though one study had looked at close to 100 different spots on the map. We used an imaging technique called scanning macro X-ray fluorescence (MA-XRF), which gave us an elemental map of the entire Vinland Map – including a graphical representation of how the various elements are distributed. We did not find iron in the map outlines or inscriptions, which means it wasn't made with iron gall ink, the usual ink used in Europe throughout the Middle Ages. But we did confirm the presence of titanium, which is not found in appreciable quantities in other medieval inks. In fact, we looked at 50 inks from the 15th century and only found tiny amounts in some documents (because titanium can be found in clay, which can become associated with the parchment during preparation).

Interestingly, we also found that an inscription on the back of the map (in titanium ink), which had sometimes been referred to as the map's "title," was written over an existing inscription in iron gall ink. We believe that the addition of text was a deliberate attempt to connect the map with a genuine medieval document. Overall, there can now be no doubt: it's a 20th century fake that was drawn on genuine 15th century parchment!

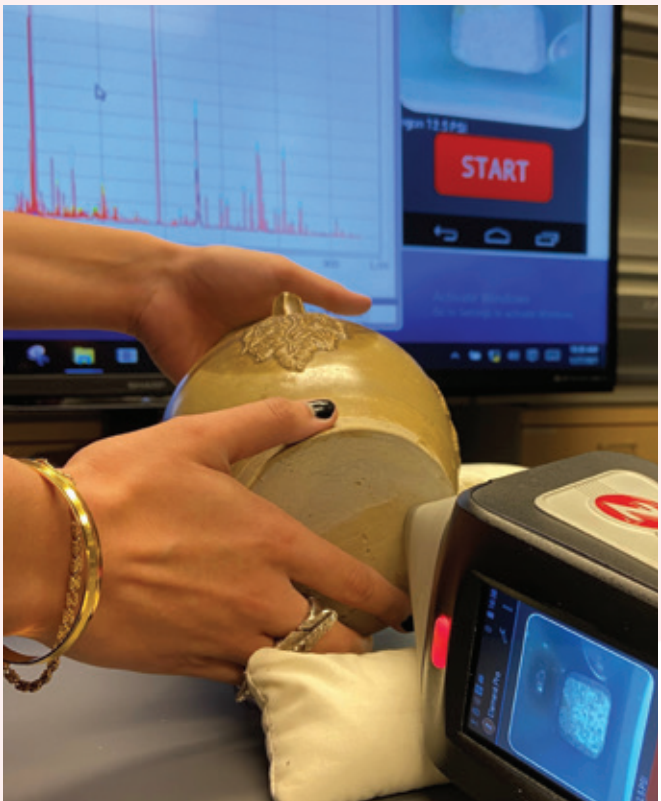
Why do you think the forgery was created?

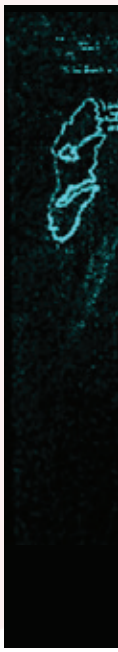
As with many such cases, it was most likely financially motivated. I believe the map was originally sold to a New Haven bookseller for $3,500 (a fair amount of money back in 1957). But a couple of years later, when evidence emerged that the map was associated with genuine medieval documents, the value went up by two orders of magnitude. The trail is difficult to follow, but some people



The Vinland Map.
Credit: General Collection, Beinecke Rare Book and Manuscript Library, Yale University

Credit: Jon Atherton and Yale University

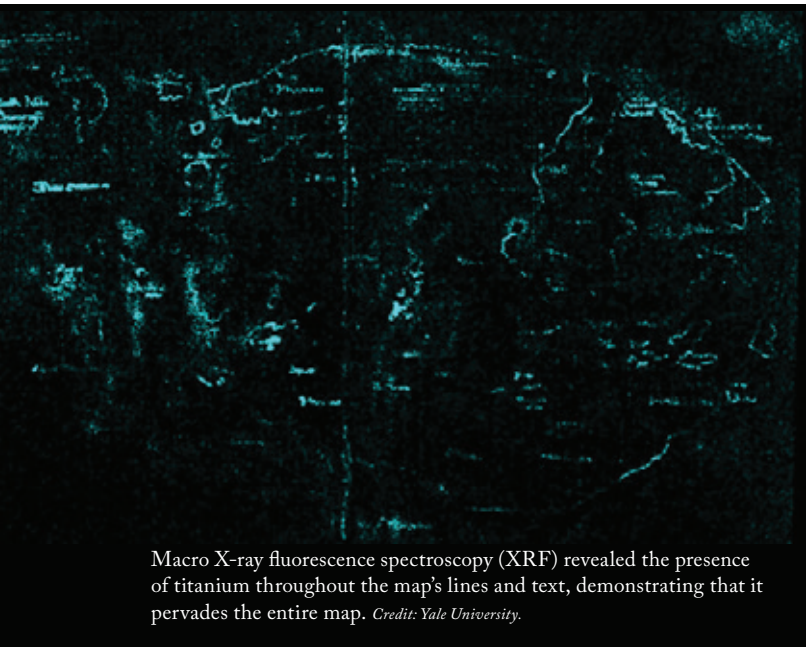




certainly made a lot of money. The New Haven bookseller wasn't forthcoming in saying what happened but his Italian counterpart (from whom he bought the book) was convicted of stealing books from the cathedral library in Zaragoza, Spain… Though that doesn't prove he was involved in making the forgery, it provides circumstantial evidence that he was not above such activities!

Does anyone still believe the Vinland Map is genuine?

The Yale Library Curator for Early Books and Manuscripts, Ray Clemens, believes the matter is concluded beyond reasonable doubt. His goal was to make sure that cartographers, historians, and other scholars would have no cause to believe anything else. And I think he's done that. In fact, there were plenty of other historical problems with the story anyway; Vikings didn't make such maps – rather, historians believe they were wayfarers who navigated by stars. I suppose you could always argue that a Viking connected with someone else who made the map, but that's a bit of a stretch… There are also problems with the Latin used in the cartography. In any case, our research should put an end to any speculation. Although science cannot definitively prove something is genuine, if we find materials that are inconsistent with the time and place that an object was supposed to be created, we can say with a very high degree of certainty that the object is not authentic.



Macro X-ray fluorescence spectroscopy (XRF) revealed the presence of titanium throughout the map's lines and text, demonstrating that it pervades the entire map. *Credit: Yale University.*

Do you get a buzz from how analytical science is able to help piece a story together?

Absolutely! If I wasn't a chemist, I probably would have been a historian. I took multiple history courses in college, and I do enjoy all those aspects. But in essence we're doing forensic science. We get clues, we look at the evidence, and then we try to interpret what it all means. The case of the Vinland Map was relatively straightforward; other times, finding an answer can be extremely difficult! And that's when art history, paleography, and analytical science must all come together to piece together the full story.

What other secrets are you trying to uncover?

One project I'm currently working on involves tarot cards – and this time we're really working with genuine 15th century objects! Most people associate tarot cards with fortune telling, but they were originally a game called trionfi and later tarocchi in Italian and are connected with our modern deck of 52 playing cards. At Yale, we have 67 out of a total of 86 cards from the Visconti di Modrone tarot deck. We're collaborating with researchers at the Metropolitan Museum of Art to study tarot cards from several Italian institutions and the Morgan Library & Museum. The latter institution has the largest number of cards from the Visconti-Sforza tarot deck, which were made at a similar time as the cards at Yale. All of these cards are hand painted – they are essentially miniature paintings – and it has been hypothesized that the two decks were made in the same workshop, perhaps even by the same artist. We've been analyzing them to understand the materials used – and how our cards at Yale differ from the ones at the Morgan. This fantastic project has involved collaborations between curators, conservators and scientists all looking at different aspects of these cards; from an analytical point of view, we've been relying on non-destructive tools such as MA-XRF and Raman spectroscopy.

I'm also working on something a little more modern. It could be said that William Henry Fox Talbot invented modern photography, as he developed the idea of making a negative and then creating multiple prints from that single negative. Talbot was also responsible for a book called The Pencil of Nature – the first commercially published book to be photographically illustrated! Published in six fascicles between 1844 and 1846, The Pencil of Nature suffered a major problem: the photographs faded very quickly, which almost torpedoed the concept of photography. We still don't know exactly why Talbot's salt prints faded so quickly – and that's what we're exploring, using handheld XRF and other analytical tools.

POSITIVELY Medieval

Portable X-ray fluorescence suggests a group of windows from Canterbury Cathedral may be the oldest stained glass windows in England – and the world

A group of windows from Canterbury Cathedral may be the oldest stained glass windows in England, according to a team of scientists from University College London and conservators from Canterbury Cathedral (1). The researchers used X-ray fluorescence spectrometry – specifically, a portable version of the technique, customized with a 3D-printed attachment – to date the windows. We spoke with the lead author of the study, Laura Ware Adlington, to find out more.

Tell us about your background in materials science...

I am a specialist within the field of archeological materials science, which uses techniques from materials science and chemistry to study archeological and cultural heritage materials. We use these approaches to study when and where things were made, but also the wider systems of technology and production, innovation and knowledge transfer, trade and exchange, and how things were used, reused and discarded. All of this is deeply entrenched in the study of the human past – based upon the idea that humans both shape and are shaped by the material world, and so, by studying material culture, we can study people. My specialism is in glass, with a particular focus on medieval stained glass, but I've also worked on various metals and ceramics.

How did you end up working on the Canterbury Cathedral project?

The panels I ultimately worked on were removed from the cathedral walls as part of emergency conservation on the stonework, which provided an opportunity for the public to see them up close in exhibitions and for specialists to study them more easily. I was working on glass from York Minster as part of my PhD and wanted to test the noninvasive technique I'd developed there on a separate case study. Fortunately, Leonie Seliger – director of the stained glass conservation studio at Canterbury Cathedral – was interested!

What were the main analytical challenges you faced?

The early stages of the data analysis were particularly labor-intensive for this project. The panels have had a complicated history, so every piece of glass had to be scrutinized to ensure that the chemical data, the style of the paintwork, and its position in the panel all agreed with the hypothesis.

Can you talk me through the analytical methods you used to date the stained glass windows?

The windows were dated using two lines of evidence that ultimately agreed. First, in the 1980s, Madeline Caviness wrote an article arguing that the stylistic characteristics of some of the panels indicated an earlier origin. We then used an analytical technique called X-ray fluorescence spectrometry, which measures elemental composition. We used a portable version of this technique (pXRF), which required a customized approach that included the design of a 3D-printed attachment to enable precise, accurate in situ analysis of medieval stained glass windows. The stylistic and chemical information agreed with each other and supported the early date.

Why did you take a portable approach with pXRF?

To take invasive samples of windows, the panels need to be removed from the wall and the individual pieces of glass removed from the lead strips (called cames) that hold them together. This is an expensive and laborious undertaking, so invasive sampling usually has to piggyback on planned conservation works. That's why it was so important to develop this in situ approach. At Canterbury Cathedral, even though the panels were removed from the walls, the glass pieces were not removed from the cames, so the in situ approach was still necessary.

Were you surprised by the results?

Honestly, yes! We could easily have had inconclusive results. The results hinged on the identification of a change in the glass source in the late 12th or early 13th century and showing that the potentially early panel predated this change in source. If there had been no change in the glass source, we would not have been able to comment one way or another. Our work at York Minster has indicated that they used some of the same glass suppliers over quite long periods of time, so it was a real possibility.

Do you have any plans to use the technique in future studies?

Yes, our team has more planned work at Canterbury and we are seeking funding to study panels at Pennsylvania's Glencairn Museum.

Was this one of the most interesting projects you've worked on?

Absolutely – but working with medieval stained glass is never dull!

