MAY 2018 # 64

the Analytical Scientist™

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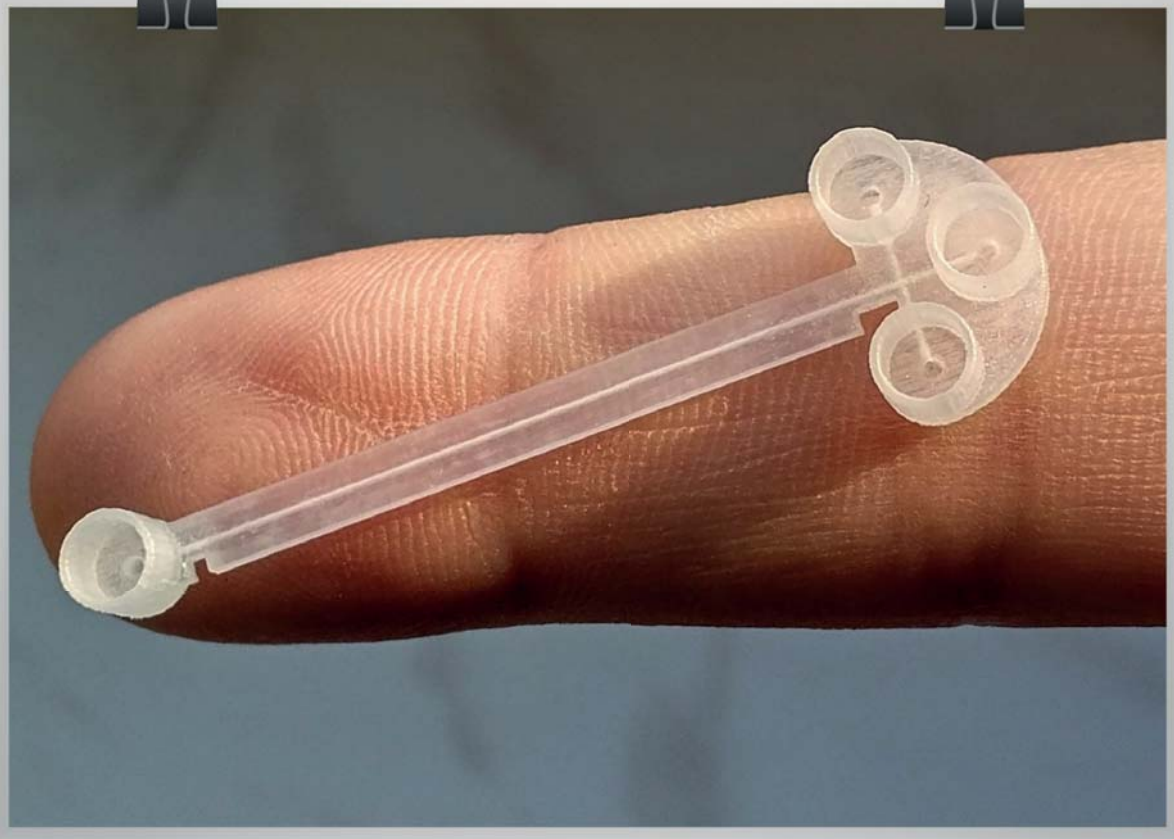
Image of the Month



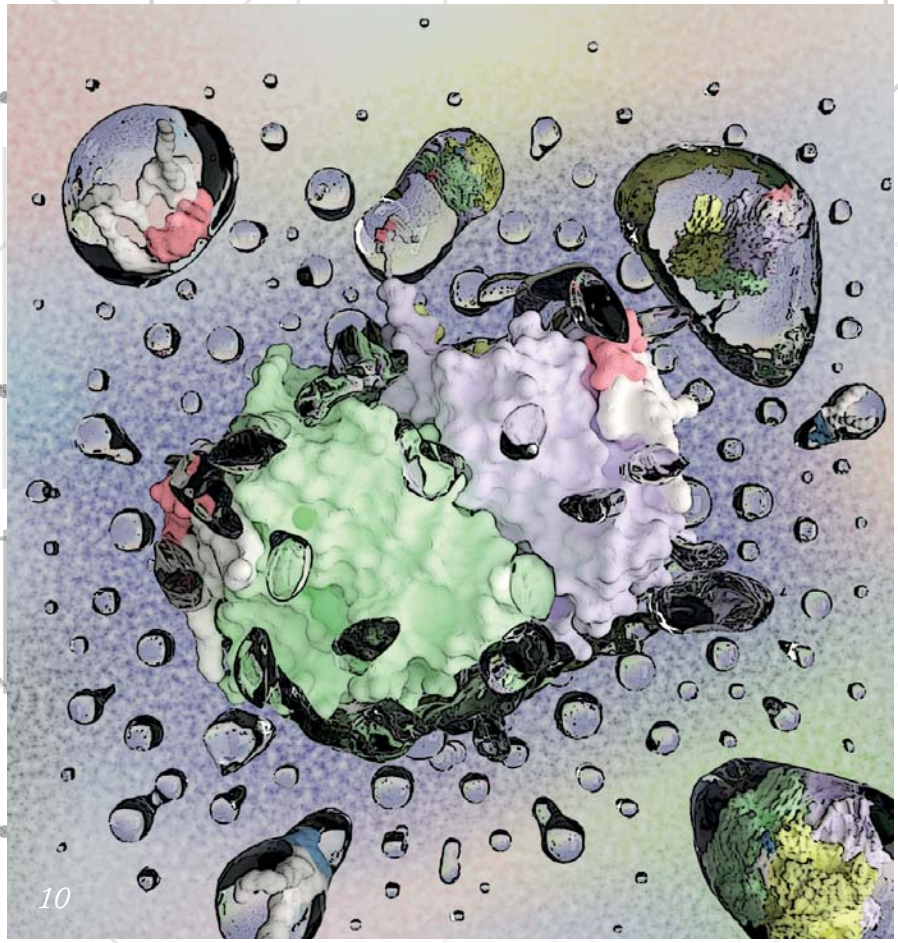
Cheap as Chips

Behold: the first ever (working) capillary gel electrophoresis chip made using high-definition inkjet 3D printing. The chip, produced by a team from the Wrocław University of Science and Technology, is capable of separating a 50–800 bp DNA ladder, and its transparency allows it to be used for laser-induced fluorescence detection. While less sensitive than those made with conventional fabrication techniques, the chip is up to 40 times cheaper, and takes hours – rather than days – to produce.

Reference: R Walczak et al., "Inkjet 3D printed chip for capillary gel electrophoresis", Sens Actuator B-Chem, 261, 474–480 (2018). Image credit: Krzysztof Adamski

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

Contents



On The Cover

A collection of images from the Maastricht MultiModal Molecular Imaging Institute.

Upfront

the Analytical Scientist

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All for (N=)One

Coming together for individualized medicine

Though our content is as diverse as ever, there is a distinct strand running through this month's issue: personalized medicine. Our trio of features all touch on tailoring therapies to patients – the promise of mass spectrometry imaging (page 22), the problems posed by inconsistent sample collection and preparation (page 32), and working towards clinical trials with N=1 (page 40).

The theme continues in the In My View section, with one doctor's view on the problem with consumer genetic tests (page 20), and concludes in our Sitting Down With interview with Kelly Zhang, Principal Scientist at targeted oncology pioneer Genentech (page 50).

Personalized (often known as precision or individualized) medicine has been a buzzword since the 1990s. But at the turn of the millennium, with the human genome project close to completion, we believed we were entering a new era of healthcare that tailored our treatment to our genomic makeup. With progress perhaps not quite as rapid as expected, President Obama launched his Precision Medicine Initiative in 2015, a 215 million dollar plan to collect genetic information from a million American volunteers to further advance personalized medicine. And there have been successes – last year the FDA made headlines with its approval of pembrolizumab based on a patient's genetic markers rather than the origin of the tumor. But doctors certainly aren't sequencing patients' genomes on a routine basis (perhaps just as well, if we're to avoid Content Director Rich Whitworth's "Gattaca"-inspired vision of the future [1]).

We now know that the relationship between genes and health is not a straight line, but rather a tangled web of proteins, metabolites and environmental factors. Consequently, personalized medicine research is moving towards a deeper understanding of the proteome and metabolome – made possible by rapid advances in mass spectrometry. The work of the Maastricht MultiModal Molecular Imaging Institute (M4I) is a perfect example.

M4I also exemplifies another core value, not just of this issue, but of this publication – cross-disciplinary collaboration. Nowhere is this more important than in translational research, where so many specialties collide. As M4I's Ron Heeren says, "Input is needed across the boundary of physics, chemistry, biology and mathematics. All of these different disciplines meet in analytical science, which is why our field lies at the base of so many great discoveries."

Are we on the cusp of a true personalized revolution in medicine? Time will tell. But if so, close collaboration between analytical scientists and clinicians will be the bedrock on which it is built.

Charlotte Barker
Editor

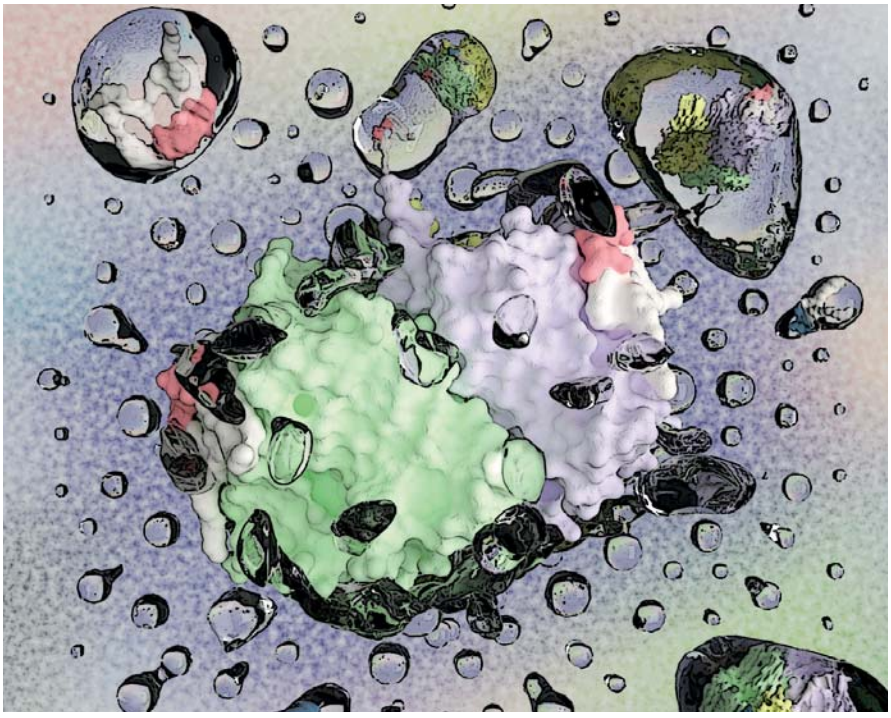
Reference

1. *R Whitworth, "G-A-T-T-A-C-A", The Analytical Scientist, 37, 7 (2016). Available at: tas.txp.to/gattaca*

Upfront

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

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



Credit: Laganowsky Laboratory, Texas A&M University

Fat Chat

Native mass spectrometry reveals how lipids and proteins communicate at cell membranes

By visualizing molecular interactions in the cell membrane with a pioneering mass spectrometry (MS) method, researchers at Texas A&M University have shown how proteins are able to recruit a specific lipid microenvironment via allostery (1). A common feedback mechanism in biology, allostery is the regulation or modulation of a biological macromolecule through the binding of an effector ligand to a binding site other than the active site.

The Texas researchers have shown that this method of communication extends to lipids. When monitoring individual lipid binding with native ion mobility MS – a technique pioneered by group leader Arthur Laganowsky that preserves non-covalent interactions – the group noted that different lipid pairs exhibit various degrees of allosteric modulation.

"It is becoming increasingly clear that membrane proteins are exquisitely sensitive to the chemistry of the lipid," said Arthur Laganowsky in a press release. "Given that lipid composition differs throughout the organs of the body, understanding how the lipid environment in these areas influences protein structure will be critical to opening new possibilities for pharmaceutical drugs designed to affect how these lipids bind with one another."

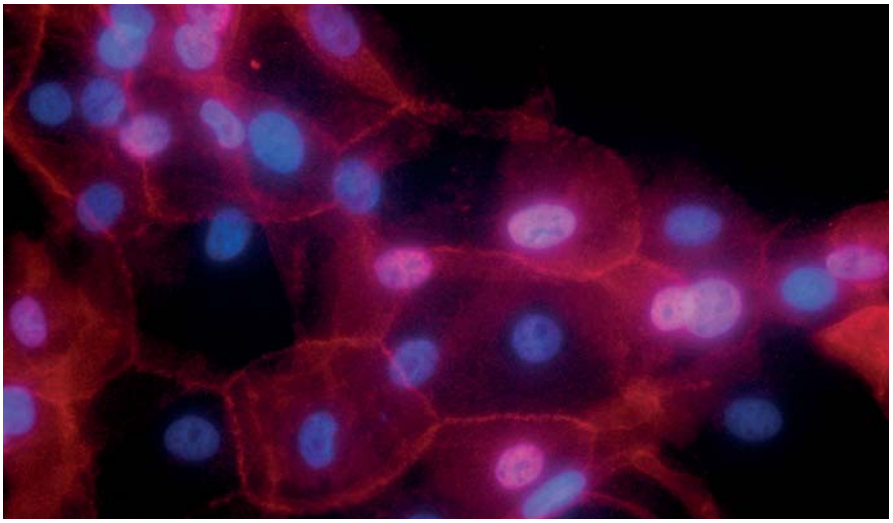
Lipid–protein interactions are generating increasing interest, as scientists uncover the key role played by lipids in the structure and function of membrane proteins; far from being inert, the lipid microenvironment of the membrane apparently plays an active part in the functioning of the cell.

Reference

1. *JW Patrick et al., "Allostery revealed within lipid binding events to membrane proteins", Proc Natl Acad Sci (2018). Available at: https://bit.ly/2HEZK46. Accessed April 30, 2018.*

The Mother of Invention

Could analysis of breast milk help diagnose pregnancy-associated cancer?



Breast cancer is the most common malignancy in women of childbearing age, and women who are pregnant or have recently given birth are particularly at risk (1). Women under 40 face lower survival rates, in part because there is no suitable screening strategy; the mammography used for breast cancer screening in older women struggles to detect changes in the denser breast tissue of younger women.

A team from Clarkson University and the University of Massachusetts set out to find a means of detecting cancer in this vulnerable group – by analyzing breast milk. "Only breast milk provides access to a large volume of breast tissue, in the form of exfoliated epithelial cells, and to the local breast environment, in the form of molecules in the milk," say the authors in their paper (2).

The team analyzed samples from eight women aged between 24 and 38 – five with a cancer diagnosis, three without. The proteins in the milk were fractionated using gel electrophoresis, before being digested using trypsin and analyzed by nanoLC-MS/MS. The data showed that levels of certain proteins differed between cancerous and control samples; for example, α1-chymotrypsin and α1-antitrypsin were upregulated, and xanthine oxidoreductase and fatty acid synthase were downregulated.

Despite the small dataset, the researchers feel the data "are supportive of the idea that molecular analysis of breast milk will identify proteins informative for early detection and accurate assessment of breast cancer risk (2)."

References

1. *Young Survival Coalition, "Breast cancer in young women: statistics and disparities", (2018). Available at: https://bit.ly/2r1P1tD. Accessed April 26, 2018.*
2. *R Aslebagh et al., "Proteomics analysis of human breast milk to assess breast cancer risk", Electrophoresis, 39, 653-665 (2018).*

Acoustic Mass Spec and Atomic Absorption

Business in brief: what's going on in analytical science?

Products and launches

Analytica 2018 saw the presentation of various new technologies, including:

- Analytik Jena's new Atomic Absorption Spectrometer.
- Microsaic Systems' latest chip-based mass spectrometer, designed for point-of-need analysis.
- SCIEX' Citrine™ MS/MS, which aims to improve on sensitivity and throughput in clinical diagnostics labs.

