Title page:

SPATIAL DISTRIBUTION OF ACOUSTIC AND ELASTIC PROPERTIES OF HUMAN FEMORAL CORTICAL BONE

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Abstract :

The purpose of this study is to quantify the spatial distribution of acoustic velocities and elastic properties (elastic constant) on Human femoral cortical bone. Four cross sections (average thickness of 2.09 ± 0.27mm) have been cut transversally between 40% and 70% of the total length and between them parallelepiped samples in each quadrant have been cut. Ultrasonic technique in transmission with immersion focused transducers at 5MHz and contact transducers 2.25MHz were used on the cross sections and parallepiped samples respectively. The first technique allows relative spatial distribution of velocities to be obtained, meanwhile the second technique allows the direct assessment of elastic constant.

For both techniques, bulk velocities were found to be lower at the posterior side with an increase along the length (from 40 to 70% total length) ($p < 0.05$). Densities and elastic constant show equivalent pattern of variation. These variations are mainly due the cortical porosity related to vascularization environment. The spatial distribution of velocities exhibits significant radial variation from the endosteal to the periosteal region. This is in agreement with variation of the porosity at that location. Same range of velocities was obtained with both techniques. The range of longitudinal velocities values varies from 3548m/s to 3967m/s and between 18.5GPa and 33.1GPa for the bulk velocities and axial elastic constant respectively. Our results are within the range with those found in the literature. However, it must be noted that the range of acoustic and elastic properties variation is concerning the same bone. So, our new results show the ability of the technique to quantify accurately local variation of acoustic and elastic properties (within the section and along the length) of human cortical bone. Furthermore, our immersion technique could be used to assess the spatial distribution of the elastic constant with the knowledge of spatial distribution of densities.

Keywords:

Mechanical Properties – Acoustic Properties – Ultrasound – Cortical Bone – Human Femur

1. INTRODUCTION

The objectives of the present study are to present an experimental investigation of mapping the acoustic and elastic properties of Human cortical bone at a resolution less than 1mm and to determine the local variation of these properties within the same bone.

Different ultrasonic methods enable to characterize the acoustic and elastic properties of Human cortical bone in vitro.

At the level of the bone tissue, some investigators (Yoon et al.,1976; Van Buskirk et al., 1981; Ashman et al., 1984; Ho Ba Tho et al., 1991) have used the transmission technique in contact with transducers at 2.25MHz; their purpose was to determine the Young's modulus of Human cortical bone derived from acoustic properties measurements. Usually cubic samples were cut in precise anatomical location and according to their symmetrical (crystallographic) axes of the bone. Furthermore the technique has been compared with conventional mechanical testing (Darmana et al., 1991). The experiment consists in measuring on the same parallelipedic samples (20 specimens) the Young's modulus deriving from ultrasonic and mechanical testing. Mean difference of axial modulus values with both techniques is about 1.2GPa (0.2-2.8GPa).

A new ultrasonic approach has been proposed by Pithioux et al. 2002, allowing to assess the elastic properties of bovine cortical bone with a rough preparation of the specimens. Two techniques were used one to measure the velocities (transmission technique with focused and immersion transducer of 1MHz) and the other one to assess the thickness (reflection technique, echo mode).

A same new commercial quantitative ultrasound technique (Sunlight Ultrasound Technologies, Rehovot, Israel) has been used by different authors. They measure the acoustic velocity using the axial transmission mode along the cortical bone. In vivo multi-site velocities have been performed on the radius, tibia, metatarsus and phalanx with respectively values of 4169m/s, 3939m/s, 3663m/s and 4047m/s of 1521 healthy women (age 20-90 years). The objectives were to evaluate the accuracy of the device and to get a database (Weiss et al., 2000). Another study consisted in the investigation of fracture discrimination by the comparison of the measurements performed on 34 women having got previously a fracture (hip, spine, ankle or forearm) and 28 healthy women (Barkmann et al. 2000). They both conclude the potential of the technique for assessment of osteoporosis and fracture risk.

Another technique called the scanning acoustic microscope (SAM) scans the surface of the bone, which allows to obtain the acoustic impedance. Thus, some authors (Meunier et al.,

1988) used this technique, which operates in reflection with low transducers between 20MHz and 50MHz in order to have a mapping of the acoustic impedance on femur Human cross sections, which supplies information at the microstructure level. Two sections of young (25 years) and old (74 years) subjects have been tested in vitro. The spatial distribution of impedance at a resolution of 1μm allowed to observe the resorption area due to the age. The section from a 25 years old subject presents lower acoustic impedance in the posterior region and an increase in the anterior area. The section from a 74 years old subject exhibits many different contrasts in closer areas with always acoustic impedance lower in the posterior and anterior sides.

Other authors investigated bone implants reflecting bone remodelling after hip arthroplasty (Zimmerman et al., 1990) through a mapping of acoustic impedance. The acoustic impedance (with transducer at 50MHz) in function of the anatomical localisation has also been performed (Weiss et al., 1998) on 10 cross sections of a femur cut between the distal and proximal levels. The acoustic impedance of the posterior side (7.55 Mrayls) is lower than the other sides (7.75 – 7.8Mrayls)(1Mrayl = 10^6 Kg/m^{2}s). Along the 20% of the femoral length the acoustic impedance of the anterior side decreases compared to the posterior side, which increases.

According to the literature review, ultrasonic transmission technique using contact or immersion focused transducers allowed to assess the elastic properties of the bone assumed anisotropic.

At the same tissue level spatial distribution of acoustic properties such as acoustic impedance was performed (Meunier et al., 1988; Zimmerman et al., 1990; Weiss et al., 1998) to evaluate the inhomogeneities of the cortical bone section.

An atlas of mechanical properties have been performed on seven subjects and on different type of bones (HoBaTho et al. 1991). The results demonstrated that mechanical properties of cortical bone varied according to the anatomical location reflecting its heterogeneity. These variations were assessed with a spatial resolution of about 5mm (sample size).

The purpose of the present study is to investigate the feasibility of assessing the spatial distribution of the elastic properties of human cortical bone at a lower spatial resolution (less than 1mm).

In order to achieve this objective, we choose an ultrasonic technique allowing to perform a mapping of acoustic velocities. This technique uses focused transducer to obtain a good spatial resolution but it is known that focused transducer provides relative velocities and subsequently elastic properties could not be deduced directly. That is why in the present study it was necessary to compare the results obtained with those of validated transmission contact technique where the elastic constants are determined from absolute velocities.

