APRIL 2020 # 86

the Analytical Scientist™

Making the "Breath Biopsy" a Reality

When it comes to cancer or infectious disease diagnosis, could we simply exhale the answers?

18 – 26

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Coming Together (Two Meters Apart)

In the face of a novel threat, how can we keep up-to-date on emerging data and still work on solutions?





It's been a while since we penned a joint editorial, but the COVID-19 pandemic very much highlights the need for combined efforts – something we wanted to replicate in house.

An estimated 20 percent of the world is now living in lockdown. As some countries begin to contemplate a return to something approaching normality, others are becoming new epicenters of the crisis. Uncertainty is one disturbing aspect of the fast-evolving situation; outcomes are difficult – if not impossible – to predict. We are currently viewing the situation from within the engine of the pandemic, unaware of the wider machinery dictating its movement. Cogs are turning – but in which direction? And to what end?

Analytical scientists live by data and its logical extrapolation into information. Right now, the data streams are variable – in quality and quantity. Never before has the word "testing" appeared so much (outside of routine analytical labs). And yet, testing is what is most seriously lacking as we write. Efforts in the USA, for example, have been hindered by faulty reagents (page 10), while regimes in other countries are crumpling under high demand. We must bring together our greatest minds to increase the reliability, speed and affordability of such tests. Until we have sufficient (quality) data, information will continue to be in short supply.

As the COVID-19 crisis deepens, we may find ourselves obsessively seeking any research that can shed light on our invisible foe. And there is a surprising amount of emerging literature. The scientific community, quick to grasp the scale of the crisis and already accustomed to collaboration, have dispensed with formalities and rivalries to share work in the public domain as rapidly as possible. To that end, the whole Texere editorial team is collaborating to keep you apprised with The COVID-19 Curator – a quick, weekly round-up of the most exciting and impactful developments (subscribe free: texerenewsletters.com/covid19newsletter).

And yet, as communities join forces, conferences succumb to cancellations across the globe (Riva – page 28 – and HPLC 2020, to name but two), trampling traditional efforts to network and exchange ideas. The Analytical Scientist team is working hard on novel initiatives that can help fill some of the gaps…

COVID-19 represents the greatest threat that mankind has faced for decades, and it will require all of our expertise and ingenuity to tackle it. To those of you working personally on the issue: we thank you dearly. And to all of you: stay safe.

Matthew Hallam
Charlotte Barker
Lauren Robertson
Rich Whitworth
Frank Van Geel

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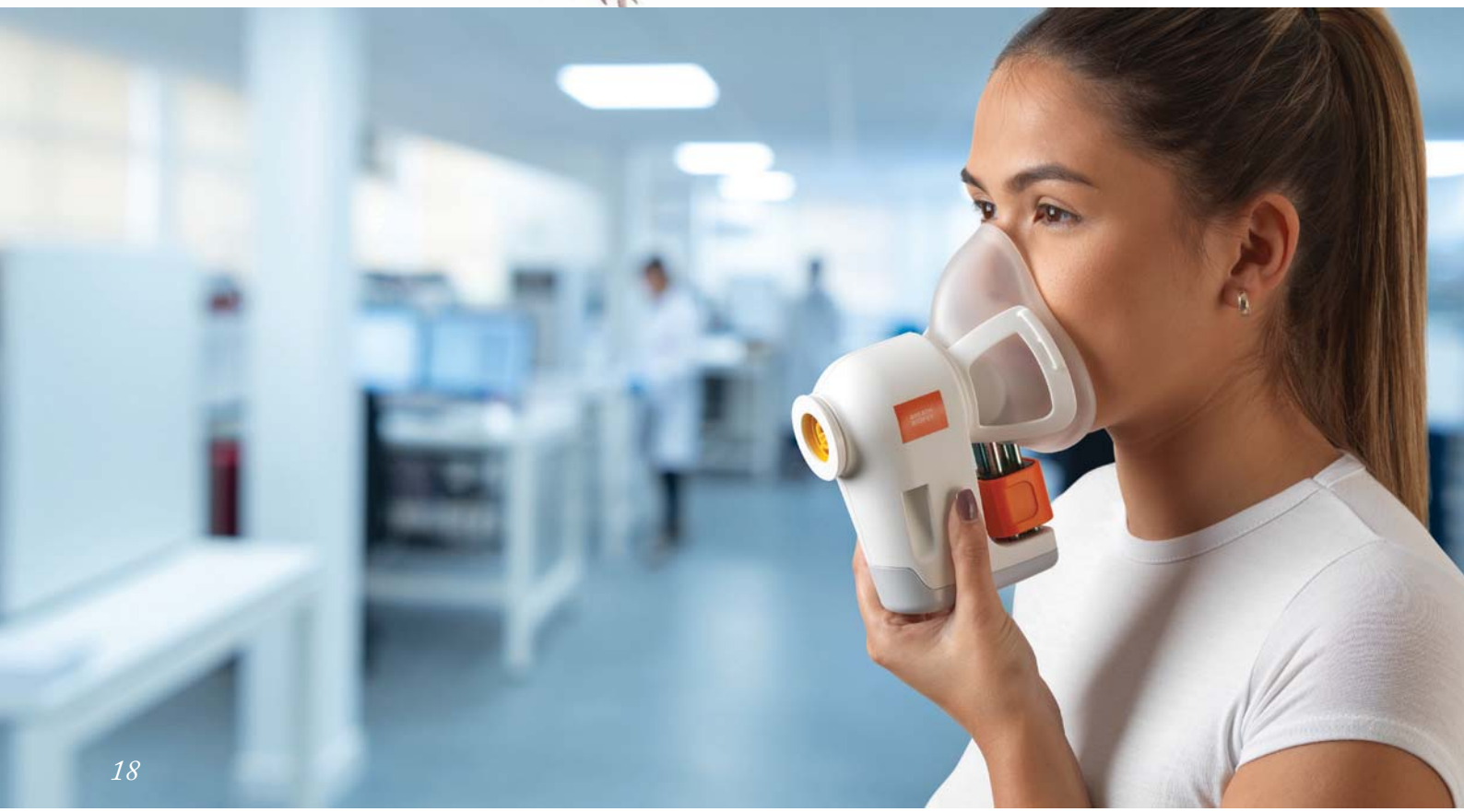

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On The Cover

A representation of the blooming field of breath diagnostics and our fight for health – in the lungs and elsewhere.

Features

Business

Sitting Down With...

the Analytical Scientist

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Upfront

Research
Innovation
Trends

SPECIAL SERIES
Advanced Clinical Analytics

Malaria Diagnosis in Hand

Point-of-care tests with smartphone analyzers could transform detection of the killer disease in resource-limited settings

The need for simplified, low-cost, point-of-care testing (POCT) systems that can diagnose infectious diseases quickly in resource-limited settings is ever-present. Developing reliable POCT diagnostics could lead to earlier detection, improved treatment, and streamlined outbreak prevention. Ideally, such a platform would make use of a user-friendly analyzer to perform data acquisition, analysis, transmission, display and storage – capabilities supported by today's smartphones.

Chong Ahn and colleagues have developed a novel microchannel capillary flow assay (MCFA) platform that can be linked with a smartphone analyzer for malaria diagnosis in developing countries. The MCFA uses chemiluminescence-based sandwich enzyme-linked immunosorbent assay to detect *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2; a known malarial biomarker) at concentrations as low as 8 ng/mL in serum samples. Optical signals indicating the presence (or absence) of PfHRP2 is then fed to the linked smartphone by a high-sensitivity detector in real-time; the detector is, in turn, powered by the smartphone.

Each of the system's three components has been carefully selected. "The lab chip is not only cheap and disposable, but can also perform autonomously with minimal user intervention," says Ahn – and this ensures ease of use regardless of technical acumen. "A smartphone then exhibits all necessary features of an analysis system, with a touch-controlled screen, high-speed processor, communication ports, wireless connectivity, storage media and rechargeable battery," he explains. Smartphones are – of course – ubiquitous, even in developing countries.

What's more, the system can be customized for the detection of different disease biomarkers – after necessary improvements to the currently achievable limit of detection. Looking forward, Ahn highlights plans to develop a totally autonomous MCFA chip for biomarker detection from whole blood, in addition to modifying the current system for the diagnosis of common mental health disorders, such as stress, anxiety and depression. "Measurement of biomarkers for common mental disorders may provide a reliable means of evidence-based diagnosis," he says. "We plan to develop a platform that combines a smartphone with polymer lab-on-a-chip devices for POCT of psychochemical stress biomarkers in saliva, while simultaneously collecting patient-reported psychosocial data."

Reference

1. *S Ghosh et al., Microsyst Nanoeng, 6, 5 (2020). Available at: https://go.nature.com/3a0YTIP DOI: 10.1038/s41378-019-0108-8*

TIMELINE

Analysts Versus COVID-19

Progress since the outbreak began

December 2019
Outbreak begins in Wuhan, China

February 16, 2020
Development of a combined optics and magnetic particles technique for high-throughput SARS-CoV-2 detection from saliva

March 10, 2020
In silico approach to accelerate the development of MS-based proteomics methods for detection of SARS-CoV-2 proteins

BUSINESS IN BRIEF

A round-up of this month's business news, from COVID-19 assays to AI-driven cancer diagnosis

- Digital Science named SciSwipe and SciFlow as the latest winners of its prestigious Catalyst Grant. The award offers up to £25,000 ($30,000) for concepts with the potential to transform scientific and academic research (1).
- Dutch biotech company Toxys raises €2 million financing for the global expansion of its animal-free safety testing for novel medicines, chemicals, cosmetics, and food. Toxys will also use the funding to speed up implementation of its toxicology assays for regulatory applications (2).
- Agilent Technologies has announced a partnership with Visiopharm to ensure standardized cancer diagnosis using artificial intelligence-driven solutions. It is hoped the comarketing relationship will lead to accelerated diagnoses for patients (3).
- The FDA has issued an emergency use authorization for a Thermo Fisher Scientific's diagnostic test for SARS-CoV-2 – the virus that causes COVID-19. The test uses Applied Biosystems TaqPath Assay technology and is designed to provide patient results within four hours (4).
- Tasso Inc has completed a $6.1 million financing round led by Vertical Venture Partners, with participation from Techstars and Cedars-Sinai. The funding will help Tasso scale its first product, Tasso OnDemand, a patient-centric sampling platform featured in our 2019 Innovation Awards, and grow their team to support significant commercial interest (5).

References

1. *Digital Science (2020). Available at: https://bit.ly/2UPjKJf*
2. *Toxys (2020). Available at: https://bit.ly/3dNpf3w*
3. *Business Wire (2020). Available at: https://bwnews.pr/2X17LLs*
4. *Thermo Fisher (2020). Available at: https://bit.ly/342j9bg*
5. *Vertical Venture Partners (2020). Available at: https://bit.ly/3aAND6y*

Behind the COVID-19 Curve

A slow diagnostic response to the coronavirus outbreak put the USA on the back foot

Efforts to ramp up diagnostic testing for SARS-CoV-2 in the USA have been hampered after a slow response to the outbreak. The original test kit distributed by the Centers for Disease Control and Prevention (CDC) contained a faulty reagent that, in many cases, reacted to the negative control, rendering results invalid (1). Hospitals and academic labs across the country were restricted from developing their own test kits until February 29.

Since then, testing has moved away from the CDC and state labs toward hospitals and commercial companies – and, as of March 14, labs using the CDC assay are no longer required to submit samples for confirmation. But where does that leave the USA? As of March 18, 37,824 tests had been conducted (2), leaving the country lagging behind others. Health officials hope that new drive-through test centers and quicker processing will help them hit testing targets.

References

1. *Science, 2020. Available at: https://bit.ly/2x8z1N7.*

March 11, 2020
WHO declares the outbreak a worldwide pandemic

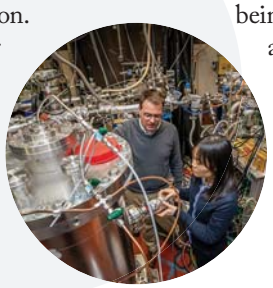
March 17, 2020
SARS-CoV-2 main protease structure solved by X-ray crystallography with MS electrophile screening

March 22, 2020
66 molecular drug targets targetable with 69 existing FDA-approved drugs identified on the SARS-CoV-2 virus using affinity purification MS

Criegee's Chemical Conundrum

Uncovering the missing link between age-related diseases and food spoilage

Autoxidation pathways of unsaturated lipids, involving biradical Criegee intermediates (CIs), could provide a link between our increased risk of developing chronic diseases or cancers as we get older, and food decomposition.



Hydroxyl radicals and other reactive oxygen species are known to cause irreversible damage to the unsaturated lipids in our bodies and our food. But Kevin Wilson (Deputy Director of Lawrence Berkeley National Laboratory's Chemical Sciences Division) and his team have discovered that these radicals have a partner in crime; so-called "Criegee intermediates," more commonly known for their role in atmospheric chemistry.

"At the start of this research, we observed unexpected 'secondary ozonides' during a hydroxyl reaction with unsaturated lipids. These are typically associated with ozone chemistry and not with reaction pathways involving hydroxyl radicals," says Wilson.

The finding stumped the team for many years, until a 2018 study showed a connection between CIs and hydroxyl radical reactions (1). Meirong Zeng (first author of the study) then designed new experiments proving that CIs play a central role in autoxidation – an autocatalytic free radical chain reaction that leads to the slow, persistent destruction of organic molecules.

"Our work points to autoxidation being initiated by hydroxyl radical addition to C=C bonds and propagated by chain reactions involving CIs, rather than the H-abstraction and peroxy radicals conventionally thought to be dominant," Wilson says.

Zeng adds, "This new pathway leads to unexpectedly rapid lipid degradation, implying that CIs could play a much more prominent role in aging and disease than previously thought (2)."

