MARCH 2016 # 38

the Analytical Scientist

Forensic Myths and Methods

Christopher Palenik and Glen Jackson tackle the "CSI effect" and the role of analytical science in forensics.

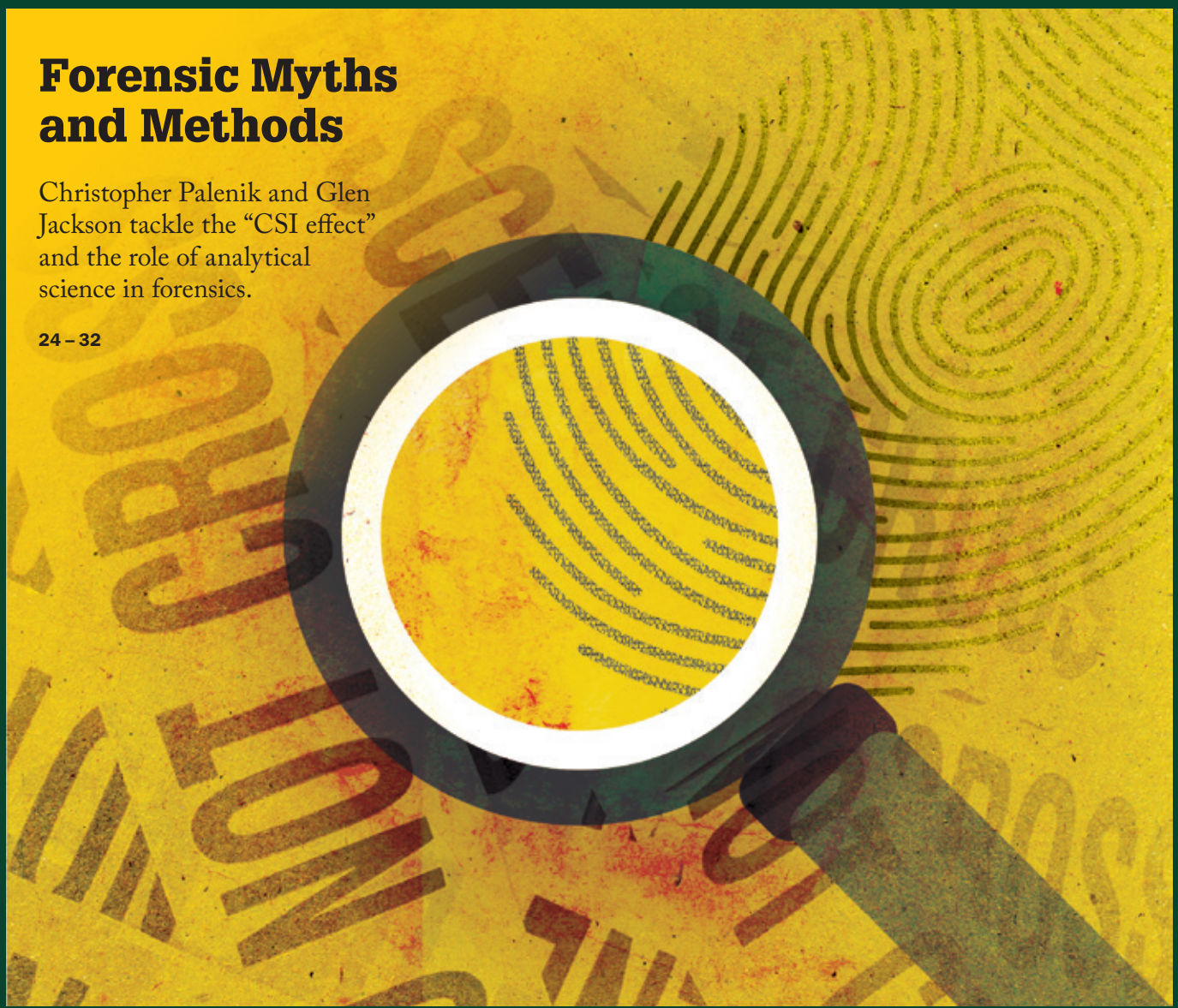

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Image of the Month

Claudia Conti, a conservations scientist from Italy's National Research Council Institute for the Conservation and Valorization of Cultural Heritage (ICVBC) holds a small piece of 'art' ahead of microscale spatially offset Raman spectroscopy (micro-SORS). "Due to micrometer-scale laser illumination and Raman collection areas, and spatial offset dimensions, micro-SORS can readily resolve thin turbid layers that are beyond the reach of both traditional SORS and conventional Raman microscopy," says Pavel Matousek, a Senior Fellow at the UK's Science & Technology Facilities Council (STFC).

For more information on art analysis with micro-SORS, visit: https://theanalyticalscientist.com/issues/0815/unraveling-art-with-analysis/

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Send it to rich.whitworth@texerepublishing.com

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On The Cover

We hold the magnifying glass over the field of forensic science.

Upfront

the Analytical Scientist

ISSUE 38 - MARCH 2016

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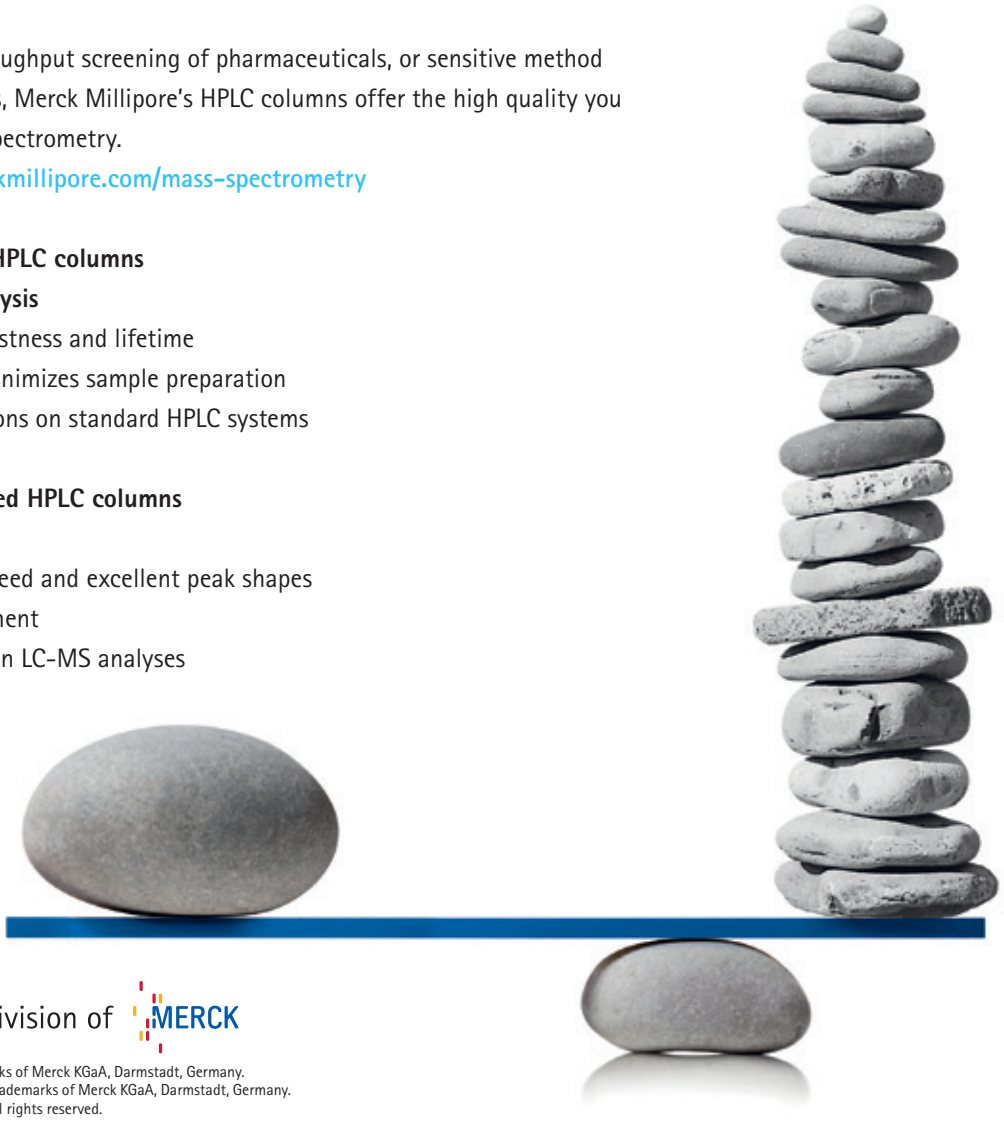

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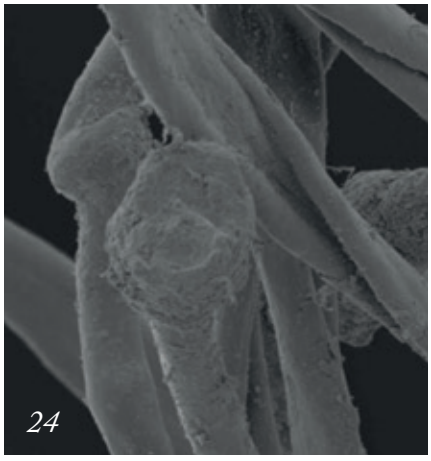

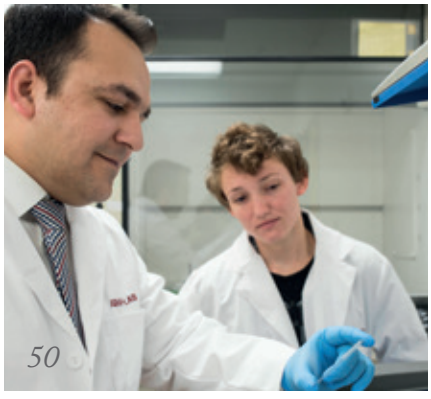

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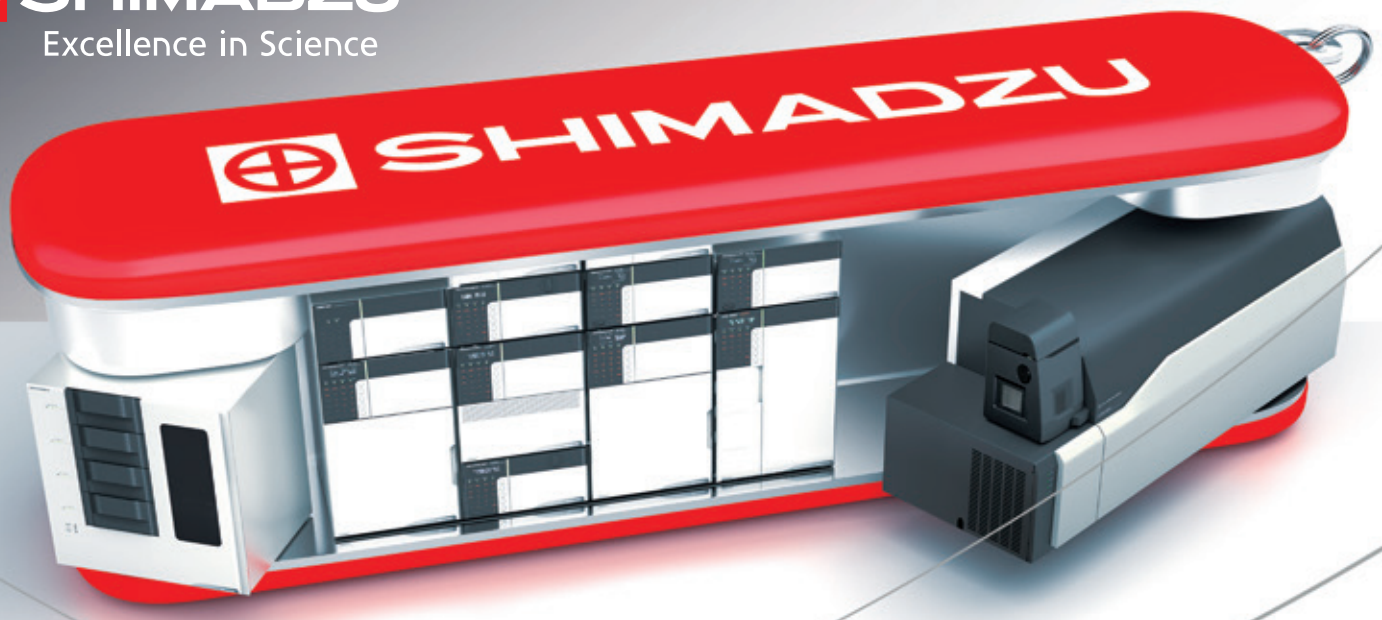

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30,000-Foot View

An over-used expression seems particularly relevant at 37,000 feet...

As I hurtle through the sky at 522 mph over the Atlantic Ocean, I am marveling – as I usually do when airborne – at the audacity of the human race. How many scientific discoveries and engineering breakthroughs were behind getting the first plane off the ground – and how many analytical scientists does it take to keep one aloft?

The woman in the seat next to me politely asks the reason for my flight. "Pittcon 2016," I say and explain that the show has everything to do with measurement science; from ensuring the safety of the always-inadequate portion of in-flight food to the quality of the jet fuel pumped into our magnificent flying machine 1715 miles ago.

I continue: "From the blood test that tells you you're vitamin D deficient – likely too long in the UK – to the amount of active pharmaceutical ingredient in the aspirin you took as a precaution to deep-vein thrombosis – we analytical scientists are everywhere!".

"That sounds simply fascinating," she says.

"Yes," I thought to myself. "Yes, it is."

Working, as you tend to do, in distinct vertical application areas or specialized horizontal techniques in the imagined analytical matrix in my mind, I suspect it's easy to slip into a state of apathy at times. When the samples seem never-ending and the reward appears only to be shifting digits in an online banking account, you could quickly forget the impact of your work on the man on the street (or the woman on the plane).

As the Editor of The Analytical Scientist, I am surrounded by the work of "fascinating" people – and it's my job to draw out the impact – but even I fall into a brief state of ennui occasionally, when the frankly miraculous becomes almost commonplace.

Looking out the window, I see blue sky and clouds, but I also imagine teams of researchers down below, collecting air samples and sending probes into the deep oceans. I look at my glass of (mediocre) red wine and ponder how the sweetness of the grapes was measured – and if my oral microbiota (see page 14) really do play such a big role in my enjoyment (they must be having an off day). And does my brightly colored "fab" popsicle really contain "no artificial colors, flavors or preservatives"? In short, I have time (over nine hours) to reflect on the wonders of analytical science and the amazing role you play in society.

Thankfully, these thoughts are actually reinforced on a regular basis by the many inspiring lectures I see at an embarrassment of conferences each year – and every time I speak to the Waseem Asghars of the world.

Rich Whitworth
Editor

Upfront

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

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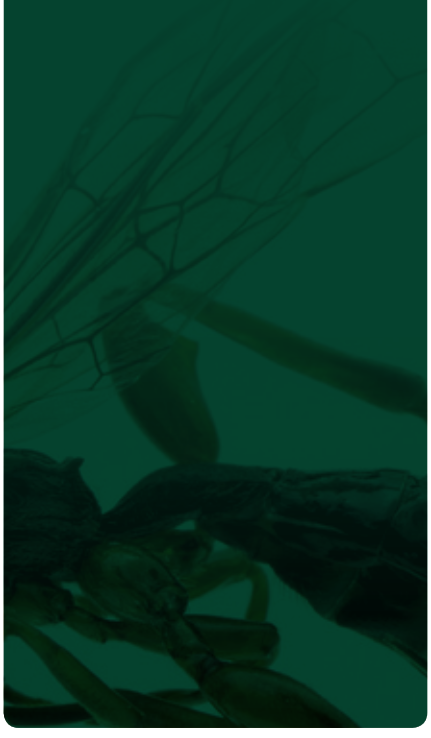


maxson.erin, via Flickr

Ahead of the Curve

Are wasp-inspired needles the neurosurgery of the future?

