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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 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:** Monitoring minocycline therapy in stroke using combined high resolution magnetic resonance and synchrotron radiation (SR) micro-CT imaging of the mouse brain	**Experiment number**: MD-499
Beamline:	**Date of experiment**: from: 01 July 2010 to: 17 December 2010	**Date of report**: March 6, 2012
Shifts: 36	**Local contact(s)**: Paul Tafforeau, Cécile Olivier, Elodie Boller, Alexander Rack	*Received at ESRF:*

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Report:

Introduction

MRI coupled with the injection of Ultrasmall particles of iron oxide (USPIO) has been successfully applied for pre-clinical and clinical studies of cerebral inflammation following stroke.[1, 2] Current limitations of this approach are represented by the difficulty of interpreting MR signal changes in terms of exact localization and quantification of USPIO. To overcome this limitation, Synchrotron Radiation phase micro computerized tomography (SR-PCT) was proposed as a new method to visualize ultrasmall superparamagnetic particles of iron oxide (USPIO) distribution into the whole brains of mice.

Materials and Methodes

All experiments using synchrotron radiation were performed on beam line ID19 at the ESRF.

The following samples were imaged : i) USPIO phantoms with concentrations in the range 0,15 nM – 1500 µM ii) 10 post-mortem brains with intracerebral injections of iron concentrations [15-1,500] µmol Fe/l; iii) 6 post-mortem brains with intracerebral injections of USPIO-labeled cells and iv) 8 post-mortem brains of mice having received intravenous injection of USPIO after induction of experimental stroke.

- *Setup and tomographic reconstruction for interferometry technique (N=1 brain)*

Imaging was performed with 23.5 keV as described elsewhere.[3] Briefly, the pixel size was set to 7.5 µm. Mouse brains were placed in a 5 cm tube filled with sucrose 30% and positioned on a rotation adapted support immersed in an aquarium filled with water. X-ray radiographs were taken and processed using the phase stepping technique, with 4 phase steps per view and an exposure time of 5 s per step. Tomographic data were acquired with 1500 projection angles over an interval of 360°. The tomography data were reconstructed by integrating the projection images line by line and then performing a standard tomographic reconstruction, with a normal filtered back projection (FBP) algorithm.

- *Setup and tomographic reconstruction for in-line phase µCT (N=18 brains)*

Specimens were glued on stands adapted to the rotation stage. Imaging was performed with 17.6 keV selected from undulator radiation using Al filters. The X-ray beam transmitted through the specimen was acquired on a detector using a LuAg scintillator screen, visible light optics and a 2048x2048 CCD detector. The pixel size was set to 8µm, which provides a field of view of 16 mm^3. The detector was positioned at one meter from the sample for the in-line phase contrast imaging. For each specimen, two adjacent scans were acquired in the vertical direction with an overlap of 0.8 mm. Finally, by merging these two data sets, a total reconstructed volume representing 15x15x20 mm^3 was obtained for each specimen. For each scan, 1999 radiographs were taken at different angles evenly distributed between 0 and 360 degrees. Finally, the Filtered Back Projection algorithm[4] was applied to obtain a reconstructed 3D volume, i.e. a stack of 2048 slices of 2048x2048. Note that since the voxel is isotropic, the slice thickness is equal to the pixel size, i.e. 8 µm.

Phase retrieval was performed from a single phase contrast image at each projection angle using Paganin's method [4]. The d/b (delta/beta)-ratio was set to 321 to correspond to the highest concentration of iron particles used in the study. This ratio was calculated using the XPOWER application in the XOP software [5]. The algorithm is implemented in-house at the ID19 beamline at the ESRF to run on graphical processing units (GPU). Images were quantized using the same interval [0.75 -1.05] for all the samples.

Absorption X-ray µCT was employed to image one sample. The same setup as the one for the phase contrast SR-µCT was used, the only difference was the samples distance from the camera (19 mm).

Results

In vitro, the relationship between iron concentrations and absorption coefficients was linear for concentrations superior to 10 µM.

Absorption X-ray grating interferometry allowed detection of USPIOs as a hyperintense signal (Figure 1a). However the image showed only marginal contrast and no brain structure could be identified. Phase contrast grating interferometry (performed on the same sample) provided exquisite brain structure depiction as well as excellent USPIO detection, but with a very long acquisition times (6h) (Figure 1b). Absorption X-ray µCT of the sample did not provide a satisfactory image neither of soft tissue nor of the contrast agent (data not shown). SR-PCT allowed both USPIO detection and identification of brain structures such as ventricles, corpus callosum, and hippocampus layers (Figure 1c) in a reasonable amount of time (40 minutes/brain) and was therefore used to image the rest of the samples.

In stereotaxicallly injected brains, SR-PCT was able to detect hyperintense signals in all mice, while allowing an accurate localization in the brain compared to T2-weighted MRI (Figure 2). Visual examination of the brain samples injected with the USPIO-labeled cells showed that all cell quantities, including the smallest, could also be detected. In stroke animals, SR-PCT provided exquisite anatomical details compared to immunohistologic slices, allowing to identify both healthy and pathological brain structures such as the ischemic lesion (Figure 3). Visualization of bright spots in the ischemic lesions co-localized with hypointense signals detected by MRI (Figure 3B2). Publication of these results is in progress.

Conclusions

Microtomography with SR-PCT showed a good sensitivity to USPIO detection, while allowing an accurate localization. Further analysis are warranted to investigate quantitative performances of the approach. In conclusion, microtomography with SR-PCT represents a promising tool for future preclinical studies of neuroinflammation, as a complement to USPIO-enhanced MRI.

