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Chemical mapping with soft X-ray spectromicroscopy

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Spectromicroscopy is a combination of spectroscopy—the way different wavelengths (colors) of light are absorbed by matter—and microscopy—imaging on a scale finer than the human eye can resolve. While using many of the same concepts, spectromicroscopy is distinct from wavelength selective imaging and microprobe analysis (small spot spectroscopy) because it uses both the spatial and spectral domains to the fullest extent possible. There are many variants of spectromicroscopy, a number of which use synchrotron light. This paper describes several recently developed techniques in which tuneable soft X-rays are used to provide chemical mapping via X-ray absorption spectroscopy[1] at a spatial resolution of more than 100 nm. Polymer and biomaterial applications are used to illustrate some of the ways soft X-ray spectromicroscopy is being used. However, these techniques have very general applicability. They are being used in virtually all disciplines in which spatially resolved chemical analysis is required—materials science, biology, medicine, environmental science, magnetic devices, electronic devices, and emerging areas of nanotechnology.

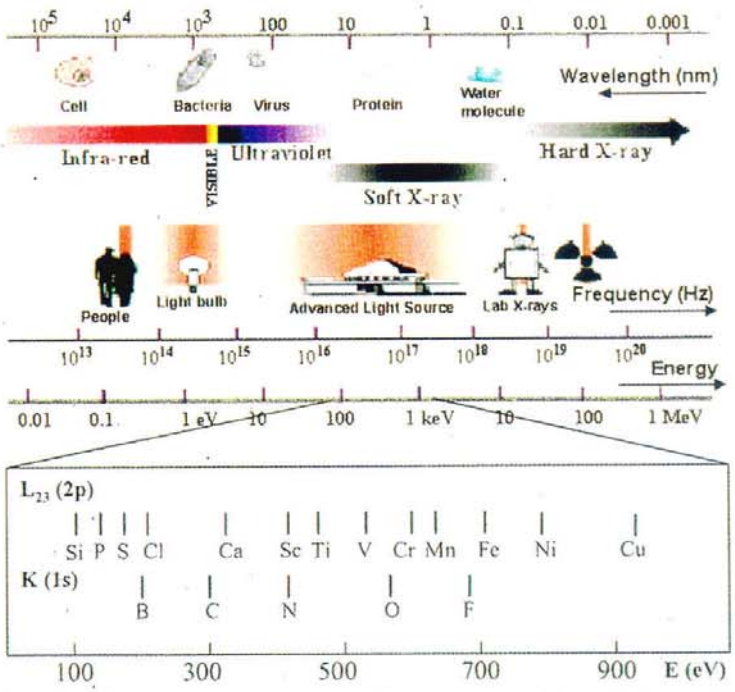

Figure 1 *Electromagnetic spectrum and key core edges in the soft X-ray region. Figure adapted from www.lbl.gov/MicroWorlds/ALSTool/EMSpec. (Image courtesy of the Advanced Light Source, Berkeley Laboratory.)*

Soft X-rays

There are a number of advantages to using soft X-ray light (wavelengths from 1 to 12 nm) rather than UV-VIS (300–800 nm) or hard X-rays (50–200 pm). At the diffraction limit, shorter wavelengths give much higher spatial resolution than longer wavelengths. X-rays use the intrinsic absorption properties of the sample and thus do not require staining or fluorescent probes, which are common in UV-VIS microscopy. There are high-contrast core edges in the soft X-ray regime for virtually all elements of the periodic table (*Figure 1*). Hard X-rays are an excellent tool for studying matter on an element-by-element basis, but the spatial resolution routinely achieved is only ~1 µm in the hard X-ray versus 50 nm or better in the soft X-ray region. In addition, the spectral resolution is much higher in the soft X-ray (~0.1 eV) than the hard X-ray (~1 eV). This enables mapping of chemical species on the basis of bonding structure rather than simply elemental content. Spectromicroscopy can distinguish chemically very similar species such as polyethylene and polypropylene because they have small but distinct differences in their carbon 1s (k-shell, or C 1s) near edge X-ray absorption spectra (NEXAFS).[1]

Achieving high spatial resolution

In scanning transmission X-ray microscopy (STXM) (*Figure 2*), light is focused to 50 nm and images are made by raster scanning the sample through the fixed focal spot while recording the intensity of the transmitted light. Fresnel zone plate focusing uses the coherent (laser-like) part of the synchrotron

