ABSTRACT

MOHANTY, KAUSTAV. Complex Tissue Characterization Using Ultrasound Multiple Scattering (Under the direction of Dr. Marie Muller).

Ultrasound imaging has been around for a very long time and exhibits high sensitivity and specificity in diagnosis of a variety of pathologies. Different ultrasound imaging modalities have the ability to characterize biological tissues and quantify macro-mechanical and micro-architectural properties. In principle, traditional ultrasound imaging is based on the concept of echolocation. There is a linear relationship between distance and time. However, in biological tissues such as the bone or the lung parenchyma, traditional imaging modalities fail due to large amounts of multiple scattering. The presence of marrow (bone) or alveolar sacs (lungs) make the ultrasound wave bounce around generating large amounts of multiple scattering, distorting images and destroying the linear relationship between distance and time. Multiple scattering and coda signals have been considered detrimental for imaging. However, as the wave is propagating in such a complex and heterogeneous media, each scattering event is an opportunity for the ultrasound wave to pick up information about the micro-architecture of the media in which it is propagating. Therefore, multiple scattering coda offers potential advantages for characterization of complex biological tissue such as the lung parenchyma or the bone.

This thesis addresses the algorithm developed for characterizing complex heterogeneous media. The approach presented here is based on ultrasound multiple scattering and exploits the complexity of ultrasound propagation in a heterogeneous highly scattering media. The acquisition of an inter-element response matrix (IRM) allows us to calculate transport parameters such as the diffusion constant D and transport mean free path L* by separating the incoherent and coherent intensities in the near field, and measuring the growth of the incoherent diffusive halo over time. In order to validate this algorithm, D and L* measurements are first carried out using FDTD (Finite
Difference Time Domain) simulations in 2-D with heterogeneous geometries with strong scatterers (Water as propagating medium and air/aluminum scatterers) with varying area fraction. The algorithm is tested in melamine sponge phantoms with varying air volume fraction. For in-vivo studies, fibrosis is induced in Sprague-Dawley rats using bleomycin and edema is induced in them using an ischemia reperfusion injury (IRI). Finally, this algorithm is applied to in-vivo dog lung clinical study for dogs suffering from congenital heart disease to identify extra-vascular lung water and detect the presence of pulmonary edema.

We then extend the algorithm in the near-field for mapping the micro-architecture of complex media based on the measurement of the local measurement of the diffusion constant D. This new algorithm for mapping D in 1D and 2D is validated with simulations and experiments. For both simulations and experiments a linear array of ultrasound transducers is used. Acquiring sub-IRMs by using subsets of elements, and calculating the growth of the diffusive halo for each sub-matrix provides an estimate of a semi-local Diffusion Constant, enabling a 1D and 2D mapping of the scatterer density or volume fraction in a strongly heterogeneous medium. This algorithm is suited to image lesions (hypoechoic) or targets (hyperechoic) in geometries, which are highly complex in nature. Standard ultrasound imaging techniques fail to detect such lesions/targets due to aberrations and the loss of linearity between distance and time, caused by multiple scattering of ultrasonic waves. In this thesis, we display a unique algorithm with the capability to predict the location as well as the size of such lesions/targets by using these multiple scattered ultrasound signals to its advantage. Lesions/targets are embedded at varying depths inside multiple scattering media with varying density of scatterers. In the simulations, aluminum scatterers are used as the source of multiple scattering and heterogeneity in the propagating media (water). In the experiments, melamine sponges are used, with air as the scattering source. Suddenly
changes in this growth indicates the presence of a region with profoundly reduced heterogeneity, indicative of the presence of a lesion/target. This methodology is combined with a depression detection algorithm enables us to predict the size and the location of lesion/targets. This methodology is then applied to quantitatively characterize the lung parenchyma and detect the presence of solitary pulmonary nodules for live imaging during intra-operative video assisted thoracic surgery (VATS).

This thesis then presents a neural network methodology to characterize osteoporosis in the cortical bone using frequency dependent ultrasound attenuation. The cortical bone is also highly scattering in nature and traditional imaging is a challenge. Using ultrasound attenuation, once can acoustically characterize the bone structure, however solving the inverse problem remains to be a major challenge. We present a method, which combines ultrasound attenuation measurement with an artificial neural network to predict micro-architectural parameters of the bone such as pore diameter ($\phi$), pore density ($\rho$) and porosity ($\nu$).

The main goal of my thesis research is to develop a clinically acceptable quantitative ultrasound imaging modality, which has the ability to characterize highly complex biological tissue such as the lung parenchyma and the cortical bone. By doing so, one can increase the sensitivity and specificity of ultrasound imaging for the diagnosis of a variety of pathology in complex biological tissue.
Complex Tissue Characterization Using Ultrasound Multiple Scattering

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**BIOGRAPHY**

I was born in Bhubaneshwar (India) to Mamun and C.P. Mohanty. Being born in an Army family, stability in terms of location was non-existent. After living across multiple states in India, and changing 11 schools, I decided on pursuing my Manufacturing Engineering at B.I.T.S Pilani, India.

After graduating with my bachelors in engineering and masters in science, I decided to go work for TATA Technologies as a CAE Engineer. Gaining HVAC modeling experiences, it only made sense to pursue a PhD in a related field. I moved to North Carolina 4 years back to study fluids and thermal engineering. However, as fate would have it, I completely shifted tracks to Acoustic Radiation and Ultrasound application in the biomedical field. As time passed, I grew more and more passionate about the biomedical ultrasound field and this dissertation is a testimony to that passion.
ACKNOWLEDGMENTS

It is time I finally acknowledge all the people who have played a major role in finishing this thesis. Getting a PhD is a mammoth task and a huge number of people had a major role to play in this journey. It is not possible to enumerate all of them but I would like to express my gratitude to some people or some things (Mathwork and GitHub).

I am particularly grateful to my advisor, Dr. Marie Muller. She gave me a chance to be her graduate student. She trusted a person with no ultrasound background with one of her first projects. Her excellent guidance, patience and mentorship has made this dissertation possible. I greatly appreciate the assistance of Dr. Thomas Egan for his support and insight into the biological aspects of my research.

After thanking the big guns, I would also take this opportunity to thank the trio at Egan Lab: “John, Mir and Behrooz”. These people were key in helping me carry out most of my in-vivo experiments as well as putting up with unreasonable requests and the dumbest questions about biological tissues. To Omid, Yasi and Micah at the Muller Lab for making this learning experience at NC State so rewarding, thank you.

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Finally, I feel greatly indebted to my parents (CP and Mamun) and my humongous joint family, whose love and faith for me has always been my inspiration for every activity that I undertake. They were always there for me when I needed them and I cannot imagine myself going through all of this without their help and support.
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CHAPTER 1 - Introduction

1.1 Challenges in Imaging Heterogeneous Biological Tissues

Sonography is a very effective technique, which uses ultrasound waves to create an image of the body for diagnosis purposes. The concept of ultrasound imaging is based on echolocation. The working principle of ultrasound imaging uses a transducer emitting a pulse of high frequency mechanical wave, which propagates through the tissue. It undergoes multiple phenomena as it propagates which can be divided into reflection, transmission and scattering. These phenomena can be seen when the wave moves from one medium into another. The beam is partly reflected back at the tissue interfaces. Backscattered signals when plotted using a Hilbert transform generates an ultrasound image. In most human tissues, ultrasound imaging is possible due to similar acoustic impedances. However, for media such as the bone (bone water matrix) or lungs (tissue air matrix) the attenuation is very high and the received signal (Figure 1.1) is very complicated making it hard to reconstruct the micro-architecture of the lung.

Figure 1.1: Multiple scattering by alveoli: the wave visits multiple alveoli before returning to the probe. The resulting signals are complex and embed information on the lung microstructure.
Lung ultrasound characterization has remained elusive due to the presence of air-filled alveoli and the very complex micro-architecture of the lung tissue. The lungs at the peripheral regions can be considered a porous media with alveolar air sacs, which act as scatterers when the ultrasound wave propagate through it (Figure 1.2). As the wave interacts with the alveolar sacs large specular reflections are also noticed due to the drastic impedance change between tissue ($\rho=990$ kg/m$^3$, $c=1540$ m/s) and air ($\rho=1.00$ kg/m$^3$, $c=340$ m/s). These specific properties of the lung tissue are responsible for ultrasound multiple scattering, a regime in which the waves do not propagate straight, and in which the linear relationship between propagation time and propagation distance is lost\textsuperscript{1-4}, which alters conventional ultrasound imaging. There is large absorption and dispersion features which are yet to be quantified and only preliminary work has been done ex-vivo\textsuperscript{4}. In this thesis, we propose an innovative method in which we exploit ultrasound multiple scattering by the alveoli to quantitatively characterize the lung parenchyma. Indeed, each scattering event can be seen as an opportunity for the wave to embed information on the micro-architecture of the parenchyma.

![Figure 1.2: Scanning electron microscopy image of normal lung. The hollow regions denote the air filled alveoli sacs.](image)
Multiple scattering and wave propagation in disordered media is a very complicated subject which has undergone tremendous change in the last 40 years. Anderson’s introductory work on wave localization phenomena was a landmark in this research\textsuperscript{5–7}. For very complex waves in disordered media, a coherent understanding has only very recently emerged. Since a localized wave does not have spatial periodicity, there was a requirement for a completely new theoretical framework. A consolidated approach was presented in the book written by P.Sheng \textsuperscript{8} which consolidated all theories explaining mesoscopic phenomena which are the ideal manifestations of wave scattering and interference effects. For infinite media, the wave propagates in a known direction with a known velocity. However, when scattering is involved, the original direction of propagation is lost and more often than not, the wave enters an Omni-directional or a radially growing wave diffusive regime\textsuperscript{9–12}. This diffusion of the wave in a scattering medium is characterized by the diffusion constant D. This D is found to have strong dependence on the wavelength $\lambda$ as well as the scatterer size $d$ in the inhomogeneous media, which is being insonicated with ultrasound. There are two major considerations for quantifying wave diffusivity and analyzing the wave diffusion regime. One is the scatterer size $d$ and its packing fraction. The second consideration is the wavelength $\lambda$. The ratio $d/\lambda$ helps in determining the average distance of coherent propagation between two scattering events. This distance is referred to as the mean free path and $d/\lambda$ is the relevant length scale in demarcating the different wave regimes. For $d/\lambda \ll 1$ the scattering is very weak. For a scale of a few mean free paths, the medium can be considered as an effective homogeneous medium. However, over multiple length scales(multiple mean free paths), this assumption does not hold true. Once the multiple scattering starts dominating, the wave enters a diffusive transport regime. For $d/\lambda > 1$, the diffusive transport regime is achieved over a length scale comparable to $d$\textsuperscript{8,9,11}. This diffusive regime can be characterized
using the coherent and the incoherent waves. It is becoming a widely studied phenomenon and both the backscattered coherent and the incoherent waves have been proved useful to characterize disordered media, exploiting coherent and incoherent effects in classical, electromagnetic or acoustic waves \(^{13-21}\).

1.2 Current State of the Art in Lung Ultrasound

Conventional lung imaging is generally done using chest radiography (CXR) thoracic computed tomography (CT) or Magnetic resonance imaging (MRI)\(^ {22-37}\). All these imaging modalities have limitations, which puts a constraint to their applicability. CXR is constrained by limited diagnostic performance, limited portability of bedside radiography and X-Ray exposure issues. CXR abnormalities are very difficult to find in patients below the age of 70, making its efficacy questionable. The diagnostic yield of CXR is very low and it is associated with radiation risks. As with any diagnostic test, there are inherent limitations to its accuracy. False-positive and false-negative results may arise for several reasons. Suboptimal technique, poor exposure, poor patient positioning, patient motion, and lack of cooperation may lead to artifacts and may obscure significant findings on the film. Even with perfect technique, lesions may be hidden by overlying shadows; also, overlapping shadows of normal structures may be incorrectly interpreted as pathologic findings\(^ {29}\). The lifetime risk of cancer death from routine CXR has also been estimated to be about 1.2 per 100,000. Interpreter variability affects the accuracy of the results. There may be an overestimation of the usefulness of CXRs to diagnose disease as the sensitivity and specificity for tuberculosis was reported as 75 and 98%, 91 and 96% for chronic obstructive pulmonary disease (COPD) and 74 and 88% for CHF\(^ {28}\). Therefore, the diagnostic yield may have errors, which may underestimate or overestimate the true incidence of the abnormality. Due to the moving thorax, the spatial resolution decreases and leads to a poor-quality X-Ray films with low
sensitivity. X-Ray beam origination is not tangential to the diaphragmatic cupola thereby hindering correct interpretation of the thoracic structures.\textsuperscript{23,30}

In the recent years, MRI has become an up and coming tool to analyze the lung parenchyma. However, MRI suffers from three main factors – low signal to noise ratio (SNR), cardiac pulsation and respiration that causes pulmonary motion and the large impedance change between tissue and air. Oxygen in air is paramagnetic and tissue is diamagnetic, which leads to a bulk magnetic susceptibility difference ($\Delta \chi = 8$ ppm) at lung–air interfaces. At each tissue interface the susceptibility difference forms a static local field gradient.\textsuperscript{32} These factors lead to magnetic field heterogeneities, which produces a very complex frequency spectrum. This overall leads to blurring of the pulmonary structures. Low spin density is a major drawback for the visualization of normal lung parenchyma and of lung diseases with loss of tissue such as emphysema. In all other lung diseases, the amount of tissue, fluid, and/or cells is increased. Thus, the number of protons is increased, and a higher signal can be obtained.\textsuperscript{38} The only way to improve SNR is to increase the proton bombarding density. For traditional lung MRI, a standard scanner with a field strength of 1.5 Tesla with parallel imaging capabilities is used. Functional MRI imaging capabilities also exist; however, special hardware is required. This combined with high costs and difficulties in the handling of equipment as well as $^3$He gas makes it a restrictive option to use MRI for regular diagnosis and treatment response.\textsuperscript{33} Even though MRI has appeared to be a promising imaging modality, currently it is the first line-indication for some lung related diseases such as pulmonary arteriovenous malformations, pulmonary sequestration, pulmonary artery hypoplasia, partial or total anomalous pulmonary venous return, persistent left superior vena cava, or pulmonary artery sling. In other more prominent lung pathologies, it acts as a secondary modality.
MRI is also associated with high acquisition times, poor spatial resolution and non-qualitative approach makes it a challenge in diagnosis of pathology.

Thoracic CT allows detection of early stage lung cancer with higher resolution than chest radiography. Chest CT plays a very important role in the critically ill patients. Pleural effusions are difficult to detect using CXR. Thus, chest CT is the only imaging modality to accurately assess for both the presence and quantify the size of the pleural effusion. CT currently holds multiple advantages over ultrasound when it comes to detecting emphysema and characterization of malignant effusions. CT is also very useful and commonly used to guide procedures such as drainage of pleural effusion. CT is more sensitive than CXR and provides valuable data for treatment response. Although thoracic CT is the gold standard for lung imaging, it is very costly, and transportation of the critically ill to the concerned department combined with radiation exposure increases the measurable risk. Many patients have relative contraindications to the use of intravenous (IV) contrast (particularly an increased risk of contrast-induced nephropathy). CT exposes the patient to substantially more radiation compared with CXR. The drawbacks to CT include the frequent use of contrast agent injection to enhance contrast resolution in the hilus and mediastinum, the use of ionizing radiation, and poor spatial resolution for reconstruction of sagittal and coronal images. Finally, CT may not always provide additional information to the nonspecific CXR, especially when the patient has diffuse airspace disease, which often yields the same differential diagnosis raised on the CXR portable study. In addition to CT, micro-CT has also been used as a tool for elucidating diseases and monitor response to treatment. Due to its resolution of 75μm, micro-CT can be used to study various lung-disease models such as emphysema and fibrosis. However, micro-CT is also restricted due to the high radiation dosage. Even though micro-CT allows 3D voxel data to be acquired, the data acquisition time is
high when acquiring data for human lung parenchyma. CT when combined with other imaging modalities increases its specificity and sensitivity. A multitude of work has been done combining CT with PET, MR and optical flow imaging which has shown promise in diagnosis and response to treatment of pathologies\textsuperscript{26,27,36}.

Over time, the use of lung ultrasound in patients in the Intensive Care Units (ICUs) had gained popularity. It has shown to exhibit higher diagnostic accuracy compared to CXR for pleural effusion, and consolidation\textsuperscript{45,46}. Lung ultrasound has also garnered a large applicability in detection of pulmonary manifestations of neonatal respiratory distress syndrome\textsuperscript{47}. Lung ultrasound is arguably the fastest and most effective method to detect diaphragmatic paralysis and diagnosing pleural effusion, especially when trying to differentiate between effusion and consolidation\textsuperscript{48}. Due to its portability and removed irradiation effects, lung ultrasound has become an option for thoracic imaging in the critically ill. The conventional approach of lung ultrasound (Figure 1.3) is based on the identification of ten standardized signs: the bat sign (pleural line), lung sliding (yielding seashore sign), the A-line (horizontal artefact), the quad sign, and sinusoid sign indicating pleural effusion, the fractal, and tissue-like sign indicating lung consolidation, the B-line, and lung rockets indicating interstitial syndrome, abolished lung sliding with the stratosphere sign suggesting pneumothorax, and the lung point indicating pneumothorax\textsuperscript{45}. 
However, reading and interpreting these signs is subjective and operator-dependent. Lung ultrasound imaging past the pleural layer is highly inaccurate because of the presence of multiple scattering in the parenchyma generated due to the drastic change in impedance from tissue to air present in the air sacs. During lung imaging, the backscattered signals are distorted, leading to artefacts and introducing large errors in reading and interpreting the images\textsuperscript{49}. These artifacts have been advantageous for interpreting pathologies and early stage detection. Soldati et al\textsuperscript{50} were successful is showing that LUS images could be a potential marker to estimate lung porosity. Soldati also managed to demonstrate how interstitial syndrome is determined by acoustic interactions in lungs of variable density and in healthy organs deflated to a non-physiologic level of density\textsuperscript{51}. Volcipelli et al\textsuperscript{52} showed the potential of bedside LUS in detection of alveolar-interstitial syndrome (AIS) and more specifically fibrosis, ARDS, pulmonary edema and pneumonia. The specific comet tail B-lines showed high sensitivity and specificity in the diagnosis of AIS when compared to radiography. Furthermore, they showed that pulmonary capillary wedge pressure (PCWP) and left ventricular filling pressure (LVFP) are two important parameters in the diagnosis and follow up of heart failure\textsuperscript{53}. Reissig et al.\textsuperscript{54} in their seminal work showed that LUS
using diffused bilateral B-Lines can be used to characterize community acquired pneumonia (CAP) and AIS. Indeed, Lung US is an easy-to-use, low-cost technology that allows accurate non-invasive bedside assessment of pulmonary pathologies. Its usefulness is related to the easy detection of certain specific vertical artefacts called B-lines. Lung US saves time and cost, provides immediate information to the clinician and relies on very easy-to-acquire and highly reproducible data.

The fundamental issue with all these works are that the interpretation of these B-Lines is highly operator dependent and qualitative in nature. We now shift our focus to highlight the quantitative work done in LUS.

1.3 Quantitative Ultrasound and Diffusion Measurements

It is surprising to note that very little quantitative work has been done in characterizing the lung parenchyma using ultrasound. This could be attributed to the complexity of the back-scattered coda obtained from the lung parenchyma or the high attenuation due to the presence of alveolar air scatterers. Floyd Dunn was one of the pioneers of quantitative ultrasound characterization of tissue. Dunn et al. in their seminal work published in 1961 first showed that a pneumonitis lung sample would have lesser absorption than a healthier lung which he attributed to fluid filling which was a lesser of an attenuating medium. They then showcased that the attenuation in lung tissue has a linear relationship with frequency. However, a bigger challenge was addressed when Dunn established a relation of the effective speed of sound with frequency as well as with inflation. The levels of inflation would modify the effective density thereby increasing or decreasing the size of scatterers and hence modulating the wave speed. Other works on the characterization of lung tissue using parameters such as attenuation and dispersion and dependence of these on levels of inflation can be found in and . Very recently, researchers have shifted away from traditional
quantification of lung tissue since the single scattering approximation does not hold true. A potential methodology for characterizing is measuring the speed of the surface wave using an external agitator to generate surface waves at different frequencies. Zhang et al.\(^{57,58}\) showed that for fibrotic lungs, the surface waves propagated at much higher velocities than for control cases. This was due to the presence of stiffer tissue. In 2016, Zenteno\(^{59}\) et al. were able to detect pneumonia even at early stages using a spectral based methodology where the frequency based attenuation was used to characterize the lung parenchyma. Although in its very nascent stages, Demi et al.\(^{60}\) recently proposed a potential quantitative method to characterize the lung using spectroscopy. They noticed that the B-Lines do not always appear for a given lung and only appear at certain frequencies. Hence it could be concluded that the B-Lines themselves are frequency dependent and this is something that should be looked into in the near future.

In highly complex media, such as the lung parenchyma, the backscattered ultrasonic signals can be processed, not for imaging but to extract quantitative parameters of the micro-architecture. J.H. Page used the diffusion constant D to characterize the porosity of strongly scattering media\(^{12,61,62}\) with steel/aluminum scatterers embedded in water as the propagating medium. Tourin et al. described a set of parameters to characterize a highly diffusive medium using parameters such as the Diffusion Constant (D) and mean free paths. These were assessed in complex media made of steel rods acting as scatterers\(^{13}\). Using ultrasound multiple scattering has also proved advantageous in providing a quantitative spatial estimate for complex bone architectures, porosities and spatial densities. Aubry et al. used multiple scattering successfully to give local measurements in the human trabecular bone which is highly complex and diffusive in nature\(^{63}\). Using a translating linear array, they were able to characterize the entire bone architecture in the along a line by mapping the diffusion constant in 1D along the transducer translation axis. The
approach developed by Aubry et al. was successfully tested on a phantom consisting of steel rods in water. $D$ is a single number characterizing a scattering medium. In a multiple scattering medium, when the wave enters the diffusive regime, $D$ is used to characterize the rate at which the diffusive halo grows. Derode, Aubry and Shahjahan then developed a methodology using singular value decomposition (SVD) to separate multiple scattering and single scattering contributions in a heterogeneous media. This allowed them to isolate the single scattering contribution, which in general is used for generating B-Mode images in sonography. Using a multiple scattering filter, they were able to identify targets/lesions, which allows visualization of defects in a system. Multiple scattering has also been used to characterize complex soft tissues. In this thesis, we demonstrated that the diffusion constant $D$ is relevant to the assessment of alveolar interstitial syndrome and more specifically pulmonary edema and pulmonary fibrosis.

### 1.4 Pulmonary Fibrosis and Edema

The first set of goals for this thesis is to establish a novel ultrasonic methodology to quantify the severity of pulmonary edema and pulmonary fibrosis as well as to monitor the response to treatment. The alveolar interstitial syndrome (AIS) can be chronic (pulmonary fibrosis) or acute (pulmonary edema). Pulmonary edema can be due to many reasons and can be either cardiogenic or non-cardiogenic. Pulmonary edema is characterized by increased extravascular lung water which causes acute dyspnea leading to high mortality rate. The increase in extravascular lung water can be seen in a CT scan with enhanced shades of grey as shown in Figure 1.4. In figure 1.4, the CT scan of a Sprague-Dawley rat is shown with the left lung being edematous and the right lung being control. Edema is induced using ischemia reperfusion injury details of which will be shared in future chapters.
Pulmonary fibrosis is a progressive, fatal, inflammatory and fibro-proliferative lung disease for which existing treatments are of limited benefit\textsuperscript{74–76}. Past data have suggested that it is the most common chronic interstitial disease.

We try to look at detection of fibrosis and edema in a very simplified manner. Both pulmonary edema and fibrosis lead to structural changes in the micro-architecture of the lung parenchyma. In fibrosis, the thickening of the alveolar walls (Figure 1.5) reduces the compliance and the volumetric intake of air due to the reduction in the effective size of the air sacs\textsuperscript{77}. In the case of edema, the alveolar sacs are filled with water, which also reduces the effective volume of the lung and its elasticity. Due to these structural changes, we hypothesize that in case of AIS, the effective amount of multiple scattering is much lower which allows the wave to diffuse deeper compared to a healthy lung. In a healthy, normal lung, the millions of air-filled alveoli are responsible for frequent scattering events, leading to short mean free paths.
In this thesis, we demonstrate, for the first time, that ultrasound multiple scattering, usually considered an obstacle to imaging highly scattering media, can be taken advantage of, in characterizing AIS in the lung parenchyma. We propose to analyze the backscattered signals to obtain the diffusivity of the lung parenchyma to characterize the lung using wave transport parameters, namely the diffusion constant D and the mean free path $^{13}$.

1.5 Lesion Detection

Small lung nodule is a common problem in pulmonary practice. The definition of a classical solitary pulmonary nodule is a single, spherical, well-circumscribed, radiographic opacity less than or equal to 30 mm in diameter that is completely surrounded by aerated lung and is not associated with atelectasis, hilar enlargement, or pleural effusion $^{78-80}$. According to the density at thin section computed tomography (CT), it is divided into nonsolid, ground-glass opacity (GGO), solid opacity $^{81}$. Localization of small, nonvisible and non-palpable nodules is challenging during Video-Assisted Thoracoscopic Surgery (VATS). Using ultrasound, it is not possible to image lesions or solitary pulmonary nodules (SPN) beyond a certain depth (10mm) and below a certain size (6mm)$^{82}$. 

Figure 1.5: CT Scans highlighting structural changes for pulmonary fibrosis.
For early lung cancer, VATS has been adopted as an important tool in the treatment of this disease through minimally invasive surgery. Low dose CT has been shown to be effective in the early detection of lung cancer, thus reducing mortality rates\textsuperscript{83–85}. However, the nodules requiring resection detected by screening are smaller. The difficulty of palpating makes VATS resection of deep-seated nodules or ground glass opacities hard. Successful VATS for the resection of pulmonary nodules depends on intraoperative nodule identification by direct visualization or palpation in the past. With the increased use of low dose CT, greater numbers of small lung lesions are detected. Not only size but also distance to the pleural surface can influence the nodule detection rate using palpation during VATS. Deep-seated solitary pulmonary nodules are difficult to palpate during VATS.

![CT-Scan of SPN in the lungs.](image)

Figure 1.6: CT-Scan of SPN in the lungs. The right lung has a peripheral lesion, which is easier to detect compared to the lesion in the left lung, which is deeper in the lung parenchyma and difficult to detect using palpation

Accurate localization is key to successful VATS lung resection, and should be done during surgery. CT-guided localization has been considered effective preoperatively \textsuperscript{86–88}. Ultrasonography during thoracoscopy to direct the resection of GGO was successful and can get
good-quality ultrasound images in 56% of patients\textsuperscript{89}. However these high number were attributed to two reasons. Firstly, these lesions were at the periphery or adjacent to the pleural line. Secondly deeper GGOs were identified using ultrasound using completely collapsed lungs. GGO is similar to adjacent normal lung tissue in density and thus localizing them with ultrasonography is hard, even for experienced chest surgeons. In a healthy, partially inflated lung, ultrasonography has been deemed unusable. Some pilot studies have measured the chance of detecting tiny pulmonary lesions including GGO with ultrasonography to define the limitation as well as help surgeons improve their skills\textsuperscript{90}. Based on the study done by Dunn and Fry\textsuperscript{3,4,91} the attenuation is very high in the lung parenchyma, even when the lung is collapsed under atmospheric pressure. Many of the clinical reports on intraoperative thoracic ultrasonography emphasize the need for thorough deflation of the lungs or atmospheric collapse of the lungs\textsuperscript{82,92–97}.

