Research article

Wavelet transform-based photoacoustic time-frequency spectral analysis for bone assessment

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ARTICLE INFO

Keywords:
Photoacoustic measurement
Wavelet transform
Time-frequency spectral analysis
Bone assessment

ABSTRACT

In this study, we investigated the feasibility of using photoacoustic time-frequency spectral analysis (PA-TFSA) for evaluating the bone mineral density (BMD) and bone structure. Simulations and ex vivo experiments on bone samples with different BMDs and mean trabecular thickness (MTT) were conducted. All photoacoustic signals were processed using the wavelet transform-based PA-TFSA. The weight-averaged mean frequency (PWMF) was evaluated to obtain the main frequency component at different times. The y-intercept, midband-fit, and slope of the linearly fitted curve of the PWMF over time were also quantified. The results show that the osteoporotic bone samples with lower BMD and thinner MTT have higher frequency components and lower acoustic frequency attenuation over time, thus higher y-intercept, midband-fit, and slope. The midband-fit and slope were found to be sensitive to the BMD; therefore, both parameters could be used to distinguish between osteoporotic and normal bones ($p < 0.05$).

1. Introduction

The increase in the incident of osteoporotic patients has been correlated with the increase in the aging population with an estimate of more than 200 million people worldwide\cite{1}. Osteoporosis causes the loss of bone mass and the degeneration of bone structure, which in turn result in increased fragility and incidence of fractures\cite{2}. Therefore, the timely evaluation of the bone mineral density (BMD) and bone structure is of great importance. The available clinical methods for osteoporosis detection mainly rely on either X-ray or ultrasound (US) techniques\cite{3}. Dual energy X-ray absorptiometry (DEXA) is regarded as the “gold standard” and is the most used and validated method for osteoporosis detection. It provides measurements of BMD at the most relevant site, the central skeleton (spine and femur)\cite{4}. However, the BMD can only explain 60\%–80\% of the variability in bone strength, and the bone microstructure information cannot be obtained. There are some specific limitations, such as ionizing radiation and high costs. Quantitative US (QUS) methods have shown greater potential in osteoporosis detection than DEXA owing to their lower cost and lack of ionizing radiation\cite{5}. QUS methods can assess the BMD, bone microstructure, and elastic properties of bone tissues but are insensitive to information relating to the chemical contents in the bone, as well as the frequency spectrum characteristics of the trabecular bone\cite{6}.

Photoacoustic (PA) detection techniques have shown great potential in disease detection owing to their ability to evaluate both the chemical and physical properties of tissue. They can present molecular
information about tissues based on the specific optical absorption spectra of different chemical components [7–9]. Moreover, the acoustic frequency spectrum of PA signals is correlated to the size/microstructure of different chemical components when they are illuminated as the absorption source [10,11]. By applying spectral analyses on PA signals, and further extracting fitted parameters – y-intercept, midband-fit and slope of the frequency domain power spectrum, microstructures smaller than the system resolution and the distribution of the absorption source in soft tissues can be obtained for disease detection [12,13]. However, cancellous bone is a dual-component porous medium, which is composed of a three-dimensional porous trabecular network filled with bone marrow in the pore. The complex structure and acoustic impedance difference lead to complicated propagation modes of the PA signal, making the assessment of the bone properties difficult. On the one hand, the physical properties of the trabeculae and bone marrow, such as light absorption, thermo-elasticity, and size, are very different, leading to the variability in the spectrum characteristics of their PA signals. On the other hand, the propagation attenuation of the PA signals is a frequency-related parameter due to acoustic impedance difference in trabecular bone and bone marrow, and act as a function of BMD [14–16]. In the past years, the effectiveness of PA techniques in the assessment of the bone properties, in particular the chemical components, microstructure, and bone strength has been investigated. Combined backscatter US and back-propagating PA measurements have been proposed to detect changes in bone mineral and collagen content, based on the measurement of apparent integrated backscatter, normalized apparent backscatter, and chromophore (absorber) density [17–19]. A hybrid multispectral PA technique was developed to quantitatively evaluate the mechanical properties of bone by measuring the speed of sound dispersion and broadband US attenuation, as well as to determine the chemical components in the bone by measuring the PA signal energy [20,21]. The multispectral PA system enabled a quantitative in-human measurement of the marrow’s blood/fat ratio, which is useful for the early diagnosis of bone pathologies [22]. Thermal PA measurements can provide the information associated with BMD, based on the dependence of the PA signal intensity on temperature [23]. Multi-wavelength PA measurements can access the microstructure and relative contents of several chemical components in calcaneus bones in vivo, including both minerals and organic materials, such as oxygennated-hemoglobin, deoxygenated-hemoglobin, and lips, which are crucial for metabolic activities and bone health [24]. PA spectral analysis and 3D PA imaging can be used to assess the bone microstructure information quantitatively [25,26].

