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*

***Reports supporting requests for additional beam time***

Reports can 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:**<br>The effect of silver nanowire diameter on genotoxicity to human fibroblast cells | **Experiment number**:<br>LS-2549 |
| **Beamline**:<br>ID16A | **Date of experiment**:<br>from: 25 January 2017 to: 28 January 2017 | **Date of report**:<br>24 February 2017 |
| **Shifts:**<br>9 | **Local contact(s)**:<br>Muriel Salome and Peter Cloetens | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

Sylvia Lehmann*, University of Grenoble–Alpes

Laurent Charlet*, University of Grenoble–Alpes

Ana Elena Prada*, ESRF and University of Grenoble–Alpes

Sylvain Bohic, ESRF

Benjamin Gilbert*, Lawrence Berkeley National Laboratory


**Report:** *This is a **Preliminary Report** because data analysis is ongoing.*
*This is a **Confidential Report** because none of the data are published.*

We accomplished all of the goals described in our beamtime proposal. The results described below are exciting new observations of nanowire toxicity. In combination with associated beamtime on ID21, and with numerous complementary laboratory studies of cytotoxicity and genotoxicity, we anticipate this work will generate a very good publication that we have begun to work towards.

***Experimental Approach***

We studied mouse fibroblast cells grown on a silicon nitride membrane and exposed to a low dose of silver nanowires (Ag NWs) for 24 hours. We studied Ag NW of similar mean length (9 μm) and two diameters (30 nm and 90 nm) to test for differences in internalization and fate. The cells were washed, rapidly frozen and studied under cryogenic conditions. We performed X-ray fluorescence mapping at 33 keV of exposed and control cells to identify individual cells containing NWs. We acquired phase contrast microscope images at two incident angles to construct stereogram images of cells containing Ag NW to verify that the NWs had been internalized. We acquired a full tomographic tilt series for a single cell to

***Major Findings***

All internalized Ag NW were morphologically altered relative to their shape in water or cell culture medium. The 90-nm NW were bent while the 30-nm NW were completely crumpled and looped. These observations show that the NWs experience significant forces during internalization that we will seek to estimate. The different in shapes could partially explain our prior findings of differential toxicity for the two NW sizes.

None of the Ag NW penetrated the cell nucleus. Thus, although we observed single- and double-strand DNA damage, the mechanism must be through an indirect chemical process.

A fraction of internalized Ag NW were co-localized with physiological elements, while a portion showed none. Based on prior fluorescence microscopy studies, we speculate that the a fraction had ruptured the endosome membrane and entered the cytoplasm.

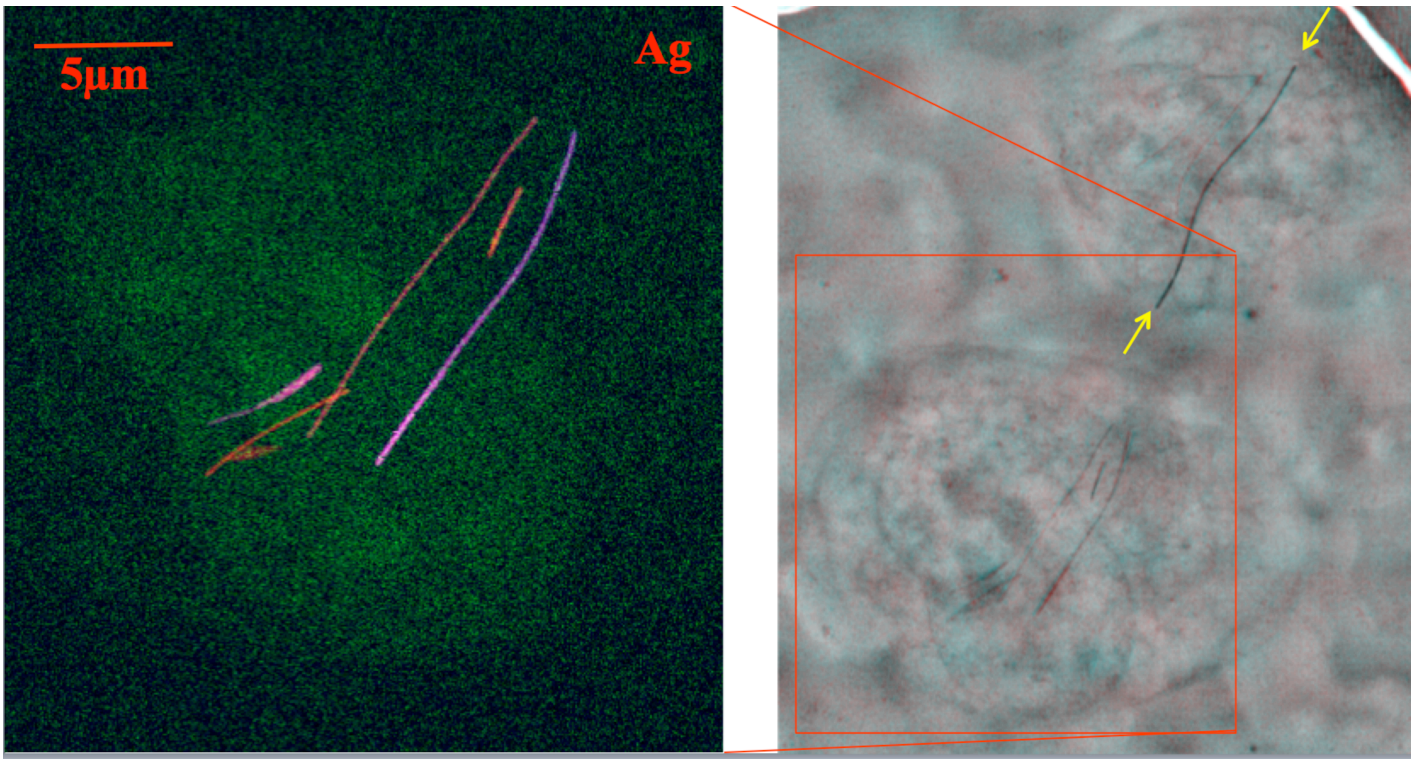


**Figure 1** X-ray fluorescence mapping and stereographic phase contrast imaging of mouse fibroblast cells containing internalized silver nanowires with mean diameter of 90 nm and length 9 µm.