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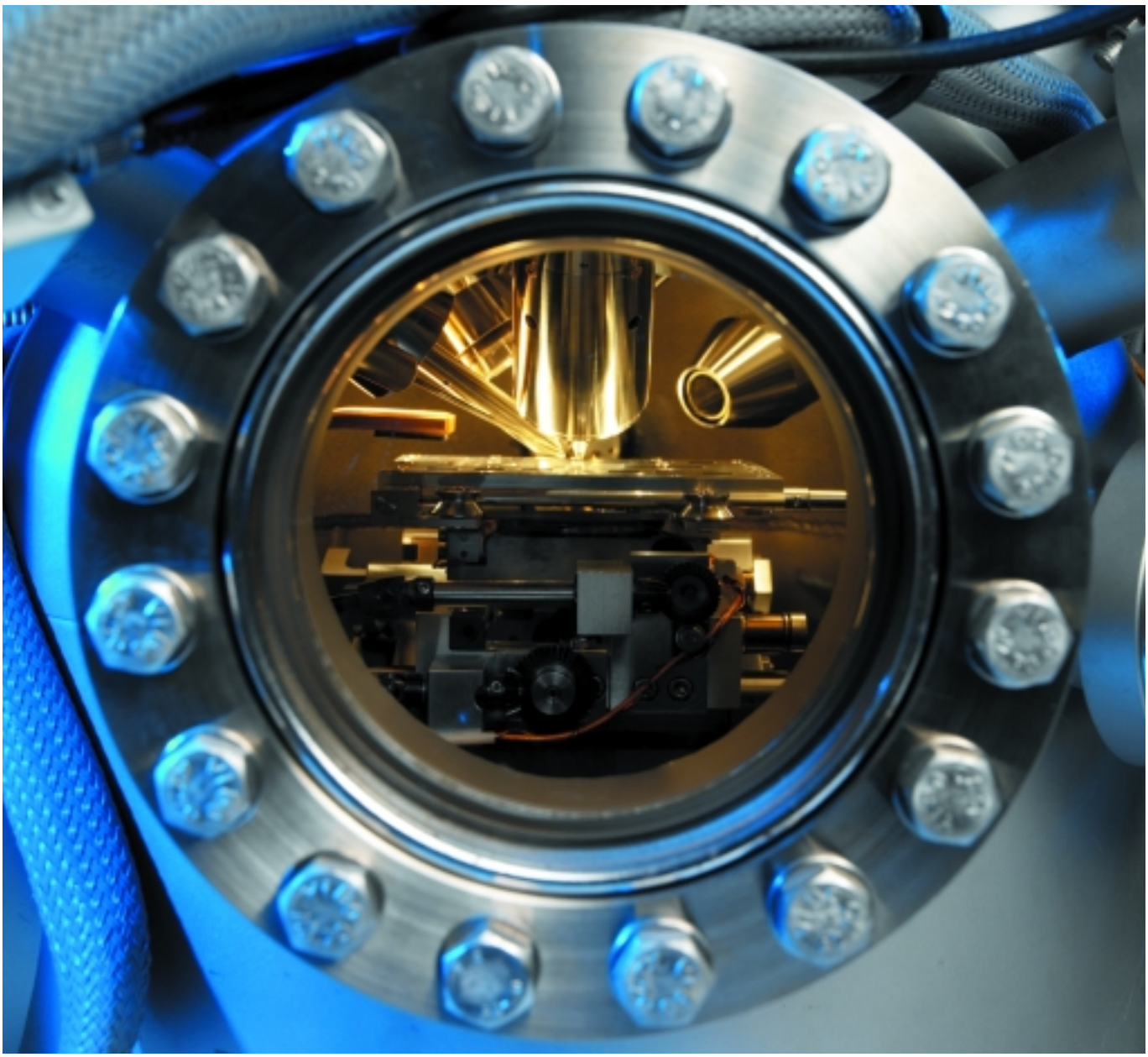

Focus on surface analysis

Uncertainty in sampling

International terminology

Beware of Humpty Dumpty!

VAM
valid analytical measurement

dti
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The DTI VAM programme:

The DTI's Valid Analytical Measurement (VAM) Programme is one of a portfolio of programmes supporting the development of the UK's National Measurement System (NMS). It covers the field of 'analytical' measurements, which are carried out widely by industry. The Programme encompasses both the traditional analytical techniques and newer ones in such areas as the analysis of surfaces at nanometre resolution.

VAM sets out the following six principles, which provide a framework to enable organisations to deliver reliable results first time, every time. Thus organisations achieve bottom line improvements through increased operational efficiency and reduction in risk.

1. Analytical measurements should be made to satisfy an agreed requirement.
2. Analytical measurements should be made using methods and equipment, which have been tested to ensure they are fit for their purpose.
3. Staff making analytical measurements should be both qualified and competent to undertake the task.
4. There should be a regular independent assessment of the technical performance of a laboratory.
5. Analytical measurements made in one location should be consistent with those elsewhere.
6. Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

Front cover photograph by Andrew Brookes

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Industrial applications of surface analysis

Ian Fletcher
ICI PLC

Introduction

The word 'Industrial', like 'Agricultural', is often used in a slightly disparaging manner intended to imply dirty or less complex situations. Yet, modern industrial analytical problems can provide significant challenges, often pushing analysts, instrumentation and techniques to the limits of what is possible.

There are many techniques available that provide chemical and morphological information from the surface regions of a sample. These include AES, UPS, RBS, LEIS, IR/Raman, XRD, XRF, AFM, SEM, TEM, EDX* and Optical Microscopy. This article will focus on the chemical characterisation of surfaces using X-ray Photoelectron Spectroscopy ('XPS', also known as 'ESCA') and Static Secondary Ion Mass Spectrometry ('SSIMS'). These are very surface specific techniques that can provide elemental, chemical and molecular information from the outermost few nanometres of a surface[1, 2]. These two techniques are often used in combination because the information generated tends to be complementary. Key analytical features of these techniques are as follows:

XPS

- Relative Quantification
- Identification of elements (excluding H) and chemistry (i.e. bonding, oxidation states)
- Sampling depth up to ca. 10 nm
- Detects 1 atom in 1000

SSIMS

- Qualitative, relative quantification possible with care
- Identification of molecular species, elements and chemistry
- Sampling depth < 1 nm
- ppm sensitivity

* *See glossary (page 6).*

Ian Fletcher obtained his PhD from Manchester University in 1984. After carrying out postdoctoral research there, he joined VSW Scientific Instruments Ltd. in 1985 and was responsible for test and development of various mass spectrometers, ion guns and XPS and SIMS instrumentation. He joined ICI in 1988 and is currently an ICI Business Scientist.

In recent years Ian's research has centred on surface analysis of coatings and coated materials, composite structures, inorganics, catalysts, polymers and pharmaceutical and biological materials for both ICI and external customers.

His current interest is characterising the distribution of molecules on surfaces and his team uses the highest quality state-of the-art XPS and ToF SSIMS instruments giving 2 microns and 100 nanometre spatial resolution, respectively.

More information may be found at www.measurementscience.co.uk or directly from the author.

Both techniques require high or ultra-high vacuum and can be applied to electrically insulating and conducting samples. Samples can also be analysed at temperatures between approximately -150 °C and +600 °C; low temperatures can facilitate the analysis of vacuum incompatible samples. For example samples with volatile or mobile components and biomaterials can be analysed almost routinely.

These are very surface specific techniques that can provide elemental, chemical and molecular information from the outermost few nanometres of a surface.

Spectra are usually recorded and these allow the identification and quantification of the various species present in the surface region. It is also possible to image using both techniques in order to reveal the 2-dimensional distribution of these species on the surface. The spatial resolution varies with technique and mode of operation but approximately 2 microns and 100 nanometres represent the best achievable with laboratory based equipment by XPS and SSIMS respectively. Both techniques make use of so-called 'Hyperspectral' imaging where each pixel in the image dataset has a spectrum associated with it. This enables spectra to be reconstructed from selected areas within the image and images to be generated from selected peaks in the spectra.

It is also possible to explore the distribution of elements and chemistry, from a few nanometres to several microns down from the surface, by depth profiling using XPS and SIMS techniques. There are several methods available to achieve this aim but it is beyond the scope of this article to go into more detail.

As with most equipment, modern surface analysis instrumentation is now more reliable and user friendly than used to be the case. Sample dimensions can now be significantly larger than the 'traditional' 1 cm square (up to 200-300 mm diameter and 20 mm thick in

some cases). Much improved sample viewing optics are also available that allow relatively easy alignment of the intended analysis area with visible defect areas or specific features on the surface, for example.

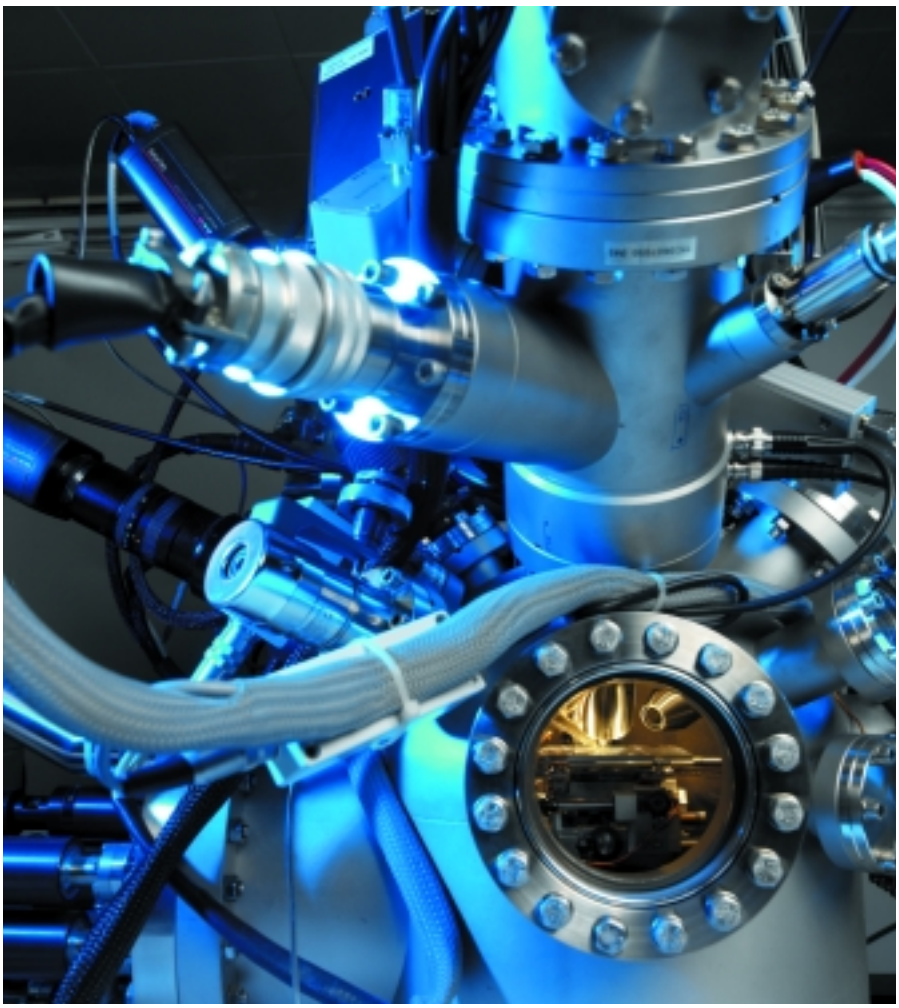
…modern surface analysis instrumentation is now more reliable and user friendly than used to be the case.

The availability of higher resolution, improved transmission and automation equates to better data quality and improved throughput, which benefits the analyst and customer alike. It is now possible to do in an hour or so what used to be impossible or that would take weeks less than 20 years ago.

XPS and SSIMS instruments are still relatively expensive though (around £0.5 - 1M for a fully specified, high-resolution instrument, for example). This is one reason why these techniques are not as widespread as IR spectroscopy, for example. If the volume of work cannot justify 'in-house' capability then outsourcing of surface analysis work to commercial service providers is a possibility.

The Surface Analysis Laboratory in ICI was the first such laboratory in the UK to obtain NAMAS (now UKAS) accreditation, back in 1990, for its XPS and SSIMS activities. Annual audits by UKAS and regular 'in-house' audits keep us on 'the straight and narrow' and also provide a framework for the continual development and improvement of our services. Such accreditation is important when supplying analytical services to ISO 9000 and similar 'quality' organisations where correct analytical results are essential. This is especially important in safety-critical areas such as automotive, aerospace, pharmaceutical, medical and quality control applications. We provide analytical services to internal and external customers throughout the world and our internationally recognised accreditation to ISO/IEC 17025 means that our results are acceptable overseas and also that potential customers have some independent assurance of our performance capability and competence.

Much of our current capability is underpinned by the fundamental work carried out by NPL[3] as part of the VAM



Secondary Ion Mass Spectrometry (SIMS).

Programme. It is reassuring to know that the fundamental issues are being addressed in a systematic and professional manner.

Applications

Practically any surface can be characterised and the applications of XPS and SSIMS are legion. They can be derived from any of the 'usual' generic areas associated with manufacturing:

- Fundamental research
- New product development
- Process development
- Technical service/problem solving
- Patent protection/legal issues
- Quality control
- Safety, Health and Environment

Most 'as-received' surfaces can be analysed along with various buried surfaces and interfaces, for example adhesive failure surfaces, fracture surfaces and various cross-sections. Care needs to be taken with cross sectioning because the cutting and polishing procedures can easily spread materials over the surface of interest.

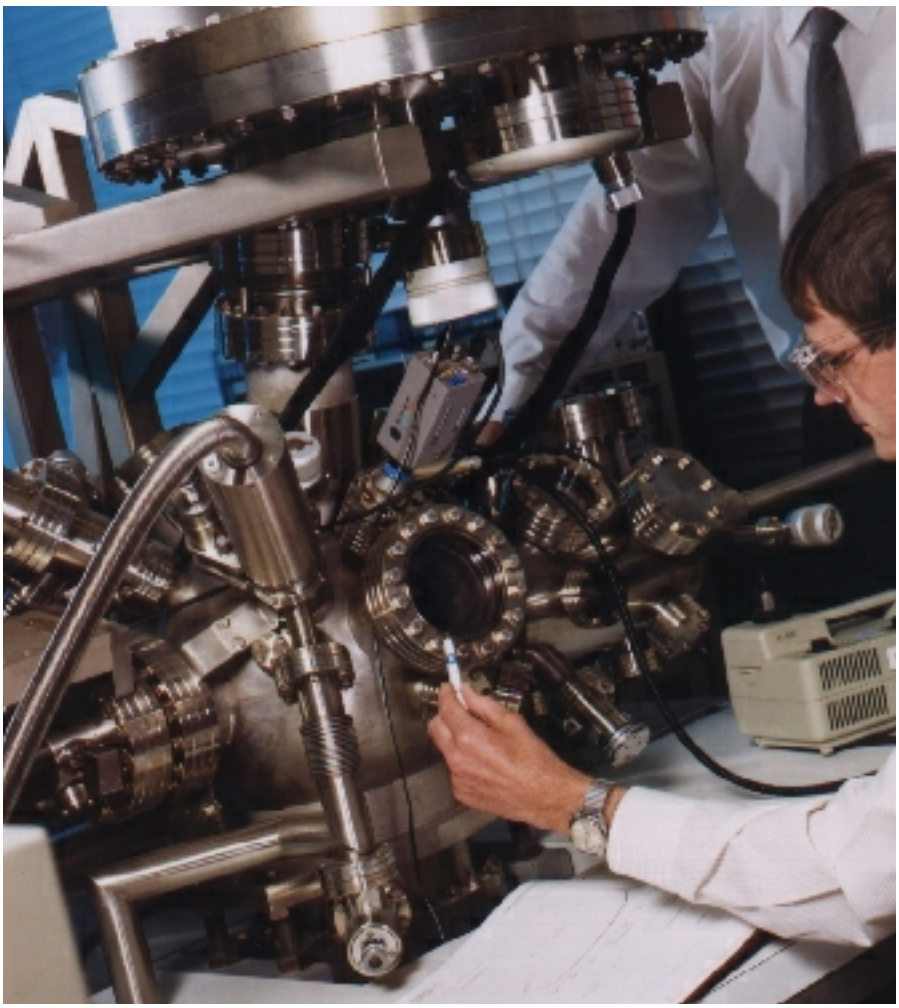
Typical analytical questions asked include specifics, such as:

- Is there any X present?
- How much X is present?
- How is the X distributed? (2-D, 3-D)

And more general questions, such as:

- What is on the surface?
- What is different between samples A and B?
- Why is the adhesion bad?
- Where is it failing?
- Why is it failing?

Throwing every analytical technique at a problem may no longer be possible financially but doing the right things at the right time can be critical to product and process developments in the modern industrial world. This needs informed decision making coupled with a focus on the benefits of the analysis rather than simply the costs of analysis. Surfaces and surface-related



X-ray Photoelectron Spectroscopy (XPS).

effects play major roles in the performance of many products in everyday use. The exploitation and development of these can generate significant income; the consequences of product failure, especially once commercialised, can be very costly indeed.

Keep it clean!

When submitting samples for surface analysis it is usually crucial to preserve the surface of interest and to avoid contaminating it. So why not put the sample into a clean polythene bag? In some cases this will be perfectly fine, but the polythene formulation almost certainly contains slip agents, anti-oxidants and, possibly, UV-stabilisers, which will migrate to the surface. It is possible to identify and quantify such additives at the surface using XPS and SSIMS, and often this is the information required when analysing polymeric systems. However, in this case the additives may transfer on to the sample surface of interest and potentially lead to an incorrect analytical result. Your friendly surface analyst will provide advice regarding the best way to package your particular samples.

High-tech rubbish

Consider if you will, the humble crisp packet! Many modern examples are laminated structures containing around 10 layers including various polymer films, metallised layers, adhesive and printed layers. The laminated structure enables the final product to have good moisture and oxygen barrier properties combined with physical strength and good heat seal and opening peel properties. The adhesion at each interface and at the heat-sealed ends of the bag is critical to the final product performance and significant development work went in to optimise the surface chemistry in these regions in order to perfect the modern bags. These high-performance, high technology devices are then thrown away without a second thought after use!

Same chemistry, different performance

This example is from a PTFE-filled Polycarbonate material for use in lubricant free bearing applications,[4] for example in the rotating parts of PC printers. The material is intended to be self-lubricating over the lifetime of the product with no maintenance being required. It was noted during wear testing against a stainless steel counterface that, after a certain sliding distance, the friction and wear properties of this material dropped to very low levels, clearly ideal for a bearing material. A transfer layer of polymer was known to form on metal counterfaces in such circumstances. However the anomalously low friction and wear properties for this material were not explained by this alone. Analysis of the unworn original and worn polymer surfaces by XPS showed no significant differences in overall chemical composition (i.e. atomic percentages of C, O and F). However, analysis by imaging XPS (Figure 1) showed that the distribution of the PTFE material was different. A PTFE-rich layer around 3 nm thick was found to form on the worn polymer surface. The low friction and wear performance was achieved once this layer and the transfer layer on the metal counterface had formed completely. This interpretation was obvious once the data were available of course!

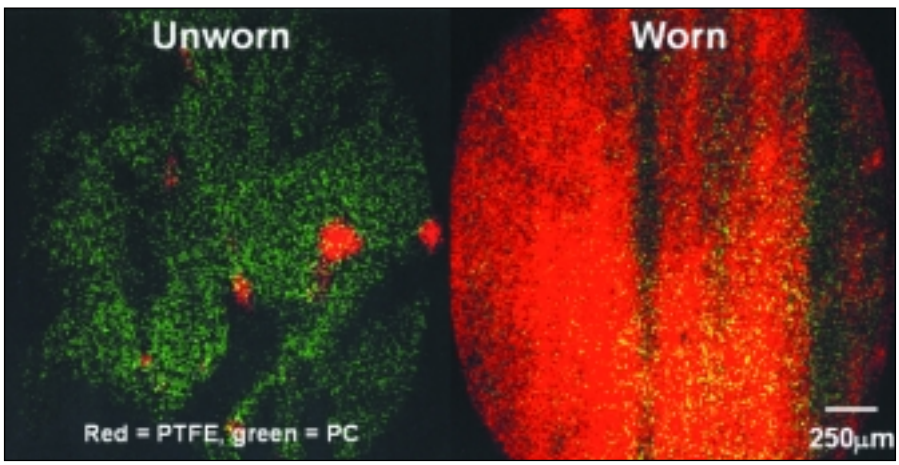


Figure 1: XPS images showing the distribution of PTFE (red) and polycarbonate (green) on unworn and worn polymer test pin surfaces.

Changing the composition of the polymer and the initial distributions of the additives in the blend could then alter the performance of the product. The understanding of such structure/property relationships is often key to successful product developments.

More resolving power, Igor!

Modern time-of-flight ('TOF') SSIMS instruments with so-called 'Reflectron' or sector-based mass spectrometers have reasonably high mass resolution performance with m/Δm of the order of 10000 or so. This is a significant improvement on the unit mass resolution available from quadrupole-based machines and on the 500 mass resolution achieved with early TOF machines.

This enables the resolution of fragment ions with the same nominal mass but different actual mass. Critically it allows the resolution of elemental and inorganic ions from organic species and also goes some way to enabling identification of the atomic composition of a fragment ion from the measured, accurate mass. The latter facilitates spectral interpretation while the former enables analyses that were previously impossible. A classic example of this is in the analysis of spent or poisoned catalysts, which usually have various organic species present on the surface of the inorganic catalyst materials. With poor mass resolution it was generally impossible to identify the presence of any inorganic species unambiguously. For example, the peak due to $^{27}Al^+$ would be obscured by the hydrocarbon fragment ion $C_2H_3^+$, the peak due to $^{56}Fe^+$ would be obscured by the hydrocarbon fragment ion $C_4H_8^+$ and so on. With high mass resolution, most of the surface chemical information is now available to us.

A picture paints a thousand words

Surface treatments are often applied to change particular properties in order to achieve a desired performance. The example in Figure 2 shows the distribution of two 'spin finish' materials applied to polypropylene carpet fibres in order to reduce friction during the carpet-making process. The manufacturers had carried out the usual 'industry standard' tests in order to determine treatment levels and corresponding friction levels. Potential customers would then be exposed to a mass of information, including various graphs and performance tables by persuasive salesmen all saying 'ours is better than theirs'. What matters in this particular case is the chemistry and distribution of the friction-reducing treatment over the surface that is actually rubbing against the machinery. The customers understand this because they have to replace expensive, worn-out parts of their machines on a regular basis. The SSIMS

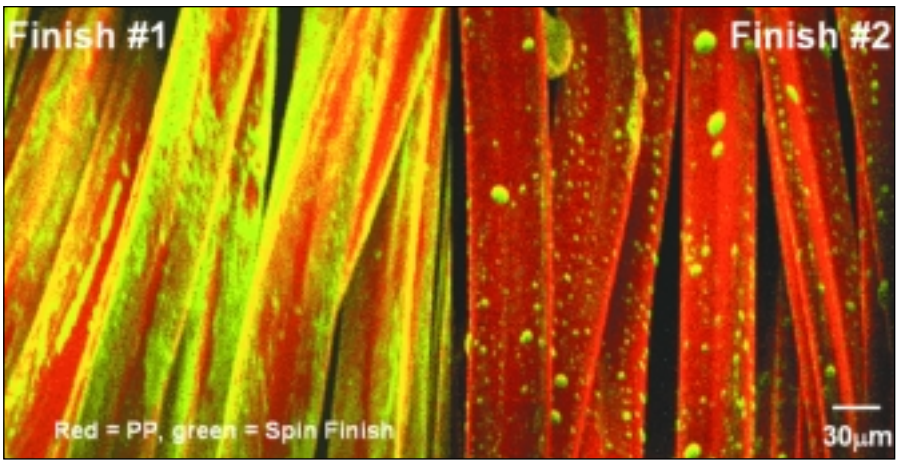


Figure 2: SSIMS images showing the distribution of two friction reducing spin finishes (green) applied to polypropylene carpet fibres (red).

images clearly show significant differences in the distribution of the two treatments, with the ICI Uniqema 'ESC' treatment (finish #1) showing spreading over the majority of the fibre surface and therefore being available to reduce friction. This correlated well with the measured low friction levels for this treatment. The competitive treatment (finish #2), even when applied at five times the level of the 'ESC' treatment, showed significantly higher friction levels. This treatment showed a tendency not to spread over the surface and formed small globules with the majority of the material forming larger globules in the 'valleys' of the tri-lobal fibres. The SSIMS images helped to get the message across and enabled the customer to make the right decision!

Conclusions

XPS and SSIMS should be part of the analytical strategy whenever elemental, chemical and molecular information is required from the outermost few nanometres of a surface. Whilst the costs of surface analysis can appear to be high, the benefits can be enormous and leverages can be as high as 100,000 times the analytical spend where significant existing products are involved. The rewards for new product developments can be even higher in the long term!

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Opinions contained in this article are those of the author and not necessarily those of ICI PLC.

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GLOSSARY

AES Auger Electron Spectroscopy
UPS Ultraviolet Photoelectron Spectroscopy
RBS Rutherford Backscattering Spectrometry
LEIS Low-Energy Ion Scattering spectroscopy
XRD X-Ray Diffraction
XRF X-Ray Fluorescence
AFM Atomic Force Microscopy
SEM Scanning Electron Microscopy
TEM Transmission Electron Microscopy
EDX Energy Dispersive X-ray spectroscopy

To find out more about surface analysis projects funded by the VAM and DTI Measurements for Biotechnology (MfB) programmes, turn to the 'Focus on surface analysis' section on page 17.

