Full-Field X-ray Fluorescence Microscopy Using a Color X-ray Camera

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Introduction

Elemental imaging of several elements simultaneously and with detection limits in the ppb range is achieved by synchrotron-based X-ray fluorescence microscopy, also often referred to as micro-X-ray fluorescence (MXRF). This has been shown, for example, by imaging Cu and U distribution in contaminated sediments [1] and P, Ca, and Zn distribution imaging of single cells and mitochondria [2]. A review on environmental application can be found in reference [3]. XRF micro-probes are available at synchrotrons all around the world and allow for 2D imaging with spatial resolution from several micrometers down to the nanometer range (30–100 nm); the latter mainly at third-generation synchrotrons.

X-ray fluorescence (XRF). Interaction of X-rays with matter is in general dominated by the absorption of photons to generate photoelectrons. Because of the relatively high energy of X-ray photons, core shell electrons are often targeted by this process. Relaxation of the core hole occurs by a transition of an outer shell electron and emission of the transition energy as either an Auger electron or a photon, usually in the X-ray energy range. The emitted fluorescent X-ray photon is characteristic of the excited element. This process is the basis for qualitative and quantitative determination of the elements present in the specimen, as well as XRF microscopy. As in other types of microscopy, MXRF can be performed in scanning mode or in full-field mode (Figure 1).

Full-field MXRF. In the full-field MXRF mode, the full sample is illuminated by the X rays from the source, and the fluorescence is guided by an optic to the fluorescence array detector. This is illustrated in Figure 1a. Horizontal and vertical slit systems can be used to shape the beam. However, most MXRF setups operate in scanning mode, which means the sample is moved through a focused primary X-ray beam that excites the fluorescent X rays. A single element fluorescence detector can be used. This is illustrated in Figure 1b. The scanning mode comes with disadvantages regarding *in situ* applications where the sample must remain fairly static or where the sample is brittle or in other ways sensitive to movements. Here full-field MXRF is advantageous. An example is the imaging of elemental distributions in droplets (10–20 µL containing Mn, Ni, Cu, and Sc) while drying. This is shown in Figure 2. The droplets were allowed to dry undisturbed while the elemental information was recorded. Full-field MXRF allows for fast imaging of large areas (for example, 12×12 mm^2 at 1,000 frames per second and 264×264 pixels) and therefore simultaneous detection of elemental changes over the entire field of view, which can be important for certain *in situ* applications. However, the detectability of each element will depend on the fluorescence yield of the element and the total counts acquired. Thus, the recording frequency will be limited by the need to acquire enough counts for detecting specific elements. Full-field MXRF also allows fast 3D elemental imaging by taking images at different depths of the sample using a sheet beam.

Materials and Methods

Energy-sensitive camera/detector. Full-field MXRF in the past has suffered from low spectral (energy resolution) and low sensitivity, which were in part caused by the event processing and the low quantum efficiency of the array detectors used, as well as by optics with low transmission for fluorescent X-ray photons. Recently full-field MXRF has become significantly more powerful by the use of a two-dimensional energy-sensing camera/detector, increasing the sensitive thickness of the

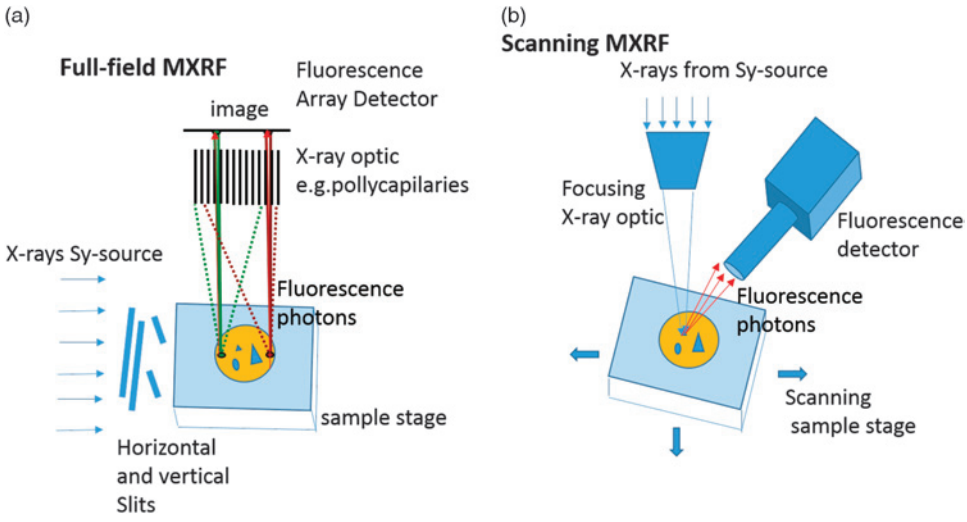

Figure 1: (a) Schematic of a typical full-field MXRF setup. The entire sample is illuminated at once, and the spatially resolved elemental information is obtained by an array detector combined with a suitable optic. (b) Schematic of a typical scanning MXRF setup. The sample is scanned through a focused beam. The primary beam is usually at 45° to the sample surface.

