

## **ULTRASONIC INVESTIGATION OF THE TRABECULAR BONE - LOW AND HIGH FREQUENCY APPROACH**

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### **1. Introduction**

Macroscopic, X-ray methods for bone quality assessment are mainly based on porous bone density measurements. The bone quality is a property that is difficult to define, as it is related to both density and structure of a bone. The principal element that determines the bone strength is the trabecular structure of a porous bone. In recent years, several new ultrasonic diagnostic methods have been developed to examine bones *in vivo*. These methods are based on measurements of velocity (SOS-speed of sound) and attenuation (BUA-broadband ultrasonic attenuation) of waves penetrating porous bone. The large interest in these methods is a result of a fact that they provide information not only about the bone density but also about their structure without using ionizing radiation. These methods are based on measurement of overall properties of trabecular bone matrix.

At higher ultrasonic frequencies micro measurements can be performed. The unique properties of the scanning acoustic microscope (SAM) make it possible to measure and to image acoustic properties of a single trabeculae, namely the acoustic impedance, density and velocity of longitudinal waves in selected areas of a porous bone.

### **2. High frequency measurements - Scanning Acoustic Microscopy**

Ultrasonic microscopy makes it possible to assess *in vitro* the quality of bones and to examine their structure [3]. At high frequencies, the acoustic microscope ensures sufficient resolution for imaging the internal structure of a trabecular bone and of a single trabecula (Fig.1 and Fig.2)

The unique properties of the acoustic microscope make it possible to measure and to image acoustic properties of a single trabeculae, namely the acoustic impedance and velocity of longitudinal waves in selected areas of a porous bone, in the case, too, of samples from patients who suffer from metabolic bone diseases, such as osteoporosis, osteomalacia and osteoidosis. Scanning Acoustic Microscope (SAM), built at our laboratory, was used for imaging and measurements. The microscope is working at the frequencies of 35, 100 and 200 MHz and make it

possible to obtain surface and subsurface images. The images are stored in the computer memory, greatly facilitating their further processing.



Fig.1 The acoustic microscope image of the trabecular structure of cancellous bone.

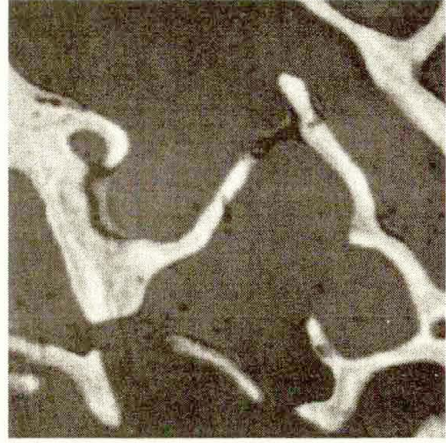


Fig.2 The acoustical microscopic image of a cross-section of trabecular bone destroyed by osteoporosis

Over the incidence angle range up to about 20 degrees, the reflection coefficient of a longitudinal wave on the water-bone boundary may be taken as constant. A 100 MHz head with a lens with a 20-degree half V-angle was used for the imaging. The application of a lens with a small V-angle allows for the normal wave incidence on the sample surface to be assumed in the model of wave and bone interaction.

### 3. Impedance and velocity determination

The brightness of images of flat samples obtained using SAM focused on the surface being imaged mainly depends on the reflection coefficient at the water-sample boundary.

The dependence between the bone impedance  $Z_b$ , water impedance  $Z_w$  and that of the reference medium  $Z_r$  can be represented as:

$$Z_b(x,y) = \frac{Z_w[A_b(x,y)(Z_r - Z_w) + A_r(Z_r + Z_w)]}{A_b(x,y)(Z_r - Z_w) - A_r(Z_r - Z_w)}$$

where  $A_b(x,y)$  is the echo amplitude at the point  $(x,y)$  of the bone image and  $A_r$  is the amplitude of the reference signal (a reflected wave focused on the surface of the sapphire sample).

