ARFI Ultrasound for the Detection and Characterization of Atherosclerosis in an FH Pig Model

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Abstract

RUSSELL H. BEHLER: ARFI Ultrasound for the Detection and Characterization of Atherosclerosis in an FH Pig Model. (Under the direction of Caterina M. Gallippi, Ph.D..)

Stroke is the third leading cause of death in the United States, with a large percentage of strokes caused by atherosclerotic rupture. Current methods of atherosclerotic detection include invasive techniques such as coronary angiography and intravascular ultrasound (IVUS), as well as noninvasive techniques such as magnetic resonance angiography and duplex ultrasound. These methods are known to be effective for detecting occlusive plaques associated with pronounced narrowing of the vessel lumen and/or blood flow obstruction. However, they are not effective for detecting nonstenotic plaques or for characterizing plaque composition. This lack of plaque compositional information prevents these imaging techniques from detecting plaque rupture risk. To accurately assess atherosclerotic plaques most vulnerable to rupture, novel detection and characterization techniques are needed.

Acoustic radiation force impulse (ARFI) ultrasound, one of several elastographic techniques under development to meet this need, uses high intensity acoustic impulses to remotely displace tissue. By assessing ARFI-induced displacement and subsequent tissue recovery, the mechanical properties of tissue can be assessed and used to characterize atherosclerosis. In order to ensure the best possible plaque detection capability, the most appropriate beam sequences must be used. Following ex vivo and in vivo demonstration of ARFI capability for atherosclerotic plaque detection and characterization, a statistical reader study of ARFI beam sequences is performed in phantoms.
as well as \textit{ex vivo} and \textit{in vivo} in an FH pig model. Finally, a serial study of ARFI is performed to assess ARFI repeatability and potential for early plaque detection.

This dissertation supports the hypothesis: in vivo, \textit{transcutaneous ARFI ultrasound will detect occlusive and nonocclusive plaques in peripheral arteries, assess plaque composition and structure and detect changes in atherosclerotic status over time.}
To Mary, for her love
and endless support.
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Chapter 1

Introduction

Atherosclerosis is a complex disease affecting a large number of Americans. The following dissertation will discuss a novel method of noninvasive atherosclerotic plaque detection and characterization, acoustic radiation force impulse (ARFI) ultrasound. We begin with Chapter 2, which describes the various stages of plaque development based on the AHA Committee on Vascular Lesions classification system and the implications on mechanical property. Additionally, the FH animal model utilized throughout the entirety of this dissertation is introduced. Chapter 3 describes the current state of imaging techniques for assessing atherosclerosis. Those techniques most widely employed, coronary angiography and duplex ultrasound, struggle at detecting non-occlusive plaques and plaque characterization. This has spurred the development of several other of the techniques described. Radiation force based imaging techniques, including acoustic radiation force impulse (ARFI) ultrasound, are described in extended detail in Chapter 4.

Chapter 5 presents the first demonstration of ARFI’s plaque detection and characterization capability in an *ex vivo* porcine artery. Collagen and elastin deposition appears to correlate with ARFI measurements, though no statistical analysis is performed. This first demonstration is then extended into *in vivo* application in Chapter
6. A control and three atherosclerotic examples are presented, with a full statistical analysis of the correlations between parametric ARFI measurement and material content, which suggests that parametric ARFI measurements can provide information on material content for characterization purposes in more advanced plaques.

Chapter 7 introduces the manipulation of beam sequence excitation and tracking methods for potentially improved atherosclerotic detection capabilities. Variations in excitation F/#, number of receive lines and number of excitation pulses are all examined. This preliminary study is then expanded in Chapters 8 and 9, where trained readers evaluated nine different beam sequences in phantoms, ex vivo and in vivo. Three methods of excitations were combined with three methods of tracking to generate the 9 sequences. Chapter 8 focuses on the plaque detection aspect of the beam sequences, while Chapter 9 focuses on the characterization aspects. Next, Chapter 10 examines three ARFI beam sequences applied at 3-month time points over a 12-month span for repeatability and serial disease detection potential. Chapter 11 provides a general discussion of the results presented and a conclusion. Finally, Chapter 12 discusses future directions for ARFI as applied to atherosclerosis. Three appendices are attached, which present some technical investigations into motion filtering, reflected wave imaging and luminal masking in Appendices A, B and C, respectively.
Chapter 2

Atherosclerosis and Stroke

2.1 Introduction: The vulnerable plaque

The third leading cause of death for both the United States and the world is stroke, which cost the United States approximately $73 billion dollars in 2010 alone. Approximately 87% of all strokes are ischemic, meaning that they involve blockage of blood supply to the brain. Either via a thrombus forming locally or forming elsewhere and embolizing, ischemic stroke is most often due to atherosclerosis [1].

Atherosclerosis is the progressive thickening and hardening of arteries. A disease that begins in early childhood, nearly everyone alive has some degree of atherosclerosis. What differs is the extent and severity of the disease and the risk associated with it, which are often two separate concepts. Atherosclerotic plaques may or may not develop into what are considered “vulnerable plaques,” a term associated with those plaques at greatest risk for rupture and subsequent formation of blood clots, which can then break off to cause heart attacks and strokes. The problem is that these vulnerable plaques are most often not the largest or the smallest of plaques, but an elusive range in the middle [2; 3; 4]. Furthermore, not all plaques are vulnerable to rupture, so the characterization of atherosclerotic plaques is of paramount importance. It is with this
goal that researchers are developing imaging techniques to better characterize plaques, hopefully resulting in both earlier and more accurate clinical diagnoses.

2.2 Stages of Atherosclerosis

2.2.1 Normal Arterial Structure

Before delving into the many ways in which atherosclerosis can develop and corrupt normal arterial structure, one must first understand what normal structure and function means. Arteries are composed of three layers, the intima, media and adventitia, organized in this order with respect to increasing radial dimension. The intima is the thinnest layer composed of an endothelial cell layer, which is in contact with blood, and a musculoelastic layer composed of smooth muscle cells and elastic fibers. Importantly, the intima provides one of the only surfaces capable of keeping blood in its liquid state [5]. As will be explained in subsequent sections, the disruption of intimal function is the main mechanism for atherosclerosis formation and as such, is the primary layer of concern when discussing atherosclerosis. At the border between the intima and media the internal elastic lamina (IEL) can be found, providing a large amount of the vessel’s elasticity. The IEL itself can be described as a network of interconnected elastin fibers arranged within the cylindrical geometry of the arterial wall, providing the vessel’s elastic backbone. Below the IEL lay the media and adventitia, which are primarily composed of collagens and smooth muscle cells, supplying the stiffness and structure to the artery. These layers are only occasionally involved with advanced atherosclerosis.

2.2.2 Type I – Initial Atherosclerosis Formation

The earliest stages of atherosclerosis are classified as Type I or initial lesions, according to the American Heart Association Committee on Vascular Lesions, which will be
used as the classification system throughout this dissertation [6]. These lesions are invisible to the unaided eye, requiring microscopy for detection [6]. Type I lesions are relatively small, focal and involve the deposition of lipoprotein and leukocytes within the intima [5; 6]. The exact nature of initial atherosclerotic formation is still the subject of investigation, but is believed to be closely tied to the initiation of inflammation within the intima, allowing binding of the first leukocytes to the arterial wall [7]. What is known, however, is the strong dependence of atherosclerotic disease formation upon the local shear stresses operating on the endothelial cell layer [8; 9].

The shear stress acting on the vessel wall is mechanotransduced into a biochemical signal that alters vascular behavior. Under normal laminar blood flow, as required for normal vascular function, the endothelial cells align with the direction of blood flow and display atheroprotective behavior, such as a reduction in endothelial cell permeability [8]. Under disturbed shear stress, as happens in bifurcations, the vasculature begins to exhibit atherogenerative behavior such as enhanced expression of vascular cell adhesion molecule (VCAM-1), resulting in increased transendothelial diffusion. When coupled with slower flow velocities, which allow increased interaction time between leukocytes and the arterial wall, leukocytes and lipoproteins adhere to the arterial wall. This results in early atherosclerotic lesions forming, which can be found in infants and children. Within 8 months, more than 45% of infants have foam cell accumulation in their coronary arteries [6]. These lesions are small and pose no overt risks at this stage.

2.2.3 Type II – Fatty Streak

Type I lesions may eventually develop into larger yellow-colored fatty streaks, or Type II lesions [6]. As with any lesion at any stage in progression, the development is nonlinear and may stabilize either permanently or only temporarily, with progression to the next stage requiring an additional stimulus [6]. With that in mind, Type II
lesions are typically the primary lesion type present in children. These lesions are in many ways simply larger Type I lesions and, as with Type I lesions, pose little to no risk at this early stage. Type II lesions can be broken into two subgroups, Type IIa and IIb, which correspond to progression-prone and progression-resistant, respectively. Type IIa lesions are the first lesions which progress to Type III and further symptomatic stages, which are those lesions subjected to decreased shear stress [6].

2.2.4 Type III – Intermediate

The development of Type III or intermediate plaques marks the point when atherosclerosis begins a transition from benign disease to advanced atheromas (Type IV). These plaques are characterized by the presence of visible extracellular lipid particles and droplets, which begin to form into pools [6]. As these plaques mark the transition from early to more advanced and risky plaques, they present an ideal time for preventative measures to begin [6]. With current imaging techniques however, detection at this stage is extremely difficult and unlikely. As this is a transitory stage of atherosclerotic development, it is worth discussing several material changes occurring within the plaque that impact the alteration of plaque mechanical property and rupture risk.

First, while collagen is present in the medial and adventitial arterial layers from the beginning, additional collagen is deposited in all stages of atherosclerotic development. A major source of arterial stiffness (up to 60% of plaque protein), collagen deposition begins early in plaque development and may represent a major mechanism of lesion progression. Collagen deposition is tied to stenosis in excessive amounts and rupture in insufficient amounts [10]. Thus, the monitoring of collagen accumulation could potentially be a vital source of information for assessing plaque development and vulnerability, a topic which will be evaluated in later sections.

Second, elastin is a significant component of blood vessel extracellular matrix and
enables arterial dilation and contraction. With atherosclerotic plaques, elastin can be found in two forms, as part of the extracellular matrix and IEL in a structured network as previously described or as free elastin deposited during atherosclerotic development. As atherosclerosis progresses, the two types of elastin change: the structural IEL elastin becomes degraded while free elastin can be deposited, something that occurs more often in advanced plaques [11].

Lastly, calcium can be deposited within atherosclerotic plaques, beginning at Type III. The impact of calcification on plaque vulnerability is still not well understood, but it has been suggested that the location of the calcification plays an important role. If the calcification is within a fibrous cap near the lumen, this may lead to increased stress and plaque rupture, while if the calcification is further away from the lumen or within a lipid pool it may even stabilize the plaque [12].

2.2.5 Type IV – Atheroma

The first lesion type considered advanced, as indicated by a predisposition towards ischemic events, is Type IV [6]. The key identifier of a Type IV plaque is an atheroma, an extensive well-defined region of extracellular lipid. Atheromas are formed by a coalescence of the smaller lipid pools formed in Type III plaques. Atheromas are typically bound by a fibrous cap of varying thickness, thickness being inversely related to plaque vulnerability for rupture [3; 4; 13; 14]. Fibrous caps are primarily made of types I and III collagen, constituting a stiff layer of protection to the plaque. If the fibrous cap is thin it may rupture, allowing thrombi to form, attaching themselves to plaque. From this point the thrombus can either be absorbed into the plaque and recovered or embolize and cause an ischemic stroke [5]. For these reasons the identification of fibrous caps and assessment of fibrous cap thickness is essential to vulnerable plaque detection.
Although Type IV+ plaques can be at a high risk for rupture, they often evade conventional methods of detection due to vascular remodeling. Vascular remodeling is a process occurring over a period of years or decades to preserve luminal diameter and, as a result, blood flow. As atherosclerotic plaques grow outward in size, they can expand until the plaques reach 40% luminal narrowing [3; 15]. As will be explained in subsequent sections, conventional imaging techniques such as x-ray angiography and duplex ultrasound rely on a reduction of blood flow for detection. However, plaques which are vulnerable can exist at a wide range of occlusions, with the majority of those plaques causing heart attack or stroke having less than 50% occlusion [7].

2.2.6 Type V – Fibroatheroma

From Type IV, the plaque may progress toward Type V, or head straight to Types VI or VII, depending on the change in plaque composition and structure [2]. The first of these, Type V, represents a plaque identified by the formation of prominent new fibrous tissue [15]. The risks of a thin fibrous cap are similar to those described for a Type IV plaque. Type V and VI begin to appear in the 20s, becoming the predominant lesion types in middle-aged and older persons [15].

2.2.7 Type VI – Vulnerable/Disrupted

Morbidity and mortality is largely due to Types IV and V lesions which have developed disruptions in the lesion surface, hematoma or hemorrhage, and/or thrombotic deposits, classified as Type VI plaques or more specifically as Type VIa, VIb, and VIc, respectively [15]. These may be combined; for example a plaque with fissures, a hematoma and a thrombus would be Type VIabc. The hematomas and thrombi may be incorporated into the lesion, contributing to the gradual narrowing of the lumen [15]. Those plaques with atheromas (Type IV and V) are especially prone to disruption,
with thrombosis being frequent from the fourth decade of life onward [15].

2.2.8 Type VII – Calcified

Although plaques from Type III onward may have calcifications present, Type VII represents those plaques, which are dominated by calcification. They typically develop directly from either Type IV or V plaques, and are sometimes classified as Type Vb.

2.2.9 Type VIII – Absent Lipid Core

A large plaque with an absent/minimal lipid core is classified as Type VIII or Type Vc. These plaques pose little risk of rupture.

2.2.10 Keys to Identifying Vulnerable Plaques with Imaging

As described in the above sections, there are many stages to plaque development, but a few key structures and material contents are essential for identifying vulnerable plaques with imaging techniques. The first of these is the ability to identify positively remodeled plaques. As mentioned above, the most widely used imaging techniques in the clinic today, x-ray angiography and duplex ultrasound, struggle to identify remodeled plaques. If a plaque is not identified, then any subsequent ability to detect vulnerability and assess risk is lost.

Assuming that a plaque is identifiable, then the identification of thin fibrous caps is key. Atheromas provide the anchor for thrombotic formation and thin fibrous caps are present in the most rupture-prone plaques. Ideally, every plaque from Type III onward would be identifiable with imaging, so as to have baseline data before atheroma formation from which to track the plaques development. If this is not possible, then identification of all Type IV+ plaques, with resolution sufficient to measure the thickness of the fibrous cap, should provide a sufficient basis for risk assessment. Additionally, as
the impact of calcium on plaque vulnerability continues to become better understood, the identification and size determination of calcium should provide useful additional information for risk assessment.

2.3 Familial Hypercholesterolemic (FH) Pig Model of Atherosclerosis

Since it is difficult to examine atherosclerosis at early stages in humans and obtain matched immunohistochemistry for validation, an animal model will be employed for the entirety of this dissertation. While many animal models of atherosclerosis exist, including mice and rabbits, a familial hypercholesterolemic (FH) pig model was chosen. These pigs spontaneously exhibit hypercholesterolemia in the range of 350–500 mg/dl when eating low fat pig feed due to a missense mutation in the LDL receptor [16; 17]. This animal model develops coronary, aortic and peripheral vascular disease in anatomic locations relevant to the human condition.

Additionally, these pigs develop atherosclerotic disease with the same histopathology seen in humans, including lesions consisting of macrophages, lymphocytes, foam cells calcification, fibrous caps and atheromas. These pigs are also of similar size to humans, weighing between 200 and 700 lbs., which will allow the use of clinical ultrasound imaging equipment, allowing these techniques to be directly translated into humans. While the carotid bifurcation in these animals is outside of ARFIs imaging capabilities due to anatomical constraints, the femoral and iliac arteries provide an ideal imaging location due to the large number of branches that provide many atherogenic imaging locations.

An animal model allows the examination atherosclerosis similar to humans in terminal studies with spatially matched immunohistochemistry providing a gold standard
for plaque characterization analysis. In addition to genetically predisposed pigs dietary hypercholesterolemic (DH) pigs were also used, which develop atherosclerosis similar to FH pigs, as well as normal cholesterolemic (NC) pigs, which have normal cholesterol, little atherosclerosis and provide a control.

2.4 Conclusions

The complexity of atherosclerotic disease both in humans and in a pig model necessitates accurate characterization of disease for vulnerability assessment. As collagen, elastin, calcium and fibrin (formed within a clot) all contribute to the structure and vulnerability of an atherosclerotic plaque, it is important to assess plaques with all of these contents in mind. The subsequent chapters will present ARFI imaging for the detection of plaques and for the characterization of these material contents.
Chapter 3

Atherosclerosis Imaging Techniques

3.1 Introduction

Conventional vascular imaging technologies rely on observation of luminal narrowing (stenosis) for the detection of atherosclerotic plaques. These technologies are either invasive, as with coronary X-ray angiography and intravascular ultrasound (IVUS), or noninvasive as with magnetic resonance angiography, duplex ultrasound and carotid intimal-medial thickness (CIMT) measurements. In addition to conventional techniques, techniques have been developed, or are under development, to attempt to provide more detailed information. These techniques include methods of tissue characterization, increased resolution through the use of optical imaging and elastographic assessment, which is separated into its own section due to its relevance to the subject of this dissertation. It should be noted that not all imaging and elastographic techniques are discussed here, only those which have been applied to the imaging and characterization of atherosclerosis.
3.2 Invasive Techniques

All of the following techniques require some type of invasive procedure, specifically a catheter injected through the femoral artery of the patient, a procedure resulting in a high rate of complications. For this reason, only the highest risk patients are typically imaged with these techniques [18].

X-ray Angiography

Also referred to as fluoroscopy, X-ray angiography is currently the gold standard for the clinical diagnosis of atherosclerosis [19]. The procedure involves using X-ray contrast agent injected into the lumen of the artery of interest, in conjunction with X-ray imaging of the region. From the acquired images, the presence of occlusive plaques can be inferred from the reduction or complete blockage of blood flow (observed as the absence of contrast agent). This technique provides no information regarding the composition of plaques and fails to detect non-occlusive plaques which have undergone vascular remodeling. Additionally, this technique has little value in preventing future coronary events due to the failure to detect less occlusive vulnerable plaques [14; 19]. CT has also been used to provide 3D imaging of atherosclerotic plaques, even identifying remodeled plaques, though it carries the same invasive risks and increased radiation dose [20].

Intravascular Ultrasound (IVUS)

As an alternative to X-ray angiography, IVUS uses an ultrasonic transducer mounted on the end of a catheter. The transducer is typically high frequency, 25 to 50 MHz, with axial resolutions <150 µm [13]. These resolutions have allowed IVUS to show some success at detecting thin fibrous caps, an important measure in assessing plaque vulnerability [4]. Additionally, IVUS is capable of detecting both occlusive and non-occlusive
plaques, a large advantage over X-ray angiography, and can measure the degree of vascular remodeling [4]. Despite the utility of the technique, the main limitation of IVUS remains the inability to characterize necrotic cores accurately and with a high degree of success, due to the lack of compositional information available using this technique [4; 13; 19]. However, this limitation is being addressed by the development of IVUS elastography, described in section 3.4.1, and IVUS VH, described below.

**IVUS Virtual Histology (VH)**

In the interest of improving IVUS plaque characterization, VH was developed to analyze the raw radio frequency (RF) ultrasound data to characterize plaque content and increase the detectability of necrotic cores [13]. By analyzing the raw data, VH is able to differentiate, fibrous, fibro-fatty, calcified and necrotic tissue. This allows VH to achieve a higher degree of success in detecting vulnerable plaques when compared to composition-less IVUS measurements, though it still has difficulty detecting some fibrous caps due to resolution constraints [19].

**Photoacoustic Imaging**

Photoacoustic imaging uses a laser pulse to induce acoustic emission from tissue due to thermal expansion, which can then be observed by high frequency ultrasound transducers resulting in resolutions in the tens of micrometers [21; 22; 23; 24]. Since optical absorbers always expand under laser excitation, prominent boundaries dominate the image with correlated signal, which suppresses interior speckle, resulting in a negligible impact of speckle on photoacoustic images. Photoacoustic imaging typically requires nanosecond laser pulses to generate optimal images, though multiple wavelengths can be used to highlight differential tissue/cell types depending on the relative optical absorption. It has already been demonstrated that combined photoacoustic/IVUS imaging
catheters are possible, and this method holds promise in providing additional tissue characterization data beyond that of IVUS alone [21].

**Optical Coherence Tomography (OCT)**

Building on the success of IVUS and IVUS VH, OCT has been implemented to leverage the superior resolution of optical imaging in the characterization of plaque [19]. OCT leverages interferometric imaging techniques to extract reflected light from infrared pulses, generating images in a similar fashion to ultrasound. With resolutions of 5-20 \( \mu \text{m} \) due to the use of infrared light, OCT has been demonstrated for characterizing vulnerable plaques [19]. This is due in part to the ability of OCT to measure the thickness of fibrous caps which would be below the resolution threshold of IVUS. This improvement in image quality is not without drawbacks, however, as the higher attenuation of optical signals by blood requires regular saline flushes or balloon occlusion of the artery in order to obtain high quality images [13; 19]. However, optical imaging is constrained by limited penetration depths of only 1-2 mm, which often fails to penetrate the entire thickness of the vessel wall [19].

### 3.3 Noninvasive Techniques

Due to the risks associated with invasive procedures, noninvasive techniques are preferable. These techniques offer little risk to the patient at the cost of limited imaging locations, resolution and/or increased expense. The only completely noninvasive technique currently used for imaging atherosclerosis is duplex ultrasound, within which B-mode and Doppler techniques are utilized, though novel techniques such as magnetic resonance angiography (MRA) and elastographic techniques are under development.
**Duplex Ultrasound**

Used extensively in the clinic for atherosclerotic assessment of the peripheral vasculature, primarily the carotid artery and carotid bifurcation, duplex ultrasound provides cheap, fast real-time imagery [20]. Duplex ultrasound combines the anatomical images of B-mode ultrasound with the blood flow images of doppler ultrasound to generate real time 2D transcutaneous images. This technique excels at identifying occlusive plaques and has shown success at measuring intima-media thickness (IMT). Increased IMT measurements in the carotid have been shown to be a strong independent predictor of cardiovascular events, with thickness increases as small as 0.1 mm increasing risk [25]. However, as the axial resolution of a typical 7.5 MHz ultrasound pulse is only 0.2 mm, and with the true resolving capability of an ultrasound image confounded by acoustic speckle, measuring IMT increases this small can be difficult [20]. Currently, Duplex and other transcutaneous ultrasound techniques are largely limited by the 2D planar images provided by conventional phased and linear arrays and can possibly miss atherosclerosis located on non-normal surfaces of the artery. Advancements in transducer design, such as 2D matrix and mechanically scanned ultrasound arrays, hold promise at removing this limitation.

**MR Angiography**

Magnetic resonance angiography has shown success at both detecting plaques and accurately assessing compositional elements such as lipids and calcium [13]. MR angiography uses specialized pulse sequences either with or without imaging contrast agent to generate images, which can differentiate plaque components on the basis of chemical composition, water content, physical state and molecular motion. This technique promises a greater possibility for detecting remodeled non-occlusive plaques when compared to X-ray angiography [20]. Additionally, this technique has been demonstrated
to detect plaques in 3D [13]. As with all MR imaging, this technique uses no radiation and can provide high resolution images, though requires a long acquisition time and is expensive. Due to the issues of cost and imaging time, it is most likely that MR angiography will be used predominantly as a secondary screening tool as opposed to providing initial diagnosis [13].

3.4 Elastography

As most currently used clinical techniques rely on stenosis and/or blood flow obstruction, they are ill suited to characterizing atherosclerotic plaque composition, a key factor in plaque risk. Furthermore, due to vascular remodeling, these methods have difficulty identifying early and remodeled plaques. Given that atherosclerotic composition is a key factor in plaque vulnerability there is a need for novel methods of characterizing plaque composition [3]. These include several imaging methods under development including IVUS elastography, noninvasive vascular elastography (NIVE) and several radiation force based ultrasound techniques.

3.4.1 Intrinsic Motion Sources

Elastographic methods using intrinsic motion sources track tissue motion using speckle tracking methods to analyze changes in the speckle pattern of arterial tissue. In the vasculature this motion is typically due to cardiac pulsation, which is used to generate strain maps and infer stiffness information regarding regions of the tissue.

IVUS Elastography

Using an intravascular catheter-based ultrasound transducer as in normal IVUS, IVUS elastography measures tissue deformation due to varying intraluminal pressure [26;
When combined with catheter pullback, this technique is capable of providing 3D reconstructions of arterial stiffness. This technique has been demonstrated both in vitro and in vivo as capable of detecting both fibrous and fatty plaques [13]. Also, since this technique uses existing IVUS catheters, and poses no additional risk to the patient, it is a promising technique for the characterization of vulnerable plaques [13; 19].

Noninvasive Vascular Elastography

While IVUS elastography has extensively documented the potential for using strain maps of the vasculature to infer atherosclerotic risk, it remains an invasive procedure. Several groups have worked to apply elastography in a noninvasive application [28; 29; 30; 31]. As with IVUS elastography, the strain is induced by cardiac pulsation, requiring no external stimulus. Reproducible results have been observed, but as with conventional 2D Duplex ultrasound, the imaging methods will require a move to 3D imaging to fully generate stiffness maps for the entire artery, something that IVUS elastography is capable of now [30].

3.4.2 Extrinsic Motion Sources

Elastographic techniques using an extrinsic motion source typically induce displacement or shear waves, which can then be observed. This can be done either through the use of a mechanical vibrator as in MR elastography or using high intensity acoustic impulses as in radiation force imaging.

MR Elastography

Although it has only preliminarily been used to assess arterial stiffness, magnetic resonance elastography (MRE) has been demonstrated in a variety of other tissues. Using
externally stimulated shear waves, MRE tracks tissue motion in 3 dimensions and provides a full 3D reconstruction of tissue modulus. This has been demonstrated for assessing stiffness in the groin [32]. The main limitations of this method are currently the high cost and time consuming nature of MR imaging.

**Radiation Force**

As an alternative to externally stimulated shear waves, sound waves themselves may be used to induce displacement and shear waves. These displacements and shear waves can be tracked and used to characterize atherosclerosis. As this pertains directly to the methodology used in this research, a more thorough description is provided in the next chapter.

