Ultrasonic Methods for Quantitative Carotid Plaque Characterization

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Doctoral Thesis

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Preface

This thesis is submitted to the KTH Royal Institute of Technology and Karolinska Institutet in partial fulfillment of the requirements for the Doctoral degree in Technology (KTH Royal Institute of Technology) and the Doctoral degree in Medicine (Karolinska Institutet). The work has been performed with Associate Professor Matilda Larsson (KTH) as the main supervisor and Kenneth Caidahl from the Department of Molecular Medicine and Surgery, Karolinska Institutet, Solna as co-supervisor with Börje Ekholm, former CEO of Investor AB, as a mentor. The research project was supported with grants from the Swedish Heart Lung Foundation, the Knut and Alice Wallenberg foundation, the Swedish Research Council, Vinnova Vinnmer Marie Curie International Qualification, foundations managed by The Royal Swedish Academy of Sciences, and the Hans Wertén Foundation.

The thesis will be publically defended at 10 AM, October 13th 2016, in the lecture hall T2, Hälsövägen 11C, Huddinge, Sweden.
Abstract

Cardiovascular diseases are the leading causes of death worldwide and improved diagnostic methods are needed for early intervention and to select the most suitable treatment for patients. Currently, carotid artery plaque vulnerability is typically determined by visually assessing ultrasound B-mode images, which is influenced by user-subjectivity. Since plaque vulnerability is correlated to the mechanical properties of the plaque, quantitative techniques are needed to estimate plaque stiffness as a surrogate for plaque vulnerability, which would reduce subjectivity during plaque assessment.

The work in this thesis focused on three noninvasive ultrasound-based techniques to quantitatively assess plaque vulnerability and measure arterial stiffness. In **Study I**, a speckle tracking algorithm was validated *in vitro* to assess strain in common carotid artery (CCA) phantom plaques and thereafter applied *in vivo* to carotid atherosclerotic plaques where the strain results were compared to visual assessments by experienced physicians. In **Study II**, hard and soft CCA phantom plaques were characterized with shear wave elastography (SWE) by using phase and group velocity analysis while being hydrostatically pressurized followed by validating the results with mechanical tensile testing. Thereafter, the phantom plaques were characterized throughout a simulated cardiac cycle. In **Study III**, feasibility of assessing the stiffness of simulated plaques and the arterial wall with SWE was demonstrated in an *ex vivo* setup in small porcine aortas used as a human CCA model. Additionally, SWE settings were optimized to maximize the shear wave bandwidth with respect to acoustic radiation force push length and number of compounded angles used for motion detection with plane wave imaging. In **Study IV**, SWE and pulse wave imaging (PWI) were compared when characterizing homogeneous CCA soft phantom plaques using phase and group velocity analysis as well as estimating the pulse wave velocity.

The techniques developed in this thesis have demonstrated feasibility to characterize carotid artery plaques and assess arterial wall stiffness *in vitro*, *ex vivo*, and *in vivo*. The results show that the techniques have the ability to noninvasively evaluate the mechanical properties of carotid artery plaques, provide additional data when visually assessing B-mode images, and potentially provide improved diagnoses for patients suffering from cerebrovascular diseases. However, additional development of the techniques is needed including a large scale *in vivo* clinical study.

**Keywords:** Atherosclerosis, Cardiovascular Disease, Carotid Artery, Elasticity, *ex vivo*, *in vivo*, Phantom, Plaque, Polyvinyl Alcohol, Pulse Wave Imaging, Shear Wave Elastography, Speckle Tracking, Stroke, Ultrasound
Sammanfattning

Hjärtkärlsjukdomar är den vanligaste dödsorsaken i världen och förbättrade diagnosmetoder behövs för att förse patienter med den mest lämpliga behandlingen. För närvarande uppskattas risken för stroke genom att visuellt bedöma plack i halskärl med hjälp av visuell analys av ultraljudsbaserade gråskalebilder, vilket är en subjektiv uppskattning av klinkern. Placks mekaniska egenskaper är korrelerade med risken för att ett plack ska brista, vilket kan leda till stroke. Därför behövs kvantitativa metoder för att uppskatta plackets mekaniska egenskaper.


Teknikerna som har utvecklats i denna avhandling har visat möjligheten att karakterisera plack i halspulsådern och bedöma stelheten av kärlväggen in vitro, ex vivo och in vivo. Resultaten visar att teknikerna har förmågan att icke-invasivt estimera de mekaniska egenskaperna i aterosklerotiska plack och minska subjektiviteten i den visuella bedömningen av B-mode bilder samt potentialen att förbättra diagnoserna till patienter med cerebrovaskulära sjukdomar. Det behövs dock ytterligare utveckling av teknikerna, inklusive en stor in vivo klinisk studie för att validera teknikernas kliniska effektivitet.
How did I end up here? That is the question I keep pondering while reflecting upon my PhD years. It has been a long journey, leaving the musician lifestyle in Chicago and trading it in for countless hours in the lab in Stockholm. It’s been a fantastic experience, which has not only taught me about medicine and science, but has also taught me about what I value in life and who I aspire to become.

In hindsight, the most important lesson I’ve learned from trying to develop diagnostic techniques for cardiovascular diseases is that as a society we should focus more resources and energy on preventive medicine. This includes providing tax incentives for healthy food, making exercise facilities accessible for all socioeconomic groups, and promoting health education continuously throughout a person’s life. No single solution is a silver bullet, but I believe making small improvements in many areas can significantly reduce the morbidity rates for cardiovascular disease, diabetes, and cancer as well as improve the quality of life during the later years in life. Sometimes the best solutions aren’t as complicated as we think.

This journey would not have been possible without the love and support of many people. First, I would like to thank my fiancé and the love of my life Anna-Mi. I was lucky enough to meet you at the beginning of my PhD, and I don’t think I would have made it without you. You challenge me and inspire me to be a better person. I wouldn’t have taken as many risks, which led to so many great things without your support. I love you.

The other pillar in my life is my mother. Mom, thanks for believing in me and giving me so many opportunities in life. Did you ever think that all that time invested in “Saturday morning homework” was going to result in a dual PhD? You’re early academic investments have significantly appreciated in value over the years! Thanks for always being there and letting me run around the world and do my thing. I love you.

I’ve been so lucky to be surrounded by great people throughout my entire PhD, and I would like to highlight a few in particular. I was lucky enough to be paired with an Albanian/Italian girl with crazy curly hair on my first day as a PhD. Elira, thanks for pushing me and making me a better scientist. I know I am a pain in the ass and stubborn as a mule at times, but thank you for putting up with me. I’ll never forget our crazy trips around the world and thank you for letting my visit you home in Italy. I think Italy has become my third home…mamma mia!

I’d like to thank my main advisor Matilda for always taking the time to talk to me and giving me the freedom to pursue my academic and extracurricular interests. I feel like I could not have had a better

Acknowledgments
advisor at KTH. It feels like a long time since we first met at that coffee shop on Södermalm. Thank you for taking a chance on me and letting me be part of the team. It changed my life and took me to places I never expected to go. I hope you’re as proud as I am of what we have built at STH.

I would like to thank my co-supervisor Kenneth for teaching me so much about atherosclerosis and stroke. I won’t forget the many interesting conversations we have had over the years. Peter Arfert is my unsung hero in the workshop. None of the experiments in this thesis would have been successful without your skilled craftsmanship. I would like to thank my mentor Börje Ekholm for taking the time to meet with me and provide career advice and opening my mind to opportunities beyond the academic world. I’d also like to thank all other great co-workers that I’ve had the pleasure of working with over the years; you know who you are. The school’s (and our department in particular) largest asset is the good people working there. You rock!

I’d like to thank all the people who I have worked with in the KTH PhD Chapter. It was truly rewarding to be chairman and I think the leadership experience changed my career path and future.

I would like to thank the Hans Wertén Foundation and the Royal Swedish Academy of Sciences for giving me the opportunity to spend part of my PhD abroad at one of the greatest universities in the world. Columbia University is an amazing place in the greatest city in the world and I had the opportunity to meet people from all over the world who pushed me hard to learn more about science, management consulting, and financial investments. It was a unique experience I’ll never forget.

I also need to give a shout out to Matt Urban at the Mayo Clinic who has taught me so much about shear wave elastography over the years. It was a fantastic experience to come and work in your lab and thank you to all the good people at Mayo that I worked with. Another international thank you goes out to Jan D’hooge at KU Leuven who helped me in the early stages of my PhD with speckle tracking.

Thanks to my friends in Stockholm. What would life be without friends? It’s been great to reconnect with you the past couple of years.

Last but not least, I’d like to thank my family. Kent, thanks for keeping Eight Bit Tiger going and writing songs when I’ve been too busy to pitch in. It’s been a blast playing shows with you around the world the past decade. Let’s keep it going another 65 years. Lena, you’re still my meal ticket. Keep saving lives and helping people! Elizabeth, thanks for teaching me about the scary things that live in the ocean. It’s safe to say that I will not venture into the deep end of the pool any time soon. Dad, thanks for being there over the years and getting several of us kids interested in medicine.

So what’s next? If you asked me this question five years ago my answer would not have been “return to Sweden to pursue a dual PhD”, so I no longer dare make any bold predictions about the future. Let’s keep a couple of pages blank in the storybook of life so we can fill them with some whimsical tales of action, adventure, and excitement. After all, you always save the best song for the encore...

Time to go put all this education to use...LET’S DO IT!

Erik Widman
Stockholm, October 2016
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>1D</td>
<td>One-dimensional</td>
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<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>4D</td>
<td>Four-dimensional</td>
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<tr>
<td>ARF</td>
<td>Acoustic Radiation Force</td>
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<tr>
<td>ARFI</td>
<td>Acoustic Radiation Force Impulse</td>
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<tr>
<td>B-mode</td>
<td>Brightness Mode</td>
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<td>CCA</td>
<td>Common Carotid Artery</td>
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<td>CTA</td>
<td>Computed Tomography Angiography</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>GPU</td>
<td>Graphics Processing Unit</td>
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<td>GSM</td>
<td>Gray-scale Median</td>
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<td>HDL</td>
<td>High-Density Lipoprotein</td>
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<td>HU</td>
<td>Hounsfield Unit</td>
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<td>ICA</td>
<td>Internal Carotid Artery</td>
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<td>IVUS</td>
<td>Intravascular Ultrasound</td>
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<td>LDL</td>
<td>Low-Density Lipoprotein</td>
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<td>MI</td>
<td>Mechanical Index</td>
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<td>Abbreviation</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>pPWI</td>
<td>Piecewise Pulse Wave Imaging</td>
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<td>PWI</td>
<td>Pulse Wave Imaging</td>
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<td>PWV</td>
<td>Pulse Wave Velocity</td>
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<td>RF</td>
<td>Radio Frequency</td>
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<tr>
<td>RMSE</td>
<td>Root Mean Square Error</td>
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<td>ROI</td>
<td>Region of Interest</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SNR</td>
<td>Signal to Noise Ratio</td>
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<td>SWE</td>
<td>Shear Wave Elastography</td>
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<td>TDI</td>
<td>Tissue Doppler Imaging</td>
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<tr>
<td>TI</td>
<td>Thermal Index</td>
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<tr>
<td>TOF</td>
<td>Time of Flight</td>
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<tr>
<td>TSI</td>
<td>Thermal Strain Imaging</td>
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1 Introduction

Cardiovascular diseases (CVDs) are the leading causes of death worldwide attributing to over 17 million annual deaths according to the World Health Organization (WHO) [1]. CVDs make up 48% of deaths caused by Noncommunicable Diseases, which is more than cancer, respiratory disease, and diabetes deaths put together. CVDs due to atherosclerosis include ischemic heart disease or coronary artery disease, cerebrovascular disease (e.g. stroke), and arterial diseases such as hypertension and peripheral vascular disease. The CVDs due to cerebrovascular disease account for approximately one third of the cardiovascular deaths and is second to ischemic heart disease (41%).

Atherosclerotic disease of the carotid arteries has been associated with the development of cerebrovascular events due to rupture of carotid plaques [2]. Cerebrovascular disease is due to the cumulative effect of several risk factors that include tobacco and alcohol use, physical inactivity, unhealthy diet, hypertension, obesity, diabetes, raised blood lipids, and genetic disposition where the slow progression of thrombotic atheroma plaque formation can take up to 20 years [3]. Carotid artery plaque formation is a complex process that does not follow a single pathway, making it possible to develop several different types of plaques. Moreover, plaque formation evolves through the progression of atherosclerosis from fatty necrotic, to mixed, to fibrous core [3]. Physicians are interested in identifying vulnerable plaques, which often have a necrotic core and a thin fibrous cap [4], as they are most likely to result in thrombosis; a blood clot obstructing the flow of blood through the circulatory system.

In current clinical practice, ultrasound duplex scanning is typically the first level of screening for atherosclerotic plaques [5]. Ultrasound is a safe and low-cost imaging modality that is widely accessible in clinics around the world. Therefore, it is the preferred first level screening method compared to computed tomography angiography (CTA) or magnetic resonance imaging (MRI). The Society for Vascular Surgery recommends a one-time ultrasound vascular screening for all men 65 years or older and screening men as early as 55 years is appropriate for those who meet the aforementioned cardiovascular risk factors [6]. The carotid artery intima-media thickness is used to identify systemic atherosclerosis and identify individuals at risk for future cardiovascular events [7-11], whereas the degree of stenosis and plaque morphology are typically assessed to determine the therapeutic approach [12-14]. Several investigations [4, 15, 16] have demonstrated that the degree of luminal stenosis is only an indirect indicator of the atherosclerotic process and that direct assessment of the mechanical properties of the plaque may be key to predict the development of future cerebrovascular ischemic events as well as determine the most suitable treatment (endarterectomy, angioplasty, or medication) [17] for the patient.
Currently, clinical non-invasive ultrasound-based methods for plaque characterization are limited to visual assessment of plaque morphology, hypoechoic area, and echo reflection in the plaque [13, 18]. There have been quantitative plaque characterization attempts by grey-scale median (GSM) measurements of the plaque [2, 19, 20] as well as using contrast-enhanced ultrasound imaging in combination with Tissue Doppler imaging (TDI) [21], however both techniques suffer from various limitations and conflicting studies [22-25]. Thermal Strain Imaging (TSI) has also been used in vivo to detect lipids in atherosclerotic plaques in the femoral arteries of rabbits [26] but the technique is not ready for clinical use. Several successful plaque characterization studies have been completed using intravascular ultrasound (IVUS) [27-29]. Moreover, non-invasive vascular elastography has also been performed with ultrasound [30, 31]. Another promising technique is acoustic radiation force impulse (ARFI) imaging, where ultrasound radiation force excites the tissue followed by measuring the tissue's axial displacement (< 10 µm). Pilot studies to characterize carotid plaques have been performed [32-38], but ARFI is a relative displacement measurement, which limits the evaluation of plaque stiffness to surrounding proximal tissue. This implies that plaques on opposing anterior and posterior arterial walls cannot be compared to each other. Additionally, it’s difficult to compare ARFI measurements between patients. Despite previous efforts, further improvements are needed to quantitative methods to characterize plaque without user-subjectivity influence.

This thesis will focus on the application of three ultrasound-based techniques to take steps toward improved plaque characterization methods for the prevention of cerebrovascular events by quantifying and assessing the mechanical properties of plaque and the carotid artery wall. The techniques include measuring the plaque strain throughout the cardiac cycle via speckle tracking on B-mode images as well as measuring the shear modulus and assessing the velocity of the pulse wave propagation of simulated carotid artery plaques using shear wave elastography (SWE) and pulse wave imaging (PWI).

