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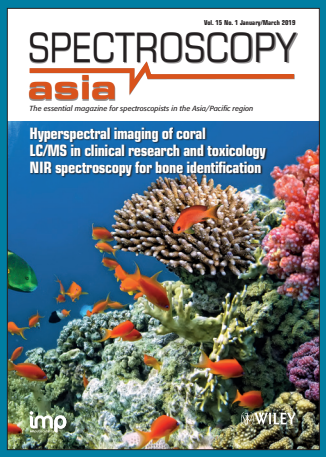

Coral reefs are suffering from the effects of climate change. A hyperspectral imaging system fitted to a submersible remotely operated vehicle has been developed and can be used to assess coral health based on induced fluorescence. Find out in the article starting on page 7.

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EDITORIAL

Coral reefs have been used as examples of climate change for decades, since the "bleaching" caused by corals stressed by heat expelling their symbiotic photosynthesising algae is such a dramatic effect. In our first article, Jonathan Teague, Jack Willans, Michael Allen, Thomas Scott and John Day describe their work in developing a hyperspectral imaging system that can be deployed on a submersible remotely operated vehicle to monitor coral health through changes in their natural fluorescence.

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 on "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, Hafiz Azeem and Tony ask whether we can smell smoke? The world is moving away from reliance on fossil fuels and towards the use of biomass, but there is a hidden danger of fires developing from bulk stores of material such as straw waste. Interestingly, smoke from burning or smouldering plant-based material has a specific signature from a marker of cellulose combustion. Hafiz has developed a technique based on thermal desorption with gas chromatography-mass spectrometry to detect very low levels of the marker, well before the smoke would set off a normal smoke detector. This can also be used to detect the burning of rainforests.

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 laboratory was representative.

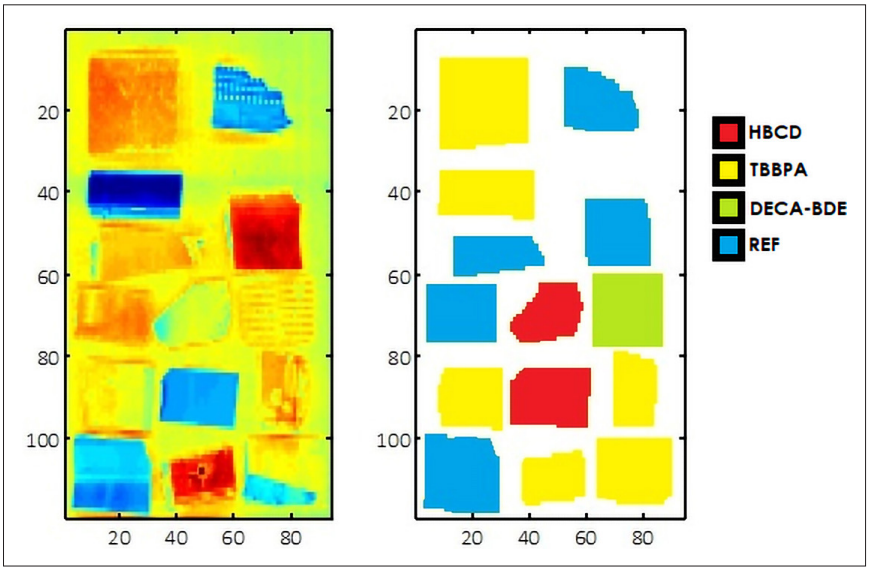
Improved plastics recycling with NIR hyperspectral imaging

If we are ever to reduce the quantity of plastics being dumped into the environment, efficient recycling is essential. Currently, many plastics cannot be economically sorted to enable their recycling. For example, many plastics contain flame retardants to increase their resistance to ignition, reduce flames spreading, minimise smoke formation and to prevent the plastic from dripping. The amount of flame retardant added to plastics and the type used can vary considerably, due to the need to tailor the plastic to its particular application and to meet safety standards. However, only plastics of the same type and with similar flame retardants can be recycled together. Therefore, before recycling can occur, plastics need to be sorted, not only by the type of plastic (acrylonitrile butadiene styrene and polystyrene in this study) but also by any flame retardant added. Without sorting, recycling cannot take place.

In a paper published in *JSI—Journal of Spectral Imaging* (doi: https://doi.org/10.1255/jsi.2019.a1), José Amigo and co-authors detail a method using near infrared hyperspectral imaging and chemometrics that can sort between different types of plastic and between different additions of flame retardant. Using an imaging technique for this recycling application is important, since it can identify individual pieces of plastic and any flame retardant they may contain from among many others, for example, on a conveyor belt in a recycling plant.

For optimum performance, any sorting technique needs to be fast and able to identify accurately the wide range of combinations of plastic and flame retardants it may encounter. The system reported in the *JSI—Journal of Spectral Imaging* paper uses the Decision Trees chemometrics technique combined with spectral data obtained from near infrared hyperspectral imaging and is able to distinguish between different plastics and flame retardants within them with 100 % accuracy.

José Amigo commented "Recycling plastics has been studied for many years.



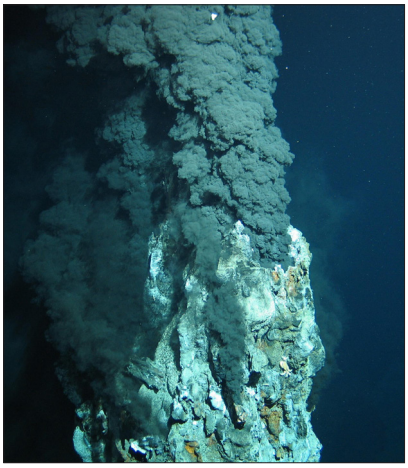
Near infrared hyperspectral imaging of samples of plastics and their classification as a function of type of flame-retardant: 1,2,5,6,9,10-hexabromo-cyclododecane, HBCD (red samples); 3,5-tetrabromobisphenol A, TBBPA (yellow samples); Pentabromophenyl ether, Deca-BDE (green samples); and reference, REF (blue samples).

Indeed, some commercial cameras separating a limited number of plastic types have been available for some time. However, in this research, we wanted to go a step further to separate plastics containing flame retardants. Moreover, the proposed methodology was tested with real samples that can be found in current recycling lines."

LIBS measurement on the deep-sea floor

For the first time, scientists at the Laser Zentrum Hannover e.V. (LZH) have succeeded in measuring zinc samples at a pressure of 600 bar using laser-induced breakdown spectroscopy (LIBS). They were able to show that the LIBS system developed at the LZH is suitable for use in the deep sea at water depths of up to 6000 m.

Locating mineral resources on the sea floor has so far been rather expensive. In order to reduce the costs, the LZH is working with eight other European partners to develop a laser-based, autonomous measuring system for underwater use by 2020. The system is intended to detect samples, such as manganese nodules, and analyse their material composition directly on the deep-sea floor.



The measurement of elements with LIBS should help to locate natural resources in a non-destructive way in the future. Photo: GEOMAR (CC BY 4.0).

For this purpose, the scientists at the LZH are developing a system for LIBS within the scope of the EU ROBUST project. In order to test the LIBS system developed by LZH under deep-sea conditions, a special pressure chamber was designed and manufactured. This can simulate a water depth of 6500 m with a pressure of up to 650 bar. The chamber is suitable for both freshwater and saltwater. Through a viewing window, the laser radiation enters the

pressure chamber with the test sample to be analysed.

New EPR method for analysing metalloproteins

A new electron paramagnetic resonance (EPR) method only needs a very small liquid sample to analyse metalloproteins. This was developed by a research team led by Associate Professor Eiji Ohmichi and Tsubasa Okamoto at the Kobe University Graduate School of Science. The findings were published in *Applied Physics Letters* (doi: https://doi.org/10.1063/1.5055743).

Metalloproteins play vital roles in our bodies for oxygen transport and storage, electron transport, oxidation and reduction. In many cases, the metal ions in these proteins are the active centres for these activities, so by identifying the exact state of these ions, we can understand the mechanisms behind their functions. EPR can be used to measure the state of electron ions in proteins. Effective EPR techniques require a certain amount of specimen volume for sensitive measurements. However, many metalloproteins are difficult to isolate and refine, so we can only obtain small samples.

Conventional EPR measurements detect the electromagnetic waves absorbed by metal ions. The notable feature of this study is the use of a trampoline-shaped device called a nanomembrane. In EPR the electron spin transitions to a high-energy state by absorbing electromagnetic waves, but at the same time the spin direction reverses, and the magnetic properties of the metal ions also change. Before the experiment the research team attached tiny magnets to the nanomembrane, so the changes in the force of attraction between the magnets and the metal ions are transformed into a force on the nanomembrane, and this EPR signal is detected. Since the nanomembrane is very thin (just 100 nm) we can sensitively measure small changes in force that accompany EPR absorption.

The solution specimen is placed in a solution cell directly above the membrane. The cell volume is 50 µL, and the team adds about 1–10 µL of solution for measurement. In order to prevent the solution from evaporating, the cell is covered with a resin lid. In this method the thin and fragile nanomembrane is independent from the solution cell, making it easy to switch specimens.

In order to evaluate the performance of this setup, the team carried out EPR measurement over a high-frequency (over 0.1 THz) for the iron-containing protein myoglobin and its model complex haemin chloride (see Figure). The team succeeded in detecting EPR signals across a wide wave frequency (0.1–0.35 THz) for a 50 mM concentration, 2 µL haemin chloride solution. They also observed a characteristic EPR signal for an 8.8 mM, 10 µL specimen of myoglobin solution. A great advantage of this method is the ability to measure across a wide frequency range, making it applicable for metalloproteins with a variety of magnetic properties.