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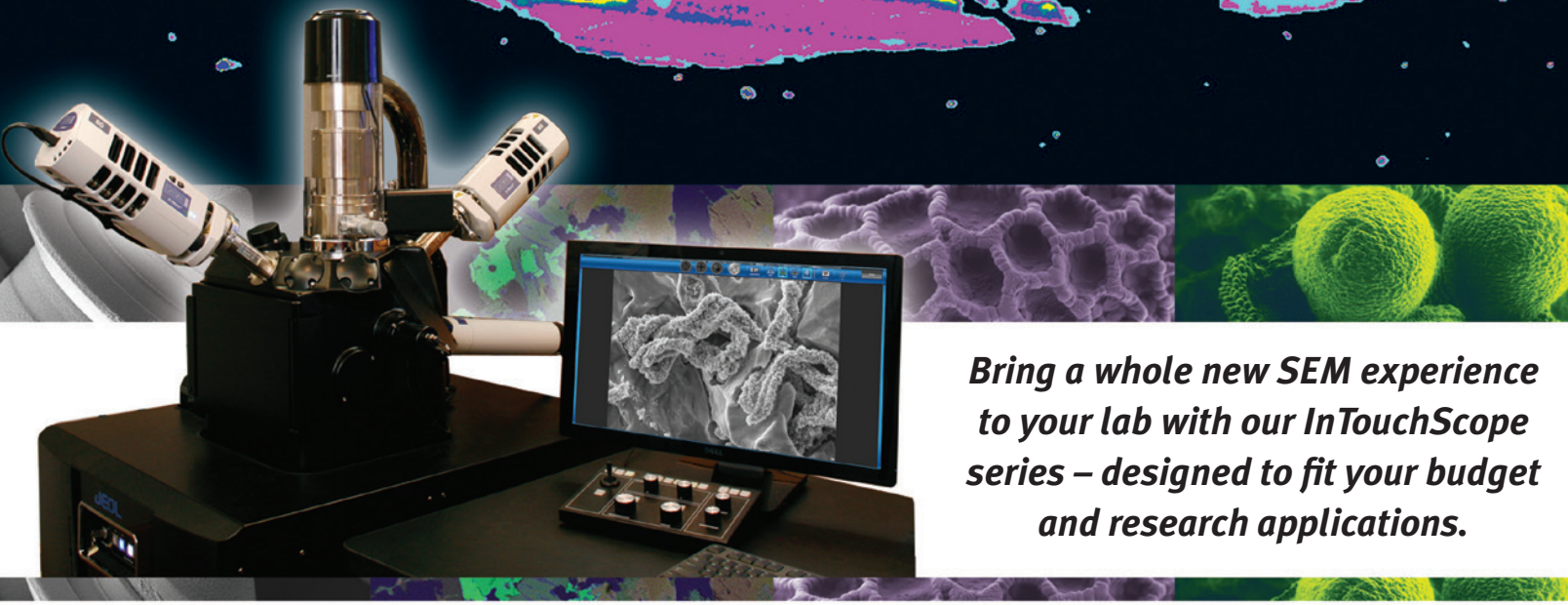

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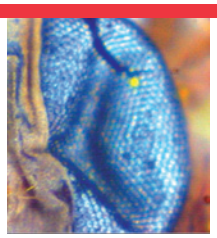

