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EMRP
European Metrology Research Programme
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1 Executive Summary

Introduction

The rate of infections and failure of implantable medical devices is relatively high and this is extremely costly, both financially and in terms of patient wellbeing. The use of novel biomaterials and surface treatments for implants can improve their interaction with the body and reliable metrology tools are required to ensure they can be manufactured effectively and certified for use. This project advanced and provided guidance in the optimal use of analytical methods for *in situ* chemical analysis and imaging of implantable medical devices such as hip liners or catheters. Specifically providing improved analytical tools to characterise thin films and buried interfaces in a reliable and traceable manner and new analytical tools with high spatial resolution and sensitivity. These enable non-analytical-experts to assess medical device advances with assured validity using state-of-art analytical tools.

The Problem

Although implantable medical devices improve quality of life for millions of people, the rates of complication, infection and device failure are unacceptably high. For example 10 % of patients using arterial stents develop severe complications, 4 % of catheter use is associated with severe infections and 10 % of artificial joints need to be replaced within 10 years. These problems can arise for a number of reasons: incompatibility with the body; introduction of infection from the implant surface; rapid wear and tear of the surfaces; or poor interaction between the implant and the body.

The Solution

Medical device companies and biomaterials scientists have developed a range of novel materials that may reduce these problems by adding: surface treatments to improve compatibility; antibacterial coatings to reduce infection; thin film over-layers and drug releasing coatings to improve their interaction and efficacy in the body. These all enable advanced product development and improved quality by precise control of near-surface chemistry, essential to ensure a reproducible biological response to implanted materials. These advances require analytical tools that are able to measure and validate the near-surface chemistry, three key areas need development to enable this:

1. Precise control of near-surface chemistry is essential to ensure a reproducible biological response to implanted materials. Since established analytical methods are often operated in high vacuum, improvements in the precision, accuracy and traceability of high vacuum nanoscale chemical images of surfaces are essential to support the industry's evolving design and manufacturing goals and ensure adherence to new regulations.
2. High vacuum analytical methods require sample preparation, have a slow turn-around and are very expensive. New tools such as ambient (non-vacuum) techniques are required that can rapidly assess faults in devices directly from the production line with little or no prior sample preparation.
3. Improved image analysis of complex geometry and mixed materials. Most medical devices consist of a wide range of geometries with grooves, loops, pins and knitted, woven or felted components that can be challenging to analyse. Various analytical methods can be used to evaluate cleanliness of medical devices, but many of the more convenient methods have limitations in terms of sensitivity or surface-specificity or are indirect methods, relying on a bulk extraction step. These methods lose knowledge of the spatial distribution, that are important to identifying how or where in the manufacturing and cleaning history that the contamination occurred and also how different surface finishes may be more or less susceptible to contamination. In addition, the analysis of explanted devices (i.e. devices removed from the body) is often required to understand the reasons for failure.

The project also developed emerging ambient techniques for in situ chemical analysis and imaging of medical devices, and applied, adapted and validated all the tools developed to real production-line medical devices.

Impact

The project aimed to advance methodologies and provide guidance in the optimal use of chemical analytical methods for *in situ* chemical analysis and imaging of implantable medical devices. As such the impact from this project needed to address a wide range of communities, from biological physicists, chemical and polymer physicists, researchers and engineers that work in the field of thin films, materials and surface physics, analytical scientists and biomedical device producers. In order to ensure this, a wide range of dissemination activities, addressing different audiences were carried out, with 11 submitted papers, 8 papers in draft, 49 presentations, 17 training events and 5 funded follow up collaborations. The 49 presentations were a mixture of oral and poster, to enable the widest audience and interactivity, and took place in over 15 countries given to an estimated audience of > 5000 participants. The 17 training events involved over 200 people including students, industrial end users, analytical specialists and research scientists.

A suite of new tools are now available at European NMIs to support medical device characterisation as well as tools and techniques suitable for research and idustrial use. These will enable improved use of chemical metrology tools for the support of biomaterial manufacture: improving reliability of analysis; application to medical devices; development of novel analytical tools; solving industrial problems. The project's outputs have been disseminated widely to: the metrology community; high-level users of precision instrumentation in research environments; regulators and medical device manufacturers.

2 Project context, rationale and objectives

The project's over-arching objective was develop the analytical tools required to measure and validate the near-surface chemistry of implantable medical devices and provide guidance on their selection and use to the medical device sector:

1. Underpinning metrology via the use of high vacuum techniques: Established high vacuum techniques, have proven indispensable in the development of advanced biomaterials but further improvements in spatial resolution, chemical speciation, reproducibility and traceable quantification (for selected methods) are needed to facilitate total quality management of devices, meet emerging regulatory requirements and more accurately control the biological response to implants. An objective of this project was to produce well characterised model systems, which represent key issues expressed by industry stakeholders. The model systems chosen were 3 different surface contaminants on both metal (representative of bone implants, for example) and on polymer substrates (representative of catheters and stents, for example). In addition model systems consisting of a protein coating and polymer thin films were also chosen (representative of the surface treatments device manufacturers employ to improve product performance). These model systems could be used to improve reproducibility and quantifiability of both the established techniques and emerging analytical methods on relevant materials.

2. Develop the use of ambient methods**:** Recent advances in ambient analytical methods offer great promise for meeting the medical device industry needs for on-line surface quality assessment during manufacture. In addition to providing detailed chemical information, these non-destructive chemical analysis tools are well suited to on-line analysis. For medical device surfaces these techniques lack the surface specificity desired, therefore new developments and advanced data analysis methods will be required to facilitate reproducible, accurate, quantitative analysis. An objective of this project was to develop emerging ambient techniques for *in situ* chemical analysis and imaging of medical devices.

3. Adaptation and validation of our analytical tool set for production line medical devices and explant analysis: the ambient methods need to be assessed against the existing accurate methodologies developed for high vacuum surface analytical methods to enable robust selections of appropriate techniques. The aim of this objective was to apply, adapt and validate the tools developed on model systems to real production line medical devices.

These objectives are of high relevance and value to the medical devices industry and will help both new product research and development and licensing, and existing product manufacture and quality control.

3 Research results

3.1 Advancing the underpinning of metrology via the use of high vacuum techniques

3.1.1 Introduction

Precise control of near-surface chemistry is essential to elicit a reproducible biological response to implanted materials. Improvements in the precision, accuracy and traceability of surface chemical measurements are essential to support the industry's evolving design and manufacturing goals. Established high vacuum techniques, including SIMS, XPS, AFM (atomic force microscopy), XRF, ellipsometry and NEXAFS spectroscopy have proven indispensable in the development of advanced biomaterials but further improvements in spatial resolution, chemical speciation, reproducibility and traceable quantification (for selected methods) are needed to facilitate total quality management of devices, meet emerging regulatory requirements and more accurately control the biological response to implants. The aim of this objective was to produce well characterised model systems which can be used to improve reproducibility and quantifiability of both the established techniques and emerging analytical methods on relevant materials. It also aimed to produce standard protocols and advanced data analysis methods to increase the precision and accuracy and improve lateral and depth resolution of SIMS, XPS, XRF and NEXAFS.

3.1.2 Develop well characterised quantitative, traceable model for relevant surface treatments and contaminants)

The aim of this research was to develop fully characterised, quantitative, traceable model systems for relevant surface treatments and contaminants. Currently, there are no available standard materials or well characterised model systems which can be used for the quantification and quality assurance of medical device surface chemistry. Nine model systems were chosen that are considered as representative of key issues expressed by our industrial stakeholders (such as Smith and Nephew and Medtronic). The model systems consist of 3 representative surface contaminants on metal (titanium Oxide, 316L stainless steel) and polymer (high-density polyethylene - HDPE) substrates. Details are given in Table 1 as model systems 1-6. In addition model systems consisting of a protein coating (model system 7) and two polymer thin films (model systems 8 and 9 were developed).

	Surface Contaminants						**Protein**	**Polymer Thin Films**	
	1	2	3	4	5	6	7	8	9
Proposed Model System (or similar)	PDMS on TiO2	PDMS on HDPE	Stearamide on 316 L stainless steel	Stearamide on HDPE	Sodium dodecyl sulphate (SDS) on TiO2	Sodium dodecyl sulphate (SDS) on HDPE	Collagen adsorbed on PTFE	High Z Doped polymer multilayers	Poly-acrylate on PDMS
Techniques	Soft and hard XRF, GIXRF, SIMS, XPS, AFM, White light interferometry, AMS, FTIR, Raman spectroscopy (SERS)						Soft XRF, GIXRF, SIMS, XPS, IR-SNOM, AFM, White light interferometry, AMS, FTIR, Raman spectroscopy (SERS)	XRF*, SIMS, XPS, SK-PFM, AFM, White light interferometry, AMS, FTIR Raman spectroscopy (SERS) *for 8 only	

Table 1 Contaminant systems that were designed and characterised.

The contaminants, N,N' Ethylene bis stearamide (referred to as stearamide or EBS from here onwards), Sodium dodecyl sulfate (SDS), and PDMS (Polydimethylsiloxane) were deposited on different relevant substrates. Stearamide is a common release agent, SDS an anionic surfactant using in many cleaning and hygiene products and PDMS occurs in lubricants and oils. They all are routinely found in the manufacture of medical devices but can cause issues with delamination of surface coatings. These contaminants were deposited on typical medical device substrates titanium dioxide (TiO_2), steel (AISI 316), silicon, and high density polyethylene (HDPE). Protocols for the preparation and characterisation of the contaminants have been developed. In brief, coatings were deposited onto substrates in a variety of manners: stearamide (3,4) was evaporated; SDS (5,6) spray coated; PDMS (1,2) spin coated; to produce a variety of film thicknesses. To prepare a protein coated sample (7), a collagen adsorption protocol was developed by NPL to enable collagen to be adsorbed on a variety of substrates.

To prepare the high Z doped polymer thin films (8), polymer layers with medium Z doping were deposited on a cardboard substrate using a complex protocol, developed by REG(LUH). Briefly, the polymer was prepared using a low viscosity gloss varnish with dispersing and defoaming additive, then the respective filler component is mixed into this varnish. A range of filler nanoparticles were used including various oxides and acrylates. To reduce the number of agglomerates, different techniques including premixing and an ultrasonic processor were applied to reduce the size of agglomerates to ~ 1 μm. The mixture was then diluted and the homogenized filler-lacquer formulations applied directly. To cure the films were exposed to UV radiation.

A protocol for the synthesis of a polyacrylate thin film coating on a model substrate (9), equivalent to those used to improve the surface properties of the urinary catheter has been developed and tested by REG(UNOTT) using different strategies to tune the polyacrylate coating and hence induce a biological response which is resistant to bacterial attachment. An optimum methodology uses an oxygen plasma treatment of the catheter surface to improve adhesion of the polyacrylate layer to the catheter substrate.

PTB developed a protocol describing how to qualify calibration standards by XRF based on the model systems for medical devices. This included preparation issues and the measurement course of action. Due to the different requirements of the various sample systems the procedure has to be slightly adapted for each. In *Figure 1*, typical XRF spectra are shown measured for different geometries and photon energies. For the analysis of stearamide, conventional XRF geometry was employed to measure the mass deposition of the involved elements and the respective stoichiometry. However, due to the small amount, for PDMS the measuring geometry was changed to total reflection XRF (TXRF), i.e. using a very shallow incidence angle to reduce the contribution from the substrate material. Here, an incidence angle of about 1° was suited for the analysis.

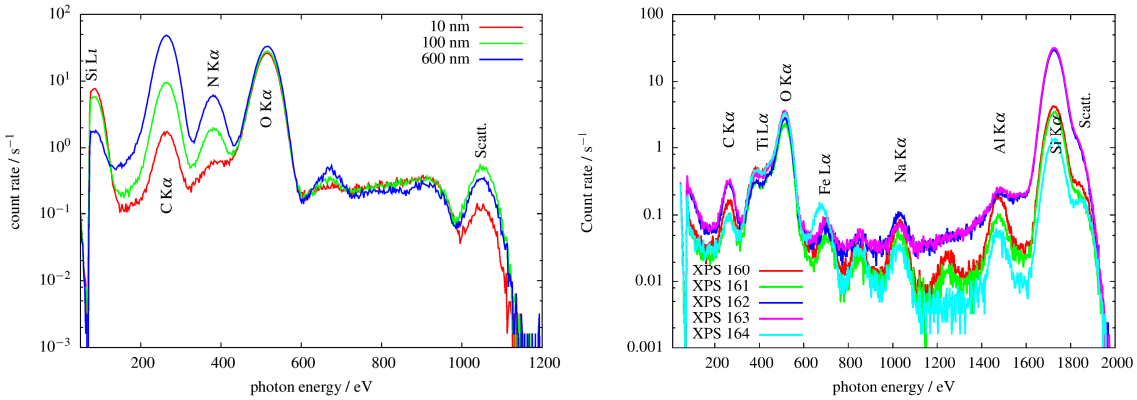

Figure 1: (left) XRF spectrum of stearamide deposited at Si recorded at an incidence angle of about 45°. The photon energy was 1060 eV to excite C, N, and O fluorescence radiation. (right) TXRF spectrum of PDMS@TiO_2@Si recorded at an incidence angle of about 1°. The photon energy was 1870 eV to excite C, O, and Si fluorescence radiation.