If we get it wrong, everyone else is wasting their time

Elaine Hughes
Jayne Arnold
Karen Bishell
and
Esther Farrar
Campden & Chorleywood Food Research Association

Chemical analysis of foods often involves the use of expensive, highly sophisticated instruments in the hands of highly skilled analysts. Ever-more powerful techniques allow the detection of smaller and smaller amounts of substances, with better accuracy and precision, and often in diverse and complex food matrices. With these kinds of developments making the headlines, it is all too easy to forget the importance of sample preparation.

The traditional method of sample handling has been for the analyst to prepare their own samples or at best for an assigned sample receipt officer to receive and book samples on behalf of a laboratory. Our team has developed an extensive sample handling service: from acquisition, through to the provision of sub-samples to analysts in several laboratories, to safe disposal after completion of the work. Centralisation of sample preparation has standardised sample handling, improved methods of recording preliminary data and led to tighter quality control, which can improve precision by minimising the uncertainty associated with sample variability. In addition, the team's approach has led to significant practical and cost efficiencies. The range of activities covered by the team is shown in Figure 1 (page 8).

Sample collection

The team serves several laboratories and often helps with sample acquisition. Although clients often submit samples, some contracts (e.g. surveillance work) require the laboratory to purchase samples from specified types of sources. Tracking down samples can be arduous and requires good knowledge of what can be found where. Team members might travel to the far South West of England or to Scotland to get exactly what is needed – and decide on which particular sample to buy, the pack type and the amount needed. By combining the collection of samples for different projects and laboratories, the buyers have built up specialist market and product knowledge, with economy-of-scale savings in time, costs and the problems of hunting out difficult-to-find samples.

Checking and logging of samples

When a sample arrives, one of the first tasks is to double-check that its condition is appropriate for analysis. This involves inspecting packaging for leakage, the product for obvious spoilage, and the label to ensure that it is within its date-mark, and, in some cases, photographing the sample for

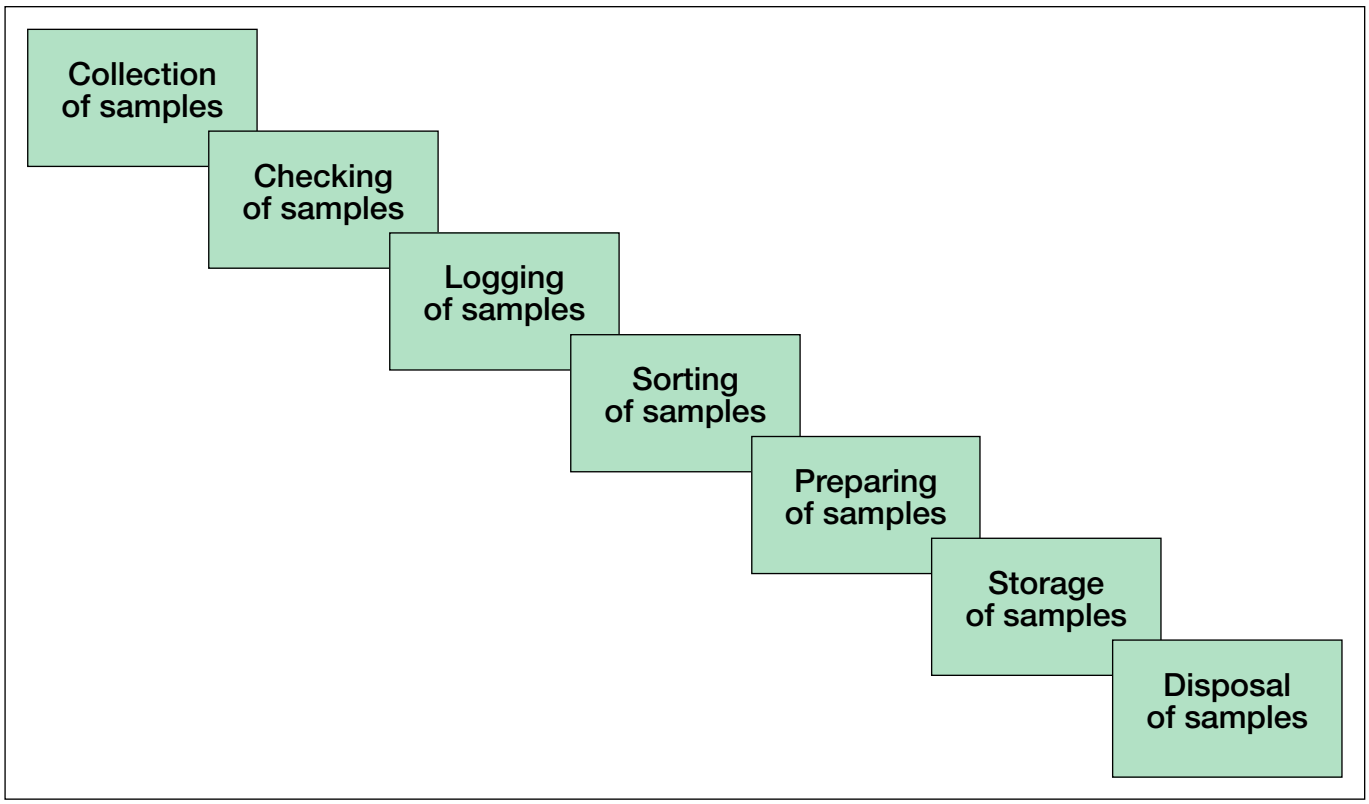


Figure 1: Sample handling activities covered by the team.

client reference. It's also important to check that the sample is representative of the material required for the test and that there is enough for the tests to be carried out. If necessary, the team will liaise with the client to obtain replacement or additional samples. These preliminary checks all save the analyst valuable time.

Standard sample details are recorded on the computerised Laboratory Information Management System (LIMS) which, apart from reducing scope for errors, has improved workload planning, reduced sample wastage, and made the information available to the analyst at the click of mouse.

Sorting and storing samples

Logged samples are sorted and allocated to appropriate secure stores to ensure appropriate conditions, co-ordinated use of storage facilities, and allocation of the right samples for the right tests and analysts. This system also means the most effective use of expensive space, with systematic but efficient quality assurance of the storage facilities (e.g. housekeeping, temperature monitoring, and record keeping). Sample control and a clear chain of custody are established at the earliest stage, so time is not wasted locating samples.

Preparation of samples and preliminary testing

Sample preparation can often most effectively be carried out by the sample handling team – either shortly after receipt of the sample or nearer to the time of full analysis. For example, preparation might involve cooking or preparing samples – often to very specific requirements (e.g. defined temperatures, times, types of cooking oil) to reflect typical domestic practices.

Preliminary testing such as visual assessment, pH and moisture measurement, determination of net sample weights or separating meat components for meat content analysis may also be needed. Many samples are blended before analysis to ensure that the sub-sample is truly representative of the original sample - essential if just a few grams out of several kilograms are to be analysed. Use of a dedicated sample preparation team can improve focus and control over blending and generate more accurate and reliable data as demonstrated by performance in proficiency schemes – to the point where the team now offers this as a service to external clients. The right-first-time approach can also bring about cost and time savings by batching of inspection and preparation work.

Disposal of samples

When all work is complete it is important that samples are disposed of in a safe and secure way. However, samples also need to be retained for specified periods, typically a minimum of three months after reporting, to comply with client requirements and to allow any further investigation that may be needed. It is therefore often necessary to liaise with clients to confirm approval for disposal and to provide disposal lists to check against. With samples centralised and under the control of a single team, the chain of custody and security is enhanced with less chance of sample mix-ups or premature disposal of samples.

Conclusions

This article has, we hope, demonstrated the crucial role that sample preparation plays in analysis. Although the examples given are based on food, the same message applies to all chemical analysis: significant cost and time savings can be made through efficient organisation of sample preparation, but if the sample preparation is not done properly everyone else in the chain is wasting their time.

Sampling as a source of measurement uncertainty – New guidance

Mike Ramsey
University of Sussex

A new Eurachem/Eurolab/CITAC Working Group, and also a new sub-committee of the Analytical Methods Committee (AMC) of the RSC*, have been set up to provide guidance on the estimation of measurement uncertainty arising from sampling. This article aims to briefly explain why uncertainty from sampling is important, and how the working of these new groups will provide various guidance documents (and even a computer game), to help in its estimation and interpretation.

Uncertainty of measurement is already recognised as the key parameter in describing the quality of measurements. What is less often recognised is that the taking of primary samples can be the largest source of this uncertainty, although it is often omitted from the process of estimation. The decision as to whether to include the primary sampling in the estimation of uncertainty depends on how the measurand is defined. The objective of a measurement may be to estimate the concentration of an analyte in a batch of material, such as aflaxotin in a bulk cargo of pistachio nuts, or iron in a batch of copper wirebars. In this case, because the measurand is defined in term of the batch, the measurement process starts with the taking of the primary sample, and all of the steps in the process contribute to the uncertainty in the final measurement, including the primary sampling. There are other situations where the measurand is specifically defined solely in terms of the sample as received by the laboratory, without any reference to how the sample was taken. In that case the primary sampling should not be included in the estimation of uncertainty, and only any sub-sampling that occurred once the sample arrived in the lab should be included.

The inclusion of primary sampling in the measurement process will give an opportunity for measurement scientists to look at the quality of the whole process, rather than just the part that is undertaken in the laboratory. It is often the case that separate organisations actually take the samples, and are responsible for the sampling quality. Sampling quality is usually approached in this cases, by simply recommending the of use of a 'correct' sampling protocol, and by training samplers to apply this protocol 'correctly', without any actual quantitative measure of the quality actually achieved. It is the measurement scientist, however, who makes the measurements that can reveal the real quality of the sampling. If the analytical community takes this opportunity, we can lead the initiative to quantify the quality of sampling, by estimating the uncertainty that it generates. This may often be performed as a service to the organisations that actually take the samples and are responsible for their quality.

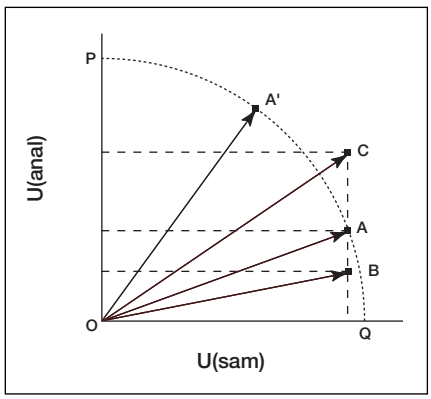
Another advantage to be gained by including sampling in the measurement process is in the judgement of whether measurements are fit for purpose. Measurements are often used to make decisions, and uncertainty in the measurement causes the potential for error in the decisions. The inclusion of the sampling uncertainty makes sure that the probability of an incorrect decision is correctly calculated. In addition the relative importance of the uncertainty contributed by the analytical method can be quantified. Where the uncertainty from the sampling is dominant, which is often the case, then it can be shown that reducing the uncertainty of the analytical method will not reduce the uncertainty on the measurement process overall. This can be a very important conclusion for analytical chemists.

Figure 1 is a diagrammatic representation of how the overall uncertainty of measurement is composed of the two contributions from the sampling and chemical analysis. Because these two contributions U_{sam} and U_{anal} add as their squares, any points such as A, A' on the circular arc PQ (centre at O) has the same combined uncertainty (by Pythagoras). The length of the vector from origin O to the point is the magnitude of this combined uncertainty. So it is easy to see visually that (for instance) halving U_{anal} at A gives B which only has a slightly smaller uncertainty. Whereas doubling U_{anal} to give C substantially increases the combined uncertainty.

…the taking of primary samples can be the largest source of uncertainty…

One of the simplest methods that will be described in the guidance for estimating measurement uncertainty that arises from sampling, is based upon the balanced design of duplicated samples[1] (Figure 2, page 10).

For a small proportion of the samples taken (e.g. 10%) a duplicate sample is also taken. The duplicate is taken using the same sampling protocol, but allowing for any ambiguity that may be present in the protocol. The duplicate may, for example, be taken at different positions around a pile of material, which is equally likely within the interpretation of the protocol. The heterogeneity that is present to some extent in all materials will then be reflected in small differences between the duplicate samples. If a separate estimate of the analytical contribution to the measurement uncertainty is required, then duplicate analysis are made on both of the sample duplicates (Figure 2). A statistical procedure called robust analysis of variance (RANOVA) is then used to estimate the overall uncertainty, and to separate out the main random components.



Michael Thompson

Figure 1: How sampling and analysis combine in variable proportions to give the overall measurement uncertainty.

* *Royal Society of Chemistry.*

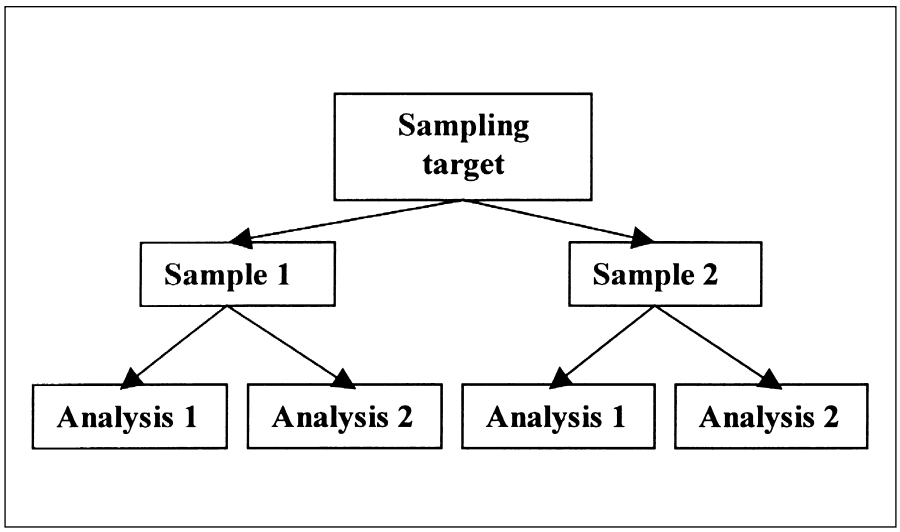


Figure 2: Balanced experimental design for empirical estimation of uncertainty.

The effect of systematic errors in the sampling are not allowed for this simple procedure, but those from the chemical analysis can be incorporated using estimates of analytical bias made using certified reference materials.

A guide looking at the estimation of measurement uncertainty arising from sampling is being prepared by the Eurachem/Eurolab/CITAC working group, and is due to be published in 2006. The aim of this guide is to explain the rationale and practical application of the methods available for the estimation of measurement uncertainty that include the contribution from primary sampling. The guide is designed to be applicable to estimating uncertainty from the full range of materials that are subject to chemical analysis. These include environmental media (e.g. rock, soil, water, air and biota), foods, industrial materials (e.g. raw materials, process intermediaries and products), forensic materials and pharmaceuticals. However, it does not include microbiological sampling, due to its extra complexity, and also estimation of uncertainty in the spatial location of areas of high analyte concentration, such as those in contaminated land.

Worked examples will be given for a range of applications, so that users will be able to follow how the procedure works, and apply them to their own situations. The Guide does not aim to recommend individual sampling protocols, which are often prescribed in other documents or regulations, but rather to estimate the measurement uncertainty generated by whatever protocol is employed.

The inclusion of primary sampling will give an opportunity for measurement scientists to look at the quality of the whole process…

The intended audience for this guide is the measurement scientist (e.g. an analytical chemist) who needs to estimate the uncertainty of measurement. As well as explaining how to estimate this uncertainty, the guide will also explain the justification for including sampling in the overall management of the measurement process, so that organisations can consider how sampling quality can be managed. Less technical documents will also be required to explain these concepts to non-specialist such as the managers responsible for organisational decisions. Several such documents are being prepared by the new AMC sub-committee, in the format of the already popular AMC Technical Briefs[2]. These briefs are short documents, usually two A4 pages, which are aimed at non-specialist scientists and give an overview of an important topic. The first one on sampling will tackle the difficult subject of terminology. The word 'sample', for example, is used erroneously by analytical chemists to mean a very wide variety of items from test portions of powder ready for dissolution, to test solutions prepared from reference materials. Until we all agree on a clearer meaning for such terms as 'sample' we will not be able to clearly quantify the role of sampling within measurement. A second form of non-specialist document is also being prepared by the AMC sub-committee. These Background Papers are intended for managers and administrators, who need to appreciate the implications of these issues for whole organisations and for policy matters. The audience may often be educated non-scientist, so the explanations have to contain less technical jargon and broader-based examples and analogies. The first of these papers is 'What is uncertainty from sampling, and why is it important?' (No.1, June 2004), and can be downloaded from the AMC website[2].

Another novel means of communicating the relative importance of sampling and chemical analysis in the measurement process, is the development by AMC of a computer game. This game is called 'Goldmine' and will soon also be downloadable from the AMC website.[2] The aim of the game is to locate a gold deposit in a fictional landscape, at the minimum cost, so as to make the maximum profit. It will enable the player to choose sampling and analytical methods with different levels of uncertainties and costs. It is possible to appreciate, therefore, that very expensive analysis, with low uncertainty, is wasted when the uncertainty of the sampling is high, due to its low cost. There is an optimal balance to be struck between the uncertainty (and therefore cost) of the sampling and the analysis, and the total number of measurements that will effectively find the location of the gold deposit. It will be interesting for the AMC to find out how popular the game is as a way of explaining the issues, compared with the more conventional method of supplying published documents.

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2 www.rsc.org/lap/rsccom/amc/amc_index.htm

The terms we use and the words we choose

International terminology for measurement in chemistry

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When we read or receive a measurement result, we are entitled to ask where this result comes from, or where it takes its authority from. In other words: what is the metrological 'trace' (or origin) of the result?

What is its 'trace-ability' and what does the term really mean?

On the present global scene, we have to agree across borders on what a given term means and have a common understanding of the concepts behind a term before we attempt to define it. Following this, we must agree the name and definition of a given term in one language, as well as its consistency with other terms before we can proceed to translate it into different languages.

Thus the justification for an International Vocabulary of Basic and General Terms in Metrology, 'VIM'[1] is evident. The VIM has been out for almost 20 years with its second edition ('VIM2') having been published in 1993. After seven years of work by the JCGM, the Joint Committee on Guides for Metrology (VIM & GUM), a draft third edition of VIM ('VIM3') has been produced and is currently available for comment via the sponsor organisations (i.e. BIPM, IEC, IFCC, ISO, IUPAC, IUPAP and OIML)* until the end of October.

In recent decades, basic concepts in measurement have evolved due to our deeper insight into measurement as a process, especially in chemical measurement. Hence, the definitions used to describe and explain concepts must be refined to accommodate these new insights. New terms for these concepts must be included or existing terms be re-defined if needed. Existing inconsistencies in the present VIM needed to be removed. Finally, the VIM had to encompass chemical measurement for the first time in history! Long overdue!

An example illustrating the need for a revised 'VIM' is the new insight in 'measurement uncertainty' as a measure of doubt[2] (end of 20th century), which is different from the 'true value/error' concept and the ensuing expression of 'confidence' (19th and most of the 20th century).

One term that could be described as being ambiguous is 'traceability'. It is the "property of a measurement result or of the value of a measurement standard ..."[1]. Yet, in common parlance, it is universally applied as a characteristic of a measurement system, or of an instrument, or of a process, or of a sample (or material), or to an institute.

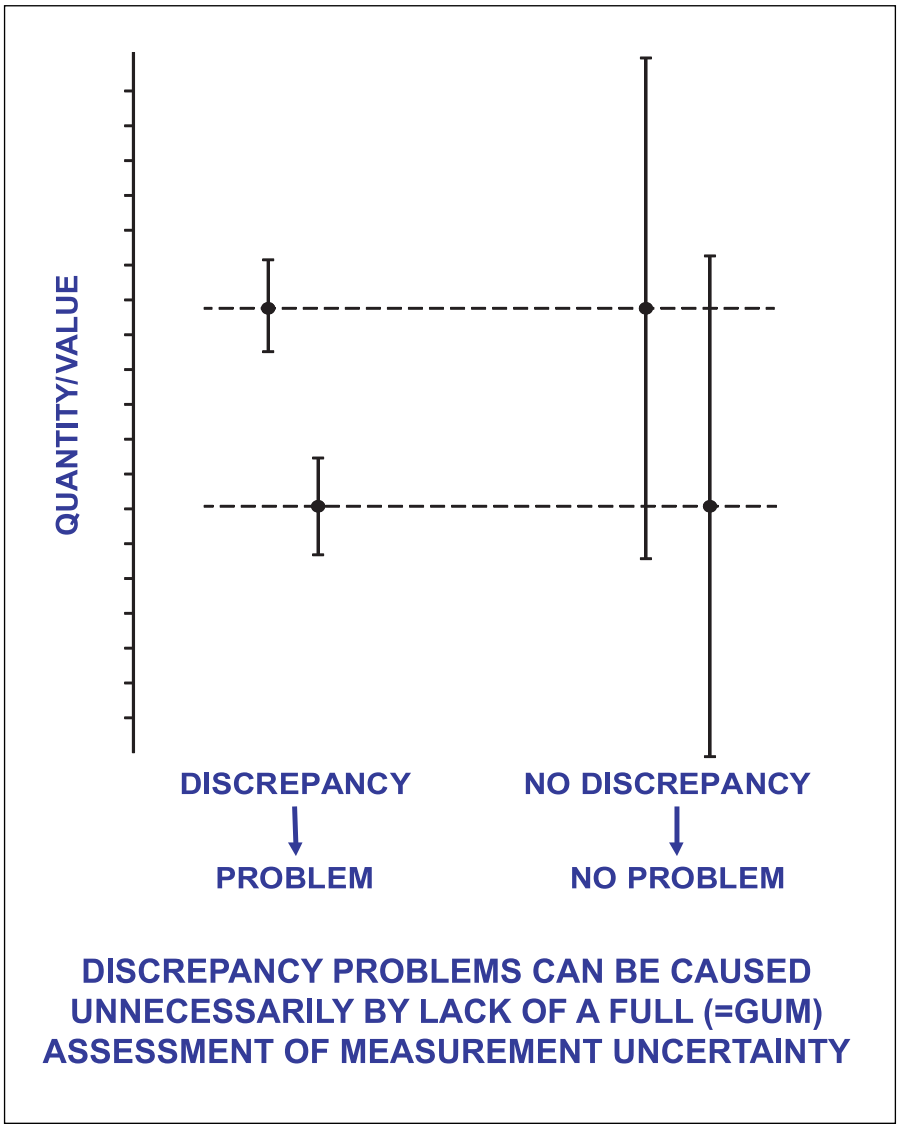


Figure 1

The same differences between quantity values of measurement results can constitute a non-significant difference, and therefore not a discrepancy, or a significant difference and therefore a discrepancy.

* *See glossary (page 13).*

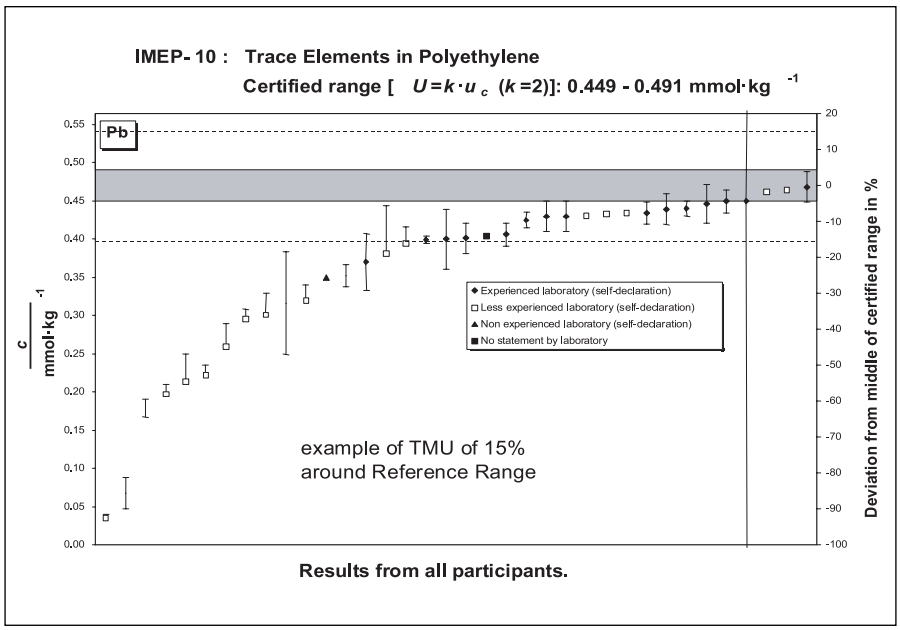


Figure 2: Display of a Target Measurement Uncertainty TMU of +/-15 % around a traceable reference value, enabling to retain a number of laboratories as being "satisfactory" according to a pre-set criterium (a relative TMU of +/- 15 %).

The TMU needs to be anchored to the metrological reference value of the interlaboratory programme in order to prevent an arbitrary 'anchoring' leading to an arbitrary selection of laboratories with 'satisfactory' performance.

