Ultrasonic Characterization of Human Coronary Arteries and Atherosclerotic Plaques

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ULTRASONIC CHARACTERIZATION OF HUMAN CORONARY ARTERIES AND ATHEROSCLEROTIC PLAQUES

by

Joseph James Hoffman

A dissertation presented to the Graduate School of Arts and Sciences of Washington University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Abstract

The development, execution, and interpretation of studies investigating the physics of the interaction of ultrasound with normal and pathological coronary artery tissues are described in this dissertation. Ultrasound is a modality capable of visualizing and characterizing the lesions that define atherosclerosis. A better understanding of the physics underlying the mechanisms by which ultrasound interacts with arterial tissue and plaques may provide benefit to patients with coronary artery disease. The two-fold goal of the studies presented in this thesis was to better understand the fundamental physics of the relationship between ultrasound and coronary artery tissue and to use this knowledge to contribute to the advancement of techniques capable of improving the diagnosis and management of atherosclerosis. The work presented in this dissertation appears to represent the most comprehensive study of the fundamental ultrasonic properties of human coronary arteries to date.

An acoustic microscopy system was developed, refined, and tested for the purposes of acquiring data from fresh (that is, not chemically fixed) coronary artery tissues and atherosclerotic plaques. Novel methods of preparing samples, imaging samples, and collecting data from samples were validated in studies of lamb tissue.
A very large data set spanning the ultrasonic bandwidth of current and near-future intravascular ultrasound (22 to 105 MHz) was acquired from human coronary artery tissues insonified in two orthogonal orientations. These data were analyzed to yield measurements of the apparent integrated backscatter from coronary arteries and atherosclerotic plaques. Studies of the radial apparent backscatter carried out in the acoustic microscope confirmed trends observed in clinical radial intravascular ultrasound; in contrast, acoustic microscopy studies of the axial apparent backscatter identified a substantially different trend. Specifically, these studies revealed that the anisotropy of apparent integrated backscatter of the media and adventitia is modest, but that the anisotropy of the apparent integrated backscatter from atherosclerotic plaque is quite substantial.

The attenuation coefficient of coronary artery tissues and atherosclerotic plaques was measured across the wide bandwidth 22 to 105 MHz. It was shown that, in the axial orientation, the attenuation coefficient is lower in the media layer than in the intima/plaque or adventitia layers. The measured axial backscatter coefficient of coronary artery media was similar to that of coronary artery intima/plaque, and smaller than that of coronary artery adventitia.

The results derived from these studies may serve to advance the understanding of the physics underlying ultrasound’s interaction with coronary artery tissues. The data may provide insights for improved clinical devices and methods, as well as serve as the basis for future measurements of the physical properties of coronary artery tissues and atherosclerosis.
Acknowledgments

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CHAPTER 1

INTRODUCTION

1.1 Background and Motivation

The broad theme of this dissertation is the investigation of the physics underlying the interactions of ultrasound with normal and pathological tissues found in the coronary arteries. These physical interactions are the primary determinant of the applicability and utility of ultrasound in the diagnosis and clinical management of persons with coronary artery disease. Furthermore, because the parameters accessible to ultrasound are directly related to the mechanical and geometrical properties of the structures interrogated, knowledge of the ultrasonic physics within coronary artery tissues might permit improved understanding of their composition and structure.

In addition to advancing the understanding of the fundamental interactions between ultrasound and the heterogeneous structures of the coronary artery, this work is
motivated by a desire to advance the understanding and treatment of atherosclerosis, currently the most deadly and expensive condition in the United States (Lloyd-Jones et al., 2009). It is hoped that the results of the studies in this thesis may play some role in the development of future devices and algorithms that improve the imaging and characterization of plaques, and thereby improve the clinical outcome of patients with atherosclerosis.

Ultrasound has been a successful modality for imaging the coronary arteries because sound with frequencies high enough to resolve the pertinent structures is capable of penetrating both the flowing blood and the superficial layers of the arteries and any plaques present. Intravascular ultrasound (IVUS) imaging scans are currently being used in the management of some patients with coronary atherosclerosis, and some fraction of these IVUS scans also include an ultrasonic tissue characterization component. The tissue characterization methods are based on extracting spectral parameters from the received ultrasonic signals and identifying features within this parameter set that correspond with specific, clinically relevant subtypes within atherosclerotic plaques. Improvement of the success of these procedures depends, in part, on improved knowledge of the fundamental properties dictating the interaction of ultrasound with coronary artery tissues and plaques.

The work of this thesis attempts to make measurements of the ultrasonic properties of coronary artery tissue over the bandwidths appropriate to current and near-future intravascular ultrasound.
1.2 Overview of the Dissertation

The dissertation is structured in such a way that each chapter represents a self-contained study or series of studies, but that later chapters build upon methods and results developed and reported in earlier chapters.

Chapter 2 provides background on the coronary arteries and a detailed description of the clinical relevance and pathophysiology of atherosclerosis. The current state of intravascular ultrasound is discussed, as well as the strengths and limitations of intravascular ultrasound tissue characterization methods. Potential effects of the anisotropy inherent in coronary artery tissue are described.

The next three chapters explain the equipment, techniques, and procedures used to gather reliable data from fresh coronary arteries. Chapter 3 describes the acoustic microscopy system, as well as its characterization and customization for the scans of this thesis.

The methodological development continues in Chapter 4, which focuses on studies performed on fresh (not chemically fixed) coronary arteries of sheep. These animal studies were conducted to refine and validate methods of acquiring data in the challenging context of fresh arterial tissue.

Chapter 5 presents a detailed description of the methods used to prepare human coronary artery segments for scanning by acoustic microscopy, and the procedures that were developed for acquiring useful data from these samples.

The next three chapters describe the ways in which the acquired data were used
to generate results regarding coronary arteries and intravascular imaging and characterization. Chapter 6 looks at the ultrasonic anisotropy of coronary artery tissue using apparent integrated backscatter as a parameter to compare backscatter data acquired in specific experimental orientations.

The attenuation of ultrasonic energy in coronary arteries and plaques is the topic of Chapter 7. The attenuation coefficient measured in these studies is an important parameter for understanding the fundamental interaction of ultrasound with the structures of coronary arteries, and is also a critical component for the reduction of the apparent backscatter to the backscatter coefficient. The process of removing the effects of attenuation and diffraction from the apparent backscatter to yield the backscatter coefficient is carried out in the studies of Chapter 8.

Conclusions and final remarks are offered in Chapter 9.
Bibliography

Chapter 2

Background

2.1 Introduction

This chapter presents background material that motivates, explains, and puts into context the studies presented in this thesis. It describes the current state of knowledge as it pertains to the work of this dissertation and indicates how the studies undertaken were designed to advance the field.

2.2 Coronary Arteries

The studies of this thesis were carried out on the coronary arteries of sheep and humans. All arteries carry blood away from the heart, and most arteries transport oxygenated blood to parts of the body where it deposits oxygen and takes up waste
products. The coronary arteries transport the blood that fuels and maintains the myocardium, the muscular component of the heart. Without adequate perfusion from the coronary arteries, the heart is at risk, and is likely to be unable to function adequately. All studies were carried out on left coronary arteries, and specifically on the anterior descending branch of the left coronary artery.

### 2.2.1 Structure of Coronary Arteries

The coronary arteries are muscular (as opposed to elastic) arteries containing three distinct layers (Barry et al., 2003; Khan et al., 2006). Starting from the lumen, the area through which the blood flows, and moving outward, one identifies the layers of the coronary artery as the tunica intima, the tunica media, and the tunica adventitia. These layers are illustrated in the graphic of Figure 5.1. Commonly, the “tunica” part of the names is omitted and the layers are simply called the intima, media, and adventitia. The intima is the innermost layer and is typically composed of a thin layer of endothelial cells bordering the lumen, followed by a fibroelastic subendothelial layer, and then by an elastic membrane called the internal elastic lamina, which separates the intima from the next layer, the media. In the absence of pathology, the intima can be as thin as several cell layers. The media is the middle layer and is composed primarily of smooth muscle cells and structural proteins. It begins just outside the internal elastic lamina and ends on the inside at the external elastic lamina. The outer layer is the adventitia, which is a loose fibrous outer coat derived from connective tissues that surround the artery (Stedman, 1976).
2.3 Cardiovascular Disease and Atherosclerosis

2.3.1 Prevalence

According to the American Heart Association’s 2009 statistical update (Lloyd-Jones et al., 2009), cardiovascular disease is the leading cause of mortality in the United States, and was responsible for approximately 34% of all deaths in the 2006. Coronary heart disease, or atherosclerosis, is the most common cause of cardiovascular disease and accounted for approximately 1 out of every 5 deaths in the United States in 2005. Atherosclerosis places significant strain on the healthcare system and the national economy. In 2004, atherosclerosis was the cause for 1.2 million hospital stays and costs totaling $44 billion, making it the nation’s most expensive condition.

2.3.2 Pathogenesis

The current knowledge of the pathogenesis of atherosclerosis is reviewed by Glass and Witztum (2001), which is summarized here. Coronary atherosclerosis develops initially when low-density lipoprotein (LDL) cholesterol, the so-called “bad” cholesterol, accumulates in the intima inside of the endothelial layer. These low-density lipoprotein particles become oxidized within the arterial wall, resulting in inflammation. The presence of inflammation triggers the recruitment of monocytes, a type of white blood cell circulating in the blood, to the area. The monocytes are initially beneficial to the artery because they differentiate into macrophages that consume the inflammation-causing low-density lipoprotein particles. As the macrophages con-
2.3 Cardiovascular Disease and Atherosclerosis

tinue to take up more cholesterol, they become “foam cells”, which are a hallmark of atherosclerosis. The pathology arises because macrophages and foam cells cannot get rid of their acquired cholesterol at great enough rate, a process thought to be controlled by circulating high-density lipoprotein (HDL). The accumulation of these macrophage foam cells triggers additional inflammation that causes the problem to worsen.

More complex atherosclerotic lesions are created when this inflammation causes smooth muscle cells to migrate across the external elastic lamina and into the intima. Once inside, they interact with the cholesterol, foam cells, other components to generate more inflammation, triggering further lesion progression. The smooth muscle cells are also responsible for depositing the structural proteins that become the fibrous component of atherosclerotic plaque.

Although the mechanism is not well understood, calcium is also deposited in the lipidic regions of atherosclerotic plaque (Wexler et al., 1996). It is thought that the calcification process may, physiologically, serve a defensive purpose, either by stabilizing the fiber structure or by creating barriers that slow the spread of the atherosclerotic lesion (Frink, 2002).

2.3.3 Ischemia, Infarction, and Vulnerable Plaque

Development of substantial atherosclerotic plaque burden can lead to significant narrowing of the coronary arteries, which can cause ischemia (low blood flow rate). However, the prevailing view is that the vast majority of myocardial infarctions (heart
attacks) caused by atherosclerosis are the result of a coronary thrombosis (blood clot) triggered by exposure of the circulating blood to the internal components of the atherosclerotic lesion, rather than as a result of progressive narrowing (Falk, 1992; Davies et al., 1993; MacIsaac et al., 1993; Lee and Libby, 1997; Naghavi et al., 2003). The plaque surface can be breached either by a rupture of a thin fibrous cap over a lipidic inner core, or by erosion of the endothelial surface (Lee and Libby, 1997).

One goal of the management of coronary artery disease is the prevention of myocardial infarctions. Because plaque rupture appears to be the primary cause of these events, successful treatment might involve identification and management of atherosclerotic plaques vulnerable to rupture. There are several types of vulnerable plaques, with each exhibiting different risks, progression, and treatment options (Naghavi et al., 2003). Providing the clinician with tools to assess and differentiate plaques may provide improved diagnosis and treatment of patients with coronary heart disease.

2.4 Intravascular Ultrasound

Intravascular ultrasound (IVUS) is one of the modalities that has shown potential for assessing atherosclerotic plaques, and specifically vulnerable plaques (Nissen, 2001; Naghavi et al., 2003). Even in the presence of flowing blood, intravascular ultrasound serves as a diagnostic tool capable of visualizing all layers of the arteries as well as the associated atherosclerotic plaques at resolutions sufficient for observing and
characterizing substructures (Crouse, 2006).

2.4.1 Coronary Angiography

The current standard of care when a patient undergoes cardiac catheterization is imaging by coronary angiography. Because this technique visualizes dye that is injected into the coronary arteries, only a silhouette of the lumen appears in the image. This information can provide an estimate of the extent of coronary artery stenosis, but does not give further information about the type or character of the plaque. Additionally, plaques that grow into the lumen (negative remodeling) and therefore cause narrowing, are thought to be of less risk than plaques that cause the artery to grow outward (positive remodeling) (Naghavi et al., 2003). Positive remodeling, by definition, does not narrow the artery, and therefore cannot be detected with angiography.

2.4.2 Visualizing Atherosclerosis

Not only does intravascular ultrasound provides information about lumen size, but because ultrasound penetrates into the underlying layers, information from within the plaque can be applied in the diagnostic process. Creating images of the layers beyond the intima and plaque may provide valuable additional knowledge. In atherosclerosis, cells from the media migrate into the intima, which aggravates the atherosclerotic lesion, and can also cause the breakdown of the internal elastic lamina and subsequent deterioration of the media. Ultrasonic monitoring of this process may be useful. The
adventitia presents an exciting target for future IVUS imaging and tissue characterization because the vasa vasorum and neovascularization originating in the adventitia are thought to play a central role in the development and progression of atherosclerosis (Ritman and Lerman, 2007). Intravascular ultrasound, specifically when used with an ultrasonic contrast agent, has shown promise in identifying the effects originating in these adventitial structures (Carlier et al., 2005; Vavuranakis et al., 2007).

2.5 Plaque Characterization

Intravascular ultrasound imaging is capable of creating images demonstrating coronary artery tissues and atherosclerotic plaques. The information used to create the image is only a fraction of the total information contained in the received signals, as is the case in most clinical ultrasonic evaluations. The envelope of the signal is often used to create a grayscale image, but the other characteristics of the received radiofrequency signal are also influenced by the sound beam’s interaction with the tissue.

Efforts to use the additional information contained in the radiofrequency signal have been particularly fruitful in the field of intravascular ultrasound. Studies applying these radiofrequency techniques to the aortae of monkeys (Jeremias et al., 1999) and the coronary arteries of humans (Komiyama et al., 2000) showed that including the RF-based information improved categorization over grayscale images alone. Kawasaki et al. (2002) demonstrated that by combining integrated backscatter (see
Chapter 6) with IVUS imaging, plaque subtypes could be identified. Other methods based on elastography (de Korte et al., 2002), wavelet analysis (Katouzian et al., 2008a), and spectral similarity (Sathyanarayana et al., 2009) have also been described.

2.5.1 Clinically Available Algorithms

Of the several intravascular ultrasound tissue characterization techniques that have been introduced, the one that is most pertinent to the work described in this thesis was introduced by Nair et al. (2001, 2002), and subsequently refined by the same group (Nair et al., 2004, 2007). This algorithm, commonly called Virtual Histology (VH), is notable for being the only radiofrequency-based tissue characterization routine currently approved by the United States Food and Drug Administration for use in patients. Rights to this proprietary method are owned by Volcano Corporation, and the commercial product is marketed under the name “VH”. Virtual histology is a machine-learning algorithm that attempts to identify tissue types by evaluating several spectral-based parameters of the received ultrasound signals based on experience acquired by comparison with data from training sets with known properties. This method has been shown to have high accuracy in matching features seen in histology (Nasu et al., 2006), but has also been shown to have some limitations and drawbacks (Katouzian et al., 2008b; Thim et al., 2010). Members of the group who developed and currently support this routine were collaborators in the studies reported in this thesis.

Outside of the United States competing technologies marketed as Integrated Back-
scatter IVUS (IB-IVUS) (Kawasaki et al., 2002) and iMap (Sathyanarayana et al., 2009), are available in addition to Virtual Histology.

2.6 Ultrasonic Characterization of Arteries

The clinically available radiofrequency-based techniques for plaque characterization are fundamentally phenomenological in nature. The assignment of regions into subtypes is based on experience from studies of previously investigated plaques. Although this method has been shown to be successful (Nasu et al., 2006; Kawasaki et al., 2002), the characteristics of the received ultrasound signals are tightly linked to the properties of the training samples and to the measurement system used to acquire the data. These features limit the utility and scalability of these algorithms. In the case of Virtual Histology, because the algorithm is only aware of a finite number of tissue subtypes, it must assign everything it sees to one of these categories. As a result, the complexity and variability inherent in tissue cannot be adequately characterized. Furthermore, introducing features not accounted for in the training, such as metal stents, can cause the algorithm to produce incorrect assignments (Thim et al., 2010). The training set results include features linked to the measurement system’s characteristics; therefore, when new instruments become available, the training sets must be reacquired, a laborious process that requires many studies on human coronary arteries.

Improvement of current methods and development of future techniques may be
2.6 Ultrasonic Characterization of Arteries

aided by knowledge of the fundamental ultrasonic properties of coronary artery tissues and atherosclerotic plaques. Previous studies of coronary arteries and peripheral arteries have attempted to address aspects of this challenge.

An early study by Rooney et al. (1982) carried out measurements of the speed of sound and attenuation in human and canine aortae. A study from our laboratory by Barzilai et al. (1987) demonstrated the feasibility of performing ultrasonic tissue characterization in atherosclerotic plaques by investigating excised human aortae. Lockwood et al. (1991) measured the ultrasonic backscatter and attenuation coefficient of femoral and iliac arteries in the radial and axial orientations (see Section 5.2). Studies in human iliac arteries (de Kroon et al., 1991a,b) showed the angle dependence of backscatter from constituent layers and plaques over a modest range of angles varying from perpendicular to the surface. Fraser et al. (2006) reported the speed of sound and attenuation coefficient of sheep aorta as measured from a pulse-echo method. The attenuation coefficient of human carotid arteries was measured by Shi et al. (2008).

More recent studies in excised human coronary arteries have measured the integrated backscatter coefficient (Machado and Foster, 2001; Machado et al., 2002), the speed of sound (Saijo et al., 2007), and the temperature dependent speed of sound (Pereira et al., 2003).

Bridal et al. (1997a,b) made estimates of fundamental ultrasonic properties from the more limited case when only backscatter data are available. These studies reported values for the attenuation coefficient of human aorta as measured from
backscattered ultrasound data.

The findings of many of the above studies, as well as studies reporting mechanical properties of coronary artery tissue and results from studies of the ultrasonic properties of blood, are reviewed by Hoskins (2007).

2.6.1 Clinical Application

One difficulty in applying the previously published results to clinical diagnosis and treatment of human subjects is that the diversity of experimental conditions compromises the interpretation of the results. Measurements made in animals may give insight to human conditions, but questions remain as to how directly results from animal models can be applied to man, given the known variations among the anatomy of different animals (Khan et al., 2006). Furthermore, the differences inherent within the human arterial system (Wickline et al., 1994; Barry et al., 2003) suggest that care must be taken in applying to coronary arteries knowledge gained from other human arteries. Studies undertaken using only human coronary arteries also require careful interpretation, because a significant amount of variation of structure and size is seen among the coronary arteries of the normal human population (Waller, 1989; Waller et al., 1992).

The studies described above were also subject to differing experimental conditions. The frequency of ultrasound used to make the measurements impacts the nature of the measurement made and the results generated (see Sections 3.1 and 6.5). Extrapolating outside the bandwidth of the experiment may provide general guidance, but is
unlikely to produce reliable values. Ultrasound measurements are typically sensitive to temperature. Many of the studies above were carried out at room temperature, although some were executed at human body temperature.

2.7 Angular Dependence

2.7.1 Coronary Artery Anisotropy

Because the ultrasonic properties of a material are dependent upon its elastic and geometric constitution, measurements of any material exhibiting anisotropy in either elastic or geometric properties will yield different results depending on the specific orientation of sound beam relative to the sample’s structure. Coronary arteries, because of their anatomical anisotropy, have been shown to exhibit some anisotropy in their ultrasonic properties. Picano et al. (1985) demonstrated in human aorta that the backscatter decreased as the angle of insonification moved from normal to the arterial wall to up to 30 degrees off axis, and also that the effect varied with plaque type in the wall. Similar results were also reported by de Kroon et al. (1991a,b) in muscular and elastic human iliac arteries. Nguyen et al. (2002) measured the ultrasonic properties of canine aortae in three orthogonal orientations. A study by Lockwood et al. (1991) measured human femoral and carotid arteries in the radial and axial directions, making it the previous work most similar to some studies in this dissertation. The results of the study by Lockwood et al. (1991) are compared to previous work and discussed in Section 6.6.3. Hiro et al. (1999) observed an angular
dependence in the circumferential direction by studying formalin-fixed human iliac arteries with an intravascular ultrasound catheter placed at different locations within the lumen.

### 2.7.2 Forward-looking Intravascular Ultrasound

Imaging of the coronary arteries with currently available intravascular ultrasound is performed in a side-looking configuration, in which the beam is projected radially out from the catheter. This type of configuration requires the catheter to pass any sites of interest in order to position the imaging device adjacent to the vessel wall. In cases in which the artery of interest is occluded, imaging becomes impossible. Currently, front looking catheters are under development with the aim of addressing this problem (Back et al., 1994; Ng et al., 1994; Evans et al., 1994; Degertekin et al., 2006; Gatzoulis et al., 2001; Courtney et al., 2008). As these devices become clinically available, a more complete understanding of the fundamental interactions between ultrasound and coronary artery tissue and plaques might aid in the interpretation of the images produced by forward-looking IVUS catheters. Views of the atherosclerotic plaques from angles other than the radial may also provide new avenues for plaque identification and quantification.
2.8 Summary

Ideally, measurements for application to human intravascular ultrasound would be carried out with as many features as possible in common with the clinical setting. In practice, pragmatic approximations are made to satisfy a number of limitations. The studies of this dissertation are also subject to such limitations, but do represent a significant advancement towards the goal of measuring the fundamental ultrasonic properties of coronary artery tissues, and generating results applicable to human clinical imaging. As is discussed in detail later, the studies presented below were performed in fresh human coronary arteries in two orthogonal configurations over an ultrasonic bandwidth that covers all currently available clinical intravascular ultrasound systems and probably the next generation of higher frequency systems. We believe that the breadth of the acquired data and the subsequently generated results provides the most comprehensive study of the fundamental ultrasonic properties of human coronary arteries to date.
Bibliography


Chapter 3

Equipment and Preliminary Studies

3.1 Background

3.1.1 Acoustic Microscopy

The resolution of an ultrasonic imaging system is related to the frequency of the propagating ultrasonic waves. As the frequency increases, the wavelength decreases, which permits the beam to interrogate a smaller spacial region. Therefore, working at higher frequencies provides better quality images. However, the downside of using a higher frequency sound wave comes in the form of attenuation that rises (linearly in tissue) with frequency. This attenuation causes the tradeoff to be between resolution and penetration depth. Clinical ultrasound systems designed to image a particular
organ or body part have fixed penetration depth requirement based on the location of the structure of interest. These instruments, therefore, are built to use the highest frequency ultrasound that will penetrate to the given depth while maintaining adequate signal-to-noise ratio.

