# SYNCHROTRON X-RAY RADIATION INDUCED DAMAGE IN BONE DURING IN SITU µCT EXPERIMENTS

Marcin Sikorski [1a], Sebastian Levin [2], Samuel McPhee [1], Lorna Sinclair [3], Yuhang Chen [1]
Christina Reinhard [3], Uwe Wolfram [2], Marta Peña Fernández [1]

[1] Institute of Mechanical, Process, and Energy Engineering, School of Engineering and Physical Science, Heriot-Watt University, UK, [2] Institute for Materials Science and Engineering, Clausthal University of Technology, Germany, [3] The University of Manchester at Harwell, UK

[a]mms10@hw.ac.uk

## Introduction

Synchrotron radiation micro-computed tomography (SRµCT) experiments have gained popularity for analysing 3D fracture patterns in bone at the microscale [1]. This technique provides exceptional spatial resolution, enabling detailed analysis of bone microarchitecture and crack initiation and propagation. However, the interaction between ionising radiation and bone tissue induces structural damage, even in the absence of mechanical loading [2]. This phenomenon creates a substantial challenge when investigating fracture mechanisms, as the observed damage may result from both mechanical testing and radiation exposure. Thus, decoupling mechanical and radiation-induced damage is crucial to identify the mechanisms influencing bone fracture resistance. Here, we investigate local mechanical and compositional changes in cortical bone at increasing radiation doses aiming to develop a damage model that incorporates radiation-induced effects to explore bone fracture mechanisms at the microscale.

## Materials and methods

Cortical bone bending samples (11 x 2 x 1 $mm^3$) from ovine femur were machined in longitudinal and transverse directions (n = 15/orientation) and notched to form an initial crack (Figure 1a). In situ SRµCT three-point bending testing was performed at beamline I13-2 (Diamond Light Source, UK) via a micromechanical loading stage (Deben CT500) with specimens immersed in PBS solution to simulate physiological conditions (Figure 1b). Specimens were loaded in ~2 N steps, and one to four SRµCT images (0.81 µm voxel size, 2250 projections, 0.2 s/projection) were acquired after mechanical relaxation, resulting in total exposures ranging between 450 s and 1800 s. The cumulative absorbed dose was simulated using the Monte Carlo code FLUKA [3] with a statistical uncertainty below 10%. The X-ray undulator spectrum used in the FLUKA subroutine were obtained with the XOPPY Python library [4] based on U22 undulator parameters [5], incorporating filters (0.95 mm pyrolytic graphite, 2 mm aluminium, 50 µm iron) and platinum mirror (4.6 mrad) with 2 x 2 mm slit size positioned 220 m from the source (200 mA storage ring current) resulting in a total flux of $3.64 \times 10^{11}$ photons/s.

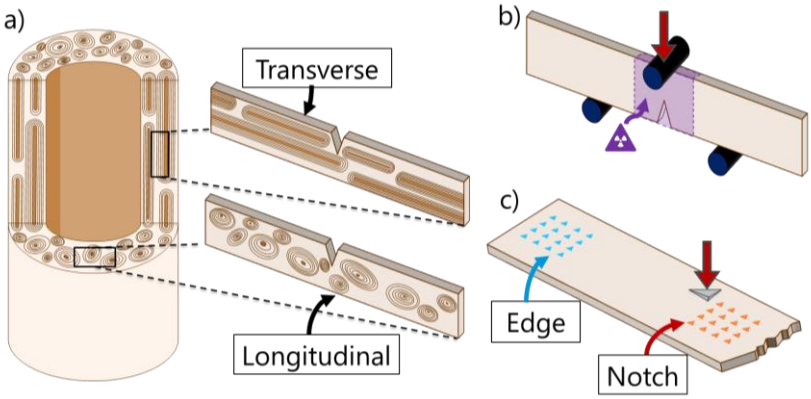


**Figure 1** a) Schematic of bone sample preparation in both transverse and longitudinal orientations with respect to the main bone axis. b) Diagram of three-point bending test conducted at beamline I13-2. c) Diagram of indentation arrays performed in regions close to the notch and edge of each sample.

Following the SRµCT experiment, wet microindentation [6] was performed with a Berkovich tip (Alemnis AG) on the high (notched) and low irradiated (edge) sides of half of each specimen (Figure 1c). The indents were arranged in a 4 × 4 array with a 50 µm separation on each side. Testing was conducted on three samples for each experimental group (exposure time group) and bone orientation (transverse and longitudinal). Welch's t-test was used to compare measured indentation modulus and contact hardness at edge and notch locations within groups and Welch's ANOVA to examine the effect of exposure time on modulus and hardness, with p-values adjusted for contrasts between exposed and control groups. Significance level was selected as $p = 0.05$.

## Results

Simulated radiation dose rates were non-uniformly distributed, with average dose rates ranging from 5 Gy/s to 30 Gy/s at the edge and notched regions, respectively, after a full SRμCT acquisition (Figure 2). The total absorbed dose ranges between 3.8 kGy and 15.5 kGy for one to four tomograms, respectively, corresponding to exposure times of 450 s and 1800 s. Microindentation measurements revealed significant changes in mechanical properties. In the transverse orientation (Figure 3b, top), significantly higher ($p<0.001$) indentation modulus and contact hardness was observed at the edge locations (6.7 kGy at 1350 s and 9 kGy at 1800 s) compared to the notch locations (40.5 kGy at 1350 s and 54 kGy at 1800 s). In the longitudinal orientation (Figure 3b, bottom), significant differences ($p\leq0.027$) were detected in the control (no exposure), 900 s exposure (edge: 4.5 kGy and notch: 27 kGy), and 1800 s exposure (edge: 9 kGy and notch: 54 kGy) groups, with significantly large variations in contact hardness across most groups ($p\leq0.004$), excluding the 450s group ($p=0.844$). The post-hoc analyses for Welch's ANOVA revealed significant reductions in indentation modulus in transverse and longitudinal directions at the notch for the longest exposure time group compared to the control ($p<0.001$), with mean reductions of 54% and 9%, respectively. Similarly, contact hardness decreases by about 73% and 41%.