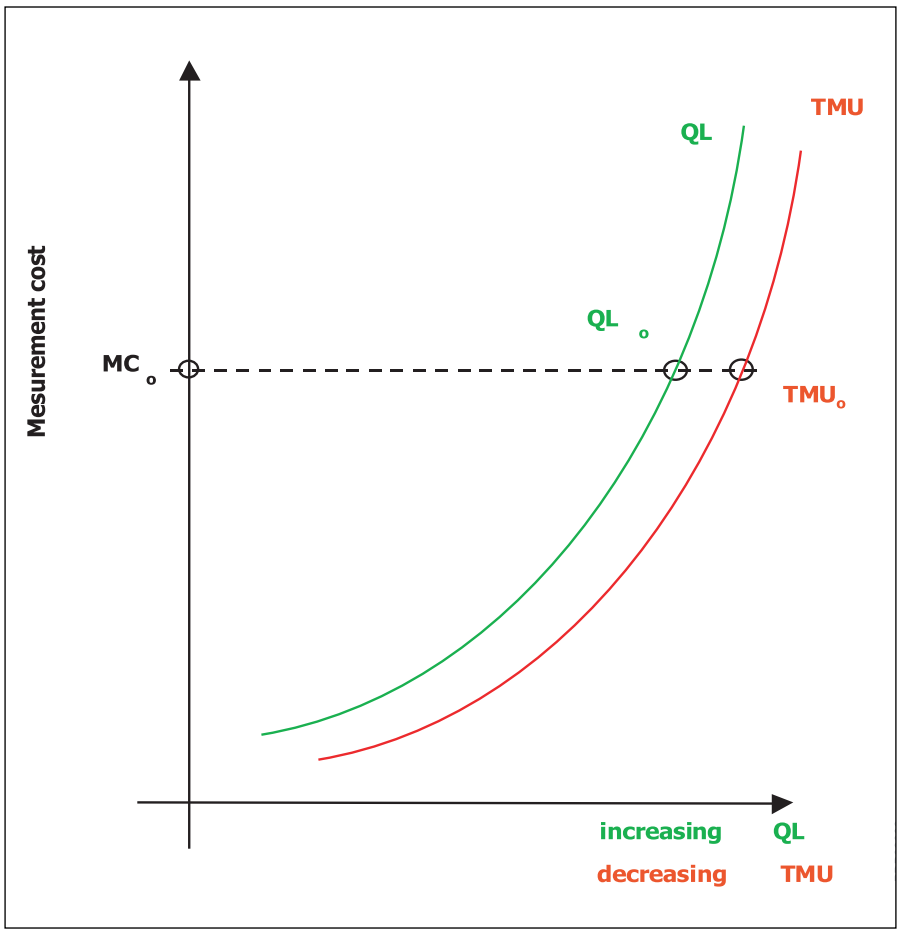


Figure 3: Relation between the Quality of Life (QL), Target Measurement Uncertainty (TMU) and measurement cost (MC).

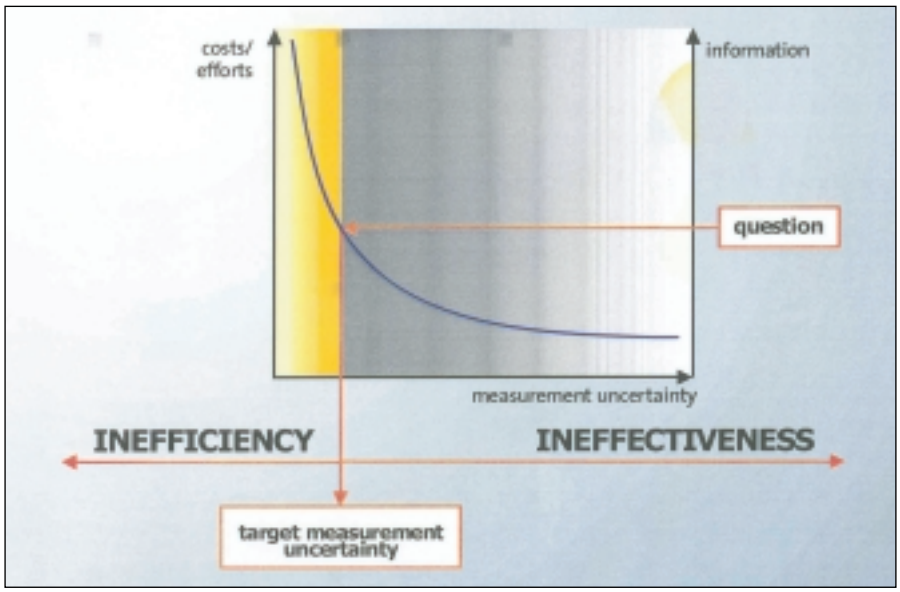
The same can be said about 'comparability': does it mean "being of the same magnitude"? Or does it mean "being traceable to the same (metrological) reference", regardless the magnitude (size) of the measurement result?

An example which is particularly relevant for chemical measurement, is the term 'measurand': "the quantity subject to measurement"[1]. 'Quantity' can be concentration, time, volume, length, electric current, mass fraction, light intensity, etc. When applied to a chemical measurement, the quantity subject to measurement is an electric current as almost all our measuring systems use electric currents. Yet we claim to have measured a 'concentration' because that is the quantity we declare to have measured. But that is not the quantity that we have actually (or directly) measured: (ratios of) electric currents.

One term that is new to VIM3 is 'target measurement uncertainty'. Figure 1 demonstrates that measurement uncertainty is an essential part of the measurement result. It should therefore enter its definition. Figure 2[3,4] demonstrates the concept of 'target measurement uncertainty' (TMU): "a measurement uncertainty needed for a specified intended use of the measurement result". That presupposes a traceable value in the measurement result that is obtained against a stated metrological reference, and which must be independent from the spread of the measurement results obtained at different measurement laboratories. It then becomes easy to identify measurement laboratories where measurement results with associated uncertainty can be found which are fit for an intended use of that result. The TMU concept also helps to decide whether an improvement of measurement results (i.e. reducing a measurement uncertainty) is needed, and whether the associated cost is justified. See Figure 3[4] and Figure 4.

The translation problem

Correct translation is known to be very difficult. It is even doubted whether it can be done 'exactly'. In any language, each term (and the words used to define it) has a semantic 'vagueness', resulting from its history and depending upon the context in which it is used. But that is also the case for the terms of the language into which a term is



P. De Bièvre / T. Walczyk

Figure 4

A Target Measurement Uncertainty (TMU) indicates whether a measurement uncertainty is inefficient or ineffective for a specified intended use. Both are extremely useful in leading to practical consequences.

being translated. It follows that it is essential that common concepts do exist, that they have a common definition, and that these are commonly accepted before any translation is even worth being attempted, let alone be suitable to fulfil its role. In 2004 this is still not the case. Insufficient clarity and lack of common understanding in the use and meaning of measurement terminology make harmonised implementation of international agreements involving measurement results a very difficult and challenging affair.

Conclusions

1. On the global scene, crystal-clear cross-border agreements are needed for fair trade. Such agreements can only last, let alone be implemented, if they are based on common understanding. Common understanding can only be built if proper communication tools are available. Such tools include the terms we use and the words we choose to define them. In agreements involving measurement results, a 'VIM' is essential.
2. In international communication, an agreed set of terms is needed. But, more importantly, agreement is needed on the definitions of concepts.
3. Several definitions of such basic concepts, and terms for these concepts in VIM2, have been refined, and in some cases re-defined, in the proposed draft VIM3, as a result of our greater insight into measurement and our understanding of the underlying concepts. New terms have also been added where deemed appropriate.

   Additional note: Extreme care has been taken to make all of the described concepts and associated terms consistent with each other. Thus VIM3 is not just a collection of terms assembled and compiled in a booklet, but a consistent set of terms and underlying concepts, tightly knitted together.
4. Unambiguous terms describing concepts are needed in one language (presumably English), in order to prevent ambiguity when translating into other languages.

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3. www.imep.ws
4. Majcen, N., Skubic, I., De Bièvre, P., *Accred Qual Assur*, **9**, pp 106–111, 2004.

GLOSSARY

BIPM Bureau International des Poids et Mesures (International Bureau of Weights and Measures)

IEC International Electrotechnical Commission

IFCC International Federation of Clinical Chemistry

ISO International Organization for Standardization

IUPAC International Union for Pure and Applied Chemistry

IUPAP International Union for Pure and Applied Physics

OIML Organisation Internationale de Métrologie Légale (International organisation for legal metrology)

Performance assessment in the molecular biology laboratory

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Introduction

It is becoming more widely recognised that there is a need for laboratories performing nucleic acid measurements to have an independent means of assessing their performance and demonstrating analytical quality to potential customers and the wider scientific community. However, there are few proficiency testing (PT) and external quality assessment (EQA) schemes for molecular analysis, and most are designed for specific analytical sectors. In the foods sector, the Food Analysis Performance Assessment Scheme (FAPAS)[1] is run by CSL, and for clinical diagnostics the UK National External Quality Assessment Scheme (UK NEQAS)[2] and the European Molecular Genetics Quality Network (EQMN)[3] both have programmes of laboratory assessment that include molecular genetic analysis. To address the increasing need for independent performance evaluation, a prototype EQA exercise was developed as part of the VAM programme, to provide the means for cross-sectoral benchmarking of laboratories performing DNA analysis[4]. The study was designed to assess laboratory competence in performance of a simple DNA extraction and polymerase chain reaction (PCR) amplification, chosen as two of the most widely used analytical techniques. The aim was to help address some of the issues surrounding the harmonisation of nucleic acid measurement techniques and processes, and to allow wider access to independent performance assessment for laboratories, regardless of their usual analytes.

* *Colony Forming Units.*

Design of the prototype scheme

A wide range of laboratories was canvassed for their opinion on the design of the scheme, including clinical and molecular microbiology laboratories, food research institutes, contract research organisations and a variety of academic research groups. There was no universally acceptable target analyte and matrix for use in the study, as most laboratories expressed a preference to work on their usual matrices. A further issue was the number of replicate measurements required to permit statistical analysis of the results from this qualitative analysis. In the final design of the scheme a balance was reached between the level of effort required from participants and obtaining sufficient replicates to allow assessment of the consistency and sensitivity of analysis within each laboratory.

The analyte used in this prototype scheme was a suspension of heat-killed bacteria, provided at high (10^8 CFU*), medium (10^7 CFU) and low (10^6 CFU) levels for DNA extraction. Participants were asked to extract genomic DNA from a total of 24 bacterial samples at varying analyte concentrations, and to amplify a proportion of the extracts using the PCR reagents provided with the samples[5]. Six positive control DNA samples were also provided to the participants for concurrent PCR amplification, and a proportion of each reaction was analysed by agarose gel electrophoresis (Figure 1).

Participating laboratories were required to interpret each sample as either positive or negative for the amplification reaction based on the gel analysis results, and to return completed results sheets and labelled photographs of their gel analysis for scoring of results.

Overall results

A total of 15 laboratories participated in the scheme, and to maintain confidentiality each laboratory was given a unique identifier code. The scheme was run in two rounds, although two laboratories (5 and 9) were unable to complete the second round because of resourcing issues. In the first round prescriptive conditions for amplification and gel analysis were supplied, to facilitate comparison of performance between laboratories. However, in response to feedback from participants, use of routine in-house methods was endorsed in round 2. In both rounds the majority of participants performed well, with laboratories scoring 79% and 74% of samples correctly overall in rounds 1 and 2 respectively. The slightly lower scores in round 2 may reflect variability introduced through increased reliance on participants' usual practices.

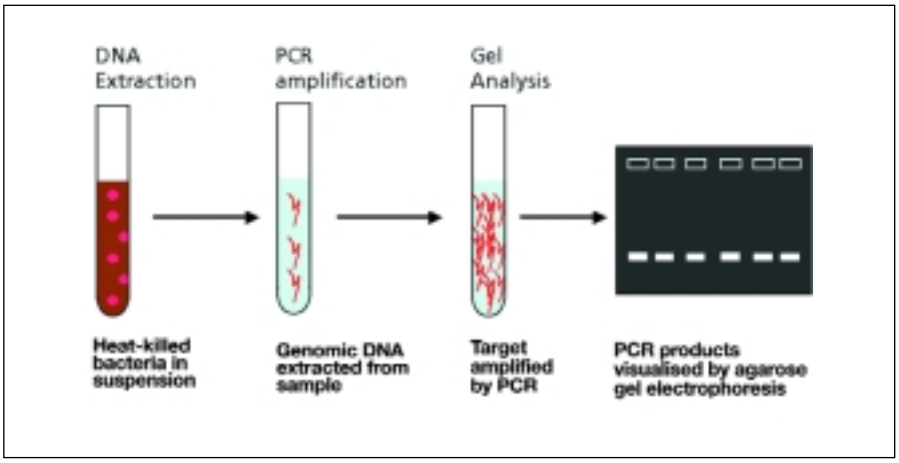

Figure 1: Schematic of the analytical process assessed.

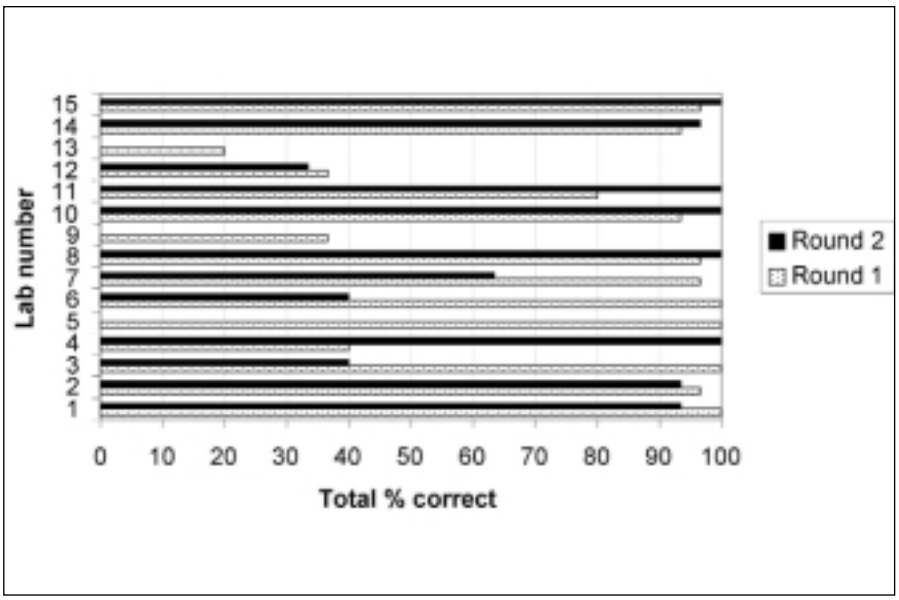

Figure 2: Summary of laboratory performance across both rounds.

The scores of each of the participants across the two rounds are presented graphically in Figure 2.

As expected, as the level of analyte decreased the participants were less able to correctly detect the bacterial target, indicating that sensitivity of the analysis was sometimes not sufficient. Overall, the high level analytes were detected correctly in 73% of samples, medium levels in 70% of samples and low level samples were successfully detected in 64% of samples over the two rounds. Compromised efficiency of either DNA extraction or PCR amplification could have affected the sensitivity of analysis.

Sources of analytical error

A number of common analytical problems were encountered, including delays in sample transit or receipt, use of incorrect sample storage conditions, low efficiency of DNA extraction, total inhibition of PCR amplification or poor sensitivity, and incorrect gel interpretation and reporting of results. A number of potential sources of experimental error are highlighted in Figure 3.

The majority of laboratories used a commercial DNA extraction or clean-up kit to perform the sample extraction, and most kits chosen were suitable for extraction of bacterial genomic DNA from cell suspensions. Use of quality controlled commercial materials should have minimised any potential downstream inhibition effects or extraction efficiency issues. However three laboratories chose inappropriate kits, which either compromised genomic DNA extraction efficiency or inhibited the subsequent PCR amplification of the extracted target, highlighting the importance of fitness-for-purpose in choice of methodology.

A probable PCR inhibition effect was observed in the results of one participant, probably through addition of a high volume (40% v/v) of DNA extract to each PCR amplification when less prescriptive conditions were allowed in round 2. Advice from a leading commercial extraction kit manufacturer has emphasised that DNA extracts should comprise less than 20% of the overall reaction volume in a PCR, to avoid potential inhibition of the reaction by high levels of EDTA which is present as a common component of elution buffers.

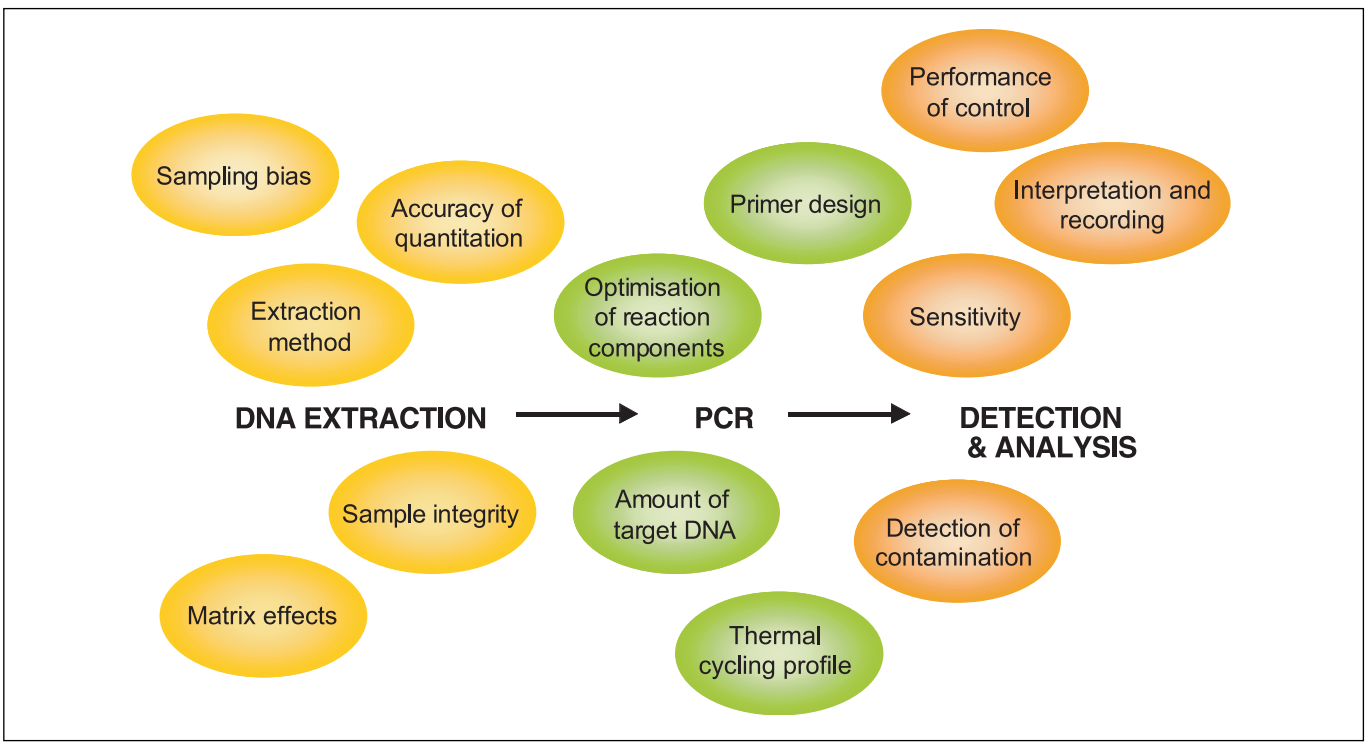

Figure 3: Common potential problems in nucleic acid analysis.

One participant experienced analytical sensitivity problems in the second round, which may have resulted from the reduction in the total volume of the PCR reaction from 25 µl to 12 µl. This small volume may have been more sensitive to any slight PCR reaction evaporation, inaccuracies in pipetting, or the presence of inhibitors in the sample extractions as the DNA eluate comprised over 40% of the total reaction volume.

In addition to problems with the practical analysis of samples, there were also some inaccuracies in interpretation of the gel results. In round 1, one of the participants reported 8 of the samples wrongly on the interpretation sheet, highlighting the value of an independent check of results to ensure accuracy of the data.

Utility of the generic assessment approach

The majority of participants in the scheme strongly endorsed the need for more widely accessible independent quality checks for commonly used procedures, and found the post-scoring feedback on performance useful.

Provision of generic analytes, to allow a variety of laboratories to participate in a single scheme, is endorsed by the observation that there were no significant differences (ANOVA at the 5% level) between the scores of laboratories dependent on either analytical sector or their usual analytes (Figures 4 and 5 respectively).

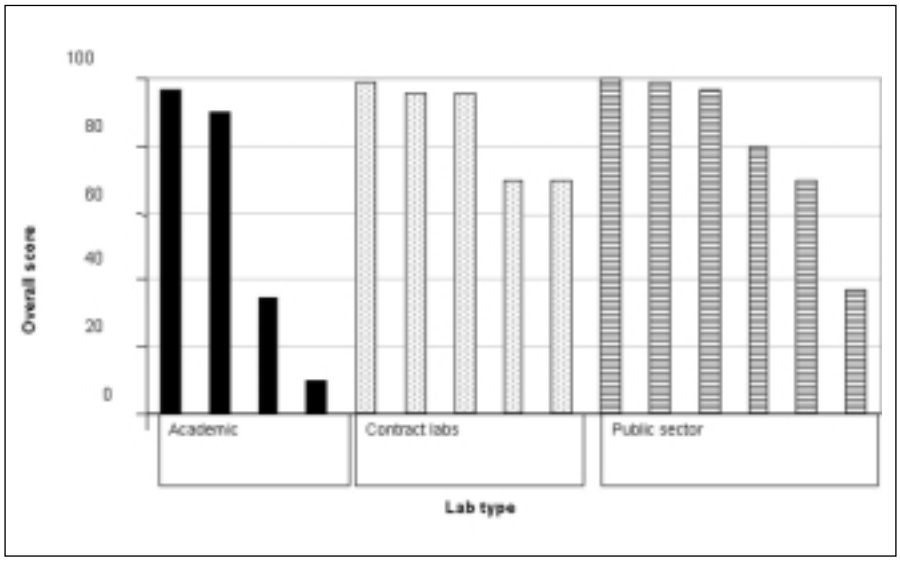

Figure 4: Overall % scores presented by laboratory sector.

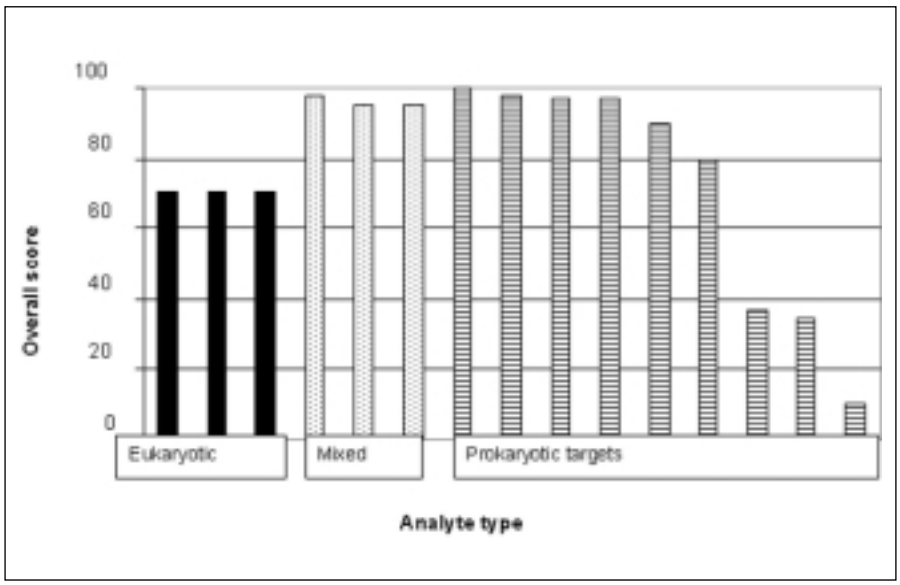

Figure 5: Overall % scores presented by routine analytes of participants.

The ability of commercial laboratories to demonstrate the quality of their analyses is fundamental to ensuring the confidence of their customers. In addition, academic laboratories will in the future be required to adhere to the recently published Joint Code of Practice for Research (2003), in order to receive continued funding from government agencies such as the Biotechnology and Biological Sciences Research Council (BBSRC), the Department for the Environment, Food and Rural Affairs (DEFRA), the Natural Environment Research Council (NERC) and the Food Standards Agency (FSA). The Code of Practice requires that researchers undergo independent audit of their research processes, and states that: *"In the longer term it is expected that most research organisations will assure the quality of their research processes by means of a formal system that is audited by an impartial and competent third party against an appropriate internationally recognised standard that is fit for purpose."* Accessible, independent means of assessing the quality of research, such as this pilot scheme, may be very valuable in fulfilling this requirement.

A second generic PT exercise for nucleic acid analysis will be run as part of the DTI's Measurements for Biotechnology (MfB) programme 2004–2007.

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G-SIMS – Direct analysis of organic surfaces

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Introduction

Surfaces are where bulk material interacts with the surrounding environment. For many advanced technology products it is this interface that is critical for correct product performance. Examples are wide ranging and far reaching, from disposable nappies to anti-cancer drug delivery systems and from food packaging to DNA screening. Consequently, the design and control of surface chemistry is of prime importance to many manufacturers. The pressing need for chemical analysis at surfaces has led to the development of powerful analytical techniques. The front line techniques are X-ray Photoelectron Spectroscopy (XPS), Static Secondary Ion Mass Spectrometry (SSIMS), Atomic Force Microscopy (AFM) and Auger Electron Spectroscopy (AES)[1]. In this article we concentrate on SSIMS which has the highest surface sensitivity (molecules at the top atom layer), highest speciation (distinguish between complex molecules) and parts per million sensitivity.

