BL20B2
Medical and Imaging I

1. Introduction

BL20B2 is a medium-length beamline with a bending magnet source dedicated to X-ray imaging. It is composed of an optics hutch, an upstream experimental hutch 1 (EH1) located 42 m from the source, and downstream experimental hutches 2 (EH2) and 3 (EH3) located 200 m from the source. The EH2 and EH3 are located in the medium-length beamline facility. A monochromatic X-ray beam from a SPring-8 standard double-crystal monochromator is available, and an energy range of 5–113 keV is covered by changing the crystals.

BL20B2 is mainly used for X-ray imaging such as X-ray microtomography and projection imaging. EH1 supports high spatial resolution and fast imaging experiments, which require a higher photon flux density. EH2 and EH3 support X-ray imaging experiments with a wide field of view using an X-ray beam with a large cross-section. In addition, phase-contrast imaging with a high spatial coherence of the beam generated by a long propagation distance from the source is performed. These activities have improved the measurement apparatus and techniques for X-ray imaging. In FY2019, 4-dimensional (4D) X-ray phase tomography using a grating interferometer was developed.

2. Development of 4D X-ray phase tomography

X-ray imaging using phase information enables observations of biological soft tissues with a higher image contrast compared with absorption-based imaging. Moreover, the phase shift is proportional to the mass density of the soft tissues in the hard X-ray region. Therefore, the density distribution in biological soft tissues can be quantitatively measured in three dimensions with a high-density resolution.

In X-ray phase tomography, most measurements were made on extracted and formalin-fixed specimens because *in vivo* measurements for organs could not fully utilize the high-density resolution of X-ray phase tomography due to the presence of bones and air, which have very different densities compared to biological soft tissues. However, for biological soft tissues that undergo dynamic changes such as tendons, cartilage, and aorta, *in vivo* measurements should provide not only structural information but also functional information. On the other hand, *in vivo* measurements using absorption contrast cannot be applied to measure slight differences in the density, which is relevant to the structure and the function of biological soft tissues due to lower sensitivity. In FY2019, a 4D X-ray phase tomography was developed to observe dynamically changing biological soft tissues in an environment that mimics *in situ* conditions, although it is not an *in vivo* measurement.

4D X-ray phase tomography was developed at EH1. A grating interferometer provides differential phase images. The grating interferometer is composed of two transmission gratings, namely a phase grating (G1) and an absorption grating (G2). The grating pitch in G1 and G2 is 2.6 μm. G1 generates a $\pi/2$ phase shift for an X-ray energy of 20 keV. The distance between G1 and G2 is set as a 3^{rd} order fractional Talbot distance of 20 keV. G2 is placed on the Piezo-driven translation stage, and the

fringe scan method retrieves the differential phase image by scanning G2. Integrating the differential phase images provides a phase image. A scanning procedure allows data for multiple fringe scans to be obtained by continuously scanning G2 in one direction. Hence, a method to acquire a series of images was developed based on the advantage that the grating pitch is nearly constant.

To scan G2 and acquire images at the proper time, a function generator (3350B, Keysight) was used. Figure 1(a) shows a schematic drawing of the scanning procedure. In this case, a simple continuous oscillation such as stretching/ compressing and its releasing is considered as a sample deformation. The deformation process of the sample is synchronized with the scanning of G2 and the acquisition of the images for phase retrievals. A

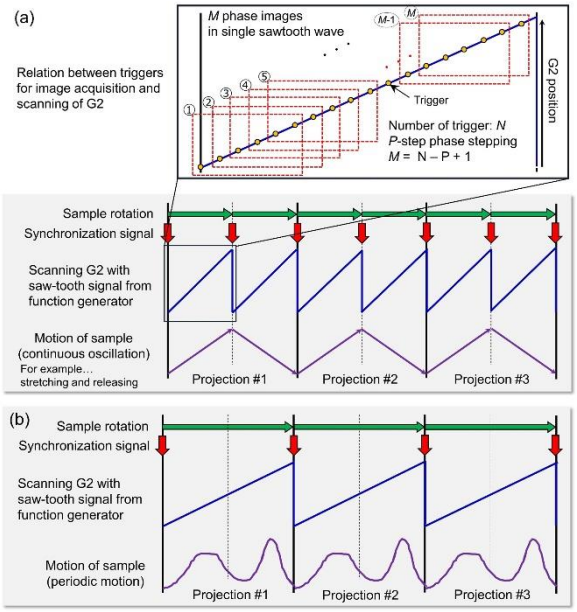

Fig. 1. (a) Schematic drawing of the scanning procedure in 4D X-ray phase tomography to observe continuously oscillating biological soft tissues. (b) Improved scanning procedure to observe more complicated periodic motion.

single projection includes two saw-tooth waves (Fig. 1(a)) [1]. The one-way deformation process of the sample is synchronized with the single saw-tooth wave. In this single saw-tooth wave, $M=N-P+1$ differential phase images (or phase images) can be obtained from N images with a P-step fringe scan. Hence, the frame rate in the image acquisition corresponds to the apparent frame rate in X-ray phase tomography.

