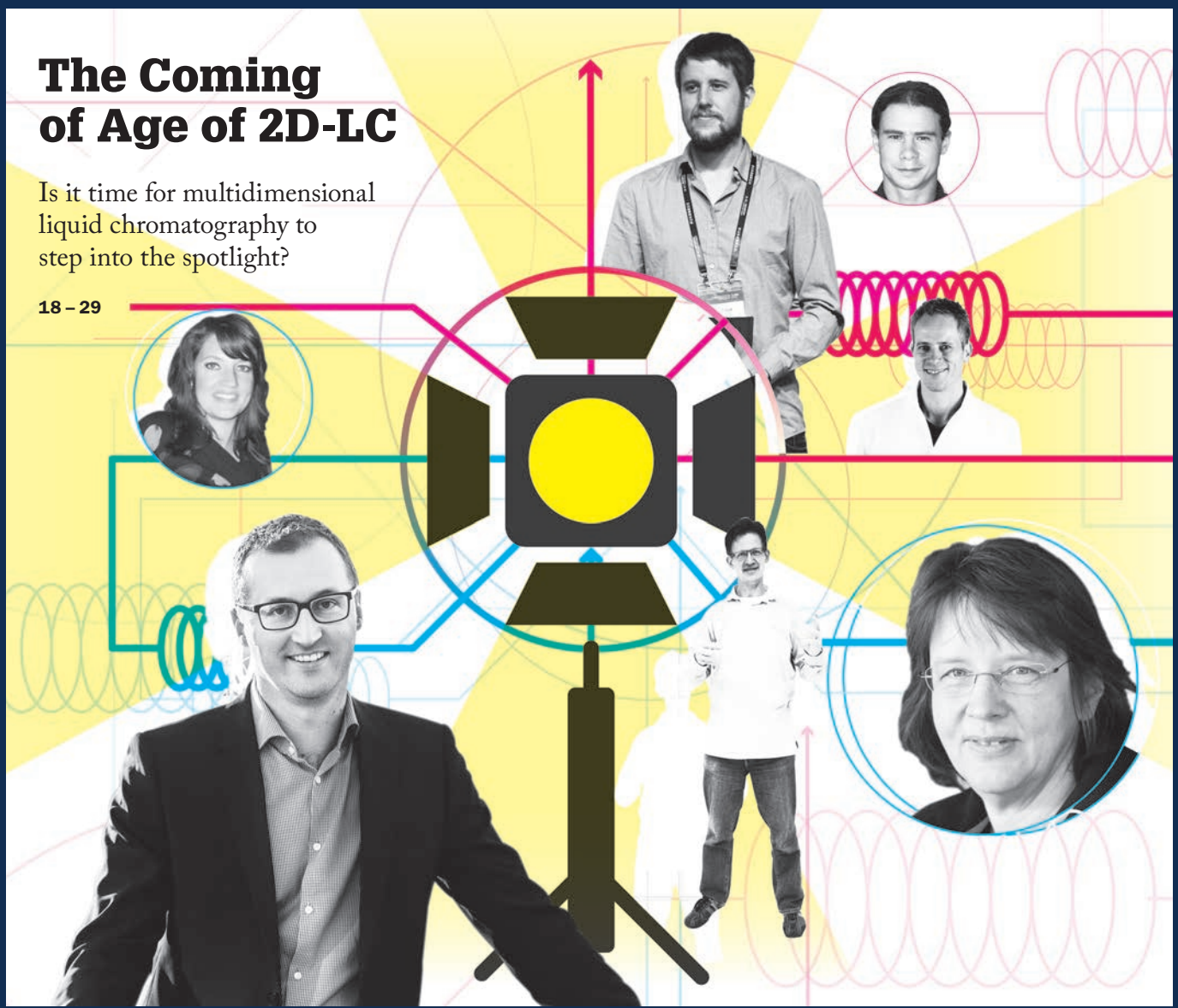
JUNE 2019 # 77

the Analytical Scientist™



The Coming of Age of 2D-LC

Is it time for multidimensional liquid chromatography to step into the spotlight?

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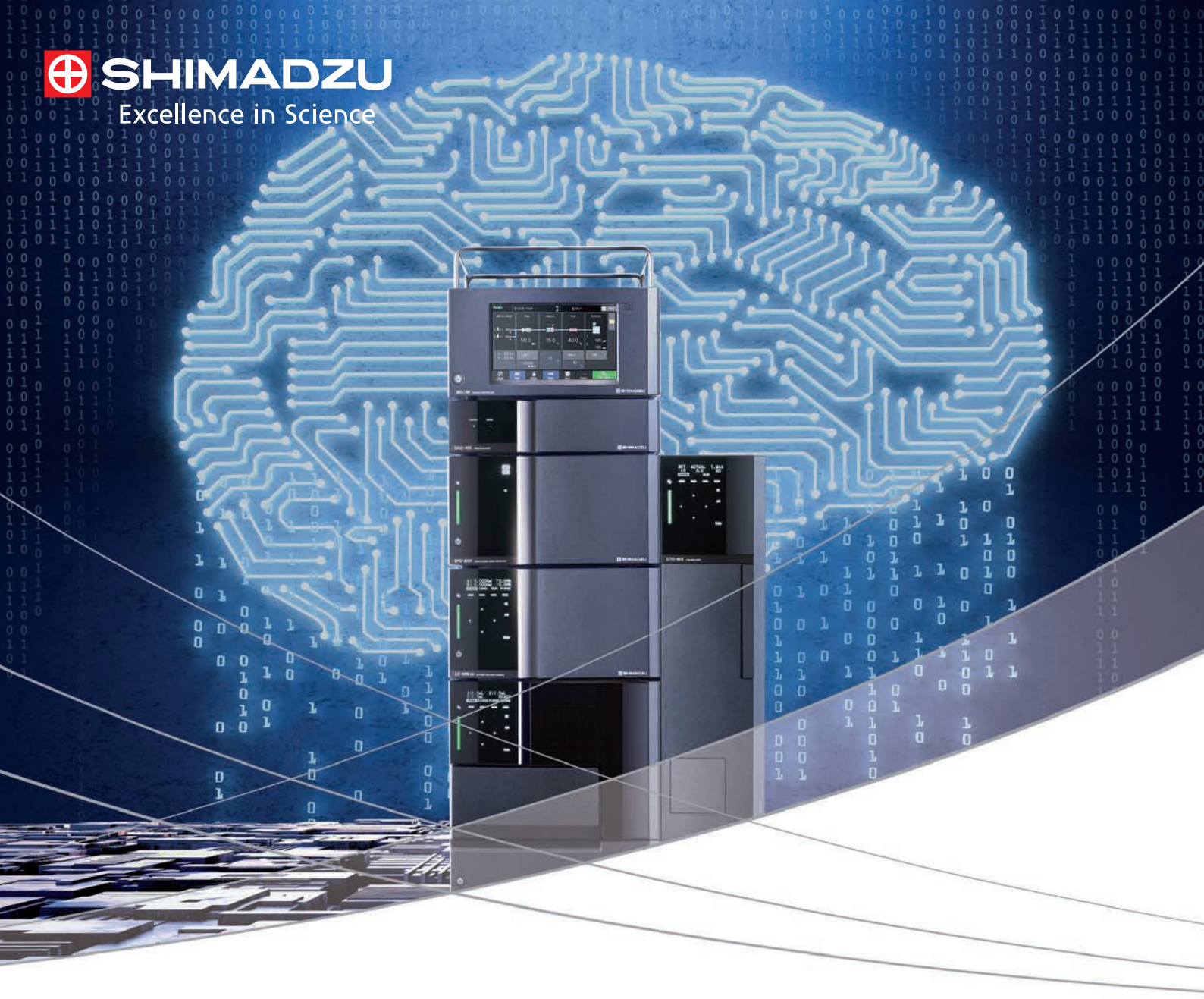

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Image of the Month

Rock On

Portable X-ray fluorescence spectroscopy (pXRF) allowed researchers to gain new insights into the pigments used in ancient rock art in Texas, USA. Previous studies at the Rattlesnake Canyon site have used ICP-MS, which requires samples of paint to be taken. With pXRF, the researchers were able to carry out elemental analysis with no risk of damaging the artwork. They reported their findings at the American Chemical Society (ACS) Spring 2019 National Meeting & Exposition.

Credit: Courtesy of Shumla Archaeological Research and Education Center

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

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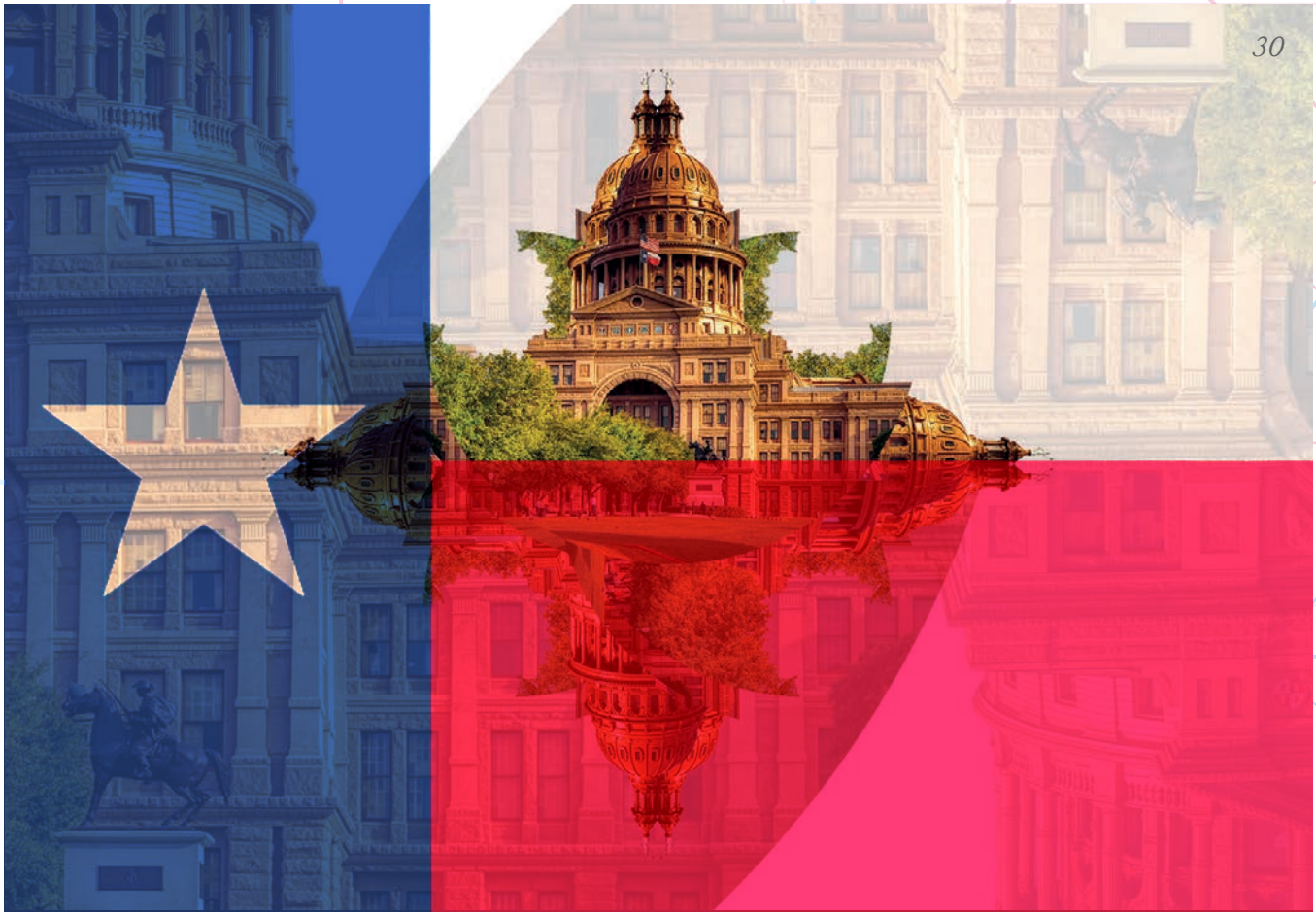

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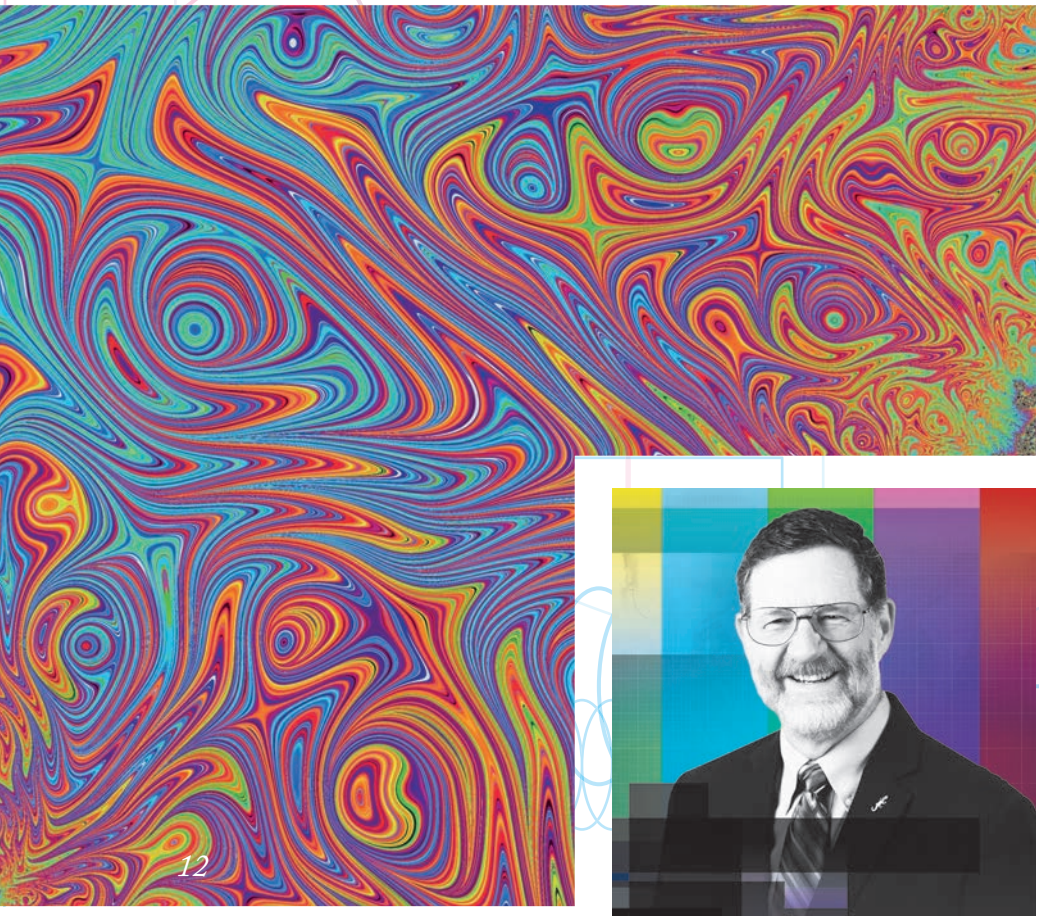

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Face Time

Are conferences still worth it in the digital age?

There is a good chance you'll be reading this issue at one of the many analytical events that fill early-summer calendars, from ASMS Atlanta to HPLC Milan.

In some ways, it's surprising that conferences still hold such sway in scientific communities. After all, in today's world, we're only ever a click away from sharing information, seeking advice, or engaging in debate with colleagues all around the world. Are we mad to fly halfway across the globe, racking up financial and environmental costs along the way? Are conferences an outdated concept in the digital world? Will the future of science be hashed out in virtual meetups and social media chatter?

Judging by the buzzing sessions and bustling exhibition halls I see every year, I'm not convinced. I'm sure we've all questioned our choice to attend an event at some point (usually while contemplating some egregious example of convention-center food or suffering the after-effects of working and playing hard). And yet, there is something so special about engaging with people face to face. The Internet has made us more connected than ever but, for most of us, a few minutes of real-life conversation can build more rapport than months of emails.

Conference-goers give a range of reasons for attending, but the overwhelming theme is connection – to meet friends and collaborators, to get feedback on their work, and to feel part of a wider community. I certainly come away from an interesting event fizzing with ideas and inspiration.

Perhaps the world will change as Generation Z comes of age; as true digital natives, they may choose new ways of connecting. But I have a hunch that modern technology will struggle to supplant the instinctive emotional pull of a face-to-face conversation.

At the same time, conferences cannot ignore how science and its communication are changing. The days of sitting in a darkened room watching a series of lengthy talks are already fading, with organizers increasingly embracing networking, social media, and discussion to stay relevant in the digital age.

