**E**UROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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:**<br>Investigation of vascular network of the limb of transgenic and wild type mice by X-ray phase contrast micro-tomography | **Experiment number**:<br>**33802** |
|---|---|---|
| **Beamline**:<br>ID17 | **Date of experiment**:<br>from: 5/03/2014 to: 10/03/2014 | **Date of report**: |
| **Shifts:**<br>12 | **Local contact(s)**:<br>Herwig Requardt | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

Michela Fratini — Centro Enrico Fermi

Sara Tavella — Universita di Genova Laboratory of Regenerative Medicine Department of Oncology Biology & Genetics

Inna Bukreeva — CNR-IPCF


**Report:**

Vascularization plays an important role in bone since embryogenesis–with endochondral ossification– to adult life–with bone metabolism. In particular, vascular niche controls the differentiation rate of hematopoietic cells before their exit to the systemic circulation. Moreover, the osteoclasts responsible for bone resorption originate from blood compartments around vessels. In reaction to physiological or pathological conditions, blood vessels may modulate the activity of bone cells, while bone cells may influence the vascular bed through an intensive molecular cross-talk. In this scenario, understanding the bone-vessel interaction is mandatory in medicine. Immunohistochemistry permits to reveal the molecular and intimate cellular relationships between these two tissues and can measure the vasculature density in a tissue, but 3D analyses are necessary to obtain reliable data on the organization of vascular networks, thus rendering these two approaches complementary. In this framework, the goal of the performed experiments was to investigate the vascular network of the limb of transgenic and wild type mice by XrPCuT (X-ray phase contrast micro-tomography). In particular, the experiment was mainly focused on the study of the geometry and size of the vessels, to obtain a quantification of the vascular network of the transgenic and wild type mice limb. For a better understanding of the functional and spatial link between bone and vessels, we used a transgenic mouse model generated in our laboratory. This transgenic mouse (LCN2-Tg) overexpresses lipocalin2 in bone. Lipocalin2 is a heparin-binging protein involved in several biological aspects, ranging from cell differentiation, to inflammation and cancer. Bone phenotype studies on these mice reveal that LCN2-Tg mice developed bone alterations [1], and our preliminary data also reveals different Vascular Endothelial Growth Factor (VEGF) expression. Since VEGF is a major angiogenic factor, in particular, we studied the vascularization in these mouse transgenic models, and compared them to controls. The comparison between the results obtained for transgenic and for wild type mice improves our knowledge on the effects of Lipocalin-2 (LCN2) in bone, with an emphasis on bone vasculature aspects. We performed these experiments using the in-line XrPCuT method. Images were obtained using an energy of about 30 keV,

and a voxel diameter of 3.5 micron was employed in order to further improve the resolution of the images. The samples ware prepared in the laboratory of experimental medicine at the University of Genova. The samples were loaded in a cell filled with physiologic salt solution placed on the beam path. We measured at least 3 samples of both transgenic and wild type mice. For each type, the samples two different contrast agents were employed: one group was filled with physiological solution, and one with the Microfill agent, particularly well suited for penetrating vessels of different diameters [2]. It was possible to image a very large volume of tissues, thus allowing for a systematic digitalization of the vessels and reaching a spatial resolution of few microns. Even though it is virtually impossible to observe blood vessels by conventional x-ray imaging techniques without using invasive contrast agents, we demonstrated that vessels as small as 20 microns can be detected using phase contrast x-ray imaging (XrPCT), providing an excellent 3D resolution of the vasculature and allowing us to differentiate between arteries, veins and capillaries in the rat limb. We obtained the 3D morphological features of microvasculature in mouse limb, using in line XrPCT without angiography for the first time with a resolution limit as small as 20 micron. Thanks to this experiment we verified the bone phenotype studies on these mice, revealing whether LCN2-Tg mice develop vascular modifications together with VEGF expression alterations.

The data analysis is finished and the first paper based on the results obtained in this experiment is in a writing stage.

## References

**[1]** Costa D, Lazzarini E, Canciani B, Giuliani A, Spanò R, Marozzi K, Manescu A, Cancedda R, Tavella S. J Cell Physiol. 2013 Nov; 228(11):2210-21

**[2]** S. Grambherr, et al Microscopy Reasearch and technique 71, 551-56, (2008)