The sample systems were analysed using NEXAFS spectroscopy in fluorescence detection mode. In *Figure 2* NEXAFS spectra for different atomic K-edges (electron binding energies of the k shell) detected for stearamide are shown. Here, the most prominent x-ray absorption edge is the nitrogen K edge characterized by resonances of the amide binding. A protocol was developed by PTB to describe how to qualify calibration standards by NEXAFS for the model systems. Due to the variety of sample systems the procedure has to be slightly adapted dependant on the system. One important factor to consider is that it is necessary to inspect the damage to the molecules that may have been caused by irradiation. Organic molecules are very sensitive to x-rays and chemical bonds may be destroyed during the analysis. For this reason, the flux of the beam line in the respective energy range has to be minimized. Starting with the lowest flux and moderate acquisition time, the NEXAFS measurement is carried out and repeated on a slightly different measurement position with a moderated increased flux. If the fine structure changes, the flux of the previous run should be used for the measurements.

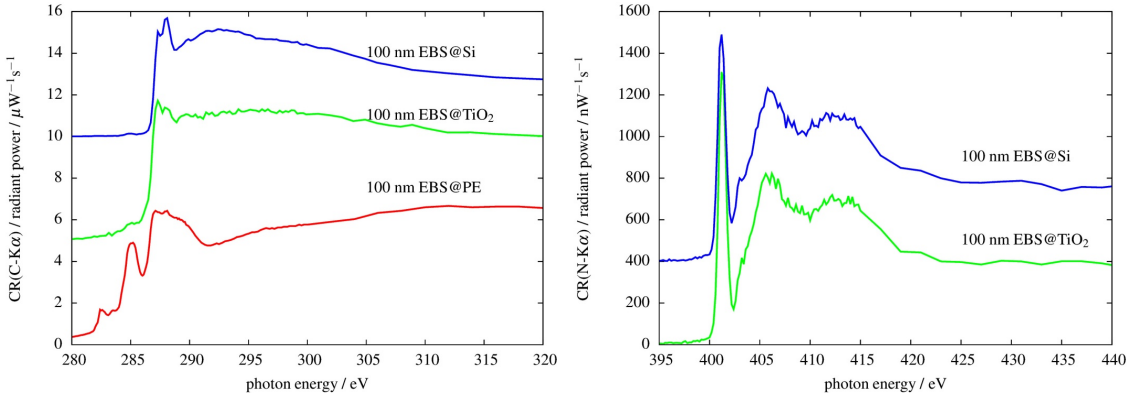

Figure 2: (Left) C K NEXAFS spectra of 10 nm stearamide deposited at different substrates measured at an incidence angle of about 10°. (Right) N K NEXAFS spectra of 10 nm stearamide deposited at different substrates measured at an incidence angle of about 10°.

The different model systems were additionally characterized by NPL regarding their uniformity, film thickness, stability, and reproducibility. The SDS model system was sprayed using a TM-sprayer (htximaging) onto a relevant substrate material. Using this simplified system of SDS deposited onto a silicon wafer, ellipsometry mapping before and after deposition, as well as SIMS imaging, have been carried out allowing us to investigate the integrity and the thickness of the contamination. The dried film of SDS on silicon (Si) is inhomogeneous, with large and small droplets and varying surface coverage. Average ellipsometry thickness measurement give a thickness of about 8.5 ± 7.0 nm for a film predicted to be 62 nm thick. Variance in thickness across the surface is reflected in the ellipsometry mapping and seen in SIMS images. The level of inhomogeneity may be worse on the hydrophobic silicon (Si) surface then on the medical devices. Spray coating is a quick way to distribute the SDS on a wide range of surfaces. However, caution is needed in assumptions about the thickness and homogeneity of the coating.

The stearamide specimens were analysed by SIMS and DESI (desorption electrospray ionisation) mass spectrometry. The respective spectra show no damage to the layer during the evaporative process. The SIMS analysis shows a uniform film across the sample with homogenous coverage of the stearamide. The analysis by XPS exhibits no contamination from calcium, fluorine, sodium, sulphur or aluminium, but the ratio of carbon to nitrogen and oxygen suggests a small amount of carbonaceous contamination. The thicknesses of the layers were measured during production with a quartz crystal microbalance (QCM), which was calibrated by spectroscopic ellipsometry.

3.1.3 Improve tools for the study of defects in thin films and at buried interfaces

Thin film coatings are being used in medical devices to improve lubrication, prevent corrosion, reduce fouling, prevent bacterial infection and promote healthy tissue integration. Defects in the films, such as pinholes or contaminants at the buried interface between coating and substrate, can result in corrosion of the substrate or delamination, cracking and spalling of the coating. The ability to detect these defects will enable an important advance in the quality management of such devices. The aim of this research was to further develop the methodologies to characterise thin films and buried interfaces (the surface between films) in a reliable and traceable manner. Protocols and methods were developed and qualified for detection of pinhole defects as well as contaminants at the film/substrate interface, on the model system of a polymer thin film (Table 1 -9) representative of a catheter coated surface. Using these methodologies, a multi-technique approach and advanced data analysis enables us to elucidate the picture at the buried interface.

To characterise and develop an appropriate polyacrylate coating with antibacterial properties, REG(UNOTT) used a polyacrylate array. An array of 116 homopolymers was produced and the adsorption of fluorescently labelled albumin was quantified on each polymer spot using a fluorescent scanner to assess protein adsorption. Multivariate SIMS image analysis of the same polymer microarrays was developed in order to predict protein adsorption. This analysis used the mass spectra to distinguish key differences within the polymer array, such as the differentiation between acrylate and methacrylate polymers, and variance specific to side groups. Partial least squares (PLS) regression analysis was then performed to identify correlations between the SIMS surface chemistry and the protein adsorption, *Figure 3*. PLS analysis identified a number of chemical moieties that correlated with high or low protein adsorption. Using PLS regression analysis, the adsorption of protein to the polymer microarray was successfully predicted. The novel application of the use of SIMS imaging datasets in the retrospective interpretation and validation of correlating protein adsorption and chemical structure of the polymer has been demonstrated. This will become an important tool for the further study of increasingly complex, multicomponent organic systems. This technique has enabled a clearer understanding of the failure mechanism of coated catheters which resist bacterial attachment and has allowed the REG(UNOTT) to develop a coating for urinary catheters, and with Camstent Ltd is looking towards a medical device approval.

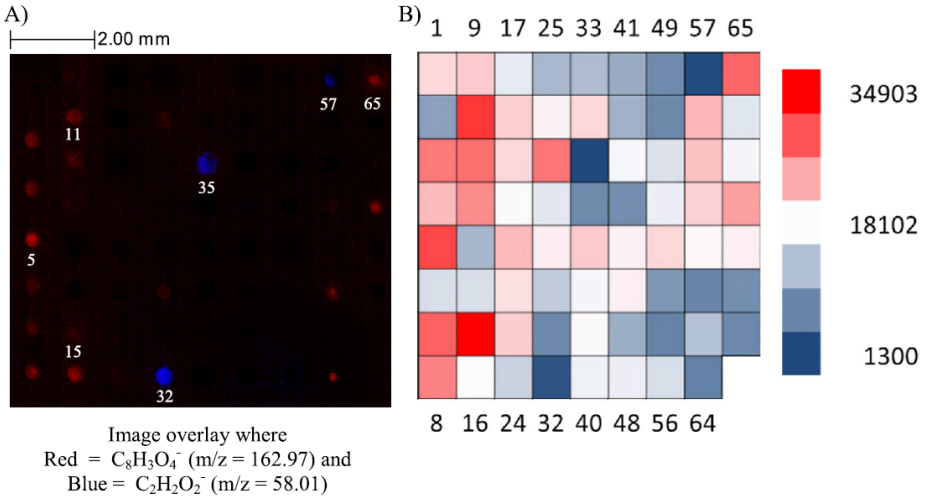

Figure 3: (a) Secondary ion images illustrating the distribution of ions found to correlate ($C_8H_3O_4^-$) and anticorrelate ($C_2H_2O_2^-$) with protein adsorption and (b) the corresponding protein adsorption heat map determined from the fluorescence data for the array. A heat scale showing fluorescence in arbitrary units is shown to the right.[from Figure 4 - Hook et al Biointerphases 10, 019005 (2015); http://dx.doi.org/10.1116/1.4906484]

Methods for manufacturing these polymer coatings of polyacrylate directly onto PDMS catheters have been developed by UNOTT involving plasma etching then dip-coating of the catheter. Plasma treatment of the substrate aids in the adhesion of the polymer coating. It was found that increasing the time for the O_2 plasma

pre-treatment of the PDMS surface from 0 to 10 minutes lead to a factor of a 1000 increase in adhesion as measured by the weighted revolutions required before delamination of the surface coating on the catheters. However, the improved lamination was destroyed as soon as the surface was wet. To understand why the delamination is occurring in the wetted coatings we need to be able to detect defects and contaminants at the interface between the coating and the underlying PDMS material. Changes in uniformity, thickness and defects in the coatings can lead to delamination, cracking and spalling of the film and ultimately device failure. Analytical tools capable of detecting these small differences at the buried interface and supplying enough information to determine their cause are required.

A procedure was developed by PTB to allow for elemental depth profiling and compositional analysis of matrix/trace elements of polymer thin films with defects by GIXRF and by NEXAFS spectroscopy. NEXAFS allows for chemical speciation and through variation of the incident angle allows for a depth sensitive analysis and reference-free quantification of the mass deposition. Chemical speciation of the PDMS catheter material was able to show the impact of different treatments on the O-K absorption edge, with the O_2 plasma treatment drastically changing the fine structure. Changes in the NEXAFS C-K edge and O-K edge with plasma treatment are consistent with a decrease in PDMS functionality at the interface. This suggests that the enhanced adhesion between the polymer coating and the O_2 plasma treated catheter are facilitated by hydrogen bonding and dipole interactions.

The 2-D and 3-D distribution of chemical moieties in the near-surface regions can be mapped using an argon (Ar) cluster ion beam to sputter through the surface region while providing SIMS analysis to produce a chemical depth profile of the polymer coating. SIMS analysis of real world samples is often hampered by surface roughness introducing topographic artefacts in depth profiles and images, leading to much poorer achievable interfacial depth resolutions then that achieved with model flat samples. To enable such high resolution SIMS analysis of buried interfaces in real world samples, an analytical protocol was developed by REG(WWU) to improve sputter depth profiling on samples with non-uniform thickness. The protocol uses PCA (principal component analysis) combined with a cross correlation to align depth profiles at each individual pixel within an image. A SIMS depth profile through the developed polyacrylate coating on a flat silicon substrate before and after applying this data correction method is shown in *Figure 4*. In *Figure 4*(a), it appears that there is the containment PDMS is present in the interfacial region, between the polymer and substrate. However, *Figure 4*(b) shows that after correcting for topographic effects, an improved depth resolution at the interface enables us to see that the PDMS is actually just present within the substrate material.

