Effect of gate choice on backscatter difference measurements of cancellous bone

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A variety of ultrasonic techniques have been developed to detect changes in bone caused by osteoporosis. One approach, called the backscatter difference technique, analyzes the power difference between two different portions of a backscatter signal. Analysis gates with a certain delay τ_d , width τ_w , and separation τ_s are used to define portions of the backscatter signal for analysis. The goal of the present study was to investigate how different choices of τ_d , τ_w , and τ_s affect four backscatter difference parameters: the normalized mean of the backscatter difference (nMBD), the normalized slope of the backscatter difference (nSBD), the normalized intercept of the backscatter difference (nIBD), and the normalized backscatter amplitude ratio (nBAR). Backscatter measurements were performed on 54 cube shaped specimens of human cancellous bone. nMBD, nSBD, nIBD, and nBAR were determined for 34 different combinations of τ_d , τ_w , and τ_s for each specimen. nMBD and nBAR demonstrated the strongest correlations with apparent bone density ($0.48 \leq R_s \leq 0.90$). Generally, the correlations were found to improve as $\tau_w + \tau_s$ was increased and as τ_d was decreased. Among the four backscatter difference parameters, the measured values of nMBD were least sensitive to gate choice ($<16\%$). © 2017 Acoustical Society of America.

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I. INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone density and microstructural deterioration of bone tissue that increases bone fragility and fracture risk. Worldwide, 158×10^6 individuals aged 50 years or over are estimated to be at a high risk for osteoporotic fracture, and the number is expected to double by 2040.¹ Approximately two-thirds of osteoporotic fractures occur at the hip and spine, resulting in chronic pain, deformity, and significant disability.² More than half of individuals who break a hip never regain post-injury status.^{3–5}

Diagnosis of osteoporosis is based on bone mineral density (BMD) measurements of the hip and spine using dual energy x-ray absorptiometry (DXA). However, ultrasound devices may be used to pre-screen patients for osteoporosis.⁶ In particular, devices that measure the speed of sound and attenuation of ultrasonic pulses propagated through the heel (calcaneus) have been used for this purpose for several years. Other clinically deployed techniques include through-transmission measurements at other skeletal locations such as the finger phalanges, axial transmission measurements of guided waves propagated along bones such as the radius and pulse-echo measurements of cortical bone thickness. New techniques are emerging rapidly.

Many research efforts are focusing on limitations associated with ultrasonic bone assessment techniques currently in use. As an example, nearly all ultrasonic devices in clinical use measure peripheral skeletal sites such as the heel.

They cannot perform ultrasonic measurements at the hip (proximal femur) and spine, locations that are considered optimal for predicting fractures and monitoring treatment.⁷ As another example, most ultrasonic devices are unable to generate diagnostic images of bone, forfeiting a potentially useful capability that ultrasound has to offer. Most importantly, ultrasonic techniques in clinical use have not demonstrated conclusively that they provide superior diagnostic information compared to that provided by DXA. Indeed, DXA remains the standard of reference for the clinical diagnosis of osteoporosis.

To address these limitations, some investigators are exploring the use of through-transmission techniques that propagate ultrasonic pulses through the neck and trochanter of the femur to measure normalized broadband ultrasonic attenuation (nBUA), speed of sound (SOS), and parameters related to the time of flight of the pulses.^{8,9} Other investigators have suggested that backscatter measurements of cancellous bone may offer a useful approach. Early backscatter studies of bone include measurements of the integrated backscatter coefficient (IBC),¹⁰ relative ultrasonic backscatter,¹¹ broadband ultrasonic backscatter (BUB),¹² the backscatter coefficient,^{13–15} the logarithm of the backscatter coefficient (LBC),¹⁶ and apparent integrated backscatter (AIB).¹⁷ Backscatter is produced when an ultrasonic wave interacts with the trabecular microstructure of cancellous bone. Backscatter measurements are convenient to perform because the same transducer can be used as both a transmitter and receiver. Clinically, this may facilitate easier access to central skeletal sites such as the hip and spine. In addition, it may be possible to perform backscatter measurements of

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bone with conventional or modified ultrasonic imaging systems.^{11,18–21}

Several studies have evaluated AIB as a bone assessment parameter. Compared to the backscatter coefficient and parameters based closely on the backscatter coefficient (e.g., IBC, BUB, and LBC), AIB is easier to measure *in vivo* because it does not require knowledge of the attenuating characteristics of tissues that lie along the path of propagation of the ultrasonic wave. AIB has been shown to be sensitive to the density, microstructure, mechanical properties, and composition of cancellous bone.^{17,22–28} In addition, AIB measurements have been performed successfully *in vivo* on human volunteers.^{29,30} However, recent studies have found that AIB possesses some significant limitations as a bone assessment parameter. For example, intervening tissues that lie between the transducer and ultrasonically interrogated region may produce errors in AIB measurements of bone.³¹ In addition, recent studies have shown that AIB may depend strongly on the portion of the backscatter signal that is analyzed.^{32,33}

AIB is determined by measuring the power in a portion of the backscatter signal defined by a “gate” that is used to select that portion for analysis. Measured values of AIB were shown to decrease by as much as 40 dB as the center of the gate was shifted to later times on the backscatter signal.³² For comparison, density variations in human cancellous bone typically produce less than a 10 dB difference in AIB.^{23,32,34} In addition, studies have found that AIB can correlate positively with bone density when the gate is located on early portions of signal, and negatively when it is placed on later portions of the signal. Intermediate gate locations may produce no correlations with density.^{32,33} These features generated confusion because investigators who used gates of different sizes and locations obtained different, and seemingly contradictory, results. It is understood now that results obtained for AIB depend strongly on gate choice. Measured values of another parameter called the frequency slope of apparent backscatter (FSAB) also were found to depend on gate choice.³² As new backscatter parameters are developed, it is important to consider the potential effects of gate choice.

