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Reports supporting requests for additional beam time

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Reports on experiments relating to long term projects

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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:** Resubmission of MD-792: In Situ 3D Damage Evolution in Cortical Bone	**Experiment number**: ME1362
Beamline: ID19	**Date of experiment**: from: 23/07/2014 to: 27/07/2014	**Date of report**: 25/08/2015
Shifts: 9	**Local contact(s)**: Dr. Alexander Oliver Rack	*Received at ESRF:*

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Scientific Background

Osteoporosis fractures constitute a major socio-economical challenge. Besides bone loss they are partly due to impaired damage repair mechanisms in bone tissue (http://www.iofbonehealth.org/). Finite element analysis (FEA) based on patient specific CT scans is a promising tool to identify patients with a high risk of fracture or to optimise treatment strategies for fractured patients. The quality of the results of those analyses depends on the quality of the used material models. The evolution of microcracks and the associated mechanical damage in bone tissue due to different loading modes is currently not well understood. Therefore, damage is most often included as a scalar variable in FEA that aim to predict bone strength (Pahr and Zysset, 2009). Given the heterogeneous, anisotropic organisation of bone (Fratzl and Weinkamer, 2007) and the loading mode dependence of the damaging process (Wolfram et al., 2011) this assumption must be revised. A lack of experimental data obviates the inclusion of more realistic damage evolutions. Therefore, the project aims at the time lapsed characterisation of the microcrack evolution due to different loading modes (tension, compression, torsion) in a macroscopic experiment. We hypothesised that distinct damage processes due to different loading modes can be identified in time lapsed synchrotron radiation micro-computer tomography (SRμCT) reconstructions.

Materials & Methods

100 parallelepipeds of approximately 3 × 3 × 20 mm were cut using a high precision band saw (EXACT, Germany) produced from cortical bone of full-grown sheep. After verifying osteonal character of the specimens they were glued into hollow aluminium cylinders. Dumbbells were lathed on a CNC lathe (Schaublin, Switzerland) to a wasted cylindrical gage length of 1.3 mm diameter, 0.5 mm height and a running in radius of 4 mm.

A custom made loading device with a 400 N and 2 Nm biaxial load cell (Novatech Measurements, England) was designed and produced. Tension and compression displacements were induced with a precision screw with a lever transmission of 1:5 and measured directly on the sample with a high precision dial indicator (±3 μm). Torsional rotations were induced with a lever arm and a micro-meter screw (Mitutoyo, Japan).

The samples were rehydrated in Hank's balanced salt solution for at least 8 hours, wiped dry on the outside and submerged in ethylenglycol. Subsequent tests were performed in a step-wise manner up to 0.12 mm, 0.17 mm and 5.0° in tension, compression and torsion, respectively. Each load step was followed by a relaxation time of seven minutes to account for creep motions. Afterwards phase contrast scans were acquired.

Phase contrast scans were performed using a harmonic 31 keV and 200 mA (7/8 multibunch mode) setup with 1499 projections over 360° angular range and a shutter time of 0.2 s, resulting in an acquisition time of approximately 480 s. No monochromators were used. Instead, the peak was isolated from the undulator source U17.6 with a specific filter combination (2 mm Aluminium + 0.25 mm Cu + gap 15 mm). Source to

detector distance was 145 000 mm and sample to detector distance was 40 mm. The transmitted X-ray beam was acquired with a 2048 × 1024 CCD FreLoN detector (Labiche et al., 2007) that was mounted behind a Gadolinium Gallium Garnet scintillator with a thickness of 10 microns and a microscope optics with a 10 × objective and a 2 × eyepiece. With this a spatial resolution of 670 nm could be realised. To facilitate fast read out, half of the CCD was blocked by lead shields and used as a buffer for the readout while the unblocked part acquired new data.

Reconstructions were performed using pyHST (provided by ESRF) and ring artefacts were corrected using a custom made MatLab software provided by ID19.

A fully automatic segmentation algorithm was developed based on Larrue et al. (2011) and implemented in ITK 4.5 for being used on a compute server (MiriQuid, MEGWARE, Germany) with 256 GB ram and two Intel Xeon E5-2690 processors with eight cores.

Images were smoothed with a Gaussian and noise was removed by an in-plane median filter. After thresholding, a closing and opening operation was performed to close cracks and cellular lacunae but not Haversian channels. This delivered a mask that allows to separate bone from the Haversian porosity. Cracks were brought to foreground by subtracting the smoothed copy from the bone mask. A steerable filter (Aguet et al., 2005) was used to enhance their contrast. The enhanced planar structures are then smoothed with a bilateral filter (Tomasi & Manduchi, 1998). Cracks were segmented based on the 95th percentile of the image grey level histogram. Finally, a connected components analysis delivered the crack maps.

Results and Discussion

Pre-tests without beam have been successfully performed and yielded 147.8±23.6 N, 306.5±29.2 N 23.88±4.9 Ncm in tension, compression and torsion. In addition, the work of Mirzaali et al. 2015 showed that those experiments are well capable to induce loading mode dependent crack families.

The image processing algorithm is able to segment micro-cracks (Wolfram et al. (2015)). However, the sheer amount of 15 TB data are not yet fully analysed so that the evaluation is unfortunately still ongoing. This is also the reason why this report is handed in so late.

Wet specimens wiped dry and submerged in ethylenglycol cannot be scanned free of artefacts. Beam interaction with the water and convection of emerging bubbles lead to motion artefacts in and around the specimen. As a work-around the specimens were dried in air in the hutch and scanning was proceeded with dry samples. Without beam drying should have increased the strength and decreased the ultimate deformation without changing the overall characteristics of such a macroscopic test. However, it was found that the initial base line scan without mechanical load reduced tensile strength and ductility already up to 90 %. Compression was less affected but suffered a strength decrease up to 60 % while torsional strength was less affected. As an immediate measure we reduced the step size in the experimental protocol.

Based on our experience it has to be concluded that *in situ* testing in combination with the acquisition of several computer tomographic 3D datasets to identify the emergence of microcracks cannot be performed on bone be it wet or dry. The induced dose was too high. Even though dose was estimated according to Barth et al. (2010) a priori and its expected impact considered in the definition of the loading it was found that the actual impact on strength and ductility of the beam was much higher.

The experiment was found to be usable to investigate the emergence of different microcrack families due to different external loading (Mirzaali et al. 2015). SRμCT is definitely the method of choice to identify the morphology of these microcrack families but only a posteriori. Therefore, some sort of labelling needs to be identified that allows to label the microcracks with respect to the loading mode and step that induced them and which can be segmented in SRμCT tomography data-sets. This would allow to do the scanning for a similar experiment in approximately 2 shifts for the same amount of specimens. However, such a labelling is currently not available and subject to ongoing research.

References

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