Reference

1. *LW Adlington, IC Freestone and L Seliger, "Dating Nathan: The Oldest Stained Glass Window in England?" Heritage, 4, 937 (2021). DOI: 10.3390/heritage4020051.*

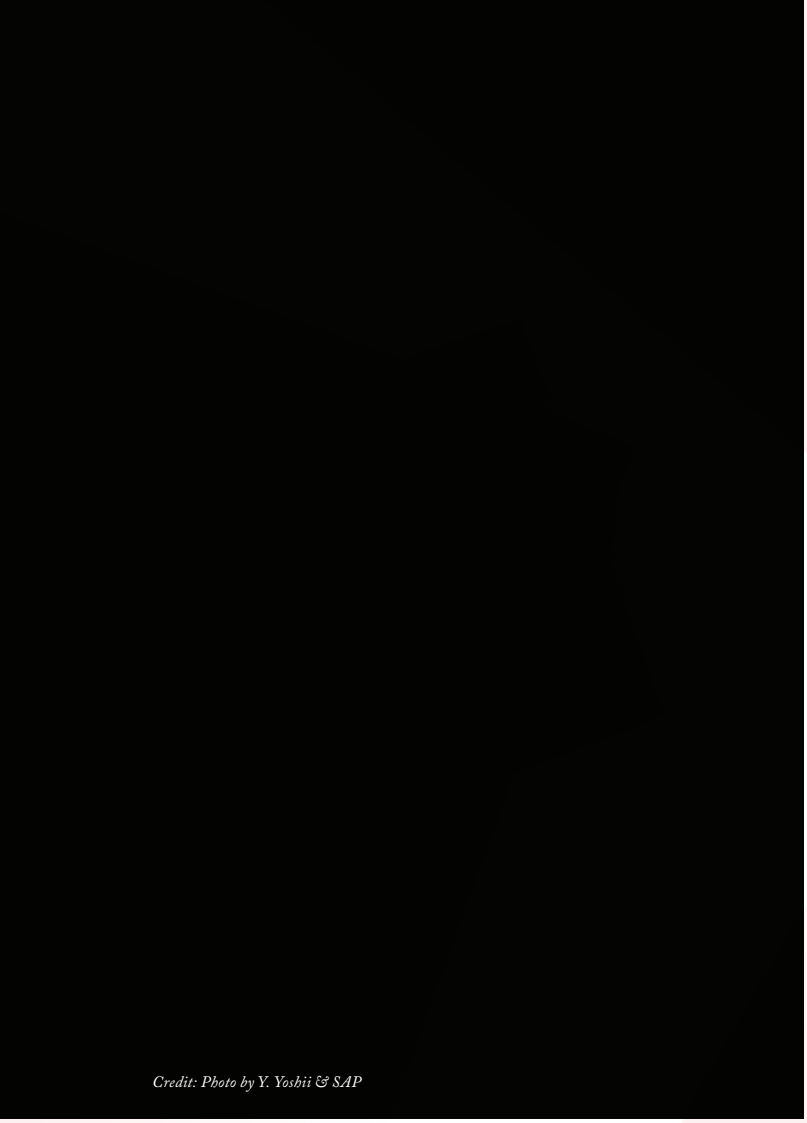
the *MYSTERY* of the *SICÁN MASK*

Analysis of the red paint preserved on the surface of a 1000-year-old gold mask excavated from a Middle Sicán tomb in Peru finds human blood and egg proteins – but why?

Thirty years ago, archeologists excavated a gold mask from a Middle Sicán tomb in Peru. Covering the gold was an unusually well-preserved layer of scarlet-red paint – still visible 1000 years after it was applied by the Sicán people. Researchers quickly identified the pigment as cinnabar (a mercuric sulfide mineral), but the identity of the remarkably effective binder remained a mystery – that is until a team of researchers applied proteomics to the problem.

James McCullagh, professor of biological chemistry and Director of the Mass Spectrometry Research Facility in the Department of Chemistry at the University of Oxford, and co-author of the study, was fascinated by the project for two reasons. "First, from a chemical perspective, how is it that the red paint was able to bind to the gold surface for such a long time?" he asks. "And second, if we could reveal what the Sicáns were using in their paints, what might that say about them as a people and a culture?"

The project was initiated by Luciana Carvalho. Carvalho graduated in chemistry in Brazil and fell in love with archeology after a trip to Egypt. She went on to study for



Credit: Photo by Y. Yoshii & SAP

"The analysis of archeological residues can provide some of the most fascinating analytical challenges due to their chemical complexity, state of degradation and the limited amount of material often available."

a degree in Egyptology and trained as an archeological conservator at UCL. There, Carvalho met John Merkel, who was part of the archeological mission led by Prof Izumi Shimada that discovered the mask.

"In 2014 I was debating whether to stay at UCL or go to Oxford to research organic residues preserved in metal corrosion, and Dr Merkel handed me a paint sample from the mask," says Carvalho. "He suggested that I go to Oxford analyze it as part of my PhD to try to solve the mystery of the binder. So that's what I did."

At first, Carvalho struggled to find the right specialists and analytical techniques to tackle the project. But she found the perfect collaborator in James McCullagh, who had started at Oxford applying MS in an archeological context. "I still have strong research links with the Research Laboratory for Archaeology and History of Art in Oxford, including Robert Hedges, Emeritus Professor of Archaeological Science, who pioneered radiocarbon dating using accelerator mass spectrometry and isotopic analysis of archeological materials," says McCullagh. "The analysis of archeological residues can provide some of the most fascinating analytical challenges due to their chemical complexity, state of degradation and the limited amount of material often available. It may not be clear what the sample contains and what we are looking for in this case. A Sherlock Holmes approach may therefore be necessary – to gather as many relevant pieces of analytical information as possible that not only provide detailed chemical information about the object, but also enable archeological scientists to make reasonable interpretations about how people interacted and used the material in the past."

Asking the right questions

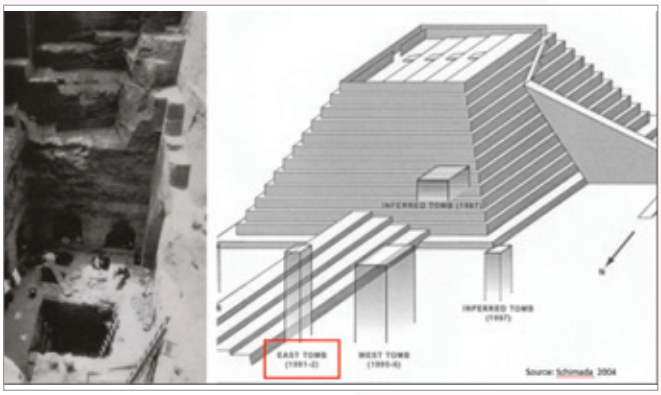
Initially, before joining McCullagh's group, Carvalho characterized the red paint with Fourier-transform infrared spectroscopy (FTIR) to have an idea of its chemical fingerprint. The results indicated the presence of proteins. Carvalho also looked for resin markers in the sample using a common protocol for lipid analysis from porous ceramics – to no avail. Either they weren't there or the sample was too small.

That's where another member of the research team – lead author Elisabete Pires, who works in McCullagh's department and focuses on proteomics – played a key role.

"Elisabete is a specialist in analyzing proteins from biological samples and she did a great job despite some challenges," says Carvalho. As part of a proteomics analytical protocol, you initially measure the amount of total protein in the sample to calculate the amount of enzyme needed for digestion into peptides. "Initially, that wasn't possible, so Elisabete had to adapt the protocol to make things work," says Carvalho.

Next, Pires used specialist software to match recovered peptides from the red paint to proteins in various databases. The initial hypothesis was that the paint could contain milk, eggs, and/or resin, which have all been used as paint binders in the past. But the initial untargeted protein search revealed the presence of blood in the red paint.

The McCullagh Group, Department of Chemistry, University of Oxford



The tomb where the mask was found

Izumi Shimada

The team found matches to human blood and bird egg proteins, including immunoglobulin heavy chain, immunoglobulin G, serum albumin, and ovomucoid. However, identifying the specific species of bird proved difficult.

"There's a big difference between having a match to a particular protein sequence that may be the same across many species, and having a match to a peptide that has a species-specific amino acid sequence." says McCullagh. "The primary amino acid sequences of some proteins are the same between species, others differ in certain places – there's some luck involved."

The chicken or the duck?

Intriguingly, the initial search against the egg database gave strong matches for chicken proteins – in fact, there was a perfect match to a particular chicken subset. The problem? It is thought that chickens were brought to the region by the Spanish several centuries after the mask was buried.

"It's fascinating," says Carvalho. "Chicken bones that date back to a time before the arrival of the Spanish have been found in Chile, but similar evidence has not been found in Peru. The genomes of chickens are very similar to those of ducks, so we hypothesized that the paint contained duck egg. However, just because we have no evidence of chickens having made it to Peru before the colonizers doesn't mean they weren't there. In archeology, we have to create stories based on very small samples of what survives."

McCullagh admits that the question of whether samples are representative is a problem for all archeologists. "When you ask questions about the past you have to be open to many possibilities. We can't rule something out just because our current interpretation of the past is a certain way. More so than many other fields of study there is often only a very limited amount of material available so we must be careful about making general interpretations."

The findings also raised further archeological questions about the significance of the human blood proteins found on the mask. The team needs data from other cinnabar-paint samples, such as from the skeleton found alongside the mask, and from other masks from the same region – and these are

"In an ideal world, we'd have access to the latest techniques but there's a cost associated with access, both in terms of running a sample and the time it takes to process data."

now the subject of follow up work. "If we could confirm that blood was used in all Sicán cinnabar paint, then we may be able to extrapolate that the Sicáns understood the importance of human blood as a requirement for life," says Carvalho. "Perhaps the reason the cinnabar paint was used for elite objects, such as gold masks, is that they thought it was important for the rebirth of their leaders in the afterlife. And we know human sacrifice was common among Latin American cultures at that time…"

The location of the tomb raises other interesting questions, says Carvalho: "The tomb was actually flooded, which meant looters could not get to it. Archeologists had to wait for the dry season to be able to reach the main chamber where the mask was found. Obviously, the river must have been dry when the tomb was created, which makes one wonder how different the climate must have been then, and whether the demise of the civilizations in Latin America had something to do with a climatic event…"

The importance of preservation

"The challenge for archeological science is that it is a poorly funded discipline," says Carvalho. "In an ideal world, we'd have access to the latest techniques but there's a cost associated with access, both in terms of running a sample and the time it takes to process data."

McCullagh notes how the technique one chooses (or has access to) drives a certain set of questions – and so the answers are not always the answers that are most useful. "GC-MS is used a lot for lipid analysis in archeological residue applications – but taking a lipid-centric view of residues by default, may not always be the right approach. LC-MS-based proteomics has been around for some time and is becoming increasingly sensitive and able to analyze smaller and smaller amounts of sample, and that lends itself very well to archeology – besides the cost."

Proteomics has been applied in archeological contexts in recent years, but rarely for residue analysis. "It has been pioneered effectively for species analysis however – to look for unique peptides in bone fragments that indicate speciation, for example," says McCullagh. "But it is rare to see proteomics used in archeology to look at residues – in fact, it has only become possible in recent years with expanding protein libraries. Luciana and Elisabete's analysis has shown very convincingly that it can be an important tool in the toolbox for answering archeological questions beyond species identification of bone.

"I'm very fond of using multi-analytical protocols – borrowing from inorganic and organic chemistry – because these are artificial boundaries. The idea is to see how the data provided by one technique can link up with a different sort of data from another technique to give a holistic view of the sample," says Carvalho. "Analytical techniques, especially in archeological contexts, can only give snapshots of the truth – not the whole truth."

