EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Mapping microbial fiber networks with extraordinary electrical conductivity	**Experiment number**: LS-3032
Beamline:	**Date of experiment**: from: 07/12/2021 to: 10/12/2021	**Date of report**: 17/01/2022
Shifts:	**Local contact(s)**: Dmitry Karpov	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Bent Smets*

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Eric Boschker

Filip Meysman

Gert Nuyts

Koen Janssens

Report:

Conclusion:
Experiment LS-3032 shows that both 2D scans and fluotomograms of cable bacteria can be obtained with high-resolution X-ray fluorescence (µXRF). The electrically conductive network of cable bacteria was successfully imaged at resolutions between 40 and 15 nm. The experiment confirmed our hypothesis that the conductive wires in cable bacteria are enriched in nickel, resulting in a striped pattern in the Ni X-ray fluorescence signal along the bacterial filament. The cell junctions did not display Ni fluorescence, suggesting that the structure in the junction is composed of a different conductive material than the wires. Determining the chemical composition of the conductive material in the cell junction could form the basis for a follow-up proposal.

Introduction:

In previous studies, it was found that the conductive wires in cable bacteria are likely enriched in nickel. However, this could not be confirmed yet due to the inability to analyze the chemical composition of these wires at nm-scale resolution. Hard X-ray µXRF overcomes this shortcoming, while simultaneously providing information on additional elements like Ca, Cu, Fe, K, Mn, P, S, and Zn. Cable bacteria filaments and the electrically conductive wires isolated from cable bacteria were deposited on silicon nitride membranes for 2D and 3D µXRF.

Results:

Preliminary 2D scans were performed to confirm the presence of the bacterial filaments in the thin ice layer on the silicon nitride membrane. These scans were recorded with a lateral resolution of 200 nm and exposure time of 50 ms to create a rough overview of the filament and identify regions of interest for imaging.
Cable bacteria filaments and the isolated conductive wires are relatively resilient to X-ray radiation and do only show signs of damage after using high doses of X-rays. Inspection of the 2D imaged regions with scanning electron microscopy (SEM) after the experiment revealed that regions exposed to X-rays suffered damage on the outer surface of the filament, but the conductive wires remained largely intact (**Fig. 1**). The regions of bacterial filaments investigated by fluotomography displayed more significant damage and deformation in the SEM images.

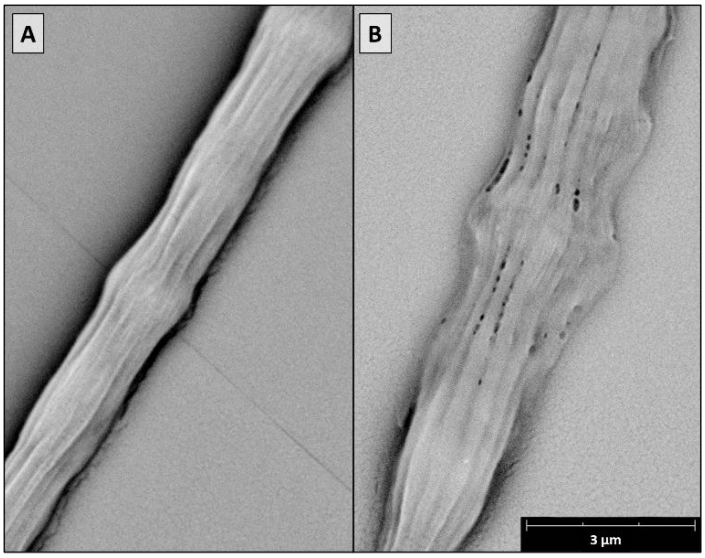

Fig. 1 – Damage in a cable bacteria filament after X-ray radiation and thawing. A) *A region of the cable bacteria filament that was not irradiated.* ***B)*** *A region of the same cable bacteria filament that was irradiated during µXRF. Damage seems limited to the outer surface of the filament.*

