APPLICATION NOTE

AX R MP Multiphoton Confocal Microscope

SHG imaging and quantitative analysis of bone collagen fibers using a multiphoton microscope

Bone is a hard tissue that supports the body, and the ratio of inorganic components (calcium etc.) in bone is only 45% by weight, with the remainder being composed of water and organic components consisting primarily of collagen. In mineralized bone, inorganic crystals mainly composed of calcium and phosphoric acid are deposited on collagen fibers, which are bundled collagen molecules. Robust and tough bone is hard and flexible. The hardness is due to inorganic components, and the flexibility is due to collagen.
Prof. Tadahiro IIMURA and Dr. Takanori SATO of the Department of Pharmacology, Faculty and Graduate School of Dental Medicine, Hokkaido University are studying the pharmacological effects of PTH (teriparatide) preparation, a drug developed for the treatment of osteoporosis. This application note introduces an example of quantitative evaluation of the arrangement of collagen fibers in bone, by combining SHG (Second Harmonic Generation) imaging using a multiphoton microscope and AI technology.

Overview

Prof. IIMURA et al. have established a method for evaluating bone formation by visualizing the collagen fibers in bones without staining, and measuring their length, area and angle. In addition, by administering the PTH preparation to an osteoporosis model cynomolgus monkey, they revealed that the PTH preparation has the pharmacological effect of aligning bone collagen fibers (see Reference).

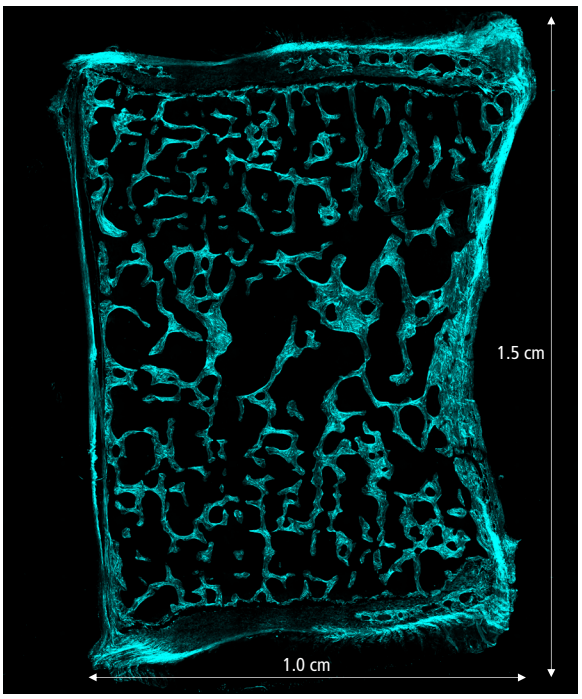

Figure 1. SHG observation of un-decalcified bone section of a monkey
Image acquisition: Resonant 1K + Denoise.ai
Objective: CFI Plan Apochromat Lambda D 10X (NA 0.45, WD 4.00)
Number of images: 1,320 (image stitching: 120 images x Z stack imaging: 11 optical slices)
Excitation wavelength: 920 nm

Experiment outline and results

To understand the characteristics of the bone, we observed the entire bone section of a normal monkey by means of SHG imaging (Figure 1). Since the bone is covered with calcium phosphate crystals, tissue observation usually requires a decalcification process to remove minerals. In contrast, SHG imaging does not require decalcification and enables us to observe the collagen fibers without staining (blue fluorescence signal shown in Figure 1). Since the bone section we observed here was relatively large (1.5 cm vertically x 1.0 cm horizontally) and the surface was not entirely flat, it was necessary to adjust focal position considering its thickness for accurate imaging. To overcome this technical issue, acquisition of images by combining stitching and Z stacking can be a solution, but it takes a long acquisition time with conventional microscopes.
The new AX R MP multiphoton microscope can capture an area about 1.4 times larger than that of a conventional model. With this new microscope, we could reduce considerably the number of images required to capture the entire bone thus shortening the acquisition time. Since the Lambda D series objectives can capture uniform images up to the edge of the field of view, creating seamless tiled images, it was also useful for observing a large sample as in this case (Figure 1). Furthermore, the combination of a high-speed resonant scanner and Denoise.ai enabled us to capture low-noise images without the need for an averaging process (Figure 2). This allowed us to acquire images several times faster than conventional microscope models. In conclusion, we were able to perform this imaging in tens of minutes, which had previously taken a few hours using a conventional model.

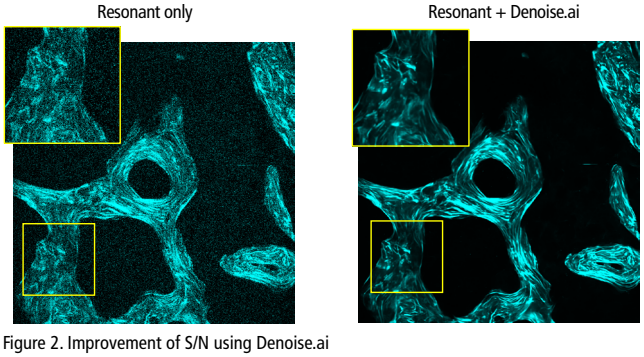

Figure 2. Improvement of S/N using Denoise.ai
Since Denoise.ai is an AI software module that has been pre-trained, it can automatically reduce the shot noise of resonant scan images.