### 3.5 Conclusions

With the wide array of imaging techniques under development, it is clear that there will soon be viable alternatives to X-ray angiography and duplex ultrasound. Clinicians will benefit from improved detection of remodeled plaques and characterization of atherosclerosis. It is unlikely that these novel methods will replace existing technologies, but rather supplement and provide additional assessment methods. The next chapter will look more closely at radiation force based imaging and introduce ARFI ultrasound.
4.1 Radiation Force Theory

Sound waves, just like other forms of wave motion, such as electromagnetic waves, exert a small force on absorbing and scattering objects in their path [33]. While most of the energy of the wave passes through tissue, a small portion of the acoustic wave’s momentum is transferred into the tissue and generates a body force. This force is given by,

$$F = \frac{W_{\text{absorbed}}}{c} \quad (4.1)$$

where $F$ (kg/(s$^2$cm$^2$)) is the radiation force transferred into the tissue as a body force, which operates on a volume of tissue as opposed to only on the surface [33], $W_{\text{absorbed}}$ (Watts/(100cm$^3$)) is power absorbed by the medium and $c$ (m/s) is the speed of sound. If plane wave acoustic propagation is assumed, then the acoustic radiation force is,

$$F = \frac{2\alpha I}{c} \quad (4.2)$$

where $\alpha$ (1/m) is the attenuation coefficient of the tissue, $I$ (W/cm$^2$) is the temporal average acoustic intensity and $c$ (m/s) remains the acoustic speed of sound in the tissue [34; 35]. Equation 4.2 provides a relation between intensity and force, in
which an acoustic intensity field translates directly into a force field, after differences in attenuation coefficient and speed of sound are taken into account. In practice, when a focused ultrasound beam is applied into tissue, this translates into a concentrated volume within which significant displacements are induced. The nature of the displacement and subsequent recovery are dependent upon tissue mechanical properties, which have been exploited in the imaging techniques described in later sections. As it turns out, the initial displacement and recovery is not the only mechanical response of interest.

Much like a pebble dropped in a pond, the initial radiation-force-induced displacement propagates perpendicularly to the direction of wave propagation as a shear wave. This propagation can be described by the following equation,

\[
\frac{\partial^2 s_x}{\partial t^2} - (c_t^2 + \nu \frac{\partial}{\partial t}) \Delta_{\perp} s_x = F
\]  

(4.3)

where \( s_x \) (m) is the component of the displacement vector along the direction of beam propagation, \( \nu \) (Pa-s) is the shear viscosity, \( c_t \) (m/s) is the speed of shear wave propagation, \( t \) (s) is time and \( \Delta_{\perp} \) is the laplacian operator acting in the transverse coordinate \( r \) (m) \[36\]. The propagation velocity of the shear wave holds particular interest in measuring for tissue, since it can be directly related to useful measures of stiffness for a given geometry. The simplest of these relations is for a homogeneous block,

\[
c = \sqrt{\frac{\mu}{\rho}}
\]  

(4.4)

where \( c \) (m/s) is the velocity of the shear wave, \( \mu \) (kPa) is the shear elastic modulus and \( \rho \) (g/m\(^3\)) is the density. The shear wave velocity (SWV) has the benefit that it retains no dependence upon acoustic intensity or attenuation coefficient, and so should be repeatable in tissue. The most glaring downside for vascular applications relates to
the dependence of the shear wave velocity-stiffness relation on geometry, as it becomes difficult if not impossible to derive an analytical relation for more complex geometries such as atherosclerotic vessels.

4.2 Methods of Acoustic Radiation Force (ARF) Imaging

Several methods have been developed to exploit acoustic radiation force (ARF) for the assessment of tissue mechanical property. These methods either examine the tissue recovery in the region of excitation, as in ARFI imaging and vibroacoustography, or shear wave propagation speeds as in SWEI/SSI.

4.2.1 Vibroacoustography

Vibroacoustography induces displacements originating from the beat frequency of two acoustic beams transmitted from a confocal transducer. The tissue then emits acoustic signal at the beat frequency, typically in the kHz range, which is recorded with a hydrophone. This has been demonstrated both in vitro and in vivo in porcine arteries [37; 38; 39], where the displacing regions were 10mm axially by 1mm in the lateral and elevational dimensions. The imaging system was able to differentiate calcifications from normal arteries with high sensitivity but low specificity. The calcified arteries, however, were generated artificially via injection of calcium hydroxyapatite, so important aspects of vulnerable plaques such as necrotic cores and thin fibrous caps were not present. Additionally, the poor axial resolution of the system may cause difficulty when attempting to resolve smaller, earlier plaques. Also, 5x5cm 2D images were generated in the coronal plane, but required separate excitations and measurements for each pixel, resulting in acquisition times in excess of 3 minutes, not accounting for cardiac gating.
To include cardiac gating, the authors estimate that it would lengthen acquisition time by 50 times using their current equipment.

4.2.2 Shear Wave Elasticity Imaging (SWEI) & Supersonic Imaging (SSI)

There are two methods currently under development to assess the shear wave velocity, SWEI and SSI [36; 40]. Both methods are very similar, with the main differences residing in the number of pushing pulses used to generate the initial radiation force. SWEI methods in current use employ only a single pushing depth, while SSI utilizes a much more complicated combination of 3+ excitation depths. SSI exploits the fact that acoustic propagation velocities are \( \sim 2 \) orders of magnitude greater than the transverse shear wave velocity to mimic a supersonic mach cone. Three or more focal ARFI excitations are focused at differing depths with the same lateral position, with some time delay. The displacements all propagate laterally with the approximate shape of a plane wave due to wave summation of the multiple shear waves. The relationship between the time delay in the transmit of the SSI excitations to the local SWV of the tissue controls the angle or ‘mach number’ of the propagating plane shear wave. SSI tends to require ultrafast imaging techniques to remain within acoustic exposure limitations. Ultrafast imaging is a technique where a plane wave transmit is followed by massively parallel beamforming (typically 128+ lines) to generate an entire B-mode image in the time of a single pulse repetition period (PRP) [40]. This allows SSI to acquire an entire FOV worth of shear wave data with only 3 ARF excitations. With 4:1 parallel receive beamforming (ParRx), as used in SWEI, more than 15 times as many excitations would be required.

With these differences between the sequences taken into account, the overall design...
of SWEI/SSI sequences is shown in Figure 4.1. Preceding Step 1 are 1-2 short high resolution reference pulses, for a baseline measurement of tissue location. This is followed by Step 1, which is the initial ARFI excitation where a high intensity acoustic pulse generates a displacement. As shown in Step 2, this is followed by a number of tracking pulses that are typically higher frequency and shorter, for better resolution, which can be compared with cross correlation techniques to track the differential displacement of the tissue (Step 3). The end result of this calculation is a 3-dimensional matrix consisting of a plot of displacement vs. time for each lateral and axial position in the image. These displacement profiles can then be compared, such as detecting time to peak displacement vs. lateral position to measure the arrival time of the shear wave from which the SWV can be calculated.

Both SWEI and SSI have already been used to assess the arterial system [41; 42]. It has been previously shown that SWEI can be used to differentiate atherosclerotic plaques from normal vessel wall and will expand on that previous work in subsequent
chapters [41]. Others have recently implemented SSI in the arterial system, though not in atherosclerotic vessels [42]. This work also attempted to generate an empirical analytical relation between the SWV and shear modulus. Based on the mathematical formulation for a leaky lamb wave with a low frequency approximation,

\[ V \approx \frac{\omega h c_T}{2\sqrt{3}} \]  

where \( V \) (kPa) is the shear modulus, \( \omega \) (rad) is the angular frequency, \( h \) (m) is the vessel thickness and \( c_T \) (m/s) is the shear wave velocity [42]. The 2 in the denominator is an empirically-derived correction factor, which gives a good approximation in their studies. However, their analysis has so far only looked at phantoms of simple plane and cylindrical geometries. It remains to be seen if this velocity approximation will hold up under the wide range of \textit{in vivo} physiological possibilities in the arterial system, such as varying arterial thickness, varying local velocity and impact of mechanical impedance changes.

### 4.2.3 Acoustic Radiation Force Impulse (ARFI) Ultrasound

In contrast to methods used to detect shear wave propagation from ARF induced displacements, ARFI ultrasound examines the tissue response in the region of excitation (ROE) itself [35; 43]. The process of ARFI imaging can be described by Figure 4.2. As with SWEI, the imaging sequence begins with 1-2 short B-mode reference pulses to establish a tissue location baseline. This is followed by a high intensity acoustic impulse to displace tissue (Step 1), referred to as an ARFI excitation or push, which is followed by an ensemble of \( \sim 60 \) B-mode tracking pulses. The ensembles are then repeated at a number of lateral positions to generate a 2D set of displacement profiles. From this data set, parametric images can be created by analyzing properties of the individual
(1) ARFI Excitation Pulse (∼70µs) induces axial displacement
(2) Conventional B-Mode pulses track induced displacement
(3) Displacements are calculated to create a displacement profile for every pixel within the image.

Figure 4.2: General method for ARFI imaging.

displacement profiles, such as the peak ARFI-induced displacement (PD) and time to 67% recovery from peak ARFI-induced displacement or recovery time (RT).

The demonstration of ARFI’s capability to detect and characterize atherosclerotic plaques ex vivo and in vivo will be thoroughly detailed in subsequent chapters, but it is worth providing a background on the applications for which ARFI has been utilized. The first demonstration of ARFI ultrasound was for differentiating fluid-filled breast cysts from other lesions [44; 45]. Since then ARFI has also been used to examine abdominal tumors in the kidney and liver [46; 47], RF ablations [48; 49], the gastrointestinal tract [50], thrombosis [51], the myocardium [52], and the prostate [53]. Additionally, ARFI was first demonstrated in human atherosclerotic arteries by Trahey et al. [54], which will be built upon in the development of ARFI ultrasound presented in later chapters.
4.3 Bioeffects and Risks Associated With Radiation Force

As with any imaging modality, safety is paramount in ARF imaging. With this in mind, clinical ultrasound imaging is regulated in the United States by limits on acoustic output established by the FDA. The two major concerns for ultrasound can be subdivided into excessive heating and cavitation, or thermal and mechanical bioeffects [55]. These two potential concerns are discussed below, with a specific focus on their relevance to ARF-based imaging.

4.3.1 Thermal Effects

As ultrasound waves propagate through tissue, they are scattered, reflected and absorbed. As they are absorbed, a portion of the ultrasound wave energy is converted into heat. The concern for imaging tissue is that excessive heating can result in detrimental effects on cell structure and function, even potentially resulting in cell death. In order to prevent potential thermal effects, the FDA currently limits ultrasound system output to a derated spatial-peak temporal-average intensity or $I_{SPTA}$ of $\leq 720 \text{ mW/cm}^2$ in non-fetal applications [56]. Additionally, the FDA’s output display standard (ODS) provides a thermal index (TI), in order to approximate the local tissue increase [55]. And, while not placing a hard upper limit on TI, manufacturers must justify TIs greater than 6 (roughly corresponding to a 6°C temperature increase). The FDA’s TI, however, does not take into account the temporal dependence of temperature increase.

The American Institute of Ultrasound in Medicine (AIUM) has developed revised recommendations for safe tissue heating due to ultrasound, based on the exposure durations and temperature increases for which biological effects have been observed. First, for temperature increases of $\leq 2^\circ \text{C}$, there have been no observed biological effects
up to exposures of 50 hours. For temperature increases between 2°C and 6°C, the safe exposures range between 4.3°C for 5 min and 6°C for 1 minute. For temperature increases greater than 6°C, a different equation is used, allowing for up to a 9.6°C temperature increase for a 5 second exposure. For even shorter duration exposures, no adverse biological effects have been observed for temperature increases of 18.6°C, 14.9°C and 12.6°C for exposure durations of 0.1, 1 and 5 seconds, respectively [56]. This last time period is of particular interest to ARF imaging as the high intensity exposures are applied for very short durations (<1 ms), suggesting that temperature increases greater than 6°C may be safe.

Examinations into the thermal effects of ARF imaging have been completed by Palmeri et al. concluding that ARFI sequences staying below the FDA acoustic output limit of 720 mW/cm² generate focal and surface heating of <1°C [57; 58]. What this means in terms of the AIUM’s most recent safety recommendations is that there may be a great deal more power and therefore increased SNR usable in ARF imaging without posing a biological risk.

4.3.2 Mechanical Effects

In addition to potential thermal bioeffects, mechanical effects are of concern to ultrasound imaging. The primary mechanical bioeffect of concern is cavitation, which is the formation and/or destruction of bubbles in tissue. If cavitation occurs, adverse effects, such as cell destruction can occur within tissue. With this in mind, the FDA specifies the mechanical index (MI) as a predictor of cavitation possibility, assuming optimally sized bubbles are already present in tissue [55]:

\[
MI = \frac{Pr.3}{\sqrt{f}}
\]  

(4.6)
where \( p_{r,3} \) (MPa) is the derated peak rarefactional (negative) pressure induced by the ultrasound pulse and \( f \) (MHz) is the center frequency of the pulse in MHz. The FDA currently limits MI to a value of 1.9 in ultrasound imaging systems. The MI, however, is only recommended for pulses <10 cycles, which ARF imaging typically exceeds. However, most tissue aside from the lung and intestine do not contain gas bubbles, and for such tissues no adverse bioeffects have been observed. For example, exposure to 10 \( \mu s \) pulses of 1 MHz at an MI of 4 in the spinal cord was shown to have no bioeffects [59]. Currently, there is no evidence suggesting that the application of ARF to tissue not known to contain gas bodies, such as the carotid and surrounding regions, will cause bioeffects at MIs below the FDA limit or even below an MI of 4 [59].
Chapter 5

ARFI Imaging for Noninvasive Material Characterization of Atherosclerosis I: Ex Vivo Demonstration of Characterization

5.1 Introduction

The need for more advanced imaging techniques to improve atherosclerotic plaque detection and characterization has been discussed in previous chapters. ARFI was first demonstrated in human atherosclerotic femoral arteries in Trahey et al., where the authors demonstrated ARFI as capable of differentiating an atherosclerotic plaque \textit{ex vivo} and of imaging a nonatherosclerotic artery \textit{in vivo} [54]. That preliminary work is built upon to demonstrate ARFI in an \textit{ex vivo} porcine model of atherosclerosis,
specifically analyzing the relationship between ARFI response and plaque material content. Increases in collagen deposition are expected result in decreased ARFI-induced peak displacement and degradation of the IEL is expected to result in larger recovery times.

5.2 Methods

5.2.1 ARFI Imaging

All imaging was performed with a Siemens SONOLINE Antares™ imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division), equipped for research purposes, and a VF10-5 linear array transducer. 2D ARFI imaging was performed ex vivo on an excised left iliac artery of an adult (1 year, 8 month old) FH pig. The vessel was tethered to a pressure apparatus in a water bath, with an intraluminal pressure of 3 kPa (22.5 mm Hg) maintained during ARFI imaging. Pressure was maintained using an air tank pressurizing a water column, which in turn pressurized the vessel. A matched B-mode frame was acquired immediately preceding ARFI imaging, and both B-mode and ARFI imaging were focused at 21 mm on the distal arterial wall. ARFI excitations (or pushes) were 300-cycles (52 µs) long at a center frequency of 5.71 MHz with an F/1.5 focal configuration. ARFI reference and tracking pulses were 2-cycles long at 6.67 MHz with an F/2 focal configuration (conventional B-mode pulses). A single ARFI ensemble consisted of 2 reference pulses, a single ARFI excitation and 50 tracking pulses. ARFI ensembles were repeated at 50 lateral positions, spaced 0.45 mm, to generate on overall field of view (FOV) of 2.3 cm. Wiperblading, a technique which entails translating the imaging focus laterally from the far left of the FOV, to the center, to one position right of the far left, to one position right of center, etc., was implemented to avoid mechanical interference and additive heating from adjacent
ARFI pushes. As described in Section 4.3.1, temperature increases were conservatively estimated to be <1°C.

Following data acquisition, the reference and tracking pulses were compared using normalized cross-correlation to measure ARFI-induced displacements and subsequent tissue recovery [60]. Parametric images for PD and RT were generated, with the color scales chosen to maximize contrast in ROIs. To facilitate image examination, a 2D mask was generated to remove all luminal and extravascular signal using a median-filtered B-mode amplitude threshold.

5.2.2 Histology

Following ARFI examination, the vessel was cut longitudinally and the luminal side was photographed en face as shown in Figure 5.1. This was then compared with B-mode and ARFI images for spatial registration. Nine tissue sections were extracted in groups of 3, each section within a group separated by 20 μm and each group separated by 0.9 mm along the circumferential length of the vessel. The group spacing approximated the elevational resolution of the imaging system, which was 1.1 mm. Within each group, 1 section was stained with hematoxylin & eosin (H&E) for baseline, 1 with Verhoeff van Gieson (VVG) for elastin (black) and 1 with Masson’s trichrome (MT) for collagen (blue). Note that tissue sections from C (Figure 5.1) include the portion of the raised focal atherosclerotic plaque that appears in spatially-matched B-mode and ARFI images.

5.3 Results

The excised left iliac artery from an adult FH pig is shown en face in Figure 5.1. The top image shows the entire vessel with regions of focal atherosclerosis outlined in red
and the near and far wall ARFI imaging locations outlined in yellow. The boxed region is shown zoomed in in the bottom image, where the distal wall of ARFI imaging can be seen, with the lateral FOV and elevational resolution drawn approximately to scale (23 mm and ~1.1 mm, respectively). The lines labeled A, B and C correspond to the histology sections in Figure 5.2 and are spaced 0.9 mm apart in the elevational imaging dimension.

The nine histology sections in Figure 5.2 are laid out with H&E for baseline in the left column, VVG for elastin in the center column and MT for collagen in the right column. The top row, corresponding to section A from Fig. 5.1, shows a small portion of the shoulder of the atherosclerotic plaque (arrow) in the rightmost region of the section. In the VVG stain, the IEL appears degraded in the region with plaque (top row, middle column, Fig. 5.2). Additionally, the MT stain shows collagen deposition in the arterial wall beneath the plaque (top row, right column, Fig. 5.2). The second row, corresponding to section B from Fig. 5.1, contains a larger portion of the atherosclerotic plaque, with the plaque extending closer to the center of histology section (black arrows). Also, a thin layer of intimal thickening extends from the raised plaque across the adjacent arterial wall (white arrows, second row, Fig. 5.2). Again, degradation of the IEL (middle row, middle column) and deposition of collagen (middle row, right column) are visible in both the focal plaque and diffuse atherosclerotic regions, though more disruption and deposition are visible under the focal plaque. Lastly, the bottom row of Figure 5.2, corresponding to the distal wall imaged with ARFI and section C in Figure 5.1, contains raised atherosclerosis throughout the section (black arrows). The left side of this section corresponds to near the shoulder of the plaque and the right side corresponds to nearer the center of the plaque. The IEL appears severely degraded (bottom row, middle column, Fig. 5.2) and collagen appears deposited throughout (bottom row, right column, Fig. 5.2).
Figure 1: Following ARFI and B-Mode imaging, the examined iliac artery was cut longitudinally to expose the vessel lumen. In Fig. 1(a), the red polygons delineate the borders of raised focal atherosclerotic plaques, with a ruler showing centimeters. The top and bottom yellow bars indicate the tissue appearing as the proximal and distal vessel walls, respectively, in matched B-Mode and ARFI imaging. The region of the interrogated atherosclerotic plaque is shown in zoom in Fig. 1(b). The yellow rectangle depicts the area of B-Mode and ARFI imaging, with axial resolution drawn approximately to scale. The spatial geometry of tissue sectioning for matched immunohistochemistry is illustrated by lines A, B, and C, each spaced 0.9 mm apart. Within each of these three regions, three sections spaced 20 µm apart were extracted for histology. Note that sections C include the raised atherosclerotic plaque that appears in matched B-Mode and ARFI images.

Figure 5.1: Excised iliac artery, en face view.
Figure 2: The nine serial horizontal sections extracted from the leading edge of the raised atherosclerotic plaque after staining with haematoxylin and eosin for baseline (H&E, left column), Verhoeff van Gieson for elastin (VVG, center column), and Masson's trichrome for collagen (right column) are shown. The sections were taken from the regions marked A (top row), B (center row), and C (bottom row) in Fig. 1(b). In the staining with H&E, pink represents cytoplasm and nuclei. In VVG stains, black indicates elastin while red indicates collagen. Collagen composition is depicted more clearly by the color blue in Masson's staining. The histology results illustrate a progressive degradation of elastin, including the internal elastic lamina, and deposition of collagen from the shoulder region toward the center of the atherosclerotic plaque (arrows).

Figure 5.2: Histology for iliac artery shown in Fig. 5.1.
Since section C spatially corresponds to a portion of the distal arterial wall imaged with ARFI, the VVG and MT stains are shown at higher magnification in Figure 5.3 for a more thorough comparison. In both stains, the histology section is broken into 5 boxed subregions, which are shown at the higher magnification, with subregion 1 corresponding to the shoulder of the plaque and subregion 5 corresponding to nearer the center of the plaque. First, the VVG stain (top, Fig. 5.3), shows that in box 1 the IEL appears largely intact (black line, arrows) and becomes progressively degraded the closer to the center of the plaque. This becomes so extensive that the black line corresponding to the IEL in box 5 is severely disrupted, appearing broken and thinned. Second, the MT stain (bottom, Fig. 5.3), shows progressively more collagen deposition from the shoulder to the center of the plaque. Interestingly the observed degradation of the IEL and deposition of collagen does not appear to progress homogeneously across the plaque. Based on the IEL degradation, collagen deposition and lack of atheroma, this plaque is a Type III or intermediate stage plaque.

B-mode and ARFI images of the atherosclerotic plaque sectioned and stained in Figures 5.2-5.3 appears in Figure 5.4 (orange arrow). Figure 5.4a shows a spatially-matched B-mode, where in the distal wall a focal atherosclerotic plaque is visible (orange arrow), beginning at ∼0 mm laterally and extending past the right side of the image. The two walls visible in the image correspond to the yellow boxes in Figure 5.4b as shown by the yellow arrows. This same plaque is visible in the ARFI images of PD (Fig. 5.4c) and RT (Fig. 5.4d). In the PD image (Fig. 5.2), where displacement in microns is mapped to color, the focal atherosclerotic plaque (boxed) displaces less (∼2.5 µm, blue) than the adjacent arterial wall (∼4-5 µm, orange-red). This corresponds with the expected increase in stiffness caused by the deposition of collagen visible in Fig. 5.3. While the PD image maps displacement to a color map, the RT image (Fig. 5.4d) maps recovery time in milliseconds. This image shows that the shoulder of the plaque
Figure 5.3: Matched histology for iliac artery shown in Fig. 5.2 displayed at higher magnification.
Figure 5.4: B-Mode, *ex vivo* image and parametric ARFI images for artery imaged *ex vivo*.

Recovery times show that the shoulder region recovers slower (~1.5 ms, blue-yellow) than the adjacent vessel wall (~1.0 ms, blue), but still much faster than the more central region of the plaque which recovers very slowly (~3-3.5 ms, red). This increase in recovery time relates with the decrease in elasticity expected from the degraded IEL seen in Figure 5.3. Note that the plaque experiences the same displacement and recovery behavior as the tissue below it.

### 5.4 Discussion

We applied 2D ARFI imaging *ex vivo* to mechanically characterize a raised focal atherosclerotic plaque in the left iliac artery of an adult FH pig, a relevant model of human CVD. The artery was examined with *ex vivo* ARFI and spatially-matched
B-mode imaging. The ARFI result was then compared with spatially-matched immunohistochemistry, where the focal plaque was confirmed.

VVG staining showed a progressively more disrupted IEL, the further toward the center of the plaque that was examined. In addition to the IEL degradation, collagen deposition was observed, also increasing toward the center of the atherosclerotic plaque. These variations in IEL continuity and collagen deposition spatially correlate with the expected ARFI result. More specifically, in regions with collagen deposition, a reduction in PD was observed. PD was the largest in regions with the thinnest layer of collagen and smallest in regions with the thickest layer of collagen. Also, in regions with a degraded IEL, an increase in recovery time was observed. As with collagen, the regions with the most degraded IEL had the largest increase in RT and regions with almost normal IEL had the smallest increase in RT.

5.5 Conclusions

This preliminary study demonstrated 2D ARFI imaging for describing the mechanical properties of a Type III raised focal atherosclerotic plaque *ex vivo*. With matched histology, a spatial correlation between collagen and elastin content and parametric ARFI-assessed tissue mechanical property was observed. In regions of enhanced elastin degradation, slower recovery rates from peak displacements were observed. In regions of collagen deposition, lower peak displacements were achieved. Although the examined plaque does not represent a thin cap fibroatheroma or other features associated with a vulnerable plaque, these results suggested that ARFI imaging was relevant for describing plaque material property in application to assessing vulnerable plaques. In the next chapter, this work will be extended *in vivo* applications, including examples of subclinical disease and a fibroatheroma.
6.1 Introduction

As explained in detail in Chapter 2, describing plaque composition, size, structure and mechanical properties are all important for the identification of vulnerable plaques. The previous chapter demonstrated ARFI ultrasound as a method for characterizing atherosclerosis \textit{ex vivo} in an FH pig model. Apparent correlations between collagen

and PD and elastin and RT were observed and ARFI images were able to assess stiffness and elasticity of the plaque. In this chapter ARFI ultrasound will be extended to the characterization of atherosclerotic plaques in vivo. Additionally, a more quantitative analysis of material content, specifically collagen and elastin, will be applied to ARFI measurement, obtaining a better understanding of the relationship between ARFI metrics and vessel material content. First a control example will be examined, to establish baseline image expectations, after which three examples of atherosclerosis will be presented, two of which are subclinical Type IV-VI and Type II plaques.

6.2 Methods

6.2.1 ARFI Imaging

Imaging was again performed with a Siemens SONOLINE Antares™ imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division), equipped for research purposes, and a VF7-3 linear array transducer instead of the VF10-5 transducer used in the previous chapter. As a result of this change, ARFI impulses were 4.21 MHz and both reference and tracking pulses were at 6.15 MHz, all pulses having a PRF of 11 kHz. ARFI impulses remained 300 cycles in duration (∼70 µs) in a F/1.5 focal configuration. The ARFI sequence consisted of 40 ensembles spaced 0.53 mm apart, generating a lateral FOV of 2.1 cm. In the last example, 15 excitations were applied, combined with 4:1 parallel receive tracking (ParRx), giving 60 effective lateral positions spaced 0.35 mm apart for a lateral FOV of 2.1 cm. Each ARFI ensemble consisted of 2 reference pulses, an ARFI excitation pulse, and 60 subsequent 2-cycle tracking pulses for a tracking duration of 6 ms. One dimensional normalized cross correlation was applied to the acquired RF data ensembles to measure ARFI-induced axial displacements. As with the previous chapter, again tissue heating is estimated to be <1°C (Section 41.
4.3.1) and employed wiperblading as an additional precaution. From the ARFI data set, images of PD and RT were generated for the assessment of arterial mechanical property. Imaging was performed \textit{in vivo} in the iliac arteries of 4 pigs: a 3 year, 6 month-old NC female, a 4 year, 4 month-old FH female, a 3 year, 1 month-old DH female and a 8 year, 4 month-old FH female.

