ESRF	**Experiment title:** μXANES and μXRF investigation of the Fe-Si relationships in tissue of subjects affected and non affected by silicosis	**Experiment number**: LS2860
Beamline: ID21	**Date of experiment**: from: 26 August 2018 to: 01 September 2018	**Date of report**:
Shifts: 15	**Local contact(s)**: Marine Cotte, Bernhard Hesse	*Received at ESRF:*

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Report:

The present experiment was focussed on the characterisation of *ex vivo* forensic samples. The purpose of the experiment was the identification of chemical trends eventually associated to the presence of silica particles in tissues pertaining to cases diagnosed by silicosis. The tissues were retrieved from the archive of the Department of Public Health, Experimental and Forensic Medicine (University of Pavia, Italy).

For the purposes of the present experiment, 6 samples were selected: 5 tissues of subject with diagnosis of silicosis, chosen on the basis of the gender, age of death, work activity, and degree of development of the illness. A sixth case was chosen as control (i.e. without histological evidence of silicosis or other pulmonary afflictions).

In order to investigate the tissue using μXRF, 10 μm-thick paraffin embedde d sections were cut from the blocks, and then mounted on an ultralene layer, which was located into the sample holder. The following section (this time 4 μm-thick), cut from the same block, was prepared for histology, i.e. mounted on a glass slide and stained with haematoxylin-eosin. The histological section was used to visualize the microscopic structure of the sample under investigation by μXRF. In the Figure 1, an exemplar microfluorescence map (a) is compared with a photograph obtained under the polarised light microscope (b).

The μXRF analysis was carried thorugh X-rays were generated by a U42 undulator magnet, whereas energy was selected by a double Si(111) monochromator. A 7.123 keV monochromatic X-ray

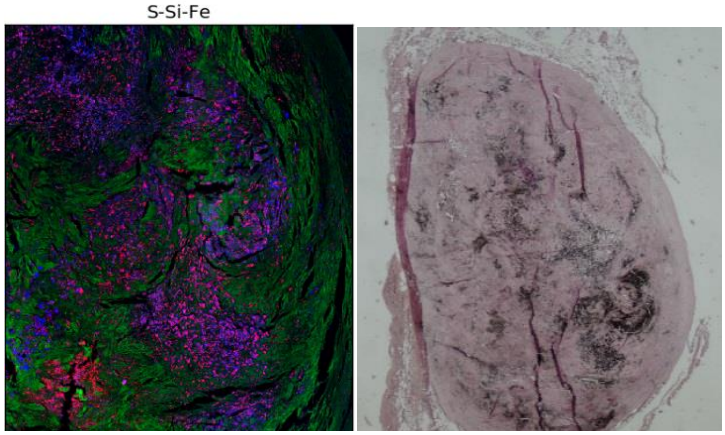

Figure 1 – (a, left) combined qualitative XRF map obtained by superposition of the previous maps (S – green; Si – red; Fe - blue); (b, right) optical micrograph of the companion histological section

beam was focused onto the samples, with a spot size of 1.3 (horizontal) × 0.35 (vertical) μm^2. The fluorescence signal was collected with a Silicon Drift Diode XRF detector (SGX Sensortech with an active area of 80 mm^2) and normalized by the incident photon intensity. Maps were recorded by moving the sample with respect to the beam incidence position and collecting the XRF photons for 100 ms/pixel.
Two types of map were registered for each sample: 1) a wide range map was collected using a square grid with step size of 5 μm in both the x and y directions. Scans were performed in continuous (zap) mode. The final size of the obtained region in this mode was of the order of some (3-8) mm^2, and the total number of pixels ranging in order of $1\text{-}5 \cdot 10^5$; 2) a short range ("high resolution") map, collected on square regions of 100 x 100 μm^2; in this case, a square grid with step size of 5 μm in both the x and y directions was used, admitting a small occurrence of oversampling in the horizontal direction. Typically, 4 to 6 type 2 maps were acquired per sample. Finally, selected XANES spectra were registered on sites, emerging from the inspection of the type 2 maps as interesting for the chemical associations.
In the Figure 2, an exemplar spectrum highlighting the average elemental composition of the map in the Figure 1a is shown.

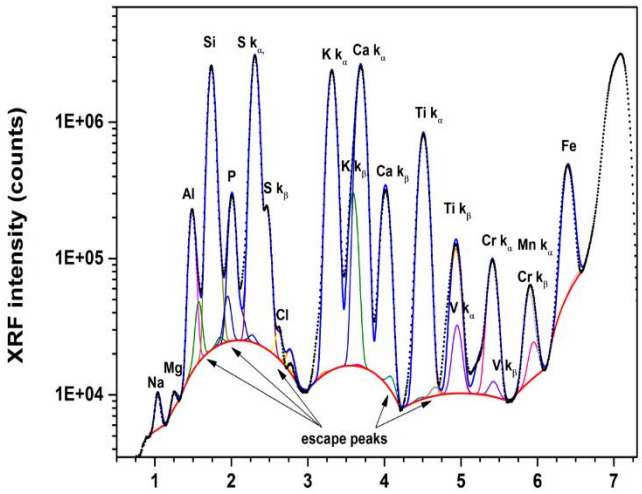

Figure 2 - Cumulative XRF spectrum of the investigated 12517 sample. The peak attribution of the main chemical components identified in the maps are indicated.

The achieved data will be merged in a unique dataset, with the aim to unravel information concerning a) the identificaiton of "natural" or spontaneous trends of association (co-localisation) of elements, with respect to the histologic evidences, also in relation with the degree of development of the illness, and b) the consistence of the chemical trends from the large range to the short range maps.
To this purposes, innovative statistical approach are going developed under CoDA (Compositional data analysis theory), including the centered (clr) logratio transformations, the replacement of the cases with zero values adopting the compositional method based on a Bayesian-multiplicative treatment, the variation array, and the the violin plot. All these analyses will drive the application of methods such as principal components analysis and cluster analysis.