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SPECTROSCOPY europe

Spectroscopy since 1975

Mapping metals in brain tissue with XRF

MS imaging in pathology applications

There's more to sampling a bag of chips…!

IMPOpen

Bags of crisps, or chips, are familiar and look straightforward but pose many sampling challenges. See the Sampling Column on page 35.

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Spectroscopy Europe is a digital magazine, published eight times a year and available free-of-charge to qualifying individuals in Europe and the Middle East.

IMPOpen

EDITORIAL

The application of spectroscopy in life science has been talked about and anticipated since I started editing this publication in the early 80s. And, of course, it is now commonplace. More recently, it has been the move into the "clinical" area—actually working on patients rather for research only—that is looked forward to. This issue really is an interesting collection (coming about by accident rather than design) of medical applications from mass spectrometry imaging, through NMR to X-ray spectroscopy.

Our first article, by Ashley Hollings and Mark Hackett, is on "Mapping metals in brain tissue with X-ray fluorescence and X-ray absorption spectroscopy at synchrotron light sources". XRF and XANES spectroscopic mapping of metal ions in biological systems, and especially the brain, has great potential. Not least in discovering the specific role that Fe, Cu and Zn play in supporting healthy hippocampal memory function, and how the loss of metal homeostasis could contribute to loss of memory and cognitive decline.

Shannon Cornett describes "The future of matrix-assisted laser desorption/ionisation mass spectrometry in pathology applications". Clinical research has led the way in the use of MALDI coupled with MS imaging in developing new applications to determine spatial data about biomolecules in tissues. MALDI imaging is a powerful, label-free analytical tool that can provide vital molecular information about protein modifications after gene expression. It also helps with visualising additional compounds like metabolites, glycans and lipids that play a part in disease pathology.

For this issue's Sampling Column, Kim Esbensen has invited David Honigs and Gary Ritchie to contribute. They have taken the interesting theme of "All that and a bag of chips" to highlight sampling problems. However, they cover far more than that, looking at what needs to be considered before a NIR analytical result can be declared valid.

John Hammond has contributed the penultimate in his series of "Four Generations of Quality", this time about the spectroscopic trio of fluorescence, NIR and Raman. He charts how they have evolved since the First Generation (pre-1975) and how standards and regulatory considerations have affected them in the years since.

There are our usual News, New Products, Applications, Diary and Product Focus (this issue on Mass Spectrometry) sections, as well as the Directory of spectroscopy suppliers towards the back.

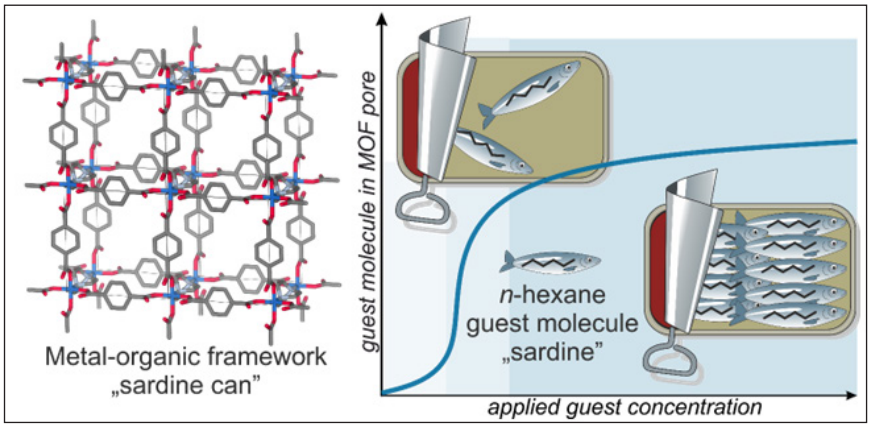
Infrared spectroscopy helps in the study of metal–organic frameworks

A spectrometer is being used to measure the MOF and guest molecule absorbance of two differently polarised types of infrared light, enabling the first measurement of both guest–guest and guest–host interactions in real-time.

Most people don't think about how molecules fit in the ultra-small spaces between other molecules, but not Professor Masahide Takahashi's research team. They study metal–organic frameworks (MOF), composed of modularly arranged metal ions and molecules (organic linkers), forming a scaffold. Metal ions act as corners connected by longer organic linkers. A MOF can be made using different metals and organic linkers, so they can be designed for specific chemical/physical properties, attractive for coating sensors in optical and electronic devices. This is because the MOF scaffold leaves a lot of internal space open. These pores can "host" numerous "guest" molecules, that can access the MOFs' huge internal surface area, which make them ideal for developing catalytic materials, gas storage, gas separation and environmental remediation.

By using an infrared spectrometer to measure the MOF and guest molecule absorbance of two differently polarised types of infrared light, the research team's method is the first to measure both guest–guest and guest–host interactions and do it in real-time. The additions to standard infrared spectrometers required for use with light polarisation use minimal materials, including easily replicable 3D-printed components. This makes the study of MOFs vastly more accessible compared to the previously used X-ray diffraction or solid-state nuclear magnetic resonance spectroscopy.

A unique property of MOFs is that they can change their conductivity and photoluminescence by increasing or decreasing the number of guest molecules that are hosted in their pores. When tightly packed in, the guest molecules can align, creating direction-dependent differences to light absorption and electrical resistance. The researchers coined this phenomenon the "sardine can" effect because the molecules in gases are not always round, differently shaped gas molecules often act like "sardines" when confined in a nanopore "can". When long molecules are added, they bump into each other until they are side-by-side, efficiently packed and pointing in the same direction just like the sardines.



The metal-organic framework forms a scaffold, with nanometer-sized pores which hold molecules (left). As long *n*-hexane gas molecules are added to the pores under pressure, the molecules align in a "sardine can" effect (right).

If you would shine a light through the side of a clear sardine can, you could get a good idea about the direction the sardines were aligned based on their shadows. However, the MOF films and guest molecules are too small to cast shadows, so the researchers used a different feature of light: polarisation. The researchers used infrared light in two polarisations and measured the absorbance of the guest molecule for each polarisation separately. As the partial pressure of the gas in the MOF film was increased, the guest molecules began to align, increasing the absorbance of one polarisation.

This allowed the researchers to find the partial pressure where the host molecules aligned and how they interacted at different pressures. The molecular bonds between different atoms absorb specific wavelengths of infrared light. By comparing which of the polarised wavelengths were absorbed, the researchers could determine the direction molecules in the MOF film were pointing. At higher pressures, when the MOF pores were full, they also discovered defects that began to appear in the MOF scaffold due to the presence of the guest molecules. When the guest molecules were removed, the defects reversed, giving the first clear observation of interactions between guest and host molecules in the MOF.

These results, published in *Angewandte Chemie Int. Edn* (doi.org/gp5tcr), are only the beginning, as this technique can be used to study different MOF films and guest molecule interactions in real-time. This new frontier of materials science has the potential to solve a lot of humanity's future challenges.

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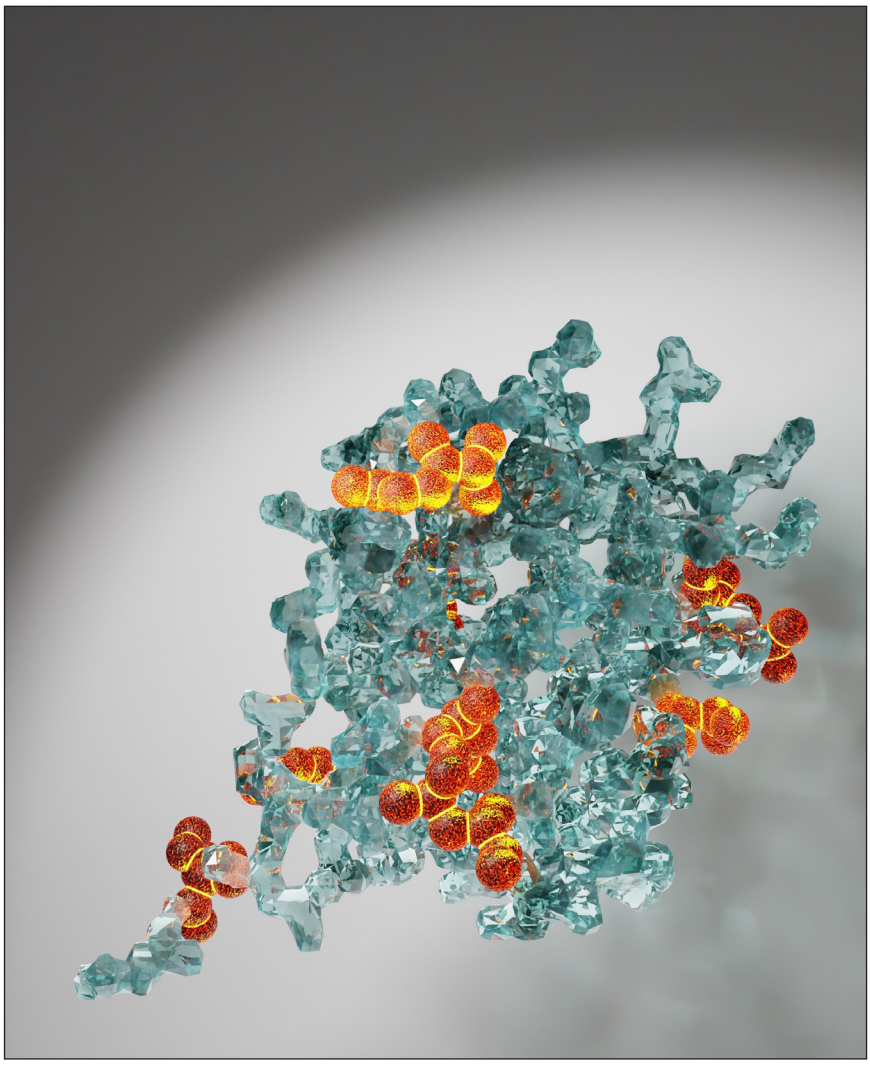
NMR spectroscopy reveals protein folding in milliseconds

Hyperpolarised water boosts signal intensities of proteins, DNA and membranes in NMR spectroscopy

A small group of researchers, including Dennis Kurzbach from the Faculty of Chemistry of the University of Vienna, have described, in *Nature Protocols* (doi.org/gp53gd), an advanced NMR method to monitor fast and complicated biomolecular events such as protein folding. For example, protein folding was long considered as one of the great mysteries of modern research. This crucial process during which amino acid chains adopt a 3D structure and functionality, takes place within milliseconds. Being this fast, protein folding events could often not be characterised by NMR spectroscopy; the standard method for studying molecular structures. Employing hyperpolarised water, researchers have now developed a method that dramatically enhances the signals of the proteins, nucleic acids and other biomolecules. This renders monitoring of processes such as protein folding possible.

With hyperpolarisation methods, more precisely dissolution dynamic nuclear polarisation (D-DNP), a signal enhancement of over 10,000-fold is possible. "The hyperpolarised water acts as a booster for the NMR signals of a protein during the measurement. The hydrogen nuclei of the hyperpolarised water are exchanged with those of the proteins, thus transferring the signal strength to the latter", says Dennis Kurzbach.

With the new method, the researchers can record an NMR spectrum every 100 ms and use it to track the 3-D coordinates of individual amino acids and how they change over time. "This allows us to monitor processes that occur in milliseconds and distinguish individual atoms", says Dennis Kurzbach.



A crystal-clear view of protein structures: hyperpolarisation makes amino acids, i.e. the protein building blocks, light up. Copyright: © Mattia Negroni

In their study the authors describe their technique in detail, from hyperpolarisation to the transfer of the hyperpolarised water to the NMR spectrometer, to the mixing of the hyperpolarised water with the sample solution, and the NMR measurement. In addition, they present six examples for method application, including the observation of protein folding or even the interactions of RNA (nucleic acids) and RNA-binding proteins as the basis for gene expressions in the cell. According to the scientists, the new method can be used for specific studies of RNA, DNA and polypeptides, especially when signal enhancement reaches the "magic" number of 1000-fold.

Characterising limestone rocks with portable Raman spectroscopy

Research shows that it is possible to classify rocks according to the size of the particles they contain during quarrying, using a portable Raman spectrometer.

The nature and potential uses of a sedimentary rock depends on the size of the particles or grains that they are composed from, and particle sizing is an important part of rock classification. A group of researchers led by Iacopo Osticioli of Istituto di Fisica Applicata "N. Carrara", Florence, Italy, has shown that it is possible to size particles and identify rock samples rapidly and accurately while they are being quarried using a portable Raman spectrometer. They published their work in *The European Physical Journal Plus* (doi.org/hvg3).

Limestone is a sedimentary, calcareous rock—that is, one made up principally of calcite and other minority minerals with variable grain dimensions. Each type produces a different quality of quicklime for specific industrial applications. It can be classified according to the sizes of the grains it is composed of, and each type has a different range of industrial uses. Previous research has shown that the intensity of Raman spectral signals, and of the background, will depend on the particle or granule sizes of the sample tested. Osticioli and his co-workers set out to quantise this effect and to use the information to see whether it would be possible to classify rocks *in situ*, in a quarry, using a portable instrument.

They examined a set of rock samples that had been classified by experts, rock pellets and crystalline calcite powder with the portable spectrometer, it showed that there was a clear correlation between Raman signal and particle size, and obtained a calibration curve. "This demonstrates that this technique can provide trustworthy information about mineral fabric", says Osticioli. The apparatus is portable and small enough to be used during quarrying, and it produces results rapidly.

Image by PublicDomainPictures from Pixabay

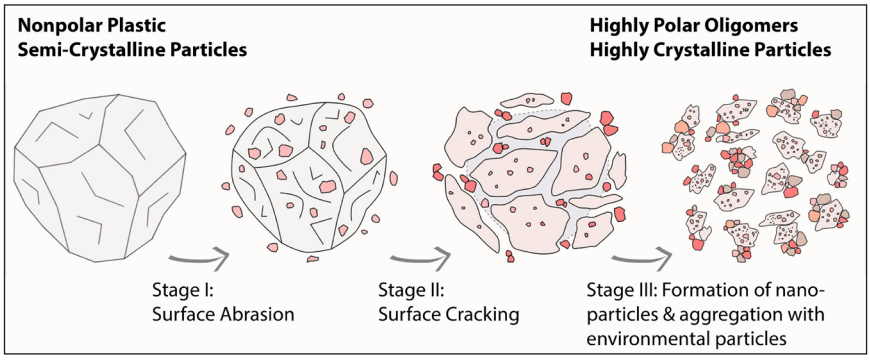
Osticioli and his team now intend to refine the calibration curve to make size assessment, and, therefore, mineral particle-size correlation, more precise. "And the technique can be extended to other minerals that are quarried for other industrial purposes," he concludes.

NMR spectroscopy reveals polyethylene degradation in the environment

Polyethylene accounts for nearly one-third of the world's plastic waste. An interdisciplinary team from the University of Bayreuth has used NMR spectroscopy to investigate the progressive degradation of polyethylene in the environment for the first time.

Polyethylene is a plastic that occurs in various molecular structures. Low-density polyethylene (LDPE) is widely used for packaging everyday consumer goods, such as food, and is one of the most common polymers worldwide as a result of increasing demand. Until now, there have only been estimates as to how this widely used plastic degrades after it enters the environment as waste. A team from the Collaborative Research Centre "Microplastics" at the University of Bayreuth has now systematically investigated this for the first time; their work is published in *Science of The Total Environment* (doi.org/hvg5). The scientists developed a novel, technically sophisticated experimental set-up for this purpose. This makes it possible to simulate two well-known and environmentally linked processes of plastic degradation independently in the laboratory: 1) photo-oxidation, in which the long polyethylene chains gradually break down into smaller, more water-soluble molecules when exposed to light, and 2) increasing fragmentation due to mechanical stress. On this basis, it was possible to gain detailed insights into the complex physical and chemical processes of LDPE degradation.

The final stage of LDPE degradation is of particular interest for studies addressing the potential impact of polyethylene on the environment. What the researchers discovered was that this degradation does not end with the decomposition of the packaging material released into the environment into many micro- and nanoplastic particles, which have a high degree of crystallinity. The reason is that these tiny particles have a strong tendency to aggregate: they attach rapidly to larger colloidal systems consisting of organic or inorganic molecules and are part of the material cycle in the environment. Examples of such colloidal systems include clay minerals, humic acids, polysaccharides and biological particles from bacteria and fungi. "This process of aggregation prevents individual nanoparticles created by polyethylene degradation from being freely available in the environment and interacting with animals and plants. However, this is not an 'all clear' signal. Larger aggregates that participate in the material cycle in the environment and contain nanoplastics do often get ingested by living organisms. That is how nanoplastics can eventually enter the food chain", says Teresa Menzel, a doctoral researcher in the field of polymer materials.



Process of the three-stage degradation of polyethylene particles. Within the environment, aggregation preferably occurs with natural colloidal systems. Credit: © N. Meides, T. Menzel, A. Mauel

To identify the degradation products formed when polyethylene decomposes, the researchers employed multi-cross-polarisation in solid-state NMR spectroscopy, which has not been widely used in microplastics research. "This method even allows us to quantify the degradation products yielded by photooxidation", says Anika Mauel, a doctoral researcher in inorganic chemistry.

Bayreuth's researchers have also discovered that the degradation and decomposition of polyethylene also leads to the formation of peroxides. "Peroxides have long been suspected of being cytotoxic, meaning they have a toxic effect on living cells. That is another way in which LDPE degradation poses a potential threat to natural ecosystems. These interrelationships need to be studied in more detail in the future", adds Nora Meides, a doctoral researcher in macromolecular chemistry.

The detailed analysis of the chemical and physical processes involved in the degradation of polyethylene made use of the interdisciplinary networking and coordinated use of state-of-the-art research technologies on the University of Bayreuth's campus. In particular, scanning electron microscopy, energy dispersive X-ray spectroscopy, X-ray diffraction, NMR spectroscopy, FT-IR spectroscopy (FT-IR) and differential scanning calorimetry.

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Terahertz multispectral imaging reveals hidden inscription on funerary cross

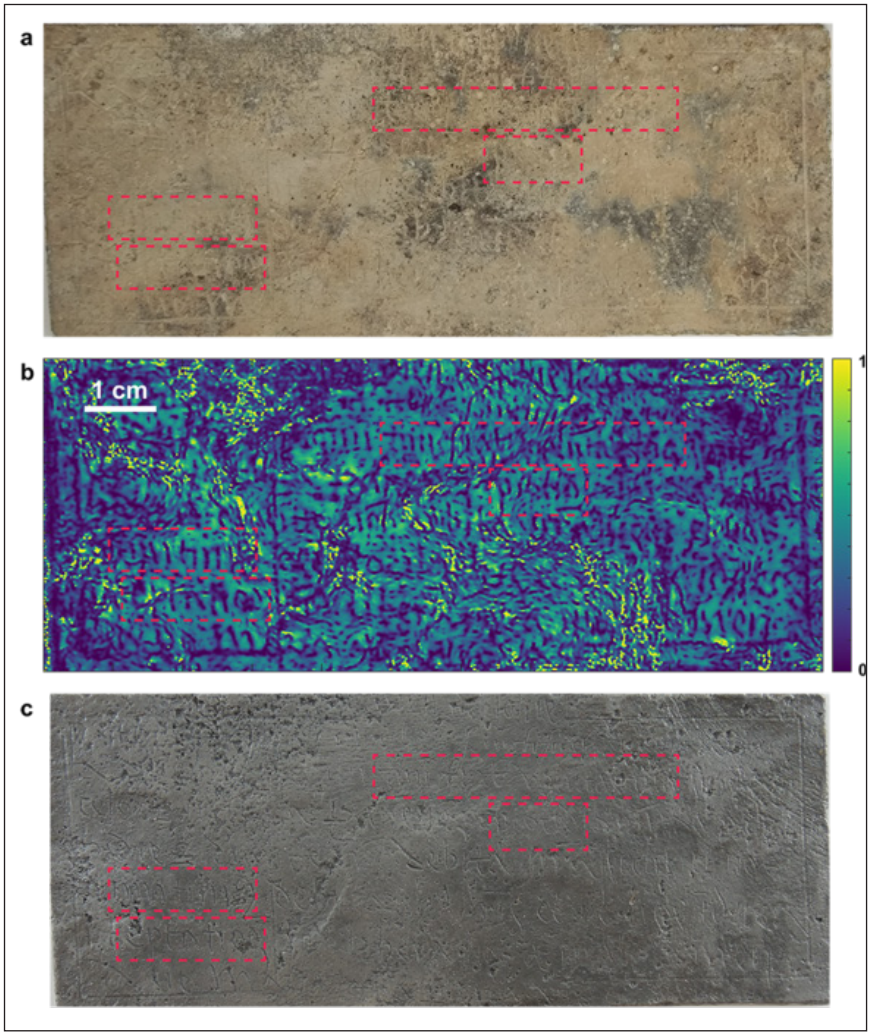
Terahertz imaging and signal processing techniques to look beneath the corroded surface of a 16th-century lead funerary cross.

In a multidisciplinary project, researchers at the Georgia Institute of Technology and Georgia Tech-Lorraine used terahertz imaging and signal processing techniques to look beneath the corroded surface of a 16th-century lead funerary cross. Led by David Citrin, a professor in the School of Electrical and Computer Engineering (ECE), the effort brought together imaging scientists, a chemist specialising in archaeological objects, and an art historian to reveal a message that had been obscured by time: an inscription of the Lord's Prayer.