Nanodroplets containing lipid molecules were used in the experiments, and the kinetics and reaction products within them analyzed in real-time

using a homemade vacuum ultraviolet (VUV) aerosol mass spectrometer. VUV photoionization allowed critical reaction products to be observed, revealing the CIs.

The next step is to investigate reaction pathways and rate constants for the hydroxyl-peroxyl radical/CI reactions, and to see whether there are broader implications for human health. "We hope the results from our study inspire further research into the biochemistry of CIs, potentially aiding both the prevention of disease and the preservation of food," says Wilson.

References

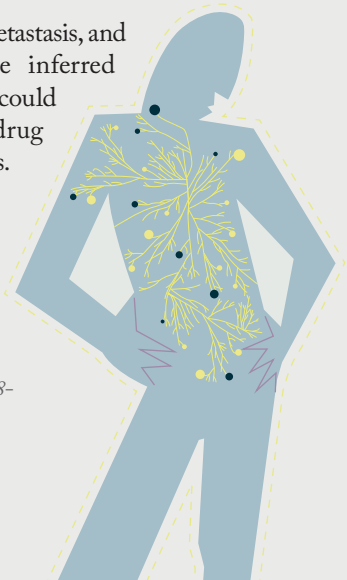
1. *X Zhang et al., J Am Chem Soc, 140, 17492 (2018). DOI: 10.1021/jacs.8b08610*
2. *M Zeng et al., PNAS, 117, 4486 (2020). DOI: 10.1073/pnas.1920765117*

Mapping Melanoma

LC-PRM-driven proteomics helps explore the mechanisms behind cancer metastasis

Targeted quantitative proteomics – using LC-parallel reaction monitoring (PRM) – is shining a light on kinome protein reprogramming during melanoma metastasis. Weili Miao and colleagues found that the expression of Janus kinase 3 (JAK3; a tyrosine kinase) is reduced in metastatic melanoma cells when compared with primary (non-metastatic) cells, by applying LC-MS and LC-MS/MS to tryptic digests. Reduced JAK3 is associated with poorer melanoma prognosis because it inhibits the activity of matrix metalloproteinases – key enzymes involved in the metastatic transition.

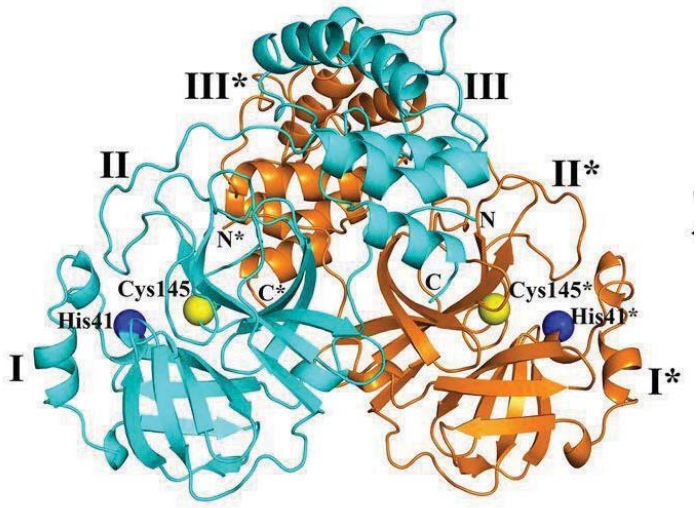
The gathered data represent the most comprehensive available for these proteins in melanoma metastasis, and the knowledge inferred by such data could inform future drug discovery efforts.



Reference

1. *W Miao et al., Sci Rep, 10, 2485 (2020). DOI: 10.1038/s41598-020-59572-5*

IMAGE OF THE MONTH



Corona in 3D

A team at the University of Lübeck, Germany, has mapped the 3D structure of the main protease of Sars-CoV-2 using the Berlin synchrotron source, Bessy II. This structural information could support the identification of COVID-19 drug candidates that inhibit the enzyme and impair viral replication.

Image credit: L Zhang et al., Science, eabb3405 (2020). DOI: 10.1126/science.abb3405

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

QUOTE OF THE MONTH

"I believe that really interesting discoveries tend to happen at the interface of different fields. Our multidisciplinary team of biologists, metabolomic scientists, clinicians, and engineers has ensured we approach challenges from unique perspectives – and allowed us to identify important biomarkers for our technology."

By Billy Boyle, CEO of Owlstone Medical, Cambridge, UK

Fluid Diagnosis – with SERS

A surface-enhanced Raman scattering approach could streamline the diagnosis of gastrointestinal tumors

Surface-enhanced Raman scattering (SERS) has demonstrated utility in the liquid biopsy of gastrointestinal tumors. "Replacing endoscopy with a non-invasive liquid strategy could tremendously improve GI tumor diagnosis by sparing patients this unpleasant procedure," says Vlad Moisiou – a spectroscopist investigating the approach.

Other advantages? "The ease with which it could be implemented as a screening strategy in clinics and large population studies," says Moisiou. But how do they do it? By mixing patient serum with metal nanoparticle colloidal solution, adding ions (in this case Ca^{2+} and Cl^-) to amplify the signal, and applying SERS. "Our efforts bridge the gap between mechanistic insights into physical processes behind SERS and clinical applications," Moisiou adds. Moving forward, the team hopes to advance their approach to a standard technique for application in hospitals and beyond.

Reference

1. *L Avram et al., J Clin Med, 13, E212. DOI: 10.3390/jcm9010212*

A Community Against Coronavirus

Scientists everywhere are going the extra mile to support each other – and our health services

In times of need, humanity shows its true colors. And in the fight against coronavirus, acts of kindness and generosity are making headlines the world over. As a reprieve from the frankly terrifying news stories that bombard us every day, we bring you a breath of fresh air...

Sharing is caring

Chemists working at the University of York, UK, have donated all suitable supplies in their possession to local hospitals. Labs from all departments have joined the effort, and their generosity hasn't gone unnoticed online.

Photo: *https://bit.ly/2xGWWDi*

And York isn't the only UK university going the extra mile. The Chemistry Department of Imperial College London have also donated all spare chemicals to produce hand sanitizing gel, as well as providing unused personal protective equipment to NHS Trust Hospitals.

See for yourself: *https://bit.ly/2QJQceW*

Meanwhile, researchers at the Indian Institute of Technology (ITT Hyderabad) are developing hand sanitizer to protect their students and staff; technicians have created 50 liters at negligible cost in just two days.

Their post: *https://bit.ly/3dwMHSS*

Collaboration calling

Alan Jarmusch of UC San Diego has issued a call for collaboration between MS experts collecting data from biofluid and human tissue relating to coronaviruses. Any researcher can access the data sharing platform – do you have data to share?

Platform: *https://bit.ly/3dvB2Du*

Tweet: *https://bit.ly/3duGg2A*

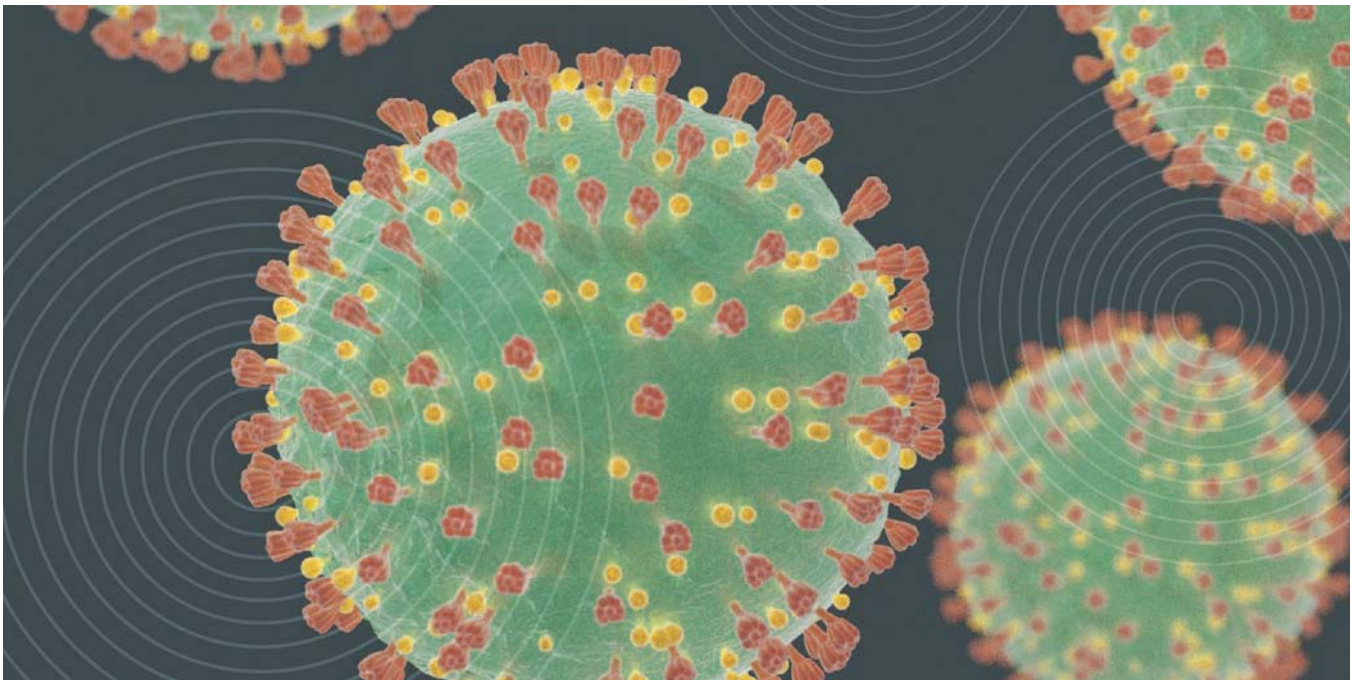
The WHO have launched a Global 2019-nCoV Clinical Data Platform, allowing Member States to contribute anonymized clinical trial data to inform public health responses, as well as publishing interim clinical care guidance for hospitalized patients and mildly ill patients isolating at home.

More info: *https://bit.ly/2UePRmm*

An educational platform

Researchers are also taking to online platforms to communicate chemical education to the public. From simple lessons as to why soap is such an effective weapon against the virus (*https://bit.ly/33J7L3S*) to graphics demonstrating how viral testing works (*https://bit.ly/3duMHTi*), the community is uniting to bring the public to our level, and to provide much-needed clarity in these uncertain times.

Want to stay up to date with research into understanding, testing and fighting COVID-19? Subscribe to "The COVID-19 Curator" – your 5-minute window into the science of the outbreak.



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In My View

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

Partnering Up for Food Harmony

We cannot underestimate the power of international collaboration when it comes to ensuring food security

By Martin Rose, Fera Science Ltd, Sand Hutton, York, UK

China is now Europe's largest source of imports, and the second most important export market overall (behind the USA). Bilateral European Union (EU)–China trade amounted to €520 billion in 2015, and this is expected to reach €700 billion by 2020. Part of this – of course – includes the trade of food items. Yet, consumer trust in the food industry across both the EU and China has been damaged by numerous accidental and deliberate food contamination and adulteration incidents, such as the 2008 Chinese melamine incident, and the 2013 EU horsemeat scandal. The ability of EU companies to export foods to and import foods from China has been hampered by these safety, traceability, regulatory, and fraud issues. And Chinese companies trying to export to Europe face similar obstacles.

Twenty-first century food supply chains are increasingly complex and highly vulnerable to safety and fraud threats. Increasing demand and growing markets then enhance the likelihood of food safety incidents and deliberate contamination, which in turn ruins consumer trust and undermines legitimate trade at domestic and international levels. Furthermore, laboratories in Europe and China are often working to different quality standards and using different analytical methods for data production towards certification and confirmation purposes, which can lead to protracted trade disputes and embargoes. An analytical partnership between the two bodies is needed to ensure consumer safety and fruitful trade.

The EU-China-Safe project aims to satisfy this need. The project is looking to develop a partnership between European and Chinese organizations involved in food control, with the aim of delivering a shared vision for food safety and authenticity based on "mutual recognition" in food standards and testing and certification (as has been achieved in other areas between these regions). There are 16 participants from the EU and 17 from China, including key research organizations from both government and industry, that will work together to combat issues with food safety and fraud known to exist between the two trading blocks.

The collaboration will be enabled through the development of an EU-China Joint Laboratory Network, operating through a state-of-the-art virtual laboratory (the virtual "Reference Laboratory 2020") with interchangeable staff from two continents, and using shared data systems to enable cooperative method development. The lab will be used as a "showcase" to communicate and demonstrate best practice through a "twinning model" that promises alignment between the two bodies. Innovative traceability tools will strengthen the most vulnerable supply chains, while improved detection of chemical and microbiological hazards and food fraud will be implemented through standard operating procedures, validation, quality control measures, and laboratory web conferences for best practice examples. Trade barriers caused by food safety and fraud issues will be analyzed, and recommendations on how to predict and prevent future events disseminated.

Where are we so far? We have started by collecting reference documents, such as a laboratory inventory, the regulations of both regions, and standard analytical methods. We identified that both regions

had partners who were interested in validating GC-MS/MS methods for dioxins analysis (as a more economical alternative to sector instrumentation), and so we have been working jointly to validate one. Major instrument manufacturers are (thankfully) lending their support, so we can work with confidence that identical technologies will be available to both regions for such exercises. The next stage will be to test the virtual laboratory where there is more variation in analytical approach.

I believe that working in partnership is always preferable to working alone. Sometimes corporate or other barriers can get in the way, but breaking these down is well worth the effort. Sometimes we have preconceived ideas or prejudices about what to expect when working with others, but my experience is to be prepared to have those illusions shattered! There are great partners out there, and there is much to be gained for all involved if you are prepared to put in that extra effort to form an effective and functioning partnership.

