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Multispectral optical imaging combined \textit{in situ} with XPS or ToF-SIMS and principal component analysis

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All X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) instruments have optical cameras to image the specimen under analysis, and often to image the sample holder as it enters the system too. These cameras help the user find the appropriate points for analysis of specimens. However they seldom give as good images as stand-alone bench optical microscopes, because of the limited geometry, source/analyser solid angle and ultra-high-vacuum (UHV) design compromises. This often means that the images displayed to the user necessarily have low contrast, low resolution and poor depth-of-field. To help identify the different regions of the samples present we have found it useful to perform multispectral imaging by illuminating the sample with narrow-wavelength-range light emitting diodes (LEDs). By taking an image under the illumination of these LEDs in turn, each at a successively longer wavelength, one can build up a set of registered images that contain more information than a simple Red–Green–Blue image under white-light illumination. We show that this type of multispectral imaging is easy and inexpensive to fit to common XPS and ToF-SIMS instruments, using LEDs that are widely available. In our system we typically use 14 LEDs including one emitting in the ultraviolet (so as to allow fluorescent imaging) and three in the near infra-red. The design considerations of this system are discussed in detail, including the design of the drive and control electronics, and three practical examples are presented where this multispectral imaging was extremely useful. Copyright © 2016 The Authors Surface and Interface Analysis Published by John Wiley & Sons Ltd.

Keywords: XPS; ToFSIMS; multi-spectral imaging; optical imaging; principal component analysis

\textbf{Introduction}

Often our collaborators bring samples for analysis in our X-ray photoelectron spectroscopy (XPS) instruments and time-of-flight secondary ion mass spectrometry (ToF-SIMS) instruments at our multi-user facility. A common occurrence is that we begin to look at the samples they bring, only for our visiting collaborator to ask 'Is that really my sample?'. The reason they ask this is that their sample looks different in the optical imaging within our XPS or ToF-SIMS instrument compared to their own microscopes. In some cases we have spent significant, expensive, instrument time searching for features or patterning that the collaborator had assumed would be obvious in any optical image of their sample. Our visiting collaborators often have extremely good optical microscopy facilities in their own laboratories.

Microscopes within XPS and ToF-SIMS instruments have improved enormously in recent years, enabled by the increased capability and reduced cost of digital imaging sensors, but still this issue persists. As time goes on we have progressed from ‘VGA resolution’, or 0.3 mega pixel cameras, to 1 megapixel, 2 megapixel, 5 megapixel and now one sometimes sees 25 megapixel Digital Single Lens Reflex (DSLR) cameras on some instruments. We are now past the point at which additional pixels will help us. The issues are rather ones of limited contrast, enforced long working-distance and the inability to adjust illumination or wavelength to particular precise requirements (e.g. UV illumination of fluorescently labelled or autofluorescent samples).

Fluorescent labelling is more likely to be an issue with ToF-SIMS than XPS. ToF-SIMS is an excellent technique for ‘label free’ analysis of biological surfaces. In validating results when applied to new biomedical problems inevitably these results must be compared with conventional techniques such as staining and fluorescent labelling of structures. This is often more of a problem than it should be: biomedical collaborators have exactly the right tools to image staining or fluorescence using appropriate light microscopes, but there is...
always an issue of digital file format conversion and image registration that makes it difficult or uncertain to overlay this on the ToF-SIMS image. This makes collaboration more time-consuming than it should be. Nevertheless, some excellent results have been achieved. For example, infrared spectromicroscopy and UV imaging experiments have been demonstrated in tandem with ToF-SIMS imaging, thereby allowing (in combination with histological staining) the identification of lipids, proteins, sugars and nucleic acids.[11]