As a demonstration of 4D X-ray phase tomography for biological soft tissues, fresh chordae tendineae extracted from a pig heart were observed under continuous stretching and releasing conditions. In this demonstration, the effective pixel size was set to 7.8 μm × 7.8 μm. The fresh sample was measured in a specially designed water cell filled with normal saline. To keep the sample fresh, the saline was kept below 7 °C using Peltier devices. The sample was set between two rotation stages facing each other using an attachment tool (Fig. 2). Then the sample was rotated under stretching by changing the distance of the rotation stages. In this case, the stretching direction is parallel to the rotational axis. A mechanical seal prevented the saline from leaking out of the water cell. In addition, a small load cell (nominal capacity: 50 N) was installed between the sample and the upper rotational stage to measure the sample load during the tomographic measurement. The chordae tendineae under observation were continuously stretched and released with an oscillation speed of 0.5 Hz. The amount of stretching was 400 μm. In this condition, the maximum load on the sample was approximately 0.8 N. The acquisition speed of images using the function generator was set to 20

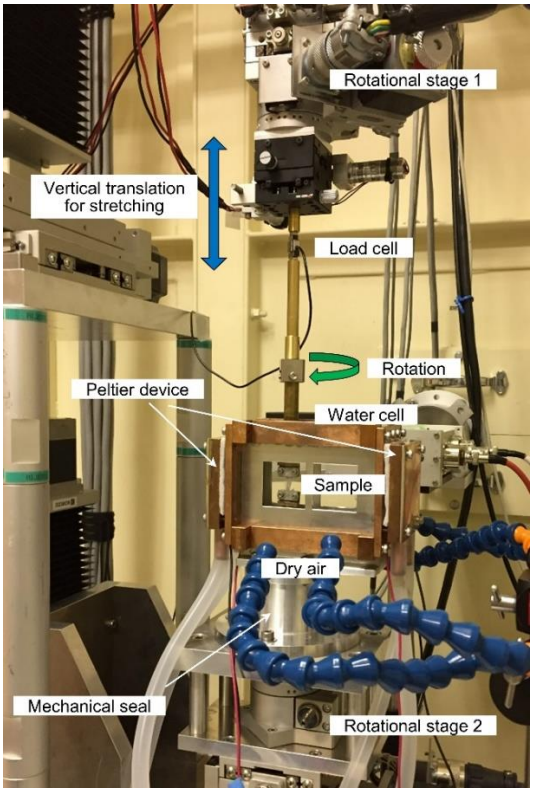

Fig. 2. Sample stage for 4D X-ray phase tomography under stretching.

Hz, where the exposure time was 40 msec. Thus, the differential phase images should be acquired at 20 frames per second. X-ray phase tomography was reconstructed from 900 projections where a 5-step fringe scan was used to retrieve a differential phase image.

Figure 3(a) shows a cross-section of chordae tendineae, which are perpendicular to the stretching direction, at the stretched and released states, where the color scale represents the mass density estimated from the phase shift. The density resolution estimated from the standard deviation in the background was 4.7 mg/cm^3. The cross-sectional shape slightly differed in the stretched and released states. Figure 3(b) shows the sequential changes of the cross-sectional area during stretching and releasing. In the measurement of the cross-sectional area, a simple threshold (=1.018 g/cm^3) was applied, and the area above the threshold was measured. Although an approximately 2.5% reduction of the cross-sectional area in P–V was observed, a clear change in the density was not.

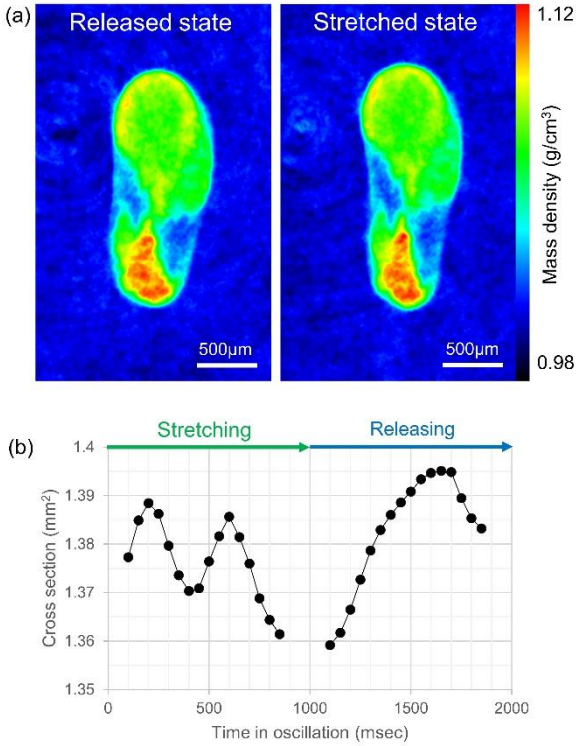

Fig. 3.(a) Cross-sections of chordae tendineae at the stretched and released states. (b) Cross-sectional area during stretching and releasing.

3. Conclusion

Four-dimensional X-ray phase tomography can be used to observe biological soft tissues with continuous oscillation, which mimics the *in situ* conditions. The X-ray phase tomographic images during stretching and releasing can be obtained at 20 frames per second. This technique can be applied to observe the deformation process in biological

soft tissues. Two saw-tooth waves accommodate each motion in stretching and releasing. To improve the flexibility in the dynamic measurement, the two saw-tooth waves can be replaced with a single saw-tooth wave (Fig. 1(b)). Although the technique is limited to periodic motion of the sample, it can measure more complex deformation processes.

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Reference:

[1] M. Hoshino, K. Uesugi, N. Yagi and T. Tsukube, *Microsc. Microanal*. **24** (Suppl 2) (2018) 30-31.