MARCH 2017 # 50

the Analytical Scientist

50 ISSUES STRONG

The Secret Life of Plants

Elena Stashenko explores the complex chemistry of Colombia's botanical treasures.

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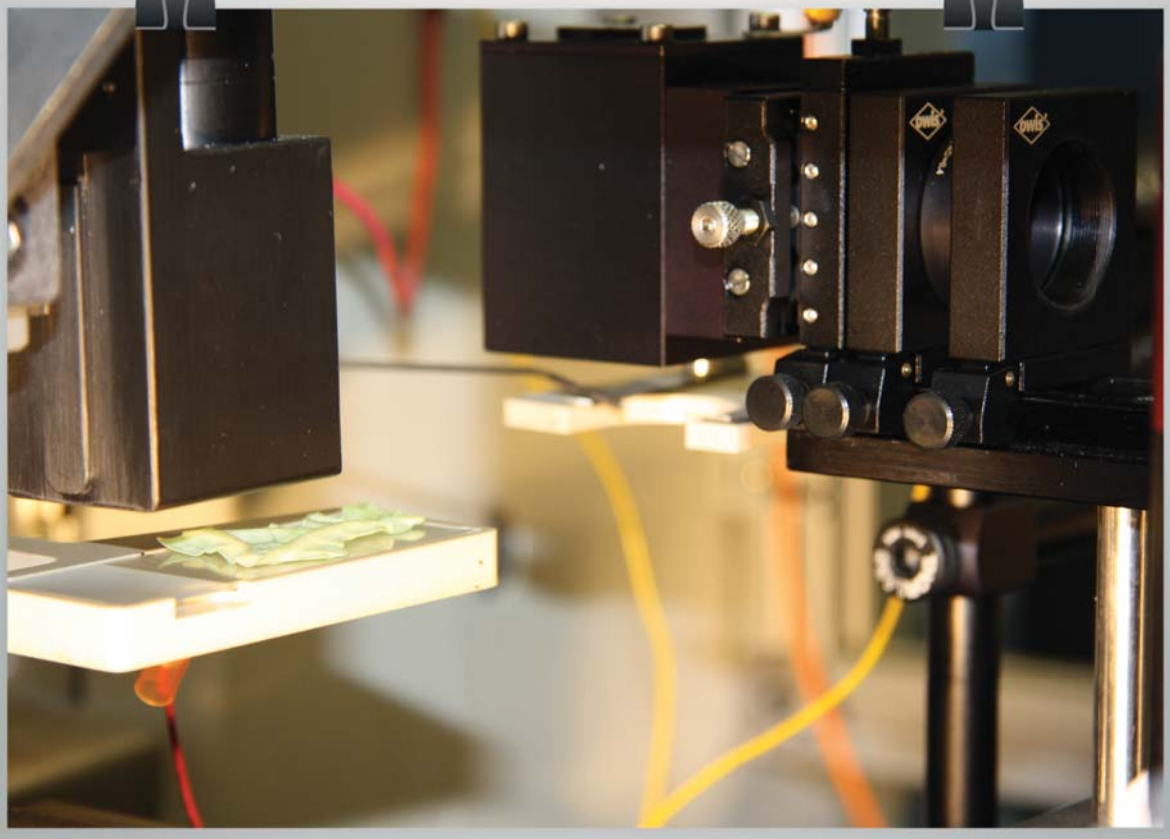

Taking the Rough with the Smooth

Researchers at the Max Planck Institute for Chemical Ecology have developed a new mass spectrometry imaging technique for reliable chemical analysis of rippled, hairy, bulgy or coarse surfaces. Laser ablation electrospray ionization (LAESI) uses a mid-infrared laser to generate vapor from the sample, which is then ionized by the electrospray source and analyzed in the mass spec. Here, their custom-built laser source is used to analyze the surface of a savoy cabbage, showing its effectiveness in assessing topographically challenging surfaces – but with its ability to analyze biofluids and metabolites, LAESI also has potential in pharma and biosciences.

Credit: Benjamin Bartels *Reference: B Bartels et al., RSC Adv, 7, 9045-9050 (2017).*

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

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On The Cover

The role of volatile compounds in pollinator–plant relationships is just one focus for analytical pioneer Elena Stashenko.

Upfront

the Analytical Scientist

ISSUE 50 - MARCH 2017

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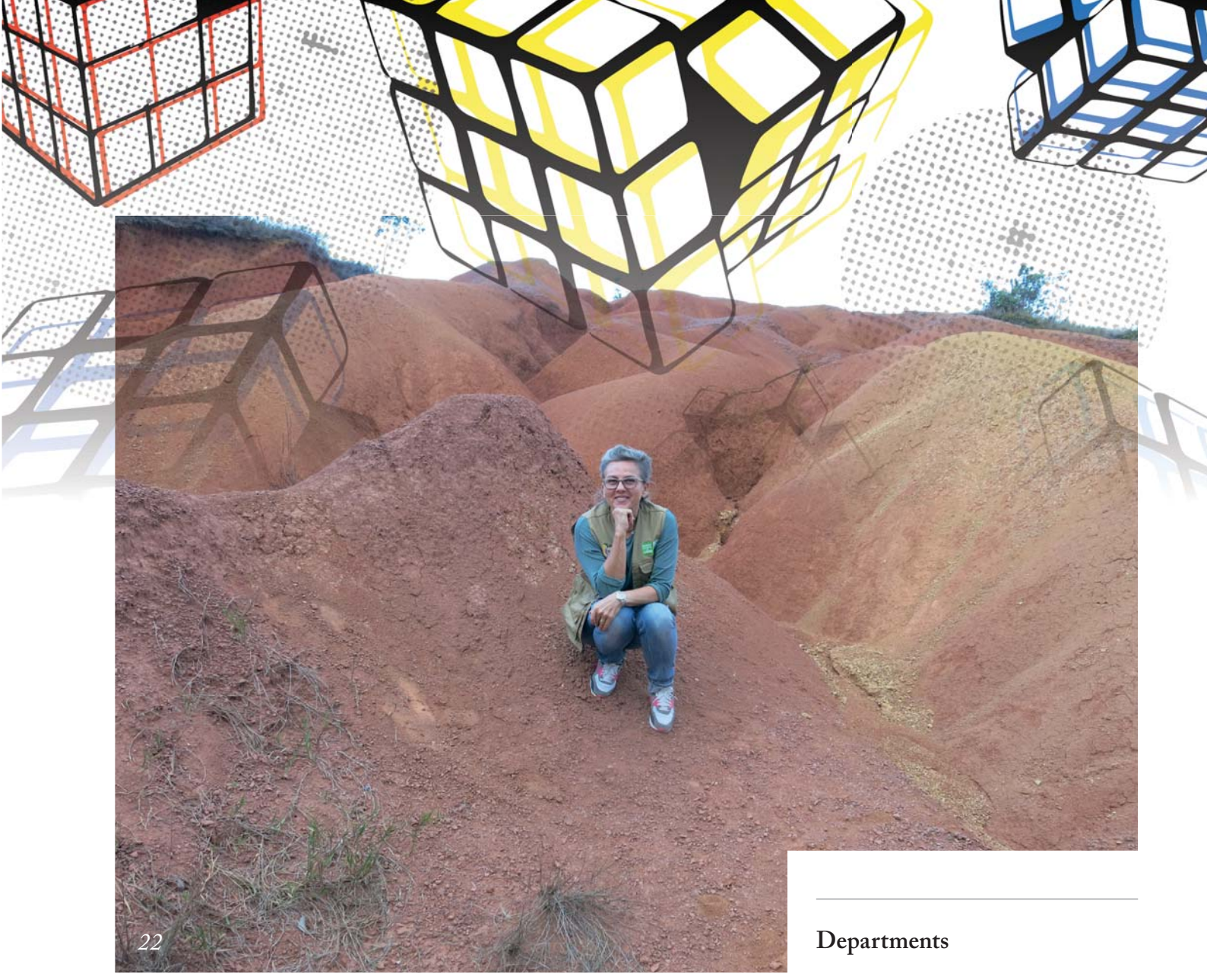

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In My View

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TOPICS COVERED INCLUDE:

- Energy, & Resource Development
- Pharmaceutical & Forensic Analysis
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- Contaminants in Food & Environment
- Biomarker Discovery & Protein Analysis
- Novel Stationary Phase Chemistry
- Atmospheric & Air Analysis
- Software & Data Analysis
- Integrated Sample Preparation
- Lab-On-A-Chip & Microfluidics
- Fundamentals and Theory
- Novel Detection Techniques
- Multidimensional Separations
- Miniaturized & Portable Systems

Pathology Needs You

Neonates represent the greatest need for simpler, more sensitive – and preferably non-invasive – analytical methods.

I am the very proud father of a brand new human; Gray Winter Whitworth was born on 9 February 2017. And I am eternally grateful to all the wonderful healthcare professionals involved. The picture of health, he aced all tests but one... jaundice. I am sure many of you have been through a similar experience, but please allow me to detail our analytical journey...

It begins with a rudimentary test: "Does he look yellow to you, Val?"

"Hmm. He does a bit – hold him near the window."

"Yes – a bit yellow-ish. What do you think, Val?"

"I'd send him down..."

"OK. Rich, May – you'll have to take him down to Rainbow Ward..."

It begins with a rudimentary test at four-days old. A friendly nurse bustles into the treatment room on Rainbow Ward, armed with a handheld, noninvasive transcutaneous bilirubinometer. I am impressed. (Later, I read that the device measures multiple wavelengths by spectral reflectance to determine the optical densities of bilirubin, hemoglobin and melanin.) Holding it to his forehead in various places and "zapping" him, she peers over her glasses at the LCD display and declares that the bilirubin level is "too high" for the device and that a heel-puncture blood sample will be required for further investigation.

Two hours later... An apologetic doctor enters the waiting room/play area to squeeze blood from a stone. Drip, drop... "OK – I'm going to have to do another little prick." Gray, having been squeezed through a birthing canal, doesn't cry at such trifles, but isn't exactly smiling. Finally, the doctor has enough blood for the lab test – she dares not send too little for fear of sample rejection...

Later that evening, the doctor dutifully calls: "... it's below the level of treatment, but too high to ignore – you'll have to come in for another blood test tomorrow..." And so it all begins again on Day 5.

The outcome of the second test? The level (a number with no units) has risen but is below the treatment threshold on "the curve." "Presumably, µmol/L," I muse, "And I'm pretty sure two data points don't represent a curve..." Back to "normality."

Conclusions. The handheld transcutaneous bilirubinometer is a fantastic idea – exactly the sort of transformative technology needed – but when it outputs an overly 'risky' reading, healthcare professionals insist on the "gold standard" test. Unfortunately, the gold standard appears to require taking too much blood from too small a person – more than once.

Though I recognize that the whole experience is entirely trivial when compared with more serious health conditions or the total lack of healthcare in other parts of the world, it did make me think: "Analytically, can we do any better?"

Rich Whitworth
Content Director

Did you know we have a sister magazine – The Pathologist – that frequently covers areas of unmet need in the field? www.thepathologist.com

Upfront

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

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

Good Things Come to Those Who Wait?

Ion chromatography helps students brew 5,000-year-old beer

Ever wondered how booze was made in ancient China? Well wonder no longer. A group of archeology students from Stanford University recently used some cunning chemistry to recreate Chinese beer using a 5,000-year-old recipe, giving them an insight into ancient culture and behavior.

But what drove the students to drink in the first place? After the excavation of the Mijiaya archeological site in northeast China, Li Liu (Sir Robert Ho Tung Professor in Chinese Archaeology) and postdoc candidate Jiajing Wang felt that the pottery assemblages from two pits – namely, the presence of funnels and stoves – could be related to alcohol making. In an attempt to prove this hypothesis, they visited Shaanxi Institute of Archaeology in Xi'an, where the artifacts were stored, and extracted residues for analysis from the interior surfaces of the vessels.

The team then used ion chromatography (IC) to analyze the residue. IC identified the presence of oxalate, which develops during the steeping, mashing, and fermentation of cereals. They also discovered traces of phytoliths from cereal husk, finding that the starch had damage consistent with being malted and mashed. In addition, the shapes and styles of the vessels showed stylistic similarities to brewing equipment of the historical period and modern ethnographic records. The conclusion? "People in China brewed cereal-based beer around 5,000 years ago – 1,000 years earlier than previously believed," says Jiajing Wang.

From there it was a 'hops', skip and a jump for Liu's students to replicate the ancient brew. The all-important taste tests revealed a sweeter, fruitier flavor than modern beers – though other

variations apparently smelt more like "funky cheese".

According to Wang, this kind of experiential archeology helps researchers make inferences about human behavior and Chinese culture at the time. "The practice of beer brewing is likely to have been associated with the increased social complexity in the Central Plain during the fourth millennium BC," says Wang. "It indicates a mix of Chinese and Western traditions – barley from the West, millet, Job's tears (a type of grass), tubers from China." They were particularly surprised by the presence of barley, as the earliest prior evidence of barley seeds in China dates to 4,000 years ago. The authors suggest that it was initially introduced to the Central Plain as an ingredient for alcohol production rather than for subsistence.

The beer that students made and analyzed will be incorporated into the research team's final findings. The team is planning to conduct more beer brewing experiments, so that they have more reference data to study ancient beer production. "This class gives students an opportunity to not only experience what the daily work of some archeologists looks like, but also contribute to our ongoing research," Wang says. But since the beer has the consistency of porridge, it's unlikely you'll see it in a bar near you… *JC*

Reference

1. *J Wang et al., "Revealing a 5,000-y-old beer recipe in China", PNAS, 113, 6444-6448 (2016)*

Here Be Dragons

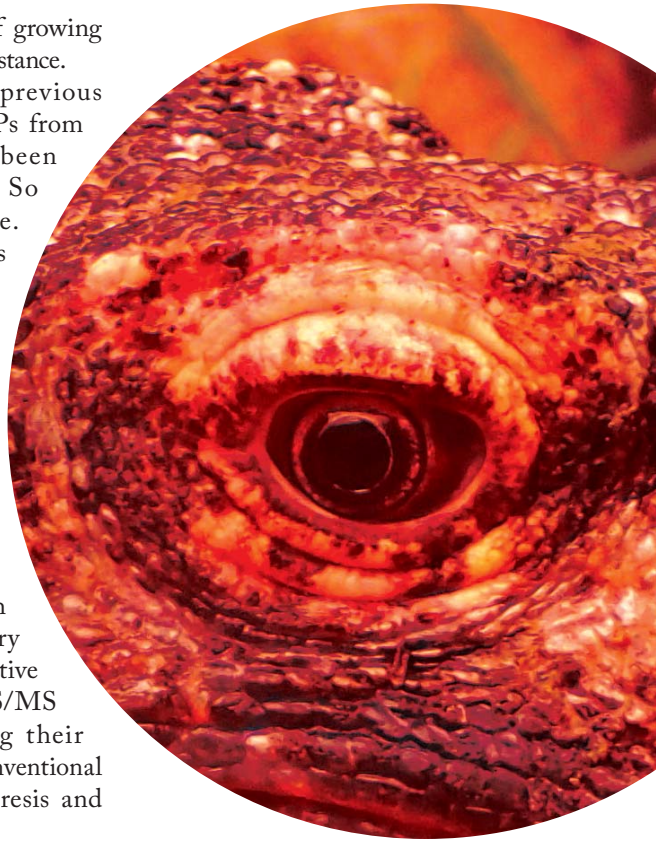
De novo-assisted sequencing with ETD-MS unearths antimicrobial peptides in Komodo dragon plasma

Komodo dragons have dirty mouths. It's not that they swear like sailors, but rather that their saliva is teeming with pathogenic bacteria. And though dental hygiene (or lack thereof) is no longer thought to be the only source of its deadly bite (the discovery of venom glands has fueled a little-known debate in reptile research circles...), the monitor lizard does need to be capable of recovering from nasty septic wounds inflicted by competing dragons, which is thought to have led to a particularly robust immune system.