References

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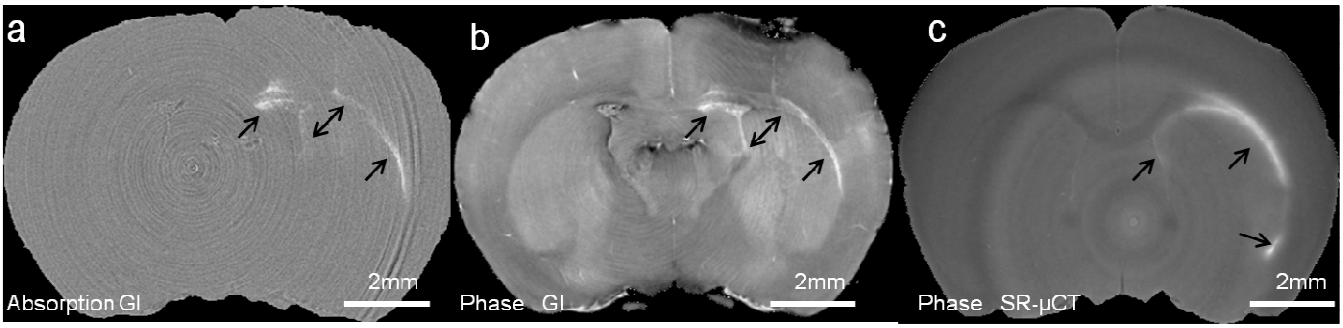

Figure 1. Examples of a mouse brain stereotaxically injected with USPIO. X-ray grating interferometry : (a) Absorption and (b) phase contrast (b) ; In-line phase μCT : (c) Phase contras. Arrows show the signals induced by USPIO

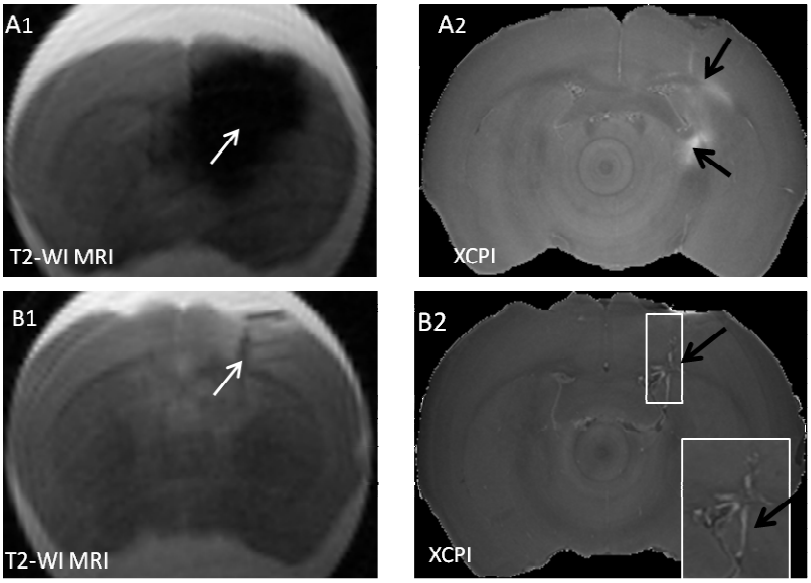

Figure 2. T2-weighted MRI (A1, B1) and SR-μCT (A2, B2) imaging of mouse brains stereotaxically injected with different concentrations of USPIO (A: 1,500, B: 15μM) Arrows show the signals induced by USPIOs. Insert: magnification of box.

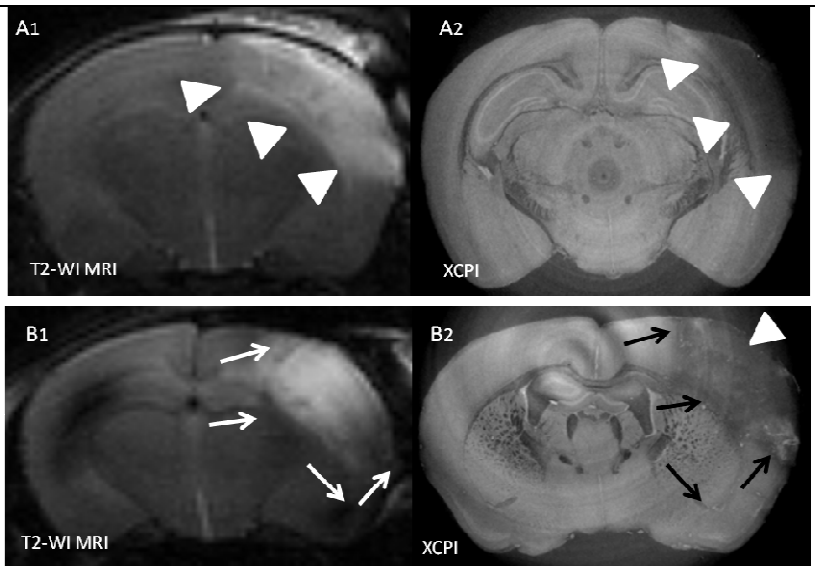

Figure 3. T2-weighted MRI (A1, B1) and SR-μCT (A2, B2) of stroke-induced mouse brains without (upper row) and with USPIO (lower row). Arrowheads show the ischemic lesions and arrows show the signals induced by USPIOs