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THE USE OF IMAGING FOR CLAIM SUPPORT IN THE HEALTHCARE INDUSTRY

Dr Chris Pickles

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SUMMARY

Over the last 10 years advances in high resolution imaging of both topographical features and chemical species' distribution have been applied increasingly in the characterization of commercially important surfaces such as hair fibres, teeth, skin and textiles. These capabilities have been used extensively to inform both the development of products and the proof of efficacy in support of performance claims for clients in several areas of the Home Care and Consumer Healthcare sectors. In this paper we describe some of these techniques and give examples of how they have been exploited in practice, from evaluating the level of protection afforded by oral health products to measuring the efficacy of skin and hair products, such as anti-wrinkle applications and conditioners.

SCOPE

There is a wide range of imaging technologies to choose from in a review of this kind. Optical microscopy is worthy of a review on its own and is used extensively in conjunction with all of the techniques addressed below. However, the focus of this white paper is on the chemical mapping capabilities of surface analytical techniques plus the surface area metrology and statistical quantification capabilities of topographical imaging techniques and the application of these to commercially important developments in the consumer healthcare sector. The author recognizes the invaluable information available from techniques such as Scanning Acoustic Microscopy (SAM), Transmission Electron Microscopy (TEM), Electron Microprobe Analysis (EMA) and many others in the study of material structure, but these are not covered in this review.

TECHNIQUES AND APPLICATIONS

WHITE LIGHT INTERFEROMETRY (WLI)

White light interferometry is an optical surface metrology technique that provides measurement of the physical characteristics of a material including micro-topography, form and texture analysis, roughness, dimensional metrology and layer thickness measurement. There are various instrumental configurations based either on focussed white light, LED or laser illumination of the sample in combination with scanning X-Y stages (scanning profilometers) or white light illumination in tandem with confocal microscope/interferometric optics (white light interferometers).

White light interferometry combines white light illumination of the sample with spatial filtering in a confocal microscope system to provide measurement of the variation in height of the sample (z-axis) thanks to the microscope optics being vertically scanned in a controlled manner. The X-Y sample stage may also be additionally scanned to provide a stitching option for larger fields of view up to 100mm x 100mm.

The resolution of the measurements are <1 µm in the x and y axes and <10nm in the z-axis. Hence micro-features and topographic variations can be monitored in detail. Sample sizes from a few mm^2 to 100 mm x 100 mm can be measured with a height variation over the sample of up to 25 mm. However, samples do not need to be presented to the instrument directly. Where 'difficult' or large samples are concerned surface replication using an air curing liquid silicone can be used to take surface replicates which can be analyzed and the data inverted to re-create the original surface topography.

Processing of the raw data allows for the generation of a range of data formats including:

PSEUDO-COLOR HEIGHT MAPS

The color scale is calibrated in nanometers, microns or millimeters. This allows 3D graphical representation of surface topography. Derived amplitude parameters allow classification of various aspects of topography - for example, Sa - the arithmetic mean of the deviations from the mean (the statistical average area surface roughness parameter) St - the total height between the highest peak and the deepest hole (the data range in z) and Sz - the mean of the distances between the five highest peaks and the five deepest holes.

PROFILOMETRY

Allows the generation of line scans across any user-defined XY plane of the sample.

CONTOUR MAP PLOTS (AXONOMETRIC PLOTS)

Regions of equivalent height are connected by color-coded lines.

3D PHOTOGRAPHS AND VIDEOS

Show the sample in xyz space with variable angle viewing, zoom and variable lighting.

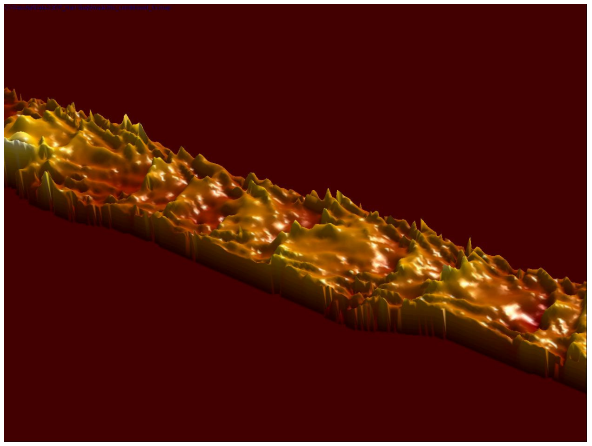
FILM THICKNESS (FOR SUFFICIENTLY TRANSPARENT LAYERS)

By focussing separately on, say, a coating surface and the substrate surface and then subtracting the data sets, a film thickness image can be generated and quantified.

