the
Analytical Scientist ®

For Research
Use Only!?

We explore the latest advances
in the clinical application
of mass spec

In our excitement for the 71st ASMS Conference on Mass Spectrometry and Allied Topics, we collected together a few interesting thoughts:

"The IM-MS based approach to identifying biomolecular signatures of disease could be applied to any number of discovery-driven and targeted endeavors due to the unbiased nature of the analysis. In addition to wound fluid, our group has looked at human breast cancer tissue extracts, cerebrospinal fluids, cell lysates, serum, and microbial extracts." – Kelly M. Hines

"If I extrapolate my experiences as a medical parasitologist, it seems we are halfway up the slope – but with a long way to the summit. There is now a rapidly growing interest, that is matched (and initiated) by increasingly sophisticated MS instruments, in the quantitative measurement of multiple proteins or metabolites in patient body fluids." – André Deelder

"We were curious whether our mass spectrometry technology was sensitive enough to compete with antibody-based tools, such as ELISA, to determine cytokine levels expressed by macrophages when exposed to a bacterial compound. Unexpectedly, we detected way more secreted proteins than we ever thought, enabling a systematic analysis of signaling adaptor functionality." – Felix Meissner

"My big dream is that one day every hospital will have an Orbitrap mass spectrometer – that would really have the greatest impact on society. There's a long way to go and, as proven by [my] story, whether it happens or not depends on many different circumstances, but also on me." – Alexander Makarov

It seems clear that mass spec and clinical applications are destined to be together. But it may surprise you to learn that all of these quotes are taken from 2013 issues of The Analytical Scientist (cheeky, we know – but we're only 10 years old and it's our birthday). How far have we come in the intervening years? Have any of the predictions, hopes, and dreams highlighted above been fulfilled? Have we come far enough? Let us know: edit@theanalyticalscientist.com

Our "Mass Spec in the Clinic" feature (page 16) pulls you all back to the future – and we feel sure that ASMS 2023 will be bursting at the seams with presentations on the full diversity of human disease (a quick glance at the program confirms our suspicions).

See you in Texas!

James Strachan, Frank van Geel, Jessica Allerton, and Rich Whitworth
Content Team at The Analytical Scientist

C Contents

06

38

46

In My View

On The Cover

Showcasing the MasSpec Pen in action as we explore mass spectrometry's latest clinical applications

Upfront

26

Feature

Core Topics

Sitting Down With

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Concrete Evidence

Why are Roman buildings so durable?

As the world's largest unreinforced concrete dome, the Pantheon is unequivocal proof of the excellent engineering skills of the ancient Romans. But we must also consider the exceptional nature of the construction materials. Specifically, how does ancient Roman concrete survive millenia, while often enduring harsh conditions? A team of researchers led by the Massachusetts Institute of Technology have used a handful of analytical techniques to solve the mystery – and the answer can be found in the complex process of "hot mixing" (1).

Previously, the process of slaking – where water is added to lime to form a highly reactive paste – was suspected as the culprit of durability – and the macroscopic lime clasts visible in the mixture were attributed to poor mixing practices. But Admir Masic, lead author of the study, had one nagging question: "If the Romans put so much effort into making an outstanding construction material, following all of the detailed recipes that had been optimized over the course of many centuries, why would they put so little effort into ensuring the production of a well-mixed final product?" (2).

Credit: Daniel Klaffke / unsplash.com

In the study, 2,000-year-old Roman mortar samples from the archaeological site of Privernum, Italy, were subjected to large-area scanning electron microscopy and energy-dispersive x-ray spectroscopy (SEM-EDS), powder X-ray diffraction, and confocal Raman imaging. SEM-EDS revealed the complex chemical composition of the mortar, and found the tiny clasts to be calcium carbonate. Spectroscopic techniques indicated that these were formed via an exothermic reaction – evidence of calcium oxide (quicklime) hydration.

From this, the MIT researchers deduced that Roman concrete was likely made by mixing quicklime directly with pozzolanic material (such as volcanic ash) and water at extremely high temperatures.

"The benefits of hot mixing are twofold. First, when the overall concrete is heated to high temperatures, it allows chemistries that are not possible if you only used slaked lime, producing high-temperature-associated compounds that would not otherwise form." said Masic in the press release. "Second, this increased temperature significantly reduces curing times since all the reactions are accelerated, allowing for much faster construction."

The hot mixing process also gives rise to lime clasts with a brittle nanoparticulate architecture that not only preferentially fractures but also enables "self-healing" of those cracks on exposure to more water.

The team hope that their findings will be considered in future cementing formulations to develop more sustainable architecture.

References
1. *LM Seymour et al., Science Advances, 9 (2023). DOI: 10.1126/sciadv.add1602.*
2. *MIT News (2023). Available at: https://bit.ly/3K4Jk8r.*

🕐 **TIMELINE**

Top 10s: The Old Guard

Continuing with our 10-year anniversary series, we look back at some of our earliest interviews with several heroes of the field

Connecting Separation Science: Pat Sandra

"The new generation should try to understand better what they are doing. They don't know or remember the fundamentals, making it difficult for them to make good selections."

Pursuing the Preparative: Georges Guiochon (1931 – 2014)

"The satisfaction in science is understanding phenomena, solving problems, and training people."

The Distinguished Scientist: Richard D. Smith

"I think of myself as having a 'passion for sensitivity', but the difference between passion and obsession is in the eye of the beholder…"

The Old Guard: Fred McLafferty (1923 – 2021)

"I have a short fuse when it comes to people who don't pull their weight, but I've been able to get away with it because people know I've seen tremendous achievements in times of adversity."

BUSINESS IN BRIEF

A quick-fire roundup of this month's business news!

- **Agilent** have announced the acquisition of e-MSion, the company behind the electron capture dissociation (ECD) technology more commonly known as the ExD cell. The move aims to make ECD technology more easily available to biopharma researchers and labs globally for accelerating drug development.
- **Waters** introduced the latest Alliance high performance liquid chromatography (HPLC) system – the Alliance iS – at Pittcon. According to Waters, the touchscreen interface, combined with in-built system health checks and guided troubleshooting can eliminate 40 percent of common lab errors (which Waters identified via customer research). The system is designed for pharmaceutical quality control.
- **Markes International** launched a new sample preparation platform at Pittcon. The new Centri 90 is a smaller

Credit: BusinessWire

version of the original Centri platform designed for sample preparation, extraction, and concentration analysis. It borrows the same functionality from the original technology, while enhancing GC-MS workflows and increasing productivity.
- **ELSIE Consortium** and **ACD/Labs** are set to combine their expertise for extractables and leachables research. Together, the companies are developing a worldwide database to support scientists in this area, which is slated for release later in 2023 via a web-based interface.
- **SCIEX** announced at SLAS 2023 that they are collaborating with **Beckman Coulter Life Sciences**. As a result, Beckman's Biomek and Echo Liquid Handlers now pair with Acoustic Ejection MS technology from SCIEX to automate sample handling. The companies are hopeful the combo will improve workflows for early drug discovery research.

Beethoven's Final Symphony

Genomic analysis of Beethoven's hair sheds (a little) light on what caused his death – and reveals a potential scandal in the family…

Ludwig van Beethoven's deafness, lifelong gastrointestinal problems, and unknown cause of death have long puzzled medical biographers.

Enter an international team of researchers, who analyzed the genome of Beethoven's hair, eliminating several genetic causes of death, including lead exposure. The team also revealed that Beethoven had a predisposition for liver disease and became infected with hepatitis B in the months prior to his death (1).

"Together with the genetic predisposition and his broadly accepted alcohol consumption, these present plausible explanations for Beethoven's severe liver disease, which culminated in his death," concluded the authors.

Surprising evidence of an affair in the direct paternal line also came to light between the conception of Hendrik van Beethoven in 1572 and the conception of Ludwig van Beethoven in 1770. And so a new mystery unravels…

Reference
1. *TJA Begg et al., Curr (2023). DOI: 10.1016/j. cub.2023.02.041.*

Mother of Ambition: Marja-Liisa Riekkola

"Well, I was the first female full professor in Finland – and when I started I was also pregnant. I proved that gender didn't matter – it is the results that count."

The Selective Chemist: Wolfgang Lindner

"I always ask tough questions, even in seminars; some people are afraid of that."

A Leading Light: Gary Hieftje

"I get a kick out of coming up with new ideas, testing them and publishing the results. But seeing students' eyes light up and watching them get involved in science is tremendously exciting."

The First Lady of Chromatography: Eva Smolková-Keulemansová

"I returned from my bad experience in the war with a key philosophy: my eyes are in the front of my head, therefore, I need to look towards the future. That's helped me throughout my whole life."

Bioanalytical Living Legend: Barry Karger

"When I started, analytical chemistry was suffering a real loss of prestige, so being aware of opportunities was important. Today, the situation has dramatically improved."

Writing the Book on GC – and Living Life: Harold McNair (1933 – 2021)

"One of my former students was asked if he had learned a lot about chemistry from me. He said, 'Chemistry? We learnt from Professor McNair how to live and enjoy life!'"

Spoiler Alert!

Introducing a paper-based biosensor that can rapidly detect putrescine levels in rotten meat

Credit: Georgia Perry

Consuming high concentrations of putrescine – a biogenic amine (BA) responsible for the foul odor of expired meat – is associated with heart palpitations and increased colorectal carcinoma risk. Many BAs do not emit a foul odor, and often go undetected. And with no regulations concerning safe putrescine limits in food, we appear to have a meaty problem…

Now, a group of researchers from Concordia University, Montreal, Canada, have presented one possible solution – a paper-based synthetic biosensor (1).

"It was serendipitous, really," says Steve Shih, co-author of the paper, reflecting on the fact that the idea for the research was sparked by a random conversation about cooking dinner. "My former Masters student, Alaa Selim and I searched the literature for methods of detecting spoiled meats and were surprised to find that there was nothing rapid and inexpensive available – for use at home or in the food industry."
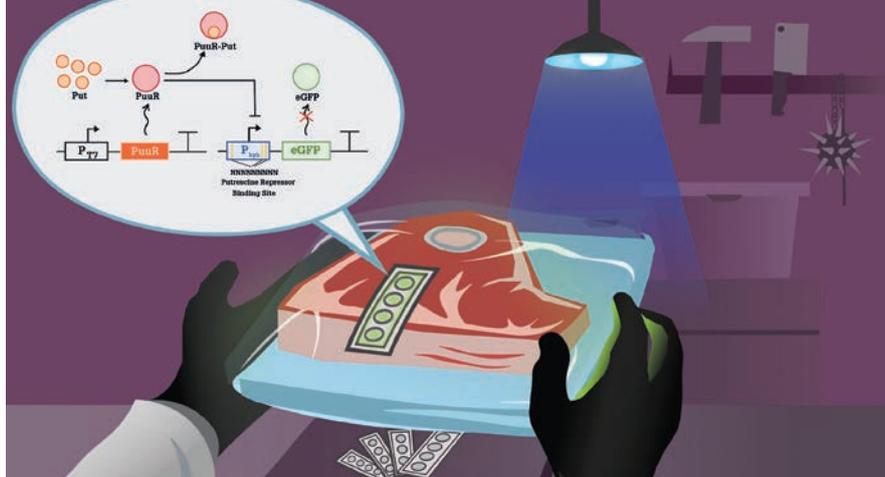
The paper-device developed by Shih and his team contains a synthetic genetic circuit that – after around four hours – turns a certain color when putrescine is detected. The intensity of the color also quantifies the amount of putrescine present in the sample. The biosensor relies on cell-free protein synthesis – which produces protein without the biological machinery of a cell. The repressor protein PuuR, which is found in Escherichia coli, was then used to assist in the identification of putrescine.

The biosensor technology was applied to pieces of beef kept under different storage conditions; the samples in the freezer and refrigerator contained low levels of putrescine, while the samples left at room temperature accumulated high levels – showing the biosensor's utility as an index for determining meat product stability. The samples were then pelleted and quantified by HPLC-MS for comparison. And although the values measured were different, due to the conversion of other biogenic amines or compounds to putrescine picked up by the sensor, they were somewhat correlated – validating the results.

"There were many challenges with this project, but the challenging ones are the most exciting," says Shih. "Optimizing a cell-free system specifically suited for our biosensor required a lot of iterative design and testing."

The team believes they've laid the foundation for many different types of cell-free biosensors that can be used in different fields. "Our lab is currently working on a cell-free paper biosensor as a diagnostic tool for infectious diseases and viruses in preparation for the next pandemic," says Shih.

Reference

1. *A.S Selim et al., ACS Appl Bio Mater, 21, 5487 (2022). DOI: 10.1021/acsabm.2c00824.*

Canadian Contamination Crisis

BPA-like chemicals in food labels are contaminating fresh produce

Bisphenol A (BPA) is a toxic chemical linked with prostate and breast cancer that is commonly found in packaging materials, such as food cans and plastics. In Canada, steps were taken to reduce BPA, but in many cases it has been replaced with similar chemicals, such as Bisphenol S (BPS).

Researchers from McGill University examined packaged fresh food in Canada and found – using a combination of ATR-FTIR spectroscopy and targeted MS/MS – relatively high concentrations of BPS in food labels that are applied with heat. For the first time, the study showed that BPS in food labels migrates through packaging and into the food contained within (1). Conversely, plastic wrapper films, pads, and trays showed very little trace of BPS.

Though Canada doesn't currently regulate the use of BPS, the amount the researchers found in fresh produce exceeded the European Union limit. "Considering the number of packaged food items sold with thermal labels, the actual dietary intake of BPS and other chemicals is likely to be high," said co-author Stéphane Bayen (2).

References

1. *XU Ziyun et al., Environ Sci Technol, 52, 12 (2023). DOI: 10.1021/acs.est.2c09390.*
2. *McGill (2023). Available at: https://bit.ly/40Dvq1Y.*

IMAGE OF THE MONTH
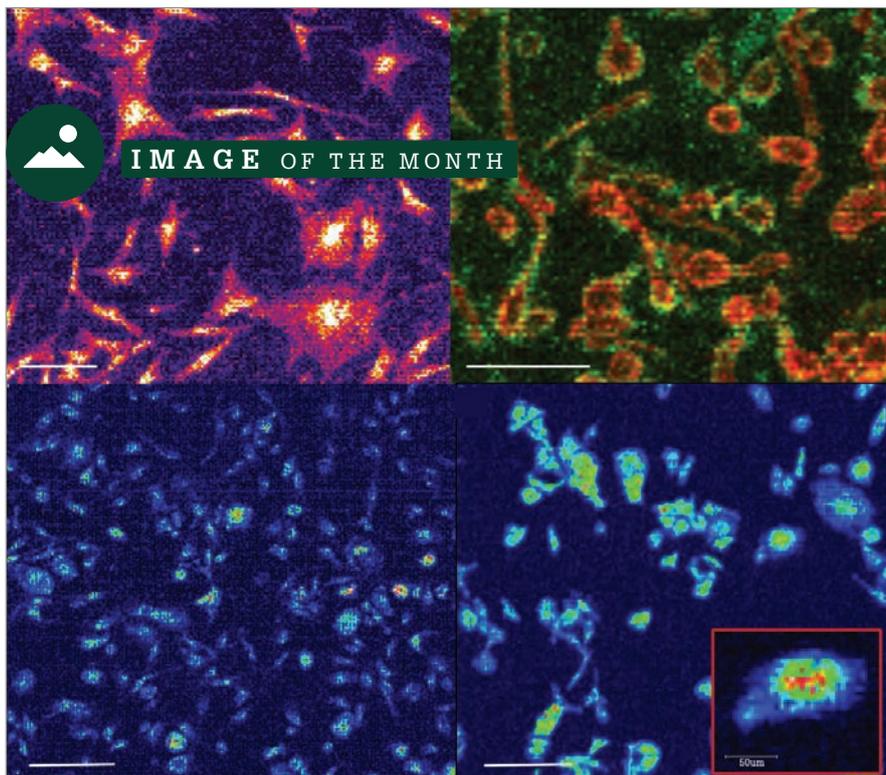
An array of dazzling cells viewed using MALDI-TOF mass spectrometry

Molecular images of single cells in 2D cultures generated on a MALDI-ToF instrument. Top: fibroblasts (left) and macrophages (right); bottom: cardiomyocytes (left) and breast cancer cells (right). Inset: a zoom in on a single breast cancer cell showing the different levels (blue, green, red) of phosphatidylcholine 36:1 in the cell. Scale bars are 200 µm; inset scale bar is 50 µm.

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TWEET OF THE MONTH

"It hurts when you don't get something you've worked towards, but take it as a learning opportunity to grow. I was a very late bloomer to a life in science, and am still learning. Don't stop dreaming and working hard to make those dreams come true."

Ben Garcia (@GarciaLabMS), Professor and Head of the Biochemistry and Molecular Biophysics Department at Washington University in St. Louis, USA.

Biological BMI Breakthrough

Multiomic blood profiling measures health more accurately than BMI

Multiomic, blood-based profiles paint a more accurate picture of obesity and metabolic disease than height-and-weight-based BMI, researchers from the Institute for Systems Biology, USA, have found.

The team studied 1,000 individuals who enrolled in a wellness program. Using UHPLC-MS/MS, they measured over 1,000 blood markers – ranging from proteins to small molecule metabolites – as well as participants' polygenic risk scores and gut microbiome profiles from ribosomal RNA (1). By analyzing the data with machine learning, the team created "biological BMI" prediction scores.

The researchers found that participants with a high biological BMI and normal traditional BMI were less healthy, but able to lose weight easier following a lifestyle intervention. And when people made positive lifestyle changes, biological BMI was more responsive and dropped earlier than traditional BMI.