Professor Ohmichi commented: "This new method makes it possible to determine to a detailed level the state of the metal ions in a tiny amount of metalloprotein solution. We may be able to apply the method to metalloproteins that previously could not be measured. For example, in our metabolisms, a metalloprotein called peroxidase plays a crucial role by converting hydrogen peroxide into water, making it harmless, but the details of the mechanism for this reactive process are still unclear. The results from this study can potentially be applied as a leading analysis method to shed light on this sort of vital phenomenon."

Mass spec of blister fluid diagnoses burn severity

Diagnosing burn depth, which can continue to increase even hours after the injury initially occurs, takes up to two weeks and often depends on the doctor's experience. Deep burns and those requiring longer than 21 days for healing typically require skin grafts. If doctors could accurately estimate burn depth and time for re-epithelialisation at an earlier stage, they might be able to reduce scarring. This is especially important for paediatric burn patients, because excessive scar tissue cannot expand with the growing child and could hamper joint movements and bone development.

As reported in the *Journal of Proteome Research* (doi: https://doi.org/10.1021/acs.jproteome.8b00355), mass spectrometry was used to analyse the proteomes of 56 samples of blister fluid from burns of different depths and re-epithelialisation times. The researchers found that the deepest burns had a different pattern of protein abundance than shallower ones. For example, haemoglobin protein levels increased with burn depth, which could result from enhanced blood cell damage. Fluid from burns that took longer than 21 days to re-epithelialise had more collagen proteins, which are involved in scar formation, than faster-healing burns. The team found that taking into account the abundance of several proteins was more accurate in predicting burn depth and time to re-epithelialisation than any protein alone. The analysis also revealed several burns that appear to have been misclassified by doctors, suggesting that the new approach could more accurately diagnose burns at an earlier stage.

Pulsed DNP greatly increases NMR sensitivity

MIT researchers have developed a way to dramatically enhance the sensitivity of nuclear magnetic resonance spectroscopy (NMR). Using this new method, scientists should be able to analyse in mere minutes structures that would previously have taken years to decipher, says Robert Griffin, the Arthur Amos Noyes Professor of Chemistry. The new approach, which relies on short pulses of microwave power, could allow researchers to determine structures for many complex proteins that have been difficult to study until now.

"This technique should open extensive new areas of chemical, biological, materials and medical science which are presently inaccessible", says Griffin, the senior author of the study. MIT postdoc Kong Ooi Tan is the lead author of the paper, which was published in *Science Advances* (doi: https://doi.org/10.1126/sciadv.aav6909). Former MIT postdocs Chen Yang and Guinevere Mathies,

and Ralph Weber of Bruker BioSpin Corporation are also authors of the paper.

The sensitivity of NMR depends on the atoms' polarisation. The greater the polarisation, the greater sensitivity that can be achieved. Typically, researchers try to increase the polarisation of their samples by applying a stronger magnetic field, up to 35Tesla. Another approach, which Griffin and Richard Temkin of MIT's Plasma Science and Fusion Center have been developing over the past 25 years, further enhances the polarisation using dynamic nuclear polarisation (DNP). This technique involves transferring polarisation from the unpaired electrons of free radicals to hydrogen, carbon, nitrogen or phosphorus nuclei in the sample being studied.

DNP is usually performed by continuously irradiating the sample with high-frequency microwaves, using a gyrotron. This improves NMR sensitivity by about 100-fold. However, this method requires a great deal of power and does not work well at higher magnetic fields that could offer even greater resolution improvements. To overcome that problem, the MIT team came up with a way to deliver short pulses of microwave radiation, instead of continuous microwave exposure. By delivering these pulses at a specific frequency, they were able to enhance polarisation by a factor of up to 200. This is similar to the improvement achieved with traditional DNP, but it requires only 7% of the power, and unlike traditional DNP, it can be implemented at higher magnetic fields.

"We can transfer the polarisation in a very efficient way, through efficient use of microwave irradiation", Tan says. "With continuous-wave irradiation, you just blast microwave power, and you have no control over phases or pulse length."

With this improvement in sensitivity, samples that would previously have taken nearly 110 years to analyse could be studied in a single day, the researchers say. One major area of interest is the amyloid beta protein that accumulates in the brains of Alzheimer's patients. The researchers also plan to study a variety of membrane-bound proteins, such as ion channels and rhodopsins. Because the sensitivity is so great, this method can yield useful data from a much smaller sample size, which could make it easier to study proteins that are difficult to obtain in large quantities.

NIR pocket-size food scanner

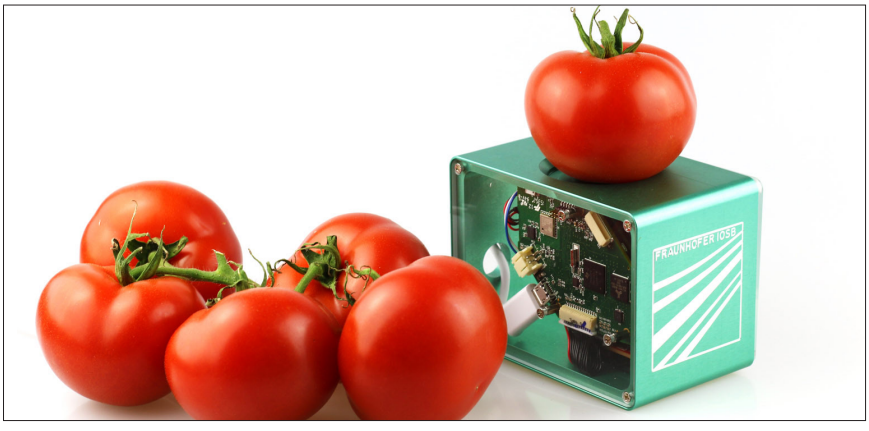
Researchers at the Fraunhofer Institute for Optronics, System Technologies and Image Exploitation IOSB, the Fraunhofer Institute for Process Engineering and Packaging IVV, the Deggendorf Institute of Technology and the Weihenstephan-Triesdorf University of Applied Sciences are developing a compact food scanner based on near infrared (NIR) spectroscopy to determine the ripeness and shelf life of produce.

"Foodstuffs are often counterfeited—for example, salmon trout is sold as salmon. Once suitably trained, our device can determine the authenticity of a product. It can also identify whether products such as olive oil have been adulterated", says Dr Robin Gruna, project manager and scientist at Fraunhofer IOSB. However, the system can only evaluate the product quality of homogeneous foods. To enable the analysis of heterogeneous products such as pizza, the scientists are investigating high-spatial-resolution technologies such as hyperspectral imaging and fusion-based approaches using colour images and spectral sensors.

To be able to determine the quality of food based on the sensor data and the measured NIR spectra, and compute shelf-life predictions, the research teams are developing chemometric methods. "Through machine learning, we can increase the recognition potential. In our tests, we studied tomatoes and ground beef", says Gruna. For example, we used statistical techniques to correlate the measured NIR spectra of ground beef with the rate of microbial spoilage and derived the remaining shelf life of the meat from the results. Extensive storage tests, whereby the research teams measured microbiological quality and other chemical parameters under various storage conditions, showed good correlation between the computed and actual total germ counts.

The scanner sends the measured data via Bluetooth to a database in the cloud for analysis. Then the test results are transmitted to an app that displays them to the user and shows how long the food item will remain fresh under different storage conditions, or indicates that its shelf life has already expired. In addition, the consumer is given tips on alternative ways of using food that is past its best-before date. A test phase is due to begin in supermarkets early in 2019, which will investigate how consumers respond to the device. More broadly, it is expected that the versatile technology will be used throughout the value chain, from raw material to end products.

The scanner has the potential to be used for many other applications where NIR spectroscopy can be applied. For example, it could be used to sort, separate and classify plastics, wood, textiles and minerals. "The range of potential applications is very wide; the device just needs to be trained accordingly," says Gruna.



ARTICLE

Applied marine hyperspectral imaging; coral bleaching from a spectral viewpoint

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With climate change and other stressors impacting our seas and oceans, coral reef degradation has been brought to the forefront of the climate change issue. Coral reefs are critically important for the ecosystem goods and services they provide. The health of these systems represents a significant concern for the 275 million people who live in coastal tropical and subtropical nations (within 30 km of corals where livelihoods and food security is largely dependent on corals.[1,2] Coral reefs can be incredibly valuable, for example Cruz-Trinidad *et al.*[3] calculated one small reef (10 × 20 km) in the Philippines had an estimated value of $38 million USD/year. This example fiscal valuation only scratches the surface of any reef's true value as an irreplaceable source of food and natural sea defence to developing countries.[4]

Corals are very susceptible to disease and conditions synonymous with climate change, as they have low tolerance to stresses such as varying water temperature, salinity and increased solar irradiation. Coral disease is one of the largest causes of reef degradation and coral death. Its incidence has been increasing worldwide since first observed in the 1970s, particularly in the Caribbean, Red Sea and Indian Ocean, linked in part to declining water quality, declining fish stocks, heat stress and, more recently, to ocean acidification driven by anthropogenic activity.[5–7] With reports of coral disease incidences increasing from 20% to 80% in a three-year period,[7] closer monitoring and protection of this valuable natural asset is essential.