If you ask the general public to name their least favorite creature, wasps are likely to be on the hit list. But in addition to their valuable pollinating activities, our unfriendly 'destroyers of picnics' have been the inspiration for a potentially ground-breaking technique to detect and treat cancerous tumors of the brain, developed by Ferdinando Rodriguez y Baena at Imperial College, London. Female wood-boring wasps use a long, flexible yet strong ovipositor to bury and protect their eggs, a little-known fact that provided an unexpected solution to the difficult task of accurate neurosurgery.

The idea for the needle came about by chance during a dinner, when Rodriguez y Baena's colleague told him about the unique physical feature of the wasp. "Suddenly, I wondered whether we could mimic this attribute in robotic medical technology to improve the delivery of treatments," Rodriguez y Baena explains. "We started by trying to blindly duplicate what nature does (and failed!) before eventually abstracting the key mechanism that governs this beautifully elegant biological insertion process to produce a viable concept."

The flexibility of the needle could have a significant impact on several medical procedures and could even enable molecular analysis, Rodriguez y Baena says. "If we are able to deploy Raman imaging along the flexible fibers embedded within our needle, we could use our steerable system to make "on the spot" measurements related to the tissue being intersected. For instance, we could differentiate between white and grey matter or identify the boundaries of a lesion."

The process from concept to reality has been a bumpy road, Rodriguez y Baena says. "During the last eight years, we have faced many challenges, ranging from how to control this unique, biologically inspired design, to its miniaturization and assessment within a realistic setup." It is expected to be another four years before it begins to make its impact on the industry. "EDEN2020 will fund the translational work and pre-clinical assessment necessary

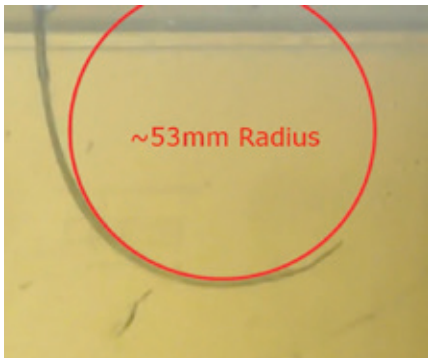


Figure 1. The wasp-inspired needle in action. To see a short video, visit: http://tas.txp.to/0316/wasp

to take this concept closer to reality. If all goes well, we could be in a position to plan a controlled clinical study in 2020."

As is so often the case in modern science, the success of the needle has been a collaborative effort. "The list of collaborators who have helped to make this project a success is long, starting with the original inspiration for a proof of concept grant funded by the EPSRC," says Rodriguez y Baena. "Since then, I have worked with several UK and European partners and a number of these are now part of the EDEN2020 consortium." His relationship with long-time collaborator Lorenzo Bello (University of Milan) is likely to intensify from the April 1, 2016, which coincides with the start of EDEN2020: "I have already known Professor Bello for a number of years. And as he is the clinical lead on the project, I expect that we will be working very closely together for the next four years at least!"

So, we now have a ready answer the next time someone asks, "What's the point of wasps?" *JC*

Conspiracy or Cock-up?

A new funding clause could leave UK scientists out in the cold

Academic communities are asking if a recent amendment to legislation is the government's attempt to prevent the influence of researchers on UK policymaking – or if it's simply a case of clumsy phrasing.

From May 2016, a new clause will be inserted into grant agreements stating that "payments that support activity intended to influence or attempt to influence Parliament, government or political parties [...] or attempting to influence legislative or regulatory action" will no longer be considered "Eligible Expenditure" (1). The new clause is based on research carried out by the Institute of Economic Affairs, which is said to have "exposed the practice of taxpayers' money given to pressure groups being diverted to fund lobbying rather than the good causes or public services".

The Cabinet Office says that the clause will "ensure that freedom of speech is protected, whilst stopping taxpayers' money being diverted away from good causes", and claims it will not "prevent organizations from using their own privately-raised funds to campaign as they see fit". However, an online petition claims the clause is an "attack on academic freedom" that will "stop grants for university research being used to influence policy-makers".

An editorial in Nature called for the UK government to reassure scientists that their advice was still welcome... (2). *JC*

Reference

1. *https://www.gov.uk/government/news/government-announces-new-clause-to-be-inserted-into-grant-agreements*
2. *http://www.nature.com/news/unintended-consequences-1.19473*

Analyzing Autism

Ion chromatography uncovers a link between iodine levels and symptoms of autism

Anna Blazewicz' interest in the determination of elements in body fluids and tissues began several years ago, whilst a chemist working in collaboration with medical clinics. Her research into autism began more recently, when in 2013 Blazewicz (pictured) initiated a project to determine various elements, including iodine, in autistic and healthy children – a project done in collaboration with the Department of Psychiatry at the Medical University of Lublin in Poland. Beyond the analytical scope of the project, Blazewicz had another strong reason: "I became interested in this very complex disorder more than 15 years ago, when my child was diagnosed with Asperger's syndrome. Essentially, I deal with autism spectrum disorders (ASD) 24 hours a day, seven days a week," she says. "In my experience, the parents of children with this disorder do not need encouragement to seek answers to the questions of what causes ASD and how it can be treated – and that means I have extra motivation, driven by the knowledge of psychiatrists, psychologists and my own experience, but also the experience of other parents with children with autism."

The team used ion chromatography (IC) to study iodine in the urine of children with severe autism (1). For the determination of iodide ions, the team applied pulsed amperometric detection (PAD), which allowed for detection in the low ppb-range, demonstrating high specificity for iodide ions.

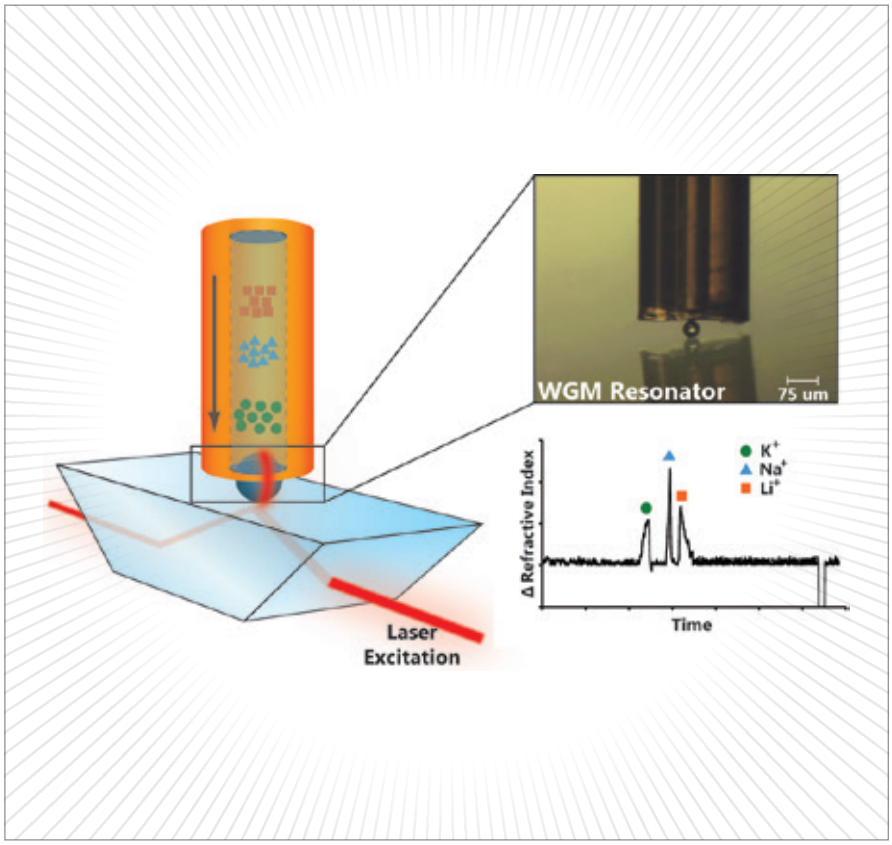
Blazewicz admits that IC "isn't very popular" in the field of medical analysis, despite several benefits. "One of the advantages of IC, when it comes to practical application, is the simplicity of sample preparation (although quite rigorous and labor-intensive procedures of sample pre-treatment are often described in literature). I've developed simpler, faster, and more effective procedures for clinical sample preparation."

In studies of this kind, selection of case study patients is an important consideration. Blazewicz says, "Factors that have to be controlled include diet, supplementation with minerals and vitamins, comorbidities, and current medication." Perhaps most important of all was the issue of collecting samples in the least invasive way possible – not an easy task in children with severe autism, who are often known to withdraw from physical contact. This was, Blazewicz says, "the greatest concern for parents."

The current research project will now be extended to take into account the influence of other analytes and factors such as diet on the symptoms of autism. Despite the huge amounts of progress made in the area of autism research, Blazewicz feels there is still some way to go. "Our knowledge about autism is growing, but not enough to understand all the etiological factors," she says. "The constant collaboration of chemists, biologists, and medical doctors is essential – and we cannot forget about the importance of proper communication with parents of children with autism." *JC*

Reference

1. *A Blazewicz et al., "Iodine in autism spectrum disorders", J Trace Elem Med Biol, 34, 32–37 (2016). DOI: 10.1016/j.jtemb.2015.12.002*



Capillary Cathedral

'Whispering-gallery' mode (WGM) boosts CE detection limits

Scientists at Kansas University have discovered that detection limits of specific analytes can be improved by combining capillary electrophoresis (CE) with 'whispering-gallery' mode (WGM) detection – so named after the effect in St Paul's Cathedral, where the circular shape of the gallery allows sound waves to propagate unusually long distances.

The illustration shows a schematic of the end column WGM resonator that senses changes in refractive index. To monitor the WGM resonance, light from a tunable diode laser is directed into a Dove prism and coupled into the resonator. The CE capillary is carefully positioned above the resonator to partially encapsulate it, while leaving enough space for fluid to flow. The inset shows a magnified view of the CE capillary outlet (75 µm ID; 363 µm OD) and WGM resonator (53 µm diameter). The WGM resonator responds to changes in refractive index as the ion bands pass by.

Reference

1. *DC Kim and RC Dunn, "Integrating whispering gallery mode refractive index sensing with capillary electrophoresis separations using phase sensitive detection", Anal Chem, 88 (2), 1426–1433 (2016).*

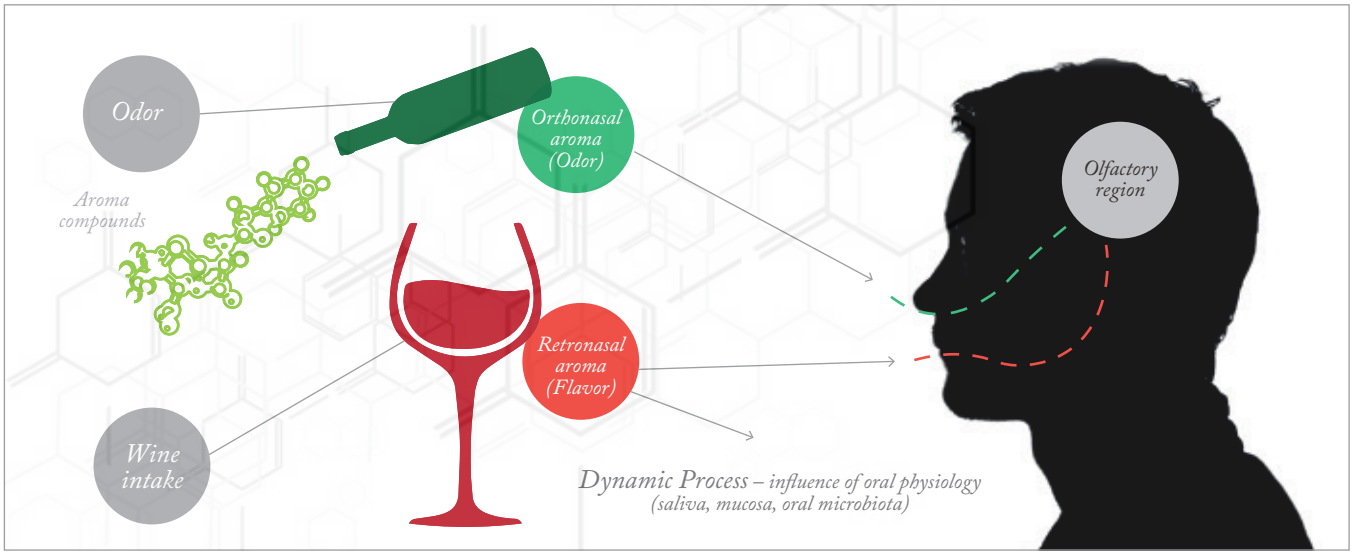


Figure 1. Two key modes play a part in aroma release and perception: (i) the immediate aroma impression, when liquids are first imbibed (the orthonasal aroma); and (ii) the prolonged retronasal aroma perception after swallowing, often called the after-taste or after-odor.

Days of Wine and Noses

Time to ditch the tasting notes? Enjoyment of wine may depend on oral bacteria...

Victoria Moreno-Arribas, of the Institute of Food Science Research (CIAL), Madrid, was aware that studies regarding aroma release during wine consumption were scarce, and also that there was little research on the role of different oral factors – saliva, oral mucosa and, in particular, the composition of oral microbiota – in aroma release during wine drinking. To that end, she embarked upon research to fill the gap – and discovered that flavor release may depend of the type of bacteria present in the mouth, something that is subject to large inter-individual variability.

Within a global research project dealing with the role of human physiology on wine aroma release and aroma perception during wine consumption conditions by using different in vitro or in vivo approaches, Moreno-Arribas' aim was to evaluate the ability of bacteria present in the oral cavity to hydrolyze grape glycosidic precursors and release the corresponding aromatic compounds ("aglycones"). The final goal was to determine if inter-individual variations in the composition and metabolic activity of oral microbiota could affect wine flavor release.

There were several stages to the research. "In a first in vitro experiment, the effect of nine representative bacteria species from oral microbiota was evaluated in terms of the biotransformation of glycosidic aroma precursors into the corresponding odorant components," Moreno-Arribas explains. "Subsequently, the same experimental procedure was performed, but using the whole human oral microbiota isolated from saliva of healthy volunteers (ex vivo)." To isolate and extract the odorant aglycones, samples from both trials were processed by head-space solid phase microextraction and then analyzed by gas chromatography mass spectrometry (GC-MS).

Though many factors are involved in our appreciation of fermented grape juice, the research gives a clearer indication of what factors affect individual enjoyment. "The results indicate the influence of oral microbiota on how aroma compounds are released in the mouth ," says Moreno-Arribas. "However, other physiological factors, such as saliva, oral mucosa, breathing, together with other sensory experiences, such as taste, texture and color, all play a part in flavor perception and come together to explain why the same food tastes very different from one individual to another."