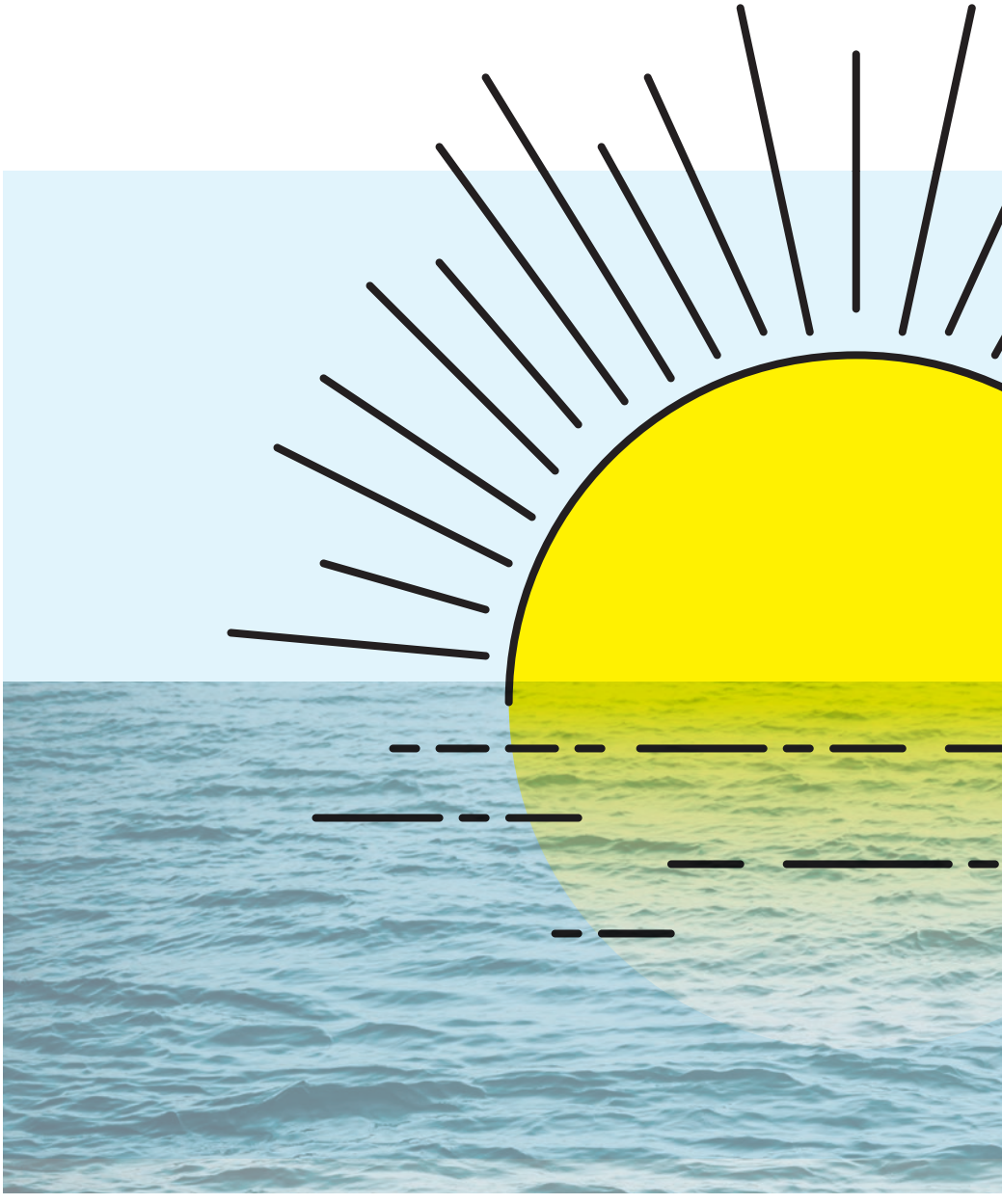
For The Analytical Scientist team, the conference calendar plays the essential role of directly connecting us to our readers and partners for feedback and further collaboration – while allowing us to keep our finger firmly on the pulse of the analytical community. And so, we look forward to meeting as many of you as we can this summer for some real face time.

Charlotte Barker
Editor

Upfront

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

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

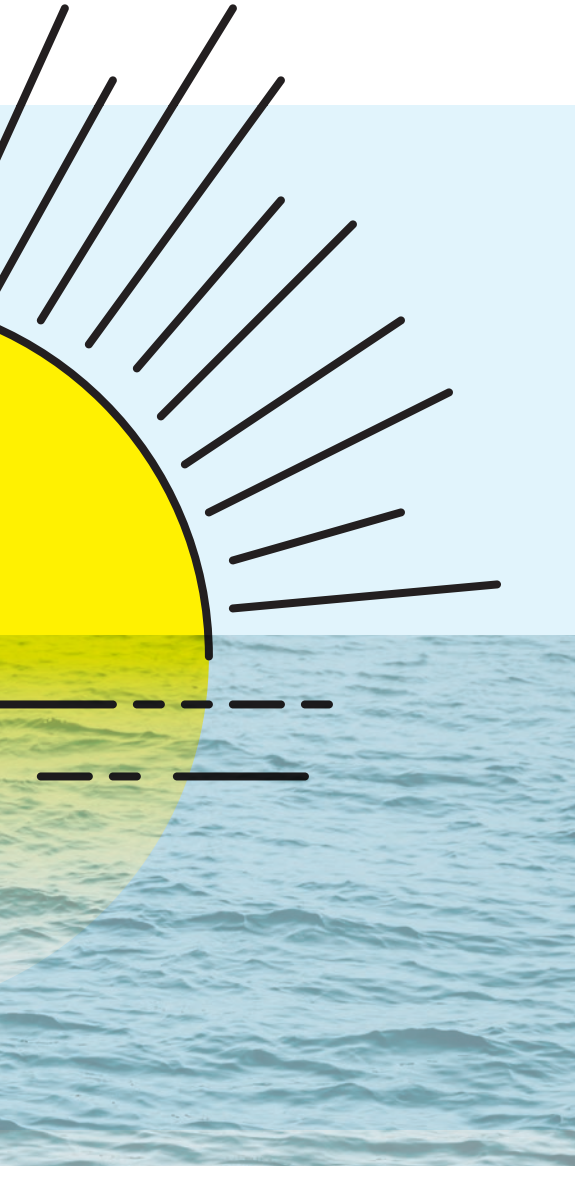


Here Comes the Sun

Characterization of algal light-harvesting protein complexes could help improve solar energy systems

Phycobilisomes act as light-harvesting antennae for cyanobacteria and red algae – some of the oldest lifeforms on the planet. As such, these protein complexes are highly evolved and incredibly efficient at capturing light for photosynthetic processes (1).

Sem Tamara and colleagues used multimodal mass spectrometry (MS) to characterize the components of B-phycoerythrin, the major phycobiliprotein in the red algae Porphyridium creuntum, and further our understanding of how the complex operates (2). This knowledge, co-investigator Aneika Leney says, has the potential to be used for "the innovative design of new light-harvesting machines



for use in bioenergy processes".

Tandem MS techniques allowed the complex structure of B-phycoerythrin – comprising 12 alpha and beta subunits linked by gamma chains – to be separated and fragmented. "Using our strategy, we were able to identify components of the light harvesting machinery that stabilize the complex and join many different protein components together. These were more heterogeneous than we initially anticipated and possess multiple different chromophores that all act to transmit light effectively from one protein to another," says Leney.

There are still gaps in our knowledge, says Leney, "We and others in the field have now characterized most of the light harvesting complex, however, questions still remain on how this complex is tethered to the membrane inside algae to complete the final energy transmission process from light to chemical energy."

The authors hope that we can eventually apply the knowledge to supercharge manmade solar cells. We certainly have a lot to learn – the light-harvesting systems of red algae operate at around 95 percent efficiency, while manmade solar panels average 10–20 percent.

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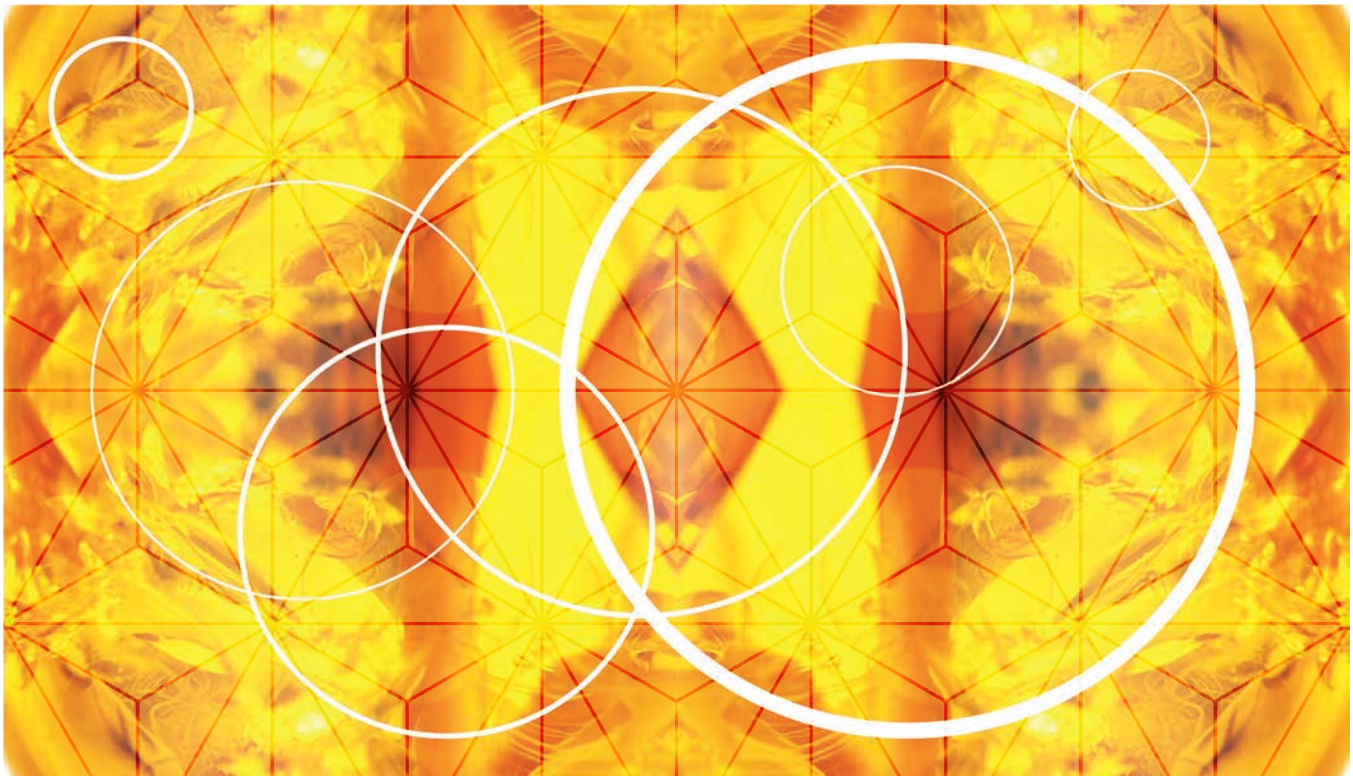
1. *L Chang et al., "Structural organization of an intact phycobilisome and its association with photosystem II", Cell Res, 25, 726–737 (2015). DOI: 10.1038/cr.2015.59*
2. *S Tamara et al., "A colorful pallet of B-phycoerythrin proteoforms exposed by a multimodal mass spectrometry approach", Chem, 5, 1302–1317 (2019). DOI: 10.1016/j.chempr.2019.03.006*



Amber Alert: The Iberian Imitation Game

Beads recovered from prehistoric burial sites reveal a secret history of counterfeiting

Amber jewellery was highly valued amongst early humans, and historians have used it to identify those of wealth or status. But not all "amber" found in prehistoric sites is quite what it seems…

Carlos P. Ordiozola and colleagues employed electron probe analysis, infrared spectroscopy, X-ray diffraction and Raman spectroscopy to study amber beads recovered from two Iberian burial sites dated from the 2nd and 3rd millennia BC (1). The team were surprised to find that neither sample produced the characteristic spectra of the Baltic or Sicilian amber traded at that time.

The researchers set out to investigate, but finding the true composition of the beads was a challenge, says Ordiozola: "We were identifying an unknown resin that has undergone 7 millennia of oxidation and polymerization processes. We do not have similar ancient resins for comparison, so an accurate identification was always going to be tricky." Nevertheless, the team were able to identify a number of interesting materials.

Infrared microscopy of the first set of beads indicated a core of aragonite, likely mollusc shell, and a coating of Pinus tree resin and apatitic calcium phosphate – thought to derive from dissolved human bone. X-ray diffraction of the second set of beads identified a core of calcium and a coating of calcite, cinnbar and an oxidized abietane. The authors identified this coating as a terpenous resin.

"We show that these communities developed complex technologies to manufacture amber equivalents, imitations or counterfeits," says Ordiozola. The discovery of these Bronze-age fakes means historians may have to rethink the relationship between amber and wealth at archaeological sites.

Though many things have changed since the 3rd millennium BC, it would seem that human ingenuity and the desire to "fake it until you make it" has remained a constant in our society.

Reference

1. *CP Odriozola et al., "Amber imitation? Two unusual cases of Pinus resin-coated beads in Iberian Late Prehistory (3rd and 2nd millenia BC)", PLoS One, 14, e0216773, DOI: 10.1371/journal.pone.0215469.*

The Power List 2019

Who are the Top 100 scientists in the field?

Nominations are now open for The Power List 2019, our annual celebration of the big names and unsung heroes of analytical science. This year we're keeping it simple and focusing on the 100 most influential analytical scientists, across all technologies and applications.

Nominate the scientists who inspire you - whether a mentor, a colleague or someone whose work you have admired from afar. Notably, we encourage you to also consider diversity.

Remember: scientists have to be nominated to be in the running, so don't hold back!

Submit your nominations at tas.txp.to/powerlist2019

The Process

- Nominations for The Analytical Scientist Power List are welcome from individuals, groups or organizations
- The full list of nominations will be put to an expert judging panel
- The panel will decide on the Top 100. The panel's decision is final and no correspondence regarding their deliberations or the final list will be entered into
- The Analytical Scientist Power List 2019 will be published in October 2019 in print and online

Experimenting with Psychedelics

Analysis of psychoactive plant residues highlights advanced botanical knowledge in ancient South America

Ayahuasca is a plant-based psychoactive drink that forms an integral part of certain shamanic rituals in South America. The effects of the potent concoction were immortalized by Terence McKenna in his 1989 book "True Hallucinations: Being an Account of the Author's Extraordinary Adventures in the Devil's Paradise", in which he described experiencing "vegetable television" (1).

While McKenna's account is certainly entertaining, a recent study by Melanie J Miller and colleagues took a rather more scientific approach to determining the components of the psychedelic brew. The team, including scientists from New Zealand, USA and Bolivia, characterized residues found on a ritual bundle from southwestern Bolivia 1,000 CE, using liquid chromatography tandem mass spectrometry (LC-MS/MS) (2).

The bundle contained a number of items, including wooden tablets for grinding psychotropic plants and camelid bone spatulas, but those chosen for study were an animal-skin pouch constructed from fox snouts, and two small pieces of dried plant material attached to wool and fiber strings. To help analyze these unusual samples, Miller brought on board toxicologist Christina Moore from Immunalysis Corporation (California, USA), whose lab specializes in illicit drug testing of biological samples.

The cross-disciplinary team identified traces of bufotenine, dimethyltryptamine, harmine, cocaine, benzoylecgonine (a product of cocaine degradation), and the possible presence of psilocin – suggesting the use of at least three plant sources. Miller says, "This is the most diverse selection of psychoactive compounds recovered from a single archaeological context in South America to date."

The compounds come from plants from geographically distant locations, including Amazonian plants. "This suggests a deep knowledge of the psychoactive and healing properties of these plants, and how to procure and use them in culturally meaningful ways," says Miller.

Miller credits advances in analytical technology with making studies like these possible by allowing the team to obtain high-quality data from tiny samples. Perhaps the next instalment of the Indiana Jones franchise should feature Harrison Ford wielding a portable mass spectrometer rather than his trusty whip!

