KRATOS ANALYTICAL
A SHIMADZU GROUP COMPANY

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A little of what we know

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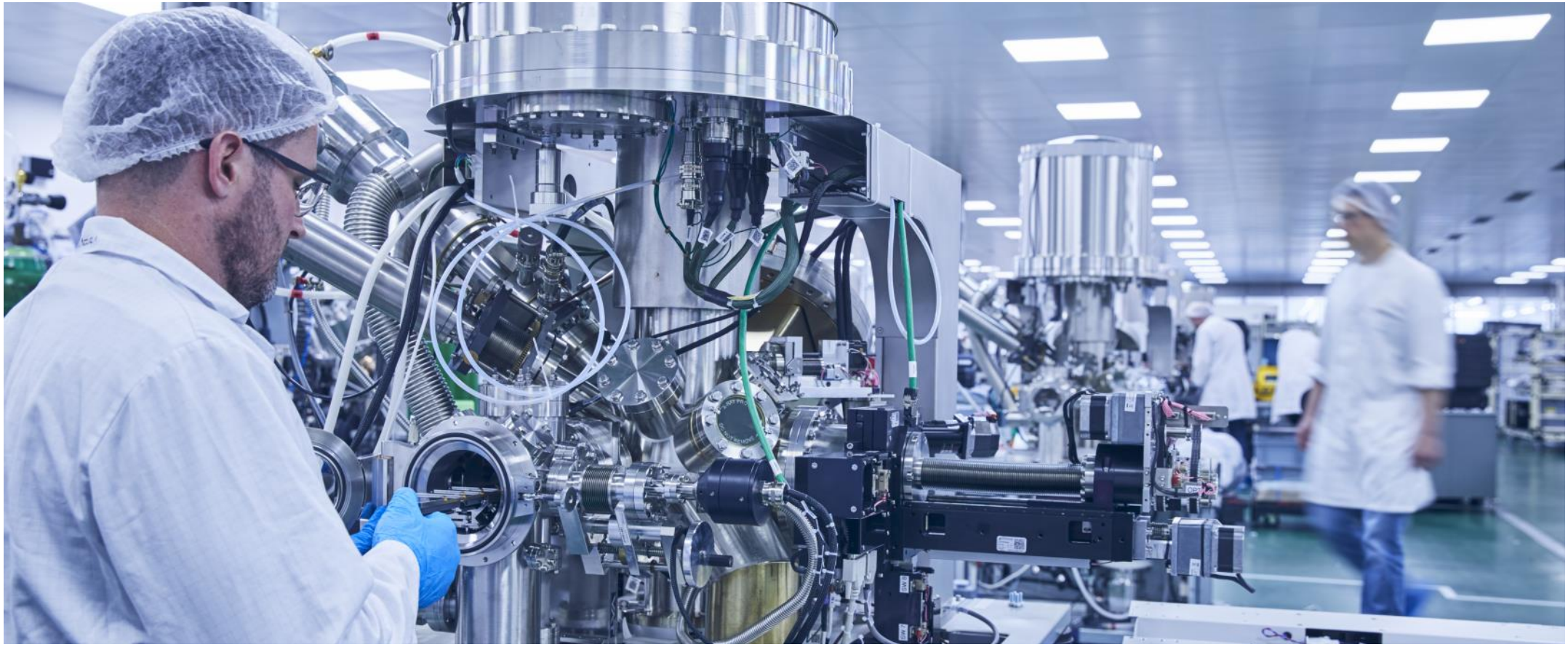
Narrowing the gap between the disciplines

Meet our Users
Dr. Andrey Shchukarev & Dr Madeleine Ramstedt, UMEÅ, Sweden

Looking back at development of Kratos spectrometers
Other Kratos firsts...



WELCOME TO THE AUTUMN KRATOS NEWSLETTER

Focus on Biomaterials

It is said 'Nature is the mother of invention'. There's a lot we can learn by synthesising or emulating nature's use of materials. The use of biomaterials, materials that interact with biological systems, is growing exponentially. So too is the surface analysis of this class of materials. In this addition of the Kratos newsletter we meet two of our Users from Umeå, Sweden who have published papers analysing biological and biomaterial samples. The theme is further explored in an article highlighting narrowing of the gap between biologists and surface analysts.

