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EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Quantification of the effects of Microbeam Radiation Therapy on brain tumor neoplasm tissue and vascular network by phase contrast microCT.	**Experiment number**: MD1037
Beamline: **Shifts:6 + 9**	**Date of experiment**: from: 27/01/18 to: 28/01/18 from: 19/04/18 to: 21/04/18 **Local contact(s)**: Alberto Bravin, Herwig Requardt, Alberto Mittone	**Date of report**: 27/02/2020 *Received at ESRF:*

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Report:

The overall aim of this research project was to investigate the potential of Phase Contrast micro-CT (PCI-microCT) as a tool to perform multiscale, quantitative comparisons of the treatment effects on brain tissue between two different experimental radiotherapy techniques, namely X-ray micro-beam radiation theraphy (MRT) and proton minibeam radiation theraphy (pMBRT). Specifically, by this experiment and the following experiment MD1121, we could successlfully achieve the carachteraization of MRT radiation-driven effects on rat brain structures via PCI-microCT. These two experiments are the continuation of a prof of concept study performed at the ESRF and published in "Barbone et al., Int J Radiat Oncol Biol Phys. 101,4 (2018)".
In the experiment MD1037, both tumor-bearing and healhty (i.e. control group) Fisher rats have been used. For the tumor-bearing ones, 9L – glioblastoma cells were injected in the right brain hemisphere following published protocols (Eur. Joor. Rad. 68S (2008) S151–S155; Phys. Med. Biol. 53 (2008) 861–878). Ten days after tumor inoculation, all the 28 rats (except for the controls) were *in vivo* irradiated according to the following list:

Group 1: unidirectional MRT - 600 Gy (peak dose), 50 μm wide, 200 μm c-t-c;
Group 2: unidirectional MRT - 400 Gy, 50 μm wide, 200 μm c-t-c;
Group 3: unidirectional MRT - 200 Gy, 50 μm wide, 200 μm c-t-c;
Group 4: unidirectional minibeams - 150 Gy, 500 μm wide, 2000 μm c-t-c;
Group 5: unidirectional minibeams - 450 Gy, 500 μm wide, 2000 μm c-t-c;
Group 6: broad beam – 5 Gy;
Group 7: broad beam – 10 Gy;
Group 8: untreated animals – control group.

Microbeams and minibeams are highlycollimated arrays of quasi-parallel X-ray beams that aregeometrically characterized by a different width of single beamlet, respectively, and different distances between the centers of two subsequent beamlets. For microbeams, we used 50 μm wide beams with 200 μm center-to-center

distance and an irradiation field of 5x8 mm^2. Minibeams were instead 5000 μm wide and had a 2000 μm center-to-center distance on a field of 6.5x8 mm^2.

After the irradiation, all animals were host at the ESRF under the control of the animal facility staff. An intermediate *in-vivo* Magnetic resonance Imaging (MRI) was done at 34 days after the irradiation to have a better control on the tumour developement. Animals were euthanized 62 days after irradiation and their brains were extracted and put in a 4% formalin solution. All these procedures were carried out following established ethical regulations.

The second part of the experiment had the purpose of evaluating *ex-vivo*, via PCI-microCT, the effects of the different irradiations on rat brains. The propagation-based phase contrast CT imaging (PBI) set-up with a 3 μm optics coupled to a PCO camera and a monochromatic X-ray beam (33 keV) were used to image the full volume of the samples. For each CT stage, 4000 projections were acquired in half-acquisition mode, over 360 degrees with equally spaced acquisition angles.
Quantitative PCI image analysis has been performed by CT reconstruction with Paganin phase retrieval algorithm and segmentation to derive a 3D visualization of brain healthy and tumor tissue, and a quantitative 3D distribution of both tumoral and healthy vasculature.
Rats irradiated with minibeams had a survival similar to that of the control animals. Probably the interbeam spacing was too big for obtaining a good therapaeutic effect and to achieve tumour sterilization.
Some examples of the results produced in this analysis are reported in the following figures.

