Ultrasonic Backscatter Signal Processing Technique for the Characterization of Animal Lymph Node

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Abstract. Quantitative ultrasonic characterization of biological soft tissues has become in recent years an essential tool in the non-invasive-non-destructive assessment of physical properties of the microstructure of tissues, due to the potential for estimating acoustic parameters associated to density characteristics, distribution and heterogeneity of histological samples, as well as making the construction of improved quantitative images that support processes of clinical diagnosis. This paper presents the implementation of computational methods based on spectral analysis techniques for the construction of parametric ultrasonic images of animal suprascapular lymph node, which is an important tissue for the analysis of animal health risk or animal health. The computational algorithms were implemented based on the estimation of the acoustic attenuation coefficient dependent of the frequency and integrated backscatter coefficient (IBC). These computational procedures automatically processed 400 ultrasonic echoes acquired in a region of interest of 4 cm^2 for each sample of lymph node, which it was exposed to an incident ultrasonic field of 2.25 MHz with bandwidth of 1 MHz @ −3 dB. The results allowed parametric identification of nodule structures as germinal nodules, which are hardly identified in conventional qualitative ultrasound images. Finally ultrasonic parametric characterization of biological study samples provides potential quantitative indicators, which are so much accurate in the estimation of histonormality.

Keywords: Backscattering · Lymph node · Ultrasound

1 Introduction

The lymph nodes are soft structures surrounded of connective tissue, which comprises an important part of the immune system, acting as a mechanical filter of the lymph (extracellular fluid responsible of collecting and returning interstitial fluid to the blood), reducing the transit of pathogenic microorganisms and infectious agents, which can alter the immune system among others [1, 2]. Due to its direct contact with invading

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pathogenic bacteria and microorganisms, the lymph node can be used as a biological reference structure to support the surveillance of animal health risks and consequently animal health [3]. In this context, the exploration of new tools aimed to the evaluation of the normality condition in biological tissue, especially in animal benefit plants, are a subject of great industrial, scientific and technological interest, due to the importance that it deserves the development of instruments and methods involved in the processes of risk control of zoonoses (infectious diseases or vectors that can be shared between animals and humans), in favor of preventing public health problems, like the overall purposes of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), in the control of diseases related to the development of biological products, food and products of animal origin, in addition to the eradication of zoonoses and the post mortem inspection of meat with commercial purposes [4]. Conventionally, the most common methods used to establish the normality of biological tissue animal and to infer in possible alterations are based on visual inspection processes, performed subjectively by a specialist in veterinary science, with procedures that include the analysis and verification of size, color and physical characteristics of the microstructure among others [5, 6]. Other procedures manual and expensive are the histological techniques used in the processing of biological tissue, in which, the disadvantage is the use of chemical reagents (hematoxylin-eosin) and invasive procedures such as tissue dehydration, staining, paraffin aggregation and microtome cutting [7–9]. In particular, the processing techniques of biological tissues have a negative effect on the environment, in addition to being susceptible to systematic error, for the variability intra-observer and inter-observer, which in some cases causes diagnostic errors. In this way, the current techniques and methods used in the histological characterization by animal benefit plants, are based in costly protocols framed in the regulations of the Ministry of Health and Social Protection, in the national context, showing the need to promote the development of support instrumentation for the diagnosis, prevention, surveillance and control of welfare of the animal health as sustainable support to public health.

In this sense, the implementation and exploration of alternative methods for the characterization of biological tissues on the basis of non-invasive non-destructive methods, has stimulated the development of techniques based in the exhibition of acoustic fields [10, 11], with potential advantages in performing analyzes, without altering the physical and chemical characteristics of tissues with an optimal relationship cost-benefit [12]. Beside this, several studies show a wide range of applications by the techniques of quantitative ultrasonic characterization, especially in soft tissues such as heart, liver, kidney among others, assuming advantages compared to tissue processing technologies like systems x-rays or technologies of characterization of laser (which presents limitations in the characterization of opaque medium with high concentrations of particles). In order to propose alternative instruments and methods to support tissue analysis, the present study evidences the implementation of a system of quantitative ultrasonic characterization of animal lymph node samples, that allows to estimate acoustic indicators associated to the normality of the tissue, with the digital processing of ultrasound signals of backscattering, based in techniques of spectral analysis and the estimation of parameters associated with the loss of acoustic energy and power of backscatter.