Physiological motion was rejected using a filter based upon the quasi-static rigid wall model (QSRW), which is described in detail in Appendix A. Briefly, this motion filter assumes that arterial wall segments experience bulk motion in response to cardiac pulsation according to the QSRW model. Over small distances (~1 cm) such as those used in ARFI wiperblading, continuous displacement of ARFI ensembles due to cardiac pulsation is assumed. ARFI ensembles were assembled into a global displacement curve, so that the final measured displacement of one ensemble became the starting displacement of the next ensemble. A 20th order polynomial was then fit to the resulting global displacement curve as an approximation for cardiac-pulsation-induced motion and subtracted to remove this motion.

A luminal mask was applied to parametric ARFI images to reject luminal blood signal. This was performed with a combination of two methods, B-mode thresholding as performed in the previous chapter and a correlation-based algorithm. The correlation-based mask used median correlation values from the last five tracking lines in each ensemble. Any location in the FOV with a median correlation value less than 0.992, an empirically derived threshold, was discarded. The two masks were combined to generate a single mask, which was then applied to parametric ARFI images. Using this mask as a basis, remaining unmasked regions of the lumen were then removed by hand.
6.2.2 FH Pigs and Matched Histology

All pigs were sedated prior to imaging in the lateral decubitus position, with the rear legs extended and physically separated to provide acoustic access to the iliac arteries in the inguinal canal. The location of the imaging transducer on the surface of the skin was marked with a tattoo following imaging by a professional sonographer. The tattoo was used for spatial registration of arterial sections corresponding to the imaging location during necropsy, which occurred within 48 hours of imaging.

As in the previous chapter, harvested vessels were cut longitudinally and opened for *en face* imaging of the vessel. Then, with B-mode and ARFI images as a guide, the matching sections of vessel corresponding to the proximal and distal wall of imaging were removed for histologic staining with assistance from a pathologist who observed imaging procedures. Each section was stained with three stains as done perviously, H&E, VVG and MT. In the case of the DH pig, Sirius Red (SR) staining was performed for differentiation of collagen subtypes I and III using polarized microscopy [61]. Additionally, for the last example von Kossa (VK) staining was performed to examine calcium content. All histology sections were imaged with transmission light microscopy and the SR stained sections were also imaged with linearly polarized light microscopy.

To spatially register histology images with ARFI images, vessel morphology was compared with gross anatomical features observed during necropsy. Minor misalignments may occur, though misregistration is not expected to be a significant source of error.

6.2.3 Statistical Analysis

The 3 examples with arteries containing plaques were spatially correlated with histological collagen and elastin content. First, the arterial wall was isolated by hand
masking that region only. The median value over the axial range of the artery was then calculated for both ARFI parameters and every lateral location. A vector of median parameters for an empirically-determined lateral sampling width $L$ (1.0-2.0 mm) was then calculated by stepping the calculation window across the length of the FOV laterally with a step size of one lateral ARFI imaging position (0.5 mm).

Second, in the corresponding histological images, the vessel wall was isolated from the background. Local percentages of collagen or elastin were calculated as follows: First, the entire wall was then subdivided into blocks of lateral span $L$, encompassing the entire thickness of the vessel. A color threshold was then applied using NIH ImageJ and the colour threshold plug-in [62]. The colors black for VVG (elastin) and blue for MT (collagen), were individually isolated. Elastin and collagen functional areas were then calculated as the percentage of black or blue pixels compared to the total number of arterial pixels in each subregion. Third, linear and exponential regression models were used to calculate the correlation between median ARFI parameter and percentage material content area. P-values were calculated using the Student’s $t$ distribution (corr function, MATLAB Statistical Toolbox, Mathworks, Inc.).

6.3 Results

In Vivo Left NC Control Pig Iliac Artery

Figure 6.1a shows the B-mode image for a NC control pig iliac artery. The walls appear clearly defined and thin with no visible intimal thickening or focal atherosclerosis. Imaging was performed, focusing on the distal arterial wall with a focal depth of 36 mm. The vessel is shown en face in Figure 6.1b, with the imaging location of the distal wall outlined by the yellow box where the width corresponds to the lateral FOV and height corresponds to elevational resolution of the imaging system. No atherosclerosis is visible
in this region or anywhere on the vessel. Spatially-matched immunohistochemistry was sectioned along the black line of the vessel (Fig. 6.1b) and stains for VVG and MT are shown in Figures 6.1c and 6.1e, respectively. Neither histology stain shows any indication of atherosclerosis, as expected, appearing with uniform thickness and no intimal thickening. An ARFI image of RT is shown in Figure 6.1e, where the distal wall is outlined by the black box. Recovery times appear relatively constant at \( \sim 2.5-3 \) ms. Similarly, the distal arterial wall, shows relatively constant peak displacements in the PD image (\( \sim 0.5-1 \) \( \mu \)m). This result is consistent with the histological result seen in Figs. 6.1c-6.1d.

**In Vivo Left FH Pig Iliac Artery**

The B-mode for the left iliac artery of an FH pig is shown in Figure 6.2a. The vessels appear similar to the NC example in 6.1a, with a small amount of intimal thickening visible, resulting in noncritical luminal narrowing of \(<10\%\) (red arrows) suggesting early atherosclerosis. The *en face* image of the vessel, shown in Figure 6.2b, indicates diffuse disease throughout the vessel, but again, shows no indication of advanced disease or vulnerable plaques. Yellow rectangles indicate the proximal and distal wall of imaging, shown near the top and bottom of the vessel, respectively. In contrast to the B-mode and *en face* images, histology sectioned along the black line of Fig. 6.2b and corresponding to the proximal wall, indicates a much more complicated picture (Figures 6.2c-6.2d).

First, a VVG stain, shown in Fig. 6.2c indicates a complex plaque, which has been divided into 3 separate boxes that together constitute the entirety of the lateral FOV. Box 1, corresponding to the left portion of the proximal wall of imaging (\( \sim 10-5 \) mm), shows a degraded IEL with increasing elastin content at the position of the IEL from left to right (stained black, black arrows). An apparent pool of foam cells,
Figure 6.1: ARFI example for NC control iliac artery.
Figure 6.2: B-mode and histology for minimally occlusive complex atherosclerotic plaque.
or possibly necrosis, is visible on the luminal side of the IEL (red arrow) covered by an apparent fibrous cap (yellow arrow), suggests that this portion of the plaque is a Type V atheroma. The region indicated by box 2 appears significantly more complex. Atherosclerosis has infiltrated the media and the region contains extensive elastin deposition throughout (black, black arrows) except in one noticeable location (green arrow). This region appears to be unbound by a fibrous cap and may represent a previously ruptured vulnerable plaque (Type VIa). Box 3 returns to more typical atherosclerotic disease with a visible, if partially degraded, IEL (black arrows) with a small atheroma visible (Type IV-V). This plaque appears highly disparate between the three boxes, but importantly, does not protrude any farther into the lumen in Box 2, despite having significant degradation of the media and quite probably increased thrombotic risk. This is an advanced nonstenotic plaque.

The MT stain is likewise divided into the same 3 boxes for analysis in Figure 6.2d. Box 1 contains collagen deposition, which increases from left to right at the inimal-medial border (black arrows). Box 3 also shows collagen deposition, though the thickness and density of deposition appears thicker and denser than box 1. Box 2 shows higher levels of collagen than boxes 1 or 3 with extensive collagen deposition visible from the luminal edge through the thickness of the vessel to the adventitia.

Parametric ARFI images corresponding to the results shown in Figure 6.2 are shown in Figure 6.3, including spatially-matched boxes to those shown for the VVG and MT stained histology (Fig. 6.2c-6.2d). First, an image of RT is shown in Figure 6.3a. Within this image, box 1 shows a progressive decrease in RT (∼4 ms to ∼2 ms), suggesting a progressive increase in elasticity, which corresponds to the progressive increase in elastin seen in histology (box 1, Fig. 6.2c). Box 2 shows a fast RT overall (∼1.5 ms), with the exception of a small region on the luminal side of the plaque in the middle of box 2 (∼4 ms). Both the overall RT and small focal region of high RT correspond
spatially to the areas of high elastin deposition and small region of no elastin deposition, respectively, seen in histology (box 2, Fig. 6.2c). Finally, box 3 shows a relatively slow but consistent RT (∼3.5 ms), which also spatially corresponds to the region of minor but constant IEL degradation seen in histology (box 3, Fig. 6.2c).

A second parametric ARFI image, this one of PD, is shown in Figure 6.3b with color denoting displacement in microns. Box 1 shows a progressive decrease in PD from left to right (∼2.5 down to ∼1.5 µm). This is spatially consistent with the progressive increase in collagen deposition seen in the MT stain (box 1, Fig. 6.2d). Box 2 shows an overall decreased PD (∼0.75–1.25 µm), consistent with the area of large elastin and collagen deposition seen in histology (box 2, Fig. 6.2d). Lastly, box 3 of Fig. 6.3b shows relatively uniform displacement (∼1 µm) that is in between the displacements observed in boxes 1 and 2, much like how the collagen deposition observed in histology is larger than box 1, but smaller than box 2 (Fig. 6.2d).

A more quantitative comparison of plaque material content with ARFI measurements is shown in Figure 6.4, where scatter plots of spatially-matched material content and ARFI property are plotted against one another. All 4 possible combinations of
Table 6.1: Correlation coefficient and P-values for ARFI and material content correlation result in all 3 diseased arteries imaged.

<table>
<thead>
<tr>
<th>Fit</th>
<th>In vivo Left FH</th>
<th>In vivo Left DH</th>
<th>In vivo Left FH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear</td>
<td>Exp.</td>
<td>Linear</td>
</tr>
<tr>
<td>RT &amp; Elastin</td>
<td>-0.705*</td>
<td>-0.710*</td>
<td>-0.273</td>
</tr>
<tr>
<td>PD &amp; Elastin</td>
<td>-0.760*</td>
<td>-0.818*</td>
<td>-0.314</td>
</tr>
<tr>
<td>RT &amp; Collagen</td>
<td>-0.905*</td>
<td>-0.901*</td>
<td>0.076</td>
</tr>
<tr>
<td>PD &amp; Collagen</td>
<td>-0.523*</td>
<td>-0.558*</td>
<td>-0.505*</td>
</tr>
</tbody>
</table>

* p ≤ 0.02

material content and ARFI measurement are shown, with least squares linear fits superimposed on the plots in red and exponential regression fits in blue. The calculated correlation values are listed in Table 6.1. Note that all 8 relationships are inverse and significant (p ≤ 0.02), i.e. as elastin content increases RT decreases, etc. This uniformly inverse relationship indicates that the collagen and elastin are co-located in this example, so that wherever there is increased elastin, there is increased collagen. So, while the expected relationships are observed, namely that more elastin results in decreased RT and more collagen results in decreased PD, these two comparisons cannot be separated. The implications of this will be discussed in the discussion session.

**In Vivo Left DH Pig Iliac Artery**

The B-mode image of a left DH pig iliac artery is shown in Figure 6.5a. The arterial wall appears thin with no intimal thickening visible, suggesting that no atherosclerosis is present. A look at the *en face* image of the vessel reveals that there are a few small Type II focal atherosclerotic plaques, shown outlined in red in Figure 6.5b. Although not visible in the photograph, these plaques were detected via palpation and visual inspection of the vessel. The region of the proximal wall of imaging is identified by a yellow box, with dimensions of the lateral FOV and elevational resolution as with previous examples. A black line within the yellow box identifies the region where
Figure 6.4: Scatter plots displaying relationships between parametric ARFI result and quantitative histological content from Figs. 6.2-6.3 with linear (red) and exponential (blue) fits to the data.
Histology was sectioned (Fig. 6.5b). Note that the plane of imaging passes through two focal plaques (green and blue arrows, Fig. 6.5b). In addition to the H&E, VVG and MT stains performed on all vessels, this section was also stained with SR for collagen subtype differentiation. Initial inspection of the histology (Figs. 6.5c-6.5e) suggests that the smaller of the two focal plaques (blue arrow, Fig. 6.5b) lacked sufficient integrity to endure the histologic sectioning and staining process, though remnants of the plaque can still be seen in several of the stains, particularly the SR stain (blue arrow, Fig. 6.5e). Higher resolution images of the three stains are shown in Figures 6.6-6.8.

The VVG stain is shown in detail in Figure 6.6, with 6 subregions shown at high resolution in boxes 1-6 to highlight the IEL (black arrows), which correspond to boxes 1-6 on the full VVG section image below. Box 1 shows a degraded IEL, with remnants of the small focal plaque visible (blue arrow). Box 2, shows a fully intact IEL, while box 3 shows intimal thickening and a heavily degraded IEL at the beginning of the larger focal plaque. This thickening increases towards the center of the plaque as visible in box 4, where a thin and highly degraded IEL is visible. By box 5, the IEL appears to be generally intact, though there is a small gap visible (red arrow). Box 6, shows a minimally-thickened intima and an intact IEL.

Figure 6.7 shows the MT stain at high resolution to highlight collagen deposition (black arrows), with the same subregions shown as in Fig. 6.6. Boxes 1-3 show a small amount of collagen deposited, with slightly higher collagen amounts of collagen visible in box 3. In the center of the larger focal plaque, box 4, a reduction in deposited collagen and deposited collagen density is actually observed (green arrows), compared with the other boxes. Box 5 shows the thickest and densest collagen deposition, while box 6 contains a modest amount of deposition, similar to that of box 3.

A further analysis of collagen composition is available in Figure 6.8, where a SR stain imaged under linearly-polarized light shows collagen subtype differentiation.
Figure 6.5: B-Mode and histology for a small focal atherosclerotic plaque.
Figure 6.6: Zoomed in regions from VVG stain (Fig. 6.5c).
Figure 6.7: Zoomed in regions from MT stain (Fig. 6.5d).
Figure 6.8: Zoomed in regions from SR stain (Fig. 6.5e), imaged with polarized light.

In this stain, the larger, thicker and stiffer collagen type I fibers appear red (white arrows), while the less stiff and thinner type III fibers appear green (blue arrows). Six subregions are shown, numbered as to how they spatially correspond to the boxes shown in Figs. 6.6 & 6.7, where box 4.5 is located in between boxes 4 and 5.

The first magnified ROI in Figure 6.8 is box 1, adventitia, illustrates the high percentage of type I fibers in adventitial tissue (white arrows), though some type III fibers are intertwined with the type I fibers. Box 1, intima, on the other hand, is largely made up of type III fibers, with only a few type I fibers visible in the medial layer of the vessel. The other, larger, plaque shown in box 4 is also primarily composed of type III fibers. Conversely, boxes 4.5 and 5 appear to have a higher content of type I fibers, with approximately equal numbers of type I and type III fibers visible.

Parametric ARFI images are shown in Figure 6.9, with boxes spatially-matched to those of Figures 6.6 & 6.7 shown. In the RT image (Fig. 6.9a, boxes 1, 3 & 4, where the
IEL appeared most degraded, it experienced longer times to recovery (~3–4 ms), while boxes 2, 5 & 6, where the IEL was largely intact experience faster recovery (~2–2.5 ms). A similar relationship between PD and collagen is shown in Figure 6.9b, where boxes 4 and 5, which had the most collagen deposition, displace the least. More specifically, box 5 displaces only ~0.25 µm, while box 4, which had a larger amount of the less stiff type III collagen, displaces more (~0.75 µm).

This analysis is again expressed in a more quantifiable way in Figure 6.10, where scatter plots of material content area and parametric ARFI measurement are shown. As there is less co-location seen in this case as compared to the previous example, the plots are more differentiable. The only significant ($p<0.02$) relationship is seen the PD vs. collagen case (linear and exponential fits, Fig. 6.10d), where an inverse relationship is observed. The relationship between RT and collagen is not only non-significant but has a nearly zero (0.076) slope. As the differences in IEL degradation are more difficult to quantify due to the lower overall signal and the fact that IEL continuity was not taken into account, it is not altogether surprising that neither of the elastin vs. ARFI property relationships were significant. Both relationships were inversely related,
however, including the expected relationship between RT and elastin. All correlation values are listed in Table 6.1.

**In Vivo Left FH Pig Iliac Artery 2**

Figure 6.11a shows a FH iliac artery with a small occlusion visible on the distal wall (orange arrow) and minimal intimal thickening visible throughout the rest of the vessel. The *en face* image confirms diffuse atherosclerosis in Figure 6.11b. As with previous examples, the yellow box corresponds to the distal wall imaging window and the black line identifies the location of spatially-matched histology. This histology is shown in Figures 6.11c-6.11f, including an additional stain of VK for calcium.

Examining the histology sections for H&E (Fig. 6.11c), VVG (Fig. 6.11d), MT (Fig. 6.11e) and VK (Fig. 6.11f) confirms diffuse atherosclerosis throughout the section. Additionally there is a focal plaque (green arrow) with a thick fibrous cap as visible in the MT stain (Fig. 6.11e) and a necrotic core (Type V). Within the necrotic core on the left side is a calcification (blue arrow, Fig. 6.11f). A second focal plaque (red arrow) with a disrupted fibrous cap (Type VI) is visible in the MT stain (yellow arrow, Fig. 6.11e). Lastly, a disruption in the media and adventitia is visible on the left side of the vessel (black arrow).

Parametric ARFI images for this region are shown in Figure 6.12. The RT image, shown in Fig. 6.12a, identifies a region of longer recovery time on the left side of the image spatially corresponding to the disruption in the media and adventitia (black arrow, ~2 ms). Also, a region of fast recovery (~0.5 ms) corresponding to the focal plaque with intact fibrous cap is visible (orange arrow). Interestingly, the necrotic core does not appear to have altered recovery, possibly due to mechanical coupling. The PD image, however, shows that the necrotic core is easily differentiable from the fibrous cap, displacing >6 µm, which is significantly more than the surrounding fibrous cap
Figure 6.10: Scatter plots displaying relationships between parametric ARFI result and quantitative histological content from Figs. 6.5-6.9 with linear (red) and exponential (blue) fits to the data.
Figure 6.11: B-mode and histology for diffuse atherosclerosis with a focal atheromatous core.
Figure 6.12: Parametric ARFI images for atheromatous plaque (Fig. 6.11).

(∼2-2.5 µm). The fibrous cap displaces similar to the rest of the arterial wall, with the exception of the disrupted fibrous cap, which displaces ∼3 µm.

Scatter plots illustrating the relationship between mechanical property and the parametric ARFI measurement are shown in Figure 6.13, with the corresponding rho and p values shown in Table 6.1. As with the previous example, the only significant result (p<0.02) is that of PD vs. collagen which has an inverse relationship (slope=-0.383) as expected. The other plots are non-significant, possibly influenced by the small variation in collagen and elastin content across the vessel in the imaging FOV. Note that black pixels corresponding to calcium, as indicated by the VK stain, were excluded from the elastin comparisons.

6.4 Discussion

The results of this pilot in vivo investigation into ARFI ultrasound for the characterization of atherosclerosis show a relationship between elastin and RT as well as between collagen and PD. First, in a control example, absent of atherosclerosis, uniform PD and RT measurements were observed (Fig. 6.1). Second, in a highly evolved and complex
Figure 6.13: Scatter plots displaying relationships between parametric ARFI result and quantitative histological content from Figs. 6.11-6.12 with linear (red) and exponential (blue) fits to the data.
Type IV-VI plaque, elastin and collagen area was correlated with ARFI measurements of PD and RT (Figs. 6.2-6.4, Table 6.1, column 1). Importantly, in this example ARFI was able to detect and characterize a minimally-occlusive plaque, differentiating the central potentially vulnerable plaque from the surrounding regions (box 2, Figs. 6.2-6.3). Based on the preliminary ex vivo studies in the previous chapter and understanding of the mechanical and physiological roles of elastin and collagen, elastin is expected to be inversely related to RT and collagen to be inversely related to PD. However, in this example it is not possible to differentiate the responses of collagen and elastin, because the relative contents of the two materials are co-located. To put it another way, not only are collagen and elastin located in the same regions, but wherever there is increased collagen, increased elastin content is also observed (Fig. 6.2).

An analysis of the scatter plots illustrating the relationships between material content and ARFI measurement in Figure 6.4, show that all four relationships, elastin-RT, elastin-PD, collagen-RT and collagen-PD show significant inverse relationships (Table 6.1). Both the linear and exponential fits provide similar correlations with similar (p<0.02) significance, so neither fit appears better suited to the data. So while the expected inverse relationships between elastin-RT and collagen-PD are observed and are statistically significant, significant inverse relationships between elastin-PD and collagen-RT are also observed. Therefore, it cannot be said definitively that these relationships are due to the expected material contents alone, but it is possible to conclude from this second example that these results support and in no way contradict the expectations regarding these relationships in atherosclerotic arteries. To fully test this hypothesis, non-co-located examples of elastin and collagen are needed to differentiate the material influence on ARFI response.

In the third example, with two small focal Type II plaques, only a significant relationship between collagen and PD was observed, though it agreed with the expectations
of an inverse relationship as with the second example. (Figs. 6.5-6.10; Table 6.1, column 2). The lack of significance in the other plots is likely due to the small amount of variability in elastin observed across the vessel, due to the early stage of atherosclerosis. Again, there was no advantage observed to using an exponential fit over a simple linear fit in this case. Additionally, a percentage area of elastin alone provides an incomplete comparison of the impact of elastin and more importantly the IEL on vascular mechanics. It is generally understood that the IEL gives arterial walls structure and elasticity and that degradation of the IEL in atherosclerosis promotes plaque vulnerability [4]. By neglecting IEL structure, an incomplete picture of the impact of elastin on the vessel mechanical response is provided. Additionally, it is likely that elastin deposition contributes to vessel elasticity less than the structured IEL. A formal ROC analysis by trained readers to evaluate slow recovery times, as well as by trained pathologists to assess both IEL structure and unstructured elastin deposition, will allow the evaluation of the relevance of IEL structure to ARFI outcomes.

As with the third example, in the fourth and last example of two Type V-VI atheromatous plaques, only collagen was significantly inversely related to PD (Figs. 6.11-6.13; Table 6.1, column 3). Although only a small variation in collagen was observed, this is potentially due to the observed disrupted fibrous cap (red arrow, Fig. 6.11). Interestingly, only the other larger atheromatous was apparent with B-mode ultrasound, which was also easily visible in the ARFI PD image due to the large PDs of the necrotic core (orange arrow, Figs. 6.11a & 6.12b). The smaller atheromatous was indistinguishable in both B-mode and ARFI. The smaller size of the atheromatous in association with surrounding collagen likely stabilized the atheromatous. Therefore, the plaque does not exhibit an identifiably different ARFI response to ARFI excitation than surrounding wall tissue. This illustrates a critical limitation of ARFI imaging of atherosclerosis: while ARFI imaging effectively exploits differences in tissue mechanical properties, in
In the context of homogeneous mechanical response with heterogeneous material content, ARFI performance may be compromised.

An additional limitation of ARFI atherosclerosis imaging with current methods is the depth of penetration. While other applications of ARFI may successfully penetrate softer tissue such as liver and kidney quite deeply, the large stiffness of the vasculature can result in displacements below the measurement threshold at much shallower depths. In terms of ARFI as applied clinically to the assessment of peripheral vessels, such as the carotid, this should not be an issue, however. Finally, while these results are promising, they remain preliminary and a larger number of animals must be examined to provide more conclusive results.

6.5 Conclusions

In a pilot study of three different atherosclerotic plaque types, (1) a complex and highly evolved Type V-VI nonstenotic plaque, (2) small, focal Type II plaques and (3) Type IV-VI atheromatous plaques, ARFI ultrasound is demonstrated as capable of noninvasively detecting and characterizing these plaques in vivo. With spatially-matched histology, a spatial correlation is demonstrated between arterial wall elastin and collagen with ARFI RT and PD results. In regions of increased elastin and collagen deposition, smaller ARFI RTs and PDs were observed. However, the study design could not isolate the impact of co-located collagen and elastin, nor could it differentiate the impact of IEL degradation separate from elastin deposition. In Chapters 8-9 this methodology will be modified to better deal with these comparisons, including the use of a larger number of animals for increased statistical significance.
Chapter 7

ARFI Beam Sequence Performance:
Preliminary Studies into the Impact
of ParRx Tracking, Excitation F/#,
and Multiple Excitations

7.1 Introduction

In previous chapters ARFI ultrasound has been demonstrated for characterizing atherosclerosis \textit{ex vivo} and \textit{in vivo}. Correlations were observed between collagen and elastin with ARFI measurements of PD and RT, respectively. Before expanding the analysis of plaque detection and characterization, the influence of different implementations of...
ARFI beam sequences was first examined. While ARFI had been applied to atherosclerotic vessels, there had been no analysis of the impact of modifications in beam sequencing technique. With this in mind, this chapter sets out to compare the impact of 4:1 ParRx compared with single A-line receive tracking (SRx), ARFI excitation F/# and whether there was any benefit to employing multiple ARFI excitations separated by a small time delay. By tailoring ARFI beam sequences to atherosclerosis imaging, it is possible that ARFI image quality can improve and as a result, plaque detection and characterization capabilities will improve.

7.2 Methods

7.2.1 Ultrasonic Imaging and Data Processing

With a Siemens SONOLINE Antares™ imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division) equipped for research purposes and a VF7-3 linear array transducer, three pig iliac arteries were imaged, a 5 year, 2 month-old FH female, a 5 year, 10 month-old FH female, and a 7 year, 1 month-old DH Female. Most imaging parameters and techniques were the same as applied in the previous chapter. ARFI excitations of 300-cycles at 4.21 MHz and tracking pulses of 2-cycles at 6.15 MHz were employed. However, some specifics of beam sequence implementation were varied. In the first example, both ParRx and SRx were employed, while the second and third examples used SRx and ParRx, respectively. For SRx imaging, 40 lateral positions spaced 0.53 mm were used and for ParRx, 15 excitations resulted in 60 tracking positions spaced 0.35 mm, as in the previous chapter. In the second example, excitation F/#’s of F/1, F/1.5 and F/3 were employed, while in the other two examples only F/1.5 excitations were used. Finally in the third example, 2 300-cycle excitations spaced 0.8 ms apart were employed (DP), while in the first and second example, only
Figure 7.1: Pressurization method for ex vivo imaging.

a single pulse (SP) was used. For SP imaging 60 track lines were employed, while only 51 track lines were used after the second excitation of DP imaging to provide the same ensemble length.