Collaborations and acquisitions

- A new Agilent-sponsored Measurement Suite will be housed within Imperial College London's Molecular Sciences Research Hub as part of a new partnership for research into biopharmaceuticals, energy and chemicals, food testing, materials research and proteomics.
- PerkinElmer has announced a collaboration with personalized genomics company Helix, to commercialize exome sequencing-based tests for consumer use.
- A collaboration between Labcyte Inc and Merck aims to utilize acoustic mass spectrometry (AMS) to further drug discovery and development. Labcyte's acoustic droplet ejection technology is able to move liquids at nanoliter resolution.
- Genomics company Freenome has partnered with proteomics company Biognosys to improve early-cancer detection. The CEO of Biognosys said, "Freenome's AI genomics platform is a natural fit for our next-generation proteomics technology, which supplies unbiased quantitative information about hundreds of proteins and thousands of peptides from a single sample analysis."

Company and people updates

- Central Europe's first genomics center will open later in 2018. Based at the Uzhgorod National University, Ukraine, it will be supported by the Beijing Genomics Institute, China.
- Brandon Ruotolo and Kristina Hakansson, from the University of Michigan, are the latest scientists to receive Agilent's Thought Leader Award for their work on protein complexes using mass spectrometry.
- Chris Elliott (pictured), Faculty Pro-Vice Chancellor and Founder of the Institute for Global Food Security, Queen's University Belfast, Northern Ireland, was presented with the Theophilus Redwood Award at Spring SciX last month. The award is given annually to a "leading analytical scientist who is also an outstanding communicator."

For links to original press releases, visit the online version of this article at: tas.txp.to/0518/BUSINESS. Read our interview with Chris Elliott at: tas.txp.to/0118/ELLIOTT.

Sub-Zero Substitute

MOFs could offer an alternative to freezing samples in low-income areas

In certain regions of the world, clinicians have limited (or no) ability to conduct analytical tests – but it's not always because of a lack of equipment or personnel; sometimes, it's down to the inability to implement a cold chain to transport samples. No matter how good the pathologist or technique, if a sample is not properly preserved, it may not be usable for testing – or worse, it may give false results (see page 22 to discover how worryingly widespread the problem is). A team of researchers from Washington University in St. Louis decided to tackle the gap in sample preservation, by enlisting the help of metal-organic frameworks (MOFs) (1).

"For the past few years, we have been working towards developing biodiagnostics for resource-limited settings," says Srikanth Singamaneni, Associate Professor in the university's School of Engineering. "As part of that effort, we have demonstrated the use of MOFs as protective encapsulants for preserving the functionality of antibodies conjugated to a biosensor surface. Following the successful completion of this work, we wondered if the technology could be used to protect protein biomarkers in the biospecimen, instead of antibodies on the sensor surface. And that led us to explore the use of MOFs for specimen preservation."

The team demonstrated their technique by encapsulating protein biomarkers in urine, blood, and plasma in a zeolitic imidazolate framework-8 (ZIF-8). When collected, samples need a MOF precursor added before being dried on filter paper. Recovering the protein for analysis simply involves dissociating ZIF-8 in a pH 6 buffer elution. Crucially, this final step doesn't affect protein analysis, meaning that workflows are minimally impacted.

The nanoporous MOF was able to preserve the proteins at both room temperature and 40°C in a comparable condition to samples frozen at -20°C. Singamaneni adds, "We have only explored proteins so far, but we would like to extend the technology to other biomarkers and test larger numbers of patient samples. We believe that the technique should be applicable to other biomarkers, such as nucleic acids and metabolites." He also notes that the reagents used are inexpensive and commercially available, meaning that the technique should be possible in even the most resource-limited areas.

Reference

1. *C Wang et al., "Metal-organic framework encapsulation for biospecimen preservation", Chem Mater, 30, 1291–1300 (2018).*

A Scientist Walks into a Bar…

And gives a presentation – as part of a global festival that invites scientists to local bars to discuss their latest research with the public over a pint

Pint of Science is a global science festival that aims to deliver "interesting and relevant talks on the latest science research in an accessible format to the public". Generally, it involves scientists giving talks at bars and pubs. Talks may focus on a variety of topics including neuroscience, medicine, geosciences, biotechnology, robotics, politics, and more.

After talking to friends, researchers Michael Motskin and Praveen Paul realized how little the general public knows about the fascinating research happening right under their noses. "Although they read/hear about science almost every day, science is often lost in translation in social and mass media. This causes people to lose faith in science because they feel that their interests or what they hear or read doesn't match reality," says Motskin. "We felt that the best way to tackle this was by connecting people to the source; to the scientists who work for the love of science, without

filters and without interpretation."

While at Imperial College London, Motskin and Paul organized an event called "Meet the Researchers" where people affected by Parkinson's, Alzheimer's, motor neurone disease and multiple sclerosis could visit their labs to see what research was being conducted. It was a big success, so they decided to branch out by taking scientists out to people in local bars to make science more accessible. Pint of Science was founded in October 2012.

"Scientists are very passionate people who love to chat about their research. The opportunity to present their research to people is one of the only occasions when they realize how impactful their work is," says Paul. "Pint of Science allows people direct access to inspiring scientists and encourages open discussion, all in the most familiar of places, the pub! Scientists drink pints too – they really aren't that different! Meanwhile, attendees have a fantastic opportunity to find out about interesting research – and get a window into how complex and challenging scientific research really is."

The festival is also very rewarding for the speakers. "Pint of Science is a fantastic opportunity to share my research with a diverse audience. For an increasing number of scientists, bringing cutting-edge research to the wider public and sharing with them our fascination with the world around us is something that gives as much pleasure as doing research itself," says returning Pint of Science participator, Jim Al-Khalili – a theoretical physicist. "The informal setting also means this is true public engagement – and the often quite challenging questions certainly keep me on my toes!"

The festival will take place May 14-16 in 300 cities across 21 countries. "We really didn't expect this festival to go viral across the globe," says co- Motskin. "It was a pet project that simply got out of hand. Our first festival in 2013 was held in just three cities, but it attracted so much attention that people emailed us asking (somewhat angrily) why it wasn't in their local city. We then started to receive emails from organizers and friends who wanted to spread the festival all over the world. Interestingly, the largest Pint of Science is now in Brazil, taking place in 60 cities (with many more asking to join)."

Take a look at the website for a full list of cities and events: https://pintofscience.com/.

Pretty Risky?

When it comes to potential health hazards in cosmetics, GC-MS comes to the rescue

What?

A group from the Guangzhou Quality Supervision and Testing Institute in China has successfully developed an analytical method to determine and quantify prohibited glycol ethers and acetates in cosmetic products.

Why?

Thought to contribute to health conditions, such as fetal toxicity and testicular deformity, the inclusion of glycol ethers and their acetates has been restricted in many countries for almost a decade, but are suspected to be still lurking in our beauty products. Although research has previously been conducted on environmental samples and food, very few have focused on cosmetics – though there are rising concerns about the health risks of chemicals in cosmetics since absorption can occur so easily through the skin (2).

How?

The analysis was performed on spiked samples using GC-MS. The analytes showed a linear relationship in the range of 0.05–25 mg/L with determination coefficients larger than 0.9987; limits of detection and quantification were in the range of 0.09–0.59 and 0.31–1.95 mg/kg, respectively.

So when it comes to detecting nasties in our nail polish, GC-MS is up to the task.

References

1. *J Huang et al., "Simultaneous determination of glycol ethers and their acetates in cosmetics by gas chromatography with mass spectrometry", J Sep Sci, [E-pub ahead of print], (2018).*
2. *California Department of Public Health, "Glycol Ethers" (2008). Available at: https://bit.ly/2HIH17F. Last accessed: April 30, 2018.*

The Power List 2018: Top 40 Under 40

Who are the best young researchers in analytical science?

Last year we celebrated the giants of analytical chemistry across 10 diverse categories. This year, we turn our attention to the rising stars of the field, with our Top 40 Under 40.

Our inaugural Top 40 were announced back in 2014, and we're looking forward to meeting a new crop of up-and-coming researchers, nominated by you – our readers.

Do you know a young researcher making a splash? Help them get the recognition they deserve.

Your nomination will be passed to our judging panel, who will select the final Top 40.

Nominate at: tas.txp.to/powerlist2018

In My View

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

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

Contact the editors at charlotte.barker @texerepublishing.com

The Road to HPLC2018 Part VI: Continuous Evolution

The pharmaceutical industry is looking to continuous manufacturing to increase efficiency and ensure safety – can separation technology keep up?

By Todd D. Maloney, Research Advisor, Small Molecule Design and Development, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA.

This year at the 47th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC2018), there will be several lectures and a tutorial session discussing best practice for integrating process analytical technologies (PATs) for continuous manufacturing.

Generally defined as a series of unit operations where materials produced in each process step are continuously transferred to the next process step for forward processing, continuous manufacturing is considered by many to be the future of pharmaceutical manufacturing. To be successful, continuous manufacturing requires the convergence of chemical engineering, analytical and organic chemistry, and process automation, to deliver robust, flexible manufacturing platforms.

A key attribute of any continuous manufacturing process is the ability to monitor product quality throughout the process. Combinations of parametric controls and PATs are frequently deployed to enable on-line monitoring of product quality attributes in continuous processes, including simple sensors (conductivity, temperature, pH) and optical spectroscopy (IR, Raman, UV-Vis). However, as pharmaceutical and biopharmaceutical processes continue to increase in complexity, the demand for information-rich experiments with the ability to track low-level impurities is driving the integration of HPLC for on-line process monitoring.

There are clearly many challenges associated with moving HPLCs into chemical or biological processing areas, including the physical space the instrument occupies, the sampling interface to the process, data management, and connectivity between analytical and engineering control systems. ATEX-rated enclosures are required to operate analytical instruments in a production environment, and can add considerable cost and complexity towards implementing on-line HPLC. Process sampling interfaces for on-line HPLC must ensure a representative sample is collected and

"There are clearly many challenges associated with moving HPLCs into chemical or biological processing areas."

that the process is not disturbed during sampling, plus sampling interfaces require thorough understanding of sample concentration, solubility, stability, material compatibility and matrix effects, to minimize the risk of fouling the sampling interface or HPLC. Communication and control between chromatography data systems (CDS) and distributed control systems (DCS) is vital to implementing on-line HPLC. Direct communication between the CDS and DCS enables on-line analysis at any time, while transfer and visualization of chromatographic data in the DCS improves process understanding and control.

At HPLC 2018, speakers from Eli Lilly and Company and GlaxoSmithKline will highlight applications of on-line HPLC in development and commercial-scale continuous manufacturing of small molecule pharmaceuticals – with speakers from Amgen, Biogen, and Merck highlighting recent applications for enhanced process control in biopharmaceutical production. The aforementioned challenges will be front and center, as each speaker presents their approach to process sampling and connectivity between the analytical and continuous manufacturing environments. Additionally, Eli Lilly will introduce novel process sampling interfaces for on-line HPLC with automated sampling, quench, and dilution. These innovative sampling interfaces enable on-line HPLCs to operate hundreds of feet away from a process, removing the instrument from the production floor, eliminating the need for ATEX enclosures, and enabling sampling and instrument control from the process DCS.

I cannot think of a better venue than HPLC2018 for these speakers to share their success stories, and to discuss the current and future challenges of on-line HPLC in a continuous manufacturing environment. With so many of the world's separation science experts in one location, there are sure to be some intriguing conversations and insights to continue instrument innovation and integration in the continuous manufacturing environment.

HPLC 2018 takes place on 29 July to 2 August in Washington, DC.
HPLC2018.org

Closing the Gap

Looking ahead as next-generation compact spectrometers close in on their benchtop counterparts

By Cicely Rathmell & David Creasey, Wasatch Photonics, Logan, Utah, USA.

The development of diode array spectrometers in the 1990s allowed spectroscopy to escape the lab by providing instant spectra in a compact, low-cost footprint. It meant that many questions requiring basic qualitative or quantitative answers could finally be measured at the point of sampling, using UV-VIS, fluorescence, Raman, or near-infrared spectroscopy. Since then, a proliferation of companies has grown up around this technology, each adding their own unique features, wavelengths, accessories, interfaces, and detectors.

As the market has matured, many spectroscopy-based applications have been commercialized in process monitoring, materials analysis, environmental monitoring, and particularly health and safety. Handheld Raman systems are now used routinely by first responders for explosives and narcotics detection, while advanced blood oxygenation systems provide precise results to guide clinical decisions in real time.

However, although many incremental gains have been made in the performance of traditional diode array spectrometers through scientific-grade detectors, cooling, and choice of optics, their performance still falls just short of their analytical lab counterparts – and applications requiring greater measurement speed, sensitivity, signal to noise ratio (SNR), and limit of detection (LOD) remain just out of reach. This gap forces many critical industrial measurements to be made offline or in remotely located labs, resulting in lost time, materials, and product. When it comes to safety and clinical outcomes, the hours, days, or weeks required for offline analytical testing have an incalculable impact.

The traditional diode array spectrometer employs a crossed Czerny-Turner design with reflective grating and mirrors, which though compact, is prone to image aberrations that limit its resolution unless designed with f/4 or higher input. This, in turn, directly limits the amount of light that can be collected at the sample and therefore sensitivity and measurement speed. Use of larger, aspheric optics can compensate to a certain point, but increases size and cost of the spectrometer.

What we need is a new breed of spectrometers – and it appears that transmissive, low f-number spectrometers are stepping up to close

this performance gap. Transmissive spectrometers are already favored for low-light applications like Raman, in this case they provide a considerable increase in sensitivity and LOD over their predecessors.

Transmissive spectrometers sidestep the limitations on f-number, correcting for aberrations with lenses, and without an increase in footprint. Commercial transmission spectrometers designed as f/2 or lower collect 4–9× more light than an f/4 spectrometer. In addition, transmission gratings (whether fused silica or volume phase holographic) can easily offer 40 percent more efficiency than reflection gratings, with more smoothly varying efficiency profiles. The benefit of this is seen in increased sensitivity, and also reduced stray light. These advantages can be optimized with gratings designed to deliver broader bandwidths and less polarization sensitivity for better performance across wavelength and in volume.

The question still remains – how do these advances in technology translate into measurable performance? As an example: our team has developed a fluorescence system capable of measuring fluorescein down to 5 picomolar concentrations, approaching the performance of benchtop fluorimeters. In addition, in the UV-VIS, the systems enable linear absorbance calibration up to 3.7 AU at 300 nm, a range typically accessible only to much larger, more expensive benchtop systems. Either system could fit on a piece of paper.

With this step change in sensitivity and SNR, the next generation of diode array spectrometers could enable analytical lab-grade measurements to be performed in entirely new environments and applications. Ultimately, we believe they will enable faster, better decisions in the plant, the field, and the clinic, closing the gap with benchtop systems and making the analytical lab a tool for confirmation rather than decision-making.

The Truth About Personal Genetic Tests

Is direct-to-consumer testing anywhere near as useful as it appears to the public – or to science?

By Suneel Deepak Kamath, Hematology/Oncology Fellow at Northwestern Memorial Hospital, Chicago, Illinois, USA.

Direct-to-consumer genetic tests like 23andMe have evolved substantially in the last decade, faster than society's ability to comprehend their medical, scientific, and ethical implications.

The path for 23andMe has been a rocky, convoluted one as it initially struggled to balance its business interests with regulatory requirements. The company started with a much larger 250+ gene assay that, in addition to testing for genetic ancestry and lighthearted traits, such as eye color or the ability to smell asparagus, tested for BRCA genes and genes associated with alcoholism, obesity, Alzheimer's and Parkinson's. In November 2013, 23andMe was temporarily shut down by the FDA for failing to prove its assays were accurate and reliable despite numerous requests. It was a harsh but necessary move by the FDA. As any physician knows, the first questions about any assay are: how reliable it is? What are the positive and negative predictive values? Is the result clinically meaningful?

The 23andMe health testing kits were reincarnated in October 2015 with a much smaller but better validated group of tests for personal genetic health risk and carrier status, with varying clinical utility. The fun trait tests for the alcohol flush reaction or sneezing with sunlight exposure are largely the same and remain good office water cooler talk but have limited health or practical value. The carrier tests include assays for sickle cell anemia, thalassemias, and cystic fibrosis, which could be useful depending on one's ethnicity and family history.

