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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** ***X-ray phase contrast and fluorescence analysis of heavy metals in bacterial biofilms***	**Experiment number**: EC-812
Beamline: ID22NI	**Date of experiment**: from: 29/06/2011 to: 04/07/2011	**Date of report**: 12/03/2012
Shifts: 15	**Local contact(s)**: PhD Heikki SUHONEN	*Received at ESRF:*

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Report:

In our experiment “X- ray phase contrast and fluorescence analysis of heavy metals in bacterial biofilms” we investigated the morphology of bacteria and the distribution of different chemical elements inside these organisms.

Soil samples were collected from Inner Mongolia and the natural bacterial population was isolated, purified via separation of soil particles and grown in artificial DEV media containing 378 µg/L Arsenic (AsV). A taxonomic profile was acquired with DNA-based PCR-DGGE fingerprint technique revealing the following composition of the natural population: *Pseudomonas stutzeri strain LC2-8, Pseudomonas sp. CO-44, Saltmarsh clone SCP-79, Filobacillus sp. MO21, Sanguibacter marinus, Cellulosimicrobium sp., Stenotrophomonas sp. J54C16*

As a control sample, a lab strain of *Pseudomonas aeruginosa* was cultivated under high nutrient conditions in DEV medium without the presence of additional Arsenic. Both types of samples were fixed and embedded in epoxy. As previous measurements had shown that heavy metals (Uranium, Osmium) conventionally used as stains in embedding protocols for transmission electron microscopy (TEM) severely influence the X-ray fluorescence signal, an embedding protocol omitting these staining steps was chosen (unpublished). The embedded bacteria were cut in thin sections for further investigation. For the pre-characterization with TEM, a thickness of 50 nm was chosen, whereas for the investigation with X-rays, slices were cut to 1 µm, 2 µm and 5 µm. TEM images (compare fig. 1) confirmed successful sample preparation and revealed – as expected – a wide distribution of size and shape within the natural bacteria population. In total, 10 different samples were prepared. Furthermore, all samples were pre-characterized by optical microscopy (compare fig. 2) in order to facilitate sample alignment during the experiment.

During the beam time at ID22NI, first, the structure of the samples was investigated in 2D by X-ray propagation based phase contrast nano-imaging, then 2D X-ray fluorescence (XRF) analysis at chosen areas was done to correlatively determine the distribution of heavy metals within the sample. On each of the 4 measured samples 6 to 8 areas were chosen to acquire sufficient statistics. Also, a standard sample was measured for calibration of the XRF spectra in order to get quantitative results. For those measurements, 8 shifts were used. The remaining 7 shifts were used for 3D tomographic measurements of the 5 µm thick samples. Both, holotomography as well as XRF tomographic scans were taken of 4 samples, again several areas on each sample were scanned.

Due to the excellent beam conditions and setup at the beamline and the dedicated support of the local contact, the beam could be focused to a spot size of 65 nm (hor) x 60 nm (vert), which allowed XRF imaging with extremely high resolution as it is only possible at very few synchrotrons. The possibility to pre-align the sample by means of a built-in optical microscope objective at the experimental station of ID22NI combined with thorough pre-characterization of the samples severely facilitated sample alignment and thus significantly contributed to an efficient use of beam time.

First results (compare fig. 3) look very promising, as individual bacteria can be distinguished in the XRF maps and it is obvious that the different elements are not homogeneously distributed among different bacteria. In future experiments, we plan to isolate different bacterial strains from the natural population in order to find out, which ones contribute to the processing of Arsenic. Combined with additional micro- and molecular-biological techniques, we thus want to shed more light on the possible pathways of drinking water pollution with heavy metals.

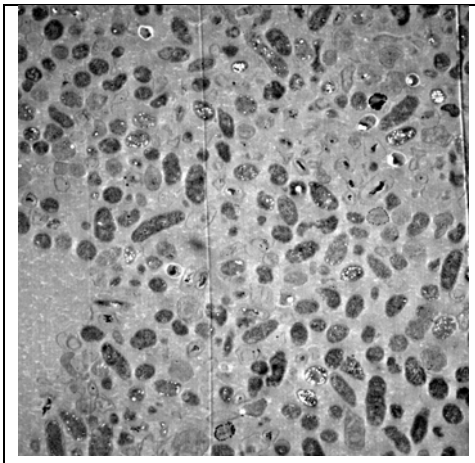

Figure 1: TEM image of the embedded bacteria, demonstating a successful embedding step. Being a natural mixed population, the bacteria show a distribution of size and shape. Magnification 3000x, field of view 14.03x14.03 µm².

Figure 2: Optical microscopy image for pre-characterization of the samples. The borders of the epoxy slice are clearly visible. Phase contrast image, 20x objective, image size 0.43x0.33 mm^2.

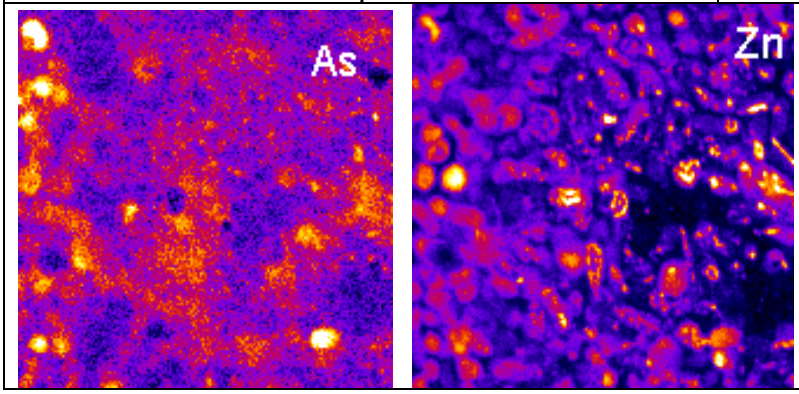

Figure 3: Elemental maps of Arsenic (left) and Zinc (right) in a natural bacterial population. Due to the excellent beam conditions at ID22NI (spot size of 65 nm hor x 60 nm vert), individual bacteria can be detected. Obviously, the different elements are not homogeneously distributed among the different bacteria. Field of view 10x10 $µm^2$, exposure time 0.4 s/pixel.