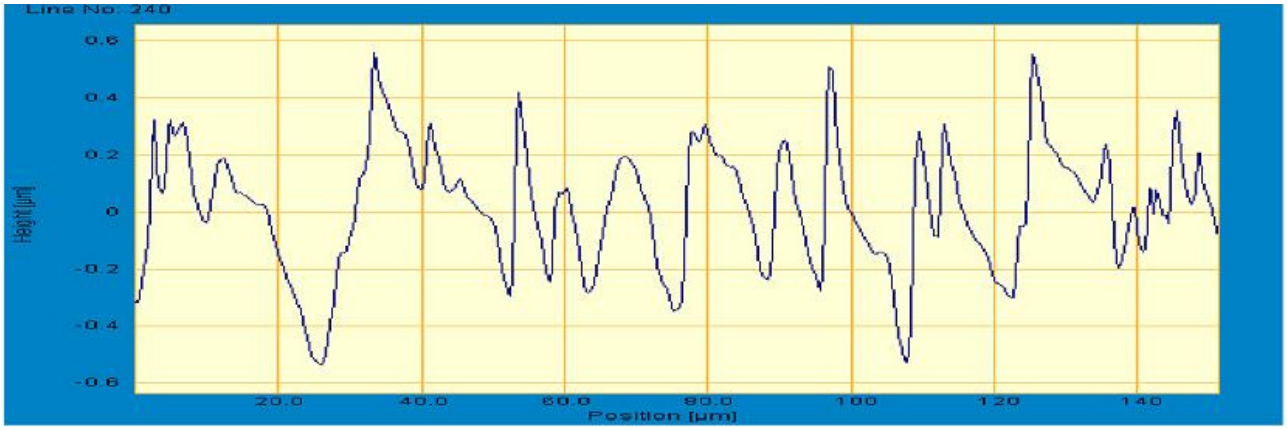
WLI APPLICATIONS

HAIR AND SKIN

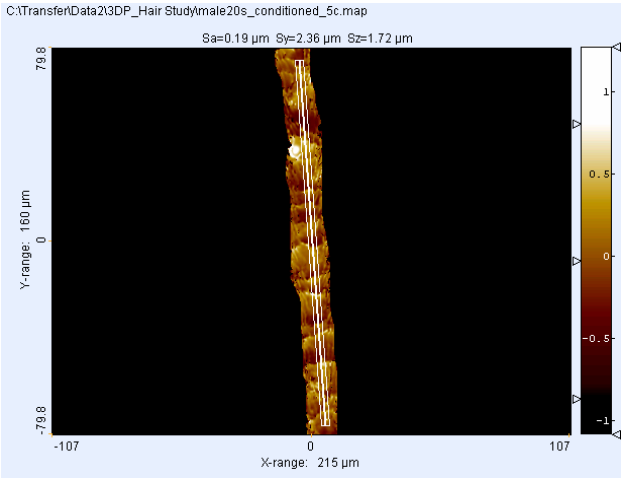
WLI has limited capability for highly curved surfaces but can be used for scale height determination on individual hair fibres using the line profile feature. As with 3DSEM, skin topographical imaging and profiling can be carried out using either replicates or mimics.



WLI 3D image of a human hair



Line-scan analysis along the length of the hair giving scale height values



3D topographical image showing scale features along a human hair

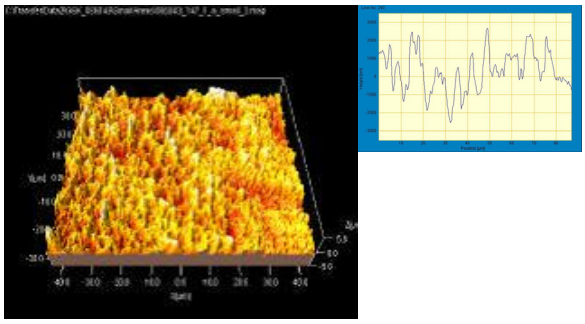
The technique is ideal for addressing the effects of hair applications, such as conditioners, on the surface structure modifications achieved in practice.

HUMAN TOOTH ENAMEL

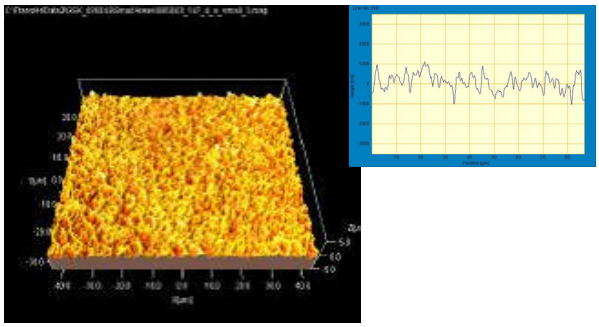
WLI has been used routinely by Lucideon to measure the enamel surface condition both before and after acid challenge and with a range of product types and treatments, including both toothpastes and mouthwashes.

A range of oral health products can be evaluated in this way. For example, the level of protection afforded by various toothpastes and mouthwashes in addition to the effect of diet among different subgroups can be measured.

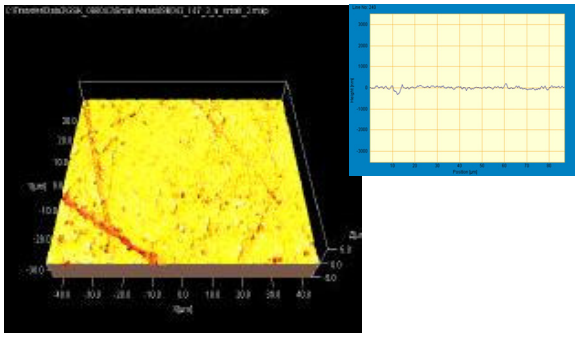
Below are 3D surface roughness images and line-scans of tooth enamel subject to acid challenge after different treatments.