"This work has the potential to significantly improve the development of predictive and preventive clinical approaches for treating metabolic disturbances," said lead author Kengo Watanabe (2).

References

1. K Watanabe et al., Nat Med (2023). DOI: 10.1038/s41591-023-02248-0.
2. Institute for Systems Biology (2023). Available at: https://bwnews.pr/3M3qr6L.

Demystifying Analytical Data Management

Analytical data is diverse, tied to many applications, and often difficult to access and share. But when data is managed well, the possibilities are endless. Here, three experts break down the key pain points and set you on the right path in your data management journey.

With Graham McGibbon, Director, Strategic Partnerships, ACD/Labs; Nichola Davies, Director, Structural Chemistry, Oncology R&D, AstraZeneca; and Mark Kwasnik, Global Product Manager, Analytical Labs, Solvay

What is the single biggest challenge in analytical data management?
Graham: The challenge of data heterogeneity is real (1) – and it was highlighted in the survey from ACD/Labs and The Analytical Scientist on analytical data management (see the infographic below for the headline figures). Labs are often dealing with a variety of different vendor technologies, software, and hardware all collecting data, which makes summarizing the information difficult. And that's just for a single technique – often people are using two or more techniques for a given analytical question.

Mark: Yes, I can relate to that! At our global Research and Innovation centers with Solvay, not only do we have a whole plethora of techniques to contend with, but often labs will have legacy equipment running – either from different vendors or even from the same vendor but with different software platforms. As a data guy, a chromatogram from seven different vendors is seven different techniques. The outputs are different, the columns are different, the headers are different – everything is different. And that makes things much more challenging.

Nichola: That problem definitely resonates with me as well. In pharma, as we look to more and more challenging targets, the molecular complexity is increasing, we need to choose the instrument that is best suited for addressing our analytical needs, which often necessitates heterogeneity.

What's the secret to a successful data management strategy?
Mark: Support from upper and middle management is absolutely essential. The people in the lab doing the leg work already have a great deal on their plates and data management does involve additional upfront work to create and fill in the data tags. So management will need to give people the extra bandwidth. But there also needs to be

The Analytical Data Management Report 2022

The headline figures from ACD/Labs' and The Analytical Scientist's survey of the analytical community

>92% OF SCIENTISTS…

- use multiple techniques
- collect data on numerous instruments
- process data using diverse software

Analytical data is managed and stored in a multitude of systems

- **80%** Microsoft applications
- **70%** Instrument software
- **34%** In-house software
- **24%** LIMS
- **13%** ELNs

40% collate analytical data from DIFFERENT INSTRUMENTS and techniques DAILY/WEEKLY

90% need analytical data on a DAILY BASIS

68% say data is HARD to access and share

70% want more INVESTMENT in data management technologies

ACD/Labs

a bottom up understanding; what's in it for the lab technicians? If you're going to make someone fill in 10 fields on the front or back end, try to include some sort of automation so they're no longer manually typing results or creating so many reports. The net result might be a similar overall workload as you move towards effective data management, which ultimately should make everyone's lives easier.

Graham: I'd also suggest making a priority list. Ask yourself, what do we most need that we can't access very easily right now? Then make sure that you're thinking strategically about how to get from point A to B. That may seem trite, but it is important to focus on value for your organization.

Nichola: We need to see a transition from thinking of data as single-use pieces of information that are consumed and then no longer usable. Data itself can be an incredibly valuable resource, especially given the pace at which digitization, AI, and machine learning are advancing. So I think it's really important to start your data management strategy with that mindset.

Are there benefits beyond efficiency?
Mark: Yes. I started down the digital journey as a lab manager, managing a team and dealing with chromatography and mass spectrometry, with purely selfish goals – making my job easier and improving team efficiency. But I discovered that there was so much more we could be doing with data. Now we treat data as an enterprise asset, like a reactor or a scientist, seeing how much we can squeeze out of it – reusing it and learning from it. For example, a report used to be something only a very small number of people with the right credentials could access in LIMS – the customer, perhaps a colleague of theirs, and the person on our end writing the report. Now, thanks to data management, scientists across the organization can learn from the data it contains.

Graham: This is a really good point that speaks to "data flow," which is a real paradigm shift. Data consumers and data generators are increasingly seen as part of the same team – and they need to generate data that the organization considers an asset. Data management strategies have to adjust to this shift if companies want to handle their data most productively.

Having traveled your own data management journey, what would you do differently?
Nichola: It is easy to underestimate the amount of time you'll need to properly dedicate to data management. For me, it isn't my main job – I've got many other pulls on my time. So if you're embarking on a big project like this, make sure it gets that management buy-in Mark mentioned!

Mark: Agreed. Plus, don't underestimate the importance of harmonization. Labs in different countries might have different languages or date formats. But it's not your job as the overarching person to dictate these things: the scientists in the different labs need to get together and figure out what works best for them.

Graham: I've learned to incorporate something we refer to as a "define and design" phase, which involves sitting down with a variety of stakeholders and addressing some of the kinds of issues Mark raised – discussing priorities and figuring out timelines. It can be at a high level, but you have to use real examples to make it concrete for people.

Dive deeper with Graham, Nichola, and Mark as they discuss challenges of analytical data management in this on-demand webinar: https://bit.ly/3mVXgaU

Reference
1. ACD/Labs, The Analytical Data Management Report 2022. Available at: https://bit.ly/3ZNKQkf

Case Study: Analytical Data Management in Practice

By Nichola Davies

Within AstraZeneca, we had two medicinal chemistry groups generating thousands of samples for testing with the task of selecting the best candidate drugs to take forward. We wanted a centralized solution, so we decided to go cloud-based and make that accessible across the organization. The solution includes data from different lab instruments, existing file shares on our lab servers, as well as the data our external collaborators share with us. That data is automatically uploaded to our cloud-based storage system, and, at that point, the relevant metadata is extracted from the data set.

We've got quite robust tagging – what the data is, where it comes from, who's running it, what the sample is, and we use that to organize and search. The centralized cloud storage enables us to have multiple consumers of that data, whether that be lab chemists accessing the data or data scientists for certain ongoing machine learning projects.

On top of that, if the data meets the minimum metadata requirements, it's also ingested into an ACD/Labs Spectrus database, which is where most of our analytical scientists access the data. We can search, visualize, and perform processing.

Squaring the Circle

Analytical science will play a pivotal role in setting up and monitoring new physical and chemical processes for plastic recycling – and the circular economy

By Harry Philipsen, Senior Expert, Structure and Analytics, DSM Engineering Materials, Geleen; Frans Beckers, Co-Founder, FBBasic–Cimar, Sittard; Anton Schotman, Innovation Broker, and Eric van Sprang, Program Manager, both at TI–COAST (Top Institute for Comprehensive Analytical Science and Technology), Amsterdam; all based in The Netherlands

In My View

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

To combat climate change, alleviate the depletion of natural resources, and reduce plastic pollution, there is a clamor for the circular use of plastics – part of a wider move towards a circular economy. However, moving away from classical, linear value chains won't be easy. All players – manufacturers of base polymers, compounders, shapers (making the final parts), end users, waste collectors, as well as companies taking care of sorting, washing and further processing – need to work together.

When starting with large amounts of mixed plastic waste, sorting is key. And though end users can play a role, waste often consists of a large number of base plastic and compounds contaminated with – for example – food remains. Some plastics can be relatively easily separated from others based on their density or other physical or chemical characteristics, but for others the task is more difficult. Relatively pure fractions are easier to recycle compared with less pure fractions, which require different

forms of further physical and/or chemical processing. And that's why we distinguish between mechanical recycling – where no chemistry is required and is thereby relatively energy (climate) friendly – and chemical recycling, which requires (much) more energy. Nevertheless, chemical recycling is difficult to avoid given that waste streams always contain fractions that are too complex or would require too much effort to mechanically sort. And materials cannot be endlessly mechanically recycled because of the chemical degradation that occurs during subsequent life cycles.

Regardless of the route chosen, analytical science must play an important role. For example, chemical recognition in sorting lines needs to be faster and more specific to get to purer plastic fractions. In the past, complex sorting plants adopted a combination of detection and separation techniques, including density and/ or magnetic separation, spectroscopic detection techniques (UV/VIS, NIR, XRF, and laser/optical sorting). Still, significant challenges lie ahead when it comes to further enhancing the specificity and speed of detection and

sorting – especially for similar materials, such as various styrene-based polymers or different polyamide types. Fortunately, we are seeing new developments in analytical techniques and methods that focus specifically on plastic recycling; for example, Netherlands-based company Veridis is working on a new detector that uses thermal identification to directly characterize a mixture of plastic flakes without the need for further homogenization. Other researchers are focusing efforts on adding tags and/or other tools to speed up identification.

However, to really speed things up, the plastic industry needs to gradually start adapting their strategies to make new materials better suited for recycling – in short, designing for recycling. Presently, recycled plastics are relatively common – in downcycled applications like traffic bollards, for example. But we are a long way from routinely using recycled plastics in high end applications that are comparable to those of the original material; for example, the needs for stringent mechanical (or other) functional properties and challenging processing

conditions, such as high temperatures.

A big challenge here is that recycled plastics are highly impure, often consisting of complex mixtures of all kinds of plastics, compounds, additives, remainders of their applications (for example, food), and partially degraded material – all of which vary over time and from batch to batch. For more demanding applications, a material's composition must be relatively constant in terms of its constituent components and their molecular characteristics, such as molar mass distribution and branching. This challenge is closely connected to the second challenge in plastic recycling: economies of scale. Or put another way, the ability to gather enough recyclate of sufficiently narrow compositional specifications that allow specific recycling processes to become economically feasible. Building such specificity poses significant challenges with regard to collection and logistics – not least the significant effort and increased carbon footprint.

Given the compositional variance and complexity of recyclate, analytical science will need to make huge strides in terms of its understanding of the relationships between impurities and the final mechanical, optical, and other physical properties of plastics. We cannot simply use specifications that have been set for virgin materials. In many cases, recyclate will not be anywhere near as pure and its composition will vary to a much larger extent. Two important questions spring to mind: Which compositional variations can we allow from the perspective of functional properties? And what about safety? Recyclates may contain components that could put people's health at risk. It's fair to state that compositional characterization of complex mixed plastic waste and investigating relationships towards next use applications is still in its infancy. Few studies are known in this area and yet we clearly need to make great strides.

But there's more: any advances in standardized characterization need to be accompanied by a uniform nomenclature of recuperated materials. On one hand, a connection to the original material, also serving REACH, needs to be incorporated; on the other hand, the nomenclature should comfort users by informing them about application families, material specifics, and quality. By speaking the same language, both in characterization and nomenclature, we can take the next step and grow the recycled volumes towards those of primary materials.

Clearly, detailed, compositional insights in recyclates are crucial, and the importance of the role of analytics cannot easily be overestimated. That said, compositional insights can be strongly assisted with paper trails – or, better, a materials passport that describes the history of waste streams, its original composition, the way materials were processed, its use in next-use applications, and its route thereafter. Such a materials passport could be of great help in getting recyclate to its next destination. Although a relatively new concept, there are already good examples of how such systems promote the building of circular value chains.

One example is a manufacturer of safety shoes that documents a completely circular process in a materials passport (C_passport), including recycling and next-use applications. Other applications include cleaning products, building and construction materials, and textiles.

To enhance awareness of the importance of compositional insights and traceability of plastic recyclate, recently TI-COAST (Top Institute for Comprehensive Analytical Science and Technology), The Netherlands, together with FBBasic and Cirmar, organized a mini symposium on "Quality assurance in the recycle chain" in Amersfoort. The organisations gathered some 70 persons throughout the value chain together, including various experts from the plastic recycling world, quality assurance organizations, and polymer producing industry, who shed light on the status quo, the developments needed, and the impact developments could have on the entire plastic industry.

During breakout sessions, the participants discussed the presented themes – namely, the dual need for a material passport and enhanced, standardized analytics – both of which could help predict the applicability of recyclates in high-end applications. However, it also became very clear that many players in the value chain currently struggle with the complexity of the matter – and so, choosing a direction and immediate next steps remains challenging. Many agreed that steering and regulation from (inter)national authorities was pivotal. Fortunately, in that regard, the EU in particular is taking a leading role by issuing mandatory guidance on the major steps required before 2030. In the Netherlands, a large initiative on circular plastics (with a focus on characterization and sorting) was given funding of more than 300 million euros by the Dutch Growth Fund in 2022.

Analytical science will play a pivotal role in setting up new, complex chemical processes for plastic recycling; analytics must be the eyes and ears of understanding how new processes work. Though essential, these processes cannot exist without a uniform nomenclature and documentation in materials passports.

TI-COAST, FBBasic/Cirmar – together with affiliated partners – are now setting up a evaluative framework for both characterization and nomenclature, fed by practical use cases. Those who are interested in joining and contributing to this development are invited to participate. For the upcoming year, new events will be organized on this topic. Updates can be found on www.ti-coast.com and www.cirmar.com.

Awards Above Suspicion

When it comes to organizing scientific committees, evaluators, and awardees, we need to employ a church and state approach – unless we want to weaken the prestige of all conference competitions

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

Many years ago, when we started organizing scientific conferences, regardless of whether they were national or international, our policy was to exclude all organizing committee members from receiving any sponsored award – including poster and oral presentations. Additionally, we did our best to exclude all students with a connection to chairpersons and/or organizers.

Some of my students weren't happy about this strict approach – they didn't want to be excluded through no fault of their own, especially considering the value of such distinctions. And it's not about the money for most (despite its almost universal significance); consider the competition for positions, where any accolade can help applicants stand out from the crowd.

Clearly, finding the right balance is tricky.

However, I have observed a new tendency of late, where at least one award is given to a researcher who appears to be actively involved in the organizing committee. Even worse, I've been to conferences where the majority of prizes are awarded to that category of participants. What's going on here?

When discussing this strange new world with colleagues, a common first reaction is to doubt the integrity of the whole process. Of course, this isn't necessarily the case – the award may be justified. But it's hard to dispel all doubt.

Are there really two schools of thought on this controversial topic within the scientific community? I decided to ask my peers for their opinion by sharing a poll on Twitter. My question: Do you believe that members of the organizing committees in scientific conferences should be practically "excluded" from presentation awards, so that non-biased processes are ensured and confirmed? In hindsight, I now recognize how my heavily weighted question may have skewed results. Nevertheless, out of 20 responses, 80 percent answered in the affirmative. The next day, I asked the same question on LinkedIn, where 72 of my connections participated and where 74 percent agreed with the need to establish a non-biased, impartial, fair process when awarding young scientists at conferences.

I breathed a sigh of relief. Apparently, I wasn't being unfair to my students by trying to insist on the need for an unbiased selection – I was simply following common sense in the eyes of an admittedly limited selection of my peers.

Clearly, there are some who think

> *"I have observed a new tendency of late, where at least one award is given to a researcher who appears to be actively involved in the organizing committee."*

we should simply have confidence in the committee's integrity and honesty, and trust that our peers assessing the presentations are using solid criteria. And I respect this argument in principle; however, given what I have seen in recent conferences, I'd suggest that this isn't always the case either.

Evaluator committee members whose task it is to review many lectures or posters do deserve our trust and respect – it isn't easy given the often-large number of excellent presentations and posters. So surely we must help them ensure the process is honest and transparent by developing and following objective, crystal-clear procedures – set at the beginning – and clear mechanisms to eliminate potential conflicts of interest. If not, we risk weakening the prestige of all such competitions, which would ultimately harm the careers of many promising young scientists who genuinely deserve recognition for their work.

To put it another way, we should keep in mind the old proverb that Caesar's wife must be above suspicion.

A Flight into the Future of (Instrument) Education

Simulators will never replace hands-on experience, but they are vital learning tools for the future – and we should treat them as such

By Charles Lucy, Professor Emeritus and 3M National Teaching Fellow at the University of Alberta, Canada

I write this on my way home from the Biennial Chemistry Education Conference (BCCE). The conference supercharged my enthusiasm with new ideas and hope that the trials of the past few years are behind us. But now – as my flight is delayed for the third time – the thunderclouds of reality begin to descend.

My teaching labs – like many others – have one or (at most two) of a particular instrument. Rarely does a student's instrument time coincide with it being discussed in lectures; some students do the experiment long before learning the theory – and some see the instrument so long after that they have forgotten the theory. And that results in a large disconnect between the lecture and the lab components.

Simulators may hold the answer to this problem, and they are available for a wide variety of analytical instruments. And, although they are not a substitute for hands-on experience, they do allow all students to explore the instrument while the theory is being taught. The speed of simulators – far faster than a chromatographic run – allows students to gain experience much quicker, moving them closer to the Gladwellian 10,000 repetitions necessary for mastery. Simulators also enable exploration of conditions that might result in long run times or damage real instruments.

Simulators can be used for dry labs, demonstrations, and guided-enquiry activities. Dry labs can replace a traditional laboratory experiment with quick runs on a simulator. Dry labs for UV/visible absorbance, fluorescence, atomic spectroscopy, X-ray fluorescence, liquid chromatography, gas chromatography, ion chromatography, cyclic voltammetry, and more were developed during the COVID-19 pandemic – and are described in the Analytical Sciences Digital Library (ASDLIB) Remote Labs site (1). They have proved to be a vital teaching resource. Instrument behavior can easily be demonstrated using these simulators.

Increasingly, I am drawn to using simulators for demonstrations to enhance active learning in the classroom. For instance, the HPLC Teaching Assistant can be used to dynamically show the effect of changing retention factor (k) from 1 to 3 to 10. After the demonstration, I invite the class to discuss what will happen when k is increased to 20. Students correctly predict that resolution increases, but are surprised by how little.