A new set of backscatter parameters was introduced recently based on an approach called the backscatter difference technique. Rather than analyzing the power in one portion of the backscatter signal, the backscatter difference technique analyzes the power difference between two gated portions of the same backscatter signal. Parameters based on this technique have been shown to correlate strongly with bone density, and to be relatively insensitive to errors caused by intervening tissues that lie between the transducer and ultrasonically interrogated region of bone.^{31,35} In addition, theoretical considerations have led to a concept called “normalization” that may reduce the dependence of backscatter difference parameters on the size and location of analysis gates applied to the backscatter signal.³⁵ These features address the major limitations of AIB as a bone assessment parameter, while preserving conveniences associated with AIB such as ease of measurement, especially *in vivo*. Consequently, interest in using backscatter difference

parameters for bone assessment purposes may increase in the future.

Given that gate choice has been a source of confusion in past studies of backscatter parameters such as AIB, it is important to carefully investigate the effects of gate choice on parameters based on the newly developed backscatter difference technique. The present study is the first study to systematically investigate the effects of gate choice over the full range of gate options allowed by signal to noise considerations. Effects of gate choice are evaluated for four backscatter difference parameters: the normalized mean of the backscatter difference (nMBD), the normalized slope of the backscatter difference (nSBD), the normalized intercept of the backscatter difference (nIBD), and the normalized backscatter amplitude ratio (nBAR). These four parameters represent all of the backscatter difference parameters that have been applied to bone and reported in the literature so far. These parameters are analyzed for 34 different gate choices applied to backscatter signals from 54 specimens of human cancellous bone.

II. METHODS

A. Specimen preparation

A total of 54 cube shaped specimens of cancellous bone were prepared from the proximal ends of 14 left human femurs (8 females, 6 males, age = 49 ± 10 years). A band saw was used to cut the specimens in the shape of cubes with side lengths of 10 mm, after which a water jet was used to remove marrow from the intertrabecular spaces. As shown in Fig. 1, the axes of the cubes were aligned with the principal anatomic axes of the proximal femur: anterior-posterior, medial-lateral, proximal-distal. The cutting procedure involved sectioning the femur into 10 mm slices perpendicular to the proximal-distal axis [illustrated by the dashed lines in Fig. 1(a)] that were further sectioned into cubes. Specimens were obtained from the neck as well as the trochanter. Two to seven specimens were chosen from each femur for ultrasonic measurements. Reasons for specimen rejection included misaligned faces, non-uniform porosity across the specimen and the presence of defects such as cortical bone inclusion. The finished specimens were stored in refrigerated phosphate buffered saline solution. Ultrasonic testing commenced approximately one month after specimen preparation was completed.

B. Ultrasonic backscatter measurements

Bone specimens were placed on the bottom of a water filled tank after being vacuum degassed for ten minutes. Ultrasonic backscatter measurements were performed using a broadband 3.5 MHz transducer (Panametrics V382, Waltham, MA, USA) with a 12.7 mm diameter aperture and a 1.8 mm beam diameter at the focal distance of 50.8 mm. The transducer was mounted to the end of a vertical arm connected to a custom three axis mechanical scanner that was used to scan the transducer over the specimen. The transducer was connected to an ultrasonic pulser/receiver (Panametrics 5077PR, Waltham, MA, USA), and the received signals were digitized

FIG. 1. (Color online) Measurements were performed on 54 cube shaped specimens of bone prepared from the proximal end of 14 left human femurs. (a) Solid lines indicate principal anatomic axes of the proximal femur. Dashed lines indicate how the femur was sectioned into slices that were further sectioned to yield 10 mm cubes of bone. (b) The axes of the finished specimens were aligned along the principal anatomic axes of the femur. (c) A photograph of two finished specimens.

by a 12-bit digitizer (PicoScope 5244A, Pico Technology, Wales, England) operating at 500 MSa/s. Ultrasonic signals were analyzed from an 8×8 mm, 40×40 point region of interest (ROI) centered on the specimen. Measurements were performed along all six directions (anterior, posterior, medial, lateral, proximal, distal). Backscatter signals were digitized from each site of the scan and saved for analysis.

C. Signal analysis

Signals were analyzed using software developed in LABVIEW (National Instruments, Austin, TX, USA). As shown in Fig. 2(a), analysis gates labeled 1 and 2 were used to select two different portions of the signal for analysis from which the power spectra $P_1(f)$ and $P_2(f)$ were determined [Figs. 2(b) and 2(c)]. The first analysis gate was delayed by an amount τ_d from the start of the signal. The two gates had the same duration (i.e., width) τ_w and were separated by an amount τ_s as shown. A rectangular windowing function was used for all gates. The spectra $P_1(f)$ and $P_2(f)$ were converted to dB and subtracted to obtain the backscatter difference spectrum $D(f)$ [Fig. 2(d)],

$$\begin{aligned} D(f) &= 10 \log_{10} P_1(f) - 10 \log_{10} P_2(f) \\ &= 10 \log_{10} \frac{P_1(f)}{P_2(f)}. \end{aligned} \quad (1)$$

The difference spectrum was divided by the time between the centers of the two gates, $\tau_w + \tau_s$, to obtain a normalized difference spectrum $nD(f) = D(f)/(\tau_w + \tau_s)$ [Fig. 2(e)]. Three backscatter difference parameters were determined

from $nD(f)$. The normalized mean of the backscatter difference (nMBD) was determined by frequency averaging $nD(f)$ from 2 to 5 MHz, a frequency range based on the -6 dB bandwidth of the transducer. The normalized slope of the backscatter difference (nSBD) and the normalized intercept of the backscatter difference (nIBD) were determined from the slope and intercept, respectively, of a line fitted to the normalized difference spectrum $nD(f)$ over the same 2 to 5 MHz frequency range.

