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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:** Tracking therapeutic cells using k-edge imaging in a murine stroke model	**Experiment number**: MD1011
Beamline: ID17	**Date of experiment**: from: December 2016 to: June 2017	**Date of report**: 26/09/17
Shifts: 12	**Local contact(s)**: Herwig Requardt	*Received at ESRF:*

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Report:

Study #1- Non-invasive imaging of transplanted therapeutic cells in the inflamed rat brain
This work was accepted for an oral presentation at RSNA 2017 (http://www.rsna.org/Annual-Meeting/).

Introduction:
Cell therapy holds promise for treatment of ischemic stroke in the chronic phase. To foster translation into the clinic, there is a need for non-invasive techniques that enable long-term follow-up of cell fate. Our purpose is to provide proof of concept of specific and quantitative in vivo imaging of therapeutic cells by an innovative, translational technique: K-edge imaging by spectral photon counting computed tomography (SPCCT). K-edge imaging with Synchrotron-produced x-rays (SXR) will serve us as a gold standard for validation of the recently developped SPCCT approach (project supported by a H2020 grant in which we are partners, http://www.spcct.eu/).

Materials & Methods:
M2-polarized anti-inflammatory macrophages were labelled with gold nanoparticles (AuNPs, 0.1 mg/mL, 15 h) and gold/cell quantified by mass spectroscopy. Gold-labelled cells were intracerebrally (IC) delivered within PBS (n=6) or a hydrogel (n=9) in a rat model of neuroinflammation, induced by IC injection of lipopolysaccharide (LPS) two weeks before treatment delivery. Other animals were injected with of AuNPs solution alone (n=1), suspended in PBS (N=1), or suspended in hydrogel (N=1). Control animals of this study included IC injection of unlabelled cell suspended in hydrogel (n=1), unlabelled cells alone (n=1), hydrogel alone (n=1), PBS (n=1), LPS injection only (n=1) and sham injection (n=1). Animals were scanned *in vivo* by SPCCT between the same day or the day after injection and then euthanized, and their heads prepared to be scanned *ex-vivo* by SXR. A subgroup of animals (i.e. gold-labelled cells in hydrogel, n=3; gold-labelled cells in PBS, n=4) were followed up *in vivo* 5 to 6 days post-injection by SPCCT and 9 to 10 days post-injection by SXR. A phantom with a concentration range of AuNPs (0 to 8 mg/mL) was scanned for calibration of

quantification with both techniques. For SPCCT acquisitions, anode tube voltage of 120 kVp and current of 100 mA were used. For SXR acquisitions, the imaging set-up at ID17 consisted of the fixed-exit monochromator coupled with the frelon camera (45 µm spatial resolution, for ex-vivo samples) or the PCO-edge camera (21 µm spatial resolution, for *in vivo* imaging). To perform K-edge imaging of gold with SXR, an absorption image is first acquired just below the binding energy of the K shell electrons (79.7 keV) and then just above the binding energy of the electrons (81.7 keV). The subtraction of these two acquisitions produces a specific image of the element. Gold K-edge images were reconstructed and gold signal was manually delineated for quantification.

Results:

K-edge imaging of gold with SPCCT specifically detected gold-labelled cells within the brain (Figure 1). Mass spectroscopy detected an amount of 165 gold pg/cell, corresponding to 82.5 µg of gold injected within cells. The measured concentrations in phantom linearly correlated with the known concentrations (R^2 = 0.99, slope: 0.82, intercept: 0.19), supporting the potential for accurate gold-cell quantification. In agreement, gold-labelled cells were also specifically detected by SXR (Figure 2). The analysis of signals volumes and contrast agents concentration for these imaging techniques are ongoing, and will be compared to the contrast agents quantification on tissue brains samples by mass spectrometry (ICP-MS).