The microscopic trabecula images obtained were processed according to the formula given above providing impedance distribution images. In the selected areas the average impedance values were calculated using 50 x 50 impedance points.

In the same area, the longitudinal wave velocity was measured using  $V(z)$  method. The  $V(z)$  technique is used for surface waves velocity determination. In the case of a bone, the focused acoustic beam excites lateral longitudinal leaky wave which propagates on the bone surface with the velocity of a longitudinal wave producing characteristic oscillations of  $V(z)$  curve. By spectral processing, the period of oscillations was determined and next used for velocity calculation.

We have examined samples of trabecular bone obtain by biopsy from patients with osteoporosis (12 cases), osteomalacia (10 cases) and osteoidosis (8 cases). In selected area of each sample average impedance and longitudinal wave velocity were measured. Then, the density of the bone sample was calculated (Tab.1).

Table 1 Mean values of impedance, velocity, density and elasticity  $=c_{11}=\rho c^2$  found for single trabeculae obtain from patients with metabolic bone disease.

	<b>impedance</b>	<b>Velocity</b>	<b>density</b>	<b>elasticity</b>
<b>osteoporosis</b>	<b>7.2 MRayl</b>	<b>3.9 km/s</b>	<b>1.8 g/cm<sup>3</sup></b>	<b>27.3GPs</b>
<b>osteomalacia</b>	<b>4.4 MRayl</b>	<b>3.4 km/s</b>	<b>1.3 g/cm<sup>3</sup></b>	<b>15.0GPs</b>
<b>osteoidosis</b>	<b>4.9 MRayl</b>	<b>3.2 lm/s</b>	<b>1.5 g/cm<sup>3</sup></b>	<b>15.4GPs</b>

Osteoporotic trabecula can be clearly distinguished from other bone samples. They are characterized by relatively high velocity, impedance and density that are close to the values found for a cortical bone. Much lower values are found for samples from patients with osteoidosis and osteomalacia. Medical description of these metabolic bone diseases explains the results found experimentally. Osteoporosis is characterized by the lack of the mass of trabecular bone but the bone building the trabecula remains almost unchanged. In case of osteoidosis and osteomalacia the mineralization process is disturbed what results in lowering acoustic velocity and density.

#### 4. Low frequency measurements

There is an increasing interest in development of noninvasive diagnostic techniques for detection of osteoporosis and predicting the risk of a bone fracture. For several years ultrasound was recognized as a very promising method, successfully competing with well-established X-ray techniques. Both methods study the trabecular structure of a cancellous bone. The surface/mass ratio of this bone is 10-fold greater than that of the cortical bone. Since the turnover of the bone is a surface-based event any disturbances of this process caused by osteoporosis or other metabolic bone disease are expressed earlier and more distinctly at cancellous sites than in the cortical bone.

Most of the publications concerning ultrasonic bone investigations describe the experiments performed in transmission mode. Also, the commercially available

ultrasonic densitometers utilize the signals transmitted through the bone. Two acoustic properties of a trabecular bone are measured. The slope of the frequency depended attenuation known as broadband ultrasonic attenuation (BUA) and speed of sound (SOS). Usually, the measurements are performed on a heel bone - calcaneus. This bone is well suited for ultrasonic investigations. It is composed mainly of a trabecular bone and it is easily accessible. The thickness of the cortical shell and overlying soft tissue is relatively low and the shape of the bone assures good penetration of ultrasound. Acoustic results were often verified by comparing bone mineral density - BMD ( $\text{g}/\text{cm}^2$ ) assessed by X-ray densitometry. It was shown [1,2], that the bone density can be measured by ultrasound as well as with X-rays and that the bone fracture risk in elderly women can be predicted based on ultrasonic results.