The backscatter signals also were analyzed in the time domain to determine a parameter called the normalized backscatter amplitude ratio (nBAR). nBAR depends on the ratio of the root mean square voltage (V_{rms}) of the first and second gated portions of the backscatter signal,

$$nBAR = \frac{\log_{10}(V_{rms1}/V_{rms2})}{(\tau_w + \tau_s)}, \quad (2)$$

where V_{rms} is defined over a time interval Δt by

$$V_{rms} = \sqrt{\int_{t_0}^{t_0 + \Delta t} V^2(t) dt / \Delta t}. \quad (3)$$

Like the other three parameters (nMBD, nSBD, and nIBD), nBAR is sensitive to the power difference between the two gated portions of the backscatter signal.

To investigate the effects of gate choice on the four backscatter difference parameters, τ_d , τ_w , and τ_s were varied in $1 \mu s$ increments. Values for τ_d , τ_w , and τ_s ranged from $2-7 \mu s$, $1-3 \mu s$, and $0-5 \mu s$, respectively. To ensure good signal to noise ratio (SNR), gate choices were required to satisfy the criterion $SNR > 10$ dB for all specimens. A total of 34 different gate choices (i.e., combinations of τ_d , τ_w , and τ_s) satisfied this criterion.

D. Signal to noise ratio measurements

The signal to noise ratio was computed in decibels according to the following equation:

$$SNR = \left(\frac{1}{f_{high} - f_{low}} \right) \int_{f_{low}}^{f_{high}} [10 \log_{10} P_{bone}(f) - 10 \log_{10} P_{noise}(f)] df. \quad (4)$$

$P_{noise}(f)$ was determined by placing a $1 \mu s$ gate on the signal starting $2 \mu s$ before the echo from the front surface of the bone specimen. $P_{bone}(f)$ was determined from a $1 \mu s$ gated portion of the backscatter signal from the specimen. The front edge of the gate was delayed ($2-10 \mu s$, $1 \mu s$ steps) relative to the front surface echo to determine SNR along different portions of the signal. The frequencies f_{low} and f_{high} were 2 and 5 MHz, respectively.

SNR decreased with increasing gate delay and specimen density. The minimum acceptable SNR was defined to be 10 dB. SNR fell below this value when the front edge of the gate was delayed by $9 \mu s$ (back edge at $10 \mu s$). Thus, to satisfy the criterion $SNR > 10$ dB for all gate choices and all specimens, the position of the back edge of gate 2 [see Fig. 2(a)] was restricted to be no greater than $9 \mu s$ ($\tau_d + \tau_s + 2\tau_w \leq 9 \mu s$).

FIG. 2. Signal analysis procedure used to determine nMBD, nSBD, and nIBD. (a) Analysis gates labeled 1 and 2 were applied to the backscatter signal to select two portions of the signal for analysis. (b) A power spectrum was obtained from the first gated portion of the signal. (c) Another power spectrum was obtained from the second gated portion of the signal. (d) The two power spectra were subtracted to obtain the backscatter difference spectrum $D(f)$. (e) The normalized backscatter difference spectrum $nD(f)$ was obtained by dividing $D(f)$ by the center-to-center separation between the analysis gates, $\tau_w + \tau_s$. nMBD was determined by averaging $nD(f)$ over the frequency range 2–5 MHz. nSBD and nIBD were obtained from the slope and intercept, respectively, of a line fitted to $nD(f)$ over the same frequency range.

E. Apparent density measurements

Apparent density was determined by dividing the total mass of each specimen by its total volume after allowing the specimen to dry in air at room temperature for 24 h. The masses of the specimens were measured using a digital balance with a precision of ± 0.01 g. Volume was determined using digital calipers with a precision of ± 0.01 mm to measure the dimensions of the specimens. Each dimension was measured three times, once with the caliper jaws positioned near one edge of the specimen, once with the jaws positioned in the center and once with the jaws positioned near the other edge. The standard deviation of these measurements averaged 0.1 mm.

F. Measurement reproducibility

To assess the reproducibility of the ultrasonic measurements, four specimens with apparent densities of 0.14, 0.35, 0.55, and 0.74 g/cm^3 were measured a second time several

weeks after the original measurements were completed. Values for nMBD, nSBD, nIBD, and nBAR were computed for all 34 gate choices and compared to the original measurements.

G. Statistical analysis

A Shapiro-Wilk test was used to analyze the normality of the data. nMBD and nBAR were normally distributed for all gate choices. nSBD and nIBD were normally distributed for all but five and four gate choices, respectively. Apparent density was not normally distributed. Thus, a Spearman correlation analysis was used to determine the correlation between the backscatter difference parameters and apparent density. Correlation coefficients reported in the present study represent Spearman's rank correlation coefficients, denoted R_s .