Figure 1.7: Schematic of VATS

Nodules adjacent to the visceral pleural surface are easily observed using ultrasonography. However, pulmonary nodules that can be identified on CT images are often much more difficult
to locate by surgeons viewing the partially collapsed lung during VATS. Surgeons may insert their finger in the thoracic cavity via another incision, bring the lung tissue to their finger and try to palpate the nodule. However, SPN can be extremely difficult to locate precisely. Small nodules might be extremely difficult to feel\textsuperscript{82,89}. Therefore, there is no guarantee that the nodule will be in the resected region of the lung parenchyma, or that the margins will be adequate. Conventional ultrasound to visualize SPNs has been described, but the lung must be completely atelectatic to visualize nodules\textsuperscript{82}. None of these methods enhances the likelihood of a clear resection margin. VATS wedge resection is being used increasingly as definitive therapy. A positive margin leaves tumor behind and substantially reduces the chance of cure.

Imaging the parenchyma using conventional B-mode ultrasound is impossible because of multiple scattering by the alveoli, which impairs the linear relationship between propagation time and propagation distance, making conventional beam forming and imaging not feasible. Accordingly, ultrasound-based techniques have until now been considered unsuitable for imaging the lung parenchyma. In this thesis, displayed is a new algorithm in which we exploit multiple scattering by strong scatterers. In any scattering medium, the source of multiple scattering or aberrations are the scatterers. The lesion or a target can be considered a homogeneous region with relatively uniform properties when compared with the rest of the heterogeneous medium. We take advantage of the unique homogeneity amidst chaotic heterogeneity to map the amount of multiple scattering or diffusivity $D$, and combine this with a depression detection algorithm, which could enable us to map and locate lesions/targets in real time. The algorithm developed for this purpose is presented in theory in this thesis. It is validated using computational data using highly heterogeneous structures where the region of lesion or target is treated as either a region of no scatterers (lesion) or a region with a very large scatterer (target).
1.6 Roadmap of Chapters

From Chapter 2 to Chapter 4, the thesis explains in details the development of the diffusion algorithm. Each chapter between 2 to 4 addresses the analytical model, data acquisition, algorithm development and its validation both computational and experimental. Chapters 5-7 then delves into the clinical application of the algorithms discussed in the previous chapters. Each chapter describes the data acquisition and challenges, and how the data was processed before the diffusion algorithms were applied on them. The diffusion constant D measures the effective properties of the media which are non-local in essence. However, we explain how we have this methodology extended to 1D imaging and further into 2D imaging to generate 2D diffusion maps. Chapter 2 will discuss how the basic Diffusion measurement algorithm was developed. Chapters 3 and 4 will describe a new diffusion mapping methodology. Chapters 5 and 6 will discuss the applications of using D to characterize the healthy lung parenchyma in rats and differentiating it from pulmonary edema and pulmonary fibrosis. Chapters 5 and 6 will also encase preliminary clinical data obtained from larger animals. Chapter 6 will then discuss an algorithm, which is developed to ensure the unequivocal diagnosis of edema and fibrosis.

In chapter 7, we will explore a methodology of characterizing bone micro-architecture, leveraging the MS properties of bone, using frequency dependent ultrasonic attenuation data which combines quantitative ultrasound with artificial neural networks (ANN) to solve the inverse problem in predicting micro-architectural properties of the cortical bone. Directions for future work will also be explored. Finally, academic accomplishments of this thesis work will be listed.
CHAPTER 2 – Diffusion Theory and Transport Parameters

2.1 Abstract

The purpose of this chapter is to introduce diffusion parameters and showcase their application to quantitatively characterize the micro-architecture of highly porous structures such as the sponge and extend it to ex-vivo lung parenchyma characterization. The lung parenchyma is a highly complex and diffusive medium for which ultrasound techniques have remained qualitative. The approach presented here is based on ultrasound multiple scattering and exploits the complexity of ultrasound propagation in the lung structure. The experimental setup consisted of a linear transducer array with an 8 MHz central frequency placed in contact with the lung surface. The diffusion constant $D$ and transport mean free path $L^*$ of the lung parenchyma were estimated by separating the incoherent and coherent intensities in the near field, and measuring the growth of the incoherent diffusive halo over time. Significant differences were observed between the $L^*$ values obtained in healthy and engineered edematous rat lungs ex-vivo. In the control rat lung, $L^*$ was found to be $332 \, \mu m$ ($\pm 48.8 \, \mu m$). The reproducibility of the measurement of $L^*$ and $D$ was tested ex-vivo and in phantoms made of melamine sponge with varying air volume fractions. 2D Finite Differences Time Domain numerical simulations were carried out on rabbit lung histology images with varying degrees of lung collapse. Significant correlations were observed between air volume fraction and $L^*$ in simulation ($r=-0.9542$, $p<0.0117$) and sponge phantom experiments ($r=-0.9932$, $p<0.0068$). Ex-vivo measurements of a rat lung in which edema was simulated by adding Phosphate-Buffered Saline showed a linear relationship between the fluid volume fraction and $L^*$. These results demonstrate the potential of methods based on ultrasound multiple scattering for the quantitative characterization of the lung parenchyma.
2.2 Introduction

The ultrasonic quantitative characterization of the lung parenchyma has remained elusive due to the presence of air-filled alveoli and the very complex micro-architecture of the lung tissue. These specific properties of the lung tissue are responsible for ultrasound multiple scattering, a regime in which the waves do not propagate straight, and in which the linear relationship between propagation time and propagation distance is lost, altering conventional ultrasound imaging. In the present paper, we propose a method in which we exploit ultrasound multiple scattering by the alveoli to quantitatively characterize the lung parenchyma. Indeed, each scattering event can be seen as an opportunity for the wave to embed information on the micro-architecture of the parenchyma. Multiple scattering is becoming a widely studied phenomenon and has been proved useful to characterize disordered media, exploiting coherent and incoherent effects in classical, electromagnetic or acoustic waves \(^{13,14}\).

Conventional lung imaging is generally done using chest radiography (CXR) or thoracic computed tomography (CT) \(^{22}\). Both these imaging modalities have limitations, which puts a constraint to their applicability. CXR is constrained by limited diagnostic performance, portability of bedside radiography and X-Ray exposure issues. Due to the moving thorax, the spatial resolution decreases and leads to poor-quality X-Ray films with low sensitivity. X-Ray beam origination is not tangential to the diaphragmatic cupola thereby hindering correct interpretation of the thoracic structures \(^{23,30}\). Although thoracic CT is the gold standard for lung imaging, it is very costly, and transportation of the critically ill to the concerned department combined with radiation exposure increases the measurable risk.

Over time, the use of lung ultrasound in patients in the Intensive Care Units (ICUs) had gained popularity. It has shown to exhibit higher diagnostic accuracy compared to CXR for pleural
effusion, and consolidation. Lung ultrasound has also garnered a large applicability in detection of pulmonary manifestations of neonatal respiratory distress syndrome. Lung ultrasound is arguably the fastest and most effective method to detect diaphragmatic paralysis and diagnosing pleural effusion, especially when trying to differentiate between effusion and consolidation. Due to its portability and removed irradiation effects, lung ultrasound has become an option for thoracic imaging in the critically ill. The conventional approach of lung ultrasound is based on the identification of ten standardized signs: the bat sign (pleural line), lung sliding (yielding seashore sign), the A-line (horizontal artefact), the quad sign, and sinusoid sign indicating pleural effusion, the fractal, and tissue-like sign indicating lung consolidation, the B-line, and lung rockets indicating interstitial syndrome, abolished lung sliding with the stratosphere sign suggesting pneumothorax, and the lung point indicating pneumothorax. However, reading and interpreting these signs is subjective and operator-dependent. Lung ultrasound imaging past the pleural layer is highly inaccurate because of the presence of multiple scattering in the parenchyma occurring from drastic changes in impedance from tissue to air in the alveoli. During lung imaging, the backscattered signals are distorted, leading to artefacts and introducing large errors in reading and interpreting the images.

In highly complex media, the backscattered ultrasonic signals can be processed, not for imaging but to extract quantitative parameters of the micro-architecture. Tourin et al. showed that it is possible to characterize a highly diffusive medium using parameters such as the Diffusion Constant (D) and various mean free paths. These were assessed in complex media made of steel rods acting as scatterers. Using ultrasound multiple scattering has also proved advantageous in providing a quantitative spatial estimate for complex bone architectures, porosities and spatial densities. Aubry et al. used multiple scattering successfully to give local measurements in the
Human trabecular bone which is highly complex and diffusive in nature. The approach developed by Aubry et al. was successfully tested on a phantom consisting of steel rods distributed in water. The diffusion constant, as demonstrated in this paper, is relevant to the assessment of lung edema, and air volume fraction in the lung. We demonstrate, for the first time, that ultrasound multiple scattering, usually considered an obstacle to imaging highly scattering media, can be taken advantage of in characterizing the lung parenchyma.

Due to the very strong impedance difference between the lung tissue and the alveoli, it is assumed that the tissue acts as the propagating medium whereas the alveoli play the role of scatterers. As reported in earlier studies, the lung parenchyma can be treated as a sponge whose volume fraction varies during inhaling and exhaling. Numerous studies have been performed using ultrasound as well as magnetic resonance imaging using gelatin sponges as a lung-mimicking phantom. Spinelli et al. used a gelatin sponge to act as a simplified structure of the lung in order to reproduce its viscoelastic properties and generate a simplified model, which can be used to reproduce mechanical, architectural, and acoustic properties of pulmonary tissues. Earlier models developed from culturing of pulmonary epithelial cells were complex and identified as a major challenge.

In the present study, we propose to take advantage of the complexity of the ultrasonic signals and of the high diffusivity of the lung parenchyma to characterize the lung using wave transport parameters, namely the diffusion constant D and the transport mean free path L*. These parameters are estimated by processing the ultrasonic signals backscattered from the lung after a pulse transmission with an 8 MHz central frequency. We provide a quantitative estimate of these transport parameters in simulations, sponge phantoms, and in the rat lung ex vivo and in synthetic model of pulmonary edema.
2.3 Diffusion Principle

2.3.1 The Incoherent wave

The objective was to measure the diffusion constant $D$ and the transport mean free path $L^*$ of the entire geometry based on the dynamic backscattered incoherent intensity. The method for processing the inter-elements response matrix, or impulse response matrix (IRM) was developed upon the work previously done by Aubry et al. The IRM ($H(t)$) has a unique feature of reciprocity ($h_{ij}(t) = h_{ji}(t)$). Using this observation, another matrix, called $H^A(t)$ was defined as follows:

for $i>j$, $h_{ij}^A = -h_{ij}$;
for $i= j$, $h_{ii} = 0$;
for $i<j$, $h_{ij}^A = h_{ij}$;

It is to be noted that $H^A(t)$ is a fictitious matrix. The temporal variation of the incoherent intensity exhibits the unique property of a growing diffusive halo, which can be visualized. The intensities were calculated for $H(t)$ by appropriately time shifting the backscattered signals so as to ensure that the received signals arrive at the same time on every transducer. The time signals $h_{ij}(t)$ were then truncated in 0.3$\mu$s overlapping windows:

$$k_{ij}(T,t) = h_{ij}(T+t) \times W_R(t)$$

with $W_R(t) = 1$ for $t \in [0,0.3\, \mu s]$ and $W_R(t) = 0$ elsewhere.
Figure 2.1: Experimental/Simulation setup showcasing an N element array placed in the near-field of the multiple scattering medium.

A short time FFT provided a response matrix called \( K(T,f) \) for each time window \( T \). Each element of \( K(T,f) \) is represented as \( k_{X_E X_R}(T,f) \) corresponding to the responses at the frequency \( f \) and time \( T \) between the emitter location \( (X_E) \) and the receiver location \( (X_R) \) as shown in Figure 2.1. The backscattered intensity \( (I(X,T)) \) was calculated by integrating the squared values of the \( k_{X_E X_R}(T,f) \) for all emitter/receiver couples (denoted by \( < |k_{X_E X_R}(T,f)|^2 > \) in Equation 2) by that are separated by the same distance \( X = |X_E - X_R| \).

\[
I(X,T) = < |k_{X_E X_R}(T,f)|^2 >_{f,(X_E,X_R)} \quad (2)
\]

The \( H^A(t) \) matrix was processed in a similar fashion to obtain the \( I^A(X,T) \). The backscattered intensity can also be obtained in the time domain by squaring and integrating \( k_{X_E X_R}(T,t) \) over time and over emitter/receiver couples separated by the same distance \( X = |X_E - X_R| \).

\[
I(X,T) = < |k_{X_E X_R}(T,t)|^2 >_{t,(X_E,X_R)} \quad (3)
\]

The post processing in the time domain is shown as below. By integrating the squared value of signals over each time window, the backscattered intensity \( (T) \) was obtained from...
\[
I_{ij}(T) = \int_{T/2}^{T+t/2} h_{ij}^2(t)dt
\]  

(4)

Where \( T \) is the time at the center of the time window and \( t \) is the width of the time window. The backscattered intensity \( I(X,T) \) was calculated by averaging \( I_{ij}(T) \) over the emitter-receiver pairs separated by the same distance \( X = |X_E - X_R| \).

2.3.2 Measuring the Diffusion constant using the Growth of the Diffusive Halo

Normalized intensities \( I_A \) and \( I \), obtained from \( H_A(t) \) and \( H(t) \) respectively, were used to evaluate the incoherent intensity using \( I_{\text{inc}} = \frac{I + I_A}{2} \).

Shown in Figure 2.2 is an example of the total backscattered intensity and its split up into its coherent and incoherent component. We seen that the coherent component is only seen when the distance between the emitter and receiver is 0, that is the emitter and receiver are the same.

![Image of total intensity, coherent and incoherent intensities](image)

Figure 2.2: Total Intensity, Coherent and Incoherent Intensities obtained from backscattered intensity measurements at any time window \( T \)
In highly scattering media, the propagation of the incoherent intensity obeys the diffusion equation and, in the near-field approximation, the incoherent intensity can be expressed as a function of the diffusion constant D:

\[ I_{inc}(X,T)=I(T)\exp\left(-\frac{X^2}{4DT}\right) \]  

(5)

Where X represents the distance between emitter and receiver. Equation 5 clearly illustrates that the incoherent intensity over time describes the growth of the diffusive halo. The incoherent intensity \(I_{inc}\) was plotted as a function of time and emitter-receiver distance (\(X=|X_E-X_R|\)). All intensities corresponding to the same emitter-receiver distance X were averaged. For each time window, the spatial spread of \(I_{inc}\) was fitted with a Gaussian plot and the variance of the Gaussian plot was calculated. The variance \(W^2(T) = 2DT\) of \(I_{inc}\) represents the dynamic growth of the diffusive halo. When \(W^2(t)\) is plotted against time, half of the slope of the linear fit is the diffusion constant D. The growing trend of the backscattered incoherent intensity is shown in Figure 2.3.

Figure 2.3: Growing diffusive halo trend depicted by growing width of the backscattered incoherent intensity
2.3.3 Mean free paths

The transport mean free path $L^*$ is associated with the diffusion constant $D$ through the equation $^{100,101}$

$$D = \frac{V_E \times L^*}{3} \quad (6)$$

Where $V_E$ is defined as the transport speed, and is taken to be the speed of sound in tissue (1.575 mm/µs). In the present study, the scatterers are the air-filled alveoli, which have been approximated as spherical air pockets in the tissue. In order to determine the relationship between the various mean free paths (transport, elastic, and scattering mean free paths), a simulation of ultrasound scattering by a circular air sac in 2-D were carried out using SimSonic, an open source simulation software based on Finite Differences Time Domain $^{102,103}$. Analysis of the pressure field by a single circular air sac was performed to estimate the average cosine of the scattering angle $\bar{\cos \theta}$ by one such scatterer $^{13,103,104}$. A plane wave of central frequency 8MHz was emitted into the medium with a single circular air scatterer of 100µm diameter. The scattered signal was recorded by placing a circular array of receivers surrounding the single alveola at a distance of 300 µm from its edges. The simulation was repeated without the scatterer. The two signals recorded on the receivers were subtracted so as to obtain only the scattered signal, providing the normalized scattered intensity as a function of the scattering angle. The average cosine of the scattering angle is calculated using the formula.

$$\bar{\cos \theta} = \frac{1}{2\pi} \int_0^{2\pi} \cos(\theta) \frac{d\sigma(\theta)}{d\theta} d\theta \quad (7)$$

Where $\frac{d\sigma(\theta)}{d\theta}$ is the differential scattering cross section and $\sigma$ is the total scattering cross section of one scatterer $^{13}$. Figure 2.4 depicts the normalized scattering cross section in the $\theta$
direction obtained as described above. This shows that air-filled alveoli are quite an isotropic scatterer.

Figure 2.4: Polar representation of the scattering cross section of a single circular air scatterer at 8MHz scattered in Θ direction with Θ=0 representing forward scattering (Also represented by the arrow).

The average cosine of the scattering angle \( \cos\Theta \) was found to be 0.0514. The isotropic scattering nature of such a circular scatterer can also be extended to 3-D. A challenge in the lung parenchyma is the existence of alveoli with varying diameters, however since the scatterers are fairly spherical air sacs, \( \cos\Theta \) will be very small for any scatterer diameter. The simulation for the air sac scatterer was performed to obtain a relationship between the elastic mean free path (\( L_s \)) and the transport mean free path (\( L^* \)). \( L_s \) and \( L^* \) are related to each other by the equation \(^{13}\)

\[
L^* = \frac{L_s}{1-\cos\theta} \tag{8}
\]

Since \( \cos\Theta \sim 0 \), it can be assumed that the transport mean free path(\( L^* \)) , the elastic mean free path (\( L_s \)) and scattering mean free path (\( L \)) are of the same magnitude \(^{13,100}\).
2.4 Validation Methodology

2.4.1 Ultrasound Experimental setup

In both simulations and experiments, ultrasound pulses were transmitted using single elements of a linear transducer array, one by one. For all numerical simulations, a 64 elements linear array with a central frequency of 8 MHz was simulated. For all experiments, a 128 element Verasonics L11-4v linear array was used, connected to a Verasonics Vantage ultrasound scanner (Verasonics, Kirkland, WA). The transducer was coupled to the lung by a layer of ultrasound coupling gel (approximately 5 mm). In both simulations and experiments, all of the elements of the array were fired one by one, transmitting a 2 cycles pulse with a central frequency of 8 MHz into the medium. For each transmitted pulse, the backscattered signals were collected on all elements of the array, which gave access to the spatial spread of the transient pressure field. This enabled the acquisition of the 128 by 128 (64 by 64 for simulations) inter-element response matrix H(t) whose individual elements are $h_{ij}(t)$ where i is the emitter number and j is the receiver number. The individual elements $h_{ij}(t)$ are the $N^2$ inter-element responses of the probe-medium system.\textsuperscript{13,16,105}

2.4.2 Sponge Phantom Experiments

For this preliminary phantom study, we used 100 cm$^3$ blocks of a commercially available melamine foam (a formaldehyde-melamine-sodium bisulfite copolymer), which cross-linked micro-architecture resembles that of the parenchyma. The melamine sponge block was saturated with controlled amounts of water with air volume fractions of 20%, 30%, 40% and 50%. This was done by assuming the dry sponge to have negligible mass, completely saturating it with water and then squeezing out the appropriate amount of water to maintain the desired volume fraction. To avoid errors related to the settling of water due to gravity, all experiments on the sponge were
carried out within 30 seconds of obtaining the desired volume fraction. Figure 2.5 is a microscopic image (NIKON eclipse LV150 optical microscope) of the melamine sponge used in its dry state. It depicts the complex network of the melamine in which water saturation reduces the number of air-filled pores. This network was assumed to mimic the lung parenchyma’s multiple scattering properties.

![Microscopic image of melamine sponge in its dry state](image)

Figure 2.5: Microscopic image of melamine sponge in its dry state depicting complex network (Scale reads 500 μm)

### 2.4.3 FDTD Simulations

Simulations of ultrasound propagation through the lung parenchyma were carried out using SimSonic, an open source simulation software based on Finite Differences Time Domain numerical methods. A histology image of the rabbit lung showing a clear depiction of the lung tissue and alveoli was used as a numerical binary map. The image was then modified by reducing the size of the alveoli to obtain varying air volume fractions as shown in Figure 2.6. The region in yellow depicts the tissue and has been given tissue properties (Density = 900 kg/m$^3$, Bulk Modulus = 2.53 GPa) whereas the region in blue denotes the alveoli and have the properties of air (Density = 1 kg/m$^3$). The dimension of the geometry is 20 × 10 mm. The numerical maps were binary images of 2000 × 1000 pixels with a grid step size of dx=0.01mm (19 data points per
wavelength). A 64 element linear transducer with a central frequency of 8 MHz and element width of 0.3 mm was simulated.

![Figure 2.6: Geometries with varying volume fractions obtained from image processing of the histology images obtained from a rabbit lung](image)

### 2.4.4 Animal experiments

First, a 300 g female Sprague-Dawley rat was used. The animal was sedated with intraperitoneal ketamine/xylazine (0.1cc/kg), and had a tracheotomy with a 14-gauge catheter, ventilated with a Harvard rodent respirator (model 681, Harvard Apparatus Co, Millis, MA) 1% isoflurane in 100% O2, tidal volume of 0.75 mL/100 gm, rate 70/min, and positive end-expiratory pressure (PEEP) 3 cm H2O. A sternotomy was performed and extended inferiorly as a small laparotomy to expose the liver. The sternal edges were retracted to expose both lungs. Intermittently, ventilation was interrupted either at the end of inhalation, or at the end of expiration by clamping the input to the tracheotomy tube to reduce lung movements. A layer of ultrasound gel of approximate thickness of 5mm was applied directly on the exposed lungs and an ultrasound
evaluation was carried out using an L11-4v linear array transducer with central frequency of 8MHz connected to a Verasonics Vantage ultrasound scanner. The IRM was acquired as described above. Heparin (600 U; Elkins-Sinn, Cherry Hill, NJ) was injected intra-hepatically. Five minutes later, the pulmonary artery was cannulated through the right ventricular outflow tract with a length of p60 tubing. The right and left atrium were incised, the endotracheal tube was clamped at end of inspiration, and the lungs were flushed with 20 ml cold (4°C) Perfadex buffered with THAM (XVIVO Perfusion Inc., Denver CO) from a height of 20 cm. The heart lung block was then excised. This removes all blood from the lungs, and is analogous to how human lungs are recovered for transplant. The blood was drained out in order to avoid clotting and to ensure that only the alveoli sacs participate in the scattering phenomena. Ideally, this would result in the absence of erythrocytes in the capillaries, however perfadex was present which would not hamper the ultrasound wave propagation due to similarities in the acoustical properties of blood and perfadex. After surgical ligation of the lungs, a polyethylene tube was inserted into the trachea, which was connected to a three way stop-cock of which one end was connected to a 5ml capacity syringe. This allowed control over the air volume fraction and avoided air leakage. Once the lungs were excised, the ex vivo ultrasound evaluation was performed by applying coupling gel directly on the surface of the lung. All ex vivo experiments were conducted within 12 hours of death of the rat. Figure 2.7 shows an image of the rat lung and the ex vivo experimental setup.
Animal Studies: This study was approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill. All animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996).

2.5 Results

2.5.1 Numerical Simulations

Pulsed waves with a central frequency of 8MHz were transmitted in the histology image of the rabbit lung (Figure 2.6) and the IRM H(t) was acquired for air volume fractions of 55%, 40%, 30%, 20% and 15%. The incoherent and coherent intensities were separated according to the method described above. The transport mean free path $L^*$ was evaluated for each case. As an example, Figure 2.8 shows the increase of the width of the diffusive halo over time, for 40% volume air fraction. The half slope of the linear fit of the variance gives the Diffusion constant D.
Figure 2.8: Temporal Evolution of the variance $W_2$ of the Gaussian fit on linc. The linear curve of the variance plot provides an accurate measurement of the diffusion constant.

$L^*$ as a transport parameter was highly correlated to the air volume fraction ($r=-0.9542$, $p<0.0117$). *Figure 2.9* shows the variation of $L^*$ as a function of increasing volume fraction ex vivo.

Figure 2.9: $L^*$ calculated from simulations for varying volume fractions.
2.5.2 Phantom experiments: Sponge study

The experiments, carried out on the 50 cm$^3$ blocks of sponge, were repeated five times so as to test the repeatability of the measurement on a given geometry. For each air volume fraction, the growth of the diffusive halo was estimated over time, enabling the measurement of $L^*$. When the incoherent intensities were plotted against time, the growth of the diffusive halo could be tracked as shown in Figure 2.10A.

![Figure 2.10 A: Normalized Incoherent intensity as a function of emitter-receiver distance X and time. B: Temporal variance evolution for 20 % and 50 % air volume fraction depicting a rapid growth of the diffusive halo for lower volume fractions.](image-url)
Figure 2.10B shows a comparison between the temporal evolution of the variance for 20% and 50% volume fraction. It is very clear from the variance plots that the growth of the diffusive halo is very restricted for higher volume fractions of air whereas it is much more rapid in lower volume fractions of air. With increasing air volume fractions, the amount of water decreased thereby reducing D and the derived L* values.

Figure 2.11 shows L* as a function of air volume fraction in the sponges. The error-bars are the standard deviation obtained from five consecutive readings obtained for each air volume fraction. A strong correlation ($r = -0.9932$, $p < 0.0068$) was also observed between L* and the air volume fraction.

2.5.3 Ex vivo Rat Lung with Varying Air Fraction

The experiments on ex vivo rat lung were carried out with the motivation of obtaining D and L* and correlating it with the ultrasound diffusivity of the lung parenchyma, for varying air volume fractions. Experiments were carried out in an iterative method where each iteration denoted the removal of 1 ml of air from the lung using the syringe. For each iteration, the
measurement was repeated 4 times in order to estimate the repeatability of the method. *Figure 2.12* shows the backscattered signals on the 64\textsuperscript{th} element of the linear probe as an example.

![Backscattered Signals](image1)

**Figure 2.12**: Backscattered received signal with central element (element 64) emitting.

$L^*$ measured in the *ex vivo* rat lung as a function of air volume fraction are shown in *Figure 2.13*. The errorbars shown on *Figure 2.13* are the standard deviation of a distribution of 4 consecutive measurements, and demonstrate the repeatability of the measurement.

![Data Points](image2)

**Figure 2.13**: $L^*$ in the ex vivo lung as a function of the volume of air removed from the specimen.
2.5.4 Ex vivo Rat Lung with PBS Addition to Simulate Edema

Instead of air, 1ml, 2 ml, and 3 ml of phosphate-buffered saline (PBS) was progressively added to the lung ex vivo via a syringe. PBS proved to be a better solution to engineer edema in the lungs because of its non-toxicity to most cells and isotonic nature. For each PBS volume fraction, the IRM was acquired and the L* values were calculated as described above. Figure 2.14 shows a plot of L* as a function of amount of PBS added to the rat lung.

![Figure 2.14: L* in the ex vivo rat lung as a function of volume of PBS added to the specimen in use.](image)

2.6 Discussion

In order to track the growth of the diffusive halo, the incoherent intensities were recorded over time. The method developed by Aubry et al. was originally developed for local measurements in the far-field, using Gaussian beamforming. In the present study, all measurements were conducted in the near-field, with the probe coupled to the lung by a layer of acoustic coupling gel (approximately 5mm). No Gaussian beamforming was performed as we were operating in the near field. It should be noted that, as mentioned in, the Gaussian Beamforming is not required for the extraction of the incoherent contribution. The simulation and phantom experiments provided values of L* in good agreement with the ex vivo and in vivo values. The
single scatterer simulations using FDTD showed that the approximation that transport mean free path, scattering mean free path and elastic mean free path are similar is reasonable, as suggested by the calculation of the average value of the cosine of the scattering angle of a single scatterer, described above 100,101.

The approach developed for analyzing the incoherent intensities showed promising results for the characterization of the lung parenchyma. By varying the amount of fluid, we were able to mimic a variety of air volume fractions. For every change of 10% in the fluid volume fraction, L* was seen to vary by an order of 100 μm. This can be attributed to the fact that when fluid saturation increased, the hollow spaces in the sponge network or in the parenchyma were increasingly occupied by fluid, which increased the distance between two scattering events on average.

