Vol. 31 No. 6 December 2019/January 2020

SPECTROSCOPY europe

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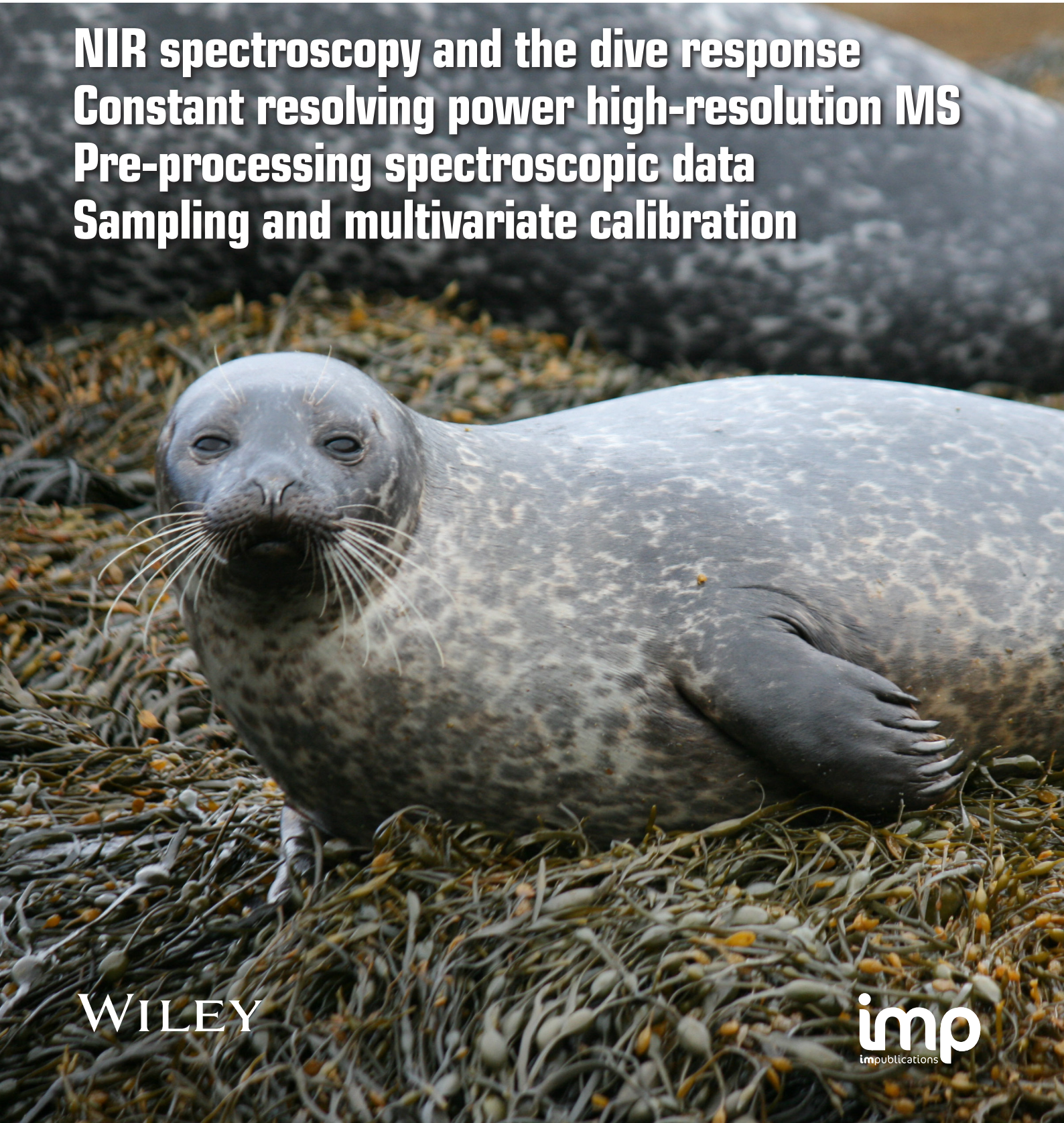
NIR spectroscopy and the dive response
Constant resolving power high-resolution MS
Pre-processing spectroscopic data
Sampling and multivariate calibration



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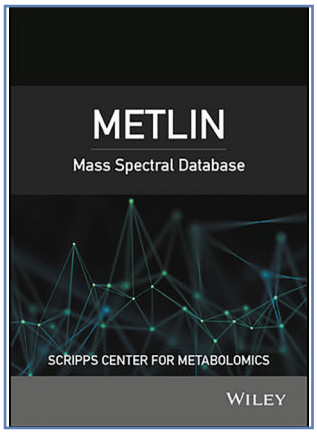
METLIN Mass Spectral Database

By Wiley*



Developed by the Scripps Center for Metabolomics, METLIN Mass Spectral Database by Wiley is the most complete MS/MS database in the field. With content divided into 3 libraries – experimental spectra, in-silico spectra, and searchable chemical structures – METLIN by Wiley has optimized its searching capabilities and validated spectrum quality to ensure the broadest coverage available.

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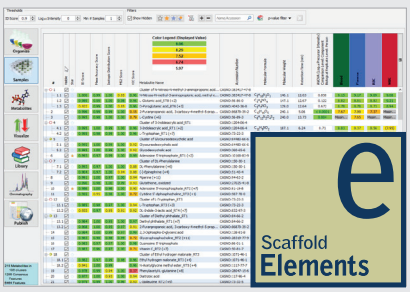

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EDITORIAL

Giving in to temptation

As we head towards Christmas, it is not unusual to allow oneself a few indulgences. I have done so in this issue, but I hope that they are treats we can all share. Back in the 1970s, I was an undergraduate at the University of St Andrews and spent my final (fourth since it is a Scottish university) year at the Gatty Marine Laboratory undertaking an investigation into the righting mechanism of Brittle Stars. A few decades later(!!), in the summer of this year, I came across work on using NIR spectroscopy to investigate the physiology of seals during diving; also carried out at the Gatty. This was featured in the News in our June/July issue earlier this year. I wanted to bring readers some more detail, and Chris Knight at St Andrews kindly agreed to help. As well as the versatility of NIR spectroscopy, I was amazed at the technical achievements involved in placing a spectrometer on a seal without harming it and recording data underwater.

The second article ("New mass spectrometry method for characterisation of the most challenging complex mixtures") has an element of indulgence, in that I have known one of the authors, Mark Barrow, for many years, since he was working in the late Peter Derrick's group at Warwick University. Now he is running his own group, which has developed a clever solution to be able to provide constant resolution, high-resolution mass spectrometry data across the spectrum. In doing so, they have assigned 244,779 unique elemental compositions: a record.

Tony Davies, Jan Gerretzen and Henk-Jan van Manen consider "Pre-processing spectroscopic data: for good or ill?". They offer many useful points to consider when using pre-processing techniques.

The Sampling Column is from Kim Esbensen and Brad Swarbrick, and looks at "Sampling for spectroscopic analysis: consequences for multivariate calibration". Kim and Brad point out that incorrect sampling is irreversible: no amount of chemometrics or further samples will be able to produce a valid model if the sampling is not representative. This applies in flowing PAT analyses as much as in static.

Finally, a reminder that Peter Jenks would greatly appreciate your input to his survey on your use of and experience with reference materials. The survey can be found at https://www.spectroscopyeurope.com/reference-material-survey-2019.

NIR spectroscopy has been used to investigate how mamamalian physiology changes during diving, and reveals more than was expected. Photo: Monica Arso Civil and SMRU

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NEWS

Miniature NMR implant measures neuronal activity

A team of neuroscientists and electrical engineers from Germany and Switzerland have developed a highly sensitive implant that enables brain physiology to be probed with unparalleled spatial and temporal resolution. The device is an ultra-fine needle with an integrated chip that combines an ultrasensitive 300-µm nuclear magnetic resonance (NMR) coil with a complete NMR transceiver. It is capable of detecting and transmitting NMR data from nL volumes of brain oxygen metabolism.

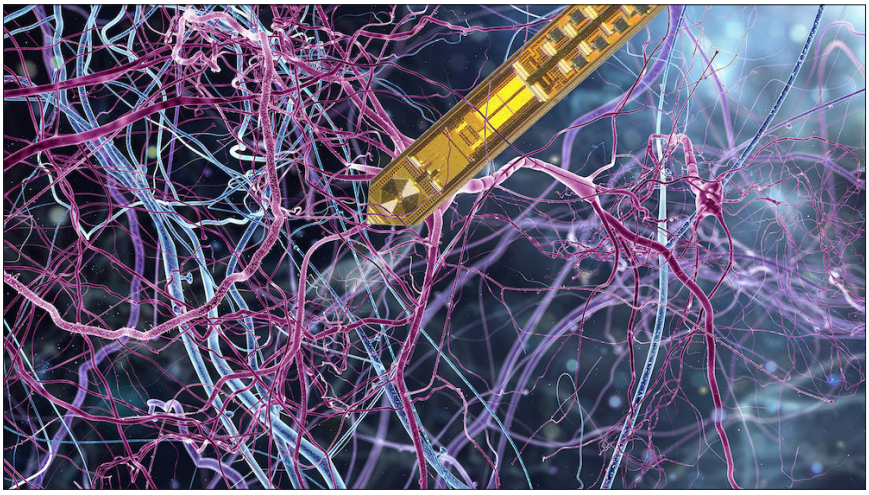


Illustration of the NMR needle in brain tissue. Credits: whitehoune - stock.adobe.com, MPI f. Biological Cybernetics, University of Stuttgart. Montage: Martin Vötsch (design-galaxie.de).

The group of researchers led by Klaus Scheffler from the Max Planck Institute for Biological Cybernetics and the University of Tübingen as well as by Jens Anders from the University of Stuttgart identified a technical bypass that bridges the electrophysical limits of contemporary brain scan methods. Their development of a capillary monolithic NMR needle combines the versatility of brain imaging with the accuracy of a very localised and fast technique to analyse the specific neuronal activity of the brain. "The integrated design of a nuclear magnetic resonance detector on a single chip supremely reduces the typical electromagnetic interference of magnetic resonance signals. This enables neuroscientists to gather precise data from minuscule areas of the brain and to combine them with information from spatial and temporal data of the brain´s physiology", explains principal investigator Klaus Scheffler. "With this method, we can now better understand specific activity and functionalities in the brain."

According to Scheffler and his group, their invention may unveil the possibility of discovering novel effects or typical fingerprints of neuronal activation, up to specific neuronal events in brain tissue. "Our design setup will allow scalable solutions, meaning the possibility of expanding the collection of data from more than from a single area—but on the same device. The scalability of our approach will allow us to extend our platform by additional sensing modalities such as electrophysiological and optogenetic measurements," adds the second principal investigator Jens Anders.

The teams of Scheffler and Anders are very confident that their technical approach may help demerge the complex physiologic processes within the neural networks of the brain and that it may uncover additional benefits that can provide even deeper insights into the functionality of the brain. With their primary goal to develop new techniques that are able to specifically probe the structural and biochemical composition of living brain tissue, their latest innovation paves the way for future highly specific and quantitative mapping techniques of neuronal activity and bioenergetic processes in the brain cells.

Their work has been published in *Nature Methods* (doi: https://doi.org/10.1038/s41592-019-0640-3).

Mass spectrometry shows how vital coral algae adapts to warming seas

The team identified that one particular species of the photosynthetic organisms called "zooxanthellae" is able to change part of its chemical make-up to survive warmers seas, which prove fatal to other similar species of zooxanthellae. The survival of these single-celled organisms is important as they help protect corals from the risk of "bleaching".

Corals live in a mutually beneficial relationship, a symbiosis, with zooxanthellae—where the tiny algae gain shelter, carbon dioxide and nutrients, while corals get photosynthetic products that can provide them with up to 90 % of their energy needs. If temperatures rise just 1˚C above the summer maximum the photosynthetic machinery of the zooxanthellae can start to malfunction and symbiosis breaks down. As a consequence, the brownish-coloured algal symbionts are lost and the coral's white limestone skeleton shines through its transparent tissue—a phenomenon known as "coral bleaching". This can lead to a coral starving, falling victim to disease and often dying.

It is already know that some species of zooxanthellae are more tolerant to heat than others, but this latest study paves the way to more accurately identifying which corals will be able to survive successfully in warmer waters of the future. Findings are published in *Coral Reefs* (doi: https://doi.org/10.1007/s00338-019-01865-x).

Scientists from the Coral Reef Laboratory at the University of Southampton worked with colleagues at University Hospital Southampton to use

the hospital's mass-spectrometry facility to analyse lipid molecules—the building blocks of the membranes of zooxanthellae cells. Among other functions, these membranes play a key role helping the algae to harness energy from the sun and convert it to food. The team compared a heat tolerant species of zooxanthellae with one which is less tolerant and found the more resilient of the two could adapt the lipid molecule composition of its membranes to withstand stress from higher temperatures. They discovered different chemical properties in the thermotolerant species as compared to their less resistant counterparts.

Lead researcher and marine biologist at the University of Southampton, Dr Cecilia D'Angelo said: "This is an important step forward in understanding how coral can handle global warming. Our findings show the coral symbionts use multiple strategies to protect themselves from excess heat. It is encouraging to see that corals have mechanisms in place to adjust to high seawater temperatures, however, not all of them have the same capacity to do so. One symbiont species studied as part of our research became severely stressed at 30°C. This temperature is considered a critical threshold for coral survival in many places of the world. The failing of defence mechanisms at temperatures above this threshold can explain observations of our previous research that only very few symbiont species are able to thrive in extreme temperature environments such as the hottest coral water of the world, the Persian/Arabian Gulf, where water temperatures hit summer maxima of up to 35°C on a regular basis. Moreover, our results will help to explore the inner workings of phytoplankton algae, the most abundant photosynthetic organisms on the planet."

Red Sea coral reef. Credit: Wiedenmann/D'Angelo

Dr D'Angelo concluded that more research can be conducted in this area: "Global warming in combination with other forms of environmental stress such as nutrient starvation has the potential to greatly reduce the diversity of coral symbionts and their hosts. Through our collaboration with colleagues at the University Hospital Southampton we can combine coral experimentation under tightly controlled laboratory conditions with molecular analysis using state-of-the-art mass spectrometry. This puts us in an excellent position to understand

the mechanisms that enable corals to respond to their environment and determine to which extend these processes will help the corals to adjust to rising seawater temperature."

FT-IR analyses microplastics during two-year, all-women research voyage of World's oceans

PerkinElmer's Spectrum Two™ FT-IR spectrometer is onboard a research trip led by eXXpedition (https://exxpedition.com/about/science/), a not-for-profit organisation running all-women sailing voyages investigating ocean plastic pollution causes and solutions. The latest mission, launched in October 2019 from Plymouth, UK, runs until Spring 2021 and features over 30 stops: from the Azores, Aruba and the Galapagos, to Fiji, Perth and Reykjavik. On each new leg, fresh crew volunteers from all walks of life will join in.

Through a Scientific Partner collaboration, the eXXpedition Round the World 2019–2021 scientist team (including members from Plymouth University) is using the portable Spectrum Two to identify and document the chemical classification of plastics found during their travels. PerkinElmer is also providing instrument and Spectrum™ 10 software training and support throughout.

"Plastic ocean pollution is one of the most visible environmental challenges we face. The accompanying and less noticeable issue is when items like plastic bottles or nets break down into microplastics to then be ingested by sea creatures and eventually us at the dinner table," said NH Kim VP/GM of Applied Markets, PerkinElmer. "Studying microplastics to accelerate scientific understanding and drive answers is paramount. We're proud to be part of the eXXpedition voyage and believe their research will generate interesting findings to help drive future environmental responses."

Emily Penn, ocean advocate, skipper and co-founder of eXXpedition added, "We're excited to have PerkinElmer's innovative technology onboard. It allows us to do real time analysis to identify polymer types and will help us explore potential solutions as research continues on dry land."