The causes of coral bleaching are widely accepted as a general response to external factors or triggers (stressors) such as elevated water temperature, usually accompanied by increased solar irradiation, ocean acidification or bacterial infection.[8] With these stressors becoming ever more present, mass bleaching events are becoming more frequent, with the first described in 1984 by Glynn and since then a further three global bleaching events have been described in 1998, 2010 and 2015/2016.[9] The most recent mass bleaching event (2015–16) affected 75% of the globally distributed locations surveyed by Hughes *et al.*[10] and is therefore comparable in scale to the then-unprecedented 1997–1998 event, when 74% of the same 100 locations experienced bleaching. With global climate-driven bleaching events coinciding with the El Niño–Southern Oscillation (ENSO) phases, average tropical sea surface temperatures are warmer today under La Niña conditions than they were during El Niño events only three decades ago.[11] This means that predicted climate change and ocean warming scenarios will herald an increase in the frequency of extreme heating events on coral reefs.

Corals primarily responsible for building modern reefs are hermatypic corals, belonging to the group Scleractinia or Stoney corals. Hermatypic corals contain photosynthetic algae specifically dinoflagellates called zooxanthellae, belonging to the genus *Symbiodinium*, that live symbiotically within its cells. The relationship is mutually beneficial, the algae provides the corals with energy from photosynthesis and in exchange receives protection and nutrients needed to conduct photosynthesis (Figure 1). Ahermatypic corals do not possess this symbiosis but instead use other means to survive.[12]

Hermatypic coral communities exhibit a natural fluorescence both from the coral itself and its symbiotic partner. The significance of this is not yet known, several studies have been conducted to try and derive meaning from this and have suggested many possibilities for the role that fluorescent proteins (FPs) play: including (i) acting as a sunscreen by providing a photobiological system for regulating the light environment;[13] (ii) as a host stress response, through their action as antioxidants; or (iii) to attract prey.[14] All that is known is that downregulation of FPs frequently occurs in injured or compromised coral tissue.[15]

The presence of FPs and their behaviour as a response to stress can potentially be exploited as a method for measuring coral health. Analysis of the natural variability in fluorescence intensity for a given species, as well as the differences between diseased and healthy specimens, enables the development of an index relating fluorescence to disease.[16]

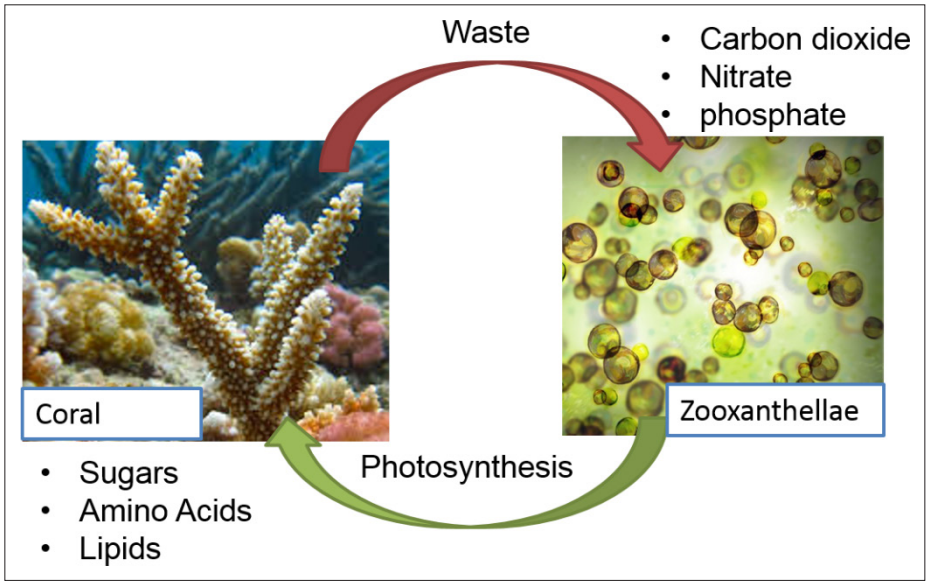

Figure 1. The symbiotic relationship between hermatypic corals and *Symbiodinium*.

Proof of concept and hyperspectral imaging

In order to characterise the subtleties of this natural fluorescence, coral samples at the London Aquarium, provided by coral aquarist Jack Willans, were imaged using a push-broom type hyperspectral camera (Headwall Nano, USA).

This hyperspectral camera captures light across the entire visible (vis) spectrum and some of the near infrared (NIR) (400–1000 nm) in each pixel, but separates the incoming photon (light) flux based on a wavelength, separating the incident light intensity into a large number (hundreds) of spectral bands whilst illuminated by a light source [fluorescence in coral requires ultraviolet (UV) or blue light (400–480 nm)]. This allows the characteristic excitation/emission peaks of coral fluorescence to be determined. Every pixel once processed in the image contains full spectral data relating to that pixel, allowing for the peak wavelength of any observed fluorescence detailed to be determined. The data was processed within the camera to produce a hypercube which could then be viewed in ENVI (Version 5.3.1) (post processing software). Spectra were extracted from various points on each coral sample to determine its emission spectrum.

As Figure 2 shows, the spectra recorded from the corals extracted from the hypercube image indicated that the corals sampled mostly fluoresced in the cyan or green wavelengths (485–512 nm). Samples were selected to represent several different types of corals (branching, plate, soft), including corals known for exhibiting fluorescence and those that were not.

The primary observed fluorescence peaks were used to correlate coral species with emitted colour. Corals exhibiting the colour "cyan" (as classed by Alieva *et al.*)[17] ranged from 459 nm to 516 nm, but with peak fluorescence intensity mainly around 480 nm. Some of the corals did not produce any fluorescence, such as C5, C7, C12, C13, C20 and C22. These made for comparison against actively fluorescing coral spectra. Some of the coral samples exhibited other weaker emission peaks, displaying the presence of dual colour fluorescence, such as C2, which displayed a main peak at 445 nm (cyan) and weaker one at 510 nm (green). Many of the corals exhibited wide emission peaks indicating that multiple colours were emitted by their fluorescence. Many of the spectra show another peak (after 643 nm) which is ascribed to the fluorescence of the coral's symbiotic partner zooxanthellae.

Bleaching experiment

Having gained proof that hyperspectral imaging had the potential to observe and quantify coral fluorescence, we looked at applying this technique for the detection of bleaching. Coral bleaching refers to the process by which the dysbiosis (breakdown of the symbiotic relationship) of zooxanthellae and the host coral occurs. The process of this dysbiosis is usually the expulsion of the zooxanthellae cells from inside the corals cells where they are typically stored. Depending on the stressor, the rate of bleaching varies but is usually a quick process (can be a matter of hours) in individual colonies and can lead to coral cell death or necrosis as a means of extraditing its once partner.

In order to observe and record the bleaching phenomenon, a laboratory experiment was devised where by coral samples (*Montipora digitata*) provided by London Aquarium, were placed into an experimental tank containing artificial sea water at a starting temperature of around 24 °C. The temperature was then incrementally increased by +2 °C each week for four weeks until the temperature reached >30 °C (this is the point at which bleaching occurs by temperature). The coral samples were imaged using the hyperspectral camera

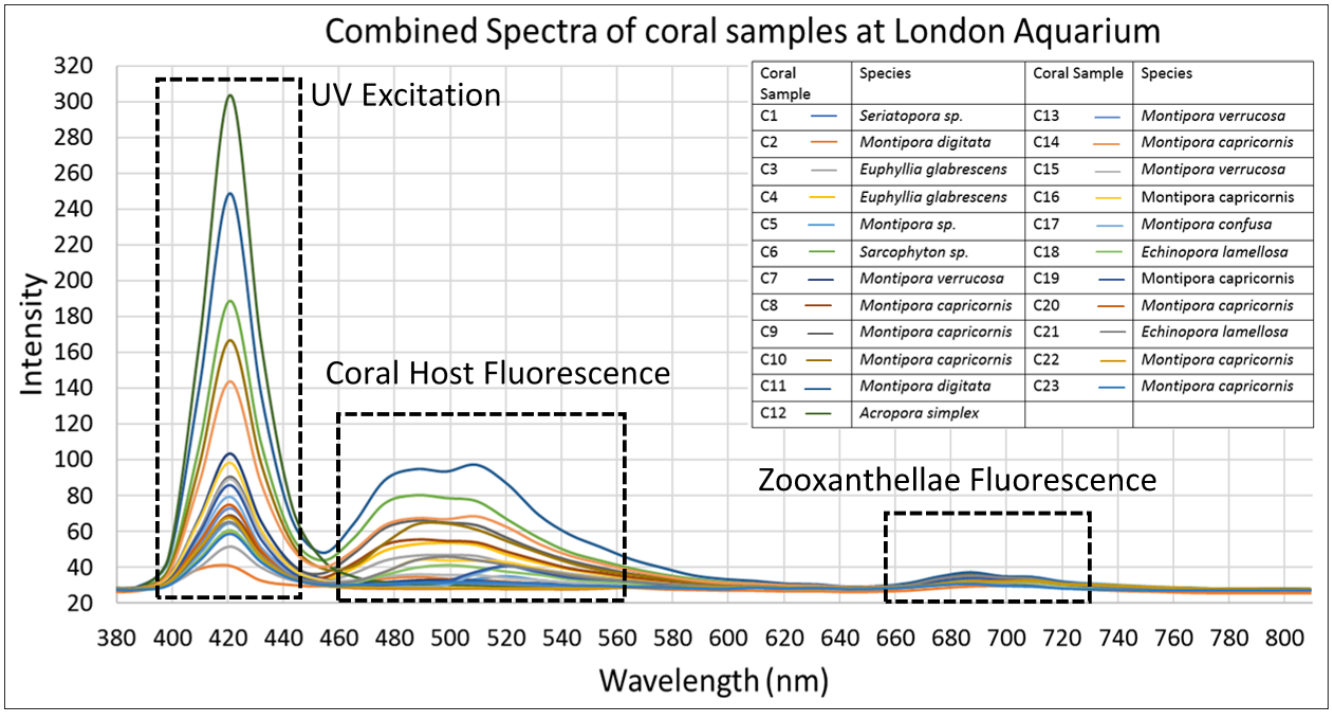

Figure 2. A combined plot of spectra generated from hyperdata generated from areas of interest highlighting all fluorescence visible. Areas of UV excitation and coral and zooxanthellae fluorescence are highlighted.

system at regular intervals throughout the course of the experiment. This purpose was to document the bleaching effect of expulsion of zooxanthellae by monitoring the fluorescence signal which was expected to decrease as they were expelled. The overall health of the coral also degrades due to the bleaching effect and this can also be monitored using the same technique, but looking at the fluorescence peak of the FPs present as opposed to the chlorophyll cells in the zooxanthellae. The collected hyperspectral data from the Headwall nano camera could then be compiled into a hypercube and loaded in to ENVI.

