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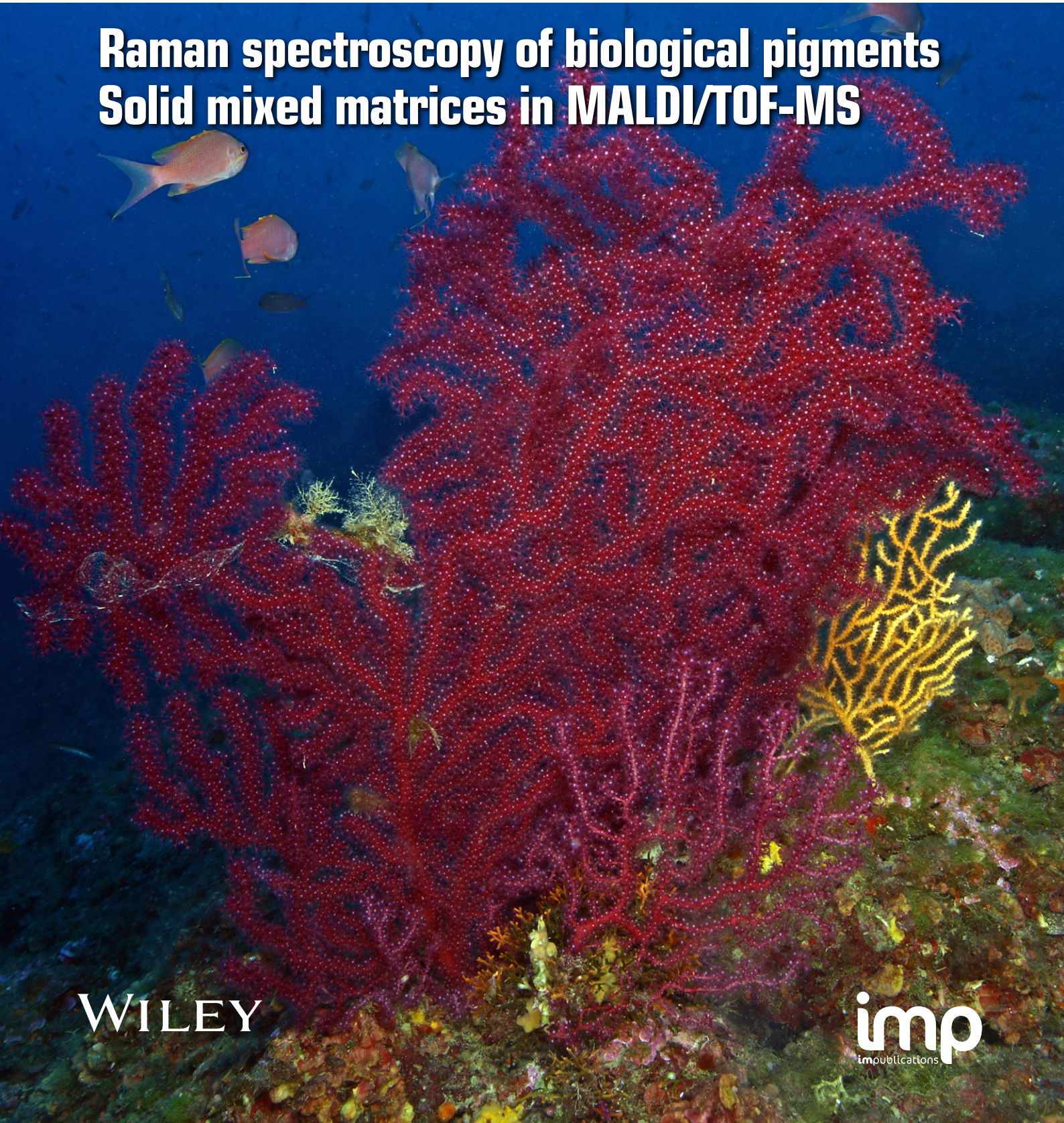
Raman spectroscopy of biological pigments
Solid mixed matrices in MALDI/TOF-MS



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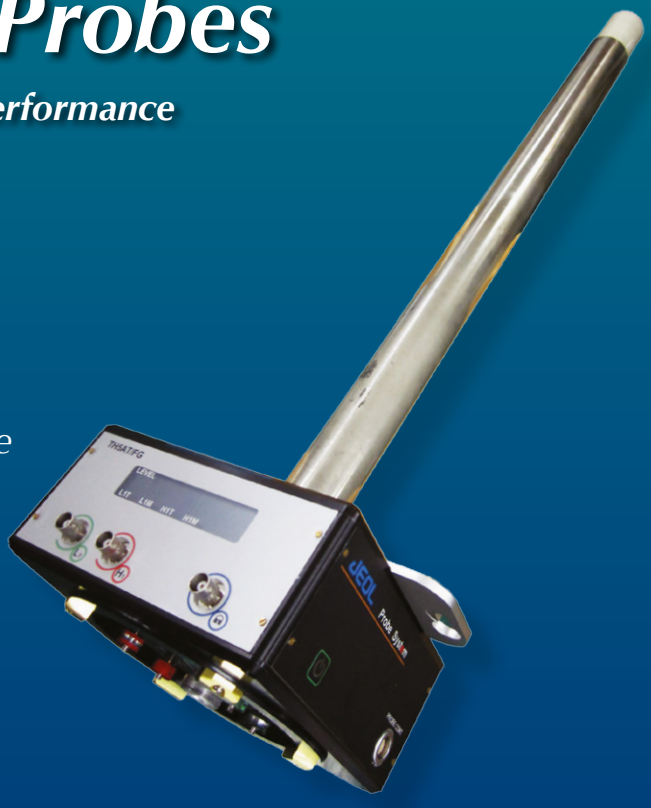

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EDITORIAL

As you will have noticed from this issue's cover, we are making a colourful start to 2016. In the first article on "The analytical niche for Raman spectroscopy in biological pigment research", Daniel Thomas and Cushla McGoverin suggest that Raman spectroscopy may have a particularly valuable role in pigment biology research. Pigments are almost universal in biology and are the basis of much of what we find attractive in flowers, birds and sea life, such as the fan corals on the cover. The authors show how Raman spectroscopy can be used to quickly confirm the presence of a pigment as well as providing more detailed knowledge about unknown pigments.

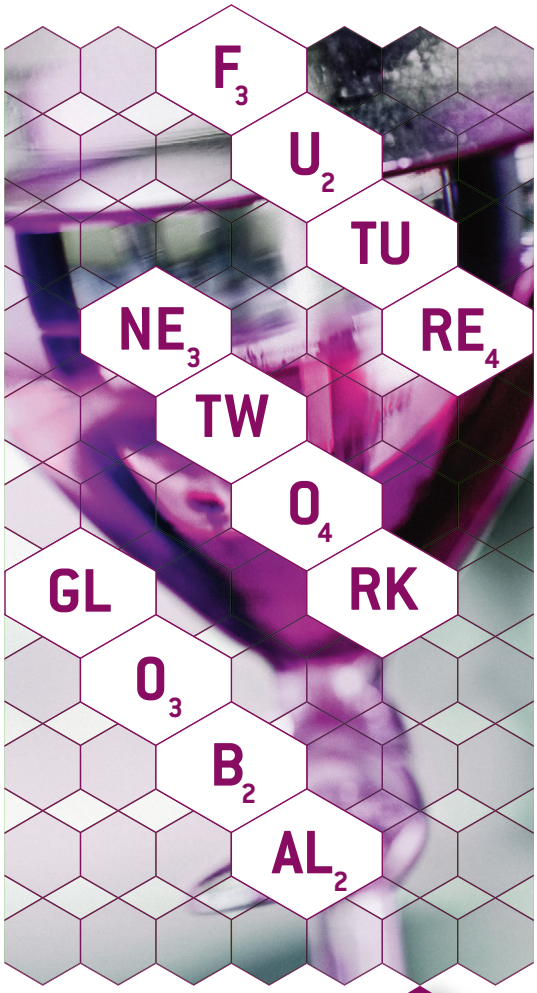
The bio theme moves to mass spectrometry in "Solid mixed matrices and their advantages in matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry" by Marek Šebela. Getting the most from various matrices for use in matrix-assisted laser desorption/ionisation (MALDI) has always been a bit of an art, and the introduction of mixed matrices increases the number of possible combinations but may improve reproducibility and so simplify analysis in the end. The author describes mixed matrices for a range of samples including proteins, peptides, oligosaccharides, oligonucleotides, lipids, polymers and even intact microbial cells!

In the Tony Davies Column, vast amounts of data and how you handle them are investigated by Tony, and Shane Ellis, Benjamin Balluff and Ron Heeren from the Maastricht MultiModal Molecular Imaging Institute. "Spectroscopic data handling at petabyte scale" shows how one institute is dealing with truly huge amounts of data, both in its collection and in its distribution to scientists for interpretation and analysis. At the same time, the institute has been able to incorporate best practice around the FAIR Data Stewardship of scientific information.

Peter Jenks looks back to the BERM 14 conference on biological and environmental reference materials in the Quality Matters Column. The next conference in the series returns to Europe: Berlin in June 2018.

In the Sampling Column, Kim Esbensen and Claas Wagner continue our education about representative sampling. In "Sampling quality assessment: the replication experiment", they provide an overview of the issue of replication, which may not be as straightforward as might be expected at first.

We also have a Product Focus on Atomic Spectroscopy, many New Products, even before the tsunami that one can expect from Pittcon next month, news of literature and nearly three pages of upcoming conferences, courses and exhibitions.



Daniel Thomas and Cushla McGoverin believe that Raman spectroscopy has a niche for the investigation of biological pigments in many organisms including these sea fan corals. Read about this on page 6.

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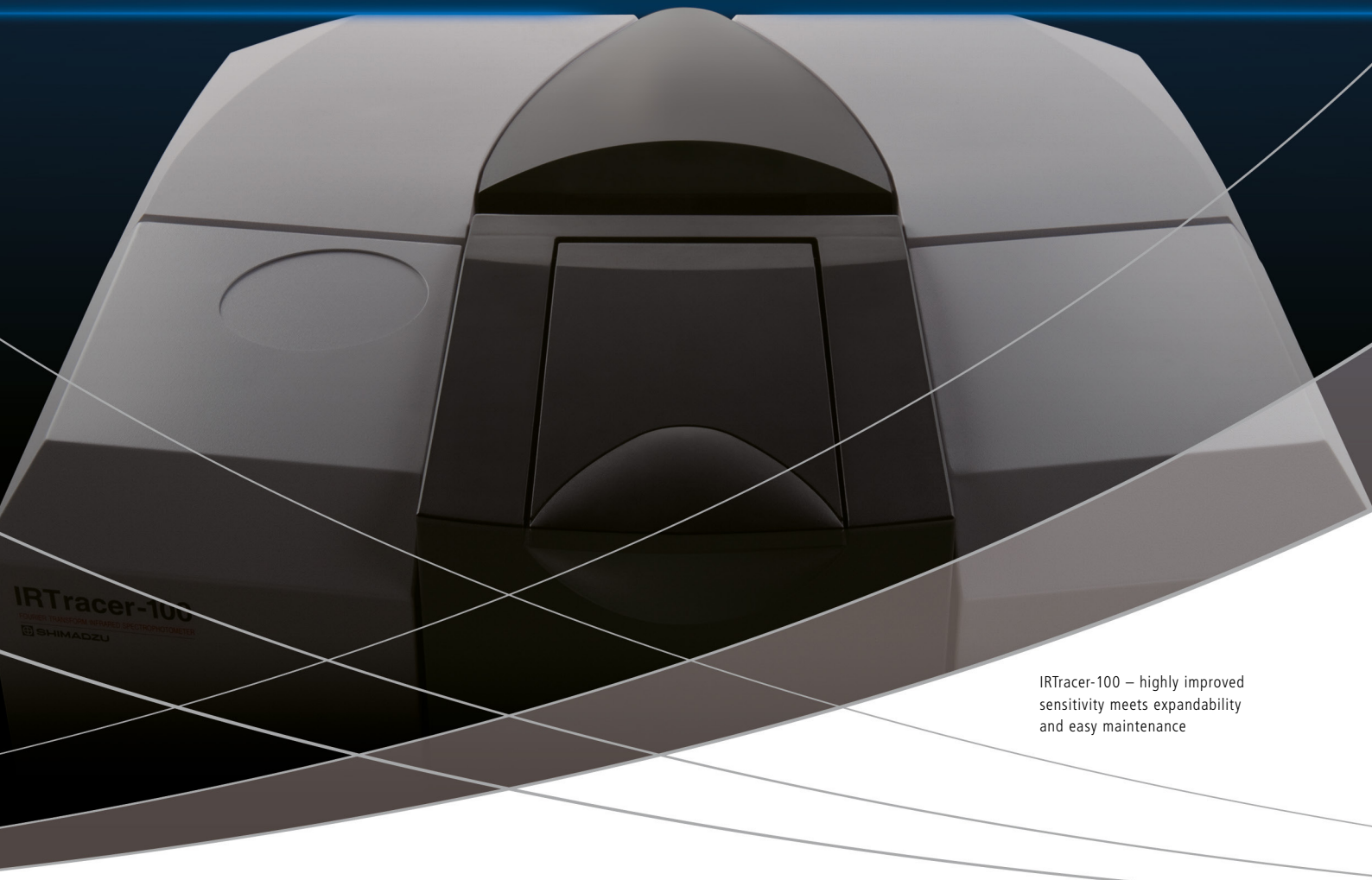

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ARTICLE

The analytical niche for Raman spectroscopy in biological pigment research

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Introduction

Pigments have important roles in the physiologies and ecologies of a diverse range of organisms, from unicellular algae to fungi, plants and animals (see, for example, Figure 1). Biological pigmentation is used for communication and camouflage, as well as maintaining health, for producing and circulating respiratory gases, and many other functions. Pigmentation research often encourages researchers with a background in biology to become familiar with analytical instrumentation more commonly used by dedicated chemists. Ultraviolet/visible spectroscopy (UV/vis) and high-performance liquid chromatography (HPLC) are staples of biochrome research, where the former is non-destructive and the latter is routinely used for chemical identification. Raman spectroscopy combines both of these advantages into a single technique, but is relatively under-utilised in pigment biology research. Raman spectroscopy provides information about functional groups in a molecule and can be used to identify biological pigments. Raman spectroscopy has the potential for wide use in pigmentation research alongside HPLC and UV/vis. The aim of this short review article is to show how Raman spectroscopy is being used in biological pigment research, beginning with studies of carotenoid pigmentation.

Carotenoids

The characteristic colours of canaries, marigolds, carrots and butterflies are all examples of the yellow through orange to red colours conferred by carotenoids. Carotenoids contain 40 carbon atoms and are divided into two groups on the basis of whether oxygen is present or not, xanthophylls and carotenes, respec-

Figure 1. Recent studies with Raman spectroscopy have focussed on anthocyanins and carotenoids as well as many other classes of biological pigment. Carotenoid pigments confer the yellow, orange and red hues to the feathers of many bird species, and anthocyanin pigments are responsible for red, pink, blue and purple colours in many flowers. Image credit: Cushla McGoverin and Daniel Thomas.

tively. Carotenoids are composed of a conjugated double bond chain which may or may not be terminated by ring structures. These shared structural traits result in three carotenoid characteristic peaks within Raman spectra: C=C stretching at 1500–1535 cm^{-1}, C–C stretching at 1145–1165 cm^{-1} and C–CH_3 deformation at 1000–1010 cm^{-1}. The structural differences between carotenoids in the terminating rings, polyene side chains and polyene length alter the Raman shift of these three dominant peaks, and introduce smaller peaks which are carotenoid specific. It is therefore possible using these peaks and their positions thereof to use Raman spectroscopy for the identification of carotenoids. This approach was recently used to non-destructively characterise the most abundant carotenoid in the feathers of 36 bird species.[1] However, these spectra were all collected from a similar matrix, keratin of the bird feather, and therefore matrix effects did not introduce a significant source of spectral variance that would perturb carotenoid characterisation when comparing the feather spectra. Raman spectra of carotenoids are environment dependent; therefore, spectra from biological tissues should not be compared to extracted carotenoid spectra for the purposes of identifying carotenoids. For example, mango tissue (*Mangifera indica*) contains a single form of carotenoid, β-carotene; the C=C stretching mode peak in the Raman spectrum of mango tissue occurs at 1529 cm^{-1}, however, in the Raman spectrum recorded from the extracted β-carotene this peak is at 1515 cm^{-1}.[2] The differences between extract and matrix-bound carotenoid spectra is, in part, a consequence of shape changes induced by the matrix. Changes in the planarity of the polyene chain result in peak shifting, particularly the C=C stretching band. In addition, the matrix may provide environments of different hydrophobicity, which will affect the extent to which the highly hydrophobic carotenoids interact with the surrounding matrix, e.g. the red carotenoid lycopene is present in tomatoes as microcrystalline aggregates due to the aqueous nature of the matrix.