Dr Winnie Courtene-Jones (University of Plymouth, UK / Science Lead eXXpedition), Emily Penn (Mission Director eXXpedition) and Sally Earthrowl (Mission Leader eXXpedition) with the Spectrum Two on board the expedition sailing vessel S.V. TravelEdge. Photo © eXXpedition and Sophie Bolesworth.

MS fingerprint test can distinguish between those who have taken or handled heroin

The fingerprint drug testing technology is based on high resolution mass spectrometry, and is now able to detect heroin, its metabolite, 6-monoacetylmorphine (6-AM) and other analytes associated with the class A drug. A team from the University of Surrey took fingerprints from people seeking treatment at drug rehabilitation clinics who had testified to taking heroin or cocaine during the previous 24 hours. A fingerprint was collected from each finger of the right hand, and the participants were then asked to wash their hands thoroughly with soap and water and then wear nitrile gloves for a period of time before giving another set of fingerprints. This same process was used to collect samples from 50 drug non-users.

The researchers found that the technology was able to identify traces of heroin and 6-AM on drug non-users in every scenario the researchers devised—whether someone directly touched the drug, handled it and then thoroughly washed their hands, or had come into contact with heroin from shaking someone else's hand.

Surrey's system cross-referenced the information from the drug non-users with the volunteers who were being treated for drug dependency and found that compounds such as morphine, noscapine and acetylcodeine—alongside heroin and 6-AM—are essential to distinguishing those who have used the class A drug from those who have not. These analytes were only present in fingerprints from drug users.

Catia Costa from the University of Surrey said: "Our results have shown that this non-invasive and innovative technology is sensitive enough to identify class A drugs in several scenarios—even after people have washed their hands. Crucially, our study shows that the process of hand washing is important when trying to assess, from their fingerprint, whether someone has used a class A drug."

Dr Melanie Bailey from the University of Surrey said: "Our team here at the

University of Surrey believes that the technology we are developing will make our communities safer and shorten the route for those who need help to beat their addictions. We also believe the technology has scope in other areas, such as confirming whether a patient is taking their medication."

They report their work in the *Journal of Analytical Toxicology* (https://doi.org/10.1093/jat/bkz088).

Development of magneto-optic effect measurement device using dual-comb spectroscopy

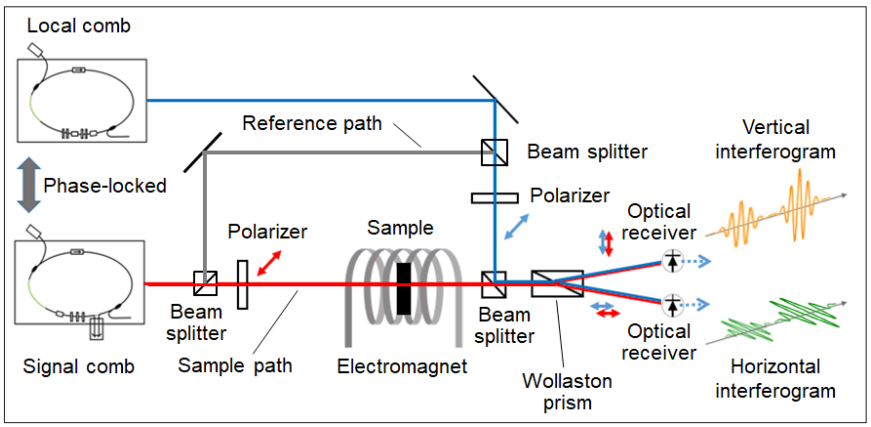
Professor Kaoru Minoshima from the University of Electro-Communications and NEOARK Corporation have succeeded in prototyping a greatly improved magneto-optic effect measurement device using dual-comb spectroscopy.

Dual-comb spectroscopy uses two precisely controlled ultrashort pulse lasers, known as optical frequency combs (optical combs). Dual-comb spectroscopy offers major improvements over conventional Fourier spectroscopy in areas including resolution, sensitivity and measuring time. So far, dual-comb spectroscopy has primarily been used for gas spectroscopy. The project that is the first in the world to develop a solid physical property evaluation technology using dual-comb spectroscopy, has demonstrated the principles in various physical property measurements.

As a first step in developing practical applications of the technique, Professor Minoshima and her colleagues developed a magneto-optic effect measurement device capable of evaluating the characteristics of magnetic materials. The optical system and signal detection system of the prototype were improved to achieve measurement performance that greatly exceeds conventional measurement methods.

The prototype achieved major progress towards practical application, featuring a magneto-optic effect measurement resolution of 0.01°, a wavelength resolution of 0.01 nm, capable of high-speed measurement through batch measurements of all wavelength components. The prototype is a desktop system, consisting of a measurement unit, a dual-comb light source and a controller. The generated magnetic field is a maximum of ±10 kilo-Oersted.

Furthermore, based on the above-mentioned solid physical property evaluation technology, the research team also developed a prototype device for measuring the complex refractive index of solids. A major feature of the prototype is its capability to measure the phase



Block diagram of Faraday effect measurement system using dual-comb spectroscopy. The signal comb (red line) passes through the sample and is superposed with the local comb (blue line). The vertical and horizontal components of the interference signal are detected by the two optical receivers. Applying magnetic field to the sample, the Faraday effect (Faraday rotation angle) is measured. Credit: ©The University of Electro-Communications, Kaoru Minoshima and NEOARK.

difference of light in addition to its intensity ratio.

Measurement devices leveraging dual-comb spectroscopy for magneto-optic effect measurement and complex refractive index measurement are expected to become important new tools for the precise measurement of polarisation and spectroscopy, and for material development. They will proceed with development targeting commercialisation in the near future.

Details are reported in *Optics Letters* (doi: https://doi.org/10.1364/OL.41.004971).

Non-invasive microscopy detects activation state and distinguishes between cell types

Most analytical methods in biology require invasive procedures to analyse samples, which can lead to irreversible changes or even their destruction. Furthermore, the sensitivity of such approaches often stems from the averaging of signals generated by a large number of cells, making it impossible to study the underlying heterogeneity of responses. Quantitative phase microscopy and Raman spectroscopy are label-free techniques used in this study to extract biomarkers based on cellular morphology and intracellular content. These approaches have been previously used to characterise specimens and identify cells from different origins; however, the measurement of finer features than the cell type with these techniques has proven to be challenging.

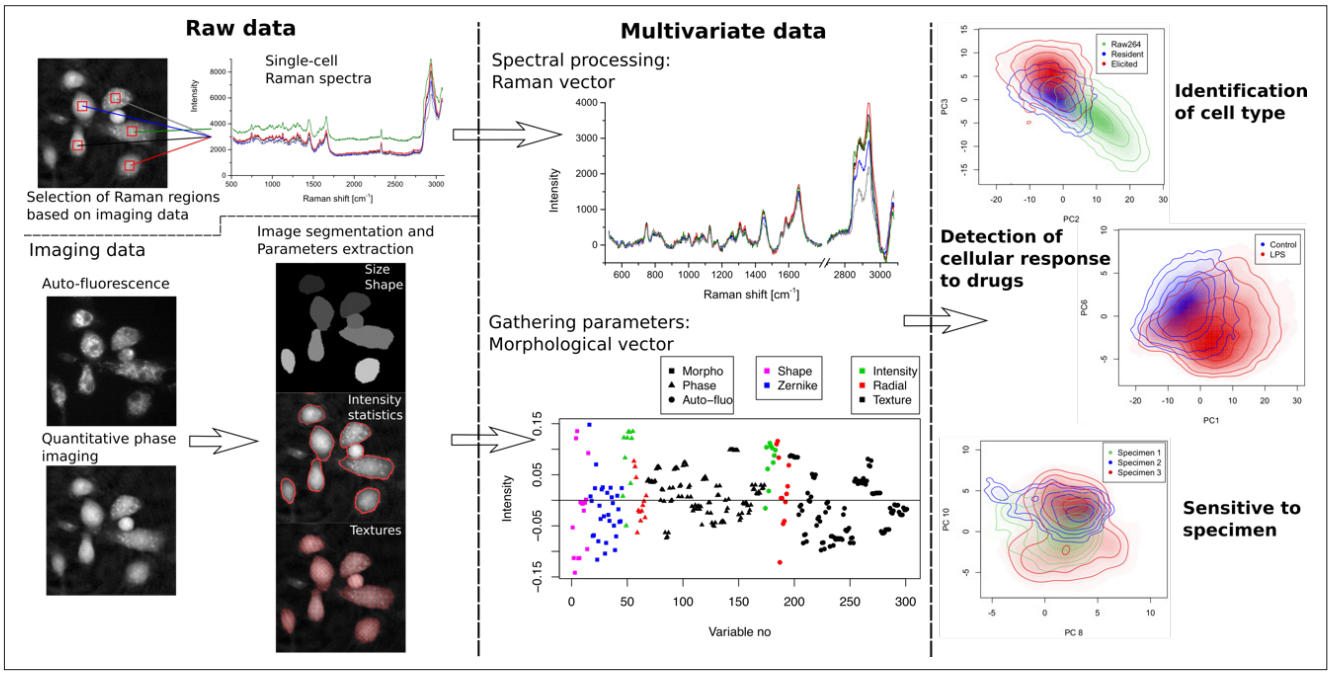
Assistant Professor Nicolas Pavillon and Associate Professor Nicholas I. Smith of the Immunology Frontier Research Center (IFReC) at Osaka University developed a label-free multimodal imaging platform that enables the study of cell cultures non-invasively without the need for any contrast agent. The pair of researchers showed how the label-free signals can be employed to create models that can detect the activation state of macrophage cells and distinguish between different cell types even in the case of highly heterogeneous populations of primary cells. "We devised specific statistical tools that allow for the identification of the best methods for detecting responses at the single-cell level, and show how these models can also identify different specimens, even within identical experimental conditions, allowing for the detection of outlier behaviours", says Associate Professor Smith.

The findings of this study show that a non-invasive optical approach, which enables the study of live samples without requiring contrast agents, can also achieve high sensitivity at the single-cell level. "In particular", says Assistant Professor Pavillon, "our results show that this method can identify different cell sub-types and their molecular changes during the immune response, as well as outlier behaviours between specimens."

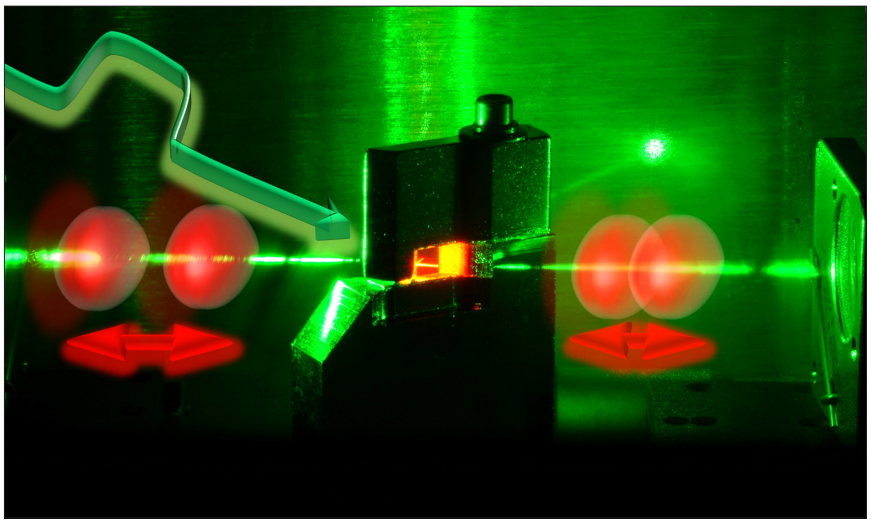
Details of the work were published in *Scientific Reports* (doi: https://doi.org/10.1038/s41598-019-53428-3).

Ultrashort laser flashes optical control

Laser pulses have long been utilised in research laboratories, industrial production and medical therapies. In these applications it is often crucial that the pulses—also known as optical solitons—occur at certain intervals. Using a special high-speed measurement technique, the researchers have now been able to show how



Methods of extracting features from label-free immune cell analysis. Multivariate label-free data, composed of both morphological and spectral parameters, are used to identify high-level features at the single-cell level such as cellular type, response to drugs, as well as response differences between specimens.



Light pulses can form pairs in ultra-short pulse lasers. The pulse intervals (red) can be precisely adjusted by making certain changes to pump beam (green). Image: UBT.

a short-pulse laser widely applied in research can be made to automatically generate pairs of light pulses separated by the desired interval. All that is required are small disturbances in the green "pump beam" (which generates the laser pulses) triggered by electric signals.

The new process centres on the targeted manipulation of solitons, wave packets that can occur in pairs in ultrashort laser pulses. "The resonance excitation and the short disturbance of soliton pairs trigger effects that can be used to specifically control ultrashort laser pulses. This opens up an exciting new area of research with a yet unforeseeable range of possible applications", said Prof. Dr Georg Herink from Bayreuth, corresponding author of the new study published in *Nature Photonics* (doi: https://doi.org/10.1038/s41566-019-0530-3). "At the right frequency, a tiny external modulation of the laser is all you need, and ultrashort laser pulses are set into reciprocal, resonant oscillation. Similar phenomena can be observed in water molecules heated in the microwave", added lead author Felix Kurtz from Göttingen.

COMPANY NEWS

Edinburgh Instruments Raman partnership with Bio-Rad and KnowItAll

Edinburgh Instruments has entered into partnership with Bio-Rad and its KnowItAll Raman Spectral Identification Partner Program. The partnership enables Edinburgh Instruments to give its customers access to Bio-Rad's Raman Spectral Database and Raman Identification Software. As a Bio-Rad Raman Spectral Identification Partner, Edinburgh Instruments' Raman Microscope customers will receive a one-year subscription to Bio-Rad's KnowItAll Raman Identification Pro, enabling them to identify spectra with Bio-Rad's patented tools.

AMETEK France opens new technology solutions centre

AMETEK France has established a Technology Solutions Centre as a regional headquarters in Élancourt City. The centre will provide access to live demos, training sessions and application support. The centre opened on 15 November.

Thermo Fisher Scientific collaborates with Owlstone Medical to advance the identification of novel biomarkers

Thermo Fisher Scientific and Owlstone Medical have entered into a collaborative partnership to advance the early diagnosis of cancer and other diseases through the discovery and validation of novel biomarkers by non-invasive breath sampling. Through the integration of Orbitrap gas chromatography mass spectrometry (GC-MS) instrumentation into Owlstone Medical's Breath Biopsy platform, the collaboration will qualify Thermo Fisher's mass spectrometers for the detection of new biomarkers via a validated discovery and routine analysis project. Developed in partnership, the new analytical methods will be used to conduct metabolomics studies of breath samples for unique biomarkers that could translate into non-invasive, routine screening solutions for improved early diagnosis of cancer and other disease.

"There is a growing need for non-invasive diagnostic solutions to support early disease detection, patient treatment and increase remission rates", said Morten Bern, Director of Marketing, Gas Chromatography, Thermo Fisher Scientific. "The combination of our Orbitrap GC-MS technology with Owlstone Medical's Breath Biopsy platform provides a unique basis to improve patient outcomes through the discovery of novel biomarkers and their incorporation into research use and clinical tests."

Billy Boyle, co-founder and Chief Executive Officer at Owlstone Medical, said: "The Orbitrap platform's ability to detect a wide range of chemicals during targeted and untargeted analyses without losing selectivity or sensitivity, promises to be of substantial benefit to our Breath Biopsy platform. With a large and rapidly expanding installed base of GC Orbitrap systems, our collaboration with Thermo Fisher Scientific represents an exciting opportunity for cross-promotion of the platform and technique, by which the benefits of Breath Biopsy can be broadly realised."