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1. *T McKenna, "True Hallucinations: Being an Account of the Author's Extraordinary Adventures in the Devil's Paradise", 1989, Rider Books, London, UK.*
2. *MJ Miller et al., "Chemical evidence for the use of multiple psychotropic plants in a 1,000-year-old ritual bundle from South America", Proc Natl Acad Sci USA, pii: 201902174 2019). DOI: 10.1073/pnas.1902174116*

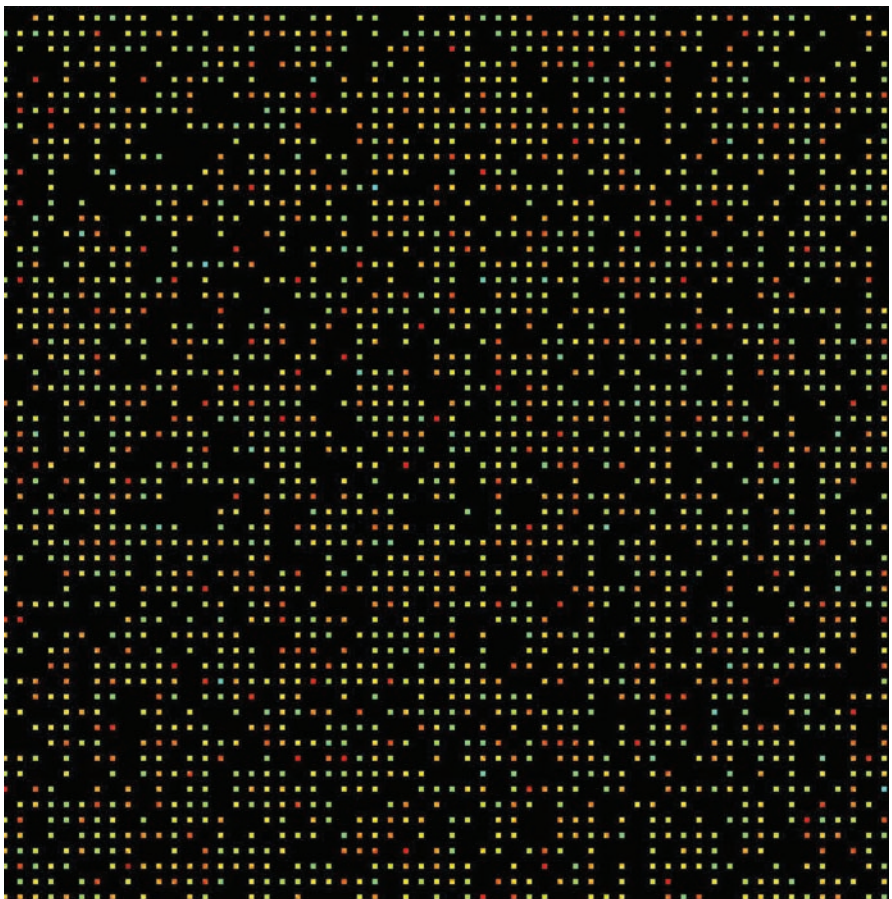
Photoacoustic Microscopy Makes All the Right Noises

A new technique uses sound waves to listen to the oxygen consumption rate of cancer cells

It may look like a giant game of space invaders, but each colored dot in the image below is an individual cancer cell within a droplet of blood. In this scanned image of single-cell metabolic photoacoustic microscopy (SCM-PAM), different colors represent the amount of oxygen dissolved in each well. To measure oxygen consumption rate, researchers place cells into individual wells filled with blood; those with higher metabolisms consume more oxygen, lowering the level remaining in the blood. But instead of tiny oxygen sensors, which can affect the metabolic rates of the cells, SCM-PAM uses a laser tuned to a wavelength that hemoglobin can absorb and turn into vibrational energy, or sound. How? Hemoglobin's ability to absorb light at that particular wavelength changes as it becomes oxygenated, meaning that its sound can indicate the degree of oxygenation in the blood. This new approach can analyze 3,000 cells in just 15 minutes, paving the way for a greater understanding of intratumoral heterogeneity.

Reference

1. *P Hai et al., "High-throughput, label-free, single-cell photoacoustic microscopy of intratumoral metabolic heterogeneity", Nat Biomed Eng, [Epub ahead of print] (2019). DOI: 10.1038/s41551-019-0376-5*



In My View

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

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

Contact the editors at charlotte.barker @texerepublishing.com

Going Native with Microfluidics

Technology that enables protein analysis in solution has the potential to unlock a deeper understanding of interactions – and how those interactions can be manipulated.

By Andrew Lynn, CEO, Fluidic Analytics, Cambridge, UK.

If you think about how (bio)therapeutics work, you will immediately note that interactions – either between proteins or proteins and other molecules – are key. Those interactions are enabled by the protein's conformation, which not only imbues each protein's unique functionality but can also change depending on the conditions in which it exists. We now acknowledge that proteins do not function alone and, therefore, should not be considered as isolated entities, but as part of complex networks that ultimately drive biological systems.

Now, let's think about how we analyze proteins. Does the protein typically exist in its native state when we study it? Are we getting a "true picture?" Techniques that rely on denaturation or digestion – or where the molecules must interact with a surface or matrix (or are fixed to a surface) – affect protein conformation (or the ensemble of conformations), which means that we are actually testing a proxy of the native solution-state protein. Indeed, the dominant techniques for measuring protein-protein interactions, such as surface plasmon resonance (SPR) or bio-layer interferometry (BLI), rely upon fixing a protein to a surface to detect binding to an interaction partner, and thus may not be an accurate representation of the interaction in the biological system.

This is the challenge we want to address at Fluidic Analytics. We want to study protein interactions with as little interference as possible. In the pursuit of this challenge we investigated the use of microfluidic technology. But first, a little background.

The fundamental principle of microfluidic diffusional sizing (MDS) came out of Tuomas Knowles' lab at the University of Cambridge. The team realized the potential of the technique in allowing native state analysis of proteins and protein complexes with high accuracy down to low concentrations. The original findings were published by Yates and colleagues back in 2015 (1).

Even in 2019, there are relatively few off-the-shelf microfluidic instruments, so most research takes place on home-built rigs with custom chips, making it the domain of experts with specialist equipment. And athough reduced sample consumption is beneficial, the small volumes and path-lengths used in microfluidic devices can create challenges around detection sensitivity. Reproducibility can be another key issue, which is why there's a movement towards standardized instruments. By standardizing the technology, researchers can harness all the benefits of microfluidics, without the headaches and time-consuming process of chasing reproducibility.

Fluidic Analytics was spun out to develop Knowles' original concept into microfluidic instruments that would allow anybody to achieve accurate and reproducible results with MDS in a "plug and play" format.

There are a number of benefits to a microfluidic system. We've already mentioned the first two obvious advantages: reduced sample consumption and greater reproducibility – both crucial to reliably gaining insights from precious samples at the early stages of development. However, there are further benefits to

MDS that are more fundamental.

At the microfluidic scale, fluids behave differently than they do in bulk. MDS actively harnesses this fundamentally different behavior to eliminate turbulence. This allows proteins to be characterized in solution, without artefacts and in a manner that preserves information about physical properties – which in turn yields critical information about protein interactions. This means that you can assess if a peptide-based biotherapeutic is suddenly getting bigger, for example. If the biotherapeutic's size has changed substantially then it's likely to be binding to a target. You can also assess if this change in size is happening at specific concentrations. We can then look at the binding constant for this interaction and check if the stoichiometry data suggests the peptide is binding to one target or more and if one is being favored over the others – allowing you to spot different mechanisms of binding.

In other words, we are bringing scientists a step closer to being able to study notoriously difficult membrane proteins and intrinsically disordered proteins in as close to a native state as possible, by generating binding affinity and stoichiometry data in real time.

As a field, microfluidics is still growing – particularly in its application to analysis of proteins. Current systems are limited in what they can analyze; for example, we may only be able to separate a mixture based on a single characteristic. But, over time, I expect to see that changing, with microfluidic systems being used for more complex manipulations or separations that offer deeper insight.

One thing seems certain: with the right tools, researchers will be able to look beyond individual proteins – even beyond individual interactions. And if they can observe the rich interplay of the proteome, they are another step closer to developing a deep, system-wide understanding of biological complexity. In turn, our ability to comprehend the full impact of perturbing these biological systems improves, which supports the development of more selective treatments.

Technology that allows us to explore proteins and antibodies binding and interacting with a host of other molecules with minimal interference helps unlock a deeper level of understanding. In turn, this may start to change the way we think about proteins – and biotherapeutics – altogether.

Reference

1. *EV Yates et al., Nat Chem, 7, 802 (2015). DOI: 10.1038/nchem.2344*

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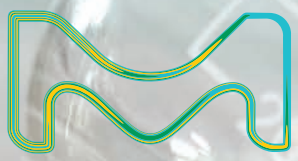

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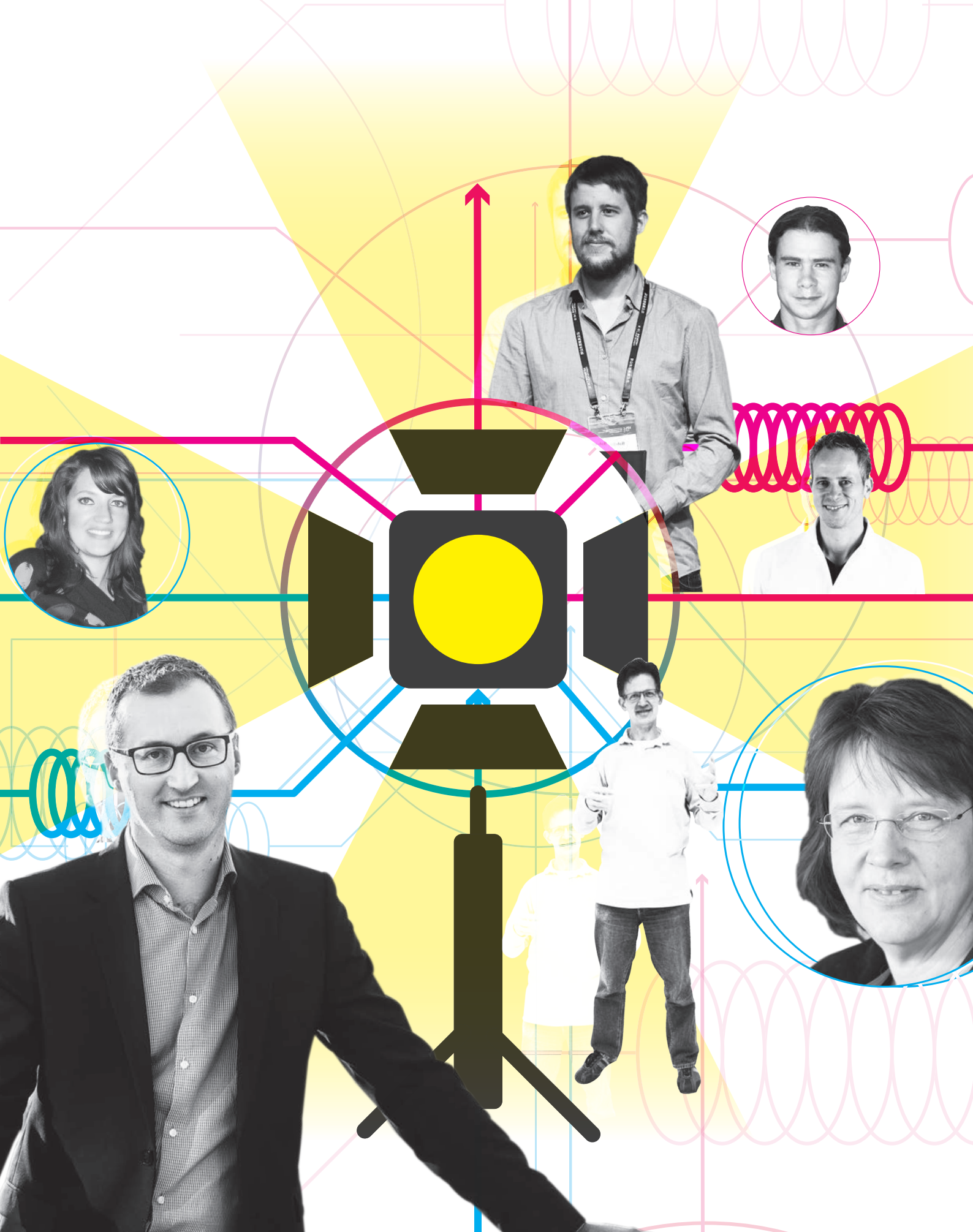

THE COMING OF AGE OF 2D-LC

Is it time for multidimensional liquid chromatography to step into the spotlight?

Though the concept has been around since the 1970s, it's only recently that 2D-LC has become a realistic prospect for more routine applications. Ahead of HPLC 2019 in Milan, we gathered seven gurus to discuss the challenges and promise of delving into the second dimension.

HOW HAVE MULTIDIMENSIONAL METHODS DEVELOPED OVER TIME?

Bob: Together with Dwight Stoll's group from Gustavus Adolphus College, we recently began compiling a free and searchable database of all existing applications of two-dimensional liquid chromatography (2D-LC; www.2DLCdatabase.com). Looking at the papers in this list, you can see the incremental stages of development unfold. Though the essential concepts of 2D-LC were introduced as early as the 1970s by Erni and Frei (1) and comprehensive 2D-LC (LC×LC) as we know it today was described in 1990 by Jorgenson and Bushey (2), it wasn't until the 21st century that development really started to pick up.

Landmark papers introducing sample dimensionality (3) and statistical overlap theory (4) convinced many scientists in academia that multidimensional chromatography was the way to obtain the vast peak capacities required for very complex samples. At the same time, an increasing number of groups started to combine 1D-LC systems for an array of applications, such as food, metabolites and pharmaceuticals.

Koen: Basic multidimensional separations (heart-cutting) have long been used to boost resolution. The breakthrough for comprehensive multidimensionality in both gas chromatography (GC) and LC was the development of high-speed separations. The latter was a tougher technical challenge (smaller particles, higher pressures), which explains the much earlier commercialization of GC×GC compared with LC×LC. The first commercial LC×LC instruments were only introduced in the past few years and this has paved the way for the full exploration of 2D-LC.

André: I believe two crucial steps have paved the way for the increasing adoption of multidimensional techniques. First, the advent of a coherent theoretical framework for multidimensional LC separations, and particularly LC×LC. Much of the theory has long been known from the work of the pioneers of the field and from GC×GC – the more established older brother of LC×LC. However, these principles had to be adapted to the unique characteristics of LC, including the need to quantify orthogonality, mobile phase compatibility issues between often-unrelated separation, and instrumental contributions to band broadening.

The second piece of the puzzle has been the availability of dedicated, quality instrumentation enabling straightforward, automated 2D-LC operation. Indeed, the column and instrumental hardware required to perform the very fast, high-efficiency separations demanded by modern LC×LC were not (commercially) available 20 years ago.

WHY IS NOW THE TIME FOR MULTIDIMENSIONAL LC?

Bob: Although work during the 1990s and 2000s established the potential of 2D-LC, its greater peak capacities came at serious cost: complex method development, reduced detection sensitivity and solvent-compatibility issues – all of which forced many researchers to combine their LC systems off-line. Meanwhile, LC and mass spectrometry (MS) established an extremely successful marriage, and peak capacity was improved by superficially-porous particle technology and tremendously powerful ultra-high-performance LC (UHPLC) systems, delaying the need for multidimensional LC.

THE ROUNDTABLE

Peter Schoenmakers, Professor and Scientific Director, Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, the Netherlands.

Bob Pirok, Analytical Chemistry Group, Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, the Netherlands.

Koen Sandra, Scientific Director, Research Institute for Chromatography, Kortrijk, Belgium; co-founder and R&D Director anaRIC biologics, Ghent, Belgium, and Visiting Professor at Ghent University, Belgium.

André de Villiers, Department of Chemistry & Polymer Science, Stellenbosch University, South Africa.

André: Much of the initial research was performed on home-built systems; the introduction of commercial, dedicated high-performance instrumentation has greatly extended the accessibility of multidimensional LC. One development of note is automated (multiple) heart-cutting instrumentation and dedicated instrumental control software. This technology is particularly promising in industries where complementary selectivity is needed to provide more information on particular sample components or fractions; for example, impurity profiling in the pharmaceutical industry.

Koen: The introduction of commercial instruments and accompanying data acquisition and data analysis software will indeed result in many new applications. Of equal importance is the availability of various interfaces that allow the scientists to select the mode best tailored towards their needs, whether LC×LC, heart-cutting (LC-LC), multiple heart-cutting (mLC-LC) or high-resolution sampling.

As André notes, the two last techniques are nowadays intensively applied in pharmaceutical analysis. Recently, interfaces have been designed to allow active solvent modulation (ASM) to facilitate transfer from first to second dimension – particularly interesting in heart-cutting and multiple heart-cutting modes.

Bob: I agree with Koen that the introduction of active modulation – in which the composition of the first-dimension effluent fraction is changed prior to injection into the second dimension – has been a major step forward. Several active-modulation techniques have been introduced, including the ASM mentioned by Koen, and stationary phase-assisted modulation (SPAM). Both techniques dramatically improve detection sensitivity, reduce analysis time and enhance applicability. For example, SPAM allows salts or organic solvents from a first-dimension ion-exchange separation to be removed prior to an aqueous reverse-phase LC separation using MS. That said, these modulators also increase the complexity of the already challenging method development for 2D-LC, and much research is devoted to solving this issue, including extensive use of computer-aided development.