Could dragon's blood, just like in some legends, be a source of medicine – or, at least, a source of pharmaceutical agents? Potentially yes, according to researchers behind a recent paper (1), who discovered 48 novel potential cationic antimicrobial peptides (CAMPs) with a custom bioprospecting approach. CAMPs play an essential role in the innate immune response, so interest in finding new peptides – and better understanding their role – has ramped up of late because of growing concerns over antimicrobial resistance.

The authors note that previous methods to discover CAMPs from biological samples have been "slow and low-yielding." So they developed a new one. Specifically, the researchers employed "custom-made microparticle harvesting of intact, functional peptides from biological samples coupled with de novo sequencing of the harvested peptides using ETD mass spectrometry." Using the approach, the researchers say they have been able to "more effectively leverage the high sensitivity of mass spectrometry and the ability to sequence native peptides based on their MS/MS fragmentation," comparing their method's power with more conventional chromatography, electrophoresis and fractionation techniques.

We're some way off Komodo monitor-derived therapeutics – but, if ever a new pharmaceutical company wishes to target a certain (fantasy-board-game-playing, Game-of-Thrones-watching) subset of the population, "Dragon Drugs Inc" is a sure winner... *RW*



Reference

1. *BM Bishop et al., "Discovery of Novel Antimicrobial Peptides from Varanus komodoensis (Komodo dragon) by Large Scale Analyses and De Novo-Assisted Sequencing using Electron Transfer Dissociation Mass Spectrometry', J Proteome Res, Article ASAP (2017). DOI: 10.1021/acs.jproteome.6b00857*

Hidden Hunger

How will future climate change impact dietary intake of selenium? ICP-MS and predictive modeling have the answer

"Humans only need selenium in a narrow range of concentrations – too high an intake can lead to toxicity and too low an intake can lead to deficiency," says Lenny Winkel (Research Scientist and Head of Environmental Inorganic Geochemistry, Eawag, Switzerland).

Nutrient deficiencies are an important health problem worldwide, explains study collaborator Prof Steve McGrath, Head of Department of Sustainable Soils and Grassland Systems at Rothamsted Research: "There are many people suffering from 'hidden hunger' across the world; people who have enough food to eat but it does not have adequate nutritional value."

Selenium is a key component of selenocysteine (the 21st proteinogenic amino acid) – an important building block of selenoproteins, such as glutathione peroxidase, which help protect cells from oxidative stress. Despite selenium's importance in our diet, we know relatively little about its concentration in soil – or the factors that most affect those levels.

Winkel and her team reviewed systematic geochemical soil surveys that had already been conducted; for example, China (1) and more recently in the UK (2). "We found that climate (rather than soil type or underlying geology) had a strong effect. And that prompted a further international collaboration to find out how climatic factors (in addition to soil and geology) affect selenium concentrations in soils across the globe," she says.

The predictive modeling was based on 33,000 data points of previously collected total soil selenium analyses, but the team also conducted further analysis on soil samples from the Rothamsted Research soil archive with inductively coupled plasma-mass spectrometry (Agilent 7500ce) to understand temporal trends in soil selenium concentrations (3).

"We used different machine-learning tools (artificial neural network models and random forest) based on all of our data as well as 26 variables that describe factors hypothesized to control soil selenium concentrations," says Winkel. "Given that our analyses showed that climate was indeed a key factor in current predictions, we decided to further analyze how changes in climate may affect soil selenium concentrations."

By the end of the century, the models predicted soil selenium losses from almost 60 percent of modeled areas – and especially from croplands.

"An important message of our work is that soil concentrations of selenium are dynamic and that changes in the soil may ultimately affect the nutritional value of crops. The relationships between selenium, climate and soil variables are complex, but one factor that played an important role in predicted future losses of selenium is aridity. In short, areas that become drier are predicted to lose relatively larger amounts."

Winkel is keen to investigate other micronutrients and trace elements to discover if they are affected by soil–climate interactions in the same way. "Another important future task is to understand how future changes in soil micronutrient content will affect the concentrations and speciation of selenium in plants. In this respect, it is also important to investigate how changes in climate may influence the speciation and thus the bioavailability of micronutrients to plants; after all, total micronutrient contents in soils are not the only factor controlling concentrations in plants."

McGrath concludes, "By developing a model that can track changes in the levels of minerals key in our nutrition, we are laying the groundwork for a solution to the problem. This model has already revealed a very important fact; that climate can be a key factor in the distribution of some essential micronutrients across the globe." *RW*

References

1. *J Chen, "An original discovery: selenium deficiency and Keshan disease (an endemic heart disease)", Asia Pac J Clin Nutr, 21, 320–326 (2012).*
2. *Y Yao et al., "Selenium, iodine, and the relation with Kashin-Beck disease", Nutrition, 27, 1095–100 (2011).*
3. *GD Jones et al., "Selenium deficiency risk predicted to increase under future climate change", PNAS, (published ahead of print; 2017).*

Products, Partnerships and a Pittcon Precis

What's new in business?

In our regular column, we partner with www.mass-spec-capital.com to let you know what's going on in the business world of analytical science. This month, we have a summary of the most exciting Pittcon product launches, and a new company joins Waters' Centers of Innovation Program.

Products

- Agilent introduces 6545XT AdvanceBio LC/Q-TOF MS system
- Bruker introduces MALDI PharmaPulse 2.0 Solution and BioPharma Compass 2.0 Software at Pittcon
- New Thermo Scientific iCAP TQ ICP-MS system at Pittcon
- Waters introduces Empower Cloud Chromatography Data System (CDS) at Pittcon
- Advion launches vAPCI Ion Source for expression CMS
- ACD/Labs previews Impurity Control Informatics System Luminata at Pittcon
- Phenomenex extends Kinet and Luna Omega LC Column Lines
- Scion Instruments announces new offerings at Pittcon 2017

Collaborations

- Alcami invests in Bruker's D8 Discover HTS XRD system
- A*STAR's Bioprocessing Technology Institute (BTI) joins Waters Centers of Innovation Program
- ChemAxon and IDBS expand partnership to advance E-WorkBook
- 908 Devices: Thermo Fisher to resell ZipChip as front-end

Investment and Acquisitions

- Thermo Fisher Scientific acquires Core Informatics, a provider of cloud-based scientific data platforms
- Thermo Fisher completes acquisition of Finesse Solutions

People

- Thermo Fisher appoints Dion Weisler to Board of Directors
- Agilent Technologies CTO Darlene Solomon elected to National Academy of Engineering
- Danaher appoints Raymond C. Stevens to Board of Directors

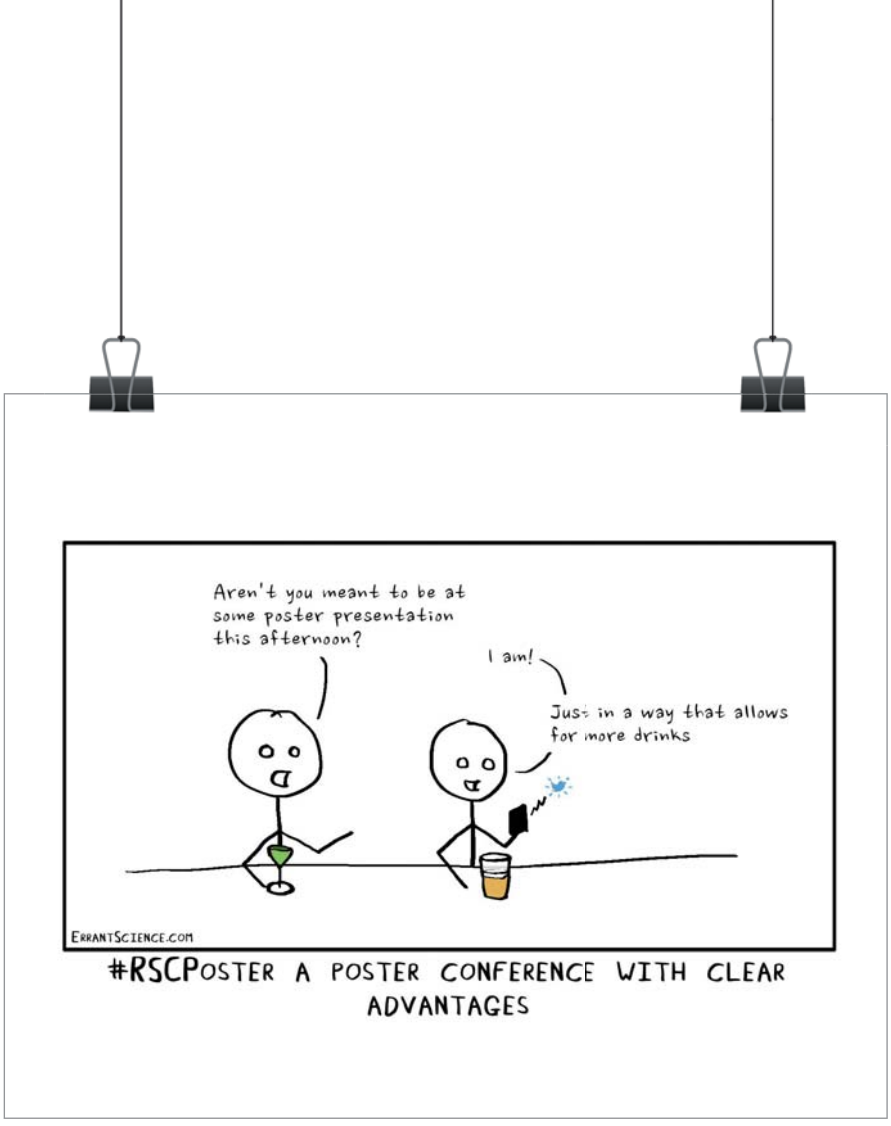
Organizations

- Launch of Shimadzu European Innovation Center in
- Duisburg, Germany
- Genedata establishes UK subsidiary in Cambridgeshire
- Antec Leyden BV and Antec (USA) Inc have announced a corporate name change to Antec Scientific

For links to original press releases and more business news, visit the online version of this article at: tas.txp.to/0317/BUSINESS

Poster Haste

In 2014, Matt Baker from Strathclyde University, Scotland, teamed up with The Royal Society of Chemistry to create 'Twitter poster sessions'. This year sees the third incarnation, and it's only growing in popularity. Baker tells us more.



How do Twitter poster sessions work?

Participants tweet an image of their poster with the title and hashtags #RSCPoster and the area (e.g. #RSCAnal) at any point throughout a 24-hour period. This means that people anywhere in the world can join in. When I had the idea I got in touch with RSC around October 2014 and they backed it. We were able to hold the first one early in 2015 and it's grown from there. For the RSC Analytical Twitter Poster Conference 2016 we had excellent engagement, with 2,670 tweets, 435 contributors, 815,866 audience members, 2.9m impressions and more than 80 posters over 24 hours.

How has it developed since the first session?

We have made a couple of changes based upon feedback and our own thoughts about the event. One was to introduce a Tumblr site to help people put up better images of their posters, in case they needed to convey important results more easily. Now in the third incarnation, we have been able to expand to all aspects of chemical sciences. I heard from scientists in other areas of chemistry who liked the idea of a Twitter poster conference, so it seemed like a natural progression.

How does it compare to a traditional poster session?

It is of course different, as you don't have someone in front of you, but the attendees have said that they enjoyed the interaction and the chance to think about question and answers. Although the brevity of 140 characters is sometimes a difficulty.

What is it like to manage?

Overall it is fun, as it is still a new idea and things are changing rapidly. With the expansion to the chemical sciences this year there is a bit more administration to sort out but I have excellent co-organisers (Ed, Sam and Craig) and great help from Philippa and Sarah at the RSC. I would like to thank the excellent scientific committee, which we have managed to expand this year – they are all volunteers and without them this would not be possible.

How important is social media for science?

It could have a very important role in communicating science and in particular, breaking down any access barriers that researchers may have – such as cost or ability to travel, which can bar attendance at other conferences. I think it would be great to crack effective networking over Twitter, too. If you can combine communication with excellent networking opportunities, it could really have an impact upon researchers and science.

What's next?

I would like it to evolve to be bigger still – we have already have requests to include chemical education research. A few of the attendees have started presenting their posters by video – so let's see what the attendees come up with this year!

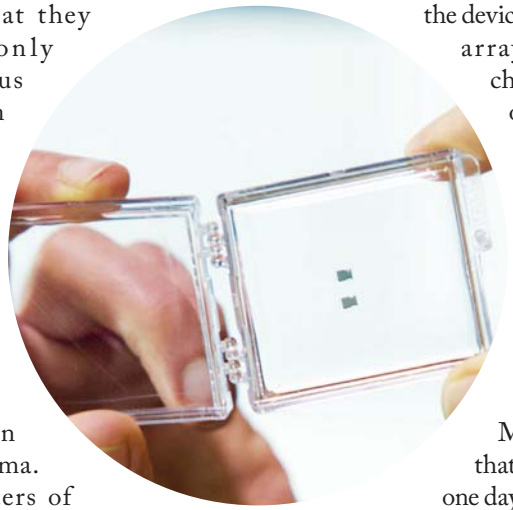
Follow Matt: @ChemistryBaker

Super Sensors

A tiny biosensor could diagnose HIV within a week of infection

A Spanish team have developed an HIV test that can detect the viral capsid protein p24 at ultra-low concentrations in human plasma (1).

Current HIV diagnostics are based on nucleic testing (NAT) or immunoassays. However, the sensitivity of the tests means that they can usually only detect the virus after it has been replicating for 2-4 weeks.



The new biosensor has a limit of detection of 10–5 pg/mL – equivalent to detecting one virion in 10 mL of plasma. That's five orders of magnitude better than the best immunoassay, and two orders of magnitude better than NAT, allowing detection within a week of infection. What's more, the results are ready in under five hours – a record for HIV testing.

"The prompt identification of individuals during the highly infectious acute or early stage of HIV infection has implications for both patient management and public health interventions," says Priscila Monteiro from Instituto de Microelectrónica de Madrid, Spain. Not least because the concentration of virus in plasma and genital secretions is extremely high during the first few weeks of infection.

Inside the rice grain-sized sensor, gold nanoparticles bind to the p24 protein. "Gold nanoparticles act as mass and plasmonic labels; the two signatures are detected by means of the microcantilever that serves as mechanical resonator for 'weighing' the mass of the captured nanoparticles and as an optical cavity that boosts the plasmonic signal from the nanoparticles," says Monteiro.

The team hope that the device will be particularly valuable in developing countries, which carry the highest burden of HIV. In this setting, cost is paramount.

"Right now, if we count the cost of the device (microcantilever array) and all the chemicals, the cost of the sensor is high," admits Monteiro. However, the components to construct the equipment can be fabricated en masse and at low cost, and Monteiro estimates that the device could one day be manufactured in bulk for less than 1 Euro: "Our nanosensor has the potential to become a cheap and user-friendly technology suitable for resource-limited settings in the future."

