Three-dimensional elemental imaging by means of synchrotron radiation micro-XRF: developments and applications in environmental chemistry

B. De Samber · G. Silversmit · R. Evens ·
K. De Schamphelaere · C. Janssen · B. Masschaele ·
L. Van Hoorebeke · L. Balcaen · F. Vanhaecke ·
G. Falkenberg · L. Vincze

Published online: 8 November 2007
© Springer-Verlag 2007

Introduction

The most brilliant sources of hard X-rays available for scientists today are provided by synchrotron radiation (SR) sources within electron or positron storage rings. These storage rings, or particle accelerators, were originally built for high-energy particle physics experiments, and the emission of synchrotron radiation, generated by the acceleration of relativistic charged particles in strong magnetic fields, was considered as an undesirable side-effect. The unique characteristics of SR (extremely high brightness/intensity, natural collimation, polarization, energy tunability, and pulsed time structure), however, were quickly recognized to be very attractive for a large number of scientific disciplines, including X-ray spectroscopic

applications with unsurpassed sensitivity and spatial resolution. The original use of synchrotron radiation in the so-called parasitic mode in the case of first-generation synchrotrons, initiated a rapid evolution towards second- and third-generation SR sources which have been optimized specifically for the production of synchrotron radiation of exceptional brilliance.

Owing to the rapid development of synchrotron sources, associated microfocusing optical elements and the increasing availability of sophisticated X-ray detectors, scanning micro X-ray fluorescence (micro-XRF) techniques have become extremely powerful tools for two- and three-dimensional (2D/3D), non-destructive elemental analysis with exceptional sensitivity. A variety of scanning XRF techniques are currently available, such as conventional or dynamic micro-XRF scanning (2D), micro-XRF computed tomography (2D/3D), and confocal micro-XRF (2D/3D) [1].

When using synchrotron sources to produce the exciting microbeam, taking advantage of the high intensity, potential monochromaticity/tunability, and high degree of linear polarisation in the storage ring plane, these techniques offer the possibility to obtain quantitative information on the elemental distributions within the sample volume with trace level detection limits and on a (sub-)microscopic scale [2, 3]. X-ray microprobes installed at the most advanced third-generation SR sources, such as the European Synchrotron Radiation Facility (ESRF) in Grenoble, offer absolute detection limits (DLs) below 10 ag for the most efficiently excited transition elements with a potential lateral resolution level better than 50 nm [4, 5].

An exciting direction in the development of non-destructive elemental imaging methods is represented by the combination of synchrotron-based scanning micro-

B. De Samber · G. Silversmit · L. Balcaen · F. Vanhaecke ·
L. Vincze (✉)
Department of Analytical Chemistry, Ghent University,
9000 Ghent, Belgium
e-mail: laszlo.vincze@ugent.be

R. Evens · K. De Schamphelaere · C. Janssen
Laboratory of Environmental Toxicology and Aquatic Ecology,
Department of Applied Ecology and Environment Biology,
Ghent University,
9000 Ghent, Belgium

B. Masschaele · L. Van Hoorebeke
Centre for X-Ray Tomography, Department of Subatomic
and Radiation Physics, Ghent University,
9000 Ghent, Belgium

G. Falkenberg
Hamburger Synchrotronstrahlungslabor at DESY,
22603 Hamburg, Germany

spectroscopic techniques with complementary X-ray imaging methods, such as X-ray absorption microtomography. X-ray absorption microtomography offers the possibility to facilitate the interpretation and aid the quantification of 2D/3D elemental imaging results by providing a full 3D absorption model, an accurate frame of reference, for the very same sample which is analyzed by SR micro-XRF techniques. Absorption microtomography provides a complementary dataset with density/mass absorption coefficient information, which not only reveals the internal structure/morphology of the examined sample but also aids the quantitative analysis of the measured 2D/3D elemental distributions. The potential of these combined micro-imaging techniques is illustrated below: by coupling information obtained by synchrotron radiation micro-XRF and absorption microtomography, it is possible to unravel the tissue-specific 2D/3D distribution of metals in situ in an essentially non-destructive manner within delicate biological samples. By the application of dynamic (fast) scanning techniques, radiation damage to the illuminated biological samples could be minimized; however, with more intense X-ray nanobeams the use of cryogenic sample stabilisation techniques may be necessary for preserving sample structural integrity.

