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EFFECTS OF PHASE CANCELLATION AND RECEIVER APERTURE SIZE ON BROADBAND ULTRASONIC ATTENUATION FOR TRABECULAR BONE *IN VITRO*

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Abstract

Phase cancellation in ultrasound due to large receiver size has been proposed as a contributing factor to the inaccuracy of estimating broadband ultrasound attenuation (BUA), which is used to characterize bone quality. Transducers with aperture size ranging from 2 to 5 mm have been used in previous attempts to study the effect of phase cancellation. However, these receivers themselves are susceptible to phase cancellation because aperture size is close to one center wavelength (about 3 mm at 500 KHz in water). This study uses an ultra small receiver (aperture size: 0.2mm) in conjunction with a newly developed 2-D synthetic array system to investigate the effects of phase cancellation and receiver aperture size on BUA estimations of bone tissue. *In vitro* ultrasound measurements were conducted on 54 trabecular bone samples (harvested from sheep femurs) in a confocal configuration with a focused transmitter and synthesized focused receivers of different aperture sizes. Phase sensitive (PS) and phase insensitive (PI) detections were performed. The results show that phase cancellation does have a significant effect on BUA. The normalized BUA (nBUA) with PS is 8.1% higher than PI nBUA while PI BUA is well correlated with PS BUA. Receiver aperture size also influences the BUA reading for both PI and PS detection and smaller receiver aperture tends to result in higher BUA readings. The results also indicate that the receiver aperture size used in the confocal configuration with PI detection should at least equal the aperture of the transmitter to capture most of the energy redistributed by the interference and diffraction from the trabecular bone.

Keywords

Phase cancellation; Broadband ultrasound attenuation; Synthetic array; Trabecular bone; Bone quality assessment; Osteoporosis; Osteopenia

INTRODUCTION

Since quantitative ultrasound (QUS) was first proposed by Langton *et al.* (1984), it has been extensively studied (Langton and Njeh 2008; Njeh *et al.* 1997) and identified as an alternative technique for assessing trabecular bone status, e.g., bone density and structure. QUS usually utilizes a through-transmission configuration, in which two axially-aligned

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ultrasound transducers, one transmitter and one receiver located on opposite sides of bone, are used to probe the bone status using ultrasound. Because of easy access, the calcaneus is the primary testing site for *in vivo* QUS. Recently, QUS was also extended to the proximal femur (Barkmann et al. 2008) and distal radius (Yamamoto et al. 2009). When broadband ultrasound propagates through bone, it loses some of its energy due to attenuation. The attenuation appears to vary linearly with frequency (Langton et al. 1984), in which the slope of the attenuation curve is called broadband ultrasound attenuation (BUA). BUA is obtained by linear fitting of the ultrasound attenuation across certain frequency range, which is usually ranged 0.2–0.6 or 0.3–0.7 MHz (Njeh et al. 1997) for *in vivo* studies. It has been found that BUA correlates well with the bone mineral density measured by dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) (Njeh et al. 1997). BUA is also a good indicator of osteoporotic fracture risk as demonstrated by several prospective and retrospective studies (Gluer et al. 2004; Hans et al. 1996; Khaw et al. 2004; Krieg et al. 2003). Because ultrasound is a high frequency mechanical wave and interacts with the macro and micro structure of the bone in a complex manner, BUA also has the potential to reflect the material, mechanical and structural properties of the bone which may not be attainable via DXA. *In vitro* studies have shown that BUA correlates well with the mechanical strength (Langton et al. 1996), microstructure (Chaffai et al. 2002), and anisotropic nature (Gluer et al. 1993; Hans et al. 1995) of trabecular bones. Compared to DXA and QCT, QUS is relatively inexpensive in terms of the device itself and the testing procedure. QUS can be portable, does not generate ionizing radiation and does not require a certified technician to operate and thus would be highly available for physicians and accessible for patients.

