Summary of Proposal 16144

Requested Access Route:	Standard
Principal Investigator:	**Dr Fabrizio Bardelli, CNR-Nanotec**
Co-Investigator(s):	Dr. Francesco Brun, Italian National Council of Researches Mr Lorenzo Massimi, CNR-Nanotec Dr. Alessia Cedola, CNR-Nanotec
Title:	**Phase-contrast nanotomography of asbestos bodies in human lung tissue**
Primary Beamline:	I13-2
Shifts Requested:	12 **RCaH requested:** No
Industry Involved:	No **Industry Group Links:** No
Science Area(s):	Medicine, Environment, Earth Science
Other Facilities:	SLS

Abstract:

The proposed experiment aims to reveal the fine details of the interfaces between the asbestos fibers and their ferruginous coating (developed after long resident time in the lungs), and between the coating and the surrounding biological tissue. This will be achieved by exploiting state-of-the-art x-ray phase-contrast nanotomography. A deeper knowledge of the interaction between asbestos fibers, their coating, and the surrounding biological tissue, can lead scientists in their effort to better understand the carcinogenic mechanism and develop more effective medical treatments for highly deadly asbestos-induced diseases, such as malignant pleural mesothelioma.

Detailed Information:

Question	Answer
What type of beam do you require?	Monochromatic
What is the typical thickness [mm] and main component of your sample?	0.05
What is the absorption of a typical sample at the choosen energy?	?
What is the required spatial resolution [µm]?	0.2 µm
What is the required field of view? [mm x mm]?	0.06 mm x 0.06 mm
Do you image weakly absorbing materials?	Yes
If you have performed radiography or Tomography experiments before, please indicate where: in lab. sources or synchrotron	ID17@ESRF
Select the reason why do you need synchrotron radiation for your experiment	highest spatial resolution
If you have any special sample environment requirements, please specify.	We will image high absorbing material (asbestos bodies, up to 20% wt. Fe) in weak absorbing biological lung tissue.

Samples:

Name	CAS No	Type	
AB		**BiologicalAgent:**	tissue sample

Experimental Method: Method

Sample Preparation:

A laser microdissector will be used to obtain tissue fragments containing asbestos bodies, with suitable dimensions for nanotomography.
Samples will be brought already embedded in paraffin blocks of suitable dimensions (50x50x50 microns); therefore, no sample preparation is foreseen at Diamond nor the use of any chemicals. Only handling and mounting the samples in the experimental chamber.
No waste samples will be produced and samples will be removed by users at the end of the experiment.
No harmful releases or dangerous conditions are foreseen.

Beamline Experiment and Environment:	The paraffin-embedded tissue blocks will be probably glued on the tip of glass capillaries for XRD or on beamline dedicated sample holders. Phase-contrast in propagation-based mode will be performed at 11.7 keV incident photon energy using Zernike phase plate. Phase-contrast tomographs will be acquired at the a highest resolution achievable on the beamline and with a field of view of 60 x 60 µm. No harmful releases or dangerous conditions are foreseen. Samples will be removed by users at the end of the experiment.

Scientific Case

In the lungs, asbestos irritate the tissue causing minerals and proteins to cluster around the inhaled asbestos fibers. These clusters are known as asbestos bodies (*AB)*, and are the product of a biomineralization process carried out by alveolar macrophages (Figs. 1a and 1b). It was generally accepted that the coating surroundings the fibers was a protective mechanism deposited by macrophages trying to segregate the cytotoxic fibers from the organic tissues[1]. However, other authors suggested that the coating material itself might 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 the DNA[4]. Earlier studies[5] suggested that the coating contained crystalline particles of the same order of size of the inorganic iron core of the ferritin molecule. On this basis, it was assumed that the crystalline material comprising the major part of the *AB* is composed of ferritin. Today, scientists converge to the conclusion that the presence of redox-active iron, either as a constituent of the asbestos crystalline structure, or adsorbed to its surface, is responsible for the genotoxic and cytotoxic effects of amphibole asbestos, and it is widely accepted that the coating consists of a protein (ferritin or hemosiderin) and mucopolysaccharides. While it is agreed that amosite and crocidolite, which contain up to 20% wt iron, are the most hazardous asbestos fiber types, the discover of an excess of mortality from mesothelioma in some villages in Central Anatolia (Turkey) due to chronic exposure to erionite[6], an iron-free fibrous mineral belonging to the zeolite group, questioned the central role of iron in the pathogenesis.

Asbestos bodies usually do not exceed 40-80 μm in length and 1-10 μm in diameter. The majority of the studies on *AB* suffer from the fact that suitable micro- or nano-probe techniques required to study small objects became widespread only recently. Bulk techniques, such as ICP-MS, require aggressive treatments of the samples (incineration or digestion in strong acids), which remove the organic component and can alter the chemical composition of the *AB*. These techniques also completely lack of spatial information. On the other hand, synchrotron radiation micro-probe tools have started to be exploited only recently to study the present topic, and only in 2D acquisition mode[7]. X-ray fluorescence maps (Fig. 1c) acquired on the *AB* in a previous experiment performed at the ESRF revealed interesting details, but the information obtained was still limited to two dimensions. Conversely, phase-contrast nanotomography has never been applied to the present subject, and has the potential to reveal the fine details of the *AB* and of their interface with the biological tissue with unprecedented level of detail in unaltered bulk lung samples.