The coral samples for the bleaching experiment were imaged in three light conditions; under white light (Aquarium light bar), blue light (Aquarium light bar 440 nm) and UV light (BlueRobotics UV light pod 405 nm). Under white light illumination, the corals' colour can be seen to decrease in intensity as bleaching progressed, which provides a baseline akin to that of a traditional coral health survey looking at light reflectance. The colour draining of the coral is a process that can be qualitively observed by eye but using hyperspectral methods we can quantify this loss. The blue light illumination elicited the highest intensity of cyan and green fluorescence but masked some of the coral fluorescence signal in the tail of the excitation peak. The UV lights provided a sharper emission peak over the blue light enabling the whole unmasked fluorescence peak to be seen. The experiment allowed us to observe the bleaching process from a quantifiable spectral data perspective as shown in Figure 3.

Conclusion and future work

This study was able to detect fluorescence in corals of numerous different species and identify the spectral intricacies as well as observing coral bleaching from a spectral perspective. The results of this initial research provide

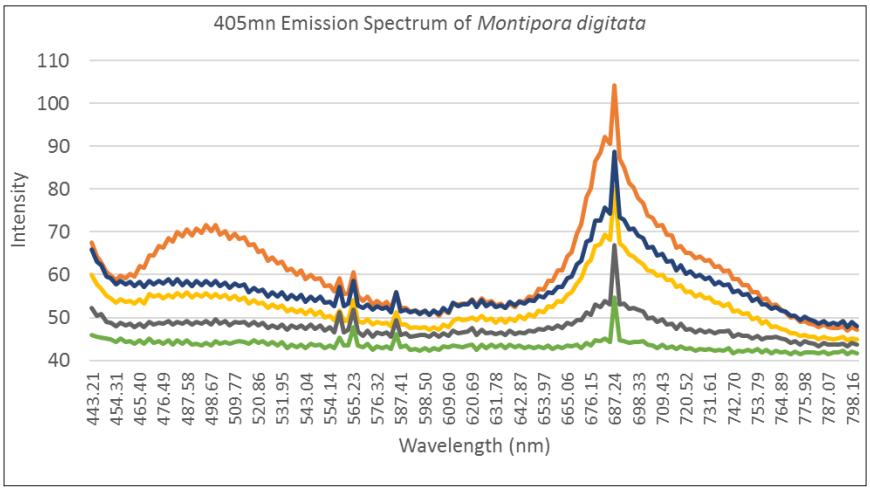

Figure 3. A spectral observation of the "bleaching effect", as the temperature increases observed fluorescence in both the host tissue and zooxanthellae decreases.

compelling evidence to prove that fluorescence may be used to indicate coral reef health. The next stage is developing a field deployable system, sufficiently cheap and semi-automated such that reef systems can be automatically and rapidly assessed on a repeated basis.

The field deployable system will be in the form of a portable fully waterproof low-cost hyperspectral imaging system (payload) that can be mounted to an ROV (remotely operated vehicle) (BlueROV2, BlueRobotics) which will act as a stable platform for obtaining images, similar to that provided by drone (UAV) systems in agricultural applications.

The system is required to be "low-cost" (<£10k) to reduce the high associated costs of commercially available hyperspectral systems and reduce the capital risk of operating in the underwater environment. In order to do this, the monochrome camera (AtikHorizon, Horizon), which ultilises a continuously variable filter, is mounted directly, using a custom 3D printed bracket, on the 16MPixel CMOS sensor. The filter (LV VIS NIR bandpass filter from Delta Optical Thin Films) has a continuous relationship between the spectral characteristics and the position. The filter has a centre wavelength range between 450nm and 850nm. Using a continuously variable filter allows for a standard camera to be modified into a "push broom" hyperspectral camera at very little cost.

The system is then to be deployed over a live reef to gather *in situ* spectral data in order to assess coral health based on the induced fluorescence (Figure 4). Data will be collected in a similar way to existing drone (UAV) technologies with raster pattern flight paths in order to fully cover the whole reef with enough overlap between images to achieve reliable stitching and production of large area maps, and photogrammetry reconstruction of reef topography to produce three-dimensional models of reefs. This will enable the creation of rapid coral health assessments and a spectral library of coral species known fluorescence emission and fluorescence signatures at various stages of bleaching.

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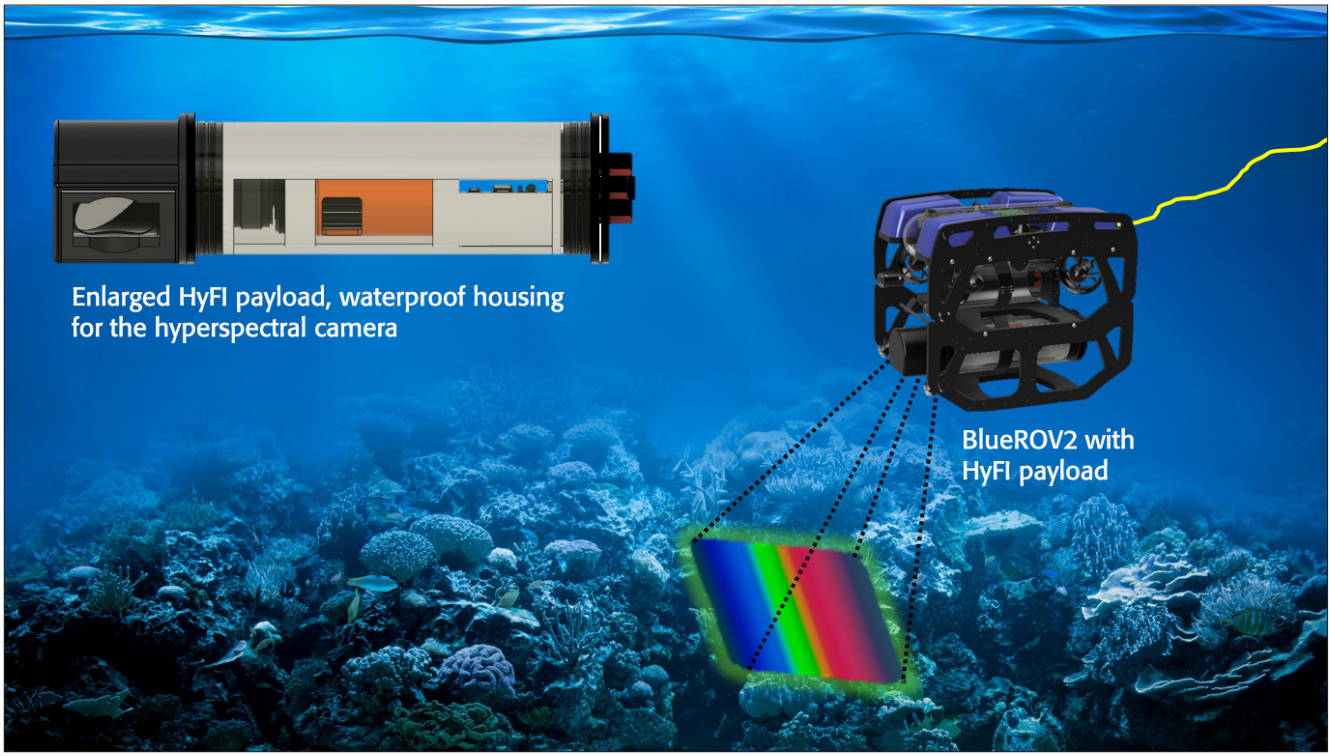

Figure 4. The hyperspectral fluorescence imaging (HyFI) payload, mounted on BlueROV2. Imaging around 1m above the reef will provide an impact free rapid health assessment. The camera is housed in a custom waterproof housing with a 90° mirrored lens to allow for perpendicular imaging of the reef.

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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 mgmL^{-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 shake" protocol: the analyte is partitioned

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

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

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easiest techniques to automate for most laboratories.