The acoustic microscope takes the opposite approach. In acoustic microscopy, one generally has a substructure of interest (muscle fiber, material flaw, microchip, etc.) that has some given length scale. Based on this scale, a frequency is selected that is capable of resolving the structure of interest. Samples are then prepared that are thin enough to permit the high-frequency ultrasound to penetrate to the target area. Sample preparation will be discussed in Chapters 4 and 5.

### 3.1.2 Intravascular Ultrasound

Intravascular ultrasound (IVUS) shares characteristics of both traditional clinical ultrasonic imaging and acoustic microscopy. Because the structures of interest, vessels, are quite small, high frequencies must be used. Fortunately, catheter-based systems permit the transducer to be positioned close enough to the tissues that the effects of the higher attenuation are manageable.

### 3.1.3 Chapter Overview

This chapter describes the equipment and materials that compose the high-frequency acoustic microscopy setup used to obtain the results of Chapters 4, 6, 7, and 8. Section 3.2 provides details of the acoustic microscope, including the transduc-
The acoustic microscope system used in these studies consists of a motion control system, a temperature-controlled specimen tank, a transducer, electronics for creating the outgoing pulse, and electronics for receiving, conditioning, and storing the incoming pulse. Custom software integrates all of these elements and permits automated scanning. The microscope can be operated with any standard immersion transducer. A schematic representation of the microscope system is displayed in Figure 3.1.

3.2.1 Transducers

The three transducers used in this study were the Panametrics V324, Panametrics V390, and Panametrics V3346 (Olympus NDT, Waltham, MA) with nominal center frequencies of 25 MHz, 50 MHz, and 100 MHz, respectively. Details of these transducers can be found in Table 3.1.

The photograph in Figure 3.2 shows, from left to right, the transducers from low to high frequency. The larger size of the 50 MHz and 100 MHz transducers is attributable to the delay line that is built into the housing. The active element in these transducers is near the small connector that can be seen on the right side of
3.2 Acoustic Microscopy System

Figure 3.1: Schematic representation of the acoustic microscopy experimental setup.
3.2 Acoustic Microscopy System

each. This active element is planar, and the pulse that it emits propagates down the delay line toward a spherical lens ground into the end of the delay line. Because the delay line’s relatively high speed of sound permits the pulse to advance quickly into the far field, the wave that impinges upon the lens is well approximated as a plane wave. The lens then focuses that plane wave to a spot, the size of which is determined by the frequency of the sound, the width and curvature of the lens, and the velocity of propagation in the host medium. A significant drawback of these transducers is that spurious multiple reflections inside the delay line are collected by the active element at the same time as actual signals. Section 5.5.3 describes how this problem is addressed.

The 25 MHz transducer has a focused active element at the end of the transducer (lower side in Figure 3.2). In this case, the excitation of the active element directly initiates propagation of a focused ultrasound pulse into the medium. This design does

<table>
<thead>
<tr>
<th>Transducer</th>
<th>V324 (25 MHz)</th>
<th>V390 (50 MHz)</th>
<th>V3346 (100 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Focal Distance</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.0 in (25.4 mm)</td>
<td>0.5 in (12.7 mm)</td>
<td>0.25 in (6.35 mm)</td>
</tr>
<tr>
<td><strong>Diameter</strong></td>
<td>0.25 in (6.35 mm)</td>
<td>0.25 in (6.35 mm)</td>
<td>0.125 in (3.175 mm)</td>
</tr>
<tr>
<td><strong>Center Frequency</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28 MHz</td>
<td>45 MHz</td>
<td>85 MHz</td>
</tr>
<tr>
<td><strong>Bandwidth</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>22-36 MHz</td>
<td>30-60 MHz</td>
<td>60-105 MHz</td>
</tr>
</tbody>
</table>

Table 3.1: Transducer specifications. <sup>1</sup>Focal Distance is in water at room temperature. <sup>2</sup>Center frequency and bandwidth can vary based on the instruments used to create the excitation pulse, and based on the specifics of the experimental setup. Values listed here are typical for the equipment and configurations of the studies described in this dissertation.
3.2 Acoustic Microscopy System

Figure 3.2: The three transducers used in the studies presented in this thesis. The nominal center frequencies of the transducers, going from left to right, are 25 MHz, 50 MHz, and 100 MHz. The 50 and 100 MHz transducers have built-in delay lines, which accounts for their larger size relative to the 25 MHz transducer.

not introduce spurious “delay line” signals.

The three transducers used in this study were chosen because their bandwidths overlap in such a way as to provide usable data over the entire range of intravascular ultrasound (IVUS) systems, either currently available or likely to be available in the near future. The bandwidths of these transducers can be inferred from power spectra of signals reflected from a nearly ideal reflector. The reflector used in this case is a highly polished stainless steel plate. Figure 3.3 shows these spectra for
3.2 Acoustic Microscopy System

Each transducer. The frequency axis (abscissa) is displayed on a log scale in both panels. The top panel of this figure shows the power spectra displayed linearly, and the bottom panel shows the same data displayed on a decibel (dB) scale. Each curve have been normalized by its maximum value, so that all peaks have a maximum value of 1 on the top plot, and 0 dB on the bottom plot. The dashed horizontal line on each plot represent the point 6 dB below this maximum value. Because the decibel as is defined as

\[ dB = 10 \times \log_{10} \left( \frac{\text{Power}_{\text{out}}}{\text{Power}_{\text{in}}} \right), \]  

\[ 6dB = 10 \times \log_{10}(0.2512). \]  

The line on the top plot is therefore at about one fourth of the maximum value in the top panel.

3.2.2 Motion Control System

The positioning of the transducer in the acoustic microscopy system is done by a Newport XPS (Newport Corporation, Irvine, CA) motion controller driving three UTM150CC1HL linear motion stages (Newport Corporation, Irvine, CA). These stages have total travel of 150 mm, and can be positioned with accuracy better than 5 µm. The XPS is programmatically controlled via ethernet connection by the computer running the acoustic microscopy system. The motion stages are configured so that each stage is perpendicular to the other two in a rectangular coordinate sys-
Figure 3.3: The frequency spectrum of a signal reflected from a steel plate placed at the transducer’s focus for each of the transducers used in the studies of this thesis. The top and bottom panels show the same data, with the top plot displaying the normalized power on a linear scale and the bottom plot displaying the normalized power on a decibel scale. The dotted lines in each plot represent the point 6 dB below the normalized peak.
tem. The stages can be seen in the left half of Figure 3.4. The x-stage is mounted on a large aluminum plate, which serves to minimize vibration and other incidental motion. On top of the x-stage and perpendicular to it is the y-stage. Both the x and y-stages travel parallel to the table top. The z-stage is attached to a bracket that fits onto the y-stage. This bracket positions the z-stage so that its travel is orthogonal to the table top.

An angled bracket is attached to the face of the z-stage which has a fitting that can accommodate any of the transducers. A gimbal tilt-table built into the bracket permits focusing of the transducer.

3.2.3 Electronics

The excitation of the transducers is controlled by a Panametrics 5900 Pulser/Receiver (Olympus NDT, Waltham, MA). On transmit, the 5900 triggers a Panametrics 5627RPP-1 remote pulser/preamplifier (Olympus NDT, Waltham, MA) to send a pulse to the transducer. The signal returning from the transducer goes back to the 5627RPP-1, which is located less than 6 inches from the transducer to minimize cable length, where it is pre-amplified with a fixed gain of $24 \pm 2$ dB. The signal is then sent to the 5900, which applies variable gain and attenuation before passing the signal on to a Tektronics 5052 Digital Oscilloscope, which performs 8-bit digitization at up to 1 Gigasamples/second. Received signals can be time averaged before recording to disk for offline analysis.

The computer that controls the acoustic microscopy system is onboard the oscil-
Figure 3.4: Photograph of the acoustic microscope experimental apparatus. The three motion control stages are shown on the left. The transducer connects to the motion stages by a gimbal tilt table on an L-bracket. The transducer cable connects to the Remote Pulser/Preamp. Photograph by Allyson Gibson (used with permission).
3.2 Acoustic Microscopy System

Figure 3.5: Photograph of the 50 MHz transducer positioned over a tissue sample in a scanning tank. The sample is mounted on a stainless steel plate that serves as a nearly ideal reflector. Photograph by Allyson Gibson (used with permission).
The software that runs data acquisition scans was custom designed and written with Labview (National Instruments Corporation, Austin, TX). A screenshot of the front panel GUI for operating the acoustic microscope system can be seen Figure 3.6.
Figure 3.6: Screen capture of the front panel GUI that permits interaction with the custom software that controls operation of the acoustic microscope.
3.2.4 Sample Tank

A coupling medium was used to minimize the acoustic impedance mismatch between the transducer and the sample. Water or a water-based solution is a very common choice of coupling medium because most tissue samples have acoustic properties very similar to water, and working with water is relatively easy. The right side of the picture in Figure 3.4 shows the acoustic microscope interrogating a sample that is mounted in one of the sample tanks used with the acoustic microscopy system. Figure 3.5 is a zoomed in view of the same setup. The sample is seen mounted to a steel plate, which serves as a nearly ideal reflector, that is itself held securely to the bottom of the tank.

3.2.5 Temperature Control

The studies described and reported in Chapters 4, 5, 6, 7, and 8 were performed, in most cases, on fresh arterial tissue. In all cases, the results were intended to be pertinent to future ultrasonic studies and diagnostic scans carried out *in vivo*. For this reason, and because the propagation of ultrasound is temperature dependent, it was decided that all studies should be done at a temperature to match human body temperature, 37°C.

Temperature-Controlled Scan Tank

A new tank was designed to carry out measurements of fresh arterial tissue at 37°C. The goal of the design was to build a tank that would keep the coupling
medium, and therefore, the tissue within about 1°C of the target temperature, without interfering with the operation of the acoustic microscope. In practice, the tank needed to be able to heat the coupling medium efficiently, and maintain temperature well enough to prevent heat gradients from being established within the coupling medium.

**Tank Design**

The temperature controlled tank built for these studies can be seen in Figure 3.7. The walls of the tank were constructed with 0.5” Plexiglas™ acrylic, which is a relatively good insulator, yet transparent, so as to allow for visualization of the sample and transducer during preparation and data acquisition. Two strip heaters (HCS-80, www.omega.com) mounted to the underside of the base plate of the tank serve to warm the coupling medium when turned on manually or cycled on by the proportional-integral-derivative (PID) controller. Anodized aluminum was chosen for the base plate because it provides a good compromise between heat transfer efficiency and corrosion resistance. The base plate and attached heaters connect to a recessed block of 2 thick Delrin™, which serves as a thermal insulator below the heaters and as a secure foundation for the tank. The left panel of Figure 3.8 shows the underside of the base plate with the strip heaters attached and the Delrin™ foundation (the while piece in the top of the left panel) removed. Two L-brackets are fixed to the top of the base plate to hold the stainless steel reflector (not shown in pictures) at a constant location in the tank. The two brass access ports on the side of the tank permit the resistive temperature detector (RTD) probe to be positioned in one of
two locations. Figure 3.9 shows the tank with the probe in place. The other port is plugged with a stainless steel rod.

**Controller Design**

Control of power to the heaters is provided by a programmable temperature controller (model CN7533, www.omega.com). Temperature feedback is provided by a resistive temperature detector (RTD) probe (model PRTF-11, www.omega.com), which is the silver cylinder shown on the far right side of Figure 3.8 and shown entering the tank through the brass port in Figure 3.9.

**Controller Construction**

To maximize safety and portability, a custom housing was built for the temperature control unit with detachable connections for line voltage, temperature probe, and heater power. The right panel of Figure 3.8 shows the housing with the top cover removed. This unit can also be operated without the heaters to monitor tank temperature.

**System Operation**

The system can be operated in an On/Off (thermostat) control scheme or with proportional-integral-derivative (PID) control. Most scans were done using the proportional-integral-derivative mode with the parameters chosen by the controller’s built-in auto-tune functionality. With these settings the tank is able to bring degassed phosphate
3.2 Acoustic Microscopy System

Figure 3.7: Photograph of temperature controlled tank constructed for the studies of this thesis. The brass ports on the side permit the temperature probe to be mounted in either of two locations. The plug connected to the base provides power to the heaters. Photograph by Joseph Hoffman.
buffered saline (PBS, see Section 3.4) from room temperature to a stable 37°C in about 35 minutes. The phosphate buffered saline remains within about ±1°C indefinitely.

Temperature Stability

Two sheets of lightweight styrofoam were used to insulate the top of the tank so that the parameters chosen for the proportional-integral-derivative controller remained appropriate as the conditions of the laboratory changed. Also, the styrofoam on the top of the tank ensured that heat was lost from the top of the tank at a much lower rate than heat was being added to the bottom, thus preventing vertical heat gradients. Styrofoam was ideal for this application because it insulated well, but permitted free movement of the transducer and easy access to the tank.

3.2.6 Scan-time Prediction Algorithm

The acoustic microscope system scanning software offers a great deal of freedom for setting the parameters of a scan. Many of these settings affect the length of time required to complete a scan. Although the qualitative effects of these choices are obvious, it is often impossible to grasp quantitatively the trade-offs in scan time one is making by selecting specific system settings. In an experiment in which the duration of scan time is critical, being able to make informed choices based on the relationship between scan time and imaging performance is very important. To address this problem, an empirical prediction model for the parameters that most significantly
3.2 Acoustic Microscopy System

Figure 3.8: Photographs of the temperature controlled tank with base remove (left) and of the temperature controller box with top removed (right). Both of these pieces were designed and built for the studies of this thesis. Photographs by Joseph Hoffman.
FIGURE 3.9: Photograph of the temperature controlled tank connected to temperature controller. The temperature probe and the heater power cord connect to the back of the temperature controller box. Photograph by Joseph Hoffman.
impact scan time in the acoustic microscope was created. For a given set of acquisition parameters, the algorithm estimates the total time required for the scan. The model is accurate to within 15%. Figure 3.10 shows the scan time prediction versus the actual scan time for a wide range of scan times. After development, the model was incorporated into the scanning software graphical user interface and used to guide all scans presented in this dissertation.

Figure 3.10: Scatter plot of actual scan time versus predicted scan time. Prediction scan time points shown with ±15% error bars. Dotted line represents perfect agreement between predicted scan time and actual scan time.
3.2.7 Peak-Detect Scanning

Peak-detect scanning is performed in advance of acoustic microscopy scanning to choose the appropriate region of interest for data collection. This rapid form of scanning records only the maximum voltage received by the transducer at each location, rather than the full voltage trace. Because the samples are very small (often much smaller than the transducer’s footprint), there is no way to align the scan visually. In this situation, the acoustic microscope itself is the only instrument capable of viewing the structures on a fine enough scale to identify the areas of interest. Therefore, a peak-detect scan is performed in which a very wide area is scanned coarsely. An image from this scan then guides the experimenter to choose a smaller area to scan somewhat less coarsely. This process can be iterated until a region of suitable size is identified.

3.2.8 Upgraded Peak-Detect Scan

Prior to the current studies, peak-detect scanning had been conducted by the same mechanism as data collection scans: the software instructed the motion controller to go to a spot, and then the software told the oscilloscope to start collecting the signals from returning from the pulser. In this configuration, the pulser pulsed continuously and asynchronously relative to the motion control and acquisition. This process is very versatile and works well for collection of data, but for the complicated structures of fresh coronary artery tissue, performing these types of scans can be very time
3.2 Acoustic Microscopy System

consuming. Because several scans were to be performed on each sample and because each scan could be up to several hours long, it was decided that upgrading the peak-detect scans was necessary in order to efficiently identify the best regions for full acquisition.

The upgraded peak-detect scan system operates by having the software send the coordinates of the peak-detect scan to the motion controller. The subsequent movement of the motion controller sends trigger pulses directly to the pulser/receiver, which in turn triggers the remote pulser to pulse the transducer. The return signal is collected by the oscilloscope, which is operating in a fast acquisition mode that permits it to store all incoming data. In this way, an entire scan line can be rapidly collected by one continuous sweep of the transducer. Once the line is finished, the software selects the peak value for each point, and peak-detect scan proceeds by scanning the next line. The improvement of this procedure comes about because for each line, the transducer makes one continuous motion in which it can collect data of almost arbitrarily high resolution. In contrast, for a data collection scan, the transducer must come to a stop at each scan site, so the more sights that are imaged in each line, the more time the scan takes. With the fast peak-detect scan, the time of the scan only increases when more lines are scanned. When performing an MxN point scan, the time required for a traditional scan is related to the product of M times N. For the fast peak-detect scan, to a first approximation, the time is only related to N, the number of lines, and not at all to M.

Having the ability to perform fast peak-detect scan permitted more accurate and
efficient data acquisition. An unexpected benefit of the fast peak-detect scan arose in the segmentation of the samples. This benefit will be described in Section 6.4.

3.3 Ultrasonic Beam Characteristics

The properties of an ultrasonic beam are determined by the characteristics of the transducer (diameter, frequency, focal distance, etc.) and by the medium in which the beam is propagating. This section investigates these properties with theoretical calculations and empirical observations.

3.3.1 Calculated Beam Properties

Focal Zone

The focal zone, sometimes called the depth of field, is the region over which the transducer’s sound beam is considered to be most tightly focused. It is commonly defined as beginning and ending at the on-axis points that are -6dB down from the maximum on-axis signal strength. The length of the focal zone is determined by the natural focusing of the transducer, expressed in the Near Field Distance, $N$ (Equation 3.3), and by the Normalized Focal Length, $S_F$ (Equation 3.4). The Near Field Distance is given by

$$N = \frac{D^2 f}{4c},$$

(Equation 3.3)

where $D$ is the diameter of the element, $f$ is the frequency, and $c$ is the speed of sound in the medium of interest (Szabo, 2004, p. 152). Table 3.1 displays these properties
3.3 Ultrasonic Beam Characteristics

for the transducers used in the studies of this thesis. The Normalized Focal Length is defined as

\[ S_F = \frac{F}{N}, \quad (3.4) \]

where \( F \) is the focal length of the transducer lens, and \( N \) is the near field distance. Using these two quantities the Focal Zone is calculated as

\[ \text{Focal Zone} = N \ast S_F^2 \left( \frac{2}{1 + 0.5S_F} \right) \quad (3.5) \]

(Panametrics-NDT, 2006, p. 42). The calculated near field distance, normalized focal length, and focal zone for all the transducers used in this study are shown in Table 3.2.

Resolution

The resolution of an imaging system is directly related to the size of the beam that is interacting with the structures of interest. For the acoustic microscopy system there are two important aspects of resolution: the lateral resolution and axial resolution. In the coordinate system of the acoustic microscope, the lateral resolution is pertinent to the x and y-directions, and the axial resolution applies to the z-direction. Therefore, C-scans, such as those described in later chapters, are only affected by the lateral resolution, whereas the B-scans are affected by the axial resolution in one dimension and the lateral resolution in the other dimension.

The lateral resolution is determined by the diameter of the beam. Because data were collected predominantly from within the focal zone, the discussion will concen-
ulate on the beam diameter at the focus. The -6 dB beam diameter, the diameter at which the intensity of the beam has fallen by 6 dB from its on-axis maximum, can be calculated by

\[
BD_{-6dB} = 1.02 \left( \frac{F_c}{fD} \right),
\]

where \( F \) is the focal length of the transducer, \( c \) is the speed of sound in the host medium, \( f \) is the frequency of interest, and \( D \) is the diameter of the lens (Krautkramer and Krautkramer, 1990). The prefactor of 1.02 is related to the choice of a -6dB cutoff and the use of a piston source. This equation is used to calculate the beam diameters of the transducers used in the current studies, which are presented in Table 3.2.

The axial resolution is most closely linked to the pulse length. As the calculation in Equation 3.3.1 shows, the beam width (and therefore the lateral resolution) is calculated for each individual frequency, so it is, in effect, a narrowband description. The temporal extent of the pulse, on the other hand, is fundamentally related the

<table>
<thead>
<tr>
<th>Transducer</th>
<th>V324 (25 MHz)</th>
<th>V390 (50 MHz)</th>
<th>V3346 (100 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near Field Distance</td>
<td>183 mm</td>
<td>294 mm</td>
<td>5282 mm</td>
</tr>
<tr>
<td>Normalized Focal Length</td>
<td>0.139</td>
<td>0.043</td>
<td>0.001</td>
</tr>
<tr>
<td>Focal Zone</td>
<td>7.1 mm</td>
<td>1.0 mm</td>
<td>0.58 mm</td>
</tr>
<tr>
<td>Beam Diameter</td>
<td>0.24 mm</td>
<td>0.068 mm</td>
<td>0.038 mm</td>
</tr>
<tr>
<td>Pulse Length</td>
<td>0.188 mm</td>
<td>0.077 mm</td>
<td>0.051 mm</td>
</tr>
<tr>
<td>Axial Resolution</td>
<td>0.102 mm</td>
<td>0.033 mm</td>
<td>0.025 mm</td>
</tr>
</tbody>
</table>

Table 3.2: Calculated properties of the transducers used in the studies of this dissertation.
3.3 Ultrasonic Beam Characteristics

Figure 3.11: Representative pulses for the three transducers used in this dissertation. These pulses are the signals captured from a steel reflector placed at the focal point of each transducer. The dotted line in each plot is the magnitude of the analytic signal of the pulse. The black bar above each pulse represents the pulse length, with the ends of the bars determined by the points at 10% of the peak of the magnitude of the analytic signal. The labels above each bar are the measured temporal pulse lengths.
3.3 Ultrasonic Beam Characteristics

bandwidth of the pulse. Axial resolution is therefore a broadband phenomenon. Because the pulse length is a complicated product of the shape and amplitude of the triggering pulse (“the big bang”), the transmission cables, and numerous properties of the transducer (piezoelectric properties, shape, impedance, backing material, etc.), it cannot be calculated from a simple equation. To estimate the axial resolution, the pulse length is measured in a configuration typical for the studies of interest. Figure 3.11 shows typical pulses from the transducers used in the studies presented in this dissertation. Also shown are the magnitudes of the analytic signals corresponding to these pulses. The pulse length is measured between the two points on the magnitude of the analytic signal that are 10% of the maximum value. The axial resolution can be estimated by asserting that pulses separated in time by the full width at half maximum (FWHM) are resolvable. By this criterion, the axial resolution is the pulse width at half of the maximum amplitude, or expressed in decibels,

$$20 \times \log_{10} \left( \frac{0.5 \times (\text{Max. Amplitude})}{(\text{Max. Amplitude})} \right) = 20 \times \log_{10}(0.5) = -6.02dB. \quad (3.7)$$

In other words, measuring the temporal 6dB down points in a pulse is a good estimate of the axial resolution. This estimate is only useful under optimal circumstances. In the more likely case that one signal is weaker than the other, the pulses must be separated by a larger distance. The bars in Figure 3.11 indicate the pulse length, and they are labeled with the pulse length in time.