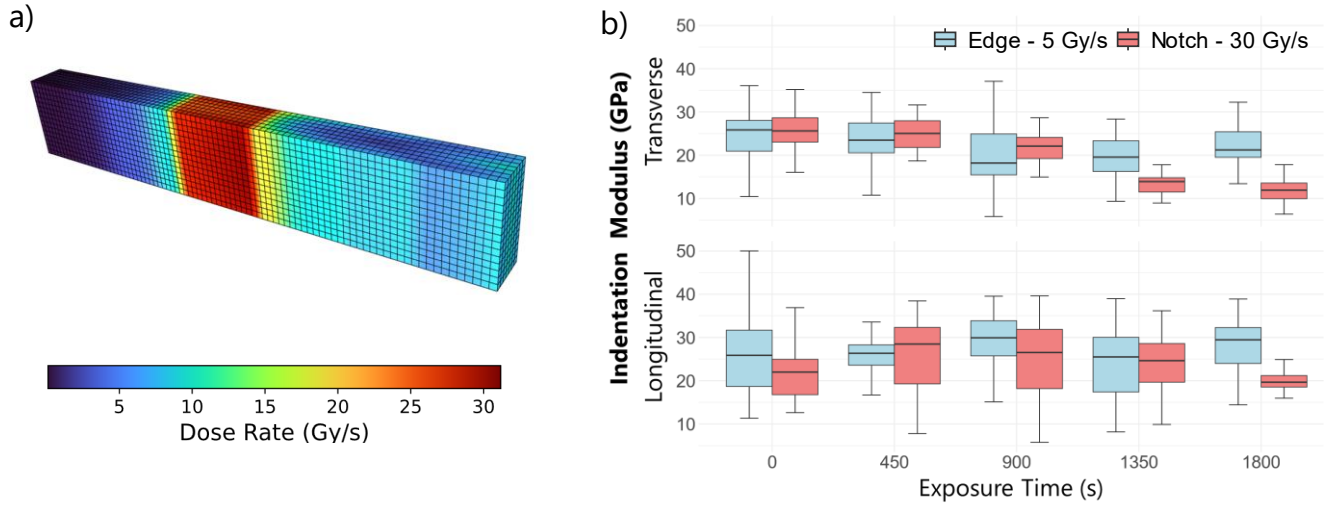


**Figure 2** a) Dose rate distribution after full SRμCT acquisition (180° rotation) from FLUKA Monte Carlo simulation. b) Indentation modulus at increasing exposure to SR X-ray radiation in transverse and longitudinal orientations (n=3 samples/exposure time group).

## Discussion

The difference in mechanical response to X-ray radiation depending on bone orientation indicates anisotropic behaviour, with the transverse orientation showing less resistance to radiation damage. The significant reduction in indentation modulus at the longest exposures indicates radiation-induced softening near the notch, which may accelerate crack propagation. This softening may result from ionisation changes in the non-fibrillar matrix [1]. Future work will combine microstructural and compositional analysis, as well as image analysis and digital volume correlation of the obtained SRμCT images (Figure 3) to further assess the effect of X-ray radiation on bone properties. Ultimately, these findings will be used to develop a computational model of bone fracture that accounts for X-ray radiation-induced damage.

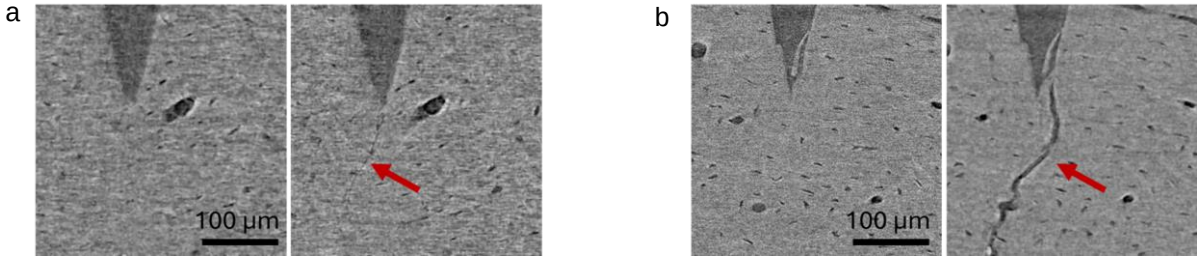


**Figure 3** SRμCT cross-sections showing crack propagation (red arrows) under the notch in (a) transverse and (b) longitudinal orientations.

**Acknowledgements** We thank Dr Leonard Turpin and Dr Kaz Wanelik for support during beamtime at Diamond Light Source (MG35848) and Dr Silvia Cipiccia for advice on Monte Carlo simulations.

**References** [1] Peña Fernández et al., Acta Biomat. 167, 2023, 83-99, [2] Sauer et al., Nature Communications 13, 2022, [3] Battistoni et al., Annals of Nuclear Energy, 2015, 10-18, [4] Sánchez del Río et al., Proc. SPIE 8141, 2011, 814115, [5] Madi et al., Nat. Biomed. Eng. 4, 2020, 343-354, [6] Mirzaali et al., Bone 93, 2016, 196-211, [7] Peña Fernández et al., JMBBM. 88, 2018, 109-11