Multispectral imaging

Multispectral imaging is gradually emerging as a valuable technique in biology and medicine.[22] Light delivered to biological tissue undergoes multiple scattering from inhomogeneities within biological structures and absorption primarily in haemoglobin, melanin and water as it propagates through the tissue. Near-infrared (NIR) wavelengths in particular can be a very useful region of the optical spectrum for biomedical imaging. NIR light penetrates biological samples, particularly lipids, within the ‘water window’ in the infrared spectrum, and gives rise to diffuse scattering within. What is absorbed in the material is not reflected, so absorbance images can have significant contrast. Multiple scattering in biological tissue can lead to a range of different contrast mechanisms at different wavelengths.[3] Scattering occurs where there is a spatial variation of refractive index, and that index can be wavelength dependent. Mitochondria have been found to be the strongest light scatterers within cells, because of the folded lipid structures within them. On a larger scale extracellular proteins (e.g. collagen) can be strong scatterers because of variations in density and therefore refractive index over a wide range of scales. The complexity of these scattering and absorption effects, together with the underlying complexity of the specimen structure, means that it is typically difficult to use the spectrum of reflected light from a specimen to identify key chemical components; nevertheless it provided a useful imaging technique to locate regions for analysis using surface analytical methods like XPS and ToF-SIMS. One needs to remember that the image comes from a much deeper region than the surface, and it should be used for navigation rather than being directly associated necessarily with the chemistry observed at the surface, unless there is good evidence that one is looking at essentially 2D structures (such as fibres cleaved perpendicular to their major axis, for example). The penetration depth of light entering tissue is also wavelength dependent, and in particular NIR radiation can generally penetrate more deeply within the ‘water window’.

For the above reasons we have implemented multispectral imaging in the entry and analytical chambers of our XPS and ToF-SIMS instruments, so as to provide the best possible optical images of the region of interest. We have also used this technique for viewing samples before introduction to our Helium Ion Microscope (HIM), also a vacuum-based instrument. We have now constructed two sets of identical electronics (each of which can be moved between different instruments and mounted to standard UHV viewports) and the software to drive it.

Choice of hyperspectral and multispectral image acquisition appropriate to surface chemical analysis

True hyperspectral images are made up of hundreds of contiguous wavelengths for each spatial position of a sample. Each pixel in a hyperspectral image contains the spectrum of that point. The resulting spectrum can be used, often by means of multivariate analysis, to characterise the composition of the set of pixels. Hyperspectral images, known as hypercubes,[46] are three-dimensional blocks of data, comprising two spatial and one wavelength dimension. The hypercube allows for the visualisation of constituents of a sample, separated into particular areas of the image, because regions of a sample with similar optical spectra may be assumed to have similar chemical composition, at least in the optically accessible region of the surface. This optically accessible region is typically much deeper than we are normally concerned with in surface analytical techniques such as XPS and ToF-SIMS; nevertheless, it may be useful depending on what is known about the sample and its surface-structure.

Instantaneous hyperspectral imaging in which each pixel of a light sensor is able to record a full spectrum at very many different wavelengths is difficult, so one of two main techniques are used to acquire the image data sequentially;

1. Line-scanning instruments record the spectrum of each pixel in a line on the sample which is simultaneously recorded by an array detector. There is one spatial dimension – the scan line – and one spectral dimension in which the light in that line is dispersed into its constituent wavelengths, sometimes called ‘pushbroom’ acquisition.[5] This is frequently used industrially (e.g. in food product quality control) where the product being scanned moves on a conveyor under the line-scanning instrument.

2. Another approach is to sequentially illuminate the sample with narrow-band wavelength light sources. As each discrete wavelength illuminates the sample a 2D image is acquired using a camera with a uniform wavelength response.

