Vol. 30 No. 6 December 2018/January 2019

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Brillouin microspectroscopy of bio systems
NIR spectroscopy for bone identification
LC-MS/MS clinical sample prep

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EDITORIAL

The questions keep coming

Last issue, I was writing about questions raised by the articles and columns. This issue, there are some answers... but yet more questions!

Kareem Elsayad and Francesca Palombo give us a fascinating update on Brillouin spectroscopy, in particular "Brillouin microspectroscopy: in search of the mechanical properties in biological systems well below a wavenumber". They show that the Brillouin measured elastic modulus clearly varies between regions of a biological sample, even if sometimes only by small amounts. Exactly what this is telling us and for what it is most useful or relevant is currently an area of active debate and remains to be seen.

Judy Stones' "A practical guide to sample preparation for liquid chromatography-tandem mass spectrometry in clinical research and toxicology" provides a valuable summary of the choice of sample clean-up methods available for the quantification of small molecules in body fluids. What are the key factors? Judy outlines the principal processing methods and provides practical advice on protocol development using quantification of serum testosterone in serum samples as the model compound.

Our third article is by Aoife Power, James Chapman and Daniel Cozzolino, "Near infrared spectroscopy, the skeleton key for bone identification". Knowledge of the origin of bones has applications in anthropology, archaeology and forensics; NIR spectroscopy, even with handheld instruments, is showing promise in being able to differentiate bones from different species.

In the Tony Davies column, Werner Barnard, Henk-Jan van Manen and Tony Davies are "Inspiring people with process analytical technology". They tell us about an initiative to promote analytical spectroscopy to a range of people from school children and their parents to Dutch chemists. PAT is the key.

In the Quality Matters column, Peter Jenks thinks about the new definition of the kilo, and other SI units, in "The kilo, the mole and the commutability of a result to activity". However, he is more concerned with maintaining accurate laboratory measurements over time, which is crucial to making data comparable.

Some answers from Kim Esbensen in the second part of "A tale of two laboratories". Kim explores further the question of whether laboratories should take wider responsibility for the samples they analyse. In particular, whether the original sampling of the materials provided to the was representative. Has he let the genie out of the bottle?

Brillouin microspectroscopy can provide a novel contrast mechanism for mapping micro-mechanics within cells and tissues, such as in sea anemones. Find out in the article starting on page 12.

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e-mail: cs-journals@wiley.co.uk

Spectroscopy Europe is a joint publication of John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, UK, and IM Publications LLP, 6 Charlton Mill, Charlton, Chichester, West Sussex PO18 0HY, UK.

Vol. 30 No. 6
December 2018/January 2019

ISSN 0966-0941

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Spectroscopy Europe is a controlled circulation journal, published seven times a year and available free-of-charge to qualifying individuals in Europe. Others can subscribe at the rate of €152 (Europe), £108 (UK), $208 (ROW, postage included) for the seven issues published in 2016. All paid subscription enquiries should be addressed to: *Spectroscopy Europe*, John Wiley & Sons Ltd, Journals Administration Department, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, UK.

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NEWS

John Chalmers wins the 2018 Norman Sheppard Award

The Norman Sheppard Award is given by the UK's Infrared and Raman Discussion Group (IRDG) and was initiated in 2012 to honour Professor Norman Sheppard FRS, who was the first recipient. Since then the award has been given annually to recognise individuals who have significantly advanced the field of vibrational spectroscopy in industry or academia, and who have demonstrated long-term support for the aims and activities of the IRDG.

We are delighted that ex Article Editor of *Spectroscopy Europe*, John Chalmers, has been announced as the 2018 winner of the Norman Sheppard Award. The IRDG say about John that he "has made outstanding contributions to industrial vibrational spectroscopy, scientific publishing, education and the IRDG during a career spanning more than 40 years". John will receive the award at the IRDG Christmas Meeting held at University College London on 20 December 2018. Many congratulations, John!

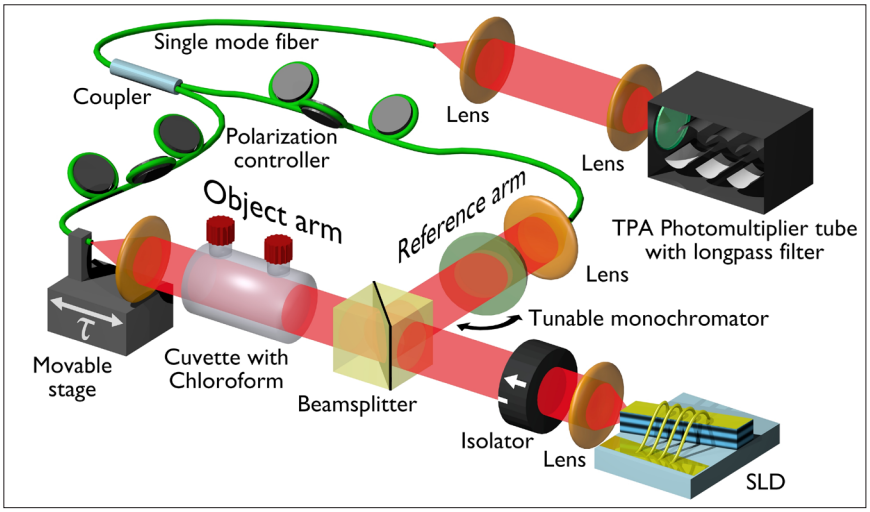
Previous winners of the Norman Sheppard Award are:

2012: Professor Norman Sheppard
2014: Professor W. Ewen Smith
2015: Professor Pat Hendra
2016: Professor Jack Yarwood
2017: Professor Bill George

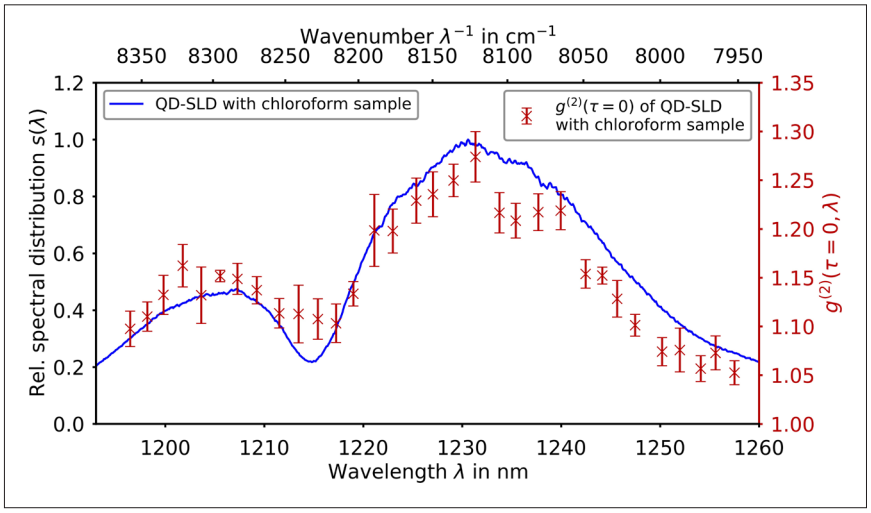
Spectrally resolving the ghost

Ghost modalities are by no means "spooky actions" but are metrology schemes exploiting intensity correlations of light based on the fundamentals of quantum optics. The "story" began in 1995 with ghost imaging (GI) using entangled photons—twin photons in the quantum mechanical sense—followed in 2004 by GI with classical correlated photons exhibiting photon bunching and generated by a so-called pseudo-thermal light source. GI is a quantum imaging modality exploiting photon correlations for the image construction, where one photon of an (entangled) pair illuminates the object to be imaged and is detected without any spatial information. Whereas the second, the so-called reference beam, is spatially analysed by scanning it; however, this beam has never seen the object. The experimentally determined correlation between both beams yields the image; therefore, it is called correlated two-photon imaging. The intensity autocorrelation or second order correlation is thus transferred into a spatial image of the object, the ghost image.

In close analogy to these spatial correlations a group at Technische Universität Darmstadt succeeded in transferring this concept into the spectral domain. In a recently published Letter in *Physical Review Applied* [P. Janassek, S. Blumenstein and W. Elsäßer, "Ghost spectroscopy with classical thermal light emitted by a superluminescent diode", *Phys. Rev. Applied* **9,** 021001 (2018); doi: 10.1103/PhysRevApplied.9.021001] the researchers exploited spectral correlations of broad-band amplified spontaneous emission light emitted by optoelectronic quantum dot superluminescent diodes. With this spectrally photon bunched light, the first ghost spectroscopy (GS) experiment with classical thermal photons was performed. In a real-world ghost spectroscopy experiment on chloroform at 1214 nm the researchers could prove the concept of classical GS. One of two wavelength-correlated beams was shone through a chloroform ($CHCl_3$) sample cell in the object arm, thus measuring the transmitted full, i.e. non-spectrally resolved, intensity. The intensity correlations were determined with a second wavelength-correlated beam, the so-called reference beam in which wavelength resolution was achieved by spectrally tunable filters. The researchers succeeded in reconstructing a ghost spectrum in terms of the second order correlation function $g^{(2)}(\lambda)$ which clearly exhibited the chloroform absorption signatures at 1214 nm.



Experimental set-up for the "ghost" spectroscopy demonstration experiment exploiting a superluminescent diode (SLD) and its application in the framework of a real-world absorption spectroscopy experiment on a chloroform sample.



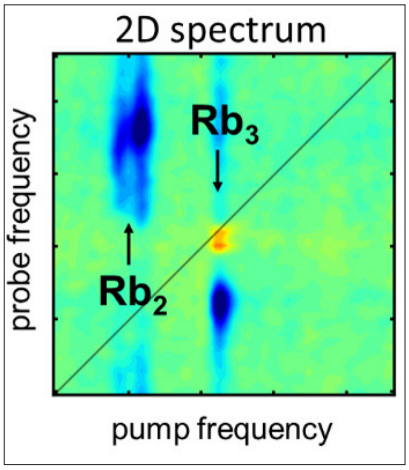
Standard spectrum of the SLD with an absorption line of chloroform (in blue) measured with an optical spectrum analyser and comparison with the second order correlation coefficient $g^{(2)}(\lambda)$ (red data), the measured "ghost spectrum".

Professor Wolfgang Elsäßer from TU Darmstadt expects that this demonstration of ghost spectroscopy with classical thermal light will further stimulate new applications of ghost modalities. He said, "I am convinced that the demonstrated and exploited analogy between ghost imaging and ghost spectroscopy will further fertilise the field, thus allowing an even deeper understanding of the experimental scheme to develop, leading to new ghost protocols and pushing the ghost modality idea into real-world applications in Chemistry, Physics and Engineering."

2D spectroscopy offers new insight into molecular processes

A research team headed by Prof. Dr Frank Stienkemeier and Dr Lukas Bruder of the University of Freiburg's Institute of Physics has succeeded for the first time in applying 2D-spectroscopy to isolated molecular systems and thus in tracing the interactive processes at a molecular level more precisely. The team has published its results in *Nature Communications* (doi: 10.1038/s41467-018-07292-w).

Coherent two-dimensional spectroscopy, which uses ultra-short laser pulses, provides a far greater amount of information than other methods, combined



2D-spectroscopy illustrates the light-induced reactions of Rubidium molecules in various colour spectra. Credit: Lukas Bruder

with a high time resolution in the range of femtoseconds. However, for technical reasons, this method has been restricted to studying bulk liquid or solid material. "In previous experiments the samples were very complex, which made it extremely difficult to isolate individual quantum-mechanical effects and study them precisely. Our approach overcomes this hurdle", explains Lukas Bruder, who headed the experiment.

In preparation for the experiment, the scientists produced superfluid helium droplets, which have no friction, in an ultrahigh vacuum. The droplets are only a few nanometres in size and serve as a substrate in which the researchers synthesise the actual molecular structures using a modular principle, in other words by combining molecular components one by one. These structures are then studied by means of 2D-spectroscopy. "In the experiments we combined various specific technologies which drastically improved the measurement sensitivity of the 2D-spectroscopy. Only by doing this was it possible for us to study isolated molecules", explains Bruder.

In an initial study, the researchers produced extremely cold molecules of Rubidium in an unusual quantum state, whereby the atoms of the molecule are only weakly bonded, and analysed their light-induced reactions under the influence of a helium environment. "Our approach opens up a range of applications, specifically in the field of photovoltaics or optoelectronics, and will eventually contribute to a better understanding of fundamental processes", says Stienkemeier.

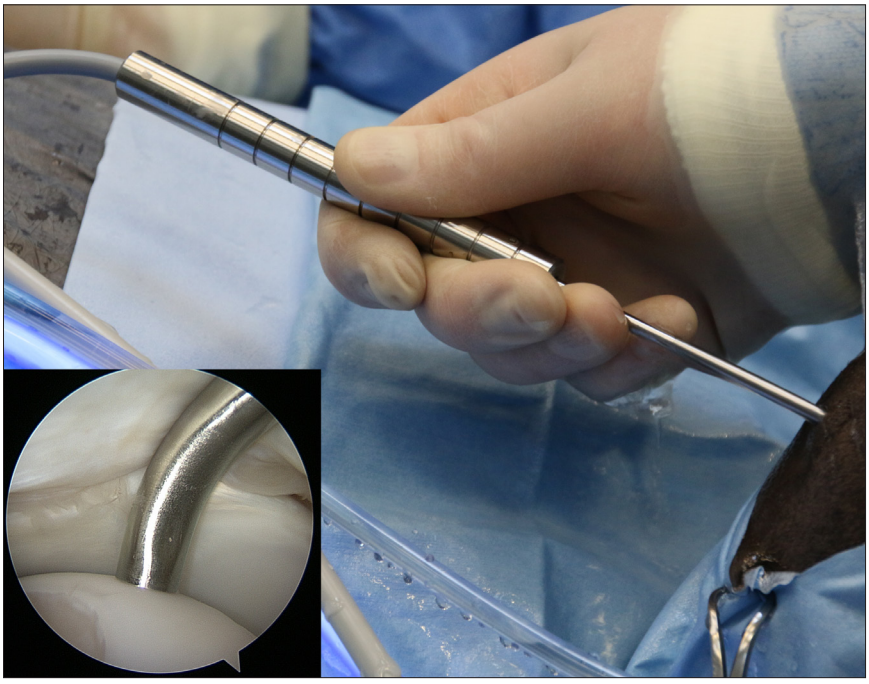
Accurate evaluation of chondral injuries by near infrared spectroscopy

Osteoarthritis is a disabling disease characterised by joint pain and restricted mobility, especially affecting the elderly. The disease generally progresses slowly, even over decades. Post-traumatic osteoarthritis, however, affects people of all ages and is initiated by joint trauma, for example, as a result of falling. The disease is most prevalent in articulating joints, such as the knee.

