2D AND 3D X-RAY IMAGING OF MICROORGANISMS IN METEORITES. L. Lemelle[1], A. Simionovici[1,2], M. Salomé[2] , B. Golosio[2] and Ph. Gillet[1], [1]Laboratoire des Sciences de la Terre, ENS Lyon, 46 allée d'Italie, 69007 Lyon FRANCE, laurence.lemelle@ens-lyon.fr, [2]ID21/ID22 group, ESRF,6 rue J. Horowitz, BP220, F-38043, Grenoble FRANCE .

Introduction: The identification of microbial cells in meteorites is a key stage in searching for extraterrestrial life traces and in studying the pristine matter of the solar system. It is essential for describing the precious extraterrestrial samples that could contribute to such questions to use non-destructive and non--penetrating techniques. In this respect, X-ray diffraction, absorption, fluorescence and associated imaging techniques at the best spatial resolutions using synchrotron facilities are among the most powerful tools to be used.

We applied state-of-the-art developments of scanning X-ray microscopy and combined X-ray microtomographies, in the 5 to 20 keV range, at the ID21/ID22 beamlines of the ESRF, in order to adress the following general questions.

- What are the possibilities of these X-ray imaging techniques if samples must be kept under some level of confinement or quarantine conditions ?
- What are the structural, mineralogical and chemical criteria necessary for assessing by these X-ray imaging techniques the presence of remnants of life forms in rocks?

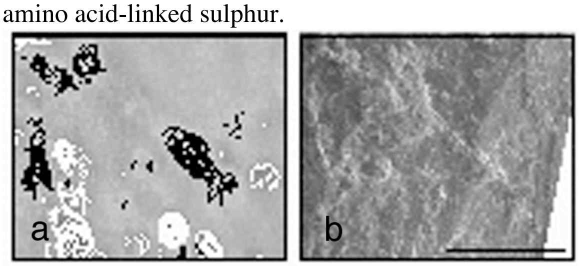
We started by looking for microorganism cells and for the aqueous alteration traces in silicate assemblages since liquid water is required for all the reactions in any terrestrial life.

2D X-ray mapping of microorganisms on the Tatahouine surface

Microorganisms were searched for among the complex terrestrial microstructures [1], [2] observed by SEM, on the surface of a fragment of the Tatahouine meteorite inherited from the Tunisian soil. Both electron and X-ray micro-beams were used to excite the intrinsic X-ray fluorescence (XRF) radiations of the putative microorganism. Interestingly, such beams allow studying *in situ* the putative microorganism and simultaneously the mineralogy of its silicate substrate. Furthermore, by tuning the energy of the synchrotron radiation X-ray beam, XANES spectroscopy can be applied for assessing the electronic structure and oxidation state of sulphur. Mapping of the redox species of S by XANES at the ID21 SXM [3] is presented below.

Micro-XANES spectra in focused mode were collected on selected positions of the sample. In this case, the zone plate was moved along the beam axis during energy scans to compensate for the change of focal length, which is energy dependent, and to keep the sample in focus. The energy was scanned between 2450 eV and 2500 eV in 0.125 eV energy steps and 2s dwell time. These spectra allowed selecting the energies corresponding to characteristic peaks, at which 2D mapping was then performed.

The SXM maps were recorded at 2473.5 eV, 2478 eV and 2482.2 eV, which are respectively characteristic for amino acid-linked sulphur, sulphite (SO_3^{2-}) and sulphate (SO_4^{2-}). The figure shows a map (Figure a) obtained by the subtraction of the map of the S-XRF excited at 2.473 keV from the one excited at 2.482 keV and the corresponding SEM image of the studied surface (Figure b□ scale bar is 20 □m). The dark zones correspond to those enriched in the amino acid-linked sulphur.

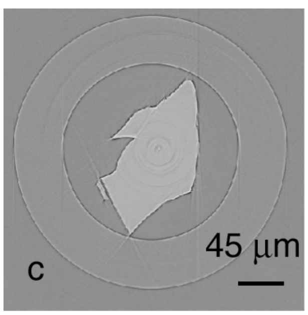


The correlation of XANES images with the distributions of the N, P and S elements is reported elsewhere [4]. It shows that these dark zones are also enriched in N and P, and have an elemental ratio (N/S) close to the one of the dehydrated . *E. coli* matter.

3D X-ray detection of microorganisms in meteorites

3D X-ray detection of aqueous alteration traces in a grain of the NWA817 meteorite□ The combination of synchrotron-based X-ray absorption and fluorescence computed tomographies (CT) is a new method [5] allowing a non-invasive and non-destructive determination of the three-dimensional (3D) mineralogy with micrometer resolution of sub-millimeter silicate grains, possibly stored in a silica holder. These CT were performed with beams of a few tens of keV from a third generation synchrotron source on one grain stored in a silica capillary. This grain is an olivine grain of the NWA817 Martian meteorite presenting a reddish alteration phase. Their mineralogy was extensively studied [6], [7].

The experiments were carried out at the ID22 beamline of the ESRF. The experimental set-up for the X-ray absorption tomography is described in Rau et al. [8]. For the fluorescence CT, a monochromatic beam of 14 keV was used, with a beamsize of 2 x 10 microns. This was obtained using compound refractive lenses, together with a 10 µm diameter pinhole, for a flux of about 10^{10} ph/s on the sample. The tomography was done in the vertical scanning geometry, recording about 120 x 120 fluorescence spectra (translations x rotations) with steps of 2.5 microns x 3 deg. The final images of the elemental maps were obtained using the algebraic reconstruction technique (ART) [9] with a resolution of 2.5 µm per pixel.



The absorption CT sections show a network of fractures in the studied grain and a few µm-thick layer formed on the grain (Figure c). The 3D facet orientation allows identifying the crystalline phase (olivine) of the studied grain; the X-ray attenuation coefficient permit to estimate its average composition ($Fo_{44\pm9}$). The fluorescence CT section reveals olivine rims enriched in iron (a major element) and/or depleted in calcium (a minor element). The CT combination shows that the µm-thick layer is preferentially formed on the (010) olivine face, has a lower density (3.5 ± 0.4 g/cm^3) than the olivine even though it is enriched in iron, and all the detectable elements composing it belong to the olivine. In conclusion, the alteration of an olivine grain is a mechanism that would have been proposed to explain the formation of the observed layer for an unknown grain. More generally, the precision presently achieved, although moderate, is sufficient to get, across a silica holder, a 3D semi-quantitative view of the mineralogy consistent with the one that can be obtained by EPMA on thin sections [10].

3D X-ray detection of microorganisms in meteorites: The distribution of a biofilm (critical thickness < 30 µm) deposited experimentally on the surface of mica grains (100 µm thick) was obtained by the tomography of the phosphorous fluorescence [11]. The interest of the absorption and fluorescence CT combination remains limited because the main components of microorganisms (C, H, O, N elements) are not detected with the current setup and the fluorescence of their minor P and S elements are strongly absorbed by the silicates (absorption is total below a 10µm slice of mica). New developments involving Compton scattering in the previous combination of CT [12], and a spiral scanning of the sample [13] should lead by their combination to an original way of describing the 3D distribution of the organic matter in complex silicate assemblages.

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