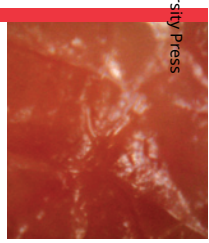

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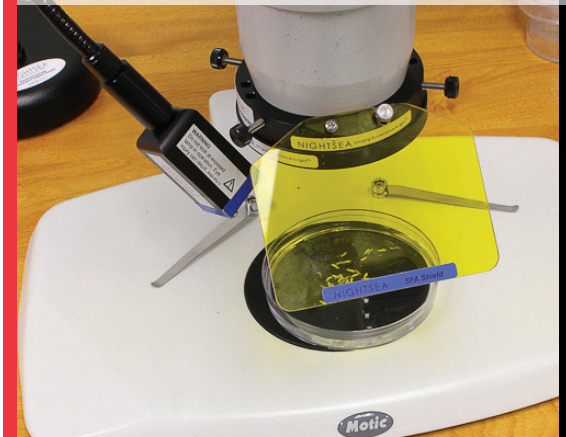

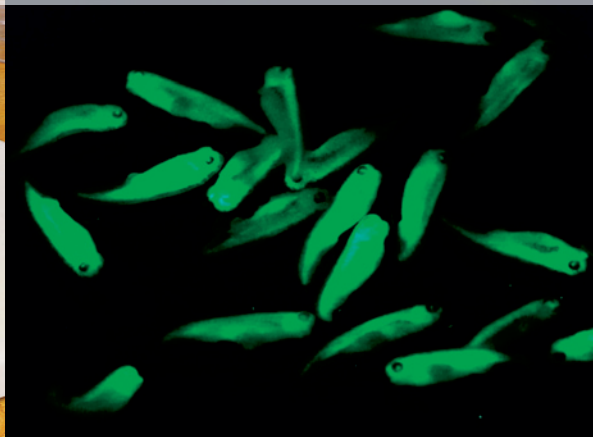

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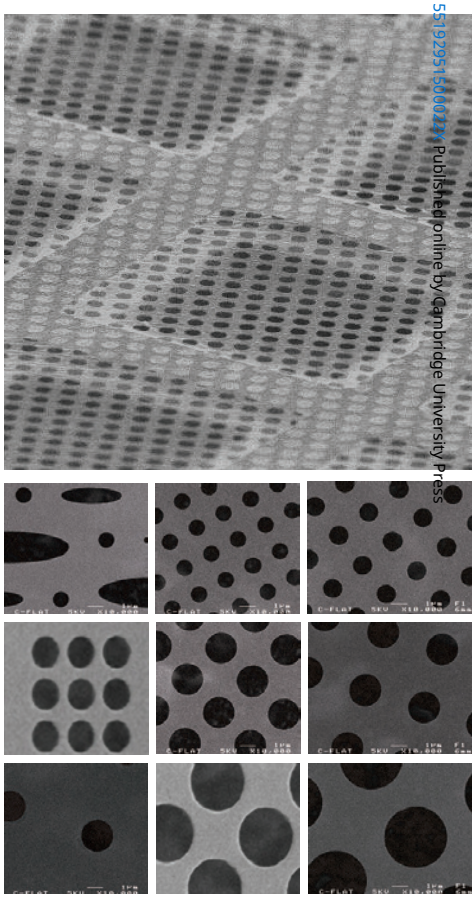

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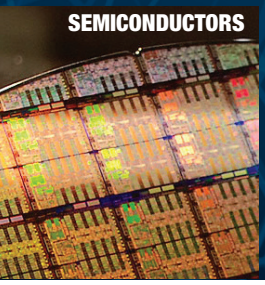