Despite of its advantages and potentials, QUS has not gained wide acceptance in the United States as a diagnostic tool since its inception. The readings from QUS, primarily BUA and speed of sound (SOS), depend on specific system parameters, such as transducer aperture and bandwidth. It is difficult to compare the readings across different devices and platforms. For *in vivo* testing, more compounding factors such as foot positioning also have a profound influence on the precision of results (Chappard et al. 1999; Cheng et al. 1999; Evans et al. 1995). While these factors can be controlled to a large extent by adopting standardized system parameters and highly-repeatable positioning system, some researchers suggested that BUA may be inherently inaccurate due to the effect of phase cancellation (Petley et al. 1995).

Phase cancellation happens at the aperture of the piezoelectric receiver where wave fronts arrive with different phases. Because of phase differences, the local charge density can either be positive or negative at different region of the transducer surface. Integrated over the transducer aperture, the overall electric response from the receiver would be reduced. The effect of phase cancellation depends on the aperture size of the receiver and becomes less significant when smaller aperture is used. Because the aperture size of the receiver in most QUS is between 12.7 mm to 29 mm (Njeh et al. 1997), which is considerably large compared to the ultrasound wavelength at 500 KHz in water (about 3 mm), thus the effect of phase cancellation on BUA readings can be significant.

To study the effect of phase cancellation, a phase insensitive receiver is needed. While this type of receiver is prohibitively difficult to manufacture, a 2-D array receiver with a small element size combined with phase insensitive (PI) and phase sensitive (PS) signal processing can be used to simulate PI and PS ultrasound detection. The array can be a physical 2-D array or synthesized by a single scanning receiver. Petley et al. (1995) first studied the phase cancellation in QUS using a receiver with a diameter of 5 mm. The receiver and transmitter (with a diameter of 25mm) were scanned simultaneously with a resolution of 1 mm. The test results on 10 subjects showed that there was a mean difference

of 31.2 dB/MHz between PI BUA and PS BUA. Strelitzki et al. (1999) used a multi-element receiver made of 4×4 elements, from which 10 elements (each with a diameter of 5 mm) were used to form a large receiver aperture. No significant differences between PS BUA and PI BUA were found from 10 *in vitro* samples. Using a commercial system with a 2-D array receiver (element size of 2 mm), Wear studied phase cancellation effects on both *in vivo* subjects (2007) and *in vitro* samples (2008) by synthesizing a 25.4 mm receiving aperture with 52 elements. In both cases, significant differences were reported. Besides experimental studies, Langton and Subhan (2001) developed simulation models in an attempt to explain the phase difference of the wave fronts at the transducer surface. In the model, the ultrasound path difference, which in turn caused the phase difference of the wave fronts, was mainly determined by the curvature of the cortical plates. By assuming a reasonable curvature, the model could explain the BUA difference between the calcaneus with end plates in place and the calcaneus with end plates sliced off. In their study, the curvature of end plates was assumed to be the primary source of phase differences. Xia et al. (2005) developed a theoretical model to explain the contributions of the end plates on the BUA readings without relying on the phase cancellation effects. In reality, the phase differences may be caused by several factors, not only the end plates but also attached soft tissues and the trabecular bone itself.

In previous studies, elements with aperture size ranging from 2 to 5 mm have been used to study the effect of phase cancellation. However, these receivers themselves are susceptible to phase cancellation because the aperture size is close to one center wavelength of the ultrasound pulse. In addition, very few studies (Wear 2008) focused on the trabecular bone itself. Furthermore, a recent study (Bauer et al. 2009) suggested that the ultrasound interferences introduced by the samples could redistribute the ultrasound energy and steer the wave front away from the receiver causing additional signal loss detected by the receiver. Such loss could be recovered by increasing the receiving aperture size. Little is known about how the receiver aperture influences the PI BUA readings for trabecular bones.

The aim of this study is to investigate the effects of phase cancellation and receiver aperture size on the estimation of BUA for trabecular bone. An ultra small receiver with an aperture size of 0.2 mm was used to avoid most of the phase cancellation by the receiver itself. In conjunction with a newly developed 2-D synthetic array system, the effects of phase cancellation and receiver aperture size on the BUA estimation could be studied simultaneously.