What Comes After the "Eureka" Moment?

Increased focus is needed when it comes to the implementation of promising technologies in appropriate application areas

By Katelynn A. Perrault, Assistant Professor of Forensic Sciences and Chemistry, Laboratory of Forensic and Bioanalytical Chemistry, Forensic Sciences Unit, Chaminade University of Honolulu, Hawaii, USA

We've always done it this way. Spend enough time convincing someone to try something new, and you will undoubtedly hear this phrase like a broken record. Yet, as analytical scientists, we pride ourselves on working on new developments at the cutting edge. Every scientist dreams of the day they will have that eureka moment – that moment when they discover a revolutionary new way of doing something. The next step, where we often fall short, is in following through to get our reluctant stakeholders to buy in. How do we take that new idea and convince people that it's worth pursuing?

Our academic backgrounds breed a culture of innovation and discovery. High impact journals only publish papers with high novelty. Awards are given for innovation in many fields of analytical chemistry. Plenary speakers are chosen based on revolutionary ideas. This early recognition is all incredibly exciting, but don't we also have an obligation to guide these innovations to fruition in applications that can benefit from them?

As a forensic chemist, I have had to think a lot about how I define my work. I believe that fundamental studies should be paired with rigorous applications-based research for the end user. I oppose the idea that I have to be either an analytical chemist or a forensic scientist (although I have always been a fan of wearing different hats for different occasions!). I spin my work depending on which audience I'm presenting to – but it often comes with criticism.

"Every scientist dreams of the day they will have that eureka moment– that moment when they discover a revolutionary new way of doing something."

In the analytical world, I am told my work is too applied; in the forensic world, too fundamental. But working at the interface of these disciplines is what makes our work exciting, and should be celebrated, rather than criticized. It is the bridge needed to shift our fundamental work in separation science to the reality of implementation in forensic investigation.

The questions I often sit up thinking about are:

- Why do we not work harder to "marry" fundamental science with applied science and practical usage?
- Why is applied research off

limits for most grants awarded in analytical chemistry?
- Are we addicted to working on the "cutting edge" and neglecting our duty to help end users?
- Are we doing enough to follow through on what we promise?

We have to make implementation easier. We have to make data and best practices available. We can't expect that the end user is going to do this for themselves, especially when they have a functioning (yet perhaps not optimal) approach at present. People are reluctant to adopt new methods due to the time taken, even if that comes with significant analytical benefits.

I would argue that this culture of novelty and working on the "cutting edge" can impede the adoption of novel techniques. We opt for publishing exciting proof-of-concept studies over rigorous validation and inter-laboratory studies, and pursue peer-reviewed publications and conference proceedings over contributing to developing standardized methods. Meanwhile, end users tell us that their existing approaches are "good enough" – no need to try something new. Industries are also applying analytical instrumentation in an increasingly "black box" manner, making it harder to convince them to invest time and money to reap the benefits of new techniques.

My field of comprehensive two-dimensional GC (GC×GC) is no exception. The technique has been around since the 1990s, yet adoption in industries that could benefit still lacks. The road to adopting new technology is not a swift one, especially in my area. There are many hurdles, and a lot of reluctance (rightly so) to adopt something new for fear it will not be admissible in court. I have to think creatively about how to market GC×GC as "worthwhile."

On the analytical side, "we've always done it this way" is not a valid excuse to ignore new technologies that provide improved answers to important questions. However, as analytical chemists, we owe it to the end users to prove our technology can work for their application, and to help them find easier ways to implement it. This endeavor requires work, and not the exciting, cutting-edge kind. It requires lengthy studies (repeating work many times, validating methods, intra-laboratory studies, inter-laboratory studies), many of which will be turned away by journals due to lack of novelty.

Working in analytical chemistry research is exciting, and thinking that we might uncover the next big innovation is probably part of what drew us to the field in the first place. But it is what comes after the "eureka moment" that determines how we use our expertise, knowledge, and discoveries to actually impact society in a meaningful way. Though this might be less glamorous, it is our duty and obligation to do it well.

Wanted: (Much) Better Bioanalytical Sample Prep

Intact protein analysis may be on the horizon, but current sample preparation methods simply aren't up to scratch

By Katarina Maráková, Assistant Professor, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

In recent years, protein analysis has – rightly – caught the attention of scientists all over the world. Medical and pharmaceutical scientists alike have recognized that various proteins present in biological samples can serve as potential biomarkers and drug targets for numerous diseases, including cancer, neurodegenerative diseases, and immune-mediated disorders. More recently, targeted proteomics of intact molecules has emerged as a promising and rapidly growing facet of this field.

Based on LC and MS, these advanced workflows host many benefits for proteomic applications versus traditional immune-based methods (1, 2). The major benefits include high accuracy, precision, and selectivity, as well as the ability to analyze several intact proteins in one run. But these approaches are not without their challenges. Low sensitivity due to poor ionization and fragmentation efficiencies, alongside non-specific binding and adsorption of proteins to surfaces and other molecules, are the primary struggles faced by the field. An efficient sample preparation protocol is therefore vital to the development of a successful workflow for intact protein analysis.

During my recent research stay at the laboratory of Kevin Schug (University of Texas at Arlington, USA), I worked to develop an analytical approach based on LC-triple quadrupole MS for the direct quantitation of multiple intact proteins (growth factors and cytokines) in complex biological matrices. The fundamental principles of single and multidimensional setup for such an approach have been published previously (3-5). During our work, however, we hit a stumbling block: the extremely limited commercial availability of sample preparation options for intact proteins – especially for cases with

limited amounts of samples.

Sample preparation workflows for intact proteins generally involve many complex, laborious, and costly steps, namely immuno-affinity purification, 2D-gel electrophoresis, use of magnetic beads and nano-particles, and size-exclusion chromatography, or any combination of these (2). Therefore, we really need a strong focus on the development of new prospective sample preparation methods with possible use for isolation and enrichment of multiple intact proteins before instrumental analysis. The need for such sample pretreatment techniques is undeniable, especially when you consider that potential proteomic biomarkers – which can have variable physicochemical properties – are present in the complex biological matrices at trace concentrations.

In my view, solid-phase extraction (SPE) and monolithic SPE spin columns are just some of the options worth testing, and may even provide a simpler and cheaper alternative for sample preparation of intact proteins. Nowadays, SPE is commonly used for sample preparation of small peptides after protein digest, but it has also been applied for the analysis of a smaller intact proteins (up to 7.5 kDa) (6). The use of organic polymer monoliths with different chromatographic mechanisms have been reported for the separation of intact proteins in traditional LC column formats, thanks to their high permeability, macropore structure and better resistance to extreme pH conditions – which are desirable characteristics when dealing with proteins that have a very high or very low isoelectric point (7).

Another interesting area in the sample preparation field is coacervation – the aggregation of amphiphilic molecules to form a separate liquid phase in aqueous media. The application of coacervates to the preparation of biological samples offers the potential to increase the concentration of low abundance analytes and the selective extraction of hydrophobic analytes from a complex matrix. Looking ahead, we plan to study the detailed application potential and conduct a systematic evaluation of these non-immunobased sample preparation methods for a larger set of variable intact proteins (different pI, GRAVY, molecular weight, abundance, and tendency to aggregate) in biological samples.

Every year, we see improvements across various performance parameters in analytical instrumentation, particularly in sensitivity. However, even with such super-sensitive instrumentation, we are not currently able to reliably target multiple intact proteins in complex biological samples due to the lack of appropriate sample preparation approaches. My hope is that, in the future, we will see more development and focus on this integral and crucial part of every bioanalytical approach.

References

1. *A Arora, K Somasundaram, "Targeted Proteomics Comes to the Benchside and the Bedside: Is it Ready for Us?", BioEssays. 41 (2019) 1800042. DOI: 10.1002/bies.201800042.*
2. *D Donnelly, et al., "Best practices and benchmarks for intact protein analysis for top-down mass spectrometry", Nat. Methods. 16 (2019) 587–594. DOI: 10.1038/s41592-019-0457-0.*
3. *E.H. Wang, P.C. Combe, K.A. Schug, Multiple Reaction Monitoring for Direct Quantitation of Intact Proteins Using a Triple Quadrupole Mass Spectrometer, J. Am. Soc. Mass Spectrom. 27 (2016) 886–896. DOI: 10.1007/s13361-016-1368-2.*
4. *D.D. Khanal, Y.Z. Baghdady, B.J. Figard, K.A. Schug, Supercharging and multiple reaction monitoring of high-molecular-weight intact proteins using triple quadrupole mass spectrometry, Rapid Commun. Mass Spectrom. 33 (2019) 821–830. DOI: 10.1002/rcm.8418.*
5. *Y.Z. Baghdady, K.A. Schug, Online Comprehensive High pH Reversed Phase × Low pH Reversed Phase Approach for Two-Dimensional Separations of Intact Proteins in Top-Down Proteomics, Anal. Chem. 91 (2019) 11085–11091. DOI: 10.1021/acs.analchem.9b01665.*
6. *K Bronsema et al., "A quantitative LC-MS/MS method for insulin-like growth factor 1 in human plasma", Clin. Chem. Lab. Med. 56 (2018) 1905–1912. DOI::10.1515/cclm-2017-1042.*
7. *J Masini, F Svec, "Porous monoliths for on-line sample preparation: A review", Anal. Chim. Acta. 964 (2017) 24–44. DOI: 10.1016/j.aca.2017.02.002.*

Embracing Rejection: the Key to Success?

Constructive criticism can open new doors when it comes to your research

By Amanda Hummon, Associate Professor, Department of Chemistry and Biochemistry and the Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA

Norm Dovichi joined our department during my second year as an assistant professor, and we soon discovered a mutual appreciation for coffee and walking. Over the next seven years, he and I would walk to the local coffee shop almost every day. We would often discuss my efforts to set up a lab that sits at the intersection of cancer biology and analytical chemistry. In particular, I catalogued – in gory detail – each rejected proposal and manuscript. Norm observed that the best thing I could do for my career (and sanity) was "to be like a duck and let it roll off your back." He pointed out that distancing myself enough from the feeling of failure would allow me to use the painful criticism to improve my science.

I often reflect on Norm's advice. It's challenging to conduct research that is meaningful to both communities. Though I trained as both a chemist and cancer biologist, I find it easier to think as the former. And that's reflected by my higher success rate when submitting manuscripts to analytical chemistry journals compared with cancer journals. My proposals also fair better at the National Institute of General Medical Sciences (NIGMS) than at the National Cancer Institute (NCI)...

Yet I continue to submit proposals to the NCI on a regular basis. Why? Am I a masochist? Maybe. The truth that I've come to realize is that if I want to make an impact and push my science in a direction that could have a positive impact on cancer patients, I need to get advice from the people at the forefront of these efforts. Clinicians have a real handle on the breakthroughs needed to improve outcomes for patients.

Over the last few years, I've submitted multiple proposals involving our favorite model system – the multicellular tumor spheroid – to both the NIGMS and the NCI. Spheroids are three-dimensional cell cultures – simple mimics of colon tumors. They can be grown from a single monoclonal cell line or by the incorporation of multiple cell types, and my lab has used them as a testbed for developing analytical methods extensively over the last ten years.

Our first rounds of proposals outlined our plans to use monoclonal spheroids. This resonated with the chemistry-based study sections at NIGMS, but hit a wall with the physicians on the NCI study sections. The medical doctors suggested that simple monoclonal cell lines were insufficient to model the complexity and heterogeneity of the epithelial tumor. I was upset when I received these summary statements (rejection is always hard) and I initially ignored the advice. But, the more I thought about it, the more I realized their points had merit. I couldn't ignore their criticisms – even if it meant overhauling our research approach.

We have expanded our studies over the last year: we now grow co-cultured spheroids. With this change has come additional complexity and numerous new challenges, but also new opportunities. The addition of endothelial cells and fibroblasts alongside colon epithelial cells means that we are now studying cell-cell communication that much more closely mimics that present in tumors in situ – a great asset in the effort to study and treat cancer.

That's not to say that I no longer get upset with every rejection – I do, but only because I care about my work. It's important to recognize that sometimes the harder path produces more meaningful results. Criticism is simply the price we have to pay.

"The truth that I've come to realize is that if I want to make an impact and push my science in a direction that could have a positive impact on cancer patients, I need to get advice from the people at the forefront of these efforts."

Making the *"Breath Biopsy"* a Reality

Non-invasive tests are needed in the battle against disease – could breath hold the answer?

From cancer to diabetes to the recent rise of COVID-19, we will all likely come face-to-face with a threat to our health. Contemporary medicine has ever-improving tools to identify and combat diseases, but there are gaps – in speed, in invasiveness, in coverage, in access, and in accuracy. Can we do better? And how can we ensure that new approaches provide the same level of diagnostic potential as those currently in service?

Here, we speak with Jane Hill, Associate Professor of Engineering, and Billy Boyle, CEO of Owlstone Medical, to find out how their research in the arena of breath analysis is opening new diagnostic doors.

BREATH BIOMARKERS OF INFECTION

With Jane Hill, Associate Professor of Engineering, Thayer School of Engineering at Dartmouth, USA

HOW DO YOU DIAGNOSE DISEASE USING BREATH?

Breath samples contain specific molecules (biomarkers) that can paint a picture of a patient's health. A given biomarker may demonstrate detectable changes, such as an increase or decrease, when a specific disease is present, and this relationship is established through statistically robust testing across large patient populations. Conducting diagnosis in this non-invasive and immediate way has a transformative potential for respiratory medicine in particular, and for improving patient quality of life.