Subsequently, I redo the demonstration pointing out the change in retention time; and then show k = 1, 3, and 10 again, this time pointing out the change in peak height; and then again to point out the change in signal-to-noise. Using these simulator demonstrations, students quickly gain a far richer appreciation for the impact of changing a variable – and such easy and rapid adjustments would be impossible with real-life instrument manipulation.

I have done such activities live, but they could alternatively be recorded as a screencast with students periodically pausing the video to do activities. In fact, guided-inquiry and screencast activities have demonstrated powerful learning gains (2). One can only imagine how much time and frustration the screencast students saved by not having to figure out how to use the simulator in real-time. Such screencast activities also allow use of simulators that students might find challenging to master.

Another pedagogical challenge is that proper technique is far easier to show than to describe. The COVID-inspired transition to electronic textbooks has enabled the inclusion of videos directly within the virtual pages of many textbooks. I prefer videos that explicitly demonstrate technique, rather than classroom-style lectures. As we transition to digital-first textbooks, I anticipate the use of such videos and simulations will continue to increase.

And though videos can provide students an excellent tour of the outside of instruments, they rarely get to see inside an instrument. Static figures in textbooks can convey only so much about instrument components. This is where augmented reality and 3D printed components (3) can open the lid on black box instruments – truly increasing students' understanding of the inner-workings.

I see the lights of home in the distance, so let me finish with some grounded comments. Simulators, videos, and augmented reality can give students greater experience with instruments than traditional lectures. But they will never replace actual hands-on experience, which students can get through undergraduate research, cooperative education work terms, or internships. So here is my message to analytical scientists: use the modern pedagogical tools described above, but also encourage your students to seek out those real-life experiential learning opportunities!

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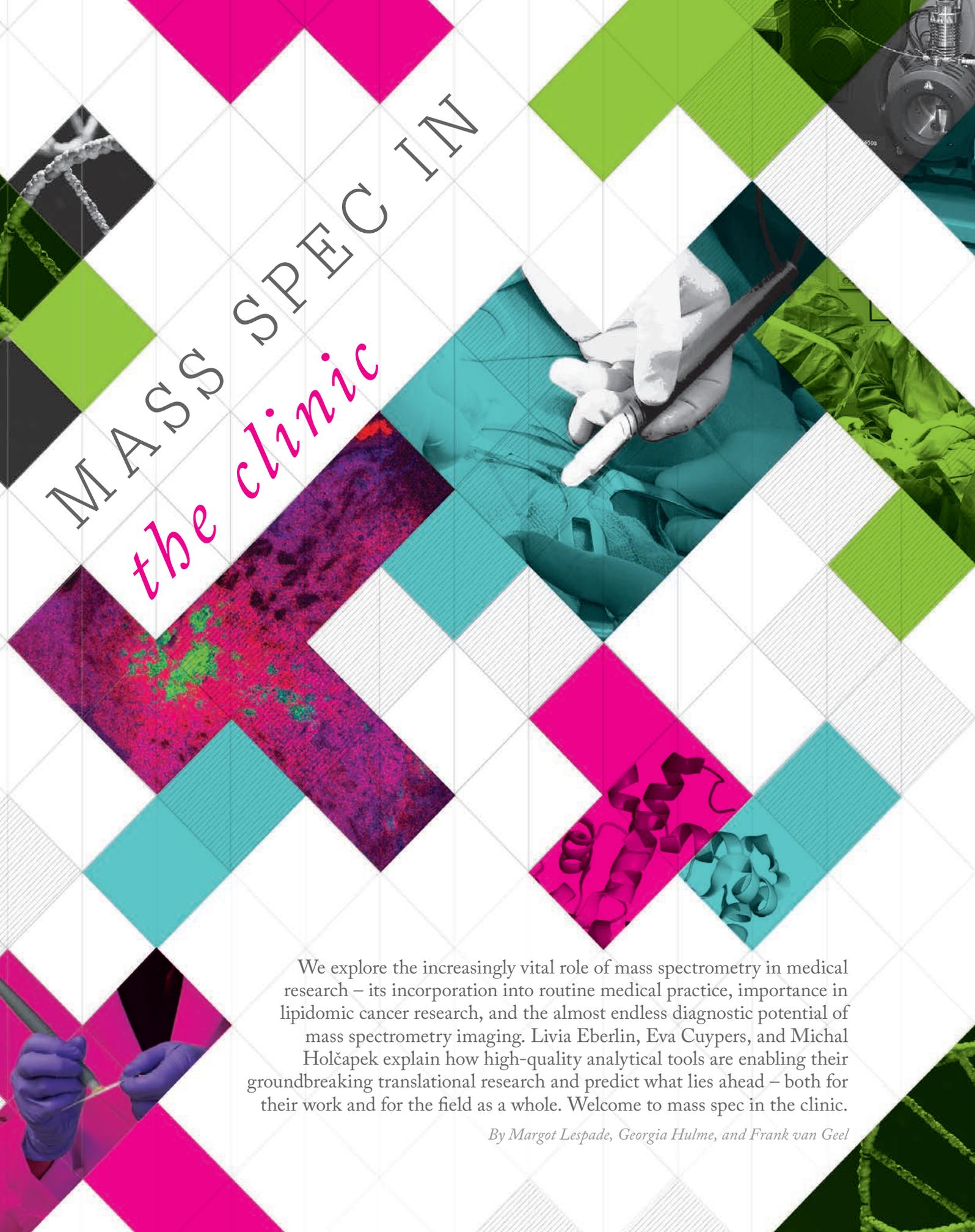
MASS SPEC IN *the clinic*

We explore the increasingly vital role of mass spectrometry in medical research – its incorporation into routine medical practice, importance in lipidomic cancer research, and the almost endless diagnostic potential of mass spectrometry imaging. Livia Eberlin, Eva Cuypers, and Michal Holčapek explain how high-quality analytical tools are enabling their groundbreaking translational research and predict what lies ahead – both for their work and for the field as a whole. Welcome to mass spec in the clinic.

By Margot Lespade, Georgia Hulme, and Frank van Geel

MSI Marvel

Eva Cuypers discusses the almost endless potential of mass spectrometry imaging (MSI) in clinical applications, her research on intraoperative diagnostics for the rapid identification of cancer cells, and the importance of researcher–clinician relationships

Mass spectrometry imaging (MSI) simultaneously acquires increasingly detailed molecular and spatial information, opening up new worlds of research possibilities. And though this rapidly-developing discipline can be widely applied to a multitude of fields, including food, environmental, and forensic analysis, its applicability to biological samples looks set to reshape the field of clinical diagnostics.

We spoke to Eva Cuypers, Assistant Professor, University of Maastricht, the Netherlands, to find out more.

What inspired your interest in diagnostic mass spectrometry?

My background as a pharmacist – specializing in toxicology and analytical science – brings basic and applied sciences nicely together. My goal has always been to create technology that moves through the translational stage and into the clinical setting or forensic field – or, in other words, research that truly makes a difference! My connections with different clinical disciplines – and the brainstorming sessions with potential end-users – enriches my development as a scientist.

Could you briefly explore some of the potential applications of MSI?

How long have you got? To give you a flavor (!), one of my PhD students is using it to investigate the spatial distribution of taste compounds in mushrooms. The effect of environmental pollution on food is also a hot topic within the MSI field; in fact, at M4i, we are researching the distribution and health effects of microplastics in fish using MALDI-MSI. In forensics, we've used MALDI and SIMS imaging to investigate drug distribution in a singular hair (1). With MSI, a full timeline of drug use can be obtained with a singular experiment and an accuracy of hours. Alternative methods, such as GC-MS, take months to gather similar results. MSI also has further forensic possibilities in fingerprints, tissue and bone samples, fibers, and ink.

Finally, in clinical research, I'd say MSI has the potential to revolutionize the field. MSI can provide valuable information about the distribution and abundance of various compounds in biological samples that can inform the development of new diagnostic tools, treatments, and therapies. For example, MSI can be used to study the distribution of drugs in tissues to determine their efficiency and safety, which can help optimize drug delivery and improve drug effectiveness.

But it can also be used directly in disease diagnosis and prognosis through the identification of specific biomarkers or compounds that are associated with specific diseases in tissues or cells from patients. Here, the focus is often on improving the accuracy of disease diagnosis and/or enabling earlier detection of disease.

MSI is also useful in tissue engineering, where it can be used to examine the distribution of various compounds in engineered tissues – such as scaffolds or implants – to determine both their composition and quality. It is also vital for toxicology and offers a way to study the distribution of various compounds in tissues to determine their toxicity.

Could you share some of the techniques you've helped develop in your career?

I have developed many standardized analytical methods during my time as a forensic toxicologist, including new extraction methods for dried blood spots and the use of ionic liquids as micro-extraction solvents for blood analysis. The research papers that I am most proud of are the ones that have had an advantageous impact on the end-user field – for example, my extraction and detection method for explosives in human blood and tissue, and the development of guidelines for intervention staff dismantling illegal cannabis plantations.

My MSI-related research on the consequences of decontamination procedures in forensic hair analysis was extremely significant. The method gave fresh insight into drug incorporation in hair and changed routine protocols around the world. I'm very proud to say that I won three international awards with this research!

My latest award-winning research was on the development of an intraoperative detection and identification method for brain tumors – the start of a new intraoperative diagnostics era. Lastly, my ongoing research on single cell MSI to connect laboratory research with clinical diagnostics is one with high impact potential. I am excited to see this research progress towards clinical implementation of MSI in digital pathology.

You've also used MSI in breast cancer research…

That's right. I developed a method where two-dimensional cells can grow on glass slides for MSI measurements – a technique that allowed us to generate molecular profiles from single cells with a spatial resolution of five micrometers. Alongside our collaborators, we built a molecular database and recognition system of 14 different breast cancer cell lines and successfully applied this system by correctly identifying the cell types in tissue samples.

Meet Eva Cuypers...

After studying pharmacy at university, Cuypers obtained a PhD degree in biomedical sciences at the School of Pharmaceutical Sciences, KU Leuven, Belgium. She then undertook postdoctoral training in Forensic Toxicology at KU Leuven and at AMOLF, The Netherlands. In 2015, she was appointed as Assistant Professor in Forensic Toxicology at KU Leuven. Her research mainly focused on developing new methods in forensic toxicology – including developing novel drug screening methods for police forces and creating a compound mix for cadaver dog training.

Cuypers' first time working with mass spectrometry imaging (MSI) was in Ron Heeren's lab at AMOLF, where she investigated drug distribution and decontamination methods for hair analysis. Having discovered the endless possibilities of MSI, she moved to the Maastricht MultiModal Molecular Imaging Institute (M4i) at University Maastricht as a Senior Researcher in 2019. In 2021, she was appointed as Assistant Professor, and became the leader of her own research group – where she now focuses on using MSI in perioperative diagnostics. Cuypers and her team are working on MSI-based methods to detect biomarkers in biological samples. They are also advancing intraoperative diagnostics for "on-the-spot" identification of tissue or disease type, tumor border, and cancer cells. According to Cuypers, her main goal is to translate molecular insights into clinical solutions for personalized medicine.

Pathological stainings and their interpretation are often still done manually by looking through a microscope – resulting in a rather "subjective" interpretation that depends on the pathologist's experience and domain of expertise. Our MSI method can indicate the cell type "on-the-fly" in an objective manner based on the molecular profiles. Moreover, the cell type recognition can be done without the laborious and time consuming stainings. The cell identifications were even possible during the MSI run – making it a fast and objective method.

The advantage of MSI here is that it is a label-free method, and therefore not limited to the amount of histological staining possible on one tissue sample. We published this research last year but also filed a patent for "digital on-the-fly pathology" and we are currently searching for a company or private partner to invest in this.

Recognizing single cells in a tissue environment – without the need for labeling – has opened up new research possibilities. Molecular changes of cell types in tumor tissue can now be detected before microscopic cell changes are even visible, which can inform therapy outcome predictions. More specifically, we showed that clinical diagnostic and prognostic markers, such as receptors, can be linked to a molecular profile, which means we can recognize them using the same single cell molecular database.

In addition, the role of specific cell types and cell–cell interactions in tumor development and progression can be investigated with a spatially detailed method that gives in-depth knowledge and further insight into specific diseases. Undoubtedly, this molecular-based, single cell recognition strategy will lead to faster and more targeted drug development.

Notably, our proof-of-concept molecular database method can be applied to any cell type. The future goal is to build cell-specific databases for different diseases and investigate whether linked recognition systems can be used for early diagnosis and prognosis. In the next few years, my research group will mainly focus on brain tumors and neurodegenerative diseases.

In short, I believe MSI will have a huge impact in personalized clinical diagnostics, prognostics, and treatment.

How will MSI techniques mesh with other emerging diagnostic trends, such as digital/computational pathology and artificial intelligence?

MSI needs to be combined with artificial intelligence (AI)

to be properly applied in routine diagnostic practice. The advantage – and disadvantage – of MSI is that it generates very detailed spatial and molecular information, which means there is simply too much data for the human brain to process in an acceptable time frame. AI can teach the system to distinguish between healthy and diseased tissue – a crucial task for the introduction of MSI into point-of-care settings.

Our research on breast cancer cells has shown us that lipids are a very important group of molecules. Lipids play a major role in cellular structure, energetics, and signaling. The characterization of changes in cellular lipid composition is a key research element for diagnostic purposes. In contrast to genetic profiling, lipidomics gives greater insight into cell activity, communication, and environmental changes occurring in the tissue. Although histological stainings are useful for different cell types or necrotic and hypoxic regions, they do not give detailed information on the cell energy level or cell signaling – both crucial parameters in cancer progression. However, there is not one, singular lipid that acts as a cancer marker. Rather, it is the change in lipid ratios that specifies cell type, and these changing ratios can only be analyzed for diagnostic purposes in a reasonable time frame when machine learning is used.

Our work on single cells is a fundamental step towards the direct translation and integration of MSI into point-of-care settings, and close collaboration with pathologists and surgeons is key to our success. MSI-based diagnostics will be used alongside current pathology workflows, and provide pathologists with more detailed molecular information. Diagnostic and prognostic interpretation should be closely developed with clinicians for direct patient implication, as it

is highly important to include end-users as early as possible to ensure clinician usability and potential.

How can analytical scientists and clinicians work more closely together?

At Maastricht University Medical Center and UZ Leuven – two clinical centers I am collaborating with – most clinicians are open to new research and diagnostic methods. I believe the key factors to a successful partnership are communication and scientific curiosity. And that means a willingness from both sides (clinicians and researchers) to put in time and effort. As a researcher, I take the time to observe and discuss surgery and diagnostic procedures. I identify the strengths and weaknesses of a clinician's methods and tools because they are the most interesting parts of my research to tackle. I am always willing to adapt proof-of-concept methods depending on clinicians' input. On the other hand, a clinician should spend time explaining procedures, testing new methods, and comparing these with existing clinical strategies.

A great example of the importance of communication can be

Below: Overlay of astrocyte staining and the MSI image.

Below: MALDI2-TOF image (TimsTOF, Bruker, positive mode) of rat brain showing some distinct lipid distributions in the tumor core (green) and surrounding invasive brain tissue (red). Spatial resolution: 10X10um.
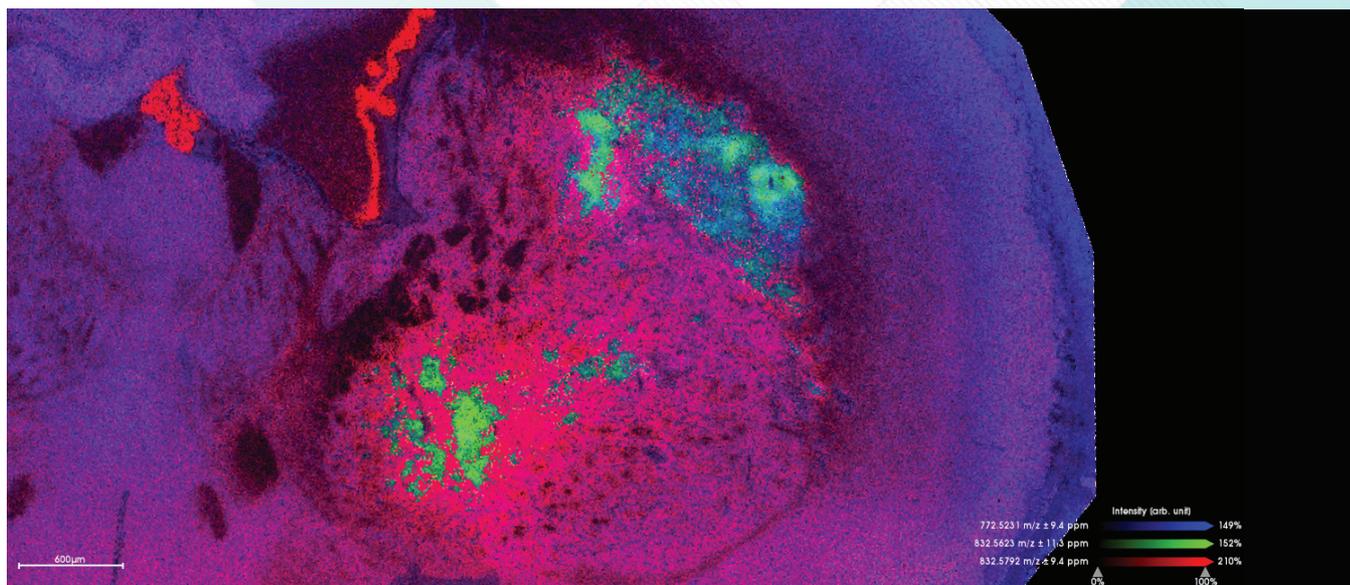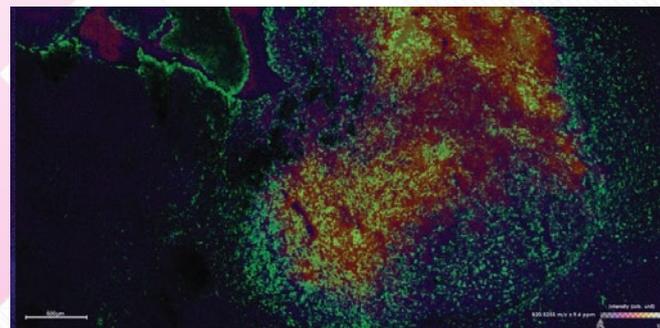
An Imaging Revolution: from CERN to the Clinic

Could recent developments in secondary ion mass spectrometry imaging revolutionize digital molecular pathology?