Although no cure currently exists for osteoarthritis, early detection of cartilage lesions could allow the disease progression to be halted by pharmacological or surgical means. Conventionally, joint health is diagnosed based on patients' symptoms, joint mobility and, if required, with x-ray and magnetic resonance imaging. Based on these examinations, joint repair surgery may be performed during arthroscopy. The decision on the optimal treatment option is made during the surgery, in which the joint health is evaluated visually and by palpating the cartilage surface with a metallic hook. These techniques are subjective and dependent on surgeons' experience and can, therefore, influence the treatment outcome.

An arthroscopic near infrared (NIR) spectroscopic probe for evaluation of articular cartilage and subchondral bone structure and composition has been developed as part of a PhD thesis at the University of Eastern Finland. The probe enables enhanced detection of cartilage injuries, as well as evaluation of the integrity of the surrounding tissue. The availability of comprehensive information on the health of joint tissues could substantially enhance the treatment outcome of arthroscopic intervention.

Clinical applications of NIR spectroscopy are still rare, however, clinical application of the technique is now possible thanks to better availability of computational power along with state-of-the-art mathematical modelling methods, such as neural networks. With these methods, the relationship between the absorption of NIR light and tissue properties can be determined. This enables reliable



The novel arthroscopic probe in an equine knee joint *in vivo* with the probe tip in contact with cartilage surface (inset).

determination of articular cartilage stiffness and subchondral bone mineral density—changes in these tissue properties are prognostic indicators of osteoarthritis.

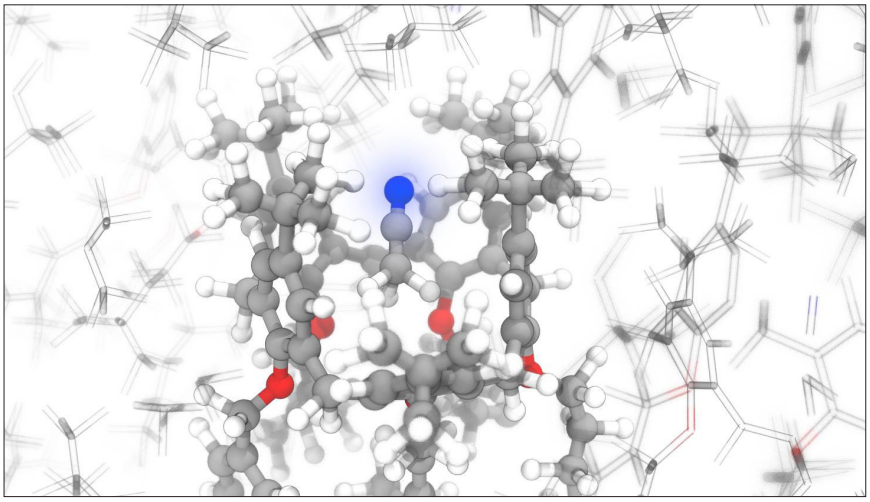
Since NIR spectroscopy is not optimal for imaging of tissues, arthroscopically applicable imaging techniques, such as optical coherence tomography and ultrasound imaging, were also used in the study. These techniques have been previously applied in intravascular imaging via specialised 1-mm diameter catheters, which are therefore well-suited for imaging narrow joint cavities. The study compared the reliability of these techniques for evaluation of chondral injuries with that of conventional arthroscopic evaluation.

"Optical coherence tomography was superior to conventional arthroscopy and ultrasound imaging. In contrast to conventional arthroscopic evaluation, optical coherence tomography and ultrasound imaging provide information on inner structures of cartilage and enable, for example, detection of cartilage detachment from subchondral bone", Researcher Jaakko Sarin from the University of Eastern Finland explains.

The doctoral dissertation, entitled *Evaluation of Chondral Injuries Using Near Infrared Spectroscopy*, is available for download at http://epublications.uef.fi/pub/urn_isbn_978-952-61-2910-5/urn_isbn_978-952-61-2910-5.pdf. The findings were originally reported in *Osteoarthritis and Cartilage*, *Annals of Biomedical Engineering* and *Scientific Reports* (doi: https://doi.org/10.1038/s41598-018-31670-5).

AI and NMR spectroscopy determine atoms configuration in record time

Many drugs today are produced as powdered solids. But to fully understand how the active ingredients will behave once inside the body, scientists need to know their exact atomic-level structure. For instance, the way molecules are arranged inside a crystal has a direct impact on a compound's properties, such as its solubility. Researchers



Credit: Michele Ceriotti / EPFL

are therefore working hard to develop technologies that can easily identify the exact crystal structures of microcrystalline powders.

A team of scientists from Ecole Polytechnique Federale de Lausanne (EPFL) has now written a machine-learning program that can predict, in record time, how atoms will respond to an applied magnetic field. This can be combined with nuclear magnetic resonance (NMR) spectroscopy to determine the exact location of atoms in complex organic compounds. This can be of huge benefit to pharmaceutical companies, which must carefully monitor their molecules' structures to meet requirements for patient safety. Their research has been published in *Nature Communications* (doi: 10.1038/s41467-018-06972-x).

Full crystal structure determination by NMR spectroscopy requires extremely complicated, time-consuming calculations involving quantum chemistry, which is nearly impossible for molecules with very intricate structures. However, the program developed at EPFL can overcome these obstacles. The scientists trained their AI model on molecular structures taken from structural databases. "Even for relatively simple molecules, this model is almost 10,000 times faster than existing methods, and the advantage grows tremendously when considering more complex compounds", says Michele Ceriotti, head of the Laboratory of Computational Science and Modeling at EPFL's School of Engineering and co-author of the study. "To predict the NMR signature of a crystal with nearly 1600 atoms, our technique—ShiftML—requires about six minutes; the same feat would have taken 16 years with conventional techniques."

This new program will make it possible to use completely different approaches that will be faster and allow access to larger molecules. "This is really exciting because the massive acceleration in computation times will allow us to cover much larger conformational spaces and correctly determine structures where it was just not previously possible. This puts most of the complex contemporary drug molecules within reach", says Lyndon Emsley, head of the Laboratory of Magnetic Resonance at EPFL's School of Basic Sciences and co-author of the study.

The program is now freely available online. "Anyone can upload a molecule and get its NMR signature in just a few minutes", says Ceriotti. Visit https://www.materialscloud.org/work/tools/shiftml to try it out.

NMR spectroscopy reveals underlying chemistry of vision in the brain

How vision and the related molecular processes in the brain work are still not fully understood. Dr Valentin Riedl of the Technical University of Munich (TUM)

Dr Valentin Riedl (left), research group leader in the Neuroradiology Department of University Hospital rechts der Isar of the TUM, with his colleague Dr Christian Sorg. (Image: K. Bauer / TUM)

and his team have now been able to show that the distribution of the two most important neurotransmitters in the brain changes as soon as we open our eyes, regardless of whether we actually see anything. The two most important neurotransmitters in the human brain, glutamate and GABA, have opposing effects: glutamate activates neurons, while GABA suppresses them. By altering the concentrations of the two neurotransmitters, the brain is able to process impressions from the eyes.

Dr Valentin Riedl, research group leader in the Neuroradiology Department of University Hospital rechts der Isar of the TUM, and his team have studied how the concentrations of the two neurotransmitters change in the visual cortex. The study is unique in that the team used magnetic resonance spectroscopy (MRS) to measure the concentrations of the neurotransmitters in detail and, above all, in parallel. The experiment consisted of three phases. The subjects first lay in the dark for five minutes with their eyes closed. They then opened their eyes and stared into the darkness. Finally, they were shown a checkerboard pattern that blinked on and off rapidly. Throughout the experiment, the concentrations of both neurotransmitters in the visual cortex were measured simultaneously.

In the resting state with the eyes closed, GABA levels were high. Surprisingly, however, concentrations of this inhibitory neurotransmitter decreased as soon as the subjects opened their eyes, despite the fact that there was still nothing to see. "The brain prepares for forthcoming stimuli as soon as the eyes are opened. This phenomenon had never previously been observed, because other studies had not measured this state", Riedl says. Only when an actual visual stimulus was perceived, i.e. the blinking checkerboard pattern, did the concentration of glutamate, the activating neurotransmitter, increase.

For the first time, the researchers also compared their MRS data with data obtained by functional MRI (fMRI), a common method for visualising human brain activity. In this technique, the consumption of oxygen is measured in specific brain regions. A high consumption is an indirect indicator of neuronal activity in a given area. They found that changes in neurotransmitter levels in the visual cortex coincided with evidence of brain activity in the fMRI scans. "The results of the two methods agreed perfectly. By combining the two techniques, we're not only able to say that there is increased activity in a region; for the first time we're also able to specifically attribute that activity to the two neurotransmitters", Riedl explains.

The findings by Riedl and his team also have clinical relevance. For example, it is suspected that the distribution of the two neurotransmitters is permanently disturbed in psychiatric disorders such as schizophrenia. "To date, however, there is no proof of this. An examination using both spectroscopy and fMRT would provide much more precise and far-reaching information on the concentrations of the neurotransmitters in patients", Riedl says.

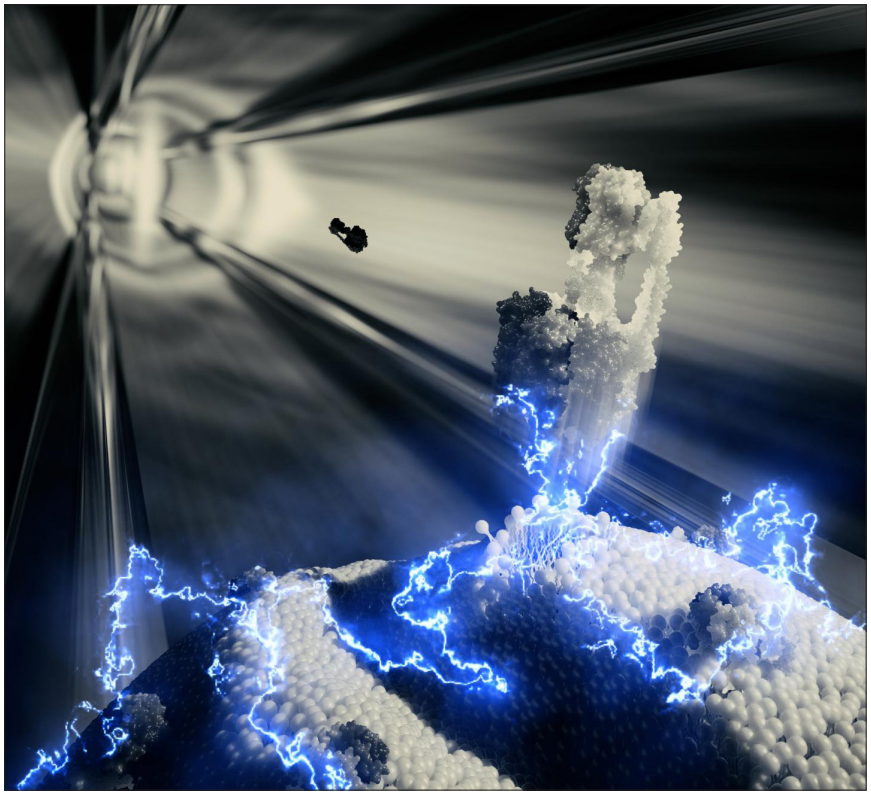
The results of the study have been published in the *Journal of Neuroscience* (doi: 10.1523/JNEUROSCI.1214-18.2018).

Direct MS analysis of cell membrane proteins

The development of a new technique to analyse cell membrane proteins *in situ* was made as part of an international research collaboration, led by Oxford University, alongside peers including Imperial College London. The technique could dramatically affect our understanding of both how cell membrane complexes work, and in the process, our approach to healthcare research. This research, published in *Science* (doi: 10.1126/science.aau0976), will enable the development of mass spectrometry in biology to be taken to a new level, enabling new discoveries that would not have been possible before.

Studying these membrane-embedded machines in their native state is crucial to understanding mechanisms of disease and providing new goals for treatments. However, current methods for studying them involve removing them from the membrane, which can alter their structure and functional properties. Lead researcher Professor Dame Carol Robinson, Professor of Physical Chemistry at Oxford's Department of Chemistry, said: "For decades, scientists have had to extract these proteins from their membranes for their studies. But imagine what you might discover if you could get proteins straight from the membrane into a mass spectrometer?"

"I wasn't sure this would ever work; I thought the membrane environment



A new technique to analyse cell membrane proteins *in situ* which could revolutionise the way in which we study diseases, such as cancer, metabolic and heart diseases. Credit: OU

would be just too complicated and we wouldn't be able to understand the results. I am delighted that it has because it has given us a whole new view of an important class of drug targets."

The technique involves vibrating the sample at ultrasonic frequencies so that the cell begins to fall apart. Electrical currents then applied an electric field to eject the protein machines out of the membrane and directly into a mass spectrometer. Not only did the membrane protein machines survive the ejection; the analysis also revealed how they communicate with each other, are guided to their final location and transport their molecular cargo into the cell.

Professor Steve Matthews, from the Department of Life Sciences at Imperial, said: "With the development of this method, the application of mass spectrometry in biology will be taken to a new level, using it to make discoveries that would not have been possible before."

Dr Sarah Rouse, also from the Department of Life Sciences at Imperial, said: "A longstanding question on the structure of one membrane machine from mitochondria has now been solved using this technique. Mitochondria are particularly interesting because there are several diseases that target them specifically, that we may now be able to design new therapies for."

Of the study's potential impact Professor Dame Robinson added: "The results are particularly exciting for mitochondrial membranes—we managed to catch a translocator in action—passing metabolites. Because mitochondrial therapeutics target a wide range of debilitating diseases, we now have a new way of assessing their effects."

Smartphone NIR

BASF has developed the Hertzstück™ PbS infrared sensor, with a wavelength range of 1000–3000 nm. Hertzstück has been developed by the startup trinamiX, a company founded by BASF researchers in Ludwigshafen, Germany, in 2015. The patented thin film encapsulation of the functional semiconductor layer is very stable and protects the sensor from environmental influences such as water and oxygen. The miniaturisation means that Hertzstück can be installed as a sensor chip on the circuit board of a smartphone.

BASF says that the first spectrometers using the new infrared sensor will be available in 2019 for industrial and semi-professional applications. They hope that the average consumer will have access to NIR spectroscopy in their smartphone by 2022.

Schematic showing how BASF envisage that a smartphone with their sensor might be used to analyse food.

ARTICLE

Brillouin microspectroscopy: in search of the mechanical properties in biological systems well below a wavenumber

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When confronted with the phrase "optical spectroscopy" either fluorescence spectroscopy, Raman spectroscopy or maybe infrared absorption spectroscopy, will come to mind. These well-established techniques, which can now readily also be performed with commercial hand-held devices, have found a broad and continuously expanding set of applications that span quality control, medical diagnostics and even art forensics. Raman spectroscopy generally measures resonances at frequency shifts in the range 500–4000 cm^{-1} (15–120 THz) relative to the excitation wavelength, and can yield detailed information on the molecular composition of a sample. But what happens when one goes to smaller spectral shifts?