Most previous studies focused on either BMD evaluation by studying the propagation attenuation in bone in the time domain or microstructure evaluation by applying the spectrum analysis on PA signals of trabeculae in the frequency domain. However, both bone mass loss and bone microstructure degeneration result in an increased incidence of fractures. The acoustic frequency spectrum of the trabeculae and the frequency-related propagation attenuation due to the acoustic impedance difference need to be studied for bone microstructure and BMD evaluation for a comprehensive assessment. The continuous wavelet transform (CWT) overcomes the shortcoming of the separation of the time and frequency domains in the usual spectrum analysis and can provide joint distribution information of the time and frequency domains of PA signals simultaneously [27]. The CWT technique has been used for signal analysis in various fields, including medical science [28,29] mechanical engineering [31–33], and geophysics [34,35]. The CWT technique can provide the time-resolved frequency spectrum of the broad-band PA signals and can be used to study the frequency spectrum distribution and frequency-related propagation attenuation of bone PA signals [36].

The complex Morlet wavelet is a complex sinusoid with a symmetric Gaussian window envelope, which has good resolution in both the time and frequency domains without introducing high-harmonic frequency components and spurious components [31,37,38].

In this work, we aim to demonstrate the feasibility of using the PA time-frequency spectral analysis (PA-TFSA) to characterize the BMD and bone microstructure information for bone assessment by performing numerical simulations and experiments on bone models with different bone loss. All the PA signals from the bone specimens were processed using the CWT technique to obtain the frequency spectrum at different times. We aim to characterize the microstructure in the frequency spectrum and characterize the BMD based on the frequency-related attenuation along the propagation distance for a comprehensive bone assessment.

2. Materials and methods

2.1. Numerical simulations

2.1.1. Bone sample models

Two-dimensional simulations of two groups of bone models were conducted to evaluate the feasibility of PA-TFSA for bone evaluation. The PA signals of the trabeculae were analyzed and compared. All the data processing was performed using MATLAB R2019b, and the simulations were performed using the k-wave toolbox [39]. By adjusting the threshold value of micro-CT images from a human calcaneus bone, the grayscale image was converted to a binary image with different area ratio of trabecular bone (ARTB). As shown in Fig. 1(a), two groups of four bone samples were chosen for the PA signal generation and propagation simulations; the white and dark areas represent the trabecular bone and bone marrow, respectively. The ARTBs for the two groups of bone samples were 22 % and 18 % [40], with a thinner mean trabecular thickness (MTT) and a higher BMD in group 2 than those in group 1. The MTT of each sample was quantified using the indirect indices of the mean trabecular plate thickness method [41].

2.1.2. Numerical simulations of PA signal generation and detection

In the simulations, the radius of the bone samples and the spacing of square grid points were set to 9 mm and 20 μm, respectively. For the soft tissue, the speed of sound and density were set to 1500 m/s and 1000 kg/m³, respectively, and for the trabecular bone, the speed of sound and density were set to 3200 m/s and 1850 kg/m³, respectively. The light was set such that it irradiated the bone samples uniformly, and the trabecular bone was set as the optically absorbing object. The initial PA pressure of the trabecular bone and that of the bone marrow and surrounding tissues were set to 1 and 0, respectively. Only the frequency spectrum of the trabeculae and the frequency-related attenuation along the propagation distance were considered. The attenuation coefficient was set to 9.94 dB/(MHz · cm). As shown in Fig. 1(b), the PA signals of each bone sample were received by 48 sensors evenly distributed around the bone samples. The distance of the sensors to the center of the bone samples was set to 15 mm, and the transducer was simulated for frequencies ranging between 0 and 37.5 MHz. Fig. 1(c) and (d) shows typical PA signals of bone samples from group 1 and group 2, respectively.