By Ron Heeren

The evolution of physical-chemical analytical instruments has traditionally focused on the improvement of resolution, separation, sensitivity, and throughput. Here, resolution refers to different parameters such as spectral resolution, molecular resolution, structural resolution, spatial resolution, and several more. In pathology based clinical diagnosis, the speed of analysis is key. Optical scanning of immunostained slides can be performed in minutes, but limited possibilities for multiplexing exist. For example, imaging lanthanide-labeled antibodies with SIMS offers the multiplexing capabilities but lacks the speed. In imaging technologies in particular, the detail that can be observed is crucial and the "resolution revolution" is strongly based on advances in detector technology and image processing. But it usually comes at the expense of throughput. Make the pixel size 10 times smaller and the same analytical area requires 100 times longer data acquisition time.

But a new development in secondary ion mass spectrometry imaging changes that paradigm – based on an innovation in mass spectrometry that takes advantage of massively parallel detection of arrival time and position capabilities, combined with an innovative detector coming from CERN: the Timepix3 system. The detector offers nanosecond timing resolution and continuous time resolved image detection. M4i researchers have coupled it to a microscope-mode mass spectrometry imaging system that allows for the detection of more than a million pixels per second – that's orders of magnitude faster than what is possible with conventional mass spectrometry imaging approaches. It uniquely combines throughput and spatial resolution with single ion detection capabilities for large m/z ions.

We've applied this new system for ultrafast SIMS based molecular imaging of large areas at submicron spatial resolution. When applied to biomedical tissue analysis, a variety of molecules can be visualized at cellular detail in a matter of minutes. I believe this approach could revolutionize digital molecular pathology, as well as perioperative diagnostics in a true clinical translational setting. In other words, bridging the translational gap between fundamental mass spectrometry research and pathology – by making tissue diagnoses more precise and rapidly improving precision medicine through more individually tailored therapies.

found in our intraoperative brain tumor project. As researchers, we thought it would be advantageous to develop a method that could identify tumor type and grade. However, after conversing with the neurosurgeons, it became clear that this was an unnecessary endeavor. Instead, neurosurgeons needed a method that could accurately and sensitively detect the glioma tumor border. So, based on their input, we changed our whole recognition system. Open communication is crucial and necessary to adapt and revise methods so they can have clinical relevance in the future.

Practical logistics are also important considerations in this line of research; M4i is situated right next to the hospital, which allows daily interaction and easy sample transfer.

Are clinicians – and especially pathologists – concerned about MSI technology encroaching on their territory?

Certainly, MSI will revolutionize clinical diagnostics; however, I'm always quick to point out that it will supplement the pathologist's job, rather than replace it. Although AI recognition methods are quickly evolving – they will always require human interpretation before decisions can be made about a patient's treatment.

What lies ahead for your work – and for MSI and lipidomics in medicine more generally?

In the past five years, MSI has evolved massively in terms of sensitivity, mass accuracy, and spatial resolution – and it is only a matter of time before the technology becomes a major part of clinical diagnostics. Single cell resolution – necessary for digital pathology – has only recently become possible. Although there are challenges – such as speed and data size – that need to be addressed, new methods are being developed by M4i to significantly speed up analysis time, while making sure single cell spatial resolution is maintained. When a sample can be scanned in a matter of seconds, a major step will be taken to adopt digital pathology worldwide. Data-mining research is also moving fast and AI will be an indispensable factor.

The MSI field is continuously evolving, and the question is no longer *if* it will move into clinical practice, but *when* will it happen?

Reference

1. E Cuypers et al., *Anal Chem*, 88, 3091 (2016). DOI: 10.1021/acs.analchem.5b03979.

Lighting Up Lipidomics

Michal Holčapek, Professor, Department of Analytical Chemistry, University of Pardubice, Czech Republic, highlights the importance of lipidomics in clinical research – and shares his work in cancer diagnosis

High-quality analytical techniques are particularly important for clinical applications…

That's right. Clinical applications have rather special requirements for the quality of the data provided. Robustness and reliability are both essential. The reason is quite obvious – the result of an analytical test applied in the medical environment can have serious consequences. Let us consider a false result of our cancer lipidomic test; a false positive result could cause severe stress for the person receiving incorrect information – and that could have negative health consequences. On the other hand, a false negative test would produce an unfounded sense of security while the tumor may be growing in the body. Both situations are clearly undesirable – but it is impossible to rule them out entirely because all existing medical tests have a certain level of false negatives and false positives. And that's why we need to develop highly reliable methods with analytical and clinical validation to reduce false results to the lowest rate possible.

How important are lipids in clinical research?

Lipids have many vital functions in cells and the entire organism. In the past, the main known roles were associated with energy deposits and the building material of cellular and subcellular membranes. Currently, we are learning much more about the roles of various lipid classes in signaling – which is very important for many metabolic pathways and cellular processes. Typically, signaling lipids are present in very low concentrations, which makes their analysis difficult. Many researchers have realized the importance of such lipid functions, resulting in enormous growth of the lipidomics field.

How does the lipidomics field compare with the proteomics and genomics fields?

The genomics and proteomics fields have relatively well-established routine methodologies for measurement and data reporting. The lipidomics field is relatively young and so still suffers from some limitations that must be overcome. For example, individual lipidomics research groups use different methods according to their preferences – the nomenclature and data reporting are not yet standardized.

> "*The broader acceptance of lipidomic measurements in patient care will be realized when different laboratories can routinely report the same concentrations measured on different platforms.*"

In 2019, the International Lipidomics Society was established to harmonize the field and provide the lipidomic community – and other related scientific communities – with well-defined minimum requirements for sample collection and storage, measurement, identification, quantitation, data reporting, and nomenclature. The broader acceptance of lipidomic measurements in patient care will be realized when different laboratories can routinely report the same concentrations measured on different platforms. We still have a lot of work to do to reach this point!

Compared with genomics, there are some interesting advantages to exploring the phenotype; the lipidome is closer to the function in the omics cascade, so it offers a higher chance of discovering biomarkers of ongoing or early disease in the human body – as already demonstrated for cancer and cardiovascular diseases.

Please briefly describe your method of diagnosing cancer using lipidomics…

Our methodology enables quantitative and high-throughput measurements of lipid concentrations in human blood. We have discovered that the lipid profiles of cancer patients are significantly different from healthy controls. However, these differences in concentration are relatively small – which is why we need accurate and robust methods. Advanced multivariate data analysis tools play an essential role in the whole workflow – as does a certain level of automation.

In terms of instrumentation, my group has introduced ultrahigh-performance supercritical fluid chromatography-mass spectrometry (UHPSFC/MS), which allows high-throughput and robust quantitation of around 200 lipids with lipid species level information – the number of carbon atoms and double bonds in particular molecules. With one UHPSFC/MS system and two operators, we can provide the capacity to measure 20,000 samples per year.

We can also use reversed-phase UHPLC/MS, which delivers lower throughput and a lower level of method automation, but
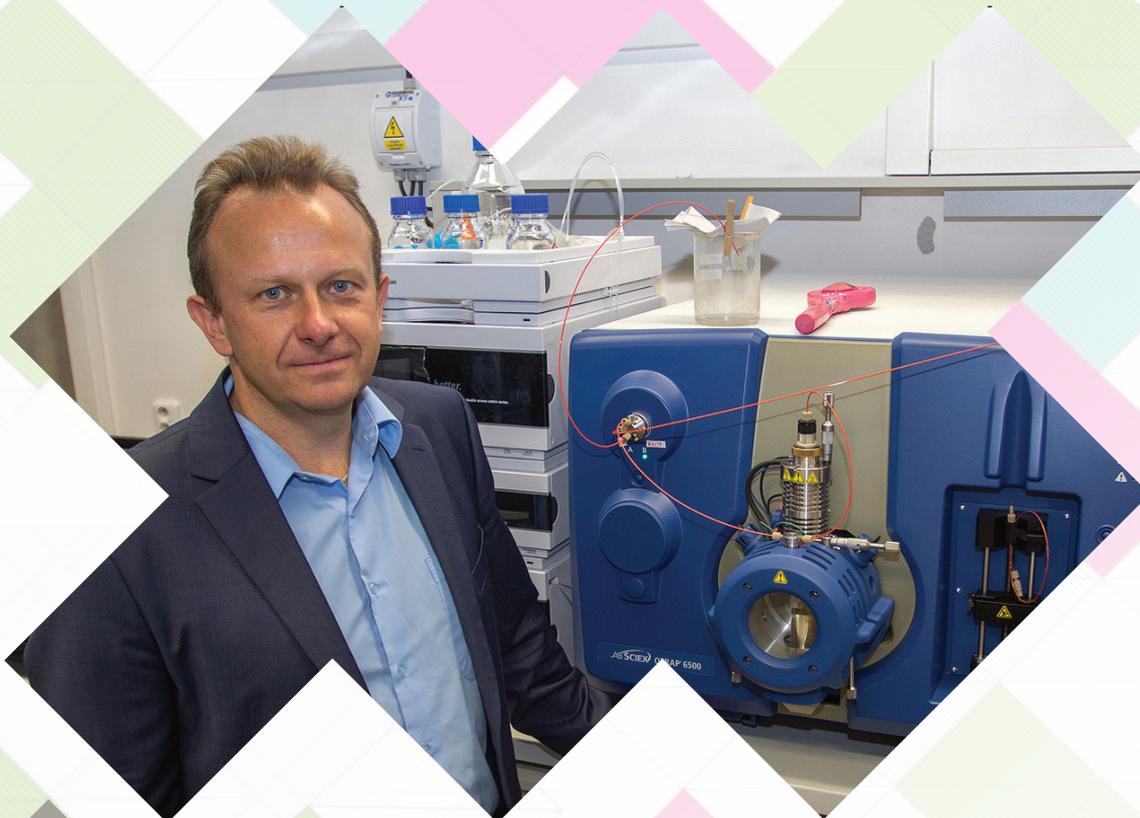
it does provide information on individual fatty acyls. This information is important for the interpretation of the biological mechanism of the observed alteration and slightly improves the accuracy of the detection of cancer patients (at the cost of reduced throughput).

The pattern of most dysregulated lipids is similar for all cancer types studied so far. In short, we see the downregulation of very-long chain sphingolipids with one double bond (ceramides, sphingomyelins, hexosylceramides, sulfatides, gangliosides, and so on) and some lysophosphatidylcholines (mainly LPC 18:2), while some phospholipids and sphingolipids containing shorter fatty acyl chain and less double bonds (typically C16:0, C18:0, and C18:1) are upregulated. It is evident that there is a potential for parallel detection of multiple cancer types because the differences among individual cancer types are relatively small.

When and why did you start exploring lipid profiles for cancer diagnoses?

In 2010, I flew from a conference in Phoenix to another one in Salt Lake City. I started reading the flight magazine – which I had

never done before – and to my great surprise, I found a really exciting article on cancer. It got me thinking about my own understanding of tumor growth. It is primarily an uncontrolled proliferation of cells, and the division of a large number of cells in an unusually short time requires the input of an enormous amount of building material for cell membranes – which are indeed lipids. I began thinking that tumor cells must have a different lipidomic composition than normal cells – which was easy to confirm. We continued with the analysis of blood and other body fluids, and we observed a similar dysregulation, which was later applied to the diagnostic area.

How does your method compare with other diagnostic methods?

Our primary focus is on pancreatic cancer (PDAC), which has a five-year survival rate of less than 10 percent. Interestingly, comparison with established diagnostic methods is impossible because there are no such methods. PDAC is an asymptomatic disease at the early stage, and so it is usually detected by chance. Sadly, when symptoms start, it is often too late to help. If our PDAC test is introduced into national screening programs for people at high risk, many lives could be saved. Our preliminary data indicates that we could also detect precancerous lesions, which could revolutionize the detection and treatment of PDAC.

To be clear, our method – though fast and inexpensive – is intended for screening purposes only; the final confirmation of the diagnosis must be made using established medical procedures.

How long do you think it will be before your method can be used in practice?

In May 2022, Lipidomics Diagnostics of Cancer (LipiDiCa) was established as a spin-off of the University of Pardubice and the FONS company in Pardubice, Czech Republic. Now the employees are trained, the UHPSFC/MS is installed, and the laboratories are equipped and operating. The next step is the last critical step before we can start the implementation of the cancer screening program: clinical validation, which means verifying method performance in a large-scale prospective cohort of high-risk subjects – and we hope to start this in a few months!

Everything must be run under the same conditions as will be used for real screening later. Alongside this, conventional clinical investigation of the presence or absence of tumor will be performed using established tools – such as endoscopic ultrasound or magnetic resonance – enabling us to determine selectivity and specificity.

It will take a few years to pass the clinical validation, and then the method can be adopted by clinicians, who will, for the first time, be able to actively detect PDAC at an earlier stage.

On a Medical Mass Spec Mission

Livia Eberlin highlights the importance of high-quality analytical tools in medical research – and details her personal mission to incorporate mass spec into routine medical practice

It almost goes without saying that high quality analytical techniques are crucial in medical and clinical applications…

Medical research would be extremely limited – if not unfeasible – without access to high quality analytical techniques, including mass spectrometry. Most critical research questions being investigated in biomedical research can only be answered by probing details of molecular processes in biological samples, which requires high-performance analytical measurements. Medical research has also become increasingly translational, with many investigators going directly to clinical studies with complex patient samples and often skipping pre-clinical studies with more controlled and less complex animal models.

Analyzing human tissues from large and diverse cohorts of patients in multi-institutional clinical studies demands the use of high-quality (reproducible and robust) analytical measurements – otherwise it would be very challenging to derive new and significant knowledge with high rigor and depth of information. One key example that comes to my mind are the many studies where investigators are trying to understand the molecular processes related to a patient's ability to respond to certain therapies, such as immunotherapies for cancer. These are very intricate questions that have many variables that could be related to the outcomes – meaning that a deep characterization of molecular patterns of disease is of the utmost importance.

Would you agree that lipidomics has gained significant ground over the last decade?

Absolutely! I love lipid analysis and have been a huge promoter of the value and depth of information that these incredible molecules carry in biomedical research. Lipids are beautiful molecules with a high diversity of both chemical structure and biological roles. Often, when we use the term "lipids" to a general audience their minds go directly to common dietary fats – but in the last decade or so, there has been rising awareness that lipids are key components to our cells' structures while playing vital signaling roles.

My personal favorite class of lipids are cardiolipins. First, because their chemical structure is quite unique with four fatty acid chains – but also because these lipids are almost exclusively found in the mitochondrial membrane, and their abundance and structure have been significantly associated with metabolic and respiratory processes that have major implications in cancer. The ability to measure these molecules has been increasingly sought after, as more people are thinking about the correlation between mitochondrial metabolism and disease state.

I believe one major reason why more people are paying attention to lipids is because mass spec techniques are now sufficiently advanced to allow direct analysis and imaging of lipids from unmodified human samples. Furthermore, we now know that genomics and proteomics alone cannot entirely explain diseases and outcomes, which has led many investigators to value lipidomics and metabolomics as an essential part of their biomedical studies.

How might recent advances in lipidomics change the day-to-day work of diagnostic professionals?

I believe it is about time that we see some real changes in clinical diagnostics by incorporating lipids into clinical analysis and decision making. There is a substantial body of scientific and clinical work that supports the translational implementation of lipidomics into routine clinical testing. There are three advances that have enabled this implementation: i) the aforementioned mass spectrometry tools that allow rapid analysis of lipids directly from patient specimens, ii) the ability to measure lipids with high chemical specificity, and iii) data analytics and machine learning algorithms that allows us to convert lipidic information into clinically actionable data.

The technology is available and ready – what we need now is standardization of methods, validation of results, and the effort to pursue the regulatory steps to make this possible.

What's your research mission?

I am extremely passionate about bringing mass spec to medical practice and enabling medical users with limited or no expertise in analytical chemistry to use mass spectrometry and its ability to gather incredible molecular information to make better decisions for their patients – especially patients with cancer.

