In-situ deformation imaging of articular cartilage using grating-based phase-contrast X-ray CT at a synchrotron light source

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Abstract
Quantification of deformation behavior of articular cartilage (AC) is crucial for understanding its mechanical function and response to mechanical stimuli. Here, we explored whether grating-based phase-contrast X-ray CT using monochromatic synchrotron light (grating-based msPXCT) enables in-situ quantification of local deformation in AC. Grating-based msPXCT of a porcine AC sample during axial compression test was conducted using a Talbot grating interferometer. Local displacements and strains were computed using a digital volume correlation method. The magnitude of axial strain decreased from the upper to middle sample zones and reached almost constant over the middle-lower zone, consistent with the depth-dependent density increase with compression. Thus, grating-based msPXCT may be suitable for quantitative analysis of AC deformation.

Keywords
articular cartilage, high density resolution, compression, digital volume correlation, strain

Introduction
Articular cartilage (AC) is a connective soft tissue that protects articulating bones by transferring and redistributing contact/impact loads. There are fluid and tissue matrix phases in AC; the fluid phase occupies 80% of the tissue volume and the matrix phase comprises mostly a fibrous network of type II collagen with entrapped proteoglycans. The fibrous network is distributed non-uniformly and anisotropically with showing structural organization that varies over the thickness direction, being oriented parallel to the AC surface in the upper zone, randomly in the middle zone, and perpendicular to the AC surface or the subchondral bone layer in the lower zone [1–3]. Such structures are deeply involved in the mechanical behaviors of AC [4–6]. Thus, the experimental study on local deformations of AC under compressive loading is crucial for comprehensive understanding of the mechanical functions and dysfunctions of AC. Furthermore, investigations of local deformations of AC may contribute to clarify the process of mechanical signal transduction to chondrocytes, which experience local deformations of AC matrix [7].

In determining the local tissue deformation in situ, displacement markers are identified before and after deformation over the tissue volume. Magnetic resonance imaging (MRI) is highly sensitive to detecting the anatomical changes of biological soft tissues, and diffusion tensor imaging, an advanced technique of MRI, has potential to provide markers for cartilage composition and structure. However, the spatial resolving power of MRI is not adequate for imaging the matrix structures of AC that can serve as displacement markers [8]. Absorption-contrast X-ray computed CT has poor image contrast for biological soft tissues because of its light element composition such as hydrogen, oxygen, carbon, etc., showing the difficulty in detecting the matrix structures based on absorption contrast.

Exploiting the phase shift in X-ray CT is a promising way to imaging of the matrix structures of AC [9, 10]. The complex refractive index of X-ray is represented as $1 - \delta + i\beta$, where $\delta$ is the phase factor, $\beta$ is the absorption factor, and $i$ is the imaginary number. Both $\delta$ and $\beta$ characterize the optical properties of the material including density. For light elements in the hard X-ray region, $\delta$ is estimated to be of the order of $10^3$ times higher than $\beta$. For example, in the case of proline, one of the major amino acids constituting collagen fibers, the value of $\delta/\beta$ at around 20-keV X-ray exceeds 2,000, calculated using a database provided by the Center.
for X-ray Optics (https://henke.lbl.gov/optical_constants). Therefore, density variations in biological soft tissues yield much stronger image contrast in phase ($\delta$)-contrast X-ray CT (PXCT) than in absorption ($\beta$)-contrast X-ray CT.

