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软 X 射线显微术和光谱显微术

J Thieme[1], S Gleber[1], G Mitrea[1], P Guttmann[2]

(1. Institute for X-ray Physics, Friedrich-Hund-Platz 1, D-37077 Göttingen, Germany;
2. Institute for X-ray Physics, c/o BESSY GmbH, Albert-Einstein-Strasse 15, D-12489 Berlin, Germany)

摘要:X 射线显微术可直接在水环境中对胶体颗粒尺寸范围内的颗粒进行高分辨率成像,将该项技术与高分辨率光谱相结合,还可用于光谱显微研究。其中,常用的两种 X 射线显微镜是透射显微镜和扫描透射显微镜,文中示出了它们的装置图。由于 X 射线显微镜能迅速拍下一物体的高分辨率图像,所以,作为一种分析仪器,扫描 X 射线显微镜更适合作光谱显微研究。作为形态学目视化的一个示例,本文用一台透射 X 射线显微镜拍摄了粘土和土壤样品的图像。根据 X 射线图像进行的低温层析实验所获得的图像得到了有关细菌构成的显微生存环境以及其它土壤胶状体的 3D 结构信息。对扫描透射 X 射线显微镜拍摄的一系列图像进行分析,得到了土壤样品的形貌特性和化学特性。

关 键 词:X 射线显微术;光谱显微术;XANES;NEXAFS;环境科学;胶体颗粒

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Microscopy and spectromicroscopy with soft X-ray

J Thieme[1], S Gleber[1], G Mitrea[1], P Guttmann[2]

(1. *Institute for X-ray Physics, Friedrich-Hund-Platz* 1, *D-37077 Göttingen, Germany*;
2. *Institute for X-ray Physics, c/o BESSY GmbH, Albert-Einstein-Strasse* 15, *D-12489 Berlin, Germany*)

Abstract: X-ray microscopy is capable of imaging particles in the colloidal size range directly in their aqueous environment with high spatial resolution. It is possible to combine this with high spectral resolution for spectromicroscopic studies. Two types of microscopes are common in X-ray microscopy, the transmission X-ray microscope and the scanning transmission X-ray microscope, whose setup is shown in this paper. While an X-ray microscope quickly takes high resolution images from an object, a scanning X-ray microscope as an analytical instrument is suited for spectromicroscopy. As an example for visualization of morphology, clay and soil samples have been imaged with a transmission X-ray microscope. Images from a cryotomography experiment based on X-ray microscopy images to obtain information about the 3D structure of microhabitats formed by bacteria and other soil colloids are shown. The analysis of a stack of images taken with a scanning transmission X-ray microscope to bring together morphology and chemistry within a soil sample is given.

Key words: X-ray microscopy; spectromicroscopy; XANES; NEXAFS; environmental sciences; colloids

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1 X-ray spectromicroscopy

In 1952 Horst Wolter pointed out that X-radiation in the energy range between the K-absorption edge of carbon and of oxygen is very well suited for microscopy purposes, calling this range "the water window"[1]. The reason becomes clear when looking at the linear absorption coefficients plotted in Fig. 1.

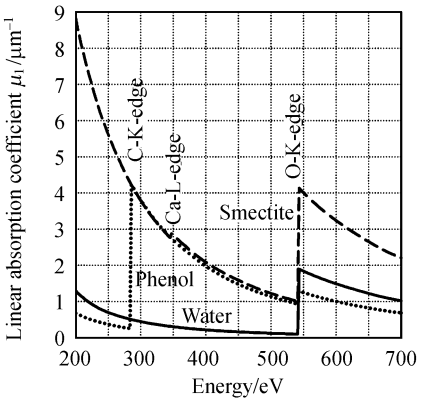


Fig. 1　Linear absorption cross-section μ_l of the clay mineral smectite, the organic substance phenol and water as a function of X-ray energy. The absorption edges of oxygen, calcium and carbon are indicated.

The linear absorption coefficients μ_l of water, of the inorganic mineral smectite and of the organic substance phenol are plotted as a function of the X-ray energy. Smectite and phenol are used here as representatives for inorganic or organic matter. It is important to note that between the K-absorption edge of carbon and of oxygen, as indicated in the graph, the μ_l of water is low compared to μ_l of the organic or inorganic matter. This gives rise to a natural amplitude contrast when imaging specimens with X-rays within this energy range. No drying, fixation or staining of a sample is necessary.