Taking into account all of the new developments, I think it is safe to say that there has been a major shift in how multidimensional LC is perceived. More attention to robustness and method development strategies is still required, but 2D-LC is no longer a technique that suffers from solvent-compatibility, cumbersome method development and reduced detection sensitivity. Instead, it is a rapidly maturing technique, with clear applications in academia and industry.

A TURNING TIDE

By Isabelle François, Principal Scientist, EU Field Sales Support, Waters, Guyancourt, France.

At the start of the 21st century, we saw an enormous focus on LC×LC at scientific conferences, but attention has now turned to heart-cut 2D-LC – a trend driven by industry (particularly biopharma), where analysts typically do not have the luxury of time-consuming method development. The rise of heart-cut 2D-LC was triggered in part by the introduction of interfaces composed of cartridges containing stationary phase in between the two dimensions, which makes the 2D-LC approach more generic, and hence more approachable for a wider audience of users.

Some chromatographers see 2D-LC and MS as competitors (see page 22) – I believe they must be seen as "partners in crime!" 2D-LC-MS offers some important benefits above and beyond 1D-LC-MS across pharma, biopharma, omics, and natural products:

1. Increased resolution: additional chromatographic resolution from the second dimension allows users to identify more compounds when analyzing highly complex samples.
2. MS compatibility: where an MS-incompatible mobile phase is used in the first dimension, an interface including stationary phase can easily be used to trap the compounds of interest and desalt, prior to sending the fractions to the second dimension and finally to MS detection.
3. Ion suppression: for "dirty" samples, the first dimension can be used as a sample clean-up step, allowing full use of the power of the MS after an orthogonal separation in the second dimension.

Ion mobility can aid in the separation of compounds, and hence also in 2DLC, but until now it has only been suitable for certain applications. The first results with the Waters Cyclic IMS (introduced at ASMS 2019) have been very exciting in this respect.

With the dramatic improvements in UHPLC over the last decade, hardware and software development for 2D-LC will (and should) focus on making instruments easier to use, more flexible and more generic, combined with easier data interpretation, so that more scientists can make use of this exciting and powerful technology.

IS THERE INDUSTRY DEMAND FOR BETTER, FASTER MULTIDIMENSIONAL ANALYSES?

Peter: The first decade of the 21st century saw the first push for 2D-LC from industry. The characterization of complex polymers that featured multiple independent distributions was an obvious fit for LC×LC because of the low sample dimensionality (resulting in structured LC×LC chromatograms) and the difficulty of applying MS for characterizing high-molecular-weight polymers.

Convinced by the need for multidimensional LC, Waters and Agilent introduced the first dedicated 2D-LC systems, making the technology more accessible to industry. We see increased availability reflected in a steep increase in the number of publications per year concerning the implementation of 2D-LC.

Koen: All fields in which both high peak capacity and high selectivity are needed could benefit from multidimensional LC, including:

- Pharmaceuticals
- Biopharmaceuticals
- Natural products
- Omics (metabolomics, lipidomics, proteomics)
- Polymers (including surfactants)
- Petrochemicals

The major driver to apply 2D-LC methods to date has been to maximize peak capacity to tackle complex samples but, as the field develops, other benefits of 2D-LC are becoming apparent, including the ability to obtain orthogonal information and make separations compatible with MS.

DO YOU VIEW MASS SPECTROMETRY AS A COMPETITOR OR PARTNER TO 2D-LC?

Bob: MS and LC-MS are immensely important analytical tools. When more quantitative information is required, the chromatography aspect is more important; if more components need to be characterized, we cannot rely so heavily on MS.

Koen: It is not logical to consider MS as a competitor – although I think that chromatographers often look at it that way. Chromatography and MS go hand-in-hand, and the majority of chromatographic systems in our lab are combined with MS. Apart from being a detector, we should not forget that MS adds resolution and so can be considered as an extra separation dimension. And the same is true for ion mobility.

André: Nowadays, MS is unarguably the most important detector in chromatography – and that is true for multidimensional LC too. Aside from the evident power of MS for identification purposes, it is especially important for the analysis of highly complex samples – the main application area of multidimensional LC – and the number of papers on multidimensional LC-MS is increasing steadily. Commercial software enabling 2D-LC-MS data analysis is available, albeit without some of the most advanced features offered for 1D-LC-MS data. However, not all MS instruments are suitable for hyphenation to multidimensional LC. For LC×LC in particular, high acquisition speeds are essential, and depending on the type of instrument, certain sacrifices have to be accepted.

COULD ION MOBILITY BE THE MISSING PIECE OF THE PUZZLE?

Koen: Of course, there is no single analytical technique that can provide the answer to all research questions. However, we do have high hopes for the superb resolution offered by ion mobility spectrometry (IMS), which has gone from a resolving power of 20–40 to greater than 200. In combination with 2D-LC and high-resolution mass analyzers, a new era of resolving power is upon us.

André: I too am excited about the incorporation of IMS in multidimensional analyses. IMS is perfectly compatible as an additional separation step of gas-phase ions between chromatographic separations and MS. Realistically, I think that IMS will rarely provide separation of isobaric species - which cannot be distinguished by MS - which also cannot be separated by multidimensional LC or LC×LC. Rather, the principal benefits of IMS in multidimensional LC are that the arrival time of ions can be used as an additional identification criterion, and mass spectral data can be filtered according to arrival time to improve the quality of MS data.

Peter: I actually disagree on this point. From the perspective of MS, ion-mobility has a lot (of selectivity) to add; from the perspective of LC-MS, I believe IMS adds very little, because the same selectivity can be more easily obtained (and exceeded) in the liquid phase.

WHAT ARE SOME OF THE RECENT APPLICATION HIGHLIGHTS?

André: To gain more widespread acceptance by industry and academia we need to show that multidimensional LC can be applied to real-world analytical problems. There are many

CHANGE ON THE HORIZON

By Stephan Buckenmaier, Senior Research Scientist, and Monika Dittmann, R&D Principal Scientist, Agilent Technologies, Waldbronn, Germany.

Two-dimensional liquid chromatography (2D-LC) is gaining popularity as an important analytical platform in many industries, because of increasingly stringent demands for identification and quantification of compounds in samples. From escalating concerns about pollutants in the environment and contamination in food, to the need for multiple attribute measurements in biopharma or chemical products, there is an increasing demand for separation power. Chromatography and (ion mobility [IM])-mass spectrometry (MS) are powerful technologies individually but their hyphenation strongly amplifies the power of the analytical platform. Multidimensional LC boosts separation power even further and allows us to make several measurements in a single analysis. Good examples can be found in the biopharmaceutical industry, where we may want to determine concentration of the API, aggregation, charge heterogeneity, structural integrity and more. For instance, Protein-A is often coupled with size exclusion chromatography (SEC) for the analysis of monoclonal antibodies (mAbs).

Another trend is to couple two methods with different selectivity for the analytes of interest. Co-eluting compounds from the first dimension are simply sent into second dimension to be resolved. Thus, the development effort for a 1D method, which for complicated samples can be time-consuming, can be dramatically reduced or even eliminated. A good example is the work of Dwight Stoll (Gustavus Adolphus College) on selective comprehensive 2D-LC (sLC×LC or multiple heart-cutting), which decouples 1D and 2D timescales by using multiple loops for sample storage of several sections across the 1D-chromatogram. The transition from full to selective comprehensive 2D-LC has been a major driver in the increasing popularity of the technique.

Right now, system hardware is robust, method setup is simplified by clever software solutions, and so 2D chromatography is becoming widely accepted. In contrast to those early days, when only a few used the technology, the ongoing exchange of ideas and experience within a growing 2D-LC community continuously amplify our knowledge, driving us all forward.

So, what's next? Future developments should focus on supporting method development as shown by Peter Schoenmakers' group at the University of Amsterdam (1). In particular, better solutions for data analysis are required when extremely complex samples (such as omics samples) are analyzed using everything on the bench (for example, comprehensive 2D-LC coupled to IMS/QTOF). We believe that forthcoming advances in these areas – and wider recognition of the incredible separation power achievable – will push 2D-LC into more routine applications.

Reference

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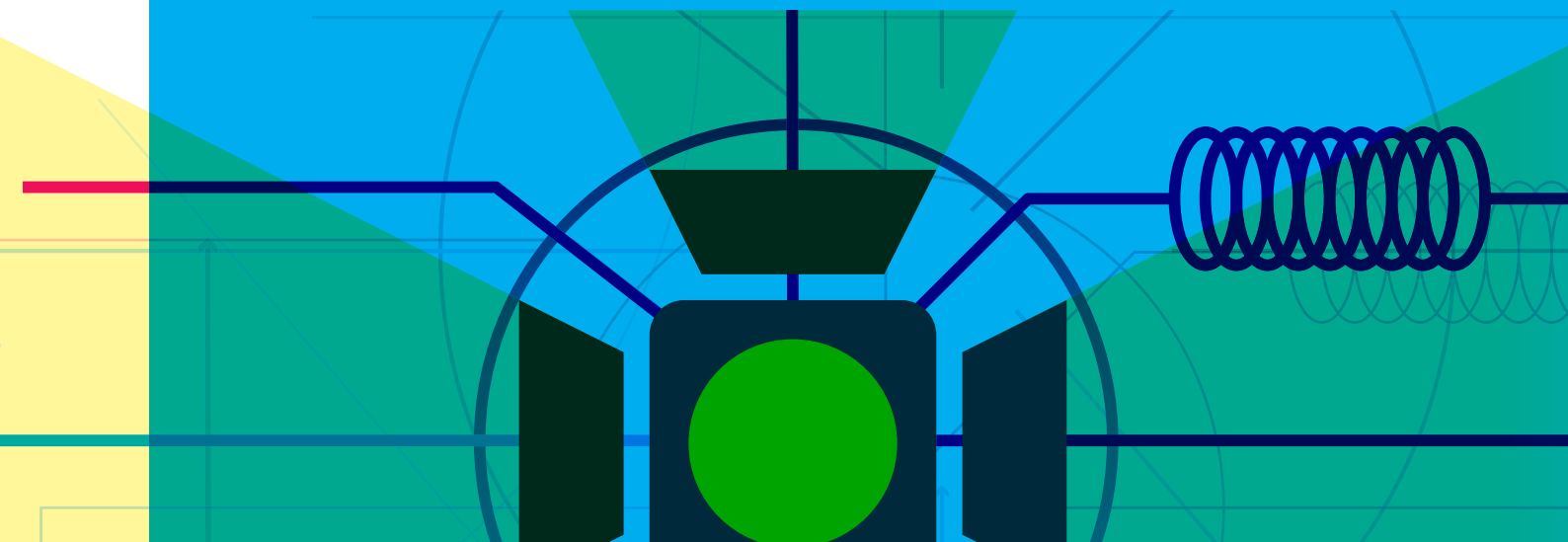


applications that admirably demonstrate the utility of the technique, including pharmaceutical analysis (for both small molecules and biopharmaceuticals), natural product analysis, polymer analysis, and the 'omics' fields (metabolomics, lipidomics and, especially, proteomics).

Koen: These are exciting times for scientists with an interest in 2D-LC. We are particularly looking forward to witnessing the developments in (bio)pharmaceutical analysis, where 2D-LC currently has the greatest momentum. Five years ago, there were often only a few LC×LC presentations on (bio)pharmaceutical applications; nowadays, these talks and posters are dominating the conferences. 2D-LC can be applied throughout all stages of drug development and the first validated methods have already become reality.

From a biopharmaceutical perspective, LC×LC has shown great value in detecting post-translational modifications in mAbs, identifying drug conjugation sites, assessing comparability between innovator mAbs and biosimilars, and so on. In heart-cutting or multiple heart-cutting mode, the technique has been used to make the incompatible compatible. As an example, mAbs and other protein therapeutics are often separated by techniques that make use of non-volatile salts, such as size-exclusion chromatography (SEC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and affinity chromatography. By combining these separation modes with reversed-phase liquid chromatography (RPLC) in front of a mass spectrometer, MS spectra can be obtained for SEC, IEX or HIC peaks, since the second RPLC dimension takes care of the desalting.

2D-LC, and recently even 3D-LC, have also been used to obtain complimentary information. By combining Protein A affinity chromatography with IEX, SEC and/or LC-MS one can simultaneously assess mAb titer as well as important structural characteristics, such as aggregation, fragmentation, and post-translational modifications, directly from cell culture supernatants.

WHAT CHALLENGES REMAIN FOR ROUTINE USE OF 2D-LC?

Koen: There are some technological improvements that would be welcome. Depending on how 2D-LC is performed, the technique can suffer from issues, such as flow/mobile-phase incompatibilities, sensitivity, reproducibility, and immature data analysis software.

The other main challenge? Education! Scientists need to understand when to use the technology – as we've seen with SFC, if they apply 2D-LC when it isn't necessary, disappointment will follow.

André: The challenges are major and often underestimated. Firstly, despite significant advances in instrumentation, it is becoming increasingly evident that loss in performance, especially extra-column contributions to zone broadening in the second dimension of LC×LC, remains problematic. The very fast and efficient separations attainable on the latest generation of columns are often severely compromised by the volume of tubing, detectors, and even connections. Plus, the extra-column volume contribution of most conventional MS instruments inevitably leads to more band broadening; in addition to insufficient data acquisition rates, this is one of the main reasons that LC×LC-MS data are currently of much lower quality than data from LC×LC-UV.

Secondly, modulation is a critical step and, despite influential developments (SPAM, ASM and even evaporative modulation interfaces) there is no universal solution for all combinations of separation modes. Indeed, one of the main drawbacks of multidimensional LC is the severe dilution experienced by analyte zones in two dimensions, which is exacerbated by the need to use narrow-bore columns in the first dimension and wide-bore columns in the second – a problem that that could be solved by effective modulation.

Finally, an important stumbling block on the path to widespread acceptance is the fact that the technique – method development and optimization in particular – is extremely complex. Important progress has been made in method development by Peter's group and others, but more should be done to make the technique accessible to non-experts.

Peter: I agree. The hardware has been extensively developed over the past decade, but – especially for LC×LC – the method development is still too lengthy and thus costly. Academic setups mostly rely on the use of trapping cartridges for SPAM. Though incredibly effective at resolving the detection-sensitivity and solvent-incompatibility issues that held 2D-LC back for a long time, they are not very robust.

WHERE DO WE GO FROM HERE?

Peter: Though ASM appears to be limited to separation systems using RPLC in the second dimension, the technique is simple and reliable. And recent reviews note that the majority of multidimensional systems do use RPLC. Combined with heart-cut 2D-LC – a technique which is relatively straightforward to use for the operator with experience in 1D-LC – this should prove a powerful tool in the analytical lab.

I expect column manufacturing techniques will change dramatically with the emergence of 3D-printing techniques – perhaps this will give rise to the next big leap in 2D-LC technology.

André: I think the priorities for the future of the field are:

1. Improved instrumentation, including high-speed, low-volume MS detectors
2. New and improved modulation strategies
3. Rapid increase in the (realistic) applications of the technique to solve real-life problems
4. More widespread understanding of the theoretical and practical aspects of multidimensional LC, and appreciation for the strengths and limitations of the technique.

Despite the remaining challenges, I suspect LC×LC will soon become a routine method for highly complex samples, just like GC×GC. And, also just like GC×GC, the next hurdle will be extracting the relevant information from such highly complex data sets. In this endeavor, chemometricians and software developers will have an important part to play, as many of the established data analysis packages for 1D-LC(-IM)-MS data are not suitable for higher-dimensional data.