As one ventures below the Raman fingerprint region (<500 cm^{-1}) there appears not to be much exciting beyond a smooth background for a while, until suddenly in the region of ~1–100 cm^{-1} (0.03–3 THz) one sees some bright structured peaks again. This is the so-called THz, or low-frequency Raman, spectral region where inelastic scattering arises from coupled molecular bond vibrations. These vibrations are slower (lower energy) than the individual bond vibrations, and though still confined to a given molecule, are a little more extended across different parts of the molecule. They can yield useful structural information and have over the last decade received much interest from among others the pharmaceutical industry for quality control applications. But what if one moves even closer to the blinding excitation laser frequency?

As we venture below the THz–Raman spectral region, again for a while one appears to only find a largely featureless spectral landscape that varies little from sample to sample. Looking onwards to even lower energies, more elaborate spectrometers are soon required, which utilise either passing a beam numerous times between scanning cavities[1] or using special high-angular dispersive optical elements.[2] Dangerously close to the excitation laser frequency at around 0.3 cm^{-1} (10 GHz frequency) one, however, now sees a single or sometimes two or three sharp peaks again. One would be forgiven for thinking this is the result of a laser instability, but it is not (in this case). This relatively poorly explored area (as far as life-science and biomedical applications go) is the territory of *Brillouin light scattering spectroscopy*. The peaks one observes here are due to the scattering from so-called acoustic phonons, or low-energy collective molecular vibrations that span hundreds to thousands of atoms.[3] These "modes", which are ever present at finite temperatures, will depend on the structure of the sample and are determined by an effective collective response (as opposed to the properties of a single molecule as is the case in the higher energy excitations). Typically travelling at a few thousands of metres per second, they have an effective wavelength on the order of ~100 nm and a relatively long lifetime, decaying over a distance of up to several microns. Seen semi-classically, the observed peak(s) in the spectrum can be understood to be the inelastic scattering of light from a density wave (or moving Bragg grating), with the observed frequency shift directly proportional to the velocity of the wave, *viz.* the Doppler shift. The velocity in turn is related to different components of the stiffness tensor (the precise components depending on the measurement geometry), and thus can be used to calculate elastic moduli (via the Christoffel equation). To this end it has been utilised for almost half a century in geology as well as condensed matter physics.

Over the last decade there has been renewed interest in using Brillouin spec-

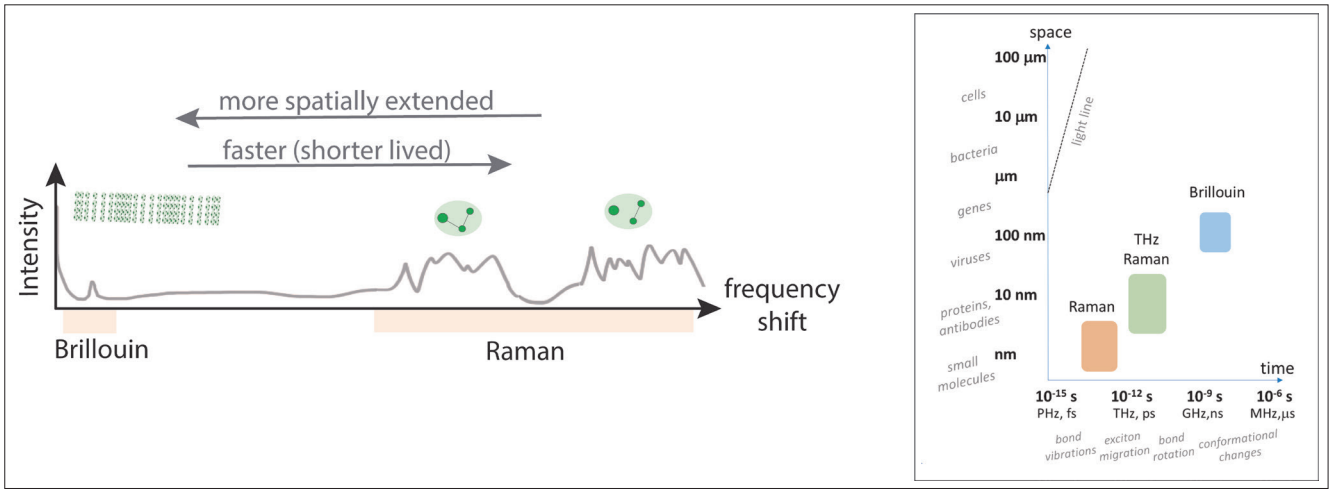

Figure 1. Sketch of combined Brillouin and Raman scattering spectra (left), and characteristic spatial and temporal scales associated with the underlying processes in relation to the sizes of different structures and time-scales of different processes (right).

troscopy to study biological specimens. This to a large extent has been driven by the realisation of a novel imaging spectrometer design, based on so-called Virtually Imaged Phase Arrays (VIPAs),[2] which allow for fast spectral measurements at laser powers conducive to studying live biological samples. Together with a confocal detection scheme, the prospect of optically measuring the elastic modulus with diffraction limited resolution and then mapping this in 3D in a live sample sounds very appealing, both for life-science research (mechanobiology), but also for potential medical diagnostic applications. There are, however, several caveats, and care has to be taken when interpreting the Brillouin derived elastic moduli. First, unlike the *Young's Modulus* measured with Atomic Force Microscopy (AFM) and other stress–strain techniques, or the *Shear Modulus* measured in microrheology, the elastic modulus measured in Brillouin scattering in the most commonly used backscattering geometry is the so-called *Longitudinal Modulus*, which also depends on the compressibility of a sample. (N.B. Longitudinal modulus derives its name from the longitudinal modes it measures, which propagate along the same direction as the incident and detected radiation.) As such, the value for incompressible materials such as liquids will be very large and may in certain cases be dominant. In addition, due to the high frequency (GHz) of the probed acoustic phonons, one is effectively probing mechanical properties also in what can be considered the high-frequency regime, where distinct mechanical relaxation rates are at play to those at lower frequencies more commonly probed with other techniques. Finally, to derive the elastic modulus from the frequency shift of the Brillouin peak requires knowledge of the ratio ρ/n^2 (where ρ is the mass density and n the refractive index of the material)—which is not always known or readily accessible in a sample, particularly in heterogeneous media, and requires parallel measurements. To a first approximation, it may be assumed that the factor ρ/n^2 is constant (Lorentz–Lorenz relation), however, the validity of this assumption may not always be justifiable. In light of all of this, extra care has to be taken both to correctly interpret the Brillouin-derived elastic moduli and when drawing any correlations with parameters obtained from other techniques.

Despite these challenges, Brillouin microspectroscopy has, over the last decade, been applied in studying various systems of biomedical interest in order to assess potential diagnostic capability. A motivation for doing so is that mechanical damage in tissue can be associated with numerous pathological states such as osteoarthritis and keratoconus, and the ability to probe these changes on a sub-micron scale using a contactless all-optical technique would prove beneficial in clinical diagnostics. Contact techniques on the other hand would present the disadvantage of decoupling engineering strain from the real strain applied to the material. But since Brillouin microscopy is contactless and probes spontaneous acoustic modes that are intrinsic to the material, cells and tissues can be analysed in their true native environment and without any mechanical perturbations. Much effort is also being devoted to making the technique more viable for optical diagnostics, such as combining with endoscopy, in a similar way as has been done with other vibrational spectroscopy techniques.

Studying samples of biological and biomedical interest, however, does not come without its own associated challenges. Biological samples are generally very complex structures with heterogeneities in composition, optical and mechanical properties on a microscopic scale, making it more challenging to extract quantitative information. Within the approximation of a homogeneous ratio ρ/n^2, which is plausible far away from electronic resonances, the Brillouin shift can, however, be expected to scale directly with the elasticity or stiffness of a material, such that large Brillouin frequency shifts can be assumed to correlate with increased rigidity and *vice versa*. In this regard, Brillouin microspectroscopy can provide a novel contrast mechanism for mapping micromechanics also within cells and tissues. Hydration is also known to have a prom-

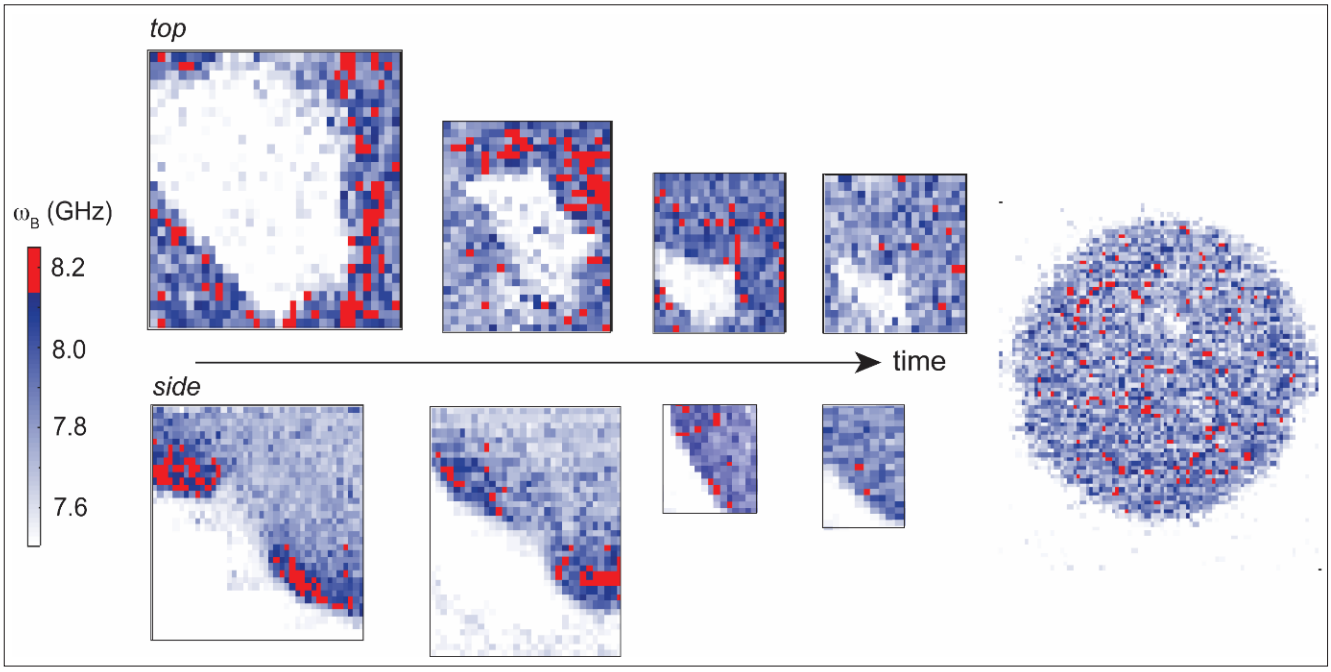

Figure 2. Time-series Brillouin frequency shift (ω_B) spatial maps of a live developing sea anemone (*Nematostella vectensis*) showing increased Brillouin shift (related to stiffness) around the invagination area at the beginning of embryogenesis (pixel size = 1 µm). Acquired using a confocal microscope attached to a dual-stage cross-dispersion VIPA spectrometer (1.3 NA objective lens, 532 nm laser power ~1.5 mW at sample with 120 ms dwell time per pixel). Sample courtesy of E. Puhlyakova and U. Technau (University of Vienna).

inent effect on the measured properties, and thus needs to be carefully controlled, together with sample preparation in general. To date, the potential biological and medical applications of Brillouin microspectroscopy have been demonstrated on a variety of cells and tissue types. These range from *ex vivo* studies on histological sections from tissue biopsies,[4,5] to *in vitro* applications in live cells[6] as well as live tissue[7] and organisms.[8] It has also been applied in the analysis of liquid biopsies (e.g. Reference 9). In regards to biomedical applications, it is probably most advanced in the field of ophthalmology—for keratoconus assessment, where it is currently undergoing clinical trials.[10] Parallel efforts have been devoted to instrument development to overcome limitations in acquisition time and to achieve high contrast for the analysis of more opaque materials.

Though much progress has been made over the last few years in terms of both instrument development and applications, there are at this point still more questions than answers, such as: How do we best understand these low energy modes probed using Brillouin scattering in complex heterogeneous biological system? What biological/biophysical relevance could their properties and the derived high-frequency elastic moduli have?

One thing that is for sure is that the Brillouin measured elastic modulus clearly varies between regions of a biological sample, even if sometimes only by small amounts. Exactly what this is telling us and for what it is most useful or relevant is currently an area of active debate and remains to be seen.

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ARTICLE

A practical guide to sample preparation for liquid chromatography-tandem mass spectrometry in clinical research and toxicology

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful technique for the quantification of small molecules in body fluids, but some sample preparation is necessary prior to analysis. With so many options available, choosing the right sample clean-up method can be bewildering for novice users. A basic understanding of the principles of sample preparation—combined with a structured approach to selecting, optimising and validating a protocol—can pay dividends in terms of time saved and more accurate and robust LC-MS/MS assays. This article looks at the key factors to consider when selecting an LC-MS/MS sample preparation strategy, outlining the principal processing methods and providing practical advice on protocol development using quantification of serum testosterone in serum samples as the model compound.

Introduction

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a well-established tool for the identification and quantification of small molecules in research and industrial settings, but the technique has only recently moved into the healthcare arena. Historically, the implementation challenge for clinical research laboratories has been the development of reliable, reproducible and cost-effective sample preparation methods but, thanks to recent technology breakthroughs, LC-MS/MS is rapidly becoming the technique of choice for many small molecule testing applications.

Why is sample preparation needed?

Small molecule analysis can be performed on a variety of sample types in a clinical research setting—including whole blood, serum, plasma, urine and cerebrospinal fluid (CSF)—and there are three main reasons that samples may need processing prior to LC-MS/MS. The most straightforward of these is to remove proteins and other constituents that may precipitate when injected into the LC mobile phase, to avoid clogging the chromatography column. Depleting or removing these matrix components prevents damage to the column and the build-up of excessive pressure within the LC system.

The next reason is to improve chromatographic performance. The volume, pH, organic solvent, buffer and aqueous composition of the liquid injected into the LC have a profound effect on chromatography, modifying LC peak shapes, peak separation and retention times (Rt). These can influence the quantitation limits, selectivity and robustness of the assay. To overcome these issues, complex biofluids often need to be exchanged for an injection solution compatible with the LC method prior to injection.

Finally, the precision and accuracy of the method, as well as the long-term stability of the LC-MS/MS instrument response, is almost always improved by selectively depleting the biological matrix to increase the analyte-to-matrix ratio. For example, phospholipids are a major constituent of cell membranes, present in serum in mg mL^{-1} amounts, which can significantly affect method performance unless depleted during sample preparation. This type of "matrix effect" is a major constraint of LC-MS/MS methods, and is discussed further in the *Assay quality* section below.