2.2. Ex vivo experiments on rabbit bone specimens

2.2.1. Experimental setup

Fig. 2(a) shows a schematic of the experimental setup. A tunable optical parametric oscillator laser (Phocus Mobile, OPOTEK, Carlsbad, CA) was used to provide laser pulses with a repetition rate of 10 Hz and pulse width of 2–5 ns at a wavelength of 690 nm. Both the hydroxyapatite in trabecular bone and the hemoglobin in bone marrow contribute to the strong absorption at the wavelength of 690 nm [42]. A dichroic mirror was used as a beam splitter to split the laser into two beams for the illumination of the bone sample and the black body for recording the laser power. At a wavelength of 690 nm, the energy of the laser beam illuminated at the bone surface was approximately 18 μJ/cm², and the diameter of the beam illuminating the bone surface was limited to 12 mm by the spatial filter. The PA signals generated by the bone samples
were received by a needle hydrophone with a bandwidth of 1–20 MHz (HNC1500, hydrophone, ONDA Corp., Sunnyvale, CA). The PA signals generated by the black body were received by a 1 MHz focused transducer (V302, Immersion Transducers, Olympus Corp., Tokyo, Japan). A standard US coupling gel was used to ensure coupling between the bone samples and the hydrophone. All the PA signals of the bone samples received a 25 dB gain through amplifiers (5072PR, Olympus Corp., Tokyo, Japan) filtered with a 1 MHz high-pass filter. They were recorded by a digital oscilloscope (HDO6000, oscilloscope, Teledyne Lecroy, USA) with a sampling rate of 2500 MHz. The external epicondyle of the femur is rich in trabecular bone, which is highly correlated with bone health. Moreover, it is also the flattest position for signals acquisition and is easy to translate clinically. Thus, each bone sample was collected at three different positions of the external epicondyle of the femur, as shown in Fig. 2(a). The PA signals collected at the three different positions were consistent.

2.2.2. Animal model

Two types of animal models with different BMDs were used. The first type was the artificial osteoporotic model. This model included rabbit...
metaphysis bone specimens before and after treatment using an ethylenediaminetetraacetic acid (EDTA) \cite{43} solution (0.5 M) for 2 days; during the treatment, the bone samples were decalcified, and the hemoglobin in bone decreased \cite{44}. Seven samples were included for each group. The second type was a well-established rabbit osteoporotic model. Ovariectomies were performed on adult female New Zealand white rabbits to obtain the osteoporotic group (n = 8). The same number of adult female New Zealand white rabbits served as controls (n = 8). After five months from the surgery, the rabbits were euthanized, and the distal femoral metaphysis of both legs of each rabbit were dissected and subjected to PA assessments. PA signals from the normal and osteoporotic rabbit distal femoral metaphysis are shown in Fig. 2 (c) to (d). Both the hemoglobin and the bone mineral were expected to absorb light with a wavelength of 690 nm. Moreover, the high frequency components are mainly from trabeculae.

2.3. PA signal processing

The PA signals generated by the bone specimens were processed as follows. First, the rising edge of the bone PA signal was selected as the starting position of the effective PA signal and was calibrated by placing the black body at the side of the bone next to the hydrophone, as shown in Fig. 3(a). Second, all the PA signals were analyzed using the CWT technique to acquire the PA time-frequency spectrum (PA-TFS) of each bone specimen, and the frequency-spectrum distribution at different times was obtained, as shown in Fig. 3(b). To better quantify the PA-TFS...

Fig. 2. Experimental set up. (a) Schematic of experimental setup for PA measurement of bone samples. (b)–(c) Typical PA signals of the bone from the normal and osteoporotic rabbit groups.

Fig. 3. PA signal processing. (a) Example of the bone PA signal. (b) An example of the photoacoustic time-frequency spectrum (PA-TFS) of the bone PA signal. (c) Power-weighted mean frequency (PWMF) and linear fit of the PA-TFS.
and obtain the main frequency component at each moment, the power-weighted mean frequency (PWMF) at different times was evaluated. The PWMF was calculated as follows:

\[ PWMF = \frac{\int f \cdot P(f) df}{\int P(f) df} \]

where \( P(f) \) is the spectrum density at each frequency \( f \). To avoid strong low frequency noise, the lower limit frequency of the interval was set at 1 MHz. The higher limit frequency of the interval was the frequency at which the maximal \( P(f) \) dropped to 20 %. The curve of the PWMF over time was fitted by linear regression, from which the parameters y-intercept, midband-fit, and slope were quantified. As shown in Fig. 3(c), the starting position of the fitting range of PWMF curve over time was at 0.5 \( \mu \)s without being affected by the cortical bone and cartilage. The region of interest (ROI) of the PWMF was chosen with a time duration of 5 \( \mu \)s, which was determined by the \(-10\ dB\) attenuation of the PA signal energy in bone.

