

# Determination of Bone Trabeculae Modulus—An Ultrasonic Scanning and MicroCT ( $\mu$ CT) Imaging Combination Approach

J.C.M. Teo · E.Y.L. Teo · V.P.W. Shim · S.H. Teoh

Received: 31 May 2005 / Accepted: 23 March 2006 / Published online: 16 May 2006  
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**Abstract** To date, there is no method to measure non-destructively the modulus of trabeculae within cancellous bone, whilst retaining its structural integrity. In this study ultrasonic scanning, coupled with microCT imaging, is employed to determine trabeculae modulus along the three major anatomical axes non-destructively. The proposed method allows cancellous bone specimens to remain intact, for possible use in subsequent studies. Volume rendering of the microCT images allows three-dimensional visualization of cancellous bone specimens to be tested. This facilitates trabeculae selection and accurate measurement of distance traveled by the ultrasonic wave, thus yielding a good degree of confidence in the acoustic velocity measured. For all the three principal anatomical directions, the measured acoustic speeds ranged from 2,115 to 3,077 m/s, giving an average of 2,505 m/s. Average wave velocities in the superior–inferior, medial–lateral and anterior–posterior anatomical directions were found to be 2,295, 2,469 and 2,754 m/s, respectively; the differences corresponding to the three directions do not appear to be significant. Subsequently, the modulus was then determined using elastic wave propagation theory.

**Keywords** Trabeculae modulus · Ultrasonic testing · MicroCT imaging · Microarchitecture

## Introduction

Cancellous bone is a cellular solid and comprises a 3D spatial arrangement, or microarchitecture, of individu-

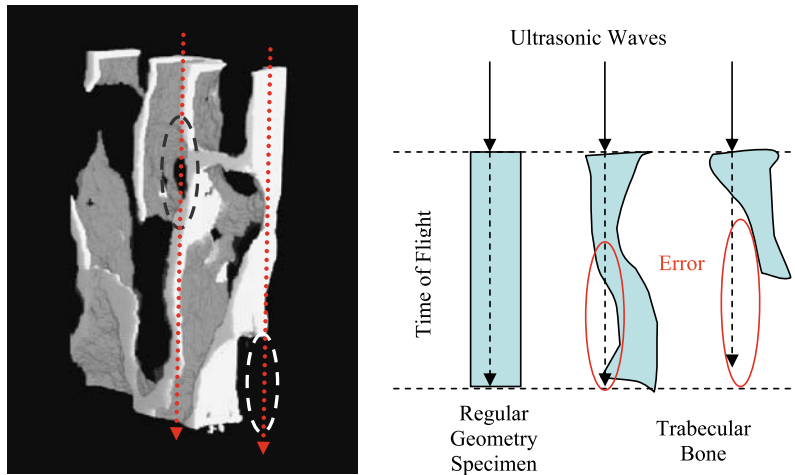
al trabeculae with interconnected pores [1]. The, apparent or bulk, mechanical properties of cancellous bone have been widely studied, using tension, compression and shear tests, as well as ultrasonic methods. Compared to the global apparent mechanical properties of cancellous bone, the mechanical properties of individual trabeculae have received less attention. This is somewhat surprising, as it has been reported that the microarchitecture of the trabeculae, together with apparent bone density, exhibits a strong correlation with the apparent mechanical properties. Nevertheless, this still leaves an unaccounted 10–28% variation in the apparent mechanical properties derived from biomechanical testing. This variance has been attributed to a lack of information on the microarchitecture of cancellous bone and the material properties of individual trabeculae [2]. Gibson and Ashby [1] reported that cortical and cancellous bones have almost the same composition; consequently, one would also expect cortical and cancellous tissue properties to be similar. However, at the onset of osteoporosis, the trabeculae within cancellous bone vary in composition, giving rise to differences in trabeculae tissue properties. This variation in composition is a result of age-related localized reduction of bone remodeling rates [3]. Investigations into the effects of trabeculae properties on apparent bulk mechanical properties of cancellous bones also appear to be scarce. Such investigations are technically challenging, as a method to measure the mechanical properties of trabeculae that does not affect the structural integrity of cancellous bone specimens, has yet to be established. This study introduces a method to overcome such technical hurdle, thus providing new insights into the investigation of bone mechanics.