We look back at some 'Kratos' firsts with pioneering development of Europe's first million volt TEM and the first magnetic sector mass spectrometer built outside the USA.

We have our regular Tips, Tricks and Thoughts from the Applications lab. where we highlight accessories for working with biological and air-sensitive samples. There's also a quick introduction to MALDI-mass spectrometry products which are also designed and manufactured here at our Manchester HQ.

If you have any ideas for articles or there's something you've always wanted to know about Kratos Analytical, please let us know.

Hoping you enjoy the read.

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TIPS, TRICKS AND THOUGHTS FROM THE APPLICATIONS LAB.

Air sensitive samples

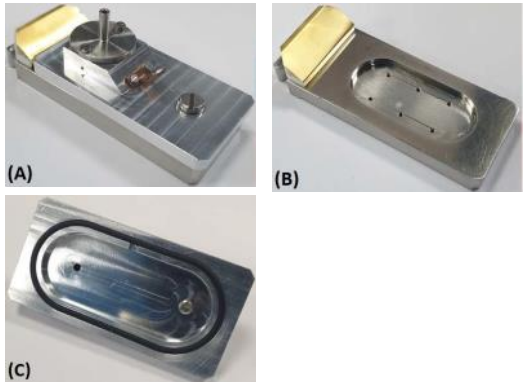
We've seen an increase in requirements for handling of air sensitive samples. Whether it's Li-containing solid electrolyte samples or preparation of frozen hydrated samples for cryo-XPS, these types of samples are linked by the requirement to limit exposure to ambient atmosphere.

An obvious solution is to prepare and mount these types of samples in a purged glove-box. For AXIS Supra/Supra$^+$ Users we can offer a simple dry-nitrogen purged glovebox that fits on the front of the flexi-lock. The flexi-lock chamber to glove box interface was incorporated into the standard design so all AXIS Supra/Supra$^+$ instruments are 'glovebox ready'. This is a simple but very effective solution, providing the capability for working with air sensitive samples.

An example of freezing a high vapour pressure sample prior to pumping is given by the XPS analysis of 1,4-dibromobenzene. Frozen to liquid N_2 temperatures before pumping the entry chamber, it was important that the dry-nitrogen environment prevented ice formation on the sample. Further details of this analysis are provided in applications note MO443(A).

The handling of air sensitive samples is further supported by the air sensitive transporter (part number WX-663). This device provides a solution to transfer a sample from a remote glove box or third party vacuum chamber to the entry chamber of a AXIS photoelectron spectrometer. This device is not pumped but the sample is isolated in the controlled atmosphere during transport. It has facilitated the research of air-sensitive samples at Harwell XPS, the UK's National Facility for XPS.

The air sensitive transporter device has recently been miniaturised into specially designed sample shuttle holders for the AXIS Supra/Supra$^+$, shown in the photographs below. The sample holders work on the principle of evacuating the volume created by placing a removable cover-lid over the samples whilst in the glove box. A small handpump is used to remove gas from the shuttle holder via a non-return valve whilst it is still in the glove box or inert environment. Atmospheric pressure holds the lid in position during transport of the shuttle holder from the glovebox antechamber to the Flexi-lock .



Plain shuttle bar with (A) lid and non-return valve in place, (B) lid removed to shown sample mounting area and (C) underside of lid, showing sealing o-ring.

A powder sample shuttle holder is also available. The powder sample holder is designed to accommodate up to 10 different powder samples pressed into wells. The wells are 1 mm deep with a diameter of 3 mm.

If you are interested to learn more about any of the air sensitive sample handling options please contact us.

MALDI mass spectrometry @ Kratos Analytical

Did you now that as well as developing, manufacturing and supporting X-ray photoelectron spectrometers from our Manchester HQ, we also design and manufacture MALDI-TOF mass spectrometers?