Moving the sensor into the clinic will be a long road, but the team are committed. "Getting treatment early will help people with HIV enjoy a longer life, and substantially reduce the risk of transmission to uninfected people," says Monteiro. *CB*

Reference

1. *PM Kosnmjaka et al., "Ultrasensitive detection of HIV-1 p24 antigen by a hybrid nanomechanical-optoplasmonic platform with potential for detecting HIV-1 at first week after infection" PLoS One, 12, e0171899 (2017).*

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 Microcalorimetry

Modern microcalorimetry certainly has great potential in biopharma development but, to make the most of any technique, it is important to understand its advantages and limitations.

By Natalia Markova, Principal Scientist – MicroCal at Malvern Instruments.

Making sure you have an optimal set of analytical techniques at your disposal is crucial whatever your research focus, but can be particularly challenging in sectors that are experiencing rapid change, such as biopharmaceutical development. Biopharmaceuticals and biosimilars are still relatively young drugs when compared to their small-molecule counterparts – and they can behave unexpectedly during manufacture.

The biopharmaceutical sector has been climbing a steep learning curve, but at last we are gaining a better understanding of which properties to monitor and how to measure them. That said, there is still room for improvement. Today, the main concerns in drug development focus on bioactivity and efficacy, stability, ease of delivery, safety and immunogenicity. What (and how) to measure when it comes to understanding these factors is still open to debate, especially as requirements can change throughout the drug development pipeline. Instrument manufacturers continue to work hard to commercialize new technologies to meet the industry's needs – and today there are many analytical solutions to choose from.

Techniques that can stay the course from formulation through to manufacture are highly desirable. In my view, orthogonality – the application of alternative techniques based on different measurement principles – is essential to secure understanding and provide the thoroughness needed to progress through development with confidence. Biopharma development is already expensive and mistakes waste precious resources.

One technique that I think is underutilized in the industry is microcalorimetry. Microcalorimetry involves the measurement of the very small heat changes that occur when a drug interacts with a target site or a protein unfolds, for example, and can help deliver information about those interactions and behaviors. Modern microcalorimetry instrumentation can detect temperature changes of as little as a millionth of a degree, which allows users to observe and quantify changes with just 10 µg of sample. But how should the biopharma community apply the technique to get the best (and most useful) results?

With isothermal titration calorimetry (ITC), heat changes are measured when a ligand, such as a drug candidate, is progressively added to a biomolecular target. The resulting heat profiles

"One technique that I think is underutilized in the industry is microcalorimetry."

generate a wealth of information that can be used to understand molecular interactions, aiding hit selection and lead optimization. ITC, therefore, lends itself to drug discovery.

In contrast, differential scanning calorimetry (DSC) detects protein unfolding/conformational change triggered by the application of a temperature ramp, thereby quantifying stability. Stability is a defining issue throughout biopharmaceutical development through to the point of drug delivery – from early screening through to quality assurance and control, and for biosimilar development. The value of the data provided by DSC therefore remains high throughout the drug pipeline.

DSC can usefully accompany a biopharmaceutical product from its earliest origins all the way to the shelf. Instrument developers must ask themselves how best to adapt DSC technology to meet requirements at every step. Current systems consume relatively little sample and are automated for higher sample throughput – important benefits, of course, that fit the technique for screening applications. To realize DSC's broader value, however, we need to ask some searching questions:

- How can we analyze DSC data as precisely as possible to maximize sensitivity?
- How can we accelerate and 'de-skill' the analytical process to make DSC more suitable for the manufacturing environment?
- How can we streamline DSC to dovetail seamlessly with orthogonal techniques, such as dynamic light scattering, which also have an established role in stability assessment?

If we can answer these challenges, DSC will be able to deliver to its full potential and build on its role as a constant companion throughout drug development and into commercial manufacture. However, more generally, these two examples highlight the need to really understand the potential of a technique to fully exploit its value. ITC boosts productivity primarily by generating a wealth of information to accelerate a single step of development – drug discovery – while DSC is a core tool across the development cycle. I believe we need to explore and embrace techniques in both camps to develop biopharmaceuticals safely and effectively.

Back to Basics

When it comes to sample prep, simple analytical ideas may prove more effective than expensive solutions.

By José Manuel Florêncio Nogueira, Researcher at the Centre of Chemistry and Biochemistry and Associate Professor at the Faculty of Sciences, University of Lisbon, Portugal.

Over the past three decades, a huge number of analytical solutions have been proposed for sample preparation in combination with chromatographic or hyphenated techniques. The complexity of many matrices, as well as the trace levels found in the samples, gave rise to what appeared to be novel ideas and modern concepts, most of them in compliance with the green analytical chemistry (GAC) principles. Good examples of well-established sample enrichment techniques are solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), introduced around 25 and 15 years ago, respectively (1).

Although these miniaturized passive sampling techniques present outstanding analytical advantages in manipulation, simplicity and sensitivity, they have several limitations. For instance, SPME is mainly associated with gas chromatography (GC) and the fibers involved are fragile and expensive, especially if dedicated to routine work. SBSE is also a costly approach in combination with GC, since a thermal desorption unit is required to desorb the analytes. Furthermore, SBSE was designed with the polydimethylsiloxane phase and, although it has excellent enrichment capacity and thermal stability, it cannot microextract the majority of polar compounds. Finally, both analytical devices were intended to be re-used, which creates difficulties, particularly if the back-extraction stage is performed through liquid desorption (LD), which requires several steps that are neither user-friendly nor compatible with routine analysis.

"Particularly in resource-poor settings, there is a clear need to focus on using resources that are available in the lab."

In the meantime, many other solutions have been suggested for routine work, including automated systems that make sampling, agitation, temperature control and derivatization very easy. Nevertheless, these sophisticated systems are useless in certain circumstances, simply because they are beyond the reach of many laboratory budgets, especially in developing countries. Therefore, we need new microextraction devices for sample prep that combine simplicity, ease of use, low costs, GAC principles and suitability for routine work.

Particularly in resource-poor settings, there is a clear need to focus on using resources that are available in the lab, rather than requiring that users rush out to buy expensive sample preparation supplies or equipment. Recently, we introduced 'bar adsorptive microextraction' (BAμE) as a novel passive sample enrichment technique that presents several advantages over previous methods (2, 3).

First, the analytical devices involved can be easily and quickly made in the lab, with very cheap materials. Second, they can be used by anybody, since the extraction stage is performed through agitation without any special requirements – it simply employs the 'floating sampling technology' concept. Third, it can be combined with conventional GC or HPLC systems, using a very simple back-extraction procedure that follows the GAC principles. Fourth, it is compatible with current GC and HPLC auto-samplers, which allows routine work without any instrumental investment.

In short, our new cost-effective and disposable BAμE device has a single LD step for the back-extraction stage, and uses only a few microliters of suitable solvents in glass vial inserts which, after sealing, are ready for instrumental analysis using conventional auto-sampler systems.

I believe that if simple, low-cost ideas like this could be implemented in analytical labs all over the world, most of the expensive solutions proposed by analytical instrument companies would be redundant. For the large number of labs worldwide without a huge budget, cheap but effective solutions are needed – and that might mean moving away from commercial pressures and influence.

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Hunting Hidden Dangers

Exposure to environmental toxins is thought to be a major cause of ill health worldwide, but there are huge gaps in our knowledge. Why is it so hard to measure the impact of these everyday chemicals?

By Tuulia Hyötyläinen, Professor of Chemistry, School of Science and Technology, University of Örebro, Örebro, Sweden.

We live our lives surrounded by potentially harmful chemicals that are used for everything from washing our hands to building cars. As a one-off, they may be harmless, but some of these chemicals in the environment eventually accumulate in humans and can cause adverse health effects. Indeed, the latest statistics indicate that toxic compound exposure may be the leading cause of human morbidity and mortality in both the developing and developed world (1). However, the threat from toxic chemicals has not been sufficiently characterized. Not only is health data available on a very limited number of chemicals, but the important role of combined exposures to multiple chemicals has not been systematically studied.

Chemical exposure studies typically use targeted methods, such as gas or liquid chromatography combined with mass spectrometry, so most of the compounds are not even measured. Lists of target compounds largely consist of those with known toxicity, such as PCBs, brominated flame retardants, pesticides, and so on. However, our chemical exposure from food packing materials, cosmetics and other everyday products are much higher. Most of the compounds in these products have little or no toxicity in themselves, but the toxicity of combined exposure may be significantly higher.

The non-targeted methods applied in areas such as metabolomics are not sensitive enough for use in exposure profiling because of the low levels of many potentially toxic chemicals. Another challenge in exposome studies is the need to measure at more than a single

"The important role of combined exposures to multiple chemicals has not been systematically studied."

"The key challenge is to find methods sensitive enough to both characterize and identify toxic compounds at low levels."

time point to fully understand whole exposure, especially for chemicals with a short biological half-life. In any case, combining metabolomics with exposure analysis allows us to link chemical contact with specific biological changes – and thus link exposure to health outcomes. And even though it is impossible to measure all exposure all the time, the biological responses of past exposure represent a level of memory that reduces the need to capture historical exposure data.

It is obvious that advanced analytical methods are needed for exposome studies, including both GC and LC combined with high-resolution mass spectrometers. The key challenge is to find methods sensitive enough to both characterize and identify toxic compounds at low levels. As the number of chemicals that can be found in the human body is huge, it is critical to identify those compounds that are drivers of adverse effects. A very useful approach is effect-directed analysis, using specific fractionation procedures and in vitro functional assays for detection of the toxic fractions. Moreover, analyzing the metabolic profiles of the cell lines tested may also allow identification of specific metabolic biomarkers of even low-level toxicity. We are currently working with this type of workflow, in close collaboration with toxicologists and bioinformaticians. Currently, the identification of unknown compounds – both metabolic markers and environmental chemicals – is the most time-consuming and challenging task, typically requiring parallel mass spectrometry methods.

Exposome studies allow me to combine two research areas that deeply interest me, namely environmental research and metabolomics. Analytical methodologies play a starring role in this research, which gives me, as an analytical chemist, many interesting challenges to work with. However, it's important to remember that novel analytical tools and non-targeted analyses produce a huge amount of data; often, the bottleneck of the analytical workflow is the preprocessing of data and data mining. Thus, one of the biggest priorities for the field must be developing better bioinformatics tools that allow us to fully exploit the data – and ultimately allow us to achieve our goal of improving global health.

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Chemometrics United

Don't choose between chemometric regression tools – combine ILS and CLS for a powerful synergistic approach.

By Neal Gallagher, Vice President and co-founder of Eigenvector Research, Manson, Washington, USA.

Chemometrics can be thought of as signal processing for measurements made on chemical systems, and the tools available range from simple to dizzyingly complex. The best tool for a given task depends both on the objective and on how the measured signal manifests. If the signal is reasonably described by the linear mixture model, it's common to rely on multivariate linear regression tools, such as partial least squares and classical least squares (CLS) for quantification. Partial least squares is one member of a broad class of inverse least squares (ILS) methods and CLS is often referred to as 'forward least squares'. In the recent past, chemometricians have favored ILS methods, dwelling on the disadvantages of CLS while ignoring the downside of ILS. I believe that a solid understanding of the pros and cons of both methods eliminates the apparent conflict between ILS and CLS, and instead allows them to be used in synergy.

"The best tool for a given task depends both on the objective and on how the measured signal manifests."

Suppose that a data set corresponding to a predictor includes signals from both a target of interest and various interferences; for example, a set of measured absorbance spectra. Also available are the corresponding measured reference values for the target (the predictand). The goal is to use an easy-to-measure predictor to predict a hard-to-measure predictand using a linear regression model. For example, in the process environment it might be of interest to replace an expensive, time-consuming, off-line wet chemistry analysis (the predictand) with a fast, inexpensive, online spectroscopic measurement (the predictor). The result is an online 'inferential sensor' that may also enable proactive control of the process. The often-stated primary advantage of ILS is that the chromophores of all analytes contributing to the signal need not be known; this is true but it's important to note that the interferences must vary in the calibration set, if the model is to account for them. Unless the interferences are varied in a way that makes their signal orthogonal to the signal of the target, there is a chance for coincidental correlation between target and interference. If the correlation remains, the ILS model can take advantage of it, but if the correlation breaks, the model will typically perform poorly in the future.

In contrast, CLS will attempt to use only the target signal and thus avoid coincidental correlation. However, without utilizing external information, CLS requires a good design of experiments (DoE). Astute readers will note that this is exactly the same DoE that would keep the ILS model from relying on coincidental correlation. So, right off the bat, understanding the two modeling approaches has provided a synergistic perspective.

A second item to note is that there is a misconception that CLS is only useful with spectra as the predictand (while citing multi-component Beer's law); in fact, CLS can be applied to other systems.

In general, ILS algorithms are fast and many tools are available to help in model identification (for example, cross-validation). Additionally, the statistics are well defined. In contrast, depending on available measurements, CLS models can be difficult to identify. Unfortunately (except for the simplest problems), interpretation of ILS models can be difficult and misleading. In contrast, CLS models tend to provide the most interpretable models – and that may well be the primary objective. During identification of an ILS model there are several useful constraints (such as non-negativity) that are not applicable during CLS model identification. The wonderful upshot is that ILS models can be used to guide CLS modeling so that both ILS and CLS can be used to their best advantage during model identification.

Because CLS allows useful constraints, provides greater interpretability and is easy to update, I anticipate expanded use of CLS in chemometrics applications in the future. However, it is the synergistic use of ILS and CLS that will enable high quality regression solutions.

The Secret Life of Plants

Transplanted from my native Moscow to the mountains of Colombia, I was astounded by the rich profusion of plant life that surrounded me – but also dismayed by the lack of investment in science. I reacted by rocking the status quo and dedicated my life's work to building up advanced analytical capability and teaching good science – all while exploring the complex chemistry of the country's native flora and harnessing its potential for the agro-industry.

By Elena Stashenko

I was born in Moscow, Russia, and was the only child of parents who were also only children; we were a very small family. My mother was a chemist, and my father was a physicist and lawyer – an expert in ballistics. With two scientist–parents, I passed through childhood between books and test tubes, and learned discipline, the conviction of the superiority of reason, and the importance of science for the progress of humanity. Although we didn't have a lot of money, I never lacked for books or culture (we often visited museums, theaters, exhibitions). The example of my parents – their love for science and reading, their responsible and dedicated work – was a defining influence both in my ideals and the path that my life took in science.

In my young life, my two great passions outside of my academic classes were sport and art, and the lessons I learned from both have enriched my life and career. For over 10 years, I practiced speed skating. Daily training taught me not only to become more disciplined and persistent, but to respect time and, when necessary, to compress it. Dedication to skating eventually displaced my painting classes, although I never lost my love for art. Today, in my classes, I try to combine art and chemistry in my presentations. Graphic design has helped me a lot in my classes to transmit scientific concepts and information to students, in a manner that is both accurate and attractive.

Choosing chemistry

At school, I had excellent teachers: serious, responsible and self-sacrificing. For the rest of my life, I have carried their memory in my heart as a true example of dedicated educators. After school, I wanted to study biology or become a veterinarian, having grown up in the company of dogs – those faithful and unconditional friends. However, my mother always wanted me to be a chemist. Ultimately, I loved and respected my mother very much, so I applied to study chemistry in the Faculty of Natural Sciences at the Peoples' Friendship University in Moscow. My father was more open when it came to my career, but he advised against his own field of criminalistics, telling me that it could be a hard and sometimes bitter profession. Nevertheless, I now teach a forensic chemistry course, which shows how instrumental analytical chemistry can be applied to solve many forensic problems and legal cases, such as drug testing, residues of explosives, arson investigation.