MATERIALS AND METHODS

Bone sample preparation

Fresh sheep trabecular bone samples were harvested from medial and lateral femur condyles (n=54, purchased from a local slaughterhouse) beneath the growth plate. Total 27 sheep femora were used, which produced two samples per femur. The samples were consistently cut into parallel trabecular bone slabs with a diamond wheel saw (MicroSlice, Metals Research Ltd, Newton Hall, Cambridge, UK) under constant water cooling along the plane transverse to the longitudinal axis of the femur. The thickness of each sample was averaged over three different sites with a micrometer (NSK, Ann Arbor, MI, USA). The averaged sample thickness is 6.26 ± 0.52 mm. The average bone volume fraction is 0.36 ± 0.10 and the main trabecular orientation and the ultrasound direction form an angle of 53.9 ± 11.4 degrees according to micro-computed tomography. Bone marrow was removed with water jetting. The samples were then wrapped with saline-soaked gauze and frozen at -20°C before testing.

Experimental setup

As shown in Fig. 1, the excitation signal was generated by a signal generator (AFG3021, Tektronix Inc, Beaverton, OR, USA) as a one-cycle sine wave pulse. The frequency of the pulse was 1.0 MHz and peak-to-peak amplitude was 1.0 V. The pulse was 50 dB amplified by a radio frequency power amplifier (E&I 2100L, Electronics & Innovation Ltd., Rochester, NY, USA). The amplified pulse drove a broadband focused ultrasound transducer (V303, Panametrics-NDT, Waltham, MA, USA) which acted as the transmitter with a focal distance of 20.32 mm, a center frequency of 1.0 MHz and an aperture size of 12.7 mm. The ultrasound wave passed through the trabecular bone sample and was received by a hydrophone (HGL-0200, ONDA, Sunnyvale, CA, USA) with an aperture size of 0.2 mm and a bandwidth of 0.25–40 MHz. The received signal was first amplified by a hydrophone preamplifier (AG-2010, ONDA, Sunnyvale, CA, USA) and then further amplified by a secondary amplifier (5072PR, Panametrics-NDT, Waltham, MA, USA). The amplified signal was digitized by a 14-bit digitizer (NI PXI-5122, National Instruments, Austin, TX, USA) at a sampling rate of 50 MHz and transferred to a PC (Optiplex Qx270, Dell Inc, Round Rock, TX, USA) for processing. A LabVIEW (National Instruments, Austin, TX, USA) program running on the PC sent the control signal to the scanner controller (NI PXI-7830R, National Instruments, Austin, TX, USA). The controller controlled the custom-made 3-D scanning system (Velmex Inc, Bloomfield, NY, USA) with a scanning step size down to 10 μ m. The ultrasound transmission, mechanical raster scan and data acquisition were all synchronized by the trigger signal generated by the scan controller.

Experimental procedure

A quantitative ultrasound phantom (M6302, CIRS, Norfolk, VA, USA) was used to calibrate and tune the newly developed ultrasound testing system (Fig 1). The bone samples were then tested one at a time with ultrasound. They were first defrosted using running water and then immersed in degassed water for 30 minutes at room temperature before ultrasound testing. As shown in Fig. 1, the bone sample or phantom was placed in the middle plane between the transmitter and receiver, which were 41 mm apart. The position of the sample was carefully adjusted until the intended region wasinsonified. Once in position, the samples were visually and carefully inspected for air bubbles. If formed, the bubbles were removed by gentle brushing and cleaning. Because the sample (about 20 mm in diameter) was much larger than the focused ultrasound beam (about 3.4 mm in diameter at central wavelength according to theoretical calculation), no sound proof material was added to surround the sample. The receiver scanned a plane (20 mm $\times$ 20 mm) parallel to the bone sample with a stepping size of 0.25 mm. The starting position of the hydrophone was determined beforehand to ensure the transmitter was centered at the middle of the scanned plane. For each sample, three scans were taken after repositioning the bone, and the gain was set to 30 dB at the secondary amplifier. Only one reference scan was made when the sample was removed, and the gain was set to 20 dB. After ultrasound testing, bone samples were wrapped with saline-soaked gauze and frozen at -20°C for further testing.