Matrix assisted laser desorption ionization (MALDI) mass spectrometry is a powerful and versatile mass spectrometry technique with a diverse range of applications. In contrast to the SIMS technique that surface analysts are more familiar with, MALDI is a soft ionisation method where a pulsed laser strikes a matrix of laser-energy absorbing small molecules (typically weak organic acids) which is mixed with the analyte material. This has the consequence of transferring the analyte to the gas phase and forming ions without fragmenting or decomposing it. Therefore, this technique is ideally suited to the analysis of biomolecules like peptides, lipids, saccharides and other large organic macromolecules such as antibodies, or synthetic molecules like polymers.

Our Shimadzu colleague, Koichi Tanaka, was honoured with a Nobel Prize in Chemistry in 2002 for developing a novel method for mass spectrometry analyses of biological macromolecules. Tanaka found that by using a mixture of ultra-fine metal powder in glycerol as a matrix, the analyte could be ionised without losing its structure, which became known as soft laser desorption.

At the time of his discovery Koichi was working at Kratos Analytical developing our KOMPACT MALDI series of instruments. Over the following 30 years Kratos has continued to develop MALDI products and provides a range of MALDI-TOF mass spectrometry products, from the ultimate performance of the MALDI-7090 for proteomics and tissue imaging to a benchtop MALDI-8020 for quality control (QC) applications.

The MALDI-7090 (top) and MALDI-8020 (bottom), designed and manufactured in Manchester, UK.

i-work

Interview with an employee

Name Sarah Flood.

Job title Repairs and Returns Supervisor.

How long have you been at Kratos?

8 years.

How would you describe your job to a 5-year-old?

We arrange to get the broken parts fixed for the factory and customers all around the world.

What is the best part of your job?

Every day is different and we get to deal with a wide variety of people from all aspects of the business, suppliers and customers.

Is this what you wanted to do when you were at primary school?

No, I wanted to be a nurse.

How did you end up at Kratos?

I had previously worked in the analytical instrument industry for 15 years in a customer service role before joining the Kratos team.

Your favourite quote / line from a film or book?

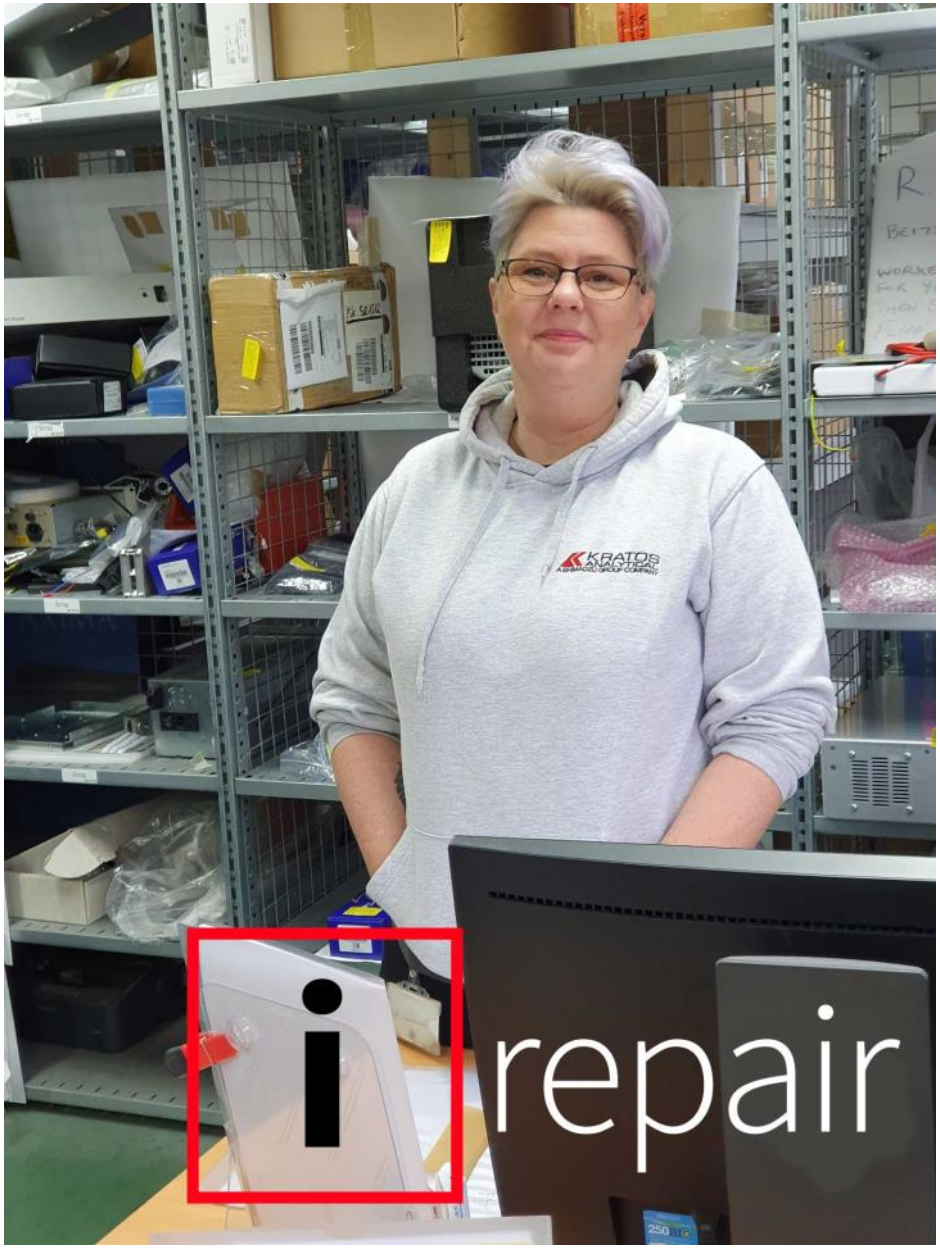
“I tell you those voices soared higher and further than anybody in a grey place dares to dream” - Shawshank Redemption, Stephen King.