This study presents two techniques allowing to investigate the spatial distribution of bulk velocities and elastic properties respectively.

2. MATERIALS AND METHODS

2.1. Specimens preparation

A cadaveric femur (male 70 years of age) was obtained from the anatomical laboratory of Hospital University of Amiens. The femur has been sectioned transversely with a low-speed diamond saw (MICROCUT 2). The femur has been cut into five sections between 40% and 70% of the total length of the femur. The average thickness of the slices is 2.09mm ± 0.27mm measured with a micrometer (Mitutoyo) which accuracy is 0.01mm.

Between the previous slices 12 parallelepiped cortical samples (5*4*13mm) have been cut parallel to the axis of the femur in the lateral, medial and posterior sides.

2.2. Calibration

The calibration of the two ultrasonic transmission techniques has been performed with different materials: copper, stainless steel, Plexi Glass, PVC.

Each sample has the same dimension with a diameter and a length of 5×5mm. The range of the theoretical velocities for these samples is between 2680m/s and 5010m/s.

2.3. Density measurement

The measurement of the apparent density of the samples has been determined by the ratio between the weight and the volume of the samples. The measurement of the weight has been performed by the use of an accurate (0.1mg) balance (Sartorius BP61S). The volume has been determined with the metric measurements, which were performed with a micrometer (Mitutoyo, 293,Japan) which accuracy is 0.01mm.

2.4. Transmission ultrasonic technique with immersion transducers (TUI)

Measurements of longitudinal velocities will be determined through an ultrasonic method, which operates in transmission. The generator delivers electrical pulses. The pulses have been transmitted to the piezoelectric transducer that transformed the electric signal into an acoustic wave. Immersion transducer at 5MHz (Panametrics, V309, MA) with a focal length of 75mm has been used. The diameter of the spot is 1.24mm.

A part of the acoustic wave is reflected back to the transducer acting this time as a receiver, which transforms the acoustic wave into an electrical pulse in order to obtain longitudinal acoustic velocities on each femoral section. The acoustic transducer is held by a column,

which is controlled by a microcomputer. In order to obtain many measurements on the surface of the specimen the transducer is translated with a step of 0.5mm with an accuracy of 1μm on the X-axis. The displacements are driven by a computer which uses a custom made program (Cactus). This equipment is installed in the Roberval laboratory located in UTC.
Each section has been laid down into a little container, which contains demineralising water (T=20°C) and is stocked in physiological serum (9%) in freezer (T = 4°C). On each point of acquisition two acoustic echoes have been recorded: the first one between the water and the material (echo 1), the other one between the material and the water (echo 2) (fig.1).

The number of points of acquisition varies from 1460 to 1734 for the focused transducer. The wave velocity (m/s) is then expressed by the length (m) difference between the specimen and the measured time delay (μs): v=2e/t, e being the average thickness of the cross section and t the time delay. Finally the computer acquires data and with another software it is obtained at the end a colour map of the acoustic velocities of the surface of the specimen.

2.5. Transmission ultrasonic technique with contact transducers (TUC)

An ultrasonic transmission technique (fig.2) developed in the biomechanics laboratory of UTC was used in order to measure velocities and elastic properties. This technique is similar to that one developed by Ashman (Ashman and al. 1984). This technique used a couple of transducers (Panametrics V106RB) at high frequencies 2.25MHz. The generator (HP 3312A) sent impulsions, which are received by the first transducer acting as a transmitter. Then the wave is propagated through the sample and the second transducer receives the longitudinal wave, which is visualized on an oscilloscope (Tektronics TDS 3032). The ratio length of the sample and the time of propagation calculate the wave velocities. In the present study, bulk velocities were measured, and then elastic properties can be deduced. This type of propagation requires that the wavelength (λ) is lower than the transversal dimension (D) of the samples (λ <<< D). The relation between the velocities and mechanical properties is:

$$\mathbf{V}_{ij}^{bulk} = \sqrt{\frac{C_{ij}}{\rho}}$$

ρ is the density (kg/m^3), Cij the elastic coefficient (Pa) of the material, i being the direction of the wave propagation and j corresponding to the movement of the particles (Dieulesaint et Royer 1974). In the present study the longitudinal wave were measured in the axial direction: $V_{ij} = V_{33}$

2.6. Statistical analysis

ANOVA test has been performed with the software Statgraphics 5.0 (Sigma Plus) in order to study the variation of acoustic and elastic properties function of the anatomical localization.

3. RESULTS

3.1. Calibration of both setups

The acoustic velocities for the different materials tested with the two ultrasonic techniques are presented table 1. The range of velocity for the material at 5MHz and 2.25MHz varied respectively from 2327m/s to 4710m/s and from 2358m/s to 4751m/s. The longitudinal velocity of cortical bone (about 4000m/s) is included in the range of theoretical velocities of these materials.

3.2. Reproducibility and accuracy of both techniques

For the transmission technique with transducers in contact, the accuracy of velocities measurements is about 60m/s (min 20 m/s – max 80 m/s) and consequently about 0.3GPa for the calculation of elastic properties. The reproducibility obtained with three measurements is about 125 m/s (84m/s-172m/s).

For the transmission technique with transducers in immersion, the accuracy of velocity is 137m/s. The reproducibility obtained with three measurements is about 140m/s.

3.3 Density measurement

Densities values varied from 1468 to 2127 kg/m^3 (1.468 to 2.127 g/cm^3). Results of the statistical analysis (fig.3) show that the density of the samples varies according to the anatomical localisation. In fact, the densities for the samples exhibit lower values in the posterior side ($p < 0.05$).

3.4. Cartography of velocities

The average of longitudinal acoustic velocities and thickness measurements for each section are summarized in Table 2. Mapping of velocities exhibits local variation of velocities if the thickness is assumed to be equal across the sample. Variation of the thickness for each sample has to be considered in order to confirm the significance of local variation. A thickness variation of 0.05mm and 0.03mm induces a variation in velocities (ΔV) about 243m/s and 175m/s respectively. It means that for each section, local variation velocities are significant if

it is higher than ΔV. The mapping of velocities for the four sections obtained at 5MHz with immersion focused transducer is illustrated from fig.4 to fig.7 with ΔV.

For all sections, the posterior side exhibits lower velocities. However one can notice significant increase of these velocities along the length (from 45% to 60% of total length of the femur).

Velocities on the medial side seemed to exhibit lower values than that of the lateral side.

Furthermore significant radial variation is observed, in fact velocities increased from endosteal to periosteal side.