Data Analysis

For both PS and PI detection, the large-aperture receiver was synthesized by the hydrophone at different scanning positions that were indexed by (i, j) . The geometric center of the synthesized receiver was determined from the reference scan by finding the maximum peak-to-peak amplitude of the scanned ultrasound field. The index for the center was denoted as (I_c, J_c) . To study the effect of receiver aperture size on BUA readings, four apertures sizes were synthesized: 2.0 mm, 6.35 mm, 12.7 mm and 19.0 mm, respectively. When the receiver aperture size was 12.7 mm (same as that of the transmitter), the synthesized virtual

receiver contained over 2000 virtual elements (each corresponding to a scanning position with a pitch of 0.25 mm).

For PS detection, a single time domain signal was obtained through beamforming by summing all signals within the aperture after the time-of-flight was compensated for the virtual focus of $F = 20.32$ mm. If the signal detected at the scanning position (i, j) is $S_{i,j}(t)$, then the synthesized signal can be expressed as,

$$S(t) = \sum_{i,j} S_{i,j}(t + \Delta t). \quad (1)$$

In Eq. (1), Δt accounts for the time of flight difference as (Lu et al. 1994),

$$\Delta t = \frac{\sqrt{(i - I_c)^2 \Delta L^2 + (j - J_c)^2 \Delta L^2 + F^2} - F}{c}, \quad (2)$$

where ΔL is resolution of the raster scan (0.25 mm); c is the speed of sound in water (1484 m/s). In addition, the index i and j in Eq. (1) and Eq. (2) have to meet the following requirement,

$$\sqrt{(i - I_c)^2 \Delta L^2 + (j - J_c)^2 \Delta L^2} \leq D/2, \quad (3)$$

where D is the aperture size and set to 2 mm, 6.35 mm, 12.7 mm and 19 mm, respectively according to the specific selection.

A single time-domain signal was synthesized for the reference, which was transmitted through water without the sample. Another time-domain signal was also synthesized for each sample. The spectra of these two signals were calculated through Fast Fourier Transform (FFT) with zero-padding. For the PS BUA calculation, the absolute spectrum of the sample was first divided by the absolute spectrum of the reference, and then linearly fitted to frequency. The BUA results was normalized by sample thickness and denoted as PS nBUA.

For PI detection, the spectrum of the each signal $S_{i,j}(t)$ was obtained through Fourier transform,

$$\tilde{S}_{i,j}(f) = F(S_{i,j}(t)). \quad (4)$$

The spectrum of the PI detected signal $\tilde{S}(f)$ is expressed as,

$$\tilde{S}(f) = \sum_{i,j} |\tilde{S}_{i,j}(f)|, \quad (5)$$

where index i and j need to satisfy Eq. (3).

The PI BUA was calculated using spectra for the reference and the sample, both valued according to Eq. (5). For PI BUA calculation, the spectrum of the sample was first divided by the spectrum of the reference and then linearly fitted to frequency. The BUA results were also normalized by sample thickness and denoted as PI nBUA.

Statistical Analysis

SPSS for Windows (Version 15, IBM, Armonk, NY, USA) was used for statistical analysis. A paired student's t -test was used for the difference between PS nBUA and PI nBUA with the same receiver size. Pearson correlation coefficients were obtained for PS nBUA with different receiver sizes, for PI nBUA with different receiver sizes and between PS nBUA and PI nBUA with the same receiver size.

RESULTS

Fig. 2 shows the PS detected time-domain signal for the QUS phantom and the reference (through water only) according to Eq. (1) for the receiver aperture size of 12.70 mm, the same as the size of the transmitter. The synthesized signal was quite clean and the noise level was low despite the aperture size of the hydrophone was ultra small. Compared to the reference signal, the synthesized signal for the phantom was weaker in amplitude and broader in pulse width due to the attenuation through the phantom. The attenuation curves of the PS and PI detected signals for the phantom are shown in Fig. 3. The curves of both PS and PI detected signals linearly attenuated with the frequency in the range of 0.4–0.9 MHz, in which the signal to noise ratio was highest for the system. The correlation coefficients of the attenuation to frequency for both PS and PI detection were $R=0.999$ and $R=0.999$, respectively. The high linearity of the attenuation demonstrated that the new system was suitable for BUA measurement. Attenuation was used here in a general sense and reflected the apparent signal loss in the detected signal. The signal loss was also caused by other effects, such as reflection at the interfaces.