All vessels were imaged within 36 hours of necropsy. Arterial branch points were sutured, and the vessels were mounted to a pressure apparatus in a 23°C saline bath. All arteries were pressurized to ~35 mm Hg, using a pulsatile flow pump as illustrated in Figure 7.1, where the vessel was pressurized using a pulsatile flow pump. Before entering the vessel however, the flow passed through a pulse dampener to remove nearly all of the flow pulsatility. Continuous flow was needed to pressurize the vessel even though the system approximates a steady state system due to saline leakage out of the branches of the vessel. The transducer (xDucer, Fig. 7.1) was then aimed over the vessel for imaging. One spatially matched B-mode frame was acquired immediately prior to each 2D ARFI acquisition for anatomical reference.

One-dimensional normalized cross correlation was applied to the acquired RF data
ensembles to measure axial ARFI-induced displacements. Motion artifacts were then removed with a linear filter. For the SP ARFI data, images of PD and RT were generated. For the DP sequence, the images generated were: peak displacement from the 1st excitation (PD$_1$), peak displacement from the 2nd excitation (PD$_2$), recovery time from the 2nd excitation only (RT), absolute difference in peak displacements ($\Delta$PD), and displacement immediately preceding PD$_2$ expressed as a percentage of PD$_1$ (%PD).

Building on the luminal mask used in the previous chapter, a mask derived from B-mode amplitudes and cross correlation coefficient variance was used to mask luminal signal. The measurement of cross correlation coefficient variance as opposed to just cross correlation coefficient was found to better differentiate the luminal signal from wall signal. Details of this and more advanced lumen filters are discussed extensively in Appendix C.

7.2.2 Histology

Following imaging, arteries were aligned with the imaging plane and sectioned for spatially-matched immunohistochemical analysis with assistance from a pathologist. Sections were taken from each vessel, spaced 10 $\mu$m apart, for H&E, VK, MT, and VVG stains.

7.3 Results

7.3.1 The Influence of Parallel Receive tracking on Spatial Sensitivity

A large focal atherosclerotic plaque is shown in Figure 7.2. A small calcification is visible near the shoulder of the plaque (black arrow, Fig. 7.2a), which measures 0.75 x
0.20 mm. In addition, the integrity of the circled region varies across histologic stains, beginning as a small hole in the VK stain (black circle, Fig. 7.2a) and progressing to a moderate disruption in the VVG stain (black circle, Fig. 7.2c), suggesting that this is a Type VIa disrupted plaque. As the stains were sectioned at the same position, but separated by \(~20–100 \, \mu m\) through elevation, this suggests a rapid change in plaque integrity over the small distance separating the three stains. The vessel has noticeable collagen deposition (green arrows, Fig. 7.2b) and moderate IEL degradation (yellow arrows, Fig. 7.2c). Finally, the wall adjacent to the vessel shows no atherosclerosis.

ARFI imaging with both SP-ParRx and SP-SRx was performed on this vessel and imaging results are presented in Figure 7.3, columns 1 and 2, respectively. B-modes for the two ARFI sequences are presented in Fig. 7.3a–7.3b and appear very similar, as expected, with a large focal atherosclerotic plaque visible from -2–10 mm. In both images a small degree of shadowing is visible (black arrow), below the location of the calcification. The adjacent wall shows no evidence of atherosclerotic development (-10–-2 mm), consistent with the histological result.

Parametric ARFI images for PD and RT are shown in Figures 7.3c–7.3d and 7.3e–7.3f, respectively. In the ParRx image (Fig. 7.3c), a region of heightened displacement (\(~15 \, \mu m\), black arrow) is observed near the shoulder of the plaque, compared to the adjacent vessel wall (\(~5 \, \mu m\)). Heightened displacement is also seen in the circled region (\(~13 \, \mu m\)), but this is not observed in the SRx image (\(~5 \, \mu m\), black circle, Fig. 7.3c). Although the circled regions appear different, the shoulder regions appear similar (black arrow), displacing \(~11 \, \mu m\) in the SRx image. Also visible in the SRx PD image is a small blue dot (blue arrow) of lowered displacement (\(<1 \, \mu m\)). The small size of this point is only visible in one lateral sample, consistent with the size of the calcification. Furthermore, this response to ARFI excitation was repeatable under multiple interrogations of multiple excitation F/#, indicating that it was likely not
Figure 7.2: Histology of a large focal plaque with a small calcification and a plaque disruption.
Figure 7.3: Comparison of SP-ParRx vs. SP-SRx ARFI in a large focal plaque with a small calcification and plaque disruption.
noise. The calcification is not visible in the ParRx PD image, however (Fig. 7.3b). This is potentially due to the methods of ParRx imaging.

A ParRx ARFI excitation is 0.55 mm wide, while the total width of the 4 ParRx lines is double that (1.1 mm). This means that the total width of the acquisition is wider than the applied force. ParRx relies on shear waves to propagate the displacement across the outer two track lines, as opposed to independently displacing each lateral sample as occurs in SRx. It’s possible that in this instance that the ParRx ARFI excitation is not laterally centered on the calcification and the subsequent shear wave propagation does not reveal it.

Lastly, the RT images for the two ARFI sequences are shown in Figures 7.3d and 7.3f for ParRx and SRx, respectively. In general, in both the ParRx and SRx images the plaque recovers more slowly than the adjacent arterial wall, corresponding to the degraded IEL observed in histology. Analyzing the same regions as in the PD images, however, further illustrates some potential differences between ParRx and SRx. In the ParRx RT image (Fig. 7.3d), the region near the shoulder of the plaque (black arrow), which was shown to displace highly in both PD images, recovers quickly (<0.5 ms). In the SRx image this same region recovers more slowly (~1.5 ms), similar to the other regions of the plaque. Within this region, the calcification is still visible in the SRx image, recovering very quickly (<0.5 ms), similar to that of the overall shoulder region in the ParRx RT image. The exact nature of this disparity is unclear from the images and histology.

The circled region in the ParRx RT image recovers quickly, while in the SRx RT image it recovers similar to surrounding regions of the plaque. This difference is visible in both the PD and RT images for the two sequences, with the region differentiable in ParRx, but not in SRx. Since the two image sequences were applied to the tissue at slightly different times, it is possible that the disparity in imaging result in this case
is due to a subtle elevational shift of the vessel during the time between when the two beam sequences were applied.

### 7.3.2 The Influence of Radiation Force F/# on SRx Image Quality

A 5 year, 10 month-old FH porcine left iliac artery imaged *ex vivo* with varying ARFI excitation F/# is shown in Figure 7.4. Spatially-matched immunohistochemistry shows a large focal atherosclerotic plaque (Figs. 7.4a-7.4c). First, the VK stain identifies that within the plaque there are extensive calcifications (black arrows, Fig. 7.4a), suggesting that this is a Type VII plaque. Second, the MT stain shows collagen deposition surrounding the calcification (Fig. 7.4b), with the exception of a small region near the shoulder of the plaque (green arrow). This region also appears to have low elastin content (green arrow, Fig. 7.4c), which upon closer inspection is a necrotic core. Adjacent to the focal plaque, the arterial wall has no atherosclerosis (blue arrow, Fig. 7.4a). Spatially matched to the histology, this arterial section is visible as the distal wall of the B-mode image in Figure 7.4d. The large focal plaque is visible from -1–10 mm with shadowing evident (black arrows) suggesting the presence of calcium. The adjacent arterial wall shows no evidence of atherosclerosis in agreement with histology (Fig. 7.4d).

SP ARFI imaging using excitation F/#’s of F/1, F/1.5 and F/3 were performed on this artery with the parametric ARFI images shown in Figure 7.4e. The PD images are shown in the top row with color scales adjusted to maximize contrast in each image. In all the PD images, the adjacent arterial wall (blue arrow) displaces more than the focal plaque (black arrows). Additionally, within the plaque the necrotic region (green arrow) displaces more than the rest of the plaque in all images. The differences in the images arise from the relative displacements of the adjacent arterial wall and necrotic region.
Figure 7.4: Comparison of F/# on ARFI Images for a large focal plaque with extensive calcifications.
In the first PD image, F/1 excitation, the adjacent wall displaces \(~12\ \mu m\), while the necrotic region displaces only \(~8\ \mu m\), a ratio of \(~1.5\). This ratio decreases to \(~1.4\) in F/1.5 and \(~0.83\) in F/3 where displacements of \(~5\ \mu m\) are visible for the adjacent wall and \(~6\ \mu m\) for the necrotic region. Only in the F/3 excitation case does the necrotic region displace further than expected. This difference in ARFI result is best explained by the change in depth of field (DOF) as F/# increases. As the F/# of ARFI increases so does the depth of field (DOF) of the excitation, with smaller F/#’s concentrating the force over a smaller area of tissue due to tighter focusing, and therefore, greater beam divergence. As the two regions in question are at two different depths (\(~18\) mm vs. \(~16\) mm), this opens up the possibility that the two depths experience different amounts of force relative to one another, causing different relative displacements as F/# changes. This is likely more pronounced due to the presence of large calcium deposits between the focal depth and necrotic region, preventing the peak ARFI-induced displacements at lower F/#’s from propagating upwards to further displace the necrotic region. Thus, this problem may not be as pronounced in all applications of ARFI, but in the case of arterial ARFI where disparate mechanical properties within a small depth range are likely, F/# appears to have a large impact.

In addition to the changing ratio in PD of the regions discussed, the visible variability in the calcification increases with F/# (black arrows, Fig. 7.4e). The small amounts of variability within the F/1 PD image become larger and more dominant as F/# increases. In the F/3 PD image, large regions with \(<0\ \mu m\) displacement are visible. As discussed in the above paragraph, the increased DOF associated with increasing F/# also results in less concentrated focusing. The decreased local focal intensity results in decreased displacement and, as a result, lower SNR visible in the calcification (Fig. 7.4e).

The associated RT images (bottom row, Fig. 7.4e) are presented on the same time
scale (0–3.5 ms) to examine the absolute changes in RT. The relative measures between the adjacent arterial wall and plaque appear relatively constant (∼1.5 vs. ∼3 ms), but the RT in the calcification appears to change significantly. The calcification changes from a highly constant response, to a highly variable RT as F/# increases (black arrow, Fig. 7.4e). The region of the necrosis changes from similar in response to the adjacent arterial wall and calcium in the F/1 case to clearly differentiable in all other F/#’s (green arrow). Upon further investigation, this appears to be related to shear wave reflections, which is further explored in Appendix B. As with the images of PD, it is clear that in the use of F/#, variability increases with F/#, likely due to decreasing SNR with increasing F/#. Additionally, the ability to clearly differentiate the various regions of the plaque increases with F/# as also observed in PD.

7.3.3 The Influence of Multiple ARFI Excitation Pulses

The third and final alteration to ARFI beam sequencing was a DP ARFI sequence with F/1.5 excitations in the left iliac artery of a 7 year, 1 month-old female DH pig, shown in Figure 7.5. Immunohistochemistry reveals a small focal atherosclerotic plaque in the left half of the vessel (Figs. 7.5a-7.5b), which is either Type II or early Type III. The MT stain shows collagen deposition within the plaque (green arrows, Fig. 7.5a), while in the VVG stain the IEL appears partially degraded in the plaque (yellow arrows, Fig. 7.5b). Immediately to the right of the plaque, the wall appears thickened with partially degraded internal structure (black arrow, Figs. 7.5a-7.5b). As this appears in multiple stains, it is not likely an artifact of histological sectioning. The plaque is visible in the spatially-matched B-mode image on the distal wall from -10 to -1 mm (Fig. 7.5c). The plaque appears small with a minor amount of intimal thickening compared to the adjacent vessel wall.
Figure 7.5: DP ARFI results for a small focal plaque.
Parametric ARFI images generated for the DP sequence are shown in Figures 7.5d-7.5h. First, the PD\textsubscript{1} image corresponding to the PD image for a SP sequence is shown in Figure 7.5d. Barring any mechanical interference effects from the second excitation, the PD\textsubscript{1} and PD images should appear identical, as they did in this example. Next, Figure 7.5e shows an image of PD\textsubscript{2}, or the PD from the 2nd excitation, on an adjusted color scale of 0-18 µm to account for the additive displacement effects of the two excitations. Both images appear very similar, based on relative measurements, and show the plaque displacing less than the adjacent wall, agreeing with the observed collagen deposition, with the center of the plaque displacing least (green arrow, Figs. 7.5d-7.5e). However, when the difference between the PD\textsubscript{1} and PD\textsubscript{2} displacements are compared in the ΔPD image (Fig. 7.5f), there are noticeable differences. The biggest of these differences (yellow arrow) corresponds to the degraded portion of the IEL in histology (between yellow arrows, Fig. 7.5b). This region of degraded elasticity recovers more slowly and thus experiences larger total displacement after the second push than the region of intact IEL. The area of collagen likely approaches the maximum inducible displacement from the first push, due to the high degree of stiffness, and as a result, yields little additional displacement from the second push, when compared with other regions of the vessel. Thus, while the PD\textsubscript{1} and PD\textsubscript{2} images appear similar at first glance, comparative measures can yield additional and potentially useful information.

Two images illustrating recovery information are presented, RT in Figure 7.5g and %PD in Figure 7.5h, which shows the percentage of PD\textsubscript{1} remaining immediately preceding the 2nd excitation. In the %PD image, regions of tissue which recovery more slowly and thus have more residual displacement will appear redder. The image of RT shows that the region of the plaque and a portion of the adjacent wall (-10–2 mm) has a slower recovery (∼1.75 ms) when compared with the rest of the adjacent wall (∼1 ms). In the region of wall which appears to have disrupted structure (black arrow,
Fig. 7.5g), the RT closely matches that of the plaque. In the %PD image, however, while the plaque still appears to recover slowly, the region of degraded structure (black arrow, Fig. 7.5h), more closely matches the %PD of the adjacent wall, appearing different than the plaque. By examining the recovery in two different ways, it is possible to differentiate this region of differential response and potentially make more detailed inferences regarding the elastic structure. Therefore, the use of both of these images in conjunction could prove more useful than either image alone.

7.4 Discussion

The results of this preliminary investigation into ARFI beam sequence optimization show that there are significant improvements that can be made to the quality of parametric ARFI images, by changing the way ARFI is implemented. In a comparison of ParRx and SRx in a large focal atherosclerotic plaque with a small calcification, the SRx image successfully differentiated the small calcification, while ParRx did not (Figs. 7.2-7.3). The small size of the calcification and the wide spacing between ParRx pushes (1.1 mm), leaves the possibility that objects such as this small calcification would not fall within the excitation focus. While ParRx does not appear to have less detection capability for larger objects, these results suggest that there may be a loss of spatial sensitivity to small local variations in material property. It is important to note that further advances in beam sequencing and data collection capabilities will likely allow for larger number of excitations with ParRx to acquire greater spatial sampling, meeting or even surpassing the spatial sampling rate observed with the SRx sequence in this example. This would likely alleviate the sampling issues observed here, assuming that bioeffects do not become a dominant issue. The lesson learned from this example is that 4:1 ParRx does not appear to be a 4:1 replacement for SRx lines, and if these results are any indication it may be significantly less so, at least with application to
atherosclerosis.

An additional disparity in this example was the region of large displacement which was apparent in the ParRx images, but not in the SRx images (black circle, Figs. 7.3c-7.3f). A further analysis of this example, focusing on wave reflections within the plaque, is detailed in Appendix B. This analysis uncovered longitudinal wave reflections occurring within the unstable region. However, the presence of wave reflections is unlikely to be seen if the region of heterogeneity is out of plane elevationally, as is likely the case here.

A second example highlights the differences in ARFI excitation F/# in a heavily calcified plaque (Fig. 7.4). In B-mode imaging, the development of dynamic receive has largely negated the impact of F/#’s, allowing higher F/#’s to be employed to maximize the DOF while resolution could then be primarily dependent upon receive beamforming. The conventional thinking up to this point in ARFI imaging had been that a lower F/# was desirable to increase peak focal displacements and as a direct result, displacement SNR. These results suggest that there are cases, such as the focal necrosis visible here (green arrow, Fig. 7.4), where F/# can play a significant role in diagnostic capability. This difference may be more important in atherosclerotic applications than in other more homogeneous applications due to the extremely high degree of heterogeneity expected. These results suggest that for anything more than the most basic and thinnest plaques, F/# can make or break diagnostic capability. If, however, the advantages of higher SNR are required, then other beam sequencing techniques such as combining multiple axial excitations with low F/#’s together could potentially be employed to achieve similar results.

In the last example the use of multiple ARFI pushes within a single ensemble was investigated to enhance differentiation in tissue response (Fig. 7.5). These results suggest that in the observed small focal atherosclerotic plaque, the region of structural
degradation was visible only via images which would not be available with SP imaging. The addition of comparative images such as ΔPD and %PD suggest that there is more information available in DP sequences that could be potentially useful. What still remains unclear at this point is the best way to present this information. While these images both appear useful, further investigation into other parametric ARFI images for superior material property differentiation may be needed. However, the increased quantity of information makes this parametric image selection more difficult.

7.5 Conclusions

Three different methods of ARFI beam sequence modification were presented and compared. These included variations in receive tracking, excitation F/# and the number of excitations within a single ensemble. The use of ParRx was observed to yield lower spatial sensitivity when compared to SRx for examining a small calcification. Results for excitation F/# illustrate a tradeoff. Lower F/#’s provide higher SNR but at the expense of decreased DOF, which may be of importance in atherosclerotic applications. Finally, DP imaging provided more information than SP ARFI that could potentially be useful for distinguishing especially stiff or inelastic structures. This work suggests that various beam sequences may be relevant to ARFI imaging. In the next chapters this work is expanded, employing three ARFI excitation methods and three tracking methods in a large number of arteries to identify those sequences best suited for plaque detection in Chapter 8 and plaque characterization of a number of material properties in Chapter 9.
Chapter 8

ARFI Beam Sequence Performance
as Evaluated by Trained Readers I:
Plaque Detection

8.1 Introduction

Previous chapters have demonstrated that ARFI is capable of both detecting and characterizing atherosclerosis in vivo. Additionally, these chapters have shown that using modified ARFI beam sequencing can provide improved detection and characterization capability. However, in both of these cases the number of examples was small, only using 4 in vivo examples and 3 alternative beam sequencing examples, so it was not possible to definitively state nor quantitatively measure the detection or characterization capability of ARFI beam sequences. In this chapter both of these studies are expanded to examine a larger number of pigs with modified ARFI beam sequencing in a quantitative evaluations study performed by trained readers.

Based on the previous beam sequence study, 9 ARFI/SWEI beam sequences were selected by combining 3 methods of excitation and 3 methods of displacement tracking.
These 9 sequences were then applied to phantoms as well as ex vivo and in vivo porcine arteries. The hypothesis was that some of these 9 sequences would perform better than the others for the detection and/or characterization of atherosclerotic plaques. With this in mind 9 sequences were used in the phantoms and then the lowest performing sequences were removed prior to the transition to ex vivo and in vivo. The results for plaque detection are presented below, while the plaque characterization results are presented in the next chapter.

8.2 Methods

8.2.1 Phantom Generation

Arterial wall mimicking agar/gelatin phantoms were constructed based on previous work by [63]. The phantoms were constructed to mimic an arterial wall with a soft or stiff inclusion as seen in Figure 8.1. The phantom was constructed with a thin layer (blue boxes, Fig. 8.1a) mimicking an arterial wall placed on top of soft tissue (red boxes, Figs. 8.1a-8.1b). Inclusions were placed within the “wall” of three widths: 0, 2.5 and 5 mm widths, outlined in Fig. 8.1a by a blue box and Fig. 8.1b by a black box. The inclusions were either soft or hard, with Young’s moduli of \(~110\) and \(~190\) kPa and were placed within layers of \(~190\) and \(~110\) kPa, respectively. The soft tissue had a Young’s modulus of \(~20\) kPa.

8.2.2 ARFI Imaging

As with previous studies, all imaging was performed with a Siemens SONOLINE Antares\textsuperscript{TM} imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division), equipped for research purposes, and a VF7-3 transducer. ARFI ensembles consisted of 2 reference lines, either 1 or 2 excitation impulses, and either 60 or 59 tracking
ARFI excitations were 300 cycles at 4.21 MHz and both tracking and reference lines were 2 cycles at 6.15 MHz. Three types of ARFI excitations were combined with 3 types of tracking to generate 9 total beam sequences, as shown in Figure 8.2. The 3 types of excitations included a single F/1.5 pulse (SP1.5), a single F/3 pulse (SP3), and two F/1.5 pulses, spaced 0.8 ms apart (DP). The three types of tracking included 1:1 SRx in the ROE, 4:1 ParRx in the ROE and 4:1 parallel receive tracking lateral to the region of excitation (SWEI) [36]. For SRx tracking, 40 ensembles spaced 0.53 mm apart were used for an overall field of view (FOV) of ∼2.1 mm, while for ParRx and SWEI, 15 excitations spaced ∼1 mm apart were used to generate 60 ARFI ensembles spaced 0.35 mm apart spanning a ∼2.1 cm FOV.

The arterial mimicking phantoms were imaged in 13 locations: each of the inclusions at 0, -3 and -6 mm lateral offsets from the center of imaging and a control with no inclusion. Imaging was performed in a water bath with water in between the transducer and the layer, mimicking the position of a distal arterial wall. The imaging focus was kept at a constant depth of 2 cm, with 2 repeated acquisitions for each of the 9 beam sequences, resulting in 234 image sets for reader evaluation.

Excised iliac arteries were pressurized to 80 mm Hg using saline for imaging as illustrated by Fig. 8.3, in similar fashion to the imaging performed in the previous
Figure 8.2: ARFI/SWEI sequences implemented in the reader study.

Figure 8.3: Revised pressurization method for ex vivo imaging.
chapter. To better simulate \textit{in vivo} imaging, imaging was performed with $\sim 20$ kPa tissue mimicking agar/gelatin phantoms placed above and below the vessel to simulate soft tissue. All 9 sequences were used in phantom studies. Based on phantom results, DP-SWEI and SP1.5-SRx sequences were excluded from the \textit{ex vivo} reader evaluation.

Six pigs were imaged in 22 total locations with 2 acquisitions per location, generating 308 total image sets. The image sets contained 12 locations focused on distal arterial walls and 10 locations on proximal walls, each evenly distributed between control and atherosclerotic imaging locations. Following imaging, biologically inert carbon particles were injected into the plane of imaging on the proximal wall for spatial registration with histology.

\textit{In vivo} imaging was performed in the lateral decubitus position with the pigs sedated, the rear legs extended and physically separated to provide acoustic access to the iliac arteries. While in Chapter 6 the surface location of imaging was tattooed, in this study sterile carbon particles were injected into the soft tissue immediately above the location of imaging. This provided superior spatial registration capabilities to tattooing. Due to the results of the \textit{ex vivo} reader evaluation study and the limitations of \textit{in vivo} imaging, only 6 sequences were applied \textit{in vivo}: SP1.5-ParRx, SP3-ParRx, DP-ParRx, SP1.5-SWEI and two sequences with 400 cycle excitations: BP-ParRx and BP1.5-SWEI. In both of these new sequences the pulse was a 400 cycle F/1.5 excitation designed to provide increased SNR in the pigs, due to small observed displacements from the original beams sequences. \textit{In vivo} imaging was performed in 14 imaging locations within 10 pigs evenly distributed between control and disease, with 6 proximal walls and 8 distal walls of imaging and 5 acquisitions per location. Of these 14 imaging locations, only 9 had the BP excitations sequences applied and 2 locations did not have SP3-ParRx due to a beam sequence malfunction. From the 14 imaging locations the 2 best acquisitions were determined by optimal vessel positioning within the FOV out of
the 5 applied for a total of 144 image sets.

8.2.3 Image Generation and Masking

For all ARFI Image sequences parametric images were automatically generated and masked. These include PD and RT for SP-non-SWEI sequences, shear wave velocity (SWV) for all SWEI sequences and peak displacement from 1st excitation (PD$_1$), second excitation (PD$_2$), recovery time from 2nd excitation (RT) and marginal peak displacement (MPD) for DP-non-SWEI sequences. MPD is given by

$$MPD = 1 - \frac{PD_1 - (PD_2 - D)}{PD_1}$$

(8.1)

where $D$ (microns) is the displacement remaining from the 1st excitation immediately preceding the 2nd excitation pulse.

Two masks for each parametric image were then generated, a mask for color scaling and a luminal mask. The color scaling mask was applied to isolate ARFI/SWEI parameter values in the arterial wall only. The median and standard deviation parameter statistics were then automatically used to create the color scales for each parametric image. The luminal mask was used to reject all luminal and reverberation signal automatically as described in Appendix C. The set of 2-4 parametric images, depending on beam sequence, constituted an image set to be evaluated by readers. Matched B-modes were acquired and used for histological registration, but were not included in the image sets for reader evaluation.

8.2.4 Reader Evaluation Study

The phantom, *ex vivo* and *in vivo* parametric ARFI image sets were evaluated by 12 trained readers. Reader training involved reviewing 10 ARFI/SWEI examples with
associated phantom structure or matched histology. Due to the large number of image sets, each image set was studied by a random subset of 6 of the 12 readers, with the image sets presented in random order. The image set evaluation was performed using a custom graphical user interface (GUI) in MATLAB® (Mathworks Inc., Natick, MA, USA). There were two GUIs used, one for phantoms and one for both ex vivo and in vivo data sets as shown in Figures 8.4 and 8.5, respectively. For phantoms only plaque detection metrics were collected, but in the actual pig arteries, both plaque detection and characterization metrics were collected simultaneously.

When presented with an image set, each reader was asked to assess the artery for image quality, plaque presence and, in the ex vivo and in vivo portions of the study, compositional elements including: collagen, calcium, elastin, and fibrin deposition, a degraded IEL, and the presence of lipid pools and fibrous caps. This was done for each lateral quarter of the image (i.e. -10 to -5 mm, -5 to 0 mm, etc.). The plaque detection reader rating possibilities are described in Table 8.1. The ratings for the phantom study were done for ‘plaque’ presence or absence only as described in the second row of Table 8.1. The rating possibilities and results of the other metrics will be described in the next chapter. Additionally, the readers were asked to measure plaque presence/absence and material content for the entire image using the roipoly MATLAB® function, allowing for multiple regions to be selected.

### 8.2.5 Histology

Following ex vivo and in vivo imaging, arteries were aligned with the imaging plane and sectioned for spatially-matched immunohistochemical analysis with assistance from a pathologist. Sections spaced 10-50 µm apart were stained with H&E for baseline, VK for calcium, Lillie’s modified Massons Trichrome (LMT) for collagen, VVG for elastin. LMT was used instead of the similar MT used previously, as it provided staining for
fibrin (green) while staining collagen in a very similar fashion to MT staining. This allowed for the ability to detect intraplaque hemorrhage or a blood clot attached to an atherosclerotic plaque, though neither of these possibilities was observed in the arterial plaques used for the reader study. Following histological sectioning, all slides were imaged using an Aperio Scanscope microscope at 20x magnification. Using the carbon particles and vessel anatomy as a reference, the microscopy images were then divided into 4 subsections corresponding to the 4 quarters of the ARFI/SWEI images. A pathologist with experience in atherosclerosis used a GUI to evaluate the histology using the criteria established by the AHA Committee on Vascular Lesions as described in Chapter 2.