The intensity I_1 of X-rays transmitted through an object can be calculated using the Lambert-Beer equation[2] $I_1 = I_0 \exp(-\mu_l d)$, where I_0 is the incident radiation, μ_l is the linear absorption coefficient, and d is the thickness of the object. The term $\mu_l d$ is the so-called optical density. When calculating the thickness where the transmitted radiation I_1 is weakened to $1/e$ of the incident intensity I_0, the resulting penetration depth $1/\mu_l$ for water ranges from 2 μm at the carbon K-edge up to 10 μm at the oxygen K-edge[3].

In the X-ray energy region of the water window the complex refractive index $n = 1 - \delta - i\beta$ is very close to unity as δ and β are very small[4]. This is the reason why scattered X-ray light will not be reflected from inner surfaces in inhomogeneous media[5]. Clear images can therefore be expected even when studying thick and heterogeneous specimen.

The spatial resolution achievable when looking with a microscope at a sample is directly related to the energy of the applied radiation. Increasing the energy implies increasing the resolution capabilities, *i. e.* the higher the energy the smaller the structures visible. The energy of X-radiation is much higher than that of visible light; therefore a much better resolution can be expected. At present, the smallest structures visible with an X-ray microscope are less than 15 nm in size[6].

Combining synchrotron radiation from an electron storage ring as a highly brilliant light source with a monochromator, it is possible to tune the energy of the X-radiation very finely. Elemental mapping and spectroscopy experiments can be performed. Images of a sample are taken with X-ray energies above and below the absorption edge of an element. Dividing both images gives rise to a map of the distribution of this element in the sample. In addition, it is possible to use near-edge resonances for NEXAFS- (or XANES-) studies. As the X-ray energy is raised to match the absorption edge, one first detects resonances, which reflect the chemical bonding state of the element, and then the step-like rise in absorption due to the element.

Important absorption edges within the energy range of the water window are listed in Tab. 1.

Tab. 1　Absorption edges of interest within the energy range of the water window

Element	Absorption edge	Energy/eV
O	K	543
N	K	410
Ca	L_2, L_3	350, 346
K	L_2, L_3	297, 295

Summarizing, it is possible with X-ray microscopy to image particles in the colloidal size range directly in their aqueous environment with high spatial resolution, and it is also possible to combine this measurement with high spectral resolution for spectromicroscopy studies.

Two types of microscopes are common in X-ray microscopy: the transmission X-ray microscope and the scanning transmission X-ray microscope. In both types Fresnel zone plates are used as high resolution optical elements[7]. Electron storage rings are used as light sources, as they generate very intense synchrotron radiation with high brilliance.

The optical setup of a transmission X-ray microscope consists of a condenser/monochromator system and a micro zone plate. The condenser/monochromator reduces the bandwidth of the synchrotron radiation coming from the storage ring and focuses it on to the object. The bandwidth is reduced to at least $E/\Delta E$ with a value of several hundred. A microzone plate behind the object acts as a high resolution X-ray objective and creates an enlarged image of the object in the image plane which is recorded by a CCD-camera. Exposure times are in the range of several seconds. The Institute for X-ray Physics in the University of Göttingen has developed several generations of these X-ray microscopes. The results presented here have been obtained with an X-ray microscope designed for and operated with the undulator U41 at the electron storage ring BESSY II in Berlin[8]. The optical setup is shown in the upper drawing of Fig. 2. The radiation from the undulator is reduced in bandwidth and focused into the object plane by an off-axis transmission zone plate. A mirror comprising one fixed and two rotating mirrors provides for aperture matching with the micro zone plate and for incoherent illumination of the sample. Using this rotating mirror condenser, the monochromaticity of the radiation has been improved to $E/\Delta E=2\ 000$, which results in a very high clarity of the images[9]. The micro zone plate creates an enlarged image of the sample in the image field where it is recorded by a backside illuminated thinned CCD camera.