The personal genetic health risk tests are largely of questionable clinical value. Three conditions tested for, Alzheimer's disease (APOε4), Parkinson's disease (LRRK2 and GBA), and age-related macular degeneration (CFH and ARMS2) are 100 percent nonpreventable in an asymptomatic person without these diseases. Though there are methods to slow their progression once the disease is established, these interventions do not prevent the disease from occurring in the first place. Thus, knowing your risk sooner won't help you prevent the disease and could be needlessly distressing. Despite common thinking, it can hurt to know more. Similarly, the hereditary thrombophilia tests (Factor V Leiden and prothrombin G20210A test) are of little value in someone with no personal or family history of thrombosis. The remaining three diseases tested for, celiac disease (HLA-DQ_A1 and HLA-DQ_B1), alpha-1 antitrypsin deficiency (SERPINA1) and hereditary hemochromatosis (HFE) have potential to be clinically actionable results. How often

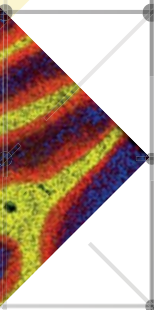
these tests detect a disease that would otherwise have been missed or detected later remains to be seen. The pervasive assumption that early diagnosis is always better isn't always true. For example, many patients with hereditary hemochromatosis don't yet have iron overload and don't benefit from early detection.

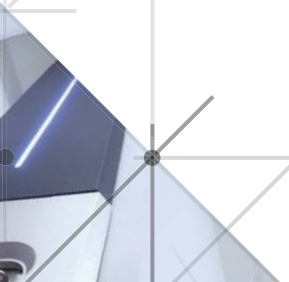
Earlier this month, the FDA authorized 23andMe to report BRCA mutations to its consumers for the first time. Given the high profile of breast cancer and BRCA mutations in the media and among the general public, I expect 23andMe kits to fly off the figurative digital shelves as a result. However, a deeper look reveals that the two BRCA1 and one BRCA2 variants tested occur most commonly in the small Ashkenazi Jewish population and are otherwise uncommon. How useful will these tests be for the general population? My greatest concern is that a negative result on a home test might dissuade women from obtaining appropriate breast cancer screening. Additionally, will women with positive results have access to affordable genetic counseling to make sense of their results? It is clear that 23andMe will profit from more consumers buying their kits to get their BRCA results – but some (perhaps even most) consumers may not benefit from this small, three-gene panel. A broader panel for a larger number of BRCA variants, on the other hand, could be instrumental in breast cancer prevention.

The future of home personal genetic testing is filled with both peril and promise. The danger lies in how much genetic data companies store and sell access to other organizations. Indeed, 23andMe shares their data with several universities, including Harvard and Stanford, companies like Pfizer and Genentech, and several Parkinson's disease nonprofits. The reports shared with consumers are a mere fraction of genetic data generated and shared with these outside organizations. If health or life insurance companies obtained the same information, it could have catastrophic financial consequences for consumers with genetic predispositions for serious or costly illnesses. Employers could also discriminate against certain job applicants based on genetic data. Government regulations currently prevent these problems, but hacking or legal maneuvering around these regulations and the informed consent process could put powerful genetic data in the wrong hands. Conversely (and much more positively), the large repository of genetic data could lead researchers to some amazing discoveries. Traditional research involves identifying patients with a disease and retrospectively looking for genetic causes of that disease. A large, population-level genetic database could help us prospectively identify subpopulations at high genetic risk for serious diseases. Pharmacogenomic data could also help us individualize drug choices and dosing to maximize efficacy and minimize toxicity.

The All of Us research program through the NIH aims to compile a large genetic database similar to that of 23andMe, but as a nonprofit, academic endeavor. Will it protect participants' genetic privacy better than for-profit companies like 23andMe?

For now, personal genetic tests are mostly cute technological novelties with limited health value. Whether they will lead to medical breakthroughs, catastrophic breaches of privacy – or both – remains to be seen.

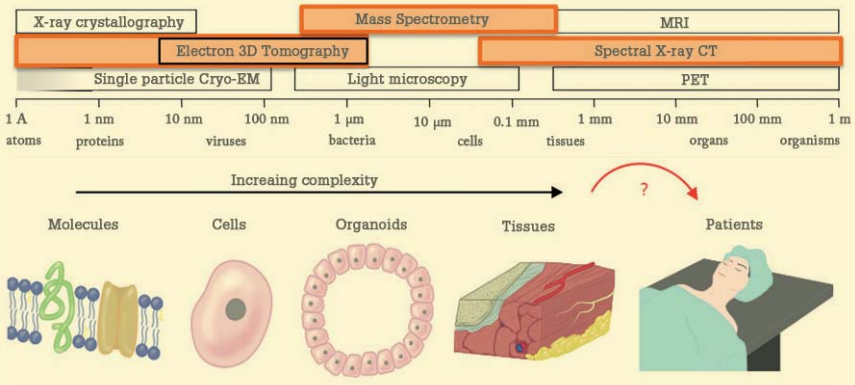




THE INSIDE STORY

The Maastricht MultiModal Molecular Imaging Institute (M4I) continues to break new ground in mass spectrometry imaging – providing doctors with better diagnostic tools, and giving us a close-up view of the complex molecular machinery that underpins health and disease.

By Ron Heeren



Breaking boundaries is a key theme of my work, and pivotal to all fields of science. The motivating force of most scientists is to break through boundaries of knowledge – seeing something that no-one has seen before is an awe-inspiring experience. Whether they are building better microscopes to visualize molecules in a cell, or better telescopes to detect far-away stars, scientists have the same drive – they want to see what they cannot yet see.

On a more down-to-earth level, breaking boundaries between disciplines is a crucial facet of our work. We need to make sure that our knowledge crosses the boundaries of our own disciplines, to answer the big questions society faces. Whether it is in life science, food, water or energy, input is needed across the boundary of physics, chemistry, biology and mathematics. All of these different disciplines meet in analytical science, which is why our field lies at the base of so many great discoveries.

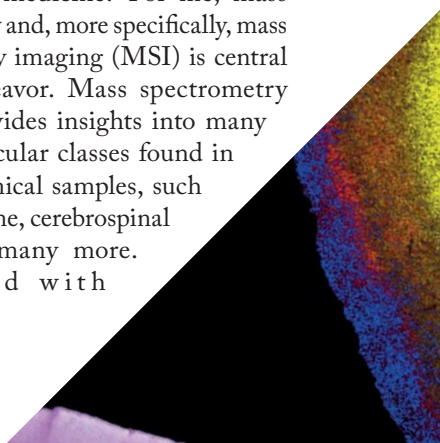
I have always been driven by curiosity; I just love figuring out how the world around me works. If the switch on my bike light stops working it's not enough to simply replace it - I want to understand the problem and try to fix it. The same curiosity that sees me dismantling my bike light also motivates my work, albeit the questions I ask are much bigger! At the Maastricht MultiModal Molecular Imaging Institute (M4I) we seek to visualize fundamental molecular processes, and apply that knowledge to improve human health.

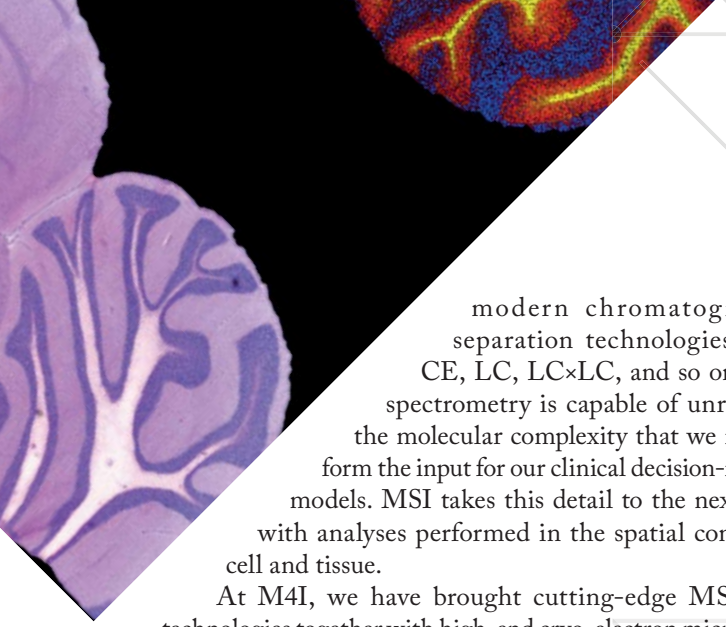
Clinicians often have very sparse information to work with – they are forced to make life-and-death decisions without all the pieces of the puzzle. It's clear to me that the future of medicine lies in clinicians gaining much more detailed information about the patient, to deliver more personalized treatment, with a better outcome. This personalized medicine – or, as scientific visionary Leroy Hood terms it, personal, predictive, preventive and participatory (P4) medicine – is where I focus my work.

To make personalized medicine a reality, we must find a way to resolve the incredible complexity of the human body and apply it in clinical decision making. And that involves gathering as much information as possible at the genome, proteome and metabolome level and coupling it to disease manifestation, treatment choices and, ultimately, patient outcome. After we gather all of this data, we can start building complex clinical decision-making models. Developments in information technology, machine learning and artificial intelligence have already started to play an increasingly important role in this process, as the sheer volume of the available personal data becomes too daunting to interpret for an individual clinician (or researcher, for that matter). Data scientists will lead clinicians, and will in turn be led by clinicians, analytical scientists and epidemiologists. It's a nice example of knowledge crossing borders to improve healthcare on many levels.

"To make personalized medicine a reality, we must find a way to resolve the incredible complexity of the human body and apply it in clinical decision making."

How do we gather the data needed by modern medicine? For me, mass spectrometry and, more specifically, mass spectrometry imaging (MSI) is central to the endeavor. Mass spectrometry already provides insights into many of the molecular classes found in complex clinical samples, such as blood, urine, cerebrospinal fluid, and many more. Combined with





modern chromatographic separation technologies (GC, CE, LC, LC×LC, and so on) mass spectrometry is capable of unraveling the molecular complexity that we need to form the input for our clinical decision-making models. MSI takes this detail to the next level, with analyses performed in the spatial context of cell and tissue.

At M4I, we have brought cutting-edge MS-based technologies together with high-end cryo-electron microscopy. In doing so, it becomes possible to image biological processes at multiple scales: a single molecule, the molecule in the context of a cell, the cells in context of diseased and healthy tissue, and that tissue in the context of the patient's biological system.

Mass effort

My group at M4I is pushing the boundaries of spatial resolution in MSI, including developing new tools to resolve molecular structures that have so far proved elusive. We are currently working on combining ion chemistry with MSI to apply imaging in a completely new way (see page 28) – if successful, this could result in a paradigm shift for the analytical application of mass spectrometry in structural biology.

Throughput is another key area for us. MSI provides orders of magnitude more detailed information for clinical diagnostics than conventional imaging techniques, but the information needs to be available to clinicians quickly. A diagnostic approach that takes hours to complete will not be adopted easily. We are working with the main MS vendors to deliver MSI-based tissue diagnoses to surgeons, pathologists and other healthcare professionals in a matter of minutes (see page 30), which will facilitate the translation of our work into personalized medicine, ultimately reducing diagnostic and treatment costs.

Whether it's fundamental research, instrument development or clinical translation, an important bottleneck is our ability to deal with the ongoing data tsunami. We need innovative data sciences and bioinformatics to digest the data as rapidly as we can now generate it (see page 31)

I believe that the team at M4I has the vision and drive to help move healthcare forward (read more about the work of some of our 'rising stars' in the following sections). But to achieve our goals, it's crucial that our research is embedded in the clinic – for example, our collaboration with nearby academic hospital, MUMC+. M4I has developed several MS-based translational medicine projects together with the MUMC+ Department of General Surgery and Pathology. After three years of building bridges, these projects are now beginning to come to fruition – not only scientifically but also clinically, with the birth of several novel diagnostic assays that are now being validated for patient care. This type of interdisciplinary research is something I am passionate about – it is absolutely crucial to advancing MSI, and science as a whole.

A beautiful example is the recently published (1) result of a collaboration between M4I and Steven Olde-Damink's group at MUMC+ on non-alcoholic steato-hepatitis (NASH). Using a mass spectrometry-based tissue imaging approach we established a novel classification model for NASH. Importantly, we found that this classification was only possible when the spatial context of the molecules was taken into account; we could not establish the classifier on a tissue homogenate in which the spatial context was lost. This study is a prime example of why MSI is needed for modern medicine.

We are entering a new era of digital pathology, and MSI fits

Mass Spectrometry Imaging 101

By Shane Ellis, Assistant Professor at M4I.

Mass spectrometry imaging is a molecular imaging technique that exploits a unique feature of every molecule – its weight. By measuring the weight (mass-to-charge ratio) of all the molecules from a small region of a sample we get can determine the spatial locations and concentrations of molecules present in the sample. By sampling many points on a sample we can build detailed images (ion distribution maps) of hundreds of molecules simultaneously. This allows us to see how the presence of certain molecules alters others in the surrounding environment, and how localized chemical processes vary across a complex and heterogeneous sample. MSI involves a variety of techniques, such as pulsed-UV laser irradiation (MALDI), focused ion beam irradiation (SIMS) or charged solvent droplets (DESI). Each method has strengths and weaknesses, and here at M4I we combine all three to help us find answers to complex biomolecular questions.

Read more from Shane on page 28.

seamlessly with innovative concepts in molecular pathology. In the future, a pathologist will order an MSI assay with similar ease to an immunohistological assay – and examine it on exactly the same platform – allowing diagnosis to be based on much more extensive molecular information. It also offers the combination of targeted and untargeted molecular diagnostics, which will be the true paradigm shift in personalized medicine and molecular pathology.

MSI-based molecular pathology can be combined with MS-based intraoperative diagnostics, as is being done with rapid evaporative ionization mass spectrometry (REIMS), with the "i-Knife" sampling device (see page 30). Our group has been the first to take all of our molecular imaging information and put it into models that classify tissue during a surgical procedure. We are building the molecular operating room of the future, to improve the quality of care and treatment outcomes of patients.

Scratching the surface

The potential impact of MSI is hard to overstate. Everything we experience, invent, touch, eat and use involves some form of surface chemistry. MSI can help us to better understand all of these surfaces and their chemical interactions with their environment, provided we are careful to ask the right questions and design our experiments in the best way.

The most obvious impact will be in medicine. MSI can be employed to more precisely define a tumor margin on a tissue section, determine the degree of ischemic damage in an

History of Mass Spectrometry Imaging

There are three main categories of mass spectrometry imaging (MSI): secondary ion MS, ambient MSI and laser-based MSI.

In the sixties, secondary ion mass spectrometry (SIMS) appeared as one of the first surface analysis technologies. Researchers employed energetic ion beams to generate secondary ions that would tell them something about the properties of a surface. At first, they focused on elementary surface composition, but they quickly realized that SIMS could be deployed to study surface chemistry, which piqued the interest of physical chemists. Modern SIMS instruments can study organic surfaces in unprecedented detail. Recent advances have included the implementation of gentle Ar-cluster beams that sputter surface without any organic subsurface damage, allowing us to build full three-dimensional molecular models of single cells. Equally revolutionary was the implementation of tandem mass spectrometry for structural identification, which moved the field from "pretty pictures" of individual m/z values to interpreted biological images. These technologies have found their way into application domains ranging from material sciences, catalysis, forensic sciences, semiconductor sciences, coating technology and, of course, biology and biomedicine.

Ambient MSI was developed when researchers realized that not all samples were suitable for the vacuum of a mass spectrometer. It took until early this century for a suitable ionization technology to be conceived – and desorption electrospray ionization (DESI) is still one of the main ambient imaging technologies for non-vacuum-compatible surfaces. It deploys a supersonic jet of charged droplets that impact the surface and pick up surface molecules. Like SIMS, it involves charged particles that impact a surface, but the desorption and ionization mechanisms are markedly different. The development of DESI resulted in a new field of imaging research and is used to directly study plant surfaces, hydrogels, water-containing polymers, drying paint and bacterial colonies on agar plates, to name just a few. It is also widely used in biomedical tissue imaging, as it requires little to no sample preparation.