The rating possibilities are listed in Table 8.2, separated by phantom, \textit{ex vivo} or \textit{in vivo}. The phantom results were not actually evaluated by a pathologist, but rather by a binary response based on the known truth of where the inclusions were placed. Also, the \textit{in vivo} histological ratings were adjusted to account for the cases used in the study, which did not include any plaques of Type III or IV and a high percentage of the plaques were Type I. Because of this, the Type I plaques were divided into three different groups, Type I with <25% thickening as measured relative to the medial-adventitial wall thickness, Type I with >25% thickening and Type I with focal thickening.
Figure 8.4: GUI for the phantom portion of the reader study.
Figure 8.5: GUI for the ex vivo portion of the reader study.
Table 8.1: ARFI Image Plaque Detection Rating Options

<table>
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<tr>
<th>Plaque Presence</th>
<th>Rating Options</th>
</tr>
</thead>
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<td>Def. not present</td>
</tr>
<tr>
<td></td>
<td>Def. - Definitely</td>
</tr>
</tbody>
</table>

Table 8.2: Phantom Gold Standard and Ex Vivo/In Vivo Histology Plaque Detection Rating Options

<table>
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<tr>
<th>Plaque Presence</th>
<th>Phantom</th>
<th>Ex Vivo</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Not Present</td>
<td>Type I</td>
<td>Type I (&lt;25%)</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>Type II</td>
<td>Type III</td>
</tr>
</tbody>
</table>
8.2.6 Statistical Analysis

The following statistical methods are as performed by a biostatistician enlisted to assist with this project, with feedback and assistance provided as necessary. Due to the nature of the acquired data, ordinal responses from multiple readers, receiver operating characteristic (ROC) curve analysis was utilized. From the ROCs, the area under the ROC curve (AUC) was extracted as a metric of beam sequence performance [64; 65; 66]. But, before describing the methodology of the ROC and AUC analysis, the data of interest must be described. The acquired data included both the readers’ image classifications and pathologists’ histological classifications, which are both recorded as ordinal data, denoted as \( X \) and \( Y \) respectively. In these experiments, possible values of \( X \) and \( Y \) are \( X = 1, 2, \ldots, m \) and \( Y = 1, 2, \ldots, n \), with larger numbers indicating more advanced disease status. According to mathematical convention, \( X \) was sometimes assumed to be continuous, while the histological result \( Y \) is always discrete and ordinal.

Conclusions were not drawn from only a few observations, but instead from many sets of readers’ analysis, which were combined into a table of size \( n \times m \). The \((i,j)\) entry of the table is the frequency of the case when \( Y = i \) and \( X = j \).

**ROC Curve and AUC**

Next, \( Y \) was assumed to be binary, (i.e. \( Y = 1 \) means disease-free and \( Y = 2 \) means diseased) and for simplicity, \( X_1 \) and \( X_2 \) are from potentially different but independent normal distributions. Additional calculations revealed that the AUC equals the probability of \( X_2 > X_1 \); this fact is the key extension to a more general scenario.

Recalling that the raw data is in the form of \( n \times m \), the \( Y \) dimension was first collapsed into binary by dichotomizing it by a chosen threshold, which turned the \( n \times m \) table into a \( 2 \times m \) table. Note that the choice of threshold might be arbitrary, but convention is to make both rows of the \( 2 \times m \) table have sufficient counts in each
entry. The remaining step was to recover the two underlying normal distributions from the 2 remaining rows.

The underlying normal random variable was denoted as $X^*_i$. Then maximal likelihood estimation (MLE) was used to estimate the following parameters: mean of $X^*_2$, variance of $X^*_2$, $m - 1$ cutoffs $c_1, c_2, \ldots, c_{m-1}$ so that $X_i = k$ if $c_{k-1} < X^*_i \leq c_k$. And, without a loss of generalization, $X^*_1$ was fixed as normal with mean 0 and variance 1 (standard normal). The estimation generally worked well, i.e. the estimated underlying normal distributions and cutoffs fit the 2 rows well, as long as there were sufficient counts in each entry of the $2 \times m$ table. There is no clear rule to tell how large is sufficient, so caution was still needed in case the $2 \times m$ table was sparse (had a lot of zeros).

Once the underlying normal distribution had been fitted, the AUC could easily be estimated, which is $Pr(X^*_2 > X^*_1) = Pr(X^*_2 - X^*_1 > 0)$, since $X^*_2 - X^*_1$ is still normal.

**Nonparametric Estimation of AUC**

As mentioned above, the result of fitting underlying normal distribution only works well when the data table has sufficient frequency counts. So, another estimation of AUC was introduced, which could apply to cases when the resultant $2 \times m$ table had many zeros and which did not need to assume the underlying distribution. In other words, it used almost nothing but the data table itself. The goal was to estimate $Pr(X_2 > X_1)$ by making a distributional assumption on $X_i$. See the brief illustration:

<table>
<thead>
<tr>
<th>Histology</th>
<th>reader</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

95
First, by calculating $X_2 > X_1$, there are $6 \times 4$ total calculations. However, it is also necessary to make the continuity correction for combinations of $X_2 = X_1$, because in the original scenario the underlying distribution was assumed to be continuous, like normal. Thus the total number of combinations with $X_2 > X_1$ should be $6 \times 4 + \frac{1}{2} \times 6 \times 1 = 27$. Dividing this number by the number of all combinations, $5 \times 6 = 30$, yields the estimated $\text{AUC} = 0.9$. And if the underlying normal distribution is used from the previous subsection, it produces $\hat{\text{AUC}} = 0.992$, which is more extreme than 0.9.

This example indicates the robustness, or in other words, the endurance from yielding very extreme AUC estimates (i.e. very close to 0 or 1) of this no-assumption approach. Other advantages of this approach include i) when the data table has moderately large counts, the two approaches give similar estimates, and ii) the calculation can be considered computation power-free compared to the previous MLE, which requires more computing time for optimization. The only drawback is that only by assuming that the data is normal (or another parametric assumption) can the analysis give us a nice-looking ROC curve.

To summarize, if the table counts are large, either method is a good estimate for AUC. If nice-looking smooth ROC curves are desired, it is necessary to assume some continuous underlying distribution for $X_i$. If the data table is sparse, then the no-assumption approach is more reliable due to its endurance from very extreme estimates.

Analysis

The general setting of this analysis was to accumulate data for each combination of beam sequences and reader. For example, in the ex vivo study there were 7 beam sequences and 12 readers; if the data was collapsed from the same reader and image
from the same beam sequence into the same table, there were 84 tables in total. Those 84 AUCs were estimated and then an analysis was conducted primarily on those 84 numbers. To test whether beam sequences had different powers of detecting disease, either traditional ANOVA or a nonparametric version of ANOVA was used; to compare two specific beam sequences, either a t-test or a nonparametric two-sample test was used. These methods are all very standard. The only concern was that if some of these 84 tables were sparse, their estimated AUC would be very extreme and consequently underpower the comparisons. When this was the case, as occurred with the \textit{in vivo} analysis, the nonparametric estimation of AUC was adopted for those specific comparisons.

\section*{8.3 Results}

\subsection*{8.3.1 Phantom Studies}

ROC curves for all readers, acquisitions and other parameters are shown in Figure 8.6 with AUCs listed on each ROC curve. First in Fig. 8.6a, both hard and stiff inclusions have comparable AUCs (0.838 and 0.849) with p=0.982, suggesting that there was no difference in beam sequence performance according to plaque stiffness. Similarly, in Fig. 8.6b both 2.5 and 5 mm wide inclusions perform comparably, with AUCs of 0.879 and 0.881. Both perform significantly better than the control (p=0.007), which with an AUC of 0.507 performs very close to the ideal control of 0.5. Lastly, Fig. 8.6c illustrates the variation in AUC for the 3 inclusion positions. In this case the AUC is observed to increase from 0.774 to 0.928 as offset from the ROE is increased, suggesting that overall beam sequence performance is offset dependent.

To better examine individual beam sequence performance, box plots of reader AUC vs. beam sequence are shown in Figure 8.7. A box plot showing reader AUC averaged
Figure 8.6: ROC Curves for Phantom Differences, AUC for each ROC is listed on each curve.
over all inclusions, repeated acquisitions and all 3 imaging locations is shown in Fig. 8.7a, while the box plot for the -6 mm lateral offset only is shown in Fig. 8.7b. Sequences are sorted from left to right, with the best performing sequence according to median area under the curve (AUC) on the left. The mean AUCs for each beam sequence are listed below the box plot of the corresponding sequence. Within the 6 highest AUC sequences, only DP-SRx and SP1.5-SRx sequences were found to be significantly worse than the top performing sequence, DP-ParRx (**, p<0.02). The SWEI sequences performed significantly worse than all 6 other sequences (***, p<0.005). This appears due, at least in part, to the inclusion overlapping with the SWEI ROE in the 0 and -3 mm lateral offsets. If only the maximum offset of -6 mm is considered (Fig. 8.7b), then the SWEI sequences are observed to yield higher AUCs, but the AUCs are still lower than those of the other 6 sequences. Reader variability, with p=0.3940 was non-significant as expected.

8.3.2 Ex Vivo Studies

Figure 8.8 displays a box plot for all 7 beam sequences applied ex vivo, averaged over the two repeated acquisitions and all examples. As with the phantom results, the beam sequences with the largest mean AUCs are listed from left to right in decreasing order. The order of the sequences is consistent with the results of the phantom study, with the exception of DP-ParRx which moves from best overall in the phantom to worst overall, among the ARFI sequences. Again, SWEI sequences have significantly lower AUCs than the ARFI sequences (***, p<0.005). It is possible that plaque location relative to the ROE may be a contributing factor in these low AUCs. The threshold used for the AUC analysis was Type III plaques, so the AUCs reported describe beam sequence performance at detecting plaques Type III+.

Among the ARFI sequences, only the two DP sequences, DP-SRx (*, p<0.1) and
Figure 8.7: AUC box plots for arterial wall mimicking phantom.
Figure 8.8: A box plot of the AUCs for ex vivo reader results.
DP-ParRx(**, p<0.02), have significantly lower AUCs when compared with the top performing sequence, SP1.5-ParRx. While SP3-SRx and DP-SRx performed slightly better than their ParRx counterparts, they were not significantly better, signifying that the fewer excitations of ParRx do not substantially decrease the ability to detect atherosclerotic plaques. Additionally, SP3-ParRx performs only marginally worse than SP1.5-ParRx, suggesting that the greater depth of field in excitation can be gained without significant loss of plaque-detecting ability, which as has been previously seen with large calcified plaques in the previous chapter, can be relevant to atherosclerosis imaging.

### 8.3.3 In Vivo Studies

A box plot of reader results from the *in vivo* study is shown in Figure 8.9, with the results of individual readers superimposed on the box plots as red circles. A threshold of Type II+ was used as a detection threshold. Notice that both SWEI sequences (SP1.5 and BP) applied *in vivo* are excluded from this plot. While the average image quality was lower overall *in vivo* compared with *ex vivo*, only the SWEI sequences had average image quality ratings below 2, meaning that the majority of image sets for these two sequences were excluded. Since the remaining sequences would fail to provide statistically meaningful results, they were excluded. Of the remaining sequences, no statistical significance was observed between sequences and the AUCs were only slightly above pure chance (0.5). Since variations in reader performance were higher than in *phantom* or *ex vivo* imaging, Table 8.3 shows the AUCs of the top 4 readers, as determined by average AUC over all examples and sequences *in vivo*, compared with the other 8 readers. While all sequences were observed to improve under this level of analysis, the largest increases in AUC were observed for SP1.5-ParRx (0.533 up to 0.642) and SP3-ParRx (0.511 up to 0.597).
Figure 8.9: A box plot of the AUCs for \textit{in vivo} reader results.

Table 8.3: AUC Values of Top 4 Readers vs. All Other Readers for \textit{In Vivo} Study

<table>
<thead>
<tr>
<th></th>
<th>SP1.5</th>
<th>SP3</th>
<th>DP</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top 4 Readers</td>
<td>0.642</td>
<td>0.597</td>
<td>0.564</td>
<td>0.555</td>
</tr>
<tr>
<td>Other Readers</td>
<td>0.478</td>
<td>0.468</td>
<td>0.522</td>
<td>0.498</td>
</tr>
</tbody>
</table>
The poor performance of the in vivo beam sequences may be due to the low occurrence of advanced plaques within the in vivo data set. Figure 8.10 shows histograms of plaques by type for both ex vivo and in vivo data sets. While in Fig. 8.10a a distribution across all plaque types is observed, Fig. 8.10b shows that the in vivo data set is heavily skewed towards Type I plaques (green). Additionally, the size of plaques in the ex vivo data set were observed to be wider, laterally, than plaques observed in the in vivo data set.

8.4 Discussion

The results of this reader-based investigation into ARFI and SWEI beam sequence performance suggest that a robust beam sequence assessment methodology has been developed. By utilizing blinded readers it was possible to perform a non-biased assessment of select beam sequences. A number of trends were observed throughout the study, including lower AUCs for SWEI sequences and high AUCs in phantoms and ex vivo.

The first trend observed throughout all studies was lower AUC values for SWEI sequences when compared to ARFI sequences. In the phantoms and ex vivo, SWEI had significantly (p<0.005) lower AUCs. Additionally, in the in vivo examination, the SWEI sequences were nearly all excluded due to low image quality. This highlights one of the difficulties of using SWEI-based approaches in the arterial system—low displacements. As radiation force displacements are already lower in the arterial system than in other softer tissues, observing shear waves propagating through highly attenuating and stiff arteries proved difficult. It is likely that if initial and subsequent displacements from the shear wave could be increased, then the performance of SWEI sequences would likely improve noticeably. However, these same increases in displacement would also improve SNR in ARFI sequences, so it remains unclear if there is a displacement magnitude...
Figure 8.10: Distribution of plaque types across histological subsections.
above which SWEI sequences would perform comparably to ARFI sequences.

Second, \textit{ex vivo} AUCs were observed to reach as high as 0.800 and no ARFI sequences were observed with AUCs below 0.724. This demonstrates that all of the ARFI sequences are effective for detecting Type III+ plaques. SWEI sequences appear significantly less capable of detecting these plaques, based on these preliminary results. Also, it appears as if there is little benefit in using DP sequences for plaque detection, considering they provide lower detection ability while using double the incident acoustic energy. While AUCs were high in the \textit{ex vivo} portion of this study, they were significantly smaller in the \textit{in vivo} examination, suggesting impaired plaque detection ability of all sequences. This may be explained, in part, by the atherosclerotic distribution in the data set and also the complex \textit{in vivo} imaging environment.

The small number of advanced plaques observed \textit{in vivo} limited the ability to measure any differences in beam sequence performance. This may also be true within the \textit{ex vivo} study, albeit to a lesser extent. Since atherosclerosis remains such a complicated and heterogeneous disease, even larger numbers of plaques may be needed before the true effectiveness of plaque detection with ARFI or SWEI beam sequences at various stages of development can be conclusively known. Second, the \textit{in vivo} results may be the most susceptible to increased performance via enhanced imaging techniques. Although only the best 2 of 5 \textit{in vivo} acquisitions were used, it’s likely that if this study was performed with real-time imaging, with which it would be possible to observe stiffness and elastic differences in real time, it would drastically improve ARFI/SWEI detection capabilities. This is due to the movement occasionally observed during imaging due to motion of the sonographer or animal being imaged. While this was not a major issue in this study, the movement still likely contributed to degradation of the data quality. Also, \textit{in vivo} the animals required deep imaging, sometimes down to 4 cm. This is near the limits of where it is possible to obtain a measurable push with the imaging
parameters used. In contrast, with \textit{ex vivo} it was possible to easily control the depth of the arterial wall and thus ensure large displacements, which would provide a high displacement SNR.

8.5 Conclusions

By examining ARFI and SWEI beam sequence performance in phantoms, \textit{ex vivo} and \textit{in vivo} it was possible to assess ARFI beam sequence performance as it relates to plaque detection. Based on \textit{ex vivo} results, it appears as if SP ARFI sequences perform best, followed by DP ARFI and then SWEI sequences. The DP ARFI sequences performed similarly to most other ARFI sequences, but the SWEI sequences showed significantly (p<0.005) less ability to detect Type III+ atherosclerotic plaques. These results suggest that this is a robust method of beam sequence comparison, but a more complete data set consisting of more advanced atherosclerotic plaques may be necessary before \textit{in vivo} comparisons may be possible.
Chapter 9

ARFI Beam Sequence Performance as Evaluated by Trained Readers II: Plaque Characterization

9.1 Introduction

In the previous chapter beam sequence performance as it relates to plaque detection was examined in a blinded reader study. In this chapter a similar analysis of beam sequence performance will be examined as it relates to plaque compositional analysis. As described in Chapter 2, it is not the size of the plaque but the presence of such compositional elements as necrotic cores, thin fibrous caps and calcium that determines plaque risk. To analyze plaque composition, trained readers evaluated the supposed impact of collagen, elastin, calcium, fibrous caps, lipid pools/necrotic cores, fibrin and the IEL on parametric ARFI image sets. Accurate assessment of these plaque contents would allow for an accurate assessment of plaque vulnerability, to the extent that plaque vulnerability can be established by plaque composition and structure.
Table 9.1: *Ex Vivo/In Vivo* Histology Assessment Instructions

<table>
<thead>
<tr>
<th>Tissue Classification</th>
<th>Peak Disp</th>
<th>Recov Time</th>
<th>MPD</th>
<th>SWV</th>
<th>Additional Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Deposition</td>
<td>-</td>
<td>-/0</td>
<td>-</td>
<td>+</td>
<td>Most Probable</td>
</tr>
<tr>
<td>Disrupted IEL</td>
<td>0</td>
<td>+</td>
<td>0/+</td>
<td>-/0</td>
<td>Most Probable</td>
</tr>
<tr>
<td>Calcium Deposition</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>+</td>
<td>Focal, Noisy</td>
</tr>
<tr>
<td>Lipid Pool/Necrosis</td>
<td>+++</td>
<td>0</td>
<td>0/+</td>
<td>-</td>
<td>Focal, Noisy SWV</td>
</tr>
<tr>
<td>Fibrous Cap</td>
<td>-</td>
<td>-/0</td>
<td>-</td>
<td>+</td>
<td>Covers Plaque</td>
</tr>
<tr>
<td>Elastin Deposition</td>
<td>0</td>
<td>-</td>
<td>+</td>
<td>0</td>
<td>Focal</td>
</tr>
<tr>
<td>Fibrin Deposition</td>
<td>+</td>
<td>-/+</td>
<td>0/+</td>
<td>-</td>
<td>Focal</td>
</tr>
</tbody>
</table>

### 9.2 Methods

The data collected from the trained readers was recorded with the same GUI as the detection data. As a result, the methods here are the same as those described in Section 8.2 of the previous chapter, with the exception of those differences mentioned below. Since this was a compositional analysis, only the *ex vivo* and *in vivo* data sets were included, the phantoms were excluded. Additionally, the compositional results from the *in vivo* data set are not presented below, even though it was collected, due to the lack of compositional diversity available in the plaques. The examples simply did not have sufficient distribution across compositional possibilities to provide a sufficient statistical basis for analysis. The readers were instructed to assess material component presence or absence within ARFI/SWEI images sets according to Table 9.1. Then, in the GUI, the readers chose among the options listed in Table 9.2. The pathologist chose among similar options for the histology, which are listed in Table 9.3.

All box plots for AUC analysis of material content were generated using the non-parametric AUC methods. For all contents except the IEL, only plaques Type III+ were considered, totaling 23 of the 84 examples imaged. Unlike all Type III+ plaques examined, there were Type II plaques which did not have IEL degradation, so these
plaques were included to make statistical analysis possible. No fibrin was observed within the data set, so there are no associated analysis to perform.

9.2.1 Multilevel Response and VUS

In addition to AUC analysis, it is possible to extend the binary response scenario to a multilevel ordinal response, that is, possible values of $Y$ are no longer restricted to just 1 and 2. By taking a 3-level ordinal response, $Y = 1, 2, 3$ for example, the true positive rate-n, $TPR_n$, was defined as $Pr(X = n|Y = n)$, which is a generalization of true positive rate (TPR, or $P(TP)$ as in fig 1) and true negative rate (TNR, or $p(TN)$ as in fig 1). By varying the thresholds for classifying $X$ into three classes, a point of $(TPR_1, TPR_2, TPR_3)$ is in $R^3$, which consists of a surface, called the ROC surface. The volume formed by this surface, $XY$-plane, $YZ$-plane, and $XZ$-plane is called the volume under the surface (VUS). Similar to the interpretation of AUC, a larger VUS indicates stronger agreement between $X$ and $Y$. Additionally this produces $VUS = Pr(X_1 < X_2 < X_3)$, where $X_i$ is the random variable of $X_i$ given the condition of $Y = i$.

The extension from ROC curve and AUC analysis to ROC surface and VUS analysis is quite straightforward, but the ROC surface cannot easily be visualized if there are more than 3 levels for $Y$. Also note that under the null hypothesis, which is $X_i$ independently following the same distribution, the AUC should be 0.5. Or in other words, random guesses from a binary response will yield AUC = 0.5. And for generalization to n-levels $Y$, random classification will yield $VUS = \frac{1}{n!}$ (i.e. 0.167 for n=3).

The estimation of VUS is also similar to AUC, so an underlying normal assumption on $X_i$’s could have been made or the combinations of $(X_1 < \ldots < X_n)$ could have been counted using the continuity corrections. The applicability of the two approaches resembles the case when $Y$ is binary, with only two cautions: i) fitting the underlying
normal is more demanding on the table counts and computational power because more parameters are to be estimated, and ii) VUS is not as interpretable as AUC due to the multilevel or high dimensional nature.

Lastly, the acquired data comprised up to 6 or 7 levels of ordinal responses. Like dichotomization into binary response (diseased or disease-free), thresholds were again needed to classify raw $Y$ into 3 or more ordinal levels, and the same convention about sufficient counts applied here as well. Due to the required diversity in responses needed for VUS analysis, it was only able to be applied to lipid pools/necrosis and fibrous caps, out of all the examined material contents.
### Table 9.2: *Ex Vivo/In Vivo* ARFI Image Assessment Categories and Rating Options

<table>
<thead>
<tr>
<th>Plaque Property</th>
<th>Rating Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Deposition</td>
<td>Def. none</td>
</tr>
<tr>
<td>IEL Degradation</td>
<td>Def. disrupted</td>
</tr>
<tr>
<td>Calcium Deposition</td>
<td>Def. none</td>
</tr>
<tr>
<td>Lipid Pool/Necrosis</td>
<td>Def. not present</td>
</tr>
<tr>
<td>Fibrous Cap</td>
<td>Not Present</td>
</tr>
<tr>
<td>Elastin Deposition</td>
<td>Def. none</td>
</tr>
<tr>
<td>Fibrin Deposition</td>
<td>Def. none</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Prob. none</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Prob. present</td>
</tr>
<tr>
<td></td>
<td>Def. present</td>
</tr>
</tbody>
</table>

**Def. - Definitely  Prob. - Probably**

### Table 9.3: *Ex Vivo/In Vivo* Histology Assessment Categories and Rating Options

<table>
<thead>
<tr>
<th>Plaque Property</th>
<th>Rating Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Deposition</td>
<td>Severe inc.</td>
</tr>
<tr>
<td>IEL Degradation</td>
<td>Intact</td>
</tr>
<tr>
<td>Calcium Deposition</td>
<td>High</td>
</tr>
<tr>
<td>Lipid Pool/Necrosis</td>
<td>Large</td>
</tr>
<tr>
<td>Fibrous Cap</td>
<td>Large</td>
</tr>
<tr>
<td>Elastin Deposition</td>
<td>Severe inc.</td>
</tr>
<tr>
<td>Fibrin Deposition</td>
<td>High</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mild inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td>Mild dec.</td>
</tr>
<tr>
<td></td>
<td>Severe dec.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
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</tr>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>None</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inc. - Increase</td>
</tr>
<tr>
<td></td>
<td>Dec. - Decrease</td>
</tr>
</tbody>
</table>

**Inc. - Increase  Dec. - Decrease**
9.3 Results

Calcium

The box plot presenting beam sequence performance in calcium is shown in Figure 9.1, ranked from left to right according to mean AUC. The mean AUCs for calcium detection, listed on the figure, ranged from 0.580 to 0.719, which are relatively close to those for ex vivo plaque detection (0.610-0.800). However, in the calcium detection results more variability is observed, and the ranking of beam sequence performance is quite different from plaque detection. SP3-SRx performs best after performing second best in plaque detection, but both SWEI sequences perform significantly better. Both SP1.5-SWEI and SP3-SWEI perform on par with all ARFI sequences, ranking second and fourth, respectively. Additionally, there appears to be no significant differences in mean AUC according to excitation method or tracking method, with the exception of both DP sequences ranking worst overall, with AUCs of 0.617 and 0.580. These two sequences are also the only two to perform significantly worse (p<0.05) than the top performing sequence.

Collagen

The box plot comparing beam sequence performance at detecting collagen content is shown in Figure 9.2. The mean AUCs range from 0.452 to 0.611, which is lower than desired and even crosses to worse than pure chance for the two lowest performing sequences (SP3-SWEI and SP1.5-SWEI). The order of performance for collagen is similar the ex vivo detection performance (Figure 8.8), with the two DP sequences performing best and both SWEI sequences performing poorly. The top performing sequence, DP-SRx has a significantly higher (p<0.05) AUC than any other sequence. Additionally, the SWEI sequences perform significantly worse (p<0.05) than all but one (SP3-ParRx)
ARFI sequence. The low AUCs of all beam sequences for collagen appears to be a result of a high false discovery rate (0.684), which is higher than the sensitivity (0.631) or specificity (0.588).

However, this plot alone may not tell the entire story. Looking back upon Table 9.1, the rating criteria for collagen and calcium appear similar. To consider the possibility of reader error in picking between these two possibilities, even if the reader understood the ARFI/SWEI image set correctly, an additional characterization grouping was examined, considering calcium and collagen together as a single entity. The box plot for reader detection of stiff objects is shown in Figure 9.3. Examining these results presents a noticeable improvement in AUCs over both collagen and calcium, ranging 0.615 to 0.795, suggesting that readers often choose wrong when deciding between collagen and calcium. Most noticeably, SP3 increased from a median AUC of 0.719 for calcium and

![Calcium AUC vs. Beam Sequence](image)

Figure 9.1: A box plot of the AUCs for calcium content, examined ex vivo.
Figure 9.2: A box plot of the AUCs for collagen content, examined *ex vivo*.