No treatment



Non-fluoride mouthwash

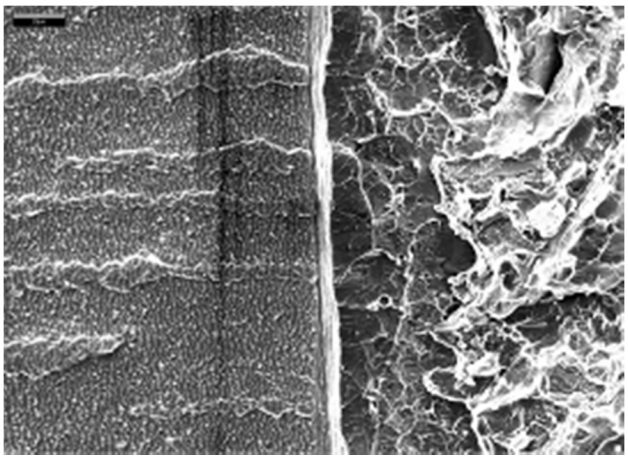


Fluoride mouthwash

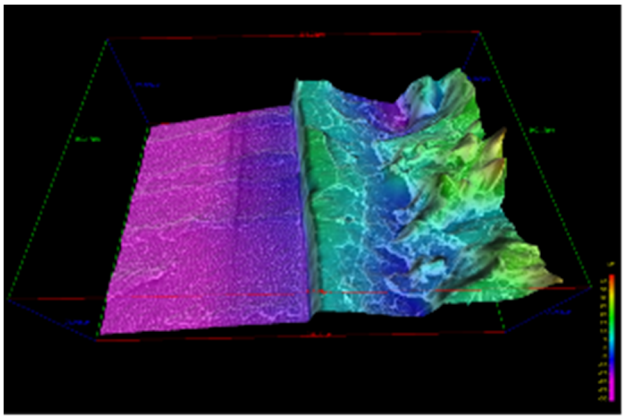
3D SCANNING ELECTRON MICROSCOPY (3DSEM)

Available software provides the capability to convert pairs of stereoscopic digital SEM images into a 3D representation of the area sampled. The resultant calibrated image contains z (height) information allowing metrology of macro and micro areas. The main benefit of this is that the metrology of materials which are difficult to determine by other methods (such as rough surfaces, angular metals, cutting tools, fibers) can be easily resolved. Furthermore, compared to white light interferometry methods, the lateral resolution in X and Y is significantly superior.

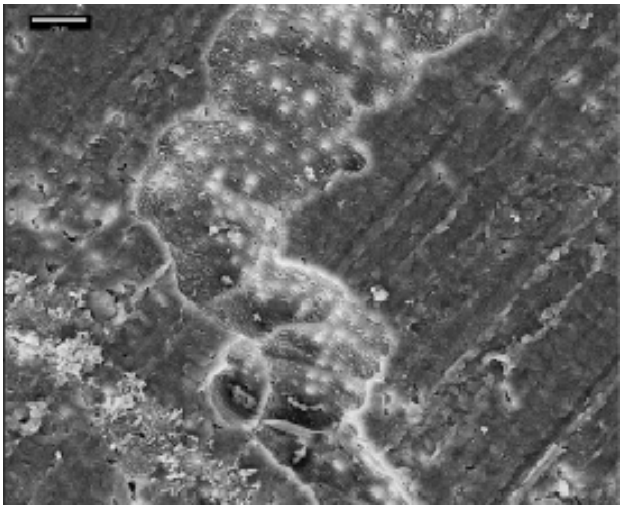
In conventional SEM the sample information is projected onto a two dimensional image plane and information about the third dimension is lost. However, with eucentric tilting of the stage, stereoscopic images can be produced with known tilt angles and working distances. The software automatically identifies points in the images which belong to the same point on the sample. From these homologous points the three dimensional coordinates are recovered, creating a true 3D image set for each pixel.



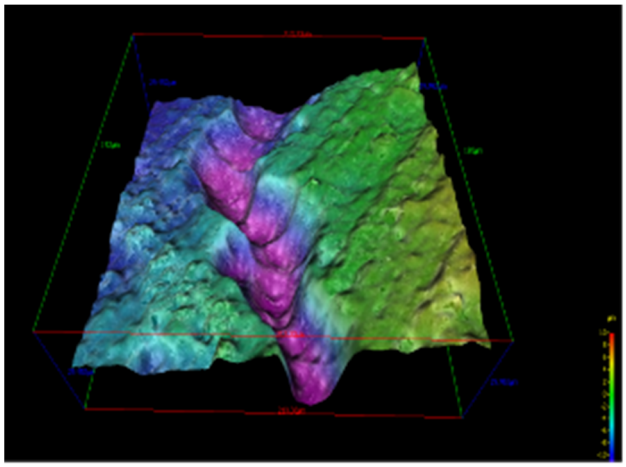
Metallic/glass interface – SEM



Metallic/glass interface - 3DSEM



Bone – SEM



Bone - 3DSEM

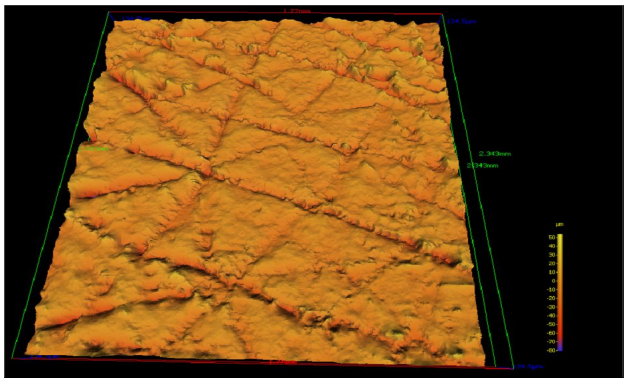
As can be seen from these comparisons the high image quality in SEM data is extended to give a high quality 3D image. From this, the full range of topographic data including line-profiles and roughness parameters can be determined. The illustrative value of 3D-SEM can be augmented with the additional topographic information and access to a wider range of materials, such as fibers and functional textiles and skin, hair and teeth, which other methods cannot supply, is also enabled.

3DSEM APPLICATIONS

Topology and metrology studies in the following areas are particularly suited to the use of 3DSEM. They represent applications where white light interferometry techniques are of limited value.

HAIR AND SKIN

Interferometric investigation of the effect of hair care and skin products is restricted by the relatively small sizes of solid additives in formulations such as the micron-sized particles used in hair-care products. 3DSEM can resolve these directly. 3DSEM is also capable of generating quantitative topographical images of human skin by taking surface replicates or, alternatively, of non-human skin mimics directly.



