Analysis of the angular influence in the spatial study of mechanical displacements in highly anisotropic media

Luis Hernández-Álvarez^{a,*}, Carlos Negreira^b, Antonio Ramos^a, Nieves Cubo Mateo^a, Luis M. Rodríguez^c, Alberto Pinto del Corral^a, Iciar González Gómez^a

^a Institute of Physical and Information Technologies (ITEFI), Spanish National Research Council (CSIC), Madrid, Spain

^b Institute of Physics, Faculty of Sciences of the University of the Republic, Montevideo, Uruguay

^c Institute of Polymer Science and Technology (ICTP), Spanish National Research Council (CSIC), Madrid, Spain

ARTICLE INFO

Keywords:

Tissue mechanical displacements
Ultrasonic velocity
Triclinic tissues
Anisotropic phantoms
Tumor malignance

ABSTRACT

Tumors with anisotropy in their structural properties experience unpredictable and asymmetric growth along certain directions that can vary over time during the tumor progression. An early recognition of such anisotropy could provide information about their malignity, facilitating early diagnostics/prognostic. However, it is difficult to be observed by classic echography. Diverse studies of the literature show 3D images of elastic properties in media with anisotropy in a certain direction, but are transversely isotropic. However, for highly anisotropy media this assumption is no longer valid and requires specific measurements of shear velocities in the 3D directions. These media with 21 independent elastic material parameters and no symmetry plane. Techniques using ultrafast focused sound scanners present difficulties in covering immediate regions around the focused area and specific information for different incident beam orientations to obtain complete 3D spatial information, which is relevant in these types of media without planes of symmetry. It is the case of malignant tumors. The current paper presents a flexible spatial study of mechanical displacements induced in highly anisotropic soft media by low frequency shaker (including printed inorganic, pseudo-organic grids and cow fiber tissues respectively). Homogeneous and heterogeneous media without planes of symmetry have been tested in the experiments by using speckle ultrasonic interferometry. Velocities associated to the induced low frequency-mechanical displacements have been analyzed at a point of interest within the sample from different angles of propagation, covering a 3D angular space. These measurements show a high spatial-dependence of results, demonstrating the relevance of making spatial angular scans to derive parameters related to the anisotropy. This technique allows detection of anisotropies in small areas of analysis, even if they are weak.

1. Introduction

Cancer is still one of the main causes of death in the world (ancer 2018 and <https://doi.org/10.1016/j.mechmat.2021.104094>). It has been extensively studied, with significant efforts made to understand the mechanisms regulating the tumor invasion by studying genetic and biochemical aspects (Herceg and Hainaut, 2007; Malandrino et al., 2018; Fares et al., 2020). However, there are still mechanisms poorly understood, such as the role of mechanical properties in tumor progression and invasion, which is still under-investigated, even ignored in many oncological and immunological reviews.

Diverse techniques are used to study mechanical properties of tumors, including optical measurements, cantilevers, elastography

(Fovargue et al., 2020), and pre-stressed surfaces (Staunton et al., 2016), but additional research on these and new techniques is still needed to understand the cell-microenvironment mechanical interaction. Recent literature describes the relationship between malignant lesions and anisotropy of breast cancer confirmed that malignant lesions are more anisotropic than benign (Sinkus et al., 2001; Skerl et al., 2016a, 2016b; Gu et al., 2020; Böl et al., 2014; Soltaninezhad et al., 2020; Soleimani et al., 2020; Yang et al., 2020; Chen et al., 2018). In 2001, R. Sinkus et al. observed a tendency of breast tumors to be anisotropic (Sinkus et al., 2001). In 2016 K. Skerl et al. found a certain correlation between anisotropy and tumor malignance in breast cancer by performing 2D shear wave elastography (SWE) measurements (Suhail et al., 2019). Chen et al. analyzed in 2018 the anisotropy of ultrasound SWE of breast

* Corresponding author. C/ Serrano 144, Madrid, Spain.

E-mail address: luis.hdez.alvarez@iec.csic.es (L. Hernández-Álvarez).

<https://doi.org/10.1016/j.mechmat.2021.104094>

Received 7 January 2021; Received in revised form 6 September 2021; Accepted 29 September 2021

Available online 4 October 2021

0167-6636/© 2021 Elsevier Ltd. All rights reserved.

lesions between two orthogonal planes, and its correlation with clinical and histopathological findings in Chinese patients (Chen et al., 2018). Anisotropy correlates with increasing elasticity parameters, BIRADS categories, core biopsy and tumor grade.

Transversal isotropy is currently assumed in some anisotropic biological tissues, such as muscle fibers (Böl et al., 2014). Some examples of anisotropy are shown in Fig. 1.