What keeps you busy when you’re not at work?

Mainly my teenage daughter!

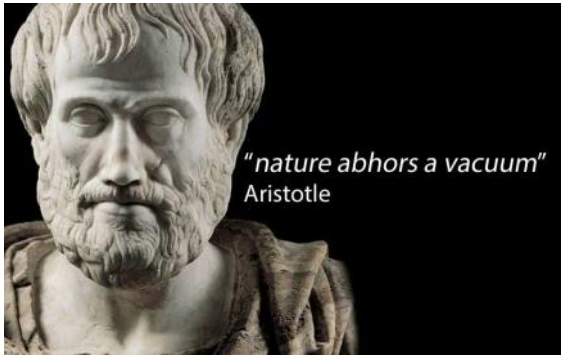
Tell us one thing that we don’t know about you?

I have recently taken up Jujitsu which I love and have just earned my yellow belt. What makes it more fun is it is run by my colleague Don Weatherburn.



BIOLOGY AND SURFACE ANALYISIS

Narrowing the gap between the disciplines



It could be argued that Aristotle's postulate that "nature abhors a vacuum" neatly describes why X-ray photoelectron spectroscopy (XPS) has not found routine application to surface characterisation of biological samples. Biomaterials are expected to operate in a hydrated environment which conflicts with the requirement of an XPS instrument to operate at ultra-high vacuum.

In the late 1980's Buddy Ratner compared a surface analyst's perception of a surface with that of a biologist. He decided that 'on the whole, biologists will not invoke surface-induced effects in their hypotheses. The surface-scientist on the other hand, will consider the problems of biology as being too complex and disorderly to be dealt with using the tools available' [1]. While the gap between the two disciplines remains, there is evidence that it is getting smaller. Biologists understand the importance of the surface of biomaterials, and surface analysis of biologically important surfaces is being undertaken.

Before we go any further, it is worth defining what we mean by biomaterials. A biomaterial is defined as a substance that has been engineered to take a form which alone, or part of a complex system, is used to direct the course of any therapeutic or diagnostic procedure by control of interactions with components of living systems [2]. We know that these materials will interact with a biological system where the bio-devices are implanted into tissues and organs, and it is the outermost surface of these materials that will interact with the biological host. Biomaterial engineering is a rapidly growing field and the importance of understanding the surface chemistry of these materials in controlling their efficacy in-vitro is vital. It may be desirable for the biomaterial to promote cell growth such as a polymer scaffold for bone regeneration. Conversely it might be detrimental to the biomaterial's application, such as biofouling of intubating tubes.