3DSEM image of human skin replicate

The efficacy of skin products such as anti-wrinkle applications can be measured directly by this methodology - if necessary using surface replication techniques. In addition, depilatories and shaving products can also be evaluated for their effects on skin topography using this technique.

CUTTING TOOLS AND BLADE EDGES

Steep-sided slopes and acute angles restrict topographical analysis to very small areas and shallow depths of field. 3DSEM provides scope for large areas (>500 µm) and significantly larger depths of field. In this application the technique can be used to quantify tool wear characteristics.

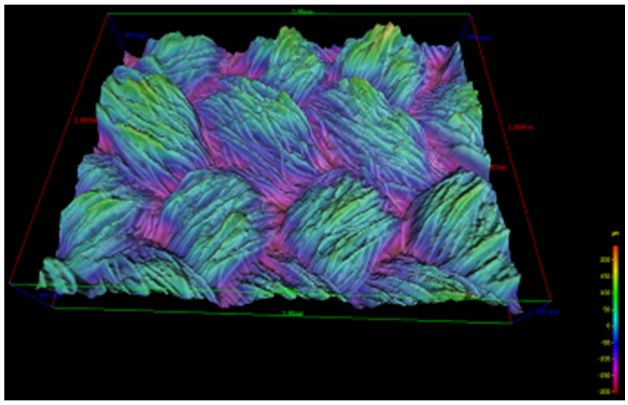
CERAMIC TILES

Tiles with a high degree of surface texturing produce poor data using white light interferometry due to their high angles and surface asperities. 3DSEM provides representative data on both macro and micro scales, enabling detailed quantitative analysis of surface treatments and slip-resistance effects.

TEXTILES

Poor reflectivity and multiple angles make it virtually impossible to generate useful information from interferometry whereas 3DSEM is able to generate useful data.

The quantification of weave (and non-woven) features allows fabric performance to be understood in terms of the fabric construction.



3DSEM image of a woven textile

ATOMIC FORCE MICROSCOPY (AFM)

The length scales of 2D spatial resolution in white light interferometry (microns) and 3DSEM (sub-micron) can be extended to the atomic scale by use of the Atomic Force Microscopy (AFM) technique. AFM exploits the repulsive/attractive force field pertaining when atoms approach each other by contacting a surface with a very sharp pointed tip (usually made of silicon using nanotechnology manufacturing techniques). This tip can be rastered over the surface whilst monitoring the separation required to keep the force between the tip and the sample surface constant. In the attractive force regime there is contact AFM and in the repulsive regime there is non-contact AFM. To avoid tip damage the cantilever holding the tip is oscillated at its resonant frequency and the shift in frequency or phase and amplitude of the driving voltage is used to generate the images.

In addition to surface topography 3D imaging, AFM can also provide information on frictional forces (lateral) and hardness (force vs distance). Moreover, and in contrast to SEM, the technique can be used in air and on submerged surfaces.

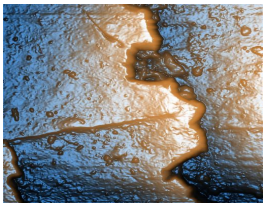
The non-contact (tapping) mode is best used for topography and involves less wear on the tip. Frictional force is measured as the cantilever twists when in contact mode as it glides over stronger and weaker attractive areas of the sample. Hardness is measured using force v distance curve as the tip is forced into the sample at each pixel in the image raster. There are other modes of imaging that use different tip materials and functionalized tips.

AFM APPLICATIONS

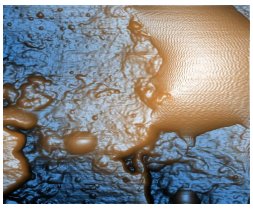
The non-contact mode topographical imaging option has been used to study the cuticle areas of human hair before and after treatments.

HAIR

The ability to sense liquid deposits using AFM has been applied to studies of hair products in terms of their spatial distribution on single hair surfaces.

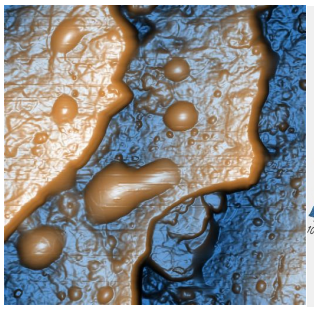


Before conditioner

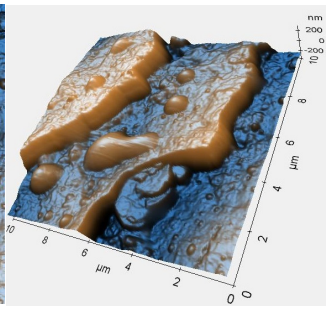


After conditioner

Above are AFM 10 micron images of the edge of a single scale on an untreated human hair “as received” and after treating with a proprietary conditioner from a sachet and rinsing in high purity water. Some of the features are smaller than the wavelength of light so cannot be seen in conventional optical instruments and the residual liquid would not be amenable to SEM imaging.



After conditioner

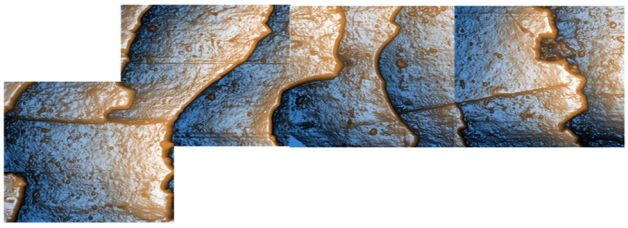


Isometric view

Above is another 10 micron area shown in plan and isometric view. The residual droplets of conditioner are clearly seen adhered to the scales and would act as a lubricant. Note the conditioner polymer is distributed as blobs all over the scales and to some extent in the corner of the staircase at the junction of the step and riser. In this case it combines to add lubricity to the surface of the scales and smooth out and lubricate the scale edges.

