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Quantitative Ultrasound and Photoacoustic Imaging for the Assessment of Vascular Parameters

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Preface

The aim of the work presented here is to develop quantitative techniques for ultrasound and photoacoustic imaging for the assessment of architectural and vascular parameters.

The works in this book can be divided into two macro areas: (1) morphological vascular studies based on the development of quantitative imaging techniques for the use with clinical B-mode ultrasound images, and (2) preclinical architectural vascular studies based on quantitative imaging techniques for ultrasound and photoacoustics.

The first section, which makes up the second and third chapters, focuses on the development and validation of quantitative techniques for the assessment of vascular morphological parameters that can be extracted from B-mode ultrasound longitudinal images of the common carotid artery. In Chap. 2, results from numerous past studies are presented, including the validation of techniques for correctly locating the CCA in B-mode ultrasound images, the development and implementation of novel completely automated techniques for the IMT measurement and plaque segmentation, and the validation and association of the automatically measured IMT value with clinical parameters. Chapter 3 focuses instead on the validation of the intima-media thickness variability parameter. Recent studies have shown that the IMT variation along the carotid artery wall has a stronger correlation with atherosclerosis than the nominal intima-media thickness value itself; hence this chapter presents an in-depth study and validation of the IMT variability (IMTV) parameter, confronting the question if manual segmentations of the lumen–intima and media–adventitia borders can be trusted as ground truth in the calculation of this parameter.

The second section, the fourth and fifth chapters, instead emphasizes quantitative imaging techniques for the assessment of architectural parameters of vasculature that can be extracted from 3D volumes, first using contrast-enhanced ultrasound (CEUS) imaging and, second, photoacoustic imaging without the administration of any contrast agent. More specifically, Chap. 4 demonstrates how the characterization and description of the vascular network of a cancer lesion in mouse models

can be effectively determined using both traditional microbubbles and liposomes. Eight mice were administered both microbubbles and liposomes and 3D CEUS volumes were acquired. Vascular architectural descriptors were calculated after a skeletonization technique was applied. Chapter 5 focuses on the development and validation of a skeletonization technique for the quantitative assessment of vascular architecture in burn wounds using completely non-invasive photoacoustic imaging, thus not requiring any contrast agent administration. It was shown how this technique could provide quantitative information about the vascular network from photoacoustic images that can distinguish healthy from diseased tissue.

A summarizing discussion (Conclusions and Final Remarks) concludes this work.

Turin, Italy Kristen M. Meiburger

Contents

Chapter 1
Introduction

Abstract Medical imaging has been forever revolutionized by the technological and digital boom that has occurred over the last few decades. The idea of quantitative analysis of medical images by a computer was first reported in the 1960s (Lodwick et al, Radiology, 80(2):273–275, 1963, [1], Meyers et al, Radiology 83(6):1029–1034, 1964, [2], Winsberg et al, Radiology 89(2):211–215, 1967, [3], Kruger et al, IEEE Trans Biomed Eng, 3:174–186, 1972, [4], Kruger et al, IEEE Trans Syst Man Cybern, 1:40–49, 1974, [5], Toriwaki et al, Comput Graph Image Process, 2(3):252–271, 1973, [6]), and at that time it was generally assumed that computers could replace medical practitioners in detecting abnormalities, because computers and machines are better at performing certain tasks than human beings are. However, growth of this sector remained initially quite limited due to the fact that computers were not sufficiently powerful, advanced image-processing techniques were not available, and digital images were not easily accessible (Doi, Comput Med Imaging Graph, 31(4–5):198–211, 2007, [7]). Since those days, along with the evolution of technology and digital imaging in general, the idea of actually *replacing* medical practitioners has also evolved, bringing forth the idea of Computer Aided Diagnosis (CAD), in which the computer output can by utilized by medical practitioners, but not replace them. This field, which is based on the idea that digital medical images are analyzed quantitatively by computers, has spread widely and quickly, becoming one of the major research subjects in medical imaging. Therefore, the development of advanced image processing techniques is required in order to obtain quantitative information (Doi, Comput Med Imaging Graph, 31(4–5):198–211, 2007, [7]).