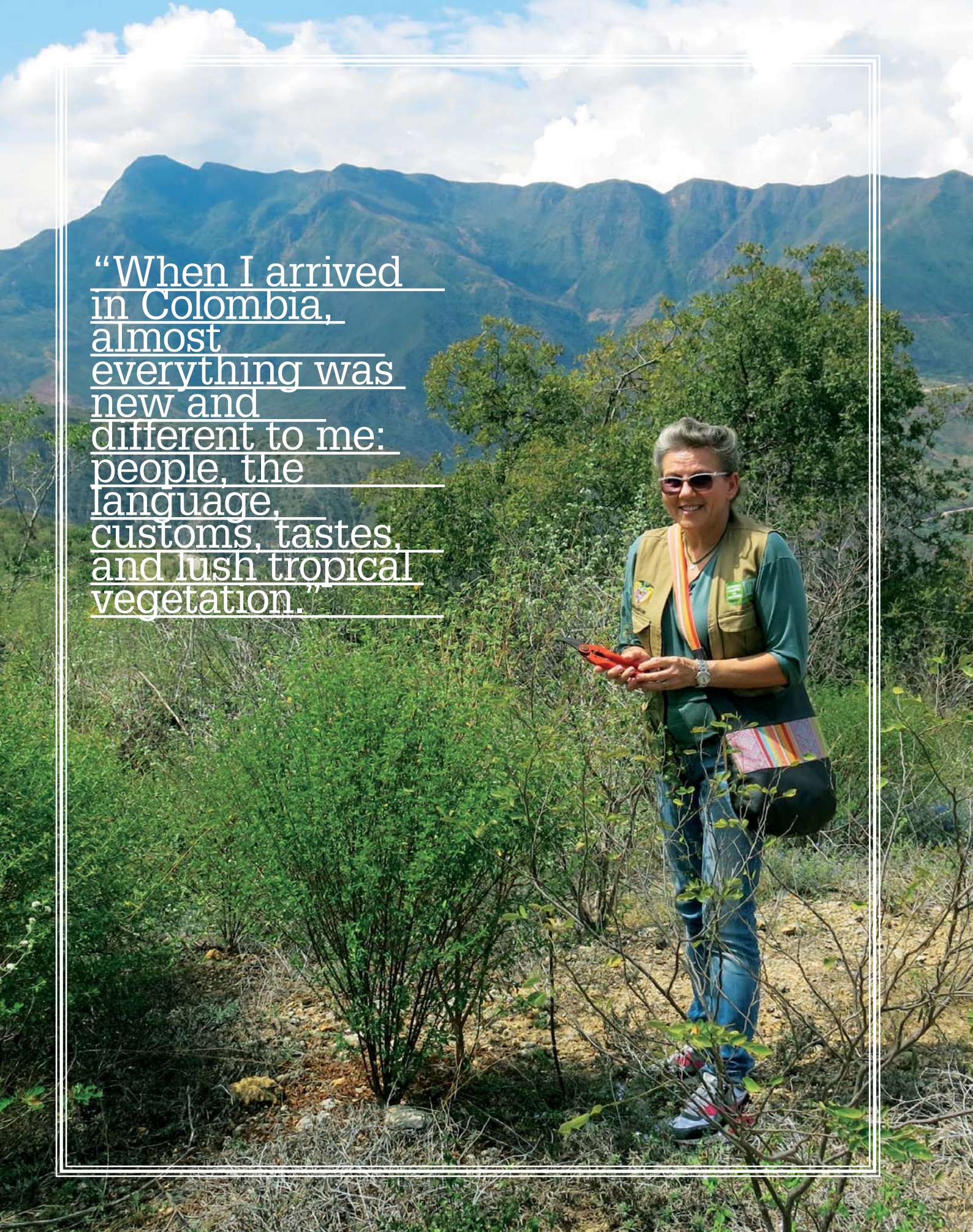
My university days were a time of many good memories, and of fascinating lectures with strict but respectful teachers. Organic chemistry was love at first glance – I felt like a demiurge, creating previously nonexistent molecules with unknown properties. Every time a shapeless mass in my flask precipitated as beautiful, shiny crystals, it felt like a miracle.

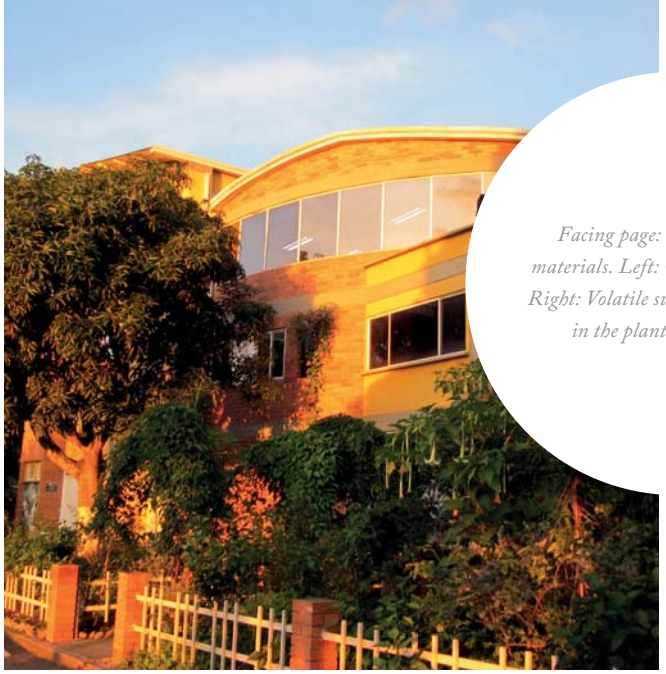
My love for instrumental analytical chemistry came much later, during a PhD devoted to mass spectrometry – a technique whose 'philosophy' is quite the opposite of organic synthesis. Molecules in the ionization chamber are destroyed and cease to exist as a whole; they dissociate and the record of the resulting fragments is the basis for establishing the original molecular structures – often unknown. It is fascinating work, similar to that of an archaeologist recombining remnants to restore an original object to its former glory. During this time (the early 1980s), the dominant themes in mass spectrometry were electron ionization, chemical ionization, fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS), along with the first experiments with glass capillary columns directly coupled to mass spectrometers. In my dissertation, I studied fragmentation patterns and stereo-specific effects of pyridine and piperidine derivatives.

A wonderful new world

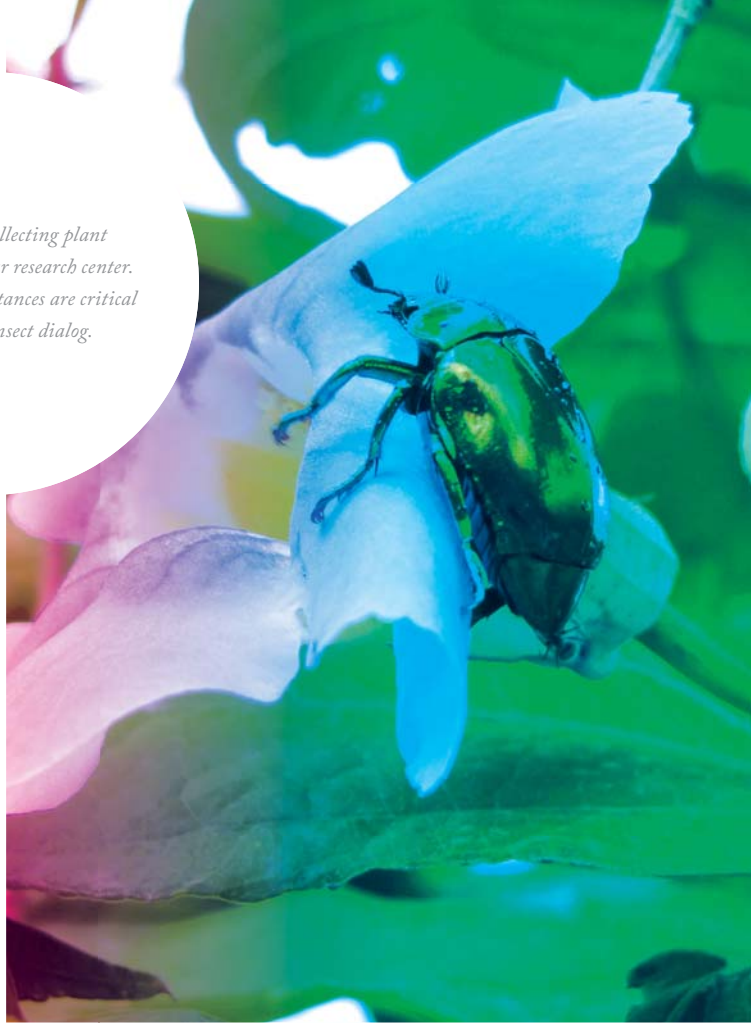
The end of my PhD studies saw a new chapter in my life begin; I fell in love, got married, and gave birth to my first daughter, Juliana. My husband was Colombian and so, at the beginning of the 1980s, I found myself a newly graduated resident of Colombia. A tropical country in the northeast of South America, Colombia has beautiful cordilleras with high and imposing mountains, wide rivers, mighty forests and expansive savannas. I came to a city surrounded by mountains, with a

Clockwise from top: In vivo sampling of flower scent with solid-phase microextraction; visiting Tumaco (SW Colombia) to learn about ethnobotanical uses of aromatic plants; the Passiflora edulis flower.


“When I arrived in Colombia, almost everything was new and different to me: people, the language, customs, tastes, and lush tropical vegetation.”



Facing page: Collecting plant materials. Left: Our research center. Right: Volatile substances are critical in the plant–insect dialog.



difficult name for a foreigner to pronounce – Bucaramanga. There, I had my second daughter, Laura, and in 1983, began my work at the Industrial University of Santander (UIS), a public university with over 20,000 students, and a strong emphasis on engineering.

When I arrived in Colombia, almost everything was new and different to me: people, the language, customs, tastes, and lush tropical vegetation. These days, we have access to the Internet, which allows us to travel 'digitally' and learn about places, their histories and customs, before ever setting foot there. But back then, a lot of things were a surprise to me. There are so many plant species – so different, rare and opulent, that a lifetime would not be enough to learn them all. Huge, hairy insects with hundreds of legs frequently enter the house without permission (and used to terrify me). There are no seasons: plants sprout and flower throughout the year. The sun rises at 6 am; at 6 pm, after a short twilight, the darkness comes. People usually go to bed early and get up very early; some classes at the university start at 6 am, when in Russia I would still be asleep. Celebrating Christmas and New Year without snow, among exuberant flowers – and in 30 °C heat – was very strange to me at first.

Students at the university, not much accustomed to foreign teachers with funny accents, were friendly, curious and very patient with my then-limited Spanish. To live in a foreign country, to understand it and to love it, you have to understand its history, geography, literature, music, and culture in general. I traveled a lot through Colombia, studying its history and customs, and fell in love with Colombian literature, especially the magical realism represented by the great Nobel Prize Laureate Gabriel García Márquez.

Science against the odds

I traveled back to my home country in 1984 to carry out doctoral studies at the University of Moscow. After graduating in the field of instrumental analysis, with emphasis on mass spectrometry, I returned to Bucaramanga, and the Industrial University of Santander, at the beginning of 1989. At this time, I could not even dream of continuing my research in mass spectrometry: in the whole country, there were only two mass spectrometry instruments and only a few chromatographs. Many students and teachers were skeptical about the possibilities of doing good science in Colombia. Equipment, infrastructure, and people with ideas and know-how are all required to develop science. The 1990s were difficult in Colombia: economically complicated, and with very little investment in education. The country was suffering at the hands of drug traffickers, who created a lot of insecurity, along with several insurgent and paramilitary groups; massacres and kidnappings were everyday news. It was a time of anxiety and distress, which led many Colombians to emigrate. The best students got their scholarships and left the country to study in the United States, Canada or Europe; most did not return to Colombia. Funding universities was not seen as a priority. Even now, there are scant resources for scientific research: in Colombia, less than 0.3 percent of GDP is devoted to the development of science and technology – much less than other Latin American countries (Brazil, Mexico, Chile, Argentina), not to mention developed countries.

The bad state of affairs led students, and even some professors, to say that it was not possible to do science in Colombia. But I felt

they were wrong. My ideal and example in life has been the Polish–French scientist Marie Skłodowska Curie, a model of dedication, scientific rigor and vehemence. With my hero in mind, I plucked up my courage, boldness and stubbornness to defy the status quo. I would not accept that it was impossible to do good basic science and good instrumental analytical chemistry in a developing country. It became my challenge and ambition to create a cutting-edge research center, to train PhD students within Colombia, and to contribute to the development of its science. Without good basic science, it would be impossible to develop the country's industry and technology, let alone innovate. Fortunately, there were several young professors who shared my conviction and joined me on my mission.

Biodiverse = paradise

When I left Moscow, I knew the names of almost all trees, flowers, insects, and animals there. At school, I used to study black and white photos in geography books, depicting strange plants and animals – anacondas and anteaters, capybaras and cacti, hallucinogenic fungi and poisonous jellyfish. I never imagined that in a few years I would be seeing many of these wonders for myself, living and working in one of the most biodiverse tropical countries in the world.

At first, I tirelessly interrogated people about the names of plants and their uses, but I soon came to realize that it is impossible to know the name of every plant in a country where there are more than 5,000 species per 10,000 m^2. During the period of the Spanish conquest, there was much ignorance (and therefore distrust) of native flora and fauna. The conquistadors brought with them many plants from their homeland, including aromatic and medicinal plants (rue, chamomile, basil, marjoram, anise, rosemary), food crops (rice, sugar cane, various cereals, apples, plum, citrus fruits, carrots, peas, beets), ornamental and 'stimulant' plants (coffee, tea). The New World, in its turn, gave to the Old World tobacco, corn, potatoes, tomatoes, blackberries, beans, cassava, rubber, cinchona trees, vanilla, cocoa, and other plant species of great economic importance. Nevertheless, most industrial crops in Colombia (palm oil, sugar cane, rice, sorghum, citrus, coffee) are introduced species. Unfortunately, Colombia's native plants have occupied a more modest place in the economy and in science, and have not been studied with due attention.

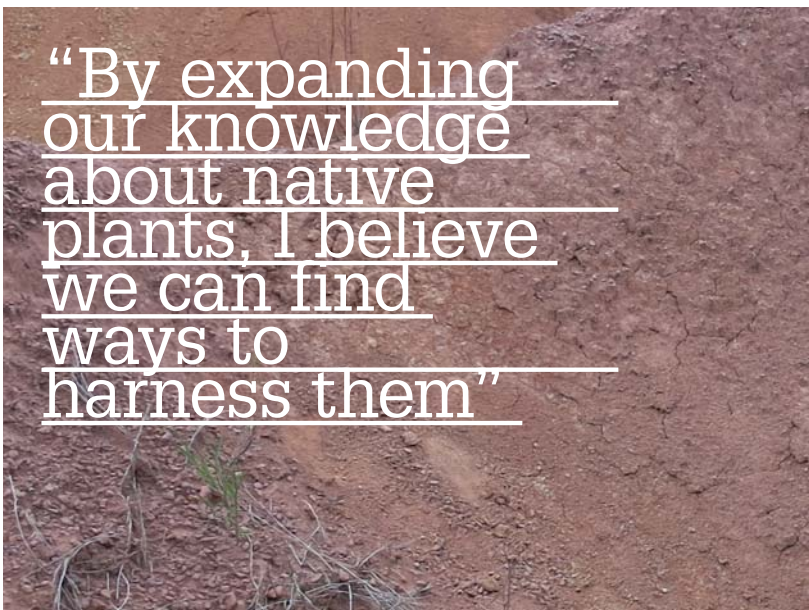
So when I asked myself, "How can I apply my analytical chemistry research here in Colombia?" the answer arose naturally: biodiversity, native plants and their metabolites. By expanding our knowledge about native plants, I believe we can find ways to harness them (wisely and sustainably), by creating products based on their oils and extracts. That was the start of a path I have followed for almost three decades.