Elemental imaging in environmental toxicology

In the field of environmental toxicology, the study of the effects of the presence of transition metals such as Co, Cu, Ni, and Zn on the health of pelagic and benthic invertebrates is an important research topic. Efforts are being made to develop increasingly accurate biotic ligand models (BLMs) [6–8], which can then be used to predict the metal toxicity as a function of the metal concentration and the physicochemical characteristics of the surface water. On the basis of reliable BLMs, scientifically more correct environmental regulations can be defined. For a further refinement and a more mechanistic underpinning of these models it is necessary to relate toxicological effects to tissue-specific and intracellular element distributions over the various internal organs of these organisms (e.g., respiratory/ionoregulatory tissues and digestive tissues), which would also allow one to determine the relative importance of different exposure routes (i.e., uptake via water or via the diet) [9–11].

The freshwater crustacean *Daphnia magna* is a frequently used model organism to investigate the mechanisms of toxicity of the metals mentioned above. Historically, it has often been difficult to link bioaccumulation to toxic effects in daphnids because total organism digestion cannot distinguish the accumulation in critical tissues from whole body accumulation. Also the small size of these organisms (2–3 mm) does not allow a straightforward determination of

the metal contents of tissues of interest by conventional techniques, such as dissection, acid digestion, and subsequent atomic spectrometry. Transformation of delicate organic samples during the necessary extraction of the metal or species of interest from the sample volume and during the subsequent sample pre-treatment and separation process remains a frequently occurring problem.

Therefore, the ecotoxicological research on daphnids is ideal to illustrate recent trends in the field of 2D/3D scanning X-ray fluorescence techniques on biological systems. The in situ analysis of the sample volume of interest with minimal influence of the X-ray microbeam allows the quantitative investigation of the accumulation of metals within specific organs with microscopic resolution and offers an elegant solution to the previously stated problem. Furthermore, this scientific case study is ideal to further improve and design synchrotron experiments in terms of sample preparation, the actual SR-XRF analysis, experimental setup, and data processing.

Figure 1 shows the employed XRF microprobe in our case study, which is installed at Beamline L of the DORIS-III storage ring of HASYLAB (Hamburg, Germany). The analytical performance of this instrument in the case of organic samples was determined using a biological standard reference material NIST SRM1577, Bovine Liver. Typical absolute detection limits at this second-generation source are in the range of 2–3 fg for the most efficiently excited elements using a monochromatic microbeam of 15 μm , and excitation energy of 20.7 keV with a measuring time of 1000 s. This translates to relative detection limits in the 0.01- to 0.10-ppm range.

Figure 2 illustrates two-dimensional scanning micro-XRF results showing the elemental distributions within two *Daphnia* samples corresponding to different levels of aquatic Zn exposure. These elemental maps correspond to the Zn distribution within an unexposed sample (left), and within a sample which was exposed to 120 $\mu\text{g L}^{-1}$ Zn during 1 week (right). Since metals can only influence physiological processes where they are effectively present, insight in the tissue-specific distribution of elements contributes to an increased understanding of possible consequences of an exposure [12]. Special preparation methods are required to avoid artifacts, especially in aquatic organisms. *Daphnia* species prepared according to a hexamethyldisilazane (HMDS) drying method revealed an excellent internal/external preservation. In this approach, the samples were dehydrated in graded acetone solutions and immersed in HMDS, which was allowed to evaporate overnight [13]. The accumulation of Zn in the different tissues is clearly visible. Both images were normalized/corrected for detector dead-time and incident beam intensity variations, and the element contents were quantified in a first-order approximation based on the elemental yields

Fig. 1 Synchrotron X-ray fluorescence microprobe installed at Beamline L, HASYLAB (Hamburg, Germany) used for obtaining the 2D projection maps and cross-sectional elemental distributions based on XRF computed tomography from the *Daphnia magna* samples shown in Figs. 2 and 3

derived from the NIST SRM1577 Bovine Liver calibration standard. A color bar, which gives the areal Zn concentration in micrograms per square centimeter for both samples, is given on the right. A distinct enrichment of Zn can be observed in (A) the gill-like osmoregulatory tissue, and in the digestive system, i.e., (B) the gut and (C) the digestive gland. The accumulation in gills may suggest that zinc accumulation may interfere with osmoregulation (as in fish). The accumulation in the digestive system may suggest that nutrient assimilation from food is a possible Zn toxicity target. It is presently unclear whether elevated levels of Zn in the gut region are located in the gut or in the

gut epithelium. More precise analysis will be required to elucidate this matter.