To determine if the backscatter difference parameters depended on measurement direction, a single factor analysis of variation (ANOVA) test was used along with a *post hoc*

analysis that compared measurements pairwise using a two tailed T -test with a 95% confidence interval. A Tukey correction was used to account for multiple comparisons.

To assess the reproducibility of the ultrasonic measurements, a two tailed T -test was used to compare measurements pairwise with a 95% confidence interval.

H. Theoretical considerations

The concept of “normalization” was introduced as a way to reduce the effects of gate choice on backscatter difference measurements of bone.³⁵ It is based on a theory developed by Sigelmann and Reid³⁶ that was adapted for tissue characterization studies by O'Donnell and Miller.³⁷ The theory expresses the power spectrum of a gated portion of the backscatter signal as

$$P(f) = \frac{4P_0(f)E(f)V(f)\eta(f)T^2}{r^2A(f)}, \quad (5)$$

where $P_0(f)$ is the power spectrum of the transmitted pulse, $E(f)$ is the two-way electromechanical power conversion efficiency of the measurement system, $V(f)$ is the gated scattering volume of the specimen, $\eta(f)$ is the backscatter coefficient of the specimen, T is the intensity transmission coefficient at the water-specimen interface, and r is the distance from the transducer to the center of the scattering volume. $A(f)$ is an attenuation term given by

$$A(f) = e^{4\alpha(f)x} \left[\frac{2\alpha(f)c\tau_w \cdot e^{\alpha(f)c\tau_w}}{e^{\alpha(f)c\tau_w} - e^{-\alpha(f)c\tau_w}} \right]. \quad (6)$$

It includes attenuation of the ultrasonic pulse up to the start of the analysis gate (through a thickness x of intervening specimen) and within the gated region. The term c is the speed of sound in the specimen, and $\alpha(f)$ is the attenuation coefficient of the specimen.

Using subscripts 1 and 2 to indicate quantities associated with the first and second gated portions of the backscattered signal, respectively, the backscatter difference spectrum $D(f)$ in dB is given by

$$D(f) = 10\log_{10} \frac{P_1(f)}{P_2(f)} = 10\log_{10} \frac{V_1(f)\eta_1(f)/r_1^2A_1(f)}{V_2(f)\eta_2(f)/r_2^2A_2(f)}. \quad (7)$$

Note that the terms $P_0(f)$, $E(f)$, and T do not appear in Eq. (7) because they are the same for both gated portions of the signal. This expression can be simplified further by assuming that the distance d between the scattering volumes V_1 and V_2 is small enough that $\eta_1(f) \cong \eta_2(f)$, $\alpha_1(f) \cong \alpha_2(f)$, $r_1 \cong r_2$, and $V_1(f) \cong V_2(f)$. Using this “small d ” approximation, Eq. (7) becomes

$$D(f) \cong 10\log_{10} \frac{A_2(f)}{A_1(f)} = (40\log_{10}e)c(\tau_w + \tau_s)\alpha(f). \quad (8)$$

The simplifying assumptions of the small d approximation yield an expression for $D(f)$ in Eq. (8) that depends on $\tau_w + \tau_s$ which is equal to the center-to-center separation between the two gates. If the assumptions of the small d approximation are valid, then these theoretical considerations indicate that the effect of gate choice can be removed by dividing the difference spectrum by $\tau_w + \tau_s$. The process

FIG. 3. Backscatter difference parameters plotted as a function of apparent bone density for one of 34 gate combinations investigated ($\tau_d = 3 \mu\text{s}$, $\tau_w = 2 \mu\text{s}$ and $\tau_s = 1 \mu\text{s}$). Error bars represent standard deviations of the mean. The four parameters shown are (a) the normalized mean of the backscatter difference (nMBD), (b) the normalized slope of the backscatter difference (nSBD), (c) the normalized intercept of the backscatter difference (nIBD), and (d) the normalized backscatter amplitude ratio (nBAR).

is called normalization, and the resulting quantity is called the normalized difference spectrum,

$$nD(f) \cong (40 \log_{10} e) c \alpha(f). \quad (9)$$

Equation (9) shows that the normalized difference spectrum $nD(f)$, and parameters based on the normalized difference spectrum, depend only on the speed of sound in the specimen and the ultrasonic attenuation coefficient. Theoretically, $nD(f)$ does not depend on gate choice. However, the result assumes that the scattering model in Eq. (5) is valid for cancellous bone and that the small d approximation is valid for the conditions of the measurements. The results of the present study can be used to test the validity of these assumptions.

III. RESULTS

A. Correlation of backscatter difference parameters with apparent density

Figure 3(a) shows nMBD plotted as a function of apparent bone density. Each point on the graph represents the

TABLE I. Spearman's rank correlation coefficients (R_s) for nMBD, nSBD, nIBD, and nBAR as functions of apparent density for 34 different gate choice combinations.