Returning to the theme of surface analysis of biological samples, early approaches were to freeze-dry the sample prior to analysis in UHV, effectively removing the water from the sample. Latterly, successful sample preparation has been developed by fast-freezing which vitrifies the water in the sample conserving spatial structure followed by XPS sample analysis at liquid nitrogen temperature. Another approach to XPS analysis of hydrated samples is the use of a specialised near -ambient pressure XPS (NAP-XPS) instrument. In their recently published paper, Kjaervik *et al.* present an informative 'comparative study of NAP- and cryo- XPS for the investigation of surface chemistry of the bacterial cell-envelope' [3]. The authors conclude that both methods allow for analysis of the hydrated bacterial cell-envelope of intact bacterial cells.

With instruments and sample preparation facilitating XPS analysis of biological and biomaterials, the types of samples analysed is increasing rapidly. It is interesting that XPS is usually just one of the techniques used to characterise a biomaterial. The technique's ability to determine quantitative surface elemental composition as well as chemical state is often applied to confirm the effect of a surface modification or sample preparation step. A good example of this is the confirmation that a conductive polymer has successfully coated a 3D silk foam-based bone tissue scaffold in the work of Hardy *et al.* [4]. This is a fascinating application of XPS in characterising a sample that is being developed so that electrical stimulation of stem cells supported on a bone tissue scaffold may be used to enhance their differentiation toward bone tissue regeneration.

A second example of XPS contributing to an understanding of biological processes is the work of Leggett *et al.* in their publication of slow polymer diffusion on brush-patterned surfaces in aqueous solution. In their work, a patterned polymer surface was used as an analogue of the structure of the cell membrane. XPS was used to confirm the successful steps in patterning the sample to create sub-micrometre scale arrays of "corrals" fabricated using double-exposure interferometric lithography, prior to further study of the structures with fluorescence correlation spectroscopy and contact angle measurements.

A final example of XPS analysis of a biological sample is presented in the excitingly titled paper 'XPS of a living tree'. Although nearly two decades since it was published, it's still striking that XPS was capable of identifying the distribution of elements and the main fibre components (cellulose and lignin) in the wood of a living tree. Shchukarev *et al.* [5] also demonstrated that XPS was able to detect the presence of inorganic elements of biological importance at the wood/bark interface.

It's hoped that these examples, although not a rigorous literature review, demonstrate the application of XPS for surface characterisation of biomaterials and biological samples. Developments in sample preparation, sample handling and modern instrumentation continue to narrow the gap between the disciplines of biologists and surface scientists.

[1] B.D. Ratner, ed Surface characterization of biomaterials, Progress in biomedical engineering, vol. 6 Elsevier, 1988.

[2] Biomaterials Journal Elsevier.

[3] M. Kjærvik, M. Ramstedt, K. Schwibbert, P.M. Dietrich and W.E.S. Unger (2021), Front. Chem. 9:666161, Surface analysis of bio-materials. DOI: 10.3389/fchem.2021.666161

[4] J.G. Hardy, S.A. Geissler, D. Aguilar Jr., M.K. Villancio-Wolter, D.J. Mouser, R.C. Sukhavasi, R.C. Cornelison, L.W. Tien, R.C. Preda, R.S. Hayden, J.K. Chow, L. Nguy, D.L. Kaplan, C.E. Schmidt, Macromol. Biosci., 2015, 15, 1490–1496. DOI: 10.1002/mabi.201500171

[5] A. Shchukarev, B. Sundberg, E. Mellerowicz and P. Persson, Surf. Interface Anal., 2002, 34: 284–288. DOI: 10.1002/sia.1301

MEET OUR USERS

Dr. Madeleine Ramstedt & Dr. Andrey Shchukarev: XPS Platform, UMEÅ, Sweden

What is the role of the XPS platform at Umeå?

The XPS platform at the University of Umeå supports XPS measurements for our university and the whole of Northern Sweden. Our instrument is the only one north of Uppsala. We encourage Users wanting to perform surface analysis to visit us and be involved with the analysis of their samples. Understandably, this has become less possible during the pandemic so we're currently running samples that have been sent to our lab. and discussing the results over the internet.