However, not all tissues respond to these geometric configurations. On the other side, the growth of tumors is usually accompanied by processes of uncontrolled cell proliferation and migration in an anisotropic manner, proportionally to their malignancy (Gu et al., 2020). The asymmetry in these tissues is described by 21 elastic constraints, complex to be derived. Investigating the deformation of tissue architecture is currently one of the most important clinical methods for cancer diagnosis (Soltaninezhad et al., 2020). Growth phenomena usually occur in living tissues under different mechano-biological stimuli (Soleimani et al., 2020). Conventional use of existing elastography technologies provide highly precise 3D images of small areas in organs and tumors, composed by thousands of scans obtained by 1D and 2D arrays of multiple transducers. However, they do not offer certain quantitative information that could be does not usually provide this information that could be relevant for a tumor diagnostic/prognosis, such as low frequency induced wave amplitudes measured at a point of study within a sample from different angles of orientation. It can be relevant to provide information of the sample anisotropy, promoted from the tumor microenvironment. A tensor attribution to certain parameters such as shear wave velocity, conventionally assumed as scalar in healthy tissues, will provide extraordinary information on the macroscopic spatial patterns of elastic properties, which in turn can be correlated to the degree of anisotropy in the medium, not always detectable by conventional techniques.

In 2011, Couade et al. performed an *In vivo* quantitative mapping of myocardial stiffening and transmural anisotropy during the cardiac cycle (Couade et al., 2011) [Couade]. They used a 128 element probe to remotely generate a shear wave thanks to the radiation force induced by a focused ultrasonic burst. The resulting shear wave propagation was tracked using the same probe by cross-correlating successive ultrasonic images acquired at a very high frame rate. They estimated the shear wave speed at each location in the ultrasonic image, impinging different points of the sample but from alleatory angles, but without taking into account a possible influence of the angle of incidence on the results obtained in different points of the samples tested.

In 2016, Schmidt et al. (Schmidt et al., 2016) [Schmidt et al., 2016]

performed a study to analyze shear waves in anisotropic tissues assumable as transversal isotropic in Brain white matter (WM). They used the technique of magnetic resonance elastography (MRE). Later, another paper published by Schmidt et al. (Schmidt et al., 2018) [Schmidt et al., 2017].

In 2018, Tweten et al. performed a study to establish the essential requirements for characterization of a transversely isotropic material by magnetic resonance elastography (MRE) et al. (Tweten et al., 2017) [Tweten et al., 2018]. These four papers refer to the use of mechanisms different than ours to induce shear waves in media that were transversely isotropic.

Unlike these studies based on the use of magnetically induced mechanisms, ultrasonic speckle interferometry technique has been used in diverse experiments of the literature (Suhail et al., 2019; Catheline et al., 2004; Gennisson et al., 2005, 2013; Benech and Negreira, 2010; Mueller et al., 2020). The wave phase velocity component (WPVC) associated to small displacements for different angles between the acoustic beam and fibers, has been measured in the current study, covering the whole 3D space. This technique makes possible to analyze local anisotropy in 3D with extreme simplicity. Comparison of 2D and 3D shear-wave elastography can be a key to differentiate benign and malignant tissue masses (Seo et al., 2018; Choi et al., 2017).

This paper presents a flexible 3D spatial study of small volume displacements in a region of interest generated by a low frequency pulse inside phantoms containing highly anisotropic soft media. Three phantoms including printed inorganic grids, pseudo-organic grids and cow fiber fabrics, respectively, have been strategically developed and tested.

2. Methodology

2.1. Experimental method: 1D transient-ultrasonic speckle interferometry

The low frequency transient mechanical excitation (100 Hz–400 Hz) is applied to the samples by means of circular paddle driven by a shaker that generates low frequency waves that propagate through the sample with higher amplitudes than longitudinal waves. These shear waves generate displacements projected onto the acoustic axis that an ultrasonic transducer attached to the shaker detects in a frequency range between 3 MHz and 5 MHz. Thus, RF ultrasonic A-scans with speeds over 1 KHz, collect information over time of the low frequency induced wave propagation at different depths within the sample, using the ultrasonic speckle interferometry technique (Hong and Gie Ohr, 1998).

The acoustic speckle signal is modified by the movements of the

Fig. 1. Some examples of anisotropic media.