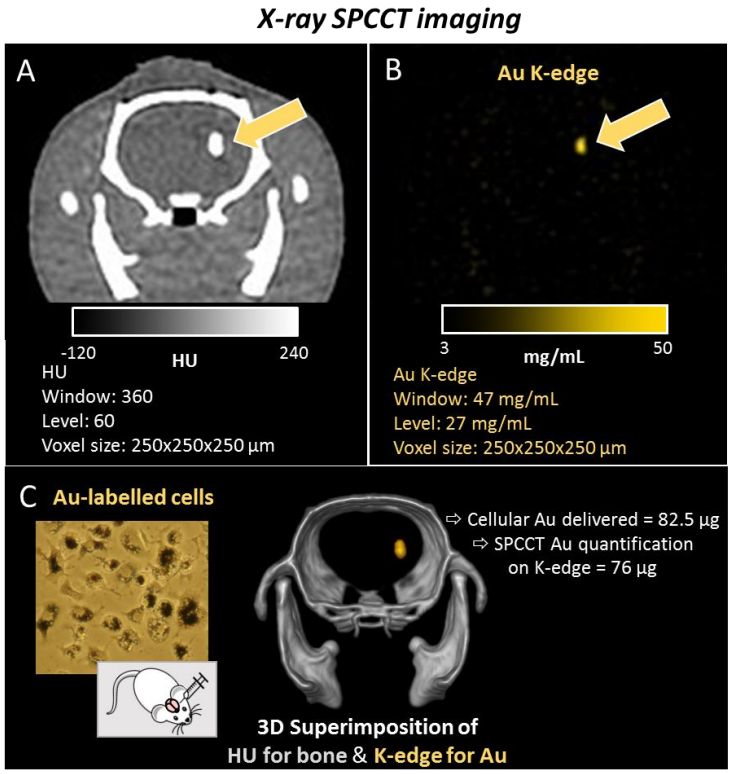

Figure 1: SPCCT imaging of therapeutic cells delivered in a rat brain. Au-labelled cells are visible in attenuation (HU, **A**) and Au K-edge (**B**) images, as indicated by the yellow arrows; images are reconstructed for the same axial acquisition. **C**. On the right, picture showing cells labelled with Au nanoparticles, visible in their cytoplasm; in the middle, 3D rendering for bone (HU, grey) and Au-cells (K-edge, yellow) for the same rat; on the left, cellular Au amount delivered and the amount detected and quantified on Au K-edge are indicated. SPCCT, Spectral photon counting computed tomography; HU: Hounsfield units; Au: gold.

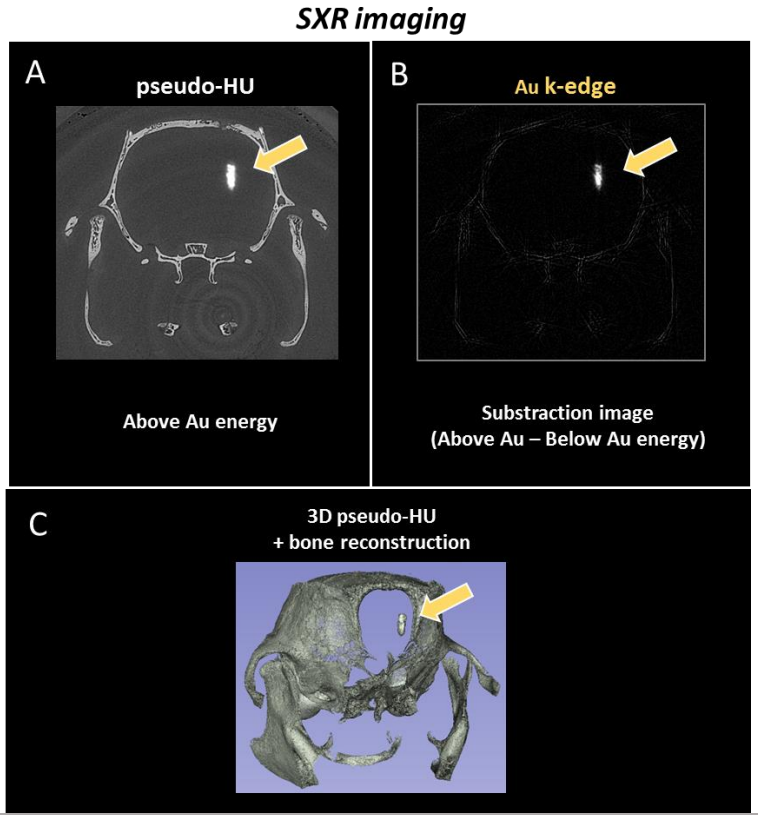

Figure 2: SXR imaging of therapeutic cells delivered in a rat brain. SXR ex-vivo imaging performed on ID17 for the fixed head of the same rat shown Figure 1. Au-labelled cells are visible and indicated by the yellow arrows. Image acquired just above the k-edge energy of Au (**A**), and the corresponding Au-specific k-edge image (**B**, substraction between the image acquired just above and that just below the Au k-edge energy) are shown. **C**. 3D rendering for the same head, where the Au-labelled cells and bone are shown. HU: Hounsfield Unit; Au: gold; SXR: synchrotron-produced x-rays.

Conclusions & Perspectives:

Our initial results provide proof-of-concept for non-invasive *in vivo* imaging of gold-labelled therapeutic cells by SPCCT, which has the advantage of being specific and quantitative. *In vivo* k-edge imaging of gold-labelled cells was also successfully performed with SXR for the first time to the best of our knowledge. SPCCT may be valuable to evaluate cell therapy in chronic stroke patients.

Aknowledgements:

This work was performed within the frame of the H2020 SPCCT project (http://www.spcct.eu/) and was supported by the European Synchrotron Radiation Facility (ESRF, project MD1011) by allocation of beamtime.

References:

1. Cuccione E, Chhour P, Hubert V, Sbaa R, Bar-Ness D, Brun E, Reymond S, Vandamme M, Crola Da Silva C, Elleaume H, Chereul E, Nighoghossian N, Berthezène Y, Cormode DP, Wiart M. Non-invasive imaging of transplanted therapeutic cells in the inflamed rat brain by spectral photon counting computed tomography (SPCCT). Abstract, Oral communication, RSNA 2017, Nov 26th – Dec 1st, Chicago (IL).