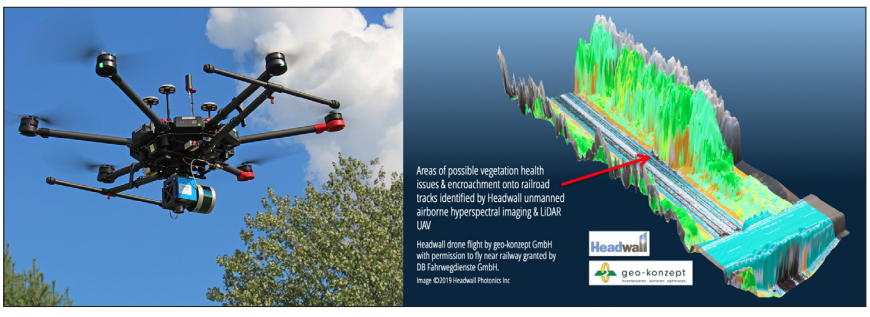
Headwall Centre for Hyperspectral Remote Sensing in Europe

Headwall BVBA, Belgium and geo-konzept of Germany have announced the formation of a Centre for Hyperspectral Remote Sensing Europe (CHRSE). This will be located at geo-konzept's headquarters in Adelschlag, Germany. The new centre will support the implementation and utilisation of hyperspectral imaging technology combined with other sensor technology, such as LiDAR and high-precision GPS, focusing on agriculture, mining, environmental monitoring and infrastructure inspection applications. The facility features large areas for unmanned drone flights and certified unmanned aerial vehicle (UAV) pilots available to test and demonstrate hyperspectral imaging technology in application-specific environments and to train the next generation of UAV operators. The centre also offers indoor meeting areas to develop applications together with researchers and users and to train data analysts utilising hyperspectral imaging technology to come to better, fact-based decisions to manage their applications.

Photothermal Spectroscopy and Bio-Rad partnership

Photothermal Spectroscopy Corp. (PSC) and Bio-Rad Laboratories have announced a partnership in which PSC will bundle Bio-Rad's IR and Raman Library and Identification software with PSC's instruments. PSC's Optical Photothermal IR Spectroscopy (O-PTIR) technique offers simultaneous sub-micron measurement of IR and Raman spectra via their mIRage+R infrared and Raman microscope. Used with Bio-Rad's KnowItAll software, the combination offers simultaneous searching of IR and Raman spectral databases for compound identification.

As part of the agreement, PSC will bundle a one-year subscription to Bio-Rad's KnowItAll IR/Raman Identification Pro, providing customers access to Bio-Rad's entire IR and Raman libraries with every PSC instrument and the ability to identify spectra with patented tools and spectral reference data available only from Bio Rad.



ARTICLE

Near infrared spectroscopy unlocks the secrets of mammalian diving success

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Diving animals, such as seals, dolphins, otters, turtles and penguins, share the same physiological constraints as we humans do when we enter back into our evolutionary home—the sea. The most instantly identifiable constraint if you put your head underwater is, of course, the inability to breath and the associated progressive asphyxia. This division between where an animal spends most of its time and where it can breathe has had a significant impact on the physiological capacities of diving animals. It is important to consider diving animals, such as seals, as "underwater animals which make only occasional brief excursions into 'our world' to breathe". They are not "animals from 'our world' that periodically dive underwater". This is more than just a semantic differentiation. Instead it is necessary to contextually understand the relationship between the physiology and lifestyle of diving animals.

One essential physiological prerequisite that equips divers, such as seals, for this profoundly different lifestyle is greater total body oxygen stores. Greater oxygen stores alone though do not make a consummate diver. A collective suite of cardiovascular responses to diving that help reduce the rate of oxygen depletion are referred to as the "dive response" and are the essential component of elite diving physiology. The most classic feature of the dive response is a reduction in heart rate—known as bradycardia. A reduction in heart rate alone does nothing to reduce the rate of oxygen consumption. Rather a reduction in heart rate couples a major redistribution of blood around the body, whereby blood flow to parts of the body like the skin, limbs and to some organs is reduced. It is this component of dive response which really conserves oxygen. Essentially this works by reducing the delivery of blood oxygen to parts of the body that would otherwise use and deplete oxygen stores. Instead the dive response helps to ensure conservation and delivery of oxygen-filled blood to priority organs, of which the brain is, of course, the most highly prioritised. Anyone one who has experienced a stroke, or fainted at the sight of a needle, will know only too well the extreme effects of an interruption of blood oxygen supply to the brain. The associated reduction in heart rate that goes along with blood redistribution during diving avoids a precipitous rise in blood pressure.

While this blood redistribution component is the essential "tool" of the dive response that facilitates diving, we know surprisingly very little about blood redistribution in diving animals. For the most part this has been the result of a lack of non-invasive and wearable technology that can measure changes in blood redistribution and what is known comes from lethal experiments of animals forced to dive involuntarily. Because of the nature of diving animals—they dive and swim—it means technology has to be wearable and non-invasive in order to be deployable on an animal that is free to choose to dive and swim of its own volition; for it's in this voluntary free-diving state where we can learn about the true dynamics of the cardiovascular system and understand how diving animals manage it to utilise their environment. Basically, what we want to do is to let the animals tell us their physiological "story" without us having to interfere at all and certainly to avoid unethical or invasive procedures. One technology that offers an insight into blood redistribution is the remarkable non-invasive, wearable, optical technology of near infrared (NIR) spectroscopy. NIR spectroscopy uses light emitted in contact with the skin to measure blood volume and oxygenation in the underlying tissue (Figure 1). These two metrics have provided a remarkable new insight in the dynamics of blood redistribution in voluntarily diving seals.

Deploying optical spectroscopy equipment on a voluntarily diving seal is far from a simple technological step and required some important mechanical adaptations. Two key adaptations necessary were marinising the equipment and making it suitable for attachment to a seal. Starting with a commercially available NIR system (Artinis PortaLite-mini, Artinis Medical Systems BV, Einsteinweg, Netherlands), we used potting techniques frequently used to house a suite of existing animal-borne instrumentation that are deployed on deep diving seals from the Artic to Antarctic.[2] The sensor body was housed in an aluminium case with a removable O-ring sealed lid which

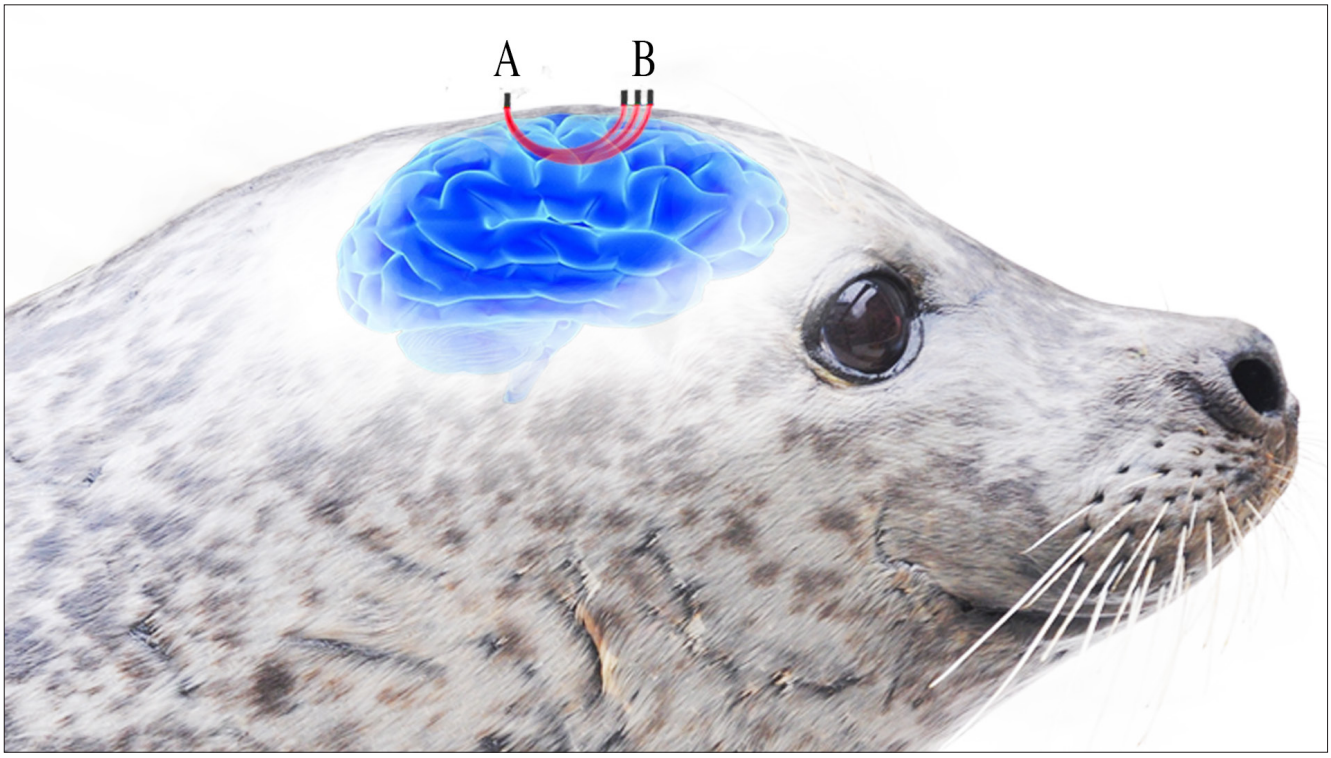

Figure 1. Three LEDs (B) emitting light around the wavelengths for oxygenated and deoxygenated haemoglobin are placed at different distances from the detector (A). The light passes through the underlying tissue before reaching the detector (A) in contact with the seal's skin. Increasing distance between the LEDs and detctor provides varying depths of optical penetration within the underlying tissue. Reproduced from Reference 1 under a Creative Commons Attribution licence.

provided water- and pressure-proofing, but also made the system robust to the "wear and tear" we expect from having a system on a non-domesticated animal. The light emitting diodes, photodiode receiver and printed circuit boards (PCBs) of the sensor head were housed in optically opaque polyoxymethylene housings filled with spectrally transparent epoxy. To ensure that epoxy encapsulated the electronics but did not cover the optical window on the optodes, the sensor head was cradled in a custom-built silicone mould. This allowed the internal components to be filled and waterproofed by epoxy but ensured the external surface and LEDs remained exposed to avoid affecting the fundamental optical properties of the LEDs.

To attach the system to a seal we used a combination of existing practices for seal instrumentation and developed novel attachment methods specifically for seal-borne NIR spectroscopy. The sensor body was bonded to the seal's fur caudal to the base of the skull using superglue—as many seal-borne devices are. The sensor head, which must provide contact between the optics and skin, required a new attachment methodology. A small section of fur was removed to the level of the skin which provided a furless optical window. Around this window of exposed skin, a 3D printed sensor head cradle was bonded to the seal's fur which secured the optics over the furless window and provided slight positive pressure on the optics against the skin.

The central focus of the study was to test to efficacy of NIR spectroscopy as an animal-borne tool. Thus, the fundamental technological and attachment issues, and finding how to overcome these problems, were central to the scope of the study. With that in mind, and the novel application of this technology, we had to rely on the assumptions that both the optical properties of seal tissues and haemoglobin were the same as those of humans, because this information hasn't yet been published. That said, the assumptions have biologically relevant basis. First, initial work from the Department of Physics at the University of St Andrews has shown that the optical properties of seal tissues, taken from freshly dead animals (skin and blubber) have optical properties which fall within the published ranges for human skin and adipose tissue. Similarly, published data[3] on the optical properties of northern elephant seal haemoglobin shows absorption peaks that are identical to cattle, sheep and multiple other mammalian species, including humans.

The marinised NIR system was deployed on voluntarily diving juvenile harbour seals (one of the two species of UK seals), see Figure 2. From the NIR data collected we were able to capture, for the first time, a level of cardiovascular control that we did not know seals possessed and frankly didn't know existed at all. Specifically, we found that in seals the blood redistribution component of the dive response is under some degree of cognitive control and

Figure 2. Harbour seals (*Phoca vitulina*). Photo: Monica Arso Civil and SMRU

not a result of the stimuli, such as facial immersion or the cessation of breathing, which activates it in other animals and humans. We found that seals started to move blood away from their peripheral tissues (in this case blubber and skin) around 15 s before they begin each dive. What this allows seals to do is to prepare for a dive and actually enter into the oxygen conservation state before they dive, so that when they dive they are not "wasting" oxygen in the early stages of the dive. And if you, as seals do, spend 90 % of your time at sea diving then these seemingly small savings total up to very significant savings over hours, days, weeks and sometimes even months of diving at sea—especially when making 2 km dives for two hours like elephant seals. In that sense, the finding of cognitive control of moving blood around the body makes a lot of sense, particularly when considering that cognitive control of heart rate has been demonstrated in seals. Seals have been shown to be able to drop their heart rate on command and also match the reduction in heart rate for the anticipated duration of the following dive. Interestingly, when a seal intends to make a longer dive the reduction in heart rate is greater and matches the intended dive duration quite well.

NIR spectroscopy technology may offer a new tool to understand the physiological envelope of diving mammals operating of their own volition at sea. Animal-borne instruments are fundamental tools in ecology and provide insight into the behaviour, movement and environment that are otherwise impossible to measure through standard observation techniques. Our understanding of the physiology of diving mammals, however, lags decades behind our understanding of behaviour. Yet, for animals that must breath-hold in order to operate in their environment, how they manage oxygen is essential to understand the responses and impacts of anthropogenic impacts on the sea. The non-invasive and small nature of NIR spectroscopy makes it an ideal tool to integrate into existing animal-borne technology.

Acknowledgements

I would like to acknowledge Steve Balfour and Sean McHugh of SMRU Instrumentation Group for developing the marinised system. Simon Moss, Ryan Milne and Matt Bivins for developing the attachment methodology. Thanks to each of my co-authors of Reference 1..

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ARTICLE

New mass spectrometry method for characterisation of the most challenging complex mixtures

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Introduction

The analysis of highly complex mixtures presents significant analytical challenges. Amongst the range of such samples, petroleum is considered one of nature's most complex mixtures. As a result, there is a strong need to better understand the chemistry of heavy petroleum and this becomes a driving force to improve analytical methodologies.

The molecular characterisation of petroleum and its derivatives by mass spectrometry has become known as "petroleomics". During the course of our research within this field, we have developed an approach for the characterisation of the most challenging complex mixtures. Based upon a combination of custom experiments and an in-house data processing algorithm, we used Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to successfully analyse a non-distillable fraction of a heavy petroleum sample, representing one of the most complex mixtures investigated to date. The approach enabled the acquisition of data at constant ultrahigh resolving power (approximately 3 million FWHM, full-width at half maximum) across the entire mass range and, in the process, we assigned 244,779 unique elemental compositions, setting a new record.

Ultrahigh resolution mass spectrometry is key

A broad view of the chemical components of heavy crude oil is key in the development of new upgrading technologies, and analytical chemistry, therefore, remains at the research forefront of petroleum upgrading. Heavy crude oils are extremely complex and most conventional analytical techniques do not offer the level of performance required to provide sufficiently detailed molecular insights into crude oil behaviour under upgrading conditions.

Ultrahigh resolution mass spectrometry, offering resolving power in the region of 10^5–10^6 FWHM and sub part-per-million mass accuracy, is perhaps the only analytical technique able to separate and assign individual compositions in complex mixtures such as crude oils. In particular, FT-ICR mass spectrometers (Figure 1) offer the highest available performance and, therefore, are the ideal analytical technique for obtaining insights into the molecular composition of crude oils. Due to the complexity of such samples, many compositions can be observed at each nominal mass, differing by small mass defects as a result of contributions from the different elements for each composition. Ultrahigh resolving power (for example, 500,000 FWHM at *m/z* 400) is often required to separate species with very small mass differences.

Figure 1. FT-ICR mass spectrometer used by the authors.