3.5. Acoustic and elastic properties of parallelepiped samples

The range of longitudinal velocities values varied from 3548 to 3967m/s and between 18.5 and 33.1GPa for the axial elastic constant. The mean and median values for the velocities are respectively 3859 ± 109m/s and 3909m/s, and respectively 27 ± 3GPa and 27GPa for the axial elastic constant.

Statistical analysis shows significant variation in velocities and C33 (figures 8-9). In fact, values of velocities (3548-3814 m/s) were found to be lower in the posterior side than the other sides (3858-3967 m/s)($p<0.05$). The range of elastic constant values in the posterior side varies from 18.5 to 25.2 GPa compared to the other sides (25.7 to 33.1 GPa).

4. DISCUSSION

Comparison of both techniques

The calibration of the two ultrasonic techniques indicates that the velocities of the different materials are within the same range and close to the theoretical velocities (ΔV about 25m/s for the TUC and 24m/s for the TUI). This difference is correct because the precision of the two techniques is about 100m/s, excepted for the stainless steel, which presents a higher velocity (about 4%) with the immersion technique.

The average differences of velocities for both techniques are equivalent compared to the theoretical velocities. The copper presents for the two techniques the same range of velocity but the value is lower (about 300m/s or 6%) than the theoretical velocity; this may be due to the quality of the material, which is not a standard one.

Range of values obtained by both techniques is within the same range for the posterior side.

For the other specimens locations (lateral and medial) maximum difference in velocities obtained with both techniques is 200m/s (mean value about 100m/s).

Values of the velocities obtained with TUC technique are lower than that of TUI technique. According to the accuracy of both techniques and calibration we can confirm the equivalence of velocities obtained with focused and contact transducers for this experimental design.

The comparison of the velocities between both techniques indicates a same variation pattern in the periphery and along the length.

Range of values and spatial distribution of acoustic properties

Despite the different experimental investigations, range of in vitro velocities obtained with our study is within the range with those found in vivo on Human long bones (Barkmann et al., 2000, Weiss et al. 2000, Antich et al., 1991). On the other hand, it is not in agreement with the study performed by Hasegawa et al. 1995. Their samples were iliac crest biopsy cortical specimens; one would expect that it differs from cortical long bones.

Using the TUC technique no significant differences were found between the lateral and medial sides velocities but the posterior side velocities were found to be significantly lower. This observation is in agreement with the results found on the elastic properties by previous authors (Meunier et al., 1988, Ashman et al., 1984, Weiss et al., 1998, Ho Ba Tho et al. 1991). The posterior region is more porous related to vascularization environment. These results were consistent according to the atlas of mechanical properties of cortical bone performed on seven subjects (Ho Ba Tho et al. 1991).

This result is confirmed by the results obtained with a morphological analysis of the microstructure (diameter of the pores of the haversian system, porosity (%)). We found that a variation of the velocities about 11% reflects a variation of pores size of a factor 2 and a factor 5 for the porosity (Bensamoun et al. 2003). A similar observation was found with the spatial distribution of velocities obtained with the TUI technique and furthermore a significant variation along the length was observed. This result allows to confirm the qualitative observation of a previous study (Weiss et al. 1998) using the mapping of acoustic impedance. Our results show that porosity in the posterior side varies along the length suggesting significant local vascularization environment influence. The advantage of the TUI technique is the assessment of spatial distribution of local velocities. The velocities for the lateral side seem to be higher than the medial side without significant difference, if a better accuracy in the thickness could be obtained, then the variation would be probably significant.
Radial velocities variation (about 200m/s) on the sections was significant. This is related to the porosity of the bone. In fact, it is well known that the endosteal side has a more porous microarchitecture, which decreases the velocities.

The red spots located on the internal boundaries of the cross sections are essentially due to some artefacts, which exhibit high velocities about of 4400m/s (fig.7). These non significant values increase the average velocities summarized in Table 2. These artefacts are related to the holes, which represent big pores (or lacunae due to pore's accumulation) at the endosteal part. These holes are more and more present when the section is located close to the metaphyseal part (section are bigger, cortical area thinner) and so may exhibit more artefacts such as section 4 (cf Figure 7). One would expect a decrease instead of an increase. In fact that is purely due to a signal processing of the detection of the second echo. The attenuation of the second echo implies a random detection close to the first echo. Subsequently, it decreases the time value between the two echos involving an artificial increase of the velocity. This demonstrates that the TUI technique is sensitive to the microarchitecture (size of pores). In fact, these artefacts would be observed depending on the ratio between the wavelength and size of the holes (or pores). We found experimentally that the longest dimension of the pores should be about three times lower than the wavelength.
In order to avoid such problem, the bone samples should not exhibit macropores about 3 times the wavelength.

Range of values and spatial distribution of mechanical properties

Spatial distribution of density and elastic constant (C33) show significant lower values at the posterior side and an increase along the length, a same pattern variation of velocities has been observed with the TUI technique. This last result confirms the ability of the TUI technique to give absolute velocities even with a focused transducer. Furthermore, the mapping velocities illustrated in figure 4 to 7 reflect a mapping of elastic constant only for the posterior side. In order to get an accurate mapping of elastic constant, the knowledge of spatial distribution of density should be assessed. Then using both data a spatial distribution of local values of elastic constant could be assessed. It should be mentioned that this is possible because the velocities obtained with focused and contact transducers were found to be equivalent in the present experimental design. Normally velocities obtained with focused transducers are relative velocities and should not be used as absolute values as the pathway is not the same due to the different beam propagation type (Bensamoun et al. 2002). Usually velocities with focused transducers are lower than those obtained with the contact one. Furthermore this technique is sensitive to the choice of the frequency. So it was important to compare these values with another ultrasonic technique, which gave absolute velocities, and elastic properties such as performed in the present study. Once this has been validated, the advantage of this new ultrasonic technique would be the direct assessment of a spatial distribution of elastic properties compared to previous study (Zimmerman et al. 1990).

One should note that it concerns only one direction meanwhile the other TUC technique allows to assess the anisotropy of the bone. In fact, the other directions were not investigated in the present study as the cutting of the specimen would be difficult to perform due to the complex geometry of the diaphysis of the femur. Besides, the transverse plane is a plane of interest for bone resorption related to bone remodelling (due to ageing process, contact bone implant or else).

To summarize, these techniques are complementary, one allows to assess spatial distribution and the other one the local anisotropy.