Line Trace

The line trace shows that the scales are ~200 - 300nm high “cliffs”. The arrow highlights material that is at the base of the cliff and may be fixing the scale down so that it no longer acts as a barb.



Stitched 10 micron images

Images can be ‘stitched’ together to generate larger fields of view.

DYNAMIC SECONDARY ION MASS SPECTROMETRY (DSIMS)

Dynamic SIMS provides quantitative information on the elemental composition of the sample surface region from a few nm to several hundreds of microns in depth. The detection sensitivity of the technique is in the ppm - ppb range for all elements in the periodic table.

In Dynamic SIMS a primary ion beam of energy 0.5 keV-20 keV is used to sputter-remove successive layers of the sample in a well-defined area ranging in size from typically 1 mm x 1 mm to 10 µm x 10 µm. The emitted positive and negative secondary ions are collected and mass analyzed in a mass spectrometer. The secondary ion currents are representative of the sample composition and with appropriate use of standard samples can be used to produce quantitative information on the lateral and depth distribution of major elements, dopants and impurities in any solid material.

Several types of analytical information can be generated:

MASS SPECTRUM

By scanning the mass spectrometer over its mass range (>500 amu) elemental and molecular information is obtained from an area of the sample.

DEPTH PROFILE

Monitoring several elemental or molecular species with time while continuously sputtering a well-defined sample area produces a plot of secondary ion intensity versus time which is processed to give concentration versus depth plots.

2D AND 3D IMAGES

Monitoring several species while the primary beam is rastered over the sample area produces chemical images of the surface. Continual acquisition of images with depth can also provide 3D information including retrospective depth profiling and sectioning.

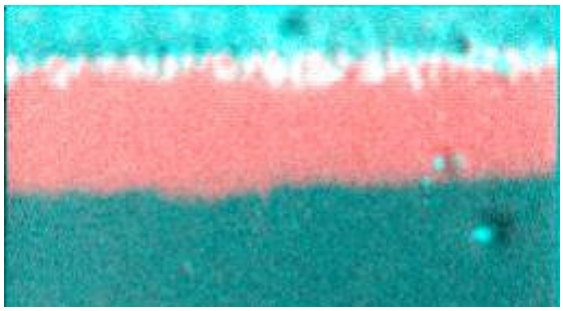
LINE SCANS

The primary beam can be scanned across surface features to produce line scans to identify distinct chemical boundaries.

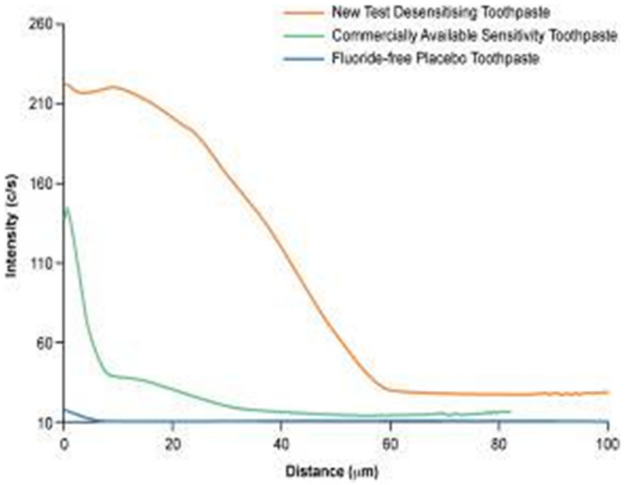
DSIMS APPLICATIONS

HUMAN TOOTH ENAMEL

Considerable work has been done in determining the depth to which fluoride penetrates the tooth enamel and this has been compared with fluoride-free treatments and advanced fluoride toothpaste formulations. With the advanced treatments significantly higher levels of fluoride penetration can be achieved compared to that of a conventional fluoride product. Both depth profiling and F species imaging have been used to confirm this.



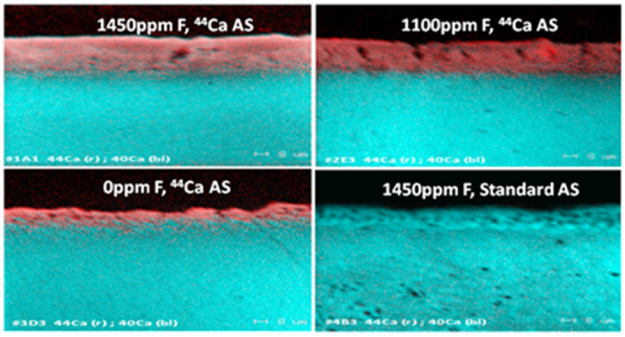
Cross-sectional DSIMS overlay image of human tooth enamel treated with an advanced fluoride formulation showing F (red) and carbon from enamel and resin (green)



Penetration distance into tooth enamel

Where products penetrate enamel, or are deposited upon it, the layer structure achieved can be correlated with practical performance experience.

Because DSIMS is a mass spectrometry technique it recognizes all the isotopic masses of the elements. This makes it particularly useful in mapping isotope doped substances and this capability has been applied to tracking the extent to which the remineralization of human tooth enamel occurs under a range of different treatment conditions.