The spatial pulse length for the studies of this thesis was calculated by multiplying the temporal pulse length by the speed of sound in phosphate buffered saline at 37°C.
3.3 Ultrasonic Beam Characteristics

(see Equation 3.8). Table 3.2 shows the spatial pulse lengths and estimated axial resolutions of the transducers used under typical conditions of these studies.

### 3.3.2 Calibration Curves

To estimate both the dynamic range and the usable bandwidth of an ultrasonic system, calibration curves are typically created. These curves are made by systematically inserting known amounts of attenuation into the system so that the response to signals of different sizes can be examined. The curves generated are the magnitude of the power spectrum for each of these progressively more attenuated signals. Ideally, the magnitude of the signals should decrease by exactly the amount of attenuation imposed on the system.

**Usable Bandwidth**

The bandwidth over which this happens is considered usable. Because most of the signal energy is concentrated near the center of the bandwidth, the edges of the band tend to fall into the noise floor sooner. The calibration curves provide an estimate of how the bandwidth varies with signal strengths.

**Dynamic Range**

The dynamic range of the system can be estimated from the calibration curves by looking at over how many dB the system responds linearly to a fixed change in the attenuation. When the signal is too strong, saturation occurs, causing a nonlinear
relative change in the power spectra. When the signal is too week, the noise in the system is comparable to the signals, and the effect of adding external attenuation is imperceptible.

**Measured Calibration Curves**

The calibration curves for the transducers used in the acoustic microscopy system for the studies of this dissertation are shown in Figures 3.12, 3.13, and 3.14. The specific system settings used are described in Chapter 5. The top panel of each figure shows the measured calibration curves, and the bottom panel shows the curves normalized by the curve corresponding to the attenuation setting typically used as the reference trace.

### 3.4 Coupling Medium Characterization

#### 3.4.1 Background

Acoustic microscopy studies rely on a liquid coupling medium to transmit sound from the transducer to and from the sample of interest. At the relatively high frequencies used for these studies, the frequency dependent effects of the coupling medium cannot be assumed negligible and must be considered in any quantitative analysis. Both the speed of sound and the attenuation of the coupling medium also vary with temperature, so this dependence must be understood and taken into account.
Figure 3.12: Calibration curves for the 25 MHz transducer. The top plot shows the power spectrum of a signal reflected from a steel plate at the transducer’s focal point for a series of 8 attenuation settings each spaced by 10 dB. The curves are displayed over the transducer’s bandwidth. The bottom plot shows the same curves normalized by the third curve from the top (red curve).
3.4 Coupling Medium Characterization

**Figure 3.13:** Calibration curves for the 50 MHz transducer. The top plot shows the power spectrum of a signal reflected from a steel plate at the transducer’s focal point for a series of 8 attenuation settings each spaced by 10 dB. The curves are displayed over the transducer’s bandwidth. The bottom plot shows the same curves normalized by the third curve from the top (red curve).
Figure 3.14: Calibration curves for the 100 MHz transducer. The top plot shows the power spectrum of a signal reflected from a steel plate at the transducer’s focal point for a series of 8 attenuation settings each spaced by 10 dB. The curves are displayed over the transducer’s bandwidth. The bottom plot shows the same curves normalized by the third curve from the top (red curve).
3.4.2 Choice of Coupling Medium

In addition to allowing sound to pass from transducer to sample, the coupling medium must also be hospitable to the sample of interest, which may be immersed for several hours. Phosphate buffered saline (PBS) at 37°C was chosen in these studies as the medium which best simulates the tissue’s environment in vivo. This choice is fortuitous because warm water is an ideal coupling medium, due to its lower attenuation and good impedance match to tissue. Phosphate buffered saline is ubiquitous in medical research, but a systematic characterization of this medium as an acoustic microscopy coupling medium is not available in the published literature. Therefore, the following studies were carried out to provide the necessary characterization.

3.4.3 Recipe

The recipe used in these studies was chosen to maintain consistency with our industrial partners working at Volcano Corporation’s Advanced Technology Lab. The recipe is as follows:

- 1.5 L Distilled Water
- 11.2 g NaCl
- 3.74 g NaH$_2$PO$_4$
- 9.82 g Na$_2$HPO$_4$
3.4 Coupling Medium Characterization

Figure 3.15: Plot of the speed of sound of different types of water as a function of frequency measured in the acoustic microscope. The line is the known speed of sound of pure water as a function of temperature (Bilaniuk and Wong, 1993, 1996). The points corresponding to degassed distilled water agree very well with the known values. Water that has either not been distilled, or not been degassed, does not agree as consistently.

3.4.4 Pure Water Speed of Sound and the Effect of Degassing

To determine the speed of sound in a given coupling medium, measurements were obtained at a series of temperatures ranging from 20°C to 50°C. A pullback experiment was carried out. At each temperature, a series of ultrasonic pulses were transmitted through the coupling medium, reflected from a steel plate, collected and recorded. After each pulse was collected, the transducer was moved a known distance farther from the reflector. Subsequently, each pulse was correlated with the first pulse to give a time-of-flight difference. This time difference was plotted against the corresponding change in distance. The slope of the linear fit to this line provided the speed of sound of the coupling medium. This method was validated by comparing the measured speed of sound of pure water as a function of temperature with its known
value. The speed of sound in pure water as a function of temperature was reported by Grosso and Mader (1972). Their data were recast after a change in the official Celsius temperature scale in 1990 by Bilaniuk and Wong (1993, 1996). Figure 3.15 shows measured values for the speed of sound in degassed tap water, distilled water, and degassed distilled water. The degassed samples had been allowed to boil at room temperature for several minutes under a vacuum desiccator before being measured. The plot shows that all measurements are close to the accepted value, but that the degassed distilled water best fits the data.

The observation the dissolved gas was substantially affecting the speed of sound led to including a degassing step in the protocol for all scans in this study. Aside from assuring the appropriate speed of sound, degassing also prevents the formation of air bubbles that appear on the transducer and sample when scanning for long periods of time at elevated temperatures.

3.4.5 Speed of Sound in Phosphate Buffered Saline

The speed of sound in phosphate buffered saline was measured in the same manner as above. The results for the phosphate buffered saline are shown alongside the values for pure water in Figure 3.16. The green curve going through the pure water data is the accepted value (Bilaniuk and Wong, 1993, 1996), whereas the red line through the PBS data is a cubic fit generated from those points. As can be seen from this plot, the saline has a greater speed of sound than pure water, but a similar temperature dependence. A 3rd-order polynomial was fit to the temperature dependent of the
3.4 Coupling Medium Characterization

Figure 3.16: Plot of the speed of sound in phosphate buffered saline and pure water as a function of temperature measured in the acoustic microscope. The measured pure water values are shown to be in agreement with the accepted values (Bilaniuk and Wong, 1993, 1996). The speed of sound of phosphate buffered saline is fit with a 3rd order polynomial (Equation 3.8).

The speed of sound of phosphate buffered saline so that the value of the speed of sound in PBS could be interpolated at the temperature useful for a given analysis. The result of that fit is displayed in Equation 3.8:

\[ v(T) = \sum_{i=0}^{3} a_i T^i, \]  

(3.8)

with the coefficients \( a_i \) given in Table 3.3.

3.4.6 Attenuation

Measurement of the attenuation coefficient of phosphate buffered saline is significantly more complicated than the corresponding speed of sound measurement. The attenuation coefficient depends on the temperature and the frequency, and any measurement of these dependencies must account for the confounding effects of the
3.5 IVUS System

Intravascular ultrasound (IVUS) scans were performed with a Volcano s5 (Volcano Corporation, San Diego, CA) system with a 20 MHz nominal center frequency array catheter (EagleEye™, Volcano Corporation, San Diego, CA) and with a 45 MHz nominal center frequency revolving single-element catheter (Revolution™, Volcano

<table>
<thead>
<tr>
<th>$i$</th>
<th>$a_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1416.9 ms$^{-1}$</td>
</tr>
<tr>
<td>1</td>
<td>5.3351 ms$^{-1}$ (°C)$^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>-0.0625 ms$^{-1}$ (°C)$^{-2}$</td>
</tr>
<tr>
<td>3</td>
<td>$2.4664 \times 10^{-4}$ ms$^{-1}$ (°C)$^{-3}$</td>
</tr>
</tbody>
</table>

Table 3.3: Coefficients of the cubic fit to the speed of sound in phosphate buffered saline as a function of Temperature (Equation 3.8).
3.6 Summary

This chapter details the equipment used to make the measurements described in this thesis, and also presents data characterizing the measurement system. The information presented here underlies and proceeding studies.
Bibliography


4.1 Background

To prepare for the ultrasonic studies of human coronary arteries, extensive preliminary studies were performed on fresh lamb coronary artery tissue. In this study, the lamb tissue was derived from sheep that were about one year old. Sheep hearts are approximately the same size as human hearts; and exhibit the same four-chambered heart structure as human hearts. These facts, in combination with the relative ease with which sheep hearts can be acquired, make sheep hearts an ideal subject for study and method validation.
4.2 Lamb Coronary Arteries

4.1.1 Chapter Overview

The first section in this chapter traces the process of the acquisition and preparation of the lamb coronary arteries from collection to isolation of the left anterior descending (LAD) coronary arteries (Section 4.2). The next section describes the methods used to prepare the coronary arteries for acoustic microscopy (Section 4.3). Tissue property measurements made on these samples are discussed in Section 4.4. Section 4.5 explains the development of a hybrid method of tissue preparation, and Section 4.6 describes other preliminary studies performed on lamb coronary arteries.

4.2 Lamb Coronary Arteries

4.2.1 Fresh Tissue Acquisition

The tissues used in the studies of this chapter were acquired from a local slaughterhouse. The hearts came from lambs being slaughtered for meat for human consumption, and were collected moments after death. The whole hearts were placed in a cooler of 0.9% saline solution at room temperature and transported (requiring about 20 minutes) to the laboratory. In the laboratory, the hearts were rinsed under cool running water for several minutes to remove any clotted blood. The rinsed hearts were placed in containers of fresh saline. The hearts were typically acquired, transported, and cleaned within 45 minutes of slaughter. Figure 4.1 shows a photograph of a representative lamb heart that has been collected and cleaned.
4.2 Lamb Coronary Arteries

Figure 4.1: Photograph of a fresh lamb heart shortly after slaughter. In this image, the left anterior descending coronary artery runs diagonally across the heart. It starts in the upper right and runs to the lower middle left. Photograph by Joseph Hoffman.

The cleaned hearts, still immersed in saline, were placed in a refrigerator for about one hour prior to further dissection. Chilling the hearts in this manner aided in the cutting and sectioning that followed.

4.2.2 Left Anterior Descending Coronary Artery

The left anterior descending (LAD) coronary artery was identified on each lamb heart. The left anterior descending branches off the left main coronary artery and
then proceeds distally down the septum. In Figure 4.1, it runs diagonally from upper
right to lower left across the heart as it is shown in this image. The left main is
concealed under the large piece of fat in the top center of the picture.

The myocardium was cut on either side of the left anterior descending coronary
artery from the level of the ostium to the apex of the heart. A final cut was made on
the inner side of the heart the down septum to remove the entire anterior descending
artery with its surrounding myocardium from the rest of the heart. The resulting
segment can be seen in Figure 4.2, and consisted of the left anterior descending
artery running all the way from the left main to near the apex of the heart on top
of a substantial amount of cardiac muscle. Frequently, substantial amounts of fatty
tissue were also present on the surface of the heart.

4.2.3 Rough Cutting the Anterior Descending

The left anterior descending coronary artery, once isolated from the rest of the
heart, is several inches long, as seen in figure 4.2. To make acoustic microscopy studies
practical, a subregion was chosen for further study. Segments closer to the proximal
end were typically chosen because more distal segments become significantly smaller
than most human arteries. The selected region was excised by making cuts in the
plane perpendicular to the axis of the artery. The distance between the cuts was
roughly 1 centimeter. One of these sections as viewed from the plane of the cut is
shown in Figure 4.4. In this figure, the arrow points to the artery, which appears as
a whitish area around a small hole.
Figure 4.2: Excised left anterior descending coronary artery from a lamb heart, with surrounding myocardium and fat. Photograph by Joseph Hoffman.
Figure 4.3: Rough cut section of left anterior descending coronary artery from lamb, viewed from the exterior surface. The proximal end of the artery is toward the top of the image and the distal end is toward the bottom. Photograph by Joseph Hoffman.
Figure 4.4: Rough cut section of left anterior descending coronary artery from lamb, viewed from the plane of the cut. The arrow in the image points to the coronary artery, which runs just along the surface of the myocardium and just below a layer of fat. Photograph by Joseph Hoffman.
4.2.4 Excising the Left Anterior Descending

The rough cut sections described above contained the left anterior descending coronary artery and a good deal of surrounding muscle and fat. This excess tissue was manually cut off by making slices progressively closer to the vessel until there was less than 1 millimeter of muscle or fat surrounding the artery. Great care was taken to minimize the amount of stress the vessel was placed under in order to reduce the chance of altering its properties. After as much surrounding muscle and fat as possible had been removed, the vessel was further prepared as dictated by what type of slice was desired for the acoustic microscopy scan.

4.3 Acoustic Microscopy Sample Preparation

4.3.1 Background

As described in Section 3.1 the acoustic microscopy system is capable of making very high resolution measurements, but obtaining reliable measurements requires that samples be prepared very precisely. For backscatter measurements, the best results are obtained with samples that have a very smooth top surface. Attenuation coefficient measurements yield the best results when, in addition to a smooth top surface, the samples also have a smooth back surface that is parallel to the front. Because the high frequency signals used are attenuated significantly with distance travelled, the samples must be very thin. To make attenuation measurements, the received pulse,
which has made a round trip through the entire thickness of the sample, must have sufficient signal strength to achieve acceptable signal to noise ratio. The best results are typically achieved with the flattest and thinnest samples. In theory, the backscatter measurements require that the sample be at least the equivalent of several pulse lengths (see Table 3.2) in thickness, but in practice, fresh tissue is sufficiently challenging to cut that the thinnest possible samples always had sufficient thickness.

This section describes the studies that were carried out to address the question of how best to measure the ultrasonic properties of fresh coronary artery tissue. Several approaches were tried, and are described below. The studies of this chapter enabled the development of the methods used on human coronary artery tissue discussed in Chapter 5.

### 4.3.2 Tissue Orientation

One of the major goals of the lamb artery studies was to determine which, if any, orientation of the coronary artery relative to the beam emanating from the acoustic microscope transducer would be possible or desirable. Each orientation results in challenges associated with preparing the tissue for acoustic microscopy. Different ultrasonic properties are available for measurement in each orientation. The anisotropic structure of coronary arteries results in an angular dependence of acoustic properties, and is discussed in detail in Chapter 6.
4.3 Acoustic Microscopy Sample Preparation

Longitudinal Slicing

The longitudinal orientation is achieved by cutting the excised artery open longitudinally relative to its axis. This was done by carefully slicing with a razor blade, or cutting with a microsurgical scissors, down the length of the artery segment. A picture of a lamb artery prepared in this manner is shown in Figure 4.5. When this artery is scanned, the ultrasonic beam strikes the inner surface of the opened vessel and penetrates into the subsequent layers of the artery.

One of the benefits of this preparation method is that the scan direction is similar to that employed in intravascular ultrasound (IVUS) measurements. By its nature, IVUS insonifies tissue in an orientation perpendicular to the direction of blood flow. The view most analogous to that employed in intravascular ultrasound can be achieved by extracting the artery from its surrounding tissue, slicing it open along the direction of flow, and laying it flat with the intimal side toward the microscope. In addition to data being acquired in a fashion similar to IVUS, this orientation also results in data being processed similarly to the way in which it is processed in IVUS; one dimension of the image is generated from transducer movement and the other from increasing penetration depth into the tissue.

There are, however, some drawbacks to the use of this orientation. The excision of the tissue must be carried out manually; and this time-consuming procedure requires extensive tissue manipulation and handling that may damage sensitive tissue. This problem is exacerbated when the procedure is attempted on tissue with relatively
unstable plaques. Ultrasonic tissue characterization is also significantly complicated by this preparation. Isolating the ultrasonic characteristics of one layer of tissue is difficult because the received signals are influenced by all intervening layers. Scans designed to measure the attenuation coefficient are nearly impossible because tissue layers through which the signal would pass. These might include variable amounts of non-arterial tissue that was not trimmed away during preparation.
Transverse Slicing

The alternative to the longitudinal preparation is to cut and image the tissue transverse to the orientation of blood flow. This method involves two transverse cuts separated by a small distance yielding a thin cross-sectional slice of the coronary artery. The cuts are typically made with the aid of a device that controls the thickness of the slice and ensures parallel cuts. Both a manual and an automated slicer were used for the lamb tissue studies. It was found that without the use of a fixative to stiffen the tissue, the manual slicer was not capable of making slices thin enough for the acoustic microscope. The automated slicer (Compresstome VF-200, Precisionary Instruments, Greenville, NC) was able to make consistent slices of fresh lamb coronary artery tissue.

The process of generating transverse slices is fully detailed in Section 5.6. Briefly, one end of the sample was affixed to a piston inside a cylinder. The cylinder was then filled with warm agarose, which quickly cooled forming a stiff medium around the tissue ideal for making high quality slices. The cylinder fit snugly into a precision slicing device that made smooth cuts and permitted very controlled advance of the piston. The result was several slices of thickness less than 1 mm over the region of previous interrogation. Although this slicing procedure has proven far more effective than any other technique, the difficulties of fresh tissue led to variation in slice quality. For each sample, a series of slices was made, and the most uniformly cut sample was chosen from among these for investigation in the scanning acoustic microscope.
The transverse slicing method is beneficial for tissue characterization because all layers of the tissue are available for direct ultrasonic investigation. However, the anisotropic nature of some of the tissue types of interest raises questions of the applicability of results gathered from this method to side-looking intravascular ultrasound. Images created from this type of acquisition can look similar to IVUS images (cross sectional view), but are formed in a fundamentally different way, making direct comparison challenging and possibly misleading.

4.3.3 Summary of Tissue Preparation

Both longitudinal and transverse cuts were used for this study. As stated, each method has advantages and drawbacks. Section 4.4 detail the methods and results of ultrasonic scans of samples prepared in these two orientations.

4.4 Tissue Measurements

4.4.1 Background

Freshly excised lamb coronary artery tissue was imaged to validate the tissue preparation and the measurement system. Investigation of fresh animal tissue provides a method for anticipating the requirements of later human tissue studies. The studies below illustrate attempts to answer some of the questions regarding how best to image fresh human coronary artery tissue.
4.4.2 Methods

As discussed in Section 4.3.2, tissue was prepared in both the longitudinal and transverse orientations. To begin to study the effects of the different choices, neighboring sections from the same artery were imaged, one cut longitudinally, the other transverse.

Data Acquisition: Transverse

The choice of data acquisition mode was dependent on the orientation of the cut. To image the layers of the artery when it was cut transverse to the direction of flow, a C-scan was carried out over the cross-sectional slice. The prepared tissue was mounted in the scanning acoustic microscope by placing it on the steel plate. To ensure stability over the course of the scan, a plastic sheet was placed over the tissue. This sheet had a hole positioned over the area of interest that permitted direct ultrasonic interrogation of the sample. The sheet was secured lightly on the tissue. The tissue's natural buoyancy in the saline coupling medium caused slight rounding of the tissue over the area of the hole. Once mounted, the tissue was scanned twice in a raster pattern; once with the focus of the transducer placed slightly below the surface of the tissue, and once with the focus of the transducer on the steel plate. The first scan collected data suitable for backscatter measurements, and the second scan collected shadowed reflector data suitable for making measurements of the attenuation coefficient in the sample. These data were recorded for offline analysis. This process was carried out with the 50 MHz and 100 MHz transducers, both of which are described in Chapter
Data Acquisition: Longitudinal

For the longitudinally prepared samples, a B-scan acquisition was performed. In this configuration, the transducer was moved across the sample in what would have been the circumferential direction. The data acquired at each site along this path were assembled into a cross sectional image of the sample. Acquiring the data in this way permitted all layers of the tissue to be imaged because the sound beam penetrated down through the superficial layers into the deeper layers. For longitudinal scans, the focal point of the transducer was positioned slightly below the surface of the sample. No shadowed reflector scans were taken because of the variable amount of surrounding myocardial tissue. Longitudinal scans were made with the 50 and 100 MHz transducer, which are described in Chapter 3.

Data Analysis

For both the longitudinal and transverse tissue slices, the data were reduced to apparent integrated backscatter, a parameter widely used to characterize tissues (Mimbs et al., 1981; Barzilai et al., 1987; Milunski et al., 1989; Thomas et al., 1989; Hoffmeister et al., 1995; Finch-Johnston et al., 2000; Gibson et al., 2007). This analysis was achieved by choosing a gated region from within the signal, applying a windowing function, transforming this windowed signal into its power spectrum, and normalizing by a power spectrum from signal reflected from a stainless steel plate at the trans-
ducer’s focus. Apparent integrated backscatter images of a lamb coronary artery created from longitudinal and transverse data can be seen in Figure 4.6.
4.4 Tissue Measurements

Figure 4.6: Apparent integrated backscatter images of lamb coronary arteries created from data acquired in two orthogonal orientations with the 50 MHz transducer. The image on the left was made from data acquired with a B-scan from a sample cut longitudinally, and the image on the right was created from data acquired with a C-scan from a sample cut in the transverse orientation. Regions-of-interest indicate the location of the media and adventitia.
4.4 Tissue Measurements

This method was first verified and refined in these studies of lamb tissue, and was then used in the studies of human coronary arteries described in Chapters 6, 7, and 8. A more detailed description of the analysis algorithm is presented in Section 6.3.

Histology

Several lamb coronary artery samples were processed for histology after acoustic microscopy scanning was complete. The tissue samples were fixed in formalin, embedded in paraffin, thinly sectioned, and stained with Hematoxylin and Eosin (H&E) stain. The images of the slides prepared from these samples aided in the identification of the layers seen in the ultrasonic images. Representative images of histology slides prepared from a lamb coronary artery are seen in Figures 4.7 and 4.8. Figure 4.7 shows the entire coronary artery, which has been cut open as described in Section 4.3.2. The dotted box indicates the area shown in Figure 4.8. Because the lambs are young and healthy, the intima layer is so thin as to be nearly invisible.
Figure 4.7: Image of histology slide created by staining lamb coronary artery tissue with H&E stain. The top pink layer with black dots is the media. The adventitia is the layer below the media. Myocardium can be seen running from the center of the image to the lower right. The red dotted box indicates the area shown in Figure 4.8.
4.4.3 Results

Figure 4.6 is representative of the images that each method provides. In both orientations, a thin bright band is typically observed bordering the lumen, followed by a darker band, followed by a bright region extending farther from the lumen.