1.1 Thesis Outline
This thesis is based on the work described in four studies, the first focusing on the application of strain assessment by speckle tracking to characterize plaque phantoms and in vivo carotid artery plaque. Studies II and III focus on characterizing plaque phantoms using SWE, validating a SWE algorithm for arterial stiffness estimation, and characterizing simulated plaques in porcine arteries in an ex vivo setup. Study IV compares SWE and PWI when characterizing homogeneous soft plaques in a phantom setup.

The thesis is organized into 12 chapters, starting with an introduction followed by the aims for each study, list of included papers, and other scientific contributions. Next, a summary is provided of the cardiovascular system followed by an overview of the principles of ultrasound as well as a description of techniques used to characterize plaques. Thereafter, Chapter 8 outlines the methods used in this thesis followed by a summary of the results, a general discussion, and a conclusion (Chapters 9-11). Future work is discussed in Chapter 12 followed by the references. The full versions of Studies I-IV are provided in the appendix.
The general aim of this thesis was to develop, demonstrate feasibility, and evaluate the potential of three ultrasound-based methods applied to the characterization of carotid artery plaques and assessment of arterial stiffness \textit{in vitro}, \textit{ex vivo}, and \textit{in vivo}. The specific aim of each study was as follows.

**Study I**
To validate a previously developed speckle tracking algorithm \textit{in vitro} to assess strain in common carotid artery (CCA) phantom plaques and thereafter applying the speckle tracking algorithm to \textit{in vivo} carotid atherosclerotic plaques and compare the quantitative strain results with visual assessments by experienced physicians.

**Study II**
To characterize hard and soft CCA phantom plaques with SWE by using phase and group velocity analysis while hydrostatically pressurizing the phantoms followed by validating the results with mechanical tensile testing. Thereafter, the phantom plaques were subjected to a dynamic environment to test feasibility of characterizing the plaques throughout a simulated cardiac cycle.

**Study III**
To demonstrate feasibility of assessing the stiffness of simulated plaques and the arterial wall in small porcine aortas used as a human CCA model with SWE in an \textit{ex vivo} setup as well as to optimize the SWE settings by maximizing the shear wave bandwidth with respect to acoustic radiation force (ARF) push length and number of compounded angles used for motion detection with plane wave imaging.

**Study IV**
To compare SWE and PWI when characterizing homogeneous CCA phantom plaques by using phase and group velocity analysis as well as estimating the PWV using PWI. Additionally, the Young’s moduli calculated from the PWV, group velocity, and phase velocity were compared in the phantom walls.
3 List of Included Papers

The thesis is based on the following four papers with full versions of the papers attached as appendixes.


3.1 Division of Labor

Paper I: \textbf{EW, ML, KC} participated in the initiation and design of the study. \textbf{EW, ML, BH, JD} participated in algorithm design. \textbf{EW, KC, ML} participated in data collection and analysis of the results. All authors read and approved the final manuscript.

Paper II: \textbf{EW, ML} participated in the initiation and design of the study. \textbf{EW, EM, DL, MWU} participated in algorithm design. \textbf{EW, EM, MWU, ML} participated in data collection and analysis of the results. All authors read and approved the final manuscript.

Paper III: \textbf{EW, ML, MWU} participated in the initiation and design of the study. \textbf{EW, EM, CA, MWU} participated in algorithm design. \textbf{EW, MWU, ML} participated in data collection and analysis of the results. All authors read and approved the final manuscript.

Paper IV: \textbf{EW, ML} participated in the initiation and design of the study. \textbf{EW, MWU, IZA} participated in algorithm design. \textbf{EW, IZA, PN, EK} participated in data collection and analysis of the results. All authors read and approved the final manuscript.
4 Other Scientific Contributions

4.1 Peer-Reviewed Papers

4.2 Conference Proceedings


4.3 Conference Contributions


4.4 Honors and Awards

2015 The Royal Swedish Academy of Sciences Engineering Sciences Scholarship

2014, 2015 Hans Werthén Foundation Scholarship

2013 Best Conference Presentation: The Swedish National Meeting for Biomedical Engineering

2012 Euroecho & other Imaging Modalities High Score abstract

2012, 2013 Knut and Alice Wallenberg Foundation Travel Scholarship

2012 Swedish Heart-Lung Foundation Travel Scholarship
5 The Cardiovascular System

This chapter provides an overview of the cardiovascular system’s anatomy and function as well as the most common CVDs. The focus of the thesis is atherosclerosis and cerebrovascular diseases.

5.1 Vascular Anatomy and Function

The cardiovascular system is composed of the heart, lungs, brain, kidneys, arteries, capillaries, veins, coronary vessels and portal veins. One of the most important functions of the cardiovascular system is the pulmonary circulation, where the blood is circulated from the right ventricle of the heart to the lungs to oxygenate the blood. Thereafter, the blood travels back to the heart through the left atrium followed by the left ventricle and is pumped through the aorta and is distributed throughout the body via the arteries, arterioles, and capillaries where it oxygenates surrounding cells. The deoxygenated blood travels back to the right atrium through the veins to complete the circulation loop. It is estimated that the blood vessels in the cardiovascular system have a total length of approximately 100,000 km [39] and that an adult contains approximately 4.7-5.7 liters of blood [40]. Moreover, it takes blood approximately one minute to travel through the circulation system.

Arteries are composed of tunica adventitia, tunica media, and tunica intima. The outermost layer, tunica adventitia, is composed of connective tissue made up of collagen fibers. The middle layer, tunica media, consists of smooth muscle cells and elastic tissue. The tunica media is the thickest layer and is the principal determinant of the arteries mechanical properties [41]. The innermost layer of the artery is a thin layer called the tunica intima composed of endothelial cells that are in direct contact with blood that flows through the artery lumen. At a young age, the tunica intima does not contribute to the mechanical properties of the artery, but over time the tunica intima thickens and becomes stiffer [42]. A cross-section of the internal structure of the artery can be seen in Figure 5.1.

The largest and most elastic arteries include the aorta and pulmonary arteries. During the systolic part of the cardiac cycle, left ventricle ejection causes a pressure increase in the aorta and connecting arteries, resulting in an expansion of the artery volume often referred to as compliance. The expansion of the artery can have a large effect on the blood pressure. The compliance of the arteries helps dampen the fluctuation of blood pressure over the cardiac cycle and is analogous to a capacitor. Arteries naturally stiffen with age, leading to a reduction of arterial compliance, which in turn increases the systolic blood pressure for a given stroke volume (hypertension). Hypertension predicts CVDs including myocardial infarction, stroke, and heart failure. Small arteries and arterioles account for the largest part of resistance in the vascular system. The arterioles adjust their diameter to regulate the blood flow.
distributed to the capillaries, thereby functioning as a resistive vessel. Red blood cells are stripped of oxygen and nutrients for the surrounding tissues in the capillaries and thereafter the blood is transported back to the heart via the veins [39].

5.2 Cardiovascular Diseases
CVDs are the leading causes of death worldwide [1]. CVDs due to atherosclerosis include ischemic heart disease, cerebrovascular disease, hypertension, and peripheral vascular disease. The focus of the thesis is atherosclerosis and cerebrovascular diseases.

5.2.1 Atherosclerosis
Atherosclerosis is a specific form of arteriosclerosis, which is the thickening and hardening of the arterial walls. The atherosclerotic process occurs naturally with age, but can be onset by the cumulative effect of several risk factors that include physical inactivity, tobacco and alcohol use, unhealthy diet, raised blood lipids, genetic disposition, hypertension, and diabetes [1, 3]. Atherosclerosis is an inflammatory process that occurs in medium to large arteries, which ultimately leads to the narrowing of the arteries from the buildup of plaque [44]. If the endothelium in the tunica media is exposed to high levels of low-density lipoprotein (LDL) cholesterol, small dense LDL particles can pass between the endothelial cells. Inside the arterial wall the LDL particles are more prone to oxidation. The endothelial cells respond by attracting lymphocyte and monocyte white blood cells from the blood stream, which penetrate into the arterial wall. The monocytes overwhelm the LDL particles and transform into macrophages. The oxidation of LDL particles by free radicals triggers an immune response, which can produce an atheroma over time if high-density lipoprotein (HDL) removal of fat from the macrophages does not keep up. The macrophages and lymphocytes absorb the oxidized LDL, forming
Plaque formation through the progression of atherosclerosis and possible end states of the disease. Reproduced with permission granted by the Creative Commons Attribution-ShareAlike 3.0 license.

If the foam cells cannot process the oxidized LDL and require HDL to remove the fats, they grow in size and eventually rupture, leaving behind oxidized materials, and fats in the artery wall. This creates a snowball effect by attracting more white blood cells to the area and continues the cycle of the inflamed artery. Over time, a fibrous cap consisting of smooth muscle and collagen is formed and the macrophages and foam cells begin to die, resulting in the formation of a necrotic core covered by the fibrous cap, known as an atheroma or plaque [45]. An illustration of the plaque formation process can be seen in Figure 5.2 with the various end states: critical stenosis, thrombosis, and aneurysm.

The progression of atherosclerosis is a slow process that occurs over decades and begins as early as childhood. The disease can be asymptomatic for decades as the arteries enlarge at all plaque locations, creating little effect on blood flow [44]. Plaques can form in the coronary arteries, leading to myocardial infarction. Plaques that form between the carotid arteries and brain can lead to cerebrovascular diseases such as stroke.

5.2.2 Cerebrovascular Diseases
Plaque composition evolves throughout the progression of atherosclerosis from fatty necrotic, to mixed, to fibrous core [3]. A plaque that is likely to rupture is referred to as a vulnerable plaque, which is characterized by surface ulcerations, a thin fibrous cap, necrotic or lipid core, intraplaque hemorrhage, and neovascularization [46, 47]. Vulnerable plaques are of particular interest as they are more likely to rupture, causing thrombosis, which can lead to ischemic stroke through occlusion if the plaque is located in the carotid artery or further down the arterial tree. Alternatively, emboli from the thrombus can drift further down the circulatory system to narrower blood vessels, resulting in transient ischemic
attack or embolic stroke [45]. Other cerebrovascular diseases include hemorrhagic stroke, which occurs when a weakened artery ruptures, either through an aneurysm or arteriovenous malformation [48]. Aneurysms can occur near a plaque as part of the artery wall is exposed to greater pressure, which can result in a wall rupture such as the one in Figure 5.2. Additionally, plaques prevent blood from penetrating the blood vessels in the artery wall to oxygenate the cells resulting in ischemia and atrophy, increasing the risk for aneurysm.
6 Ultrasound Imaging

6.1 Principles of Ultrasound
Ultrasound refers to sound pressure waves that extend beyond the audible human range of hearing (greater than 20 kHz) and medical ultrasonic devices typically operate from 1 to 70 MHz. Ultrasound is used to detect material flaws in nondestructive testing applications, used for cleaning and mixing in industrial applications, and used in nature by porpoises and bats to locate prey and obstacles. To the common man, ultrasound is primarily associated with prenatal sonography, but medical applications of ultrasound extend well beyond fetal ultrasound imaging and include visualizing organs and tissues for diagnosing different diseases as well as therapeutic applications of ultrasound.

In medical ultrasound imaging systems, the sound pressure wave is transmitted and received by a transducer. The waves are generated by exciting piezoelectric crystals with a voltage, which in turn deforms the crystals and creates pressure waves that couple to the tissue through ultrasound gel and propagate through the tissue. The waves travel through the tissue and partially reflect back to the transducer when they encounter two tissues of different acoustic impedance ($Z$) \[ Z = \rho c \] (6.1)
where $\rho$ is density and $c$ is the speed of sound of the tissue. The amplitude of the reflected wave is dependent on the difference in acoustic impedance between the two tissues, where a large difference results in a large reflected wave and a small difference results in a small reflected wave. The location at which the reflected wave occurs can be calculated by measuring the time of flight (TOF) between the transmission of the ultrasound wave and reflected echo. This is referred to as the pulse-echo technique. By transmitting several pulse-echoes, a brightness mode (B-mode) image can be created where the echo amplitude corresponds to the pixel intensity and the TOF determines the pixel location in the image. The speed of sound is determined by the density and stiffness of the tissue, but the variation in speed of sound is very small in soft tissues and is typically assumed to be constant at 1540 m/s [50]. As the wave propagates through the tissue it is continually absorbed, leading to weaker reflections, which results in darker B-mode images at greater depths. This is typically corrected electronically by increasing the amplitude of echoes further away from the transducer. Moreover, scattering of the propagating wave occurs as it travels through the tissue and encounters an irregular surface or if the investigated object is smaller than the wavelength of the transmitted wave. The scattered echoes interfere with each other causing constructive and destructive wave interference, resulting in a fluctuating wave pattern known as a speckle pattern, which can be considered a form of acoustic noise in the image.
The most common clinical transducers include linear and phased array transducers. Linear arrays typically operate in the 4-15 MHz range and benefit from superior image quality, while phased array transducers operate in the 1-3 MHz range and can image a larger area and deeper depths. Ultrasound machines can acquire 2D (static images), 3D (moving cineloops), and 4D (3D cineloops) B-mode images. While B-mode is the most common imaging modality, M-mode (motion mode) and Doppler mode (measures blood flow) are also frequently used.

The level of detail provided by the ultrasound system is determined by the spatial and temporal resolution. The in-plane spatial resolution is composed of axial and lateral resolution and determines the ability of the ultrasound system to resolve adjacent objects. The elevational resolution is the ability to distinguish imaging planes lying behind or in front of the imaged plane and is dependent on imaging frequency and beam geometry. The axial resolution (along the axis of the beam) is determined by the frequency of the transmitted wave, where higher frequencies can resolve smaller objects, but cannot propagate as deep into the tissue as low frequency waves. Additionally, the axial resolution can be improved by shortening the transmission pulse by decreasing the number of transmitted cycles. The lateral resolution (perpendicular to the beam) is determined by the imaging depth and the width of the transmission beam, also known as the aperture, where a narrower beam increases the lateral resolution and is dependent on the number of excited piezoelectric crystals. The temporal resolution is determined by the frame rate (the number of images that can be acquired per second) of the ultrasound system and a high frame rate is needed to detect fast moving objects. The frame rate can be increased by imaging at shallower depths or by e.g. selecting fewer scan lines per frame.

In linear arrays, B-mode images are constructed using the pulse-echo method by exciting a small group of piezoelectric crystals in the array (creating a focused beam by using electronic time delays), waiting for the reflected echoes, thereafter exciting the next small group of piezoelectric crystals and repeating this process for the length of the array. The time to construct the image \( T_{image} \) is

\[
T_{image} = \frac{2Nd}{c}
\]  

where \( N \) is the number of scan lines in one image, \( d \) is the depth, and \( c \) is the speed of sound. The maximum frame rate \( FR\_{Max} \) is

\[
FR\_{Max} = \frac{1}{T_{image}}
\]  

For a linear array with 128 crystals imaging at 4 cm depth, the maximum frame rate would be approximately 150 Hz. The frame rate can be drastically increased by exciting all the piezoelectric crystals in the array in parallel, creating a full image from one single transmission. This type of imaging is referred to as ultrafast imaging or plane wave imaging and enables measuring tissue motion with frame rates in the kilohertz range [51]. Technology breakthroughs such as increased computing speeds and powerful graphic processing units (GPUs) have enabled this type of parallel processing with a satisfactory level of performance, which was previously not possible to achieve. A disadvantage with plane wave imaging is lower image quality [52], which mainly affects the image contrast rather than the resolution [51]. However, the loss in image quality is acceptable when tracking shear or pulse waves as the propagating waves are tracked based on phase differences in the radio frequency (RF) signal and not from the B-mode image [51, 53, 54].
To overcome the limitation of reduced image quality and signal to noise ratio (SNR) when using plane wave imaging, several plane waves are typically transmitted into the tissue at different angles and thereafter coherently summed to reconstruct the image. This method is referred to as coherent compounded imaging [54]. The focalization step, which is typically done during the transmission phase for regular B-mode imaging, is retrospectively created during summation of the compounded imaging. Therefore, the image quality is dependent on the number of compounded angles used to reconstruct the image and a trade-off is made between the maximum plane wave imaging frame rate and the image quality, where more imaging angles increases the image quality.