Gate Choice [τ_d, τ_w, τ_s] (μs)	nMBD	nSBD	nIBD	nBAR
[2,1,0]	0.79 ^a	0.40 ^b	0.22	0.80 ^a
[3,1,0]	0.79 ^a	0.33 ^b	0.21	0.76 ^a
[4,1,0]	0.75 ^a	-0.05	0.45 ^a	0.69 ^a
[5,1,0]	0.77 ^a	0.20	0.19	0.70 ^a
[6,1,0]	0.77 ^a	0.30 ^b	0.12	0.70 ^a
[7,1,0]	0.65 ^a	-0.04	0.26	0.48 ^a
[2,2,0]	0.86 ^a	0.60 ^a	0.51 ^a	0.86 ^a
[3,2,0]	0.86 ^a	0.36 ^b	0.54 ^a	0.84 ^a
[4,2,0]	0.86 ^a	0.25	0.49 ^a	0.84 ^a
[5,2,0]	0.88 ^a	0.38 ^b	0.38 ^b	0.82 ^a
[2,3,0]	0.88 ^a	0.64 ^a	0.63 ^a	0.88 ^a
[3,3,0]	0.88 ^a	0.48 ^a	0.66 ^a	0.87 ^a
[2,1,1]	0.85 ^a	0.63 ^a	0.45 ^a	0.84 ^a
[3,1,1]	0.84 ^a	0.29 ^b	0.51 ^a	0.82 ^a
[4,1,1]	0.84 ^a	0.17	0.51 ^a	0.83 ^a
[5,1,1]	0.84 ^a	0.44 ^a	0.28 ^b	0.80 ^a
[6,1,1]	0.82 ^a	0.14	0.38 ^b	0.74 ^a
[2,2,1]	0.88 ^a	0.57 ^a	0.67 ^a	0.87 ^a
[3,2,1]	0.87 ^a	0.49 ^a	0.61 ^a	0.86 ^a
[4,2,1]	0.89 ^a	0.46 ^a	0.67 ^a	0.86 ^a
[2,3,1]	0.89 ^a	0.68 ^a	0.72 ^a	0.88 ^a
[2,1,2]	0.87 ^a	0.52 ^a	0.65 ^a	0.86 ^a
[3,1,2]	0.86 ^a	0.48 ^a	0.59 ^a	0.84 ^a
[4,1,2]	0.88 ^a	0.46 ^a	0.61 ^a	0.85 ^a
[5,1,2]	0.86 ^a	0.32 ^b	0.49 ^a	0.82 ^a
[2,2,2]	0.88 ^a	0.64 ^a	0.66 ^a	0.88 ^a
[3,2,2]	0.89 ^a	0.58 ^a	0.81 ^a	0.88 ^a
[2,1,3]	0.88 ^a	0.52 ^a	0.66 ^a	0.87 ^a
[3,1,3]	0.89 ^a	0.58 ^a	0.69 ^a	0.87 ^a
[4,1,3]	0.88 ^a	0.27 ^b	0.68 ^a	0.87 ^a
[2,2,3]	0.90 ^a	0.66 ^a	0.78 ^a	0.89 ^a
[2,1,4]	0.90 ^a	0.66 ^a	0.68 ^a	0.89 ^a
[3,1,4]	0.88 ^a	0.61 ^a	0.81 ^a	0.87 ^a
[2,1,5]	0.89 ^a	0.60 ^a	0.76 ^a	0.89 ^a

^a $p < 0.001$.

^b $p < 0.05$.

mean value of nMBD for a particular specimen determined by averaging values over all measurement sites on the specimen and all measurement directions (1600 measurements per side, 6 sides per specimen). Error bars represent standard deviations of the mean. Similar graphs for nSBD, nIBD, and nBAR are shown in Figs. 3(b), 3(c), and 3(d), respectively. The same gate choice was used for all four graphs in Fig. 3: $\tau_d = 3 \mu s$, $\tau_w = 2 \mu s$, and $\tau_s = 1 \mu s$. Spearman's rank correlation analysis was used to determine how well the ultrasound parameters correlated with apparent density. Table I reports Spearman's rank correlation coefficients (R_s) for all 34 gate choices. Table II reports mean and standard deviation values for nMBD, nSBD, nIBD, and nBAR averaged over all 54 specimens for each of the 34 gate choices.

B. Anisotropy

Statistical analysis with ANOVA found a significant ($p < 0.05$) anisotropy for 31 of 34 gate choices for nMBD, 8 of 34 gate choices for nSBD, 18 of 34 gate choices for nIBD, and 28 of 34 gate choices for nBAR. Figure 4 shows mean values of

TABLE II. Mean $\pm$ standard deviation for the backscatter difference parameters nMBD, nSBD, nIBD, and nBAR averaged over all 54 specimens for 34 different gate combinations.