In SSIMS, energetic primary ions are fired towards the sample surface where they impact, travelling some ten nanometres into the bulk before losing their energy and coming to rest. As the ion travels through the material it collides with target atoms setting them in motion known as a cascade. Some of the energy of the cascade travels back towards the surface and results in desorption of surface species called sputtering. Most of these sputtered fragments are neutral but a small fraction, typically 1%, are ionised and are subsequently mass analysed according to their mass to charge ratio. Most sputtered ion fragments come from the topmost layer and this, coupled with the high signal levels, gives the technique high surface sensitivity, able to detect femtomoles of molecules at surfaces. Additionally, the ion fragments give detail related to the functional groups present at the surface.

For many years SSIMS was a promising technique that gave variable results that were difficult to interpret. This irreproducibility

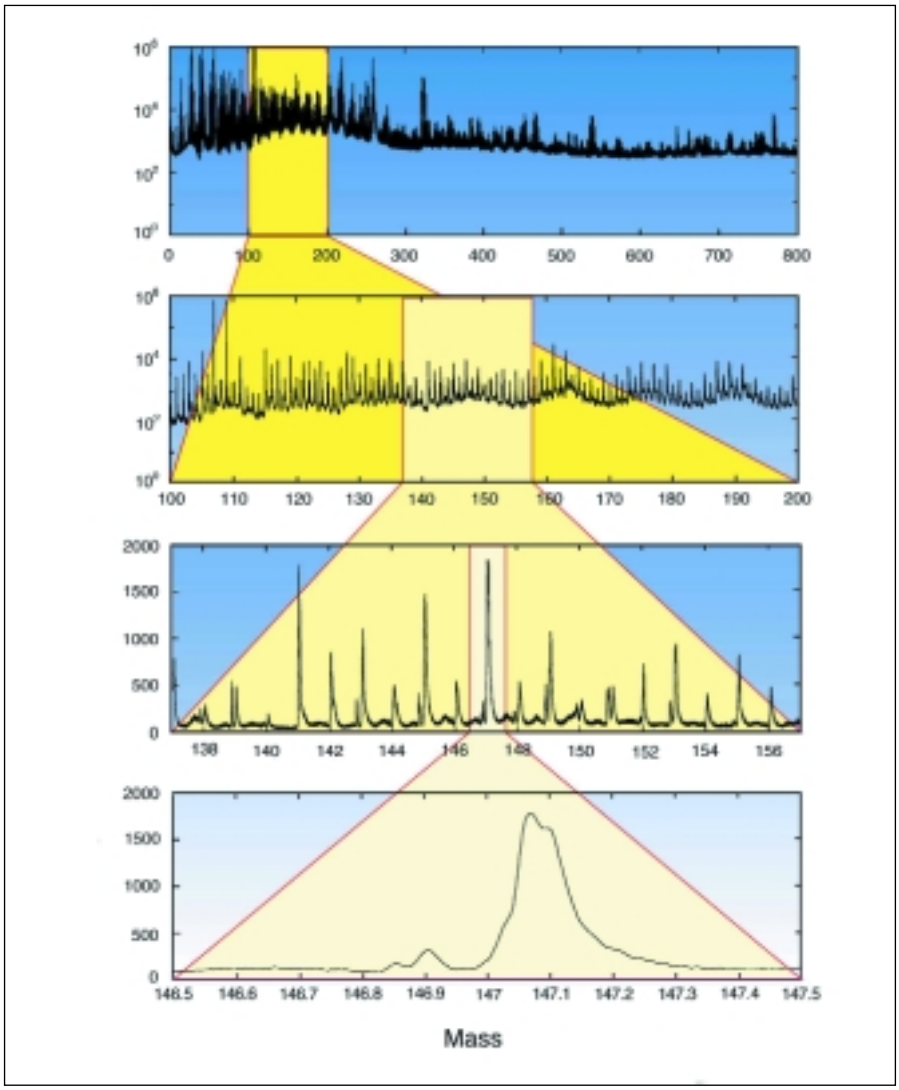


Figure 1: The positive ion static SIMS spectrum of a surface layer of Irganox 1010 molecules on silver illustrating the complexity of the mass spectrum.

was tolerated since the information content in SSIMS was not available via other routes. However, this restrained the method to a few academic institutions where experts could publish work. In the last decade this has changed significantly through improvements in instrumentation and the use of reference procedures and methods. In 1996 NPL conducted a major interlaboratory study[2] showing that, on average, instrument repeatability was then 10% – a significant advance on earlier work, which could vary by two orders of magnitude. In 2002 an eagerly awaited second study[3] with a protocol for analysis[4] was conducted. Results show that over 85% of instruments now have excellent repeatabilities of < 1%. This outstanding level of control positions SSIMS at a new gateway for the analysis of molecular orientation, molecular structure, automated analysis and as we shall see, direct interpretation of data via G-SIMS.

SSIMS instrumentation has developed considerably over recent years driven by industrial demand and academic innovations. Modern high performance instruments use time-of-flight analysis with a mass resolution of 15000 and high transmission and ion detection efficiency. Innovations in cluster ion beam technology have now opened up organic analysis at 150 nm spatial resolution. Recently, a new bench top instrument has been launched broadening the accessibility of the technique[5].

SSIMS Interpretation

The strength of SSIMS, and also one of its drawbacks, is that the mass spectra are extremely rich with hundreds of mass peaks, many of which clearly contain aspects of the molecular information. As we shall see later, many of these peaks are degraded fragment ions that, unfortunately, do not relate directly to parent molecules. These ions lead to confusion. Figure 1 (page 17) illustrates the issue with a mass spectrum of an industrial antioxidant, Irganox 1010, used in the polymer industry, on a silver substrate.

SSIMS…spectra are rich in chemical detail

A molecular mechanics representation of a single molecule on the Ag surface is shown in Figure 2. Note that the spectrum of Figure 1 is for a surface coating of molecules not an individual molecule. It is clear that there are many peaks and by progressively zooming in on smaller and smaller regions we see the complexity of the spectrum.

A Compound-specific library can only be a first aid measure.

Traditionally, to identify a material, the SSIMS spectrum is used as a fingerprint for comparison with library spectra. Such libraries[6,7,8] are very useful and, through the pioneering work of Briggs, have allowed the technique to become established in the industrial analysis of polymers. However, the growth and coverage of libraries is, by necessity, limited. Since the publication of the first library in 1989, the combined content only contains spectra for 600 materials. In comparison to the range of industrially relevant materials this is a tiny fraction as illustrated in Figure 3. For new and rapidly growing industrial sectors such as pharmaceuticals, biomaterials, sensors and displays the problems are acute. A compound-specific library can only be a first-aid measure. A library-independent method is required providing direct interpretation, accessing the full space of measurement capability indicated in Figure 3. Hence, G-SIMS was developed at NPL as part of the VAM programme.

G-SIMS

We envisage the impact of a single primary ion schematically in Figure 4 (page 20). Typically for an ion beam of energy 10 keV, that energy is dissipated in the surface of the material over a range of approximately ten nanometres (around 30 atom layers). The energy distribution of excited atoms at the surface is approximately a 2D-Gaussian in shape, centred on the original impact site with a FWHM of around 10 nm. In this scheme we consider a monolayer of molecules covering the surface, folic acid for instance. It is clear that those folic acid molecules along the peripheral zone of the impact site are more gently liberated from the surface and have a higher probability of remaining intact. As we move closer to the centre of the impact site, the energy rises and the molecules are more fragmented, so that at the centre, the emitted ions have high internal energy and most probably consist of small degraded fragments. The measured SIMS spectrum is the volume integral of all these fragment ions and so contains some intact molecular ions amongst a dominant background of smaller degraded ions. We see an example of this in Figure 1 (page 17). The small fragment ions are not

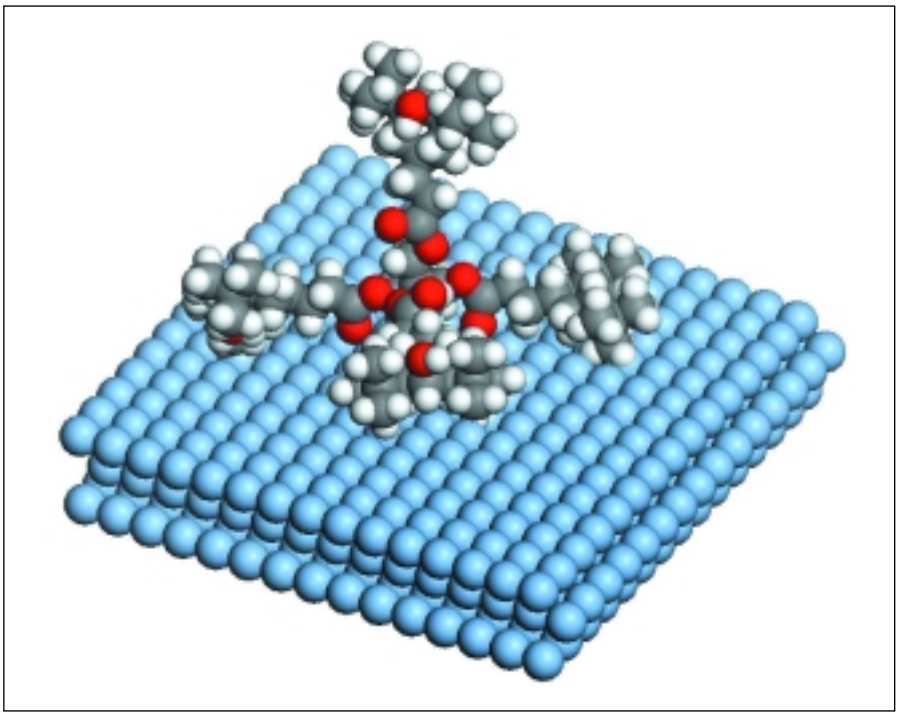


Figure 2: A molecular model representation of a single Irganox 1010 molecule on a silver surface.

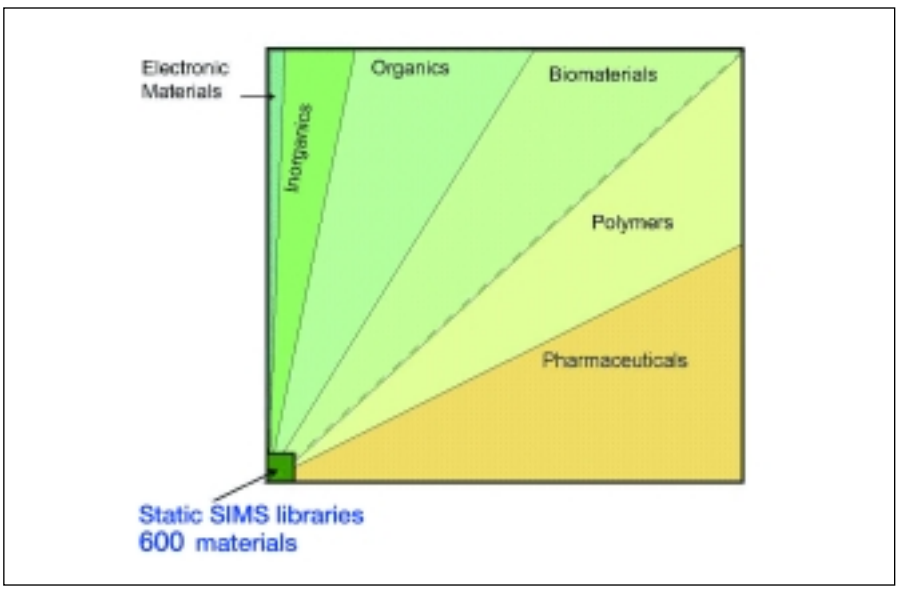
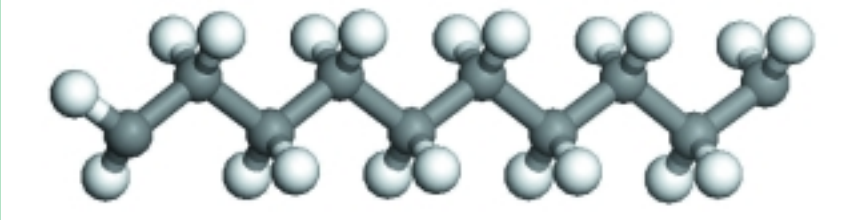


Figure 3: Illustration of the comparatively minute size of SSIMS libraries compared with the industrial need.



We consider the fragmentation of the above hydrocarbon molecule into the series C_nH_{2n}, C_nH_{2n-1}, C_nH_{2n-2}, etc and note that the energy, Δu, to remove each successive hydrogen atom is approximately the same. Thus, the number of fragments, N_i, of composition C_nH_{2n-i}, derived from N_0 components of composition C_nH_{2n}, is given by the simple partition function relation:

$$N_i = N_0 \exp\left(\frac{-i\Delta u}{kT_p}\right) \quad (1)$$

If we now consider these at the two ion beam energies, E_1 and E_2 with associated fragment surface plasma temperatures T_{p1} and T_{p2}, we get:

$$\frac{N_i(E_2)}{N_i(E_1)} = \frac{N_0(E_2)}{N_0(E_1)} \exp\left[\frac{-i\Delta u}{k}\left(\frac{1}{T_{p2}} - \frac{1}{T_{p1}}\right)\right] \quad (2)$$

The ratio of $N_i(E_2)$, for a low ion beam energy, E_2, to those at high energy, $N_i(E_1)$, gives a factor, F_x, where x is the index relating to each mass peak. This F_x term is related to the effective surface plasma temperatures T_{p1} and T_{p2} from Equation (2). Since T_{p1} and T_{p2} are of a similar magnitude and T_{p2} is less than T_{p1} by ΔT, we may first consider the factor F_x^2, which would be the F_x value for surface plasma temperatures T_{p1} - $2\Delta T$ and T_{p1}. Thus, one could use F_x^{13} or some high power to deduce the result at a significantly lower surface plasma temperature than that relevant to either of the recorded spectra.

We may now generate a low surface plasma temperature SSIMS spectrum by multiplying an existing spectrum, N_x, with the factor F_x^g. This forms the G-SIMS spectrum with intensities I_x given by:

$$I_x = M_x N_x F_x^g \quad (3)$$

where g, the G-SIMS index is often set at 13. The additional factor M_x, the mass of the emitted fragment, is found useful to enhance the natural fall in emission with mass.

Box 1: The G-SIMS Equation

helpful for identification, as they are ubiquitous amongst many different material types. In many cases, larger, more stable ions such as polycyclic aromatic ions may form, which may be misleading and, if used, give poor identification and quantification.

We now consider how fragmentation of a molecule leads to daughter products and how their intensities are related. In the recoil from the primary ion impact, the surface zone may be characterised by a surface plasma temperature, T_p, as a function of the radius, r, from the point of impact. The plasma temperature is also a function of the bombarding ion species and the impact energy, E. If we reduce the plasma temperature by adjusting the primary beam energy, the relative intensities of the more intact molecular fragments would be expected to increase. In principle, given the relative intensities of ions at two different values of T_p it is possible to extrapolate to the relative spectral intensities at a lower surface plasma temperature. This is the basic principle of G-SIMS, which is explained in more detail in Box 1 and in references 9, 10 and 11.

G-SIMS examples

In practice, to generate G-SIMS spectra, all that is required are two subsequent spectra acquired from the same area using different ion beam conditions that provide as large a difference in surface plasma temperatures as possible. A detailed analysis in reference 9 shows that selecting primary ions with high and low mass is best. Modern instruments controlled by computer are well equipped to do this. We use a commercial dual beam source delivering 10 keV Cs^+ (131 amu) or 10 keV Ar^+ (40 amu) along the same ion optical column. Thus ensuring co-incidence on the sample. The instrument computer is programmed to acquire a first spectrum using Cs (low fragmentation) followed by Ar (high fragmentation). The total fluence of ions is kept below 1×10^{16} ions/m^2 so that ion-induced damage effects are small[12].

For G-SIMS, 100% of peaks lead to direct interpretation and identification

As an example of the power of G-SIMS for direct analysis we show in Figure 5 the SSIMS and G-SIMS spectra for the polymer polystyrene (PS). The dominant intensity ions in the SSIMS spectra are polycyclic aromatic ions with complex linked cyclic arrangements that exhibit little direct relevance to the PS structure. These structures are very stable ions produced from the high energy fragmentation cascade, either as recombination events or the end result of a decay process for a molecule originally with excess energy. These "characteristic" peaks are common to many other materials. In contrast, the G-SIMS spectrum is dominated by ions with pendant phenyl groups exactly as would be expected from the molecular structure giving direct identification without the need for library data. G-SIMS has been tested extensively on many materials, including polymers such as PS, PC, PTFE, PMMA, and complex molecules including Irganox 1010, caffeine, cholesterol, glucose, poly-L-lysine, folic acid and proprietary liquid crystals. In Table 1 (page 20) we summarise the effectiveness of G-SIMS compared with SSIMS in the identification of peaks that lead to direct interpretation of a series of crystallisable organic molecules. Typically, for SSIMS only one or two peaks above, say, 1% of the maximum peak intensity provide direct interpretation out of several hundred peaks. For G-SIMS the situation is quite different, spectra typically contain five peaks with 100% leading to direct interpretation and identification. This step change opens the door to applications in areas where the fingerprint approach of collating spectra is not practicable. In some cases, particularly large complex molecules, the parent mass alone does not identify the molecule. An extension of the G-SIMS concept is being developed to deduce the

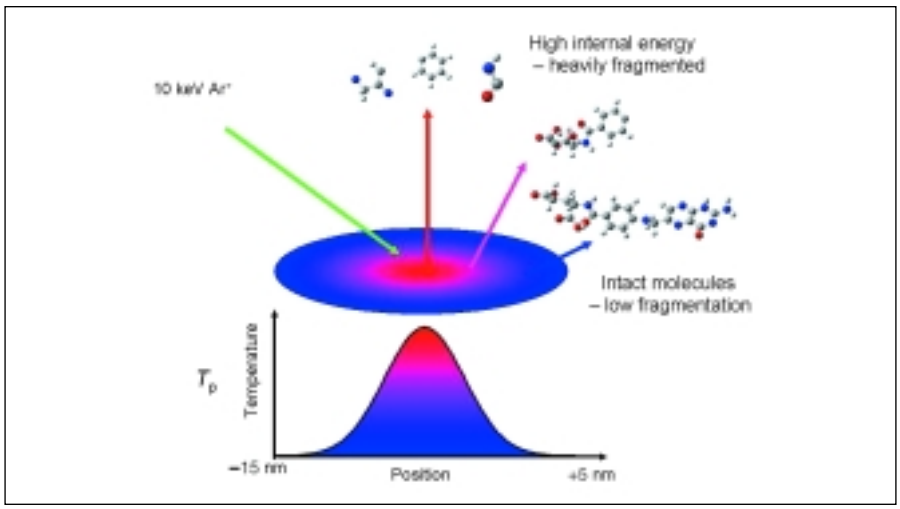


Figure 4: Schematic illustration of the surface energy distribution from a single ion impact on a target material with a surface monolayer of folic acid molecules. Typical fragmentation products are shown.

Crystallisable organic	SSIMS		G-SIMS	
	Number peaks > 1%	Percentage of peaks leading to direct interpretation	Number peaks > 1%	Percentage of peaks leading to direct interpretation
Poly-L-lysine	60	1.7	5	100
Cholesterol	241	0.8	3	100
Liquid Crystal (proprietary)	165	0.6	2	100
Caffeine	68	2.9	5	100
Sucrose	200	1.0	4	100
Irganox 1010	142	3.5	13	100
Folic acid	50	0.0	5	100

Table 1: Summary of percentage of peaks leading to direct interpretation for SSIMS and G-SIMS for 7 organic molecules.

molecular structure by studying the evolution of the fragmentation pathways as T_p is varied. Further work in the new VAM programme is developing the approach for mixed multi-organic materials.

Conclusions

G-SIMS is a powerful method for the direct interpretation of SSIMS spectra. The G-SIMS spectra are equivalent to the spectra that would be obtained from a very low collision cascade surface plasma temperature. The G-SIMS spectra therefore exhibit peaks that are more directly related to the material structure. All that is necessary to generate a G-SIMS spectrum are two spectra, either at different ion beam energies, or more effectively, using two different primary ion species. This is straightforward in modern commercial instrumentation. To assist analysts to produce G-SIMS spectra a VAM "Easy G-SIMS" Excel spreadsheet will soon be available from the surface and nanoanalysis website for download[1] free of charge. The spreadsheet gives users an indication of the instrument repeatability and outputs the G-SIMS spectrum.

New work develops the significant potential of G-SIMS to elucidate the molecular structure of complex ions at the surface and supports the analysis of mixed multi-organic surfaces.

This work provides a major platform for the development of static SIMS and G-SIMS for the analysis of complex molecules at surfaces.

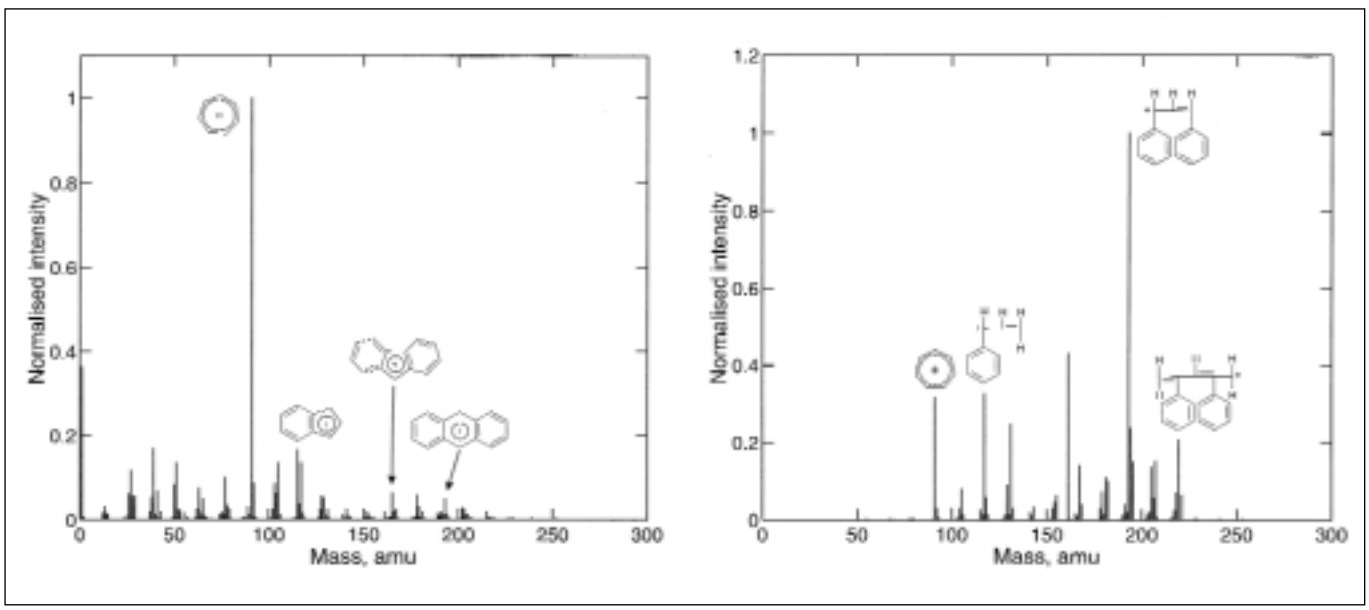


Figure 5: Examples of (a) Static SIMS and (b) G-SIMS spectra of polystyrene (PS).

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VAM G-SIMS wins IoP Paterson Medal

Congratulations goes to the author, Ian Gilmore, who was awarded the Institute of Physics (IoP) Paterson Medal and Prize for 2004, for his major contributions to the analysis of molecules at surfaces; in particular for the development of G-SIMS. The medal was presented to Ian at the IoP's awards dinner in January by the President, Professor Sir David Wallace.

The Paterson medal, named after Sir Clifford Paterson, founder of the GEC Research Laboratories, is awarded annually for outstanding contributions to the utilisation and application of physics, particularly in the development, invention or discovery of new systems, processes or devices, which show the successful, commercial exploitation of physics. The award is intended to encourage younger physicists.

IoP President, Professor Sir David Wallace, presents the Paterson Medal to Ian Gilmore at the IoP Awards dinner in January.

Nano-analysis using Atomic Force Microscopy

AFM enables identification of nano-regions via modulus measurement.

Charles Clifford
Peter Cumpson
and
Martin Seah
National Physical Laboratory

Introduction

The atomic force microscope (AFM), first described by Binning *et. al.*[1] in 1986, has become industry's most popular scanning probe surface analytical tool. A commercial AFM works by scanning a microfabricated silicon or silicon nitride tip attached to a cantilever across the surface. The deflection of the cantilever is detected using a laser and is used to build up an image of the surface. The AFM can operate in a number of different modes, each giving different information about a surface at sub 20 nm spatial resolution and, for certain samples, atomic resolution can be routine. Figure 1 (page 22) shows a two-phase polymer blend imaged using the AFM in force modulation mode[2] allowing qualitative information on the relative stiffness of the two polymers at a higher resolution than any other method. Without the AFM, the current interest in nanotechnology simply would not have developed as it has.