But what does this mean for our enduring love of wine? Moreno-Arribas believes it may make choosing that bottle of red a lot easier: "We are still a way away, but in the future it may be possible that by analyzing your saliva, your nasal mucosa and the composition of bacteria that make up your oral microbiota, you can find out which wine is likely to provide you with more pleasure – one which your palate, your nose and your brain will enjoy the most. I hope we can get to that point one day." On the other hand, you could just try before you buy... *JC*

Analytica & Humanity

Munich will play host to the 2016 Humanity in Science Award winner – and much more

This year sees the 25th incarnation of Analytica in Munich. As well as an array of presentations on hot topics in the analytical sciences, Analytica will also provide a platform for Waseem Asghar, winner of the 2016 Humanity in Science Award to present his prize-winning work: "Development of a new paper and flexible material-based diagnostic biosensing platform that could be used to remotely detect and determine treatment options for HIV, E-coli, Staphylococcus aureas and other bacteria." We 'sit down' with Waseem on page 50.

Here are but a few of the sessions that we have our eyes on:

May 10

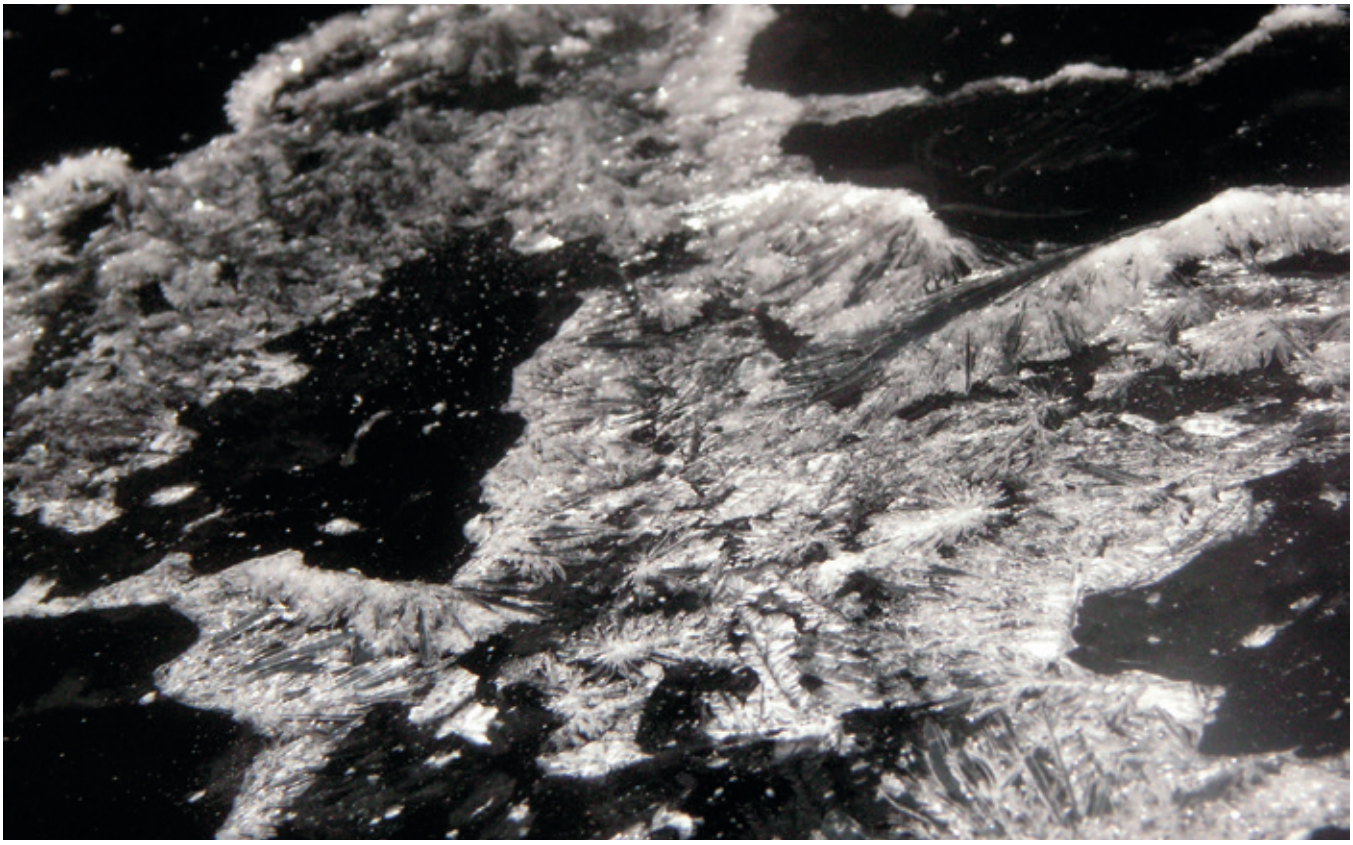
- 10.30. Using mass spectrometry to understand cystic fibrosis as a protein misfolding (John Yates III)
- 14.30. Towards the comprehensive analysis of metabolome (Thorsten Teutenberg)
- 16:30. Recent examples for the utility of chromatography, ion mobility, and mass spectrometry in sports drug testing (Mario Thevis)

May 11

- 10.30. Recent developments and perspectives in foodomics (Alejandro Cifuentes)
- 14.00. Towards the comprehensive analysis of metabolome (Guowang Xu)
- 16.00. Pyrrolizidine alkaloids - a new threat for food and feed safety? (Christopher Gottschalk)

May 12

- 11.30. Cell-derived vesicles on microfluidic platforms for pharmaceutical and diagnostic applications (Petra Dittrich)
- 14.00. Spectroscopic analytical technology for bioprocesses – challenges, limitations and possibilities (Dörte Solle, see feature on page 36)
- 15.00. Studying oxidation reactions of nucleic acids with electrochemistry-mass spectrometry (Herbert Oberacher).



Hello, Ketty

A new test for ketamine aims to improve the accuracy of toxicology screens

Ketamine is an animal tranquilizer that has found recreational (and unforgivable) uses in less innocent times. To allow quicker diagnosis of abuse, toxicologists at University of Beira Interior (Portugal) and forensic scientists from the National Institute of Legal Medicine and Forensic Science (INMLCF in Portugal) and University of Santiago de Compostela (Spain) have developed a more accurate method for detecting ketamine in human urine (1). Eugenia Gallardo explains the context to the research.

What motivated you to develop a test for ketamine?

Ketamine works as a sedative and provides pain relief in humans, but also causes memory loss and can cause users to hallucinate. When people with ketamine intoxication attend hospital emergency services, their symptoms may be mistaken easily for alcohol intoxication, resulting in them being given the wrong treatment. Therefore, developing new tests to detect psychoactive substances in biological specimens is essential to keep up with – and even get ahead of – trends in drug use. The new study describes, for the first time, a fast, simple and fully validated method to detect ketamine in urine and plasma. These drugs are difficult to analyze; and we see a lot of versatility in the molecules and new drugs are appearing almost every month. Traffickers are always one step ahead of the authorities.

How does the test work?

The drug is extracted from small amounts of biological specimens (0.25 mL), which pass several times through a previously conditioned cartridge (MEPS). The approach is basically a miniaturization of traditionally used procedures for sample preparation, and has the advantages of using small amounts of sample (more tests can be run on the provided sample, whose volume is often limited, especially in forensic scenarios), lower amounts of organic solvents (more environmentally friendly) and reduced preparation times (in our case, extraction takes place within 10 minutes, and the result is obtained after 20 minutes). Compared with existing methods, this new procedure

is faster and more cost effective, which helps doctors ensure the right treatment is administered more rapidly. The low limits of detection and high amounts of the compounds extracted from very small samples also makes this procedure suitable for laboratories performing routine analysis in the field of toxicology; for example, in forensic and clinical scenarios.

What was the biggest hurdle during development?

The optimization of the whole process was undoubtedly the most time-consuming task, because all parameters that affect sample preparation must be previously screened and optimized to decrease the number of interferences and get better process efficiency.

What next?

We are still working in this field, developing rapid methods to detect other new psychoactive substances. These compounds are extremely dangerous, since many of them are labeled "not for human consumption" but they are designed to provide similar symptoms to those observed when illegal drugs are used. Their sale through Internet sites at low cost, and the uncontrolled production and distribution have raised concerns about potential damage and addiction. For instance, 255 novel psychoactive substances (NPS) were detected in Europe between 2012 and 2014. Consequently, we feel that it is important to perform strict investigations to better understand their epidemiology and pharmacology, using toxicological analytical screenings.

Reference

1. *I Moreno et al., "Determination of ketamine and its major metabolite, norketamine, in urine and plasma samples using microextraction by packed sorbent and gas chromatography–tandem mass spectrometry", J Chromatogr B Analyt Technol Biomed Life Sci, 1004, 67–78 (2015). DOI: 10.1016/j.jchromb.2015.09.032*

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 edit@texerepublishing.com

Standing Up for GC-MS – and Good Science

Though a recent paper about the data-distorting potential of gas chromatography in metabolomic studies is far from perfect, it does at least draw attention to the absolute criticality of robust experiment design – on both sides of the argument. And that's got to be a good thing.

By Karl Burgess, Head of Metabolomics, Glasgow Polyomics, University of Glasgow, Scotland.

Mingliang Fang's paper in Analytical Chemistry, "Thermal Degradation of Small Molecules: A Global Metabolomic Investigation" (1), has been producing significant controversy since its appearance – and the paper's conclusions are upsetting many people. Fang's paper queries the entire concept of gas-chromatography mass spectrometry – a methodology used routinely in metabolomic investigations. By heating samples in a mimic of GC separation (GC generally uses a heat gradient to separate biomolecules) or derivitization followed by liquid chromatography, they observed significant changes in the metabolic profile observed – breakdown of triphosphates to monophosphates, for example.

On the face of it, it's a scary concept; many of the metabolites we've been measuring for decades are being incorrectly assigned! However, there are a few important caveats regarding the methods used: whether the water in the resuspension solvents is an appropriate solvent for derivitized compounds and whether there was any evaporation of the derivitized samples, which might lead to some of the results obtained using LC-MS. There's also the observation that some of the compounds of importance discussed, such as ATP, that break down under the heat treatment, don't fly using conventional GC-MS techniques.

More generally, though, this is actually a problem with GC-MS 'untargeted metabolomics' – a type of metabolomics where one looks for metabolic changes across the whole of biochemistry (or at least as much of biochemistry as it's feasible to measure). My group has focused on untargeted metabolomics – using LC-MS rather than GC-MS – for many years. It's a fascinating and challenging area of research that has come under a lot of pressure over the last few years. Metabolomics – especially the 'omics' part of it – has the goal, for me, of analyzing the entire metabolome without bias. In reality, we're very unlikely to ever achieve this goal (every methodology is biased in some way), but the possibility of discovering entirely new biochemistry is a significant draw.

Temperature is a consideration in LC-MS too - we spray the sample through a heated needle, into a heated interface. Thermal degradation products and complex adducts that increase the complexity of spectra were one of the first stumbling blocks in untargeted LC-MS metabolomics, but because of their elution at the same time as the molecular ion, we can track those changes much better than in GC-MS. However – and very importantly – we view untargeted metabolomics as either a hypothesis-generating experiment or as additional verification of a result

"Metabolomics – or any analytical method for that matter – is inherently flawed. We are viewing reality through a cracked lens."

obtained from another experiment. Validating the changes that we detect with metabolomics using alternative methods are key elements of good science in general, and this is equally true for GC-MS.

I know of few groups doing GC-MS metabolomics who don't go down the targeted route of carefully analyzing standards to check retention times and fragment patterns to build up a method, and I would hope that anyone thinking of performing metabolomics as hypothesis verification would validate their method carefully, choosing appropriate controls and standards.

Metabolomics – or any analytical method for that matter – is inherently flawed. We are viewing reality through a cracked lens. But if we understand how the cracks distort the image, we can obtain useful information. As scientists, we're trained to be skeptical and not just accept a result on face value. As such, Fang's paper is an interesting, if not valuable, contribution to that skepticism.

I'm not going to throw away my GC-MS just yet though…

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Teaching Rights and Wrongs

Ethics in science reflects the ethos of scientists. How important is the role of professors in academia when it comes to the behavior of scientists in the real world?

By Victoria Samanidou, Professor of Analytical Chemistry, Aristotle University of Thessaloniki, Greece.

Ethics in research should be sine qua non (a prerequisite) – and the same should apply to all aspects of life. It's common sense, right?

Children learn to distinguish between 'good' and 'bad', between 'right' and 'wrong' early on. Older family members typically instill moral values in children and, when that is not enough, behavior (or character) can be further amended by teachers (to some degree). As people 'grow up' they continue to develop – a process that can be influenced by numerous external factors. And as scientists mature, they pass through various stages that inevitably affect their professional attitude. Fortunately, if they are open minded, they can be inspired and guided by their mentors.

And here arises the first question. Do we academic professors spend sufficient time and effort when it comes to contributing to the creation of conscientious and responsible scientists? Undoubtedly, we provide our students with knowledge on core science, on applied science, on laws in science... But do we satisfactorily – or, more importantly, do we successfully – teach ethics in science?

According to Aristotle, "ethos" (the Greek word for character) covers issues of wisdom, virtue and good will. What is our role in the construction of the ethos of developing scientists in terms of 'politically correct' behavior in the scientific community? Are we

"What is our role in the construction of the ethos of developing scientists in terms of 'politically correct' scientific behavior?"

responsible for their conduct beyond graduation and, if so, to what extent? Do we provide them the right standards to follow? In my view, we certainly have the power; unfortunately, power can be tough to handle, so we must constantly question if we are using it well – and in the right way.

In the earliest semesters, students learn that they must cooperate with each other – a skill that they will increasingly rely on and need to improve as they work towards common targets. In modern

"What happens when bad behavior is 'copy-pasted' from the world of publishing and into the real world?"

science, teamwork is very often required – but rivalry in the professional arena can be so cruel that scientific skills are not the only ones that are acknowledged...

Scholars immediately confront 'research' when they are asked to carry out undergraduate, postgraduate or PhD theses. From the beginning, they are taught that research is the tool to promote science. But they should also be taught that certain rules must be followed to promote research. Even earlier, young scientists may misbehave by cheating in exams. It may appear a mild misdemeanor to professors, but it is unfair to other students. It could also be the first step down a slippery slope of continued deviation from the rules. The result? Researchers who fabricate or falsify results, researchers who don't publish results that don't fit to the initial hypothesis, researchers who give no credit to other scientists or steal ideas and works, authors who commit plagiarism or don't properly cite previous work, and so on...

What happens when bad behavior extends beyond "the world of publications", but is 'copy-pasted' into the real world as well? What would happen if results are altered in the pharmaceutical industry to promote an insufficiently (or inappropriately) developed drug formulation? And what about the use of adulterants and clandestine additives in the food industry with no concern for the risk to consumer health? The addition of melamine to milk and infant formula to skew protein analysis – with the lethal consequences – is a well-known example. And it was a scientist's idea.

Can we prevent similar situations in the future? Animal welfare, human rights, privacy, health and safety, environmental consequences, and so on, should always be respected – in every aspect of life. To that end, we must continually reinforce these values to ensure that ethics is an attitude to life and not a legal obligation.

Using the special words of Dionisios Solomos – a national poet of Greece – we should all be striving for "Omorfos kósmos, ithikós, angeliká plasménos." – a beautiful world, ethical, created by angels.

Magnetic Attraction

Liposomes have great utility in bioanalysis and drug delivery. Could bringing the two fields together provide new analytical tools?

By Katie Edwards, research associate/ lecturer, Department of Biological and Environmental Engineering, Cornell University, Ithaca, New York, USA.