Ultrasonic testing is a non-destructive method based on elastic wave propagation theory, and enables the

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J.C. Teo (✉) · E.Y. Teo · V.P. Shim · S.H. Teoh  
National University of Singapore,  
Singapore 117576, Singapore  
e-mail: mpctcmj@nus.edu.sg

**Fig. 1.** Schematic diagram showing possible sources of error: trabeculae struts that do not span the full length of the parent bone cube (*within white dashed lines*), and trabeculae that intermittently span the full length of the parent bone cube (*within black dashed lines*). Red dotted arrows show schematically the path of ultrasonic waves



measurement of elastic moduli. Elastic wave theory relates the elastic modulus and the density a continuum specimen to the longitudinal elastic wave speed via equation (1). By measuring the density of a specimen and the time taken (time of flight), for ultrasonic waves to propagate through it, the elastic modulus can be ascertained.

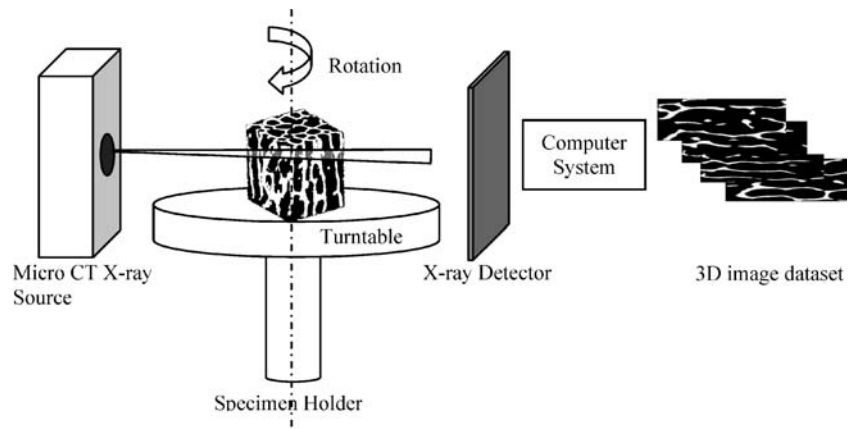
$$\nu = \sqrt{\frac{E}{\rho}} \quad (1)$$

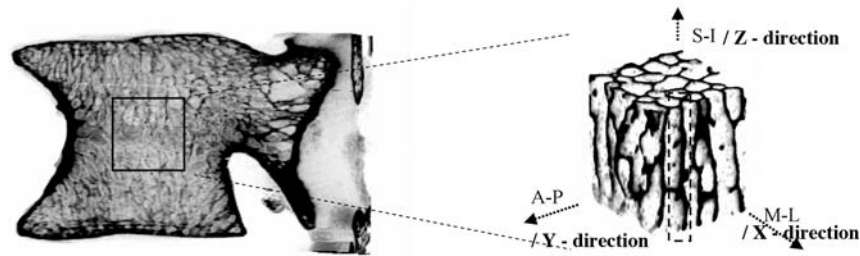
Ultrasonic testing is very attractive, as the results are not affected by factors inherent in other testing methods. For instance, results from compression, tensile or shear testing of cancellous bones are dependent on specimen geometry [4], boundary [5] and loading conditions [6]. Ultrasonic testing therefore possesses good potential for measuring the modulus of biological samples. Rho and his co-workers are strong proponents of ultrasonic testing for determining bone modulus, and have investigated the modulus of cancellous bone cubes as a whole [7, 8] as well as individual trabeculae [7, 9, 10, 11]. Typically, they used

ultrasonic frequencies of 50 kHz and 2.25 MHz for scanning cancellous bone cubes and individual trabeculae, respectively. At these frequencies, the wavelengths of the ultrasonic waves are much longer than the characteristic dimension of the structure being scanned [10]; this is a requirement based on elastic wave propagation theory [7]. In their earlier investigation of trabecular modulus, cylindrical cancellous specimens were extracted from bovine femora, with trabecular orientations approximately parallel to the longitudinal axis of cylindrical specimens. (No detailed explanation of the scanning method was provided.) A later study was conducted, whereby individual trabeculae were excised from human proximal tibia using micro-tweezers, and then ultrasonically tested [8]. This method ensures accurate measurement of the trabeculae length, as well as the propagation path of the ultrasonic waves. This method is undesirable, as it does not leave the cancellous bone intact for subsequent studies.