1.1 Quantitative Imaging

Healthcare continues to seek improved quantitative medical imaging biomarkers with which to better diagnose, treat, and monitor the health of patients. In fact, quantitative imaging (QI) is becoming an increasingly common tool in modern medicine, advancing from research trials to clinical reading rooms [8].

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But what is quantitative imaging? According to the Quantitative Imaging Biomarkers Alliance (QIBA) [9]:

> Quantitative imaging is the extraction of quantifiable features from medical images for the assessment of normal conditions or the severity, degree of change, or status of a disease, injury, or chronic condition relative to normal. Quantitative imaging includes the development, standardization, and optimization of anatomical, functional, and molecular imaging acquisition protocols, data analyzes, display methods, and reporting structures. These features permit the validation of accurately and precisely obtained image-derived metrics with anatomically and physiologically relevant parameters, including treatment response and outcome, and the use of such metrics in research and patient care.

Quantitative imaging can theoretically be applied to any digital imaging modality, and currently has important clinical applications in ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), nuclear medicine, including positron emission tomography (PET), and more recently, photoacoustic (PA) imaging. Quantitative imaging is obviously enhanced by volumetric data sets, which facilitate assessments of morphological, functional, parametric, and other quantitative features [8]. The use of contrast agents and imaging over a period of time also enhances quantitative imaging possibilities, providing the enhancement of structures that normally produce low image contrast, and also allowing a comparison between pre- and post-contrast images and the assessment of the rate and pattern of enhancement or washout over time [8].

1.1.1 Ultrasound

Ultrasound imaging is a non-invasive imaging methodology which provides real-time information about tissue anatomy and function. It is based on the emission of ultrasound waves that interact with tissue and are reflected back to the probe and therefore detected. Image contrast in ultrasound imaging is based on the difference of adjacent tissue impedances, with a large difference of impedance producing a higher amplitude ultrasound wave that is reflected and therefore detected. This methodology is portable, real-time, inexpensive, and does not use ionizing radiation; however it suffers from being operator-dependent, it has a small field of view and is limited by the large impedance difference of bone and air compared to other soft tissues. There are various ultrasound imaging modes, but the prevalent ones are B-mode ultrasound imaging, Doppler imaging, and contrast-enhanced ultrasound imaging.

Gray-scale B-mode ultrasound images are commonly used to obtain size and distance measures, providing the basis for diagnosis in much of obstetric and cardiac imaging [8]. This modality is commonly used for the screening and evaluation of atherosclerosis, as the carotid artery wall is easily imaged with ultrasounds and permits the assessment of the artery wall thickness and/or plaque stenosis [10–15]. It is also often used for fast-look follow-up examinations. Doppler ultrasound has been used for quantitative characterization of vascular disease as well [16]. Contrast-enhanced ultrasound imaging is also widely used, mainly to enhance the vasculature

of structures. Studies have shown how this technique can distinguish malignant from benign tumors [17–19] and it has been used for perfusion studies of organs after transplantation [20].

1.1.2 CT

Computed tomography, which is used to refer to X-ray computed tomography, is a non-invasive imaging technique that provides information about internal organs, bones, soft tissue, and blood vessels. It combines a series of X-ray views taken from many different angles and computer processing to create cross-sectional images. It is a modality based on ionizing radiation (i.e., x-rays) that can produce adverse effects, but the modality has many benefits that can outweigh potential risks. CT images are formed by emitting x-ray photons from an x-ray tube which interact with the imaged area of the body, and then exit the patient and are detected. Contrast in CT depends mainly on tissue attenuation properties.

The standardization of the CT pixel value with the Hounsfield unit (HU) scale allows the characterization of tissue density [21]. HU measures allow lesion characterization using a region of interest (ROI)-based measurement of average density or voxel-counting based on a threshold value [22]. Dual-energy CT scanners have recently emerged, bolstering the clinical role of QI. Using this modality, improved characterization of tissue has been made possible thanks to the differential absorption of x-rays by tissues of differing chemical composition at various energies [23], and it has been used to determine the composition of renal calculi [24].