Liposomes have been widely investigated and used for drug delivery applications since the 1960s (1–3). Their interior volume has been employed to entrap hydrophilic therapeutics, relying on encapsulation to provide a large payload, confer stability to sensitive molecules, and facilitate delayed release. Moreover, the exterior of the lipid bilayer has been functionalized with molecules capable of providing targeting of liposomes to specific tissue types using antibodies, for example. The incorporation of PEGylated lipids can help minimize clearance of liposomes by the reticuloendothelial system. Tailoring the size of liposomes helps target the leaky vasculature of tumor tissues as oppose to healthy tissues. Additionally, the incorporation of pH or thermosensitive lipids gives greater control over the release of the payload.

Many properties that make liposomes advantageous for drug delivery also make them attractive for analytical applications. For example, high concentrations of hydrophilic molecules, such as visible or fluorescent dyes, may be encapsulated, while the exterior

"Many properties that make liposomes advantageous for drug delivery also make them attractive for analytical applications."

may be functionalized with antibodies to serve as a more sensitive alternative to the traditional enzyme/substrate-

based amplification in enzyme-linked immunosorbent assays (ELISA) (4). The lipid bilayer can support non-traditional biorecognition elements, such as gangliosides, to allow targeting of toxins, while entrapping DNA within liposomes can serve as templates for signal amplification in immunoPCR-type applications (5). These and a wide array of other in-vitro analytical techniques using liposomes have been thoroughly reviewed in other resources (6, 7).

One of the key challenges with particle-based labels, including liposomes, in bioanalytical techniques is overcoming the mass-transfer limitations that lead to suboptimal detection limits and prolonged assay times. Recently, a method was developed that combined the encapsulation of high concentrations of fluorophores with the ability to direct DNA probe and oleic acid-iron oxide functionalized liposomes via a magnetic field to an underlying binding surface in a sandwich hybridization assay (8). This microtiter plate-based approach yielded a lower limit of detection, decreased reagent usage, and a shorter assay time compared with the same assay performed in the absence of a magnetic field – and it could be applied to standard ELISA-type formats. In this example, a magnetic field provides direction to the underlying surface to promote binding events by the liposomal signaling species (8). However, other analytical uses of magnetic liposomes include their utility as biocompatible cell-sorting options (9) and as vessels to store and pre-concentrate substrate molecules for flow injection-based enzyme assays (10).

In vivo, liposomes using entrapped or membrane-embedded paramagnetic or superparamagnetic species have been used to provide contrast in magnetic resonance imaging (MRI) applications (11). And there have been investigations with magnets to direct iron oxide functionalized liposomes to tumor sites (12) as well as to provide localized magnet-induced hyperthermia (13). Other studies have combined magnetic species with thermosensitive lipids in liposomes to afford content release caused by localized heating (14).

Historically, great parallels have existed between the drug delivery realm and bioanalytical techniques using liposomes, and yet although these two areas have drawn from each other, they have advanced independently. When I consider coupling recent advances in magnet-enhanced

drug delivery with developments in biorecognition and the functionality that can be engineered in microfluidic systems, it seems that – through combined efforts – a whole realm of yet unrealized analytical tools are possible.

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Don't Neglect Protein QC

Protein samples need – and deserve – special treatment, if you expect the best results from your experiments.

By Dieter Braun, Professor of Systems Biophysics, Ludwig-Maximilians-Universität München, Munich, Germany.

We hear it all too often: the study of biological systems is becoming increasingly complex. However, I'd like to ask, is it also becoming quantitative? Not in the sense of measuring more and more parameters, but in measuring basic parameters with an increased level of precision? Sadly, this is probably not the case.

From my own experience and that of my colleagues working in protein binding biotechnology, it is clear that little consideration is given to the handling of proteins in both academia and industry. A red warning light should be flashing for anyone needing to rely on quantitative, experimental data!

The evidence suggests that, all too often, even simple rules in protein preparation have been forgotten or, even worse, ignored due to limited time or resources. Scientists think that they can get away with not implementing even basic quality control when handling proteins. Nevertheless, proteins are special and need special care to yield solid and reliable results.

"Ask yourself: are there enough test measurements at hand to be sure that the protein is still happy and intact?"

For example, proteins are obtained from other laboratories or bought directly from suppliers. And, without even checking them internally, it is assumed

"In reality, taking proper care of your protein samples is fundamental to performing solid science"

that the proteins "should work". But, sometimes a very small change of buffer conditions lead to agglomeration or dysfunctional, unfolded states. A couple of simple questions need answering: did I really check the robustness of the buffer system? Does the melting temperature or circular dichroism of the protein indicate that it is in a well-defined folded state?

Everybody knows that checking the effects of freezing and thawing on protein samples is cumbersome, but it is necessary to guarantee the reproducibility of the subsequent measurements. Ask yourself: are there enough test measurements at hand to be sure that the protein is still happy and intact? In bad cases, proteins may not survive storage for one hour at room temperature...

The list of possible problems when handling proteins is long and well known to people in the field, but because we get used to ordering oligonucleotides on a large scale, it appears that some people think we can treat proteins the same. It is not sufficient to buy a lyophilized protein, dissolve it, and begin experiments. Even if you are lucky, only 10 percent of the protein might be in an active conformation.

I have the impression that lab throughput has become so important that checking protein loss, purity, activity, agglomeration by gels, circular dichroism and folding melting temperature (to assess the quality and buffer conditions for the protein) has become a luxury. And let's not forget the need to address and evaluate protein losses from pipetting and adsorption.

In reality, taking proper care of your protein samples is fundamental to performing solid science. Despite all the progress, proteins remain quite tricky to handle. I dearly hope that basic quality control in protein handling will gain the attention it deserves and start to improve again. Otherwise, bionalysis will not become more complex or quantitative, it will simply become noisier.

Forensic Myths and Methods

Don your purple nitrile gloves and grab a swab or two, as we delve into the realities of crime scene investigation.

Everyone's a forensics expert – or so our viewing habits have led us to believe. On both the small and silver screens, DNA evidence has long been presented as the fulcrum on which a case balances – the final nail in the coffin of guilt. The result of this altered reality? Illogical or inadequate questioning from lawyers and unrealistic expectations from juries...

Far from being the climactic clincher in murder trials, forensic science is complex and laborious – and is often put to use in far more prosaic settings than the criminal court. Certainly, for forensic specialists, the reality is far more grit than glamor, with work more likely being characterized by delays, frustration and contamination – an estimated 300,000 backlogged cases across the USA alone is cause for concern.

With scope for improvement, is it time for analytical scientists to rise to the challenge? Christopher Palenik (Vice President and Senior Research Microscopist at Microtrace) and Glen Jackson (Professor of Forensic and Investigative Science at West Virginia University) talk about the methods and the myths in forensics – and the changing role of analytical science.

The Elegant Application of Science

With Christopher Palenik

Effectively growing up within the discipline of forensic science, I've been able to watch it from a relatively unique perspective. Some of my earliest memories involve sitting with my father in his microscopy laboratory in our family's basement, watching him work and hearing him talk about cases, instruments and the elegance of scientific problem solving. As a child, we had scientists from around the world sleep over at our home as they passed through in the course of their business. Visitors included detectives, lab directors, and scientists from Scotland Yard, the USSR and Germany. The dinner conversations were very often amazing. I remember hearing the late Robin Keely of Scotland Yard discussing how he analyzed an entire handgun in an scanning electron microscope (SEM). Since those early days, much of my education and many of my summers were focused on pursuing this path. I've always enjoyed pure scientific research, but by the end of graduate school I knew that I wanted to work on questions of a more applied nature.

At Microtrace, we work on a wide range of interesting and unusual questions, ranging from art and antiquities to capital murder cases. Our projects are often topical, many are high profile, and (to my enjoyment) many require some aspect of scientific research.

The "protocolizing" problem

There is a lot of good work being done both by researchers and practitioners to advance the capabilities of science in forensic disciplines. As a discipline, the most significant changes taking place in our country are the setting of national guidelines and standards both for laboratories and the practice of forensic science, and great efforts are being placed into ensuring that the significance and interpretation of results are maximized while at the same time remaining firmly rooted in science. What concerns me is that the process of standardizing and "protocolizing" is attempting to fit everything into a step-by-step process. For some types of analyses, such as drugs and DNA, where each sample is processed similarly, this works well. Crime labs are best equipped to deal with the routine, and – for the most part – they do a good job. Unfortunately, these standardized approaches can begin to break down in more complex cases, such as those involving the synthesis of disparate items of physical evidence or evidence that is presented without comparison samples. Trace evidence is a discipline that can encounter literally anything, from dust to pollen to building materials to nanoparticles – and many of these materials are encountered only sporadically. Indeed, the full suite of materials that may be encountered makes a "standard" analysis impossible, and the application of less thoughtful approaches can reduce significance or lead to unsupported conclusions. Many investigations would benefit from a generalist forensic overview to direct and synthesize results in more complex investigations.

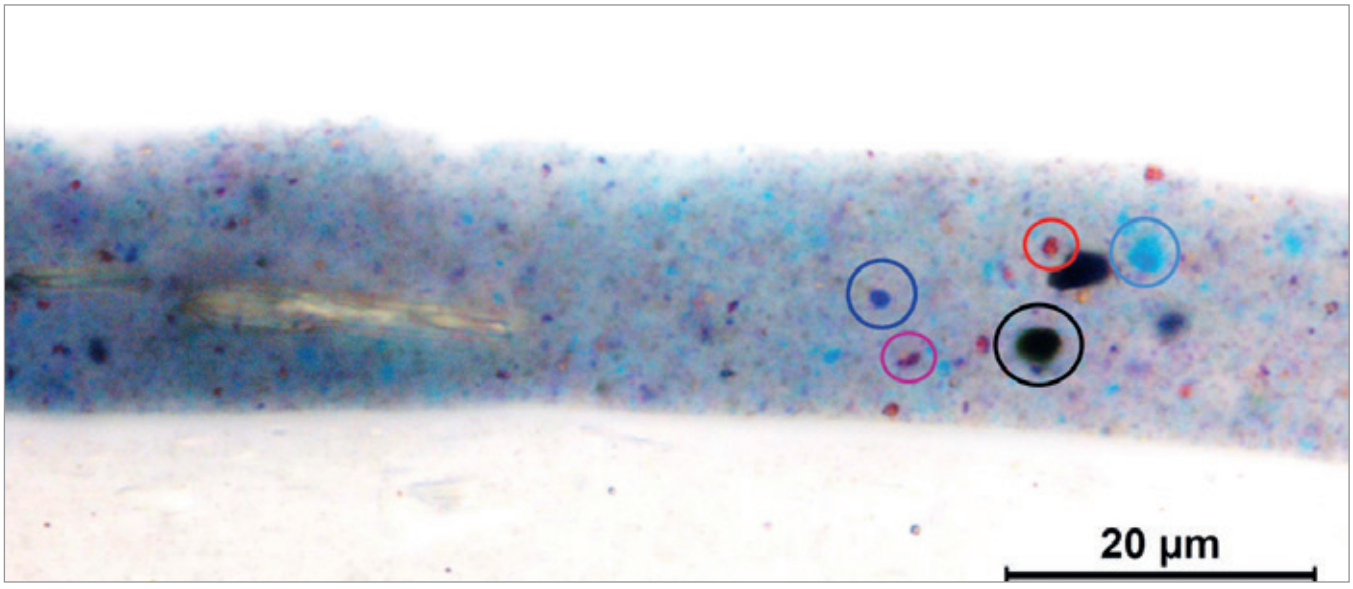

Figure 1. Thin section of a blue automobile paint observed under a light microscope. A number of different colored pigments (circled) have been combined to produce the visible paint color. Confocal Raman microspectroscopy provides a means by which these individual pigments can be specifically identified.

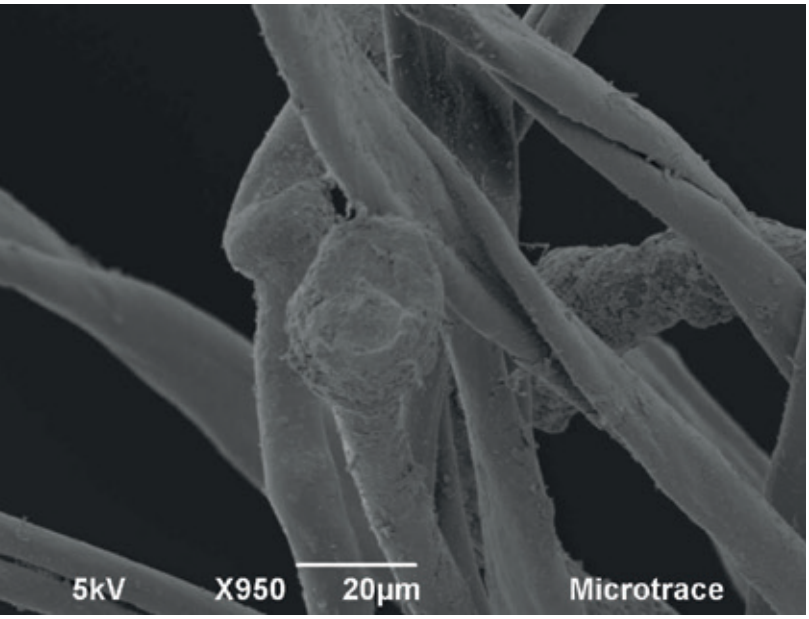

Figure 2. Scanning electron microscope image showing the characteristic "bulbous" morphology of a polyester fiber that resulted from the recoil of the fiber after being stretched and severed by a bullet as it penetrated a sweatshirt.

"So many of the most cited historical forensic successes have hinged on the clever and often elegant application of science to an unusual set of circumstances."

For me, the most interesting changes in the field involve analyzing these unusual samples and conducting research in new areas applicable to forensic science. The majority of trace evidence analysis is conducted by a handful of techniques that include light microscopy, SEM/EDS, and FTIR spectroscopy. The instruments utilized are wholly capable of proving a great deal of information; however, there is almost always room to

Forensic Tech to Watch

In the discipline of trace evidence, the National Institute of Justice supports a variety of applied forensic research aimed at expanding the scope of the discipline. Some of the research focused around more difficult types of evidence, such as sand and soil, colorants (pigments in paint and dyes in fabrics), and nanoscale evidence. The techniques supporting this research rely on relatively new (to the forensic field) instruments, such as Raman microspectroscopy and higher-resolution electron microscopy, as well as the automation of these methods. For example, pigments and dyes are responsible for the color of nearly every item on and around you. Yet these colorants are present typically at levels of about 1 percent, which has made them traditionally very difficult to analyze. The maturity of confocal Raman microspectroscopy as a robust, bench-level method, provided the first means by which pigments could be analytically identified on a reliable basis.

With the assistance of an NIJ grant, we have had the opportunity to systematically study a large population of pigments and dyes, which has resulted in a foundation that permits scientists to identify these pigments in a practical manner. We have successfully applied this pigment identification to casework and utilize it on a regular basis. Other labs that are beginning to acquire Raman microscopes will have the ability to implement this approach as well; however, new approaches can take years or decades to develop into a mature technology that is ready for broad adaptation. A panel at the 2016 American Academy of Forensic Sciences on "Transitioning new technologies," characterized the challenges that exist from research through discipline wide adaptation.

Despite the challenges, the ultimate goal of forensic research is to provide new types of data – and more data – that can be used to improve the significance of evidence and provide better constraints on the uncertainties of results, while providing more efficient analyses.

Christopher Palenik

With an educational background at the University of Chicago and University of Michigan that spans chemistry, geology, materials science, and nuclear engineering, Chris' formal education culminated in a PhD thesis focused on the world's only naturally occurring nuclear reactor. This academic basis was counterbalanced by practical internships at the Bundeskriminalamt in Germany (the German Federal Police Crime Laboratory), the Internal Revenue Service National Forensic Laboratory, and a post-doctoral fellowship at the Federal Bureau of Investigation. Through this educational background, Chris developed an intimate familiarity with a wide range of materials and microanalytical approaches.

