Advances in acoustic microscopy and high resolution ultrasonic imaging: from principles to new applications

Roman Gr. Maev
The Institute for Diagnostic Imaging Research, University of Windsor
688 University Avenue W. Windsor, Ontario, CANADA N9A 5R5

ABSTRACT

The goal of this lecture is to provide an overview of the recent advances in high-resolution ultrasonic imaging principles and techniques and their biomedical applications. This lecture will offer a number of new results from leading research groups worldwide who are engaged in aspects of the development of novel physical principles, new methods, or the implementation of modern technological solutions into current high resolution imaging techniques and methods. Together with the abovementioned academic and practical avenues in high resolution ultrasonic imaging research, intriguing scientific discussions, which have recently surfaced and will hopefully continue to bear fruit in the future, will be reviewed. Another goal of this lecture is to encourage a new generation of researchers to be more involved in research and development in the field to realize the great potential of high resolution acoustic imaging and advance the progress into its various biomedical applications.

Keywords: Acoustic microscopy, tissue characterization; quantitative ultrasound

1. INTRODUCTION

Ultrasonic imaging is a powerful tool in medical diagnostics, with a wide variety of ultrasonic scanners available in clinical practice and research. Sophisticated instruments give detailed live images of internal organs and provide measurements of vital characteristics. Safety and simplicity of use make them a convenient and widespread diagnostic tool for non-invasive testing. Ultrasound waves propagate easily in soft biological tissues with relatively low attenuation, even at frequencies on the order of a hundred megahertz. The major characteristic of ultrasound related to tissue material is the speed of sound. It is determined by elastic properties of the tissue and its density. From an acoustical point of view, most soft tissues are similar to water, but have slightly higher elastic coefficients and a corresponding increase in the speed of sound (1480 – 1620 m/s for various tissue components). These small variations cause differences in acoustic impedance between areas of tissue and corresponding variations in reflection and scattering of ultrasound waves. All ultrasound imaging techniques are based on receiving and analyzing these secondary waves. The difference between them is methods and algorithms of analysis[1].

Compared to widely-used medical ultrasound, acoustic microscopy operates with high frequencies at relatively short distances. The acoustic beam emitted by the transducer passes through a coupling liquid (water) and is reflected back by inhomogeneities in the sample structure. The strength of the reflected signal is determined by the change in characteristic acoustic impedance at such an inhomogeneity. The reflections captured in the digitized received signal (A-scan) represent a one-dimensional property distribution along the acoustical beam. The ultrasonic image is digitally constructed from a series of A-scans acquired during lateral scanning. Most clinical ultrasound devices use phased array technology with beamforming and angular scanning. At the current technological level, array manufacturing and phasing operation is very problematic for high frequencies. That is the main reason acoustic microscopes usually scan by the mechanical motion of a single acoustic lens along the sample surface. The main types of images obtained by acoustic microscopes are B-scan and C-scan. More complex data processing algorithms produce enhanced images that can also be attributed to these two categories. A B-scan represents the 2D distribution of amplitudes along the axial and the lateral coordinates (vertical cross-section). A C-scan represents the 2D distribution of amplitudes in a lateral plane at a certain time delay after the initial pulse has been emitted from the transducer (horizontal cross-section). Both types of scans may

Medical Imaging 2014: Ultrasonic Imaging and Tomography, edited by Johan G. Bosch,
Marvin M. Doyley, Proc. of SPIE Vol. 9040, 904007 · © 2014 SPIE
CCC code: 1605-7422/14/$18 · doi: 10.1117/12.2044402

contain noticeable electronic noise, which increases with axial depth, specific artifacts and speckles. Elimination of this signal debris is an essential part of instrument tuning and data processing algorithms.