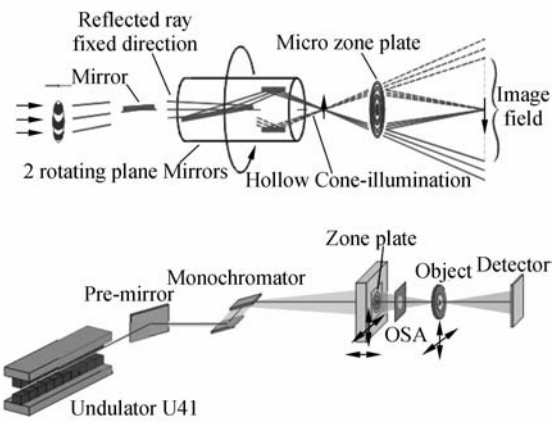


Fig. 2　Optical setups of the transmission X-ray microscope (top) and the scanning transmission X-ray microscope (bottom) at the electron storage ring BESSY II in Berlin.

In a scanning transmission X-ray microscope, the polychromatic synchrotron radiation is reduced in its bandwidth by a grating monochromator to $E/\Delta E$ of several thousand. A micro zone plate focuses this radiation in a very small focal spot on to the sample. A detector records the radiation transmitted through the object and the signal is stored in a computer. By scanning either the focal spot through the sample or the sample through the focal spot and recording the transmitted signal time, an image can be generated in the computer. Due to the scanning process, exposure times are much longer than

with an X-ray microscope. Tuning the energy of the X-ray light with the monochromator and keeping the position of the focal spot yields a spectrum from this specific object point. Using a segmented detector it is possible to detect the signal not only in brightfield mode but also in darkfield and in differential phase contrast mode at the same time. The lower drawing in Fig. 2 shows the setup of the scanning transmission X-ray microscope at BESSY II[9], which is located side by side with the transmission X-ray microscope. The monochromator consists of a plane mirror and a plane grating with variable line density[10]. The latter provides for reduction of the bandwidth of the radiation and for a coherent illumination of the micro zone plate. This zone plate creates a small spot, diffraction-limited in its size. The zone plate and thus the spot are moved by a piezo scanning device in very fine steps across the sample behind it. To choose beforehand regions of interest on the sample, it can be moved coarsely by stepper motors. A pn-CCD camera behind the object acts as a configured detector, collecting the transmitted signal.

In conclusion, while an X-ray microscope quickly takes high resolution images from the object to be studied, it is possible using a scanning X-ray microscope as an analytical instrument to perform spectromicroscopy.

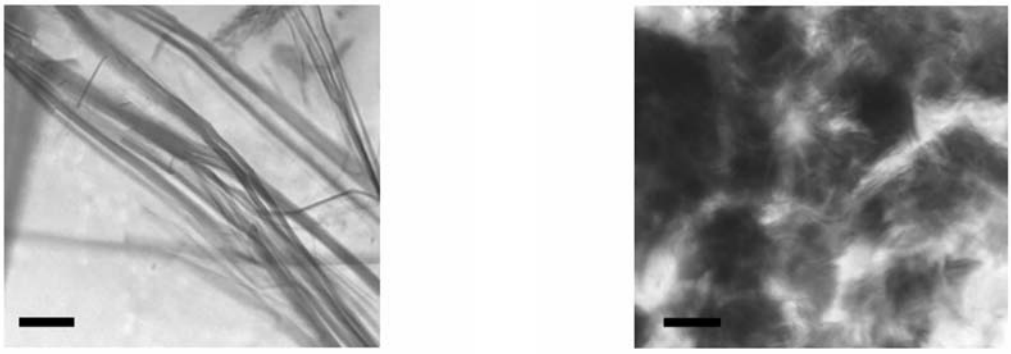
2 Visualization of nanoparticles

Clay and soil samples have been imaged with the transmission X-ray microscope. The aim was to image specimens from the environment in aqueous dispersions and to see the morphology of colloidal clusters. Two clay samples, Na^+-montmorillonite and nontronite (Fig. 3), and two soil samples, luvisol and gleysol (Fig. 4), have been imaged. The images clearly show the desired morphology of clusters of colloids or nanoparticles. A micro zone plate with $dr_n = 25$ nm has been used as microscopic objective, resulting in a resolution in the images of about the same value. As a result, single particles within the clusters can easily be identified.

The clay samples have been chosen to demonstrate the great variety in shape of clay particles. Na^+-montmorillonite consists of small particles stacked together, whereas nontronite particles are much larger in size, forming structures much more open.