3. Results

3.1. Numerical simulations of two groups of bone samples

Fig. 4(a) and (b) shows the PA-TFS of two groups of bone samples with different ARTBs, thus different MTTs and BMDs. Their PA-TFSs intuitively reflect the frequency spectrum distribution at each moment and the propagation attenuation of acoustic frequency over time. There are higher frequency components as the MTT decreases, which is consistent with the theory that PA signals produced from the thinner trabeculae have higher acoustic frequency components [26,45]. The high-frequency components in the PA-TFS of the two groups of bone samples attenuated over time. In contrast, the high frequency components attenuated rapidly as the BMD increases [46], as indicated by the yellow dotted line in Fig. 4(a) and (b). The representative PWMF curve and quantization parameters are shown in Fig. 4(c)-(f). With higher frequency and lower frequency-related propagation attenuation over time, the bone specimens with thinner trabeculae and lower BMD have higher y-intercept, midband-fit, and slope.

3.2. Experimental results of EDTA-treated bone model

Fig. 5(a) and (b) shows the PA-TFS of the untreated bone sample and EDTA solution-treated bone sample, respectively. From the figure, it can be seen that the PA-TFS of the bone after the EDTA treatment had higher frequency components and lower frequency-related attenuation compared to that of the untreated bones. Fig. 5(c)-(f) shows representative PWMF curves and quantization parameters. As shown in the figure, with higher frequency and lower frequency-related propagation attenuation over time, the bone specimens after the EDTA treatment with thinner trabeculae and lower BMD has higher y-intercept, midband-fit, and slope than those of the untreated bones. The statistical results at 690 nm show significant differences in the y-intercept, midband-fit, and slope between the groups of rabbit metaphysis before and after treatment with EDTA.

3.3. Experimental results of animal models with osteoporosis

3.3.1. DEXA imaging for BMD analysis

DEXA imaging was conducted on the femoral metaphysis of each rabbit model. Fig. 6(a) shows a photograph of the experimental setup; Fig. 6(b) shows the location of the bone chosen for the bone density calculation, and Fig. 6(c) shows the BMD results quantified from the DEXA images. There was significant bone loss in the osteoporotic group, confirming the pathologic conditions of the osteoporotic group, as well as the difference between the two groups of rabbit subjects.

3.3.2. PA-TFSA of two groups

The PA-TFS of the control and osteoporotic groups are shown in Fig. 7(a) and (b), respectively. The osteoporotic bone samples showed a higher frequency and lower frequency-related propagation attenuation over time than those of the normal bones. The representative PWMF curve and quantitative parameters are shown in Fig. 7(c)-(f). The osteoporotic bones have higher frequency and lower frequency-related propagation attenuation over time with thinner trabeculae and lower BMD. There is a significant increase in the measured slope, y-intercept and midband-fit in the osteoporotic rabbit bone specimens when compared to the normal bone specimens as observed in Fig. 7(d)-(f). The midband-fit and slope parameters were found to be sensitive to BMD and could be used to distinguish the osteoporotic and normal bones.
3.3.3. PA-TFS parameters association with BMD

The analysis results of the correlations between the PA parameters $y$-intercept, midband-fit, and slope and the BMD of bone samples of the two groups are shown in Fig. 8 (a)–(c), respectively. The midband-fit and slope were significantly and negatively correlated with the BMD, with Pearson correlation coefficient distributions of 0.59 and 0.54, respectively. A good correlation is also observed between the BMD and PA-TFS parameters in terms of midband-fit and slope.

4. Conclusion and discussion

The wavelet transform-based PA-TFSA could effectively characterize the BMD and bone microstructure information for bone assessment at a wavelength of 690 nm. Both simulations and ex vivo experiments on bone samples with different BMDs were conducted. Higher frequency components and lower frequency-related propagation attenuation over time were observed in the PA-TFS of the osteoporotic bones with thinner trabeculae and lower BMD. The PA-TFS parameters midband-fit and slope can distinguish the osteoporotic bones form the normal bones. The results of the ex vivo experiments were in good agreement with the results of the simulations. The results demonstrate the considerable potential of the PA-TFSA for the noninvasive and nonradiative clinical assessment of bone health.