2. RESOLUTION AND CONTRAST MECHANISMS

The essential feature of soft tissues is the absence of strongly reflective boundaries inside. Significant reflected signals are produced only by tissue surfaces – i.e. interfaces between the sample and surrounding media. For acoustic microscopy biological material should be sectioned at an appropriate thickness and attached to the substrate – ex. normal slide glass or high molecular polymer material used in dishes for cell cultures. The choice of sample thickness is determined by the operating frequency and analysis algorithm[2]. Reflections from both interfaces, coupling liquid to tissue and tissue to substrate, in classical acoustic microscopy, should be separated and analyzed individually. Axial resolution in this case is entirely determined by the duration of the signal and the bandwidth of the receiver. The use of short sound pulses approaches theoretical limits – approximately half a wavelength. The contrast of an acoustical C-scan comes from variations of the reflective coefficient, i.e. variations of specific acoustic impedance across the tissue sample. However, advanced numerical processing allows working with thin samples and extracting important information in case of interleaving signals. Their interference is determined by acoustic properties of biological materials: interference in frequency is determined by the thickness and sound speed of the sample; interference in intensity by the surface reflection coefficient and sound attenuation in the material. Quantitative measurement of the sound speed in this case is based on analysis of interference of frequency-dependent characteristics. It can be obtained either by serial measurements with varying frequencies or by fast Fourier transform of a single broadband pulse.

Lateral resolution of ultrasonic imaging is determined by the width of the acoustic beam. Spherical focusing enables reducing it down to the theoretical limit, which is roughly equal to the wavelength multiplied by the angular aperture. High resolution requires waves with short wavelength and corresponding high acoustic frequency. As a rule of thumb, the size of visualized objects entirely determines minimal operational frequency of the ultrasonic imaging system. Attenuation of ultrasound waves in tissue sharply increases with frequency, which limits imaging depth for ultrasonic devices. A proper compromise between the opposing demands for a large penetration depth and good spatial resolution has to be found for each particular application.

Classical acoustic microscopy expects achievement of maximal resolution only at certain depth, determined by the focal distance of the acoustic lens. Imaging the entire volume of thick samples requires repeated scanning with the focus located at different depths in order to maintain resolution along the axial direction. The final image should be composed from subsequently acquired short B-scans. Synthetic Aperture Focusing Technique (SAFT) is another approach for depth-invariant imaging, which involves numerical processing of the B-scans from the aggregate of acquired data. According to this technology, the focal point is modeled as a point source of acoustic far-field with the transducers opening angle[3] – each individual point of the image is calculated by summing up echo signals with a corresponding delay. Further, 2D scanning in both lateral and elevation directions allows obtaining images with constant high resolution along both coordinates. As an additional benefit, this method allows for significantly extended range of the system beyond the focal depth of the transducer. Taking into account diffraction effects provides further improvement of acoustical images – ex. a noticeable increase in contrast and signal-to-noise ratio (SNR)[4].

A variation of this method is Limited Angle Spatial Compounding (LASC) technique. It includes multidirectional echo measurement and incoherent superposition of B-mode images from different directions. Instead of linear scanning in the elevation direction, the focused transducer is tilted in the plane of the B-scan. The sequence of SAFT-processed B-mode images obtained by lateral scanning at various tilting angles should then be combined into one output image. The compound B-mode image is obtained by the superposition of scan-converted B-mode images[5]. Advantages of this approach are significant improvements in contrast, suppression of speckle and electronic noise, and reduction of image artifacts.

3. ADVANCED METHODS OF ACOUSTIC MICROSCOPY

Classic acoustic microscopy is a rather qualitative approach aimed at visualizing the sample morphology. Tissue characterization requires quantitative analysis of echo signals with the goal of assessing histological tissue information. Several quantitative techniques have been developed for the measurement of tissues parameters. One of the most popular

methods called V(z) is based on recording the output voltage of a focused transducer as a function of distance between the focal point and the surface of the specimen[1]. In a quasi-monochromatic system the interference between directly reflected waves and leaky surface waves produces an oscillating dependency of the output signal amplitude from the elevation position of the lens. The phase velocity and propagation attenuation of surface waves, as well as a reluctance function for the specimen-water interface, can be obtained from the recorded V(z) data. This method is directly applicable and quite effective for hard tissues[6,1] – ex. bones and dental materials. The heterogeneity and anisotropy of bone tissue render measurement and data interpretation more difficult. The necessary defocusing increases the interrogated surface area and the spatial resolution drops dramatically. Soft tissues require a special arrangement due to the close impedances of the specimen and coupling – attachment of thinly sectioned samples to the solid substrate. Additional load on the substrate surface changes the V(z) curve. The computer parameter-fitting technique with Fourier analysis provides tissue parameters: sound speed, attenuation and elastic modules[7,1].