The melamine sponge saturated with water doesn’t perfectly replicate tissue properties of the lung parenchyma. However, the basic micro-architecture of the cross linked sponge generates a multiple scattering medium which replicates the strong multiple scattering effects observed in the lung parenchyma due to the presence of air. The purpose of the melamine sponge experiments was to work in media with a controlled amount of saturating fluid. This enabled us to validate our quantitative approach for the calculations of the transport mean free path. The present study demonstrates that changes by 10% of the water volume fraction were easily detected, and lead to variations in the mean free path that were much larger than the error-bars. A thorough sensitivity study remains to be conducted in order to determine the lowest possible change of water volume fraction detectable by the method, and will be the subject of a further study.

There is however a limitation to these results. In all experiments (phantom, ex vivo), an increase in water volume fraction resulted in a decrease in air volume fraction, which in turn resulted in an increase of L*. We suggest that this could be used to detect pulmonary edema in
vivo. However, other pathologies such as pulmonary fibrosis are likely to result in similar changes in the air volume fraction, although the water content in the fibrotic lung does not change. Compared to the acoustic impedance of the air-filled alveoli, the acoustic impedance of fibrotic tissue is likely to be similar to that of water. As a consequence, discriminating between pulmonary fibrosis and pulmonary edema using the present method might be challenging. This will be investigated in a further study, where we will compare in vivo models of fibrosis and edema in the rat. The change in L* due to addition of water was more prominent compared to the change in L* due to addition of air. This can be attributed to the near incompressibility of biological tissues.

Another limitation of the approach presented in this study is the spatial resolution of the measurements. The estimation of L* reflects the average micro-architecture of the lung over a region of interest corresponding to multiple mean free paths. The measurements of L* presented here are therefore not local, which impairs the possibility of imaging. This would have to be taken into account for the evaluation of edema, as the excess fluid tend to settle at the bottom of the lungs due to gravity. This issue also raises the question of the depth of penetration of the ultrasonic signals in the lung tissue. Indeed, the high diffusivity of the lung microstructure could prevent sufficient penetration. In the rat lung, which was around 10 mm thick, we were able to verify that through propagation was occurring, as demonstrated by the specular reflection at around 18μs, shown in Figure 2.12, attributed to the interface at the back of the lung. The rat lung was chosen because of the availability of a model for edema, however, its small size is a limitation. Because of this, the growth of the diffusive halo could only be tracked for time period of about 18μs. For an application to the human lung, such measurements would only be relevant if the growth of the diffusive halo could be tracked over longer time periods, in order to explore larger regions of interest. To test whether it would be feasible, we conducted a feasibility study in the pig
lung *ex vivo*. The pig lung was connected to a ventilator and fully inflated in order to maximize the air volume fraction. *Figure 2.15* shows that the growth of the diffusive halo can be tracked over 25μs. No specular reflection from the back of the lung could be observed in the backscattered signals. The calculated D value was observed to be 0.17mm²/μs and the corresponding L* value was found to be 323μm.

Figure 2.15: Temporal variance evolution for Pig lung depicting growth of diffusion halo over a large range of time.

2.7 Conclusion

In this paper, we exploit ultrasound multiple scattering to characterize the micro-architectural properties of a highly complex and diffusive biological medium: the lung parenchyma. We propose a near field method based on the assessment of the growth of the diffusive halo. We demonstrate that by separating the coherent and incoherent backscattered intensities, it is possible to measure the diffusion constant and transport mean free path in the lung, which are related to the air volume fraction in the lung parenchyma. We validated the results obtained with this approach using numerical simulations and phantom experiments, which demonstrated its accuracy in quantitatively predicting the mean free path. This method could be
utilized to non-invasively and quantitatively assess the lung parenchyma. It could eventually be used to quantify the extent of edema and/or fibrosis for bed side lung ultrasound.
CHAPTER 3 - 1-Dimensional quantitative micro-architecture mapping of multiple scattering media

3.1 Introduction

Multiple scattering, is an obstacle to imaging but carries a lot of potential for the characterization of the microarchitecture of complex heterogeneous media. Multiple backscattering signals carry information about the diffusivity of disordered media, when quantified, enables the characterization of their micro-architecture (density or porosity). A method described by Tourin et al. based on coherent back-scattering in the far field, measures the diffusion constant D. The information carried by the coherent contribution to scattering is relevant if the scattering is weak (weak localization). Furthermore, the coherent contribution can only be exploited in the far field. In highly disordered media, the incoherent contribution to the backscattering embeds information on the micro-architecture that can be exploited as well. This letter focuses on applications where far-field conditions can’t be achieved. Hence, incoherent backscattered contributions, which have been observed in various applications becomes the key parameter to predict the diffusivity of a medium.

This study exploits the incoherent contribution of the backscattered signals measured in the near-field. We propose to use subsets of elements of linear arrays transducers to evaluate D of a complex medium semi-locally along a line, in the near field. Aubry et al. extracted D from the incoherent intensity by performing Gaussian Beamforming of the incident and backscattered wavefields to artificially create sources on the surface of the sample. In the present paper, we show that it is possible to extract D from the incoherent intensity under strictly near field conditions and without Gaussian Beamforming, which would find more straightforward applications, especially for medical diagnosis and lung characterization. One such example would be endobronchial
ultrasound (EBUS) to characterize the lung parenchyma for cancer staging\textsuperscript{109} using multiple scattering or detect solitary pulmonary nodules/lymph nodes\textsuperscript{16}. Currently EBUS is used to perform needle biopsies of peribronchial lymph nodes. However, if EBUS was used to characterize the lung parenchyma, near-field assumptions would be necessary. For EBUS lung characterization or peripheral nodule imaging, far-field conditions cannot be achieved and multiple scattering starts occurring approximately at the transducer surface. The assumption behind the present method is that in the near field, the incident and backscattered waves are directive enough and Gaussian Beamforming is not required. If the source and the receivers are directive enough and located in the near-field, the incoherent intensity exhibits the growth of the diffusive halo. Placing the transducer at the surface of the sample corresponds to a near-field configuration with the incoherent backscattering intensity showing a well characterized time dependence\textsuperscript{21}. We demonstrate that such a typical behaviour can also be observed with conventional linear array transducers. We use finite difference time domain (FDTD) simulations of circular plastic scatterers in water and show experimental validation with plastic (Z=2.31MRayl) cylindrical scatterers in water. Since plastic is not a strong scatterer, we also validate this near field approach using aluminium (Z=17.1MRayl) circular scatterers in water using FDTD. All FDTD simulations are carried out in 2-D.

3.2 Materials and Methodology

Both, experiments and simulations are performed in the near field, with the transducer array placed at the surface of the scattering medium. In the experiments, a Philips ATL/L7-4 linear array is used. The width of the array enables to cover a region of 39.68mm. A complex medium was fabricated, constituted of Acrylonitrile Butadiene Styrene (ABS) plastic rods randomly distributed with a volume fraction (VF) varying spatially along the length of the transducer (Figure 3.1). This
4cm x 4cm phantom is divided in 5 sections with VF of scattering rods of respectively 5%, 10%, 15%, 20% and 25%. Due to the lower resolution of the metal printer available, the diameter of the scattering rods (0.5mm) and relatively high VF, the phantom had to be 3D printed using ABS plastic instead of metal, which would have provided stronger scattering.

Finite differences time domain (FDTD) simulations were carried out for two separate media. The geometries consisted of circular Aluminium scatterers ($\rho=2.7\text{g/cm}^3$, Speed of Sound=$6.32\text{mm/\mu s}$, $E=70\text{GPa}$) and ABS circular scatterers ($\rho=1.05\text{g/cm}^3$, Speed of Sound=$2.25\text{mm/\mu s}$, $E=1.4\text{GPa}$), randomly distributed in water ($\rho=1\text{g/cm}^3$, Speed of Sound=$1.5\text{mm/\mu s}$), with controlled VF. The 4cm x 4cm geometry was divided into 5 sections with varying VF 5%, 10%, 15%, 20% and 25%. The purpose of simulations is twofold. Firstly, using plastic scatterers, we validate this methodology and compare with experiments. Secondly, using aluminium scatterers, we also highlight the efficiency of this methodology for strong scatterers. Simulations were performed in 2D using SimSonic\textsuperscript{102,110}, an open-source FDTD simulation tool. The spatial grid step was 0.02mm (approx. 15 points per wavelength). The CFL (Courant-Friedrichs-Lewy) condition was set to 0.999. The time step was defined according to the scatterer.

Experiments were conducted in a water tank. Signals were acquired using a 128 element ultrasonic array (L7-4) connected to a Vantage Verasonics (Verasonics, Kirkland, WA) operating at a central frequency of 5.1MHz. A voltage of 30V was used to improve signal to noise ratio (SNR) since acoustic pressures are low due to single element emission. In the current in vitro and ex vivo experiments, the SNR on the backscattered signals was high enough and backscattered intensities could be computed. If this was not the case, for example in vivo, groups of elements could be used to transmit and receive instead of single elements. The transducer array was placed at a distance of approximately 1mm from the phantom to emulate near-field conditions. The size
of the phantom and the transducer was the same. The transducer lateral axis was placed along the varying VF.

![Image](image.png)

Figure 3.1: Schematics of the Geometry used for Simulations and 3D Printed part used for experiments.

Both the experimental and simulation setups consisted of a 128 element linear array transducer with pitch 0.31mm. Given the dimensions of the transducer elements, and analytical calculations by Marini and Rivenez\(^{111}\), the near field was between \(0.35\lambda\) and \(88\lambda\). In the present case, the transducer was approximately 1mm away from the surface of the medium, with either water or a thin layer of coupling gel in between. This corresponds to approximately three wavelengths in water, which ensures near field conditions. An IRM (inter-element Response Matrix\(^{21,108}\)) was acquired by emitting 2-cycle pulses at 5.1MHz with single transducer elements one by one, and by recording the backscattered echoes on all elements for every transmit, for 40\(\mu\)s. The process was repeated until the backscattered echoes resulting from consecutive transmits from all elements had been acquired. The IRM matrix \(H(i,j,t)\) had \(128 \times 128 \times N\) elements, where \(N\) was Number of time steps. Each time trace of the IRM can be represented by \(h_{ij}(t)\)\(^{16,17,21,63}\). The time traces were time-shifted to ensure that the first back-scattered signal arrival occurred at \(t=0\) for all emitter-receiver couples. This compensated for differences in the emitter-receiver distance. It
would also compensate for an uneven surface of the inspected sample, which was not the case in
the present experiments and simulations, but could be relevant in many situations.

The backscattered intensity $I(r, T)$ was calculated by averaging the square of $h_{ij}(t)$ over
overlapping time windows $T$ of 0.3 μs and also over the emitter-receiver couples that were
separated by the same distance, $r = |X_E - X_R|$ \(^{16,63}\), where $X_E$ and $X_R$ were respectively the
locations of the $i^{th}$ emitter and $j^{th}$ receiver in mm. The width of the time window was chosen based
on the central frequency and the a priori estimated mean distance between scatterers, such that it
spanned over roughly one scattering interaction. It was taken to correspond to 1.5 wavelengths. A
smaller time window (<0.5λ) would not capture complete scattering events leading to inaccurate
backscattered pressure calculations. In order to separate the coherent and incoherent contributions
to the backscattered intensity, H matrix was transformed into an antisymmetric matrix $M$ as
described in\(^ {16}\). Backscattered intensities $I(r, T)$ and $I^A(r, T)$ were calculated from the matrix $H$ and
its anti-symmetric matrix $M$. The coherent and incoherent intensities were separated by adding or
$I(r, T)$ and $I^A(r, T)$ as shown in equations 1 and 2. Assuming the propagation in such a complex
medium to verify a diffusion process, the incoherent intensity can be expressed by equation 3.

$$I_{coherent}(r, T) = \frac{I(r, T) - I^A(r, T)}{2} \quad (1)$$

$$I_{incoherent}(r, T) = \frac{I(r, T) + I^A(r, T)}{2} \quad (2)$$

$$I_{incoherent}(r, T) = I(T) \exp \left( -\frac{r^2}{4DT} \right) \quad (3)$$

The measurement of $D$ is achieved by fitting the incoherent intensity profile
$I_{incoherent}(r, T)$ with a Gaussian curve with variance $W^2$, at each time window $T$ \(^ {16,21,63,108}\). $D$ is
obtained from the linear fit of $W^2(T)$. 
When the entire IRM of $128 \times 128$ elements is considered, this method gives an average measurement of $D$ for the whole insonicated medium. For more local measurements, the IRM was split into sub-IRMs. First, the entire $128 \times 128 \times N$ IRM was acquired as described above. Then it was split into sub-IRMs. Each sub-IRM provided information about the complex medium in the sub-region in front of it. These sub-IRMs can be perceived as sub-arrays of controlled apertures. The first IRM consisted of backscattered echoes from pulses emitted one by one and received by the first 33 elements and had dimensions $33 \times 33 \times N$. The choice of the size of a sub-IRM is application dependent. By averaging $I(r,t)$ over the same element-to-element spacing over 33 elements, one loses the ability to have localization laterally for the sub-region facing this group of 33 elements. However, by using series of sub-arrays with 33 elements each, we are able to evaluate $D$ in the sub-regions facing each of these sub-arrays. This does provide a lateral localization. The smaller the number of elements in each sub-IRM, the higher the localization along the transducer axis. However, choosing too few elements can lead to speckle fluctuation not being completely averaged out and impairing the estimation of $D$. Choosing too many elements can lead to global averaging which may lead to incorrect local prediction of $D$. For the particular media investigated here, averaging over 33 elements was a good compromise between localization and robustness of the $D$ values. However, the number of elements for the sub-array is a function of the amount of scattering and will therefore depend on the medium investigated.

In the near-field, we assume the beam to be directive enough to measure $D$. Combining all sub-IRMs enabled us to generate a 1-D map of $D$ along the linear array. In the work by Aubry et al\textsuperscript{16,63}, the array was translated along the sample to measure $D$ along a line. In the work reported here, the transducer array is stationary and can map the geometry in one single IRM acquisition, without performing any beamforming. However, the span of the mapped region was restricted to
the width of the transducer array. In order to map larger geometries the array could be translated along the sample. This 1-D mapping method is to quantify changes in the medium diffusivity along the transducer axis. The D value measured for each sub-array represents the diffusivity of a sub-region facing the sub-array, for which the depth is unknown and depends on the amount of scattering by the heterogeneous structure. This is the reason why a full 2D image of D cannot be achieved for the time being.

3.3 Results and Discussion

Figures 3.2 A and 3.2 B show FDTD simulations results in distributions of ABS plastic (weak scattering) and Aluminium (strong scattering) scatterers respectively. To generate error bars, for each simulation, three realizations of random scatterer distributions were generated, with the VF varying along the transducer axis as shown in Figure 3.1 A. The resolution of the proposed method is a function of the width of the sub-array (33 elements), and of the amount of multiple scattering in the medium, which will influence the size of the region insonicated by a single pulse transmit. The errorbars, along with the width of the sub-arrays, are an indication of the resolution, specific to the media investigated here. Additionally, FDTD simulations were performed for homogenous scatterer distributions (each distribution had a fixed VF which was not varying spatially), referred to here as stand-alone distributions. These stand-alone cases refer to FDTD simulations done separately on 5 geometries with fixed volume fraction, (5, 10, 15, 20 and 25%). This validates the mapping algorithm by verifying that the mapped values at specific locations (Figure 3.1 A) are similar to those obtained from geometries with fixed, corresponding VF of scatterers. As expected, in Figure 3.2 A, the diffusion constant decreases with increasing VF. At 5% VF, the D values have been evaluated to be 1.45mm²/μs whereas at 25%, D=0.44mm²/μs. Similarly, Figure 3.2 B shows comparison of 1D mapping of D in the presence of Aluminium
scatterers. As the VF varies from 5% to 25%, D varies from 1.94 to 0.5345. For the Aluminium scatterers, the local Diffusion Constants estimated in the different regions of the sample with a gradient of VF was very close to the Diffusion Constant estimated in the stand-alone distribution of the corresponding VF. For the plastic scatterers, at 10% porosity, the maximum difference between D estimates in mapping and stand-alone simulations was 27%. This can be attributed to the fact that for weaker scattering, the wave is not as localized and the diffusive halo grows faster. This introduces wave diffusion through adjacent regions and the measurement of D captures more features of the surroundings regions (such as the domain with VF 5%). On the other hand, when scattering is stronger, the diffusive halo does not grow as fast, and D reflects more local characteristics.

Experiments also exhibited the same trend, with increasing Diffusion Constants for decreasing VF (Figure 3.3). There is a very good agreement between experiments and simulations for the 1D mapping of the geometry in the ABS plastic distribution with a maximum deviation of 14.8 % occurring at 15% VF.

Figure 3.2: Diffusion Constant values calculated from simulations
The present approach proposes to work in the near-field without any beamforming of the incident wave. We established that 1D mapping of the Diffusion Constant in the near-field was possible for a highly scattering medium. This can also be used to detect regions with low concentrations or absence of scatterers. 2-D FDTD simulations were run in a medium containing 20% by area circular Aluminium scatterers (diameter 100μm) immersed in water. At a depth of 15mm, a region of diameter 8mm was inserted containing no scatterers. The simulation map is shown on Figure 3.4 A and the corresponding Diffusion Constant 1D map along the transducer axis on Figure 3.4 B. D is significantly higher in the region containing no scatterers than in the neighbouring regions. This can be attributed to the fact that the wave diffused faster in the region of the inclusion in the absence of scatterers\textsuperscript{21,63,108}. The increase in D is gradual instead of sudden due to the local averaging of the medium properties.
Figure 3.4: Simulation and Diffusion Constant (D) map for detection of absence of heterogeneity.

An application of the proposed method is the detection of tumor or the presence of tumours in highly scattering organs such as the lungs. In the lung parenchyma, conventional ultrasonic imaging is impossible due to multiple scattering from the air-filled alveoli \(^{82,90,112}\). The lung parenchyma is a highly scattering medium compared to the nodule. Because the lung alveoli are air sacs with known material properties and dimensions, the ability to map D would allow to evaluate relative changes in the scatterer density in both the healthy parenchyma and in the tumor, and to get an estimate of the location of a nodule along the transducer axis. An edematous pig lung (3-5 weeks old) was harvested and connected to a handheld disposable resuscitator, which kept the alveoli filled with air. To mimic the presence of a nodule, 0.8cc of heated Vaseline was injected at a depth of 15mm from the surface in the left middle lobe using a 2ml syringe\(^{113}\). The size of the generated nodule (approximately 11mm) was determined by the volume of Vaseline injected. The lung was then made airtight using an umbilical clamp and inflated to mimic in-vivo conditions. Since the knowledge of the nodule location was known in priori, the linear array was placed in the region of interest such that the nodule was approximately at the centre of the linear array. The array was placed approximately 2mm from the parenchyma and coupled using ultrasound gel.
Figures 3.5A and 3.5B show the experimental set-up and the results obtained from 1D mapping. Figure 3.5C shows a conventional B-Mode ultrasound image. The nodule cannot be imaged conventionally due to multiple scattering by the alveoli.

As expected, in the region of the nodule, due to the absence of alveoli, the wave diffused faster, without scattering, thereby increasing D. The nodule diameter retrieved was 11.6mm, comparable to the actual size of the Vaseline nodule. The proposed method overestimated the size of the lesion by approximately 10%. This could be attributed to the local averaging of D. Here, we show that it is possible to detect and predict the size of a solitary pulmonary nodule in an inflated lung.

![Figure 3.5: Application to SPN size estimation in Pig Lungs](image)

**3.4 Extension to Circular Arrays for Intravascular Applications**

This methodology was extended to check its feasibility for circular transducer arrays with 128 elements and a similar methodology was applied to check if this algorithm has the ability to predict spatial changes in the measurement of D. Shown in Figure 3.6 is a schematic of a multi-element circular array transducer. This study was mainly done to check for the feasibility of intravascular transducer arrays such as the Volcano array. Intravascular ultrasound (IVUS) has enhanced our understanding of atherosclerotic plaque morphology, and provides a unique opportunity to guide cardiovascular interventions and evaluate the results of these interventions\textsuperscript{114}.
Simulations were carried out with water as the propagating medium and aluminum scatterers. The distribution/porosity of the scatterers varied along the clockwise direction unlike the previous case. Once the IRMs were acquired, the IRMs were split and the D values were obtained and plotted as a polar plot. Shown in Figure 3.6 and 3.7 are the schematic of the geometry and the results with aluminum scatterer size as 100 microns, operating frequency =5MHz and dx=0.02mm. The area fraction of each region is depicted in the figure and the center blue line is the location of the probe. Although the diameter of this probe is 12mm, these results could be extended to probes of smaller sizes.

Figure 3.6: Circular Transducer Set 1
It can be seen that even though the 1-D circular mapping doesn’t clearly demarcate the regions where the area fraction changes. It is still able to identify regions of high and low $D$. The 1-D measurements can never be perfectly localized since we take a subset of the IRM which is bound to record multiple scattering coda from the nearby regions.

### 3.5 Proof of Concept: Can two 1-D maps generate a 2-D map?

In the previous sections we have shown that using sub-IRMs and 1-D mapping, we can detect lesions or targets in a highly scattering medium. However, this method has limitations. We are not able to detect the location of the lesion along the depth axis. We propose to generate a 2D image of the lesion using compounding. By acquiring IRMs in two perpendicular direction and obtaining two 1-D maps of $D$, we can generate the 2-D image. For a proof of concept, we carried FDTD simulation similar to the ones mentioned in the previous sections (3.3). The simulation domain was a 4cm by 4cm water ($\rho=1g/cm^3$, Speed of Sound=1.5 mm/$\mu$s) region with circular aluminium ($\rho=2.7g/cm^3$, Speed of Sound=6.32mm/$\mu$s, E=70GPa) (diameter = 340$\mu$m ) and area
fraction of 20%. The operating frequency was set to 5Mhz and the input was a Gaussian pulse with a 60% bandwidth. The lesion size was set to 8mm and IRMs were acquired in two perpendicular directions as shown in Figure 3.8.

Figure 3.8: Schematic of simulation domain for perpendicular compounding

Shown in Figure 3.9 and Figure 3.10 and the two 1-D maps and the final 2-D image of the lesion formed after taking a cross product of the two 1-D maps.

Figure 3.9: 1-D diffusion maps in perpendicular direction
We see that from this perpendicular compounding method we are able to image the lesion with high accuracies in terms of localization and size prediction. This methodology has promise in lesion and target detection for highly scattering media with its advantage being its simplicity and robustness.

3.6 Conclusion

The measurement of D cannot be completely local because the transducer emits a spherical wave and the path of this wave in a scattering medium cannot be tracked, hence artefacts from surrounding regions may creep in. Even in the near field, perfect direction and collimation cannot be fully achieved. It is best to use frequencies corresponding to wavelength of the order of the scatterer size to promote multiple scattering and eventually diffusion. As the wave enters the diffusive regime, the propagation paths are random. However, the diffusion of energy can be tracked using the incoherent backscattered intensity. D provided a good indication of relative changes in the volume fraction of scatterers of highly scattering media, which could not be imaged.
using conventional ultrasound imaging. This study shows the feasibility of assessing the spatial
distribution of the diffusivity in highly scattering media in the near field without beamforming.
CHAPTER 4 - Lesion Imaging and Target detection in Multiple Scattering (LITMUS) Media

4.1 Abstract

In this chapter, we present an ultrasound algorithm (LITMUS) suited to image lesions (hypoechoic) or targets (hyperechoic) in media, which are highly complex in nature. Standard ultrasound imaging techniques fail to detect such lesions/targets due to aberrations and the loss of linearity between distance and time, caused by multiple scattering of ultrasonic waves. LITMUS has the capability to predict the location and size of such lesions/targets by using these multiple scattered ultrasound signals to its advantage. In our experimental and computational studies, we use a linear array of transducers/receivers. Lesions/targets are embedded at varying depths inside multiple scattering media with varying density of scatterers. In the simulations, aluminum scatterers are used as the source of multiple scattering and heterogeneity in the propagating media (water). In the experiments, melamine sponges are used, with air as the scattering source. For multiple locations along the transducer, the incoherent backscattered intensity of the backscattered signals are extracted and the linear growth of the diffusive halo over time is tracked. Sudden changes in this growth indicates the presence of a region with profoundly reduced heterogeneity, indicative of the presence of a lesion/target. This methodology combined with a depression detection algorithm enables us to predict the size and the location of lesion/targets. Despite the presence of strong heterogeneity and multiple scattering, LITMUS displays high fidelity in predicting the location and size of the lesions.
4.2 Introduction

Ultrasonic imaging using linear arrays or non-destructive testing are based on the linear relationship between propagation time and propagation distance between the transducer element and the scatterer, \( t = \frac{2d}{c} \). When a wave propagates into a porous, heterogeneous and strongly scattering medium, the received echoes have two major contributions: Single scattering and multiple scattering. Linearity between distance and time can be assumed in cases when single scattering is dominant. However in the presence of a high scattering density, multiple scattering plays a major role in the propagation of the wave, impairing the linearity between time and distance and leading to a diffusive process. In these cases, classical imaging methods fail. Imaging a porous heterogeneous media has remained a challenge due to the failure of traditional imaging modalities, higher attenuation, dominance of multiple scattering over single scattering and loss in distance-time linearity. These challenges have significantly limited the application of ultrasound in non-destructive evaluation (NDE) of materials, crack size imaging and prediction in polycrystalline materials, lung nodule imaging etc… Given all challenges in analysis of a complex media, multiple scattering has become a well-studied phenomenon especially in characterizing micro-architectures of disordered media. Work previously done by a variety of researchers has showcased the ability to use multiple scattering to calculate the diffusion constant (D), group velocities etc. to characterize disordered media. Diffusion constant was then correlated with transport parameters such as the transport mean free path and the scattering mean free.

Algorithms have been developed to image strongly scattering complex media. Holmes et al. developed a full matrix capture (FMC) approach which was tested on aluminum blocks with notches placed at different depths. The FMC approach combined with the total focusing method (TFM) improved the SNR and has shown good results in imaging complex media. Since it has
been established that it is the multiple scattering contribution which leads to the failing of traditional imaging modalities, Aubry & Derode’s work on separating the single and multiple scattering contributions in backscattered waves played a major role in the further development of imaging techniques for random media. Once the separation of single and multiple scattering is performed, one can applied the DORT (decomposition of the time-reversal operator) method masked with a SSF (single scattering filter) in order to remove any multiple scattering contribution. This algorithm was tested in a medium containing randomly distributed Aluminum rods (diameter of 0.8mm). The target to be detected was placed beyond the random medium whose thickness was more than 3 times the transport mean free path ($l^t$). Shahjahan et al then extended the algorithm developed by Aubry and Derode by combing the DORT and TFM approaches with a multiple scattering filter (MSF) to image polycrystalline media and detect the presence of a flaw/crack or notch. Their results showed that the TFM approach alone had a low flaw detection rate. However, applying DORT along with a multiple scattering filter exhibited high detection fidelity with acceptable SNR. To image a heterogeneous medium, reducing the multiple scattering contribution is very important. Hence, multiple element arrays provide a great improvement and flexibility. In medical applications, beamforming and TFM improve the single to multiple scattering contribution. However, in media where the scattering is purely dominated by multiple scattering, conventional beamforming fails. In such media, the wave diffuses slowly and the diffusive process can be characterized by the diffusion constant $D$, which can be extracted by analyzing the coherent or incoherent contributions to the wave. Previous work done by Aubry and Derode and Mohanty et al showcased the applicability of extracting the incoherent intensity of the backscattered wave to calculate $D$ and characterize heterogeneous media such as the lung parenchyma and melamine sponge foams. Mohanty et al also showed
that using smaller sections of an ultrasound linear array, one could map $D$ along the transducer axis to detect heterogeneity in a porous medium and identify location of anechoic lesions in the lung parenchyma.