scatterers. From these speckle modifications, it is possible to reconstruct the motion of diffusers which, in turn, is the motion of the medium itself in a first approach. The ratio between the distance travelled by diffusers along the direction of the ultrasound beam and the time at which the transducer receives the echo, makes possible to calculate the velocity component in the that direction. In this hypothesis an A-Scan records a snapshot of the position of the scatterers for all depths selected within the medium. The time shift can be detected by comparing two echo signals with each other. This task is accomplished by a correlation algorithm between consecutive echo signals. This inspection mode allows a very accurate detection of the displacement, with a resolution much lower than the acoustic wavelength. This procedure measures the displacements of the small volumes of diffusers of the medium (some tens of microns) in windows of tens of millimeters in the axial direction. A primary analysis of the errors in the accuracy of the method indicates that the resolution of the displacement estimation could be around 2–5 μm . From these displacements on the acoustic axis, we can evaluate the wave phase velocity component (WPVC) of the low frequency induced wave on the acoustic axis at different depths. Thus, It will be possible to derive values for the shear elastic modulus from this parameter in isotropic or transversely isotropic media, approximately one third of the Young's modulus of the medium in soft tissues (Soleimani et al., 2020; Yang et al., 2020; Chen et al., 2018; Skerl et al., 2016b; Suhail et al., 2019). However, the well-known expression of said modulus is no longer valid for triclinic media and, furthermore, a mathematical expression has not yet been derived to relate it to the transverse velocity from the elastic Christoffel matrix in soft tissues or similar to them, to obtain the shear elastic modulus, which for this type of media is space-dependent and proportional to approximately one third of Young's modulus of the medium (Benech and Negreira, 2010). The measurements were made at room temperature, constant during the time required for each experiment, of less than an hour. The displacement results obtained for the elementary volumes of the tested samples are not affected in a first approach by small temperature changes, smaller than 0.5 $^{\circ}\text{C}$.

2.2. Experimental models and sample preparation

Three types of samples with a high anisotropy degree have been developed for the experiments: polylactic acid (PLA) printed grids, cow fiber meat slices and biocompatible hydrogel respectively. These pieces were embedded into a gelatin spheres with diameters of 50 mm containing agar particles. The different nature of the materials involved required different procedures for their manufacture. The size of the fibers varied from piece to piece.

2.2.1. Sample preparation

Three different phantoms were selected to mimic representative tissues in mammal organisms. They were strategically designed and developed to obtain samples progressively mimicking organic tissues

with a high degree of anisotropy that could not be assumed as transversally isotropic media.

According to our purpose of measuring low-frequency induced wave amplitudes related to the sample anisotropy, the samples were designed and printed with long fibers oriented in one direction in linear grids (Fig. 2a and 2b) to be placed within the gelatin spheres, except the cow slices, which were selected in the marked with a preferred direction of their fiber orientation (Fig. 2c). A biogel grid was 3D-printed to obtain homogeneous (the same physical properties as the gel sphere in which they were layered) but anisotropic samples to be tested. We aimed to mimic not a malignant tumor stroma but its anisotropic elastic properties.

Manufacture of gelatin spheres containing the anisotropic samples. The spheres were manufactured by assembling two half-spheres. Milli-Q water (purified and deionised), gelatine from type A pigskin and agar particles were mixed to create the medium of the spheres as follows: 1) A mixture of 30 g of pig gelatine and 400 ml of Milli-Q water was formed at 70 $^{\circ}\text{C}$ to become homogeneous (between 15 and 20 min); 2) 15 g of agar and 100 ml of Milli-Q water were added to the mixture keeping the temperature constant until the mixture became viscose; 3) the mixture was placed in a mold to obtain the desired half-spherical shape; 4) the anisotropic pieces to test were placed over one of the half-spheres before its solidification (with the exception of control samples, which did not include any sample to test); 5) the molds were placed in a cold container and surrounded with ice for one day to obtain solid semi-spheres (Fig. 2c); 5) finally, both semi-spheres were joined by a heat gun to form a complete sphere. They were kept in a cold environment to ensure proper maintenance.

Hard Polymers-Thermoplastics. Linear grids were designed in openSCAD (Free software, General Public License version 2, GPL2) as thin cylindrical wires with a diameter of 50 μm . Parallel strands of 500 μm in thickness were laid separated at a random distance (500–1, 500 μm , center-center distance), and piled up to 2 mm. Additive manufacturing (3D print-ing) was selected to fabricate these linear grids, using a 1.75 mm filament of blue PLA as material (BQ, Madrid, Spain). A Prusa i3 (Hephestos1, BQ, Madrid, Spain) without a heated platform was used.

Fibrous Meat Slices - Skeletal Muscle. Fibrous meat obtained from the knuckle of cow was manually sliced to form a cylinder-like structure of 2 mm of high. On this occasion, the linear grids and, consequently, the directionality, were defined by the natural fibers presented in the piece. Since this was a manual procedure, the size of the diameters and the fibers varied from piece to piece.

Soft Biopolymers - Hydrogels. Linear grids designed in openSCAD as cylindrical wires with 30 mm-diameters. Parallel strands of thickness-840 μm were laid separated at a random distance (840–2, 520 μm , center-center distance), and piled up to 2 mm in height. In this case, an in-house-modified Prusa i3 (Hephestos1, BQ, Madrid, Spain) equipped with a syringe extruder (based on Harry's Paste Ex-truder by

Fig. 2. a) Linear grid sample constructed with blue PLA; b) Hydrogel linear grid during its fabrication process; c) Fibrous meat slice, already embedded in one of the semi-spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

“yoursstreuliis” and licensed under the Creative Commons Attribution-Share-Alike license) was used. A paste of 9% autoclaved methylcellulose (MC) and 3% autoclaved alginic acid sodium (alginate) dissolved in sterilized distilled water was used as grids materials. Alginic acid sodium salt from brown algae (M/G ratio 1:2) and methylcellulose (M0512, Sigma, USA, molecular weight ≈ 88 kDa, $4.000 \text{ cP} \times \text{s}$) were purchased by Sigma-Aldrich (Germany).