Unsubstituted linear polyacetylenes

Bright biochromes are also common in marine environments, e.g. the red skeleton of *Corallium* sea fan corals. Sea fans and many other octocorals (i.e. corals with eight-fold symmetry) can also have yellow, pink and purple skeletons, and these exclusively-marine animals have a long history of being harvested for use in jewellery and other ornaments. Despite the centuries of tradable value, the pigmentary basis for octocoral skeletons has remained largely unknown. Relatively recent studies of pigmented octocoral skeletons with Raman spectroscopy sought an end to the mystery and recovered spectra with major peaks around 1100–1120 cm^{-1} and 1500–1520 cm^{-1}, but the spectra did not have a strong peak at 1000 cm^{-1}. These spectra suggested that octocoral pigments may be "psittacofulvins", a group of pigments that had previously only been reported from parrot feathers.[3,4]

Parrots are unusual among birds in that the red, orange and yellow colours of their plumage are not the result of carotenoid pigmentation. Instead, parrots achieve brightly coloured plumage with a group of polyunsaturated linear aldehydes (polyenals), pigment compounds for which the structure was first predicted with Raman spectroscopy.[5] As with carotenoid molecules, the conjugated carbon backbones of psittacofulvins give rise to strong C=C (e.g. 1500–1520 cm^{-1}) and C–C (e.g. 1100–1120 cm^{-1}) peaks in Raman spectra. The backbones of psittacofulvins are not methylated as they are in carotenoids though, and the C–CH_3 deformation mode (e.g. 1000 cm^{-1}) that helps identify a carotenoid is absent in spectra from parrot feathers (see Figure 2). In contrast, spectra from octocorals have a minor peak around 1004–1020 cm^{-1} attributed to C–CH_3 rocking. Although a fully resolved structure for the octocoral pigment is yet to be reported, the presence of a C–CH_3 rocking peak in the former is strong evidence that octocorals and parrot feathers have distinct pigments. Raman spectroscopy has shown that octocoral and parrot pigments have similar architecture, an unsubstituted linear polyacetylene backbone,[4] but that "psittacofulvins" are still a parrot-specific plumage pigment.

Tetrapyrroles

Birds achieve vivid colouration with a suite of chemically-distinct pigments,

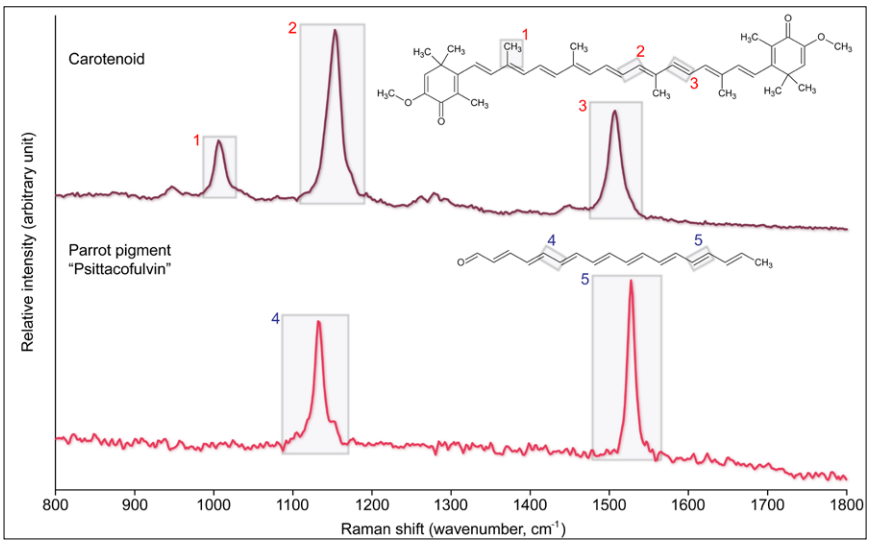


Figure 2. Raman spectrum from a Purple-breasted Cotinga (*Cotinga cotinga*) feather pigmented with carotenoids (upper spectrum) and from a Dusky Parrot (*Pionus fuscus*) feather pigmented with polyenal "psittacofulvin" pigments (lower spectrum). The peak around 1000 cm^{-1} in the upper spectrum identifies C–CH_3 deformation and represents a clear difference between the two spectra. We can therefore conclude that the conjugated backbone of the polyenal "psittacofulvin" pigment is not methylated.

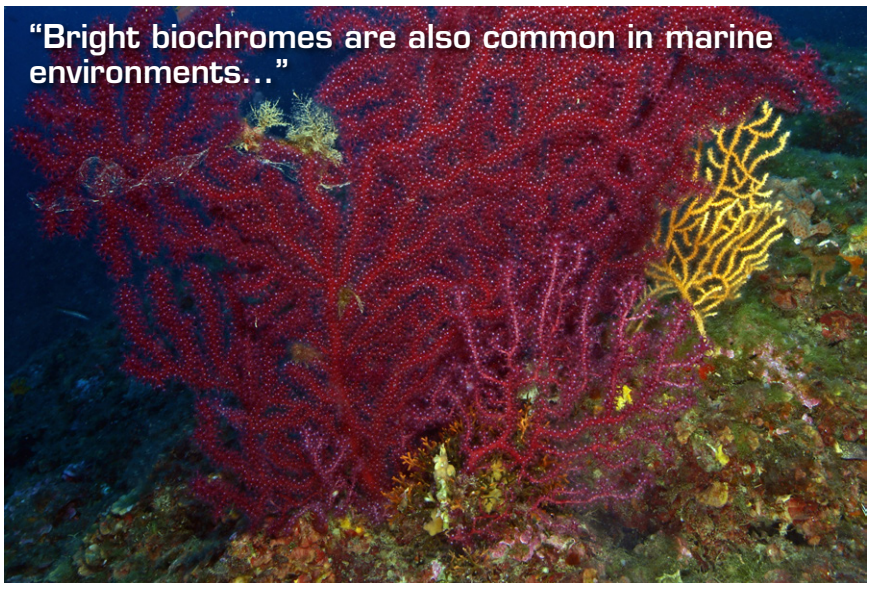
including the tetrapyrrole pigments that colour the surfaces of eggshells. Brown eggshells are coloured with protoporphyrin IX (e.g. chicken eggs), the precursor macrocycle for haeme, and the classic "eggshell blue" and blue-green shell colours are conferred with biliverdin, a linear tetrapyrrole pigment broken down from haeme. Blue-green and brown pigments help to camouflage eggs and can aid parents in distinguishing their own eggs from an egg laid by a brood parasite (e.g. cuckoo). While most pigmented eggshells are brown, blue-green or a mixture thereof, some comparatively rare eggshells are pink, orange or bright green.[6] The bright green eggshells of an Elegant Crested Tinamou (*Eudromia elegans*) were recently studied with Raman spectroscopy.[7] Seven peaks in Raman spectra from the Elegant Crested Tinamou eggshells both identified a tetrapyrrole pigment and distinguished biliverdin from protoporphyrin (1619, 1588, 1467, 1295, 1248, 1174 and 970 cm^{-1}). The two most intense peaks from the pigment were at 1295 cm^{-1} and 1248 cm^{-1} which identified the C=C and C–N stretching of the pyrrole subunits. Hence, Raman spectroscopy showed that biliverdin is responsible for a wide blue to green colour gamut in eggshells. Moreover, the ability to identify biliverdin in eggshells without destroying the specimen is a useful application for studying ancient remains (i.e. zooarchaeology).

Moa are extinct ostrich-like birds that inhabited New Zealand until the 15th century. Eggshell fragments from upland moa (*Megalapteryx didinus*) are olive-green or faint blue and suggest that biological pigments can be preserved in eggshells over extended time scales. Ancient eggshell fragments with pigmentation are rare and destructive analyses are not always viable. Instead, faint blue moa eggshell fragments were analysed with non-destructive Raman spectroscopy, confirming the presence of biliverdin.[7] Beyond presence or absence data, information about pigment concentration could provide valuable insight into the ecology of these extinct birds. Pigment concentrations could potentially be gained from Raman spectral peak ratios calibrated with concentration data from HPLC. The strongest peak in a Raman spectrum of a pigmented eggshell is attributed to the symmetric stretching of the carbonate anion in calcium carbonate.[7] The relative intensities of carbonate and pigment peaks are expected to change with concentration, providing a non-destructive method for quantifying pigment levels.

Unresolved pigments

Colourful animals held in museum collections are excellent resources for studying evolutionary patterns in biological pigmentation. Raman spectroscopy is ideally suited for surveying the pigments of museum specimens as the technique requires no specialised sample preparation and analyses are rapid (i.e. specimen handling time is short). Known pigments in biological tissues can be quickly identified (e.g. carotenoids in feathers), and there is the potential for new pigments to be discovered. For example, an unusual yellow feather pigment was recently identified in the feathers of some penguin species.[8] The solubility, light absorption and fluorescence properties of the penguin pigment were revealed to be substantially different from any known plumage pigment. More information about the chemical identity of the penguin pigment was sought with Raman spectroscopy.

Functional groups in the penguin pigment identified with Raman spectroscopy were consistent with a nitrogen-bearing heterocyclic ring.[9] A distinct peak at 1466 cm^{-1} was attributed to the stretching of bonds in a lactam ring (i.e. cyclic amide) and peaks at 1285 cm^{-1} and 1351 cm^{-1} were attributed to C–N stretching and C–C stretching. Nitrogen heterocycles occur in several types of pigment including pterins, porphyrins and linear tetrapyrroles. The yellow penguin pigment and the tetrapyrrole pigment bilirubin have broadly similar Raman spectra, except that the penguin pigment has a peak at 1577 cm^{-1} with the highest relative intensity.[9] The Raman spectrum from the penguin pigment does not otherwise match any other published spectrum. The penguin pigment has proven challenging to extract from the feather for mass spectrometric analyses and structural elucidation. Resolving the structure of the yellow penguin pigment will provide deep insight into the function and evolutionary origins of the unusual yellow pigment.



Anthocyanins

Raman spectroscopy is widely used to confirm the presence of a known compound in a particular sample. However, when the sensitivity and chemical specificity of standard Raman spectroscopy are insufficient for an analytical problem, the surface enhancement effect may be needed. In surface-enhanced Raman spectroscopy (SERS) specific molecular vibrations are enhanced when

the molecule is adsorbed to, or in proximity of, a coinage metal surface with nanometric roughness (e.g. silver or gold nanoparticles, silver or gold nanopatterned surfaces). Surface-enhanced Raman spectroscopy has recently been used to characterise plant sources of anthocyanins (red, purple and blue plant pigments).[10] Anthocyanins have a long history of use as dyes particularly for textiles. SERS spectra were recorded from plants pigmented with anthocyanins (bilberry, elderberry, purple corn, sumac and hollyhock) and extracts from aged and unaged dyed textiles. Spectral peaks characteristic to anthocyanins were observed in the spectra recorded from each plant species sample.

Moreover, pigments extracted from wool fibres (as little as 0.5 mg) produced SERS spectra with anthocyanin-identifying peaks.[10] However, even the sensitive SERS spectra were insufficient for identifying specific anthocyanins or plant sources in the wool spectra. This experiment indicated that it is possible to identify the presence of anthocyanins in textiles even after the colour has faded; a result that has implications for the analysis of these dyes in historical textiles.

Conclusion

Raman spectroscopy is used in biological pigment research as a method for quickly confirming the presence of a known biochrome. Researchers have also used the technique to identify particular pigments beyond the generalised pigment class, to investigate the chemical identities of unknown pigments, and although not fully explored here, to measure pigment concentrations. Raman spectroscopy is ideal for studying pigments *in situ* (i.e. without extraction from biological tissues) as there is no specialised sample preparation required. We feel that Raman spectroscopy could be more widely used in biology research, and we hope that this brief review encourages pigment researchers to explore the value of this technique for their own study systems.

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ARTICLE

Solid mixed matrices and their advantages in matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

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Introduction

This review article describes the use of solid mixed matrices in matrix-assisted laser desorption/ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS). These are prepared by combining common matrix compounds and applied particularly to analyse peptides, proteins, oligosaccharides, oligonucleotides, lipids, synthetic polymers or intact cells. The biggest advantage of this approach is that it typically yields homogeneous sample spots on the target plate and provides highly reproducible measurements accompanied by increased sensitivity and resolution.

Since its discovery towards the end of the 1980s, MALDI, which is usually associated with a TOF mass analyser in commercial instruments, has become a common technology in biological MS.[1] MALDI-TOF MS has found its application not only in the analysis of peptides and proteins, oligonucleotides, oligosaccharides, technical polymers and small polar compounds, but also viruses or even intact microbial cells. Initially there was an opinion that it would never be possible to analyse large biomolecules using mass spectrometry. It was assumed that, as first it is necessary to vaporise and then ionise the sample to perform MS, large molecules would be decomposed long before their vaporisation. Moreover, mass spectrometers were originally designed for small molecular masses not exceeding 1 kDa.[2]

Laser desorption/ionisation for biomolecules was independently introduced by Michael Karas and Franz Hillenkamp (Germany) for solids in a crystalline environment[3] and Koichi Tanaka (Japan) in the liquid phase.[4] The use of lasers to generate ions dates back to the 1960s when a majority of the initial experiments was done with inorganic compounds. The primary role for the laser was to produce a very rapid heating, and the energy transfer was considered to occur via the metallic substrate.[5] Karas and Hillenkamp, who actually coined the term MALDI, first recognised that a small organic compound, which strongly absorbed at the laser wavelength they used and exhibited a soft ionisation, could enhance the ionising laser desorption process. During analyses with amino acids, the highly absorbing tryptophan functioned well as a matrix for alanine, a non-absorbing amino acid, when they were present together in the form of a 1 : 1 mixture.[3] Later on, Karas and Hillenkamp worked with nicotinic acid as a matrix with excitation at 266 nm. Having been aware of results of Koichi Tanaka and his coworkers,[4] they successfully turned their increased effort with a new post-acceleration detector into a finding of the possibility to ionise and detect proteins with molecular masses above 10 kDa.[6] In contrast, Tanaka and his coworkers relied on an ultrafine cobalt powder with glycerol for laser desorption and ionisation and to their satisfaction, they could measure proteins up to about 35 kDa.[4] Nowadays, this method is rarely used compared with the approach by Karas and Hillenkamp. Nevertheless, Koichi Tanaka was awarded the Nobel Prize for Chemistry in 2002 together with John Bennett Fenn, who in parallel discovered electrospray ionisation (ESI), another soft ionisation technique, which is also well suited to studying biological macromolecules.[2]

The role of matrix in MALDI process

Competition between the two soft ionisation techniques saw ESI-MS pushed forward in its development as it offered the advantage of being able to be coupled on-line with high-performance liquid chromatography. In the 1990s, the acceptance of MALDI MS as an analytical tool was raised by introducing both delayed extraction of ions (resulting in high resolution spectra) and post-source decay fragmentation (allowing one to obtain structural information on selected ions).[5] The process of MALDI is achieved in two steps.[7] First, the analyte

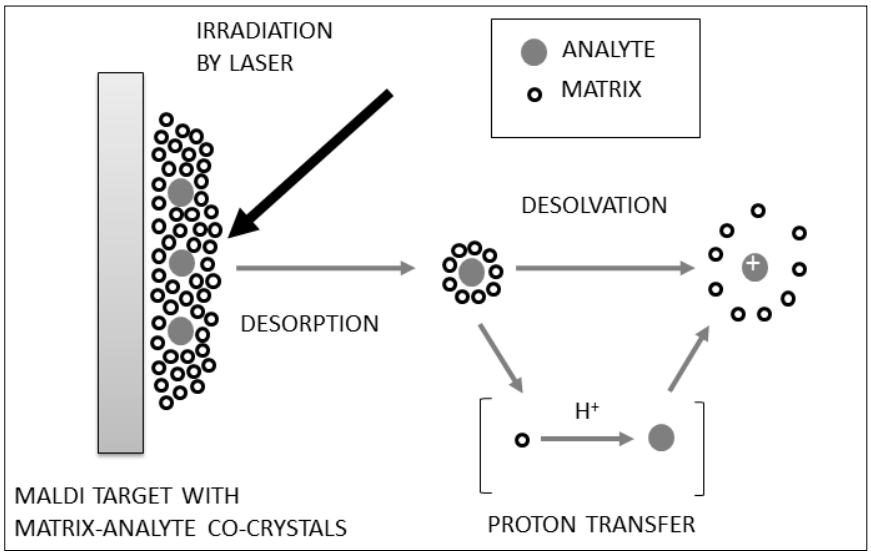

Figure 1. A scheme of the principle of MALDI (adapted from a previous publication[7]).

to be investigated is typically dissolved in a solvent, which contains an appropriate matrix compound. On the target plate, a "solid-solution" deposit of analyte-doped matrix crystals is formed on evaporation of the solvent. As a result, molecules of the analyte are embedded in an excess of matrix molecules.[7] In the second step, which takes place under vacuum conditions in the ion source of the spectrometer, the co-crystals are ablated in portions by intense laser pulses (see Figure 1). The exact mechanism of the formation of ions is not completely understood. Definitely, the matrix is indispensable in this process. The rapid heating by laser irradiation causes the accumulation of energy through excitation of the matrix molecules. A dense plume of material containing both the matrix and matrix–analyte clusters expands into the vacuum of the ion source.[8] The most widely accepted mechanism of primary ionisation involves generation of charged species within a cluster ablation process or gas-phase proton transfer in the expanding plume from photo-ionised matrix molecules.[7,8] The ions in the gas phase are then accelerated by an electrostatic field and move towards the analyser.