Three noninvasive ultrasound-based techniques have been applied in Studies I-IV to quantify the mechanical properties of simulated in vitro and in vivo carotid artery plaques. The techniques included in this thesis are SWE, speckle tracking, and PWI.

6.2 Shear Wave Elastography

The objective of medical elastography is to detect hard or soft tissue to diagnose the presence or severity of a disease. Historically, palpation has been used as early as 1500 BC to detect abnormalities in tissues by searching for hard masses within organs. However, palpation suffers from several limitations: the technique can only be applied to superficial tissues and organs, tissues surrounding the investigated organ may distort the examination, and it is a qualitative technique that is subject to individual interpretation by the physician. Medical elastography techniques aim to address these limitations.

The basic principle of elastography is to apply an external static force and measure the deformation of the tissue from which the Young’s modulus can be calculated. Hard tissues have a larger Young’s modulus compared to softer tissues and therefore tissues of different pathological states can be detected. If a transient impulse force is applied, rather than static compression, compressional and shear waves will be generated in the tissue which are associated with the medium’s bulk and shear moduli, respectively [55, 56]. Compressional waves propagate very quickly (1540 m/s) in the tissue in the direction of the applied transient impulse whereas shear waves propagate much slower (1-10 m/s) by comparison in the orthogonal direction to the applied transient impulse. Moreover, there is little variation between the bulk modulus of tissues whereas the shear modulus varies greatly between different biological tissues [57].

Ultrasound is one of several imaging modalities that can be used for elastography. SWE is an imaging modality that uses the shear modulus of the tissue as a contrast mechanism. Instead of applying an external transient impulse, SWE uses an ARFI to noninvasively generate shear waves inside the investigated tissue (Figure 6.1(a)). The waves are imaged using high speed plane wave imaging (Figure 6.1(b)) and tracked with cross-correlation techniques where the propagation speed of the shear wave is proportional to the tissue stiffness. In large uniform tissues it is assumed that the shear wave propagates in a homogeneous, incompressible, isotropic, linear elastic material [57] and the shear modulus (μ) and Young’s modulus (E) are calculated as

\[ \mu = \rho c_g^2 \]  \hspace{1cm} (6.4)

\[ E \approx 3\mu \]  \hspace{1cm} (6.5)

where \( \rho \) is the tissue density and \( c_g \) is the group velocity of the propagating shear wave. SWE has successfully been applied in a variety of tissues where it has detected tissue changes related to disease or
Figure 6.1 a) A linear transducer applying an acoustic radiation force impulse (ARFI). b) The resulting shear wave propagates orthogonally to the applied ARFI and is imaged using high speed plane wave imaging. The speed of the propagating shear wave corresponds to the stiffness of the tissue.

physiological processes in liver [58], breast [59], kidney [60], arterial wall [61], myocardium [62, 63], thyroid [64], and muscle [65].

The assumptions applied to large uniform tissues do not hold when applying SWE to arteries or plaques and other analytical solutions are required to estimate the shear modulus. The primary limitation is that the geometry of the artery creates shear wave frequency dispersion, implying that different frequencies of the shear wave are propagating at different speeds. Therefore, an analysis that takes the phase velocity of the shear wave into consideration is required.

As previously mentioned, plaques develop in various morphological configurations. To simplify the analysis, the plaque is considered to be part of the arterial wall and the artery is considered to be a hollow cylinder. There is currently no analytical solution for a shear wave propagating in a hollow cylinder [66], but it is possible to approximate the shear wave propagation with a model of a fundamental antisymmetric Lamb wave mode traveling through an infinite plate submerged in incompressible water like fluid [61, 67-69]. The approximation is valid as the dispersion curves for a lamb wave traveling through a hollow cylinder and an infinite plate converge at higher frequencies [61]. The wave dispersion equation for a homogeneous elastic plate submerged in incompressible water like fluid is

\[ 4k_L^2 \beta \cosh(k_L h) \sinh(\beta h) - (k_x^2 - 2k_L^2)^2 \sinh(k_L h) \cosh(\beta h) = k_L^2 \cosh(k_L h) \cosh(\beta h) \]  \hspace{1cm} (6.6)

\[ k_L = \frac{\omega}{c_L} \]  \hspace{1cm} (6.7)
\[ k_s = \omega \sqrt{\frac{\rho}{\mu}} \quad (6.8) \]

\[ \beta = \sqrt{k_L^2 - k_s^2} \quad (6.9) \]

where \( \omega \) is the angular frequency, \( \rho \) is the tissue density, \( c_L \) is the frequency dependent Lamb wave velocity, \( k_L \) is the Lamb wave number, \( k_s \) is the shear wave number, \( h \) is the half-plate thickness and \( \mu \) is the shear modulus. Equation 6.6 can be fit to experimental data where \( \mu \) can be estimated. Phase velocity analysis is applied in Studies II-IV for estimating the shear modulus in phantom and \textit{ex vivo} arteries with simulated plaques.

### 6.3 Speckle Tracking

The objective with speckle tracking is to estimate tissue motion or deformation by tracking a group of pixels, known as a speckle pattern, within a region of interest (ROI) over time. This is accomplished by block matching kernels, a small region of pixels, across imaging frames and tracking their displacement. A kernel containing a unique speckle pattern is selected (typically with a percent overlap with respect to other kernels) and is tracked within a searchable area from frame \( t \) to frame \( t+1 \). The size of the searchable area is typically defined by the maximum velocity of the tracked objects. For every location in the searchable area, a similarity function is calculated to estimate the agreement between the position of the kernel in frame \( t \) and the new kernel location in frame \( t+1 \). The size of the searchable area is calculated as \((2m + 1) \times (2n + 1)\). The maximum value of \( C_{NCC} \) corresponds to the best possible kernel match between frames \( t \) and \( t+1 \) and the displacement of the kernel can be estimated between the two frames. Spline interpolation can be used to detect sub-sample motion between frames. After the best match has been found, new kernels are selected and the motion estimation is performed until the entire region of interest is investigated. Thereafter, the procedure is repeated on all successive frames in the cineloop and the displacement is tracked throughout the cardiac cycle. An illustrative example of the principle of speckle tracking is shown in Figure 6.2.

Motion and deformation can be used to identify vulnerable plaques, assuming that a vulnerable plaque with a soft necrotic core will display greater deformation compared to stable plaques with a calcified or fibrous core throughout the cardiac cycle. Similarly, increased arterial stiffness in atherosclerotic arteries will show reduced compliance to the blood pulse wave and this reduced motion can be detected.
Figure 6.2 An illustrative example of the principle of speckle tracking. A kernel (red box) with a specific speckle pattern is tracked over two successive frames, frame $t$ and frame $t+1$, within a search area outlined by the green rectangle in frame $t+1$. A similarity function is calculated for every position of the kernel in the search area. The location with the best similarity measure is the estimated displacement between the two frames for the kernel.

by speckle tracking. The Cauchy strain (%) is a common measure used to quantify elasticity in cardiovascular applications and can be calculated as

$$\varepsilon = \frac{L - L_0}{L_0}$$  \hspace{1cm} (6.11)

where $L_0$ is the initial length of the segment and $L$ is the displacement. In Study I, the peak strain was calculated on in vivo and phantom carotid artery plaques. Speckle tracking has been previous used to estimated strain in the wall of a carotid artery in a sheep [70], detect aortic aneurysms [71], used in echocardiography [72], and IVUS applications [27, 73, 74].

6.4 Pulse Wave Imaging

Pulse wave velocity (PWV) is a measure of arterial stiffness that has become the clinical golden standard for assessing aortic vascular health and has been adopted by the European Society of Hypertension and the European Society of Cardiology [75]. Pulse waves are generated during the cardiac cycle by the ejection of blood from the left ventricle and travel throughout the arterial tree [76]. The speed of the propagating wave is referred to as the PWV and is a widely accepted clinical measure for aortic stiffness and an indicator for overall arterial stiffness [77, 78], where a faster PWV corresponds to stiffer arteries.

The PWV can be estimated by measuring the pulse wave TOF between two points and dividing the result by the measured distance. Clinically, the PWV is typically measured between the carotid and femoral arteries [78]. This measurement has two main sources of error. First, the pulse wave in the carotid and femoral arteries are traveling in opposing directions confusing the measurement from a physiological perspective. Second, it is difficult to accurately measure the distance between the carotid and femoral arteries, resulting in a measurement error [79, 80]. Despite these two limitations, the
technique is widely accepted as the clinical standard assessment for aortic health. It is common to calculate the Young’s modulus ($E$) based on the PWV from the Moens-Korteweg equation

$$E = \frac{2rpPWV^2}{h}$$

where $\rho$ is the blood density, $r$ is the inner radius of the artery, and $h$ is the wall thickness. However, there are several limitations with the Moens-Korteweg equation. The equation assumes an infinitely long, straight, isolated, and cylindrical vessel with elastic, isotropic, and homogenous walls, containing a homogenous, incompressible and nonviscous fluid [77]. Many of these assumptions are invalid, and the assumptions are even more incorrect when a plaque is present in the vessel wall. Additionally, the equation does not take into account a dynamic wall thickness and artery radius throughout the cardiac cycle.

Noninvasive imaging-based methods have been attempted to measure PWV using MRI [81-83] and ultrasound [84-86], but the techniques suffer from poor temporal or spatial resolution. PWI is an ultrasound-based technique that provides regional estimates of the PWV with high spatial and temporal resolutions [84, 87]. In PWI, the pulse wave is tracked throughout the length of the ultrasound transducer and is visualized in a spatiotemporal plot from which the PWV can be estimated as the slope of the propagating pulse wave. Additionally PWI provides a more localized PWV measurement than the clinical measurement between the carotid and femoral arteries. PWI can be implemented with conventional B-mode imaging with a limited number of scan lines (to increase the frame rate) or high speed plane wave imaging, similar to SWE, to achieve a high temporal resolution. PWI was applied in Study IV to characterize the stiffness of phantom plaques.
7 Imaging Techniques for Plaque Characterization

7.1 Ultrasound Applications in Screening for Cerebrovascular Diseases

Cerebrovascular disease is currently clinically screened by visually assessing carotid vulnerable plaques with ultrasound duplex scanning where the stenosis degree and plaque morphology are assessed to determine plaque vulnerability [5]. The ultrasound screening can be followed up by an angiographic examination (e.g. CTA, MRA, invasive angiography), which provides information regarding the plaque size, location, and degree stenosis [88, 89], but provides no information about the plaque composition or its mechanical properties. Depending on the aforementioned plaque characteristics, appropriate therapies include exercise, dietary changes, statins, angioplasty surgery (stenting), or endarterectomy [12, 90].

It is possible to classify plaques based on their composition on the Gray-Weale scale when visually assessing B-mode images of carotid artery plaques. The Gray-Weale four-level scale consists of type 1: mainly echolucent lesions; type 2: primarily echolucent lesions with some areas of echogenicity; type 3: dominantly echogenic lesions; and type 4: uniformly echogenic lesions. Generally, types 1 and 2 are considered to correspond to vulnerable plaques with a soft core, whereas types 3 and 4 are considered to correspond to more stable fibrous plaques [91].

Several studies show that assessing the degree of lumen stenosis is an indirect indicator for determining plaque vulnerability [4, 15, 16]. Since vulnerable plaques have a soft necrotic or lipid core, it makes sense to study the mechanical properties of the plaque to distinguish vulnerable plaques with a soft lipid core from those with a more stable fibrous or calcified composition. Therefore, quantitative techniques for assessing the mechanical properties of plaques are needed.

The aforementioned ultrasound techniques can theoretically distinguish vulnerable and fibrous plaques as well as noninvasively quantify the plaque’s mechanical properties. Speckle tracking can potentially be applied to measure the strain of carotid artery plaques throughout the cardiac cycle, where one expects a soft vulnerable plaque to exhibit greater peak radial strain compared to a more stable fibrous plaque. Previous attempts to assess plaque strain using radiofrequency based ultrasound speckle tracking have been performed [92, 93]. SWE can potentially estimate the shear modulus of a plaque to determine its composition, where vulnerable plaques would have a lower shear modulus than a fibrous plaque. SWE has previously been attempted to detect vulnerable plaques in vivo [94-96], but the studies suffer from
several shortcomings including lack of validation and neglecting the frequency dispersion effects of the shear wave propagating through the plaque. PWI can potentially measure the PWV in the plaque, where pulse waves would propagate slower in soft vulnerable plaques and faster in harder more fibrous plaques. PWI has been attempted to monitor atherosclerotic arteries [98] but has not been attempted to characterize vulnerable plaque.

### 7.2 Additional Noninvasive Ultrasound-based Techniques

Many other noninvasive ultrasound-based methods have been attempted to characterize the mechanical properties of carotid artery plaques. One of the more common techniques is to measure the GSM of plaques, where the median pixel intensity from the B-mode image is measured inside the plaque [99-101]. Shortcomings of the technique include variability from different ultrasound machine settings or variability between different ultrasound machine manufactures and several studies show conflicting results using GSM as an indicator for increased risk of stroke [102-104].

Other techniques have been attempted, including assessing the axial strain ratio, attenuation coefficient, and scattering size to distinguish fibrous, calcified, and lipid ex vivo plaques, but no in vivo experiments have been performed [105]. TSI has been used in vivo in a rabbit study to detect lipids in plaques [26] and ex vivo in excised porcine coronary arteries [106]. Lipids exhibit a temperature dependent ultrasound speed compared to water-bearing tissues causing the reflected ultrasound RF signals to shift in TOF, which produces a thermal strain. To the author’s knowledge, TSI has not been attempted in a human in vivo study. Contrast-enhanced ultrasound imaging has been used to image neovascularization in carotid artery plaques as neovascularization is associated with plaque vulnerability [107-110]. While this technique is successful at imaging neovascularization in carotid artery plaques, not all vulnerable plaques develop this biomarker and thus go undetected. Others have used contrast-enhanced ultrasound imaging to detect the presence of the vasa vasorum in carotid artery plaques, but no large-scale clinical studies have been performed [111, 112]. Moreover, the vasa vasorum of carotid artery plaques is not widely recognized as a clinical biomarker for vulnerable plaques.
Contrast-enhanced ultrasound imaging has been used with TDI in a case study to detect and evaluate carotid stenosis [113]. Additionally TDI has been applied to measure arterial wall motion in normal and diseased carotid arteries, but the technique suffers from usefulness due to high variability and a complex protocol [114].

ARFI imaging has been applied to characterize in vivo carotid artery plaques in small pilot studies [115-117]. ARFI imaging is similar to SWE in that the technique uses an ARF to create a tissue displacement. The techniques differ in that ARFI imaging characterizes the tissue based on the induced relative strain compared to the surrounding tissue instead of tracking the speed of the resulting propagating shear wave. Since ARFI measures relative stiffness differences to nearby surrounding tissues, plaques located in the anterior and posterior carotid artery walls cannot be compared as well as measurements between patients. Additionally, since ARFI is a relative measurement, repeatability over time may be difficult. To date, small successful pilot studies have been performed with validation by histology but no large scale clinical trials have been performed [38].