AS : My experience of XPS started in 1983 so with well over 30 years experience I make the claim that I have probably seen all solid samples in the Universe, even samples of moon rock.

Can you describe a typical day at work/for the instrument?

AS: It is my responsibility to run the instrument and generate data for our Users. I would always start the day with a quick check of the instrument, vacuum, water and computer. A quick visual health-check. Then I would generally start sample analysis. Typically, I leave samples pumping in the load-lock over night so that they have out-gassed and are ready to analyse. There may be some routine maintenance requirements, our instrument is 22 years old, so it needs to be treated with some care and respect. Depending on the number of samples and the type of analysis required a working day can start as early as 7 am and finish at 10 pm at night.

MR: Our cryo experiments result in long working days. There's a lot of preparation of the samples and pre-cooling with liquid nitrogen before we can start the analysis.

AS: Before we start any analysis, I always ask the customer, what do you want to know about the surface of your sample? Probably about 50% of the customers simply want to know the surface composition which requires routine analysis. However, every sample is unique, and the analysis can identify unknowns which allows me to highlight further questions that they may need to consider answering to get a better understanding of their samples. The exciting samples are those that need some consideration. The samples that start with the question 'how are we even going to attempt the analysis of these samples?' are the most engaging.

You never know how living matter such as bacteria, fungi, viruses will behave for surface analysis.

Our early experiences with bacteria were startling. Our collaboration with Laura Leone on the XPS analysis of bacteria involved fast-frozen samples that 'jumped' from the sample holder. The samples didn't have sufficient adhesion once frozen and ended out of the sample holder. As you can imagine this was detrimental to the UHV – a real disaster!

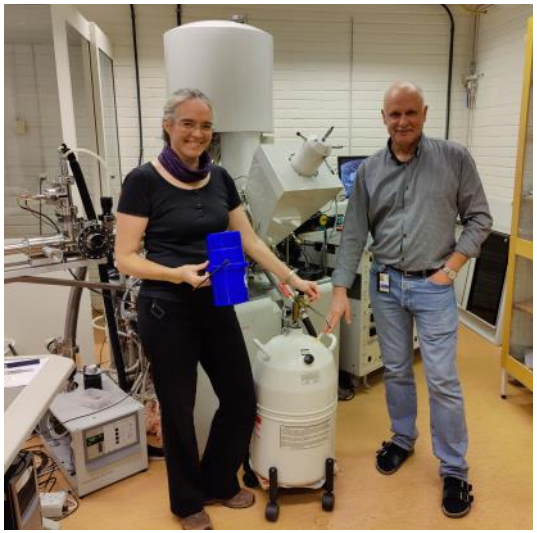
You've developed some novel methods for analysing biological samples.

The bio sample analysis started after we developed cryo-methods for the studies of mineral suspensions. This was driven by an interest to study bio-geo chemistry. We determined that we could interpret the data in terms of the electric double layer and the behaviour of the minerals in suspension. Having worked with these carefully prepared frozen samples, we collaborated, as mentioned previously with Laura to study bacteria surfaces. We undertook a similar approach to the mineral samples, determining the coordination and double electric layer information. As we looked at these types of samples more carefully, it was apparent that we could gain information on the composition of bacteria cell walls. The freezing stabilised the sample, retained the water and was less disruptive than previous freeze-drying techniques. We have also concluded that the cryo-sample preparation is less time consuming and maintains better sample cleanliness than the freeze-dry approach.

Why do you think XPS and surface analysis important in materials development?

MR: There are so many surface-related questions when it comes to biology, it's very exciting to be moving towards analysis of biological samples. Such samples are challenging as they are so dynamic, but we believe that XPS has a role in characterising these the cell interface. Even in the cases where XPS cannot fully answer the questions, it is a useful tool to indicate the need to use further analysis techniques.

AS: Industrial and material developments are moving toward nano scales, so the surface



becomes more and more important and is responsible for the properties of the material. Many chemical questions related to surfaces can be answered by XPS.

What has surface analysis taught you?

AS: I have developed a life philosophy based on surface analysis. Interacting with people is very similar to surface analysis. To get answers from people, you must probe, just as you do with surface analysis.