2 Theoretical Fundament

The ultrasound use as tools in the noninvasive and nondestructive characterization (low power), it has driven the analysis of tissue properties, heterogeneous structures and the assessment of liquids and materials. This is because the ultrasound pressure variations during propagation process in interrogated media provide records of ultrasound backscattering signals, which contribute to estimates of quantitative parameters associated to properties of the characteristic media such as density spatial distribution and concentration heterogeneities and so on.

The acoustic attenuation is the combination of physical effects such as reflection, dispersion and absorption during the mechanical propagation processes in elastic media waves. In procedures of acoustic tissue characterization describes the energy loss when it is exposed to an ultrasound field, proving that the attenuation estimation can be used like a quantitative indicator to differentiate states of histonormality in biological media. For purposes of this study, the absorption is not considered because the characterizations experiments were performed using low power and indeed there is no temperature increase of the sample study. Thus, the attenuation effects are strictly caused by reflection and scattering process related to acoustic impedance variations from spatial distribution in the media. The mathematical model that describes the acoustic attenuation is obtained from the general dÀlembert solution unidimensional [12, 13]. One important specific solution in the frequency domain from which the estimation of the attenuation can be derived as described in (1):

$$\log\left[\frac{S_f(f)}{S_i(f)}\right] = -\beta f^n x, \tag{1}$$

where the power law $\alpha = \beta f^n$ is the attenuation coefficient (expressed in dB/cm), which relates the frequency - dependent attenuation coefficient β (expressed in dB/cm MHz), the frequency f and the index n that describes the frequency dependence, usually considered linear for soft biological tissues. $S_f(f)$ is the power spectral density for ultrasonic backscatter signals from sample study and $S_i(f)$ is the power spectrum of the ultrasonic incident wave and x is the propagation medium distance, equivalent to twice x because the characterization methods is performed in pulse-echo mode.

On the other hand, the acoustic scattering is a phenomenon of multidirectional propagation caused by interaction mechanisms between incident waves and heterogeneity characteristics from the propagation medium [17]. For experimental effects of ultrasonic soft tissues characterization, the scattering processes can be described in terms of the backscattering coefficient $\sigma_b(f,x)$. This parameter is used for measuring the power of backscatter that generates a volume of biological tissue. Its mathematical model is expressed in (2) according to [13]:

$$\sigma_b(f,x) = \frac{R^2}{A_0 \Delta x} w(f,x) \tag{2}$$

where R is the focal length of the transducer, A_0 is the effective radiation area of the transducer, Δx is the propagation distance (tissue thickness) and $w(f,x)$ is the rate between the spectral average power density from backscattering ultrasonic signal on the sample of study and the average power spectrum from a reference medium (material with known acoustic properties). Considering that $\sigma_b(f,x)$ can be integrated into the bandwidth of the ultrasonic transducer, the integrated backscattering coefficient (IBC) is the most used parameter to describes structural features of tissues from the average of spectral energy backscattered per unit of volume and incident energy unit for a frequency range. The mathematical model is seen in (3):

$$IBC = \sum_{f=\min}^{f=\max} \frac{\sigma_b(f,x)}{f_{\max} - f_{\min}} \quad (3)$$

where $f_{\min} - f_{max}$ represent the bandwidth of the ultrasonic transducer.

