EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 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:** High-resolution fluorescence and phase-contrast imaging of tissue samples	**Experiment number**: MI-1029
Beamline:	**Date of experiment**: from: 16 Apr 2010 to: 22 Apr 2010	**Date of report**: 02/03/16
Shifts:	**Local contact(s)**: H. Suhonen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

H. Suhonen*

F. Xu*

L. Helfen*

P. Cloetens*

Report:

We have developed a technique for correlative 3D imaging of both structure and elemental composition for extended objects and applied to visualize the subcellular response in rat lung tissue to toxic stress after exposure of the lungs to nanotubes.

Nanoscale electron density and elemental composition laminography was implemented at the nano-imaging endstation ID22NI of the European Synchrotron Radiation Facility (ESRF). A Kirkpatrick-Baez mirror system was used to focus x-rays of 17 keV energy to a focal spot of 47 (V) × 82 (H) nm^2 (FWHM) with a flux of more than 10^{12} photons/s in the focus. To get laminographic images, we set the sample rotation axis at a 60° inclination with respect to the incoming beam. For electron density imaging, the x-ray focus was used as a virtual source, and magnified images of the sample (at 60 nm pixel size) were obtained with x-ray projection microscopy. Phase retrieval was then performed on the images with the contrast transfer function method [1] to get the x-ray phase modulation due to the sample. The lateral field of view was 90 × 90 μm^2 with the thickness of the sample that could be imaged being approximately equal to the lateral field of view. The elemental composition was imaged with the sample at the x-ray focus. An energy sensitive detector, placed at 90° from the incident beam direction, was used for detecting the characteristic fluorescence photons. The horizontal rotation axis ensured a minimised path of the emitted photons inside the specimen, reducing self-absorption effects. Scans of 100 × 100 points with a step size of 0.5 μm were repeated acquired for 64 rotation angles.

Rats were anesthetized and were intratracheally instilled with SWCNT dispersed with bovine serum albumin (BSA) as previously described [2]. Seven days later the rats were sacrificed. Tissue specimens from the lungs were fixed in 10% formaldehyde and processed routinely for embedding in paraffin. Slices of 40 μm thickness and 3×3 mm^2 cross section were cut from the paraffin blocks and were used for 3D imaging.

The main results are shown in Fig. 1 and presented according in our principal publication [3] where more information can be found.

A lung specimen was first screened by a low resolution phase contrast imaging (Fig. 1a) and coarse resolution 2D fluorescence scan over the area of about 0.2 × 0.3 mm^2 with step size of 1.8 μm (Fig. 1b). Areas with hotspots of iron were apparent in the fluorescence image, and we zoomed into one of these areas for high resolution imaging. A fluorescence data set with step size of 500 nm, and a phase contrast data set with pixel size of 60 nm were recorded in this ROI for laminographic 3D reconstruction.
Two slices were chosen for closer inspection as illustrated in Fig. 1c. In Fig. 1d, 1e and 1f slice #1 from the lung specimen is seen to contain an excess of iron inside cellular structures. Based on the structural images, the size (about 12 μm), location (close to alveolar surface), and shape of the nucleus (single, round) indicate that the cells in question are alveolar macrophages (AM). The fact that excess iron is localized inside some of the AMs indicates the presence of nanotubes within the AMs, confirming the previous finding from electron microscopy that AMs are able to phagocyte most of the nanotubes from the lungs. Figures 1g, 1h and 1i show slice #2 with a part of an alveolar wall structure, showing hotspots of iron inside cellular structures of the wall. Based on the shape of the cells, they can be identified as type-1 pneumocytes and one AM. The iron concentration is more than a factor of 10 lower than in the AMs of slice #1, but still higher than in the surrounding tissue. Although the iron could be endogeneous, comparing the different cells in the lung wall shows that some have a much higher quantity of iron than others. The images suggest for the first time on an *in vivo* experiment that nanotubes have entered in small amounts inside some of the pneumocytes.

Nanolaminography thus allowed focusing on a region of the lung that was found to be highly different from the surrounding tissue based on the low resolution fluorescence scan. This approach brings benefits to biology and other applications where the specimen's heterogeneity makes a single ROI unrepresentative of the entire specimen, and where the interesting regions may be far apart from each other.