The slopes of the linearly fitted lines (Fig. 3) were defined as PS BUA and PI BUA and listed in Table 1. The BUA results for the phantom with different receiver apertures were calculated and listed in the table. The BUA readings became smaller when a larger receiver aperture was used. For PS detection, the change was smaller compared to the change for PI detection. When the receiver aperture was 12.70 mm, the PI BUA was 54.19 dB/MHz which is close to the manufacturer-specified BUA of 51.80 dB/MHz.

Fig. 4 shows the typical distribution patterns of the ultrasound fields through water and a bone sample. The intensity of the pixel in the images represents the peak-to-peak amplitude of the received signal by the hydrophone at each scanning position. The distribution pattern of the ultrasound fields reflected the energy distribution a receiver would see, and the aperture size of the receiver would determine how much energy it can collect. For the water (Fig. 4a), the pattern was regular and round. Most energy was located within a circle with a diameter of 12.70 mm corresponding to the transmitter aperture size. For the bone sample (Fig. 4b), the distribution pattern was irregular and broadened. Significant amount of energy was steered away from the geometrical center.

Similar to Fig. 2, Fig. 5 shows the PS detected time-domain signal for the bone (as in Fig. 4b) and the reference (as in Fig. 4a). Fig. 6 shows the attenuation curves of the PS and PI detected signals for the bone sample. Once again, the attenuation curves for both PS and PI detected signals changed linearly with the frequency. The correlation coefficients were $R=0.961$ and $R=0.979$ for PS and PI detection, respectively, for this bone sample.

The BUA results for all bone samples are illustrated in Fig. 7. The mean PI nBUA for bone samples became smaller when a larger receiver aperture was used. Similarly, PS nBUA became smaller with increasing receiver aperture, except for the aperture size of 19.00 mm. As the aperture size increased, so did the difference between PS and PI nBUA.

The detailed BUA results are listed in Table 2. Overall, the correlation coefficients between the attenuation and the frequency were over 0.950, except for PS detection with an aperture of 19.00 mm ($R=0.886$). The paired student's t-test showed the PS nBUA and PI nBUA for each aperture size was significantly different except for the aperture size of 2.00 mm. For the aperture size of 12.70 mm, PS nBUA was 8.1% higher than PI nBUA.

Fig. 8 shows the average PS and PI attenuation of all bone samples versus frequency. Although the standard deviation at each frequency was quite high, an obvious trend can be observed. As expected, the attenuation of the PS detected signal was higher than the PI detected signal on average. However, the PS and PI nBUA were well correlated, and the correlation coefficients were higher than 0.950 (Table 2) except for the aperture of 19.00 mm. Fig. 9 shows the scatter plots and linear regression between PS and PI nBUA for the aperture size of 12.70 mm.

Fig. 10 and Fig. 11 show the average attenuation versus frequency with different apertures size for PI and PS detection, respectively. For clarity, the errors bars (like those in Fig. 8) were not shown. For PI detection, the average attenuation changed linearly with the frequency for all aperture sizes. Attenuation increased as the receiver aperture was reduced. These trends were also true for PS detection, except for the aperture size of 19.00 mm. At this aperture size, the attenuation visually deviated from the fitted straight line. The deviation was also reflected by the relatively low mean correlation coefficient ($R=0.886$ as listed in Table 2) between the attenuation and frequency for all bone samples.

Table 3 and Table 4 list the correlation coefficients between PS BUAs and PI BUAs for different aperture sizes, respectively. For the neighboring aperture sizes, the correlation was high. When the difference between aperture sizes increased, the correlation decreased.

