the Analytical Scientist

SPECIAL SERIES:

Spectroscopy

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UPFRONT

Losing (Cognitive) Control

High-demand cognitive work causes a build up of glutamate in the brain – according to real-time magnetic resonance spectroscopy analysis – which, in turn, may alter economic decision making

Mental fatigue has long puzzled scientists. Why do we feel it? What generates it? These questions nagged researchers from Pitié-Salpêtrière University in Paris, France, as they contemplated why machines can do cognitive tasks continuously without fatigue but the brain cannot. They hypothesized that fatigue arises from an increase in the cost of exerting cognitive control – which stems from glutamate accumulation in the brain (1).

To test this theory, two groups of participants executed either high- or low-demand cognitive tasks, interlaced with economic decisions, while researchers measured the levels of metabolites in their brains. The researchers found that, when intense cognitive work is prolonged for several hours, some potentially toxic byproducts of neural activity, such as glutamate, accumulate in the prefrontal cortex. "This alters the control over decisions, which are shifted towards low-cost actions (no effort, no wait) as cognitive fatigue emerges," explains Antonius Wiehler, co-author of the paper. Other signs of fatigue in the group performing mentally challenging tasks included pupil dilation and increased levels of glutamate in the synapses of the inferior prefrontal cortex.

In previous studies, the researchers used fMRI – but this technique could not explain why the cost of cognitive control increases over time. Instead, the team used magnetic resonance spectroscopy (MRS), which meant that they could measure brain metabolites while human subjects carried out the tasks.

Although the study had minor limitations – such as the low spatial and temporal resolution of MRS – and the results were only correlational, they do offer an explanation as to why cognitive control is harder to mobilize after a strenuous workday. Wiehler hopes that, in the future, "prefrontal metabolites could be monitored using MRS to detect cases of severe fatigue/burnout in many different situations, such as employees after work, athletes during heavy training programs, or students during revisions before their exams."

In further studies, the researchers hope to learn why the prefrontal cortex is more susceptible to fatigue and glutamate accumulation than other brain regions. Wiehler seeks to understand how metabolite buildup could be prevented or removed from the synapses. He adds, "Going forward, we may also be exploring whether markers of fatigue are predictive of clinical outcome across diseases such as cancer or depression."

Reference

1. A Wiehler et al., Current Biology, 32, 1 (2022). DOI: 10.1016/j.cub.2022.07.010

UPFRONT

The Key to Chirality

Is electronic circular dichroism-circularly polarized Raman the way forward for chirality detection?

Credit: by fdecomite / CC BY

Researchers from the University of Alberta have developed a new form of chiral Raman spectroscopy: eCP-Raman (1). They claim that the technique, which combines electronic circular dichroism (ECD) and circularly polarized Raman (CP-Raman), can identify chirality with greater sensitivity than current methods.

There are a number of techniques researchers use to study chirality, but they all have limitations. X-ray crystallography requires a single crystal; NMR and HPLC require specific chiral shift agents/ stationary phases; chiral tag-molecular rotational resonance (MRR) is only for small molecules; and ECD and optical rotatory dispersion (ORD) may not be sensitive or unique to all stereogenic centers – and the theoretical modeling needed to extract chirality information can lack accuracy.

Vibrational circular dichroism (VCD) and Raman optical activity (ROA) are both highly sensitive to chirality and can be done in solution directly, but their signals can be weak and often require long acquisition times to obtain a good signal-to-noise ratio. The Alberta researchers showed that eCP-Raman can detect chirality with a sensitivity often much greater than regular ROA and comparable to ECD. eCP-Raman can also be measured using a regular ROA instrument.

"Until now, researchers had not recognized the existence of eCP-Raman in ROA experiments, and our discoveries provide a new mechanism for understanding this chiral phenomenon, and may help with the design of new chirality sensors," says Yunjie Xu, a professor in the Department of Chemistry at Alberta and corresponding author of the study.

In a study demonstrating the technique, the team untangled different chirality transfer mechanisms reported previously using ROA measurements. According to Xu, there have been a good number of reports about amplification of ROA features in complicated systems, including those with nanostructures. She says, "Whether such amplification is caused by the specific inter/intramolecular bonding interactions with the resonating chiral solute or not could be difficult to evaluate and we show that such specific interactions are not always needed for the large amplification observed."

Xu and her colleagues also showed that resonance ROA signals are generally contaminated by eCP-Raman signals. "The current work not only points out this crucial issue, but also offers ways to remove such contamination," says Xu. "So the discovery should greatly aid the current theoretical development of resonance ROA."

What's next for eCP-Raman? Though the technique is new, some recent applications have already been reported; for example, using it for the molecular structure information of a series of atropisomeric naphthalene diimides (2). Xu adds, "We are currently focusing on extracting resonance ROA of the Ni complex and related systems, and working together with theorists to evaluate/further develop theoretical models for resonance ROA."

REFERENCES AVAILABLE ONLINE

Introducing eCP-Raman

By Yunjie Xu

eCP-Raman is a combination of ECD and CP-Raman and can be measured using a regular ROA instrument. This phenomenon was first discovered in the measurements of Raman and chiral Raman of a resonating Ni complex. This Ni complex has very large magnetic dipole moments and, therefore, a large dissymmetry factor g (the intensity ratio of ECD over UV-vis). Nowadays, the majority of ROA measurements are done using a back scattered circular polarized design. In the case of Ni, a strong ECD absorption causes a noticeable imbalance of the right versus left circularly polarized light (RCPL vs LCPL). This imbalance leads to CP-Raman. Such CP-Raman can happen for both chiral or achiral molecules, and is collected in the same manner as ROA, IRCPL - ILCPL.

This is an important discovery because researchers have not recognized the existence of eCP-Raman in ROA experiments. It provides a new mechanism for chirality transfer and a new way of monitoring chirality.

UPFRONT

Tackling TD-fNIRS Head On

Kernel Flow: How a new wearable brain scanner plans to make brain imaging mainstream

Although time-domain functional near-infrared spectroscopy (TD-fNIRS) is currently considered the pinnacle of non-invasive optical brain imaging techniques, it can be complex, cumbersome, and costly, and, therefore, is not yet widely employed in the field. Sensing a gap in the market, engineers from Kernel, a US neurotechnology company, designed a wearable brain-imaging device called Kernel Flow (1). Weighing in at 2.05 kg, the TD-fNIRS headset contains 52 modules arranged in eight plates that fit on either side of the head. Using picosecond laser pulses and detectors to estimate photon scattering and absorption in tissues, Kernel Flow measures changes in blood oxygenation that correlate with groups of neurons firing.