The apparent integrated backscatter images were quantified by drawing regions-of-interest in the layers identified with histology. The values of apparent integrated backscatter were averaged within these regions to yield a value for the layer. In each image, the media and adventitia were identified. The results of this analysis at the two different frequencies (50 MHz and 100 MHz) are shown in Figure 4.9. The height of each bar represents the mean of 6 lamb arteries, and the error bars represent the standard error of the mean.

4.4.4 Discussion and Conclusions

The investigations of fresh coronary artery tissue from lambs were designed and executed for two purposes: to validate methods for subsequent use on human tissue and to begin gaining an understanding of the ultrasonic properties of arterial tissue.

The lamb studies facilitated development of methods and procedures that were successfully applied to human coronary artery tissues. These methods are described in Chapter 5, and the resulting studies are described in Chapters 6, 7, and 8.

At the time the results of the lamb studies were first generated, it was unclear exactly what was being observed. The plot in Figure 4.9 shows that the adventitia is
4.4 Tissue Measurements

Figure 4.8: Zoomed in view of a section of media from lamb coronary artery histology slide stained with H&E. The media is the dense pink layer on the top. The loose layer behind the media is the adventitia. This region is noted by the red box in Figure 4.7.
4.4 Tissue Measurements

Figure 4.9: Apparent integrated backscatter from fresh lamb coronary arteries measured from two orthogonal directions over two frequency ranges. The height of the bars represents the mean of 6 samples, and the error bars represent the standard error of the mean.
brighter than the media in both orientations, and that there is very little difference between the frequency bands. Most notably, these data show no significant difference between the transverse and longitudinal orientations. Subsequent studies of human arterial data have revealed that many of these trends observed in lamb arteries are similar to those found in human arteries.

4.5 Hybrid Approach

The study of Section 4.4 involved preparing and measuring tissue in both the longitudinal and transverse orientations. However, even samples from the same artery can display variation over its length. Therefore, to better understand the ultrasonic properties of the coronary arteries, samples were prepared and imaged in the longitudinal orientation, and these same samples were subsequently prepared and imaged in the transverse orientation. This procedure, while more complicated, permits direct comparison of individual regions insonified from two orthogonal orientations.

4.5.1 Feasibility

Preliminary efforts in lamb tissue demonstrated that it is possible to image a sample of fresh coronary artery tissue in two orientations. Once demonstrated, this method became the dominant method employed in studies of coronary artery tissue. All of the data acquired for the studies of Chapters 6, 7, and 8 were collected from samples prepared in this way. Chapter 5 lays out the details of how this procedure
4.6 Other Preliminary Studies

4.6.1 Tissue Degradation

Because the ultrasonic properties of tissue depend on the tissue’s temperature, the results most relevant to living patients must be acquired near human body temperature. *In vivo*, the constant perfusion of the tissue serves to maintain homeostasis. When the tissue is removed from the living animal it begins to degrade. The concern was that the degradation of freshly excised tissue would be accelerated in the warm phosphate buffered saline in which the ultrasonic scans were performed. If significant degradation occurred, the tissue’s elastic and geometric properties might change, causing significant alteration of the ultrasonic parameters.

Procedure

To quantify the degree to which the tissue’s ultrasonic properties change over the course of a scan, two representative ultrasonic parameters from six tissue samples were measured over many hours in the heated tank. On each sample, a 49-point grid of sites was measured once an hour for at least seven hours. The grid was chosen to include several types of arterial tissue. The six samples were divided into three groups and each of the groups was studied with a transducer of nominal center bandwidth of 25 MHz, 50 MHz, or 100 MHz. Phosphate buffered saline (see Section
**4.6 Other Preliminary Studies**

Figure 4.10: Signal loss versus time in a heated water tank for six segments of lamb coronary artery tissue. Each data point represents the mean of 49 sites within the segment and the error bars represent the standard deviations of those means. Of the six segments, two each are measured with the 25, 50, and 100 MHz transducers.

3.4) was used as the coupling medium for all studies. The parameters chosen for investigation were apparent integrated backscatter and signal loss at the middle of the bandwidth. These are intermediate parameters that could be further reduced to the more fundamental backscatter coefficient and attenuation coefficient, respectively. As a result of using intermediate parameters, the overall levels remain significantly dependent on experimental factors such as tissue thickness and coupling medium attenuation. However, further reduction was not performed for this study because the relative change of the intermediate parameters provides all of the information necessary to determine the extent of tissue degradation.
4.6 Other Preliminary Studies

Figure 4.11: Apparent integrated backscatter versus time in a heated water tank for six segments of lamb coronary artery tissue. Each data point represents the mean of 49 sites within the segment and the error bars represent the standard deviations of those means. Of the six segments, two each are measured with the 25, 50, and 100 MHz transducers.
4.6 Other Preliminary Studies

Results

As can be seen in Figures 4.10 and 4.11, none of the samples showed drastic changes in either signal loss or apparent integrated backscatter over the course of the study. There is some variability as time progresses, but it is substantially smaller than the variability between sites (indicated by the error bars, which represent the standard deviations of the 49 sites). This study demonstrated that reliable measurements can be made on fresh tissue in a 37°C saline bath even if data acquisition spans several hours.

4.6.2 Tissue Freshness

Fresh lamb tissue was used as a proxy for the fresh cadaveric tissue that was studied in the work of Chapters 6, 7, and 8. For this preliminary study lamb tissue was been studied within the first three days after slaughter. In seeking to quantify the effects that the time between initial excision and ultrasonic investigation has on tissue properties, data were acquired from fresh lamb hearts on the day of slaughter, one day later, and two days later. The observations from these studies suggested that the samples’ ultrasonic properties are not drastically altered during this time. The inherent inter-sample variation among hearts did make resolving the effects of time difficult due to the relatively small number of samples.

Due to the length of time required to collect all necessary data, scans were performed on the day of slaughter and for the following two days. Because all longitudinal
4.6 Other Preliminary Studies

Figure 4.12: Bar chart showing the apparent integrated backscatter of samples measured one day after slaughter compared to (different) samples measured two days after slaughter. Bar heights represent the mean of 5 samples and the error bars are the standard error of the mean.

(B-scan) data were acquired on the day of slaughter (Day 0), the transverse (C-scan) data that were either collected on the day after slaughter (Day 1), or two days after slaughter (Day 2) have been compared. As can be seen in Figure 4.12, the integrated backscatter of hearts measured on Day 1 is very similar to that measured on Day 2. Performing Student’s T-test reveals that none of these comparisons reach a level of significance of $p < 0.05$. Although these results do not prove that no changes occur, they indicated that any changes are likely to be modest.
4.6.3 Fresh vs. Fixed

Chemical fixation by formalin (aqueous solution of formaldehyde) preserves tissues by inducing protein cross-linking. However, several studies have demonstrated that this form of fixation can cause alterations to ultrasonic tissue properties (Steen et al., 1992; Sasaki et al., 2003; Baldwin et al., 2005, 2007). The effects of formalin on coronary artery tissue remains largely unknown. To examine the relationship between the properties of tissue before and after fixation, acoustic microscope measurements were performed on freshly prepared samples, then repeated on the same samples after they had been fixed them in formalin for one week. All measurements were made with the 50 MHz transducer described in Chapter 3 and were carried out in phosphate buffered saline at 37°C. The Bland-Altman plot (Bland and Altman, 1986) in Figure 4.13 shows that formalin fixation generally increases the level of backscatter from the tissue. This study confirmed that measurements of the ultrasonic properties of coronary artery tissues should be made on fresh tissue.

4.7 Conclusions

The lamb studies described in this chapter provided the opportunity to develop and evaluate methods for preparing and measuring coronary artery tissue in ways that had not previously been attempted. The fresh lamb tissue offered the chance to validate methods without using human tissue. The methods developed from these studies were subsequently successfully applied to human coronary arteries. The data
4.7 Conclusions

Figure 4.13: Bland-Altman comparison of the apparent integrated backscatter measurements between fresh lamb coronary artery tissue and the same tissues after one week of formalin fixation. The horizontal axis shows the mean of the apparent integrated backscatter measured in the fresh and fixed samples. The vertical axis shows the difference between the apparent integrated backscatter measurements made from the fresh and formalin-fixed samples.

acquired on lamb tissue yielded results that have now been shown to be consistent with similar measurements in human tissue.
Bibliography


5.1 Introduction

Preparing appropriate samples is one of the great challenges of acoustic microscopy studies. The preliminary lamb studies of Chapter 4 developed and validated the methods explained in this chapter. The methods described herein were used on all human coronary artery samples processed in the course of this study.

5.1.1 Chapter Overview

This chapter details the methods used to collect the data that are analyzed and discussed in the later chapters of this thesis. It builds upon the work described
in Chapter 4, and specifically, uses the hybrid method of coronary artery scanning referenced in Section 4.5. The next section of this chapter, Section 5.2, lays out the coordinate system that will be used to describe the coronary arteries. Section 5.3 provides details of sample acquisition and storage. Intravascular ultrasound (IVUS) scans are discussed in Section 5.4, and acoustic microscopy scans are described in Section 5.5 (radial) and Section 5.6 (axial). Histology preparation of the samples is the topic of Section 5.7.

5.2 Definition of Coordinate System

For the sake of terminology, the coronary arteries are idealized as cylinders, and will be described in terms of a cylindrical coordinate system. As illustrated in the left panel of Figure 5.1, arterial blood flows in what will be referred to as the axial direction. The radial direction originates from the central axis and points outward in all directions. The circumferential direction is normal to both of these directions, and therefore points around the perimeter of the cylinder.

In this conception, a side-looking intravascular ultrasound (IVUS) catheter interrogates tissue in the radial direction. An IVUS catheter that projected a sound beam strictly forward would image in the axial direction, and oblique angle IVUS catheters would insonify in between the radial and axial directions.

When the cylinder of the coronary artery is splayed open as described in Section 5.3, the layered cylinder becomes a layered sheet. The naming conventions are re-
tained when the artery is in this form. As shown in the right panel of Figure 5.1, the axial direction now points along the long edge of the sheet, the circumferential direction points along the short edge of the sheet, and the radial direction points into the depth of the sheet.
5.2 Definition of Coordinate System

Figure 5.1: Graphic illustrating the coordinate system used to describe the coronary arteries investigated in the studies described in this thesis. The panel on the left shows the intact coronary artery, which is modeled as a cylinder with the blood flow in the direction of the cylinder’s axis. The figure on the right illustrates the same artery segment having been cut axially and opened. The labels from the cylindrical coordinate system are retained and mapped to the new rectangular coordinate system of the opened artery.
5.3 Specimens

5.3.1 Sample Acquisition

The samples used in this study were sections of the left anterior descending (LAD) coronary artery from human hearts collected at autopsy. All tissue was gathered and used in compliance with regulations of the Washington University Human Research Protection Office. At autopsy, the coronary artery was removed from the rest of the heart along with a substantial amount of cardiac muscle in such a way that the entire length of the artery is preserved. The remaining heart tissue provided stability and rigidity as the artery was scanned with the IVUS catheters. The samples received from the coroner were similar in size and shape to the excised lamb coronary artery shown in Figure 5.2.

In all, nineteen human left anterior descending arteries were collected, prepared, and measured between July 2009 and May 2010. Of these nineteen (19), seventeen (17) were male and (2) were female. The individuals ranged in age from 36 to 87 years with mean age of 51.7 years. The gender and age corresponding to all samples is displayed in Table 5.1.

5.3.2 Sample Storage

Even though chemical fixation seems to have minimal or mixed effects on the ultrasonic properties of some tissues, such as liver and kidney (van der Steen et al., 1991, 1992; Sasaki et al., 1996; Sasaki, 2003), there is evidence that fixatives such
5.3 Specimens

Figure 5.2: Lamb left anterior (LAD) coronary artery. The pictured vessel, along with surrounding myocardium and fat, has been dissected from the rest of the heart. The yellow pin is very close to the left main coronary artery, from which the anterior descending artery branches. Photograph by Joseph Hoffman.
5.3 Specimens

as formalin do significantly affect the properties of myocardium (Hall et al., 2000; Baldwin et al., 2007) and arterial tissue (Wilhjelm et al., 1997). Therefore, in this study, all ultrasonic scans were completed before chemically preserving the tissue for histology processing.

Some previous studies have frozen arterial tissue prior to scanning (Lockwood

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<td>19</td>
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Table 5.1: Demographic information of the individuals whose coronary arteries were studied.
et al., 1991; Saijo et al., 2007), but because each round of ultrasonic scanning in the current study was performed at 37°C, a protocol of freezing between scans would have involved several freeze/thaw cycles. This process would have required the tissue to be repeatedly transitioned from -20°C to 37°C. It was therefore determined that storing the tissue in refrigerated saline at approximately 2°C between scans would best maintain the samples during data acquisition periods. All scans were performed as soon as feasible, typically beginning one day after death, and always completed no later than 8 days after death.

5.4 IVUS Scanning

Side-looking intravascular ultrasound scans were performed with the system and catheter-borne transducers described in Section 3.5. The left anterior descending arteries were mounted in a paraffin tray by securing the surrounding tissue with pins. The side branches were ligated and occluded and the vessel was cannulated so that it could be perfused with phosphate buffered saline (see Section 3.4) at physiological temperature, pressure, and flow rate. This setup has been used previously by collaborators in this work (Nair et al., 2002, 2007).

An IVUS scan of the entire artery was performed with each catheter to identify regions of interest. Data were collected over the region of interest with an automated axial pullback, which collected cross-sectional side-looking IVUS images every 0.5 millimeters. A representative image acquired with the 45 MHz IVUS catheter can be
Figure 5.3: Intravascular ultrasound (IVUS) image of a left anterior descending coronary artery created by a 45 MHz IVUS catheter. The transducer is in the center of the image. The arrow points to the plaque. This is the same artery as is shown in Figure 5.4 and Figure 5.5.
5.4 IVUS Scanning

Figure 5.4: Intravascular ultrasound (IVUS) image of a left anterior descending coronary artery created by a 20 MHz IVUS catheter. The transducer is in the center of the image. The arrow points to the plaque. This is the same artery as is shown in Figure 5.3 and Figure 5.5.
5.4 IVUS Scanning

**Figure 5.5:** Intravascular ultrasound (IVUS) image of a left anterior descending coronary artery created by a 20 MHz IVUS catheter (same image as Figure 5.4), overlaid with Virtual Histology plaque type identification. The dark green color indicates fibrous tissue; the light green indicates fibro-fatty tissue; the red indicates necrotic core tissue; and the white indicates dense calcium.
seen in Figure 5.3. In this image, the arrow points to the plaque. The same section was imaged with the 20 MHz IVUS catheter, and an image from this scan is found in Figure 5.4. At this time, radiofrequency (RF) data were also collected which can be input into the proprietary VH algorithm (Volcano Corporation, San Diego, CA). The VH stands for virtual histology, and this algorithm uses spectral parameters of the returned signals in concert with a machine learning routine to attempt to identify plaque subtypes (Nair et al., 2001, 2002, 2004, 2007). Figure 5.5 shows the IVUS image of Figure 5.4 overlaid with the plaque subtype results from the Virtual Histology. Segments such as this one that were decided to be of particular interest were marked with a suture to guide subsequent acoustic microscopy studies. The suture was also visible on the IVUS image and served to confirm collocation of the acquired IVUS data.

All IVUS scans were performed at Volcano’s Advanced Technology Laboratory in Cleveland, Ohio. When IVUS scanning was completed, the sections of interest were rough cut out of the full artery length. The resulting segment is similar in size and shape to the rough cut segment of lamb artery seen in Figure 4.3. A suture was placed in the myocardium on the proximal side of the sample to keep track of the segment’s orientation. A rough cut section of human left anterior descending coronary artery with a suture in the proximal end is shown in Figure 5.6. The segments were sealed in jars of phosphate buffered saline, packed in ice and sent overnight to the laboratory in St. Louis, Missouri for acoustic microscopy preparation and scanning.
Figure 5.6: Rough cut section of fresh human coronary artery with surrounding tissue viewed from the proximal side. The coronary artery is seen in the opening in the upper part of the tissue. The black suture indicates that this side is the proximal side. Photograph by Joseph Hoffman.
5.5 Radial Acoustic Microscopy

5.5.1 Radial Sample Preparation

The rough-cut vessels that arrived in St. Louis had at least two sutures: one in the myocardium on the proximal side, and one or more along the top indicating sites of substantial plaque burden. Images of a rough cut segment of the segments can be seen in Figures 5.6 and 5.7. Figure 5.7 shows the external side of the sample with the suture indicating an area of substantial plaque. Figure 5.6 shows the same sample viewed from the proximal side. The suture in this image simply serves to distinguish the proximal from the distal end of the sample. The locations of the suture or sutures along the top, in combination with the previously acquired IVUS images, were used to guide further sample preparation.

Surrounding myocardium and fat were removed by making a series of cuts located progressively closer cuts to the vessel wall. This process continued until less than one millimeter of surrounding tissue remained. The distal end of the excised sample was marked with black India ink to maintain knowledge of the vessel’s orientation relative to the heart. A micro-surgical scissors was used to cut axially down the length of the artery, permitting it to be opened and laid flat. Figure 5.8 shows a human artery that has been cleaned of surrounding tissue, cut down the length of the vessel, and opened. The artery in this image was relatively free of atherosclerotic plaque, but the whitish area down the center from top to bottom does indicate some thickening of the intima.
Figure 5.7: Rough cut section of fresh human coronary artery with surrounding tissue viewed from the external surface. The suture indicates where along the vessel a substantial plaque was observed under IVUS imaging. Photograph by Joseph Hoffman.
Figure 5.8: Opened section of fresh human coronary artery. This section has been excised from most surrounding tissue, cut axial and splayed open. The intimal surface is shown. Photograph by Joseph Hoffman.
5.5 Radial Acoustic Microscopy

Figure 5.9: Human coronary artery sample mounted for radial acoustic microscopy. The tissue is visible through the window in the plastic sheet. Photograph has been digitally edited to minimize glare. Photograph by Joseph Hoffman.

5.5.2 Radial Scanning

The opened artery was placed on a stainless steel plate with the intimal surface facing up. The segment was held in place by a plastic sheet with a rectangular hole that permitted direct access to the tissue by the ultrasonic beam. A picture of the sample mounted in this way is shown in Figure 5.9.

The steel plate and mounted artery were placed in the sample tank described in Chapter 3, which had previously been filled with degassed phosphate buffered saline (see 3.4) and preheated to 37°C. In this configuration, the lumen side of the artery
### Table 5.2: Radial data acquisition settings.

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</table>

was facing upward and presented a relatively uniform surface for ultrasonic scanning. This configuration is called the radial orientation because the acoustic beam strikes the arterial tissue from the radial direction (see right panel of Figure 5.1). The data acquired in this way are analogous to IVUS data because IVUS also interrogates arterial tissue radially.

The acoustic microscopy setup that was described in Chapter 3 was configured with the settings shown in Table 5.2. With these settings, radiofrequency (RF) data were acquired from the sample and recorded. Side-looking IVUS and axial acoustic microscopy as described below create images that display a cross-sectional view of the artery. These cross sectional images display the artery in the radial/circumferential plane (see Figure 5.1). Therefore, to maximize resolution in the imaging dimension, the scan steps in the circumferential direction were chosen to be small enough that
the sound beam at successive locations overlapped by at least half of its diameter. In this configuration, the axial scan step size corresponds to the inter-slice distance, and is not related to image resolution. Therefore, the step size in the axial direction was much larger (300 µm).

5.5.3 Radial Data

On each sample investigated radially, a backscatter data scan was taken with each of the three transducers described in 3. For the backscatter data scan, the focus of the transducer was placed roughly 250 µm below the intimal surface. RF data were collected from the sound backscattered from the region near the focal point of the transducer. Before and after each backscatter data collection scan, a reference trace was collected. The reference used for all studies was the signal reflected from a stainless steel plate located at the transducer’s focal point.

Delay Line Signals

For backscatter measurements made with the 50 MHz and 100 MHz transducers, the presence of spurious signals from within the transducer’s delay line is unavoidable (see Section 3.2.1). To account for these reflections, a trace was recorded immediately after each backscatter data acquisition with all of the same settings, except that the transducer had been pulled back from the tissue by one centimeter. This movement ensured that there will be no actual backscatter in the recorded trace, and that only the signals from within the delay line are recorded. This trace was then
5.6 Axial Acoustic Microscopy

5.6.1 Axial Sample Preparation

Preparation for Automated Slicing

After the completion of the radial acoustic microscopy scans, the tissue sample was removed from the sample tank. The distal end of the sample was glued to the end of plastic piston. Figure 5.10 shows the sample glued to the piston. The piston fit snugly into an aluminum cylinder, which is also shown in Figure 5.10.

After the glue had set, the piston was lowered in the cylinder until the proximal end of the artery sample was just below the top of the cylinder as seen in Figure 5.11. The tissue was held upright with a small tweezers while liquid agarose (Sigma-Aldrich, St. Louis, MO) at a temperature of approximately 45°C was poured into the cylinder. As the agarose cooled to room temperature, it solidified. The aluminum cylinder containing the artery sample encased in solid agarose is shown in Figure 5.12.
5.6 Axial Acoustic Microscopy

Figure 5.10: Fresh human coronary artery segment affixed to plastic piston in cylinder with superglue. Photograph by Benjamin Johnson (used with permission).
Figure 5.11: Coronary artery segment attached to plastic piston with aluminum cylinder raised in preparation of agarose pouring. Photograph by Benjamin Johnson (used with permission).
Figure 5.12: Coronary artery encased in agarose inside aluminum slicing cylinder. Photograph by Benjamin Johnson (used with permission).
5.6 Axial Acoustic Microscopy

Automated Slicing

The aluminum cylinder shown in Figures 5.10, Figure 5.11, and Figure 5.12 is designed to fit tightly into the precision tissue slicer described in Chapter 3. Figures 5.13 and 5.14 show the cylinder containing the tissue affixed into the tissue slicer. Figure 5.13 shows the sample in the cutter from the front, and Figures 5.14 shows the sample and tank from the side. In these pictures, the end of the cylinder is seen to extend into a small tank. This tank is filled with saline during cutting. The tissue slicer has a microtome behind the piston that permits the user to precisely advance the piston while the aluminum cylinder remains in place. A razor blade attached to the vibration assembly is aligned with the end of the cylinder. The agarose and tissue are advanced beyond the end of the aluminum cylinder by a set amount, and then the mechanical cutting process is engaged to make a smooth cut of the tissue. The tissue and agarose plug can then be further advanced and cut again to make a slice of known thickness.