As a well-known example, two elemental compositions can differ through the presence of C_3 rather than SH_4—a difference of only 0.00337 Da! It is essential to be able to resolve this mass difference (or "mass split") in order to observe and assign sulfur-containing compounds that can poison catalysts, act as atmospheric pollutants and prove to be corrosive at elevated temperatures.

Traditional FT-ICR-based analysis can be used to assign the individual molecular species across a wide mass-to-charge ratio (*m/z* 150–1600), which includes components that are sufficiently heavy to be less amenable for gas chromatography. It is well known, however, that the ability to resolve individual compositions using FT-ICR MS decreases as the *m/z* increases; for a given *m/z* range, the

resolving power is inversely proportional to the *m/z*. Therefore, small mass differences might be not individually determined at higher mass. Unfortunately, it is also at higher *m/z* that it is possible to have a greater number of compositions per nominal mass, and so this is the region where ultrahigh resolving power is most needed.

FT-ICR instruments must also deal with a balance that determines the complexity of the data they can produce. Such mass spectrometers typically need approximately 100 ions per *m/z* (i.e per ion cloud) to successfully record a peak, but there is also an upper limit on the number of ions that can be stored within an ICR cell during an experiment: typically of an order of a few million ions, before space–charge effects become too severe. This essentially means that FT-ICR mass spectrometers can detect a maximum of tens of thousands of peaks within a single experiment, thus limiting the dynamic range.

Overcoming space–charge limitations

Those performance limitations are particularly important when heavier, low volatility fractions of heavy crude oil are analysed. The aim of our work was to analyse the "maltenes" (a fraction of petroleum soluble in *n*-heptane) of a truly non-distillable fraction (boiling point above 687°C at atmospheric equivalent temperature) of a heavy crude oil. Following experiments using different experimental set-ups and methods of sample preparation, successful acquisition of a full mass spectrum remained elusive.

The reason for this turned out to be the extraordinary complexity of the sample, basically overwhelming the instrument. In order to detect signal, it was necessary to start by isolating and detecting a very narrow *m/z* range. In this experimental set-up, a narrow *m/z* range of ions is transmitted through a quadrupole and accumulated in a collision cell, prior to transfer to the ICR cell for excitation and detection. This decreases the number of ions been detected in a single experiment, reduces the deleterious space–charge effects and increases the dynamic range. By performing this experiment, it was found that approximately 300 individual molecular compositions could be detected per nominal mass. By acquiring data using multiple, narrow windows, it is possible to "stitch" the data to generate a single mass spectrum. This method has been previously demonstrated and led to the previous record number of unique compositional assignments for a single sample: 126,264 molecular species for an "asphalt volcano" sample.[1]

Although space–charge effects are reduced and dynamic range is increased, the resolution achieved by traditional stitching methods, however, suffers from the well-known decrease in resolving power with increasing *m/z*, in similarity with traditional broadband experiments. This means such stitching experiments do not offer sufficient improvement in performance to resolve and assign compositions at the higher *m/z* region.

Additionally, traditional stitching methodologies have influenced the overall mass envelope as a consequence of different instrument parameters for the different windows. Anticipating molecular compositions of up to *m/z* 2000 present in the truly non-distillable fraction, a traditional stitching method will not have enough resolving power to individually assign molecular species with a mass difference of less than 0.0011 Da, for example, above *m/z* 1000. A fundamental modification of the stitching method was therefore necessary.

The mass resolving power of FT-ICR MS instruments can be increased by increasing the magnetic field, which means buying larger magnets and is extremely expensive, or by increasing the acquisition time of the experiment, as long as the signal can be sufficiently long-lived. To overcome the limitations of resolving power, narrow *m/z* windows were acquired but after prior planning, a target resolving power was calculated and the segments were acquired with increasing time domain data length with increasing *m/z* (i.e. the data were detected for longer periods for consecutive windows). By doing so, it was possible to ensure each *m/z* window was produced at the same resolving power (Figure 2).

The new approach also incorporates in-house software named Rhapso[2] that trims the data, determines the best position for overlapping the many segments, corrects the relative abundances of the ions in the segments (due to "edge effects" for windows when using a quadrupole for the isolation) and then automatically stitches the segments. The result

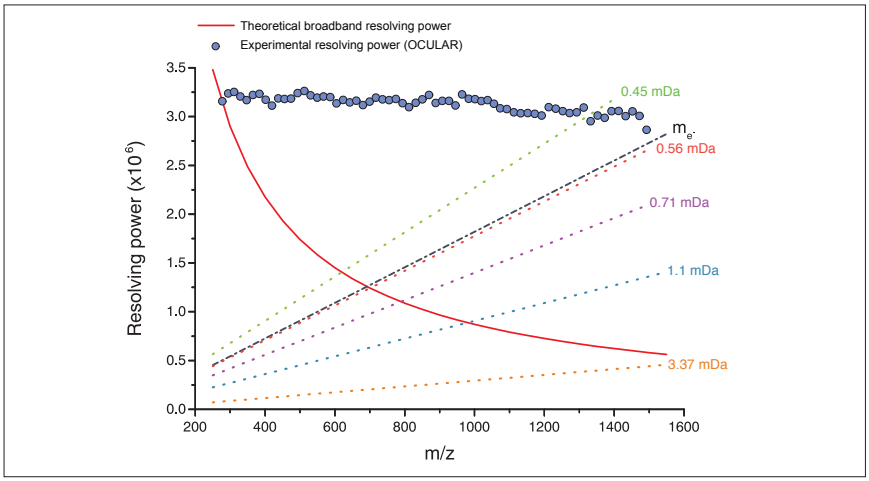

Figure 2. Red line: mass resolving power calculated for a mass spectrum acquired in broadband mode under ideal conditions, such as a perfect vacuum and no space-charge effects. The dotted lines represent the calculated minimum mass resolving powers required to resolve two peaks of comparable abundance, separated by the mass difference listed. The data points mark the mean experimental resolving power per 20 Da window. The resolving power afforded by the OCULAR method is sufficient to resolve peaks separated by a difference equivalent to only the mass of one electron (me, continuous black line; 0.0005485 Da) across the full mass range. Reproduced from Reference 3 under a Creative Commons Attribution licence.

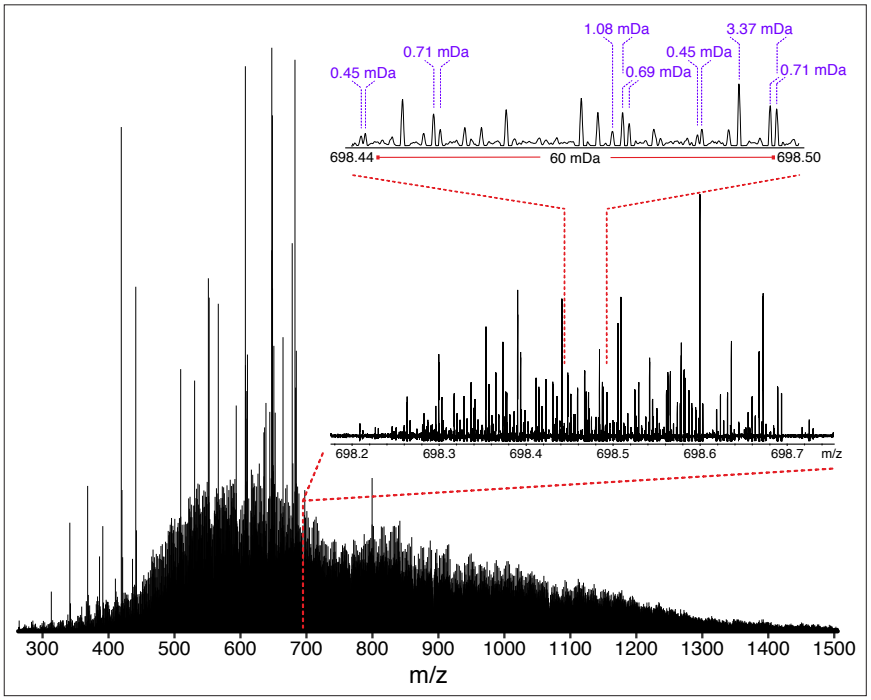

Figure 3. Stitched mass spectrum of the non-distillable fractionof a heavy crude oil, with peaks spanning *m/z* 260–1505. The spectrum was obtained by stitching 65 windows, each with an *m/z* width of 24, with steadily increasing acquisition time per segment to result in near constant resolving power across the *m/z* range. An enlarged region, showing assignments for *m/z* 698, is shown. Reproduced from Reference 3 under a Creative Commons Attribution licence.

is a single mass spectrum which was produced at near constant resolving power across the full *m/z* range. This approach has been termed "operation at constant ultrahigh resolving power" or "OCULAR". Using OCULAR, it becomes possible to acquire a mass spectrum with a constant ultrahigh resolving power, improved mass accuracy, reduced space–charge effects and increased dynamic range (Figure 3). In the published example, it was possible to assign a total of 244,779 individual molecular compositions in a truly non-distillable fraction of a heavy crude oil: the largest number of unique elemental compositions detected in a single sample to date.[3]

The approach is flexible and the performance that can be achieved using OCULAR depends on the number of windows and the target resolving power. For instance, a mass spectrum with an increased resolving power could be achieved by the acquisition of a minimum of two segments and, in this case, the mass spectra could be acquired in approximately 30 min. Heavier fractions of crude oil, however, will require higher performance and, therefore, a higher number of narrow windows in order to sufficiently boost performance. In the example of the truly non-distillable fraction, 65 windows were acquired with a mass range of 24 Da each in order to produce the complete mass spectrum.

A constant ultrahigh resolving power of approximately 3 million FWHM was achieved across the range of *m/z* 260–1505. This level of performance has enabled the resolution of species which differ in mass defect by less than the mass of an electron and has allowed the assignment of molecular compositions with sub-ppb (part-per-billion) mass accuracy. The extraordinary number of compositions spanned dozens of heteroatomic compositions, contained up to 114 carbon atoms, and represented up to 51 double bond equivalents (DBE). The high number of species with heteroatomic compositions (species containing sulfur, nitrogen and/or oxygen atoms) helped to explain the extremely low volatility of the truly non-distillable fractions of the crude oil and highlight the enormous challenges that will need to be addressed to upgrade heavier crude oils.

Further work

While the OCULAR approach has been used to successfully characterise a petroleum sample which had previously been too challenging due to its complexity, its applicability is not confined to petroleum samples. With the flexibility of the method, it is also suitable for the analysis of other complex mixtures and challenging samples, with anticipated applications in the fields of metabolomics, medical research, polymers, environmental analysis and renewable energy, amongst others. At the University of Warwick, usage and development of OCULAR is on-going and further publications utilising OCULAR will follow.

Acknowledgements

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Pre-processing spectroscopic data: for good or ill?

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At a recent international conference, I attended a good lecture by a scientist using Ion Mobility Spectrometry (IMS) in a food analysis application. During the talk, one slide mentioned that they had used Savitzky–Golay smoothing on the IMS data and that started me wondering. I asked why they had decided that they needed to smooth the IMS data and was told that as they did it routinely for infrared spectra they just applied it to the IMS data as well.

I thought a better approach might have been to decide what data processing was really required and be able to justify the additional data manipulation steps in terms of improving on an analytical figure of merit, for example. You really need to start by accepting that the spectroscopic data you have just measured isn't fit-for-purpose. Now measuring data of insufficient quality for the role it must play can have as many good (for "good" read unavoidable) reasons as bad.

Why is my raw data not fit-for-purpose?

One common reason is that you do not have enough sample. This may be unavoidable if there simply isn't more available, but can also arise by failure to prepare enough during sub-sampling. Surprisingly often it is worth going back to the source of the sample and simply asking if you can have a specific amount required to carry out your analysis. This can sometimes lead to 5 kg sacks of material requiring disposal at the end of the work, but remember in many settings the people carrying out the sampling normally work in tonnes not in milligrams. Lack of sample amount can also make the answer to the analytical question less reliable if you do not have enough to carry out a number of full-method replicates of the analysis to deliver a good estimate of the error in your result. For a fuller discussion on sampling and errors, see the Sampling Column in this issue.

Another can arise by not paying enough attention to the resolution settings on the spectrometer or method being run on the instrument. Be aware of the settings on instruments which are automatically averaging several scans for each data point they are recording as well as the actual number of data points being recorded across the width of the narrowest peak in the spectrum. Depending on the type of spectrometer being used, taking a setting which records too high a resolution can mean the scan time for each spectrum becomes long if a reasonable signal-to-noise ratio is required. This can also cause issues if the spectrometer is liable to drift, meaning there is not an infinite amount of time available for each of the independent measurements.

For hyphenated methods, such as gas chromatography/ion mobility spectrometry (GC/IMS) data which triggered this article, this resolution consideration will also include the time axis for the sample separation step (Figure 1).

With the introduction of the much more rapid ultra-high-performance liquid chromatography (UPLC® or UHPLC) systems, much effort was spent in increasing the speed at which the attached spectrometers were capable of scanning. This was so that sufficient data points could be obtained to properly define each peak, since analytes were eluting off the columns an order

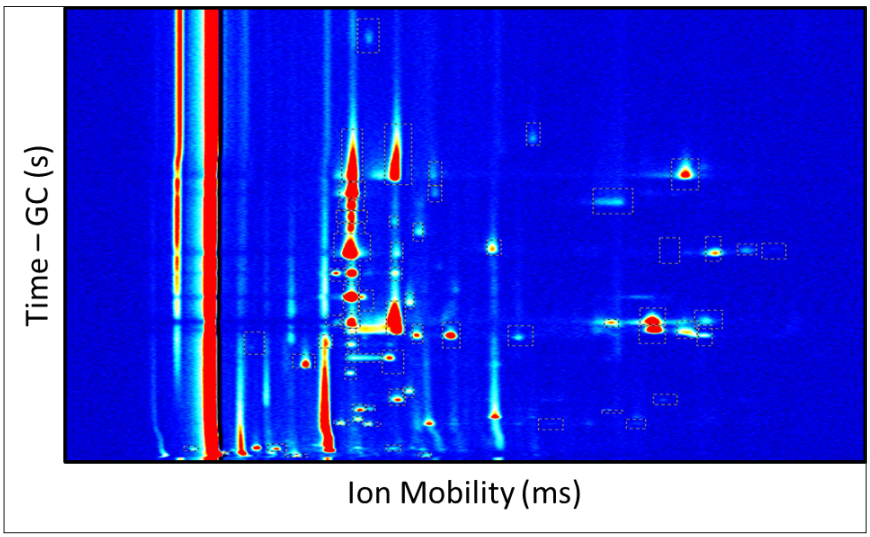

Figure 1. A somewhat typical GC/IMS analytical run showing relatively complex peak shapes compared to infrared spectroscopy.

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of magnitude faster, delivering much narrower, more intense peaks.

It is often the case that a system being studied is changing as it is being measured and this dynamic change is what you are studying. Clearly the time available for each independent measurement is constrained by the rate at which the system is changing, so it may not be possible to acquire many scans for each time point in order to achieve excellent signal-to-noise ratios.

A review by Engels and co-workers sums up some of the issues which lead to a demand for spectroscopic data pre-processing to remove unwanted artefacts in data sets under the headings of noise, baseline offset and slope light scatter temporal and spectral misalignment, normalisation, scaling and element-wise transformations, supervised pre-processing methods and finally artefacts in hyphenated techniques.[1] This is an excellent starting point if you wish to go deeper into the subject than this column's space allows. The authors acknowledge how extremely difficult it can be to determine which method or pre-processing methods can successfully be applied. It is important to take into account the specific data set characteristics emphasising that the identification of which artefacts are present among which properties of the spectroscopic data is of considerable importance that cannot be ignored in this choice of pre-processing strategies.