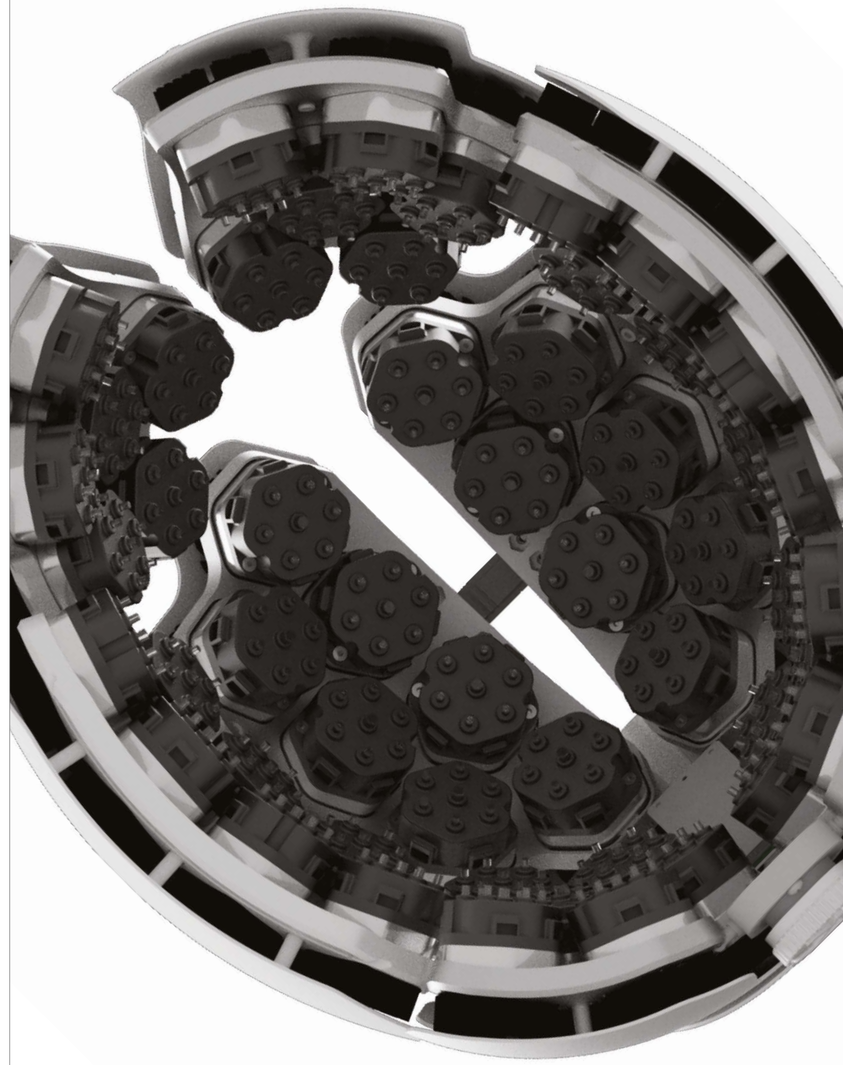
When developing Kernel Flow, the engineers evaluated other non-invasive brain imaging techniques, including EEG, ultrasound, fMRI and MEG, but ultimately homed in on TD-fNIRS - "the perfect combination of scalability, temporal resolution, spatial resolution, and wearability to build a mainstream brain interface," according to Ryan Field, Kernel's chief technology officer. [LR2] Importantly, the team wanted to overcome the limitations of traditional TD-fNIRS, while maintaining the performance of a research grade system. "Our goal was to build a scalable brain interface that could someday be used by anyone and everyone, and to create the infrastructure to enable the mainstream adoption of brain measurement," says Field.

The team used standardized methods of assessment for brain imaging instruments and a commonly used validation task to assess the system's performance; the results showed that the Kernel Flow headset demonstrated performance comparable to existing TD-fNIRS benchtop systems. Although these results are very promising, Kernel highlights the need for future work, including the collection of additional human neuroscience data and the evaluation of system performance with different hair and skin types. Both are things the team at Kernel is currently working on.

Kernel has expressed interest in applying their system to novel drug discovery, pain measurement and management, healthy brain aging, cognitive changes, and elite performance. "We believe that a large amount of high-quality data collected in a standardized way will be the key driver of innovation in applying brain measurements to personal insights," explains Field. "We also hope that, one day, using a brain interface will become as common as picking up your phone."

Reference

1. Ban et al., Journal of Biomedical Optics (2022). DOI: 10.1117/1.JBO.27.7.07471



UPFRONT

Positively Medieval

Portable X-ray fluorescence suggests a group of windows from Canterbury Cathedral may be the oldest stained glass windows in England – and the world

A group of windows from Canterbury Cathedral may be the oldest stained glass windows in England, according to a team of scientists from University College London and conservators from Canterbury Cathedral (1). The researchers used X-ray fluorescence spectrometry – specifically, a portable version of the technique, customized with a 3D-printed attachment – to date the windows. We spoke with the lead author of the study, Laura Ware Adlington, to find out more.

Tell us about your background in materials science…

I am a specialist within the field of archaeological materials science, which uses techniques from materials science and chemistry to study archaeological and cultural heritage materials. We use these approaches to study when and where things were made, but also the wider systems of technology and production, innovation and knowledge transfer, trade and exchange, and how things were used, reused and discarded. All of this is deeply entrenched in the study of the human past – based upon the idea that humans both shape and are shaped by the material world, and so, by studying material culture, we can study people. My specialism is in glass, with a particular focus on medieval stained glass, but I've also worked on various metals and ceramics.

How did you end up working on the Canterbury Cathedral project?

The panels I ultimately worked on were removed from the cathedral walls as part of emergency conservation on the stonework, which provided an opportunity for the public to see them up close in exhibitions and for specialists to study them more easily. I was working on glass from York Minster as part of my PhD and wanted to test the noninvasive technique I'd developed there on a separate case study. Fortunately, Leonie Seliger – director of the stained glass conservation studio at Canterbury Cathedral – was interested!

What were the main analytical challenges you faced?

The early stages of the data analysis were particularly labor-intensive for this project. The panels have had a complicated history, so every

"The panels have had a complicated history, so every piece of glass had to be scrutinized to ensure that the chemical data, the style of the paintwork, and its position in the panel all agreed with the hypothesis."

piece of glass had to be scrutinized to ensure that the chemical data, the style of the paintwork, and its position in the panel all agreed with the hypothesis.

Can you talk me through the analytical methods you used to date the stained glass windows?

The windows were dated using two lines of evidence that ultimately agreed. First, in the 1980s, Madeline Caviness wrote an article arguing that the stylistic characteristics of some of the panels indicated an earlier origin. We then used an analytical technique called X-ray fluorescence spectrometry, which measures elemental composition. We used a portable version of this technique (pXRF), which required a customized approach that included the design of a 3D-printed attachment to enable precise, accurate in situ analysis of medieval stained glass windows. The stylistic and chemical information agreed with each other and supported the early date.

Why did you take a portable approach with pXRF?

To take invasive samples of windows, the panels need to be removed from the wall and the individual pieces of glass removed from the lead strips (called cames) that hold them together. This is an expensive and laborious undertaking, so invasive sampling usually has to piggyback on planned conservation works. That's why it was so important to develop this in situ approach. At Canterbury Cathedral, even though the panels were removed from the walls, the glass pieces were not removed from the cames, so the in situ approach was still necessary.