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

ARTICLE

Near infrared spectroscopy, the skeleton key for bone identification

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Introduction

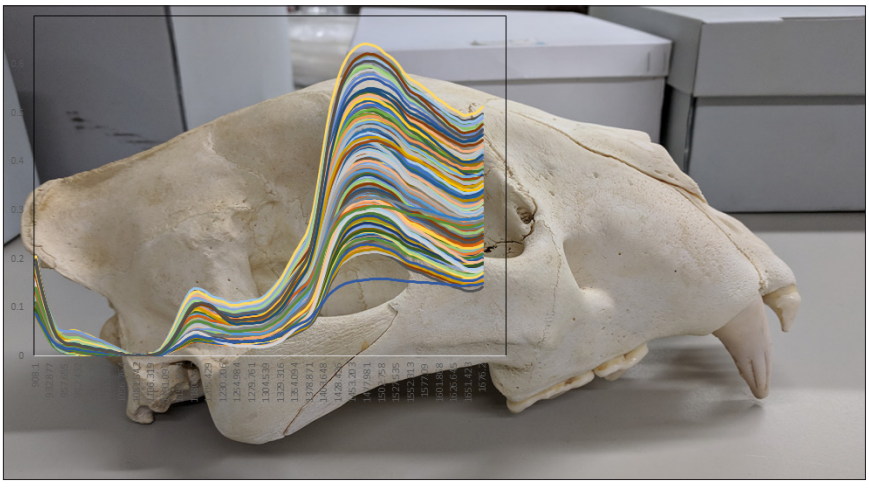
New developments in the field of optics and electronics have resulted in an increasing number of new applications of spectroscopic analysis, which is further augmented by the introduction of portable and compact spectrophotometers.[1–3] This miniaturised instrumentation allows analysts to explore unique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



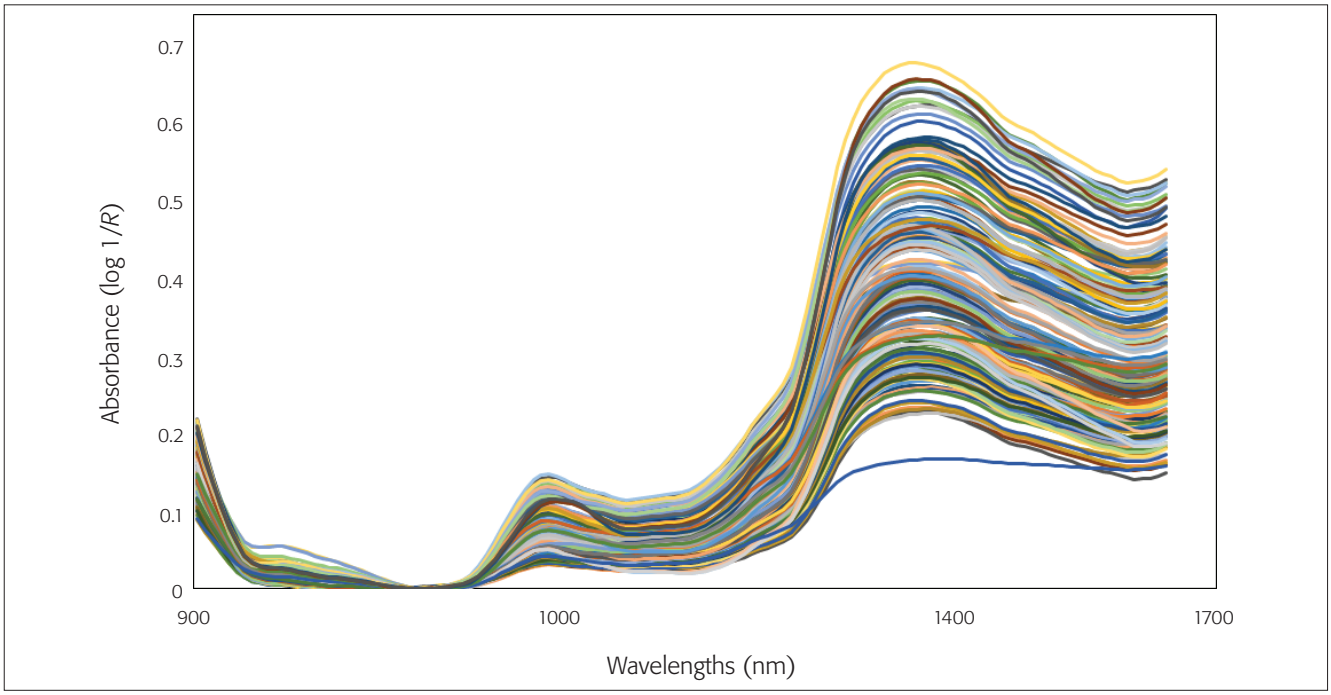


Figure 1. Near infrared spectra of skull samples.

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]

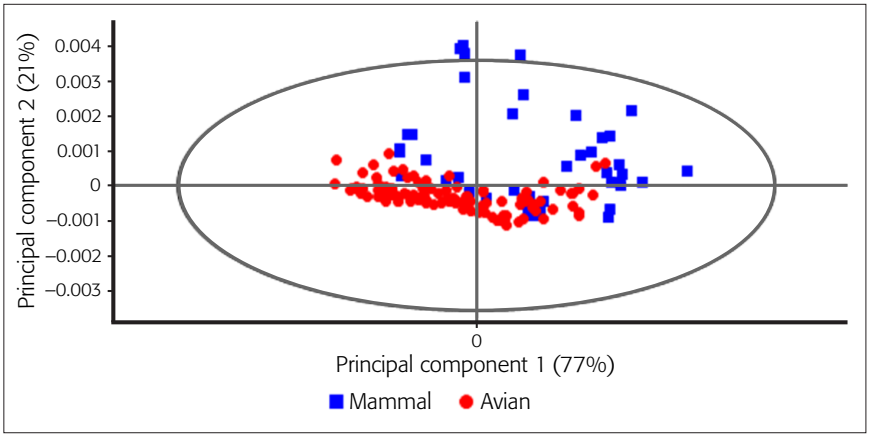


Figure 2. Principal score plot for the classification of avian and mammalian skull bones samples based on NIR reflectance spectra.

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

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TONY DAVIES COLUMN

Can you smell smoke?

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Hafiz Abdul Azeem recently presented some interesting results from his work on atmospheric aerosols. Following their capture, he combined the optimisation of the extraction process with chromatographic separation and mass spectroscopic detection to identify various sources of pollution through their emission marker fingerprints.[1] One spin-off of this work has been the use of a specific biomarker from cellulose combustion to potentially warn of low-heat smouldering in, for example, agricultural materials in bulk storage.

Aerosols don't just come out of cans!

Atmospheric aerosols are everywhere. They are a mixture of very small particles and liquid droplets whose size means that they can be transported in the atmosphere for huge distances, changing their chemical composition as they are exposed, for example, to UV radiation and ozone during their lifetimes. You have been exposed to aerosols your whole life, and their origin (human-made or natural) affects how they interact with your body. Which molecules are observed making up such aerosols depends very much on the original source. Aerosols formed from the burning of agricultural biomass waste (or illegal slash and burn operations in rainforests!), for example, are different to those produced from motor vehicles, commercial shipping or oil-fired heating systems in buildings.

As humans, we need to be interested and aware of their presence and activity in our environment as there can be potential serious health issues. Their very small size means that they can easily gain access to our lungs. But, as a parent of twins who both had breathing difficulties at their early years, I know aerosols can be used very beneficially as a simple way to conduct very small amounts of medicines deep into the lungs of babies and small children, depositing these medicines at exactly the right locations to be most effective in re-opening the airways.[2–6]

Size is everything!

Not all aerosols make it into the lower reaches of the lungs. Our whole airway from the nose all the way down to the tiny alveolar spaces is littered with our defence mechanisms against contaminated air. These serve to clean out the contaminants in what we have breathed in before this air reaches the innermost parts of our lungs. In healthy humans, who have not destroyed these defences by, for example, smoking or working in contaminated environments for long periods, there are several different defence mechanisms targeting different sizes of contaminants. Starting with your nasal hair, and progressing to the upper respiratory tracts, the larger aerosol particles, 10 µm or larger, tend to be stopped by wall collisions. Their larger mass and fast airflow speeds mean that they don't follow the curves and bends of your airways. The heavier particles that make it past these obstacles can then be lost as gravity takes control where the airflow slows down your airway. Your defence mechanisms include traps such as small hair-like cilia, mucus and your cough reflex. Small, light aerosols can make it past your body's defences. However, if they are too small (0.5–5 µm), Brownian motion in the low-air flow in the lower regions of the lungs may mean that they stay in the airway not colliding with the walls of the lungs, just waiting to be expelled out as you exhale. Between these two extremes, aerosols containing medicines (or unwanted polluting chemicals) can make it past your defences and into the bronchus and eventually the alveoli.

Identifying aerosol origins

The ability to analyse the chemicals present in aerosols not only can deliver important information on the potential toxicity of what we are breathing, but also the distribution of these chemicals can provide important information on the origins of the aerosols.[1]

For example, phenolic compounds and sugars indicate aerosols formed from biomass burning whereas carboxylic acids and polyaromatic hydrocarbons, depending on the species identified, indicate human activity, be it motor vehicle emissions or fossil fuel use such as coal burning.

Hafiz investigated the best methods of sample preparation such as supercritical fluid extraction, liquid–liquid microextraction or hollow-fibre liquid-phase microextraction and concentrated on liquid chromatography, supercritical fluid chromatography or gas chromatography/mass spectrometry analyses (with derivatisation where necessary) for data collection and anal-

ysis leading to the identification of the individual marker substances in the aerosols. As emission marker fingerprints can be highly diverse in their chemical nature, unfortunately there is no all-in-one solution available that can be used for the detection of all of them at the same time. It requires hard-core analytical chemistry skills and access to a number of instrumental techniques for comprehensive study of various emission marker fingerprints.

It is, however, possible to suggest faster, cheaper and greener methods of analysis for certain emission markers of interest, based on their chemical nature. For example, thermal desorption hyphenated to gas chromatography/mass spectrometry can be used to study certain sugars. Handheld portable samplers are available in the market to capture aerosols on desorption tubes. These tubes can then be placed in a thermal desorption unit and analysed by online derivatisation followed by online gas chromatography/mass spectrometry analysis (Figure 1).