Careful examination of the microstructure of cancellous bone shows that the trabeculae are not regularly shaped (Fig. 1). Because of this irregularity,

**Fig. 2.** Schematic diagram of Micro Computed Tomography (Micro CT) arrangement





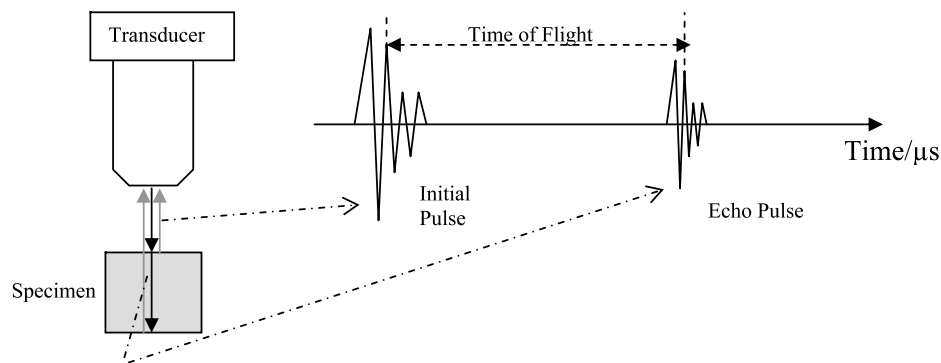
**Fig. 3.** (Left) Volume rendered  $\mu$ CT image of a vertebral body, sagittally sectioned to expose the microarchitecture of cancellous bone. (Right) Approximate position of extracted cube (white solid box) and corresponding 3D view, with appropriate anatomical axes. Also marked (within dashed rectangle) is a typical trabecular that spans the entire length of the specimen

the path taken by the ultrasonic wave may pass through bone intermittently (within black dashed lines). From the resultant ultrasonic waveform, it is difficult to determine the time of flight through bone only; the interfaces between bone and non-bone material are indistinguishable. There may also be trabeculae that do not span the entire length of the parent cancellous bone cube, thus giving rise to errors in the calculated ultrasonic velocity. Error in the second case can be rectified easily if the actual distance the ultrasonic waves propagate through can be measured. Prior evaluation of the microstructure of cancellous bone may allow ultrasonic testing to yield better results.