2.2.2. Experimental setup and procedure

The experimental setup used in this work is schematically shown in Fig. 2(a). A 5 MHz central frequency ultrasonic transducer is attached a mechanical vibrator (mini-shaker, type 4810, Bruel & Kjaer, Narum, Denmark) working from 100 to 400 Hz acts on the phantom's surface, generating shear waves inside the sample (Fig. 3a). An US-KEY High-performance ultrasonic device including transmitter, receiver and digitizer of ultrasound pulses (Lecoeur électronique, France) is connected to the single transducer to emit and receive ultrasonic signals at an ultra-fast framerate (up to 5000 frames/s). The PC controls electronics and allows synchronization between the ultrasonic emission and the LF (low frequency) excitation. Two single transducers were used for different measurements working at 3.5 and 5.0 MHz respectively. The ultrasound electronics allows us to record up to 90 A-lines sampled at 40 MHz with 1400 points each. It represents 26 mm of measuring length inside the medium (with a sound speed of $1.5 \text{ mm } \mu\text{s}^{-1}$). The pulse repetition frequency (PRF) can be adjusted from 700 Hz to 6000 Hz.

Local WPVC maps associated with the small displacements measured on the acoustic axis induced by the shaker are obtained with a conventional ultrasound speckle interferometry technique. As the piston strikes the surface of the dummy, two types of waves are generated in the near field, a longitudinal wave and shear wave respectively. It is known that shear waves acquire higher amplitudes than longitudinal waves at low frequencies in soft solids, therefore shear waves are firstly detected. Shear waves are purely transverse in the far field (where they can be considered as plane waves), but they have a longitudinal component in the near field of the piston due to diffraction effects. Fig. 3. b shows a global image of the whole system, including the actuator/receptor device, gelatin spheres containing the samples to be tested, angular positioner and computer with an ultrasonic processor to order and process the acoustic signals. An angular positioner allows setting separately angles θ and φ respectively, with a precision obtained of less than 0.5° . Nine different angular positions of tapping/reception were

selected with respect to the spheres containing the anisotropic samples. These positions were defined by polar coordinates taking as reference the center of the spheres where the samples were layered. The angle φ refers to the degree of inclination from the vertical z-axis and θ as a horizontal rotation angle respect to the x-axis (see diagram of Fig. 3). The x-axis was selected as parallel to the direction of the lines or fibers in the sample, which means $\theta = 0^\circ$.

Each experiment required a week for completion, except in control tests, which did not include introduction of anisotropic samples into the gelatin-sphere. 25 percussions were made at a single point on the gelatin sphere containing the anisotropic sample, all of them with the same orientation angle. An average value for the low frequency induced WPVC generated in the sample was determined from the 25 quantified results.

The number of excitations, as well as the initial position and the width of the working space window, were checked using MATLAB (version 7.10.0 R2010a) and modified according to the position of the shaker in each measurement. The method was applied three times in each position. It should be remarked that the cow tissue did not cover completely the circular area in the middle of the gelatin sphere. However, it did not affect the results of the experiments as only a small area in the center was measured.

3. Results

3.1. Control experiments

Results obtained in the Control experiments are detailed in Table 1, which refers to induced low frequency wave velocity amplitudes measured at two frequencies: $f_1 = 3.5$ MHz, and $f_2 = 5.0$ MHz to analyze their frequency dependence and stability.

These results obtained in the Control samples (only gelatin isotropic spheres) at a frequency $f = 3.5$ MHz are displayed in Fig. 4, showing a shear velocity of the elastic wave that remains almost constant at the two frequencies tested for any orientation of the ultrasonic beam (θ and φ angles), according to the isotropy of the sample (gelatin). These coherent WPVC (wave phase velocity component) amplitudes measured on the Control samples validate the feasibility of the system and method for the purpose of this work. These results were used as reference in the experiments with anisotropic samples, with which were compared the results.

Fig. 3. a) Scheme of the measurement procedure from different 9 angular positions ($\varphi = 0^\circ; 45^\circ; 90^\circ$ and $\theta = -45^\circ; 0^\circ; 45^\circ; 90^\circ$ respectively; b) image of the device. The screen in the computer shows low-frequency displacement field as a function of time and z where a propagating low frequency wave associated to the displacements induced by the shaker, and a graphic over imposed showing the resulting travel time of this induced wave computed from the phase of the displacement field.