And this leads us back to a sugar called levoglucosan. This tracer compound has been seen as an ideal marker substance for biomass burning as the pyrolysate contains significant quantities of this sugar.

So here is the idea... the more we, as a society, are forced to move away from oil-based fuel sources the more we need to retain and store alternative sustainable biomass such a straw waste. The more we store for longer periods the greater the risk from catastrophic fires caused by undetected slow-temperature smouldering conditions in the stored material. These low-temperature, smouldering fires can occur spontaneously and can rapidly escalate to huge problems when the material is moved and exposed to fresh unlimited oxygen supplies during handling.

A challenge

So here is a challenge… as we clearly cannot deploy tens of thousands of hyphenated sample analysis gas chromatography high-vacuum mass-spectrometer laboratory analytical systems of the scale used in this method optimisation study, we need dedicated chemical-specific sensors to monitor environments such as those described above at a very low cost to supplement the failing smoke detectors. Because we need to quite urgently "smell" smouldering low-temperature fires rather than wait for the non-existent smoke to reach the smoke detectors, by which time it's probably too late! Madsen and Hafiz reported a proof of concept that aerosol signature, in the form of detection of levoglucosan evolved from smouldering fire, could be used for early detection of smouldering fire.[8] Specialised electronic noses and sensors are required for real-time detection of smouldering.

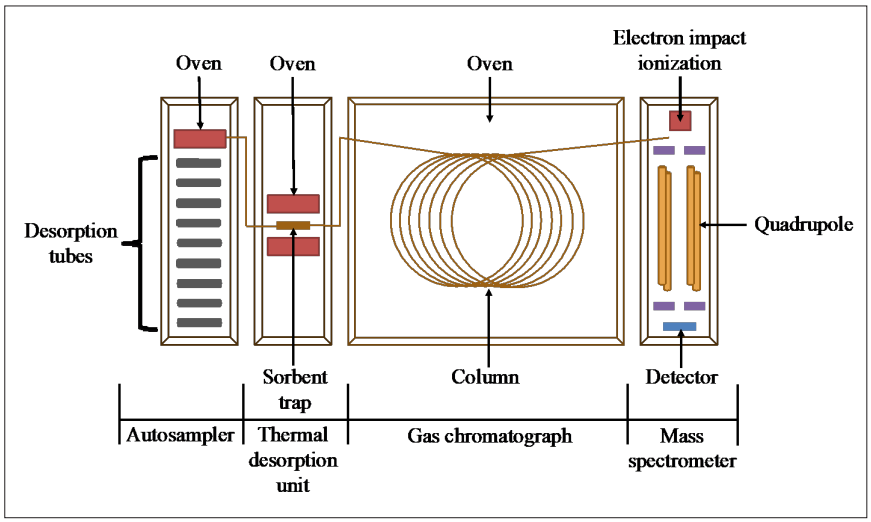

Figure 1. Illustration of various steps of thermal desorption hyphenated to gas chromatography/mass spectrometry, adapted and modified from Materic *et al.*[7]

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SAMPLING COLUMN

A tale of two laboratories II: resolution

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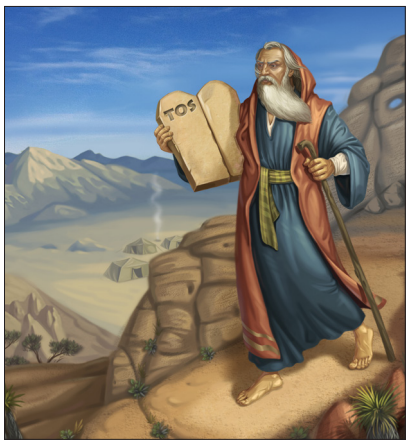
This column completes the tale of two fictional laboratories both facing the issue: "How can the Theory of Sampling (TOS) help the commercial laboratory to improve its reputation and to increase its business"? For decades, Laboratory A has been in fierce market competition with Laboratory B, and indeed 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!

SAMPLING COLUMN

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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ATOMIC

Analytik Jena enhances PlasmaQuant MS series

Analytik Jena has added features and models to its PlasmaQuant MS series. Customers will be able to configure every system in the ICP-MS series to suit their individual requirements. Further, two additional models have been introduced. The PlasmaQuant MS Q is optimised for high-throughput applications, such as quality control for consumer goods, food inspection or environmental monitoring. The PlasmaQuant MS Elite S has been developed for routine analysis of ultratrace components and is very sensitive. It is especially suitable for industries where analysis depends on the lowest detection limits and the highest recovery rates, such as applications in the semiconductor industry, quality control for high-purity chemicals or in geochemistry and geochronology.

Analytik Jena

http://link.spectroscopyasia.com/31-010

Advion introduces the SOLATION ICP-MS analyser

Advion has released the SOLATION inductively coupled plasma mass spectrometer. It has a 90° quadrupole deflector that lowers interference by ensuring that the analyser and detector are not in line with the plasma beam, preventing neutrals and particles from entering the analyser, improving signal-to-noise and preventing contamination. The SOLATION uses triple-cone ion extraction with the sample and skimmer cones available in Ni or Pt. The third extraction cone, followed by an Einzel lens, are electrically controlled to maximise transmission of ions into the vacuum system. Dual function detectors measure in both analogue and pulse detection modes with seamless transmission between the two, to allow measurement of high and low levels in a single analysis with $>10^9$ linear dynamic range. Pulse detection captures ions generating pulses <20 ns, and is accurate and linear to a minimum dwell time of <100 µs. Analogue detection is used for higher ion signals while deactivating pulse detection to extend detector lifetime.

Advion

http://link.spectroscopyasia.com/31-003

ICP spectrometer with new torch configuration

SPECTRO Analytical Instruments has introduced the new SPECTROGREEN inductively coupled plasma optical emission spectrometry (ICP-OES) analyser, which includes a new Dual Side-On Interface (DSOI) technology. DSOI technology uses a vertical plasma torch, observed via a new direct radial-view technology. Two optical interfaces capture emitted light from both sides of the plasma, using only a single extra reflection, for added sensitivity and elimination of issues plaguing newer vertical-torch dual-view models. As a result, DSOI provides twice the sensitivity of conventional radial systems and yet avoids the complexity, drawbacks and cost of vertical dual view models.

The new instrument saves on consumables with a low-purge optic design (a UV-PLUS option offers a no-purge feature) and requires no added cooling. ORCA optical technology maximises light throughput, stability and sensitivity, and a new GigE readout system significantly boosts spectra processing and transport speeds for faster analysis speeds and shorter sample-to-sample times.

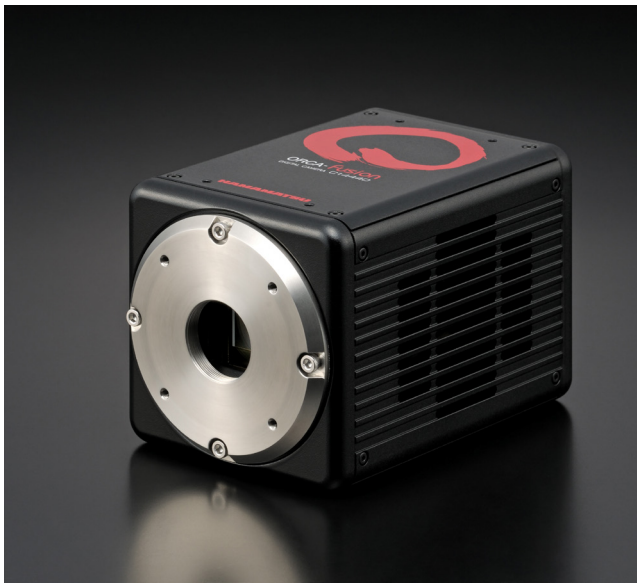
SPECTRO Analytical Instruments

http://link.spectroscopyasia.com/31-011

NEW PRODUCTS

IMAGING

New sCMOS camera



Hamamatsu Photonics have developed a new sensor designed that combines the strengths of EMCCD and sCMOS sensors, the ORCA-Fusion. Recently, sCMOS cameras have developed into the dominant technology for imaging applications where high sensitivity and high speed are required, gradually replacing cameras based on CCD or EMCCD technology. The disadvantage of sCMOS in comparison with previous sensor technologies has always been the uniformity of the sensor in terms of gain, offset and readout noise. A broad distribution of readout noise limits the visual appearance and data quality of a camera, particularly in low-light conditions. Hamamatsu Photonics have used their experience in using offset and gain correction to improve the uniformity of sCMOS cameras and coupled this with a new sensor designed to limit noise distribution. The ORCA-Fusion is thus able to provide images and robust data at all light levels, especially in tough low-light conditions.

Hamamatsu Photonics

http://link.spectroscopyasia.com/31-005

INFRARED

Bruker INVENIO-S research FT-IR spectrometer

Bruker has launched the INVENIO S Fourier transform infrared (FT-IR) research spectrometer that replaces the previous TENSOR spectrometer series. The INVENIO S focuses on productivity in routine and advanced laboratory analysis. Its optional Transit Channel™ allows instantaneous, software-controlled switching between measurement techniques by providing an additional, easily accessible sample compartment. The compact design allows bench space for additional, external accessories, expanding its capabilities to include IR microscopy and imaging, thermogravimetric analysis, high-throughput screening or vibrational circular dichroism. The infield upgradability to INVENIO R provides access to spectral range expansion (from far-IR to visible) and time-resolved spectroscopy (Rapid Scan, Step Scan, Interleaved TRS) when they are needed. An integrated touch panel provides intuitive guidance with typical workflows from routine to advanced applications in R&D. The INVENIO S meets all requirements of Good Laboratory Practice (GLP), and additional validation options are available.

