Microstructural characterization of trabecular bone using ultrasonic backscattering and diffusion parameters

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Abstract: Finite differences time domain methods were utilized to simulate ultrasound propagation and scattering in anisotropic trabecular bone structures obtained from high resolution Computed Tomography (CT). The backscattered signals were collected and the incoherent contribution was extracted. The diffusion constant was calculated for propagations along and across the main direction of anisotropy, and was used to characterize the anisotropy of the trabecular microstructures. In anisotropic structures, the diffusion constant was significantly different in both directions, and the anisotropy of the diffusion constant was strongly correlated to the structural anisotropy measured on the CT images. These results indicate that metrics based on diffusion can be used to quantify the anisotropy of complex structures such as trabecular bone.

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1. Introduction

Various quantitative ultrasound methods (QUSs) have been developed for the assessment of trabecular bone. Some are based on the measurement of the speed of sound (SOS), others on the measurement of the frequency-dependent attenuation (Broadband Ultrasound Attenuation or BUA), which have been shown to be correlated with bone density.^{1–3} Some devices are based on the measurement of parameters related to the fast and slow longitudinal waves modeled by Biot,⁴ which propagate in trabecular bone.^{5,6} These methods provide measurements related to bone stiffness, porosity, and density.^{7–10} However, the assessment of bone density alone is not enough for an early diagnosis of bone degradation. Although bone mineral density is a strong predictor of bone strength, other parameters such as trabecular microarchitecture influence bone mechanical competence. Two structures with different microarchitectures can have very different mechanical competences, and even more so in bone, in which selective improvements of mechanical properties will occur in the areas where mechanical strains are the greatest.¹¹ As a consequence, the bulk of research in the last decade has led to an updated definition of osteoporosis, now characterized by low bone mass and micro-architectural deterioration of bone tissue, which consists in changes in trabecular separation, thickness, connectivity, and anisotropy.^{12–15} A study showed a higher anisotropy in trabecular bone from individuals who suffered hip fracture than in bone from unfractured individuals, after controlling for bone volume.¹⁶ Although they measure parameters related to density, stiffness, and porosity, the QUS techniques described above generally do not provide metrics related to specific features of the trabecular micro-architecture. Measurements of the backscattered power in different propagation directions have been significantly correlated to the anisotropy of the apparent density,^{17,18} but only moderately ($p < 0.5$). Wear *et al.* showed that multiple regression models combining BUA, ultrasound velocity, and backscatter coefficient slightly improved the prediction of microstructural parameters.¹⁹ In another study by Chaffai *et al.*, the correlation between the ultrasound backscatter coefficient with parameters of the microstructure was higher and more significant ($r = 0.79$, $p < 10^{-4}$ between the backscatter coefficient and the trabecular separation). However, in this study, no significant independent association was found between structural parameters and the backscatter coefficient after adjustment for density.²⁰ In the present paper, we propose to use ultrasound multiple scattering (MS) to measure the anisotropy of trabecular structures. MS has been shown to occur in bone at 3 MHz for

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propagation distances greater than 8 mm,²¹ and models of MS have been proved to fit experimental data in bone mimicking phantoms.²² When MS occurs during propagation in heterogeneous structures such as bone, ultrasound backscattered signals contain both single scattering (SS) and MS contributions. In SS, the incident ultrasonic wave is subjected to only one scattering event before traveling back to the transducer. In this case, a clear relationship between time and distance exists, determined by the equation $d = c \cdot t/2$, where c is the wave velocity in the homogenized medium and t is the time of flight. In MS, the ultrasonic wave is subjected to many scattering events by the microstructure before returning to the receivers. In this case, one cannot rely on a direct relationship between propagation distance and time of flight. This makes imaging very difficult and traditional ultrasonic imaging is based on the assumption of SS, usually neglecting MS. This assumption is justified in soft tissue in the megahertz range, where MS is much weaker than SS. In bone however, when the contrast between the fluid and solid phases is large, MS can be exploited in the megahertz range.²³ During propagation in a complex medium such as trabecular bone, each scattering event is an opportunity for the ultrasonic wave to collect information on the micro-architectural and elastic properties of the microstructure. Over time, the backscattered signals are increasingly complex but also increasingly full of information.