The aim of the present work is to provide an imaging algorithm capable of detecting lesions/targets in a highly scattering media. For very strong scatterers such as air pockets in water (similar to the lung parenchyma), traditional B-Scan modality fails due to presence of multiple scattering and aberration effects. In addition, TFM and DORT methodologies individually fail when multiple scattering dominates. In the presence of strong scatterers (Aluminum, air etc…), if the target is placed behind a distance significantly larger than the scattering mean free path, imaging remains a challenge. We propose a methodology, which takes advantage of multiple scattering events and aberrations in order to map the complex medium and detect the size and shape of a target/lesion. We demonstrate the proof-of concept in both simulations and experiments. In simulations, we use water as a propagating medium and aluminum scatterers as the heterogeneities, with varying scatterer area fractions (AF) of 10% and 20%. The target to be imaged here is either an anechoic lesion (a region of absence of scatterers) or a very large Aluminum target whose size and depth will be varied. For experiments, we use a 128-element linear array kept in the near field of a sponge/melamine foam media. The foam is modified to vary the air volume fraction (VF) of 10%, 20% and an anechoic lesion made of petroleum jelly is embedded at a depth of 15mm from the surface of the sponge.

The incoherent backscattered intensity was extracted, giving access to the spatial spread of the diffusive halo as a function of time. A depression detection filter previously developed by Winslow et al. is then applied to identify the regions of depression of the spatial spread, thereby giving an estimate of the location and size of the target.
4.3 Materials and Methods

4.3.1 Data Acquisition

For the data acquisition process, we consider an \( N \) element linear array transducer (\( N=96 \) for simulations and \( N=128 \) for experiments). The transducer was placed at a distance of 3mm (near-field) from the edge of the random medium. A 2 cycle Gaussian pulse with central frequency 5MHz was transmitted from the emitter \( i \) and received by all the transducers \( j=1:N \). This enabled the acquisition of an Inter-element response matrix (IRM). The IRM is represented by \( H(t) \) whose dimensions are \( N^*N^*t \), the individual elements of which are \( h_{ij}(t) \). The individual element \( h_{ij}(t) \) are the \( N^2 \) inter-element responses of the probe-medium system\(^{13,16,21}\).

Once the IRM has been obtained, the IRM was split into sub-IRMs with number of elements \( P \) (Odd number only) (\( P = 33 \) for simulations, \( P=43 \) for experiments). Let us assume that for all explanation purposes \( P=33 \) and \( N=96 \). When the IRM is split into sub-IRMs, we refer to each of those as \( H_z(t) \) where \( z \) ranges from \( \frac{P+1}{2} \) to \( \frac{2N-P+1}{2} \) (\( 17 \leq z \leq 80 \)). Hence the first the sub-IRM will be referred to as \( H_{17}(t) \) and each element of this sub-IRM is \( h^{17}_{ij}(t) \) where \( i \) ranges from 1:33 and \( j \) from 1:33. Hence the dimension of \( H_{17}(t) \) is \( 33^*33^*t \). The superscript in \( h^{17}_{ij}(t) \) has been set to 17 since that is the central element of the sub-IRM, and that is where the counting would begin from. If \( P=43 \), the counting would start from 22. \( H_{17}(t) \) when further processed (see below), will give the growth of the diffusive halo in the area in front of the transducer element number 17. This is equivalent as having a small linear array with 33 elements translated across the medium to get a semi-local assessment of the diffusive properties. This is described in Figure 4.1. It should be noted that the region in front of elements 1-16 and 81-96 will have no representation by any sub-IRMs and will remain inaccessible for image generation and backscattered intensity calculation.
4.3.2 Mapping the spread of the diffusive halo

Once all the sub-IRMs were been obtained, the main objective is to calculate the backscattered intensity and split it in its 2 constituents, the coherent and the incoherent intensities. The incoherent backscattered intensity gives access to the diffusion constant $D$ according to

$$I_{inc}^z(X,T) = I^z(X,T)\exp\left(-\frac{x^2}{4DzT}\right) \quad (1)$$

Where $X$ represents the distance between emitter and receiver and $D_Z$ is the local diffusion constant. The method for processing the sub-IRM was developed on the basis of the work done by Aubry et al and further extended to the near field by Mohanty et al. Every sub-IRM $H_z(t)$ has a specific reciprocity feature: $h_{ij}^z(t) = h_{ji}^z(t)$. Based on this property we defined an anti-sub-IRM $H_A^z(t)$ wherein: for $i > j$, $h_{ij}^{zA} = -h_{ij}^z$; for $i = j$, $h_{ij}^{zA}=0$ and for $i < j$, $h_{ij}^{zA} = h_{ij}^z$. The backscattered
The backscattered intensity is then calculated by squaring and integrating $k_{ij}^z(T, t)$ over time and over emitter/receiver couples separated by the same distance $X = |i-j|$. It was then summed over all frequencies to obtain the given equation.

$$I^z(X, T) = \langle \left| k_{ij}^z(T, f) \right|^2 \rangle_{f\in[i,j]} \quad (3)$$

The backscattered intensity can also be obtained in the time domain by squaring and integrating $k_{ij}^z(T, t)$ over time and over emitter/receiver couples separated by the same distance $X$.

$$I^z(X, T) = \langle \left| k_{ij}^z(T, t) \right|^2 \rangle_{t\in[i,j]} \quad (4)$$

Where $i,j = (z - \frac{p-1}{2}, z + \frac{p-1}{2})$ for a fixed value of $z$. The same procedure can be applied to obtain $I^A(X, T)$ which is the backscattered intensity for the anti-sub-IRM. Note here that $I^A(X, T)$ doesn’t have physical relevance and is purely made up for separating the coherent and incoherent
intensities. The incoherent intensity $I_{inc}^{z}(X,T)$ is obtained by taking an average of $F^{A}(X,T)$ and $F(X,T)^{16,21,63}$.

$I_{inc}^{z}(X,T)$ is a 2D intensity matrix and is a function of T and $X=|i-j|$. It can be seen from equation 1 that the exponential can be fitted by a Gaussian distribution. At each time window, the incoherent intensity profile is fitted with a Gaussian curve and its variance $(W_{z}^{2}(T)=2D_{z}T)^{16,63}$ is plotted against time. The linear trend in the variance can be explained by the observation that, in a highly scattering medium, the wave follows a diffusive regime and the diffusion constant represents the rate of growth of the diffusive halo over time.

This is repeated for all transducer element positions $z$ ranging from $\frac{P+1}{2}$ to $\frac{2N-P+1}{2}$ to obtain a 2D variance map which is denoted by $W^{2}(z,T)$. A change of variable by associating $T$ with the depth of propagation using an effective speed of sound $C_{eff}$ leads to a final 2D variance map denoted by $W^{2}(z,y)$ where $z$ will represent the transducer axis and $y$ represents the depth in the medium.

### 4.3.3 Lesion/Target Detection Using Image Processing

The growth of the variance with time $(W_{z}^{2}(T))$ when fitted with a linear slope gives the local diffusion constant $D_{z}$. The linear trend is well captured which showcases the growth of the diffusive halo. However, when the wave encounters an anechoic lesion or a region with no scatterers, the variance deviates from its linearly increasing trend. As the wave enters the region without scatterers, the diffusion constant increases and so does the slope of the variance curves$^{16,17,21,108}$. The localized increase in the variance is what enables us to track the location of the anechoic lesion. To identify the location of this rapid change, these deviations from a linear behavior are treated as outliers and isolated. To do so, we define two new matrices or 2D fields.

1. **Linear Fit Field (L $(z,y)$)**: Map generated from a line by line, linear regression fit of $W^{2}(z,y)$.
2. Delta Field ($\Delta(z,y)$): Map showcasing the outliers in the variance map.

$$\Delta(z, y) = 1 - \max(W^2(z, y) - L(z, y)) \tag{5}$$

The delta field is a representation of the outlier map. In an ideal case, the outliers will form a closed enclosure which needs to be extracted in order to map the anechoic lesion. The delta map is first treated with a 2-D median filter. Then, we apply a depression detection filter, based on the work by Winslow et al\textsuperscript{119} whose original purpose was to detect lakes on a topological surface. The filter treats the outside edges of the closed loop depression as the edges and the depth of the depression is also evaluated. The filter identifies all possible closed loop depressions and based on the depth of the depression, it assigns values between 0 and 1, where in 1 is the depression with the maximum depth. The LITMUS algorithm is a purely qualitative lesion detection algorithm and hence the magnitude of the depression does not have any physical significance at the current moment apart from the fact that it indicates the quality of the closed loop trend. Given below is the implementation procedure of the depression detection filter.

1. Determine the indices and pixel values of all the local minima’s in the delta map $\Delta(z,y)$.
2. For each minimum, determine the indices of the neighboring 8 or 4 points.
3. If the value from the minimum to neighboring points (emanating radially outwards) increases, it is considered an upward slope and a potential lesion location.
4. Move to the next neighboring points and measure change in value. If no increase in value is detected, we just found an edge.
5. Increment index of the local minima
6. Repeat steps 2,3,4 and 5 till you reach the edges of the delta field, stop
A binary threshold was then applied on the normalized matrix post-depression detection filter (Figure 4.5) to isolate the lesion and represents its location (Figure 4.6). A normal Gaussian filter of order 4 is applied in order to obtain an estimate of the actual size of the lesion.

### 4.3.4 Numerical Simulations

For all numerical simulations, we use SimSonic, an open-source simulation software based of finite difference time-domain (FDTD) numerical methods\textsuperscript{120}. Heterogeneous structures of varying area fractions (AF) (10\% and 20\%), lesion size (6mm and 8mm) and depths (10mm and 20 mm) were generated. Given in Figure 4.2 is an example of such a structure used in simulation. A water layer 3 mm thick was simulated between the ultrasound probe and the heterogeneous medium to simulate ultrasound gel. The lesion depth estimated here are the distance between the lesion surface and the surface of the scattering media.

![Lesion = 6mm, Depth = 10mm](image1)

![Target = 6mm, Depth = 15mm](image2)

**Figure 4.2:** Binarized simulation structure.

The image shown in Figure 4.2 is binary. The white portion is treated as water (density = 1000 kg/m\(^3\) and speed of sound = 1500 mm/µs) and the circular scatterers (diameter = 120 ± 30 &mu;m) have been given properties of Aluminum (density = 2700 kg/ m\(^3\) and speed of sound 6.3
mm/μs). In this example, the scatterer area fraction is 20%. The lesion here is simulated as a domain of pure water without any scatterers. The dimensions of the structure are 40 x 40 mm. The grid step for the numerical simulation was $dx = 0.02\text{mm}$ (15 points per wavelength) and the time step was $dt = 0.0022\text{ μs}$.

To show the ability of LITMUS to identify targets, we generate a binarized structure containing aluminum scatterers (Density $= 2700\text{ kg/m}^3$) with area fraction 10% and 20% and the propagating medium is water. We place a larger aluminum target (diameter $= 6\text{mm}$) which we wish to identify using LITMUS. This aluminum target was placed at a depth of 15mm from the beginning of the scattering medium (18mm from transducer surface). All simulations were carried out by simulating a 40mm long linear array transducer, which was split into 96 elements (element width $= 0.41\text{mm}$), emitting 2 cycle Gaussian pulses with a central frequency of 5MHz. An IRM was acquired as described above by firing each element one by one and receiving from all the elements of the linear array for each transmit. 96 simulations (one for each transmit) were carried out to generate an IRM for a given heterogeneous structures. The acquired IRM $H(t)$ has dimension of 96 x 96 x Time. The individual elements of $H(t)$ can be expressed as $h_{ij}(t)$ wherein $i$ is the emitter index and $j$ is the receiver index.

4.3.5 Experimental Setup: Sponge

For the experimental validation of the proposed method, we performed a sponge phantom study similar to that conducted by Mohanty et al\textsuperscript{21}. We used a 50-cm$^3$ block of commercially available melamine foam. The melamine sponge was first saturated with water and then the sponge was shaken so as to evenly distribute water, and expel some water from it to obtain desired air volume fraction of 10% and 20%, which was controlled by weighing the sponge. Once the desired air volume fraction was obtained, an anechoic lesion was implanted by injecting petroleum jelly
at the desired location using a syringe with an 11-gauge needle. The volumetric amount of the petroleum jelly embedded into the sponge is 1ml or 1cm$^3$. This volumetric quantity corresponds to lesion size of approximate size 10mm-12mm depending on the shape of the lesion.

Two samples of air volume fraction 10%, 20% each were obtained and the lesion was planted at a depth of 15mm from the surface of the melamine foam. The lesion was planted using a 5ml 11-gauge syringe, which was filled with petroleum jelly. The transducer was placed on the foam with a layer of ultrasound gel of around 1mm. For all experiments, we used a 128-element L7-4 linear array transducer with a central frequency of 5.2MHz, connected to a Verasonics Vantage ultrasound scanner (Verasonics, Kirkland, WA, USA). The transducer was coupled to the surface of the sponge using a thin layer of ultrasound gel. The IRM was acquired by firing all the 128 elements of the transducer/emitters one by one and acquiring from all receivers simultaneously. The obtained IRM $H(t)$ had dimensions of 128 x 128 x Time. This $H(t)$ was then split up in to sub-IRMs to obtain the final variance map, as described above.

4.3.6 Error Analysis

The signal to noise ratio was calculated post the depression detection phase and pre applying the threshold to isolate the lesion in question. All other depressions were treated as noise and the maximum intensity of these aberration depressions was treated as noise.

$$SNR = 20\log\left(\frac{I_{max}}{I_{max noise}}\right)$$ (6)

Since the lesion size prediction is a challenge, we measured our accuracy based purely only the central location (z-coordinate and y-coordinate) of the predicted lesion.

$$\%E_y = \left(\frac{\text{Lesion YCoord at max intensity} - \text{Lesion centre YCoord from image}}{\text{Lesion centre YCoord from image}}\right) \times 100$$ (7)
4.4 Results

To summarize, we ran the following simulations and experiments, results for which are shown in this section.

Simulations:

1. 8mm lesion placed at 20mm depth for 10% and 20% area fraction of aluminum scatterers.
2. 6mm lesion placed at 10mm depth for 10% and 20% area fraction of aluminum scatterers.
3. Elliptical lesion placed at 15mm depth for 20% area fraction of aluminum scatterers.
4. 6mm target placed at 15mm depth for 10% and 20% area fraction of aluminum scatterers.

Experiments

1. 10mm lesion placed at 15mm depth in sponges with air volume fraction of 10%
2. 10mm lesion placed at 15mm depth in sponges with air volume fraction of 20%

4.4.1 Simulations: Lesion Detection

Figure 4.3 shows the Diffusion Constant as a function of Aluminum scatterer volume fraction in heterogeneous media not containing any lesion. The diffusion constant significantly decreases with increasing scatterer volume fraction.
We next show results from the implementation of the LITMUS method for the case of an 8mm lesion size placed at a depth of 20mm for the 20% area fraction. The variance field $W^2(z,y)$ and the linear fit field $L(z,y)$ of the mentioned case are shown in Figure 4.4.

Figure 4.3: Plot of Global diffusion constant (D) vs AF for all simulations

Figure 4.4: Surface plots for the variance field and linear fit field for the 8mm lesion size, 20mm depth, 20% AF
It can be seen that with the increase in time (depth), the variance increases. In Figure 4.4, growth in the diffusive halo can be seen, however it is not linear (presence of deviations from the linear trend). The normalized variance field was fitted with a linear regression model, line by line, to obtain the linear fit field and the delta field as described above. was obtained, the nodule location was isolated. It can be seen from the normalized delta field in Figure 4.4 that a lesion is present at a depth of 23mm. However, other closed loops are also present. After the application of the depression detection filter, the image obtained should contain only the lesion. However due to large amounts of multiple scattering, the DDF detects not only the lesions but other closed loop. This is displayed in Figure 4.5.

Figure 4.5: 2D intensity map the normalized delta field after applying the depression detection filter for the 8mm lesion size, 20mm depth, 20% scatterer fraction.

DDF is then applied to isolate the lesion followed by a Gaussian filter of the order of 4 to obtain the final lesion/target image(Figure 4.6 C).After the depression detection filter, thresholding and applying the appropriate order Gaussian smoothing, we obtain the final image for the lesion location and size prediction as shown in Figure 4.6. Also shown in Figure 4.6 are results obtained for the 8mm lesion size for 10% AF with the lesion located at 20 mm depth.
Figure 4.6: A: Final lesion location intensity map. B: Simulation structure. C: Comparison of lesion image with actual structure.

Figure 4.7 shows the lesion detection images for the 6mm lesion cases placed at a depth of 10mm and 10% and 20% AF of aluminum scatterers.

Figure 4.7: A: Final lesion location intensity map. B: Simulation structure. C: Comparison of lesion image with actual structure.
Similarly the % error along the z coordinate could also be calculated where z-coordinate is the transducer axis and y-coordinate is the depth axis. Shown in Table 4.1 and Table 4.2 are the compilations of the error % and the SNR values of the 4 cases shown in Figure 4.6 and Figure 4.7.

<table>
<thead>
<tr>
<th>Table 4.1: Error Tabulation for 8mm Lesion Size placed at a depth of 20mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion Size=8mm, Depth=20mm</td>
</tr>
<tr>
<td>10%</td>
</tr>
<tr>
<td>20%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4.2: Error Tabulation for 6mm Lesion Size placed at a depth of 10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion Size=6mm, Depth=10mm</td>
</tr>
<tr>
<td>10%</td>
</tr>
<tr>
<td>20%</td>
</tr>
</tbody>
</table>

The lesions described so far all had a circular shape. To determine whether other shapes could be detected, an elliptical lesion (dimensions 10mm by 5mm) was investigated. Shown in Figure 4.8 are the results obtained on an elliptical lesion placed at 15mm depth (18mm from surface of transducer) in a scattering medium with aluminum area fraction of 20%. This example highlights the ability of the LITMUS method to detect the shape of a lesion located in a scattering medium.

Figure 4.8: Lesion detection for 10mm by 5mm elliptical Lesion, 15mm Depth and 20% AF. A: Final lesion location intensity map. B: Simulation structure. C: Comparison of lesion image with actual structure.
4.4.2 Numerical: Target Detection

*Figure 4.9* highlights the results of target detection.

![Figure 4.9: A: Target location intensity map. B: Simulation structure. C: Comparison of lesion image with actual structure.]

4.3 are the tabulated errors for target detection. From table 4.3, it can be seen that the location of the target is being predicted with high accuracy by the LITMUS algorithm.

<table>
<thead>
<tr>
<th>Target Size=6mm, Depth=15mm</th>
<th>YCoord</th>
<th>ZCoord</th>
<th>SNR(dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>7.0%(1.48mm)</td>
<td>3.4%(0.68mm)</td>
<td>6.93</td>
</tr>
<tr>
<td>20%</td>
<td>11.29%(2.37mm)</td>
<td>9.8%(1.96mm)</td>
<td>6.35</td>
</tr>
</tbody>
</table>

4.4.3 Experimental: Sponge

Shown in *Figure 4.10* are the results obtained for a 10 mm lesion placed at a 15 mm depth for two different air volume fractions of respectively 10% and 20%. The LITMUS algorithm was first applied and a preliminary location of the lesion was determined based on $C_{eff}$ =1540 m/s. The LITMUS generated image was then overlapped with the sponge cross-section and the $C_{eff}$ was
recalculated to achieve perfect overlap between the sponge lesion image and the LITMUS generated image.

Figure 4.10: Lesion detection for 10mm Lesion obtained from experimental results, A: Final lesion location intensity map. B: Cross sectional view of the sponge implanted with petroleum jelly.

Based on data fitting to calculate $C_{\text{eff}}$ for an optimum overlap between the LITMUS predicted lesion and cross sectional image of the sponge with nodule lesion, the $C_{\text{eff}}$ values were found to be 1.24 mm/μs and 1.12 mm/μs for the 10% and 20% VF cases respectively. It was seen that in the sponge, the lesions were isolated with an SNR of 2.4dB and 1.3 dB for the 10% and 20% air volume fraction cases respectively.

4.5 Effective Speed of Sound

As described above, a priori knowledge of the effective speed of sound in the complex medium is required to implement the LITMUS algorithm. For the experimental validation, $C_{\text{eff}}$ of 1.54 mm/μs was an incorrect assumption in the sponge phantom and these $C_{\text{eff}}$ values were recalibrated using data fitting. We envision the LITMUS algorithm to be applied to solitary pulmonary nodule detection in the lung parenchyma. A large amount of previous work indicates
the speed of sound drops in the lungs\(^2\)\(^-\)\(^4\). Air scatterers in tissue exhibit a reflection coefficient 0.99 due to the high impedance mismatch between tissue/water (\(\rho=1000\text{kg/m}^3\), \(\text{SOS} = 1500\text{m/s}\)) and air (\(\rho=1/\text{kg/m}^3\), \(\text{SOS} = 340\text{m/s}\)). In order to gain insights on wave propagation in media containing air scatterers, we carried out 2-D FDTD numerical simulation using SimSonic. Porous 2D structures were generated with 0\% (pure water), 10\% and 20\% air area fraction with circular air scatterers (diameter) 300\(\mu\text{m}\). The effective Speed of Sound (SOS) \(C_{\text{eff}}\) was calculated for a total propagation distance of 30mm in SimSonic using an approach similar to that adopted by Meziere et al \(^{121}\). A time-distance matrix was generated and \(C_{\text{eff}}\) was evaluated using a linear regression fitting. Show in Figure 4.1L is the time distance relationship and the effective \(C_{\text{eff}}\) values obtained for air scatterers in a tissue matrix. Shown in Figure 4.1L is the time distance relationship for structures with area fraction 10\% and 20\% with aluminum scatterers (120 ± 30 \(\mu\text{m}\)). In the case of aluminum scatterers, it can be observed that \(C_{\text{eff}}\) remains constant at 1.54 mm/\(\mu\text{s}\). For the air scatterer case, as the volume fraction increases, \(C_{\text{eff}}\) drops. In addition, it is interesting to note that as the wave propagates deeper, it slows down further due to scattering. Therefore, \(C_{\text{eff}}\) is a variable not only dependent on the porosity fraction of the media but will also be dependent on the depth of the lesion location. This could result in a challenge to associate the depressions in the normalized delta field map (Figure 4.4) to an actual lesion/target location in structures where the lesion is located beyond a depth of ~2cm. Due to this non-linear relationship between distance and time for higher air area fractions at larger depths, it is possible that the LITMUS algorithm could produce inaccurate results due to the lack of knowledge of the \(C_{\text{eff}}\) at that depth.
For the air scatterer case (Figure 4.11), these simulations give \( C_{\text{eff}} \) as a function of nodule location and air fraction. From these, \( C_{\text{eff}} \) was evaluated for a total propagation distance of 30mm and was found to be 1.41 mm/μs and 0.88 mm/μs for 10% and 20% air scatterer fraction. We would like to point out here that we don’t use the \( C_{\text{eff}} \) values obtained from simulation for the sponge experiments for a variety of reasons. Firstly, SimSonic as an FDTD tool is ideal for wave propagation in solids and liquids and not for air scatterers since it is second order in space and that affects its ability to resolve the large discontinuity in the acoustical properties between water and air. Secondly, the geometry in which the simulations were carried out are very different from the sponge micro-architecture. The purpose of these simulations was only to highlight the slowing down of the waves in the presence of air scatterers.

4.6 Memory Effect and Its Impact

In highly multiple scattering media, it remains a challenge to image due to speckle and large amounts of aberrations. Due to the presence of aberrations, the linearity between distance and time is lost. Another very important thing to note is the analysis backscattered RF signals from the concept of the concept of memory effect. In a nutshell, memory effect is the phenomenon in which as the wave is backscattered from deeper regions, while on its way back to the probe, it may forget the information of deeper regions and pick up information about the region last traversed.
by it. Backscattered ultrasound waves tend to provide information only of the region it last travels through or traverses.

Therefore, in the context of this chapter, if the lesion is placed very deep, the region that the backscattered wave has to travel after interacting with the lesion is very large. Similarly, once the ultrasonic wave, after interaction with the lesion is travelling back to the probe and experiences a domain with very high porosity, it may end up forgetting information that it was previously carrying. Both these contributions of memory effect hinder imaging of highly multiple scattering media. The concept of memory effect is shown in Figure 4.12.

Figure 4.12: Schematic of memory effect

In order to validate the theory of memory effect, FDTD simulations were conducted. Geometries with gradually increasing porosities were generated in a simulation domain of 20mm by 20mm as shown in Figure 4.13. A Gaussian pulse centered at 5MHz was used and a grid size of \( dx = 0.015 \text{mm} \) (20 points per wavelength). The scatterer density (initial scatterer size = 120\( \mu \text{m} \))
was kept constant and the scatterer size was increased to increase the area fraction of the geometry. The gradient of geometry is measured in terms of \( \frac{\text{% Increase In Area Fraction}}{\text{cm}} \).

Figure 4.13: FDTD Simulation Geometries with varying porosities along the depth

The linear array used was a 64 element linear array with the size of each element =0.3mm. The simulations were carried out for a total of 25µs with a CFL value of 0.99. Once the IRM was acquired, the diffusion constant D was evaluated based on the methodology explained in chapter 2. Show in Figure 4.14 are the variance plots for the cases shown in Figure 4.13. Tabulated in table 4.4 are the D values for the same.
The effective porosity was calculated by taking a ratio of the total number of pixels occupied by the scatterers and the total number of pixels in the entire geometry. Clearly, we see that with increase in porosity (Effective porosity) the D values drop. This is attributed to the restricted growth of the diffusive at higher porosities. With increased area fraction due to the larger scatterers, the growth of the diffusive halo is restricted.

We also generated 4 more geometries with effective porosities at 3, 10, 15 and 20%. However, these geometries had uniform scatterer size distribution. As the porosity increases from 3% to 10% the number of scatterers dint change. The size changed. These homogeneous geometries are shown in Figure 4.15.
Figure 4.15: FDTD Simulation Geometries with constant porosities along the depth:

For these 4 geometries also, FDTD simulations were carried out and IRM was extracted and the D values were tabulated as shown in table 4.5.

<table>
<thead>
<tr>
<th>D($\text{mm}^2/\mu\text{s}$)</th>
<th>Porosity Grad %/cm</th>
<th>Effective Porosity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.71</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>0.73</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>0.62</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>0.55</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 4.5: Diffusion constant values for memory effect validation for homogeneous geometries
Let us now look at how the $D$ changes with change in porosity. Shown in Figure 4.16 is the dependence of $D$ on the effective porosity for cases shown in Figure 4.13 and Figure 4.15. It can be seen that as porosity increases, the $D$ decreases. However, the rate at which $D$ drop for constant porosity case is higher than the case with varying porosity along depth. The initial portion of all geometries shown in Figure 4.13 have low porosities. As the wave travels back after interacting with high porosity regions, it is carrying information which will yield low $D$ values. However, as it gets scattered by the low porosity region at the beginning, it acquires information from that domain which ends up increasing the $D$ values. Hence, for all varying porosity cases, the $D$ values appear to be much larger. As the wave is backscattered, the growth of the diffusive halo is restricted by high porosity regions, while traversing back, the diffusive halo is allowed to grow more rapidly due to the presence of low porosity regions. This memory effect can be summarized as a phenomena in which the $D$ values technically doesn’t give information of the whole geometry as an average. Rather, it tends to retain information of the last set of multiple scattering events that it went through. The memory of an acoustic wave can be considered to be ephemeral in nature and doesn’t retain all the information. This is a disadvantage in lesion and target imaging.
As the wave has interacted with the lesion or the target, as it travels back, it ends up forgetting information of the lesion/target that it had interacted with previously. This effect puts a limitation on the LITMUS algorithm in terms of the extent of porosity that can be imaged and the depth of lesion.

4.7 Discussion

As the area fraction of scatterer increases, the ultrasonic wave propagation becomes more and more random due to the increased number of scattering events. This phenomena is also highlighted in Figure 4.3, where with increasing area fraction the diffusion constant D decreases linearly. This can be looked at from the perspective of the growing diffusive halo. In a structure with a high area fraction of scatterers, the growth of the diffusive halo is restricted by the high density of aluminum scatterers.