Bruker

http://link.spectroscopyasia.com/30-W-120

High-temperature transmission FT-IR cell

Aabspec has developed the #CXS, a new version of their #CXX transmission FT-IR cell. The #CXS keeps the features of the #CXX including ultra-low internal volume, a flow path ensuring that the reactive gases first strike the catalyst where it is exposed to the optical beam, temperatures to 800 °C etc. External surfaces are water cooled. The mounting system supplied by Aabspec with the #CXS is spectrometer specific. Temperature control is provided by the programmable Aabspec #STP-6 offering multi-ramps, multi program memory etc.

Aabspec

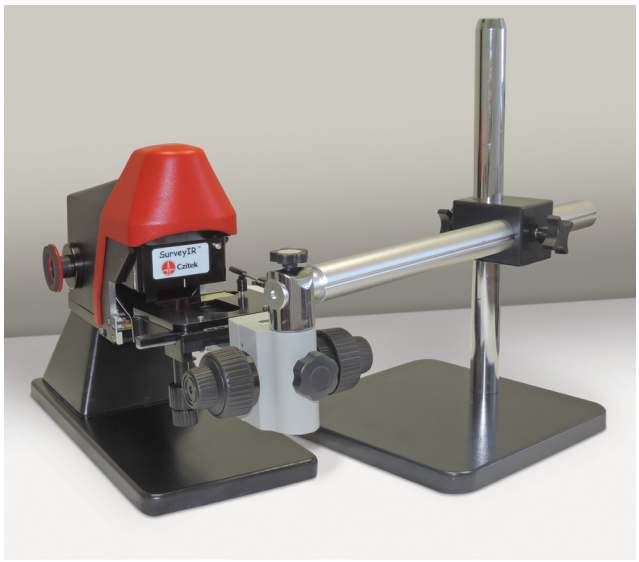
http://link.spectroscopyasia.com/31-007

FT-IR microanalysis accessory for bulky samples

Czitek has introduced a version of their SurveyIR FT-IR microanalysis accessory for use with bulky samples, the SurveyIR$_{VS}$. The design of the SurveyIR$_{VS}$ decouples the stage from the optical microscope, allowing analysis of a large range of sample geometries along with research-grade visual images from a high-resolution colour video camera. A high depth-of-field enables rapid specimen location and manipulation in reflection and ATR viewing modes. There is good viewing quality through the diamond ATR, which simplifies target alignment and helps ensure good sample/ATR coupling including visualisation of the contact. The user can also view the sample whilst collecting data, allowing visualisation and interaction with the sample whilst observing the IR spectrum. eSpot software provides visual image display, manipulation, capture, documentation and storage. It also enables illumination mode selection and remote image mask size chosen by the user from six sample aperture options.

Czitek

http://link.spectroscopyasia.com/30-W-119



MASS SPECTROMETRY

LC-TOF-MS system for analysis of large molecule biologicals

The pharma industry is moving towards large molecule biologicals, which are much more complex and require more sophisticated analytical tools. Waters has introduced the BioAccord™ System that can be operated by almost anyone in the lab. BioAccord is a liquid chromatography-mass spectrometry (LC-MS) instrument that uses the ACQUITY UPLC™ I-Class Plus with the ACQUITY RDa™ Detector; a small footprint time-of-flight mass spectrometer specifically developed for the BioAccord. Dedicated workflows for intact protein mass, released glycans and peptides have been developed for the system. System integration and application test is performed at the Waters factory, so the system is ready to go on installation. There are automated setup and self-diagnosis routines, and the BioAccord runs under UNIFI™, Waters' compliance-ready LC-MS informatics platform.

Waters

http://link.spectroscopyasia.com/31-008

NIR

Fibre optic multiplexer for NIR spectrometers

Galaxy Scientific has introduced a fibre optic multiplexer for use with NIR spectrometers, which allows a single spectrometer to automatically switch between up to ten different sampling devices. This enables NIR measurements from multiple sampling points or multiple product streams. It is designed to accept standard SMA905 low-OH optical fibre inputs (commonly used for connecting a NIR spectrometer to a process probe) and is designed to switch 600 µm solid core fibre. Smaller core fibre or larger core fibre bundles are also compatible. The multiplexer is factory configurable at the time of order to have any number of channels between 2 and 10 and more channels can be added after purchase. The unit can be placed close to the sample points and distant from the spectrometer, reducing the cost of long fibre optic runs while still protecting sensitive instrumentation.

Galaxy Scientific

http://link.spectroscopyasia.com/31-004

New fNIR optical imaging systems

BIOPAC Systems, the global distributor for fNIR Devices, has announced the latest generation of their functional near infrared (fNIR) optical imaging systems. The new high-density imaging systems provide in-lab or real-world cognitive function assessments for physiology researchers looking to understand brain activity without fMRI. The extra-lightweight sensor fits comfortably on the forehead where it monitors relative changes in oxy or deoxy haemoglobin as a proxy for the brain activity in the prefrontal cortex.

The new fNIR systems are available in three versions. The fNIR 2000C is a stationary unit that collects data from up to 18 optodes. The fNIR 2000M is a wireless and mobile imager that collects data from up to 18 optodes and can be used while subjects are performing tasks in the lab or in the real world. The fNIR 2000S is a 54-optode capable imager with advanced features for recording up to three subjects simultaneously. A new through-the-hair sensor will allow researchers to assess other areas of the brain such as motor and visual cortices. Adding in

NEW PRODUCTS

different parts of the brain increases understanding of brain activity without the complications of fMRI.

All systems include fNIRSoft and COBI software for data collection and analysis. Data can be synchronised with and imported into AcqKnowledge Software to understand the physiological response, including EEG, EDA, ECG, BP and other important physiology signals.

BIOPAC Systems

http://link.spectroscopyasia.com/31-002

PHOTONICS AND OPTICS

UV-NIR neutral density filters

Acton Optics & Coatings has introduced a new series of UV-NIR neutral density filters. Thanks to patented UV coating processes, these can include broadband UV-NIR neutral density filter performance down to 190 nm. The new ND filters, which are suitable for use with broadband sources like xenon, deuterium and tungsten halogen, have been designed to optimise the utility of precision optical systems, spectrometers and medical systems.

Various standard densities are available from 0.3 OD to 2.5 OD and the new filters can be stacked to create additional or deeper densities. The exclusive filter coatings can also be deposited on custom-sized substrates for OEM applications and, if required, can be designed for other optical density values.

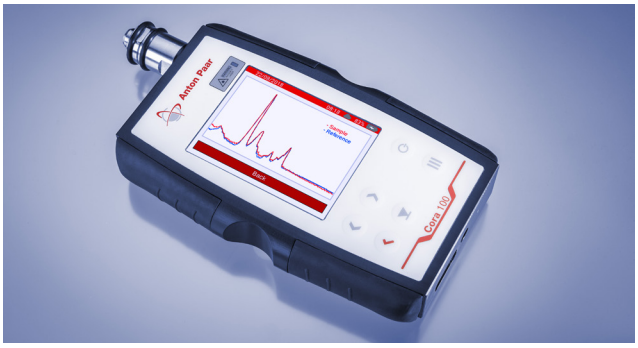
Acton Optics & Coatings

http://link.spectroscopyasia.com/31-001

RAMAN

Cora 100 handheld Raman spectrometer from Anton Paar

Anton Paar has introduced the Cora 100 handheld Raman spectrometer for on-the-spot identification of explosives, narcotics and hazardous materials. The intuitive software and accessories can be used by anyone, without formal training in Raman spectroscopy. The small footprint (16 × 10 × 3 cm), small weight (700 g) and rugged construction make it suited to single-handed operation and on-site identification of substances. The Raman analyser has successfully been tested according to military specifications, MIL-STD 810G, as well as European tests for dust and water, and is classified as IP67 waterproof. Spectral libraries for narcotics, hazardous materials, explosives and chemical warfare agents are available. Customised libraries can also be created by the user. The collected data is saved and stored in the spectrometer for further report generation. The point-and-shoot adapter is suited for measurements through bottles or plastic bags for identifying



liquids and powders. Quick non-contact analysis without moving samples, for example potential explosives, can be performed with the right-angle sampling adapter.

Anton Paar

http://link.spectroscopyasia.com/30-W-118

X-RAY

Optical and XRF imaging and analysis

HORIBA Scientific has released the XGT-9000 X-ray analytical microscope (μXRF), which simultaneously performs elemental analysis and optical observation of samples. Incorporating HORIBA's proprietary X-ray technology, the XGT-9000 not only screens foreign objects in a range of production processes, including semiconductor integrated circuits, it also measures film thickness and adherence amounts with a high degree of accuracy. By integrating the features of a high-resolution microscope and high-intensity X-ray beams, the XGT-9000 performs non-destructive foreign-object analysis on samples, switching between high-speed analysis mode for rapid screening of foreign objects, and detailed analysis mode using the micro beams first incorporated in earlier models. The XGT-9000 is equipped with three types of optical illuminations: bright field coaxial, dark field and transmission. Combining bright field coaxial and dark field illuminations enables clear observation of the samples with flat or uneven areas, such as semiconductor wafers and films. The XGT-9000 offers highly accurate and fast foreign-object analysis, enabling it to detect both visible foreign objects and invisible ones down to several microns in size.

Irradiating X-rays are coaxial with optical observation images, which avoids any misalignment. Other improvements include shortened analysis time, enhanced mapping and image processing, as well as ease of combination with other analysis equipment.