Breath testing uses a number of highly sensitive techniques and specialist equipment, including secondary electro-spray ionization MS (SESI-MS), an ambient ionization technique with MS detection that facilitates the analysis of trace concentrations of vapors, and GC×GC-MS. These techniques are particularly useful in the case that a biomarker has not yet been established for a particular disease, allowing us to investigate a large range of molecules and identify subtle yet informative changes in breath composition.

HOW DID YOU COME TO WORK IN BREATH ANALYSIS?

My first introduction to the analysis of vapor came from an interest in how proteins were charged and attracted into a mass spectrometer. This led me to SESI-MS volatile analysis as a science, and developing approaches to apply to medical applications. Naturally, the most obvious application areas are respiratory infections, which are traditionally difficult to diagnose (it is hard to get a sample from the depths of the lungs) – never has this been more obvious than with the coronavirus pandemic.

Of course, COVID-19 only appeared recently, but other respiratory infections are plentiful. Tests of disease etiology mainly rely upon sputum samples and, although some tests are relatively rapid, extracting meaningful information from sputum can take weeks. Moreover, around a third of the population is unable to produce sputum, and both children and HIV patients struggle to produce lower lung samples further complicating reliable diagnoses. Many people, particularly our most vulnerable, therefore go without diagnosis, potentially leading to serious consequences, including, sadly, death. Much of our work falls under pediatric medicine for this reason; the fast detection time and non-invasive nature of a breath test offers clear advantages at the point of care. We also work in intensive care units, where we diagnose acute respiratory infections, as well as diagnosing and monitoring chronic illness and patient treatment responses.

WHAT APPLICATION AREAS ARE YOU WORKING ON RIGHT NOW?

Our focus is primarily diseases of the lung. One of our main interests is in tuberculosis, which is the biggest killer of all the infectious diseases, killing around 1.5 million people each year. Tuberculosis diagnosis is challenging for a number of reasons. For instance, up to half of patients co-infected with HIV can go undiagnosed as current tests falter when HIV is present.

We also work on other bacterial species, including related mycobacteria, that are difficult to identify with standard clinical methods. The other major area of our work is patients who have cystic fibrosis, where current diagnostics face unique challenges. Unlike the majority of other lung conditions, infections in cystic fibrosis are polymicrobial, meaning that

"Much of our work falls under pediatric medicine for this reason; the fast detection time and non-invasive nature of a breath test offers clear advantages at the point of care."

identification of the primary pathogens in the milieu of benign organisms can be challenging. Our ability to monitor changes in a patient who is currently undergoing treatment for cystic fibrosis also allows us to interrogate the effectiveness of their prescribed eradication therapies. However, the current diagnostic procedures rely on sputum samples which, with new treatments designed to clear mucous, will become harder to access. Our breath analysis circumvents this issue.

Testing for Travel: SARS-CoV-2

Could MS facilitate lung disease testing at our borders?

The COVID-19 pandemic highlights a dire need for improved testing. Today, crucial transport loci represent a key frontier in our battle to maintain health… And MS of exhaled breath could provide answers.

Researchers at Missouri S&T are developing an MS-based system for the detection of airborne biohazards at airport security checkpoints as part of the University of Missouri System's NextGen Precision Health Initiative (1). Using exhaled breath and trained on machine learning systems, the test is designed to first screen for the presence of a virus. When virus particles are detected, the breath will then be passed to an MS detector for further analysis to discern what type of virus it is – all taking place in less than a minute. Their target? Differentiation of cold, flu, and coronavirus.

Besides the obvious benefit of identifying infection and thus preventing further spread, the test also offers the opportunity to mitigate the impact of canceled flights in pandemic scenarios, potentially saving the aviation industry billions. But the test will not be found in our airports any time soon: clinical trials are due to begin in around a year's time, with the full system expected to take significantly longer.

Reference

1. *Spencer Chin, "Airborne biohazard system screens travellers" (2020). Available at: https://bit.ly/3awwjzw*

WHAT ARE THE PRINCIPLE CHALLENGES?

The challenges associated with delivering a reliable breath test are many and tough to overcome. We've had to carefully consider every aspect of test development, from sampling techniques to clinical study design, instrumentation, and data analysis, all before we could even start to consider the choice of biomarkers that will drive the test.

We know that volatile organic compounds can be sampled quickly and non-invasively from breath. When these compounds are produced through normal metabolic processes within the body, detectable changes may indicate the presence of a specific disease. If this change is limited to a single molecule and can be verified statistically across a clinical population, you have an ideal biomarker for use in a diagnostic system. Unfortunately, such definitive biomarkers are rare in our complex biology, and we have to look more expansively at multiple changes in several detectable chemicals, which increases the necessary sophistication surrounding our studies.

AND FOR THE FUTURE?

There are a lot of infectious diseases out there – with COVID-19 being a particularly topical one... We hope to explore the opportunity to combine a number of technologies to drive COVID-19 identification, such as our breath analysis system and a nucleic acid amplification method. Viruses are traditionally harder to diagnose, and patients present with non-specific signs and symptoms due to generalized inflammation. We have had success in this area, though – we were recently able to differentiate influenza A from B using breath analysis in animal subjects. Next? Establishing the potential efficacy of this approach in humans, hopefully allowing subsequent application in triaging for coronavirus carriers.

The future of such tests will rely on clinical translation, both for diagnostic use and for tracking treatment response. Reducing these tests to handheld devices represents a major focus. The ability to rule out tuberculosis infection using a handheld device in the field would have a dramatic effect on our healthcare approach, and on our ability to contain infections. This also holds true for vulnerable groups, such as lung transplant patients and children with cystic fibrosis, for whom early diagnosis represents a top priority. There are certainly challenges to address in reducing the systems to such small devices, but we hope that these questions will be answered in time.

Owlstone Medical's ReCIVA® device in action.
Image courtesy of Owlstone Medical.

THE BUSINESS OF THE BREATH BIOPSY

With Billy Boyle, CEO, Owlstone Medical, Cambridge, UK

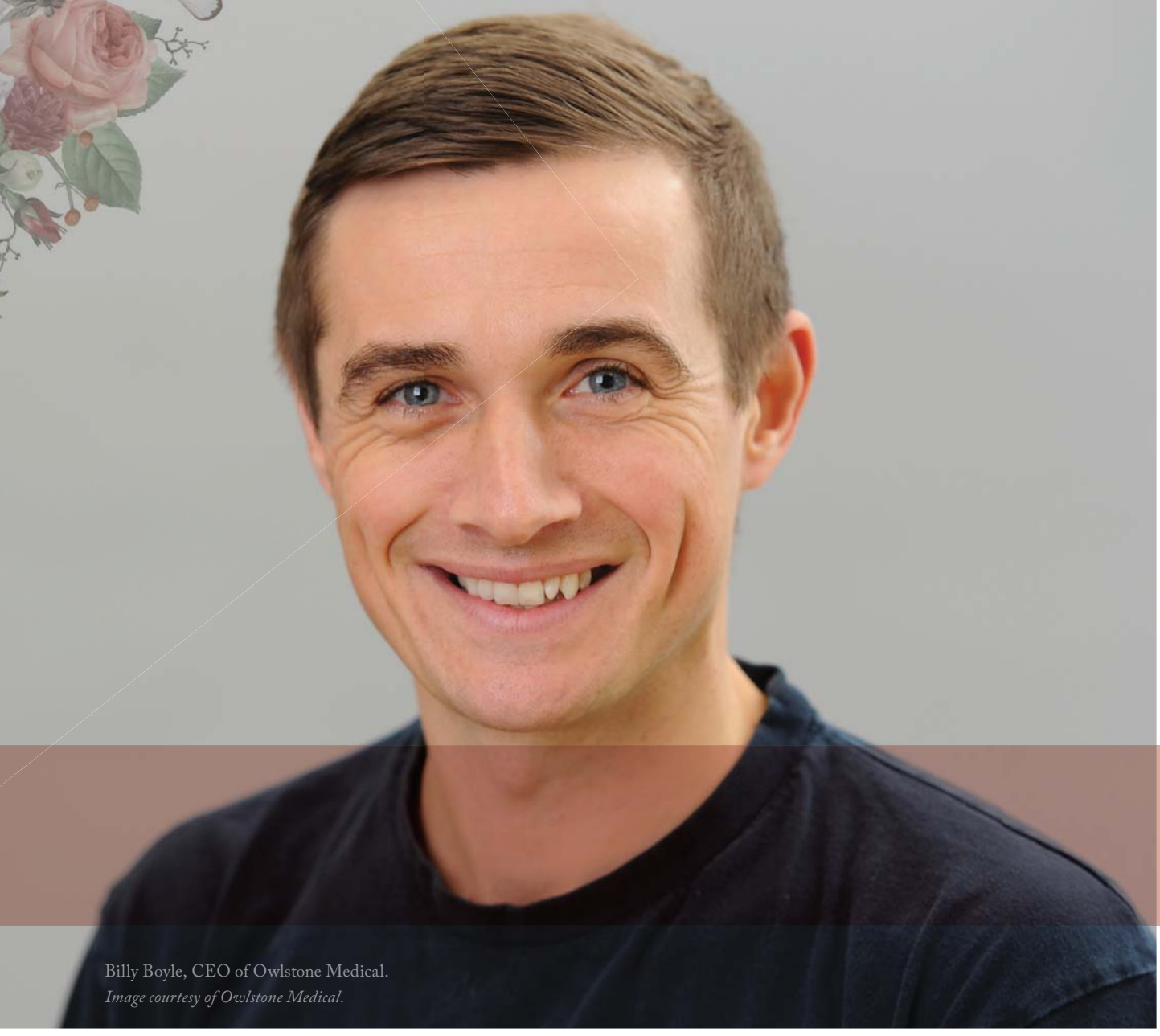
WHERE DID IT ALL BEGIN?

Growing up in Belfast, I was a typical geeky kid who loved computer programming and electronics. Engineering was an obvious choice for my undergraduate degree, and the engineering degree at Cambridge University appealed to me because you do two years of general engineering before you specialize. Plus, I loved Cambridge as a city – my brother studied there and I had happy memories of visiting him.

HOW DID YOU MAKE THE TRANSITION INTO BUSINESS?

Towards the end of my course, my director of studies offered me a summer research project on micro- and microelectromechanical systems (MEMS). I jumped at the chance, largely because I owed the college money and was at risk of not being allowed to graduate – this was a way to clear my debt!

Working with him was phenomenal. I had a great time and happily agreed to join the group for a further three years. It was through the Cambridge MEMS group that I met David Ruiz-Alonso and Andrew Koehl, who worked on superconductors and chemical sensors, respectively. We all had an interest in starting a business, and they ultimately went on to become my co-founders at Owlstone Inc. Today, I'm CEO of Owlstone Medical, which was born out of our expansion from industrial chemical detection and sensing.



Billy Boyle, CEO of Owlstone Medical.
Image courtesy of Owlstone Medical.

WHAT'S YOUR MISSION STATEMENT AT OWLSTONE MEDICAL?

Simple: to save 100,000 lives, and £1.5 billion in healthcare costs. Our vision is equally concise: to be the global leader in Breath Biopsy® for early detection and precision medicine.

HOW DID THE COMPANY BECOME WHAT IT IS TODAY?

We had wanted to focus on ubiquitous chemical sensing since the company's inception, which soon led us in the direction of instrument development, and eventually to a full-service testing model. Later, we decided to focus on the issue of cancer detection – an issue very close to my heart after my wife's cancer diagnosis. It's incredible that survival can be up to ten times more likely with early detection – it seems clear this should be an absolute focus of more research. Coupled with an increasing number of publications on volatile organic compounds (VOCs) associated with cancer, we recognized an opportunity to position our business to make a real difference for patients.

HOW DOES ONE GO ABOUT IDENTIFYING DIAGNOSTIC BIOMARKERS?

When you're faced with a question like "is there a biomarker for cancer?", there are two components you must juggle to answer

"It's incredible that [cancer] survival can be up to ten times more likely with early detection."

it. First, you must discern if there is an actual underlying biomarker – some kind of altered metabolism linked to a plausible biological rationale – that can be detected. The next consideration is how these markers can be monitored, if they do exist. We opted to volatilize the metabolome in exhaled breath.

A number of specialists collaborate to answer these questions. The breath omics community (comprising data scientists, biologists, metabolomics experts and so on) is mostly interested in the analytical side of things, while clinicians identify a clinical need and work backwards to the analytical approach needed to fulfil it. An engineering mentality bridges these approaches by asking the right questions at the right time. That's one of the truly exciting components of building our diagnostic platform and business – bringing together an interdisciplinary team that covers discovery capability and speedy biomarker identification.

TALK US THROUGH THE DEVELOPMENT OF YOUR "BREATH BIOPSY" PLATFORM…

The platform consists of two streams, one for when we know what chemicals we are looking for, and one for when we do not. For biomarker discovery, we use GC-MS to separate and identify VOCs, but development is ongoing and associated with many challenges. First, the VOCs reported previously in literature demonstrated little agreement between them – many of these results also come from pilot studies, lacking validation in an independent cohort. Second, there is the technical challenge of collecting a high-quality, reproducible breath sample. We are tackling the former through a robust discovery workflow. The latter I'm pleased to say we've ticked off the list with a specialized collection device – the ReCIVA® Breath Sampler. Development of the sampler alone relied on investment of around £2 million. When we do know what chemicals we are looking for, for example, once biomarkers

A Broader Look at Breath Diagnostics

A quick round up of ongoing research in breath diagnosis

Breast Cancer

Researchers at New York Medical College, USA, have employed ultra-clean breath collection balloons coupled with GC-MS or GC-surface acoustic wave detection to identify volatile organic compound (VOC) biomarkers of breast cancer (1), achieving accuracy as high as 90 percent. The team hopes that the technique could not only diagnose breast cancer patients but also stratify them into low-, intermediate- and high-risk groups, while reducing the need for mammograms.