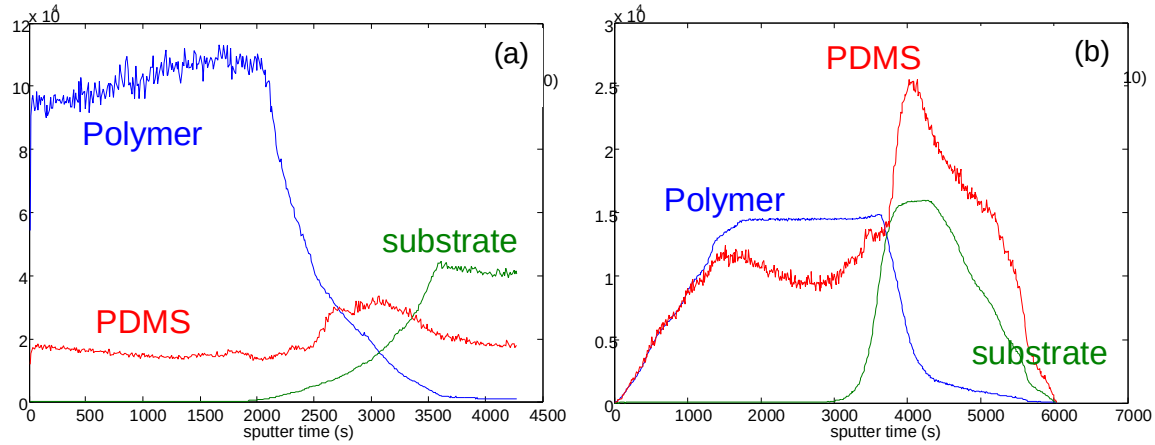

Figure 4: SIMS depth profiles showing ion intensities from the polymer, the contaminant PDMS and the substrate with sputter depth. (a) Raw data, (b) after data processing to remove topographic effects.

3.1.4 Improve the spatial resolution for 2-d and 3-D chemical state imaging

The 2-D and 3-D distribution of chemical moieties in the near-surface regions is critical to the performance of many types of medical devices such as drug delivery devices, tissue engineering scaffolds and bio-resorbable bone implants. Here, a system that models drug eluting stents is focused on. State-of-the-art drug-eluting stents feature a two-layer coating system over a stainless steel mesh substrate. The first coating deposited on the stent metal encapsulates the stent to eliminate interaction between the metal and the coronary artery tissue and blood. The second coating consists of a biodegradable, resorbable polymer blended with an anti-inflammatory drug and is designed to release the drug at a controlled rate during the initial weeks of implantation. For proper function of the drug eluting stents, the bonding between the metal and the encapsulating coating, the interface between the encapsulating coating and the drug-eluting coating, and the 3D distribution of the drug in the biodegradable matrix must all be precisely controlled.

Medtronic provided a set of state-of-the art drug-eluting stents and respective flat model system consisting of corrosion-resistant alloy (MP35N) that is coated with 1µm parylene C and the polymer layer polylactic acid (PLA) including the drug rapamycin. The ratio between Rapamycin and PLA has been varied: 50:50, 25:75, 13:87, as well as the thickness of the entire layer.

XPS and SIMS methodologies were employed by NPL and BAM for characterisation of the flat drug-elution stent model systems, without the additional challenge of the wire geometry of a real stent. For the assessment and the selection of suitable SIMS peaks for polymer and drug a procedure has to been developed to recommend characteristic peaks of rapamycin and PLA that exhibit a linear change in peak intensity with drug/polymer concentration. Overall 10 peaks are found to pass these criteria. The shortlisted peaks exhibit a linear relationship between intensity and drug loading making them best suited for reliable quantitation**.** The SIMS depth profile of the flat model stent system is shown in Figure 5 as reconstructed 3D images (REG(WWU). Figure 5 shows the ions indicative of: the substrate Cr^+ (red); a salt contaminant K^+ (blue); the polymer matrix $C_3H_4O^+$ (orange); the drug $C_5H_{10}N^+$ (green). The observed structure correlates to the optical image, which shows a high topographic coating of the film. SIMS indicates that the drug and the matrix is homogeneously distributed throughout the entire coating film. This implies that no significant degradation of the coating signals in deeper layers occurs. Instead of the Parylene C layer between the coating and the substrate, potassium (K^+) is strongly detected and located at the interface region.

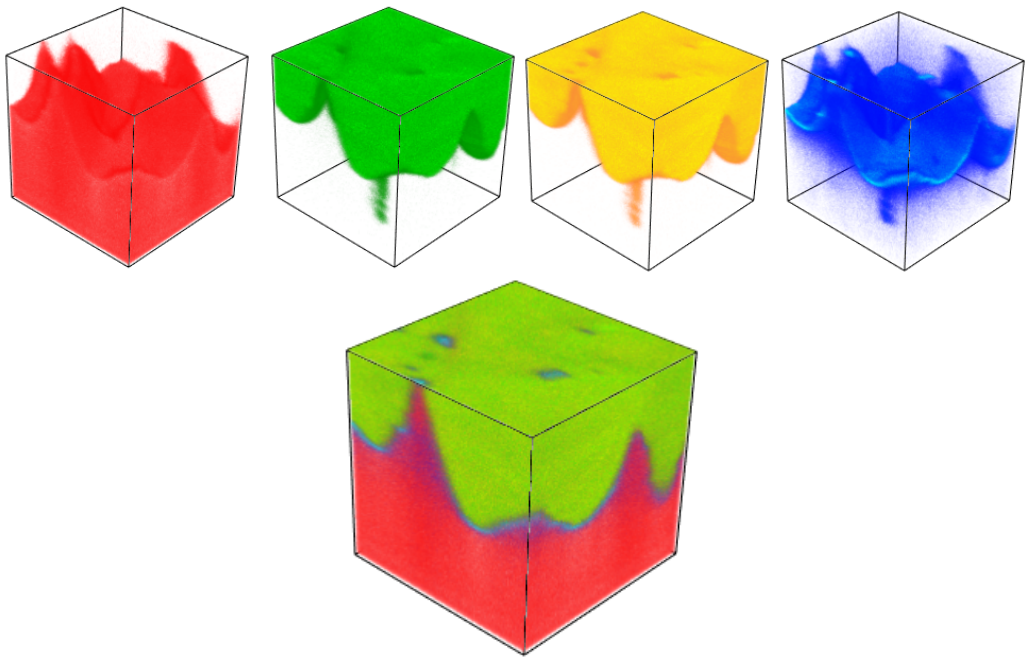

Figure 5: 3D images of a Rapamycin/PLA flat model stent system (Rapamycin/PLA wt% radio: 50/50); area 150 x 150 µm²; Cr^+ (red), K^+ (blue), $C_3H_4O^+$ (orange), and $C_5H_{10}N^+$ (green).

In order to understand the corrosion of a bio-degradable implant, knowledge about the elemental distribution and the chemical binding state on the micrometer scale is necessary. To reliably determine the spatial composition and binding state, robust metrological tools need to be considered for the analysis. For the 2D lateral characterization of the elemental and species distribution with x-rays a methodology was developed (by PTB) by combining µ-XRF and NEXAFS to access the soft x-ray region. Soft x-rays gain access to the light elements as magnesium (Mg), aluminium (Al) and sodium (Na). Furthermore, a validation procedure for obtaining spatial information capabilities with µ-XRF / NEXAFS is proposed.

There are several possibilities for focusing of the soft x-rays ranging from zone plates (providing spot sizes about 1µm) and smaller or mono capillary optics (providing spot sizes about 10 µm). The drawback of zone plates is the requirement to use a restricted energy range, therefore, it was decided to use a mono capillary optic with a larger spot size, but that allows for small energy changes necessary for NEXAFS analysis. Another issue when using a highly focussed beam is the significantly increased divergence that makes it more difficult to use the reference-free approach to obtain quantitative XRF. For the development and validation of this procedure for µ-XRF and NEXAFS in the soft x-ray range, slices of explanted rabbit bone tissue were analysed by PTB. After different incubation times the Mg alloy pin was removed. In Figure 6, µ-XRF mapping of rabbit femur for the magnesium concentration exhibited is recorded at a photon energy of about 1622 eV. The spot size of the focussed beam is ~ 15 µm x 40 µm. Further elements such as C, N, O, Al, and Na are also detected and additional µ-NEXAFS measurements at the Mg K absorption edge obtain chemical state information.

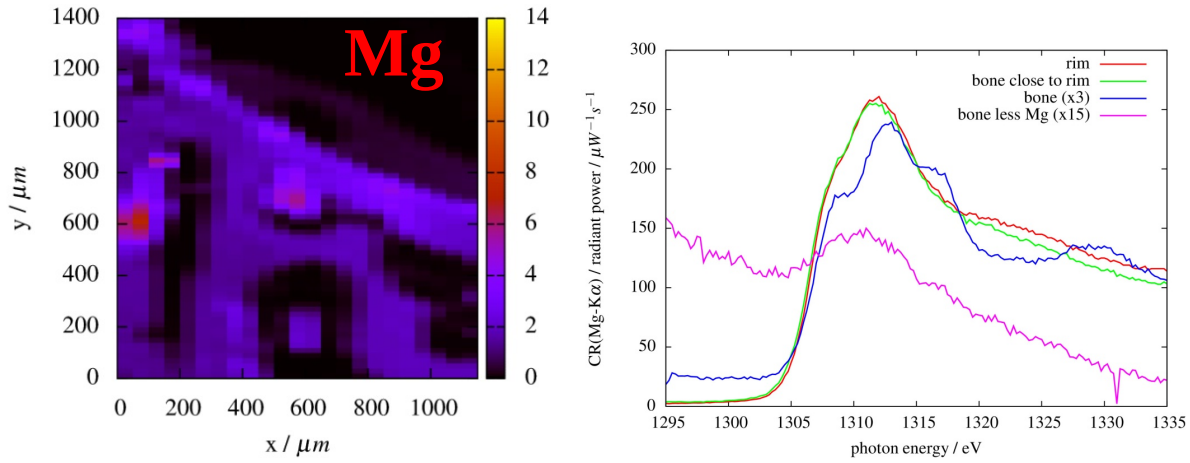

Figure 6: (left) the µ-XRF mapping of rabbit femur for the magnesium concentration is shown recorded at a photon energy of about 1622 eV. The spot size is about 15 µm x 40 µm. Further elements C, N, O, Al, and Na have been measured as well. (right) µ-NEXAFS measurement at the Mg K absorption edge are carried out

3.1.5 Develop the underpinning metrology for the characterisation of metal nano-particle coatings

Infections caused by bacterial biofilm formation on the surfaces of implants are the most common complication associated with implanted medical devices. Once established, biofilm based infections are generally impossible to eradicate without removing the medical device. Metal thin films and nano-particle coatings, including silver (Ag), copper (Cu) and zinc (Zn), are being widely used to reduce bacterial biofilm formation on catheters and other device surfaces but their effectiveness is highly variable. Better metrological tools are necessary to improve performance of these devices. The aim of this task was to further develop X-ray based analytical tools such as TXRF and GIXRF for the analysis of chemical elements, bonding states, quantities and, possibly, size dependencies of the nanoparticle systems related to the biomedical materials of interest. The focus was on an improvement of XRF under grazing incidence conditions as a reference-free quantitative analysis method for size-resolved nanoparticles. Therefore, relevant influence parameters, such as incident photon energy, particle size contributions as well as particle

deposition density shall be studied. Furthermore, these techniques shall be made compatible with complementary instrumentation for on-site and in-situ analysis studied in Objective 3.2 (Advancing the underpinning of metrology via the use of high vacuum techniques).

Two different procedures were identified for the deposition of Cu nanoparticles on a Si wafer, using the preparation proposed by Betancourt [O. E Rivera-Betancourt, IEEE Sensors Journal 10, 699 ,2010] and Asefa [Asefa, Langmuir 26, 7469-7474, 2010], respectively. In addition, different kinds of relevant stabilizers were used, potassium chloride (KCl) and other ACS (American Chemical society) grade chemicals**.**

The procedure for the elemental and dimensional analysis of nanoparticles deposited on flat surface is based on the effect of incident and reflected beam are interfering at flat surfaces. In particular, for X-rays solid materials are optically thinner than vacuum or air. Thus, under very shallow grazing angles, X-rays are totally reflected at flat surfaces. Under this condition an X-ray standing wave field (XSW) occurs above the surface, which significantly influences the excitation condition for emitting fluorescence radiation of the material deposited on that surface. Using this effect, TXRF analysis can be used as a surface-sensitive analytic technique that is sensitive on the nanometre scale. The periodicity of this XSW and the X-rays penetration depth into the sample can be varied continuously by changing the incident angle. Taking advantage of the XSW tunability, dimensional information on the nanoscale sample systems can also be obtained. The measured fluorescence intensity as a function of the varying incident angle is very distinctive for samples of different dimension and composition. This can be used to determine size information of particles that have been deposited on flat substrates. However, the relative excitation intensity at any fixed angle has to be considered carefully when trying to achieve reference-free quantitative TXRF measurements of particles with known dimensions.