Many modifications of the V(z) techniques employing various types of excitation signals, focused transducers, and processing algorithms of the analog and digital signals have been proposed[1]. A continuous wave Doppler system provides data for a single particular frequency. In contrast, the pulse mode reveals properties of the thick specimen over a wide frequency band[7]. In this case, signals corresponding to reflections from both sample surfaces and to surface waves are separated in time, and the difference in time of flight can be directly determined from output data along with their amplitudes. The analysis of time-resolved pulse echo V(z) data has several advantages compared to measurement with amplitude-detected signals. The major benefit is that all the information is kept in the signal and can be used for analysis. Some processing steps are necessary for reliable analysis, including band-pass filtering, amplitude detection, time-of-flight based defocus correlation, etc.

The speed of sound can be calculated from the time delay between pulses reflected from the first and second sample boundaries. However, the distance between these boundaries, i.e. the sample thickness, should be known with high accuracy. This usually presents problems even for ideally flat samples and becomes an especially hard task for samples coming out of standard slicing procedures. Fortunately, both speed of sound and thickness can be simultaneously assessed by referring to the sound speed of the coupling, i.e. water. If the sample thickness is less than the wavelength, then the resulting output signal will be the interference of the two reflections. Intensity and phase spectra are calculated by Fourier transforming the waveform. Positions of minimum and maximum points on the intensity spectrum provide phase differences, which further may be used for calculations of sound speed and thickness. Two peaks corresponding to both reflections may be separated by using proper window functions and other acoustic parameters such as attenuation and acoustic impedance may be calculated and visualized as a function of the frequency[8].

The majority of well-known acoustic phenomena are associated with linear elasticity of the materials described by Hooke's law. However, in the event of high intensity of the input signal or when materials exhibit specific properties, the stress-strain relation becomes nonlinear and produces a variety of additional effects such as wave shape distortion, generation of higher harmonics, etc. The presence of inhomogeneities and microbubbles dramatically enhances local nonlinear properties of the specimen. Effective nonlinear distortion of ultrasonic wave was demonstrated in a number of publications; harmonics up to the 4th order were detected and analyzed. The main benefit for practical use of nonlinear effects is improved resolution, traded, however, for decreased signal-to-noise ratio and as result, lower sensitivity. The nonlinear visualization technique was further developed by replacing standard transducer of acoustical microscope with an assembly of three or even four active elements attached to the buffer of the acoustic lens. Generation of sum- and difference- frequency waves occurs in common focal point where individual beams meet. It distinguishes the nonlinear phenomena originating in the sample material from the ones associated with coupling liquid and transducer. The obtained distribution of nonlinear properties in the tissue characterizes the resonance response of certain cell groups. The cellular structure of biological tissues shows certain anisotropy of elastic parameters. Anisotropic nature is clearly visible for muscle tissues, which have a distinctive orientation of fibers. While the change in sound speed may be just a few percent, the attenuation in the direction parallel to the muscle fibers is almost twice that of the perpendicular direction. This effect may also be used for tissue characterization.

Especially useful anisotropy measurements can be made in the case of hard biological materials – ex. bones and dental tissues. Anisotropic by nature, these materials reveal strong angular dependence of elastic coefficients. While the size of a single mineralized fibril is still smaller than the sound wavelength for frequencies up to the Gigahertz range, larger structural elements can be resolved by an acoustical microscope. Using a cylindrically focused acoustic lens, anisotropic properties can be easily mapped and visualized in the process of scanning. Image analysis detects areas with distorted anisotropy (demineralization, etc.). Another option is the use of a shear wave acoustic lens[9].