Either approach could, conceivably, be implemented in a UHV system such as an XPS or ToF-SIMS instrument, because they require only optical access. The second approach is particularly simple to implement in surface analysis instruments because these typically already include cameras and/or optical microscopes to navigate the sample being analysed. We used these existing cameras and optics but added narrow-wavelength illumination from commercially available LEDs. Of course all XPS or ToF-SIMS systems come with some form of illumination, these days usually using high-brightness white LEDs, as shown in Fig. 1. Our multi-LED system in most cases results in only a minor modification of this system, as shown in Fig. 2. The extra cost of such a system is minimal; the range of wavelengths available from commercial LEDs has progressively widened over the last 20 years, and in the last few years LED lighting has become popular in commercial and domestic settings, creating a market for high-power narrow-band LED devices. Somewhat similar LED multispectral systems have been developed by a number of authors in recent years,[6,7] although our work seems to be the first time this method has been applied to surface analysis. As examples of other applications of LED illuminated multispectral imaging, Shrestha et al.[8] recently proposed a LED based spectral film scanner. Tominaga and Horiuchi[9] proposed capture and illumination using LED light sources. How the spectrum emitted by LEDs varies with angle and the age of the LED has also been studied.[10] Multispectral imaging in the optical and IR range has been used to identify inhomogeneity in polymers and authenticate documents.[11] There are many applications of multispectral imaging in the analysis and conservation of paintings and other heritage materials.[12] At least one commercial LED-based multispectral imaging instrument[13] is available for operation in air.

It is very useful to be able to perform Multispectral Imaging (MSI) in situ in surface analysis instruments. The most important three capabilities that this adds are as follows;
Ability to image features having contrast outside the human optical range (e.g. near IR images of fats and lipids in biological samples),

Imaging physico-chemical processes that modify rather than simply reflect light (most often fluorescence)

Identifying different metamers – materials that look the same in a RGB image but in fact have different reflectance spectra. Colours that match this way are called metamers.

For example, Chlorophyll a and b are near metamers, but appear distinctly different in MSI.

**Design of a multispectral imaging system for surface analysis instruments**

Most multispectral LED imaging systems, with a few exceptions, have used monochromatic cameras. These typically are more...
accumulating 14 RGB images for processing. We settled on a design in which the controlling computer switches on a series of 14 LEDs in sequence of wavelength, longer wavelength. This could be done using a monochromatic camera and optical filters; however, this increases the complexity of the system. We settled on a design in which the controlling computer switches on a series of 14 LEDs in sequence of wavelength, taking an image from an existing RGB camera each time, thereby accumulating 14 RGB images for processing.

LED-based MSI is now very practical, but to deliver light to a specimen in vacuum one must first direct it through a suitable glass viewport, as shown in Fig. 2. Type 7056 ‘Kodial’ borosilicate glass is typically used for viewports on modern UHV instruments. This has good transmission over the entire range of wavelength commonly accessible using commercial LEDs, from 365 nm to over 1000 nm. Therefore no special UHV viewport is typically needed, and multispectral illumination can be retrofitted very easily to existing instruments. In most cases all that is required is the replacement of a single white-light source (often a high-brightness white LED in modern instruments) with a printed circuit board (PCB) containing multiple high-brightness, narrow wavelength LED devices.

The wavelengths of our main LED system comprise nominal emission specifications of: 390, 447.5, 465, 485, 505, 535, 590, 615, 620, 630, 655, 740, 850 and 940 nm. The manufacturers are Cree, Lumileds, Vishay and OSA Opto Light, although in this fast developing LED lighting market individual products are updated and specifications change very rapidly. Because of high absorption of IR by water for wavelengths above 1350 nm, we have only collected and analysed wavelengths below 1300 nm, and for most of our work we have used LEDs of nominal wavelength 940 nm and below. The main printed circuit board (PCB) containing the LEDs is shown in Fig. 3, and in Figs. 4 and 5 with two of the LEDs operating at low current. This double-sided PCB is populated with a variety of different LEDs in miniature surface-mount packages in order to achieve the required range of wavelengths. Double-sided construction combined with ample use of vias and large thermal planes ensures that heat generated by the LEDs during operation is removed effectively. The PCB dimensions have been designed to mate directly to standard 2.75-inch conflat-type UHV viewports commonly used throughout most surface analysis instrumentation, enabling simple transfer between multiple instruments.

We have found that the cameras typically used in commercial XPS and ToF-SIMS instruments have optical filters to remove UV and IR light, so that often no image can be captured directly from UV or IR illumination (though UV induced fluorescence in the optical range can). Inexpensive USB microscopes are available with reasonable resolution and both UV and IR sensitivity, and we have incorporated one of these in our HIM (Zeiss, US) and (temporarily) on the entry chamber of our ToF-SIMS IV instrument (IONTOF, Munster, Germany) with good results.