Were you surprised by the results?

Honestly, yes! We could easily have had inconclusive results. The results hinged on the identification of a change in the glass source in the late 12th or early 13th century and showing that the potentially early panel predated this change in source. If there had been no change in the glass source, we would not have been able to comment one way or another. Our work at York Minster has indicated that they used some of the same glass suppliers over quite long periods of time, so it was a real possibility.

Do you have any plans to use the technique in future studies?

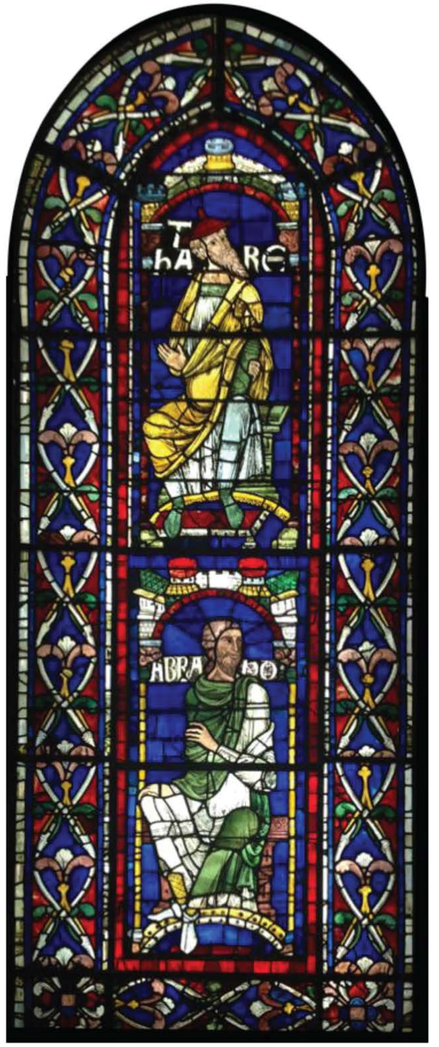
Yes, our team has more planned work at Canterbury and we are seeking funding to study panels at Pennsylvania's Glencairn Museum.

Was this one of the most interesting projects you've worked on?

Absolutely – but working with medieval stained glass is never dull!

Reference

1. LW Adlington, IC Freestone, and L Seliger, Heritage, 4, 937 (2021). DOI: 10.3390/heritage4020051



Before 1790, the Ancestor figures were originally positioned in pairs, one over the other, in the upper windows (clerestory) of Canterbury Cathedral. Two of the extant figures (pictured here), which are now housed in the Great South Window at Canterbury Cathedral, are seen displayed as they would have been originally in an exhibition in the Canterbury Chapter House in 2015.

APPLICATION NOTE

Kinetic Dye Degradation

Measuring absorbance and transmittance of Allura Red azo dye

Allura Red is a powdered azo dye used in the food, cosmetic, drug, leather, and paper industries. If they are systemically absorbed, azo dyes can be metabolized via azoreductases of intestinal microflora by liver cells and skin surface bacteria. This metabolism leads to aromatic amines that can be hazardous. They can also be released into ecosystems through water waste, for example, in the textile industry.

With the help of a spectrometer, it is possible to retrieve important data on dye degradation. You can, for example, measure the dye concentration in water and analyze under which conditions it is broken down the fastest. This data can support quality control and safety regulations.

Allura Red is known by many names, but the most familiar is Red 40 or E129. Azo dyes are synthetic coloring agents that contain an azo group (-N=N-) in their chemical structure. Kinetics is the study of how quickly or slowly a chemical reaction occurs. This experiment applies kinetics to dye degradation. For the oxidizing agent, we used bleach to degrade the color of Allura Red. The active ingredient in bleach is sodium hypochlorite (NaClO). The sodium hypochlorite changes the chemical compound of Allura Red, causing the red color to disappear.

In this experiment, we measured the absorbance and transmittance of an Allura Red solution mixed with a bleach solution. Allura Red absorbs green coloring (480- 560nm) and transmits red coloring (640-700 nm). Using Beer-Lambert's Law, we used the absorption measurement conversions to solve for the unknown changing concentrations of Allura Red.

Curious about the outcome of this experiment? Click the link to download the full PDF.

DOWNLOAD THE FULL APPLICATION NOTE

FEATURE

Exciting Times For… Spectroscopy

We ask five leading spectroscopists what they think is the single-most exciting development in spectroscopy today

Could you give me a broad overview of some of the biggest developments in spectroscopy over the past decade?

Karen Esmonde-White: The application of some more sophisticated chemometric and machine-learning artificial-intelligence technologies are definitely up there. Even as somebody who doesn't use them, I can still see from a distance the impact they're having by allowing people to get useful information out of spectra more easily. That's really exciting to watch!

Rob Lascola: I agree. The integration of artificial intelligence and machine learning with spectroscopy is amazing. They're enabling real-time analysis and intelligent mapping of observable areas, which promises to give significantly faster and more accurate analysis and results for some applications, such as cancer detection. And it can really help make point of care treatment and diagnostics more feasible. It'll be fascinating to see how this progresses going forward.

Jean-Francois Masson: The ability to sense complex molecular patterns in clinical samples or monitor specific molecules in blood has been a game changer in the field. The plasmonic sensing field always promised to achieve that – but, for the first ten to fifteen years, the promise was not really coming to fruition…

However, we've witnessed a stark change in the past decade. Sensors working in serum are now routinely reported and we have been able to use spectroscopy to classify different types of cells and tissues

"Another key area of development is the role molecular spectroscopy plays in detecting and monitoring key environmental challenges, such as the consumer-led concern around microplastics."

based on spectral differences. This was made possible by advances in instrumentation, surface chemistry, and data processing using more advanced algorithms such as machine learning.

Juergen Popp: A comprehensive answer to this question is difficult – or even impossible – because the field of optical spectroscopy has developed rapidly in recent years. Therefore, I would like to focus here especially on the development of Raman spectroscopy, which, in my opinion, has become one of the most important optical analytical methods in the last 10-20 years – next to fluorescence spectroscopy.

Though Raman spectroscopy was still reserved for specialists in the early 1990s, it has, in recent years, become a fairly routine method, with applications extending into all areas of the natural sciences – and also into unexpected disciplines like art history. Raman spectroscopy has even left the earth and is flying to Mars!

The main reasons for this are rapid advances in instrumentation, the availability of small, easy-to-use lasers (mainly diode lasers) that no longer require special electrical connections or cooling, the development of the CCD camera as a powerful multi-channel detector, and especially the existence of efficient filters to suppress the elastically scattered Rayleigh light. These advances in instrumentation have led to the availability of easy-to-use, commercial Raman instruments and have greatly expanded the range of applications.