7.3 Additional Plaque Characterization Techniques

IVUS has been attempted in combination with speckle tracking to characterize carotid artery plaques [73, 74, 118, 119]. The disadvantages with this technique are that it is invasive and not as cost-effective as a conventional non-invasive ultrasound screening. Plaque characterization has been attempted by other imaging modalities as well. MRI has been used to characterize plaques and while it does provide superior spatial resolution compared to ultrasound, it is expensive and time consuming [120-122]. Additionally, MRI is a high risk factor for initiating recurrent plaque rupture events [122]. CTA has been attempted to characterize plaque based on the Hounsfield unit (HU) values [123, 124], however the technique is limited because the HU level significantly changes according to the applied kiloelectron volt. Dual-energy CT has been attempted [124-127] to detect plaque and measure carotid stenosis, where it was found that the dimension of calcified plaque and carotid artery decreased with increased energy output. Moreover, further large clinical studies are required.
8 Methodology

The methodologies used in Studies I-IV are described in this chapter to achieve the aims listed in chapter 2. First, a description is provided regarding the construction of carotid artery plaque phantoms and the study subjects involved. Next, descriptions of image acquisition, visual image analysis, and software development are provided followed by post-processing techniques, reference methods used, statistical analysis, and a summary of the methodologies for Studies I-IV.

8.1 Carotid Artery Plaque Phantoms and Study Subjects

8.1.1 Phantom Design

Carotid artery phantoms with a plaque mimicking inclusion were designed for Studies I, II, and IV (schematic shown in Figure 8.1(a)). The phantoms were created from a mixture (w/w) of 87% deionized water, 10% polyvinyl alcohol (PVA) with a molecular weight of 56,140 g/mol (Sigma-Aldrich, St. Louis, MO, USA) and 3% graphite powder with particle size < 50 μm (Merck KGaA, Darmstadt, Germany). The solution was heated to 55 °C and stirred until the mixture thickened and was fully dissolved. It was subsequently poured in a mold with a bronze rod with a 6 mm diameter placed in the center as seen in Figure 8.1(b) resulting in a hollow cylindrical-shaped vessel phantom with fixing collars. To construct the plaque mimicking inclusion, the rod had a 50 mm long and 1.5 mm thick extension attached to create a cavity in the phantom wall. The mold was frozen for 24 hours at approximately -23 °C and then thawed for 24 hours at room temperature (≈ 22 °C). To increase the phantom stiffness, the mold was repeatedly frozen and thawed. The number of freeze-thaw cycles was varied depending on the desired phantom wall stiffness.

To create soft plaque mimicking inclusions, the bronze rod with attachment was removed and the phantom wall cavity was filled with an identically prepared PVA-graphite solution and the entire phantom was exposed to an extra freeze-thaw cycle. A B-mode image of the resulting phantom with soft plaque inclusion can be seen in Figure 8.1(c).

Phantoms with hard plaque mimicking inclusions were constructed for Study II by creating hard plaque inclusions followed by embedding them in PVA mixture to create vessel phantoms (schematic shown in Figure 8.2(a)). The hard plaque inclusions were created by pouring the same PVA mixture into an acrylic mold as seen in Figure 8.2(b) (50 mm length, 1.5 mm thickness, and partial cylindrical shape with 120° circumference). Thereafter, the mold underwent seven freeze-thaw cycles creating hard plaque inclusions. The inclusions were placed in the vessel phantom mold and filled with a new batch of PVA mixture encompassing the plaque inclusions and thereafter the mold was exposed to three freeze-
thaw cycles. The resulting phantom had three freeze-thaw cycles for the vessel wall and ten cycles for the hard plaque inclusion. A B-mode image of the resulting phantom with hard plaque inclusion can be seen in Figure 8.2(c). A summary of the number of phantoms used in each study can be seen in Table 8.1 and the number of freeze-thaw cycles for each type of phantom can be seen in Table 8.2.

For the mechanical testing in Study II, two control carotid artery phantoms without plaques were created for both the soft and hard plaque phantoms by using the vessel phantom mold with a rod (without an extrusion) creating a symmetrical vessel phantom without a plaque inclusion. The control phantoms were created because the mechanical testing required geometric symmetry and material incompressibility in order to calculate the shear modulus. To validate the SWE measurements in the soft plaque and vessel wall with mechanical testing, the control phantoms had the same number of freeze-thaw cycles (one and three freeze-thaw cycles, respectively) and were constructed from the same PVA batch as the soft plaque phantoms. Similarly, the hard plaque and vessel wall control phantoms had ten and three freeze-thaw cycles, respectively and were also constructed from the same batch of PVA as the hard plaque phantoms.

The PVA phantoms with plaques were attached to a fixture with the plaque positioned in the anterior wall as seen in Figures 9.3(a) and (b). For Study II the fixture had an adjustable short end, allowing the length of the cavity to be adjusted. The phantoms were pre-stretched to avoid curvature of the vessel wall when attached to the fixture. In Study I and IV, the fixture was not adjustable. In Study I, sonomicrometry crystals were super-glued (Loctite, Düsseldorf, Germany) to the plaque lumen and posterior phantom walls to measure a reference strain throughout a simulated cardiac cycle. The phantoms were embedded in mixture (w/w) consisting of 3% agar (Merck KGaA, Darmstadt, Germany), 4% graphite power, and 93% deionized water by mass, which was heated while continuously stirred to 73 °C, then cooled to 40 °C, and subsequently poured into the fixture. In Study I and the dynamic measurements in Study II, the phantoms were connected to a programmable pump (CompuFlow 1000MR; Shelley Medical Imaging Technologies, Ontario, Canada) to simulate a carotid flow profile at a rate of 60 cycles/min. In Study I, a blood-mimicking solution of 60% deionized water and 40% glycerin (Merck KGaA, Darmstadt, Germany) mixture was pumped through the phantoms at peak flow rates 10, 20, 30, and 35 mL/s. In Study II, the same solution was pumped through the phantoms at a peak flow rate of 30 mL/s.

For the static measurements in Study II the phantoms were surrounded by water and all air bubbles were removed in the phantom lumen by filling the phantom while holding it at an approximate 45° angle, allowing the air bubbles to escape the phantom lumen and rise to the surface. The absence of air bubbles was verified with B-mode ultrasound scanning. Next, the movable wall of the fixture was adjusted 8 mm to remove bulging from the water filled phantoms.

| Table 8.1 Overview of the number and types of phantoms in Studies I-IV. |
|-----------------|-----------------|
|                 | Soft Plaque     | Hard Plaque    |
| Study I         | 4               | -              |
| Study II        | 3               | 3              |
| Study III       | -               | -              |
| Study IV        | 3               | -              |
Figure 8.1 a) A long-axis cross-sectional schematic of the soft plaque phantom with dimensions. b) Phantom mold with bronze rod with an extension to create the plaque cavity in the vessel wall. A rod used for creating the control phantoms (without an extension) is placed in front of the mold. c) B-mode image of the PVA phantom with a soft plaque inclusion to the left in the anterior wall.

Figure 8.2 a) A long-axis cross-sectional schematic of the hard plaque phantom with dimensions. The hard plaque (black) is inserted into the phantom mold in Figure 8.1b followed by pouring PVA around the plaque insert. b) Mold for creating five hard plaque inserts. c) B-mode image of the PVA phantom with a hard plaque inclusion to the left in the anterior wall.

<table>
<thead>
<tr>
<th>Table 8.2 Number of freeze-thaw cycles per type of phantom.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Plaque Phantoms (wall/plaque)</td>
</tr>
<tr>
<td>Number of freeze-thaw cycles</td>
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</table>
In Study IV, the phantoms were embedded in a gelatin-based material. The gelatin solution was prepared (w/w) by mixing 45% boiling deionized water with 10% gelatin (Now Foods, Bloomingdale, IL, USA). The solution was stirred for 5 minutes until the gelatin was dissolved. Next, 45% deionized water (room temperature) was added to the solution and the mixture was stirred until it reached 40 °C. The gelatin solution was poured into the fixture and thereafter cooled in a refrigerator (~ 2 °C) for 24 hours to allow the gelatin to stiffen. The phantoms were connected to a hose, filled with water, and pressurized to 20 mmHg prior to data acquisition.

8.1.2 Porcine Arteries

In Study III, five thoracic aortas were surgically removed from small Swedish Yorkshire pigs (mean weight 35 ± 1.6 kg, 2 male, 3 female) to be used as a model for a human carotid artery after the pigs had been sacrificed for medical education. Fascia surrounding the arteries was removed and the arteries were cut to a length of approximately 10 cm. To simulate a region with increased arterial stiffness or calcified plaque, half of the artery was soaked in a bath of 36.5% formaldehyde solution (Sigma-Aldrich, St. Louis, MO USA) for 20 minutes. Hereafter, the stiffened arterial regions will be referred to as simulated plaques. Next, the intercostal arterioles were glued shut with universal hybrid glue (Loctite, Düsseldorf, Germany) and the specimens were attached to a fixture using thread with the intercostal arterioles facing downward, pressurized with saline, and checked for leaks. Thereafter, the arteries were stretched approximately 5% of their unpressurized length to minimize buckling during pressurization. The fixture was attached to a pressure sensor followed by a saline filled column and placed in a saline bath.

8.1.3 Humans

In vivo experiments were conducted in Study I. The data were collected at Karolinska University Hospital in Huddinge, Sweden by an experienced ultrasound technician with consent given by the patients and approval by the ethical review board (2011/331-31/3, Stockholm, Sweden). The patient mix consisted of 4 males and 3 females with a mean age of 77 ± 6 years. Patient medical data were collected including sex, age, and blood pressure.
8.2 Image Acquisition

Different ultrasound systems were used for Studies I-IV. For Study I, a GE Vivid E9 ultrasound machine (Horten, Norway) was used with a handheld 9LD linear array transducer and the cineloops were recorded by an experienced sonographer. The cineloops were acquired at 3.5 cm depth, with a center frequency of 10 MHz, image compounding turned off, and two focal points on the arterial walls resulting in a frame rate of 42 Hz. Three long-axis and short-axis cineloops were collected over 3 consecutive cardiac cycles for the CCA, the bulb, and the internal carotid artery (ICA). The same settings were used for in vitro image acquisition and the transducer fixed above the phantom to avoid probe motion.

In Study II, an Aixplorer ultrasound machine (Supersonic Imagine, Aix-en-Provence, France) with a custom shear wave research package was used together with a fixed SL15-4 linear array transducer to collect in-phase and quadrature (IQ) data. Three consecutive short ARF pushes were made at a center frequency of 6 MHz and a push length of 150 µs, resulting in a total push length of 450 µs. After generating the ARF pushes, the resulting shear wave was tracked using ultrafast plane wave imaging at a frame rate of 8 kHz with a center frequency of 7.5 MHz with no angle compounding at an imaging depth of 3 cm. Three data acquisitions were collected in the plaque and wall for each phantom.

Two ultrasound systems were used in Study III. An Aixplorer ultrasound machine with a fixed SL15-4 linear array transducer was used to image the porcine arteries from which the arterial wall thickness was measured from the B-mode images. Long-axis images were acquired with a center frequency of 7.25 MHz, 3.5 cm depth, a focal point on the anterior arterial wall, using harmonic imaging with no image compounding resulting in a frame rate of 32 Hz. The lumen diameter was measured at 60 mmHg at three locations in both ends of the artery and averaged. The anterior wall thickness was measured

Table 8.3 Ultrasound systems and settings used for Studies I-IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>Ultrasound System</th>
<th>Transducer</th>
<th>Imaging Center Frequency (MHz)</th>
<th>Frame Rate (Hz)</th>
<th>Push Frequency (MHz)</th>
<th>Push Length (µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>GE Vivid E9</td>
<td>9LD</td>
<td>10</td>
<td>42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Study II</td>
<td>Supersonic Imagine Aixplorer</td>
<td>SL15-4</td>
<td>7.5</td>
<td>8000</td>
<td>6</td>
<td>3 x 150</td>
</tr>
<tr>
<td></td>
<td>Supersonic Imagine Aixplorer / Verasonics Acquisition System</td>
<td>SL15-4 / L7-4</td>
<td>7.25 / 5</td>
<td>32 / 10000</td>
<td>4.09</td>
<td>100-700</td>
</tr>
<tr>
<td>Study III</td>
<td>Verasonics Acquisition System</td>
<td>L7-4</td>
<td>5</td>
<td>10000 / 8333</td>
<td>4.09</td>
<td>100</td>
</tr>
</tbody>
</table>

29
from 20-120 mmHg in increments of 20 mmHg at three locations within the shear wave propagation region and averaged together.

In studies III and IV, the shear waves were generated and imaged with a Verasonics acquisition system (Verasonics, Kirkland, WA, USA) using a fixed linear L7-4 probe (Philips Healthcare, Andover, MA) with an in-house script to generate shear waves with an ARF push and collect IQ data using plane wave imaging. The ARF push had a 4.09 MHz center frequency with an F-number of 1 and the resulting propagating shear wave was measured with high speed plane wave imaging with a center frequency of 5 MHz resulting in a frame rate of 10 kHz. The push length was varied from 100-700 μs and the number of imaging angles were 1, 3, and 5 compounding angles. Three data acquisitions where collected in the plaque and wall for each phantom. The pulse waves in Study IV were imaged with the same system with high speed plane wave imaging at a frame rate of 8333 Hz and three data acquisitions where collected in the plaque and wall for each phantom. Table 8.3 summarizes the ultrasound systems and settings for Studies I-IV.

8.3 Visual Image Analysis

The in vivo cineloops in Study I were visually analyzed and scored by two experienced physiologists using EchoPAC (GE Healthcare, Little Chalfont, United Kingdom). The physicians outlined the plaque borders in diastole, which functioned as boundaries for ROI placement during speckle tracking analysis. The cineloops were assessed with four different measures. First, the plaques were classified homogeneous or heterogeneous. Second, the plaques were classified if the surface was smooth or irregular based on visual interpretation of B-mode and color Doppler images [128, 129]. Third, the plaques were classified on a Gray-Weale four-level scale [91]. Fourth, the GSM of the plaques were measured on a single frame of the cineloop in systole and used as an alternative measurement of echogenicity compared to the Gray-Weale visual assessment. The GSM was measured with Automated Measurement System (AMS), a software program developed by the Department of Signals and Systems at Chalmers University of Technology, Gothenburg, Sweden and the Physiology group at the Wallenberg laboratory, Gothenburg University, Gothenburg, Sweden.

8.4 Software Development

All of the software development in Studies I-IV was performed in MATLAB (MathWorks, Natick, MA, USA). In Study I, cineloops were exported from EchoPAC and imported to MATLAB 2011a as input data. Unprocessed IQ data was exported from the Aixplorer ultrasound machine and post-processed in MATLAB 2012b in Study II. For Studies III and IV, IQ data from the Verasonics acquisition system was post-processed in MATLAB 2012b and 2015b, respectively.

8.4.1 Speckle Tracking

In Study 1, an in-house speckle tracking algorithm based on normalized cross-correlation was used to perform motion tracking and estimate radial and longitudinal strain in plaque phantoms [130]. The algorithm was originally developed for measuring strain in the arterial wall but was fine-tuned to measure strain in plaques by reducing the kernel size. Axial and lateral motion estimation was performed on 3 consecutive pump cycles on both the RF signal and the envelope-detected signal with a kernel length and width of three and five wavelengths (λ), respectively. The kernel search area was defined to allow maximum velocities of 2 cm/s with a 60% axial and lateral overlap using spline interpolation to detect sub-sample motion. Subpixel motion was accounted for by implementing linear interpolation when accumulating the displacement maps throughout the cardiac cycle to assess the
8.4.2 Shear Wave Elastography

The shear wave propagation speeds were analyzed with phase velocity analysis to account for frequency dispersion. Group velocity analysis was also performed where all frequencies of the shear wave were assumed to propagate at the same velocity.