0.522 for collagen to an AUC of 0.795 at characterizing stiff materials together and performed significantly (*p*<0.05) better than all but SP1.5-ParRx. The other sequences displayed smaller improvements or stayed roughly the same, but no other significant differences were observed.

**IEL**

The box plot for beam sequence performance at characterizing the IEL is shown in Figure 9.4. As mentioned in the methods section, Type II plaques are included in this analysis. The mean AUCs ranged from 0.544 up to 0.664. The two SWEI sequences performed poorly, ranking third and second to last. SP3-ParRx had the highest AUC of 0.738 and performed significantly better than both SWEI sequences, SP1.5-ParRx and DP-ParRx. The DP sequences were split between second highest AUC, DP-SRx (0.628), and lowest AUC, DP-ParRx (0.544). Additionally, DP-ParRx and SP1.5-SWEI
Figure 9.3: A box plot of the AUCs for collagen and calcium content combined, as examined *ex vivo*.

had significantly lower AUCs than the top 4 sequences, including DP-SRx and all three SP ARFI sequences.

**Lipid Pool/Necrotic Core**

Both AUC and VUS box plots are presented for lipid pools/necrotic cores in Figure 9.5. The AUC box plot is shown in Fig. 9.5a, with median AUCs ranging from 0.591 to 0.710. These AUCs are all relatively high, suggesting a high degree of success at detecting lipid pools, on par with plaque detection (0.610-0.800). However, all sequences performed similarly, with the exception of SP3-SRx, which performed significantly better (p<0.05) than all other sequences. In contrast to the plaque detection analysis, both SWEI sequences perform similarly to most ARFI sequences, though they ranked second to last and last. The two DP sequences perform fourth and fifth, though still on par with most other sequences.
Figure 9.4: A box plot of the AUCs for IEL degradation, examined *ex vivo*.

This material content was also examined with VUS analysis, which should provide a more robust analysis of material content due to the more sophisticated nature of the analysis. The VUS box plot, shown in Figure 9.5b, was completed by breaking the histological response into three groups. As described in the methods, this means that the pure chance VUS result would be $\frac{1}{3!}$ or 0.166. All sequences perform above this level, with VUSs of 0.216 to 0.365. The biggest change in terms of sequence performance is DP-ParRx, which improves from fifth under AUC analysis to second with VUS analysis. SP1.5-SWEI also increases in AUC analysis to best in VUS analysis, and SP3-SRx drops from first to sixth. Other sequences remained in the same order. These results suggest that SWEI (performing first and fourth under VUS analysis) perform quite well at detecting lipid pools and necrotic cores, a key component of advanced atherosclerosis and vulnerable plaques.
Figure 9.5: AUC and VUS box plots for lipid pools/necrotic cores, examined *ex vivo*.
Fibrous Cap

The other plaque element to have both AUC and VUS analysis performed is fibrous caps, with box plots shown in Figure 9.6. As with lipid pools, fibrous cap detection is key to plaque vulnerability assessment, with AUC results shown in Figure 9.6a. The three SP ARFI sequences performed best, with SP3-SRx having the highest AUC and performing significantly ($p<0.05$) better than four sequences. The SWEI sequences performed fifth and sixth, while the two DP sequences performed fourth and last. The mean AUCs ranged from 0.559 to 0.674, slightly lower than those seen in lipid pools/necrotic cores and plaque detection.

The VUS sequence results are shown in Figure 9.6a. As with the lipid pool/necrotic core results, above 0.167 is considered a positive result. Only one sequence falls below that, SP1.5-SRx, with a VUS of 0.151. SP1.5-SWEI moved up five spots to claim the highest VUS (0.287), and many of the changes in order of sequence mirror those seen in the lipid pool/necrotic core VUS box plot. SP3-SWEI increases one spot to fourth and surprisingly, SP3-SRx drops from first to last. It is interesting that the same sequences make similar shifts in the same direction in both the fibrous cap analysis, and lipid pool/necrotic core analysis, but this may be related to the interrelationship between the two compositional elements more than anything.

Elastin

The AUC box plot for elastin deposition is shown in Figure 9.7. The mean AUCs range from 0.463 up to 0.588, with only DP-SRx falling below an AUC of 0.5. For the first time, DP-ParRx has the highest AUC (0.588), performing significantly ($p<0.05$) better than SP3-ParRx and DP-SRx, which performs worst. The two SWEI sequences perform second and third best, while the three SP ARFI sequences all perform comparably to each other. Additionally, DP-SRx performs significantly worse ($p<0.05$) than the
Figure 9.6: AUC and VUS box plots for fibrous caps, examined ex vivo.
top four sequences, which includes the other DP sequence. Free elastin deposition is expected to occur much less frequently (it is only seen in less than 25% of cases, in contrast with collagen (75%) and calcium (62%)), suggesting that infrequency of extensive elastin deposition may be impacting this analysis.

### 9.4 Discussion

When beam sequence performance for plaque characterization is examined, it is clear that no one beam sequence is clearly the best. In fact, it appears as if there are few, if any, sequences which perform similarly across all plaque compositional elements as there were five different sequences performing best across the 9 box plots presented. The most consistent of these was SP3-SRx, which performed best in 4 of the box plots (calcium, collagen/calcium together, lipid pool/necrosis AUC and fibrous cap AUC). Perhaps
most surprising, given the level of significance seen in the plaque detection results, are the SWEI sequences, which actually perform best in both of the material contents analyzed with VUS. It is clear from these results that SWEI cannot be discounted, as was suggested by the last chapters results, but perhaps SWEI can be used in association with ARFI sequences for the best possible characterization capability, while ARFI alone is used for detection. Anecdotal feedback from the readers suggested that the SWEI sequences were more confusing and provided lower average image quality, though scientific support for this has not been fully examined at this time. If this is indeed the case and a greater number of SWEI image sets were excluded due to poor image quality, then this could have impacted the results. It does appear as if DP sequences provide no discernible benefit, while utilizing double the incident acoustic energy to all other sequences.

Overall, high AUCs were observed, typically in the range of 0.7-0.8, for the top performing sequences, suggesting that the best sequences have a high rate of success at characterizing plaques. However, unexpectedly low AUCs were observed in the case of collagen, which when combined with calcium deposition, resulted in some of the highest AUCs observed. This suggests that, while overall the readers performed well when assessing the images and translating those interpretations into plaque compositions, there still is room for improvement in how the readers decided upon stiff compositional elements. This may suggest that the assessment instructions, as shown in Table 9.1, could be improved. When generating that table all of the data collected prior to the initiation of this study was used, though the total of all that data combined was less than the size of the data sets that the readers evaluated. Before translating this study into humans or expanding this study this table should be revised, including the new data.

Additionally, the variability among reader performance, especially at characterizing
calcium and collagen, suggests that some of the readers may have been insufficiently trained. The readers enrolled in this study began with varying levels of experience with ARFI; only a few had experience with ARFI imaging of atherosclerosis. When the readers were trained they were provided with several examples of ARFI images of atherosclerotic plaques, though for most compositional elements there were only 1 or 2 examples in the training set. Expanding the training set and providing expanded training for the readers would likely improve the readers’ ability to accurately characterize atherosclerotic plaques and reduce inter-reader variability.

As noted in the methods, this analysis only included Type III+ plaques, since below that threshold detectability is a greater concern than characterization. A preliminary analysis into including Type II or Type I+ plaques suggested that had these plaques been included, beam sequence performance across the board would have improved noticeably. Though, as the prime concern was with differentiating beam sequence performance, the inclusion of Type III+ plaques should provide a more realistic view as these are the complex and potentially risky plaques most important to assess.

Lastly, due to the extreme complexity and heterogeneity of atherosclerosis development, even larger studies than this would likely provide a better assessment of beam sequence performance. 23 atherosclerotic examples were included in this analysis, which provides a broad spectrum of atherosclerotic manifestations, but nowhere near the wide range of possibilities available. This is evidenced by the lack of fibrin in any of the included examples, preventing any assessment of beam sequences performance for intra-plaque hemorrhage or thrombus formation. Additionally, this would provide the possibility of performing the more robust VUS analysis on all compositional elements.
9.5 Conclusions

This chapter presents a reader-based study of plaque characterization for 7 ARFI/SWEI beam sequences. No sequence had consistently the highest or lowest AUC, though SP3-SRx did perform best in 4 of 9 cases and SWEI sequences performed best in the two material contents analyzed with VUS analysis. These results suggest that overall ARFI/SWEI sequences perform well at characterizing plaques, including lipid pools/necrotic cores, fibrous caps and calcium, three key identifiers of vulnerable plaques. However, additional and better reader training may improve these results further. Additionally, a larger data set would produce more robust results that likely include blood clots and/or intra-plaque hemorrhage, allowing for the inclusion of fibrin, another key identifier of plaque vulnerability and thrombosis. Lastly, DP sequences provided little to no discernible improvement in plaque characterization to other ARFI sequences, while doubling the incident acoustic energy to the patient.
Chapter 10

Feasibility of ARFI for a Longitudinal Analysis of Disease Progression

10.1 Introduction

The previous chapters have primarily focused on optimizing ARFI ultrasound for the detection and characterization of atherosclerosis, especially subclinical disease. Next, another important clinical aspect of ARFI ultrasound is investigated: detecting changes in disease progression over time. In this examination two aspects of serial imaging are studied, repeatability of ARFI and the ability of serial ARFI interrogations over longitudinal time points to detect changes in disease status. This chapter examines the variation and repeatability of ARFI measurements at 3-month time points over a 1 year span in 11 porcine arteries to determine longitudinal imaging feasibility and atherosclerotic detectability.
10.2 Methods

10.2.1 ARFI Imaging

ARFI imaging was performed with a Siemens SONOLINE Antares\textsuperscript{TM} imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division), equipped for research purposes, and a VF7-3 transducer. In contrast to the two previous chapters, only 3 sequences were used in this examination: SP-ParRx, DP-ParRx and SP-SWEI. Sequence parameters were the same as those used in Chapters 8-9, using 300-cycle excitations and with a $\sim2$ cm FOV. Spatially matched B-modes were acquired immediately preceding each ARFI data acquisition.

For this longitudinal study, 12 pigs (8 FH, 4 NC) were imaged at 5 time points: Baseline (BL), 3 months, 6 months, 9 months and 12 months. Given the 2 arteries imaged per pig and the 2 wall locations per artery, this resulted in 48 arterial wall imaging locations, each imaged with 5 repeated acquisitions. From these 48 locations, 11 locations from 8 pigs were selected for analysis, according to the presence/absence of disease, quality of ARFI images and quality of histological sections. The 11 locations included 4 proximal and 7 distal arterial walls and were a subset of the in vivo locations used in the in vivo portion of Chapters 8-9; only those 3 locations not repeated at multiple time points were excluded.

For each image acquisition, the arterial wall was isolated with a mask of the variance of the second time derivative of displacement, similar to the color scaling mask applied in Chapters 8-9 and described in detail in Appendix C. This mask was then examined and, if necessary, tweaked by hand to remove any non-arterial signal. The resulting mask was then applied to images of ARFI parameters, averaging the signal over 4 sub-sections corresponding to the lateral quarters of the image. The parameters analyzed were: PD and RT for SP-ParRx, PD\textsubscript{1}, PD\textsubscript{2}, $\Delta$PD, MPD and RT for DP-ParRx and
SWV for SP-SWEI. All PD measures (PD, PD\textsubscript{1}, PD\textsubscript{2} and ∆PD) were scaled for anticipated relative maximum displacement according to focal depth calculated from ARFI application in a ∼20 kPa agar-gelatin phantom.

To compare with the ARFI parameter results, a trained sonographer measured the femoral IMT (FIMT) thickness for each artery at each time point. The measurements were made 4 times and averaged to obtain a single FIMT measurement for each artery.

As these were the same locations examined in Chapters 8-9, the histological analysis was the same as described in Section 8.2.5. For this study, both plaque type and material content results were utilized for comparison. The ratings were the same as those listed for the \textit{in vivo} analysis of Chapters 8-9 (Tables 8.2 and 9.3) and were used on an ordinal scale according to pathologist rating.

### 10.2.2 Statistical Analysis

Two levels of statistical analysis were performed, a measure of relative standard deviation (RSD) across control examples over time and a correlation of ARFI parameter measurement with histological result. To calculate the average subsection measurement for each parameter at each time point, the median measurement across acquisitions was taken. Instead of the more common standard deviation, the RSD was used, due to the variation in measurement magnitude across serial acquisitions. The RSD was calculated according to

\[
RSD = \frac{SD_{tp}(\text{Mean}_{\text{subsect}}(\text{Parameter}))}{\text{Mean}_{tp}(\text{Mean}_{\text{subsect}}(\text{Parameter}))}
\]

for all examples with an average histological result across the vessel for all ≤Type I plaques. In both the numerator and denominator the mean across subsections is first calculated (Mean\textsubscript{subsect}), followed by calculating the standard deviation (SD\textsubscript{tp}) and mean across time points (Mean\textsubscript{tp}), respectively for the numerator and denominator. The RSD for the FIMT measures was calculated in a similar way, excluding the
averaging across subsection.

To calculate the correlation of ARFI parameters with histological result, the mean ARFI parameter measurements of RSD versus time were used to calculate a slope for each ARFI parameter and imaging location. The slopes were then correlated with the mean histological result on an ordinal scale. For example, a positive slope in PD, would suggest that PD variability increased with time, as heterogeneity would increase with more advanced atherosclerosis.

10.3 Results

The first question investigated was the repeatability of ARFI measurements at multiple time points for control examples. The spatially-matched B-mode images for one of the pig arteries imaged is shown in Figure 10.1. In this Figure, two anatomical markers are circled in red in each B-mode image. The soft tissue has identifiable echogenic features which connect to the arterial wall (circles). These features appear relatively repeatable with the maximum translation being between the 3 month and 12 month time points (Figs. 10.1b & 10.1e), where the left red circle is observed to translate from $\sim0$ mm to $\sim15$ mm, respectively, for a total shift of $\sim1.5$ cm. This amount of motion is comparable with the magnitude of motion observed during the multiple acquisitions of a single time point. Considering the difficulties endured during imaging porcine arteries \textit{in vivo}, this amount of motion is not entirely surprising. However, since the amount of translation observed between these images is greater than the subsection width of 5 mm, a direct comparison of ARFI parameters across time points was deemed unreliable and instead a relative comparison of subsection measurements using RSD was utilized.

The RSD for each measured ARFI parameter (as well as FIMT) is shown in a box plot in Figure 10.2. The ARFI parameter measures for the three beam sequences are shown along with FIMT. As only $\leq$Type I plaques were included in this calculation,
Figure 10.1: B-mode images repeated at 5 time points illustrate localization repeatability.
the tissue response is expected to remain homogenous across subsections, resulting in low RSDs (\(\sim 0\)). Looking at the measured ARFI parameters, the PD measures (PD, PD\(_1\), PD\(_2\) and ΔPD) and MPD all have relatively low median RSDs of \(\sim 0.2\). Both RT measures from SP-ParRx and DP-ParRx have higher median RSD measures of \(\sim 0.45\), suggesting that RT is more variable across time point. While SWV is expected to have a relatively high RSD based on experimental observations, the median RSD is lower than RT at \(\sim 0.3\). It should be noted that the SP-SWEI sequence experienced issues with SNR, and only a very small percentage of the arterial wall yielded acceptable SWV measurements. This low sample number (when compared to other sequences) may explain the lower than expected RSD. Lastly, the FIMT measurement had a median RSD of \(\sim 0.25\), which was comparable with the ARFI PD measures, though the variability across imaging example appears smaller.

The high degree of repeatability observed over time with ARFI lays a solid foundation for detecting changes in disease over time. Unfortunately, however, in this data set, less advanced disease was observed than would be ideal for a robust analysis of ARFI detection across time. A histogram of plaque type is shown in Figure 10.3, where the columns are colored according to plaque type. As this data set is a subset of the data used in Chapters 8-9, this figure will appear quite similar to Figure 8.10b. Recall that due to skewing of the data toward early stage plaques, the rating scale was adjusted to better account for the high frequency of Type I plaques observed, differentiating the Type I plaques based on the plaque thickness as a percentage of the medial-adventitial thickness (\(<25\%\), \(>25\%\) and Diffuse or \(>25\%\) and Focal). And as with the results in those chapters less than optimal results were anticipated in correlating ARFI parameters over time with histological result, due to the lack of a robust plaque Type distribution observed.

The correlation results between ARFI parameter (as well as FIMT) and histological
Figure 10.2: Variation in parameter measurement across time point for plaques ≤Type I at final time point.
Figure 10.3: Distribution of plaque types across histological subsections.
result are shown in Table 10.1. Those parameters which correlated significantly with histological result are listed in bold. The first significant result observed is FIMT ($FIMT_a$) which had a correlation of 0.760 with $p<0.02$. However, this result was largely due to the impact of a single imaging location, whose FIMT slope was approximately an order of magnitude greater than the others. If this location is excluded ($FIMT_b$), then the correlation is no longer significant and changes drastically, becoming negative. The only other set of significant correlations are $PD_1$ and $PD_2$, which are significant when correlated with both lipid pools and fibrous caps ($p<0.1$). As none of the fibrous caps were disrupted in this data set, the two histological parameters had identical ordinal ratings. None of the other correlations were significant, though the sign of the correlations (typically positive for PD and negative for RT) aligned with expectations, suggesting that a more robust data set could potentially yield significant results.

### 10.4 Discussion

In this study, an analysis of ARFI parameter reproducibility over time was conducted for plaques with very little disease. There were RSDs similar to that of FIMT for all PD measures, while RT and SWV measures had higher RSDs. To completely interpret these results, the imaging conditions must be taken into account. Large pigs were imaged while positioned in the lateral decubitus position, which involved applying a fair amount of pressure on the transducer to compress adipose tissue and hold the transducer in place. In a number of cases, however, a moderate amount of motion was observed both within and between ARFI acquisitions. This resulted from a number of sources including fatigue on behalf of the sonographer, normal respiratory motion of the pig and sudden kicking/jerking motions if the pig was not fully sedated.

To help alleviate physical fatigue on the sonographer, approximately 18 months before the completion of the study, an articulating arm with a molded transducer
holder was implemented. This was observed to greatly (but not completely) reduce the amount of motion observed both within and between ARFI acquisitions. The arm was clamped to a 50 lb. dumbbell for stability in conjunction with light force by the sonographer to hold the transducer in place. However the arm was only implemented in a portion of the imaging time points, as the pigs were enrolled in the study on a rolling basis. As a result, these problems and difficulties introduced a larger than desirable amount of motion during in vivo imaging.

Additionally, due to the off-line nature of the data processing and parametric image generation, it was sometimes difficult to align the transducer for repeated acquisitions, since precise physiologic markers (such as the echogenic regions in Fig. 10.1) were not always visible. It's expected that by implementing real-time ARFI imaging the success at detecting and re-localizing developing plaques would increase significantly.

Lastly, as described in Figure 10.3, a less than optimal amount of advanced atherosclerosis was observed in the animal model. During the duration of the study, all FH pigs were placed on a high fat diet to increase the atherosclerotic development. While atherosclerosis was observed to increase compared with those animals not on a high-fat diet, this was not as often the case in the imaging location. Due to the abundance of adipose tissue in the pig model and anatomical limitations in imaging the iliac artery, the area which could be imaged was limited. This is something that, due to the atherogenic and accessible nature of the carotid bifurcation in humans, would likely not pose as great a problem. Additionally, the short time span over which the pigs were placed on a high fat diet (12 months), compared with the long time over which humans have to develop disease, will likely result in a greater number of more easily detectable plaques (Type III+).
10.5 Conclusions

8 pigs in 11 imaging locations were imaged at 3 month time points for a year. In those imaging locations with $\leq$Type I disease, good repeatability in ARFI parameters from SP-ParRx, DP-ParRx and SP-SWEI beam sequences was observed when compared with FIMT measurements. Only a small number of advanced plaques were present in the data set, resulting in few correlations between ARFI/SWEI/FIMT parameters and histological result. These results were promising and suggest that in a study of humans with more advanced disease, real-time ARFI could potentially be used for tracking longitudinal disease progression.
<table>
<thead>
<tr>
<th>Sequence</th>
<th>SP-ParRx</th>
<th>DP-ParRx</th>
<th>SP-SWEI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>PD</td>
<td>RT</td>
<td>PD1</td>
</tr>
<tr>
<td>Plaque Type</td>
<td>-0.391</td>
<td>-0.201</td>
<td>-0.198</td>
</tr>
<tr>
<td>Collagen Deposition</td>
<td>0.111</td>
<td>-0.202</td>
<td>0.206</td>
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<tr>
<td>IEL Degradation</td>
<td>0.228</td>
<td>-0.066</td>
<td>0.447</td>
</tr>
<tr>
<td>Calcium Deposition</td>
<td>0.174</td>
<td>-0.170</td>
<td>0.359</td>
</tr>
<tr>
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<td>0.538*</td>
</tr>
<tr>
<td>Fibrous Cap</td>
<td>0.491</td>
<td>-0.022</td>
<td>0.538*</td>
</tr>
<tr>
<td>Elastin Deposition</td>
<td>0.356</td>
<td>-0.146</td>
<td>0.354</td>
</tr>
</tbody>
</table>

*p<0.1  **p<0.02
Chapter 11

Conclusions and Future Directions

The results presented in this dissertation demonstrate that ARFI is capable of both detecting and characterizing atherosclerotic plaques. Furthermore, the results of Chapters 8 and 9 show that ARFI and SWEI beam sequences are capable of detecting and characterizing those plaques, from Type III onward. This includes plaques which have already ruptured, and are therefore most vulnerable to thrombosis formation and subsequent stroke, but also those which not even developed atheromas or fibrous caps and are much earlier on the progression to high risk of rupture. Additionally, ARFI was demonstrated as capable of detecting both occlusive and minimally-occlusive plaques, another advancement over current non-invasive technologies.

It is clear from these results that real-time ARFI imaging is vital to leveraging the full capability of ARFI. This technology has already been developed, but the importance of real-time imaging is clear, and should be adopted for future studies, if possible. Real-time ARFI would allow sonographers and clinicians to further optimize plaque detection and characterization, by utilizing feedback on the elastographic images provided by ARFI to perfect alignment of the atherosclerotic plaques with the imaging plane. Additionally, while SWEI did not perform as well at plaque detection, real-time imaging would allow for placing the ROE at an optimal lateral position for
plaque detection, potentially improving SWEI plaque detection capability. Real-time imaging would allow for optimized ROE placement near a region of interest, as opposed to simply placing it in the center of the FOV, providing improved image quality due to larger displacements where they are most needed. Real-time plaque identification would also potentially allow for multiple SWEI ROEs centered around a point of interest. Advanced atherosclerosis is quite complex and can contain mechanical impedance mismatches such as atheromas and calcifications, which would reflect and distort propagating shear waves. Placing SWEI ROEs on each side of a plaque of interest may allow for better characterization capability by observing shear waves propagating in multiple directions and thus reducing reflection artifacts.

While SWEI performed poorly at plaque detection, it did perform well in the reader study at characterizing lipid pools/necrotic cores, fibrous caps, calcifications and elastin deposition. This suggests that while SWEI may not be optimal for detecting plaques, SWEI and ARFI together may be the best tool for characterization, in association with ARFI alone for the detection. However, it does not appear as though DP ARFI provides worthwhile benefits for atherosclerosis imaging. DP ARFI doubled patient exposure without performing significantly better than SP ARFI sequences. DP ARFI performed significantly below SP ARFI sequences in plaque detection and on par with or below SP ARFI sequences for plaque characterization. The increased acoustic power used by DP ARFI could be better utilized by a SP ARFI sequence to increase displacement SNR with increased pulse intensity, an expanded FOV or for multiple focal depths. These possible alternatives should be investigated further in future studies.

It was also not possible to conclusively state whether SP1.5 or SP3 ARFI performs best for plaque detection and characterization, as in most cases, SP3 and SP1.5 were not significantly different from one another. The same can be said for ParRx vs. SRx; no overarching trends were observed in the ex vivo analysis to suggest that either is
best in most situations. However, there are other aspects of larger F/#s and ParRx that must be taken into consideration as well. While F/3 excitations did not appear to provide a significant benefit in most cases, not many of the plaques imaged \textit{ex vivo} were more than $\sim$3 mm thick axially. Chapter 7 showed that as plaques get bigger, large F/#s may be vital for detecting atheromas in plaques more than 4 mm thick, suggesting that in the clinic, where 50%+ occlusion is regularly seen, that this could tip the scales into favoring larger F/#s. Additionally, the use of 4:1 ParRx came at no significant difference in plaque detectability or characterization capability in most cases, suggesting that these techniques can likely be employed to reduce patient exposure without causing loss of ARFI detection and characterization capability.

Another trend that has emerged from these results is the importance of displacement SNR. Aside from movement during imaging, which would become less of a factor with real-time imaging, small displacements were the largest issue observed with \textit{in vivo} plaque detection and characterization. As focal depths approached 4 cm, displacements fell to near the noise floor in some cases. This could be alleviated with advancements in the transducers and imaging parameters used to perform ARFI imaging. Specialized transducers for ARFI, optimizing for both pushing and tracking, would likely improve image quality. Additionally, 1.5D or 2D matrix arrays could be used to increase elevational focus capability, better concentrating the excitation pulses and increasing focal displacements. Combining ParRx with increased acoustic power and/or excitation pulse length could provide increased displacements and greater penetration depth without increasing patient exposure. Lastly, a better understanding of the bioeffects associated with radiation force imaging may allow for higher intensities to be used, without fear of negative bioeffects.

Another aspect of ARFI imaging investigated was the feasibility of serial ARFI imaging. Serial ARFI imaging does appear feasible and only stands to benefit from real-time
imaging and improved displacement SNR. As with the reader analysis of in vivo plaque detection and characterization, serial in vivo imaging suffered from a lack of sufficient data for a full analysis of plaque development over time. The use of a more complete data set, consisting of larger numbers and more diverse atherosclerotic manifestations would likely provide a stronger base, from which a more complete analysis could be performed. If a similar study were to be performed again in pigs, then older pigs or a longer high-fat diet would likely prove beneficial for developing advanced atherosclerosis more often. If ARFI is first translated into humans, then older adults would likely provide a wide range of advanced atherosclerosis. The translation to the carotid bifurcation in humans will likely also provide increased image quality, due to the ease with which the carotid is accessed in humans, in contrast to the difficulty in imaging the iliac arteries in 700 lb. pigs.