All samples in this study were cut to a thickness of either 1.0 mm or 750 µm. As many slices as possible were made from the length of the segment. The tissue slicing process had a high success rate, but did not always produce ideal results. Despite the best efforts at preparation, the tissue slicer on occasion would shred and rip the tissue rather than smoothly cut it. Each slice was examined after cutting to evaluate and record the success of the slicing operation. The best slices were chosen for use in axial acoustic microscopy scanning. At least one good slice was obtained from each
Figure 5.13: Agarose-encased coronary artery segment inside automated tissue cutting apparatus viewed from the front. The aluminum cylinder is in the center of the image, and the razor blade mounted on the cutting arm is above the sample and to the left. Photograph by Benjamin Johnson (used with permission).
Figure 5.14: Agarose-encased coronary artery segment inside automated tissue cutting apparatus viewed from the side. The aluminum cylinder is seen protruding into the cutting tank, and the razor blade mounted on the cutting arm is above the sample and to the left. Photograph by Joseph Hoffman.
Table 5.3: Axial data acquisition settings.

<table>
<thead>
<tr>
<th>Setting</th>
<th>25 MHz</th>
<th>50 MHz</th>
<th>100 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raster Step Size</td>
<td>300 ( \mu \text{m} )</td>
<td>300 ( \mu \text{m} )</td>
<td>300 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Remote Pulser Energy</td>
<td>1 ( \mu \text{J} )</td>
<td>1 ( \mu \text{J} )</td>
<td>1 ( \mu \text{J} )</td>
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<tr>
<td>Remote Pulser Damping</td>
<td>20 ( \Omega )</td>
<td>50 ( \Omega )</td>
<td>20 ( \Omega )</td>
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<td>Number of Averages</td>
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<td>Recorded Trace Length</td>
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<td>5000 points</td>
<td>5000 points</td>
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<td>Digitization Rate</td>
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</tr>
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<td>Pulse Repetition Rate</td>
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<td>5.0 kHz</td>
<td>5.0 kHz</td>
</tr>
<tr>
<td>Receiver Highpass Filter</td>
<td>1 kHz</td>
<td>1 MHz</td>
<td>10 MHz</td>
</tr>
<tr>
<td>Receiver Lowpass Filter</td>
<td>50 MHz</td>
<td>100 MHz</td>
<td>200 MHz</td>
</tr>
</tbody>
</table>

5.6.2 Axial Scanning

The samples chosen from the slicing procedure were again placed in the acoustic microscope by mounting them on the stainless steel plate. In contrast to the case of radial acoustic microscopy, the samples in this case were held down by two thin flat plastic straps, which provided more flexibility in mounting. An image of an axial sample mounted on the steel plate is shown in Figure 5.15.

Rapid Peak-Detect Scan

To identify the region on the tissue from which to acquire data, a rapid peak-detected scout scan was carried out over the entire tissue surface. This functionality was added to the acoustic microscopy system for these scans and is described in
Figure 5.15: Human coronary artery segment prepared and mounted for axial acoustic microscopy. The sample is held down to the steel plate by two black plastic straps running from left to right in the picture. A portion of the agarose disk can be seen around the tissue. Photograph by Joseph Hoffman.
Section 3.2.7. The rapid peak-detect scans were essential for correctly targeting the regions for the full data scans. The resulting images were used to register the histological images with the acoustic microscopy image as described in Section 6.4.

**Acquisition**

Using the acoustic microscopy setup that was described in Chapter 3 with the settings as indicated in Table 5.3, radiofrequency (RF) data were acquired from the sample and recorded. Because the tissue was cut in the radial/circumferential plane, which is also the plane of the desired image, a raster scan was used to acquire the data. In these raster scans, the step size was the same in both the \( \hat{x} \) and \( \hat{y} \) directions. In Table 5.3 this distance is referred to as the Raster Step Size.

### 5.6.3 Axial Data

Axial data were acquired with each of the three transducers described in Chapter 3. For each sample, two full scans were made in the axial orientation. A backscatter data scan was made that was similar to the backscatter scan made in the radial orientation (Section 5.5.3). For this scan, the focal point of the transducer was placed approximately 250 µm below the sample’s surface. The other scan was a shadowed reflector scan in which the reflection from the stainless steel plate on which the sample sits was recorded at each point. This scan provides information regarding the manner in which the tissue at the scan site attenuates the beam. Reference traces of the reflection from a stainless steel plate (unshadowed) located at the focal point of the
transducer were collected before and after each set of scans.

As is explained in Section 5.5.3, spurious signals arise from the delay line when using the 50 MHz and 100 MHz transducers. The same method described in Section 5.5.3 is used for suppressing the resulting artifacts in the axial data.

5.7 Histology

After the completion of the ultrasonic scans, slices were fixed in 10% buffered formalin for several days. They were then embedded in paraffin, sliced, and stained with movat pentachrome stain. An photograph of one of the slides prepared in this way is shown in Figure 5.16.

5.8 Summary

The methods described in this chapter represent a novel experimental technique for investigating the ultrasonic properties of fresh human coronary artery tissues from two orthogonal angles. The procedures were optimized on lamb tissue and then successfully applied to the study of human coronary artery tissue.
FIGURE 5.16: Representative image of a histology slide made from a coronary artery segment stained with movat pentachrome. The artery has been cut open as described above. The bright pink layer is the media. A substantial plaque is seen in the middle lower left.
Bibliography


Chapter 6

Wide Bandwidth Characterization of the Anisotropy of Coronary Arteries and Atherosclerotic Plaques using Apparent Integrated Backscatter

6.1 Introduction

This chapter describes studies of the apparent integrated backscatter from fresh human coronary arteries. The data that are analyzed, presented, and discussed in this chapter were acquired using the methods described in Chapter 5. As explained in Chapter 5, data were acquired in two orthogonal orientations from each sample.
This chapter compares the results measured from those two orientations. Because the radial scan configuration did not permit attenuation measurements, there is no way to reduce radial data beyond apparent (that is, not compensated for the effects of attenuation) integrated backscatter. Apparent integrated backscatter is therefore the best parameter to facilitate comparison between data acquired in the radial and axial orientations.

This limitation of radial scanning is shared by most clinical ultrasound, in which no information about the tissue’s attenuation is typically available. So, in this sense, reduction to apparent integrated backscatter is a suitable choice because it provides the greatest parallel with the clinical environment.

6.1.1 Overview of Chapter

This chapter describes the reduction of acoustic microscopy data to apparent integrated backscatter, presents the results, and discusses the implications of these results. Section 6.2 provides background information, including goals and motivation for the study. Details of the analysis of the raw data are given in Section 6.3. Section 6.4 explains the process by which the intima/plaque, media, and adventitia layers are identified, delineated, and measured. The results, discussion of the results, and conclusions are presented in Sections 6.5, 6.6, and 6.7, respectively.
6.2 Background

6.2.1 Goals and Motivations

Current clinically available intravascular ultrasound (IVUS) scans are side-looking, in the sense that if the catheter is aligned with the guidewire, a pliable wire used to navigate the arterial network, then the IVUS probe projects sound out of the “side” of the catheter. In this configuration, the ultrasound pulse originates near the center of the artery, propagates through the flowing blood, strikes the catheter wall approximately normal to its surface. With the cylindrical coordinate system of Section 5.2 and Figure 5.1, side-looking IVUS transmits and receives ultrasound in the radial direction.

The side-looking orientation has many advantages for IVUS imaging. Because the sound strikes the layers of the arteries approximately perpendicularly to the interfaces, the significant acoustic impedance difference at the boundaries make these layers relatively easy to distinguish. Additionally, the cross-sectional images it produces are straightforward to interpret.

A catheter capable of imaging in a more forward-looking direction might be able to overcome the primary limitation of the side-looking configuration, the requirement that the guidewire be able to move beyond any structure to be imaged. A forward-looking system would potentially be able to image arteries with a chronic total occlusion.

When these front-looking catheters become available, the images generated may
be quite different than what is currently seen with side-looking IVUS. Because the coronary arteries (and associated atherosclerotic plaques) have an anisotropic structure (Waller et al., 1992), the direction at which the ultrasound scatters from these structures will impact the received signals. In myocardium (Wickline et al., 1991; Gibson et al., 2007) and tendon (Hoffmeister et al., 1995), it is known that when the beam is perpendicular to the predominant fiber direction, more sound is backscattered than when the beam is parallel to this direction. Even though coronary arteries are obviously quite different from heart or tendon, the media layer of coronary arteries and many plaques have at least some fibrous character. Therefore, there is reason to expect that the apparent integrated backscatter, and by analogy clinical images, may be affected by the angle of the sound beam relative to the tissues.

6.2.2 Coordinate System

As described in Section 5.2 the coronary arteries are approximated as cylinders. In this convention, the blood flows down the axis of the artery. This direction, the direction of blood flow, will therefore be referred to as the axial direction, which in the convention of the cylindrical coordinate system would be the \( \hat{z} \)-direction. The radial direction, the \( \hat{r} \)-direction, points outward in all directions from the central axis. Finally, the circumferential direction, the \( \hat{\theta} \)-direction, is orthogonal to both of these directions and runs around the cylinder with sign specified by the right-hand-rule. This coordinate system is illustrated in Figure 5.1.
6.2 Background

6.2.3 Samples and Sites

As described in Section 5.3, nineteen fresh human left anterior descending coronary arteries were studied. From these nineteen vessels, forty-four sites were imaged in both the radial and axial orientation. Not all artery tissue subtypes (intima/plaque, media, adventitia) were visible in the images acquired from all sites. Tables 6.1 and 6.2 show a list of all sites and which tissue types were found in each. The analyses of this chapter only include those sites where each given tissue type was found. For example, because intima/plaque was only seen in 38 of 44 sites at 50 MHz, only those 38 data values are included in the results presented in Section 6.5.

Inclusion Criteria

A tissue subtype was said to be present in a sample if a substantial region-of-interest could be drawn that did not include border regions with neighboring tissue types.
Table 6.1: Part 1 of 2. Artery tissue subtypes found in each site at each axial scan frequency: Intima/Plaque (I/P), Media (M), Adventitia (A). Each artery, numbered in the order in which it was imaged, is listed in the far left column. The second column, “Site”, is the number given to each site when it is sliced for axial scanning. These numbers are used solely for record keeping. For each frequency, a “Y” means that artery tissue type was visible in the image made with that transducer. A “N” means that no substantial amount of that tissue type was present. For the Intima/Plaque (I/P) subtype, a “N” indicates that no substantial plaque was visible. Continued in Table 6.2.
6.2 Background

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Table 6.2: Part 2 of 2. Artery tissue subtypes found in each site at each axial scan frequency: Intima/Plaque (I/P), Media (M), Adventitia (A). Each artery, numbered in the order in which it was imaged, is listed in the far left column. The second column, “Site”, is the number given to each site when it is sliced for axial scanning. These numbers are used solely for record keeping. For each frequency, a “Y” means that artery tissue type was visible in the image made with that transducer. A “N” means that no substantial amount of that tissue type was present. For the Intima/Plaque (I/P) subtype, a “N” indicates that no substantial plaque was visible. Continued from Table 6.1.
6.3 Data Analysis

6.3.1 Background

Quantitative measurements of the local values of ultrasonic backscatter were reported in terms of apparent integrated backscatter, an imaging parameter that has been employed extensively for tissue characterization (Mimbs et al., 1981; Barzilai et al., 1987; Milunski et al., 1989; Thomas et al., 1989; Hoffmeister et al., 1995; Finch-Johnston et al., 2000; Gibson et al., 2007). Apparent integrated backscatter can be generated from diverse data sets and can be mapped to gray scale in a fashion that approximates the image mapping produced by clinical systems.

6.3.2 Apparent Backscatter Transfer Function

To quantitatively assess the data from the acoustic microscope scans, the power backscattered from the sample, $P_{\text{sample}}(f)$, was normalized by the power returned from an ideal reflector (highly polished stainless steel plate) located in the same location in the field, $P_{\text{steel}}(f)$:

$$ABTF_{\text{linear}} = \frac{P_{\text{sample}}(f)}{P_{\text{steel}}(f)}.$$  (6.1)

This quantity is known as the Apparent Backscatter Transfer Function (ABTF), and removes many system-dependent affects from the results. Because the reflection from the steel is much brighter than the backscatter from soft tissue, the apparent
6.3 Data Analysis

Figure 6.1: Power spectrum of the signal backscattered from a representative site (bottom, labeled “Sample”), and power spectrum of a signal reflected from a stainless steel plate (top, labeled “Reference”). Data acquired with the 50 MHz transducer.

The backscatter transfer function is commonly expressed in the log domain:

$$ABTF_{dB} = 10 \log_{10}(P_{sample}(f)) - 10 \log_{10}(P_{steel}(f)).$$  \hspace{1cm} (6.2)

6.3.3 Apparent Integrated Backscatter

To generate a single parameter from each site, and to smooth out the physiologically insignificant variations over the bandwidth, the apparent backscatter transfer function is averaged over the usable bandwidth to generate the apparent integrated backscatter (AIB):

$$AIB_{dB} = \frac{1}{\Delta f} \int_{f_L}^{f_H} ABTF_{dB}(f) \, df,$$  \hspace{1cm} (6.3)
6.3 Data Analysis

Figure 6.2: Apparent backscatter transfer function (solid line), and the average of this over the bandwidth, the apparent integrated backscatter (dotted line). This plot created from the data shown in Figure 6.1.

where the bandwidth is $\Delta f = f_H - f_L$. The high ($f_H$) and low ($f_L$) points used for each transducer are specified in Table 3.1. These points were chosen so that the bandwidths of the three transducers would connect while remaining above the -6 dB down point. The normalized power spectra of all three transducers and the -6 dB line are shown in Figure 3.3.

6.3.4 Windowing

For each scan site, a window, $W_{t_1 \rightarrow t_2}(t)$, was be applied to the received RF signal, $V(t)$, and the power spectrum was calculated by taking the magnitude squared of the
Fourier transform of this windowed signal:

\[ P(f)_{t_1 \rightarrow t_2} \propto \left| \mathcal{F} \mathcal{F} \mathcal{T} \left\{ V(t) \times W_{t_1 \rightarrow t_2}(t) \right\} \right|^2, \quad (6.4) \]

where \( W_{t_1 \rightarrow t_2}(t) \) is a Tukey window, given by

\[
W_{t_1 \rightarrow t_2}(t) = \begin{cases} 
0, & \text{if } t < t_1 \\
\sin \left[ \frac{\pi}{2} \left( \frac{t - t_1}{w (t_2 - t_1)} \right) \right], & \text{if } t_1 \leq t < \left( t_1 + \frac{w}{2} (t_2 - t_1) \right) \\
1, & \text{if } \left( t_1 + \frac{w}{2} (t_2 - t_1) \right) \leq t \leq \left( t_2 - \frac{w}{2} (t_1 + t_2) \right) \\
\cos \left[ \frac{\pi}{2} \left( 1 + \frac{t - t_2}{w (t_1 + t_2)} \right) \right], & \text{if } \left( t_2 - \frac{w}{2} (t_1 + t_2) \right) < t \leq t_2 \\
0, & \text{if } t_2 < t.
\end{cases} \quad (6.5)
\]

A Tukey window is a rectangular window with sinusoidal tapering on either end. The percentage of the window that is tapered is controlled by \( w \), which was set to \( w = 0.2 \) for the studies of this dissertation. Figure 6.3 shows such an 80% Tukey window \((w = 0.2)\) with \( t_1 = 0 \) and \( t_2 = 1000 \). The dotted lines represent the locations of the tapering. This is called an 80% Tukey window because the central 80% of the window is equal to unity.

The length of the window used depended on the transducer that had collected the data. For the 25 MHz transducer a 0.3 \( \mu \)s window \((t_2 - t_1 = 0.3 \ \mu s)\) was used, and for the 50 MHz and 100 MHz transducers a 0.15 \( \mu \)s window \((t_2 - t_1 = 0.15 \ \mu s)\) was used.
6.3 Data Analysis

Figure 6.3: Tukey window as defined in Equation 6.5 with \( w = 0.2 \), \( t_1 = 0 \), and \( t_2 = 1000 \). With these parameters, the turn-on taper ends at \( t = 100 \) and the turn-off taper begins at \( t = 900 \). These points are indicated by the dashed lines.

6.3.5 Axial Apparent Integrated Backscatter Analysis

For the axial data sets, which were acquired by raster scanning over the face of the tissue as described in Section 5.6, only one apparent integrated backscatter value is extracted from each scan line. In other words, the value for \( t_1 \) in Equation 6.5 is fixed for all traces. For each sample the window was placed approximately 200 \( \mu \text{m} \) behind the front wall of the tissue sample to avoid any ringdown from the surface. A trace showing a representative backscattered signal and window placement is shown in Figure 6.4.
6.3 Data Analysis

6.3.6 Radial Apparent Integrated Backscatter Analysis

Radial scanning as carried out in these studies insonifies intima/plaque, media, and adventitia at every scan location. The ultrasonic wave propagates through the intima/plaque to access the media, and subsequently, through the media to access the adventitia. To construct images showing all of these layers, the radial data were analyzed in a B-scan fashion. In this analysis the entire A-line was used to form the image. A sliding window approach was used, in which the apparent integrated backscatter for each point in the line was calculated as in eq. 6.3, but with the gate in a different position. The apparent integrated backscatter at each position ($AIB(t_i)$) is a function of the power spectra windowed at a corresponding location ($P_{t_i\rightarrow t_{i+n}}$) with $n$ being the number of points in the gate.
6.3 Data Analysis

For the studies of this thesis, the beginning of the gate was placed at the first point in the trace, the integrated backscatter was calculated, the gate was advanced one point, the apparent integrated backscatter for this location was calculated, and this process was repeated until the the end of the gate reached the last point in the trace. The resulting integrated backscatter line was shorter than the original trace length by the gate length. This “boxcar averaging” process, which is illustrated in Figure 6.5, was carried out for each line, and the lines were assembled into radial images. The apparent integrated backscatter values were mapped to grayscale as shown in the bottom of Figure 6.5.
Figure 6.5: Illustration of the creation of apparent integrated backscatter images from radial acoustic microscopy data. The top panel shows the raw RF data with the sliding window represented by the dashed lines. The apparent integrated backscatter calculated at every gate position generated the second plot. These values are then mapped to the grayscale shown in the bottom axes. Multiple lines like this one were assembled into images.
6.4 Segmentation

6.4.1 Registration

To generate quantitative values from the apparent integrated backscatter images, the regions in the images that corresponded to the tissue types of interest were identified. This process was guided by the histology images, such as the one seen in the bottom panel of Figure 6.6.

For the axial acoustic microscopy images, the histology images were registered with the rapid peak-detected scout scan images acquired immediately before the axial acoustic microscopy (see Section 5.6.2). Because the rapid peak-detect scan images, like the histology images, provide a view of the entire sample, registering the two is more straightforward than trying to match the acoustic microscopy image directly to the histology. A representative peak-detect scan image is shown in the top panel of Figure 6.6. The coordinates of fast peak-detect scans and the coordinates of the data scans were recorded, so that the data region could be superimposed on the fast peak-detect scan image as indicated by the blue box in the top panel of Figure 6.6.

Interpretation of the radial acoustic microscopy images was achieve more directly because the layers are more distinct, as a result of the fact that in this orientation the beam is perpendicular to the elastic laminae. For the radial images, the media is a distinct dark layer between the intima/plaque and the adventitia. This definite landmark aids in registering the images with histology.
Figure 6.6: Peak-detect scan (top panel) and histology (bottom panel) images of a human coronary artery with an atherosclerotic plaque. The box on the top panel indicates the region from which axial acoustic microscopy data were acquired. Images not on the same scale.
6.4.2 Region-of-Interest Selection

In each image, an attempt was made to identify three regions: intima/plaque, media, and adventitia. As shown in Tables 6.1 and 6.2, most images yielded some areas of all tissue types. The regions-of-interest chosen for analysis were selected based on confidence of identification. That is, only areas that could be identified with certainty were included in the regions-of-interest. Care was taken to avoid any areas near an interface between two tissue types. These areas typically contain cells from the tissue types on either side of the boundary, which confounds the results, and these regions contain membranes (elastic laminae) that can cause ultrasonic interference. Figure 6.7 shows representative regions-of-interest for axial images, and Figure 6.8 shows the regions-of-interest for the same sites in the radial direction.

6.5 Results

The apparent integrated backscatter of all the points within the regions-of-interest drawn on the images was averaged to generate a value for each tissue type. Figure 6.9 is a box and whisker plot displaying the results of this quantitative segmentation of the radial and axial acoustic microscopy images. The left panel shows the data generated from the 25 MHz transducer, with the integration carried out from 22 to 26 MHz. The center panel corresponds to the 50 MHz transducer with an integration bandwidth of 30 to 60 MHz. The right panel shows data from the 100 MHz transducer integrated from 60 to 105 MHz. In each panel, the left three data sets are the intima/
6.5 Results

Figure 6.7: Axial acoustic microscopy images at three frequency bands shown without and with regions-of-interest used for analyzing the intima/plaque (green), media (red), and adventitia. The same sites are shown in Figure 6.8.
Figure 6.8: Radial acoustic microscopy images at three frequency bands shown with regions-of-interest used for analyzing the intima/plaque (green), media (red), and (adventitia). The same sites are shown in Figure 6.7.
plaque, media, and adventitia for the radial acoustic microscopy scan, and the right three data sets are the same regions measured with axial acoustic microscopy. Each data point represents the mean ± standard deviation of the relevant sites. For the 25 MHz transducer, the number of sites included was 37, 41, and 39 for the intima/plaque, media, and adventitia, respectively. For the 50 and 100 MHz transducers, the number of sites included was 38, 44, and 42 for the intima/plaque, media, and adventitia, respectively.
Figure 6.9: Apparent integrated backscatter from all sites. Integration bandwidths are 22-36 MHz (left panel), 30-60 MHz (center panel), and 60-105 MHz (right panel). The left three data sets in each panel are the intima/plaque (green), media (red), and adventitia (blue) for the radial acoustic microscopy scan, and the right three data sets are the same regions measured with axial acoustic microscopy. Each data point represents the mean ± standard deviation of the relevant sites. For the 25 MHz transducer, the number of sites included was 37, 41, and 39 for the intima/plaque, media, and adventitia, respectively. For the 50 and 100 MHz transducers, the number of sites included was 38, 44, and 42 for the intima/plaque, media, and adventitia, respectively.
6.5 Results

6.5.1 Radial and Axial Trends

Because radial acoustic microscopy insonifies tissue in the same orientation as side-looking IVUS, it was anticipated that the typical “bright-dark-bright” pattern of backscatter from the intima/plaque, media, and adventitia, respectively would be seen in the radial acoustic microscopy data. In each of the three panels of Figure 6.9 the radial data display this pattern. For all three transducers, the backscatter is measured from the media is the smallest, and the backscatter from the intima/plaque and adventitia are significantly larger.

The backscatter from the axial scans demonstrated a markedly different pattern. In this orientation, much more backscatter was observed from the media than from the intima/plaque. The adventitia also backscattered significantly more than the intima/plaque.