8.4.2.1 Phase Velocity Analysis

The phase velocity analysis algorithm, summarized in four key steps in Figure 8.4, was used to estimate the shear modulus based on the phase velocity of the propagating shear wave. The IQ data were divided such that the left and right propagating shear waves could be studied independently. First, a 2D autocorrelator [131] measured the axial displacement by detecting the phase-shifts between consecutive pulse-echo measurements. The axial displacement data were bandpass filtered to remove motion cumulative strain. Radial and longitudinal speckle tracking strain curves were obtained by linear regression in a ROI and thereafter low pass filtered with an averaging window three samples in length to remove noise.

The ROI size when estimating strain of in vivo plaques was based on the size of the plaques during diastole. The ROI for radial strain was approximately 50% of the plaque height and 1.5 mm wide. The longitudinal ROI width was approximately 4 mm wide and 1.5 mm in height.

Figure 8.4 Summary of the shear modulus estimation algorithm by phase velocity analysis.

IQ Data

1. B-Mode
   - 2D Autocorrelator
   - Select Shear Wave Tracking Lines
   - Bandpass Filter (Dynamic Test)

Axial Disp. Map

2. K-space Map
   - 2D FFT
   - Quadrant Crop
   - Amplitude Mask

Phase Velocity Map

3. Phase Velocity Calculation
   - Identify Max Intensity
   - High & Low Frequency Cutoff Limits

Phase Velocity Curve

4. Full Bandwidth
   - Lamb Wave Model
   - Least Squares Fit
   - Upper Half-Bandwidth

Shear Modulus

Shear Modulus
artefacts in the dynamic test with cut-off frequencies at 100 and 2000 Hz. The motion was averaged between three to five lateral pixel lines in the center of the investigated medium depending on the thickness of the investigated object. Next, an axial displacement plot (Figures 9.9(d)-(f)), representing the time (ms) versus distance (mm), of the propagating shear wave was created. Data along the time axis were removed once the shear wave had fully dissipated to remove noise and late wall reflections.

In the second step of the algorithm a 2D fast Fourier transform (FFT) was performed on the axial displacement plot to convert the shear wave propagation to the frequency domain, referred to as the k-space representation. The k-space plot was either cropped to the upper or lower right quadrant depending on if the right or left shear wave was analyzed. Next, noise was removed by a threshold filter that retained 90% (Study II) or 97% (Study III and IV) of the maximum signal intensity.

The third step involved creating a phase velocity map by multiplying each paired frequency and wave number for all k-space locations. The dominant antisymmetric Lamb wave mode curve was found by finding the maximum magnitude for each frequency. Cut-off limits were introduced to eliminate erroneous data. In Study II the high cut-off frequency was set to the frequency where the intensity had decreased to less than 30% of the maximum k-space intensity. The low cut-off frequency was set to the frequency where the phase velocity between neighboring data points were greater than 50%. In Studies III and IV the cut off limits were selected manually.

The fourth step of the algorithm involved fitting a model for an antisymmetric Lamb wave propagating in a plate (equation 6.6) to the phase velocity curve using a least squares fit [61, 67] from which the shear modulus ($\mu$) was calculated. The fit was performed two ways. First, on the full phase velocity data set and thereafter on the upper half-bandwidth of the phase velocity data set as cylindrical- and plate-based dispersion curves converge at high frequencies [61].

8.4.2.2 Group Velocity Analysis
The shear wave propagation speed, shear modulus, and Young’s modulus were calculated from the shear wave group velocity in Studies II and IV. The group velocity was calculated one of two ways. In Study II, the group velocity was estimated by performing a Radon transform on the shear wave axial displacement plots. This was accomplished by summing the axial displacement data along line trajectories around the matrix center to determine the trajectory of the wave motion. The group velocity was calculated as the slope of the peak Radon sum trajectory [132-134]. In Study IV, the group velocity was calculated by finding the maximum displacement for each row of the axial displacement plot followed by calculating the slope of the maximum shear wave axial displacement. The shear and Young’s moduli were calculated by equation 6.4 and 6.5, respectively.

8.4.3 Pulse Wave Imaging
In Study IV, the PWV was calculated by PWI in the anterior vessel wall or plaque of PVA phantoms. First, the axial wall or plaque velocity were estimated with a 1D normalized cross-correlation algorithm [135] using a window size of 10$\lambda$ and 90% window overlap. To reduce noise, the image was smoothened by a Gaussian filter. Thereafter, the plaque and wall borders were manually outlined and the phantom wall and plaque were semi-automatically segmented using a Sobel-based edge detection method followed by fitting a smoothing spline through the resulting segmentation. A pulse wave tracking line was placed in the center of the phantom wall and plaque. A 2D spatiotemporal plot of the PWI axial velocities was plotted as a function of the time to visualize the propagating pulse wave. The 50% downstroke point was selected as the tracking feature to estimate the PWV. To calculate the PWV, a linear regression was calculated on the 50% down-stroke point arrival time plotted against the pulse wave.
travel distance. The PWV was calculated as the inverse of the slope. The Young’s modulus was calculated from the Moens-Korteweg equation.

8.5 Reference Methods
In Studies I and II speckle tracking and SWE were compared to sonomicrometry and mechanical testing, respectively.

8.5.1 Sonomicrometry
Three sonomicrometry crystals were glued to the phantom plaques. Crystal one was glued to the apex of the plaque in the lumen, while crystals two and three were glued on the exterior of the phantom plaque approximately 1 cm apart in the same horizontal plane, forming a triangle with crystal one. The sonomicrometry data from Study 1 was processed in MATLAB 2011a. First, outliers from the inter-crystal displacement curves were removed by thresholding, thereafter the resulting signals were filtered with a 10-sample median filter. Five pump cycles of data were averaged to obtain the final displacement curves. The radial displacement was calculated by the law of cosines and the longitudinal displacement was the distance between crystals two and three. The strain ($\varepsilon(t)$) was calculated as

$$
\varepsilon(t) = \frac{D(t) - D_0}{D_0}
$$

(7.1)

where $D_0$ is the initial distance between the crystals and $D(t)$ is the distance between the crystals at time $t$.

8.5.2 Mechanical Testing
Three control phantoms were mechanically tested in Study II as a validation for the shear modulus estimated by the SWE phase velocity analysis in the hard and soft plaque inclusions as well as the phantom vessel wall. A custom fixture was created allowing the phantoms to be simultaneously filled with water while performing a tensile test using an Instron 5567 (Instron Worldwide, Norwood, MA, USA) with a 100 N (calibrated 0.05% maximum error) electro-mechanical single range load cell.

The phantoms were pre-stretched 8 mm to mimic the test conditions from the SWE experiments. Thereafter, the phantoms were axially loaded up to 10% strain at a rate of 10 mm/min and the test was repeated three times. The stretch ($\lambda_z$) and applied Cauchy stress ($\sigma_z$) were calculated as

$$
\lambda_z = \frac{\delta_z + L_i}{L_i}
$$

(7.2)

$$
\sigma_z = \frac{F_z \lambda_z}{A_i}
$$

(7.3)

where $\delta_z$ is the deformation, $L_i$ is the initial length, $F_z$ is the axial force, and $A_i$ is the cross-sectional area of the sample. Hooke’s law states that the axial stress of a homogeneous linear material may be expressed as
\[ \sigma_z = E \varepsilon \]  

where \( E \) is the linear elastic modulus of the material and \( \varepsilon \) the applied Cauchy strain. A mean linear elastic modulus (\( \overline{E} \)) was calculated by using thirty sub-segments along the strain axis of the stress-strain curve. For each sub-segment, an elastic modulus (\( E_i \)) was derived by a local least-squares fit linearization [136]. The shear modulus was calculated as

\[ \mu = \frac{E}{3} \]  

assuming that the material was perfectly incompressible.

### 8.6 Statistical Analysis

Advanced statistical data analyses were performed in MATLAB while simple statistics such as means and standard deviations (SD) were calculated in both MATLAB and Excel (Microsoft Corporation, Redmond, WA, USA). To evaluate the quality of the speckle tracking strain to the reference sonomicrometry strain, the RMSE was calculated between the two methods. The RMSE was additionally used to evaluate the quality between the shear wave phase velocity curve and Lamb wave curve fit in Studies II and III.

\[ \text{RMSE} = \sqrt{\frac{1}{N} \sum_{n=1}^{N} (\varepsilon(n) - \hat{\varepsilon}(n))^2} \]  

In Study I, \( \varepsilon(n) \) is the sonomicrometry strain, \( \hat{\varepsilon}(n) \) is the strain estimated by speckle tracking, and \( N \) is the number frames in one simulated cardiac cycle. In Studies II and III, \( \varepsilon(n) \) is the shear wave phase velocity, \( \hat{\varepsilon}(n) \) is the curve fit of the Lamb wave propagation speed, and \( N \) is the number of data samples.

In Study I, Bland-Altman plots were used to illustrate similarity between sonomicrometry and speckle tracking strain. Limits of agreement were calculated as the average distance \( \pm 1.96 \) SD of the difference in the Bland-Altman plots. Thereafter, Pearson correlation analysis was performed. A paired t-test was performed to compare the plaque and wall peak strain where \( p \leq 0.01 \) was considered significant.

In Study IV, the coefficient of determination (\( r^2 \)) was calculated to estimate the quality of the linear regression to the 50% down stroke points.

Means and SD were calculated for age, artery length, plaque thickness, diameter, strain, shear modulus, Young’s modulus, RMSE, phase velocity, group velocity, frequency, SNR, PWV, and \( r^2 \). The coefficients of variation were calculated on the shear modulus estimates in the wall and plaque in Study IV to assess how consistent the repeated measurements were.
8.7 Summary of Methodologies

Study I: Ultrasound Speckle Tracking Strain Estimation of in vivo Carotid Artery Plaque with In vitro Sonomicrometry Validation

The accuracy of an in-house speckle tracking strain estimation algorithm was tested in PVA carotid artery plaque phantoms by validating the strain estimates against sonomicrometry. After validation, a small in vivo pilot study was performed on seven patients with carotid atherosclerotic plaques where strain was calculated on the plaques and compared to quantitative visual assessment by two experienced physicians.

Study II: Shear wave elastography plaque characterization with mechanical testing validation – a phantom study

The shear moduli were estimated using phase velocity analysis of the propagating shear wave in PVA carotid phantoms with hard and soft plaque inclusions. The shear wave estimates were compared to shear moduli calculated from mechanical tensile testing. Finally, the plaque phantoms were tested in a dynamic environment by connecting the phantoms to a programmable pump and measuring the shear modulus in the plaque throughout a simulated cardiac cycle.

Study III: Shear Wave Elastography Quantifies Stiffness in ex vivo Porcine Artery with Stiffened Arterial Region

Porcine aortas with stiffened segments were used as models of the human carotid artery with stiff plaques. The arteries were attached to a fixture and pressurized with a water column. Thereafter, the bandwidth of the shear wave was maximized with respect to the ARF push length and number of compounded angles. The plane wave imaging frame rate was 10000, 3333, and 2000 Hz for 1, 3, and 5 imaging angles, respectively for the angle compounding experiment. For 3 and 5 compounded angles the imaging angles were [-1, 0, 1] and [-2, -1, 0, 1, 2] degrees, respectively. Next, shear moduli were measured in the arterial wall and simulated plaque at static pressures ranging from 20-120 mmHg. Finally, the stiffness was incrementally increased in the arteries to evaluate the sensitivity of SWE.

Study IV: Carotid Plaque Characterization in a Phantom Setup: A Comparison of Shear Wave Elastography and Pulse Wave Imaging

PVA phantoms with homogeneous plaque inclusions were created, attached to a fixture, and pressurized to 20 mmHg. The wave propagation speeds for SWE and PWI were compared in the vessel wall and plaque. Thereafter, the Young’s moduli were calculated by group velocity, phase velocity, and PWV using equations 6.4, 6.5, and 6.12, respectively.

An overview of Studies I-IV, the applied techniques, and the number of phantoms, arteries, and in vivo plaques can be seen in Table 8.4.
Table 8.4 Overview of the studies, applied techniques, and number of phantoms, arteries, and in vivo plaques.

<table>
<thead>
<tr>
<th>Method</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speckle Tracking</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SWE Phase Velocity Analysis</td>
<td>-</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SWE Group Velocity Analysis</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>PWI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>Mechanical Tensile Testing</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sonomicrometry</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Studied Object                |         |          |           |          |
| PVA Phantoms Soft Plaque      | 4       | 3        | -         | 3        |
| PVA Phantoms Hard Plaque      | -       | 3        | -         | -        |
| Porcine Arteries              | -       | -        | 5         | -        |
| Patients with Plaques         | 7       | -        | -         | -        |
The results are summarized for Studies I-IV and complete results can be found in the full versions of the papers in the appendices.

9.1 Study I
The correlation plots for peak strain calculated by speckle tracking vs. sonomicrometry for radial (r = 0.96, p < 0.001) and longitudinal (r = 0.75, p ≤ 0.01) measurements can be seen in Figure 9.1(a) and (b), respectively. The results show that there was strong correlation between speckle tracking and sonomicrometry in both the radial and longitudinal directions, with slightly stronger correlation between the measurement techniques in the radial direction. Bland-Altman plots for the radial and longitudinal directions can be seen in Figures 9.2(a) and (b), respectively. Limits of Agreement (LOA) were -4.40% to 3.48% in the radial direction and -2.29% to 2.78% in the longitudinal direction. There was 35.1 ± 16.9% greater radial (p < 0.001) and 88.6 ± 72.0% greater longitudinal (p < 0.001) peak strain in the plaque compared to the vessel wall when measured with speckle tracking.

![Figure 9.1](image)

Figure 9.1 Correlation plots for negative radial (a) and positive longitudinal (b) peak speckle tracking (ST) strain versus peak sonomicrometry (Sono) strain in four PVA phantoms with plaques with peak flow rates 10, 20, 30, and 35 mL/s.
Sixteen plaques were found in the patient population, but seven data points were omitted due to poor image quality or too small plaque size (diameter < 2 mm). The plaque Gray-Weale classification, number of plaques, means and SDs of peak strain values in radial and longitudinal directions as well as the pulse-pressure adjusted strain values can be seen in Table 9.1. The pulse-pressure adjusted results show increased peak radial and longitudinal strain in the heterogeneous echolucent plaques (Gray-Weale types 1 and 2) compared with the echogenic Gray-Weale type 3 plaque. The results show that echolucent plaques, which are more likely to be vulnerable plaques with a soft necrotic or lipid core, show greater strain in the radial and longitudinal directions compared to plaque with a more fibrous composition. The mean and SD plaque GSM sorted by Gray-Weale classification was: type 1 GSM 40 ± 0; type 2 GSM 84 ± 9; and type 3 GSM 53.

### 9.2 Study II

Figures 9.3 (a) and (b) indicate agreement between the shear modulus estimation by phase velocity analysis on the upper half-bandwidth of the propagating shear wave and mechanical testing results in the soft and hard phantom plaques as well as the vessel walls. The mean shear modulus estimated by phase velocity analysis in the soft plaque was 5.8 ± 0.3 kPa whereas the mechanical testing measured

<table>
<thead>
<tr>
<th>Gray-Weale Classification</th>
<th>Number of Plaques</th>
<th>Radial Strain (%)</th>
<th>PP Adjusted Radial Strain (x10² mmHg⁻¹)</th>
<th>Longitudinal Strain (%)</th>
<th>PP Adjusted Longitudinal Strain (x10² mmHg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>-5.4 ± 1.5</td>
<td>-9.9 ± 2.7</td>
<td>1.3 ± 0.7</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>-2.7 ± 1.4</td>
<td>-5.0 ± 2.9</td>
<td>1.3 ± 0.5</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-2.6</td>
<td>-2.9</td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure 9.3 Mean shear moduli estimates (kPa) using phase velocity analysis on the shear wave upper half-bandwidth for the plaque and wall in the soft (a) and hard (b) plaque phantoms. The bar chart compares the estimates for the left shear wave (LSW, orange), right shear wave (RSW, red), mean of left and right shear wave (Mean SW, blue), and mechanical testing (MT, green).