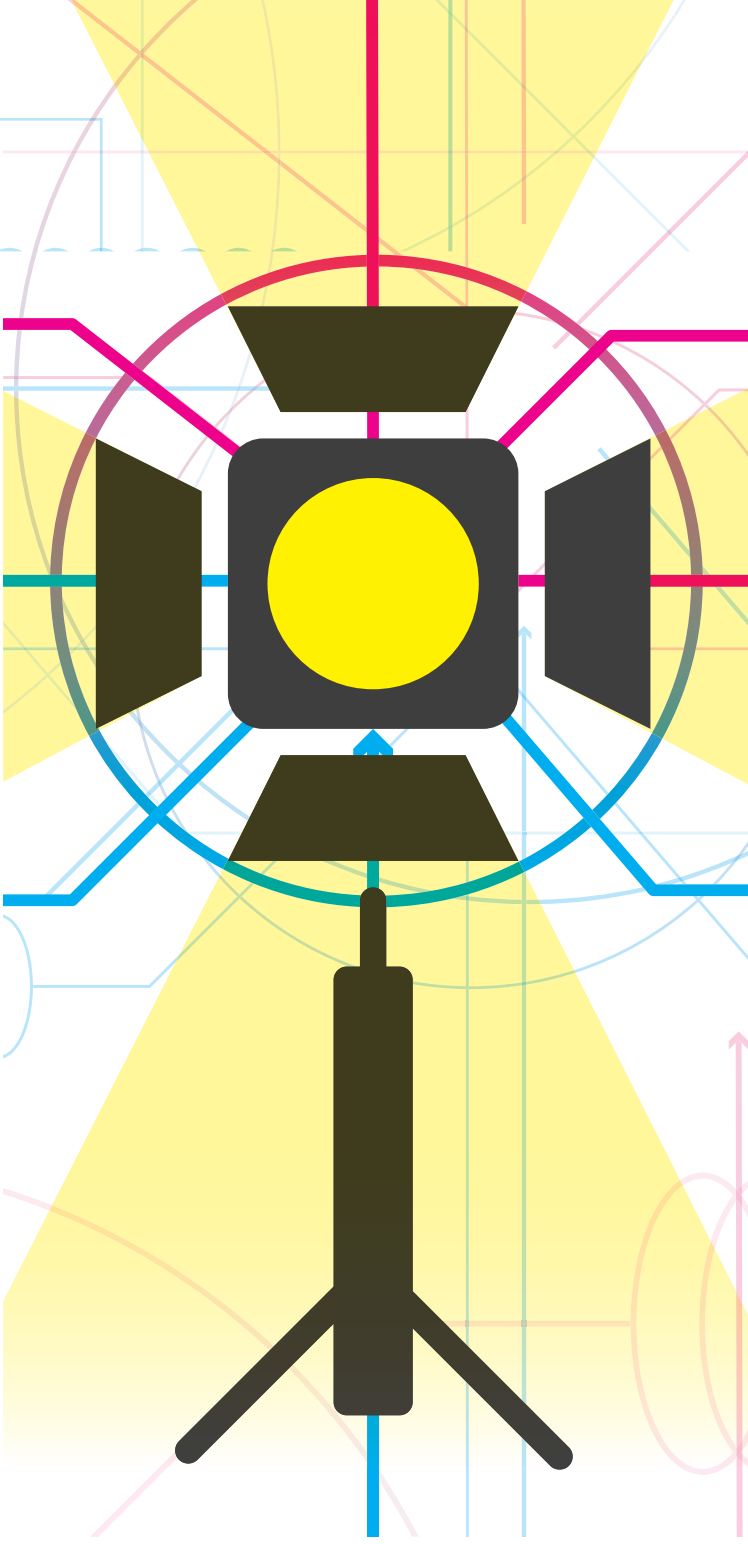
For me, research in 2D-LC has been a highly rewarding experience, and I look forward to whatever the future holds with excitement and anticipation.

Koen: In the near-future, I believe we will see multidimensional LC being widely used in (bio)pharma R&D labs and starting to enter QC labs. I think we can expect to see a number of developments:

1. Many software advances to facilitate method development and data analysis, and further research into overcoming incompatibilities between dimensions; for example, using ASM.
2. Increasing use of 3D-LC (in space/time), which will put extra pressure on method development, detection and data analysis.
3. Miniaturization and other instrumental advances to cope with the sensitivity issues in LC×LC.
4. Incorporation of reactors in multidimensional set ups.

In biopharmaceutical analysis, peptide mapping is required to identify first-dimension peaks. When peaks are collected off-line, peptides can easily be generated by adding trypsin but in an online set-up this is more complex. Several papers have described online digestions in multidimensional set-ups.

During a panel discussion at a recent biopharmaceutical analysis conference, an attendee representing a pharmaceutical company asked: "People have been talking about 2D-LC for so many years, when will it finally become applicable in the biopharmaceutical industry?" My response: "The time has come."



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THE COMING OF AGE OF PETER SCHOENMAKERS

Peter Schoenmakers will be known to many readers as a visionary scientist, generous collaborator, entertaining lecturer and enthusiastic dancer. He recently received the prestigious Dal Nogare Award at Pittcon and will mark his 65th birthday at HPLC 2019 in Milan. To celebrate, we asked some of Peter's colleagues, mentors and students to share their memories.

By Bob Pirok, Van 't Hoff Institute for Molecular Sciences, Analytical Chemistry Group, University of Amsterdam, the Netherlands and Hans-Gerd Janssen, Unilever Research and Development Vlaardingen, the Netherlands.

Peter obtained his PhD in 1981 under the supervision of Leo de Galan in Delft, the Netherlands, and Barry Karger in Boston, Massachusetts, USA, focusing on the modelling of gradient reversed-phase liquid chromatography (RPLC). Wolfgang Lindner from the University of Vienna remembers these early days well: "I've known Peter for more than 40 years, since I was a postdoc at Barry Karger's lab in Boston and Peter was there as guest student during his PhD. This stay at Barry's lab influenced his whole life, as he met his wife Dana there!"

Afterwards, Peter started working for Philips Research in Eindhoven, the Netherlands. Here, Peter continued to work on developing models for liquid chromatography (LC), but also entered the field of supercritical fluid chromatography (SFC). Hans-Gerd Janssen says, "At Philips Research in Eindhoven, Peter ran two large research projects; one developing an expert system for optimization in LC and the other exploring SFC. I worked as a young PhD student in Karel Cramers' group on capillary SFC at Eindhoven University, just 5 km down the road. We decided to join forces and I spent half my time

working with Peter at Philips. This really shaped my career – I sometimes wonder where I would have been without his guidance during those years."

Dr. Mary Ellen McNally, now a Technical Fellow at FMC in Newark, Delaware, but then at DuPont, also remembers Peter's revolutionary approach to SFC. "In the early days of SFC (1986 through 1995), there was a battle between the capillary and packed column experts on which was the best technique to move forward. The debate raged among academics and instrument company experts but industrial chemists, like Peter and me, just wanted to use the technique to solve problems."

Peter would later move to Shell in Amsterdam before spending a few years in Houston, Texas. Together with Jan Blomberg and Jan Beens, Peter Schoenmakers contributed significantly to the development of comprehensive two-dimensional gas chromatography (GC×GC). The subsequent success of the technique in the field of petrochemical analysis was largely the result of their pioneering work.

Indeed, Peter has left his mark across many areas of chromatography, something which Sjoerd van der Wal, emeritus professor at Van 't Hoff Institute for Molecular Sciences, underlines: "The common thread throughout his research is the study and description of different forms of chromatography with easy-to-understand models that do not frighten users with their complexity. From modelling gradient RPLC during his PhD, to investigating the application of SFC in industry, and developing GC×GC, Peter has contributed on multiple fronts."

The research component of his career was always a priority for Peter and in the late 1990s he took a post at the University of Amsterdam alongside his job at Shell. Describing this experience, Peter says "I always assumed 50/50 meant evenly dividing the working week across two jobs but, in reality, it meant 50 hours in one place and 50 hours in the other."

In 2002, he became full-time professor of polymer analysis and analytical chemistry at the University of Amsterdam, succeeding world-renowned chromatographer Hans Poppe. Here, Peter immediately boosted the impact of polymer HPLC, expanding the scope beyond size-exclusion chromatography towards other separation modes and multidimensional separations. Ron Peters, Principal Scientist at DSM Coating Resins in Waalwijk (the Netherlands) and part-time professor at the University of Amsterdam, says, "Peter has made tremendous, ground-laying contributions to the characterization of polymers by his work on the application of multidimensional separations to complex polymeric systems."

Ever since, his research focus has been on comprehensive two-dimensional (liquid) chromatography and applications of

analytical chemistry in forensic science. Peter has published more than 300 papers and several book chapters, and his book, "Optimization of chromatographic selectivity – a guide to method development," is still fundamental reading.

Peter is the chairman of the Separation and Characterization of Macromolecules (SCM) symposium series, a role which he embraces, says Harald Pasch, a professor at the University of Stellenbosch, South Africa: "My group always appreciates attending the SCM meetings and presenting our research – particularly the graduate students in the group who come to learn about the latest developments and speak to the most prominent scientists in the field. Along with the scientific program, the students thoroughly enjoy the conference party and I must give credit to Peter as being one of the most dedicated dancers at this event!"

Peter is also a member of the Permanent Scientific Committee of the HPLC series of conferences and editor for the Journal of Chromatography A and takes an active role in both. "I've worked with him on the HPLC Conference series and the Journal of Chromatography A. In both settings, he naturally took a leading role through his common sense, thoughtfulness, and positive approach," says Robert "Bob" Kennedy, a professor at the University of Michigan.

Xiaoli Wang, R&D Section Manager at Agilent Technologies, draws attention to the many awards Peter has received, which demonstrate the huge impact he has had: "Peter's scientific accomplishments in the area of separation science are evident from the long list of honors on his CV. Some of the most prestigious awards include the Silver Jubilee medal from the Chromatographic Society in 1989, the EAS award for excellence in separation science in 2010, and the Csaba Horvath Memorial Award in 2015." Other recent international awards include the CASSS Award (2015), the John H. Knox Medal of the RSC (Belgium, 2014) and the Martin medal of the Chromatographic Society (2011).

In 2016, Peter successfully applied for a European ERC Advanced grant for the project STAMP (Separation Technology for a Million Peaks) in which he and his team are developing three-dimensional microfluidic systems using 3D-printing technology, retention modelling, computational fluid dynamics, monolithic stationary phases and advanced imaging techniques.

Peter is a keen supporter of public–private partnerships, and was instrumental in founding COAST, an organization bringing together analytical chemists in industry and academia.

Despite his scientific accomplishments, Peter always makes time for young scientists, whose successes he sees as his true legacy. Within COAST, Peter instituted educational programs to allow talented students at all levels to receive additional training from analytical experts in industry and academia.

Asking current and former PhD students for their stories about Peter generated many tales of his support and encouragement – and his directness. "He will tell you immediately when you have made, or are about to make, a mistake," agree Dorina Adamopoulou, Noor Abdulhussain and Pascal Breuer, PhD students within the large STAMP project. "But this constructive criticism is immediately followed by a positive note with suggestions and ideas for improvement."

A young PhD student can learn a lot from Peter, as the authors know from experience. When Bob Pirok was nervously rehearsing his first conference lecture for HPLC2015 late at night in his hotel room, he received a text message from Peter ordering him to "Get down to the 'beer tent' now!" The next morning, encumbered by a horrible hangover, Bob looked accusingly at Peter, who retorted cheerfully, "Hey, at least you are no longer nervous!" To Bob's surprise, it worked.

Although we cannot recommend that other young scientists take this particular recommendation, Peter is certainly well qualified to advise on giving a memorable conference presentation. "Peter is a great speaker. I always enjoy his talks because I know that I will learn something, hear a visionary idea, and get a good laugh! His lectures provide vision and clarity for the field, and his excellent sense of humor permeates his talks," says Bob Kennedy.

"I always enjoy Peter's talks because I know that I will learn something, hear a visionary idea, and get a good laugh!"

Ton Brooijmans, molecular characterization expert at DSM Coating Resins, Waalwijk (the Netherlands), agrees: "Peter is a dedicated participant in national and international meetings where he has always presented amazing analytical topics in a very amusing way. The combination Peter utilizes in his lecture: hardcore science, humor, inspiration and publicly giving credit to those who earned it, garnered my immediate respect and admiration."

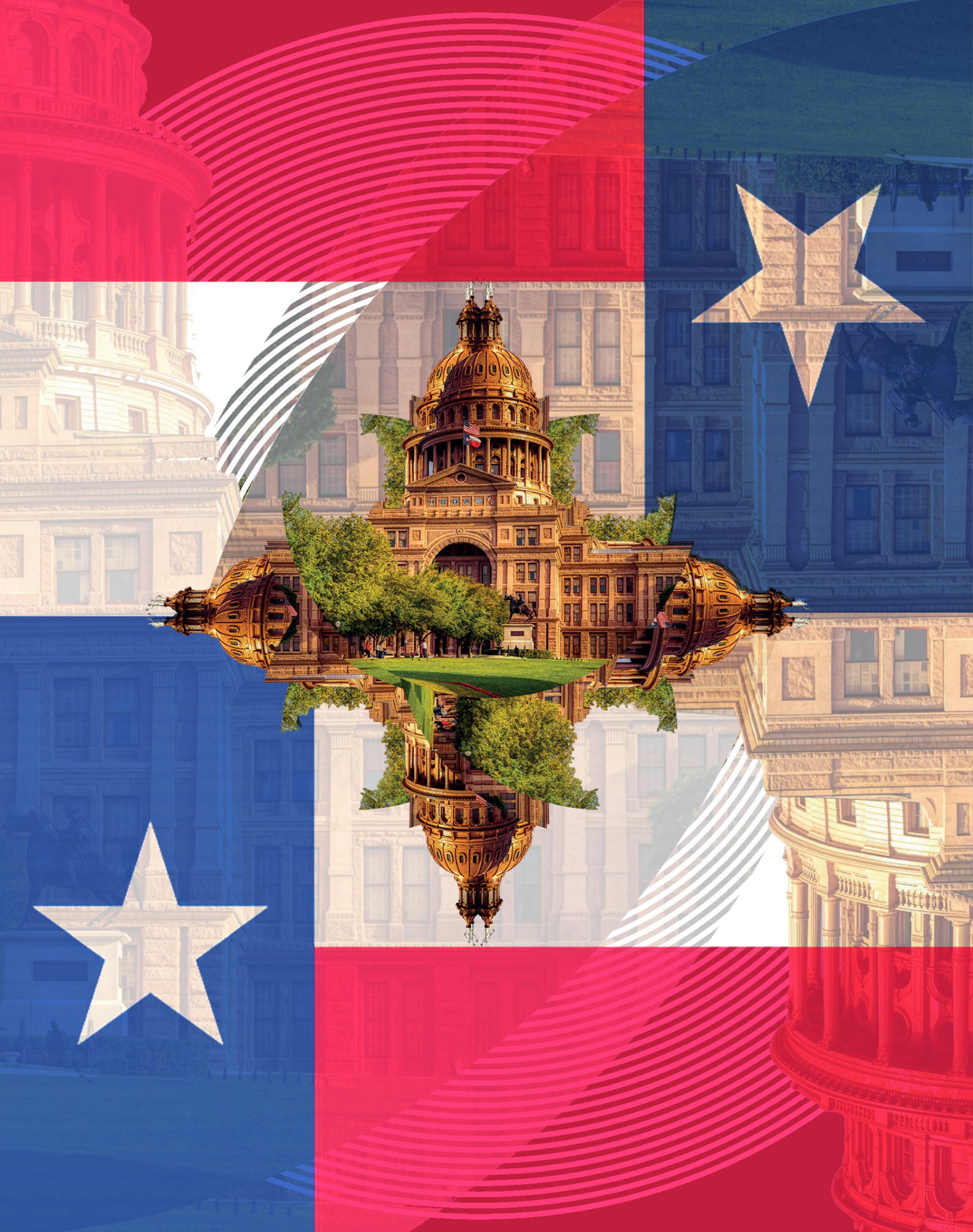
Amongst his collaborators, Peter is also known for his spontaneous creativity, which can transform everyday meetings into stimulating brainstorm sessions. Ira Lurie, Research Professor of Forensic Sciences at George Washington University, says "Peter and I were sitting in my office when I pointed out the need for some semi-quantitative means of describing the value of 2D-LC for improving the specificity of retention time measurements. Within 15 minutes, Peter derived an equation on my whiteboard to estimate the decrease in uncertainty of retention time measurements when employing multidimensional LC."

Xiaoli Wang had a similar experience during his time as PhD student in Peter Carr's group. "When Peter visited us in Minnesota, he told us about his work on isocratic Poppe plot, which was invented by another world-renowned Dutch chromatographer – Hans Poppe. That discussion immediately led us to an idea for a collaboration to extend the concept of Poppe plot from isocratic to gradient elution. We spent hours on making a Poppe plot. He was so meticulous about the graphical details. I was amazed by that!"

Peter's ability to bring people together is a gift, says Sjoerd van der Wal: "With his kind personality, spontaneity, (sometimes bold) humor and tremendous effort, Peter collaborates with a large, international network."

During the Dal Nogare Award session at Pittcon 2019, Peter laid out his vision of the future in chromatographic separations, and the pivotal role for multidimensional chromatography. He believes three-dimensional chromatography will one day be commonplace; however, he noted that complex method development already renders 2D-LC chromatography too costly for routine implementation in the industrial analytical lab and made a plea for better computer-aided method optimization tools to make 2D-LC more accessible.

Bob Pirok was delighted to contribute to the session by presenting an algorithm developed during his PhD, commenting: "It's amazing to think that this work goes back to a paper Peter published in 1978, during his PhD, and algorithms he developed on a calculator. Even then, Peter realized the need for modelling retention in order to facilitate rapid and effective implementation of the complex chromatographic systems we need tomorrow. Looking at the conference program of HPLC2019 in Milan, we can see that this topic is now more relevant than ever."