DISCUSSION

A new synthetic array system was developed to study the effects of phase cancellation and receiver aperture size on BUA measurements for trabecular bone *in vitro*. An ultra small receiver with an aperture size of 0.2 mm was used to reduce the phase cancellation at the physical receiver itself. To the best of our knowledge, this was the first to study the phase cancellation effects on the QUS with a confocal configuration, where two confocal transducers were employed as the transmitter and receiver, respectively, to focus on a spatial point within tested tissue. The results showed that trabecular bone itself (without bone marrow, cortical shells, and soft tissues) introduced significant phase aberrations to the ultrasound wave. Phase cancellation on the surface of the focused receiver had significant effects on the BUA readings. As shown in results (Fig. 8), PS detection tended to have higher attenuation at a specific frequency. On average, the PS nBUA was 8.1% higher than PI nBUA when size of the receiver aperture and transmitter aperture was the same (12.7 mm). The difference was statistically significant ($p<0.001$) according to the two-tailed paired student's t-test. The results also showed that the receiver aperture size had a significant effect on PI nBUA and PS nBUA of trabecular bones. The scanned ultrasound fields revealed that the energy of the ultrasound wave was redistributed possibly due to interference and diffraction from the trabecular architecture. The broadened and redistributed sound waves require the receiver aperture size to be appropriately large to collect most of the energy and to estimate BUA accurately in bone tissue characterization.

With the confocal configuration, the PS nBUA was 8.1% higher than PI nBUA when both transmitter and receiver aperture size was 12.7 mm. This result was close to the *in vitro* result by Strelitzki et al. (1999) for intact heel bones and lower than the *in vitro* result by Wear (2008) for heel bones with cortical shells and bone marrow removed. In addition, two previous *in vivo* studies found that the PS BUA was 21% (Wear 2007) and 60% (Petley et al. 1995) higher than PI BUA. The results by Petley and colleagues (1995) might not be typical, since the transmitter and receiver were scanned at the same time which was different from most QUS systems (Langton and Njeh 2008; Njeh et al. 2000). Furthermore, the ceramic receiver they used had a central frequency of 5 MHz, much higher than 1 MHz of the transmitter, and might not have sufficient bandwidth to detect attenuated signal with high signal to noise ratio at the frequency range of 0.2–0.6 MHz at which BUA results were obtained. Nevertheless, the difference between PS BUA and PI BUA in current study was relatively low compared to above-mentioned results. This might be primarily due to the fact that the aperture size of present study, 12.7 mm, was smaller than those in previous studies (at least 25 mm). Larger aperture could suffer more phase cancellation effects, which was easily observable in Fig. 7. In addition, several other factors could also contribute to this difference. First, trabecular bone samples in present study were from a different species. The trabecular bone in the sheep femoral condyles was dense, and the bone volume fraction was around 0.36 ± 0.10 . Second, the ultrasound tests were conducted in the proximal-distal direction instead of medial-lateral direction. The main trabecular direction and ultrasound testing direction formed an angle of 53.9 ± 11.4 degrees. Third, a confocal configuration was used in present study which maximized the acoustic energy at the focal area. The effects of bone volume fraction (Serpe and Rho 1996), orientation of the trabecular bone (Njeh et al. 2001) and transducer configuration (Nicholson et al. 1997) on PS BUA have been studied. However, further investigations are needed to clarify how these factors influence the degree of phase cancellation.

The PS nBUA and PI nBUA for trabecular bones were well correlated although the values were statistically different. As shown in the results (Fig. 9), the correlation coefficient was 0.962 (receiver aperture size of 12.7 mm in Table 2). Two previous *in vitro* studies (Strelitzki et al. 1999; Wear 2008) also found that correlation coefficient was close to 0.95, implying that over 90% of the variability in PI BUA could be explained by the PS BUA in *in vitro* experiments without soft tissue. The correlation between PS BUA and PI BUA *in vivo* was around 0.71 (Wear 2007), suggesting that the soft tissue and cortical bone introduced additional phase aberrations to the wave fronts. These additional aberrations might add to the phase cancellation effects introduced by the trabecular bone, increasing the variability of PS BUA that could not be explained by PI BUA. For *in vitro* applications, PS detection doesn't introduce too much new information. However, under *in vivo* applications the situation could be different.