Interestingly, nearly all of the matrix compounds that are routinely used today were discovered in the early days of MALDI.[5] In 1989, Beavis and Chait introduced cinnamic acid derivatives, such as sinapinic, ferulic and caffeic acids (SA, FA, CA, respecively),[9] which performed excellently for the analysis of proteins using ultraviolet lasers with maximum wavelengths at 355 nm (Nd:YAG) or 337 nm (nitrogen laser).[5] After a few years, the same researchers discovered that α-cyano-4-hydroxycinnamic acid (CHCA) was efficient for MALDI-TOF MS analysis of peptides and glycopeptides in the molecular mass range of 500–5000.[10] 2,5-Dihydroxybenzoic acid (DHB) has become another matrix suitable for peptides and glycopeptides, which was first examined at that time.[11] Currently, there are many compounds available which can be applied for this purpose according to the specific needs, but only a few of them are really good. Anyway, matrix choice and optimisation of the sample preparation protocol are the most important steps in MALDI experiments.[7] The final selection is always related to the chemical nature of the analyte and the respective laser wavelength. The most effective MALDI matrix must simultaneously meet a number of general requirements:[7] strong absorbance of the laser light at a given wavelength, ability to sublime, vacuum stability, promotion of analyte ionisation, solubility in analyte-compatible solvents and lack of reactivity. The choice of matrix is also important for the control of fragmentation, especially in the case of proteins and peptides carrying post-translational modifications. A small number of compounds have been studied with respect to their propensity to induce fragmentation. They are thereby classified as "hot" (assisting the formation of ions with a high internal energy) or "cold". For example, Karas *et al.* found that post-source decay (= metastable fragmentation) decreases in the order CHCA or SA > DHB mixed with 2-hydroxy-5-methoxybenzoic acid (so-called "super DHB") > 3-hydroxypicolinic acid (HPA) for protonated glycoproteins.[12] However, it has been shown that the assignment of "hot" and "cold" matrix may reverse as the analyte or laser energy changes.[13]

Mixed matrices for proteins, peptides, oligosaccharides and oligonucleotides

Various approaches have been explored to enhance matrix selectivity, such as the use of additives, but they do not directly affect the ionisation and rather influence co-crystallisation or exclusion of salt impurities. A big potential resides in combining more compounds into binary or ternary matrix mixtures. The above-mentioned "super DHB" is a mixture of DHB and 2-hydroxy-5-methoxybenzoic acid in a weight ratio of 9:1. This binary matrix provides higher yields and signal-to-noise ratios for analyte ions in the high mass range: typically it is applicable to intact proteins. The reason is elucidated by the formation of a disorder in the DHB crystal lattice resulting in "softer" desorption.[14] Another combination based on DHB involves CHCA as the second matrix component (in a weight ratio of 1:1).[15] This mixed matrix proved to be well suited for peptides and intact proteins (an improved signal intensity and resolution was found especially for glycoproteins). The crystallisation of samples with CHCA/DHB results in a homogeneous pattern of CHCA-derived crystals in the central part of the sample spot with small DHB crystals at the perimeter. When applied, CHCA/DHB provides both significantly improved spot-to-spot reproducibility of mass spectra and reduced noise compared

with the use of either component individually, which simplifies fully automated acquisition. An interesting binary matrix for measuring phosphopeptides was discovered by combining HPA with CHCA in an optimised weight ratio of 1:1 (see Figure 2). As a result, phosphopeptide signals can be acquired reproducibly in either the positive- or negative-ion mode at a lower laser power than with DHB (a common matrix for phosphopeptides) or CHCA alone. In addition, the analysis time is shortened by avoiding searching for the so-called "sweet" spots as is necessary when working with DHB. Concurrently, neutral losses of phosphate group (–80 Da) and phosphoric acid (–98 Da) are reduced. The mixed matrix is also applicable to intact phosphoproteins (as has been demonstrated with molecules up to 30 kDa).[16]

Oligosaccharides represent a challenge for MALDI-TOF MS as there are typically many problems with low sensitivity and strong background noise. Neutral oligosaccharides are detected in the positive-ion mode as sodium- or potassium-charged pseudomolecular ions for which DHB usually represents the matrix of choice. Oligosaccharides from human milk have been studied with numerous matrices, their binary mixtures and single-matrix mixtures with various additives.[17] DHB with 2,4-dinitrobenzoic acid and a diluted NaCl solution was found optimal for standard analyses with neutral compounds. 5-Chloro-2-mercaptobenzothiazole proved to be an excellent first layer for DHB and 6-aza-2-thiothymine (ATT; by the way this is perfect for investigations on acidic oligosaccharides in the negative-ion mode) or could be used alone.[17] Combining DHB with the basic aminopyrazine in a weight ratio of 3:1 allowed an excellent data acquisition not only for neutral monosaccharides and oligosaccharides (including the content of a real plant extract) but also maltodextrins up to 40 glucose units.[18] In contrast, the presence of salts is undesirable when performing MALDI-TOF MS with oligonucleotides because of the formation of adducts between the phosphate backbone of DNA and counter ions (Na^+, K^+). Nowadays, oligonucleotides are largely utilised as gene probes in molecular biology. When the quality evaluation of synthetic preparations by MALDI-TOF MS is necessary, HPA, ATT or 2′,4′,6′-trihydroxyacetophenone are suitable matrices.[7] Diammonium hydrogen citrate is used as a common additive. A major obstacle is the relatively poor mass resolution, which increases in parallel with the increasing molecular mass accompanied by the decrease in signal intensity and sensitivity. To cope with these troubles, the use of a mixture of HPA with pyrazinecarboxylic acid (4:1, v/v, for saturated solutions) has been described, which provides better spectral parameters as well as higher reproducibility and tolerance to impurities.[19]

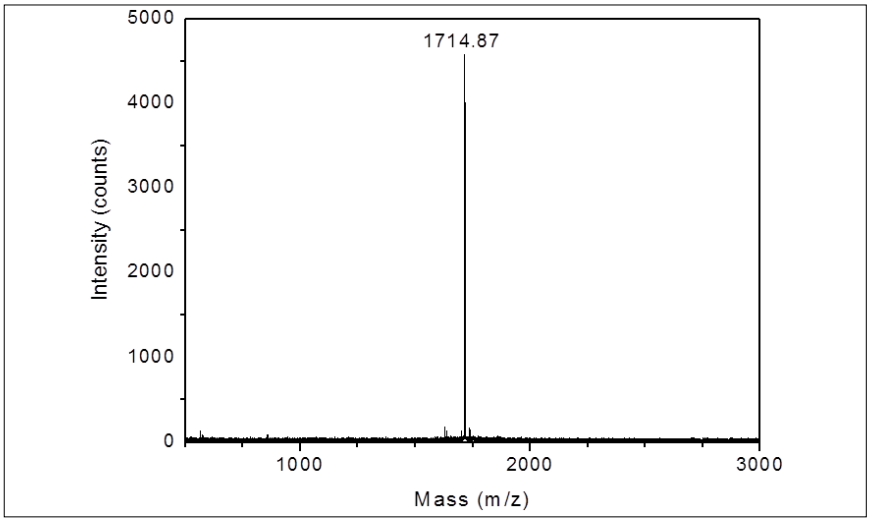

Figure 2. MALDI-TOF mass spectrum of a synthetic phosphopeptide carrying phosphorylation at a serine residue. The spectrum was acquired using HPA/CHCA mixed matrix.[16]

Mixed matrices for lipids, low-molecular-weight compounds, polymers and intact microbial cells

Dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) is recommended for MALDI-TOF MS experiments with lipids.[7] A ternary matrix containing dithranol, DHB and CHCA (in hexane/isopropanol/dimethylsulfoxide; mixed in an equimolar ratio with the addition of sodium iodide) has recently been employed to analyse lipid impurities in biodiesel, which is composed mainly of fatty acid methyl esters.[20] The biggest advantage of the ternary matrix mixture resided in the formation of smaller and more homogeneous co-crystals with samples (compared with the use of individual matrices), which had a positive impact on reproducibility and sensitivity. Moreover, semi-quantitative information could be obtained using a calibration with standards. Similarly, a binary matrix consisting of CHCA and DHB in a weight ratio of 1:1 in 70% methanol/0.1% TFA (trifluoroacetic acid) containing 1% piperidine was found efficient for MALDI imaging MS of phospholipids.[21] The binary mixture was deposited onto a sliced rat brain tissue and, importantly, it crystallised homogeneously, which resulted in increased reproducibility and signal intensities as well as a higher number of signals. The matrix was superior to conventional matrices in its performance and functioned well in both positive- and negative-ion modes: more than 100 glycerophospholipids and sphingophospholipids could be identified by MS and tandem MS.[21] As a certain disadvantage, a strong background at masses below 500 Da appeared which fortunately did not disturb the use of CHCA/DHB for phospholipids. The problem of background signals at low masses was addressed by Guo and He[22] in 2007: a binary matrix combining CHCA and basic 9-aminoacridine (the compounds differ from each other in their affinity to protons) significantly reduced the number of background matrix peaks in both positive- and negative-mode detection of small molecules. In addition, better signal-to-background ratios were observed for negatively

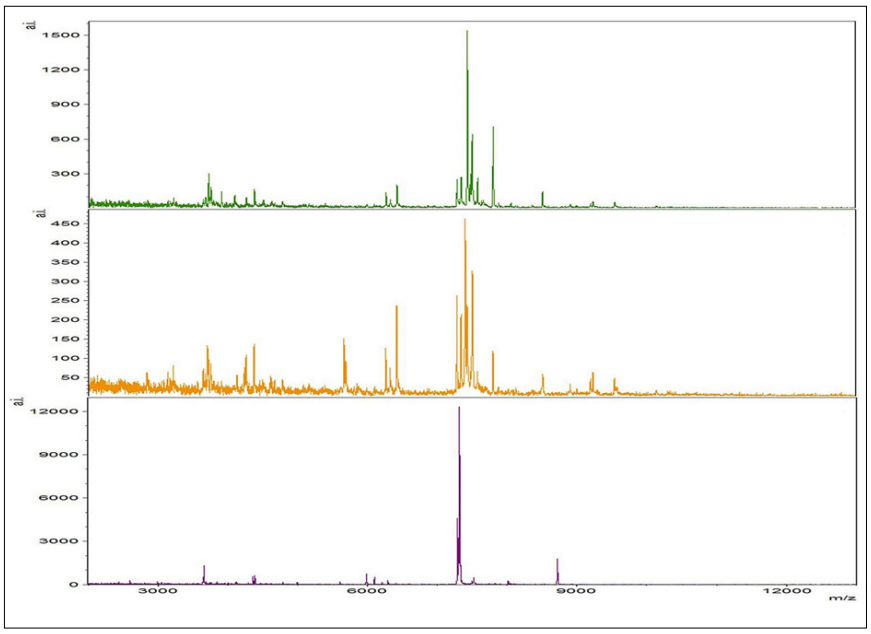

Figure 3. MALDI-TOF mass spectra of intact fungal cells. Each spectrum, acquired using FA/SA mixed matrix,[25] belongs to a different plant pathogen species of the same genus.

charged inositol phosphates in complex plant extracts. Various binary matrices (e.g. DHB + CHCA, DHB + SA, CHCA + SA and others based on 2,6-dihydroxybenzoic acid as an isomer of DHB) or ternary mixtures such as DHB + CHCA + SA or 2,6-dihydroxybenzoic acid + CHCA + SA allowed one to achieve an improved sensitivity in the analysis of polyethylene glycols with molecular masses ranging from 1 kDa to 10 kDa (the reason probably resides in more homogeneous matrix-sample co-crystal patterns).[23]

FA is a commonly used matrix for intact cell/spore MALDI-TOF MS of fungi. In respect of the number of ion species and signal intensities, FA was shown to be superior, for example, to CHCA, SA, 2-hydroxy-5-methoxybenzoic acid or 2′,4′,6′-trihydroxyacetophenone when *Fusarium* species were analysed despite involving additives or applying binary matrix combinations such as DHB/2-hydroxy-5-methoxybenzoic acid and CHCA/DHB.[24] One major drawback of FA is the inhomogeneous look of its co-crystals with the analyte resembling snow-covered branches. In our laboratory, we have a long and extensive experience in the use of binary matrices for intact fungi. To find the optimal matrix, CA, CHCA, DHB plus FA were evaluated individually and then also in the form of various binary mixtures such as CHCA/FA, CHCA/SA and FA/SA (different mixing ratios and solvents). The mixture of FA and SA in a weight ratio of 1:3 (specifically 5:15 mg mL^{-1}) in acetonitrile/2.5% (v/v) TFA, 7:3, v/v, was finally proven to be optimum for working with fungi and oomycetes.[25] It allows a reproducible acquisition of signals (because of the homogeneous crystallisation pattern) with relatively high signal-to-noise ratios, which are distributed evenly over a large mass region (see Figure 3). Also a binary mixture CA/SA or ternary mixture with CA, FA and SA represent good choices for working with fungal cells (unpublished results).

Conclusions

The preparation of samples for MALDI-TOF MS is an exciting experimental chemistry. The choice of the best matrix, solvents and sample preparation technique are crucial steps on the way to achieving reliable results. To overcome certain imperfections in the use of common matrix compounds, there is a possibility of applying additives or making binary or ternary matrix systems. Combining more compounds into a final mixture allows optimisation of the different properties required of the matrix by varying the identity and/or concentration of components. As a consequence, the sample preparation step provides more homogeneous crystallisation on the target plate, which ensures higher reproducibility of measurements and is typically accompanied by both higher sensitivity and better resolution of signals.

Acknowledgements

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TONY DAVIES COLUMN

Spectroscopic data handling at petabyte scale

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For many analytical spectroscopists, data handling challenges arise every few years when the space on the USB stick used to move data between the spectrometer and the office computer becomes full. With the founding of the Maastricht MultiModal Molecular Imaging Institute M4I at the Brightlands Maastricht Health Campus and the associated appointment of two new Professors, the interests of Professor Ron Heeren with the Division of Imaging Mass Spectrometry and Professor Peter Peters and the Division of Nanoscopy, a perfect storm of data has been created. At the largest molecular imaging centre in Europe, Ron Heeren's group study high-resolution molecular imaging of biological systems and polymers through the development and application of state-of-the-art mass spectrometry based molecular imaging approaches for nanomedicine and biomedical research. Peter Peters' team use techniques such as high-resolution cryo-electron microscopy to investigate complex protein structures in cells. This far-sighted strategic decision by Maastricht University has attracted significant funding to the location and has allowed an unrivalled capability to be established which is still growing.

Size of the data storm

Working together with some of the major instrument vendors in our field they are now generating data at the rate of 100s of GBytes/day. These large amounts of data need to be rapidly and securely stored in a location which is also designed to be able to serve this data back to the individual researchers when they need to begin the task of data analysis and processing, a significant challenge within itself. An example of the state-of-the-art development work being undertaken with commercial companies is the beta testing of a new parallel imaging MS/MS nanoTOF II from Physical Electronics (PHI) (Figure 1) where the TOF-SIMS spectrum (MS1) and the MS/MS spectrum (MS2) are acquired in parallel. This high-information-volume methodology allows researchers to directly compare spectra, images or depth profiles from MS1 and MS2 of the same three-dimensional volume containing hundreds of thousands or more pixels.

On another new instrument, the Bruker rapifleX MALDI Tissuetyper™ time-of-flight (TOF) which offers acquisition rates up to 50 times faster than other MALDI imaging systems, they have generated results from experiments that were performed on brain sections with pixel sizes ranging from $10 \times 10\,\mu m^2$ to $50 \times 50\,\mu m^2$. The data generated in both positive- and negative-ion modes yielded information-rich and complementary lipid spectra revealing the spatial changes of the lipidome composition throughout the mouse brain. The speed of the instrument allowed an entire mouse brain to be imaged consecutively in both positive- and negative-ion mode in ~35 minutes.[1] These high acquisition speeds allow work on new classes of matrices that are unstable under high vacuum for MALDI-MSI studies, but, of course, this means large amounts of data are now acquired much faster, thus placing further demands on IT infrastructure. A typical experiment from this instrument

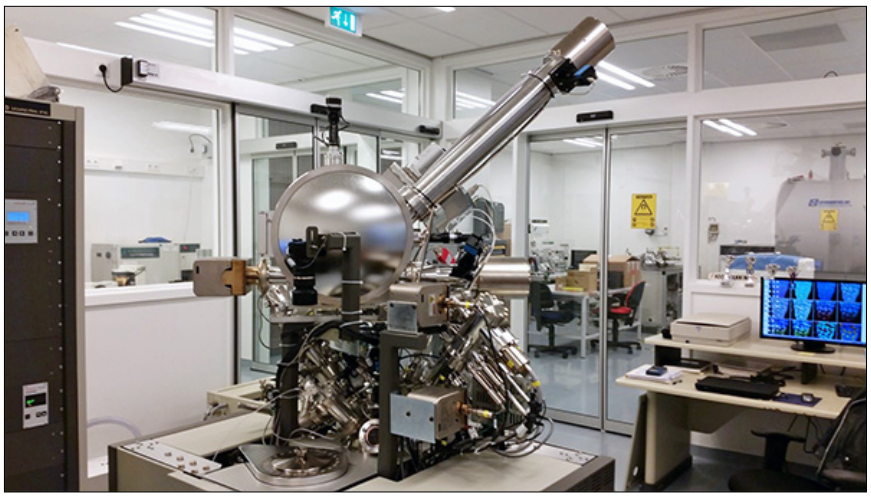

Figure 1. The new Physical Electronics NanoToF II tandem SIMS system in operation at M4I in Maastricht.