Texas Reflections

We catch up with six stand-out speakers from the 43rd International Symposium on Capillary Chromatography and 16th GC×GC Symposium in Fort Worth to unearth the hottest topics in Texas.

Get with the Program

With Tadeusz Górecki, Professor, University of Waterloo, Canada.

What's the latest from your lab?

We are continuing our efforts to develop a practical system for temperature programming of the second-dimension column in GC×GC, which can increase the 2D peak capacity by as much as 50 percent. We have developed a universal system in which a single metal 2D column serves three roles, as separation column, heater, and temperature sensor.

What are the key trends in GC×GC?

After a long period of relative stagnation, several new modulators have been introduced recently. While none of them are truly ground-breaking, they provide the users with more options. Plus, there are numerous interesting applications of GC×GC, as well as further advances in data handling and processing.

What challenges face your field?

The perceived complexity of the technique makes many users shy away from it, not helped by the difficulties with handling and processing huge data files. While GC×GC can produce a lot of data in a short time, extracting useful information from these data is not easy. On top of that, many users spend very little time on optimizing the separation itself. This can only be overcome by education on the one hand, and advanced "big data" techniques (involving artificial intelligence) on the other.

Predictions and aspirations?

It is more a dream than a prediction, but within my lifetime I would like to see GC×GC used for the most challenging samples in every gas chromatographic laboratory. As for my research, the goal has always been to provide the users with better tools to perform GC×GC separations, and I plan to carry on doing this until I retire.

Cracking the Case

With Katelynn Perrault, Assistant Professor, Chaminade University of Honolulu, Hawaii, USA.

What's the latest from your lab?

Our focus is applying GC×GC in the forensic sciences. We are working on demonstrating chromatographic parameters related to calibration and peak quality when converting a traditional gas chromatography-quadrupole mass spectrometry (GC-qMS) instrument into a comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry/flame ionization detection (GC×GC-qMS/FID) instrument. This is accomplished via retrofitting with a reverse fill/flush modulator and coupling the two detectors to get the best selectivity and quantification. One of the major obstacles for forensic laboratories in implementing multidimensional approaches is the lack of quality assurance parameters. By retrofitting commonly used instruments, we hope to provide benchmark data to help laboratories justify the adoption of GC×GC. This is of the utmost important in the forensic sciences, as calibration data and quality assurance are critical components of demonstrating valid science in a courtroom.

What are the key trends in GC×GC?

Many industries are now starting to implement GC×GC as a core component of their workflow, sparking debate in the community regarding areas for improvement. Key topics discussed in Texas this year were the need for uniform reporting in publications, more published full validation studies, and tools for understanding the complexity of batch data. There was a constructive dialogue between industry professionals, academic researchers, and vendors that I believe will have a positive impact moving forward.

Flow modulation – both reverse fill/flush (RFF) and diverting flow modulation – seems to be gaining ground compared with the thermal modulation seen in most published studies to date. The emergence of commercial tools for flow modulation will open new doors for the field of GC×GC; this will be interesting to observe in the coming years.

What challenges face your field?

High-quality GC×GC separations can reveal a wealth of data that was previously "hidden". We have moved from single sample analysis studies to studies that involve large batches, longitudinal analysis, multiple channel detection, and so on. This complexity in study design leaves us wading through mountains of data, trying to extract meaning. A discussion group held during the GC×GC symposium highlighted the wide range of data treatment strategies currently used, ranging from commercial software tools to a variety of in-house custom software. I believe we need to make data handling and interpretation simpler and more meaningful, but maintain high analytical quality and valid statistical foundations – a tricky path to navigate.

Predictions and aspirations?

My goal has always been to rely on strong foundations of chromatography but bring an "end user" flair to the way we think about GC×GC. We can do the best chromatography in the world, but if GC×GC isn't adopted for the applications it can benefit most, we have not done our job. Our next phase of research will provide data on hardware tools, statistical interpretation techniques, and communication strategies to allow the adoption of GC×GC in crime laboratories. I hope to see forensic investigations put the wealth of information that GC×GC can provide to good use in investigating homicides, missing persons cases, and mass disasters.

To learn more about GC×GC and how it could benefit your laboratory, join me at the 11th Multidimensional Chromatography Workshop in Honolulu, Hawaii on January 5-7, 2020. It's free to register and a great opportunity to network with experts in the field. www.multidimensionalchromatography.com

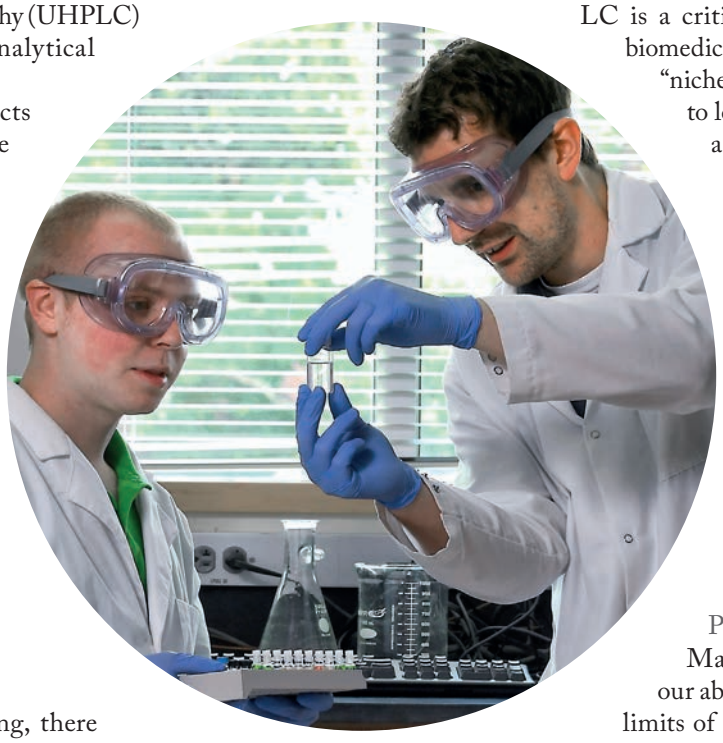
Under Pressure

With James Grinias, Assistant Professor, Rowan University, Glassboro, New Jersey, USA.

What's the latest from your lab?

As our lab continues to grow, two main research directions have emerged: fundamental studies in ultrahigh pressure liquid chromatography (UHPLC) and miniaturization of analytical measurement platforms.

One of our newer projects sits at the interface of these two areas: utilizing portable LC instrumentation for targeted analysis in point-of-care and field settings. As a young graduate student, I always envisioned trying to work with a system like this; based on our initial testing, I think a lot of exciting new developments will be achieved over the coming months and years.



What are the key trends in UHPLC?

At this year's ISCC meeting, there were many posters focused on sample preparation, especially utilizing supercritical fluids and solid-phase microextraction. The use of new detectors was also a prominent topic, especially vacuum ultraviolet detection and catalytic reactor technology coupled to FID for GC and light-emitting diode sources for LC and ion chromatography (IC) separations. In terms of applications, monoclonal antibodies and antibody–drug conjugates remain hot topics for separation scientists, with a lot of work focusing on mapping the glycosylation of these biomolecules.

Throughout the meeting, the close relationship between capillary LC and MS for omics applications was apparent. As part of the capillary LC panel discussion, involving a number of leaders in the field, the need for a better interface between capillary LC columns and MS inlets was identified as a major goal for the next few years.

What challenges face your field?

While commonly employed by researchers and in core facilities, the perception of capillary LC as "less robust" and "difficult to use" by chromatographers will limit its entry into routine analysis. New strategies for integrated chromatographic systems using capillary LC will help overcome these hurdles, but it will still take some time for the solutions to become fully integrated into standard workflows. Capillary LC is a critical separation technique in biomedical research, but is still seen as a "niche tool" by some in industry due to low sales relative to analytical- and prep-scale columns.

Across all research areas, experimental reproducibility is a growing issue that plagues the scientific endeavor. Our use of open-source strategies to share all details of new platforms with potential users, along with non-proprietary data formats, is one way to combat this issue in the realm of analytical instrumentation.

Predictions and aspirations?

Many of the hurdles that limit our ability further push the pressure limits of LC are in-depth engineering problems that may require entirely new approaches to how we imagine LC instruments. Thus, I believe that chromatography researchers will shift some of their focus back towards examining unique stationary phase selectivities. While C18 will likely continue to dominate, other reversed-phase column types and HILIC-based methods will find favor in specific application areas.

In my lab, we will continue to refine the ways that current instrumentation can be used to increase the throughput of widely-used methods. We have just started to scratch the surface on integrating single-board computers into low-cost platforms, and hope to make further advances in this area in the coming years. Finally, we are excited by the newfound ability to perform separations with a battery-operated, field-portable LC – my students and I are dreaming of all the different ways we can utilize this new tool!

Switch Up

With Peter Q. Tranchida, Associate Professor, University of Messina, Italy.

What's the latest from your lab?

The lab is involved in the development and use of both heart-cutting and comprehensive 2D chromatography systems (GC, LC and supercritical fluid chromatography [SFC]) combined with powerful forms of detection. My own research focuses on the use of state-of-the-art GC-MS instrumentation, with particular focus on GC×GC-MS systems. At the moment, I am working on the development of consumable-free forms of modulation, comprising both pneumatic and thermal desorption devices.

What are the key trends in multidimensional chromatography?

Conference presentations on multidimensional chromatography development are gradually being replaced by talks on applications of multidimensional chromatography-MS. Inevitably, instrumentation is becoming smaller, more powerful, automated and stylish, equipped with do-it-all software and leaving little to the imagination and the intervention of the operator.

What challenges face GC×GC?

Although the benefits have been recognized for decades, we have yet to see widespread acceptance of GC×GC, or incorporation in official methods. In my opinion, there are several reasons for this:

- GC×GC is seen as complicated technology, mainly confined to high-tech research laboratories
- 1D chromatography combined with MS remains a sufficiently powerful approach in many instances
- High cost of purchase and operation
- The revolutionary nature of the technology

Predictions and aspirations?

I hope to develop a unified GC-MS/GC×GC-MS instrument that is easy to use and affordable. I believe the ability to switch between 1D- and 2D-GC in a single instrument would lead to a much wider diffusion of GC×GC.

Decoding Data

With Pierre-Hugues Stefanuto, Research Scientist, University of Liège, Belgium.

What's the latest from your lab?

Our lab has always been focused on the development and implementation of comprehensive two-dimensional gas chromatography-high resolution time of flight mass spectrometry (GC×GC-HRTOFMS) solutions for a range of applications, mostly in the forensic and petrochemical arenas. Over the past few years, we have grown more involved in medical research, specifically in volatilomics. We use a multi-sample and multi-technique approach in combination with convergent data processing tools to take an untargeted view of important medical questions. Right now, we are trying to understand inflammation processes in the lung, using exhaled breath analysis (GC×GC-HRTOFMS, SIFT-MS and others).

What are the key trends in GC×GC?

For me, data science for processing and method development is the real hot topic. Analytical methods are more and more multidimensional, and the data generated are more and more complex. New tools are needed to help users to get the best out of their analyses. On the hardware front, the general trend is away from expensive cryo-fluids for the modulation process and towards solutions-based flow or cryo-free thermal modulators, which should make GC×GC to more attractive for routine applications.

What challenges face your field?

The next big step for GC×GC is the launch of tailormade integrated solutions. Now that we have robust and user-friendly hardware, we must develop the software to allow more specialized GC×GC methods. These software solutions will have to integrate hardware control, optimization tools, and data processing in a single package. We need to make the transition from GC to GC×GC simpler and faster.

Predictions and aspirations?

My goal is to continue our work on data processing solutions to merge data from GC×GC and other analytical platforms and obtain true untargeted analysis. I believe that the key role of the analytical chemist is now at the border between chemical analysis and data science. High-quality chemical data will allow us to build robust models using advanced data science tools. This powerful combination is the only way to achieve robust omics screening to answer the big challenges of science.

Single-Cell Science

With Michelle L Kovarik, Assistant Professor, Trinity College, Hartford, Connecticut, USA.

What's the latest from your lab?

My lab uses microelectrophoretic separations to look at cellular stress responses in the social amoeba Dictyostelium discoideum. This organism has a unique social lifecycle, in which the normally unicellular organisms aggregate and differentiate into a multicellular superorganism when deprived of nutrients. We use capillary electrophoresis to make quantitative measurements of enzyme activity in the cells during this process. Now, we are transferring our capillary separations to a microchip format, with the goal of making single-cell measurements.

What are the key trends in bioanalytical separations?

Biological systems have great chemical complexity, and as analytical scientists move from the genome to the proteome to the metabolome the complexity only increases. Fred Regnier's keynote address at ISCC was a great example of how separations can help us to capture the full range of biomolecular information. Glycomics and glycoproteomics also featured prominently at ISCC this year, including talks covering MS, CE-MS, labeling, and enzymatic processing, to name just a few. Another trend was highlighted by a great session on new tools, with many talks eschewing complex systems and instead presenting simple, custom solutions for exciting new applications. Vince Remcho's work on paper devices, Adam Woolley's work on truly microscale 3D printing, and Jim Grinias' overview of the history and current state of open-source instrumentation are all great examples of this approach.

What challenges face the field?

A key challenge in analyzing biological samples is their complexity, so maximizing peak capacity, developing multidimensional separations, and improving specificity are all important goals. As biological applications of mass spectrometry (MS) grow and mass spectrometers become more powerful and more sensitive, the ability to couple separations to MS is ever more important.

Another challenge is working with the "big data" generated by multidimensional separations coupled with MS-MS detection. These data sets often need sophisticated statistical analysis to draw out trends or identify key features that distinguish sample groups. Analytical educators need to make sure that the next generation of separation scientists knows how to select and implement effective data analysis methods from the long list of those available.

Predictions and aspirations?

Working with microfluidics has shown me how a technology can develop from "bleeding edge" science to a mainstream tool, and I think we'll see the same maturation of many of the new technologies presented at ISCC. When you develop a new tool it's exciting be the only lab in the world capable of a certain measurement, but if these advances are restricted to specialists, they will never meet their full potential. I hope to continue to develop the robustness and accessibility of new tools until they are available in all sorts of labs.


ERROR

REPLICATING FAILURE

What's causing the "reproducibility crisis" – and is there a cure?

By Peter T Kissinger

Over the past five years, there has been a steady increase in the number of papers withdrawn from top journals – largely after the results were found to be impossible to replicate. This phenomenon has been dubbed a "reproducibility crisis."

In government, forensic labs have been caught "dry labbing" data, while more than one pharmaceutical manufacturer has been shut down by the FDA after quality control labs have been found to be "testing to compliance" by throwing out data that missed the mark. In some cases, so-called "predatory journals" with weak or nonexistent peer review have been implicated. In others, scientific fraud has been discovered, careers ruined, and coworkers embarrassed.

In some fields of science, such as psychology and oncology, the challenges have been especially numerous. In biology, there have been incidental errors with cell lines and sourcing antibodies on which assays depend; quality was assumed but not validated, and instruments have been trusted but not calibrated. In other cases, solutions have been used long after their expiration date, or clinical trial data tampered with. All things considered critics have been given every reason to be hard on scientists. Fake news annoys, but fake science destroys.

What is causing the crisis? An amalgamation of factors are at play – these include more complex science, more sophisticated instruments with which many users are unfamiliar, a highly competitive funding climate, pressure to publish positive results (and to do so ahead of competitor labs), and also – of course – pleasing the boss.

Enemies of reproducibility

Three overlapping tendencies account for much of the poor reproducibility observed in published science, the first of which is natural variance. When mixed with confirmation bias – that is, bias that leads us to interpret data in a way that aligns with our existing beliefs – many experiments may never be considered fully repeatable, especially given that individual scientists vary in so many respects. We see the impact of natural variance in meta-analyses of existing reports; in these instances, larger data sets are examined, but often little thought is given to variances introduced by their differing methods.

The second driver of poor reproducibility is sloppiness in experimental design and execution. Various aspects of an experiment, including the instruments, reagents, cell cultures and antibodies used, and the use of inadequately validated methods, can contribute to this sloppiness. What's more, there is often little training given in maintaining all these factors; important variables are sometimes not accounted for because experimenters are unaware of them.

The third and rarest factor is willful fraud, which is arguably much more visible than the first two influences. Humans have a tendency to lie and have done so throughout history – especially when the temptation overwhelms the consequences of being caught. Most researchers start with good intentions, but there are various stressors that may break a researcher's character. An overbearing lab supervisor or financial executive who conveyed too much promise to investors can lead to scientists wrongfully taking the fall.

