Life science applications and research potential of the TwinMic spectromicroscopy station at ELETTRA

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Abstract. Since its opening to the users in late 2007, the TwinMic beamline at the Elettra synchrotron has been attracting the interest of a broad life science community. The recent development and implementation of a low energy x-ray fluorescence detection mode have increased the capability and the versatility of the scanning mode of the TwinMic microscope. Now using simultaneously the x-ray transmission and emission detection systems one can perform elemental, absorption and phase contrast imaging that provides complementary chemical and morphological information for the sample under investigation. The recent achievements in the performance of the instrument using combined acquisition modes for characterization of nano and micro-structured biomaterials will be illustrated with selected exemplary results.

1. Introduction
Understanding the biochemistry and the processes occurring in complex biological systems at their natural subcellular length scales requires an interdisciplinary research approach. X-ray microscopy using a synchrotron light that combines high resolution x-ray imaging with element sensitive spectroscopies, namely x-ray fluorescence (XRF) and x-ray absorption spectroscopy (XAS) is one of the powerful techniques that has been gaining the life scientists interest in the recent decade [1, 2]. Both type of X-ray microscopes, full-field imaging and scanning, are in operation at most of the synchrotron facilities using soft or hard X-rays. The major advantage of the microscopes using hard x-rays is their high penetration depth and micro-XRF ability to detect trace elements but for soft matter the absorption of hard x-rays is very weak and for low Z elements the fluorescence signal using hard x-rays is extremely low. This paper emphasizes the potential of soft x-ray microscopy, combing x-ray imaging and low energy x-ray fluorescence (LEXRF) imaging and microspectroscopy for analysis of materials at submicron scale. In particular, we present short description of the instrument and methodology and selected results from the ongoing research related to the cellular distribution of CoFe²O⁴ nanoparticles in fibroblast cells and composition and structure of the asbestos body developed in human lung tissues after inhalation of asbestos, which illustrate its potential in bio-medical research.

2. The TwinMic spectromicroscopy station
TwinMic instrument [3] is a soft x-ray microscope operated at the Elettra synchrotron (Trieste, Italy) in the x-ray range 400 and 2200 eV. It consists of two modules that host a full-field
transmission x-ray microscope (TXM) and in scanning x-ray microscope (SXM), respectively, with easy switch between the two [3]. Here we report some recent results obtained using the SXM, where the sample is raster scanned under a microprobe formed by zone plate diffractive optics. The transmitted x-rays providing absorption and differential phase contrast images and also micro-XAS spectra are collected by using a fast readout CCD camera or a photodiode [4]. The X-ray fluorescence photon emission, which provides elemental images and LEXRF micro-spot spectra can be simultaneously detected by 8 silicon drift detectors (SDDs) in an annular configuration [5]. The LEXRF mode of TwinMic detects the K lines of elements from B to P and the L lines of chemical elements with emission in the 400-2100 eV energy range (such as Fe, Ni, Cu, and Zn) with submicron spatial resolution [6]. The LEXRF set up has been extensively requested for life science applications since it permits elemental mapping of light elements, namely C, N, O, Mg, Na, that are the main constituents of life matter and the elemental maps can be correlated to the morphology of the specimen revealed by the absorption and phase contrast images.

3. Results

The TwinMic microscope has been successfully used in different research fields including fuel cell technology [7], biotechnology, biomaterials, food science and nanotoxicology, neuroscience and clinical medicine. Recent research efforts have provided new insights into (i) the morphology distribution and correlation of the elements as result of plant’s growth under various environmental conditions and in presence of toxic substances [8, 9], (ii) concentration dependence of penetration of magnetic nanoparticles in different cell organelles and changes in the nanoparticle chemistry inside the cells [10,11], (iii) cellular internalisation and degradation of magnetic nanoparticles in digestive gland epithelium [12], (iv) reaction of lung tissue in the presence of inhaled asbestos fibres [13], (v) glucose metabolism in neurons [14], and many others. In the paper we report two examples of ongoing bio-related studies that illustrate the potential of the instrument in relevant research domains.