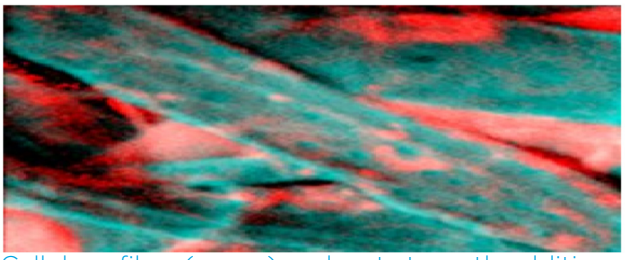


44Calcium DSIMS images from cross sections of eroded human enamel after treatment with NaF or Fluoride-free dentifrices, followed by incubation in artificial saliva (AS)

Samples were treated with toothpaste slurries followed by remineralization using artificial saliva. In order to identify new calcium uptake during the remineralization phase an artificial saliva was prepared which contained enhanced levels of 44Ca. DSIMS imaging was used to determine the extent of new calcium uptake into the enamel as shown above for four different treatments.

TEXTILES

The DSIMS technique is capable of generating chemical images for diverse species and has not only high sensitivity (ppm-ppb) but also excellent spatial resolution (micron pixellated). This has been used to effect in looking at non-woven products such as paper towel where the location of the wet strength additives relative to the fibre crossing nodes has been investigated.



Cellulose fibre (green) and wet strength additive (red)

The advent of 'functional fabrics' where performance effects are delivered by the deposition of surface chemistry-modifying materials has benefited greatly from the application of these surface analysis methods. Water repellence, breathability and antimicrobial functionality are just a few of the areas where they have been applied successfully.

TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY (TOFSIMS)

In ToFSIMS a sample is bombarded with a beam of pulsed primary ions. Secondary ions are emitted from the sample surface and these are mass analyzed to provide detailed surface chemical information on elements, chemical groups, molecules and polymer fragments. ToFSIMS analysis can be carried out under so-called '*static*' conditions using such low primary ion doses that the sample is effectively undamaged by the analysis. It is also highly surface sensitive in this *static* regime where the sampling depth is only 1 - 2 monolayers (often <3 nm). ToFSIMS is analytically highly sensitive (often trace detection levels) but it is not quantitative although it can provide some semi-quantitative information.

For *static* ToFSIMS analysis the technique usually provides mass spectra and secondary ion images. Where necessary, the retrospective data processing capability of the instrument is particularly useful when a selected area of the sample is scanned in *spectrum-per-point* mode. In this mode a mass spectrum is acquired at each (µm scale) pixel point for the field of view and the

resulting two-dimensional array of spectra is stored.

Retrospectively, a number of operations can be performed, including:

REGION-OF-INTEREST (ROI) ANALYSIS
For defined regions (any shape and size) within the analyzed area, it is possible to sum the mass spectra at all of the pixel points within each defined region.

IMAGING ANALYSIS
For the analyzed area chemical maps can be created for any of the spectral peaks within the mass range. For weak signals, where there is not enough individual peak intensity to produce meaningful images, it is possible to add related peak intensities, thereby improving the image statistics.

The retrospective feature allows data to be analyzed/re-analyzed at a later date and ROI spectra from different regions of a sample can be produced or different chemical maps created, without the need for re-analyzing the sample in real time.

Some of the instrument capabilities and features include:

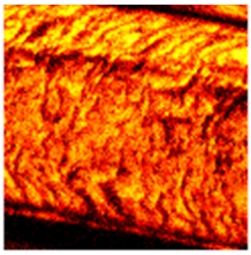
MASS SPECTRA
Positive (+) and negative (-) ion spectra can be routinely acquired for both conducting and insulating samples.

ION IMAGES (CHEMICAL MAPS)
Images (+ and -) can be acquired for both conducting and insulating samples, down to submicron spatial resolution.

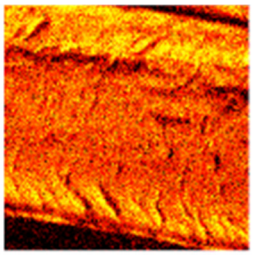
AREA ANALYSIS
Sample areas are routinely scanned with an electronic raster which permits a maximum area of analysis of 500 µm x 500 µm. For larger areas, the motorized sample stage is “rastered” (which allows a maximum area of analysis of 9 cm x 9 cm).

TOFSIMS APPLICATIONS

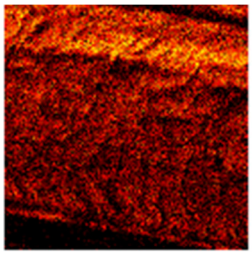
HAIR
The versatile chemical mapping of molecular species available from the ToFSIMS technique has been applied to the study of residual substances on the surface of human hair. These can be from either natural (e.g. sebum) or synthetic (e.g. hair products) sources. Human hair has a diameter of around 60 - 70 µm. The sub-micron capability of the SIMS technique generates spectacular high resolution images showing the spatial distribution of residues with ppm sensitivity (brightness indicates abundance).