And that means developing and implementing user-friendly mass spectrometry techniques that can rapidly measure the incredibly rich lipid and metabolic composition of human specimens. We are very passionate about using high-performance mass spectrometry to characterize patterns of lipids or specific lipid molecules that can be correlated to disease state, subtype, outcomes, and response to treatment. We use refined data analytics and biostatistical tools to translate that information to

*"I am extremely passionate about bringing mass spec to medical practice and enabling medical users with limited or no expertise in analytical chemistry to use mass spectrometry."*

actionable outputs that can guide medical doctors in treating patients. We are, of course, also very interested in accessing lipid information from human tissues and correlating the mass spec data with data from other molecular assays (for
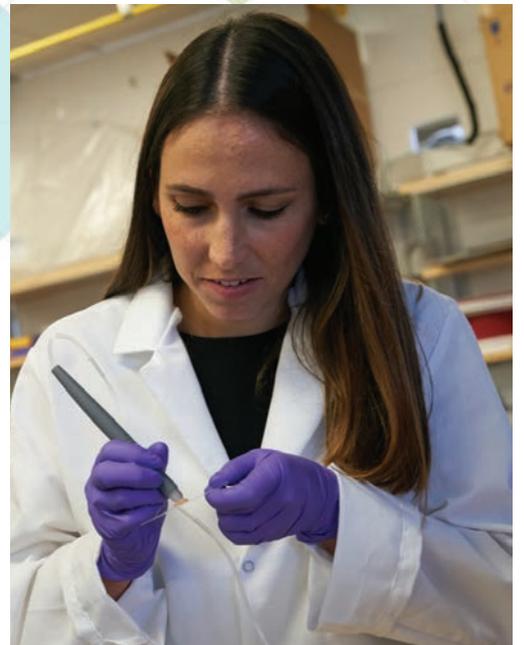
example, proteins and genes) to better understand biological processes related to disease development, progression, and treatment outcomes.

Could you please share some recent research highlights?

A major research effort in our lab involves several projects in which we are translating and testing the MasSpec Pen technology in clinical practice to help surgeons and pathologists identify and diagnose tissues during surgical procedures. The MasSpec Pen is a handheld device that enables gentle and rapid analysis of tissues in vivo or ex vivo. The device is easy to use and allows non-expert users to perform direct mass spec analysis of lipids and metabolites from tissues in seconds, with minimal training. The pen employs a very basic chemical principle for fast and efficient lipid analysis from tissues: it deposits a solvent droplet onto a tissue, which extracts lipids and metabolites, and then transfers the droplet containing the molecules to a mass spectrometer for immediate analysis. This molecular analysis is non-destructive – meaning that there is no damage to the tissue from the gentle lipid extraction, meaning that the tissue can still undergo standard clinical analysis. The non-destructiveness of the techniques has also allowed us to do repetitive tissue analysis in vivo, allowing molecular-based tissue identification prior to excision, which is truly transformational to clinical practice.

Over 20 surgeons have now used the MasSpec Pen in the operating room in ongoing clinical studies at MD Anderson Cancer Center and Baylor College of Medicine at the Texas Medical Center, US, across many diseases including breast, pancreas, thyroid, ovarian, and lung cancers. In a recent study that we published in PNAS (1), we used the MasSpec Pen to analyze 157 banked human tissues, including pancreatic ductal adenocarcinoma, pancreatic, and bile duct tissues – yielding rich molecular profiles characterized by high abundance of metabolites, fatty acids, and lipids. We then used the molecular data to generate classification models and achieved an overall 91.5 percent agreement with pathology for discriminating normal pancreas from cancer – including histologically complex samples with low tumor cell concentration evaluated within our test set. Key molecular predictors in the classifiers included lipids species, including glycerophosphoinositols. We then translated the MasSpec Pen to the operating room and predicted on in vivo and ex vivo data acquired during 18 pancreatic surgeries, and achieved 93.8 percent overall agreement with final postoperative pathology reports. Notably, when we integrated the banked tissue data with intraoperative data,

the agreement improved agreement to 100 percent.

We also have many studies in which we are using desorption electrospray ionization mass spectrometry imaging (DESI-MS imaging) to better understand cancer progression and outcomes related to treatments. For example, we recently published a study (2) in which we used DESI-MS imaging to investigate alterations in lipid profiles in tissues related to anti PD-1 treatment. We identified specific lipid alterations associated with the degree of response to anti-PD-1 treatment – including a significant increase of long-chain polyunsaturated lipids within responsive tumors following anti-PD-1 therapy. Immunofluorescence imaging of tumor tissues also demonstrated that the altered long-chain polyunsaturated lipids associated with treatment response were localized to dense regions of tumor immune infiltrates. These results indicate that effective anti-PD-1 therapy modulates lipid metabolism in tumor immune infiltrates, and provide evidence that further investigations of the related immune-metabolic pathways may be useful for better understanding success and failure of anti-PD-1 therapy – which is really exciting.
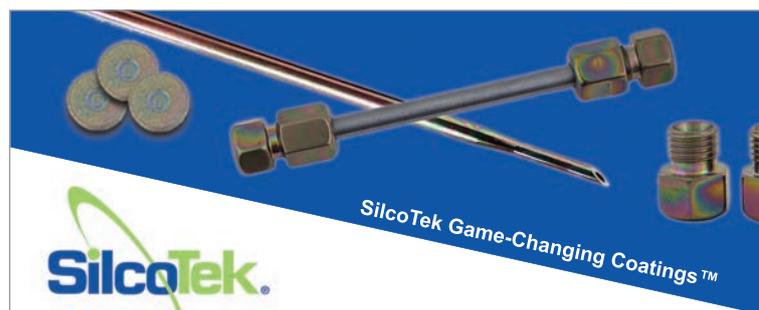
You are evidently excited and optimistic about the potential impact of your research…

The research that my lab and other groups are pursuing in direct lipid analysis using innovative mass spectrometry technologies have enormous potential in guiding treatment decisions for patients. The molecular data acquired in clinical studies shows that we can provide a rapid assessment of lipid profiles to immediately inform disease state and stratify cancer – but we also know that the depth of this data provides new biological knowledge of the molecular processes that are altered in these tissues to identify novel targets for therapies.

In my lab, we are particularly excited about the MasSpec Pen – and the innumerous applications for clinical practice in enabling rapid and non-destructive assessment of tissues, providing access to molecular data from living human tissues in a way that has been very limited before.

And is that what makes your research so rewarding?

It is really amazing to see how the lipid-based molecular information (acquired in seconds with mass spec techniques) can be used clinically to make a huge impact on treatment decisions for patients. It is also extremely rewarding to witness the excitement from medical professionals who gain access to a high-performance technology – such as mass spectrometry – through user-friendly systems that can help them improve their practice and how they treat their patients in a way that was not possible before.

In clinical practice, "molecular" studies are still most commonly associated with genetic or proteomic research, and I would love

"Lipids are incredible molecules that can also provide highly diagnostic molecular information – and they are easily measured with mass spectrometry techniques! Incorporating these technologies and molecules into clinical decision making should provide a real advantage in our ability to better diagnose and treat patients."

for doctors to understand that there is a lot more to "molecular diagnostics" than DNA sequencing, q-PCR of RNA, or assays for protein analysis. Lipids are incredible molecules that can also provide highly diagnostic molecular information – and they are easily measured with mass spectrometry techniques! Incorporating these technologies and molecules into clinical decision making should provide a real advantage in our ability to better diagnose and treat patients.

There is a pressing need for our scientific community to conduct rigorous studies that help validate and standardize methods across institutions and research protocols to truly show the impact that lipid analysis by mass spectrometry can have in the clinic. Rigor in data and statistical analysis is absolutely essential – but we also need larger studies with broad and diverse patient cohorts to show the clinical utility of our methods in improving patient care across the world.

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Mass Spec Technology Forum

Mass spectrometry is a powerful analytical tool used to study the properties of chemical substances and their interactions with other molecules. In this forum, you will learn more about mass spectroscopy and its potential applications to the proteomics field.

Our expert speakers will share their knowledge and experience, and also discuss the latest advances in the field. Their respective talks are titled:

- Torsten Muller (Bruker): "Unleashing the Power of 4D-Proteomics™ Ecosystem for Plasma Analysis: Pioneering Precision Medicine Research"
- Yuehan Feng, PhD (Biognosys): "Taking plasma biomarker discovery to the next level with TrueDiscovery™, a DIA-based platform for deep and unbiased proteome profiling"
- Dorte Bekker-Jensen (Evosep): "Pushing the boundaries for robust and high-throughput single cell analysis with Whisper Flow technology powered by dia-PASEF"
- Dr. Garwin Pichler (Preomics): "Streamlined & automated sample processing for in-depth proteomic analyses"

Whether you are a student, researcher, or an analytical science professional, this forum is an excellent opportunity to learn more about mass spectroscopy and its potential (plasma) proteomics applications.

Don't miss this chance to gain valuable insights and expand your knowledge in the fascinating field of mass spectrometry. Register now and secure your spot!
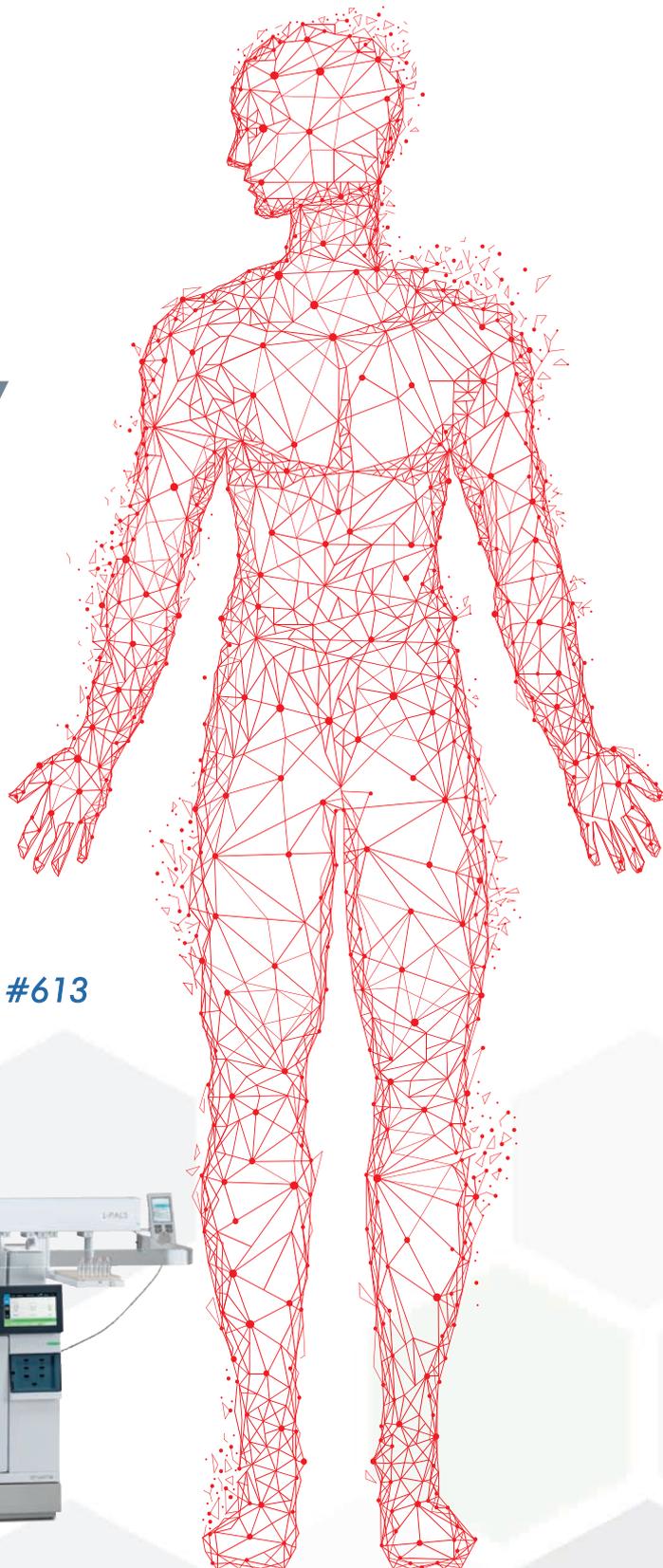
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Superfast muscles. Using a combination of MSI and LC-MS, researchers have recently discovered a type of "superfast" muscle fibers, which are known for their ultra-high speed and endurance, and are usually found in human eyes or hummingbird wings – but have never been documented in a mammalian limb before. According to the authors, although clinical applications are a long way off, these findings are particularly exciting for regenerative medicine in neuromuscular diseases.

Pinpointing *Candida auris*. You may have heard about *Candida auris* in the news recently – with outbreaks occurring in hospitals and care homes across the world. Unfortunately, identification of newly emerging lineages of this fungus remains a diagnostic challenge, and often results in the improper identification of yeast pathogens. And that's why researchers have recently developed a method using HRMS to rapidly and accurately identify *C. auris*. They examined 102 strains from all five clades and were able to correctly identify all *C. auris* strains with an identification accuracy of 99.6 percent. This method also provided a faster turnaround time compared with current technology – making it an attractive alternative to conventional methods.

There must be something in the water. Researchers have recently completed the first large-scale wastewater-based epidemiology study in the UK. Using multiple mass spectrometry-based methods, they analyzed wastewater from 10 cities for both chemical and biological markers of health – including pesticides, pharmaceuticals, and disease-causing viruses. Among other things, results indicated localized outbreaks of norovirus, COVID-19, and influenza – along with spikes in usage of over-the-counter painkillers. According to the authors, routine monitoring of wastewater could provide early warning for the next epidemic.

Mosquito shutdown. Researchers from the University of California, Riverside, USA, have mapped the sperm proteome of *Culex pipiens* – or the common house mosquito – to identify elements essential for mosquito reproduction. Mature sperm from *Culex pipiens* were isolated and analyzed by mass spectrometry – revealing the mechanisms that activate and maintain sperm motility, and identifying specific proteins that maintain sperm quality and activate them to swim. This study has important implications for population control of *Culex pipiens* and may help slow transmission of brain-swelling encephalitis and West Nile Virus – the team also hopes that these findings will apply to other species of mosquitoes, particularly those that carry malaria.

References available online

IN OTHER NEWS

Machine learning-based diagnostic tool shows promise in detecting tuberculosis and drug-resistant tuberculosis using nanoparticle-enhanced laser desorption/ionization mass spectrometry (NPELDI MS).

Preliminary results of UHPLC/MS/MS analyses suggest that chronic generalized inflammation caused by immuno-metabolomic changes drives headaches in long COVID patients.

According to international panel of scientists, spectroscopy and mass spec technology on Mars may lack the necessary sensitivity to detect life.

Fragmentation followed by deep fractionation with LC-MS-MS allows detection of protein isoforms in human proteome, generating comprehensive catalog of human protein diversity.

Fighting for Mass Spectrometry

Alexander Makarov reflects on the development of the Orbitrap technology, gaps in the mass spec toolkit, and the emergence of rival technologies

Reflecting on the development of the Orbitrap analyzer, what do you think were the most important factors in its success?

One contributing factor to the Orbitrap success is that it was a matter of survival. When we started developing it, I was working in a very small company in Manchester, UK, during a recession, on a very low salary. We started to realize that we couldn't survive on contract R&D projects alone – we needed our own products. Even with very limited knowledge of the market, it was clear that we needed something really out of the box.

This was when my first internal treatise of the Orbitrap theory came about. I explained my idea to my colleagues – who took one look at my huge stack of paper and said, "Okay, that looks thick enough – we believe this will work." At this point, we very fortunately got a £50,000 grant from the UK Department of Trade and Industry. We were then under a lot of pressure because we had a limited amount of time to work on the Orbitrap development, but also had to continue R&D projects for our company to stay afloat.

Other problems arose around my ideas for the Orbitrap design. Originally, my idea was actually very complicated and when I told our electronics engineer what I needed, he told me it was impossible – in fact, what I wanted then, we still cannot do today. At the time, I did not appreciate the complexity of what I was asking for. This resulted in me being left with, essentially, several pieces of metal and no electronics to drive my design.

I had to come up with something quickly – and I decided to work with pulsed ions produced by a laser. And that meant I didn't need all these complex electronics, but I did need to change the mechanics. We had to update everything, change designs, order new parts – and this became a recurrent theme throughout the whole Orbitrap development. There was never a single idea, we had to adapt continuously – even if it meant throwing away years of work. I would say that there were at least seven or eight instances where most people would have given up the project. But we always had alternatives and we always tried Plan B before the project could be stopped.

As the project progressed and we demonstrated performance, it gained more traction and we were able to build a wider team. We also had excellent managerial support, which allowed us to develop the Orbitrap technology in a relatively short time – although it did take nine years from the inception to the commercial launch. But, in my opinion, the absolute key factor to the Orbitrap success was perseverance – and many sleepless nights!

When you worked with us in 2013, you said that the Orbitrap age was just beginning. Have your expectations been met?

Yes and no. My main idea for the Orbitrap analyzer was that I wanted it to become a routine detector. The trap itself – the basic device made up of three pieces of metal – in its simplicity, lends itself well to routine analysis. I essentially wanted it to become as standard as a single quadrupole. There are thousands of single quadrupoles produced each year and used for all types of routine analysis.

But, somehow, the Orbitrap analyzer remained a higher performance technique. And this is reflected in its price positioning – essentially it is considered a premium

product. But I do hope that, one day, this commoditization is still going to happen.

Following this analogy with quadrupoles, with the latest family of Orbitrap Exploris instruments we have already taken this step by reducing size and complexity to that of triple quadrupoles. The Orbitrap Exploris MX detector in particular will be useful for routine LC analysis – and this is where we have the largest volume and penetration in analytical sciences.

Honestly, I thought this would happen faster – but, of course, I didn't take into account the way instrument companies operate. This has been a huge learning curve for me over the past 10 years – it is not enough to create a perfect technology and produce it, you also need to have solid and innovative business models in place to support its expansion.

Yes. There are many ways in which we could enhance the information that we provide. In ion mobility spectrometry, for example, although the cross-section information obtained is limited, it is already extremely useful. However, people are desperate for more information about the structure of molecules. The more we go beyond these limitations – using, for example, spectroscopy of ions or optical methods – the more we can analyze, and the better we can determine what is happening with our mixtures. In my opinion, there is massive leeway to explore – but it will require a combination of many complicated spectroscopic techniques.

We also have a lot of mass spectrometry aspects which are still in development – like fragmentation or ionization methods. We are only at the beginning, and we still have to compensate for shortcomings through better informatics – but I personally would like to see improved methods to address the structure of molecules.