### **5. In vivo detection of bone disease with ultrasound-comparison with bone densitometry**

In our in vivo experiments 0.3 MHz - 0.7 MHz frequency ultrasound was used. The corresponding wavelength (5mm-2mm) at the upper frequency limit approached characteristic dimensions of the trabecular structure (trabecula thickness 0.1mm - 0.4mm, trabecula spacing 0.5mm - 2mm). We believe that characterization of a bone by scattered signal and determination of a trabecular structure crosssection function introduces a new quality into ultrasonic assessment of bone status.

BUA (Broadband Ultrasonic Attenuation) results are well correlated with BMD (Bone Mineral Density). Since the scattering of acoustic waves by trabecular structure is a fundamental component of attenuation, the scattering should also correlate with BMD. This dependence was confirmed by P.Laugier et al [4], who found a moderate correlation between the integrated backscattered coefficient and BMD.

In our approach we were searching for new indicators of trabecular bone properties. Two of them, the shift of an amplitude spectrum of a transmitted wave and a TSC (Trabecular Structure Crosssection) function were found to be good candidates for further study.

### **6. Instrumentation**

We built a system for in vivo heel examination (Fig.3). The system consisted of a pair of wideband, flat, composite transducers (diameter = 25mm) was operating at a central frequency equal to 0.58 MHz.

Transducers were mounted in small tanks filled with water. The wall of the tank opposite to the transducer face was made of a thin latex membrane. The foot under examination was placed between the tanks. By positioning the acoustic heads (1mm step) the area of measurement could be selected. Then, by increasing the pressure in the tanks, the heel was surrounded by a " water balloon", which fits the foot shape. Good transmission at the skin/rubber interface was assured by applying ultrasonic coupling gel.

The transmitting/receiving system was driven by a computer. The transmitter was developed in our laboratory. It generated the burst like signal of one period duration at the center frequency of 0.5 MHz and peak to peak amplitude of 100V. The pulse repetition rate was equal to 1kHz. Signals from the receiving transducer were amplified by a wide band receiver (0.1MHz - 1MHz) and were next captured by an A/D converter (12 bit, 20 MHz). Up to 32 successive echoes were averaged and stored in the computer for further processing.

In order to assure the shortest possible ultrasonic pulse (the wide band transmission) a set of transmitting-receiving transducers was carefully designed. Both transducers were fabricated out of composite material (diced PZT filled with epoxy resin) resulting in their relatively low acoustic impedance (12.2 MRayl), and high coupling coefficient ( $k_t = 0.6$ ). Acoustic backing together with the front quarter wave matching to water assured the overall 6dB bandwidth better than 400 kHz around 0.58MHz. A great effort was put into eliminating any spurious reflections coming from the backing of a transducer, which could disturb scattered waves. Both transducers could operate in transmission and receiving modes.

The following set of data was collected for each patient: 1° The reference signal, which is a signal transmitted through water only, 2° the signal transmitted through the heel, 3° two signals reflected from both sides of a heel and 4° two signals scattered from the selected heel area, corresponding to the position of trabecular bone.

The transmitted signals were used for BUA, shift of the spectrum and velocity evaluation. They were used also for some experiments with velocity dispersion assessment but we could not find any rational interpretation of the results and they are not presented in this paper. The reflected signals allowed us to measure the thickness of a heel and to select the trabecular bone area. The scattered signals were used for trabecular structure crosssection function determination.

## 7. Transmission mode study

Signals transmitted through the water and through the heel (Fig.3) were used to calculate frequency dependent attenuation and a shift of signal spectrum. The ratio of amplitude spectrum of a through bone-transmitted signal ( $S_b$ ) and through water ( $S_w$ ) determines the attenuation (Fig.4). The slope of the attenuation curve for a given frequency range defines the BUA (Fig.5).

Frequency dependent attenuation influences the spectrum of transmitted pulses. Since the higher frequencies in the spectrum are more attenuated than the lower ones, the spectrum moves to the lower frequencies. We have found it interesting to determine the shift of the mean frequency of the spectrum ( $\Delta f$ ) for the signal transmitted through the trabecular bone (Fig.4).