"Our approach enabled us to read a text that was hidden beneath corrosion, perhaps for hundreds of years", said Alexandre Locquet, an adjunct professor in ECE and researcher at Georgia Tech-CNRS IRL 2958, a joint international research laboratory at the Georgia Tech-Lorraine campus in Metz, France. "Clearly, approaches that access such information without damaging the object are of great interest to archaeologists."

The cross, cut from a sheet of lead, was found in a burial plot at an abbey in Remiremont, France—a couple hours drive from the Georgia Tech-Lorraine campus. Known as a croix d'absolution, it is a type of funerary cross that dates to the Middle Ages and has been found at sites in France, Germany, and England.

"This type of cross typically bears inscriptions of prayers or information about the deceased", said Aurélien Vacheret, director of the Musée Charles-de-Bruyères in Remiremont and co-author on the study. "It is thought their purpose was to seek a person's absolution from sin, facilitating their passage to heaven."



Comparison of the inscription on (a) the original cross before corrosion removal, (b) the final terahertz image after post-processing and (c) the cross after corrosion removal. Credit: Georgia Tech-Lorraine

The museum loaned the cross to Citrin's lab in hopes that the team could use imaging techniques to make the invisible visible. Citrin and his group specialise in non-destructive evaluation and develop techniques that allow for detailed examination of an object's hidden layers without changing or damaging its original form. Although their work often has industrial applications, such as detecting damage to airplane fuselages, the group embraced the opportunity to inspect the cross—a chance to further explore their technology's applications for archaeological purposes.

The team used a commercial time-domain terahertz system to examine the cross every 500 µm across the object. First, the scanner

Introduction to the Theory and Practice of Sampling

Kim H. Esbensen

with contributions from Claas Wagner, Pentti Minkkinen, Claudia Paoletti, Karin Engström, Martin Lischka and Jørgen Riis Pedersen

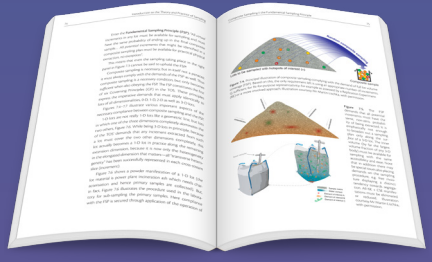
"Sampling is not gambling". Analytical results forming the basis for decision making in science, technology, industry and society must be relevant, valid and reliable. However, analytical results cannot be detached from the specific conditions under which they originated. Sampling comes to the fore as a critical success factor before analysis, which should only be made on documented representative samples. There is a complex and challenging pathway from heterogeneous materials in "lots" such as satchels, bags, drums, vessels, truck loads, railroad cars, shiploads, stockpiles (in the kg–ton range) to the miniscule laboratory aliquot (in the g–µg range), which is what is actually analysed.

This book presents the Theory and Practice of Sampling (TOS) starting from level zero in a novel didactic framework without excessive mathematics and statistics. The book covers sampling from stationary lots, from moving, dynamic lots (process sampling) and has a vital focus on sampling in the analytical laboratory.

"I recommend this book to all newcomers to TOS"

"This book may well end up being the standard introduction sourcebook for representative sampling."

"One of the book's major advantages is the lavish use of carefully designed didactic diagrams"



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sent short pulses of terahertz electromagnetic radiation over each section of the cross. Some waves bounced back from the layer of corrosion, while others penetrated through the corrosion, reflecting from the actual surface of the lead cross. This produced two distinct echoes of the same original pulse. Next, the team used an algorithm to process the time delay between the two echoes into a signal with two peaks. This data revealed how thick the corrosion was in each scanned point. The measurements of the light beams that reflected from the underlying metal were then collected to form images of the lead surface below the corrosion.

Although crucial data was gathered during the scanning process, the raw images were too noisy and jumbled and the inscription remained illegible at the time. But Junliang Dong, then a PhD student in Citrin's lab, had the insight to process the images in a special way to eliminate the noise. By subtracting and piecing together parts of the images acquired in different frequencies, Dong was able to restore and enhance the images. What was left was a surprisingly readable image containing the text.

Using the processed images, Vacheret was able to identify multiple Latin words and phrases. He determined they were all part of the paternoster, commonly known as the Our Father or the Lord's Prayer.

The team also worked with a conservationist to chemically reverse the corrosion on the cross, confirming the paternoster inscription. Comparing their images to the clean cross, the team found their images had revealed parts of the inscription not observable on the original cross. By uncovering additional aspects of the inscriptions that were previously undocumented, their work was able to offer deeper understanding of the cross and further insight into 16th-century Christianity in Lorraine, France.

"In this case, we were able to check our work afterwards, but not all lead objects can be treated this way", Citrin said. "Some objects are large, some must remain *in situ*, and some are just too delicate. We hope our work opens up the study of other lead objects that might also yield secrets lying underneath corrosion." Citrin's group has also used terahertz imaging to look beneath the surface of 17th-century paintings, elucidating paint layer structure and providing insights into techniques of master painters. They are currently investigating surface coatings on ancient Roman ceramics.

The cross project, published in *Scientific Reports* (doi.org/hvg7) illustrates that success requires more than just accurate measurement, but also careful data processing and collaboration between researchers from disparate fields. The team's approach opens new perspectives for terahertz imaging analysis and could produce great boosts for the fields of digital acquisitions and documentation, as well as character recognition, extraction and classification.

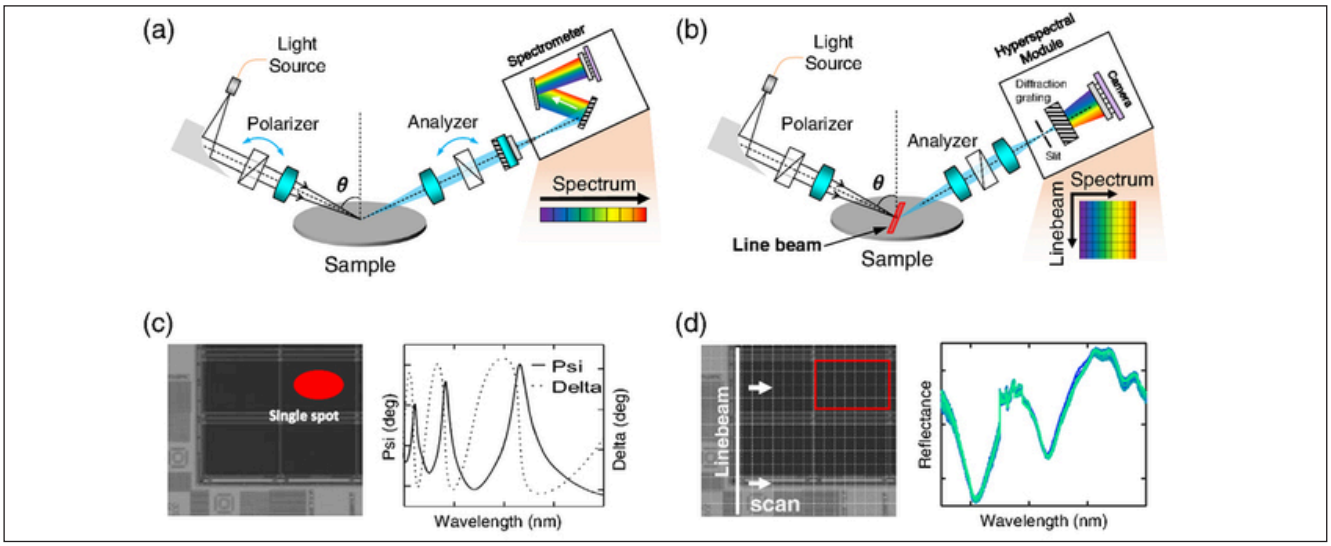
"Despite three decades of intense development, terahertz imaging is still a rapidly developing field", said Locquet. "While others focus on developing the hardware, our efforts concentrate on making the most of the data that is measured."

New hyperspectral imaging technique to monitor modern semiconductor devices

Researchers at Samsung developed a novel approach to inspect and measure the critical dimensions of semiconductor devices using hyperspectral imaging, with higher speed and resolution than conventional methods.

Semiconductor fabrication technology, used commonly in modern electronics, has been steadily advancing for decades. Today, semiconductor devices have shrunk to miniature sizes but are capable of performing unprecedented complex functions. However, manufacturing semiconductors at the scale of a few nanometres has its fair share of technical challenges. For semiconductor devices to operate as intended, manufacturers need to guarantee the uniformity of what is known as "critical dimension" (CD). Manufacturing processes should be capable of accurately replicating the finest details of semiconductor structures down to the CDs, since even minor imperfections could cause devices to malfunction.

Unfortunately, one of the main problems of manufacturing ever-smaller semiconductor devices is that there are currently no fast, reliable and accurate techniques available to measure CD uniformity in cells, chips and wafers. Even though technologies like transmission electron microscopy and atomic force microscopy offer high spatial resolution, their scanning speed is too slow to make their application feasible for high-speed manufacturing. Fast measurement methods do exist, such as optical critical dimension (OCD) spectrometry (which measures uniformity in semiconductor devices by analysing the spectrum of light reflected off their surface). But such methods



(a, c) The OCD spectroscopy approach. Though practical, it has limited spatial and spectral resolution and long scan times. (b, d) The LHSI approach developed by the research team, with a shorter scanning time and much higher spatial and spectral resolution. Each square in the grid shown in (d) corresponds to an observed "pixel." Credit: Yoon *et al.*, doi: 10.1117/1.JMM.21.2.021209

often have a low spatial resolution, putting them at a disadvantage.

Against this backdrop, a team of researchers led by Dr Myungjun Lee, head of the Inspection Solution Group at Samsung Electronics, recently developed a novel approach called "line-scan hyperspectral imaging" (LHSI). This method, reported in *Journal of Micro/Nanopatterning Materials and Metrology* (doi.org/hvg9) can potentially solve the issue of speed and resolution in measuring CD uniformity. The LHSI system is designed to deliver a better resolution than OCD along with a much higher throughput. The key difference between OCD and LHSI is the way in which the surface of the semiconductor is scanned. In OCD, the system focuses a beam of light to produce a bright spot on the wafer. The reflected light is then captured by a spectrometer and the acquired spectrum is analysed. Because the bright spot is rather large, OCD systems have a low resolution, making it hard to visualise small details in the semiconductor's structure.

In contrast, the LHSI system illuminates the wafer's surface with a narrow rectangular beam, an approach known as "line scan." The reflected rectangular beam is relayed into a hyperspectral module, which consists of an arrangement of mirrors, slits, gratings and cameras. The gratings spatially decompose the incoming beam into its constituent frequencies, which are then captured by cameras optimised for different spectral ranges.

The LHSI approach allows the cameras to capture the spectral information of each vertical "pixel" of the scanning rectangular beam in one shot. As a result, a large amount of data can be generated quickly, speeding up the CD uniformity measurements. "Our system enables the simultaneous collection of massive amounts of spectral and spatial information with an extremely large field of view of $13 \times 0.6\,mm^2$", says Dr Lee. "Additionally, our method has an improved throughput of up to 10,000 times compared to the standard OCD method."

In addition to its speed, the LHSI system offers a spatial resolution of 5 µm and a spectral resolution of 0.25 nm over a wide range of wavelengths (350–1100 nm). This allowed the researchers to clearly observe fine details on various semiconductor devices, outperforming other conventional approaches by a remarkable margin. "We believe that there is currently no technology other than LHSI that can be used to analyse CD uniformity at such high levels of throughput and resolution", says Dr Lee. "It could be the tool we need to overcome current measurement limitations in the field of high-volume semiconductor manufacturing. However, even though we successfully demonstrated the potential of LHSI, the critical modules, including a more stable light source, higher sensitivity cameras, higher precision optics etc. need to be further improved by suppliers."

Overall, these results suggest that LHSI is a promising solution to one of the pressing problems in semiconductor manufacturing. Perfecting this technology further will help accelerate semiconductor development and fabrication processes, reducing their costs and enhancing the performance of the electronic devices we rely on so much today.

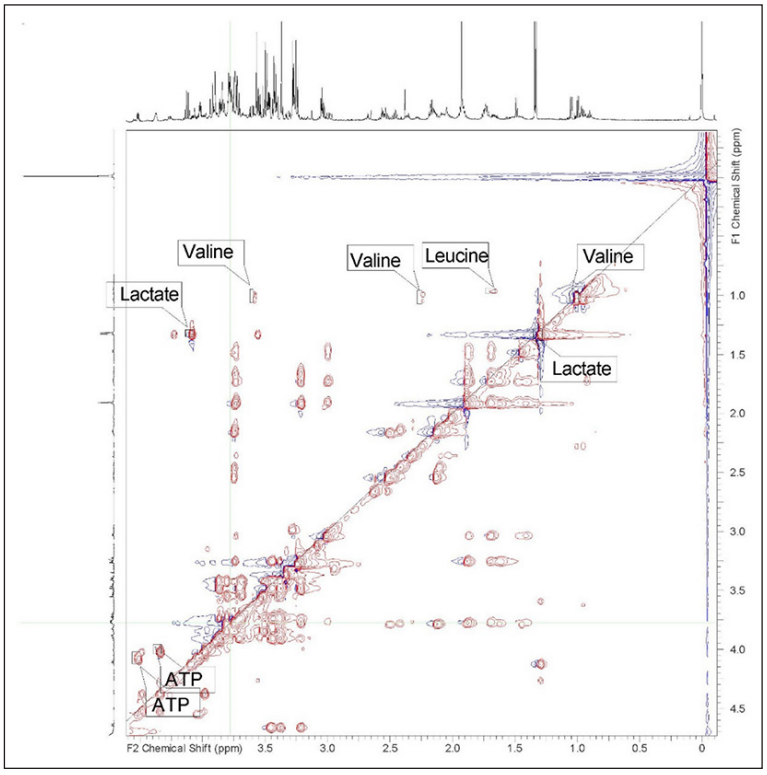
NMR assessment of metabolites in African elephants

Researchers have used NMR spectroscopy to conduct the first assessment of metabolites in African savanna elephants, an important step in understanding the relationship between their metabolism and health.

North Carolina State University researchers have conducted the first assessment of metabolites in African savanna elephants (*Loxodonta africana*), an important step in understanding the relationship between metabolism and health in these endangered animals.

"The bottom line is that we have taken the first step in what will be a lengthy process to advance our understanding of the relationship between metabolites and elephant health", says Michael Stoskopf, a professor of clinical sciences at NC State.

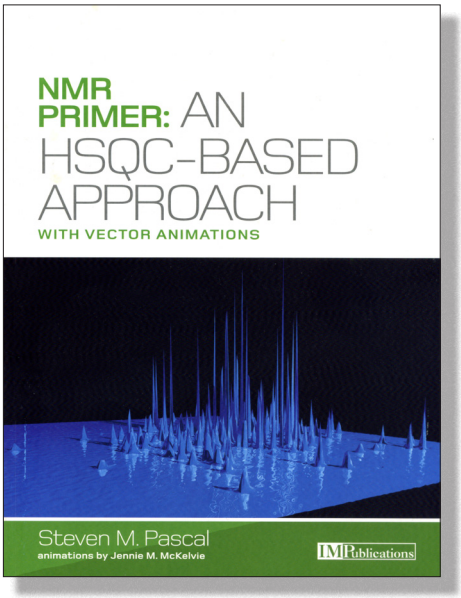
For the study, published in *Metabolites* (doi.org/gp4wmv) researchers collected blood samples from six African savanna elephants at the North Carolina Zoo. All of the elephants were healthy adults and received the same diet. Analysis was carried out with 1D and 2D ^{1}H NMR spectroscopy methods.



2D TOCSY proton NMR spectra of the polar extract of whole blood from an 18-year-old female African savanna elephant (*Loxodonta africana*). Representative labelled peaks are valine, lactate, leucine and adenosine triphosphate (ATP). Reproduced under a CC BY licence from https://doi.org/10.3390/metabo12050400

"We've established a technique that allows us get an accurate snapshot of metabolites in these elephants", Stoskopf says. "We found little difference in metabolites among the six elephants, likely due to their sharing a common diet. This work represents a good start, there were no particular surprises here, but establishing the metabolites present in elephants that are on a very specific diet gives us a snapshot of metabolism in these animals."

"Elephants are highly adaptable, and live in a wide variety of environments on a wide variety of diets", says Kimberly Ange-van Heugten, a teaching associate professor of animal science at NC State. "This study should serve as an excellent jumping off point for studies that can offer additional insights. For example, with precise analytical and research techniques we could potentially see how changes in diet might affect an elephant's metabolomics."



NMR PRIMER: AN HSQC-BASED APPROACH

(with vector animations)

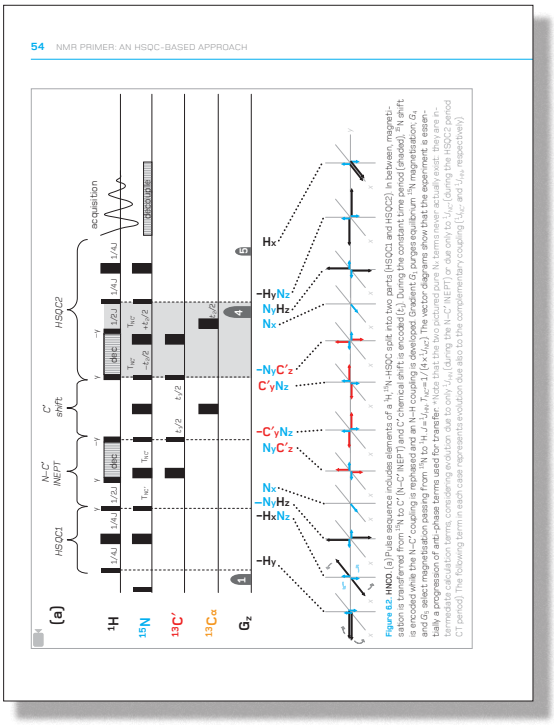
by Steven M. Pascal

This book has one aim: to explain the key two-dimensional protein NMR experiment, the $^{1}H,^{15}N$-HSQC, along with variants and extensions, in a generally accessible manner. Vector diagrams of one-, two- and three-dimensional pulse sequences are provided, along with accompanying animated versions. The animations allow the evolution of net magnetisation during the course of the experiments to be visualised and directly compared with the corresponding spin operator terms.

First, a brief introduction to spins, populations, the NMR experiment and relaxation is provided. Evolution due to J-coupling is next described and used to explain magnetisation transfer in the HSQC experiment and several variants. The extraction of structural, sequential and dynamic information is then illustrated via various extensions of the HSQC. Extensive footnotes and appendices introduce several more advanced concepts, such as sensitivity enhancement and the TROSY effect.

ANIMATIONS

The animations were originally created in Flash, which is no more. The animations have been converted to animated GIFs which enable them to be viewed easily with any browser. Control of these animations works best in Google Chrome using the GIF SCRUBBER extension: this allows pause/restart/reverse/speed control/etc.



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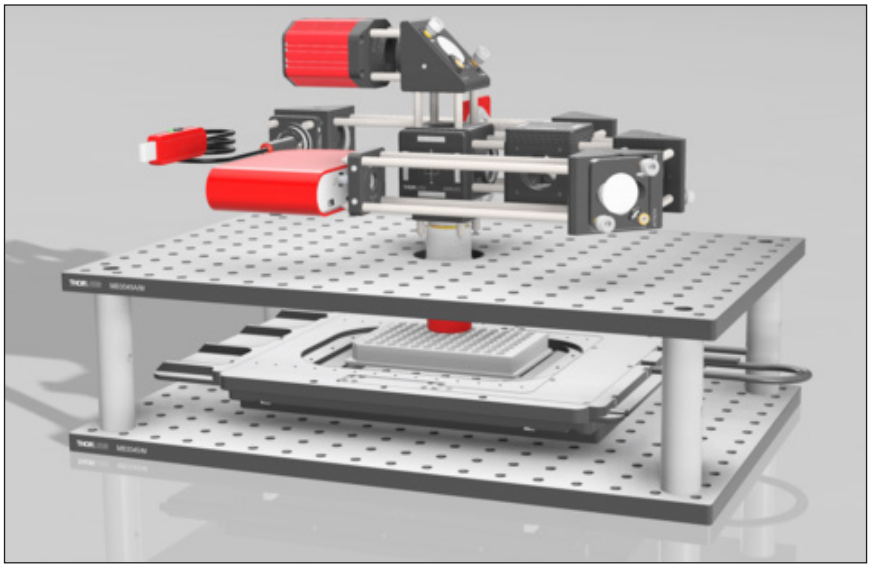
Rapid Raman method shown to detect infection in cystic fibrosis

The new Raman spectroscopy methodology has the potential to detect infections in cystic fibrosis patients in minutes and could be expanded to target a variety of diseases and counter anti-microbial resistance.

Researchers at the University of Southampton have demonstrated a quick and accurate method to diagnose bacterial infections using a new multi-excitation Raman spectroscopy methodology. The technique has the potential to detect infections in cystic fibrosis patients in minutes rather than days. In future, the simple analysis could be performed on hospital wards to deliver faster and more effective treatment. The approach could also be expanded to target a variety of diseases and counter anti-microbial resistance.