Unfortunately, PTB found TXRF measurements with varying incidence angles exhibit that these samples are not suited for the further development of this technique because the nanoparticles are strongly agglomerating. Here, further experiments are necessary with a strongly reduced concentration of Cu nanoparticles. For a development of this methodology, the coverage should be sparse with no agglomeration. The analysis regarding the chemical binding state is still possible and with the results reflecting the analysis of an ensemble of particles. *Figure 7* shows the Cu $L_{3,2}$ NEXAFS spectra of nanoparticles prepared with the Betancourt protocol using different stabilizers.

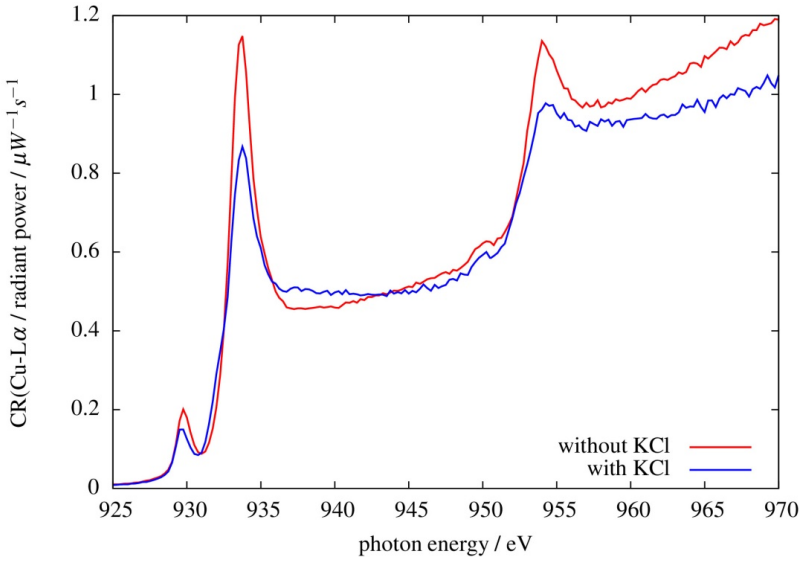

Figure 7: Cu-$L_{3,2}$ NEXAFS spectra of Cu nanoparticles deposited at a Si substrate by using different stabilizers.

3.1.6 Conclusions:

In order to advance the underpinning metrology via the use of high vacuum techniques, the project:

- Developed well characterised quantitative, traceable model systems to enable quantification and quality assurance of medical device surface chemistry at NMIs.
- Improved tools to characterise thin films and buried interfaces in a reliable and traceable manner. We achieved this by:
    - Developed a multivariate SIMS image analysis of polymer microarrays to predict protein adsorption. Using PLS regression analysis, the adsorption of protein to a specific polymer chemistry was successfully predicted from the surface chemistry. These methods improve the rapidity of selecting polymers with antibacterial properties and will continue to be used by UNOTT and have been reported to enable use by the wider research community.
    - Using GIXRF and by NEXAFS spectroscopy to enable elemental depth profiling and interfacial compositional analysis of polymer thin films with defects. This analysis enabled, for example, a catheter model system to be studied to demonstrate the enhanced adhesion between the polymer coating and the O_2 plasma treated catheter are facilitated by hydrogen bonding and dipole interactions.
    - Using argon (Ar) cluster 3D-SIMS to look at the sputter depth profiling of polymer thin films. An analytical protocol was developed to improve depth resolution on samples with non-uniform thickness. This has been reported to enable use by the wiser research community.
- Improved the spatial resolution for 2d and 3d chemical state imaging. We did this by:
    - Developing protocols for the depth characterisation of drug distribution in bioabsorbable matrices (using model flat drug eluting stent systems) using XPS and SIMS.
    - Through the use of post-acquisition ad hoc data analysis and data fusion of multiple techniques to enhance the veracity and reduce the effect of noise in results. This improved depth resolution by over a factor of 2.
    - The development and validation of the use of mono capillary optics for high resolution μ-XRF and NEXAFS to improve spatial resolution from mms to μms.

3.2 Development of emerging ambient techniques:

3.2.1 Introduction

Medical device companies and biomaterials scientists have developed a range of novel materials that may reduce complications and failures of implanted devices by using surface treatments, thin film over-layers and drug eluting coatings. Although the established surface analysis methods, such as XPS and SIMS, have demonstrated their value for the development of these advanced biomaterials, they are very poorly suited for routine monitoring of medical devices because they must be operated in high ultra-high vacuum, often require special sample preparation, have a slow turn-around and are very expensive.

Recent advances in FTIR (Fourier transform infrared spectroscopy) and Raman micro-spectroscopy offer great promise for meeting the medical device industry needs for on-line surface quality assessment. In addition to providing detailed chemical information, FTIR and Raman are non-contact, non-destructive chemical analysis tools well suited to on-line analysis. For medical device surfaces these techniques lack the surface specificity desired, therefore new developments and advanced data analysis methods will be required to facilitate reproducible, accurate, quantitative analysis.

Ambient Mass Spectrometry (AMS) includes a variety of methods that are able to obtain mass spectra from samples in their native state, at ambient pressure and temperature. Methods include PADI (plasma assisted desorption ionisation), DESI and AP-MALDI (atmospheric pressure matrix assisted laser desorption

ionisation) mass spectrometries, and are becoming increasingly important among manufacturers in many industries due to the high sensitivity of detection of molecules, high speed and the requirement for little or no sample preparation. Although these techniques show enormous promise for high speed analysis of medical devices, considerable research is required to improve reproducibility and accuracy. Metrology for AMS is in its infancy and the techniques are, at the present state-of-the-art, non-quantitative. The surface sensitivity and specificity are poorly understood and detection limits are not known for most systems. Reproducibility is generally poor (20 % - 30 %) and accurate data interpretation can be challenging because fragmentation and ionisation processes are poorly understood. There is now an urgent requirement to develop the metrology to ensure measurements are valid and accurate and to qualify the technology for use in the sensitive and highly regulated medical device industries and this is the aim of this objective.

3.2.2 Enhance sensitivity and reproducibility for analysis of surface layers and contaminants

The aim was to develop the foundation metrology needed to provide robust, reproducible, surface sensitive analysis of medical device materials using AMS, FTIR Microscopy and Micro-Raman Spectroscopy. These techniques were used to perform chemical analysis of the molecular composition of surface layers of model and real systems. Discussions with industrial stakeholders influenced the development of traceable model systems for relevant surface treatments and contaminants. Some of the well characterised model systems developed in Objective 3.1 were used as reference materials in order to advance the metrology.

Films at different thicknesses of surface contaminants such as (N',N'-ethylene bis(stearamide) (600 nm, 100 nm, 50 nm and 10 nm on HDPE and silicon), SDS (50nm on silicon, steel and HDPE), PDMS (deposition at different concentrations on Ti/TiO_2 and HDPE) were analyzed on different substrates in order to test the specificity of detection, the sensitivity of detection, the spatial resolution and the versatility of each ambient technique. A detailed comparison between all ambient techniques was performed by NPL. Moreover, different real sample systems i.e. hip liners and wound dressings provided by Smith & Nephew contaminated with stearamide and SDS, and catheters coated with acrylate based antibacterial polymers provide by REG(UNOTT), were employed to test the efficiency of detection of ambient techniques on a real biomedical devices with complex geometries.

Use of vibrational spectroscopic datasets from both FTIR and Raman techniques allowed implementation of 2D correlation data analysis tools in the spectral region from 3400 cm^{-1} to 2800 cm^{-1}, and additionally for FTIR data in the spectral range between 1700 $cm^{-1}$and 1500 cm^{-1}. The use of this correlative tool enabled the study of complementary molecule-specific features of the different layer thicknesses as spectral changes in terms of intensity modifications could be assessed. Hence 2D correlation was successfully exploited for visual decomposition of spectral complexity delivered by these complementary methodologies. The 2D correlative analysing tools in combination with indispensable ambient techniques such as IR and Raman spectroscopy provide further qualitative characterisation options of surface contaminants on in~/organic substrates. The layer thickness-sensitive modes unravelled by 2D correlation maps provide further opportunities for fast quantification of organic surface contaminants with reliable identification in order to support the quality control of production-line biomedical devices.

Synchrotron radiation based FTIR spectroscopy with a grazing incidence (GIR) and micro-Raman mapping were also performed by PTB and INRIM to study the contaminant distribution on the surface at the micrometer scale. This provided chemical images of the provided model systems proving the existence of a homogeneous surface layer coating, demonstrating their applicability as reference standards.

Stearamide was identified in FTIR and Raman spectra for all films at different thickness and a linear relationship was found between spectral response and layer thicknesses, both on Si and HDPE substrates. The results show that the Stearamide model samples successfully functioned as prerequisites for the construction of calibration curves, either based on band integral ratios or simply band integrals, with modest regression coefficients. Consequently, these curves could be used for the quantification of unknown coating thicknesses on real sample systems.

Typical vibrational modes were identified by FTIR and Raman for SDS on Si, steel, and HDPE proving the versatility of both techniques. SDS displays a quite homogenous surface layer with small round-shaped

convex (and concave when the substrate is hydrophilic) protrusions of SDS aggregates occurring on all types of substrate surfaces.

FTIR and Raman studies on PDMS-coated Ti/TiO_2 and HDPE samples revealed that the PDMS layer can be detected on both substrate types. A PDMS layer on Si and on HDPE of ca. 10^{-6} M was detected. The sample system with a concentration of c~10^{-7}M was barely detected, due to the low PDMS concentration only two bands could be ascribed to the surface coating. The model samples with a concentration of c~10^{-8} M did not exhibit any contributions from PDMS, defining the detection limit of the FTIR and Raman methodologies for the detection of PDMS surface contaminants. The GIR-FTIR (Grazing incidence reflection-FTIR) profiles displayed a homogeneous PDMS surface layer while micro-Raman mapping proved a non-homogenous distribution of the contaminant at the sub-micrometric scale. This relates to the different spatial resolution of the vibrational spectroscopy techniques.

SDS and stearamide samples were also analysed with DESI and PADI mass spectrometry by NPL. Modifications were made to the DESI and PADI systems to try to enhance detection. In particular, a homemade DESI spray head, that provides a more powerful gas flow than the commercial Prosolia spray head, was used to successfully detect stearamide. A thermal desorption stage was used with PADI to aid desorption; this has previously helped the detection of non-volatile molecules. However, in this study characteristic ions were still not observed, possibly due to the substrates used. DESI is able to detect SDS and stearamide on all substrates, with modifications to the spray head needed for the analysis of stearamide. PADI is not suitable for the analysis of these contaminants on the selected substrates (polyethylene, stainless steel and silicon). In DESI analysis, stearamide was detected from 10 and 50 nm thin films on polyethylene and silicon substrates, while SDS was detected from 5 and 50 nm thin films on polyethylene and stainless steel substrates. A repeatability between 5 and 36% was calculated for 3 repeat measurements.

Production line medical devices were also tested by NPL, PTB and INRIM to assess main issues of ambient techniques relating to the complex geometry and different materials. SDS was used to contaminate hip liners as a potential residue of cleaning and hygiene products. Stearamide was used to contaminate hip liners and wound dressing as a result of contamination in the fabrication process. In AMS experiments, liquid extraction surface analysis (LESA) was used to sample directly from a wide range of complex geometries and DESI was able to sample even from adsorbent surfaces. Both LESA and DESI can detect SDS from a range of medical devices. LESA demonstrated a significantly better repeatability. FTIR and Raman were also employed in the characterization of these biomedical devices and they were able to detect both SDS and stearamide on hip liners in inside and outside surfaces, handling the complex geometry of the samples. However, in the analysis of stearamide contaminated wound dressing both FTIR and Raman were not able to detect the contaminant due to the overlapping of vibrational signals of the substrate background.

3.2.3 Develop statistics and informatics tools to improve the accurate identification of organic surface species including both contaminants and engineered surface components

The aim was develop and improve informatics tools that allow for the accurate identification and quantification of molecules. An understanding of instrument noise is used to improve accuracy and reliability of the multivariate results. Multivariate methodologies provide detailed and precise information in a short time scale to support, improve and optimise the quality control of biomedical devices with respect to surface modifications, contamination and controlled drug release. This allows for fast identification of defective products and for the fast exploration of production failure rates.