What are the options of sample preparation?

In the ideal world, sample preparation should be simple, low cost and allow matrix depletion with the option to concentrate the analyte(s) of interest. The most commonly used techniques for small molecule LC-MS/MS sample preparation can be broadly divided into eight categories, each of which is briefly outlined below and summarised in Table 1.

Table 1. Overview of LC-MS/MS sample preparation protocols.

Protocol	Analyte concentration[a]	Relative cost	Relative complexity	Relative matrix depletion
Dilution	No	Low	Simple	Less
Protein precipitation	No	Low	Simple	Least
Phospholipid removal	No	High	Relatively simple	More[b]
Liquid-liquid extraction	Yes	Low	Complex	More
Supported-liquid extraction	Yes	High	Moderately complex	More
AC Extraction Plate	Yes	High	Relatively simple	More
Solid-phase extraction	Yes	High	Complex	More
Online SPE	Yes	High	Complex	More

[a]without matrix concentration, [b]phospholipids and precipitated proteins are removed, but not other matrix components

Dilution

Dilution or "dilute and shoot" methods simply involve the addition of purified water or the LC mobile phase to the patient sample prior to LC-MS/MS analysis. This technique is widely used for low protein matrices (e.g. urine or CSF) because it is fast, simple and inexpensive. Ideally, the sample, internal standard and diluent are pipetted directly into the autosampler vial or microplate well, then mixed, centrifuged and loaded straight onto the LC-MS/MS for automated analysis. This ensures a streamlined workflow and minimises resource and reagent use.

Protein precipitation (PPT)

Protein precipitation or "protein crash" is analogous to dilution methods, but is intended for high protein matrices, such as serum, plasma or whole blood. PPT has many of the same features that make dilution a popular sample preparation protocol, being fast, simple and cheap. The sample, internal standard and a precipitating agent, such as acetonitrile or methanol/$ZnSO_4$, are mixed together, then centrifuged or filtered to separate out the precipitated proteins before the supernatant is injected into the LC-MS/MS system.[1]

Liquid–liquid extraction (LLE)

Liquid–liquid extraction has been used in sample preparation workflows for many years, and involves the partitioning of analyte(s) from an aqueous biofluid into a water immiscible organic solvent based on polarity. It offers a number of benefits for LC-MS/MS assays, as it allows the concentration of analytes, enhancing sensitivity, and depletion of matrix components, increasing selectivity. Unfortunately, this multi-step process is relatively labour intensive, requiring the partitioning of the analyte(s) into the organic solvent, separation of the organic and aqueous layers, evaporation of the organic solvent, and reconstitution of the analyte(s) in a solvent mixture that is miscible with the LC mobile phase.

Phospholipid removal media (PLR)

Although 96-well format plates for filtering protein precipitates have been available commercially for many years, the last decade has seen the development of filtration plates designed to capture and remove phospholipids. The post-precipitation supernatant flows through a bed packed with moieties, e.g. zirconia-coated silica, that retain phospholipids. This offers greater selectivity while maintaining the simplicity of PPT protocols.[2]

Supported or solid-supported liquid extraction (SLE)

Supported liquid extraction is similar to LLE, but the partitioning of analytes from the aqueous biofluid into an immiscible organic solvent occurs in a particulate bed, composed of diatomaceous earth or synthetic particles, packed into a cartridge or 96-well plate. The diluted biofluid is slowly added to the bed and becomes dispersed in an ultra-thin layer coating the particles. An immiscible organic solvent is then passed through the media, causing high efficiency partitioning of non-polar analytes into the solvent. This method offers many of the sensitivity/selectivity advantages of LLE,[3] while being less labour intensive and resulting in more consistent extraction.

Solid-phase extraction (SPE)

Solid-phase extraction uses a selective stationary phase which binds or partitions the analyte(s). This phase often requires pre-treatment for optimal extraction, then the diluted biofluid flows through the stationary phase, which captures the analyte(s) while allowing other matrix components to flow to waste. Following several wash steps, an elution solvent is used to recover the analyte(s). Samples may then require eluate evaporation and reconstitution with an LC-MS/MS compatible solvent before analysis.

AC Extraction Plate™ (ACP)

The AC Extraction Plate[a] (Tecan) is a "smart" extraction consumable that works on the same principles as SLE, partitioning non-polar analytes from aqueous biofluids into a more non-polar phase. The difference is that the ACP uses a proprietary polymer, which is coated onto the plate wells, as the stationary phase. The advantage of this approach is that it uses a "pipette and

[a]For research use only. Not for use in diagnostic procedures.

shake" protocol: the analyte is partitioned into the non-polar stationary phase, the extraction residue is discarded. Then, following a wash step, a relatively non-polar elution reagent is used to partition the analyte(s) back into the liquid phase. By eliminating the flow-through process used in most SLE or SPE protocols, the ACP workflow is easily automated. It also has the capacity to concentrate analytes and deplete matrix components, leading to enhanced specificity and sensitivity.[4]

Online SPE (O-SPE)

Online SPE uses an LC "trap" column—analogous to the SPE cartridge or plate—to capture the analyte while matrix components flow to waste. Reversal of the flow then elutes the target analyte(s) directly onto the analytical LC column. This approach minimises hands-on time, but requires a more sophisticated LC set-up and a high level of expertise to ensure consistent performance.

Why invest in additional sample clean-up?

Table 1 clearly indicates that dilution and PPT are the most straightforward and inexpensive sample preparation techniques, although greater complexity and/or increased costs are necessary to selectively concentrate analytes and deplete matrix components. The value of analyte concentration seems obvious—increasing sensitivity—but what are the benefits of matrix depletion? There are two main reasons to perform enhanced sample clean-up; improving assay quality and enhancing process reliability.

Assay quality

Atmospheric pressure ionisation (API) is the chemical process that converts uncharged analytes in a liquid phase to ions in the gas phase to allow detection by MS. It is well established that the presence of residual matrix components in the LC eluate causes interference with the ionisation process within an API source. This "matrix effect" differs between samples and analytes, potentially causing quantification errors.

As mentioned previously, phospholipids are a major source of unacceptable matrix effects in serum, plasma and whole blood samples. Co-eluting a stable-isotope labelled internal standard (SIL-IS) can be used to effectively compensate for matrix effects, but consensus guidelines and accreditation requirements for clinical research laboratories often require detailed evaluation of these matrix effects during method validation.[5–9] For example, the College of American Pathologists' checklist[5] states that the average matrix effect determined from at least 10 different matrix sources must be less than 25%, and the coefficient of variation (relative standard deviation) due to matrix effects must be less than 15%, or "validation studies must include data to demonstrate that matrix effects do not affect assay accuracy". As a result, a sample preparation protocol that efficiently depletes matrix components will be necessary for many assays.

Robust operations

Another reason for depleting matrix components during sample preparation is to preserve the cleanliness and, therefore, performance of the mass spectrometer. Each injection of an extracted biological sample deposits some residual matrix material on the hardware of the mass spectrometer, and these deposits gradually degrade the handling of ions. Over time, this will result in fewer ions reaching the detector, reducing sensitivity until the instrument fails system suitability testing and must be cleaned. Each cleaning cycle (venting to atmospheric pressure, cleaning the hardware and pumping back down to high vacuum) can take up to 24 hours, resulting in a significant loss of instrument time. Cleaner extracts can help to lengthen maintenance-free intervals, leading to more uptime and greater productivity.

Effective pre-analytical sample clean-up can also make the need for instrument maintenance and servicing more predictable, ideally limiting servicing to scheduled six-month preventive maintenance visits. Compared with a sudden, unexpected loss of sensitivity due to insufficient sample preparation, this avoids batch failures and unplanned downtime, which have the knock-on effects of more sample repeats, turnaround time delays and higher production costs.

It is also worth remembering that the mass spectrometer is not the only component of your system that may be degraded by the presence of residual matrix materials. Excessive pressure due to clogging of injection valves, tubing, guard columns and columns can cause an LC system to shut down without completing a run, leading to more repeat testing and delays. Investment in additional sample clean-up can also help to extend the operational life of guard columns and columns, while enhancing chromatographic performance.

How to choose a sample preparation technique?

There is no one size fits all solution for LC-MS/MS sample preparation. When selecting the most appropriate technique for your assay and workflow, consider these factors:

1) Analyte chemistry: polarity (Log P, Log D), charge (pKa), thermal stability and molecular weight.
2) Analyte concentration: is concentration or dilution of the analyte(s) needed to achieve the desired lower limit of quantitation (LLoQ)?
3) Known challenges: specific applications have widely recognised difficulties, such as achieving appropriate sensitivity for serum steroid hormones, sufficient selectivity for opiates/metabolites in urine, or a robust protocol for high throughput tests such as serum 25-hydroxy vitamin D.
4) Workload: sample volume constraints, batch size expectations, and throughput and turnaround time requirements.
5) Laboratory resources: automated versus manual liquid handling, experience with LC multiplexing and O-SPE automation, availability of extraction equipment (solvent evaporators, positive pressure or vacuum extraction modules, heating blocks, multi-vortexers etc.), expertise available for sample preparation during development, validation and production.

To demonstrate how a systematic approach can be applied in practice, consider the selection of an extraction

protocol for serum testosterone. A recent publication details the use of ACP to develop a serum testosterone analysis suitable for specimens from women and infants.[4] The laboratory's key considerations for the new workflow were good precision, better selectivity than automated immunoassays and ease of automation.

1) Testosterone is a thermally stable, non-polar, neutral steroid (Log P=3.32) with molecular weight of 288.42. Extraction protocols that work particularly well with neutral non-polar small molecules are LLE, SLE and ACP. PPT, SPE and O-SPE are also options, but with some caveats. Mixed mode SPE (combining reverse phase and ion exchange stationary phases) is highly selective for weak acids and weak bases, because both polarity and charge can be used for analyte retention/matrix removal. In contrast, retaining an uncharged analyte such as testosterone on a simple non-polar C18 stationary phase, while simultaneously attempting to remove non-polar matrix constituents, is more challenging.

2&3) Much lower quantification limits are required when measuring testosterone in paediatric and female patients compared to adult males. A challenging LLoQ of 1–5 ng dL^{-1} is desirable, and typically requires concentration. Dilution and PLR protocols do not concentrate analytes, and dilution does not remove serum proteins. PPT protocols involve dilution and, if evaporated after the precipitation step, will concentrate matrix components as well as the analyte, making it more difficult to maintain an appropriate LLoQ. LLE, SLE, ACP, SPE and O-SPE are more appropriate, with some theoretical preference for LLE, SLE and ACP.

4) LLE has the lowest cost of materials, and works well for small batches, but requires excellent manual technique, is too labour intensive for large workloads and can be difficult to automate. O-SPE requires minimal hands-on time, but in-house expertise is needed to maintain a high throughput O-SPE set-up. SLE, ACP and SPE are all well-suited to high throughput applications and automated liquid handling in 96-well plates. Comparing ease of automation, ACP uses less equipment (only an orbital shaker) and does not require a positive pressure or vacuum manifold, while SLE has fewer steps than SPE.

5) Resources and available expertise are usually the decisive factors. For laboratories with access to in-house or external expertise, personal preference, prior experience and availability of extraction and automated liquid handling equipment will all come into play. As a result, there are diagnostic laboratories using LLE, SLE, SPE, O-SPE (as well as the most recent option, ACP) for testosterone analysis. For those developing their expertise in-house, there is extensive literature for LLE[10–13] and vendors of SLE, SPE, O-SPE and ACP consumables offer extensive application support. ACP and SLE would be the easiest techniques to automate for most laboratories.

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Judy Stone obtained her PhD at the University of Toronto, and has been working with clinical chromatography and mass spectrometry since 1984. Her research interests are in mass spectrometry method development and automation for clinical toxicology, TDM and endocrinology. She teaches a short course twice yearly on "Getting started with quantitative clinical LC-MS/MS" at MSACL meetings in the United States and Europe, and was the faculty chair for the AACC online certificate course (1st edition) in clinical mass spectrometry.

Following this process, ACP was chosen as the preferred sample preparation technique, due to the technology's potential to reduce variability and improve batch-to-batch stability through optimisation of the extraction protocol. The resulting, extensively validated method has yielded excellent precision, accuracy and robustness.[4]

Summary

Sample preparation is a "necessary evil" for sensitive and reproducible LC-MS/MS analysis, and also offers operational benefits in terms of instrument uptime and maintenance scheduling. Many clinical research laboratories are now looking to take advantage of LC-MS/MS for small molecule quantification, but each lab must choose which sample clean-up technique is most appropriate to its analytical goals and workload.

For the serum testosterone example discussed, the ACP method chosen provided a combination of good sensitivity and precision, improved selectivity over existing immunoassay methods and ease of automation.[4] The availability of newly developed extraction consumables, such as the AC Extraction Plate, provide innovative and cost-effective alternatives to traditional liquid- or solid-phase techniques, offering laboratories the potential to improve assay performance and reliability while reducing the burden on busy laboratory staff.[14]

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ARTICLE

Near infrared spectroscopy, the skeleton key for bone identification

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Introduction

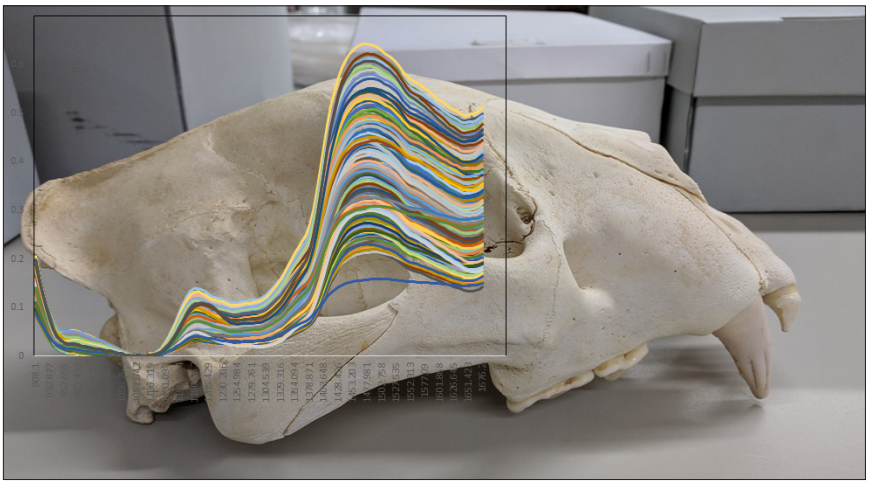
New developments in the field of optics and electronics have resulted in an increasing number of new applications of spectroscopic analysis, which is further augmented by the introduction of portable and compact spectrophotometers.[1–3] This miniaturised instrumentation allows analysts to explore unique practices in a diverse of fields including anthropology and forensics.[4–11]

Bone and bone materials are complex structures comprising of minerals (approximately 55–65 wt %), organic materials (approximately 25–35 wt %) and approximately 10 wt % water.[3] The mineral component of bone consists of carbonated hydroxyapatite particles embedded in the organic matrix, a combination of collagen and non-collagenous proteins.[3] The ratio of these inorganic and organic components will differ depending on the type, origin and function of the bone. Moreover, the environment of the subject will also influence the bone's makeup, with numerous factors impacting, for example the host's diet.[3]

The application of near infrared (NIR) spectroscopy for the discrimination of bone materials—either pulverised (bone meal) or as whole (bone fragments), in the study of archaeological materials with a primary focus on the conservation of delicate materials and as a forensic tool in art preservation—has been evaluated in a number of studies.[4–11] More recently, the technique has been applied to a wide range of palaeontological studies, which has opened up an exciting opportunity for palaeontologists to employ non-destructive analytical techniques combined with chemometrics.[4–11] This study aims to evaluate the capability of a portable NIR instrument to classify and identify the origin of skull bones from a number of different animal species (mammalian, avian and reptile).

