

**Measurement of wave velocity distribution  
in a trabecula by micro-Brillouin scattering**

顕微 Brillouin 散乱法による骨梁中の音速分布測定

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**1. Introduction**

Bone has complicated properties which depend on the multi-scale structures. Considering structure in a large scale, bone has two main parts, cortical and trabecular bones. Furthermore, this trabecular bone is very important because it exhibits initial symptoms of osteoporosis. The conventional ultrasonic wave evaluation induces the information of the multi-scale elastic properties in the large area, where the ultrasonic wave passed through. In order to understand the microscopic elastic properties without the effect of macroscopic structures, we have succeeded elastic anisotropy in a small part of bone using a micro-Brillouin scattering technique.<sup>[1]</sup>

The objective of this study is to investigate bone elastic properties in a minute area without the effect of macroscopic structures. Especially focusing on the wave velocity, we have estimated the elastic distribution in a trabecula.

**2. Experiment system**

Micro-Brillouin scattering measurements were performed by a six pass tandem Fabry-Perot interferometer (JRS scientific instruments) using an argon ion laser with the wave length of 514.5 nm. The micro-Brillouin scattering system contains microscope for Raman scattering. The actual diameter of the focused laser beam in the sample was approximately 10 μm. This spot diameter enables to evaluate elastic properties without the effect of bone structure.

The RIOA scattering geometry used is shown in Fig. 1. The interaction of incident and scattered lights enables the simultaneous measurement of the phonons that propagate in each direction of wave vector of  $q^{\Theta A}$  and  $q^{180}$  in one measurement. This geometry is attained by attaching a flat metal to the reverse side of the sample films as a reflector.

**3. Specimen and measurements**

In this study, the trabeculae in bovine femoral trabecular bone in the distal part (27 or 29- month-old) were used as specimens. Figure 2 shows the

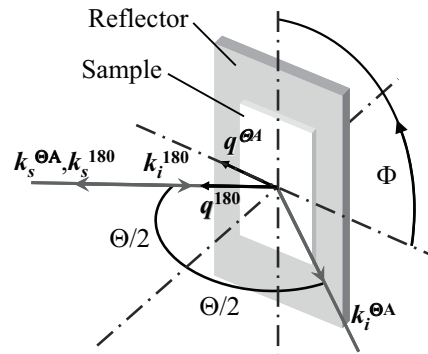


Fig. 1 RIOA scattering geometry:  $k_i$ : the wave vector of the incident light,  $k_s$ : the wave vector of the scattered light  $q_r$ : the wave vector of the sound wave,  $\Theta/2$ : the angle between the incident laser beam and the normal line of the sample surface.

specimen used. We prepared two types of specimens, where trabecular aligns along the bone axis direction (Fig. 2(a)) or anterior-posterior direction (Fig. 2(b)). In order to obtain enough transparency, thinly sliced specimens were well polished. The thickness of the specimens was around 150 μm.

We measured wave velocities in the trabecular alignment direction at 12 different positions in each trabecula. Furthermore, we defined average velocity of a trabecula. In addition, the orientation direction of each trabecula was compared with the wave velocity. Finally, wave velocity was measured by 5 μm intervals to examine details of the wave velocity distribution of a trabecula.

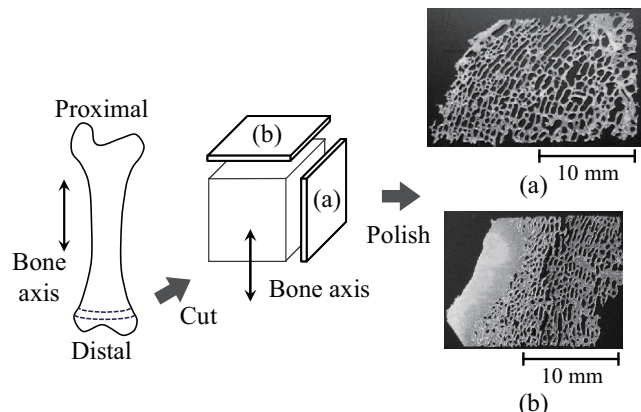


Fig. 2 The thin trabecular specimens.

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#### 4. Results and discussion

**Figure 3** shows a typical Brillouin scattering spectrum obtained from a trabecula. The longitudinal wave velocity in a trabecula was estimated as  $4.92 \times 10^3$  m/s. Wave velocity measurement error was approximately 1%.