Gate choice [τ_d, τ_w, τ_s] (μs)	nMBD (dB/ μs)	nSBD (dB/MHz/ μs)	nIBD (dB/ μs)	nBAR (μs^{-1})
[2,1,0]	3.09 $\pm$ 1.04	0.400 $\pm$ 0.352	1.68 $\pm$ 1.38	0.149 $\pm$ 0.048
[3,1,0]	3.17 $\pm$ 0.96	0.439 $\pm$ 0.333	1.63 $\pm$ 1.37	0.151 $\pm$ 0.046
[4,1,0]	3.08 $\pm$ 0.70	0.476 $\pm$ 0.294	1.42 $\pm$ 1.40	0.143 $\pm$ 0.031
[5,1,0]	3.06 $\pm$ 0.78	0.286 $\pm$ 0.387	2.06 $\pm$ 1.58	0.141 $\pm$ 0.033
[6,1,0]	2.77 $\pm$ 0.67	0.401 $\pm$ 0.343	1.36 $\pm$ 1.30	0.124 $\pm$ 0.028
[7,1,0]	2.76 $\pm$ 0.59	0.265 $\pm$ 0.375	1.83 $\pm$ 1.61	0.123 $\pm$ 0.025
[2,2,0]	3.21 $\pm$ 0.89	0.535 $\pm$ 0.185	1.34 $\pm$ 0.69	0.151 $\pm$ 0.040
[3,2,0]	3.18 $\pm$ 0.76	0.520 $\pm$ 0.160	1.36 $\pm$ 0.78	0.147 $\pm$ 0.032
[4,2,0]	3.08 $\pm$ 0.66	0.417 $\pm$ 0.164	1.63 $\pm$ 0.77	0.139 $\pm$ 0.026
[5,2,0]	2.94 $\pm$ 0.62	0.373 $\pm$ 0.175	1.63 $\pm$ 0.69	0.130 $\pm$ 0.023
[2,3,0]	3.20 $\pm$ 0.78	0.536 $\pm$ 0.139	1.33 $\pm$ 0.57	0.148 $\pm$ 0.033
[3,3,0]	3.14 $\pm$ 0.71	0.474 $\pm$ 0.121	1.48 $\pm$ 0.57	0.142 $\pm$ 0.028
[2,1,1]	3.13 $\pm$ 0.94	0.420 $\pm$ 0.197	1.66 $\pm$ 0.73	0.150 $\pm$ 0.044
[3,1,1]	3.13 $\pm$ 0.76	0.458 $\pm$ 0.174	1.52 $\pm$ 0.87	0.147 $\pm$ 0.035
[4,1,1]	3.07 $\pm$ 0.67	0.381 $\pm$ 0.165	1.74 $\pm$ 0.86	0.142 $\pm$ 0.028
[5,1,1]	2.92 $\pm$ 0.66	0.344 $\pm$ 0.201	1.71 $\pm$ 0.82	0.132 $\pm$ 0.026
[6,1,1]	2.76 $\pm$ 0.54	0.333 $\pm$ 0.202	1.60 $\pm$ 0.83	0.124 $\pm$ 0.021
[2,2,1]	3.19 $\pm$ 0.81	0.520 $\pm$ 0.136	1.37 $\pm$ 0.63	0.149 $\pm$ 0.035
[3,2,1]	3.13 $\pm$ 0.71	0.461 $\pm$ 0.133	1.51 $\pm$ 0.60	0.143 $\pm$ 0.029
[4,2,1]	3.00 $\pm$ 0.63	0.412 $\pm$ 0.119	1.56 $\pm$ 0.57	0.134 $\pm$ 0.023
[2,3,1]	3.16 $\pm$ 0.75	0.492 $\pm$ 0.117	1.44 $\pm$ 0.52	0.145 $\pm$ 0.031
[2,1,2]	3.11 $\pm$ 0.82	0.439 $\pm$ 0.142	1.58 $\pm$ 0.67	0.148 $\pm$ 0.037
[3,1,2]	3.10 $\pm$ 0.73	0.401 $\pm$ 0.137	1.70 $\pm$ 0.69	0.145 $\pm$ 0.032
[4,1,2]	2.97 $\pm$ 0.63	0.388 $\pm$ 0.116	1.61 $\pm$ 0.59	0.136 $\pm$ 0.025
[5,1,2]	2.86 $\pm$ 0.58	0.318 $\pm$ 0.135	1.75 $\pm$ 0.64	0.129 $\pm$ 0.022
[2,2,2]	3.15 $\pm$ 0.76	0.476 $\pm$ 0.128	1.48 $\pm$ 0.56	0.145 $\pm$ 0.032
[3,2,2]	3.06 $\pm$ 0.67	0.446 $\pm$ 0.108	1.49 $\pm$ 0.47	0.138 $\pm$ 0.026
[2,1,3]	3.10 $\pm$ 0.78	0.401 $\pm$ 0.130	1.70 $\pm$ 0.62	0.146 $\pm$ 0.034
[3,1,3]	3.02 $\pm$ 0.68	0.401 $\pm$ 0.110	1.62 $\pm$ 0.51	0.140 $\pm$ 0.029
[4,1,3]	2.92 $\pm$ 0.58	0.357 $\pm$ 0.100	1.67 $\pm$ 0.57	0.133 $\pm$ 0.022
[2,2,3]	3.09 $\pm$ 0.72	0.461 $\pm$ 0.114	1.47 $\pm$ 0.47	0.141 $\pm$ 0.029
[2,1,4]	3.03 $\pm$ 0.73	0.401 $\pm$ 0.122	1.63 $\pm$ 0.53	0.142 $\pm$ 0.031
[3,1,4]	2.97 $\pm$ 0.63	0.374 $\pm$ 0.085	1.66 $\pm$ 0.47	0.136 $\pm$ 0.025
[2,1,5]	2.99 $\pm$ 0.68	0.378 $\pm$ 0.102	1.66 $\pm$ 0.49	0.139 $\pm$ 0.028

FIG. 4. Backscatter difference parameters determined along the six measurement directions (anterior, posterior, medial, lateral, proximal, distal). Measurements were averaged over all 54 specimens and all 34 gate choices for each measurement direction. Columns represent the means, and error bars represent standard deviations of the means. Horizontal lines indicate significant differences between measurement direction pairs. The four parameters shown are (a) the normalized mean of the backscatter difference (nMBD), (b) the normalized slope of the backscatter difference (nSBD), (c) the normalized intercept of the backscatter difference (nIBD), and (d) the normalized backscatter amplitude ratio (nBAR).

nMBD, nSBD, nIBD, and nBAR for each measurement direction averaged over all 54 specimens and all 34 gate choices. Error bars represent standard deviations of the mean. Horizontal lines on the graphs indicate statistically significant ($p < 0.05$) differences between pairs of measurement directions.

C. Measurement reproducibility

No significant difference ($p > 0.05$) was found between repeated measurement trials for any of the four parameters and any of the 34 gate choices.