The approach shown in this paper combines a depression detection filter applied over a 2D variance (diffusive halo growth) map to identify the location of the lesion or target. This methodology has shown promise in accurately predicting the location of the lesion. We use the multiple backscattered signal to calculate the incoherent cone as a function of time/depth. When the wave propagates back after interacting with the lesion/target, due to the presence of scatterers on its backtracked path, it loses some of the information. Rather, it gains information of the scattering media. This is a major obstacle in imaging any media with very strong scatterers. Shown in Figure 4.4, is a roadmap of the steps taken by the LITMUS algorithm to finally isolate or predict the location of the lesion of size 20mm present in a 20% area fraction scatterer geometry. Once the DDF is applied on the normalized delta field shown in Figure 4.4, the lesion is isolated. It can be seen from figure 4.5, that the DDF on its own predicts the location of lesion accurately however predicting the size and the boundaries accurately is still a challenge. The DDF also ends up
detecting other closed loops, which are not the lesion. These closed loops are eliminated using a thresholding as mentioned in the methods section. However, applying a threshold does not completely remove the aberrations and this can be witnessed in Figure 4.6 and 4.7. The LITMUS algorithm detects lesions of a varied size range, depths and area fraction with high accuracies.

From Tables 4.1 and 4.2, it can be seen that the lesion is isolated with a reasonable SNR. SNR for the 6mm lesion size, 10mm depth cases are 11.3 dB and 14.8 dB for the 10% and 20% area fraction cases. These values are almost twice as large as in the 20mm depth cases. The deeper the nodule is placed, the harder it becomes for LITMUS to detect the lesion and separate it from the noise. Low SNR values for the 20mm depth indicate that the lesion depression is almost of the same order of magnitude as that of noise, making it a challenge to detect lesions placed at larger depths. This can also be seen in figure 4.6, where even after the application of the threshold, ghost lesions are detected. In addition, the percentage error in the lesion location coordinates increases with the increase in the quantity of scatterers. The denser the scatterers, the more difficult it is for the backscattered wave to retain memory of the target or the lesion. For cases with porosities greater than 20% and lesion size below 5mm, LITMUS failed to identify the location of the lesion.

After circular lesions, we shift our focus to more complex shapes such as ellipse. We see that LITMUS identified the elliptical shape of the lesion which gives us strong reasons to believe that LITMUS is sensitive to shape. It may not be able to identify very complicated lesions with curly boundaries, but it is able to differentiate between a circular and an elliptical lesion.

The main purpose of this algorithm was to have a unified methodology, which detects lesions as well as targets. We defined a lesion as a region with no scatterers whereas a target as a much larger scatterer. In more realistic applications, the lesion will not be perfectly homogeneous and will contribute to backscattered intensities but those backscattering signals would be
negligible compared to the multiple backscattering contributions due to aluminum scatterers. We see that the LITMUS algorithm was able to detect a 6mm target with acceptable SNR values of 6.93dB and 6.35dB for 10% and 20% area fraction cases. The deviation in the target’s centre coordinates was within 2.5mm. We would like to remind the readers again that in multiple scattering, it is very difficult to localize measurements. Given the multiple scattering regime and high diffusivity, obtaining perfectly localized diffusion measurements is not possible. In addition to that, each Rf line generated by a sub-IRM is not completely local. It shares emitter and receivers with other sub-IRMs which were used to generate the neighboring Rf lines. Therefore, deviations of the order of 2.5mm are acceptable and showcase the ability of LITMUS to isolate lesions and targets in such a diffusive media.

After computational validation, experiments were carried out in a melamine foam/sponge saturated completely with water. The water was then expelled by shaking to obtain desired volume fractions of air. 1cc of Vaseline was used to generate the lesion at the center of the foam at a depth of 15mm. The size of the lesion was under-predicted by LITMUS. The percentage errors in the nodule size with respect to that determined from image processing was found to be 18% and 26% for the 10% and 20% VF cases respectively. These errors observed can be attributed to multiple causes. Given the fact that the scattering here was caused by air, which are far stronger scatterers than aluminum, the localized changes in the variance map which correspond to the lesion size were not strong enough. It is also possible that the Gaussian filter of order 4 was not the appropriate smoothing order to achieve accurate lesion size predictions.

One of the major strengths of LITMUS is its ability to detect lesions/targets at depths beyond 10mm, however its limitations lies in the size of the lesion. Using LITMUS, we were able to detect lesions of size 8mm at depths up to 20mm. LITMUS uses the aberrations generated from
multiple scattering to its advantage which otherwise would be a burden in classical imaging techniques. It was displayed that LITMUS is sensitive to lesion shape (circular or elliptical). LITMUS is unique due to its ability to image geometries with such high porosities. Since the effective speed of sound didn't change much when the scatterers were aluminum (low impedance shift compared to air), LITMUS has shown higher fidelity in identifying lesions/targets in geometries with aluminum scatterers. Hence, by utilizing the multiple scattering, which earlier was seen to be a burden, we were not only able to characterize and estimate the porosity of the medium but also identify discontinuities and lesions with high fidelity.

4.8 Limitations

One major limitation is its dependence on the effective speed of sound $C_{\text{eff}}$. This methodology could potentially generate incorrect results for a porous media with air-scatterers if the lesion is placed at a large depth as the effective speed of sound $C_{\text{eff}}$ drops sharply as the wave propagates deeper and deeper into the medium, which was already shown in Figure 4.11. The major downside of the thresholding after applying the DDF is the fact that if there are two lesions of different sizes lying near each other, the threshold will ignore the existence of the secondary anechoic lesion considering it noise. Hence, one could infer from this that the LITMUS works very well for solitary lesion systems. Placing the 6mm nodule at a 20 mm depth for computation showed the inability of LITMUS to detect the location of the lesion. This could potentially be due to memory effect. As the backscattered wave propagates through a multiple scattering regime, it could potentially lose information on the regions it previously tracked, thereby losing memory of the lesion region \textsuperscript{122}. 
4.9 Conclusion

The goal of this work was to develop a method for detection of targets and lesions embedded in a highly scattering heterogeneous media. It requires the acquisition of IRMs, which is split into smaller sub-IRM$s$. The backscattererd incoherent intensity is mapped and local deviations in the linear variance trend allows us to image lesions and targets. In this study, either simulations were carried out in a distribution of aluminum scatterers, which had a lesion, or a target embedded in them. LITMUS was able to detect lesions and targets with high fidelity by locally tracking the changes in the variance. The combination of a DDF and diffusive halo 2-D map allowed for the prediction of the lesion location. The first set of results were highly encouraging: 2-D imaging of such highly scattering media up until now has been virtually impossible. LITMUS was able to detect 6mm lesions placed at a 10mm depth (SNR=3.97dB) and an 8mm lesion placed at 20mm depth (SNR=11.3 dB) for scatterer distribution of 10\% and 20\%. When the 6mm lesion was placed at 20 mm depth, LITMUS failed to isolate the fluctuations in the localized diffusive halo map. LITMUS was also applied to target detection (SNR=6.35dB) and the preliminary results are very promising. It was interesting to discover that indeed LITMUS is sensitive to shape and can accurately differentiate between a circular and an elliptical lesion.

The experimental results have shown high fidelity compared to the current imaging methodologies in imaging highly scattering media. However, the dependence of imaging in media with air scatterers does indeed require a priori knowledge of the $C_{\text{eff}}$, which is a shortcoming. To overcome this, new experimental tests will be used to perform parametrical studies of determining $C_{\text{eff}}$ as a function of depth, air volume fraction and frequency in order to precisely determine the position of the lesion.
CHAPTER 5 - Fibrosis Assessment: An In-Vivo Rodent Study

5.1 Abstract

Idiopathic pulmonary fibrosis (IPF) affects 200,000 patients in the U.S. IPF is responsible for changes in the micro-architecture of the parenchyma, such as thickening of the alveolar walls, which reduces compliance and elasticity. In this study, it is proposed to verify the hypothesis that changes in the micro-architecture of the lung parenchyma can be characterized by exploiting multiple scattering of the ultrasound waves by the lung parenchyma. Ultrasound propagation in a highly scattering regime follows a diffusion process, which can be characterized using the Diffusion Constant. We hypothesize that in a fibrotic lung, the thickening of the alveolar wall reduces the amount of air (compared to a healthy lung), thereby minimizing the scattering events.

Pulmonary fibrosis is created in Sprague-Dawley rats by instilling bleomycin into the airway. The Rats are studied in groups of n=6 (3 male and 3 female) 2, 3, and 4 weeks after bleomycin administration. This was expected to create a range of severity of pulmonary fibrosis for assessment. Using a 128-element linear array transducer operating at 7.8MHz, in-vivo experimental data is obtained from Sprague-Dawley rats and the Diffusion Constant is calculated. Right after the ultrasound measurement, the rats are euthanized and Computed Tomography scans are performed to validate the degree of fibrosis created. Significant differences (p<0.05) in the D values between control and fibrotic rats showcase the potential of this parameter for diagnosis and monitoring of IPF.
5.2 Introduction

The alveolar-interstitial syndrome (AIS) in the lung includes heterogeneities which most of the times leads to severe respiratory failure. AIS leads to conditions which may be chronic (e.g., pulmonary fibrosis) or acute (e.g., acute respiratory distress syndrome (ARDS), acute pulmonary edema, interstitial pneumonia). Pulmonary edema is caused due to many reasons and can be cardiogenic or non-cardiogenic. Pulmonary edema is characterized by increased extravascular lung water which causes acute dyspnea leading to high mortality rate\textsuperscript{123}. Pulmonary fibrosis is a progressive, fatal, inflammatory and fibro-proliferative lung disease for which existing treatments are of limited benefit. Past data have suggested that it is the most common chronic interstitial disease accounting for a majority of new interstitial lung diseases \textsuperscript{74}. The main histopathological features of usual interstitial pneumonia, best seen at low magnification, is a heterogeneous appearance with areas of sub-pleural and para-septal fibrosis and honeycombing (i.e., cystic fibrotic airspaces lined by bronchiolar epithelium and often filled by mucin and variable numbers of inflammatory cells) alternating with areas of less affected or normal parenchyma (spatial heterogeneity). In patients with heart failure, (edema) interlobular septa are thickened by water. In pulmonary fibrosis, interstitial lobular septa are thickened by collagen tissue accumulation. Most of the fibrosis consists of eosinophilic collagen with few associated inflammatory or stromal cells. This collagen deposition thickens alveolar septa and forms patchy scars. It also accompanies areas of honeycomb change. The latter are characterized by thick walls containing collagen and varying amounts of chronic inflammation\textsuperscript{124}.

Conventional chest radiography and high-resolution computer tomography (HRCT) are the most common techniques to detect pulmonary fibrosis as well as to assess treatment efficiency.
High-resolution CT (HRCT) of the chest has been found a sensitive and reproducible method to assess the extent and the pattern of pulmonary fibrosis\textsuperscript{125–127}. However, they are associated with high radiation, high costs and very low portability.

Lung ultrasound (LUS) is a non-invasive, cheap and a portable technique which has developed over the past 50 years to potentially detecting AIS\textsuperscript{52}. It is only very recently that it has garnered importance as a valid criterion for assessing pulmonary edema and fibrosis compared with HRCT as the gold standard. Chest US has multiple uses, both in diagnosis as well as intervention. Studies have started showing that LUS, as a consequence of its advantages over radiography and HRCT (no radiation exposure, cost effective and high portability) can play the primary role in diagnosis and monitoring of fibrosis\textsuperscript{128}. LUS in the lung has always been considered poorly resolved because of the air sacs and the thoracic cage. The latest development of lung ultrasound is based on new applications and discovery of the significance of sonographic artifacts, The conventional approach of lung ultrasound is based on the identification of ten standardized signs\textsuperscript{45,129}. However, reading and interpreting these signs is subjective and operator-dependent. Amongst these ten lines, pulmonary fibrosis is characterized by the ULCs (Ultrasound Lung Comets). ULCs are an echographic image detectable with chest sonography\textsuperscript{125,130}. This image consists of multiple comet tails fanning out from the lung surface as shown in Figure 5.1\textsuperscript{125,131}. ULCs are generated by the reflection of the ultrasound beam from the thickened sub-pleural interlobular septa. In patients with heart failure, interlobular septa are thickened by water, and ULCs represent an early sign of pulmonary interstitial edema, well related to the increase in cardiac peptides, radiographic signs of pulmonary congestion, invasive measurement of extravascular lung water and pulmonary capillary wedge pressure\textsuperscript{131–133}. In pulmonary fibrosis, interstitial lobular septa are thickened by collagen tissue accumulation and, therefore, ULCs are generated by the
same anatomical interface as in heart failure, i.e. a thickened sub-pleural interlobular septa, although the physical scatterers are represented by lung air fibrosis and not by air–water impedance mismatch\textsuperscript{134}.

![Diagram of normal lung and pulmonary fibrosis](image)

Figure 5.1: Reflections of the ultrasound beam by thickened interlobular septa give rise to ULCs\textsuperscript{134}.

The quantitative tissue characterization of the lungs has remained a challenge using acoustic and ultrasound techniques. The presence of air sacs make the lung a highly diffusive, aberrating and scattering medium. The diffusive nature of the lung destroys the linear relationship between propagation time and propagation distance thereby making imaging of the lung inaccurate. Increase in the sophistication of the equipment clubbed with the ever increasing image resolution has made it possible to obtain high quality RF data. Tourin et al. in their previous work, defined a set of parameters such as diffusion constant (D) and transport mean free path (L*) which are dependent parameters to characterize complex and diffusive media\textsuperscript{13}. These were assessed in a diffusive medium made of steel rods acting as scatterers in water. This methodology was then
applied to the human trabecular bone by Aubry et al. to give local measurements of the porosities of the bone micro-architecture \(^{16,63}\). This methodology was then tested by our group in a previous study\(^ {21}\)[Chapter 2 and Chapter 3] wherein we validated this methodology on melamine sponge with varying air volume fraction. The main goal of this chapter is to determine if using ultrasound multiple scattering and diffusion measurements, it is possible to characterize and stage fibrosis. Fibrosis is induced in the lungs using a bleomycin rodent model, details of which are discussed in the upcoming sections. The in-vivo rodent study is done by performing a sternotomy so as to remove the rib cage and ultrasound IRM data is acquired using a L11-4v linear array probe.

5.3 Materials and Methods

5.3.1 Bleomycin Rat Model

Pulmonary fibrosis was created in Sprague-Dawley rats by instilling bleomycin into the airway. After sedation, rats were intubated with a 12-gauge catheter. Bleomycin 2mg/kg, dissolved in 100 \(\mu\)L sterile PBS, was administered into the trachea. The rat was extubated and allowed to recover. This bleomycin dose causes development of pulmonary fibrosis within 2-3 weeks \(^ {135-137}\)(Fig.2). Rats were studied in groups of \(n=6\) (3 male and 3 female) 2, 3, and 4 weeks after bleomycin administration. This was done provide a range of severity of pulmonary fibrosis for assessment\(^ {138}\). Six rats (3 male, 3 female) who received no treatment served as controls. A total of 24 Sprague Dawley rats (12 male and 12 female) were used in this study (349±91.76 gms).
5.3.2 Animal Preparation for Ultrasound

After sedation, a tracheotomy was performed. Rats were ventilated with a Harvard rodent ventilator. Anesthesia was maintained with titrated isoflurane. A sternotomy was performed, both pleural spaces were opened, and the sternal edges spread maximally to expose both lungs. The incision was extended inferiorly into the abdomen to expose the liver. Heparin was administered intrahepatically to prevent clotting after the lungs were removed. For the ultrasound measurements, ultrasound coupling gel was applied directly onto each lung. Ultrasound measurements are taken in rat lungs in vivo as described below. After the ultrasound data is collected, the rat was euthanized by cardiectomy under anesthesia. The trachea was clamped at end-inspiration. The heart-lung block was excised and was immersed in cold phosphate buffered saline (PBS) for an ex-vivo CT scan (described below). Following the CT scan, the heart-lung block was subjected to inflation fixation for histologic interpretation (described below).

5.3.3 Data Acquisition methodology

All the in-vivo experiments were conducted with an L11-4v (128 element Linear Array Transducer) connected to a Verasonics Vantage ultrasound scanner. The transducer was placed on the exposed lung using a 1-2mm layer of coupling gel. All the elements of the array were fired one
by one transmitting a 2 cycle pulse with a central frequency of 7.8 MHz (300kPa) into the medium. The backscattered signals were collected on all 128 elements of the array and this gave us access to the spatial spread of the transient pressure field. The sampling frequency of the data acquired was 62.5 MHz and the total data acquisition time was set to 40 μs. This enabled the acquisition of a 128 by 128 by 2500 impulse response matrix (IRM) \( \mathbf{H}(t) \) whose individual elements \( h_{ij}(t) \) are the \( N^2 \) impulse responses of the medium. \( \mathbf{H}(t) \) was then processed using the methodology earlier described by Tourin et al and Aubry et al.\(^{13,16}\). For simplicity purpose, the origin time \( (t=0) \) was set to the arrival of the first backscattered wave for every single receiving transducer. The IRM’s reciprocity feature was exploited to generate the anti IRM represented by \( \mathbf{H}^A(t) \). This was done using a simple matrix manipulation as shown.

- for \( i>j \), \( h_{ij}^A = -h_{ij} \);
- for \( i=j \), \( h_{ii} = 0 \);
- for \( i<j \), \( h_{ij}^A = h_{ij} \);

\( \mathbf{H}(t) \) and \( \mathbf{H}^A(t) \) were then processed to obtain \( D \) from the incoherent intensity. Analytically, the incoherent intensity can be represented as

\[
I_{inc}(X, T) = I(T) \exp \left( -\frac{X^2}{4DT} \right),
\]

Where \( X \) represents the distance between emitter and receiver. Equation (1) clearly established that when the incoherent intensity was plotted as a function of \( X \) and \( T \), \( D \) would be an indicator of how diffusive the multiple scattering medium was. The measurement of the diffusion constant from experimentation can be done by plotting \( I_{inc} \) as a function of \( T \) and \( X \). The intensity was averaged over all emitter receiver couples separated by the same distance. At each time window, the backscattered incoherent intensity \( I_{inc} \) was fitted with a Gaussian curve and the
variance of the Gaussian fit represents the dynamic growth of the diffusive halo given by $W^2(T) = 2DT$. Once $D$ was extracted, the transport mean free path $L^*$ was evaluated based on equation 2. The details of this methodology can be found in Chapter 2 as well.

$$D = \frac{V_e \times L^*}{3}$$

### 5.3.4 High Resolution CT Scanning and Scoring

In order to optimize the resolution, ex-vivo high resolution CT scans were performed rather than imaging lungs in the live, sedated, breathing animal. A high resolution preclinical CT system (CT 120, TriFoil Imaging, Inc. Chattsworth, CA) was used to acquire micro-CT images on lung specimen. The entire lung was removed from deceased rats, inflated, and closed at the airway. Images were immediately taken after lung collection with x-ray energy of 100 kVp, current of 50mA, 100 ms of exposure time, and 2x2 binning. Images were reconstructed using Feldkamp reconstruction algorithm to create isotropic CT images with nominal resolution of 50 um. Final images were converted to DICOM format with Hounsfield unit (HU). Before imaging, the lung block was inflated manually with air by syringe to a volume that visually approximates the volume of the lung block at end-inspiration when it was removed. Compliance will be different for each lung block, depending on the amount of fibrosis. Inflation at the imaging facility to a particular inspiratory pressure or precise volume is not possible. Volume will change because lung cells remain viable for hours after circulatory arrest, so oxygen consumption is ongoing. With a respiratory quotient of 0.8 (CO2 production to O2 consumption), the volume of air in the lung block will diminish with time, so inflation is required. Imaging were taken within 10 min of inflation for all the specimens. Severity of lung fibrosis was scored blindly by a radiologist from 0-4 based on visual assessment of fibrotic tissue volume, with 0 being no fibrosis, 1 being small
local fibrosis affecting less than 15% lung volume, 2 being local medium fibrosis affecting up to quarter of lung volume, 3 being large fibrotic tissue affecting up to 50% lung volume, 4 being diffusive fibrosis affecting multiple lung lobes with more than 50% lung volume.

5.3.5 Histology and Scoring

After each CT scan, the lung blocks were subjected to inflation-fixation. The lung blocks were submerged in paraformaldehyde for 24-48 hours, then washed and stored in 70% ethanol. A paraffin section of lung, stained either by hematoxylin and eosin (5 micron sections stained with H&E) or by a trichrome method, is systematically scanned in a microscope using a x10 objective. Each successive field was individually assessed for severity of interstitial fibrosis and allotted a score between 0 and 8 using a predetermined scale of severity (table 1)\textsuperscript{139–141}. These scores were mapped to the histology images as shown in Figure 5.4\textsuperscript{140}. Severity of lung fibrosis was studied on the Ashcroft scale (0-8 scaling). After examining the whole section, the mean score of all the fields were taken as the fibrosis score for the section and expressed correct to two decimal places. A veterinary lung pathologist assessed these in a masked manner.

Table 5.6: Ashcroft scoring protocol

<table>
<thead>
<tr>
<th>Grade of Fibrosis</th>
<th>Sample Photograph</th>
<th>Ashcroft Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Figure 1A</td>
<td>Normal lung</td>
</tr>
<tr>
<td>1</td>
<td>Figure 1B</td>
<td>Minimal fibrous thickening of alveolar or bronchiolar vessels</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>Moderate thickening of walls without obvious damage to lung architecture</td>
</tr>
<tr>
<td>3</td>
<td>Figure 1D</td>
<td>Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>Total fibrous obliteration of the field</td>
</tr>
<tr>
<td>5</td>
<td>Figure 1F</td>
<td>Severe distortion of structure and large fibrous areas; “honeycomb lung” is placed in this category</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Figure 1G</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Figure II</td>
<td></td>
</tr>
</tbody>
</table>
5.3.6 Data Analysis and statistical methods

The diffusion constant was calculated from IRM acquisitions as described in Chapter 2. Differences between L* values obtained from control and fibrotic lungs were tested using the Kruskal–Wallis test with Dunn’s post-test (data was not normally distributed, so non-parametric analysis was chosen). All data are presented as mean ± standard deviation. Statistical significance was set a priori at \( p<0.05 \) and is graphically depicted on all figures as (*) for \( p<0.05 \), (**) for \( p<0.01 \) and (***) for \( p<0.001 \) as well as NS to denote non-statistically – significant comparisons. Statistics were performed in MatLab 2018a.

Due to the very low penetration in control rat lungs, only a certain number of points (range of points depicting start time and end time) were selected to calculate the variance. This was automated using the `findchangepoints` command in MatLab. For a trend to qualify, The \( R^2 \) of the trend should have been greater than 0.4 and a negative variance trend for the time being is rejected. Data sets were also rejected for post processing if no multiple scattering was observed. This can
be attributed to the lung operating at full volume capacity. Due to this reason, it is advisable to acquire data at the exhaling cycle to ensure penetration and eventually witness multiple scattering.

## 5.4 Results and Discussion

Shown in Figure 5.4 are examples of the variance plots obtained for a control lung and a 3Wk fibrotic lung.

![Figure 5.4: Variance growth for in-vivo rodent data Control and Fibrosis](image)

We see that for the 3Wk fibrosis case, the variance or the width of the diffusive halo increases more rapidly than the case for control. This can be attributed to the thickened alveolar interstitial regions, which reduces the capacity of the lungs, thereby effectively reducing the size of the air scatterers and allowing the wave to diffuse more freely. In the case of the control rat lungs, the growth of the diffusive halo is highly restricted due to the large number of scatterer present in the lungs.

Shown in Figure 5.5 are the L* values obtained for all control and fibrotic lungs. It can be seen that we are able to differentiate between control and fibrosis (2Wk, 3Wk and 4Wk) with high
statistical significance (p<0.001). The $L^*$ for control, 2Wk fibrosis, 3 Wk fibrosis and 4 Wk fibrosis was found to be $466\pm109\ \mu\text{m}$, $773\pm304\ \mu\text{m}$, $690\pm191\ \mu\text{m}$ and $729\pm245\ \mu\text{m}$ respectively. The large error bars can be attributed to two reasons. First, these readings are in-vivo and our attempt to acquire at the exhaling stage based on visual hints could be a potential source of error. Secondly, the lungs (size=3cm) were smaller than the transducer (3.8 cm). This could potentially allow signals from nearby regions to creep in artificially and increase the $L^*$.

**Figure 5.5: $L^*$ Values for in-vivo rat**

Staging was the objective for this chapter, and the results seem to imply that staging using this methodology is not possible. In order to verify whether staging can be done, it is important to compare the data with CT and histology scores. Shown in Figure 5.6 and Figure 5.7 are the fibrosis severity scores mapped to their respective CT and histology images.
As fibrosis becomes more and more dominant in the lungs, the tissue starts thickening which can be observed in histology images as well as the CT images. At score 4 of CT, we see higher shades of gray which characterized a thickened septa which is also corroborated by histology images. Shown in Figure 5.8 and Figure 5.9 are the boxplots for the fibrosis severity score for control, 2,3 and 4wk fibrosis obtained from CT and histology images.
It can be seen from Figure 5.8 that the maximum amount of fibrosis occurs after 2 weeks of bleomycin treatment, after which, fibrosis starts subsiding. Histology also supports a similar claim wherein fibrosis does peak after 2 weeks of bleomycin administration. Show in Table 2 are the average severity scores obtained from histology and CT.

<table>
<thead>
<tr>
<th>Rat Type</th>
<th>CT Score</th>
<th>Histology Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2Wk</td>
<td>2.8</td>
<td>2.6</td>
</tr>
<tr>
<td>3Wk</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>4Wk</td>
<td>2</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Table 5.7: Average severity scores
We hypothesize that the bleomycin model is not a permanent model for inducing fibrosis in the rodents. The bleomycin rodent model hits a peak fibrosis at 2 weeks and eventually starts subsiding. The blind CT and histology severity scores were also compared to check if both methodologies quantified fibrosis in a similar manner. Shown in Figure 5.10 is the comparison between CT and histology scores.

![Figure 5.10: Comparison between CT and Histology severity scores](image)

The trend between the CT severity and histology severity score is a positive one with p<0.05 which suggests that both quantify the extent of fibrosis in a similar fashion. The L* values were compared with histology scores as shown in Figure 5.11.

![Figure 5.11: Comparison between L* values and Histology severity scores of all 24 rats](image)
It can be seen from Figure 5.11 that the transport mean free path $L^*$ has a positive statistically significant trend with the histology severity scores. This then counters the previous hypothesis that $L^*$ cannot be used to estimate the degree of fibrosis.

### 5.5 Conclusion

We successfully demonstrated that for a Sprague-Dawley rat lung, we were able to differentiate control from fibrosis. $L^*$ was obtained by separating the coherent and the incoherent backscattered intensities and calculating $D$ which represented the dynamic growth of the diffusive halo. $L^*$ is a representation of how the wave diffuses in the lung parenchyma. In control lungs, due to the large air scatterers and large air volume, the growth of the diffusive halo is restricted leading to low $L^*$ values. However in fibrotic rat lungs, the extensive thickening of the interlobular septa allowed for the wave to diffuse freely in lung parenchyma thereby accentuating the value of the diffusion constant $D$ and $L^*$.

After the acquisition of ultrasound data, the rats were euthanized and underwent high resolution CT followed by H&E stained histology analysis. The histology and CT images were blind scored by lung pathologist and compared with $L^*$. From Figure 5.11, it is evident that with increase in $L^*$, the severity of pulmonary fibrosis as predicted by histology images also increased. This suggests the potential of the diffusivity method to detect and quantify fibrosis. A staging capability could allow for monitoring response to treatment. Currently the in-vivo data acquisition is has a few challenges. Firstly the rat lungs are smaller than the probe, allowing information from the periphery of the lungs to creep in and over-predict $L^*$ values. This can be seen in the high standard deviations for $L^*$. Secondly, it would be advisable to trigger the Verasonics along with the rodent respirator to ensure that data is acquired at the end of the exhaling cycle ensuring maximum ultrasound penetration into the lung parenchyma. We believe that in larger animals and
with a more controlled experimental environment (acquisition at exhaling), these error bars can be reduced.