4. BIOMEDICAL APPLICATIONS OF ACOUSTIC MICROSCOPY

Visualization and representation of acoustical data as easily perceived images is a major benefit of acoustic microscopy. Starting with pioneering works, acoustic microscopy researchers demonstrated wide opportunities for ultrasonic visualization of cells, their microstructure and development. Currently, investigation of the microstructure and mechanical properties of individual cells and cell cultures is still one of most interesting and fruitfully developing areas of applications for acoustic microscopy. High contrast of the acoustic images can be reached without fixation and staining, which offers a unique opportunity to study cells in vivo. It was established that neither high-frequency ultrasonic irradiation nor mechanical scanning causes damage or significant changes in the cell behavior. Cell damage by low-frequency ultrasound was observed only for extremely high intensities and long exposure time, which far exceed the normal operation of an acoustic microscope. Individual cell can be resolved at frequency ranges starting from approximately 300 MHz and detailed information about internal structure is visible around 1 – 1.5 GHz. A two-dimensional distribution of the ultrasonic intensity and related mechanical properties, visualized in various experiments, provides a solid basis for analysis of the cell structures, such as the nucleus in the central part and cytoskeleton at the peripheral zone.

A sequence of acoustic images, taken at some time intervals, may be used to animate dynamic changes in the cytoskeleton and cell adhesion to the substrate during cell motion over the substrate[10]. Details of biomechanics and cell behavior under various external stimuli may be easily observed and analyzed. For example, model experiments have demonstrated the efficiency of acoustic microscopy in measuring the effect of heating on the size and contact area of cells grown on substrate[11]. The reactions of individual cells on the surrounding chemical composition have a great importance for treatment monitoring.[12]

Basic acoustic parameters, impedance and attenuation, characterize the elastic state of the cell cytoskeleton and its components, such as filament proteins. The ultrasound velocity and absorption were measured and mapped in various cell structures; their dependence on the distribution of actin fibrils was studied[13]. The strained cytoplasm state, through contraction of actinomyosin complex, increases the velocity from 1600 to 1800 ms^{-1}. The topographic distribution of ultrasound velocity values is aligned with the direction of forces supporting the cell structure[14].

Undoubtedly, supravital noninvasive investigation of tissues and organs is the most interesting and practically important application of acoustic microscopy. The technique of sample preparation for high frequency acoustic microscopy studies has much in common with methods of classical histological microfabrication. For direct comparison of optical and acoustic images, samples should be prepared in the same way – i.e. thin sections should be cut either after fixation, water removal and enclosure in paraffin, or after freezing on a microtome. This procedure is dictated by the requirement of obtaining thin parallel samples necessary for both examinations. Such treatment essentially affects acoustical properties of the tissues, but keeps the tissue structure visible. Studies based exclusively on ultrasound do not require the above described procedure and may deal with fresh samples. Moreover, the real elastic parameters should be measured on unprocessed tissues.

The development of quantitative investigation techniques has made it possible to obtain more accurate data on the acoustic properties of the components of tissue structures. Correlation between the composition of a tissue and its acoustic properties is the most important problem in tissue studies. It was found that mechanical properties on the tissue level are controlled by water and fat content[15], and conjunctive tissue properties[16]. Pressure in the blood vessels and of the interstitial liquid, peculiarities in the intercellular material aggregation and the interaction of cells also give a noticeable influence.

An acoustic microscope operating at a frequency range of 100 – 400 MHz was demonstrated to be an effective tool for differential medical diagnosis of various types of cancer. Liver carcinoma, gastric cancer and breast cancer were actively investigated by many research groups[17]. It was found that tumor tissues have a different sound speed and attenuation in comparison with healthy tissues, but the magnitude and sign of these changes vary for different cancer pathologies. While cancer cells themselves show reduced sound speed and attenuation, the average values for a tumor can be higher than the surrounding normal tissue. This phenomenon can be explained by the active involvement of rigid collagen fibers in the process of tumor formation.

Acoustical transparency of soft tissues opens the possibility to use thick samples when wave penetration is more than several wavelengths. It may be convenient for practice in low frequency (25–70 MHz) ultrasound in order to avoid thin

cutting (microtoming) and related procedures of fixation/paraffin injection. A sandwiching between a rigid cover plate and a highly reflective substrate will remove the effect of surface roughness of the specimen. Two reflected signals, from the plate-sample and from the sample-substrate, may be used for C-scan image generation. For the first reflection the contrast will originate in lateral variations of acoustic impedance, while for the second it mostly represents lateral variations of attenuation. Quantitative data can be further extracted by waveform analysis and the Fourier transform[18]. For example, in the frequency range 15 -50 MHz, the relative acoustic attenuation for melanoma tumor tissue estimated by this method is seen to be approximately 30+ dB higher than normal tissue. That fact opens possibilities for express diagnostics of cancer boundaries during surgery.