Samples, spectra collection and analysis

Skull bone samples were sourced from different animal species belonging to the collection of skulls stored at the Science lab (CQU, Rockhampton, Queensland, Australia). Their origin was recorded as per the label in the box and they were stored at room temperature (26 °C ± 5). The NIR spectra of the samples were obtained from measurements at three random positions on the skull with a MicroNIR OnSite (Viavi, Santa Rosa, CA, USA) in the spectral range of 950–1650 nm. Whole samples were scanned and the resulting spectra analysed using principal component analysis (PCA) and partial least squares discriminant analysis regression (PLS-DA). More details about the methodology and samples can be found in a recent article.[3]



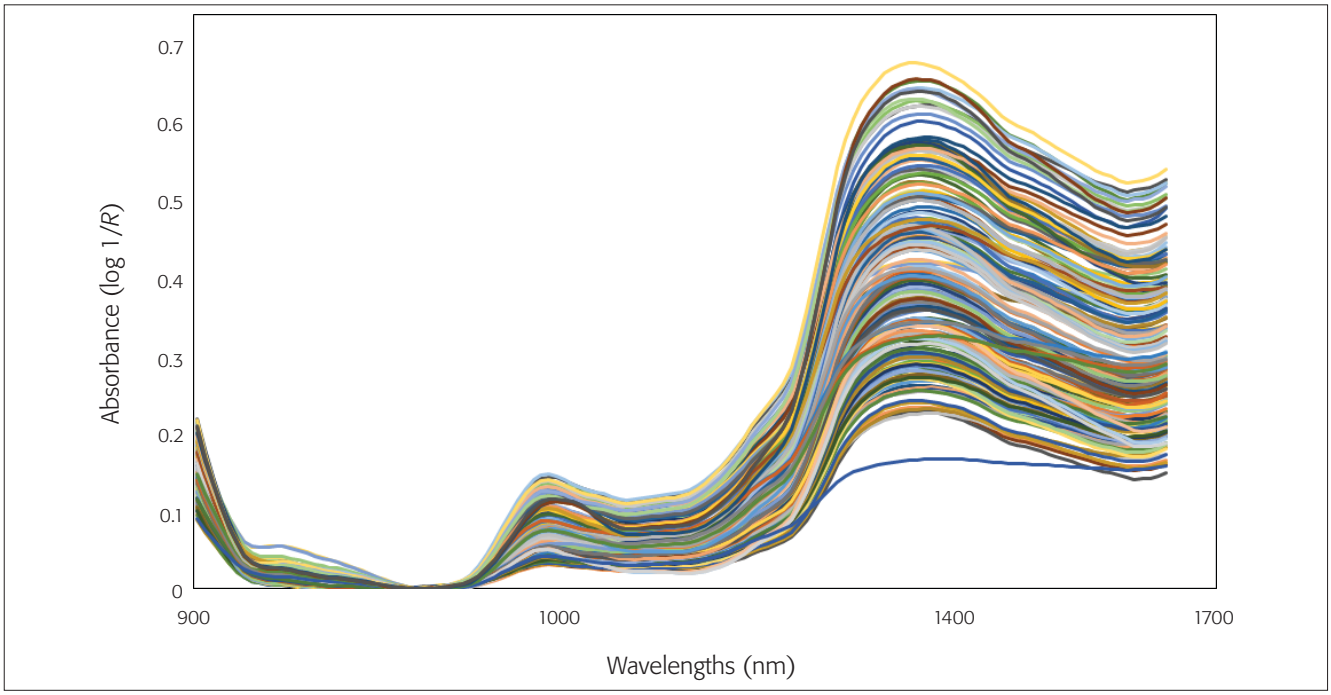


Figure 1. Near infrared spectra of skull samples.

Results and discussion

The NIR spectra of skull bones samples from each of the animal classes analysed in the region between 950 nm and 1650 nm are shown in Figure 1. The NIR spectra show variation in the intensity of some of the wavelengths. In particular, in absorption bands around 982 nm, which are related to combination vibrations of H–O–H bend and O–H stretch of water, and around 1186 nm with C–H bond vibrations.[3,12] Differences between samples were also observed around 1400 nm associated with O–H bonds (1470 nm).[3,12] Absorption bands near the O–H bonds were reported by other authors to be associated with the degradation of hydroxyapatite in external regions of bones and considered a very useful indicator of diagenetic processes in the bone.[3] Figure 2 shows the PCA score plot for the classification of avian and mammalian skull bones samples. It has been observed that PC1 accounted for 77% of the total variance while PC2 explained a further 21% of the variance, demonstrating that NIR spectroscopy was capable of differentiating between the two animal classes. The loadings (not shown) highlighted the influence of wavelengths at 1007 nm, 1180 nm and 1447 nm associated with the stretching vibrations of the carbonated hydroxyapatite matrix of the bone, as described in the raw spectra.[3]

The classification rates based on the NIR spectra and PLS-DA analysis were 96% and 81% for the correct classification of skull bone samples as avian and mammalian, respectively. Overall, a 91% correct classification rate was obtained for the classification of skull samples according to the class (mammalian and avian) which is equivalent or better than the classification rates reported in similar studies.[3]

The classification results obtained in this study infer that differences in the chemical composition of the bones might be responsible for the observed differences in the NIR spectra and thus in the classification results obtained. Differences in metabolism, nutrition and environment effects (sunlight degradation, season) might explain the observed differences between the skull bones from different families and species.[3]

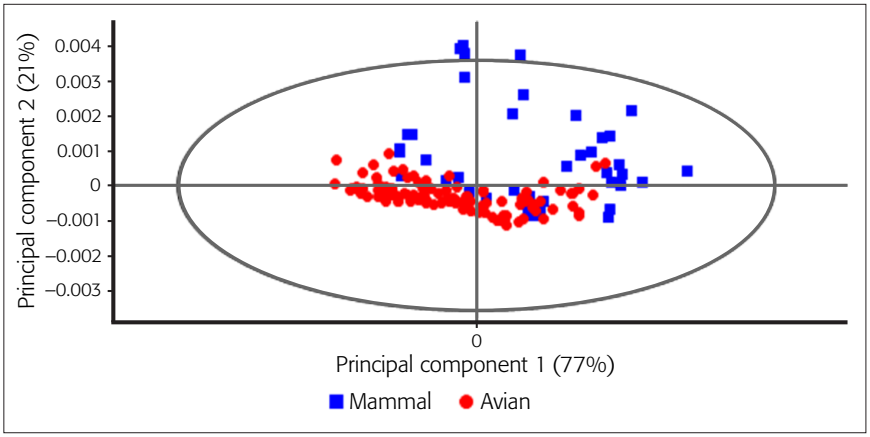


Figure 2. Principal score plot for the classification of avian and mammalian skull bones samples based on NIR reflectance spectra.

However, this information was not available at the time of the analysis.

There is a wealth of information that can be obtained from vibrational spectroscopic examination of fossils and bones, ranging from taxonomic identification of enigmatic microfossils, fossil preservation mechanisms, diagenetic and thermal alteration pathways and histories of fossils, to chemical composition. Moreover, the results of this study indicated that the requirement to test large numbers of samples is not a prerequisite to validate and confirm the ability of NIR spectroscopy to differentiate/class bones of different species. Therefore, the results of this study have added to the state of the current knowledge in the application of NIR spectroscopy to analyse bones from a diverse range of animal species.

Given the non-specificity of the technique, these positive preliminary results indicate that this method of analysis has the potential to identify any animal bone sample. The non-invasive nature of this analysis ensures the quality of the sample is preserved. This contrasts favourably with traditional methodologies, which are expensive, time consuming and often require highly specialised operators and instrumentation.[3,12] Therefore, the rapid nature, lack of consumables and sample preparation required results in a far more time- and cost-effective analysis per sample.[3]

Conclusion

This study demonstrates the potential of NIR spectroscopy coupled with chemometric data processing as a means of rapid, non-destructive classification of skull bone samples. It is apparent that this approach can readily distinguish between various animal classes and species of mammals and birds. The study highlighted the potential usefulness of the technique in the field as more accessible instruments appear in the market. The authors envision that further optimisation of this technique could lead to significant advances in the field of anthropology, archaeology and forensics. Potential applications include identification of bone fragments or even items fabricated from animal bone, such as ornamental figures or other artefacts made with bone fragments.

Acknowledgements

The supply of the skull bones by Central Queensland University is acknowledged.

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14. For more information on the applications discussed here, contact agnieszka.sitarska@tecan.com.

TONY DAVIES COLUMN

Inspiring people with process analytical technology

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In recent months, we have promoted analytical spectroscopy at Open Days and at the biggest Chemistry conference in the Netherlands. At both events we were challenged to create demonstrations to inspire parents, teenagers as well as students and postgraduates into the world of industrial chemistry with a specific twist of process analytical technology (PAT). This article will show what we created and how the platform can be used for educating people and colleagues at many different levels of prior knowledge.

From health & safety to advanced chemometrics

The advantage of telling such a story is that the arguments for deploying process analytical spectroscopy are so compelling that they are easily understood by people with different levels of prior understanding. If you start by discussing removing people from the act of having to take samples from an active chemical manufacturing plant, the advantages are so simple to understand, from a health and safety perspective, as to be obvious to an audience with no technical training at all.

For audiences with a greater interest in the financial drivers, discussions around improving the manufacturing process by stopping at exactly the right moment for the best profit from a batch are easy to follow. As is obtaining a better understanding of the whole manufacturing process, so that you can adjust the manufacturing parameters as a run proceeds. Obviously better than discovering something was not quite right when the results from the final lab-based testing comes in. Such capabilities can also make an enormous contribution to the overall sustainability of chemical manufacturing and the reduction in waste.

For those who like to talk, we can go on for hours, but for many of our visitors it is better to see equipment in action to understand some of the finer aspects of the work of our teams in this area. We put together a very simple demonstrator for our Open Days showing different near infrared (NIR) spectroscopic fibre probe types in a single reaction vessel (Figure 1). These types of probes are, of course, the pre-eminent workhorses in the PAT arsenal.

We chose three NIR optical probe configurations to exemplify some of the investigations that need to be made as part of a PAT deployment project.

Running demonstrators

In both the Open Days and conference displays we chose to highlight the work of a scientist in a process analytical team through the example of different operational environments that require the selection of transmission, transflection or reflection probes to deliver the best analytical results (Figure 2).

Here, the team executed reaction monitoring experiments against three calibration models individually created for the different probe types where the monitoring "results" from each probe were displayed in real time on a single monitor system.

This experiment has the advantage that it is relatively simple to set up and dismantle after use, the chemometric models are quite stable for live demonstrations and the figures we show around the location of the display simple for all to understand.

Figure 1. The setup used at the Open Days with the three probes inserted from above for simultaneous monitoring of the reagent being dosed from the rear port.

Upgrading for exhibitions

Following the same theme, we have created a more complex version of this process analysis demonstrator for the Dutch chemists' conference. Here the audience is generally at a much higher scientific level than during a normal open

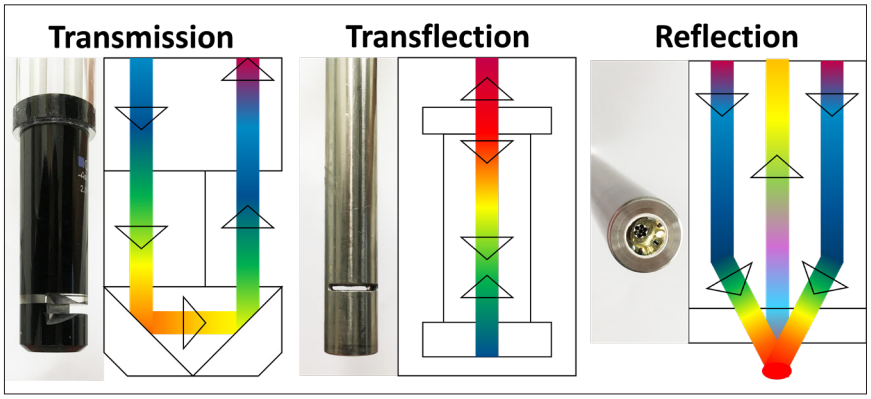

Figure 2. Three fundamentally different probe designs used during a selection process for different environments the probes may be exposed to.

day and so a number of new additional features were introduced.

Instead of just running a dosing experiment, we introduced the concept of having to work with chemical processes where reactor contents are not just pure clear liquids but contain particles or crystallising products. This required a change to the "reactor vessel" with a bigger piece of glassware selected, which was deeper and also wider at the top to allow more ports to be built into the lid. The additional depth allowed the simulation to add complexity with the addition of solid particles. When the stirrer was switched on these particles span up into the body of the reactor and their effect on the different probe designs could be followed in real-time. These particles had to exhibit some very specific properties. To be able to repeat the demonstration multiple times per hour the particles needed to be heavy enough to settle quickly to the bottom of the reactor once the power to the stirrer was removed. The particles also had to be of a specific size to demonstrate interference with the process analytical measurement from certain probe designs.

With the increase in vessel size we were also now able to demonstrate the benefits of online analysis as opposed to the "inline" variants we had been showing in the open-day version. Using one of the extra ports in the reactor lid we could take a flow out of the reactor in bypass mode; this we equipped with a transmission NIR probe. Equipping the inlet with a filter meant we could show one of the strategies for continuing to use transmission probes even in environments where the particulates in the main flow or reactor means that in-line monitoring is impossible.

By building this demonstrator on a metal frame on wheels we were able to make transportation to the exhibition relatively simple. The size of the frame was specified that when not in use for exhibitions the frame will fit neatly into our normal process analysis floor to ceiling fume cupboards allowing it to be a useful resource in our day-to-day work. For the exhibition we were also able to take the bypass flow through two further glass condensers to simulate more complex separation and processing steps which gives a more balanced picture.