In this paper, we propose to exploit the MS contribution to backscattered signals in order to retrieve the anisotropy of bone microstructures. The *Simsonic* FDTD freeware was used as a tool to simulate ultrasonic propagation and scattering in two-dimensional (2D) and three-dimensional (3D) microstructural maps of a trabecular horse bone obtained from high resolution μ -computed tomography (CT) images. A simulated 40-element transducer array was used to transmit ultrasonic pulses with a central frequency of 5 MHz, and receive ultrasonic backscattered responses. The coherent and incoherent contributions to the backscattered signals were separated according to a method described by Aubry and Derode.²⁴ The temporal and spatial variations of the incoherent intensity are a growing diffusive halo whose width grows over time as $\sqrt{2Dt}$. Tracking the growth of the diffusive halo over time therefore enabled the measurement of the diffusion constant D , for the propagation in each direction. The anisotropy of the diffusion constant was quantified and compared to the structural anisotropy of the trabecular microstructures. As a comparison, the anisotropy of the SOS was measured for propagation in both directions and was found to be much less sensitive to the structural anisotropy than the diffusion constant.

2. Materials and methods

2.1 Horse bone microstructures

Trabecular horse bone from the femoral epiphysis was imaged using propagation phase contrast Synchrotron microtomography. The horse bone specimen was scanned at the ID19 beamline of the European Synchrotron Radiation Facility (Grenoble, France) according to a method described in Mézière *et al.*,²³ with a pixel size of 12.64 μm . The images were reconstructed using algorithms described by Paganin *et al.*²⁵ and Mirone *et al.*,²⁶ and binarized using MATLAB (Mathworks®, Natick, MA) in order to keep only the trabecular bone structure, according to a method described in Ref. 23.

Twenty-five microstructural maps of trabecular bone were used to simulate ultrasonic propagation and scattering. Each simulation map had 1301×1301 grid steps. The spatial grid step Δx was set to 10 μm . The microstructures exhibited different degrees of anisotropy: as an example, the microstructure shown in Fig. 1(a) is relatively isotropic, while the microstructure shown in Fig. 1(b) is highly anisotropic. The structural anisotropy of each map was quantified by calculating the normalized autocorrelation function of the images in the x and y directions, respectively. A threshold of 50% was set to measure the widths of mean autocorrelation functions measured along the x and y directions for each map. The normalized ratio of widths of autocorrelation functions in both directions, denoted as $R_{st} = |W_x - W_y| / (W_x + W_y)$ is a metric of the structural anisotropy of microstructures. Theoretically, the R_{st} value should be close to 0 for an isotropic microstructure, and between 0 and 1 for an anisotropic structure. As an example, the R_{st} values measured for the structures shown in Figs. 1(a) and 1(b) are 0.05 and 0.36, respectively.

2.2 Simulation of ultrasound propagation in models of horse bone

2D and 3D simulations of ultrasound propagation in these structures were performed using the Finite Differences Simsonic Freeware.²⁷ The CT scans were used to create binary maps representing trabecular bone structures in water, for which the material properties, obtained from Ref. 28 are described in Table 1. Simulations of ultrasound

Fig. 1. Example of CT slices used to simulate the ultrasound propagation and scattering in isotropic (a) and anisotropic (b) bone.

propagation were run independently in these 2D (single CT slice) and 3D structures (stack of 300 CT slices). Isotropic and anisotropic maps were visually identified and the 3D maps were rotated such that the main direction of anisotropy was parallel to the x axis. A linear transducer array with 40 elements was simulated with a central frequency of 5 MHz and a 100% -6 dB bandwidth, and successively placed 5 mm away from the sample, parallel to the x and y axis to transmit ultrasonic pulses into the medium. The Impulse Response Matrix was acquired according to the following method described in Derode *et al.*²¹ An ultrasonic Gaussian pulse with a central frequency of 5 MHz was emitted into the scattering medium from element $\#i$ of the transducer array. The backscattered signals were recorded on all of the elements of the transducer array. The process was repeated and all elements were successively used to transmit, which led to an $N \times N \times t$ ultrasonic impulse response matrix $H_{ij}(t)$ ($i, j = 1, \dots, 40$). The matrix $H_{ij}(t)$ is symmetric due to spatial reciprocity. The received backscattered signals were time-shifted to compensate for the arrival time difference due to varying distances between transmitters and receivers, so that the first backscattered wave front arrived at $t=0$ on each element. To characterize the anisotropy, the transducer was successively placed along the x and y axes, to simulate propagation in the x and y directions, respectively.