Do you have any major concerns for the mass spec field?

Yes. There are two areas where mass spectrometry is under emerging pressure from new technologies. The first is proteomics – where methods based on antibodies, next generation sequencing, or single molecule protein sequencing are attracting more investment than mass spectrometry instrumentation got over its 100-year history. With all this effort and investment, these new technologies are increasingly competing with mass spec. Another area is tissue imaging and spatial-omics, where non-mass spec methods are developing very rapidly. In this sense, there is an existential danger for mass spectrometry. However, I do not think there is imminent danger when it comes to small molecules, pesticides, metabolites – where these new methods are not working as well.

I believe we can still offer a good fight against all emerging methods even in

"Mass spectrometry has steadily accommodated greater and greater sample complexity overall – and this is what keeps mass spectrometry competitive against other emerging methods."

those two fields, if we switch to different business metrics, such as cost of analysis, which better suits mass spec due to lower consumables expense. I think this will enable mass spec to be competitive against other methods in future – although for now, they are still orders of magnitude away from the separation capabilities of LC/MS.

When you think about mass spectrometry as a whole, what do you think the most important development has been over the past 10 years?

I think democratization of mass spectrometry has been vitally important. Many companies now have mass spec technology that is much more accessible to users with no prior knowledge of mass spec. But I think we still have some way to go! And I think we need to make that happen before other competing methods do.

The rise of data-independent acquisition is also definitely up there, and, more generally, the increase of complexity of samples that mass spec can turn into actionable data. Data-independent acquisition is a specific example, which is usually used for proteomics, but it has implications way beyond proteomics. These types of breakthroughs are mainly driven by development of informatics, and neural networks – but they have also been complemented by an increase in speed of analyzers, as well as large improvements in liquid chromatography and other aspects of the complete workflow.

Mass spectrometry has steadily accommodated greater and greater sample complexity overall – and this is what keeps mass spectrometry competitive against other emerging methods. If we organize our instruments and our business models correctly, I think mass spectrometry will remain competitive. If we don't, we will face increasing pressure from other methods based on next generation sequencing or other new techniques.

The Mixture Matcher

Arun Moorthy and Edward Sisco share details of the mass spectral search program designed specifically for seized drug analysis using DART-MS

What are the main challenges of mass spec data interpretation in general and in DART-MS?

Mass spectra are indirect measurements of molecular structure, and so data interpretation – which can also be thought of as compound identification or structure elucidation – can be challenging if you don't know what you're looking for. Peaks in a mass spectrum characterize the aggregate abundance of ions of specified mass-to-charge ratios, which means each peak could represent multiple substructures from the same molecule.

In traditional chromatography-based mass spectrometry approaches, you can usually be reasonably certain the peaks in a given mass spectrum are substructures of a single molecule. However, with ambient ionization mass spectrometry techniques – like DART-MS – that don't lean on chromatography, interpretation is more difficult because each peak could be representative of multiple substructures from multiple molecules.

And that's why you developed a data interpretation tool?

The NIST/NIJ DART-MS data interpretation tool (DIT) is a simple application that allows users working in seized drug analysis to compare their measured mass spectra – which are usually mixtures – to a library of over 1,000 compounds of interest (1). What differentiates the DIT from other mass spectral library search programs is that it was

Left to right: Arun Moorthy and Edward Sisco

specifically designed to work with mixture mass spectra (those collected without chromatography). It was also designed to leverage spectra of the same mixture measured at multiple in-source collision induced dissociation (IS-CID) energy levels – which enables analysis of both molecular ions and major fragment ions.

To operate this tool, the user loads up to three IS-CID mass spectra (measurements at a low, medium, and high fragmentation level) of their unknown mixture. At the press of a button, the algorithm that underpins the DIT – the inverted library search algorithm – then looks for partial pattern matches between the query mass spectra and the pure compound library mass spectra. The results of the search are then compiled into tables that summarize possible compounds in the mixture with metrics a user can use to make decisions. It

is a very focused tool with limited features, but this focus also keeps it simple to use.

Another unique aspect of the DIT is that we developed it with continuous feedback with several end-users from US Federal, State, and Local forensic laboratories, and so the specific features it does have – like report generation – are really useful to forensic practitioners. The first version of the DIT was released to the public in October 2021, and we've since updated the software with version 2 released in February 2022. We have plans to release a version 3 in the upcoming months.

The DIT is available for download as a packaged R Shiny Application from the NIST Public Data Repository (https://data.nist.gov/od/id/mds2-2448); the source-code is available at the same link and we hope other researchers, practitioners, and vendors will look to

customize or extend the DIT to meet their needs.

You appear to have simplified something rather complex – presumably implementation was not straightforward…

Funnily enough, the biggest challenge we faced was probably "development-adoption stalemate," which is when a potentially useful technology is stunted in its development because of an apparent "lack of market" – yet, at the same time, the shortage of users could just as accurately be attributed to a lack of development!

The NIST Mass Spectrometry Data Center regularly builds and evaluates a variety of mass spectral libraries and requires a large investment in dedicated instrumentation and staff. In 2013, after a short run, NIST stopped updating the DART-MS Forensic Database because, at the time, there just weren't enough people using the library to justify the resources required to maintain it. But in late 2019, we suggested that people weren't using the library because it lacked spectra of some of the newer (and very important) drugs. Additionally, the existing mass spectral data interpretation tools were designed for a very different type of analysis – like compound identification with EI mass spectra – which made them cumbersome to use with DART-MS mass spectra.

And that's when we decided to combine our technical aptitudes, and simultaneously update the DART-MS Forensic Database with relevant compounds while also creating new algorithms and software specifically for working with IS-CID mass spectra of mixtures. By mid-2020, we had an updated library and prototype search software. We reached out to several forensic labs to see if there was any promise with what we were doing – and the feedback was incredibly positive! We then increased our efforts, acquiring new funding and expanding our team to include personnel dedicated to the development and maintenance of the library and software.

Now, we have a regularly updated DART-MS forensic database, a user-friendly software tool, and a growing number of users. It took a lot of effort, but we are incredibly proud that we were able to break through the stalemate – for now.

Have you considered other applications of the tool?

We have recently begun using the DIT as part of the Rapid Drug Analysis and Research (RaDAR) program at NIST to help public health and public safety officials across the country monitor the illicit drug landscape in near real-time.

But we can see the DIT being useful in any application area where people are using ambient ionization mass spectrometry techniques for compound identification, including and beyond DART-MS. The only real requirement is that there be an appropriately formatted search library with pure compounds of interest to the application space. Though our focus has been illicit drug analysis, we recognize there are many other application areas where this capability would be useful (such as environmental spaces, food safety, or pharmaceuticals) so we are cleaning up our library building and evaluation pipeline to make it user-friendly, with the goal of releasing it to the public within the next year.

We have also been working on incorporating additional search options in the DIT for applications where a user may not be looking to identify mixture components per se, but trying to find complete matches to their mixture mass spectrum in a library of mixture mass spectra – essentially matching mass spectral fingerprints. One application that we've been particularly interested in is the identification of wood species in the timber trade.

Mass spectra are rich measurements with layers of information. Even for experienced mass spectrometrists, having tools that can help peel back that information can aid their mass spectral interpretation. The DIT is one of several tools that are available to help with data interpretation. We tried to streamline the application such that users of ambient ionization mass spectrometry techniques like DART-MS could work with our library (and future libraries) with as few challenges as possible. We look forward to seeing it applied across new areas in the future – to that end, if anyone has questions or would like to collaborate on building new is-CID mass spectral libraries and extending the DIT, they can contact us at *DARTdata@nist.gov*.

"We have a regularly updated DART-MS forensic database, a user-friendly software tool, and a growing number of users."

Arun Moorthy is a Research Scientist at the Mass Spectrometry Data Center, Biomolecular Measurement Division, National Institute of Standards and Technology, Gaithersburg, MD, USA.

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Powering Polymers

The key to meeting the demand for new and more complex polymeric materials? High-performance and application-tailored GPC/SEC systems.

By Christian Schmidt

Gel permeation chromatography (GPC) and size exclusion chromatography (SEC) separate analytes by size, using special columns with defined porous particles in the stationary phase. In doing so, they open up interesting possibilities – especially in the analysis and characterization of polymers, for which there is rising demand. This increasing interest is not only limited to industrially produced materials, but is also applicable to natural polymers, such as cellulose, starch, rubber, or even proteins. These methods are also used in sample preparation to separate high-molecular and low-molecular components for further analysis.

Over the years, KNAUER has adapted its HPLC systems – based on extensive discussions with leaders in the field and fruitful cooperation with manufacturers and users – to the unique requirements of GPC/SEC. For example, the AZURA SEC systems incorporate special seals and ball valves for long-lasting performance, paired with outstanding flow precision and ultra-low pulsation.

These specialized systems allow users to easily analyze samples, perform purification tasks, or collect fractions. In the polymer and food industries in particular, we can draw on an extensive pool of applications. However, we can also offer even more specialized solutions that, for example, enable the analysis of polycations that are used in the cosmetics industry, for water treatment, or in paper production.

To give a specific example, customers have used KNAUER's AZURA SEC systems in the field of biopolymer engineering. Molecular weight distribution in this area is a critical parameter that can impact behavior in various applications, and, as such, it is an important consideration when selecting biopolymers for specific uses. Customers have also used AZURA SEC systems to monitor the degradation of biopolymers over time to better understand product design, processing, and long-term performance – for example, as biodegradable packaging materials, as food supplements, or in medical applications.

But it is important to note that each application is different – and that means GPC/SEC systems should be optimized and equipped with easy-to-use, modern software. Selecting the right column-eluent combination is also essential for the quality and robustness of analyses. Thanks to a highly modular concept, the AZURA SEC systems can be fully tailored to customer requirements. In addition, our experienced experts can support customers in finding the best AZURA SEC system solution for their GPC/SEC applications.

One major challenge for GPC is ensuring an optimal separation mechanism in the desired molecular weight range; optimization involves selection of a suitable combination of column material, solvent, and further parameters, such as temperature, flow rate and sample concentration. And although these conditions are known for the most common synthetic polymers, it is often necessary to develop a method for novel materials to find optimal parameters for the analysis. Again, KNAUER's team of experts are available to guide you through these challenges at any point in your method development.

Looking to the future, it is likely that the demand for new – and, by extension, more complex polymeric materials – will continue to rise. In particular, sustainable polymers, more specialized and smarter engineering polymers, and polymeric materials for biomedical and pharmaceutical applications will all require increasingly detailed data on the molecular structure and the internal distribution of the building blocks. Here, classic GPC/SEC methods typically reach their limits. Researchers are currently applying new and more complex chromatographic and detection methods to the development of these materials, which will slowly become established in routine analysis – at which point a high degree of automation will be required; fortunately, this is one of KNAUER's strengths!

We certainly expect interesting innovations in the GPC/SEC area in the future – and we're excited to support customers through their unique journey!

Christian Schmidt is Product Manager at KNAUER
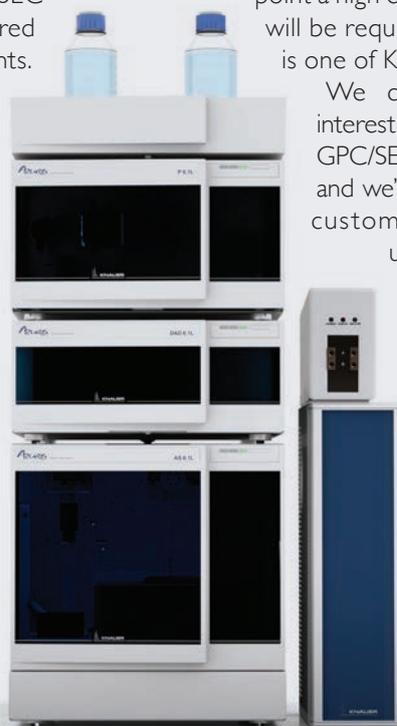
Goodbye chromatography? MIT engineers have devised an alternative to chromatography for lower-cost protein purification, using nanoparticles functionalized with bioconjugates to rapidly crystallize proteins. The standard chromatography-based approaches require specialized resins and strict quality control, increasing the cost of purification. The researchers say the new method can rapidly crystallize proteins at much lower protein concentrations. "The goal is to reduce the cost so that this kind of drug manufacturing becomes affordable in the developing world," said senior author Kripa Varanasi in a press release.

Green diarylethene? With the popularity of diarylethene-based photoswitches across chemistry, materials science, and biotechnology industries, researchers at The State University of New York set out to improve the purification process. HPLC and supercritical chromatography (SFC) methods were developed to separate photoswitchable isomeric compounds, which allowed for quicker analysis while maintaining a sufficient baseline solution for separated compounds. The end result, the researchers say, is much less solvent waste.

Cooking with crickets. Growing environmental and food scarcity concerns are turning the world's attention to insects as a cheap and sustainable protein-rich source of nutrition. With this in mind, scientists from the US Department of Agriculture, the Department of Grain Science, and Kansas State University have combined size-exclusion chromatography with SDS-PAGE to characterize cricket protein powders and products. They found that differences in processing changed the functionality of the products, which impacted their interactions when added to a wheat dough-based system.

Fermentation: the future of coffee? Researchers at Zurich University of Applied Sciences, Switzerland, have discovered that the fermentation of coffee creates flavors akin to fruit juices, according to unpublished data revealed ACS Spring 2023. They divided arabica beans into groups and prepared them in three different ways: washing the beans with water, removing the skin from the bean, and fermenting the whole coffee fruit in stainless steel tanks with carbon dioxide. After each group was brewed, the samples were analyzed with gas chromatography (GC) olfactometry-mass spectrometry. The team identified six compounds that contributed to the fruity flavor and raspberry scent. Samo Smrke, a research associate in the team's lab, said in a press release: "There's still quite a lot of unknowns surrounding this process" – and that could lead to further interesting coffee flavors in the future.

References available online

A Positive Sum

Success in analytical sciences requires a good grasp of the basic principles – and the van Deemter equation has underpinned separation science methodologies for decades. Has it become dogma? We say, "No! The van Deemter equation is here to stay!"

A conversation between Deirdre Cabooter and Gert Desmet

Gert Desmet: It's not correct to say that the van Deemter equation is dogma. Quite the reverse – the plate height curve described by the equation actually helps undermine dogmatic practices in the field. For example, why do people persist in running proteomic separations at 300 nL per minute? We know this flow rate is not necessarily the best for mass spectrometry, and we now have columns designed to operate at velocities from 50 to 1000 nL per minute. But because the original proteomics studies, from the 1990s, specified 300 nL per minute, it's stuck – people just keep repeating the original method! The same mindset is at work when people replace a 4.6 mm column with a 2.1 mm column, but neglect to adjust the flow settings. Inevitably, they get pressure errors, and the separation doesn't work as expected. If operators were more familiar with the van Deemter curve, they would understand how to optimize flow rates for each specific separation set-up. Because that's what the van Deemter does – it defines optimal flow rates.

Deirdre Cabooter: It's the basis of everything in separation sciences. If you are not familiar with the van Deemter equation, you won't get the most out of your instruments. It's like having a Ferrari, but never moving out of first gear. You have the capacity but you have no idea how to use it.

Gert: And using an advanced column in the wrong way, in the wrong set-up, is like putting a Ferrari engine in a Volkswagen Beetle – it's just not optimal! In the same way, when people try separating a large molecule using flow rates designed for small molecules, their process won't be efficient, because – as van Deemter shows us – the optimum velocity is much lower for larger molecules.

Deirdre: Exactly – the nature of the compound you are studying will affect optimal system parameters. And because the optimum flow rate is not the same for all molecules, there's not just one plate height curve – there are many, according to the analyte and the conditions it needs. Most chemistry MSc courses, however, just show the textbook van Deemter curve; students are never told that the shape of the curve depends on the analyte and the mobile phase. It's not put in context.

Gert: And the results of that uncontextualized, imperfect understanding can be unfortunate. At the least, it can result in operators wasting a lot of time and money in trial-and-error method development. More seriously, consider a situation where somebody new takes over an established quality control method. They see an error message, so they put in a new column, the first they across. If this column has a different particle size and they don't adjust the system settings,

"Most chemistry MSc courses just show the textbook van Deemter curve; students are never told that the shape of the curve depends on the analyte and the mobile phase."

the system would no longer be working at the optimal flow rate. And this might result in impurities slipping under the main peak, so they don't get noticed. This can be critically dangerous, and is a very good reason for operators to ensure they are familiar with the practical implications of the van Deemter equation.

Deirdre: Importantly, the van Deemter equation not only allows us to define the

optimum conditions for a given process, but also to understand why they are optimal. Fundamentally, it shows the effect of flow rates and particle size – two important variables in method development. Once the chemistry has been optimized, people want to speed up the method, and that must be done with reference to van Deemter. Otherwise, processes will be inefficient, and possibly inadequate. Unfortunately, there is now a lack of separation scientists who are truly familiar with basic principles, which means that industry is sometimes forced to recruit from other disciplines. But these workers are not equipped to rigorously analyze the separation problem, and so may unthinkingly follow existing procedures – which is when things can go horribly wrong. So we really need to get students to understand the importance of the van Deemter equation – it's hugely important; for example, it helps people make cost-effective purchasing decisions rather than being persuaded to buy column technology they don't need.