Table 1Results of induced low frequency wave velocity amplitude obtained at 3.5 MHz and 5.0 MHz respectively from different θ and φ orientation angles.

f = 3.5 MHz	θ	$\varphi = 0^\circ$		$\varphi = 45^\circ$		$\varphi = 90^\circ$	
		Average Value (m/s)	Standard deviation	Average Value (m/s)	Standard deviation	Average Value (m/s)	Standard deviation
f = 5.0 MHz	45°	1.606	0.685	1.533	0.610	1.113	0.611
	0°	1.606	0.685	1.529	0.541	1.162	0.590
	-45°	1.606	0.685	1.528	0.573	1.046	0.537
	-90°	1.606	0.685	1.525	0.587	1.627	0.587
	45°	1.738	1.099	1.758	1.154	1.755	1.084
	0°	1.738	1.099	1.810	1.940	1.807	1.004
	-45°	1.738	1.099	1.781	1.080	1.817	1.129
	-90°	1.738	1.099	1.653	0.868	1.802	1.200

Fig. 4. 3D Visualization of measured induced low frequency WPVC amplitudes measured in Control gelatin spheres; a) at 3.5 MHz; b) at 5 MHz.

3.2. Anisotropic samples

3.2.1. PLA material

Table 2 includes the results obtained from the PLA anisotropic linear grids embedded in the gelatin sphere at frequencies of 3.5 and 5.0 MHz, respectively.

A considerable variation of WPVC amplitudes can be observed with the angle of orientation of the shaker focusing a central area in the anisotropic sample embedded in the gelatine-sphere at both frequencies, reaching maximal amplitudes for percussion and measurements at a position of $\theta = 0$ and $\varphi = 90^\circ$, parallel to the sample fibers, at the two tested frequencies. On the contrary, it decays down to a minimum when percuting and sensing at $\varphi = 0^\circ$ (zénit/pole of the sphere), perpendicular to the 2D fibers direction. Intermediate values with similar WPVC amplitudes were found at $\theta = 45^\circ$ and $\theta = -45^\circ$ respectively, which is consistent with the direction of fiber arrangement in the anisotropic samples. Higher amplitudes were found with a rise of the angle φ (increasing toward the horizontal plane where the sample is layered

inside the sphere), independently on the θ angle. Results of Table 2 and graphically represented in Fig. 5a and 5.b respectively.

These results provide quantitative information of the sample anisotropy, showing variations in the magnitude of the velocity components toward maximum values when the orientation of the wave generator/transducer approaches the direction of arrangement of the sample fibers in the plane where they are displayed.

3.2.2. Meat slice

The samples consisted of commercial slices of cow meat consisting of skeletal muscle were placed in the middle plane of the gelatin-sphere. Collagen fibers exhibit different orientations of collagen and muscle fibers were randomly distributed in these organic samples. Results of measured values from different θ and φ angles are displayed in Table 3 and graphically represented in Fig. 6a and 6.b for the two frequencies tested of 3.5 MHz and 5.0 MHz respectively.

The measured WPVC amplitudes show apparent angular dependence, with the exception of those measurements made from the zenith

Table 2Average induced WPVC values measured on PLA samples embedded in gelatin spheres at 3.5 MHz and 5.0 MHz from different θ and φ orientation angles.

f = 3.5 MHz	θ	$\varphi = 0^\circ$		$\varphi = 45^\circ$		$\varphi = 90^\circ$	
		Average Value (m/s)	Standard deviation	Average Value(m/s)	Standard deviation	Average Value (m/s)	Standard deviation
f = 5.0 MHz	45°	1.018	0.627	1.544	0.899	1.677	1.088
	0°	1.018	0.627	1.797	1.181	1.970	1.316
	-45°	1.018	0.627	1.404	0.911	1.682	1.161
	-90°	1.018	0.627	0.961	0.457	1.301	0.885
	45°	1.027	0.265	1.643	1.017	1.872	1.152
	0°	1.027	0.265	2.241	1.204	2.727	1.431
	-45°	1.027	0.265	1.404	1.005	1.793	1.099
	-90°	1.027	0.265	1.085	0.766	1.241	0.885

Fig. 5. Spatial pattern of measured low frequency induced wave velocity amplitudes on PLA samples a) at 3.5 MHz; and b) $f = 5.0$ MHz.

Table 3

Average low frequency induced WPVC values measured on meat slice at 3.5 MHz and 5.0 MHz from different θ and φ orientation angles.