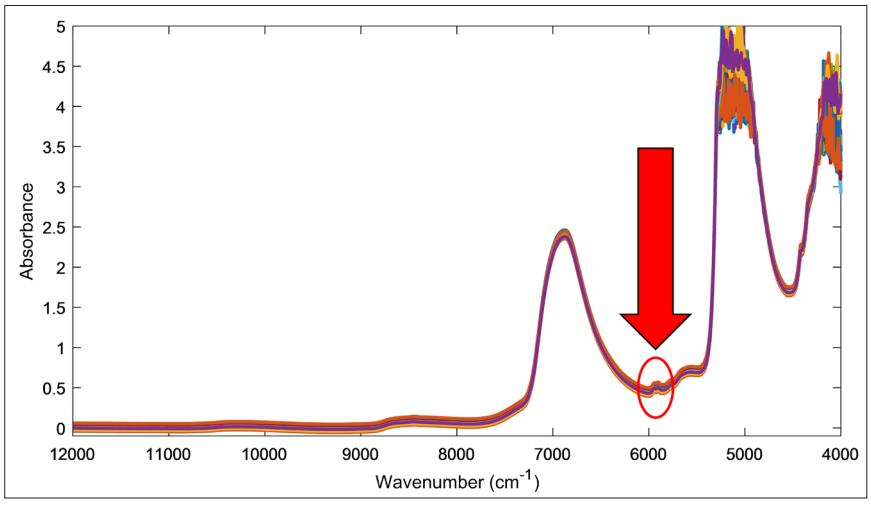

Figure 3. A simple example why chemometrics is required to find and measure the analyte needle in the spectroscopic haystack.

Explain the benefits of chemometrics

The chemometrics analysis is often difficult to explain briefly. However, one image the team generated brought the message of the power of chemometric data processing to extract information from the surrounding noise which we added to the presentation of the process analytical work (Figure 3). From a purist's perspective, this does not necessarily show extracting data from impossible sets, but it does show analytical peak sizes people can relate to and depending on your particular audience you can discuss this figure at many different levels.

Conclusion

So, having preached in several recent columns about the need for better training and interaction of more advanced technologies to inspire our future generations, it was fun and educational to put this relatively simple setup together as model of probe selection for a PAT implementation project.

Acknowledgements

The authors would like to thank Thibaut Février, Herman Strijdveen as well as Rob Bloemenkamp, Kristiaan Klijnstra and Ali Ghamati for invaluable help and bright ideas in realising this project.

QUALITY MATTERS

The kilo, the mole and the commutability of a result to activity

Peter J. Jenks, FRSC
The Jenks Partnership

For all of my scientific life, weight has been traceable to a lump of platinum alloy sitting under a series of glass bell-jars and located in Paris, France. In my early days, amount of substance was expressed as mg L^{-1} or ppm, in many labs these units are still commonly found. In 1971 the Mole was made the seventh SI base unit and slowly the idea of molar solutions permeated analytical chemistry.

Now, all that is set to change because on 16 November this year the 26th General Conference on Weights and Measures (CGPM) voted unanimously in favour of revised definitions of the SI base units, a change that the International Committee for Weights and Measures (CIPM) had proposed earlier in the year. The new definitions will come into force on 20 May 2019.

As a direct consequence the kilogram, ampere, kelvin and mole will then be defined by setting exact numerical values for the Planck constant (h), the elementary electric charge (e), the Boltzmann constant (k) and the Avogadro constant (N_A), respectively. This brings them into line with the metre and candela, which are already defined by physical constants, subject to correction to their present definitions and does not change the size of any units, so maintaining continuity with existing measurements.

The changes

The kilogram, symbol kg (lower case) is the unit of mass: the previous definition is that it was equal to the mass of the international prototype of the kilogram.

2019 definition: The kilogram is now defined by taking the fixed numerical value of the Planck constant h to be $6.62607015 \times 10^{-34}$ when expressed in the unit J·s, which is equal to $kg \cdot m^2 \cdot s^{-1}$, where the metre and the second are defined in terms of c and $\Delta\nu_{Cs}$.

A consequence of this change is that the new definition of the kilogram is dependent on the definitions of the second and the metre. A second consequence is that a new definition for the mole is required.

The mole, symbol mol, is the SI unit for amount of substance. The previous definition was that it is the amount of substance of a system that contains as many elementary entities as there are atoms in 0.012 kg of carbon-12. When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles or specified groups of such particles.

2019 definition: One mole contains exactly $6.02214076 \times 10^{23}$ elementary entities. This number is the fixed numerical value of the Avogadro

constant, N_A, when expressed in the unit mol^{-1} and is called the Avogadro number.

A further consequence of these changes is that the current defined relationship between the mass of the ^{12}C atom, the dalton, the kilogram and the Avogadro number will no longer be valid.

As the wording of the ninth SI Brochure[1] implies, the mass of a ^{12}C atom is exactly 12 dalton then the number of daltons in a gram cannot any longer be the numerical value of the Avogadro number: (i.e., $g/Da = N_A \cdot mol$).

These changes are all very interesting at the academic level, but what do they mean to chemical metrology and the use of reference materials? In truth very little, as they are designed to ensure continuity with all existing measurements that in turn underpin the certified values of the CRMs.

A far greater concern is the issue of commutability, a subject I have touched on in the past. It was the laboratory medicine community who first expressed concern about the commutability of data, but with the move to accredit medical diagnostic laboratories to ISO/IEC 17025 the concept has migrated into the world of general analytical chemistry. Why is commutability so important?

Maintaining accurate laboratory measurements over time is crucial to making data comparable. This is generally achieved by the use of an accredited quality system, typically ISO/IEC 17025, and establishing traceability to a reference system, the SI. Reference materials are key components of such reference systems and for establishing traceability this means that commutability of reference materials is a critical property to ensure they are fit for use.

Commutability is defined as the equivalence of the mathematical relationships between the results of different measurement procedures for a reference material and for representative samples taken from the area to be analysed. This material characteristic is of special importance for measurement procedures that are optimised for measuring analytes directly in real-world samples. It becomes even more important when the reference material is certified for some aspect of biological quantity and not an amount of substance.

For example, how do we produce reference materials for use in DNA measurements? The measurement of specific DNA fragments to demonstrate the presence, or absence, of a biological entity has become a key part of food quality measurement and is creeping into microbiology, food, environmental and clinical. Many will recall the 2014 adulteration of beef with horse meat. As a direct consequence, LGC developed new reference materials to underpin the detection of alien species within a defined processed meat. The materials were analysed using three different approaches—DNA sequencing, a PCR-based method and an immunoassay method—to confirm the expected meat species in the samples and the absence of possible species cross-contamination. The limit of detection is below 1% of one meat species in the presence of another. The Reference Materials were produced by weighing out previously verified pure meat and mixing them in the defined proportion. No subsequent analysis was made. When using Sanger sequencing it is common to use mitochondrial genes, for example MT-RNR1 which encodes RNA 12s and MT-CYB which encodes cytochrome b. Ultimately the identity of the genes is validated against known DNA Sequence Databases.

It seems to me that traceability to a proprietary sequence database is a long way away from traceability to the SI and that the new definitions for amount of substance mean little in this context. The question remains unanswered, just how can traceability and commutability be maintained when an assay of a complex biological material is validated by comparing a sequence with data on a database, most probably supplied by the instrument maker?

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SAMPLING COLUMN

A tale of two laboratories II: resolution

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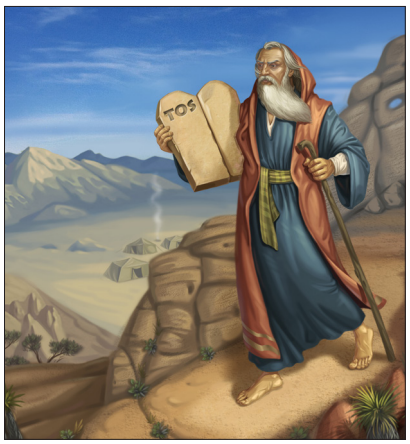
This column completes the tale of two fictional laboratories both facing the issue: "How can the Theory of Sampling (TOS) help the commercial laboratory to improve its reputation and to increase its business"? For decades, Laboratory A has been in fierce market competition with Laboratory B, and indeed several others on the global market, which has resulted in a "healthy" business-oriented science, technology and expertise drive that has served all laboratories well. Both laboratories are keenly aware of the necessity to be in command of TOS for all their in-house activities involving sampling, sub-sampling, mass-reduction and sample splitting. But, whereas Laboratory A has availed itself of the services of TOS strictly within its own regimen only, as is indeed the case for most laboratories, one fine day the manager of Laboratory B had an epiphany that made her see potential advantages of applying TOS in full, which involve a distinctly "beyond-the-traditional-laboratory" scope. What happened? And how did it help Laboratory B to do better in the market?

Scope. In addition to the column author's own take, three other contributors from science, commerce and economics have been asked to give their suggestions on what could possibly have been the contents of Laboratory B head's epiphany? Let's start on the lighter side…

Epiphany interpretation I: knowingly closing one's eyes or not?

"A vision of a white-bearded figure carrying a tablet comes down from the mountain. The CEO can barely make out the writing, but there are the letters 'TOS' at the top … As the figure spoke of primary sampling error effects not taken proper care of, she became terrified at the thought of potential implications for her laboratory … culpability, and the ultimate terror … litigation."

Indeed, starting out on the lighter side, this interpretation turns decidedly serious right away… culpability, litigation… because of what? This can only relate to consequences of decisions made based on the analytical results. Which is why all commercial laboratory analytical reports carry a disclaimer, in one or many other



forms, the contents of which are identical. However, "The analytical results reported here, and their analytical uncertainty, pertain to the samples delivered." For emphasis "…pertain to the *samples* delivered". This disclaimer has the clear aim to absolve the analytical laboratory of legal responsibility regarding any-and-all consequences of decisions made based on the analytical results. Such decisions are made by the client.

Most laboratories (including A and B) are undoubtedly fully aware of the risk of relatively minor sampling errors affecting the Total Analytical Error (TAE) stemming from in-house sub-sampling, sample preparation, mass-reduction etc. in the pathway from "samples received" to analysis. All of which are very seriously taken care of in any commercial laboratory enterprise whose reputation and livelihood are directly associated with the most professional command of all aspects of the science, technology and practise of *analysis*.

But the effects of the dominating primary sampling errors, if/when not taken proper care of (see previous column) are still looming in nowhere land; nobody is willing to take responsibility. The manger realised that the consequences for believing blindly in the analytical report would be borne only be the client.[1]

Epiphany interpretation II: the economic dilemma

"The CEO of Laboratory B realised that a new business opportunity no other laboratory so far had tapped into, would be to encompass the whole process, from lot to aliquot, i.e. taking care of proper counteractions w.r.t. ***both*** *TSE and TAE."*

She felt particularly satisfied to avoid the negative statement: "Primary

sampling is outside Laboratory B's responsibility",[1] being fully aware though, that by identifying this risk element, the largest uncertainty component, Laboratory B would actually demonstrate its deliberate unwillingness to acknowledge the consequences hereof. Which would, therefore, still have to borne by the client alone—yet this risk, and its demonstrably dire economic consequences, are well known. Increasingly, knowledge of these negative effects seems like a burden … …

"Also: increase market share! She was well aware of this challenge, since no one had so far gone the whole way. And she understood the reason. Typically, clients of the laboratory only ask for the result of the aliquot analysis because they need to document the analytical results for **their** *clients in turn."*

A-ha, laboratories often exists in a broader perspective: from-lab-to-client-to-client. As an example, think: analytical laboratory → consulting engineering company (e.g. responsible for environmental surveys) → regulatory authority. There are many other *similar* situations in which the entity responsible for the primary sampling is an *outsourced* entity by the ultimate end-user. In such a case, there is typically no direct communication between the lab and the end-user. The market has *faith* that TAE pertains not only to the laboratory results but also the TSE part—to the degree that this "technicality" is known (which *may* well be to only a very small degree, viz. current experiences). The immediate client of the laboratory has no interest in correcting this, since this would only increase costs unilaterally (in order to start performing representative primary sampling). This is the traditional economic argument.[1]

Of course, in a market economy, companies (commercial laboratories are no exception), each being micro-economic ventures on their own, primarily feel responsible for their own economy. They feel that they **must** look to maximise profit before anything else. So the conventional wisdom goes in the harsh real-world of market economics.

There are two components in this aspiration: increase earnings and/or limiting costs, both defining the gap for profitability. In her dream the CEO felt very sure of being in command of this *narrow*, microeconomic competence—but, of course, just going along as usual was not really the issue…

"Laboratory B CEO's epiphany was a realisation that the whole package TSE + TAE was not ***in demand*** *by the client, because the clients-of-the-client believe this is included already. The CEO realised a critical need for finding tangible, compelling examples of what will happen in case of the omission of TSE, specifically in terms of economic impacts for commerce but also other less directly tangible impacts for the public. It was felt essential to facilitate an efficient awareness (perhaps even public intervention) of these matters, lest 'Sampling… is gambling'!"*

Laboratory B therefore needs also to address the *clients-of-the-client* in creating an **explicit demand** for a more responsible behaviour by the primary laboratory client, and indeed of the laboratory itself. This will require a two-fold exercise i) an *augmented* marketing strategy and ii) becoming involved in fostering *increased awareness* w.r.t. TOS in general, the dire economic effects of continuing to neglect the primary sampling error effects in particular.[2] But, even in her dream trying to break free of traditional bonds, the CEO could hear voices repeating the "board room" argument: why should Laboratory B be the one to accept larger costs for delivering the exact same quality analytical results?

Speaking of dreams, epiphanies, nightmares—the latter often comes in the form of a *dilemma*: "I am doomed (economically) if I undertake larger costs than all of my competitors" and "I am doomed (morally) if I neglect the new insight that neither the client nor the client-of-the-client care one bit whether TSE is included—so long as this is **not known** by the end-user". Clearly, this is an untenable situation in any time perspective.

"What is common to dilemmas is ***conflict****. In each case, an agent regards herself as having moral reasons to do each of two actions, but doing both actions is not possible. Ethicists have called situations like these* ***moral dilemmas****. The crucial features of a moral dilemma are these: the agent is required to do each of two (or more) actions; the agent can do each of the actions; but the agent cannot do both (or all) of the actions. The agent thus seems condemned to moral failure; no matter what she does, she will do something wrong (or fail to do something that she ought to do)".*

Epiphany interpretation III: the moral resolution

There were some powerful statements in the epiphany, almost as if *written in stone*:

i) The client, and the client-of-the-client, *deserves* to know about the risk of severe economic (and other) consequences if neglecting the $TSE_{primary\ sampling}$ effects.

ii) In case this is not known to the client and/or the client-of-the-client, everybody in-the-know, Laboratory B of course included, has a *moral obligation* to rectify this, to fill-in this factual lacuna. It cannot be right deliberately to keep one's client in the dark regarding issues that have a very high

risk of severely influencing its bottom line adversely.

iii) WHAT will happen the day the clients find out about this wilful omission?

iv) Integrity: doing what is right, regardless of whether this is known or not. Integrity is a characteristic that comes from within, based on awareness and knowledge.