Furthermore, an increased interdisciplinary dialogue between spectroscopists and end users, such as clinicians, has resulted in Raman spectroscopy entering a new era. This application push of Raman spectroscopic bioanalytics over the past 10 years has led to both new hardware and software advances that include new Raman fiber probe designs, field-deployable easy to-use Raman microscopes, and, most importantly, novel data processing techniques that exploit artificial intelligence (AI) for automated analysis of data sets.

The latter is very important – but does not only apply to Raman spectroscopy. The success of spectroscopic methods for medical diagnosis and therapy (and other applications, such as in the life sciences, process analytics, pharmaceuticals, or environmental analysis) is closely related to the development of tailored data evaluation algorithms. In short, measurement data must be translated into qualitatively and quantitatively usable information for the end user – and significant progress has been made in this area in recent years. In fact, we have developed a universally applicable Raman data analysis software called RAMANMETRIX (see: https://docs.ramanmetrix.eu/). This software allows robust and reliable data analysis of Raman spectroscopic data with the click of a button.

Over the past 10-20 years, Raman spectroscopy has evolved from a purely scientific research method to a mature analytical tool with a wide range of potential applications.

Tell me about your current work and how it fits into the trends you're seeing…

Lascola: Compared to graduate school, my work is much less technically


Geoff Winkett


Jean-Francois Masson

complex; it's now a lot of absorbance spectroscopy, conventional linear Raman spectroscopy, and even some X-ray work. But what's gotten more complicated is the application area. I'm studying nuclear materials, and doing measurements in our nuclear materials processing facilities. Essentially, I've now traded experimental setup complexity for being able to make measurements in highly radioactive or highly acidic or basic environments. It's been challenging in a completely different way but also very rewarding because I get to see how my efforts affect the work of others.

Esmonde-White: It's interesting that Rob notes how instrument complexity is decreasing but the application complexity is increasing, because that's exactly what we're seeing in Raman. Over the past 20 years, we've observed complex instruments that took a long time to master becoming increasingly accessible and easier to use. We see it with infrared, we see it with Raman, and certainly with near-infrared spectroscopy. Increased accessibility allows users to address more complicated applications – particularly in the biology space. Much of the progress has to do with the knowledge of the field, but also with emerging technologies.

What is the most exciting development in molecular spectroscopy today?

Geoff Winkett: There are many exciting developments underway in molecular spectroscopy right now, but one area of particular interest is growth in the biopharma market. Given the amount of investment going into biologic drugs, there is a strong belief that large-molecule pharma will outweigh traditional small-molecule pharma in the years to come. This creates a significant opportunity (and challenge!) for molecular spectroscopy products to support customers in this area with their testing needs.

Molecular spectroscopy solutions based on techniques such as Fourier-transform infrared (FTIR), fluorescence, ultraviolet-visible, and Raman spectroscopy can complement other techniques such as high-performance liquid chromatography (HPLC) in multiple areas of the biopharma value chain, from early discovery all the way through to quality assurance (QA), quality control (QC), and production. Such techniques are powerful tools for application areas such as protein quantification, protein stability, oligo QA/QC, raw materials identification, and more – uses that can help customers in the molecular spectroscopy field improve time to market, reduce the cost of ownership, and ensure compliance with regulatory bodies.

Another key area of development is the role molecular spectroscopy plays in detecting and monitoring key environmental challenges, such as the consumer-led concern around microplastics. The infiltration of plastics in our environment and their impact on human health has been a hugely important topic in recent years. As a result, we've seen increased demand from customers who need to be able to identify these particles in water supplies.

Masson: I view two major fields as being exciting. The first one involves the use of spectroscopic sensors as point-of-need devices – and eventually wearable devices. Though it has been a promise of the field for the past 20 years, all the tools are now available for analytical scientists to design sensors that will fulfill this promise and help humanity. The technology could cause a paradigm shift in healthcare, as there are so many ailments that would benefit from a rapid response and increased accessibility. However, one challenge we are experiencing is the need to transition from sensors working in serum to whole blood and to design long-lasting, miniature, wearable, optical devices that are insensitive to fouling.

The second field is optophysiology, which marries photonic devices with the measurement of physiological parameters. To date, optophysiology has been mainly driven by untargeted chemical measurements using general spectral responses, but I believe the ability to specifically capture complex molecular changes at the cellular or tissue level is the next frontier in optophysiology. I dream of the opportunity to reveal minute metabolite changes at the cellular level that will help to better understand disease progression and treatment efficacy. Designing nanosensors able to do this is what drives my current research.

Lascola: There are many choices, but I'm really impressed with spatially offset Raman spectroscopy. It's exciting because we're able to see light and make measurements in environments where you wouldn't expect to. Normally, when you're shining light on a sample, you have a very clear


Juergen Popp


Karen Esmonde-White

path for the light to get to it. But there are many situations where you don't have a clear path; for example, if you have a sample inside a bottle or if you're trying to measure tissues inside the body. Normally, the signal from the embedded sample is dominated by the surrounding material. But if you monitor the scattered light at a small offset from the position where the excitation light hits the material, the sample's scattered light becomes more prominent. You have a lot more sensitivity to that embedded signal, ultimately giving you more power in your spectroscopic analysis.

Esmonde-White: I would agree with Rob on this. The realization that we can do Raman spectroscopy in turbid media and in layered samples and collect spectra from buried layers has been revolutionary for Raman spectroscopy. It's especially important in the biomedical field where now you can get measurements through the skin to analyze subsurface bone or precancerous lesions, for example. It's very exciting to see the potential of spatially offset Raman spectroscopy in this area. On a different note, it's also starting to have an impact in art and conservation technologies; now, you don't necessarily need to take a cross section of the sample to collect data from subsurface layers, which I think is really interesting!

Popp: In my opinion, the latest developments in IR spectroscopy (the sister method to Raman spectroscopy) are particularly exciting. For many years, a major problem in IR spectroscopy was the lack of suitable IR excitation sources with a high photon density. This problem has since been solved with the introduction of quantum cascade lasers as highly brilliant light sources; indeed, using these lasers as an excitation source for IR spectroscopy or imaging in the spectral range from 950 to 1800 cm-1 can be seen as a significant milestone. For one thing, they partially compensate for the appearance of strong IR water absorption bands in the IR spectra of biomedical and biological samples that mask other relevant bands. And as the intensity of quantum cascade lasers is several orders of magnitude higher than that of thermal emitters in FTIR spectrometers, large-scale and uncooled microbolometer arrays can be used as detectors instead of the smaller MCT-based liquid nitrogen-cooled FPAs. A spectrometer or interferometer for spectral information acquisition is not needed because quantum cascade lasers are tunable.

Another highly exciting development is photothermal IR microscopy, which is based on the non-radiative transformation of absorbed energy into heat. The use of tunable quantum cascade lasers for IR-excitation causes absorbed heat to locally expand and thereby change the refractive index of the sample, which can be detected with optical systems in the visible range. This allows IR images of aqueous samples (such as living cells) to be acquired with submicrometer spatial resolution. This feat has not been possible before and will significantly expand the application range of IR spectroscopy/microscopy, especially as it relates to biomedical issues.