Gert: In my classes, I illustrate the van Deemter impact with computer animations. I give students a model situation, and ask them to improve the process by altering variables like particle size and pressure. Of course, it would be ideal if each student could modulate settings physically rather than virtually, via access to a real instrument, because learning through experience is best. Unfortunately, this approach is unaffordable with large classes, so visualization tools are the most realistic option. For example, to explain the physics behind van Deemter, I use the analogy of a series of mixing units; in each unit, a molecule has a probability of remaining in the unit or leaving for the next one. The more units per length you have, the narrower the distribution in residence time eventually becomes. The larger the units, the more time it takes for a molecule to leave and hence the larger the plate height becomes. Overall, my experience is that computer animations really aid understanding, especially for the current generation of students. And there are some very useful chromatogram simulators on the internet.

Deirdre: That's a good point – I have

seen excellent animated tools in other fields, including videos that illustrate convolutional neural networks and other complex systems. Why don't we construct similar kinds of visual concepts for the van Deemter equation?

Gert: Yes! In fact, we could integrate this approach into teaching – we could ask the students themselves to make a short video clip wherein they demonstrate one of the core principles. Instead of just providing them with a computer animation, we give them an assignment that requires them to make one! That would play to the strengths of today's students, while at the same time helping them grasp the fundamental importance and significance of the van Deemter equation – to the great benefit of both the students and the industry that will employ them. Watch this space!

Gert Desmet is a Full Professor and Department Head, Vrije Universiteit Brussel, Belgium

Deirdre Cabooter is a Professor, University of Leuven, Belgium

The Cutting Edge of Biopharma Analytics

How can new LC-MS tech help biopharma manufacturers? We speak with one of our Innovation Award winners.

Ying Qing Yu is Director, Biopharmaceutical Sciences, in the Scientific Operations Department at Waters Corporation, whose BioAccord System with ACQUITY Premier device won a spot on both The Analytical Scientist 2021 Innovation Awards and The Medicine Maker 2021 Innovation Awards.

The system is designed to solve the prime problems of cost and complexity faced by all biopharma companies taking a crack at liquid chromatography-mass spectrometry (LC-MS) adoption. In this interview, Yu runs us through both the workings of the technology and its place in the wider context of mass spectrometry for biopharmaceutical companies.

What makes your work exciting?

I lead a group of scientists that develop new and innovative LC-MS analytical solutions to improve the safety and efficacy of biotherapeutics. The work we do is exciting because the biopharma industry we support is always evolving and always growing. Biotherapeutics have a huge positive impact on people's lives, and I know that improving the health and wellbeing of mankind is the best way for me to apply my expertise and knowledge.

In a nutshell, what does the Waters system do?

The BioAccord LC-MS – which is controlled by our compliance-ready software, waters_connect – is an integrated, benchtop LC-MS system. It consists of an ACQUITY Premier UPLC system and an ACQUITY RDA time-of-flight mass detector. The system includes optical detectors for tunable UV and fluorescence that are in-line with the mass detector, and embedded SmartMS technology, which automates setup and self-diagnosis, lowering the usability barrier for non-expert MS users.

It's suitable for late-stage drug development, process control, and quality control (QC) settings in both regulated and non-regulated environments for intact protein, released glycan, and peptide monitoring applications.

How were you involved in the development of the system?

For the development of BioAccord LC-MS System, I led a team of biopharma application scientist from the very early stages of the project. Along with the rest of the team, we were very involved in all the major milestones of the project: the drafting of the user request documentation, the alpha and beta system testings (to which we invited external biopharma thought leaders), commercialization, pre- and post-launch application development, marketing, and customer support.

What's the origin story of the system?

A few years ago, when Waters was developing its next generation LC-MS systems, we examined the needs of the biopharmaceutical industry.

From FDA reports, we learned that almost every BLA filing contains mass spectrometry data. The key product attributes measured with MS have increased every year over the past two decades. Though we found examples of LC-MS for QC use, LC-UV was predominantly used for product release testing. We wanted to understand the reasons why QC labs were reluctant to use mass spectrometry for release testing, so we conducted hundreds of interviews with industry scientists.

We found that the top six criteria for LC-MS system deployment in the QC labs are: robustness, assay-to-assay reproducibility, ease of use, small footprints, integration, and compliance-ready informatics with a streamlined workflow

Equipped with this information, we assembled a cross-functional team

(of which I was a part of) to develop the BioAccord system, which we successfully launched in 2019. At the same time, we were working on the development of a new chromatography surface technology for the ACQUITY UPLC that minimizes non-specific interactions between the metal surfaces of the LC and a variety of molecules, such as oligonucleotides, phospholipids, acidic peptides, and glycans. This latest LC surface enhancement goes under the trade name MaxPeak High Performance Surface (HPS) technology, and it was commercialized in 2021 with the introduction of the ACQUITY Premier LC for the BioAccord System.

The BioAccord system features what we call SmartMS technology. The system has a color-coded front panel display showing the system status. When the status is green, it indicates that the system is either ready to go, or running an analysis. When it is orange, it signals that the system may need to be checked for minor issues. If it is red, it indicates that it may be time for a service call.

The system's software automatically checks the performance of the BioAccord system between injections by checking system performance with reference standards, including the MS peak resolution, the absolute and relative MS response, and the mass accuracy. This automatic system monitoring process is designed to make sure that the system performs to specification, and to ensure that it adjusts system settings accordingly.

What specific challenges can the system solve?
The system is easy to use and maintain, and is designed for non-expert MS users. It is a compliance ready, high-performance system that can be deployed in both regulated and non-regulated environments for the routine analysis of a variety of biotherapeutics (protein, peptide, glycan, oligonucleotides and cell culture media).

In 2021, Waters Corporation and Sartorius entered into an agreement to work together and bring LC-MS into the upstream bioprocessing laboratory, where there is a real need for both an at-line product and process quality attribute analysis. Today, it can take 2–4 weeks for bioprocess engineers to receive results from a core analytical laboratory on samples taken from a bioreactor (e.g., a full plate with 48 samples). This slows down the clone selection process considerably. The BioAccord System, however, can generate the same information in a matter of hours.

This is an ideal application for the system, where those responsible for bioprocess development needn't be mass spectrometry experts, and where the information provided by LC-MS can make a difference in deciding the best cell line and clone for expressing a biotherapeutic, for monitoring product attributes of the drug, and for monitoring cell culture media.

What are the main challenges faced when developing analytical systems?
Correctly understanding user requirements and defining the right product requirements are some of the biggest challenges. Engineering teams can come up with innovations, but understanding what a fit-for-purpose system looks like and which improvements customers value most is critical. Developing complex analytical systems involves several large teams, and this in itself is another challenge. Nobody ever said that getting large teams of electrical, mechanical, software, quality, test, and system engineers plus chemists, service teams and applications support teams to work toward a common goal was easy!

Why is it important for companies to keep apace with changes in technology?
It is understandable that many companies want to stick with older systems, despite advancements made in analytical technology. Their most likely reason for this is the disincentive of upfront capital equipment costs. Another consideration is the time and effort demanded by the validation of new methods.

However, there are good reasons for laboratories to pursue upgrades. If new technologies can improve analytical throughput effectively, or measure multiple critical quality attributes of a drug product from a single LC-MS assay, or measure the product attributes of new modality therapeutics during development, then the long-term benefits of upgrading to the newest, state-of-the art analytical technologies will easily outweigh the initial burden of installation and training.

How will analytical technology continue to evolve?
Over the next decade we'll see two main areas of improvement. One is on advancing high resolution mass spectrometry technology, enabling the mass measurement of very large and complex molecules accurately. For example, charge detection mass spectrometry (CDMS) for the analysis of very large molecules holds a great deal of exciting potential. The second area is to continue to improve ease of use for LC-MS instruments for the routine measurement of different modalities. Lowering the skill barrier for LC-MS operation, and data processing would help to improve laboratory productivity.. I would like to see improvements in integrated informatics systems that are optimized for automatic workflow-driven data acquisition, processing, reporting, and sharing. Advances here would facilitate faster and more accurate decision-making and lower the development costs of biotherapeutics.
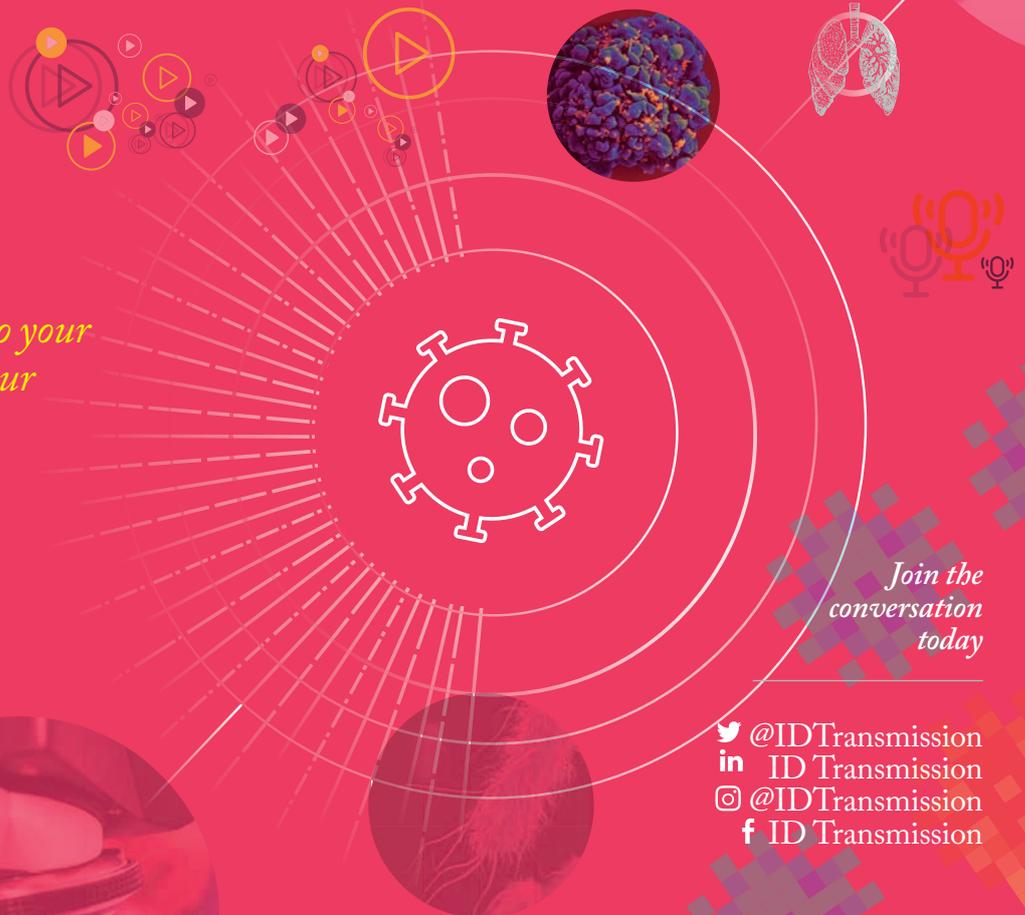
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Core Topic
Spectroscopy

Getting energized. Despite being one of the most well known and well studied processes across the globe, a team at The University of Cambridge were surprised to find there may still be a lot we don't know about photosynthesis. Researchers used in vivo ultrafast transient absorption spectroscopy to understand more about the early stages of photosynthesis and discovered new ways to extract energy from the process. "The fact that we didn't know this pathway existed is exciting, because we could be able to harness it to extract more energy for renewables," said co-author Laura Wey in the press release.

Spectroscopy stars. Two spectroscopy awards were presented at Pittcon last week. First, the Pittsburgh Spectroscopy Award was given to Robert Tycko, Acting Chief, Laboratory of Chemical Physics, NIH, USA, who has spent the past three decades advancing the fundamentals of magnetic resonance spectroscopy. Second, Craig Prater, CTO at Photothermal Spectroscopy Corp, USA, was awarded the 2023 Williams-Wright Award for his involvement in the development and commercialization of many AFM technologies and instruments, which are in widespread use in academic and industrial research. Nominations can now be submitted for the 2024 award.

BrAIn power. DeepGlioma is an AI-based diagnostic screening system that uses stimulated Raman histology to analyze tumor samples and improve brain cancer diagnosis. In a recent study with 150 active participants, DeepGlioma's accuracy was over 90 percent. "Rapid methods for molecular classification hold great promise for rethinking clinical trial design and bringing new therapies to patients," said Daniel Orringer, a senior author of the paper, in the press release.

Crab shell spectroscopy? Researchers from Ateneo de Manila University have turned crab shells into a bioplastic for diffraction gratings – optical components that could enable portable spectrometers. The scientists prepared the crab shells through a soft lithography replication process, using microscopy and diffraction experiments. The resulting lightweight bioplastics – called chitosan – are also biodegradable. "Conventional gratings are typically made of heavy materials such as glass," said Raphael A. Guerrero, a team leader in this study, in a press release. "But gratings made of chitosan could be used to make lighter and less expensive spectrometers."

References available online

IN OTHER NEWS

A surface-enhanced Raman spectroscopy-based nanostructure sensor can identify arsenic-containing molecules at low concentrations in water, food, and soil.

Researchers outline the first experimentally quantifiable approach to defining molecular assembly, which they believe could pave the way to using spectroscopic techniques to detect alien life.

A procedure using two types of spectroscopy could improve accuracy of deep brain stimulation treatment for Parkinson's disease; Mireille Quémener, Université Laval, Canada, will show this research at Optica's Biophotonics Congress on April 27.

Advances in monochromated STEM and monochromated EELS enable researchers to understand the correlation of lattice vibrational properties with local atomic configurations within different materials.

LIBS: Five Years On

We last spoke to Rick Russo five years ago, covering all things laser-induced breakdown spectroscopy (LIBS). Where are we now? What progress has been made? Do the same issues still crop up? Russo gets us up to speed.

Could you briefly tell us a bit about your current research?

My current focus expands beyond elemental analysis – using LIBS to measure isotopes at atmospheric pressure.

What are the applications?

There are two primary applications of isotope analysis with LIBS. Number one is uranium and plutonium analysis of nuclear material samples. Previously, this type of analysis has been conducted with mass spectrometry. In fact, the community has spent 70 years developing MS to analyze isotopes. However, it is difficult to take isotope measurements in the field with a mass spectrometer – and although researchers have been working on portable MS technology for over half a century – they still haven't quite mastered it. Even if these measurements are taken in the laboratory, MS requires a lot of sample preparation. My focus has been on analyzing nuclear isotopes in the field for agencies, such as the Department of Energy, the IAEA, and the nuclear industry. LIBS is also important for nuclear forensics, where isotopes are needed and obtained in a field environment. Lastly, LIBS can be applied to geochemistry – the field is very interested in isotope ratios for prospecting and age dating. My primary focus has been on isotopes for nuclear related measurements.
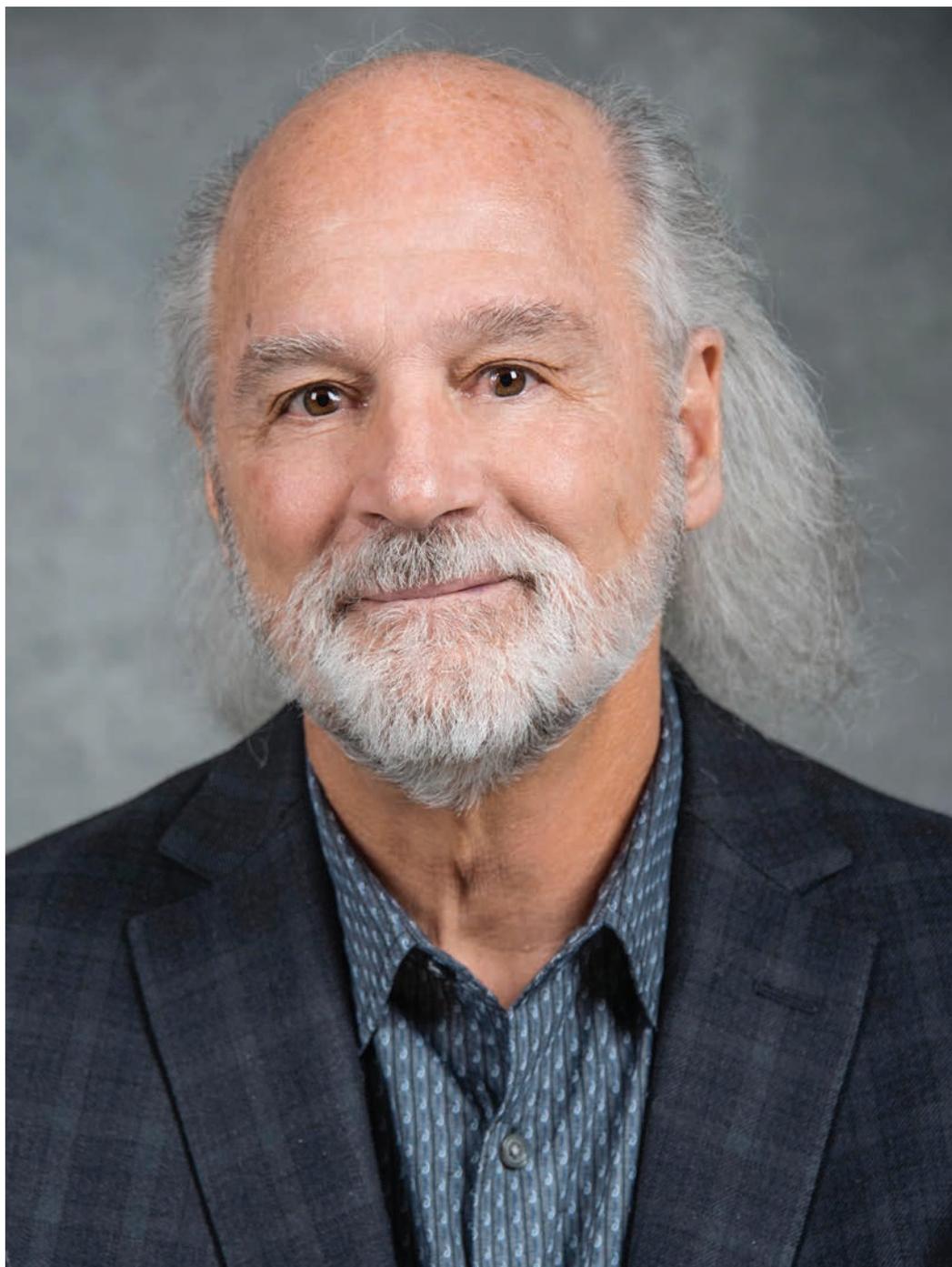
What's the most significant change in LIBS technology over the past five years?