Following his formal education, Chris has had the fortunate opportunity to continually expand his knowledge while applying it to a wide variety of unusual investigations. With projects that have included capital punishment cases, military court martials, and civil litigation and clients from pharma, food, environmental and nanotechnology industries, he has encountered a range of scientifically fascinating and newsworthy cases. Some of the more unusual samples have included a holocaust era lamp shade allegedly made of human skin, baseball bats and balls signed by the likes of Joe DiMaggio and Shoeless Joe Jackson, materials from unlicensed cosmetic surgeries, and a wide assortment of alleged pills, animal parts, and other miscellanea allegedly encountered during the consumption of food. The application of microscopy to a variety of unusual questions through a rigorous application of the scientific method provides an elegant link among this seemingly disparate ranges of clients, materials, and fundamental sciences. The outcomes of these investigations have been accepted in courts, published in peer reviewed journals, presented at meetings, and featured in media ranging from the National Geographic channel to NBC's Today show. Chris is a fellow of the American Academy of Forensic Sciences, and serves in appointed positions on the North Carolina Forensic Science Laboratory Advisory board and the National Institute of Justice Forensic Science Standards Organization (OSAC).

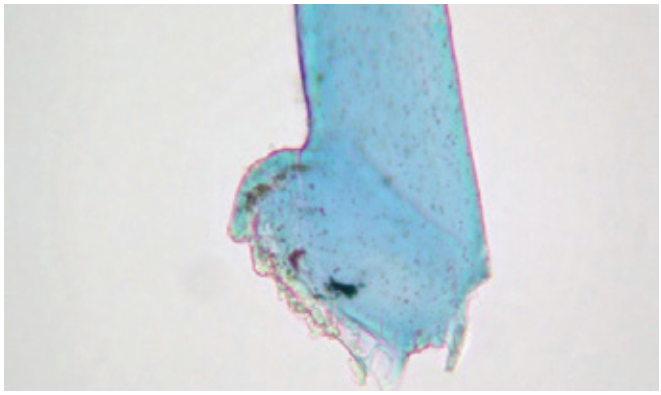

Figure 3. Light micrograph of a polyester fiber after being severed by a bullet. The characteristic "bulbous" morphology of the fiber is apparent as are fine metal particles of lead that were transferred from the bullet to the severed end of the fiber during the brief bullet-fiber contact; a prime example that illustrates that Locard's exchange principle applies to even the briefest and most minute contacts between materials.

improve the quality and extent of information provided through better sample preparation and more detailed analysis and higher level interpretation. You may notice that these areas of improvement center around the ability of the analyst more than the instruments and standardized method – and they are not unique to forensic applications.

Similarly, the life span of most trace evidence laboratory equipment is dictated more by the age of the attached computer than by technology improvements or failure of the instruments themselves. Due to budget constraints, many labs do not have concrete plans for updates and replacements of instruments. To that end, our laboratory at Microtrace was founded with the goal of providing a resource for those seeking answers to difficult problems that can be approached through the thoughtful application of scientific methods of investigation. We have been providing scientific assistance in criminal, civil and industrial forensic investigations for over 20 years, with analytical services that are utilized by prosecutors, defense attorneys, police, forensic laboratories, and the news media. Our analytical expertise in the identification of single small particles and traces of microscopic evidence permit us to exploit remnants of almost anything that might be left at a crime scene or carried away from it.

Pushing the right buttons?

Forensic practitioners come from a wide range of training and educational backgrounds. While many forensic scientists originate from traditional scientific programs (for example, chemistry, biology, geology), the rise of specialized forensic programs has resulted in more and more scientists with specialized training in forensic science. Depending on the program, this can be good or bad. In some cases, the forensic training is heavily based in the fundamental sciences, so students end up with a fundamental background in not only the forensic application of analytical methods but also their theory. There are also programs that are much more applied, which can result in graduates who know how to "press the right buttons" (so to speak), but don't have a strong understanding of the theory behind these instruments or the properties of the materials that they encounter. The latter situation can make students appealing job candidates, since they already know how to use common instruments; however, such limitations inevitably show up as weaknesses either in the course of daily work or in court testimony.

"If anything needs to be improved in analytical forensics, it's the right kind of thinking."

I certainly think that forensic science is often seen as a more relevant and accessible science than traditional sciences, such as chemistry or physics. I'm pretty certain this is, in part, due to the popularity of the discipline with the media and its seemingly more direct applicability to topical issues. The positive upshot is that few forensic science programs have difficulties drawing students. But the counterpoint is that many students come into the program more fascinated by the social aspects of the discipline than the technical and scientific efforts required to obtain this information...

If anything needs to be improved in analytical forensics, it's the right kind of thinking. So many of the most cited historical forensic successes have hinged on the clever and often elegant application of science to an unusual set of circumstances. As long as humans are involved in the process of analysis, there is room for creativity in the application of the scientific method at the bench level. Unfortunately, quality systems in laboratories tend to discourage this type of creativity, because deviations from protocols require additional effort on the part of both the analyst and supervisor – both of whom are generally already backlogged...

Christopher Palenik is Vice President and Senior Research Microscopist at Microtrace, Illinois, USA.

Creativity Versus Conservatism

With Glen Jackson

Like most academic researchers, my responsibilities include teaching, research and service. In research, my goal is to try to advance the capabilities of forensic science – and bioanalytical chemistry – through the development of novel instrumentation. More specifically, the instruments and capabilities we develop are related to mass spectrometry.

I have mixed feelings about how well analytical chemistry is helping the practice of forensic science. On the one hand, there are plenty of examples of amazing capabilities being developed in a wide variety of analytical disciplines. On the other hand, the conservative nature of forensic science generally discourages the adoption of new technologies. Because of its cautious nature and disconnect with advances in academia, forensic science practice struggles to benefit from exciting new developments the way that other disciplines do.

How to battle the backlog

Crime labs are having a difficult time trying to keep up with the challenging demands of modern seized drugs, which often contain novel synthetic psychoactive compounds like cannabinoids and bath salts (cathinones). Labs are struggling to identify the exact isomeric nature of these new psychoactive substances, and struggling to meet legal demands like structural or functional analogs of existing controlled substances. In other areas, it will be interesting to see if the establishment of the NIST OSAC organization and their standards leads to any notable changes or improvement in the practice of forensic science. I've been serving on the seized drug subcommittee of OSAC throughout 2015 and my colleagues and I are uncertain as to what will become of our effort. Will the standards be required or legally enforceable or simply used as optional guidance, like the current SWGDRUG guidelines?

GC-MS and FTIR spectroscopy are the bread and butter of most forensic labs, and SWGDRUG guidelines virtually require the use of one or both when confirming the identity of drugs. As noted, there are an increasing number of drug seizures involving novel psychoactive substances in which GC-MS and FTIR may only narrow the identity to two or four isomers of a drug. In such cases, chiral chromatography (GC or LC) or GC-IR/LC-IR will be necessary to reach isomer differentiation. I'm still amazed that fast-GC has never caught on the way I thought it would. Labs complain a lot about backlogs, but they could easily purchase a narrow-bore capillary column for their existing GCs and halve the time for their separations, without any loss in chromatographic performance. For unknown reasons, fast-GC just never caught on...

The CSI effect(s)

My colleagues and I have written before about the so-called "CSI effect". Two important factors about "the CSI effect" are that: i) there are actually many different effects, not just one; and ii) the effects of legal and forensic dramas started well before CSI first aired in 2000. For example, applicants to forensic science BS degree programs accelerated in the early 1990s when X-files and Law and Order were new and very popular. However, it is also true that the number of universities offering degrees in forensic science certainly has correlated with the popularity of the CSI series, and there are now many forensic science programs in each state.

Forensic dramas like CSI have done an excellent job at turning students on to STEM degrees like forensic science, pathology, anthropology and entomology. Most students understand the differences between reality and fiction, but there's always a minority of students with unrealistic expectations of themselves and the limits of science. (Unrealistic expectations by the public are another example of the CSI effect.) Graduates from our programs (BS or MS in Forensic and Investigative Science at WVU) report satisfactory job placements or continued educational advancement, so we're comfortable with continuing to educate students in these majors. Plus, they are actually employable in a variety of industries, so we're comfortable with their employment opportunities.

Generally speaking, criminalists and forensic chemists are well

> "Forensic science could establish itself as a core scientific discipline with the ability to help elevate the other sciences instead of just borrowing their techniques."

Glen P. Jackson

Glen Jackson is the Ming Hsieh Distinguished Professor of Forensic and Investigative Science at West Virginia University. He is also co-editor in chief of the newly-launched Elsevier journal "Forensic Chemistry", a fast, high-impact journal devoted to all basic and applied areas of forensic chemistry. He also holds appointments in Biology and the C. Eugene Bennett Department of Chemistry. Before moving to WVU, he was an Associate Professor of Chemistry and Director of the Forensic Chemistry Program at Ohio University. Dr. Jackson is the author or co-author on two patents, more than 55 publications, and more than 100 presentations.

Jackson is currently the chair of the Forensic and Security Interest Group at ASMS and recently served as Program chair for the 2015 ASMS Sanibel conference on Forensic and Security Applications of Mass Spectrometry and SciX Conference 2015. He is a member of the NIST OSAC subcommittee on Seized Drugs, teaches several forensic-related mass spectrometry workshops each year and is an active forensic chemistry consultant.

prepared for their casework. Graduates from FEPAC-accredited programs are generally well-rounded scientists, especially if they have a master's degree in addition to their bachelor's degree. I would say that there is currently a significant absence of expertise in method development and method validation in the workforce; the lack of experience stems from the fact that, until very recently, there were virtually no forensic science PhD programs to foster rigorous scientific experimentation skills.

"Forensimetrics" and the future

There is a lot of confusion in the forensic community about uncertainty of measurements. Many practitioners think that qualitative measurements – like drug identifications – cannot be reported with an error because there are no numerical answers. However, this opinion ignores the fact that qualitative determinations are not perfect and therefore must have some uncertainty. Although error reporting for quantitative measurements is quite well understood by practitioners, there is simply no agreement on how or whether to report errors for qualitative determinations. It would be great if the NIST OSACS could provide guidance on such error reporting, but that's not likely to happen any time soon.

Most analysts are not really challenged scientifically when testifying. It is simply not realistic to expect defense lawyers to challenge an expert witnesses about their knowledge of fundamental concepts in chromatography, mass spectrometry or FTIR. However, defense lawyers are becoming more educated in cross-examining expert witnesses, and I'm sure we will soon see examples of inadequate and embarrassing testimony by analysts about instrumental methods of analysis. (see Making a Murderer from last month's issue: tas.txp.to/0316/MAM)

Right now, we're in an exciting position – at the birth of forensic informatics or "forensimetrics". I honestly think that by solving problems within their own discipline, forensic scientists could provide solutions with equally profound applications in other disciplines. When you think about it, all science is evidence based because we experiment with the world around us. Forensic scientists are becoming very proficient at using Bayesian networks to combine and weight complicated and seemingly unrelated evidence to compare one hypothesis with another. Almost every other discipline that depends on scientific evidence to make inferences about a sample could benefit from this sophisticated understanding. It is but one example of how forensic science could establish itself as a core scientific discipline with the ability to help elevate the other sciences – instead of just borrowing techniques.

Glen Jackson is Professor of Forensic and Investigative Science at West Virginia University, USA.

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Chasing the Dioxin Detection Dragon

For over 50 years, we have been continually striving to push the limits of quantitation for dioxins. But where do we stand today – and what's our next plan of attack?

By Jean-François Focant

Before I consider how far we have come in the chase after the lowest detectable level for dioxins, I think it's good to look back to see where we started. The topics I discuss in this article are based on work from several colleagues, and I'd like to acknowledge them here: B. L'Homme, C. Calaprice, D. Krumwiede, H. Mehlmann and, finally, D. G. Patterson Jr, who also wrote a recent article on a similar topic – To Attograms and Beyond – in The Analytical Scientist towards the end of last year (http://tas.txp.to/0216/Patterson).

Patterson delved into biomonitoring studies, so I'll be brief and just note that they aim to discover how much of a particular contaminant – dioxins in this case – actually end up in our bodies and can be done in two ways, either by using environmental measurements and complex models to predict or by direct human sample measurement. The second option makes biomonitoring easier, because you have a direct measurement and don't need to do any modeling. But it's also more challenging for analytical chemists. The levels are much lower, so detection limits become increasingly important. It's a balancing act then, but I believe direct biomonitoring is the way to go.

In the early days

Back in 1945 the spraying of DDT was tested on beaches in the state of New York with the aim of eradicating mosquitoes – while children gleefully played in a fog of the chemical. In the 1950s, on aircraft returning from exotic locations DDT was sprayed in the cabin. Of course, in such exposure incidents the route is obvious and we need less sensitive methods. (As an aside, even in 2015 you may have been in an aircraft when the doors have closed and the pilot has stated, "Don't be afraid [...] it's harmless" as the flight attendants pass through the cabin with their best smiles, waving aerosol cans of some chemical – probably permethrin. Maybe in another 50 years we will look back with surprise on this practice as well.)

Following these DDT "tests" – and the dawn of realization – researchers published papers in the 1960s on pesticide storage in human fat tissue with limits of detection in the ppm range (1). Not bad for the day (and I dream of dioxins at ppm levels from an analytical chemistry point of view!) In 1965, researchers measured DDT and DDE pesticide residues in human milk as well, using GC-ECD for quantitation (2). However, DDT is just one molecule and the levels were high so the work was relatively straightforward. Thankfully, the levels of all persistent organic pollutants (POPs) have been decreasing since the 1960s. And though the decrease is positive for humankind, it does represent an analytical challenge.

Winds of change

In 1988, Patterson et al. published a landmark paper showing the correlation between adipose tissue and serum levels of 2,3,7,8-TCDD – and that's why we no longer use adipose samples (3). I'm surprised this paper has not been cited more often for that very reason. (Any group doing serum analysis should be citing this paper as a validation of their work.) But along with a shift to serum analysis, demand for sensitivity increased yet again. After all, there is more fat in milk (>5 percent) than in serum (<0.5 percent), which results in a respective shift from ppt levels to ppq levels in serum. On the plus side, participation rates in volunteer studies are on the up...

Lower limits of detection demand the best chromatography and highest sensitivity afforded by magnetic sector MS systems. And at the same time, we must not lose sight of reproducibility. Currently, when measuring dioxins at the femtogram level, we can expect RSD values of 20–30 percent. So, if we consider a move to the attogram level, what variation can we expect? Assuming an adapted Horwitzian "trumpet" curve approach, we could predict 50–60 percent, which is unacceptable.

In today's routine biomonitoring labs, we

can expect that for 2,3,7,8-TCDD at the ppt level (it's actually lower) in a 5 ml serum sample we will actually inject around 15 fg (with a 60 percent recovery). So we have 20–30 percent RSD.

But let's not forget the drive towards smaller samples. If I ask you to choose between giving 10–20 ml of blood or taking a finger-prick test, I can guess which you would prefer. Certainly the right direction – no surgery, no hospital, no syringe, no fear – but now we're talking about 20–50 µl, and our need for sensitivity just increased again. At such sample volumes, we gain the potential to study those who are not typically included, such as the very young and very old, or isolated populations – or even dolphins.