References

1. Cloetens, P. *et al.* "Holotomography: Quantitative phase tomography with micrometer resolution using hard synchrotron radiation x rays". *Appl. Phys. Lett*. **75**, 2912 (1999).
2. Elgrabli, D. *et al.* "Effect of BSA on carbon nanotube dispersion for in vivo and in vitro studies". *Nanotoxicology* **1**(4), 266–278 (2007).
3. Feng Xu, Lukas Helfen, Heikki Suhonen, Dan Elgrabli, Sam Bayat, Péter Reischig, Tilo Baumbach and Peter Cloetens, "Correlative Nanoscale 3D Imaging of Electron Density and Elemental Composition in Extended Objects", *PloS ONE* **7**(11), e50124 (2012)

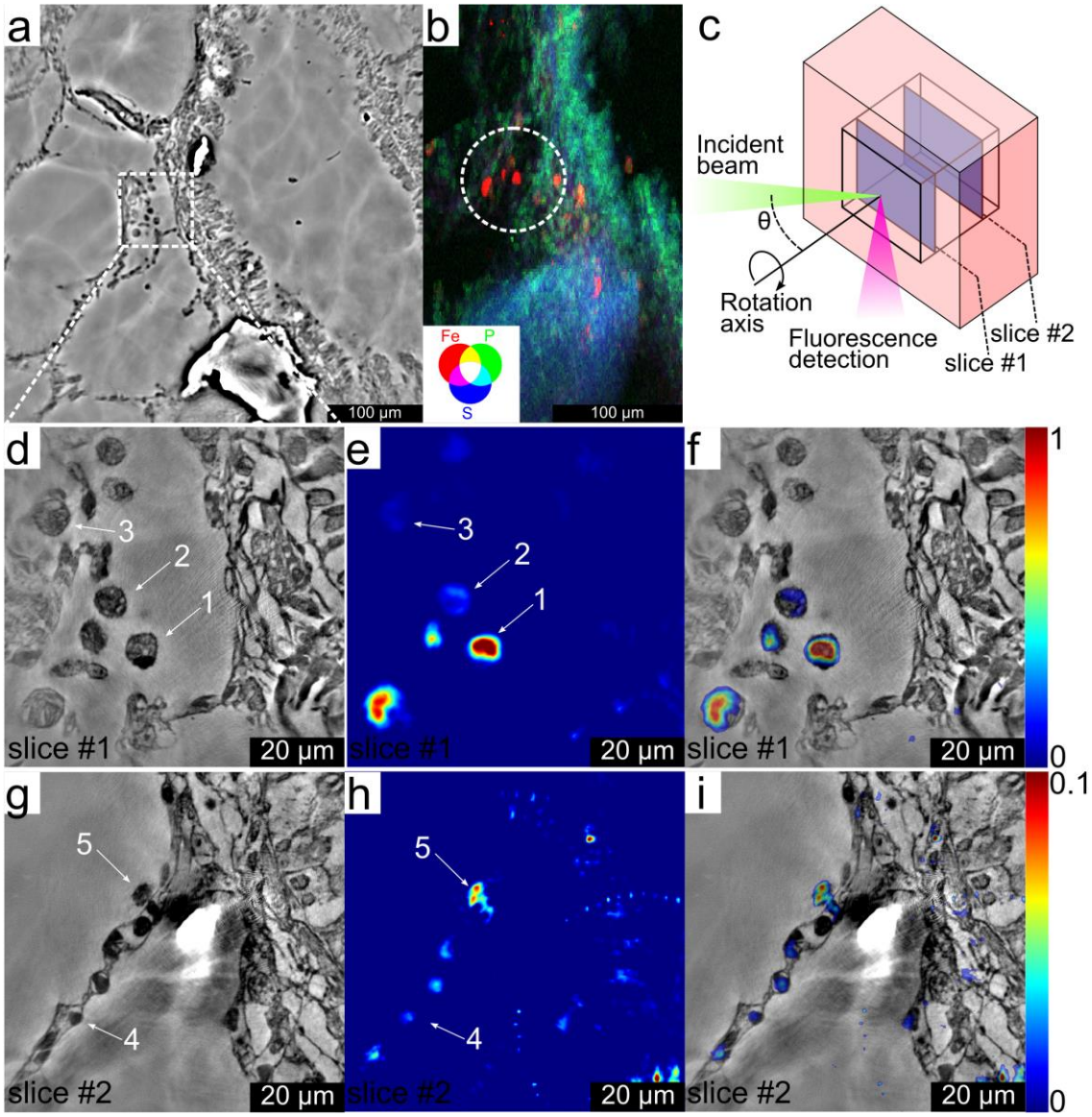

Figure 1: Imaging of carbon nanotube contamination in a lung tissue. a, Slice from a low resolution electron density volume. b, Low resolution 2D fluorescence image of the specimen showing iron, phosphorus and sulfur distributions. The circle indicates the ROI that was chosen for higher resolution imaging. c, A schematic representation of the imaging geometry, showing the ROI inside the sample and the two slices that were chosen for further analysis. Phase contrast (d), Fe fluorescence signal (e) and correlative image of Fe and phase contrast (f) for slice #1 in the lung specimen. Numbers 1, 2, and 3 indicate positions of alveolar macrophages. Phase contrast (g), Fe fluorescence signal (h) and correlative image of Fe and phase contrast (i) for slice #2 in the lung specimen. Number 4 indicates the position of a type 1 pneumocyte, while number 5 indicates the position of an alveolar macrophage. Notice the factor of 10 difference in the relative fluorescence signal between parts (e, f) and (h, i).