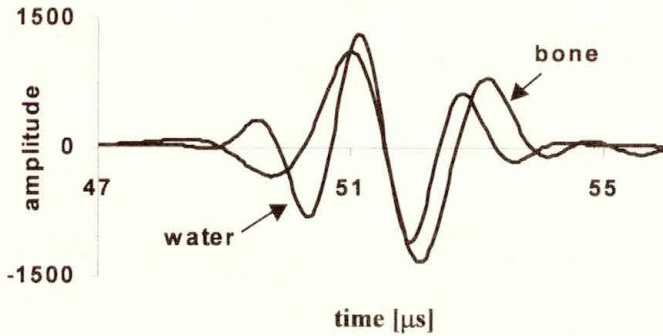


Fig.3. Ultrasonic pulses transmitted through the heel (multiplied by a factor of 63) and through water.

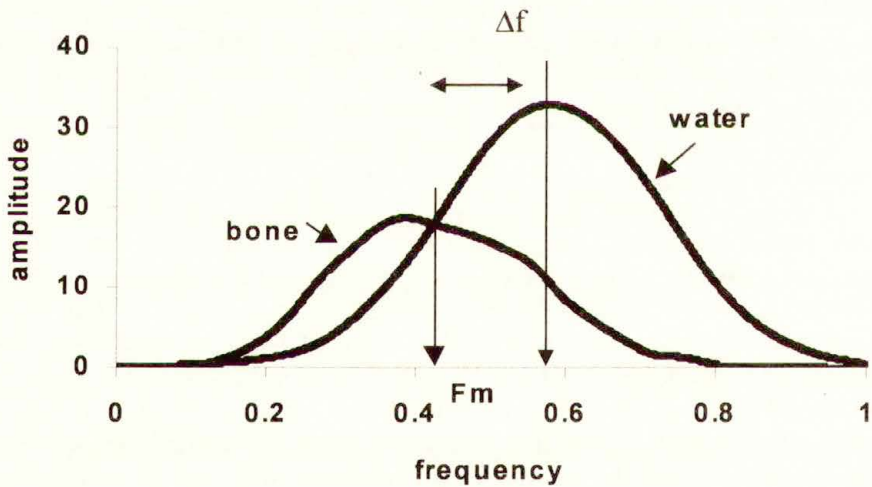


Fig.4. Amplitude spectra of transmitted pulses.  $F_m$ -value of a mean frequency of a through bone transmitted signal spectrum. Bone spectrum magnified 30dB.

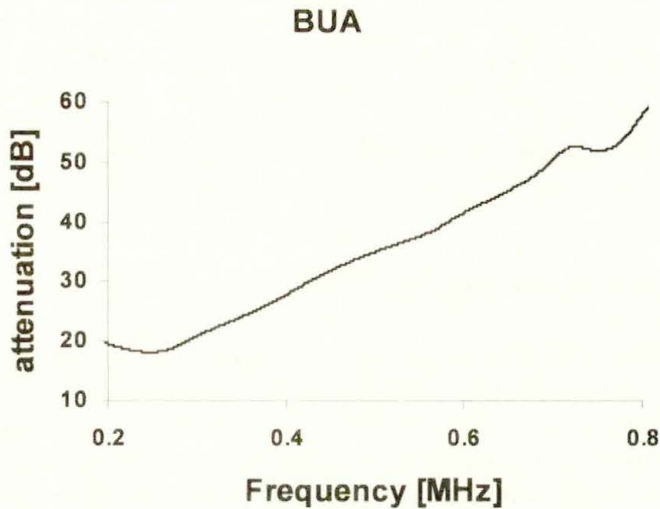


Fig.5 The attenuation/frequency dependence for a heel bone. A linear regression of this curve determines BUA.

### **8. Advantages of Probing the Trabecular Bone with Golay Coded Ultrasonic Excitation**

The use of coded signals in medical ultrasound allows both frequency and penetration depth to be increased while retaining image resolution and avoiding the need to augment the transmitted peak pressure amplitude. Such approach minimizes the potential for bioeffects even if the average transmitted energy is increased. The goal of this work was to examine noise immunity and the bandwidth requirements of Golay coded waves used to determine frequency dependent attenuation of highly absorbing and scattering biological tissues such as bones.