Lastly, another issue identified in the reader-based study is the importance of reader training. As already stated, atherosclerosis is extremely complex and analyzing parametric ARFI images of the disease can be confusing to those without much experience. While the impact of training on reader performance was not specifically investigated, readers did appear to have some difficulty deciphering parametric images, specifically the SWEI images. More extensive and comprehensive training is recommended for ideal reader detection and characterization capability, both in future studies and the clinic. Additionally, a further analysis of the data set used for the reader study would likely provide a better understanding of the impact of plaque content on ARFI response. In the instructions given to the readers, the directions for translation from ARFI parametric result to material content were based on past experience and ARFI results. However, a quantitative analysis of the nature of parametric ARFI measurements, such as the magnitude of the change, the size of change in ARFI versus the actual size of the compositional element, etc, for the now known combinations of plaque composition
would provide a more robust basis for future ARFI interpretation and reader training.

Although substantial work remains before ARFI can be translated into wide clinical use, this work suggests that atherosclerotic plaque detection and characterization is not only possible, but high levels of plaque detection and characterization are achievable with ARFI and SWEI imaging.
Appendix A

Evaluation of A Long Wave Approach to Physiological Motion Rejection

A.1 Introduction

In ARFI imaging, the shape of induced tissue displacement-recovery profiles measured in the region of radiation force excitation is relevant to discerning tissue structures. In SWEI imaging, time to peak tissue displacement induced by shear waves propagating away from the region of radiation force excitation is pertinent to measuring differences in shear wave velocities and estimating Youngs modulus.

In both ARFI and SWEI imaging, physiological motion corrupts measurements of impulsive radiation force induced displacements. In particular, physiological motion in arterial walls due to dilation and contraction with cardiac pulsation are an order of magnitude greater than radiation force induced wall displacements. However, considering physiological motion as additive noise, it may be rejected by simple subtraction of an appropriate noise describing function. Physiological motion in ARFI imaging has previously been approximated as a straight line on the timescale of a single ARFI

displacement profile (generally \(\sim 5\) ms). In reality, the shape of physiological motion on ARFI profile time scales is not a perfect line, and so this approach may result in incomplete filtering. To address this problem, Fahey et al recently introduced a quadratic physiological motion filter [67], which subtracts a second order polynomial (rather than a first-order line) fit to initial and anticipated recovery time points of ARFI displacement profiles. The researchers found that the quadratic filter outperformed the linear filter in the presence of tissue acceleration, but both filters performances were compromised by temporally short tissue tracking intervals relative to tissue recovery times. Fahey et al also introduced a model-based motion filter that uses a finite element model to predict physiological motion [68]. The model-based motion filtered yielded performance improvements when the temporal length of tissue tracking was short, but it requires a priori information regarding tissue mechanical properties and homogeneity, which is generally not available for in vivo atherosclerotic arteries.

A new approach to physiological motion rejection in arterial radiation force imaging is proposed that extends the time scale over which filtering methods operate by exploiting the long wave model for arteries [69; 70; 71]. The long wave model is based on the linear assumption for long waves, which is described by [69]:

\[
0 = -\frac{t^+}{a} + p + \frac{T\delta\xi}{a\delta x} \tag{A.1}
\]

\[
exp\{i\omega(t - \frac{x}{c})\} \tag{A.2}
\]

where \(\xi\) is radial wall displacement, \(x\) is the longitudinal dimension of the artery, \(p\) is the pressure, \(t^+\) is the perturbation stress, \(T\) is the circumferential tension per unit length, \(a\) is the radius of the tube, \(c\) is the pulse wave velocity, \(\omega\) is the circular frequency, and \(t\) is time. \(\xi\) varies with (A.2). As a result of (A.1), the vessel can be
considered to oscillate radially as if it were a rigid tube. In other words, radial vessel
wall motion can be approximated as uniform across a finite lateral distance determined
by the pulse wave wavelength. The wavelength of the pulse wave is assumed to be 1 cm,
the maximum lateral spacing between consecutive ARFI displacement profiles acquired
using wiperblading. Therefore, under the long wave assumption, consecutively acquired
ARFI displacement profiles may be aligned end-to-end to represent bulk arterial wall
pulsation over the entire time course of 2D ARFI data collection. This provides a
long (~0.24 s) snapshot of physiological wall motion, as opposed to the short (~6 ms)
snapshot of physiological motion described in the time course of a single displacement
profile. The longer snapshot supports better approximation of physiological motion
coincident with radiation force induced motion and therefore may enable more complete
filtering. This study investigates several motion filtering techniques that exploit the
long wave model and compares them to comparable motion filtering methods that
instead operate on the time frame of a single ARFI displacement profile.

A.2 Methods

A.2.1 Simulated Data

Simulated arterial wall RF data including respiration and cardiac pulsation were gen-
erated using Field II [72; 73]. The simulation employed a 60 element linear array
transducer with an 11 kHz pulse repetition frequency (PRF), a 6.15 Hz center fre-
quency, and a 40 MHz sampling frequency. The simulated artery was centered at 30
mm in depth and had a 4 mm radius with a 0.3 mm wall thickness. Arterial wall
motion due to respiration and cardiac pulsation were modeled by approximations de-
scribed by [74]. RF data was acquired over a 20 mm axial range of observation in 40
lateral positions spaced 0.53 mm apart. In each lateral position, a tracking ensemble of
6 ms was acquired for a total data acquisition time of 0.25 s. The simulated RF data was then processed with 1D cross-correlation to measure axial displacements over time. The simulated physiological motion displacement profiles were then added to ARFI and SWEI displacement profiles experimentally measured in an agar-graphite tissue mimicking phantom with an approximate Youngs modulus of \(~19\) kPa. Experimental ARFI imaging parameters were as described above for the simulated data but with a 300 cycle excitation impulse applied at each lateral position prior to tracking. All experimental ARFI data was acquired with a VF7-3 Transducer on a Siemens SONOLINE Antares\textsuperscript{TM} imaging system (Siemens Medical Solutions USA, Inc., Ultrasound Division). Experimental SWEI imaging parameters were also as described for the simulated data but with the 300 cycle impulse applied at the center of the field of view to facilitate the observation of laterally propagating shear waves.

A.2.2 In Vivo Data

ARFI and SWEI imaging were performed in the iliac arteries of one FH pig and two NC pigs and to demonstrate the relevance of the evaluated filtering methods to in vivo imaging. The FH pig was aged 7 years 3 months. Immunohistochemistry acquired after the completion of imaging confirmed atherosclerosis, but no plaques were present in the examined artery. The NC pigs were aged 12 months and 10 months, and immunohistochemistry confirmed the lack of atherosclerosis in the examined arteries. The FH pig was imaged before, during, and immediately after euthanasia (with normal, slowed, and stopped heart rate, respectively) for comparison of filter performance with normal, reduced, and no physiological motion. While the FH pig was imaged at multiple time points, the NC pigs were imaged at a single, live time point. All imaging was performed by a sonographer trained in peripheral vascular ultrasound.

2D ARFI and SWEI imaging were performed with 300-cycle excitation impulses
centered at 4.21 MHz and 2-cycle tracking lines (F/# of 1.5) centered at 6.15 MHz. For ARFI imaging, tracking ensembles of 6 ms in duration were acquired in each of 40 lateral focal positions. For SWEI imaging, 6 ms tracking ensembles were collected using 4:1 parallel receive processing in each of 15 lateral locations. All procedures were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

A.2.3 Filters Investigated

Two types of filters were investigated: (1) those that operated on the time course of a single ARFI or SWEI displacement profile (6 ms), and (2) those that operated along the time course of total data acquisition (0.24 s) under the long wave assumption.

Filters operating on individual displacement profiles were a linear regression filter, a quadratic regression filter, a real principal component (PC) regression filter, a complex PC (cPC) regression filter, and an FIR high-pass filter. The linear filter was applied by subtracting a linear fit from the reference displacement to the displacement three time points before the end of the ARFI or SWEI profiles (which were inclusive of physiological motion). The quadratic filter was applied by subtracting a second order polynomial fit from the reference displacement to the last several displacement samples (5, 10, 15, 20, or 25) from ARFI and SWEI profiles. The FIR filter was applied using a Type II Chebyshev high-pass filter with the stop-band extending to 55 Hz. We anticipate poor FIR filter performance because radiation force induced and physiological motion spectra overlap.

PC and cPC regression filters were applied by first calculating the PCs for each tracking ensemble. Then, a subset of PCs were selected to span physiological motion by observation of PC time projections, as described by Gallippi and Trahey [75; 76]. Physiological motion was incompletely separated from ARFI/SWEI induced motion,
so a second order polynomial was fit to the first and last 10 points of the selected PCs to capture only the represented physiological motion. Physiological motion was then rejected by subtracting the projection of the data onto the polynomials. It should be noted that PC and cPC regression filters differed in that PC filters operate on only real displacement profiles, while cPC filters operated on complex displacement profiles (generated by the Hilbert transform). Because cPC filters exploit both complex magnitude and phase information, more complete signal separation is theoretically supported in the context of multi-directional motion. In the case of cPC filters, incomplete ARFI/SWEI and physiological motion separation was accommodated by fitting a second-order polynomial to each of the real and the complex portions of the selected PCs, generating complex polynomials.

Filters operating along the time course of total data acquisition under a long wave assumption were polynomial regression, PC regression, cPC regression, and FIR high-pass. Prior to filtering, ARFI or SWEI profiles were organized by acquisition time. This was done by aligning the reference measurement for each ARFI or SWEI profile to the final displacement measurement of the preceding profile for each axial depth position, resulting in a 2D data (long wave) matrix with dimensions depth and time. The content of this matrix is shown for a single depth position in Figure A.1 using simulated 2D ARFI data. The solid line corresponds to sequentially aligned ARFI profiles that are exclusive of physiological motion, and the dashed line corresponds to sequentially aligned ARFI profiles that are inclusive of physiological motion.

For motion rejection by polynomial regression filtering, an nth order global polynomial (n=3, 5, 10, 15 or 25) was fit to the last 10 points of each of the 40 sequentially aligned displacement profiles (for a total of 400 fit points). A unique global polynomial was fit to displacement profiles acquired at each depth position. The global polynomial was then partitioned into 40 subsections temporally corresponding to each
Figure A.1: Simulated ARFI motion profiles of physiologic motion assembled under the rigid wall assumption.
ARFI or SWEI displacement profile. Each subsection was initialized to zero and then subtracted from the ARFI or SWEI profiles.

Long wave assuming FIR, PC, and cPC filters were generally applied as described above, but for operation along the time course of total data acquisition rather than along a single ensemble. In the case of PC and cPC filtering, nth (n=3, 5, 10, 15 or 25) rather than second order polynomials were fit to selected PCs to accommodate incomplete ARFI/SWEI and physiological motion separation.

For all examined filters, a follow-up linear filter applied along the time course of a single displacement profile rejected remnant physiological motion. All filter performance data is shown after the addition of the follow-up linear filter.

A.2.4 Validation

The simulated data described above was used as gold-standard validation for filtering results. Filtered simulated physiological motion plus experimental ARFI/SWEI displacement profiles were compared to the original experimental ARFI/SWEI displacement profiles (that were exclusive of physiological motion) by sum of absolute difference (SAD) measures. SAD scores were computed in terms of differences between original and filtered full displacement profiles, ARFI peak displacements, ARFI recovery times, and SWEI times-to-peak displacement for axial positions near the simulated imaging focal depth. ARFI peak displacement is the largest tissue displacement induced by ARFI excitation in a given axial and lateral position. It is measured as the maximum of the tracked ARFI displacement profile. ARFI recovery time is the time required for the tissue to recover from its peak radiation force induced displacement. It is measured as the time at which tracked displacement magnitude equals 1/3 of the measured peak displacement. SWEI time-to-peak is the time required for tissue beside the region of excitation to achieve its largest radiation force induced displacement. It
is measured as the time at which a tracked displacement profile reaches its maximum value. It is important to note that if insufficiently rejected, physiological motion distorts the measured tissue displacement profile shape, which can directly impact all of these parameters of interest. We illustrate ARFI displacement profiles before and after physiological motion rejection below.

Qualitative in vivo validation was also performed by comparing ARFI peak displacement and recovery time outcomes in the FH pig pre-injection, post-injection, and post-mortem. Arterial pressure decreased, and so ARFI induced peak displacement and recovery time values changed, as the heart slowed and finally stopped during euthanasia. Despite differences in absolute ARFI parameter values, relative differences in peak displacement and recovery time parameters were compared before and after filtering of normal (pre-injection), slowed (post-injection), and no (post-mortem) physiological motion.

A.3 Results

A.3.1 Simulation

Representative results of polynomial regression filter assuming a long wave model are shown in Figure A.2. Gross motion before physiological motion filtering is shown in the solid line, motion after 5th order polynomial regression filtering is shown in the dashed line, and motion after final linear filtering is shown in the dash-dotted line. Note that the dash-dotted curve is difficult to see due to the small deviations from the ideal ARFI displacement profiles, which are shown in the dotted line. Two individual displacement profiles are shown in zoom to offer a closer view of the effects of motion filtering.

Similar results were obtained from the long wave assuming PC and cPC regression filters. The time projections of the four most energetically significant real (top) and
Figure A.2: Tissue motion before and after polynomial regression filtering.
complex (bottom) PCs computed from simulated ARFI data are shown in Figure A.3. In both PC and cPC cases, PCs 1 and 2 (solid) primarily span physiological motion; however, some ARFI information is incompletely separated. In the case of cPC filtering, this is observed in terms of both cPC magnitude and phase. The fitted polynomials (dashed) are intended to capture only the physiological motion portion of the PCs. The projection of ARFI data onto the fit polynomials is shown in Figure A.4. Notice that the net description of the physiological motion component improves as the number of selected PCs increases from 1 (dashed) to 2 (dash-dotted), with subtle improvement gained by selecting the 3 most energetic PCs to span the physiological motion subspace (dotted).

The SAD scores measured for all examined filters are shown in Tables A.1 and A.2. The results for each filter are provided both with and without a final linear filter being applied before the validation measurement is taken. The Peak result is the average error in peak displacement and is given in microns, the Recovery is the average error in recovery time and is given in milliseconds, and Profile is the average error in the full displacement profile, given in microns. The columns in each table are sorted increasing from left to right according to the average displacement profile SAD values with a linear filter applied (bottom row). For filters in which multiple implementation options were tested (i.e. multiple orders of polynomials), the best performance is reported. This corresponded to using the final 10 displacement profiles points for the quadratic filter, and an order of n=5 for the long wave assuming polynomial regression, PC and cPC filters. Finally, calculation times using a 2.4 GHz Core 2 Duo processor with 4GB of RAM running MATLAB (Mathworks Inc., Natick, MA, USA) are reported in Table A.3.
Figure A.3: Time projections of the four most significant PCs and cPDs with corresponding polynomials.
Figure A.4: Projection of ARFI data onto polynomials fit to PCs.
<table>
<thead>
<tr>
<th></th>
<th>Average SAD Per:</th>
<th>Polynomial (LWA)</th>
<th>PC (LWA)</th>
<th>cPC (LWA)</th>
<th>Linear (Profile)</th>
<th>Quadratic (Profile)</th>
<th>FIR (Profile)</th>
<th>FIR (LWA)</th>
<th>PC (Profile)</th>
<th>cPC (Profile)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Without Linear Filter</td>
<td>0.017 0.016 0.016 0.019 0.025 0.652 0.454 1.237 1.237</td>
<td>0.017 0.016 0.016 0.019 0.025 0.652 0.454 1.237 1.237</td>
<td>0.020 0.015 0.015 0.020 0.025 0.413 0.413 0.120 0.120</td>
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<tr>
<td></td>
<td>With Linear Filter</td>
<td>0.016 0.016 0.016 0.019 0.025 0.664 0.465 0.616 0.616</td>
<td>0.016 0.016 0.016 0.019 0.025 0.664 0.465 0.616 0.616</td>
<td>0.013 0.013 0.013 0.020 0.025 0.413 0.413 0.140 0.140</td>
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Table A.1: ARFI Errors

<table>
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<tr>
<th></th>
<th>Average SAD Per:</th>
<th>Polynomial (LWA)</th>
<th>PC (LWA)</th>
<th>cPC (LWA)</th>
<th>Linear (Profile)</th>
<th>Quadratic (Profile)</th>
<th>FIR (Profile)</th>
<th>FIR (LWA)</th>
<th>PC (Profile)</th>
<th>cPC (Profile)</th>
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<tbody>
<tr>
<td></td>
<td>Without Linear Filter</td>
<td>0.254 0.256 0.171 0.106 0.130 0.670 0.523 3.365 3.365</td>
<td>0.254 0.256 0.171 0.106 0.130 0.670 0.523 3.365 3.365</td>
<td>0.119 0.119 0.070 0.010 0.008 0.599 0.642 0.908 0.908</td>
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<td>29.546 28.789 20.353 7.315 7.476 43.693 48.223 162.766 162.766</td>
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<tr>
<td></td>
<td>With Linear Filter</td>
<td>0.074 0.078 0.078 0.106 0.128 0.714 0.545 0.688 0.688</td>
<td>0.074 0.078 0.078 0.106 0.128 0.714 0.545 0.688 0.688</td>
<td>0.008 0.008 0.010 0.010 0.010 0.658 0.635 1.078 1.078</td>
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<tr>
<td></td>
<td></td>
<td>5.167 5.257 5.278 7.315 7.513 43.548 46.512 101.979 101.979</td>
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Table A.2: SWEI Errors

<table>
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<th>Polynomial (LWA)</th>
<th>PC (LWA)</th>
<th>cPC (LWA)</th>
<th>Linear (Profile)</th>
<th>Quadratic (Profile)</th>
<th>FIR (Profile)</th>
<th>FIR (LWA)</th>
<th>PC (Profile)</th>
<th>cPC (Profile)</th>
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<td>7.5</td>
<td>9.1</td>
<td>10.7</td>
<td>57.5</td>
<td>67.2</td>
<td>109.5</td>
<td>236.2</td>
<td>1253.8</td>
</tr>
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</table>

Table A.3: Computation Time
A.3.2 In Vivo

For brevity, only in vivo results from the best performing filters are shown. Figure A.5 illustrates in vivo ARFI displacement profiles acquired in a left femoral artery of the FH pig prior to lethal injection. The profiles are assembled through time under the long wave assumption. The profiles are shown inclusive of physiological motion and after motion filtering with a linear filter. Figures A.6 and A.7 report the result of imaging in the left femoral artery of a FH pig before, during, and after euthanasia. The linear (third rows), quadratic with a follow-on linear (fourth rows), and long wave assuming 5th order polynomial with a follow-on linear (fifth rows) filters are compared. The filters are applied at three distinct time points: prior to injection of euthanizing agents (left columns, Pre-Euthanasia),
following injection of euthanizing agents but prior to the cessation of cardiac pulsation (middle columns, During Euthanasia), and after cardiac pulsation had ceased as assessed by a veterinary surgeon (right columns, Deceased). Figure A.6 shows the resultant ARFI peak displacement images with brightness corresponding to peak displacement magnitude in microns according to the adjacent color bars. Note that the images are shown on different color scales to highlight consistency in relative peak displacement outcomes despite differences in absolute peak displacement magnitude. This is discussed further in the Discussion section below. Figure A.7 illustrates the resultant ARFI recovery time images with brightness corresponding to recovery time in milliseconds. Again, the images are displayed on different color scales to highlight relative similarities and differences.

Figure A.8 displays original and filtered displacement profiles measured at points in the left and in right portions of the imaging filed of view (FOV) for the arterial wall shown in Figures A.6 and A.7. Linear (dot), quadratic with follow-on linear (dash-dot), and long wave assuming 5th order polynomial with a follow-on linear (dash) filters are compared. Profiles acquired before injection with euthanizing agents (right column), during euthanasia (middle column), and post-mortem (right column) are shown.

Results of motion filtering ARFI and SWEI data obtained in NC pigs are shown Figures A.9-A.11. Figure A.9 shows images of peak ARFI-induced displacement for the acquired data with no filter applied (Fig. A.9a), filters operating only on single profiles (Figs. A.9b-A.9c) and filters operating under the long wave assumption (Figs. A.9d-A.9f). The filters with no long wave assumption shown are the linear (Fig. A.9b) and quadratic plus follow-on linear (Fig. A.9c). Peak displacement images following long wave assuming 5th order polynomial regression plus follow-on linear filter (Fig. A.9d), PC regression plus follow-on linear filter (Fig. A.9e), and FIR plus follow-on linear filter (Fig. A.9f) are also shown. Brightness denotes peak displacement in microns, with the
Figure A.6: *In vivo* ARFI peak displacement images from the left femoral artery of an FH pig.
Figure A.7: *In vivo* ARFI recovery time images from the left femoral artery of an FH pig, exactly matched to the peak displacement images of Figure A.6.
Figure A.8: Unfiltered and filtered ARFI displacement profiles before injection with euthanizing agents (right column), after during euthanasia (middle column), and deceased (right column).

vessel wall appearing bright (arrows). Peak displacement images are all adjusted to the same displacement scale to highlight differences in filter performance. Figure A.10 shows images of ARFI recovery time for the same unfiltered (Fig. A.10a), single profile filtered (Figs. A.10b and A.10c) and long wave assumption filtered (Figs. A.10d-f) results. Brightness represents time to recovery from peak displacement in ms.

In addition to the peak displacement and recovery time images generated from 2D ARFI, a separate example demonstrating the filters in a Sinclair pig using a SWEI beam sequence are presented in Figure A.11. In this example the images display the measured laterally propagating shear wave velocity in m/s. As in the previous example, unfiltered result (Fig. A.11a) as well as the results of single profile filters (Figs. A.11b and A.11c), are shown. The long wave assuming filters (Figs A.11d-A.11f) are also shown.
Figure A.9: In vivo ARFI peak displacement images in the right iliac artery of an NC pig.
**In Vivo 2D ARFI Images of Peak Displacement**

**Single Profile Filters**

- a. Unfiltered
- b. Linear
- c. Quadratic

**Rigid Wall Assuming Filters**

- d. Polynomial
- e. PC
- f. FIR

Figure A.10: *In vivo* ARFI recovery time images in the right iliac artery of an NC pig.
In Vivo 2D SWEI – Shear Wave Velocity

Single Profile Filters

a Unfiltered  
b Linear  
c Quadratic

d Polynomial  
e PC  
f FIR

Rigid Wall Assuming Filters

d Polynomial  
e PC  
f FIR

Figure A.11: \textit{In vivo} SWEI shear wave velocity images in the right iliac artery of an NC pig.
A.4 Discussion

In the analysis of simulated data, the long wave assuming polynomial, PC, and cPC regression filters yielded the lowest SAD scores, while the FIR (both single profile and long wave assuming) as well as the single profile PC and cPC filters yielded the largest SAD scores. The impact of the additional linear filter (applied after both single profile and long wave assuming polynomial regression, PC, cPC, and FIR filters) was significant. For example, in the case of 2D ARFI imaging (Table A.1), the additional linear filter rendered long wave assuming polynomial, PC, and cPC filter SAD scores measured along the entire profile lower than their single-profile filter counterparts. Recovery time error was also reduced by the additional linear filter; however, long wave assuming filters out-performed single profile filters even in the absence of the additional linear filter. The additional linear filter has little impact on SAD scores for measured peak displacements, which were relatively small for all of the examined filters.

In the case of SWEI imaging, long wave assuming filters alone produced larger SAD scores than single profile filters alone. Diminished long wave assuming filter performance relative to the ARFI imaging case may be attributed to the shorter overall SWEI imaging time period. While ARFI displacement profiles were acquired over a period of 40 x 6 ms = 240 ms, SWEI displacement profiles were acquired over a period of 15 x 6 ms = 90 ms. Although long wave assuming filter performances improved with the additional linear filter, the improved long wave results were similar to single-profile filter performances. This suggests that the long wave assuming filters do not make a substantial improvement over the single-profile linear filter alone. However, it should be noted that shear wave velocities were computed by a simple time-to-peak divided by distance-from-excitation calculation. More sophisticated methods of shear wave velocity determination were not investigated here, although they may benefit from long wave assuming filters.
In general, filter performance was highly consistent between the three top performers (the long wave assuming polynomial, PC and cPC filters), although associated computation time varied considerably (Table A.3). The linear filter required the least computation time (3 seconds per 2D ARFI data set) versus 239 seconds for PC and nearly 21 minutes for cPC filters. The worst performing filters were the PC and cPC filters that operated on single displacement profiles as well as the FIR filter. The most likely reason for poor PC and cPC filter performance when operating on a single profile is the short time frame, which did not support separation of ARFI/SWEI displacements from physiological motion. This lack of separation appeared to skew the fitted polynomials. Poor FIR filter performance was predicted given that ARFI/SWEI and physiological motion spectra overlap. Interestingly, the quadratic filter yielded larger SAD scores than the simple linear filter. This is manifested in the overall lower measured recovery times and higher shear wave velocities in the in vivo examples (Figures A.10c and A.11c, respectively), although focal variations are conserved. The quadratic filter may have adverse affects such as decreasing contrast in parametric radiation force images. Similar trends were observed by [68] in their analysis of the quadratic filter.

The relevance of the simulated data to in vivo data is evidenced by the in vivo unfiltered and filtered ARFI displacement profiles shown in Figure A.5. Note the similarity in the shape of the profiles to the simulated data profiles shown in Figure A.1.

In the analysis of the in vivo qualitative validation data (obtained in a FH pig pre-injection with normal physiological motion, post-injection with slowed motion, and post-mortem with no motion), single profile linear and quadratic with follow-on linear filters yielded ARFI peak displacement and recovery time images that were highly consistent with those of the long wave assuming polynomial with follow-on linear filter. In the peak displacement images of Figure 6, the post-mortem image with no motion
filter (right column, second row) shows a region of smaller peak displacement in the
distal arterial wall (boxed) between -10 to -2 lateral mm (arrow). This region of smaller
peak displacement is preserved in the case of linear filtering, quadratic with follow-on
linear filtering, and long wave assuming polynomial with follow-on linear filtering. All
three filters preserve the region of smaller peak displacement in the context of normal
(left column), slowed (middle column), and no (right column) physiological motion.
Importantly, obvious motion artifact (arrow) in the pre-injection, no filter image (left
column, second row) is removed by all three filters.