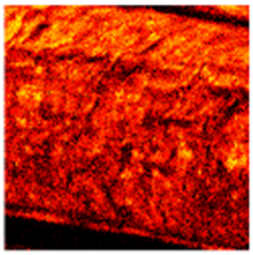
Keratin



Silicone

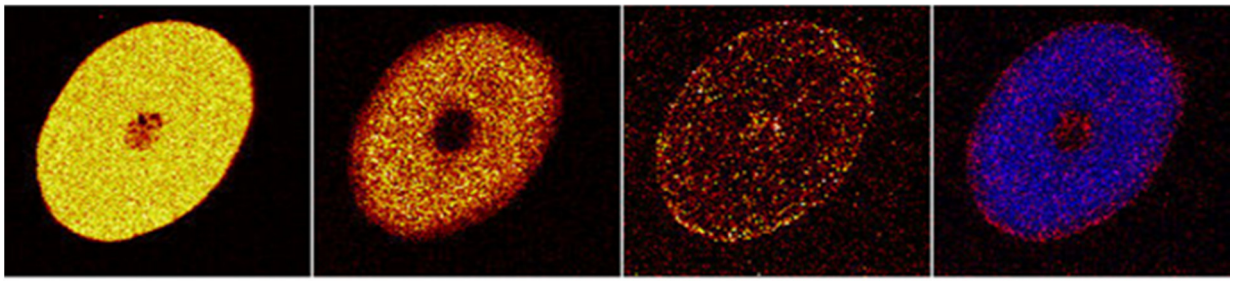


Fatty Acid



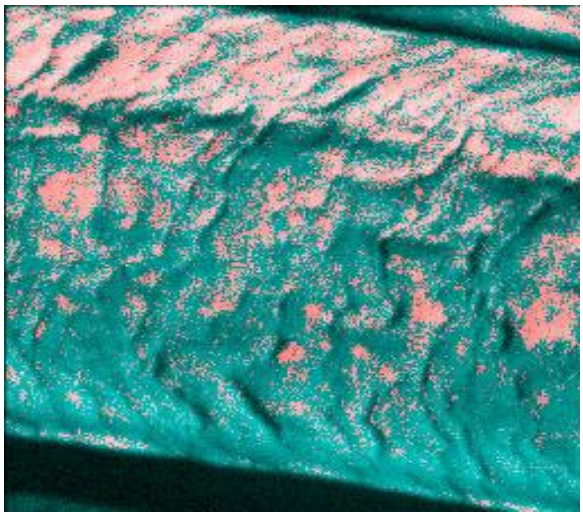
Alkyl Sulfate

Similar images can also be generated on cross-sections of hair:



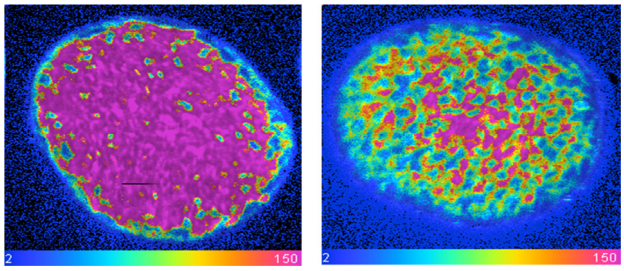
Protein Alkyl Sulfate Oleate Total Ion Map

Product residues can be readily identified and their spatial distribution followed using this technique. This is important in hair product development not only from an efficacy standpoint but also in terms of ensuring potential adverse effects are addressed effectively.



False color overlay of alkyl sulfate (red) on keratin (green)

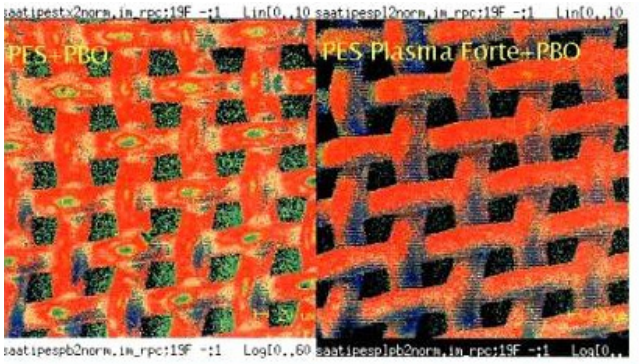
In addition to the surface chemical mapping of hair by ToFSIMS the technique can also be used to determine the extent of chemical species penetration into the hair body by the mapping of hair cross sections.



Oil penetration into hair fibre. Images show more intense color where the concentration of the active material is higher.

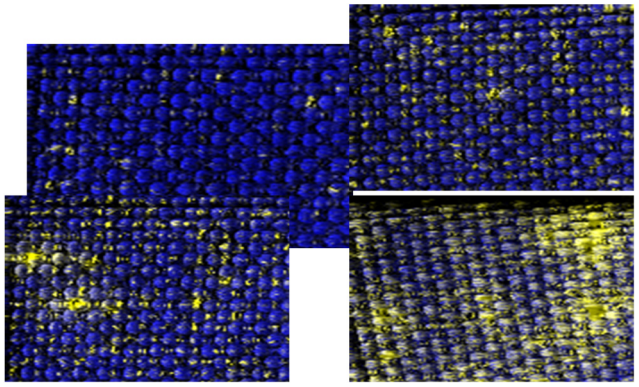
TEXTILES

Fabric treatments have become widespread with the advent of ‘smart textiles’. The effects delivered by surface modifications and by deposited ‘effect’ chemicals are dependent on the extent and/or level of treatment or deposition achieved. These can be established directly by ToFSIMS imaging. In the case below, pre-treatment improved subsequent deposition.



Fluoro-polymer distribution on polyester substrate. Plasma treatment enables high, uniform coverage of fluorocarbon (right). Exposed node regions without plasma treatment (left)

Surface coverage as a function of the process control variables can also be explored:



Coverage of a plasma deposited antimicrobial (yellow) on a polyester substrate (blue) at four different process conditions

X-RAY PHOTOELECTRON SPECTROSCOPY (XPS)

X-ray Photoelectron Spectroscopy (XPS) also known as ESCA (Electron Spectroscopy for Chemical Analysis) is an extremely surface-specific and non-destructive technique that provides quantitative surface chemical state information for all elements except hydrogen and helium. It is the most established (since the mid-1960s) and widely used surface analysis technique.