Cystic fibrosis is an inherited condition that causes sticky mucus to build up in the lungs and digestive system. This causes lung infections and problems with digesting food. It affects around 1 in every 10,000 births in the UK. Treatments are available to help reduce the problems caused by the condition. Yet recurring infections still dramatically reduce the quality and length of life. The current methods for diagnosing immediate (acute) and longer-term (chronic) infections are complex and time-consuming in the laboratory. For biofilm infections, it can take days from collecting and processing a patient's sample to achieving a result. This delays effective treatments and impacts patient outcomes.

A multi-disciplinary team from the University of Southampton and University Hospital Southampton set out to develop a diagnostic tool that would be rapid, accurate and simple-to-use for doctors. They have developed a new chemical analysis technique called multi-excitation Raman spectroscopy. Professor Mahajan continued: "Our new Raman spectroscopy-based method offers many advantages over resource-intensive, culture-based methods, allowing rapid and label-free analysis. It is reagent-less and avoids complex sample-preparation steps with sophisticated equipment. Here, we have developed a method that is highly accurate yet rapid and neither requires nanoscale materials for enhancing signals nor fluorophores for detection." The work is published in *Analytical Chemistry* (doi.org/hvhh).



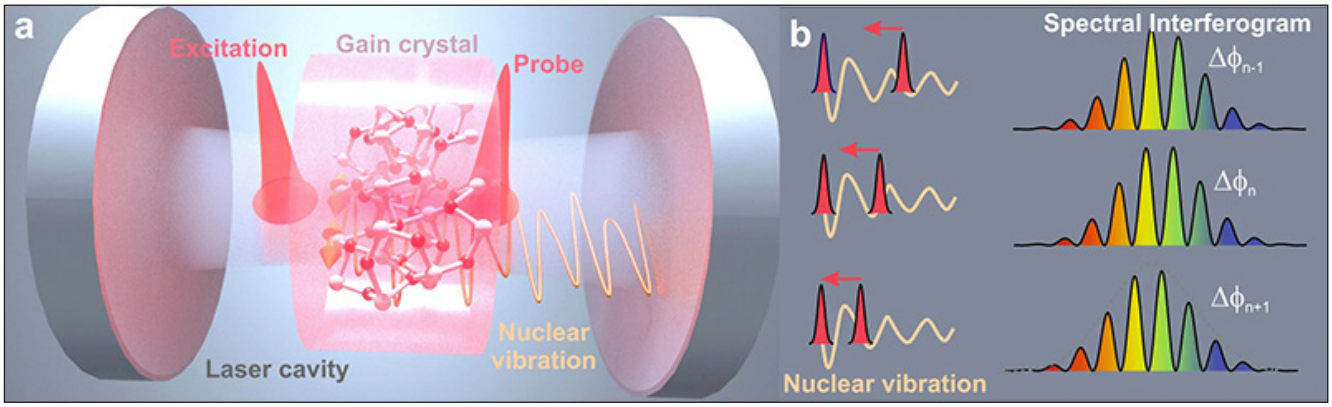
Prototype of the spectroscopy device. Credit: University of Southampton

Long term infections in the lungs of people with cystic fibrosis are extremely hard to treat. There is evidence that the *Pseudomonas aeruginosa* bacteria exists as biofilms in the body, protecting the bacteria from antibiotic action and driving antimicrobial resistance. This increases the urgency for rapid and effective treatment. The Southampton research showed 99.75 % accuracy at identifying *Pseudomonas aeruginosa* and *Staphylococcus aureus* across all studied strains. This included 100 % accuracy for drug-sensitive and drug-resistant *Staphylococcus aureus*.

Prof Faust, Director of NIHR Southampton CRF, said: "Our study demonstrates an important step toward a rapid and reagent-less diagnostic tool requiring only simple or routine sample preparation. Such a platform could also prove useful in a variety of other disease areas and help address the mounting challenge of anti-microbial resistance."

Atomic terahertz-vibrations solve the enigma of ultrashort soliton molecules

Optical solitons often combine into pairs with very short temporal separation. Introducing atomic vibrations in the terahertz range, researchers at the Universities of Bayreuth and Wrocław have solved the puzzle of how these temporal links are formed.



a Two ultrashort solitons (red) circulate inside an active laser cavity: the leading soliton excites a coherent nuclear vibration (orange) in the gain crystal, the trailing pulse samples the refractive index modulation. b During the soliton approach, the trailing soliton encodes the temporal waveform of the nuclear vibration in its phase (left). The relative phases at each roundtrip n, $\Delta\phi_n$, are detected via spectral fringes of single-shot interferograms (right). Credit: *Nature Communications*, 10.1038/s41467-022-29649-y, reproduced under a CC BY licence.

Stable packets of light waves, called optical solitons, are emitted in ultrashort-pulse lasers as a chain of light flashes. These solitons often combine into pairs with very short temporal separation. Introducing atomic vibrations in the terahertz range, researchers at the Universities of Bayreuth and Wrocław have now solved the puzzle of how these temporal links are formed. The dynamics of the coupled light packets can be used to measure atomic vibrations as characteristic "fingerprints" of materials in an extremely fast manner.

In ultrashort-pulse lasers, optical solitons can form particularly tight spatial and temporal bonds. These are also called ultrashort "soliton molecules" because they are stably coupled to each other, similar to the chemically bonded atoms of a molecule. The research group in Bayreuth used a widely used solid-state laser made of a sapphire crystal doped with titanium atoms to find out how this coupling occurs. First, a single leading flash of light stimulates the atoms in the sapphire's crystal lattice to instantly vibrate. This characteristic motion oscillates in the terahertz range and decays again within a few picoseconds. In this extremely short time span, the refractive index of the crystal changes. When a second flash of light immediately follows and catches up with the first, it senses this change: it is not only slightly affected by the atomic vibrations, but can also stably be bound to the preceding soliton. A "soliton molecule" is born.

"The mechanism we discovered is based on the physical effects of Raman scattering and self-focusing. It explains a variety of phenomena that have puzzled science since the invention of titanium-sapphire lasers over 30 years ago. What is particularly exciting about the discovery is that we can now exploit the dynamics of solitons during their generation in the laser cavity to scan atomic bonds in materials extremely rapidly. The entire measurement of a so-called intracavity Raman spectrum now takes less than a thousandth of a second. These findings may help to develop particularly fast chemically sensitive microscopes that can be used to identify materials. In addition, the coupling mechanism opens up new strategies to control light pulses by atomic motions and, conversely, to generate unique material states by light pulses", explains Dr Georg Herink, head of the study, which was published in *Nature Communications* (doi.org/gpzgnw).

In parallel with the analysis of experimental data, the researchers have succeeded in developing a theoretical model for soliton dynamics. The model allows to explain the observations obtained in experiments and to predict novel effects of atomic vibrations on the dynamics of solitons. The interactions of solitons in optical systems and their applications for high-speed spectroscopy are currently being investigated in the DFG research project FINTEC at the University of Bayreuth.

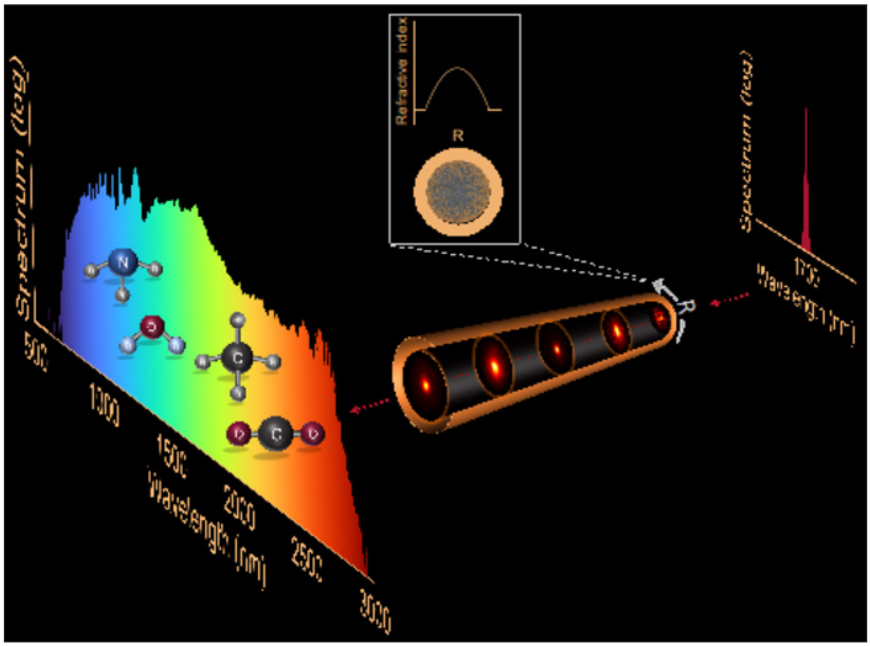
New self-cleaning optical fibre as a supercontinuum light source into the mid-IR

A type of optical fibre with a refractive index that varies continuously across the fibre structure has been shown to yield a dramatic increase in supercontinuum power, while still preserving a smooth beam intensity profile.

When a high-power ultrashort pulse of light interacts with a material such as a glass optical fibre, a range of highly non-linear interactions take place that cause complex changes in both the temporal and spectral properties of the injected light. When taken to the extreme, such interactions can lead to the generation of a rainbow laser of light commonly referred to as a supercontinuum light source. Since its first demonstration in a special type of optical fibre in 2000, supercontinuum laser light has revolutionised many areas of science, ranging from metrology and imaging at unprecedented resolution to ultrabroadband remote sensing and even the detection of exoplanets.

The bottleneck with current supercontinuum sources, however, is that they are based on optical fibres that support a single transverse intensity profile or mode, which inherently limits their optical power. What's more, conventional optical fibres are made of silica glass with transmission limited to the visible and near infrared region of the spectrum. Extension of supercontinuum light to other wavelength regimes such the mid-infrared requires optical fibres made of so-called soft glasses, but these possess a lower damage threshold than silica, limiting even more the power of the supercontinuum beam.

Recently, a different type of optical fibre with a refractive index that varies continuously across the fibre structure has been shown to yield a dramatic increase in supercontinuum power, while still preserving a smooth beam intensity profile. "The refractive index variation of such graded-index optical fibres leads to periodic focusing and defocusing of the light inside the fibre that enables coupling between spatial and temporal non-linear light–matter interactions. This leads to a self-cleaning mechanism that yields supercontinuum light with high power and a clean beam profile. As well as their many applications, they also provide a means of studying fundamental physics effects such as wave turbulence", says Professor Goëry Genty, the leader of the research group at Tampere University.



Utilising two glasses with a different refractive index and stacked with a specific arrangement has allowed researchers to develop for the first time a multimodal fibre with a parabolic refractive index with transmission up to the mid-infrared and high non-linearity. The spectrum of short pulses of light injected into the fibre massively broadens to span from the visible to mid-infrared. Significantly, unlike in conventional multimode fibres, the light beam remains smooth as the result of self-cleaning dynamics induced by the parabolic refractive index. Such a light source with ultrabroad spectrum, smooth beam and high power finds applications in, e.g., environmental sensing or high-resolution imaging for medical diagnostics. Credit: Tampere University

While these fibres have recently attracted significant attention from the research community, their use has been, up to now, restricted to the visible and near infrared. In collaboration with the group of Profs Buczynski and Klimczak at the University of Warsaw (Poland) and the group of Prof. Dudley in the University of Burgundy France-Comté (France), the Tampere team demonstrated for the first time the generation of a two-octave supercontinuum from the visible to mid-infrared in a non-silica graded-index fibre with a self-cleaned beam.

"This problem has now been solved by using a particular design that utilises two types of lead–bismuth–gallate glass rods with different refractive indices drawn

to yield a nanostructured core. The result is a graded-index fibre with an effective parabolic refractive index profile with transmission up to the mid-infrared, and, as cherry on the cake, enhanced non-linear light–matter interactions", says researcher Zahra Eslami.

The mid-infrared is of crucial interest as it contains the characteristic vibrational transitions of many important molecules.

"The novel solution will lead to more efficient supercontinuum light sources in the mid-infrared with many potential applications, e.g., for pollutant tagging, cancer diagnostics, machine vision, environmental monitoring, quality and food control", explains Genty.

The researchers anticipate that this novel type of fibre will very soon become an important and standard material for the generation of broadband sources and frequency combs.

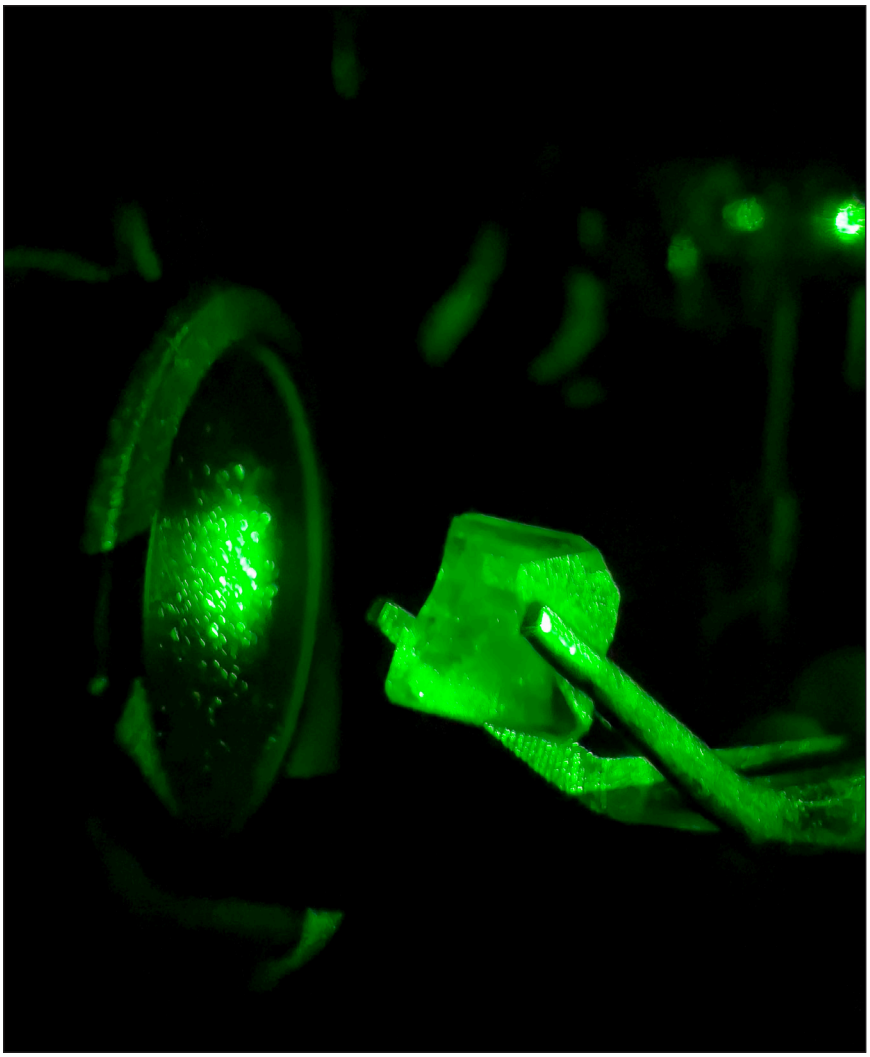
The research was carried out at Tampere University and within the Academy of Finland Flagship for Photonics Research and Innovation (PREIN), and published in *Nature Communications* (doi.org/hvhm).

Raman images the birth of crystals

A team from the University of Geneva has succeeded in visualising crystal nucleation with Raman microspectroscopy that was invisible until now.

At the interface between chemistry and physics, the process of crystallisation is omnipresent in nature and industry. It is the basis for the formation of snowflakes but also of certain active ingredients used in pharmacology. For the phenomenon to occur for a given substance, it must first go through a stage of so-called nucleation, during which the molecules organise themselves and create the optimal conditions for the formation of crystals. While it has been difficult to observe this pre-nucleation dynamics, this key process has now been revealed by the work of a research team from the University of Geneva (UNIGE). The scientists have succeeded in visualising this process spectroscopically in real time and on a micrometre scale, paving the way to the design of safer and more stable active substances.

In the first phase of nucleation, molecules begin to arrange themselves to form a "nucleus", stable clusters of molecules, which leads to the development and growth of a crystal. This process occurs stochastically, meaning it is not predictable when and where a nucleus form. "Until now, scientists have been struggling to visualise this first stage at the molecular level. The microscopic picture of



The scientists used lasers to reveal the molecular structure at work during nucleation, but also to induce nucleation and observe its spectral fingerprint. © Oscar Urquidi

crystal nucleation has been under intense debate. Recent studies suggest that molecules seem to form some disordered organisation before the formation of "nuclei". Then how does the crystalline order emerge from them? "That is a big question!", explains Takuji Adachi, assistant professor in the Department of Physical Chemistry at the UNIGE Faculty of Science.

Takuji Adachi's team, supported by two researchers from the Department of Chemistry at McGill University (Nathalie LeMessurier and Lena Simine), has taken a decisive step by succeeding in observing the nucleation process of an individual crystal at the micrometre scale by optical spectroscopy. "We have succeeded in demonstrating and visualising the organisation and formation of molecular aggregates that precede crystallisation", explains Johanna Brazard, a researcher in the Department of Physical Chemistry.

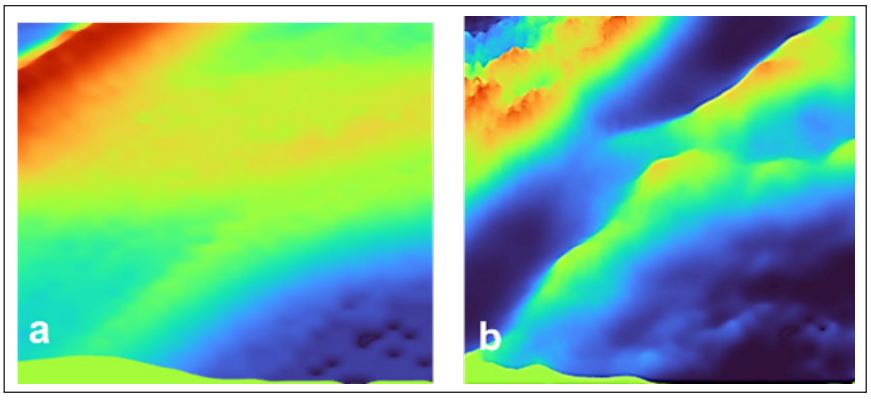
To observe this phenomenon, the scientists combined Raman microspectroscopy and optical trapping. "We used lasers to highlight the molecular structure during the nucleation but also to induce the nucleation phenomenon and thus be able to observe it and record its spectral imprint," explains Oscar Urquidi, a doctoral student in the Department of Physical Chemistry. The model substance chosen to conduct these experiments was glycine, an amino acid that is an essential building block of life, dissolved in water.

"Our work has revealed a stage of crystallisation that was previously invisible", says Takuji Adachi. "Visualising more precisely and better understanding what is happening at the molecular level is very useful for directing certain manipulations more effectively." In particular, this discovery could make it easier to obtain purer and more stable crystal structures for certain substances used in the design of many drugs or materials. It has been published in *Proceedings of the National Academy of Sciences* (doi.org/hvhq).

Expanding infrared microspectroscopy with computational reconstruction

Computational imaging technologies have substantially reduced the costs of imaging systems and at the same time significantly improved their performances. Use of a Lucy–Richardson–Rosen computational reconstruction method with infrared microspectroscopy has produced 3D images.

Computational imaging technologies have substantially reduced the costs of imaging systems and at the same time significantly improved their performances, such as three-dimensional imaging capability, multispectral imaging with a monochrome sensor etc. However, computational imaging methods are not free of challenges. Most, if not all, computational imaging methods require special optical modulators such as scatter-plates, Fresnel zone apertures and coded apertures that map every object point into a special intensity distribution. A computational method reconstructs the recorded intensity distribution into multispectral, multidimensional images. Since an intermediate reconstruction step is involved, computational imaging methods are termed indirect imagers while conventional lens-based imaging systems are direct imagers. The need for special optical modulators in computational imaging is due to the limitations in the reconstruction mechanisms. Furthermore, even though the above computational methods can render additional information than conventional lens-based imagers, the quality of reconstruction has never been to the level of a lens-based imager.



Direct imaging of a bundle of silk threads in 3D space, showing focused and out-of-focus objects. b. Reconstruction result using Lucy-Richardson-Rosen algorithm.

In this research work, published in *Opto-Electronic Science* (doi.org/hvhv), a novel computational holography method has been developed by combining two well-known deconvolution methods namely the maximum likelihood algorithm developed by Lucy and Richardson and non-linear correlation

developed by Rosen. This Lucy–Richardson–Rosen algorithm is capable of deconvolving intensity distributions obtained from direct imagers such as Cassegrain objective lenses. This development links direct and indirect imaging methods creating a major impact. When the imaging condition is satisfied, a direct image of the object is formed and when the imaging condition is disturbed, the computational reconstruction method is applied. The new method was applied to image chemical samples at the infrared microspectroscopy system of the Australian synchrotron. From a single camera shot of the chemical sample and the known three-dimensional point spread functions of the Cassegrain objective lenses, a complete three-dimensional image of the chemical sample is generated by the Lucy–Richardson–Rosen algorithm.