Surface mass spectrometries often use non-linear detection of the ions produced from the surface to create the mass spectra. This can lead to dead-time effects within the spectra where intensities shown do not relate to the actual amount of ions reaching the detector. This can lead to confusion when analysing complex data sets, such as 2D or 3D images where there is a detailed mass spectra associated with every single pixel (often looking at >10^8 pixels). The use of multivariate analysis (MVA) methods are applied to such data to aid in their interpretation, but dead-time effects within the spectra lead to potentially confusing artefacts within the final results which are hard to trace back due to the large volume of data. Some of these artefacts can be eliminated by applying an advanced Poisson dead-time correction that accounts for the signal intensity in the dead-time window. Because this correction is nonlinear, it changes the noise distribution in the data. Here,

the accuracy of this dead-time correction and the noise characteristics of the corrected data has been analysed and a simple but accurate equation for estimating the errors in the data developed. *Figure 8* illustrates the effect of carrying out PCA analysis on corrected and uncorrected data for a SIMS image on a model system. When the data are not corrected for dead-time effects, physically unrealistic artefacts in the image *Figure 8* (a) are seen. This complicates interpretation of the images. These artefacts in the PCA images are dramatically reduced for dead-time corrected data as can be seen in *Figure 8* (b).

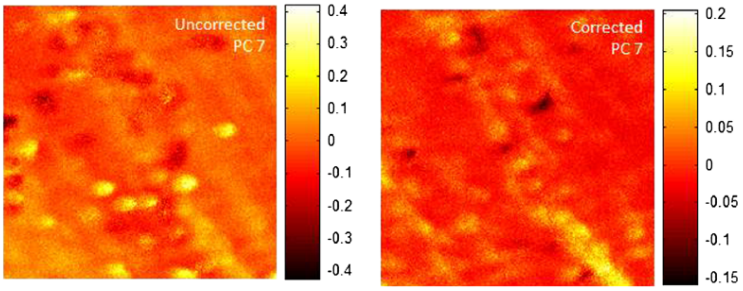

Figure 8: PCA image analysis of 390 selected peaks from SIMS for the uncorrected (a) and corrected (b) data. [Surf. Interface Anal. 2014, 46, 581–590]

Using these recommendations for using the advanced dead time correction and through review of the literature and experienced knowledge we make a number of guidelines for the best way to optimise the use of multivariate analysis with complex data sets in order optimize the outcomes of MVA in surface mass spectrometry. An overview of the best practices has been developed into an interactive flow diagram by NPL with support of PTB. This gives simple, practical advice to industrial users in the effective and best use of PCA for surface mass spectrometry data.

3.2.4 Improve both the lateral and in-depth spatial resolution for 3-D chemical state imaging with Ambient Mass Spectrometry

Mass spectrometry imaging (MSI) has the ability to map the detailed chemical composition of a drug and/or its metabolites or the distribution of an additive in a polymer - a powerful tool for product development. SIMS (secondary ion mass spectrometry) provides high-sensitivity small molecular information and offers excellent spatial resolution (200nm or less are routinely observed) and 3D images with a depth resolution of better than 10 nm. However, ambient MSI can be quicker and has opened up whole new areas to MSI. MALDI imaging can be carried out in vacuum and at atmospheric pressure (AP-MALDI), can detect large molecules such as proteins and achieve a routine spatial resolution of ~10 µm in vacuum. DESI imaging compliments SIMS and MALDI in that it can be used for the analysis of both large and small molecules, and involves little or no sample preparation. Yet it has relatively poor spatial resolution (routinely 200µm) in comparison to other techniques. For all the MSI techniques useful spatial resolution is limited by the speed of analysis required, the type and size of molecule to be analysed, the ionisation efficiency and transmission and ultimately the number of molecules available in a given pixel. Here, we develop ambient MSI techniques for high resolution 3D imaging.

3D ambient MSI requires the development of depth sensing and profiling with the techniques, something not presently well understood or carried out. Our initial experiments used PADI to simultaneously remove layers of and identify polymers. Ellipsometry was used to determine the film thicknesses and measurements of the craters left after analysis used to calculate the rate of desorption. These profiles showed that PADI-MS was able to profile the polymers, PMMA and PLA, with erosion rates of 0.6 and 2 nm/s respectively. Using this technique we were able to profile a dual layer of PMMA spun cast on top of PTFE tape and differentiate the two layers with depth.

To further our understanding of depth sensing, compound detection and ionisation across a range of ambient mass spectrometries, AP-MALDI, DESI, LESA and PADI a set of well characterized model systems were designed. These used drug and embedding media commonly found in drug eluting stents (Rapamycin, Probucol, Paxlitaxel and Polylactic acid - PLA).With the exception of PADI, which was only able to detect

ions characteristic of PLA, all compounds were detected by all methods. By comparing the mass spectra and changes in the sample surface after analysis, sampling aspects of each technique can be considered such as: fundamentals of different desorption/ionisation mechanisms; spatial and depth resolution; mixing and suppression effects. Assessing the number of ions detected vs molecules sampled (i.e. the molecules detected per pixel) present sensitivity and achievable spatial resolution is measured. Knowledge of these aspects is essential to optimize sensitivity and erosion rates to achieve higher depth and spatial resolution.

The challenge of achieving high resolution 3D ambient MSI requires and improvement on the present achievable spatial resolution (at best 40µm). A novel AP-MALDI source for imaging ambient mass spectrometry with best possible laser focus of 800 nm has been built by NPL, Figure 9. The initial build of this novel source is now complete showing proof of concept and imaging capabilities. The new prototype system has been tested for the range of model systems and results have been extremely positive, with molecular ions detected from all these samples. The current sensitivity is a limiting factor in accessing the highest resolution possible. The best possible laser focus on a sample in the new system is 800 nm, Figure 9 (a), but improved sensitivity is needed to access this resolution for molecular ion imaging. The source has been built with an open construction, Figure 9 (b), to enable: variable focusing of laser; 'Sniffer' heating and possible modification and addition of post-ionisation. These have allowed optimisation to improve sensitivity of molecular ion detection. The use of a plasma source for post ionisation gave improvements in sensitivity for the model drug systems of up to a factor of 3200 %, Figure 9 (c and d). This instrument has enabled a dramatic shift in spatial resolution and sensitivity for AP-MALDI and ambient IMS.

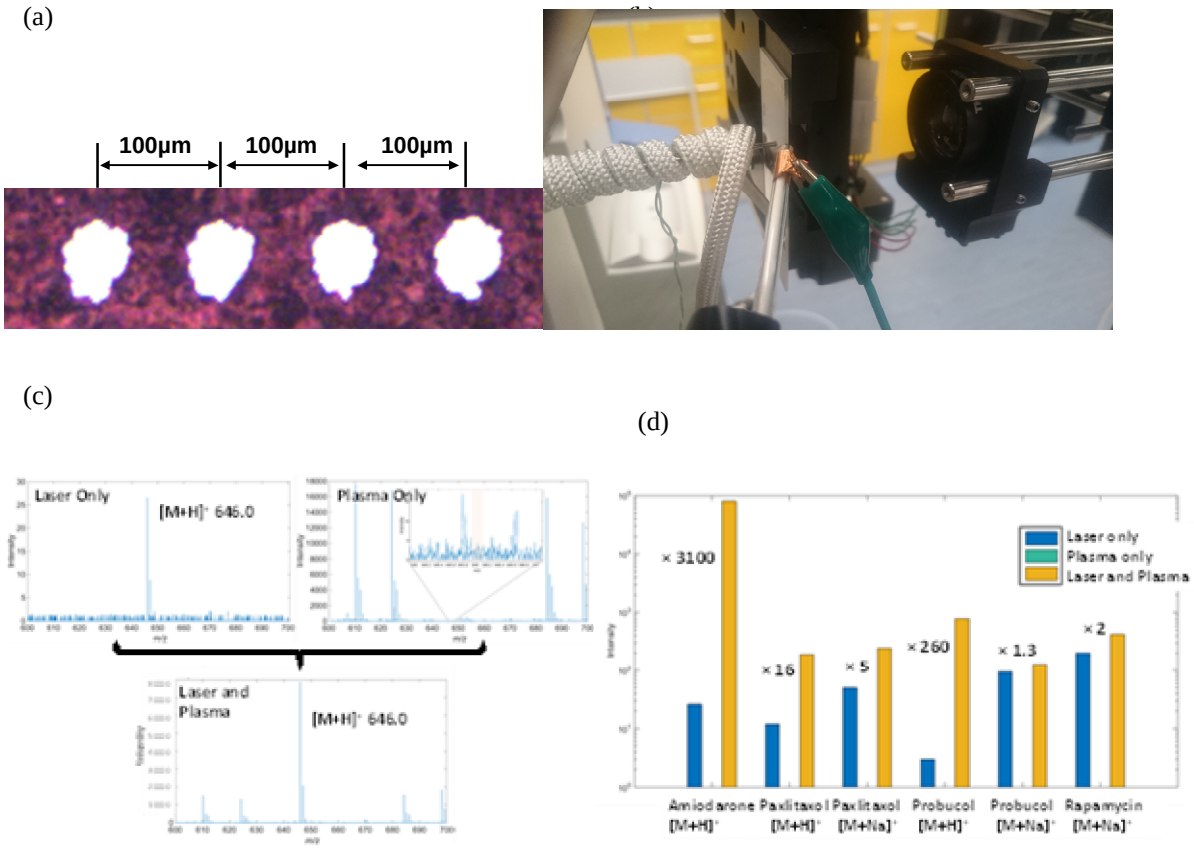

Figure 9: Results from the new AP-MALDI source showing:(a)laser spot size at a focus of 50 nm; (b) the open source and transmission mode sample stage; (c) mass spectra of Amioderone with DHAP matrix showing the molecular ion for three conditions: laser only; with the laser off and plasma on; with both the laser and plasma on and (d) the molecular and sodiated ion yield detected for 4 different drugs with: laser only; plasma only; laser + plasma.

3.2.5 Develop the underpinning metrology for the characterisation of metal nano-particle coatings

The aim was to characterise metal nanoparticle (NP) coatings with respect to their physicochemical properties via implementing ambient methodologies. SR-based FTIR microspectroscopy can be exploited in terms of studying the vibrational fingerprints delivered by functionalised, biochemically modified NP coatings. In addition, enhanced vibrational spectroscopy on nanoparticle coatings and functionalised surfaces of the biomedical devices was also performed.

Different SEIRA (Surface-Enhanced Infrared Absorption Spectroscopy).nanoparticulate biolabels and their related components were studied by INRIM by means of synchrotron radiation (SR-) based FTIR spectroscopy. These SEIRA biolabels comprised different antibody-fluorophore conjugates being chemisorbed to gold nanoparticles the latter of which functioned as SEIRA nanoantennas for enhancement of molecular absorption. The different biolabels were characterised by FTIR micro-spectroscopy, enabling hyperspectral data-acquisition in the mid-infrared spectral region. The aim of this approach was to implement SR-based SEIRA methodology for the selective, reliable and sensitive spectroscopical readout in order to qualify the complex biolabels for multiplexed biosensor approaches and bio-medical assay applications. Uni- and multivariate statistics tools were implemented for systematically studying the enhanced/unenhanced molecular fingerprints of biolabels and related biochemical target components. This systematic approach illustrates that the both the SEIRA and FTIR molecular makeup of the biolabels could be distinctively separated and discriminated into clusters by means of principal components analysis.

Different sizes of gold and silver nanoparticles (NPs) were produced by direct and seeded growth methodologies. SERS analysis was first performed in solution using a diagnostic biomarker (creatinine) in order to test the SERS effect of the produced nanoparticles and to calculate their enhancement factor. Gold and silver NPs with a diameter of 50nm provided the highest enhancement factor in solution due to their optimized surface area for SERS analysis. Gold Nanoparticles were also anchored on glass microscope slides using a silanization chemistry in order to obtained SERS substrates. These tests were conducted at INRIM by soaking the SERS substrate in a 1 ppm solution of creatinine and collecting Raman signals in dry conditions. Traditional Raman analysis turned out to be not sensitive enough to detect creatinine at such small concentrations. In traditional RS a detection limit of 500 ppm was calculated while in the optimized SERS analysis the detection limit is around 50 ppb. These studies allowed to develop reproducible and reliable procedures for gold NPs fabrication and to test their efficiency of detection in respect to their surface chemistry features.

In order to develop reproducible SERS sensitive methods for *in-situ* detection of contaminants on biomedical devices, different deposition strategies for gold nanoparticles were first studied. In particular, a) incubation of the sample in NPs solutions and NPs deposition on the surface by b) drop coating, c) vacuum drying and d) evaporation in a saturated atmosphere of ethanol were tested. Vacuum drying procedure resulted to be the most efficient in terms of time of deposition and homogeneity distribution of the NPs on the surface. This optimized deposition method was applied on model systems and tested for SERS analysis. SDS model systems on silicon, steel and HDPE and SDS contaminated hip liners were used for these analysis. Gold NPs with different dimensions, shape and shell were compared. Spherical gold nanoparticles with a diameter of 120nm demonstrated the highest enhancement factor in SERS analysis. In situ detection of SDS by SERS was achieved on silicon and steel but not on HDPE. Even if the sensitivity of detection was increased in respect to the traditional Raman Spectroscopy, with a calculated Enhancement Factor (EF) of 3, the interaction of gold NPs with the contaminant induces a spectral distortion of the Raman fingerprint of the SDS, making very difficult its discrimination on the surface.