It was also noticed that with passing of time after bleomycin treatment of the rats, fibrosis effects peaked at two weeks as predicted by CT, histology and L* values. Lastly, out of the 242 IRMs acquired, we were only able to extract trends with $R^2 > 0.4$ 69% of the times. The other 31% of the data sets exhibited trends with $R^2$ below 0.4. This could be due to a few reasons. Firstly, if the data was acquired at the peak inhaling stage, the reflection would be too strong and hence penetration would be weaker than what is required for an incoherent trend to be visible.
CHAPTER 6 - Edema diagnosis in Rodents and Pigs

6.1 Abstract

The current gold standard for diagnosing alveolar interstitial syndrome such as edema or fibrosis is either X-Ray or CT. Using lung ultrasound for the detection of these pathologies is only applicable in the critically ill and is done using the BLUE protocol. The current techniques for LUS are based purely on artefacts, which are qualitative in nature and are limited by the presence of alveoli and multiple scattering. However, there is a need from pulmonologists to quantify edema. Quantification would enable monitoring progress from treatment. This chapter presents the in-vivo quantification of pulmonary edema in the lung parenchyma using ultrasound multiple scattering by measuring the diffusivity of ultrasonic waves in complex media. This is followed by the unequivocal discrimination of edema and fibrosis (previous chapter) using a spectral based decomposition of plane wave data acquisition. The experimental setup consisted of a linear array transducer L11-4v connected to a Vantage Verasonics. In-vivo data was acquired for six edematous rats and compared with the data obtained from control and fibrotic rats as shown in the previous chapter. We show that by measuring $L^*$ from IRM allowed us to identify the existence of a pathology (Edema and fibrosis) and measuring the backscattered frequency shift (BFS) from plane wave acquisition allowed for unequivocal discrimination. This methodology is also extended to larger animals such as porcine model where we hypothesize the interaction of ultrasonic wave with an edematous lung.

Key Words: Quantitative Ultrasound, Multiple Scattering, Lung Parenchyma, Pulmonary Edema, fibrosis, Spectral based detection, Image feature extraction.
6.2 Introduction

The alveolar-interstitial syndrome (AIS) in the lung includes heterogeneities which most of the times leads to severe respiratory failure. AIS leads to conditions which may be chronic (e.g., pulmonary fibrosis) or acute (e.g., acute respiratory distress syndrome (ARDS), acute pulmonary edema, interstitial pneumonia).

Figure 6.1: CT Scan of Edema with diffused ground glass areas and gravity dependence

Pulmonary edema may be classified as increased hydrostatic pressure edema, permeability edema with diffuse alveolar damage, permeability edema or mixed edema. The relative amounts of intravascular or extravascular fluid in the lung are mostly controlled by the permeability of the capillary membrane as well as the oncotic pressure. Pulmonary edema can be due to many reasons and in general is either cardiogenic or non-cardiogenic. Pulmonary edema is characterized by increased extravascular lung water which causes acute dyspnea leading to high mortality rate. Diagnosis for edema is most commonly based on physical examination, radiographs and echocardiography. However identifying edema radiographically at its nascent stages still poses issues. Findings of pulmonary edema on chest radiographs have an accuracy as low as 69%. 

CT scans are also used for detection of edema and are considered as the gold standard. They exhibit inter- and intralobar septal lines with diffused ground glass areas and a gravitational anteroposterior gradient as shown in Figure 6.1.

The conventional approach of lung ultrasound is based on the identification of ten standardized signs\(^\text{45,145}\). Out of these 10 signs, edema is characterized by the B-line artefact. These B-Lines (Figure 6.2) are defined as laser-like hyperechoic reverberation artifacts that arise from the pleural line and extend till the end of the screen (depends on depth chosen in the imaging settings) without fading (If fading and diffusion occurs, its fibrosis). There has been seen direct correlation between these artifacts and extravascular lung water, ILD (interstitial lung disease) and non-cardiogenic lung edema\(^\text{45,49,52,70,131,133,146}\). However, reading and interpreting these signs is subjective and operator-dependent. These signs are purely qualitative and poorly defined. Lung ultrasound imaging past the pleural layer is highly inaccurate because of the presence of multiple scattering in the parenchyma occurring from drastic changes in impedance from tissue to air in the alveoli. During lung imaging, the backscattered signals are distorted, leading to artefacts and introducing large errors in reading and interpreting the images\(^\text{49}\).
In highly complex media, the backscattered ultrasonic signals can be processed, not for imaging but to extract quantitative parameters of the micro-architecture. Tourin et al. showed that it is possible to characterize a highly diffusive medium using parameters such as the Diffusion Constant (D) and various mean free paths. These were assessed in complex media made of steel rods acting as scatterers. Using ultrasound multiple scattering has also proved advantageous in providing a quantitative spatial estimate for complex bone architectures, porosities and spatial densities. Aubry et al. used multiple scattering successfully to give local measurements in the human trabecular bone which is highly complex and diffusive in nature. The approach developed by Aubry et al. was successfully tested on a phantom consisting of steel rods distributed in water. The diffusion constant, as demonstrated in this paper is relevant to the assessment of lung edema, and air volume fraction in the lung. We demonstrate, for the first time, that ultrasound multiple scattering, usually considered an obstacle to imaging highly scattering media, can be taken advantage of in identifying and quantifying pulmonary edema in the lungs.
Due to the very strong impedance difference between the lung tissue and the alveoli, it is assumed that the tissue acts as the propagating medium whereas the alveoli play the role of scatterers. In the present chapter, we propose to take advantage of the complexity of the ultrasonic signals and of the high diffusivity of the lung parenchyma to characterize edema using wave transport parameters, namely the diffusion constant $D$ and the transport mean free path $L^*$. The diffusivity effectively allows us to predict presence or absence of a pathology giving an indication of the air fraction in the lung parenchyma. The proposed approach of quantitatively detecting and staging edema is based on the hypothesis that pulmonary edema leads to structural changes in the micro-architecture of the lung parenchyma. In edema, the alveolar sacs are filled with water, which reduces the effective volume of the lung and its elasticity. Due to these structural changes, we hypothesize that in case of edema, the effective amount of multiple scattering is much lower which allows the wave to diffuse more freely compared to a healthy lung. The scattering mean free path ($L^*$) is an indicator of the diffusivity of the medium which is derived from the diffusion constant $D$. By using $L^*$, we can quantify the extent of edema. In a healthy, normal lung, the millions of air-filled alveoli are responsible for frequent scattering events, leading to short SMFPs. In contrast, in pulmonary edema, due to reduced volume of air, and increased volume of tissue, less scattering should be observed. Therefore, the $L^*$ is expected to be significantly larger in fibrotic and edematous lungs than in normal lungs. We also showcase the applicability of another method developed by Zenteno et al. which is a spectral based pneumonia detection algorithm and follows the principle of extracting bandwidth frequency shift of a plane wave over the depth of the ultrasound signals. They calculate the frequency downshift, which acts as a quantitative parameter to distinguish control from pneumonia. However, we apply this technique to differentiate pulmonary edema from fibrosis since $L^*$ as a single parameter is not enough to completely
characterize the lung parenchyma. Once the presence of a pathology has been identified, a spectral breakdown of plane waves allows us to predict whether the identified disease is pulmonary edema or fibrosis (previous chapter).

6.3 Materials and Methods

6.3.1 Ischemia Reperfusion Injury (IRI) and Animal Preparation for Ultrasound

Pulmonary edema was created in the left hilum of six Sprague-Dawley rats (3 male, 3 female, 349±91.76 gms) using ischemia-reperfusion injury (IRI). After sedation, a tracheotomy was performed, rats were ventilated with a Harvard rodent ventilator and anesthesia was maintained with titrated isoflurane. A left thoracotomy was performed in the 4th interspace. The hilum of the left lung was encircled and clamped with a clip, and was removed after one hour of ischemia to produce left lung edema due to IRI. Ultrasound coupling gel was then applied directly onto the right lung and the IRM was acquired. Following the IRM acquisition, plan waves were emitted to calculate the backscatter frequency shift. Heparin was administered intrahepatically to prevent clotting after the lungs were removed. After the ultrasound data was collected, the rats were euthanized by cardiectomy under anesthesia. The trachea was clamped at end-inspiration. The heart-lung block was excised. The heart-lung block was immersed in cold phosphate buffered saline (PBS) for an ex-vivo CT scan. Following the CT scan, the heart-lung block was subjected to inflation fixation for histologic interpretation.

6.3.2 Wet to Dry Ratio

In order to corroborate the L* values, the wet to dry ratio (W/D) for the edematous and control lungs were calculated. W/D ratio is the gold standard for measuring lung edema in animal experiments. It is almost never used in humans except in destructive testing using human lungs. W/D ratio was calculated by taking a piece of the lung or a lobe, weighing it when its fresh (wet),
then drying it in an oven for 24-28 hours and then again weighing the lobe. The ratio is a measure of how wet the lung is. In a small lung (rat), it is critically important to take the same area of the lung. In large animals, the non-dependent portions (anterior) are much less edematous leading to a lower W/D ratio compared to the dependent portions (posterior) which exhibits higher W/D ratios. For the experiments conducted in-vivo for edema detection, after ultrasound evaluation, W/D ratios were evaluated for both edema and control lung lobes.

6.3.3 Data Acquisition methodology - IRM

All the in-vivo experiments were conducted with an L11-4v (128 element Linear Array Transducer) connected to a Verasonics Vantage ultrasound scanner. The transducer was placed on the exposed lung using a 1-2mm layer of coupling gel. All the elements of the array were fired one by one transmitting a 2-cycle pulse with a central frequency of 7.8 MHz (300kPa) into the medium. The backscattered signals were collected on all 128 elements of the array and this gave us access to the spatial spread of the transient pressure field. The sampling frequency of the data acquired was 62.5 MHz and the total data acquisition time was 40 μs. This enabled the acquisition of a 128 by 128 by 2500 impulse response matrix (IRM) $H(t)$ whose individual elements $h_{ij}(t)$ are the $N^2$ impulse responses of the medium. $H(t)$ was then processed using the methodology earlier described by Tourin et al and Aubry et al.\textsuperscript{13,16}. For simplicity purpose, the origin time ($t=0$) was set to the arrival of the first backscattered wave for every single receiving transducer. The IRM’s reciprocity feature was exploited to generate the anti IRM represented by $H^A(t)$. This was done using a simple matrix manipulation as shown.

- for $i>j$, $h_{ij}^A=-h_{ij}$;
- for $i=j$, $h_{ii}=0$;
- for $i<j$, $h_{ij}^A=h_{ij}$;
**H** (t) and **H**<sub>A</sub> (t) were then processed to obtain D from the incoherent intensity. Analytically, the incoherent intensity can be represented as

\[ I_{inc}(X, T) = I(T)\exp\left(-\frac{X^2}{4DT}\right), \]

Where X represents the distance between emitter and receiver. Equation (1) clearly establishes that when the incoherent intensity is plotted as a function of X and T, D would be an indicator of how diffusive the multiple scattering media is. The measurement of the diffusion constant from experimentation can be done by plotting \( I_{inc} \) as a function of T and X. The intensity was averaged over all emitter receiver couples separated by the same distance. At each time window, the backscattered incoherent intensity \( I_{inc} \) was fitted with a Gaussian curve and the variance of the Gaussian fit represented the dynamic growth of the diffusive halo given by \( (W^2(T) = 2DT) \). Once D was extracted, the transport mean free path \( L^* \) was evaluated based on equation 2. The details of this methodology can be found in Chapter 2 as well.

\[ D = \frac{V_e \times L^*}{3} \]

**6.3.4 Data Acquisition methodology – Backscattererrd Frequency Shift (BFS)**

In order to evaluate the BFS, RF samples were acquired using L11-4v (128 Linear Array Transducer) operating at central frequency of 6.25MHz and a sampling frequency of 25 MHz. Plane waves were emitted using all 128 elements simultaneously and the RF data was recorded for all transducers. The depth of the data acquisition was set to 4cm. The post processing of the acquired RF data is based on the work previously done by Zenteno et al. \(^{59}\) Spectral information was estimated from the RF data. Each RF line was considered to be a block. Hence, the entire RF data can be considered to contain 128 blocks. Once the RF data was obtained, the region of interest
(ROI) was demarcated to ensure that the wave is backscattered only from the lungs and not the neighboring region as shown in Figure 6.3.

Figure 6.3: Schematic for calculating backscattered frequency shift

Each block, which has a width of 0.29mm was split into 50% overlapping time windows (Figure 6.3) and the power spectra of the block was estimated using a Hanning Window as a gating function. The length of each time window was set to 0.5μs. Once the power spectral data of each block was obtained, the spectral information was averaged over all blocks. In addition, a function measuring the rate of decay over depth of the higher frequency within the -16dB bandwidth from the ultrasound signals was estimated. These averaged functions were then plotted against depth of wave propagation and the slope value was used to describe the spectral variation along the depth as shown in Figure 6.3. The slope of this linear fit gives us the BFS. This algorithm developed in 2016 was used as a pneumonia detection algorithm and was based on the measurement of the fundamental bandwidth shift of the central frequency of the signal due to absorption or scattering.
6.3.5 Data Analysis and statistical methods

The diffusion constant was calculated from IRM acquisitions. Differences between L* values obtained from control and fibrotic lungs were tested using the Kruskal–Wallis test with Dunn’s post-test (data was not normally distributed, so non-parametric analysis was chosen). All data are presented as mean ± standard deviation. Statistical significance was set a priori at p<0.05 and is graphically depicted on all figures as (*) for p<0.05, (**) for p<0.01 and (***) for p<0.001 as well as NS to denote non-statistically – significant comparisons. Statistics were performed in MatLab 2018a.

Due to the very low penetration in control rat lungs, only a certain number of points (range of points depicting start time and end time) were selected to calculate the variance or the growth of the diffusive halo. This was automated using the findchangepoints command in MatLab. For a trend to qualify, The R² of the trend should have been greater than 0.4 and a negative variance trend for the time being is rejected because it corresponds to noise. Data sets were also rejected for post processing if no multiple scattering was seen. This can be attributed to the lung operating at full volume capacity. Due to this very reason, it is advisable to acquire data at the exhaling cycle to ensure penetration and eventually witness multiple scattering.

Edema is generally characterized by larger penetrations of the ultrasonic waves and higher R² values. The edema data was processed a little differently than it was done for control. A B-Mode image from the Rf image was generated and only these regions where B-Lines (Figure 6.2) weren’t witnessed were considered in calculating the L* as shown in Figure 6.4. In Figure 6.4, for calculating the SMFP, only the region in green was considered for evaluating the L* (Region in Red boxes are the b-lines), reasons for which will be discussed later on in this chapter. Although since a minimum of 64 elements were used to calculate the L*, we were not able to completely
remove the effects of B-Lines from calculating the L* since finding a continuous region of no B-Lines for a span of 64 elements (2cm) was not possible.

Figure 6.4; ROI definition for evaluating edema

6.4 Results and Discussion

Shown in Figure 6.5 are examples of the variance plots obtained for a control lung and an edematous lung after 60 minutes of reperfusion.

Figure 6.5: Variance growth for in-vivo rodent data Control and Edema
We see that for edema, the variance increases more rapidly than the case for control. This rapid increase can be attributed to the fact that with edema, fluid gets accumulated in the interlobular septa as well as the alveolar sacs. Non cardiogenic pulmonary edema is due to injury to the alveolar septa. Primary injury to the vascular endothelium or damage to the alveolar epithelial cells produces an inflammatory exudate that leaks into the interstitial spaces and in more severe cases, the alveoli. This effectively changes the hydrostatic pressure and the effective size of the alveolar sacs during breathing is reduced. This allows the wave to diffuse freely in the lung parenchyma leading to larger diffusivity and larger L*. Shown in Figure 6.5 is the comparison of L* values between control, fibrosis (previous chapter) and edema. The L* for control, 2Wk fibrosis, 3 Wk fibrosis, 4 Wk fibrosis and edema was found to be 466±109 μm, 773±204 μm, 690±191 μm, 729±245 μm and 722±354 μm respectively.

Figure 6.6: L* values for in-vivo rodent data (Control, Fibrosis and Edema)
From Figure 6.6, we can see that the diffusion methodology is able to differentiate between control and edema with high statistical significance (p<0.001). This was also corroborated by the wet to dry ratio as shown in Figure 6.7. L* as a parameter was also able to stage edema as shown in figure 6.7. It can be seen from Figure 6.7 that the transport mean free path L* has a positive statistically significant trend with the wet to dry ratio.

![Figure 6.7: Wet to dry ratio for control and edematous lungs and comparison with L*](image)

The high error bars can be attributed to three reasons. First, these readings are in-vivo and our attempt to acquire at the exhaling stage based on visual hints could be a potential source of error. Secondly, the lungs (size=3cm) were much smaller than the transducer (3.8 cm). This could potentially allow signals from nearby regions to creep in and increase the L*. This could result in an over prediction of L* which can eventually be fixed in larger animal models. Lastly, and the
most important reason for why we see extremely large error bars are the b-lines. It has been witnessed that a b-line generates a purely coherent backscattererd intensity profile even after separating the coherent and incoherent contribution. It is still unclear why these b-lines are witnessed and why we see these b-lines in our IRM in only some of the cases. However, the purpose of this chapter is to try understanding these unique b-lines and build a hypothesis.

This methodology predicts very similar values between control and fibrosis. L* as an individual parameter is not sufficient to identify whether the pathology in the lungs is edema or fibrosis. This differentiation is tackled by doing a spectral decomposition of a plane wave emission and calculating the backscattered frequency shift (BFS) as explained in the methodology section. Shown in Figure 6.8 are the functions measuring the rate of decay over depth of the higher frequency within the -16dB bandwidth from the ultrasound signals for edema and fibrosis.

![Figure 6.8: Spectral functions measuring the frequency decay over depth for edema and fibrosis](image)

We see here that for fibrosis, the decay in the frequency is steeper than in the case for edema. We hypothesize that BFS (MHz/cm) is a parameter, indirectly describing frequency dependent attenuation, which shows a negative slope along the depth of the propagating medium.
The negative slope indicates that as the wave propagates and gets backscattered, there is a downward shift in the spectral frequency domain which is a function of the depth. This shift occurs due to the following reason. As the wave propagates deeper, the ultrasonic wave attenuates due to multiple scattering and due to absorption. Given that both exhibit similar L* values, scattering takes a back seat and absorption plays a major role here. In edema, the alveolar spaces are now occupied by water, which is a less absorbing media. However, in fibrosis, due to the scarring and thickening of tissue, the effects of absorption is more profound leading to steeper drops in the spectral frequency decay. Shown in Figure 6.9 are the values of BFS obtained for control, fibrosis and edema.

![Figure 6.9: BFS value comparison for control, edema and fibrosis](image)

In Figure 6.9, the difference in the values obtained for BFS for edema and fibrosis (2Wk) are statistical significant with values of 0.39±0.15 MHz/cm and 0.67±0.12 MHz/cm respectively. The high values in BFS for fibrosis is attributed to thickening of the interlobular septa, which attenuates higher frequencies at faster rates thereby showing larger values.
6.5 Observation of Super Coherence in Incoherence (SCIC)

The purpose of this particular section is to understand why in Edema we see such large error bars in the L* and what is its relationship to observing b-lines. To understand this better let us consider Figure 6.10 and understand what b-lines are. B-Lines are laser like super hyper-echoic artefacts that continue to the end of the red ROI in the figure 6.10. We see two distinct b-lines generating from the top of the lung pleural line^{69,149,150}

![Figure 6.10: B-Mode image of edematous rat lung obtained from IRM acquisition](image)

Rather than considering all 128 elements to calculate the L*, the IRM is split into two matrices of 64 x 64 x Time. Therefore, from one IRM, we obtain two IRMS where the first IRM represents only the red ROI and the second IRM represents the green ROI.
Figure 6.11: Incoherent intensity and variance growth in regions with and without B-Lines. A: With B-Lines, B: Without B-Lines

We see that in Figure 6.11 B2, the width of the backscattered incoherent intensity grows gradually which leads to a freely growing diffusive halo shown in Figure 6.11 B1. This growing incoherent cone is an indication of multiple scattering and the obtained $L^*$ value is $729\mu m$. However, for the region demarcated in the red ROI in Figure 6.10, the growing backscattered incoherent cone is not witnessed. Rather a coherence is witnessed for the region with B-Lines, which is counter intuitive given that we have accounted for separation of coherent and incoherent intensity. In Figure 6.11 A2, the incoherent intensity does not spread and neither does the width of the diffusive halo, generating an $L^*$ of $8\mu m$ which is even less that what is witnessed for control. This in a way can be considered to be supercoherence\textsuperscript{12,148,151} which we are not able to remove.
even after separating the coherent and incoherent backscattererd intensities. We hypothesize that for the green ROI, the wave goes through traditional multiple scattering generating a growing diffusive halo which is characterized by an increasing variance. However, in the red ROI, the ultrasonic wave is probably bouncing off the same region in a continuous loop or is being guided through the interlobular septa with occasional reverberations, which leads to a super coherent wave that does not separate from its incoherent counterpart.

6.6 Edema in Pig Lungs and observation of Super Coherence

Edema was observed in porcine lungs during ex-vivo lung perfusion (EVLP) for lung transplantation from non-heart beating donors. These pig lungs were used to characterize edema. Due to the heterogeneity of edematous lungs, specific regions were identified in pig lungs which were relatively less wet and could be potentially treated as control. These pig lungs were connected to either a hand-held resuscitator or a ventilator (7ml/kg tidal volume). Pulsed waves with a central frequency of 5.1MHz were transmitted using the L7-4v probed connected to a 128 channel vantage Verasonics and the IRM $H(t)$ was acquired. The coherent and incoherent intensities were separated and based on the method described in the previous chapters, the transport mean free path ($L*$) was calculated. The data was acquired during the exhaling cycle to ensure maximum penetration of the ultrasonic waves into the lung parenchyma for optimum characterization. Shown in Figure 6.12 are examples of the variance plots obtained for a control lung and an edematous lung after 60 minutes of reperfusion.
As shown in previous sections and chapters, we see that the diffusive halo represented by the variance grows more rapidly in the edema lung compared to the control lung, which is attributed to the presence of extra vascular lung water, which effectively reduces the size of the alveolar sacs and allows the ultrasonic wave to diffuse freely. Shown in Figure 6.13 are the $L^*$ values obtained for 3 control and 5 edematous regions in the pig lungs. For each region, three sets of data were acquired.
The $L^*$ values obtained for control and edema had high contrast and the difference between them was statistically very significant ($p<0.001$). The $L^*$ values for control and edema were found to be $231\pm157\ \mu m$ and $852\pm561\ \mu m$ respectively. We see that here also, edema exhibits very large error bars which can again be attributed to the observation of SCIC. To understand this phenomena better, we look at a 1-D map (Chapter 3) of the $L^*$ values for an edematous pig lung. The sub-aperture for the 1-D mapping was chosen to be 48 elements. Shown in Figure 6.14 A is the b-mode (obtained from IRM) of an edematous porcine lung where clear b-lines were not observed. Shown in Figure 6.14B is the 1-D map of the $L^*$.

Figure 6.14: 1-D map of $L^*$ in edematous porcine lung without B-Lines
We see that without the presence of B-Lines, the 1-D map of the L* values obtained for porcine lungs all show that the lung is highly edematous with no observation of SCIC. Shown in Figure 6.15 A is the b-mode (obtained from IRM) of an edematous porcine lung where clear b-lines were observed and are demarcated by the red boxes. Shown in Figure 6.15 B is the 1-D map of the L* for the same IRM.

![Image](image_url)

Figure 6.15: 1-D map of L* in edematous porcine lung with B-Lines

We see that in regions (green Box) without the presence of B-Lines, the 1-D map indicates high L* values in the range of 600 – 800 μm. However as the 1-D map approaches the B-Lines, due to the presence of SCIC we observe almost negative L* values.
This raises questions regarding why very low $L^*$ values are observed at transducer position of -5mm even though no B-Lines are observed. As mentioned in chapter 3, diffusion constant and $L^*$ is an averaged parameter over space and time and is not completely localized. Hence when the $L^*$ for -5mm was calculated, the sub-IRM considered for the calculation ranged from -12.5 mm to 2.5 mm. This in addition to the diffusive nature of multiple scattering are the reasons for observing low $L^*$ even when discrete b-lines are not seen. This goes out to say that using 1-D mapping we indeed capture the essence of the B-Lines and the effect they have on diffusivity. Shown in Figure 6.16 are the backscattered incoherent intensity for 2 discrete regions of transducer position at -15mm (no b-lines) and transducer position 8mm (b-lines).

Figure 6.16: Incoherent intensity and variance growth in regions with and without B-Lines. A1: Without B-Lines, A2: With B-Lines, B-Diffusive halo growth
In Figure 6.16 A1, we see that the diffusive halo grows linearly and freely indicating edema. However in Figure 6.16 A2 we see a coherent like growth of the diffusive halo which is impervious to the separation of the coherent intensity from the incoherent intensity thereby displaying super coherence in incoherence (SCIC). The $L^*$ value for the transducer locations of -15mm was found to be 833 μm whereas in the transducer location of 8mm the $L^*$ was found to be -43μm. A very low $L^*$ does indeed indicate the existence of SCIC which needs to be studied further.

From a pure diagnosis perspective, it is indeed exciting to see that $L^*$ can differentiate between control and edema. Also it can detect regions with B-Lines and regions without B-Lines. We hypothesized that edema would lead to an increase in the $L^*$ due to rapidly growing diffusive halo. This was because of the reduced air fraction. However due to the presence of B-Lines, the $L^*$ values are suppressed. In edema, the $L^*$ values are affected by two unique physics which have opposite effects on the diffusive halo growth. The increased fluid build-up increases $L^*$ whereas the B-Lines suppress the $L^*$.

6.7 Compliance Measurements in Porcine Lungs

Lung compliance is a measure of the lungs ability to stretch and expand. Ex-vivo compliance measurement experiments were conducted with edematous pig lungs. Control (N=1) and edematous (N=2) pig lungs were connected to a ventilator with low breathing rates (5 breaths/minute) and the tidal volume (7ml/kg, weight of pig~30kg) was varied between 100ml to 700 ml (with realistic air intake values at 210 ml). At discrete tidal volume steps, Pulsed waves with a central frequency of 5.1MHz were transmitted using the L7-4v probed connected to a 128 channel vantage Verasonics and the $IRM_H(t)$ was acquired. The coherent and the incoherent intensities were separated and based on the method described in the previous chapters, the transport
mean free path (L*) was calculated. Shown in Figure 6.17 are preliminary data obtained from the compliance study.

From Figure 6.17 we see that with change in tidal volumes, L* for edema changes more rapidly than for control. Hence, the slope of the L* vs tidal volume can be used as a potential parameter to characterize the compliance of lungs.

6.8 Conclusion and Future Work

We successfully demonstrated that for a rodent model of edema in Sprague-Dawley rat lung, we were able to differentiate control from edema. L* was obtained by separating the coherent and the incoherent backscattered intensities and calculating D which represented the dynamic growth of the diffusive halo. L* is a representation of how the wave diffuses in the lung parenchyma. In control lungs, due to the large amounts of air scatterers and large air volume, the growth of the diffusive halo is restricted leading to low L* values. However in edematous rat lungs, the extravascular lung water allowed for the wave to diffuse freely in the lung parenchyma thereby accentuating the value of the diffusion constant D and L*. Howbeit, L* individually was not enough to quantify or characterize the lung parenchyma tissue structure. Due to this limitation, we
introduce a co-dependent parameter, the BFS (MHz/cm) which is calculated using a frequency spectral downward shift algorithm. The negative slope of the spectral downshift in the frequency was hypothesized to be dependent on two phenomenon, i.e. multiple scattering and absorption (more dominant). On the other hand, edema exhibited the lowest BFS values due to low absorption. High values in fibrotic lung tissue were attributed to an enhanced absorption, which could have occurred due to the thickening and scarring of the tissue. It was observed that the BFS values obtained for control and fibrosis were of similar magnitude. However using BFS as an independent parameter to differentiate between fibrosis and edema proved to be relevant. A major hindrance in our experiments is the inability to map the extent of edema in the lungs. Since edema is gravity dependent, we would want to map and quantify the extent of edema in different regions of the lung. However, since the size of the rat lung is smaller/comparable to the size of the transducer, the spatial resolution becomes a challenge. After the ultrasound acquisition, the rats were euthanized and underwent high resolution CT followed by H&E stained histology analysis (data not available). The wet to dry ratio of the edematous lungs were also evaluated and they corroborated the presence of edema in the rat lungs.