2.3 Incoherent backscattering intensity—Measurement of the diffusion constant

Using the Impulse Response Matrix, the incoherent backscattering intensity was computed according to a method described by Aubry and Derode.²⁴ The reciprocity of the Impulse Response Matrix was exploited and an antisymmetric matrix $H_{ij}^A(t)$ was created such that $H_{ij}^A = H_{ij}$ for $i > j$, $H_{ii}^A = 0$ for $i = j$, and $H_{ij}^A = -H_{ji}$ for $i < j$. The intensities I^A and I were obtained from $\mathbf{H}^A(\mathbf{t})$ and $\mathbf{H}(\mathbf{t})$, respectively, by integrating the squares of the backscattered signals over 1 μ s long time windows. The incoherent backscattered intensity was given by $I_{\text{inc}} = I + I^A/2$, as described by Aubry and Derode.²⁴ The temporal and spatial variations of the incoherent intensity are a growing diffusive halo which width grows over time as $\sqrt{2Dt}$. Tracking the growth of the diffusive halo over time therefore enabled the measurement of the diffusion constant D , for a propagation in each direction. The anisotropy of the diffusion constant was defined as $R_D = |D_x - D_y| / (D_x + D_y)$. The diffusion constants could not be measured accurately in 2 of the 25 maps, due to the presence of SS signals with large amplitude, impairing the growth of the diffusive halo over time.

2.4 Speed of sound measurements

Plane waves (1 and 5 MHz) were transmitted through the microstructures in the x and y directions using all 40 elements of the simulated transmitter. On the other end of the sample, a large receiver was simulated, in order to receive the coherent wave. The time of flight of the first arriving signal was measured, which allowed to derive the effective SOS in the structures in both directions. The anisotropy of the SOS was defined as the

Table 1. Density, elastic constants, wave velocities in bone and water (values from Ref. 27).

	Density (mg/mm ³)	c_L (mm/us)	c_{11} (GPa)	c_{12} (GPa)	C_{66} (GPa)
Water	1.0	1.27	2.37	2.37	0
Trabeculae	1.85	4.0	29.6	17.6	6

normalized ratio of the time of flight of the first arriving signal in both directions:
 $R_V = |V_x - V_y| / V_x + V_y$.

3. Results

Figure 2 shows the evolution of the width of the incoherent part of the signal as a function of time when ultrasonic wave propagated along the x (circles) and y (squares) axes of the microstructural maps shown in Figs. 1(a) and 1(b), respectively, for 2D simulations. It can be observed that, as expected, the MS contribution increases progressively with propagation time, as the width of the diffusive halo progressively grows. The diffusion constant can be retrieved from the slope of these curves. In the relatively isotropic microstructure shown in Fig. 1(a), the slopes are very similar, which indicates that the diffusion constant is very similar for waves propagating in the x and y directions. On the contrary, in anisotropic microstructures such as the one shown in Fig. 1(b), the diffusion constant for a propagation along the y axis is much higher than for a propagation along the x axis. This indicates a stronger contribution of MS in the y direction, which can be attributed to a smaller trabecular separation in the y direction and results in smaller diffusion constants when the waves propagate along the y axis, in anisotropic maps. The metric of anisotropy R_D defined above was compared to the structural anisotropy ratio R_{st} .

Figure 3 shows the relationships between the structural anisotropy R_{st} and the scattering anisotropy measured using R_D for 2D simulations. A strong and significant correlation was found between this metric and the structural anisotropy ($r=0.87$, $p=7.6 \times 10^{-8}$).

No SOS measurements could be performed at 5 MHz, which was the frequency used to assess the diffusion constant. This is due to the fact that, at this frequency, the receiver was not large enough to cancel the signal fluctuations due to MS, which impaired the accurate measurement of a time of flight. At 1 MHz, the time of flight for a plane wave through transmission could be accurately measured. The anisotropy of the SOS R_V was only moderately correlated to the structural anisotropy ($r=0.65$, $p=0.0043$).