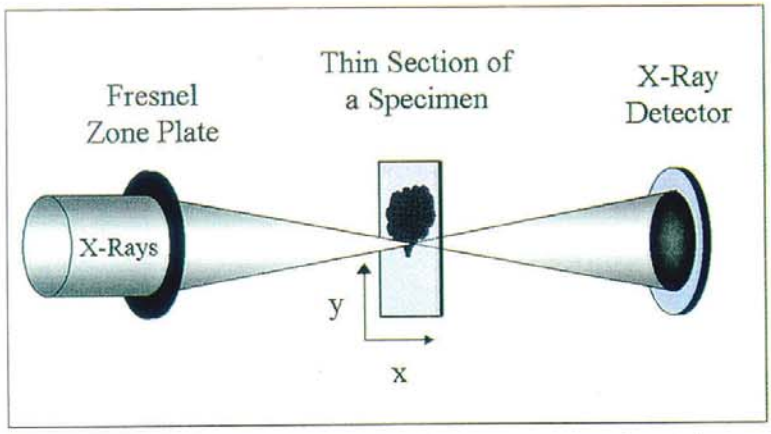

Figure 2 *Concept of a scanning transmission X-ray microscope. The incident synchrotron X-rays are monochromated, then focused to a small spot by a Fresnel zone plate. X-ray images are accumulated point-by-point by measuring the transmitted X-ray intensity as the sample position is raster scanned through the focused beam.*

light beam. For this reason, a third-generation light source with a relatively large coherent fraction, such as the Advanced Light Source (ALS) (Lawrence Berkeley National Laboratory), is preferred. In fact, STXM was developed and the most advanced technical program is still an undulator line on the X-ray ring in the National Synchrotron Light Source (NSLS). Zone plates are also used in full-field transmission X-ray microscopy (TXM). Here, the sample is illuminated with a wide angular range of X-rays, and the transmitted and refracted X-rays are magnified by an imaging zone plate and recorded with an X-ray-sensitive camera. TXM currently has higher spatial resolution (~20 nm) than STXM (~40 nm), but has generally inferior chemical analysis capabilities, since all TXMs are mounted on lower-energy-resolution beamlines.

A second soft X-ray spectromicroscopy technique, X-ray photoelectron emission microscopy (X-PEEM), images the electrons produced by X-ray ionization with a column of electromagnetic lenses similar to those used in electron microscopes. X-PEEM detects photoejected electrons that can only escape from a very thin surface layer (1–2 nm). This surface sensitivity can be very useful. However, X-PEEM is restricted to samples that can be studied in a high vacuum, are very flat, and have some conductivity. It is ideal for investigations of thin film coatings. The chemical sensitivity of both STXM and PEEM is obtained by the dependence of the images on the X-ray wavelength used.

Deriving chemical maps from spectromicroscopy image sequences

For spectromicroscopy, images are recorded at a number of energies that are selected in order to differentiate the chemical components of the system. While the number of energies can be as small as the number of components in the system, it is generally better to sample a large number of energies. Full spectral sampling is particularly useful in studies of poorly characterized materials in which the number and identity of the components are unknown. The set of recorded images forms a 3-D volume of data (x, y, E) called an image sequence. *Figure 3a* shows a few images of a ~100-image sequence (280–300 eV) of core-shell polymer microspheres of which the core is poly(divinylbenzene-55) (DVB-55) and the shell is mainly poly(ethylene glycol dimethylacrylate) (EGDMA). These microspheres are being developed by Harald Stöver and co-workers (McMaster University, Hamilton, Ontario, Canada) as possible improved materials for