One of the most accessible and feature-rich objects for histological study is human skin. Skin carries out extremely important biochemical functions in the human organism and possesses a complex mechanical microstructure. Plenty of materials have been collected and published. The comparative analyses of acoustical and optical images have been performed in the range 20 – 100 MHz with resolution down to 10 mkm[19]. Tumors, inflammatory processes, wounds, and burn scars have been intensively studied. Three main layers of the skin can be clearly distinguished in acoustical images: epidermis (outer dense keratinizing stratified epithelium); a more transparent basal layer of epithelial cells; and finally a dermis, consisting of a dense network of entangled collagen and elastin fibers. Acoustic images exhibit variations in impedance and absorption that arise both from difference in elasticity and viscosity of individual components and from different scattering at the interfaces. Sweat glands and hair follicles are easily recognizable on the scans. Pathological changes also are well distinguished on histological samples[20]; that fact gives possibility to the introduction of in-vivo ultrasonic diagnostics. Skin structures are easily accessible from the surface even for high-frequency techniques. Non-invasive acoustical imaging allows in-vivo visualization of skin morphology valuable for clinical practice.

Small animal imaging is another field of application. A large number of experiments are conducted on rats and mice in biomedical research, and in many cases step-by step observation of progress is highly desirable. Ultrasonic imaging provides a powerful tool for documenting experiment development. Several research groups proposed various systems and methodologies for that purpose. Real-time imaging of a mouse heart was the goal of extremely high frame-rate systems[22]. Advanced processing utilizing the above-discussed multidirectional limited angle spatial compound imaging approach together with the PSF-SAFT reconstruction technique[21] allows for high quality B-scans of the stomach, liver, ribs and spine of the animal.

CONCLUSION

Acoustic microscopy is ready to step from experimental setups into everyday usage. Several companies are manufacturing commercially available devices[2,8,23]. The development of methodology and establishment of clearly formulated procedures will spread their usage. Together with operation of stationary or desktop systems, development of small portable devices will provide a fast and convenient way to get important information about an object. This can be achieved by the use of small hand-held scanners built by classical schemes or by incorporating multi-transducer high-speed electronic systems, having compact size and greatly reduced time of scanning[23]. Recent developments such as ultrasound speed microscopy, 3D ultrasound imaging and high frequency array transducers may realize clinically acceptable hand-held acoustic microscopy in the near future.

REFERENCES

[1] Maev, R. Gr., [Acoustic microscopy. Fundamentals and applications] Wiley-VCH, Weinheim, (2008).

[2] Jones, J. P., “Applications of acoustic microscopy in diagnostic medicine,” Int. J. Imag. Syst. Technol. 8, 61-68 (1997).

[3] Opretzka, J., Vogt, M., and Ermert, H.. “A model-based synthetic aperture image reconstruction technique for high-frequency ultrasound,” Proc. IEEE Ultrason. Symp. 377-380 (2009).

[4] Opretzka, J., Vogt, M., and Ermert, H., “A synthetic aperture focusing technique with optimized beamforming for high-frequency ultrasound,” Proc. IEEE Ultrason. Symp. 2303-2306 (2010).

[5] Vogt, M., and Ermert, H., “Limited-angle spatial compounding imaging of skin with high-frequency ultrasound,” IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 55(9) 1975-1983 (2011).

[6] Raum, K., Kempf, K., Hein, H.J., Schubert, J., and Maurer, P., „Preservation of microelastic properties of dentin and tooth enamel in vitro – a scanning acoustical microscopy study," Dent. Mater. 23 1221-1228 (2007).

[7] Tittmann, B.R., Miyasaka, C., Maeva, E., and Shum, D., "Fine mapping of tissue properties on excised samples of melanoma and skin without the need for histological staining" IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 60(2) 320-331 (2013).