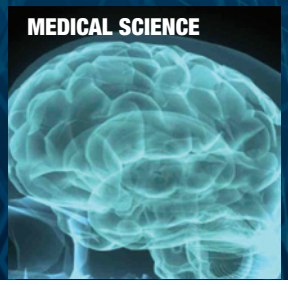

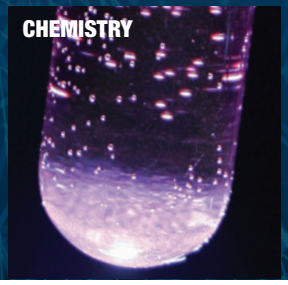

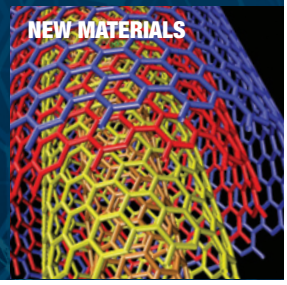

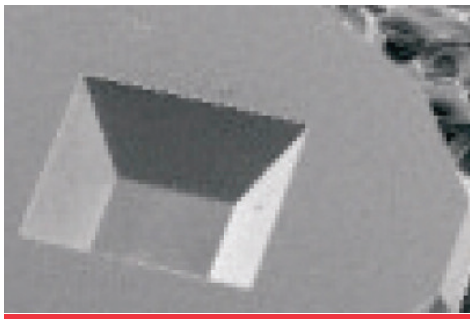

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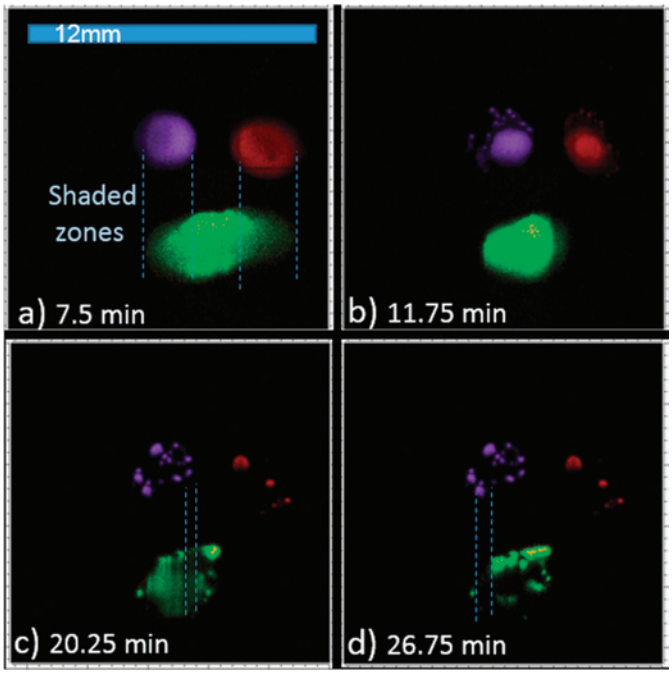

Figure 2: Elemental images collected from droplets while drying using an X-ray color camera in TXRF illumination geometry. The droplet consists of two 10 µL droplets and one 20 µL droplet: Mn (violet), Ni (red), and Cu+Sc (green). The X-ray source is located at the top of each picture (shaded regions are indicated by dotted lines). (a) Image taken after 7.5 min drying, (b) image taken after 11.75 min of drying, (c) image taken after 20.5 min, and (d) image of completely dried specimen after 27 min. Measurements were carried out at the Beamline/BESSYII instrument at Helmholtz-Zentrum Berlin.

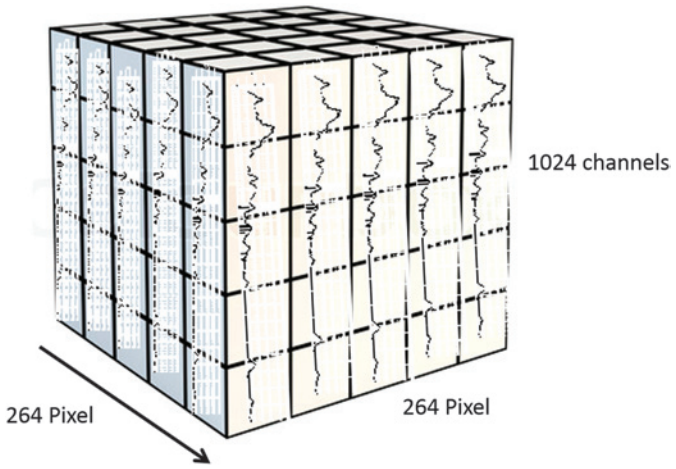

Figure 3: Schematic showing multiple dimensions in the acquisition of an XRF spectrum at each pixel. Similar data cubes may be acquired in both scanning and full-field microscopy modes. Reprinted with permission from Oliver Scharf.

detector, using arrays of capillary optics (>200 k capillaries) to guide more fluorescence photons to the detector array, and improving event processing software.

Modern full-field array detectors like the SLcam® provide spectral (energy) resolution of < 160 eV [4], comparable to single-chip silicon drift detectors (SDDs). At each pixel a full spectrum comprising an energy range of >10 keV is acquired. Figure 3 shows the typical data cube for a spectrum image with a 264×264 array and spectra collected over 1,024