$f = 3.5$ MHz	θ	$\varphi = 0^\circ$		$\varphi = 45^\circ$		$\varphi = 90^\circ$	
		Average Value (m/s)	Standard deviation	Average Value(m/s)	Standard deviation	Average Value (m/s)	Standard deviation
$f = 5.0$ MHz	45°	1.025	0.616	1.765	1.167	2.359	1.414
	0°	1.025	0.616	2.219	0.732	2.713	1.064
	-45°	1.025	0.616	1.805	1.178	2.293	1.440
	-90°	1.025	0.616	1.653	0.858	1.948	0.984
	45°	1.027	0.265	1.643	1.017	1.872	1.152
	0°	1.027	0.265	2.241	1.204	2.727	1.431
$f = 5.0$ MHz	-45°	1.027	0.265	1.404	1.005	1.793	1.099
	-90°	1.027	0.265	1.085	0.766	1.241	0.885

Fig. 6. Spatial pattern of measured low frequency induced WPVC amplitudes on cow meat slice samples a) at 3.5 MHz; and b) $f = 5.0$ MHz.

($\varphi = 0^\circ$), as expected (where any variation has not a singular physical meaning). They reach a maximal amplitude at the angular position of $\theta = 0^\circ$ and $\varphi = 90^\circ$ at the frequency of 5.00 MHz, denoting higher sensitivity with the frequency rise within the range considered.

3.2.3. Biogel grid samples

Table 4 presents quantified results of the measured low frequency induced WPVC average and their standard deviations in Biogel grid samples at 3.5 MHz and 5.0 MHz respectively. These samples have

Table 4Average low frequency induced WPVC values measured on Biogel grid samples at 3.5 MHz and 5.0 MHz from different θ and φ orientation angles.

f = 3.5 MHz	θ	$\varphi = 0^\circ$		$\varphi = 45^\circ$		$\varphi = 90^\circ$	
		Average Value (m/s)	Standard deviation	Average Value(m/s)	Standard deviation	Average Value (m/s)	Standard deviation
f = 5.0 MHz	45°	0.817	0.501	0.919	0.243	1.084	0.497
	0°	0.817	0.501	0.861	0.315	1.378	0.449
	-45°	0.817	0.501	0.735	0.271	1.071	0.436
	-90°	0.817	0.501	0.892	0.187	0.842	0.522
	45°	0.817	0.501	0.919	0.243	1.084	0.497
	0°	0.817	0.501	0.861	0.315	1.378	0.449
	-45°	0.817	0.501	0.735	0.271	1.071	0.436
	-90°	0.817	0.501	0.892	0.187	0.842	0.522

physical properties similar to those of the gelatin sphere, mimicking homogeneous spheres with anisotropy referred to the fiber alignment.

Results of the acoustic velocity measured from different angles of orientations θ and φ are displayed in Fig. 7a and 7.b for the two frequencies under study.

4. Discussion

During the experiments, small variations of the WVPC amplitudes were found on the control isotropic samples tested from any angle φ (with respect to the horizontal plane where the sample was placed) and θ (in the horizontal equator plane of the sphere, plane angle formed between the shaker and fibers orientation of the anisotropic samples layered within the gelatin sphere). This is coherent with the isotropy of the control spheres without a sample inside, which should provide full homogeneity in the acoustic displacement/velocity amplitude measurements.

On the contrary, variations in the acoustic response was found depending on the sample structural and geometric properties, as well as frequency conditions. Apparent changes in the WPVC amplitude were found on the spheres containing anisotropic samples when measuring from different angles φ and θ respectively. Specifically, higher values were measured with increasing angle φ , i. e., when the direction of percussion/reception became closer to the horizontal plane including the anisotropic samples in the equator of the gelatin spheres. Measured WVPC values reached the highest amplitude in this plane at $\theta = 0^\circ$, for a full alignment of both, fibers and the acoustic axis.

The cow meat samples have been used to analyze results on real biological tissue explants. These samples showed the greatest differences of the induced WPVC amplitudes with the angle variations, followed by the PLA linear grids. On the contrary, the smallest variations of the

WPVC were obtained on the biogel structures, with identical structural properties to those of the gelatin sphere but including a printed fiber alignment to provide anisotropy in the geometrical configuration. Similar results were obtained on the Control and Biogel samples, in which it was very difficult to identify the direction of the fibers.

In all the samples, experiments made at $f = 5.0$ MHz provided WVPC higher amplitudes than those obtained at $f = 3.5$ MHz at certain elevation angles over the plane containing the sample φ and different angles of orientation θ of the shaker/receiver transducer with respect to the sample fiber direction. It means a higher sensitivity with the frequency rise within the frequency range and sample sizes considered for the study.

In the experiments, the obtained standard deviations are particularly large. It is due to some mechanical noise induced in the received echo signal traces, as a logical consequence of the transducer face itself being used as a striker on the surface of the sample under study. Since it is necessary to perform a number of percussions (10–15) for each measurement in order to obtain the average for each angular setting this introduces some noise in the A-scans.

The very low amplitude and short times of the pulsed ultrasonic waves used to perform the experiments measuring the low frequency wave velocity induced by a single percussion applied from the site of the transducer do not affect the elastic properties, but instead, measure some parameters associated to them. In the same way, the time of flight of the waves is very short in each experiment, of milliseconds, which do not induce temperature changes in the sample. It would be affected for samples continuously exposed to the ultrasonic waves during long times of minutes.