Asthma

We have previously reported on Jef Focant's work in the asthma diagnostics space – his team applied GC-time-of-flight MS (GC-TOFMS) and GC×GC-TOFMS to validate five VOC biomarkers (2). Elsewhere, Alexander Schmidt of Christina Davis' lab at the University of California, Davis, has identified six potentially useful exhaled metabolites using LC-MS and LC-MS/MS – four of which (all prostaglandin-related compounds) were found to be specific to asthma patients (3)..

Pneumonia

Olanrewaju Lawal of the University of Manchester, UK, and team recently applied thermal desorption-GC-MS to identify novel VOC markers (2-cyclopenten-1-one, cyclopentanone, cyclopentanol, and 1-hexanol) associated with ventilator-associated pneumonia pathogens (4). The results, obtained from artificial sputum medium, require further validation in prospective patient populations, but aim towards improved point-of-care diagnostics on a pathogen-specific basis.

Reference

1. *M Phillips et al., Breast Cancer Res Treat, 170, 343 (2020). DOI: 10.1007/s10549-018-4764-4*
2. *M Hallam, "Diagnosis: Asthma", The Analytical Scientist (2019). Available at: https://bit.ly/2JJVdQI*
3. *AJ Schmidt, J Breath Res, 142, 026001 (2020). DOI: 10.1088/1752-7163/ab35b5*
4. *O Lawal et al., J Breath Res, 12, 026002 (2018). DOI: 10.1088/1752-7163/aa8efc*

Image courtesy of Owlstone Medical.

"People have been talking about breath biopsies for a long time, but we haven't witnessed any real breakthrough successes."

have been identified and validated, we are able to deploy our own proprietary analysis system based on Field Asymmetric Ion Mobility Spectrometry.

We were fortunate to receive an SPRI grant for our lung cancer studies, and that gave us the time and space to gather a team of engineers for sampler development, eventually leading to the breakthroughs that became the foundation of the aforementioned "Breath Biopsy" platform. Today, these biopsies are used to identify VOCs associated with not only lung cancer, but also colon cancer – and we have active clinical trials in both of these areas. LuCID is a multi-centre prospective trial for lung cancer screening; Phase I is complete, Phase II is ongoing. And we are currently running a patient trial called InTERCEPT, which is assessing the potential of a screening test for the early detection of colorectal cancer. We are also working with commercial and academic partners to explore the utility of Breath Biopsy in liver disease, respiratory disease, environmental exposure, and other areas.

WHY BREATH?

Because it's easy – at least for the patient! If testing is less invasive, patient compliance is increased. Compliance is a huge issue across all areas of medicine, but is particularly prevalent in tests for conditions like colon cancer, where compliance can be 50 percent or lower, or in lung cancer, where, in the United States screening guidelines are in place but where compliance

is under 4 percent. A simple breath test is, of course, much preferred to colonoscopy for example, and the underlying biology remains sound. Breath is, however, a highly dynamic sample, in which analytes can vary in their concentration by up to five orders of magnitude. Tests with high-resolution and accurate mass measurement are needed.

WHAT'S THE STATE OF BREATH DIAGNOSTICS TODAY – DO YOU THINK THIS WILL CHANGE TOMORROW?

People have been talking about breath biopsies for a long time, but we haven't witnessed any real breakthrough successes. The exhaled urea test for *Helicobacter pylori* is one example of a breath test in regular use, but this is not VOC-based. An initial success would push the field forward by creating a solid set of data that future ventures can feed off. At the moment, companies are relying largely on chemical sensors with inherent technology limitations, such as issues with sample acquisition. And that's the very issue we set out to solve first with our ReCIVA collection device.

WHAT'S NEXT?

Much of the project has evolved as we've developed an understanding of the nature of the challenge in front of us – I anticipate this will continue. One of the main tasks we face is continually reducing the chemical noise – there's a never-ending cycle of improvements that can be made regarding the quality of sample obtained. Biomarker identification will also expand in terms of both top-down and bottom-up approaches.

GURUS OF GAS CHROMATOGRAPHY

With ISCC & GC×GC 2020 sadly canceled, we invite leading gas chromatographers to discuss the current state of the art – and to make predictions for the technique's future in our "Virtual Riva Roundtable."

What is the state of GC and GC×GC today?

Giorgia: From my point of view, GC and GC×GC are both now "mature" techniques, and their growth curve is beginning to plateau. State-of-the-art GC machines are of very high quality. In particular, MS detectors are improving in performance exponentially, making GC and GC×GC coupled with MS a powerful and versatile tool in many areas. Some improvements are still necessary, or desirable, such as new column stationary phases, better inertness of connections, and more intuitive hardware, but I doubt these will have a revolutionary impact on the field.

One current downside is the still-limited acceptance of GC×GC as a routine technique. It is still seen as mainly suited for research purposes, but it is actually mature and robust enough to be used more routinely. However, the power of GC×GC is actually essential for very challenging applications, and this will hopefully help boost its acceptance and use.

Peter: I'd agree with Giorgia that GC is a rather mature technology – and I'd say it's poised to produce a profusion of compact devices with low energy consumption, mostly within the context of GC-MS. The role of MS is becoming understandably dominant, and it occupies a central role in our expanding molecular-level knowledge of the world.

Apart from the introduction of GC×GC itself, there have been many noteworthy developments, including fused-silica capillary GC, Deans Switch multidimensional GC, improved speed, introduction of new stationary phases, increased power and decreased device size (in some cases miniaturized), novel injection approaches, new detectors, and many more. I believe that GC×GC, fused-silica capillary GC and GC-MS evolution have been particularly important.

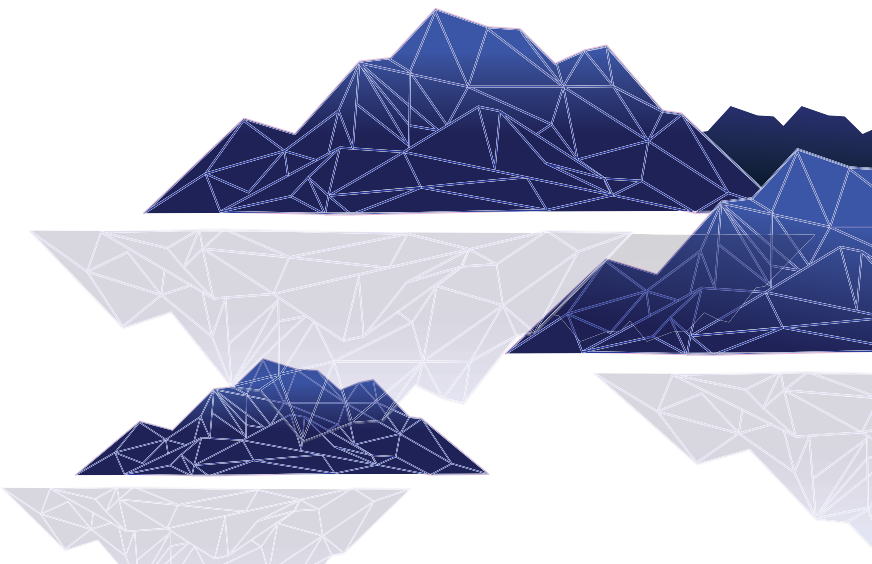
Kevin: It feels like the GC industry is currently trying to reinvent itself with more compact and user-friendly systems. Indeed, there have been exceptional developments in detector power, but front-end automated sample preparation systems are becoming more advanced and capable, as well. Now more than ever, we have more choices of different accessories and detectors to upgrade GC systems and make them more capable and more powerful.

How does GC fit into your research?

Peter: I work primarily with GC×GC – a technique that grabbed my attention from my first encounter with it. Now, having witnessed its evolution over almost two decades, I'm fortunate enough to work with five to six of these systems, using different modulators and MS detectors, to support my everyday research.

Such approaches are, of course, essential for expanding our understanding of the world we live in at a molecular level. However, focusing more closely on foods, as I do in my own research, novel sample preparation approaches and

> "The role of MS is becoming understandably dominant, and it occupies a central role in our expanding molecular-level knowledge of the world."



Meet the Gurus

Kevin Schug

My research focuses on the development of new methods for monitoring environmental samples affected by unconventional gas and oil operations, evaluating different oilfield wastewater treatment technologies, and bioanalytical approaches for clinical and pharmaceutical applications. As the Shimadzu Distinguished Professor of Analytical Chemistry in the Department of Chemistry & Biochemistry at the University of Texas Arlington (UTA), I use what I would call "cool toys" to solve these problems – gas, liquid and supercritical fluid chromatography instruments coupled with MS and vacuum ultraviolet spectroscopy, with an emphasis on online coupling and multidimensional separations.

Giorgia Purcaro

I was appointed the post of Professor of Analytical Chemistry at the Gembloux Agro Bio Tech Department of the University of Liége, Belgium, in 2018, following doctoral and postdoctoral specialization in hyphenated chromatographic techniques (under the supervision of leaders such as Phil Marriott, Luigi Mondello and Peter Tranchida), numerous international experiences, and the receipt of prestigious awards. My current research interests include the development of advanced multidimensional and comprehensive chromatography techniques, as well as miniaturized sample preparation approaches for food quality and safety applications.

Peter Tranchida

I'm an Associate Professor in Food Chemistry at the University of Messina, with a passion for advanced GC-MS methods – particularly GC×GC-MS. Analytical objectives in food analysis are highly variable, ranging from pure curiosity on food composition to evaluations of quality, authenticity and safety, making these methods essential. On the GC×GC-MS front, I've contributed to a wide range of instrumentation-, optimization- and application-based studies. In recent years, I have focused a lot on novel forms of modulation.

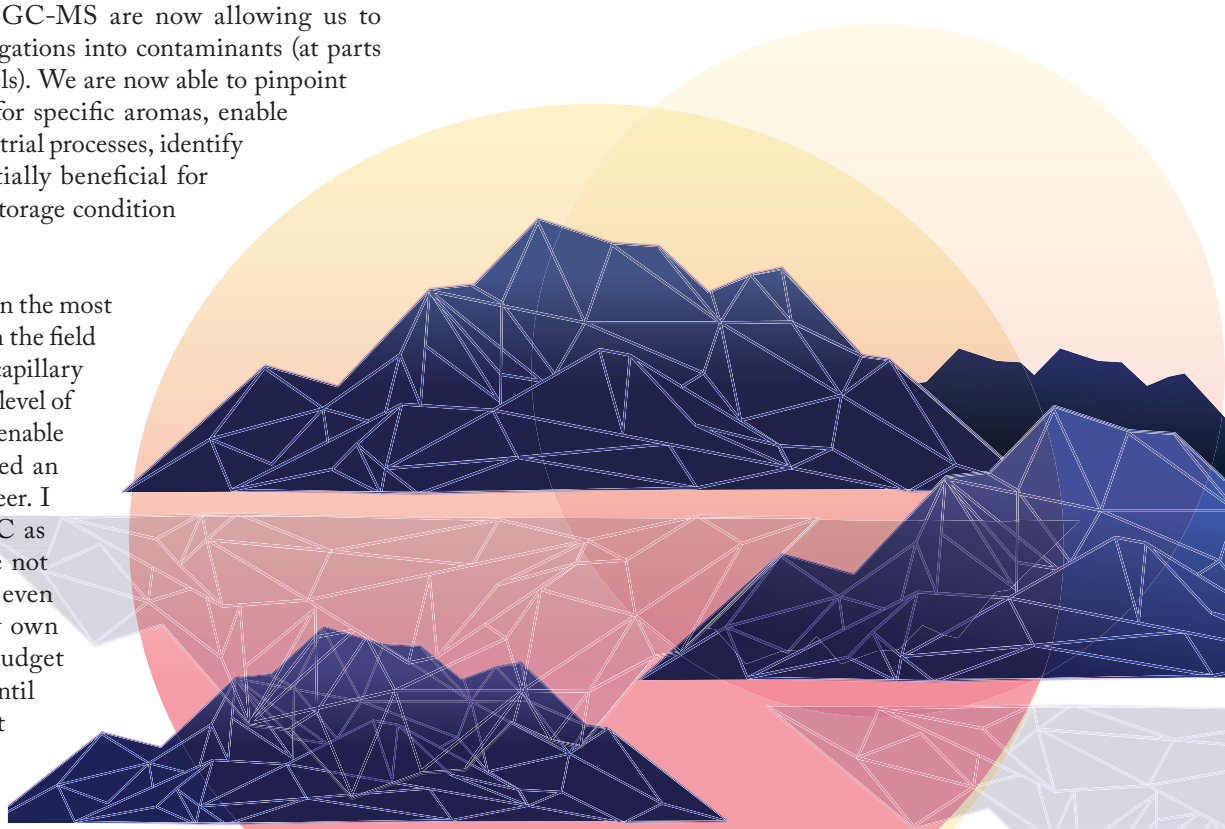
"GC×GC has been the most revolutionary invention in the field since the introduction of capillary columns, providing a new level of understanding of GC-amenable samples"

the application of GC×GC-MS are now allowing us to perform in-depth investigations into contaminants (at parts per billion or trillion levels). We are now able to pinpoint compounds responsible for specific aromas, enable fine optimization of industrial processes, identify food components potentially beneficial for human health, monitor storage condition effects, and more.

Giorgia: GC×GC has been the most revolutionary invention in the field since the introduction of capillary columns, providing a new level of understanding of GC-amenable samples. And it has played an important role in my career. I first experienced GC×GC as a PhD student and have not strayed from it since – even when I had to build my own modulator because of budget restrictions. From then until now, it's given me great satisfaction in terms of my personal career and research advancements. In fact, GC×GC has altered many paradigms in the field of food analysis.

