Working towards early detection of osteoarthritis

Related publication: Madi K., Staines K. A., Bay B. K., Javaheri B., Geng H., Bodey A. J., Cartmell S., Pitsillides A. A. & Lee P. D. In situ characterisation of nanoscale strains in loaded whole joints via synchrotron X-ray tomography. *Nat. Biomed. Eng.* **4**, 343 (2020). DOI: 10.1038/s41551-019-0477-1

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There are no effective methods for the early detection of osteoarthritis (OA), a condition that affects a large part of the world's population. The subtleties of how early-stage OA affects the shape (morphology) and mechanical behaviour of different joint tissues are not well understood.

Researchers wanted to measure the morphology of the different tissues in 3D with cellular-level resolution, while the joint was under lifelike loading. They needed to measure the strains within these tissues at the level of 100's of nanometres, without using staining or too high a flux, both of which damage the tissue and change its mechanical behaviour.

Using the 'Diamond Light Source - The University of Manchester Collaboration' Imaging Branchline (I13-2), they were able to take time-resolved 3D images, which they then analysed to resolve the strains and displacements within the tissue with 100 nm resolution. They were able to image joint morphology at a cellular level and resolve strains with 100 nm accuracy in physiologically realistic loading conditions for the first time. Using a combination of three key advances (low dose, pink phase contrast imaging, a unique nano-precision loading device and bespoke Digital Volume Correlation analysis software) enabled them to characterise how the articular cartilage adapts to spread load during the early stages of OA.

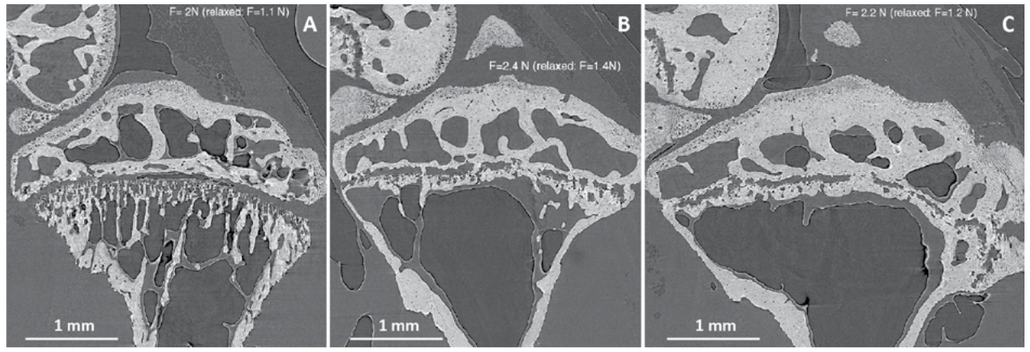

Figure 1: Vertical sections through tomography data of knee joints under load for (a) 8-week, (b) 36-week and (c) 60-week-old STR/Ort representing pre, incipient, and advanced stages of arthritis. Significant changes in overall joint morphology and mineralised tissue characteristics are readily apparent.

The technique developed may be used to determine the efficacy of new treatments for OA at very early stages.

Osteoarthritis is one of the largest contributors to global disability among all medical conditions, and the societal health care burden is increasing dramatically as populations age[1]. It is a pernicious medical condition with a host of contributing factors and comorbid conditions, and suitable treatment strategies have long been sought. But progression from initial disease identification through management of symptoms to eventual surgery (total joint replacement) remains common and is burdensome both in terms of years lived with disability and pain, and direct and indirect cost of care. A significant barrier to understanding of disease etiology and evaluation of treatment efficacy is the disparate perspectives taken by researchers and the variety of methodologies they employ. This study employed three key advances to help bridge those gaps: low dose, pink phase contrast imaging (on I13-2); a unique nano-precision loading device; and bespoke Digital Volume Correlation analysis software.

The behaviour of cells, the structure and composition of tissues, the organisation of tissues within bones, and the mechanical function of synovial joints are all relevant but involve very different spatial scales and research techniques. Without a consistent perspective and integrated research context it is difficult to unify results into a coherent picture. This project was organised around a classic medical research approach of small-animal disease models. The STR/Ort mouse strain exhibits age-related knee arthritis in a manner very similar to that seen in humans and is an established model of the disease[2]. Comparison with the healthy CBA control mouse strain as a function of animal age provides a basis for identifying key differences associated with the pathology.

A primary outcome of this study was attainment of high-resolution tomography data for native-state (fully hydrated, unfixed, unstained) intact knee joints subjected to compressive *in situ* loading (Fig. 1), at a resolution sufficient to reveal tissue and cellular levels of detail, enabling simultaneous functional and morphological evaluation. Study of intact joints is essential in establishing proper boundary conditions and maintaining the overall residual stress state of the mechanical system. Resolution to tissue and cellular levels of detail connects through to biological research that addresses tissue characteristics, calcified cartilage in particular, and microenvironments of the embedded chondrocytes and osteocytes.