The research group of Prof. Saulius Juodkazis, at Swinburne University of Technology, has developed a new computational holography technique for rapid imaging of biochemical samples. The infrared microspectroscopy unit uses a nitrogen-cooled Mercury–Cadmium–Telluride single-pixel detector, a tightly focusing Cassegrain objective lens pair and a point-by-point scanning approach to record two-dimensional information of a sample. The scanning method is time-consuming, limiting the number of samples that can be studied during a synchrotron beam-time project.

In this project, the single-pixel detector was replaced by a focal point array detector, and a weaker Cassegrain objective lens was used to increase the beam diameter in the sample plane. This method enabled single-shot two-dimensional imaging of the samples. Computational imaging methods such as coded aperture correlation holography can transform conventional imagers into three-dimensional imagers.

Unlike the previous computational imaging methods, in the proposed method, direct imaging and indirect imaging can co-exist. When the imaging condition is satisfied, the system behaves as a direct imager, and when the imaging condition is not satisfied, the system behaves as an indirect imager requiring computational reconstruction. A new reconstruction method was designed by combining two well-known reconstruction methods, namely the maximum likelihood algorithm developed by Lucy and Richardson and the non-linear reconstruction method developed by Rosen. The new Lucy–Richardson–Rosen algorithm reconstructed three-dimensional information of samples from a single camera shot of the samples and pre-recorded three-dimensional point spread intensity distribution. Consequently, the developed method significantly improved the speed of imaging using the infrared microspectroscopy unit.

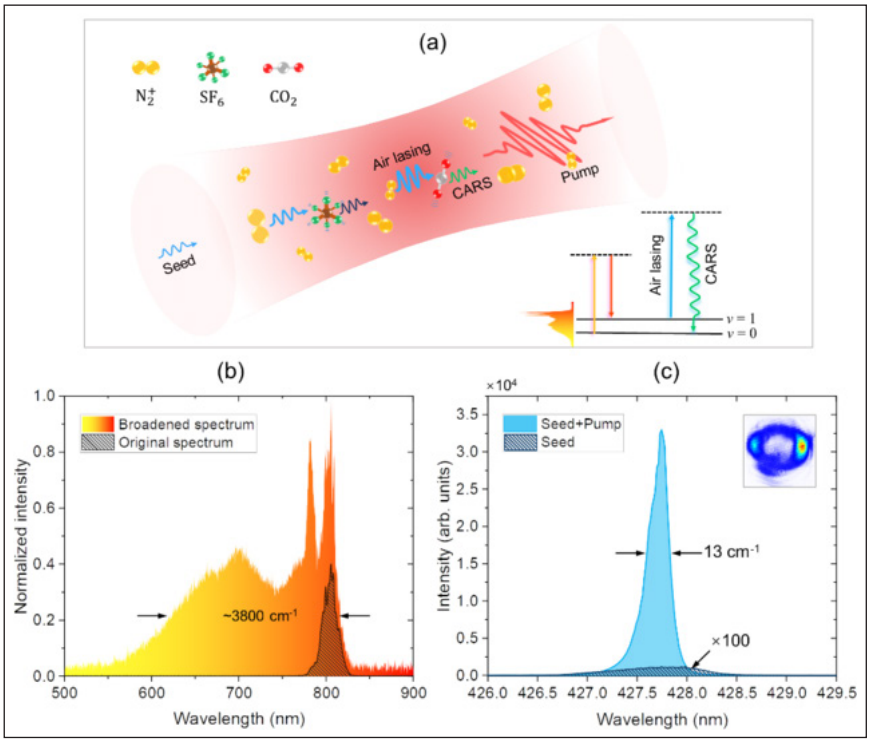
While the new algorithm aided computational imaging technique have transformed the conventional infrared microspectroscopy unit into a three-dimensional infrared microspectroscopy unit, further investigation on the algorithm revealed surprising aspects of the algorithm. The algorithm was able to deconvolve numerous deterministic optical fields significantly better than existing computational reconstruction methods. It is believed that the new reconstruction algorithm will revolutionise the field of computational imaging where scattering fields can be replaced by deterministic ones with a better signal-to-noise ratio and lower photon budget.

Air lasing: Raman spectroscopy for atmospheric detection

Air-lasing-assisted coherent Raman spectroscopy can provide quantitative measurement and simultaneous detection of two greenhouse gases, as well as the identification of CO_2 isotopes.

Innovative advances in ultrafast laser technologies provide new strategies for remote sensing of atmospheric pollutants and hazardous biochemical agents. The high-energy femtosecond laser can propagate a long distance without diffraction via femtosecond laser filamentation. Besides, abundant secondary radiations, e.g. supercontinuum white light, air lasing and fluorescence, provide natural probe sources for atmospheric detection at a remote location. Particularly, the discovery and intensive investigation of air lasing induced by intense femtosecond laser pulses open an exciting perspective for atmospheric remote sensing due to its ability to generate cavity-free light amplification in the open air. Although significant efforts have been paid to air-lasing-based remote sensing, the realistic application still remains challenging due to the limit of the detection sensitivity and signal stability.

Recently, the research team from the Shanghai Institute of Optics and Fine Mechanics (SIOM) of the Chinese Academy of Sciences proposed an air-lasing-assisted coherent Raman spectroscopy, realising the quantitative measurement and simultaneous detection of two greenhouse gases, as well as identification of CO_2 isotopes. The detection sensitivity can reach



Basic principle for the greenhouse gas detection with air-lasing-based Raman spectroscopy. (a) The generation scheme of air lasing and coherent Raman scattering; (b) the original and broadened spectra of the pump laser; (c) the spectrum and spatial profile of air lasing. Credit: Ultrafast Science

0.03 % and the minimum signal fluctuation is about 2 %.

In the new Raman spectroscopy technique, reported in *Ultrafast Science* (doi.org/hvhx),a femtosecond laser excites the optical gain of molecular nitrogen ions and achieves a seed amplification of more than 1000 times, resulting in 428 nm air lasing with a linewidth of 13 cm^{-1}. Meanwhile, the spectral width of the pump laser reaches 3800 cm^{-1} after non-linear propagation, enabling excitation of molecular coherent vibrations of most pollutants and greenhouse gases. When air lasing encounters coherently vibrating molecules, it will effectively produce coherent Raman scattering. By recording the frequency difference of Raman signal and air lasing, the molecular "identity information" can be known. Thus, such a Raman scheme combines the advantages of the femtosecond laser and air lasing, it thus can meet the needs of multi-component measurement and chemical specificity.

Some specific designs used in this work, especially the optimisation of pump-seed delay and the choice of perpendicular polarisation, ensure a high detection sensitivity and signal stability. It was shown that the minimum detectable concentrations of CO_2 and SF_6 can reach 0.1 % and 0.03 %, respectively. The minimum signal fluctuation reached the level of 2 %. The research team also demonstrated that the technique can be applied for simultaneous measurement of CO_2 and SF_6. More importantly, the measured Raman spectrum can well distinguish $^{12}CO_2$ and $^{13}CO_2$.

The simultaneous measurement of various pollutants, greenhouse gases as well as the detection of CO_2 isotopes are of great significance for tracing the sources of air pollution and studying the carbon cycling. This is a significant advantage of the proposed technique as compared to traditional remote sensing methods. However, for realistic application of trace gas remote detection, it is necessary to improve the detection sensitivity to the ppm or even ppb level, as well as extend the detection distance from the laboratory scale to the kilometre scale. It is expected that such a goal can be realised in the near future with the development of high-repetition, high-energy femtosecond laser technologies.

ERC grant for single-molecule force spectroscopy research

Nearly €2.5M over a period of five years has been awarded to develop research that aims to better understand how mechanical forces and chemistry affect each other by studying the rupture and reformation of basic chemical bonds.

Anne-Sophie Duwez, Professor and Director of the NanoChem Laboratory at the University of Liege has been selected to receive an ERC Advanced Grant from the European Research Council for her ChemForce project. This funding, nearly €2.5M over a period of five years, was awarded to develop research that aims to better understand how mechanical forces and chemistry affect each other by studying the rupture and reformation of basic chemical bonds. A research programme that could find applications in the field of green chemistry and the development of materials with unique mechanical properties.

Photo : Université de Liège / S. Seyen

In 1952, Schrödinger wrote that we would never experiment with a single electron, atom or molecule. Forty years later, methods derived from scanning probe microscopes (SPMs) allowed us to manipulate single atoms and molecules, and even single bonds. Single-molecule force spectroscopy (SMFS), which consists in trapping and stretching a molecule between an atomic force microscopy (AFM) tip and a surface, enables to probe (and/or induce) molecular processes *in situ* and in real time by the application of mechanical forces. Such experiments have provided unprecedented insights into the structure and function of many biological systems, including DNA, proteins, enzymes, molecular machines etc. The ability to observe one molecule at a time allows us to ask and answer questions that are impossible, or extremely difficult, to address using conventional ensemble techniques.

Anne-Sophie Duwez, Professor of Chemistry and Director of the NanoChem Laboratory (MolSys Research Unit / Faculty of Science) at the University of Liege is one of the researchers working with these advanced techniques. "SMFS has contributed to major advances in biology and biophysics. It has been exploited to a much lesser extent by chemists, mainly in the field of polymers. It remains largely under-exploited in chemistry." This observation led the researcher to take an interest in the way in which forces and chemistry influence each other. "The mechanics of chemical bonds is still in its infancy and could benefit greatly from SMFS. Major questions that could not been addressed so far, such as the mechanical reversibility of chemical bonds and the lifetime of bonds under mechanical load, could be elucidated. SMFS offers remarkable opportunities to advance our fundamental understanding of chemical bonds. It can also open up avenues for exploiting the ability of mechanical loads to affect chemistry and guide thinking in the design of new materials, reactions and processes, in a framework other than thermodynamics in solution."

However, the implementation of single-molecule mechanics on small synthetic molecules remains a major challenge due to the very small scale of the processes involved compared to large biological systems. The difficulty comes from the need to develop adequate tools and to prepare suitable molecules that can be interfaced with the device, especially when one wants to probe the reversibility of bonds. "SMFS is very demanding and only a few laboratories in the world have the expertise to design, perform and interpret advanced custom SMFS experiments. Over the past few years, my group has acquired the necessary expertise and developed a series of pioneering approaches in the field of SMFS that now allow us to tackle this big question, which requires a considerable joint effort between synthetic chemists, physical chemists and engineers."

The ChemForce project aims to broaden the scope of SMFS

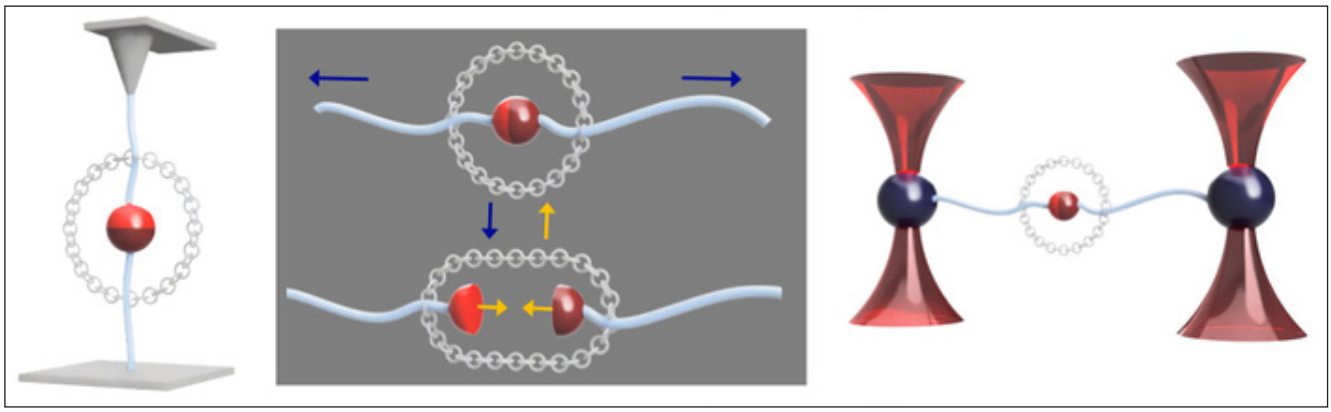


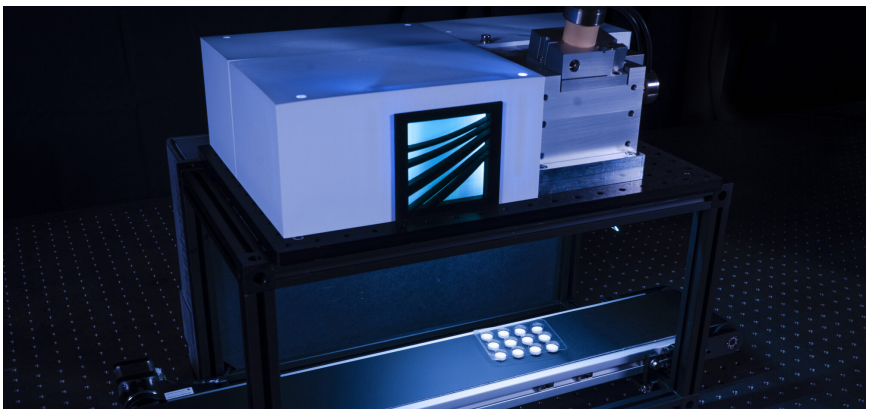
Figure 1. Schematic of the general objective of ChemForce. The concept of tethered bonds is shown in the middle section. The partners of the bond remain in close proximity after the bond has been broken open and can rebind. Single-molecule force spectroscopy by AFM (left) or Optical Tweezers (right) is used to monitor the process in various environments (solvent, pH, presence of competing reagents, stimuli, ...). Credit: A.S. Duwez / University of Liège

and adapt it to obtain a detailed picture of the interaction between mechanical forces and chemistry at the single molecule level. "We will overcome one of the major failure of SMFS over the past 25 years, namely the impossibility or extreme difficulty of probing the reformation of bonds after their rupture. To this purpose, we will synthesise and probe a series of molecules containing tethered chemical bonds to study their mechanical stability as a function of their geometry and environment, including the time they can withstand a defined force, and their reversibility (Figure 1). The tethered structure ensures that the components of the bond remain in close proximity after the bond rupture, leaving the possibility of reforming the bond and of studying how mechanical forces and proximity can trigger a chemical reaction." The five-year project will provide a detailed understanding of how mechanical forces can modify the free energy surface of chemical reactions. This is basic research, but it could have many applications, particularly in the field of mechanochemical synthesis, and could provide the keys to the development of much more effective and better controlled self-healing materials.

Inline-capable spectroscopic 100 % inspection for industrial QA and process control

Fraunhofer IAF has developed an integrable measurement system which uses machine vision to detect samples and verify them using laser-based infrared spectroscopy.

With an inline-capable, laser-based infrared spectroscopy measurement system, researchers at the Fraunhofer Institute for Applied Solid State Physics IAF want to support industrial companies from the pharmaceutical, chemical and food industry in making quality assurance measurements and process control more reliable and at the same time more efficient. The system has a flexible design and a high spectral scanning speed that allows it to be integrated into existing visual inspection systems, enabling full spectroscopic inspection.



Demonstrator of an inline-capable infrared spectroscopy measurement system of Fraunhofer IAF for applications in the pharmaceutical, chemical and food industry. © Fraunhofer IAF

"The measurement system vividly demonstrates the great benefit of laser-based infrared spectroscopy for industrial sectors in which products have to be verified or differentiated reliably and quickly", emphasises Dr Marko Härtelt, responsible project manager at Fraunhofer IAF.

The specific advantages of the system result from the backscattering spectroscopy method in the mid-infrared with wavelengths in the range of 4–12 µm. Since molecular compounds have very characteristic absorption and emission lines in this spectral range, they can be clearly identified. One of the core components of the measurement system is a broad-emitting and spectrally fast-scanning laser module. It combines quantum cascade lasers (QCLs) from Fraunhofer IAF and micro-opto-electro-mechanical grating scanners (MOEMS) from the Fraunhofer Institute for Photonic Microsystems IPMS. The high brilliance of the light source and the unique properties of the MOEMS scanner enable infrared spectroscopy at a rate of one kilohertz.

A neural network analyses measurement results, and enables the system to drastically minimise the error rate and at the same time the required measurement time.

Shimadzu Denmark managing director

Joachim Holm is the first local managing director of the Shimadzu office in Denmark. On 1 April, he took over the reins at the office established 18 months ago in Ballerup on the outskirts of Copenhagen, which was previously managed centrally by the management of Shimadzu Europe and locally by Morten Thorslund, sales and service manager.

Joachim Holm is an experienced senior sales leader specialising in pharma, life science and medical products. Holm studied pharmacy and business management in Copenhagen and has held several management positions at notable medical technology and pharma companies in recent years, including ChemoMetec and Specific Pharma. Thanks to his role as manager with responsibility for the Nordic countries—at Medtronic, among other places—he is familiar with the entire Scandinavian market. The 43-year-old lives with his family in the Copenhagen metropolitan area.

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ARTICLE

Mapping metals in brain tissue with X-ray fluorescence and X-ray absorption spectroscopy at synchrotron light sources

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The hippocampus (Figure 1A) is a brain region critical to spatial learning and memory. The hippocampal formation contains a highly organised architecture of neurons and neuron–neuron connections, which have been intensively studied by neuroscientists for many decades. In fact, the hippocampus is often referred to as the "Rosetta Stone" of neuroscience, with many believing elucidation of hippocampus circuitry and cell function will unravel the inner workings of the brain.

A fascinating fact of the hippocampus is that it appears to be relatively enriched in transition metal ions, particularly Fe, Cu and Zn (Figure 1B). The Zn enrichment was discovered by scientists developing histochemical methods to detect labile metals in brain tissue (e.g., works of Danscher and others),[1,2] with work led by Frederickson definitively demonstrating that the characteristic pool of labile metal ions observed in the hippocampus was Zn.[2–4] Of great interest, experiments aimed at depleting the labile Zn pool in the hippocampus subsequently revealed behavioural and cognitive deficits in mice,[2] consistent with facets of memory loss observed during neurodegenerative diseases of ageing, such as Alzheimer's disease.[5] Consequently, a plethora of lines of research enquiries emerged, aiming to uncover the physiological and chemical pathways through which transition metal ions might be implicated in healthy memory function, and also memory loss.

While the classical Timm's histochemical stain has been invaluable to study labile Zn in the hippocampus (and brain in general), a number of important advances in this field have now been made using direct spectroscopic mapping. Specifically, the provision of intense (bright) and tuneable X-ray sources at synchrotron facilities has revolutionised the biological applications of X-ray techniques, especially X-ray fluorescence spectroscopy (XRF) and X-ray absorption spectroscopy (XAS). Key advantages of XRF are its ability to simultaneously and directly detect (map) elemental distribution at cellular resolution (and sometimes sub-cellular resolution), *in situ*. The direct *in situ* detection capabilities of XRF are

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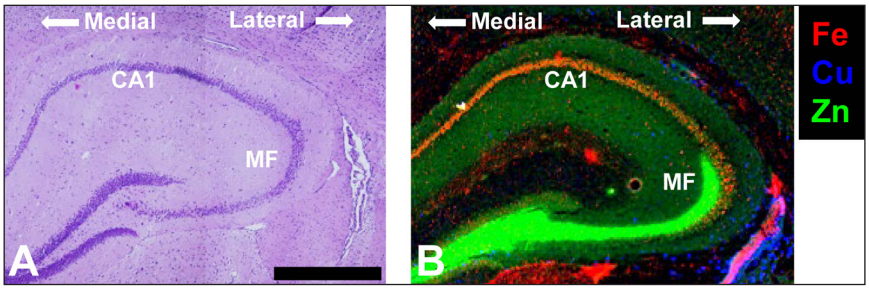

Figure 1. (A) Haematoxylin and eosin histology of the hippocampus showing the characteristic organisation of brain cells (purple dots). (B) XRF elemental maps highlighting two key sub-regions of the hippocampus, the Fe-enriched neuron layer Corpus Ammonis 1 (CA1) and the Zn enriched mossy fibre region (MF) that contains numerous neuron–neuron connections (synapses). The medial to lateral orientation of the tissue is shown. Scale bar = 500 µm. Data was collected at the X-ray fluorescence microscopy beamline at the Australian Synchrotron, and is adapted with permission from Reference 12.

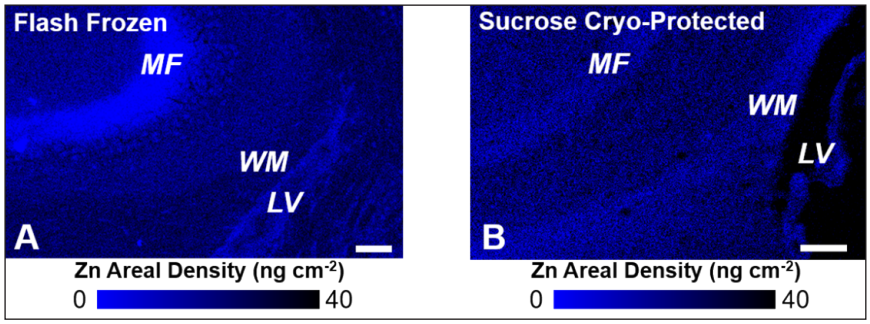

Figure 2. XRF elemental mapping of Zn distribution in (A) non-fixed flash frozen hippocampal tissue, and (B) formalin-fixed sucrose cryo-protected tissue. A substantial redistribution of Zn is observed as a consequence of formalin fixation and sucrose cryo-protection. Specifically, Zn is lost from the mossy fibres (MF, neuron–neuron connections) and redistributed to white matter tissue (WM) adjacent to the brain lateral ventricles (LV). Scale bar = 100 µm. Images adapted with permission from Reference 7.

critical when studying brain tissue, as chemical fixation and/or addition of staining reagents, which are common place in other microscopies, are now known to drastically alter the metal ion content and distribution within brain tissue (Figure 2).[6–8]

Several important early examples of the use of XRF to study brain tissue include a definitive demonstration that the Timm's histochemical stain reveals the presence of a labile pool of hippocampal Zn[4] and the observations that transition metal ions co-localise with amyloid-β plaques (a hallmark of Alzheimer's disease).[9] More recent studies have highlighted that while amyloid-β plaques within the hippocampus appear to become enriched with metal ions in models of Alzheimer's disease (e.g., Zn), the brain tissue surrounding the plaque becomes metal deficient.[10] This has then raised an interesting research question, does metal accumulation in plaques contribute to disease pathology, or is metal deficiency a contributing factor, or both?