Distribution of burial contents in cross-section – mask at the bottom of item #10

Simulated Moving Bed: IMPRESSively Sustainable

How KNAUER contributes to an EU sustainability initiative with chromatographic knowledge and the application of simulated moving bed technology to recycle solvents and reduce waste

By Svea Stephan, Application Specialist at KNAUER Wissenschaftliche Geräte GmbH

In the past, the core components needed to produce materials such as polyesters could only be sourced from fossil fuels. But things are changing as the industry recognizes the need to move towards more sustainable practices – especially new biorefining approaches for the production of intermediary chemicals. The EU's IMPRESS Project brings together 10 leading companies and several research and educational organizations to create a process in which these intermediates can be produced using renewable resources, such as byproducts from the forestry and agricultural industries.

Sustainability is a key part of the corporate philosophy at KNAUER, and we have established environmental-friendly processes in all of our departments. To name just a few examples: the entire company uses only green electricity, we optimize manufacturing processes through life cycle assessment, and we always consider the ecological impact of new products during development. Our philosophy and actions, in combination with our expertise in chromatography, meant we were a great fit for IMPRESS – and we have been involved since September 2019.

Many goods can be produced using second-generation biomass, which is first pretreated and converted into sugars and sugar alcohols using process technologies, such as fermentation and filtration. Chromatography is then used for the separation and purification of these products – and that's where KNAUER steps in.

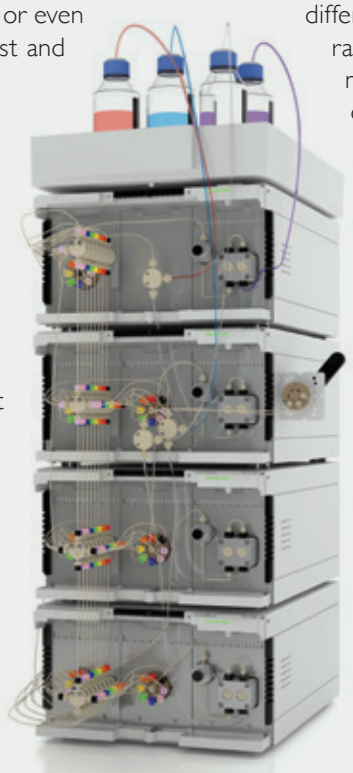
In fact, KNAUER has a wealth of experience in the use of different chromatographic techniques for the separation of sugars. And we knew of a technology that was not only very well suited to the application – but also highly sustainable and able to slot into a continuous process: simulated moving bed (SMB).

Introducing SMB

SMB systems continuously separate a product stream into two fractions. The continuous mode of operation is a huge advantage at the production scale because the need for equipment maintenance and cleaning is very low. Once the process reaches a steady state, an SMB system can run efficiently for days or even weeks. In short, it's fast and productive.

But that's not all: SMB technology is also highly sustainable. The majority of the solvent used is recycled in the system and only a low level of dilution is required. Specifically for the IMPRESS project, most of the necessary sugar and sugar alcohol separations can be performed using water as the mobile phase.

KNAUERs SMB systems are incredibly flexible, and can be used in many different separation modes, allowing them to be configured for varying separation tasks. In addition, they are also available in different materials and pressure ranges. And once a separation method has been developed on our SMB system it can be quickly scaled up to pilot scale.



Through our involvement in the IMPRESS project, KNAUER is even better positioned to develop individual and innovative solutions for your needs – including those involving SMB – whatever your industry.

To learn more about SMB and the IMPRESS Project, watch this video: https://www.youtube.com/watch?v=YLnQG_RzuH8

How We Work

KNAUER develops and manufactures a wide range of scientific instruments for chromatographic separations for use in both analytical laboratories and in production sites. Offerings include systems and components for analytical HPLC/UHPLC, preparative HPLC as well as fast protein liquid chromatography (FPLC) and multi-column systems, such as SMB.

Our lab has a team of interdisciplinary application specialists from a variety of professional backgrounds, enabling us to look at complex separation problems from several angles and to find the best solution for each task, to help with method development, or to optimize an existing method.

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Core Topic
Mass Spec

Dialing down the noise. High-resolution mass spectrometry (HR-MS) has been gaining impressive traction in recent years, particularly with respect to its application in proteomics. However, with increasingly large datasets being produced, distinguishing true, quantitative peptide peaks from the background noise can be challenging, often requiring manual inspection. In a new study, researchers from Kitasato University in Japan used a combination of six machine learning algorithms to successfully extract a higher number of peptide peaks with higher accuracy and precision than conventional methods. Crucially, their strategy focused on reducing the false-positive peaks seen in other machine learning approaches by assigning unanimously selected true peaks.

Seeing (and predicting) resistance. Antimicrobial stewardship describes a systemic effort within healthcare settings to limit overuse of antibiotics. A key part of this effort is selecting the optimal treatments for a particular disease, chiefly to limit the use of broad spectrum antibiotics and to improve patient outcomes. Current antimicrobial resistance testing methods are culture-based and slow. MALDI-TOF is already extensively used in microbiology labs to identify microbial species, but the lack of a comprehensive catalog of marker masses for pathogen–drug combinations has kept the approach out of the clinic. Now, a group of scientists from Switzerland have created a large-scale, publicly available database called DRIAMS, which combines mass spectra from clinical isolates with their respective laboratory-confirmed antibiotic resistance profile.

An IMS drug test. In the world of anti-doping, MS-based methods coupled to chromatography have reigned supreme. But emerging analytical techniques like ion mobility MS (IMS) could potentially offer unique advantages to the field, particularly in identifying novel or unknown anabolic steroids (AAS) – one of the most prevalent classes of performance-enhancing drugs banned by the World Anti-Doping Agency (WADA). Analytical scientists have now established an IMS workflow that meets WADA's minimum required performance levels for the detection of AAS in human urine.

Tapping ion trap potential. Hybrid quadrupole ion trap/TOF-MS instruments have the potential to offer high sensitivity, resolution and MS/MS capability in a number of fields, but analytical performance has been limited by ion trap parameters. In a recent paper, researchers studied the relationship between mass resolution, sensitivity and extraction phase angle by implementing a square waveform phase modulation strategy. By eliminating the influence of the extraction phase angle, the team were able to improve both the mass resolution and sensitivity.

IN OTHER NEWS

PreOmics, a spin-out from Matthias Mann's lab that develops sample prep tools for MS-based proteomics analysis, has announced €13.5m of series B funding provided by Bruker Corp.

Waters launches new MS Quan application for Waters Xevo mass spectrometers, allowing analysis of hundreds of small molecules in food and environmental samples in a single run.

Researchers discover a new ionization phenomenon – triboionization – that could improve transport efficiency and signal intensity in miniature mass spectrometers.

Gold nanoparticles/thiol-β-cyclodextrin-functionalized TiO_2 nanowires could enhance the performance of surface-assisted laser desorption/ionization mass spectrometry (SALDI MS) and MSI for imaging natural products.

References available online

Essential Mass Spec

We asked MS experts from the 2021 Power List: Is there a particular instrument you would not have been able to live without over the past 10 years?

Richard van Breemen

As a biomedical mass spectrometrist for 40 years, I have been fortunate to have had access to a wide variety of instruments including time-of-flight (ToF), ToF-ToF, ion trap, ion trap-ToF, quadrupole, quadrupole-ToF (Q-ToF), ion mobility Q-ToF, triple quadrupole, magnetic sector, FT-ICR, and orbitrap mass spectrometers. Every type of mass spectrometer has its strengths and weaknesses, and no one instrument has been able to address every analytical question. Therefore, necessity continues to drive innovation such that mass spectrometers of all types are becoming more widely applicable to diverse biomedical applications, while becoming more sensitive, more automated, and smaller.

Over the last 10 years, my research laboratory has relied primarily on two types of tandem mass spectrometers – triple quadrupole and Q-ToF instruments, both of which are fast and sensitive for biomedical applications. We use triple quadrupole mass spectrometers interfaced with UHPLC systems for quantitative analysis of small molecules, such as drugs, drug metabolites, natural products, and biomarkers. Equally important and essential to our natural products research as well as to our drug discovery and development effort has been the Q-ToF mass spectrometer interfaced with UHPLC, due to its high resolution and accurate mass capabilities.

Joseph Loo

I've been blessed to have access to a variety of different mass spectrometers, including Orbitraps, quadrupole time-of-flight with ion mobility, and Fourier transform ion cyclotron resonance instruments. To echo what Richard said, each has their strengths and weaknesses for the types of research projects they can address. Because my group is interested in a wide variety of areas, we like having all kinds of different instruments, and I

feel that my students benefit from the variety of tools we have. But my favorite instrument was a magnetic sector-ion trap hybrid mass spectrometer with an electrospray ionization source we had when I worked in industry. It took forever to tune, and it required two separate data systems to operate it, but it was a joy to work with. You felt like you had total control of the ions as they traveled through the instrument. My students today would have hated it – slow, very little automation, and sometimes finicky. But I had fun!

Ruedi Aebersold

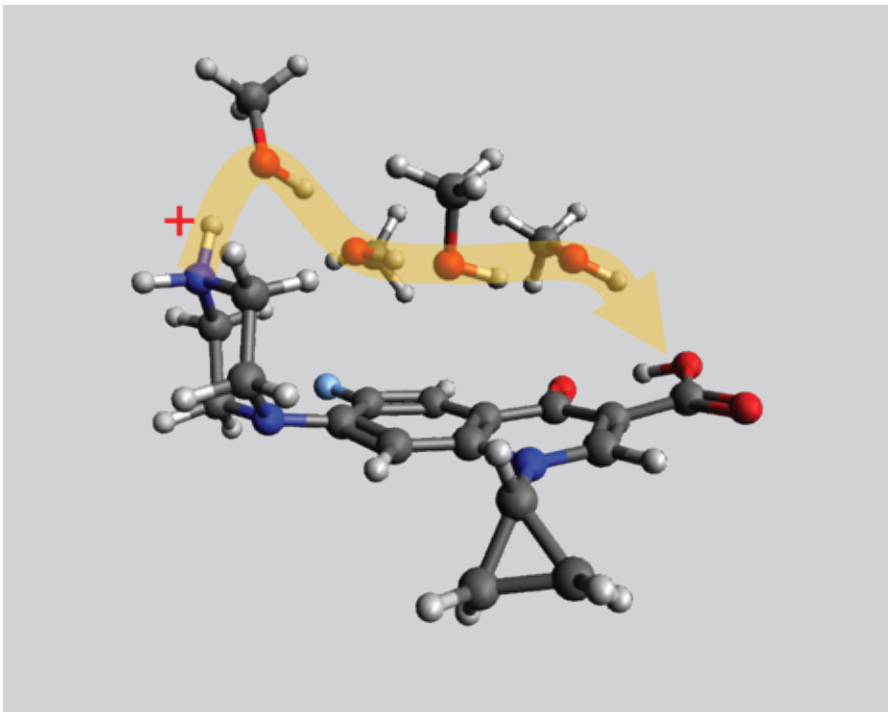
I'd say an ESI tandem mass spectrometer. Essentially all our work depended on these types of instruments – without them, proteomics as we know it today would not be possible. The progress in the performance of the instruments over the last couple of decades has been incredible. Today, even the protein contents of single cells can to some extent be analyzed. Some speculate that other powerful approaches will displace MS as the key technology in proteomics. I don't think that this will happen anytime soon. For sure, some aspects of proteomics will be taken over by faster, simpler or cheaper techniques, which are now emerging. However, the incredible flexibility of MS to not only identify protein species but also determine their functional state, their interactions with other proteins or nucleic acids, or to indent the site and type of PTM's will remain key tasks for MS.