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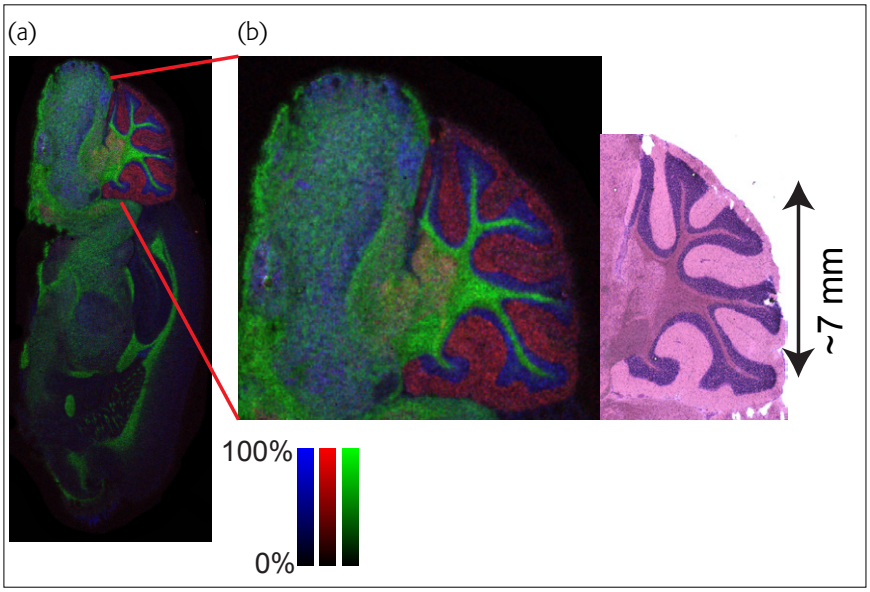

Figure 2. (a) Positive-ion images of $[PC(40:6)+K]^+$, $[PC(38:6)+K]^+$ and $[PC(36:1)+K]^+$ observed at *m/z* 972, 844 and 826 and shown in red, blue and green, respectively, acquired with a 20 × 20 µm raster. This image contained 181,723 pixels. (b) Enlarged region showing the complementary distributions of these ions in the cerebellum. The corresponding H&E-stained section is shown on the right. Reproduced from Reference 1 with permission; © 2015 John Wiley & Sons Ltd.

(see Figure 2) yields data at around 10–100 GB per tissue. In this particular case the raw data stream is made up of over 181,000 individual mass spectra measured at a resolution of 20 µm. Of course such advancements open the way to analysis of large tissue cohorts for clinical studies. In this respect TBs of raw data are expected which must be treated carefully along with the confidential, associated patient data.

Another area of large data production and analysis in this group at Maastricht is from the team working on developing the medical applications of the Waters iKnife Rapid Evaporative Ionisation Mass Spectrometry (REIMS) systems and their associated databases. This system allows for molecular analysis of surgically removed tissue in real-time during the cutting process by collecting the smoke produced and introducing it into a mass spectrometer (in this case a Xevo system from Waters). It relies heavily on the generation and access to tissue and disease-specific databases that are compared to the molecular profile of the tissue in contact with the surgical knife. It thus provides real-time feedback to surgeons as to the type of tissue they are cutting and allows the differentiation of tumorous and healthy tissue. This critical information, based on a series of collected mass spectra, helps ensure all tumorous tissue is removed and minimises the need for follow-up surgery.

Weathering the storm—data handling infrastructure

In order to efficiently master the in-house data tsunami and to provide the researchers with the opportunity of actually interpreting the data volumes and converting them into knowledge (with the associated publications of course!) the following infrastructure has been put in place (see Figure 3).

The demands on that IT-infrastructure in data handling are two-fold: on one side huge amounts of data have to be stored somewhere (storage space), as the data produced surpasses standard PC storage possibilities. And on the other hand, this amount of data has to be moved in a short amount of time from and to the storage (network speed).

At the M4I, a petabyte centralised storage system from Hitachi Data Systems has been installed which is connected via Gigabit–Ethernet connections to the instruments, data analysis clients and university network. In order to reduce the data transfer rates between the storage and the data analysis units, the mass spectrometry imaging (MSI) data is processed and reduced on the fly during acquisition. The latter can lead to a 100- to 1000-fold reduction, depending on the type of data, enabling acceptable response times for the analysis by the researcher. Also MSI data can benefit tremendously from parallelised processing, as an MSI dataset is a collection of individual mass spectra where each spectrum can be treated separately. Hence, commercial as well as in-house developed software make use of multi-core processing systems. At the Maastricht University there are currently two nodes of 64 cores and each with 512 GB RAM memory available. As a partner in the Dutch Life Science Grid, it is possible to upscale to greater computational power using clusters of other participating centres.

Another important pillar for data analysis, successful interpretation and generation of relevant results, is an IT-infrastructure for the integration of the data with other data. In the context of projects that run in collaboration with the Academic Hospital of Maastricht (AZM), this can be clinical data or other types of data that has been obtained by other techniques from the same sample/patient (e.g. genomic data, MRI scans etc.) Other data can also be meta-data related to the experiment such as instrument settings during data acquisition or the sample preparation protocol. This IT-infrastructure of storage and integration also enables to fulfill the requirements of the FAIR data criteria.

FAIR data

The M4I is, with other Dutch-based research groups, a member of the Dutch Techcentre for Life Sciences (DTL) who are promoters of the FAIR Data approach (http://www.dtls.nl/fair-data/). Long-term readers of this column will have no difficulty in recognising and welcoming the ideals behind the FAIR data approach. As they describe it data should be:

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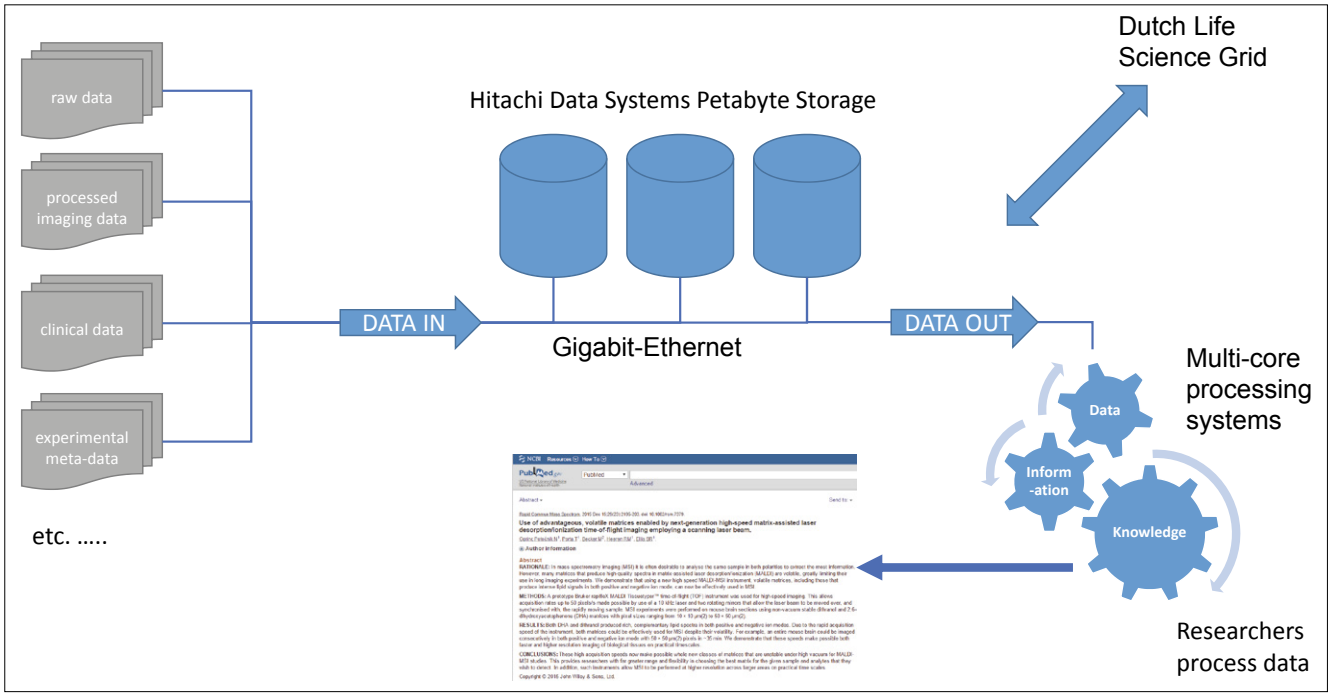

Figure 3. Very rough outline of the data generation to publication pathway at M4I.

- Findable—easy to find by both humans and computer systems and based on mandatory description of the metadata that allow the discovery of interesting datasets;
- Accessible—stored for the long term such that they can be easily accessed and/or downloaded with well-defined license and access conditions (Open Access when possible), whether at the level of metadata or at the level of the actual data content;
- Interoperable—ready to be combined with other datasets by humans as well as computer systems;
- Reusable—ready to be used for future research and to be processed further using computational methods.

As such the DTL is working with similarly interested international bodies on the FAIR Data Stewardship of scientific information (https://www.force11.org/group/fairgroup/fairprinciples).

These lay down exactly what steps an organisation needs to take in order to meet the ideals of the FAIR data approach. This is still work in progress but is very well aligned as general principles for sensible Big Data archiving not only in the bio-spectroscopy fields but for all of us regardless of our specific areas of interest.

To be Findable:

F1. (meta)data are assigned a globally unique and eternally persistent identifier

F2. data are described with rich metadata

F3. (meta)data are registered or indexed in a searchable resource

F4. metadata specify the data identifier

To be Accessible:

A1. (meta)data are retrievable by their identifier using a standardised communications protocol

A1.1. the protocol is open, free and universally implementable

A1.2. the protocol allows for an authentication and authorisation procedure, where necessary

A2. metadata are eternally accessible, even when the data are no longer available

To be Interoperable:

I1. (meta)data use a formal, accessible, shared and broadly applicable language for knowledge representation.

I2. (meta)data use vocabularies that follow FAIR principles

I3. (meta)data include qualified references to other (meta)data

To be Re-usable:

R1. meta(data) have a plurality of accurate and relevant attributes

R1.1. (meta)data are released with a clear and accessible data usage license

R1.2. (meta)data are associated with their provenance

R1.3. (meta)data meet domain-relevant community standards

Conclusions

In conclusion it is very pleasing to see not only significant investment into advanced spectroscopic techniques being made in Europe during economically difficult cycles, but also that the longer-term future of the spectroscopic data is also at the forefront of the minds of those fortunate enough to be receiving this support and a key enabler of their strategy and, we are confident, of their future success.

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QUALITY MATTERS

BERM 14 retrospective: autumn in Maryland, USA

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The 14th Biological and Environmental Reference Material symposium (BERM14) was held at the Gaylord National Resort and Conference Centre, National Harbour, Maryland, USA, from 11 to 15 October 2015. It was, based on the feedback received, a resounding success, both scientifically and socially. The weather was perfect and the location, well, impressive as was the local organisation.

Before looking at the BERM 14 Meeting highlights it is important to put BERM into context: it is the original symposium looking at biological and environmental reference materials. The first meeting, then known as "BRM" was organised by Dr Wayne Wolf and held in Philadelphia, USA, in September 1983: 26 years ago! It was a delight to see that Wayne was part of BERM 14, supporting AOAC with their exhibition booth. Another BERM stalwart, Dr Stephen Wise, was present in his capacity of Scientific Chair. Steve told me that he had attended the early BRM meetings as a (then) young NIST Scientist. Steve's enduring support for BERM was marked by the presentation of an Award Shield by Hendrik Emons, on behalf of everyone who attended BERM Symposia over the years.

Since then, BERM meetings have alternated between Europe and North America, with a few variations in that BERM 13 followed BERM 12 in Europe. So after a gap of nine years it was high time BERM returned to the USA. The last meeting held in the USA, BERM 10, took place between 30 April and 5 May 2006 and attracted close to 200 delegates. The venue for BERM 14 was chosen to ensure there would be no space restrictions, no need to go outside during the day and with plenty of dining and social options in the local area. Feedback from attendees was universally positive and the organisers can be congratulated for a job very well done indeed!

Dr Stephen Wise, from NIST and the meeting Scientific Chair, together with his wife proudly displaying the plaque given to them to mark more than 30 years involvement with the BERM series of symposia.

Back in 1983, that really is 32 years ago, just 25 people shared 16 presentations. Indeed, back then the idea of the application of sound chemical metrology principles to biological matrices was somewhat novel. For BERM 14 the ever increasing level of interest in reference materials fuelled interest: key features from BERM 14 include:

- 281 attendees: a new record
  - NMIs, government agencies, commercial providers, non-profits, accreditation bodies, users of reference materials
  - Scientists from 27 countries attended
- 78 invited and contributed oral presentations in 14 sessions
- 108 posters
- Almost four full days of presentations, with parallel sessions in the morning and afternoon, ran each side of 21 plenary and keynote speakers who helped set the tone for the following oral presentations.
  - In total 99 oral presentations
- Two poster sessions allowed 108 posters to be presented to the delegates.
- Twenty-three organisations, most of them producers of CRMs and or PT supported the event by sponsoring a range of opportunities.

Over time the biological aspect of BERM has grown, and this time was no different. Indeed with six main sessions looking at all aspects of metrology and reference materials in biological systems there was much to challenge a traditional analytical chemist.

Notable sessions included:

Reference Materials for Biosimilars, Pharmaceuticals and Bioanalysis, kicked off by a challenging Keynote talk by Bary Cherney from Amgen.

BERM delegates winding down in the roof top bar after a gruelling day in scientific sessions.

The BERM 14 programme demanded parallel sessions: the photo shows a speaker from ANAB talking about the development of ISO 17034.

CRM Developments for Clinical Analyses which included two fascinating talks by Hongmei Li and Liuxing Feng from the National Institute of Metrology in China looking at the application of "hyphenated" analytical techniques such as HPLC-ICP-MS and IDMS to some challenging bio molecules.

Commutability of Clinical CRMs, lead by Ingrid Zegers from IRMM, looked an area which is going to get more and more important as the demand for and use of CRMs in clinical and biological metrology increases.

CRM Developments for Food & Dietary Supplements got off to a wonderfully informative and amusing start with a plenary talk by Mark Blumenthal from the American Botanical Council in which he highlighted the many challenges associated with bringing good analytical metrology to a rapidly evolving and creatively marketed sector of the health care industry.

Reference Materials and Microbiology was kicked off by Raymond Cypess from ATCC who showed just how CRMs and best practices are essential to close the reproducibility gap that so often appears in biological measurements.

Confidence in Identification for Preparation of Biological Reference Materials asked, in a number of different ways, the fundamental question: how do you know for certain what you are measuring?

The meeting was brought to a close by a thoughtful and final plenary session by Dr Derek Craston, who holds the position of Government Chemist to the United Kingdom Government, effectively the "Supreme Court" for metrology disputes between the UK Government and UK Business! With the weight of such responsibility Derek has some very clear ideas about the need for and use of CRMs!

So, BERM 14 is over: BERM 15 is due to take place during June in 2018 and will be in Berlin, Germany, hosted by BAM and supported by IRMM. Dr Ulrich Panne, a long-time supporter of BERM, will be the Symposium Chair.

And so it is almost over: just time to look forward to BERM 15, which will be in Berlin in 2018.

SAMPLING COLUMN

Sampling quality assessment: the replication experiment

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This column gives an overview of an issue that has not received proper attention for decades, the issue of "replication". This issue turns out to be complex and there has been a lot of confusion in the literature. Three answers to what is often stated in response to the fundamental question: "what is replicated exactly?" are i) replicate samples, ii) replicate measurements or iii) replicate analysis (replicate analytical results). Upon reflection it is clear that these three answers are not identical. The often only implied understanding for all three cases is that a beneficial averaging is carried out with the connotation that important insight can be gained by "replication". By replicating the specific process behind replicated samples, measurements and results, some measure of variability is obtained; but a measure of what? There are many vague prerequisites and imprecise assumptions involved, which need careful analysis. For starters, i) addresses the **pre**-laboratory realm, while ii) and iii) play out their role **in** the analytical laboratory—but even here: are replicate analysis the same as replicate measurements?

Background

From the discipline of design of experiments (DOE) comes a strict conceptual understanding and terminology because of the controlled surrounding conditions. In the situation of chemical synthesis influenced by several experimental factors, temperature, pressure, concentration of co-factors for example, it is easy to understand what a replicate experiment means: one is to repeat the experimental run(s) under *identical* conditions for all controllable factors, taking care to randomise all other factors, in which case the variance of the repeated outcome, be it small or large, will furnish a measure of the "total experimental uncertainty", which will be larger than the strict analytical **repeatability**. In routine operations in the analytical laboratory, variability also reflects effects from other uncertainty contributions stemming, for example, from small-scale sampling of reactants involved, which may not necessarily represent completely "homogeneous stocks". Added uncertainty contributions may also occur from resetting the experimental setup—to what precision can one "reset" temperature, pressure, concentration levels of co-factor chemical species after having turned the setup off and cleaned all the experimental equipment? Still, such uncertainty contributions are usually considered acceptable parts of the total analytical error (TAE). Often all of the above turn out to be of small, or vanishing, effect because of the regular conditions surrounding a controlled DOE situation.

Stepping back one step, however, one might find it equally relevant to repeat the experiment by another technician, researcher and/or in another laboratory, enter the well-known analytical concept of **reproducibility**. There may be more, smaller or larger effects in this widened context, and careful empirical total effect estimations must always be carried out in order to arrive at a valid estimate of the augmented, effective TAE.

Behold the whole lot-to-analysis pathway

Below we address more external issues, not always on the traditional agenda for replication, in fact quite often left out, or forgotten.

There are in fact many scenarios that differ from a nicely bracketed DOE situation. Indeed most data sets do not originate exclusively from within the complacent four walls of an analytical laboratory. What will be described below constitutes the opposing end of a full spectrum of possibilities in which the researcher/data analyst must also recognise *significant* sampling, handling and other errors in addition to the effective TAE. The total sampling error (TSE) will include all sampling and mass-reduction error effects, all incurred *before* analysis. It is self-evident that these errors must also be included in realistic analytical error assessments; TAE alone will not give a relevant, valid estimate of the total effective effects influencing the analytical results. We are forced to be able to furnish a valid estimate of the total sampling-handling-analysis uncertainty estimate (GEE: = TSE + TAE).

The description below is supposed to deal comprehensively with the many different manifestations surrounding the replication issue, such that most realistic scenarios are covered. At the heart-of-the-matter is a key question: what is meant by "replicate samples"? This issue will appear more complex than may seem the case at first sight and will receive careful attention w.r.t. definitions and terminology. It will also transpire that this issue is intimately related to *validation* in data analysis, chemometrics and statistics.