5. CONCLUSION

This study shows the assessment of spatial distribution of the acoustic velocities by using the TUI technique at 500µm spatial resolution. This technique allowed to get a more precise spatial distribution of acoustic and mechanical properties (for example in the radial and peripherical direction). These results found within a same bone suggest the importance of the measurements according to the anatomical location and local heterogeneities for the same subject. These local variations from the endosteal to the periosteal related to bone remodelling would be of interest for finite elements models.

This demonstrated the ability of the TUI technique to assess the spatial distribution of velocities and elastic properties around the periphery and along the length of the bone. However, technical considerations are required such as a perfect parallelism in order to have a good interpretation of the data. Furthermore, sensitivity to the inhomogeneities at the level of the microstructure should be considered for the experimental design. Once these points are under control, quantitative assessment of spatial distribution of elastic properties could be deduced from a velocity and density mapping.

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Table 1. Acoustic velocities for metal samples used for the calibration of both techniques

Table 2. Minimum, maximum and average values with standard deviation of thickness and velocities obtained with a focused transducer at 5MHz for each section (1, 2, 3, 4).

Samples (5*5*5mm)	Longitudinal velocities (m/s) 5MHz	Theoretical Velocities (m/s)	Longitudinal velocities (m/s) 2.25MHz
Copper	4710 ± 28	5010	4731 ± 23
Stainless steel	6041 ± 41	5790	5674 ± 46
Plexi Glass	2720 ± 14	2680	2879 ± 24
PVC	2327 ± 12	-	2358 ± 8

Tab 1. Acoustic velocities for metal samples used for the calibration of both techniques

Section number	Thickness (mm)	Femoral Length (%)	5MHz V_F (m/s)
1	1.728 ± 0.054	44.8	3400 - 4400 4158 ± 291
2	1.934 ± 0.030	50.13	3400 - 4400 4064 ± 203
3	2.142 ± 0.026	55.71	3700 - 4600 4147 ± 141
4	2.192 ± 0.0256	59.95	3400 - 4600 4344 ± 143

Tab 2. Minimum, maximum and average values with standard deviation of thickness and velocities obtained with a focused transducer at 5MHz for each section (1, 2, 3, 4)

Fig.1 Propagation of the echos through the section

Fig.2 Ultrasonic transmission technique in contact

Fig 3. Illustration of density function of the anatomical localisation (* : $P < 0.05$)

Fig.4 Velocities mapping of section 1 (44.8% of femoral length) obtained with focused transducer at 5MHz ($\Delta V = 243$m/s) (M: medial, L: lateral, P: posterior)

Fig.5 Velocities mapping of section 2 (50% of femoral length) obtained with focused transducer at 5MHz ($\Delta V = 175$m/s) (M: medial, L: lateral, P: posterior)

Fig.6 Velocities mapping of section 3 (55.7% of femoral length) obtained with focused transducer at 5MHz ($\Delta V = 175$m/s) (M: medial, L: lateral, P: posterior)

Fig.7 Velocities mapping of section 4 (60% of femoral length) obtained with focused transducer at 5MHz ($\Delta V = 175$m/s) (M: medial, L: lateral, P: posterior)

Fig 8. Range of acoustic velocities of parallepiped samples around the periphery of the diaphysis (*: $P<0.05$ **: $P<0.001$)

Fig 9. Range of axial elastic constant function of the anatomical localisation (*: $P<0.05$ **: $P<0.001$)

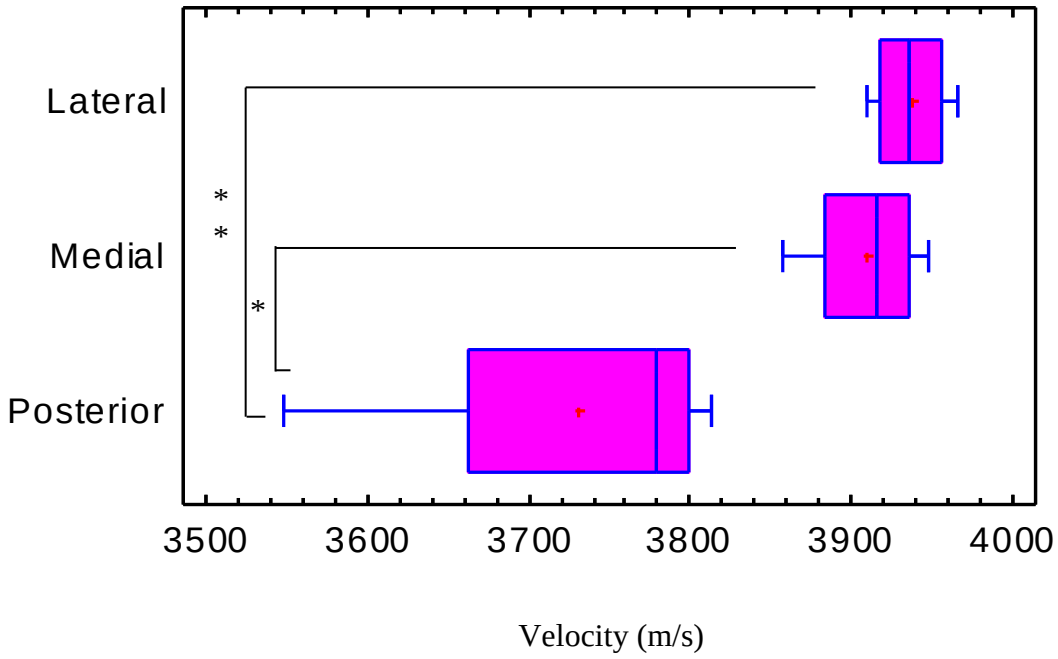
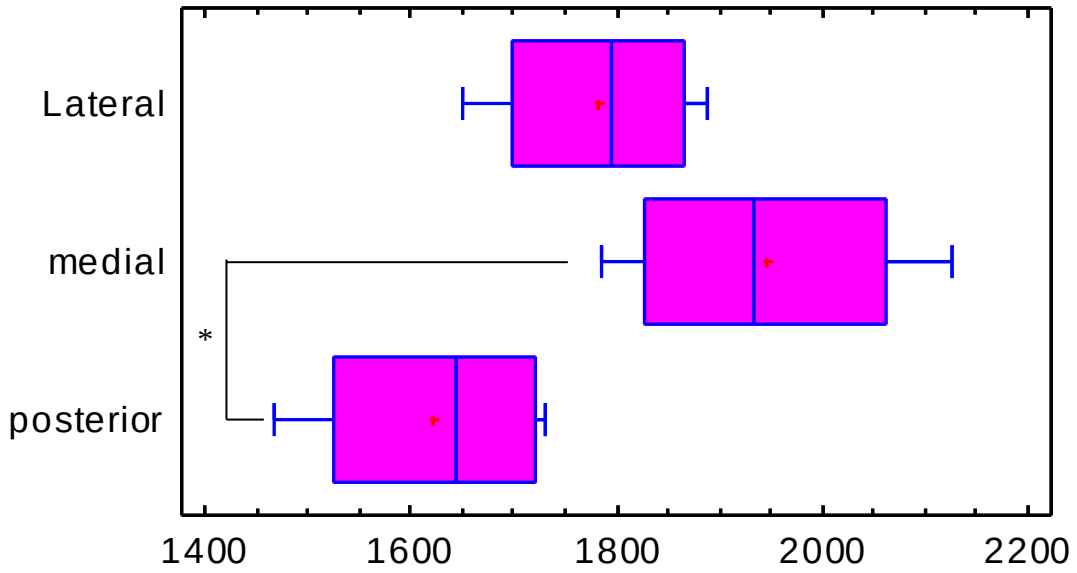
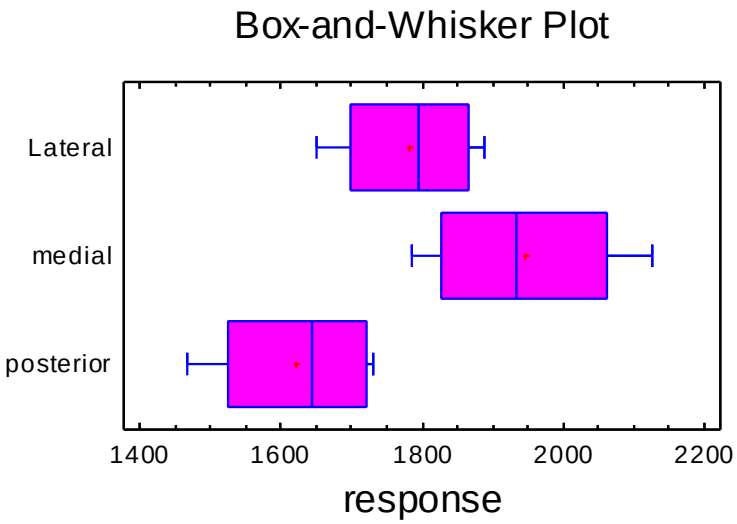