Receiver aperture size had a significant effect on PI BUA for trabecular bones. The mean attenuation for all bone samples at a specific frequency decreased as the receiver aperture size increased from 2.00 mm to 19.00 mm (Fig. 10). The PI nBUA results (Fig. 7 and Table 2) also reflected the same trend with the mean PI nBUA decreasing from 46.19 dB/MHz/cm to 30.43 dB/MHz/cm. The correlation between PI nBUA values at two different aperture sizes dropped significantly as the size difference between them was increased (Table 4). These effects of receiver aperture size on PI BUA were directly related to the amount of energy captured by the corresponding aperture. The receiver aperture size for PI detection should at least equal the transmitter aperture size since the distribution pattern of ultrasound field (Fig. 4b) for trabecular bones was much broader than the pattern for water (Fig. 4a). The redistribution effects were directly observed on trabecular bone samples. Similar effects were previously observed only on phantoms by Bauer et al. (2009). In their study, one phantom with step discontinuity in thickness was used to introduce interference and

redistribution of ultrasound energy, while another phantom without discontinuity was used as control. They found that the attenuation curve of PI detected signal deviated significantly from the curve of the control phantom when the receiver aperture was significantly smaller than the transmitter aperture. Therefore, an appropriately large aperture size was needed to accurately measure the true PI BUA (measured from the control) with the presence of the discontinuity. While the true PI BUA for the bone samples would be difficult to determine in the current study, similar trends in attenuation curves suggested that the trabecular structure was also a significant source for ultrasound interference.

Receiver aperture size also had a significant effect on PS BUA for trabecular bones. The mean attenuation at a specific frequency increased with reduced aperture size when aperture sizes were smaller than 12.70 mm, and became unpredictable after the receiver aperture size increased to 19.00 mm (Fig. 11). A previous study (Strelitzki and Truscott 1998) also found that it was difficult to predict trend of the PS BUA based on the receiver aperture size. However, the inter-correlations (around 0.9) between BUA for different aperture size in that study were significantly higher than correlations in the current study (Table 3). Such discrepancy might be caused by configuration differences. Instead of unfocused transducers, a confocal transducer pair was adopted in present study. Focusing caused the wave front to unevenly spread out and reduced the inter-correlation between different receiver apertures. Furthermore, the physical mask used in the previous study might reflect some energy back to the receiver and increase the inter-correlation, while a physical mask was not needed in current study.

There were several other factors might also influence the BUA results for both PS and PI detection. First, the exact value of BUA might change with the frequency ranges used to fit the straight line (Lin et al. 2009). In current study, the frequency range was chosen as 0.4–0.9 MHz because the signal to noise ratio was highest in this range for the experimental setup. For most *in vivo* studies (Langton and Njeh 2008; Njeh et al. 1997), the frequency range was set to 0.2–0.6 MHz or 0.3–0.7 MHz. On the other hand, attenuation has been shown to linearly depend on frequency up to 2 MHz (Chaffai et al. 2000; Wear 2001) for *in vitro* measurements. Second, relatively thin trabecular bone samples (average 6.26 ± 0.52 mm) were used to improve the signal to noise ratio because of the small receiver aperture size of 0.2 mm in the setup. The nBUA results reported in this study were normalized by the sample thickness since BUA has been found to linearly depend on sample thickness (Langton et al. 1984). However, normalization is not routinely adopted and recommended in clinical practice (Wu et al. 1995). Finally, the three measurements on each sample were treated as independent measurements in statistical analysis making the effective sample size 162 instead of 54. Treating each measurement independently was justified by the fact that the transmitter focal point was small (~ 3.4 mm in diameter at central frequency) and the sample was repositioned within the ultrasound field for each measurement. For the same sample, the BUA could be quite different in different regions (Laugier et al. 1996). In future experiments, the excitation signal for the transmitter can be changed to a discharging pulse, and the amplitude of the signal can be increased to improve the signal to noise ratio at the frequency range at 0.3–0.7 MHz so BUA can be evaluated at that frequency range. With a higher driving signal, the sample thickness may be increased to around 10 mm. The scanning speed of stage can also be improved to make more measurements for each sample. In addition, a second motorized stage could be added to precisely position the bone sample. With calibration, the position of ultrasound beam relative to the bone sample can be determined. Site-matched studies on the bone properties extracted from micro-computed tomography and BUA values can be conducted and may reveal if phase cancellation has degrading effects on the clinical effectiveness of quantitative ultrasound.