Fig. 7. spatial pattern of measured low frequency induced wave WVPC amplitudes on cow Biogel samples a) at 3.5 MHz; and b) $f = 5.0$ MHz.

5. Conclusions

In this paper we present a simple method to measure the angular-dependence that requires only a single ultrasonic transducer and angular positioning device. However, it is efficient to identify types of anisotropy, providing measurements of the shear velocity in the 3D space. It allows to identify directions of anisotropy of the medium under study and quantify its elastic properties in any angular direction.

The current work provides a simple and flexible method to measure a 3D spatial pattern of low-frequency wave phase velocity component (WPVC) in small regions of anisotropic samples. Our proposal to measure velocities as a function of the orientation of the transducer acoustic beam axis relative to the fiber orientation allows a good approximation of the WPVC/anisotropy ratio of the three samples analyzed.

Three types of samples with different configurations of structural properties and similar fiber directionality were designed for the current study: a hard polymer grid (thermoplastic), a beef slice (skeletal muscle) and a soft polymer printed grid (hydrogel). They have different densities, viscosities and sound speeds.

Homogeneous gelatin spheres without containing any sample inside were used as control in the experiments, where the WPVC amplitude remained constant for any angular orientation of the transducer acoustic beam axis in each small region measured. However, anisotropic media induce variations in the WPVC amplitude. A angular dependence is observed in these samples with the orientation of the transducer acoustic beam axis. Different WPVC were obtained as a function of the relative orientation of the acoustic beam axis with respect to the sample fibers direction. In the experiments we found smaller displacements associated to a shear velocity induced in the rigid polymeric grids, reaching maximal values of 1.94 m/s at $\theta = 0^\circ$ (within the plane in which the simple is embedded in the gelatin sphere) and $\varphi = 45^\circ$ (angle between the acoustic axis and fibers alignment). This amplitude was approximately 0.74 times smaller than the highest velocity found in the soft samples tested, of 2.73 m/s reached in both PLA and cow meat slice samples at $f = 5.0$ MHz for $\theta = 0^\circ$ and $\varphi = 90^\circ$ respectively. Thus, smaller acoustic displacements associated to the induced shear wave velocity projected onto the acoustic axis were measured in the rigid polymeric grids.

Our results show that conventional assumption of transverse isotropy for several tissues, used in some elastographic studies, cannot be applied in media with high-order anisotropy, where each measurement angle provides different displacements.

The present work is complementary to other studies in the literature referring to inhomogeneous media with established full triclinic anisotropy. This demonstrates the need to obtain quantitative 3D spatial patterns of tissue elastic properties, which could be relevant in early diagnostic and prognostic medical applications. This implies that in the physical model, 21 variables in the Christoffel tensor should be used to obtain these velocities for anisotropic media.

Therefore, the simple method based on the use of a single transducer could be very effective in finding anisotropies in dynamically progressive media. According to several studies correlating anisotropy and malignancy of solid tumors, this approach may be useful during benign/malignant differentiation of solid breast masses. The 3D measurements of the low-frequency WPVC proposed in this work allow the detection of anisotropies in small, albeit weak, areas of analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work has been done in the framework of the projects: Spanish National Plan RETOS-N° DPI2017-90147- R in collaboration with the Oncology Department of the Ramón y Cajal Hospital of Madrid; ERANET-EMHE 200022; and CYTED-DITECROD-218RT0545. We want to thank the Faculty of Sciences of the University of the Republic of Montevideo, Uruguay, for the techno-logical contribution in the experimental equipment, and to Prof. Dr. Lance Munn from the Harvard Medical School for his helpful discussion and assessment on mechanical properties of tumors.