We cannot forget our uncertainty; in 20 µl of blood there will be only 0.1 fg of 2,3,7,8-TCDD – that's 100 ag, a real challenge. And even though it is at very low levels, it's also the most toxic, so it serves as an excellent benchmark. Another Patterson paper appeared in 1996 that boosted sensitivity with GC×GC, getting down to around 335 ag for 2,3,7,8-TCDD (4). We later revisited this work with modern instrumentation in 2011 using cryogenic zone compression (CZC) with a loop modulator (5) on a high-resolution magnetic sector MS system. And in doing so, we are edging closer to our 0.1 fg goal.

Limits to limits

Working at such low levels poses a number of challenges. Some are instrumental; in particular, the trade off between sensitivity and accuracy (I go into more detail in a presentation I gave at the 10th International Symposium on Recent Developments in POPs Analysis in 2015: http://info1.thermoscientific.com/pops-analysis). But at these levels, we can also be confounded by isobaric species contamination of our standards. And as Ferrario et al. noted (6) "It is ironic that the advances in technology that have allowed the progressive lowering of detection limits have reached a limit imposed by the very contaminants the technology was designed to measure." In other words, even if an instrument has a limit of detection of 50 ag, without a dedicated cleanroom, ultrapure standards, and due care, the limit is essentially unattainable.

How do we fight against the challenges? First, we need to intensify our efforts in sample preparation. As noted, DBS analysis followed by micro liquid-liquid extraction or micro-extraction by packed sorbent (MEPS) is one option. Another is volumetric absorptive micro-sampling (VAMS), a method published in 2014 (7) that is very interesting in terms of reproducibility, especially given that quantitative analysis is the aim. I go into detail about the pros and cons of these methods in my presentation, but I'd like to note that although the VAMS method is not as precise as current routine methods, it certainly feels like a step in the right direction.

After sample preparation, we must find novel ways to optimize our instrumental measurements. We need to use the most sensitive instruments – today, that is still magnetic sector MS systems – but there is still room for further improvement. Scientists, including some manufacturers, are exploring a number of areas with a view to improving sensitivity, including ion volume geometry and emission current to improve ionization efficiency; others offer alternative ionization methods, for example, APCI GC-HRMS; and the potential for multi-collector GC-HRMS. And I've already mentioned the real potential of CZC to optimize the GC separation step. Thermo Fisher Scientific is also working to further improve its time-controlled (t)-CZC approach, which hopefully becomes commercially available in the future, to enhance the signal of certain selected peaks (8) – these are all moves in the right direction.

So, where do we stand today? The good news is that a renewed focus on sample preparation and the evolution of technology are coming together to the point where the >0.1fg TCDD target is reachable; however, we must not forget that continual evaluation of measurement uncertainty is essential as we explore the attogram world.

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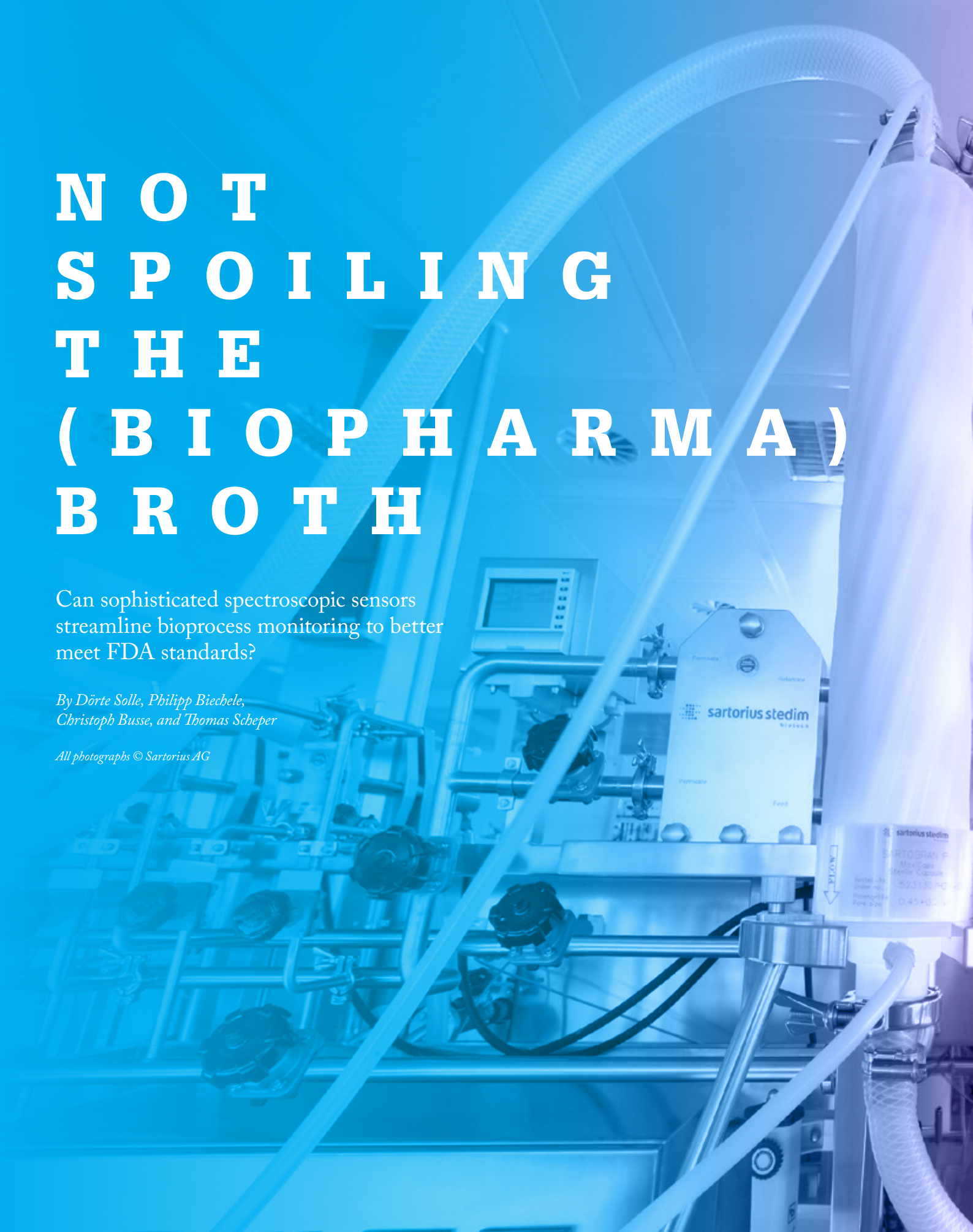
NOT SPOILING THE (BIOPHARMA) BROTH

Can sophisticated spectroscopic sensors streamline bioprocess monitoring to better meet FDA standards?

By Dörte Solle, Philipp Biechele, Christoph Busse, and Thomas Scheper



The ability to measure all process variables is of great importance in the field of bioprocess monitoring, control, documentation and approval. In 2002, the FDA launched a new initiative to promote innovation of process analytical technologies within the pharmaceutical industry. The aim? To create processes that generate products of ensured quality. How? By using effective and suitable sensor systems for measuring quality-related process variables.

Process analytical technology (PAT) has been part of biotechnology, biopharma production, and the food industry for some years, with some of the sensor technologies used for bioprocess monitoring well established and reliable. And yet, despite newer systems being available for bioprocess monitoring, many are still not commonly used – most likely because the implementation of new sensor systems into already approved processes would lead to a time-intensive and expensive re-approval of the process.

Three's a charm

Most bioprocesses are three-phase systems; the cells are dispersed as a solid phase in a liquid medium phase, which is aerated by a gas phase (1 – please note that all references can only be found in the online version of this article: tas.txp.to/0316/sensors). The interactions among these three phases are complex, and there are several types of variables to consider among these three phases: physical (for example, pressure, temperature), chemical (for example, pH, pO_2, nutrients, and metabolites) and biological (for example, biomass concentration, cell morphology). Monitoring and control of physical variables are common during bioprocesses, with chemical variables like pH and pO2 also being established. For nutrients, metabolites and the biological components, however, sensors are either not established or are not available.

Biological components often react very sensitively to environmental changes, sometimes resulting in adverse effects on activity or reproducibility of the process. Detailed analysis and monitoring of the three phases – combined with deep process knowledge – is therefore necessary to control and optimize cultivation processes for high product concentration and quality, as well as for documentation purposes.

At present, most variables are monitored off-line by sample taking or by at-line HPLC. Off-line sensors are possible, but less desirable because of infrequent sampling and long response times; without closely following important process dynamics, efficient control of the process is not possible (2). In-line or in-situ sensors are essential in bioprocesses so that the actual state of the bioreactor can be controlled and monitored at all times. However, developing sensors for bioprocesses is a complicated challenge, because sensors interfaced directly with a bioreactor must be robust enough for the harsh condition of sterilization, and must not be affected by fouling or by interference with the medium. Subsequently, not all analytical tools from the laboratory are suitable for in-line monitoring of bioprocesses in an industrial environment.

"There are several advantages to using spectroscopic sensors. No sampling is needed (except for calibration), there is no interaction between the sensor and analytes, and several different process variables can be determined simultaneously."

State-of-the-art tech

In bioprocesses, changes in the concentrations of several gases, especially oxygen and carbon dioxide, provide information about cell growth, metabolism, and productivity, and can be monitored by off-gas analytics – very common in bacterial and yeast cultivations.

The concentrations of dissolved gases, including oxygen and carbon dioxide, as well as various nutrients, metabolites and products, need to be monitored in the liquid phase. Classical electro-chemical sensors can be used for pH, pCO_2 and pO_2 measurements in steel bioreactors, using standard ports. For disposable bioreactors, optical chemosensor systems – also called optodes – can be used for those variables. Optodes are based on the interaction of a matrix-embedded indicator and the analyte (3) and can be pre-sterilized within the disposable containers by γ-radiation and can be connected to optical fibers via transparent materials, such as glass. Such optical chemosensors are used to monitor chemical variables, but for nutrients and other biological variables, spectroscopic measurement is recommended. Common spectroscopic methods for bioprocess monitoring are focused on the spectral range from UV to MIR, including fluorescence and Raman spectroscopy. Various bioprocess variables can be measured in different spectral ranges (4–16) (see Figure 1).

There are several advantages to using spectroscopic sensors. No sampling is needed (except for calibration), there is no interaction between the sensor and analytes, and several different process variables can be determined simultaneously. However, chemometric data analysis is required for spectroscopic bioprocess monitoring to extract relevant process information.

The most important variable in bioprocesses is the biomass – the solid phase in the complex, three-phase system. The biomass can be characterized by its concentration or by its metabolic activity, and different analytical methods exist to determine both of these. The measurement of the optical

density (OD) by turbidity is one of the most frequently applied technologies for biomass monitoring, but impedance measurements can also provide metabolic information about culture condition. And through so-called in situ microscopy (ISM) – microscopy directly in a bioreactor – it is possible to acquire pictures of the suspended organisms and to analyze the cell concentration, cell size, cell distribution, and morphology automatically by image-processing algorithms (42).

In addition to the biomass, the concentration of viable, metabolically active cells is of special interest in bioprocesses because they are the only ones able to grow and produce the desired product. The determination of bioactivity is possible by certain sensors; for example, turbidity probes allow inferences about cell size and morphology, impedance sensors can be used for the observation of lipid storage in yeast (43), and image analysis by ISM systems makes information on cell size and morphology accessible. However, special systems have been developed specifically to analyze the metabolic activity of cells. The oxygen uptake rate (OUR) is a robust indicator of the determination of cellular activity, and as one of the fundamental physiological characteristics of aerobic culture growth, it has been used frequently for the optimization of bioprocesses (44–46).

Getting on-line

Several sensor technologies provide an enormous amount of data, especially when spectra are generated via in-line sensors at high frequency. The data must be correlated to important

From UV to MIR: the biomass monitoring spectrum

Infrared spectroscopy includes spectral areas of near infrared (NIR, 740 nm to 1300 nm) and mid-infrared (MIR, up to 15000 nm). In general, IR light excites different vibrational modes of molecules. Each organic and inorganic compound has a special spectral IR signature from these vibrations. IR spectroscopy offers very fast, robust and sensitive multi-analyte information from the culture broth of bioprocesses. It is a non-invasive process analytical technology, applied in-line by direct beam or optical fiber.

MIR radiation excites fundamental rotational vibrations of functional groups from organic compounds. Molecules such as glucose, lactate, fructose, acetic acid, ammonia, and even antibodies (17) have a characteristic absorption spectrum which can be used to identify single components in bioprocesses quantitative, sensitive, and specific.

A high degree of water absorption appears in MIR spectra. However, in-line measurement in aqueous solutions is possible using appropriate fiber optic probes that incorporate attenuated total reflection (ATR) technology and Fourier transformation (18–20). The measurement principle of ATR probes results in a very short (only few µm) path length and cells cannot be detected because they are too large to enter the measuring zone.

NIR spectroscopy is also based on different vibrational modes, overtone and combination vibrations after excitation. Important targets are the O-H, C-H and N-H bonds. The NIR range is thus suitable for monitoring of substrates such as glucose and lactate, biomass, and the products of a bioprocess (17, 21). As a result of the lower energy of the NIR and the resulting overtone vibrations, the bands are much broader, often overlapping, and not as specific as in MIR spectroscopy (20). Thus, NIR spectroscopy has a more qualitative character, compared to the more precise and quantitative MIR spectroscopy. NIR spectroscopy offers a more global view to a bioprocess, e.g. by batch trajectory (22).

Due to its higher robustness, NIR spectroscopy is better applied for monitoring industrial production processes. MIR spectroscopy is well suited for process development and optimization due to its multiplexing technology and the fact that fragile ATR fibers are used.

UV/Vis spectroscopy uses ultraviolet and visible light (10–740 nm) to excite electrons of molecules, the observable transitions taking place at unsaturated bonds, such as in aromatics (11). A variety of analytes, substrates, metabolites, and products can be determined with UV/Vis spectroscopy, which has high sensitivity, and high resolution spectrophotometers can be compact, inexpensive, and robust, making these instruments interesting for industrial process applications (21). However, UV/Vis spectroscopy does not currently play a major role in bioprocess monitoring (23) despite the use of CCDs or photodiode arrays making UV/Vis spectroscopy even more attractive.

Fluorescence spectroscopy facilitates monitoring and control of many important molecules for bioprocesses, including proteins with aromatic amino acids (tryptophan), NAD(P)H (biomass), ATP, pyruvate, vitamins, pyridoxines, coenzymes, and flavins (12, 21, 24–27). Each fluorescence-active compound has a specific pair of excitation and emission wavelengths. Simultaneous measurement of several different fluorophores in the culture broth is possible by 2-D fluorescence spectroscopy (13, 24, 28–31).

Raman spectroscopy is another form of vibrational spectroscopy. It is based on shifted wavelength scattering of molecules, after excitation by monochromatic light, usually produced by adjustable lasers (32). Several analytes, including glucose, lactate, acetate, formate, glutamine, and glutamate, can be measured (1, 15, 21, 33–37). The use of Raman spectroscopy is limited by the strong fluorescence activity of several biological molecules in the culture broth (34). This fluorescence signals overlay the Raman bands. To avoid fluorescence, low energy lasers can be used, but then heating effects can occur (21, 24, 38).