1.1.3 MRI

Magnetic resonance imaging (MRI) is a non-invasive imaging technique, which provides information about in-vivo tissue anatomy, function and metabolism. This modality does not rely on ionizing radiation, but it is quite expensive. MRI makes use of hydrogen atoms predominantly originating from tissue water to generate images. By manipulating the magnetic moments of the hydrogren nuclei with radio frequency (RF) fields, images can be produced [8]. Contrast in MRI depends mainly on three parameters: proton spin density (PD), the longitudinal relaxation time (T_1), and the transverse relaxation times (T_2 and T_2*).

MR signal intensity units lack inherent meaning, being influenced by sequence parameters as well as hardware and software selection. However, some advanced MRI sequences and postprocessing techniques allow for the computation of parametric maps in which the pixel values are used for diagnosis. Some examples are: calculation of the T_2* relaxation time which is used in liver imaging as a marker of the presence and severity of hepatic iron deposition [25]; Diffusion weighted (DW) MRI for the apparent diffusion coefficient (ADC) of tissue [26]; MRI spectroscopy for the

extraction of information regarding the presence and concentration of chemicals in a Region-Of-Interest (ROI) [27].

1.1.4 Nuclear Medicine

Nuclear medicine, and Positron emission tomography (PET) [28] in particular, is a functional imaging technique that produces a three-dimensional image of functional processes in the body. The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. Three-dimensional images of tracer concentration within the body are then constructed by computer analysis. This modality requires the administration of an ionizing contrast agent, yet it still remains a powerful tool for evaluating tissue metabolism.

PET imaging allows the calculation of the standardized uptake value (SUV) which represents the concentration of radioactivity within a tissue, normalized by dividing it by the ratio between the decay-corrected injected radioactivity and that patient's body weight, lean body mass, or surface area [8, 29, 30]. This parameter is used to determine the likelihood of malignancy of a lesion and to predict tumor aggressiveness and treatment response. Other PET-based parameters that are being applied in clinical decision-making include the metabolic tumor volume, total lesion glycolosis, and heterogeneity index [8].

1.1.5 Photoacoustics

Photoacoustic imaging is a relatively new imaging modality that can provide information about in-vivo tissue composition, function and metabolism. It is based on the illumination of tissue with a pulsed laser light, usually in the near infrared (NIR) spectrum, that is absorbed by the tissue which undergoes a rapid conversion to heat which produces a small temperature rise. This effect results in the emission of broadband low-amplitude acoustic waves which can be detected by an ultrasound receiver and processed to form an image. Contrast is based on tissue absorption properties at different wavelengths. It is often coupled with ultrasounds to provide the anatomical structure image.

The spectroscopic properties of oxygenated haemoglobin and deoxygenated haemoglobin provide a natural endogenous contrast agent for this technique, but still many exogeneous contrast agents exist and are undergoing constant evolution. Some photoacoustic contrast agents that are used are methylene blue, ICG, and gold nanoparticles, to mention a few [31–35]. This imaging modality, especially with the application of contrast agents, has a strong presence in preclinical imaging [34–37], but it is quickly making its way to clinical studies, as various research groups have recently used this modality for the evaluation of breast cancer [38–40].

As can be noted from the previous paragraphs, there are many different modalities that can be taken advantage of for quantitative imaging. However, both nuclear medicine and CT imaging modalities require the use of ionizing radiation, which should, if possible, be avoided. MRI does not require ionizing radiation, but the exam is far from being portable and is expensive. Ultrasound imaging, on the other hand, thanks to its non-invasive nature, low cost, broad diagnostic applicability and easy handling, is the second-most used imaging modality in clinical practice after conventional x-ray radiography [41, 42]. Photoacoustic imaging, which is undergoing an exponential growth in research, also does not use ionizing radiation and can provide important functional information, thanks to the spectroscopic properties of this imaging modality. For these reasons, the work in this final work focused on the development of quantitative imaging techniques for ultrasound and photoacoustic imaging, two fundamental biomedical imaging modalities that are explained in more detail in Sects. 1.2 and 1.3.