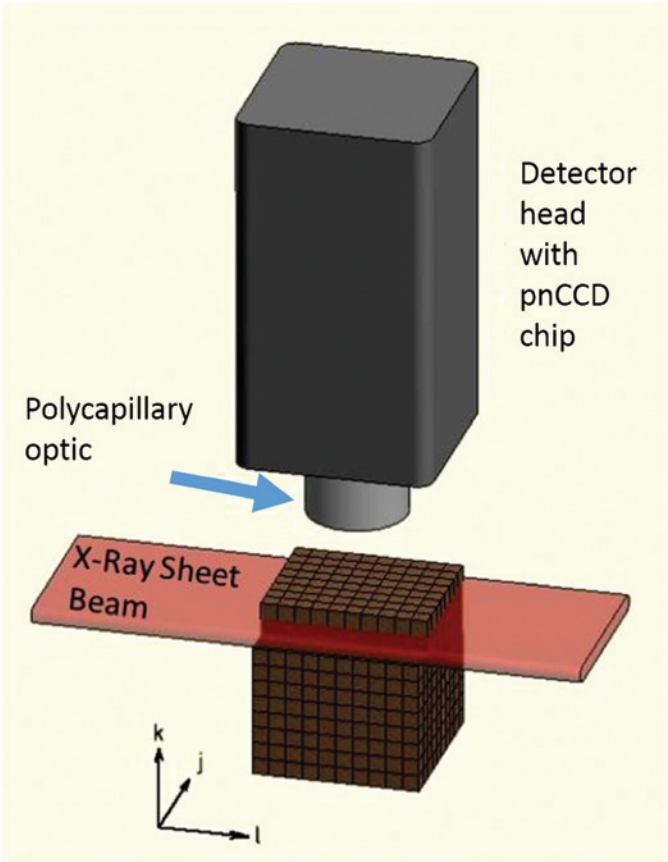

Figure 4: Schematic showing a sheet beam exciting a slice of specimen and an energy-dispersive detector detecting elemental signals. Reprinted from [5] with permission from the Royal Chemical Society.

channels. The counts acquired for a specific element line can be extracted and displayed to show the spatial distribution of this element in an X-ray map. However, these array detectors do not achieve the high count rates of SDD detectors, for example, the SLCam® array camera count rates currently are only about 22 cps/pixel. The spatial resolution obtained with full-field setups is in the single-digit micrometer range and is comparable to spatial resolutions achieved in scanning mode at second-generation synchrotron facilities.

Excitation and detection. In full-field X-ray microscopy, the sample is illuminated with a grazing incident beam, a total reflected beam, or a sheet-like beam [5, 6]. A schematic of the setup using a sheet-like X-ray excitation beam from Radkte et al. [5] is shown in Figure 4. A 3D image of an object can be obtained by translating the object through the sheet beam, where the in-depth image resolution is given by the thickness of the sheet beam. Elemental specific XRF data can be obtained from this excitation. Semiconductor array detectors such as charge coupled devices (CCDs) are used to achieve an elemental analysis as the object passes through the sheet beam. CCDs are in general energy-dispersive; however, to achieve energy (spectral) resolution comparable to SDDs, sophisticated devices like the silicon-based SLcam® (developed by PNSensor GmbH, Munich, and IFG, Berlin, with other partners) have to be used [4]. Such devices are often referred to as color X-ray cameras, or CXCs, because they provide energy resolution sufficient to discriminate

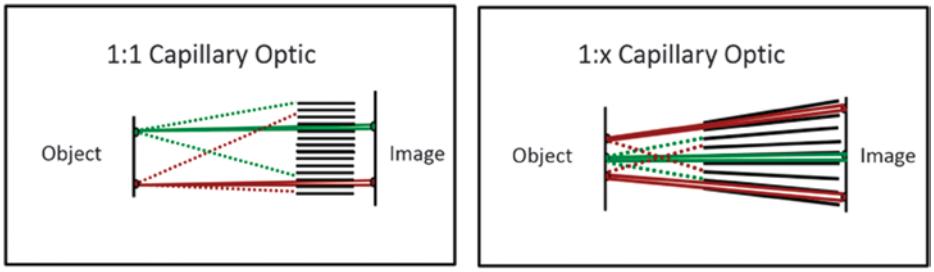

Figure 5: Schematic diagrams of a 1:1 capillary optic and a 1:x optic. Reprinted with permission from Oliver Scharf.

elemental fluorescence lines (comparable to different colors in the light optical regime).

Full-field X-ray microscopy is more restricted than X-ray absorption microscopy regarding the optics set between the array detector and the sample to be imaged [7]. This is because the optical setup needs to be fairly achromatic to guide photons of different energy accurately, which is necessary in MXRF. Pinholes have been successfully used [8, 9]. However, polycapillary optics allow for very high transmission of X rays of various energies. These optics may be used as guiding optics, achieving the resolution given by the array detector (Figure 5a), or they may project an enlarged image on the detector (Figure 5b). For example, the spatial resolution achieved with the SLcam® is about 50 µm using a 1:1 optic, but it can be improved by enlarging the image to 1:5 and even 1:8 [4]. By using an algorithm it is possible to achieve sub-pixel resolution, better than 5 µm [10].