Our interest was to determine Broad Band Attenuation (BUA) coefficient because it is widely [4] accepted as indicator of trabecular bone density and is helpful in osteoporosis diagnosis. The attenuation of the ultrasonic signals in bone tissue often results in echoes from deep structures being virtually buried in noise. The work described in the following aimed at the evaluation of the possibility of using GC waves to obtain BUA data measured in trabecular bone at the frequency range beyond 0.5 MHz. This frequency is commonly used in the commercially available ultrasonic densitometers, which operate in transmission mode. In this mode, ultrasound energy is interrogating heel bone being positioned between an acoustic source and receiver. In contrast we wished to examine the feasibility of using GC waves in pulse-echo mode. As evidenced below, GC significantly improves signal-to-noise ratio and the GC based measurement system holds promise to provide the osteoporosis screening using pulse-echo mode approach.

This is because the use of GC signals allows both frequency and penetration depth to be increased while retaining image resolution and avoiding the need to augment the transmitted peak pressure amplitude [6]. Moreover, the GC pulse-echo approach minimizes the potential for bioeffects even if the average transmitted energy is increased. The development of the pulse-echo system applicable to bone tissue is of great importance as it would allow early diagnosis of selected, critical bones; non-ionizing, non-invasive diagnosis of bone such as hip is currently not available.

## 9. Golay Complementary Sequences

The properties of the code examined in this work were based on Golay complementary sequences first suggested in [7]. These sequences are composed of pairs of binary codes, and belong to a larger family of sequences called complementary pairs, which consist of two sequences of the same length  $N$  of which auto-correlation functions have side-lobes equal in magnitude but opposite in sign. Summing them up results in a composite auto-correlation function with a peak of  $2N$  and zero side-lobes.

We have chosen a Golay complementary sequences to be used in our ultrasonic transmitter because of their unique properties; the side lobes after decoding at the receiver side are cancelled.

Our GC transmitter used in this work is capable to produce 8 bits and 16 bits sequences. We have not found any significant differences in attenuation estimations using the reconstructed signals for 8 or 16 bits transmissions, however the S/N ratio almost doubled when the 16 bits signal was used. Therefore, for attenuation measurements (in transmission and backscattering or pulse-echo modes) we have used 16 bits sequences only and, accordingly all results presented below correspond to this code length.

## 10. Methods

The attenuation versus frequency measurement system was prepared initially for *in vivo* heel examinations and for the bone samples inspection. The system consisted of a tree pairs of the wideband, focused transducers operating at center frequencies equal to 0.5 MHz, 1MHz and 2 MHz (-6dB bandwidth = 60%, focal length=50 mm), respectively. Each transducer set was custom built and carefully selected in order to eliminate any possible spurious reflections originating in the backing of the transducers. This process optimized transducers' performance.

For the purpose of *in vivo* measurements we have used our bone scanner described in detail in [8]. The measurements of bone samples were performed in a water tank equipped with a sample holder and manual positioning system.

The sinusoidal and coded signals at the frequencies of 0.5MHz, 1MHz, and 2 MHz were synthesized using Arbitrary Function Generator ( LeCroy 9109, USA) The signals were routed through power amplifier (ENI 3100LA, USA) to the transmitting transducer mounted on the scanning system. The received signals were



then amplified by Ritec Broadband Receiver RB-640 and stored using Infinium HP 54810A Oscilloscope. The decoding of the received signals was performed off-line.

The measurements were conducted *in vivo* on volunteer's heels (three individuals-12 measuring sites) and *in vitro* on three human heel bones with a cortical layer removed. Prior to measurements the bone samples were defatted and saturated with water in vacuum chamber.

For transmission measurements a standard procedure of BUA determination was applied and the BUA coefficient was calculated as a slope of a linear regression fit of a ratio of amplitude spectra of a pulse transmitted through the bone and a reference pulse transmitted in water.