Previous studies showed that the spectral parameter slope can quantitatively evaluate the trabecular thickness for bone health evaluation, as the thinner trabeculae can generate higher PA frequency by comparing with the thicker trabeculae [45,47]. Therefore, the osteoporotic bones with thinner trabecular bone have higher frequency components of PA-TFS, thus higher $y$-intercept and midband-fit than control bones. Furthermore, bones with lower BMD have lower US attenuation [48–50]. Therefore, bones with lower BMD leads to the lower US attenuation in both the time and frequency domains of the PA-TFS and have higher slope. The osteoporotic bones with lower BMD have lower frequency-related propagation attenuation over time [46,51] and thus have higher midband-fit than normal bones. For the EDTA-treated bone models, all the parameters, namely the $y$-intercept, midband-fit, and slope can distinguish the untreated bones from the treated bones at a wavelength of 690 nm. For the well-established rabbit models, the midband-fit and slope were found to be more sensitive to BMD variation than to variations in the $y$-intercept. This may be due to several reasons. First, the properties of EDTA-treated bone models are different from those of rabbit osteoporosis models. In the EDTA-treated bone model, both the bone mineral and hemoglobin decreased after the treatment [44]. In contrast, in the well-established rabbit osteoporosis model, only the bone mineral decreased in the osteoporotic bone. Second, the parameter $y$-intercept is much more sensitive to the changes in chemical contents than the other parameters [52]. Thus, large changes in the chemical components lead to large changes in the parameter...
y-intercept in EDTA models. There are red blood cells in the red bone marrow in the distal femur, which are rich in hemoglobin [53]. Upon illumination with a wavelength of 690 nm, there are less low frequency components from bigger red bone marrow clusters in the EDTA bone models than in the rabbit osteoporosis model. Third, there is a negative correlation between changes in the y-intercept and the slope. Therefore, the y-intercept of the EDTA group is significantly higher than that in the untreated group and can be used as an indicator of the group.

The results of this study show that the PA-TFSA can successfully characterize both the microstructure and BMD for a comprehensive bone assessment. However, the PA-TFSA has some limitations when applied in human cancellous bone in vivo. First, the frequency components in human cancellous bone are lower than those of the metaphysis in rabbits. The trabeculae of human cancellous bone are thicker than the metaphysis of rabbits, so there are lower frequency components when it is illuminated as the absorption source. Furthermore, there is strong acoustic attenuation in human cancellous bone, especially for high frequency components. The applicability of the PA-TFSA parameters for BMD and bone microstructure evaluation needs to be further validated at low frequencies. To compensate for the strong acoustic attenuation, we can use a dual backscattered US and PA system for measuring the acoustic propagation attenuation in human cancellous bone. The backscattered PA modality is used to measure the size of the trabecular bone, while the backscattered US modality is used for US attenuation compensation [24]. Second, both bone mineral and hemoglobin contribute to strong absorption at a wavelength of 690 nm. The lower frequency components produced by hemoglobin in bigger bone marrow clusters have a bad influence on the analysis of the size of trabecular bone. The spectrum of bone marrow clusters needs to be studied for trabecular bone spectrum correction at its specific absorption peak. Third, cancellous bone is an anisotropic medium. PA signals from more receiving angles need to be averaged for a more accurate assessment. Furthermore, more bone specimens are needed to study the correlation between the PA-TFS parameters and the BMD and MTT.

Cancellous bone is a porous medium composed of trabecular bone and bone marrow clusters. Lipid, hemoglobin, and water are the main components of bone marrow, which has important roles in the internal environment of bone and influence bone mineralization [54,55]. Osteoporosis significantly changes not only nonorganic components, such as bone mass and bone microstructure, but also organic components, such as the amount of lipid. Magnetic resonance imaging studies have also show that osteoporosis is associated with an increased bone marrow fat mass and that the bone marrow fat fraction is negatively correlated with the BMD [56]. Therefore, both organic and nonorganic components needed to be studied for a comprehensive bone assessment. There are several PA methods for detecting changes in the chemical components and microstructures of bones. Multi-wavelength PA analysis can quantitatively assess the relative content of chemical components in bone by

**Fig. 7.** Results of *ex vivo* photoacoustic time-frequency spectral analysis (PA-TFSA) of the normal and osteoporotic rabbit bone specimens at a wavelength of 690 nm. (a) Photoacoustic time-frequency spectrum (PA-TFS) of a normal bone sample. (b) PA-TFS of an osteoporotic bone sample. (c) Representative power-weighted mean frequency (PWMF) of different bones from the control group and osteoporotic group, respectively. (d) Y-intercept of PWMF in two groups of bone specimens. (e) Midband-fit of PWMF in two groups of bone specimens. (f) Slope of PWMF in two groups of bone specimens. (*p* < 0.05).