The imaging challenges in achieving suitable tomography quality within this context were manifold. Load was applied through the robust, nano-precision P2R *in situ* system that allowed careful monitoring of sample relaxation before projection acquisition[3]. Customised sample grips were developed through additive manufacturing from laboratory tomography pre-scans of representative samples and positioned within the loading system with precision x-y stages. Beam configuration and scan parameters were critical in limiting acquisition time and minimising sample damage whilst maintaining suitable resolution. Efforts by scientists of the I13-2 branchline were essential in establishing the overall imaging protocol that proved effective.

Prior work with mouse bone samples produced excellent resolution of microstructural details using a monochromatic step-scan strategy, but estimated sample X-ray dose of 157 kGy was excessive, embrittling the tissue and hampering functional assessment. A shift to 20 keV 'pink beam', continuous acquisition and increased filtering (Carbon 950 µm, Aluminum 2 mm, Silver 75 µm, Platinum) to eliminate the more damaging low-energy X-rays, proved effective. Imaging of similar resolution to monochromatic scanning was produced but with dose reduced to approximately 100 kGy. A final adjustment - primarily a reduction in the number of projections from 2400 to 600 - reduced exposure to 27 kGy and total acquisition time to 1.1 minutes. Both of these factors, low dose and low scan time, were critical for project success. Resolution alone is not enough for *in situ* loading studies of biological samples as beam damage and sample motion must be controlled.

Functional assessment was conducted through digital volume correlation (DVC) analysis, which compares an initial unloaded tomography data volume with subsequent volumes generated under *in situ* loading to quantify deformation within the samples[4,5]. The full joint was evaluated in this way (Fig. 2), demonstrating internal deformation patterns within both the femur and tibia. The displacement measurement accuracy was assessed by correlating repeat image volumes in the unloaded state and was determined to be 240 – 480 nm for the full joint, and 80 – 160 nm for the articular calcified cartilage region.

An additional loading methodology using a 200 µm radius diamond tip indenter in place of the femur was employed to create uniform mechanical input to the medial condyle of the tibia. This allowed more spatially resolved deformation measurement and a closer association with microstructural detail (Fig. 3). Deformation localised within the articular calcified cartilage region of

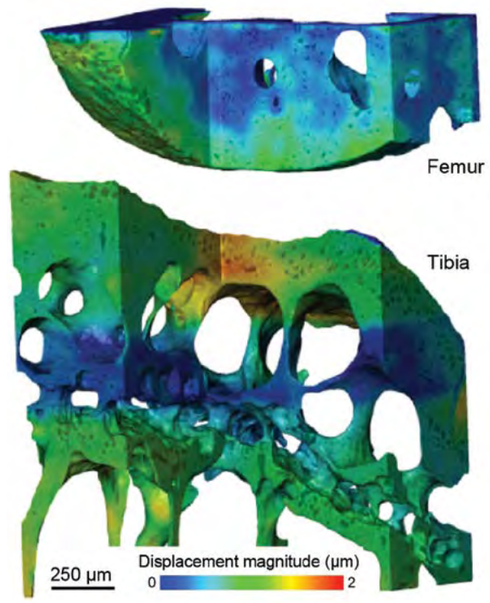

Figure 2: Digital Volume Correlation (DVC) generated, nanometre accuracy, displacement field for a 36-week old STR/Ort knee joint under compressive loading. Large-scale view reveals the influence of overall tissue structure on joint compliance.

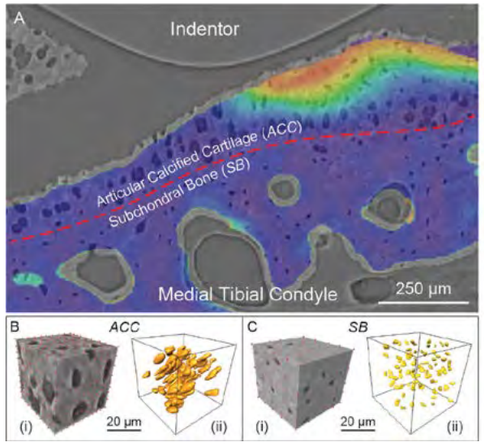

Figure 3: Combined morphological and functional assessment under indentation loading. Displacements concentrate within the articular calcified cartilage region (a). Hypertrophic chondrocyte lacunae (b(i)) provide texture for DVC tracking, and morphological analysis (b(ii)) quantifies pore volume, shape and orientation. Similar features arise from osteocyte lacunae within the subchondral bone region (c(i)), (c(ii)).

the articular surface is a key insight into joint function. The same imaging data allows visualisation and quantification of the small lacunae hosting individual cells within the tissue. While further study is required to fully elaborate the structure/functional relationships within this system, this synchrotron-based *in situ* imaging approach applied to intact bones and joints is a promising approach with no analogue within other existing research methodologies.

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