1.2 Ultrasounds

Ultrasounds, as the name itself already implies, are sound waves that oscillate at a frequency which is greater than the upper limit of human hearing ($f > 20\,\text{kHz}$). Since ultrasounds are mechanical waves of compression and rarefaction, the only type of energy that they transfer to biological tissues is mechanical, which stimulates the tissue molecules. The propagation speed of ultrasounds varies depending on the elasticity of the material and has the following relation with the sound cycle frequency:

$$v = \lambda f \tag{1.1}$$

where v is the propagation speed, f is the frequency, and λ is the wavelength.

Another important parameter that can be calculated for various materials is the acoustic impedance (Z) by the following equation:

$$Z = \rho v \tag{1.2}$$

where v is the propagation speed and ρ is the medium density.

As Table 1.1 shows, many tissues and organs that are quite different from one another can still show similar density and acoustic impedance values. It can also be observed that most biological tissues have propagation speeds that are close in value, so generally a typical value of 1540 m/s is assumed.

Ultrasounds are generated due to the piezoelectric effect. When an electrical impulse is applied to a piezoelectric element, it vibrates and produces the ultrasound. Vice versa, when a piezoelectric element is vibrated, it produces a pulse of electricity. An ultrasound transducer contains on its surface an array of piezoelectric crystals, which each produce an ultrasound wave. The summation of all of the generated waves forms the ultrasound beam. Ultrasound waves are generated in pulses and each pulse

Table 1.1 Tissue and organ characteristics

Material	Density [kg/m^3]	Propagation speed [m/s]	Acoustic impedance [kg/m^2/s $\cdot 10^{-6}$]
Air	1.2	330	0.0004
Water	1000	1480	1.48
Soft tissue (mean)	1060	1540	1.63
Liver	1060	1550	1.64
Muscle	1080	1580	1.70
Fat	952	1459	1.38
Brain	994	1560	1.55
Kidney	1038	1560	1.62
Lung	400	650	0.26
Bone	1912	4080	7.80

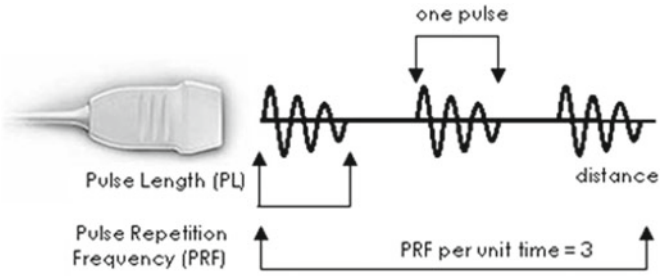

Fig. 1.1 Ultrasound pulse parameters. Image available from: http://www.usra.ca/

generally consists of 2 or 3 sound cycles of the same frequency. The pulse length (PL) is the distance travelled per pulse, while the pulse repetition frequency (PRF) is the rate of pulses emitted by the transducer (number of pulses per unit time). These parameters can be seen in Fig. 1.1.

As an ultrasound wave propagates through matter, it interacts in various ways. First of all, the ultrasound pulse continuously loses energy. The *attenuation* that it undergoes is defined by the following equation:

$$A(z) = A_0 \mathrm{e}^{-\alpha z} \tag{1.3}$$

where $A(z)$ is the amplitude of the pulse at the depth z, A_0 is the initial amplitude of the pulse, and α is the absorption coefficient of the matter which increases the higher the frequency is. Furthermore, the attenuation (I) of the ultrasound wave (in dB) as it passes from one medium to another can be defined as follows:

$$I = 20 \cdot \log_{10} \frac{A_2}{A_1} \tag{1.4}$$

When an ultrasound pulse meets an interface between two different materials, the pulse is not only attenuated as described above, but it also undergoes two interactions of fundamental importance for ultrasound imaging: *reflection* and *transmission*. These two phenomena are generally expressed through the reflection coefficient R, and the penetration coefficient T:

$$R = \left(\frac{Z_1 - Z_2}{Z_1 + Z_2}\right)^2 \tag{1.5}$$

$$T = 1 - R \tag{1.6}$$

So, if the two materials present similar values of acoustic impedance, very little of the pulse is reflected. On the other hand, if the two materials have very dissimilar values of acoustic impedance (as what happens if one of the materials is air), almost all of the pulse energy is reflected and, therefore, does not penetrate into the tissue which is found below.

