

# Brillouin Spectrum of a Bovine Bone

The mechanical properties of a polished bovine bone were optically probed using the LightMachinery Brillouin system. This technique analyzes the inelastic scattering of photons following interactions with acoustic phonons in a material.

April 2024

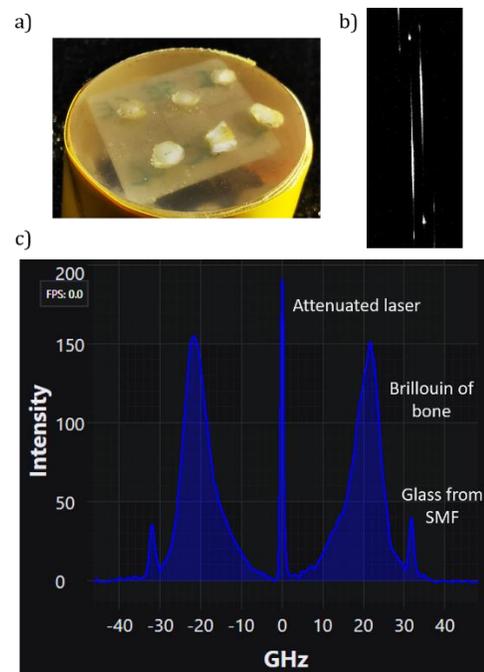
For questions, contact [hyperfine@lightmachinery.com](mailto:hyperfine@lightmachinery.com)

**Background.** Physical changes at the tissue and cellular levels are involved in the progression of several diseases including osteoporosis. Better characterizing these mechanical deregulations could advance our understanding of such pathologies<sup>1,2,3</sup>. Brillouin scattering spectroscopy is a contactless, label-free, and high-resolution technique that can probe the stiffness and viscosity of biological materials at the cellular level<sup>4</sup>. In this short note, we show that the LightMachinery Brillouin system is well adapted to probe opaque biological samples such as bones.

**Experiment.** The bone samples (provided by MSTAT LLC) were mounted in epoxy and polished down (5  $\mu\text{m}$  grit size) to obtain a flat and smooth surface (see Fig. 1 a). A Brillouin confocal microscope (HF-9000) with a 20X/0.4NA objective was employed to focus 20 mW of laser power (Cobolt 04-01 Series,  $\lambda = 532 \text{ nm}$ ) on the sample, and to collect the Brillouin signals in a backscattering geometry. To reduce the pump signal, the sample was tilted with respect to the excitation beam. The collected scattered light was coupled into a single mode fiber and connected to a LightMachinery HF-8999-PK-532 spectrometer (0.5 GHz resolution, > 110 overall effective contrast). Lorentzian profiles were fitted to extract the Brillouin peak positions.

**Results and discussion.** Brillouin scattering measurements were acquired at different locations within the bovine bone samples. A representative camera image and spectrum are presented in Fig. 1b-c. The extracted Brillouin frequency shift for this sample location is  $21.60 \pm 0.01 \text{ GHz}$  and the linewidth is  $7.29 \pm 0.02 \text{ GHz}$  (well resolved given the 0.5 GHz FWHM resolution). Upon sampling different positions, the bone samples appeared non-uniform in composition. This is most likely due to the complex microstructures composing the bone, which can be discerned and resolved given that the lateral size of our excitation

volume was approximately  $1 \mu\text{m}$ . Future work includes mapping the bone samples and comparing the frequency shift and linewidth maps with the brightfield images in search of both feature correlations and departures. We hope that the LightMachinery Brillouin system will help research teams to improve our understanding and diagnostic capabilities of bone diseases.



**Figure 1.** a) Mounted bovine bone samples (picture from D. Pederson). b) Small area of the camera image showing the Brillouin signals (broad peaks) and mostly suppressed pump peaks (dots). c) Corresponding Brillouin spectrum.

## References.

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4. Robert Prevedel et al. *Nature methods* 16, no. 10 (2019)