Typically, in the past, the industrial use of AFM has been focused on generating quantitative ultra-high resolution topographical images to examine, for example, surface roughness. This has allowed new levels of topography to be observed. In skilled hands, however, a range of further attributes have been realised at atomic or near-atomic resolution that have enabled researchers to develop the ideas that drive much of today's frontier nanoscience developments for new nanotechnology products. Under the VAM programme, the Surface and Nano-Analysis team at NPL is developing calibration methods and protocols for those in industry who are applying these quantitative measurement techniques, beyond the topographical, using

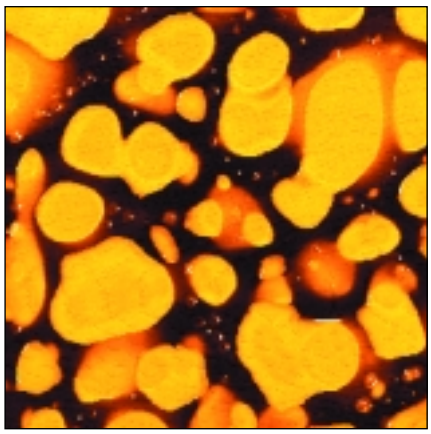

Figure 1: AFM force modulation image of a two-phase polymer blend with 10 µm field of view.

AFM identifies soft materials at surfaces with higher spatial resolution than any other method. It can be used to image polymers with 10 nm resolution.

AFM. This is vital in the healthcare, aerospace, packaging, medical and pharmaceutical industries where high resolution non-invasive quantitative analytical techniques are necessary.

Initially, this work at NPL has been focussed in two areas. The first involves developing a method to identify, at the nano-scale, polymers at surfaces with high spatial resolution[3]. This is done using modulus measurement. To do this effectively we identified the need to calibrate, among other parameters, the AFM cantilever spring constant. This leads to the second area, which is the development of a compact and easy-to-use MEMS (micro electro-mechanical) calibration device for calibrating AFM cantilever spring constants[4,5,6,7]. This new device, called an Electrical Nanobalance, is calibrated using a method that allows traceability to the SI for the first time. For the AFM user, the device offers a simple, accurate and traceable method for AFM cantilever spring constant calibration.

Nano-scale modulus measurement

AFMs can been used to map the nanomechanical properties of surfaces using techniques such as force modulation mapping, as shown in Figure 1, and force-displacement analysis. Quantification of the force and elastic parameters are critical to the nanomechanical analysis and positive identification of materials at the nano scale and for assessing behaviour at surfaces. We have addressed the quantification issues in modulus measurement at surfaces for homogeneous materials using force-displacement analysis and indicated how to do this with sufficient accuracy to identify materials[3]. We have developed two routes to quantitative modulus measurement using both the AFM on its own and the AFM combined with a nanoindenter to deal with two different measurement requirements. The first involves the direct measurement of modulus using a fully calibrated instrument and allows depth analysis. The second uses indirect measurement through calibration by reference materials of known reduced modulus, E^*. We therefore measure the nano-scale properties of polymers in four ways. Here, because of space constraints we will detail just two. These being the direct route using a calibrated AFM and the second using a nanoindenter and reference materials of known modulus.

In the direct method for the AFM, the calibration includes the tip shape, the cantilever deflection and the spring constant. In our work, the AFM tip is first deliberately blunted by scanning quickly and repeatedly over silicon. This provides a tip that is constant throughout the duration of the indentation measurements. The tip shape is measured by scanning over very sharp silicon spikes. This generates an image that is essentially that of the tip rather than the sharp spikes. In one case, for example, the AFM tip is found to be effectively cylindrical in shape with a radius of 176 ± 20 nm. The spring constant of the cantilever is measured by landing it on a pre-calibrated cantilever of known spring constant[4]. From the result of that experiment and the result of pressing the test cantilever onto a very stiff sample, the spring constant of the cantilever can be determined and is found to be 125 ± 12 N/m. For this study, nine polymer samples are chosen with moduli between 0.1 and 3.5 GPa. Their bulk moduli were measured using dynamic mechanical analysis (DMA). For nano-scale modulus, the AFM tip is pressed into the sample and a force-displacement curve produced. This is converted into a force against indentation depth curve by subtracting the position of the original surface, obtained from pressing the cantilever tip against a very stiff sample where no indentation occurs. From fitting a function dependent on the tip shape to the force-indentation depth curve, the nano-scale modulus can be determined as a function of depth. Four of the nine polymers can be analysed using the AFM with this cantilever. Higher modulus materials require a stiffer cantilever. In general, the cantilever needs to

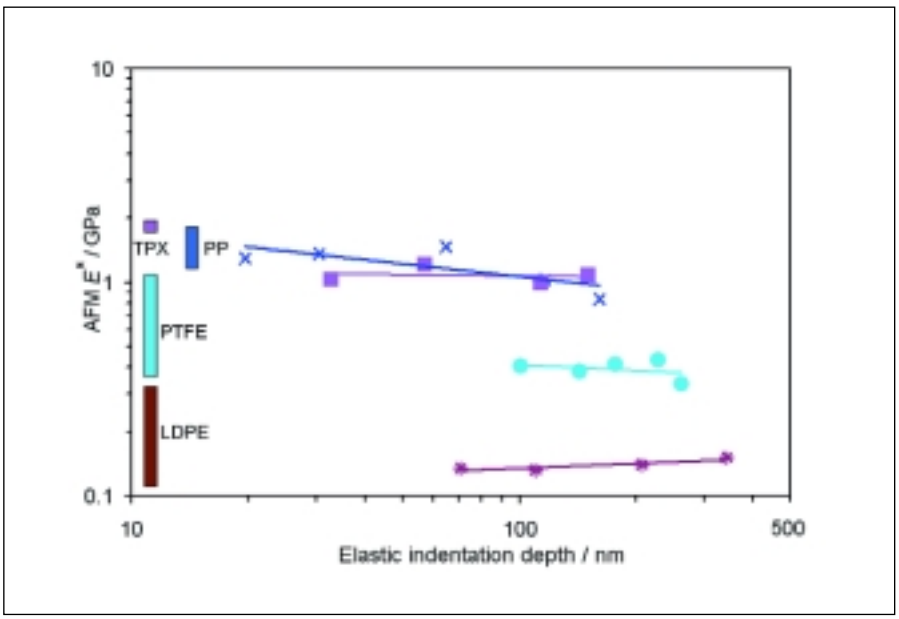

Figure 2: Direct measurement of nanoscale reduced modulus, E^*, by AFM for PP(x), TPX(■), PTFE(●)and LDPE(★).

Inset to the left are the manufacturer's bulk modulus values.

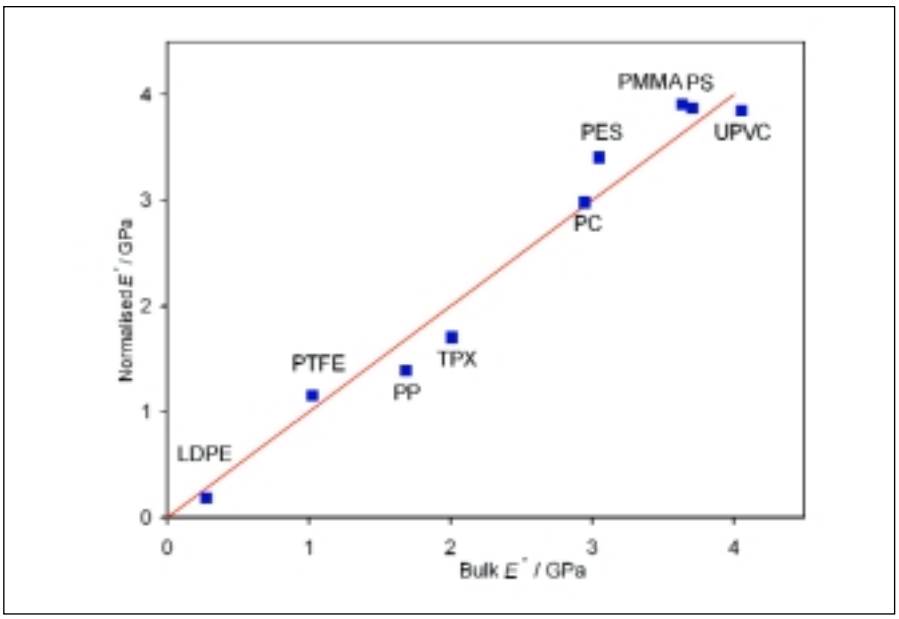

Figure 3: Measurement of E^* for the nanoindenter using reference materials at all depths between 140 – 400 nm referenced to the bulk modulus values.

The solid line shows an ideal correlation.

be matched to the stiffness of the sample. The AFM determined values for the nanoscale modulus for four polymers are shown in Figure 2. This shows little variation of reduced AFM modulus with depth and is in broad agreement with the range of manufacturer's values for each polymer shown to the left. However, the AFM nanoscale reduced modulus values are currently 33% lower, on average, than those of the bulk reduced reference moduli and 35% lower than the centre values of the manufacturer's reduced modulus values shown to the left in Figure 2. There are a number of reasons why the nanoscale modulus is lower, the most significant of which is the non-ideal cylindrical nature of the AFM indentation tip. This bias is being evaluated to define the accuracy of any absolute result and also of the depth dependence. Critical issues here are the determination of the tip shape and the measurement of the cantilever spring constant. This latter issue is dealt with below.

The second route, the reference material method, does not rely on absolute calibration of an AFM or nanoindenter but instead it relies on the use of reference materials of known reduced modulus. It is important, however, that the key instrumental parameters remain constant throughout the measurements. For the AFM, this includes the spring constant and the tip shape. For the nanoindenter, whose results are detailed below, this includes the tip shape and the nanoindenter instrumental constants calibration parameters. The nine polymer samples mentioned earlier are used as reference materials and are assumed to be homogeneous and to have a known reduced modulus as a function of depth. Figure 3 shows the calibration plot for the nanoindenter for nine polymers. An excellent fit is obtained for eight polymers with a root-mean-square relative uncertainty of 12%. The relative error of LDPE is ignored as it has a very low nanoscale modulus value. The bias seen in the absolute approach via the direct method has now been removed. This is an excellent plot that is independent of specific knowledge of the tip shape and other calibration values. From this plot, an unknown polymer may be analysed at the nanoscale, its modulus determined and, providing the moduli of the polymers to be identified or distinguished differ by more than 20%, they may be identified with 95% confidence. For depth analysis by this second route, these reference moduli need to be known as a function of depth. We recommend that users evaluate a set of reference samples using a traceable nanoindenter via the first route, and then use these to calibrate the AFM by the second route for identification of nano-regions using the AFM.

Calibrating the AFM Cantilever Spring constant

Calibration of AFM cantilevers is essential for the measurement of nanonewton and piconewton forces, which are critical to analytical applications of AFM in the analysis of polymer surfaces, biological structures and organic molecules. The AFM can be used to press into a surface to determine the elastic properties of polymers and other materials. Alternatively, the AFM can be used to pull molecules away from surfaces to examine for example, the unravelling of DNA, the unfolding of multi-domain proteins or the force associated with specific bonds breaking. All these applications need a quantitative value of the AFM cantilever spring constant, which is used to convert the cantilever deflection from a voltage signal into a force in Newtons.

Manufacturers' nominal values for the spring constant can be a factor of two or more in error. Therefore, a simple, quick and accurate calibration method is necessary. There are three principal methods to determine the spring constant; these are "dimensionally" from geometric data and materials constants, "static experiments", and "dynamic experiments"[8]. The dimensional methods involve measuring the cantilever dimensions and knowing the material properties of the cantilever to determine the spring constant either via simple equations or finite element analysis (FEA). Static experimental methods typically involve applying a constant force to the cantilever and measuring its deflection. Dynamic experimental methods generally involve finding the cantilever's resonant frequency and combining that with other measurements. This can involve attaching masses to the cantilever to measure the change in the resonant frequency. A review of the current methods available[8] has shown that the static experimental methods are best and easiest to use but still have large uncertainties. These methods are cumbersome and not very accurate. To overcome this problem, NPL has developed[5,6] a MEMS electrical nano-balance[7], microfabricated from polycrystalline silicon. The means to design and make prototype MEMS devices was developed at NPL as a Strategic Research project beginning in 2001. This ensured that by October 2003,

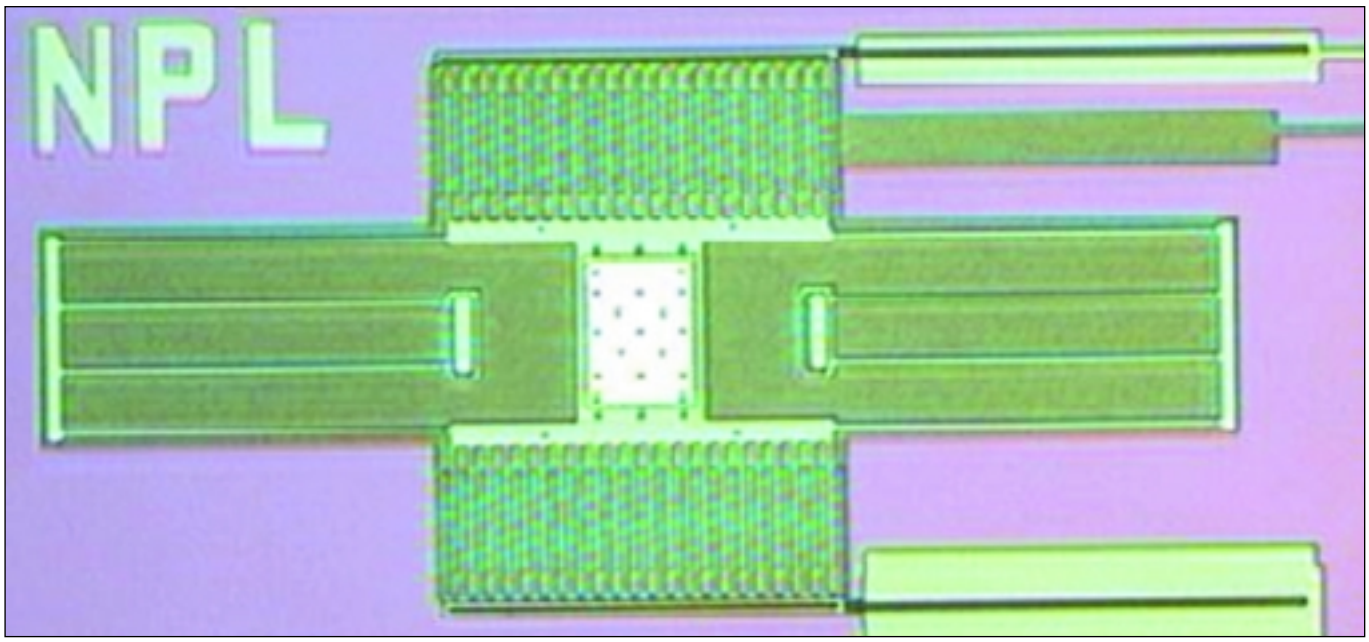

Figure 4: Optical micrograph of a prototype MEMS (micro electro-mechanical system) nanobalance device for AFM spring constant calibration[7]. The field of view is around 1 nm wide.

this capability was firmly in place to meet VAM needs. The electrical nanobalance is designed to be used to calibrate AFM cantilevers using the static experimental method. Here, the user will simply land and press their test AFM cantilever onto a nanobalance, which is traceably pre-calibrated. The nanobalance is essentially a capacitor with one fixed electrode and one moveable electrode. The moveable electrode is suspended on a spring having a spring constant similar to that of the AFM cantilever to be calibrated (see Figure 4). The basic principle of operation is inherited from work at NPL in recent years on electrical realisation of much larger forces by the Watt Balance method[9].

The calibration of the Nanobalance, to be conducted at NPL before dissemination, comprises two steps performed in vacuum and requiring only electrical measurements and optical interferometry. In step 1, the static displacement of the moveable electrode is measured as a function of d.c. potential. The force displacing the spring is proportional to the gradient of capacitance as the movable electrode is displaced. This capacitance gradient is difficult to calculate with sufficient accuracy from the geometrical dimensions of the device alone, given the relatively large fractional uncertainties inherent in micromachining. Instead, in step 2, we measure this capacitance gradient in the following way. A small a.c."dither" signal is added to the d.c. voltage to set the device into mechanical resonance, with an amplitude typically in the range 5 to 20 nm. We measure the small a.c. current through the device, and the velocity amplitude of the device simultaneously. Doppler interferometry allows us to measure this nanoscale vibration with much greater accuracy than other forms of optical interferometry (better than 1%, equivalent to an uncertainty in amplitude of less than

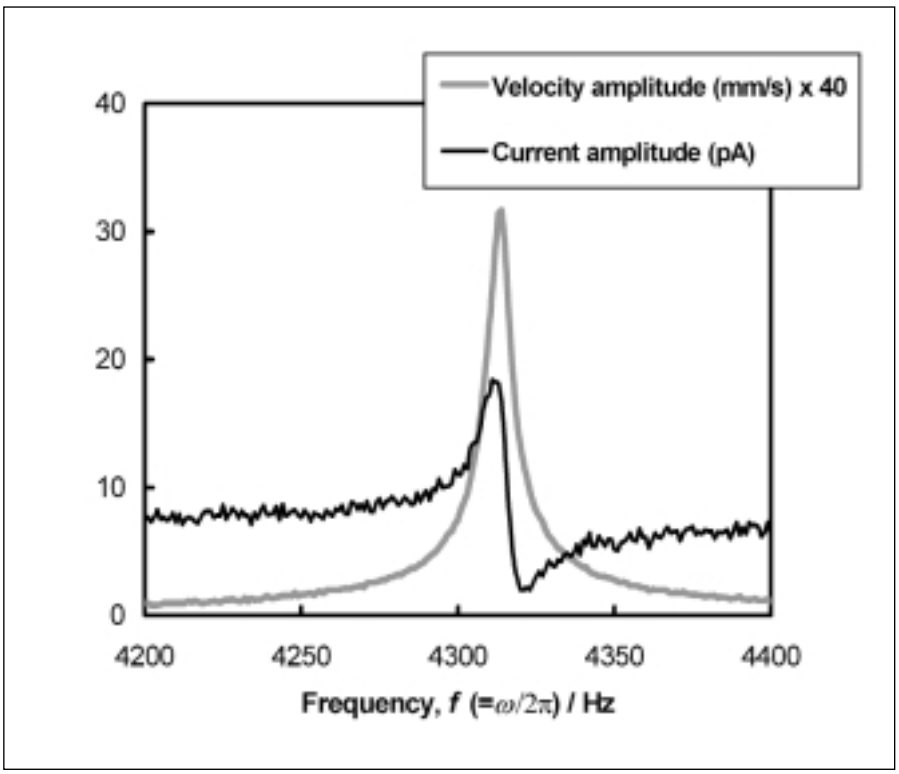

Figure 5: Normal velocity and measured current near the fundamental mode of vibration.

The changing phase of the mechanical response through the resonant peak gives rise to the feature in the measured current, which allows the capacitance gradient and hence the spring constant of the device to be found; this particular device has a spring constant of 0.193±0.01 N/m[7].

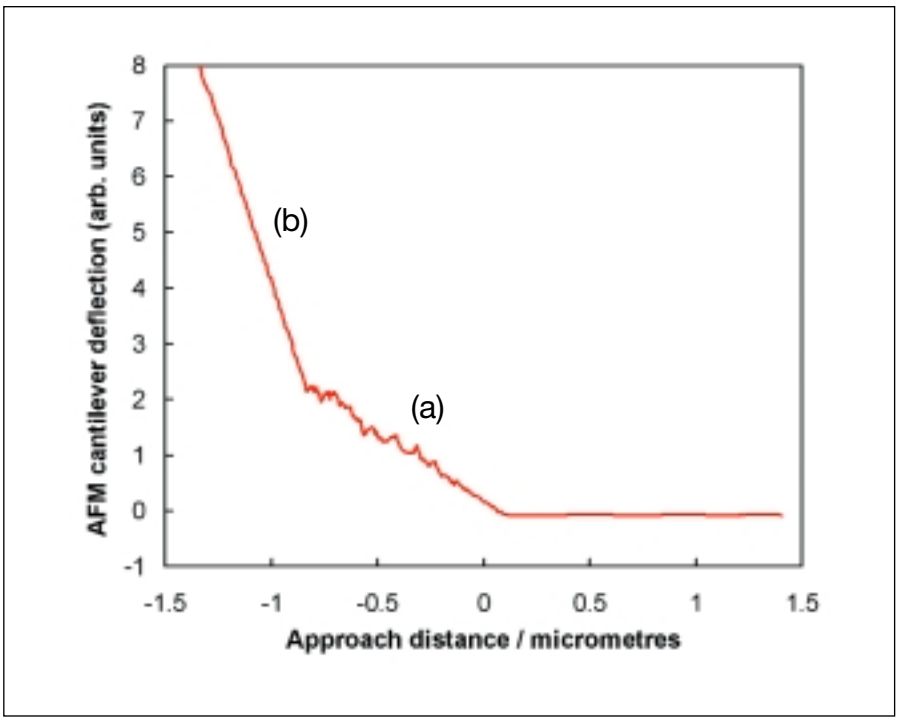

Figure 6: Approach curve generated by landing and pressing an AFM test cantilever onto a prototype NPL electrical nanobalance.

The spring constant of the test AFM cantilever is determined from the ratio of the two gradients of linear segments (a) and (b).

an Angstrom). The current arises from the change in capacitance that occurs as the separation of the electrodes of the capacitor varies (see Figure 5). Combining the dither amplitude with the measured current gives us the capacitance gradient, and hence the force on the spring. Combine this in turn with the displacement measured in step 1 and we obtain the spring constant of the spring supporting the moveable electrode. The electrodes of the capacitor are then permanently connected to each other electrically, and the calibrated device is sent to the AFM user.

The calibrated Nanobalance can then be used as a reference spring by the AFM user within their own AFM instrument to calibrate the spring constant of the cantilever under test[7]. This involves landing the test cantilever on the device and pushing onto it. This generates a curve similar to Figure 6. From the ratio of the gradients of the two slopes the spring constant of the test cantilever is determined. Devices are currently under test for robustness, shelf life and usability and should become available in 2005.

Conclusion

We have demonstrated the use of the Atomic Force Microscope as an essential and developing tool for Nano-Analysis. Two methods to calibrate the nano-scale modulus to allow identification of polymers at surfaces have been detailed. For the direct method, a variety of instrumental factors need prior calibration. The most important of these, for the AFM, being the tip shape and the spring constant of the cantilever. These parameters can have large uncertainties. The data for four polymers show a very small dependence of modulus on depth but were on average 33% lower than the reference bulk values. For the reference material method the above factors are not calibrated but instead a factor proportional to the modulus is determined by comparison with reference polymers of known modulus, obtained from the DMA results. From the nanoindenter calibration plot, an unknown polymer may be analysed at the nanoscale, its modulus determined and, providing the moduli of the polymers to be identified or distinguished differ by more than 20%, identified with 95% confidence.

To improve upon the calibration of AFM spring constants, NPL has designed the electrical nanobalance. This is based on a calibrated reference spring that can be made traceable to the SI. The electrical nanobalance is straightforward to calibrate, either by an AFM manufacturer or a calibration laboratory using a combination of Doppler velocimetry[10] and electrical current measurement. By matching the spring constant of the electrical nanobalance to the approximate spring constant of the AFM cantilever under test, it should be straightforward to design electrical nanobalances capable of calibrating AFM cantilevers having spring constants between 0.01 N/m and at least 90 N/m with relative ease and higher accuracy than available to date.

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Biometrology for Atomic Force Microscopy Imaging

Claire Madden
Molecular Profiles Ltd

Introduction

Arguably the biggest advance in imaging since the introduction of electron microscopy is the development of scanning probe microscopy (SPM). Of this family of instruments, Atomic Force Microscopy (AFM), developed by Binning and co-workers in 1986 has emerged as the most versatile technique. AFM provides nanoscale resolution imaging of surfaces for both conducting and insulating materials without the need for complex sample preparation. Additionally the imaging environment is highly flexible enabling most systems to be studied in air, under solution, at controlled temperature and humidity.

Advances in the structural understanding of biological systems are governed by the use and development of such sophisticated instruments. AFM offers a powerful technique for studying biological samples, primarily because it can achieve single molecule resolution in pseudo-native environments. The temporal resolution of the technique also allows dynamic biological processes to be probed and visualised. However, due to the complex interplay of forces that frequently occur between an AFM tip and biological samples, validation and metrology is complicated and hence is one area of focussed research. For example, Cumpson and Hedley[1] have worked towards improved instrument calibration for biological measurements, where the force of interaction between probe and sample typically falls in the nano-picoNewton range. Under the VAM programme they have developed an electrical nanobalance enabling the accurate and traceable spring constant calibration of AFM cantilevers (see page 21).

Here we explore the complementary development of reference standards to enable the rigorous exploration of the effects of AFM imaging parameters on apparent single biomolecular dimensions. This 12 month research project was undertaken as part of the DTI's Measurement for Biotechnology (MfB) Programme.

AFM Basics

The basic components of an AFM are displayed in Figure 1a. During imaging, a sharp probe, typically made of silicon or silicon nitride and mounted on the end of a flexible cantilever is raster scanned across a sample surface with the aid of a piezoceramic crystal. The forces of interaction between the probe and surface are monitored through the accurate measurement of the deflection of the cantilever as the probe interacts with features on the sample surface. The extent of cantilever deflection can be determined by a number of methods with one of the most common systems reflecting a laser off the back of the cantilever and measuring the angle of reflection using a position sensitive quadrant-diode photodetector. From this measurement a three dimensional image of the surface topography can to be generated.