Though SIMS and ambient techniques have been valuable, the laser-based technologies have arguably had the biggest impact on MSI. In particular, MALDI-MSI has revolutionized MS-based molecular pathology. The key advantage over the other two technologies is that MALDI-MSI can offer information on a much wider variety of compounds, ranging from metabolites, lipids, peptides, proteins and intact polymer molecules directly from complex surfaces. Even though every molecular class requires a different sample preparation protocol, the breadth of molecular coverage, even within a single class, is still unsurpassed. Spatial resolution has evolved over the

organ for transplantation, classify the severity of a disease, and so much more. The pharmaceutical sector will benefit from detailed information on local drug metabolism – researchers will be able to see if a drug reaches a target, without the need for labels that could interfere with its mode of action. Better information on local drug metabolism and pharmacokinetics (DMPK) will be crucial to innovation in drug development.

Food is another area where MSI could make a major impact. For example, we are collaborating with a local organic wine farmer to understand how his method of spraying a microbial extract on his vineyard improves the quality of his plants, his soil, and ultimately his wine. We have scanned leaves from his vineyard throughout the season with DESI-MSI to get a picture of what is happening on the surface of the plant. These type of studies will lead to innovative biological pest control methods and reduce the chemical footprint of the farmer on the environment – only one of the many ways in which MSI will contribute to sustainable agriculture.

The impact of MSI on science and society is already tremendous and can only grow. If the number of published papers is an indication of the impact of MSI, the best is yet to come! Read on to find out more about the young researchers breaking through scientific and technical barriers at M4I.

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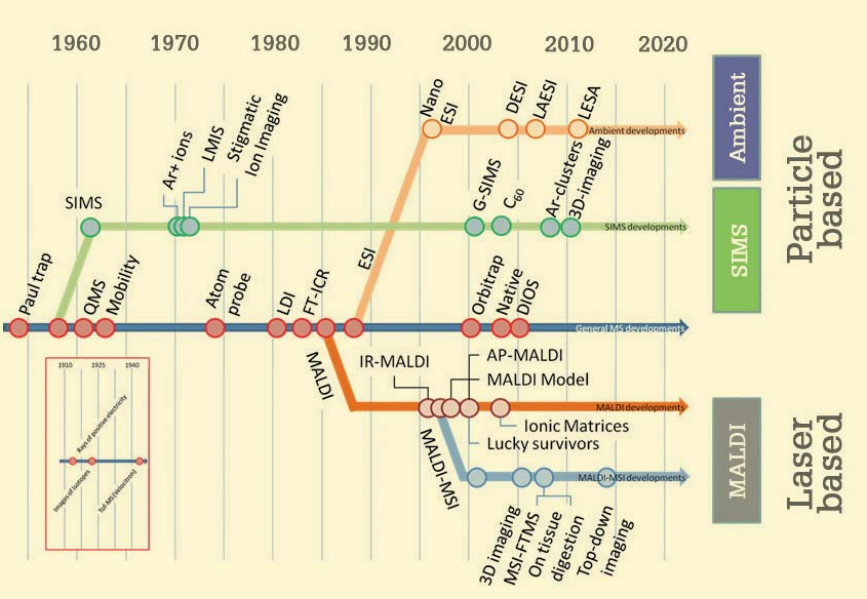
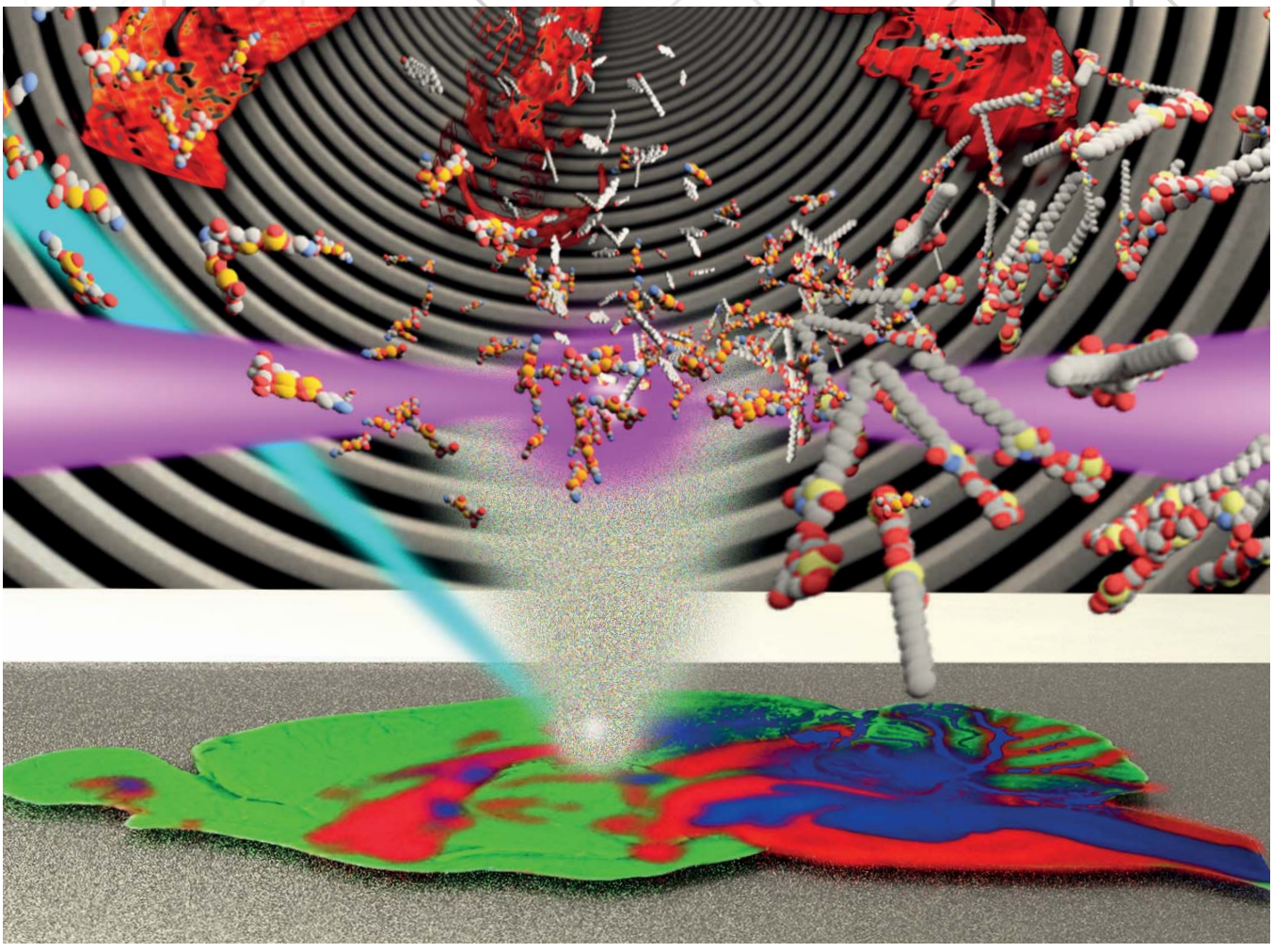
Ron Heeren is the Director of the Maastricht MultiModal Molecular Imaging Institute (M4I) and Division Head of Imaging Mass Spectrometry at Maastricht University, Maastricht, the Netherlands.

years from hundreds of micrometers to just 1–5 micrometers, making the technology compatible with morphological features of interest to pathologists.

All three types of imaging, whether particle- or photon-based, have benefitted from the technological advances in mainstream mass spectrometry. MSI can now be routinely performed on modern hybrid high-resolution instruments such as Fourier transform ion cyclotron resonance mass spectrometry and Orbitrap systems. In addition, developments in time-of-flight (ToF) mass spectrometry have provided new high-throughput approaches that allow us to screen a tissue section in 10–15 minutes, dependent on size and required spatial resolution. These two methods combined – high-throughput MS with high-resolution MS – are the cornerstones of MSI-based clinical diagnostics. At M4I we routinely use them back to back – high-throughput MALDI-ToF-MSI to screen tissues from large patient cohorts, complemented with high-resolution FT-based MSI on selected samples to identify the molecular profiles found. New methods are surfacing fast; three years from now, the MSI field will undoubtedly look very different to today. As more and more disciplines adopt (and adapt) our technologies, I believe we will move from evolutions to revolutions in the years to come.



Reproduced from: RMA Heeren, "Getting the picture: The coming of age of imaging MS", Int J Mass Spectrom, 377, 672-680 (2015).



Artist's impression of MSI using laser-induced post-ionization (MALDI-2)

The Imaging Innovator

Shane Ellis is an assistant professor in imaging innovation and structural imaging. We caught up with him to find out how M4I is pushing imaging technology to its limits – and beyond.

What is the aim of your research?

My group develops new instrumental methods and applications to improve the chemical information we can extract from imaging data. I have a strong focus on lipids, and find it fascinating that in almost every tissue studied with MSI, a heterogeneous spatial distribution of lipids is seen, and yet the underlying reasons behind these distributions are not known. More generally, very little is known about the roles of individual lipids in cell metabolism and function – I want to add to our knowledge.

How are you improving MSI?

As a chemist, I want to know exactly what molecules we are seeing in MSI, but this goal is complicated by the presence of isomers. We are combining new MS/MS methods (such as selective gas phase ion/molecule reactions) with imaging, to resolve structural isomers and discover exactly what molecules are contributing to a signal. By breaking down an image into the isomeric contributors, we can begin to understand the biochemical origin of MSI data. By combining this with the power of high-mass–resolving-power MSI using FT-based analyzers to reduce the search space for structural assignments, we would one day like to gain a cell-by-cell view of all active metabolic processes and how they are altered with disease.

We are also working on adding temporal data to MSI. With MSI, we acquire a static snapshot of a heterogeneous sample – but in reality, the molecules we detect are the result of a variety

of dynamic processes. To capture this change over time, we are infusing isotope labels into animals so that we can monitor uptake of the isotope label into biochemical processes. This means we can directly view the turnover and synthesis of new molecules, and differentiate new molecules (synthesized since label introduction) from old molecules (synthesized before label introduction). This provides a powerful and as yet largely untapped resource to image the kinetics of biochemical conversions within tissues.

Speed is also very important, especially for clinical applications of MSI. Modern time-of-flight (ToF) systems allow us to acquire data up to 20 times faster than a few years ago, but these methods are now at the physical limits of conventional ToF technology. M4I is heavily involved with the Medipix consortium at CERN, working on semi-conductor-based detectors for stigmatic imaging. With the Timepix detector and dedicated ion optics within stigmatic imaging mass spectrometers, we can acquire thousands of pixels in parallel (rather than one at a time). Instead of one detector we have 262,144 detectors, each capable of recording both ToF and impact position (1–3).

What's next?

The typically low ionization efficiencies of many molecules mean that we may only detect one in every 10,000-1,000,000 occurrences of a given molecule – this is the ultimate limitation in sensitivity. Work is ongoing at M4I and elsewhere to finally overcome this key challenge, either using targeted derivatization methods to convert certain molecules into more detectable forms (for example by adding a fixed charge), or using the MALDI-2 method, where a second laser is fired into the MALDI plume. Pioneering work by the University of Muenster (4) and later by M4I (5) has shown this method can enable up to two orders of magnitude greater sensitivity for certain molecules and significantly improves the depth of molecular coverage for an MSI experiment (see Figure 1).

I also think the use of MS/MS methods (both conventional and new variants) will continue to gain momentum, moving towards true molecular identification of signals observed in MSI. High mass resolution has been a huge advance, but ultimately this provides little structural information beyond elemental composition. For structure determination, MS/MS is needed. The challenge is that MS/MS is typically a targeted approach, and it's not yet clear how best to combine this with the untargeted nature of MSI.

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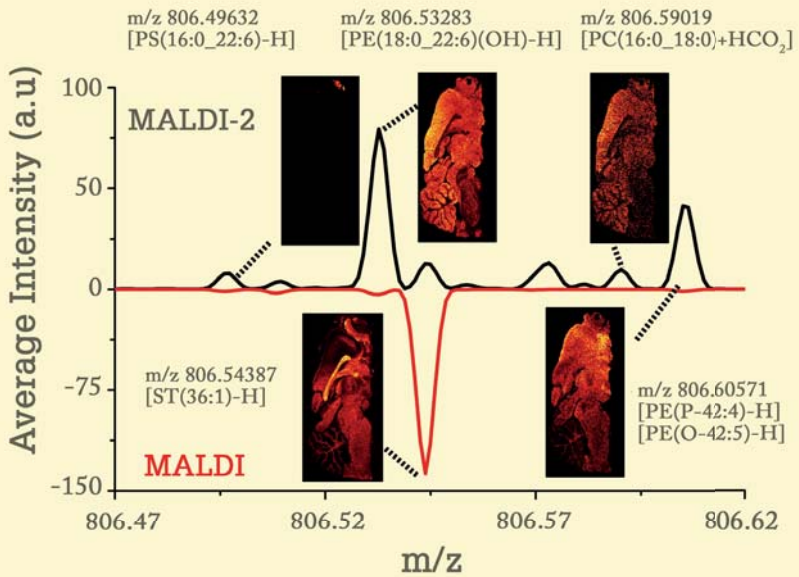

Figure 1. Comparison of MALDI coupled with laser post-ionization (MALDI-2, black trace) and conventional MALDI (red trace) for MSI of lipids from mouse brain tissue. MALDI-2 dramatically improves the ionization efficiency sensitivity for many lipid classes. All lipid assignments are performed with a mass error tolerance of 2 ppm, and in the majority of cases supported with on-tissue MS/MS. Reproduced from (5).

The Clinical Collaborator

Tiffany Porta, an assistant professor at M4I, tells us how the institute is translating MSI technology into the operating theater.

What is your goal?

My research group focuses on translational research and clinical imaging. My main interest is to provide new clinical diagnostic tools, with a focus on intraoperative diagnostics. Therefore, I am strongly connected with the hospital and working very closely with surgeons and pathologists. Our ultimate, joint goal is to improve the clinical decision-making process (surgical and medical) that results in better patient outcome. This is what drives me and my research.

What problem do you hope to solve?

Surgery is the best hope of a cure in 80 percent of diagnosed cancer cases. Whether a cure can be achieved usually depends on the quality of the surgical resection (removal) of the tumor. At the moment, it is hard for surgeons to find the edges of the tumor and remove all the cancerous tissue. To find out if any cancer remains, frozen cut tissue sections are evaluated by a pathologist; results are often not available for several days, and sometimes prove inconclusive. There is ample evidence that improving the accuracy of surgical resection would reduce the number of patients requiring further surgery and improve overall outcomes. This is where molecular profiling based on mass spectrometry comes in – by using a tissue (disease)-specific database we can provide real-time and specific molecular analysis of tissue and assist the surgical decision-making process. We can also use rapid molecular pathology of resected tissue to assist pathologists in their diagnosis.

Tell us about the iKnife…

The technology behind "iKnife" or "intelligent scalpel" is rapid evaporative ionization mass spectrometry (REIMS). – developed by Zoltan Takats for the rapid classification of human tissue via MS analysis. It analyses aerosols released during electrosurgical dissection using electric scalpel or forceps. The smoke generated during electrosurgery is very rich in molecular information, including tissue-specific profiles that discriminate between the tumor and surrounding tissue – data not available to the naked eye of the surgeon. The electrosurgical aerosol collected in real-time is compared with a reference model to determine (within seconds) the type of tissue being cut (for example, tumor versus non-tumor). In a clinical setting, the data would be provided interactively to the surgeon as they cut the tissue. Through this rapid, on-line analysis, surgeons get immediate feedback to help them resect the tumor accurately, leaving no cancerous tissue behind. The beauty of this approach is that existing surgical devices need no modification to combine them with REIMS, the surgical procedure remains the same, and surgeons need no extra training. And for me, these are key points to accelerate the translation of the technique into clinical practice.

Currently, reference models are built ex vivo, which permit the creation of spectral databases for prospective use. In Maastricht, we are currently building databases on breast, colorectal liver metastasis, sarcomas, and head and neck tumors, which are validated histologically by expert pathologists. Our next step is to move our work in vivo and go into the operating theatre, where we will work closely with the clinical staff and surgeons. The goal is to validate the databases that we are currently building ex vivo and work with clinicians towards integration of the iKnife in clinical routine.

What developments lie ahead?