Two main examples from my research are the application of GC×GC to fingerprinting the volatile profile of foods, opening the door to omics disciplines in this space for quality and authenticity control, and the detailed characterization of food contaminants. In the latter case, GC×GC is crucial for elucidating mineral oil saturated hydrocarbons and alkylated mineral oil aromatic hydrocarbons (MOSH and MOAH) – key food contaminants that can be differentiated only by using this technique.

Kevin: When I was a graduate student, I had the opportunity to decide whether my graduate work would focus on GC or high-performance liquid chromatography (HPLC). I chose HPLC, because my assessment at that time was that it was a much more exciting field in which to focus. Indeed, LC-MS continued to be strong and still is today. It was many years before GC became a regular part of my research activities. However, focusing on the environmental impact of unconventional oil and gas extraction in Texas provided many opportunities for methodological innovation. Today, we've developed a suite of methods for measuring water contaminants, such as the use of headspace GC and GC-MS for the monitoring of volatile



The Criticality of Sample Prep

With Elia Psillakis

In my role as Professor of Aquatic Chemistry at the School of Environmental Engineering of the Technical University of Crete, Greece, I develop new and rapid sample preparation methods to detect "emerging" organic pollutants, and monitor their fate in natural and treated water. In my lab, we do not develop new materials and extracting phases for sample preparation. Instead, we push methods to extremes, speeding up extraction and forcing analytes to take the "fast lane." To develop such methods, we first need to understand the underlying processes, so we are also very much involved in the fundamentals behind sample preparation and microextraction, in particular.

Several meaningful and practical developments have been introduced to GC in the past decades. In parallel with this (r)evolution in analytical instrumentation, sample preparation is also blooming - although perhaps unnoticed. Different sample prep technologies are mature and still far from their decay phase, meaning the science underlying them is well understood and their initial faults have been removed, or are being reduced by ongoing development. Currently, a number of sample preparation methods are in their growth phase, and we should expect to see an explosion in new techniques and their widespread use in the near future.

We all need to conduct some form of sample preparation before GC, and this creative science relies on rapid, selective, sensitive and powerful analytical tools that are practiced sustainably. Accordingly, current advances opt for the use of "green" procedures that exclude or minimize solvent use and energy consumption, as well as taking many other, similar factors into consideration.

Though we realize the criticality "of" sample preparation, there is still a way to go in realizing the criticality "in" sample preparation. If mistakes are made, we cannot backtrack and fix them, and some errors can also go unnoticed. Sample preparation looks easy but its internal workings and the fundamentals of extraction, involving natural and frequently complex samples, are much less developed and understood when compared with the physicochemically simpler systems, such as GC. Overlooking the complexity of sample preparation is critical.

Both the fundamental scientific problems and the challenges we face are complex and often require cross-disciplinary solutions. Technological developments will continue to happen but, to facilitate real progress, we need to break down the single-discipline silos of separation science and embrace trans-disciplinary collaboration. As attention focuses ever more intently on complex worldwide problems (the so-called "nexus" of challenges around food, energy, water, and the environment), it makes increasing sense for us to combine forces and address these problems.

As for the future, I wish (rather than expect) to see the approach of "open source" and "open science" in separations – and sample preparation in particular. Within the context of citizen science, I hope to see active public engagement in research, data monitoring, and collection. Until then, our community is – thankfully – more than aware of the criticality of sample preparation. This is highlighted by the EuChemS Sample Preparation Task Force, which I have the honor of leading.

and semi-volatile organic compounds in groundwater and treated oilfield wastewater. These methods have been developed within my team using combined targeted and untargeted analysis conditions – made necessary by the complexity of oil samples and tendency to use proprietary chemicals in unconventional oil and gas extraction.

> "I'd like to see more effort on automation and miniaturization of sample preparation at a reasonable cost, to match and complement the high performance achievable with modern GC instruments."

How do the International Symposium on Capillary Chromatography and the Comprehensive Two-Dimensional Gas Chromatography (ISCC & GC×GC) symposia typically support your work – and the overall field?

Giorgia: The Riva meetings are, for me, a place of inspiration and confrontation, where the most passionate and advanced scientists gather together to exchange ideas and opinions. The pleasant, cozy environment of Riva del Garda facilitates networking and professional exchanges, making these meetings unique! After every meeting, I come back to my lab with a bunch of new ideas to explore.

Kevin: The ISCC meeting in Riva del Garda, Italy was the first international conference I ever attended. It was my PhD advisor's favorite, and it quickly became mine as well. Riva is the premier international meeting for all things GC, for capillary LC, for microscale and electrically-driven separations, and even for multidimensional separations — not just GC×GC, but LC×LC, as well. One of my proudest moments was being able to introduce the scientific community to the vacuum ultra-violet (VUV) detector for GC for the first time at the 2012 Riva del Garda meeting.

Peter: I am involved in the general organization of the GC×GC Symposium. Apart from enjoying the beauty of the location and meeting with long-time friends, I usually leave Riva with an abiding admiration for the best presenters and presentations. It's a very important venue for exchanging ideas about the whole field.

Riva 2020 has sadly been canceled. What impact will this have, and how can the community respond?

Peter: The impact of the coronavirus pandemic on the economy is extreme, including the cancellation of Riva 2020. In fact, many events around the world are being postponed by a year. I would

like to think of the next "RIVA" being a greatly attended event, with an enhanced pleasure of just being together as a community and truly appreciating the wonderful location. I hope to see the general scientific community intensely engaged in avoiding a similar situation in the future, and also united in facing the environmental challenges awaiting us in the future.

Kevin: It's difficult to forecast the future of the event with this year's cancellation. The ongoing pandemic will change many things, including a large number of conference cancellations and postponements. But, we still have the US meeting in Fort Worth 2021 to look forward to, and hopefully we'll be back in Riva for 2022!

Giorgia: The cancellation of Riva is an unfortunate situation, which will have a negative impact on exchange both among scientists, and between scientists and industry. The relaxed environment of Riva del Garda has always guaranteed fruitful interactions, while the disconnect offered by leisure time outside the conference allows attendees to focus totally on networking while attending the event itself. The technology and connectivity available to us today may compensate in part for this missed opportunity, but it will not be the same. In this period of uncertainty, I think the community will act to strengthen our virtual connections until we can meet again.

What do you see in your crystal ball for GC's future, and what developments will help it get there?

Giorgia: In my opinion, two main factors continue to cause a bottleneck in the field: sample preparation and data handling. I'd like to see more effort on automation and miniaturization of sample preparation at a reasonable cost, to match and complement the high performance achievable with modern GC instruments. On the other side, data handling still remains an unavoidable hurdle. Algorithms need to be improved to make integration more robust, and, at the same time, they should provide support to extract useful information from the overwhelming amount of data generated by the multidimensional and ultra-sensitive techniques available today.

Peter: I expect further major developments in MS and sample preparation, but fewer in chromatography. Benefits will spring from the use of faster, more compact and environmentally-friendly automated machines. The work of analysts will be made much easier, along with decreasing emphasis on their role in operating the instrumentation.

Kevin: I see the merging of more advanced data science techniques and analytical measurements. Given the power and choices of front-end sample preparation, high-efficiency chromatographic separations, and back-end detection, researchers have a myriad of means to create methods to differentiate samples of interest or perform ultratrace analysis. Tools like machine learning and artificial intelligence will also become more routine in chromatography systems. GC can profile volatile and semi-volatile compounds in just about any sample; as Giorgia says, we need more and more robust and user-friendly means to process data, and I think those tools are emerging quickly.

Business

Economic drivers
Emerging trends
Business strategies

Breaking into the Separations World

Making Sepax Technologies a commercial success was anything but straightforward, but – as they say – the customer is always right

By Xueying Huang, President, Sepax Technologies, Newark, Delaware, USA

Following the end of the Great Cultural Revolution in 1976, China was a very poor country. My parents – who worked as farmers at the time – earned less than one US dollar each day. Fertilizers were essential to grow rice, but such chemicals were government-controlled and very difficult to buy. I watched my parents stand in never-ending lines from the early morning, only to return empty handed on a regular basis.

I decided that I would study chemistry so that I could produce my own fertilizer to improve life for my parents.

I arrived in the US with 60 dollars in my pocket (borrowed) in the summer of 1993 and joined Mary Wirth's group at the University of Delaware as a graduate student. Mary asked me to study organic molecular diffusion and rotation on LC surface stationary phases, using laser spectroscopy to understand mechanisms of chromatographic separation at the molecular level. Thus began my career in chromatography.

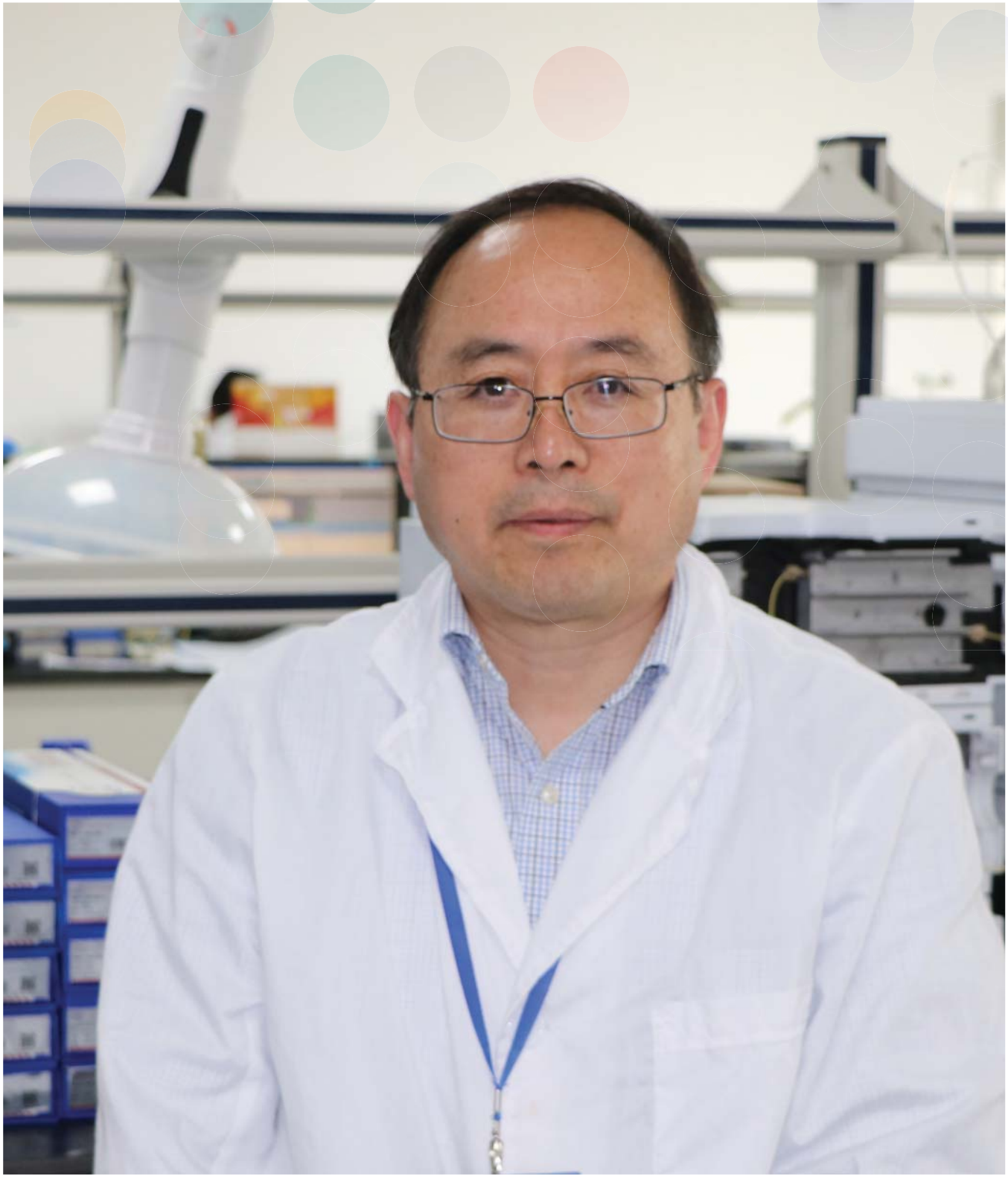
In Chinese culture, even today, parents, teachers, and the government judge a person's value based on obedience. In contrast, Mary encouraged me to adapt to Western culture, guiding me to fearlessly explore the unknown. Under her supervision, we pioneered the production of uniform polymer brushes on a solid surface with controlled chain length and density using atom transfer radical polymerization (ATRP – a living polymerization reaction that controls the length and molecular weight distribution of polymer chains) in 1996. By applying this surface technology to silica gel or polymer beads, we produced completely new stationary phases – and opened the door to a new field now followed by many groups.

A tough transition

I went on to a postdoc in Michael Fayer's group at Stanford University, where I studied the nanodomain structure of blended polymer materials with picosecond laser spectroscopy. This was all very new to me – requiring a background in theoretical calculation and chemical physics – and I soon became frustrated. I thought, "Life is short – do something you're good at!" So I did. On completing my postdoc, I slipped back into experimental research in nanomaterials at DuPont Central R&D – a company with a glorious history. They invented many revolutionary technologies, from Nylon to Teflon to Kevlar, which have all had a great impact. I filed more than 20 patents and published a number of papers during my time there, but none of the technologies I invented were commercialized. For a large corporate like Du Pont, commercialization is a big deal; smaller ideas often went under the radar. I began to worry. Does my work have value?

"In Chinese culture, even today, parents, teachers, and the government judge a person's value based on obedience. In contrast, Mary encouraged me to adapt to Western culture, guiding me to fearlessly explore the unknown."

Spring 2005 marked the beginning of my life as an entrepreneur. I decided to start up a company. But what should I do? During the time I worked at



"Reaching out to our customers by giving technical seminars and discussion sessions allowed us to understand their needs and challenges."