Slow and steady wins the race

We started with small projects, funded by government science agency COLCIENCIAS. In 1993, after more than three years of waiting, we bought our first pieces of analytical equipment from Hewlett-Packard: a HP 5890 GC and a HP 5972 GC with mass selective detector, both still working until recently. With these tools, we were admitted into the kingdom of tropical plant research, studying the secondary metabolites behind their intriguing characteristics.

Gradually, with funding from the Colombian government, COLCIENCIAS, and the Ministry of Agriculture, as well as the funds we had generated ourselves by running courses and providing analytical services, we began to stock the research center with the necessary tools: extraction equipment, analytical instruments, gas chromatographs with various detectors (TCD, FID, ECD, NPD, FPD), liquid chromatographs with different detection systems (UV-Vis, DAD, FLD, ELSD), GC-MS and LC-MS systems, low-resolution (Q) and high-resolution (rTOF, Orbitrap) mass spectrometers and tandem systems (QqQ). Initially, we focused on GC-MS for analysis of essential oils, but today we largely employ LC-MS analysis to study polar molecules, flavonoids, alkaloids, anthocyanins, and other metabolites of native plants.

In 1998, we started the National School for Chromatography and Related Techniques, to provide training to anyone who wanted to learn how to use chromatography and mass spectrometry, and apply them in their own field. Since that time, more than 250 courses have been given to about 2,500 people. The Research Center also offers analytical services to Colombian industry, to projects developed by other researchers, or to governmental control entities (food, environmental, forensic). It's a different facet of work, requiring a high degree of rigor and responsibility to ensure confidence in the



Clockwise from top: Our research group, with over 50 members; essential oils; collecting plant materials in the field.

Case Studies

Playing Chicken

A few years ago there was a spate of chicken fatalities in local poultry farms, and we set out to find the cause. It looked like the deaths were caused by toxic contaminants in food; however, standard analytical procedures to test for organo-chlorine and organo-phosphorous pesticides found nothing in the sorghum-based chicken feed used in the farms. Tests for aflatoxins and pathological bacteria also came up negative. We subjected the sorghum grain to various extraction procedures, before concentrating the grain extract tenfold and analyzing it with GC-MS, which showed the presence of fatty acids characteristic of sorghum, and indole traces. We had recently installed a GC-MS instrument equipped with the pulsed-splitless injection mode, which reduces the residence time of the injected sample at high (250 °C) temperatures. The analysis of the same extract, using pulsed-splitless injection, discovered a pyrrolizidine alkaloid. These alkaloids are thermolabile and had not been detected in previous analyses because they decompose at the injection port. Close examination of the sorghum grains revealed that around four percent were intruder grains - the same size, but a different color and shape. They were carefully separated and subjected to soxhlet (CH_2Cl_2) and SFE extractions. GC-MS analysis of these extracts showed the presence of indole and monocrotaline, a very harmful pneumotoxic and hepatotoxic compound. The intruder seeds were planted in our experimental garden and were botanically identified as Crotalaria retusa. The solution was simple: farmers were warned to eliminate Crotalaria from their sorghum crop before processing the grains for chicken food.

The Sweet Smell of Success

The chemistry of flowers and their volatile substances is a particular passion for me. Flowers showcase the power of evolution and play diverse roles in plant physiology. Their main responsibility is to perpetuate the plant and they use various strategies to attract pollinators: different forms and colors, rising temperature, and release of volatiles. The emission rate and the amount and type of substances released change with the time of day, light, and whether the plant has already been pollinated. It takes weeks or months for a synthetic organic chemist to produce a single compound, but a flower can synthesize tens of them in a few minutes. What's more, the substances released by different flower parts (e.g., petals, sepals, feminine and masculine organs) are not the same. Determining these profiles constitutes an important part of our analytical studies, required by biochemists, entomologists, ecologists and biologists who study pollination and plant fertilization. Extraction techniques such as purge and trap, and solid-phase microextraction (SPME), combined with comprehensive chromatography (GCxGC), play a fundamental role in our studies. We are also studying the relationship between flower color and the antioxidant activity of flower extracts, and we have already found a clear correlation between red color and superior antioxidant activity.

The Essentials

Essential oil production requires large volumes of vegetation. Typically, around 1 kg of essential oil is obtained from 100 kg of biomass. Many of our projects in the last 10 years have been geared to production of the required biomass in the countryside by small farmers' associations. During the projects, the farmers learn how to cultivate aromatic plants, while adhering to clean agricultural practices, plus how to carry out post-harvest tasks and obtain the essential oil using stills designed by our group and the School of Mechanical Engineering. Lemongrass, citronella, mountain oregano (Lippia origanoides), rosemary, prontoalivio (Lippia alba), damiana, palmarosa, sage and ylang-ylang are some of the species included. These pilot production projects are particularly important because they provide an alternative to coca plantations, which have declined notably in recent years. Many aromatic plants provide three or four harvests per year and the resulting essential oil is an added-value, small-volume product that can be used in many sectors of the economy. It is an example of the application of basic scientific research to benefit populations that have had fewer development opportunities. Once the essential oils have been characterized, the knowledge of their biological and physicochemical properties permits the design of products such as mouth washes, moisturizing creams and oils, massage oils, insect repellents, and many more. Thus, through the study of the plant's chemistry we arrive at functional products based on natural ingredients. In turn, the production of these ingredients enhances the living standards of the farmers and their families.

Top: Moringa oleifera flower fragrance sample in vivo. Bottom: Flowers change their volatile profile upon pollination.

analytical results obtained. We obtained the Accreditation of the Laboratory Quality System 10 years ago. The funds from analytical services are reinvested in financing theses, scholarships, the purchase of reagents, accessories, equipment and their maintenance, as well as for participation in international symposia and conferences.

In late 2004, several research groups in the country came together to develop a national program for the study of biodiversity in Colombia; in particular, tropical plants. Chemists, microbiologists, plant physiologists, botanists, agronomists, biochemists, and chemical, mechanical and industrial engineers have all participated in this program. The study of plants and their metabolites needs a multidisciplinary team, as it requires an understanding of both behavior and chemistry. Together, we must interpret the 'language' of the plants, learn their 'temperament', and assess their relationships with other plants or insects, their metabolism and adaptation strategies.

The Research Center of Excellence CENIVAM (Research Center for the Agro-Industrialization of Tropical Medicinal

A Chemist's Best Friend

Loyal and caring, dogs have accompanied mankind throughout history. Unfortunately, many dogs live on the streets of Colombian cities in miserable conditions. They barely survive. Although government and non-government organizations exist to protect them, they are not enough. We have developed an interesting program with our students that aims to help.

We have helped to find homes for many of the stray dogs that have come to our university. In fact, a few of them have remained and live in the Institute; they are part of the staff, and students, professors and researchers contribute to provide their food, care and veterinary needs. The dogs go to classes; always punctual, they sit in the front row and participate in their own way in our seminars. We also allow graduate students to bring their own dogs into the Institute, so they are not preoccupied by leaving their pets home alone all day.

Some people would say that it is impossible to have dogs in the laboratory, in the library, or in class – especially dogs that came from the streets. But our dogs have dramatically changed the work atmosphere. They have introduced a sense of harmony, relaxation and positive energy, with their wagging tails, happy disposition, and quickness to thank, love, and forgive. They have taught our heterogenous group of students, researchers and office workers a lot. Dogs act as emotional buffers, sometimes even as lightning rods. It has been an amazing experiment, a twist of socio-biology. To me it feels natural that we, who study biodiversity, share our work environment not only with a wide range of plants, but also our canine friends, who engender unity, cooperation, and positive emotions.

and Aromatic Vegetal Species) was created at the beginning of 2005, dedicated to the study of tropical plants and their agro-industrialization. This government-supported project has proved very successful, bringing together more than 150 researchers (including undergraduate and graduate students, professors, and young researchers) from 20 different research groups of 10 universities in the country, coordinated by our Research Center for Biomolecules here at the Industrial University of Santander.

Essential beauty

The study of biodiversity begins with botanical expeditions and the taxonomic identification of the plant species collected. More than 1,200 botanical samples have been collected and taxonomically identified (by the Colombian National Herbarium at Bogota), during more than 30 trips to different regions in Colombia. Back at the laboratory, we carry out extractions to obtain volatile fractions, essential oils, supercritical extracts, and hydro-alcoholic extracts, which are tested for biological activity. Around 700 essential oils and 500 extracts (obtained with ethanol–water blend or supercritical CO_2) have been derived from the collected plants, many of them never before studied.

The secondary metabolites of the plants are highly complex mixtures. Their constituents have different volatilities, polarities, and concentrations; their extraction requires the use of various techniques, headspace, solid-phase microextraction (SPME), purge & trap (P&T), steam distillation or hydrodistillation, solvent or supercritical fluid extraction, or matrix solid-phase dispersion. Analysis of the fractions, oils or extracts obtained requires the use of gas chromatography, liquid chromatography and detection systems with high-resolution mass-spectrometric analyzers. After their characterization, essential oils and extracts are sent to different laboratories to study their biological properties. After performing some 5,500 assays, around 45 percent of all oils or extracts tested positive for one of the biological properties examined (antibacterial, antiviral, antifungal, anti-inflammatory, antigenotoxic, photoprotective, among others).

Research in my group follows several avenues. One is the study of essential oils, their production, their physicochemical analysis, and the determination of their biological properties. Despite

> "After nearly three decades, it is still exciting to study the biodiversity of tropical plant species through the prism of their chemical constituents."

the rich botanical diversity in Colombia, most essential oils are currently imported. Rural Colombia remains wedded to traditional agriculture, with little technological advancement or innovation. By strengthening the alliance between the university (know-how), business (technological capacity) and countryside (biodiversity) we hope to develop the natural ingredients industry in Colombia. Eventually, essential oils and extracts may not only supply the internal demand, but could also be exported. We are also making final products based on essential oils and extracts, including air fresheners, repellents, creams, mouthwashes, soaps, and antiseptic gels.

Of particular note is our natural insect repellent, which contains essential oils that we are already producing with a group of farmers. It is a very important product, especially now, with the rise of dengue, Zika, and chikungunya, all transmitted by the Aedes aegypti mosquito. We have patented a number of our discoveries, including a mobile essential oil still for field use, and the biotransformation of citronellol to hydroxycitronellol by means of a fungus. Another eight patent requests have been filed recently.

Other lines of research include the isolation and identification of toxic alkaloids in tropical plants, plus the study of polyphenolic compounds (flavonoids, anthocyanins) in tropical flowers and their antioxidant activity. I find the study of tropical flowers particularly fascinating. Flowers use many strategies to attract pollinators – extremes of shape and color, sweet nectar, and intense and constantly-changing fragrances. They can even vary their temperature and color over the course of the day. This wonderful complexity means that the study of flower metabolomics requires an ingenuous combination of extraction methods and highly sensitive analytical techniques. High-resolution technologies (GCxGC, GC-TOF-MS, LC-TOF-MS, and Orbitrap-related techniques), as well as tandem configurations (QqQ, Q-TOF) are needed for the complete and reliable description of secondary metabolites, which can serve as a biologically relevant signal for a pollinator at ppb or even ppt concentrations.

Insect predators or pollinators of plants are worthy of study in their own right, too. It is interesting to discover how some flower secondary metabolites are transferred to insects and can even become defensive tools; for example, some caterpillars devour plant leaves that contain pyrrolizidine alkaloids and so become toxic to their natural enemies. The study of these complex chemical relationships would not be possible without a good base of high-resolution chromatographs and mass spectrometers.

'Good science in a developing country' is not oxymoronic

I am passionate about doing science in a developing country that is building its STEM sector – a country that suffers many socio-economic and political problems, but where young people are eager for knowledge and progress. I believe that by creating laboratories with cutting-edge technology and diverse and modern extraction systems, we are contributing to an exciting and growing science economy in Colombia.

Curiosity and motivation are both needed to push research forward in such settings – something that university teachers must inculcate in students. More than 300 undergraduate students have completed their program at CENIVAM in recent years, along with 50 Master's degrees and 20 PhDs. In my classes, I try to convey my message in an entertaining way and pass on my love of the field. Having more 'fans' of instrumental analytical chemistry is very important, because this discipline permeates so many fields of science – medicinal chemistry, forensic, environmental, food chemistry, natural products, petroleum chemistry, geochemistry, and many more.

After nearly three decades, it is still exciting to study the biodiversity of tropical plant species through the prism of their chemical constituents – the products of their secondary metabolism. And discovering – through chemical analysis – a plant's structures, origins and functions remains a great challenge. This fantastical journey begins with reliable instrumental chemical analysis, and that makes our work as rewarding as it is important.

Elena Stashenko is Director of the Research Center for Biomolecules at the Industrial University of Santander in Bucaramanga, Colombia.

BREAKING IT DOWN: THREE GURUS OF LIBS

Our experts take stock of the last 20 years of laser-induced breakdown spectroscopy – and wonder if it's finally ready to spread its wings...

What have the past 20 years of LIBS brought us?

David Hahn: The last 20 years have brought a much deeper understanding of the physics behind LIBS, notably with regard to the fundamental processes of analyte transport (such as dissociation and diffusion), excitation, homogeneity within the plasma, and equilibrium. We can draw a parallel to the development of ICP.

Vincenzo Palleschi: I've been working in LIBS for more than a quarter-century (my first LIBS paper was published in 1990), and the progress in understanding the processes involved in LIBS has been outstanding. In many cases, the theoretical framework was already present in the literature; however, the peculiar characteristics of the LIBS technique (based on the complex interplay of laser, plasma and sample) have required great efforts to interpret. As a consequence, we can now tune our systems according to the application, optimizing the experimental parameters, such as laser wavelength, pulse duration, focal length of the focusing system, and so on. Today, LIBS analysis has moved to many fields that were difficult to imagine 25 years ago. But this is not necessarily a good thing…

Richard Russo: I guess I'm the odd one out here, because I feel that the theory has only somewhat evolved, yet applications are proliferating. The community has delved into fundamental physics of laser plasmas and gained a good understanding of time resolved phenomena; however, we still cannot predict the response. We do not know what amount of mass will be ablated and what percentage will be heated to emission – a complicated problem considering the spatial and temporal aspects of the transient plasma.

The LIBS technique is straightforward from an experimental point of view, which is both a blessing and a curse – as Vincenzo alludes to. Why? Because anyone can focus a pulsed laser on any sample and create a plasma. But good analytical chemistry requires experience of how the plasma spectroscopy relates to the sample chemistry. LIBS is no different than arc/spark spectroscopy in principle – it is all atomic emission spectroscopy. To advance LIBS for routine applications, we need to use the same principles that have made arc/spark a mainstay in many applications.

The Experts

Richard E Russo

Rick Russo has studied the fundamental properties of laser material interactions and related applications for over 30 years. Since 1982, he has held various positions at the Lawrence Berkeley National Laboratory in Berkeley, California, where he is currently a Senior Scientist. Rick is also CEO and founder of Applied Spectra.