HORIBA Scientific

http://link.spectroscopyasia.com/31-009

Conferences

2019

6–10 April, Orlando, United States. **Experimental Biology 2019.** eb@faseb.org, https://experimentalbiology.org/2019/home.aspx.

15–18 April, Sao Paulo, Brazil. **XII Workshop on Sample Preparation (XII WPA).** http://www.iq.usp.br/wpa2019/.

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

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

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

6–8 May, CapeTown, South Africa. **SETAC Africa 9th Biennial Conference.** setac@setac.org, https://www.setac.org/events/EventDetails.aspx?id=1165114&group=.

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

19–22 May, San Antonio, TX, United States. **AAPG 2019 Annual Convention and Exhibition (ACE).** convene2@aapg.org, https://ace.aapg.org/2019/about/about-ace.

20–24 May, Vienna, Austria. **15th International Symposium on Isotope Hydrology.** Olive Kracht, o.kracht@iaea.org, https://www.iaea.org/events/international-symposium-on-isotope-hydrology-2019.

20–21 May, Vancouver, Canada. **International Conference on Hyperspectral Imaging and Remote Sensing (ICHIRS 2019).** https://waset.org/conference/2019/05/vancouver/ICHIRS.

21–25 May, Toronto, Canada. **7th Georgian Bay International Conference on Bioinorganic Chemistry (CANBIC-7).** Martin Stillman, canbic@uwo.ca, http://canbic.ca/index.html.

22–23 May, Rotterdam, Netherlands. **The 3rd International Conference and Exhibition on Petrochemical and Oil Analysis (PEFTEC 2019).** info@ilmexhibitions.com, https://www.ilmexhibitions.com/peftec/.

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

26–30 May, Chiba, Japan. **Japan Geoscience Union (JpGU) Meeting 2019.** http://www.jpgu.org/meeting_e2019.

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

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

3–7 June, Quebec, Canada. **102nd Canadian Chemistry Conference and Exhibition.** http://www.csc2019.ca/.

8 June, Ottawa, Ontario, Canada. **Laurentian SETAC 23rd Annual General Meeting.** setac@setaceu.org, http://www.laurentiansetac.ca/.

9–14 June, Mexico City, Mexico. **Colloquium Spectroscopicum Internationale XLI (CSI XLI).** info@csi2019.mexico.com, http://www.csi2019mexico.com/.

9–14 June, Mexico City, Mexico. **Latin American Meeting on Laser-Induced Breakdown Spectroscopy (LAMLIBS).** mayo.villagran@icat.unam.mx, http://www.csi2019mexico.com/index.php/lamlibs.

11–12 June, Muenster, 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.

17–19 June, London, United Kingdom. **4th International Congress on Organic Chemistry and Advanced Drug Research.** https://organicchemistry.pulsusconference.com/.

25–27 June, San Jose, California, United States. **OSA Optical Sensors and Sensing Congress.** https://www.osa.org/en-us/meetings/osa_meetings/optical_sensors_and_sensing_congress/.

25–28 June, Dorval, Quebec, Canada. **Spectr'Atom 2019.** Diane Beauchemin, diane.beauchemin@chem.queensu.ca, http://www.csass.org/SpectrAtom2019.html.

25–28 June, Dorval, Canada. **63rd International Conference on Analytical Sciences and Spectroscopy (ICASS).** Diane Beauchemin, diane.beauchemin@chem.queensu.ca, http://www.csass.org/ICASS.html.

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/.

15–18 July, Honolulu, Hawaii, United States. **15th International Congress of Toxicology (ICTXV).** sothq@toxicology.org, https://www.toxicology.org/events/ict/index.asp.

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28 July–2 August, Singapore, Singapore. **16th Annual meeting Asia Oceania Geosciences Society (AOGS).** info@asiaoceania.org, http://www.asiaoceania.org/society/index.asp.

28 July–2 August, Yokohama, Japan. **International Geoscience and Remote Sensing Symposium (IGARSS 2019).** https://igarss2019.org/.

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

12–13 August, Auckland, New Zealand. **7th Asia Pacific Congress on Chemical and Biochemical Engineering.** asiachemical2019@gmail.com, https://www.chemicalengineeringconference.com/.

25–30 August, Berlin, Germany. **21st International Society of Magnetic Resonance (ISMAR) Conference joint with EUROISMAR 2019.** euroismar2019@fmp-berlin.de, https://conference.euroismar2019.org/event/1/.

25–29 August, San Diego, CA, United States. **258th American Chemical Society (ACS) National Meeting & Exposition.** NationalMeetings@acs.org, https://global.acs.org/events/258th-acs-national-meeting-exposition/.

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 (COLA 2019).** 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/.

15–19 September, Cartagena, Colombia. **SETAC Latin America 13th Biennial Meeting.** setac@setac.org, https://sla2019.setac.org/.

16–18 September, Melbourne, Australia. **International Conference on Materials Science and Engineering 2019.** conference@materialsoceania.com, https://www.materialsconferenceaustralia.com/.

22–25 September, Phoenix, Arizona, United States. **2019 GSA Annual Meeting.** meetings@geosociety.org, http://www.geosociety.org/GSA/Events/Annual_Meeting/GSA/Events/2019info.aspx.

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

24–26 September, Sao Paulo, Brazil. **6th Analitica Latin American Congress.** analitica@nm-brasil.com.br, https://www.analiticanet.com.br/pt/perfil-do-evento.

29 September–3 October, Portland, United States. **2019 Materials Science and Technology Conference (MS&T19).** metsoc@cim.org, http://www.matscitech.org/.

6–11 October, Mendoza, Argentina. **15th Rio Symposium on Atomic Spectrometry.** secretary@15riosymposium.com, https://www.15riosymposium.com/.

13–18 October, Palm Springs, United States. **SciX 2019 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.

3–7 November, Toronto, Canada. **SETAC North America 40th Annual Meeting.** https://toronto.setac.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/.

1–6 December, Boston, United States. **Materials Research Society 2019 Fall Meeting (MRS 2019).** https://www.mrs.org/fall2019.

9–13 December, San Francisco, United States. **2019 American Geophysical Union (AGU) Fall Meeting.** meetinginfo@agu.org, https://meetings.agu.org/upcoming-meetings/.

2020

12–18 January, Tucson, Arizona, United States. **2020 Winter Conference on Plasma Spectrochemistry.** Ramon Barnes, wc2020@chem.umass.edu, http://icpinformation.org.

17–22 February, Anaheim, California, United States. **2020 American Academy of Forensic Sciences (AAFS) 72nd Annual Scientific Meeting.** https://www.aafs.org/home-page/meetings/future-past-aafs-meetings/.

24–28 May, Chiba, Japan. **Japan Geoscience Union (JpGU) Meeting 2020.** http://www.jpgu.org/en/articles/20171208meetingplan.html.

Courses

2019

13–20 September, Dresden, Germany. **5th International Summer School Spectroelectrochemistry.** https://www.ifw-dresden.de/news-events/scientific-events/summer-school-spectroelectrochemistry/.

Exhibitions

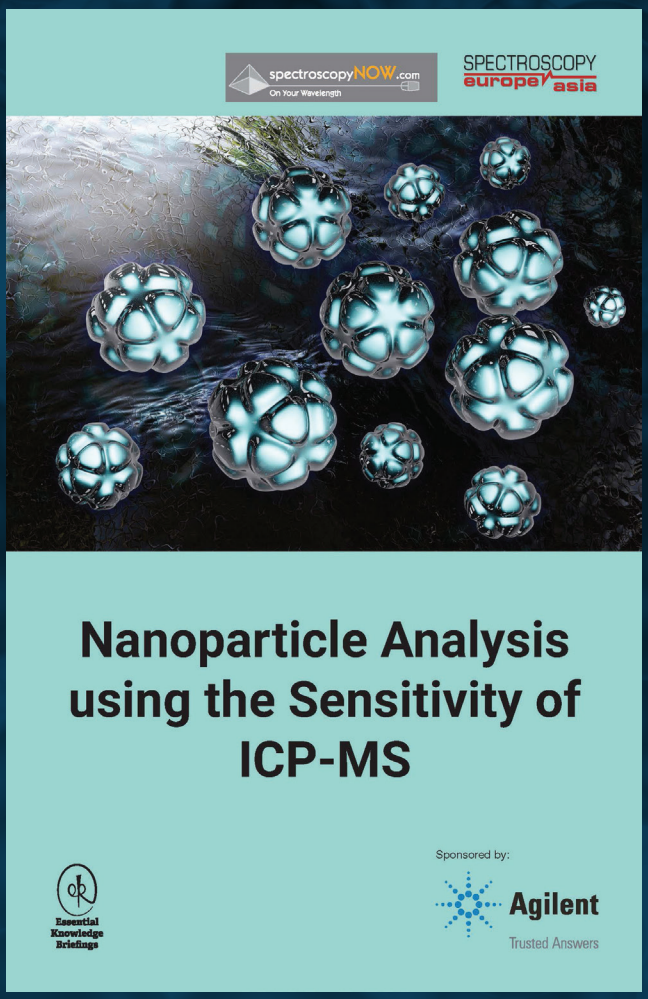
2019

20–22 March, Shanghai, China. **Laser World of Photonics China 2019.** info@world-of-photonics-china.com, https://world-of-photonics-china.com.

3–5 April, Ho Chi Minh, Vietnam. **analytica Vietnam 2019.** https://www.analyticavietnam.com/.

7–9 May, Beijing, China. **AchemAsia 2019.** https://www.achemasia.de.

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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