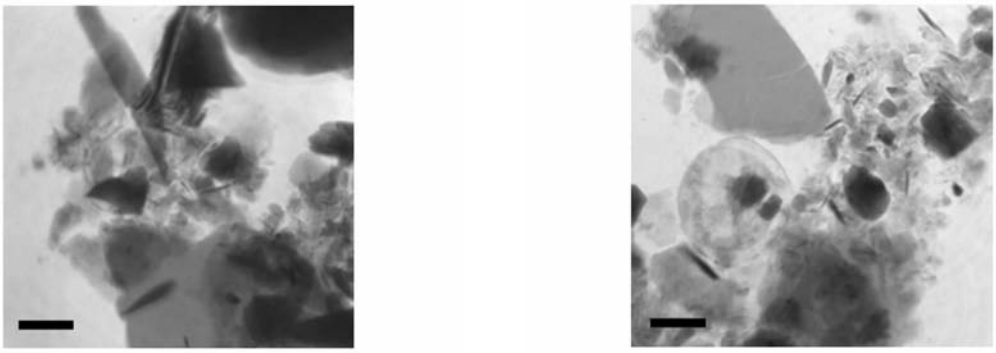
The soil samples show that in the colloidal range clusters of particles feature many different appearances. The trained eye, however, quickly identifies in each sample particles of similar shapes. In this size range all samples mainly consist of clay particles making these similarities understandable. In addition, micro-organisms are very often attached to the particles. They can be found in the images as ellipsoidal or round shapes with a distinct membrane.

Clusters of colloids in soils consist mainly of clay particles, oxides, and organic substances. They attach to each other in a heterogeneous way controlled by the chemical conditions within the soil, forming its micro- and nano-pore system. Within this pore system nutrients are stored, *e. g.* as cations adsorbed to the charged surfaces of the colloidal particles. Bacteria in search for these nutrients attach themselves to the surfaces of single colloidal particles as well as to the clusters formed. As X-ray microscopy images normally are 2D projections, it is not possible to clarify the exact spatial arrangement of bacteria and soil colloids. Particles and bacteria are clearly visible, but the question is whether the bacteria are inside or outside of the clusters. Tomography based on X-ray microscopy images can be used to find the answer. For these studies the transmission X-ray microscope of the Center for X-ray Optics at Beamline 6. 3 of the Advanced Light Source, Lawrence Berkeley Laboratory, Berkeley, has been used[12]. To obtain information about the 3D structure of the micro habitats formed by bacteria and other soil



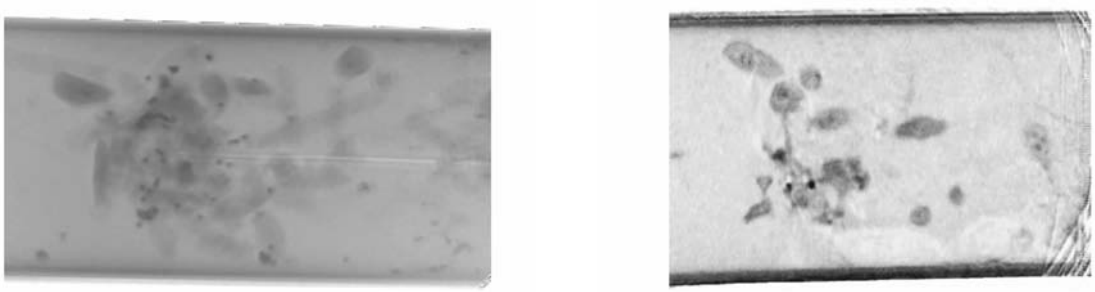
(a) (b)

Fig. 3 Transmission X-ray microscopic images of two clay types, nontronite (a) and Na^{+}-montmorillonite (b), imaged in aqueous media using the X-ray microscope at BESSY II with a resolution of about 25 nm. The scalebar indicates 2 μm.



(a) (b)

Fig. 4 Transmission X-ray microscopic images of two soil types, luvisol (a) and gleysol (b), imaged in aqueous media using the X-ray microscope at BESSY II with a resolution of about 25 nm. The scalebar indicates 2 μm.



