



# Application of a micro-Brillouin scattering technique to characterize bone in the GHz range



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## ABSTRACT

The evaluation of elastic properties of bone matrix has been investigated using several techniques such as nanoindentation and scanning acoustic microscopy (SAM). These techniques make use of good spatial resolution, which can prevent effects due to microstructures at the level of several hundreds of microns. In this paper, micro-Brillouin scattering ( $\mu$ -BR) is introduced as another possible technique to characterize the elastic properties of bone. This technique is well known as a non-contact and non-destructive method to evaluate viscoelastic properties of transparent materials in the GHz range. Using thin, translucent bone specimens with thicknesses of around 100  $\mu$ m, and the reflection induced optical geometry, ultrasonic wave velocities in the GHz range were obtained. Because this technique optically measures thermal phonons in the specimen, we can easily measure in-plane anisotropy of wave velocities by rotating the specimen. In a single trabecula, the site matched data between SAM and  $\mu$ -BR showed good correlation, revealing the applicability of this technique to characterize material properties of bone. Some recent results on the anisotropy in a trabecula and the elasticity evaluation of newly and matured bones are also introduced.

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## 1. Introduction

Brillouin light scattering is generally referred to as an interaction between light and thermally excited elastic waves called acoustic phonons. It was first investigated by Brillouin and

independently by Mandelshtam early in the 20th Century [1,2]. Because Brillouin scattering is an inelastic process, it is commonly used to measure spectral changes of scattered light, which can provide information on the properties of phonons in the medium. Because spectral changes are generally in the GHz range, the introduction of monochromatic laser light and high-contrast spectrometers are important tools for such measurements.

The characterization of material using Brillouin scattering or Brillouin spectroscopy has become a powerful tool for obtaining

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viscoelastic properties, which makes use of the non-contact and non-destructive characteristics of the technique [3–5]. However, most Brillouin spectroscopy studies only investigate transparent materials except for studies of surface acoustic waves in opaque solids [6,7] because light should interact with bulk acoustic phonons inside the material. The trend of Brillouin spectroscopy has thus been focused upon the characterization of transition processes, structural monitoring and environmental sensing of transparent materials. Of course, the ability of Brillouin scattering to obtain viscoelastic characteristics is very important for the investigation of biological tissue. Changes in the rheological properties of biological tissue can often be a symptom of disease, such as the increase in elasticity of arterial walls due to arteriosclerosis. After the early work of Harley [8], Cusack and Miller used Brillouin scattering to determine the elastic constants of rat-tail tendon collagen, which is assumed to be transversely isotropic [9]. Other transparent biological tissues, such as the lens of the eye, have also been investigated. Recently, a new *in vivo* Brillouin spectroscopy technique has been developed [10].

The first trial using Brillouin scattering technique for bone was reported by Lees et al. [11]. In addition to tendons, they performed measurements on deer antler and cow tibia using a high-performance Fabry–Perot interferometer. They reported velocities of 4.86 km/s at 11 GHz for a dried cow tibia specimen, which was much higher than the data of animal leg tendons and deer antler. The most important issue for Brillouin spectroscopy of bone is specimen transparency, which requires specimens that are sufficiently thin and have a smooth surface.

In this article, some recent Brillouin scattering studies on the characterization of bone are discussed. The optical microscope with a suitable optical geometry for very thin specimens have provided information on elastic properties with high spatial resolution. This enables analysis of relatively homogeneous volumes, which is expected to provide information on bone matrices. For *in vitro* measurements, nanoindentation and scanning acoustic microscopy (SAM) are also well known, and these methods provide information on elastic modulus, hardness and acoustic impedance in a minute area ( $\mu\text{m}$  order) [12–16]. The target area sizes of these measurements are similar to those of the micro-Brillouin scattering technique ( $\mu\text{-BR}$ ). Therefore, in this article, we first introduce a comparative study of site matched data of SAM and  $\mu\text{-BR}$  using an identical specimen. The article also followed other results of bone BR studies.