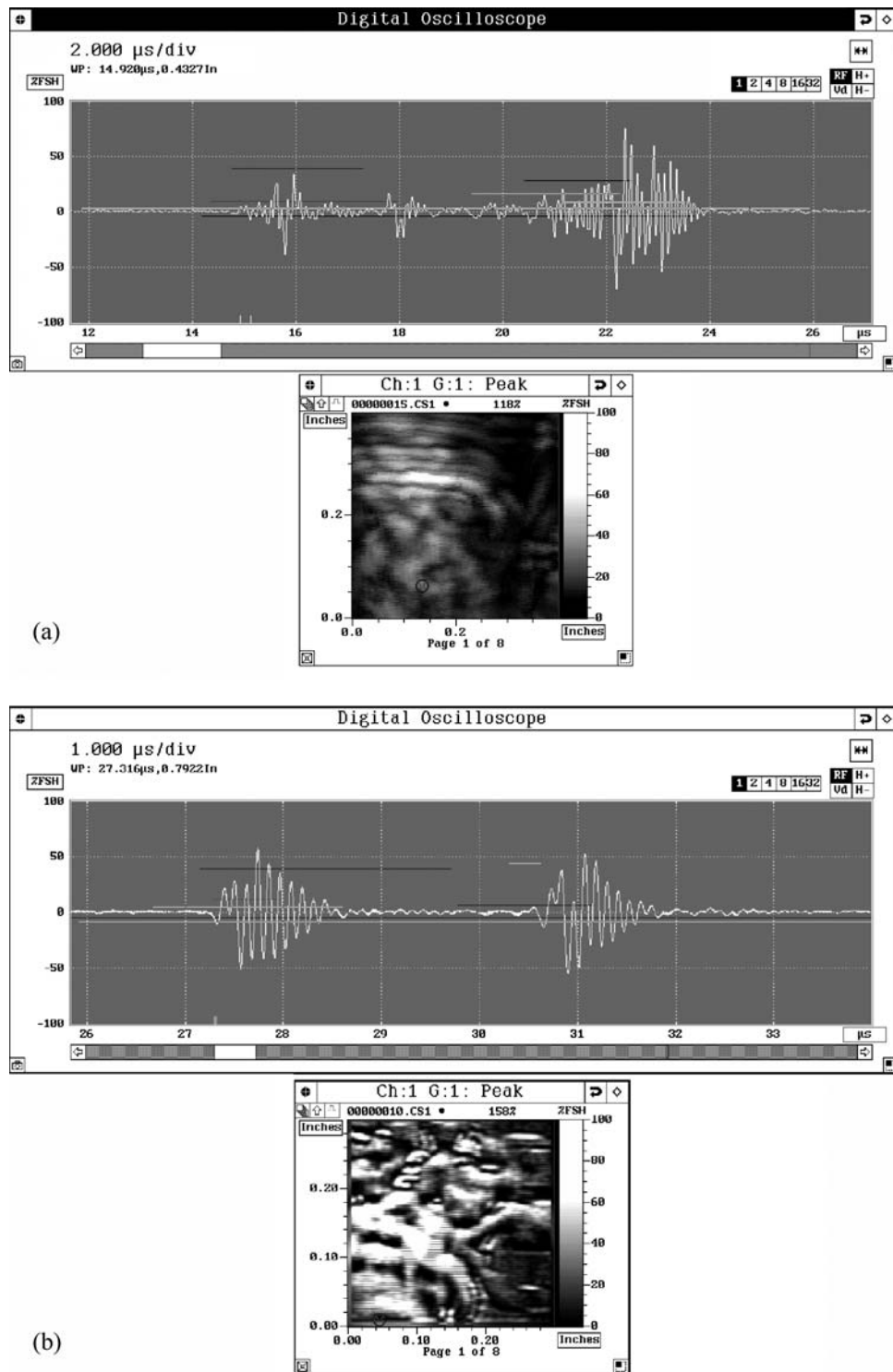
Histological sectioning is a commonly used method to evaluate the microstructure of cancellous bone [6]. Destructive serial sectioning is performed and in between each section, a microscopic image is taken using cameras and the resultant images stored as a three-dimensional dataset. From this dataset, standardized methods to evaluate the microarchitectural parameters are employed [13, 14]. Micro-computed tomography (CT) is a non-destructive method for evaluation of cancellous bone microarchitectural parameters. Like conventional clinical-CT scanners used for clinical evaluation, micro-CT scanners radiate X-rays through a specimen to obtain a series of images

to form a three-dimensional dataset. The X-ray emitter and detector are placed opposite each other in a micro-CT, while the specimen is rotated between them. In contrast, a patient lies still in clinical-CT and the emitter/detector rotates around the patient. The former arrangement is employed in the present micro-CT approach, as it allows the distance between the emitter and specimen to be varied, yielding different focus sizes. In general, micro-CT scanners differ from their clinical-CT counterparts in terms of having a smaller focus size, smaller output dose, and radiation that can penetrate denser materials. These differences allow microstructures to be imaged; however, it requires a longer acquisition time. A micro-CT dataset is similar to the dataset obtained from histological sectioning, except that the process is non-destructive; a micro-CT dataset can be processed to evaluate microarchitectural parameters such as bone volume (BV), thickness of trabeculae (Tb.Th), size of pores (Tb.Sp) and the density of trabeculae (Tb.N). In addition, with the use of volume rendering software, the dataset facilitates examination of cancellous bone specimens, allowing 3D visualization of the microarchitecture and pre-selection of particular trabeculae to be ultrasonically tested.

There is no accepted standard method to verify the results obtained from ultrasonic testing. Rho et al. [8]



**Fig. 4.** Schematic diagram of pulses captured on an oscilloscope during transmission of ultrasonic waves. TOF is the time between successive pulses. Actual pulses obtained when a single trabeculae is subjected to ultrasonic testing [Fig. 5(a)]



**Fig. 5.** (a) The upper image shows the signal obtained from ultrasonic testing of a random trabeculae located by visual inspection based on an ultrasonic surface scan; the signal is very noisy and it is difficult to ascertain the echoes through bone alone. (b) The lower image is a clearer signal obtained using the present method. Passage of the ultrasonic wave through bone alone is ensured as the location for targeting of the pulse was identified using microCT imaging

conducted another study and compared the results obtained from microtensile testing with that obtained from ultrasonic measurements. The specimens tested comprised engineering materials such as aluminium,

stainless steels and individual trabeculae excised from the proximal region of a single human tibia. In each case, it was observed that the average values of the elastic modulus measured ultrasonically were slightly