Figure 3 shows the result of the “cross-fertilization” between laboratory X-ray absorption microtomography and SR micro-XRF techniques (2D dynamic scanning and XRF computed tomography). The reconstructed 3D image allows a thorough investigation of the tissue structures and their corresponding elemental contents. The colors red, green, and blue are the scaled Ca, Zn, and Fe intensities, proportional to the elemental concentration. A color triangle at the right top of the image renders the colors which indicate the combined presence of elements. The very

Fig. 2 Micro-XRF dynamic scans on *Daphnia magna* samples subjected to different exposure levels of Zn, using a microbeam of approximately 15 μm (FWHM) which defines the attainable resolution during this experiment. The left image corresponds to an

unexposed sample, while the sample on the right was exposed to $120 \mu\text{g L}^{-1}$ Zn during 1 week. Both samples were dried using an HMDS drying technique previously used for SEM analysis [13]

distinct accumulation of Ca in the exoskeleton, Fe in the gill-like tissue, and Zn in the gut and eggs can be clearly observed. For an example of quantification of a single element (e.g., Zn), we refer to Fig. 2.

One disadvantage of scanning X-ray fluorescence techniques is that the recorded analytical response is only element-specific and not chemical state (species)-specific. However, by using an energy-tunable synchrotron microbeam, the method can be easily extended towards a species-sensitive method by the combination of X-ray fluorescence tomography or confocal XRF with X-ray absorption spectroscopy (XAS) techniques. This allows the collection of complementary information on the local chemical environment of the elements of interest, e.g., to obtain information with respect to their oxidation state. In the specific case study on *Daphnia magna*, micro-XAS experiments in fluorescence mode on trace metals within specific tissues can provide insight into the transformations that the target elements undergo after uptake.

The previously discussed micro-XRF and micro-CT data are perfectly complementary to tracer experiments already being performed using stable isotopes for evaluating the importance of different exposure routes of metals on daphnids, whereas the hyphenation of separation methods

such as HPLC, capillary electrophoresis, and/or 2D gel electrophoresis with inductively coupled plasma mass spectrometry (ICP-MS) is expected to give complementary information to chemical state mapping using XAS techniques [14, 15].

Outlook and summary

Owing to the increasing availability and rapid evolution of third-generation synchrotron radiation sources, associated X-ray micro/nano-focusing optics and various types of X-ray imaging (2D) and energy dispersive spectroscopy detectors, a new generation of synchrotron X-ray micro/nanoprobes are being planned and realized at various SR facilities. As mentioned earlier, an interesting trend at such microprobes is the use of combined methodologies, coupling complementary techniques such as scanning micro-XRF, micro-XAS/XRD, and micro-CT, providing elemental, chemical state, and structural/morphological information, potentially in a three-dimensional manner. We illustrated here an example of synchrotron micro-XRF measurements coupled with high-resolution laboratory micro-CT for in situ non-destructive elemental imaging in biological systems with resolution levels on the 3–15 μm range.

In terms of highly focused X-ray beams, at third-generation synchrotron sources, it is now possible to obtain beam sizes in the sub-micron range down to a few tens of nanometers [4, 5]. Examples of third-generation synchrotron radiation facilities implementing hard X-ray nanoprobes on insertion device sources are the ESRF (beamlines ID13 and ID22), the Advanced Photon Source (APS) (beamline 26-ID), and the PETRA-III facility in Hamburg, which is currently under construction. With lateral resolution in the 30- to 100-nm range, we expect to see a rapid evolution towards in situ elemental imaging on the nanoscopic scale in various application areas, such as Earth and environmental science, materials science, archaeology, medical science, and functional biology.

With respect to biological imaging, shifting from the microscopic scales (organ/tissue level) towards a (sub-) cellular field of view is expected. Using nanobeams of X-rays coupled with state-of-the-art scanning systems and multi-element detectors, a detailed 3D structure of the elemental distributions within individual cells will be detectable, which is likely to provide new insights in the role of metals in cellular metabolism. For example, within the framework of micro analysis on daphnids detailed above, assessing the 3D elemental distribution within individual cells of *Daphnia* with nanoscopic resolution is expected to allow a more profound insight into the cellular detoxification process.