Koen Sandra

Without a doubt, high resolution mass spectrometry. It is unbelievable how many analytical questions we have addressed and keep addressing every day using this technology. From the in-depth characterization of large and heterogeneous therapeutic proteins like antibody-drug-conjugates, to the untargeted discovery of blood plasma metabolite and lipid biomarkers for the early detection of various diseases, to the identification of small molecule impurities in food additives. The evolution these instruments have been through over the last 10 years is enormous. Resolution is one thing, although often overemphasized, but I'm especially fond of the advancements in sensitivity, robustness, and data handling capabilities. Despite the latter, my advice to all mass spec geeks out there: don't forget to examine the data yourself; there is so much precious information to be found – and search algorithms won't pick up everything.

Understanding ESI

How exactly do neutral molecules become protonated in electrospray ionization mass spectrometry?



Some analytical scientists say mass spectrometry can be divided into two eras – before and after the invention of electrospray ionization (ESI); one such person is Adam Trevitt, who leads a laser chemistry laboratory at the University of Wollongong, Australia. In a recent paper (1), Trevitt and his team set out to better understand how neutral molecules become protonated during positive-ion ESI by investigating methods of detecting and separating protonation isomers.

Specifically, they looked at the antibiotic ciprofloxacin, which is known to have two possible protonation sites. Their findings showed that the resulting protomers could be resolved by differential ion mobility spectrometry. Here, Trevitt shares more details of the study.

What were you trying to achieve?

It is almost impossible to overstate the impact soft ionization processes like ESI have had on MS and chemical analysis. Generally speaking, soft-ionization means the process delivers molecules into the gas-phase intact or with limited fragmentation while leaving the molecule with charge (or multiple charges). A common ionization process is protonation or deprotonation of a neutral molecule. Since the site of protonation can affect fragmentation pathways, ion conformation and mobility, as well as ionization efficiency, we were motivated to investigate the ESI process. To do this, we have been developing methods to detect and separate protonation isomers – ions that only differ by the site of protonation.

Talk us through your approach…

We used a triple-quadrupole mass spectrometer (Sciex QTRAP 5500) equipped with a planar differential mobility spectrometer (DMS) cell (SelexION), which is situated between the electrospray source and the MS. A small adaptation to the throttle gas line enabled solvent vapor delivery after the mobility cell, which was used to deliver gas-phase solvent molecules to intercept these selected ions. The DMS operates as a filter and can be set to select a target ion population.

Once we proved that the DMS could separate the ciprofloxacin protonation isomers, we could select each isomer and expose it to solvent vapors. This approach allowed us to deduce how each protonation isomer responded to different solvent vapors and track any isomerization.

"It is almost impossible to overstate the impact soft ionization processes like ESI have had on MS and chemical analysis."

What are the key takeaways from your research?

One takeaway point from this study is that care must be taken in MS/MS experiments with polyfunctional molecules, as variations in protonation isomer populations can influence the product ion distribution and confound interpretation of fragmentation spectra (particularly, for example, in automated workflows). Secondly, the choice of solvents used in ESI MS experiments can play a large role in the generation and selective suppression of some protonation isomers.

Reference

1. *B Ucur et al., J Am Soc Mass Spectrom (2022). DOI: 10.1021/jasms.1c00331*

Lipid Characterization – Transformed

Using the advanced capabilities of the SCIEX ZenoTOF 7600 system to determine double bond positions in phospholipid fatty acid chains

Presenting highlights from Zhengzheng Zhang's webinar "Positional determination of the double bonds in fatty acyl chains of phospholipids using the electron activated dissociation (EAD) capabilities of the ZenoTOF 7600 system," which is now available to view on demand at: https://bit.ly/3qWT1KP

The field of lipid research has grown immensely over the last few decades. Lipids were initially identified as structural components of cellular membranes. However, researchers have come to realize that these diverse molecules are active players in many biological processes, including human disease states, such as Alzheimer's, metabolic syndrome, and lysosomal storage disorders. Key to understanding their roles is the precise determination of their structures – no simple task given that mammalian cell membranes are known to contain more than 1000 different phospholipids alone.

Although lipid species generally fall into classes that share specific subgroups and configurations, the diversity of lipid molecules is enormous. Phospholipids, also known as phosphatides, are a class defined by a hydrophilic "head" composed of a phosphate group and two hydrophobic "tails" derived from fatty acids. These are joined by an alcohol residue (usually a glycerol molecule) and form lipid bilayers through their amphiphilic characteristic. Phospholipids are a key structural component of all cell membranes, providing both a two-dimensional fluidity and mechanical strength protecting against rupture.

Characterization of lipids is a complex matter. Molecular composition is critical – but details about individual components, such as class, head groups, lengths of different fatty acids, modifications, attachment points, numbers, positions of double bonds, and even cis/trans configurations are all required for a complete description. As a result, the full structural description of lipid molecules is generally an arduous task that involves a series of characterization steps based on different methodologies.

Finding the double bond in the lipid haystack

The fatty acid residues attached to phospholipid molecules are one of the fundamental components that drive their biological activity. Fatty acid residues present as long carbon chains. Their lengths vary greatly, and they can be fully saturated or contain areas of unsaturation with single or multiple double bonds. The chain length and the presence and location of any unsaturation are both known to have a direct effect on both the lipid structure and function. Therefore, understanding their biological activity requires a full characterization of the fatty acid portion.

Because of this complexity, several techniques must be employed; there is currently no single commercial technology available to fully characterize lipids. However, to help simplify the process, we can employ the technique of electron activated dissociation (EAD) with a commercial system – namely, the ZenoTOF 7600 system from SCIEX.

Electron activated dissociation versus lipid characterization

EAD is a general term that describes a number of fragmentation techniques that take advantage of ion-electron or ion-ion reactions. A beam of electrons reacts with the molecular ions in the sample, creating an intermediate radical state that breaks into measurable fragments. Altering the energy of the electrons yields different fragment profiles, inducing fragmentation in different molecules. Using EAD with the ZenoTOF 7600 system and its Zeno trap allows the simultaneous capture of ions and electrons with high speed and efficiency – and the electron kinetic energy and density can be finely tuned. In short, the ZenoTOF 7600 system gives us access to versatile reagent-free electron fragmentation with high sensitivity and high reproducibility.

In fact, the flexibility of the ZenoTOF 7600 system and the sensitivity of the output allows us to identify the deeper structural components of the lipid and, importantly, the fatty acid tail groups. EAD can produce diagnostic fragments of the lipid head group allowing identification of head group variations. However, it can also reveal the composition of the lipid backbone (glycerol/sphingoid) and provide information on the regioisomerism. Importantly, it can also describe the chain structure further by identifying chain length, double bond position, and the associated stereochemistry. How? EAD produces a rich set of chain fragments originating from the intact precursor ion with a sequential loss of CH_2 from the fatty acid backbone. These fragments generally span the entire length of the fatty acid chains and help to confirm the lengths of the chains themselves. Inspection of the peak pattern observed at each sequential carbon loss can reveal the location of a double bond.

Analysis in perspective

To understand more about the relationship between a lipid's structure and its biological activity in health and disease, we need sensitive, reproducible, and ideally single-system methods for lipid characterization. With advancing technology like the ZenoTOF 7600 system, automation of the identification process is nearing a reality – and that will transform lipid identification and research.

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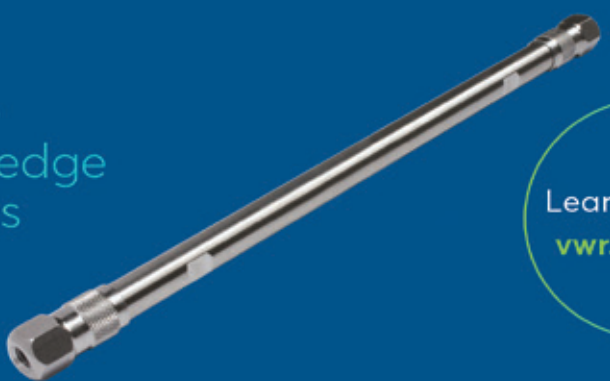

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Core Topic
Chromatography

Considering the Current State of Gas Chromatography

International GC×GC Symposium Chair and University of Alberta Professor James Harynuk argues that gas chromatography is very much alive

What is the state of GC today?

GC is doing well! You often hear phrases like, "GC is a mature technology" – people may take that to mean boring or dead, but I disagree. GC is far from dead – especially when we move to the area of GC×GC. In GC, there is interesting work on new stationary phases coming along, vacuum outlet GC is becoming more mainstream thanks to some plug-and-play columns that have been released recently, and when we get to GC×GC things are definitely looking up.

People are realizing that GC×GC isn't hard, but another platform you should use, at least initially, when looking at complex problems. In the area of GC×GC-MS, there is some legitimate competition on the instrumentation side, which is great for the field because competition brings innovation – good for everyone! There are more commercial options today because the community is growing. Five or so years ago, I could do a search for all the GC×GC papers published in a year and I would know pretty well all of the corresponding authors and many of the other authors on every paper. Those days are long gone, and that is great!

There are researchers working on all aspects of the technique, and GC×GC is becoming more of a routine tool to support interesting research projects in all kinds of areas. At last year's virtual GC×GC conference, we had over 400 people attending every day of the meeting.

What do you see ahead for GC in your crystal ball – and what developments will help it get there?

Looking into the GC and GC×GC future, I think that the communities are going to start demanding better access to data. I know a number of people in the field who are frustrated when they look at the relative ease with which LC-MS data can be accessed using third-party and custom-built tools. The GC-MS community needs to push vendors in this area. The community needs better tools to process data (especially from big studies with thousands of samples) and to develop custom solutions for processing data. To get there, we need easy, efficient, and headache-free access to raw instrumentation data.

You made the decision to go virtual with this year's GC×GC. Can you speak to the challenges of chairing an event in the current uncertain climate?

When you're planning an in-person event, by far the biggest challenge is predicting how many people will come in person. We did everything we could to make it as easy for people, but there are many factors that are outside of anyone's control. I think that people are realizing that international travel will not be as simple or inexpensive as it was before COVID, and a lot of people are willing to accept some extra hassle and extra costs. However, there is a point where people will say that the extra restrictions are too much. It is a very complex situation – you're trying to figure out if we will have enough people to justify an in-person meeting and to satisfy commitments with the venue. This was a big challenge.

In the end, we decided on the virtual route. Though this brings its own challenges, such as the significant learning curves with running our ticketing and online platforms. Even before the decision to go virtual, we had planned a significant virtual component as part of a hybrid event. We're also doing everything with volunteer time from members of the committee (thanks to all of you!) because we want to keep the meeting affordable for our attendees.

Popular Reflections: ProEZGC – Modeling on the Go

In 2019's "A GC in Your Laptop," Jaap de Zeeuw discussed ProEZGC, a web program that simulates separations allowing trainees or potential users to "play" with gas chromatography optimization. Here, we catch up on the easy GC journey.

Are you still using the ProEZGC web program to help students and inexperienced users?

Yes – and it is still spreading. I've been presenting workshops at universities for the past four years. These are two 90 minute sessions, which include exercises. Session one is method translation and optimization. Using the same stationary phase, the goal is to get the exact same chromatogram (peak elution order), but faster (for example by trading in some efficiency for speed) or at lower operational cost (like using N2 instead of He or shorter columns). I offer them five different situations in which they have to use the method translation software and they must choose from multiple-choice answers. Session two is modeling using the ProEZGC. Here, the phase is not defined; we want to find the phase that offers the best separation. After the phase is found, students can experience the impact of flows, carrier gas type, temperatures, and column dimensions. Normally in the lab, a GC needs to be configured – you need to purchase different columns and you need someone on the job full time. If that's all available, one can do 4–5 experiments in an afternoon. Now, with the modeling, one can do 400–500 experiments! In this session, they have to experience what happens with some peaks if you change program conditions, choose the best stationary phase for a set of 10 analytes and get the shortest possible run times by working with the options that the modeler provides.