4. Discussion

The significant correlations obtained between R_D and R_{st} suggest that measuring parameters derived from ultrasound MS can provide access to the structural anisotropy. The anisotropy of the SOS was a poorer indicator of the structural anisotropy than the anisotropy of the parameters related to MS. This could be related to the fact that the SOS measurements were performed at a lower frequency (1 MHz instead of

Fig. 2. Width of the diffusive halo as a function of time. The rate of the width of the diffusive halo over time gives access to the diffusion constant. Top: propagation in the isotropic map shown in Fig. 1(a). The slopes giving the diffusion constant are identical for a propagation along the x and y axes. Bottom: propagation in the anisotropic map shown in Fig. 1(b). The slopes giving the diffusion constant are different for a propagation along the x and y axes. The circles and the squares represent ultrasonic propagation along the x and y axis, respectively.

Fig. 3. Correlation between the structural anisotropy (widths ratio of the autocorrelation functions of the CT images in both directions) and the scattering anisotropy parameters. In both cases, the relationship seems to be linear, and the correlation is significant. Left: correlation with the RMSS ratio ($r=0.82$, $p=1.3 \times 10^{-6}$). Right: correlation with the RD ratio ($r=0.87$, $p=7.6 \times 10^{-8}$).

5 MHz). Nevertheless, these results suggest that parameters related to MS such as the diffusion constant are better suited for the assessment of the trabecular anisotropy than more conventional parameters such as the SOS. In the present study, we find that the structural anisotropy is moderately correlated to the velocity measured at 1 MHz ($r=0.65$, $p=0.0043$). These results are consistent with the results published by Gluer *et al.*²⁹ and by Hans *et al.*,³⁰ which also showed moderate ($r=0.36$, $p<0.007$)²⁹ or insignificant³⁰ correlations between the structural anisotropy and the SOS around 500 kHz and at 11.25 MHz, respectively.

The frequencies chosen in this study (5 MHz) are higher than the frequencies typically used for bone assessment (1 MHz or less). By choosing a higher frequency range, the objective was to promote MS in the structures. Ultrasound MS had previously been observed in trabecular bone at 3 MHz for propagation distances larger than 8 mm.²¹ Although the presence of dispersion due to scattering is known to have an effect on sound speed measurements,³¹ the time-of-flight measurements performed here were only compared for different directions of propagation.

As expected, the diffusion constant grew faster with time in the 3D simulations than in the 2D simulations, as MS in all directions was allowed. 3D simulations still allow the observation of differences in the diffusion constants when the waves propagate along or across the direction of anisotropy. However, these differences are not as marked as in the 2D simulations, and the directions of propagation had to be aligned parallel and perpendicular to the preferential direction of anisotropy. This is not unrealistic in bone, where the direction of anisotropy is known *a priori*: the skeleton is subjected to anisotropic stresses due to gravity, and the horizontal trabeculae are the first to disappear.¹⁶

The objective of this study was to establish the proof of concept for a new approach for the assessment of anisotropy in trabecular bone, based on ultrasound MS. We have proposed a metric R_D based on the diffusion constant, which was found significantly correlated to the structural anisotropy of trabecular structures obtained using CT scans. Measuring this metric will be useful in *in vitro* studies focusing on anisotropy. For *in vivo* applications, the impact of a layer of soft tissue will be established in a subsequent study. The effect of the cortical layer on MS has not been the focus of the present study. However, because the approach presented here relies on the assessment of the growth of the diffusive halo over time, it is likely that the addition of a cortical layer to the trabecular structure will only delay the growth of the diffusive halo, and will not impact the value of the measured diffusion constant. The calcaneum would be an appropriate site for measurements around 3 or 5 MHz. This study has a few limitations: first, the direction of anisotropy must be known *a priori*, as the ultrasound pulses must be transmitted along and across this direction. A second limitation of this study is that the simulations did not account for absorption, which might impact the amount of MS. The results found in the literature on the effect of absorption on propagation in trabecular bone are contradictory. Haiat *et al.*,²² using a model of MS, found that accounting for absorption in the trabeculae had very little effect on

the dispersion curves, but that accounting for absorption in the marrow had a stronger impact. On the contrary, the experimental results of Pakula *et al.* showed that replacing the marrow by water had no effect on the propagation.³² However, it is undeniable that in a higher frequency range such as the one used in the present study, the losses over the long propagation paths involved in MS could be important. An experimental study remains to be conducted in order to account for losses by absorption and for true 3D propagation and scattering. This will be the focus of our future work. If the propagation paths were getting shorter due to losses, preventing MS, this could be circumvented by using groups of elements for transmit instead of single elements. The method presented here is innovative and we show that ultrasound MS has the potential to quantitatively characterize micro-architectural properties of trabular bone such as anisotropy.