(a) (b)

Fig. 5 (a) X-ray micrograph of a cluster of colloidal soil particles in a 1% dispersion from the AH-horizon of a chernozem soil. The sample is filled in a capillary with a diameter of 8 μm and kept at cryogenic temperature. (b) Computer-generated slice through the capillary containing a cluster of colloidal soil particles. The diameter of the capillary is approx. 8 μm, thus the cluster itself is about 6 μm in diameter. The thickness of the slice is 100 nm.

colloids, cryo-tomography based on X-ray microscopy images with about 45 nm resolution was performed. The tomographic reconstructions shown in Fig. 5 reveal the spatial arrangement of bacteria and other soil colloids which cannot be obtained from 2D projections. The results show that X-ray nanotomography is a very powerful tool for examining the 3D structure of clusters of colloidal particles[13].

3 Morphology and chemistry

To bring knowledge about the morphology of a sample together with information about the chemistry within the sample, it has been proven very useful to take a set of images at closely spaced energies throughout the near-edge region of the desired element[14]. As an example, some of a stack of images from a soil sample taken with the scanning transmission X-ray microscope at BESSY II are shown in Fig. 6, taken around the K-absorption edge of carbon within the energy range of 283～308 eV and with energy steps of 0.5 eV. The images have to be aligned to each other afterwards because of possible drifts or movements within the instrumentation. After that, a spectrum can be obtained from each selected object point by plotting the optical density of that point of all energies at which images have been taken. Spectra can be obtained not only from discrete pixels, but from larger regions by integrating the optical density of all pixels within a chosen area. This method to learn about the chemistry of a sample is more time consuming than simply taking a spectrum, but it is true spectromicroscopy, producing spatially and spectrally resolved data of an object at the same time. The radiation dose applied to each point of the sample is the same for point spectra as well as for stacks. An otherwise well characterized chernozem soil from an area close to Göttingen[15] has been taken as a first sample for demonstration. Chernozem is an alkaline soil (pH＝8.2～8.4) with a high organic content (4.1 %). A small drop of a 1% dispersion in deionized water has been placed on a 100 nm thick Si_3N_4-membrane, and let dry. As a result, small clusters of soil colloids will be found on the membrane.

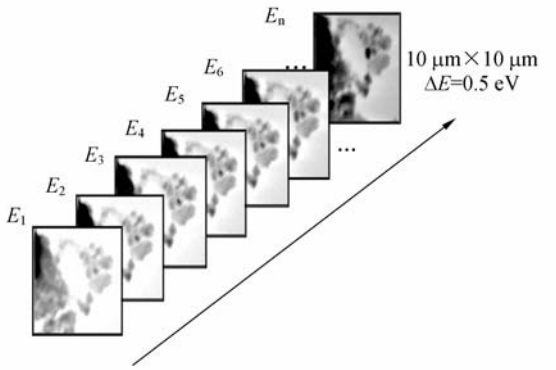


Fig. 6　Stack of images as a function of energy taken with the scanning transmission X-ray microscope at BESSY II, showing colloidal structures within a chernozem soil. The image size is 10 μm×10 μm with 100 pixel×100 pixel and dwell time of 10 ms/pixel. Energy range for the full stack is 283～308 eV, ΔE＝0.5 eV.

Images of a chernozem sample have been taken around the K-absorption edge of carbon. In Fig. 6, a stack of images taken between E＝283 eV and E＝308 eV can be seen. Fig. 7 shows one image of colloidal particles from a whole stack and three regions marked in light grey. Integrating the transmission signal I_1 of each region as a function of energy and using the regions marked dark gray as I_0 the spectra below each image is obtained as $-\ln(I_1/I_0)$, which yields the optical density. The left image and the appendant spectrum below clearly reveal the L_{II}- and L_{III}-absorption features of potassium, a strong indication for clay particles. The spectrum in the middle reveals the organic origin of

the particle marked in the image. The set at the left shows the result of the sum of all particles from that flock. The differences in the spectra are clear proof for the need of spatially resolved data.