Results

Drying of aqueous drops. A major advantage of full-field XRF imaging over scanning MXRF is rapid recognition of the major features in elemental distributions. The field of view is usually large, for example, 12×12 mm [4], and therefore an overview of the sample and "non-targeted" results are obtained. Full-field observation is also favorable for imaging objects *in situ*, especially in environments where liquids are involved or when the specimen must remain static. Imaging of droplets while drying requires a non-destructive probe operating under ambient conditions. The experiment shown in Figure 2 could not have been accomplished in an electron microscope that typically must place the specimen under vacuum. That experiment also had a temporal aspect. While XRF images were acquired every 15 seconds, only a selection of images taken over the 27-min drying process is displayed in Figure 2. Simultanous imaging of spatially separated areas can also be realized using a full-field setup, allowing the observation of process changes over time. Time-resolved measurements, however, are limited by the acquisition rate and count rate [11].

Ancient Phoenician object. A Phoenician ivory (eighth-century BCE) from the Badisches Landesmuseum, Karlsruhe, Germany, was examined by full-field XRF imaging using synchrotron radiation, a straight polycapillary optic (Figure 5a), and the SLcam® energy-dispersive camera/detector by Reiche et al. [12]. This non-destructive analysis provided distributions of the major, minor, and trace elments on the surface of the carved object (Figure 6). Of the major elements in the global spectrum from the front surface of the object, Ca and Sr are known to be from the ivory, Cu is likely a pigment that once decorated part of the design, and Fe could be either from a pigment outling the design or picked up from the burial sediments. These assumptions about the elements in the object were largely derived from the elemental images produced by the SLcam®. The assumptions about the Fe distribution were gleaned from the manner in which some Fe deposits were located in the deep crevices of the carving, whereas other Fe deposits appear to follow the the surface cracks. These elemental results help to produce a hypothesis concerning the colors employed in the original ancient artwork.

Shadowing effects. A static position of the sample is indispensable for diagnostics in total reflection X-ray fluorescence (TXRF) analysis. The term TXRF decribes a certain geometry in XRF elemental analysis that allows for trace element determination in minute amounts of a sample. In TXRF the excitation beam inpinges at a very small angle (in the range of 0.1°) onto the sample carrier surface. The shadowing of parts of the sample by rough surface features is an interference in TXRF, and better understanding of shadowing would improve the method significantly. Imaging of shadings in the TXRF geometry was possible using a full-field micro-XRF setup [6]. Figure 7 shows shadows in a Cu fluorescence image of a copper plate caused by roughness and particles as the plate was illuminated in total reflection excitation geometry from the bottom of the figure. The Cu image was captured using a color X-ray camera.

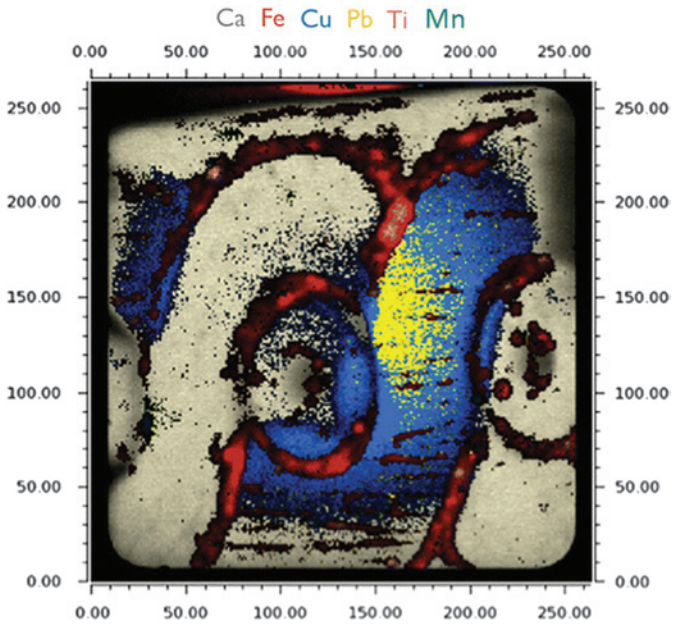

Figure 6: Analysis of an ancient Phoenician carved ivory object. Elemental images overlay of Ca K-alpha image (ivory), Cu K-alpha image (blue), Pb L-alpha image (yellow), Ti K-alpha image (purple), and Fe K-alpha image (red). Full width = 13 mm. Reprinted from [12] with permission from the American Chemical Society.