CONCLUSIONS

A scanning ultrasound measurement system was developed for bone assessment using a receiver with an ultra-small aperture size to limit the phase cancellation effects on the physical receiver itself. The effects of phase cancellation and aperture size on BUA estimation were studied with trabecular bones *in vitro*. Results show that phase cancellation does have a significant effect on BUA (e.g., PS nBUA is 8.1% higher than PI nBUA), while PI BUA is well correlated to PS BUA. Receiver aperture size also influences the BUA reading for both PI and PS detection. Smaller receiver aperture tends to have higher BUA readings. In confocal configuration with PI detection, the receiver aperture size should at least be equal to the size of the transmitter to capture most of the energy redistributed by the interference and diffraction from the trabecular bone.

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Fig. 1. Schematic diagram of the experimental setup for the ultrasound measurement.

Fig. 2.

The synthesized time-domain signal (a) through water, (b) through phantom. Aperture size of the synthesized receiver is 12.70 mm.

Fig. 3. The attenuation curves of the PS and PI detected signals for the phantom. Aperture size of the synthesized receiver is 12.70 mm.

Fig. 4. The distribution of the ultrasound field detected by the synthesized 2-D array (a) through water, (b) through a bone sample. The size of the images is 20 mm $\times$ 20 mm.

Fig. 5.

The synthesized time-domain signal (a) through water, (b) through the bone sample. Aperture size of the synthesized receiver is 12.70 mm.

Fig. 6.
The attenuation curves of the PS and PI detected signals for the bone sample. Aperture size of the synthesized receiver is 12.70 mm.

Fig. 7.

The average PS and PI nBUA of bone samples with different synthesized receiver apertures.

* indicates significant difference by paired t-test, $p < 0.001$.

Fig. 8. Average PS and PI attenuations of bone samples versus frequency. The error bar denotes the standard deviation, and the receiver aperture size is 12.70 mm.

Fig. 9.

Correlation between PS nBUA and PI nBUA for bone samples. Aperture size of the synthesized receiver is 12.70 mm.

Fig. 10.
Average PI attenuations of bone samples versus frequency with different receiver aperture sizes.

Fig. 11. Average PS attenuations of bone samples versus frequency with different receiver aperture sizes.

Table 1

BUA results for the phantom

Aperture (mm)	2.00	6.35	12.70	19.00
PS BUA (dB/MHz)	63.53	62.50	61.27	60.90
PI BUA (dB/MHz)	62.16	59.95	54.19	45.98

Table 2

BUA results for the bone samples

Aperture (mm)	2.00	6.35	12.70	19.00
PS nBUA (dB/MHz/cm)	Mean	46.24	42.79	37.75
	SD	15.18	14.01	14.17
	R	0.959	0.968	0.958
PI nBUA (dB/MHz/cm)	Mean	46.19	41.77	34.94
	SD	14.46	12.13	10.33
	R	0.962	0.972	0.974
PS nBUA- PI nBUA (dB/MHz/cm)	Mean	0.05	1.03*	2.80*
	SD	1.06	2.96	5.09
	95% CI	(-0.11,0.22)	(0.57,1.49)	(2.01,3.59)
PS nBUA&PI nBUA	Correlation	0.999	0.985	0.962
				0.727

* significant difference by the paired t-test, p<0.001

Abbreviations: SD=standard deviation; R=mean correlation coefficient between attenuation and frequency; CI=confidence interval.

Table 3

Correlation between PS nBUA

Aperture (mm)	2.00	6.35	12.70	19.00
2.00	1	0.964	0.789	0.669
6.35	-	1	0.909	0.764
12.70	-	-	1	0.878
19.00	-	-	-	1

Table 4

Correlation between PI nBUA

Aperture (mm)	2.00	6.35	12.70	19.00
2.00	1	0.963	0.820	0.716
6.35	-	1	0.929	0.838
12.70	-	-	1	0.964
19.00	-	-	-	1