AFM can be operated in a number of different modes depending on the nature of the interaction between the probe and surface. For imaging biomolecular species, Tapping Mode™ AFM, combines the high-resolution capability of contact mode with the nondestructive nature of non-contact imaging. Here the probe vibrates close to its resonant frequency, as in non-contact imaging. However, the amplitude of the oscillations are large enough to allow the probe to periodically contact the surface. During this intermittent contact, forces acting between the probe and sample cause a change in the vibrational characteristics of the cantilever (amplitude, phase and resonance frequency). For example, energy loss due to the probe contacting a high surface feature causes a reduction in the probe oscillation amplitude. This change can be continually monitored during scanning and translated into a topographical three-dimensional image, (Figure 1b).

A basic requirement for successful imaging of any single or multi-component biomolecular system is the immobilisation of the sample to a rigid substrate in discrete entities, avoiding aggregation. This can be achieved through chemisorption or physisorption and has been a topic of research for many years. For example, a generally accepted methodology for DNA adsorption is the inclusion of low levels of transition metal salts to help loosely tether

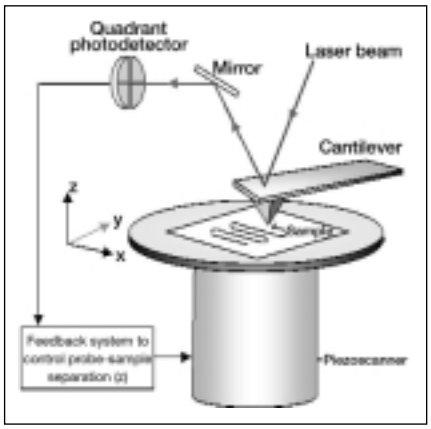

Figure 1(a): Schematic representation of the atomic force microscope.

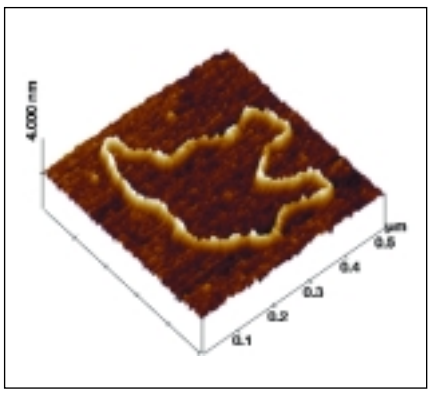

Figure 1(b): Resultant image of a single molecule of plasmid DNA, imaged in air by tapping mode AFM.

samples to atomically flat samples such as mica through electrostatic forces of interaction[2].

Towards reference standards for bimolecular AFM imaging

For crystalline and hard physical materials, the high signal-to-noise ratio achieved with AFM can provide image resolution at the atomic scale and as such, well-characterised AFM reference and calibration standards exist. In contrast single biomolecular species can be considered mechanically "soft" often resulting in a complex and convoluted response to imaging depending on a combination of factors including environment, supporting substrate, AFM tip type and instrumental set-up. Hence, applicability of traditional standards and imaging guidelines are limited and no recognised biological reference materials or validated guidelines exist to assist measurement.

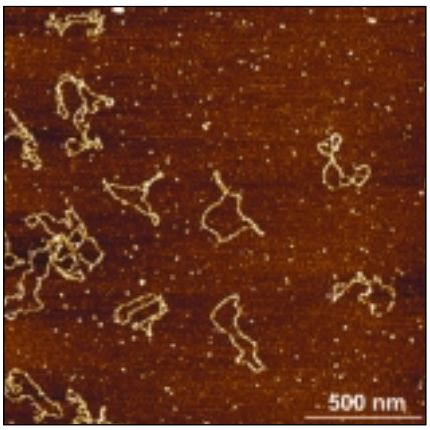

Figure 2(a): Typical deconvoluted AFM image obtained for plasmid DNA (and Au Nanoparticles) adsorbed onto mica and imaged under solution, z scale = 5 nm.

Clearly, solving difficulties in the convolution of tip shape and tip induced structural distortions with soft biological samples will allow the potential of AFM to be fully realised. Here variability in image quality and biomolecular dimensions for two systems has been considered. Firstly a simple one-component system, based on DNA and studied in air and secondly the development of a novel three-component system for imaging under solution. In the latter system, the three components include incompressible colloidal gold, plasmid DNA and a bio-responsive protein. Each component was incorporated to respond to AFM imaging in a different but complementary fashion to enable deconvolution of tip geometry and the subsequent study of the effect of instrumental parameters on apparent dimensions. Criteria for the exact selection of the reference materials were based on universal availability, and well-characterised systems existing in the literature. Probe geometry and instrumental parameters such as scan speed, size, drive amplitude and feedback gains were all varied systematically.

All data were recorded in Tapping Mode™ on a Digital Instruments Nanoscope IIIa AFM, utilising a liquid cell for solution work, size E scanner head and either triangular contact mode silicon nitride tips for solution, (Santa Barbara, CA) or Tapping Mode™ etched silicon tips for dry conditions (Olympus).

The incorporation of plasmid DNA into a reference standard provides both a check of spatial calibration and effect of tip geometry. Here, the well-characterised double stranded circular pBR322 from *E. Coli* (4365 bp, ca 1.5 µm contour length, 2.4 nm diameter) has been used. The theoretical three-dimensional structure falls within the size range that is most affected by the finite size of the probe. This can lead to systematic enlargement of dimensions and poor lateral sample tracking as a function of scan speed. For example the diameter of plasmid DNA has been reported in the AFM literature to vary from 3.5 to 24 nm, apparently 2 – 12 times wider than theoretically expected[3,4,5].

With the instrument fully calibrated according to the manufacturer's recommendation the DNA contour length was assessed as a possible internal x-y check of the instrument calibration. The contour length varied by up 3% depending on the scan size where four scan sizes between 1.5 x 1.5 µm to 10 x 10 µm were studied. Figure 2a gives a typical AFM image obtained for the plasmid DNA. Average height and width measurements were observed to be 1.1 ± 0.3 nm and 9.2 ± 0.3 nm respectively under solution and 0.7 ± 0.1 nm and 12 ± 2.1 nm respectively for DNA imaged under dry conditions. Noticeably this exhibits the expected deviation from the theoretical value of 2.4 nm. This can be attributed to factors such as residual salt layers present on the mica surface, electrostatic interactions between tip and DNA and complicated image convolution of tip and biomolecule even after algorithm deconvolution.

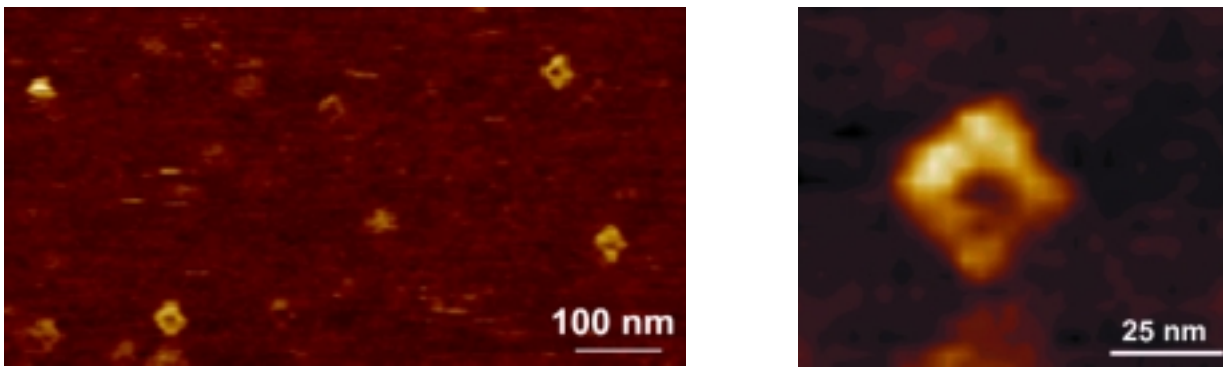

Figure 2(b): AFM image of GroEL molecules under solution, deposited onto a mica substrate as single molecules rather than 2-D arrays, z scale = 4.3 nm.

The presence of globular proteins on a reference standard is equally as important. At the single molecule level, rather than protein arrays, high-resolution imaging becomes difficult without chemical fixation, due to the greater susceptibility of macromolecular structures to lateral and vertical forces of the probe. GroEL, the chaperonin of *E. coli*, is a suitable reference biomolecule for AFM studies due to its well-defined shape. It is a supramolecular complex composed of 14 identical subunits arranged in two sevenfold symmetric rings stacked in the form of a barrel[6]. The height of the barrel is 14.6 nm, the diameter 14 nm, while the diameter of the internal cavity is about 4.5 nm. The combination of lateral convolution and tip penetration into the cavity of GroEL offers a direct evaluation of the tip convolution effect on images of macromolecular samples. Here the lyophilised GroEL, Chaperonin 60 powder, containing Tris buffer salts, was simply diluted with water (2 µg mL^{-1}) before deposition to a mica surface (Figure 2b). Average diameter, height and inner diameter was observed to be 30.2 ± 1.5 nm, 2.4 ± 0.2 nm and 8.5 ± 1.7 nm respectively, indicating significant lateral and vertical distortions exist for such responsive materials.

The inclusion of incompressible colloidal gold nanoparticles (5 nm diameter) (Figure 3a) is two-fold: firstly acting as an internal check of the instruments z calibration and secondly as a robust method for assessing the tip geometry in-situ[7]. The latter can be achieved through basic methods similar to reported blind reconstruction methods[8] here using SPIP tip characterisation software, version 3.1.0.3 (Image Metrology A/S 1998-2003, Lyngby Denmark) (Figure 3b). Robust to repeat imaging and stable in a number of environments the co-adsorption of spherical colloidal gold particles together with the biomolecules has allowed reconstruction of the tip geometry and subsequent correction for widening effects. Under optimised preparation procedures the deposition of the three components with regions of individual entities can be achieved and deconvolved for tip geometry (Figure 4).

With reproducible systems in place it has been possible to start to correlate observed molecular dimensions for these well-characterised species as a function of specific instrumental factors. Scan rates for both

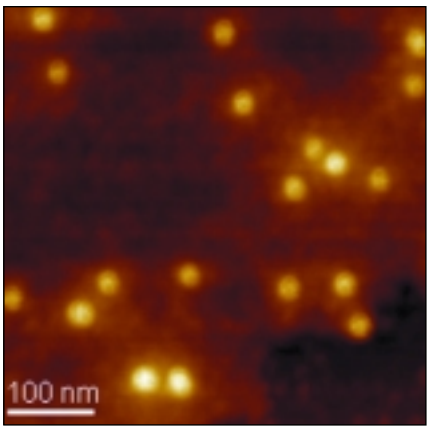

Figure 3(a): AFM images of Au 5 nm particles on mica taken under deionised water, z scale = 7.4 nm.

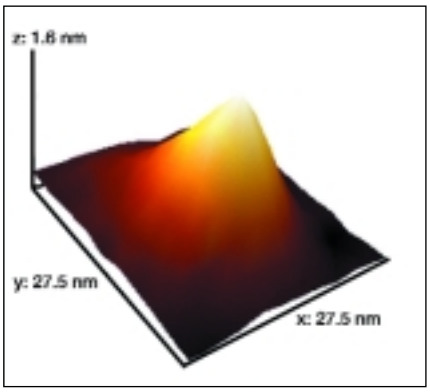

Figure 3(b): Blind reconstruction of tip profile obtained from gold nanoparticles.

systems have been varied from 0.5 – 15 Hz to assess tip tracking as it moves across a sample (Figure 5a). It is observed that an increase in gold and DNA width occurred with higher scan rates as expected due to reduced accuracy of tip tracking. GroEL shows little change in width with increasing scan rate, however the average overall width (measured at half-height) for the protein was observed to be 30.2 nm ± 1.5 nm, over two times the expected value. This indicates that the lateral force experienced by the loosely bound macromolecule is not trivial and can result in a significant image artefact. Generally a scan rate between 3 – 7 Hz gave optimum imaging whilst not being so slow as to result in lateral drift causing unacceptable distortion in imaging.

One of the most important operational parameters in tapping mode is the oscillation amplitude and set-point which controls the level of interaction of the tip with the sample and is therefore critical to measurement accuracy of "soft" biomolecules. The tapping strength can be expressed as r_{sp} in the equation:

$$r_{sp} = A_{sp} / A_0$$

Where r_{sp} is the set-point ratio, A_{sp} is the set-point amplitude (cantilever oscillation amplitude to be maintained during imaging by the feedback loop) and A_0 is the drive amplitude. Commonly defined parameter ranges are r_{sp} = 0.8 - <1 for light tapping, r_{sp} = 0.7 - 0.5 corresponds to moderate tapping, and r_{sp} < 0.4 is considered hard tapping. In addition, increasing the drive amplitude whilst maintaining a constant r_{sp} will also increase the strength of tip-sample interaction. Figure 5b gives an indication on the effect on image quality whilst Figure 5c indicates the effect on measured dimensions for the plasmid DNA. Generally it is observed for both systems that even under optimised conditions and after tip deconvolution the measured dimensions for the components will vary significantly to theoretical and x-ray crystallographic determined values. Care must therefore be taken in the analysis of biological based systems when quoting

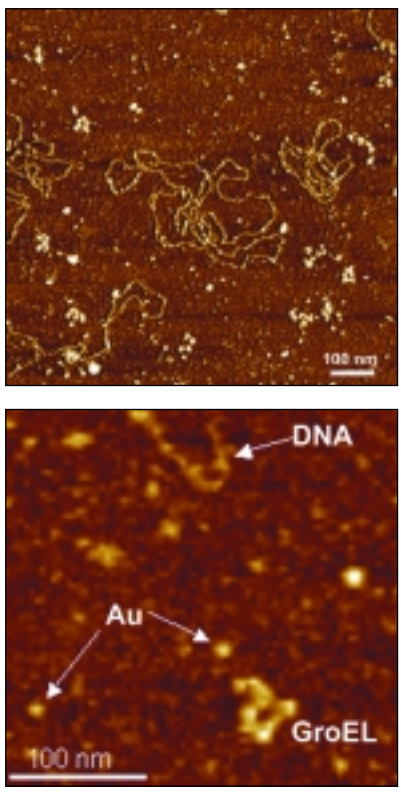

Figure 4: Deconvolved AFM images of combined gold, DNA and GroEL entities on a mica surface, z scale = 4.5.

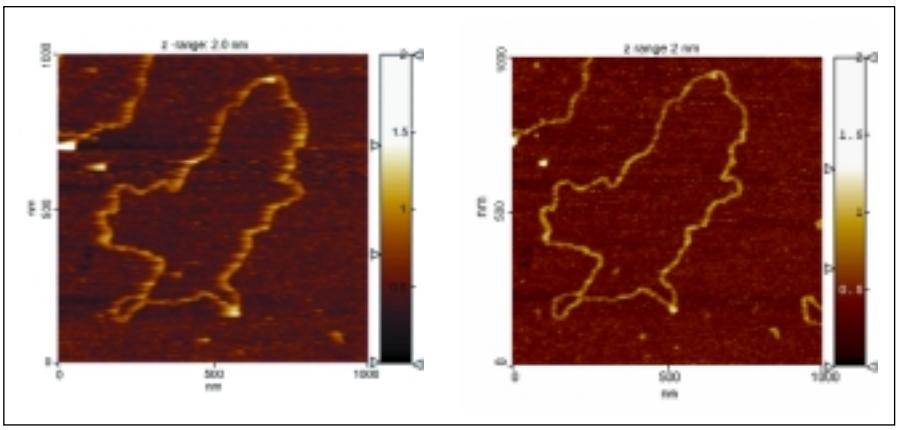

Figure 5(a): Left: poor tracking at 15 Hz. Right: improved tracking at 7 Hz.

measurements of complex samples should indeed remain a goal for future research.

Acknowledgements:

The author wish to acknowledge those who have collaborated on this half of the programme of research. Acknowledgement is made to Dr Clive Roberts and Dr Stephanie Allen at the School of Pharmacy, The University of Nottingham and Dr Jianzin Zhang and Dr Hosam Abdelhady of Molecular Profiles Ltd. Additionally we would like to thank the DTI for sponsoring this research.

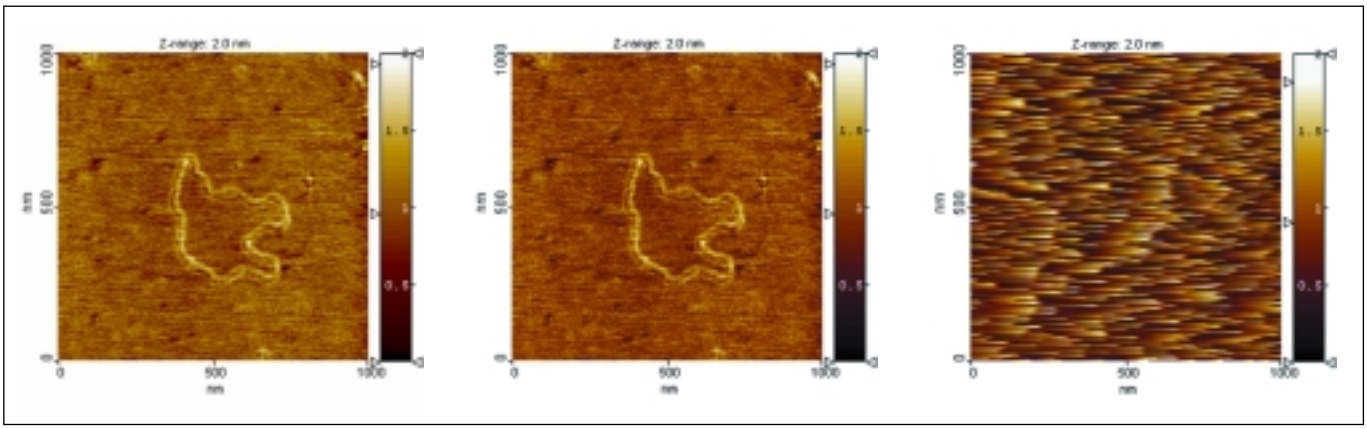

Figure 5(b): AFM images comparing the effect of oscillation amplitude and set point on image quality (left: hard tapping, middle: light tapping, right: poor sample contact).

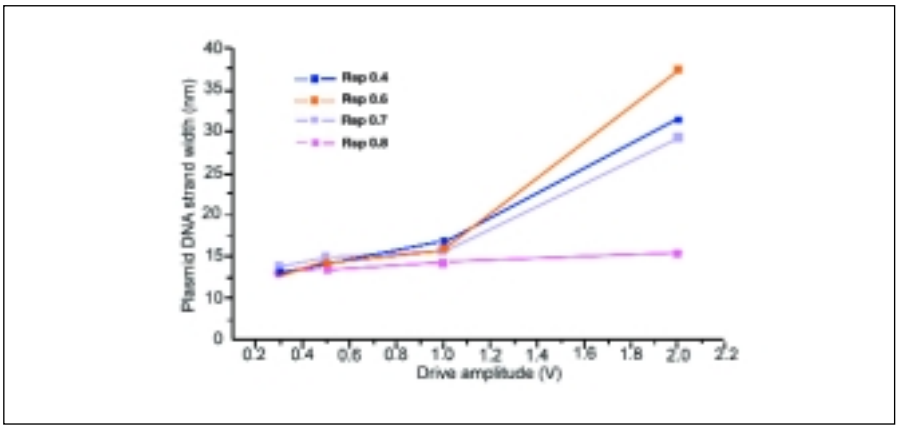

Figure 5(c): Effect of the set point and drive amplitude on the observed width of pDNA.

measured dimensions, and comparison to a standard validated system with species of known dimensions would be advantageous.

The complex interplay of forces with biomolecular species will have significant impact on measured dimensions even after careful optimisation. Single component standards can be used to generate optimised imaging parameters and measurement limits for standard operating systems. Multi-component systems, can be achieved, but emphasise the unique nature of biomolecular species in response to AFM analysis. Additionally with images, especially in the size region of single biomolecular species, being a complex convolution of sample and tip, the need for routine tip deconvolution should be promoted. Well-characterised and validated reference materials against which to compare

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MBE for VAM Working Group member

Don Munns [Picture], a key member of the VAM Working Group, which advises DTI on the VAM Programme, has been awarded the MBE in the Queens Birthday Honours list. The award is principally for his work on international standards for measuring air pollution, as well as his work at the Environment Agency and its predecessors.

Don joined the Industrial Air Pollution Inspectorate in 1976, as Technical Assistant to the Chief Inspector and manager of six teams responsible for monitoring emissions from major industrial processes in England and Wales. He progressed to District Inspector (Luton) and Manager for the Anglia Region of Her Majesty's Inspectorate of Pollution (covering about a fifth of England and Wales). For many years Don was also chairman of the Minerals and Incineration Industry Group (MINIG), and presented many papers throughout Europe and America on incineration. Following the formation of the Environment Agency Don was promoted to National Manager for Integrated Pollution Control, Regulatory Policy. Don now works as a Pollution Officer responsible for regulating major industrial sites in North London, Bedford and Hertfordshire.

Since 1974, and completely separate from his day job, Don has been a member, and for the last 15 years, Chairman of the British Standards Institute Committee, EH2: responsible for four sub-committees on air pollution measurement methods. Don was Leader of the UK delegation to annual meetings of CEN/TC 264 (The Technical Committee of CEN, Comite European de Normalisation/The European Committee for Standardisation on air pollution measurement methods), and ISO 146 ('International Organization for Standardization' committee for air measurement methods). In 1997 he was elected Chairman of CEN TC264 for 2 consecutive terms. As chair of CEN TC264, Don represented his committee on the European Committee, CAFÉ (Clean Air For Europe), looking at the implementation of air quality directives in Europe. He also represented TC 264 at international conferences throughout Europe.

"During his time with the [Environment] Agency and its predecessors Don's work has resulted in significant reductions in the emissions of species such as dioxins, dusts and acid rain gases from many major industrial processes, both directly through his actions as an inspector and through his management of teams of inspectors".

Extract From the citation

"Don is highly regarded by his peers for his work on improving air quality and particularly both ambient and stack emission monitoring methods in the UK and Europe. Colleagues in both the [Environment] Agency and outside, at for example, the National Physical Laboratory, pay testament to his ability to enable people from different organisations and countries to work together to make improvements to the environment".

Extract From the citation

Leading VAM scientist is awarded major international prize

NPL's Dr Martin Seah [Picture] has been awarded the International Union for Vacuum Science, Technique and Applications (IUVSTA) Prize in Technology, for his contribution to the science, technology and application of surface chemical analysis. The award was presented at the recent IVC-16 conference in Venice, where Dr Seah gave an invited plenary lecture entitled, "The Development of Quantitative Analysis by AES and XPS".

Dr Seah is a leading authority on surface chemical analysis with many major contributions to different projects within the VAM programme.

IUVSTA, is a union of national member societies whose role is to stimulate international collaboration in the fields of vacuum science, techniques and applications and related multi-disciplinary topics including solid-vacuum and other interfaces. Most developed countries are involved. In the UK, the member body is the British Vacuum Council that operates out of the Institute of Physics. A large proportion of the work of IUVSTA is concerned with the solid surface which many years ago was surrounded by vacuum but now this is often gas or liquid.

IUVSTA sponsors many small meetings as well as one major conference every three years. This year's conference featured 1600 papers presented in 13 parallel sessions covering topics from "Plasma Processes for Microelectronics, Semiconductors and Optoelectronics" to "Nano-Bio and Self-Assembly".

Further details can be found about the IUVSTA awards at: www.iuvsta.org/whatisIU2.html

Follow: 'Structure', then 'The IUVSTA Prizes' and '2004 IUVSTA Prize Winners'.

LGC's Steve Ellison elected as new Eurachem Vice Chair

After a ballot of Eurachem member nations undertaken earlier this year, the Eurachem General Assembly ratified the election of LGC's Dr Steve Ellison [Picture] as its new Vice Chair, at its annual meeting in Prague in May. From this position, he will automatically become Eurachem Chair in two years' time.

Dr Ellison has been involved with Eurachem for over 10 years; first as Secretary to the Eurachem Measurement Uncertainty and Traceability Working Group, then as a UK representative to the Eurachem General Assembly, and now as Eurachem Vice Chair. Dr Ellison also chairs the Eurachem Qualitative Analysis Working Group.

In response to his election, Dr Ellison said: *"I am very honoured that Eurachem members feel able to place their confidence in me as their Vice Chair."*

Established in 1989, Eurachem is a network of laboratories in 31 European nations, working in co-operation to establish an internationally recognised system of traceability for chemical measurement and promoting good quality practice. During the past 15 years, it has made a significant impact on the European analytical scene, by providing internationally accepted guidance on subjects including measurement uncertainty, method validation and accreditation for chemical, microbiological and R&D laboratories. It also works in collaboration with other bodies, including AOAC International, CITAC, Eurolab and EA, on common issues and in lobbying and influencing major regulatory and political bodies; including the EU.

"Eurachem is a mature organisation with a solid reputation for authoritative technical guidance and for effective promotion of good measurement practice", said Dr Ellison. "It has a well-considered future strategy and I hope that Eurachem will continue to grow and work closely with current and new members, as well as international metrology organisations, accreditation bodies, regulators and others, to achieve wider harmonisation of measurement practices and regulations."

LGC was one of the original laboratories involved in the establishment of Eurachem. With funding from the VAM Programme, LGC provided the Secretariat function for Eurachem until 1997. In its role as the UK's National Measurement Institute for chemical measurement and bioanalysis, LGC continues to provide one of two UK representatives to the General Assembly, while others from LGC participate fully in Eurachem Working Groups and associated projects.