Any tips or tricks for surface analysts?

Floating the sample, removing the electrical contact to the spectrometer, can solve a lot of problems that can arise due to sample charging during XPS analysis.

Care taken in sample mounting can greatly simplify sample analysis. One approach we have found very helpful for cryo-XPS is to use a holder with a 5-millimetre diameter copper stub and put a metal mesh on which we place the wet sample or indeed drop liquid for cryo-analysis. The mesh structure increases the sample contact area, speeds up the freezing, and 'anchors' it to the holder.

Other Kratos firsts...

Eighteen months ago, we were marking the fiftieth anniversary of the first commercially available **X-ray photoelectron spectrometer**, the ES-100. This instrument was produced by Associated Electrical Industries (AEI), which later became Kratos Analytical. But this was only one of numerous 'firsts' in scientific instrumentation that can be linked to Kratos and our preceding companies, Metropolitan Vickers (MV) and AEI.

In the same year that the XPS instrument was delivered to Durham University, AEI Scientific Apparatus completed the first commercial **one million-volt electron microscope** to be built in Europe for delivery to the Atomic Energy Research Establishment at Harwell [1]. The TEM was massive by conventional standards and extended over three floors. The million-volt accelerator and Cockcroft-Walton generator were housed on the middle floor and the upper floor contained the travelling crane used to dismantle the heavy pressure vessels which contain the high voltage insulating gas.

AEI/Kratos 1 MV transmission electron microscope console.

The photograph (left) shows only the lower floor level containing the microscope console mounted on its heavy anti-vibration block. In principle, the design of the column was similar to that of a conventional instrument except that the high energy of the electron beam necessitates special precautions against leakage of X-rays. Radiation shielding was accomplished by using a large diameter column and installing depleted uranium collars and lead castings at critical points. The final image screen was viewed safely through an eleven inch-thick lead glass window. A true feat of scientific engineering.

Another first in our history was the first commercial **mass spectrometer** built outside the USA, the MS1. In 1944, Metropolitan Vickers received a contract to manufacture four mass spectrometers for the Tube Alloys Directorate, which was responsible for the British development of the atomic bomb (before the project was replaced by the Manhattan project in collaboration with the USA). At the time, MV was already known for engineering innovation, particularly in vacuum technology and high energy physics. The MS1 instruments were 6 inch radius 90° magnetic sector mass spectrometers with an electron impact (EI) ion source and 2 kV accelerating voltage. They were based on the 1940 glass mass spectrograph designs by US physicist, Alfred Nier, and specifically made for measurements of 235/238 Uranium isotope abundance. Pivotal to the design and production of MS1 was Jack Blears, research and development lead at MV, who was keen on mass spectrometry since reading Nier's 1936 paper whilst in a WW2 air-raid shelter! As Jack would later describe 'nothing important was left to chance. At the time, there were no stabilized power supplies, no emission regulators, no suitable electrometer valves, no 10 Mohm resistors and only an engineer's knowledge of chemistry and physics'. The first MS1 delivery was made to James Chadwick in the Physics Department of Liverpool University, in March 1946. Chadwick would later lead the British delegation to the Manhattan project in the US.

MS1 (1946): Analysis of isotope ratios in uranium hexafluoride (UF_6) contained in gas cylinders on the side of the instrument by mathematician Anne Mettrick from MV. This is likely to be the fourth MS1 which remained at MV and was used to develop MS2.

In parallel with developments in electron and ion optics, Kratos also pioneered the application of computers for the acquisition and processing of mass spectral and X-ray photoelectron spectra. In this area Kratos can boast many and fundamental patents, the most fundamental being the **connection of a computer to a mass spectrometer** collector for recording high resolution mass spectrum scans. DS10 was the first commercial mass spectral data acquisition system. Introduced in 1967, it ran on a PDP8 central processor and allowed acquisition of high-resolution scans, calibration, determination of accurate masses and calculation of elemental compositions.

With such rich heritage, it's easy to understand why Kratos continues to lead, not only in the field of surface analysis with our state-of-the-art AXIS Supra$^+$, but also in latest generation mass spectrometries with our MALDI-MS instruments.

[1] Times Newspaper Oct 3rd 1969.