In the backscattering mode two segments of the echo gated at two different axial distances or depth were used to calculate BUA coefficient. We have found that the amplitude spectrum of a gated backscattered signal often contained many random peaks and valleys and consequently could not be directly used for BUA coefficient calculation. Therefore, we have developed a special procedure to smooth out the spectrum. First, a portion of the echo signal corresponding to trabecular bone was selected. Next, two gates of the same length and separated by a constant time distance were used to extract two brief transients from the echo signal. The corresponding amplitude spectra of the transients were compared and the values of attenuation versus frequency were calculated. Then, the "double gate" was moved to another position within the trabecular bone scattering area and a new value of BUA was determined. This procedure was repeated until the BUA values were found for sixteen gates' positions. The BUA values were eventually averaged and a slope of a linear regression fit for the averaged BUA values was used to calculate BUA coefficient.

## 11. Measurements in transmission mode

The measurements of BUA coefficient were performed in four selected areas of each bone sample at the frequencies of 0.5 MHz, 1 MHz and 2MHz. For those measurements the S/N ratio was equal to 15 dB because for *in vitro* experiments we could increase the transmitted power without the risk of introducing any (potential) biological effects. Results of the measurements obtained for transducer excitation with 16 bits Golay code were compared with those obtained for 2 cycles tone burst excitation, which were then considered to be a common reference. For 0.5 MHz excitations BUA was calculated in the frequency range from 0.3MHz to 0.7MHz. At 1 MHz the useful data were obtained between 0.6 MHz and 1.2MHz.

We have found a good correlation ( $r = 0.95$  and  $r=0.93$ ) for BUA values corresponding to 0.5 MHz and 1MHz measurements. In addition, at 1 MHz we have established a good ( $r = 0.91$ ) correlation for both 1 MHz GC excitation and 0.5MHz 2 cycles sine burst excitation measured at 0.5 MHz range (i.e from 0.3-0.7MHz). Fig.1 shows that the 1MHz BUA curve follows closely the GC curve in the 1MHz range while the 0.5MHz BUA curve is identical with GC one in the 0.5 MHz frequency range.

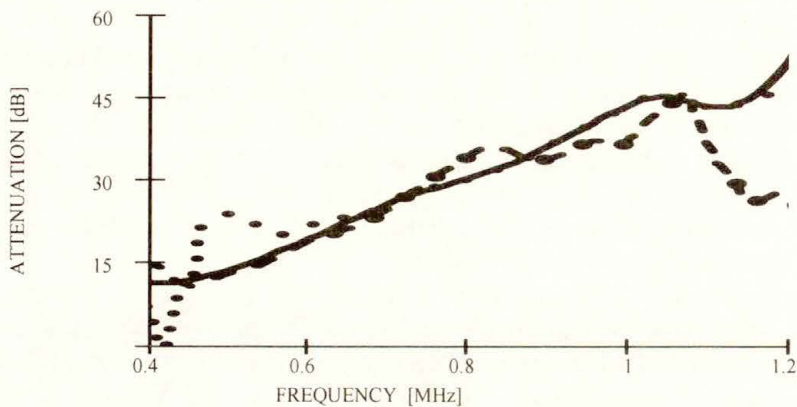


Fig.6. Frequency dependent attenuation measured for two excitation schemes. Solid line: GC at 1MHz, dotted line: sine burst at 1MHz, and dashed line: sine burst at 0.5MHz.

Similar measurements were performed *in vivo*, but here when 1 MHz frequency was used, to assure the safe exposure conditions, the voltage applied to the transducer was 30 dB lower than in the experiment with bone samples. Consequently, the S/N ratio was very low, close to 0 dB. At 0.5 MHz the correlation between BUA coefficient measured with sine burst excitation and using Golay coding was very good ( $r = 0.92$ ).