In the lab

Another factor in the lack of reproducibility of academic research in particular is that so little is at stake. The mission is education, including the enhancement of critical thinking, and graduates are the product. While graduates certainly can pose a risk to themselves and others, universities do not sell them, drive them, dose them, cook them, ingest them, or spread them on our faces, so unlike other products they are not subject to regulation by

Keep in Mind…

"There are lies, damned lies, and statistics." **Benjamin Disraeli**

"There's pretty much never been a great idea that didn't begin as a heresy that offended someone." **H.L. Mencken**

"Imagination is more important than knowledge." **Albert Einstein**

"In times of profound change, the learners inherit the earth, while the learned find themselves beautifully equipped to deal with a world that no longer exists." **Eric Hoffer**

"However beautiful the strategy, you should occasionally look at the results." **Winston Churchill**

"There is the risk you cannot afford to take, and there is the risk you cannot afford NOT to take." **Peter Drucker**

"If you already know it's going to work, it's not an experiment, and only through experimentation can you get real invention. The most important inventions come from trial and error with lots of failure, and the failure is critical, and it's also embarrassing." **Jeff Bezos**

"What people say, what people do, and what they say they do are entirely different things." **Margaret Mead**

"It is not necessary to change. Survival is optional." **W. Edwards Deming**

the FDA. While we as teachers invest in students, they invest far more in themselves, and neither of these investments are monitored by the Securities and Exchange Commission.

Especially for early-career researchers, the focus is on doing something new, and whether it is something that matters is too often considered a 'nice to have', not a 'must have.' In such work, there is a dangerous potential for students to make 'stretch claims' that may not be backed by their data. Plus, new graduate students generally have trouble applying critical thinking to published work. Students in their later years of study are better at assessing papers for their relevance and this is a true sign of advancing scientific education.

Compared with 50 years ago, published methods sections are substantially shorter today, despite the fact that there are now added details to include; that means it is often impossible to reproduce a published protocol without some degree of guesswork. To confuse matters further, the most careful work is often done at companies and is never published. Publishing means relatively little in many environments – better instruments, intellectual property and the threat of regulations can all matter a lot more in industry. In academia, fraud occasionally rears its head to support a degree or promotion, but by far the most common problem is the aforementioned lack of care regarding measurement details. More care is taken in industry because more is at stake in terms of public safety, potential legal action and regulatory intervention from bodies like the EPA, FDA, USDA or FBI.

Scientists also like to make things "look better". We can do this with log plots, averaging data and playing with confidence limits. Simple tricks, such as changing the origin of the Y-axis to change the appearance of the individual bars with respect to each other are very popular. The temptation to partake in such practices is especially pressing in clinical trials, where the ambiguities of biology meet the hope of recovering the large sums required to conduct the studies in the first place.

Beyond the lab

The number of channels available to disseminate research findings has increased dramatically in recent years. Both

Reproducibility Fail #1: Sloppiness

Problems:

- Calibration
- Appropriate standards (purity)
- Algorithms/software
- Significant figures
- P-values
- Misleading averages
- Not understanding instrument limitations
- Publishing claims not supported by data

Solutions:

- Maintain curiosity about reagents, tools, instruments, and software (trust but verify)
- Quality assurance programs
- Calibration schedules
- Preventive maintenance schedules

Reproducibility Fail #2: Wishful Thinking

Problems:

- Selecting data (if it doesn't fit, it must be wrong)
- Pretty pictures (beauty contests for cells and mice)
- Redrawing figures for clarity
- Dry lab data (fitting unrealistic expectations)

Solutions:

- Double-blind trials
- Sharing data and methods
- among laboratories
- Validation rules

Reproducibility Fail #3: Ethics

Pressures:

- Money (collecting it or saving it)
- Promotion
- Fame/hype
- Speed (winning a race, impatience)
- Sent abstract before doing the science
- Editors filling pages
- Peer reviewers inattentive
- Pleasing a supervisor or investor (fear)

commercial and society-based journals have exploded, quality content is in relatively short supply and peer review is badly stretched. Moreover, we are experiencing an 'open-access movement' (in my view, the right to open access is comparable to a right to free beer) and the existence of 'predatory' journals complicates matters further, while online forums and webinars provide yet another avenue to get your work into the public domain. For those tempted by shortcuts in the lab, the bottom line is that any work can be published somewhere, no matter how weak or irrelevant.

So how can we make science reproducible again? Life science leadership at all levels must set the tone to improve the situation. The progress of good science is easily broken when stressed by perverse incentives that allow speed and money to trump quality. Lab managers and executives should be careful to not shoot the messenger delivering inconvenient data. A careful analysis of the experimental design with appropriate controls, validated methods, and reagents is necessary. When tenure or grant renewals depend on publications, make them good, and when a round of venture funding or releasing a manufactured lot depends on a result, get it right. The pressure is real, but it's how this pressure is managed that matters.

In 2017, my friend and fellow curmudgeon, Ira Krull, commented on the reproducibility crisis in these pages (tas.txp.to/Krull1 and tas.txp.to/Krull2). Krull suggests the imposition of rules and associated courses on analytical method validation (AMV), analogous to the GMP, GLP, and GCP standards that we apply in biopharma once we move from the discovery phase to the development phase of a new therapeutic. I disagree. It can be debilitating to add rules and independent QA systems to the discovery phase within academia or industry. The systems are expensive to put in place, maintain, and follow.

Good academic scientists are cautious. Be sure to try things more than once, and be circumspect about the claims you make – if you don't, your reputation will suffer. Over time, the signals will rise and the noise will sink into oblivion. Pledge to do better, but don't let perfect be the enemy of good. The current system needs your help.

Peter Kissinger is a Professor at Brown Laboratory of Chemistry, Purdue University, and a founder of Bioanalytical Systems, Inc. (BASi), Prosolia, Inc., and Phlebotics, Inc. Indiana, USA.

the Analytical Scientist™

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the Spectroscopist

INSIDE

Finding the Source

We catch up with Jacob Shelley, Professor at Rensselaer Polytechnic Institute, about the most exciting new advances in spectroscopy and mass spectrometry – including his group's quest for better ionization sources.

What is the focus of your research?

I would say that we have two main branches of research. First, we want to speed up analysis, largely by producing portable mass spectrometry (MS) instrumentation. Second, we are developing 'smart' automated data processing tools that use algorithms to extract useful information from spectra. We are also working towards developing a ubiquitous ionization source that can be used to detect elemental species, small molecules and large biopolymers; these systems could fragment molecules to obtain structural information and then break it down even further to look at atomic ions. We have actually accomplished this using an ionization source called solution-cathode glow discharge (SCGD).

What advances do you find most exciting?

In terms of instrument development, there's an emphasis on compact and portable systems, as well as combined systems. We are also seeing the design of analytical instruments for real-world applications, such as the use of MS in medical diagnosis and even in operating theatres; researchers like Livia Eberlin (University of Texas) and R. Graham Cooks (Purdue University) are working directly with surgeons on intra-surgery tumor imaging. Another exciting development is that people are taking commercial, off-the-shelf items like cell phone cameras and developing them into powerful spectroscopic and imaging tools. In fact, Aydogan Ozcan at UCLA is converting cell phone cameras into high-resolution microscopes capable of imaging individual red blood cells.

Are analytical scientists closely involved in these medical applications?

Healthcare professionals and analytical scientists are working closely together to develop the technology, but there is rarely an instrument expert present in the operating theatre. Smart software can help non-expert users operate the instrument and interpret the outcomes, but ideally the operators should have some understanding of the underlying science in case of an algorithm failure or other malfunction.

Has artificial intelligence (AI) had a big impact on the field?

Absolutely, and especially on the optical spectroscopy side. The use of and reliance on AI, deep learning and machine learning is expanding. One example is the work of Garth Simpson at Purdue University, whose team has developed a deep learning approach to achieve target levels of spatial resolution in chemical images in a much shorter time. There is also Igor Lednev at the University of Albany-SUNY, who's using support vector machines and artificial neural networks alongside Raman spectroscopy to diagnose diseases like Alzheimer's in the early stages. His team is also working towards forensic applications for these techniques, which just emphasizes how widespread the applications of AI could become within spectroscopy.

Is there an area you are particularly passionate about?

The resurgence of atomic spectroscopy is very close to my heart. It's great to see because this area was thought to have peaked with the success of inductively coupled plasma-MS (ICP-MS), but there remains a need to measure elements and heavy metals. My group is currently working on a portable mass spectrometer for atomic analyses – for now, this is being geared towards nuclear applications by

"Researchers are starting to tackle longstanding problems"

studying uranium isotope ratios. We are close to achieving the detection levels seen with ICP-MS by using a different plasma source on a smaller MS platform; this is the SCGD I mentioned earlier. It has demonstrated detection levels comparable with ICP-MS at the parts-per-quadrillion level and we're able to get isotope ratio precisions for elements like uranium and lead that are at least as good as with multicollector ICP-MS.

Any other exciting applications in the field right now?

Researchers are starting to tackle longstanding problems, and the use of atomic spectroscopy and MS to detect halogens is a particularly interesting one. Past detection limits with ICP-MS have been very poor in the positive ionization mode and manufacturers don't make ICP-MS instruments that can record negative ions. Kaveh Jorabchi (Georgetown University) recently developed a very clever method, called PARCI-MS, to detect fluorine. Fluorine-containing molecules are persistent organic pollutants and a global issue – just down the road from my lab at Hoosick Falls (tas.txp.to/Hoosick) there's been major contamination of water supplies with perfluorooctanoic acid – and the trouble is that there's no bona fide way to quantify these species. It's a sure sign of progress that we're finally ready to start tackling this type of problem.

CSI XLI is coming up – what's your experience of this conference?

The first CSI I went to was in Budapest (2009) and I was immediately impressed, mainly because of the quality of the science and the community that it brought together. CSI hosts scientists involved with many techniques, from Raman to atomic spectroscopy and MS, all of whom attend to share their passion for analytical science. A lot of interesting collaborations have come out of this meeting and it's also paved the way for strengthening other spectroscopy conferences.

Jacob will be presenting on "Simultaneous Elemental and Molecular Detection via Optical and Mass Spectrometries in Search for the Chemical Origins of Life" at CSI XLI, 9–14 June 2019, Mexico City, Mexico.

Pick of the Papers

We asked Jacob to list three recent publications that have caught his eye...

Ultrasonic sculpting of virtual optical waveguides in tissue

M Chamanzar et al., Nat Comm, 10, 92 (2019). DOI: 10.1038/s41467-018-07856-w

What?

Using ultrasound waves to improve the retrieval of light by optical imaging tools in biological contexts – in this case, in mouse brain tissue.

Why?

The imaging of biological events in vivo (in the body) has invaluable applications such as the simulation of brain activity.

Yet, current approaches cannot maintain high spatial resolution when studying deep tissue due to light absorption, scattering and diffraction. Ways to improve resolution, such as implantable waveguides or optical fibers, may damage the tissue and cause complications; thus, a non-invasive way to steer light into deep tissue is needed.

How?
Pressure-induced density gradients were established in the tissue using ultrasound waves – these gradients create a refractive index contrast and light is confined in the areas of higher refractive index. The ultrasound waves act as waveguides, guiding light to these areas and acting to counter scattering and diffraction of the incoming beam of light.

Towards in-baggage suspicious object detection using commodity Wi-Fi

C Wang et al., IEEE Conference on Communications and Network Security, 2018.

What?
Applying existing Wi-Fi devices and usual Wi-Fi networks to identify dangerous objects in unopened containers.

Why?
Baggage checking requires specialized instruments and substantial manpower, both of which are expensive. The authors present a method to assess the contents of containers, such as bags, with relatively low cost and without invading the privacy of individuals. The method could be useful in large public spaces, where baggage checks are ineffective, inefficient or both.

How?
The team designed a novel system based on channel state information (CSI) measurements readily available in Wi-Fi devices. The system, comprising a simple Wi-Fi transmitter and receiver, performs both CSI phase adjustment and reconstruction; the CSI measurements were then subjected to a process of material classification and risk estimation, based on shape imaging and volume estimation.

Tip-enhanced Raman imaging of single-stranded DNA with single base resolution

Z He et al., J Am Chem Soc, 141, 753-757 (2019). DOI: 10.1021/jacs.8b11506

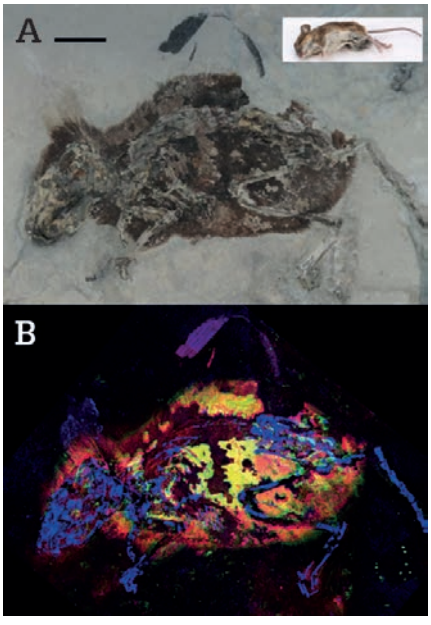
What?
Tip-enhanced Raman scattering (TERS) was used to resolve single nucleotide bases (the building blocks of the genetic code) in a single-stranded DNA molecule.

Why?
To demonstrate the subnanometer resolution of TERS – important if it is to fulfil its promise for chemical imaging and sensing at single-molecule scales.

How?
Single-stranded viral DNA was uncoiled and attached to a substrate by its phosphate groups to expose the nucleotide bases. A silver tip was subsequently scanned along the strand at steps of 0.5 nm, comparable to reported distances between stretched DNA bases, to produce a Raman spectrum that could be interpreted based on the expected readouts of individual bases – adenine, thymidine, guanine and cytosine – at each step. The accuracy of the resulting sequence was confirmed by comparing it with the known sequence using a string-matching algorithm.

Of Mice and Methylation

Our quick-fire round up of the most eye-catching spectroscopy research



Simply Red

The pigments in ancient fossil samples can provide us with valuable insights into evolutionary processes, but methods to study them are scarce. Researchers recently developed a method to attribute red fossil pigmentation to organosulfur-Zn complexes using synchrotron rapid-scanning x-ray fluorescence and both sulfur and zinc x-ray spectroscopy. Using this method, the team could see that the fur of a three-million-year-old mouse fossil from Germany was probably a similar reddish color to modern field mice (Figure 1).
Read the article: https://go.nature.com/2HQH56C

Figure 1. Optical and X-ray images of Apodemus atavus "lateral" fossil. a Optical image with inset of extant A. sylvaticus in the upper right for comparison (scale bars = 1 cm). b False-color SRS-XRF image reveals exceptional preservation of integument as well as bone. Reproduced from P Manning et al., Nat Communications, 10, 2250 (2019). DOI: 10.1038/s41467-019-10087-2

Monitoring Methylation

DNA methylation guides gene expression in cells and has important implications in disease and embryonic development. Current methods to study global methylation are invasive, but Raman spectroscopy may provide an easier alternative. By using Raman to monitor methylation in human colon carcinoma cells (high methylation), a standard cell line and murine embryonic stem cells (low methylation), Ruben Daum and team demonstrated how Raman can act as a real-time, marker-independent tool for detecting DNA methylation.
Read the article: https://go.nature.com/2QfP1lK

Washing Wastewater

Adsorbent materials developed from biomass are helpful in removing heavy metal pollutants from industrial wastewater. Using inductively coupled plasma-optical emission spectroscopy, a team from Islamic Azad University (Iran) were able to measure the removal of Cr(VI) and Ni from wastewater by the powdered bark of Platanus orientalis. At optimized pH, average removal power was 90.15% for Cr(VI) and 65.76% for Ni, and the authors suggest that the bark may provide a cheap biosorbent for this purpose.
Read the article: https://bit.ly/2McjRgC

There's Something in the Air

Traffic-related air pollutants (TRAP) may contribute to childhood anxiety. Kelly J Brunst and colleagues used magnetic resonance spectroscopy to study brain metabolites – largely myo-inositol – in 145 adolescents. By linking the measurements to anxiety symptoms, the team were able to identify significant associations between TRAP and myo-inositol levels, and between myo-inositol levels and generalized anxiety symptoms.
Read the article: https://bit.ly/2wfLgU8

Quantitative Cannabidiol Oil Analysis with CDS Pyroprobe

This application note demonstrates multi-step analysis of CBD oil, along with a reproducibility study and a cannabidiol calibration curve on concentration.