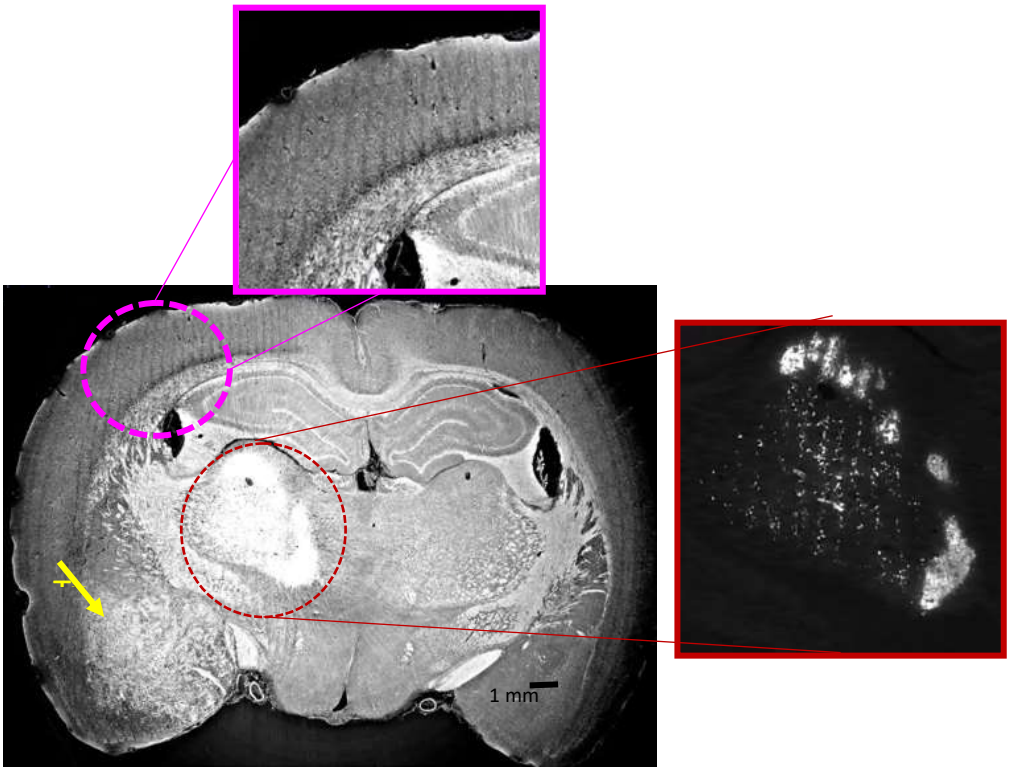

Figure 1: post-mortem PCI-CT image of a 600 Gy MRT irradiated glioblastoma-bearing rat. The yellow arrow points out the tumour while the pink circles zooms the tissue ablations due to the minibeams. On the right, an adjusted windowing image shows the formation of hiperdense structures along the beam path both as microdeposits and clusters. Unpublished data

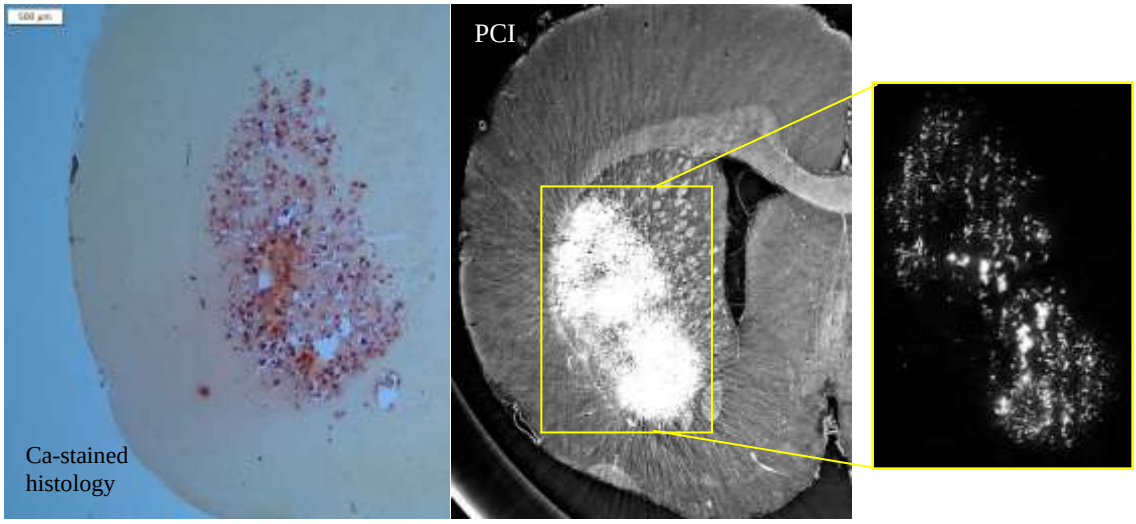

Figure 2: : post-mortem PCI-CT image of a 400 Gy MRT irradiated healthy rat. A comparison between PCI and Ca-stained histology shows th Ca deposits along the beam path in the Caudate Putamen. The ajdusted-windowing image agrees with the histology showing clusters and microcalcifications. Unplublished data

Figure 1 represents a glioblastoma-bearing rat irradiate with MRT of 600 Gy as peak dose. The remaining tumour after 62 days of survival is pointed out by the yellow arrow while microbeams tissue ablations and microcalcifications along the MRT paths in the thalamus are presented in separate windows. Figure 2 shows the case of an healthy irradiated rat with MRT 400 Gy as peak. On the left, a Ca-stained histology highlights the microdeposits and their clustering that are also depicted with great contrast and detail in the adjusted windowing PCI-microCT image on the right.

The analysis of the collected PCI-CT data is in progress and we are performing the last histology and immunohistochemistry examinations to validate the results. This experiment has focussed on investigating late-term effects of the administered radiation treatments and the obtained results will be complement by other PCI-CT datasets acquired at different synchrotron facilities by using smaller pixel sizes. This multiscale approach will allow a detailed study of the morphological changes wihtin the irradiated tissues. Nevertheless, tissue ablations and the Fe and Ca deposits that have been observed, attracted the interest of our collaborators expert in neuropathology, who were unable to determine the specific nature of the visualized lesions, and thus recommended to study the produced lesions at earlier stages (e.g. at two weeks post irradiation). For this reason, a new beamtime will be requested at the ESRF. In addition, in order to correctly classify and, lately, understand the mechanisms leading to the observed microcalcifications (Fe and Ca deposits), we also requested and got granted an experiment of Small and Wide Angle X-ray Scattering (SAXS & WAXS). This experiment is planned in the next months at the Swiss Light Source (SLS). The results of all this multi-scale and multi-technique study will be collected in a manuscript.

Acknowledgements

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