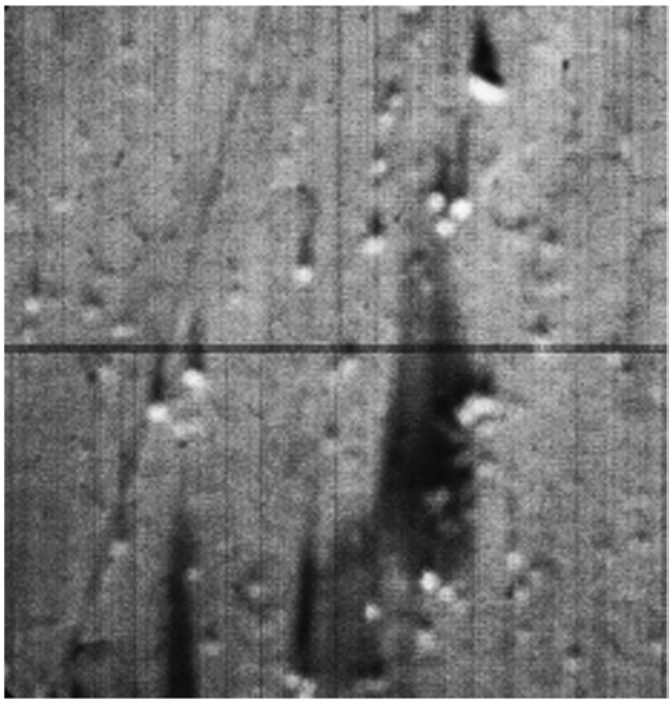

Figure 7: Shadows on a Cu plate caused by roughness and particles recorded in total reflection geometry with an SLcam® color X-ray camera. Reprinted from [6] with permission from Elsevier.

Shading is also observed in the drying droplets shown in Figure 2. Changing shading patterns (blue dotted lines) are caused by the changing physical shape of the specimen recorded in the drying experiments of droplets. Shadows are clearly visible, and they change in dimensions during the drying process.

Reconstructing 3D images from sheet-beam slices. Three-dimensional imaging can be achieved with full-field XRF by slicing the object with a sheet beam and reconstructing the image slices, a method introduced by Radtke et al. [5]. In general the synchrotron beam can be collimated into a sheet beam vertically and horizontally by slit systems. In their study two different geometries were tested. A sidewards positioning of the camera did not provide optimal flux on the sample. By positioning the camera horizontal looking down on the sample, higher flux was obtained because in such geometry the second multilayer of the double multilayer monochromator (DMM) can be bent to focus and generate an excitation beam of 50 µm height. Figure 8 shows an example of a hornet imaged with a sheet beam. The specimen was chosen because insects are often used as biomonitors of metal contamination in the environment. An animation of the three-dimensional distributions in this image can be found in the supporting material of [5]. Data from 200 layers, corresponding to about 6 ms per voxel, were measured. The total measurement time was about 24 hours.

Absorption near-edge structure. In synchrotron-based full-field emission XANES microscopy, a narrow energy range on the excitation side, ΔE less than 1 eV, may be achieved using crystal monochromators. The absorption near-edge (XANES) features of one selected elemental fluorescence line can be imaged. This type of analysis can provide information on the distribution of a specific species of an element because the near-edge fine structure changes for different chemical species of the regarded element. Although laboratory XANES point analysis has become quite powerful, XANES imaging suffers from low intensity of the analytical signal. Synchrotron sources can provide a small ΔE, efficient imaging optics, and now the color X-ray camera, SLcam®. Full-field fluorescence mode micro-XANES was demonstrated recently by Tack et al. [13]. Figure 9 shows differential imaging of Fe^0 and Fe^{3+} in an iron test sample containing both Fe foil and Fe_2O_3 powder.