Recent developments have concentrated on miniaturization of the system and making it minimally invasive. For example, integration of REIMS with an endoscopic polypectomy snare to allow in vivo analysis of the gastrointestinal tract is a promising methodology to explore internal structures in a minimally invasive way (1). High diagnostic accuracy for tumor type and known histological features of poor prognostic outcome in colorectal cancer was reported, based on a multivariable analysis of the mucosal lipidome. The potential of this approach for other minimally invasive procedures has also been demonstrated by combining real-time MS with surgical laser systems where aerosol is generated by thermal ablation. The molecular patterns generated are specific to the cellular phenotypes and can easily distinguish benign from malignant regions in patient biopsies, which opens the door for applications in a wide range of clinical areas. Additionally, the cavitron ultrasonic surgical aspirator (CUSA), which is widely used for brain and liver surgery, can also be combined with the REIMS technology for intraoperative diagnostics (2).

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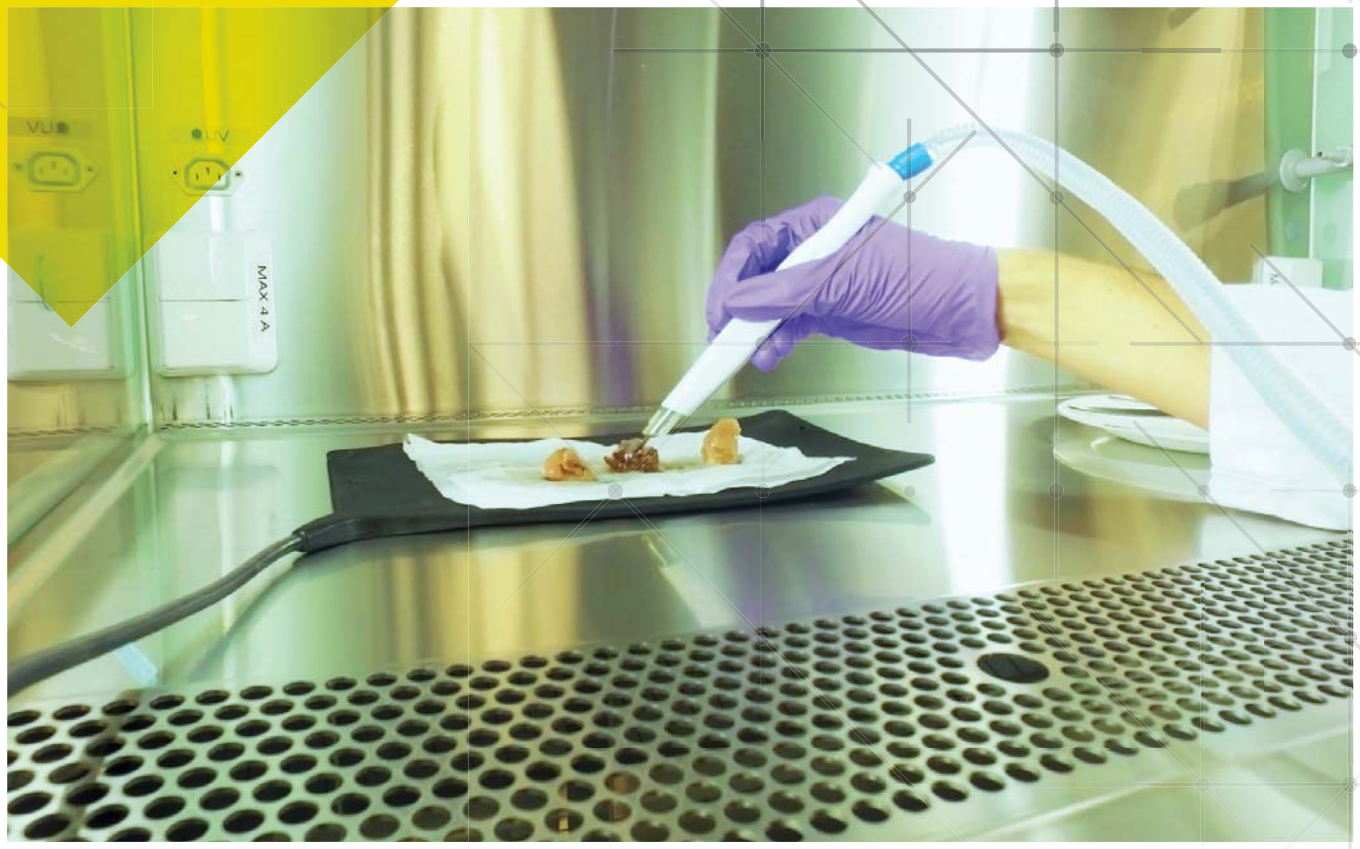


The Big Data Explorer

Assistant professor Benjamin Balluff develops innovative bioinformatics approaches that allow M4I researchers to master their data.

What is your goal?

I develop advanced data analysis methods for MSI of cancer tissues – revealing molecular heterogeneity within apparently homogeneous tumors. Intra-tumor heterogeneity plays an important role in therapeutic failures and progression of the disease. I want to use MSI to pinpoint clinically detrimental tumor subpopulations for further in-depth investigation.

What are the challenges?

The analysis of MSI data is challenging in many ways – ranging from the optimal processing of gigabyte-sized data to selecting the correct statistical analysis. The rapid advance of instrument capabilities has increased demands on computational power and memory. What's more, this data delivers a degree of detail that the human brain is unable to process without the help of algorithms and clever data visualization tools. I develop new methods and algorithms that help to interpret the data and ultimately find answers to urgent biomedical questions. Integration of different (imaging) data modalities is a prerequisite for successful personalized medicine.

What new advances excite you?

With the rising popularity of MSI, more bioinformatics groups from outside of our field have become interested in this type of data, which in turn leads to an acceleration in the development of useful tools for the analysis of MSI data, including commercial solutions. I hope this widespread interest will help us, as a community, to achieve our aim of making MSI a robust diagnostic tool in a clinical setting.

Our focus is, of course, mainly on MSI, but there is a wider trend in life science to integrate data on the same subject from different modalities. We have to work together with different specialties and disciplines, and find a way to combine heterogeneous data (of different sizes and optical resolutions, at different scales, in different storage formats, and so on) using tailored software solutions.

GARBAGE IN, GARBAGE OUT

Preanalytical error is unwelcome in all labs – but in the clinic it can delay diagnoses or even cost lives. Here, we share lessons learned from a pathologist pushing for greater awareness of the issue in medicine.

By Carolyn Compton

Error. It's a subject no physician (or researcher, or analyst) wants to think about, especially when it comes to their ownpractice. And yet errors still occur. Research is still irreproducible; clinical tests still show false positives and false negatives; results still sometimes make no sense at all. Why? In the medical laboratory, at least, the problems may not be integral to the test itself – rather, they may arise from the way a sample was treated before it ever underwent testing: the preanalytical phase.

PREANALYTICAL ERROR IN PATHOLOGY

As a pathologist, I perform analytical tests on patient specimens to make diagnoses. The testing process is often separated into three familiar phases: preanalytical, analytical, and post-analytical (also known as the interpretative or consultative phase).

Preanalytical	*Analytical*	*Post-analytical*
The preanalytical phase includes any actions or factors involved in acquiring, handling, transporting, and processing a patient specimen prior to the actual analysis.	The analytical phase includes all factors related to the test platform and to the testing process itself.	The post-analytical phase refers to the interpretation of the test results in light of our expertise as physicians to formulate a diagnosis (or differential diagnosis) to guide patient management.

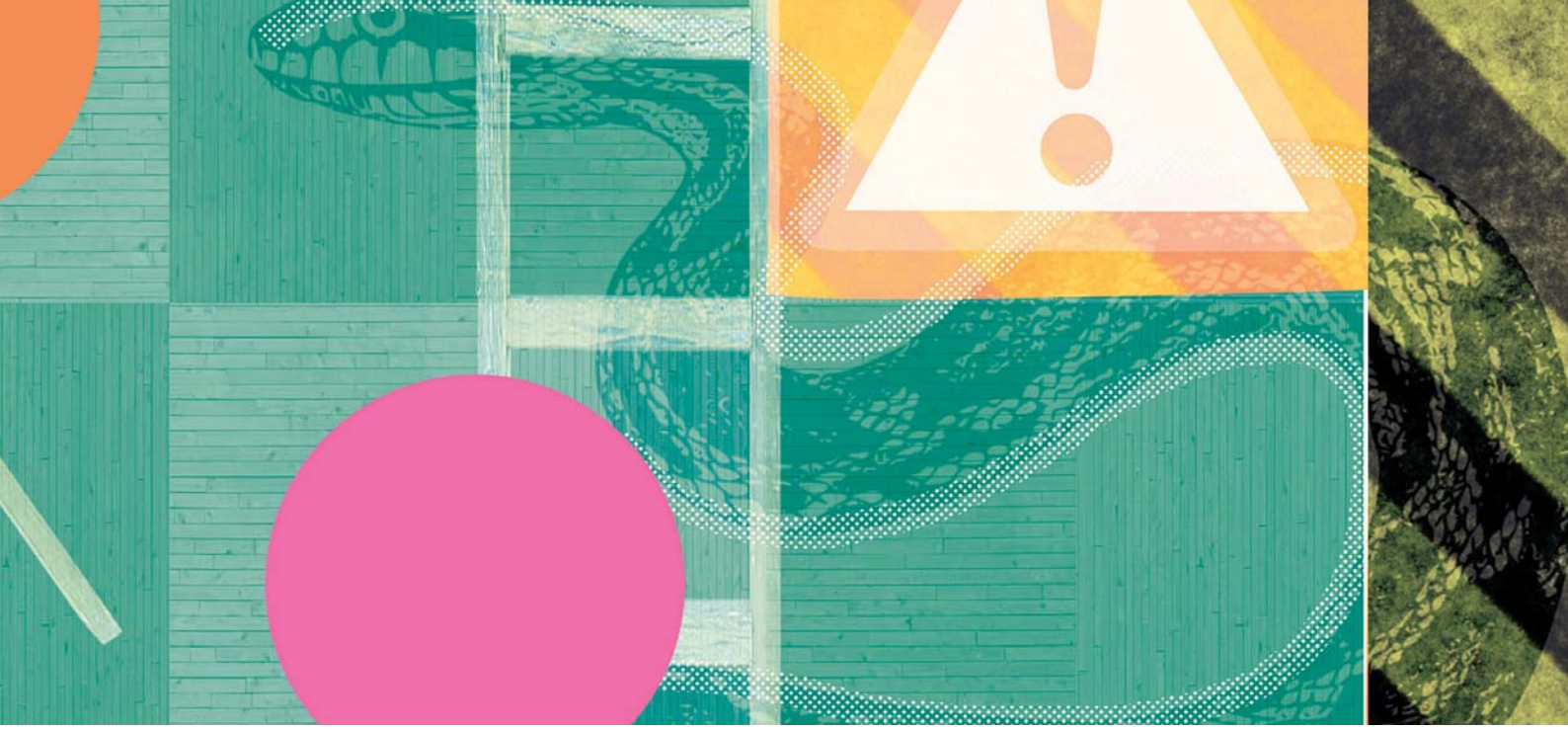
We strive for precision and validity in all of our analyses so that the data we generate reflects the true biological state of the patient. It has been estimated that data from the pathology laboratory comprises as much as 80 percent of the objective, quantitative disease information that exists in a patient's medical record – and much of this data directly guides patient management. This leaves little room for error. Flawed results mean flawed medical decision-making. In short, an incorrect answer from even a single test can have serious consequences for a patient.

Some preanalytical errors – specimen mislabeling, for example – are clerical; others are related to factors that compromise the quality of the specimen and may reduce or even destroy its suitability for certain types of testing. In other words, a particular test could be highly specific and sensitive, but would yield a spurious result if the analytes in the specimen of interest were artifactually altered or corrupted. For example, one research group has shown that a delay in time to stabilization (also known as "cold ischemia time") can artifactually render a HER2-positive breast cancer specimen negative on Herceptest® analysis (1)(2)(3). When the result of a companion diagnostic test such as Herceptest® functions as a gateway to targeted therapy, artifactually induced false negative test results could incorrectly rule out treatment with a potentially life-saving drug – a devastating consequence.

QUALITY BEGETS QUALITY

In this era of "precision medicine," diagnosis, prognosis, prediction, and treatment are often based on the molecular characteristics of the patient and on the molecular features of the disease. These characteristics are typically determined directly from the analysis of representative biospecimens – which means that, if we want to generate high-quality molecular analysis data, we need high-quality specimens. In fact, the increased power of modern molecular analysis technologies has raised the bar for the molecular quality of patient specimens; the better our testing methods get, the better our sampling methods must be to keep up. No matter how dazzling new analytical technologies may be, the "garbage in, garbage out" paradigm still applies to the data they produce. No technology can spin straw into gold!

Preanalytical issues are central to specimen integrity and molecular quality. The myriad steps involved in acquisition, handling, processing, transportation, and storage can have



profound effects on both the composition and quality of different molecular species in patient biospecimens. Safeguarding their molecular integrity in the preanalytical period is an immediate challenge; it can't be delayed or disregarded. Once compromised, a specimen's molecular quality cannot be retrieved.

The molecular quality of a specimen at the time of fixation, when its biological activity is stopped, determines its fitness for testing. After that, if the specimen is well-preserved and carefully stored, its quality may remain essentially unchanged; otherwise, it will only further diminish as the specimen degrades over time. Therefore, preanalytical factors that directly impact a specimen's molecular integrity can have an adverse effect on both real-time patient management and future decisions based on reanalysis of the same specimen.

Additionally, if the patient enters a clinical trial and their specimens are used for correlative scientific studies or discovery research, the downstream consequences of bad data and irreproducible study results can be profound. We are just beginning to appreciate the fact that a huge amount – more than half, in fact (4) – of published biomedical data cannot be reproduced. No one has yet looked closely at the degree to which poor or unknown patient specimen quality may contribute to this problem. I suspect that, when we do, it will be significant.

A MATTER OF STANDARDS

Why are there currently no established or enforced standards around preanalytics? It's a difficult question – with a complicated, multifactorial answer.

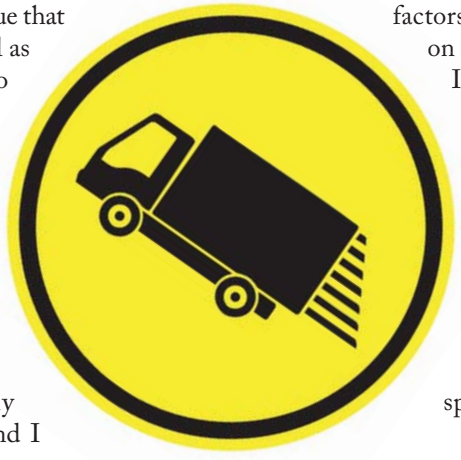
First, I see a lack of awareness and a need for education about preanalytics throughout the medical community. Pathologists, surgeons, and every other professional who is part of the specimen chain of custody (radiologists, pathology assistants, nurses, phlebotomists, medical technologists and much more) need to be educated about preanalytics. It's vital that they all understand the role they play as links in an unbreakable quality chain.

Second, there is a dearth of biospecimen science data upon which to build evidence-based procedures for preanalytics that affect precision medicine. This kind of information is focused on the specimen itself and how it is affected by different preanalytical factors, alone or in combination. It's the data that everyone wants – but no one wants to pay for! We need much more biospecimen science to fully understand the impact of different preanalytical factors on different biomolecular specimens of different sample types. Furthermore, specific analytical platforms may have different requirements for analyte molecular quality – something else that I fear may often be overlooked. These data are foundational for precision medicine, and yet, at the moment, they are sadly lacking.

Third, old practice habits are hard to break. Legacy systems in medical institutions may be difficult to redesign to accommodate changes in preanalytical workflows. By and large, we are still handling patient specimens the same way we have for decades, with no sign of change on the way. In addition, patient specimen preanalytics cross many professional domains, and there are no cross-cutting standards to assure that key preanalytical steps are controlled and documented in an end-to-end fashion.

Fourth, there is no specific reimbursement for the professional time, expertise and effort required to address preanalytics in real time – as they should be. This issue must be addressed to assure compliance with preanalytical standards across the board. People typically do what they are paid to do, even if they don't fully understand the scientific reasons behind the mandates.