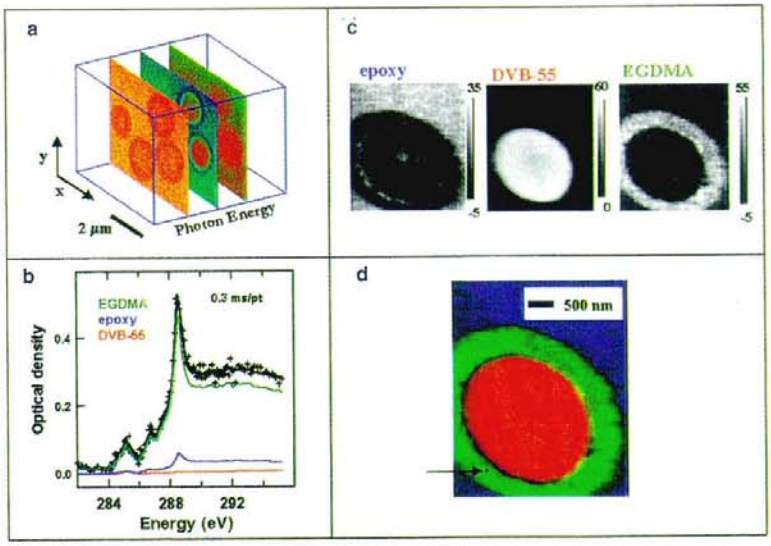

Figure 3 *Example of chemical mapping by soft X-ray spectromicroscopy. a) Images at three energies of four polymer microspheres[2] recorded with the scanning transmission X-ray microscope at the Advanced Light Source. b) Analysis of a single pixel (sampled for 0.3 msec/wavelength) as a weighted combination of the reference spectra of the three components. c) Derived quantitative chemical maps of the three components. The gray scale gives the component thickness in nanometers. d) Color-coded composite map. The dot at the end of the arrow is the pixel whose spectrum is analyzed in (b).*

chromatography or for slow-release delivery applications. The few-micron-diameter microspheres are embedded in an epoxy (Spurr's resin) and ultramicrotomed to form an X-ray translucent specimen about 60 nm thick (sample preparation for C 1s STXM is similar to that used for transmission electron microscopy). The image contrast changes with X-ray energy due to different wavelength dependence of the absorption of the sample components. The image sequence is analyzed by decomposing the spectrum at each pixel into a weighted sum of reference spectra. If the intensity scales of the analyte and the reference spectra are expressed as linear or mass absorption coefficients, then the weights provide a quantitative chemical map of each component. An example of a single pixel fit is shown in Figure 3*b*. The weights from each fit provide individual component maps (Figure 3*c*), which can be combined into a color-coded composite map to display the spatial relationship of the chemical components (Figure 3*d*). The sample in Figure 3 is one of a series of microspheres with the same core composition but different compositions of the shell. Detailed analysis of this series has been used to demonstrate that STXM can provide quantitative analysis of the majority components (those with >5 vol. % in the region sampled) of polymer systems with a few percent accuracy at ~100-nm spatial resolution.[2]

In work by other groups, STXM is being used to study environmental remediation issues, the internal structure of cells, meteorite chemistry, as well as many problems in polymer microstructure. Since it is a photon-in/photon-out technique, it is relatively easy to adapt STXM to a wide range of sample environments, including wet specimens, chemical exposure, mechanical stress, and variable temperatures.

Studies of protein adsorption on polymers

In collaboration with John Brash (Chemical Engineering, McMaster University), an expert in polymers used for blood contact medical applications, STXM is being developed for application to biomaterials. The technique is used to investigate possible preferences in the site of first attachment of selected proteins to complex polymers that have a number of different components exposed at the surface.[3] *Figure 4* shows the chemical map of a system in which fibrinogen, a blood protein, has been adsorbed from buffer solution onto a polyurethane containing two different aromatic filler particles—styrene acrylonitrile (san) and polyisocyanate polyaddition product (pipa). The C 1s spectromicroscopy shows that the fibrinogen prefers to attach to the matrix near the san particles rather than the pipa particles. The interesting thing about the sample shown in Figure 4 is that the data were recorded with a 10-μm overlayer of water. This indicates that it may be possible to use STXM to study protein attachment to polymer surfaces under conditions similar to real biological applications. However, the cryomicrotome method used to prepare the polymer sample shown in Figure 4 leaves the san particles protruding several times the size of a protein molecule from the rest of the surface. This, combined with clustering of the protein at the sides of the san particles, suggests that mechanical as well as chemical interactions may be operative.

In order to obtain a model system that presents only chemical interactions, spun-cast polystyrene-polymethylmethacrylate (ps-pmma) blends, which form chemically differentiated surfaces

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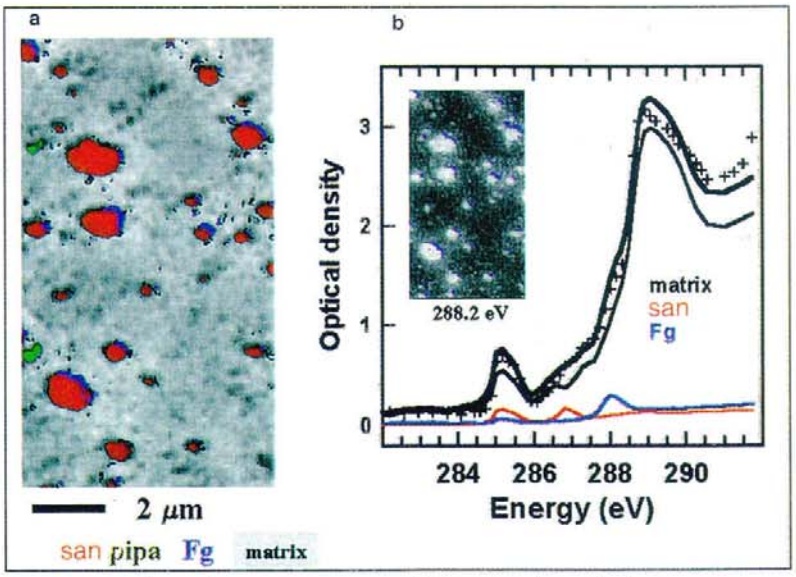