Finally, I would like to highlight the exploration of a completely new method of IR absorption spectroscopy: field-resolved infrared spectroscopy. In contrast to conventional FTIR spectroscopy, this novel method measures the coherent field emitted by the vibrationally excited molecules after excitation with an ultrashort MIR pulse of a few optical cycles. The detection of this field allows a significant lowering of the detection limit compared with FTIR spectroscopy and thus the analysis of low-concentration molecules in strongly absorbing mediums.

Is there an application area that will benefit most from these developments?

Masson: I think the point-of-need sensor field is obvious. The current workflow of sending samples to a lab works well in large cities and in higher-income communities that have access to affordable healthcare – but it fails miserably in remote communities or lower income places that lack infrastructure. I think we have a duty to address this with the tools we develop.

For optophysiology, the possibilities are vast. I believe that medicine could benefit the most, as optophysiology can provide new means to perform diagnostic measurements, it can provide molecular details necessary to better understand diseases, and it could also serve to determine treatment efficacy. This is already happening, but the next generation of optophysiology sensors will bring the field to a new level. I predict that it will be an essential tool in personalized medicine.


Rob Lascola

Popp: Clinical diagnostics will benefit most – and, in fact, are already benefiting – from all of the above-mentioned developments. The sharp increase in cancer (aging society) and the rapid spread of life-threatening infectious diseases and antibiotic-resistant germs (increasing global mobility and ill-considered use of broad-spectrum antibiotics) represent areas of unmet medical need.

There is a great need for new methods that enable earlier diagnosis of these diseases and, therefore allow initiation of targeted therapy as soon as possible. In recent years, spectroscopic methods have shown their potential to provide clinicians with relevant information to address these medical challenges.

We've discussed exciting developments, but what major challenges is the field facing today?

Winkett: Significant challenges laboratories and analytical scientists face are data integrity, security, and compliance with regulatory authorities' requirements. These are complex, quickly evolving areas and customers

need to have peace of mind when using testing equipment. As a result, we have made key investments to help our customers navigate these challenges. In fact, we recently released an updated version of the software behind our Cary 3500 UV-Vis spectrophotometer, which builds controls around signatures, access administration tools, electronic signature workflows, and audit trails into the software – a reflection of the industry's increasing data security concerns.

Popp: A major challenge is the clinical translation of spectroscopic approaches for routine clinical diagnostics. Although research on clinically suitable compact light sources, ultra-sensitive detectors and their linkage with modern concepts for AI continues, translational research in Europe is facing major challenges, especially with regard to the EU Medical Device Regulation (MDR) 2017/745.

Currently, this EU regulation significantly hinders the testing of spectroscopic approaches on patients in the form of preclinical or clinical studies. For example, Raman spectroscopy has proven its potential in proof-of-principle studies for certain diagnostic and therapeutic questions, but the actual performance has not yet been demonstrated under routine clinical conditions in the form of comparative studies in a large cohort of patients.

New types of funding are urgently needed to build on proof-of-concept research to establish follow-up studies according to the above-mentioned MDR guidelines. Special infrastructures that offer open user platforms – for example, under the umbrella of a university hospital – and bundle the expertise of renowned players from science and industry are necessary to accelerate the translation of new diagnostic and therapeutic procedures in the long term. The Leibniz Center for Photonics in Infection Research, Jena, Germany – which was recently included in the national roadmap by the German government – shows what such translational research could look like in concrete terms.

Masson: There are many wonderful ideas to create new sensors to help humanity. However, the gap between new ideas and the market remains significant, meaning that only a small fraction of these ideas become commercial sensors. The development of sensors still requires new chemistry, new technology and significant data processing ability, but I think standardization will be one of the next challenges. We might develop different variations of sensing technologies, but we will need to identify the most promising ones and push them to the market.

Another challenge concerns the increased complexity of the data generated by sensors. There are many spectroscopic sensors that capture broad spectral signatures associated with disease but processing data with standard linear methods is becoming increasingly difficult and must be facilitated by machine learning methods; educating analytical scientists in these chemometrics and machine learning methods is direly needed.

Lascola: With recent advances allowing instrumentation to be simplified and brought into a production environment, it is becoming increasingly easy to get data. And though you can't expect every person who's going to use these instruments to be a spectroscopic expert, there still has to be some degree of knowledge. And this is another area where the instrument design can start to incorporate real-time evaluation of the quality of data. Some handheld instruments do incorporate such elements, but I'd like to see more intelligent guidance for acquiring data – aiding the "casual user" or somebody whose expertise is in a different area. Helping users get the right data so they can make the right analyses and the right decisions is something that will really help spectroscopic technologies come into more widespread use.

Esmonde-White: Rob said that perfectly; I completely agree!

Juergen Popp is Scientific Director at the Leibniz Institute of Photonic Technology, Germany; Jean-Francois Masson is a Full Professor at the University of Montreal, Canada; Geoff Winkett is General Manager and Vice President of Molecular Spectroscopy at Agilent; Rob Lascola is Senior Fellow Scientist at the Savannah River National Laboratory, USA; Karen Esmonde-White is Product Manager at the Endress+Hauser Group, USA

ONLINE

The Decade's Biggest Spectroscopy Breakthroughs

What's the biggest spectroscopy breakthrough in the last 10 years – and why? Four spectroscopists from our 2021 Power List have the answers.

With Karen Faulds, Professor, Head of Bionanotechnology and Analytical Chemistry, Department of Pure and Applied Chemistry, University of Strathclyde, UK

Rohit Bhargava, Founder Professor and Director, Cancer Center at Illinois, University of Illinois at Urbana-Champaign, USA

Rachel Popelka-Filcoff, Rock Art Australia Minderoo Chair in Archaeological Science, School of Geography, Earth and Atmospheric Sciences, Faculty of Science, The University of Melbourne, Australia

Duncan Graham, Distinguished Professor and Head of Department, Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK

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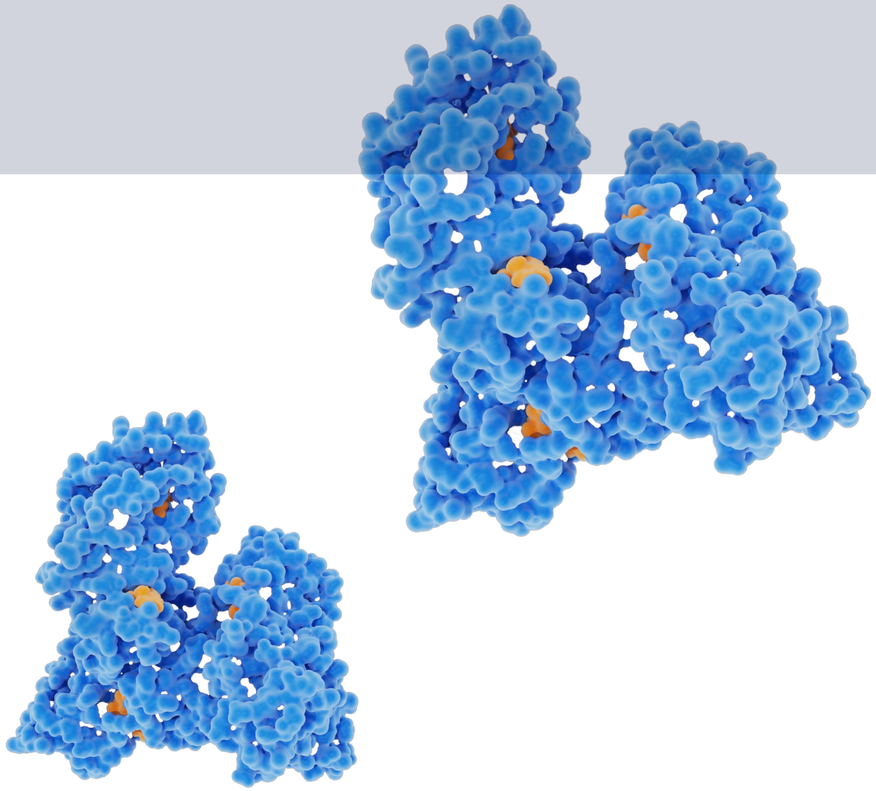
APPLICATION NOTE

Spectroscopy Techniques for Protein Analysis

A high-level overview of how spectroscopic techniques are utilized for protein analysis



Quick and accurate protein content quantification is critical for modern biology, biochemistry, and biophysics labs worldwide. Not only are researchers interested in detecting the total protein content, but they are also interested in protein classification/identification and proteomics. Although researchers have many tools at their disposal for understanding the complexities of proteins, the cornerstone of any protein laboratory is absorbance and fluorescence spectroscopy. We have discussed both methodologies in a previous application note "Biomedical Applications for Spectroscopy," which is a great resource for method fundamentals and therefore not covered in this app note. Instead, this application note will provide a high-level overview of how these spectroscopic techniques are utilized for protein analysis.

To demonstrate how powerful these techniques are for protein analysis, we focus our attention on one particularly interesting classification of pronates known as albumin. Human serum albumin is the most common protein in the body. It regulates osmotic pressure and plays a crucial role in maintaining overall blood pressure. Human serum albumin also serves as a carrier for a wide range of hydrophobic molecules like steroids in the bloodstream. In addition to the medical importance of human serum albumin, bovine serum albumin (BSA) is also extremely important because of its wide range of uses in the lab. BSA is used in applications ranging from stabilizing enzymes and antibodies, to preventing other proteins from adhering to the inner walls of microcentrifuge tubes. But the primary reason BSA is so popular, is its use as the standard by which all other proteins are measured.

Curious about the full application note? Click the link to download.

DOWNLOAD THE FULL APPLICATION NOTE

FEATURE

Frontline Analysis in the War Against Spice

How MANDRAKE gathers intelligence, shares information with the police and public, develops new technologies – including a portable benchtop NMR enabled by a unique pattern recognition algorithm – to combat the UK's synthetic cannabinoid epidemic

By Oliver Sutcliffe

A few years ago, "Spice" – a drug containing one or more synthetic cannabinoids – rose to prominence in the UK after images of people (often homeless) stood frozen or collapsed in the street were published on the front pages of national newspapers. The city of Manchester, where I work, has been referred to as the "spice capital" of the UK, but, in truth, the abuse of synthetic drugs is endemic in every major city in the UK and in many cities throughout the world.

Let me take you back to Manchester in 2013; the police were aware of psychoactive substances circulating in what were referred to as "head shops" – specialized shops selling "legal highs" that are structurally similar and mimic the effects of controlled drugs. In 2016, the UK government passed the Psychoactive Substances Act, which made the supply and production and import of new psychoactive substances illegal. The pet shops shut overnight, but it wasn't long before a black market was established.

With the birth of a robust black market, individuals on the streets started exhibiting much more severe effects than had been seen with the "legal" synthetic cannabinoids, including withdrawal and the catatonic states featured in the headlines. The problem was compounded by the fact that many police officers were uncertain as to the legal status of the synthetic cannabinoids and struggled to identify them on the street – moreover,

"The work that we do to identify substances and rapidly issue warnings feeds into our broader role as an intelligence gathering service, which is crucial to fighting the spice epidemic."

prevalence and potency varied widely. Violence and collapses in the city surged. One report claimed that 95 percent of the homeless community in Manchester were using spice regularly (1); and a 2016 survey found that 33 percent of inmates in UK prisons had used spice in the last month (2).

As part of a series of initiatives to combat the problem, we at Manchester Metropolitan University joined forces with Greater Manchester Police to co-create MANchester DRug Analysis and Knowledge Exchange (MANDRAKE). The aim was to expand our understanding of what is actually in the products people were taking, as well as exploring the human effects through our academic collaborations. Ironically, the ban on legal highs meant that many academic groups could no longer purchase and test these drugs. Indeed, many groups across the country stopped looking for these compounds because in some cases required specificHome Office licenses – an expensive and time-consuming process – to conduct their research on the now-illegal substances.

Criminologists in other parts of the country had spoken to users, asking about drugs on the street, what they'd been using, whether the drugs were getting stronger or weaker. But it was all subjective. We needed a way to identify and monitor what people were taking. Our lab was fully licensed to hold controlled drugs, and to even make new drugs to use as reference materials. So, what began as an informal knowledge sharing arrangement grew into a formal drug testing facility, running continuously and covering the whole of Greater Manchester. And it was unique in the UK.

The power of NMR

There are three main components to MANDRAKE: reducing harm, gathering intelligence, and developing new technologies that can be used on the front line staff in the police or prison service. If, for example, the police seize a batch of pills and their presumptive tests (normally a colorimetric test) are inconclusive, these samples can be potentially sent to MANDRAKE for some initial presumptive tests, using infrared spectroscopy or nuclear magnetic resonance (NMR) spectroscopy, which are followed up by confirmatory tests and quantification using gas chromatography-mass spectrometry. The final results are usually disseminated within two-and-a-half hours of us receiving the initial sample.

The benchtop NMR device we use is interesting. We were originally approached by a instrument manufacturer, Oxford Instruments, who thought we might be interested in purchasing a benchtop NMR to use when teaching. They demonstrated how it worked by testing different alcohols. I asked whether they'd thought about using it for forensics; they hadn't. But they had explored meat speciation during the UK's horsemeat scandal (an exciting decade!) using an algorithm that could fingerprint different leads, which we thought could work in drug-testing context. So, they funded us to develop a similar algorithm for forensics using our in-house library of substances. In the resulting paper, we presented a 93 percent accuracy rate (3) – excellent for a single component for our patented approach.

The main advantage of our pattern recognition algorithmic NMR approach is that it's extremely easy to use, which is vital if we want prison guards or police officers to use it. Frontline responders aren't scientists and need a simple discriminator they can interpret – a a red light or a green light so to speak, which was our assumption from the beginning and informed how we developed the algorithm and user interface. In addition, by removing any complicated spectral analysis from the process, you also remove any potential operator bias – which we know exists.