[8] Hozumi, N., Yamashita, R., Lee, C.K., Nagao, M., Kobayashi, K., Sajio, Y., Tanaka, M., Tanaka, N., and Ohtsuki, S., "Time-frequency analysis for pulse driven ultrasonic microscopy for biological tissue characterization," Ultrasonics, 42 717 – 722 (2003).

[9] Tittmann, B.R,, Miyasaka, C., Maeva, E., and Maev, R., "Acoustic imaging of isotropic and anisotropic thick tissue" Proc. 30th Int. Acoustical Imaging Symp., Monterey, CA, Mar. 1–4, 113–117. (2009).

[10] Hoppe, M., and Bereiter-Hahn, J., "Applications of scanning acoustic microscopy – survey and new aspects," IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 32(2) 289-301 (1985).

[11] Tittmann, B.R., Miyasaka, C., Mastro, A., and Mercer, R.R., "Study of cellular adhesion with scanning acoustic microscopy" IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 54(8) 1502-1513 (2007).

[12] Brand, S., Weiss, E.C., Lemor, R.M., and Kolios, M.C., "High frequency ultrasound tissue characterization and acoustic microscopy of intercellular structures," Ultrasound Med. Biol. 34 (9) 1396-1407 (2008).

[13] Luers, H., Hillmann, K., Litniewski, J., and Bereiter-Hahn, J., "Acoustic microscopy of cultured cells; Distribution of forces and cytoskeletal elements," Cell Biophysics, 18 279-293 (1992).

[14] Karl, I., Bereiter-Hahn, J., "Cell contraction caused by microtubule disruption is accompanied by shape changes and an increased elasticity measured by scanning acoustic microscopy," Cell Biochem. Biophys. 29 225-241 (1998).

[15] O' Brien, W.D., Erdman, J.W., and Hebner, T.B., "Ultrasonic propagation properties (at 100 MHz) in excessively fatty rat liver," J. Acoust.Soc. Amer., 83 1159-1166 (1988).

[16] Saijo, Y., Tanaka, M., Okawai, H., Sasaki, H., Nitta, S., and Dunn, E.. "Ultrasonic tissue characterization of infracted myocardium by scanning acoustic microscopy," Ultrasound Medicine Biology, 23 77-85 (1997).

[17] Chen, D., Malyarenko,E., Seviaryn, F., Yuan, Y., Sherman, M., Bandyopadhyay, S., Gierach, G., Greenway, G.W., Maeva, E., Strumban, E., Duric, N., and Maev, R., "Characterization of human breast cancer by scanning acoustic microscopy" Proc. SPIE 8675, (2013).

[18] Maeva, E., Severin, F., Miyasaka, C., Tittmann, B.R., and Maev, R.G., "Acoustic imaging of thick biological tissue," IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 56(7) 1352-1358 (2009).

[19] Altmeyer, P., Gammal, S., and Hoffmann, K., [Ultrasound in Dermatology], Springler, Berlin (1992).

[20] Miyasaka, M., Sakai, S., Kusaka, A., Endo, Y., Kobayashi, M., Kobayashi, K., Hozumi, N., and Tanino, R.. "Ultrasonic tissue characterization of photodamaged skin by scanning acoustic microscopy," Tokai J Exp Clin Med., 30(4) 217-225 (2005).

[21] Opretzka, J., Vogt, M., and Ermert, H.. "A high-frequency ultrasound imaging system combining limited-angle spatial compounding and model-based synthetic aperture focusing," IEEE Trans. Ultrason., Ferroelectr. Freq. Control, 58(7) 1355-1365 (2011).

[22] Foster, F.S., Zhang, M.Y., Zhou, Q., Li, G., Mehi, J., Cherin, E., Harasiewicz, K.A., Starkoski, B.G., Zan, I., Knapik, D.A., and Adamson, S.L. " A new ultrasound instrument for in vivo imaging of mice," Ultrasound Med. Biol., 28(9) 1165-1172 (2002).

[23] Edited by Roman Gr. Maev [Advanced in Acoustic Microscopy and High Resolution Imaging. From Principles to Applications, 14 Chapters] Wiley-VCH, Weinheim, (2013).