6.5.2 Trends Across Frequencies

The physical properties of the beam produced by the three transducers used to generate these measurements can affect results. Aside from the attenuation in the tissue, which is not accounted for in apparent integrated backscatter, there are also affects due to the unique diffraction patterns of each transducer. These features make inappropriate direct comparison of values of apparent integrated backscatter between transducers and between orientations. Nevertheless, relative comparisons of the trends observed between points can be made.
6.5 Results

The same basic patterns are seen at each frequency range in Figure 6.9, but there are also some trends observed as the frequency rises. For the intima/plaque and media, there seems to be a slight rise moving from 25 MHz to 50 MHz to 100 MHz. Conversely, there seems to be a slight decrease in the apparent integrated backscatter from the adventitia moving from 25 MHz to 50 MHz to 100 MHz. For the most part, these trends act independently of orientation.

6.5.3 Trends by Layers

To observe the effects of insonification direction on the individual layers of the coronary artery tissue, all data values acquired at each frequency were grouped together for each tissue type. These pooled data are displayed on the histograms seen in Figures 6.10, 6.11, and 6.12. Figure 6.10 shows overlapping histograms for the radial and axial data collected at all frequencies from the adventitia. Although the distributions in this plot are significantly different, there is a substantial amount of overlap. Likewise with the distributions of Figure 6.11, which show the radial and axial apparent integrated backscatter from the media at all frequencies. There is separation between the distributions, but also substantial overlap. The histograms for the apparent integrated backscatter from the intima/plaque (Figure 6.12) are very well separated and overlap very little. The axial apparent integrated backscatter from the intima/plaque is very significantly lower than the apparent integrated backscatter from this same layer in the radial orientation.
Figure 6.10: Histogram of the apparent integrated backscatter from the adventitia at all sites. Solid bars represent sites imaged axially, and open bars indicate sites imaged radially. Data from all three transducers are included, with each transducer’s acquisition being counted as a separate site.
Figure 6.11: Histogram of the apparent integrated backscatter from the media at all sites. Solid bars represent sites imaged axially, and open bars indicate sites imaged radially. Data from all three transducers are included, with each transducer’s acquisition being counted as a separate site.
6.5 Results

**Figure 6.12:** Histogram of the apparent integrated backscatter from the intima/plaque at all sites. Solid bars represent sites imaged axially, and open bars indicate sites imaged radially. Data from all three transducers are included, with each transducer’s acquisition being counted as a separate site.
6.6 Discussion

6.6.1 Clinical Implications

Side-looking IVUS scans are routinely performed in the coronary arteries to assess atherosclerosis and plaque progression, and increasingly to quantify plaque types for evaluation of risk individual lesions have to rupture. A review of the literature indicates that intravascular ultrasonic devices are being developed that will permit imaging in other orientations (Back et al., 1994; Evans et al., 1994; Gatzoulis et al., 2001; Yeh et al., 2006). When these devices become available for clinical use, it seems likely that some of the intuitions developed from side-looking IVUS will require reevaluation (Courtney et al., 2008).

The apparent integrated backscatter images created in this study by imaging fresh coronary arteries using radial scans correspond very well with traditional side-looking IVUS images, in which the media backscatter less sound than the intima/plaque and adventitia. One might therefore anticipate that integrated backscatter images created by axially scanning coronary arteries would provide some additional insight to the forward-looking IVUS catheters of the near future. Angles between front-looking and side-looking are expected to yield results somewhere between these two extreme cases.

Specifically, this study indicates that in the axial direction, the coronary artery layers are likely to be resolvable, but will probably appear quite different than they appear in the current side-looking format. The media, typically dark in side-looking IVUS, may be slightly brighter under forward-looking IVUS. Because the adventitia
seems likely to scatter as brightly in the axial direction as in the radial direction, the contrast between the media and adventitia is likely to be similar in forward-looking IVUS as it is in side-looking IVUS; however, this resolution may be somewhat frequency dependent. In any case, resolving the internal and external elastic lamina should be possible, and therefore, the layers should be distinctly visible.

One of the benefits that may come about from forward-looking IVUS is a potential substantial improvement in plaque quantification. One currently available method for plaque-type discrimination relies on a decision-tree method in which many spectral parameters are used to distinguish plaques (Nair et al., 2001, 2002, 2004, 2007). Adding an additional orientation of investigation (i.e. forward-looking IVUS), adds new parameters, and permits comparisons between radial and axial measurements of the same region. Because many of the tissues in arteries are highly directional, anisotropy is likely to be particularly useful for distinguishing tissue types. It should be noted that the axial scans of this study represent the extreme limit of angle-dependent ultrasonic imaging, and that in vivo scans would likely be executed at an oblique angle. Although the current study does not provide a direct analogy to studies at these acute angles, the significance of the changes observed over a ninety degree rotation implies that there will be a measurable impact if the imaging angle varies from normal to the arterial wall.
6.6 Discussion

6.6.2 Ultrasonic Anisotropy

The results presented above (specifically Figures 6.11 and 6.10) suggest that from 22 MHz to 105 MHz, the anisotropy of apparent integrated backscatter of the media and adventitia is modest. The anisotropy of the apparent integrated backscatter from the atherosclerotic plaque, on the other hand, is quite substantial (see Figure 6.12). These results are consistent with a model of the coronary artery in which the media is composed of fibers running circumferentially, and hence scatters similarly in the radial and axial directions, and in which the adventitia is a loose network with little order. In this simple model, the plaque would therefore exhibit some predominant orientation longitudinally down the artery. The fibrous components of the atherosclerotic plaques tend to be somewhat random, but these results suggest that there may be a global preference to orient down the axis of the artery.

6.6.3 Correspondence with Previous Work

As shown in Figure 6.9 and Figure 6.10, apparent integrated backscatter from the adventitia was similar in the radial and axial directions. This finding is consistent with previous work of de Kroon et al. (1991a,b), and with the idea that the loose connective tissue that forms the adventitia is not preferentially oriented in the axial or radial directions.

The results of some aspects of this study do not appear to parallel the results of earlier work in femoral arteries of Lockwood et al. (1991). In that study, it was
reported that in both the radial and axial orientations, thickened intima scattered less than media, which, in turn, scattered less than adventitia. The current work indicates that this trend holds in the axial orientation, but not in the radial orientation. This apparent disagreement may be attributable to the many differences between that work and the current study. Later work from the same group on coronary arteries (as opposed to femoral arteries) investigated radially does parallel the results of the current work and is consistent with the bright-dark-bright layered pattern seen in side-looking IVUS (Machado and Foster, 2001; Machado et al., 2002).

### 6.6.4 Limitations

The methods and therefore results of this study are subject to some practical limitations. The trends observed seem robust, but the relatively small sample size must be considered when interpreting these results. Additionally, precise preparation and positioning of minute tissue samples in very specific orientations across numerous imaging modes poses challenges to the collocation of data from these scans.

The time required for data collection necessitates that studies be performed over several days. No gross changes in tissue properties were observed over this period, but small changes cannot be ruled out.

Because this study attempts to make measurements pertinent to clinical intravascular ultrasound imaging, the apparent integrated backscatter results presented have not been compensated for the attenuation of overlying layers. The attenuation properties of these samples will be discussed in Chapter 7. The comparison most affected
by attenuation is between the axial and radial data for the media and adventitia. The current experimental configuration is such that these layers must be measured through overlying layers in the radial orientation, but directly in the axial direction. In a clinical setting, the media and adventitia cannot be measured directly, and the presence of overlying tissue may further alter the appearance of these layers. Additional clinical complications may arise from the presence of flowing blood and the variability of plaque composition and thickness.

Because calculation of the apparent integrated backscatter does not require one to compensate for diffraction effects, the different focal characteristics of the catheter-borne transducer array and the acoustic microscopes single element transducer can impact the results. Furthermore, the diffraction field effects also differ when using the same transducer in either a radial or axial orientation. This difference arises because the same region of tissue encounters different parts of the interrogating field in each image-forming mode (B-mode for radial; C-mode for axial).

6.7 Summary and Conclusions

Freshly excised human coronary arteries were imaged with three ultrasound modes. Two of these modes, side-looking IVUS and radial acoustic microscopy, insonify arterial tissue from the lumen through the intima into the deeper layers. Apparent integrated backscatter images made from the radial acoustic microscopy data display the same relative layer brightness as side-looking IVUS. In contrast, the third mode,
axial acoustic microscopy, generated images with a reversal of the relative brightness between the media and the intima/plaque layer. This unexpected result, the magnitude of which is substantial enough to alter image appearance qualitatively, may prove to be valuable for image interpretation and tissue characterization as forward-looking or oblique angle IVUS systems become clinically available.

Additionally, these results give insight into the fundamental anisotropic properties of coronary artery tissue components. Knowledge of these properties may shed light on the pathogenesis, progression, and clinical management of coronary atherosclerosis.
Bibliography


Chapter 7

Ultrasonic Attenuation of Coronary Artery Tissues and Associated Atherosclerotic Plaques

7.1 Introduction

This chapter describes studies of the manner in which ultrasound attenuates as it propagates through fresh human coronary arteries. The parameter used to quantify each tissue layer’s ability to attenuate sound is the attenuation coefficient, a frequency-dependent measure of the loss of signal amplitude as function of distance traveled in a given medium. The data that are analyzed, presented, and discussed in
7.2 Background

this chapter were acquired using the methods described in Chapter 5. As explained in that chapter, data were acquired in two orthogonal orientations from each sample. Unlike Chapter 6, which reported values of an uncompensated parameter (apparent integrated backscatter), this chapter presents absolute measurements (the attenuation coefficient). Measurement of this intrinsic property comes at the cost of data from the radial orientation, which cannot be fully reduced to the attenuation coefficient with the currently available measurement system. Therefore, this chapter will concentrate entirely on data acquired in the axial configuration.

7.1.1 Overview of Chapter

Along with background information relevant to the attenuation coefficient in coronary artery tissues and atherosclerotic plaques, background details of the measurements are presented in Section 7.2. Section 7.3 discusses the methods employed for data analysis and reduction. Measurements of the sample thickness and speed of sound are explained in Section 7.4. Image segmentation and region-of-interest quantification are the topics of Sections 7.5 and 7.6, respectively. The results generated from these studies are presented and discussed in Section 7.7.

7.2 Background

The attenuation coefficient plays a significant role in shaping the ultrasound images created by all laboratory and clinical scans, including intravascular ultrasound
(IVUS). Because the attenuation of sound with increasing distance limits penetration depth at a given frequency, it is the attenuation coefficient that is primarily responsible for dictating which frequencies can be used to image a given structure. In that sense, attenuation of the sound within the medium of interest is the controlling factor for imaging system resolution and performance. If a medium could be found with an attenuation coefficient of zero, arbitrarily high frequencies would be used to generate images of high resolution.

Contrast in clinical backscatter images is generated because some regions within the tissue backscatter more sound and are mapped to “brighter” pixels. When interpreting the resulting images, the observer can discern structures based on this local contrast, but the presence of attenuation prevents the intrinsic backscatter properties from being mapped to the image. Few conclusions can be drawn about non-adjacent regions of equal brightness on a clinical ultrasound image, because the differing paths the sound beam followed to get to and from those locations subjected the beam to different amounts of frequency-dependent attenuation. For this reason apparent integrated backscatter is an excellent parameter for analyzing clinical data from which information about the attenuation is not available. Apparent integrated backscatter permits comparison within and among similar images, but it cannot easily provide meaningful comparisons among results acquired under different experimental conditions.
7.2 Background

7.2.1 Motivations and Goals

Attenuation in Intravascular Ultrasound

In intravascular ultrasound (IVUS) the effect of attenuation is complicated by the layered nature of vascular tissue. Images and measurements of the deeper layers are influenced by the intervening layers. These layers can vary tremendously from person to person, and from site to site within the same person. If the artery also has atherosclerotic plaque, that buildup presents another inhomogeneous layer through which the sound must travel. When imaging patients, the sound must also travel through the flowing blood, which significantly attenuates ultrasound at intravascular ultrasound frequencies.

As demanding as the situation sounds, there is also hope. Compared to other ultrasonic imaging applications such as echocardiography, intravascular ultrasound presents relatively manageable attenuation. Unlike echocardiography, the coupling is consistent, the entire path length is visualized, and there is a known number of layers. Unraveling the attenuation properties of all the intervening structures between the transducer and the moving heart in echocardiography is a nearly impossible task. In IVUS, a knowledge of the attenuation coefficients of the plaque, intima, media, adventitia, and blood, along with the thicknesses of each might be enough to fully compensate for the effects of attenuation in the images.

The studies of this chapter were undertaken, in part, as a step toward this goal. Currently, little is known about the attenuation of the tissues that make up coronary
7.2 Background

arteries, and the work herein begins to address that lack of knowledge.

**Attenuation for Tissue Characterization**

The other significant motivation for investigating the attenuation properties of coronary arteries is to identify and measure the fundamental physical properties of the constituent tissues. Attenuation has been shown to be an effective means of studying, among other things, the anisotropy of myocardium (Baldwin *et al.*, 2006), the fiber structure of fetal hearts (Gibson *et al.*, 2008), the evolution of protein cross linking (Hall *et al.*, 2000; Baldwin *et al.*, 2007), and the nature of atherosclerotic plaques in human aortae (Bridal *et al.*, 1997). Accurate measurements of the attenuation coefficients of coronary may provide insight into the intrinsic structure of coronary artery tissue, and specifically may shed light on the mechanisms of atherosclerotic plaque growth and development.

**Attenuation for Plaque Identification**

As described in Chapter 2, efforts are being made to identify plaque and coronary artery tissue with ultrasound in the clinical setting. Understanding the attenuation properties of these tissues may improve existing techniques, but may also lay the foundation for new techniques that incorporate the attenuation properties into the identification algorithms.
7.2 Background

7.2.2 Coordinate System

The same coordinate system and naming convention are maintained as were employed in previous chapters. As described in Section 5.2 the coronary arteries are approximated as cylinders. In this convention, the blood flows down the axis of the artery. This direction is referred to as the axial direction. The radial direction points outward in all directions from the central axis. The circumferential direction is orthogonal to both of these directions and runs around the cylinder. This coordinate system is illustrated in Figure 5.1.

7.2.3 Samples and Sites

As described in Section 5.3, nineteen fresh human left anterior descending coronary arteries were studied. From these nineteen vessels, forty-four sites were imaged in both the radial and axial orientation. The studies of this chapter will make use only of the axial data. Not all artery tissue subtypes (intima/plaque, media, adventitia) were visible in the images acquired from all sites. Tables 6.1 and 6.2 show a list of all sites and indicate which tissue types were found in each. The analyses of this chapter include only those sites in which all three tissue types were found. For the 50 and 100 MHz transducers, 37 sites were found to have all three layers. For the 25 MHz transducer, 36 sites demonstrated all three layers. All results presented in Section 7.7 are generated from these sites.
7.3 Data Analysis

7.3.1 Background

Propagation of a plane acoustic wave through a medium can be expressed as

\[ y(x, t) = e^{i(kx - \omega t)} e^{-\alpha x} \]  \hspace{1cm} (7.1)

where \( y(x, t) \) can represent either the particle velocity or the pressure at a spatial location, \( x \), at an instant in time, \( t \). For this formulation, and assuming a linear system, the characteristics of a wave at a given frequency, \( \omega = 2\pi f \), are entirely determined by the propagation medium’s intrinsic properties contained in the wave number, \( k \), and the attenuation coefficient, \( \alpha \). The first exponential in Equation 7.1 oscillates with variation in \( x \) and \( t \) and controls the wave’s propagation. The second exponential provides damping with propagation distance, with the magnitude of the loss dictated by the attenuation coefficient. This parameter, \( \alpha \), is the focus of the remainder of this chapter.

The attenuation coefficient depends on frequency, and for soft tissues this dependence has been observed to be approximately linear over a wide range of frequencies (Goss et al., 1978; Mimbs et al., 1980; Mottley and Miller, 1990; Tu et al., 2003; Baldwin et al., 2006, 2007; Gibson et al., 2008). If the attenuation coefficient is assumed to rise linearly with frequency

\[ \alpha(f) = \beta f. \]  \hspace{1cm} (7.2)

The value \( \beta \) is called the slope of attenuation, and if the assumption underlying
Equation 7.2 is valid, $\beta$ provides a complete description the medium’s attenuating properties.

### 7.3.2 Axial Scan Data

Section 5.6.3 explains that for each sample two full scans were made of the tissue in the axial orientation. For the first scan, the focal point of the transducer was placed slightly below the surface of the tissue and the sound backscattered from the region near the focal point was recorded. The data from this scan were used to estimate properties related to backscatter from within the tissue. The other scan was acquired over the exact same region but with the focal point placed on the stainless steel reflector. The acquired data consisted of the signal reflected from the steel but shadowed by the tissue, rather than the backscattered signals from within the sample. Figure 7.1 shows a schematic of the shadowed reflector experiment. The left side represents the reference trace and the right side represents collecting the signal reflected from the steel plate with the tissue placed in the path. The shadowed reflector scans were acquired with all three transducers described in Section 3.2.1.

### 7.3.3 Attenuation Coefficient Determination

The attenuation coefficient is determined by comparing the shadowed reflector scan with a reference trace which has been reflected from a steel plate at the same location, but with only coupling medium in the path. The power spectrum of this
Figure 7.1: Experimental configuration of the shadowed reflector measurements described in this chapter. On the left, a reference trace is collected by positioning the transducer so that the distance from it to the steel reflector (d) is equal to the focal length. On the right, the shadowed reflector measurement records the reflection from the steel plate with the tissue sample interposed between the transducer and the reflector. The thickness of the tissue is represented by x.
received reference signal, \( P_{ref}(f) \), is given by

\[
P_{ref}(f) = S(f) \cdot \Gamma(f) \cdot \left( e^{-\alpha_{host}d} \right)^2 \cdot R_{h \rightarrow r}^I \cdot \left( e^{-\alpha_{host}d} \right)^2,
\]  

(7.3)

where \( f \) is the frequency, \( S(f) \) is the power spectrum of the transmitted pulse, \( \Gamma(f) \) represents the frequency-dependent measurement system effects, \( \alpha_{host} \) is the attenuation coefficient of the host medium (phosphate buffered saline for all studies of this thesis), \( d \) is the distance between the transducer and the reflector (see Figure 7.1), and \( R_{h \rightarrow r}^I \) is the intensity reflection coefficient from the host medium to the reflector.

The attenuation term appears twice to indicate that the attenuation acts as the pulse travels from the transducer to the receiver, and again during the return trip. Each attenuating term is squared because the equation is written in terms of power rather than amplitude. More succinctly, Equation 7.3 can be written

\[
P_{ref}(f) = S(f) \cdot \Gamma(f) \cdot e^{-4\alpha_{host}d} \cdot R_{h \rightarrow r}^I.
\]  

(7.4)

When the sample is inserted into the path, shadowing the reflector as illustrated in the right side of Figure 7.1, the power received becomes

\[
P_{shadowed}(f) = S(f) \cdot \Gamma(f) \cdot \left( e^{-\alpha_{host}(d-x)} \right)^2 \cdot T_{h \rightarrow s}^I \cdot \left( e^{-\alpha_{sample}(x)} \right)^2 \cdot R_{s \rightarrow r}^I \cdot \left( e^{-\alpha_{sample}(x)} \right)^2 \cdot T_{s \rightarrow h}^I \cdot \left( e^{-\alpha_{host}(d-x)} \right)^2,
\]  

(7.5)

where \( x \) is the thickness of the sample, \( T_{h \rightarrow s}^I \) is the intensity transmission coefficient going from the host to the sample, \( T_{s \rightarrow h}^I \) is the intensity transmission coefficient going from the sample to the host, \( R_{s \rightarrow r}^I \) is the intensity reflection coefficient from the sample to the reflector, and \( \alpha_{sample} \) is the frequency-dependent (amplitude) attenuation.
7.3 Data Analysis

coefficient of the sample. As before, the exponential terms are squared because this equation is written in terms of power. Each attenuation term appears twice because of the round trip. Two transmission coefficients appear because the sound crosses the boundary between host and sample on the way to the reflector and the same boundary in the opposite direction on the return trip. Equation 7.5 can be simplified by combining exponentials and by realizing that $T_{2→1} = T_{I_2→I_1}$ because of the symmetry in the definition

$$T_{I_2→I_1} = \frac{4|Z_1 \cdot Z_2|}{|Z_1 + Z_2|^2}$$

(7.6)

where $Z_1$ and $Z_2$ are the complex acoustic impedances of the two layers. Equation 7.5 now becomes

$$P_{\text{shadowed}}(f) = S(f) \cdot \Gamma(f) \cdot e^{-4\alpha_{\text{sample}}(x)} \cdot e^{-4\alpha_{\text{host}}(d-x)} \cdot (T_{h→s})^2 \cdot R_{I_h→I_r}$$

(7.7)

Dividing the shadowed power spectrum $P_{\text{shadowed}}(f)$ from the reference power spectrum $P_{\text{ref}}(f)$ eliminates the effects described by the term $\Gamma(f)$, and the dependence on the transmitted pulse power spectrum, $S(f)$, yielding

$$\frac{P_{\text{ref}}(f)}{P_{\text{shadowed}}(f)} = e^{-4\alpha_{\text{host}} d} \cdot e^{4\alpha_{\text{sample}}(x)} \cdot e^{4\alpha_{\text{host}}(d-x)} \cdot \frac{R_{h→r}}{(T_{h→s})^2 \cdot R_{I_h→I_r}}$$

(7.8)

The exponentials can be rearranged and simplified to eliminate $d$

$$\frac{P_{\text{ref}}(f)}{P_{\text{shadowed}}(f)} = e^{4x(\alpha_{\text{sample}} - \alpha_{\text{host}})} \cdot \frac{R_{h→r}}{(T_{h→s})^2 \cdot R_{I_h→I_r}}$$

(7.9)

Taking the natural log of both sides gives

$$\ln\left(\frac{P_{\text{ref}}(f)}{P_{\text{shadowed}}(f)}\right) = 4x(\alpha_{\text{sample}} - \alpha_{\text{host}}) + \ln\left(\frac{(T_{h→s})^2 \cdot R_{I_h→I_r}}{R_{h→r}}\right)$$

(7.10)
Solving for the attenuation coefficient of the sample yields

\[ \alpha_{\text{sample}} = \alpha_{\text{host}} + \frac{1}{4x} \cdot \ln \left( \frac{P_{\text{ref}}(f)}{P_{\text{shadowed}}(f)} \cdot \frac{(T_{h\rightarrow s})^2 \cdot R_{s\rightarrow r}^l}{R_{h\rightarrow r}^l} \right) \]  \hspace{1cm} (7.11)

Equation 7.11 expresses the attenuation coefficient in its natural units of inverse distance (typically cm\(^{-1}\) or mm\(^{-1}\)), but the attenuation coefficient is typically calculated by performing a log-domain subtraction of the power spectra expressed in decibels. To recast this equation, the base-e logarithm (\(\ln\)) in Equation 7.11 is converted to a base-10 logarithm (\(\log\)) using standard logarithm rules

\[ \ln a = \log_{10} a = \log a. \]  \hspace{1cm} (7.12)

Rewriting 7.11 as

\[ \alpha_{\text{sample}} = \alpha_{\text{host}} + \frac{1}{4x} \cdot \frac{\ln 10}{10} \cdot 10 \log \left( \frac{P_{\text{ref}}(f)}{P_{\text{shadowed}}(f)} \cdot \frac{(T_{h\rightarrow s})^2 \cdot R_{s\rightarrow r}^l}{R_{h\rightarrow r}^l} \right) \]  \hspace{1cm} (7.13)

permits the power spectra to be expressed in decibels

\[ \alpha_{\text{sample}} = \alpha_{\text{host}} + \frac{\ln 10}{20} \cdot 10 \log \left( \frac{P_{\text{ref}}^\text{dB}(f) - P_{\text{shadowed}}^\text{dB}(f)}{(T_{h\rightarrow s})^2 \cdot R_{s\rightarrow r}^l} \right). \]  \hspace{1cm} (7.14)

### 7.3.4 Reduction to Attenuation Coefficient

**Shadowed Reflector to Signal Loss**

Equation 7.14 was used to reduce the acquired shadowed reflector data to the attenuation coefficient. Figures 7.2 and 7.3 demonstrate the steps involved in the reduction algorithm for a representative region-of-interest. The top panel of Figure
7.3 Data Analysis

7.2 shows in light gray the power spectra from many nearby scan sites within a representative segment of atherosclerotic plaque. Because there is substantial variation from site to site, even within the same tissue type, the power spectra were averaged to generate a global value for the tissue of interest. The averaged power spectrum is the thick curve in the top panel. The middle panel of Figure 7.2 repeats the averaged shadowed reflector power spectrum and includes the reference power spectrum obtained from a reflection from the unshadowed steel plate. All power spectra in the first two plots are expressed in decibels. As guided by Equation 7.14, the shadowed reflector power was subtracted from the reference power to generate the frequency-dependent signal loss plotted in the bottom panel of Figure 7.2.