Table 9.2 Means and SDs (n = 6) of shear moduli (µ) for the soft and hard plaque phantoms’ vessel walls and plaques with phase velocity analysis estimates (left and right shear waves averaged together) on the full and upper half-bandwidth of the propagating shear wave. The results are compared to mechanical testing. All units are in kPa.

<table>
<thead>
<tr>
<th></th>
<th>µ Plaque Full BW</th>
<th>µ Plaque upper HBW</th>
<th>µ MT Plaque</th>
<th>µ Wall Full BW</th>
<th>µ Wall upper HBW</th>
<th>µ MT Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPP</td>
<td>3.4 ± 1.1</td>
<td>5.9 ± 0.6</td>
<td>3.3 ± 0.5</td>
<td>25.9 ± 8.0</td>
<td>25.0 ± 1.2</td>
<td>29.7 ± 0.5</td>
</tr>
<tr>
<td>HPP</td>
<td>98.2 ± 16.8</td>
<td>106.2 ± 17.2</td>
<td>98.3 ± 3.4</td>
<td>22.4 ± 5.6</td>
<td>23.3 ± 2.1</td>
<td>29.7 ± 0.5</td>
</tr>
</tbody>
</table>

SPP = Soft Plaque Phantom, HPP = Hard Plaque Phantom SWE = Shear Wave Elastography, BW = Bandwidth, HBW = Half-Bandwidth, MT = Mechanical testing

Table 9.3 Means and SDs (n = 6) of RMSE (m/s) for the full bandwidth and upper half-bandwidth in soft and hard plaque phantoms and phantom walls.

<table>
<thead>
<tr>
<th></th>
<th>RMSE Full Bandwidth</th>
<th>RMSE Upper Half-Bandwidth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Plaque Phantoms</td>
<td>0.17 ± 0.06</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Hard Plaque Phantoms</td>
<td>0.73 ± 0.86</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td>Phantom Wall</td>
<td>0.58 ± 0.20</td>
<td>0.10 ± 0.04</td>
</tr>
</tbody>
</table>
Table 9.4 Comparison of shear wave velocities (m/s) and shear moduli (kPa) estimates in the soft and hard plaque phantoms (n=6) for phase velocity and group velocity analysis. The frequency (Hz) at which the phase velocity was evaluated once the phase velocity curve had plateaued. Left and right shear waves are averaged together.

<table>
<thead>
<tr>
<th></th>
<th>Phase Velocity Frequency</th>
<th>Phase Velocity Shear Modulus Upper HBW</th>
<th>Group Velocity Frequency</th>
<th>Group Velocity Shear Modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Plaque</td>
<td>1.9 ± 0.04</td>
<td>5.9 ± 0.6</td>
<td>2.2 ± 0.1</td>
<td>5.2 ± 0.0</td>
</tr>
<tr>
<td>Hard Plaque</td>
<td>5.5 ± 0.24</td>
<td>106.2 ± 17.2</td>
<td>5.0 ± 0.5</td>
<td>26.8 ± 0.3</td>
</tr>
<tr>
<td>SPP Wall</td>
<td>3.7 ± 0.06</td>
<td>25.0 ± 1.2</td>
<td>3.4 ± 0.1</td>
<td>12.4 ± 0.0</td>
</tr>
<tr>
<td>HPP Wall</td>
<td>3.5 ± 0.13</td>
<td>23.3 ± 2.1</td>
<td>3.4 ± 0.1</td>
<td>12.4 ± 0.0</td>
</tr>
</tbody>
</table>

SPP = Soft Plaque Phantom, HPP = Hard Plaque Phantom, HBW = Half-Bandwidth
*The hard plaque phase velocity curve never fully plateaued

3.3 ± 0.5 kPa. The mean plaque shear modulus in the hard plaque measured by phase velocity analysis and mechanical testing was 106.2 ± 17.2 kPa and 98.3 ± 3.4 kPa, respectively. In the vessel wall of the soft plaque phantoms, the mean shear modulus measured by phase velocity analysis and mechanical testing was 25.0 ± 1.2 kPa and 29.7 ± 0.5 kPa, respectively. Similarly, the vessel wall shear modulus in the hard plaque phantoms was 23.3 ± 2.1 kPa measured by phase velocity analysis and 29.7 ± 0.5 kPa by mechanical testing. Table 9.2 shows the mean shear moduli and SDs calculated by phase velocity analysis over the full and upper half-bandwidth of the propagating shear wave compared to mechanical testing for the wall and plaque in the soft and hard plaque phantoms. Table 9.3 shows the means and SDs of the RMSE (given in m/s) in the plaques and walls of the hard and soft plaque phantoms for the full spectrum and upper half-bandwidth.

A comparison of the shear wave velocities as well as the shear moduli estimated by phase and group velocity in the plaques and walls of the soft and hard plaque phantoms can be seen in Table 9.4. The frequency at which the phase velocity was evaluated at (once the phase velocity curve had plateaued) is also given. The results show that the group velocity underestimates the shear modulus, particularly in the hard plaque.

The results from the dynamic experiment are shown in Figure 9.4 where a normalized carotid artery flow profile curve (a) from the programmable pump has eight consecutive data acquisitions highlighted on the curve (marked 1-8). The corresponding shear moduli estimates for each acquisition can be seen for the soft and hard plaque phantoms in Figures 9.4(b) and (c), respectively. A change in shear moduli throughout the simulated cardiac cycle can be seen in both plaques, which approximately correspond to the changes in the flow profile curve in Figure 9.4(a), with exception for ARF push 2 in the hard plaque phantom.

9.3 Study III
The average length and wall thickness for the five porcine arteries was 9.68 ± 0.32 cm and 1.34 ± 0.17 mm, respectively. The results from the ARF push length analysis with respect to shear wave bandwidth and shear moduli estimation in an ex vivo artery with a stiffened region simulating a plaque can be seen in Figures 9.5(a) and (b), respectively for 60 (dashed lines) and 120 mmHg (solid lines) in the arterial wall (blue) and simulated plaque (red). Peak bandwidths of 2030 ± 15 Hz in the simulated plaque and 1920 ± 5 Hz in the wall were measured. As the ARF push length increased the shear wave
bandwidth decreased, resulting in shear moduli overestimates. A push length of 100 µs was therefore selected to maximize the bandwidth for the angle compounding, pressure, and sensitivity experiments (see summary of methodologies section for descriptions of experiments).

The results show (Figure 9.6) that as the number of compounding angles increased the shear wave bandwidth rapidly decreased. Moreover, the low imaging frame rate made it not possible to track the propagating shear wave in the arterial wall at 120 mmHg with 5 imaging angles, in the simulated plaque at 60 mmHg with 5 imaging angles, or in the simulated plaque at 120 mmHg with 3 or 5 imaging angles. Therefore, one imaging angle was selected for the pressure and sensitivity experiments to maximize the shear wave bandwidth.
Figure 9.5 Shear wave bandwidths (a) and shear moduli estimates (b) with respect to the ARF push length in a porcine artery wall (W60, blue dashed line) and simulated plaque (P60, red dashed line) at 60 mmHg and 120 mmHg (W120, solid blue line, P120, solid red line).

Figure 9.6 Shear wave bandwidth with respect to the number of compounded imaging angles for a porcine wall (blue) and simulated plaque (red) at 60 mmHg (striped columns) and 120 mmHg (solid columns). Missing data at 3 and 5 imaging angles was due to an insufficient imaging frame rate making it not possible to track the propagating shear wave.
Figure 9.7 Shear moduli estimates with respect to arterial pressure for the simulated plaque (a) and arterial wall (b) in five arteries labeled A1-A5.

The results for the static pressure experiment for the five arteries (labeled A1-A5) are shown in Figure 9.7 for the simulated plaque (a) and arterial wall (b). The shear moduli estimates increased with respect to pressure.

The sensitivity of phase velocity analysis of the shear wave is shown in Figure 9.8 where the shear moduli estimates increased with respect to the time that the artery soaked in formaldehyde. The technique detected a small change in stiffness after 2 minutes, a larger change after 4 minutes, and the stiffness gradually plateaus as the time increases.

Figure 9.8 Shear moduli estimates with respect to the time the porcine artery soaked in formaldehyde. The error bars represent the SD from repeated measurements at each data collection point.
Figure 9.9 B-mode images of the phantom plaques with PWI tracking lines (red lines) and SWE push locations (blue circles) in phantom 1 (a), phantom 2 (b), phantom 3 (c) with the maximum plaque diameter and degree of stenosis given for each plaque. Both SWE and PWI were evaluated in the region highlighted by the yellow dashed line. (d, e, f) Sample shear wave spatiotemporal plots of the shear wave propagating to the right through phantoms 1-3 (maximum displacement of ± 2 µm given in yellow and blue colors) with the corresponding Lamb wave model curve fits (blue lines) on the upper half-bandwidth and the phase velocity of the shear waves (red dots) (g, h, i). Spatiotemporal plots (j, k, l) of the pulse-wave propagation with the linear regression of the 50% down-stroke markers overlaid on each plot for phantoms 1-3. Corresponding linear regression of the 50% down-stroke markers (m, n, o) with PWV estimates and $r^2$. 
Figure 9.10 Comparison of the mean phase velocity in the upper half-bandwidth of the propagating shear wave (blue), the shear wave group velocity (orange) and the pulse wave velocity (gray) in the plaque inclusion (a) and wall (b) for the three PVA plaque phantoms.

9.4 Study IV

A comparison of SWE and PWI in the three PVA plaque phantoms can be seen in Figure 9.9. As seen in the B-mode images (Figures 9.9(a)-(c)), the plaque morphology and thickness varied from phantom to phantom. Sample spatiotemporal plots (Figures 9.9(d)-(f) and (j)-(l)) and curve fits for SWE (Figures 9.9(g)-(i)) and PWI (Figures 9.9(m)-(o)) for the three PVA plaque phantoms are shown illustrating the difference in wave speeds between the plaque phantoms. The PWV measurements where performed approximately in the same regions as the SWE propagation (Figures 9.9(j)-(l)) and the effect of the plaque thickness on the PWV estimates can be seen in Figure 9.10(a). Additionally, Figure 9.10(a) shows that the mean velocity of the upper half-bandwidth of the phase velocity curve is similar to the group velocity of the shear wave and that the pulse wave propagates faster than the shear wave in the plaque. The wave velocities between SWE and PWV were similar in the phantom wall (Figure 9.10(b)), but the mean velocity of the upper half-bandwidth of the phase velocity was slightly faster than the SWE group velocity and PWV. The high $r^2$ values in Table 9.5 indicate high quality linear regressions for the PWV data for both the wall and plaque. The Young’s moduli (Table 9.6) were estimated by using the data in Figure 9.10 and Equations 6.4 and 6.5 for the group velocity, the shear moduli and Equation 6.5 for the phase velocity, and the PWV and Equation 6.12 for the Young’s modulus estimate using the Moens–Korteweg equation.

Table 9.5 Mean and SDs for PWV coefficient of determination ($r^2$) for the linear regression performed on the 50% down-stroke arrival time data plotted against the pulse wave travel distance in the vessel wall and plaque for phantoms 1-3.

<table>
<thead>
<tr>
<th></th>
<th>Phantom 1</th>
<th>Phantom 2</th>
<th>Phantom 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall $r^2$</td>
<td>0.99 ± 0.00</td>
<td>0.99 ± 0.00</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>Plaque $r^2$</td>
<td>0.98 ± 0.01</td>
<td>0.96 ± 0.02</td>
<td>0.94 ± 0.04</td>
</tr>
</tbody>
</table>
Table 9.6 Means and SDs of Young’s moduli (kPa) estimates in the plaque and wall for phantoms 1-3 estimated by using SWE phase velocity, group velocity, and PWI and Equations 6.4, 6.5, and 6.12.

<table>
<thead>
<tr>
<th>Location</th>
<th>Analysis</th>
<th>Phantom 1</th>
<th>Phantom 2</th>
<th>Phantom 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque</td>
<td>Phase Velocity</td>
<td>14 ± 1.4</td>
<td>12 ± 0.0</td>
<td>19 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>Group Velocity</td>
<td>7.5 ± 0.1</td>
<td>6.8 ± 0.5</td>
<td>9.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>PWI</td>
<td>5.2 ± 0.5</td>
<td>11.2 ± 1.1</td>
<td>40.8 ± 1.1</td>
</tr>
<tr>
<td>Wall</td>
<td>Phase Velocity</td>
<td>77 ± 1.4</td>
<td>59 ± 1.4</td>
<td>77 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Group Velocity</td>
<td>39.1 ± 0.3</td>
<td>34.6 ± 0.8</td>
<td>44.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>PWI</td>
<td>30.9 ± 0.8</td>
<td>35.8 ± 2.5</td>
<td>37.2 ± 2.4</td>
</tr>
</tbody>
</table>
10 Discussion

CVDs are the leading causes of mortality in the world [1] and a combination of increased awareness of preventive care, improved diagnostic techniques, as well as better treatment are needed to reduce mortality and morbidity rates.

Preventive care is simple in theory, but complex to implement in practice as it relies on a combination of personal responsibility, access to healthy dietary options, support from government social programs, support from employers, and changing social norms. There are four preventive measures individuals can take to reduce the likelihood of developing CVDs. First, avoid tobacco use or being exposed to secondhand smoking as tobacco use is associated with increased risk for CVDs [1, 3]. Second, limit alcohol consumption. The American Heart Association (AHA) recommends that if an individual does consume alcohol, limit the daily consumption to 1-2 drinks for men, and 1 drink for women [137, 138]. Third, to improve overall cardiovascular health, the WHO and AHA recommends at least 30 minutes of moderate-intensity activity at least five days per week and a moderate- to high-intensity muscle-strengthening activity at least 2 days per week for additional health benefits [139, 140]. Fourth, many studies have shown that a Mediterranean diet - which includes produce, fish, whole grains, and healthy fats - reduces the risk for CVDs [141-144]. By successfully implementing these four strategies in large populations, CVD mortality and morbidity rates will decrease, life expectancy will increase, and the quality of life at older age will improve. Moreover, other diseases such as cancer and diabetes will decrease as they are known two have similar risk factors. To implement these strategies for long-term success, education is required for different age and social segments in society. Governments need to focus on early childhood education regarding health and wellness in combination with daily physical exercise classes as well as providing healthy lunches in schools. Health education should also be provided to the public through national health campaigns on a continuous basis, focusing on socioeconomic challenged geographic regions, which often have higher rates of CVDs and lower life expectancy. Aside from education, other measures could include improving access to healthy food, subsidizing vegetables and produce, imposing higher taxes on unhealthy foods, and providing access as well as subsidizing memberships to exercise facilities. No single solution is a “silver bullet”, but many small changes can lead to an overall improvement in health resulting in substantial savings in healthcare costs overtime [145-147].

Mortality rates due to CVDs could be reduced further by improving diagnostic methods to identify vulnerable plaques. The work outlined in this thesis has focused on improving the assessment methods for reducing the incidence of cerebrovascular events. As previously stated, vulnerable plaques are identified and visually assessed by physicians in current clinical practice. A more optimal procedure
would be to identify potential vulnerable plaques at an early stage, prior to showing symptoms of CVDs (shortness of breath, fatigue, angina, feeling lightheaded or fainting, leg pain) and intervening with changes to diet and exercise habits or medication, rather than relying on more complicated procedures at later more advanced stages of CVDs, such as endarterectomy, angioplasty, or stent retrievers. The methods included in this thesis are intended to take steps towards this goal. Ultrasound has several advantages over other imaging modalities, which include being noninvasive, safe, widely available, and relatively low cost. One of the main disadvantages with ultrasound is that it is difficult to quantitatively evaluate the risk of CVDs. Therefore, physicians have relied on visual interpretation of B-mode images. It is important to clarify that quantitative ultrasound methods are not intended to replace visual assessment of CVDs, but aimed to provide physicians with an understanding of the mechanical properties of the investigated tissue. Using this information in combination with the location of the plaque, surrounding tissues, and patient risk factors, the likelihood of cardiovascular events and best possible treatments can be determined. Additionally, it is not the author's intent to argue that the ultrasound-based techniques included in this thesis are superior to other quantitative ultrasound plaque characterization techniques. Most techniques have both advantages and disadvantages, and currently there is no gold standard for ultrasound-based quantitative plaque characterization. However, it is possible that the techniques included in this thesis could be used together or in combination with other techniques to provide a more comprehensive assessment during plaque characterization.