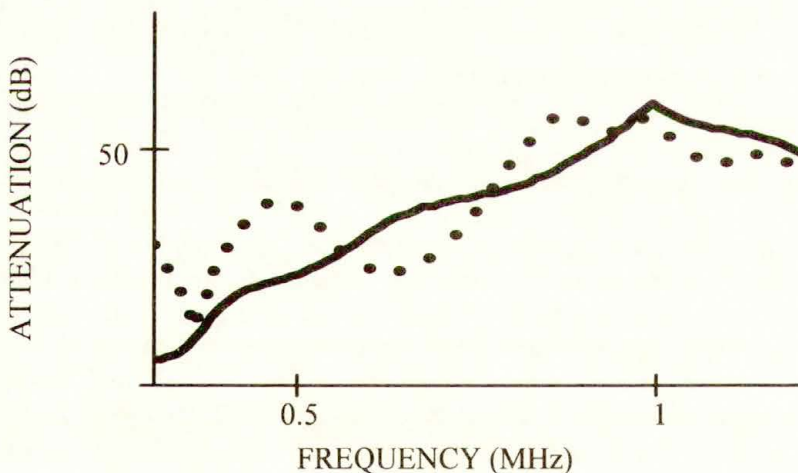


Fig.7 Frequency dependent attenuation measured for S/N ratio = 0dB. Solid curve: GC excitation, dotted line: sine burst excitation.

This correlation decreased to the value of  $r = 0.4$  for the frequency of 1 MHz. Analyzing the data shown in Fig. 2., we have found that for low S/N ratio and sine burst excitation the BUA coefficient could not be determined correctly. However,

for GC transmissions under same conditions we were able to determine BUA coefficient successfully.

## 12. Determination of BUA coefficient using backscattered waves (pulse-echo mode).

To validate the backscattered or pulse-echo approach, the BUA coefficients determined using Golay coding in transmission mode and those measured in pulse-echo mode were compared. First, as described in the previous section the bone samples were measured *in vitro*. At 0.5 MHz the correlation coefficients were low ( $r=0.52$  and  $r=0.51$ ) for both the sine burst and GC excitation. A closer examination of the results indicated that at this frequency the useful backscattering distance (i.e. penetration depth) in the trabecular bone was not sufficiently long in comparison to the pulse length and resulted in unsatisfactory averaging.

The same measurements were then repeated at 1 MHz frequency and relatively good correlation coefficients ( $r=0.82$ , and  $r=0.71$ ) for GC signals and sine burst excitation, respectively, were obtained. The penetration depth of coded signals was markedly deeper in comparison to that achieved using the burst signals. This extended penetration resulted in higher correlation between the transmission and pulse-echo mode measurements.

The *in vitro* results encouraged us to perform *in vivo* comparison for 1MHz Golay coded transmission. We obtained the values of correlation lower than in the case of bone sample measurements *in vitro* ( $r = 0.64$ ). A subsequent inspection of decoded signals indicated the undesirable existence of a minute time shift or delay between the two correlated sequences. This shift was caused by small changes in heel position of the examined subject during the time needed to switch transmitter between the two codes and store the received echoes. Off-line correction of this time delay increased the correlation coefficient to the value of  $r = 0.72$ .

## 13. Measurement at 2 and 3 MHz

To gain additional insight into penetration depth properties of Golay coded signals in pulse-echo mode we have carried out *in vitro* and *in vivo* measurements at 2 and 3 MHz. We have obtained good S/N ratio for reconstructed signals and sufficient penetration depth to determine frequency dependent attenuation (see Fig.9).

In transmission mode, the conventional way of BUA calculation was not possible as the mean frequency shift of a spectrum received from the bone was larger than the bandwidth of reference spectrum (see Fig.8). To alleviate this issue, other indicators of a bone status, for example the value of the frequency shift could be used [8] or, alternatively, the reference signal would have to be changed.