We can measure the number of searches performed and we see a constant increase in usage. I also know that some universities have already adopted the program in their chromatography classes. I spoke with a university course director who wanted to pay the licence fee for using the program. He was amazed and happily surprised that there was no fee! In addition, Restek hosted a student from the latter university as part of his undergraduate thesis. He spent the summer performing experiments for the further development of ProEZGC at Restek's headquarters in Bellefonte, Pennsylvania, USA.

Have there been any advances in separation simulation technology since you wrote the article in 2019?

The accuracy of the libraries have been improved and over 2000 compounds and half a dozen different new columns have been added. We also worked on the addition of PLOT columns. We did a poster recently on the method translation for alumina columns using H2, Ne and N2. The matches were very good, so it is likely that modeling is also possible.

With regard to training and education, has the COVID-19 pandemic changed how you view the importance of modeling technology?

For me, COVID-19 has opened a new world of teaching. I did hundreds of webinars and never could reach so many people in so many countries.

The cool thing is that, with ProEZGC, you can do modeling anywhere – indeed, you have a "GC in your laptop" so you can do a lot of work at home or in isolation. I had access to a prototype eight years ago when I was at a conference in Singapore. During a discussion, someone stated that "nitrogen is a dinosaur gas" and should not be used – and I felt challenged to prove the opposite! N2 has a low optimum velocity but it's cheap! So, I used the modeler to simulate separations with N2 and found that you can replace a standard 30m x 0.25mm using helium for a 20m x 0.15mm using nitrogen and get exactly the same separation, in the same amount of time! I remember it was on a Saturday and I sent an email to my colleague Jack Cochran, who was in the US. He was curious and went in the lab on Sunday, took the most challenging column combination (using a Stabilwax column – when it works with a polar phase it will for sure work with a non-polar phase). He came back the next day sharing data that showed it was a perfect match. Later, this was tested with four other phases and four different component groups and it always worked.

Is there anything else you'd like to mention?

I am still excited about working with and presenting the program. I would like to see an option to show a T0 peak (methane), as well as a way to add some different peak heights for close eluting peaks. We can also consider different detectors and responses. If I can choose a solvent type, this peak could also be displayed. Also, the initial oven temperature depends on the solvent used and especially if I use the splitless injection technique.

Finally, I'd like to thank Chris Nelson and Chris English for their input.

The Decade's Biggest Chromatography Breakthroughs

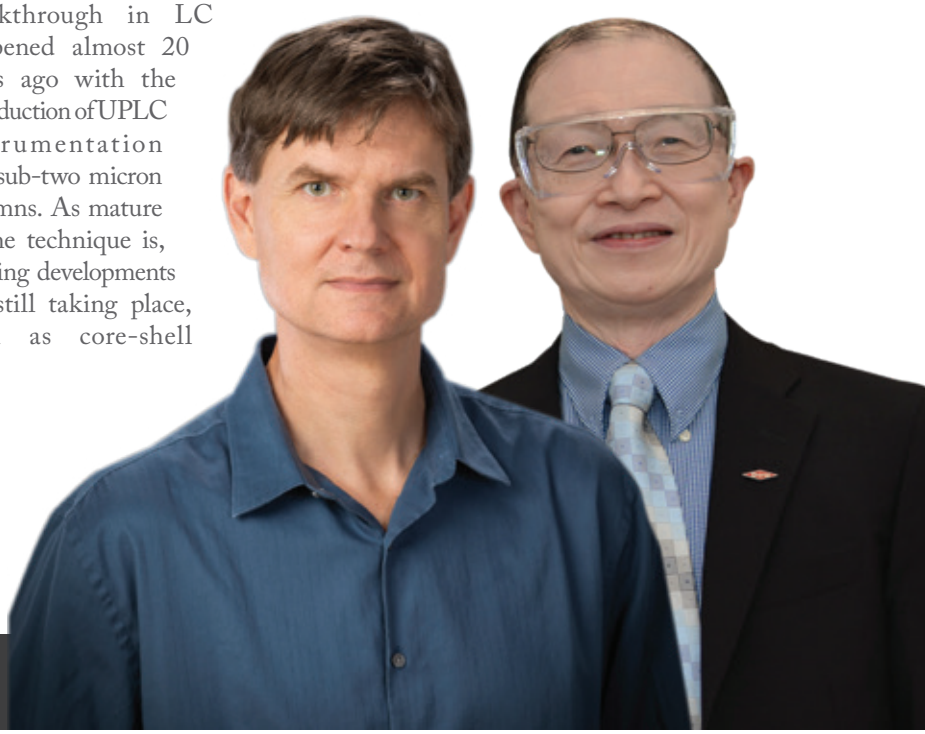
We asked chromatography experts from the 2021 Power List: what has been the biggest breakthrough in the field over the last 10 years – and why?

Ian Wilson: I'm afraid I have a long memory, so may I stretch the question to the last two decades? If so, I'd say the introduction of sub-two micron particles combined with pumps that could deliver the high pressures that make UPLC possible was truly transformative. HPLC had been languishing, slowly moving from five-micron packings to 3.5 (but frankly why bother: the gain in performance wasn't that great…) and 4.6 mm id to 3.0 or (if you were brave...) 2.1 mm i.d., columns. The gains were often marginal – a minor increase in sensitivity and a reduction in solvent consumption. Then UPLC exploded onto the scene with the chromatograms looking more like GC than LC. The data produced were game changing for complex mixture analysis. So, as a result of UHPLC, you could have the same number of peaks in a fraction of the time, or really explore the complexity of your sample with the same run time as your (now outdated) HPLC run. The field never looked back.

Paul Haddad: Method development in chromatography has traditionally been a laborious pastime because of the huge number of stationary phases and mobile phases available, coupled with the necessity to equilibrate the system with each new set of conditions before reliable retention times can be obtained. This has provided impetus for intensive research into methods for prediction of retention times without the need for experiment. This prediction process, entitled Quantitative Structure-Retention Relationships, enables retention to be predicted on the basis of chemical structure alone, without experimentation. The level of accuracy of these predictions only needs to be sufficient for deciding the type of stationary phase and approximate mobile phase composition most likely to yield the desired separation. A limited number of experiments can then be conducted to optimize the conditions.

Martin Gilar: For the most part, liquid chromatography (LC) is a mature technology (it's older than me!) and big breakthroughs are slow coming. Like Ian, I think the last major breakthrough in LC happened almost 20 years ago with the introduction of UPLC instrumentation and sub-two micron columns. As mature as the technique is, exciting developments are still taking place, such as core-shell technology, mixed-mode columns, or compact MS detectors. But it's fair to say that the improvements we are making in LC technology are incremental as opposed to ground-breaking. Perhaps the biggest tectonic shift over the last 10 years is the focus on the analysis of biopolymers, such as proteins, peptides, nucleic acids, oligonucleotides, glycans and lipids.

Jim Luong: As a field of practice and despite its maturity, the role of GC has not diminished or been replaced. Instead, its use increases with continual developments. Some examples of enabling technologies driving GC to new heights over the last ten years include digital transformation, advances in electronics for highly accurate pneumatic control, passivation chemistries to improve overall system inertness, microfluidic devices for the minimization of system void volume, and, most recently, the incorporation of catalysis and 3D printing to enhance flame ionization detection capability.



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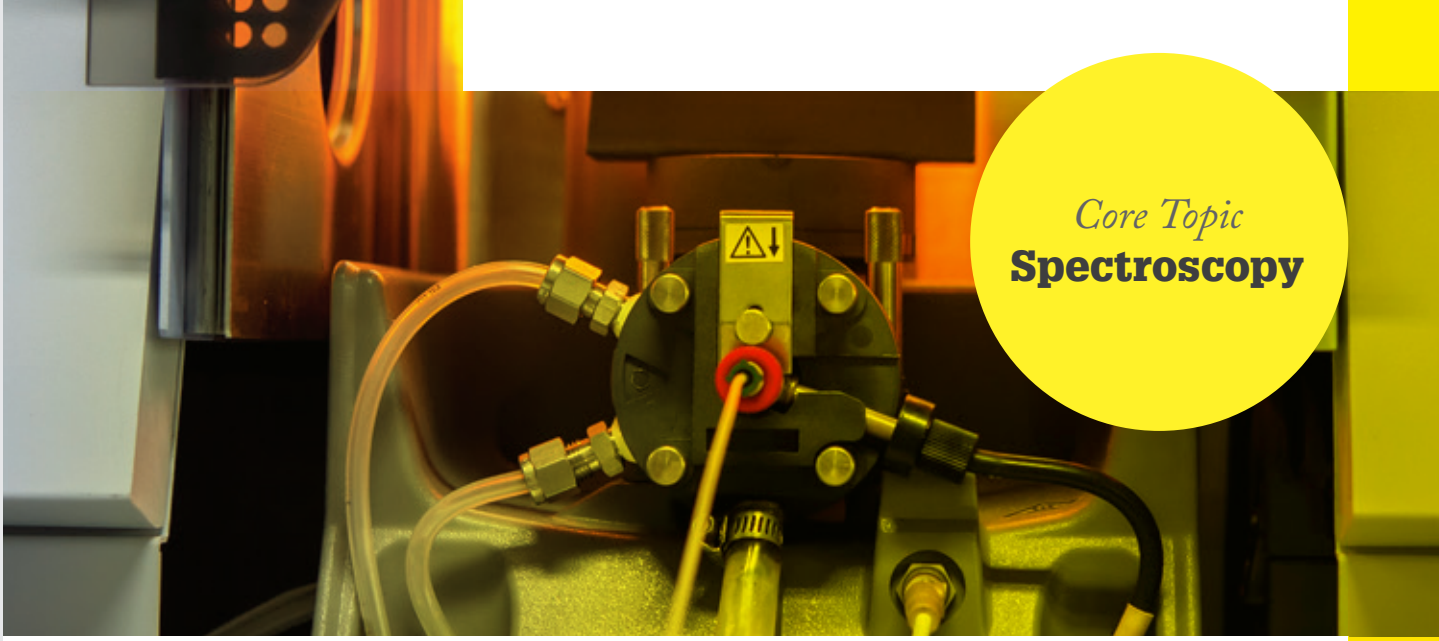

Core Topic
Spectroscopy

All hands to the pump. What connects quantum computers and migratory birds? They both rely on radical pairs… The observation of spin dynamics in radical pairs remains challenging, despite recent technological advances. Now, researchers from the Universities of Konstanz, Würzburg, and Novosibirsk have developed a new way to monitor the spin evolution in radical-pairs and read out the singlet/triplet ratio. Termed "pump-push spectroscopy," the technique allows "snapshots" of the radical pair's spin state to be captured at specific points in time. The researchers hope their work will lead to a better understanding of how migratory birds use the Earth's magnetic field to navigate across vast distances. It could also open up new avenues in quantum computing and in the field of organic solar cells.

Fragility glows. Photoluminescence spectroscopy could help reveal early signs of damage in concrete structures that usually escape detection via normal imaging inspections. Researchers from Rice University and the Kuwait Institute for Scientific Research discovered that Portland cement contains microscopic crystals of silicon emitting near-infrared photoluminescence. They applied a layer of opaque paint to a cement block and compressed it to induce microcracks, exposing the substrate's near-infrared emission and revealing the fracture locations, pattern, and progression. And because the luminescence varies slightly with the type of cement, the technique could also be useful for identifying different cement formulations.