Vincenzo Palleschi

Vincenzo Palleschi is a Professor at the University of Pisa and Head of the Applied and Laser Spectroscopy Laboratory at the Institute of Chemistry of Organometallic Compounds, Pisa CNR, Italy. He has been working with LIBS for over 25 years and is founder of the LIBS conference series and Chair of EMLIBS 2017 in Pisa.

David W Hahn

David Hahn is Professor and Department Chair at the University of Florida, Department of Mechanical and Aerospace Engineering, where he heads the Laser-Based Diagnostics Laboratory. The laboratory is dedicated to the development and application of advanced diagnostics techniques, including LIBS, Raman, light scattering and LIF. He has been working on fundamentals and applications of LIBS for over 20 years.

What challenges does the field face?

VP: One of the recurring questions I face when presenting LIBS to non-specialists is: "If the LIBS technique is so powerful, why is it only used in a few research laboratories?" My answer: "Poor marketing". Twenty-five years of LIBS experience has taught me that it does not pay to oversell a technique above its actual capabilities. As a community, we have often presented the LIBS technique as a 'Swiss knife' able to do everything, instead of looking for specific applications in which LIBS would excel with respect to other analytical techniques. The great expectations created around some proposed LIBS applications failed miserably when faced with the reality, which did not contribute to the acceptance of the technique among the analytical science community.

RR: I agree with Vincenzo that the biggest problem for the field has been failure to manage expectations. A single failure can outweigh 99 successes, so we need to be patient and establish widespread successful industrial applications.

Over my 40 years working with lasers, I have seen interest in LIBS wax and wane. The current upswing seems more durable, mainly due to improved instrument components (lasers, spectrometers) and wider interest from industry. We have also learnt that for LIBS, like all technologies, one configuration does not fit all. There are tradeoffs between sensitivity, precision, speed, spatial resolution, and so on. Matrix effects aren't always a bad thing. Unlike solution analysis, where the solution is the matrix and the elements of interest are a minor part of the matrix, for direct solid sample analysis (LIBS) – the elements of interest are the matrix. For classification analysis, the matrix is your friend – the unique spectra become a barcode for that particular sample. For sensitivity, absolute limits of detection are spectacular, with femtogram and less capability. The community needs to remember not to compare bulk analysis to microscale analysis.

DH: LIBS has most certainly expanded greatly, and successful applications are emerging. I believe the greatest pitfall facing LIBS is its 'misuse' in systems of very high non-homogeneity or significant unknown composition, often resulting in unacceptable uncertainty. Applying LIBS to a well-defined analytical space makes the most sense.

Chemometrics have been a big boost to LIBS; however, simply over-training within a given set of standards and then classifying within that set does not necessarily speak to the success of LIBS. I remain highly skeptical of LIBS for areas like cancer detection, as the classification with high-

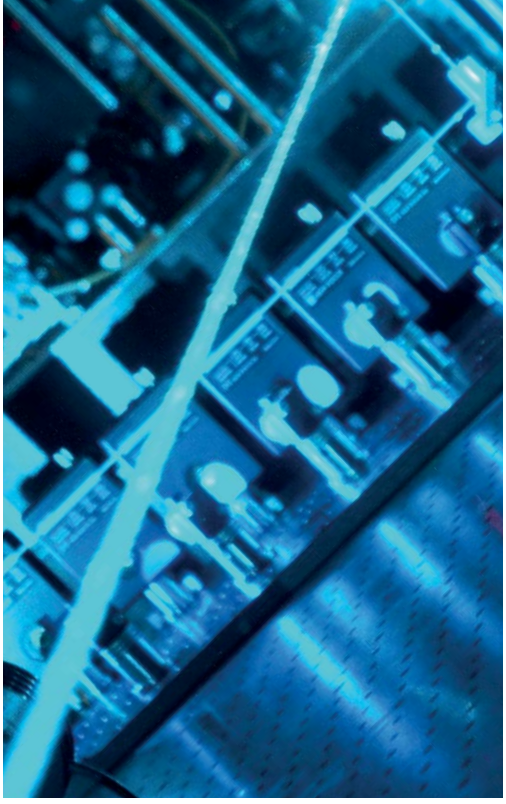
order chemometric methods is often tagged to contaminants or simply concomitant elements (for example, sodium or calcium), which have no bearing on the physics of the problem at hand. That is truly a potential trap for any analytical scheme.

What have been the milestones in LIBS?

VP: I might be a little bit biased because I was directly involved in it but, in my opinion, the most important milestone in LIBS as a field was the first LIBS Conference in 2000. That event cemented an active and lively LIBS community. In terms of technological leaps, the introduction of double-pulse LIBS and calibration-free analysis were important milestones.

RR: LIBS was first demonstrated as a potential analytical technique in 1963, when it was called laser plasma spectroscopy. Commercial instruments were available in the 1970s and 1980s but components – especially lasers – were not as reliable as today's versions. Handheld LIBS with successful applications could be considered a milestone, but we still need to establish suitable applications. Hyphenated approaches that couple LIBS with Raman or ICP are milestones in expanding capabilities. Laser ablation molecular isotopic spectroscopy (LAMIS; developed in my group) is a milestone in providing the ability to measure isotope ratios at atmospheric pressure and at remote distances – no other technology can provide this capability (See "More on LAMIS"). The NASA Curiosity Chemcam project was a milestone in terms of increasing exposure to LIBS outside the academic community.

DH: The implementation of LIBS on Mars was an engineering accomplishment for the decades. I say engineering, because the fact it's being done on a remote planet (which can't be praised enough) is more revolutionary than the actual science of LIBS on Mars. The use of chemometrics and multivariate analyses are also significant milestones.



"Much of the effort is in R&D and the publication rate is phenomenal. However, displacing existing technologies is not easy unless there is compelling value added."

In what applications has LIBS contributed most?

RR: Much of the effort is in R&D and the publication rate is phenomenal. However, displacing existing technologies is not easy unless there is compelling value added. The real breakthrough will come when industry has a killer application that cannot be addressed using other, more established or recognized technology. The crucial question is: what does LIBS offer that industry cannot do using current ICP, arc/spark or XRF?

VP: At the moment, I cannot think of any LIBS applications that have obtained results significant enough to be worthy of special note. Over the decades, we have seen a lot of 'proofs of principle' but – with the exception of academic research – the technique has yet to find its ideal application.

DH: I believe one of the best applications is in metals recycling and processing, where LIBS can make significant contributions as a real-time sensor.

What did the Mars project do for LIBS?

RR: The science community does not need to be convinced. They are on board already. However, the funding and commercial sectors need to see the capabilities and demand, and NASA showed that LIBS is worthy of the investment. ChemCam cost millions of dollars and is a beautiful system, but I'm not sure if we have seen the full benefits of NASA's effort on the commercial or funding sectors yet.

VP: The Mars mission has been a great boost for the popularity of LIBS. However, the results obtained so far don't seem to

More on LAMIS
By Richard Russo

Laser ablation molecular isotopic spectroscopy (LAMIS) is an advanced implementation of LIBS, which measures molecular spectra appearing later after the ablation laser pulse. These are not the same molecular spectra as measured by Raman, but heated molecules that form after the hot atoms/ions collide with species in the plasma. With LAMIS, the isotopes of light elements (C, N, H, Cl) become part of the analytical package. Tandem LA-LIBS/LAMIS-ICP can do the job of GD, XRF, carbon and mercury analyzers, OES and MS at the same time. Rapid chemical imaging, analysis and depth profiling with high spatial resolution at atmospheric pressure are benefits that underlie the complete elemental and isotopic analysis capabilities of this technology.

The LIBS–LAMIS combination is an all-optical elemental/isotopic analytical instrument, well suited for real-time standoff measurements. NASA proved that the LIBS technology can work on Mars with a standoff distance of about 7 meters – a very successful demonstration of real-time elemental analysis in a challenging environment. Many industrial applications can benefit from standoff in-line elemental analysis. Couple LIBS with LAMIS for standoff, and you have the only approach that can measure isotopes at a distance.

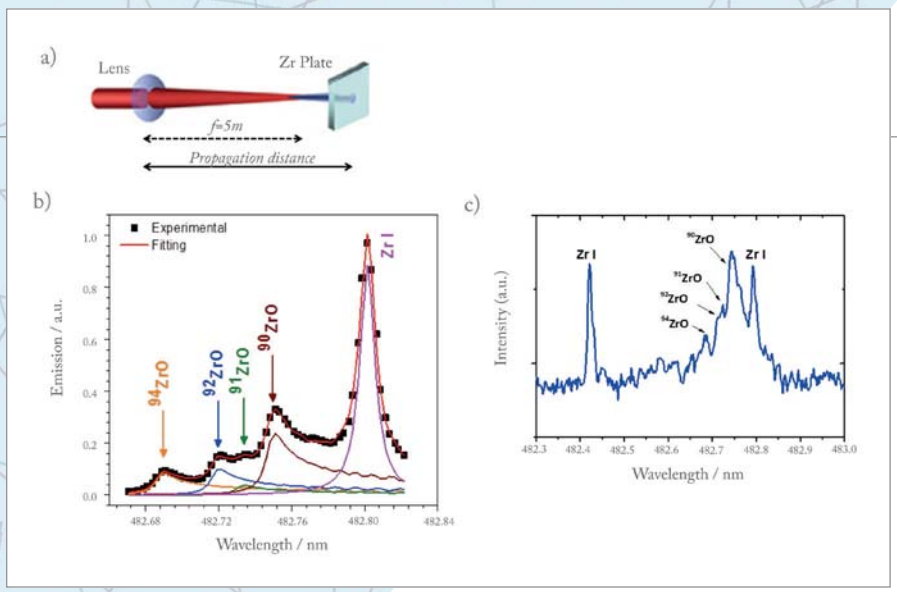


Figure 1. LAMIS measurement at remote distances using femtosecond laser induced filaments. a) F2-LAMIS experimental setup, b) Zr isotope measurement at 9 m, and c) 74 m. Note: Spectrometer resolution is not the same.

Partnership in Pisa
By Vincenzo Palleschi

The 9th Euro-Mediterranean Symposium on LIBS (9th EMSLIBS) will be organized by the Institute of Chemistry of Organometallic Compounds of the National Research Council of Italy (CNR-ICCOM) in Pisa, Italy, from 11 to 16 June 2017.

Recent EMSLIBS conferences have transcended their original regional scope and acquired a global reputation, which will be enhanced this year by being co-organized with the Colloquium Spectroscopicum Internationale (CSI), a conference now celebrating its 40th anniversary. Together these events represent a historic forum to discuss developments in fundamentals and applications of all branches of spectroscopy.

The joint organization of EMSLIBS and CSI will have a positive impact on both conferences by stimulating collaboration, as all participants and exhibitors will attend both events with a single registration. Moreover, the two conferences will share the Plenary and Award lectures, and all social events.

Several 'hybrid' sessions will be organized on specific applications, such as environmental analysis, industrial diagnostics, geology, and cultural heritage, making the joint event one of the most important for the whole analytical spectroscopy community.

Notably, the LIBS event won't be held again in the Euro-Mediterranean area until 2022; the EMSLIBS conference is ready to fill this gap, offering researchers who cannot travel to America or Asia a complete and up-to-date picture of the most recent developments in LIBS research.

Read more about the event and register at www.emslibs.org

have met the high expectations of the proposers. In my opinion, the most important result of LIBS on Mars has been the flourishing of portable LIBS systems here on Earth.

DH: I agree with Rick and Vincenzo – the engineering involved in making LIBS work remotely on another planet is fantastic, but the main benefit has been to boost the profile of LIBS.

How do you expect to see the field develop in the near future?

VP: In scientific research, there is always room for surprises and technical jumps. However, I feel we are already exploring the limits of the LIBS technique. The application of chemometric methods could improve the reliability of LIBS quantitative analysis, but (for intrinsic reasons) I'm not expecting to see LIBS competing with existing laboratory techniques in terms of reproducibility, sensitivity and precision in the near future. The brightest prospects for LIBS will probably be along two promising lines: micro-LIBS imaging in the lab and fast remote diagnostic of industrial or environmental processes outside the laboratory. Nanoparticle-enhanced LIBS, the current darling of the field, is extremely interesting to study from a fundamental point of view, but I'm not really sure that the improvements in sensitivity would compensate for the rather complex sample treatment required.

DH: I believe new breakthroughs in repetition rate in lasers and detectors will be the next big thing. LIBS is still highly susceptible to sample inhomogeneity, and one way to counter that is massive ensemble averaging. The ability to rapidly record and process thousands or tens of thousands of spectra to 'homogenize' samples will be significant for many applications. Processing of tagged tissue samples (for example, adding an antigen-tagged molecule with a unique metallic signal) is a promising area for LIBS.

RR: From a commercial standpoint, successful industrial applications are critical; we need to know how LIBS can solve industrial problems, if we are to displace mainstay technologies – in other words: what is the value added?

The value proposition for this technology is compelling – every element and isotope with a single instrument, no sample preparation and rapid turn-around time. Yet, several commercial applications have struggled, even where there are tangible benefits. Companies have tried for years to get LIBS accepted by the pharmaceutical industry. Even with all the benefits and successful demonstrations, it seems companies like PharmaLaser could not get this community to adopt LIBS. Why not? I once read that it took over 20 years for society to give up on typewriters and completely switch over to computers. Computers had to be proven in successful areas before mainstream society adopted them.

"Understand the pros and cons of competing techniques, and propose the use of LIBS only where it has a real advantage."

What advice would you give the LIBS community?

RR: Don't extrapolate! Each application needs an optimized method. This is a requirement with every other analytical technology (consider the effort that goes into dissolution procedures that have been around for ages). Some in the LIBS community might like to think it is not the case for LIBS. But it is.

My goal is to have one instrument capable of measuring every element and isotope on the periodic chart. This can only be achieved by combining LIBS with other techniques such as ICP-MS and LAMIS.

VP: Understand the pros and cons of competing techniques, and propose the use of LIBS only where it has a real advantage (price, simplicity, performance). Don't advertise results that you only hope to obtain. A good scientist should know the limitations of his or her technique, before exploiting its advantages.

DH: Seek out the 'right' applications of LIBS – the 'low-hanging fruit' that can benefit from its unique characteristics (low sample prep, in situ analysis, rapid processing, elemental analysis). LIBS is not an analytical panacea, and never will be – no analytical technique is. In addition, attention to the physics of detection with chemometrics remains essential. Making classifications based on spurious signals that are not rooted in fundamental physical (elemental) differences in the target space does not advance the cause of LIBS as an accepted analytical scheme.

Profession

Leadership
Talent Development
Career Planning

The Science of Sugar: Lessons Learned with Pauline Rudd

Pauline Rudd's passion for glycans started early – as a teenager she experimented with extracting sugars from natural products in her kitchen. Today, she is a principal investigator at NIBRT – Ireland's National Institute for Bioprocessing Research and Training. Here, she reflects on her early interest, and the complex but crucial role of glycobiology in biosimilar development.

Chemistry is fascinating... but wasn't my first choice

As a child, I wanted to be a physicist. My uncle was a physicist and he and I used to talk physics every time we met. I joined the British Junior Astronomical Association, but it was very male dominated at the time; there were 48 boys... and me. I was never allowed to look down the telescope. I got into chemistry, and specifically sugars, because I could do it at home in my kitchen using very simple ingredients, like potato starch. I used to beg a few grams of this and that from the pharmacies in my hometown for my experiments. Eventually, a pharmacist suggested that I talk to his son, telling me: "He's as crazy as you are!" We had similar interests, and while still in school started a company called Wessex Biochemicals to make rare sugars and sugar phosphates. I was about 14 years old and it was tremendous fun. Our main piece of equipment at the time was a washing machine with a heater and a side paddle, which we used to extract trehalose from hot ethanol and baker's yeast.