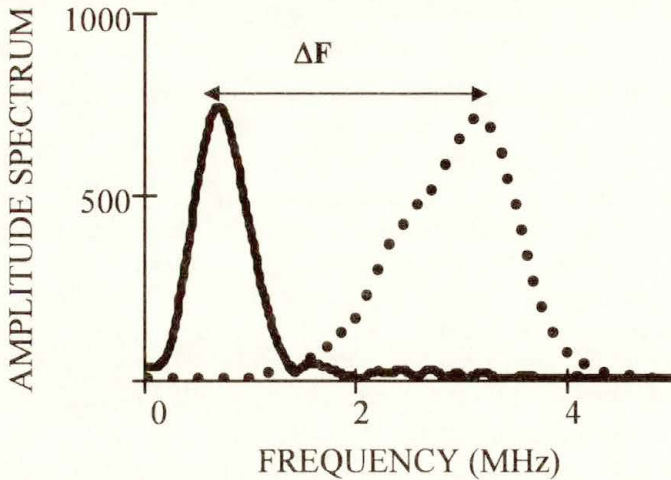


Fig.8 Amplitude spectra for 3MHz Golay coded transmissions trough water (dotted) and trough the bone (solid), bone spectrum amplified 80 dB

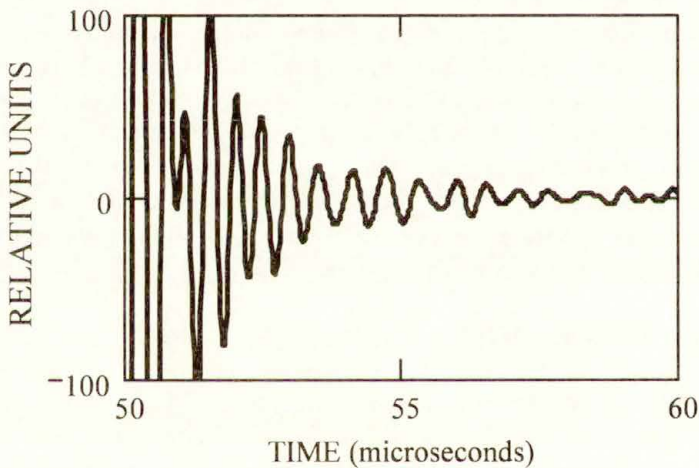


Fig.9 2MHz signal backscattered from a heel bone. Penetration depth = 6 mm. BUA coeff. calculated for this case (frequency range 1.3-2.4MHz) was equal to 3.1dB/MHz-mm

#### 14. Conclusions

The results presented above indicate that in comparison with the conventional, transmission mode of bone characterization using ultrasound, the use of coded signals allows enhancement of interrogating frequencies and provides augmented penetration depth without the need of increasing the peak pressure amplitude of the wave probing the tissue.

In transmission mode we have also examined the bandwidth and noise immunity requirements of Golay coded waves. We have found that in this mode Golay coded

signals retained all information related to frequency dependent attenuation and at the same time, doubled the frequency range in which ultrasonic attenuation could be determined. We have also found that the GC signals are highly noise immune; they allowed the attenuation properties of a bone to be calculated at  $S/N = 0$  dB. As noted previously under such conditions the conventional sine-burst excitation failed completely.

Our pulse-echo experiments confirmed that the BUA coefficient could be determined from backscattered echo signals. Thus, the use of GC signals holds promise to extend the usable frequency range for trabecular bone investigation, and to enable the diagnosis of the bones accessible from one side only. Also, they provide enhanced bone penetration depth, which enables application of averaging technique necessary for frequency dependent attenuation determination.

As noted above, the reconstruction of coded signals is very sensitive to time delay of the decoded components. It is possible to transmit two Golay code sequences almost simultaneously, so the influence of a small-uncontrolled bone movement that degraded decoding could be eliminated. To this end a new coder for our transmitter is being developed. Instead of switching from one code component to another, the two sequences will be sent together (separated by 0.5 ms) and the process of data collection for one measurement will be done below 1ms; this time is sufficiently short to ignore uncontrolled bone movements.

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