Fig 8. Range of acoustic velocities of parallepiped samples around the periphery of the diaphysis (*: P<0.05 **: P<0.001)



Density (kg/m^3)



: P < 0.05)

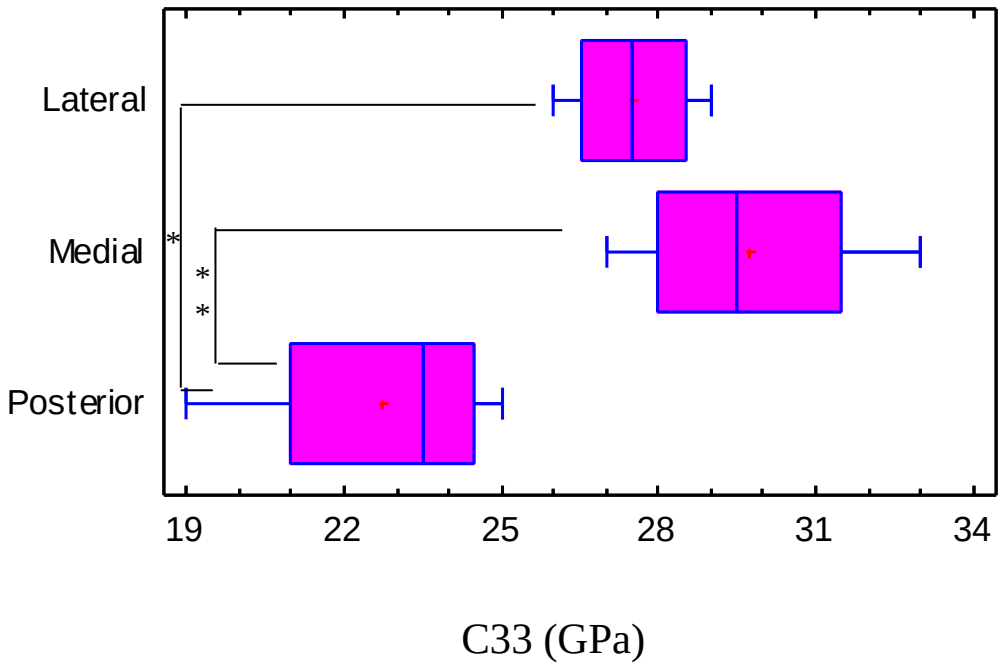
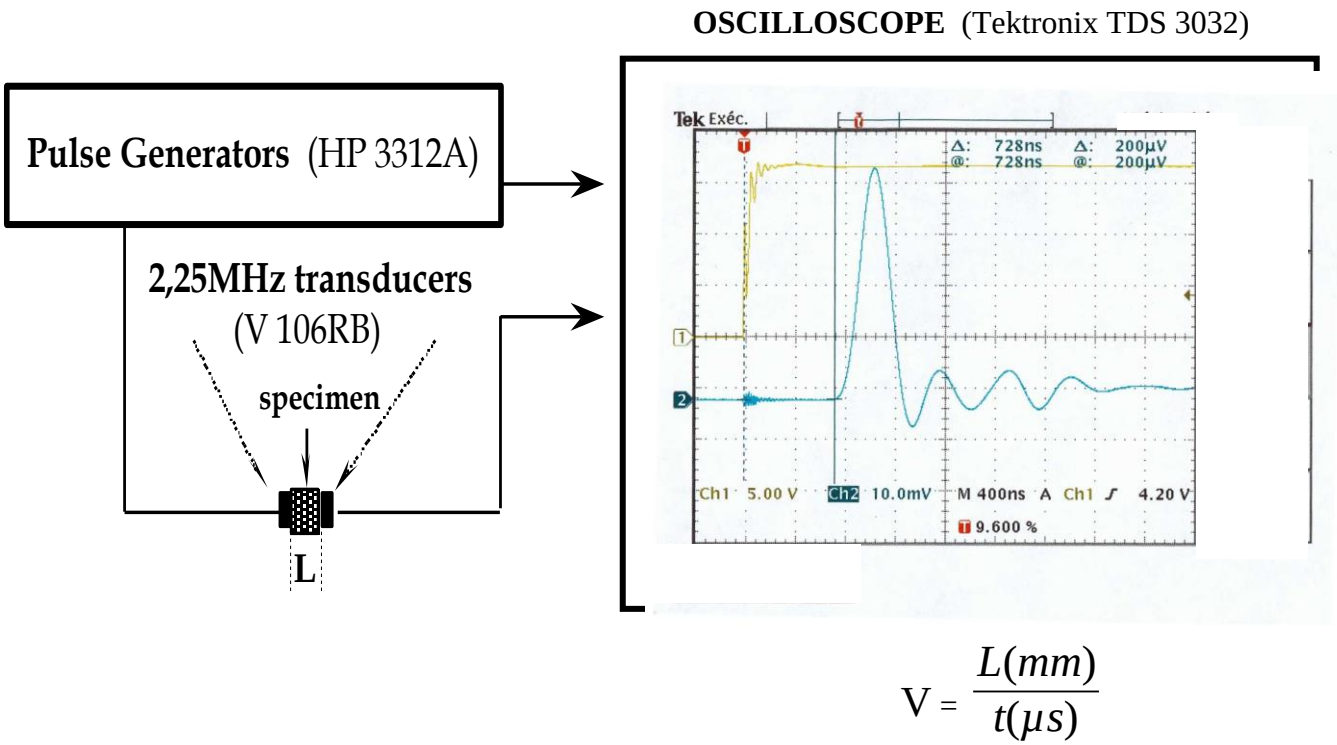