In addition to mapping metal ions associated with disease states, synchrotron XRF has been applied to characterise metal ion distribution in the healthy hippocampus, identifying hippocampal subregions locally enriched in Fe, Cu or Zn (Figure 1).[11,12] Intriguingly, XRF revealed that the healthy rodent hippocampus contains an especially high Fe content within a region of hippocampal neurons (the "CA1" sector) known to display high vulnerability to neurodegeneration (e.g., neurodegenerative disease, stroke, brain trauma). Even more fascinating, the neurons within the CA1 sector that are closest to the middle of the brain (medial) contain more Fe than the neurons closer to the outside of the brain (lateral).[11] The lateral-to-medial trend of increasing Fe content matches the pattern of neurodegeneration seen within this highly vulnerable brain region (i.e., medial CA1 neurons are more vulnerable than lateral CA1 neurons). Not surprisingly, a number of stroke research groups are actively using synchrotron XRF to study the relationship between Fe and neurodegeneration after stroke.[13]

Building from elemental mapping, a rapidly developing application of XRF and XAS beamlines at synchrotron facilities is the *in situ* study of metal ion speciation (oxidation state, coordination geometry, types of ligands). Continued advances at 3rd and 4th generation synchrotron facilities, combined with improvements in X-ray optics, detectors and electronics makes it now possible to collect hundreds, or even thousands, of micro-XAS spectra, to map metal speciation within biological samples. Metal speciation is most commonly studied by using a small region of the XAS spectrum, known as X-ray Absorption Near-Edge Structure (XANES).

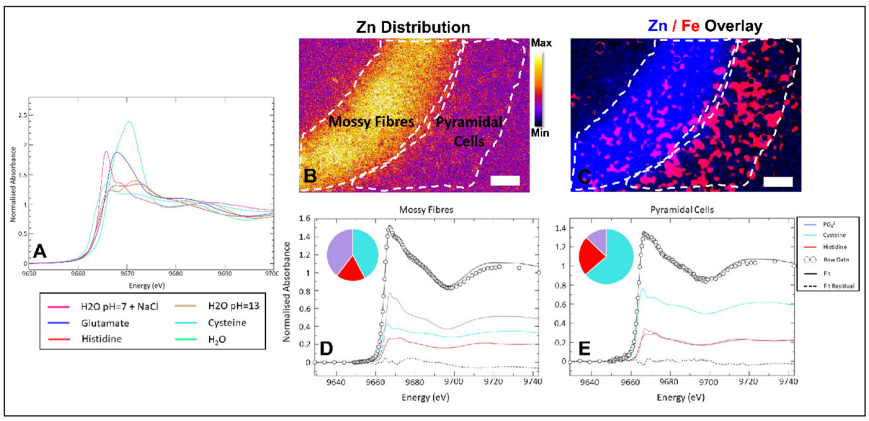

Figure 3. Development of XANES spectroscopic mapping to study Zn speciation in the hippocampus. (A) XANES spectra from standard solutions of Zn^{2+} in the presence of different biological ligands, showing the characteristic "richness" of the XANES spectral region to differences in coordination environment. (B, C) Hippocampal Fe and Zn distribution revealing the locations of the MFs (neuron–neuron connections) and the adjacent neuron layer (pyramidal cells). (D, E) Micro-XANES spectra from the MF layer (D) and the pyramidal neurons (E) highlighting spectroscopic differences indicative of the different chemical forms of Zn present in each region. Scale bar = 50 µm. Figure reproduced with permission from Reference 19.

Initial applications of XANES spectroscopic mapping of biological systems include mapping the distribution of different oxidation states of S, Se and As compounds in plant tissue.[14,15] The methods have now been adapted to map Fe and Cu speciation in *Caenorhabditis elegans*,[16,17] Fe speciation in brain tissue[18] and a Zn method development is in progress (Figure 3).[19]

A number of analytical challenges still need to be resolved for XANES-mapping of brain tissue to reach its full potential. These challenges include the development of suitable spectral libraries that adequately model the different chemical forms of metal ions found in the brain, in addition to understanding the effects of sample preparation on metal ion speciation. At this stage, a great deal of work has been done by this community to optimise sample preparation to preserve elemental distribution in brain tissue, but much less is known about how oxidation state and coordination environmental of metal ions might be changing in brain tissue during the various stages of sample preparation. While challenges still exist, continued advancement in XRF and XANES spectroscopic mapping of metal ions in biological systems, and especially the brain, is an exciting prospect. Indeed, there is likely much yet to be learned about the specific role that Fe, Cu and Zn hold in supporting healthy hippocampal memory function, and how the loss of metal homeostasis could contribute to loss of memory and cognitive decline.

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Ashley Hollings is completing her PhD at Curtin University under the supervision of Dr Mark Hackett, sitting at the interface of analytical chemistry and neuroscience, specifically focussed on developing new spectroscopic protocols to image brain metals and biochemistry during ageing. Ashley's PhD incorporates a multi-modal imaging approach, using X-ray absorption near edge structure (XANES) spectroscopy alongside X-ray Fluorescence Microscopy (XFM), synchrotron radiation Fourier Transform Infrared (SR-FT-IR) microscopy and confocal Raman microscopy. Ashley hopes that the combination of X-ray spectroscopy and vibrational spectroscopy will help to reveal links between metal dis-homeostasis and altered brain biochemistry during ageing and dementia.
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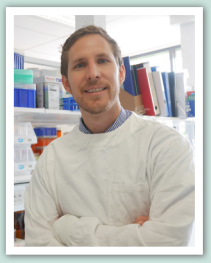

Dr Mark Hackett is an analytical chemist interested in the development and application of spectroscopic tools to study metal ions in biological systems. Dr Hackett primarily uses X-ray spectroscopies at synchrotron light sources, such as X-ray fluorescence and X-ray absorption spectroscopy to determine elemental distribution and speciation, *in situ*. These analyses are coupled with a multi-modal workflow, incorporating other spectroscopic tools (Fourier transform infrared spectroscopy, Raman spectroscopy, optical microscopy) to help reveal metal homeostasis in the context of a "holistic biochemical picture". Dr Hackett received his PhD from The University of Sydney (2011), which was followed by post-doctoral fellowships at the University of Saskatchewan, Canada. Dr Hackett is currently an ARC Future Fellow at Curtin University, Western Australia.
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ARTICLE

The future of matrix-assisted laser desorption/ionisation mass spectrometry in pathology applications

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Clinical research has led the way in the use of matrix-assisted laser desorption/ionisation (MALDI) coupled with mass spectrometry imaging (MSI) in developing new applications to determine spatial data about biomolecules in tissues. MALDI imaging is a powerful, label-free analytical tool that can provide vital molecular information about protein modifications after gene expression. It also helps with visualising additional compounds like metabolites, glycans and lipids that play a part in disease pathology. Together with the emergence of new MALDI tools for targeted protein imaging, MALDI imaging offers the potential for scientists to fill in the gaps left by spatial transcriptomics and genomics in molecular investigations on tissue samples.[1,2]

This article describes MALDI imaging's potential uses in pathology applications, and the benefits of the technique to map hundreds of biomolecules (proteins, lipids and glycans, for example) in a label-free, untargeted manner or for imaging target proteins using a modified immunohistochemistry protocol, often from a single tissue section. We also discuss the more recent influence of technological advances such as trapped ion mobility spectrometry (TIMS) and laser-induced post-ionisation (PI), as well as the impact on cancer research and disease management and how expanding the depth of available molecular information can translate into benefits for patient care.

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Current use of biomolecule spatial data

The discovery of specific molecular markers of disease via molecular labels and probes has gradually expanded the traditional diagnostic histopathology of tissues. After the completion of the Human Genome Project in 2003, The Cancer Genome Atlas (TCGA) programme generated over 2.5 petabytes of genomic, epigenomic, transcriptomic and proteomic data from the molecular characterisation of over 20,000 primary cancer and matched normal samples, which spanned 33 cancer types. That data, which is available publicly, has already improved the ability to diagnose, treat and prevent cancer by connecting morphological information with genetic and molecular insights.

From these developments, the field of clinical molecular diagnostics emerged, aided by technological advancements such as sequencing technology, reverse transcription polymerase chain reaction (rt-PCR), immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH) methodologies. One perspective[3] about these developments in the field summarised that: "Pathologists will become pilots for precision cancer therapy through their unique ability to combine morphological and molecular findings". Indeed, the adoption of epidermal growth factor receptor (EGFR) mutation testing by pathology departments fits that narrative. By demonstrating the efficacy of kinase inhibitors erlotinib and gefitinib in the treatment of EGFR-mutated lung adenocarcinomas, the field's paradigm shifted and began to emphasise the development of spatial omics techniques.

Current diagnostic tools

With the establishment of spatial information as a key component of analysing the microenvironment of disease in tissues, researchers turned to a suite of diagnostic tools such as IHC, spatial transcriptomics

and imaging mass cytometry. While these tools serve multiple disease pathologies, which notably include neurology and cardiology, there is tremendous potential in the field of oncology where identifying the location and interactions of cellular components can dictate disease outcomes.

While these tools produce useful data about proteins in tissues, each one also brings challenges. The key limitations of all three tools include the inability to capture the variety of post-translational modifications (PTMs) in the cellular proteome, or to visualise the vast cellular lipidome and metabolome in context of tissue pathology, all of which would offer a broader molecular insight and stronger basis for classification compared to considering only the pre-translational proteome.

Impact of MALDI imaging

MALDI imaging is a powerful tool for mapping the distribution of molecules—ranging from small metabolites to large proteins—from a thin section of tissue without need for molecular tags or labels. A single MALDI imaging measurement can yield up to several thousands of distribution maps, or ion images, that reveal greater insight and understanding of molecular makeup and regional heterogeneity in the tissue. Due to its untargeted nature, it captures information about the spatial proteome and additional spatial omic signatures that are unique to the local cell neighbourhood without prior knowledge of the compounds. Many researchers agree that moving beyond protein biomarkers is important for next-stage clinical understanding, with one recent publication[4] noting, for example, that: "Lipids play a significant role in the manifestation of cancer. However, research into lipid biomarkers of cancer is still in its infancy."

For oncology applications, untargeted MALDI imaging enables the visualisation of tumour-associated biomolecules that are missed at the gene level. From a practical point of view, the MALDI imaging workflow is compatible with standard histological procedures, it maintains spatial resolution at around 10 µm, and the tissue section under test is preserved for further study. The result is a powerful complementary technique capable of discovering and spatially mapping important features at a deeper molecular level (Figure 2).

Routine oncology applications

MALDI imaging offers a great deal of potential for use in oncology. Detailed spatial proteomic, lipidomic and metabolomic insights can complement traditional genomic and transcriptomic methods. Often, knowing where molecular expression changes can be just as important as knowing if expression changes. This can be especially true if certain compounds are highly spatially concentrated or if molecules co-distribute in specific compartments because vital information is lost when examining only homogenised samples. The identification of new predictive or prognostic biomarkers, and the classification of heterogeneous tumour subpopulations, give important contextual clues to tissue-level communication networks that are integral to cancer growth and treatment success.

Many recent studies demonstrate the advantage of integrating MALDI imaging with traditional immuno techniques for tissue pathology applications. For example, Yagnik *et al.* reported the development of a new method based on novel photocleavable mass-tags (PC-MTs) for facile antibody labelling, which enables highly multiplexed IHC based on MALDI imaging (MALDI-HiPLEX IHC). The same technique, MALDI imaging, effectively projects untargeted imaging results into the familiar context of immuno-based cellular classification. The author's conclusions were that the new combination shows promise for use in the fields of tissue pathology, tissue diagnostics, therapeutics and precision medicine.[5]

Work published in 2019 by Randall *et al.* demonstrated how MALDI imaging of lipids and metabolites in tissue samples accurately reflected a patient's prostate cancer stage, as defined by traditional histologic evaluation using the "Gleason score". This is the current standard of care; however, this time-consuming process is prone to intra/inter-observer variability, and provides no information about altered metabolic pathways

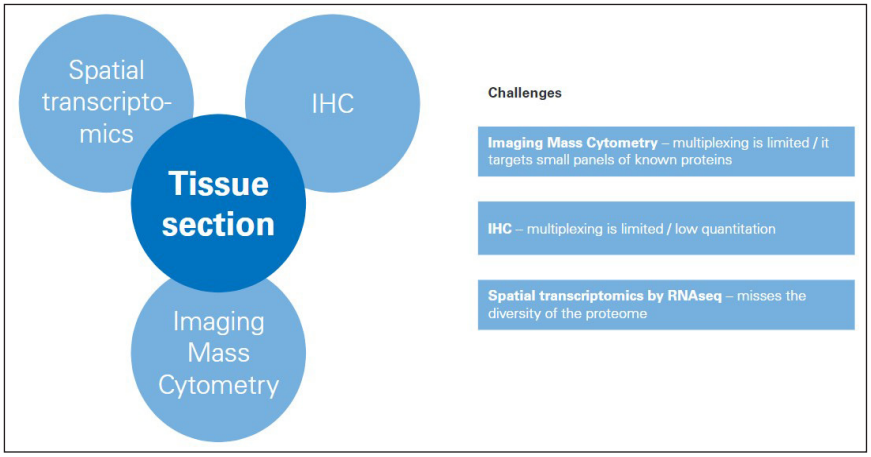

Figure 1. Challenges for tools for classifying/mapping cellular regions in tissue.

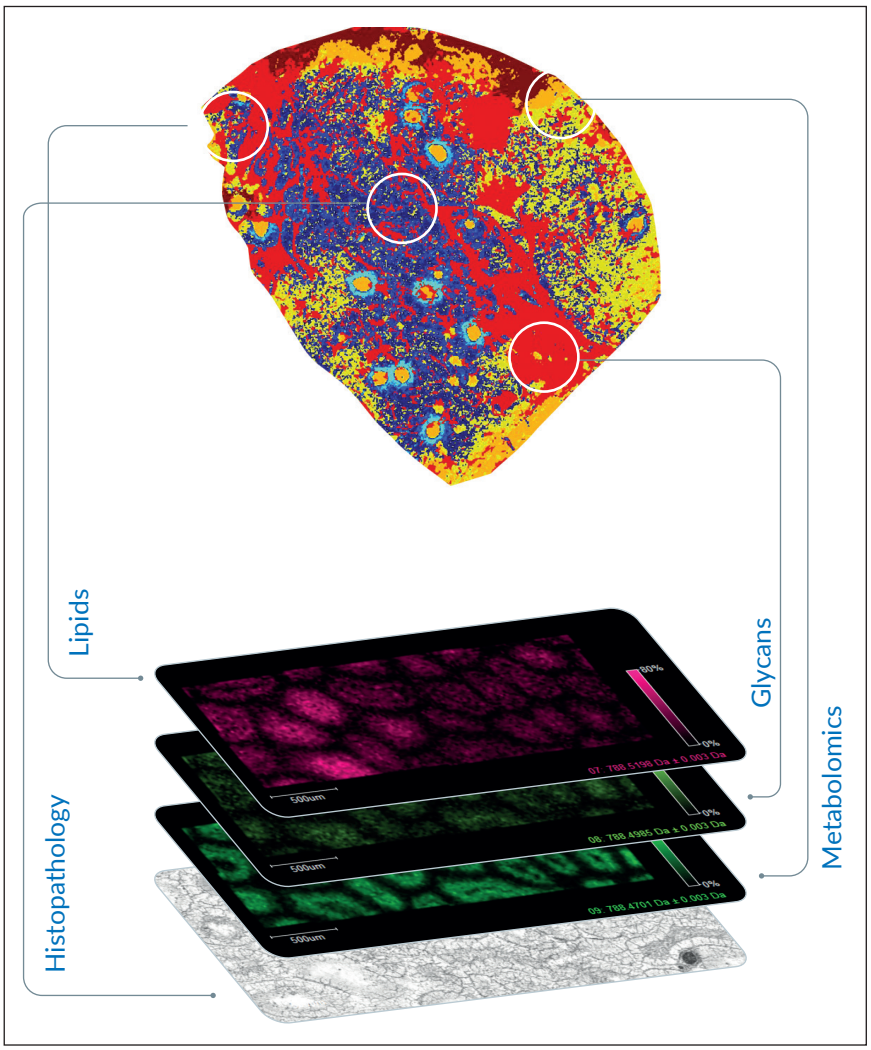

Figure 2. Additional disease insights available from MALDI imaging.

or altered tissue architecture. They concluded that MALDI imaging could be used as a potential clinical tool to support more objective and faster diagnosis.[6]

To assess the suitability of MALDI imaging as a front-line technique, Bassu *et al.* recently reported on "real-time" molecular assessment of tumour margins. The goal was to discriminate surgical resection specimens from patients in a workflow that was rapid enough to directly impact surgical decisions.[7] By adapting various stages of a conventional 30-minute protocol for MALDI imaging, they developed a reliable and reproducible 5-minute workflow that they concluded placed MALDI imaging firmly in the realm of routine clinical decision making (Figure 3). Furthermore, they suggest that by using an artificial intelligence (AI) step in the workflow, the MALDI imaging data could be analysed directly, without visual review, using previously established machine-trained models.

New technological advancements

Two recent breakthroughs in technology have significant implications for the future of MALDI imaging. First, ion mobility separation (IMS) has greatly broadened the range of biomolecules that can be analysed by pre-separation ahead of mass analysis. Of the many IMS technologies, Trapped IMS (TIMS) offers significant benefits for MALDI imaging as well as traditional omics. The timsTOF flex instrument (Bruker, Billerica, MA, USA) is one example, which adds a high spatial resolution MALDI imaging source to deliver a spatial dimension to omics analyses. The use of the technique was demonstrated in a joint project between Bruker and University of Maastricht, The Netherlands, that illustrated how MALDI-guided spatial omics uncovers proteomic diversity in lipid-segmented subpopulations of breast cancer that would otherwise have been missed using traditional bulk sampling.[8]

Second, novel laser-induced post-ionisation (PI) technology has delivered an increase in MALDI imaging sensitivity, by up to three orders of magnitude. Termed MALDI-2, this technology has now been applied to the timsTOF fleX and, together with TIMS, provides for the separation and mapping of even more analytes, such as lipids and glycans, in the complex tissue environment.[9] Currently, this exciting development is being explored in research projects. However, it is easy to see how this technology could, in time, offer next-level performance to pathology applications too.

Conclusion

MALDI imaging provides significant advantages for molecular analysis of tissue specimens in greater detail. Crucially, MALDI imaging maintains the spatial relationships of analytes in tissues, allowing improved translational and clinical insights. That capability expands the accessibility of a broad spectrum of analytes in tissue including proteins, lipids, glycans and other analytes, in both targeted and untargeted manners.