Taking time out to raise a family doesn't mean the end of your career

I went to the University of London to study chemistry and when I returned home, we continued to build the company, which was later sold to Sigma London. We bought the site, which Sigma still occupy in Poole, and continued to run the science. After I had children, a lab was built for me at home so that I could combine work and motherhood. But eventually we moved and I couldn't take the lab with me, so I was out of the lab for 15 years.

I did a lot of the things in the interim while looking after the children, including commercial analytical work and some forensic science projects. After the fourth child started school, I went back to full-time work in Oxford and was fortunate enough to obtain a place in Professor Raymond Dwek's lab. Later, this became the Oxford Glycobiology Institute – of which he remains Director. After such a long career break, I'd never imagined that I'd be able to go back to working with sugars, so I was very happy! That said, I had to work very hard to advance. I started out in Oxford as a glass washer, but eventually I was able to form my own group. Some 23 years later, 11 of us moved to Ireland to work with NIBRT – the National Institute for Bioprocessing Research and Training in Dublin. NIBRT provides unique training courses for people to learn how to operate the plants that produce new therapeutic drugs. My group mainly focuses on developing advanced glycoanalytical technologies, some of which have been commercialized by Waters Corporation, to analyze glycosylation in biotherapeutics and in systems biology, which sets out to link glycans with classes of molecules such as genes, proteins, transcription factors and lipids. This gives us information about the pathways that molecules take as they are made by cells. If a pathway is damaged in disease we can see the effects in the glycan structures.

Looking back, I have been very fortunate. Today, I think it is much harder to find a career path, even if you love science.

Photo credit: Simon C Rudd.

Sugars are complex – and they matter in drug development

At least 60 percent of natural proteins have sugars attached to them. These sugars are huge – often bigger than the proteins to which they are bound; they are molecules in their own right. They play a role in protecting proteins, but are also important in cell communication, signaling pathways, and in the immune system.

Many biotherapeutics are glycosylated proteins. Given that they are often delivered to the patient in large quantities, you need to be absolutely sure they will activate in the right place in the body and not cause unwanted effects. For example, many drugs target tumor cells; the antigen binding sites locate the tumour epitope and then the rest of the molecule can initiate a killing reaction. There are many interactions that an antibody can engage with once it's bound to the target though, so it is important to design an antibody that will not have adverse effects.

The sugar molecules, or oligosaccharides, are made up of branching chains of monosaccharide residues. The sequence and the way in which they are linked, as well as their number, can affect the protein in various ways, including its efficacy, stability and safety, so removing or changing sugars can often have quite drastic effects. For example, erythropoietin, which is used to treat anemia and which has been associated with doping in cycling, has three huge oligosaccharies with four branches terminating with sialic acid. The drug can stay in the patient for three hours, but if you remove this acid, it will be gone in three minutes – which doesn't make for a very efficacious drug! In the worst case scenario, the wrong glycosylation profile can mean the patient has to receive high levels of the drug, which can cause them to raise an unwanted immune response.

When developing a biologic, attention must be paid to the structure of the molecule to ensure that it is safe. However, biologicals are sensitive and unwanted post-translational modifications can occur during bioprocessing. There is always some batch-to-batch variability in biomanufacturing, but you need to ensure that your glycosylation profile remains within a safe window. Consistent glycosylation is generally a very good marker of the consistency of a bioprocess – and it is something that regulators pay close attention to. When submitting a drug for approval, you need to submit data around critical features of glycosylation.

Similarity comes in many shades

For those who aren't chemists, the topic of glycosylation can often seem daunting and complicated, but it is actually not difficult to understand the basic science, since many sugars are members of families of nested structures. In fact, I think we need more people to understand the topic, especially given the increasing number of small companies and start-ups that want to get into the biosimilar space. Not everyone appreciates how challenging matching a glycosylation profile of a biosimilar to an innovator product can be.

Start-up companies focusing on biosimilars sometimes leave glycosylation studies until the very last minute. Developing a biopharmaceutical is incredibly challenging, and sometimes there is an assumption that developing a biosimilar is easy in comparison, since you are copying an already-developed product. The difficulty comes in ensuring that your biosimilar has the same protein and glycosylation profile as the originator drug, within specified limits. In this regard, the innovator company perhaps had the easy job – they made the drug and showed that it was safe and non-toxic. The glycosylation profile came out as it did, and there was no need to match it to anything else. Often, a biosimilar developer may think they have copied a biological drug, but their process may be very different – and so too may the glycosylation profile. Given all the different variables in processing, you can end up creating hundreds of

Pauline Rudd's Reading Recommendation

For those wanting to know more about glycosylation, I recommend the following review, which was written by a group that I am privileged to be associated with in the Bioprocessing Technology Institute in A*Star, Singapore; I am a visiting investigator.

P Zhang et al., "Challenges of glycosylation analysis and control: an integrated approach to producing optimal and consistent therapeutic drugs", Drug Discov. Today, 21, 740–765 (2016). PMID: 26821133.

clones that aren't actually similar to the innovator product at all, which wastes a lot of time. Scientists do try to think about the problem rationally, but working with biological products is always challenging. If you're new to the area (and even for experienced scientists), understanding and controlling your glycosylation profile can be a nightmare – you'll need to dredge scientific literature to understand what conditions promote the glycosylation you need, and learn to understand your sugars and how they affect your drug.

All of this said, you also have to bite the bullet and try out some process conditions or gene editing! The innovators aren't going to tell you what they did, so you have to try things out and learn for yourself how they affect glycosylation.

Analytical technology is always advancing

The most common techniques for analyzing glycosylation profiles are liquid chromatography (LC) and mass spectrometry (MS). Capillary electrophoresis is also important. In a nutshell, glycan analysis is all about separation. Different separation techniques give you different information. LC, for example, separates oligosaccharides on the basis of shape, charge, and hydrophobic and hydrophilic surfaces. Mass spectrometry, on the other hand, provides information about composition; it will tell you how much your sugar weighs and you can work out which mono-saccharides are there, but it won't always tell you whether it's glucose or galactose, or the way in which they are linked together. For that, you need more sophisticated technology that can fragment or break the sugar into pieces. These pieces give you the sequence and linkage of the sugars.

Large companies can afford to have many instruments to provide different information, but smaller companies can find it more difficult to invest in equipment. In my group at NIBRT, we have access to a lot of equipment so we are often asked to help out smaller companies (as well as larger ones).

One challenge with the newest analytical equipment, however, is the sheer volume of data generated. It is important to remember that some aspects of glycosylation may not really affect the product – what the regulators care about are the critical features that affect safety and efficacy, such as antigenic epitopes.

Several vendors have developed really good workflows for their equipment (some of which we've helped to establish). We worked with Waters Corporation to establish an effective LC-MS workflow, where every sample goes straight from the LC onto the coupled MS, and then the information from both are lined up by the informatics program to give orthogonal confirmation of structure. This type of continuous bioinformatics is very important to interpret large data sets and to obtain GMP compliant information.

There's always room for improvement

There are reliable glyco-technologies available for every stage of bioprocessing, including at- or on-line automated sample prep, which can be attached to a sterile sampler. In the past few years there has been a step change in the development of robust technologies, and the drive now is to miniaturize and develop faster approaches.

Areas for future exploration include micro-analytics and sampling through the whole reactor. There is even the possibility of a swarm of micro samplers in the processor, which can radio out glycosylation features and create a 3D picture of conditions in the reactor. We know that cells have altered glycosylation at different phases in the cell cycle and in hypoxic conditions, and adding real-time analysis would help to understand where this was happening in the reactor. We also need better bioinformatics to aid data analysis, particularly for mass spectrometry data and advanced technologies that involve fragmentation.

Wanted: Winning Project for 2017

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2016 Winner: Cheaper HIV Diagnostics

Waseem Asghar, Assistant Professor at the Departments of Computer Engineering & Electrical Engineering, Computer Science, and Biological Sciences, Florida Atlantic University, USA, was given 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 aureus* and other pathogens."

HUMANITY IN SCIENCE AWARD

the Analytical Scientist

Solutions

Real analytical problems
Collaborative expertise
Novel applications

Taming the Third Dimension

In an ambitious bid to boost separation performance, spatial 3D liquid chromatography prototypes aim to leave an impression.

By Jelle De Vos and Sebastiaan Eeltink

The problem

The resolving power provided by contemporary 1D- and 2D-LC technology is insufficient to tackle the complex sample mixtures encountered in modern life-science studies, where each sample may contain hundreds of thousands of proteins. Furthermore, when high-peak capacity separations can be realized, it is often only achieved at the expense of very long analysis times, making the technology intrinsically unsuitable for biomarker discovery and validation studies.

Background

Since the introduction of the first commercially available HPLC column in the early 1970s, there has been a trend towards using smaller particles to increase the separation efficiency and sample throughput. Using state-of-the-art packed columns operated at ultra-high-pressure conditions, the maximum peak capacity of high-resolution one-dimensional LC is currently limited to about 1,000 in a few hours (1). To boost separation performance, two-dimensional liquid chromatography (2D-LC) separation technology has been developed. Conventional on-line 2D-LC separations are performed by coupling two columns using a high-pressure switching valve to modulate fractions eluting from the first-dimension (^{1}D) column and directing these to the second-dimension (^{2}D) column. Provided orthogonal separation mechanisms are employed, the maximum peak capacity that can be realized is the product of the two individual developments. In this way, LC×LC separations yielding peak capacities of up to 2,100 in 60 min have recently been developed (2). However, a disadvantage of conventional 2D-LC is that the fractions collected are analyzed sequentially.

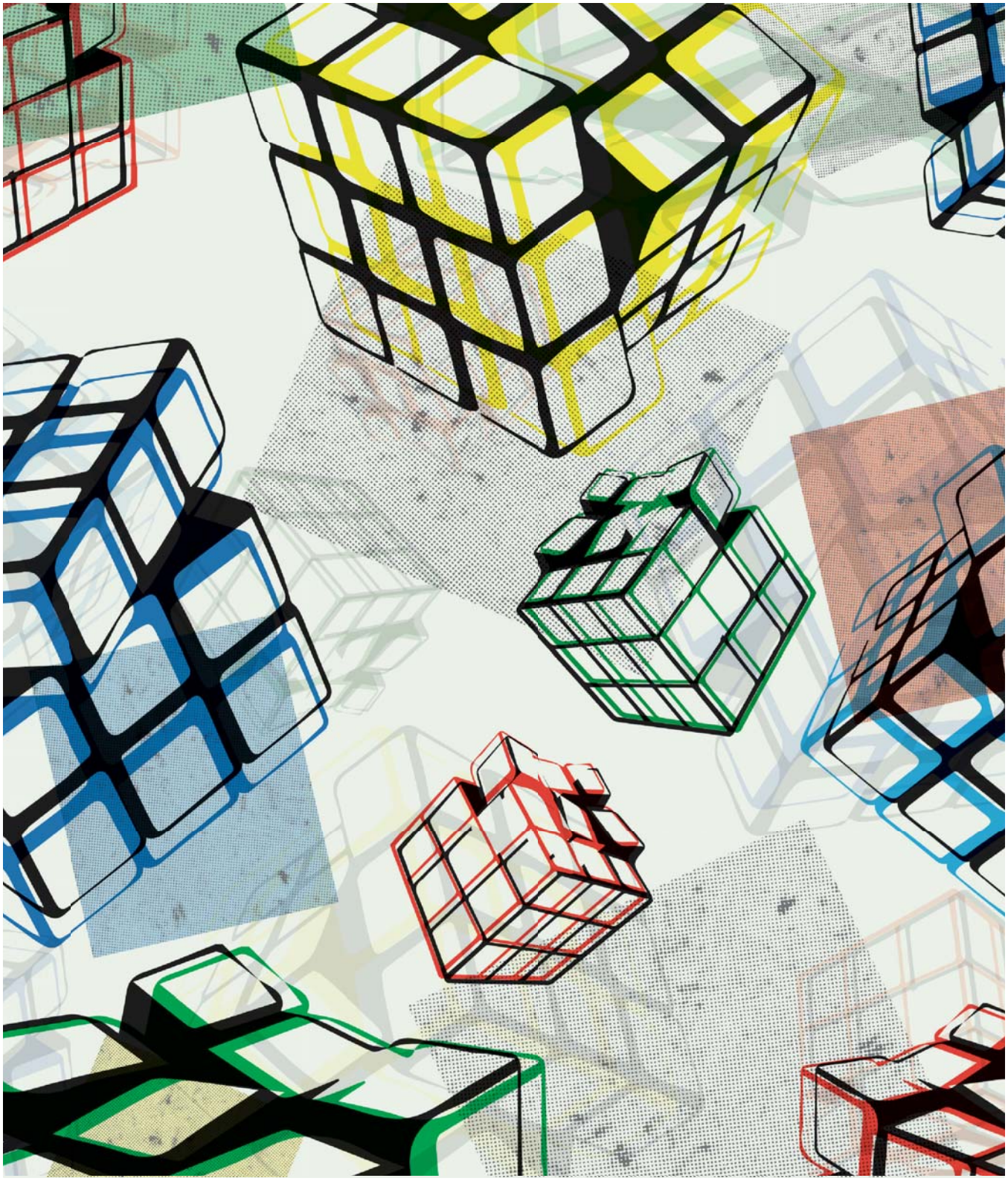
The solution

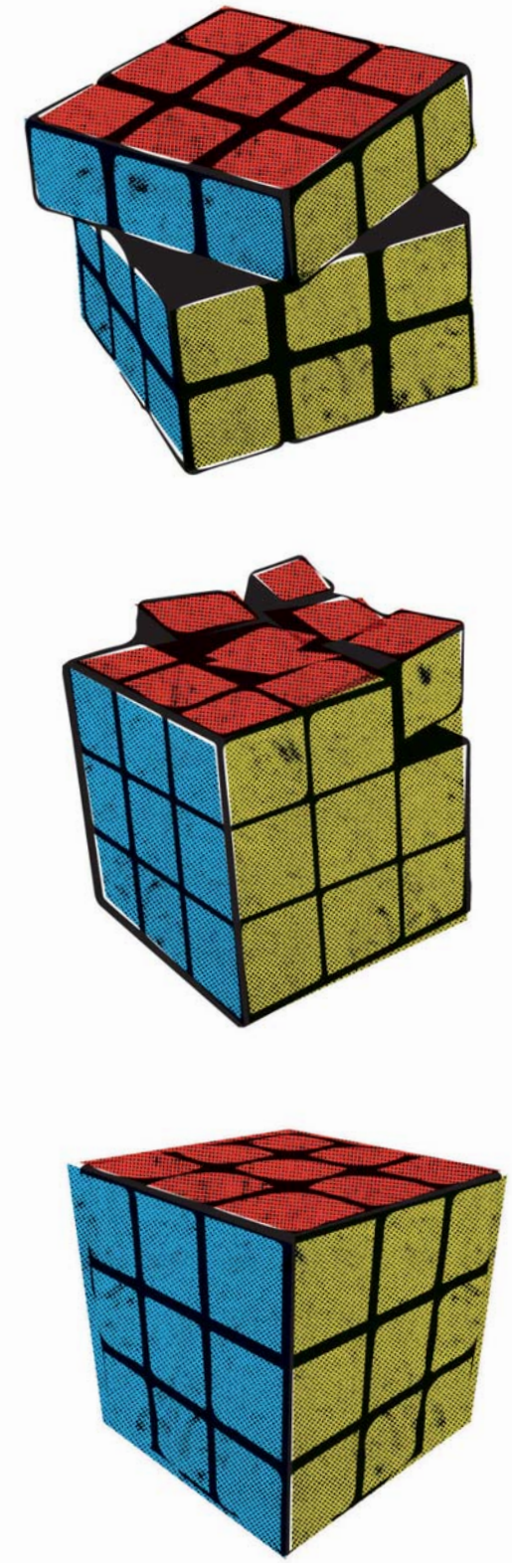
Several years ago, Peter Schoenmakers from the University of Amsterdam, the Netherlands, explained a new chromatography concept to us, with the potential to deliver unmatched separation performance in terms of the maximum peak capacity and peak-production rate – spatial 3D liquid chromatography (3D-LC). Spatial 3D-LC separations are performed by forcing analytes to migrate to different positions in a 3D body (see Figure 1). In brief, after injecting the sample mixture into one corner of the device, three subsequent separations take place in the X, Y and Z directions, which means that each peak is characterized by its X, Y and Z coordinates in the 3D separation body.