Fig 9. Range of axial elastic constant function of the anatomical localisation (*: P<0.05 **: P<0.001)



$$V = \frac{L(mm)}{t(\mu s)}$$

Fig.2 Ultrasonic transmission technique in contact

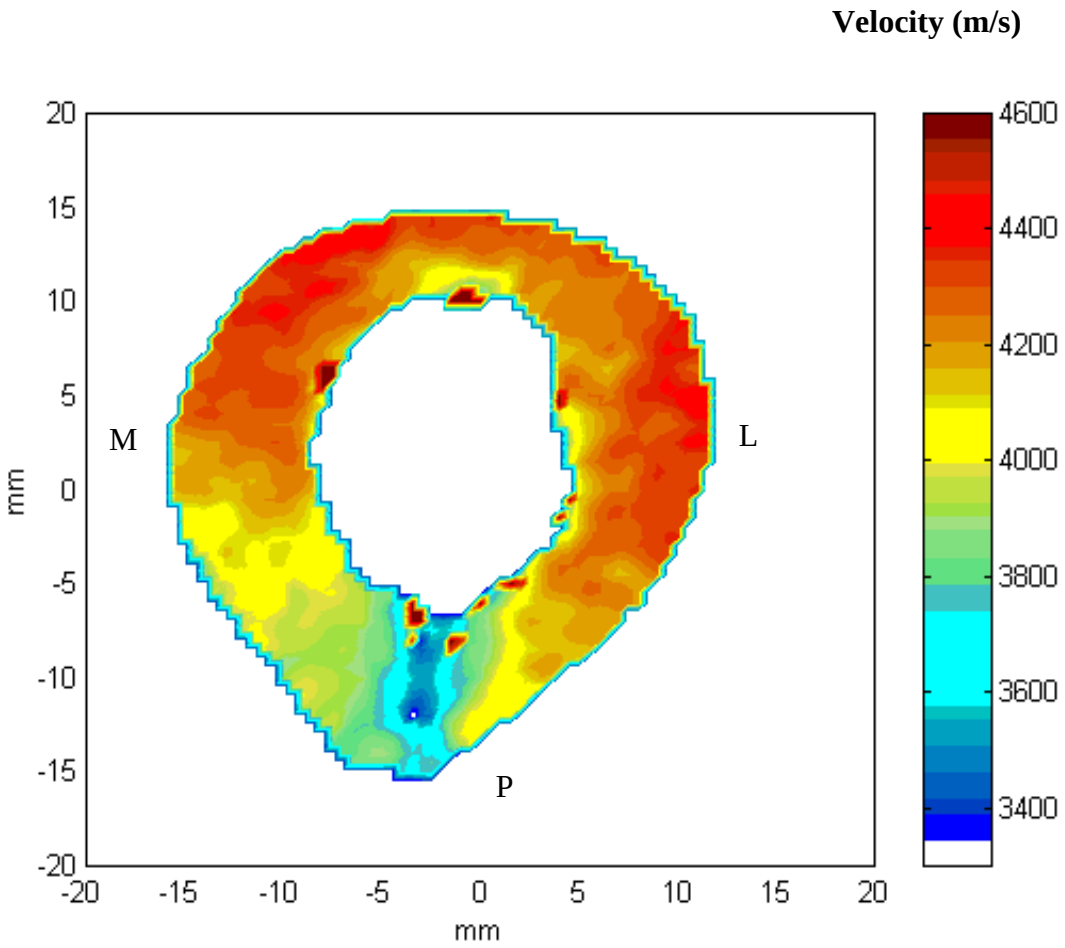


Fig.4 Velocities mapping of section 1 (44.8% of femoral length) obtained with focused transducer at 5MHz (ΔV = 243m/s) (M: medial, L: lateral, P: posterior)

