EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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*

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report")*,
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Imaging joint biomechanics in growth and osteoarthritis	**Experiment number**: LS-3124
Beamline: BM05	**Date of experiment**: from: 9th May 2023 to: 15th May 2023	**Date of report**:
Shifts: 12	**Local contact(s)**: Dr Paul Tafforeau and Dr Joseph Brunet	*Received at ESRF:*

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Scientific Background

Osteoarthritis (OA) is a progressive, age-related degenerative musculoskeletal disease and a leading cause of pain and functional disability in older adults. OA affects approximately 7% of the global population with 10% of men and 18% of women over the age of 60 presenting with its symptoms, thus bearing significant socio-economic and healthcare burdens (1, 2). Despite being hallmarked by the loss of articular cartilage, subchondral bone thickening and formation of osteophytes, the development of these pathological features are often proceeded by significant pain, a symptom that often results in the diagnosis of advancing OA. In this instance, there is a critically unmet need to identify those that are predisposed to OA and monitor the progression of those with OA as no predictive imaging biomarkers of this disease or disease modifying therapeutics currently exist. While the tissues of the knee joint retain their distinct functions throughout life, how these structures physiologically and biomechanically interact to drive OA pathogenesis continues to be poorly understood and remains at the forefront of OA research. We have previously developed novel approaches that have enabled the characterisation of nanoscale strains from full-field synchrotron X-ray computed tomography (sCT) images, acquired using the Diamond-Manchester imaging branchline, i13-2 (3, 4), at the Diamond Light Source, UK upon in-situ compressive loading by digital volume correlation (DVC) (5). Using the STR/Ort mouse, a spontaneous murine model of human OA along with its non-OA CBA parental-strain mouse (6-9), we aimed to i) characterise the hierarchical distribution of strains across the structures of the knee joint, ii) define the intimate link between joint anatomy and microstructure and strain patterning and finally iii) assess whether this relationship changes during OA progression. Collectively, our studies across growth, adult and ageing in healthy and OA-prone mouse strains will help untangle the interplay between hierarchical changes in the joint tissue structures and their mechanical behaviours. Further, the novelty in our approach will aid in the discovery of novel imaging biomarkers have the potential to be used for the identification of localised tissue degeneration prior to OA diagnosis and in monitoring the efficacy of disease-modifying therapeutics in the clinic.

Experimental technique

Whole fresh-frozen ex-vivo mouse hindlimbs from male STR/Ort and CBA at ages prior to the onset of OA (8-11 weeks), at disease onset (20 weeks) and at advanced OA (40 weeks) (10) (N=6 per group), were thawed to room temperature before being mounted on a Deben CT500 compressive loading rig (Suffolk, UK), consisting of an open frame with two carbon-fibre pillars, between bespoke sample cups (figure 1A). High-resolution sCT images of murine knee joints were acquired using a filtered white X-ray beam (2.33 mm aluminium and 0.41 mm copper) with an average energy of 63 keV on the BM05 beamline, at an effective pixel size of 1.45 µm, magnified with a 5x objective, and detected using a 50 µm LuAG:Ce scintillator in conjnction with an PCO.edge 4.2 camera (2048 pixels x 2048 pixels) (imaging set up shown in figure 1B). 6000 projections were collected per scan in half-acquisition fly-scan mode (11) over a continuous rotation of 360 degrees, to provide a final field of view of 5.21 mm x 2.97 mm. Exposure time was set to 18 milliseconds which equated to ~3.5 minutes and a radiation dose of approximately 7.2 kGy per scan. Propogation distance was set at 250 mm to give optimum in-line phase contrast required to resolve the hard and tissue compartments of the knee joints. For in-situ compressive loading, whole CBA and STR/Ort knee joints were subjected to an initial 2 N holding load and imaged at room temperature after a stress-relaxation period of 15 minutes to minimise sample motion during scanning. Successive scans were taken upon incrememental displacement of 20 µm, 50 µm and 100µm, proceeded with 15 minutes of stress-relaxation per step in order to investigate strain distribution in the soft tissues of the joint (figure 1C). Reconstruction of tomographic sCT datasets were achieved using the in-house PyHST2 software (12) in which the following steps were applied; paganin phase-retrieval (δ/β=500), application of an unsharp mask on the projection phase maps to retrieve the edges blurred by the paganin method, filtered back-projection, and ring artefact removal (13). Further 3D analyses of strain patterns and hierarchicial anatomical structures in reconstructed datasets were performed in Avizo 3D (version 2022.2, Thermofisher Scientific, USA).

