	Experiment title: Detection of remnants of bacterial activity on micrometeorites	**Experiment number**: **ME-402**
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Report:

This work is in press. The abstract is pasted bellow. The full reference of the corresponding paper is: Lemelle L., Simionovici A., Susini J., Oger P., Chukalina M., Rau Ch., Golosio B., Gillet P. (in press) Journal de Physique IV .

Abstract. X-ray imaging techniques at the best spatial resolution and using synchrotron facilities are foreworn as powerful techniques for the search of small life forms in extraterrestrial rocks under quarantine conditions. Absorption and fluorescence X-ray microtomographies on submillimeter silicate assemblages inside a container reveal the mineralogical microenvironments were life should be looked for in priority. Limitations with respect to bacteria detection are due to the difficulties to obtain information about light elements ($Z \leq 28$), major constituents of biological and silicate samples. The X-ray signature of a “present” bacteria on a silicate surface was defined by X-ray mapping, out of a container, as coincident micrometer and oval zones having strong P and S fluorescences (S-fluorescence being slightly lower than P-fluorescence) and an amino-linked sulfur redox speciation. The detection of a single cell along with new procedure to calculate tomographic views will allow considerable improvements of 3D detection of life by X-ray techniques.

1. INTRODUCTION

The search for small life forms in extraterrestrial rocks started with the report of some evidence of bacteria remnants in the martian meteorite ALH84001. The debate has now moved to more general questions. What are the structural, mineralogical and chemical criteria necessary for assessing the presence of remnants of life forms in rocks from the Earth, meteorites and Mars? These criteria must be further refined in the forthcoming years for the characterization of the Martian samples which should be available in 2010. One of the fundamental issue concerning these samples is to verify whether or not life exists and has existed on Mars. Beside this fundamental issue, these exceptional samples, available in only very small quantity, must first be characterized under quarantine conditions to limit the contamination of both the samples and the terrestrial atmosphere. It is also essential for studying rare extraterrestrial samples to describe them using non-destructive techniques. In this respect, it has been emphasized that X-ray diffraction, absorption, fluorescence and associated imaging techniques at the best spatial resolution using synchrotron facilities are among the most powerful tools to be used for these small samples. We applied them to adress two kinds of questions:1- What are the possibilities and also the limits of X-ray imaging techniques if samples must be kept under some level of confinement or quarantine conditions? 2- What are the natural or experimental aqueous alteration traces of bacteria activities left on the silicates?

2. SAMPLES

2.1 Meteorites presenting natural aqueous alteration traces

The olivine crystals of the Martian meteorite “North West Africa” (NWA817) contain secondary mineralization attributed to a preterrestrial alteration. The pyroxene crystals of the meteorite “Tatahouine”. contain secondary mineralization: rims iron stains and carbonate rosettes. Carbonates are due to infiltration by liquid water of the Tatahouine soil and bacteria-like forms are observed on their surface.

2.2 Silicates altered experimentally by aqueous bacterial suspensions

Biofilms of *E. coli* and *Agrobacterium rhizogenes* were formed on the (001) surface of micas.

3. 3D X-RAY IMAGING UNDER CONFINEMENT OF METEORITES PRESENTING AQUEOUS ALTERATION TRACES

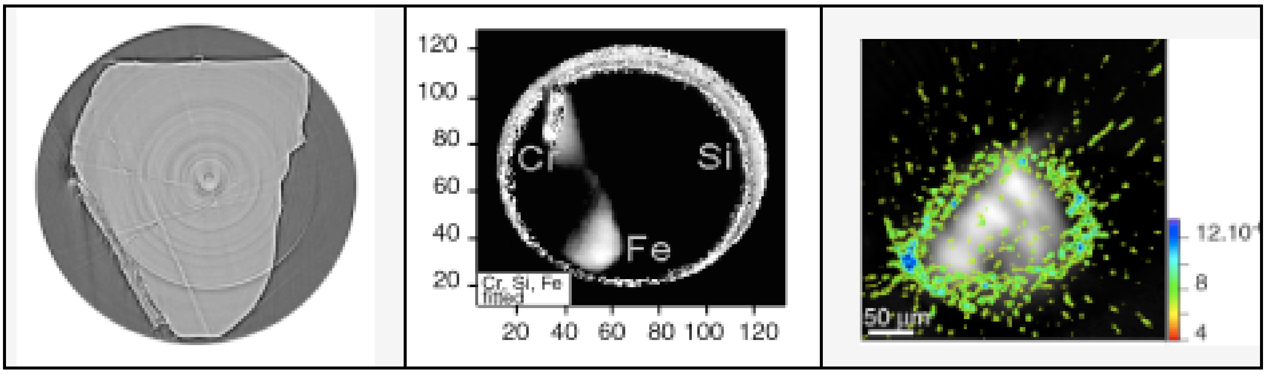
3.1 X-ray absorption tomography

This technique reveals the 3D texture of sub-millimeter silicate grains with a sub-micrometer resolution. The tomogram that can be obtain by 2D superimposed sections as the one shown in figure a, shows the shape of the grain, its exact position and configuration in such container. Two crucial data for the search of life are obtained: (1) the 3D distribution of an internal network of fine fractures (0.33 μm < thickness < 1 μm) and (2) the topology of the contact between silicate and alteration phases (even those poorly contrasting as the thin alteration layer on an NWA817 olivine). Indeed, microorganisms will be looked for preferentially there as they are presumably favorable pathways for water circulation.

3.2 X-ray fluorescence tomography

This technique reveals some bulk chemical images of a sub-millimeter capillary/meteorite assemblage with a micrometer resolution (Figure b). It thus also provides images of the shape and texture of silicate grain having a worth resolution than by X-ray absorption tomography. Semi-quantitative chemical 2D cross-sections were obtained for all the major elements having a Z≥ 40, using a procedure developed by Chukalina et al.. Fitting and background subtraction are performed for all the X-ray lines using an online version of the Axil program. Absorption of the incident beam and of the fluoresced photons in the sub-millimeter assemblage (capillary/silicate grain) are corrected step-by-step with an Algebraic Reconstruction Technique.

This technique provides, thanks to the bacterial P-fluorescence, the accurate 3D distribution of a biofilm (critical thickness < 30 μm) deposited on the surface a 100 μm-thick mica (Figure c). Nevertheless, a special care must be taken to interpret the apparent structures and absolute counts of P-photons observed on the reconstructed image of the biofilm. Indeed, statistic of the P-photons counts is poor as indicated by the radiative P-artefactual structures. In addition calculations of the absorption coefficients show that P-photons should not be detected below a 10 μm-thick layer of mica.



a) b) c)

Figure 1: a: A reconstructed cross-section of an NWA817 grain stored in a pure silica capillary (170 μm-internal diameter, and 45 μm-thick walls) by X-ray aborption tomography. Only the image of the internal content of the capillary is reported here and it was not filtered to conserve maximum resolution. b: Superposition of the cross-sections of a Tatahouine pyroxene grain viewed through a pure silica capillary and reconstructed from the fluorescence of Si (K_α = 1.8 keV) and Fe (K_α = 6.4 keV). The pixel size is 2 x 2 μm^2. Reconstructed tomographic cross-sections of a mica grain covered by a K84-biofilm distributed on the external surface. Image was obtained by the superposition of the cross-sections reconstructed from the fluorescence (backgrounf free) of P (K_α = 2.0 keV) and transmission signal. Cross-sections were calculated with an FBP procedure.