**Signal Loss to Attenuation Coefficient**

The signal loss (see bottom panel of Figure 7.2) is an intermediate result that incorporates the loss incurred both in the bulk and at the interfaces, and is uncompensated for the attenuation induced in the coupling medium. To remove the effects of the interfaces and to identify the per length bulk loss, the final term in Equation 7.14 involving reflection and transmission coefficient was subtracted from the signal loss and the result was divided by the path length ($2x$, in this case 2 mm). This process yielded the plot in the top panel of Figure 7.3, which would be the attenuation coefficient in decibels per millimeter if the attenuation coefficient of the host medium were negligible. To account for the non-negligible attenuation of phosphate buffered saline at the frequencies of these studies, the attenuation coefficient of the
7.3 Data Analysis

host medium (in $dB \cdot mm^{-1}$) (see middle panel of Figure 7.3) was added to the curve in the top panel of Figure 7.3 to produce the attenuation coefficient of the sample, shown in the bottom panel of Figure 7.3. As expected the resulting attenuation coefficient is fit well with a straight line over the bandwidth. The attenuation coefficient can be transformed to units of $mm^{-1}$, or equivalently nepers per centimeter ($Np \cdot mm^{-1}$), by multiplying by the conversion factor $(\frac{\ln 10}{20} \approx \frac{1}{8.69})$.

7.3.5 Attenuation Coefficient Images

To create images based on the attenuation coefficient, it can be calculated at every scan site individually, rather than averaging nearby sites together as shown above. Performing the analysis at individual sites is not optimal for determining tissue properties, but can produce useful images that visually indicate the samples’ attenuation properties. Such images were created for every segment with each transducer. Because each calculation produces the attenuation coefficient as a function of frequency, the mapping to grayscale was carried out with two different (but related) parameters extracted from the attenuation: the slope of the linear fit over the bandwidth, and the value of this fit at the middle of the bandwidth. Ideally, these parameters would produce identical images because the ideal fit line would go through zero. However, because of the variability inherent in performing the analysis at a single scan location, not all fit lines crossed the 0 MHz point at zero. Representative images of the slope of the attenuation coefficient and the midband fit to the attenuation coefficient can be seen in Figure 7.4 for all three frequency bands.
Figure 7.2: Reduction of shadowed reflector signals to Attenuation Coefficient, Part 1 of 2. The top panel illustrates averaging the power spectra from nearby sites (gray) to generate an average shadowed reflector power spectrum (black). The middle panel shows the average shadowed reflector power spectrum (black) and the reference power spectrum from the reflection from the steel plate (blue). The bottom panel is the total signal loss (purple), which was determined by subtracting the shadowed reflector power spectrum from the reference power spectrum.
Figure 7.3: Reduction of shadowed reflector signals to Attenuation Coefficient, Part 2 of 2. The top panel is the normalized signal loss, which has been compensated for the effects of interface losses and has been normalized by the path length in the tissue. The middle panel is the accepted theoretical value for the attenuation coefficient in the coupling medium. The solid green line in the bottom panel is the result of adding the top and bottom panels, and is the attenuation coefficient of the sample. The dotted line is a linear fit over the bandwidth. The slope of this fit line is displayed on the graph.
Figure 7.4: Images of a human coronary artery and atherosclerotic plaque derived from the attenuation coefficients at three frequency ranges. The left column of images is the slope of attenuation and the right column is midband value of the linear fit to the attenuation coefficient. All images are of the same sample, but registration between images acquired by the three transducers may not be exact.
7.4 Sample Thickness and Sound Velocity

7.4.1 Thickness Uncertainty

The automated tissue slicing process described in Section 5.6 permitted selection of slice thickness; however, the soft and yielding nature of the samples caused the overall slice thickness to vary from the target thickness, and local inhomogeneous in stiffness caused variations in thickness over the slice. In general, slices tended to be thicker than intended, and typically thinner near the intima than near the adventitia. It is speculated that the fatty nature of the tissue adjacent to the adventitia presented a greater challenge to the automated slicer than did the more fibrous and rigid tissue near the plaque and intima.

As seen in Equations 7.11 and 7.14, the thickness of the sample is related to the attenuation coefficient in such a way that an error in the thickness is directly propagated into the final value of the attenuation coefficient. For these reasons, it was not assumed that samples were cut to the precise thicknesses intended by the automated slicer.

7.4.2 Thickness Measurement

Direct measurement by mechanical means of the tissue thickness is impossible because of the small size and semisolid consistency of the samples. Therefore, the thickness was measured ultrasonically using a method describe by Sollish (1979). This method can be formulated in many ways, but with the experimental configuration
of this study, the tissue thickness was measured by comparing the times of flight of an ultrasonic signal that reflected from the top surface of the tissue and a signal of the unshadowed reflection from the steel plate. Using these two timing marks, the sample thickness ($x_{\text{sample}}$) was determined

$$x_{\text{sample}} = \frac{v_{\text{host}}}{2} (t_{\text{ref}} - t_{\text{front}}),$$  

(7.15)

where $v_{\text{host}}$ is the speed of sound in the coupling medium (in this case, phosphate buffered saline), $t_{\text{ref}}$ and $t_{\text{front}}$ are the round trip times-of-flight from reflections from the unshadowed reflector and the front of the sample, respectively.

### 7.4.3 Velocity Measurement

Inclusion of a third timing mark, the round trip time-of-flight for the shadowed reflection from the steel plate ($t_{\text{back}}$), permitted determination of the speed of sound in the sample. Because the tissue was sitting on the steel plate, the reflection from the steel was equivalent to the reflection from the back wall of the sample. With this timing mark, as well as the sample thickness found with Equation 7.15, the speed of sound was calculated

$$v_{\text{sample}} = \frac{2x_{\text{sample}}}{t_{\text{back}} - t_{\text{front}}} = v_{\text{host}} \left( \frac{t_{\text{ref}} - t_{\text{front}}}{t_{\text{back}} - t_{\text{front}}} \right).$$  

(7.16)

### 7.4.4 Timing Marks

The timing marks were identified from the data acquired for backscatter analysis, because these data included the entire sample’s thickness rather than just the reflec-
tion from the shadowed reflector. For each sample, a B-mode image was created from the backscatter data so that a two-dimensional slice of the sample could be visualized.

Because of the volume of data acquired for this study was so large (approximately 1.5 million axial scan locations), the front and back wall timing marks could not be estimated manually. A semiautomated algorithm was created that made initial guesses as to the location of these landmarks, and then permitted operator-interactive adjustment to verify correct identification. The algorithm located the back wall by seeking out the highest level of backscatter in each trace, which in most cases was from the steel plate. It then checked that the timing marks selected at all sites were within a fixed number of standard deviations from the mean location within the sample. The specific number of standard deviations was adjusted by the user. This check compensated for any spurious large signals. The front wall detection required the user to input the approximate number of points in the record before the tissue was encountered. This value was called the offset. The algorithm calculated the average value of the points within the offset (presumably random noise), and then looked for the first site beyond the specified offset that had a value more than a fixed number of standard deviations above this mean. Again, the specific number of standard deviations was adjusted by the user. The algorithm then performed a user-specified number of passes of a two dimensional binomial filter over the sample to assure continuity of the estimated surfaces.
Figure 7.5: Images of a human coronary artery and atherosclerotic plaque derived from the attenuation coefficients at three frequency ranges with segmentation overlaid. The left column of images is the slope of attenuation and the right column is midband value of the linear fit to the attenuation coefficient. In each image, the intima/plaque, media, and adventitia are indicated by green, red, and blue lines, respectively.
7.5 Image Segmentation

Identification of the regions (intima/plaque, media, and adventitia) within the images was carried out in the manner described in Section 6.4. The regions were initially identified on the histology images (see Figure 6.6), and these images were compared to the rapid peak-detect images (see Section 5.6.2) acquired from the same sites. Because the peak-detect images were acquired in the acoustic microscope, the recorded scan coordinates permit registration with the attenuation coefficient images. Registering the attenuation coefficient-based images with the peak-detect images is more straightforward than registering with backscatter-based images because the peak-detect process is essentially a simplified shadowed reflector scan. Therefore, the peak-detect images are based on the same information that the slope of attenuation and midband fit to the attenuation coefficient images are based on.

Once the tissue layers (intima/plaque, media, and adventitia) had been identified in the images, a region-of-interest was drawn in each layer. The regions-of-interest were selected such that the included pixels were unambiguously part of the targeted layer. Boundary regions between layers were avoided. Portions of the media layer that were shown by histology to have plaque infiltration were also avoided. Figure 7.5 shows representative regions of interest superimposed on the attenuation coefficient-based images.
7.6 Region Quantification

To obtain the most accurate values of the attenuation coefficient within each region-of-interest, the received power spectra from all of the sites within the region of interest were averaged as exhibited in the top panel of Figure 7.2. The analysis of the region-of-interest proceeded as described in Section 7.3.4 and as illustrated in Figures 7.2 and 7.3. This process permitted a single attenuation coefficient to be generated for each layer within each sample.

7.7 Results and Discussion

7.7.1 Attenuation Coefficient

The attenuation coefficients for intima/plaque, media, and adventitia measured from 22 to 105 MHz are shown in Figures 7.6 and 7.7. Figure 7.6 expresses the attenuation coefficient in decibels per millimeter (dB·mm$^{-1}$) and Figure 7.7 shows the same results in nepers per millimeter (Np·mm$^{-1}$). As explained in Section 7.2.3 only sites in which all three tissue layers could be identified were included in the analysis. The complete breakdown of which layers were seen in which sites can be found in Tables 6.1 and 6.1. The solid lines represent the averages 37 sites for the 50 and 100 MHz transducers, and 36 sites for the 25 MHz transducer. The dotted lines indicate plus and minus one standard deviation from the mean. Good agreement is seen between the three frequency bands, which form a relatively continuous trend across
Figure 7.6: Attenuation coefficients for each tissue type over the entire bandwidth in decibels per millimeter (dB/mm). The solid lines are the mean of 37 sites for the 50 and 100 MHz transducer values and 36 sites for the values from the 25 MHz transducer. The dotted lines indicate plus and minus one standard deviation from the mean.
Figure 7.7: Attenuation coefficients for each tissue type over the entire bandwidth in nepers per millimeter (Np/mm). The solid lines are the mean of 37 sites for the 50 and 100 MHz transducer values and 36 sites for the values from the 25 MHz transducer. The dotted lines indicate plus and minus one standard deviation from the mean.
the entire bandwidth. In these figures, the frequency axis is plotted logarithmically to more accurately represent the ranges covered by each transducer. The 100 MHz transducer covers a wider range of frequencies, but it’s fractional bandwidth is similar to those of the other two transducers.

<table>
<thead>
<tr>
<th>Band</th>
<th>Tissue</th>
<th>Slope (\text{db/mm-MHz})</th>
<th>DC Intercept (\text{db/mm})</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-36 MHz</td>
<td>Intima/Plaque</td>
<td>0.26</td>
<td>1.87</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>0.05</td>
<td>1.82</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>Adventitia</td>
<td>0.22</td>
<td>2.67</td>
<td>0.995</td>
</tr>
<tr>
<td>30-60 MHz</td>
<td>Intima/Plaque</td>
<td>0.31</td>
<td>0.39</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>0.13</td>
<td>-1.52</td>
<td>0.9998</td>
</tr>
<tr>
<td></td>
<td>Adventitia</td>
<td>0.26</td>
<td>1.76</td>
<td>0.998</td>
</tr>
<tr>
<td>60-105 MHz</td>
<td>Intima/Plaque</td>
<td>0.13</td>
<td>11.42</td>
<td>0.923</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>0.12</td>
<td>-1.27</td>
<td>0.9996</td>
</tr>
<tr>
<td></td>
<td>Adventitia</td>
<td>0.13</td>
<td>8.24</td>
<td>0.961</td>
</tr>
<tr>
<td>22-105 MHz</td>
<td>Intima/Plaque</td>
<td>0.20</td>
<td>5.02</td>
<td>0.948</td>
</tr>
<tr>
<td></td>
<td>Media</td>
<td>0.10</td>
<td>0.01</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>Adventitia</td>
<td>0.16</td>
<td>5.80</td>
<td>0.932</td>
</tr>
</tbody>
</table>

Table 7.1: Linear fit parameters for measured attenuation coefficients. A line was fit to the attenuation coefficient (see Figure 7.6) of each tissue type over each band, yielding a slope, 0 MHz (DC) intercept, and an R-squared value. For the wide band fit shown in the bottom row, the data from 22-36 MHz came from the 25 MHz transducer, the data from 36-60 MHz came from the 50 MHz transducer, the 100 MHz transducer provided the data from 60-105 MHz.

### 7.7.2 Linear Fit to Attenuation Coefficient

As stated in Section 7.3.1, the frequency dependence of the attenuation coefficient in soft tissue is commonly fit with a line. Table 7.1 shows the results of a linear fit to
the attenuation coefficient measured in each tissue layer over the bandwidths of each transducer and over the full bandwidth. Each fit results in a slope of the attenuation value and a value of the intercept at 0 MHz (DC Intercept). The R-squared values are also presented to indicate the quality of each fit. In all cases, the R-squared value is greater than 0.9.

At every frequency range studied, the media has the smallest slope of attenuation, a finding that is consistent with the images (see representative images in Figures 7.4 and 7.5. The intima/plaque and adventitia have a similar slope in all bands. Over the bandwidths of the 25 and 50 MHz transducers, the slope of attenuation of the intima/plaque is slightly larger than that of the adventitia.

7.7.3 Wide Band Fit

The last row of values in Table 7.1 shows the linear fit to the attenuation coefficients measured over the entire band. For this fit, the data from 22-36 MHz came from the 25 MHz transducer, the data from 36-60 MHz came from the 50 MHz transducer, the 100 MHz transducer provided the data from 60-105 MHz. The discontinuities at the regions of transition between bandwidths resulted in a fit of slightly lower quality (see R-squared values in Table 7.1); however, fitting over the largest possible bandwidth provides an estimate of the most broadband attenuation trends.
7.7 Results and Discussion

7.7.4 Intercept from Linear Fit

Ideally, the DC intercept of each linear fit would be close to zero. Table 7.1 shows that in most cases the intercept is close to zero, but in a few instances it is quite large. The intima/plaque and adventitia measurements made with the 100 MHz transducer exhibit an intercept value of approximately 10 dB/mm. The plots of the data to which these lines were fit (see top and bottom panels of Figure 7.6) show that between 80 and 90 MHz the rate of rise of the attenuation coefficient noticeably decreases. This flattening causes the slope of the fit to decrease and the value for the 0 MHz intercept to increase.

The measurements made in the highly attenuating regions (intima/plaque and adventitia) at the highest frequencies (100 MHz transducer) pose the greatest experimental challenge, and therefore produce the least reliable data. If the launched signal travels through 2 mm of plaque tissue that attenuates at 25 dB/mm, a 50 dB loss is incurred (ignoring smaller order effects). To perform a data collection scan on, and create an image of, a sample that includes both this plaque-filled region and also the neighboring media, which may only impose 15 dB of loss, the imaging system must have a dynamic range of at least $50dB - 15dB = 35dB$, and ideally more to acquire data with good signal-to-noise ratio. The calibration curves of Section 3.3.2 demonstrate the the acoustic microscopy system has over 40 dB of dynamic range, but probably no more than 50 dB at the highest frequencies of this study. These facts imply that the data corresponding to the regions in the attenuation coefficient
curves that show a flattening were collected with an acceptable, but relatively modest signal-to-noise ratio.

The physics of the interactions between the ultrasound beam and the tissue structures in the highly attenuating regions may also contribute to the change in character of the attenuation coefficient at the high end of the bandwidth. At 90 MHz, the wavelength is just under 20µm, which is a length comparable to the substructures within coronary artery tissue. The flattening of the attenuation coefficient might be indicating that the fundamental interactions between the ultrasound and the tissue are shifting character as the wavelength approaches some characteristic length scale.

### 7.7.5 Integrated Attenuation Coefficient

The slope of attenuation is often used as a single-number summary of a tissue’s attenuation features, but because not all tissue regions exhibit a linear fit that goes through zero, the slope of the fit does not accurately indicate the overall level of attenuation in the medium. Integrated attenuation coefficient has been used previously (Bridal et al., 1997) as a parameter that summarizes the attenuation induced by a medium over a certain frequency range. Although not as theoretically satisfying, or as easy to extrapolate from, as slope of attenuation, the integrated attenuation coefficient provides an intuitive measure of a material’s loss under specific experimental conditions. It is determined

\[
\alpha_{\text{integrated}} = \frac{1}{f_{\text{high}} - f_{\text{low}}} \int_{f_{\text{low}}}^{f_{\text{high}}} \alpha_{\text{sample}}(f) df
\]  

(7.17)
where $\alpha_{sample}(f)$ is the measured attenuation coefficient and $f_{\text{low}}$ and $f_{\text{high}}$ are the low and high ends of the bandwidth, respectively. The integrated attenuation coefficients are displayed graphically in Figure 7.8 and in tabular form in Table 7.2. The plot of Figure 7.8 clearly demonstrates the relatively uniform rise in attenuation coefficient with frequency range, and further exhibits that the media layer attenuates less than the intima/plaque and adventitia.

These results parallel the previously published attenuation measurements made in aorta by Bridal et al. (1997). In that study it was found that the media attenuated less than all plaque types. Adventitia was not included in these measurements. The values reported in this previous study for the integrated attenuation of atherosclerotic plaque are very similar to the measured value for intima/plaque. The current measurements for the integrated attenuation of the media of coronary artery are lower than the previously reported values for aorta by approximately a factor of two. This difference may be attributable to the intrinsic differences between the medial layers of muscular arteries such as the coronary arteries, and elastic arteries like the aorta. The experimental configuration and data reduction were also quite different between the two studies. The results from Bridal et al. (1997) were derived from radial backscatter data, whereas the results in this thesis are calculated from axial shadowed reflector data.
Figure 7.8: Graphical representation of the integrated attenuation coefficient values at each bandwidth for each tissue type. The data points are mean ± standard deviation.

Table 7.2: Tabular representation of the integrated attenuation coefficient values at each bandwidth for each tissue type. The data are reported as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Integrated Attenuation Coefficient (dB/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22-36 MHz</td>
</tr>
<tr>
<td>Intima/Plaque</td>
<td>8.1±2.6</td>
</tr>
<tr>
<td>Media</td>
<td>3.2±1.0</td>
</tr>
<tr>
<td>Adventitia</td>
<td>7.8±2.3</td>
</tr>
</tbody>
</table>

7.7.6 Anisotropy of Attenuation Coefficient

The above results present the attenuation coefficient in coronary artery tissue for the case of ultrasound propagating axially through the tissue (see Figure 5.1). These
values are of interest for understanding of the intrinsic properties of these important structures and may be valuable for the development of new technologies; nonetheless, measurements of the attenuation coefficient for the case of radial propagation would be more immediately applicable to current clinical side-looking intravascular ultrasound. Despite the fact that no quantitative claims can be made regarding the radial attenuation coefficients, inferences about these properties can be made from the axial data and a knowledge of other anisotropic tissues. Assuming that the uniaxial fiber model to used to understand the ultrasonic anisotropy in myocardium (Mottley and Miller, 1988) is also applicable to arterial tissue, some general comments about the relative trends in the attenuation coefficient as a function of frequency can be made, based on what is known about the anisotropy of apparent backscatter in the arteries. The apparent integrated backscatter studies of Chapter 6 demonstrated that the uncompensated backscatter from the media is similar in the radial and axial directions. It is speculated that the predominantly circumferential direction of the fibers that compose the media introduce a symmetry between the radial and axial directions, both of which are perpendicular to this fiber direction. This symmetry suggests that the attenuation coefficient in the radial direction may be similar to the attenuation coefficient measured axially. The intima/plaque layer demonstrated significant anisotropy in the measured values of apparent integrated backscatter (see Figure 6.12). The uniaxial fiber model implies that a predominantly axial fiber structure is present within the intima/plaque. Studies of myocardium (Madaras et al., 1988; Mottley and Miller, 1988; Hoffmeister et al., 1995; Verdonk et al., 1996; Sos-
novik et al., 2001; Baldwin et al., 2006; Gibson et al., 2007, 2008) have shown that apparent backscatter is largest perpendicular to the myofibers and relatively smaller parallel to the fiber direction. Conversely, these earlier studies have shown that the attenuation coefficient is larger in the parallel orientation and smaller in the perpendicular orientation. Based on this analogy, it can be predicted that the radial attenuation coefficient in the intima/plaque may be smaller than that attenuation coefficient measured axially.