Fifth and finally, there are still many who discount the importance of preanalytics, which I find very hard to comprehend. Worse still, they may discount the importance of specimen quality or reject the premise of "garbage in, garbage out" altogether! There are those who believe that, through the wonders of technology and data science, data quantity can overcome the challenges of poor data quality. In my opinion, this kind of thinking is unrealistic and unacceptable – even potentially dangerous – at the level of the individual patient. I would argue that it is misplaced at the population data level as well. If precision is truly the goal, there is no conceivable situation in which preanalytical variation is truly unimportant and can be confidently disregarded – and thinking so can only lead to disaster.



SOURCES OF ERROR

In a December 2014 think tank sponsored by the National Biomarker Development Alliance (NBDA), my private and public sector colleagues and I established a ""Top 10" list of key contributors to preanalytical error – the top five for tissue specimens and the top five for blood samples. For tissues, the top five sources of error are:

1. Cold ischemia time
2. Method of processing (section thickness, temperature, fixative volume to tissue mass ratio)
3. Type and quality of fixative
4. Total time in formalin
5. Storage conditions

For blood and serum specimens, the top five are:

1. Time to processing
2. Method of draw (draw order, tube type, tube fill volume)
3. Method of stabilization (tube inversions)
4. Method of processing (centrifugation speed, centrifugation time, temperature)
5. Storage conditions

Every one of these factors can have innumerable variations in routine practice in different practice settings, or even from day to day in the same practice setting. In other words, each is variably variable! And because there is no requirement to document any of these things on a specimen-by-specimen basis, these preanalytical factors are unknown for any given patient specimen. As a consequence, the molecular laboratory – and the person who actually performs molecular analyses – has no way of knowing whether or not a given specimen is fit for purpose and will yield reliable results. This, of course, means that the veracity of the readouts from the test platforms is also unknown – and yet, because they're all we have, we report them anyway.

Our challenge for precision medicine is to decrease, as much as possible, the variation in the "Top 10" factors by following recommendations founded on the current state of biospecimen science. In addition, the actual performance metrics related to the top 10 must be documented in daily practice – or, at the very least, every deviation from the recommended guidelines must be recorded. Otherwise, how can we know the provenance of a patient specimen? We need to change standard operating procedures in every laboratory so that preanalytical data are a part of each specimen's permanent record.

SMALL CHANGES, BIG RETURNS

Based on the independent review the PPMPT has conducted over the past two years of the scientific literature related to tissue and blood preanalytics, the team has made five recommendations for each sample type.

For tissues, the areas where new approaches can deliver the greatest value are:

Area of concern	*Recommendation*
Time to stabilization	60 minutes or less
Method of processing	Section thickness ≤5 mm Volume/mass ratio ≥4:1 (optimal ≥10:1) Transport temperature: room temperature (20–25°C)
Method of stabilization	Type of fixative: 10% neutral phosphate-buffered formalin (pH tested daily) Optimal time in fixative: 6–24 hours (includes time in formalin in processor); a maximum of 36 hours may be acceptable or even required for fatty tissues like breast
Tissue processor variables	Maintenance schedule: manufacturer's recommendation or a validated deviation Paraffin type: low melt <60°C Total time in processor: 7.5–8 hours (forbid nonstandard practices such as "topping off" with nonstandard solutions)
Storage conditions	Room temperature (20–25°C) Dry conditions

For blood, the areas of greatest value are:

Area of concern	*Recommendation*
Time to first processing step	60 minutes or less
Specimen acquisition	Tube type: • if processing time >2–3 hours, use acid-citrate-dextrose (ACD) tube • for proteomics studies, use EDTA • for coagulation studies, use sodium citrate • do not use lithium heparin for nucleic acid amplification studies Volume of tube fill: manufacturer's recommendation (if less than specified for tubes with additives, document variance) Draw order: • culture bottles • light blue (citrate) • gold (gel, serum) • red (no gel, serum) • green or tan (heparin) • lavender or tan (EDTA) • royal blue (EDTA) • grey (sodium fluoride) • tubes with other additives
Method of stabilization	Tube inversions: manufacturer's recommendation
Method of processing	Centrifugation speed and time: variable, depending on validated protocol and biomolecule of interest Temperature: room temperature, unless validated protocol dictates otherwise
Storage conditions	Freeze-thaw cycles: ≤ 1 for nucleic acids and proteins (use aliquots)

At the moment, quality assurance is close to completely absent from the preanalytical phase. Now that we've set out some recommendations and guidelines, our next step is to implement our generalized, five-point action plan to ameliorate preanalytical variability (see "Time to Act"). It's our hope that, by making recommendations and devising ways to achieve them, we can begin the process of establishing a quality assurance ecosystem.

A BETTER BIOMARKER

The future of medicine depends on the development of molecular biomarkers. They can provide more precise diagnosis and patient stratification, detect early disease, elucidate risk of disease, predict disease outcome, response to therapy, and therapeutic toxicities and permit monitoring of therapeutic management. Unfortunately despite its importance, biomarker development has historically been fraught with failure. The majority of biomedical discovery research has proven irreproducible or invalid, and very few qualified biomarkers have been produced in the last decade. Failures in biomarker science have translated into failed clinical trials and, ultimately, the inability of biomedicine to deliver on the emerging promise of precision medicine.

Rigorous adherence to standards that are consistent, and consistently applied across the development process, is required to achieve the reproducibility we currently lack. Of primary importance, therefore, is the quality of the starting materials – the biospecimens used for analysis. Development of complex biomarker approaches represents an even higher bar. Preanalytical artifacts may abrogate any ability to define biological effects of interest or distinguish biological signatures of importance in patient samples. This problem is especially consequential when the biomarker assay is a companion diagnostic and the gateway to access to a therapy. Neither a false positive nor a false negative biomarker test is tolerable in that circumstance.

Regulatory approval of new biomarker assays is now also focused on specimen quality as it relates to the quality of the data on which approvals are based. The biomarker qualification programs of the US Food and Drug Association and the European Medicines Agency emphasize the need to document the biospecimen quality of diagnostic biomarkers used for either drug or device (assay) development. It is imperative that the entire biomedical community addresses the need for standardized processes and fit-for-purpose biospecimens to accelerate the delivery of accurate, reproducible, clinically relevant molecular diagnostics for precision medicine.

A RECIPE FOR FAILURE

The NBDA, a part of the Complex Adaptive Systems Institute at Arizona State University, for which I serve as Chief Medical Officer, has intensively studied the process by which biomarkers are currently developed and has identified the root causes of most biomarker development and validation failure. The most significant among these include the following issues:

- Discoveries often start with irrelevant clinical questions – that is, questions that may be biologically interesting, but are not useful in clinical practice.
- Biomarker discoveries are often based on "convenience samples" – biospecimens of unknown or poor quality.
- Rigorous, end-to-end, appropriately powered statistical design is often lacking.
- Technology standards are either lacking or disregarded if they exist.
- Data and metadata quality and provenance are often inadequate to poor.
- Analysis and analytics are often inappropriate or inadequate for the sophistication of the clinical question and/or design.

All of these issues must be simultaneously addressed if the biomarker failure rate is to be reversed. We need cross-cutting standards that support biomarker development in an end-to-end fashion. At the moment, the development process is siloed and disjointed, adding to the likelihood of failure as we proceed from discovery through development to regulatory approval and clinical implementation. We need to collaborate across disciplines if we want to see biomarker development succeed.

LESSONS LEARNED

The amount of clinically meaningful and biologically significant data that we can generate from biospecimens has increased by orders of magnitude in recent years. As our analytical methods and technologies have evolved, however, quality assurance concerns have been focused primarily on how we test specimens – with little or no attention paid to the specimens themselves.

Ultimately, no matter how sophisticated and technologically advanced our analytical platforms, the quality of the data can never be higher than the quality of the starting materials – the analytes.

It is now possible to generate petabytes of bad data from bad specimens – and we can do it with unprecedented speed. The stakes are higher than ever. But regardless of how much effort is involved and how far we have to go to ensure full quality control, we need to remember that it's all worth it for one reason: our patients. They are counting on us.

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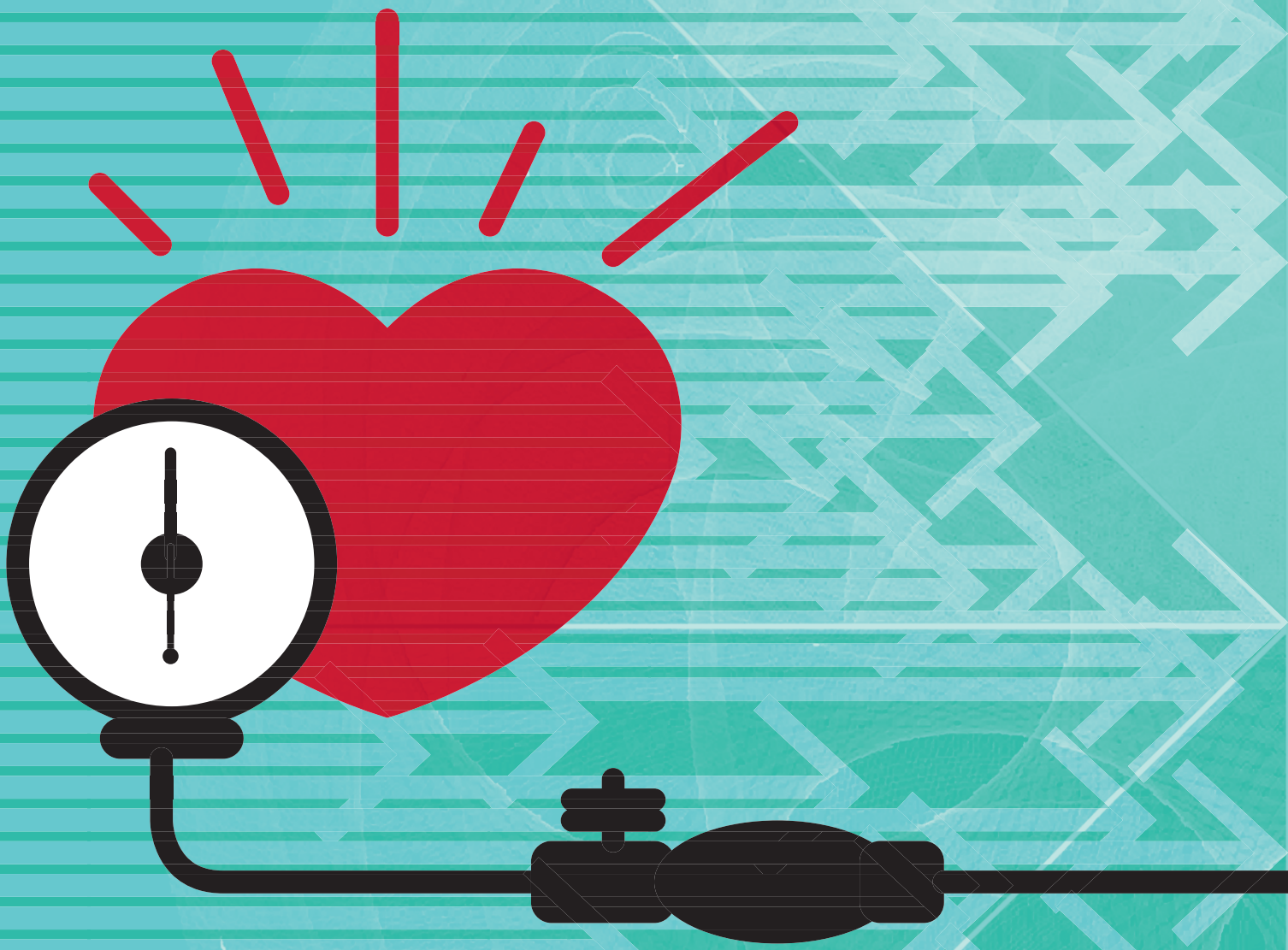
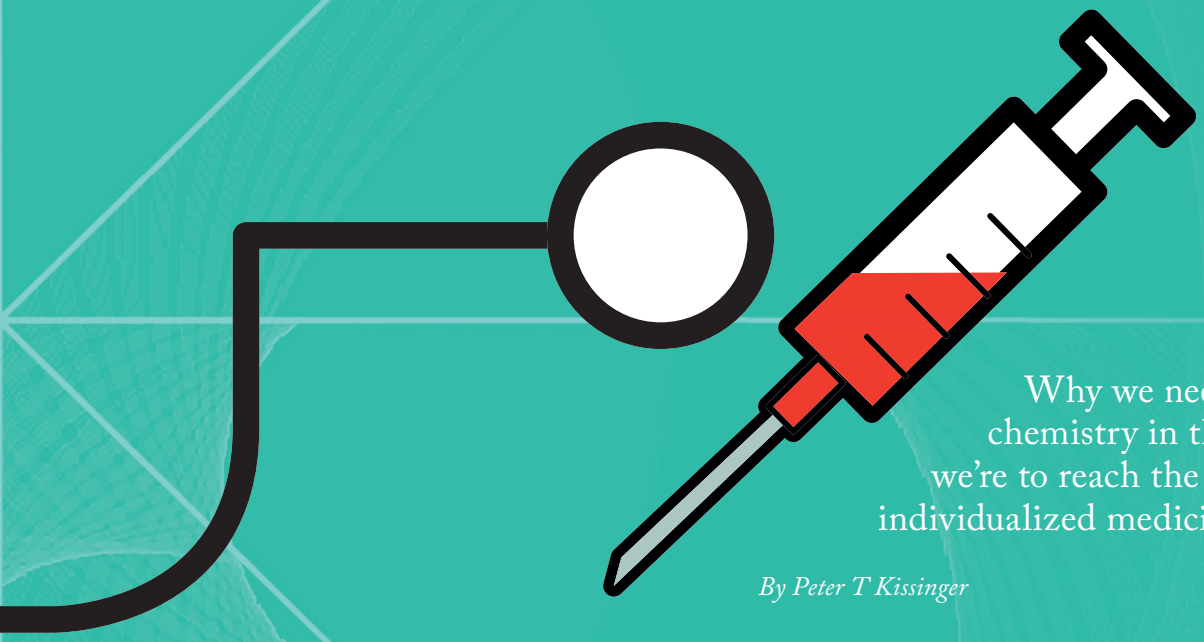
TIME TO ACT

The five objectives of our generalized action plan to ameliorate preanalytical variability are:

1. Verify the "Top 10" preanalytics from the published literature and translate these into practice metrics – and then, of course, publish our findings.
2. Propose accreditation checklist questions to CAP's Laboratory Accreditation Program with the goal of enforcing the Top 10 through the College's laboratory accreditation process.
3. Educate pathologists about the Top 10 list, its scientific basis, and the practice metrics that need to be met to control and record them.
4. Educate other professional groups – such as surgeons, nurses, pathology assistants and other healthcare professionals – about patient specimen preanalytics. Assist them, individually as needed, in developing their own practice guidelines to assure specimen quality and in helping to orchestrate overall concordance among practice guidelines throughout the biospecimen chain of custody, from patient to analysis.
5. Seek financial support from payors and professional support from regulators and funders to implement and sustain the practices that control – and the infrastructure to document – patient specimen molecular quality for precision medicine and translational research.



CLINICAL CHEMISTRY: THE ROAD TO N=1

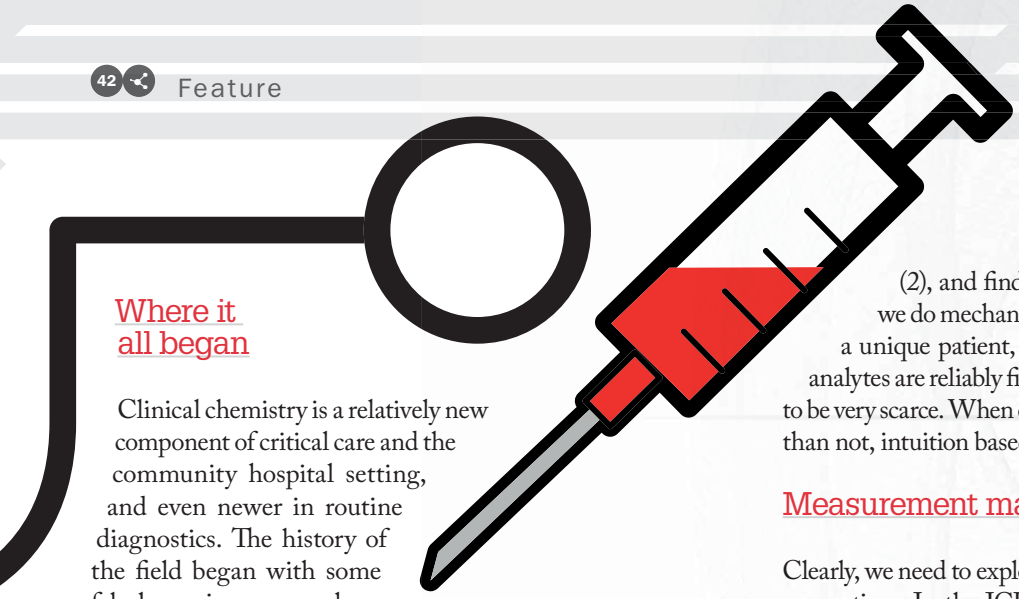


Why we need more chemistry in the clinic if we're to reach the goal of individualized medicine.

