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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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: Anatomy of Central Nervous System Immune Privilege**	**Experiment number**: MD-1230
Beamline: ID17	**Date of experiment**: from: 10 Dec 2020 to: 12 Dec 2020	**Date of report**: 14 Jan 2022
Shifts: 6	**Local contact(s)**: Alberto Bravin	*Received at ESRF:*

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Dr. Alberto Bravin

Report:

Three experimental sessions were scheduled for this experiment for use of the beam:

- **Experimental Session 1 (10-12 Dec 2020): Remote access post-mortem imaging**
- Experimental Session 2 (12-13 Oct 2021): Pilot in vivo experiment
- Experimental Session 3 (18-20 Nov 2021): Full in vivo experiment

Experimental Session 1 (10-12 Dec 2020): Remote access post-mortem imaging

On-site user operation or use of the animal facilities was not possible in December 2020 due to the COVID-19 situation. Instead, fixated post-mortem samples were submitted to ID17 and handled by beamline staff (Alberto Bravin, Michael Krisch) while staying in communication with the user group via Zoom. Scans were started by beamline staff or remotely by users via Guacamole. The purpose of this experiment was to:

1. Narrow down the selection of six available types of X-ray contrast agents for *in vivo* experiments
2. Investigate the difference in results between injection into the ventricle or cisterna magna
3. Evaluate the need for K-edge subtraction for the final results
4. Identify technical issues

Due to considerations of possible leakage of contrast agent out of the CSF spaces, high molecular weight (high MW) and nanoparticle-based blood pool contrast agents were evaluated along with standard low molecular weight (low MW) angiography contrast agents (Table 1).

Table 1: Overview over the six tested X-ray contrast agents

Contrast agent name	**Type of compound**	**Radiopaque element**	**K-edge**
Accupaque	Low MW organic molecule	Iodine	33.2 keV
ExiTron P	High MW organic molecule	Iodine	33.2 keV
Gadodiamide	Low MW chelate complex	Gadolinium	50.2 keV
GadoSpin P	High MW chelate complex	Gadolinium	50.2 keV
ExiTron nano 12000	Nanoparticles with hydrophilic coating	Barium	37.4 keV
Aurovist 15 nm	Nanoparticles with hydrophilic coating	Gold	80.7 keV

Each of these contrast agents were injected into the mouse cerebrospinal fluid (CSF) space, either into a lateral ventricle or into the cisterna magna. 12 mouse cadavers covering all these combinations plus 12 duplicates as a reserve were submitted to the beamline, as well as 3 cadavers without contrast agent injections for comparison.

These were mounted into the first iteration of the user-designed, additively manufactured mouse holder for *in vivo* skull measurements. This holder was designed for correct positioning of the skull to minimize bone density in beam direction, minimal number of holder parts in the field-of-view, full plastic construction, mechanical skull fixation and body heating (Fig 1A). Mouse skulls were imaged with monochromatic beam at photon energies above and below the absorption edge of the respective X-ray contrast agent. 4000 projections were recorded per scan with 100 ms exposure time at 3.1 µm voxel size, using a half-acquisition scheme with asymmetric rotation axis to extend the field of view of the 2560 pixel wide pco.edge 5.5 camera. The resulting scans allowed us to determine optimal parameters for the *in vivo* experiment:

1. ExiTron nano 12000, the barium nanoparticle-based X-ray contrast agent had the most even distribution throughout the CSF space and provided sufficient contrast for segmentation (Fig. 1B, C).
2. Contrast agents injected via the cisterna magna had difficulties reaching the ventricular spaces, while ventricular injections showed good distribution throughout the entire CSF space.
3. ExiTron nano 12000 showed sufficient contrast in scans above the K-edge that it was possible to automatically segment CSF spaces separately from the skull. K-edge subtraction imaging was therefore not required to achieve the study goals, simplifying further experiments.
4. Necessary design changes for the mouse holder were identified. Placing of teeth and ear bars without breaking parts was difficult for newly trained personnel, increasing time for mounting, which would be problematic for the *in vivo* experiment. An increase in robustness, tightness of fit and speed of mounting an animal will be the focus of the revision.

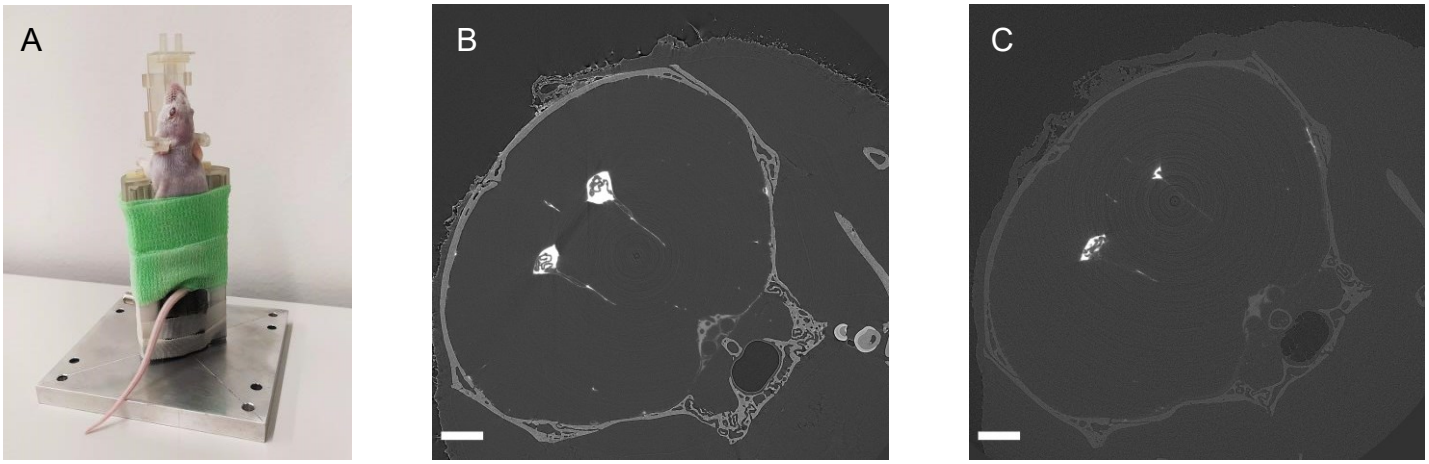

Figure 1A: Photograph of the custom-designed mouse holder used for this session. 1B: Single slice showing mouse skull and CSF spaces injected with barium nanoparticles. Both lateral ventricles are clearly visible. 1C: Mouse skull injected with gold nanoparticles. While higher contrast can be observed compared to the skull, as expected of the higher photon energy, lateral ventricles do not appear fully filled. Scale bars: 1 mm.