Approaches to spectroscopic data pre-processing: or "my boss told me to do it" syndrome

In some laboratories there are preferences for carrying out certain types of pre-processing as standard, and this includes standard ordering of the pre-processing steps. These have often been handed down over the years and the original reasons for these workflows are no longer known by the current laboratory staff.

Jan Gerretzen and co-workers at the University of Nijmegen working under the Dutch COAST initiative carried out some work to try and eliminate the "black magic" around the selection of the data pre-processing steps and the order in which they should be carried out. They adopted a systematic Design of Experiments approach to varying baseline, scatter, smoothing and scaling pre-processing steps for reference data sets in Latex monitoring (quantifying butyl acrylate and styrene) as well as corn data sets for their moisture content.[2] In a separate report the approach was tested on data from a near infrared (NIR) spectrometer monitoring NaOH, NaOCl and Na_2CO_3 concentrations in a waste treatment system of a chlorine gas (Cl_2) production facility. The gaseous waste effluent of this facility contains chlorine, which is removed by a caustic scrubber where the waste gases are led through a solution containing NaOH.[3]

Selection of pre-processing strategies

Quite often text books or spectroscopic data processing packages will describe the effect of individual pre-processing algorithms. However, there is little support around the consequences of applying multiple pre-processing steps during data analysis. Even the order that the pre-processing steps are applied can have a drastic effect on the quality of the analysis, let alone how the parameterisation of each step impacts subsequent steps or the final result.

Table 1 shows an experimental design used in this approach. A full factorial design was selected to evaluate the influence of each pre-processing step. The response variable measuring the model improvements from the pre-processing steps was the root-mean-square error of prediction figures.

Figure 2 shows how close the rapid Design of Experiments approach came to determining the best sequence and parameterisation of various pre-processing strategies, compared to identifying the absolute best strategy determined by Brute Force number crunching of every possible variable (over 5000 solutions required to be calculated).

Most authors highlight the fact that their work can really only be deemed applicable to the types of data and particular types of samples they are analysing. In Reference 1, the application of variable selection and data pre-processing were only observed to improve the model performance when they were carried out simultaneously[2] and the conclusion was that although the specific "best-case" data pre-processing solutions were found, the more general applicability of this work was in defining a successful generic approach to scientifically decide on the best spectroscopic data pre-processing methodology to use.

Peter Lasch looked at spectral pre-processing for infrared and Raman

Table 1. Data preprocessing Design of Experiments derived from Reference 1.

Experiment	Baseline	Scatter	Smoothing	Scaling
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	No
3	Yes	Yes	No	Yes
4	Yes	Yes	No	No
5	Yes	No	Yes	Yes
6	Yes	No	Yes	No
7	Yes	No	No	Yes
8	Yes	No	No	No
9	No	Yes	Yes	Yes
10	No	Yes	Yes	No
11	No	Yes	No	Yes
12	No	Yes	No	No
13	No	No	Yes	Yes
14	No	No	Yes	No
15	No	No	No	Yes
16	No	No	No	No

Data smoothing
Often used to reduce random noise where further data accumulation is not possible. Depending on the data set data smoothing can damage the data set leading to distorted picture of the results. Some typical data smoothing methods include Moving Average across a number of data points, the number of points averaged is adjustable and Savitsky–Golay smoothing which fits a polynomial to segment of the data set. In Savitsky–Golay smoothing the order of the polynomial can be changed (first-order = Moving Average) as well as the range of data to be fitted.

ATR correction
Correction of mid-IR spectra sampled using the Attenuated Total Reflectance (ATR) technique for the penetration depth dependence related to the frequency in the spectrum. It does not attempt to correct for the refractive index differences between the sample and the crystal that can lead to "derivative-like" spectra.

Multiplicative Scatter Correction (MSC)
Rinnan and co-workers took a critical look at a range of pre-processing methods in NIR spectroscopy chemometric modelling including a group of scatter-corrective pre-processing methods includes Multiplicative Scatter Correction using a reference data sets. They also looked at how different pre-processing methodologies impacted on the quality of prediction results for six different spectrometers using filter, dispersive and Fourier transform technologies. In whichever combination they applied pre-processing they could only achieve at best a 25% improvement in the prediction error—and the concluded with a warning about the risks associated with incorrectly setting the parameters for the window size or smoothing functions.[5]

Derivative filters
Quite a popular pre-processing strategy to enhance the resolution of complex spectra assisting in identifying overlapping peaks and also assists in minimising the influence of baseline effects. For instruments that acquire signals in the time domain such as Fourier transform infrared spectrometers several techniques exist to apply filters to enhance resolution and reduce noise in the time domain before the data is transformed to the frequency domain.

spectroscopic techniques used in the field of biomedical vibrational spectroscopy and microspectroscopic imaging.[4] Here techniques including cleaning the datasets (outlier detection), normalisation, filtering, detrending, transformations like ATR correction and "feature" selection are discussed. The article contains some interesting explanatory graphics and longer discussions on water vapour correction, different strategies for normalisation, baseline correction and data filtering for noise removal or spectral resolution enhancement (use of derivative filters). Raman-specific spectroscopic data pre-processing is also addressed, covering topics such as the removal of cosmic ray artefacts and fluorescence background signals. The author acknowledges that a combination of pre-processing steps is usually required to obtain the best results and bemoans the sparsity of systematic investigations in which the effectiveness of different ways of applying pre-processing workflows to the specific needs of subsequent quantitative or classification analytical procedures is investigated. The author acknowledges that it is one of the main data analysis tasks to adapt and optimise these workflows, but this is still more an art rather than a science!

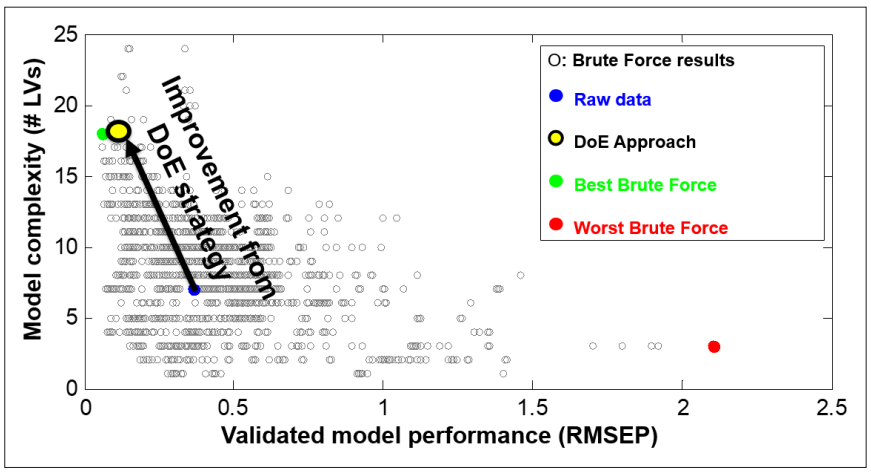

Figure 2. Successful application of a Design of Experiments approach to spectroscopic data pre-processing for model optimisation (data taken from the work reported in Reference 3).

Conclusion
I think it is clear that we are often constrained from measuring the ideal spectra for our tasks and that data pre-processing can eliminate or mitigate some of the problems arising from having to handle sub-optimal measurements. However, it is also clear that these pre-processing steps need to be carried out with our eyes wide open and after giving the problem some thought. The computing power now commonly available allows us to also use the Design of Experiments approach to find the best pre-processing strategy for our specific data sets—and that this pre-processing strategy needs to be re-assessed for each individual problem and not blindly copied across from one spectroscopic field to another.

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SAMPLING COLUMN

Sampling for spectroscopic analysis: consequences for multivariate calibration

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An alternative title for this column could have been: "What's in an analytical datum?" Analytical results, and with them multivariate chemometric models, cannot be validated in isolation; analytical results have a pedigree, a history, which influences the quality of determination just as much as the technicalities of the analytical method itself—in fact, often more so. The relevant issue is representativity **with respect to the primary target material, the lot. Analytical aliquots, or direct analytical signals, are the end-products of a compound "lot-to-analysis" pathway in which** all **preceding sampling/signal acquisition operations must be representative in order for analytical results (data in the chemometric world) to be valid renditions of the original lot material. The incurred sampling, sub-sampling, sample preparation and sample presentation errors, collectively constituting the Total Sampling Error (TSE_{TOT}), are typically 10–25+ times larger than the spectroscopic measurement errors alone (TAE_{SPEC}), i.e. TSE_{TOT} dominates the total measurement uncertainty budget. Focussing on analysis alone (TAE_{SPEC}) is, therefore, a breach of due diligence when seen from the point of view of the** user **of analytical results, which forms the basis for critical decision making in science, technology and industry. This column surveys the proper context for all critical steps** before **spectroscopic analysis and their impact on multivariate modelling of spectroscopic signals, irrespective of whether the TSE_{TOT} contributions are large, intermediate or small. All cases must be treated identically, including sensor-based solutions from the Process Analytical Technology (PAT) realm.**

Introduction

The key issue of "sampling" is material and lot *heterogeneity* and how to **counteract** its adverse influence on sampling/signal acquisition, sub-sampling and sample preparation/presentation processes, all of which demonstrably take place **before** analysis. The Theory of Sampling (TOS) is the guiding framework for meta-analysis of all spectroscopic modalities. The TOS emphasises the Fundamental Sampling Principle (FSP), which states that all potential units from an original material must have an equal probability of being sampled in practice, and that samples are not altered in any way after sampling. Units can be particles, particle fragments or collections-of-units making up the practical sampling unit, termed *increments*. In the realm of quantitative spectroscopic analysis, compliance with the FSP is rather often a hidden elephant in the room; far from always properly acknowledged. In this light, many potential pitfalls exist regarding analysis in the lab as well as in Process Analytical Technology (PAT) applications, which **must** be avoided, lest unnecessary Total Sampling Error (TSE_{TOT}) will be produced. These errors will uncontrollably inflate the total Sampling-and-Measurement Uncertainty (SMU).

The present column focuses on the adverse influences that may crop up in the chemometric data modelling "on the other side" of production of analytical results, if the basic representativity demands from TOS are not heeded. The TOS needs only minimal presentation is this column.

Theory of Sampling, TOS

The FSP is the first of six Governing Principles (GP) and four Sampling Unit Operations (SUO), which must be honoured in order to guarantee sampling and analysis representativity. In previous *Spectroscopy Europe* columns, and within the chemometric and spectroscopic communities (the NIR realm in particular), the TOS has been presented extensively to any depth desired, as a unified, systematic framework for all principles and practical operations needed before analysis. While it is often argued that the analyst is only responsible for TAE_{SPEC}, someone else must then be responsible for controlling TSE_{TOT}. This is a most unfortunate division, however, that positively invites a serious sin-of-omission: who is really in charge of guaranteeing representativity of the analytical result, if/when most of the uncertainty is incurred outside the complacent four walls of the analytical laboratory? We here argue that it is better to view the "lot-to-analysis" pathway as a unified whole, as a common responsibility, which **includes** the quantitative analyst (of any spectral modality) as well as the data analyst, whether of chemometric or statistical inclination.

This column is a reasoned call for a *holistic* view of sampling, analysis and data modelling as an integrated whole. The relevant literature is numerous, and presents the minimum TOS competence

necessary to be scientifically, technically and managerially responsible for guaranteeing relevant, meaningful and reliable analytical results—and for multivariate calibration models in all contexts, under all circumstances and for all types of materials that can be subjected to spectroscopic analysis. The comprehensive core reference list is all one needs.[1–8]

Reference 1 is a treatise answering the question: "WHY we need the Theory of Sampling (TOS)". Reference 2 is the most recent presentation of the TOS, and Reference 3 is of particular relevance for quantitative spectroscopic analysis. Reference 4 is the *de facto* international standard on the general principles for representative sampling, often accompanied by Reference 5 for full coverage of the relationship between the TOS and Measurement Uncertainty (MU). Reference 6 introduces readers to the key process technology interplay between the TOS and PAT. Reference 7 is the most recent chemometric textbook, in which the critical pre-sampling realm is fully integrated, including its bearings on proper model validation (issues not covered by any other chemometric textbook). Reference 8 is a feature on the first order issues related to the application of on-line NIR to predict pharmaceutical powder composition, as an example of a study following the holistic call. This column takes up this scope and will in particular deal with the consequences of TOS non-compliance for the chemometric community, for which "data" are usually considered sacred entities—in the sense that nobody cares much about the pre-analytical realm: "Chemometricians analyse and model the data—basta!"

Only two TOS elements are needed for the present purpose:

Fundamental Sampling Principle (FSP): "TOS—the missing link in PAT",[6] amplified by Reference 8, explained the difference between physical extraction of **samples** (representative) or **specimens** (non-representative) relative to TOS-compliant spectral interaction with a stream of matter (representative "PAT process sampling"), and how this difference results from failure to comply with the FSP as applied to flowing streams of matter (all explained more fully below).

Sampling Bias: Failure to eliminate the complement of Incorrect Sampling Errors (ISE), wholly or partly, will unavoidably lead to a sampling bias, of unknown magnitude, which cannot be corrected for as presented in many of this column's References. It will appear that within the PAT approach there are several major pitfalls if/when the pertinent TOS principles are not heeded (or are perhaps unknown).

In medias res

For the purpose of chemometric multivariate calibration/validation/prediction, we are at first interested in the relationship between:

- "From-lot-to-aliquot" (sampling + analysis, i.e. traditional physical sampling), and
- "From-lot-to-spectrum" (sampling via *in-* or *on-line* application of spectral analysis, PAT)

The traditional domain sampling + analysis needs only little comment. Seen from the point of view of the professional analytical laboratory, "samples" arrive in the lab, which is hired to produce the requested analytical results. Preferably representative samples, of course, but it is no mystery why many professional laboratories declare that the relevance, validity, quality and representativity status of primary samples is solely the responsibility of the client who supplies them, as this conveniently saves the day w.r.t. decisions made on the basis of the analytical results produced. Not surprisingly, we see in many cases that results supplied by Quality Control (QC) laboratories are understood as the absolute truth. And apparently with good reason, the analysis **is** representative of the sample delivered, but all issues about whether this means that it is representative of the entire lot from which it was extracted have disappeared. The demarcation between QC and production is never more pronounced than in this situation.

But it **is** also fair to say that such critical pre-analysis issues have begun to appear on the agenda, at least for some laboratories: "We know about the potential for gross sampling errors, that may very well jeopardise the objective of the client. Shall we tell him, or not?" There is (very) much more to discuss concerning the complex relationship between client, in this case production, and laboratory, and this was recently subject to an extensive analysis earlier in the Sampling Column.[9,10]

The in-, on-line realm (analysing *while* sampling) is of particular interest to the current column. Historically there has been a trend within PAC (Process Analytical Chemistry) and PAT to consider installing PAT *sensors* into a pipeline as synonymous with: "No sampling needed—spectra are acquired directly", but this is a mistake of the highest order! Reference 6 was the first to

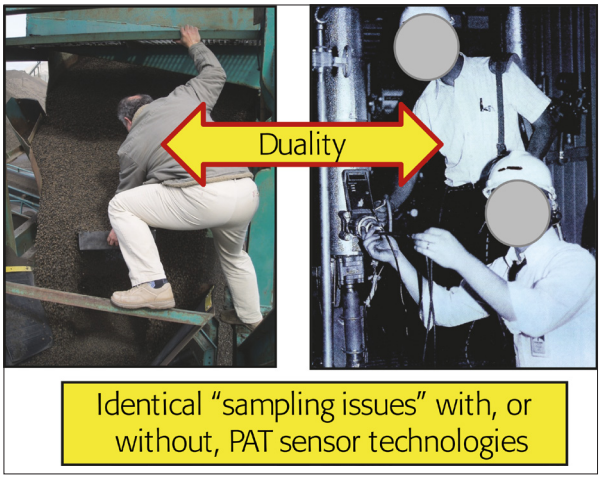

Figure 1. The fundamental sampling **duality**. Physical **or** optical grab sampling incur identical sampling error effects (ISE, CSE). Illustration copyright KHE Consulting, reproduced with permission.