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Use this ref to explain: Kyrieleis, A., Ibison, M., Titarenko, V. & Withers, P. J. Image stitching strategies for tomographic imaging of large objects at high resolution at synchrotron sources. *Nucl. Instrum. Methods Phys. Res. Sect. Accel. Spectrometers Detect. Assoc. Equip.* **607**, 677–684 (2009).

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We do the paganin on the projections, that gives you phase maps, then we do unsharp mask on it to get rid of the blurred of the paganin and then you reconstruct using the filtered back proj

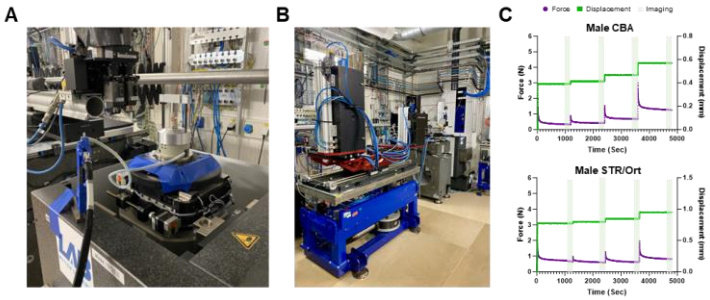

Figure 1: In-situ loading of murine CBA and STR/Ort knee joints on BM05. Experimental set up of the Deben CT500 compression rig on BM05 tomography stage (A) with complete imaging set up shown (B). Representative force curve showing force versus displacement over time from our sequential compression-based loading regime for CBA (top) and STR/Ort knee joints (C).

Preliminary findings

Using our ex vivo methods for knee joint loading with characterisation of strains from full-field sCT images, we find that applied loads predominate in the posterior region of the medial tibial plateu (10), which we have been able to isolate ahead of regionalised analyses following displacement by DVC (figure 2A-C). Initial observations in reconstructed sCT images have confirmed that the pathological phenotype observed in the knee joints presents exclusively in male STR/Orts with advancing age compared to the CBA control strain (figure 3), and are representative of that seen in OA in this model. Such phenotype is characterised by erosion of the articular cartilage (yellow asterisk, figure 2H), increased thickness of the articular calcified cartilage (regions with lower grey values), thickening of the subchondral bone (blue arrowhead) and reduction in joint space (red asterisk) in the posterior region of the medial tibia. Comparatively, the male CBAs display normal knee joint architecture, with our observations in line with previous reports that have shown that this strain does not exhibit signs OA with age (5, 10). Further in-depth analyses of these sCT images will involve anatomical characterisation into the tibial growth plate (e.g. number of bridges and ariel density), and the underlying subchondral (e.g thickness, porosity and osteocyte number) and trabecular bone (e.g. marrow space volume, trabecular thickness and separation).

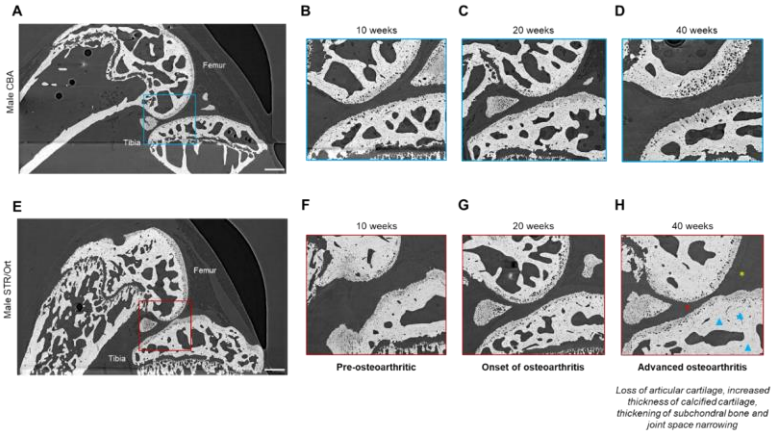

Figure 2: sCT confirms OA phenotype in STR/Ort mice with age. Representative sCT images of male CBA (A) knee joints with zoomed images at the posterior medial tibia (blue box) at 10 weeks (B), 20 weeks (C) and 40 weeks (D) show conservation of healthy joint anatomy with age. In STR/Ort mice (E), joint architecture appears to be conserved at 10 weeks (F) and 20 weeks (G) with noticeable degeneration, encompassing loss of articular cartilage (yellow asterisk), joint space narrowing (red asterisk), subchondral bone thickening (blue arrowheads) and thickening of the articular calcified cartilage (H), visible at 40 weeks of age. Scale bars represent 500 µm.