Figure 4 *a) Color-coded composite map of fibrinogen located relative to two types of filler particles—styrene acrylonitrile (san) and polyisocyanate poly addition (pipa). b) Detailed analysis of the most fibrinogen-rich pixels, which are highlighted in blue in the insert. While the amount of protein signal indicates multilayer adsorption, other work has demonstrated monolayer sensitivity.*[3]

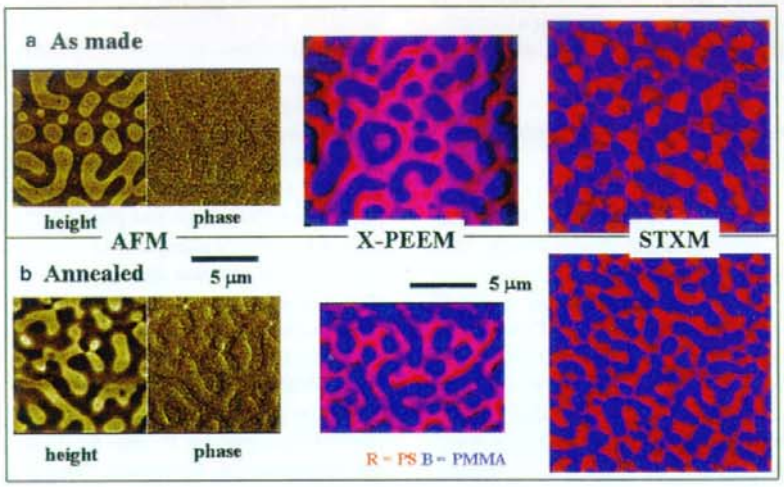

Figure 5 *Height mode and phase mode atomic force micrographs, compared to color-coded chemical maps (red = polystyrene, blue = polymethylmethacrylate) derived from surface-sensitive X-PEEM and bulk-sensitive STXM image sequences. a) Results for the as-made 30/70 ps-pmma thin film. b) Results for a similar film after annealing for several hours above the glass transmission temperature.[4] The STXM and X-PEEM data were recorded at the Advanced Light Source.*

that are flat to within 5 nm, are being used. STXM, X-PEEM, and atomic force microscopy (AFM) are being applied to the study of these samples.[4] *Figure 5* compares height mode and phase mode AFM images with chemical composite maps derived from STXM and X-PEEM image sequences of as-made and annealed 30/70 ps-pmma (wt%/wt%) films. The soft X-ray spectromicroscopy unambiguously identifies the domains. The AFM images had initially been misinterpreted by assuming that the majority pmma component would form the continuous phase. Interestingly, the bulk-sensitive STXM shows clear changes with annealing. In the color composite map of the as-made sample, there are three levels of shading that correspond to ps-rich, pmma-rich, and mixed regions, probably consisting of ps-rich and pmma-rich domains stacked on top of each other. After annealing, which allows the ps and pmma to phase segregate more completely, the STXM map exhibits two levels corresponding to ps-rich and pmma-rich regions. In contrast, the surface-sensitive AFM and X-PEEM show little change with annealing.

In work by other groups, X-PEEM is being used to study surface and thin-film phenomena such as protective films formed by oil additives in car engines, magnetic structures such as those used in the computer recording industry, and mechanisms of self-assembly and phase segregation in thin-film polymer mixtures.

For further information, recent reviews of X-ray microscopy can be found in Refs. 5 and 6. Currently, STXM instruments exist at the NSLS (xray1.physics.sunysb.edu/) and the ALS (www-als.lbl.gov/) in the U.S., the Berlin Synchron Centre (BESSY) in Germany, and the Pohang Light Source in Korea. X-PEEM instruments exist at the ALS, the Synchrotron Radiation Centre of the University of Wisconsin-Madison (www.src.wisc.edu/, www.src.wisc.edu/mephisto/default.htm), and synchrotron laboratories in Europe and Japan. STXM and X-PEEM facilities are being developed at the Canadian Light Source (CLS) (cls.mcmaster.ca/beamlines/). The CLS will provide fast turnaround, fee-for-service research to facilitate prompt access to these powerful techniques without the need of in-house expertise.

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