SAMPLING COLUMN

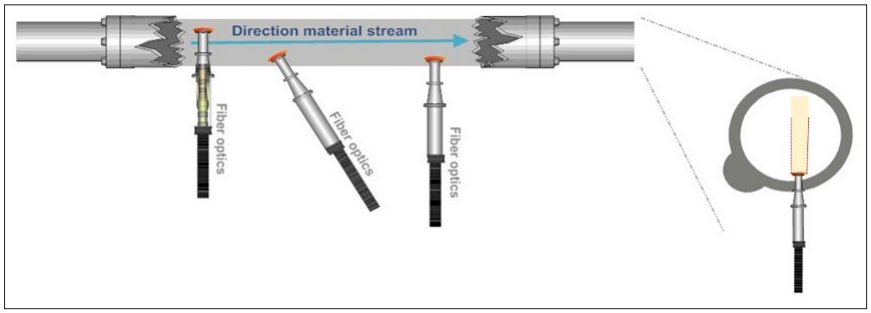

Figure 2. The "PAT sensor application solves all sampling issues" fallacy, which follows because PAT sensors are not "seeing" a volume corresponding to a full cross-sectional **slice** of a moving stream of matter, see also Figure 3. Illustration copyright KHE Consulting and Martin Lischka, reproduced with permission.

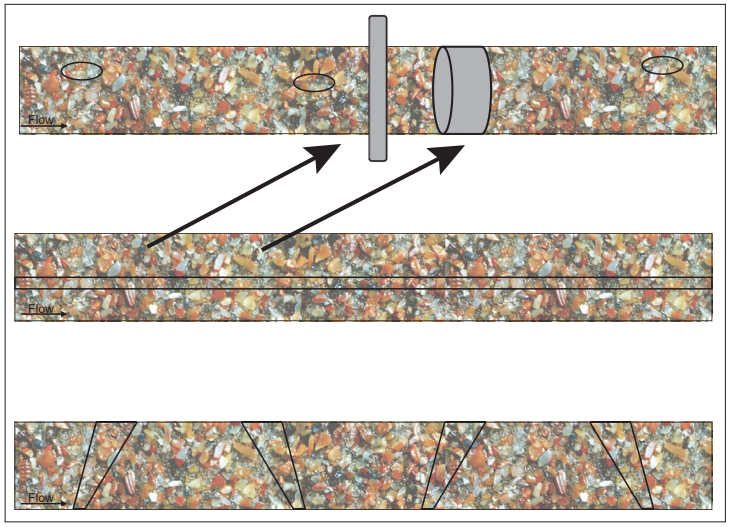

Figure 3. Massive ISE as a result of **non**-compliance with TOS' principles. This figure can alternatively be understood as looking down on the top of moving conveyor belts, or as representing a longitudinal section of a ducted flow in a pipeline. "TOS-correct" delineation and extraction of cross-sectional increments, or sliced stream volumes, are shown in grey in the top panel. All other indicated increments give rise to a significant sampling bias. Illustration copyright KHE Consulting and Martin Lischka, reproduced with permission.

deal intensively with what is a sampling *duality*, Figure 1.

This "no sampling" fallacy optimism is illustrated by showing how "direct" application of a PAT sensor does **not** eliminate the occurrence of massive ISE, Figures 2 and 3.

It seems difficult to understand why this fallacy has originated, and why it has been propagated during at least two decades in the PAT realm. The strict truth is that **only** a full slice of the stream of matter qualifies as the proper volume/mass *support* for a representative increment/signal.[a]

The above leads directly to a fundamental distinction in this context between:

1) A *sample* cell (sample: noun) and,
2) A *sampling* cell… (sampling: verb)

Upon reflection, it is the act of *simultaneous* sampling-and-analysis that distinguishes the sampling cell—and which in a sense may appear as making (physical) sampling superfluous. But this latter is critically dependent on representative sampling, a proviso of overwhelming importance. If sampling is **not** representative, all manner of unknown, inconstant sampling bias will still be part of the equation, totally destroying the "no sampling" claim. On the other hand, if/when a sampling-and-analysis cell complies with TOS' demand for representativity, conditions are right for reaping the powerful advantages of the PAT revolution, but only then. Reference 6 treats these issues in detail.

A cursory survey of relevant industrial process technology and dedicated PAT literature from the last 10 years or so does not impress. Unwitting neglect of the "full slice" dictum can be found in abundance (but there are moments of satisfaction as well). The mission here is **not** to identify which are which, but only to direct attention to the critical need for a certain minimum TOS competence in the PAT realm.

Proper application of TOS' relevant GP and SUO in the pre-analysis realm is a mandatory requirement in order to **guarantee** that samples, or the spectral acquisitions from matter streams, can be proven to be representative. Failure to live up to this demand will result in *compromised* analytical samples/signals with which to begin a subsequent chemometric data analysis or modelling.

As a prominent contemporary example, consider the rapidly expanding case of continuous manufacturing (CM) in the pharmaceutical industry sector. Figure 5 shows the many locations in the CM pathway where NIR spectroscopic characterisation finds very good use. Figure 5 also shows where one would easily lose one's way were not a modicum of TOS competence involved in the design, implementation, validation and operation

[a]The present column presents the strict demands for representative increment extraction/signal acquisition. The reader will realise that often there are severe practical difficulties involved when trying to comply herewith, for example that the effective NIR path length is ~30 mm while the effective duct diameter can be larger (much larger), say 100 mm or more? "Smartly" implemented reflectance probes *may* go a certain way to remedy this shortfall, but are essentially bracketed by the same path length maximum. A bypass duct will quickly become of significant interest a.o. The issues raised in the present column will be addressed in the form of **solutions** in the next Columns in this series.

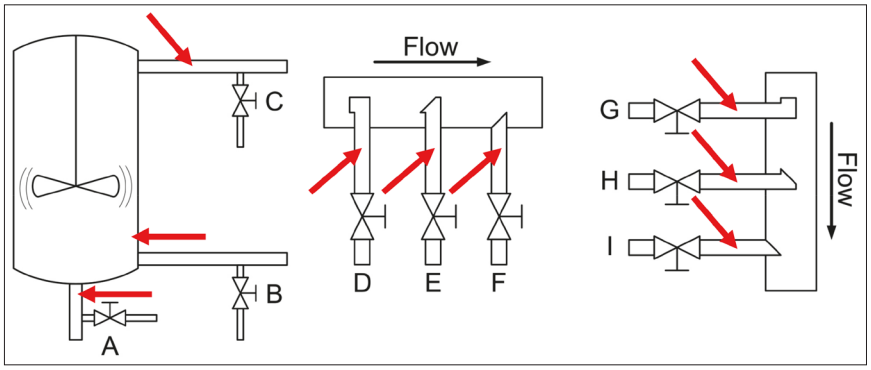

Figure 4. Overview of the many possibilities for installing "sample extraction valves" and "PAT sensors" in traditional process industry. Note that all configurations shown here will lead to incorrect, i.e. biased samples or spectral signals as regards representativity because their support volumes do not correspond to full stream slices. Reference 6 treats these process sampling issues in depth w.r.t. **solutions** to the problems emphasised. Illustration copyright KHE Consulting, reproduced with permission.

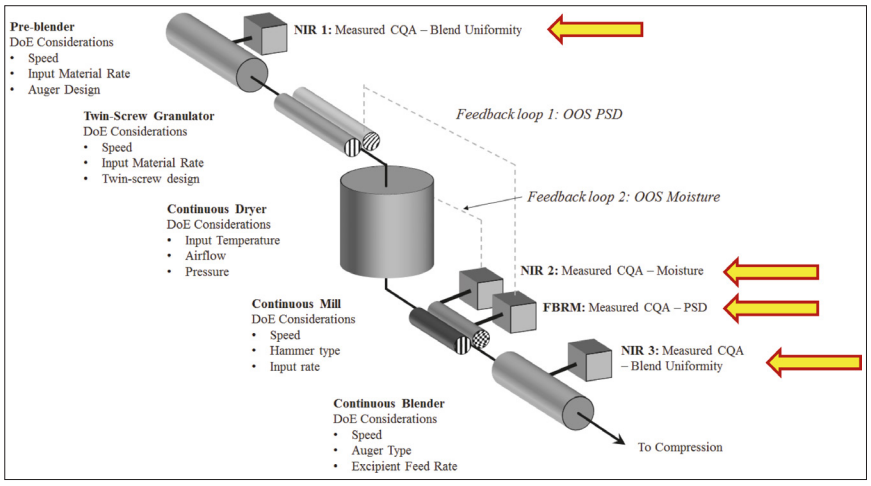

Figure 5. Four process analytical locations involved in CM, based on in-line sampling-and-NIR spectroscopic prediction of pharmaceutical API composition, moisture, PSD and blend uniformity. In addition to ISE associated with incorrect support volumes, which will affect the quality of spectral X-data in multivariate calibration, there is also the equally important issue of the quality of corresponding Y-data, for which representative reference samples must be obtained. But **where** exactly, and **how**, should these be extracted? This is a classical sample extraction issue, far from always properly acknowledged and far less satisfactorily solved, primarily because TOS continues to be a partly (largely) little acknowledged critical success factor in process technology, spectroscopy and chemometrics. Illustration copyright QbD Consultancy, reproduced with permission.

phases of starting up using this manufacturing approach.

While it may often *seem* to be relatively easy to obtain "direct spectral data" *via* inserted PAT sensors, as indicated in Figures 4 and 5 (X-data in regression), these are nevertheless critically *dependent* on whether the ISE issues outlined above have been successfully eliminated, or not. Failure to comply with this requirement is the by far the most often met with deficiency within a trigger-happy chemometric community; the literature is full of illustrative examples, but we shall here refrain from identifying journals, papers, authors—the task here is to sound a warning against continuing to be unaware (or to wilfully neglect) the critical support volume dictum. Fortunately, a lot of work has been performed by the pioneers of CM systems to address such sampling issues and the fact is that a CM system essentially reduces a traditional 3-D sampling plan (traditional approach to manufacturing) with a 1-D sampling situation. This is the optimal, TOS-correct understanding from which to begin to look for solutions to the sampling issues warned about.

In addition, in order to perform *proper* multivariate calibrations for one or more y-variables, it is necessary to extract relevant, valid and representative reference samples (Y-data in regression). Indeed, this also applies for proper test set samples to be used for *validation* of the desired multivariate calibration models with which to carry out on-line prediction of blend uniformities (real-time compositional variation), moisture, PSD… .[7] A documented facility for representative acquisition of **both** sensor signals (X-spectra) and reference samples (reference data) **must** be present, or multivariate calibration/validation models for prediction will forever continue to suffer "impossible to reduce" prediction errors etc. These issues are often described in suspiciously murky fashions in the literature; one is tempted to interpret this as if authors actually do understand the fundamental ISE issue here, but are at a complete loss to come up with solutions that work. In fact, "sweeping the problem under the carpet" has led to many process failure investigations and "incomprehensible results", which in reality is fighting fires that simply are just not there in the first place.

Many skills needed

This column has the purpose to introduce all elements from the diverse disciplines of i) the TOS, ii) process engineering, iii) spectroscopic analysis, iv) sensor technology, v) PAT and vi) chemometric data analysis. All need to acknowledge that analytical results pertaining to heterogeneous materials and systems have a **history** in which some degree of sampling (primary, secondary, tertiary[b]) is always present.[c] For this fundamental

[b]i.e. sub-sampling/splitting in several stages; these are *bona fide* sampling processes in their own right.

[c]Exceptions, for example uniform materials, de Beer dilutions etc. cannot establish a basis for solving the infinitely more complex issues surrounding the kind of significantly heterogeneous materials treated here.

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reason "What's in an analytical datum?" will always include a significant complement of error effects and uncertainties stemming from this pre-analysis realm (smaller or larger, but never absent). The point is that this state-of-affairs must be acknowledged by analysts and data analysts; this point has been forcefully argued in Reference 5. It is no longer appropriate to pass the responsibility onto someone else. If PAT is going to be implemented successfully by an organisation, all involved in quality, production, regulatory affairs, as well as management, must be on the same page.

Chemometric data modelling

"Compromised samples or signals"—in what sense? Compromised, because extraction of physical samples or acquisition of spectral signals will be associated with a significant sampling bias. The nature of a sampling bias is that it cannot be corrected for by any means, data analytical or statistical, as distinct from an analytical bias, which can be subjected to a statistical bias-correction. This, perhaps surprising, distinction is treated in full in References 2–7.

The effect of this on chemometric data analysis and modelling is like the proverbial elephant in the room, generally unnoticed. Put simply, no manner of data analysis, data modelling etc. from the chemometric and the statistical domains will be able to correct for a sampling bias; see References 6 and 7 for a full argumentat for *why* this is not possible; also see Figure 6.

What is the specific effect on multivariate data analysis, modelling and calibration?

First: There will be an *inflated* total MU associated with every analytical result, very often significantly larger than the specific analytical error itself (which *may* occasionally also be significant of course, but only as a result of an analytical method not in proper control; such an issue will eventually be brought under control, GLP a.o.).

Second: There **will** always be a component of the multivariate data errors so effectively screened away by the powerful bi-linear data modelling approaches in chemometrics that must be taken into account when appropriate. Figure 7 shows a principal illustration of chemometric decomposition of multivariate data into systematic data structures (principal- and PLS-components) and decoupled multivariate data errors (εs in chemometric parlance).

A tacit understanding within chemometrics has been that bi-linear errors (εs) would turn out to include TOS-errors stemming from sampling deficiencies, in which case it would actually be possible to correct for ISE after all. Indeed, multivariate data analysis would then appear on the scientific scene with an unbelievable power, not even known or foreseen in the genesis and development of chemometrics. Alas, this is not so!

The effect of sampling bias inflation of the total sampling + analysis uncertainty level is such that both data analytical *components* as well as their complementary errors (εs) are affected by the inconstant bias effect. Because TOS-errors are expressed for single variables in turn, the bias will affect each individual variable differently. The sum-effect of an unresolved sampling bias is such

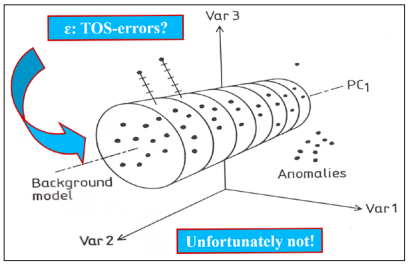

Figure 7. Chemometric bi-linear data model of pervasive data structures, PCA or PLS-components (schematic sketch). The multivariate data model errors (εs) are often optimistically thought of as "TOS-errors" (TSE_{TOT} + TAE), but they are not, see text for clarification.

that TSE_{TOT} will vary every time a new analytical determination is attempted on a new sample. This means that every new sample added to an already existing data matrix, think of a training data set, will each add its own, varying contribution to the total data variance—and thus also to the total data set covariance. Thus, both components and errors will be affected. These issues are described in more detail in Reference 7.

For completion, the complement of Correct Sampling Errors (CSE) will also affect each variable individually, after elimination or maximal reduction of ISE.