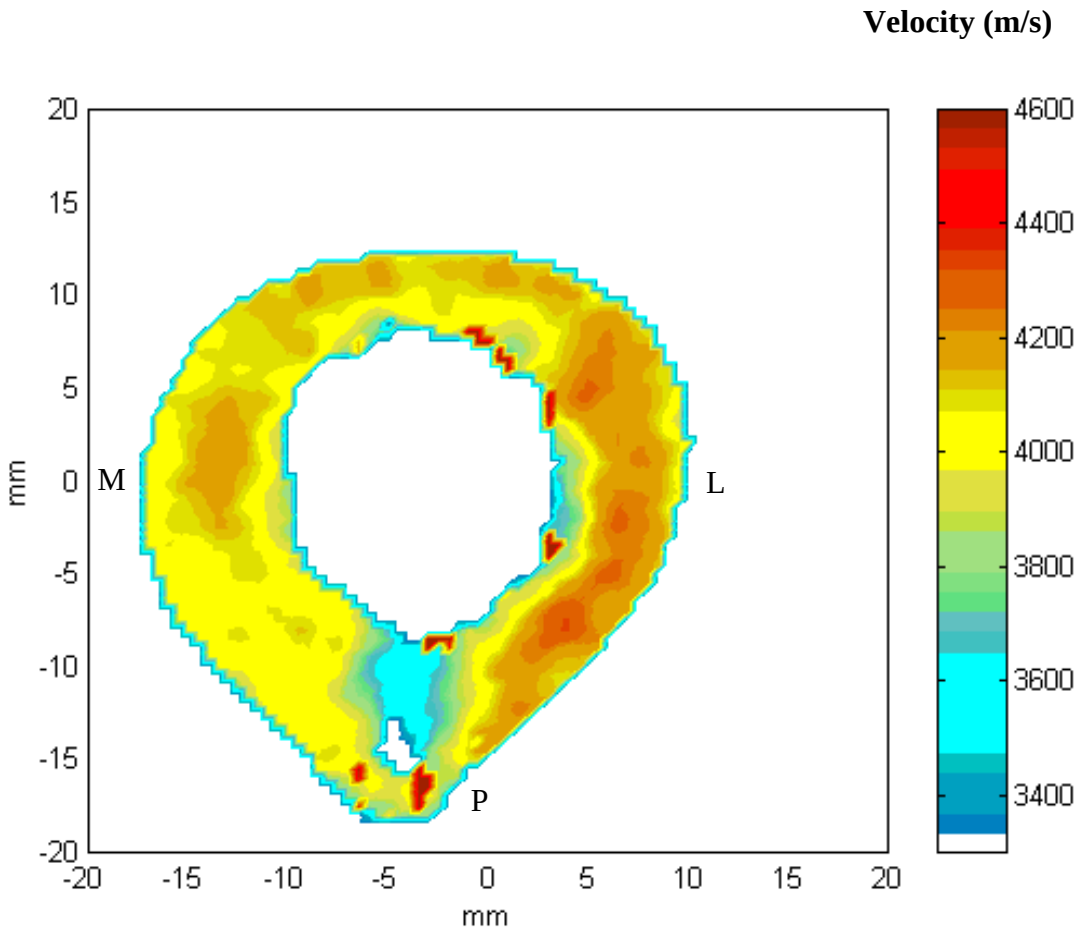


Fig.5 Velocities mapping of section 2 (50% of femoral length) obtained with focused transducer at 5MHz (ΔV = 175m/s) (M: medial, L: lateral, P: posterior)

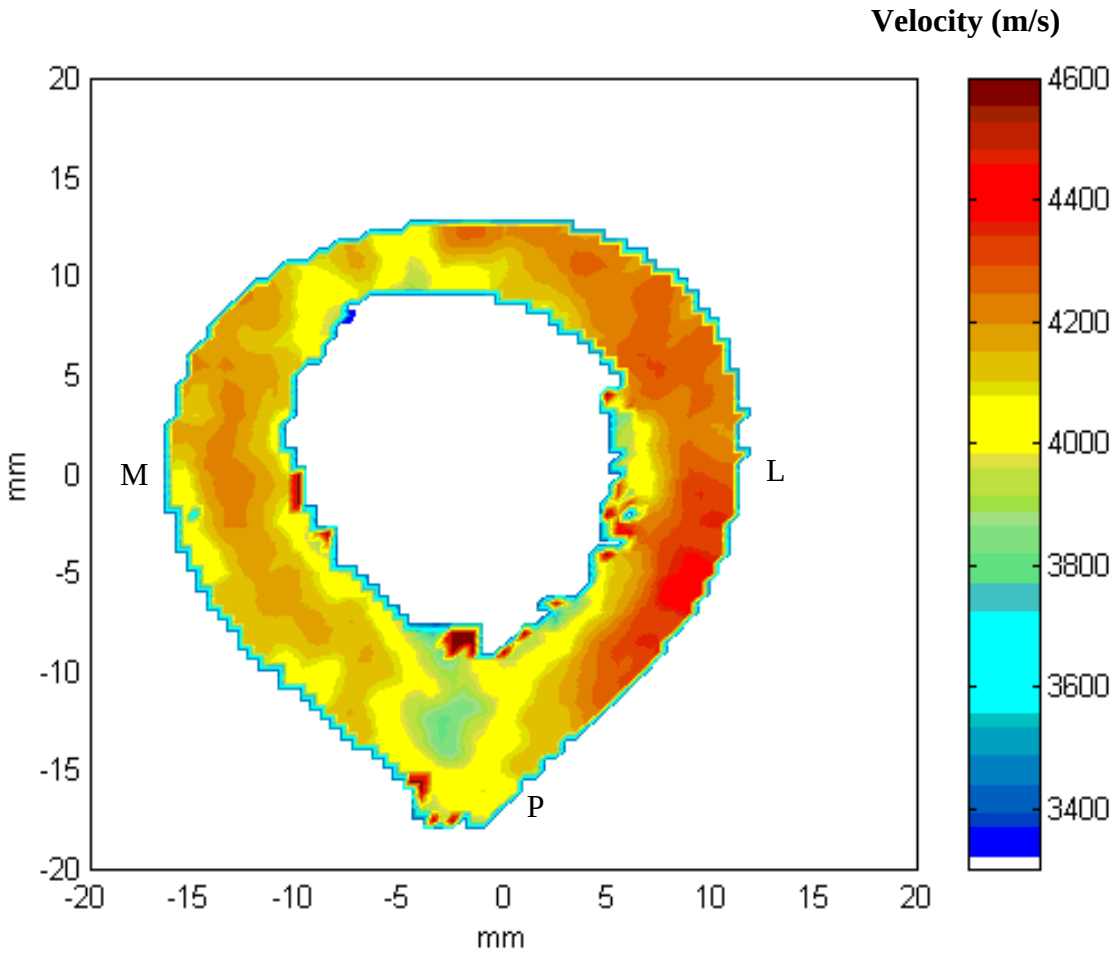


Fig.6 Velocities mapping of section 3 (55.7% of femoral length) obtained with focused transducer at 5MHz (ΔV = 175m/s) (M: medial, L: lateral, P: posterior)