1.2.1 Ultrasound Imaging

Ultrasound imaging is based on the fact that the ultrasound pulse is partly reflected when it meets an interface between two materials with different acoustic impedance. The same transducer that emits the ultrasound pulse also receives the reflected echoes of the pulse, and the depth at which the echo is produced (d) can be found by measuring the time required for the pulse to travel to the reflecting site and for the echo pulse to return (Δt):

$$d = \frac{1}{2} c \Delta t \tag{1.7}$$

where c is equal to the average propagation speed in biological tissues (1540 m/s).

So, by emitting a sequence of ultrasound pulses along different scansion lines and by listening to the return echoes produced (Fig. 1.2), an image that shows the spatial distribution of the discontinuities of the irradiated tissues can be generated.

The most common visualization strategy that is used is B-mode, which is based on brightness. In B-mode ultrasound images, a certain pixel of the image is brighter if the amplitude of the return echo in that point is greater. So, strong specular reflections give rise to bright pixels (*hyperechoic*), weaker diffuse reflections produce gray pixels (*hypoechoic*), and no reflection produces dark pixels (*anechoic*).

The bi-dimensional image exhibits along the horizontal axis the width extension of the tissue that is irradiated, while the vertical axis reveals the depth of the pulse that generated the return echo.

A fundamental parameter in all imaging techniques is *spatial resolution*. Axial spatial resolution depends on the wavelength of the sound cycle that is emitted. In ultrasound imaging, however, the emission of an ultrasound pulse consisting of only one sound cycle is hardly ever used, as discussed previously. In order to have a

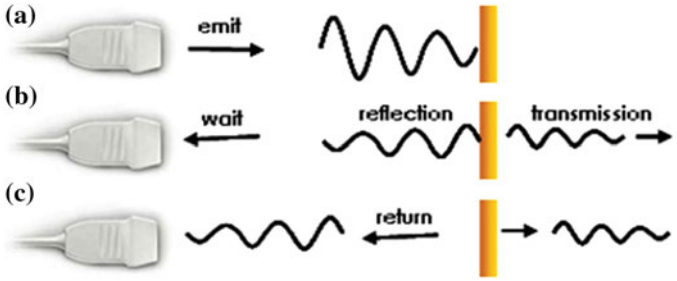

Fig. 1.2 Example of emission of ultrasound pulses and waiting for return echoes. Image available from: http://www.usra.ca/

narrow bandwidth of frequencies with a minimum power loss, an ultrasound pulse consisting of two or three sound cycles is emitted. In this way, the axial spatial resolution depends not on the wavelength of the sound cycle but on the pulse length. If the distance between two points is greater than the pulse length, then the points will be seen as two distinct objects.

With these considerations, it can be observed that the higher the frequency is (therefore, the shorter the wavelength is, considering the same propagation speed), the better the axial spatial resolution. High frequencies are, therefore, optimal for a clearer image, but there is the disadvantage that tissues absorb more energy at higher frequencies, putting a limit on the possible scansion depth [43].

1.2.2 Contrast Enhanced Ultrasound Imaging (CEUS)

CEUS is an enhanced form of ultrasound scan that uses intravenous administration of a contrast agent, typically microbubbles. This contrast agent is an intraluminal tracer and can be used to obtain angiography-like images of vasculature. Ultrasound scan contrast agents were introduced in clinical practice in the early 1990s. The currently approved and used agents include SonoVue (Bracco SpA, Milan, Italy), Optison (GE Healthcare, Princeton, NJ), Definity (Lantheus Medical Imaging, N. Billerica, Mass), and Levovist (Schering AG, Berlin, Germany). The contrast agents consist of microbubbles (approximately 1–8 μm), generally filled with a perfluorinated gas that has a low solubility, and stabilized with a phospholipid or protein shell to improve circulation time [44]. Because of the size of microbubbles, they cannot leave the intravascular compartment, or in other words, they cannot extravasate. The microbubble shell is eliminated from the body through the reticuloendothelial system when the gas is exhaled. Contraindications for microbubble contrast agents are unstable angina, acute cardiac failure, acute endocarditis, known right-to-left shunts, and known allergy for microbubble contrast agents. Microbubble contrast agents have been administered in millions of patients and are safe; side effects are extremely rare [45].