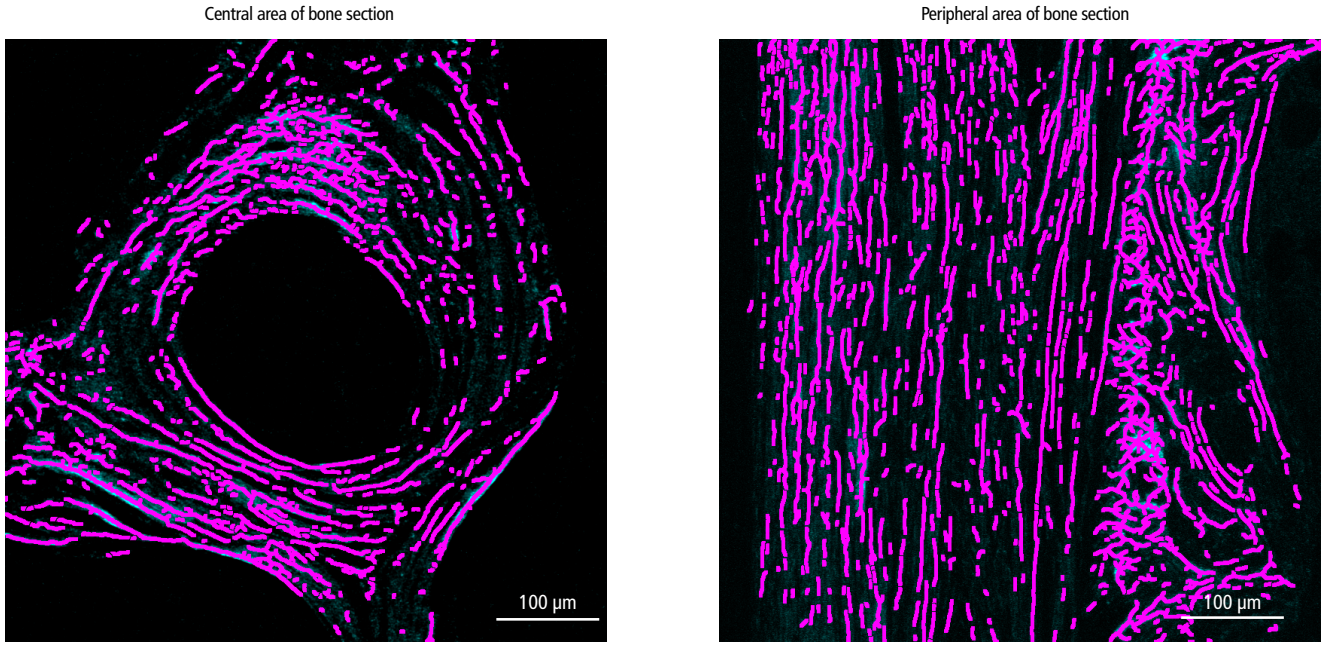

Figure 3. Binarized images of collagen fibers from SHG image
Image acquisition: Resonant 2K
Objective: CFI75 Apochromat LWD 20XC W (NA: 1.00, WD: 2.8 mm)
Excitation wavelength: 920 nm

Figure 3 shows binarized images of collagen fibers in the central and peripheral area of the bone section taken with a 20X objective. High-speed Z stack imaging with the resonant scanner minimized photobleaching. In the AX R MP, an image resolution of up to 2K can be selected for the resonant scanner. As a result, individual fibers could be detected clearly. The characteristics of bone formation can be quantitatively evaluated by measuring collagen fibers with several parameters such as length and area. Figure 4 shows the measurement result of the orientation of fibers. The angle of collagen fibers was classified into six groups (0-30°, 30-60°, 60-90°, 90-120°, 120-150°, and 150-180°) with the horizontal as 0°, and the proportion of each was obtained. From this result, it was found that collagen fibers extended at various angles in the central area of the bone section, and few fibers extended in the vertical direction (90°). On the other hand, in the peripheral area of the bone section, fibers extending in the vertical direction accounted for a large proportion of the fibers.

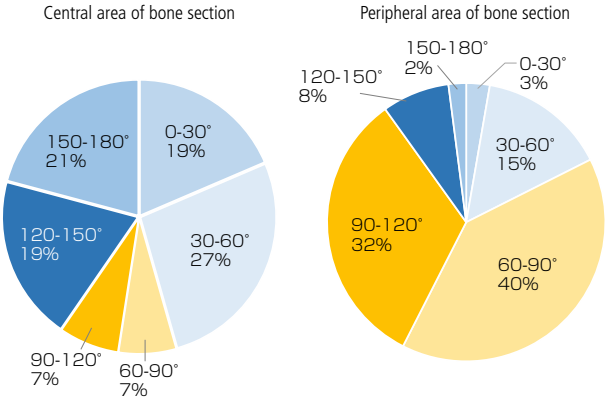

Figure 4. Measurement result of orientation of collagen fibers in bone section

Summar

In the papers of Prof. IIMURA and Dr. SATO, it is reported that collagen fibers are aligned linearly and regularly in bone sections treated with PTH preparations, forming long, thick collagen fibers. With the administration of the PTH preparation, the mechanism that releases force applied to bone is facilitated, and tough, flexible bone is formed. The observation method using SHG imaging is very beneficial as a new standard for evaluating anti-osteoporotic drugs.

Reference

Microscopy (Oxf). 2021 Nov 24; 70(6): 498-509. doi: 10.1093/jmicro/dfab020.
A quantitative analysis of bone lamellarity and bone collagen linearity induced by distinct dosing and frequencies of teriparatide administration in ovariectomized rats and monkeys
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Product information

AX R MP Multiphoton Confocal Microscope

The AX R MP achieves deep imaging at high speeds and high resolutions with a large field of view, and ensures a large space for flexible sample placement.

- Large FOV: 22 mm field of view
- High speed: Max. 720 fps (2048 x 16 pixels)/resonant
- High resolution: Max. 8K pixels/Galvano, max. 2K pixels/resonant