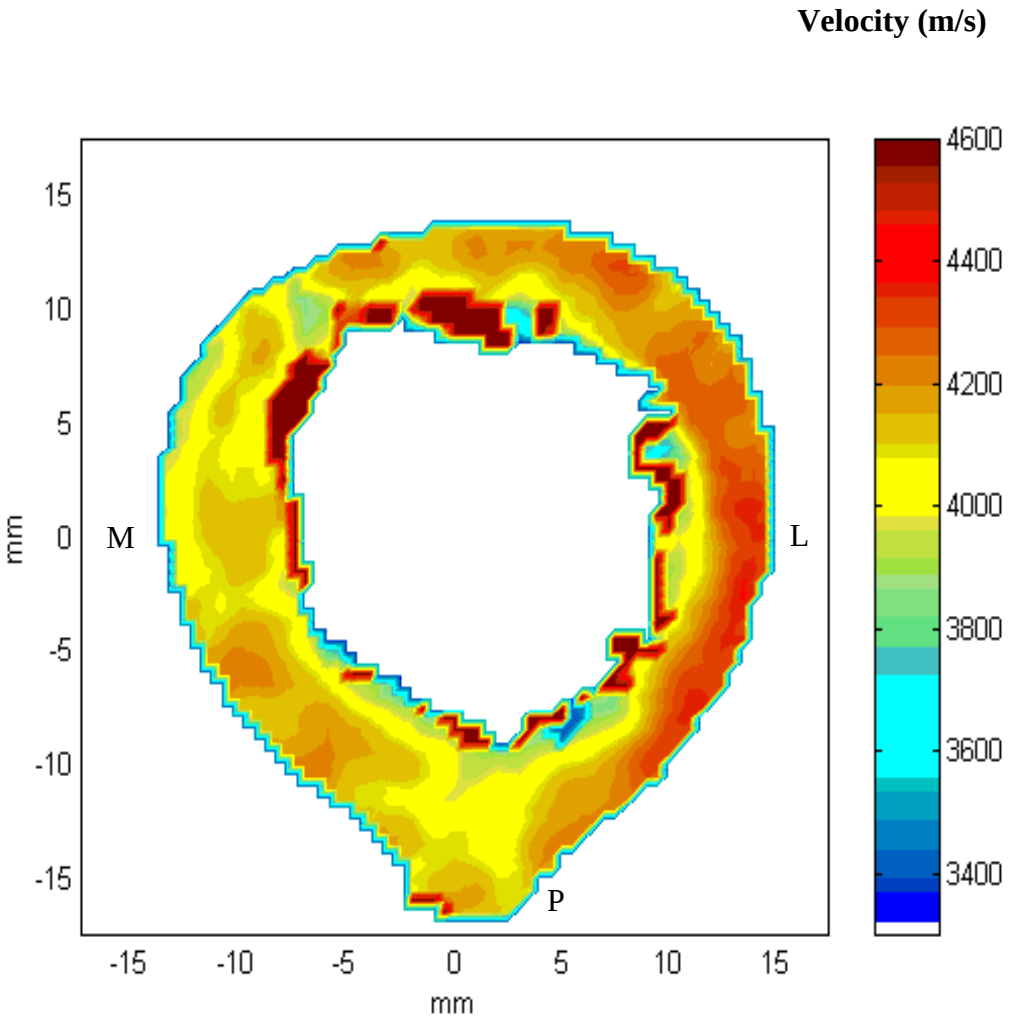


Fig.7 Velocities mapping of section 4 (60% of femoral length) obtained with focused transducer at 5MHz (ΔV = 175m/s) (M: medial, L: lateral, P: posterior)

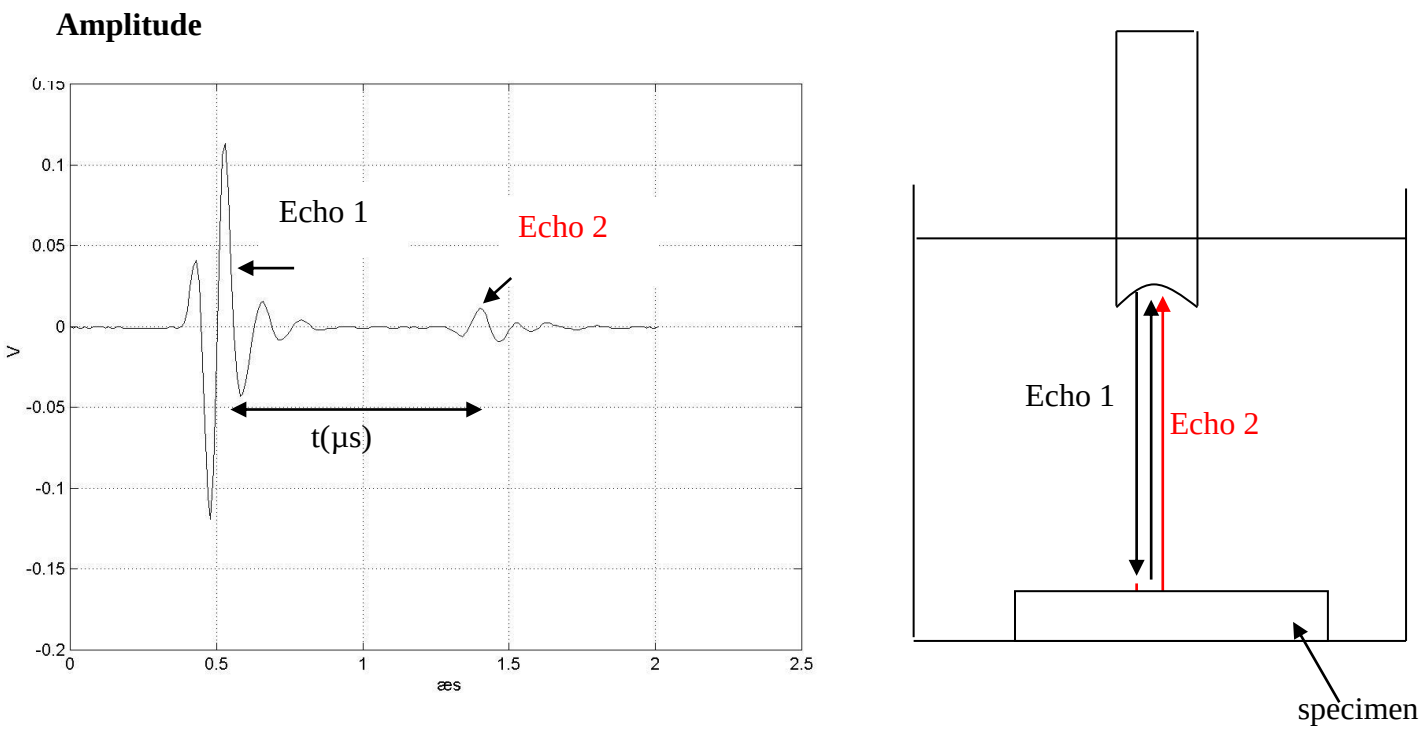


Fig.1 Propagation of the echos through the section