Preliminary DVC analysis has revealed the progressive evolution of compressive strains in the articular calcified cartilage layer of medial tibial condyle in the STR/Ort tibia following initial displacement with 0.02 mm (figure 3A), with regionalised bands of higher compressive strain (~2%) in the region of the tibial epiphysis directly under the femoral condyle evident following 0.05 mm displacement (figure 3B) and 0.1 mm displacement (figure 3C). Further DVC analysis will be performed on the underlying subchondral bone to investigate compressive strain distribution, and moreover whether these strain patterns emerge consistently across the knee joints with age or exclusively as the result of joint degeneration in the STR/Orts.

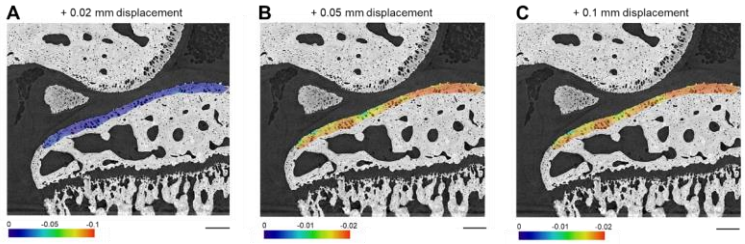

Figure 3: Distribution of compressive strains across the calcified articular cartilage in the medial tibia of STR/Ort mice resolved by digital volume correlation. Representative zoomed sCT images of the medial tibia overlaid with compressive strains localised to the articular calcified cartilage (third principal strain) following 0.02 mm displacement (A), 0.05 mm displacement (B), and 0.1 mm displacement (C). Scale bars represent 250 µm.

Further work and impact

Integrating the hierarchical anatomy of the knee joint with nanoscale strain patterning attained by DVC, made possible by high-resolution sCT imaging will enable us to further dissect the complex interplay between tissue physiology and biomechanics that contribute to joint demise in OA. Our preliminary analyses thus far have yielded interesting results and provide an indication that specific structural parameters could be correlated with localised strains across the tibia. Further studies will focus on strain analyses within the lateral and medial condyle of the femur (9, 14) using the sCT images acquired as part of proposal LS-3124. We then aim to establish the link between strain distribution within key structures of the joint with whole tibial and femoral shape analyses using BoneJ (15) as described in (14) microCT images of complete, intact whole hindlimbs that were imaged at ESRF. As part of our our validation studies, we will next prepare knee joints for histological and histomorphometric examinations to quantify the severity of OA and assess the matrix phenotype of the bone and cartilage in line with published methods (16, 17). In addition we will employ Raman spectroscopy, a label-free and non-destructive fingerprinting modality, to study the molecular chemistry of the joint to assess whether age-related changes to osteochondral matrices underpin localised changes in strain distribution. We envision that by uniquely taking an correlative approach, we can establish both i) an imaging biomarker that is linked to abnormal strain pattering with OA progression and ii) an spectral biomarker that could be used to non-invasively and non-destructively identify and grade OA in patients within clinical settings.

In future studies, we plan to apply for more beamtime to explore the sexual dimorphic mechanisms that enable the protection of female CBAs and STR/Ort mice from the spontaneous development of OA as seen in the male counterparts following knee joint loading. Given the recent Extremely Brilliant Source (EBS) facility upgrade at ESRF, we envision that the improvements to brilliance, flux and coherence of the X-ray beams will allow us to undertake pioneering studies into the sex-specific ultrastructure of i) the soft tissues structures within the knee joint in females and ii) investigate how they deform in response to compressive stress by in-situ loading and sCT imaging.

We have already drafted figures for two papers. The first “Synchrotron resolved strains during the onset and progression of murine knee osteoarthritis”, and the second “Anatomical variations in joint tissues in osteoarthritis predisposition” which we intend to submit to Nature Biomedical Engineering (impact factor = 29.234). Further expected outcomes from our studies include internal, national and international conference presentation (Osteoarthritis Research Society International, April 2024 in Vienna, Austria; Bone Research Society Annual Meeting, June 2024 in Sheffield, UK; European Society of Biomechanics, date TBC in 2024 in Edinburgh, UK), local image analysis workshops and scientific outreach events.

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