Among other PXCT, grating-based PXCT is highly density-sensitive, possibly allowing density-based structure imaging of biological soft tissues. Using monochromatic synchrotron light in grating-based PXCT (grating-based msPXCT), Momose et al. [11] estimated its detection limit of density deviation at a few milli-grams per cubic centimeter. The hydrated collagen microfibril is estimated at 1.19 g/cm$^3$ [12], which is larger than the densities of surrounding cells and fluids. Therefore, grating-based msPXCT enables distinguishing between the collagen matrix and the surroundings with densities close to the water density. Several studies demonstrated in-situ imaging of local structure of biological soft tissues or a light-element material by grating-based PXCT with monochromatic [13, 14] or white synchrotron light [15], indicating its potential for the quantification of static and dynamic deformation. However, it is remained to be investigated whether grating-based msPXCT can be used for in-situ quantitative analysis of AC deformation. This study was thus undertaken to investigate the availability of grating-based msPXCT for yielding regional image structures that serve as in-situ displacement markers in AC. In compression test of a porcine AC sample, the tracking of regional structures was tested using a digital volume correlation (DVC) method [16, 17].

**Materials and Methods**

**Sample preparation**

Weight-bearing AC tissue was harvested from the porcine medial femoral condyle, purchased from a local abattoir. Using a 3-mm biopsy punch, the column sample was cored out perpendicular to the articular surface to obtain flat cylinders. The AC sample was wrapped with moistened physiological saline gauze and stored at –30°C just before the experiment.

**Grating-based phase-contrast X-ray CT with monochromatic synchrotron light**

Experiments were performed in the first experimental hatch of beamline BL20B2 at a synchrotron radiation facility, SPring-8 (Hyogo, Japan), where a bright monochromatic light is available for achieving the excellent image quality and quantity in grating-based PXCT. The system for grating-based msPXCT consisted of a rotational sample stage, Talbot interferometer, and X-ray detector (Fig. 1a). The Talbot interferometer, composed of a phase grating (G1) and an absorption grating (G2), was optimized for the 20-keV X-ray. The grating pitch of G1 and G2 was both 2.4 μm. The pattern thickness of G1 was designed to generate π/2 phase shift. To generate moiré images, G2 was placed at 232 mm (5th fractional Talbot distance) from the G1 and aligned parallel to the lines of G1. The moiré image reflects the sample-induced phase shift, and it can be determined using three or more moiré images. To obtain multiple moiré images, G2 was mounted on a piezo-driven stage and allowed to move step by step in the direction orthogonal to the lines [13]. A fiber-coupled sCMOS camera (ORCA Flash4.0, Hamamatsu Photonics) equipped with a beam monitor 2 (AA40P, Hamamatsu Photonics) and a 10-μm-thick P43 (Gd2O2S:Tb) phosphor screen was used as the X-ray detector. The view size is 9.0 mm (H) × 5.5 mm (V), and the effective pixel size was 4.46 μm.
**In-situ imaging of sample deformation under compression**

The AC sample was thawed in physiological saline at 25°C and placed with the surface up on the stage of rotation in a container filled with 25°C physiological saline. The compression force was applied on the surface with an acrylic plug with a diameter of 10 mm. The vertical motion of the plug for AC sample compression was controlled by a mechanical translation stage driven by a linear stepping motor, and the position information of the plug is obtained by a cantilever-type displacement transducer (CE-5, Tokyo Measuring Instruments Laboratory). The compressive load was measured with a load cell (TCSS-5L, Toyo Sokki) built into the axial shaft.

The AC sample was imaged at pre-loaded and compressed states. Scanning started after 10-min period of stress relaxation. Five moiré images (a five-step fringe-scanning method) were acquired to obtain one differential phase-shift image at each angular position of the sample rotation (Fig. 1b). The five-step fringe-scanning method is demonstrated to be suitable for grating-based msPXCT rotation (Fig. 1b). The exposure time per fringe scan was 200 ms. The sample was rotated over an angular range of 180° in 0.2° steps, resulting in total 900 differential phase-shift images. Images of sCMOS dark current and direct beam were also steps, resulting in total 900 differential phase-shift images.

**Density calculation based on phase contrast**

The relationship between \( \Delta \phi \) and the phase factor increment \( \Delta \delta \) is represented by the following equation:

\[
\Delta \phi = \frac{2\pi}{\lambda} \cdot \frac{1}{c_f} \cdot \Delta \delta \tag{1}
\]

where \( \lambda \) is the X-ray wavelength, \( t \) is the pixel size, and \( c_f \) is the calibration factor depending mainly on the X-ray detector. The calibration factor was determined using by phantom solutions of NaCl at various concentrations. In this experiment, \( c_f \) was set to 1.033.