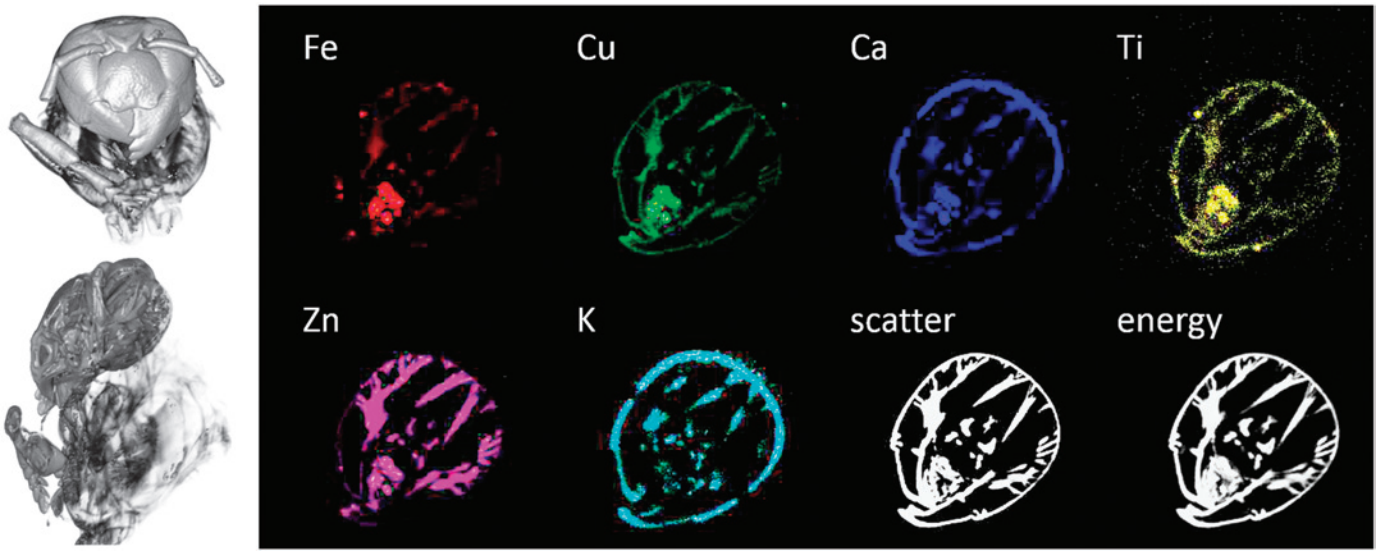

Figure 8: Elemental images of a hornet acquired with full-field XRF microscopy, sheet beam excitation, and an SLcam® color X-ray camera. This shows the left of the reconstruction of the surface and a look inside the sample from the scatter signal. Color images show the distribution of elements in the 53rd layer with a resolution of (50 × 50) µm². Additionally the scattered intensity and the total deposited energy per pixel are shown. The deposited energy is equivalent to the measurement with a conventional CCD without energy resolution. Reprinted from [5] with permission from the Royal Chemical Society.

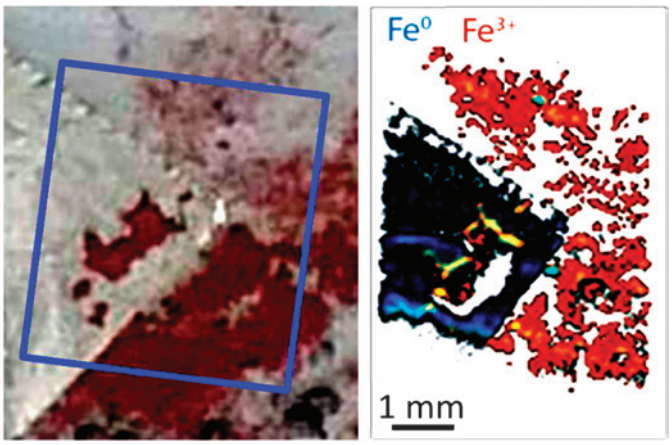

Figure 9: Left: Photograph of an iron test sample composed of a 4 µm thick Fe foil, FeO particles, and Fe_2O_3 powder. The blue box shows the area that was investigated using the SLcam®. Right: Result of differential imaging of the iron test sample after 20 min and monitoring the Fe Kα signal at 7,120 and 7,143 eV. Spatial resolution 48×48 µm². Reprinted from [13] with permission from the American Chemical Society.

Conclusion

Full-field XRF microscopy using a color X-ray camera is a powerful tool to quickly image large areas (up to about 100 mm²) with spatial resolution from 50 µm to 5 µm. It is ideally suited to non-destructive study of fragile samples, such as those found in cultural heritage research, and for *in situ* imaging.

Acknowledgment

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