The use of MALDI imaging offers new opportunities to create a top-down, disease-centric pathological view of tissues at the molecular

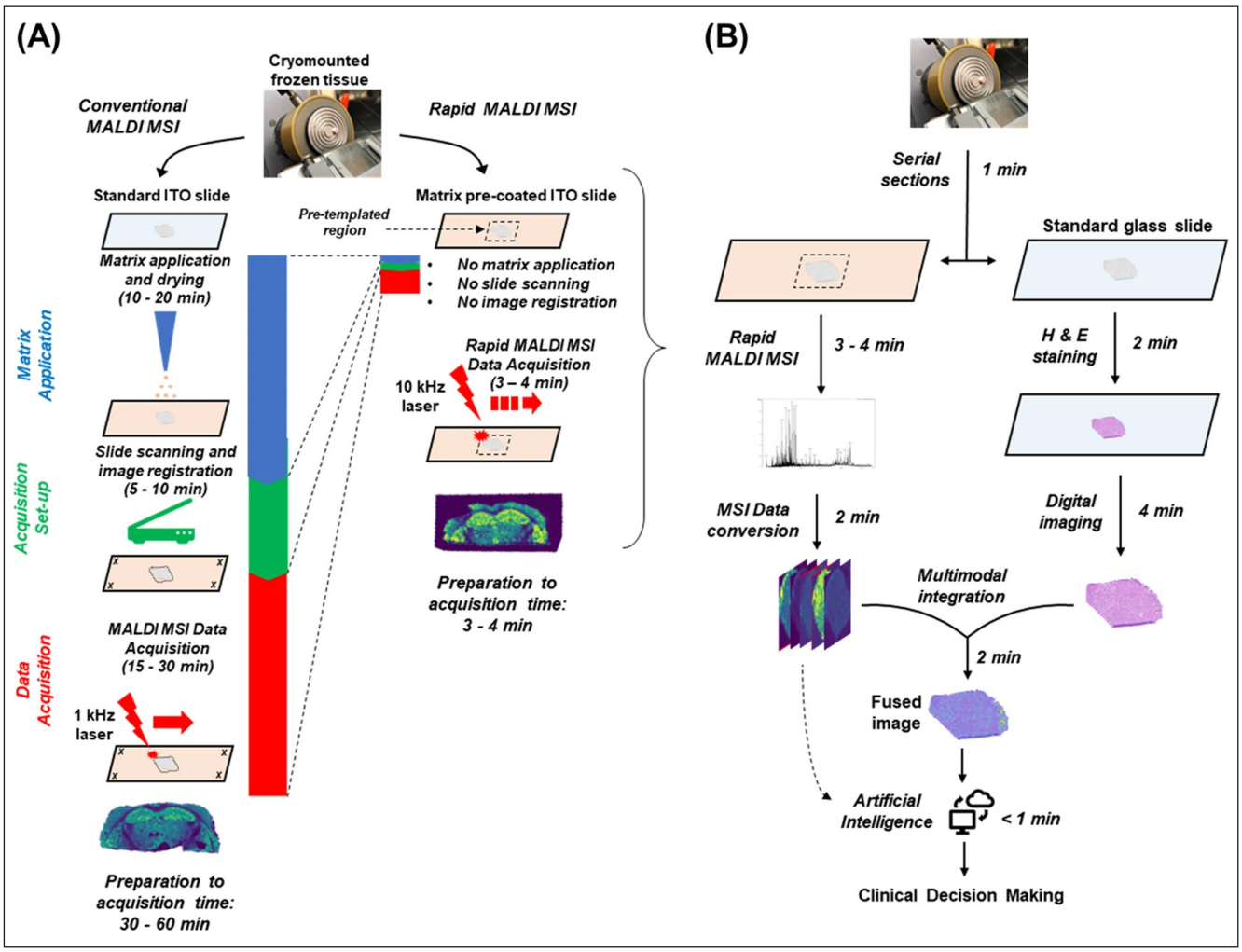

Figure 3. (A) Comparison of conventional vs rapid MALDI imaging. (B) Proposed workflow for MALDI imaging in the frozen section room. Figure reproduced from Reference 7 under a CC BY licence.

level that can inform therapeutic strategies, support diagnosis and improve patient outcomes. Further developments to the technology will provide faster measurement speeds, increased sensitivity without compromising spatial resolution, and even deeper molecular content—important factors that will accelerate the adoption of MALDI imaging in the routine pathology environment.

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All that and a bag of chips

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This column has invited two world-renowned experts in near infrared (NIR) spectroscopy to let the world benefit from decades of leading-edge experience, especially regarding sampling for quantitative NIR analysis. Our colleagues jumped at the opportunity with a didactic exposé of issues to consider before a NIR analytical result can be declared valid. Two very practical examples shed a penetrating light on the interconnectedness of analytical technology *sensa stricto* and the use of the final analytical results. And not only that, the reader is also invited to a brief tour of the Bayesian statistical world view along the way …—Kim H. Esbensen (Editor)

Introduction

"All that and a bag of chips". It's a common enough saying, but things get messy real fast if you just try to sample a bag of chips/crisps. There are several fundamental issues that formally jump at your throat. First of all, based on the Theory of Sampling (TOS), there is the "Fundamental Sampling Principle (FSP)", which compels us: "All virtual increments in any lot must be susceptible to sampling and must have the same probability of ending up in the final composite sample".[1] This means that a NIR analyst *must* start with the opening *assumption* that everything in the original lot (every component) has had an equal probability of being selected to appear in the "sample" delivered to the analytical laboratory (and in the correct proportions too). That's sort of reminiscent of how the statistician **Bayes** developed his famous probability theory world view: "In the absence of knowledge, everything is equally probable". But then "knowledge" starts to show up and it kicks us, and our equal probabilities, in the teeth. Let's follow how this happens.

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Critical issues to consider

We've all had a bag of crisps or chips in our hands. And we have all probably noted that the crumbs in the bottom of the bag are different from the intact chips. They are oilier, have more salt and more seasoning, and are definitely harder to pick up, i.e. they possess an inherent reluctance towards sampling. Right here at the outset this doesn't bode well, since we are mandated (FSP) to have equal probability of picking up components. But with dramatically unequal sizes and physical properties it is quite challenging to pick them with equal likelihood; how is it possible to do this in the correct proportions?

And equal probability based on what criterion? Equal based on volume or mass? That depends on what you want to know. Do you want to know the analysis of the average chip experience, or do you

Figure 1. There are chips—and there are chip fragments "all the way down the grain size scale" … Photo: Kim H. Esbensen

want to know the analysis of what is in the package for nutritional labelling say? By volume or by weight? Where did all these questions come from?

The questions come from the fact that a little knowledge changes our equal probability assumption irrevocably, just like a bit of knowledge changes Bayesian prior probabilities. So, let's settle this question by focusing on mass. We want equal probability by mass. What do we do next?

We can reduce everything to the same particle size and try to randomly select from that. This sounds straight out of the TOS' playbook, but there is a serious catch: for what common particle size do we aim with a comminution operation? Push the size of the resulting ensemble of ground-down particles far enough, and you'd, for example, be left with a single grain of salt, which cannot ever be representative of the whole bag. We can easily reach a particle, and a sample size, which is simply *too small* to represent the whole (bag). Well then, we can reduce everything down to the smallest particle size, and mix this state thoroughly and then select a high enough number of them so that the probability of having a nonsensical event like a salt crystal doesn't change the overall result. Some would say *randomise* all the ground-down particles, but we must be aware that a fully homogenous end-state for poly-component mixtures does not exist—there will always be a residual, non-compressible heterogeneity.[1]

Or, we can *stratify* our approach. We can separate the sample into the large chips vs the crumbs. We can easily weigh these two groups—and sample and analyse these two groups separately, and then balance the two analytical results by their correct mass fractions to get back to equal probability. Stratifying the material based on our knowledge that smaller pieces have more surface area and pick up more oil and that the seasoning falls off and resides in the small pieces preferentially makes it an easier problem. It might perhaps seem counter-intuitive, but breaking the problem into parts, analysing the parts and then putting it all back together appropriately weighted, is actually more accurate in many cases. Why? Because we are again working from a knowledge base, and we use that knowledge to change the problem definition. "Random selection" works when you don't know things, but peek while you are doing that so-called random selection, and your pesky brain changes your operative performance and, therefore, also your results.

Regardless, now our bag of chips is separated into two piles, the larger original pieces and the crumbs. We have the comparison weights. Now *how big* should the sub-samples (increments) be and *how many*? How big a.o. depends on the reference analytical technique as every analyst know, but it also depends on the material characteristics of the material you are sampling, and this is one of the core issues that we have the TOS to helps us resolve.[1]

Some provocative thoughts: all for the good cause

How many increments is an easier question to answer. What follows is deliberately a bit provocative, but it serves the purpose well: The principal answer to *how many* is three. What? How come "three"? Because three is the minimum number of data to be able to estimate a standard deviation. One result gives you a *mean*. Two gives you a *difference*. Three is the minimum for getting to an estimate of a standard deviation. We can of course, and often *should*, involve a higher number of results—but while we *can* get more and more measurements, their individual impact on improving the mean goes down. Improving our knowledge for the least amount of work peaks at three. With three we have a rough idea of how consistent or inconsistent our equal probability increments are and can begin to figure out how many we really need to get our uncertainty down to where we would like it to be. All the while we do this, it is imperative to know *how to* extract increments in an unbiased fashion—this is where the TOS comes in with fatal consequences for those who have not vested a minimum effort in getting to this competence level.[1]

So, three gets us the first knowledge with which to figure out how many we really need, again based on our desired precision. So, with three increments of the chips (you *may* call these "sub-samples" if you like, but you are blurring the precise terminology recommended in the TOS) and three of the crumbs and their results, we can make an educated guess with error bars and the whole shebang of the likely content of the whole bag of chips. Six increments, sub-samples, as a minimum will get us started (yes, many would insist on a higher number of observations, but that's another story).

So, what do most people do in practice? Well, lots of times they throw the whole bag into a Cuisinart food processor, "mix well" and then proceed to take one sample of the ground-down mixture and go from there. And they are fortunate in their ignorance. Because if they had worked in pharmaceuticals, for example, and learned about mixing and unmixing, component density and the like, they would not be anywhere as confident that they had analysed a representative sample. Knowing things again changes the rules and the probabilities.

The above example helps us to understand how the TOS can be used to logically and systematically think through how to use the NIR spectroscopy analytical powerhouse correctly—to improve

the accuracy and precision of the analytical result. The realm of "before analysis" is a critical success factor for representative and, therefore, reliable analysis, a realm that always must be reckoned with.[1–3]

TOS basics

Besides the first fundamental principle of the TOS, FSP, there are five more governing principles that have a bearing on how suitable for their intended use your NIR measurements will be. The most important of these, together with a few salient focus issues are:[1,4–6]

1) **Lot dimensionality:** Defined as the number of effective dimensions that need to be *covered* by the sampling process. The TOS shows how there are overwhelming advantages in sampling from elongated 1-D lots, most often in the form of moving 1-D lots (process sampling).
2) **Sampling Invariance:** Refers to the fact that all materials are made up of "constituent units" pertaining to three scales, e.g. starting from the absolutely smallest scale:[7]
   - Atoms and molecules. While this scale level is generally not of interest for the macroscopic sampling normally occurring in technology, industry and society, this is central to NIR analysis (see Reference 2 for details).
   - The critical scale level commensurate with the sampling tool volume, defined as the sampling increment, in which the constituent units can be grains, particles, fragments thereof, as well as aggregations, particle clumps a.o., coherent enough so as not to be fragmented in the sampling process).
   - The largest scale of interest is the observation scale of the sampling target itself, the *lot scale*.
3) **Sampling Correctness (bias-free sampling):** The TOS uses this term to denote that all necessary efforts have been executed which has resulted in successful elimination of the so-called "bias-generating errors", a.k.a. the Incorrect Sampling Errors (ISE).[4]
4) **Sampling Simplicity (primary sampling + mass-reduction):** This principle specifies the multistage nature of all sampling processes, stating that there is always a primary operation, followed by a series of representative mass reductions (sub-sampling or splitting operations) until a representative analytical aliquot has been produced.[2] This principle allows all stakeholders to optimise the individual sampling and analytical stages independently of each other.
5) **Heterogeneity Characterisation:** Heterogeneity is attributed as the primary source for effects from the two so-called "correct sampling errors", and a specific sampling process may itself result in effects from up to three additional ISEs.[1,5,6]

Specifics of NIR analysis

In performing NIR experiments, the first non-negotiable criterion is that the sample to be analysed must be representative of the target material from which it has been extracted. It makes no sense to analyse a sample (aliquot) that cannot be documented to be representative of the whole lot from which it originates.[1]

Ritchie presents the Analytical Method Triangle for the modern NIR experiment, which takes into account sample Design of Experiment (DoE),[8] Figure 2.

NIR characteristics can be visualised as three legs of a triangle:

1) Instrument Qualification
2) Method Validation
3) Sample DoE

… with a fourth component being *analyst knowledge* (education, qualification and training). Meeting the regulatory requirements for an analytical method requires that critical parameters for instrument and method performance be evaluated. Similarly, samples must be evaluated for their appropriate properties and response for NIR measurements, e.g. that they have

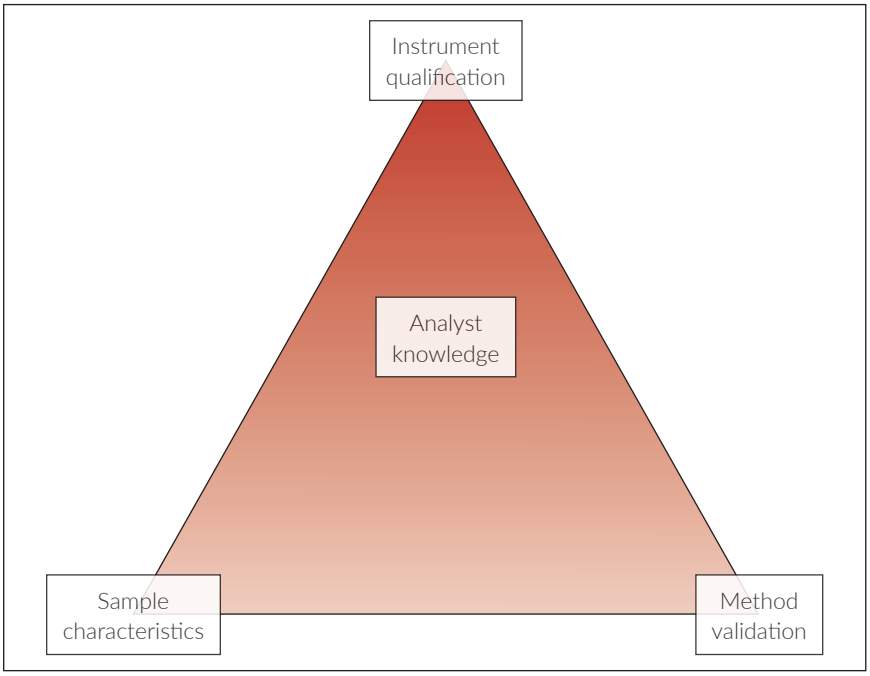

Figure 2. Analytical Method Triangle; see text for explanation.

been sufficiently "homogenised" a.o.

The NIR measurement is unlike most other analytical determinations for several reasons.[3] Because NIR analytical results are based on a *correlation* of spectra to reference values determined from a valid reference method which is sensitive, specific and selective for the analyte, NIR measurements are *indirect measurements*. As a result, NIR measurement errors arise primarily from calibrations based on the NIR measurements plus the laboratory values obtained from the reference method.[9,10] The sample carries with it two major sources of error—in addition to the various sampling errors governed by compliance, or rather by non-compliance with the representativity demands from the TOS. The total combined error contributes to the *bias* (difference) observed between the calibrated NIR and compendial reference methods. Also, NIR measurement spectra carry with them three qualities that reflect the effective degree of heterogeneity of a sample:

1) The physical dimensions of the sample itself, expressed as particle size, due to diffuse scattering energy interacting with the sample material and the nature of diffuse reflectance interacting with the detector. Differences in the physical composition of the sample lead to scattering of non-absorbers which interfere with the absorption spectrum of the analyte of interest.
2) The chemical composition of the sample as a result of the overtones and combination vibrations of molecules, lead to absorptions in the NIR portion of the electromagnetic spectrum. Contaminated absorption spectra will arise from a heterogenous sample outside the specifications for the sample under study and will, therefore, lead to erroneous results.
3) The NIR measurement and the samples temporal and spatial positioning while spectra are being acquired and whether the sample is static or moving also has a definite effect on the final spectra.

While the heterogeneous nature of a material in a specific comminution state is scale invariant, the physical, chemical compositional and positional (spatial) characteristics for solids are magnified in material exhibiting NIR absorption and the heterogeneous nature of materials makes itself evident in the following ways:[2]

Non-absorbing components in the joint particle domain exhibit their effects as multiplicative and additive scatter. This is heterogeneity-exhibiting physical effects which leads to calibration and prediction error in the calibration model. Furthermore, heterogeneity contributes to sampling error due to compositional effects because particles in general may well be composed of *different grades* of the analyte in question, also contributing to the analytical error component of the model. In addition, samples that are moist or wet, will evaporate water and this will cause the spectra to appear to *shift*. Last, temperature will have a similar shifting effect on spectra if care is not taken in controlling the sampling of hot or cold samples. See more on these issues in measurement uncertainty in, e.g., References 2, 3 and 11.

As all NIR analytical techniques require that method validity, accuracy, precision and linearity through appropriate DoE have been demonstrated, the sampling processes involved before analysis should *also* be subject to a process akin to DoE that informs the analyst about the sources and magnitude of sample heterogeneity, and the sources of other sampling errors—all of which have to be counteracted by the universal procedure called *composite sampling*.[1,2,4–7,11] In addition, samples being measured in a moving process should be studied using variographic analysis.[1,2]

All NIR experiments should be accompanied by an analytical sampling plan. The minimum requirement shall be that the total sampling error and the total analytical error have been successfully *decomposed* and individually quantified.[1,2,6,11]

In practice

Another example follows below that illustrates TOS principles as they apply to the modern NIR experiment.

This time, instead of sampling a bag of chips, let's work through how you sample *soymeal*. The first major difference is that a trade association called the National Oilseed Processors Association (NOPA) exists, which has produced sampling guidelines for the industry. Better follows these, or else? This dictum will, of course, depend on whether these guidelines contribute towards representativity, or *not*.

What lot size are we sampling now? We are going to sample a truck, or a rail carload. How do we extract a sample from this "enormous" lot size? Well, this is a perfunctory example of the dictum: "Best to sample a *moving* stream of material, because this is a 1-D lot configuration" (see above)!

For example, as the soymeal is falling into the vehicle through a chute, a travelling *sample cutter* translates across the chute stream periodically. It's a lot easier to sample the undisturbed material as it is being loaded into the vehicle than *after* it has deposited and segregated therein, which entails separating fines from the lighter fluffy particles big time (Figure 3). How big a sample do we need to extract? Well, most people will *grab* the NOPA guidelines recommended amount, and quickly store this amount in a NOPA bag approved for this application—*et*

Figure 3. Particle size distribution in soymeal.

voila, job done! With this approach they would be following scores of colleagues who, unfortunately *wrongly*, operate from the question: "How big shall my sample be in order for it to be representative?" The assumptions behind this evergreen question have been thoroughly debunked, however, sample mass is *not* the driver for sample representativity. For readers of this column, it suffices to refer to References 1, 2, 4–7 and 11 for full explanation and documentation.

Where the money comes in

Why is sampling of the commodity soymeal so important that it has a solid description and procedure? Soymeal generally has a targeted, or contractually promised, protein content. You need to prove you've hit the specified protein minimum content. All the while you are busy complying with this demand, soybean meal also has a maximum allowed moisture level, so you also need to show you didn't exceed that—or there is a 1:1 penalty. 1% over the threshold moisture, and you get penalised 1% of the price (plus that fraction of the shipping, if you have that in the contract as well). Finally, there is also fibre in soybean meal, mostly left from the hulls. Removing the hulls is clearly important because if you are leaving some in and try to sell hulls at soymeal prices—is not fair tradecraft. The penalty for excess fibre is not 1:1 for hulls, but a *multiple*. It soon gets very pricey to have too many hulls if/when you get caught. The hulls are very light and will separate to some degree while being transported and loaded/offloaded, so correct sampling is ever so important!

Sampling *evenly* as the transportation vehicle is filling is very smart, because you'll be safe in that the entire lot volume (the entire lot mass) will be available for your incremental sampling, and you will assuredly be able to produce a fit-for-purpose representative composite sample. However, a word of caution from the world of practice: the authors have seen dust bags that are being filled with airborne particles be *cleaned* by dumping this dust back *onto* the product. In one way this makes sense: this dust is a bona fide part of soybean meal, but **if** you were just grabbing a sample from the lot *without thinking* and happened to grab some of these dusty fines, your analytical validity would be *off*—because those fines, and the fines from the bag of chips, share something in common: they are assuredly not representative of the *bulk* composition. In soybean meal analysis, this thorny issue is solved by enforcing a *random sampling over time*, a scheme which no operator can mess with. No brains, no prior knowledge is involved, so the sampling can be truly random. This is one reason why studies are done double-blind, or you need to have iron-clad enforced procedures. Give someone time to think about how they are sampling things—and randomness is right out the window. Thinking TOS-correctly comes first, and later it is all just action, i.e. sampling.

How not to do it

The final step doesn't have NOPA approval, and some people skip it. You have your NOPA bag and you now sally forth to your NIR instrument. But don't just arbitrarily grab "enough" material to fill your aliquot cup and make a measurement. This would be the cardinal grab sampling *sin*,[1,2,11] writ very small; but this is still *grab sampling!*

Instead extract three analytical aliquots; you are now replicating the analytical sampling + analysis three times. These three readings can be compared, and they carry a lot of information. From these few results you can figure out the estimated magnitude of this final stage NIR aliquot *sampling variability* and make sure it is in line with your *a priori* set precision threshold. See above regarding analytical threeness and its importance. You'll find a full description of the *replication experiment*, here executed for just three analytical results, in References 1–3: this is essential knowledge for analysts of any ilk, not only NIR.

NIR is not alone

Much can be learned by stepping outside the NIR domain. For example, concerning how to arrive at a reliable analytical result

Figure 4. How to arrive at a reliable analytical result for a much more complex lot, e.g. incinerator bottom ash. A simple food processor comminution is not enough, much more powerful crushing technologies are needed, as well as another analytical technique (XRF), but the necessary separation into size-classes is identical to what is needed also for a bag of chips, even though the primary sampling step is clearly also critical. Photo credits: Center for Minerals and Materials (MIMA), GEUS (https://www.geus.dk).

for complex lots of quite different size, composition and spatial heterogeneity, e.g. incinerator bottom ash (Figure 4), surely this is another issue all together. For one thing, bottom ash is a heavily segregated material and there is no way NIR analysis can do analytical justice to this kind of material [you'll need X-ray fluorescence (XRF)]. Yet the pre-analysis issues are *identical* to those for a bag of chips, only more accentuated and at a different scale. Here, much more powerful crushing technologies are needed, but the necessary separation into size-classes is identical. The critical pre-analysis realm is treated in detail in References 1–3 and 11.