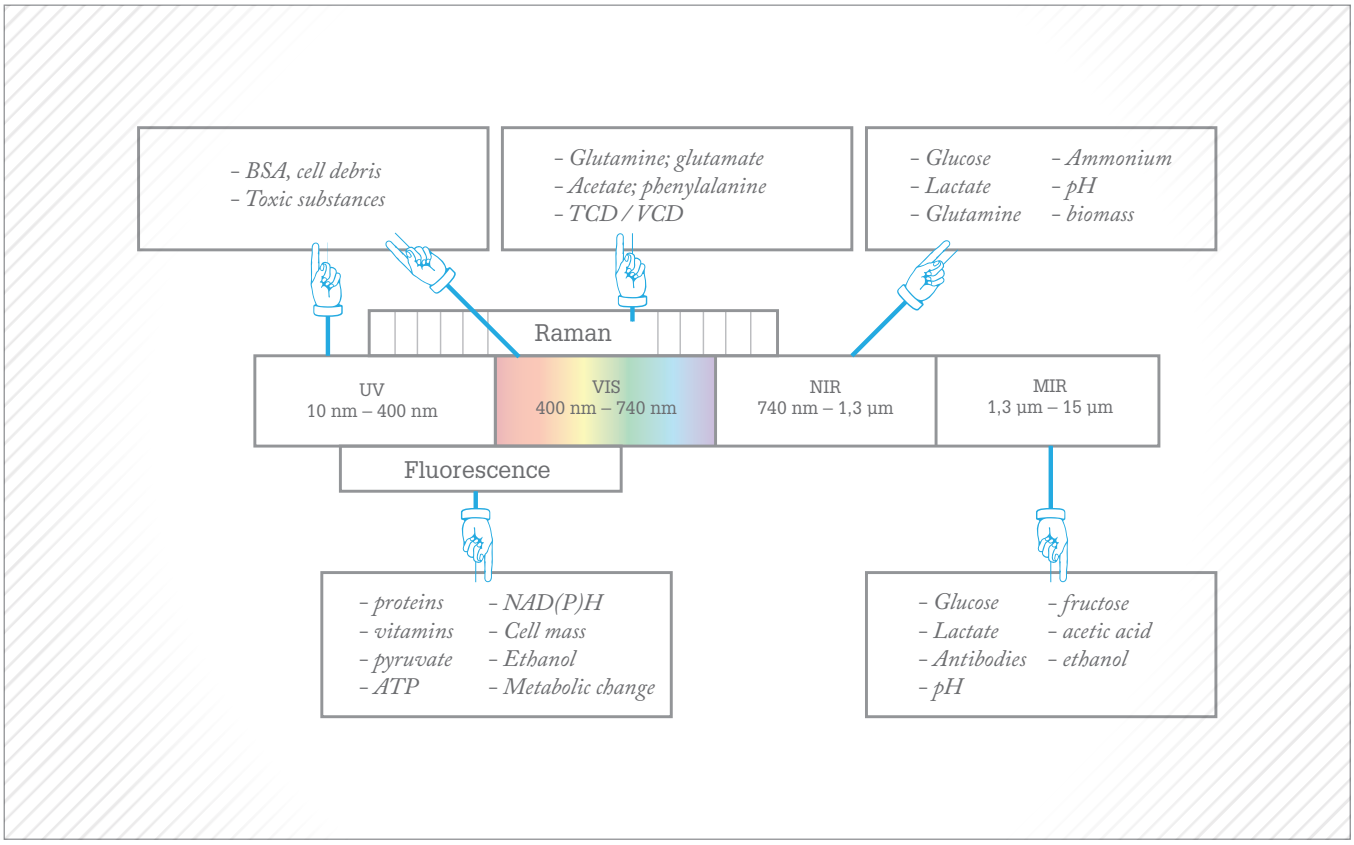


Figure 1: Spectral range for bioprocess monitoring with accessible variables.

process variables, like substrate concentration, or to the actual process status in a calibration model. Afterwards, the model can be used to predict the variables or the process status from on-line spectroscopic data. For these correlations, multivariate data analysis must be applied because the relevant information is distributed over the entire data set and it cannot be found in a subset of a spectrum nor in only one spectrum (47, 48). Such chemometric methods are used to provide interpretable information from the enormous amount of spectroscopic data of a bioprocess.

Data pre-processing is a sensitive and powerful tool for spectroscopic data (49), with the method of choice depending upon the spectral data. After data pre-processing, multivariate data analyses are performed to extract qualitative or quantitative process information from the spectral data. Many established applications are based on principal component analysis (PCA), which assesses the main components of spectral variance induced by changes during the process. With PCA, a classification of raw materials, batches, or the process status is possible (50).

All of these qualitative methods can be used to monitor a bioprocess as defined by PAT (51), and high process reproducibility and product safety can be provided by this type of process supervision (52). A process target line, or trajectory, can be identified out of similar ideal process runs (53–56), with different measurements, multivariate spectroscopic data, or univariate classical process data being pooled to generate a holistic view of the bioprocess (57).

In contrast to those qualitative methods, quantitative models are needed to describe correlations between single analytes and spectral data. Using PLS, the values of different variables can be predicted from a spectroscopic measurement by chemometric models (58, 59). For a PLS calibration, representative process data are needed – including both spectral data and the corresponding reference values. The data need to be distributed over the entire process, describing the variance inside a single process run as well as different process runs. Both variabilities need to be considered for calibration, in order to calculate a reliable PLS model with satisfying prediction quality to unknown process data (60–63). The process of model

construction is sensitive and extensive, but based on this, broad on-line monitoring in terms of the PAT is possible (64–68).

Novel optical sensors are of course among the major developments in bioprocess monitoring, with spectroscopy increasingly being used for determining variables in-line in the liquid phase – and having strong advantages despite the significant requirement for calibration and data treatment via chemometric tools.

Part of the (bio)reactor

If we are to meet the special requirements of these modern single-use reactor systems, we need a new sensor philosophy. Conventional sensors were mostly built as reusable devices for long-term operation, but cannot be inserted directly into single-use bioreactors. In comparison to reusable devices, the lifetimes of such single-use sensors can be shorter, but still must be long enough for long-term, continuous production processes. Furthermore, these sensors must be cheap (owing to their single-use nature), small, and modular (21, 28).

Optical sensors and semiconductor devices (for example, ISFETs) are well suited for such purposes (69). Sensor patches or other measurement systems can be connected with reusable external equipment; therefore, the material of disposable reactor systems must be permeable to the sensor signal; for example, glass windows are a common way of transmitting optical signals from the inner space of the reactor to connected external devices, such as optical fibers and detectors. The observable trend of modern bioprocessing toward single use, disposable systems will help to promote the development of new sensor systems or adapter systems that enable the connection of "classical" sensors to disposable reactors.

Quality first

The FDA specifies that "quality cannot be tested into products; it should be built-in or should be by design". "Built-in" bioprocess quality is enabled by combining process analysis, process knowledge, and process modeling, with tools like multivariate data analysis, bioprocess modeling, Design of Experiments (DoE), and new sensor technologies to reach defined quality goals and to document the process. The process information generated can provide deeper process knowledge for the safe handling of all quality-related variables; the ability to monitor and control critical process parameters (CPP) is the path towards holistic control. The upshot? Quality can be ensured during all manufacturing steps and makes real-time release of products feasible via process validation.

Optical and spectroscopic sensors meet these requirements, as well as offering the possibility to monitor various compounds simultaneously. The downside is that these sensors require complex data handling via chemometric models to derive valid process information. The variety of such sensors described in research is huge, and transfer to broader applications in industrial biotechnology in the near future seems likely. If the biopharmaceutical industry is committed to a total process overview and the ongoing improvement of processes by on-line monitoring (ultimately aiming to meet the goals of the PAT initiative), modern sensors must be embraced – and further development is inevitable.

Dörte Solle, Philipp Biechele, Christoph Busse, and Thomas Scheper are all based at the Institute of Technical Chemistry, Leibniz University, Hannover, Germany.

References can be found in the online version of this article: tas.txp.to/0316/sensors

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Profession

Leadership
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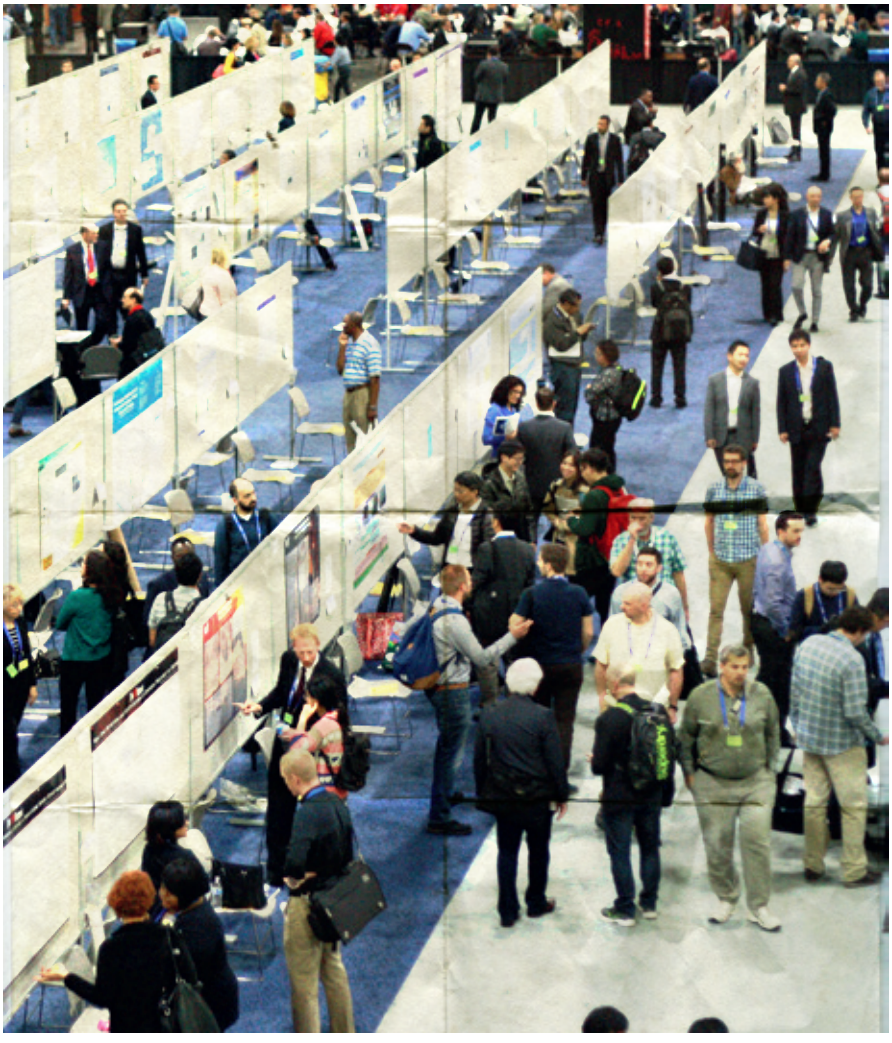
Poster Appraisal

Are poster sessions still relevant in 21st century scientific meetings or are they just filling time and space – and justifying the cost of sending students to events? Here, Deirdre Cabooter (HTC organizer), Greg Klunder (SciX organizer), Hamed Eghbali (HPLC organizer and judge), and grad student Cory Stiner make the case for laminated works of science.



What are your personal experiences of poster presentations?

Deirdre Cabooter: They are a great way to interact and share your research with like-minded people. I was very nervous for my first poster presentation, but because I had a number of viewers at my session, my tension eased – it was great practice! Since then, I've always enjoyed discussing my research during poster sessions; you have more time to elaborate on the work and get some valuable feedback.

Greg Klunder: For me, a good poster needs to be easy to scan and read, yet convey all of the important points in a limited space – and putting all this together is more challenging than preparing a talk. It's not as simple as taking your viewgraphs and laying them down in the space (although, I have done so in the past!) Once the poster is printed, the hard work is over; then it's just talking about the work with the viewers. Fortunately, I've had some good experiences. Everyone who has stopped to talk to me about the work has been truly interested and we have had great discussions. One challenge is offering to explain to a passer-by – trying to hook them with an 'elevator speech'!

Hamed Eghbali: I have fond memories of

"There is a constant challenge, however, in making poster sessions attractive to attendees and presenters, so that they encourage interaction and don't just provide a social gathering."

my early sessions. I have to admit that I was never nervous, as I'd put most of the effort in before the conference. My first poster presentation (miniaturized separation columns) was at SCM (2007) in Amsterdam where the focus was on polymer separations. At the end of the conference, my poster was among the 10 best posters, which was great motivation. Since then, I have been very fortunate with my posters scoring among the top 10 posters at each event, and winning several prizes over the years. As a result, at HPLC 2011, the organizing committee asked me to join the poster jury. This was a great honor. In 2013, I joined Dow Chemical and I was able to attend HPLC 2015, where I presented a poster and was asked to join the poster jury in the final round. It was a nice experience.

Cory Stiner: The first time I presented a poster, I was nervous. I struggled with my delivery, because I was unsure of the best way to present my research. As the poster session continued, I began to become more comfortable, and I figured out what worked best for me. I had many viewers stop by to ask questions about my research, and I was able to answer most of the questions. For the most part, I received a lot of positive feedback and even some suggestions on experiments that I could try, so overall, I would have to say that my first poster session was a success. In fact, I could say that every poster presentation has been a success, because I have had great feedback that I would not have received otherwise.

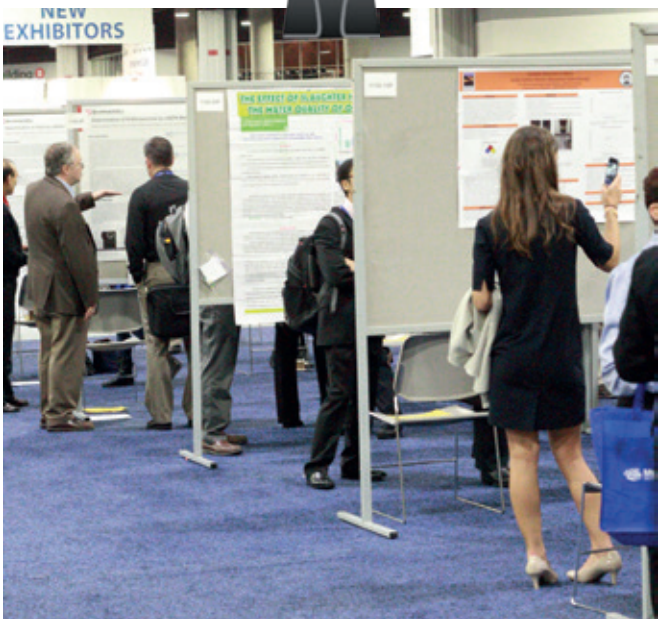
What about the value of posters at scientific meetings?