From the porcine lung edema model (Figure 6.14), we see that we can map the L* in 1-D and analyze the extent of edema in different regions of the pig lung and understand its heterogeneity and localization. The ability of the 1-D map to quantify the extent of edema in different regions is promising. This proves the efficacy of the diffusivity method to detect lung pathologies like edema, quantify it and eventually use it for staging of edema. This staging capability could allow for monitoring response to treatment. The hypothesis of observing SCIC is very intriguing and needs to be studied further. It was noticed that in regions of b-lines strong coherent backscattererd intensities were observed in the incoherent backscattered cone. This
resulted in very low L* or negative L*. It would be of high interest to characterize negative L* and correlate them to SCIC. Lastly, no b-lines were observed in IRM acquisition of fibrosis data. We hypothesize here that since the b-lines in fibrosis are diffused, we were unable to see them due to multiple scattering.

Currently the in-vivo data acquisition is associated with a few critical issues. Firstly the rat lungs are smaller than the probe, allowing information from the periphery of the lungs to creep in and over-predicting L* values. This has resulted in the high standard deviations for L*. Secondly, it would be better to trigger the Verasonics along with the rodent respirator to ensure that data is acquired perfectly at the end of the exhaling cycle ensuring maximum ultrasound penetration into the lung parenchyma. We believe that in larger animal models, combined with a more controlled experimental environment (acquisition at exhaling), these error bars can be reduced. One major limitation of data acquisition and observing of growing diffusive halo trends is the high attenuation and lack of penetration of the ultrasonic waves into the lungs. If the data was acquired at the peak inhaling stage, the reflection would be too strong and hence penetration would be weaker than what is required for an incoherent trend to be visible.
CHAPTER 7 - Artificial neural network to extract micro-architectural properties of cortical bone using ultrasonic attenuation: a 2-D numerical study

7.1 Abstract

The goal of this study is to estimate micro-architectural parameters of cortical porosity such as pore diameter ($\phi$), pore density ($\rho$) and porosity ($\nu$) of cortical bone from ultrasound frequency dependent attenuation using an artificial neural network (ANN). First, heterogeneous structures with controlled pore diameters and pore densities (mono-disperse) were generated, to mimic simplified structure of cortical bone. Then, more realistic structures are obtained from high resolution CT scans of human cortical bone. 2-D dimensional finite-difference time-domain simulations are conducted to calculate the frequency-dependent attenuation in the 1-8MHz range. An ANN is then trained with the ultrasonic attenuation at different frequencies as the input feature vectors while the output is set as the micro-architectural parameters. The ANN is composed of three fully connected dense layers with 24, 12 and 6 neurons, connected to the output layer. Our dataset is trained over 6000 epochs with a batch size of 16. The trained ANN exhibits the ability to predict the micro-architectural parameters with high accuracy and low losses. ANN approaches could potentially be used as a tool to help inform physics-based modelling of ultrasound propagation in complex media such as cortical bone. This will lead to the solution of inverse-problems to retrieve bone micro-architectural parameters from ultrasound measurements for the non-invasive diagnosis and monitoring osteoporosis.

Key Words: Quantitative Ultrasound, Multiple Scattering, Cortical Bone, Osteoporosis, Neural Networks
7.2 Introduction

Osteoporosis is the most common metabolic bone disorders. It affects cortical as well as trabecular bone. It is characterized by low bone mass, tissue degradation, deteriorated macroscopic mechanical properties and altered micro-architecture. Loss in bone mass leads to frequent fracturing, higher mortality and reduction in life expectancy by 1.8 years. For these reasons, early diagnosis and monitoring of osteoporosis is crucial. Several imaging modalities provide semi-quantitative to qualitative analysis of the bone. DXA (Dual X-Ray Absorption) correlates well bone mineral density (BMD). High resolution peripheral quantitative computed tomography (HR-pQCT) and magnetic resonance imaging (MRI) based techniques are also used for the characterization of bone. However all these methods have shortcomings. MRI lacks resolution, and CT based methods are associated with radiation doses making it impossible for frequent use and monitoring. DXA is less ionizing but BMD measured with DXA insufficiently correlated with fracture risk. However, the assessment is qualitative with high variability from person to person and suffers from radiation issues. MRI and CT are also associated with high costs and low portability.

Traditional ultrasound imaging is based on the concept of echolocation. However, in highly heterogeneous media such as bone or the lungs, traditional imaging methods fail. This is due to the high heterogeneity and large impedance changes within the propagating media. The presence of fluid-filled pores introduces multiple scattering and aberrations, making bone elusive to ultrasound imaging and pathology detection. Quantitative ultrasound (QUS) techniques are better suited for such applications. In highly scattering media such as bone or the lungs, the wave enters diffusion regime which need to be characterized using quantitative methods. As the ultrasonic wave travels through a complex heterogeneous medium, its propagation is affected
Correlating changes in the cortical micro-architecture to ultrasonic parameters such as frequency dependent attenuation, phase velocity or backscattering coefficient could provide insight into the early onset of osteoporosis and help quantify the cortical bone structural parameters. QUS can also be used to extract macroscopic changes such as elasticity by measuring the axial speed of sound, which is recognized to be a significant parameter in overall bone quality. The fact that ultrasound is a mechanical wave makes them sensitive to macro-mechanical changes of the cortical as well as trabecular bone. A multitude of research studies have been performed to investigate the dependence of ultrasonic parameters on the micro-architectural properties of the trabecular. Analysis of parameters such as frequency dependent attenuation, wave velocity (fast and slow), backscatter coefficient and diffusion constant has shown promise. Various QUS methods have been developed to study cortical bone thickness and the wave speed, which can then be associated to its macro-mechanical properties. Zheng et al. applied a spectral ratio method to estimate the broadband ultrasound attenuation (BUA) in cortical bone. Similar work was done by Xia et al., wherein they calculated the normalized BUA (nBUA) to characterize cortical bone. Recent work by Yousefian et al. showed that a phenomenological power law model of the frequency dependent attenuation could potentially be used to characterize the micro-architectural properties of complex porous structures, giving access to pore diameter, pore density and porosity. Data-driven predictions combined with machine learning enables us to generate prediction outcomes and is currently being extensively used for clinical applications. Clinical level classification accuracies of skin cancer and breast cancer are now achievable using Convolutional Neural Networks (CNN). Neural networks have the ability to identify patterns and relationships from complex data sets and predict outcomes. In supervised learning, a labelled training data
set is used to map the input to the output. Here, we propose to use neural networks to map ultrasonic data (the frequency-dependent attenuation) to microstructural features of cortical bone (pore diameter, pore density and porosity).

However the purpose of this paper is not only to attempt to develop a potential inverse problem solving method using machine learning, but also to use machine learning to explore the dependence of ultrasonic attenuation to these micro-architectural properties. We envision that neural networks will inform physics-based modelling efforts by putting the spotlight on the specific features that are the most relevant to the relationship between ultrasound parameters and microstructural parameters. We could then potentially draw on information obtained from machine learning to refine models of ultrasound propagation in cortical bone.

In this study, finite difference time domain (FDTD) simulations are conducted to calculate the frequency dependent attenuation (1MHz-8MHz) in 2-D geometries resembling simplified cortical bone structures (mono-disperse). Pore diameter and pore densities are modified to obtain attenuation data for a wide range. In addition, realistic cortical bone structures (poly-disperse) are obtained from high resolution CT scans of human cortical bone and using image processing techniques, micro-architectural parameters are extracted. The attenuation data over the range of frequencies are mapped to the corresponding micro-architectural parameters and used as the data set. An artificial neural network (ANN) is then trained over the acquired data set to predict micro-architectural parameters. In section II, we will discuss the data collection methodology for both mono and poly-disperse cases. Section III will present how the ANN model was formulated followed by the results and conclusion in sections IV and V.
7.3 Data Collection and Methodology

7.3.1 FDTD Simulation

All simulations of ultrasound propagation through structures mimicking cortical bone were carried out using SimSonic, an open source simulation software based on FDTD numerical methods\cite{102,110}. The simulated media were solid slabs with fluid filled pores resembling bone tissue (marrow inside cortical bone). The solid phase was given the properties of pure bone whereas the fluid filled pores were given properties of water. Absorption coefficients were attributed to both solid and fluid phases. Both scattering and absorption (visco-elasticity) were responsible for the attenuation of the ultrasonic wave \cite{242}. The material properties can be seen in Table 7.1. Simulations were carried out in the structures in the 1–8MHz range with 1MHz frequency intervals. The transmitted wave was a Gaussian ultrasonic pulse with a -6 dB, 20% bandwidth. Shown in Figure 7.1 is an example of the input signal in the time and frequency domains for a 3MHz pulse.

![Figure 7.1: 3MHz Signal in time and frequency domain](image-url)
Table 7.8: Material properties for FDTD simulations

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</table>

With a highest simulated frequency of 8MHz, the smallest wavelength is equal to 500 μm. All simulations were carried out in 2-D with a grid step size of $Δx = Δy = 10μm$ (50 points per wavelength). Perfectly Matching Layer (PML) boundary conditions were applied at both ends of the simulation domain in the direction of wave propagation to minimize the effect of boundary reflections. Symmetry boundary conditions were applied in the direction perpendicular to wave propagation to simulate a plane wave and to eliminate effects of diffraction. In order to satisfy the CFL (Courant-Friedrich Levy) condition, the CFL number was set to $0.99^{243}$ and the sampling time step $Δt$ was chosen as

$$Δt = 0.99 \frac{Δx}{\sqrt{d}c_{max}} \quad (1)$$

Where $c_{max}$ is the maximum speed of sound in the simulation domain and $d$ is the dimension of the space ($d=2$ for 2D).

7.3.2 Mono-Disperse Geometry

Simple mono-disperse structures resembling cortical bone were generated using a Monte Carlo method for each combination of pore diameter ($ϕ$) and pore density ($ρ$). Fluid filled pores were randomly distributed in the solid bone slab while ensuring that there are no interconnected or
overlapping pores. The pore diameter \((\phi)\) and pore density \((\rho)\) ranges from 20-120 \(\mu m\) and 3-16 pores/mm\(^2\) respectively. The porosity \((\nu)\) was derived from pore diameter \((\phi)\) and pore density \((\rho)\) based on Equation 2.

\[
\nu = \frac{\pi \phi^2}{4} \rho
\]

Using Equation 2, the porosity ranges from 0.001 to 0.18 (zero being no fluid and one fluid only). The generated structures have a dimension of 10mm by 10mm (1000 pixels by 1000 pixels). Shown in Figure 7.2 are examples of two mono-disperse structures generated for FDTD simulations. 110 unique combinations of pore diameter \((\phi)\) and pore density \((\rho)\) were generated and FDTD simulations were carried out. For each combination, three different realisations were generated to form 330 simulations in total (3 (realisations) x 110 (cases)).

Figure 7.2: Mono-disperse bone schematic geometry, A: \(\phi = 50 \mu m, \rho = 3 \text{ pores/mm}^2\), B: \(\phi = 120 \mu m, \rho = 16 \text{ pores/mm}^2\)

### 7.3.3 Poly-Disperse Geometry

The structure presented in the previous section is a simplified representation of bone micro-architecture. In order to obtain more realistic cortical bone simulation domains, 2-D images were obtained from high resolution CT scans of a human cortical bone. 3-D CT scans of resolution 6.5 \(\mu m\) were obtained and 2-D planar cross sections were randomly taken for FDTD simulations. The
CT scans were normalized and binarized based on a threshold which demarcated the bone matrix and the fluid filled pores. The porosity (ν) of the images was obtained by dividing the number of pixels associated with the liquid phase to the total number of pixels in the cross-section. To measure the average pore diameter and pore density, every single closed surface within the segmented bone cross-section was labelled. The number of labels indicated the total number of pores, and the ratio between pore number and the entire bone area provided the pore density (ρ) in pores / mm². To estimate the mean pore diameter, pores were assumed to have circular shape. The area of each pore was equated to the area of a circle and diameter of each pore was calculated. By measuring the area of each pore and averaging the calculated diameters, the mean pore diameter (ϕ) and its standard deviation was obtained. FDTD simulations were carried out on 964 cross sectional structures with the pore diameter (ϕ), pore density (ρ), porosity (ν) and standard deviation (SD) of the pore dimeter ranging from 27-115 μm, 9-22 pores/mm², 0.01 – 0.2 and 16-82 μm respectively. Shown in Figure 7.3 are examples of poly-disperse structures obtained from CT scans after image processing.

Figure 7.3: Poly-disperse bone schematic geometry, A: ϕ =50 μm, ρ = 16.85 pores/mm², ν=0.047, SD=33 μm, B: ϕ =71 μm, ρ =14.3 pores/mm², ν=0.081, SD = 47 μm
7.3.4 Attenuation Measurement: Time-distance matrix approach (TDMA)

Plane waves were transmitted through structures (mono and poly-disperse) using FDTD simulations. Receivers were placed at 30 consecutive longitudinal positions along the structure in the direction of wave propagation. The receiver size was the same as the simulation domain size (10 mm for mono-disperse and 3.7 mm for poly-disperse) hence covering the height of the slab. Time domain signals were recorded by each receiver in a time-distance matrix \( s(\bar{t}, x) \), which was then converted into the frequency domain \( S(f, x) \). In the time domain, the attenuation is assumed to have an exponential decay over time and the attenuation-dependent frequency was calculated based on Equation 3:

\[
|S(f, x)| = e^{-\alpha(f)x}
\]  

(3)

Using a frequency sweep from 1 to 8 MHz, the attenuation coefficient \( \alpha(f) \) was calculated, details of which can be found in the work by Yousefian et al. Shown in Figures 7.4 and 7.5 are frequency dependent attenuation plots for the mono-disperse and poly-disperse cases.

![Figure 7.4: Frequency dependent attenuation for mono-disperse FDTD simulation](image-url)
It can be clearly seen that with an increase in pore diameter, the attenuation at a given frequency increases. This is attributed to additional losses in the ultrasonic wave due to stronger scattering. Similarly, with an increase in pore density, the attenuation increases, due to an increased number of scatterers, accentuating the attenuation.

### 7.4 Neural Network Model

The methodology adopted was acquiring of data from FDTD simulations and using it to train an ANN. The ANN model built for supervised training consisted of five fully connected layers, as shown in Figure 7.6. It should be noted that Figure 7.6 is not an accurate representation of the number of neurons in each layer, but rather is a pictorial representation of how the neurons in each layer are connected. In supervised learning, a labelled set of data is used to map the input data to the desired output. In a regression problem, the output is not a classification but discrete output values, which are mapped to the input feature vector. The ANN was trained based on back-propagation, with the input layer having 8 neurons and the output layer having 3 or 4 neurons depending on whether standard deviation of the pores has been considered as an output or not. The
number of neurons in the hidden layers have been set as 24, 12 and 6. We deliberately kept the number of neurons low in this problem statement to avoid over-fitting. The $\alpha(f)$ at frequencies 1-8MHz was used as the input feature vector $\vec{X}$, whereas the output layer $\vec{Y}$ gave the predicted pore diameter ($\phi$), pore density ($\rho$) and porosity ($\nu$). Hence, $\vec{X}$ is an 8-dimensional space whereas $\vec{Y}$ is a 3 or 4-dimensional space. All the ANN modelling was done in the Python API of TensorFlow.

Figure 7.6: Schematic of the ANN structure. The arrows depict connection between neurons of 2 layers.

Once all the data was acquired, the total data set was split into training and test data (test size =0.2). The training data (Both $\vec{X}$ and $\vec{Y}$) was scaled using the StandardScaler transformation in Python. While training and compiling the ANN model, we used the Adam optimizer. Furthermore, the training data was then split into training and validation sets (cross validation size=0.2) and the validation loss (mean squared error) was monitored for convergence or fine-tuning of the hyper-parameters. The batch size while training was set to 16 samples and the ANN was trained over 6000 epochs. The validation loss was calculated based on the equation shown below

$$Loss = \frac{1}{n} \sum_{i=1}^{n} (Y_i - \vec{Y}_i)^2$$

(4)
Where $Y_i$ is the actual output, $\hat{Y}_i$ is the prediction from the model and n is the total number of data sets over which the loss is being calculated. The performance of the ANN model was evaluated based on the validation loss.

For this study, we built three separate ANNs. The first ANN only took into account the mono-disperse geometries. The second ANN took only the poly-disperse geometries into account. An finally an unified ANN model was trained using all mono and poly-disperse cases. For the Mono-disperse case, the output parameters were set as pore diameter ($\phi$), pore density ($\rho$) and porosity ($\nu$). We acquired 330 (110 x 3 (realisations)) sets of data. For a given realization, 110 sets were acquired by varying the pore diameter ($\phi$) and pore density ($\rho$) in ranges of 20-120 $\mu$m (11 discrete steps) and 3-16 (10 discrete steps) pores/mm$^2$ respectively. Synthetic data was then generated from these 110 sets of data using spline interpolation. 110 sets of data were interpolated to 1130 sets of data with steps in pore diameter ($\phi$) and pore density ($\rho$) set to 2 $\mu$m and 0.23 pores/mm$^2$. This was considered a reasonable assumption since at a given frequency, attenuation increased monotonously with increase in pore size and pore density as shown in Figure 7.7.

![Figure 7.7: Attenuation trends at Frequency =8MHz. A: Fixed pore diameter, B: Fixed pore density](image)

Figure 7.7 depicts the trend in attenuation at a central frequency of 8 MHz. For a fixed pore size ($\phi$), the attenuation increases with increasing pore density ($\rho$). Similarly, for a fixed pore density ($\rho$), the attenuation increases with increasing pore size ($\phi$). The main reason for this interpolation to generate synthetic data was to have enough data sets for training. For the poly-disperse cases, enough data sets were readily available via the CT slices and no such interpolation was conducted. The output variables were set as pore diameter ($\phi$), pore density ($\rho$), porosity ($\nu$) and standard deviation of the diameter distribution (SD). For the third unified neural network, which combines both the data sets, the output vector had four variables with SD being set to zero for the mono-disperse cases. Shown in Table 7.2 are the details of all the neural networks. The difference in the number of trainable parameters arises from the difference in output variables.

<table>
<thead>
<tr>
<th>DNN Model</th>
<th>Dimension of Input Feature Vector</th>
<th>Output Variables</th>
<th>Trainable Parameters</th>
<th>Total Data Sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono-Disperse</td>
<td>8</td>
<td>3</td>
<td>615</td>
<td>3390</td>
</tr>
<tr>
<td>Poly-Disperse</td>
<td>8</td>
<td>4</td>
<td>622</td>
<td>964</td>
</tr>
<tr>
<td>Combined</td>
<td>8</td>
<td>4</td>
<td>622</td>
<td>1294</td>
</tr>
</tbody>
</table>

### 7.5 Results and Discussion

The convergence of the ANN for the mono-disperse model over 6000 epochs is shown in Figure 7.8. Even though the training losses are 0.07, the validation loss was determined to be 0.13. By increasing the number of neurons, the validation loss is bound to get lower; however, over-fitting might occur if too many neurons are used in the hidden layer. Shown in Figure 7.8 are the results obtained from the ANN.
Figure 7.8: ANN Results for Mono-Disperse data only

The x-axis depicts the true values whereas the y-axis depicts the values predicted by the ANN post training. The normalized root mean square deviation (NRMSD) for the test data was calculated based on equations (5) and (6).

\[
RMSD = \sqrt{\frac{\sum_{i=1}^{N} (y_{predicted} - y)^2}{N}} \quad (5)
\]

\[
NRMSD = \frac{RMSD}{\bar{y}} \quad (6)
\]

Where, \(y\) is the true value, \(N\) is the total number of readings and \(\bar{y}\) is the mean of the actual values. NRMSD was found to be 0.06, 0.18 and 0.065 and \(R^2\) were found to be 0.98, 0.99 and 0.8 for pore diameter (\(\phi\)), pore density (\(\rho\)) and porosity (\(\nu\)) respectively. It can clearly be seen that for prediction of pore density (\(\rho\)), the ANN does not perform as well as it does for the other two parameters. At low pore diameter (\(\phi\)), with change in pore density (\(\rho\)), the frequency dependent attenuation does not change much. It is hypothesized that at low pore diameter (\(\phi\)), scattering is
weak and attenuation is dominated by absorption. However, at high pore diameters ($\phi$), the attenuation changes more significantly with change in pore density ($\rho$). In this stronger scattering regime, the porosity affects attenuation more significantly. This is illustrated in Figure 7.9. It is interesting to note that pore density ($\rho$) is the controlled variable along with pore diameter ($\phi$). The porosity is obtained from equation 2 and is derived from pore diameter ($\phi$) and pore density ($\rho$). The ANN is capable of predicting porosity with high accuracies, however it fails to do so with pore density ($\rho$). This could also be attributed to the fact that porosity has a dependence of $\phi^2$ and hence the determining factor for porosity becomes the pore diameter ($\phi$) and the effect of pore density ($\rho$) is reduced.

![Attenuation plots for varying pore diameters and pore sizes.](image)

Figure 7.9: Attenuation plots for varying pore diameters and pore sizes.

Shown in Figure 7.10 are the results obtained using the ANN on the poly-disperse structures. Normalized root mean square deviation (NRMSD) for the test data was found to be 0.069, 0.071, 0.043 and 0.055 for porosity ($\nu$), pore diameter ($\phi$), pore density ($\rho$) and standard deviation (SD) respectively. The fact that the ANN predicts the SD (obtained from image processing of CT scans) shows its sensitivity to the kind of pore distribution that exist in real cortical bone. For the poly-disperse case, the porosity ($R^2=0.99$) and the pore density ($R^2=0.94$) are both predicted accurately and independent of each other. For the poly-disperse case, porosity
(\nu) is not derived from pore diameter (\phi) and pore density (\rho) and is obtained from image processing as detailed in section II.

Figure 7.10: ANN Results for Poly-Disperse data only

Figure 7.11 shows the results obtained from an ANN with input data as the data obtained from both mono-disperse and poly-disperse simulations. The loss and validation loss for the combined ANN was 0.05 and 0.15 respectively. The results showcased in Figure 7.11 exhibits high accuracy in predicting porosity (\nu), pore diameter (\phi) and standard deviation with R^2 values as 0.97, 0.94 and 0.98 respectively. NRMSD for the test data was found to be 0.09, 0.10, 0.21 and 0.11 for porosity (\nu), pore diameter (\phi), pore density (\rho) and standard deviation (SD) respectively. One can clearly see in Figure 7.11, the pore density predictions are less accurate in the low range of \rho values between 3 and 9 pores/mm^2; however, for mid-range and higher pore densities, the ANN is sensitive and can predict \rho more accurately. Note here that for true SD values of zero
(mono-disperse), the ANN predicts SD values between 0-3 μm. This demonstrates that the ANN has the potential to identify the type of distribution and even predict the standard deviation of the distribution of scatterers in the cortical bone with high fidelity.

Figure 7.11: ANN Results for Mono and Poly-disperse combined data (Unified Model)

7.6 Conclusion

An ANN was trained on FDTD simulated data of ultrasound propagation cortical bone mimicking structures with controlled porosity ($\nu$), pore diameter ($\phi$) and pore density ($\rho$). The ANN was trained for mono-disperse (created using a Monte Carlo approach) and poly-disperse (obtained from human femur CT scans) structures. The two structures were then combined and a unified ANN was established. The trained model, taking the ultrasonic attenuation data as the input feature vector, predicted the micro-architectural properties. Agreement between the predicted values and true values ranges from good to excellent. The ANN consistently predicted porosity ($\nu$) and pore diameter ($\phi$) with $R^2$ values greater than 0.9 and slopes of magnitude 1. A limitation of
the ANN model was its lower $R^2$ values and higher NRMSD for predicting pore density ($\rho$). From the unified ANN, it can be said that the ANN is sensitive to the range of pore density. Lower pore densities were more difficult to predict and the NRMSD for $\rho$ ranging in the 3-10 pores/mm$^2$ was found to be 0.48. On the other hand, the ANN showed potential in solving the inverse-problem by accurately predicting the porosity ($\nu$), pore diameter ($\phi$) and standard deviation (SD). The lack of sensitivity towards $\rho$ does indicate that, in the near future, one could shift towards only looking at porosity ($\nu$) and pore diameter ($\phi$) in the ANN model for predicting the onset of osteoporosis and characterizing the cortical bone. The unified ANN, was able to differentiate between the mono-disperse and poly-disperse cases, exhibiting its ability to predict the type of pore distribution in the micro-architecture. A large number of studies suggest significant correlations between ultrasound parameters and bone parameters. It is now necessary to develop inverse problems in order to retrieve these bone parameters from ultrasound measurements. However, phenomenological models and relationships based on observation are sub-optimal for the development of solving of inverse problems. It will therefore be necessary to develop physics-based models. We propose to use data driven predictions to inform the development of these physics-based models, due to their ability to point out the relevant parameters that need to be focused on.

In the near future, the ANN can be trained with the ultrasonic attenuation data to predict more traditional micro-structural parameters such as bone mineral density (BMD) and bone volume/Total Volume (BV/TV). This in combination with porosity ($\nu$), pore diameter ($\phi$) and pore density ($\rho$) could help us establish a holistic prediction model for the cortical bone.
CHAPTER 8 - Conclusion

8.1 Review of Thesis

Chapter 1:

- Challenges in ultrasonic imaging of heterogeneous biological tissues and porous media
- The current state of the art in imaging of the lungs
- Ultrasound as a potential tool to characterize the lung parenchyma
- Target pathologies

Chapter 2:

- Introduction to diffusion theory and multiple scattering
- Methodology development and its validation
- Phantom validation and ex-vivo measurements

Chapter 3:

- Extending diffusion theory to generate 1-D maps of diffusivity
- Computational and experimental validation of the 1-D mapping

Chapter 4:

- Extending diffusion theory to generate 2-D images of diffusivity
- Using 2-D diffusion imaging for lesion and target detection
- Computational and experimental validation
- Introduction to effective speed of sound concept

Chapter 5:

- Introduction to fibrosis and micro-architectural changes due to fibrosis
- Hypothesis development of ultrasound interaction with fibrotic lungs
• Comparison of L* with histology and CT scoring

Chapter 6:

• Introduction to edema and micro-architectural changes due to edema
• Hypothesis development of ultrasound interaction with edematous lungs
• Backscattererd frequency shift as a parameter for unequivocal detection of fibrosis and edema
• Observation of super coherence
• Large animal results and compliance measurements

Chapter 7:

• Quantitative ultrasound in cortical bone
• Application of ANN for inverse problem solving
• Micro-architecture parameter prediction of cortical bone.

8.2 Key Contributions

Scattering fundamentally limits ultrasonic inspection of biological tissue. It restricts the achievable performance of ultrasonic imaging in biological tissues such as the lung parenchyma and bone. Whilst there seems to be no direct solution, quantitative ultrasound has stepped up to provide unique parameters such as diffusion constant and mean free paths that may help us characterize such complicated biological tissues. Multiple scattering, which earlier was considered random coda could now be post-processed in a unique way to extract information about the domains it has traveled in. This thesis revolves around understanding how multiple scattering is an indication of the diffusivity of the wave and then extracting relevant information from the multiple scattering coda. Previous studies within this field often relied on single scattering assumptions and generating images from single scattering signals, and thus limiting us
to relatively ‘weak’ scattering regimes. It is now evident that in order to advance quantitative ultrasound characterization of biological tissues, we must also consider multiple scattering environments.