Upon hearing the decision of the Eurachem General Assembly, LGC's Chief Executive, Dr Richard Worswick said: *"I am delighted that Steve has been elected as Eurachem's Vice Chair. The appointment indicates the high regard members have for Steve, who has been active within Eurachem for many years. LGC supported the establishment of Eurachem in 1989 and continues to be a strong supporter of its activities."*

Incoming Eurachem Chair, Professor Dr Wolfhard Wegscheider (University of Leoben, Austria) said: *"I am very pleased to find myself working closely with Steve Ellison as Eurachem Vice Chair. Steve, like me, is a passionate supporter of Eurachem and I believe that we will work well together in implementing Eurachem's strategy for its future development, and that the organisation will be even stronger when Steve's term as Eurachem Chair is finished."*

Steve's term of office as Eurachem Vice Chair will last until spring 2006 when he will automatically succeed Professor Dr Wegscheider as Eurachem Chair.

To find out more about Eurachem visit www.eurachem.com. To participate in Eurachem activities in the UK, join the Eurachem UK Network by visiting the 'Clubs and Networks' area on the VAM Website.

GLOSSARY

AOAC InternationalAssociation of Analytical Communities (www.aoac.org)

CITAC Co-operation on International Traceability in Analytical Chemistry (www.citac.cc)

EA European co-operation on Accreditation (www.european-accreditation.org)

NPL and VAM represented at the Royal Society's Summer Exhibition 2004

Analytical scientists from NPL and Imperial College presented high profile VAM research as part of NPL's exhibit at the Royal Society's Summer Exhibition.

As the UK national academy of science founded in 1660, the Royal Society plays a crucial role as the champion of top quality science and technology to the academic, political and industrial sectors as well as increasingly to the general public. The Royal Society's Summer Science Exhibition takes place in early July each year. It offers an outstanding opportunity for visitors to discover the best of the UK's science and technology research, and for researchers to display their work in an extremely high-profile forum. The event is made unique by the chance visitors get to meet and talk to the researchers who are behind the work on display. NPL's proposal to produce an exhibit based on trace quantitative sensing work using Surface Enhanced Raman Spectroscopy (funded under the VAM Programme) was chosen from around 80 proposals. The stand was on display at the Royal Society in July and was visited by an estimated 3,500 members of the public, schoolchildren, teachers, journalists, politicians, academics and fellows of the Royal Society.

The exhibit, 'Seeing Single Molecules' aimed to highlight NPL's pioneering approach to single molecule detection using raman spectroscopy carried out in collaboration with Imperial College. The emphasis was placed on the story of the development of new ways to detect single molecules that allow researchers to probe biological processes occurring within cells, highlighting the potential impact on the quality of everyday life and demonstrating the magnitude of the scientific achievement. Through a variety of interactive, hands-on exhibits the stand conveyed the concept of the numbers of molecules around us everyday, the number we come into contact with on a regular basis and the magnitude of the challenge of seeing a single molecule in even a very small sample.

All visitors during the week were enthusiastic and engaging and received the stand and its message extremely well. Teachers, in particular, were excited about the methods of expressing the concept of orders of magnitude in science and technology, which are accepted to be difficult topics to address in education. The exhibit was an exceptional opportunity to promote the science undertaken by VAM to a wider audience and proved a great success.

The NPL exhibit with several visitors during one of the public sessions.

Reference materials producers launch new European initiative

In May this year, three major European producers of reference materials joined forces at a new level of international co-operation. The Institute for Reference Materials and Measurements (IRMM) (an institute of the European Commission's Joint Research Centre), LGC (UK) and the Federal Institute for Materials Research and Testing (BAM) (Germany) established the European Reference Material (ERM®) concept and launched it at a press conference at Analytica 2004 in Munich.

Certified Reference Materials with the new trademark ERM® fulfil harmonised quality criteria based on modern international guidelines. Speaking at the press conference, Professor Hendrik Emons (IRMM) explained the aims and opportunities of the ERM® concept. Moreover, he illustrated, using the example of ERMs for genetically modified food, how such reference materials contribute to the surveillance of the implementation of EU legislation.

Dr John Marriott (LGC) gave an overview of the range of ERMs developed to support measurements related to environment and health, with a specific focus on the development of reference materials for low sulfur fuels. In order to reduce environmental pollution, the level of sulfur in fuels is being progressively reduced by European legislation. In collaboration with the oil and petroleum industry, the ERM® partnership is developing a range of materials to help with sound analysis at the lower levels, thus supporting corresponding environmental regulations.

Professor Irene Nehls (BAM) introduced different kinds of metals and alloys as Certified Reference Materials. Two of the newest materials that fulfil the ERM® quality criteria are based on the alloys used for the production of the new Euro coins. The exact compliance with the legal specifications for these alloys forms the basis for the verification of adulteration of coinage and for the identification of coins in vending machines. Therefore, the reference materials are important instruments for coin producers to ensure the quality of their products and to control the correctness of their specifications.

For further information about this new European partnership, visit The ERM® website at www.erm-crm.org

Global Watch secondments offer vital support for UK SMEs

DTI Global Watch secondments offer small and medium-sized businesses (SMEs) in the UK financial support to acquire technology, knowledge and advanced skills from overseas organisations.

Grants of up to 50% of the overall secondment costs are available from the DTI. This financial support enables SMEs to make major, strategic improvements in their skills, capabilities and competitiveness which otherwise would normally be beyond their reach.

Global Watch is a DTI initiative, which seeks to highlight overseas technology opportunities and foster new partnerships. Global Watch secondments offer UK companies the opportunity to exploit knowledge from almost any country in the world: to date secondments have been made to over 25 countries, including: Australia, Azerbaijan, China, Cuba, Japan, Norway, South Africa, Ukraine and the USA.

Two types of secondments are available – outward and inward.

An outward secondment enables a UK SME to send a key member of staff abroad for between three and 12 months to work in one or more companies or other centres of excellence. The secondee is expected to return with new knowledge and apply it to improve his or her own business's performance.

An inward secondment gives a UK SME access to knowledge and expertise, which is unavailable in the UK or can be obtained only from abroad. The overseas secondee can spend between three and six months working in the UK business.

After completing a secondment, a business will be required to share the knowledge it has gained with other interested UK organisations, with the overall aim of increasing the flow of information and technology transfer into the UK.

Global Watch secondments – vital support for UK SMEs

Any UK SME may apply for a DTI Global Watch secondment as long as its proposed project meets the following criteria:
A project requiring the acquisition of advanced skills and/or knowledge primarily scientific or technological in nature, not readily available in the UK, needed for the development or improvement of a product, process or service by a UK-based SME and which has the potential to generate excellent commercial returns.

For more information about how to participate in a Global Watch secondment contact:

Lorna Davies
Global Watch Secondment Co-ordinator

Tel: 01367 245210
secondments@globalwatchonline.com
www.globalwatchonline.com/secondments

Publications for the analytical scientist from the Royal Society of Chemistry

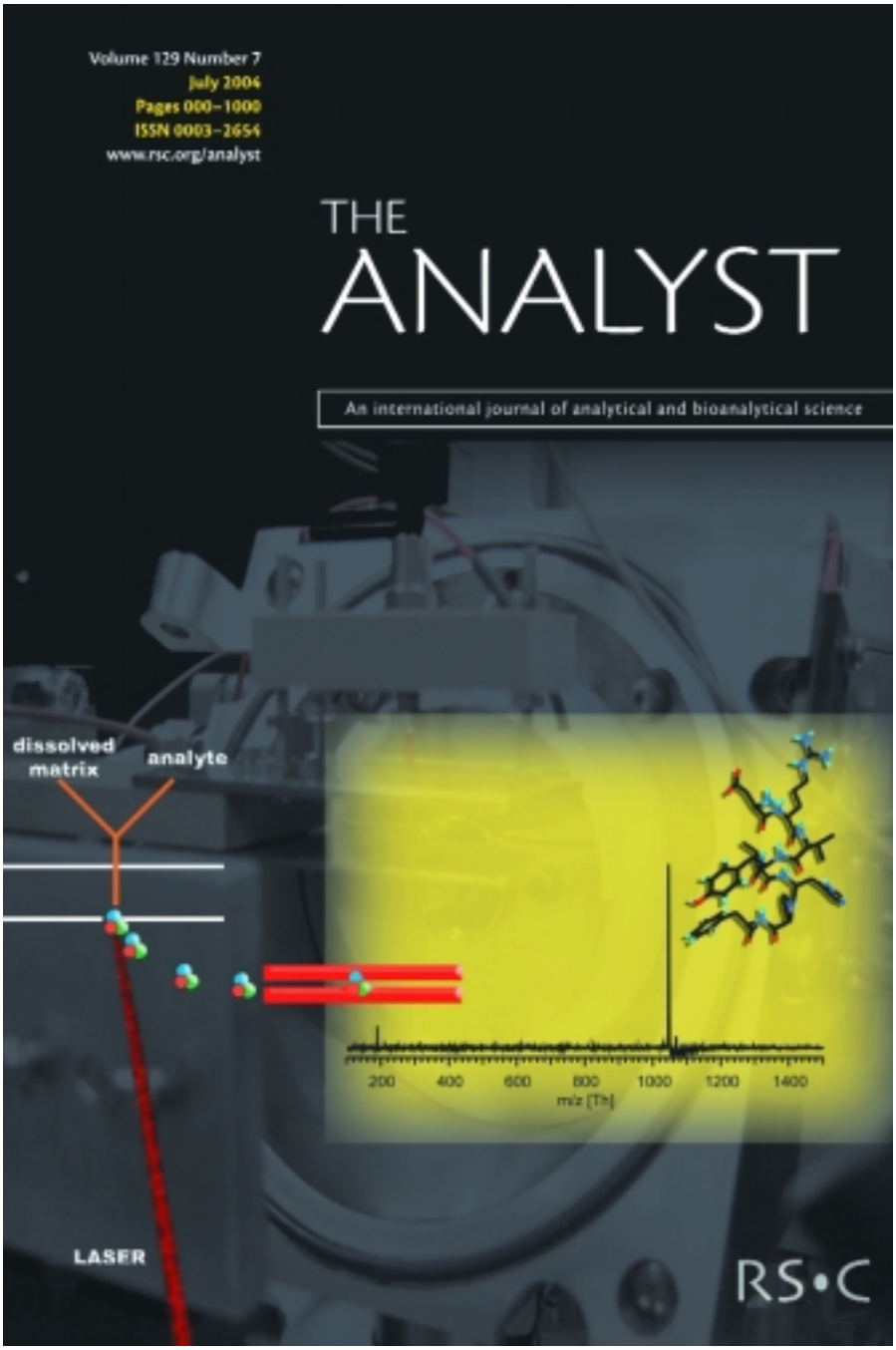
Christopher Marshall
Royal Society of Chemistry

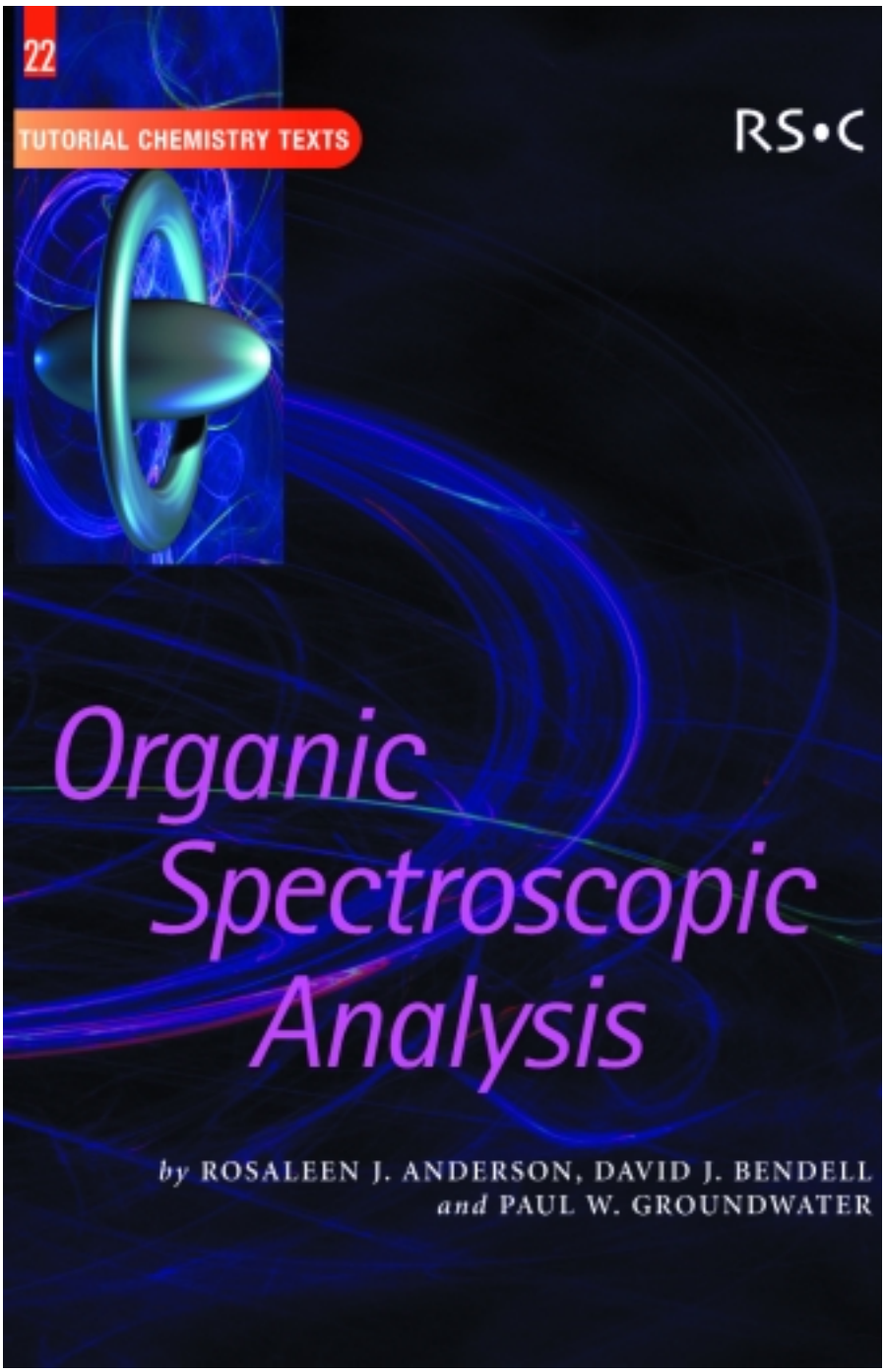
The Royal Society of Chemistry is the largest organisation in Europe for advancing the chemical sciences and has a long tradition of publishing books and journals for analytical scientists worldwide. The monthly journal, '**The Analyst**', for example, was first published in 1876.

More recent journals include the '**Journal of Analytical Atomic Spectrometry (JAAS)**', an international journal concerned with the development and application of spectrometric techniques to elemental analysis, which began in 1986, and the '**Journal of Environmental Monitoring**', which celebrates five years of publication this year.

Access to the full text of issues of 'The Analyst' published between 1876–1996 and issues of 'JAAS', published between 1986-96, is now possible via the RSC Journals Archive (www.rsc.org/archive), which enables the rapid location of information via full-text searching.

In addition to peer-reviewed journals, the RSC publishes '**Analytical Abstracts**', the only abstracting service designed specifically to meet the information needs of analytical scientists. It provides comprehensive coverage of new techniques and applications and unique indexing and is available as a printed monthly journal, with free site-wide access to the companion web database, '**Analytical WebBase**', which contains 25 years of data. More than 100 international journals are scanned for items for inclusion in 'Analytical Abstracts' (www.rsc.org/aa). Around 1,400 abstracts are included in each





monthly issue of the printed journal allowing analysts to scan the current worldwide analytical literature conveniently and rapidly.

The Royal Society of Chemistry publishes many books on analytical chemistry and spectroscopy for practitioners and students.

For practitioners there are conference proceedings, such as **Plasma Source Mass Spectrometry: Applications and Emerging Technologies**. (Edited by J G Holland, University of Durham and S D Tanner, PerkinElmer SCIEX, Canada, published 2003 in hardcover, at £99.95) and **Environmental Radiochemical Analysis II** (Edited by P Warwick, Loughborough University, UK, published 2003, in hardcover, at £99.95). There are also books in various series:

- RSC Analytical Spectroscopy Monographs; e.g. **Chemometrics in Analytical Spectroscopy** (Second edition published 2004, in hardcover, at £99.95) and **Glow Discharge Optical Emission Spectrometry** (T Nelis and R Payling, University of Newcastle, Australia, published 2004, in hardcover, at £79.95).

*"**Cyclodextrins in Chromatography** [is]...valuable for anyone working with cyclodextrins in chromatographic methods, especially readers wishing to study the overview and historical background of cyclodextrins as either a mobile phase additive or a stationary phase."*

Analytical and Bioanalytical Chemistry

Box 1

- RSC Chromatography Monographs;

  e.g. **Cyclodextrins in Chromatography** (Published 2003, in hardcover, at £99.95) and **Hyphenated Techniques in Speciation Analysis** (Edited by J Szpunar and R Lobinski, both from Centre National de la Recherche Scientifique, France, published 2004, in hardcover, at £79.95).

- RSC Food Analysis Monographs.

These regularly receive high commendation, as can be seen from the above extract from a recent book review in box 1.

The Royal Society of Chemistry also publishes VAM books, which are designed to be used by practising analysts, laboratory managers, analytical quality controllers, university lecturers and their students.

Books aimed at students include:

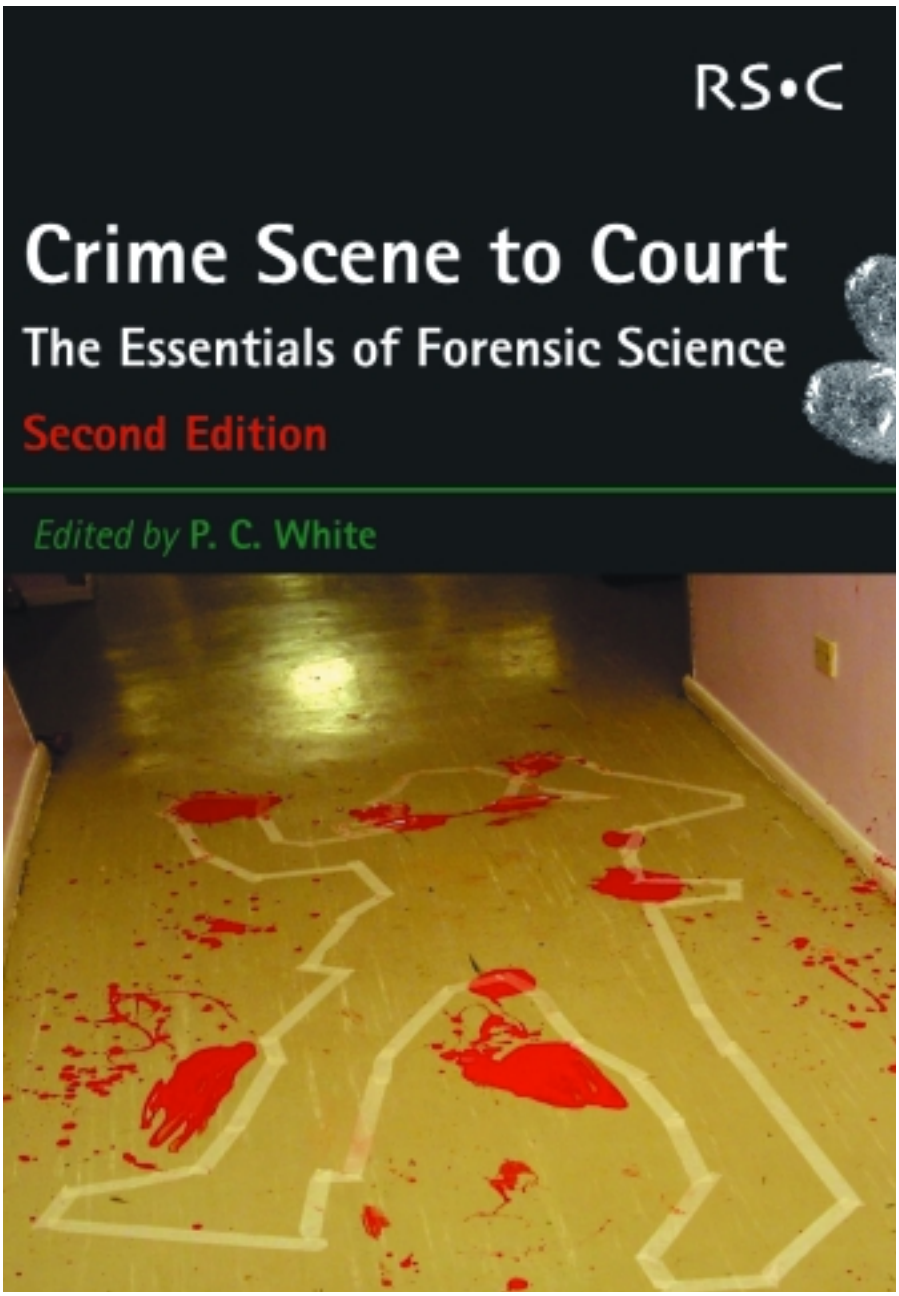
- **Basic Atomic and Molecular Spectroscopy and Organic Spectroscopic Analysis** (Both in the *Tutorial Chemistry Text* series at £14.95 each);

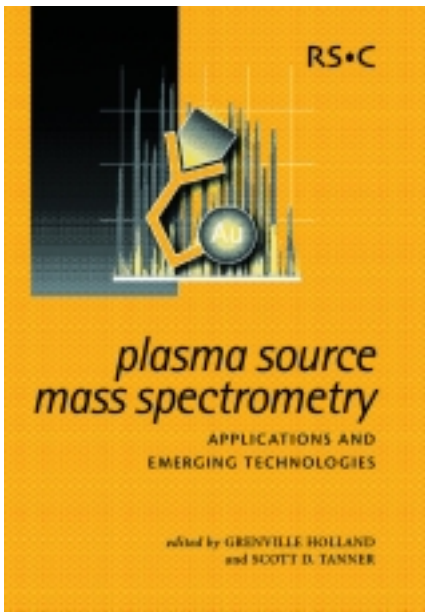
- **Statistics for the Quality Control Chemistry Laboratory** (E Mullins, Trinity College, Dublin, Ireland, published 2003, at £34.95);

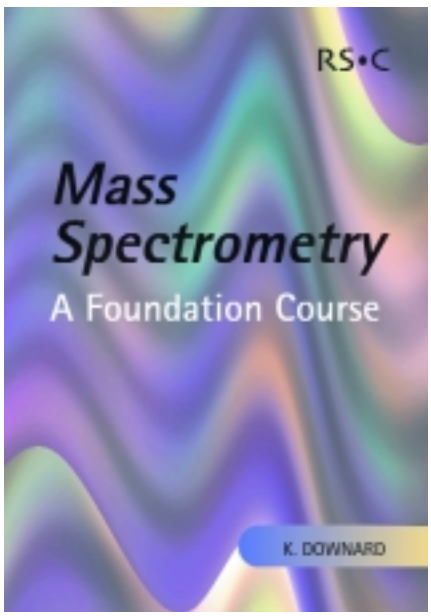
- **Mass Spectrometry: A Foundation Course**
  (K Downard, University of Sydney, Australia, published 2004, at £39.95).

Mass Spectrometry: A Foundation Course is a new textbook covering the field of mass spectrometry across the chemical, physical, biological, medical and environmental sciences. Sufficient depth is provided for the reader to appreciate the reasons behind and basis for particular experiments. Highly readable, easy-to-use and logically presented, this book is an ideal text for students.

Leading the 'bestseller' field is undoubtedly **Crime Scene to Court: The Essentials of Forensic Science**, the second edition of which has just been published in softcover, at £24.95. Since the first edition was published in 1998, **Crime Scene to Court** has been purchased in tens of thousands by forensic scientists, police and lawyers and is now used by many lecturers and students as the standard text for forensics and related courses.







A complete listing of all Royal Society of Chemistry books on analytical chemistry can be found on on the RSC's website at www.rsc.org/books. Scientists can also keep up to date with the RSC Analytical and Spectroscopy products by registering their interest on the website, www.rsc.org/CFReg.htm

All the books mentioned in this article can be ordered via:
Sales & Customer Care
Royal Society of Chemistry
Thomas Graham House,
Science Park,
Cambridge, CB4 0WF

Tel: 01223 432360
Fax: 01223 426017
sales@rsc.org
www.rsc.org/shop

Inspiring the analysts of tomorrow

Vicki Barwick
LGC

Proficiency testing competition for schools and colleges

It is important that all analysts master basic laboratory skills such as using pipettes and preparing standard solutions. To give students the opportunity to practice and improve their laboratory skills, LGC and the Nuffield Curriculum Centre organise an annual chemistry proficiency testing (PT) competition for schools and colleges. The competition has been running since 1996 and continues to grow in popularity. 2004 was the most successful year yet in terms of the number of students taking part. The competition is aimed at students studying AS/A2 or equivalent courses. The students work in teams to determine the concentration of an acid solution, supplied by Kodak Ltd Analytical Laboratories. Each team is given a z-score based on how close their result is to the actual concentration of the solution. The students also have to complete report sheets describing how they carried out the experiment and how they calculated their final result. In particular, the students are asked to describe the steps they took to ensure that their results were valid and also to think about the sources of uncertainty in their final result. The winning school is chosen based on the z-scores and the quality of the report sheets. To help students complete the activity, each participating centre is supplied with copies of the VAM resources 'Basic Laboratory Skills' and 'Introducing Measurement Uncertainty'.

