ESRF	**Experiment title:** First combined nano x-ray phase contrast and fluorescence tomography of human lung tissue samples impacted with asbestos fibers	**Experiment number**: LS2548
Beamline: ID16A	**Date of experiment**: from: 23-11-2016 to: 28-11-2016	**Date of report**: 22-02-2017
Shifts: 15	**Local contact(s)**: Peter Cloetens, Sylvain Bohic	*Received at ESRF:*

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Report:

Project summary: The aim of the experiment was to combine phase-contrast and fluorescence tomography to obtain complementary information on asbestos fibres embedded or extracted from human lung tissue. After prolonged stay in the lungs, these fibres develop a ferruginous oating that is believed to enhance their toxicological outcome. Revealing the fine details and the composition of the coating can help undertanding its formation mechanism and, in turn, the carcinogenic mechanism of asbestos. The data acquired in this experiment are part of a project that has recently been funded by the European Commission [1], and that led to a publication on Scientific Reports [2].

Samples: Lung tissue samples from two individuals were 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 were cut using a laser microdissector. The fragments were then glued on the thin tips (5 μm) of borosilicate capillaries with a diameter fitting the beamline sample holders. Other samples were prepared by digesting the lung tissue with NaClO and recovering the fibres by filtration on porous membranes. These samples served as comparison with the fibres embedded in the tissue.

Phase-contrast tomography measurements: High resolution (60-70 nm) tomographs were collected on 5 fibres embedded in the original lung tissue plus 6 fibers deposited on porous membranes.

Fluorescence tomography measurements: High resolution (100-130nm) fluorescence tomography or 2D maps were collected on the very same fibres where phase-contrast tomographs were acquired. Nevertheless,

due to limited beamtime and to the long time required to acquire fluorescence tomographs (~8h for tomographs collected at 30 different angles), the latter were acquired only on 4 fibres. To save time, 2D fluorescence mapping was performed on the remaining fibres.

Preliminary results: The experiment was fully successful, the only limitation being the relatively small number of sample measured, which was due to the long acquisition time required by fluorescence tomography measurements. The analisys is still ongoing, but preliminary processing of the data clearly show that it will be possible to obtain quantititive complementary information from phase-contrast and fluorescence data (see **Figure 1**). In particular, besides the high resolution imaging of the fibres with their coating, phase-contrast data can allow estimating the average density of the asbestos bodies (i.e. the asbestos fibres plus their ferruginous coating). This is required to perform a elemental quantification using fluorescence data [3]. It is worth noting, in fact, that reliable elemental quantification has always been prevented because of the lack of reliable values on the thickness and density of single asbestos bodies. The data acquired during this experiment will finally allow the knowledge of these two quantitities for each of the asbestos bodies measured. These data will also complement phase-contrast tomography data measured at ID17 (LS2480) at lower resolution (3.5 µm), but on large tissue volumes.

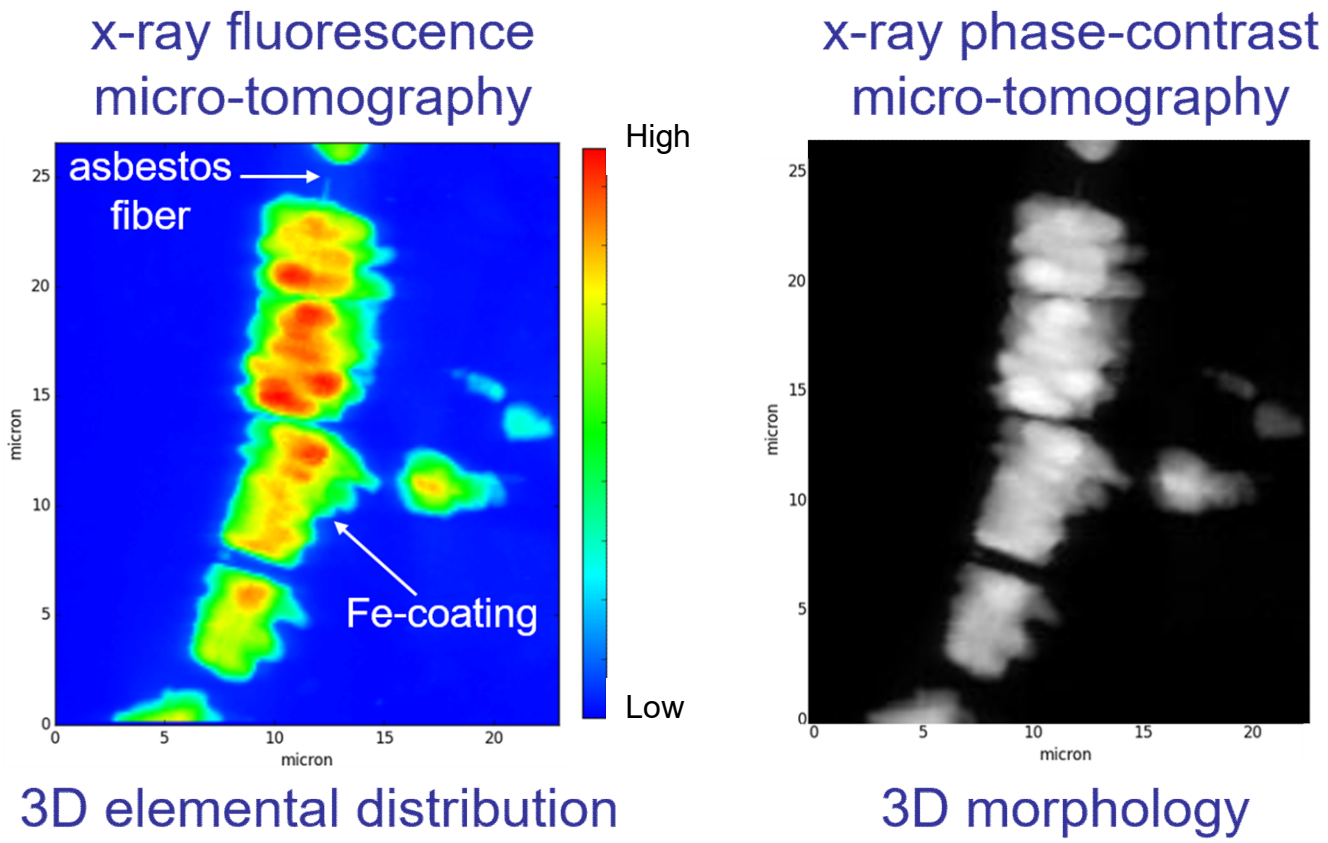

Figure 1. Left panel: fluorescence tomography of an asbestos body. Right panel: phase-contrast tomograpy of the very same asbestos body.

[1] http://biominab3d.altervista.org/
[2] F. Bardelli et al. New insights on the biomineralisation process developing in human lungs around inhaled asbestos fibres. *Scientific Reports* (in press).
[3] E. Kosior et al. Combined use of hard X-ray phase contrast imaging and X-ray fluorescence microscopy for sub-cellular metal quantification. *Journal of Structural Biology* 177 (2012).