**Table 1** Modulus obtained from ultrasonic scanning of engineering materials

Material	Calibration value from user's manual (GPa)	Bumrerraj and Katz [17] (GPa)	Experimental value (GPa)
Aluminum	109.56	105.81	101.02–109.95
Teflon	4.14	2.40	3.62–3.97
Copper	126.10	196.60	130.58
PMMA	–	8.68	6.90–7.28

greater than those measured by the microtensile testing method. However, this could be attributed to the very low strain rates used in the microtensile tests, compared to the strain rates in ultrasonic testing, as suggested by Carter and Hayes [15]. In the present study, microtensile testing was not employed because of difficulties in manufacturing specialized mountings to clamp small specimens and also because this would destroy the microarchitecture.

Consequently, in this study, a novel method, that combines ultrasonic scanning with micro-CT imaging to determine the modulus of individual trabeculae within a cancellous bone cube is proposed. This enables the modulus of trabeculae to be obtained non-destructively, so that the specimen remains intact, allowing subsequent examinations to be carried out.

## Experimental Procedure

### Specimen Preparation

Porcine vertebral bodies, from levels T10–L2 of the spine, were extracted from two mature pigs (weighing 40 and 55 kg). After all soft tissue was removed, under constant irrigation, a slow speed circular diamond saw was used to cut 5 mm cancellous bone cubes from the vertebral bodies. A total of 40 specimens were obtained. Anatomical orientations of the samples—superior–inferior (SI), anterior–posterior (AP) and medial–lateral (ML)—were marked with colored ink

and bone marrow was removed using a low impact water jet [7, 16]. The cubes were stored in individual containers to minimize thermal cycling.

### Location of Trabeculae Using MicroCT Imaging

The bone cubes were placed in a specially fabricated jig filled with phosphate buffered solution (PBS) to prevent deterioration, and isotropically scanned at 14  $\mu\text{m}$  resolution using an SMX-100CT microCT scanner (Shimadzu, Japan). The X-ray parameters were set at 47 kV and 80  $\mu\text{A}$ . With these settings and a 0.5 mm aluminum plate as the X-ray detector, good contrast was achieved between the PBS and trabeculae (Fig. 2).

From each anatomical plane of the resultant image dataset, locations of trabeculae that span the entire length of the cube were identified and their coordinate values determined using voxel resolution. The objective was to ultrasonically scan five trabeculae per anatomical direction. Cartesian coordinate values were calculated with reference to an origin corresponding to an indentation made at one corner of the bone cube specimen; this was easily identifiable in the  $\mu\text{CT}$  dataset (Fig. 3). The coordinate system was used to guide the ultrasonic transducer from the origin to ascertain the locations of full-length trabeculae.

### Ultrasonic Testing

An ultrasonic C-Scan machine (Sonix Inc., USA), fitted with a 2.25 MHz transmitter/receiver probe, was used to determine the “time-of-flight” (ultrasonic wave propagation time) through individual trabeculae of cancellous bone specimens submerged in water (Fig. 4). A frequency of 2.25 MHz was selected to ensure that the wave propagated through individually identified trabeculae and not the bone specimen as a whole. Linear traversing in three orthogonal directions was facilitated by worm gears attached to AC motors and controlled digitally by a computer. Each traversing axis had a resolution of up to 1  $\mu\text{m}$ . Prior to testing, the validity of results from the C-Scan machine was checked. Common engineering materials of various

**Table 2** Ultrasonic wave speed and modulus values obtained

Source	Wave speed, $v$ (m/s) (Std. dev.)			Modulus, $E$ (GPa) (Std. dev.)			Trabecular density, $\rho$ ( $\text{kg}/\text{m}^3$ ) (Std. dev.)
	SI	AP	ML	SI	AP	ML	
1	2474.51 (298.52)	2602.30 (411.79)	3077.68 (786.78)	15.78 (4.49)	17.53 (5.15)	25.35 (12.93)	2513.66 (182.60)
2	2115.55 (558.88)	2335.19 (595.02)	2430.72 (653.55)	13.87 (4.32)	17.42 (4.91)	17.17 (4.22)	2597.55 (328.91)



