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:** Uptake of Co nanoparticles and distribution in C. elegans following exposure experiments	**Experiment number**: EV71
Beamline: ID16A	**Date of experiment**: from: 18/6/2014 to: 24/6/2014	**Date of report**: 15/08/2015
Shifts: 18	**Local contact(s)**: Remi Tucoulou Tachoueres, Alexandra Pacureanu	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Simone Cagno* Norwegian University of Life Sciences
Ole Christian Lind*, Norwegian University of Life Sciences
Gert Nuyts*, University of Antwerp
Frederik Vanmeert*, University of Antwerp
Dag Anders Brede, Norwegian University of Life Sciences
Koen Janssens, University of Antwerp
Brit Salbu, Norwegian University of Life Sciences

Report:

In this experiment we have investigated the speciation and internal distribution of engineered nanomaterials, and in particular cobalt nanoparticles, within exposed *Caenorhabditis elegans* (*C. elegans*) by utilizing the nanotomography and nano-XRF set-up at ID16A.

Caenorhabditis elegans is a model organism for exposure experiments because it is facile, has a short life cycle and is easy to propagate in large populations. Being transparent, it allows optimal observation of its internal organs. The small size (~0.2 mm x 1 mm), make nematodes ideal specimen for high resolution imaging of entire individuals and their organs

The nematodes used for this study were exposed to Co nanoparticles in standardized soil pore water for 96 hours and subsequently preserved fixed in paraformaldehyde/glutaraldehyde and dehydrated using ethanol and hexamethyldisilazane (HMDS). Comparison with non exposed individuals was performed as well.

The experiment has followed the following schedule: first, phase contrast CT and 2D-have been performed on entire individuals and target organs of C. elegans in order to characterize the distribution of Co in the nematodes and relate it with the morphology; in a second phase 3D nano-XRF has been performed on the most relevant areas (indicated by the previous step) in order to prove/disprove effective uptake of Co-nanoparticle, i.e. presence of univocally identified Co-particles within the inner boundary of target organs.

The nano-CT and nano-XRF performed on exposed *C. elegans* yielded unprecedented high quality images showing the inner organs of the nematode and the extent of the Co distribution. Co was univocally proven to be inside the nematode. An example of the images obtained in experiment EV71 is shown in Fig 1; an explanatory light microscope picture is shown in Fig. 2.

The image in Fig.1 was created by phase contrast absorption X-ray nanotomographic imaging of a preserved nematode mounted inside a quartz capillary (diameter 0.1 mm) at ID16A Nano Imaging. Beamsize was 20 nm x 37 nm and the X-ray energy 17 keV.

What we see in Fig.1 is two embryos in the uterus of an adult hermaphrodite *C. elegans* nematode. Behind the embryos we can see abundant Co nanoparticles in the intestine of the nematode. This level of detail can only be obtained at nano-imaging beamlines. The presence of Co was confirmed by X-ray fluorescence tomography performed at the same location.

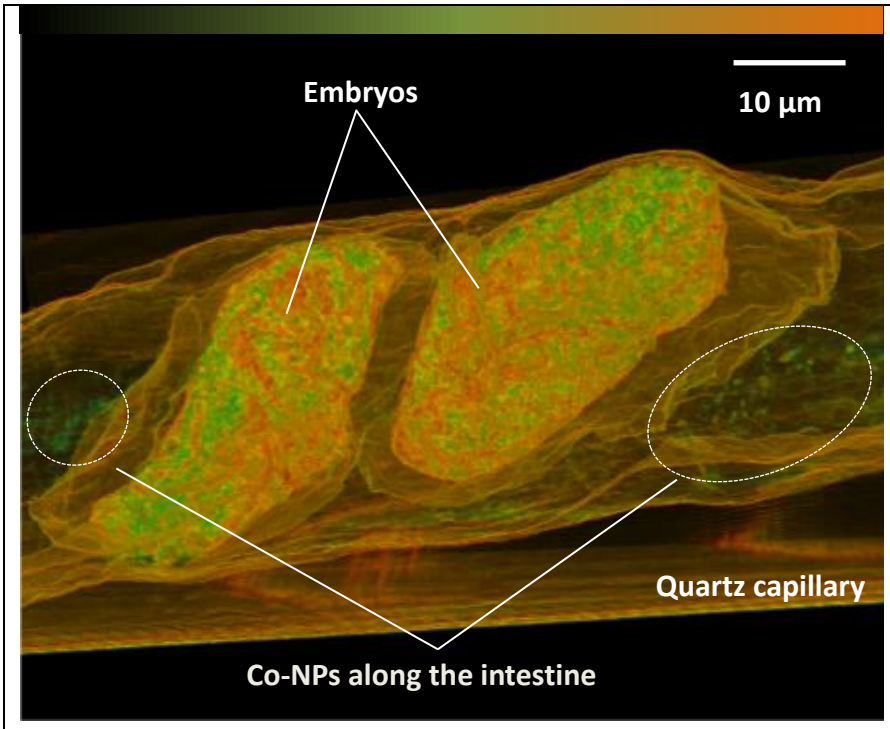

Fig. 1. Phase contrast CT 3D rendering of a segment of nematode showing Co particle aggregates in the intestine, next to the embryos

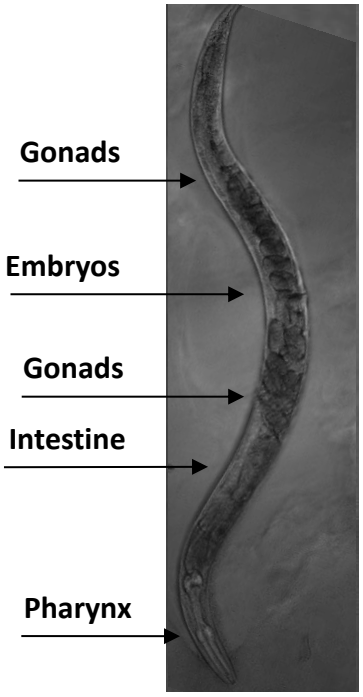

Fig. 2. Light microscope image of a *C. elegans* individual

The results of EV71 have demonstrated the enormous potential for studying the fate of nanoparticles in small model organisms such as *C. elegans* that can be performed at beamline ID16A-NI. The present results will serve as an important input to nanotoxicology. Moreover, samples have been produced that could be tested in the near future to determine the crystallographic/molecular/oxidation state information pre-andpost-uptake by means of micro XANES and XRD.

This study will be fully described in Brede et al. (2015), *State-of-the art nanoimaging of Co nanoparticle distribution within exposed Caenorhabditis elegans*, in preparation. Moreover, we are proud to report that these results were selected for a Beauty of Science item on ESRF News, July 2015, no. 70.