The LEDs are driven by a purpose-built USB-controlled 15-channel LED driver board of our own design, connected to the USB interface. The separate intensity control for each LED is achieved at low current. This double-sided PCB is populated with a variety of different LEDs in miniature surface-mount packages in order to achieve the required range of wavelengths. Double-sided construction combined with ample use of vias and large thermal planes ensures that heat generated by the LEDs during operation is removed effectively. The PCB dimensions have been designed to mate directly to standard 2.75-inch conflat-type UHV viewports commonly used throughout most surface analysis instrumentation, enabling simple transfer between multiple instruments.

Figure 3. Printed circuit board containing high-power single-colour LEDs for multispectral imaging. The PCB is designed to fit a 2.75-inch conflat UHV viewport.

Figure 4. Multi-LED PCB as shown in Fig. 2, this time with the 485-nm LED illuminated.
being used for imaging, given that we wish to use this system on several different instruments where the camera parameters are not easily under our control. The LED driver uses a programmable precision current source to provide the forward current required to illuminate the LEDs. The current set-point can be programmed from 0 mA to 400 mA in increments of 0.1 mA. A bank of metal-oxide semiconductor field-effect transistors (MOSFETs) selects which LED the drive current is to be routed through, as shown in the schematic in Fig. 6. The drive current then flows through a sense resistor which allows negative feedback to be applied around the current source. This closed-loop control scheme ensures that the LED current is always exactly as programmed, and removes errors because of variables such as the supply voltage and the forward voltage-drop of the different coloured LEDs. It also avoids excessive wasteful dissipation in drain resistors that would otherwise be needed.

Figure 7 shows the LED illumination system in operation on our Thermo Scientific Thetaprobe XPS instrument (East Grinstead, UK).

In this instrument the camera looks vertically downward at the horizontal sample in the analysis chamber. The PCB shown in Fig. 3 can be seen attached to a viewport in Fig. 7, while a separate smaller PCB is used for the shortest wavelength LED alone. This UV LED has a nominal wavelength of 365 nm and can dissipate almost 10 W of power thermally, so has its own separate heatsink, necessitating the separate PCB. It is, however, controlled by the same driver board.

Measurement of emission spectra and dependence on the drive current

Figure 8 shows measurements we have made of the spectral emission from 13 of the LEDs in our system, using a CLS00A illuminance spectrophotometer. These are reasonably well-spaced in the wavelength range. Figure 8 also makes clear the wavelength-range of each LED, which in some cases can be quite narrow.

Figure 9 shows the response of three of these LEDs to three different levels of applied forward current through them. Ideally the spectral shape of the emission from each LED would be unchanged as the current is increased, and only the emitted power would increase. In fact there can be significant shifts in the centre-
wavelength of LED emission as power increases. The direction of shift – whether to longer or shorter wavelengths is not consistent, with some LEDs having emissions that shift up, some LEDs that shift down in wavelength, and some LEDs showing very little shift at all. This can make quantitative comparison of images acquired at different currents quite difficult. In most cases such quantitative comparisons are not needed for use in surface analysis where the main aim is to identify the best regions for XPS or ToF-SIMS study. Nevertheless multivariate analysis of such images really requires the current levels for individual LEDs to be constant throughout the dataset, even if (for example) some LEDs are operated at higher current than others.

**Multispectral imaging in analyser and entry chambers**

It is useful to have MSI on both analysis and entry chambers of an XPS or ToF-SIMS instrument. In the analysis chamber this allows *in situ*, sub millimetre-scale highlighting of inhomogeneities suitable for study by XPS or ToF-SIMS (e.g. differentiating between inks, copolymer systems etc). NIR spectra of some polymer components are significantly different and useful. Also fluorescent imaging, e.g. of labelled biological species. MSI can be useful in the entry-lock of an XPS or ToF-SIMS instrument prior to introduction to vacuum to highlight water content of regions that, once dry in vacuum, show little optical contrast.