Clarification

Upon reflection it will be appreciated that "replication" can concern the following alternatives in the lot-to-aliquot pathway from primary sampling to analytical result:

1. Replication of the primary sampling process
2. Replication starting with the secondary sampling stage (i.e. first mass reduction)
3. Replication starting with the tertiary sampling process (i.e. lab. mass reduction)
4. Replication starting with aliquot preparation (e.g. powder compactification)
5. Replication starting with aliquot instrument presentation (e.g. surface conditioning)
6. Replication of the analysis (measurement operation) only (TAE)

The last option is the situation corresponding to "replicate measurement" in the most restricted case. But does this mean that the analytical aliquot (the vial) stays in the analytical instrument all the time while the analyst simply "presses the button" say 10 times? Possibly; in which case this furthers a strict estimate of TAE *only*. However, it seems equally relevant to extract the vial and insert it in the instrument repeatedly, allowing a possible temperature variation to influence on TAE because this is a more *realistic* repetition of the general work and measurement process in any laboratory than simply leaving the test portion in the instrument. This is a first foray into what is known as "Taguchi thinking",[1] which opens up a focus on potentially influencing factors which are not embedded in the experimental design explicitly. Clearly this kind of external thinking is relevant in many situations and should therefore be included in the replication approach. One important dictum of Taguchi's is: do not necessarily look only for optimal results (which *may* have large variability), but to results where the response variability is low over a large span of the experimental domain (even if less optimal). This is a clever way of gaining more information about the process involved, be this a production or manufacturing process, or the analytical process itself. Certain scepticisms have been voiced regarding the merits of this approach, but we will let the reader decide on this matter.

Opening up for the relevance of this type of *perturbation* of the analytical process, to another analyst it may appear

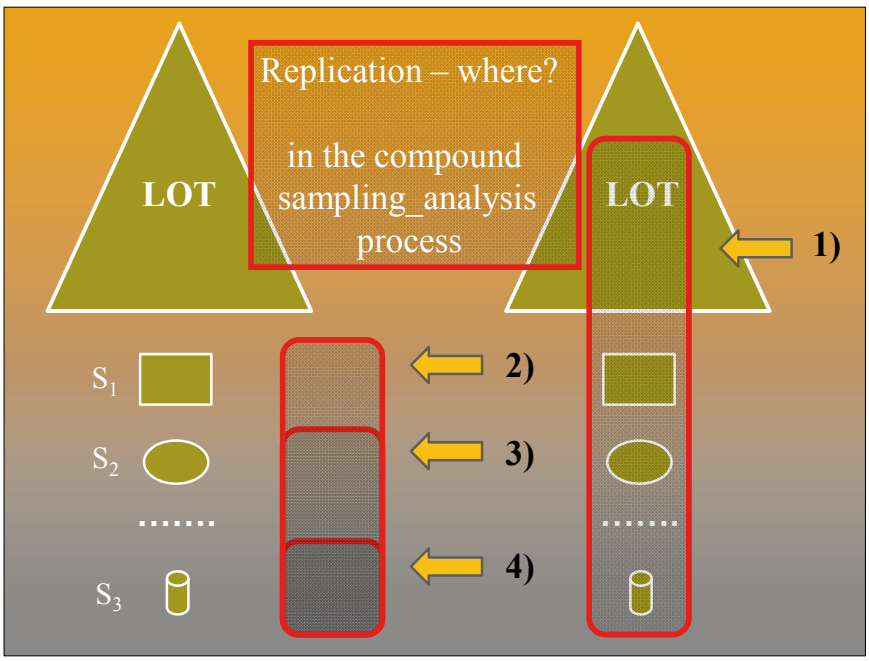

Figure 1. Replication can be performed at many stages in the full lot-to-aliquot pathway, but which is the most realistic situation pertaining to the general operations not **only** in the analytical laboratory? It turns out that all replication must meaningfully start "from the top".

equally reasonable to include some, or all, of the "sample preparation" procedures in the replication as well, because these part-operations cannot necessarily be performed in completely identical fashion. This effect should then also be repeated, say 10 times (stages 4 and/or 5 above) in order to acquire a measure of its variance contribution.

But having broadened the horizon this far, it is a logical step to follow up with still further realistic perturbations of the measurement process, which broadly means including also the tertiary, secondary and in the full measure of things, even also primary sampling errors in the replication concept. Why? Because these are *de facto* uncertainty contributions that will have been in play for any-and-all analytical aliquot, ever subjected to measurement! Following the full impact of the *Theory of Sampling (TOS)* and its detailed treatment of the phenomenon of *heterogeneity*, it is clear that the only complete "sampling-and-analysis" scenario that is guaranteed to include **all** uncertainty contributions must start with replication of the primary sampling ("replication from the top"). Any less comprehensive replication scenario is bound to be incomplete.

Repeating the primary sampling, again say 10 times (preferentially more when needed), means that each of 10 individually sampled primary samples is being subjected to an identical protocol that governs **all** the ensuing subsampling (mass-reduction), sample handling and preparation stages and procedures in the laboratory. From the logic of this full representativity pathway, "from lot-to-analytical aliquot", this is the only procedure incorporating the complete ensemble of uncertainties and errors encountered of whatever nature (sampling, handling, preparation, presentation). The point is that for each replicated primary sample, all potential errors will be manifested *differently* ten individual times giving rise to an accumulated variance which is the most realistic estimate of the **total** measurement uncertainty (MU).[2] In particular this estimate is bound to include the full sampling error effects (TSE), which will often dominate.

In clear contrast, starting at **any** other of the levels in the list above, stages 2–6 will guarantee an incomplete, inferior

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TSE + TAE estimation, which is structurally destined to be too low, i.e. unrealistic.

Should one nevertheless feel compelled to "shortcut" the full replication procedure by not starting "from the top", one is **mandated** to describe the rationale behind this choice and to provide a **full report** of what was in fact done, lest the user of the analytical results has no way of knowing what was implicated in the umbrella term "replication". "Users" and decision makers, acting on the analytical data, do not like to be kept in the dark.

Undocumented or unexplained application of the term "replicate experiments" (or "repeated experiments") has been the source of a significant amount of unnecessary confusion in the past. Many times s^2(TAE) has simply been *misconstrued* to imply s^2(TSE + TAE), a grave error, for which *someone* or *somebody* (or some ill-considered, incomplete protocol) is responsible. But we are here not interested in pointing fingers at any entity (private or legal); it suffices to stop continuing such practice.

The above scenario illustrates an unfortunate responsibility compartmentalisation, which is sometimes found in scientific, industrial, publishing or regulatory contexts:

"The analyst is not supposed to deal with matters *outside* the laboratory (e.g. sampling)"

"This department is *only* charged with the task of reducing the primary sample to manageable proportions, as per codified laboratory's instructions"

"Sampling is automated and carried out by process analytical technology (PAT) sensors; there is no sampling issue involved here"

"I am not responsible for sampling, I only analyse/model the *data*"

... and similar *excuses* for not seeing the complete measurement uncertainty context. All too often the problem belongs to "somebody else", with the unavoidable result that the problem does not receive further attention. Therefore this stand ("not our responsibility") is always potentially in danger of being perpetuated and if so "replicate analysis" will still take its point of departure at stage 3 (maybe stage 2), but never from stage 1, the primary sampling stage. This is not an acceptable situation. There are many occasions in which authors, reviewers and even editors have missed cracking down with the necessary firmness on such demonstrable ambiguities regarding "replication", with the certain result that the reader is not able to understand what was intended, nor what was indeed carried out, because of incomplete descriptions in the "Method" sections of scientific publications and technical reports. The issue is therefore far from trivial, indeed grave errors are continuously being committed. But rather than address the obvious first question: who is responsible, the way forward shall here be constructive. The focus shall be on ways and means to put an effective end to the confusion surrounding the replication issue, and indeed put it to good use instead.

Quantifying total empirical variability—the replication experiment

Above was outlined how a realistic estimate of the *total* TSE + TAE, a replication experiment (RE) must always start "from the top". This is where replication starts, be this primary sampling in nature, in the field, sampling in the industrial plant, or it can be sampling of any target designated as the primary lot (examples follow below).

Figure 2 shows the scenario in which an avid sampler is facing a large lot with the objective of establishing a realistic estimate of the average lot concentration for one (or more) analytes. It is abun-

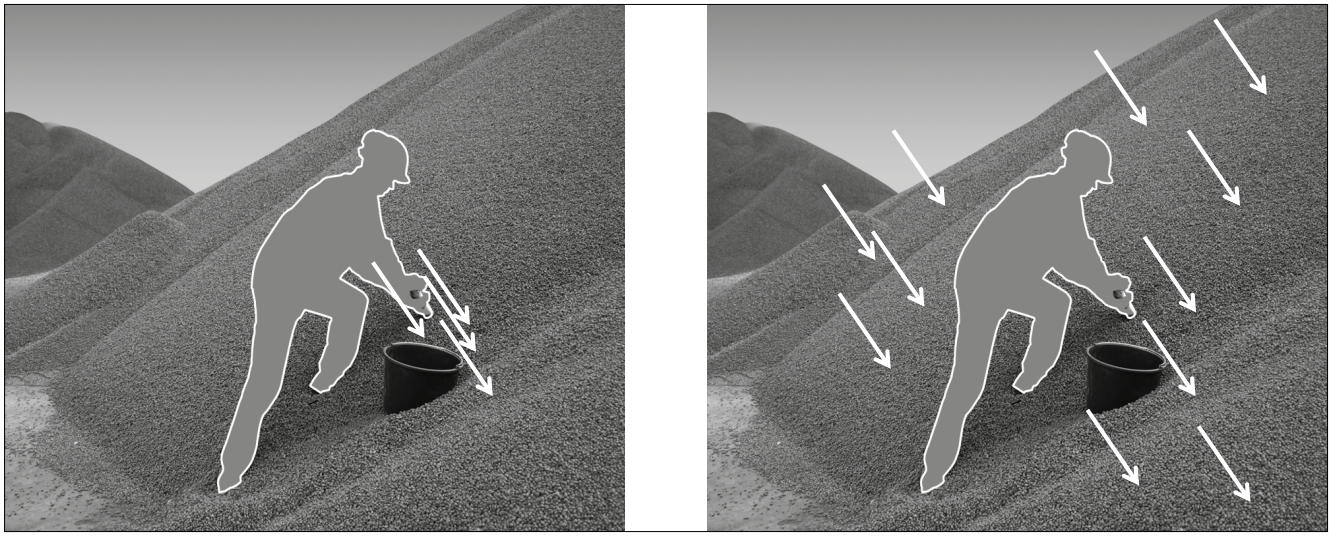

Figure 2. A primary sampler approaching a significantly heterogeneous lot with a grab sampling RE approach, but deployed with two very different coverage footprints. The left side realises the RE on an irrationally narrow footprint in relation to the full geometrical scale of the lot. The right side attempts to take account of the (hidden) lot heterogeneity by employing a wider footprint as a basis for the RE. These alternative scenarios will result in different relative sampling variability estimates because of the different lot heterogeneities covered. (N.B. neither of these primary sampling procedures succeeds to sample the interior of the lot, so both are not honouring the fundamental sampling principle (FSP).

dantly clear that a single grab sample stands no chance of ever being able to do this job because of the intrinsic distributional heterogeneity of the lot. It does not matter whether the lot is small, intermediate or large, the point being that this intrinsic heterogeneity is *unknown* at the moment of routine sampling. The sampler therefore has no other option than to act as if it is significantly large. There is no problem assuming this rational stance, the TOS furnishes all necessary governing principles and practical procedures and equipment assessment possibilities so as always to be able to deal with significant lot heterogeneity, e.g. Esbensen & Julius (2009).[3]

By deploying a RE, Figure 2 (right), the sampler now has access to a first estimate of the effective variability of the sampling procedure, but with TOS it is also clear that there is a grave breach of the fundamental sampling principle (FSP).

Relative sampling variability

It has been found useful to employ a general measure of the sampling variability as expressed by a RE, enter the *RSV*: the relative sampling variability.

The variability of any number of replications can be quantified by extracting and analysing the analytical results from a number of replicate primary samples. These specifically shall have the aim to *cover* the entire spatial geometry of the lot *as best possible*, i.e. spanning the geometrical volume of the primary lot in an optimal fashion (given the circumstances), and calculating the resulting empirical variability based of the resulting analytical results a_S. Often a relatively small number of primary samples may suffice for a first survey, though never less than 10. It is essential that the sampling operations are fully realistic replications of the standard routines, i.e. they shall **not** be extracted at the same general location, Figure 2 (left), which would only result in a *local* characterisation not at all able to relate to the effects of the full lot heterogeneity. What is meant here is that the successive primary sampling events shall take place at other, *equally likely* locations where the primary sampling is to be replicated. The RE shall be carried out by a fixed procedure that specifies precisely how the following sub-sampling, mass reduction and analysis are to be carried out. It is essential that both primary sampling as well as all sub-sampling and mass-reduction stages and sample preparation is replicated in a completely identical fashion in order not to introduce artificial variability in the assessment.

Note that when these stipulations are followed it is possible to conduct a RE for any sampling procedure, for example a grab sampling vs a composite sampling procedure.

It has been found convenient to employ a standard statistic to the results from a RE. The relative coefficient of variation, CV_{rel} is an informative measure of the relative magnitude of the standard deviation (*STD*) in relation to the average (X_{avr}) of the replicated analytical results, expressed as a %:

$$CV_{rel} = \left[\frac{STD}{X_{avr}}\right] \times 100 = RSV \qquad (1)$$

RSV is called the relative sampling variability (or relative sampling standard deviation). *RSV* encompasses all sampling and analytical errors combined as manifested by a minimum 10 times replication of the sampling process being assessed. *RSV* therefore measures the total empirical sampling variance influenced by the specific heterogeneity of the lot material, *as expressed by the current sampling procedure*. This is a crucial understanding. There can be no more relevant summary statistic of the effect of repeating the full lot-to-aliquot pathway procedures (10 or more times) than a RE-based *RSV*.

In the last decade there has been a major discussion in the international sampling community as to the usefulness of a singular, canonical *RSV* threshold; opinions have been diverse. In the last few years a consensus has emerged, however, that *indicates* a general acceptance threshold of 20%. *RSV*s higher than 20% signify a too-high sampling variability, with the consequence that the sampling procedure tested must be improved so as better to counteract the inherent heterogeneity effects in the lot material. Should one elect to accept a *RSV* higher than 20% this shall have to be justified and made public to ensure full transparency for all stakeholders.

The usefulness of the *RSV* measure cannot be underestimated. For *whatever*

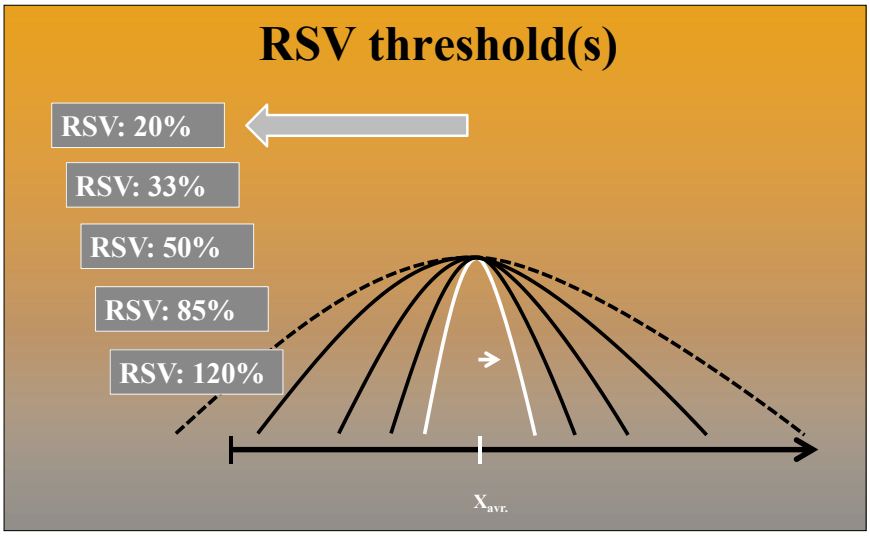

Figure 3. Schematic illustration of replication experiment thresholds *RSV*, e.g. 20%, 33%, 50%, 85% and 120%. Very large relative standard deviations (higher than approximately 85%), when interpreted as representing a standard normal distribution, apparently give rise to negative concentration values. This has no physical meaning, however, and need not cause any untoward worry; these are but model fitting artefacts, of no practical consequence. The essential information for the sampler is manifest already when *RSV* transgresses >20%, i.e. when the sampling procedure is operationally too variable and **must** be improved upon (TOS).

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lot material, sampled by *whatever* procedure, the specific lot/procedure *combination* can be very quickly assessed. There are no untoward practicalities involved which might militate against performing a RE assessment; indeed anybody can perform RE assessment on any sampling procedure, or for any sampling equipment etc. It should never be possible to argue for, or against, a specific sampling procedure without a transparent quantitative assessment. RE numbers speak for themselves. The "difficult" issue of sampling is put on a fully understandable, and very simple operational basis—the RE.

Based on an extensive practical experience over 50 years from many applied sectors and fields within science, technology and industry, there are very many cases on record in which the 20% threshold is exceeded (not infrequently by significant deviations); but there are also an important number of cases in which the existing procedure is vindicated. A few illustrative examples are given below. But first: what information is residing in a simple *RSV* level?

Figure 3 illustrates how *STD* is expressed as a fraction of the general level quantified by X_{avr}. In this illustration the white distribution has a *STD* which is exactly 20% of X_{avr}. Also indicated are cases where the empirical *STD* forms, e.g. 33%, 50%, 85%... The issue clearly is, at what %-level is one no longer comfortable with the quantification resolution, e.g. for *RSV*=50% the signal-to-noise ratio is 1 : 1 only, likely not an acceptable situation under any accounts.