**Table 3** Specimen dimension details

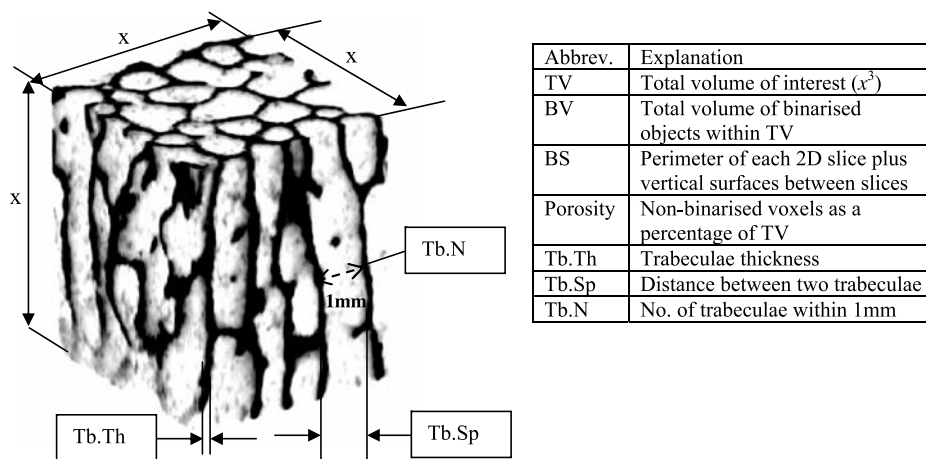
Microarchitectural parameter	Units	Anatomical direction	Mean		Standard deviation	
			Source 1	Source 2	Source 1	Source 2
Bone volume (BV)	mm <sup>3</sup>		25.07±6.69	23.95±5.75	4.58	4.77
Tissue volume (TV)	mm <sup>2</sup>		115.91±11.43	123.90±9.41	7.61	6.43
Bone surface area (BS)	mm <sup>2</sup>		649.77±274.22	694.91±251.93	198.55	132.21
BS/BV	mm <sup>-1</sup>	SI	17.94±5.61	20.45±4.12	4.62	4.13
		AP	29.41±12.74	32.96±9.35	7.30	5.28
		ML	29.48±10.67	33.12±6.83	7.43	5.58
BS/TV	mm <sup>-1</sup>		5.61±2.15	5.60±1.77	1.67	1.01
BV/TV	%		21.68±5.15	19.30±4.46	4.11	3.52
Bone porosity	%		78.32±5.15	80.70±4.46	4.13	3.52
Trabecular thickness (Tb.Th)	µm	SI	110.36±29.50	101.13±20.87	29.59	17.66
		AP	72.13±26.13	62.00±13.00	19.01	8.69
		ML	71.88±26.88	61.88±14.12	17.26	8.95
Trabecular number (Tb.N)	mm <sup>-1</sup>	SI	1.93±0.43	1.94±0.53	0.58	0.36
		AP	3.21±1.59	3.15±0.93	1.04	0.58
		ML	3.21±0.77	3.16±1.04	1.05	0.63
Trabecular separation (Tb.Sp)	µm	SI	1068.50±618.5	1012.25±458.75	373.14	259.45
		AP	656.81±378.81	624.44±280.44	257.60	164.01
		ML	653.56±383.56	625.19±291.19	248.76	177.15

thickness (1.5–20.0 mm) and known wave propagation speeds, were ultrasonically scanned for comparison.

The pulse-echo inspection method, also known as the reflection mode, is used in this study. A transducer emits an ultrasonic signal into the sample and the signal is reflected back into the transducer. The transducer serves as both emitter and receiver. When an ultrasonic pulse reaches the top surface of the specimen, part of the wave is reflected and captured by the transducer as the initial pulse and the rest of the pulse is reflected when it reaches the base of specimen; this is captured as the echo (Fig. 4). The time-of-flight (TOF) is the basis of ultrasonic testing and it is the time difference between the initial signal and the echo. From the TOF

and the specimen thickness ( $t$ ) the ultrasonic velocity, or elastic wave velocity ( $v$ ) can be determined.