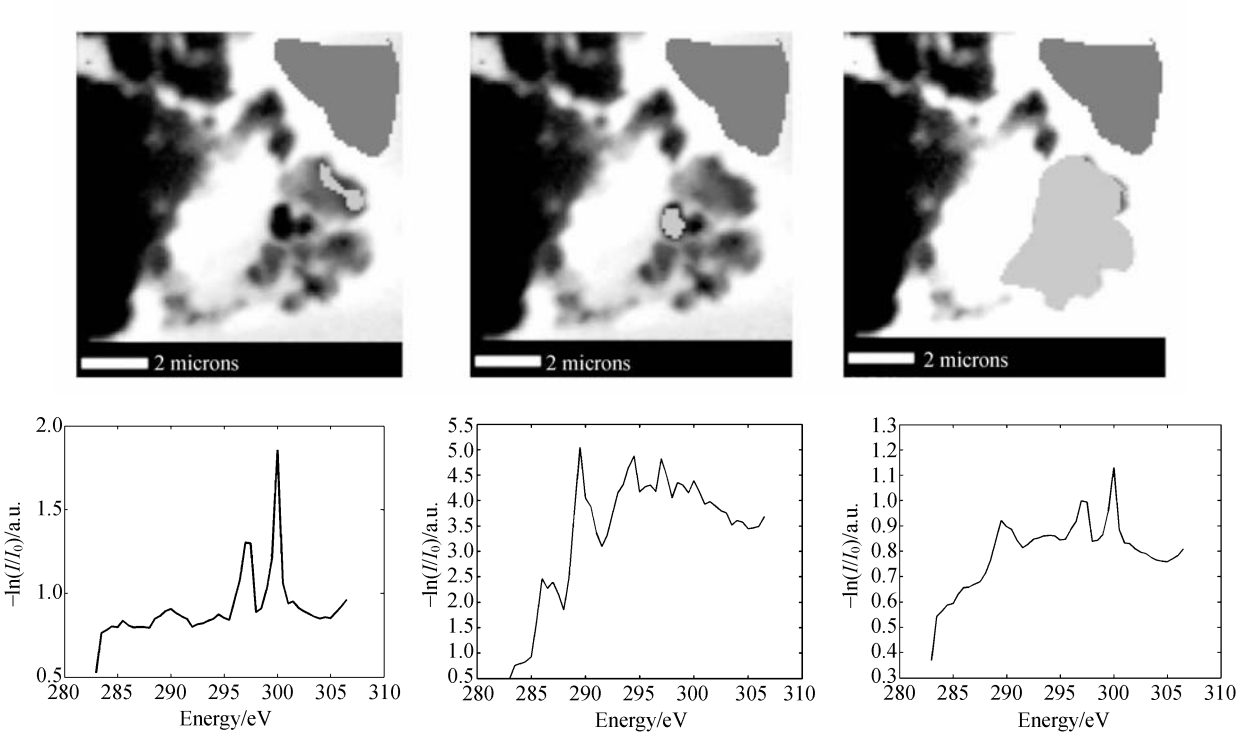


Fig. 7 Selected regions marked in light grey from a stack of images and the corresponding spectra at the carbon K-absorption edge. The region used for I_0 is marked dark gray.

4 Summary

The examples of X-ray spectromicroscopy studies presented here have demonstrated that X-ray microscopy is a very suitable tool for examining colloidal structures in their original aqueous matrix. Imaging as well as tomographic reconstruction are very powerful methods for examining the morphology of clusters of colloidal particles. The analysis of samples from environmental science, which are very heterogeneous physically as well as chemically, is a real challenge for X-ray spectromicroscopy but achievable. Spatially-resolved studies allow the characterization of structure and chemistry of clusters of soil colloids in relation to other elements. The results presented here have documented the possibilities for studies comparing not only spectra obtained from bulk samples but looking in detail into the microstructure formed by colloids.

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Authors' biography: Juergen Thieme, born in 1955 received his Physics Diploma in 1984 and his PdD in 1988 at the Georg-August-University Goettingen in X-ray microscopy with zone plate optics. After this he was research assistant and since 1996 he is research scientist in the Institute for X-ray Physics at the Goettingen university with special interests in the field of Nanostructure technology, X-ray optics and environmental science. He is involved in the design, construction and use of the Götingen X-ray microscopes (STM) at the BESSY I and since 1998 BESSY II storage ring facilities in Berlin to be used primarily for spectromicroscopic investigations in environmental sciences. He used other storage ring facilities such as NSLS, ESRF, ELETTRA, APS, and ALS and consulted the HESYRL microscopy project and the laboratory X-ray microscope project in Lund employing a laser plasma X-ray source. He organised international workshops in the fields of X-ray Microscopy and Environmental Sciences. E-mail: jthieme@gwdg.de