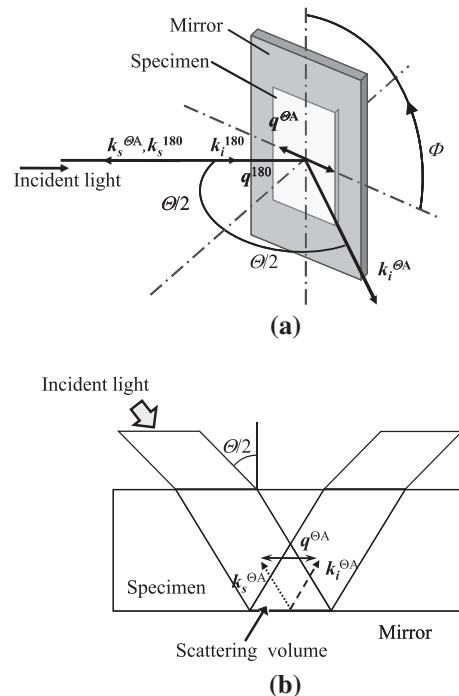
## 2. Brillouin scattering technique

As mentioned above, Brillouin scattering is the interaction between light and thermally excited acoustic phonons. Here, the thermal elastic wave (an acoustic phonon) is a modulation in the dielectric constant of the medium, which can be regarded as a moving diffraction grating for the incident light wave. Therefore, Brillouin scattering can be explained by the familiar concepts of Bragg reflection and Doppler shift. Brillouin spectroscopy measures spectral changes of scattered light. In other words, the light picks up viscoelastic information of the medium as a shift in frequency. Note that a frequency shift of Brillouin scattering photons are measured at a fixed phonon wavelength, whereas wavelength at a fixed ultrasonic frequency is measured using a conventional ultrasonic technique. In addition, measurement errors mainly result from the configuration of the optical incident angle of laser light and the evaluation of very weak Brillouin scattering peaks from thermal phonons. The difficulty of the measurement is to isolate the weak Brillouin scattered peaks from very intense Rayleigh peaks (elastic scattering) in the observed spectrum. Usually, the frequency shifts of Brillouin scattering are on the order of GHz, which

is too small to resolve with conventional spectrometers. In the 1980s, this spectral separation was successfully accomplished through the use of high-finesse scanning Fabry–Perot interferometers and then angle-dispersive etalons. One of the most famous interferometer systems is the six-pass tandem Fabry–Perot interferometer (TFPI) with piezoelectric scanning on a dynamic vibration isolation system. The exceptional long-term stability of the system allows for measurements of very weak scattering from thermal phonons.

Because Brillouin scattering is an interaction between phonons and photons, measurement requires a suitable choice of scattering geometries. In some geometries including back scattering, we need the refractive index  $n$  of the medium. The most convenient geometry for thin layers and films is the Reflection Induced  $\Theta$  Angle (RI $\Theta$ A) geometry developed by Krüger [17]. This geometry is an extension of right angle (90A) geometry and has a unique configuration with a mirror. In this geometry, we do not need the value  $n$  to obtain the wave velocity and we can measure both longitudinal and shear phonons simultaneously. As shown in Fig. 1, the reflected incident light and light scattered at the surface of the mirror interacts with phonons propagating in the in-plane direction of the specimen layer. Because the specimen is attached to a mirror, the heat conduction through the mirror helps to avoid an increase in the temperature of the specimen.

In this RI $\Theta$ A geometry, the interaction between incident and scattered light enables the measurement of longitudinal and shear phonons that propagate in each direction of the wave vectors  $q^{\Theta A}$  and  $q^{180}$  in one measurement.  $q^{180}$  can also be observed in conventional back scattering geometry. Here, the wave velocity of  $q^{\Theta A}$  is measured in the area where incident and reflected lights interfere. From the measured spectra, we obtain the frequency shifts  $f^{\Theta A}$  and  $f^{180}$ , which give us the wave velocities as:



**Fig. 1.** Configuration of the RI $\Theta$ A scattering geometry. (a) Optical geometry.  $k_i$  is the wave vector of the incident light,  $k_s$  the wave vector of the scattered light,  $q^{180}$  is the wave vector of the ultrasonic wave traveling in the direction of back scatter,  $q^{\Theta A}$  is the wave vector of the ultrasonic wave traveling in-plane,  $\Theta$  is the angle between the incident laser beam and the normal line of the specimen surface and  $F$  is the rotation angle in the plane. (b) The RI $\Theta$ A scattering geometry inside the specimen.

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