EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** **Material and shape maturation dynamics in the long bones of young healthy laboratory mice**	**Experiment number**: MD-852
Beamline: ID 19	**Date of experiment**: from: 05. Sept. 2014 to:07. Sept. 2014	**Date of report**:
Shifts: 6	**Local contact(s)**: Dr. Alexander Reck (alexander.rack@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Background

Within the first 14 days after birth, mice grow significantly in length and weight. To withstand increasing load/force on the limbs, the long bones must swiftly adapt morphologically and stiffen structurally. Although mice bones have become an important model organism for the study of bone healing, aging and disease (e.g. osteoporosis) surprisingly only little is known about the normal tissue formation dynamics in these important research models. Our aim is to understand soft to hard-tissue transformation in the early development stages in healthy mice. The dynamics that lead a porous foam-like femur-bone rim in the young to expand and later "densifiy" to form a solid cortical shell within only several days, will be unraveled by comparing 2D soft-tissue histology and 3D tomography helping understand structural transformation at mm and micrometer length scales.
The aim of the performed experiment was to benefit from the narrow bandwidth radiation that ID19 provides, to obtain information about the 3D mineral deposition/distribution/density. Furthermore, phase contrast should be used to reveal the morphological changes occurring near blood-vessels, as well as providing information about the soft tissue (non-mineralized) spaces (size, shape, proportion and orientation). This information will provide the foundation to later understand abnormal bone structural changes in mammals, in 3D.

Experiments and Setup at ID 19

Healthy mouse femora of different aged mice (1, 3, 7, 10 and 14 days after birth, n = 5 samples/age) were extracted with the soft tissue partially removed. Samples were fixed with ethanol or paraformaldehyde to prevent tissue degradation and stored in sealed polyethylene vials in an ethanol environment. Whole bone overview scans with a low resolution (10µm effective pixel size) were obtained using a laboratory x-ray source to identify the regions of interest for the high resolution scans obtained at the ESRF.

High resolution (effective pixel size 0.647µm) synchrotron radiation computed tomography scans were performed at the ID 19 beamline with x-ray energies of 20 – 30keV. Samples were imaged at 360 degrees using a rotation step size of 0.09 degrees, resulting in 4000 projections per sample. Each sample was imaged at multiple detector distances to obtain absorption and phase-contrast enhanced information. Combining the different imaging modes will allow holotomographic reconstruction.

Analysis and preliminary Results

We were successful in imaging all samples with the different imaging modes. We could thus obtain absorption (see Fig. 1 A) as well as phase-contrast high resolution images which we used to reconstruct holotomographies (see Fig. 1 B) that allow for direct tissue density extraction This should help us to reveal the mineral density changes occurring during the massive structural changes occurring in the bones within the first two weeks of a mouse life. Also, due to the very high contrast of these imaging modalities, small pore extraction and characterization and thus information on the changes in the porosities occurring in the mouse femur maturation can be obtained.

Although the reconstruction and analysis is still ongoing, we obtained very promising preliminary results so far.

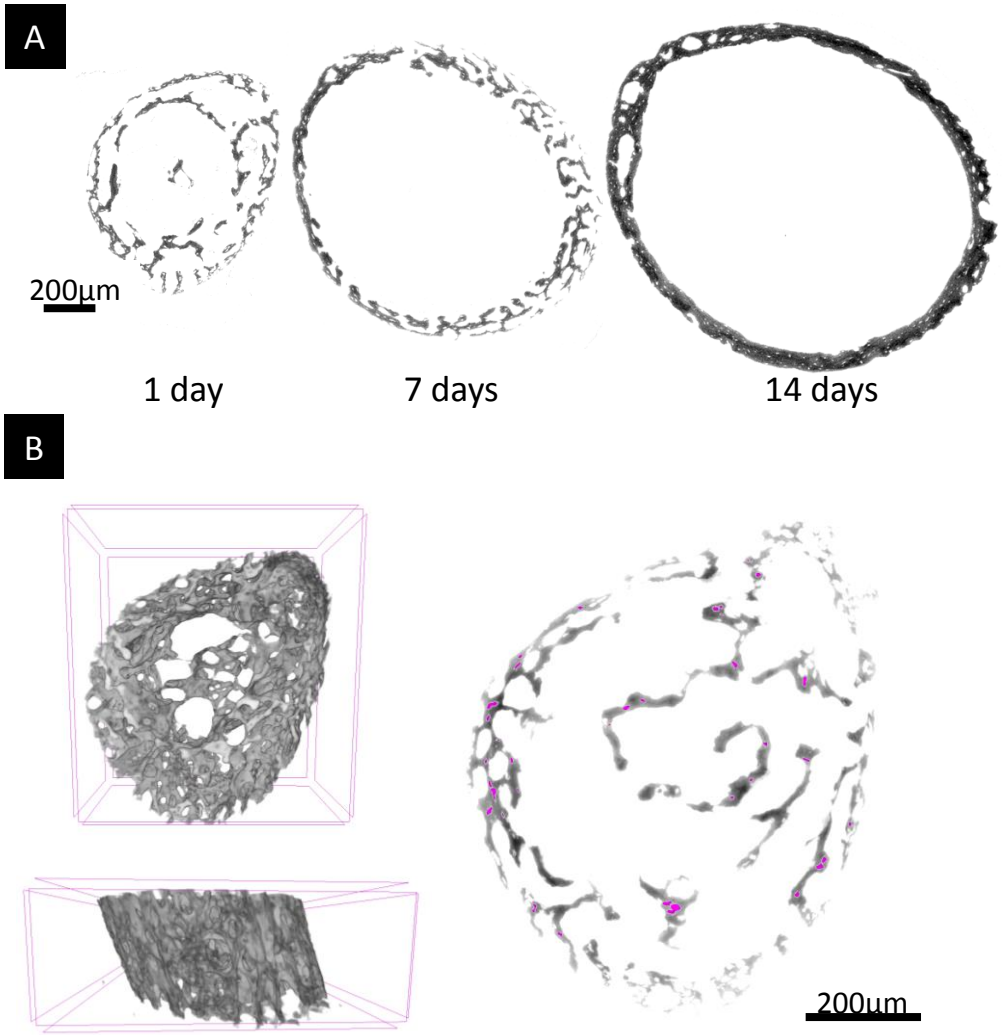

Figure 1 A) Cross sections of femora of different aged mice (1, 7 and 14 days after birth) obtained with apsorption tomography. Images reveal massive macro-structural changes occuring in the midhsafts within the first two weeks after birth. B)3D rendering and cross section of a 3 day old femur reconstructed using a holotomographic approach. The cross-section image nicely shows the density-differentces within the tiny, mineralized struts present in theses young bones. Additionally, the images exhibit a high contrast, allowing for pore segmentation, shown here with a magenta overlay.