3.1 Cellular distribution of CoFe2O4 nanoparticles in fibroblast cells

One of the ongoing nanotoxicology-related research efforts focuses on the analysis of the effects of magnetic nanoparticles on cells. In particular, we have investigated Balb3T3 mouse fibroblast cells grown on Si3N4 windows after exposure for 24 hours to different concentrations (40 and 1000 µM) of CoFe2O4 nanoparticles (NPs) with average NP diameter of 30 nm. We analysed at least 3 cells for each condition. These results represent the first attempt of evaluating the behavior of CoFe2O4 NPs at sub-cellular level by x-ray spectromicroscopy. The information contained in LEXRF maps and micro-spot XRF spectra [10], acquired after treatments using different NPs concentration exposure have revealed that, for concentrations below 500 µM, Co and Fe are localized in the cytosol, i.e. in the perinuclear region, whereas at higher NPs concentrations, although the most of the NPs are still localized in the cytosol, Fe and Co XRF peaks were also observed in the nuclear region of the cell. Moreover, the Fe and Co XRF intensities retained the stoichiometric ratio of the intact NPs, whereas the Fe/Co ratio in the nucleus indicated a sensible accumulation of Co. As an example Figure 1 shows the absorption, phase contrast, and elemental XRF maps of a fibroblast cell grown in a medium containing CoFe2O4 NPs with concentration of 1000 µM. While the C signal delineates the cell structure and morphology, the Fe and Co maps indicate the distribution of the NPs. Since oxygen is also present in the cell constituents the O signal inside the cell becomes stronger where the particles are located. The absorption and phase contrast images clearly show the presence of a white corona delimiting the cell nucleus that becomes particularly visible at high NPs
concentrations, which is associated with the formation of liquid droplets [10].

Fig. 1 Absorption (a) and phase contrast (b) images (40µm x 40µm) of a fibroblast cell exposed to 1000 µM concentration of CoFe₂O₄ NPs and corresponding elemental maps of C, O, Fe and Co, acquired at a photon energy of 1.1 keV and with a spot size of 670 nm.

3.2 Asbestos body morphology and composition in human lung tissues
Asbestos toxicity and its carcinogenic effect are known since a few decades but the mechanism involved in the inflammation and toxicity process are still not well understood. The results obtained with TwinMic [12] represent the first attempt for exploring the morphology and composition of an asbestos body in the lung of exposed patients using untreated paraffinized histological lung tissues by a combination of soft x-ray microscopy approaches. The observed specimen morphology and differences in the local distribution of specific chemical elements (namely Si, Fe, O and Mg) have provided distinct fingerprints for the core asbestos fibre, where Si is the main constituent, and for the ferruginous bodies contained in the tissues, characterized by a strongly increased content of Fe, Mg and O compared to the surrounding tissue. The results have shown that, along with Fe, Mg also plays an important role in the formation mechanisms of asbestos bodies that can be related to processes arising as a response of the lung tissue to the asbestos toxicity [13].

Summary of the asbestos-relevant results is shown in Figure 2. The absorption and phase contrast images of an asbestos body in lung tissue coupled with the corresponding elemental maps of Si, Mg and Fe, reveal that the darkest, highly absorbing regions are the one containing Fe, which is the principle constituent of the body developed around the fibre that is well visible in the Si map. The presence of Mg coincides with the location of Fe but some accumulation of Mg can be clearly found also in the tissue in the vicinity of the body.

This first study was carried out at low energy (2 keV) by using the TwinMic XRF setup. Further combined investigations (TwinMic beamline in Elettra and ID21 beamline in ESRF) at higher energy will be presented in a future manuscript in preparation.

4. Conclusion
The present paper briefly describes the research potential of the TwinMic spectromicroscope in the life science field by concentrating on two case studies of on-going research. The
combination of x-ray imaging (absorption and phase contrast) with elemental analysis (XRF) have provided new insights in the biological events occurring at submicron scale. The planned future developments aim at complementing the XRF with XANES spectroscopic analyses, also available at TwinMic microscopy station (see for instance ref. 7), where speciation will add new information to morphological and elemental analyses.

![Absorption and phase contrast images of an asbestos body in lung tissue.](image)

**Fig. 2** Absorption (a) and phase contrast (b) images (25µm x 32µm) of an asbestos body in lung tissue. The corresponding elemental maps show the distribution of Si, Mg and Fe in an area surrounding the asbestos body (29µm x 35µm, 1.95 keV, 500nm beam diameter).

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