In total, **12 2D µXRF scans** and **two fluotomograms** were performed. 2D scans were done at a spatial resolution of 25 or 15 nm. The fluotomograms were composed of 74 projections, recorded at 40 nm resolution. These scans provide information on the distribution of Ca, Cu, Fe, K, Mn, Ni, P, S, and Zn throughout the bacterial filaments.
In the 2D µXRF scans, Ni and Cu display a particular distribution in the cell. The Ni concentration is highest in the conductive wires that run along the length of the filament, resulting in a (green) striped pattern (**Fig. 2A**). The cell junctions, which also contain a conductive structure, do not display high Ni X-ray fluorescence. Instead, the junctions are enriched in copper (red). The S signal was expected to display a similar pattern as Ni, but this pattern is not observed.

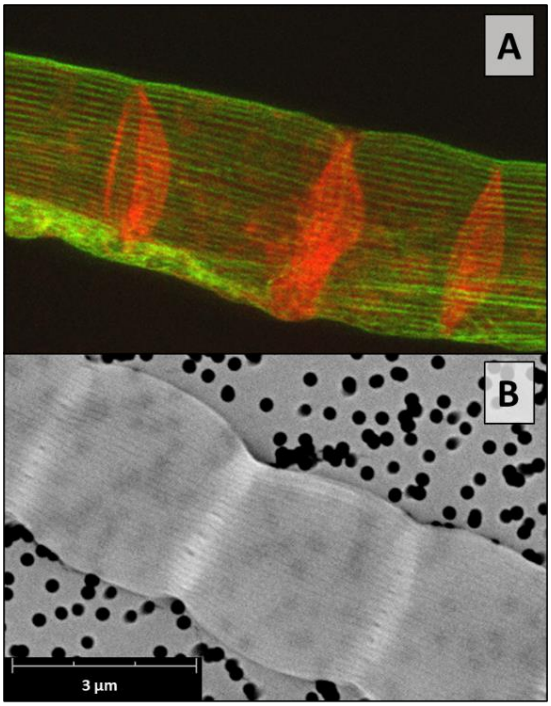

Fig. 2 – 2D map of nickel and copper in the conductive wire network (A) and scanning electron microscopy image of cable bacteria filament for comparison (B). A) *The nickel signal (green) displays an increased intensity in parallel lines along the length of the filament. These lines coincide with the electrically conductive wires visible as ridges on the surface of the cable bacteria filament in the electron microscopy image. The copper signal (red) is concentrated in the cell junctions, where a ring-like structure seems to connect to the conductive wires.*

The fluotomograms are characterised by distortions due to the deformation of the bacterial filaments during the measurement, hampering the reconstruction of the 3D images. However, the striped pattern as a result of the Ni X-ray fluorescence can be vaguely observed in some projections. The structure in the cell junction that is observed in the 2D scans is not visible in the tomograms.

Discussion:

The resilience of cable bacteria to X-rays could be attributed to the conductive wires. These wires are made up of a strong, unknown protein that can endure various chemical treatments and high temperatures. Therefore, damage to the cell seems to be limited to the outer surface. The damage is likely the result of a combination of radiation and thawing of the samples after the experiment.
The striped pattern in the Ni fluorescence signal along the filament confirms that the electrically conductive wires in cable bacteria are enriched in Ni. The cell junctions do not display an increased Ni fluorescence signal, indicating that the conductive material in the junction has different chemical composition than the conductive wires. The Cu X-ray fluorescence signal in the junction suggests that the conductive structure that interconnects the wires is likely composed of copper-containing proteins. The S X-ray fluorescence signal was expected to display a similar pattern since both Ni and S are theorized to be involved in a sulphur-ligated nickel group in the conductive wires. The absence of this pattern is likely the result of the high abundance of S in other proteins and molecules in the cell, contributing to a more diffuse signal across the cell. Removing the cytoplasm and membranes so that only the conductive wires remain could resolve this problem and would likely yield a S fluorescence signal similar to the Ni signal.
Determining the composition of the conductive structure in the cell junction and visualizing it in cross-sections could be investigated in a future proposal.