In order to overcome these issues and to develop more reproducible and reliable tools of detection, a LESA approach in combination with homemade and commercial Klarite SERS substrate was set up SDS was quickly extracted and aspirated from the surface using a proper solvent and subsequently deposited on SERS substrate for Raman analysis (Figure 10). SDS was successfully detected on silicon, steel, HDPE and hip liner biomedical device with no spectral distortion (Figure 11). Quick identification of the contaminant was also achieved by using commercial Raman libraries with a similarity match higher than 85%. An EF close to 10 was calculated in LE-SERS analysis for SDS detection.

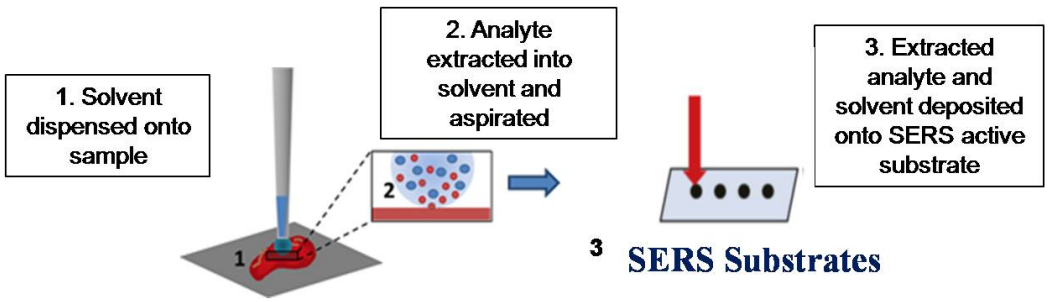

Figure 10: Scheme of contaminant detection on the surface of a biomedical device by a combination of LESA and SERS.

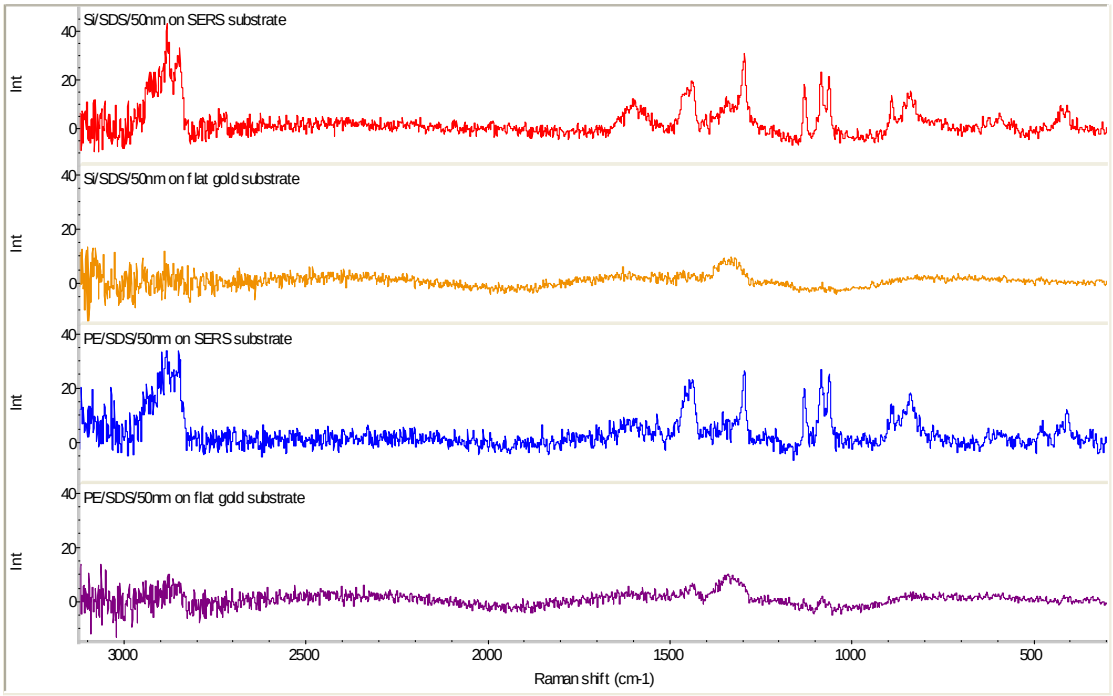

Figure 11: Raman spectra of SDS deposited on Klarite SERS and flat gold (used as control) substrates after liquid extraction on Silicon and HDPE.

3.2.6 Conclusions:

For production-line medical devices reliable tools such as AMS and FTIR, Raman and SERS provide appropriate high quality signals/fingerprint and fast data analysis tools in order to unravel the complex spectral features of contaminants for the sake of distinct and fast identification during industrial processing and to underpin industrial quality control. These techniques are suited for studying the physicochemical treatments on biomedical device samples in order to support the fundamental research on polymer-based science of surface coatings.

In order to *develop the use of emerging ambient techniques*, the project:

- Successfully illustrated the power of a combined FTIR and micro-Raman approach to:
    - Explore the physicochemical as well as surface and interfacial properties to reveal homogeneity and physicochemical properties of surface contaminants.

- Qualify model reference materials delivering characteristic signals for the respective layer thickness for use in the construction of calibration curves.
- Qualitatively envisage different concentrations of surface contaminants in combination with a micro-spectroscopic setup allowing the surface pattern to be characterised

- Developed post analysis procedures and methods to improve the use of multivariate and informatics tools for the interpretation of complex data sets. This involved:
  - Removal of spectral artefacts in SIMS imaging by applying an advanced Poisson dead-time correction
  - Recommendations on pre-processing for different types of data and identification
  - Development of a best practice guide to the use of PCA in surface spectroscopy
  - Delivery of overview and training tutorials at several European meetings
- For the first time in the world illustrated the use of PADI-MS for depth profiling polymers with erosion rates of nanometres per second.
- For the first time in the world characterised the erosion rates, spatial resolution and sensitivity of 4 different ambient mass spectrometries for PLA and drugs relevant to drug delivery coatings on stents.
- Built a novel AP-MALDI imaging source for high resolution (potential of nm rather than μm spatial resolution) and sensitive ambient mass spectrometry that incorporated:
  - A variable focussing of the laser able to achievable spot sizes of better than 800 nm
  - A heated inlet sniffer to enhance sensitivity and show the detection of molecular ions from a range of materials
  - Addition of plasma post ionisation to enhance sensitivity by up to 3200%
- Developed a SR-based SEIRA methodology for the selective, reliable and sensitive spectroscopical readout to qualify the complex biolabels for multiplexed biosensor approaches and bio-medical assay applications.
- Developed reliable and reproducible protocols for the fabrication of Gold NPs and SERS substrates for bio-analytical applications.
- Developed a reliable and sensitive procedure based on LE-SERS approach (novel to Europe) for the identification of surface contaminants on biomedical devices.

3.3 Adaptation and validation of metrological tools for the production line medical devices and explant analysis:

3.3.1 Introduction

The aim of this objective was to apply, adapt and validate the tools developed in Objective 3.1 and Objective 3.2 on model systems to real production line medical devices. The complex geometry of most medical devices creates analytical challenges. Catheters have a convex exterior surface and a concave interior lumen. Artificial hips have corners, grooves, a convex ball and a concave cup. Stents consist of a wire mesh tube and pacemaker leads have a wide range of geometries with grooves, fittings, loops, and pins that can be difficult to access non-destructively. Heart valves and artificial arteries often include knitted, woven or felted components. In addition to the geometric challenges, device development and failure analysis often

requires the analysis of explanted devices and the associated biological tissues. Metrology tools developed in previous objectives were validated and adapted on real industrial problems.

Existing methodologies developed from the analysis of model systems in Objective 3.1 for state of the art high vacuum surface analytical methods (including XPS, SIMS and Synchrotron based spectroscopies) were used alongside those developed in Objective 3.2; established ambient methods such as Raman and FTIR spectroscopy and emerging methods ambient surface mass spectroscopies. This multi-technique approach across multiple laboratories enabled comparisons to be drawn across the techniques so that robust technique selection and validity of information could be considered and published, along with good practice recommendations. These objectives are of high relevance and value to the medical devices industry and will help both new product R&D (research and development) and existing product manufacture and QC (quality control) to use optimal analytical methods, thereby saving both time and money. Ultimately achievement of these aims and adoption of the outputs will improve the competitiveness of the medical devices industry.

3.3.2 Adapt, integrate and test the metrological tools developed for analysis of contaminants on production line medical devices

The aim of this research was to adapt and validate the methodology (methods and procedures) developed on the model systems for use on intact production line medical devices. In particular assessing the techniques for the detection of common manufacturing contaminants that can cause device failure and for the detection of surface defects. This are critical tools needed for identifying and solving causes of product failure.

Through consultation with industrial partners SMN and Medtronic, and NPL, PTB, BAM and REG(LUH), a total of 12 Medical devices were obtained, representing a diverse range of product types:

- PVD silver coated wound dressing; Polyurethane dressing film Physical Vapour Deposition coated with a mixed silver/silver-oxide antibacterial surface layer
- Drug eluting stents; Rapamycin-Polylactid-acid stent systems
- Drug eluting stents; Coroflex® ISAR 2.5 and 3.0 × 24 mm sirolismus (rapamycin) eluting stent systems, Braun Melsungen AG, Berlin-Germany
- Calcium hydroxyapatite coated bone implants; 2x samples of Ti6AL4V implant with hydroxyapatite coated sintered beaded surface, ovine bone explant at 12 weeks, distal femur, fixed in 10% neutral buffered formalin, followed by IMS dehydration and then embedded in Technovit 7200 VLC resin
- Uncoated implants; 2x samples of CoCrMo alloy implant with sintered beaded surface, ovine bone explant, fixed in 10% neutral buffered formalin, followed by IMS dehydration and embedded in Technovit 7200 VLC resin
- Polyethylene hip liners; 20x R3 XLPE acetabular cup liners
- Elastoplast Wound dressings; Elastoplast Antibacterial silver dressings
- Contact Lenses
- Hydrocolloid dressings; Elastoplast Hydro-colloid Blister, Boots Hydro-colloid dressings
- Alginate Dressings; Savlon Alginate dressings, Boots Alginate Burn dressings.

The selected production line medical devices were analysed with AMS by NPL for contaminants and surface defects, and contrasted with SIMS analysis. Protocols developed in Objective 3.2.2 using the model systems were tested and adapted as necessary for analysis of the complex geometry devices. The level of detection/effectiveness was determined for each method. In summary: SIMS, DESI (AMS) and LESA (AMS) have been able to analyse medical devices with varying effectiveness. SIMS could detect stearamide and

PDMS contamination from medical devices easily. DESI and LESA could detect PDMS and SDS contamination with LESA most sensitive and repeatable for detecting SDS. The usefulness of each analytical method was evaluated and this is summarised in Figure 12.

	Wound dressings				Contact lens	Hip liner
	Hydrocolloid	Alginate	Antibacterial	Gentle		
SIMS	✗	✗	☺	☺	✗	☺
LESA	☺	☺	✗	✗	☺	☺
DESI	☺	☺	✗	☺	☺	☺

Figure 12: Guide to optimal surface analysis method selection across high vacuum and ambient surface analyses for different medical device products. The table aims to give an indicator of ease of use of the different techniques when studying a particular product.

The production line medical devices were also analysed with optical micro-spectroscopy (SR-FTIR) by PTB for contaminants and surface defects. Procedures developed using the model systems in Objective 3.1.3 were tested and adapted as necessary for analysis of the complex geometry devices. Samples tested included PVD silver coated wound dressings (3 samples), drug eluting stents, hip liners and explanted (from rabbit) resorbable Mg alloy pin implants, Deliberate SDS contamination was detected across multiple locations and geometries, flat and curved doped Polyethylene hip liners. The thickness of the contaminant was also successfully estimated. A deliberately applied Stearamide contamination was detected on hip-liners and on antibacterial silver coated dressings using SR-FTIR spectroscopy and the thickness of the contaminant determined. Thickness measurements via FTIR were in agreement with those estimated from the deposition process.