By Peter T Kissinger

Sixty years ago, as an elementary school student, I was required to complete a physical examination in order to join an athletic team or participate in summer camp. At the time, such exams were fully in the domain of physics. The available tools measured height and weight and included a chilly stethoscope, a blood pressure cuff, a rubber hammer, and a mercury thermometer. There was a little device with a bright light used to peer into my ears, nose and throat (otoscope/auriscope). Virtually no chemical measurements were made beyond looking at the clarity of urine and a semi-quantitative test for sugar therein.

Following a recent morning encounter with my physician, I told a class of premeds that I'd just had a "pchem" exam. I related how a "physical" had become a "physical chemistry" exam, with the doctor showing me tables of numbers on a tablet computer, enabling comparison with reference ranges and my own longitudinal data. Those same data are now available to me anywhere on planet Earth. Clinical chemistry has come a long way in my lifetime, and it is advances in instrumentation that have had the biggest impact on medicine. The microscope and the thermometer got us started, but even these are recent advances considering our history of several hundred millennia.



Where it all began

Clinical chemistry is a relatively new component of critical care and the community hospital setting, and even newer in routine diagnostics. The history of the field began with some fabulous pioneers, such as Donald Dexter Van Slyke (1883-1971), Joseph J. Kleiner (1897–1974), Arnold Orville Beckman (1900-2004), Wallace H. Coulter (1913-1998), Leland C. Clark (1918-2005), Solomon Aaron Berson (1918-1972), Lenard Tucker Skeggs, Jr. (1918-2002), Rosalind Sussman Yalow (1921-2011), and John Wendell Severinghaus (b. 1922). These great minds were tinkerers – they did not follow a strategic plan, create PowerPoint slides or speak of reimbursement codes or third party payers. There is no room here to dig deep into the history of clinical chemistry, but a great place to start learning more is a short review by Larry Kricka and John Savory, published in 2011 (1). My point: clinical chemistry is largely a post-WWII phenomenon which in many respects did not accelerate until the 1970s. Diabetics had no means to monitor glucose at home even modestly well until 1980, 50 years after insulin became a drug. The American Association of Clinical Chemists began in 1948 and about thirty years later, just as I joined, the name was changed to the American Association for Clinical Chemistry, implying advocacy and welcoming a wider demographic.

The age of complexity

With the human genome project, we were thought to be on the cusp of a great advance in diagnostics, but we now know that knowledge of genes alone are not enough. Next, at the turn of the millennium, we thought that the proteome would be the answer. The terms biomarker and molecular diagnostics were invented, but once again, the new dawn of diagnostics failed to materialize. Now, we are moving onto metabolomics – will it deliver? Only time will tell. All of these areas have potential to develop further, but it will require more investigative effort than was initially thought.

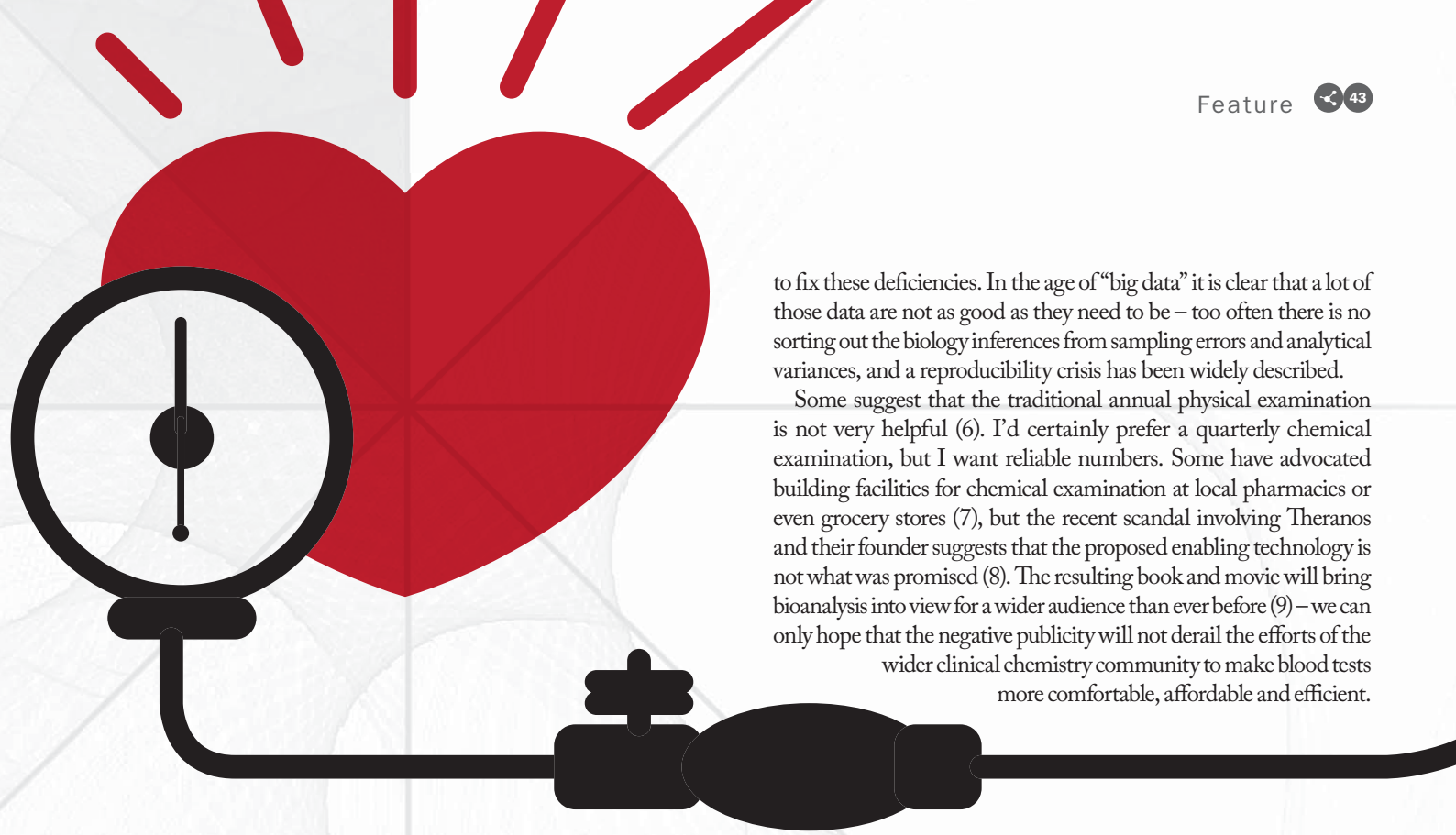
Each person is unique and defined by much more than their genome, which itself is less stable than we thought. Our proteins are in constant post-translational flux, depending on the time of day, the time we last ate, and the time a drug entered the body. What we consume, the variability of our microbiome, and the state of various organs are not reliably programmed at birth. Yet we largely operate with the tyranny of averages – we chase p-values (2), and find more correlations and probabilities than we do mechanisms. When a physician is confronted with a unique patient, averages aren't much help. While some analytes are reliably fixed in a homeostatic fashion, these appear to be very scarce. When one doctor sits with one patient, more often than not, intuition based on experience matters most.

Measurement matters

Clearly, we need to explore the virtue of more chemistry measures versus time. In the ICU, displays still mainly focus on physical metrics. The only routine chemical measure is oxygen saturation. But physical measures of temperature, blood pressure and heart rate are all responding to chemistry. When they wander too far, we take a blood sample, but could the problem have been anticipated? We dose a drug based on such crude notions as 10 mg for all or mg/kg or mg/m2. Shouldn't we be dosing to achieve a measured exposure? Isn't concentration in circulation a better concept of dose than a pill swallowed or a bolus infused? Shouldn't drug monitoring be the most common companion diagnostic, especially in critical care where drug–drug interactions and organ system deficiencies are likely? Getting the right drug at the right dose at the right time is not often a genomics problem. Likewise, every child matures biochemically and physiologically in a way that does not follow a consistent timeline – shouldn't we be measuring more? Is it not odd that a bioanalytical chemist who has lived seven decades has never had a single measurement of the circulating concentration of a prescribed drug? I've never even been tested for glucose tolerance. Pianos get tuned more often. My doctor tells me my hemoglobin A1C is average, but averages can come from an infinite number of data sets. I want to know my variance, the method variance, and a subpopulation variance (old men, in my case) (3).

Testing, testing…

So much for venting. Things are improving – we are doing more point-of-care testing, although it is still limited. We are getting closer to N=1 personal reference ranges and we have access to our own electronic health data. We can make measurements in smaller volumes of blood than ever before and can now do a lot of tests with 0.1 mL, a few with 0.01 mL and some with less than 0.001 mL. However, we still frequently take far more blood than we need. There have been several reports of anemia resulting from too many blood draws with cardiac patients (4,5) and we've all heard of excessive ordering of diagnostic tests. I suspect that most of the volume of those blood draws was thrown away, and this can be confirmed by a visit to your local clinical chemistry laboratory – more than one major lab has told me "all of our automation is based on sample tubes large enough



to hold a bar code". The patients are waiting for bioanalytical chemists, clinical chemists, and pathologists to improve this situation. The tools are getting better, and among them are mass spectrometers, which in the clinical world are now at the stage where the Skeggs' AutoAnalyzers were in the 1960s.

Mass spectrometry as an analytical resource is older than pH meters, oxygen electrodes and immunoassays, but is relatively new to diagnostics. Performance is good, but there remain significant challenges for quantitative work in clinical chemistry, including many nonlinearities whereby variable matrix components influence the response for the desired analyte(s). Many do not fully understand this matrix effect and its impact on method validation. Mass spectrometry technology is not yet economically competitive for random access, allowing for rapid examination of different analytes in each of a series of samples using a single instrument. This is especially impactful for intensive care clinical applications where rapid turnaround time can be critical. On the other hand, when samples are numerous for a single analyte or panel, and time is not critical, there is no better performance for the price.

Sample quality

A major worry in clinical chemistry today is the difficulty in finding properly collected and characterized samples from carefully controlled biology. Sampling matters – every bio sample comes with a set of attributes, which too often are incomplete, with time (chronobiology), nutrition, polypharmacy, and comorbidities rarely available in any detail. Understanding of the problem, the will to do better, and the money to improve are generally in short supply, but the time has come to fix these deficiencies. In the age of "big data" it is clear that a lot of those data are not as good as they need to be – too often there is no sorting out the biology inferences from sampling errors and analytical variances, and a reproducibility crisis has been widely described.

Some suggest that the traditional annual physical examination is not very helpful (6). I'd certainly prefer a quarterly chemical examination, but I want reliable numbers. Some have advocated building facilities for chemical examination at local pharmacies or even grocery stores (7), but the recent scandal involving Theranos and their founder suggests that the proposed enabling technology is not what was promised (8). The resulting book and movie will bring bioanalysis into view for a wider audience than ever before (9) – we can only hope that the negative publicity will not derail the efforts of the wider clinical chemistry community to make blood tests more comfortable, affordable and efficient.

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

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Solutions

Real analytical problems
Collaborative expertise
Novel applications

See-Through Science

Allowing non-destructive chemical identification through opaque materials, the TASIA-winning STRam(an) represents an evolution in Raman technology.

By Katherine A. Bakeev

The problem

Raman spectroscopy, now widely available in handheld and portable formats, is used for measuring the molecular fingerprint of a sample, and is particularly useful for rapid, nondestructive material identification. The specific molecular information supplied by a Raman spectrum has proved invaluable in chemical, material, pharmaceutical and biomedical research, and in medical diagnostics. But the technique has limitations – samples can only be measured directly, or through transparent containers. Raman identification through opaque packaging would make the technology easier to use for incoming raw materials in warehouses and for first-responders, customs agents and others who need to rapidly identify materials without touching them. In addition, conventional Raman typically has a very small sampling area with a high power density at the laser focal point on the sample, which means that only a limited portion of a sample is measured, and samples may heat or burn. We reasoned that if we could design a system that overcomes these issues, Raman could be used more widely and give more repeatable results for heterogeneous samples, such as mixed powders or natural products.

Background

The STRaman technology, invented by Jun Zhao and Jack Zhou of B&W Tek, expands the capability of Raman spectroscopy to measure samples beneath diffusely scattering packaging material. Jun also developed the identification algorithm and led the product development, and I helped get the workflow in place and design the user interface.

We set out to design a system with a much larger sampling area than the conventional Raman spectroscopy

"To make measurements beyond the surface layer we knew that we needed to get more power to the sampling point."

confocal approach, to enhance the relative intensity of the signal from the deeper layers, thereby increasing the effective sampling depth and allowing the measurement of materials inside visually opaque containers. Raman spectroscopy is often used for material identification in pharmaceutical manufacturing, as well as by law enforcement for testing unknown materials, so we knew that sample measurement and identification through different packaging materials (without having to open containers) would give much more flexibility, reduce exposure to the samples and avoid sample contamination – ultimately making it quicker to get an actionable result.

The larger sampling area of STRaman technology has the added advantage of preventing sample damage by reducing the power density at the point of measurement, as well as improving measurement accuracy by eliminating the variability detected when measuring with a small spot size on a heterogeneous sample. The combined benefits of lower power density and greater penetration depth make the STRam system a suitable analysis tool for biomedical samples such as living tissue (such as under the skin's surface).

The solution

To make measurements beyond the surface layer we knew that we needed to get more power to the sampling point. We began by increasing the throughput of our portable Raman system to provide greater sensitivity and decreased measurement times, while still giving predominantly a surface measurement. The result is the STRam, a portable Raman instrument that has see-through capability.

The system is comprised of: the patent-pending probe, a high-throughput spectrometer, and specialized algorithms for the identification of the samples from the spectrum, which has contributions from the surface layers and underlying sample. Our design uses a coaxial excitation and collection path of the signal that is utilized in conventional Raman to measure beyond the diffusely scattering layers that cover a sample.

The STRam probe is designed to

cover a larger surface area and increase the sampling depth compared with conventional Raman, so that the Raman spectrum beneath diffusely scattering opaque layers can be measured without being overwhelmed by the Raman signal of the surface layer.

The STRam is operated through an embedded touchscreen computer with software that walks users through the measurement steps and gives a match result in seconds. Our customers – who work in forensics, customs, border agencies, and pharmaceutical companies – wanted the ability to use commercial spectral libraries, as well as the ability to develop custom libraries. So we developed libraries and software to fulfill this demand, including a full narcotics library and 21CFR part 11 compliant ID software.

Many pharmaceutical companies already rely on handheld Raman for raw material testing through bottles and plastic drum liners. Chemical suppliers are able to verify the contents of their containers without breaking the seal, and because measurements can be done in seconds, can test a large number of packages in short time. Customs agents and postal inspectors who encounter suspicious envelopes can use the STRam to determine if they pose a threat without exposing anyone in the process.

Beyond the solution

Customers are already asking for the technology to be extended to a handheld instrument, so that is one of our next challenges.

Another challenge for us is to expand the capability of the instrument to work

"We would like to see the STRam adopted widely for nondestructive, noninvasive inspections across the transportation industry."

at different laser excitations. Currently, the instrument comes with a 785 nm laser excitation; we want to offer 532 nm (carbon material analysis) and 1064 nm (fluorescence avoidance).

We would like to see the STRam adopted widely for nondestructive, noninvasive inspections across the transportation industry – including logistics and shipping companies and customs and border agencies. This has the potential to help verify that materials are what they should be and to identify unknowns, increasing everyone's safety.