Elastic wave propagation theory defines the relationship between tissue modulus ( $E$ ), tissue density ( $\rho$ ) and ultrasonic velocity via equation (1). Again, this is only valid if the wavelength of the propagating wave is larger than the cross-sectional dimension of the trabeculae and smaller than that of the cancellous bone specimen tested. The ultrasonic testing device is able to generate a two-dimensional image of the surface scanned. The image generated is based on reflection of ultrasonic pulses and is used for visual positioning of the transducer [lower window in Fig. 5(a) and (b)]. Without microstructural examination of



**Fig. 6.** Schematic diagram and microarchitectural parameters evaluated from three-dimensional micro-CT image datasets. Detailed explanation of the parameters can be found in literature [14]

a specimen, the trabeculae structure below the surface is unknown; this may yield erroneous results, difficulty in determining the TOF [Fig. 5(a)], and possible inaccuracy in determining the wave propagation distance. As trabeculae are never perfectly straight, even with the proposed technique, minor adjustments must be made to obtain a clear ultrasonic echo [Fig. 5(b)]. Manual adjustment of the specimen support platform was limited to  $\pm 5 \mu\text{m}$  in the  $x$ - $y$  plane.

## Results

### Verification of Ultrasonic C-Scan Technique

To verify the suitability of utilizing ultrasonic scanning with a 2.25 MHz transducer, materials of known elastic wavespeed were scanned, and the results of the elastic modulus derived were compared with values from Bumrerraj and Katz [17] as well as a calibration table provided in the User's Manual accompanying the ultrasonic tester (Table 1).

### Trabeculae Modulus

A total of 40 specimen cubes were extracted from porcine spines and subjected to ultrasonic testing along the three primary anatomical axes—Superior–Inferior (SI), Anterior–Posterior (AP) and Medial–Lateral (ML). Micro-CT imaging was used to aid in location of specific trabeculae, which span the length of each specimen cube. At least six locations with full-length trabeculae were tested for each direction and the average values computed; the actual thicknesses of these trabeculae were measured from  $\mu\text{CT}$  images. Trabeculae density was determined by dividing the mass of the cancellous bone cubes (after removal of bone marrow) by the total trabeculae volume. The tissue modulus is then evaluated using equation (1). Table 2 summarizes average trabeculae modulus values obtained from cancellous bone extracted from porcine vertebrae.

Micro-CT imaging also enables the cancellous bone volume (BV) and average trabeculae thickness (Tb.Th) to be calculated, along with other microarchitectural parameters (Table 3 and Fig. 6). The mass of each bone cube was measured using a digital scale; trabeculae density was subsequently determined by dividing the measured mass by the corresponding BV. An average trabeculae density of  $2,555.6 \text{ kg/m}^3$  was obtained; the highest value for human trabecular bone material density reported in literature is  $2,055 \text{ kg/m}^3$  [9] and the lowest is  $1,600 \text{ kg/m}^3$  [18]. For equation (1)

to be valid, the wavelength has to be smaller than the bone cube, but larger than the cross-section of trabeculae. The Tb.Th values were examined to verify this.

## Discussion

Based on elastic wave propagation theory, calculation of the modulus requires knowledge of the wave speed and material density. The wave speed is in turn determined by measuring the “time of flight” of the ultrasonic propagation and the distance which it propagates through. Ashman and Rho [7] first used ultrasonic testing to determine the modulus of individual trabeculae while preserving the structural integrity of cancellous bone. Distance was assumed to be the characteristic length of the cancellous bone specimen. On closer inspection of cancellous bone, due to its microarchitecture, it has been shown that ultrasonic testing of trabeculae is not straightforward. The aim of this study is to determine non-destructively, the modulus of trabeculae within a cancellous bone specimen. A novel method that couples ultrasonic testing with micro-CT imaging has been presented. This approach allows non-contact, non-invasive assessment of trabeculae modulus whilst leaving the structural integrity of bone specimens intact.