MR spectroscopy for multiple sclerosis diagnosis. Early detection of multiple sclerosis (MS) is crucial for patient prognosis. Although conventional magnetic resonance imaging (MRI) can detect white matter lesions, alternatives are needed for improved detection of changes at the biochemical level. To that end, a team of researchers at the Medical University of Vienna used magnetic resonance (MR) spectroscopy with a 7-tesla magnet to compare the neurochemical changes in MS patients with healthy controls. They were able to visualize pathologic findings beyond lesions, with metabolic abnormalities detected in normal-appearing white matter and cortical gray matter. Based on the results, the researchers suggest 7-tesla spectroscopic MR imaging could be a valuable new tool in the diagnosis and treatment of MS patients.

Unusual collaboration for safe salmon supply. Lightsense Technology recently announced a partnership with Pure Norwegian Seafood AS to develop new multi-spectral instruments based on Enhanced Photodetection Spectroscopy (EPS) technology. The aim? To detect bacterial pathogens in salmon processing and, therefore, reduce the financial impact of food contamination (4). EPS technology has already been used to identify coronaviruses in human saliva and to detect bacterial pathogens in water.

IN OTHER NEWS

A heat resistant polymer has been developed with superior performance and at a lower cost, significantly expanding the scope of applicability of surface-enhanced Raman spectroscopy.

Hubble spectroscopy suggests black-hole outflows triggered star formation in dwarf galaxy Henize 2-10.

A wearable time-domain functional near-infrared spectroscopy (TD-fNIRS) device paves the way for more accessible noninvasive optical brain imaging.

Donald J. Douglas, a pioneer of inductively coupled plasma-mass spectrometry, receives the Lifetime Achievement Award at the 2022 Winter Conference on Plasma Spectrochemistry.

References available online

The Decade's Biggest Spectroscopy Breakthroughs

What's the biggest spectroscopy breakthrough in the last 10 years – and why? Four spectroscopists from our 2021 Power List have the answers.

Karen Faulds

There have been so many but I think an important one in surface-enhanced Raman spectroscopy (SERS) is that systems can be controlled to allow quantitative detection of bioanalytes which, coupled with the development of portable Raman spectrometers, has enabled so many great SERS assays to be developed that have real potential for translation into sensitive, quantitative, point of use clinical applications. Another area is the development of deep Raman approaches, pioneered by Pavel Matousek at Rutherford Appleton Laboratory, which opens up the possibility of meaningful Raman measurements at depth and in the future in vivo – and in humans.

Rohit Bhargava

The biggest breakthrough in our field has been the ready availability of tunable lasers across the mid-IR spectral region. Over the prior four decades, IR spectroscopy had become synonymous with Fourier transform (FT) spectroscopy. With the advent of the fast FT and computation, the multiplexing advantages of wide bandwidth IR spectroscopy made data recording so efficient that FT-IR spectroscopy became unparalleled in performance. With the availability of quantum cascade lasers, a widely tunable, high intensity source became available. This technology has changed our field – spurring the development of new instrumentation, fast imaging, and novel capabilities – and it promises to be the basis of many more advances.

Rachel Popelka-Filcoff

All the various forms of X-ray spectroscopy and their associated instrumentation have been invaluable to my research. From hand-held portable X-ray fluorescence (PXRF), SEM-EDS, to high-resolution X-ray microscopy (XFM), these methods each have their own advantages in compositional analysis and elemental mapping. In the field of archaeological science, techniques that are non-destructive and/or non-invasive are vitally important – especially when it's not possible to take samples from cultural heritage materials.

Duncan Graham

I really like the creation of new probes for sensing conditions/molecules inside cells using Raman and/or stimulated Raman scattering – I think this is really game changing. This approach makes use of the vibrational frequency of alkynes being in the cell silent region and then changing in response to different stimuli, for example, pH or glutathione. Amazing work has been coming out of Japan and the US in this area.

Quick Tips for LIBS

Laser-induced breakdown spectroscopy (LIBS) is high throughput, you can see the elemental distribution of heterogeneous materials, and you also can analyze samples in situ without any sample preparation. But that doesn't mean you can forget good scientific practice!

Laser-induced breakdown spectroscopy (LIBS) is a technique I've used to study geological specimens, forensic samples, as well as cultural artifacts. It allows me to see all elements, and it is especially good for the lighter elements – and there aren't many techniques that allow one to do that. LIBS also has a high sample throughput, while offering insight into the elemental distribution of heterogeneous materials. Finally, and a big plus for many, it can be performed with no sample preparation – you can analyze materials where you find them. All of these benefits attracted me to LIBS, but I was also aware that none of these benefits allow me to ignore good scientific practice.

Recently, I was helping a group of archeologists who had just purchased a handheld LIBS device; certainly, they could operate the instrument – but would the data they planned to gather be of any value? When you buy an instrument from a manufacturer, they'll tell you how to turn it on, how to adjust the settings, and so on. It is all very helpful, but they can't tell you how to do your research. I suppose it's a little like buying a new stove and having it professionally installed; if you don't know how to cook (and fail to consult a recipe), the results will likely range from inedible to dangerous. Likewise, I've reviewed papers from researchers who think they have interesting LIBS results to share, but clearly haven't fully understood the nuances. For example, one paper was trying to draw some exciting conclusions from an inadequately sized sample suite– but their results could never convince a reputable reviewer. I wish I could have whispered some things in their ear about experimental design before they entered the publication process…

Tip 1. Your data are only as good as the quality of your sample library. If you want to use LIBS in a qualitative sense, where you utilize machine learning tools to build classifiers for identification (something we've done for geological materials), you need a suitable dataset. And that means collecting representative data from a sample suite of appropriate size. In the beginning, I'll admit that I assumed I could zap a bunch of samples from various places to get a clear picture of provenance. But in reality, it's not that easy. LIBS can provide those answers, but only if you have sufficient numbers of representative samples.

Tip 2. Become a chemometrics expert – or collaborate with one. Looking back, I'm sure we can all find an example of where we were so excited about our results that we lost focus on the need for robust data analysis. Equally, if we revisit any past research, we might now approach data analysis differently, applying knowledge and lessons learned in the meantime – or benefiting from advances. I was trained as a synthetic organic chemist – and that's a long way from LIBS and data science! I've made mistakes but I also learned and evolved. In short, good science must be at the heart everything we do – whatever the technique or method.

Spectroscopic Diagnosis on the Fly?

How do spectroscopy-based devices fare among today's portable point-of-care diagnostic technologies?

Today, we wouldn't think twice about using a smartphone to take a high-resolution family photo – but what about a diagnostic image down to the single-molecule level? The camera technologies in many of today's smartphones, and other miniature microscopy- and spectroscopy-based devices, have these capabilities – and Qingshan Wei, Assistant Professor of Chemical and Biomolecular Engineering, and her colleagues at North Carolina State University, USA, set out to review them. We caught up With Wei to find out more about the current state of point-of-care diagnostic technologies.

Which approaches, in your view, are currently most promising?

It is difficult to beat the sensitivity of fluorescence microscopy – so we believe that mobile fluorescence-based detection platforms are the most viable approach to point-of-care single-molecule detection. Smartphone-based fluorescence microscopes have already been used to image single viruses and DNA molecules. Similarly, fluorescence spectroscopy could see more development in the future. Spectroscopy has not been as heavily explored as microscopy methods, but that is rapidly changing with the development of smartphone-based fluorescence and Raman spectrophotometers and other portable spectrometers.

What are the most promising spectroscopic approaches?

Fluorescence spectroscopy is able to elucidate the unique signals of different fluorescent tags for multivariate analysis. Raman spectroscopy has also recently been demonstrated on portable smartphone-based devices, and it is useful for label-free analysis of samples by examining the vibrational scattering signals of biomolecules of interest.

Which spectroscopic approach is currently the most advanced?

Fluorescence spectroscopy has been demonstrated on mobile systems with the use of plasmonic nanomaterials that enhance analytes' fluorescence signals. Plasmonic enhancement has also brought traditionally less sensitive techniques – such as Raman spectroscopy – to point-of-care devices. Though great progress has been made with fluorescence spectroscopy, Raman and other spectroscopy methods have yet to achieve the single-molecule level of detection on true portable systems.

What are the main barriers to the development and commercialization of single-molecule spectroscopic methods?

Sensitivity and resolution are always key limiting factors in the performance of analytical tools. For imaging single molecules on true portable platforms, the sensitivity and resolution must be extremely high and the background noise must be eliminated to the fullest extent. This can be easier said than done on a portable platform that may be used in the field. Additionally, the platforms require much more power than a smartphone alone due to the use of lasers and other components. Powering the devices with portable sources can present obstacles to applying these devices in resource-limited settings.

How far away are we from a widely available, low-cost, point-of-care tool for single-molecule analysis?

Our review covers several new technologies that are able to demonstrate single-molecule and particle detection on true portable platforms. We expect these devices to be available to healthcare personnel first before becoming available to the public as commercial at-home testing devices. One example of a device that could see rapid implementation in public health would be single-particle fluorescence imaging on mobile platforms for detecting single nucleic acids or particles of SARS-CoV-2.

Hyperion II: Next Level Microscopy

The Hyperion is one of the most successful infrared microscopes in history – but innovation can never stand still. We spoke with Tom Tague, Applications Manager at Bruker Optics, to find out how he and the Bruker Optics team took Hyperion to the next level...

How did you approach developing the Hyperion II?

When contemplating how to take Hyperion to the next level, we realized it had the potential to make a big difference in the microanalysis community, but we needed to go back to the drawing board and incorporate several new technologies.

One of the major issues in spectroscopy is that you're always working in one of two different regimes: noise-limited and signal-limited. If you're in a noise-limited regime, the molecules are all illuminated and you need to see the average to improve your detection limits. Here, the noise of the detector and the system in general is crucial; the sensitivity for getting more photons isn't important. In a signal-limited regime, you're looking at potentially thick samples (in microfluidics, for example, where water is absorbing and impeding flow) and you need to "turn on the lights" with a longer pathlength. This requires a brighter source.

Our bright spark originated from our acquisition of IRM^2 four years ago. IRM^2 developed the important interface to quantum cascade laser (QCL) technology we incorporated into Hyperion II. This technology delivers brightness orders of magnitude greater than before, vastly improving the pathlength. As a result, users can analyze large areas rapidly even in a signal-limited regime – the size of a business card in a few minutes, for example, which is previously unheard of at five microns' spatial resolution.

What other features does the Hyperion II have?

Bright sources tend to be narrow in spectral coverage. So if you're not in a signal limited regime, why not use a glowbar? It has low noise, with noise being further reduced by averaging and has excellent spectral coverage. We felt strongly that the Hyperion II needed to be capable in both signal- and noise-limited regimes. So, with the flick of a switch in the software, you can collect an image with either traditional FTIR source-based imaging or the QCL laser source – and the software seamlessly manages handling the data.

It's also worth noting that these are large datasets – you're talking millions of spectra. If you're looking at microplastics or tissue characterization, the file sizes can also be very large. To combat this, we implemented another new feature – active image processing. As an image comes off the hopper, one monitor shows the results while the computer simultaneously does a metric analysis.

Who will benefit most from these new features?

We did not develop Hyperion II for innovation's sake alone. The combination of FT-IR and QCL technology will provide new opportunities for researchers in a wide range of fields, including pharmaceuticals, forensics, polymers and plastics, semiconductors, and more. But there's one field in particular that will benefit – microplastics, one of the greatest obstacles currently facing mankind. In fact, throughout the development of this product, we worked closely with groups in the US and Europe to ensure the device could be used by non-experts in the microplastics field, who need to be able to analyze a lot of data quickly, accurately, and with a high signal-to-noise ratio.

What does having been involved in the Hyperion II's development mean to you personally?

Having been involved in trying to incorporate bright sources into a spectrometer for over a decade, I can tell you that simultaneously ensuring stability, coverage over the spectral range, and cost-effectiveness is not easy! To have accomplished this goal is a real technical achievement, and I'm proud to have been part of the team.