3 Ultrasonic Tissue Characterization System

The ultrasound characterization system for the exploration of acoustic indicators from animal lymph node can be seen in the Fig. 1. The system comprises a cartesian microcontroller positioner with millimetric resolution located at the top of an acoustic tank, a pulser - receiver system (*Olympus* pulse generator *PR 5072*) to emission of an ultrasonic field in pulse–echo mode with pulse repetition frequency of 100 kHz, an ultrasonic transducer *Panametrics-NDT* with resonance frequency of 2.25 MHz and 1 MHz @ −3 dB bandwidth, an oscilloscope for acquisition and signals digitization.

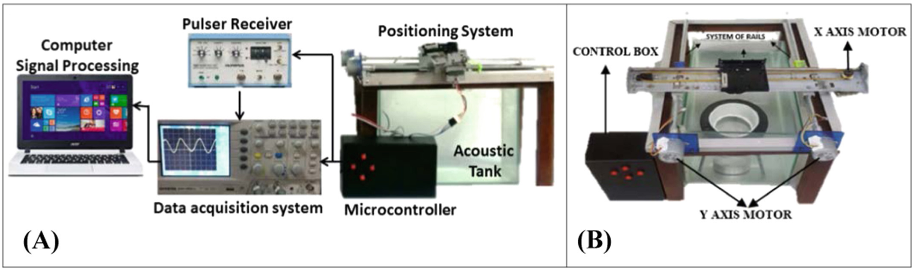

Fig. 1. The implemented system is composed by: **(A)** System of emission, acquisition and signal processing. **(B)** The acoustic transducer positioning system (cartesian robot).

The acquisition of the ultrasonic signals was carried out using an oscilloscope *Gw Instek G,* with rate sampling of 25 m/s, which allows the storage backscatter signals to enable its posterior processing by computational algorithms in the workstation. The implemented computational procedures for processing of ultrasonic signals were performed in MATLAB version R2014A.

3.1 Biological Samples and Ultrasonic Signal Processing

The bovine supra-mammary lymph nodes selected for the ultrasonic characterization were collected with the consent of veterinary services of the animal slaughterhouse. In this study lymphatic cells in a medullary region from four nodules were acoustically inspected. The delimited RoI was of 2 × 2 cm.

On the other hand, an immersion method was used aiming the ultrasonic characterization by the system described in Fig. 1. Lymph node samples were exposed to a 2.25 MHz ultrasonic field inside acoustic tank, which uses water as coupling medium for the optimal ultrasonic energy transfer to the tissue. The focal length transducer was estimated at 12 cm according to far field technical specifications. In this way, four hundred twenty ultrasonic backscattered signals were stored during the inspection space of 4 cm^2 for each lymph node. The signals were recorded by the oscilloscope used as signal acquisition system. An example of ultrasonic backscattered signal acquired from RoI is seen in Fig. 2.

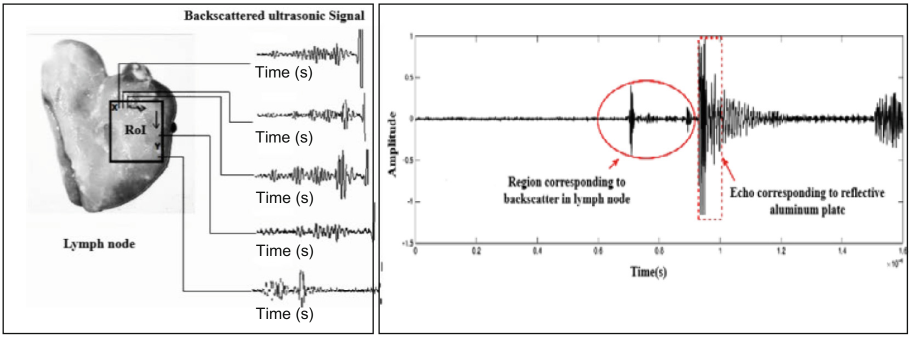

Fig. 2. Signals obtained from lymph node RoI during acoustic inspection area of 2 × 2 cm using to cartesian positioning system.