Consequences for chemometrics

Because there are many influential agents involved for each sample extracted, or for each signal acquired by a PAT instrument, it may easily be an unhelpful simplification to understand all "data" as but identical realisations of variables, each with a systematic information content to which is added a stochastic

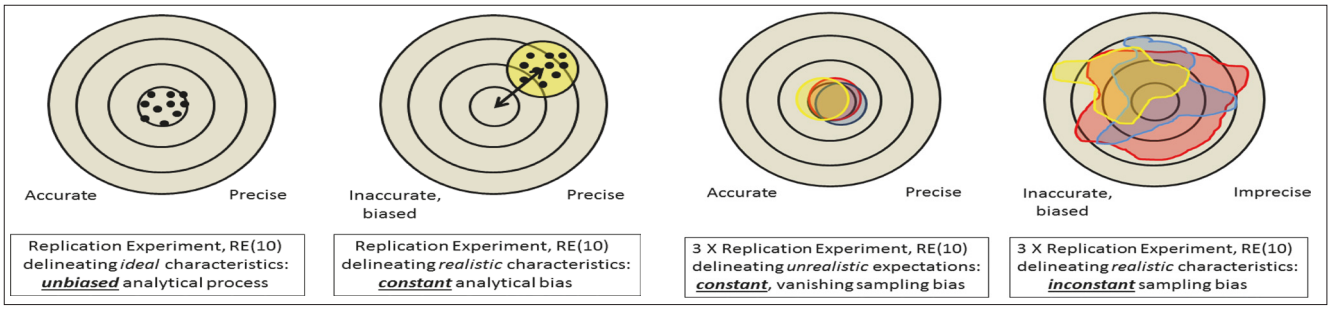

Figure 6. Analytical bias (left) vs sampling bias (right). Bias effects are shown as manifested by Replication Experiments (RE).

error complement. Within chemometrics the former can be successfully modelled by data analytical "components" and the latter can be conveniently identified, quantified—and then discharged, so this would be "all one needs to know" **if** data are always, *ipso facto*, representative and reliable. This is not so, however!

This column has argued that the background for each individual datum, and for analytical data collected as matrices must be appreciated in a more fully developed setting in which significant parts of the traditional "measurement error" also contain contributions, often large contributions, related to the specific history of each sample, aliquot or spectral signal support. The data analyst must be mindful of this intricate relationship, lest glib, simplistic interpretations of "measurement error" will run a grave risk of not reflecting the more complex reality.

The archetypal manifestation of these relationships is shown in Figure 8, which highlights the fact that any chemometric prediction model falling short of sufficient performance, for example as evidenced by a "too high" $RMSEP_{validation}$, can **only** be improved upon by caring about the TSE incurred for all data, critically based on the full understanding that the error complement is overwhelmingly made up of contributions by the TSE_{TOT}.

Thus, it is not a guaranteed successful strategy to care only for the "data" as such, with an aim of optimal data analytical modelling systematics (chemometric components), *perhaps* acknowledging a minor measure of accidental analytical error in addition. This will, therefore, **not** include the major determinants stemming from unrecognised, or deliberately overlooked, sampling errors and their incurred uncertainties. Interpretation of standard regression-prediction figures-of-merit, e.g. $RMSEP_{validation}$ must be based on a modicum of TOS knowledge and competence in order to be able to *improve* on unsatisfactorily "imprecise" prediction performance statistics. In our collective experience many data analysts skip straight to the R^2 value of a fitted regression line **as if** this was some form of magical truth statistic, but it only relates to model fitting.

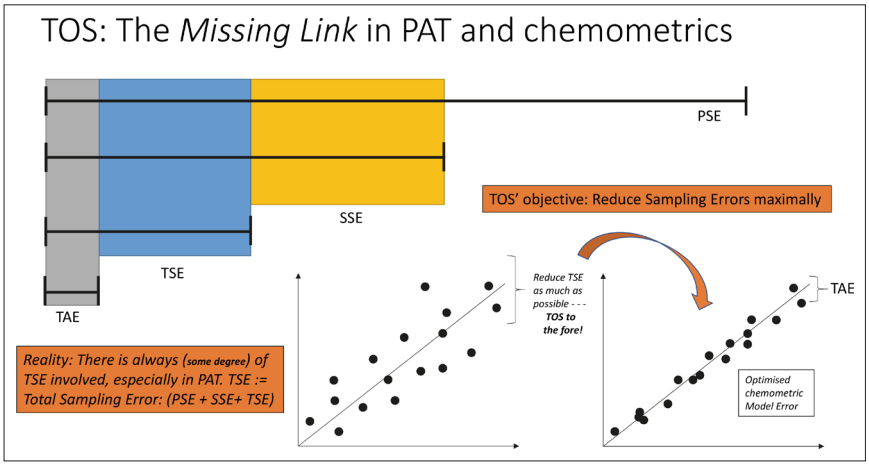

Figure 8. Theory of Sampling (TOS)—the missing link in PAT and chemometrics. Key effects shown here concern decoupling of TSE_{TOT} into contributions from primary sampling errors (PSE), secondary and tertiary sampling (SSE, TSE) and the analytical error s.s. (TAE). The lower panels illustrate how an unsatisfactory $RMSEP_{VALIDATION}$ of a multivariate prediction model manifests itself on a "predicted vs reference" plot. The TOS is the only approach that outlines solutions that lead to reduction of TSE_{TOT}.

Here is the most relevant criterion for evaluation: for an optimal bi-linear model, the RMSEP errors should be statistically *comparable* to the reference chemistry errors and this situation should be achieved with a relatively small number of model components. The implicit modelling of random artefacts in the spectral data to overcome material heterogeneity and non-representative sampling **only** relates to the miniscule test volumes involved. If a large number of model components is required to achieve your error target (if this is even at all possible), this is only modelling a *mirage*. If a chemometric model cannot be brought below your *a priori* established RMSEP error threshold, it is telling you there is a fundamental problem *outside* of the analytical realm.

This is true information—for which no amount of calling for "more samples", more spectra (to average), more model components will ever help. The unpleasant situation, Figure 8, simply means that you must focus on improving your sampling practices. TOS to the fore!

Conclusions

The one sure way **not** to be able to reduce the uncertainty elements behind data analytical models that does not comply with desired prediction performance goals, is the traditional call for more data (an approach very often cited in the literature and observed in practice). More data, meaning more samples for analysis, will always display the same TSE_{TOT} characteristics as the samples already included in the contemporary training data set, see Reference 7. The number of times this futile call has been heard in practice is overwhelming, and is usually preached to those with little experience in the PAT/chemometrics fields in order to avoid the more difficult problems revealed here. Focussing on the root cause, i.e. *why* the samples and their analytical result do not match with reality is a simple sampling issue, however, and must be treated as such.

In fact, most of the initial efforts in PAT implementation and data modelling should be focused on improving and optimising sampling—way before analysis and data analysis. As the saying goes: "if the data already contain the information, then the chemometrics will succeed". However, if the data are swamped by sampling noise, even applying implicit or explicit "correction functions" will still not improve the accuracy of the analytical results, because this inaccuracy can never be modelled away.

Chemometrics is not a black box, "push button" approach where the modelling will automatically do the rest!

SAMPLING COLUMN

Automated chemometrics routines in software packages should be outlawed and never used! How dare a vendor provide software to replace the many years of competent chemometricians' experience around the world by reducing our collective practice down to a single automated routine! And when this approach does not work, the same vendors tell their clients to collect "more samples" to avoid the issue at hand. Chemometrics is not a supermarket of models, but a scientific expertise area where all sources of variation must be understood such that the model is interpretable and validateable.

Barring trivial, accidental TAE mishaps (which are always special cases, only of interest to themselves), the only way to reduce unsatisfactory (TSE + TAE) levels is by reducing TSE_{TOT}. Thus, the **only** way to be able reduce the "troublesome", apparently incompressible uncertainty contributions behind unsatisfactory multivariate data analytical models, Figure 8, is to master the necessary basics of the TOS.

Chemometrians are not exempt from these scientific insights. There is no longer an excuse to hide behind "I don't need to learn chemometrics, the superior software will sort it out for me". Like with CGMPs for the 21st Century, we also need to take a 21st Century approach to the full sampling–analysis–data analysis pathway, otherwise we will be travelling the same merry go round, always chasing our own tail and never progressing.

The promise

We shall address the many issues pointed to in this column from the point of view of solutions in the next columns in this series.

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The authors

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Brad Swarbrick is owner of Quality by Design Consultancy (www.qbd-consultancy.com) and Co-founder of KAX Group (www.kaxgrp.com). He is a world recognised expert in the application of chemometrics and design of experiment methodology to Process Analytical Technology (PAT) applications in Quality by Design (QbD) environments.

ATOMIC

Agilent announces new ICP-OES systems

Agilent Technologies has announced a new generation of ICP-OES spectrometers, the 5800 ICP-OES and 5900 ICP-OES systems, that incorporate new smart capabilities that provide insight into samples, processes and operational status. Greater instrument intelligence enables labs to avoid unplanned down-time and take a more pro-active approach to reduce the need to remeasure samples.

Agilent's IntelliQuant feature captures data from the entire wavelength range, identifies spectral interferences and provides recommendations to ensure correct analysis. Sensors and counters tell the user when maintenance is needed. The Neb Alert feature continuously monitors the nebuliser, and alerts the user when the nebuliser needs cleaning or is leaking. The ICP Expert software includes data analytics and smart algorithms that help with method development and automate troubleshooting, including Fitted Background Correction (FBC), Fast Automated Curve-fitting Technique (FACT), Inter Element Correction (IEC) as well as IntelliQuant.

Agilent Technologies

http://link.spectroscopyeurope.com/31-114

New OES analyser for complete metals analysis control

Hitachi High-Tech Analytical has introduced the OE750, a new optical emission spectrometer for verifying incoming materials specifications in metal processing facilities. It covers the entire spectrum of elements in metal and has low detection limits. New optical concepts, with four patents pending, using CMOS detector technology is behind the high level of optical resolution and large dynamic range of the OE750. As a result, this new analyser has a very wide wavelength range, meaning it can measure the entire range of elements within metals at low ppm levels. The analyser can meet the requirements of relevant standards and specifications, such as the ASTM E415 test method for carbon and low-alloy steel and ASTM E1086 the Standard Test Method for the analysis of Stainless Steel by Spark Atomic Emission Spectrometry.

One advantage of the OE750's new optics design is the fast start-up time. The instrument is ready for use in less than an hour, thanks to the low volume occupied by the optics. This aids high throughput production, where facilities need to check 100% of the material supplied. In addition to the new optics design, the OE750 has a new sealed spark stand with optimised laminar flow; this reduces argon consumption, reduces the likelihood of contamination and reduces maintenance requirements. A mid-pressure system with low-pressure argon purge cuts down the pump usage. This reduces the power consumption of the pump by 90% and enables an oil-free design to be used, increasing reliability and instrument uptime.

In addition to new hardware, the new OE750 includes software such as the Hitachi GRADE Database that includes more than 12 million records for over 339,000 materials from 69 countries and standards. As an option it comes with a SPC/LIMS package enabling an easy an effective monitoring of processes involved with the instrument and the processes to be controlled with it. This can be used to meet demands from standards such as IATF 16949.

Hitachi High-Tech Analytical Science

http://link.spectroscopyeurope.com/31-111

NEW PRODUCTS

FLUORESCENCE

Microscope connection for PicoQuant's FluoTime 300

PicoQuant have introduced the FluoMic accessory that enables the FluoTime 300 time-resolved fluorescence spectrometer to be used with remote samples without requiring any lengthy alignment or coupling procedures. The FluoMic's pre-aligned fibres allow the shining of excitation light from both pulsed and steady-state sources of the spectrometer to a microscope, such as the Olympus BX43, with a special microscope coupler unit. Emission is collected from a small sample area (down to 2 µm spatial resolution) and guided via a fibre to the detection arm of the FluoTime 300.

PicoQuant

http://link.spectroscopyeurope.com/31-112

IMAGING

Hyperspectral camera

NIREOS, a spin-off from the Politecnico di Milano University, has introduced a new hyperspectral camera, HERA. This is a compact device (15.7 × 15.5 × 11 cm) and can capture a continuous spectrum in the 400–1000 nm region of each pixel of a scene in just a few seconds. HERA is based on a patented Fourier-transform technology, which dispenses with filters or gratings, which typically limit the light throughput. Instead, HERA employs an interferometer with a 1 cm clear aperture that ensures acquisition of images even in challenging conditions (such as low illumination light, fluorescent hyperspectral imaging etc.). HERA has <1 nm resolution at 400 nm wavelength. When it is not required, the user can choose via software to perform quicker measurements with worse spectral resolution. The software immediately provides the hyperspectral processed image and offers the capability to perform a first data analysis, enabling the user to check and compare the spectra of selected pixels.

NIREOS

http://link.spectroscopyeurope.com/31-119

OPTICS

New max power mode for picosecond pulsed laser driver

PicoQuant has upgraded its Taiko PDL M1 picosecond pulsed laser driver, which is now capable utilising the full power of its diodes. The new max. power mode of operation allows running all existing and new laser heads at the maximum pulse energy possible for any repetition rate setting. The selection of laser heads is expanded with modules emitting in the green (530 nm, 560 nm and 595 nm) as well as a new generation of high-powered multimode diodes covering the visible to NIR range.

PicoQuant

http://link.spectroscopyeurope.com/31-117

New picosecond pulsed lasers

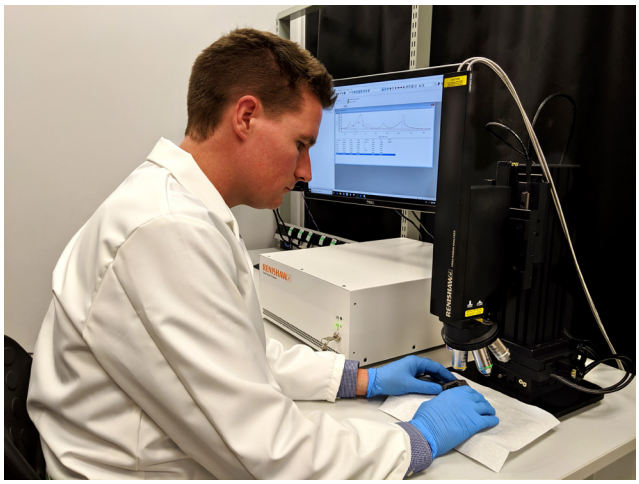
PicoQuant's VisUV platform of high-powered, picosecond pulsed lasers has been expanded with two modules, the VisUV-280-560 and VisUV-295-590. They generate picosecond light pulses at 280 nm/560 nm or 295 nm/590 nm with maximum average output powers in the UV of more than 1 mW or more than 0.5 mW, respectively. Both modules are suitable for exciting molecular probes, nanoparticles or quantum dots in time-resolved spectroscopy and microscopy applications, including STED super-resolution microscopy.

PicoQuant

http://link.spectroscopyeurope.com/31-120

RAMAN

Renishaw's new Virsa Raman analyser



Renishaw's Virsa Raman Analyser is a fibre-optic-coupled Raman spectroscopy system designed for remote analysis. It enables the expansion of applications of Raman spectroscopy to a new range of samples and environments beyond the confines of a laboratory Raman microscope. The system includes a spectrometer with one or two internal lasers—the dual excitation option enables the user to avoid fluorescence by switching between wavelengths at the touch of a button. Users also have a choice of Video Fibre Probes (VFPs): the VFP10 for general and bulk sampling and/or the VFP20C for high spatial-resolution confocal measurements. Renishaw-supplied VFPs can be used separately or stacked to share a common objective lens to enable concurrent analyses at different laser wavelengths.

The Virsa Raman Analyser has a modest footprint. It can be used on a benchtop or mounted in an industry standard 19" rack providing a transportable option. The Virsa Raman Analyser supports a range of third-party probes. It can also be integrated with third-party systems, enabling users to analyse samples using two or more techniques at the same time without having to transfer them between instruments. Additional sampling options are also available, including the SB100 three-axis probe positioner, immersion probes and macro sampling kits.

Renishaw

http://link.spectroscopyeurope.com/31-109

Virtual TruScan RM app enables method validation in the cloud

A new virtual companion to the Thermo Scientific TruScan RM handheld Raman analyser is available for pharmaceutical and biopharmaceutical manufacturers that continue to move toward digitisation and cloud computing. It enables reprocessing of spectra using TruScan RM's algorithm in the cloud and allows for method validation without a physical sample. The Virtual TruScan RM (VTR) app is designed to save pharmaceutical manufacturers time and money by accelerating the release of new materials into production and reducing costs related to existing global method validation processes. This digital twin also strengthens manufacturers' ability to assess falsified and substandard medicines.