This combination of technological innovation in microscopy and real practical application is what has made working on the development of the Hyperion II one of the highlights of my career. I'm excited to see what doors it will unlock for researchers in the near future!

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Core Topic
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Mass photometry in AAV characterization. A big challenge facing the gene therapy field is developing analytical tools to ensure purity and safety of the vectors used to deliver genes – such as recombinant adeno-associated viruses (AAVs). Now, researchers from the National Heart, Lung, and Blood Institute, USA, have applied the recently developed single-molecule technique, mass photometry (MP) – which measures mass distributions of biomolecules – to AAV characterization. They found that MP can measure heterogeneity, relative species content, packing efficiency, and other attributes of AAVs accurately, reproducibly, quickly, and with minimal sample preparation. "Those attributes make MP an attractive tool for the biophysical characterization of viral vectors in industry and academic applications," the researchers concluded.

Separating the genes from the chaff. Another challenge in AAV production is the presence of capsids that lack the required gene of interest, causing purity issues and potentially immune responses that reduce the effectiveness of the therapy. In a recent study, a team of Pall researchers used a novel anion exchange chromatography elution method to separate empty and full AAVs. Initially the results did not meet the team's separation expectations, which they thought was related to membrane chromatography devices tending not to have a sufficiently large number of theoretical plates. So they switched to a step gradient, which provided better AAV enrichment.

Agilent teams up with Lonza. Sticking with analytical challenges in advanced medicine, Lonza and Agilent are collaborating to integrate new analytical technologies into Lonza's Cocoon – an automated and closed platform for cell therapy manufacturing. The idea is to bring real-time information on Critical Quality Attributes (CQA) so that the manufacturing process can be "directed" in real time. This approach could be particularly valuable for autologous cell therapies, such as approved CAR T-cell therapies Yescara and Kymriah, because of the inherent variability of the starting material – cells taken from a patient, which are then genetically modified and infused.

Best practices for aggregate analysis. The cell and gene therapies mentioned above look to large-molecule therapies – especially protein therapeutics – as a model for rapid progression from an emerging to an established therapy. As such, many analytical techniques that help ensure the quality of protein therapeutics are now well established; for example, analytical ultracentrifugation (AUC) is used to characterize soluble aggregates that can reduce drug efficacy and safety. In a recent review article, industry and academic researchers explore the key considerations and best practices for aggregate analysis – summarized in a handy table.

IN OTHER NEWS

Protein Metrics will sell their Byos software with Agilent's liquid chromatography-mass spectrometry (LC-MS) systems and will work on common applications as part of collaboration.

Researchers use high-speed fluorescence image-enabled sorting to isolate cells with complex cellular phenotypes.

Depixus, a biotech developing technology that immobilizes DNA within a flow cell to detect changes, such as base modification patterning, raises €30.6 million in Series A financing.

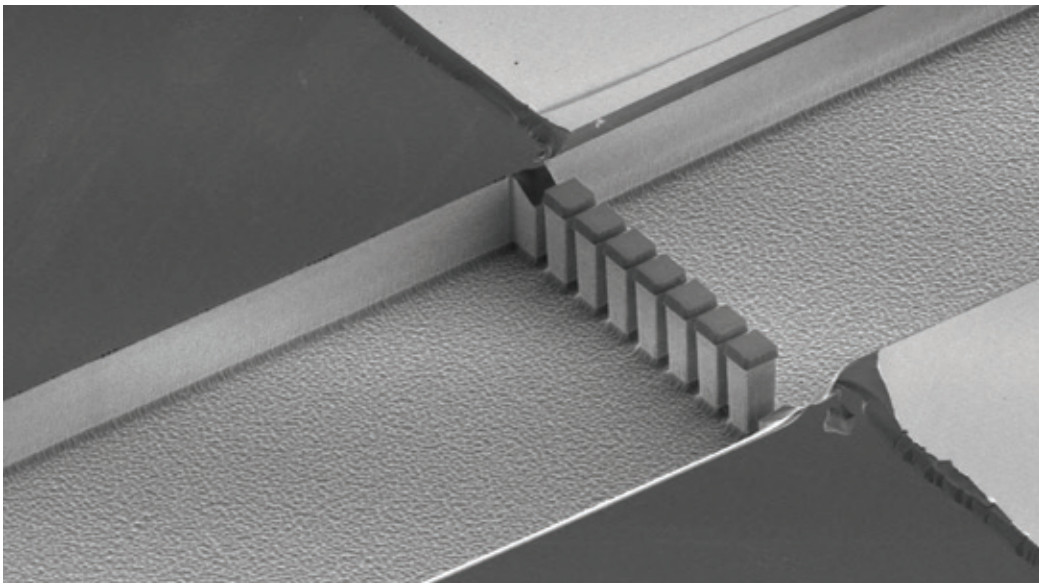
Scientists at Scripps Research use high-resolution, low-temperature electron microscopy (cryo-EM) to rapidly characterize antibodies – elicited by a vaccine or infection – that bind to a desired target on a virus at the atomic level.

References available online

Dynamic Analytics for Advanced Medicine Manufacture

How an integrated nESI-MS-based sample-to-analysis platform aims to automate quality monitoring in cell therapy bioprocesses

Credit: Georgia Institute of Technology. The cell immobilization features inside the cell processing device



Cell-based therapies have been described as revolutionary for their ability to treat – and even cure – life-threatening diseases, including cancer. But the absence of real-time analytical techniques to understand what is happening in cells at the biochemical level during manufacturing is a challenge in successful development. What's more, the critical quality attributes (CQAs) that correlate to therapeutic function, performance and potency are unclear and the critical process parameters (CPPs) that impact quality throughout cell therapy manufacturing are largely unknown (1).

"Current real-time process analytical technologies (PATs) are necessary to maintain culture viability, but do not provide information on CQAs that more accurately predict cell state," says Andrei Fedorov from the Georgia Institute of Technology, USA. "Real-time PATs involving Raman spectroscopy have been effective in monitoring certain biomolecules, but these PATs lack the sensitivity, selectivity and dynamic range to detect a diverse set of biomarkers at low concentrations. The most effective methods to identify CQA biomarkers – namely, high performance liquid chromatography mass spectrometry (HPLC-MS) and enzymatic assays (for example, ELISA), are generally offline, which limits their utility as inputs for feedback control."

These issues have led to a substandard, low-yield system that relies on analytics at the end of production, which is not only expensive but also time-consuming – and time is a luxury that many patients do not have.

Noting the clear need for new analytical technologies – especially in the manufacture of advanced therapies, Fedorov and his colleagues developed the Dynamic Sampling Platform (DSP) (2) – an integrated sample-to-analysis system that combines a sampling interface, cell processing device, and detection by nanoelectrospray ionization mass spectrometry (nESI-MS).

The team applied their DSP to an intracellular analysis of 1500 human umbilical vein endothelial cells (HUVEC) and were able to detect nearly all proteogenic amino acids, as well as almost all key metabolites identified as HUVEC specific biomarkers.

"The platform has demonstrated the capability to rapidly detect clinically relevant intracellular biomarkers – compounds that were previously identified via conventional HPLC ESI-MS and a long analysis time," says Fedorov. "DSP also replaces numerous manual handling steps; works with ultra-small cell samples; is capable of self-regeneration for long-term, continuous operations; and is suitable for integration into cell growth bioreactors for direct-from-culture analysis."

Given its ability to identify new CQAs and monitor known CQAs in biomanufacturing, the researchers believe DSP could help in the development of cell therapies with increased safety, efficacy, potency, and overall quality.

"The ultimate vision is to provide fully automated, multi-omic monitoring of cell manufacturing workflows," says Fedorov. "The resulting multimodal technology platform would enable biochemical readout of both the extracellular and intracellular environments in real-time. And it would represent a crucial milestone in enabling fully automated quality monitoring with integrated feedback control in cell-based therapy manufacturing – two challenges that must be overcome if the transformative potential of cell therapies is to be fully realized."

Waters and Sartorius Collaborate to Accelerate Bioprocess Development

Is collaboration the key to accelerating cell line clone selection? We caught up with Davy Petit, Senior Director of Global Pharmaceutical and Biomedical Research Business at Waters, to find out.

What are your main aims for the project?

Biopharmaceuticals are long overdue for faster, more effective, and more accurate clone selection and process development – and the collaboration between Waters and Sartorius is specifically focused on optimizing cell line clone selection. We want to maximize drug product quality, yield, and manufacturing efficiency – all while reducing the development timeline. Ultimately, we want to fully integrate process control, monitoring, and product quality testing into the manufacturing environment.

The pivotal process of clone selection typically takes up to 10 weeks. We want to cut that time by developing a solution – based on Sartorius' Ambr multi-parallel bioreactors and Water's small footprint LC-MS instrument, BioAccord (1) – that will provide bioprocess scientists with vital upstream analytical data about recombinant proteins within 48 hours (compared with the 3-4 weeks that it takes today). We believe our approach has the potential to accelerate bioprocess development and reduce the number of cell culture runs from six to perhaps one or two.

"Ultimately, we want to fully integrate process control, monitoring, and product quality testing into the manufacturing environment."

Do you think mass spectrometry is underused in biopharma process development?

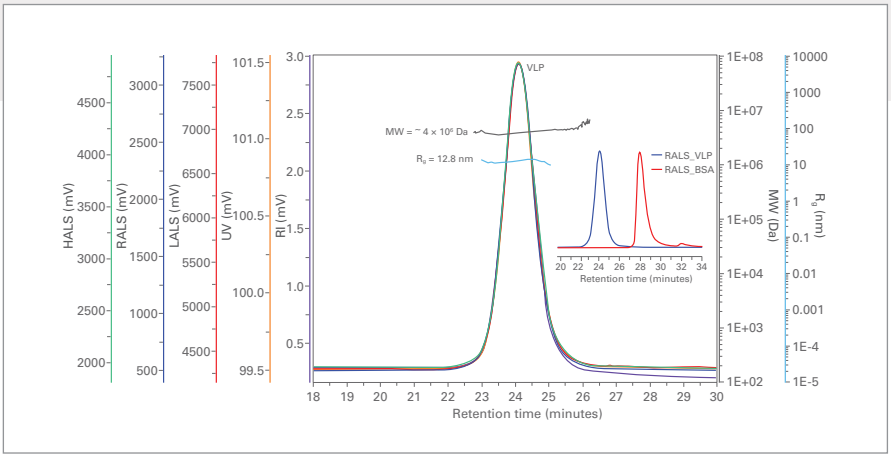
Well, analytical groups use mass spectrometry to support their process development colleagues, but the separation of process and analytical functions often causes inefficiencies. As process labs do not have direct access to MS instruments, they are at the mercy of another laboratory to generate data.

I think wider adoption is limited by misperception; mass spectrometers are often assumed to be large, complex, and difficult to use – and that can sometimes be the case. And it's also why we are working with Sartorius to develop appropriate applications for clone selection and upstream process development. Theoretically, users should be able to seamlessly adopt the solution and see the direct benefit of MS workflows without the complexity.

Do you have any advice for other biopharma companies who might be interested in implementing more advanced analytical instrumentation in their processes?

Many companies are looking at more advanced use cases, such as online feedback control in later stage pilot or production environments, but the adoption cycles are long, and the solutions require customization. If they start with early-stage process development with standardized at-line solutions, they can benefit more rapidly from the technology. And the experience they gain can facilitate the adoption of more advanced use-cases in later-stage production environments.

Molecular Weight and Size Determination of VLPs Using Multi-Angle Light Scattering



Virus-like particles (VLPs) – protein structures that mimic native viruses, but are non-infectious – are of increasing interest as potential candidates in new vaccines and gene therapy products. But thorough characterization of VLPs is required for their development and final product quality control.