For the 2004 competition, 134 sets of samples were sent out to 113 schools and colleges. We received back 339 sets of results from 80 centres. At least 1268 students took part in the competition. 66.1% of the participants achieved a z-score (absolute) of less than 2. This indicates a good experimental result. 6.2% of participants achieved a z-score of between 2 and 3. The students in this group 'could do better'. They perhaps need to exercise a little more care in their practical work to improve their results. 27.7% of participants got a z-score of greater than 3. A score greater than 3 indicates a poor result. Often, such high z-scores are nothing to do with the practical abilities of the students but are the result of transcription or calculation errors. A common mistake is to forget that the sample

was diluted before being analysed. This illustrates the importance of taking care at every stage of the analytical process.

Choosing the winners for this year's competition proved extremely difficult. There were 15 centres who were good enough to be considered for a prize. In the end it was not possible to discriminate between two of the centres so this year's prize is shared by Nottingham High School, and Gorseinon College (last year's winners). Both centres will receive a monetary prize and a framed certificate. Six other centres are worthy of a mention for their very good results and reports. Unfortunately this time they did not qualify for a prize. They are: Abingdon School, Cheltenham Ladies College, Halesowen College, Hills Road VIth Form College, Salesian College and The Perse School for Girls.

The competition continues to receive positive feedback from teachers. When asked how well the competition fits in with their course, responses included, *"Excellent fit"*, *"Perfect – I used this exercise to introduce volumetric analysis along with grades of glassware etc."* and, *"Fits perfectly with AVCE Unit 8 – Chemical Detectives"*.

The competition will be running again in Autumn 2004/Spring 2005. To register your interest please visit the Training & Education section of the VAM website.

Chemistry in Action workshop for teachers

In June, LGC and Loughborough University, with support from the RSC Analytical Chemistry Trust Fund, organised a successful hands-on workshop for teachers of post-16 science courses. The 'Chemistry in Action' day aims to give teachers the opportunity to use a range of instrumental techniques such as gas chromatography, high performance liquid chromatography, flame photometry, infra red and UV/visible spectroscopy. Analytical techniques such as chromatography and spectroscopy feature in the specifications for many science courses.

However, schools and colleges are often unable to provide teachers or students with experience of their use. As a result, many teachers have to teach a range of modern analytical techniques without ever having had the chance to gain hands-on practical experience themselves.

During the workshop the participants had to use a range of analytical techniques to solve a 'murder mystery' case. At the end of the workshop the teachers had to present their findings and agree on a solution to the case. In addition to gaining valuable experience of a range of techniques, the participants also received an extensive resource pack including a range of VAM materials to help promote quality in analytical measurement back in the classroom. The workshop received many favourable comments, with teachers welcoming the opportunity to get to grips with many of the analytical instruments that aren't available in schools and colleges.

We plan to run a similar event in 2005. Details will be published in the Events section of the VAM website.

Photography courtesy of Bryony Booth, Loughborough University

Teachers at the 'Chemistry in Action' workshop gaining hands-on experience with flame photometry.

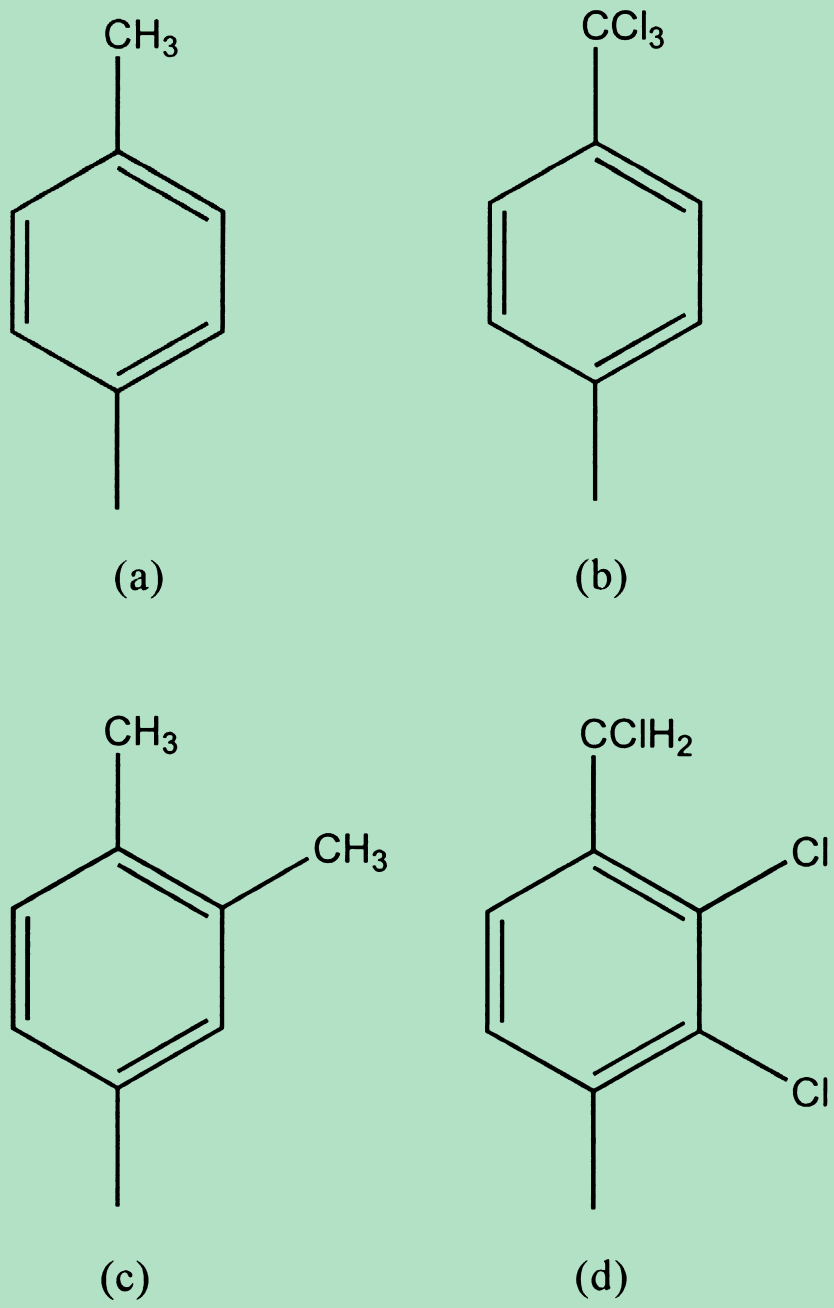
Beware of Humpty Dumpty!

Kevin Thurlow
LGC

Last time, Ted Godly examined the workings of the rule-makers in nomenclature circles. IUPAC continues to produce recommendations and the new "Preferred IUPAC Name" ("PIN") book, covering organic chemistry, should be published soon. This welcome addition to the genre should clarify some of the issues raised by the publication of the 1993 'Guide' to IUPAC organic nomenclature, which superseded some of the 1979 'Blue Book', but merely offered advice in other areas. Of course, there are many different types of chemical name, and a long systematic name is not always convenient for general use, so people do create short names. This is understandable, but there are problems if nobody else knows what you mean by a name. The 'Guide' sought to remove some long standing and popular 'trivial' names of radicals, like 'tolyl' and 'xylyl', and use the systematic alternatives.

Even with the fairly simple examples (Figure 1(a)-(c)), it may be seen that the systematic approach makes the name somewhat longer, and traditionalists preferred the old names. This is understandable, when you consider that the above examples only constitute fragments of what might be very large molecules. However, many users might be perplexed by the 'α,α,α-trichloro'! It could be much worse. Consider Figure 1(d). There is a significant difference between the Guide and pre-Guide names, and the attraction of the older system becomes very apparent.

In fairness to IUPAC, they did state that the 'Guide' was just what its name implied, and should be treated accordingly. The new 'PIN' book is intended to provide the 'preferred' name for those who want it, but allowance is made for people who want to continue with the old rules. IUPAC realises that many chemists will continue to use 'acetic acid' (likely to be replaced by 'ethanoic acid') and 'acetone' (likely to be replaced by 'propanone'). LGC has been providing



	Guide	Pre-Guide
(a)	4-methylphenyl	*p*-tolyl
(b)	4-(trichloromethyl)phenyl	α,α,α-trichloro-*p*-tolyl
(c)	3,4-dimethylphenyl	3,4-xylyl
(d)	2,3-dichloro-4-(chloromethyl)phenyl	α,2,3-trichloro-*p*-tolyl

Figure 1

IUPAC names for pesticides for many years to ISO. Many of these have 'tolyl' or 'xylyl' in the name, and this is reflected in the indexing. If we suddenly used 'methylphenyl' instead of 'tolyl', it would cause a great deal of confusion. Anybody wanting to see how many pesticides had 'tolyl' in the name would have to look up 'methylphenyl' as well. So for the sake of consistency, we have stuck to 'tolyl'. It is easier to continue to use 'tolyl' than go back and change them all to 'methylphenyl', especially as lots of lists will have the old name. It does make sense to have a single, preferred name for a chemical, but most chemicals have a variety of names or reference numbers, which are used in different circumstances. As long as you have a way of looking up synonyms, you can live with that.

IUPAC has to keep looking at the naming procedures to keep up with the practical chemists. As chemistry evolves, so does nomenclature. For example, the advent of fullerenes caused a few problems as the existing rules gave no indication of how to name them. It should be remembered that the aim of the IUPAC name is to provide an unambiguous name for a chemical structure. It is unlikely that this name will be the one you use in general conversation. Everybody will use the name 'abamectin' rather than the full systematic name, but you cannot deduce the structure from the name 'abamectin'. (There is not room for the structure and systematic name here! Interested readers may care to look them up.)

The organic committee has blazed a trail with its preferred names, and the inorganic committee is following suit. Preliminary discussions on the subject were held in late August to try to determine the best way forward. Even something as apparently simple as $CuSO_4$, copper sulfate, may be named in a variety of ways. The name 'copper sulfate' is not great, as there is also a Cu_2SO_4, so how about calling the former 'cupric sulfate' and the latter 'cuprous sulfate'? Then you have to remember the difference between '-ic' and '-ous'. You could use stock notation (copper(II) sulfate) or the charge number (copper(2+) sulfate), both of which are unambiguous here. You could even use the full systematic name (viz. copper tetraoxosulfate), which describes the structure exactly. It shows the structure has one copper, four oxygens, and one sulfur. Admittedly, it is long-winded, but it does distinguish it from Cu_2SO_4, which would be dicopper tetraoxosulfate. All this might seem unnecessary for a simple example, but could be useful with more complicated structures. For example, how many people can remember the difference between phosphinic, phosphorous, phosphonic, phosphoric, orthophosphoric, diphosphoric, metaphosphoric and hypophosphoric acids? A more systematic approach could be beneficial there. An added complication is that 'meta' and 'ortho' do not have the same meanings as in organic chemistry.

Of course the main aim is that names of any kind must be unambiguous. This is particularly important in official documents, especially legal documents. Humpty Dumpty commented (in 'Alice Through the Looking Glass') that, "When *I* use a word, it means just what I choose it to mean."

Unfortunately, many users of chemical names have chosen to emulate him. The people drafting a legal document wanted to call a very complicated compound 'azobenzene', and took some convincing that since 'azobenzene' (Figure 2) represented only a fragment of their compound, it would be confusing and just plain wrong, to call their structure by the same name. Once they realised that use of the wrong name would compromise any legal action, they bit the bullet and used a sensible name.

"When I use a word, it means just what I choose it to mean."

Similarly, 'dioxin' is widely used as a synonym for a compound, which has a dioxin ring as a small part of the structure. There is a hysterical reaction every time 'dioxin' is mentioned in newspapers, but although some 'dioxins' are extremely toxic, others are fairly harmless. One highly respected writer stated that "dioxins are organochlorine compounds", which came as quite a surprise to those of us who thought a 'dioxin' was a ring system with two oxygens and four carbons. Official sources do make some extraordinary statements - for example, "nitrous oxide is not the same as oxides of nitrogen" and "pyridine is a chlorinated nitro-paraffin". Probably the most egregious example is the following gem, "....actual methylene, that is to say raw methyl alcohol....". Methylene is a radical with the formula '-CH_2-'.

So don't let Humpty Dumpty loose on chemical regulations.

For advice on Chemical Nomenclature, please contact:
The VAM Helpdesk
LGC

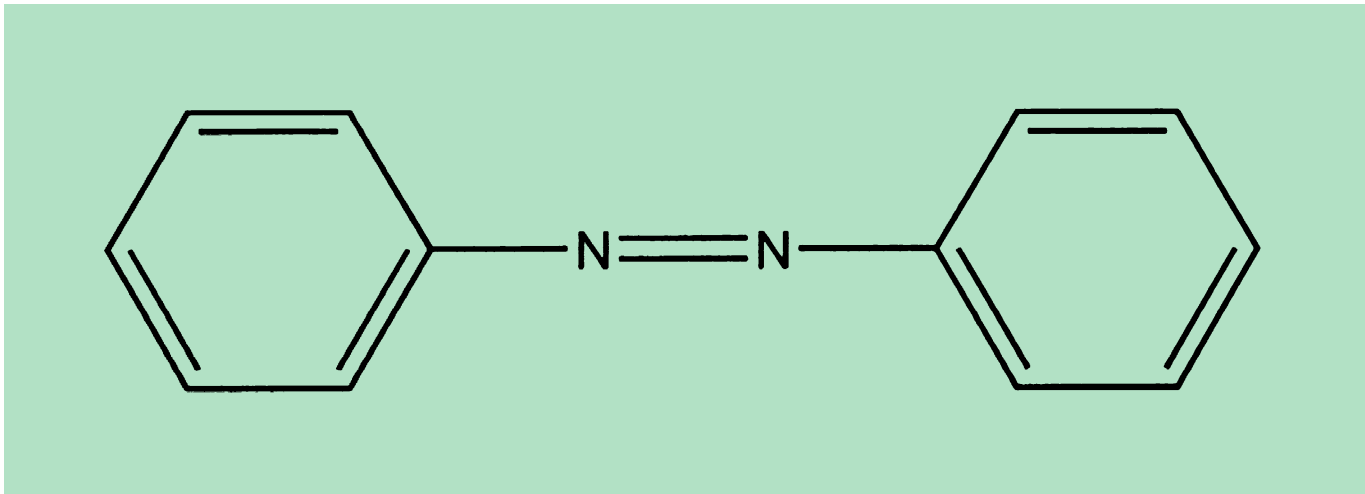


Figure 2

Nano-molecular Analysis for Emerging Technologies

Sponsored by the VAM Surfaces projects at NPL

National Physical Laboratory, Teddington

2–3 November 2004

Across Europe there are new funding initiatives for micro and nanotechnology as well as emerging technologies such as tissue engineering, diagnostic arrays and sustainable technologies. The analysis of complex molecules at surfaces at the highest possible spatial resolution is critical. At this meeting, therefore, we focus on the frontier issues for SPM nano-analysis and SIMS measurement as well as state-of-the-art capability. The meeting will engage analysts from both industry and academia.

The meeting will cover the following topics:

Day 1: Frontiers in SPM Nano-Analysis

- Advances in SPM imaging techniques
- Nano-mechanical and Nano-chemical analysis
- Spectroscopic methods using SPM
- Protein unravelling and Molecular pulling

Day 2: Frontiers in SIMS measurement

- Rapid and reliable data interpretation, PCA, neural networks and G-SIMS
- Molecular structure, orientation and identification of molecules at surfaces using SIMS
- Cluster ion beams: Potential benefits and applications
- Measurement requirements and capability development for biological and bio-nanotechnology surfaces

Evening Plenary and Poster Session

After the oral presentations on the 2nd November, a dedicated poster session and manufacturers' exhibition will be held. There will also be a plenary talk, which will be given by **Professor George Smith FRS (University of Oxford)** entitled, *"Total analysis of nano-volumes"*, before the conference meal.

For further details please contact:

Charles Clifford
National Physical Laboratory

charles.clifford@npl.co.uk
www.npl.co.uk/nanoanalysis

LGC's analytical quality training programme

Analytical quality is of paramount importance to anyone making chemical measurements and to those making decisions based on the results from such measurements. There are increasing burdens on companies to meet regulatory, trade and quality requirements and this has resulted in greater emphasis on method validation, measurement uncertainty and traceability. This is endorsed by the international accreditation standard ISO/IEC 17025, used in the UK by UKAS as a basis for laboratory accreditation. The standard contains detailed requirements for these topics. Evidence of training and competence in the topics mentioned is a requirement of the standard and of customers of analytical results.

The range of courses offered under LGC's analytical training programme is designed to meet the increasing need for laboratory managers and analysts to demonstrate competence and to keep abreast with quality assurance issues and practises. New courses are regularly added to ensure that the training programme continues to meet the needs of the analytical community.

All the courses consist of lectures and workshop sessions, providing opportunities for group discussions and to practise the newly acquired knowledge. To ensure maximum benefit from each course, delegates work in small groups for the workshop sessions, with a tutor present for each group. They will be run mainly in South-West London, either at LGC's facilities at Teddington or at the Lensbury Conference Centre, Teddington Lock. However, they can be customised to suit the needs of an individual company that require in-house training for a group of staff.

For further information on LGC's Analytical Quality Training Programme please contact:

Lorraine Didinal
LGC

Tel: 020 8943 7631
Fax: 020 8943 7314
training@lgc.co.uk

Achieving traceability in chemical testing

21 October 2004
19 May 2005
18 October 2005

Reliable measurements depend on competent staff, validated methods and equipment, comprehensive quality systems and traceability to appropriate measurement standards. Laboratory accreditation to ISO/IEC 17025 is a demonstration that these requirements have been met. Establishing traceability is a new requirement of accreditation and recent European directives. To achieve comparability of results over time or from one location to another, it is essential to link all the results to some common, stable reference or measurement standard. The results can be compared through their relationship to that reference. Traceability is the process of linking results to a reference. Traceability is required for results obtained from all types of method, standard, in-house and those where the results is defined by the method (empirical method).

Principles and practice of measurement uncertainty in chemical testing laboratories

9–10 November 2004
9–10 February 2005
14–15 June 2005
8–9 November 2005

The ability to estimate measurement uncertainty is now a requirement of testing laboratories accredited to ISO/IEC 17025. This course is in line with ISO principles and with the Eurachem/CITAC guide 'Quantifying Uncertainty in Analytical Measurement'. The first day introduces the principles of evaluating uncertainty and the second day goes on to provide the tools for identifying uncertainties and using validation data. The lectures and workshops take delegates through the process of evaluating uncertainty. Completion of this course should provide sufficient training to enable analysts to carry out an uncertainty evaluation for their own laboratory methods.

Using proficiency testing in the analytical laboratory

23 November 2004
26 April 2005
15 November 2005

Participation in PT schemes gives laboratories an objective, independent measure of the quality of their output, and is a highly effective diagnostic tool for a laboratory's quality system. It is therefore important for analytical laboratories to obtain the optimum benefit from participation in proficiency testing. This course will help those with the responsibility for deciding on which PT schemes are appropriate, as well as customers and auditors, to:

- understand how proficiency testing works and the different types of scheme that are available;
- select the most appropriate scheme(s) in which to participate;
- interpret and use effectively the results and evaluation from PT schemes;
- understand the statistical approaches used in proficiency testing.

Basic statistics and experimental design for biological scientists

30 November 2004
5 April 2005
6 September 2005

The application of statistical concepts to experimental data is essential for biological scientists wishing to maximise the information from experimental studies. This requires all biologists to design experiments with statistics in mind. Good experimental design and statistics are fundamental in ensuring that reliable conclusions can be drawn from a data set. This course will help biologists, who wish to design experiments and interpret data efficiently and effectively, to:

- effectively design experiments amenable for statistical analysis;
- develop understanding of significance testing and hypotheses;
- perform common statistical analyses on experimental data;
- interpret results of the analysis.

Quality systems in testing laboratories

2 December 2004
7 June 2005
1 December 2005

This course provides an introduction to quality systems appropriate for use in a testing laboratory. The main standards in use, GLP, ISO/IEC 17025 and ISO 9001 will be described and their selection and implementation discussed. Their similarities and differences will be highlighted. This course will help laboratory staff and quality managers:

- understand the benefits of a quality system;
- identify the factors to consider when selecting the most appropriate quality system to meet your needs;
- develop procedures to minimise duplication when more than one standard is in use in your organisation;
- prepare for the implementation of a quality system.

Method validation

2–4 December 2004
8–10 March 2005
19–21 July 2005
6–8 December 2005

Method validation is the process that provides evidence that a given analytical method, when correctly applied, produces results that are fit for purpose. No matter how well a method performs elsewhere, analysts need to confirm that the method is valid when applied in their laboratory. There is now a much greater emphasis on method validation in the ISO/IEC 17025 accreditation standard. Through a number of workshops, delegates build a validation protocol for a method of their choice. This three-day course will also help analytical chemists and potential or existing laboratory managers who are involved in method development and validation to:

- understand method validation and its requirements;
- select and apply the statistics required during method validation;
- select and use the appropriate types of method validation studies;
- appreciate and understand the links with measurement uncertainty and equipment qualification.

Statistics for analytical chemists

24 February 2005
12 July 2005
25 October 2005

The quality of analytical data is a vital aspect of the work of an analytical chemist. The application of statistics is central to the assessment of data quality and an understanding of statistics is essential to the interpretation of analytical results. This computer-based course provides an introduction to the basic statistical tools that analytical chemists need for their work. It will help them:

- understand some of the most important statistical concepts used by analytical chemists;
- calculate the most common statistics;
- apply significance testing;
- use linear regression in calibration.

The course starts from looking at data and then explains the most common statistical parameters and how to calculate them.

UK events

Further statistical tools for analytical chemists

12 April 2005
13 July 2005
29 November 2005

The ever-increasing amounts of data generated in the course of analytical measurements means there is a greater need to use statistical tools to assess the quality of the data and to assist with their interpretation. Appropriate use of these tools improves the chances of making correct decisions. This course builds on the material in the 'Statistics for analytical chemists' course and will help analysts:

- deal with normal and non-normal distributions;
- identify cases of normally distributed data with outliers;
- calculate statistical parameters in the presence of probable outliers;
- identify where two-way ANOVA is appropriate;
- use some of the more advanced regression tools.

Laboratory News Forum

ICC, Birmingham
18–19 October 2004

The Laboratory News Forum is the first independent multi-platform event for the UK laboratory professional. Bringing together key laboratory personnel in an educational environment, where a short-course programme sits side-by-side with plenary sessions, product briefings, an exhibition and the well-established Laboratory News Awards.

LGC's Dr Steve Ellison and Vicki Barwick will be hosting two short training courses on:

- Analytical Method validation (day 1)
- Statistics (day 2)

Further information about these and other analytical training courses will be available at LGC's stand at the exhibition. There will also be an opportunity at LGC's stand (number 23) to view the latest information available from both the DTI VAM and MfB programmes.

For further information or contact:
Laboratory News Forum
Quantum Business Media
Quantum House
19 Scarbrook Road
Croydon, CR9 1LX

www.labnewsforum.co.uk

Molecular analysis: Right first time

Teddington
25–26 November 2004

This two-day workshop focuses on developing core skills for molecular biological analysis, by raising awareness of quality issues, experimental design and good laboratory practice. The course is aimed at recent graduates, but would be of benefit to most analytical scientists in the molecular biology laboratory. Consisting of a mixture of practical laboratory-based activities, discussion sessions and seminars, the course will cover PCR design, set up and performance, relevance of quality systems, statistical analysis and interpretation of results. The benefits of traceable analysis and ensuring that experiments are 'right first time' will also be highlighted.

For further information, please contact:
Jacquie Keer
LGC

Telephone: 020 8943 7449
jacquie.keer@lgc.co.uk

International events

Proficiency testing in analytical chemistry, microbiology and laboratory medicine

Portorož, Slovenia
(25)-26–27 September 2005

Organised by Eurachem (in co-operation with CITAC, EQUALM, Slovenian Chemical Society, Eurachem Slovenia, Metrology Institute of the Republic of Slovenia (MIRS), SILAB and Slovenian Accrediation (SA)), the aim of this workshop is to address current practice, problems and future directions of interlaboratory comparisons. Focus is on proficiency testing (PT) and external quality assurance/ assessment (EQA) in analytical chemistry, microbiology and laboratory medicine.

The workshop will be preceded by a training course on 25 September 2005, looking at the practical implementation of uncertainty in PT. The training course is open only to workshop participants.

Both the workshop and training course are targeted at organisers of PT/EQA schemes, technical assessors, QA managers, accreditation bodies, participants to PT and the laboratories' customers.

For further information, contact:

The workshop secretariat

Tel: +32 14 571 232
Fax: +386 3 734 21 57
eurachem.pt2005@email.si

Contact points

For advice on:

- Analytical quality assurance;
- Chemical nomenclature;
- Proficiency testing;
- Reference materials;
- Statistics.

Contact:

The VAM Helpdesk
LGC
Tel: 020 8943 7393
vam@lgc.co.uk
www.vam.org.uk

For information and advice on:

- Traceable gas standards;
- Gas analysis and analyser performance testing;
- Analysis of Gas Awareness Club;
- Gas proficiency testing;
- Trace water vapour and odour;
- Industrial process monitoring;
- Quality assurance of ambient air measurements;
- Surface and nano-analysis;
- European standards (norms) on gas analysis.

Contact:

Neil Harrison
Analytical Measurements Group
National Physical Laboratory

Tel: 020 8943 6443
neil.harrison@npl.co.uk
www.npl.co.uk/npl/environment

Further information on the VAM programme

The VAM Helpdesk
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www.vam.org.uk

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LONDON SW1W 9SS
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www.dti.gov.uk

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www.aeat.co.uk

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www.hdda.co.uk