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References and links

- ¹B. Fournier, C. Chappard, C. Roux, G. Berger, and P. Laugier, "Quantitative ultrasound imaging at the calcaneus using an automatic region of interest," *Osteoporosis Int.* **7**(4), 363–369 (1997).
- ²C. Cepollaro, S. Gonnelli, A. Montagnani, C. Caffarelli, A. Cadirni, S. Martini, and R. Nuti, "In vivo performance evaluation of the Achilles Insight QUS device," *J. Clinical Densitometry* **8**(3), 341–346 (2005).
- ³C. M. Langton, S. B. Palmer, and R. W. Porter, "The measurement of broadband ultrasonic attenuation in cancellous bone," *Eng. Med.* **13**(2), 89–91 (1984).
- ⁴M. A. Biot, "Theory of propagation of elastic waves in a fluid-saturated porous solid. II. Higher frequency range," *J. Acoust. Soc. Am.* **28**(2), 179–191 (1956).
- ⁵T. Otani, "Quantitative estimation of bone density and bone quality using acoustic parameters of cancellous bone for fast and slow waves," *Jpn. J. Appl. Phys.* **44**(6S), 4578–4584 (2005).
- ⁶I. Mano, K. Horii, S. Takai, T. Suzuki, H. Nagaoka, and T. Otani, "Development of novel ultrasonic bone densitometry using acoustic parameters of cancellous bone for fast and slow waves," *Jpn. J. Appl. Phys.* **45**(5S), 4700–4702 (2006).
- ⁷C. F. Njeh, R. Hodgkinson, J. D. Currey, and C. M. Langton, "Orthogonal relationships between ultrasonic velocity and material properties of bovine cancellous bone," *Med. Eng. Phys.* **18**(5), 373–381 (1996).
- ⁸G. Haiat, F. Padilla, R. Barkmann, S. Dencks, U. Moser, C. C. Glüer, and P. Laugier, "Optimal prediction of bone mineral density with ultrasonic measurements in excised human femur," *Calcified Tissue Int.* **77**(3), 186–192 (2005).
- ⁹F. Jenson, F. Padilla, V. Bousson, C. Bergot, J. D. Laredo, and P. Laugier, "In vitro ultrasonic characterization of human cancellous femoral bone using transmission and backscatter measurements: Relationships to bone mineral density," *J. Acoust. Soc. Am.* **119**(1), 654–663 (2006).
- ¹⁰R. Barkmann, S. Dencks, P. Laugier, F. Padilla, K. Brixen, J. Ryg, and C. C. Glüer, "Femur ultrasound (FemUS)—First clinical results on hip fracture discrimination and estimation of femoral BMD," *Osteoporosis Int.* **21**(6), 969–976 (2010).
- ¹¹C. H. Turner, "Three rules for bone adaptation to mechanical stimuli," *Bone* **23**(5), 399–407 (1998).
- ¹²J. F. Griffith, K. Engelke, and H. K. Genant, "Looking beyond bone mineral density," *Annals N.Y. Acad. Sci.* **1192**(1), 45–56 (2010).
- ¹³M. Hahn, M. Vogel, M. Pompesius-Kempa, and G. Delling, "Trabecular bone pattern factor—A new parameter for simple quantification of bone microarchitecture," *Bone* **13**(4), 327–330 (1992).
- ¹⁴NIH, "NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, March 7–29, 2000: Highlights of the conference," *South Med. J.* **94**(6) 569–573 (2001).
- ¹⁵M. Brandi, "Microarchitecture, the key to bone quality," *Rheumatology* **48**(4), iv3–iv8 (2009).
- ¹⁶T. E. Ciarelli, D. P. Fyhrie, M. B. Schaffler, and S. A. Goldstein, "Variations in three-dimensional cancellous bone architecture of the proximal femur in female hip fractures and in controls," *J. Bone Min. Res.* **15**(1), 32–40 (2000).
- ¹⁷B. K. Hoffmeister, S. A. Whitten, and J. Y. Rho, "Low-megahertz ultrasonic properties of bovine cancellous bone," *Bone* **26**(6), 635–642 (2000).
- ¹⁸K. A. Wear, "Anisotropy of ultrasonic backscatter and attenuation from human calcaneus: Implications for relative roles of absorption and scattering in determining attenuation," *J. Acoust. Soc. Am.* **107**(6), 3474–3479 (2000).
- ¹⁹K. A. Wear, S. Nagaraja, M. L. Dreher, and S. L. Gibson, "Relationships of quantitative ultrasound parameters with cancellous bone microstructure in human calcaneus in vitro," *J. Acoust. Soc. Am.* **131**(2), 1605–1612 (2012).