The last word

In summary, **think** first. **Know** about the *critical* "before analysis" sampling issues (TOS).

Then **execute** according to the plan developed[5] (**think no more**).

Now **analyse** the aliquot you worked so hard to be representative; in fact, analyse *three* aliquots.

Be happy that you involved at least *some* sampling + analysis validation in your procedures.

And then go **reward yourself** for a job well done with a drink—and a bag of chips.

(*You may also reward yourself by gorging on the plethora of relevant references below.*)

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QUALITY MATTERS

Four Generations of Quality: a spectroscopic trio

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Introduction

In the last article,[1] we concentrated on how the "screwdriver in the spectroscopist toolkit" the UV/visible spectro(photo)meter, and its associated software has evolved during our Four Generations. In this article we'll now look at three other related spectroscopic techniques/tools in the box, namely, Fluorescence, near infrared (NIR) and Raman; and discuss the "what", "where" and "how" of these techniques are being used to improve the quality of the measurement processes associated with them.

This article, therefore, uses the Four Generations in the previously described time periods and does uncover some interesting points for discussion. This chronology effectively plots the evolution of these techniques from Research to Analytical Quality Assurance (QA), and the associated Quality requirements associated with them.

However, with due deference to the multitude of reference texts available, let's quickly state that it is not the intention of this article to discuss the theory of the appropriate science, and if this is of interest to the reader, then any of the excellent, and well-known texts, some of which are referenced below, should be consulted.

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1st Generation: the years before 1975

During this period, the essential theory was established, and in some cases, instrumentation developed in a purely research orientated environment. However, unlike their UV/visible counterpart(s), the instrumentation that today populates our laboratories, and, as we shall see, increasingly other technical arenas will rely on later developments. For example, improvements in the quality and/or size of fundamental components, data processing etc. and these innovations will be discussed as appropriate in the chronology.

Fluorescence

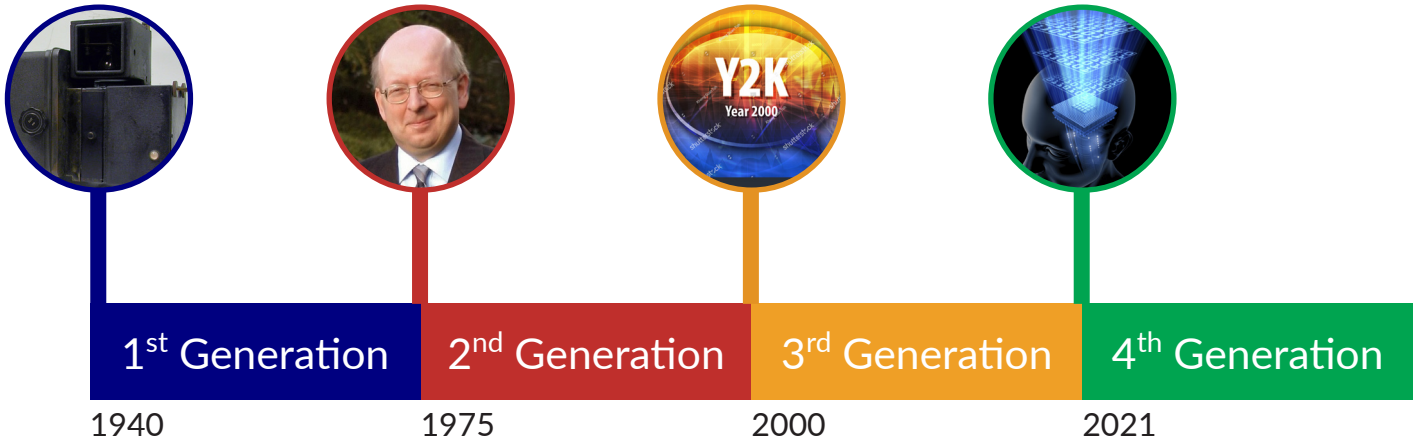
In the 1950s the US National Institutes of Health's Dr Robert Bowman developed a spectrophotofluorometer, or "SPF", that allowed scientists to use fluorescence to identify and measure tiny amounts of substances in the body. Those familiar with the application of fluorescence will recognise the name, particularly as it is found in the Aminco–Bowman name of US-manufactured Series 2 (AB2) systems, referenced as the measurement instrument in many of the scientific papers of the period.

NIR

The near infrared (NIR) spectral region lies between 780 nm and 2500 nm (4000 cm^{-1} to 12,800 cm^{-1}) bridging the more well-known and analytically used regions of the UV-Vis (190–780 nm) and the infrared (4000–600 cm^{-1}). Until relatively recently, it has been called the "forgotten" region.

Vibrational spectroscopy in the NIR region is dominated by overtones and combinations that are much weaker than the fundamental mid-IR vibrations from which they originate. Because molar absorptivities in the NIR range are low, radiation can penetrate several millimetres into materials, including solids, and it is this capability that has allowed the development of many recent applications. Many materials, such as glass, are relatively transparent in this region. Fibre-optic technology is readily implemented in the NIR range, which allows monitoring of processes in environments that might otherwise be inaccessible.

The most common measurements performed in the NIR spectral range are transmission and reflection spectrometry. Incident NIR radiation is absorbed or scattered by the sample and is measured as transmittance, T, or reflectance, R, respectively. Transflection spectrometry is a hybrid of transmission and reflection wherein a reflector is placed behind the sample so that the optical path through the sample and back to the detector is doubled compared to a transmission measurement of a sample of the same thickness. Transflection is used to describe any double-pass transmission technique. The light may be reflected from a diffuse or specular



(mirror) reflector placed behind the sample. This configuration can be adapted to share instrument geometry with certain reflection or fibre-optic probe systems in which the source and the detector are on the same side of the sample.

As mentioned in the last article,[1] the Cary Model 14 UV-VIS Spectrophotometer was a double beam recording spectrophotometer designed to operate over the wide spectral range of ultraviolet, visible and near infrared wavelengths (UV/Vis/NIR). This included wavelengths ranging from 185 nm to 870 nm, and the Cary Model 14B, almost identical in exterior appearance, measured wavelengths from 0.5 nm to 6.0 µm.

Raman

Although the inelastic scattering of light was predicted by Adolf Smekal in 1923, it was not observed in practice until 1928. The Raman effect was named after one of its discoverers, the Indian scientist C.V. Raman, who observed the effect in organic liquids in 1928 together with K.S. Krishnan, and independently by Grigory Landsberg and Leonid Mandelstam in inorganic crystals. Raman won the Nobel Prize in Physics in 1930 for this discovery. Systematic pioneering theory of the Raman effect was developed by Czechoslovak physicist George Placzek between 1930 and 1934. The mercury arc became the principal light source, first with photographic detection and then with spectrophotometric detection.

In the years following its discovery, Raman spectroscopy was used to provide the first catalogue of molecular vibrational frequencies. Typically, the sample was held in a long tube and illuminated along its length with a beam of filtered monochromatic light generated by a gas discharge lamp. The photons that were scattered by the sample were collected through an optical flat at the end of the tube. To maximise the sensitivity, the sample was highly concentrated (1 M or more) and relatively large volumes (5 mL or more) were used.

Modern Raman spectroscopy nearly always involves the use of lasers as excitation light sources; however, lasers were not available until more than three decades after the discovery of the effect, the first laser being produced in 1960.

2nd Generation: the years 1975 to 2000

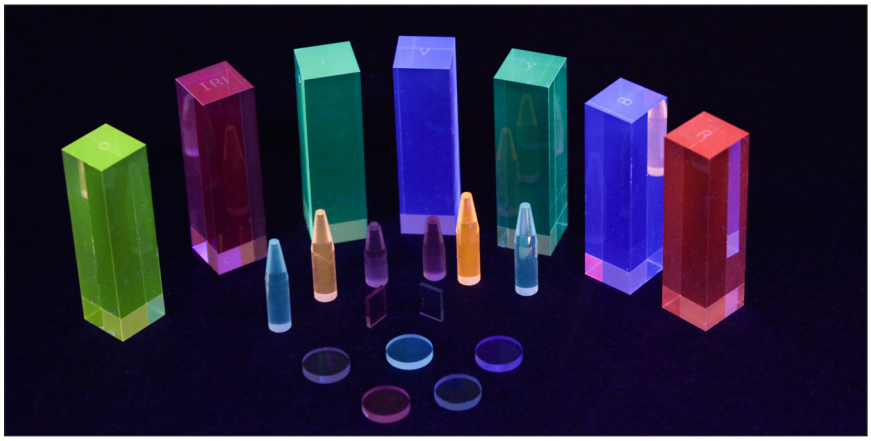
During this period, as in so many of the articles in this series, we see the rapid evolution into the number generating systems we are now so familiar with, and associated with this explosion of data, the requirement to prove the accuracy of these values.

Fluorescence

From a personal perspective, fluorescence was a spectroscopic technique that formed the subject of a dissertation for my degree course, but, in practice, it wasn't a technique encountered until towards the end of the millennium. This resurgence in general interest in the technique was no doubt initiated by the publication of two reference volumes relating to fluorescence, namely a standards publication by the UVSG in 1981,[2] and a technique reference,[3] which, now in its 3rd edition, has become synonymous by name only. From a technical perspective, the high sensitivity of the technique promoted its use in application areas requiring lower detection limits. However, this increased application into multiple areas, invariably involved uses in regulated industries such as water quality, pharmaceuticals etc. which, as discussed for other techniques, also prompted the initial drafting of the appropriate standards by international regulatory bodies. Also, in a related development, Polymerase Chain Reaction (PCR) was invented in 1983 by the American biochemist Kary Mullis at Cetus Corporation; Mullis and biochemist Michael Smith, who had developed other essential ways of manipulating DNA, were jointly awarded the Nobel Prize in Chemistry in 1993—but more of this application in the next Generation.

NIR

The NIR (780–2500 nm) region was largely neglected by analysts for many years because of the complex nature of the spectra produced by



Fluorescence standards

water, proteins etc. in this region. However, thanks to the many and varied multivariate mathematical calibration approaches made available at the time, broadly described by the term "chemometrics", it found new and important applications, in the grain and foodstuffs industry for raw material and QA procedures; being promoted by Technicon with their "Infralyser", registered in April 1986.

Additionally, it was the arrival of FT-NIR instruments in the 1990s and the increasing awareness of NIR's unique information content in the signal that can provide both physical and chemical information, which catalysed the deployment of NIR spectrometry systems for both qualitative and quantitative purposes within the many industries.

These applications often involve comparing an NIR spectrum from a sample to reference spectra and assessing similarities against acceptance criteria developed and validated for a specific application. In contrast, applications of quantitative analysis involve the development of a predictive relationship between NIR spectral attributes and sample properties. These applications typically use numerical models to quantitatively predict chemical and/or physical properties of the sample based on NIR spectral attributes. Examples include moisture content, content uniformity, hardness, particle size, packing density etc.

Raman

In this period, Raman was still essentially the tool of the research scientist, and it's only in the next generation that we see it "come of age" as an investigative and/or QA technique. Technological advances made Raman spectroscopy much more sensitive, particularly since the 1980s. The most common modern detectors now being charge-coupled devices, replacing the previously used photodiode arrays and photomultiplier tubes. The advent of reliable, stable, inexpensive lasers with narrow bandwidths has also had an impact with respect to the availability of the technique.

3rd Generation: the years 2000 to 2020

Fluorescence

As stated above, fluorescence detection is used extensively in PCR and the technique became fundamental to many of the procedures used in genetic testing and research, including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes. During this period, PCR became a common and often indispensable technique used in medical laboratory research for a broad variety of applications including biomedical research and criminal forensics. In addition, the latter half of this period, saw the implementation of fluorescence into the clinical and diagnostic marketplace, which culminated in the extensive use of PCR testing for Covid-19 diagnosis.

In addition, coupled with multiwell plate formats, microscopy and optical screening fluorescence has seen a dramatic expansion in its application areas, due in no small part to the development and use of a vast array of highly stable chemical fluorescent probes and markers.

From a personal perspective, this development is analogous to the array, no pun intended, of chemical colorimetric reagents developed in the 1950s to allow the accurate quantitation of metallic species by visible spectro(photo)metry, long before the development of atomic absorption spectroscopy; the most well-known of which is probably Dithizone.

Also, during this period, in the 1980s, we saw the development of Quantum Dots, a uniquely fluorescently active species, where the colour produced on irradiation by "white" light is related to the size (in nm) of the particles; but, like so many developments we have discussed here and in previous articles in the series, interest didn't really take-off until they became commercially available in the next generation.

From a regulatory perspective, the drafting of the required standards, for example, ASTM International begun at the end of the previous Generation, were completed in this session.[4]

NIR

It is the versatility of the available sample presentation modes in the NIR, i.e. fibre-optic probe-based systems, and latterly, small handheld units, combined with the availability of powerful signal process electronics, that revolutionised the use of the technique in two main

Quantum dots

pharmaceutical areas outside of the Quality Control laboratory during this period.

First, in the Goods-In receiving area of the Raw Materials warehouse, bulk materials could now be checked for identity and compliance to specification, immediately on receipt. As is the case with other spectroscopy measurements, interactions between NIR radiation and matter provide information that can be for both qualitative and quantitative assessment of the chemical composition of samples. In addition, qualitative and quantitative characterisation of a sample's physical properties can be made because of the sample's influence on NIR spectra. Example application uses include the identification of fundamentally chemically different compounds, Active Pharmaceutical Ingredients (APIs), excipients, dyes, packaging material, polymorphs, isomers and physical characteristics, such as crystalline vs amorphous.

Second, NIR spectrometry can now provide an invaluable tool in assisting compliance with the pharmaceutical PAT initiative, because probes can easily be mounted within process streams, mixers etc. to allow qualitative analysis of, for example, reaction pathways, drying of a product, granulation, blending and or content uniformity, monitoring of blister-packed final product etc.

Raman

Seen as a complementary technique to NIR in many of the above application areas described for NIR, Raman has found uses in screening systems, from airport security, to identifying drugs, both elicit and counterfeit.

Unsurprisingly, therefore, during this period the United States Pharmacopeia (USP) expanded their spectroscopy General Chapters to include the appropriate 87x and 187x chapters, for both NIR and Raman, as previously described.[5]

4th Generation: from 2021 forward

As we enter this Generation, we can see that these three spectroscopic techniques are now well developed as mature techniques, with the associated references and regulatory control standards in place. However, we are all aware of the impact of the Covid-19 pandemic over that last two years, and (hopefully) as we begin to exit its dramatic impact we can reflect on the resultant significant shift towards more clinical and/or biological applications of spectroscopy in general.

These changes will be discussed more fully in the next, and last, article in the series, as we "journey into the future" and discuss whether the science fiction of the last decades of the previous millennium is becoming science fact?

References

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3. J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Edn. Springer (2006). https://link.springer.com/book/10.1007/978-0-387-46312-4
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John Hammond is an experienced analytical scientist, spectroscopist and technical marketing professional, skilled in the development, production and marketing of analytical systems into highly regulated and controlled industries. A Fellow of the Royal Society of Chemistry (FRSC), executive member of ISO/TC334 and an Expert Advisor to the United States Pharmacopeia, General Chapters, Chemical Analysis committee.
j.p.hammond@starna.com

Multi-element analysis of drinking water

The analysis of elemental content is critical to ensuring the quality and safety of drinking water. Regulations such as Directive (EU) 2020/2184 and ISO 17294 provides guidelines for using ICP-MS for water analysis, aiming to protect human health from the adverse effects of its contamination.

ICP-MS technology is ideal for water analysis thanks to its multi-element detection capability combined with low detection limits and high-speed of analysis. However, plasma and matrix-based polyatomic interferences and doubly charged species need to be accounted for by applying mathematical corrections and/or collision/reaction mechanisms. The PerkinElmer NexION® ICP-MS with Universal Cell technology, which can be operated in both Collision and Reaction modes to tackle polyatomic interferences enables laboratories to meet and/or exceed the ISO and EU directives specifications.

PerkinElmer

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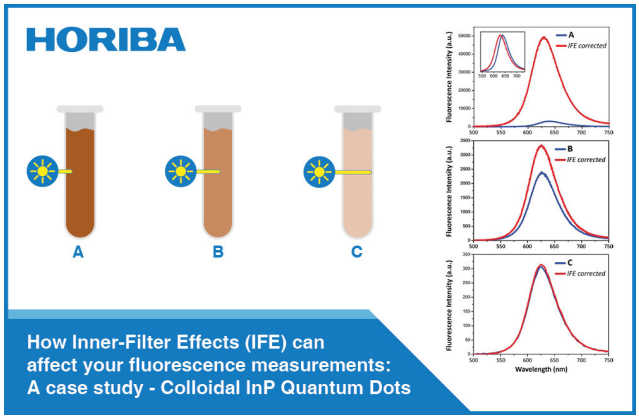

How Inner-Filter Effects (IFE) can affect your fluorescence measurements

The applications of quantum dots (QDs) are rapidly expanding, i.e. *in vivo* imaging, light-emitting devices, photodetection or solar energy conversion, and they require precise knowledge of the QDs emission properties. The Inner Filter Effects (IFEs) can significantly affect fluorescence emission spectral profiles, distorting their general shape, shifting the spectral position of the peak maxima and decreasing the emission intensities. In this application note, the Duetta™ 2-in-1 fluorescence and absorbance spectrometer can measure true molecular fingerprints, which requires the simultaneous acquisition of fluorescence and absorbance, correcting for IFE in real-time.

HORIBA Scientific

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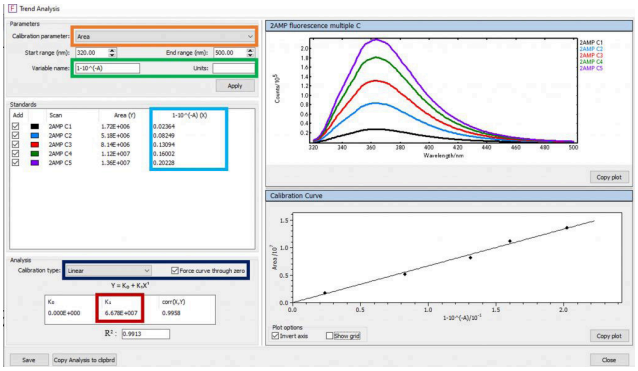

Relative quantum yield of 2-aminopyridine

Quantum yield is a fundamental photophysical parameter that describes a sample's fluorescence efficiency and is defined as the ratio of the number of photons emitted to the number of photons absorbed by a sample. Accurate and reliable quantum yield measurements are important for a broad range of applications including displays, solar cells, bioimaging and drug development. There are two optical methods for measuring the quantum yield: the absolute method and the relative method. In the absolute method, the quantum yield is measured directly using an integrating sphere, while in the relative method the fluorescence intensity of the unknown sample is compared with the fluorescence intensity of a standard sample to calculate the quantum yield of the unknown. In this application note, an Edinburgh Instruments FS5 spectrofluorometer is used to measure the quantum yield of 2-Aminopyridine (2AMP) via the relative method. 2AMP in sulfuric acid (H_2SO_4) has been previously used as a quantum yield reference standard in the UV-visible range. The quantum yield of 2AMP was measured to be 60 % in 1968 and 66 % in 1983. These literature quantum yield reference values are now decades old, and this note presents a reinvestigation and revaluation of the quantum yield of 2AMP in 1 M H_2SO_4 using quinine bisulphate (QBS) in 1 M H_2SO_4 as the reference standard with a modern spectrofluorometer.

Edinburgh Instruments

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Analysis of total chlorine in oil by XRF at 1500 W

In order to demonstrate the analysis of chlorine (Cl) in oil, a calibration curve has been constructed according to ASTM D4929 method except that a rhodium anode tube has been used. Five standard samples were prepared in accordance with the norm to construct a calibration curve. This method proposes the use of net peak intensities; hence a background position was chosen. The counting time on the Cl peak and the background position are identical. From a concentrate product which contained 1000 ppm of Chlorine, a mother solution was prepared by diluting this concentrate with Isooctane. This mother solution was then used to prepare several samples with various Chlorine concentrations.

Thermo Fisher Scientific

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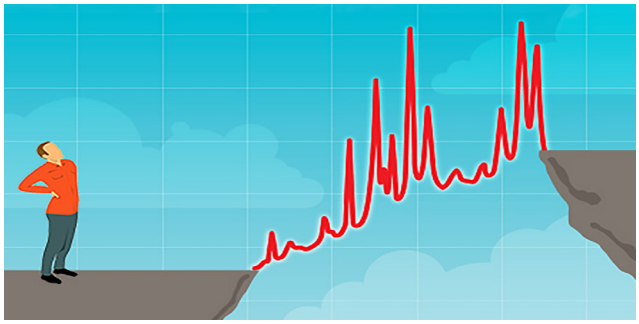

Extending Raman's Reach

Raman has come into its own over the last decade, making the leap from lab to industry and the field with handheld and field-portable analysis systems. As with many technologies, however, the reduction in size has often meant a compromise in performance as compared to traditional benchtop Raman systems. This has limited the applications in which portable Raman spectroscopy can be applied, and/or the detection limit that can be achieved. It is, however, possible to extend the reach of portable Raman to close this gap and enable new applications through careful product design that optimises sensitivity, size and wavelength. This tech note looks at design options to optimise each, and how they can stretch the limits of applied Raman spectroscopy by providing a step change in the performance traditionally available through compact spectrometers.