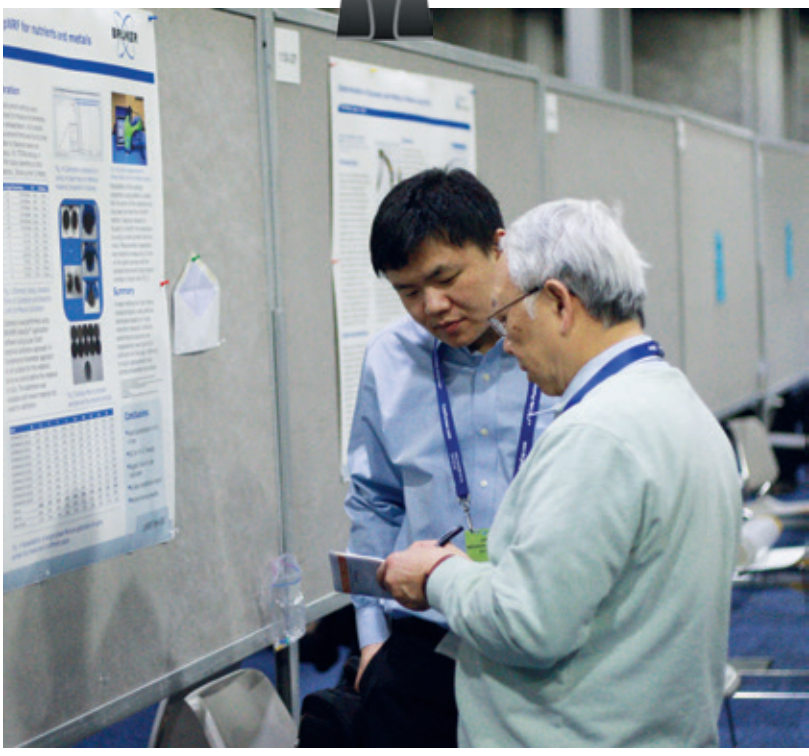
DC: Posters are definitely useful. You get to interact and discuss experimental results (there is often no time to do this after an oral presentation) and you can get valuable feedback from people who are not within your circle of trust. Sometimes people will not be supportive, but this is also a great opportunity to learn to defend your views and work!

Given the limited number of slots for oral presentations, posters are critical for attendees to present their work. There is a constant challenge, however, in making poster sessions attractive to attendees and presenters, so that they encourage interaction and don't just provide a social gathering.

HE: I think posters play a key role, as presenters get the opportunity to develop their presentation skills and network with people from all over the globe. I am convinced that poster sessions help with establishing cooperation between different groups. In addition, the presenters get the opportunity to hear the opinion of the viewers. Sometimes, people who are not so familiar with the research topic can offer the presenter new perspectives or ideas for future consideration.

Posters are also important because viewers get to see the most recent





Top Ten Poster Tips

1. Use a short, punchy title to grab attention
2. Make sure important information is eye-catching to passers-by
3. Don't be overly verbose – say what you need to say and no more
4. Use a clear font that's easy to read for the main text
5. Break up the text with bullet points and sub-headlines
6. Remember: a picture paints a 1000 words – make the most of attractive and information-rich figures
7. Consider the overall design – does your poster stand out from the crowd?
8. Use a logical layout that's easy to follow
9. Don't forget to include your name, institutional affiliation, and contact details
10. Remember to include acknowledgements – or it may be your last poster session...

results in the field. When you are listening to a talk, it is not always easy to focus your attention constantly throughout and you can miss important information. However, when you go to a poster session, you have the chance to view each one at your own pace, which allows you to extract important information more easily. Also, you may see a poster that could be of interest to your colleagues and you can tell them to go to see it. Talks aren't shared so easily.

CS: I think they are very important. I believe that poster sessions are a win-win situation for both the presenter and the viewer, because there is an exchange of ideas between the two people that can lead to new possibilities such as collaborations, advances in research projects, and new research projects. For presenters, posters are a quick way to present your research to many people, so it allows a person to determine whether your research is interesting. The presenter of the poster gains so much knowledge and advice from the questions they receive. The viewers will gain exposure

"The content represents the reputation of the presenter and the institution so it's highly important."

to different types of current research in the field that may spark new ideas to implement in their own research or for starting a new research project. Finally, if people aren't interested, they can move on to the next poster; in an oral presentation, they'd most likely have to sit through the whole presentation...

Do you expect your students or employees to participate in poster sessions?

DC: Yes! All our students get to go to conferences to present posters. They deserve the exposure for their own work.
GK: Most definitely. Presenting a poster is an excellent way to display hard work and it helps to justify their attendance at the conference. Attending meetings, seeing other work, and interacting with colleagues is as important, if not more so, than presenting their own work. Of course, we all want our work to be interesting to others and have a chance to showcase it – and sometimes a poster is the only opportunity.
HE: Certainly, as posters are good for both the visibility of the institution and the presenter. It allows them to meet new people and start new collaborations. In addition, I would also encourage those who are less interested to consider presenting because it can be a very positive learning experience. Institutions should select the best topics/results and make sure that they give a good representation of the institution, which is far better than presenting a large number of posters just for the sake of it.

How important is poster content – should it always focus on something new?

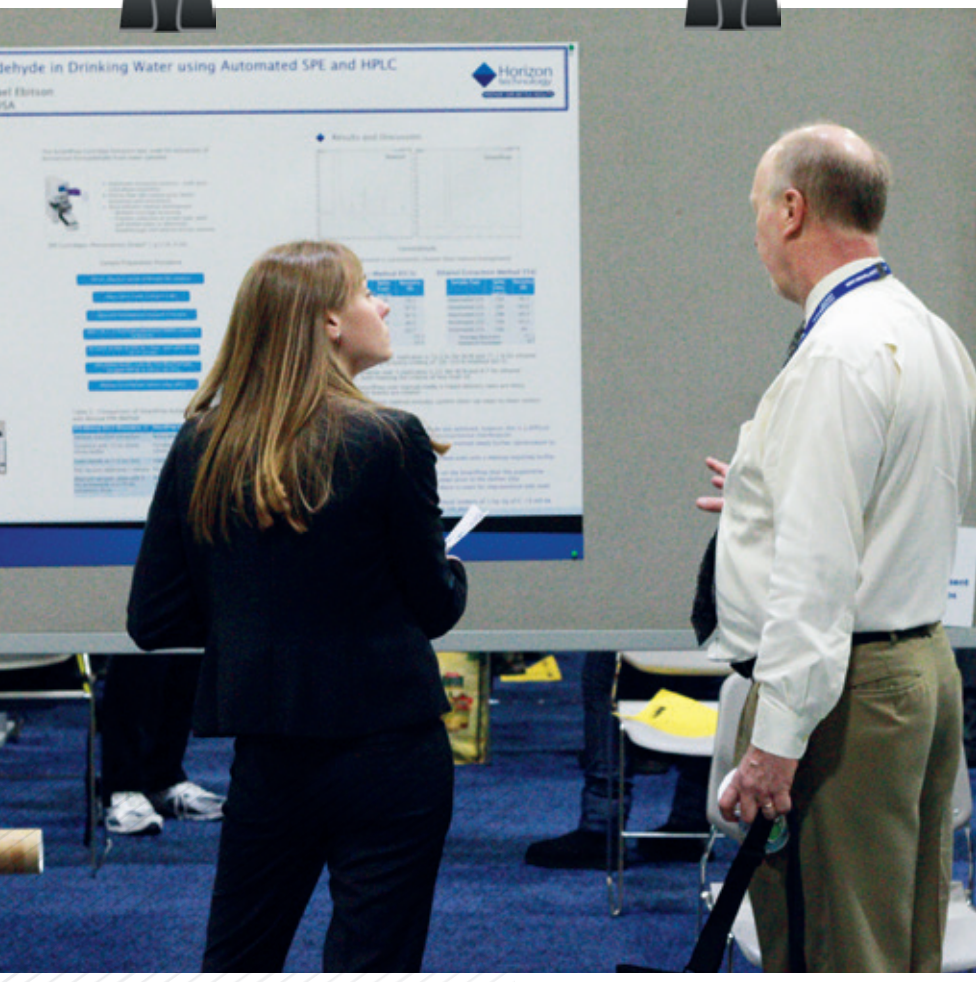
DC: It is all about the content – and visual presentation is important too. I think many interesting new developments are discussed on posters (often to get a feel of the general opinion on new topics) before they are launched...
GK: Compiling your work into a presentable format is, in many cases, the first step toward getting the work ready for publication.
HE: The content represents the reputation of the presenter and the institution so it's highly important. The novelty of the content varies from poster to poster. Some only give a description of the activities within a research team. In contrast, other posters represent new scientific findings, which are often the preliminary work for a future scientific publication, so the content has major value. The majority of posters you see at a conference are of the second type.
CS: A poster shares the story of your research and so the content encourages the viewers to ask questions. If the presenter does not give an introduction, research goals, experiments, results, conclusion, and future work, how can the person viewing the poster understand the importance of the research? Doing this brings attention to the presenter and their organization for future collaborations and endeavors with other people.

Is the importance of poster content sufficiently appreciated?

GK: Unfortunately, to some attendees, posters have the stigma of being 'work that wasn't good enough for an oral presentation'. Since there is limited availability for oral presentations, the conference organizers work hard to find balance in the talks and, sometimes, top quality work gets presented as a poster. For example, once the preliminary program has been set, SciX keeps abstract submissions open for late breaking research developments that can only be accommodated as poster submissions.
HE: Most often, I think people who view posters are hoping to benefit their own work. A typical example is that people working on a similar subject or for a competitor visit the sessions to look at what the other side is doing.

Is it a good idea to provide awards for the best posters?

DC: It's a great idea and, in fact, we had an award for the three most innovative poster contributions at HTC-14. Students deserve to get exposure for the work they have done, and giving them a few minutes to talk about it is



Poster "Elevator Pitch"

Do you plan to present a poster in the coming months? You could offer a sneak preview to readers of The Analytical Scientist by sending your "elevator pitch" (no more than 200 words and a single figure) by email – we'll share the best in our Upfront section along with your contact details (if you wish) and the conference you'll be attending.

Submit your Poster Elevator Pitch now, by emailing the Editor: rich.whitworth@texerepublishing.com

an excellent way of doing that.

GK: Poster sessions are a constant work in progress and anything that can be done to improve the experience for the attendee and presenter should be considered. Providing a venue to display recorded short elevator speeches, incorporating more poster content into the online program or mobile app, awards for top presenters, invited poster presentations, are some ideas that I have heard being considered. It's important to keep similar content together so that the session has a consistent theme. Awarding presenters with the ability to give an oral presentation only works well if they can be placed in a session with similar topics.

HE: The prize is not a bad idea. It motivates the presenters to do their best and it also increases excitement towards the end of the congress when the award winners are announced. I think that poster sessions are quite well organized, but this does not mean that there is no room for further improvement. More specifically, the special (presentation) session at HPLC 2015 was a nice experiment, which was a great success from my point of view. The participants are also required to show another set of skills, namely their oral presentation skills, which provides another dimension to the whole event. The presentation session can be tense for the participants but at the same time, it makes it exciting.

CS: I believe that awards should be provided for the best posters. People deserve to be recognized for their hard work and contributions to the field.

What are your views on poster sessions – do they need to be dragged into the 21st century? Do you have a funny anecdote to share? Let us know online in the comments or email joanna.cummings@texerepublishing.com

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Waseem Asghar

Meet the Winner

Waseem Asghar

Waseem Asghar, Assistant Professor at the Departments of Computer Engineering & Electrical Engineering, Computer Science, and Biological Sciences, Florida Atlantic University, USA, has been chosen as the winner of the 2016 Humanity in Science Award for "development of a new paper and flexible material-based diagnostic biosensing platform that could be used to remotely detect and determine treatment options for HIV, E-coli, Staphylococcus aureas and other bacteria."

Waseem will be presented with a humble prize of $25,000 during an all-expenses paid trip to Analytica 2016 in Munich, and his work will feature in an upcoming issue of The Analytical Scientist.

Could it be you in 2017?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

Has your own work had a positive impact on people's health and wellbeing? Details of the 2017 Humanity in Science Award will be announced soon.

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Bioengineered Humanity

Sitting Down With... Waseem Asghar,
winner of the 2016 Humanity in Science Awards and Assistant Professor, Department of Computer & Electrical Engineering and Computer Science, and Biological Sciences, Florida Atlantic University, USA.

Congratulations on winning the 2016 Humanity in Science Award!

Thank you so much. It's wonderful news – but it will take some time to absorb... I'm certainly very happy and honored to be chosen for such a prestigious award, and it will no doubt push me to work even harder. Such encouragement at any point in one's career is extremely welcome, and it will provide me with a boost of positive energy for quite some time!

Tell us about the winning project...

The main focus of my work is developing point-of-care (POC) devices with applications in several diagnostic areas. Our nomination for the Humanity in Science Award fits within that research and describes the development of a transparent, cellulose paper-based microfluidic biosensing platform. The motivating thrust for the project was to develop low cost diagnostics, specifically for HIV viral load and CD4 quantification – but we expanded into detecting other viruses, bacteria and specific cells in blood – all integrated with mobile technology.

Right now, there are about 36 million people infected with HIV, but common diagnostic tools are based on PCR or flow cytometry – expensive instrumentation and expensive tests with a requirement for a state-of-the-art lab. In the developed world, such tests may be perfectly acceptable, but they are not feasible in developing nations – which account for around 75 percent of all those infected.

What inspired the direction you took?

We looked at glucometers – handheld devices facilitating self-management of diabetes – and wanted to replicate the functionality for a broader spectrum of diseases for millions of potential patients. Next, we needed to find the most appropriate materials, all the while focusing on cost and the potential to be locally produced.

Where does your passion for meeting unmet diagnostic needs stem from?

I see people suffering around me and it compels me to act. Going back to diabetes, I have a 19 year-old cousin with the disease who is now managing it well because she can monitor her own glucose levels. My mother also had diabetes for many years, and I remember as a child knowing that she was unsure about administering the correct dose of insulin. An inexpensive portable device has transformed the disease for patients. My work location has a high number of new HIV cases, so I can see a need around me; POC devices in community centers could make a real difference.

Consider Ebola and Zika; one big problem is that the diagnostics are not cheap and simple, which makes disease management almost impossible. And what about the next disease burden around the corner? Simpler detection methods can rapidly be modified for upcoming infections.

What stage is your work at?

We have tested our devices with real clinical samples (blood and serum), and we are now in the process of evaluating the devices with patient samples. The next step will be to move forward with FDA approval. And although our device gives very rapid results (25-30 mins) for viral load in a clinically relevant range, we are also continuing to optimize sensitivity.

How did you come to combine engineering and biology?

When I was a kid, my parents always used to say, "Our Waseem will be an engineer or a scientist," and, in a way, I think I followed a path partly to fulfill that prophecy! It certainly matched my interests – I loved tinkering with car engines and 'reverse engineering'...

At first, I wasn't particularly interested in biomedical engineering until I took one course during my PhD that sent me on a new journey into bioengineering. I worked hard outside of my PhD – I took a lot of extra courses, studied online, and read many books – all to build my knowledge in biology and biochemistry, so that I could succeed in what I felt was my new career direction. A post-doc at Harvard Medical School exposed me to clinical work and challenges – as did another at Stanford Medical School. Now, I am very satisfied; I'm doing what I want to do. I'm helping humanity in some way, and there's no greater goal.

Which scientists do you respect?

Harvard's George Whitesides springs to mind first; I really like the work he's done so far. Langer is another inspirational character. But there are many other people doing great work, such as Samir Iqbal (University of Texas), Utkan Demicri (Stanford University), Mehmet Toner (Harvard Medical School), and Rashid Bashir (University of Illinois at Urbana-Champaign). Reading their papers keeps me motivated – it's where I want to see myself.

Do you think engineers tackle problems differently?

Absolutely. There are many wonderful scientists in all fields, but I do believe engineers typically have a different perspective; we focus on making things workable. There are some engineers in fundamental research, but the majority of us are involved in applied technologies – integrating what's out there in electronics, biology and engineering – to address an unmet need or to improve a process. That said, I don't really consider myself an engineer as such, but simply a researcher. It shouldn't matter what the problem is – with dedication, you can solve it. One thing is certain: diversity is always a good thing. Take the device area, where so much can be achieved, but where slow progress, I believe, stems from lack of collaboration with engineering disciplines. Multidisciplinary teams are more commonplace today, and I can see many positive changes coming in the future.

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We look forward in welcoming all of you to Limassol for EPRW 2016!

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