Recognition! Adoption takes a long time, but I think – thanks to the success of the ChemCam instrument on Mars – LIBS has been more widely acknowledged. Instead of solely injecting funds into MS advancements, people have realized that there are alternatives, and have started to lean on LIBS a little more. Many researchers have stopped deeming LIBS "too complicated" with "too many problems," and are finally asking how LIBS technology can be tailored to their application requirements. Everything has its idiosyncrasies and it's nice to see that people aren't immediately

ruling out LIBS. After all, it can do things that other techniques cannot. I would say that the technology hasn't changed that much, but there is much more emphasis on data analytics from processing the LIBS signals. Artificial intelligence and machine learning have greatly benefited LIBS, and I believe that they will continue to help unravel the quality of measurements.

What are you most excited about in the LIBS field?

LIBS grants us the ability to conduct measurements that cannot be done any other way. It's one of the few technologies that can actually do standoff chemical analysis – detection without physically collecting the sample – which NASA demonstrated by having the Mars Rovers operate remotely. Further, the measurement itself is standoff and can easily be adopted for advanced inline measurements.

Back in 2017, you said it is important that we figure out the value proposition of LIBS and how it could solve industrial problems. It sounds like we're closer…

I think we are at the early adopter stage of LIBS technology. There are five stages – innovators, early adopters, early majority, late majority, and the laggards. Implementation of any technology takes decades. We definitely have more early adopters in industry than we did five years ago. As soon as a major company uses LIBS' unique capabilities to improve their manufacturing, they'll gain a competitive edge – and more will follow.

I recently read an article published by the World Health Organization that said we need to improve the way we monitor salt in food. I always say there's no error bars on food labels because of the way laboratory-based measurements are done – they're extremely slow. But if you could measure salt as you're processing the food – which is possible

with LIBS – you'd immediately improve accuracy. When the industry realizes that we need better tolerances on particular measurements, and that we can't retrieve them without real-time inline measurements, a switch will flick in terms of technological uptake.

Has LIBS been accepted by the pharmaceutical industry yet?

No. Integration into the pharmaceutical industry remains a tough challenge. Whether that's because there are serious issues with heavy metal contamination or an issue with ingredients, the pharmaceutical industry is hard to crack. My guess is that they would probably adopt the technology when the majority does, rather than stepping up as early adopters.

Where do we go from here? What do you want to see happen in the future?

I would like to see LIBS technology recognized as standard protocol and for it to become more mainstream. Ideally – in the next ten years – we'll reach a level where we aren't struggling to convince people of its potential and scalability. Adoption is accelerating, and my company alone has gone from selling one or two instruments a year to selling ≥40/year. The early adopters are the laboratories within industrial places. However, they're not buying LIBS for process control – they're buying it to use as a laboratory technique for raw material analysis, failure analysis, or general composition analysis. We have huge amounts of infrastructure going into the electrification of the world. But what about the chemistry of the batteries? LIBS is ideal for measuring the chemistry of batteries. I want people to understand how valuable this technology is and how it can solve their problems. That would be the real win – and I think machine learning and AI will help us get it there.

MORE BROADLY...

Do you have any concerns for the spectroscopy field or analytical science in general?

I don't really have any big concerns. Chemical analysis is a fundamental part of everyday society! Everything is based on the elements on the periodic chart – you have to know what you're eating, if something changes color, or if a component isn't working correctly in any device. Everything comes down to chemistry. And so analytical chemistry will never go away. And the more complex the world gets, the more analytical science will be needed.

If you weren't working in analytical science, what would you be doing?

If I could answer that question, maybe I'd try a different hobby! I have a drum set sitting next to me, a Spanish course on my computer, and I've even tried surfing, but – everything gets boring faster than science. I retired from Berkeley in 2019 with the idea of pursuing other things, but I got bored real fast! Now I'm back to being (gratefully) overwhelmed at Applied Spectra every day. I think – for me – there is no limit to learning. I like continuing to grow my mind.

Lastly, do you have any advice for any young analytical scientists beginning their career?

Keep an open mind, know what's been done, and be innovative. And don't expect things to happen overnight!

Picking Out the Bad Apples

Luis Rodriguez-Saona of Ohio State University discusses the spectroscopic techniques at the forefront of food analysis – and the hurdles involved in translating new technology from initial development through to application

What are your main spectroscopy research interests?

I apply novel analytical technologies to agricultural product testing, with a focus on developing portable and handheld sensors based on vibrational (infrared and Raman) spectroscopy to support quality, safety, and nutritional monitoring of food and agricultural products. In collaboration with leading optical sensing companies, my molecular vibrational lab has combined spectroscopy with chemometrics for food safety and quality assurance. We have also developed predictive models for the rapid detection, identification, and classification of chemical and microbial contaminants as well as food components with biological activity.

What are the main applications – and specific advantages – of vibrational spectroscopy in food analysis?

FT-NIR and mid-IR spectroscopy are mainly used for the rapid and non-destructive detection of major components in food – including moisture, protein, fat, and carbohydrates. Dairy, grains, beverage, and meat sectors have all embraced the technology. Vibrational spectroscopy provides spectral information from diverse functional groups present in different samples, allowing us to obtain unique profiles from food analytes

that can be used to quantitate target molecules or identify any potential tampering contaminants. NIR has been a benchmark for developing predictive models that estimate major component levels. While mid-IR provides higher fingerprinting capabilities and detection levels to screen for contaminants and adulteration, NIR can deeply penetrate materials – allowing the analysis of intact and non-homogeneous material. Mid-IR attenuated total reflectance (ATR) can only penetrate a few microns deep and requires homogenization of the sample.

Could you tell us a little about the use of optical sensor technology for food analysis?

The miniaturization of spectrometers – particularly using NIR spectroscopy – has opened doors for field analysis and allowed us to make measurements in-situ without transporting samples to the lab. There is now a broader range of wavelength selection technology employed to disperse polychromatic light, such as MEMS, diffraction grating, volume phase holographic grating, LVF, the Fabry-Pérot interferometer and detectors, such as CCDs or InGaAs. Our work uses the technology to

Raman spectroscopy has revolutionized handheld systems and given access to unique fingerprinting capabilities for remote sensing. We have been working on applications of different systems equipped with excitation lasers in the NIR (1064 nm, Wasatch and Rigaku) and visible (785 nm, Metrohm) regions. Raman equipped with a 1064 nm excitation laser effectively limits background fluorescence but weakens the energy for molecular vibrations of biological samples. Our research on SERS has shown limited signal by employing a 1064 nm excitation laser as opposed to a laser in the visible region.

What are the main analytical challenges that manufacturers face in the food and beverage industry?
The complex nature of foods often makes it difficult for the technology to be implemented in some application areas. In the fruit and beverage sector, the high content of water is a challenge because of its strong dipole nature. The water signal masks important features in the spectrum – making it difficult to develop quantitative and classification algorithms.

The development of robust predictive models using chemometrics is another challenge. Models need to capture the variance of the samples and often require a large data set to provide robustness. Further, models need to be independently validated to

collect spectral information in the 1100 to 2500 nm range so we can access unique information from combination modes that appear in polyatomic molecules. We have explored the use of ATR systems operating in the mid-IR region (Agilent 4500 and 5500 series) for quality assurance, phenotyping of breeding materials, and identifying food contaminants.

determine their predictive ability. The field of chemometrics and machine learning is advancing at a fast pace, which will help us extract information from convoluted spectral data and create powerful algorithms that obtain maximum information from the raw data.

What are the most cutting-edge techniques in food analysis?
I've already name dropped several exciting technologies in this space! NIR handheld technology is advancing at a fast pace – and using micro-electromechanical systems – spectra collection can be widened to the short wave IR and help develop predictive models. Surface enhanced Raman spectroscopy (SERS) can improve detection limits into the ppb level; the Raman signal of a target analyte can be enhanced when it is in proximity to the SERS substrate – depending on the shape, size, and orientation of the nanoparticles. And there is exciting research on functionalized SERS substrates with unique recognition ligands and encapsulation. Nevertheless, improvements in SERS substrates are still required for consistent detection of analytes in complex environments. XRF is also an interesting technology for in-field elemental analysis – and detection limits are improving.

Analysis of Phosphorylated Peptides Using a Bioinert YMC-Accura Triart C18 Column
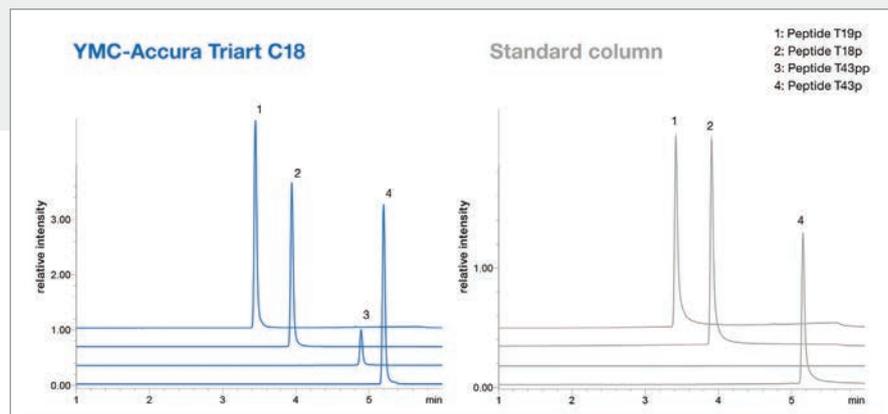

Figure 1. Extracted ion chromatograms (EICs) of the phosphopeptide mixture separated using the bioinert YMC-Accura Triart C18 column or the equivalent stainless steel column hardware equilibrated with 10 injections.

For phosphate group-containing biomolecules such as (phospho-)peptides, nucleotides, and oligonucleotides the analysis with standard column hardware is challenging. YMC-Accura column hardware has been designed to eliminate any interaction between sample and stainless steel due to a strict coating of the column body and the frits. It allows sharp peaks, stable recoveries, and eliminates

consequential carry-over effects. This makes it a great choice for working at trace-levels.

Four phosphopeptides T19p, T18p, T43p, and T43pp were analysed with the bioinert YMC-Accura Triart C18 and the corresponding stainless steel column at 60 °C. Using the bioinert column led to higher intensities and peak areas for all peaks. Additionally, the high recovery rate from the YMC-Accura Triart C18

column also enabled the detection of the challenging phosphopeptide T43pp, which contains two phosphate residues. In contrast, the analysis with the standard column hardware showed no signal, even after thorough equilibration using several sample injections.

Full method details can be accessed here: https://ymc.eu/d/brDoQ

SEC-MALS Characterization of Copolymers Used for Drug Delivery

Figure 1. RI chromatogram and molecular weight traces obtained for PEG-PGA by light scattering (LS) and conventional calibration (CC)

Biodegradable polymers are an important class of macromolecules that can be employed as drug-delivery agents to solubilize hydrophobic therapeutic molecules in water. The pharmacokinetics of the polymer within the body is greatly dependent on their molecular weight and size.

Size exclusion chromatography (SEC) has been widely used to study the molecular characteristics of polymer-drug systems. A series of PEO hom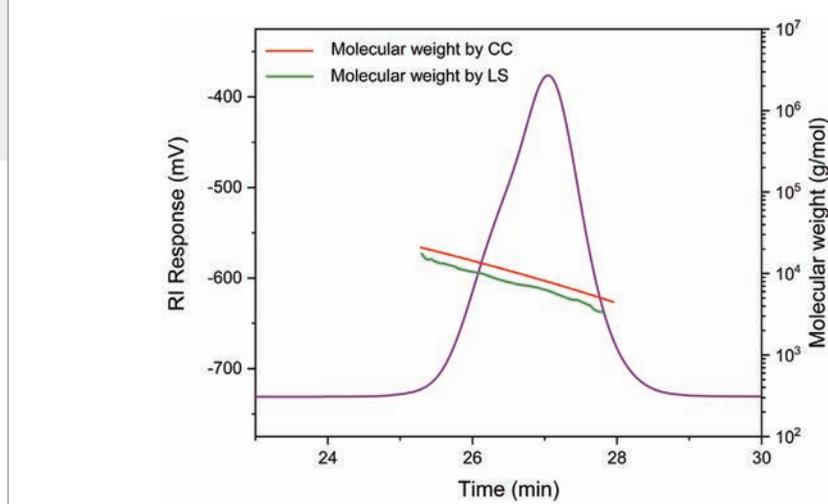opolymers and a PEG-PGA copolymer were analyzed and compared using SEC with conventional calibration and multi angle light scattering. The use of conventional calibration provides accurate molecular weight if the structure and the chemistry of the calibration standards and the samples are identical. The PGA block in the copolymer modifies the conformation of the PEO chain, resulting in an overestimated molecular weight when using a PEO calibration. The use of a light scattering detector is required to determine the true molecular weight of polymers, regardless of their chemistry and structure.

Find out more at:
http://bit.ly/Copolymer-characterization

Mentor, Collaborator, UVPD Pioneer

Sitting Down With… Jenny Brodbelt,
Professor, Department of Chemistry,
University of Texas, Austin, USA

How did you get into analytical science?

Math was always my best subject in high school – but science offered more options! Fortunately, I had enthusiastic teachers, so I had a good foundation for college. I majored in chemistry with a minor in math. Many of my friends were pre-med, but that was a no-go for me – I could not handle the blood and gore of surgery nor the frantic stream of patients as a physician. I was first captivated by analytical chemistry when I took an instrumental analysis class – the lab portion was a round robin in which students would partner up and rotate through different spectrometers and analytical methods each week. The unusual part: most weeks one of the partners had to donate urine to provide a "real sample" for the experiment. That sealed my interest in analytical science!

Interestingly, my undergraduate research project focused on isolation of natural products in plants, so it was on the fringe of analytical science – but I knew that pursuing more advanced analytical chemistry and focusing on the amazing field of mass spectrometry was my best path in graduate school.

When did you know you made the right career choice?

As soon as my first mass spectrometer arrived and was unpacked in the lab, I knew I would enjoy my first years as an assistant professor. But the real clincher was when a couple of brand-new grad students joined the lab and I witnessed first-hand how ideas can propagate and veer off in new directions.

Please share a little about your research group…

My group is excited about advancing mass spectrometry as an analytical tool through development of instrumentation and new methods. In particular, we have focused on developing ultraviolet photodissociation (UVPD) and expanding its applications to characterizing biological molecules. UVPD is a method of energizing ions through absorption of photons, which causes fragmentation.

The dissociation patterns produced by UVPD are chock-full of informative and unusual fragment ions; it creates a very rich molecular fingerprint. And that's how we've been able to use UVPD to characterize big molecules like proteins (to pinpoint post-translational modifications) and small molecules, like lipids (to localize double bonds and other features). More recently, we have also started using UVPD to dissect multimeric protein complexes with the aim of understanding how proteins interact and assemble into functional complexes.

As for the Brodbelt Research group, well, it's a diverse mix of mostly graduate students, one or two postdocs, and a small handful of undergraduates. Each person has a unique perspective and passion – whether it be analyzing lipids, or characterizing proteins, or modifying mass spectrometers, or developing new data analysis tools.

We collaborate extensively with bio-oriented groups, like those in biochemistry or molecular biology, to pursue more complex biological problems.

In short, I would say our special expertise is the analytical science of mass spectrometry, and we partner with biological scientists to gain their insight and expertise to increase the impact of our work.

What's the most challenging experience of your career?

There are many challenges in an academic science career. One challenge is figuring out how to motivate others to achieve project goals and surpass expectations. Another ongoing challenge is the management of advanced instrumentation, which requires endless care, teamwork, lots of trouble-shooting, and consistent maintenance.

And the aspect you're most proud of?

Mentoring. It is amazing to watch group-members (grad students, undergraduates, and post-docs), who have thrived in science and been incredibly innovative in the lab, taking scientific risks, jumping into new projects without a clear path ahead, and facing and clearing obstacles along the way. My group-members really drive the science and are the innovators. One example is my group's effort to integrate lasers with mass spectrometers to enable photodissociation for MS/MS. There were many technical hurdles – fortunately, enthusiastic grad students were willing to lead this effort and make it successful. They were fearless!

Thinking about mass spectrometry as a whole, what has been the most important development over the past decade or so?

There are so many great options. I would say that the broader availability of high accuracy, high resolution, high mass analyzers has transformed the information we can harvest from mass spectra, and this accessibility has accelerated many areas of biological research.

Do you have any concerns for the mass spec field – particularly in terms of biological applications?

We have many more new users and new applications, but fewer people gaining expertise in the fundamentals of mass spectrometry and fewer trained in the design and hands-on building/modification of mass spectrometers. Many will argue about whether it is the biological questions that drive the mass spectrometry science or whether advances in mass spectrometry drive the biological questions – but it doesn't really matter. We must continue cultivating interest in instrumentation and core knowledge in mass spectrometry to keep the field moving forward.

If you weren't a scientist, what would you be doing instead?

I enjoy writing and the creative process, so maybe a novelist. I'd likely focus on mysteries and creepy melodramas; absolutely no slashers or sci-fi!