3.3.3 Adapt, integrate and test the metrological tools developed for analysis of stents

The aim was to adapt and validate the methodology (methods and procedures) developed on the model stent systems for use on intact stents with complex geometry. Metrological tools developed on flat surfaces were tested and adapted as needed for device characterisation. The methodology for lateral and in-depth characterisation developed in Objective 3.1.4 was adapted to real stents by BAM in conjunction with NPL. The real eluting stent systems were analysed to establish traceable measurements and quantitative characterisation tools that will satisfy existing and emerging regulatory requirements.

Firstly, a Rapamycin PLA stent systems were analysed by REG(LUH). PLA, Rapamycin and Amide I spectral signatures were detected using SR-ATR-FTIR and MIR across stents with different ('medium' ca. 10µm and 'thin' ca. 5µm) coating thickness and varying polymer/drug %wt ratios of 12.5/87.5%, 25/75 %, and 50/50% (See *Figure 13*) In addition, a drug eluting stent from Braun of PLA and Probucol was analysed by XPS Compared to the flat drug model systems, these stents show complex mesh geometry offering much smaller areas for analyzing. However, adaptation of methodology to enable angle resolved XPS measurements has

been proven to be a suitable method for depth profiling (in depth characterization) of real stents. As on the model samples a surface accumulation of silicon has been measured on the real stent samples. With SIMS, when moving from flat surfaces to the 'real stent' systems a number of issues were highlighted associated with the topography of the stent. A number of these obstacles were identified and ways to overcome them recommended through careful consideration of: sample mounting; analytical conditions; data analysis have been outlined in best practice guidelines for stent analysis. This is crucial to obtaining meaningful 3D images.

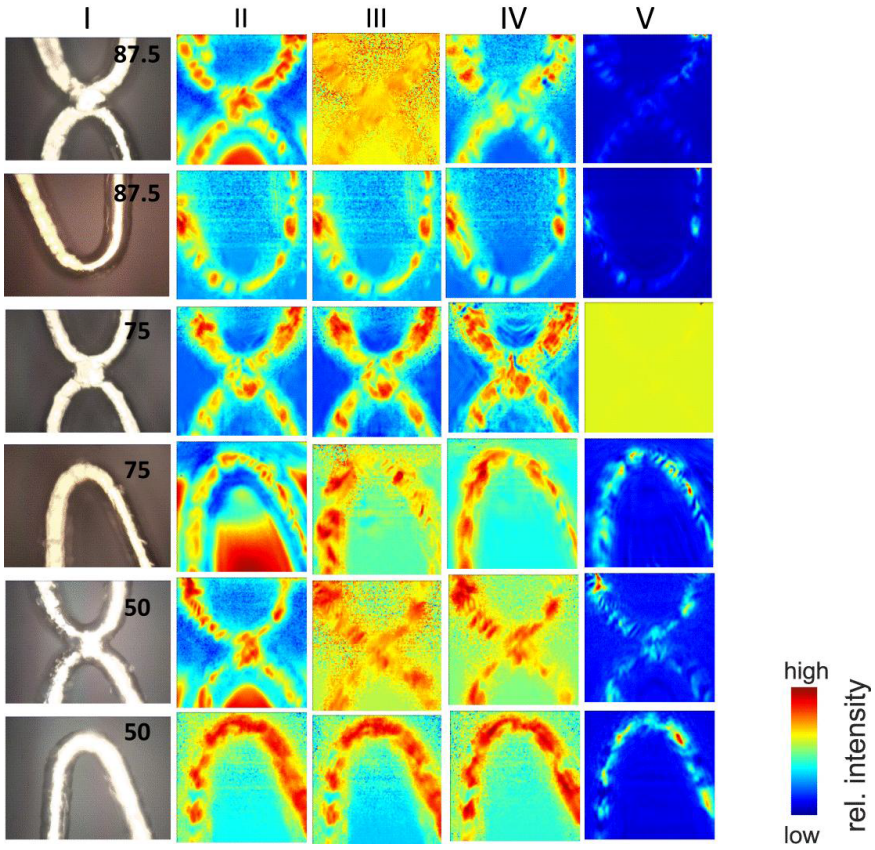

Figure 13 : Example of 'thin' stent samples (coating thickness ca. 5 µm) with their respective polymer-drug wt-% ratios 12.5/87.5%, 25/75 %, and 50/50 % at two different regions. Chemical maps were constructed by calculation of II: Integral@1750 cm-1, III: Integral@1641 cm-1, IV: integral@1186, V: Band ratio II/III. Spectral analysis took place between 3900 cm-1 and 900 cm-1, applying a Globar light source for FPA investigations.

3.3.4 Establish utility of the tools developed for failure analysis of explanted devices

The aim of this research was to use multiple techniques, such as imaging SIMS, micro-Raman, and FTIR micro-spectrometry (with advanced µ-XRF and NEXAFS methodologies in the soft x-ray range) in order to enable the 2-D chemical imaging of explanted bone implants and cardiovascular stents. By combining the complementary data from these multiple techniques from Objective 3.1.3, chemical speciation, quantifiability and lateral resolution will be enhanced. The goal was to quantitatively investigate the lateral distribution and migration of bioresorbable implants and coatings and eluting drugs into the bone and tissue environment with a high dynamic range of detection sensitivity.

An explanted resorbable Mg alloy pin implants from rabbit, provided by SMN were analysed with a SR-based FTIR spectroscopic analysis (by PTB) and by micro-X-ray fluorescence on different explanted rabbit tissue sections. Figure 14 shows several example elemental maps obtained by micro-X-ray fluorescence of the

resorbable magnesium alloy/rabbit bone explants. SR-based FTIR spectroscopic analysis modes that refer to implant-adhesive proteins (Amide I, II: 1661 cm-1 and 1541 cm-1) within the bone tissue can be observed (2 week sample). Further, we can detect phosphate (1179-1017 cm-1) and type B carbonate bands (1077 cm-1), Figure 15, originating from the hydroxyapatite ($Ca_5(PO_4)3OH$) matrix. Adhesive proteins can be detected nearby the former Mg pin region, known for their influence on corrosion process and migration into the corrosion layer within the implant-tissue interface. PTB also developed several cluster analysis based approaches to produce chemical maps, using Principal Components Analysis and Hierarchical Cluster Analysis.

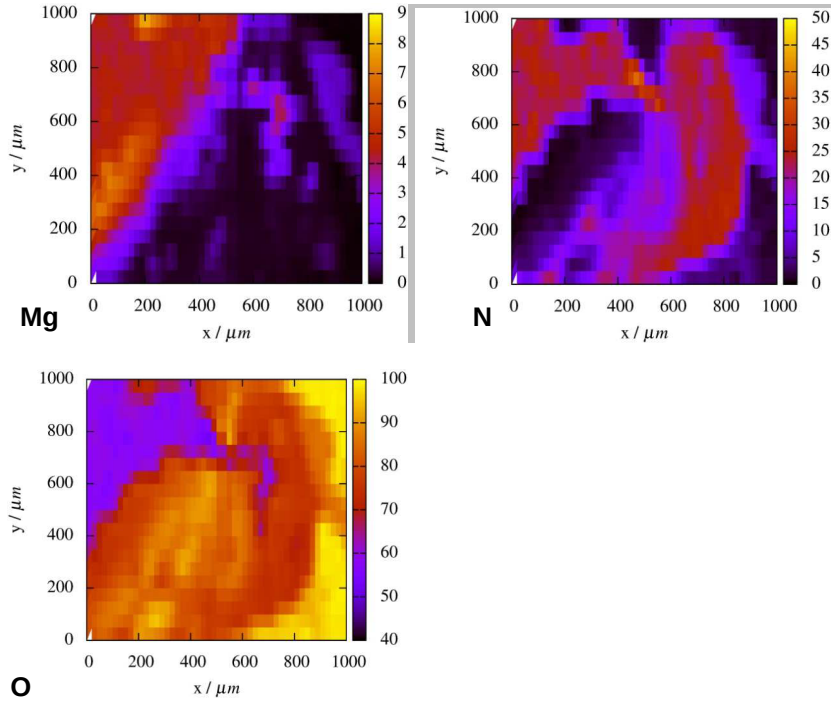

Figure 14: Example of chemical speciation across rabbit bone/magnesium alloy sample explanted after 3.5 years, analysed using SR-micro X-ray fluorescence.

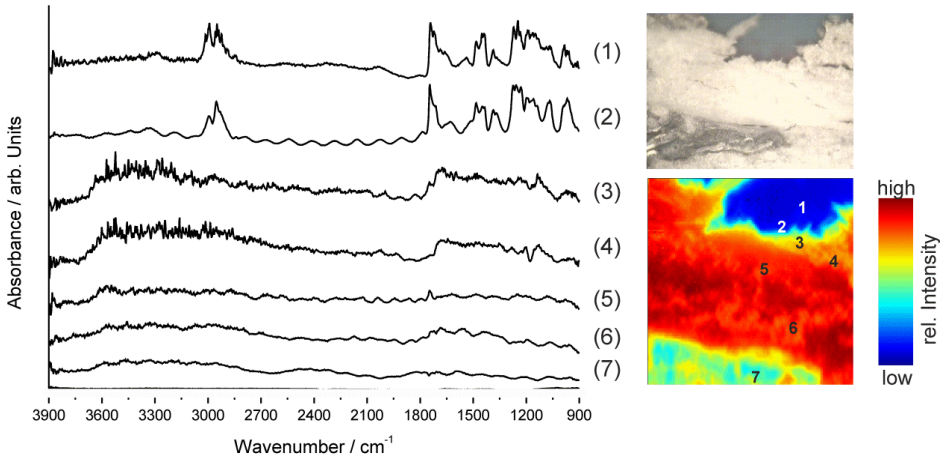

Figure 15: Example of Focal Plane Array (FPA) chemical map and MIR spectra of rabbit bone tissue section with Mg-Alloy implant (3.5 years) at seven different regions, see FPA-chemical map at right (integrated region between 1680 cm-1 and 1620 cm-1. Map dimensions: 680µm × 580µm.

Procedures have been developed for the spatial specification of bone implant coating tissue systems using SIMs and SR-micro-SRF. Work carried out at NPL resulted in an Argon-sputter pre-cleaning method being developed to prepare an embedded explant samples for the multiple surface analyses undertaken by several of the project partners, including the SIMS work. This pre-cleaning procedure also provided a robust and reliable method for co-analysing the same areas of explant samples for the purposes of chemical/elemental speciation, yielding directly comparable data from SIMS, XPS, SR-μ-XRF, SR-FTIR/MIR and Raman imaging. This additional methodology will be the subject of a forthcoming peer-reviewed paper.

Following cleaning, a large SIMS stage raster allows assessment of both implant and surrounding region, Figure 16. XPS data was also successfully obtained, but due to the relatively poor XPS resolution the data was summed to produce a line scan covering the centre of the metal to the outer bone of the explant and this could be used to verify the similarly summed SIMS data, Figure 17. XPS provides complementary quantitative atomic compositions giving Ca/P and O/P ratios similar to expected for hydroxyapatite and native bone.

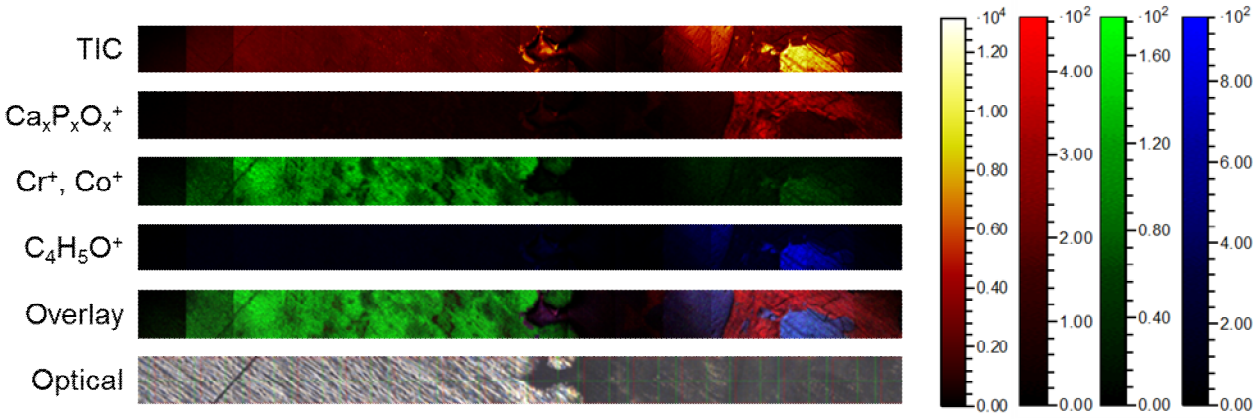

Figure 16: SIMS chemical image (bottom) of spatial distribution of implant alloy, calcium phosphates (mineralised bone) and embedding resin; optical image of analysed region shown at top, with total ion count image at centre.