NMR also has some advantages over Raman spectroscopy – though I will say that Raman has advanced since we first started working with NMR. We found that when firing Raman at a tablet or a piece of paper we would get results that just didn't make sense because it was picking up the excipients or something in the background. With NMR, you extract the drug out of whatever matrix is in there, which produces a better match.

And because the device was portable, easy to use and rapid in terms of its turnaround time, we were able to trial frontline analysis. We decided to focus on MDMA initially because it is the most common controlled drug in the nighttime economy. And because the algorithm allows us to identify a drug without using a reference standard material (meaning we didn't have to carry around a sample of MDMA, which would be illegal!) we were able to deploy at a nightclub in Manchester. We get the results back in 2.5 minutes and are also able to detect anything dangerous, both in terms of potency or another substance cut with a pill. During this pilot project, we found the world's

strongest MDMA tablet – four to five times the usual dose. We issued a warning on social media, which allowed individuals to dispose of the tablets before taking them and putting themselves in serious danger.

The work that we do to identify substances and rapidly issue warnings feeds into our broader role as an intelligence gathering service, which is crucial to fighting the spice epidemic. We are able to provide quantitative data on the changes in spice potency over time, which the police can use to paint a more comprehensive picture of the market. For example, we've seen sudden increases in potency after manufacturing errors and decreases following the arrest of major players. We now know which strains are on the street, how their potency is changing over time. So, if we hear reports of sudden increases in potency or of new strains, we can test those, issue warnings and create reference materials to use in the future. Overall, it allows the police to be more proactive in dealing with drugs.

In one notable example, an individual, who had smoked spice and then felt very unwell, decided to submit the rest of it to find out what it was – a legal move. We were able to synthesize the compound to confirm the structure – a completely new cannabinoid that had not been seen in the UK before (it had only been found in the US). Such information feeds into the national and international surveys on drugs and can help the police understand the wider situation.

In addition to testing batches of drugs, we're also involved in developing new ways of finding out whether someone has taken spice. For example, last year we – alongside the University of Bath – received a £1.3 million research grant to help refine a portable device based on a simple fluorescence-spectral-fingerprinting-based saliva test, developed by the Bath group in 2019 (4).

The battles to come

Fortunately, the legislative changes we've seen to combat new psychoactive substances – both in the UK and in China – have reduced the number of new drugs on the market. A few years ago, we were running samples and seeing completely new substances on a regular basis, but that has subsided. The main challenge now is understanding local variations in potency so that we can identify unusually strong batches on the streets and issue warnings in a matter of hours.

Many of the devices out there are black and white in terms of "is it or isn't it there?" For example, we got a sample from the police that they believed was MDMA, but the infrared kept returning a negative result. It turns out it was 6-bromo-MDMA, but it has an additional bromine on the aromatic ring in the drug, so it didn't initially match exactly what was in the database. Our system was able to say that, although it is a new substance and isn't in the database, it is an MDMA derivative. So, if people are collapsing at an event or on the street, we can pass that information to welfare teams or paramedics who can treat people in a similar way to how they might treat someone who took the derivative drug. If we can increase the number of field-deployable devices that are able to do this, we could access more robust quantification and therefore more effective harm reduction and knowledge gathering.

I've also had conversations with people looking at forensics. In the UK, we have major backlogs in forensic services, which leads to a lot of additional police time. Hypothetically, if a police officer arrests someone because they've found a bag of white powder in their possession. Instead of waiting days or weeks for the results of testing the sample, they could run it through the NMR and get a result the same day – and potentially find out that their suspect was actually selling crushed paracetamol, for example.

There are many ways we can use rapid, portable, easy-to-use analytical tools.

Oliver Sutcliffe is a Senior Lecturer at Manchester Metropolitan University and Director of MANDRAK

REFERENCES AVAILABLE ONLINE

FEATURE

Live, Laugh, LIBS

Richard Hark, conservation scientist at Yale's Institute for the Preservation of Cultural Heritage, argues that chemometric tools are what's needed to take LIBS to the next level

By Richard Hark, conservation scientist at the Institute for the Preservation of Cultural Heritage, Yale University, USA

Are you using laser-induced breakdown spectroscopy (LIBS) in any of your projects?

We've used it for a few projects. We utilized LIBS to find out whether a piece of Chinese jewelry – a gigantic necklace in a series of interconnected little silver cones – was silver plated or solid silver. XRF can detect silver but cannot penetrate deeply into the metal. We decided to apply LIBS in a very inconspicuous spot, slowly making a tiny crater the width of a human hair down through the silver to explore the stratigraphy of the metal. We were able to determine that it was silvered and not solid silver. Admittedly, there may be other methods to achieve the same goal, but LIBS was also a really convenient way of gaining an approximate thickness of the silver coating at the same time.

"I would love to see more development of chemometric tools for non-expert users, to increase accessibility."

We've also analyzed over 100 Chinese ceramics for a book being written by one of the curators at the Yale University Art Gallery. For this project, we've been using a combination of LIBS and XRF because they are so complementary. LIBS allows us to detect some elements that XRF cannot, but XRF is actually better at seeing other elements that fall below the detection limit of LIBS. We also use chemometric tools to see if there are patterns in elemental composition.

What important advances have been made in portable LIBS?

I remember using a backpack unit that was developed by Ocean Optics. It looked (and felt) like an army backpack – and it came with a wand that looked a little like a metal detector. You could zap things on the ground, which was satisfying. It actually worked quite well for some applications, but it wasn't always practical for some geological or archeological applications I was interested in. Since then, we've seen great developments in handheld units driven primarily by industrial applications – using LIBS for scrap metal sorting, or for positive material identification, to make sure you're using the right component in a manufacturing process, or even looking at the carbon content of steel. Now there are five or six companies that make handheld LIBS systems that work much the same way as a handheld XRF device – and that's remarkable. These devices have only been made commercially available over the past five years or so and represent a significant improvement on previous portable approaches.

What advances would you still like to see in LIBS?

When we use LIBS, we're often looking for clues that tell us whether an object is from a particular region – our Chinese ceramic project is a good example of this need. But NIST standards do not exist for such work, so you have to use chemometrics and machine learning tools to group similar things together. Right now, you need to become a competent data scientist or collaborate with one to access the power of LIBS. I would love to see more development of chemometric tools for non-expert users, to increase accessibility. It would be great if a scientist could start using LIBS without in-depth data science knowledge and instead simply input data into a program that tells them whether there's any clustering. I believe there are some companies working on such data analysis tools – and they will really complement handheld LIBS.

ONLINE

Quick Tips for LIBS

Laser-induced breakdown spectroscopy (LIBS) is high throughput, you can see the elemental distribution of heterogeneous materials, and you also can analyze samples in situ without any sample preparation. But that doesn't mean you can forget good scientific practice!