The CEO realised that the integrity of Laboratory B was at stake!

The CEO realised that she would rather be CEO of a company with scientific integrity, than continue to avoid a societal and moral obligation, now knowing well the adverse consequences for her company's clients!

The CEO was thus now convinced that *honesty, integrity and transparency* must be the motto for Laboratory B's behaviour in the "analysis for sale" market. This has a necessary corollary obligation for her company. It is critically necessary to partake in a campaign for increased TOS awareness directed at everybody involved. This includes companies where sampling plays a critical role in general (**quite** a few it turned out, after just a few moments' thought) and analytical laboratories specifically (commercial as well as academic).[3] It also includes all relevant entities in society at large, e.g. monitoring and regulatory authorities, department and governmental advisors and agencies, scientific outlets, NGOs.[4]

As but one example of importance, the EFSA (European Food Safety Agency) is charged with safeguarding the public regarding food safety and public health in all of the EU's member states. What would happen if representative sampling was **not** one of its most important priorities? N.B. of course an entity like EFSA has a series of major other obligations and objectives, but many of these would suffer were not proper sampling also taken seriously. Most routine and advanced analytical characterisation of, e.g., food, feed, plants, GMOs…. are completely at the mercy of whether the relevant primary "samples" are indeed representative samples, or not. As all readers of these columns will know intimately, this is of imperative importance and cannot be overlooked without severe risks of adverse consequences, certainly not only of economic character, but infinitely more important, consequences for **public health** in its most broad perspective. What would happen, *hypothetically*, if the European populace one day were to find out that their public health safeguarding is not backed by absolute competence and total diligence? To be absolutely clear, the example of EFSA is *imaginary*, and only used here to focus the perspective, viz. the recently published comprehensive report specifically on sampling.[5]

Laboratory B's new vision and mission

The CEO laid out a new vision and mission for Laboratory B; the following mottos would henceforward now be the message to its customers:

- Laboratory B trusts and supports employees to take personal ownership and accountability, and learn from their experiences …
- Laboratory B is partnering with customers to enhance their productivity and performance …
- Laboratory B is listening to customer challenges and actively anticipating their future requirements …
- Laboratory B will do the right thing even if it means losing business …

In the market place there would be no mercy for a company's reputation, if it was revealed and proved that the company engaged in a willing omission of disclosure and co-responsibility for the primary sampling error dominance w.r.t. the total Measurement Uncertainty ($MU_{sampling + analysis}$). The market would not be kind in the face of: "but we are simply seeking maximise our own profit—in a stark competition".

On the said "fine day" (see previous column), the CEO instigated a vigorous campaign for total scientific and economic responsibility and transparency.[6] Among other initiatives she immediately made contact with appropriate TOS experts and educators in order to collaborate on this new mission. By doing this she was sure of minimising her own costs while maximising the benefits for clients—**and** clients-of-clients.

Can this really lead to increased commercial success?

How can one make sure that one's favourite commercial analytical laboratory, or company producing instrumental analytical equipment and "solutions", observe due diligence w.r.t. the overwhelmingly largest contributor to the **total** Measurement Uncertainty ($MU_{sampling + analysis}$)?

Easy—even a cursory visit to relevant company web sites clearly reveals whether there is the appropriate awareness, or not. The reader is encouraged to do exactly that—and observe which company/companies instil confidence and trust in the mind of the website reader w.r.t. the so-often forgotten critical sampling issue.

The genie is out of the bottle, it is only a matter of who will be the first mover…?[7] Will it be your laboratory?

Acknowledgements

The column author is grateful for suggestions and input re. possible epiphany interpretations from three individuals, greatly appreciated. Due to certain, often large, artistic licenses taken in the interest of the tale, these individuals are thanked profusely but shall remain anonymous; they know who they are.

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LUMINESCENCE

FluoTime 250 plug and play time-resolved spectrometer

PicoQuant have introduced the FluoTime 250 fluorescence lifetime spectrometer. Comprehensive software wizards allow even novice users to perform complex measurements such as fluorescence decays or time-resolved anisotropy studies in a very short time. Advanced users get full access to all instrument capabilities via a point-and-click interface or integrated scripting language. All components in the optical beam paths are motorised and controlled by the EasyTau 2 software. Emission wavelength selection is via a motorised filter wheel equipped with various cut-off or bandpass filters. An optional monochromator is available for the UV/vis spectral range. By adding an optional monochromator, the FluoTime 250 can also measure time-resolved emission spectra (TRES). The FluoTime 250 uses picosecond pulsed lasers diodes and LEDs covering a spectral range from 255 nm to 1550 nm. All excitation sources are controlled via a PDL 820 laser driver, which allows output power and repetition rates (up to 80 MHz) to be varied and supports burst mode for selected laser heads.

PicoQuant

http://link.spectroscopyeurope.com/30-W-113

IMAGING

New QCL chemical imaging system from Agilent

Agilent's 8700 Laser Direct Infrared (LDIR) chemical imaging system combines quantum cascade laser (QCL) technology with rapid scanning optics and Agilent Clarity software. Unlike other QCL imaging systems that use 2D focal plane array (FPA) detectors, the 8700 LDIR employs a single-element electrically cooled detector to eliminate laser coherence artefacts from images and spectra. It has the ability to survey and image large sample areas and then interrogate smaller areas of interest in more detail without changing any optics. The field of view can be changed from microns to centimetres or the pixel size from 1 µm to 40 µm. ATR imaging data can be acquired with a pixel size down to 0.1 µm, and unknowns can be identified using either commercial or custom libraries from the ATR spectra. The 8700 LDIR works in either reflectance or ATR mode, automatically switching between these two modes by directing the incident beam to the appropriate objective. The movement of the sample relative to the beam is fully automated, this process yields a high-quality two-dimensional molecular image in a short time. The 8700 LDIR has two visible channels: a large field of view camera to obtain an entire view of the sample and a microscope grade objective to

capture high magnification detail. No requirement for liquid nitrogen reduces operating costs and simplifies maintenance, whilst the instrument's small footprint saves laboratory bench space.

Agilent Technologies

http://link.spectroscopyeurope.com/30-W-108

SpectraPro HRS-750 imaging spectrograph

Princeton Instruments' SpectraPro HRS-750 is a new 750 mm focal length spectrograph and scanning monochromator that features an astigmatism-corrected optical design, a mechanical scanning range of 0–1500 nm, as well as resolution of 0.05 nm or better. New technology built into the HRS-750 includes Princeton Instruments' spectral deconvolution technology, ResXtreme, which is provided with every SpectraPro HRS spectrometer. ResXtreme improves the spectral resolution, peak intensities and consistency across the 2D focal plane by as much as 60 %. The SpectraPro HRS-750 can also take full advantage of Princeton instruments' patented IntelliCal® wavelength and intensity calibration system. Every SpectraPro HRS-750 can utilise up to three interchangeable triple-grating turrets and also includes Princeton Instruments' exclusive AccuDrive, which yields significant improvements in wavelength accuracy and repeatability over previous scan systems and increases grating-to-grating wavelength precision to sub-pixel repeatability.

SpectraPro HRS-750 applications include Raman spectroscopy, photoluminescence, fluorescence, LIBS, plasma diagnostics, transmission, absorption and microspectroscopy. All SpectraPro HRS-750 spectrographs are supported by Princeton Instruments' 64-bit LightField® imaging and spectroscopy software. LightField provides control of the SpectraPro HRS-750 as well as comprehensive data analysis capabilities.

Princeton Instruments

http://link.spectroscopyeurope.com/30-W-109

NEW PRODUCTS

INFRARED

New FT-IR spectrometer

The Thermo Scientific Nicolet iS20 FTIR spectrometer, an upgrade over the existing Thermo Scientific Nicolet iS10 FTIR spectrometer, features LightDrive Optical Engine technology. The redesigned optical system provides higher spectral resolution (0.25 cm^{-1}) and single-to-noise ratio (50,000 : 1). The new IR source eliminates hot spot migration, resulting in more consistent spectral data, especially through an ATR accessory. The user interface has a touch panel with a multi-coloured LED scan bar providing visual feedback on instrument status (ready, scan, alert). A 10-year warranty is offered on the source, laser and interferometer, whilst the LightDrive interferometer provides a five-fold increase in relative lifetime over current interferometer technology.

Thermo Fisher Scientific

http://link.spectroscopyeurope.com/30-W-116

MAGNETIC RESONANCE

JEOL integrates CRAFT data processing

JEOL has expanded the quantitative and statistical analysis capability of its NMR spectrometers through collaboration with the developer of the CRAFT (Complete Reduction to Amplitude-Frequency Table) data processing technique. In conjunction with the recent JEOL DELTA NMR software release 5.3.0, CRAFT for DELTA V1.0 provides direct time domain-to-spreadsheet analysis, allowing for more objective extraction of quantitative information for compounds of interest, provides statistical analysis for metabolomics, reaction monitoring or quality control. Peaks that are too close to effectively quantify by traditional integration are completely resolved sinusoids in the time domain with separate information of frequency, amplitude, phase and decay rate available as numbers in a table.

Pioneering work for CRAFT direct time-domain analysis of NMR data was begun by Dr Krish Krishnamurthy and first published in 2013, and the work was extended to 2D NMR in 2016. By utilising Bayesian analysis of the time domain spectrum, CRAFT bypasses many issues brought about by Fourier transform to a visual frequency dimension.

JEOL

http://link.spectroscopyeurope.com/30-W-111

MASS SPECTROMETRY

PerkinElmer's QSight 400 Series triple quad mass spectrometer

PerkinElmer has announced the launch of its QSight® 400 Series triple quad mass spectrometer, designed for rapid identification and quantitation of pesticides, mycotoxins and emerging contaminants in complex food, cannabis and environmental testing applications. PerkinElmer has developed a single LC/MS/MS method for use with the instrument that meets pesticide and mycotoxin regulatory requirements. This uses electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) for low level analysis of pesticides and mycotoxins. Using dual source (ESI/APCI) technology eliminates the need for any other analytical method, including GC/MS/MS, and enables five times greater detection capabilities than current industry methods.

Key features of the QSight 400 Series include a patented StayClean™ and hot-surface-induced desolvation (HSID™) technology that reduces the amount of scheduled cleaning and maintenance, helping improve throughput by 15%. The dual source technology with two separate independent ion inlets, which can be set to ESI and APCI sources, enables detection of a wider list of contaminants, including chlordane and pentachloronitrobenzene.

PerkinElmer

http://link.spectroscopyeurope.com/30-W-117

LEYSPEC residual gas analyser for vacuum systems

Leybold has launched the LEYSPEC for residual gas analysis in vacuum systems. The product series is available in six variants covering basic and extended residual gas analysis methods in high and ultra-high vacuum applications. The analyser has an integrated display and, at the touch of a button, can display the partial pressures of the relevant gases at any time. If the user is interested in an additional gas in the process, another channel can be individually assigned to it. LEYSPEC software enables a wide range of applications, from simple operations to complex analyses. The software always displays the total pressure. Additional test procedures and functionalities are pre-installed, such as the helium leak test or the setting of warnings and error limits for certain gases. Simple gas analyses can be performed without connecting the LEYSPEC to a computer.

The range offers products for 100, 200 or 300 amu, depending on process requirements. The LEYSPEC view version is suitable for residual gas analyses in high-vacuum pumping stations, in research and development applications as well as for environmental tracking and gas impurity analyses. The LEYSPEC ultra variant is designed for sophisticated residual gas analyses with higher sensitivity, reliable detection of very low partial pressures and higher bake-out temperatures.

Leybold

http://link.spectroscopyeurope.com/30-W-112

NEW PRODUCTS

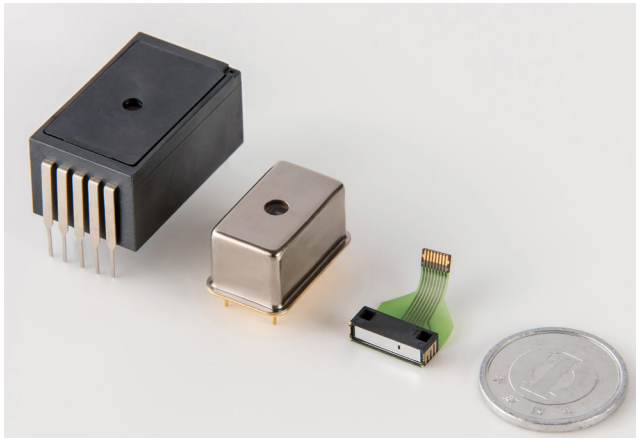
NEAR INFRARED

Miniature grating spectrometer

Hamamatsu Photonics has introduced their smallest grating spectrometer, the SMD series mini-spectrometer C14384MA. This offers high near infrared sensitivity, compact size, light weight and low cost. Compared to Hamamatsu's existing range of MS series mini-spectrometers, the C14384MA is about 1/40th of the cubic size and 1/30th of the weight. It also offers sensitivity in the same near infrared range, but around 50 times higher. This makes the C14384MA suitable for applications where real-time, on-site measurement is required; for example quality inspection of food or agricultural crops and environmental analysis from quadcopters to drones.

Hamamatsu Photonics

http://link.spectroscopyeurope.com/30-W-106



POLARIMETRY

High-throughput microplate reader for circular dichroism

Bio-Logic and Hinds Instruments have introduced the EKKO®; the first high-throughput circular dichroism (CD) microplate reader. It can screen a 96 well plate in under two minutes, as opposed to several hours for a traditional CD spectrometer linked to an auto sampler. The device is also more cost-efficient, using 10 times less nitrogen than a standard spectropolarimeter with an auto sampler and 4 times less sample at a comparable light path. It is able to identify enantiomeric excess (ee) rates as well as yield, resulting in data that can prove highly beneficial to the screening process.

While CD instruments have been available for many years, they are designed to measure the CD of a single reaction product at a time. The EKKO® can rapidly determine the enantiomeric composition of 96 reaction products in a standard laboratory micro well plate, equating to thousands of readings per day. The device can therefore help reduce bottlenecks in the drug discovery process and is suited to parallel/combinatorial synthesis techniques, where more test combinations can be carried out in less time. Although designed with evaluating enantiomeric excess in mind, the device is flexible, and its optics enable the device to work down to the far-UV at 185 nm, making it suitable for the determination of secondary protein structures, for biotherapeutic development or formulation optimisation.