Figure 3(A) describes the computational procedures implemented for the estimation of the attenuation coefficient frequency - dependent β and IBC aiming the ultrasonic tissue characterization. The algorithm calculates firstly the angular coefficient β on the transducer bandwidth from the best linear adjustment of the logarithmic spectral subtraction between the tissue and reference normalized spectra respectively, showing spectral energy loss because biological tissues produce effects of low pass filters. The β estimation for each node allowed generating a data matrix, which is used to construct a parametric ultrasonic image taking into account first the logarithmic data compression and a spline interpolation. On the other hand, Fig. 3(B) represents the construction of IBC parametric images from the following steps: (1) Enlistment of ultrasonic backscatter signals acquired from tissue cross section during acoustic inspection; (2) Spectral calculation by the periodogram technique from tissue backscatter signals fragmented with an overlapping rate of 50% on the RoI. The ultrasonic signal partition in RoI is performed considering a partition size equivalent to the temporal pulse width from the ultrasonic incident signal. Subsequently it is selected in order to calculate the fast fourier transform (FFT); (3) IBC calculations use theoretical models previously described; (4) For generating IBC parametric images, each data matrix IBC is interpolated by spline method and is fitted with a meshgrid, according to the dimensions of the RoI of the tissue, which was inspected with a step size of 1 mm per backscatter signal collected.

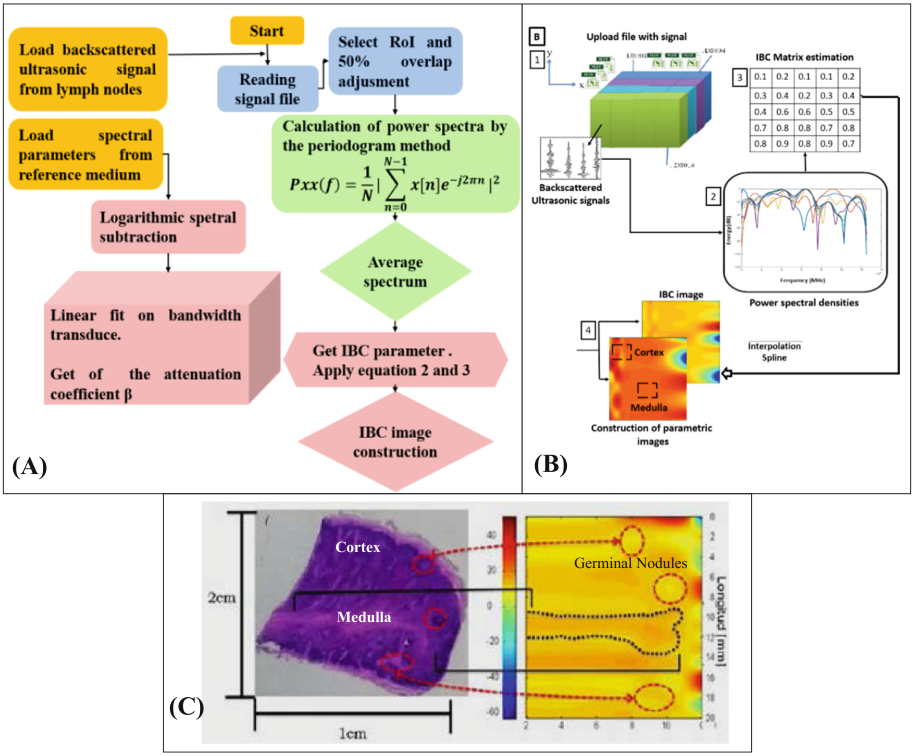

Fig. 3. (A) Computational procedures used to estimate spectral parameters. (B) Parametric image construction from IBC. (C) Histological plate of lymph node supra mammary Inspected (left). Parametric IBC image of cross-section of tissue (right).