**Fig. 8.** Correlation analysis between PA parameters and bone mineral density (BMD). (a) Y-intercept, *R* = −0.26. (b) Mid-band fit, *R* = −0.59. (c) Slope, *R* = −0.54.
using an unmixing procedure over an optical spectrum ranging from 680 to 950 nm [44,57]. The coherent frequencies of the combined US and PA coherent backscattering signals are strongly correlated with the scattering mean-free time in the bone, which can characterize the trabecular spacing [58]. However, few studies have been conducted on the characterization of both the content and microstructure of the organic and inorganic chemical components. In the future, the extra multi-wavelength PA-TFSA method can be used to obtain both the content and size information of chemical components, such as collagen, hemoglobin, and lipids, at their specific absorption peaks for a more comprehensive assessment of bone quality.

Multi-angle PA-TFSA can also help to locate osteolytic metastasis for guiding treatments. The bone marrow within the trabecular pore space becomes larger as to as to local bone defect in the early stage of osteolytic metastasis [59]. By performing multiple angles scanning on the bone at the absorption peak of bone marrow and analyzing the PA signals with PA-TFSA, the joint time-frequency spectrum at each scanning position can be obtained, as shown in Fig. 2(b). When illuminated at the wavelength of the absorption peak of bone marrow, higher frequency components are expected from smaller bone marrow clusters at the location of bone metastases. The relative position and size of the bone metastases can be obtained from PA imaging.

Compared with DEXA imaging, the PA-TFSA can provide more comprehensive information for bone assessment, including the BMD and bone microstructure. For the gold-standard DEXA imaging, soft tissues have an influence on the measurement of the BMD. Moreover, DEXA requires the use of ionizing radiation and cannot give bone microstructure information, which is highly related to bone health. The parameters y-intercept and midband-fit of PA-TFSA can provide bone microstructure information and the slope can provide frequency-related propagation attenuation over time, which is related to the BMD. Combined with multi-wavelength technology, this non-invasive and non-irradiating PA-TFSA method can provide information of chemical component changes, BMD, and microstructure of bone for a comprehensive assessment of the bone health.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgment

This project was supported by The National Natural Science Foundation of China under grant numbers 20130415, 11827808, 11674249 & 11704188, National Key Research and Development Program of China under grant numbers 2017YFC0111400 & 2016YFA0100800, Natural Science Foundation of Jiangsu, China (no. BK20190826), and Postdoctoral Science Foundation of China under grant number 2019M65166.

We would like to thank Editage (www.editage.cn) for English language editing.