DuPont, it was often difficult to find a vendor who could supply a product that provided good separation of biological molecules and nanomaterials. I identified this as an area of opportunity, and started Sepax Technologies.

Now came the difficult part – the one that I had to tackle on my own. Starting my own business meant that nobody would provide my paychecks, and I had no health insurance or security. Yet I needed to pay everything: salary, rent, supplies, electricity, and so on. No matter how hard I worked, the young company was short of money to pay the employees' salaries, especially at the end of the year for the first few years, even though I wasn't paying myself at the time. There was no way that we could access a loan; the only way we could survive was to get some business from the customers. Lack of money for salaries, however, was just the beginning of the challenging journey ahead. I was encouraged by "I have fought the good fight, I have finished the race" (2 Timothy 4:7).

The customer is always right

In the early days of business, I visited most of the major US biopharmaceutical companies – Amgen, Biogen, Genentech, BMS, Pfizer, Merck, and so on. Reaching out to our customers by giving technical seminars and discussion sessions allowed us to understand their needs and challenges. I was even fortunate enough to receive good advice from acting scientists on exactly what products they needed.

This insight allowed us to rapidly launch a number of new products in quick succession from 2007 to 2009, including Nanofilm® Size Exclusion Chromatography (SEC) for membrane protein separation (1), the first 3µ SEC columns (Zenix® series), and CNT™ SEC specially for length sorting of DNA wrapped carbon nanotubes. The main benefits for us? Customer orders flooded in, solving our cash flow issues. Even more importantly though, it laid a foundation that allowed us to further target each market fragment in biopharma (such as monoclonal antibodies, , antibody-drug conjugates, bispecific antibodies, insulin, vaccines, virus-like particles, and liposomes).

Sustainable business requires solid products.

The word I heard most from customers was "consistency." LC column products are frequently used for quality control in the pharmaceutical industry, and

Business in bullets

What do we want to achieve in five years?

- Continue to focus on customer needs and challenges.
- Establish long-term relationships with key customers (treat them as business partners).
- Establish leadership and ability to influence the industry.
- Expand our business in emerging markets, biopharmaceuticals and medical diagnostics.

Key learnings

- Life is short. Do something you are good at.
- If you wish to become an entrepreneur, do not hesitate. Take action!
- Create value for customers.
- Focus on an area of technology when the business is small.
- Team building is a constant endeavor and critical to business growth.

industrial standards form a key part of these product specifications. And that underscored a move on our part to focus much more energy on lot-to-lot consistency studies rather than on new chemistry development. In 2013, we received ISO 9001 certification across all functions of the company.

Adapt and expand

Focusing on customer needs, we conduct weekly joint meetings for sales, marketing, tech support and R&D, which focus on outstanding product-related or technical issues… Otherwise known as new opportunities! This philosophy is the driving force behind development of our new products – effectively forming a bridge between customer needs and new business through a mixture of serendipity and good old-fashioned curiosity.

As an example, chemists often don't talk to medical professionals, but our opportunity program allowed us to identify a need for high-resolution LC columns to separate glycated hemoglobin (HbA1c) and its multiple charge variants in diabetes testing. In response, we developed a high-resolution ion-exchange column able to separate HbA0, HbA1c, Pre-HbA1c, HbA1a1, HbA1a2, HbA1b, and HbF with incredible precision to meet this demand: the GlyHb™.

Customers later got in touch to request a faster analysis time – down to 1 minute from 3 minutes. Of course, we developed a new column product to meet this need. We then developed appropriate reagents, then instruments, then a total solution for HbA1c test in blood samples. In short, we had developed a totally new business in the field of in vitro diagnostics.

The story today

Started in 2005 in Newark, Delaware, Sepax Technologies is today a fast-growing technology company and an emerging leader in the biological separation industry. In short, our main achievements are as follows:

- Developed and commercialized more than more than 1000 LC column products and 100 types of bulk media for process chromatography.
- Over 30 patents on LC materials and processes, owning central IP.
- Over 10 trademarks.
- ISO 9001:2015 quality management system.
- Over 10,000 customers worldwide, many at renowned companies.
- 170 employees worldwide.
- Three business platforms: analytical, process, and medical.
- Over 800 publications and references using our products.
- Established global R&D, production supply chain and sales and distribution channels.

"In short, we had developed a totally new business in the field of in vitro diagnostics."

With innovations heavily focused on chromatography separation materials, we have acted as a leading provider of HPLC columns for bioanalytical solutions, bulk media for process purification, and medical diagnostics. Founded in 2009, Suzhou Sepax Technologies focuses on the emerging market of China. Suzhou Sepax's 26,000 m^2 state-of-the-art facility serves as the R&D, manufacturing, sales and marketing center for the rapidly growing China market, with an additional 15,000 m^2 bulk resin manufacturing facility for the global process chromatography market – meaning we look forward to an exciting future ahead.

Reference

1. *CB Roth et al., "Stabilization of the β2-adrenergic Receptor 4–3–5 Helix Interface by Mutagenesis of Glu-1223.41, A Critical Residue in GPCR Structure", J Mol Bio, 376, 1305. DOI: 10.1016/j.jmb.2007.12.02*

NTS: an Analytical Goldmine

Non-targeted screening takes us outside of the box to present numerous opportunities – and offer untold rewards. Here's how we're increasing access to the challenging hardware and software workflows required.

By Stefan Bieber, Co-founder of The Analytical Research Institute for Non-Target Screening (AFIN-TS GmbH), Augsburg, Germany

My interest in non-targeted screening (NTS) began very early in my academic career. I completed my undergraduate thesis as part of Thomas Letzel's research group at the Technical University of Munich; they had been using NTS for some years before, and had considerable experience in the field. It was there I saw the potential to apply NTS in many different research projects, such as red wine (1), beer or waste- and surface waters (2) – and also realized how much I enjoyed sharing my analytical expertise with others. Combining a passion for NTS with the joy of teaching seemed, to me, the perfect opportunity.

A holistic solution

At the time, many labs and institutions wanted to implement NTS, but they did not have the time or resources to establish their own workflows. Together, Thomas and I created a comprehensive business concept, covering knowledge transfer and support for advanced analytical topics, including polarity-extended separations like supercritical fluid chromatography (SFC), as well as education in basic analytic topics (GC- and LC-MS, for example) for non-academic employees in analytical labs.

Several years later, we cofounded AFIN-TS, which provides services to numerous organizations: instrument vendors, separation phase vendors, analytical companies, software engineers, and educational facilities in numerous fields. Our philosophy is simple – to provide a comprehensive and holistic solution to consumer needs, not only restricted to NTS.

To achieve this, we combine broad and efficient analytical techniques with robust and reproducible data evaluation workflows. We use polarity-extended separation techniques to isolate non-polar to very polar compounds in one run, and couple these with different ionization techniques and sensitive MS – all optimized through chemometrics.

> *"Combining a passion for NTS with the joy of teaching seemed, to me, the perfect opportunity."*

The subsequent data evaluation is then expressed in a tailored workflow – which is important, because data evaluation is both time-consuming and a significant source of potential error.

Many tools are available for data evaluation, but none can solve every

Mass Spectrometric Non-Targeted Screening 101

NTS is an unfocused but comprehensive analytical strategy based on MS. It requires the separation of a sample and subsequent detection of separated compounds by high-resolution and accurate tandem-MS to record fragment spectra signals.

All analytical steps are adjusted to detect as many compounds as possible. Special attention must be paid to the choice and optimization of individual analytical techniques, and data evaluation is conducted using specific software tools for peak finding and feature alignment. Ideally, these tools combine all the signals produced by a compound, including isotopes and adducts. These features consist of the accurate mass of the detected compound, its retention time, and – if available – the fragment spectrum. Using representative background measurements, one can then identify sample-relevant features.

NTS has applications in nearly every analytical field: investigating the origin of red wine; identifying trace organic compounds in surface water; finding the cause of product alterations, and many more. The procedure is the same – the difference is the subsequent data evaluation strategy. NTS allows you to learn so much more about samples and their background, and to work – as they say – "outside of the box."

problem – not yet, at least. We like the idea of combining existing tools to create a comprehensive data evaluation system that is fit for purpose; the online NTS platform FOR-IDENT represents significant movement towards the realization of this concept, and we are now extending it further. Initially, FOR-IDENT was just applicable to LC- and SFC-MS-derived data, but – thanks to our partners – we have been able to eliminate this restriction. We've adjusted our data evaluation workflows and strategies accordingly, and launched last year our ready-to-use GC-Soft-Ionization-MS NTS system with comprehensive data evaluation support, which we continue marketing now.

"Our mission? To increase the applicability and use of NTS, and to transfer the required analytical knowledge in an effective and sustainable way."

What makes a business tick?

Of course, there are many important things to consider when evaluating what makes a business successful. In addition to a well-thought-out business concept and competent partners, you must also be willing to learn a lot of new things. We offered a broad range of services from the start, because we would never have been able to pay our bills with

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Cannabinoid Quantitation via Rugged and Adaptable HPLC/UHPLC Method Development

April 23, 2020, 12pm EDT / 9am PDT / 5pm BST

Potency testing demands in the cannabis industry have exploded and are continuing to expand in product formulations, applications, and plant cultivations. Of the hundreds of known cannabinoid molecules, six have been of primary interest to quantitate (THC, THC-A, CBD, CBD-A, CBG, and CBG-A). With various regulations, such as the Farm Bill, distinguishing between Marijuana and Hemp through levels of intoxicating cannabinoids, specific and accurate quantitation of these cannabinoids is ever more critical and even more challenging with a rising number and level of minor cannabinoids potentially present from chemical reactions or novel cultivars. Along with the sheer number of potentially interfering cannabinoids, their chromatographic profiles are a challenge to maintain as their retention profiles can have significant shifts from minute changes in common chromatographic conditions. Here we'll cover the key underlying chromatographic factors to ensure robust and rugged potency method development that can be adapted readily to accommodate the dynamic landscape.

Learning Objectives

- Mobile phase factors applied to method accuracy and ruggedness.
- Instrument and method transfer considerations in robustness.
- Critical cannabinoids resolved chromatographically.

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FOR-IDENT

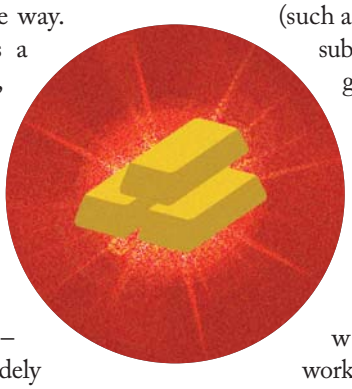
FOR-IDENT (water.for-ident.org) is an online software platform for MS NTS applications that was created as part of a research project funded by the German Ministry of Education and Research. It combines compound databases and data evaluation tools in powerful NTS workflows. The databases contain anthropogenic (water relevant) compounds (STOFF-IDENT) or biogenic compounds originating from plants (PLANT-IDENT). Users can upload their NTS data to FOR-IDENT and apply the integrated workflows, using MS, MS/MS and retention time information for the identification of candidate molecules. This open-access approach ensures that companies, authorities and scientific institutions can access reproducible and reliable NTS workflows free of charge and without any barriers.

NTS alone. However, the combination of NTS consulting, seminars, training and LC or SFC method development services – as well as the support we offered in general analytics and molecule identification – has allowed us to move forward.

When starting any business, the most important consideration is funding. As a consulting company, we do not fit the description of a typical start-up; we were not marketing scalable products with mappable growth potential. Thus, financing our business by venture capital or business angels was almost impossible. We were fortunate to find some extraordinary partners who supported our ideas and concepts from the beginning, giving us the freedom to grow in a sustainable way and building our business step-by-step. And still, remain open to new potential partners wanting to collaborate and help evolve NTS further.

NTS for all

Our mission? To increase the applicability and use of NTS, and to transfer the required analytical knowledge in an effective and sustainable way. We see ourselves as a professional consulting, education, and research institute – and that doesn't apply only to NTS, but to a wide array of advanced analytical techniques. As a PhD student, I worked a lot with SFC – a technique that is not widely established but can be extremely powerful in NTS. To increase its usage, knowledge of the technique must be shared. There's a clear lack of skilled and trained staff in many institutions; we offer a portfolio of seminars and advanced training that we hope will go some way to countering this current lack of know-how. Demand for training is one of our success metrics – if there is no more demand for education on a particular topic, then it will have become a widely applied strategy. Our goal will have been met.



I'm very optimistic about the future of NTS generally, and our business in particular. In 2020, we are creating a framework with our partners to further extend what we offer in terms of teaching. Within the next three years, our main topics will be tailor-made NTS solutions, the programming of analytical platforms, and sustainable knowledge transfer. Long-term, our own research interests will shift towards holistic concepts, with increased integration of advanced data evaluation, such as machine learning and neural networks. There's much potential and demand for automation in data evaluation, while the need for NTS is increasingly obvious in many analytical areas, including metabolomics (3), environmental analysis, and pharma. In the next few years, however, I believe food analysis will lie at the forefront of NTS development – the community using NTS to detect food fraud and to identify food contaminants (such as non-intentionally added substances) only grows as time goes on.