The laser power can be adjusted to as low as one percent, meaning it can be used in biomedical research and tissue analysis to increase understanding and early diagnosis of disease, with less chance of sample damage. The STRam's more widely dispersed power could also prove useful in archaeology and conservation to contribute to the understanding of provenance, authenticity, and degradation – samples and artwork are less likely to be damaged and more representative measurements can be made of the samples.

The ability to measure samples inside packages, eliminating the need for sample preparation, is one of the major advantages of Raman. Going that step further and measuring through opaque packages – from white plastic bottles to fiber sacks, envelopes and even skin – allows easy adoption of this fundamental spectroscopic tool in many working environments, in the laboratory or in the field. This could open Raman to many new potential users, for whom it has not previously been a viable tool.

Katherine A. Bakeev is Director of Market & Customer Development at B&W Tek, Newark, Delaware, USA.

Rapid Analysis of Organochlorine Compounds in Water using Automated SIFT-MS

Combining the power of direct analysis using selected ion flow tube mass spectrometry (SIFT-MS) and GERSTEL automation, headspace analysis of chlorinated volatile organic compounds (VOCs) in water is greatly simplified. This application note demonstrates the linearity and repeatability achievable with automated SIFT-MS.

Mark J. Perkins[1], Vaughan S. Langford[2]
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[2]Syft Technologies Limited, Christchurch, New Zealand

Organochlorine compounds such as chloroform, trichloroethylene (TCE), and tetrachloroethylene (perchloroethylene, PCE) found widespread use in diverse industries in the 20th century. Although usage is now greatly reduced, these compounds continue to be significant contaminants in air, soil, and water. The purge and trap approach is most commonly applied for analysis of these species in water, followed by gas chromatography analysis coupled with either electron capture detection (GC-ECD) or mass spectrometry (GC-MS). Not only are these methods slow, the GC requires moisture to be removed. Application of SIFT-MS can accelerate analysis through direct headspace analysis as the need to purge, trap, and dry are all eliminated.

Standards containing chloroform, trichloroethylene, and tetrachloroethylene were prepared from a 1,000-ppm solution in methanol. From this stock solution, 10 µL was transferred into 10 mL of water to make a 1-ppm standard solution. The solutions for linearity and repeatability measurements were prepared by taking 20 µL, 50 µL, 100 µL, 250 µL, 500 µL, 750 µL and 1,000 µL aliquots and filling them to a total of 10 mL in water in 20-mL headspace vials. This yielded solutions ranging in concentration from 2 to 100 ppb.

The solutions were analyzed using a Syft Technologies Voice200*ultra* SIFT-MS instrument integrated with a GERSTEL Multipurpose Sampler (MPS) (GERSTEL, Mülheim an der Ruhr, Germany) equipped with a GERSTEL agitator/incubator and headspace vial racks. The solutions were incubated for 15 minutes at 60 °C. Headspace was sampled using a 2.5-mL headspace syringe (heated to 150 °C; fill speed of 200 µL s^{-1}) and injected into the SIFT-MS instrument's inlet at a flow-rate of 50 µL s^{-1} (giving a total flow rate of ca. 420 µL s^{-1}).

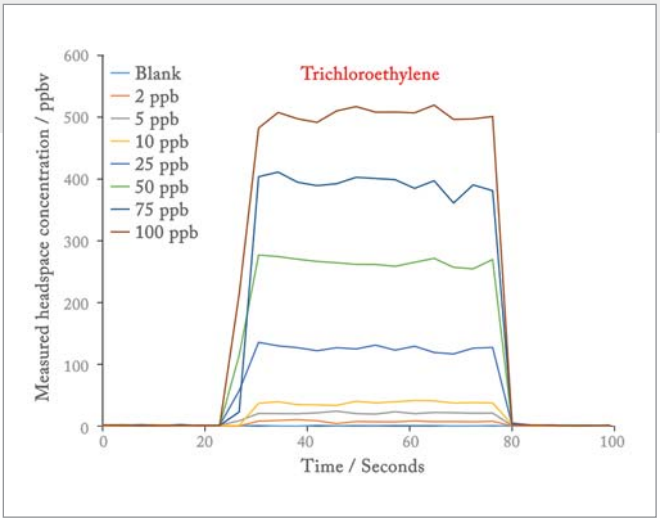


Figure 1. SIFT-MS selected ion mode analysis of trichloroethylene as headspace from the standard solutions is slowly injected into the instrument.

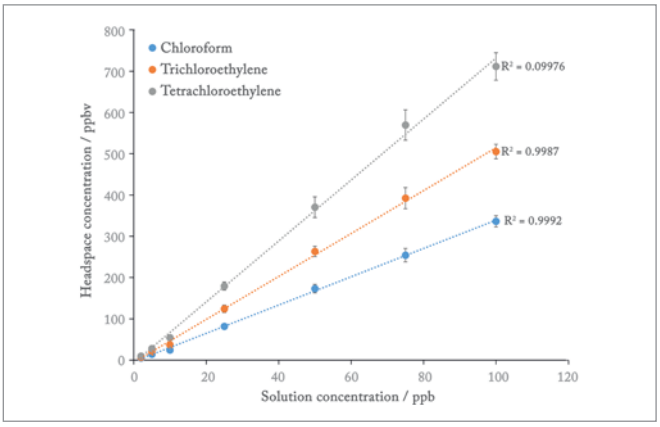


Figure 2. Linearity of water headspace analyses for chloroform, trichloroethylene, and tetrachloroethylene using SIFT-MS.

Figure 1 shows the results obtained for trichloroethylene analysis, illustrating very stable injection by the autosampler and measurement by the SIFT-MS instrument. These data, together with that obtained simultaneously for chloroform and tetrachloroethylene, are plotted as linearity curves in Figure 2. Linearity is excellent. Error bars correspond to the measurement uncertainty across the repeated measurements during injection (Figure 1). Repeatability was investigated at a solution concentration of 50 ppb. The RSDs obtained with six replicate injections were 3.3 percent, 1.8 percent, and 1.5 percent for chloroform, trichloroethylene, and tetrachloroethylene, respectively.

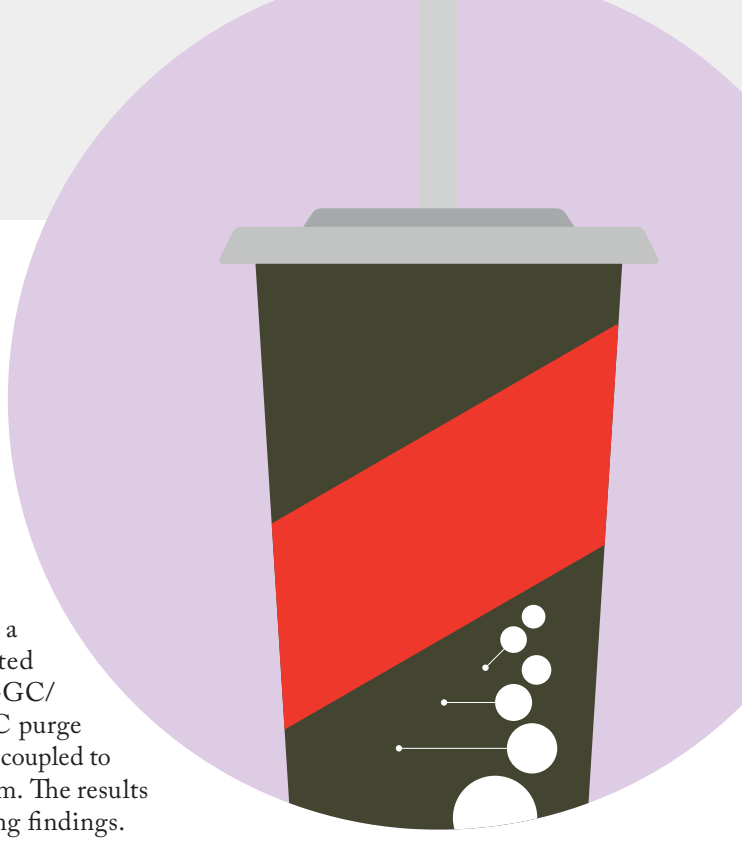
This study demonstrates that SIFT-MS is a very powerful new technique for rapid determination of chlorinated VOCs in water. Direct analysis using automated SIFT-MS is sensitive, linear, and repeatable. SIFT-MS provides substantial throughput increases over traditional purge and trap-GC methods, since no preconcentration or drying is required.

Download the full application note: tas.txp.to/0518/SYFT

Purge and Trap of Soft Drink - Ethanol Found

A non-alcoholic beverage is tested by the most sensitive purge and trap method.

By Xiaohui Zhang



It's no coincidence that the human nose is directly over the mouth, nor that there is an "aromatic" class of organic compounds. While smell is not strictly speaking a component of taste, it certainly can have a significant effect.

On the quantitative analytical testing side, the Purge and Trap method can extract and concentrate volatile flavor compounds from a liquid sample for subsequent analysis by GC/MS. In this application, a soft drink was tested with purge and trap-GC/MS on a CDS 7000C purge and trap concentrator coupled to PAL RTC 850 System. The results reveal some interesting findings.

Figure 1 tagged the seven most significant peaks from a soft drink sample. Of particular interest was that ethanol was found at sub hundred ppb level. Typically, beverage manufacturers' recipes and formulations are trade secrets. Based on general industry knowledge and analytical experience it is highly unlikely that ethanol is added as a formulary compound. Since the sample contained sugar, low-level ethanol is hypothesized to be the result of fermentation. Ethanol is among the compound list, supporting findings by previous researchers and of concern to consumers, although at a very low level. Figure 2 depicts a more detailed look, with more than sixty compounds identified in the purge and trap-GC/MS chromatogram.

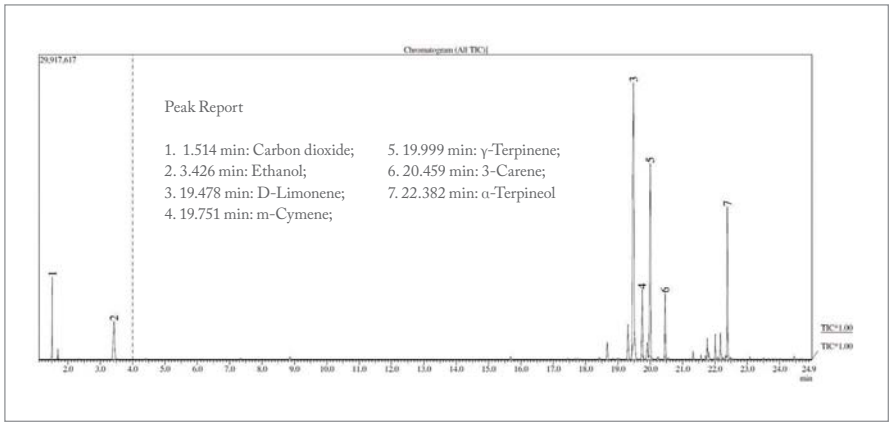


Figure 1. Most significant peaks from a soft drink sample shown in the purge and trap-GC/MS chromatogram.

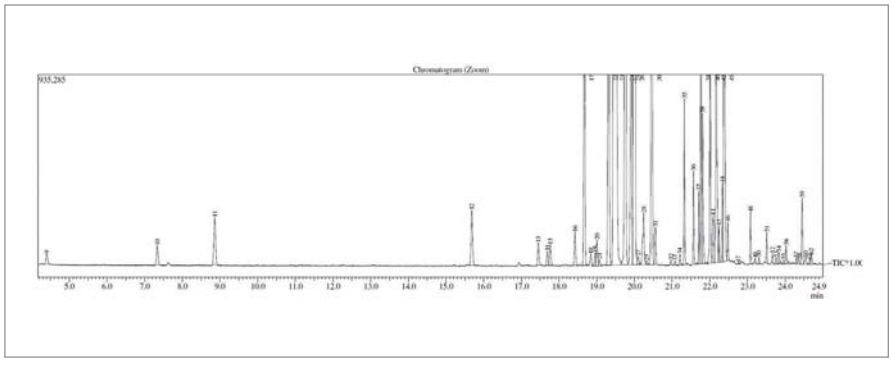


Figure 2. More detailed peak profile in the purge and trap-GC/MS chromatogram.

The data presented here is a simple capability demonstration. As a routine practice, quantitative P&T-GC/MS is performed in EPA Methods 525 and 8260 for aqueous samples. Potential applications for ethanol content include process monitoring/optimization and comparative sample analysis for diet beverages.

Contact: Carol Byrd +1 610 932 3636
Download the full application note: tas.txp.to/0518/CDS

A Driving Force

Sitting Down With... Kelly Zhang, Principal Scientist, Genentech, South San Francisco, California, USA.

Why analytical chemistry?
I've been interested in science since high school – one of my teachers had a great influence on me, and he always said that "science is beauty". During my studies, I explored several different areas including polymers, organic synthesis, and even programming, but ultimately analytical chemistry was the area I enjoyed the most. Everything starts with a good analytical tool.

What's your role at Genentech?
As an analytical scientist, I partner with different groups in drug research and development, including chemists, formulation scientists, toxicologists, regulatory affairs, and clinicians, to move new drugs from discovery, to clinical and ultimately to commercialization. I also work on new analytical technology evaluation and development. As a manager, I spend time hiring the best people and working together with them on their career development, to amplify the impact we can have.

What drew you to the pharmaceutical industry?
It is an area of science that has an immediate impact. My work brings me close to patients and I can see that the drugs we have made are making a difference to people's lives – it makes me feel that all the blood, sweat and tears are worth it.

What made you choose a career in industry rather than academia?
In industry, you have less freedom than in academia on choosing the topics you want to study, but there is a tangible reward. An example that springs to mind is a cancer patient who came to talk to us and shared how a Genentech drug, which I was involved with for 7 years, saved her life. We hugged and thanked each other. The feeling you get when you know patients are counting on you is quite different from other achievements.

That's not to say that academia isn't important and rewarding. I believe that to move knowledge and technology forward, industry, academia and instrument vendors need to collaborate.

What are the analytical challenges in pharma?
In pharma, we always want things better and faster – we want best-in-class and first-in-class drugs, and we want better and faster technologies to deliver the enabling data. The drug R&D timeline is still too long. In a dream world, we would just push a button and get all of the data we need! So we need methods that are quicker, but also more robust – when working with patients there's no room for compromise in quality. Drug modalities are getting more and more complex, so we need better analytical technologies to help us characterize drugs and predict drug activity. Analytical technology is moving forward, but compared to other fields, the progress is slow. From an R&D point of view, we need to think of more creative ways to handle the challenges facing us.

How can we speed things up?
People have to be creative and take smart risks. We have to communicate and collaborate more – there shouldn't be any boundaries. You may fail – but if you don't try, you will never know. At Genentech, we say we live in the future, because we are creating things for ten years ahead. We value mastery, we encourage people to be innovative, and we try to foster creativity. We said, "We need to have the guts to rewrite the textbook" – and it did happen.

You have spoken before about the importance of a multidimensional approach…

"We have to communicate and collaborate more – there shouldn't be any boundaries."

The samples we are working on are increasingly complex – there isn't one method that can handle it all, and you can lose a lot of time on method development and sample characterization. With multidimensional separations, if something co-elutes on the first column, you can use a specific column for that group of compounds in the second dimension for further separation. This cuts down on the time needed for method development and lets us analyze complex samples quickly. It helps us better understand the chemistry and interactions of the drug, and improve the formulation and drug delivery technology.

Where would you like to see technology heading?
Firstly, I think miniaturization is the future. Miniaturization not only means saving our lab space, but also tech that is faster and more environmentally friendly, producing less waste. Secondly, it would be exciting to have integrated technology that is multiplexed-platform and generalized – it is time-consuming and inefficient to have multiple separate methods and detectors for one sample. Thirdly, I'd like to see smarter, more user-friendly software, with deep learning capability. Smarter software would mean we start every day a little further ahead, based on past data.

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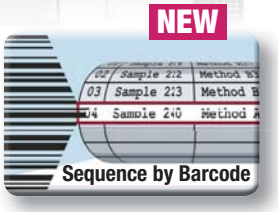
SPME, SBSE, Thermal desorption

Headspace & Dynamic Headspace (DHS)

Sample Prep by mouse-click (MAESTRO)



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