IV. DISCUSSION

A. Effect of gate choice on the measured values of the backscatter difference parameters

Inspection of Table II shows that the measured values of the backscatter difference parameters do not exhibit a strong dependence on gate choice. For example, the measured values for nMBD (when averaged over all measurements on all specimens) are approximately 3 dB/ μ s, independent of gate choice. Without normalization, the resulting non-normalized parameter (MBD) would range from approximately 3 dB (for $\tau_w + \tau_s = 1 \mu$ s) to approximately 18 dB (for $\tau_w + \tau_s = 6 \mu$ s) for the gate choices considered in this study. This result demonstrates that the normalization procedure is reasonably effective at compensating the measured values for the effects of gate choice.

Given the effectiveness of the normalization procedure, the assumptions underlying the procedure appear to be valid up to $\tau_w + \tau_s = 6 \mu$ s which corresponds to an approximate distance $d = 4.5$ mm between gate centers, assuming a speed of

sound of 1500 m/s for cancellous bone. Note, however, that this result applies to the conditions of the present study. The upper limit for $\tau_w + \tau_s$ depends on the validity of the small d approximation which assumes that the ultrasonic properties of the specimen are sufficiently uniform so that $\eta_1(f) \cong \eta_2(f)$ and $\alpha_1(f) \cong \alpha_2(f)$ over a distance d representing the ultrasonically interrogated region of bone, and that the ultrasonic beam (which is influenced by focusing and diffraction effects) is sufficiently uniform over the same region so that $V_1(f) \cong V_2(f)$.

While the normalization procedure is effective at removing most of the effects of gate choice on the measured values of the backscatter difference parameters, some systematic variation with gate choice is evident. Overall, gate delay appears to have the largest effect. Figure 5 shows the four backscatter difference parameters plotted as a function of gate delay. nMBD, nSBD, and nBAR demonstrate a highly significant ($p < 0.001$) decrease as the gate delay τ_d is increased. The reason for this decrease is not clear, but it may be associated with attenuation of the ultrasonic wave which increases as τ_d is increased. The attenuating characteristics of cancellous bone cause the tissue to behave like a low-pass filter to ultrasonic waves. Increasing the gate delay redistributes the power to lower frequencies which may influence the measured values of the backscatter difference parameters.

B. Effect of gate choice on the correlations of backscatter difference parameters with apparent density

As seen in Table I, nMBD and nBAR yield stronger correlations with apparent density than nSBD and nIBD for any given gate choice. This result is consistent with findings

FIG. 5. Measured values of (a) the normalized mean of the backscatter difference (nMBD), (b) the normalized slope of the backscatter difference (nSBD), (c) the normalized intercept of the backscatter difference (nIBD), and (d) the normalized backscatter amplitude ratio (nBAR) plotted as a function of gate delay τ_d . Each data point represents the mean value of the parameter averaged over all measurements on all 54 specimens for a particular gate choice.

from previous studies.^{20,31,35} The correlation coefficients listed in Table I tend to increase as the gate delay τ_d is decreased and as the center-to-center separation between the gates $\tau_w + \tau_s$ is increased. These trends are apparent in Fig. 6 which shows plots of the correlation coefficients versus the quantity $\tau_w + \tau_s - \tau_d$. The correlations become notably weaker when this quantity becomes negative (i.e., when $\tau_w + \tau_s < \tau_d$). A possible explanation for this result is that the power difference between the gates becomes smaller and thus more difficult to measure as the center-to-center separation is reduced (by reducing gate separation τ_s and/or gate width τ_w). Another consideration is that the SNR decreases as gate delay τ_d is increased. Reduced SNR may negatively impact the measured correlations.

The results of the present study suggest that the correlation of all four backscatter difference parameters with apparent density is optimized by minimizing the gate delay and maximizing the center-to-center separation between gates. This result assumes that the second gate is not placed so far away from the first gate that SNR becomes a problem. In addition, it should be noted that the gate width τ_w affects the frequency resolution of the difference spectrum. Gate width should not be so small that the frequency resolution falls below acceptable levels. For the present study, the maximum desired frequency spacing between points in the difference spectrum was 1 MHz which required the gate width to be no less than 1 μs .

C. Anisotropy

Alignment of the trabeculae in cancellous bone may produce a directional dependence of the ultrasonic

measurements. As seen in Fig. 4, effects of anisotropy are evident. The finding that the backscatter difference parameters may be sensitive to trabecular orientation suggests that they also may be sensitive to the microstructural characteristics of cancellous bone which can be measured directly using x-ray micro computed tomography techniques. This would be an interesting direction for future research.

The effects of anisotropy on backscatter difference measurements of cancellous bone have been addressed in previous studies.^{20,35} Overall, the effects were found to be modest, similar to the findings of the present study. In the present study, most results (Figs. 3 and 5 and 6, and Tables I and II) are based on measurements averaged over all six measurement directions. Averaging was performed to maximize the volume of the specimen that was ultrasonically interrogated. Measurements along a single direction are localized to an $8\text{ mm} \times 8\text{ mm} \times z$ region of bone where z ranges from approximately 1.5–5.3 mm, depending on gate choice. Thus, measurements along a single direction interrogate a volume of $96\text{--}339\text{ mm}^3$ which is much less than the total volume (1000 mm^3) of the specimen. This can lead to errors in the analysis of correlations with apparent density, a quantity that is based on the total volume of the specimen.