References

[1] T. Sozen, L. Ozkizik, N. Calik Basaran, An overview and management of osteoporosis, Eur. J. Rheumatol. 4 (2017) 46–56.
[2] Francisco José López, New approaches to the treatment of osteoporosis, Clin. Obstet. Gynecol. 4 (4) (1987) 383–393.
[3] P. Pisani, Screening and early diagnosis of osteoporosis through X-ray and ultrasound based techniques, WJR 5 (2013) 398.
[4] C.V. Albanese, F. De Tervelzi, R. Passarello, Quantitative ultrasound of the phalanges and DXA of the lumbar spine and proximal femur in evaluating the risk of osteoporotic vertebral fracture in postmenopausal women, Radiol. Med. 116 (2011) 92–101.
[5] C.F. Nghé (Ed.), Quantitative Ultrason: Assessment of Osteoporosis and Bone Status, Martin Dunitz [s.l.], London, 1999.
[6] K.-Y. Chin, S. Ima-Nirwana, Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect? Int. J. Med. Sci. 10 (2013) 1779–1783.
[7] H.-W. Wang, N. Chai, P. Wang, S. Hu, W. Dou, D. Umulis, L.V. Wang, M. Sturek, R. Lucht, J.-X. Cheng, Label-free bond-selective imaging by listening to vibrationally excited molecules, Phys. Rev. Lett. 106 (2011), 238106.
[8] L.V. Wang, J. Yao, A practical guide to photoacoustic tomography in the life sciences, Nat. Methods 13 (2016) 627–638.
[9] R.C. Herman, B. Hofstadter, Vibrationspectra and molecular structure V. infra-red studies on light and heavy acetic acids, J. Chem. Phys. 6 (1938) 534–540.
[10] M.N. Fadhel, E. Hysi, J. Zalew, M.C. Kolios, Photoacoustic simulations of microvascular bleeding: spectral analysis and its application for monitoring vascular-targeted treatments, J. Biomed. Opt. 24 (2019) 1.
[11] G. Xu, J.B. Fowlkes, C. Tao, X. Liu, X. Wang, Photoacoustic spectral analysis for microstructure characterization in biological tissue: analytical model, Ultrasound Med. Biol. 41 (2015) 1473–1480.
[12] L. Hysi, M.N. Fadhel, M.J. Moore, J. Zalew, E.M. Stromh, M.C. Kolios, Insights into photoacoustic speckle and applications in tumor characterization, Photoacoustics 14 (2019) 37–48.
[13] R.E. Kumon, C.X. Deng, X. Wang, Frequency-domain analysis of photoacoustic imaging data from prostate adenocarcinoma tumors in a murine model, Ultrasound Med. Biol. 37 (2011) 834–839.
[14] N. Seeha, Z.E.A. Fellah, M. Fellah, E. Ogam, A. Virgin, F.G. Miriti, C. Depollier, W. Lauriks, Ultrasonic characterization of human cancellous bone using the Biot theory: inverse problem, J. Acoust. Soc. Am. 120 (2006) 1816–1824.
[15] T.J. Haire, C.M. Langton, Biot theory: a review of its application to ultrasound propagation through cancellous bone, Bone 24 (1999) 291–295.
[16] Z.E.A. Fellah, J.Y. Chapelon, S. Berger, W. Lauriks, C. Depollier, Ultrasonic wave propagation in human cancellous bone, Application of Biot theory. J. Acoust. Soc. Am. 116 (1) (2004) 61–73.
[17] L. Yang, B. Lashkari, J.W.Y. Tan, A. Mandelis, Photoacoustic and ultrasound imaging of cancellous bone tissue, J. Biomed. Opt. 20 (2015), 076016.
[18] L. Yang, B. Lashkari, A. Mandelis, J.W.Y. Tan, Bone osteolytic metastasis diagnostics: photoacoustics versus ultrasound, Int. J. Thermophys. 36 (2015) 862–867.
[19] B. Lashkari, L. Yang, A. Mandelis, The application of backscattered ultrasound and photoacoustic signals for assessment of bone collagen and mineral contents, Quant. Imaging Med. Surg. 5 (2015) 12.
[20] I. Steinberg, A. Eyal, I. Gannot, Multispectral photoacoustic method for the early detection and diagnosis of osteoporosis. Photonic Technologies and Diagnostics IX, International Society for Optics and Photonics, 2013.
[21] I. Steinberg, N. Turko, O. Levi, I. Gannot, A. Eyal, Quantitative study of optical and mechanical bone status using multispectral photoacoustics, J. Biophoton. 9 (2016) 924–933.
[22] I. Steinberg, L. Shiloh, I. Gannot, A. Eyal, First-in-human study of bone pathologies using low-cost and contact dual-wavelength photoacoustic system, IEEE J. Sel. Top. Quantum Electron. 25 (2019) 1–8.
[23] T. Feng, K.M. Kozloff, C. Tian, J.E. Perozky, Y.-S. Hsiao, S. Du, J. Yuan, C.X. Deng, X. Wang, Bone assessment via thermal photo-acoustic measurements, Opt. Lett. 40 (2015) 1721.
[24] T. Feng, Y. Zhu, R. Morris, K.M. Kozloff, X. Wang, Functional photoacoustic and ultrasonic assessment of osteoporosis: a clinical feasibility study, BME Frontiers 2020 (2020) 1–15.
[25] T. Feng, K. Kozloff, M. Cao, Q. Cheng, J. Yuan, X. Wang, Study of photoacoustic measurement of bone health based on clinically relevant models. SPIE Photonics West, International Society for Optics and Photonics, 2016.
[26] T. Feng, K.M. Kozloff, G. Li, S. Du, J. Yuan, C.X. Deng, X. Wang, Characterization of bone microstructure using photoacoustic spectral analysis, Opt. Express 933 (19) (2015) 25217–25224.
[27] I. Daubechies, The wavelet transform, time-frequency localization and signal analysis, IEEE Trans. Info. Theory 36 (3) (1990) 900–905.
[28] J.N. Watson, P.S. Addison, G.R. Clegg, M. Holzer, F. Sterz, C.E. Robertson, A novel wavelet transform based analysis reveals hidden structure in ventricular fibrillation, Resuscitation 43 (2000) 121–127.
[29] M.P. Wachowiak, D.C. Hay, M.J. Johnson, Assessing heart rate variability through continuous wavelet transform, Explor. Geophys. 40 (2009) 233.
[30] J.N. Watson, P.S. Addison, G.R. Clegg, M. Holzer, F. Sterz, C.E. Robertson, A novel wavelet transform based analysis reveals hidden structure in ventricular fibrillation, Resuscitation 43 (2000) 121–127.
[31] M.P. Wachowiak, D.C. Hay, M.J. Johnson, Assessing heart rate variability through continuous wavelet transform, Explor. Geophys. 40 (2009) 233.
[32] J.N. Watson, P.S. Addison, G.R. Clegg, M. Holzer, F. Sterz, C.E. Robertson, A novel wavelet transform based analysis reveals hidden structure in ventricular fibrillation, Resuscitation 43 (2000) 121–127.
[33] M.P. Wachowiak, D.C. Hay, M.J. Johnson, Assessing heart rate variability through continuous wavelet transform, Explor. Geophys. 40 (2009) 233.
[34] J.N. Watson, P.S. Addison, G.R. Clegg, M. Holzer, F. Sterz, C.E. Robertson, A novel wavelet transform based analysis reveals hidden structure in ventricular fibrillation, Resuscitation 43 (2000) 121–127.
[35] M.P. Wachowiak, D.C. Hay, M.J. Johnson, Assessing heart rate variability through continuous wavelet transform, Explor. Geophys. 40 (2009) 233.
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T. Feng, Y. Zhu, K.M. Kozloff, B. Khoury, Y. Xie, X. Wang, M. Cao, J. Yuan, D. Ta, Q. Cheng, Bone chemical composition assessment with multi-wavelength photoacoustic analysis, Appl. Sci. 10 (2020) 8214.