Size exclusion chromatography (SEC) coupled with multi-angle light scattering (MALS) provides valuable molecular weight (MW) and size (radius of gyration, Rg) determination while enabling VLP detection even at extremely low concentrations.

Parvovirus VLP was analyzed on various TSKgel PW$_{xl}$ SEC columns connected to a LenS$_3$ MALS detector, in addition to RI and UV detectors. In one experiment, the molecular weight and Rg values were measured and compared with literature values. In the other experiment, multiple dilutions of the VLP solution were made and analyzed to assess the limit of detection of the LenS$_3$ MALS detector.

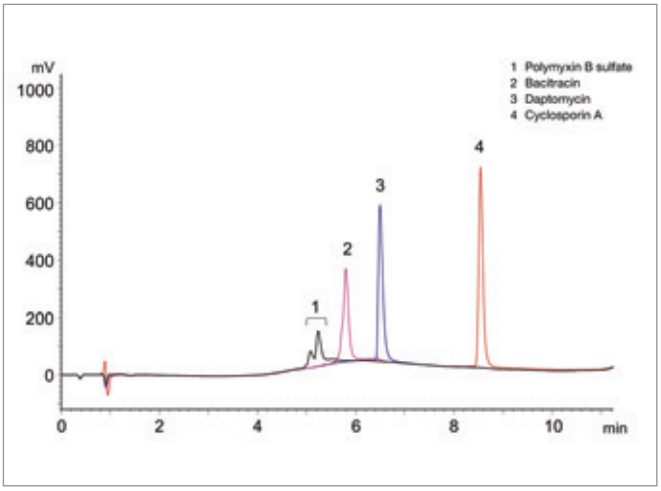
https://www.separations.eu.tosohbioscience.com/OpenPDF.aspx?path=~/File%20Library/TBG/Products%20Download/Application%20Note/a21i08a.pdf

UHPLC Analysis of Cyclic Peptides Under LC/MS Compatible Conditions

Cyclic peptides are characterised by polypeptide chains that are arranged in a cyclic ring. This structure offers many advantages over their linear counterparts; for example, higher thermal stability and a higher resistance to digestion – whilst their rigid structure results in a better biological activity due to improved binding to the target molecule (1).

Cyclic peptides have various applications too. The peptides in this application note, polymyxin B sulphate, daptomycin and bacitracin are antibiotics, whereas cyclosporin A is used as an immunosuppressant.

Based on the hydrophobicity of the peptides, a YMC-Triart Bio C4 column was chosen for the separation. Since a relatively high concentration of acetonitrile is needed for elution, a C4 modification is a good choice as it provides longer retention times. As is the YMC-Triart Bio C4, due to the fact that sharp peaks are obtained using a LC/MS compatible mobile phase containing formic acid at an elevated temperature of 70°C.



Reference

1. *DA Horton, J Comput Aided Mol Des, 16, 415–430 (2020).*

Download the application note with the full method details here (https://ymc.eu/d/brDmO).

Spotlight on... Technology

WITec alpha300 Ri – Inverted Confocal Raman Imaging Microscope

The alpha300 R*i* offers 3D confocal Raman imaging through an inverted beam path to enable sample investigation from below. This is a great advantage when working with aqueous solutions, oversized samples and environmental enclosures. All modular components and upgrade possibilities for the alpha300 series are compatible with the R*i* version.
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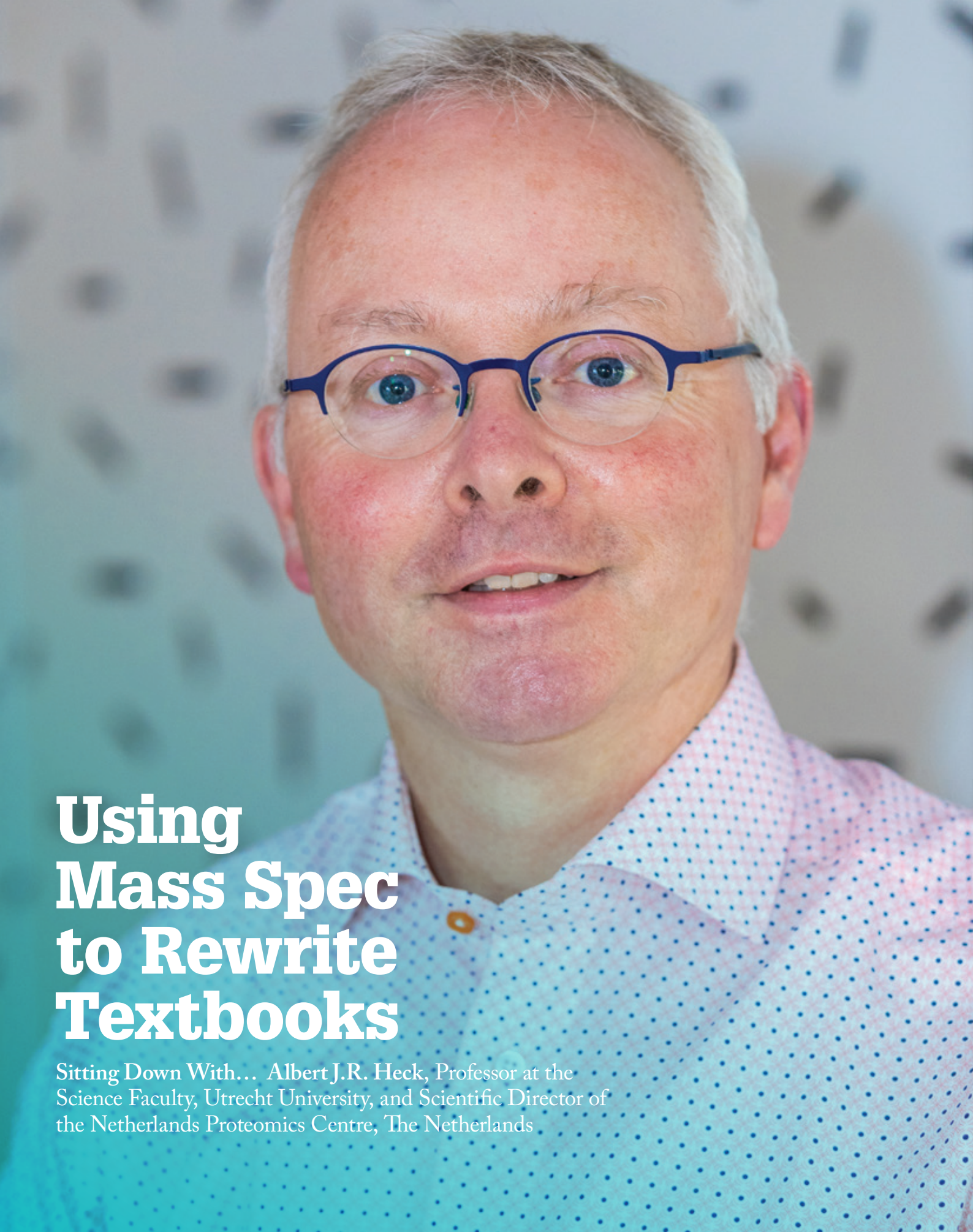
Transforming Chromatographic Data into Meaningful Results

ChromCompare+ is an easy-to-use data analysis and chemometrics platform for aligning and comparing chromatographic data in automated workflows. A batch of GC–MS or GC×GC datafiles, in a wide range of formats, can now be compared in minutes instead of days – or even weeks.
Find out more at https://chem.sepsolve.com/Spotlight/CC



Using Mass Spec to Rewrite Textbooks

Sitting Down With… Albert J.R. Heck, Professor at the Science Faculty, Utrecht University, and Scientific Director of the Netherlands Proteomics Centre, The Netherlands

Tell me a bit about your work at the Netherlands Proteomics Centre.

The Netherlands Proteomics Centre is one of the largest mass spectrometry labs in Europe, and I'm currently heading up a team of around 60 people. We're a diverse bunch of people – with 19 different nationalities represented – but we're also diverse in terms of our academic backgrounds. Many in our team are analytical chemists, but we also have biochemists, immunologists, engineers, bioinformaticians and programmers.

And what are the main areas you work in?

Again, it's pretty diverse. At the broader level, I'd say there are two main spaces we work in: i) technology innovation and ii) fundamental knowledge in biology and medicine. On the one hand, we work to develop better tools for MS – or try to do things that have never been done before with MS, whether that's intact protein analysis, crosslinking MS, or the analysis of post-translational modifications. Here, we may work closely with vendors to help them improve their instruments or ask them to implement certain useful features.

On the other hand, we work on basic research questions in science, like cell signaling events or immune responses. And that means the application aspect of our work is quite broad. One exciting area at the moment (for me at least) is measuring the proteomes of single cells. Why do certain cells react differently to different drugs or changes in the environment? Here, the sensitivity of mass spectrometers is something we are really keen to develop. It's also extremely exciting to see both our group and others making single-molecule measurements by MS. We are now developing tools to make this even easier and to ensure the technology is more accessible.

What drives you?

I'm a mass spectrometrist at heart. So I'm driven by a (perhaps naïve) view that MS can answer all sorts of questions in biology. I'm constantly asking, what's the next big question we can answer with MS? Is there a question we never even thought about answering with MS? Outside of MS, I'd say what really drives me is a yearning to understand how life works. There's so much we don't yet understand – but whenever we do understand something new it seems all the more beautiful.

Where did your initial thirst for all things MS come from?

Honestly, I don't know. I never woke up as an eight year old kid and said, I want to work in proteomics! It's something that has developed naturally by being presented with chances and challenges – and by making decisions. I trained as a physical chemist – I was more interested in how atoms worked and how chemical bonds were formed. After making a few decisions about my career, I entered the field of analytical chemistry; initially, I wasn't even sure how interesting it would be! At the time, I was more intrigued by physical chemistry – possibly because there seemed to be too many unanswered questions in biology. But over the years, the ability to get real-world data has really taken off in biology – and I think that triggered my move into these fields. People often ask me if I'm a biochemist or an analytical scientist or an immunologist… Basically, I'm MS-based – but I want my expertise to be useful across almost every field.

Would you say you've been successful in your career?

I think it's always tough to measure your own success. What I'm most proud of is watching young people come into our group, get trained up, grow and mature, and then leave to do their own thing. They often go on to become experts in industry or academia and become very successful in the next step of their career – it's great to see what they've all achieved over the years. And I guess that's one way of measuring success.

And if you can change the textbook – on biology or immunology say – then that's also a real success. Going against the grain and making a significant contribution to our understanding of how the world works is truly amazing. We've been lucky enough to make these sorts of contributions – we've shown that posttranslational modifications of proteins, such as phosphorylation and glycosylation, are way more diverse and complicated than what was traditionally believed in the textbooks. We've also recently shown that, contrary to popular belief, the human body makes a limited repertoire of antibodies that are abundantly present in our blood to fight invading pathogens.

What are the areas you are most excited about?

I think we all hope – and expect – MS to play an even bigger role in personalized healthcare moving forward. I don't know exactly what that role will be, but already we've seen how MS is used to analyze biomarkers. There's the example of heel-prick testing in babies for metabolic diseases. Soon, we should be able to expect MS to be used for personalized diagnostics and to monitor the health of individuals. There are then bigger questions to be answered around whether we want this and how useful it will be… I see it as our job to show whether it can be done first. I actually think it will be very useful; for example, personalized proteome profiling can help us understand whether a certain therapy will work for an individual. It can then also help you understand how altering the treatment alters the response. In the future, I think we'll be able to use MS to not only monitor disease, but to monitor healthy people as well.

Any advice for those taking their first steps in the field?

It's good to have goals and plans, but don't be too rigid. Stay creative and remember that not everything will go exactly to plan. Always be ready for the unexpected. And be sure to take the chances that come your way – and then make the most of them.