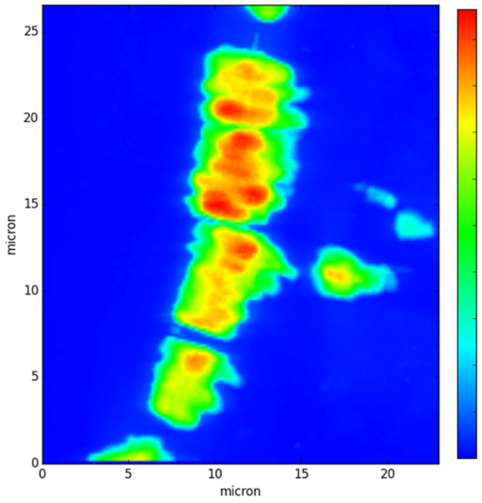
EUROPEAN SYNCHROTRON RADIATION FACILITY

ESRF User Office
CS 40220, F-38043 GRENOBLE Cedex 9, France
Delivery address: 71 avenue des Martyrs, 38000 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

Application for beam time at ESRF – Experimental Method

Proposal Summary

After prolonged stay in the lungs, asbestos fibres develop a ferruginous coating that is believed to enhance their toxicological outcome. Revealing the fine details and the composition of the coating will help understanding its formation mechanism and, in turn, formulating a solid model of the carcinogenesis.
The proposed experiment is a continuation of experiment LS-2548 (see experimental report). The aim of the experiment was to combine phase-contrast and fluorescence tomography to obtain complementary information on asbestos fibres embedded in human lung tissue. The experiment was fully successful (Figure 1); the only limitation was the relatively small number of samples measured, which was due to the long acquisition time required by fluorescence tomography measurements. In this new experiment, the same measurement strategy will be applied to study lung tissue samples from individuals subjected to a different occupational exposure to asbestos with respect to the samples studied in the first experiment.



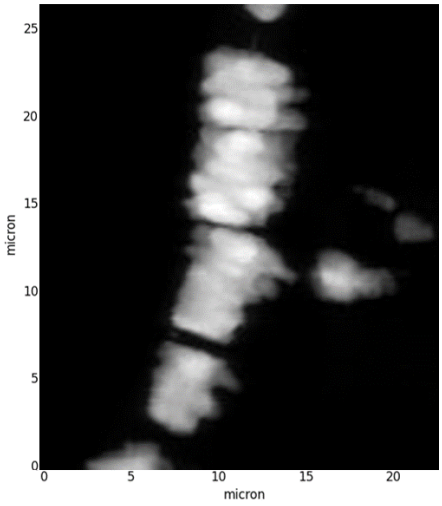


Figure 1.

Left panel: X-ray fluorescence tomography showing the distribution of Fe on an asbestos body (red = higher concentration, blue = lower concentration). Pixel size 130 nm.

Right panel: Phase-contrast tomography of the same asbestos body shown in the let panel. Pixel size 60 nm.

Scientific background

When it is inhaled, asbestos irritates the tissue causing minerals and proteins to cluster around the foreign fibers. These clusters are known as ***asbestos bodies*** (*AB)*, and are the product of a biomineralization process induced by alveolar macrophages. It was generally accepted that the coating was a protective mechanism to segregate the cytotoxic fibers from the organic tissues[1]. However, other authors suggested that the coating material itself may enhance the cytotoxic properties of asbestos by increasing the generation of free radicals[2]. These studies also demonstrated that the iron contained in the coating is catalytically active[3] and can induce modification in DNA[4]. Earlier studies[5] suggested that the coating of the *AB* is composed of ferritin. Nowadays, scientists converge to the conclusion that the presence of redox-active iron, either as a constituent of the crystal, or adsorbed to its surface, is responsible for the genotoxic and cytotoxic effects of amphibole asbestos, and that the coating consists of an iron protein (ferritin or hemosiderin) and mucopolysaccharides. The size of the *AB* is usually 40-80 µm in length and 1-4 µm in diameter. The majority of the studies on *AB* suffer from the fact that suitable nano-probe techniques required to study the composition of such small objects became widespread only recently. Bulk techniques, such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), require aggressive treatments of the samples (incineration or digestion in strong acids) that remove the organic component and can alter the chemical composition of the *AB*. These techniques also lack of spatial information. Microprobe tools based on synchrotron radiation, on the other hand, have started to be exploited only recently to study the present topic and only in 2D acquisition mode[6]. X-ray fluorescence maps acquired on *AB* in previous experiments

performed at ID18F and ID21 at the ESRF revealed interesting details[7], but the information obtained was still limited to two dimensions. Conversely, X-ray fluorescence and phase-contrast tomography have the potential to reveal the tridimensional elemental composition and distribution, and the morphology of the *AB* at the nanometric scale, pushing further the characterization of these intriguing objects.

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc)

Lung tissue samples from a former worker of a steel plant and from one of an asbestos mine, both affected by asbestosis and pleural mesothelioma, were collected after forensic autopsy and preserved in formalin (10%). Non-neoplastic portions of lung tissue were examined to estimate the number of *AB* per gram of dry weight (g_{dw}) by optical microscopy and scanning electron microscopy. In both specimens the burden of *AB* (~3.4 and ~$5.6 \cdot 10^4$) largely exceeded the amount established to indicate a high level of occupational exposure to asbestos ($1 \cdot 10^3/g_{dw}$)[8]. Following the preparation method used for the first experiment, lung tissue samples will be brought to the ESRF in the form of histological sections. To facilitate locating the asbestos fibers in the lung tissue, small areas (50-100 µm in diameter) centred on the fibres will be cut using a laser microdissector (i.e. a laser cutter coupled to an optical microscope). The fragments will then be glued on thin tips (5 µm) of borosilicate capillaries with a diameter fitting the beamline sample holders (Huber pin). Samples will be measured in vacuum, which will favor the stabilization of biological samples and allow the detection of the fluorescence signal of lighter elements (in particular of Si, which is the main constituent of the asbestos fibers). Measurements will be performed at 17 keV incident photon energy, which will allow the simultaneous detection of Si, Ca, and Fe, the main constituents of the *AB*. To obtain high quality tomographic reconstructions, phase-contrast projections will be acquired at 1500 different angles with a pixel size of 60-70 nm. To save time, fluorescence tomographs will be acquired at only 30 different angles with a pixel size of 130 nm.

Beamline(s) and beam time requested with justification

Being a continuation experiment LS-2548, this new experiment should be performed at ID16A beamline. In addition, this beamline is currently the only one that allows combining x-ray phase-contrast and fluorescence tomography at the nanoscale, and in vacuum acquisitions. Considering the number of samples (10) and the acquisition time required by fluorescence tomographs, at least 15 shifts will be necessary to complete the experiment.

Results expected and their significance in the respective field of research

The acquired data will reveal the composition and fine morphological details of the *AB*. In addition, phase-contrast data will allow estimating the average density of the *AB*, which, in combination with fluorescence data, will allow performing for the first time a reliable and spatially resolved elemental quantification[9]. The results of this experiments will add essential pieces of information that can help revealing the formation mechanism of the *AB*, and, in turn, the carcinogenic mechanism. The research project underlying this experiment has been funded by the European Commission[10].

References

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