The canonical *RSV* threshold, 20%, serves as a general indication only in the case where *nothing* is known *a priori* as to the heterogeneity of the material involved. Materials and material classes certainly exist that may merit a higher, or a lower, threshold, for which the proposed general *RSV* value can, of course, no longer serve. For such cases, a material-dependent quantification can be developed, dependent upon the sampler's own competence and diligence. The mandate in the sampling standard DS 3077[4] is clear: **all** analytical results shall be accompanied by an appropriate *RSV*, voluntarily described and reported in full.

Figure 4. Examples of replication experiments (RE) that are easily set up. On the left is a dynamic process sampling situation, at the right sampling from a stationary lot. Both sampling scenarios can be assigned an objective *RSV* quality index. In order that no misunderstanding may occur, it is only necessary to perform a proper, calibrating RE **once**, as part of surveying and characterising the intrinsic heterogeneity of a specific lot material.

While it is acceptable to level criticism against the suggested threshold (20%), this also entails the obligation to perform empirical due diligence in the form of a RE. Recent industrial, scientific and technological history is flush with examples of major surprises brought about by such simple replication experiments and their attendant *RSV*. It is either the intrinsic material heterogeneity which is underestimated or, at other times, the sampling procedure turned out to be much less universal than assumed.

The purpose of a RE is often to assess the validity of an already existing sampling procedure. In practice, the RE can only perform and test a current sampling procedure as it *interacts* with a specific lot material. *Should* a *RSV* for this exploratory survey exceed the canonical, or case-specific, threshold, the need for complete fulfilment of the TOS has been documented and is therefore mandated, no exceptions allowed. There may be good reasons to start validation by testing an existing sampling procedure—there is always the *possibility* it may turn out to fall below the pertinent threshold, and thus be acceptable as is. But in all other cases, TOS-modifications must be implemented, no exceptions.

One can therefore view *RSV* as a flexible and relevant sampling procedure *quality index*, scaled with the inherent heterogeneity encountered. *RSV* is particularly useful for initial characterisation of sampling from *stationary lots*, while it is much more customary to use a dynamic, process sampling augmented approach, called *variographics* when sampling from dynamic lots. *RSV* and *variographics* are closely related approaches fundamentally quantifying the same heterogeneity; the latter approach is much more powerful, however, due to the fact of its more elaborate experimental design which allows full decomposition of GEE, see, for example, References 5–8. Variographic heterogeneity characterisation of dynamic lots is the subject of a later column in this series.

All examples described above pertain to issues related to sampling and other error contributions **before** analysis. It is noteworthy that some analytical procedures can have significantly large TAE, e.g. of the order of 10–20% or more,

which is then already factored into the empirical *RSV* level. The principle issues from the few examples given here can be generalised to many other material and lot types. The GEE=TSE+TAE issues are identical for all lot systems.

The following examples illustrate how a specific sampling equipment can be assessed with respect to several different materials (with specific heterogeneities), which may result in both pass and fail.

RE is a general facility that can in fact be deployed at all stages in the lot-to-aliquot pathway, i.e. also a stages later than the primary sampling stage. If the objective were to assess and compare the two splitters in Figure 6 specifically, the RE may well be initiated at this sub-sampling stage directly (in such a case it is of course still critical to add the sampling error effects from the preceding stages in the final evaluation).

The replication experiment (RE) is a powerful and highly versatile sampling/analysis quality assessment facility that can be deployed with great flexibility. It is necessary to be fully specific as to what is meant by "replication" in the situation at hand, i.e. at what stage in the lot-to-analysis pathway is replication to commence. We shall have occasion to employ replication experiments many times in these columns.

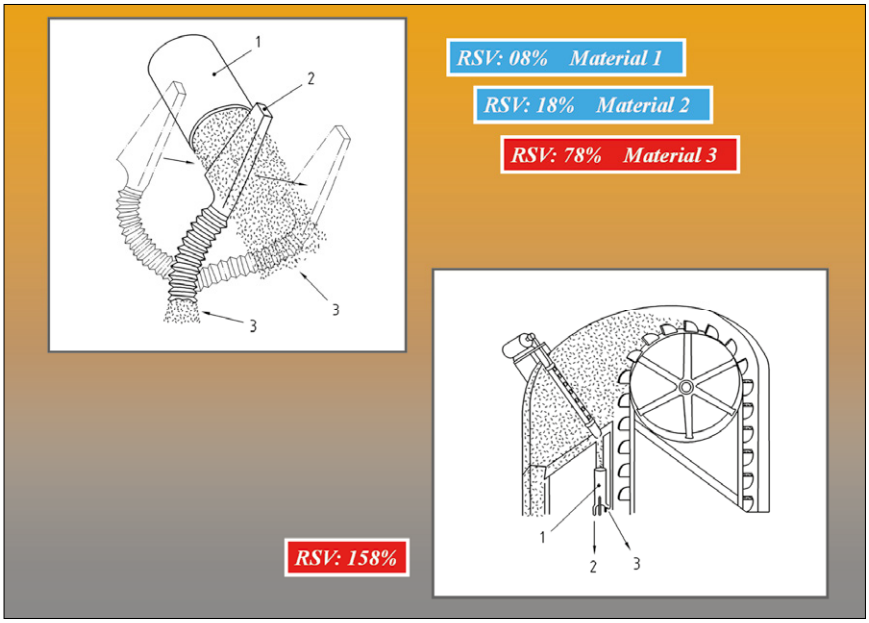

Figure 5. Upper left: primary process sampler assessed for three different materials, one of which does not pass the test of the dedicated RE (*RSV*=78%). Lower right: a complex primary sampler being subjected to a RE with the distinctly worrisome result of *RSV*=158%. N.B. illustrative examples only, no specific sampler is endorsed, nor renounced. Samplers are sketched only in order to illustrate how RE may be used for quantitative assessment.

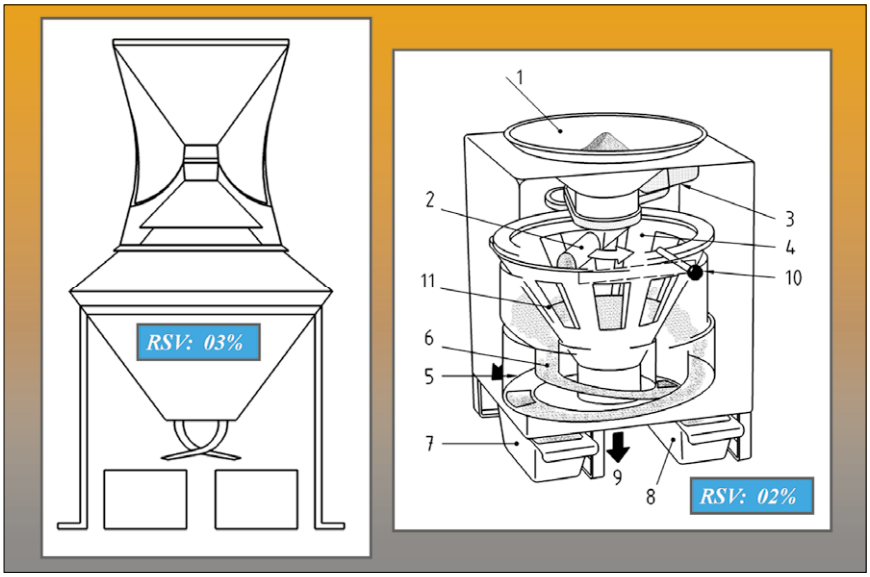

Figure 6. Two laboratory equipment (splitters) subjected to RE assessment, showing highly satisfactory quantitative results. N.B. illustrative examples only, no specific sampler is endorsed, nor renounced. Samplers are sketched only in order to illustrate how RE may be used for quantitative assessment.

Notes and references

1. Taguchi approach: http://en.wikipedia.org/wiki/Taguchi_methods
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Adrok Ltd.

Tel: +44(0)131 555 6662
rgallagher@adrokgroup.com
www.adrokgroup.com

PRODUCT: **Adrok ADR Test Chamber**
APPLICATIONS: Atomic Dielectric Resonance (ADR) for identifying sub-surface geological features ▪ Applications in the oil, gas and water industries.
KEY FEATURES: Adrok provides information on what lies beneath the earth's surface with no need to drill cores ▪ Low energy wave forms penetrate and return data from the interior of materials, rather than gleaning information from the surfaces of solids ▪ Technology differentiates materials based on several parameters, with dielectric permittivity measurement being one of the major functions used for sub-surface mapping

Inorganic Ventures

Tel: +1-540-585-3030
info@inorganicventures.com
inorganicventures.com

PRODUCT: **AACU1**
APPLICATIONS: ICP-MS ▪ ICP-OES ▪ Atomic Absorption Spectroscopy
KEY FEATURES: ISO 9001 ▪ Traceable to NIST ▪ TCT Packaging for longer shelf life

PRODUCT: **AAFE1**
APPLICATIONS: ICP-MS ▪ ICP-OES ▪ Atomic Absorption Spectroscopy
KEY FEATURES: ISO 9001 ▪ Traceable to NIST ▪ TCT Packaging for longer shelf life

LOT-Quantum Design Ltd

Tel: +44 01372 378822
info@lotoriel.co.uk
www.lot-qd.co.uk

PRODUCT: **Dual AR Coated Back-Illuminated CCD Cameras**
APPLICATIONS: Raman spectroscopy ▪ UV/NIR photoluminescence ▪ Multi-track spectroscopy ▪ Fluorescence experiments ▪ Plasma studies
KEY FEATURES: Dual AR coating for increased broadband response ▪ Back-illuminated CCD for high quantum efficiency ▪ TE cooling for low dark current ▪ Deep depleted sensor for NIR fringe suppression ▪ USB 2.0 connectivity

PRODUCT: **High Resolution Shamrock Spectrographs for Atomic Spectroscopy**
APPLICATIONS: Raman spectroscopy ▪ Photoluminescence ▪ Multi-track spectroscopy ▪ Fluorescence experiments ▪ Plasma studies
KEY FEATURES: 0.1 nm spectral resolution ▪ Multi-track capability for optical fibre input ▪ Double detector output ▪ Image astigmatism correction optics ▪ Motorised grating turret

PerkinElmer, Inc.

Tel: 203-925-4602
info@perkinelmer.com
www.perkinelmer.com

PRODUCT: **NexION® 350 ICP-MS**
APPLICATIONS: Environmental monitoring ▪ Food quality/safety testing ▪ USP 232/233/2232 (elemental impurities in pharmaceutical and nutraceuticals) ▪ Speciation analyses ▪ Biomonitoring ▪ Nanomaterials characterisation
KEY FEATURES: World-class data acquisition rates for complete sample characterisation ▪ Unrivaled stability provides increased uptime and unparalleled productivity ▪ Simple operation with new workflow-based Syngistix™ cross platform software ▪ Real-time single nanoparticle acquisition with optional Nano Application Module

PRODUCT NAME: **Optima® 8x00 ICP-OES**
APPLICATIONS: Environmental monitoring ▪ Food quality/safety testing & nutritional elemental analysis ▪ Pharmaceutical & nutraceutical testing ▪ Geochemical/mining ▪ Used oils/lubricants analysis
KEY FEATURES: Flat Plate plasma technology lowers operating and maintenance costs ▪ Dual View for high and low concentrations in the same run ▪ PlasmaCam™ viewing camera aids in method development and productivity ▪ Simple operation with new workflow-based Syngistix™ cross platform software

PRODUCT NAME: **PinAAcle™ 900 AA Spectrometers**
APPLICATIONS: Environmental monitoring ▪ Food quality/safety testing ▪ Biomonitoring
KEY FEATURES: Flame only, furnace only or space-saving designs featuring both ▪ Choice of deuterium or longitudinal Zeeman background correction ▪ TubeView™ color furnace camera simplifies autosampler operation ▪ Simple operation with new workflow-based Syngistix™ cross platform software

PRODUCT NAME: **PinAAcle 500 Flame AA Spectrometer**
APPLICATIONS: Geochemical/mining ▪ Nutritional elemental analysis ▪ Environmental monitoring
KEY FEATURES: Completely corrosion resistant, minimising maintenance and reducing operating costs ▪ Robust and reliable without compromising on performance ▪ Flexible operation through easy-to-use Syngistix Touch™ or more comprehensive Syngistix™ for AA software ▪ Lowest cost-per-element analysis using a FAST Flame-enhanced system

PRODUCT FOCUS

Shimadzu Europa GmbH
Tel: +49 (0) 203-76870
shimadzu@shimadzu.eu
www.shimadzu.eu

SHIMADZU Excellence in Science

PRODUCT: Atomic Absorption Spectrophotometer AA-7000
APPLICATIONS: Environmental ▪ Metallurgy ▪ Food and agriculture ▪ Pharmaceuticals ▪ Petrochemistry
KEY FEATURES: Dual atomiser double-beam optics ▪ Highest sensitivity ▪ Dual background correction functions ▪ Advanced safety technology ▪ Evolving system configuration with smallest footprint

PRODUCT: ICPE-9800 series
APPLICATIONS: Environmental ▪ Food ▪ Water ▪ Pharmaceuticals ▪ Petrochemistry ▪ Quality Control
KEY FEATURES: High-speed simultaneous analysis of multiple elements ▪ Low operating costs ▪ Minimum argon consumption ▪ Fast rinse time ▪ Reduced memory effects increase analytical throughput

Teledyne Leeman Labs
Tel: +1-603-886-8400
leemanlabsinfo@teledyne.com
www.teledyneleemanlabs.com

PRODUCT: Hydra II Series Mercury Analysers
APPLICATIONS: Mercury analysis ▪ Cold vapour atomic absorption (CVAA) ▪ Cold vapour atomic fluorescence (CVAF) ▪ Thermal decomposition ▪ Amalgamation

PRODUCT: Inductively Coupled Plasma (ICP-OES and ICP-AES)
APPLICATIONS: Elemental analysis ▪ Atomic spectroscopy
KEY FEATURES: Advanced solid-stated array detector (L-PAD) ▪ Full wavelength coverage, low stray light optics ▪ Full frame imaging ▪ Radial axial and dual view configurations

NEW PRODUCTS

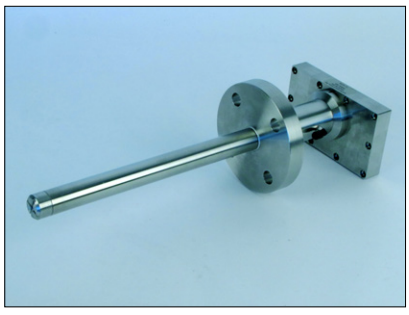
INFRARED

23-mm diamond ATR probe

Axiom Analytical has introduced the DMD-373 diamond ATR probe, which is similar to the existing DMD-370 but in a smaller (23mm) diameter, making it suitable for a wide range of laboratory and on-line process applications. The mid-IR transmission of the new probe exceeds 10%, providing high performance with FT-IR spectrometers with room-temperature detectors. In common with the DMD-370, the new probe features a diamond ATR element, Hastelloy C-276 construction and energised PTFE seals, allowing it to function over wide ranges of temperatures and pressures, and with aggressive chemical systems, including strong acids and bases. The use of metallic internal lightguides provides performance stability and long term reliability.

Axiom Analytical

▸ link.spectroscopyeurope.com/28-01-100



Axiom Analytical's DMD-373 diamond ATR probe is suitable for a wide range of laboratory and on-line applications.

Process ATR probe

Axiom Analytical's new DPR-212 ATR probe offers similar advantages to their DPR-210 ATR probe but in a straight, 30-cm long configuration designed to provide optical transmission of over 20%. The earlier DPR-210 includes a right-angle joint which allows it to be inserted vertically into a sample container when mounted in a traditional spectrometer sample compartment. In contrast, the straight DPR-212 is ideal for use with instruments such as the Bruker Alpha or Thermo-Fisher iS Series which allow the probe to point straight down out of the sample compartment. When used with a more traditional instrument, it can be inserted into a sample vessel through a suitable sealing fitting. In common with the DPR-210, the new probe offers a selection of interchangeable ATR materials, such as ZnSe, ZnS, AMTIR-1 and germanium.

Axiom Analytical

▸ link.spectroscopyeurope.com/28-01-101

MASS SPEC

Single quad GC-MS

Shimadzu has released the GCMS-QP2020, a high-end single quadrupole gas chromatography mass spectrometer. A newly developed, large-capacity, differential exhaust turbomolecular pump enables hydrogen and nitrogen as well as helium to be used

The GCMS-QP2020 from Shimadzu is a high-end single quadrupole GC mass spectrometer.

NEW PRODUCTS

as carrier gas. It also allows switching between ionisation modes without stopping the mass spectrometer. In the GCMS Insight software, the Smart SIM method creation function automatically creates programs to obtain data only during the elution time of the target component, thereby achieving high sensitivity. The system allows simultaneous analysis of more than twice as many components as previous models. In addition, measurements that used to be split into multiple methods can now be integrated into one. The GCMS-QP2020 is available in a number of configurations to suit the components targeted and the phase of the samples. A variety of databases are available including environmental analysis, foods analysis, volatile compound analysis, biological sample analysis and forensic analysis.

Shimadzu

link.spectroscopyeurope.com/28-01-102

Mass spectrometer for bioreactor quantification

The Hiden HPR-40 DSA mass spectrometer systems are designed for monitoring dissolved gaseous content in aqueous solutions. All systems feature a media interface with semi-permeable membrane to enrich the transition of gases and vapours and simultaneously inhibit the transfer of water vapour. Applications include diverse areas of microbiological study including fermentation culture analysis, biofuel research, methane generation, soil core sampling, seawater and freshwater evaluation. The systems enable real-time monitoring of dissolved gaseous species in bioreactor operation and in evolved fermenter off gas, including continuous measurement of CO_2, O_2 and N_2 organic vapours. Interface types include slim immersion probes for static media and circular flow through membrane carriers for flowing media, with choice of membrane and membrane area to optimise sensitivity for specific gaseous species. For photo-responsive studies, cuvette-style vessels are available with integral agitation and capacities ranging from 2 mL to 500 mL.