The VTR App expands the capabilities of the TruScan RM and can be linked to Connect, Thermo Fisher's platform for secure, cloud-based data storage, scientific analysis apps and peer collaboration tools.

Thermo Fisher Scientific

http://link.spectroscopyeurope.com/31-115

UV/VIS

Measuring the flux of UV LEDs

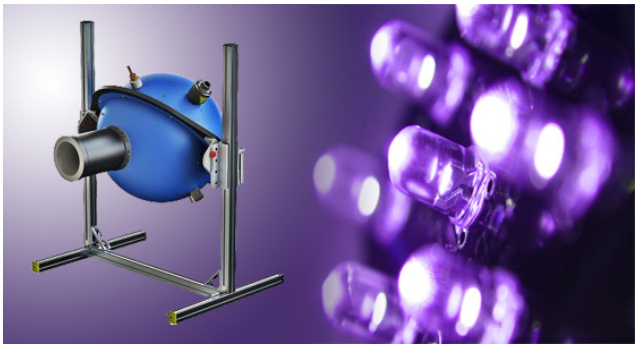
Integrating spheres are widely used to measure the total light output (radiant or luminous flux) of light sources such as lamps, luminaires and LEDs, but few (if any) come calibrated for measuring the output in the ultraviolet (UV) part of the electromagnetic spectrum below 350 nm. This has presented those working with specialist UV LEDs operating in the UVC (200–280 nm), UVB (280–315 nm) and UVA (315–400 nm) bands with something of a problem. The new Labsphere UV LED Flux Spectroradiometer available from Pro-Lite comes calibrated to NIST standards from 200 nm to 980 nm. It is purpose designed for measuring the spectral radiant flux in Watts of UV-VIS-NIR

NEW PRODUCTS

LEDs. It combines a compact integrating sphere for total hemispheric collection of the emitted light with a fast array spectrometer with an advanced stray light correction algorithm for accurate readings in the UV. Each system is supplied with a NIST-traceable spectral flux standard. The system can be configured with a temperature-controlled sample holder for studying the variation of light output as a function of temperature. LIV sweeps may also be performed to investigate the full gamut of electrical and optical characteristics of the LED under test. For visible wavelength LEDs, the spectroradiometer will of course report photometric and colorimetric values (lumens, CCT, CRI etc.).

Pro-Lite

http://link.spectroscopyeurope.com/31-118



PoliSpectra M116 provides up to 32 channels in UV-NIR spectroscopy

HORIBA Scientific has announced a new fibre-coupled multispectra system capable of simultaneous measurement of up to 32 channels. The PoliSpectra® M116 MultiTrack Fiber Spectrometer features a concentric optical design with UV extended spectral range (below 185 nm with optional N_2 purge) and a customized fibre bundle providing high throughput and excellent imaging quality with minimal crosstalk. This new product offers a high speed and low-noise 2-D scientific back-illuminated CMOS sensor running at 94–188 frames per second which can be configured with 8, 16 or 32 fibre input channels for simultaneous acquisition of UV-NIR spectra (2048 pixels per spectrum). This design enables PoliSpectra M116 to provide spectral resolution of 1 nm, combined with high sensitivity. Additionally, the high QE

sCMOS sensor (95 % in the visible) and an integrated order sorting filter allow wavelength coverage beyond 1 µm.

HORIBA Scientific

http://link.spectroscopyeurope.com/31-113

X-RAY

Handheld XRF analyser for identifying ionic salt raw material

The Thermo Scientific IonicX portable X-ray fluorescence (XRF) analyser verifies the identity of ionic salts. It can identify and authenticate the top five salts used in biopharmaceutical and pharmaceutical manufacturing: NaCl, KCl, $MgCl_2$, $CaCl_2$ and NaOH. This capability can reduce the time and cost of pharmaceutical materials testing while helping to maintain regulatory compliance and quality standards. The IonicX meets good manufacturing practices (GMP) and the 21 CFR Part 11 requirements. Designed specifically for the pharmaceutical industry, the IonicX XRF analyser is easy-to-use, portable, fast and requires minimal sample preparation.

Thermo Fisher Scientific

http://link.spectroscopyeurope.com/31-110

SciAps introduces new X-ray analyser

SciAps' X-550 is a new handheld X-ray analyser, which weighs 2.8 lbs with the battery. The X-550 supports alloy applications, including low silicon (Si) for sulfidic corrosion, residual elements (API 751) and API 5L alloy chemistry requirements. The "nose" of the analyser is narrow allowing it to access tight spaces. The X-550 analyses common alloys in 1 s or less. For alloys requiring longer test times or two-beam analysis, pre-configured on-board apps assure the correct testing by every operator.

SciAps

http://link.spectroscopyeurope.com/31-116

Conferences
2020

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

27–29 January, Liege, Belgium. **Chemometrics 2020 Conference.** chemom2020@sciencesconf.org, https://chemom2020.sciencesconf.org/.

29–31 January, Ghent, Belgium. **16th International Symposium on Hyphenated Techniques in Chromatography and Separation technology.** https://kuleuvencongres.be/htc16/.

6 February, Guildford, United Kingdom. **6th BMSS Ambient Ionisation Special Interest Group (SIG) Meeting.** Andrew Ray, andrew.ray@astrazeneca.com, https://www.bmss.org.uk/bmss-ambient-ionisation-sig-meeting/.

16–21 February, San Diego, United States. **2020 Ocean Sciences Meeting (OSM).** meetinginfo@agu.org, https://www2.agu.org/ocean-sciences-meeting/.

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/.

23–27 February, San Diego, United States. **The Minerals, Metals & Materials Society (TMS) 2020 150th Annual Meeting.** mtgserv@tms.org, https://www.tms.org/tms2020.

22–26 March, Philadelphia, United States. **259th American Chemical Society National Meeting.** natimtgs@asc.org, https://www.acs.org/content/acs/en/about/governance/committees/cwd/meetings.html.

4–7 April, San Diego, United States. **Experimental Biology 2020.** eb@faseb.org, https://experimentalbiology.org.

26–29 April, Oviedo, Spain. **The 5th International Glow Discharge Spectroscopy Symposium.** Peter Robinson, pete@masscare.co.uk, https://www.ew-gds.com/.

3–8 May, Vienna, Austria. **2020 European Geosciences Union (EGU) General Assembly.** secretariat@egu.eu, https://www.egu2020.eu/.

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

24–26 May, Rome, Italy. **8th CMA4CH Meeting, Measurements, Diagnostics, Statistics in Environment and Cultural Heritage fields.** infocma4ch@uniroma1.it, http://www.cma4ch.org.

24–28 May, Winnipeg, Canada. **103rd Canadian Chemistry Conference.** http://www.ccce2020.ca/.

27–28 May, Graz, Austria. **chii2020.** http://www.chii2020.com/.

31 May–4 June, Houston, Texas, United States. **68th ASMS Conference.** https://www.asms.org/conferences/annual-conference/future-annual-conferences.

4–5 June, Muenster, Germany. **2nd Workshop on Laser Bioimaging Mass Spectrometry.** Michael Sperling, ms@speciation.net, https://bit.ly/2VbCvoH.

7–10 June, Loen, Norway. **10th Nordic Conference on Plasma Spectrochemistry.** Yngvar Thomassen, yngvar.thmassen@stami.no, http://nordicplasma.com/.

15–17 June, Bali, Indonesia. **International Conference on Materials Science and Engineering 2020.** materialsasia@prismscievents.com, https://www.materialsconferenceasia.com/.

21–26 June, Honolulu, Hawaii, United States. **2020 Goldschmidt Conference.** helpdesk@goldschmidt.info, https://goldschmidt.info/2020/.

21–26 June, Courmayeur, Italy. **18th Chemometrics in Analytical Chemistry Conference (CAC2020).** ludovic.duponchel@univ-lille.fr, https://cac2020.sciencesconf.org/.

24–26 June, Warsaw, Poland. **European Symposium on Atomic Spectrometry 2020.** Ewa Bulska, esas2020@uw.edu.pl, http://www.esas2020.uw.edu.pl/.

28 June–4 July, Gangwon, South Korea. **AOGS 17th Annual Meeting.** info@asiaoceania.org, http://www.asiaoceania.org/society/public.asp?view=up_coming.

29 June–1 July, Manchester, United Kingdom. **The 20th Biennial National Atomic Spectroscopy Symposium (BNASS 2020).** Dr Phil Riby, philip.riby@manchester.ac.uk, http://www.rsc.org/events/detail/40623/bnass-2020-the-20th-biennial-national-atomic-spectroscopy-symposium.

5–8 July, Skagen, Denmark. **International Association for Spectral Imaging (IASIM) 2020.** 2020@iasim.net, https://2020.iasim.net/.

25–31 July, Chambersburg, United States. **International Diffuse Reflectance Conference (IDRC) 2020.** info@cnirs.org, http://www.cnirs.org/.

23–28 August, Boston, MA, United States. **XXIX International Conference on Magnetic Resonance in Biological Systems (ICMRBSXXIX).** John Markley, jmarkley@wisc.edu, http://www.icmrbs.org/.

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- ☐ NMR, ESR, EPR
- ☐ Photonics and Optics
- ☐ Raman
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6–10 September, Singapore, Singapore. **SETAC 8th World Congress.** setac@setac.org, https://singapore.setac.org/.

7–11 September, Heraklion, Crete, Greece. **NanoBio Conference 2020.** info@nanobioconf.com, https://nanobioconf.com/.

8–10 September, Sheffield, United Kingdom. **41th British Mass Spectrometry Society Annual Meeting 2019—BMSS41.** Mark Mcdowall, mark_mcdowall@icloud.com, https://www.bmss.org.uk/41st-bmss-annual-meeting/.

13–16 September, Orlando, United States. **134th AOAC International Annual Meeting & Exposition.** meetings@aoac.org, http://www.aoac.org.

20–25 September, Kyoto, Japan. **11th International Conference on Laser-Induced Breakdown Spectroscopy (LIBS2020).** Yoshihiro Deguchi, ydeguchi@tokushima-u.ac.jp, http://www.fm.ehcc.kyoto-u.ac.jp/Sakkalab/member/sakka/LIBS2020/index.htm.

20–26 September, Aachen, Germany. **17th International Symposium of Trace Elements in Man and Animals (TEMA17).** Prof. Dr. Lothar Rink, immunologie@ukaachen.de, https://www.ukaachen.de/kliniken-institute/institut-fuer-immunologie/institut.html.

30 September–2 October, Amsterdam, Holland. **Hyperspectral Sensing meets Machine Learning and Pattern Analysis (HyperMLPA).** http://www.spectroexpo.com/hypermlpa/.

30 September–2 October, Amsterdam, Holland. **11th workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing (WHISPERS).** http://www.spectroexpo.com/whispers/.

2 October, Amsterdam, Holland. **2nd Symposium on Short Wave Infrared Imaging and Spectroscopy (Swllms).** http://www.spectroexpo.com/swiims/.

4–8 October, Pittsburgh, United States. **2020 Materials Science and Technology Conference (MS&T20).** metsoc@cim.org, http://www.matsc-itech.org/.

11–16 October, Reno, NV, United States. **47th Annual Conference of Federation of Analytical Chemistry and Spectroscopy Societies (SciX2020).** scix@scixconference.org, https://www.scixconference.org/event-3326054.

25–28 October, Montreal, Canada. **2020 GSA Annual Meeting.** http://www.geosociety.org/.

15–20 December, Honolulu, Hawaii, United States. **The International Chemical Congress of Pacific Basin Societies 2020.** https://pacifichem.org/.

2021

31 January–5 February, Ljubljana, Slovenia. **2021 European Winter Conference on Plasma Spectrochemistry.** Johannes T. VanElteren, http://www.ewcps2021.ki.si/.

15–21 February, Houston, United States. **2021 AAFS 73rd Annual Scientific Meeting.** https://www.aafs.org/home-page/meetings/future-past-aafs-meetings/.

6–10 June, Philadelphia, PA, United States. **69th ASMS Conference.** https://www.asms.org/conferences/annual-conference/future-annual-conferences.

20–24 June, Duesseldorf, Germany. **51st International Symposium on High Performance Liquid Phase Separation and Related Techniques.** Michael Lammerhofer, michael-laemmerhofer@uni-tuebingen.de, https://www.hplc2021-duesseldorf.com/.

5–9 June, Minneapolis, Minnesota, United States. **70th ASMS Conference.** https://www.asms.org/conferences/annual-conference/future-annual-conferences.

Courses

2020

13–17 January, Genoa, Italy. **School of Multivariate Analysis.** chimicanalitica@difar.unige.it, http://www.difar.unige.it/images/Chimica_Analitica/MultivAn_depliant_Jan2020.pdf.

26 April–1 May, Seattle, United States. **Eigenvector University 2020.** https://eigenvector.com/events/eigenvector-university-2020/.

Exhibitions

2020

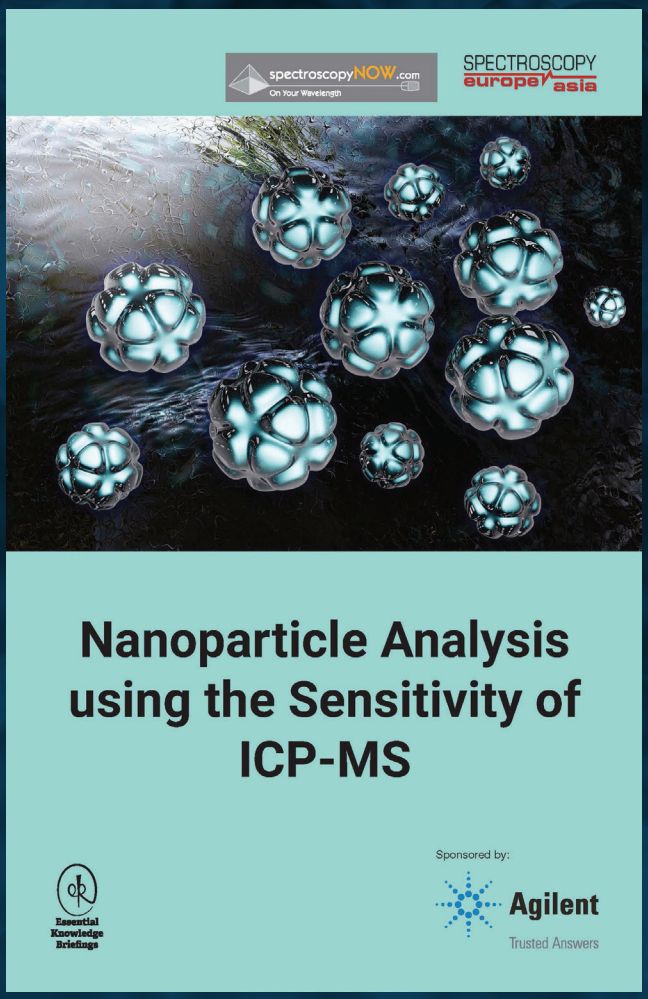
1–5 March, Chicago, United States. **Pittcon 2020—Conference on Analytical Chemistry and Applied Spectroscopy.** pittconinfo@pittcon.org, https://pittcon.org/.

16–18 March, Dubai, United Arab Emirates. **ARABLAB 2020.** info@arablab.com, https://www.arablab.com/.

31 March–3 April, Munich, Germany. **analytica 2020: 27th International Trade Fair for Laboratory Technology, Analysis, Biotechnology and Analytical Conference.** https://www.analytica.de/.

2021

7–11 March, New Orleans, United States. **Pittcon 2021—Conference on Analytical Chemistry and Applied Spectroscopy.** pittconinfo@pittcon.org, https://pittcon.org/.



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