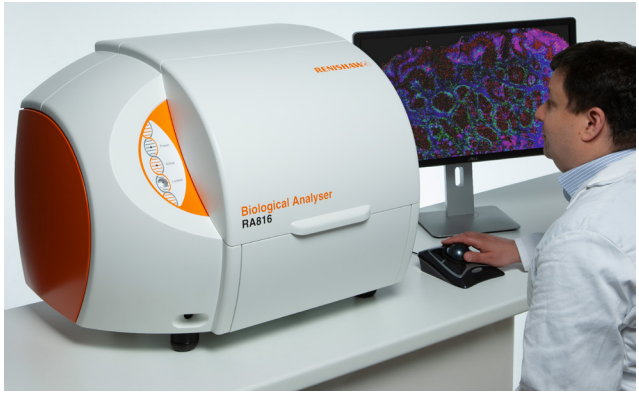
Bio-Logic

http://link.spectroscopyeurope.com/30-W-114

RAMAN

RA816 Biological Analyser

The new Renishaw RA816 Biological Analyser enables the rapid collection of detailed information from a range of biological samples, including tissue and biofluids. It allows biologists and clinicians to identify and assess biochemical changes associated with disease formation and progression. They can obtain the full range of biochemical information without prior knowledge of specific molecular targets or labelling or staining. The instrument obtains detailed information on the distribution and amount of biochemical species within biological samples, including tissue biopsies, tissue sections and biofluids. Users are able to reveal detailed information from biological samples; from the distribution of exogenous and endogenous compounds within tissue, to the detection of protein secondary structure changes due to drug interaction and tissue injury. The analyser has been extensively tested at multiple locations including the Neuro-oncology Department at Oxford Radcliffe Hospital, UK and Humanitas Hospital in Milan, Italy, where they have been studying brain tissue for the genetic classification of glioma tumours. The analyser has the potential to aid surgeons in tailoring their surgical strategy based on a patient's specific tumour genetics.



Renishaw

http://link.spectroscopyeurope.com/30-W-115

NEW PRODUCTS

UV/VIS

New Cary customisable UV/vis spectrophotometer

Agilent Technologies has introduced the Cary 3500 UV/vis spectrophotometer. The Cary 3500 UV-Vis is designed as clip-together spectrophotometer engine and modules. The engine is common to each configuration and can be paired with one of five different modules to create a customised instrument. It contains a long-life, fast xenon flashlamp as well as the optics that make the high-resolution and high-absorbance measurements possible. Configurations include the Cary 3500 Multicell UV-Vis for measuring up to seven samples and a reference (or other combinations in the eight cell positions). The Multicell spectrophotometer is available in an ambient, temperature-controlled, or multizone configuration. The Cary 3500 Compact UV-Vis is designed for measuring a single sample and reference, and is available in either an ambient or a temperature-controlled configuration.

Agilent Technologies

http://link.spectroscopyeurope.com/30-W-107

X-RAY SPECTROMETRY

ED XRF analyser for the petrochemical industry

Bruker has launched the S2 POLAR, multi-element benchtop analyser based on polarised energy dispersive x-ray fluorescence (ED XRF). The S2 POLAR offers the same analytical precision for quality control in the petrochemical industry as typically achieved by more expensive wavelength dispersive x-ray fluorescence instruments. It analyses ultra-low sulfur content and achieves detection limits in the sub-ppm range for gasoline, kerosene and diesel. Its ability to measure multiple elements simultaneously, including chlorine for corrosion prevention and phosphorus against residue build-up, makes the S2 POLAR suited to the analytical demands of refineries, as well as for the downstream supply chain of pipelines, oil terminals and petrol stations.

The S2 POLAR is compliant with all relevant ASTM, DIN, IP, JIS and ISO norms. Bruker's multilingual TouchControl™ interface and factory calibrated application packages for the ASTM norms D7220, D4294, D6481 and D7751 ensure "plug-and-play" operation. SampleCare™ component protection supports high instrument uptime and robustness.

Bruker AXS

http://link.spectroscopyeurope.com/30-W-105

Optical spectrum from 8 eV to 2000 eV from three gratings

McPherson's Model 251MX is easy to use and operates over the 0.5–150 nm range by indexing diffraction gratings. It is optimised for high-energy photons including soft x-ray and extreme UV. The Model 251MX does not scan with point detectors, it works exclusively with microchannel plate intensifiers and/or direct detection CCD array detectors. Regardless of type, the detector is located on a flat focal plane. The diffraction gratings are from holographic masters and their laminar groove profile, that looks like a square wave, results in diffraction efficiency in even orders of light lower than either sawtooth or sinusoidal profiles. This helps keep a high-energy spectrum clean and more easily interpretable—especially at short wavelengths.

McPherson

http://link.spectroscopyeurope.com/30-W-110

Conferences
2019

24–28 January, San Diego, CA, United States. **Pacific Conference on Spectroscopy and Dynamics PCSD 2019.** https://www.westernspectroscopy.org/

24–27 January, Fort Myers, FL, United States. **Sanibel Conference on Mass Spectrometry, Chemical Cross-linking and Covalent Labeling: From Proteins to Cellular Networks.** https://www.asms.org/conferences/sanibel-conference

30 January, Huddersfield, United Kingdom. **5th Ambient Ionisation SIG Meeting.** Andrew Ray, andrew.ray@astrazeneca.com, http://www.bmss.org.uk/SIG_ambient-ion.shtml

30 January–1 February, Montpellier, France. **Chimiométrie 2019.** chemom2019@sciencesconf.org, https://chemom2019.sciencesconf.org/

2–7 February, San Francisco, United States. **Photonics West 2019.** http://spie.org/conferences-and-exhibitions/photonics-west?SSO=1

2–6 February, Washington, United States. **Society for Laboratory Automation and Screening (SLAS2019).** https://www.slas2019.org/

3–8 February, Pau, France. **European Winter Conference on Plasma Spectrochemistry-EWCPS-2019.** Ryszard Lobinski, ewcps2019-chair@winterplasma2019.com, https://winterplasma19.sciencesconf.org/

19–20 February, Prague, Czech Republic. **European Congress on Pharmaceutics & Pharmaceutical Technology.** pharmaceutics@pharmaeuroscicon.com, https://pharmaceutics.euroscicon.com/

25–27 February, Prague, Czech Republic. **PHOTOPTICS 2019: 7th International Conference on Photonics, Optics and Laser Technology.** photoptics.secretariat@insticc.org, http://www.photoptics.org/

3–7 March, Hilton Head Island, SC, United States. **7th Annual Practical Applications of NMR in Industry Conference (PANIC-2019).** https://www.panicnmr.com/

3–6 March, Bethesda, United States. **International Foundation Process Analytical Chemistry (IFPAC-2019) Annual Meeting.** http://www.ifpac-global.org/

18–20 March, Edinburgh, United Kingdom. **17th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems (Pharmaceutica 2019).** pharmaceutica@pharmaceuticalconferences.org, https://novel-drugdelivery-systems.pharmaceuticalconferences.com/

25–26 March, Budapest, Hungary. **EuroSciCon Conference on Biosimilars 2019.** kennedypeyton001@gmail.com, https://biosimilars.eurosciconference.com

25–28 March, Berlin, Germany. **2nd International Plant Spectroscopy Conference (IPSC-2019).** https://ipsc-2019.julius-kuehn.de/

31 March–4 April, Orlando, FL, United States. **257th American Chemical Society (ACS) National Meeting & Exposition: Chemistry for New Frontiers.** NationalMeetings@acs.org, https://www.acs.org/content/acs/en/meetings.html

6–10 April, Orlando, United States. **Experimental Biology 2019.** http://experimentalbiology.org/2017/About-EB/Future-Meetings.aspx

9 April, London, United Kingdom. **Advances in Hyphenated Mass Spectrometry.** mark_mcdowall@icloud.com, http://www.bmss.org.uk/games2019/games2019.shtml

17–18 April, Osaka, Japan. **9th International Conference and Exhibition on Spectroscopy and Analytical Techniques.** lcms2018hk@gmail.com, https://spectroscopyconference.massspectra.com/registration.php

22–26 April, Phoenix, Arizona, United States. **2019 Materials Research Society (MRS) Spring Meeting & Exhibition.** https://www.mrs.org/spring2019

30 April–2 May, Chester, United Kingdom. **APACT19.** https://apact.co.uk/

1 May, Sheffield, United Kingdom. **The Sixth Mass Spectrometry Imaging One-Day Meeting.** Dr Jamie Young, jamie.young@shu.ac.uk, https://www.eventbrite.com/e/bmss-sig-imaging-symposium-2019-tickets-51956350844

5–10 May, San Jose, United States. **Conference on Lasers and Electro-Optics (CLEO).** confserv@osa.org, https://www.cleoconference.org/home/

6–9 May, Beijing, China. **The 9th World Conference on Sampling and Blending-WCSB9.** http://www.wcsb9.com/

13–15 May, Edinburgh, United Kingdom. **Challenges in Analysis of Complex Natural Mixtures: Faraday Discussion.** http://www.rsc.org/events/detail/29574/challenges-in-analysis-of-complex-natural-mixtures-faraday-discussion

20–21 May, Zurich, Switzerland. **21st Annual European Pharma Congress.** pharmaeurope@pharmaceutical-conferences.org, https://www.ideaconnection.com/conferences/3879-21st-Annual-European-Pharma-Congress.html

22–24 May, Berlin, Germany. **2nd International Symposium on Single Photon based Quantum Technologies.** Kerstin Wicht, events@picoquant.com, http://www.quantum-symposium.org

2–6 June, Atlanta, United States. **67th ASMS Conference on Mass Spectrometry.** office@asms.org, http://www.asms.org/conferences/annual-conference/future-annual-conferences

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2–5 June, Nara, Japan. **15th International Symposium on Applied Bioinorganic Chemistry (ISABC 15).** http://web.apollon.nta.co.jp/isabc15/

9–14 June, Mexico City, Mexico. **Colloquium Spectroscopicum Internationale XLI (CSI XLI).** http://www.csi2019mexico.com/

11–12 June, Münster, Germany. **5th International Workshop on Electrochemistry/Mass Spectrometry (ElCheMS 2019).** martin.vogel@uni-muenster.de, https://www.uni-muenster.de/Chemie.ac/en/karst/workshops/elchems.html

16–20 June, Split, Croatia. **5th International Sclerochronology Conference (ISC2019).** Melita Peharda, isc2019@izor.hr, http://jadran.izor.hr/isc2019/index.html

17–20 June, Oslo, Norway. **16th Scandinavian Symposium on Chemometrics (SSC16).** ssc16@nofima.no, http://ssc16.org/

27–29 June, Amsterdam, Netherlands. **18th Annual Congress on Pharmaceutics & Drug Delivery Systems.** clarajane567@gmail.com, https://pharmaceutics.annualcongress.com/

30 June–3 July, Warsaw, Poland. **7th International Symposium on Metallomics.** Ryszard Lobinski, sekretariat@metallomics2019.pl, http://metallomics2019.pl/

8–12 July, Auckland, New Zealand. **International Conference on Advanced Vibrational Spectroscopy (ICAVS10).** ICAVS Secretariat, Podium Conference Specialists, 2661 Queenswood Drive, Victoria, BC, Canada, V8N 1X6. http://www.icavs.org/2019-conference/

5–9 August, Lombard, IL, United States. **68th Annual Denver X-ray Conference (DXC 2019).** http://www.dxcicdd.com

18–23 August, Barcelona, Spain. **GOLDSCHMIDT2019.** https://goldschmidt.info/2019/

25–30 August, Berlin, Germany. **21st International Society of Magnetic Resonance (ISMAR) Conference joint with EUROMAR 2019.** https://www.weizmann.ac.il/ISMAR/

3–5 September, Manchester, United Kingdom. **40th BMSS Annual Meeting 2019.** bmssadmin@btinternet.com, http://www.bmss.org.uk/bmss2019/bmss2019.shtml

8–11 September, Denver, United States. **133rd AOAC International Annual Meeting and Exposition.** meetings@aoac.org, https://www.aoac.org/aoac_prod_imis/AOAC_Member/MtgsCF/19AM/AM_Main.aspx?WebsiteKey=2e25ab5a-1f6d-4d78-a498-19b9763d11b4

8–13 September, Maui, Hawaii, United States. **15th International Conference on Laser Ablation.** Vassila Zorba, vzorba@lbl.gov, https://cola2017.sciencesconf.org/resource/page/id/11

15–20 September, Gold Coast, Australia. **NIR-2019.** nir2019@yrd.com.au, http://www.nir2019.com/

23–26 September, Freiberg, Germany. **Colloquium Analytical Atomic Spectroscopy 2019: CANAS 2019.** canas@chemie.tu-freiberg.de, http://www.canas.eu

24–26 September, Amsterdam, Netherlands. **10th Workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing-WHISPERS.** http://www.ieee-whispers.com

13–18 October, Palm Springs, United States. **2019 SciX Conference (formerly FACSS): Annual National Meeting of Society for Applied Spectroscopy (SAS)/The 46th Annual North American Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies..** scix@scixconference.org, http://www.scixconference.org

5–8 November, Prague, Czech Republic. **9th International Symposium on Recent Advances in Food Analysis (RAFA 2019).** jana.hajslova@vscht.cz, http://www.rafa2019.eu/pdf/rafa%202019_1st_flyer.pdf

Courses 2019

19–22 February, Modena, Italy. **2019 Chemometrics Tools for Process Monitoring School.** Dr. Marina Cocchi, marina.cocchi@unimore.it, http://155.185.32.100/MCMP/SMCMP_home.html

19–21 February, Berlin, Germany. **11th Annual Short Course on Time-Resolved Microscopy and Correlation Spectroscopy.** https://www.picoquant.com/events/workshops-and-courses

11–15 March, Gembloux, Belgium. **Training on Vibrational Spectroscopy and Chemometrics.** Juan Antonio Fernández, j.fernandez@cra.wallonie.be, http://www.cra.wallonie.be/en/annual-spectroscopy-and-chemometrics-training

29 April, Chester, United Kingdom. **APACT19 Pre-Conference Courses.** https://apact.co.uk/pre-conference-courses

Exhibitions 2019

12–14 March, Dubai, United Arab Emirates. **ARABLAB 2019.** info@arablab.com, https://www.arablab.com/

17–21 March, Philadelphia, United States. **Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy—Pittcon 2019.** pittconinfo@pittcon.org, https://pittcon.org/

9–11 July, Johannesburg, South Africa. **Analytica Lab Africa.** Barbara Kals, barbara.kals@messe-muenchen.de, https://www.analytica-africa.com/

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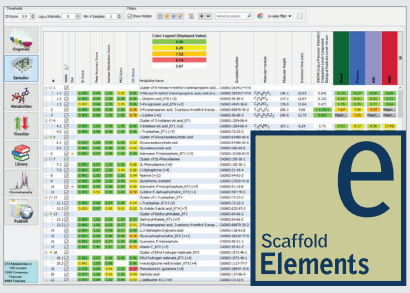

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