Hiden Analytical

link.spectroscopyeurope.com/28-01-103

NMR

Reaction monitoring with benchtop NMR

Magritek have released new solutions that enable straightforward reaction monitoring on the Spinsolve benchtop NMR spectrometer. One of the largest growth areas for benchtop NMR is reaction monitoring where it is being broadly applied in chemistry and pharmaceutical sciences. NMR has a range of advantages for reaction monitoring. Spectra

Magritek have released new solutions that enable reaction monitoring on the Spinsolve benchtop NMR spectrometer.

are chemically specific, enabling measurements to be sensitive to molecular structure and reaction kinetics. The signal has a linear response to concentration and it is generally not sensitive to the matrix. Results are representative of the complete sample and the measurement is non-destructive. To enable the Spinsolve spectrometer for reaction monitoring Magritek have created solutions with either glass flow cells or PTFE flow tubing that fit through the completely clear bore of the instrument. The glass flow cell option offers a large sample volume in the sampling region of the spectrometer and a narrow internal diameter outside of that zone. This gives the maximum signal-to-noise output with minimal fluid in the flow system. The hardware kit is completed with a peristaltic pump, appropriate tubing and a stand to provide access below the spectrometer. Complementing the hardware is full experiment scripting capability and pump control available in the latest instrument software. Stop-flow or continuous flow monitoring is straightforward to set up.

Magritek

link.spectroscopyeurope.com/28-01-104

PHOTONICS

Femtosecond fibre laser

TOptica have introduced FemtoFiber ultra NIR, the third generation of their femtosecond fibre laser. It provides pulses of <150 fs at a repetition rate of 80 MHz and with >500 mW output power at a wavelength of 780 nm. The laser has a compact design and does not need water cooling.

TOptica

link.spectroscopyeurope.com/28-01-105

Terahertz systems

TOptica's new time-domain terahertz platform, TeraFlash, and the CW-terahertz spectrometer, TeraScan, both have a peak dynamic range >90 dB. The TeraFlash generates THz spectra with a bandwidth of >5THz, allowing layer thick-

The TeraFlash, TOptica's new time-domain terahertz platform.

ness measurements down to 20 µm. The TeraScan provides frequency resolution better than 5 MHz, which enables precise spectroscopic measurements of trace gases.

TOptica

link.spectroscopyeurope.com/28-01-106

Ultra-compact CW laser modules

Integrated Optics introduced an upgrade to its MatchBox series of ultra-compact CW laser sources for spectroscopy, sorting and illumination. The MatchBox2

The MatchBox series of ultra-compact CW laser sources from Integrated Optics.

series features improved ruggedness, extended operational temperature range, new serial UART interface, laser control software and a smaller footprint. The series of OEM laser sources offers more than 25 different wavelengths often with both SLM (single-longitudinal mode) or non-SLM spectral options. Four options of output types are available: free-space, MM, SM and PM fibre. All types of lasers are offered in the same matchbox-size enclosure, unified +5V DC power input and serial control interface. All lasers with minor exceptions feature more than 500 : 1 polarisation contrast or >20 dB polarisation extinction ratio from a polarisation-maintaining fibre. SLM lasers typically feature around 50 dB side-mode suppression ratio (SMSR) with superior centre wavelength stability of few picometres over the temperature range from 15°C to 35°C. The lasers of the MatchBox2 series are self-contained, however, a number of accessories are offered to make test and installation more convenient, especially in scientific setups.

Integrated Optics

link.spectroscopyeurope.com/28-01-107

High quantum efficiency photocathode

Photonis Netherlands has released a new photocathode designed to provide a combination of extremely low dark counts, fast response time and a high quantum efficiency. Hi-QE Photocathodes are engineered to provide heightened spectral sensitivity in specific wavelength ranges for use in photon-starved applications. The Hi-QE Photocathode increases quantum efficiency (QE) 50% when compared to standard S20 photocathodes, while lowering dark counts as much as 10× with near-symmetrical pulse height distribution. Currently the spectral ranges cover UV, blue and green wavelengths. The new photocathodes are offered as an option to a number of Photonis photon counting products, and the Hi-QE option can also be specified for the Imaging Photon Camera.

Photonis

link.spectroscopyeurope.com/28-01-108

Single photon counter for time-correlated applications

In time-correlated single photon counting (TCSPC), single photons are not only counted but the time of detection is also determined based on a reference signal. Here, a laser pulse generally serves as a reference. This method is a statistical counting method. TCSPC is used in particular in fluorescence lifetime measurements. This method is often compared to a stop watch: a laser pulse excites a sample (time start); just a few pico- or nanoseconds later, a "fluorescence photon" is released (time stop). This time is recorded in a histogram. After many start-stop passes, a conclusive histogram is created that displays the intensity of the fluorescence depending on time. The COUNT T is equipped with an avalanche photodiode (active area of 150 µm) and features a high detection efficiency of >80% and a temporal resolution of up to 350 ps. In addition to fluorescence lifetime measurement (FLIM), the timing module is used in time-resolved fluorescence and single-molecule spectroscopy, as well as LIDAR applications.

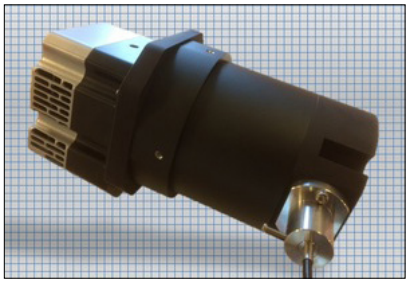
Laser Components

link.spectroscopyeurope.com/28-01-109

RAMAN

OEM Raman spectrometer

The Eagle Raman-S spectrometer platform from Ibsen Photonics is designed for OEM integration into weak intensity Raman solutions at 785 nm or 830 nm excitation. It has low stray light level and



The Eagle Raman-S OEM spectrometer platform from Ibsen Photonics.

high efficiency, and optical resolution as good as 4 cm^{-1} with a wavelength range of 800–1100 nm. It can be equipped with either a –10°C cooled BT-CCD or a –60°C LDC-DD cooled camera.

Ibsen Photonics

link.spectroscopyeurope.com/28-01-110

Raman-AFM system

Renishaw have added Bruker's Dimension Icon atomic force microscope to the range of instruments supported by their inVia confocal Raman microscope. The combined instrument has a flexible arm linking the two instruments, which couples light between them with mirrors. The arm uses Renishaw's StreamLineHR high-resolution mapping technology that can map areas up to 500 µm × 500 µm, with position encoders ensuring 100-nm repeatability. Bruker's PeakForce QNM complements StreamLineHR by providing even higher resolution nano-mechanical information.

Renishaw

link.spectroscopyeurope.com/28-01-111

SAMPLE PREP

Dioxin and PCB cleanup

The GO-xHT clean-up system from Miura Institute of Environmental Science is available in Europe through Shimadzu. The GO-xHT is targetted at dioxins and PCBs analyses of food and feedstuffs. It offers high throughput whilst saving solvents and eliminating cross contamination: 1 mL extracts use less than 100 mL of solvent per sample. Further, this consists of 85 mL of hexane and 2 mL of toluene and no dichloromethane. Three GO-xHT versions are available with two, four or six columns in parallel. The system can be purchased

NEW PRODUCTS

Shimadzu are distributing the GO-xHT clean-up system for dioxins and PCBs.

on its own or packaged with Shimadzu's GCMS-TQ8040 triple quadrupole mass spectrometer, the combination of which fulfills the new EU 589/2014 regulation on analysing dioxins and PCBs.

Shimadzu

link.spectroscopyeurope.com/28-01-112

SOFTWARE

Online chemometrics

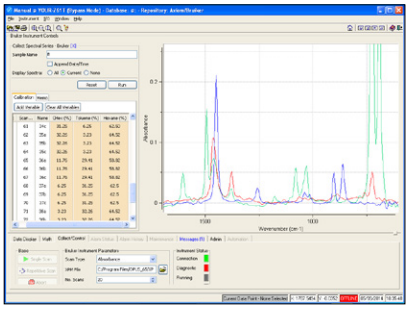
MKS Data Analytics has released SIMCA-online 14, which now has batch evolution OPLS that provides improved interpretation of process monitoring and the evolution level process model. An enhanced Control Advisor works with multiple phases for batch evolution models, while supporting continuous processes, to provide forecasts and advice on how to improve the outcome of the process. An improved interface includes login using Windows credentials through Active Directory, summaries of all configurations into one view, and groupings of continuous data by size and colour for easier and faster visual interpretation. A dedicated simplified workflow assists in updating models and keeping track of configurations and project files.

MKS Data Analytics Solutions

link.spectroscopyeurope.com/28-01-113

Automation of data collection and chemometric model development

Symbion Systems has introduced the EDX Experiment Design and Execution Module. The new module automates the process of spectroscopic data collection and chemometric model development.



The EDX Experiment Design and Execution Module from Symbion Systems.

It allows spectra to be collected using Symbion LX, DX or RX and transferred directly to Symbion QT chemometrics without the need for duplicate manual data entry. During collection, the user can add any number of variables, create a spreadsheet of component values and annotate a memo block for each spectrum. All of this information is transferred intact to Symbion QT to facilitate the building of any number of multivariate calibrations. On deployment, EDX allows the user to add multiple trends based on calculations such as peak height, peak ratio or integral as well as chemometric predictions.

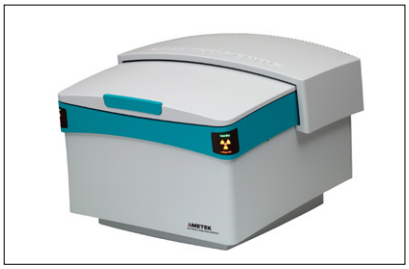
Symbion Systems

link.spectroscopyeurope.com/28-01-114

X-RAY

New line of ED-XRF spectrometers

Spectro Analytical Instruments have introduced a new line of Spectro Xepos spectrometers. They include developments in adaptive excitation, and tube and detector technologies that improve sensitivity (often by 10× or more). The X-ray tubes in the new instruments remain powered on between measurements to prevent on/off variations from affecting readings. This provides long-term stability and precision up to 3× better than before. If speed is prioritised over precision, the new spectrometers can reduce measurement times achieving analyses of most samples within a few minutes. Operating software has been redesigned and the new TurboQuant II software analyses practically any unkown liquid, powder or solid sample.



Spectro have introduced a new line of their Xepos ED-XRF spectrometers.

Spectro Analytical Instruments

link.spectroscopyeurope.com/28-01-115

Very large area CCD for direct X-ray detection

Andor Technology has introduced the new iKon-XL "open-front" ('SO') 16-Megapixel CCD camera platform for vacuum ultraviolet (VUV) and soft X-ray direct detection. This new camera platform is based on back-illuminated CCD231-84 and CCD230-80 sensor variants from e2v delivering up to 95% QE, with deep depletion options for enhanced sensitivity in the 4–10 keV region, down to 2 e^- read noise and up to 350,000 e^- pixel well depth. Its maintenance-free deep-TE cooling down to –75°C minimises sensor dark current and enables the use of long exposure times for weak signal detection, without the need for liquid nitrogen or cryo-coolers. The iKon-XL "SO" is suited for large field-of-view laboratory-based or synchrotron-based X-ray diffraction, X-ray plasma imaging and spectroscopy or X-ray microscopy experiments. New "Extended Dynamic Range" technology allows simultaneous access to the lowest noise and maximum well depth within one scan and is complemented by up to 18-bit digitisation capability. Flexible connectivity is standard through either USB 3.0 or a long distance direct fibre-optic interface. Images can be read out at a range of readout speeds via either single or quad ports, the latter benefiting from the design attention given to electronic stability and image quadrant balancing. The iKon-XL "SO" comes with a standard DN160CF/8" CF/CF-203 rotatable flange and a metal-to-metal knife-edge sealing interface for seamless integration with ultrahigh vacuum chambers.

Andor Technology

link.spectroscopyeurope.com/28-01-116

Clinical applications

Shimadzu has published an application handbook titled *Clinical*. Its near 140 pages cover 47 real life applications including Vitamin D, steroids, immunosuppresants, catecholamines and amino acids analysis, as well as relevant technologies and solutions including chromatography, mass spectrometry, spectroscopy and life science instruments. The book can be downloaded for free from www.shimadzu.eu/clinical.

Shimadzu

link.spectroscopyeurope.com/28-01-120

Steel analysis

Rigaku has published a new application report describing the analysis of low-alloy steel using a benchtop wavelength dispersive X-ray fluorescence (WDXRF) spectrometer. Rigaku Application Note # XRF 1042 details the quantitative analysis of elements in low-alloy steels, with complete information regarding sample preparation, method calibration and repeatability. Alloy steel is amalgamated with various elements to improve its mechanical properties. Steels comprised of up to 8% alloying elements are called low-alloy steels. Low-alloy steels are typically designed to achieve better hardenability. Their mechanical properties are determined by the concentrations of the different elements added to the steel, some of which are at very low levels. The concentrations of elements in molten steel are adjusted during the steel making process, typically in electric furnaces. Rapid analysis of the elemental composition is therefore essential. As part of the control process, analyses of slag and raw materials such as quicklime and ferroalloys are also required. X-ray fluorescence spectrometers are routinely employed due to their rapid analysis capabilities and their ability to measure both bulk metal and powders. For the analysis detailed in the report, certified standard reference materials of low-alloy steel provided by NIST and JSS (Japan Steel Standard) were used to establish the calibration, and measurements were performed using the Supermini200 with a 200 W Pd target X-ray tube.

Rigaku

link.spectroscopyeurope.com/28-01-121

Overcoming matrix effects with XRF

Spectro have published a white paper explaining why overcoming matrix effects associated with X-ray fluorescence (XRF) analysis is critical to achieving consistent high-accuracy results. *Mitigating Matrix Effects with Advanced Spectra-Handling Functionality When Using XRF for High-Accuracy Elemental Analysis* is available to download. A great advantage of energy dispersive X-ray fluorescence (ED-XRF) analysis for rapid screening analysis is its ability to measure samples directly with a minimum of preparation. Realising this benefit, however, requires eliminating potential errors that can result when atoms in the sample matrix influence the fluorescence of others and thus the intensities measured by the spectrometer are influenced. Such effects, which include absorption and enhancement, when taken collectively, are referred to generally as matrix effects. For quality control applications, when the sample matrix is known or can be matched, a variety of standards-based XRF calculation procedures are available to compensate for undesirable matrix effects.

However, creating the right basis for consistently high-accuracy results requires additional spectra handling functionality to determine the correct net intensities of the measured spectra. The new white paper explains why this additional functionality is a critical aspect of overcoming matrix effects and ensuring those consistently high-accuracy results.

Spectro

link.spectroscopyeurope.com/28-01-122

DIARY

Conferences 2016

13–17 March, San Diego, California, USA. **251st American Chemical Society National Meeting.** natimtgs@acs.org, www.chemistry.org.

14–18 March, Baltimore, Maryland, USA. **American Physical Society March Meeting.** www.aps.org/meetings/march.

17 March, York, UK. **RSC NMR Discussion Group (NRMDG) Spring Meeting: *In situ* Monitoring by NMR: What is it all About?** www.nmrdg.org.uk.

20–22 March, Long Beach, California, USA. **OSA Optics and Photonics Congress: High-Brightness Sources and Light-Driven Interactions.** www.osa.org/en-us/osa_org/highbrightnessopc.

21–23 March, Cambridge, UK. **Faraday Discussion: Advanced Vibrational Spectroscopy For Biomedical Applications.** www.rsc.org/events/detail/16963/advanced-vibrational-spectroscopy-for-biomedical-applications-faraday-discussion.

28 March–1 April, Phoenix, Arizona, USA. **Materials Research Society Spring Meeting.** www.mrs.org/spring2016.

31 March–2 April, Eger, Hungary. **European Symposium on Atomic Spectrometry (ESAS 2016).** Ms Beatrix Schenker, c/o Hungarian Chemical Society, H-1015 Budapest, Hattyú u. 16. II/8. Hungary, esas2016@mke.org.hu, www.esas2016.mke.org.hu.

1–6 April, San Diego, California, USA. **Experimental Biology 2016.** eb@faseb.org, http://experimentalbiology.org.

3–7 April, Colchester, UK. **The 49th Annual International Meeting of the ESR Spectroscopy Group of the Royal Society of Chemistry.** Dr Dima Svistunenko, svist@essex.ac.uk, www.esr-group.org/conferences/2016-conference-essex.

4–7 April, Brussels, UK. **SPIE Photonics Europe 2016.** http://spie.org/conferences-and-exhibitions/photonics-europe.

6–7 April, Prague, Czech Republic. **3rd FoodIntegrity Conference.** foodintegrity2016@vscht.cz. www.foodintegrity2016.eu.

10–15 April, Pittsburgh, Pennsylvania, USA. **57th Experimental Nuclear Magnetic Resonance Conference.** www.enc-conference.org.

10–15 April, Oxford, UK. **IBBI 2016–9th Conference on Isolated Biomolecules and Biomolecular Interactions.** www.ibbi-conference.org.

16–19 April, Salt Lake City, Utah, USA. **American Physical Society April Meeting.** www.aps.org/meetings/april.

17–21 April, Baltimore, Maryland, USA. **Photonics Innovations for Advanced Technologies and Applications.** stephaniek@spie.org, http://spie.org/x94875.xml.