**Biological example**

In our laboratory we are interested in organisms that exhibit cryptobiotic states as examples for analysis by ToF-SIMS and imaging by Helium Ion Microscopy. Tardigrades (also known as ‘water bears’) exhibit a cryptobiotic state in which, under very dry conditions such as exposure to vacuum, their bodies produce trehalose in large quantities. It is conjectured that the purpose is to preserve their proteins and protein higher structure. Eventually metabolism ceases. In this cryptobiotic state they have been shown to survive ultra-high vacuum conditions and other environmental extremes, and have a high probability of returning to a viable metabolic state when rehydrated. For example in September 2007, tardigrades were taken into Earth orbit on the FOTON-M3 mission and for 10 days were exposed to ambient vacuum and radiation of low earth orbit. After they were returned to Earth, many survived rehydration and some were even still capable of reproduction. This may suggest that the tardigrade in its cryptobiotic state can more easily be analysed by UHV imaging mass spectrometry techniques at room temperature than other types of animal tissue. However, one very practical problem remains, in that identifying the tardigrade within the complex organic surrounds, food sources and detritus which forms its habitat is a challenge for the limited optical microscopy facilities typically available on surface analysis instruments.

We have added a USB microscope to our Zeiss Orion Nanofab HIM at the entry lock. Figure 10 shows an image taken under white light in which a tardigrade is present but completely impossible to see. Figure 11, taken a moment later under illumination by the narrow-wavelength 485-nm LED, shows the tardigrade very clearly, largely because of the greatly reduced reflectance from the algae surrounding it.
Multispectral imaging has a long history in the study of heritage materials, especially paintings, but has added value when combined with chemically specific analysis techniques such as XPS and ToF-SIMS. Figure 12 shows an Indian miniature painting which we have studied in our Iontof IV ToF-SIMS instrument. This is a watercolour painting on an old legal document from Rajasthan, India. A currency unit (the anna) mentioned in the court-fee stamp was superseded in 1957, so that the paper (and therefore the writing still discernible on it) were at least 58 years old at the time our work was done. The painting itself, however, is considerably newer, and we believe at most a few years old. An IR-sensitive US microscope has been used at the horizontal flange window of the entry chamber to this instrument, giving a top-down view of samples brought into vacuum in our Iontof IV time-of-flight secondary ion mass spectrometer system. This allowed images to be recorded at a working distance of about 8 to 10 cm through the Type 7056 ‘Kodial’ borosilicate glass window.

Multivariate analysis of multispectral images

Multivariate approaches (such as Principal Component Analysis, PCA) have been applied in XPS, ToF-SIMS and Auger spectroscopy since the 1980s. Similar multivariate methods are also used in the analysis of MSI optical images, especially in the remote sensing community. Multivariate analysis, and even ultimately ‘data fusion’ approaches that make use of optical, photoelectron and mass spectra may be very attractive, but need to be approached with some caution. Cameras chosen by instrument manufacturers for imaging on existing XPS and ToF-SIMS instruments have been specified to operate as imaging devices, and have not had the detailed consideration of linearity and calibration that has been applied to the photoelectron or mass spectrometry components of the instrument. Multivariate techniques typically assume a high degree of linearity of response that may not be present and was never expected to be needed in the cameras used. In particular there may be any combination of nonlinearities arising from;

1. Intrinsic nonlinearity of the sensor (CCD, CMOS)
2. Significant ‘dark current’ – a background signal intrinsic to the imaging sensor that may vary across the field of view (often largest in certain pixel rows or columns of the sensor).
3. Discretisation error as images are converted to formats with low colour depth.
4. Hardware or software ‘auto gain controls’, gamma or ‘white balance’ adjustments, some of which often cannot be switched-off by the user.
5. Chromatic aberration in the lens optics can make it difficult to keep all wavelengths in focus, especially those at the extremes or beyond the visual range (e.g. while some NIR or UV wavelengths may be transmitted by such optics and may even be detected by the camera, this may not have been considered important by the designer, and therefore such images may not be in focus, and multivariate treatment of the data may need to account for this).
We have nevertheless had some success in applying PCA to MSI images acquired in our XPS and ToF-SIMS instruments. This typically requires some adjustment of the intensity of illumination by setting the forward current through LEDs appropriately. We apply the RV1 algorithm we previously applied in ToF-SIMS for PCA to the entire set of red, green and blue images acquired from 14 LEDs, i.e. $3 \times 14 = 42$ images in total. This typically takes a few seconds to calculate, but produces ‘score images’ that bring-out the main variations within the field of view very effectively.