**Figure 4** shows the wave velocity distribution in one trabecula. Complicated distribution was found in this result. There was no characteristic pattern in the velocity distribution. The measured longitudinal wave velocities were different at each measurement point, and the maximum difference of velocity was 250 m/s. From results of other trabeculae, there were velocity dispersions in range of 160-500 m/s. The measured wave velocities were in the range of  $4.58$ - $5.16 \times 10^3$  m/s in the trabeculae along the bone axis direction, whereas those along the anterior-posterior direction were  $4.60$ - $5.09 \times 10^3$  m/s. The average velocities are shown in **Fig. 5**. The difference was not statistically significant due to the trabecular direction, telling that the observed velocities in each trabecula did not depend on the trabecular direction.

**Figure 6** shows the wave velocity distribution measured by 5  $\mu\text{m}$  intervals in middle part of trabecula. Thus, we find that the wave velocity distribution is complicated in a minute area. One reason for this seems to come from inhomogeneous composition the collagen and hydroxyapatite in a trabecula. Rupin et al., also pointed the complicated distribution of acoustic impedance in a trabecula by scanning acoustic microscopy.<sup>[2]</sup>

#### 5. Conclusion

Wave velocities in a trabecula were measured by a micro Brillouin scattering method. The value was around  $4.92 \times 10^3$  m/s. Wave velocity distributions in trabeculae were complicated, and the effects of trabecular direction on the velocity were not statistically significant. This tells us the possibility that the average elastic properties are similar in all trabeculae.

#### 6. Acknowledgments

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#### References

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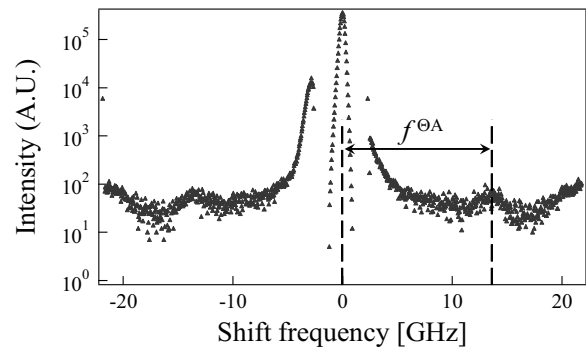


Fig. 3 Measurement spectrum of a trabecula.

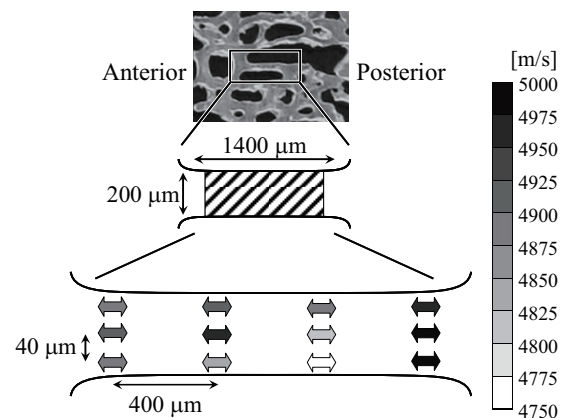


Fig. 4 Wave velocity distribution in a trabecula.

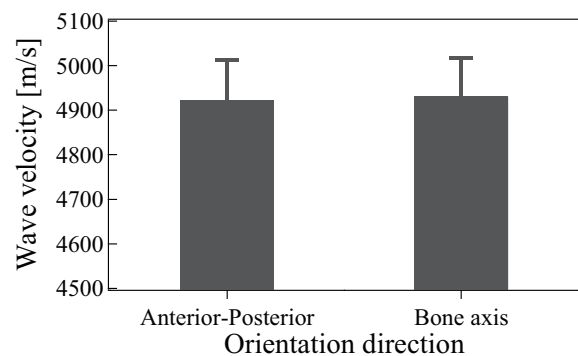


Fig. 5 The relations between the orientation direction and average velocity.

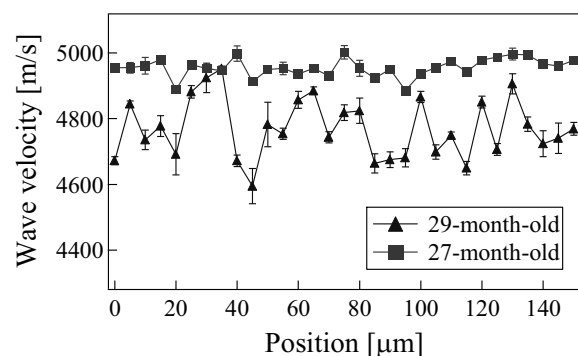


Fig. 6 Detailed wave velocity distributions of two trabeculae aligned in the anterior-posterior direction.