The relation between \( \Delta \delta \) and local density of AC sample \( \rho \) can be given by the following equation:

\[
\Delta \delta = \frac{\lambda^2 r_e N_A Z}{2\pi M} \left( \rho - \rho_p \right) \tag{2}
\]

where \( r_e \) is the classical electron radius, \( N_A \) is Avogadro’s constant, \( Z/M \) is the ratio of atomic number-to-mass, and \( \rho_p \) is the density of physiological saline. For biological soft tissues, \( Z/M \) could be 0.55 (NIST database, http://physics.nist.gov/PhysRefData/XrayMassCoef/tab2.html).

**Digital Volume Correlation Method**

The reconstructed images at the pre-loaded and compressed states were converted to 8-bit gray-scale and registered for a five-layer region adjacent to the top surface of sample stage, which could be regarded as a fixed boundary because the sample compression is unlikely to cause deformation in these layers. Then, displacements of local gray level patterns between the two images were determined using a DVC method [16, 17], implemented into Amira Software (v. 2021.1; Thermo Fisher Scientific). First, the pre-loaded sample images were decomposed into sub-volumes (286 × 286 × 286 voxels), and the same-sized volume that best matches the gray level pattern of each sub-volume was found within the compressed sample image by means of cross-correlation (local matching). During the process of local matching, the sub-volume was iteratively translated and deformed in 6 degrees of freedom (rotation and shearing), using interpolation, to achieve the highest cross-correlation [18]. The first-order shape function was used to determine the displacements of voxels within the final deformed sub-volumes.

Second, using the result of local matching as the initial step, global matching was conducted. The 4-noded tetrahedral mesh (6580 nodes) was generated within the pre-loaded sample image (see Fig. 4a). Within the mesh, a cubic spline gray level interpolation was used. Then, in an iterative process, the mesh was deformed so that the sum of squared difference of gray levels of voxels between the mesh volume and the corresponding volume within the compressed sample images was minimized. Finally, the displacement distribution within the mesh volume was calculated using a trilinear interpolation.

**Results and Discussion**

Figures 2a and 2b show longitudinal and cross-sectional images of the AC sample, respectively, reconstructed at the pre-loaded state and at the 6.9%-compressed state after full relaxation, generating the mean reaction forces of 0.51 N and 0.74 N, respectively. Differences between mean reaction forces measured in the first and last 1-minute scanning periods were 0.66% and 0.61% at the pre-loaded and compressed states, respectively. The cross-sectional images of the upper, middle, and lower zones, respectively, are located at the same heights between the two states. Reconstruction was redigitized such that \( \rho \) \( [g/cm^3] \) = 0.991 + (0.667 × 10\(^{-3}\)) × 8-bit gray-scale value. The density resolution based on NaCl solution phantoms was 2.5 mg/cm\(^3\). Overall density through the layers between \( l \)- and \( l' \)-cross sections (Fig. 2a) was 1.094 g/cm\(^3\) at the pre-loaded state and 1.100 g/cm\(^3\) at
the compressed state, where \( l \) - and \( l' \)-cross sections are equidistant from the plug surface and the surface of the rotational sample stage, respectively. In both states, the density increased toward the lower layers as consistent with an earlier study [19]; the within-layer densities of the upper, middle, and lower layers (Fig. 2b) were, respectively, 1.076 ± 0.243, 1.095 ± 0.200, and 1.112 ± 0.176 g/cm\(^3\) at the pre-loaded state and 1.091 ± 0.266, 1.100 ± 0.217, and 1.113 ± 0.164 g/cm\(^3\) at the compressed state. The density increase accompanying the sample compression was most pronounced in the upper zone, implying the occurrence of exclusive compressive deformation and fluid drainage there (see also Fig. 4c).