Finally, improved evidence-based medicine is needed for the management and treatment of CVDs [148]. Currently, treatments for carotid stenosis include change in diet and increased exercise activity, medication (blood thinners and aspirin), endarterectomy, thrombolysis, and angioplasty [149-152]. There are new medical devices entering the market for the treatment of stroke, such as stent retrievers [153, 154] and vacuum catheters [155, 156] for the removal of thrombosis. For stent retrievers, a collapsed wire mesh is inserted into the artery via a catheter and pulled through the thrombus. The wire mesh is then expanded, attached to the thrombus, which is thereafter removed by retracting the wire mesh through the artery. Emboli may dislodge from the thrombus during removal, and therefore vacuum catheters are being developed to try to minimize the amount of emboli that may drift further down the arterial tree during the procedure. To prevent cerebral sequelae, time is critical and measures should be taken within 6 hours from the time of stroke. Large clinical trials are being performed to evaluate the efficacy of stent retrieval systems [157-160].

The results from Studies I-IV are discussed in detail for each technique. Thereafter, the general limitations of the techniques are described followed by future perspectives on plaque characterization.

10.1 Speckle Tracking

Speckle tracking was used in Study I to measure the strain throughout a simulated cardiac cycle in carotid artery phantoms with plaque inclusions and the strain was thereafter validated against sonomicrometry, an alternative ultrasound-based technique to measure strain. Next, the strain was measured in nine in vivo carotid artery plaques attempting to identify vulnerability with the hypothesis that vulnerable plaques exhibit increased strain throughout the cardiac cycle compared to more stable plaques. The results were then compared to visual assessment of plaque vulnerability by two experienced physicians using the Gray-Weale scale.

The phantom study showed agreement between strain measured by speckle tracking and sonomicrometry. The correlation was stronger in the radial direction ($r = 0.96$) compared to the longitudinal direction ($r = 0.75$). It is more difficult to track the longitudinal motion for two reasons. First, lower correlation in the longitudinal direction is expected as the lateral resolution is inferior to the
axial resolution. Second, there is less longitudinal motion compared to radial motion due to the nature of the compliance of the simulated plaque as the pulse wave from the cardiac cycle travels through the vessel exerting greater radial force compared to longitudinal force on the arterial walls. Additionally, the 2-D motion tracking algorithm does not take into account out of plane motion, which may affect radial and longitudinal motion estimation.

When comparing the speckle tracking and sonomicrometry peak radial strains in the Bland-Altman plot in Figure 9.2(a), it can be seen that the speckle tracking strain overestimates at low peak strains (between -5% and -10%) and underestimates at high peak strains compared to sonomicrometry. Whereas in the longitudinal direction the speckle tracking algorithm underestimates for low peak strains and overestimates for large peak strains compared to sonomicrometry. The results also show that the radial strains were approximately 5 times larger than the longitudinal strains. The Bland-Altman plots confirm that speckle tracking and sonomicrometry estimate similar strains in the phantom plaques with margins of error from 3.48% to -4.40% in the radial direction and 2.78% to -2.29% in the longitudinal direction.

The main difference between this study and other studies that have attempted to measure strain in plaques using speckle tracking is that other studies are invasive and use IVUS [27, 73, 74, 92, 93, 105, 161], whereas our method is noninvasive and uses conventional ultrasound. Moreover, many other IVUS techniques lack validation against other strain measurement techniques. It is also important to point out that many of the referenced studies focused on arterial wall strain [92, 93, 161]. Study I is an important contribution to the field since it noninvasively focuses on evaluating plaque strain to assess the likelihood of plaque rupture.

The in vivo study showed agreement between the increased pulse-pressure adjusted peak radial and longitudinal strains measured in carotid artery plaques and the Gray-Weale visual assessment where plaques rated 1 and 2 were considered more likely to be associated with vulnerable plaques. The strain measurements were normalized according to the patient’s blood pressure as it was one of the driving forces for radial and longitudinal strain (in addition to shear forces from blood flow and tethering forces from the heart) and variation in the blood pressure could bias the results. Three shortcomings of the study include that the sample size was small (n = 9), only one plaque was considered a more heterogeneous fibrous plaque, and the visual assessment was not validated by histology. Still though, the results indicate that measuring plaque strain by speckle tracking is feasible and that different types of plaque can be distinguished by type based on the peak radial and longitudinal strains. Given a larger sample size with more fibrous and calcified plaques, this distinction would be more evident. The technique is not suitable for all plaques as seven of the plaques where omitted from the study due to insufficient diameter size (less than 2 mm) or poor data quality due to probe motion during acquisition. The study also showed that it was not possible to characterize plaques based on the GSM measurement, which differs from previous studies [162]. Using the GSM of plaques to determine the plaque vulnerability has been disputed where recent studies [163] show no additional value in using GSM to evaluate vulnerable plaque, which conflicts with previous studies [164].

10.2 Shear Wave Elastography

SWE was used in Studies II, III, and IV to characterize hard and soft plaque phantoms, demonstrate feasibility in ex vivo arteries, and compare plaque characterization ability in phantoms to PWI. In Study II, SWE was used to characterize PVA carotid artery phantoms with plaque inclusions in a static setup and the results were validated by mechanical testing. Thereafter the PVA phantoms where connected to a pump and the shear modulus was measured using SWE in a dynamic environment.
In the static experiment there was agreement between the shear moduli estimated by SWE and mechanical testing as shown in Figure 9.3. There was greater variability in the SWE shear moduli estimates for the hard plaque compared to the soft plaques, as seen in Figure 9.3 and Table 9.2, as the shear waves propagate faster through the stiffer material. Hence, less ultrasound frames recorded the shear wave propagation making it more difficult to track with the autocorrelator algorithm. The upper half-bandwidth fits were in general better than the full bandwidth fits between the Lamb wave model and the phase velocity data as indicated by the RMSE values in Table 9.3.

Three observations can be made from the results in Table 9.4. First, similar group and phase velocities were measured in the soft plaques and vessel wall, indicating that the phase velocity curves plateau near the shear wave group velocities. The phase velocities for the hard plaques never fully plateaued, making their values less meaningful. Second, the shear moduli estimates calculated from phase and group velocity were significantly different in the hard plaques and phantom walls, despite similar phase and group velocity shear wave speeds. This may be due to the effects of frequency dispersion caused by geometry as the hard plaque was embedded in the phantom wall. Moreover, the assumptions of a homogeneous, isotropic, incompressible, infinitely large medium when using equation 6.4 are invalid when calculating the shear modulus using group velocity. This results in significant underestimates and indicates that phase velocity analysis is necessary. Third, similar shear moduli were estimated in the soft plaque by phase and group velocity indicating that the assumptions of a homogeneous, isotropic, infinitely large medium hold for the soft plaque allowing the shear modulus to be calculated by group velocity in this experimental setup. The results are not expected to transfer to *in vivo* conditions where the heterogeneous composition of vulnerable plaques must be taken into consideration and a study must be performed to investigate if group velocity can estimate the shear moduli in vulnerable plaques.

In the dynamic test, the shear moduli estimates for the soft and hard plaques seen in Figure 9.4(b) and (c) varied throughout the cardiac cycle, but not as much as expected. For the soft plaque, the shear moduli varied approximately between 4 to 5 kPa and followed the shape of the simulated carotid flow profile. The hard shear moduli varied approximately between 87 to 99 kPa and followed the shape of the simulated carotid flow profile for the most part except the second data point. This variation in shear moduli was significantly less than the shear moduli variations Couade, *et al.*, [61] recorded *in vivo* where he measured a dynamic range of approximately 50 kPa throughout a cardiac cycle. The difference in results could be due to the fact that the elastic properties of PVA differ to that of the arterial wall or that the programmable pump could not generate a large enough transient pressure increase. Moreover, Couade, *et al.*, measured the shear modulus in the arterial wall compared to measuring the shear moduli in hard and soft PVA phantom plaques, as in our experiment. Despite the difference, the experiment showed it is possible to filter out the background motion from a dynamic environment and perform SWE measurements in simulated plaques. This is possible because the background motion results in low frequency noise in the frequency domain, which was filtered out using a bandpass filter.

The shear moduli values of the PVA plaques created in Studies II and IV compare favorably to the shear moduli measured in human plaques using tensile and compressional testing ranging from 7-100 kPa [165]. While similar shear moduli values can be achieved by varying the number of freeze-thaw cycles, the dimensions of the PVA phantoms were larger than that of the human carotid arteries. The wall thicknesses of the phantoms were 3 mm while the wall thickness of a human carotid artery is approximately 1 mm. This difference in thickness made it easier to track propagating shear waves in the phantoms compared to *in vivo* measurements. The plaque inclusions were large and offset to one side of the phantom due to limitations of the manufacturing technique. It would have been more preferable to create small plaques in the center of the phantom to reproduce a more realistic plaque configuration.
Measuring the shear modulus of human plaques using SWE will be more challenging compared to PVA phantom plaques due to the variation of morphology and composition of in vivo plaques.

In addition to demonstrating feasibility in ex vivo arteries with simulated plaques, Study III focused on addressing two limitations from Study II, namely studying the relationship between ARF push lengths, the number of coherent compounded imaging angles, and shear wave bandwidth. The push length experiment showed that bandwidths of approximately 1500 Hz are required to obtain consistent shear moduli estimates as seen in Figures 9.5(a) and (b). For bandwidths less than 1500 Hz, which is a result of push lengths longer than 300 µs, the shear moduli estimates increase rapidly due to insufficient data points when applying the Lamb wave model to the upper half-bandwidth of the phase velocity data. This is likely the reason why the phase velocity shear moduli estimates for the hard and soft plaque phantoms in Study II (push length 450 µs) overestimated compared to mechanical testing (Figures 9.3(a) and (b)). Figures 9.5(a) and (b) also show that larger bandwidths are required for simulated stiff plaques at high pressures for consistent shear moduli estimates. Finally, it was more difficult to obtain consistent estimates in the simulated plaques compared to the artery wall since the bandwidth dropped faster with respect to push length.

Figure 9.6 shows that it is more important to have a high imaging frame rate in order to track the propagating shear wave rather than improving the image quality by increasing the number of compounded imaging angles at the expense of reduced frame rate, especially in stiff plaques and regions of increased arterial stiffness. The need for a high frame rate is evident since the shear wave is tracked based on phase differences in the RF signal and not from the B-mode image. Hence, one would not expect performance improvements due to slightly enhancing the image quality with coherent compounded imaging. It was not possible to track the shear wave propagation at 3 imaging angles for the plaque at 120 mmHg. Moreover, it was only possible to track the shear wave propagating through the wall at 60 mmHg at 5 imaging angles. For 3 and 5 imaging angles the bandwidth was drastically reduced by approximately 1500 Hz, making it not possible to provide reliable shear moduli estimates, as seen from the ARF push length results (Figures 9.5(a) and (b)).

The shear moduli estimates increased with respect to an increase in pressure in the simulated plaque and arterial wall, as can be seen in Figures 9.7(a) and (b), respectively. For many of the arteries, the shear moduli increased approximately linearly with respect to in vivo pressures (60-120 mmHg). The linear response is in agreement with Bergel’s results [166], where an approximate linear relationship between static pressure and Young’s modulus was found in canine thoracic aortas for pressures up to 120 mmHg. It is unlikely that this linear relationship between static pressure and shear modulus holds for in vivo conditions, which is possibly due to the experimental setup. Rapid changes in pressure would likely induce a nonlinear response in the arterial wall. The increase in shear modulus with respect to pressure differs from Bernal’s, et al., results [67], where a similar experiment in porcine carotid arteries was performed measuring the shear modulus with respect to static pressure from 10-100 mmHg and measured a slight decrease in the shear moduli as the pressure increased. Couade, et al., [61] found that the shear modulus varied between $130 \pm 15$ kPa in systole and $80 \pm 10$ kPa in diastole (the blood pressure was not provided) in a carotid artery wall of a healthy human, which is in agreement with our results of $97 \pm 10$ kPa at 120 mmHg and $50 \pm 5$ kPa at 60 mmHg as shown in Figure 9.7(b). Additionally, Couade measured a difference of approximately 50 kPa in wall stiffness for the healthy arterial wall throughout the cardiac cycle, which matches our mean difference of 56 kPa in wall stiffness (Figure 9.7(b)) between systolic and diastolic pressures.
Figure 9.8 shows the ability of SWE to detect small stiffness changes in the artery, which could potentially allow for early detection of atherosclerosis. The new biomarker could allow for earlier detection, compared to measuring intima-media thickness, assuming that a change in arterial stiffness occurs prior to a visually detectable thickening of the arterial wall. The ability to detect small changes in plaque stiffness suggests that SWE may be suitable for plaque characterization, and distinguish between many different types of plaque with a lipid, necrotic, fibrotic, or intraplaque hemorrhage core.

The SWE results from Study IV are discussed in section 10.3 as they are compared to the PWI results.

To avoid plaque rupture, patient safety must be taken into consideration when exciting in vivo plaques with ARF. It would be most ideal to focus the ARF next to the plaque so that the resulting propagating shear wave travels through the nearby plaque, but there may be instances where the generated shear wave attenuates and does not enter the plaque and the ARF push has to be focused in the plaque directly. Doherty, et al., [34] simulated exciting various types of plaque with ARFI and showed that the stress induced by the ARFI is approximately three orders of magnitude less than the force imposed by blood pressure. Additionally, several ARFI studies have been performed on in vivo plaques where the plaques are excited with ARFI directly and no complications have been reported [32, 33, 38]. Exceeding thermal index (TI) limits and damaging the tissue due to overheating is unlikely with SWE or ARFI as the bursts of ARF are very short (hundreds of µs), and the patient scan time can be performed in a few minutes. The mechanical index (MI) is primarily a concern when using contrast agents as high MI values can result in cavitation of the contrast bubbles, but contrast agents are not typically used for SWE measurements. Cui, et al., [167] compared using a contrast agent to a normal SWE measurement when measuring the shear wave velocity in liver, but found no difference in the results. [167]

### 10.3 Pulse Wave Imaging

To the best of our knowledge, Study IV is the first study comparing SWE and PWI for characterizing plaque in a phantom setup. When comparing different techniques, it is difficult to create an experimental setup with identical test conditions for both techniques to make a fair comparison as the techniques have different underlying mechanisms and estimate different measurements (shear modulus versus PWV). SWE and PWI are similar in a sense that both methods track waves propagating along an artery (or through a plaque), although the waves are of different type (shear waves versus pressure waves) and generated by different sources (acoustic radiation push versus ventricular ejection).