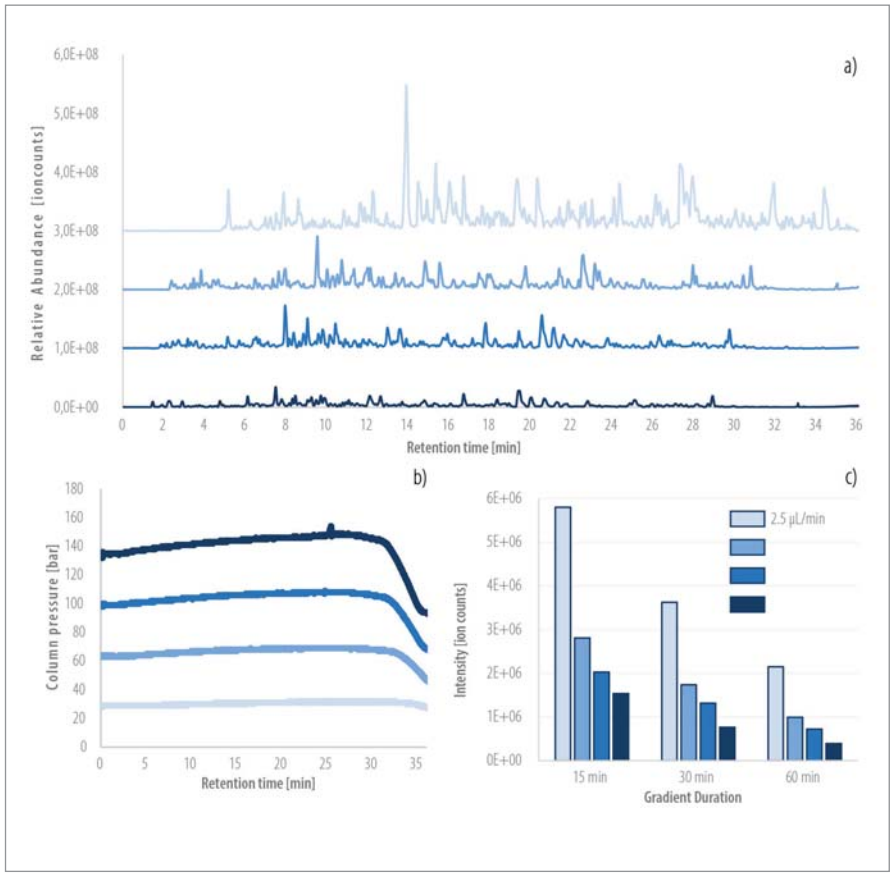
Of course, there are plenty of challenges to overcome. We will need to find ways to simplify data evaluation; combined with different tools and platforms, we must develop robust workflows. To truly master this, we need NTS users from various fields talking with MS vendors. We need to collaborate to address the challenges and develop clever solutions. So, I end with a simple message to all NTS users: let's get together!

References

1. *G Greco et al., "Serial coupling of reversed-phase and zwitterionic hydrophilic interaction LC/MS for the analysis of polar and nonpolar phenols in wine", 36, 1379 (2013). DOI: 10.1002/jssc.201200920*
2. *S Bieber et al., "RPLC-HILIC and SFC with Mass Spectrometry: Polarity-Extended Organic Molecule Screening in Environmental (Water) Samples", 89, 7907 (2017). DOI: 10.1021/acs.analchem.7b00859*
3. *R. Wahman, J. Grassmann, P. Schroeder and T. Letzel: Plant Metabolomics via RPLC-HILIC-TOF-MS. LCGC N. America and Spectroscopy supplement: Current Trends in Mass Spectrometry (2019).*

Bottom-up Proteomic Profiling Using µPAC™ CapLC Columns

Combining improved sensitivity with analytical flow robustness and throughput



In contrast to conventional LC columns that contain randomly packed beads as their stationary phase, microchip-based pillar array chromatography columns have a separation bed of perfectly ordered and freestanding pillars obtained by lithographic etching of a silicon wafer. The regular mobile phase flow pattern through these microchip pillar array columns adds very little dispersion to the overall separation, resulting in better peak resolution, sharper elution and increased sensitivity. The freestanding nature of the pillars also leads to much lower back pressure buildup, and makes it possible to operate longer columns.

As a consequence of the low column backpressure inherent to µPAC™ column backbones, chromatographic separation can be performed over a wide range of flow rates (1–15 µL/min). MS base peak chromatograms of 30-min gradient separations (500 ng HeLa protein digest standard) are described for four different flow rates (2.5, 5, 7.5 and 10 µL/min). Whereas the first eluting peptide peaks are observed at a retention time of 5 min at 2.5 µL/min, operating the column at 10 µL/min allows reducing the column void time down to 1 min, which can result in a significant increase of sample throughput for bottom-up proteomics experiments. Besides a high flow rate flexibility and low column backpressure, excellent peptide peak shapes were obtained with the µPAC™ capLC column. Based on 15 reference peptides from the Pierce™ Retention time calibration (PRTC) mixture, average peak widths measured at half maximum (FWHM) between 0.10 and 0.11 min were achieved for the 30-min gradient separations at all flow rates.

When comparing the µPAC™ capLC column to a packed bed alternative, striking differences in retention time stability were observed. Whereas an average coefficient of variation on retention times (including all 15 PRTC peptides) of 0.73 percent was observed for the packed bed column, variation in retention time was reduced almost three-fold (down to 0.26 percent CV) by working with the µPAC™ column. This highlights again the positioning of the µPAC™ capLC column as a robust and reliable alternative to the traditional packed bed capillary flow columns frequently used in high-throughput proteomics research.

Considering the overall number of identified protein and peptide groups, it becomes clear that the µPAC™ capLC column outperforms the traditional packed bed column. For a 30-min gradient at a flow rate of 7.5 µL/min, a relative gain of more than 20 percent at the protein level and more than 40 percent at the peptide level was found for the µPAC™ capLC column. But also for the other applied gradient times (15–60 min) and flow rates (2.5–10 µL/min), the PharmaFluidics µPAC™ capLC column outperforms conventional state-of-the-art columns, resulting in a higher proteome coverage (both at protein and peptide level).

Jeff Op De Beeck, Geert Van Raemdonck, Bo Claerebout, Kurt Van Mol, Natalie Van Landuyt and Paul Jacobs

Spotlight on... Technology

Robust 785 nm lasers for Raman by Cobolt

HÜBNER Photonics, a leading supplier of all laser wavelengths to Raman, introduces the Cobolt 08-NLDM 785 nm, with enhanced spectral purity. HÜBNER Photonics now offers the widest selection of Cobolt lasers for Raman spectroscopy and all with the same footprint. Run your system or experiment knowing the laser is dependable.
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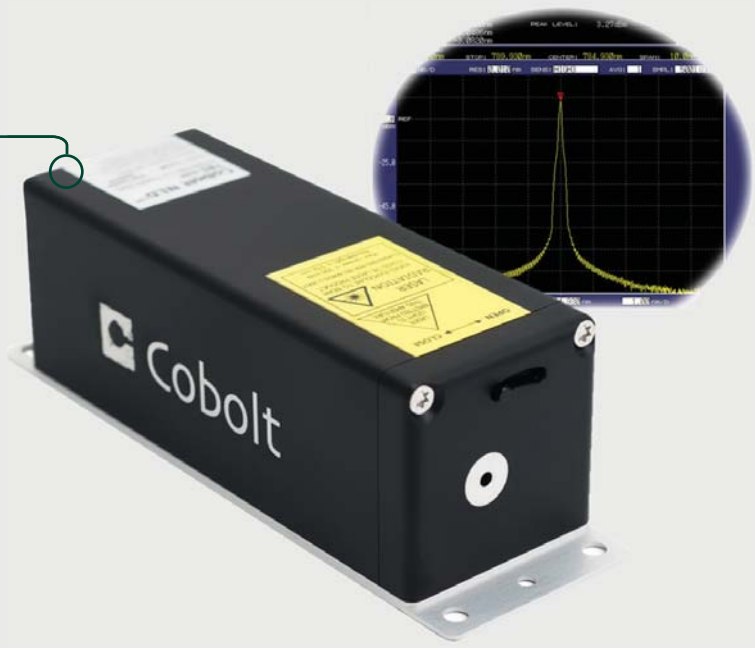

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A Comprehensive Success

Sitting Down With… Phil Marriott, Professor in the School of Chemistry, Monash University, Melbourne, Australia

What makes you tick – in science and elsewhere?

I thrive on challenges – and on supporting others. That feeds into my enjoyment of empowering analysts through formal teaching activities, journal publications and conference presentations. It also underscores my love of languages (I learned French – albeit poorly – at school, learned some Bahasa Malaysia, speak Cantonese – courtesy of my Malaysian wife – and studied Mandarin Chinese some years ago). And being a proud grandfather to my six grandchildren provides me with constant drive – and lots of books to read to them.

Though my "ability to tick" may have been somewhat reduced by a bad bike accident some years ago, I manage the issue in my stride. Unfortunately, my other great love – running – is also largely behind me, but I have competed in both triathlons and marathons. Peter Schoenmakers and I have shared this hobby for some time. I sent him a video detailing our various runs around the world to celebrate his 65th birthday in 2019!

Tell us more about your teaching activities…

I teach undergraduate students at Monash University, run a research group that has hosted seventy-five overseas researchers, and conduct GC×GC workshops across the globe (which in turn means lots of great food experiences). I enjoyed extended visits in South Korea, China and Brazil through scientific academy research exchange programmes, involving numerous lectures to keen students. Their rationale for attending? To see the capabilities of leading-edge GC×GC technologies.

An important consideration in my teaching is the broad set of GC applications present across countries; Singapore is a hub of petrochemical research, Brazil has a thriving natural product and essential oil research community, and Portugal is home to a wealth of environmental research. Despite differences, they share common issues, for example, access to multidimensional equipment.

Riva 2020 has been canceled - what's so special about the conference?

The ISCC meeting in Riva del Garda is by far the best specialist conference on capillary separation methods, and leads the way in GC innovation. You need only attend once to understand the attraction. I vividly recall the first time I attended. Especially the mountains with the chapel and church nestled part way up – and the Friday afternoon challenge of scaling the heights. Alongside great food, great science, and great people, it's a truly memorable experience.

The GC×GC Symposium (of which my workshop is a part) has been a regular feature at Riva since 2005. I'd say there has never been less than 30 attendees, and – in the past few years – this has climbed to as high as 70. This is, in part, due to Pat Sandra's input; he was instrumental in bringing the GC×GC Symposium to Riva. This provided a bona fide reason to attend for the GC community, and led to a huge spike in conference attendance. Luigi Mondello – another great GC×GC contributor – has now taken over Pat's role.

What's so special about comprehensive GC (besides the "pretty pictures")?

I'd say the "pretty pictures" are definitely part of the intrigue. I organized a "GC×GC Grand Master Exhibition" at Riva one year, highlighting researchers' GC×GC images as works of art. But the real value lies in being able to observe total sample compositions via the total volatile profile. In doping analysis, for example, this means that cheats have no place to hide – each component is displayed, with its own unique combination of retention times and MS outputs.

Resolution of potentially interfering matrix peaks allows precise quantification and qualification of analytes, and we can observe phenomena that are obscured or unapparent with GC. GC×GC is also used to screen samples when developing and characterizing new processes, acting as a sentinel method to inform which of the available GC technologies is best suited to continued analysis.

How widely is comprehensive GC used at present – do you anticipate this will change?

Almost all major GC research areas have also been investigated with GC×GC, but there is still much to investigate. Petrochemical applications are compelling due to the complex nature of the samples. In environmental analysis, GC×GC is effective in the sensitive and selective detection of pesticides, polychlorinated biphenyls, and dioxins. I may be biased, but I'd say that GC×GC could support at least one stage of the analysis cycle in any area of volatile compound research.

In the future, I expect to see applications for GC×GC in metabolomics, and further complex samples like essential oils. There is work yet to be done, however, to improve metabolite coverage for such samples. GC doesn't display compounds that don't resolve or are present in small amounts, and teasing them from the matrix is a devilish task. GC×GC might – or should – resolve them more effectively, especially in the case of minor compounds that are swamped by major analytes. MS structure identification also requires improvement, but this can be very difficult if spectra are not sufficiently specific.

What will it take for comprehensive GC to really take off?

The $64,000 question! A summit of users, researchers, manufacturers and regulators may help to recognize barriers to adoption, but more extensive standardized methods, improved reproducibility, enhanced user training, and employer commitment to the technique will also be necessary. We also need to standardize nomenclature and reduce costs; this will require across-the-board collaboration from industry, regulators and researchers.

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3 simple steps from data to answers

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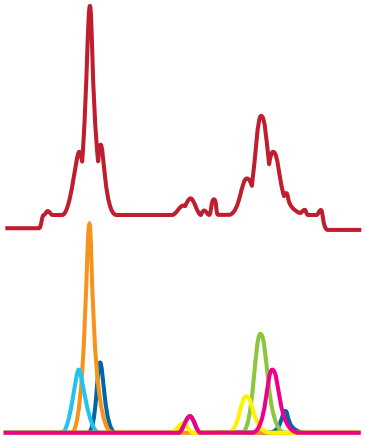
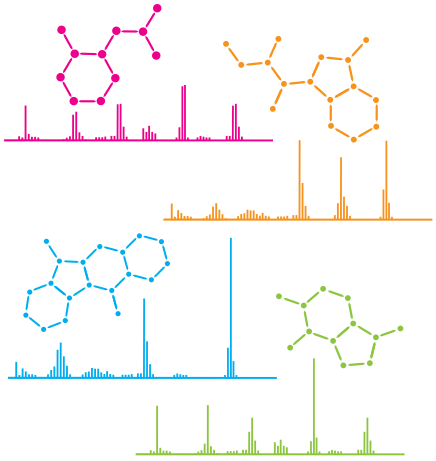
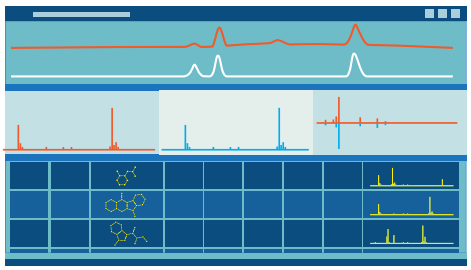


Automatically extract and separate components



Search commercial and proprietary libraries to quickly identify structures



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