For clinical applications, microbubble contrast agents have been registered for tissue perfusion imaging and cardiac chamber border enhancement [44]. Most commercially available ultrasound systems provide specific pulse sequences, such as amplitude modulation or pulse inversion, that retrieve nonlinearities at low acoustic power, which are only exhibited by microbubbles [46]. These techniques enable the suppression of tissue in the image and allow the specific identification of the microbubbles signal.

1.3 Photoacoustics

Photoacoustic (PA) imaging, also known as optoacoustic imaging, is a biomedical imaging modality that has seen exponential growth in the last couple of decades [47]. This hybrid modality, which is based on the use of laser-generated ultrasound, combines spectroscopic-based specificity of optical imaging with the high spatial resolution of ultrasound imaging.

The discovery of the photoacoustic effect dates back to 1880 when Alexander Graham Bell first observed the generation of sound owing to the absorption of modulated sunlight [48]. Despite the early discovery of this physical phenomenon, very little active scientific research or technological development took place, mainly due to the lack of an adequate light source. In fact, it was only with the development of the laser in the 1960s that sensing applications began to be investigated, since the laser source finally provided the necessary high peak power, spectral purity and directionality that PA sensing applications require [47].

Still, even after the development of the laser, applications were first more geared towards the exploitation of the indirect gas-phase cell type of PA detection, and the first uses of the direct detection of laser-induced ultrasound waves were focused on characterizing solids as a potential non-destructive testing tool. The first investigations of using the photoacoustic effect for biomedical imaging were only in the mid 1990s and the first biomedical images appeared soon thereafter [36, 49–51]. With these first images, the field of photoacoustic imaging continued to progress steadily but really only began to see its exponential growth once the first truly compelling *in-vivo* images began to be obtained in the early to mid 2000s [47].

1.3.1 The Photoacoustic Effect

As already mentioned, the photoacoustic effect is based on laser-generated ultrasound. As can be seen in Fig. 1.3, there is first an optical path, in which a pulsed light source irradiates the tissue which absorbs the light. The absorption by tissue chromophores is then followed by a rapid conversion to heat which produces a small temperature rise. This effect leads to the acoustic path, since there is an initial pressure increase which then relaxes, resulting in the emission of broadband low-amplitude

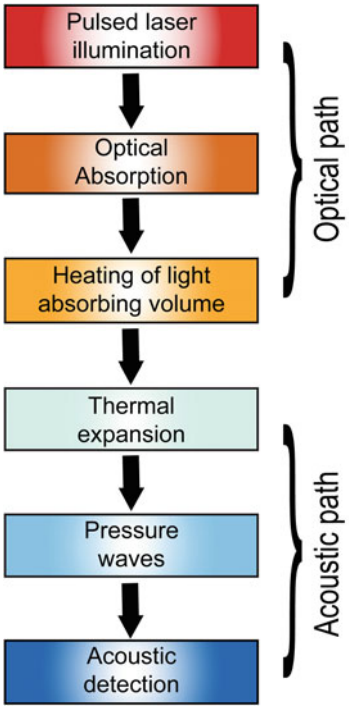

Fig. 1.3 The photoacoustic effect

acoustic waves which can be detected by an ultrasound receiver and processed to form an image [52–54].

So, the mechanism of signal generation can be described as one in which the optically induced initial pressure distribution, called p_0, is encoded onto a propagating acoustic wave which is then converted to a time-resolved electrical signal upon detection by the ultrasound receiver. From this explanation, it is clear that the resulting photoacoustic image is therefore a representation of p_0. In order to generate acoustic waves, the thermal expansion needs to be time-variant [55], which therefore requires that the acoustic propagation time is small compared with the length scale of the heated volume [47]. A typical laser pulse duration in photoacoustic imaging is less than $10ns$, which respects both the thermal and stress confinement times, which are explained in more detail in [55]. In this case, with simple thermodynamic considerations and assuming 1D plane wave propagation in a homogeneous medium, it can be shown that p_0 at a certain point z is proportional to the absorbed optical energy $H(z)$, as shown in Eq. 1.8.

$$p_0(z) = \frac{\beta {v_s}^2}{C_p} H(z) \tag{1.8}$$