XPS analysis involves irradiation of a solid or low vapor pressure liquid sample with soft X-rays in an Ultra High Vacuum (UHV) chamber, typically operating at a pressure of 10^{-9} mbar. Electrons are emitted from the energy levels of the elements in the sample with characteristic kinetic energies. These energies are such that only those electrons emitted from atoms close to the surface can escape the sample into the surrounding vacuum, where they are analyzed, producing a spectrum of photoelectron intensity vs. energy.

Chemical state and functional group information are manifested by subtle shifts in peak position of as little as a few tenths of an electron volt. Monochromatic X-rays and/or curve-resolving techniques are used to separate overlapping features.

The analysis depth is 2-10 nm depending upon sample orientation. Surface elemental compositions are expressed as atomic% and the detection limit is typically 0.1 at.% (1000 ppm).

Various types of analysis are available:

XPS SPECTROSCOPY
Linescans and chemical state imaging can be performed over a range of analysis areas from ~50 µm to several mm. Analysis times are dependent upon the analysis area and the detail of information required.

XPS IMAGING
Quantitative XPS imaging of element or chemical state distributions can be performed in scanning or parallel imaging modes over areas from ~500µm to 5mm squares at spatial resolution down to *ca* 5 µm. The image colors represent the composition at each pixel in at% and regions of interest can be quantified and tabulated.

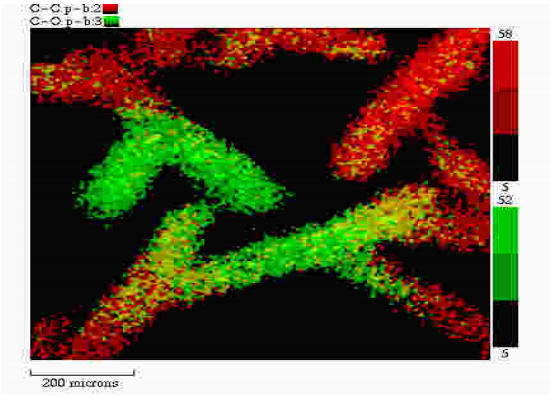
ANGLE-DEPENDENT XPS DEPTH PROFILING
By varying the sample orientation with respect to the analyzer, the variation of surface chemistry with depths in the range 2-10 nm can be probed non-destructively.

SPUTTERED DEPTH PROFILING
A locally destructive technique which measures changes in sample composition as a function of depth (up to 1 µm below the surface) by removing surface material with a focussed argon ion beam.

XPS APPLICATIONS

TEXTILES

XPS imaging has not been widely used in healthcare applications although it is the only technique which can give quantification of the species being imaged and, as such, could be used to establish the distribution of soils or deliberate fabric treatments or other surface deposits such as antimicrobials where the substance loading was of interest. In one study on a surface treated polypropylene tissue support mesh the coverage of a polyethylene oxide oligomer (diglyme) was measured using XPS imaging. The polypropylene signal was monitored by using the high resolution C-C electron energy and the diglyme signal was monitored using the high resolution C-O electron energy.



Open mesh polypropylene (PP) tissue support coated with diglyme - PP substrate (red) diglyme coating (green)

SUMMARY AND FUTURE PERSPECTIVES

Surface chemical mapping and topographical profiling has been used to support several areas of healthcare research, development, failure investigation, manufacturing issues and product claim studies over the last 10-15 years and particularly over the last 5 years. Lucideon has been at the forefront of these developments and is the only provider in Europe with the capability to deliver qualitative and quantitative chemical composition imaging by four separate techniques in addition to quantitative physical form imaging in 2D, 3D and video formats. Together with the other surface characterization techniques we fully expect the application of chemical and physical surface imaging technologies to spread more widely in the healthcare sector as their power to inform product and process performance phenomena becomes more widely recognised.

ABOUT LUCIDEON

Lucideon is a leading international provider of materials development, testing and assurance.

Through its offices and laboratories in the UK, US and the Far East, Lucideon provides materials and assurance expertise to clients in a wide range of sectors, including healthcare, construction, ceramics and power engineering.

The company aims to improve the competitive advantage and profitability of its clients by providing them with the expertise, accurate results and objective, innovative thinking that they need to optimise their materials, products, processes, systems and businesses.

ABOUT THE AUTHOR

DR CHRIS PICKLES - CONSULTANT TO LUCIDEON

EXPERTISE IN: AUTOMOTIVE; POLYMERS; SURFACES & COATINGS

Chris holds a Degree in Chemistry, a PhD in Polymer Science, and a Postdoctoral Fellowship.

AEROSPACE

Chris has been supplying surface analysis capabilities to the aerospace industry for over five years with particular emphasis on carbon reduction programs involving composite developments, coating analysis and lubricant developments in relation to the introduction of biofuels.

AUTOMOTIVE

Chris has worked in both the aftercare sector as a Company Technical Manager and in tier one supply chain manufacturing as Managing Director.

Chris has been responsible for the plastic injection moulding and blow moulding manufacture of automotive component systems including highly technical mouldings such as fuel tanks and 3D spoilers. In addition Chris has also managed an integral supply chain utilizing Toyota production system protocols.

POLYMERS

During his career, Chris has spent four years researching copolymer design for bulk property manipulation and the statistical mechanics of PVC to determine conformational sequencing. Chris's knowledge also encompasses plastics manufacturing, including injection moulding of glass-filled nylon and co-extrusion blow moulding of complex 3D components.

SURFACES AND COATINGS

In the field of surface science, Chris has conducted research projects on alternative material sources for surfactants and detergent product re-formulation. These include the re-launch of a branded fabric washing product in Brazil and the design of a surfactant system utilizing renewable resources. As Technical Manager in the automotive aftercare industry he has managed the development and quality control of spray paints for high speed aerosol filling.