References

- WHO cancer 2018, [https://www.who.int/news-room/fact-sheets/detail/cancer#:~:text=Cancer%20is%](https://www.who.int/news-room/fact-sheets/detail/cancer#:~:text=Cancer%20is%20).
- Benech, N., Negreira, C., 2010. *Phys. Med. Biol.* 55, 1753–1765.
- Böl, M., Ehret, A.E., Leichsenring, K., Weichert, C., Kruse, R., 2014. *Acta Biomater.* 10 (7), 3225–3234. <https://doi.org/10.1016/j.actbio.2014.03.003>.
- Catheline, S., Gennisson, J.-L., Delon, G., Fink, M., Sinkus, R., Abouelkaram, Culioli, J., 2004. *J. Acoust. Soc. Am.* 116, 3734–3741.
- Chen, Y.L., Gao, Y., Chang, C., Wang, F., Zeng, W., Chen, J.J., 2018. *Cancer Imag.* 18 (1), 11. <https://doi.org/10.1186/s40644-018-0144-x>. PMID: 29622044; PMCID: PMC5887177.
- Choi, H.Y., Sohn, Y.M., Seo, M., 2017. *Clin. Radiol.* 72 (10), 878–886. <https://doi.org/10.1016/j.crad.2017.04.009>.
- Couade, M., Pernot, M., Messas, E., Bel, A., Ba, M., Hagege, A., Fink, M., Tanter, M., 2011. *IEEE Trans. Med. Imag.* 30 (2), 295–305. <https://doi.org/10.1109/TMI.2010.2076829>.
- Fares, J., Fares, M.Y., Khachfe, H.H., et al., 2020. Molecular principles of metastasis: a hallmark of cancer revisited. *Sig Transduct Target Ther* 5, 28. <https://doi.org/10.1038/s41392-020-0134-x>.
- Fovargue, D., Fiorito, M., Capilnasiu, A., Nordsletten, D., Lee, J., Sinkus, R., 2020. *Sci. Rep.* 10, 1–12.
- Gennisson, J.-L., Cornu, C., Catheline, S., Fink, M., Portero, P., 2005. *J. Biomech.* 38, 1543–1550.
- Gennisson, J.-L., Deffieux, T., Fink, M., Tanter, M., 2013. *Diagnostics and Interventional Imaging* 94, 487–495. <https://doi.org/10.1016/j.diii.2013.01.022>.
- Gu, J., Polley, E.C., Ternifi, R., Nayak, R., Boughey, J.C., Fazzio, R.T., Fatemi, M., Alizad, A., 2020. *Breast* 54, 248–255. <https://doi.org/10.1016/j.breast.2020.10.013>.
- Herceg, Z., Hainaut, P., 2007. *Molecular oncology* 1, 26–41.
- Hong, S.-Kyoung, Gie, Y., 1998. *J. Phys. D Appl. Phys.* 31, 1392–1396, 1392–1396, 1998.
- Malandrino, A., Kamm, R.D., Moeendarbary, E., 2018. In vitro modeling of mechanics in cancer metastasis. *ACS Biomater. Sci. Eng.* 4 (2), 294–301. <https://doi.org/10.1021/acsbomaterials.7b00041>.
- Mueller, S., Mueller, J., El Shaarawy, O., 2020. Springer, pp. 495–508. https://doi.org/10.1007/978-3-030-40542-7_42.
- Schmidt, J.L., Tweten, D.J., Badachhpe, A.A., Reiter, A.J., Okamoto, R.J., Garbow, J.R., Bayly, P.V., 2018. *J Mech Behav Biomed Mater* 79, 30–37. <https://doi.org/10.1016/j.jmbbm.2017.11.045>.
- Schmidt, J.L., et al., 2016. *J. Biomech.* 49, 1042–1104.
- Seo, M., Ahn, H.S., Park, S.H., Lee, J.B., Choi, B.I., Sohn, Y.M., Shin, S.Y., 2018. *J. Ultrasound Med.* 37 (1), 99–109. <https://doi.org/10.1002/jum.14309>.
- Sinkus, R., Lorenzen, J., Kuhl, C., 2001. Conference: radiological society of north America. Scientific Assembly and Annual Meeting, 2001.
- Skerl, K., Vinnicombe, S., Thomson, K., McLean, D., Giannotti, E., Evans, A., 2016a. *Acad. Radiol.* 23 (1), 53–61. <https://doi.org/10.1016/j.acra.2015.09.016>. Epub 2015 Nov 10. PMID: 26564483.
- Skerl, K., Vinnicombe, S., Thomson, K., McLean, D., Giannotti, E., Evans, A., 2016b. *Acad. Radiol.* 23 (1), 53–61. <https://doi.org/10.1016/j.acra.2015.09.016>.
- Soleimani, M., Muthyala, N., Marino, M., Wriggers, P., 2020. *J. Mech. Phys. Solid.* 144, 104097. <https://doi.org/10.1016/j.jmps.2020.104097>.
- Soltaninezhad, M., Bavali, A., Nazifinia, Z., Soleimani, V., 2020. *Biomed. Opt Express* 11, 2996–3008.
- Staunton, R., Vieira, W., Fung, K.L., Lake, R., Devine, A., Tanner, K., 2016. *Cellular and molecular bioengineering* 9 (3), 398–417.
- Suhail, Y., Cain, M.P., Vanaja, K., P. A. K., Levchenko, A., Kalluri, Kshitiz, 2019. *Cell Systems* 9, 109–127.
- Tweten, D.J., Okamoto, R.J., Bayly, P.V., 2017. *Magn. Reson. Med.* 78 (6), 2360–2372. <https://doi.org/10.1002/mrm.26600>.
- Yang, H., Xu, Y., Zhao, Y., Yin, J., Chen, Z., Huang, P., 2020. *BMC Cancer* 20 (1), 930. <https://doi.org/10.1186/s12885-020-07423-x>.