We strongly believe in the potential of spatial multi-dimensional LC; together with Schoenmakers, patents were filed describing various aspects of the 3D separation body, including flow control elements and detection.

At the Vrije Universiteit Brussel, we have been developing the microfluidic chip technology necessary to realize spatial 3D-LC separations since 2010. In that time, we have delved into many different aspects of the technology and how it can be applied in prototype chips. One such prototype chip for spatial 3D-LC developed in our lab is depicted in Figure 2. The concept is easy to grasp

"The analysis time is greatly reduced with spatial 3D-LC when compared with conventional coupled-column multidimensional approaches."





with a cube in mind: after finalizing a ^{1}D dimension separation in the X direction, the ^{2}D separation stage is performed simultaneously in 16 parallel Y channels. In a final step, all the compounds situated in the X–Y plane are separated in 254 parallel ^{3}D channels (Z direction). The maximum peak capacity ($^{3D\text{-}LC}n_c$) that can be realized is the product of peak capacities (*nc*) achieved in the three individual separation stage, according to:

$$^{3D\text{-}LC}n_c = {}^{1}n_c \times {}^{2}n_c \times {}^{3}n_c$$

Crucially, because of the parallel nature of the separations in the second and third dimensions, the total analysis time ($^{3D\text{-}LC}$time) is only the sum of the three separation stages:

$$^{3D\text{-}LC}\text{time} = {}^{1}\text{time} + {}^{2}\text{time} + {}^{3}\text{time}$$

The upshot is that the analysis time is greatly reduced with spatial 3D-LC when compared with conventional coupled-column multidimensional approaches, in which sampled fractions are analyzed sequentially.

But getting there is no simple task. One major challenge was the need to address flow control during subsequent separation stages. To that end, we explored the use of physical barriers to confine the flow between the individual stages, and controlled flow with ^{2}D and ^{3}D flow distributors (3,4). We also needed to demonstrate proof-of-principle when it came to creating polymer-monolithic stationary phases in-situ in the microfluidic chip. To confine monolith formation to the desired location, we developed a UV-initiated polymerization approach in combination with the application of photomasks.

Beyond the solution

Many challenges remain in the full realization of this spatial 3D-LC concept. For example, flow control can still be improved, orthogonal retention mechanisms should be established, and detection is currently a bottleneck. To address the latter problem, it is envisioned that detection of analytes can be realized via a 'printing technique', which is to say by immobilizing the effluent from the final separation on a suitable substrate at regular intervals. The result of the 3D separation will be a series of 2D time images, from which 3D images can be reconstructed after (mass spectrometric) imaging. Recently, Jelle De Vos obtained a postdoctoral grant from the Research Foundation Flanders to extend our research and further develop spatial 3D-LC technology. But we are also open to establishing collaborations with both academic groups and industry partners to join us in our journey from concept to three-dimensional reality.

Jelle De Vos is a postdoctoral researcher and Sebastiaan Eeltink is a research professor at the Vrije Universiteit Brussel (VUB), Department of Chemical Engineering, Brussels, Belgium.

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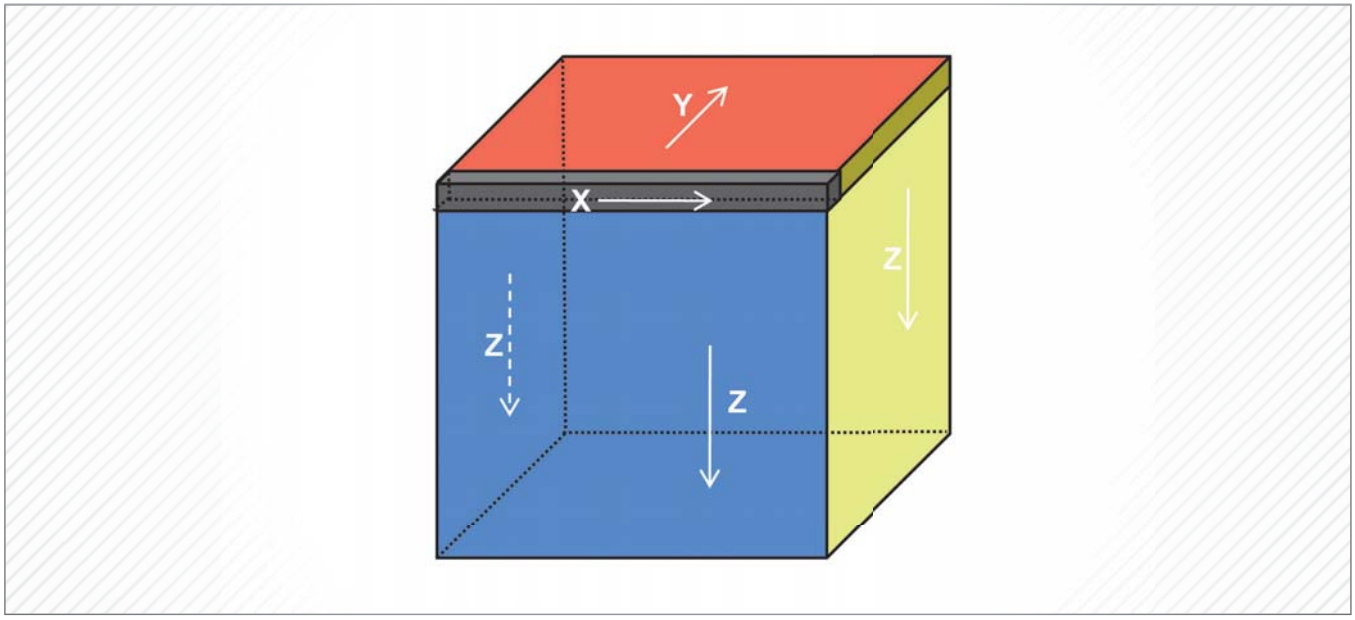

Figure 1. Concept of spatial 3D-LC. Components are separated in the space domain via three subsequent developments, with each peak being characterized by its (X,Y,Z) coordinates in a 3D separation body.

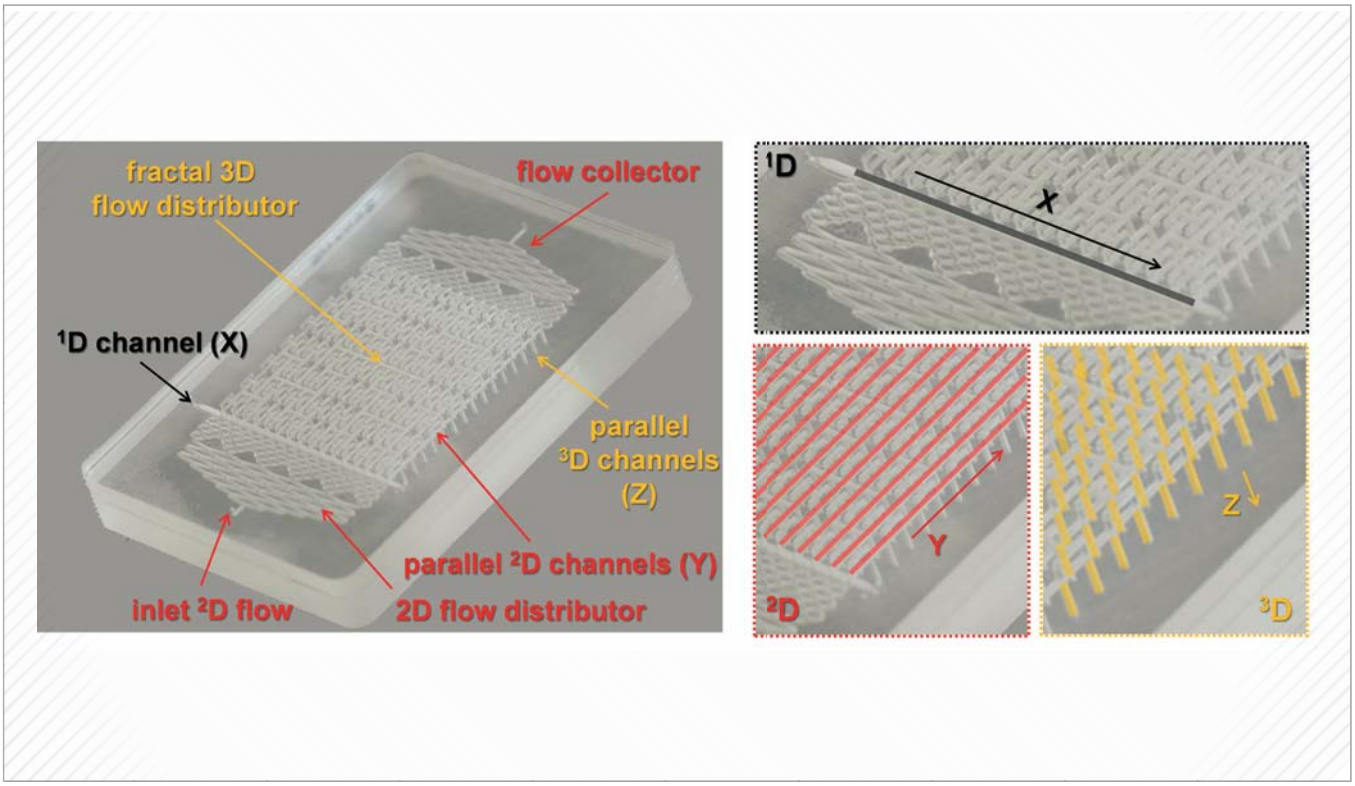

Figure 2. Prototype chip for spatial 3D-LC developed at the VUB. After sample injection, three subsequent developments can be realized in the X, Y and Z directions, respectively. The radially-interconnected 2D and fractal 3D flow distributors allow for homogeneous flows during the 2D and 3D developments.

Collaborating for the Clinical Win

Sitting Down With... Ron Heeren, Director of Maastricht MultiModal Molecular Imaging Institute (M4I), Distinguished Professor and Limburg Chair at Maastricht University, the Netherlands.

Is there a common theme to your career?
Change and passion. I always told myself that every ten years I would do something different. I was trained as a physicist, became a professor in chemistry, and now I am working in a molecular imaging institute housed in a medical department. I stay enthused about what I am doing by making it worthwhile – and changing my environment and research topics helps to keep my passion for science alive.

So, what motivates you?
Curiosity, enthusiasm, passion – wanting to be an explorer. I'm very lucky to have been able to set up this wonderful institute within the University of Maastricht. A collaborator of ours said to me recently, "I feel like a kid in a candy store!" and that's exactly the environment that we wanted to create – it encourages people to do great analytical science and great molecular imaging. Here, I get to define the questions I'd like to ask and the best tools for answering those questions, and so explore the world around me. How much better does it get?

Tell us about your current research...
Our major focus is using molecular imaging based on mass spectrometry to assess the molecular content of tissues, so we can provide clinicians and medical researchers with feedback on the cellular phenotype. Say a surgeon operates on a patient and removes a tumor; it's sent to a pathologist, who takes a section for hemotoxylin–eosin staining and inspection, and we take an adjacent section for mass spec imaging analysis. Half an hour later, we aim to provide the surgeon and pathologist with our findings and see how they match. In a second clinical research project – intraoperative diagnostics – we are analyzing the smoke from laser surgery to give surgeons the information they need in real time.Most of our research is biomedical, but we also use MS imaging to study new biomaterials, regenerative medicine, drug distribution and metabolism, forensics, and even historical paintings.

And recent findings?
In a study on cholangiocarcinoma we identified several peptides, proteins and lipids that distinguish transient neoplastic tissue (on its way to becoming a tumor) from full-blown tumor cells and healthy tissue. In other words, we can assess a single piece of tissue and define different cellular tissue phenotypes, which helps us to assess how clean the surgical margin of a tumor is; has the surgeon removed enough? Is there any cancerous or pre-cancerous tissue remaining?

How close are these tools to the clinic?
We've developed technology and methods for a number of diseases. The next step is validation – we need to work with large patient cohorts to make sure that the markers we have found in ten organs are stable and robust in 100 organs or 1,000 organs. That's one reason the group is based in Maastricht, which gives us access to a dynamic clinical environment with a large volume of samples.

To establish a validated clinical diagnostic test, several major clinical studies and a lot of administrative and legal paperwork are needed. It's difficult to predict how long it will take, but I hope that within 3-5 years some of these tests will be routinely available. For now, they are still in the research phase.

What is the role of analytical scientists in clinical collaborations?
Typically, the analytical scientist provides the technology and data needed to make a clinical assessment. The analytical scientist also needs to understand the questions or challenges faced by medical practitioners, and how new techniques could fit into the clinical workflow. They are the axle in the wheel – a project manager, analytical scientist and communicator all in one. As an analytical scientist, you are too often forced into the role of a service provider, and that's not the way we work. Our ethos is CORE – collaborative, open research and education.

"Changing my environment and research topics helps to keep my passion for science alive."

What's the secret to successful collaboration?
Crucial to the success of this type of multidisciplinary research is a willingness to give something up to ultimately gain a lot more. Sometimes we generate great results, but rather than presenting them at an analytical science conference ourselves, we ask the surgeons on the team to present them at a surgery conference. We may give up a little visibility in our own community but, in the long term, we have a much bigger impact in the clinical field – where it really matters.

What are your career highlights, so far?
From a research perspective, the best thing has been seeing how the ideas we had ten years ago are being realized in the clinical environment. I've always believed our work would make a difference for patients, and seeing that start to happen is wonderful. Talking about it at TEDxMaastricht recently was an absolute highlight for me, personally. Molecular structure plays a much bigger role in disease than we previously thought, and being able to visualize that with the technology we've developed is fantastic. It's also great to have the chance to improve the instruments we all rely on, by collaborating with the companies who make them.

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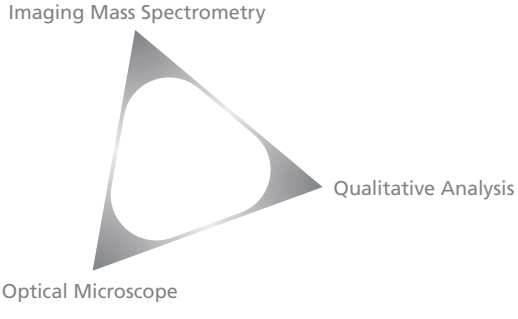

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