Phase-contrast in propagation-based mode will be performed at 11.7 keV incident photon energy using Zernike phase plate. Phase-contrast tomographs will be acquired at the highest resolution achievable on the beamline and with a field of view of 60 x 60 μm. The data acquired on I13 will be combined with lower resolution/higher field-of-view tomographic data recently acquired at ID17 at the ESRF (Fig. 1d), so to obtain information at different spatial scales.

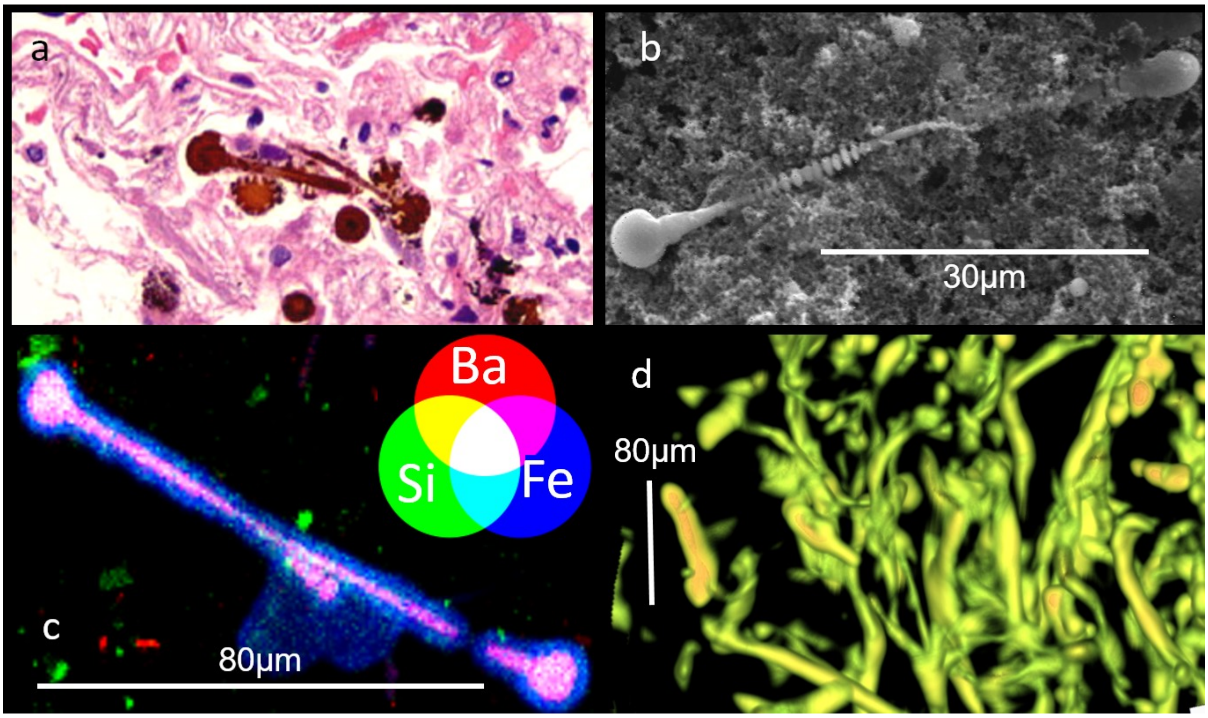

Fig. 1. (a) Optical microscope image of an AB in a histological section (400x). (b) SEM image of an AB extracted from the lung tissue. (c) Micro x-ray fluorescence map of an AB extracted from the lung tissue highlighting the co-localization of Si, Ba, and Fe (pixel size $0.5x0.5\mu m^2$). (d) Volume rendering of lung tissue with AB obtained from phase-contrast data acquired at ID17 at the ESRF with voxel size of 1.34µm.

The I13 beamline at Diamond will allow performing high resolution phase-contrast tomography on a relatively large number of samples, being therefore the ideal beamline to perform the proposed experiment. Considering the number of samples (5 to 10 AB for for each of the 4 individuals = 20-40 tomographs), and the time required to optimize the beamline components (non-standard setup is required), as suggested by the beamline scientists, a minimum of 12 shifts will be necessary to complete the experiment,

References

[1] A B Kane. Asbestos bodies: clues to the mechanism of asbestos toxicity? *Human Path.* 34, 735 (2003);

[2] H Pezerat *et al.* Man-made mineral fibers and lung cancer. *Toxic. and Ind. Health* 8, 77 (1992);

[3] A J Ghio *et al.* Ferruginous bodies: implications in the mechanism of fiber and particle toxicity. *Toxic. Path.* 32, 643 (2004);

[4] L G Lund *et al.* Iron associated with asbestos bodies is responsible for the formation of single strand breaks in φ X174 RFI DNA. *Occupat. & Environ. Medicin* 51, 200 (1994);

[5] F D Pooley. Asbestos bodies, their formation, composition, and character. *Environ. Res.* 5, 363 (1972);

[6] I. Baris *et al.* Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: a four year study in the Cappadocian region of Turkey.Int. *J. Cancer* 39, 10 (1987);

[7] L. Pascolo *et al.* The interaction of asbestos and iron in lung tissue revealed by synchrotron-based scanning X-ray microscopy. *Scientific reports 3*, 1123 (2013);