While the results reported in Figure 6 show little filter impact on peak displacement,
the results of Figure A.7 demonstrate that the quadratic filter diminishes measured
ARFI recovery times. The unfiltered, post-mortem ARFI recovery time image (right
column, first row) shows a region of prolonged time to recovery in the distal arterial
wall (boxed) between -10 to -2 lateral mm (arrow). All three examined filters retain
this region of prolonged recovery time; however, the quadratic plus follow-on linear
filter uniformly decreases measured time to recovery in the distal arterial wall across
the imaging FOV. In the pre-injection case, linear and long wave assuming polynomial
with follow-on linear filters yield recovery time measurements of ∼3-3.5 ms across the
distal vessel wall, while the quadratic with follow-on linear filter yields ∼2-3 ms recovery
times. This trend is also observed in post-injection and post-mortem cases, and it is
consistent with expectations. Obvious motion artifacts (arrows) that are apparent in
the no filter, pre- and post-injection images (left and middle columns, first row) are
removed by all three filters.

Figure A.8 looks more closely at the impact of motion filtering on individual ARFI
displacement profiles in the in vivo qualitative validation data. Pre-injection, the phys-
iological motion component is large relative to the magnitude of ARFI displacements.
Both profiles (from the left of the imaging FOV lateral mm, top, and the right of the
imaging FOV [23.2 mm, 8.6] lateral mm, bottom) show that all three filters perform similarly, but with the quadratic filter resulting in the smallest displacement magnitude. Similar trends are observed post-injection and post-mortem, where transducer motion may be present.

In the case of measured peak displacements for 2D ARFI for the in vivo NC example (Figure A.9), the unfiltered result differentiates the arterial wall (arrows). As with the recovery time images for this example, the single profile linear (panel b) and quadratic plus follow-on linear (panel c) as well as the long wave assuming polynomial plus follow-on linear (panel d) and PC plus follow-on linear (panel e) filters yield consistent peak displacement results, differentiating the arterial wall from surrounding tissue. In this case, the quadratic filter does not substantially distort measurement of peak displacement. The long wave assuming FIR plus follow-on linear filter (panel f) yields a lower overall peak displacement result, although the filter was able to differentiate the arterial wall clearly.

In the in vivo results for 2D ARFI recovery time in the NC example (Figure A.10), the unfiltered result shows inconsistency in peak displacement and recovery time values as well as poorly distinguished arterial wall from surrounding tissue (arrows). This is contrasted with the single profile linear filter (panel b) and the long wave assuming polynomial plus follow-on linear (panel d) and PC plus follow-on linear filters (panel e), which yielded more consistent peak displacement and recovery time results and clearer differentiation of the arterial wall (arrows). Notably, the long wave assuming filters resulted in greater spatial variability than the linear filter, especially in recovery time images. With no gold-standard, it is difficult to qualitatively determine the significance of this difference. As mentioned above, the quadratic filter provides similar results to the other highly performing filters, although with an overall decrease in the measured recovery time. To demonstrate the detrimental impact of poorly performing filters, the
long wave assuming FIR plus follow-on linear filter is presented in panel f. The FIR filter adversely affects the recovery time result, although some arterial wall differentiation is still possible.

In the case of in vivo SWEI imaging in the NC example, the unfiltered data shows severe distortion of shear wave velocity (Figure A.11, arrows). The linear (panel b), quadratic plus follow-on linear (panel c), long wave assuming polynomial plus follow-on linear (panel d) and long wave assuming PC plus follow-on linear (panel e) filters yielded a more consistently measured shear wave velocity and allowed clearer differentiation of the arterial wall from the surrounding tissue. The shear wave velocity for the linear, polynomial and PC filters is relatively consistent (7-8 m/s, arrows), while the quadratic filter results in a slightly higher shear wave velocity (8-9 m/s, arrows). The FIR filter appears to have removed sufficient data that a measurement of shear wave velocity is no longer possible.

By numerical metrics alone, one may conclude that the long wave assuming polynomial, PC and cPC regression filters subtly outperform the linear filter. However, the qualitative analysis of in vivo data demonstrates that subtle differences in performance metrics do not translate to meaningful differences in imaging outcomes. Long wave assuming filters resulted in ARFI and SWEI images that were highly similar by visual inspection to those generated after motion filtering with the linear filter alone. Given its computational efficiency, the linear filtered cannot be discounted.

A.5 Conclusions

Long wave assuming polynomial, PC and cPC filters with a follow-on linear filtered yielded the lowest SAD scores in our simulated data experiments. However, qualitative analysis of filtered in vivo 2D ARFI and SWEI images showed little performance difference between the single profile linear filter and long wave assuming polynomial, PC,
and cPC filters. Given the computational efficiency of the linear filter, it remains an attractive filtering option. This work shows that saving on computation time does not come at the expense of compromised performance. It should be considered, however, that this analysis has been limited to fairly simple arterial morphologies. Long wave assuming filters may offer a greater advantage over the linear filter in more complicated arterial morphologies, such as those associated with advanced atherosclerosis.
Appendix B

Reflected Wave Imaging

B.1 Introduction

ARFI ultrasound has been demonstrated for distinguishing soft lipid-rich or necrotic plaque regions, fibrous caps, calcium deposits, and other plaque components, both \textit{ex vivo} and \textit{in vivo} in previous chapters. Upon close inspection of the the examples shown in Figures 7.2-7.5, wave reflections were observed within both ARFI data sets. As waves propagate in tissue, they are reflected at mechanical impedance interfaces, with the strength of the reflection related to the difference in impedances. Due to the large differences in material mechanical impedance possible in atherosclerosis, there is a high probability of wave reflections in ARFI imaging of atherosclerosis. Thus we hypothesize that these shear wave reflections can be detected by tracking in the region of ARF excitation and that the reflections are indicative of nearby plaque components with different mechanical impedances.

B.2 Methods

B.2.1 ARFI and SWEI Imaging

ARFI data were acquired with a Siemens SONOLINE Antares™ imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division) equipped for research purposes and a VF7-3 transducer. Each ARFI ensemble consisted of 2 reference lines, a single excitation impulse, and then 60 tracking lines. Reference and tracking lines were 2 cycles in duration at 6.15 MHz acquired with 4:1 parallel receive. The excitation impulse was 300 cycles in duration at 4.21 MHz and F/1.5. Sixty ARFI ensembles were acquired, each spaced 0.35 mm, for a 2.1 cm lateral FOV. A spatially matched B-Mode image was acquired immediately preceding each ARFI acquisition. SWEI imaging was performed similarly to ARFI imaging, but with displacement tracking in positions lateral to a constant ROE.

B.2.2 Reflected Wave Imaging (RWI)

RWI was performed as an adjunct to conventional ARFI imaging. Shear and longitudinal wave reflections (WRs) were detected as inflections in median-filtered (0.4 ms time window) 1D axial displacement profiles measured in the ARF ROE. The amplitude of SWR was positive or negative depending on the difference in mechanical impedances at the reflecting interface:

\[ R_s = \frac{Z_1 - Z_2}{Z_1 + Z_2} \]  \hspace{1cm} (B.1)

where \( R_s \) is the reflection coefficient, \( Z_1 \) is the impedance of the incident medium, and \( Z_2 \) is the impedance of the transmission medium [77]. When the transmission medium has a higher mechanical impedance (\( Z_1 < Z_2 \)), \( R_s \) is negative. Parametric RWI images showing number of wave reflections were rendered.
B.2.3 Shear Wave Velocity Estimation

In RWI, shear waves induced in the ARF ROE travel laterally to the reflecting interface and then back to ROE, so distance calculations must consider round-trip travel in a manner analogous to a pulse-echo imaging system. Shear wave velocity (SWV) can be measured by RWI by two methods. First, if the reflecting interface is a known distance d from the ROE, RWI SWV can be measured as

\[ \frac{2d}{\Delta t} \]  

(B.2)

where \( \Delta t \) is the time after the ARF excitation the WR is observed in the ROE. Second, if the position of the reflecting interface is unknown, RWI SWV can be measured as the slope of the propagating reflected shear wave front across lateral distance and time:

\[ SWV_{RWI} = \frac{2\Delta d}{\Delta t} \]  

(B.3)

where \( \Delta d \) is the distance between two lateral positions in which the propagating reflected shear wave front is observed, and \( \Delta t \) is the time difference in the arrival of the reflected shear wave at those two lateral positions. In this paper, we have assumed that the position of the reflecting interface is unknown and calculated RWI shear wave velocity by equation B.3. Note that shear wave velocity measurement in SWEI does not involve round-trip shear wave propagation and thus does not include a factor of 2 in the numerator:

\[ SWV_{SWE} = \frac{\Delta d}{\Delta t} \]  

(B.4)

where \( \Delta d \) is the distance between two observed lateral positions, and \( \Delta t \) is the arrival time difference of the shear wave in the observed lateral positions.
B.2.4 Histology

Following imaging, arteries were aligned to the imaging plane and sectioned for spatially matched immunohistochemical analysis with assistance from a pathologist. Sections spaced 10-50 $\mu$m apart were stained with H&E for baseline, VK for calcium, MT for collagen, and VVG for elastin. Histological sections were then scanned with 20x microscopy for comparison with ARFI/SWEI result.

B.3 Results and Discussion

B.3.1 RWI versus SWEI in a Tissue Mimicking Phantom

Figure B.1 presents the results of a preliminary phantom experiment comparing RWI and SWEI SWV estimation. Figure B.1a shows a matched B-Mode image of a homogeneous ($\sim$48 kPa) phantom with a 3.5 mm glass bead inclusion. Figures B.1b and B.1c respectively show parametric RWI and SWEI images of displacement (color) given time versus lateral position. The lateral range in Figs. B.1b and B.1c is indicated by the white line in Fig. B.1a. A negatively reflected shear wave front is apparent in both the RWI and SWEI images (black lines), with the slope of the RWI reflected shear wave front approximately twice that of SWEI. The calculated SWVs for RWI and SWEI were $3.92 \pm 0.08$ m/s and $3.97 \pm 0.07$ m/s, respectively, showing good agreement between RWI and SWEI.

B.3.2 Left Iliac Artery with a Large Calcification, Ex Vivo

Figure B.2 demonstrates RWI in a 5 year, 10 month-old FH pig left iliac artery, ex vivo. The spatially matched immunohistochemistry (Figs. B.2a-B.2c) shows a large Type VII heavily calcified focal atherosclerotic plaque in the right half of the vessel with
large calcifications visible the VK stain (black arrows, Fig. B.2a). Additionally, a soft necrotic region (green arrow, Figs. B.2b-B.2c) with low collagen (Fig. B.2b) and elastin (Fig. B.2c) content is visible within the plaque, which appears to be covered by a fibrous cap. A spatially matched B-Mode image (Fig. B.2d) shows a large focal atherosclerotic plaque from -1 to 10 mm laterally with shadowing suggesting the presence of calcium; the adjacent vessel wall (-10 to -1 mm laterally) has no visible plaque.

A parametric image of ARFI PD (Fig. B.2e) shows that the necrotic region undergoes relatively large displacement (∼15 µm, green arrow, Fig. B.2e), but the calcified regions undergo relatively low displacement (1-5 µm, black arrows, Fig. B.2e). The spatially matched RWI image (Fig. B.2f) shows detected wave reflections (# of WRs, in color) in the necrotic region and in areas surrounding the calcium deposits. Representative 1D axial ARFI-induced displacement profiles measured in positions labeled
Figure B.2: RWI visualizes shear wave reflections within a heavily calcified plaque.
1-3 in Fig. B.2f are shown below the panel. In the necrotic region (1), the profile has two detected negative reflections (arrows). We hypothesize that these reflections are caused by the interfaces of soft necrotic cells with adjacent stiffer fibrous cap and calcium deposits. Profiles (2-3) also show negative shear wave reflections (black arrows), which we hypothesize are due to nearby calcium deposit interfaces.

Figure B.3 shows SWEI SWV results for this same vessel calculated using equation B.3 with $\Delta d$ and $\Delta t$ measured relative to the ROE. From approximately 1 to 5 mm laterally, the SWEI SWV is roughly 10 m/s; however, an abrupt change in SWV to roughly 5 m/s is observed from 5 to 10 mm laterally. It is probable that this change is not representative of the true tissue property but rather an artifact resulting from a reflected shear wave front. SWEI displacement profiles measured from 0 to 10 mm laterally showed a second peak $\sim$0.6ms following the initial induced peak, beginning in the ROE and traveling in the positive lateral direction (data not shown). We hypothesize that the second peak was a positive shear WR from the plaque-lumen interface on the left side of the plaque. As the initial and reflected shear waves propagated, they broaden and merge. At approximately 5 lateral mm, they were no longer distinguishable as two peaks, so the estimated $\Delta t$ was delayed, resulting in a lower estimated SWV. This result suggests that while both RWI and SWEI are relevant to measuring SWV, SWEI as implemented here may be susceptible to error from shear WRs.

**B.3.3 Right Iliac Artery with a Heterogeneous Plaque, *Ex Vivo***

Figure B.4 presents *ex vivo* RWI performed in a 5 year, 2 month-old FH pig right iliac artery with a large focal atherosclerotic plaque. The VK stain shows a small calcium deposit in the plaque (black arrow, Fig. B.4a). The MT stain shows collagen deposition, particularly at the position of the internal elastic lamina (Fig. B.4b). The VVG stain
shows minor degradation of the internal elastic lamina (Fig. B.4c). These stains also show highly heterogeneous composition in the circled portion of the plaque (Fig. B.4a-B.4c). Over the sectioning thickness (∼50-200 µm), what begins as an apparently small hole in the vessel wall (VK stain, circle, Fig. B.4a) grows into two larger holes (MT stain, circle, Fig. B.4b) and ends with a single, still larger hole with a thin cap (VVG stain, circled, Fig. B.4c). This region of heterogeneity is not apparent in the spatially matched B-Mode image (Fig. B.4d). However, it does appear in the ARFI PD image (Fig. B.4e) as a region of spatially variable peak displacements (circled, Fig. B.4e).

The heterogeneous composition of this region of the plaque is further identified in the RWI image (Fig. B.4f). Multiple WRs were detected, including more than three in the area labeled (2) and two in areas labeled (1) and (3). Representative 1D axial displacement profiles extracted from areas 1-3 show multiple reflections (arrows, panels (1-3)). This is especially evident in area (2), where several reflections are visible before the tissue recovers (panel (2)).

To more closely examine wave reflections in area (2), two parametric M-Mode style images are shown (Figs. B.4g-B.4h). Fig B.4h shows displacement in color given depth versus time (axial slice), recorded in the position of the white vertical line in (Fig. B.4e).
Figure B.4: RWI identifies longitudinal wave reflections in a disrupted plaque.
After the initial ARFI-induced displacement at \( t=0 \), a displacement wave propagates from \( \sim 16.5 \) axial mm down to \( \sim 18 \) axial mm, where it is reflected, and then back up to \( \sim 16.5 \) axial mm, where it is reflected again, etc. The amplitudes of the reflections are positive at \( \sim 18 \) axial mm (the bottom edge of the hole observed in Figs. B.4a-B.4c) and negative at \( \sim 16.5 \) axial mm (the luminal edge of the hole) (Fig. B.4g). This is consistent with a longitudinal wave reflecting at the interface of low-to-high mechanical impedance at \( \sim 18 \) axial mm and at the interface of high-to-low mechanical impedance at \( \sim 16.5 \) axial mm. Note that reflected longitudinal wave amplitude is the opposite sign of reflected shear wave amplitude [77]. These data suggest the presence of a relatively low mechanical impedance tissue structure between \( \sim 16.5 \) and 18 axial mm. These data also highlight that both shear and longitudinal wave reflections can be exploited to enhance delineation of tissue structures.

A parametric image of displacement (color) given time versus lateral position (lateral slice) recorded in the position of the white horizontal line in (Fig. B.4e) is shown in (Fig. B.4h). A positively reflected shear wave propagates from \( \sim 6.3 \) to \( \sim 8.5 \) lateral mm (arrow), suggesting the presence of a lower mechanical impedance tissue structure to the left of \( \sim 6.3 \) lateral mm. Combined with the data of panel (Fig. B.4g), we hypothesize that a soft, perhaps lipid-rich tissue region is position between approximately 16.5 to 18 axial mm and to the left of 6.3 lateral mm. This location spatially corresponds to the observed holes in the matched immunohistochemistry (Figs. B.4a-B.4c) and to the position of high PD in the ARFI image (Fig. B.4e).

### B.3.4 Left Iliac Artery with an Atheromatous Core, *In Vivo*

Figure B.5 shows RWI in an 8 year, 4 month-old FH porcine iliac left iliac artery, *in vivo*. The artery contains a Type V atheromatous plaque (black arrows, Figs. B.5a-B.5c) with an intact, thick fibrous cap and a small calcium deposit in the lower left
of the atheromatous core (green arrow, Fig. B.5a). The atheromatous nature of the plaque is not visible in the matched B-Mode image (Fig. B.5d), but its core appears as a region of heightened displacement (∼15 µm) in the ARFI PD image (white arrow, Fig. B.5e).

A RWI image of the # WRs (Fig. B.5f) shows an area spanning above to below the atheromatous core with two detected reflections (circled). Three reflections are detected in a smaller area (arrow). Axial and lateral slice images corresponding to the positions of the vertical and horizontal white lines, respectively, in the ARFI PD image (Fig. B.5e) provide further information regarding shear wave propagation. In the axial slice, a negative WR is evident at ∼24 axial mm, suggesting the presence of a tissue structure with relatively high mechanical impedance. The lateral slice image also shows a negative shear WR at ∼5-6 lateral mm, suggesting an interface with a tissue structure of relatively high mechanical impedance at ∼4 lateral mm. These data are consistent with the position of the calcium deposit observed in the matched immunohistochemistry (Figs. B.5a-B.5c). RWI measured a SWV of 5.0 ± 0.8 m/s in the atheromatous core.

**B.4 Conclusions**

RWI identified soft, necrotic or lipid-rich plaque regions near fibrous cap, calcium, and collagen deposits. RWI supports SWV estimation, which may be useful for quantitative ARF-based imaging. These pilot results suggest that RWI allows for material and structural discrimination of atherosclerotic plaques, *in vivo* and *ex vivo*, based on both shear and longitudinal WRs.
Figure B.5: RWI applied to an atheromatous plaque with calcification.
Appendix C

Reverberation Artifact Rejection and Luminal Masking in Arterial ARFI Imaging

C.1 Introduction

In arterial ultrasound imaging, interpretation of plaque geometry may be distorted by reverberation artifacts in the lumen. Although clutter artifact rejection has been extensively studied using frequency [78], blind source separation [79; 80; 81], harmonic imaging [82], and other methods, such clutter rejection approaches may not be effective for separating reverberation and wall signals because these signals tend to be correlated. However, their decorrelation rates vary. We hypothesize that variability in 1D axial cross-correlation (CC) and displacement measures can be exploited to reduce reverberation artifact while preserving wall signal in arterial ARFI imaging. To test this hypothesis, this study examines the performance of novel luminal masking and reverberation rejection methods by applying them to ARFI data sets acquired in a pig model.

C.2 Methods

C.2.1 ARFI Acquisition

All ARFI data sets were acquired with a Siemens SONOLINE Antares™ imaging system (Siemens Medical Solutions USA, Inc. Ultrasound Division) and a VF7-3 transducer. ARFI ensembles consisted of 300-cycle excitations at 4.21 MHz followed by 60 2-cycle track lines at 6.15 MHz. 40 ARFI ensembles were spaced 0.53 mm for a lateral span of 2.1 cm. A spatially matched B-Mode was acquired immediately preceding each ARFI acquisition.

C.2.2 Cross-correlation (CC) and Displacement Masks

Input Data and Theshholding

The reverberation rejection filters operated along the slow time dimension of 3D data matrixes of CC or displacement, considering only the last 30 time steps (3 ms). An illustration of the operation of these masks is shown in the flow chart of Figure C.1, with the example data shown taken along axial range at ∼4.2 lateral mm in panel C.1A (black box).

Variance of Time Derivative

For 3-D matrices of either CC or displacement (with matrix dimensions of axial range, lateral span, and slow time), the median (through slow time) CC and displacement values were calculated for each voxel. The variances of the 0th, 1st, or 2nd order slow time-derivative were also calculated. Note that these calculations produced 2-D data matrices. Panel C.1B shows normalized variance of 0th, 1st and 2nd order CC time derivatives versus axial range for a sample lateral position. The vertical axis is logarithmic. As the order of the time derivative increases, normalized variance decreases
Figure C.1: Flow chart for lumen filtering and reverberation detection approaches.
in the positions of the arterial walls, with the wall variance \( \sim 2 \) orders of magnitude lower in the 2nd versus the 0th order CC time derivative. In panel C.1B, the normalized variance of the 2nd CC time derivative is shown with the locations of the arterial walls (black), lumen (red), and reverberation (green) identified.

**Adaptively Determine Thresholds**

The median or variance values for each column in the resulting 2-D data matrices were sorted, and a threshold value was empirically determined for each column as a percentage of the values. While the percentage remained the same for each column, the threshold adapted to the relative variance of each ARFI ensemble. These adaptively determined thresholds were then applied column-wise to the 2D data matrices to either generate a binary rejection mask (direct mask, C.1C), or cluster the median or variance values (clustering mask, C.1D).

**C.2.3 Direct Mask**

**Apply Threshold and Result**

To apply the direct mask, the adaptively determined threshold values were applied to the 2D data sets column-wise, such that all pixels with median or variance values above the threshold were rejected as shown in panel C.1D. This had the effect of rejecting both lumen and soft tissue signals, as shown in panel C.1E.

**C.2.4 Clustering Mask**

**Cluster Variances**

As in the direct mask, the threshold was applied column-wise, but the remaining low median or variance pixels were then spatially clustered using 8-connected components.
with bwlabel in MATLAB (The Mathworks, Inc.). The cluster with the largest mean median or variance value was then considered to be the lumen. Therefore, the result of this clustering mask is referred to as lumen detection. The median axial index of the accepted pixels in each column was considered to be the centerline of the lumen. A sample cluster is shown in panel C.1F.

**Apply Threshold**

Using the vector of luminal centers, the algorithm then searched up and down from the center point in each column to determine the near and far boundaries of the lumen, as shown in panel C.1G.

**Lumen Masking Result**

The lumen identified in the previous step is rejected as shown in panel (H). As opposed to the direct masking result in panel C.1G, only the signal from $\sim 20$ to $26$ mm is rejected, and the soft tissue signal above $20$ mm is retained.

**Cluster Variances II**

While the above-described clustering mask isolates the arterial lumen, it rejects pixels corresponding to both reverberation and blood signal. However, reverberation pixels may be separated from blood pixels by repeating the first step in the clustering mask using a higher threshold (white arrow, panel C.1I).

**Reverberation Detection**

To then isolate reverberation pixels, the lumen masking result and cluster from the previous step are subtracted. The difference is the set of reverberation pixels (panel C.1J). Reverberation can then be identified in the corresponding ARFI peak displacement.
image as in panel C.1J, for example.

C.2.5 B-Mode Amplitude Thresholding Mask

In addition to the CC and displacement masks, a simple B-mode thresholding mask was also applied for comparison. The input data was a 2-D matrix of B-mode amplitudes as opposed to a 2-D matrix of median or variance used in the CC & displacement masks. The filtering operations were otherwise the same, with the mask applied both directly and with clustering.

C.3 Results and Discussion

All masks were applied to six NC porcine iliac arteries with no known atherosclerosis. Varying degrees of reverberation artifact were observed, with selected results presented in Figure C.2. The first column of Fig. C.2 displays B-Mode images spatially matched to ARFI images. The second column shows unmasked ARFI-induced peak displacement images with displacement shown in microns. Arterial walls generally exhibit small displacements (blue-green, ≈1 microns) and the lumen exhibits large displacements (yellow-red, ≈2+ microns). However, measured ARFI-induced displacements are lower in the positions corresponding to reverberation in the lumen.

The third and fourth columns show results utilizing the 2nd time-derivative of displacement for both direct (third column) and clustering (fourth column) masks. In the direct mask examples, both lumen and soft tissue were removed while retaining the arterial walls. In examples 1-5 (first through fifth rows), nearly the entire lumen is rejected, but so is a substantial amount of soft tissue. In the sixth example (sixth row), with particularly large reverberation artifact, both masking methods failed to reject the majority of lumen. All masking methods, including those not shown, performed
Figure C.2: Lumen masks and reverberation detection applied to 6 in vivo pig arteries.
poorly on this example due to the extent of the reverberation artifact.

The fourth column shows lumen detection (clustering) mask results. The most obvious difference between the clustering and direct mask results is that the clustering mask retains the soft tissue. While the clustering mask generally performed as well or better than the direct mask at isolating the lumen, this was not the case in the second (second row) and sixth examples. These examples highlight a limitation of the clustering mask. This limitation stems from the methods axial search from the identified lumen centerline for the first values below the adaptively determined threshold. If low median or variance values occur away from the arterial wall, the entire region between the position of the value and the wall will be retained (both lumen and reverberation artifact). Methods to overcome this limitation will be pursued in future work.

Lastly, the fifth column shows the reverberation artifact identified clustering. The isolated reverberation regions are generally spatially consistent with the observed locations of reverberation artifact in the B-mode images.

To quantify and compare masking performance, proximal arterial wall, distal arterial wall, and lumen regions were hand delineated in each image. The outcome of each masking method using varying threshold levels was then compared to the hand delineated regions. The highest performing mask was considered to be the mask that removed the largest amount of the lumen while retaining at least 98% of the pixels in the arterial walls. Note that neither soft tissue retention nor reverberation signal isolation are included in this quantitative analysis. The effectiveness of a subset of the examined masking methods is shown in Fig. C.3, including those that threshold based on B-mode amplitude, median CC, the variance of 2nd derivative of CC, and the variance of 2nd derivative of displacement. The performances of both direct and clustering masks are reported. Thresholding based on the variance of the 2nd derivative of CC and of displacement (with either direct or clustering masks) perform the best, followed
by the direct amplitude mask. Note that only thresholding based on the variance of 2nd derivative of displacement with a clustering mask was statistically significantly better than the amplitude-based direct mask (paired t-test, Table C.1). Interestingly, the clustering and direct masks performed comparably when thresholding based on the variance of the 2nd time derivative of either CC or displacement. Thus, the clustering mask yielded lumen rejection and reverberation artifact identification without soft tissue rejection. Lastly, thresholding based on the variances of 1st and 2nd derivatives of CC and displacement performed comparably with no statistically significant difference (data not shown).
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Table C.1: Paired T-Test Results (p<0.02 items bolded)
C.4 Conclusions

Thresholding based on the variances of the 1st and 2nd derivatives of CC and displacement for the clustering mask were found to be the most effective approaches for automatic isolation of lumen reverberation artifact without rejection of surrounding soft tissue. Clustering also allows for separation of reverberation signal from blood signal. This preliminary study suggests that temporal correlation and displacement information may be exploited to achieve reverberation rejection in arterial ARFI ultrasound. Due to it’s speed and ease of application, the variance of the 2nd derivative of displacement is used in Chapters 8-9 for reader evaluation of ARFI/SWEI image sets.
Bibliography