With the aid of micro-CT imaging, individual cancellous trabeculae that span the entire length of the specimen were subjected to ultrasonic testing. The wave speeds measured from the ultrasonic testing of the trabeculae in the three anatomical directions ranged from 2,115 to 3,077 m/s, with an average value of 2,505 m/s. Literature indicates an average ultrasonic velocity of 2,898 m/s for trabeculae from the human proximal tibia (S.D. 84.5) [8]; average values for human and bovine femur are about 2,704 m/s (S.D. 142) and 2,501 m/s (S.D. 168), respectively [7]. Ultrasonic tests by Nicholson et al. [9] using a 1 Mhz transducer, yielded wave speeds of 2,250 m/s (S.D. 140). Their usage of such a frequency is questionable; at such high frequencies resultant wavelength is smaller than the cross-sectional area of trabeculae struts. Modulus obtained may be focused on the components (lamellae, lacunae and canaliculi) of trabecular bone instead. It can be seen that the speed of ultrasonic wave propagation through trabeculae material lies between 2,000 to 3,000 m/s. The cylindrical cancellous bone specimens employed by Ashman and Rho [7] did not allow orthotropic ultrasonic testing. Cubic specimens enable ultrasonic testing in the SI, ML and AP directions. The average wave velocities in the SI, ML and AP directions were found

**Table 4** Wavelength and trabeculae thickness from tests with a frequency ( $f$ ) of 2.25 MHz

Source	Average velocity, $v$ (m/s)				Average wavelength, $\lambda$ (mm) $\lambda=v/f$			
	SI	AP	ML	Average	SI	AP	ML	Average
1	2474.51	2602.30	3077.68	2,718.16 Tb.Th (mm)	1.1 0.110	1.15 0.072	1.37 0.072	1.21 0.08
2	2115.55	2335.19	2430.72	2,293.82 Tb.Th (mm)	0.94 0.10	1.04 0.062	1.08 0.062	1.02 0.07

to be 2,295, 2,469 and 2,754 m/s, respectively; the difference does not appear to be significant.

The ultrasonic wave speeds obtained were combined with trabecular density to calculate the modulus. A caveat of this study is the assumption of a common trabecular density throughout a given cancellous bone specimen. The volume was derived from micro-CT datasets with a 14  $\mu\text{m}$  resolution and there was inherently already some approximation of the actual volume; although the scan resolution is smaller than the average trabeculae thickness of 80  $\mu\text{m}$ , there will be geometries that are smaller than the scan resolution and would be approximated. Other researchers have employed Archimedes Principle to determine trabeculae volume; however the present study found that this method has its disadvantages. As the geometry of cancellous bone is highly intricate, trapped air bubbles within the structure are difficult to remove even after rigorously shaking each specimen in water. Evaluation of trabeculae thickness (Tb.Th) with respect to the ultrasonic wavelength is important in ultrasonic testing. For elastic wave propagation theory to apply, the wavelength has to be larger than the cross-sectional dimension of trabeculae, but smaller than the overall size of the bone specimen [7, 9]. In the present study, the ultrasonic wavelength is at least ten times larger than the average Tb.Th (Table 4) and five times smaller than the overall dimensions of the bone specimen. Equation (1) is therefore valid; these details have not been reported previously.

A method of verifying the trabeculae modulus derived should be established, and it is suggested that the use of  $\mu\text{FE}$  analysis be examined. Kabel et al. [19] have used  $\mu$ -scale finite element analyses as an inverse approach to calculate tissue modulus, and obtained an average value of 5.6 GPa for whale trabeculae. Their cancellous bone cube specimens were subjected to compressive testing and the bulk elastic stiffness obtained. Prior to testing,  $\mu\text{CT}$  imaging was undertaken and used to construct  $\mu\text{FE}$  models. The  $\mu\text{FE}$  models were assigned boundary conditions similar to that in compressive testing, and simulations were run based

on varying isotropic trabeculae properties until a similar overall elastic stiffness was obtained. This method can be used for trabeculae modulus verification; however, inherent errors, machine compliance, specimen placement and viscoelastic effects from compressive testing tend to result in underestimation of values [19].

One recommendation is the coupling of three-dimensional visualization software with control of the motors that drive translational movement of the ultrasonic testing machine. With this integration, identification of the best trabeculae to be ultrasonically tested and positioning of the ultrasonic probe can be effected simultaneously.

## Conclusion

A novel method to determine the elastic modulus of bone trabeculae using ultrasonic testing coupled with microCT imaging has been proposed. MicroCT imaging enables the identification of appropriate specific trabeculae in cancellous bone specimens for scanning. The proposed method has the advantage of preserving the structural integrity of extracted cancellous bone specimens, thus facilitating subsequent further investigation into the dependence of cancellous bone mechanical properties on trabeculae modulus.

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