By Karen Sam

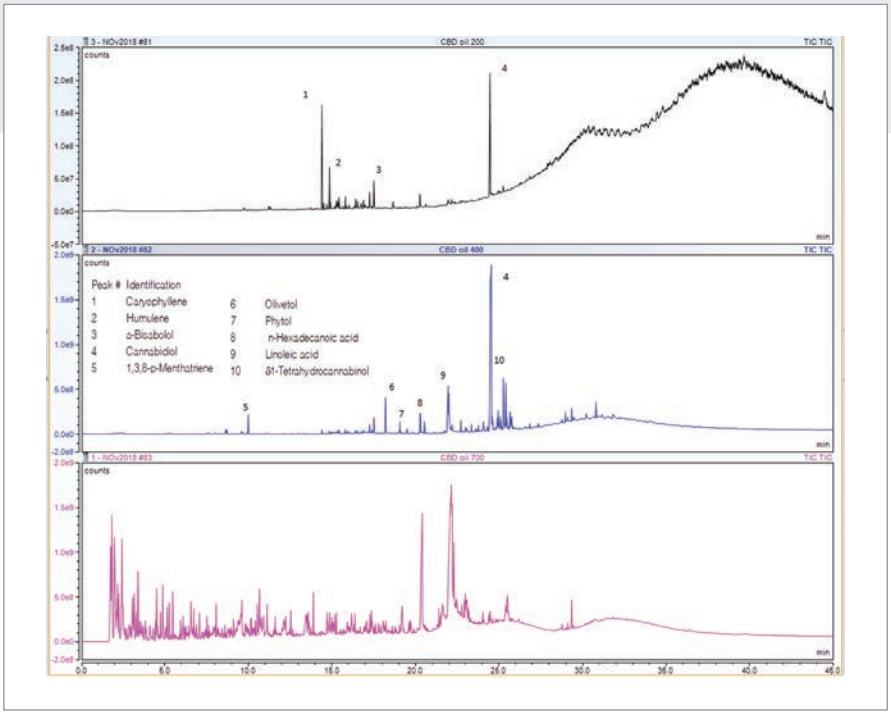


Figure 1. Multi-step analysis of CBD oil, 200°C (top), 400°C (center), 700°C(bottom).

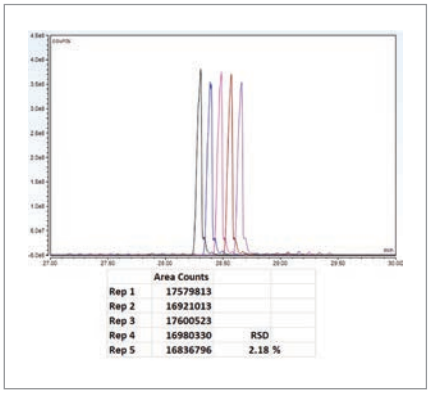


Figure 2. Time offset replicate chromatograms (m/z 231.2) and calibration curve for CBD.

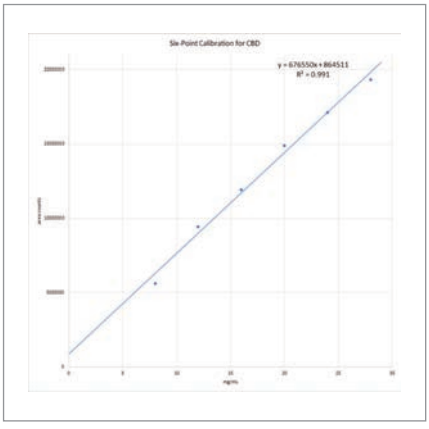


Figure 3. Calibration plot for CBD.

Cannabidiol (CBD) oil, from the cannabis plant, is being credited for helping treat many medical issues and has gained interest in nutraceutical and pharmaceutical industries. Analytical testing using the CDS Pyroprobe can both qualify ingredients in CBD oil and quantify analytes.

A 500 µg quantity of CBD oil was first screened with a multi-step temperature sequence of 200˚C, 400˚C, and then 700˚C on a CDS 6000 Series Pyroprobe to reveal specific active compounds including sesquiterpenes α-Bisabolol, α-Caryophyllene, and cannabinoids (CBD and δ9-Tetrahydrocannabinol) (Figure 1).

After choosing 300˚C as a thermal extraction temperature for CBD, a reproducibility test was performed. Five 0.5µL aliquots of CBD oil diluted in half with hexane (for easy syringe additions) were each run at a setpoint of 300˚C for 30 minutes. Replicate chromatograms are shown in Figure 2; area counts yield an RSD of 2.18 percent.

After confirming the reproducibility, a CBD calibration curve was created by running 4, 6, 8, 10, 12, and 14 µg of a CBD standard, equivalent to concentrations of 8, 12, 16, 20, 24, and 28mg/mL from a sample with fixed volume of 0.5µL. A linear regression with r2 >0.99 was observed, also shown in Figure 3. By comparing the average area of the CBD in the replicates in relation to the calibration curve, it was determined that the sample has 24mg/mL of CBD. As the sample was diluted in half, the original CBD oil has 48mg/mL of CBD, close to what was claimed by the manufacturer (50mg/mL).

Experimental Parameters

Samples were pyrolyzed in a DISC tube, using a CDS Model 6200 Pyroprobe.

6200 Pyroprobe

Mode:	*Trapping*
DISC Final:	*300˚C hold 20min*
Trap Rest:	*50˚C*
Trap Final:	*300˚C 10min*
Interface:	*300˚C*
Oven:	*300˚C*
Transfer line:	*300˚C*

GC/MS

Column:	*5 percent phenyl (30m x 0.25mm)*
Carrier:	*Helium, 50:1 split*
Injector:	*320˚C*
Oven:	*80˚C for 10 minutes 10˚C/min to 300˚C*
Ion Source:	*230˚C*
Mass Range:	*35-600amu*

Spotlight on... Technology

CDS 6000 Series Pyroprobe

The 6000 Series Pyroprobe is the 6th generation pyrolyzer from CDS Analytical. With the revolutionary Drop-In-Sample-Chamber (DISC) system, it offers easy sample loading by the DISC tube, unparalleled reproducibility at 1.5% RSD for Polystyrene, superior resolution with up to C55 for Polyethylene, and expandability with TD and DHS.

Learn more at www.cdsanalytical.com/pyrolysis-6200

CALYPSO: The most advanced LCMS N2 generator on the market (Four models)

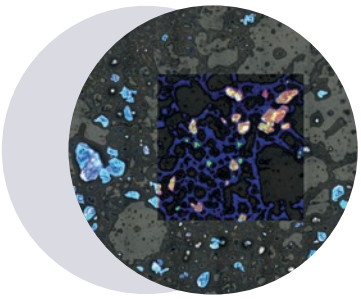
The CALYPSO Nitrogen range provides a nitrogen solution for LCMS instrument with an increase pressure of 8 bar (116 psi). Key features: without or with integrated oil free air compressor using Energy Saving Technology mode (EST), Color touch screen display with remote access and Smart Triple Filtration and Advanced technology PSA.
Learn more at https://www.f-dgs.com/en/produit/calypso-nitrogen-gas-generator-lcms/

Obtain Direct Mass Analysis from TLC Plates with the Advion Plate Express™

The Advion Plate Express provides a simple, automated means of obtaining mass spectra directly from TLC plates. Paired with the expression Compact Mass Spectrometer (CMS) this creates the TLC/CMS technique. From this, users can quickly and confidently identify products, even in complex mixtures, without additional sample preparation.
Learn more at www.advion.com

IonSense DART-OS with OpenSpot – Simple, Fast Identification

Couple the DART®-OS with the OpenSpot® Sample Cards for simple sampling and near-instantaneous results. Apply a small amount of liquid or powder on the sample card, insert into the DART-OS, and identify your sample in a few seconds. Ideal for food QA, forensics analysis, and organic synthesis.
Watch the Video at https://youtu.be/5iTSUEEFvMA



WITec ParticleScout: Detect, Classify & Identify Particles with Raman Imaging

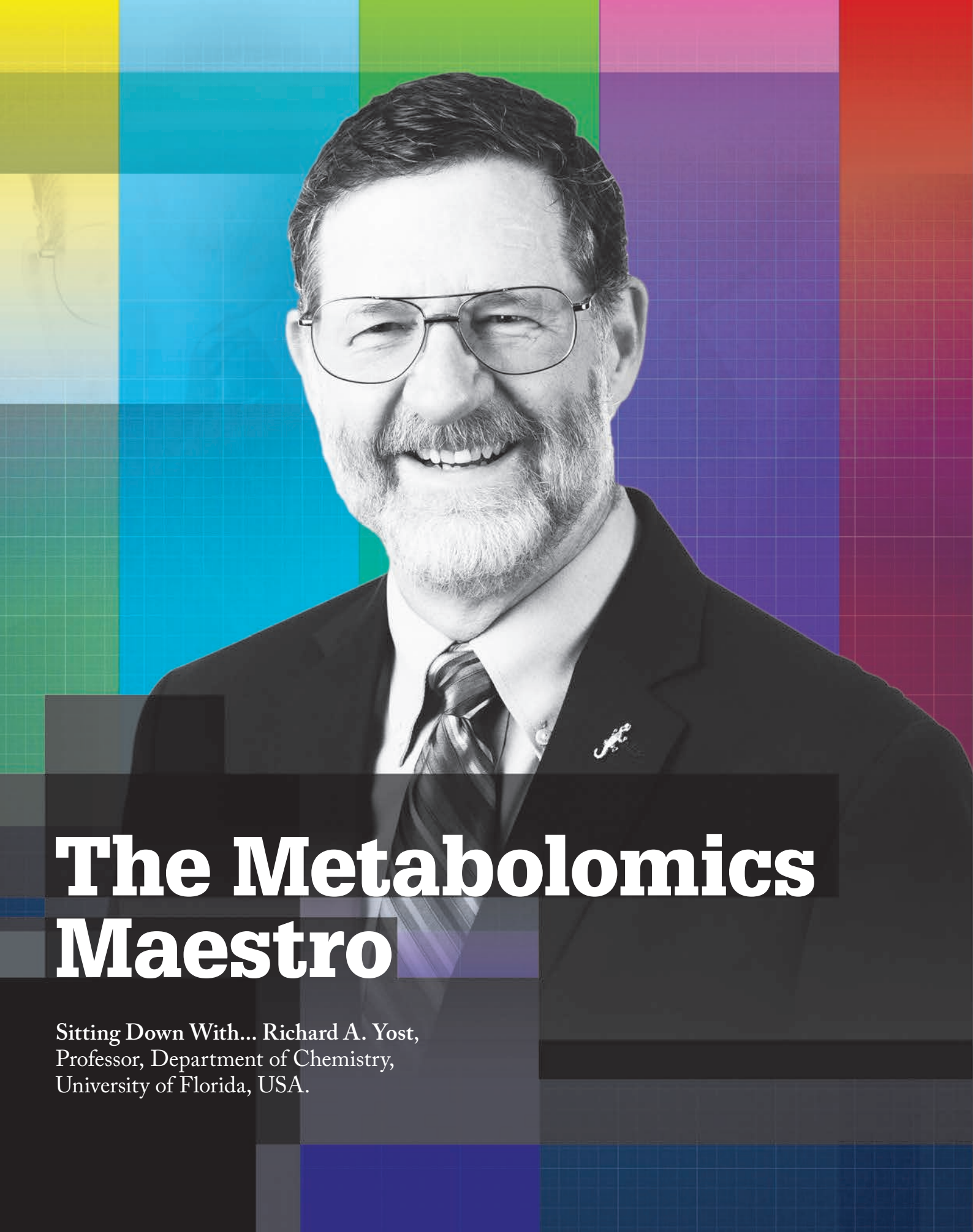
WITec's ParticleScout is an advanced analytical tool for the alpha300 Raman microscope series that surveys, classifies and identifies particles over even large sample areas. Automated routines sort particles, acquire their Raman spectra and generate detailed reports. Microparticles are of ever-increasing interest in environmental science, cosmetics, packaging and many other fields.
www.witec.de/products/accessories/particlescout/

Bruker's timsTOF PRO with PASEF

Mass spectrometry (MS)-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. Bruker's proprietary timsTOF Pro system uses Parallel Accumulation Serial Fragmentation (PASEF) acquisition method to provide extremely high speed and sensitivity to reach new depths in shotgun proteomics and phosphoproteomics, using low sample amounts.
Learn more at https://bit.ly/2uJ41ys

Vocus PTR-TOF

The TOFWERK Vocus PTR-TOF mass spectrometer measures trace volatile organic compounds (VOCs) in real-time. TOFWERK's novel Vocus proton transfer reaction cell and high-performance time-of-flight technology combine to offer market-leading sensitivity and separation power for online analysis of industrial, laboratory, and environmental VOC samples.
https://www.tofwerk.com/products/vocus-ptr-tof/



The Metabolomics Maestro

Sitting Down With... Richard A. Yost,
Professor, Department of Chemistry,
University of Florida, USA.

What are your major responsibilities?

I'm a professor and the Head of Analytical Chemistry at the University of Florida. I also serve as the Director of the Southeast Center for Integrated Metabolomics (SECIM) and I'm the Director of the Metabolomics Consortium Coordinating Center (M3C), which coordinates the metabolomics research funded by the NIH Common Fund. My favorite role is as a teacher and mentor – my next PhD to graduate will be my 100th! I consider the primary responsibility of a professor to be producing outstanding scientists who can go out and have a big impact on the world.

What makes a good PhD student?

In my mind, the ideal PhD student has at least two of three characteristics: i) 'brilliant,' with the intellectual capacity to handle complicated concepts about analytical chemistry, instrumentation and measurements; ii) hardworking, committed and dedicated; iii) gifted in the laboratory, with a talent for the practical aspects of the work. If you aren't brilliant, you had better be hardworking and good in the lab. Likewise, if you don't plan to work hard in your PhD, then you had better be both brilliant and practically adept. Other skills also factor into the success of PhD students, though; for example, a number of my graduates with exceptional social skills and an entrepreneurial spirit have gone on to have successful careers as CEOs of start-up companies.

What drew you to mass spectrometry?

MS is a fantastic technique that can be applied to solve unique problems in metabolomics and beyond. When I was a new assistant professor at the University of Florida, MS wasn't considered an analytical technique, but rather was used largely by organic chemists for determining the structure of new molecules and by physical chemists for fundamental measurements. Today, the applications are extraordinary. Even molecules as large as antibodies can be measured by LC-MS, and methods to detect molecules at trace levels are expanding.

How have advances in MS contributed to research?

The development of commercially available LC-MS/MS with high resolution MS in the final stage has had a huge impact on the field. For untargeted metabolomics, having tandem MS as well as the accurate mass at high resolution for the second stage has been invaluable. The addition of further stages of separation has been an exciting development, and ion mobility is leading the way in this space. Ion-mobility-enabled mass spectrometers are widely available, and are useful for separating mixtures that can't be handled with chromatography or MS alone. In fact, we used ion mobility with LC-MS to examine two epimers of Vitamin D. We resolved sodiated ions of the epimers using ion mobility – one epimer adopted an open conformation and the other a closed conformation, each with a distinct drift time.

ASMS2019 is in full swing as we go to press – what is your role at ASMS and what is the aim of the meeting?

I'm the current President of ASMS after having spent two years organizing the conference program. About 8,000 people attend each year, with up to 4,000 papers presented. I particularly like the focus on poster presentations at ASMS, as it provides an opportunity for dynamic discussions; much more so than oral presentations, which often leave little time for questions. Moving forward, we'd like to encourage even more interaction between fundamentalists, who are concerned with things like bond energies, and those involved in the applications of MS. To this end, we have introduced a new oral session called "Fundamentals for Everyone", which we hope will be engaging for the entire MS community.

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

The goal is innovation. I like nothing more than to hear a new idea and think, "Wow, I've never heard that before" or "I never knew that would work." I have no doubt I will continue to be surprised on a regular basis because of the tremendous room for innovation in this field. MS is unlike many other analytical techniques in that it both separates and analyzes, which makes MS very powerful and is one of the main reasons for its continued growth. During my 40 years on the faculty at the University of Florida, I've watched MS grow while a lot of other analytical techniques that showed promise have lost their way; I'm confident that MS will continue to grow for the next 40 years.

What would you consider your greatest achievement?

That's a difficult question! I'm proudest of watching the careers of my PhD graduates flourish. But I'm probably best known for conceiving and inventing the first triple quadrupole mass spectrometer, with Chris Enke at Michigan State University. Reviewers from the National Science Foundation thought it would never work, but Chris and I were stubborn enough to do it anyway. Today, it's the most common mass spectrometer in the world, which is pretty funny for an idea that was dismissed and deemed unworthy of funding 40 years ago. What's more, it's now used to screen millions of newborn babies for 50 to 100 inherited diseases every year, saving thousands of them from an early death. Not a bad legacy.

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Soar to new heights of certainty, clarity, and confidence, even if your lab has limited mass spec experience. The Agilent InfinityLab LC/MSD iQ system is self-aware and keeps a focused eye on your lab operations through its revolutionary intuitive technology. Transition your staff from UV to mass detection in the quickest, easiest way possible with the newest addition to our family of powerfully perceptive mass spectrometry instruments.

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