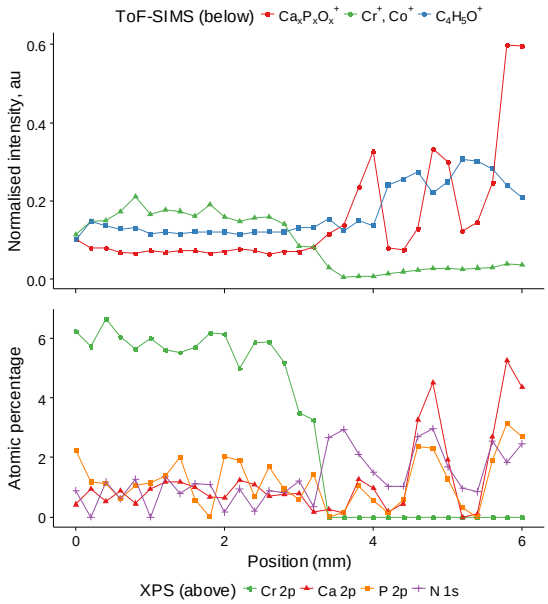

Figure 17: Complementary XPS (top) and SIMS (bottom) lateral profiles

Micro-Raman mapping at INRIM was able to provide a profile distribution of some main components on the samples such as hydroxyapatite, collagen and embedded resin materials over the analysed surface. No Raman signals were recorded on the region of the alloy. Embedded resin signals were very intense in the Raman spectrum and they tend to cover interesting signals related to proteins and bone, whose main vibrational bands are present around 1640 and 960 cm^{-1}, respectively. In order to overcome this issue, a 'Mathematica' based tool was developed by INRIM to subtract resin signals from the Raman map and to obtain a more consistent chemical imaging for collagen and hydroxyapatite (Figure 18). However, because of a very thick resin layer, only Raman signals of the resin were detected, thus limiting the application of Raman analysis in this field.

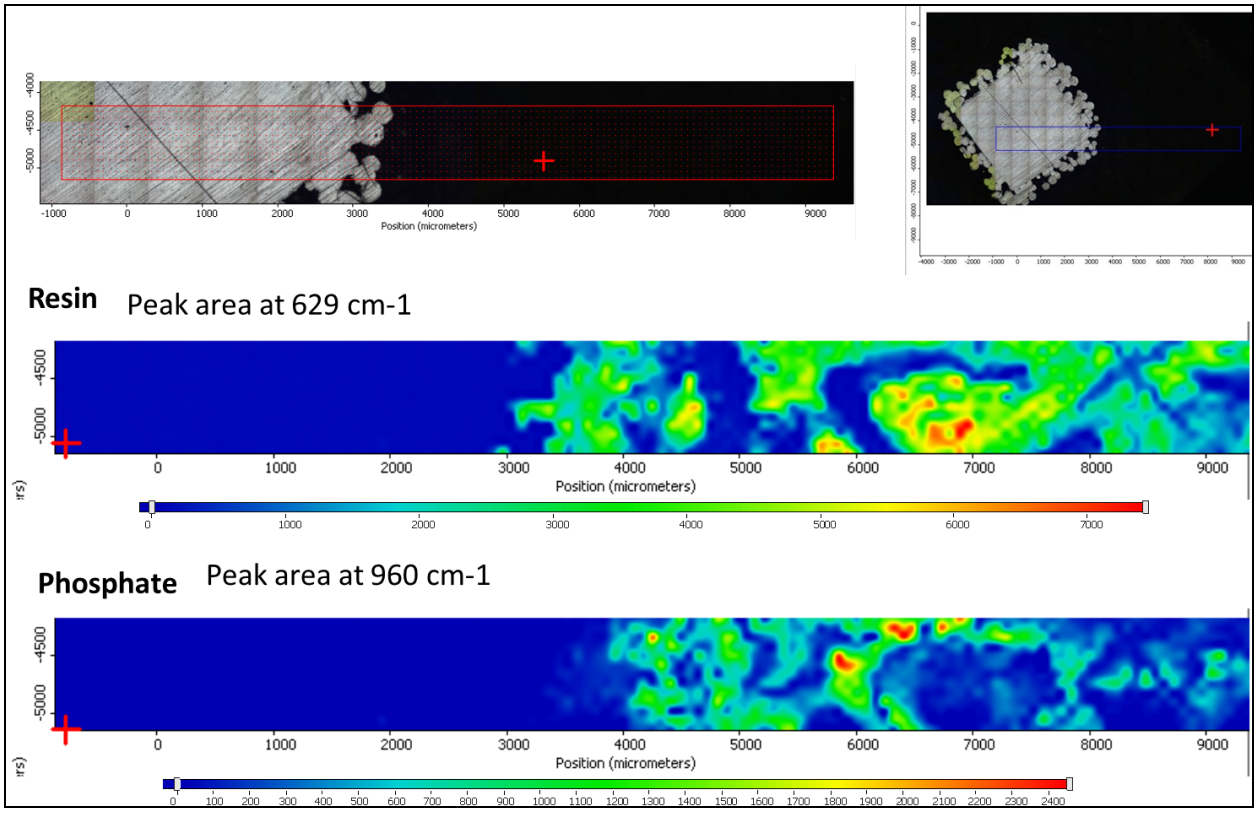

Figure 18: Raman maps of CoCrMo explant sample, showing distribution of embedding resin and phosphate (calcium hydroxyapatite) detectable by Raman (based on 'Mathematica' calculated subtraction spectra).

SR-FTIR/MIR analysis of the CoCrMo alloy implant was able to detect characteristic Amide I (1655 cm^{-1}) and Amide II (ca. 1548 cm-1) modes and a spectral feature at 1247 cm^{-1} (v C-N, v C-O) that originate from accumulated proteins nearby the bone-implant interface. Further, bands can be observed that are characteristic for carbonate stretching vibrations which refer to the bone tissue matrix. These spectra were resolvable distinctly from the embedding resin spectra.

A comparison analysis between 2D chemical images obtained with Raman, FTIR, XPS and SIMS was conducted to describe the sensitivity of detection and imaging resolution of all these different techniques. The preparation protocol developed enabled the possibility of data fusion between techniques (SIMS, XPS and possibly optical spectroscopies). For fusion of the SIMS and Raman data (seen in Figure 18) a dual PCA data fusion algorithm was developed for combining mass spectrometry and Raman imaging data. In brief, this involves performing PCA on both imaging data sets, then performing an additional target factor rotation to maximise correlation between scores in each dataset, swapping correlated components (retaining scores from the high spatial resolution data and loadings from the high spectral resolution data) and finally projecting the fused components back into the original space.

This research illustrates and enhances the power of using different techniques for analysis of explanted implants from bone and the advantages gained from the complementarity of using a multi-technique approach to obtain relevant information about bone growth adjacent to the implant and corrosion of the implant into the surrounding tissue. This is critical to develop implants which complement the natural environment and lead to longer implant lifetimes reducing the need for removal or replacement.

3.3.5 Conclusion:

the project was able to successfully integrate and test metrological tools developed for analysis of production line medical devices. This was only possible with a combined approach across the NMIs using the wide range of techniques available.

The project has successfully established a utility of tools developed for failure analysis of production line medical devices and explanted medical devices

- The first ever comparative work using AP-MALDI of production line medical devices, as compared with the analyses of a diverse range of production line medical devices via SIMS (high vacuum), LESA and DESI (both ambient methods). This multi-technique approach using adaptations of existing methods promises greater analytical yields and enhanced sensitivity, yet with the convenience and higher sample throughput related to ambient methods and is a logical extension of the validation work from the second objective. Recommendations for industrial use of these techniques for surface mass spectrometry of medical devices to detect surface defects is presently being written up for a peer reviewed paper.

- Methodologies for the use of different surface mass spectrometries and FTIR for the detection of common contaminants direct from medical devices have been developed. Comparisons and recommendations of the appropriate use and application of the 5 different techniques are available to be applied in industrial measurement.

- Good comparative work on SIMS, XPS, Raman and SR-FTIR/MIR methods applied to drug eluting stents will be published, including guidance on optimal analytical parameters and technique selection.

- The power of a range of different analytical techniques for the analysis and subsequent development of implanted medical devices has been shown for assessment post-implantation of implant integration into live bone regions. This is the first time in the world a single implant sample has been measured by the range of techniques and data compared in this way.

- Methods for multiple analyses of single samples and subsequent data fusion have been developed to allow all available data to be easily used to assess the success of an implant. This aims to aid and support other research and measurement teams to carry out this type of analyses.

In summary, the successful completion of these objectives have provided both further knowledge and guidance pertaining to the surface analysis of real medical device products and new practical approaches to optimising and utilising existing and developing methods. Importantly, this includes provision of greater confidence in the use of recently available and more practicable ambient analytical methods in an industrially relevant setting, with validated improvements to reproducibility achieved by the Project Partners.

4 Actual and potential impact

The project aimed to advance methodologies and provide guidance in the optimal use of chemical analytical methods for *in situ* chemical analysis and imaging of implantable medical devices. As such the impact from this project needed to address a wide range of communities, from biological physicists, chemical and polymer physicists, researchers and engineers that work in the field of thin films, materials and surface physics, analytical scientists and biomedical device producers. In order to ensure this, a wide range of

dissemination activities, addressing different audiences were carried out, with 11 submitted papers, 8 papers in draft, 49 presentations, 17 training events and 5 funded follow up collaborations. The 49 presentations were a mixture of oral and poster, to enable the widest audience and interactivity, and took place in over 15 countries given to an estimated audience of > 5000 participants. The 17 training events involved over 200 people including students, industrial end users, analytical specialists and research scientists.

A suite of new tools are now available at European NMIs to support medical device characterisation as well as tools and techniques suitable for research and idustrial use. These will enable improved use of chemical metrology tools for the support of biomaterial manufacture: improving reliability of analysis; application to medical devices; development of novel analytical tools; solving industrial problems. The project's outputs have been disseminated widely to: the metrology community; high-level users of precision instrumentation in research environments; regulators and medical device manufacturers.

Early Impacts:

The improvement in analytical techniques developed in the project and the guidance on appropriate selection of technique has already lead to a number of examples of improved industry capability:

- Analysis developed in this project by NPL and University of Meunster has enabled a clearer understanding of the adhesion and failure mechanisms of coated catheters which resist bacterial attachment. This has enabled the University of Nottingham, another project participant to develop a coating of novel urinary catheters with Camstent Ltd who are working towards medical device approval.
- A global medical device company recently made use of ambient methods when determining the source of contamination along a production line. This resulted in a rapid turnaround which enabled production to be resumed quickly with minimal loss of earnings.
- A global prosthesis manufacturer is working with NPL in a feasibility study to improve performance by plasma immersion ion implantation to reduce sliding frictional losses internal to the device and providing more efficient actuation by surface treatment. The study will address processing challenges relating to surface characterisation, process design & scale-up and demonstrates techniques developed as a part of this project being used in a product development lifecycle that, if successful will lead improved patient wellbeing.
- This project enabled the use of different techniques for analysis of implants removed from the body to assess bone growth adjacent to the implant and corrosion of the implant into the surrounding tissue. The advantages gained from using a multi-technique approach is essential to obtain the relevant information, and a number of medical device companies have shown interest on these approaches. Ultimately, this understanding has the potential to aid both product development and the meeting of regulations.

These early examples of improved industry capability illustrate that the surface characterisation techniques and guidance developed as part of this project have the ability to contribute to the quality assurance, product development and regulatory requirements of the medical device industry. This will ultimately have a direct impact on the health and wellbeing of patients requiring medical implants (an estimated 100 million people in the EU have a permanent implanted medical device) and will contribute to the continued growth of the EU medical device industry.

5 Website address and contact details

http://projects.npl.co.uk/IND56-Q-AIMDS/

JRP Contact: Fiona Moriarty (Fiona.Moriarty@npl.co.uk)

6 List of publications

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- Al Hook, DJ Scurr, ToF-SIMS analysis of a polymer microarray composed of poly(meth)acrylates with C6 derivative pendant groups, *Surface and Interface Analysis*, **2016**, 48(4), pp 226-236

  http://dx.doi.org/10.1002/sia.5959