7.8 Summary and Conclusions

This results generated from the studies presented in this chapter represent the most comprehensive measurements of the axial attenuation coefficient of fresh (that is, not chemically fixed) human coronary artery tissue and atherosclerotic plaque to date. The measured attenuation coefficients were shown to be fit well over the appropriate bandwidths by a straight line. It has been demonstrated that the axial attenuation coefficient at the frequencies of current and near future intravascular ultrasound is the lowest in the media and higher in the intima/plaque and adventitia. These result may be useful for future work modeling the ultrasonic and mechanical properties of coronary artery layers and plaques, and may be relevant for the design and application of new intravascular ultrasonic imaging devices.
Bibliography


Bibliography


Chapter 8

The Backscatter Coefficient of Coronary Artery Tissues and Atherosclerotic Plaque

8.1 Introduction

This chapter builds upon work described in previous chapters by compensating the measured apparent backscatter (see Chapter 6) with the experimentally determined attenuation coefficient (see Chapter 7) and with theoretically derived approximations of the experimental configuration (see Section 8.3) in order to generate an estimate of the intrinsic backscatter properties of human coronary artery tissues and atherosclerotic plaques. These innate properties are described by the backscatter coefficient.
8.2 Background

8.1.1 Chapter Overview

Background information regarding the ultrasonic backscatter coefficient is provided in Section 8.2. Section 8.3 explains the method by which the backscatter coefficient was determined from experimental data and theoretical models. The verification of this procedure in tissue-mimicking phantoms is presented in Section 8.4. The human coronary artery data are discussed in Section 8.5 and the results are exhibited and interpreted in Section 8.6.

8.2 Background

The ultrasonic backscatter coefficient conveys the intrinsic properties of a medium as they relate to the backscatter of sound. This parameter dictates how much energy incident upon a medium will be backscattered per unit distance of propagation. Stated as an equation

\[ P_{\text{backscattered}}(\omega) = \eta(\omega) \cdot l \cdot P_{\text{incident}}(\omega), \]  

(8.1)

where \( \omega = 2\pi f \) is the angular frequency, \( \eta(\omega) \) is the backscatter coefficient, \( l \) is the propagation length, and \( P(\omega) \) is ultrasonic power.

8.2.1 Motivation and Goals

The backscatter coefficient represents a comprehensive and versatile measure of a medium’s backscatter properties. Understanding these properties in coronary arteries
and plaques may provide knowledge regarding these tissues’ physical characteristics that might, in turn, permit improved imaging and characterization of atherosclerosis. Currently available clinical ultrasonic intravascular imaging systems create images by displaying the relative apparent (not compensated for attenuation and diffraction) backscatter. Future systems capable of detecting not only these uncompensated relative changes in backscatter, but also fundamental backscatter properties may provide additional diagnostic value.

The work of this chapter seeks to advance these goals by making measurements of the backscatter coefficient of coronary artery tissues and atherosclerotic plaques.

8.2.2 Coordinate System

The same coordinate system and naming convention are maintained as were employed in previous chapters. As described in Section 5.2 the coronary arteries are approximated as cylinders. In this convention, the blood flows down the axis of the artery. This direction is referred to as the axial direction. The radial direction points outward in all directions from the central axis. The circumferential direction is orthogonal to both of these directions and runs around the cylinder. This coordinate system is illustrated in Figure 5.1.

8.2.3 Samples and Sites

As described in Section 5.3, nineteen fresh human left anterior descending coronary arteries were studied. From these nineteen vessels, forty-four sites were imaged in both
the radial and axial orientation. The studies of this chapter will make use only of the axial data. Not all artery tissue subtypes (intima/plaque, media, adventitia) were visible in the images acquired from all sites. Tables 6.1 and 6.2 show a list of all sites and indicate which tissue types were found in each. The analyses of this chapter include only those sites in which all three tissue types were found. For the 50 and 100 MHz transducers, 37 sites were found to have all three layers. For the 25 MHz transducer, 36 sites demonstrated all three layers. The results presented in Section 8.6 were generated from these sites.

8.3 Data Analysis

8.3.1 Backscatter Coefficient Determination

In theory, the backscatter coefficient is the origin of the physical phenomena that underlie the backscatter experiment. In practice, the backscatter coefficient is the endpoint of a calculation that seeks to isolate these physical phenomena. Laboratory measurements of backscatter are complicated by attenuation within samples and by the finite aperture of the transducer, which results in diffraction of the ultrasonic field. Determination from experimental data of the backscatter coefficient, a parameter independent of these effects, requires compensation for diffraction and attenuation.
8.3.2 Diffraction Compensation

To compensate the acquired data for the effects of diffraction in the field, the method introduced by Chen et al. (1997) was used. This method has been applied to data acquired at frequencies similar to those used in echocardiography (1-10 MHz) (Chen et al., 1998; Marsh et al., 1998; Wear et al., 2005; Yang et al., 2007; Yang, 2007). Its application to higher frequency measurements has been verified by Machado and Foster (1999). The method can, in general, be applied to many different pulse-echo experimental configurations, but the presentation below concentrates on the specific case applicable to the studies reported in this dissertation—a spherically focused piston transducer. The initial discussion of this section presents results valid for a medium with no loss. The effects of attenuation will be incorporated in Section 8.3.3.

The result of the analysis by Chen et al. (1997) is that in an experiment using a spherically focused broadband ultrasonic transducer the backscatter coefficient (neglecting attenuation) is given by

\[ \eta(f) = \frac{\langle |V_S(\vec{r} \in \mathcal{V}; \omega)|^2 \rangle |D_{\text{ref}}(z_{\text{ref}}; \omega)|^2}{|V_{\text{ref}}(z_{\text{ref}}; \omega)|^2 l \cdot \bar{D}_{S}(\vec{r} \in \mathcal{V}; \omega)} \] (8.2)

where \( \langle |V_S(\vec{r} \in \mathcal{V}; \omega)|^2 \rangle \) is the frequency-dependent ensemble average power spectrum of the backscattered signal from points at location \( \vec{r} \) within the volume \( \mathcal{V} \), \( |V_{\text{ref}}(z_{\text{ref}}; \omega)|^2 \) is the power spectrum of the signal reflected from an ideal reflector located on-axis at \( r = z_{\text{ref}} \), \( |D_{\text{ref}}(z_{\text{ref}}; \omega)|^2 \) is the acoustic coupling function, \( \bar{D}_{S}(\vec{r} \in \mathcal{V}; \omega) \) is the mean diffraction correction, and \( l \) is the length of the gate used.
8.3 Data Analysis

to analyze the backscattered signal. The mean diffraction correction function accounts for the diffraction effects in the field arising in the backscatter experiment. It is obtained as

\[ \bar{D}_S(\vec{r} \in V; \omega) = \left( \frac{\pi a^2}{r^2} \right) E_\infty \exp \left( -\frac{E_\infty}{\pi} \frac{k^2 a^4}{4r_0^2} \left( \frac{r_0}{r} - 1 \right)^2 \right) \]  

(8.3)

where \( a \) is the transducer radius, \( \vec{r} \) is the field point, \( k \) is the wave number, \( r_0 \) is the focal length of the transducer, and \( E_\infty \) is the ratio between the effective cross-sectional area of the beam of a corresponding planar transducer in the far field to the surface area of that planar transducer. For a piston source \( E_\infty = 0.46 \). Because the experiments of this thesis were carried out very near the focal point, it can be assumed that \( r = r_0 \), which permits simplification of the mean diffraction correction to

\[ \bar{D}_S(\vec{r} \in V; \omega) = \left( \frac{\pi a^2}{r_0^2} \right) E_\infty. \]  

(8.4)

The acoustic coupling function accounts for the diffraction effect encountered in the reference experiment in which a signal is reflected from a steel plate, by coupling the transducer’s beam pattern with the planar surface of the reflector. Under the assumptions that the transducer is focused and that the reflector is at the focal point, the acoustic coupling can be written

\[ |D_{ref}(z_{ref} = r_0; \omega)|^2 = \exp \left( -\frac{2}{\pi} \sqrt{\frac{2\pi r_0}{ka^2}} \right). \]  

(8.5)

Inserting Equations 8.4 and 8.5 into Equation 8.2 provides a formula for compensating for the diffraction that is given entirely in terms of experimentally measurable and
known parameters

\[
\eta(\omega) = \frac{\langle |V_S(\vec{r} \in V; \omega)|^2 \rangle}{|\bar{V}_{ref}(\vec{z}_{ref}; \omega)|^2} \cdot \frac{1}{L} \left( \frac{r_0^2}{\pi a^2} \right) \frac{1}{E_\infty} \exp \left( -\frac{2}{\pi} \sqrt{\frac{2\pi r_0}{ka^2}} \right).
\] (8.6)

The first fraction in this equation, the ensemble average backscattered power spectrum normalized by the power spectrum of a reflection from a steel plate, is the backscatter transfer function (ABTF) discussed in Section 6.3. The remaining terms compensate the transfer function for the effects of diffraction in the field and are calculated from known parameters of the experiment.

### 8.3.3 Attenuation Compensation

In addition to the effects of diffraction, which are accounted for in Equation 8.6, all real-world experiments are also subject to the effects of loss of energy in the field. The loss originates from two sources: loss at the boundaries, which is frequency-independent if the beam impinges upon the interface normally, and frequency-dependent attenuation in the bulk of the material. The interface losses are calculated by applying the appropriate reflection and transmission coefficients, in a manner similar to that outlined in Section 7.3.3. In the current experiment the acoustic impedance of tissue is similar to that of the coupling medium, so the surface losses are insignificant in comparison with the bulk losses. Accounting for the bulk losses makes use of the measured attenuation coefficient of the medium, but is complicated because loss within the analysis gate must be considered as well as loss incurred on the round trip to and from the gate. The appropriate compensation was introduced by Sigel-
8.3 Data Analysis

mann and Reid (1973) and was adapted for single-pulse broadband measurements by O'Donnell and Miller (1981). It is given by

\[ F(\alpha, z_0, v, \tau) = e^{4\alpha z_0} \frac{2\alpha v \tau e^{\alpha v \tau}}{e^{\alpha v \tau} - e^{-\alpha v \tau}} \]  

(8.7)

where \( \alpha \) is the frequency-dependent attenuation coefficient, \( v \) is the speed of sound in the material, \( \tau \) is the temporal length of the analysis gate \( (\tau = \frac{2L}{v}) \), and \( z_0 \) is the distance the signal propagates through the material before reaching the front of the analysis window. The first exponential in Equation 8.7 accounts for the loss within the overlying material and the remaining terms describe the loss incurred within the analysis gate.

Figure 8.1 shows the attenuation coefficient and corresponding attenuation compensation for a representative region-of-interest in a medial section of human coronary artery. To generate the plot in the bottom panel, the plot in the top panel was converted to nepers per millimeters by dividing by \( \frac{20}{\ln 10} \approx 8.69 \), and was then inserted into Equation 8.7 along with the appropriate experimental parameters \( (z_0, v, \tau) \).

8.3.4 Reduction to Backscatter Coefficient

The acquired data, in the form of the apparent backscatter transfer function, were reduced to the backscatter coefficient by applying the compensations described above. The reduction formula can be generated by appending the attenuation compensation
8.3 Data Analysis

**Figure 8.1:** Attenuation coefficient (top panel) and corresponding attenuation compensation factor (bottom panel) for a representative region-of-interest from the media of a coronary artery. The curve in the bottom panel is generated by converting the curve in the top panel to units of Np/mm and applying Equation 8.7.
(Equation 8.7) to the diffraction corrected apparent backscatter transfer function

$$\eta(\omega) = \frac{1}{l} \cdot \left[ \frac{\langle |V_S(\bar{r} \in \mathcal{V};\omega)|^2 \rangle}{|V_{\text{ref}}(z_{\text{ref}};\omega)|^2} \right] \left[ \frac{\pi a^2}{r_0^2} \right] E_\infty \exp \left( -\frac{2}{\pi} \sqrt{\frac{2\pi r_0}{ka^2}} \right) \left[ e^{4\alpha_0 z_0} - \frac{2\alpha v \tau}{e^{\alpha v \tau} - e^{-\alpha v \tau}} \right]. \quad (8.8)$$

In this equation, the term in the first bracket is the measured apparent backscatter transfer function, the term in the second bracket corrects for the effects of diffraction, and the final term is the attenuation compensation factor.

### 8.3.5 Implementation of Backscatter Coefficient Reduction

The process of extracting the backscatter coefficient from the acquired data is described mathematically by Equation 8.8, and is illustrated graphically in Figure 8.2. All axes on this plot are displayed on a log scale. Each vertical axis spans one decade, but the absolute level is shifted from panel to panel. The top panel in this figure is the mean apparent backscatter transfer function from a representative region-of-interest. Specifically, these data were acquired with the 50 MHz transducer from the sample shown in Figure 6.7, and the pertinent region-of-interest is the media region in the middle right panel.

The second panel of Figure 8.2 shows the experimentally determined attenuation correction. This function was derived from the attenuation coefficient (see Chapter 7) according to Equation 8.7.

The diffraction compensation term (the second bracketed term of Equation 8.8) is pictured in the third panel of Figure 8.2. This compensation appears to be nearly
Figure 8.2: Illustration of the determination of the backscatter coefficient (bottom panel) for a representative region within the media of a human coronary artery sample. The plot in the top panel is the apparent integrated backscatter. The second panel’s curve is the experimentally determined attenuation compensation factor. The calculated diffraction compensation is shown in the third panel. Multiplication of the data in the first three panels and normalization of the product by the gate length yields the bottom panel. All vertical and horizontal axes are plotted on a log scale.
frequency-independent on the scale of the figure, but it is, in fact, weakly frequency
dependent (proportional to $e^{-\sqrt{\frac{T}{\omega}}}$).

The final plot is the backscatter coefficient for this region-of-interest. Multiplying
the top three plots and then dividing by the gate length, $l$, yielded this curve.

8.4 Verification of Reduction Scheme

To validate the reduction to backscatter coefficient, the algorithm prescribed above
was applied to an ultrasonic tissue-mimicking phantom. The goal was to use the
three transducers described in previous chapters to make frequency-dependent mea-
surements of the backscatter coefficient across the entire bandwidth. Ideally, the
compensations described above would account for the substantial differences between
the three transducers (see Section 3.3) and yield a continuous function across the
entire range.

8.4.1 Tissue-Mimicking Phantom

The phantom measured in these studies was created by mixing graphite scatterers
into a polyvinyl alcohol cryogel background. The polyvinyl alcohol cryogel was made
by following the procedure of Fromageau et al. (2007). After mixing, the phantom was
subjected to two freeze/thaw cycles. A piece approximately equivalent in thickness
to the arterial samples was cut from the solidified phantom. Scanning was performed
on the phantom sample in the same manner as was done on the arterial segments (see
8.4 Verification of Reduction Scheme

Sections 5.6.2 and 5.6.3).

This homogenous phantom provided an ideal medium because it contained scatterers significantly smaller than all wavelengths of ultrasound used in this study, and because its backscatter properties were similar to arterial tissue.

8.4.2 Phantom Results

The four panels of Figure 8.3 show the measurements and calculations that go into the backscatter coefficient, as well as the coefficient itself for the phantom study. The top panel shows the measured apparent backscatter transfer function. The second panel shows the experimentally determined attenuation coefficient. The theoretically derived diffraction compensation factor is shown in the third panel. The fourth panel displays the estimated backscatter coefficient of the polyvinyl alcohol cryogel phantom. The diffraction compensations in the third panel demonstrate that the 25 MHz transducer has a substantially different diffraction field than the 50 and 100 MHz transducers.

The estimate of the backscatter coefficient of the polyvinyl alcohol phantom made with the approach detailed above yielded a curve that is continuous across the entire bandwidth of the studies of this dissertation. This success implies that the employed method is likely valid in the current application.
8.4 Verification of Reduction Scheme

Figure 8.3: Estimated backscatter coefficient of polyvinyl alcohol cryogel phantom (bottom panel) across the full bandwidth and the intermediate results (top three panels) from which it was calculated.
8.5 Human Coronary Artery Data

The backscatter coefficient analysis was performed on the human coronary artery data acquired as described in Chapters 5, 6 and 7. The segmentation described in the later two chapters provided the regions-of-interest in which the backscatter coefficient was calculated.

8.6 Results and Discussion

The measured backscatter coefficients averaged over those sites in which all tissue layers were present are displayed in Figure 8.4. The three panels are, from top to bottom, the intima/plaque, media, and adventitia. The overall values of the backscatter coefficients form these three layers suggest that the adventitia intrinsically backscatters more than the media and intima/plaque layers. This result is in general agreement with the trend observed in femoral arteries by Lockwood et al. (1991), in which the adventitia has a larger axial backscatter coefficient that the media at 50 MHz.

8.6.1 Transition Regions

These plots demonstrate moderately good continuity across the bandwidths, with the transition between the 25 MHz transducer and the 50 MHz transducer less well matched than the transition between the 50 and 100 MHz transducers. This seems likely to be attributable to the more substantial differences in the diffraction field
8.6 Results and Discussion

Figure 8.4: Backscatter coefficients estimated in the three tissue layers from all three transducers. The solid lines represent the average of the sites in which all three layers were observed, and the dashed lines represent this average plus one standard deviation.
created by the 25 MHz relative to the 50 and 100 MHz transducers.

8.6.2 Frequency Dependence

The intima/plaque and media plots show a rise with frequency, whereas the adventitia plot does not indicate such an increase with frequency over the widest band (from 22 to 105 MHz). This behavior may be attributable to the inclusion of fat cells in the adventitial regions-of-interest. The relatively large size (approximately 0.1 mm diameter) of these cells makes inappropriate modeling them as point scatterers. The inclusion in the analysis of scatterers whose diameters were on the order of the wavelength would be expected to cause deviation from the trend seen in the more common case of scatters much smaller than the wavelength.

8.6.3 Correspondence with Previous Studies

Previously published measurements made axially over a smaller bandwidth (35-65 MHz) have demonstrated that the backscatter coefficient of media is lower than that of adventitia, and that the backscatter coefficient of plaque components can be range from being higher than the adventitia to being lower than the media (Lockwood et al., 1991). The current work agrees with the relative trend between the media and adventitia and finds that the combined intima/plaque has a similar backscatter coefficient to the media, thereby confirming the trends observed in femoral arteries by Lockwood et al. (1991).

One previous study performed in the media of canine aortae found the axial back-
scatter coefficient to be of the same order of magnitude as the results presented in this thesis (Nguyen et al., 2002). An older study found the radial backscatter coefficient in “normal” aortic wall to be on the same order as the media and intima/plaque layers of the current study (Picano et al., 1985). It should be noted that the “backscatter coefficient” calculated by Picano et al. (1985) was compensated for diffraction by a different method, and was not compensated for the effects of attenuation.

Previous work done in human coronary arteries presented the integrated backscatter coefficient acquired radially at 50 MHz (Machado and Foster, 2001). This study reported that the mean integrated backscatter coefficient for the media was lower than the adventitia. The data presented in Figure 8.4 agrees with this trend. The study by Machado and Foster (2001) shows substantial variation in the integrated backscatter coefficient of the thickened intima, yet in general finds it to be higher than the integrated backscatter coefficient of the media. This disagreement with the current result may be attributable to the anisotropy of the intimal structures, which would be consistent with the findings of Chapter 6, or may be attributable to the biological variation between a thickened intima and a mature atherosclerotic lesion. Additionally, this previous study acknowledged that the use of a global attenuation compensation for the layered structure of the coronary artery is only a first-order approximation.
8.6.4 Comparison with Apparent Integrated Backscatter

Measurements presented earlier of the axial apparent integrated backscatter (see Figure 6.9) revealed that the intima/plaque seems to backscatter much less than the media. However, when this result is compensated for other pertinent effects (primarily attenuation), it is observed that the intima/plaque and media have similar intrinsic scattering properties. This comparison serves to reinforce the point that apparent trends (intima darker than media) may originate from the combination of two or more fundamental features of these tissue layers. Hence, techniques for evaluating the fundamental properties of coronary artery tissue and plaques may add additional diagnostic value beyond that provided by current clinical ultrasonic images.

8.7 Conclusions

The results extracted from the studies of this chapter represent a significant advancement in the understanding of the fundamental scattering properties of coronary artery tissues and atherosclerotic plaques over the bandwidth of current and near future intravascular ultrasound imaging. The backscatter coefficient underlies all clinical and laboratory ultrasound measurements, so knowledge of its relationship to anatomical structures might provide guidance for the development of new techniques and technologies.
Bibliography


Chapter 9

Summary and Concluding Remarks

This dissertation described the development, execution, and interpretation of studies that investigated the physics of the interaction between coronary artery tissues and ultrasound. The two-fold goal of these studies was to better understand the fundamental features of the physics underlying the relationship between ultrasound and coronary artery tissue and to use this knowledge to contribute to the advancement of techniques capable of improving the diagnosis and management of atherosclerosis.

Chapter 2 provided background information pertinent to atherosclerosis of the coronary arteries, as well as background material describing relevant previous studies of the interactions between ultrasound and arterial tissues and plaque. Methods for performing ultrasonic tissue characterization in coronary artery tissue and plaques were also discussed.

Chapter 3 described the acoustic microscopy experimental system that was em-
ployed to collect the data presented in this thesis. Measurements carried out to characterize the ultrasonic parameters of this system were presented. Specific upgrades and improvements made for coronary artery imaging and characterization were explained.

Studies carried out on lamb tissues were the focus of Chapter 4. These studies were used to develop methods of preparing and measuring fresh (that is, not chemically fixed) coronary artery tissue. The methods validated in lamb tissue were subsequently applied to human coronary artery tissue.

Chapter 5 detailed the methods by which data were collected from human coronary arteries. The process involved the preparation of samples from fresh (that is, not chemically fixed) arteries for acoustic microscopy and the means by which data useful for the extraction of fundamental parameters were obtained.

The data collected as described in Chapter 5 were analyzed to yield measurements of the apparent integrated backscatter from coronary arteries and atherosclerotic plaques in Chapter 6. These values were compared in several orientations, and it was found that the backscattering trends well known from clinical intravascular ultrasound carried out in the radial orientation were altered when the tissue was imaged from an axial orientation.

Chapter 7 compared the attenuation properties of human coronary artery tissue and plaques by reducing the acquired data to the attenuation coefficient. These studies showed that measurements of the attenuation coefficient across a wide bandwidth can be carried out in human coronary arteries. The resulting values indicated that,
in the axial orientation, the attenuation coefficient at frequencies of current and near-future intravascular ultrasound is lower in the media layer than in the intima/plaque or adventitia.

Chapter 8 exhibited the techniques for and results of determining the backscatter coefficient of coronary artery tissues and atherosclerotic plaques. This chapter demonstrated that consistent measurements of the backscatter coefficient can be made in coronary artery tissue. Furthermore, the measured axial backscatter coefficients of coronary artery media were similar to those for coronary artery intima and plaques, suggesting that the effects of attenuation may be a dominant feature of the relative axial backscatter trend observed in Chapter 6.

Viewed in total, the studies of this dissertation represent an advancement of the understanding of the physics underlying ultrasound’s interaction with coronary artery tissue, and may provide insights for improving clinical arterial ultrasonic characterization as well as successful techniques for making measurements of the physical properties of normal and pathologic vascular tissue.