The approach described in Chapter 2 is validated with partially saturated melamine foams. The dependence of diffusion constant with the volume fraction of air in the sponge is established. The methodology developed in Chapter 3 is believed to be the first delivery of a near field 1-D diffusion mapping methodology for quantitative characterization of highly heterogeneous media with strong scatterers. This methodology provides a robust quantitative evaluation of multiple scattering media with the ability to identify lesions or targets in the 1-D transducer axis space. Other features of the 1-D mapping were also introduced such as generating a 2-D diffusion map using coherent compounding with 2 perpendicular IRM acquisition and its application to intravascular circular probes. This enabled us to establish future advances with objective measures of the progress that new methods achieve.

Whilst the 1-D mapping was a success, 2-D imaging with access to the depth using diffusivity was still a challenge given the large amounts of multiple scattering and memory effect. In Chapter 4, we attempted to image the local diffusivity in the transducer axis and in the depth axis by identifying local fluctuations in the variance and combining it with a depression detection filter. This methodology showed promise in accurately predicting the location of the lesion; however, its accuracy while predicting the boundaries and the size is still up for improvement. For the first time, a computational study of the memory effect was also introduced which showcased the memory behavior during multiple scattering. This memory effect is a hindrance in isolating the lesion location along the depth axis. It remained uncertain whether this 2-D imaging methodology could potentially image in geometries with air volume
fraction greater than 20% and for lesions located at a depth greater than 20 mm and lesions smaller than 6 mm.

After establishing the methodology of characterizing sponge phantom, it was of paramount importance to check for its feasibility to the quantitative characterization of lung pathologies such as edema and fibrosis. These questions were answered in Chapter 5 and 6 where the mean free path is revealed to capture the micro-architectural changes that occur in the lungs due to edema and fibrosis. A roadblock was met when the $L^*$ values of edema and fibrosis seemed to lie in the same region denoting a similar scattering regime. The effective drop in the porosity of the lungs was not enough to characterize edema or fibrosis. The diffusion approach combined with the backscattered frequency shift was able to provide an unequivocal identification of edema and fibrosis using the diffusion methodology. The diffusion approach in general was shown to be successful, and could potentially aid in characterizing complex biological tissues and other heterogeneous media by the interpretation of multiple scattering coda in the future.

The focus was then shifted from characterizing lung tissue using quantitative ultrasound to characterize the cortical bone using quantitative methods. An ANN was trained on FDTD simulated data of ultrasound propagation cortical bone mimicking structures with controlled porosity ($\nu$), pore diameter ($\phi$) and pore density ($\rho$). The ANN was trained for mono-disperse (created using a Monte Carlo approach) and poly-disperse (obtained from human femur CT scans) structures. The two structures were then combined and a unified ANN was established. The trained model, taking the ultrasonic attenuation data as the input feature vector, predicted the micro-architectural properties.
8.3 Thesis Publications, Submitted Papers and Future Submissions


[7] K. Mohanty, and M. Muller, “Lesion Imaging and Target detection in Multiple Scattering (LITMUS) Media,” submitted to IEEE UFFC.
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APPENDICES
A- Separation of Single and Multiple Scattering

A.1 Abstract

When ultrasonic waves are propagating in a heterogeneous media, they contain single scattering (SS) and multiple scattering (MS) contributions. Traditional ultrasound imaging is completely dependent on single scattering. However, the multiple scattering contributions cannot be ignored. Especially when one is dealing with quantitative characterization of biological tissues.

In order to separate the SS and MS, we replicate a random matrix theory approach based on the work done by Aubry in 2011 (Insert Citation). The IRM is acquired using the method described in chapter 2. The SS contributions show a peculiar deterministic coherence along the anti-diagonals of the IRM, no matter what the distribution of the scatterers are. This deterministic coherence is taken advantage of and using a singular value decomposition (SVD) approach, the SS contribution is isolated. This methodology is shown for a melamine foam at 20% air fraction. The data is acquired at 5.1 MHz using a L7-4 128 element linear array connected to a vantage Verasonics. This appendix shows the math and the numerical modeling that goes behind separating the SS and MS contribution.

A.2 Introduction

Standard imaging works based on echolocation. Several sources emit an ultrasound wave into a medium. As the wave propagates, due to the impedance mismatch, it is reflected by the inhomogeneities or the scatterers in that media. The reflected waves are then measured by the same ultrasound transducers which then converts the time signal to intensity and generates traditional images. In single scattering, the incident wave undergoes only one scattering event before coming back to the ultrasound probe. Due to the linear relationship between distance and time $t = \frac{2d}{c}$ ($c$ is the sound velocity), the SS is used for image generation. A multiple scattering contribution
occurs when the wave undergoes multiple scattering events before reaching the ultrasound probe. When the extent of heterogeneity is very high, the multiple scattering contributions ruin the ability to image that media and cannot be ignored. Thus, classical imaging fails in multiple scattering media [4–7]. Standard imaging techniques rely on the single-scattering assumption (first Born approximation). Recently, an original technique was demonstrated to separate the multiple scattering from the single scattering contributions. The method relied on SVD to find the cut-off Eigen vector which would separate the single and multiple scattering contribution. In this chapter we apply the separation technique to a sponge phantom to see if we can isolate the SS and MS contributions. The method is based on a random matrix approach. The interest of this work is twofold. First, once single and multiple scattering contributions are isolated, we can use the SS contribution to simply measure the diffusion constant. Second, using a ratio of the MS and SS contributions we want to see if 2-D imaging is possible in strongly scattering media, which is discussed in Appendix B. The experimental results we present here is from a sponge phantom, which is completely saturated with water and then shaken to expel water and obtain 20% air fraction. We operate at a 5.1 MHz frequency connected to the vantage Verasonics.

**A.3 Methodology and Algorithm**

For calculation ease, rather than using N=128 elements, we use N=125 elements for emission and reception since N+3 is a multiple of 4. This reasoning will be understood better in the upcoming explanation.

**Step 1 : Data Acquisition (Refer Chapter 2 or Chapter 3)**

An IRM is acquired for a sponge phantom of 20% air volume fraction using an L7-4v linear array transducer.

**Step 2 : Conversion to Frequency domain and Time Windowing**
One the IRM H(t) has been acquire dimensions of which are 125 x 125 x Time, the following needs to be done. The time signals \( h_{ij}(t) \) were then truncated in 1.2 µs overlapping windows:

\[
k_{ij}(T, t) = h_{if}(T + t) * W_R(t) \quad (1)
\]

with \( W_R(t) = 1 \) for \( t = [0, 1.2 \mu s] \) and \( W_R(t) = 0 \) elsewhere.

Figure A.1: Experimental/Simulation setup showcasing an N element array placed in the near-field of the multiple scattering medium.

A short time FFT provided a response matrix called \( K(T, f) \) for each time window T. Each element of \( K(T, f) \) is represented as \( k_{ij}(T, f) \) corresponding to the responses at the frequency \( f \) and time \( T \) between the emitter location \( i = X_E \) and the receiver location \( j = X_R \) as shown in Figure 1. As an example, the \( K \) matrix at a given time window T and frequency exhibits coherence along its anti-diagonals as shown in Figure 2.
Figure A.2: Real part of matrix K obtained in a sponge at time T = 27 µs and 4.9 MHz

\( K(T, t) \) converted to \( K(T, f) \) whose elements are given by \( k_{ij}(T, f) \), where for the sake of simplicity we also say that

\[ k_{ij}(T, f) = k_{ij}^S(T, f) + k_{ij}^M(T, f) \]  \hfill (2)

**Step 3 : Rotation of matrix data**

The key to separate these two components is the peculiar coherence of the \( K^S(T, f) \) along its antidiagonals. It consists of building two matrices \( A_1 \) and \( A_2 \)

\[
A_1 = [a_{1uv}] \text{ of dimension } (2M - 1) \times (2M - 1), \\
\text{such that } a_1[u, v] = k[u + v - 1, v - u + 2M - 1] \]  \hfill (3)

\[
A_2 = [a_{2uv}] \text{ of dimension } (2M - 2) \times (2M - 2), \\
\text{such that } a_2[u, v] = k[u + v, v - u + 2M - 1] \]  \hfill (4)

where \( M = (N + 3)/4 \). Here \( N = 125 \) and so \( M = 32 \) is an even number. \( A_1 \) and \( A_2 \) correspond to the antidiagonals of \( K(T, f) \). For \( A_1 \), dimensions are \( LA_1 = 2M - 1 \) and for \( A_2 \), dimensions are \( LA_2 = 2M - 2 \). We will invariably now use \( A \) for both \( A_1 \) and \( A_2 \) and \( L \) for both \( LA_1 \) and \( LA_2 \).
Step 4: SVD

The $A$ matrix can be split into its two components of SS and MS.

$$A = A^S + A^M \quad (5)$$

The SVD of $A$ is written as

$$A = U \Lambda V^* = \sum_{k=1}^{L} \lambda_k U_k V_k^* \quad (6)$$

However, since there are only $M$ non-zero Eigen values, we replace $L$ with $M$.

$$A = U \Lambda V^* = \sum_{k=1}^{M} \lambda_k U_k V_k^* \quad (7)$$

Step 5: Cutoff Eigen Value for determination of SS and MS contributions

This step aims at identifying the cut off Eigen value at which the intensity can be separated into the SS and MS contribution. This iterative process is shown in the flow chart in Figure 3 below.
Figure A.3: Principle of the separation between the single and multiple scattering contributions

**Step 6 : Obtaining $K^{S,M}$ from $A^S$ and $A^M$**

The third step is the reverse of the third step. From $A^S$ and $A^M$, two matrices $K^S$ and $K^M$, are built with a change of coordinates.

- if $(i - j)/2$ is an integer, then, $k^{S,M}[i, j] = a_1^{S,M} [(i - j)/2 + M,(i + j)/2]$

- if $(i - j)/2$ is not an integer, then, $k^{S,M}[i, j] = a_2^{S,M} [(i - j - 1)/2 + M,(i + j - 1)/2]$

$K^S$ contains the single scattering contribution (plus a residual multiple scattering contribution) and $K^M$ contains the multiple scattering contribution.
Step 7: Obtaining Backscattered Intensity $I^S$, $I^M$ and $I^{Total}$

The backscattered intensities can be obtained using the equation 8

$$I^{S,M,Tot} = \langle |k_{i,j}^{S,M,Tot}(T,f)|^2 >_{f,(i,j)|m=j-i} \rangle (8)$$

The above equation denotes that the amplitudes in the $K$ matrix and squared, summed over all frequencies and averaged over all source/receiver couples separated by the same distance $X$.

A.4 Validation Results

The single and multiple scattering contributions can be written as $S$(Signal) and $N$(noise) subspace. However, in our case, the Noise $N$ is actually the multiple scattering contribution. The multiple scattering is not random in our case and has a high contribution to the total intensity. Shown in Figure 4 is the ratio of the Multiple scattering to single scattering plotted against time. We see that MS has a large contribution to the total intensity making it clear that this is not noise and neither is it random.

![Figure A.4: Ratio of MS and SS over time](image-url)
Shown in Figure 5 are the normalized backscattered intensities (SS, MS and Total) obtained for the sponge phantom. We can clearly see the growing diffusive halo which can be mapped using a growing variance and the diffusion constant can be evaluated.

![Figure A.5: Backscattered Intensities for the sponge case.](image)

A.5 MATLAB Code

```matlab
clc;
clear all;
filename=('data_L7_4_20171107T130649.mat');
load (filename);
Rf=Rf(:,1:125,1:125);
index=size(Rf);
num_el=index(2);
Rf=permute(Rf,[3 2 1]);
sig_beg=500;% beginning of the relevant signal in points
sig_end =4992;
thresh_crit=0.5;
fc=5.1;
dt=1/62.5; % time step
```
duree=1.4; % duration of time windows in micros
c=1.5; % average speed of sound
trans_size = 0.3;
probe_size = index(2)*trans_size;

%% Time Shifting
H1=Rf(:, :, sig_beg:sig_end-500);

%% TimeWindow Creation
nbt0=length(H1); % duration of the signal in points
fe=1/dt; % sampling frequency
deb=1; % beginning of time windows in number of points (leave at 1)
inter=duree/2; % interval between successive time windows in micros
debut=deb+inter*fe; % debut des fenetres temporelles en nombre de points
fin=deb+floor(duree*fe); % fin des fenetres temporelles en nombre de points
nbt=length(debut(1):fin(1)); % duree de la fenetre
f = fe*(0:(nbt/2)-1)/nbt;

%% Generating the K Matrix
for i=1:size(debut,2)
  for ll=1:size(H1,1)
    for mm=1:size(H1,2)
      temp=fft(squeeze(H1(ll,mm,debut(i):fin(i))));
      K_Caps{ll,mm}(i,:)=temp;
    end
  end
end

%% Visualize
for ll=1:size(H1,1)
  for mm=1:size(H1,2)
    freq_zone=8;
    time_zone=40;
    visualize(ll,mm) = K_Caps(ll,mm)(time_zone,freq_zone);
  end
end
imagesc(real(visualize));caxis([-10000 10000])
colormap gray
xlabel('i'); ylabel('j');
set(gca,'FontSize',25)

%%
M=(num_el+3)/4;
A1=zeros(2*M-1,2*M-1,size(debut,2),nbt);
for i =1:2*M-1
  for j=1:2*M-1
    A1(i,j,:,:)=K_Caps{i+j-1,j-i+2*M-1}(:,:,);
  end
end
A2=zeros(2*M-2,2*M-2,size(debut,2),nbt);
for i =1:2*M-2
  for j=1:2*M-2
    A2(i,j,:,:)=K_Caps{i+j,j-i+2*M-1}(:,:,);
  end
end
end
LA1=2*M-1;
LA2=2*M-2;
%%
for ii=1:size(debut,2)
    for kk=1:nbt
        lambdaA1{ii,kk}(:)=svd(A1(:,:,ii,kk));
        lambdaA2{ii,kk}(:)=svd(A2(:,:,ii,kk));
    end
end
%%
for ii=1:size(debut,2)
    for kk=1:nbt
        q=1;
        while q<=32
            lambdamaxA1_Q=1+sqrt((M-q+1)/LA1);
            lambdaA1_Norm_Q= lambdaA1{ii,kk}(q)/sqrt(sum(lambdaA1{ii,kk}(q:M).^2)/(M+1-q));
            if lambdaA1_Norm_Q> lambdamaxA1_Q
                q=q+1;
            else
                rankofseparationA1(ii,kk)=q-1;
                break
            end
        end
    end
end
for ii=1:size(debut,2)
    for kk=1:nbt
        q=1;
        while q<=32
            lambdamaxA2_Q=1+sqrt((M-q+1)/LA2);
            lambdaA2_Norm_Q= lambdaA2{ii,kk}(q)/sqrt(sum(lambdaA2{ii,kk}(q:M).^2)/(M+1-q));
            if lambdaA2_Norm_Q> lambdamaxA2_Q
                q=q+1;
            else
                rankofseparationA2(ii,kk)=q-1;
                break
            end
        end
    end
end
%%
for ii=1:size(debut,2)
    for jj=1:nbt
[UTempA2 STempA2 VTempA2]=svd(A2(:,:,ii,jj));

A1_Temp_Summation=zeros(2*M-1,2*M-1);
A2_Temp_Summation=zeros(2*M-2,2*M-2);

for tt=1:rankofseparati
    A1_Temp=STempA1(tt,tt)*UTempA1(:,tt)*VTempA1(:,tt)'
    A1_Temp_Summation=A1_Temp+ A1_Temp_Summation;
end

for tt=1:rankofseparationA2(ii,jj)
    A2_Temp=STempA2(tt,tt)*UTempA2(:,tt)*VTempA2(:,tt)'
    A2_Temp_Summation=A2_Temp+ A2_Temp_Summation;
end
A1_SS(:,:,ii,jj)=A1_Temp_Summation;
A2_SS(:,:,ii,jj)=A2_Temp_Summation;
end
A1_MS = A1-A1_SS;
A2_MS=A2-A2_SS;

for i =1:2*M -1
    for j=1:2*M -1
        if mod((i-j)/2,1)==0
            K_Single(i,j,:,:)=A1_SS((i-j)/2+M,(i+j)/2,:,:);
            K_Multiple(i,j,:,:)=A1_MS((i-j)/2+M,(i+j)/2,:,:);
        else
            K_Single(i,j,:,:)=A2_SS((i-j-1)/2+M,(i+j-1)/2,:,:);
            K_Multiple(i,j,:,:)=A2_MS((i-j-1)/2+M,(i+j-1)/2,:,:);
        end
    end
end
K_Total=K_Single+K_Multiple;

figure
subplot 121
imagesc(real(K_Single(:,:,40,8)));caxis([-10000 10000])
colormap gray
xlabel('i'); ylabel('j');
set(gca,'FontSize',25)
subplot 122
imagesc(real(K_Multiple(:,:,40,8)));
colormap gray
xlabel('i'); ylabel('j');
set(gca,'FontSize',25)

%%
XSpread=(-size(A1_Temp)+1:size(A1_Temp)-1);
K_Single_Abs=abs(K_Single).^2;
K_Multiple_Abs=abs(K_Multiple).^2;
K_Total_Abs=abs(K_Total).^2;

I_MS=zeros(length(XSpread),size(debut,2));
I_SS=zeros(length(XSpread),size(debut,2));
I_Total=zeros(length(XSpread),size(debit,2));

%%
for ii = 1:size(debit,2)
    for ll=1:size(XSpread,2)
        counter=0;
        data_MS_Temp=0;
        data_SS_Temp=0;
        data_Total_Temp=0;
        for jj=1:LA1
            for kk=1:LA1
                if jj-kk==XSpread(ll)
                    counter=counter+1;
                    data_MS_Temp=data_MS_Temp+sum(K_Multiple_Abs(jj,kk,ii,:));
                    data_SS_Temp=data_SS_Temp+sum(K_Single_Abs(jj,kk,ii,:));
                    data_Total_Temp=data_Total_Temp+sum(K_Total_Abs(jj,kk,ii,:));
                end
            end
        end
        I_MS(ll,ii)=data_MS_Temp/counter;
        I_SS(ll,ii)=data_SS_Temp/counter;
        I_Total(ll,ii)=data_Total_Temp/counter;
    end
end

for i=1:size(debit,2)
    I_MS_Norm(:,i)=I_MS(:,i)/max(I_MS(:,i));
    I_SS_Norm(:,i)=I_SS(:,i)/max(I_SS(:,i));
    I_Total_Norm(:,i)=I_Total(:,i)/max(I_Total(:,i));
end

%%
for i = 1:size(debit,2)-1
    for j=1:2*M-1
        for k=1:2*M-1
            tempsignal_Single =squeeze( ifft(K_Single(j,k,i,:)));
            tempsignal_Multiple =squeeze( ifft(K_Multiple(j,k,i,:)));
            Rf_Single(j,k,(i-1)*nbt/2+1:i*nbt/2)=tempsignal_Single(1:nbt/2);
            Rf_Multiple(j,k,(i-1)*nbt/2+1:i*nbt/2)=tempsignal_Multiple(1:nbt/2);
        end
    end
end
for i = 1:size(Rf_Single,1)
    tempsingle=Rf_Single(i,i,:);
    tempsingle=mag2db(abs(hilbert(tempsingle)));
    tempmultiple=mag2db(abs(hilbert(Rf_Multiple(i,i,:))));
    Bmode_Single(i,:)=tempsingle;
    Bmode_Multiple(i,:)= tempmultiple;
end

%% Cone Image Generation

X_Spread_Post_CoordChange=linspace(-probe_size/sqrt(2),probe_size/sqrt(2),size(I_MS_Norm,1));
Depth_Spread = debut*dt;
subplot 121
imagesc(Depth_Spread,X_Spread_Post_CoordChange,I_MS_Norm);
colormap hot
xlabel('Time(\mus)'); ylabel('Normalized Intensity MS');
set(gca,'FontSize',25)

subplot 122
imagesc(Depth_Spread,X_Spread_Post_CoordChange,I_SS_Norm);
colormap hot
xlabel('Time(\mus)'); ylabel('Normalized Intensity SS');
set(gca,'FontSize',25)

subplot 133
imagesc(Depth_Spread,X_Spread_Post_CoordChange,I_Total_Norm);
colormap hot
xlabel('Time(\mus)'); ylabel('Normalized Intensity Tot');
set(gca,'FontSize',25)
B.1 Methodology and Algorithm

Step 1: Data Acquisition

For the data acquisition process, we consider an $N$ element linear array transducer ($N=128$). The transducer was placed at a distance of 2mm (near-field) from the edge of the random medium. A 2 cycle Gaussian pulse with central frequency 5MHz was transmitted from the emitter $i$ and received by all the transducers $j=1:N$. This enabled the acquisition of an Inter-element response matrix (IRM). The IRM is represented by $H(t)$ whose dimensions are $N^2*t$, the individual elements of which are $h_{ij}(t)$. The individual element $h_{ij}(t)$ are the $N^2$ inter-element responses of the probe-medium system.

Once the IRM has been obtained, the IRM was split into sub-IRMs with number of elements $P$ (Odd number only such that $P+3$ is divisible by 4) ($P = 41$). Let us assume that for all explanation purposes $P=41$ and $N=128$. When the IRM is split into sub-IRMs, we refer to each of those as $H_z(t)$ where $z$ ranges from $\frac{P+1}{2}$ to $\frac{2N-P+1}{2}$ ($17 \leq z \leq 80$). Hence the first the sub-IRM will be referred to as $H_{21}(t)$ and each element of this sub-IRM is $h_{21,i}(t)$ where $i$ ranges from $1:41$ and $j$ from $1:41$. Hence the dimension of $H_{21}(t)$ is $41^2*41*t$. The superscript in $h_{21,i}(t)$ has been set to 21 since that is the central element of the sub-IRM, and that is where the counting would begin from. If $P=37$, the counting would start from 19.

$H_{21}(t)$ when further processed (see below), will give the growth of the diffusive halo in the area in front of the transducer element number 21. This is equivalent as having a small linear array with 41 elements translated across the medium to get a semi-local assessment of the diffusive properties. This is described in Figure B.1. It should be noted that the region in front of elements...
1-20 and 109-128 will have no representation by any sub-IRMs and will remain inaccessible for image generation and backscattered intensity calculation.

Figure B.1: Schematic diagram of splitting the IRM (H(t)) in to sub-IRMs (H_s(t))

**Step 2 : Processing each sub-IRM**

For P=41, we obtain 88 sub-IRMs, with each sub-IRM having the potential to generate information about the Rf data in the depth axis, in front of the central element of that corresponding sub-IRM. For each sub-IRM the data is processed as shown in Appendix A. Corresponding to appendix A, each sub-IRM has a dimension of 41*41*T. For each sub-IRM, M=(P+3)/4=11. The exact same process is followed where the sub-IRM is converted to a corresponding (T, f). Separating the SS and MS contributions gives us access to the normalized backscattered intensities in the SS, MS and Total backscattering regimes.
MATLAB Implementation.

For dd=1:N-P+1
parameter=cone_SS.^-1; \textit{(Ratio of MS and SS)}
ImageNorm = parameter;
ImageNorm=imgaussfilt(ImageNorm,2);
ImagePrelim=ImageNorm;
Image = ImagePrelim(:,beg_data:end_data); \textit{(Defining ROI in the time domain)}
LineImageSS(dd,:)=mean( Image((resolution+1)/2 -(el_con-1)/2:((resolution+1)/2+(el_con-1)/2,:),1); \textit{(Taking standard deviation at each time window of the line vector which defines the geometric spread of the ratio of MS and SS )}
LineImageSS(dd,:)=LineImageSS(dd,:)/max(LineImageSS(dd,:));
end

\textbf{Step 3 : Extracting Lesion Location from 88 Rf Lines}
MATLAB Implementation.

ImageNormalized_PreProcess= LineImageSS /max(max(LineImageSS));

ImageNormalized =imgaussfilt(ImageNormalized_PreProcess,2);
ImageNormalized =wiener2(ImageNormalized_PreProcess,[2 2]);

[X11 , X22]=findDepressions(ImageNormalized); \textit{(Calculating closed loop depressions formed which signify the existence of a lesion)}
X22=medfilt2(X22,[4 4]);

\textbf{for} zz=1:index(1) \textbf{(Remove NAN values)}
\textbf{for} tt=1:index(2)
\textbf{if} isnan(X22(zz,tt))
X22(zz,tt)=0;
\textbf{end}
\textbf{end}
\textbf{end}

X22=X22/max(max(X22));

\textbf{for} zz=1:index(1)
\textbf{for} tt=1:index(2)
\textbf{if} X22(zz,tt)<threshold
X22(zz,tt) = 0;
\textbf{end}
\textbf{end}
\textbf{end}

\textbf{for} i =1:size(X22,1)
\textbf{temp} = X22(i,:);
X22(i,:) = imgaussfilt(temp,4);
\textbf{end}

\textbf{for} i =1:size(X22,2)
\textbf{temp} = X22(:,i);
X22(:,i) = imgaussfilt(temp,4);
B.2 Sponge Nodule Detection

The IRM used here are the same that were used in chapter 4.

B.2.1 Lesion at 15mm Depth, 10Vf, Ceff=1.44 mm/μs

Figure B.2: Lesion at 15mm Depth, 10Vf
B.2.2 Lesion at 15mm Depth, 20Vf, Ceff=1.31 mm/μs

Figure B.3: Lesion at 15mm Depth, 20Vf

B.3 Application to Lung Nodule Localization

The lungs of one edematous Yucatan minipig were provided by Dr Tim Nichols (UNC Chapel Hill). A nodule was created by injecting Vaseline via a needle inserted through the apex of the lower lobe. The nodule was placed 15 mm below the lung surface. Ultrasonic data was acquired and processed similarly to the phantom study. IRM was acquired using an L7-4v operating at 5.1MHz. CT imaging was performed to visualize the nodule. Ceff was used as 1.1mm/μs. The Vaseline nodule, although not a cancerous lesion, has in common with actual tumors that it is a solid area free of alveoli. A conventional ultrasound B-mode image is presented for reference (Figure B.8). The nodule is not visible on the B-mode, but is clearly visible on the picture reconstructed with the proposed method (Figure B.9). This justifies the need for a new ultrasound-
based method to image PNs. The diameter of the nodule was 9.8 mm on the reconstructed ultrasound MS picture and 11.3 mm on the CT. Ultrasound Nodule Depth was 12.04mm compared to the CT Scan Nodule Depth which was 14.9mm. This discrepancy was observed probably due to inflation of the lungs before CT.

A major challenge in nodule imaging is the attenuation and the depth of penetration of the ultrasonic waves. Based on the study done by Dunn and Fry\textsuperscript{3,4,91} the attenuation is very high in the lung parenchyma, even when the lung is collapsed under atmospheric pressure. Ultrasonography
during thoracoscopy to direct the resection of GGO was successful and can get good-quality ultrasound images in 56% of patients\textsuperscript{89}. However these high number were attributed to two reasons. Firstly, these lesions were at the periphery or adjacent to the pleural line. Secondly deeper GGOs were identified using ultrasound using completely collapsed lungs. GGO is similar to adjacent normal lung tissue in density and thus localizing them with ultrasonography is hard, even for experienced chest surgeons. In a healthy, partially inflated lung, ultrasonography has been deemed unusable. Some pilot studies have measured the chance of detecting tiny pulmonary lesions including GGO with ultrasonography to define the limitation as well as help surgeons improve their skills\textsuperscript{90}. Based on the study done by Dunn and Fry\textsuperscript{3,4,91} the attenuation is very high in the lung parenchyma, even when the lung is collapsed under atmospheric pressure. Many of the clinical reports on intraoperative thoracic ultrasonography emphasize the need for thorough deflation of the lungs or atmospheric collapse of the lungs\textsuperscript{82,92–97} However the goal of this algorithm is to be able to localize lesion for VATS in the lungs either at the residual volume or simply at atmospheric collapse which is a common clinical practice.