Fig. 3 Three-dimensional rendering of the unexposed *Daphnia magna* sample shown in Fig. 2 (left side). The grayscale dataset gives a full 3D absorption reconstruction of the daphnid (3- μm resolution) obtained by the UGCT micro/nano CT setup at Ghent University. Two RGB composed micro-XRF datasets obtained at HASYLAB, Beamline L are also incorporated in the image: a micro-XRF 2D dynamic scan (height 175 $\times$ 20 μm , width: 122 $\times$ 20 μm) and a micro-XRF computed tomography cross section (width 165 $\times$ 20 μm) through the gill tissue, eggs, and gut

Acknowledgements This work was supported by the European Community-Research Infrastructure Action under the FP6 “Structuring the European Research Area” Programme (through the Integrated Infrastructure Initiative “integrating Activity on Synchrotron and Free Electron Laser Science”). B. De Samber is a Ph.D. student supported by the Research Foundation - Flanders (FWO), Belgium. G. Silversmit is Postdoctoral Fellow of the Research Foundation - Flanders (FWO), Belgium. L. Balcaen and K. De Schampelaere are Senior Research Assistants of the Research Foundation - Flanders (FWO), Belgium.

References

1. Vincze L et al (2004) X-ray fluorescence microtomography and polycapillary based confocal imaging using synchrotron radiation. In: Bonse U (ed) Developments in X-ray tomography IV. SPIE-Bellingham, Washington, pp 220–231
2. Vincze L, Vekemans B, Brenker F, Falkenberg G, Rickers K, Somogyi A, Kersten M, Adams F (2004) Three-dimensional trace element analysis by confocal x-ray microfluorescence imaging. *Anal Chem* 76:6786–6791
3. Vincze L et al (2004) Monte Carlo simulation for x-ray fluorescence spectroscopy. In: Tsuji K, Injuk J, Van Grieken R (eds) X-ray spectrometry based on recent technological advances. Wiley, Chichester, pp 435–462
4. Pfeiffer F, David C, Burghammer M, Riekel C, Salditt T (2002) Two-dimensional x-ray waveguides and point sources. *Science* 297:230
5. Schroer C et al (2005) Hard x-ray nanoprobe based on refractive x-ray lenses (2005). *Appl Phys Lett* 87:6951
6. Paquin P, Gorsuch J, Apte S, Batley G, Bowles K, Campbell P, Delos C, Di Toro D, Dwyer R, Galvez F, Gensemer R, Goss G, Hogstrand C, Janssen C, McGeer J, Naddy R, Playle R, Santore R, Schneider U, Stubblefield W, Wood C, Wu K (2002) The biotic ligand model: a historical overview. *Comp Biochem Physiol C Toxicol Pharmacol* 133:3–35
7. De Schampelaere K, Janssen C (2004) Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environ Toxicol Chem* 23:1365–1375
8. Van Sprang P, Janssen C, Heijerick DG, De Schampelaere K (2005) Development of a chronic zinc biotic ligand model for *Daphnia magna*. *Ecotoxicol Environ Saf* 62:1–10
9. De Schampelaere K, Janssen C (2002) A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium and pH. *Environ Sci Technol* 36:48–54
10. Heijerick D, De Schampelaere K, Janssen C (2002) Predicting acute zinc toxicity for *Daphnia magna* as a function of key water chemistry characteristics: development and validation of a biotic ligand model. *Environ Toxicol Chem* 21:1309–1315
11. De Schampelaere K, Vasconcelos D, Tack F, Allen H, Janssen C (2004) Effect of dissolved organic matter source on acute copper toxicity to *Daphnia magna*. *Environ Toxicol Chem* 23:1248–1255
12. De Schampelaere K, Canli M, Van Lierde V, Forrez I, Vanhaecke F, Janssen C (2004) Reproductive toxicity of dietary zinc to *Daphnia magna*. *Aquat Toxicol* 70:233–244
13. Laforsch C, Tollrian R (2000) A new preparation technique of Daphnids for scanning electron microscopy using hexamethyldisilazane. *Archiv für Hydrobiol* 587–596
14. Balcaen L, De Schampelaere K, Janssen C, Moens L, Vanhaecke F (2007) Development of a method for assessing the relative contribution of waterborne and dietary exposure to zinc bioaccumulation in *Daphnia magna* by using isotopically-enriched tracers and ICP-MS. *Anal Bioanal Chem* (in press)
15. Vanhaecke F et al (2003) Detection by ICP-MS. In: Cornelis R, Caruso J, Crews H, Heumann KG (eds) Handbook of elemental speciation: techniques and methodology. Wiley, Chichester, pp 281–312