In vivo, it is unlikely that backscatter difference measurements can be performed along multiple orthogonal directions as performed in the present *in vitro* study. The impact of this limitation on clinical applications of the backscatter difference technique is not clear. At the hip and spine, the anterior direction may be the most likely measurement direction. When data from the present study are analyzed only in

FIG. 6. Correlation of (a) the normalized mean of the backscatter difference (nMBD), (b) the normalized slope of the backscatter difference (nSBD), (c) the normalized intercept of the backscatter difference (nIBD), and (d) the normalized backscatter amplitude ratio (nBAR) with apparent density plotted as a function of the difference between the center-to-center separation between analysis gates ($\tau_w + \tau_s$) and gate delay (τ_d).

the anterior direction, the correlations of nMBD and nBAR with apparent density decrease by approximately 20% and 24%, respectively. It seems reasonable to attribute this effect to errors associated with basing the correlations on an ultrasonically interrogated volume that is much smaller than the total volume of the specimen, but other explanations are possible. For example, measurements in opposite directions (e.g., anterior and posterior) may help cancel errors associated with local variations in density across the specimen. For some gate choices, measurements in opposite directions may effectively interchange the regions of bone tissue that correspond to the two gated portions of the backscatter signal. The result may be a reduced sensitivity to errors caused by small scale density variations within the ultrasonically interrogated region.

V. SUMMARY AND CONCLUSIONS

Efforts to devise new backscatter techniques for ultrasonic bone assessment are motivated by several factors including: (1) the goal to address limitations associated with previously investigated backscatter parameters, (2) the desire to perform measurements at clinically interesting sites such as the hip and spine, (3) the need to identify diagnostic techniques that are more predictive of fracture risk, and (4) the desire to develop imaging based ultrasonic bone assessment techniques. Recent *in vitro* studies suggest that ultrasonic

parameters based on the backscatter difference technique may address some of these goals. For example, one study showed that backscatter difference measurements at the neck of intact proximal femurs correlate with BMD measured at the same location using DXA.²⁷ Another study showed that nMBD and nSBD are relatively insensitive to errors produced by intervening tissues, a characteristic that may help facilitate *in vivo* measurements at the hip and spine.³¹ A third study demonstrated that backscatter difference measurements can be performed with a modified clinical imaging system.²⁰

Given these early successes, future ultrasonic studies of bone involving the backscatter difference technique seem likely. It is therefore important to understand how backscatter difference parameters depend on the portions of the backscatter signal chosen for analysis. Studies involving other backscatter parameters such as AIB have demonstrated that gate choice can have a large influence on the measured results, and on conclusions drawn from those results. The present study is the first study to systematically investigate the effect of gate choice on backscatter difference parameters over the full range of options allowed by signal to noise considerations. Four backscatter difference parameters are investigated: the normalized mean of the backscatter difference (nMBD), the normalized slope of the backscatter difference (nSBD), the normalized intercept of the backscatter difference (nIBD), and the normalized backscatter amplitude ratio (nBAR).

The main findings of the study are the following. Among the four parameters tested, nMBD and nBAR consistently demonstrate stronger correlations with apparent bone density than nSBD and nIBD for all gate choices, suggesting that nMBD and nBAR may be more sensitive to changes in cancellous bone tissue caused by osteoporosis. For most gate choices, nMBD and nBAR produce similar correlations suggesting that time domain analysis of the signals (nBAR) is nearly as effective as a frequency domain analysis (nMBD). In addition, the study finds that correlations of the backscatter difference parameters with apparent density are optimized by minimizing the gate delay τ_d and maximizing the center-to-center separation $\tau_w + \tau_s$. However, care should be exercised to ensure that τ_d is sufficiently large to avoid the echo from the bone surface and that the SNR in any analyzed portion of the signal is acceptable.

Another important finding of the present study is that the normalization procedure is effective at reducing the effects of gate choice on the measured values of the backscatter difference parameters over a wide range of gate choices. The normalization procedure is based on theoretical considerations which show that the effects of gate choice on the backscatter difference spectrum $D(f)$ can be removed by dividing $D(f)$ by the center-to-center separation between the gates, $\tau_w + \tau_s$. However, the normalization procedure is based on several assumptions about the uniformity of the ultrasonic beam and the uniformity of the ultrasonic properties of the specimen over the region corresponding to the two gated portions of the backscatter signal. As the center-to-center separation between the two gates is increased (by increasing the gate width τ_w and/or gate separation τ_s), the assumptions may become less valid. One goal of the present study was to test the validity of these assumptions. The present study finds that the normalization procedure is most effective for nMBD and nBAR whose measured values vary by less than 16% and 21%, respectively, over the full range of gate choices allowed by signal-to-noise considerations. The main effect of gate choice on the measured values of nMBD and nBAR appears to be a tendency of these parameters to decrease as the gate delay τ_d is increased. This feature may be caused by the frequency dependent effects of ultrasonic attenuation of cancellous bone as discussed earlier.

In conclusion, the backscatter difference technique may offer a useful approach for ultrasonic bone assessment that addresses limitations found in other approaches. Of the four backscatter difference parameters investigated, nMBD and nBAR demonstrate the strongest correlations with apparent density, suggesting that they may be most sensitive to changes in cancellous bone caused by osteoporosis. The correlations between all four backscatter difference parameters and bone density are found to improve as gate delay τ_d is decreased and the center-to-center separation $\tau_w + \tau_s$ between the gates is increased. The normalization procedure applied to these parameters is effective at reducing the influence of gate choice on the measured values of these parameters, especially for nMBD and nBAR.

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