- ²⁰S. Chaffai, F. Peyrin, S. Nuzzo, R. Porcher, G. Berger, and P. Laugier, "Ultrasonic characterization of human cancellous bone using transmission and backscatter measurements: Relationships to density and microstructure," *Bone* **30**(1), 229–237 (2002).
- ²¹A. Derode, V. Mamou, F. Padilla, F. Jenson, and P. Laugier, "Dynamic coherent backscattering in a heterogeneous absorbing medium: Application to human trabecular bone characterization," *Appl. Phys. Lett.* **87**(11), 114101 (2005).
- ²²G. Haiat, A. Lhemery, F. Renaud, F. Padilla, P. Laugier, and S. Naili, "Velocity dispersion in trabecular bone: Influence of multiple scattering and of absorption," *J. Acoust. Soc. Am.* **124**(6), 4047–4058 (2008).
- ²³F. Mézière, P. Juskova, J. Woittequand, M. Muller, E. Bossy, L. Malaquin, and R. Boistel, "Experimental observation of ultrasound fast and slow waves through three-dimensional printed trabecular bone phantoms," *J. Acoust. Soc. Am.* **139**(2), EL13–EL18 (2016).
- ²⁴A. Aubry and A. Derode, "Ultrasonic imaging of highly scattering media from local measurements of the diffusion constant: Separation of coherent and incoherent intensities," *Phys. Rev. E* **75**(2), 026602 (2007).
- ²⁵D. Paganin, S. Mayo, T. Gureyev, P. Miller, and S. Willkins, "Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object," *J. Microscopy* **206**, 33–40 (2002).
- ²⁶A. Mirone, E. Brun, E. Gouillart, P. Tafforeau, and J. Kieffer, "The PyHST2 hybrid distributed code for high speed tomographic reconstruction with iterative reconstruction and *a priori* knowledge capabilities," *Nucl. Instrum. Methods Phys. Res. B* **324**, 41–48 (2014).
- ²⁷E. Bossy, F. Padilla, F. Peyrin, and P. Laugier, "Three-dimensional simulation of ultrasound propagation through trabecular bone structures measured by synchrotron microtomography," *Phys. Med. Biol.* **50**(23), 5545–5556 (2005).
- ²⁸R. N. McCarthy, L. B. Jeffcott, and R. N. McCartney, "Ultrasound speed in equine cortical bone: Effects of orientation, density, porosity and temperature," *J. Biomech.* **23**(11), 1139–1143 (1990).
- ²⁹C. C. Gluer, C. Y. Wu, M. Jergas, S. A. Goldstein, and H. K. Genant, "Three quantitative ultrasound parameters reflect bone structure," *Calcified Tissue Int.* **55**(1), 46–52 (1994).
- ³⁰D. Hans, C. Wu, C. F. Njeh, S. Zhao, P. Augat, D. Newitt, and H. K. Genant, "Ultrasound velocity of trabecular cubes reflects mainly bone density and elasticity," *Calcified Tissue Int.* **64**(1), 18–23 (1999).
- ³¹K. A. Wear, "The effects of frequency-dependent attenuation and dispersion on sound speed measurements: Applications in human trabecular bone," *IEEE Trans. Ultrason., Ferroelect., Freq. Control* **47**(1), 265–273 (2000).
- ³²M. Pakula, F. Padilla, and P. Laugier, "Influence of the filling fluid on frequency-dependent velocity and attenuation in cancellous bones between 0.35 and 2.5 MHz," *J. Acoust. Soc. Am.* **126**(6), 3301–3310 (2009).