18–20 April, Liverpool, UK. **Third International Glow Discharge Spectroscopy Symposium (IGDSS2016).** Peter Robinson, pete@masscare.co.uk, www.ew-gds.com.

18–21 April, Moama, New South Wales, Australia. **17th Australian Near Infrared Spectroscopy Group (ANISG) / New Zealand NIRS Society (NZNIRSS) Conference.** www.anisg.com.au/conference-2016.

19 April, Wotten-under-Edge, UK. **Inside Raman UK Seminar.** www.renishaw.com/en/inside-raman-uk-seminar--18268.

20–25 April, Baltimore, Maryland, USA. **SPIE Next Generation Spectroscopic Technologies IX.** http://spie.org/SIC/conferencedetails/next-generation-spectroscopic-technologies.

22–26 April, Xi'an, China. **11th International Conference Series on Laser-Light and Interactions with Particles (LIP 2016).** Dr Jiajie Wang, lip2016@xidian.edu.cn, http://lip2016.csp.escience.cn.

24–27 April, Gothenburg, Sweden. **European Conference on Nonlinear Optical Spectroscopy (ECONOS 2016).** Stina Hemdal, econos2016@chalmers.se, www.chalmers.se/en/conference/econos2016.

25–28 April, Fort Lauderdale, Florida, USA. **OSA Optics and Photonics Congress: Biomedical Optics.** www.osa.org/en-us/meetings/optics_and_photonics_congresses/biomedical_optics.

2–3 May, Seattle, Washington, USA. **Center for Process Analysis & Control (CPAC) Spring Meeting.** Mel Koch, kochm@uw.edu, http://cpac.apl.washington.edu/event/2016+CPAC+Spring+Meeting.

2–6 May, Lille, France. **European Materials Research Society (E-MRS) 2016 Spring Meeting.** www.european-mrs.com/meetings/2016-spring-meeting.

8–11 May, Bagnols-sur-Cèze, France. **EMAS 2016—12th Regional Workshop on Electron Probe Microanalysis of Materials Today—Practical Aspects.** luc.vantdack@uantwerpen.be, www.microbeamanalysis.eu/events.

9–13 May, Mendoz, Argentina. **International Meeting on Photodynamics and Related Aspects.** http://photodynamics9.wix.com/phd9#!program/cfcg.

9–13 May, Poznan, Poland. **The 24th Annual World Forum on Advanced Materials.** office_polychar24@put.poznan.pl, www.polychar24.divisia.pl.

10–11 May, Monheim, Germany. **Young Investigators in Lipid Science Meeting.** www.dgfett.de/meetings/aktuell/duesseldorf2016/#ort.

13–14 May, Tokyo, Japan. **16th Symposium on Molecular Spectroscopy.** http://regulus.mtrl1.info.hiroshima-cu.ac.jp/~molspec/e-index.html.

14–17 May, Zlatni Pyasatsi, Bulgaria. **Developments and Applications of Solid State NMR to Materials Science, Chemistry and Engineering Conference.** www.zing-conferences.com/conferences/solid-state-nmr.

18–20 May, Lisbon, Portugal. **CEM 2016.** www.cem.uk.com.

19–20 May, Berlin, Germany. **4th International Conference on Advanced Applied Raman Spectroscopy (RamanFest 2016).** http://ramanfest.org.

23–25 May, Ormylia, Chalkidiki, Greece. **12th Biennial International Conference of the Infrared and Raman Users Group (IRUG12).** s.sotiropoulou@artdiagnosis.gr, http://www.irug.org.

25–26 May, London, UK. **Analytical Lab Informatics 2016.** http://massinformatics.co.uk/ali/

25–28 May, Antalya, Turkey. **3rd International Conference on New Trends in Chemometrics and Applications (NTCA 2016).** Dr Remziye Güzel, chemometrics@ankara.edu.tr, http://ntca.ankara.edu.tr.

6–9 June, San Francisco, California, USA. **BIO International Convention (BIO 2016).** exhibit@bio.org, http://convention.bio.org/2016.

5–9 June, Halifax, Nova Scotia, Canada. **99th Canadian Chemistry Conference and Exhibition (CSC 2016).** www.csc2016.ca.

5–10 June, San Jose, California, USA. **Conference on Lasers and Electro-Optics (CLEO).** www.cleoconference.org.

5–9 June, San Antonio, Texas, USA. **64th ASMS Conference on Mass Spectrometry.** office@asms.org, www.asms.org.

5–8 June, Loen, Norway. **8th Nordic Conference on Plasma Spectrochemistry.** yngvar.thomassen@starni.no, www.nordicplasma.com.

6–9 June, St Augustine, Florida, USA. **17th International Workshop on Physical Characterization of Pharmaceutical Solids (IWPCPS-17).** www.assainternational.com/workshops/iwpcps-17.

6–10 June, Barcelona, Spain. **XVI Chemometrics in Analytical Chemistry.** rtaqam@idaea.csic.es, www.cacbarcelona.com.

6–10 June, Berlin, Germany. **7th International Conference on Spectroscopic Ellipsometry (ICSE-7).** http://www.icse-7.de.

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6–9 June, Fort Myers, Florida, USA. **14th Pharmaceutical Powder X-ray Diffraction Symposium (PPXRD-14).** www.icdd.com/ppxrd.

7–10 June, Karlsruhe, Germany. **13th International Conference on the Applications of Magnetic Resonance in Food Science (FOODMR 2016).** Leo Nick, Conference Bureau, Forschungs-Gesellschaft Verfahrens-Technik e.V. Theodor-Heuss-Allee 25, D-60486 Frankfurt am Main, Germany, mrfood@gvt.org, https://mrfood2016.gvt.org.

9–15 June, Novosibirsk, Russia. **12th International GeoRaman Conference.** georaman2016@gmail.com, http://georaman2016.igm.nsc.ru.

12–15 June, Todi, Italy. **6th CMA4CH Mediterraneum Meeting.** http://www.cma4ch.org/index2.html.

19–24 June, Andover, New Hampshire, USA. **Gordon Research Conference on Multiphoton Processes.** www.grc.org/programs.aspx?id=11703.

19–24 June, Ludwigsburg, Germany. **Spectroscopies in Novel Superconductors (SNS 2016).** www.fkf.mpg.de/SNS2016.

19–24 June, Torun, Poland. **23rd International Conference on Spectral Line Shapes.** icsls23@fizyka.umk.pl, http://icsls23.fizyka.umk.pl.

19–24 June, Gothenburg, Sweden. **European Conference on X-Ray Spectrometry (EXRS2016).** EXRS2016, Sweden MEETX AB, SE-412 94 Gothenburg, Sweden, exrs2016@meetx.se, www.exrs2016.se.

20–24 June, Champaign-Urbana, Illinois, USA. **71st International Symposium on Molecular Spectroscopy.** Birgit D. McCall, International Symposium on Molecular Spectroscopy, University of Illinois, 306B Noyes Laboratory, 505 South Mathews Avenue, Urbana, IL 61801, USA. birgit@isms.illinois.edu, http://isms.illinois.edu.

26–29 June, Inuyama, Japan. **7th International Workshop on Plasma Spectroscopy, IPS 2016.** ips2016@plasma.engg.nagoya-u.ac.jp, www.ips2016inuyama.com.

26–30 June, Montreal, Canada. **SPEC 2016.** www.spec2016.com.

27 June–1 July, St Petersburg, Russia. **Laser Optics 2016.** conference2016@laseroptics.ru, www.laseroptics.ru.

29 June–1 July, Groningen, The Netherlands. **8th International Conference on Coherent Multidimensional Spectroscopy (CMDS 2016).** www.cmds2016.org.

3–7 July, Aarhus, Denmark. **EUROMAR 2016.** Prof. Thomas Vosegaard, Interdisciplinary Nanoscience Center and Department of Chemistr, Aarhus University, Denmark, tv@chem.au.dk, http://euromar2016.org.

3–6 July, Chamonix Mont-Blanc, France. **6th International Conference of the International Association for Spectral Imaging (IASIM 2016).** http://iasim16.sciencesconf.org.

4–6 July, Liverpool, UK. **18th Biennial National Atomic Spectroscopy Symposium (18th BNASS).** www.rsc.org/events/detail/19910.

10–14 July, Stony Brook, New York, USA. **14th International Conference on Surface X-ray and Neutron Scattering (SXNS14).** Gretchen Cisco, sxns14@bnl.gov, www.bnl.gov/sxns14.

16–17 July, Biddeford, Maine, USA. **Gordon Research Seminar on Vibrational Spectroscopy.** www.grc.org/programs.aspx?id=16690.

17–22 July, Santa Fe, New Mexico, USA. **OSA Topical Meeting: International Conference on Ultrafast Phenomena.** www.osa.org/en-us/meetings/topical_meetings/international_conference_on_ultrafast_phenomen.

17–22 July, Biddeford, Maine, USA. **Gordon Research Conference on Vibrational Spectroscopy.** www.grc.org/programs.aspx?id=12221.

17–22 July, Breckenridge, Colorado, USA. **58th Annual Rocky Mountain Conference on Magnetic Resonance.** www.rockychem.com.

17–23 July, Sanya, Hainan Island, China. **24th Annual International Conference on Composites/Nano Engineering (ICCE-24).** Professor David Hui, Department of Mechanical Engineering, University of New Orleans, New Orleans, LA 70148, USA, dhui@uno.edu, www.icce-nano.org.

17–21 July, Istanbul, Turkey. **World Polymer Congress (MACRO 2016).** Prof. Yusuf Yagci, y.yagci@macro2016.org, http://macro2016.org/default.asp.

18–22 July, Mauritius. **International Conference on Pure and Applied Chemistry (ICPAC 2016).** icpacmru@gmail.com, http://sites.uom.ac.mu/icpac2016.

19–21 July, Hamburg, Germany. **International Symposium on Environmental Analytical Chemistry (ISEAC39) "Environmental and Food Monitoring".** Prof. Dr Jose Broekaert, University of Hamburg, Institut für Anorganische und Angewandte Chemie, Lehrstuhl für Analytische Chemie heterogener Systeme, Martin-Luther-King Platz 6, 20146 Hamburg, Germany. jose.broekaert@chemie.uni8hamburg.de, www.iaeac.com.

24–28 July, Columbus, Ohio, USA. **Microscopy & Microanalysis 2016.** www.microscopy.org/MandM/2016.

25–27 July, Glasgow, UK. **BSPR 2016 BSR 2016 — Proteomic Approaches to Health and Disease.** Karl Burgess, karl.burgess@glasgow.ac.uk.

25–28 July, Heidelberg, Germany. **OSA Optics and Photonics Congress: Imaging and Applied Optics.** www.osa.org/en-us/meetings/optics_and_photonics_congresses/imaging_and_applied_optics.

30 July–5 August, Chambersburg, Pennsylvania, USA. **18th International Diffuse Reflectance Conference (IDRC 2016).** www.idrc-chambersburg.org.

1–5 August, Rosemont, Illinois, USA. **65th Annual Denver X-ray Conference (2016 DXC).** www.dxcicdd.com.

14–19 August, Fortaleza, Brazil. **25th International Conference on Raman Spectroscopy (ICORS 2016).** www.icors2016.org.

14–19 August, West Dover, Vermont, USA. **Gordon Research Conference on Molecular Structure Elucidation.** www.grc.org/programs.aspx?id=17262.

20–26 August, Toronto, Canada. **21st International Mass Spectrometry Conference.** contact@imsc2016.ca, www.imsc2016.ca.

21–25 August, Philadelphia, Pennsylvania, USA. **American Chemical Society National Fall Meeting & Exposition.** www.acs.org.

21–24 August, Los Angeles, California, USA. **8th Workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing (WHISPERS).** www.ieee-whispers.com.

24–26 August, Reims, France. **14th Biennial HITRAN Database Conference combined with the 13th Atmospheric Spectroscopy Applications (ASA) Meeting.** Maud Rotger, maud.rotger@univ.reims.fr, www.univ-reims.fr/site/evenement/asa-hitran/home-accueil,18642,32042.html.

28 August–1 September, San Diego, California, USA. **SPIE Optics + Photonics.** http://spie.org/x30582.xml.

28 August–2 September, Irkutsk, Russia. **Asia-Pacific EPR/ESR Symposium (APES 2016).** Dr Dmitriy Polovyanenko, apes2016@nioch.nsc.ru, www.apes2016.org.

30 August–3 September, Prague, Czech Republic. **24th International Conference on High Resolution Molecular Spectroscopy (PRAHA2016).** Professor Štepán Urban, University of Chemistry and Technology, Faculty of Chemical Engineering, Technická 5, CZ-16628 Praha 6, Czech Republic, praha16@vscht.cz, www.chem.uni-wuppertal.de/conference.

31 August–2 September, Edinburgh, UK. **Ultra Fast Imaging of Photochemical Dynamics.** www.rsc.org/events/detail/19765.

4–8 September, Torino, Italy. **10th Conference of the European Federation of EPR Groups (EFEPR).** efepr2016@unito.it, www.efepr2016.unito.it.

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11–16 September, Antwerp, Belgium. **5th International Conference on Vibrational Optical Activity.** Prof. Dr Christian Johannessen, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium, voa5@uantwerpen.be, http://www.voa5.org.

11–14 September, Dresden, Germany. **20th European Symposium on Polymer Spectroscopy (ESOPS 20).** www.ipfdd.de/en/events/conferences-and-workshops/20th-european-symposium-on-polymer-spectroscopy-esops.

12–16 September, Chamonix-Mont Blanc, France. **9th International Conference on Laser Induced Breakdown Spectroscopy (LIBS 2016).** Conference office, Université Claude Bernard Lyon 1, Cellule Congrès de l'UCBL, LIBS 2016, 43, bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. contact@libs2016-france.org, www.libs2016-france.org/en.

13–15 September, Eastbourne, UK. **37th Annual Meeting of the British Mass Spectrometry Society (BMSS 2016).** www.bmss.org.uk/meetings.shtml.

18–23 September, Santa Fe, New Mexico, USA. **2016 SciX Conference (formerly FACSS): Annual National Meeting of the Society for Applied Spectroscopy (SAS) / The 43rd Annual North American Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies.** facss@facss.org, www.facss.org.

25–30 September, Copenhagen, Denmark. **1st International Conference on Infrared, Millimeter, and Terahertz Waves (IRMMW-THz 2016).** www.irmmw-thz2016.org.

26–28 September, Ulm, Germany. **13th Confocal Raman Imaging Symposium.** Dr Karin Hollricher, WITec GmbH, Lise-Meitner-Str. 6, 89081 Ulm, Germany, Karin.Hollrichr@witec.de, http://www.witec.de.

12–13 October, Coventry, UK. **Photonex 2016 Exhibition Roadshow & Conference.** www.photonex.org/index.php.

8–9 November, Berlin, Germany. **6th Annual Lab Automation & Robotics (ELA 2016).** https://selectbiosciences.com/conferences/index.aspx?conf=LABAR2016.

14–16 November, Somerset, New Jersey, USA. **Eastern Analytical Symposium and Exposition (EAS 2016).** www.eas.org.

22–25 November, Taipei, Taiwan. **17th International Symposium on Luminescence Spectrometry (ISLS 2016).** www.isls2016.com.tw.

30 November–3 December, Kagoshima, Japan. **ANS2016: The 5th Asian NIR Symposium.** Prof. Satoru Tsuchikawa, Graduate School of Bioagricultural Sciences, Nagoya University, Japan, office@ans2016.org, http://ans2016.org/index.html.

2017

5–10 March, Chicago, Illinois, USA. **The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon 2017).** http://www.pittcon.org.

5–9 June, Copenhagen, Denmark. **ICNIRS 2017.** icnirs2017@mci-group.com, www.icnirs2017.com.

7–10 July, Victoria, Canada. **9th International Symposium on Two-Dimensional Correlation Spectroscopy (2DCOS-9).** www.icavs.org/icavs-9.

11–16 July, Victoria, Canada. **9th International Conference on Advanced Vibrational Spectroscopy (ICAVS-9).** www.icavs.org.

8–13 September, Santa Fe, New Mexico, USA. **2017 SciX Conference (formerly FACSS): Annual National Meeting of the Society for Applied Spectroscopy (SAS) / The 44th Annual North American Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies.** facss@facss.org, www.facss.org.

Courses

2016

29 February–4 March, Gembloux, Belgium. **Annual Spectroscopy and Chemometrics training.** Juan Antonio Fernández Pierna, j.fernandez@cra.wallonie.be, www.cra.wallonie.be/en/events/training-in-vibrational-spectroscopy-and-chemometrics.

5–8 April, Matera, Italy. **European Fourier Transform Mass Spectrometry Workshop.** www.eftms2016.it.

13–16 June, Todi, Italy. **Multivariate Analysis Course, School for Novices.** http://www.cma4ch.org/frames-cou.html.

13–17 June, Warwick, UK. **Interpretation of Infrared and Raman Spectra.** James A. de Haseth, IR Courses, Inc., 165 Sunnybrook Drive, Athens, Georgia 30605-3347, USA, dehaseth@ircourses.org, www.ircourses.org/schedUK.html.

11–15 July, Brunswick, Maine, USA. **Infrared Spectroscopy I: Interpretation of Infrared and Raman Spectra.** www.ircourses.org.

Exhibitions

2016

20–23 March, Dubai, United Arab Emirates. **ARABLAB 2016.** www.arablab.com.

12–14 April, Moscow, Russia. **Analitika 2016.** www.analitikaexpo.com.

25–28 April, Dalian, China. **AnalytiX—Annual Conference and Expo of AnalytiX 2016.** www.bitcongress.com/Analytix2016.

10–13 May, Munich, Germany. **Analytica 2016—25th International Trade Fair for Laboratory Technology, Analysis, Biotechnology and Analytica Conference.** http://www.analytica.de.

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