The degree of similarity between a density-based reconstruction and a light micrograph stained with hematoxylin and eosin at nearby cross-sections of the lower zone is presented in Fig. 3. Regions of tissue matrix, stained dark pink, roughly correspond to rather high-density (light) regions, while compartments including chondrocytes with dark blue-stained nuclei correspond to low-density (dark) regions.
Fig. 3 Portions of a phase-contrast cross-sectional image (a) and a light micrograph of a 5-μm-thick formalin-fixed section, stained with hematoxylin and eosin (b). Both were derived from the same sample of AC. The phase-contrast image is not the same as the region shown by the light micrograph but depicts a very nearby cross-section in the lower zone. Bar, 500 μm.

Fig. 4 (a) Four-noded tetrahedral mesh (6580 nodes) generated within the reconstructed image of the articular cartilage sample at the pre-loaded state. Only surface mesh is shown for clarity. (b) Nodal displacement vector fields in the whole specimen and in the 0.3-mm-thick longitudinal section σ. All vectors are scaled in length to a reference vector. (c) Plots of the normal strain in the z direction (ε_{zz}) and the density (ρ) at the pre-loaded and compressed states along the z-compression axis from top to bottom of the mesh volume. Both ε_{zz} and ρ are shown by mean +/- sd of the central 1 × 1 mm²-region of each layer.
There have been reported multiple chondrocytes that orient in a columnar arrangement in the lower zone of AC [20]. Thus, grating-based msPXCT allows making the distinction between the matrix and chondrocyte compartments, and the heterogeneous density pattern (Fig. 2) reflects structural organization of matrix and chondrocytes that varies by layer [1–3].

The density-based structure is blurred due to the density gradient at the structure interfaces; however, its regional speckle-like patterns (Figs. 2b and 3a) could serve as a carrier of deformation information for DVC [21]. Figure 4a depicts 4-noded tetrahedral mesh for DVC analysis defined within the reconstruction of the pre-loaded sample (only surface mesh is shown), and Fig. 4b shows the displacement field generated by compression in the whole specimen and in the longitudinal section. As seen in the longitudinal section, the nodal displacement vectors were oriented predominantly in the compression (z) direction in the area near the center, and their magnitude was larger in the upper zone than in the other zones. Near the left-lower side surface, relatively large deformation occurred due to the irregular sample shape. The tendency of outward small displacement in the right-lower area is the result of the influence of the subchondral bone at the bottom of the specimen (see Fig. 1b). Such displacement field leads to the strain distribution depending on the z-direction. As shown in Fig. 4c, the mean magnitude of axial strain (normal strain in the z direction) in the central 1 × 1 mm²-region ($\varepsilon_{zz}$) of each layer decreased rapidly from the upper to middle zones and became almost constant over the middle and lower zones. In an earlier study using second harmonic generation microscopy [22], the similar depth-dependent trend of strain was observed in a study using second harmonic generation microscopy [22], the similar depth-dependent trend of strain was observed in a study using second harmonic generation microscopy [22].

Concluding Remarks

We demonstrated the potential of grating-based msPXCT for deformation analysis of AC. This high density-sensitive method enables in-situ imaging of structural organization of tissue matrix and chondrocyte compartments, and its regional patterns are trackable as displacement markers. In the compression test of the AC sample, grating-based msPXCT combined with the DVC method achieved quantification of regional deformation, demonstrating that the axial strain and the compression-induced density increase varied compatibly over the thickness direction. The method proposed here will allow time-resolved evaluation of AC deformation under periodic compression (dynamic viscoelastic test) by synchronizing image acquisition, sample rotation, and grating movement with the compression cycle [14, 15]. Mechanical signals invoked by local AC deformation affect the function of chondrocytes and the pathophysiology of osteoarthritis [23]. Since the local deformation across the AC thickness differed between dynamic and static compression [22], it is significant to extend this method to dynamic viscoelastic testing of AC and characterize local deformation for understanding of health and diseases of AC.

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