Figure 3 shows histological plaque generated for a nodule by a processing and paraffin inclusion together with a staining of hematoxylin eosin. Figure 3 also shows a parametric image generated by the estimation algorithm of IBC parameter. In Fig. 3 (C), two structurally different sections are observed. In the upper left of the parametric image is evidence subtly small nodular areas corresponding probably effects of germinal nodules that are located preferably in the lymph node cortex. In the second instance in Fig. 3(C) also can be displayed in the middle of the spatial distribution, medullary sinusoids of a lighter color and including medullary trabeculae. The density variations of structural tissue are better detailed by generation of ultrasound parametric images ultrasound, which are more sensitive to the characteristics of the tissue microstructure, in contrast with the generation of ultrasound images in mode B, because in the latter it is lost information by the transformation of high-frequency backscatter signals to low-frequency signals, representing the qualitative characteristics of an irradiated sample. Figure 3(C) shows the medullary sinuses that presents a lymph node and are coherent with the structures seen by visual inspection in a histologic cross-section of the node. In the middle region of the image is possible to appreciate a region more uniform that for distance corresponds to the medulla of the sample study, this uniformity corresponds to the properties of echogenicity of this structure. From the calculation of data matrices, statistical analyzes are carried out to correlate acoustic parameters associated to the regions of the medulla and cortex of lymph nodes. Thus Fig. 4 shows for the medulla structure a percentage difference of acoustic parameters of 33% between normal and abnormal classes. Likewise, for cortex exist a percent difference of 24.9% for these classes, that shows a clear difference of the parametric acoustic indicators (IBC and attenuation) for normal and abnormal structures.

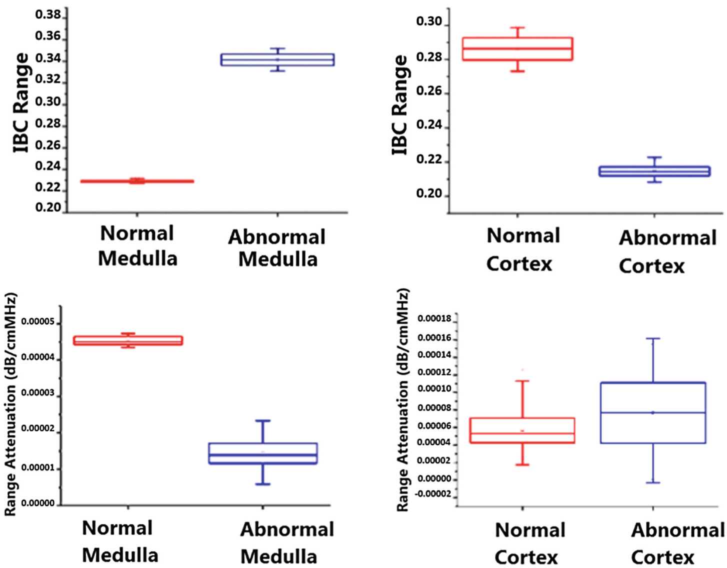

Fig. 4. Comparison of distribution of IBC parameters and attenuation between normal and abnormal lymph node samples.

The results obtained from ANOVA variance test show a statistically significant difference through the acceptance of the alternative hypothesis with an acceptance rate of 95%, which demonstrates that pixel values of parametric images in regions of medulla and cortex are useful to discern between nodules considered histologically normal and abnormal. Based on the parametric ultrasonic images different structures of the lymph nodes can be identified due to differences in echogenicity of the same.

4 Conclusions

The present study demonstrates how parametric characterization techniques by ultrasound, favor non-invasive and non-destructive analysis in the evaluation of animal soft tissue structures. The parametric ultrasound images of IBC allow a better visualization of structures such as cortex and medulla of lymph nodes in comparison with conventional ultrasound images in B mode, which difficult to identify in detail medullar structures or germinal centers. Finally, it is convenient to mention that the techniques of parametric ultrasonic characterization can contribute to technify process related with of histological analysis and the minimization of contamination risks evidencing the acoustic exposimetry techniques like an unconventional potential tool in analysis of biological tissues.

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