Wasatch Photonics

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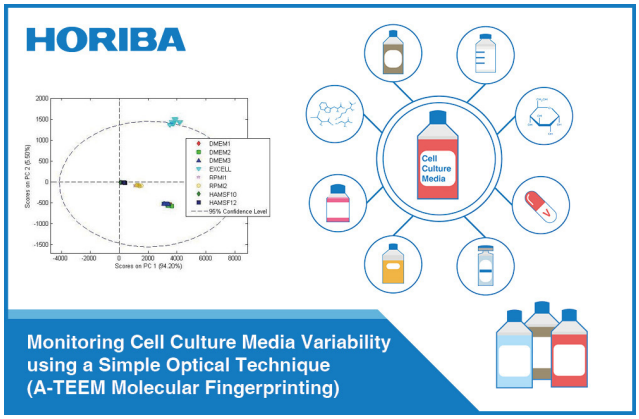

Monitoring cell culture media variability using A-TEEM

Cell culture media for bioreactors provide everything a cell line needs for optimal growth and product yield. It is important to maintain their composition consistent as even subtle variations could have a noticeable impact on the growth rate of the cell culture and its yield. The biopharmaceutical industry has begun to turn to spectroscopic methods, such as fluorescence, for cell culture media characterisation analysis due to the speed of testing, minimal sample handling requirements and relatively lower cost when compared to mass spectrometry and chromatography. In this application note, HORIBA presents A-TEEM (Absorbance Transmission Excitation-Emission Matrix) fluorescence spectroscopy to characterise cell media and the effect that storage conditions have on their compositions.

HORIBA Scientific

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Total oxide X-ray analysis

This note describes total oxide X-ray analysis with Thermo Scientific ARL 9900 IntelliPower Series simultaneous/sequential X-ray fluorescence spectrometers. Wavelength-dispersive X-ray fluorescence (WD-XRF) allows measurement of up to 83 elements of the periodic table in samples of various forms and nature: solids or liquids, conductive or non-conductive. Advantages of XRF over other techniques are speed of analysis, generally easy sample preparation, very good stability, precision and wide dynamic range (from ppm levels to 100 %).

Accuracy of analysis of powders can be impaired by particle size effects and mineralogical effects. Although inhomogeneities and particle size effects can often be minimised by grinding below 50 µm and pelletising at high pressure, often mineralogical effects cannot be completely removed, or harder particles cannot be broken down below the required size.

Fusing these oxidic materials is the best way of completely removing both grain size and mineralogical effects. Essentially, the procedure consists of heating a mixture of sample and a borate flux, namely lithium tetraborate and/or lithium metaborate at high temperature (1000–1200°C) so that the flux melts and dissolves the sample. The overall composition and cooling conditions must be such that the product after cooling is a one phase glass.

Thermo Fisher Scientific

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Product Focus on Mass Spectrometry

Advanced Chemistry Development, Inc. (ACD/Labs)
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MS Workbook Suite

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- Handle MS, LC/MS and GC/MS data acquired with instruments from any major vendor
- Auto-annotate peaks and associate compound structures
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MS Structure ID Suite

Software to help generate structure candidates

- Deconvolute complex LC/MS and GC/MS data into individual component traces
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Predict mass spectral fragmentation in seconds. Have more confidence in your compound IDs, learn about fragmentation mechanisms and publish your results easily.
It's as simple as this:

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- Select the ionisation polarity and fragmentation options
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Bal Seal Spring-Energised Seal

Our Bal Seal® static and dynamic seals, which combine proven Bal Spring® canted coil spring energiser technology with advanced polymer formulations, are proven performers in spectrometry equipment. They seal consistently for more cycles, making better sample resolution and faster throughput a reality.

MORE INFORMATION »

Bal Spring® Canted Coil Spring

Our Bal Spring® canted coil spring mechanically fastens, conducts electricity and shields sensitive electronics from EMI/RFI. Its independent coils, which serve as multiple contact points for optimal current carrying capability, ensure consistent, reliable connection—leading to less contamination, more accurate *m/z* measurement and minimal downtime.

MORE INFORMATION »

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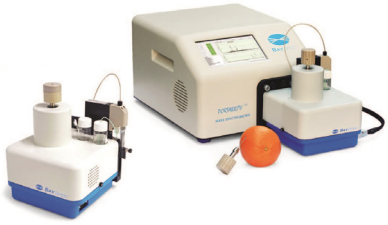

Portability™ – Portable Mass Spectrometer

- Portable and compact
- Detection limit: low ppb
- Miniature linear ion trap
- Real-time results and direct sample analysis
- Compatible with atmospheric ionisation techniques including ESI, TD-ESI, ESI, TD-APCI, APCI

MORE INFORMATION »

Continuity™ – Portable Mass Spectrometer

- Portable and compact
- Higher sensitivity & larger mass range
- Miniature linear ion trap with MS/MS capability
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- Compatible with atmospheric ionisation techniques including ESI, TD-ESI, ESI, TD-APCI, APCI

MORE INFORMATION »

PRODUCT FOCUS

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ionRocket
The ionRocket is a thermal desorption and pyrolysis (TDP) device coupled with DART®-MS, capable of directly heating the sample from room temperature to 600 °C. The system can be utilised to identify additives in the lower thermal desorption region and polymer matrices in the higher pyrolysis region.

MORE INFORMATION »

Hiden Analytical
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QGA Compact Tool for Quantitative Gas Analysis
The QGA system is a high-performance gas analyser configured for real-time continuous monitoring of multiple species with an extremely wide dynamic range. With a compact footprint and simplified operation, the Hiden QGA satisfies the needs of users in research and production scale applications alike.

MORE INFORMATION »

pQA Portable Quadrupole Analyser
The pQA portable gas analyser is a versatile mass spectrometer featuring interchangeable sampling inlets. MIMS inlets are offered for analysis of dissolved species in ground water, sediments, fermentation cultures, soil samples and low volume gas analysis.

MORE INFORMATION »

HPR-40 DEMS Differential Electrochemical Mass Spectrometer
The HPR-40 DEMS is a system for analysis of dissolved species in electrochemistry. The system includes two differential electrochemical mass spectrometry 'DEMS' cell inlets, designed for material/catalysis studies, cell type A, and electrochemical reaction studies, cell type B.

MORE INFORMATION »

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NexION 5000 Multi-Quadrupole ICP-MS
PerkinElmer's NexION® 5000 ICP-MS is a four-quadrupole instrument designed to remove the most complex interferences and address the most challenging applications in trace-elemental testing, delivering exceptionally low ppt BECs and outstanding detection limits for accurate and repeatable results.

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MALDI-8030

Affordable Class-leading Workhorse: The MALDI-8030 is the latest in a long line of MALDI-TOF products from Shimadzu and part of the successful benchtop series. Instrument performance specifications is extended from those of the MALDI-8020 benchtop to cater for compounds best suited to analysis in negative ion mode. This dual-polarity, benchtop linear MALDI-TOF mass spectrometer delivers outstanding performance in a compact footprint, making it an ideal choice for today's increasingly demanding laboratories.

Key features:

- Dual mode (positive/negative ion) MALDI-TOF
- 200 Hz solid-state laser, 355 nm for quick analysis
- Load-lock chamber for fast sample introduction
- UV laser-based source cleaning (patented)
- Flexibility with slide targets options
- Small footprint/benchtop design saving you laboratory space
- Quiet operation (<55 dB) for comfort
- Sample tracking using barcode reader and slides
- MALDI Solutions software rated by users as extremely easy to use (includes data integrity features)

Applications:

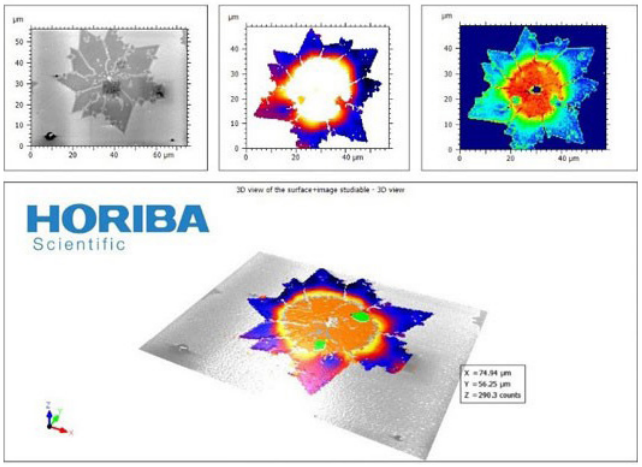
- Polymers
- Oligonulcleotides
- Peptides, proteins and antibodies for BioPharma
- Lipids
- QC analysis

MORE INFORMATION »

NEW PRODUCTS

IMAGING

HORIBA and Digital Surf launch graphYX software range



HORIBA Scientific and Digital Surf, creator of the Mountains® software platform for image and surface analysis in microscopy and metrology, have released graphYX, a new software range for users of HORIBA's Raman spectroscopy solutions, comprising two product levels: graphYX and graphYX-3D.

graphYX is an app included in HORIBA's LabSpec 6 software suite that allows users to highlight features of their samples by combining multimodal images obtained from SEM, Raman, CL, AFM, NanoRaman, EDX, EBSD, FT-IR and other techniques. It will be delivered as standard on instruments such as the HORIBA AFM-Raman and nanoGPS navYX. graphYX software, when combined with nanoGPS navYX, provides a complete solution for quickly relocating points of interest and overlapping map data on the sample surface. nanoGPS navYX is a multimodal and multiscale solution that facilitates sample study and collaboration between researchers using different analytical tools at different locations.

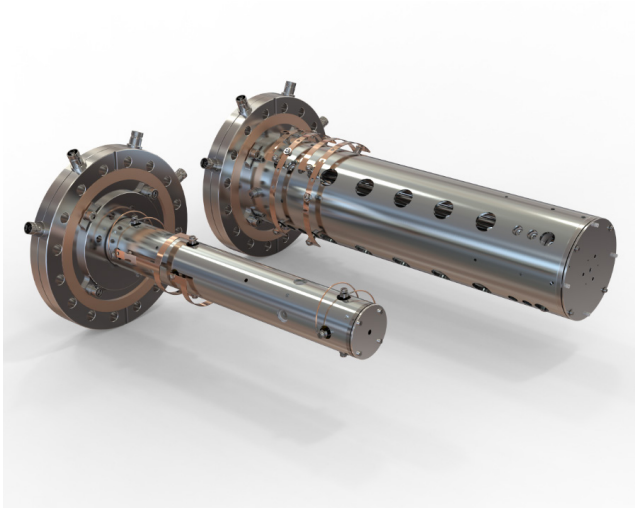
graphYX-3D adds 3D topographic image rendering for techniques such as AFM and AFM-Raman.

HORIBA Scientific

- https://link.spectroscopyeurope.com/673-P1-2022

MASS SPEC

New mass spectrometer series for high mass analysis



The Hiden Cluster series is a range of quadrupole mass spectrometry systems and components, developed specifically for high mass analysis that the analysis of nanoparticles requires. Systems include the 9 mm EPIC system, as well as the 20 mm DLS-20 system, for analysis of species up to 20,000 amu. Components include 9 mm and 20 mm pole diameter quadrupole assemblies with high-power RF operating at optimum frequency to accommodate high mass transmission required in cluster analysis. The Cluster series has precision tri-filter quadrupole assemblies, 90° ion beam deflector option and are air gauge tested for conformity.

Applications include nanoparticle analysis, molecular beam analysis, precursor and contaminant analysis for high mass species. Systems are equipped with Hiden's multi-level software package, offering simple control of mass spectrometer parameters and complex manipulation of data and control of external devices. Multiple sampling configurations are offered to suit research requirements, EPIC and IDP systems offer mid axis potential for negative-ion detection and thermal

desorption studies. The Cluster series are incorporated into the Plasma and SIMS analysis systems for plasma and surface characterisation. For pulsed deposition processes, time-resolved measurements are offered to 50 ns time resolution.

Hiden Analytical

- https://link.spectroscopyeurope.com/672-P2-2022

Industrial process mass spectrometer

Process Insights has expanded its industrial mass spectrometer range with the new Extrel MAX300-RTG 2.0 with touchscreen for process applications. It can report the complete stream composition and has a multi-port stream selector for up to 160 samples. The MAX300-RTG 2.0 features an integrated customisable 15" touch screen display and user-friendly GUI interface making it easy to use with minimal training. It has the speed necessary to analyse the total composition of a sample in seconds and can be fully automated to measure several points in a process, or multiple production lines, with a single analyser. Due to its ability to monitor the operation of several process units, the MAX300-RTG 2.0 can be used to replace multiple gas chromatograph systems. It has an uptime of >99 %, which is achieved through a combination of low maintenance requirements, a modular design including pre-assembled service replacement parts and a plug-and-play ioniser that reduces cleaning requirements.

Process Insights

- https://link.spectroscopyeurope.com/2431-P1-2022

X-RAY

Sample automation and new capabilities for the X-Pulse benchtop NMR spectrometer

Oxford Instruments has launched the all-new X-Pulse benchtop broadband NMR spectrometer with X-Auto, an automatic sample changer. Newly added X-Pulse functionalities significantly increase ease of use, throughput and remote working capability while further reducing ongoing costs. This has been achieved by removing any need for deuterated solvents using novel external signal frequency lock technology, adding automated software switching between nuclei, improvements in sensitivity and unattended sample exchange. Continuous flow monitoring and advanced sample temperature control are coupled with the only true broadband nuclei selection to address a diverse range of analytical chemistry needs across industries, from batteries to pharmaceuticals. The unique modular architecture makes this new X-Pulse fully configurable and a highly cost-effective investment for core science, advanced analytical research, quality control optimisation and teaching.

The combination of the new X-Auto sample changer, which allows up to 25 samples to be preloaded, and new functionality in the SpinFlow 3.1 software, ensures that users can add individual experiments or long queues to each sample with a few simple clicks. By reordering experimental queues, short duration experiments can be prioritised on all samples to quickly determine the value of continuing with longer queued experiments, maximising efficiency. Through a remote connection to the instrument, all pre-loaded samples can be changed, and new experiments added or tailored to the user's analysis requirements. This minimises time with the instrument and maximises remote working productivity. Automated software switching between chemical nuclei is particularly advantageous for applications requiring the selection of a wide range of NMR active nuclei, including those in the battery, polymer and fine chemical markets.
Oxford Instruments

- https://link.spectroscopyeurope.com/1338-P1-2022

Conferences

2022

1–4 June, Primošten, Croatia. **Magnetic Moments in Central Europe 2022 (MMCE 2022).** https://mmce2022.hkd.hr

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

12–15 June, Leon, Norway. **10th Nordic Conference on Plasma Spectrochemistry.** yngvar.thomassen@stami.no, http://nordicplasma.com

19–23 June, Dublin, Ireland. **12th International Conference on Clinical Spectroscopy.** http://spec2022.org

19–23 June, Valencia, Spain. **18th International Conference of the Metabolomics Society.** https://www.metabolomics2022.org

20–23 June, Prague, Czech Republic. **29th Symposium on Plasma Physics and Technology.** sppt2020@plasma-conference.cz, https://www.plasmaconference.cz

20–24 June, Champaign, IL, United States. **75th International Symposium on Molecular Spectroscopy.** https://isms.illinois.edu/

24–29 June, Memphis, United States. **31st International Conference on Ion Mobility Spectrometry (ISIMS 2022).** https://www.isims.info/conference-2022

27–29 June, Online, UK. **BNASS 2022.** https://www.rsc.org/events/detail/40623/bnass-2022-the-20th-biennial-national-atomic-spectroscopy-symposium

28 June–1 July, Paris, France. **inArt 2022: 5th International Conference on Innovation in Art Research and Technology.** inart2022@sciencesconf.org, https://inart2022.sciencesconf.org

3–6 July, Esbjerg, Denmark. **International Association of Spectral Imaging Conference (IASIM-2022).** https://2020.iasim.net

3–6 July, Oxford, UK. **British Society for Proteome Research Annual Scientific Meeting.** secretary@bspr.org, http://www.bspr.org

24–28 July, Chicago, United States. **2022 American Association for Clinical Chemistry (AACC) Annual Meeting.** https://www.aacc.org/meetings-and-events/annual-meeting-dates-and-locations

30 July–4 August, Chambersburg, United States. **2022 International Diffuse Reflectance Conference (IDRC).** idrc@cnirs.org, https://cnirs.org/content.aspx?page_id=22&club_id=409746&module_id=500874

8–10 August, Kingston, Canada. **64th ICASS Conference on Analytical Sciences and Spectroscopy.** diane.beauchemin@chem.queensu.ca, http://www.csass.org/ICASS.html

21–25 August, Chicago, United States. **American Chemical Society (ACS) National Fall 2022 Meeting.** natimtgs@asc.org, https://www.acs.org/content/acs/en/meetings/acs-meetings/about/future-meetings.html

26 August–1 September, Scottsdale, United States. **AOAC International Annual 2022 Meeting and Exposition.** meetings@aoac.org, https://www.aoac.org/events/2022-aoac-annual-meeting/

28–31 August, La Jolla, United States. **SMASH 2022—Small Molecule NMR Conference.** https://www.smashnmr.org/

28 August–1 September, Lisbon, Portugal. **8th EuChemS Chemistry Congress.** euchems2022@chemistry.pt, https://euchems2022.eu/

29 August–1 September, Reims, France. **19th European Conference on Spectroscopy of Biological Molecules-ECSBM.** https://www.univ-reims.fr/ECSBM-2022/

29 August–2 September, Rome, Italy. **18th Chemometrics in Analytical Chemistry Conference (CAC 2022).** https://cac2022.sciencesconf.org/

4–8 September, Singapore, Singapore. **SETAC 8th World Congress/12th SETAC Asia-Pacific Biennial Conference.** barbara.koelman@setac.org, https://singapore.setac.org

4–9 September, Brno, Czech Republic. **2022 European Symposium on Analytical Spectrometry (ESAS) & 17th Czech–Slovak Spectroscopic Conference (CSSC).** esas2022@spektroskopie.cz, http://esas-cssc2022.spektroskopie.cz/

6–9 September, Prague, Czech Republic. **10th International Symposium on Recent Advances in Food Analysis (RAFA 2022).** rafa2022@vscht.cz, https://www.rafa2022.eu

12–14 September 2022, Cairns, Australia. **Australian Near Infrared Spectroscopy Group Conference.** thebattens@bigpond.au, https://anisg.com.au

13–15 September, Manchester, UK. **42nd BMSS Annual Meeting.** https://www.bmss.org.uk/media-centre/news/bmss42-first-announcement/

13–16 September, Rome, Italy. **12th Hyperspectral Workshop, WHISPERS.** info@ieee-whispers.com, https://www.ieee-whispers.com/

14–16 September, Chester, United Kingdom. **Advances in Process Analytics and Control Technologies (APACT 22).** admin@cpact.com, https://apact.co.uk/

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15–16 September, Paris, France. **9th International Conference on Advanced Applied Raman Spectroscopy (RamanFest2022).** https://www.ramanfestconf.com/2022/index.php

2–7 October, Cincinnati, United States. **Annual Conference of Federation of Analytical Chemistry and Spectroscopy Societies, SciX 2022.** facss@facss.org, http://www.scixconference.org

9–12 October, Denver, United States. **2022 Geological Society of America (GSA) Meeting.** meetings@geosociety.org, http://www.geosociety.org

16–19 October, San Diego, United States. **PANIC 2022.** https://panicnmr.com/conference-schedule-sandiego-2022/

12–16 December, Chicago. **2022 AGU—Advancing Earth and Space Science Fall Meeting.** meetinginfo@agu.org, https://www.agu.org/Events/Meetings/Fall-Meeting-2022

2023

29 January–3 February, Ljubljana, Slovenia. **2023 European Winter Conference on Plasma Spectrochemistry.** http://www.ewcps2021.ki.si

19–22 March, Philadelphia, PA, USA. **Pittcon 2023.** https://www.pittcon.org

21–27 August, Innsbruck, Austria. **NIR-2023.** https://www.spectroscopyeurope.com/events/nir-2023

17–20 September, Baverno, Italy. **SMASH 2023 - Small Molecule NMR Conference.** https://www.smashnmr.org

Courses

2022

25–30 September, Erice, Italy. **International School on Mass Spectrometry (IntSMS).** http://www.spettrometriadimassa.it/intsms2022

15 October, Obergurgl, Austria. **Advanced Study Course on Optical Chemical Sensors (ASCOS).** Christian.W.Huck@uibk.ac.at, http://ascos.org/

Exhibitions

2022

21–24 June, Munich, Germany. **analytica 2022.** https://www.analytica.de

22–26 August, Frankfurt, Germany. **ACHEMA.** https://www.achema.de

13–16 September 2022, Rome, Italy. **Fourth Spectro Expo.** https://www.spectroexpo.com

15–17 September, Hyderabad, India. **analytica Anacon India.** https://www.analyticaindia.com

24–26 November, Istanbul, Turkey. **Turkchem.** http://www.chemshoweurasia.com

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