Figure 14 shows a powder sample immobilised on carbon-loaded conductive tape, as seen using the built-in camera and white light illumination of our Thermo Scientific Theta Probe XPS instrument. Using comparison images from a white ceramic tile we have normalised the image at each wavelength. Under multispectral imaging, and PCA processing of these images, the second principal component (as shown as the ‘scores’ image in Fig. 15) indicates that there are in fact two components to this apparently homogeneous white powder. The first principal component represents the powder at the top right of the image, whereas the powder at the bottom left is optically distinguishable, but only by multispectral imaging.

Figure 15 shows a ‘scores’ image resulting from multivariate analysis. The ‘loadings’ from such an analysis would be expected to contain absorption spectrum information, at least with limited wavelength resolution, from components of the sample. We have not found it easy to interpret the loadings. Clearly there is a mixture of several optical scattering and absorption contrast mechanisms responsible for reflectance at any particular wavelength. We had hoped that the

Figure 14. Powder sample viewed in white light using the camera in our Thermo Scientific Theta Probe XPS instrument. The scale is the same as in Fig. 15.

Figure 15. First Principal Component ‘scores’ image calculated from 12 optical images recorded using the same camera as Fig. 12, but with LED illumination at 370, 390, 447, 465, 485, 505, 535, 590, 615, 620, 630 and 655 nm. This clearly shows that there is more than one type of white powder in the field of view, and that the powder distributed in the top right has a different optical response to that in the bottom left, even though the two powders look the same in white light.

Figure 16. XPS survey spectra of the two powders shown in Fig. 15. Powder B was identified as having a different composition by multispectral imaging (Powder B appears in Fig. 16). In particular note the presence of barium peaks in powder B that emphasise its different chemistry.
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loadings would allow us to extract UV/visible absorption spectra that may be correlated with databases of spectra for organic materials (for example). However because of the complexity of the chemistry and scattering processes taking place in real samples this remains firmly a topic for future work.

Comparing Figs. 14 and 15, notice that PCA effectively removes the isolated bright spots of specular reflection, and the diffuse optical imperfection at the top left of the field of view. Figure 16 shows XPS spectra taken from points within the regions of coverage of the two powders. From this figure we observe that while some elements are detected in both powders, such as carbon and oxygen, the presence of barium (for example) in powder A and calcium in powder B confirms that these are very different chemically.

Conclusions

We have demonstrated successful multi-spectral imaging (MSI) in situ within surface analysis instruments. MSI may be very useful in identifying for analysis and locating suitable analysis positions, especially for fluorescent materials or fluorophore-labelled species. The relatively narrow wavelength emission spectra, high brightness and low cost of commercially available LEDs make MSI very practical in UHV equipment. The transmission of ordinary UHV viewports is very good in this range, so that no changes need to be made within the vacuum system. Multivariate analysis can be applied to these MSI images, provided care is taken over the performance of cameras and chromatic aberrations in their optical systems.

Options for us in developing MSI in surface analysis in future include;

1. Adding LED boards wired in parallel so that both entry lock and analyser chamber are illuminated and therefore one can switch between imaging one or the other from the software by simply switching the camera source.
2. Addition of filters with sharp edges in their absorption spectra, so as to increase the spectral resolution. Filter wheels are available that are USB controlled; however, the present simplicity and lack of moving parts of an LED-only system is also very attractive.
3. More complete scheme in which combinations of XPS, ToF-SIMS and perhaps even Raman spectroscopy image data sets are combined with MSI images in multivariate analysis.

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