Y. Yang, S. Wang, C. Tao, X. Wang, X. Liu, Photoacoustic tomography of tissue subwavelength microstructure with a narrowband and low frequency system, Appl. Phys. Lett. 101 (2012), 034105.

A. Hosokawa, T. Otani, Ultrasonic wave propagation in bovine cancellous bone, J. Acoust. Soc. Am. 101 (1997) 558-562.

Yuehling Wang, Ting Feng, Meng Cao, J.E. Pervosky, K. Korollf, Qian Cheng, Jie Yuan, Photoacoustic measurement of bone health: a study for clinical feasibility, in: 2016 IEEE International Ultrasonics Symposium (IUS), IEEE, Tours, France, 2016, pp. 1–4.

S. Chaffai, F. Peyrin, S. Nuzzo, R. Porcher, G. Berger, P. Laugier, Ultrasonic characterization of human cancellous bone using transmission and backscatter measurements: relationships to density and microstructure, Bone 30 (2002) 229-237.

O.T. Baran, A.M. Kelly, A. Karellas, M. Lionet, M. Price, D. Leachey, S. Strutterman, B. Mcherry, J. Roche, Ultrasound attenuation of the Os calcis in women with osteoporosis and hip fractures, Calcif. Tissue Int. 43 (1988) 138–142.

S. Han, J. Rho, J. Medige, I. Ziv1, Ultrasound velocity and broadband attenuation over a wide range of bone mineral density, Osteoporos. Int. 6 (1996) 291–296.

K.I. Lee, H.-S. Roh, S.W. Yoon, Acoustic wave propagation in bovine cancellous bone: application of the modified biot-attenborough model, J. Acoust. Soc. Am. 114 (2003) 2284–2293.

M.L. Oelze, J.F. Zachary, W.D. O’Brien, Characterization of tissue microstructure using ultrasonic backscatter: theory and technique for optimization using a Gaussian form factor, J. Acoust. Soc. Am. 112 (2002) 1202–1211.

A. Piñé, A. Torricelli, P. Taroni, A. Bassi, E. Chikozoe, E. Giambattistelli, R. Caveddu, Optical biopsy of bone tissue: a step toward the diagnosis of bone pathologies, J. Biomed. Opt. 9 (2004) 474.

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