ONLINE

Analytical Mythbusting

Richard Hark, LIBS expert and conservation scientist at Yale's Institute for the Preservation of Cultural Heritage, reveals his detective work in the cultural heritage sphere

SPOTLIGHT ON...

Technology

Compact, Stabilized Halogen Light Source

The AvaLight-HAL-S-Mini is a compact, stabilized halogen light source with adjustable focusing of the fiber connection, maximizing output power at the desired wavelength. Besides that, this versatile light source also has an adjustable electrical power output to provide extra power or longer bulb life.

MORE ABOUT THE AVALIGHT-HAL-S-MINI

High-Sensitivity Spectrometer with Cooled, Back-Thinned Detector

The AvaSpec-ULS2048x64TEC-EVO is the ideal instrument for low-light applications. Due to its cooled, back-thinned detector, this spectrometer has a high level of sensitivity and can catch as many photons as possible, while the cooling allows for a longer integration time with low-noise levels.

MORE ABOUT THE AVASPEC-ULS2048X64TEC-EVO

High-Resolution Spectrometer: AvaSpec-ULS4096CL-EVO

With 4096 pixels, the AvaSpec-ULS4096CL-EVO is an ideal instrument for high-resolution applications like plasma measurements and LIBS. Due to the CMOS detector, fast microprocessor, improved memory capacity, USB3 power supply, and Gigabit Ethernet communication, this spectrometer is completely future-proof!

MORE ABOUT THE AVASPEC-ULS4096CL-EVO

SITTING DOWN WITH

Public Service Spectroscopy

Sitting Down With… Ramon Barnes, Professor Emeritus of Chemistry, University of Massachusetts, Amherst, USA

How did you become interested in spectroscopy?

Howard V. Malmstadt at the University of Illinois was a big influence during my PhD research – which was on the basic concepts of time-resolved spark spectroscopy – he really got me interested in analytical and measurement sciences. I then spent some time at NASA while in the Army, continuing with spark studies of refractory alloys, followed by some plasma work on pre-inductively coupled plasma (ICP) discharges, which, to be honest, I found rather unsatisfactory. But then I read articles by Stanley Greenfield and Velmer Fassel on ICP and immediately realized it was a winner. I actually spent some time with Fassel at Iowa State – and this was a pioneering time for ICP. Then, at the University of Massachusetts, where I eventually became a tenured professor, we built our own ICP sources and continued to develop the technique in terms of fundamentals, computer simulations, applications, and instrumentation.

What excited you about ICP then – and does it still hold its own?

As Fassel pointed out many years back, ICP is sensitive, fast, has relatively few matrix effects – especially in optical emission spectroscopy – and covers the entire periodic table simultaneously. Today, ICP is used everywhere: anything that needs trace analysis uses either ICP emission spectroscopy or ICP-MS, depending on the concentration levels and other criteria. It's very popular in clinical, geochemical, environmental, forensic, pharmaceutical, and industrial analysis. Though it isn't as portable as X-ray fluorescence or as high resolution as nanoSIMS, it complements other technologies and has become central to elemental analysis. Today with laser ablation ICP-MS bioimaging, single cell nanostructures, and mass cytometry are pushing new frontiers of elemental measurements. Combined with separation and fractionation techniques, ICP detection has advanced speciation analyses significantly as well.

You've worked with some giants of spectrochemical analysis – what made them special?

Both Fassel and Malmstadt were innovative, creative thinkers. In particular, they had a knack for spotting areas of need for development and creating new concepts and ways of applying instrumentation. Malmstadt really encouraged us to think about first principles of instrumentation and measurement – really, he was a measurement scientist. Just glance through the list of people who grew from his research programs: Jim Winefordner, John Walters, Willard Harrison, Gary Hieftje, Gary Horlick, Bonner Denton, Stan Crouch, and their "second –generation" students just to name a handful. He inspired the whole community – students and colleagues.

Please tell us about the Plasma Spectroscopy/Spectrochemistry Winter Conference

I look at my career as one of public service – be it academic, industrial, or educational. Back in the 1970s, the ICP mission was growing, but Fassel had to work hard to battle opposition and convince instrument makers; it was almost 10 years between the technique being described in the literature to the development of the first commercial instrumentation. The big question was whether focusing on ICP was worthwhile, especially when atomic absorption and other techniques were doing so well – they were solving problems and making progress, but not without limitations. Leo de Galan decided to organize two discussion workshops in Holland in the mid 1970's to gather a small group of both basic researchers and instrumentation developers together to discuss the possibilities and uses of ICP. I felt that the discussions needed to continue, so we set up another workshop as well as a larger conference in San

"The big question was whether focusing on ICP was worthwhile, especially when atomic absorption and other techniques were doing so well – they were solving problems and making progress, but not without limitations."

Juan, Puerto Rico, early January 1980, when around 150 people participated. And that worked very well in terms of answering the burning questions at the time and charting the direction of ICP. Some of the first ICP-OES instruments were exhibited. That grew into the Winter Conference on Plasma Spectrochemistry, which has been going for 40 years in North America followed by sister meetings in Europe and Asia-Pacific countries.

How has the conference changed over the years?

It has certainly evolved and shifted emphasis. It still covers all aspects of plasma-spectrochemistry, and now people are discussing immunology, bioimaging, single cell/particle analysis, accurate isotopic analysis – with a real focus on ultra-trace elements, fast elemental analysis, high-resolution 2D and 3D imagining, as well as the fundamentals of the techniques and instrumentation developments including those with glow discharge devices, microwave plasma systems, laser-assisted technologies, sample preparation approaches, and advanced solid state detectors and software. And of course it has grown: now we have 400–500 attendees and between 200–300 presentations. For example, the 2022 Winter Conference is planned for Tucson, Arizona, January 17-22, and will be preceded by almost 50 professional development short courses.

Speaking of public service, you also continue to publish the ICP Information Newsletter…

Yes, the newsletter – in combination with the Winter Conference – is a great way to highlight new problems and developments in the field. Both are classified as non-profits entities, which means any revenue we generate must go to support science education, spectrochemical developments, and other activities to help encourage plasma-spectrochemistry through research and travel grants, for example.

What are your thoughts on the future of ICP?

It is clear that there's a continuing need for better, faster, smaller, smarter, and more sensitive techniques. ICP has stepped up and maintained its capabilities over the years – along with some other plasma sources to account for better detection capability, faster analysis, micro and nano resolution capabilities, as well as isotopic analysis in essentially all commercial and academic research fields. And that's exactly what many industries are increasingly looking for – the semiconductor industry is always pushing the boundaries of detection limits for example. I believe ICP, along with other plasma sources, will continue to predominate – at least for another decade until we come up with something easier, faster, and smarter.

Is there anything else you'd like to share?

Yes, I'd like to mention my wife, Dorothy. We've been married for over 50 years, after meeting as graduate students at the University of Illinois. She's much smarter than I am and certainly much more of a people-person! I've learned a great deal from her, and I appreciate all her encouragement and patience over the years. Without her, I think I'd not be doing what I'm doing. She deserves plenty of credit!