The three phantom plaques in Figures 9.9(a)-(c) had varying degrees of stenosis, varying maximum plaque diameters (measured from the B-mode image), and different morphology. The plaque size and shape was a result of how much PVA was inserted into the wall cavity during the manufacturing process. While the different geometries had little effect in the shear wave group and phase velocity propagation speeds (Figure 9.10(a)), it had a larger impact on the PWV (Figures 9.9(m)-(o) and Figure 9.10(a)). The PWV increases as the plaque diameter decreases. This indicates using PWV to characterize in vivo plaques may be difficult if the results vary due to plaque thickness and morphology making it difficult to determine the contribution of change in PWV based on the variation of plaque composition versus the variation of plaque thickness. Moreover, the variation in plaque morphology leads to complex wave reflections for the shear waves as seen in Figures 9.9(d)-(f). The reflections made it difficult to get good lamb wave fits for the shear waves (Figures 9.9(g)-(i)). The pulse wave also reflected off the fixture and reverberated in the phantom (Figures 9.9(j)-(l)), which is an artifact of the experimental setup. These types of reflections can occur near the carotid artery bifurcation in vivo but are typically not as strong as in this experiment [168, 169].
The mean upper bandwidth velocity of the shear wave propagated at similar speeds as the mean group velocity in the plaques (Figure 9.10(a)). This implies that the shear wave phase velocity plateaus near the group velocity and the thickness of the plaque (≈6mm) is large relative to the wavelength of the propagating shear wave (≈1.8 mm). The plaque, relative to the shear wave wavelength, can be considered an infinite large medium and Equation 6.4 and 6.5 can be applied to calculate the shear and Young’s modulus. Similar wave propagation speeds where found in the wall for SWE and PWV. While the shear wave group velocity and PWV are functions of the density, modulus of the vessel wall, and geometry [77], the similarity between the shear wave velocity and PWV is not an expected result as the wave velocities are a function of different mechanisms. The shear wave velocity is a function of the density, shear modulus, and thickness of the vessel wall while the PWV is dependent on the Young’s modulus, wall thickness to vessel radius ratio, and blood density [77]. The results in Figure 9.10(b) are a coincidence as the ratio of the lumen size to wall thickness produces similar PWV and SWE wave velocities. Table 9.5 shows high values for the coefficient of determination for the linear regression performed on the 50% down-stroke arrival time PWV data indicating high quality PWV estimates.

The Young’s moduli values in Table 9.6 were calculated with Equations 6.4, 6.5, and 6.12. In the wall, the Young’s moduli were higher when calculated with the phase velocity data compared to the group velocity and PWV estimations. The Young’s moduli estimates based on phase velocity are believed to be the most accurate in the wall since the shear moduli values were validated with mechanical testing in similar phantoms in Study II. There is great variation in the PWV-based Young’s moduli estimates in the plaque between the three phantoms, which is due to the difference in PWV estimates as a result of the varying plaque thickness. It is clear that the Moens-Korteweg equation is not suitable to estimate the Young’s modulus in plaques and that it is more appropriate to compare wave velocities.

The Young’s modulus results differ from previous work. Oviedo, et al., [170] measured the PWV using PWI in polyacrylamide gel concentrations and compared the results to mechanical testing and found good agreement in soft (E = 51 kPa) and stiff (E = 251 kPa) vessel phantoms, but a -18% underestimation in normal stiffness (E = 112 kPa). Vappou, et al., [169] found mixed agreement between the Young’s modulus calculated from PWI and mechanical testing in soft PVA phantoms with varying concentrations of PVA. Bernal, et al., [67] found similar agreement between the Young’s modulus calculated from the shear wave group velocity and from the pulse wave for a urethane tube. None of the mentioned studies had vessel phantoms with plaque inclusions.

Both SWE and PWI have benefits and disadvantages regarding in vivo heterogeneous plaque characterization. It is worth highlighting that PWI has the benefit of tracking pulse waves, which are easier to track due to larger tissue displacements (40 µm) [169] compared to a shear wave push, which only displaces the tissue 2-10 µm [61, 171, 172]. This may be advantageous for in vivo measurements.

10.4 Limitations
There are several general limitations that affect the performance of speckle tracking, SWE, and PWI. The level of effectiveness of each technique is in part due to the experience of the sonographer and their ability to obtain high quality images of the carotid artery and plaques. Each technique is dependent on image quality and therefore is sensitive to probe motion and requires a steady hand while acquiring data. This limitation could potentially be reduced by designing a probe holder to be used during acquisition. Speckle tracking was in particular sensitive to probe motion in our in vivo experiments as the patient was required to hold their breath for 3 seconds during acquisition, which is difficult for some elderly sick patients. There is also a difference in acquisition time between speckle tracking and SWE,
where 1 s of data are collected for speckle tracking (for one cardiac cycle) and a few milliseconds of data are collected for SWE, inherently making speckle tracking more sensitive to probe motion.

The three techniques are dependent on the image acquisition frame rate; in particular SWE in order to obtain sufficient bandwidths when performing phase velocity analysis. While a high frame rate leads to greater temporal resolution, the trade-off between spatial resolution and imaging frame rate was not explored in Study IV. Speckle tracking, unlike SWE and PWI, did not use plane wave imaging, but acquired data with traditional B-mode linear scanning. It may be possible to improve the performance of the technique by implementing plane wave imaging to increase the imaging frame rate when tracking the plaque strain throughout the cardiac cycle, but there is a risk of incorrect sub-pixel motion estimation if the speckles have not moved enough from frame to frame.

The techniques studied in this thesis were 2D techniques. Thus, the speckle tracking algorithm did not consider out of plane motion and SWE and PWI did not consider out of plane propagation of the shear and pulse waves, respectively. Moreover, since the plaque core can vary in composition in 3D, it is possible that these 2D techniques characterize a part of the plaque that is stable, while missing part of the plaque that may be vulnerable. The sonographer must therefore acquire short-axis and long-axis images of the plaque or 3D methods of these techniques must be developed.

Intuitively, the potential to characterize plaques by all three methods is limited by the plaque’s size. In Study I, the in vivo plaque diameter had to be greater than 2 mm to measure the plaque strain. The limitation of plaque size was not formally studied for SWE or PWI. However, based on our experience, reliable shear modulus estimates can be obtained in a shear wave propagation region 1 cm in length for SWE. As aforementioned, the plaque size limitation for PWI will be dependent on the imaging frame rate and center frequency, but piecewise Pulse Wave Imaging (pPWI) estimates have been performed on 2-4 mm wall segments of murine normal, atherosclerotic and aneurysmal arteries in mice [98]. The simulated plaques in the study were modeled in the carotid artery, but in practice it is more common for the plaque buildup to occur in the carotid bifurcation [173]. Additionally, many of the PVA plaque phantoms were modeled as homogeneous hard or soft plaques, but it is more common for real in vivo plaques to consist of mixed composition [3].

In Study I, there were several study specific limitations worth mentioning. The phantom plaque had a significant radial strain gradient as a result of greater plaque movement near the lumen versus the movement facing the posterior artery wall. The gradient was a result of the experimental setup and was not as prominent in vivo. To avoid the extremes, the ROI height was selected to be 50% of the vertical plaque size and the ROI was placed in the center of the plaque in order to measure an average value of the strain in the plaque. For the in vivo study it was not intuitive where to place the ROI in heterogeneous plaques with mixtures of echolucent and echogenic regions. To average the strain value in both regions we attempted to place the ROI in both echoluent and echogenic regions. Additionally, comparing the plaque strain to plaques visually assessed on the Gray-Weale scale is not a gold standard reference method as the visual assessment is subjective. A better reference method would have been to compare plaque strains to histology, but it was not possible to obtain the plaques post-surgery in this study. Moreover the in vivo study was performed in a small population and only one type 3 plaque was included. A larger patient population with more diverse plaques would have been desirable. Finally, circumferential strain was not considered in this study, which may potentially contain clinically significant information.
In Studies II-IV, modelling the shear wave propagation through the plaque as a Lamb wave propagating through an infinite plate submerged in a viscous fluid can be questioned due to lack of physical connection. The model of a Lamb wave propagating through a plate and a model of a shear wave propagating in a thin hollow cylinder converge a higher frequencies (above 1 kHz) [61] and therefore accurate results are achieved when curve fitting the upper half-bandwidth of the fundamental mode of the shear wave to the Lamb wave model. Due to the complexity of an analytic solution for a wave propagating in a hollow cylindrical structure submerged in a fluid, the plate model is the current best choice when performing phase velocity analysis to find the shear modulus.

In the phase velocity analysis, only the fundamental antisymmetric Lamb wave in a plate was studied and data was cropped out once the shear wave had fully dissipated in order to reduce noise when performing the FFT. The wave reflections could possibly provide plaque composition information and other studies have investigated higher order shear wave modes [67]. Characterizing plaques using SWE will be more difficult in vivo for two reasons. First, additional ARF may be required to generate a sufficiently strong shear wave to propagate through a plaque, particularly soft vulnerable plaques as seen in Study II, and the current limits for ultrasound output intensity may have to be increased. Second, in Studies II-IV the shear wave was tracked horizontally on single pixel lines. The carotid artery is often angled or curved in vivo and tracking methods on a curved surface must be developed.

In Study II, it was difficult to make a direct comparison between SWE and mechanical testing shear moduli estimations since different phantoms were used for each measurement. However, special care was taken to mimic the conditions in the static SWE experiment to produce an identical mechanical stress state between the setups. Although the mechanical test was used as a reference value, it was also subject to error, which should be taken into consideration.

It was not possible to obtain arteries with real plaques in Study III. The stiffened arteries aimed to mimic increased arterial stiffness or homogeneous stiff plaques located in the arterial wall due to the limitations of the plaque fabrication technique. The plaque model has limitations due to the geometry and lack of increased echogenicity of the simulated stiff plaques. The porcine aorta intima-media-adventitia thickness was slightly thicker (average thickness 1.34 mm) than human carotid intima-media thickness (approximately 0.8 mm) reported in literature [9]. Porcine carotid arteries were not available and the aorta was selected as the artery of choice due to availability and similar dimensions to the human carotid artery.

In Study IV, the Moens-Korteweg equation is a limitation when estimating the Young’s Modulus as the equation assumes an infinitely long, straight, isolated, and cylindrical vessel with elastic, isotropic, and homogenous walls, containing a homogenous, incompressible and nonviscous fluid [77]. Due to the plaque in the vessel wall, many of the assumptions are invalid. Additionally, the equation does not take into account wall thickening or a dynamic radius throughout the cardiac cycle. Taking this into consideration, in addition to that the assumptions for Equation 6.4 are not truly met when calculating the shear modulus from the group velocity, makes the Young’s Moduli calculated from the different techniques not a one-to-one comparison.

In Study IV, A single pulse wave was generated by pinching the rubber tube connected to the phantom fixture. It would have been more realistic to connect the phantom to a programmable pump, which was attempted but resulted in reflected waves continuously propagating in the phantom due to the experimental setup making it difficult to track the desired pulse wave.
10.5 Future Perspectives on Plaque Characterization

The techniques developed in this thesis have the potential to provide more accurate plaque characterization through quantitative assessment of the mechanical properties of carotid plaques. This will enable physicians to provide more accurate diagnoses, select better treatment for patients, and avoid unnecessary surgery (such as endarterectomy and angioplasty) when not needed. This could lead to improved morbidity rates for CVDs. Additionally, speckle tracking, SWE, and PWI can be used for the early detection of CVDs reducing the time for early intervention, although SWE must first be tested in vivo. The plaque characterization accuracy will additionally improve as the techniques are expanded to 3D ultrasound, eliminating out of plane motion and wave propagation. Finally, the techniques will reduce the subjectivity when visually characterizing plaque and shorten the learning curve when interpreting ultrasound-based examinations.

To realize these techniques in a clinical setting several improvements must be made. First, all of the techniques involve many manual steps in the processing of that data. Therefore, more automated post-processing must be developed to make the techniques more user-friendly. Second, it is difficult to design a “one-size-fits-all” system that accurately characterizes every plaque. Hence, smart algorithms are needed that change certain parameters (such as the kernel size or number of tracking lines) based on the size and location of the plaque. Third, the algorithms require large amounts of computational power and the post-processing is currently performed offline after the examination. Thus, processing speed can be improved by optimizing the post-processing code and reducing the collected data to the minimum data required for accurate characterization. Furthermore, processing times will be reduced with the advancement of increased computational power.
11 Conclusions

The studies presented in this thesis have developed, demonstrated feasibility, and evaluated the potential of three ultrasound-based methods to characterize carotid artery plaques and assess arterial wall stiffness in vitro, ex vivo, and in vivo. The techniques included in this thesis have the potential to noninvasively evaluate the mechanical properties of carotid artery plaques and may reduce subjectivity when visually assessing B-mode images, which would improve diagnoses for patients suffering from cerebrovascular diseases. The specific conclusions for Studies I-IV are summarized below.

Study I A previously developed speckle tracking algorithm was validated by sonomicrometry in CCA plaque phantoms in vitro and demonstrated feasibility of radial and longitudinal speckle tracking strain estimation in carotid artery plaques in vivo. The results show potential for assessing plaque vulnerability based on strain, where increased radial and longitudinal strain can be measured in dominantly echolucent and primarily echolucent plaques with some areas of echogenicity.

Study II Hard and soft CCA phantom plaques were characterized with SWE by using phase and group velocity analysis while being hydrostatically pressurized followed by validating the results with mechanical testing. Phase velocity analysis was necessary in the hard plaque and vessel wall, whereas group velocity analysis provided similar results in the soft plaque. Moreover, the shear modulus was successfully measured in the phantom plaques throughout a simulated cardiac cycle indicating the possibility to apply the technique in vivo.

Study III An approximate linear response in stiffness with respect to realistic in vivo diastolic and systolic pressures was found in in five porcine arteries with simulated increased arterial stiffness or stiff plaques using SWE. A 100 µs ARF push length resulted in the largest bandwidth and a minimum bandwidth of approximately 1500 Hz is necessary for consistent shear modulus estimates. Additionally, a high imaging frame rate is more important than a lower frame rate with better image quality when using SWE to estimate arterial wall and plaque stiffness.

Study IV Both SWE and PWI were able to distinguish the soft plaque from the wall in the plaque phantoms, but the PWV was higher than the shear wave propagation speed in all plaque phantoms. SWE had lower standard deviations than PWI and the PWV estimates were more sensitive to the plaque thickness and morphology compared to SWE. However, PWI has the benefit of examining a larger plaque and tracking a stronger wave
propagating over a longer region compared to SWE. The techniques can be used to characterize plaque and monitor the progression of atherosclerosis in the arterial wall.
12 Future Work

While the studies included in this thesis primarily focused on the development and feasibility of speckle tracking, SWE, and PWI, work still remains to implement the techniques in a clinical setting. Potential future work is listed below.

- Perform a larger *in vivo* study for SWE, PWI, and speckle tracking with a more diverse population of plaques. The study should include patients undergoing an endarterectomy and be validated against histology. If a large plaque strain database is collected then physicians could use this database as a reference to assess vulnerable plaques.

- Improvements to the speckle tracking algorithm include adding a contrast agent that could possibly improve the edge detection of the plaques, leading to better outlines by the physicians which may result in better motion tracking. Additionally the tracking can be improved using a high frequency probe and the temporal resolution could be increased by implementing plane wave imaging for speckle tracking.

- Perform an *in vivo* study using SWE to characterize CA plaques and validate the results against histology. Develop a user friendly interface for physicians and determine if estimating the shear modulus is necessary to evaluate plaque vulnerability or if estimating the shear wave speed is sufficient.

- Perform additional tests in plaque phantoms with different geometries and heterogeneous compositions.

- Extend SWE and speckle tracking to short-axis images and test in plaque phantoms.

- Improvements to the SWE algorithm include tracking the shear wave propagation along a curved surface for an easier *in vivo* implementation and using a model of a wave propagating through a cylinder when performing phase velocity analysis.

- An *in vivo* comparison of SWE and PWI could be performed to understand how the techniques can complement each other when characterizing real carotid plaques.

- All techniques could benefit from designing a probe holder to reduce transducer motion during data acquisition.
Finally, all techniques could be implemented in 3D to account for out of plane motion and wave propagation.
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